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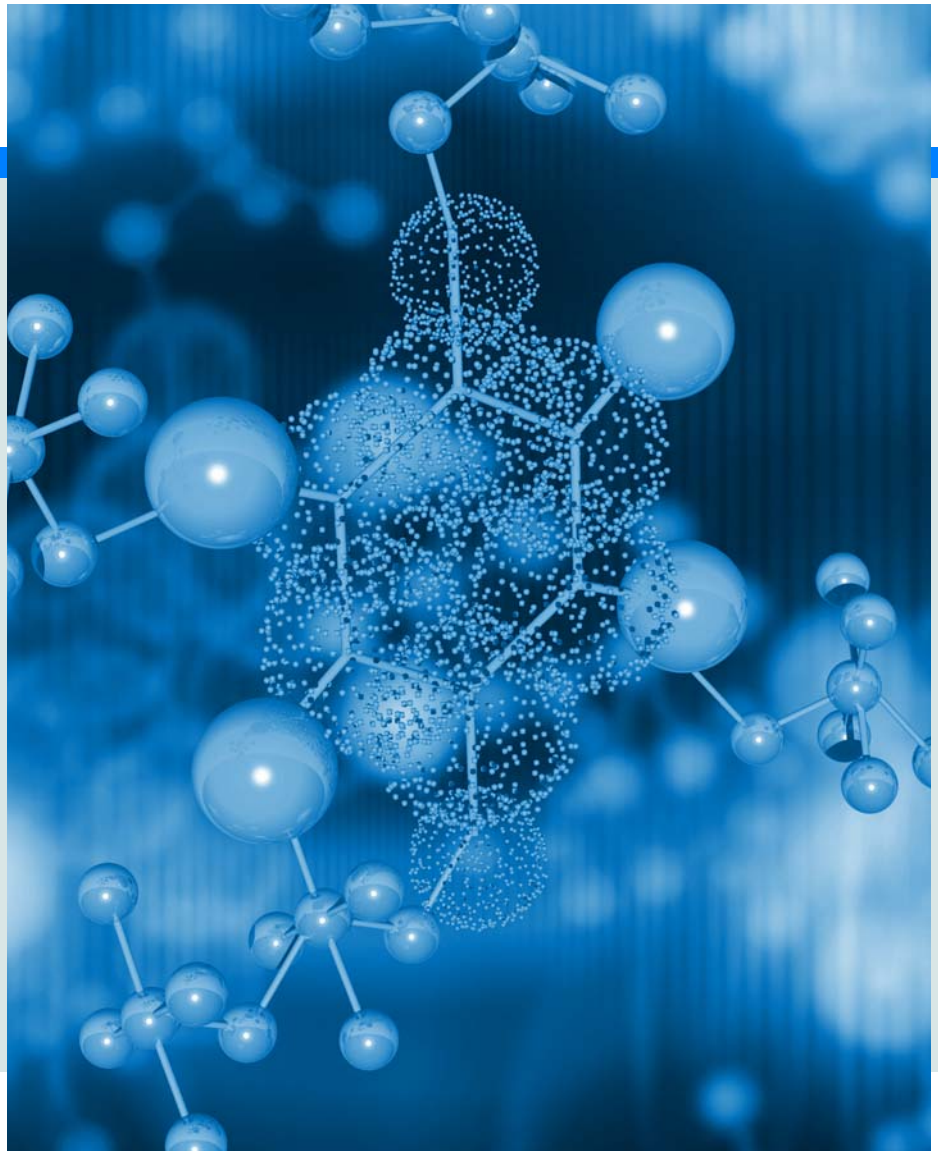

SHELXTL

• Software Reference Manual

Part Number: 269-015901

Publication date: November 1997

think forward

SCD

This document covers the SHELXTL software program.

References to this document should be shown as 269-0159xx SHELXTL Software Reference Manual.

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1 Introduction to SHELXTL

SHELXTL is an integrated system for the determination of crystal structures from diffraction data. The programs have been highly optimized to minimize calculation times, memory, and disk space requirements. All calculations are valid for all space groups, in conventional settings or otherwise, and there is almost no limit on the number of reflections per structure. The number of unique atoms is limited to 5000 for structure refinement and 10000 or more for all other calculations.

SHELXTL is based on the widely used public domain SHELX-97 program system. It contains a very large number of improvements and additional facilities (e.g., automatic space group determination, absorption corrections, statistical analysis of reflection data, and in particular extensive interactive molecular graphics).

SHELXTL is available for a variety of computer and operating systems including PCs running MS-DOS, Windows NT or Linux, as well as UNIX systems including Silicon Graphics and IBM workstations. The user interface is designed to have as similar a “look and feel” as possible on these different systems; the major differences are the resolution of the monitor and the appearance of the text font. The MS-DOS and UNIX versions are both designed to be able to read UNIX and DOS format files, thus making the use of shared disks (e.g., on NFS networks) very simple. The NT version is run under the control of the Bruker XSHELL user interface, and so NT-specific questions are discussed in 269-0230xx XSHELL User Manual.

Version 5 of SHELXTL was a major rewrite in order to incorporate and exploit modern structure solution and refinement algorithms and recent advances in computer technology. Version 5.1 includes advances in refinement techniques and a new program XPRO that acts as an interface for protein applications. The 1997 requirements for the submission of papers to Acta Cryst. in CIF format are also fully implemented. Although the programs are primarily designed for use with the Bruker line of X-ray diffractometers and area detectors, they can also be used with any other source of single crystal diffraction data.

2 SHELXTL Programs and Files

SHELXTL consists of five major programs which use files of the type ‘name.ext’, where ‘name’ is a name of up to eight characters describing the structure or problem, and ‘ext’ is a three-character filename extension. Files for a given problem always have the same ‘name’ but different extensions. These extensions define the function of each file and are generated or recognized automatically by all the SHELXTL programs.

The major programs are:

- **XPREP —** Automatic space group determination, absorption corrections, scaling and merging of different datasets, index transformations, reflection statistics, reciprocal space plots and contoured Patterson sections. XPREP reads the raw data file name.raw and the parameter file name.p4p written by the the Bruker XSCANS diffractometer control program or the Bruker SMART CCD-detector system, and writes the crystal data file name.ins and reflection data file name.hkl for use by the programs XS and XL. In addition, XPREP writes a log of all operations to the file name.prp and writes a CIF format file name.pcf.
- **XS —** Structure solution by ‘phase annealing’ direct methods or automated Patterson interpretation based on superposition minimum functions. XS reads name.hkl and name.ins and writes the solution in the form of crystal data plus an atom list to the file name.res and a listing file name.lst.
- **XL —** Least-squares structure refinement. XL reads name.ins (obtained by editing the name.res file from XS or a previous XL job, e.g.,via XP) and writes a new results file name.res and listing file name.lst. In addition XL can produce the CIF format files name.cif (crystal data, atoms and refinement results) and name.fcf (observed and calculated structure factors).
- **XP —** Interactive molecular graphics and publication quality diagrams. XP reads the file name.res from structure solution and refinement and can write name.ins for the next refinement run. XP also creates plotfiles (name.plt), save files name.sav (local snapshot files of current XP parameters) and orthogonal coordinate files name.ort.
- **XCIF —** Preparation of tables for publication via CIF format. XCIF reads the CIF format files name.cif, name.pcf and name.fcf and writes tables either directly to the printer or to a file name.tex.
- **XPS —** Structure solution by fragment search. XPS reads name.inp (similar to name.ins) and name.pat (Patterson file written by XS) and writes the results to name.rep (crystal data and atoms) and a listing file name.lst.
- **XPRO —** Interface for protein applications. XPRO provides extensive facilities for interacting with widely used protein programs and for analyzing the results of protein refinements using XL, e.g., in the form of Postscript plots.
- **XWAT —** This is a shell program that calls XL iteratively to perform 'automatic water divining' for macromolecules.

In addition, the program **SPRINT** is provided for printing on PCs.

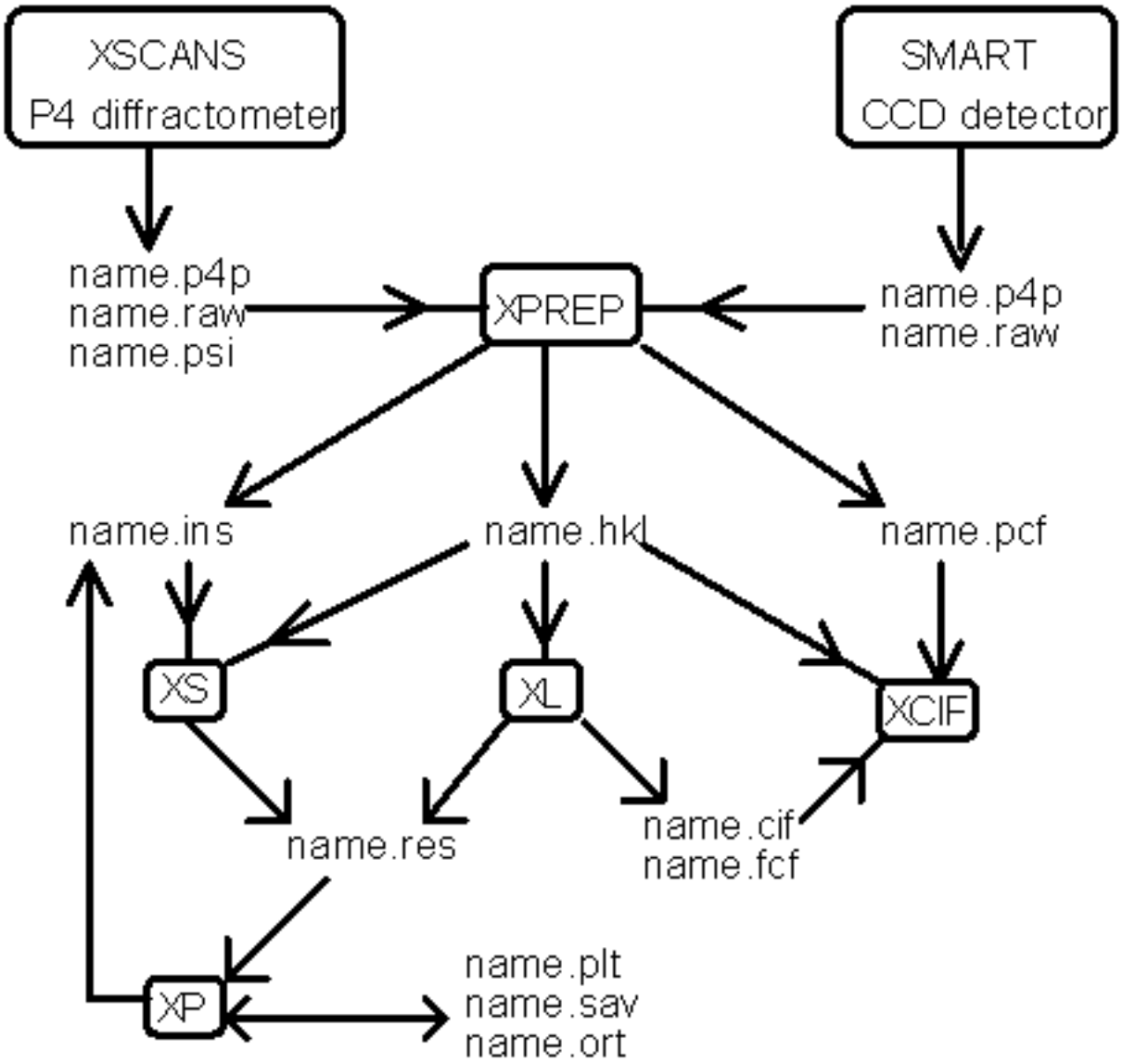

Figure 2.1 – Major SHELXTL programs and files (the listing file name.lst written by XS and XL and less frequently used programs and files are omitted for clarity). The protein interface program XPRO is able to read and write most of these files.

2.1 Program Organization (Illustrated for XL)

To run XL (or XS) only two input files are required (atoms/instructions and reflection data); since both these files and the output files are pure ASCII text files, it is easy to use the program on a heterogeneous network. The reflection data file (name.hkl) contains *h*, *k*, *l*, F^2 and $\sigma(F^2)$ in standard SHELXTL format (Section 2.3); the program merges equivalents and eliminates systematic absences; the order of the reflections in this file is unimportant. Crystal data, refinement instructions and atom coordinates are all input as the file name.ins; further files may be specified as “include files” in the .ins file (e.g., for standard restraints), but this is not essential. Instructions appear in the .ins file as four-letter keywords followed by atom names, numbers, etc. in free format; examples are given in the following chapters. There are sensible default values for almost all numerical parameters. XL is normally run on any computer system by means of the command:

```
xl name
```

where `name` defines the first component of the filename for all files which correspond to a particular crystal structure. On some systems, `name` may not be longer than 8 characters. Batch operation will normally require the use of a short batch file containing the above command etc. The executable program must be accessible via the “PATH” (or equivalent mechanism). No environment variables or extra files are required.

A brief summary of the progress of the structure refinement appears on the console, and a full listing is written to a file name.lst, which can be printed or examined with a text editor. After each refinement cycle a file name.res is (re)written; it is similar to name.ins, but has updated values for all refined parameters. It may be copied or edited to name.ins for the next refinement run. The MORE instruction controls the amount of information sent to the .lst file; normally the default MORE 1 is suitable, but MORE 3 should be used if extensive diagnostic information is required. The ACTA instruction produces CIF format files for archiving or electronic publication, and the LIST 4 instruction (generated automatically by ACTA) produces a CIF format reflection data file (name.fcf). For PDB deposition of macromolecular results, WPDB and LIST 6 should be used. The program XPRO should then be used to complete the PDB file.

Two mechanisms are provided for interaction with a XL job which is already running. The first is used by the MS-DOS and some other ‘on-line’ versions: if the [ctrl-I] key combination is hit, the job terminates almost immediately, but without the loss of output buffers etc. which can happen with [ctrl-C] etc. Usually the [Tab] key may be used as an alternative to [ctrl-I]. If the [Esc] key is hit during least-squares refinement, the program completes the current cycle and then, instead of further refinement cycles, continues with the final structure-factor calculation, tables and Fourier etc. Otherwise [Esc] has no effect. On computer consoles with no [Esc] key, [F11] or [Ctrl-[] usually have the same effect.

The second mechanism requires the user to create the file name.fin (the contents of this file are irrelevant); the program tries at regular intervals to delete it, and if it succeeds it takes the same action as after [Esc]. The name.fin file is also deleted (if found) at the start of a job in case it has been accidentally left over from a previous job. This approach may be used with batch jobs under most operating systems.

2.2 The .ins Instruction File

All instructions commence with a four (or fewer) character word (which may be an atom name); numbers and other information follow in free format, separated by one or more spaces. Upper and lower case input may be freely mixed; with the exception of the text string input using TITL, the input is converted to upper case for internal use in XL. The TITL, CELL, ZERR, LATT (if required), SYMM (if required), SFAC, DISP (if required) and UNIT instructions must be given in that order; all remaining instructions, atoms, etc. should come between UNIT and the last instruction, which is always HKLF (to read in reflection data).

A number of instructions allow atom names to be referenced; use of such instructions without any atom names means ‘all non-hydrogen atoms’ (in the current residue, if one has been defined). A list of atom names may also be abbreviated to the first atom, the symbol ‘>’ (separated by spaces), and then the last atom; this means ‘all atoms between and including the two named atoms but excluding hydrogens’.

2.3 The Reflection Data File name.hkl

The .hkl file consists of one line per reflection in FORMAT(3I4,2F8.2,I4) for h, k, l, F_o^2, $\sigma(F_o^2)$, and (optionally) a batch number. This file should be terminated by a record with all items zero; individual data sets within the file should NOT be separated from one another - the batch numbers serve to distinguish between groups of reflections for which separate scale factors are to be refined (see the BASF instruction). The reflection order and the batch number order are unimportant. This ‘.hkl’ file is read each time the program is run; unlike version 4 of SHELXTL, there is no facility for intermediate storage of binary data. This enhances computer independence and eliminates several possible sources of confusion. The .hkl file is read when the HKLF instruction (which terminates the .ins file) is encountered. The HKLF instruction specifies the format of the .hkl file, and allows scale factors and a reorientation matrix to be applied. Lorentz, polarization and absorption corrections are assumed to have been applied to the data in the .hkl file. Note that there are special extensions to the .hkl format for Laue and powder data, as well as for twinned crystals that cannot be handled by a TWIN instruction alone.

In general the .hkl file should contain all measured reflections without rejection of systematic absences or merging of equivalents. The systematic absences and R_{int} for equivalents provide an excellent check on the space group assignment and consistency of the input data. Since complex scattering factors are used throughout by SHELXTL, Friedel opposites should normally not be averaged in preparing this file; an exception can be made for macromolecules without significant anomalous scatterers. Note that XS always merges Friedel opposites.

2.4 Refinement Against F^2

XL always refines against F^2, even when F-values are input. Refinement against ALL F^2-values is demonstrably superior to refinement against F-values greater than some threshold [say $4\sigma(F)$]. More experimental information is incorporated (suitably weighted) and the chance of getting stuck in a local minimum is reduced. In pseudo-symmetry cases it is very often the weak reflections that can discriminate between alternative potential solutions. It is difficult to refine against ALL *F*-values because of the difficulty of estimating $\sigma(F)$ from $\sigma(F^2)$ when F^2 is zero or (as a result of experimental error) negative.

The diffraction experiment measures intensities and their standard deviations, which after the various corrections give F_o^2 and $\sigma(F_o^2)$. If your data reduction program only outputs F_o and $\sigma(F_o)$, you should correct your data reduction program, not simply write a routine to square the F_o values! It is also legal to use HKLF 3 to input F_o and $\sigma(F_o)$ to XL. Note that if an F_o^2 value is too large to fit format F8.2, then format F8.0 may be used instead. - the decimal point overrides the FORTRAN format specification.

The use of a threshold for ignoring weak reflections may introduce bias which primarily affects the atomic displacement parameters; it is only justified to speed up the early stages of refinement. In the final refinement ALL DATA should be used except for reflections known to suffer from systematic error (i.e., in the final refinement the OMIT instruction may be used to omit specific reflections - although not without good reason - but not ALL reflections below a given threshold). Anyone planning to ignore this advice should read Hirshfeld & Rabinovich (1973) and Arnberg, Hovmöller & Westman (1979) first. Refinement against F^2 also facilitates the treatment of twinned and powder data, and the determination of absolute structure.

2.5 Initial Processing of Reflection Data in XL

XL (and XS) automatically rejects systematically absent reflections. The sorting and merging of the reflection data is controlled by the MERG instruction. Usually MERG 2 (the default) will be suitable for small molecules; equivalent reflections are merged and their indices converted to standard symmetry equivalents, but Friedel opposites are not merged in non-centrosymmetric space groups. MERG 4, which merges Friedel opposites and sets δf" for all elements to zero, saves time for macromolecules with no significant dispersion effects. Throughout this documentation, F_o^2 means the EXPERIMENTAL measurement, which despite the square may possibly be slightly negative if the background is higher than the peak as a result of statistical fluctuations etc. R_{int} and R_{sigma} are defined as follows:

$$R_{int} = \Sigma \mid F_o^2 - F_o^2(\text{mean}) \mid / \Sigma [F_o^2]$$

where both summations involve all input reflections for which more than one symmetry equivalent is averaged, and:

$$R_{sigma} = \Sigma [\sigma(F_o^2)] / \Sigma [F_o^2]$$

over all reflections in the merged list. Since these R-indices are based on F^2, they will tend to be about twice as large as the corresponding indices based on F. The ‘esd of the mean’ (in the table of inconsistent equivalents) is the rms deviation from the mean divided by the square root of (n-1), where n equivalents are combined for a given reflection. In estimating the $\sigma(F^2)$ of a merged reflection, the program uses the value obtained by combining the $\sigma(F^2)$ values of the individual contributors, unless the esd of the mean is larger, in which case it is used instead.

For some refinements of twinned crystals, and for least-squares refinement of batch scale factors, it is necessary to suppress the merging of equivalent reflections with MERG 0.

2.6 Least-squares Refinement using XL

Small molecules are almost always refined by full-matrix methods (using the L.S. instruction in XL), which give the best convergence per cycle, and allows esd's to be estimated. The CPU time per cycle required for full-matrix refinement is approximately proportional to the number of reflections times the square of the number of parameters; this is prohibitive for all but the smallest macromolecules. In addition the (single precision) matrix inversion suffers from accumulated rounding errors when the number of parameters becomes very large. An excellent alternative for macromolecules is the conjugate-gradient solution of the normal equations, taking into account only those off-diagonal terms that involve restraints. This method was employed by Konnert & Hendrickson (1980) in the program PROLSQ; except for modifications to accelerate the convergence, exactly the same algorithm is used in XL (instruction CGLS). The CGLS refinement can be also usefully employed in the early stages of refinement of medium and large 'small molecules'; it requires more cycles for convergence, but is fast and robust. The major disadvantage of CGLS is that it does not give esds.

For both L.S. and CGLS options, it is possible to block the refinement so that a different combination of parameters is refined each cycle. For example after a large structure has been refined using CGLS (without BLOC), a final job may be run with L.S. 1, DAMP 0 0 and BLOC 1 (or e.g., BLOC N_1 > LAST for a protein) to obtain esds on all geometric parameters; the anisotropic displacement parameters are held fixed, reducing the number of parameters by a factor of three and the cycle time by an order of magnitude.

2.7 R-indices and Weights

One cosmetic disadvantage of refinement against F^2 is that R-indices based on F^2 are larger than (more than double) those based on F. For comparison with older refinements based on F and an OMIT threshold, a conventional index R1 based on observed F values larger than $4\sigma(F_o)$ is also printed.

$$wR2 = \{ \Sigma [w(F_o^2 - F_c^2)^2] / \Sigma [w(F_o^2)^2] \}^{1/2}$$

$$R1 = \Sigma | |F_o| - |F_c| | / \Sigma |F_o|$$

The Goodness of Fit is always based on F^2:

$$GooF = S = \{ \Sigma [w(F_o^2 - F_c^2)^2] / (n - p) \}^{1/2}$$

where n is the number of reflections and p is the total number of parameters refined.

The WGHT instruction allows considerable flexibility, but in practice it is a good idea to leave the weights at the default setting (WGHT 0.1) until the refinement is essentially complete, and then to use the scheme recommended by the program. These parameters should give a flat analysis of variance and a GooF close to unity [there was a bug in version 5.0 of XL that can occasionally cause the program to abort when trying to estimate the new weighting parameters, though it appeared to happen only with poor quality data or the wrong solution]. If the weights are varied too soon, the convergence may be impaired, because features such as missing atoms are ‘weighted down’. For macromolecules it may be advisable to leave the weights at the default settings; and to accept a GooF greater than one as an admission of inadequacies in the model.

When not more than two WGHT parameters are specified, the weighting scheme simplifies to:

$$w = 1 / [\sigma^2(F_o^2) + (aP)^2 + bP]$$

where P is $[2F_c^2 + \text{Max}(F_o^2,0)] / 3$. The use of this combination of F_o^2 and F_c^2 was shown by Wilson (1976) to reduce statistical bias.

It may be desirable to use a scheme that does not give a flat analysis of variance to emphasize particular features in the refinement, for example by weighting up the high-angle data to remove bias caused by bonding electron density (Dunitz & Seiler, 1973).

2.8 Fourier Syntheses

Fourier syntheses are summarized in the form of peak-lists (which can be edited and re-input for the next refinement job), or as ‘line printer plots’ with an analysis of non-bonded interactions etc. It is recommended that a difference electron density synthesis is performed at the end of each refinement job; it is quick and of considerable diagnostic value. In contrast to SHELX-76, XL finds the asymmetric unit for the Fourier synthesis automatically; the algorithm is valid for all space groups, in conventional settings or otherwise. Before calculating a Fourier synthesis, the Friedel opposites are always merged and a dispersion correction applied; a value of R1 is calculated for the merged data (without a threshold). Reflections with F_c small compared to $\sigma(F_o)$ are down-weighted in the Fourier synthesis. The rms density is calculated to give an estimate of the ‘noise level’ of the map.

2.9 The Connectivity Array

The key to the automatic generation of hydrogen atoms, molecular geometry tables, restraints etc. is the connectivity array. For a non-disordered organic molecule, the connectivity array can be derived automatically using standard atomic radii. A simple notation for disordered groups enables most cases of disorder to be processed with a minimum of user intervention. Each atom is assigned a ‘PART’ number *n*. The usual value of *n* is 0, but other values are used to label components of a disordered group.

Bonds are then generated for atoms that are close enough only when either (a) at least one of them has *n* = 0, or (b) both values of *n* are the same. A single shell of symmetry equivalents is automatically included in the connectivity array; the generation of equivalents (e.g., in a toluene molecule on an inversion center) may be prevented by assigning a negative 'PART' number. If necessary bonds may be added to or deleted from the connectivity array using the BIND or FREE instructions. To generate additional bonds to symmetry equivalent atoms, EQIV is also needed.

2.10 Tables

For small structures, bond lengths and angles for the full connectivity array may be tabulated with BOND, and all possible torsion angles with CONF. Although hydrogen atoms are not normally included in the connectivity array, they may be included in the bond lengths and angles tables by BOND $H. Alternatively HTAB produces a convenient way of analyzing hydrogen bonds. It is also possible to be selective by naming specific atoms on the BOND and CONF instructions, or by using the RTAB instruction (which was designed with macromolecules in mind). Least-squares planes and distances of (other) atoms from these planes may be generated with MPLA. Symmetry equivalent atoms may be specified on any of these instructions by reference to EQIV symmetry operators. All esds output by SHELXL take the unit-cell esds into account and are calculated using the full covariance matrix. The only exception is the esd in the angle between two least-squares planes, for which an approximate treatment is used. Note that damping the refinement (see above) leads to underestimates of the esds; in difficult cases a final cycle may be performed with DAMP 0 0 (no damping, but no shifts applied) to obtain good esds.

The HTAB instruction has been introduced in SHELXL-97 to analyze the hydrogen bonding in the structure. A search is made over all hydrogen atoms to find possible hydrogen bonds. This is a convenient way of finding the symmetry operations necessary for the second form of HTAB instructions (needed to obtain esds and CIF output), and also reveals potential misplaced hydrogens, e.g.,because they do not make any hydrogen bonds, or because the automatic placing of hydrogen atoms has assigned the hydrogens of two different O-H or N-H groups to the same hydrogen bond. In the second form of the HTAB instruction, HTAB is followed by the names of the donor atom D and the acceptor atom A; for the latter a symmetry operation may also be specified. The program then finds the most suitable hydrogen atom to form the hydrogen bond D-H•••A, and outputs the geometric data for this hydrogen bond to the .lst file and the .cif file (if ACTA is present).

3 SHELXTL Hardware and System Considerations

3.1 MS-DOS Version

SHELXTL requires a PC with at least 8MB main memory, one of the following SVGA graphics cards:

1. Tseng ET-3000

2. Tseng ET-4000 or ET-4000/W32

3. Western Digital WD90C31, Paradise or newer Cirrus

4. older Cirrus

5. ATI or

6. VESA (a software driver or the shareware “Display Doctor” driver may be required),

and a color monitor capable of 256 colors and a resolution of 800 × 600 (or better). A 486-DX or Pentium (or fully compatible) processor is required, and there should be adequate (i.e., at least 50 MB) free disk space; the version of MS-DOS should be 5.0 or later. Usually the programs will be able to ‘auto-sense’ which, if any, of the above graphics processors is present; occasionally it is necessary to overrule this by setting the environment variable `SAXI$GRAPHCARD` to the appropriate number from the above list (e.g., the line ‘`SET SAXI$GRAPHCARD=4`’ in the file AUTOEXEC.BAT would specify a Cirrus chip).

The environment variable `SXTL` should be set to the full path and name of the SHELXTL initialization file; if this environment variable is not set, it is assumed to be `C:\SAXI\SXTL\SXTL.INI`. The SXTL environment variable will normally be SET in the AUTOEXEC.BAT file, but the MS-DOS ‘SET’ command may also be used for testing purposes or to switch between different versions of this file, e.g., to switch between network printing on a color Deskjet and local printing on a Laserjet.

The initialization file defines the names of the three directories containing SHELXTL programs; the standard settings are:

- **C:\SAXI\SXTL** - All SHELXTL executables and the files sxtl.ini, xl.dic, xcif.def, xcif.ang, xcif.met and xcif.ger. This directory MUST be in the PATH (also defined in AUTOEXEC.BAT).
- **C:\SAXI\SXTL\HELP** - All SHELXTL help files. These ASCII files are intended for online browsing, and contain a subset of the information given in this manual. The file xp.hlp is also used by the HELP instruction in XP.
- **C:\SAXI\SXTL\EGS** - All example files, including those referred to in this Manual and those required for the XP demo loop.

3.2 UNIX Versions

SHELXTL should run on any Silicon Graphics system running IRIX 5.3 or later equipped with at least 32 MB main memory and an 8-bitplanes color graphics system. For IBM RS/6000 systems a '3D' (e.g., GT4) graphics adaptor with 8-bitplanes is required, with at least 32 MB main memory and AIX 3.2 or later.

The environment variable `SXTL` should be set to the full path and name of the SHELXTL initialization file; if this environment variable is not set it is assumed to be `/usr/saxi/sxtl/sxtl.ini`. The SXTL environment variable will normally be set in the in the global or local .profile files, but the UNIX `export` (Korn shell) or `setenv` (C shell) commands (see below) may also be used for testing purposes or to switch between different versions of this file, e.g., to switch between network printing on a color Deskjet and local printing on a Laserjet. The initialization file defines the names of the three directories containing SHELXTL programs; the standard settings are:

- **/usr/saxi/sxtl/** - All SHELXTL executables and the files sxtl.ini, xl.dic, xcif.def, xcif.ang, xcif.met and xcif.ger. This directory should either be in the PATH or aliases should be defined as indicated below.
- **/usr/saxi/sxtl/help/** - All SHELXTL help files. These ASCII files are intended for online browsing, and contain a subset of the information given in this manual. The file xp.hlp is also used by the HELP instruction in XP.
- **/usr/saxi/sxtl/egs/** - All example files, including those referred to in this manual and those required for the XP demo loop.

For the Korn shell, the following could be included at the end of the global (/etc/profile) or local (.profile) user initialization files:

```
set sxtl='/usr/saxi/sxtl/'
alias -x xp=$sxtl'xp'
alias -x xprep=$sxtl'xprep'
alias -x xs=$sxtl'xs'
alias -x xl=$sxtl'xl'
alias -x xcif=$sxtl'xcif'
alias -x xps=$sxtl'xps'
alias -x xpdb=$sxtl'xpdb'
export SXTL $sxtl'sxtl.ini'
```

whereas for the C shell the following should be inserted in /etc/cshrc (global) or .cshrc (local):

```
set sxtl='/usr/saxi/sxtl/'
setenv SXTL $sxtl'sxtl.ini'
alias xp $sxtl'xp'
alias xprep $sxtl'xprep'
alias xs $sxtl'xs'
alias xl $sxtl'xl'
alias xcif $sxtl'xcif'
alias xps $sxtl'xps'
alias xpdb $sxtl'xpdb'
unset sxtl
```

3.3 Printers and Plotters

The programs XP and XPREP are able to output raster plots to printers compatible with HP LaserJet and DeskJet but not PaintJet printers. Installation-dependent parameters (e.g., remote printing over a network) are set in the sxtl.ini file.

4 Reciprocal Space Exploration

The XPREP program provides extensive menu-driven facilities to aid the user in determining the correct cell and space group and to prepare the files for structure determination. It was written specially for SHELXTL and has no public domain counterpart. XPREP is designed to process data from the Bruker XSCANS and SMART systems for X-ray data collection, but may in principle be used with diffraction data from other sources, provided that it is in one of the formats supported. XPREP may even be used without any reflection data in order to check a unit-cell to see whether it can be transformed to a higher metric symmetry. XPREP is designed to perform all operations that involve analysis and manipulation of intensity data, e.g., space group determination, reciprocal lattice displays, empirical and face-indexed absorption corrections, intensity statistics, scaling and merging of different datasets, derivation and filtering of anomalous differences, contoured Patterson (Harker) sections, and preparation of the input files for structure solution with the SHELXTL program XS etc.

At each strategic decision point the program presents the user with all available information and a selection of possible options, plus a recommended course (which may be chosen with the [Enter] key). It is thus useful both for users with little crystallographic experience as well as for an expert with a problem structure (or in a hurry). The program can display weighted and color-coded reciprocal lattices (like precession photographs), with considerable diagnostic and educational value, and incorporates the LePage (J. Appl. Cryst. 15 (1982) 255-259) algorithm for the detection of higher metric symmetry. XPREP is completely general for all 230 space groups, and makes any necessary reorientation of the axes in order to bring the data into a ‘standard setting’.

Since XPREP is essentially interactive and is entirely menu-driven, the best way to get to know it is by means of examples. Since the determination of the correct space group is a non-trivial operation which is critical to the success of all subsequent stages in crystal structure determination, this will be illustrated first here in some detail. Four test data sets are provided to illustrate typical problems in space-group determination; the user is recommended to run through these four examples on the computer. First the corresponding data files *.hkl should be copied to a working directory. The following notes were reconstructed from the files *.prp which provide a log of all operations using XPREP.

4.1 Examples of Space Group Determination

Even experienced crystallographers have some hesitation in assigning cubic space groups, since they occur relatively infrequently, especially for organic structures such as in the first example. First of all XPREP is started by entering:

```
xprep mpi2
```

If the data had been collected using XSCANS or SMART, it would have been possible for XPREP to extract the unit-cell etc. from the.p4p file; as it is, the cell 16.776 16.776 16.776 90 90 90 must be entered by hand. After this, it is only necessary to press the [Enter] key repeatedly to find the space group. The following information appears in the menu displays and is written to the file mpi2.prp:

```
 Original cell in Angstroms and degrees:

   16.776   16.776   16.776    90.00    90.00    90.00

  6934 reflections read from file MPI2.HKL         Mean(I/sigma) =   10.33

 Lattice exceptions: P     A     B     C     I     F    Obv    Rev    All

 N (total) =         0   3470  3466  3464  3476   5200   4626   4630   6934
 N (int>3sigma) =    0   1515  1525  1528  1426   2284   2123   2102   3220
 Mean intensity =  0.0  122.5 122.9 122.7  99.2  122.7  131.6  131.4  124.8
 Mean int/sigma =  0.0   10.1  10.1  10.1   9.1   10.1   10.6   10.6   10.6

 Lattice type P chosen           Volume:       4721.34
-------------------------------------------------------------------------
Determination of reduced (Niggli) cell

 Transformation from original cell (HKLF-matrix):
   1.0000  0.0000  0.0000   0.0000  1.0000  0.0000   0.0000  0.0000  1.0000

 Unitcell:      16.776   16.776   16.776   90.00   90.00   90.00

 Niggli form:    a.a =   281.43      b.b =   281.43      c.c =   281.43
                 b.c =     0.00      a.c =     0.00      a.b =     0.00
 -------------------------------------------------------------------------
```

```
 Search for higher METRIC symmetry
 ---------------------------------------------------------------------------
 Option A:  FOM = 0.000 deg.  CUBIC      P-lattice  R(int) = 0.058 [  5767]
 Cell:  16.776 16.776  16.776  90.00  90.00   90.00    Volume:      4721.34
 Matrix: 0.0000 1.0000 0.0000 0.0000 0.0000 1.0000 -1.0000  0.0000  0.0000

 Option A selected
---------------------------------------------------------------------------
Space group determination

 Lattice exceptions: P     A     B     C     I     F    Obv    Rev    All

 N (total) =         0   3470  3466  3464  3476   5200   4626   4630   6934
 N (int>3sigma) =    0   1515  1525  1528  1426   2284   2123   2102   3220
 Mean intensity =  0.0  122.5 122.9 122.7  99.2  122.7  131.6  131.4  124.8
 Mean int/sigma =  0.0   10.1  10.1  10.1   9.1   10.1   10.6   10.6   10.6

Crystal system C and Lattice type P selected

Mean |E*E-1| = 1.047 [expected .968 centrosym and .736 non-centrosym]

Chiral flag NOT set

Systematic absence exceptions:

          41/43  42=21  a--   b--   n--   --n
N          72     24    786   786   816   564
N I>3s     18      0    223     3   220   285
<I>      62.7    1.1   76.5   1.5  73.5  92.2
<I/s>     9.9    0.5    9.2   0.6   8.9  16.6

Option  Space Group  No.  Type  Axes  CSD  R(int)  N(eq)  Syst.  Abs.  CFOM

[A] Pa-3            #205 centro  2     68  0.058   5767   0.6 /  8.9  3.27

Option [A] chosen
```

It will be noticed that the program checks the lattice absences and confirms the lattice is primitive. It then calculates the mean value of $|E^2–1|$ which suggests that the structure is centrosymmetric. The systematic absences are then checked, and a “b” glide plane found (which also causes a 4_2 axial absence). The mean Intensity divided by σ(I) (<I/s>) should be about unity (or less) for a systematically absent set of reflections. Note that the program knows which absences are relevant for each combination of lattice type and Laue group, so (for example) does not complicate the statistics here by analyzing for a 'd' glide which could only arise for a centered lattice. Similarly it is not necessary to consider (say) a 2_1 absence which is generated automatically by centered lattice conditions.

At first sight the b glide found here might indicate a space group “Pb-3”, but there is no such space group! This solution to this problem is that the axes must be anti-cyclically permuted to produce the standard space group Pa-3. The program works this out (“Axes 2”) and since the space group is in fact unambiguous only one choice is offered. The merging R-index “R(int)” is acceptable for this Laue group, but was presumably above the default threshold for the higher-symmetry cubic Laue group. 5767 reflections were eliminated on merging, so R(int) is very reliable. The space group is centrosymmetric and occurs 68 times (after eliminating doubtful assignments) in the Cambridge Structural Database.

We proceed by defining the empirical formula so that the program can deduce the unit-cell contents. The formula may include nested brackets but the numbers must always follow the element symbols or brackets. Several standard groups (here ethyl) are allowed; the first symbol of an element or group symbol must be in upper case, the second (if any) in lower case.

```
Determination of unit-cell contents

 Formula: [C9H10N2]6 EtOH

 Formula weight =    923.22
 Tentative Z (number of formula units/cell) =   4.0  giving rho = 1.299,
 non-H atomic volume =  17.1  and following cell contents and analysis:

 C     224.00    72.86 %              H     264.00     7.21 %
 N      48.00    18.21 %              O       4.00     1.73 %

 F(000) =    1976.0        Mo-K(alpha) radiation        Mu (mm-1) =   0.08

--------------------------------------------------------------------------------
 File MPI2A.INS set up as follows:


 TITL MPI2A in Pa-3
 CELL 0.71069  16.776  16.776  16.776  90.00  90.00  90.00
 ZERR    4.00   0.002   0.002   0.002   0.00   0.00   0.00
 LATT  1
 SYMM .5-X, -Y, .5+Z
 SYMM -X, .5+Y, .5-Z
 SYMM .5+X, .5-Y, -Z
 SYMM Z, X, Y
 SYMM .5+Z, .5-X, -Y
 SYMM .5-Z, -X, .5+Y
 SYMM -Z, .5+X, .5-Y
 SYMM Y, Z, X
 SYMM -Y, .5+Z, .5-X
 SYMM .5+Y, .5-Z, -X
 SYMM .5-Y, -Z, .5+X
 SFAC C H N O
 UNIT 224 264 48 4
 TREF
 HKLF 4
 END
```

In this case, we have chosen a new file name (mpi2a) so that the reorientation of the axes does not lead to confusion; the program creates the files mp2ia.ins and mpi2a.hkl, ready for structure solution with XS. Although this space group is the only cubic space group for which it may be necessary to permute the axes, the problem frequently arises in the orthorhombic system.

XPREP provides a great deal of useful diagnostic information and should always be run as a check even when the space group appears to be clear-cut. As a bonus the input files for XS and XL are produced automatically. Sometimes it happens that there is more than one possible space group and that the different possibilities involve different standard settings; in such a case different filenames should be assigned for each space group, so that XPREP automatically creates a new .hkl file with the transformed indices for each. The different possibilities can then be tested separately with XS etc., as in the next example of a less clear-cut space group.

The test structure AGS4 (which is also plotted in the course of the standard XP demo) may be processed in a similar manner, producing the following output:

```
 Original cell in Angstroms and degrees:

    8.381    8.381    6.661    90.00    90.00    90.00

   1475 reflections read from file AGS4.HKL         Mean(I/sigma) =   29.08

 Lattice exceptions: P    A     B     C      I      F     Obv    Rev    All

 N (total) =         0   738   738   742    738   1109    984    985   1475
 N (int>3sigma) =    0   633   633   604    626    935    862    855   1283
 Mean intensity =  0.0 480.9 488.5 225.5  493.8  398.0  520.7  510.0  495.8
 Mean int/sigma =  0.0  28.2  28.7  20.4   28.7   25.8   29.3   29.5   29.1

 Lattice type P chosen           Volume:        467.88

------------------------------------------------------------------------------

 Determination of reduced (Niggli) cell

 Transformation from original cell (HKLF-matrix):
  0.0000  0.0000  1.0000   1.0000  0.0000  0.0000    0.0000  1.0000  0.0000

 Unitcell:       6.661    8.381    8.381   90.00   90.00   90.00

 Niggli form:    a.a =    44.37      b.b =    70.24      c.c =    70.24
                 b.c =     0.00      a.c =     0.00      a.b =     0.00
(Sample printout continued on next page.)

------------------------------------------------------------------------------
 Search for higher METRIC symmetry
------------------------------------------------------------------------------
Option A:  FOM = 0.000 deg.  TETRAGONAL  P-lattice   R(int) = 0.027 [  885]
Cell:    8.381   8.381   6.661   90.00   90.00   90.00   Volume:     467.88
 Matrix:1.0000 0.0000 0.0000 0.0000 1.0000 0.0000 0.0000 0.0000 1.0000

 Option A selected

------------------------------------------------------------------------------
```

```
 Space group determination
 Lattice exceptions: P    A     B     C     I     F    Obv   Rev   All

 N (total) =         0   738   738   742   738  1109   984   985  1475
 N (int>3sigma) =    0   633   633   604   626   935   862   855  1283
 Mean intensity =  0.0 292.4 297.0 137.1 300.2 242.0 316.6 310.1 301.4
 Mean int/sigma =  0.0  28.2  28.7  20.4  28.7  25.8  29.3  29.5  29.1

Crystal system T and Lattice type P selected

Mean |E*E-1| = 0.800 [expected .968 centrosym and .736 non-centrosym]

Chiral flag NOT set

Systematic absence exceptions:

          41/43  42   n--   -b-   -c-   -n-  -21-   --c
N           8     5   130   152   143   149    20    59
N I>3s      8     5   107   137   135   134    20    58
<I>     669.8 585.3 248.9 188.1 485.3 498.9 629.4 922.3
<I/s>    73.8  59.7  28.1  28.5  39.6  39.1  62.0  57.4

Option  Space Group  No.  Type  Axes  CSD  R(int)  N(eq)  Syst.  Abs.  CFOM

[A] P-4           # 81  non-cen  1    23  0.027    885   0.0 / 28.1   4.93
[B] P4/m          # 83  centro   1    13  0.027    885   0.0 / 28.1  10.33
[C] P4            # 75  chiro    1     4  0.027    885   0.0 / 28.1  20.77
[D] P4/mmm        # 123 centro   1    17  0.252   1115   0.0 / 28.1 167.26
[E] P422          #  89 chiral   1     4  0.252   1115   0.0 / 28.1 167.26
[F] P4-42m        # 111 non-cen  1     2  0.252   1115   0.0 / 28.1 192.63
[G] P4-4m2        # 115 non-cen  1     2  0.252   1115   0.0 / 28.1 192.63
[H] P4mm          #  99 non-cen  1     0  0.252   1115   0.0 / 28.1 259.29

Option [A] chosen
```

The crystal is primitive tetragonal and exhibits no systematic absences, and the merging R-index favors the lower symmetry tetragonal Laue group 4/m. The statistics are closer to non-centrosymmetric but are not really decisive, and should be regarded with caution here because the structure contains heavy atoms on special positions. Of the three possible space groups the program recommends P-4, which is non-centrosymmetric and more common in the Cambridge Database than the other two (but this is an inorganic structure!). As it happens P-4 is indeed the correct space group, but it would probably have been advisable to consider P4/m too, for which the combined figure of merit CFOM, which takes into account all the available information, is almost acceptable. Usually a CFOM of less than 1 is a decisive indication that the proposed space group is correct, and a value greater than 10 is rather unlikely to be correct. A good approach in such a case is to prepare a set of files - with different names - for each plausible space group, and to run all through XS to solve the structure before making a choice.

In a case such as this, where the structure was solved in a non-centro-symmetric space group, it should be checked carefully that the solution is not consistent with the corresponding centrosymmetric space group (here P4/m) by checking for unexpected relations between the coordinates of independent atoms (and large correlation coefficients in the least-squares refinement).

Had it been known that the compound is optically active (it isn't), the "chiral flag" could have been set on determining the space group. This would force the choice of P4 or P422 here; these are the only possible space groups which can accommodate chiral molecules. The reciprocal space plots for this structure ('R' from the main menu followed by 'Z' and then layer number 0) show a convincing four-fold axis in reciprocal space, but no further symmetry, as expected for this Laue group. We can now proceed with the determination of Z and the preparation of the input files for structure solution with XS:

```
Determination of unit-cell contents
 Formula: Ag(CNS2)4 AsF6

 Formula weight =    657.33
 Tentative Z (number of formula units/cell) =   1.0  giving rho = 2.333,
 non-H atomic volume =  19.5  and following cell contents and analysis:

 C       4.00     7.31 %               N       4.00     8.52 %
 F       6.00    17.34 %               S       8.00    39.02 %
 As      1.00    11.40 %               Ag      1.00    16.41 %

 F(000) =     314.0        Mo-K(alpha) radiation        Mu (mm-1) =   3.73
--------------------------------------------------------------------------------
 File AGS4.INS set up as follows:

 TITL AGS4 in P-4
 CELL 0.71069   8.381   8.381   6.661  90.00  90.00  90.00
 ZERR    1.00   0.001   0.001   0.001   0.00   0.00   0.00
 LATT -1
 SYMM -X, -Y, Z
 SYMM Y, -X, -Z
 SYMM -Y, X, -Z
 SFAC C N F S AS AG
 UNIT 4 4 6 8 1 1
 TREF

 HKLF 4
 END
```

The following dataset (usm2.hkl) was collected by mistake as C-centered monoclinic but could not be solved. In fact it should be rhombohedral, but a bug in the P3 diffractometer control program (present in all DG and VMS versions prior to 1990, but only active under extremely unusual circumstances) led the IT command to suggest the apparently unexceptional monoclinic cell.

```
 Original cell in Angstroms and degrees:

   16.207   26.937    6.823    90.00   106.32    90.00

    1850 reflections read from file USM2.HKL        Mean(I/sigma) =   25.73

 Lattice exceptions: P      A     B     C      I      F     Obv    Rev    All

 N (total) =         0    926    926     0    925    926   1232   1238   1850
 N (int>3sigma) =    0    737    737     0    734    737    987    998   1477
 Mean intensity =  0.0  122.7  122.7   0.0  123.3  122.7  120.2  125.4  123.9
 Mean int/sigma =  0.0   25.7   25.7   0.0   25.7   25.7   25.5   26.2   25.7

 Lattice type C chosen           Volume:       2858.68

-------------------------------------------------------------------------------

 Determination of reduced (Niggli) cell

 Transformation from original cell (HKLF-matrix):
    0.0000 0.0000 -1.0000  -0.5000 -0.5000  0.0000  -0.5000  0.5000  0.0000

 Unitcell:       6.823   15.718   15.718  117.93   98.33   98.33

 Niggli form:    a.a =    46.55      b.b =   247.07      c.c =   247.07
                 b.c =  -115.73      a.c =   -15.54      a.b =   -15.54

-------------------------------------------------------------------------------


 Search for higher METRIC symmetry
-------------------------------------------------------------------------------
 Option A: FOM = 0.020 deg. RHOMBOHEDRAL  Obv. hex.  R(int) = 0.020 [  583]
 Cell:  26.937  26.939   6.823  89.98  90.00 120.00      Volume:    4288.02
 Matrix: 0.0000 1.0000 0.0000 -1.5000 -0.5000 -1.0000 0.0000 0.0000 1.0000
-------------------------------------------------------------------------------
 Option B: FOM = 0.020 deg. RHOMBOHEDRAL P-lattice   R(int) = 0.020 [  583]
 Cell:  15.718  15.718  15.718 117.94 117.94 117.94      Volume:    1429.17
 Matrix:-0.5000 0.5000 0.0000 -0.5000 -0.5000 0.0000 1.0000  0.0000  1.0000

 Option A selected

-------------------------------------------------------------------------------
```

The figure of merit gives the largest deviation in degrees of a lattice two-fold axis from its expected position. The merging R-index of 0.020 provides a convincing check that the LAUE symmetry, as well as the metric symmetry, is rhombohedral. In rhombohedral cases the user is always offered the choice between the obverse cell on hexagonal axes and the rhombohedral primitive cell. All SHELXTL programs are equally at home with either, but here we choose the former since the latter is extremely non-orthogonal (this has disadvantages for the Fourier grid-point interpolation in the peak-search routine). If the rhombohedral alpha angle had been around 90 degrees, the latter would have been a better choice. The space group determination and preparation of the XS input files causes no further problems:

```
Space group determination

 Lattice exceptions:  P     A      B      C     I     F     Obv    Rev    All

 N (total) =          0    925    926    928    925   1387    0   1224   1850
 N (int>3sigma) =     0    743    737    730    744   1105    0    996   1477
 Mean intensity =   0.0  129.5  122.7  129.7  119.4  127.3  0.0  124.7  123.9
 Mean int/sigma =   0.0   25.7   25.7   26.0   26.2   25.8  0.0   26.3   25.7

 Crystal system H and Lattice type O selected

 Mean |E*E-1| = 0.997  [expected .968 centrosym and .736 non-centrosym]

 Chiral flag NOT set


 Systematic absence exceptions:

          61/65  62=31   63     -c-    --c

 N           1     0       1    102     38
 N I>3s      1     0       1     90     32
 <I>    2523.3   0.0  2523.3  177.8  241.7
 <I/s>   141.6   0.0   141.6   35.2   35.0

Option  Space Group  No.  Type  Axes  CSD  R(int) N(eq)  Syst. Abs.   CFOM

 [A] R-3            #148  centro   1   232  0.020   583   0.0 / 25.7   0.90
 [B] R3             #146  chiral   1    85  0.020   583   0.0 / 25.7   8.38

 Option [A] chosen
--------------------------------------------------------------------------------
Determination of unit-cell contents

 Formula: Et2NC4O3(NH)2

 Formula weight =    198.20

 Tentative Z (number of formula units/cell) =  18.0  giving rho = 1.382,
 non-H atomic volume =  17.0  and following cell contents and analysis:

 C     144.00    48.48 %              H     216.00     6.10 %
 N      54.00    21.20 %              O      54.00    24.22 %

 F(000) =    1890.0        Mo-K(alpha) radiation        Mu (mm-1) =   0.10

--------------------------------------------------------------------------------

 File USM2H.INS set up as follows:

 TITL USM2H in R-3
 CELL 0.71069  26.938  26.938   6.823  90.00  90.00 120.00
 ZERR   18.00   0.004   0.004   0.001   0.00   0.00   0.00
 LATT  3
 SYMM -Y, X-Y, Z
 SYMM -X+Y, -X, Z
 SFAC C H N O
 UNIT 144 216 54 54
 TREF
 HKLF 4
 END

 Writing new reflection file: USM2H.HKL

--------------------------------------------------------------------------------
```

The following structure (roe119.hkl) was collected as orthorhombic since the three cell angles were close to 90 degrees. Axial photographs were not taken, but an empirical absorption correction was applied to the data before running XPREP. The original space group determination proceeded as follows (to reproduce this, it is necessary to override the ‘H’ option suggested by the program with 'S'). Note that the angles have been left at their unconstrained values rather than rounding off, which later proves to be useful. If the cell is read from the data collection files this will be done anyway:

```
Original cell in Angstroms and degrees:

  10.161   14.394   30.428    90.01    90.00    89.96

  1849 reflections read from file roe119.hkl        Mean(I/sigma) =   32.60

 Lattice exceptions: P     A      B     C      I      F     Obv    Rev    All

 N (total) =         0    917    917     0    906    917   1239   1231   1849
 N (int>3sigma) =    0    716    716     0    717    716    975    964   1453
 Mean intensity =  0.0  120.7  120.7   0.0  116.0  120.7  120.5  123.9  121.8
 Mean int/sigma =   0.0  32.4   32.4   0.0   31.8   32.4   32.6   32.9   32.7

 Lattice type C chosen           Volume:       4450.32

-------------------------------------------------------------------------
... etc. ...

 Mean |E*E-1| = 0.931 [expected .968 centrosym and .736 non-centrosym]

 Chiral flag NOT set

 Systematic absence exceptions:

          c--    n--    -c-    -n-    --a    --b   --21
 N        180    180     88     88     34     34     25
 N I>3s   123    123     55     55     17     17      2
 <I>    108.6  108.6  134.3  134.3  182.6  182.6    1.2
 <I/s>   27.4   27.4   38.2   38.2   33.9   33.9    1.0

 Option  Space Group  No.  Type  Axes  CSD  R(int) N(eq)  Syst. Abs.   CFOM
 [A] C222(1)        # 20  chiral   1   155  0.111   191   1.0 / 27.4   4.80
 Option [A] chosen
-------------------------------------------------------------------------
Determination of unit-cell contents

Formula: [(CF3)3C6H2]2BiCl

Formula weight =    806.63
Tentative Z (number of formula units/cell) =   8.0  giving rho = 2.408,
non-H atomic volume =  14.6  and following cell contents and analysis:

 C     144.00    26.80 %              H      32.00     0.50 %
 F     144.00    42.39 %              Cl      8.00     4.40 %
 Bi      8.00    25.91 %
 F(000) =    2992.0        Mo-K(alpha) radiation        Mu (mm-1) =   8.17
```

There were five independent hints that not all is well:

(a) One of the cell angles deviates by more than usual (0.04 degrees) from 90 degrees.

(b) The statistics are clearly centrosymmetric, but only a non-centrosymmetric space group is consistent with the systematic absences.

(c) Reciprocal space plots (the option ‘P’ followed by ‘Z’) show the expected C-centered lattice, but the zero layer (hk0) looks very strange and appears to have extra systematic absences that do not fit any known space group.

(d) The calculated Patterson cannot be explained on the basis of the expected one unique heavy atom in C2221.

(e) Neither direct nor Patterson methods could solve the structure.

Since only reflections of type h,k,l and a few -h,-k,-l were collected, the merging R-index is not able to help. If a few equivalents (e.g., with one index negative) had been collected here, the result might well have been the message that no space group is consistent with the given information! Again the ‘higher’ symmetry cell search ‘H’ provides a clue (and shows that the symmetry is in fact lower!); however it is first necessary to tighten up the tolerance on the metric symmetry check, so that cells with lower than orthorhombic symmetry are considered. This is done by selecting ‘T’ in the main menu, then ‘T’ again from the tolerance sub-menu, and trying a value of 0.02 degrees. In other cases one might reduce the maximum R(int) for terminating the cell search (option ‘R’ from the sub-menu).

```
 Determination of reduced (Niggli) cell

 Transformation from original cell (HKLF-matrix):
-0.5000  0.5000  0.0000   0.5000  0.5000  0.0000   0.0000  0.0000 -1.0000

 Unitcell:       8.807    8.812   30.428   89.99   89.99   70.44

 Niggli form:    a.a =   77.56      b.b =    77.66      c.c =   925.86
                 b.c =    0.04      a.c =     0.04      a.b =    25.99
-------------------------------------------------------------------------
 Search for higher METRIC symmetry
-------------------------------------------------------------------------
 Option A: FOM = 0.041 deg. ORTHORHOMBIC  C-lattice  R(int) = 0.111 [  191]
 Cell:  10.161  14.394  30.428   89.99   90.00   90.04    Volume:   4450.32
 Matrix:-1.0000 0.0000  0.0000 0.0000 1.0000 0.0000  0.0000  0.0000 -1.0000
-------------------------------------------------------------------------
 Option B:  FOM = 0.010 deg.  MONOCLINIC  P-lattice  R(int) = 0.111 [  191]
 Cell:   8.807  30.428   8.812   89.99  109.56   90.01    Volume:   2225.16
 Matrix: 0.5000 -0.5000 0.0000 0.0000 0.0000 -1.0000  0.5000  0.5000 0.0000

-------------------------------------------------------------------------
 Option C:  FOM = 0.040 deg.  MONOCLINIC   C-lattice  R(int) = 0.111 [ 191]
 Cell:  14.394  10.161  30.428   90.00   90.01   89.96    Volume:   4450.32
 Matrix:0.0000 -1.0000 0.0000 -1.0000 0.0000 0.0000  0.0000  0.0000 -1.0000
-------------------------------------------------------------------------
 Option D: FOM = 0.041 deg.  MONOCLINIC   C-lattice  R(int) = 0.111 [  191]
 Cell:  10.161  14.394  30.428   89.99   90.00   90.04    Volume:   4450.32
 Matrix:-1.0000 0.0000  0.0000 0.0000 1.0000 0.0000  0.0000  0.0000 -1.0000

-------------------------------------------------------------------------
```

Of the four possible choices, B was chosen by hand since it has the best figure of merit (ignoring the triclinic cell, which must have a zero figure of merit). It leads to a convincing space group assignment (note the much lower CFOM!) and acceptable reciprocal space displays. Incidentally the program would have reoriented P2(1)/a to P2(1)/c, but left P2(1)/n unchanged, since this non-standard setting is often used when it results in a beta angle closer to 90 degrees, which implies that either IT has been run when collecting the data on a P3 diffractometer, or the 'H' option has been used in XPREP (as here), or both (best), so that the metric cell is standard.

```
Space group determination

Lattice exceptions:  P     A      B      C      I      F     Obv    Rev    All

N (total) =          0    916    763    911    917   1295   1226   1236   1849
N (int>3sigma) =     0    707    617    700    716   1012    962    974   1453
Mean intensity =   0.0  120.6  124.9  120.2  120.7  121.7  122.4  122.9  121.8
Mean int/sigma =   0.0   32.3   33.2   31.8   32.4   32.4   32.9   32.5   32.7

 Crystal system M and Lattice type P selected

 Mean |E*E-1| = 0.951 [expected .968 centrosym and .736 non-centrosym]

 Chiral flag NOT set


 Systematic absence exceptions:

        -21-    -a-    -c-     -n-

 N        25     41     39     34
 N I>3s    2     20      3     17
 <I>     1.2  151.7    0.6  182.6
 <I/s>   1.0   28.4    0.7   33.9

 Option  Space Group  No.  Type  Axes  CSD  R(int) N(eq)  Syst. Abs.   CFOM

 [A] P2(1)/c        # 14  centro   1 19410  0.111   191   1.0 / 28.4   0.39

 Option [A] chosen
--------------------------------------------------------------------------
 File roe119m.ins set up as follows:

 TITL ROE119M in P2(1)/c
 CELL 0.71069   8.807  30.428   8.812  90.00 109.56  90.00
 ZERR    4.00   0.002   0.006   0.002   0.00   0.03   0.00
 LATT  1
 SYMM -X, .5+Y, .5-Z
 SFAC C H F CL BI
 UNIT 72 16 72 4 4
 TREF
 HKLF 4

 END

 Writing new reflection file: roe119m.hkl
```

The conclusion is that the true space group is P2(1)/c and that ONLY HALF the necessary data have been collected. Fortunately the crystal had survived and the data could be recollected (and merged with the existing half data set using XPREP); the structure could be solved and refined in P2(1)/c with no further (serious) problems. Note that it is not necessary to reestablish the unit-cell contents when changing the cell, since the program automatically keeps track and changes the Z value accordingly.

In addition to preparing the .ins and (if required) .hkl files, XPREP also writes a .pcf file in CIF format. This file may be merged with the CIF file (.cif) from XL when preparing tables using XCIF when the structure determination is complete. Since all the necessary files have been prepared by XPREP, the structure can now be solved by direct methods by the command: `xs name` (where 'name' is the filename with extension omitted) at the operating system prompt. If a Patterson solution is required, 'TREF' must be edited to 'PATT' in the .ins file first.

4.2 XPREP Space Group Notation

In addition to suggesting possible space groups, XPREP also allows a space group to be entered by the user (option 'S' from the main menu, followed by option 'I' from the space group determination menu). The program then checks the systematic absences, outputs the frequency in the Cambridge DataBase, etc., as it does when it suggests the space group itself. The space group MUST be in the conventional setting, except that Pn, P2/n and P2(1)/n are accepted as alternatives to Pc, P2/c and P2(1)/c so that axes can be chosen with beta close to 90 degrees. For all rhombohedral space groups both the rhombohedral primitive cell and the hexagonal obverse setting are permitted (the program uses the cell dimensions to decide which is intended). In the monoclinic system the unique axis must be y. The short space group symbol is entered exactly as in volume A of the new International Tables, with the conventions that an inverse axis is indicated by a minus sign immediately before the digit (instead of by a bar across the top), and for a screw axis the translation factor should be given in round brackets rather than as a subscript. Examples are: P-1, P2(1)/c, Pna2(1), Fdd2, I4(1)/acd, R32, P-3c1, Pa-3, F-43c.

4.3 Input of Chemical Formulas into XPREP

It is necessary to enter the proposed chemical formula into XPREP to predict the Z value, cell contents, density etc. It is not critical if it is not quite right - the direct and Patterson methods are not very sensitive to whether the cell contents have been given correctly - and there is an opportunity to change the formula and recalculate the cell contents etc. when running XL. XPREP will prompt for the formula if the 'C' option is chosen from the main menu.

The conventions for entering the formula are as follows. Chemical elements are represented by a capital letter followed, if necessary, by a lower case letter. The following common groups may also be entered directly: Me (methyl), Et (ethyl), Pp (propyl - Pr cannot be used because it is an element symbol!), Bu (butyl), Cp (cyclopentadienyl: C_5H_5) and Ph (Phenyl: C_6H_5). Brackets (of all three types) may be employed to any depth, and numbers may be integer or decimal. Spaces are ignored, but may be added for clarity. The only important restriction compared with normal chemical use is that numbers MUST appear AFTER the corresponding symbols or brackets, and never before them. A typical example is: [(Ph3P)2PtBr]2 (EtOH)0.5

4.4 Resetting the Tolerances for the XPREP Decisions

The tolerances for space group determination and identification of higher metric symmetry have been carefully tuned, and it will not normally be necessary to alter them. Some of the values are determined by the program on the basis of the intensity data that have been read in, others have standard default values. The current values may be examined and - if necessary - changed by taking the ‘T’ option from the main menu. In the space group determination the program takes into account that systematic absence indications are relatively unreliable if there are only a very small number of reflections in the group, and that the diagnostic value of R(int) in determining the correct Laue group depends on the number of equivalents which were merged to obtain it.

The actual application of these tolerances and the calculation of the CFOM figure of merit for space group determinate is a complex empirical process which has been “fine-tuned” on the basis of a large number of structures. The program attempts to combine all available information in reaching a probability decision in much the same way as an experienced crystallographer would do. The procedure is not infallible, but the chances of success may be improved by collecting equivalent reflections and by performing absorption corrections and then repeating the space group determination.

Note that by increasing the metric and R(int) tolerances it is possible to obtain the matrices for possible pseudo-merohedral twinning.

4.5 Reciprocal Space Plots

Layers of reciprocal space may be displayed on the screen by choosing the ‘R’ option from the main menu, followed by specifying the axis (X, Y or Z) perpendicular to the layers. The cursor keys may be used to change the layer number and which axis is perpendicular to the layers. Symmetry equivalents (or just Friedel equivalents) may be included; this is useful for checking the completeness of the data. Two circles, at default resolutions of 1.5 and 1.0 Å, are superposed upon the plots so that the resolution of the data can be seen. The size of the reflection spots corresponds to their intensities, and the color indicates their intensity to sigma(intensity) ratio. Thus dark blue means “less than 1.5 sigma”, light blue less than 3 sigma, green less than 5 sigma, yellow less than 10 sigma and white greater than 10 sigma. If there are few white spots between the two concentric circles, the resolution of the data is not adequate for direct methods to succeed (direct methods assumes ‘atomic resolution’!). If there is a blank patch or the data extend more in one direction than another, perhaps the index limits were set wrongly for data collection or perhaps the cell has been entered incorrectly. Unexpected patterns of absent reflections may indicate a wrong space group assignment or twinning. To make a hard copy of the diagram currently being displayed, hit [Ctrl-P]; it may be necessary to use the H option first to specify the type of plotter, if this does not agree with the information read by XPREP from the initialization file sxtl.ini The H option also enables the reciprocal space display to be written to a SHELXL plot file.

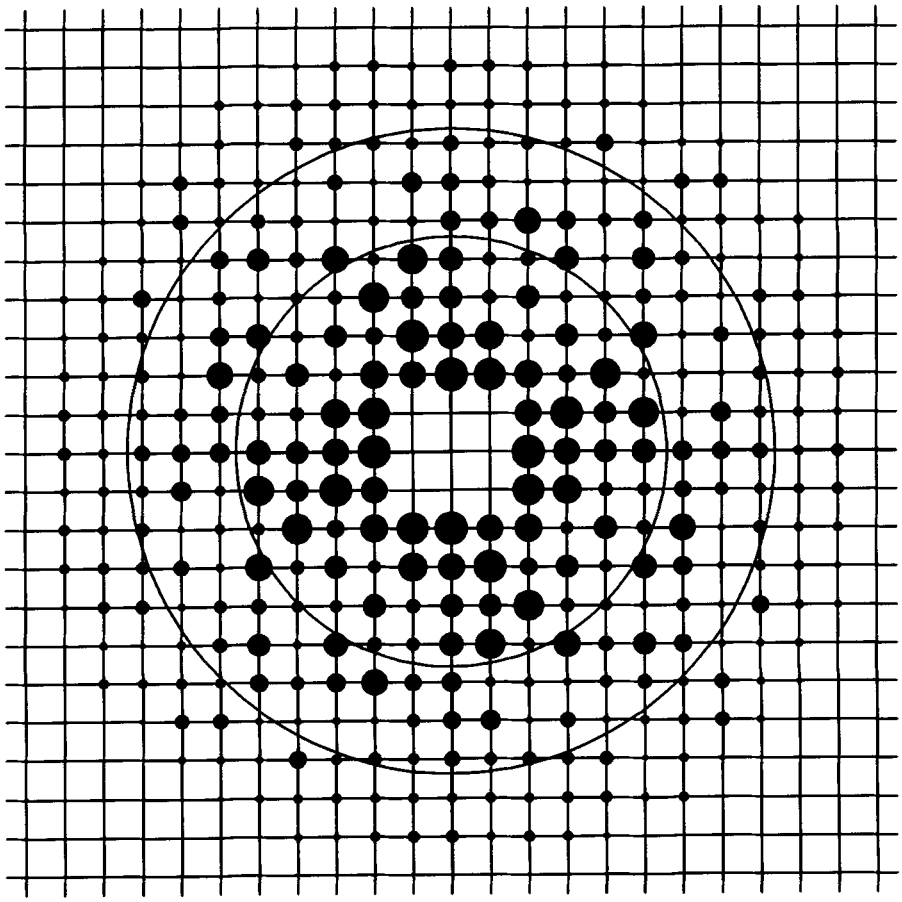

Figure 4.1 – Reciprocal Space Plot for AGS4 (layer hk0)

4.6 Contoured Patterson Sections

Harker (and other) Patterson sections may be contoured by choosing the option ‘P’ from the main menu. This enters the Patterson submenu, where it is possible to set the contour levels and define which layer is to be plotted. As with the reciprocal space plots, hitting [Crtl-P] whilst a plot is displayed creates either a direct hard-copy or a SHELXTL plot file; the H option is used to set up the type of hard-copy required. The highest interpolated peaks in the section are output to the console and to the .prp file. There are options for super-sharpening [coefficients $\sqrt{(E^3F)}$], for origin removal [the coefficients are multiplied by $(E^2\text{-}1)/E^2$] and for weighting down weak reflections [the coefficients are multiplied by $F^4/(F^4+g\sigma^2(F^2))$ where E is a normalized structure factor and g is input by the user (the default of zero gives unit weights)]. Peak heights are expressed relative to an origin peak of 999 and in sigma units. In order to estimate the 1 sigma level (rms deviation from zero) of the map, a specified section, chosen to avoid Harker and other strong peaks, may be specified.

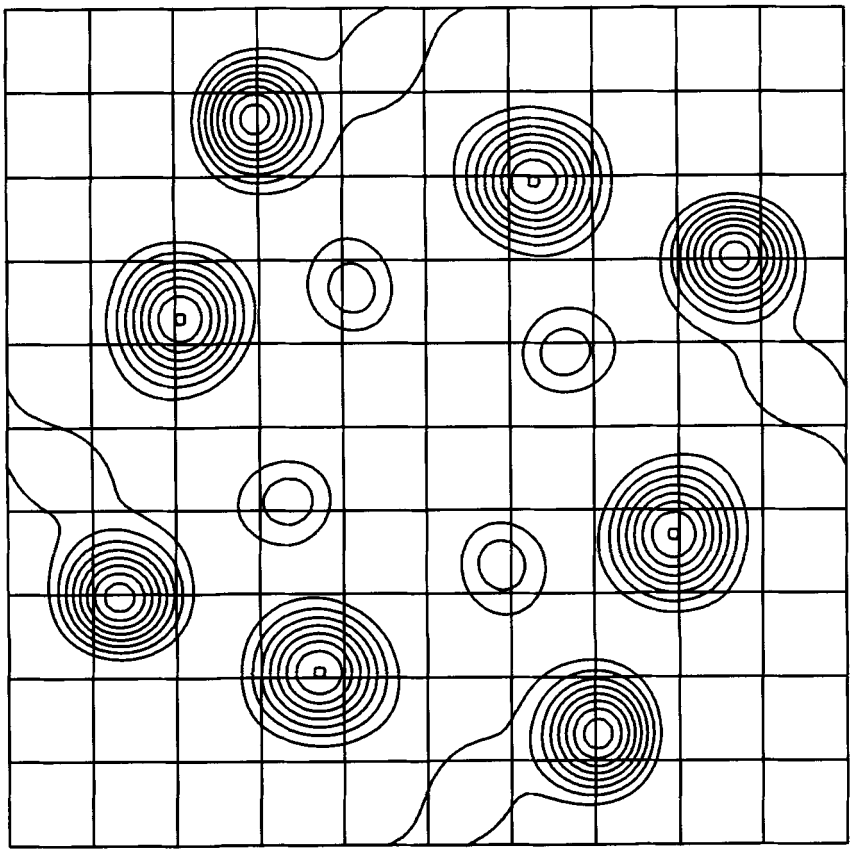

Figure 4.2 – The z=0.5 Layer of the Patterson for AGS4

4.7 Empirical Absorption Corrections (Diffractometer Data)

Data collected with the Bruker SMART CCD detector (or the HI-STAR multiwire detector) should in general be processed with SAINT and then SADABS. In addition to providing an empirical absorption correction (should this be required), SADABS also puts the standard deviations of the measured intensities onto an absolute scale. For specimens that do not require absorption corrections, and it is not necessary to reorientate the axes, the .raw file written by XSCANS may be renamed to .hkl and input directly to the XS or XL programs. If absorption corrections are required for diffractometer data, XPREP first reads the .raw file to establish the space group (and orientation of the axes), and the .raw file (and possibly a .psi file) is then read again to apply the absorption corrections (XPREP does not store the direction cosines from the .raw file because this would halve the number of reflections that could be stored simultaneously). It is best to collect the .raw and .psi files relative to the same unit-cell, but XPREP is able to handle any necessary reorientation. In addition there is an option to read a file containing direction cosines and re-output it with transformed reflection indices and direction cosines for dealing with pathological cases.

The empirical absorption corrections are based on reflection measurements at different azimuthal angles and/or measurements of equivalent reflections. Thus for area detector data, the .raw file is read twice; the first time to select suitable reflections for refining the absorption parameters, and the second time to apply the corrections. In the case of diffractometer data a .psi file will normally be required. The reflection measurements in the .psi file are normal two-theta/omega or omega scans, and the increment in the azimuthal angle psi is usually 10 to 30 degrees. This file should contain at least 200 but not more than about 500 measurements. The space group needs to be established before performing empirical absorption corrections, because XPREP needs to be able to work out which reflections are equivalents; these equivalents do not need to be contiguous in the .psi file.

Two separate empirical treatments are available:

1. Six parameters are refined to define a pseudo-ellipsoid which is then used to calculate the corrections. In order to improve the theta dependency of the correction (which would otherwise be completely indeterminate for a sphere because all equivalents have the same 2θ) the mean value of μr (in dimensionless units, i.e., if the linear absorption coefficient μ is in mm^{-1} as printed out by the program XS, then r must be in mm) is restrained to an input value. When estimating this average, much more weight should be given to the smallest dimensions. In practice μr does not need to be measured very accurately. This method was originally intended for the ellipsoidal crystals which result when one has tried to grind a crystal to a sphere, but in practice it gives reasonable results also for crystals with well-developed faces provided that they are not thin plates.

2. Three parameters (the value of mu*t and two “edge effect” fudge factors) are refined to fit a thin plate to the data. The program requests the indices h, k, l of the principal face. For strongly absorbing crystals which are good approximations to thin plates, this method can produce spectacular improvements. A minimum glancing angle may be specified to eliminate reflections for which the incident or diffracted beams lie very close to the plane of the lamina.

For both methods, the merging R-index before and after applying the correction is printed. The hgcl5 example illustrates the ellipsoid correction (you will need to copy the files hgcl5.raw and hgcl5.psi to your working directory before running this test). XPREP is started with: 'XPREP hgcl5' (which reads the .raw file because no .hkl file is found) and the cell 7.521 7.521 46.111 90 90 120 is input. The space group (R-3c, hexagonal axes) is determined in the usual way, and the data massaging / absorption correction sub-menu entered (by 'A' or 'D' from the main menu). Option 'P' is then chosen for psi-scan corrections. The first five questions can be answered with [Enter], then the lamina (thin plate) correction is selected with 'L'. The face indices should be given as 0 0 1 and the minimum glancing angle set to 3 degrees. The absorption parameters are then refined, reducing the merging R(int) from 0.3225 to 0.0503 (!). To use the absorption parameters to correct the data in the file hgcl5.raw, the remaining questions can be answered with [Enter].

The crystal used for the above test was a thin plate, and experience indicates that the empirical method is more effective in such cases, since it is difficult to measure the critical dimension (the crystal thickness) accurately enough for the face-indexed Gaussian integration method. The empirical parameters can also partially correct for bending of the plate.

The facility for rejecting reflections for which the incident or diffracted beams lie very close to the plane of the lamina is useful, since it is difficult to estimate a good correction in such cases (for example, it would be necessary to take the beam divergence into account). The minimum glancing angle test eliminates 17 data from the .psi file and 27 .raw data from the raw file, but reduces the final R-indices for the least-squares structure refinement. In such cases it is advisable to measure equivalents, so that fewer reflections are missing from the final merged data set.

4.8 Face-Indexed Absorption Corrections

The Bruker XSCANS and SMART data reduction systems provide excellent facilities for indexing and measuring crystal faces. These are written to the .p4p file and so are read by XPREP. To perform face-indexed (Gaussian) absorption corrections, 'A' from the main menu should be followed by 'F' from the absorption correction / data massaging sub-menu. The user is then given the opportunity to input and/or edit the list of crystal faces. The program then suggests a value for the absorption coefficient (calculated from the cell contents; option 'C' from the main menu) and the user may also set the precision. This calculation is relatively slow, especially when very high precision is requested (which requires more grid points for numerical integration).

For very strongly absorbing samples it is difficult to measure the distances of the faces from the reference point accurately enough, so a good strategy is to perform .psi scans and to use these to hand optimize the distances of the faces before correcting the full data. This involves performing the face-indexed absorption corrections on the psi-scan data and then calculating the merging R-indices (option S) for various combinations of crystal face distances in order to find the optimum values. The shortest distances are the most critical.

4.9 Statistics and Data Massaging

The absorption and data massaging sub-menu (entered by ‘A’ or ‘D’ form the main menu) provides facilities for reading, writing and merging datasets, derivation of filtered anomalous differences (for calculating anomalous Pattersons) and statistics showing the resolution dependence of R(sigma) and R(int) and the completeness of the data. It is possible to store several different datasets simultaneously; all other operations are performed on the ‘current dataset’ which may be specified in this sub-menu. Most of these operations require knowledge of the space group, so it should be determined before entering this sub-menu. The facilities for reading and writing files allow transformation matrices to be specified, so it is possible to merge datasets collected relative to different unit-cells etc. The isotropic scaling and merging option allows the refinement of relative overall isotropic temperature factors as well as scale factors, so that datasets collected at different temperatures may also be combined.

Area detector data processed by SADABS should be merged in XPREP rather than XS and XL, because a more sophisticated algorithm that allows for downweighting of outliers is employed in XPREP. A resolution cutoff may be applied (option H in the data massaging submenu) by XPREP (or by using SHEL in XS and XL).

An example of the statistics obtained for a routine small-molecule data collection on a SMART system follows:

```
                INTENSITY STATISTICS FOR DATASET # 1  sad.hkl

  Resolution #Data #Theory %Complete Redundancy Mean I Mean I/s R(int)  R(sigma)

   Inf - 2.10    65     66     98.5    13.35     901.2   91.89  0.0195   0.0086
  2.10 - 1.65    64     64    100.0    15.27     518.1   89.95  0.0218   0.0077
  1.65 - 1.40    78     78    100.0    14.81     480.1   83.23  0.0227   0.0087
  1.40 - 1.25    77     77    100.0    12.47     327.8   72.45  0.0244   0.0093
  1.25 - 1.15    78     78    100.0    10.86     241.3   67.98  0.0248   0.0105
  1.15 - 1.05   110    110    100.0     9.85     233.2   63.21  0.0306   0.0118
  1.05 - 1.00    72     72    100.0     9.11     174.2   50.13  0.0274   0.0127
  1.00 - 0.95    90     90    100.0     8.68     190.8   55.83  0.0308   0.0128
  0.95 - 0.90   104    104    100.0     7.97     134.4   45.85  0.0311   0.0144
  0.90 - 0.85   136    136    100.0     7.49     110.3   42.86  0.0355   0.0158
  0.85 - 0.80   162    162    100.0     6.77      86.0   38.51  0.0368   0.0187
  0.80 - 0.75   222    222    100.0     5.99      67.4   32.75  0.0449   0.0227

  0.85 - 0.75   384    384    100.0     6.32      75.3   35.18  0.0407   0.0208
   Inf - 0.75  1258   1259     99.9     9.23     227.7   54.69  0.0249   0.0113

 Merged [S],  lowest resolution =  8.54 Angstroms,    476 outliers  downweighted
```

Note that R(int), which reflects the square root of the variance of equivalents, is appreciably higher than R(sigma), which is a measure of the precision of the resulting mean intensities. In this case, with the help of the relatively high redundancy, high quality complete data (except for one reflection behind the beam-stop) were obtained to 0.75Å resolution.

The A option is often followed by the calculation of Harker sections of anomalous Pattersons. For a standard P6 myoglobin test using the 2K Bruker CCD, Cu-Kα rotating anode and Göbel mirrors, the super-sharp origin-removed Patterson gave the two unique double weight and one unique single weight Harker peaks as the strongest peaks in the z=0 Harker section (except for some residual noise near to the origin); each of these peaks is observed six times in the section because of the 6-fold symmetry axis of the Patterson. The mean peak heights, relative to an origin height of 999 (with the value in multiples of the sigma level in brackets), were: 80.7 (24.6), 74.6 (22.7) and 32.8 (9.9), and XPREP produced the following plot:

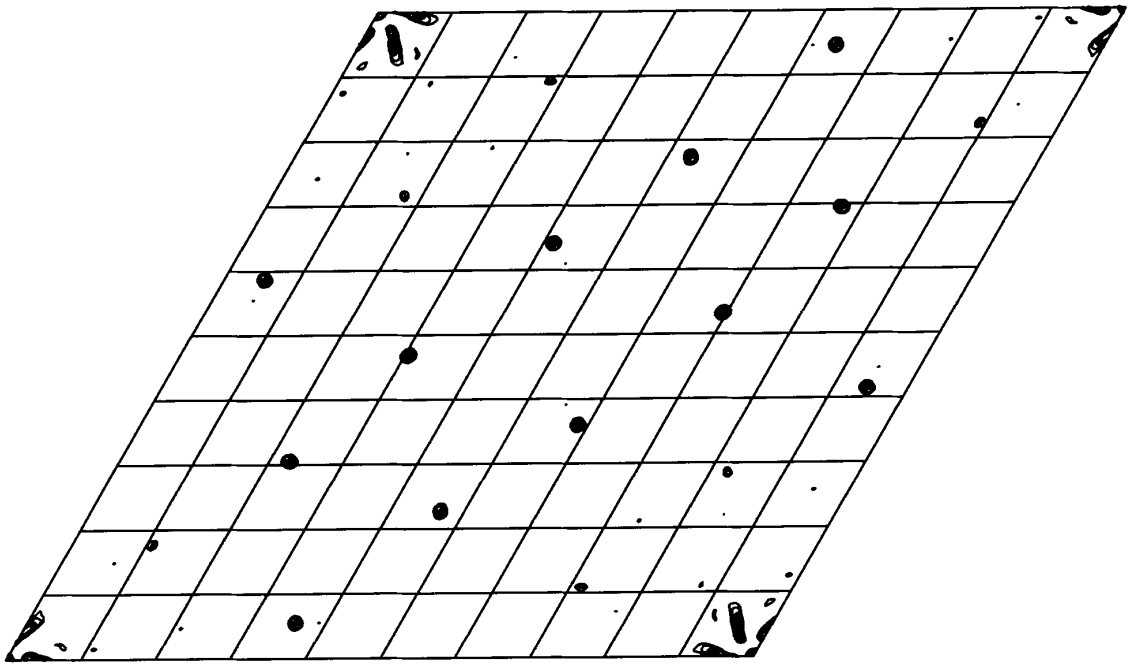

Figure 4.3 – The z=0 super-sharp origin-removed Harker section for P6 myoglobin

5 XS - Structure Solution by Direct Methods

XS is the Bruker SHELXTL module for the solution of crystal structures from X-ray diffraction data, and is primarily designed for single crystal data from small structures (1-500 unique atoms) at atomic resolution, but is also useful for the location of heavy atoms from macromolecular isomorphous or anomalous ΔF data. The direct methods part of XS implements the 'phase annealing' procedure described in *Acta Cryst.* **A46** (1990) 467-473, and the Patterson interpretation is explained in *Crystallographic Computing,* **5** (1991) 145-157. For the successful application of this program to the solution of protein structures from very high-resolution native data alone see *Acta Cryst.* **D49** (1993) 18-23. This chapter describes the direct methods applications of XS; Patterson interpretation is discussed in the next chapter.

XS is general and efficient for all space groups in all settings, and there are no arbitrary limits to the size of problems which can be handled, except for the total memory available to the program. All instructions are in machine independent free format, with extensive use of default settings to minimize the amount of input required from the user. Instructions and data are taken from two standard (ASCII) text files, so that input files can easily be transferred between different computers.

5.1 Running the XS Program

XS may be run on-line by means of the command:

```
xs name
```

where ‘name’ defines the first component of the filename for all files which correspond to a particular crystal structure. On some systems, ‘name’ may not be longer than 8 characters. On UNIX systems, all filenames (including XS) MUST be given in *lower case*. Batch operation will normally require the use of a short batch file containing the above command etc.

Optionally, for the PC version of XS only, a switch `/B` may be used to indicate that XS is being run in batch mode by the Bruker diffractometer control program XSCANS. This causes XS to add instructions to the .res file so that the latter may be read into XP to produce a (PERS) picture of the solution automatically.

Before starting XS, two files — name.ins and name.hkl — MUST have been prepared, almost always using the SHELXTL program XPREP. name.ins contains instructions, crystal and atom data etc., and name.hkl contains the reflection data; the format of this file (3I4,2F8.2) is standard for all SHELX and SHELXTL programs. The name.hkl file should be terminated by a record with all items zero. The reflection order is unimportant. This .hkl file is read each time the program is run; if data from several crystals need to be scaled together, this should be done with XPREP before using XS. XS ignores batch numbers, direction cosines or wavelengths if they are present in the name.hkl file.

A brief summary of the progress of the structure solution appears on the console, and a full listing is written to a file name.lst, which can be printed or examined with a text editor. After structure solution a file name.res is written; this contains crystal data etc. as in the name.ins file, followed by potential atoms. It may be copied or edited to name.ins for structure refinement using XL or partial structure expansion with XS.

Two mechanisms are provided for interaction with an XS job which is already running. The first, which it is not possible to implement for all computer systems, applies to 'on-line' runs. If the [Ctrl-I] key combination is hit, the job terminates almost immediately (but without the loss of output buffers etc. which can happen with [ctrl-C] etc.). If the [Esc] key is hit during direct methods, the program does not generate any further phase permutations but completes the current batch of phase refinement and then proceeds to E-Fourier recycling etc. If the [Esc] key is hit during Patterson interpretation, the program stops after completing the calculations for the current superposition vector. Otherwise [Esc] has no effect. On computer consoles with no [Esc] key, [F11] or Ctrl-[usually have the same effect.

The second mechanism requires the user to create the file 'name.fin'; the program tries at regular intervals to delete this file, and if it succeeds it takes the same action as after [Esc]. The file is also deleted (if found) at the start of a job in case it has been accidentally left over from a previous job. This approach may be used with batch jobs.

5.2 The .ins Instruction File for XS

Three types of general calculation may be performed with XS. The structure of the .ins file is extremely similar for all three (and the .hkl file is always the same). The .ins file always begins with the instructions TITL..UNIT in the order given below. There follows TREF (for direct methods), PATT (for Patterson interpretation) or TEXP plus atoms (for partial structure expansion). The final instruction is usually HKLF.

Direct Methods	Patterson Interpretation	Partial Structure Expansion
TITL ...	TITL ...	TITL ...
CELL ...	CELL ...	CELL ...
ZERR ...	ZERR ...	ZERR ...
LATT ...	LATT ...	LATT ...
SYMM ...	SYMM ...	SYMM ...
SFAC ...	SFAC ...	SFAC ...
UNIT ...	UNIT ...	UNIT ...
TREF...	PATT	TEXP
HKLF 4	HKLF 4	atoms
		HKLF 4

Table 5.1 – .ins file structure

Although these standard settings should be appropriate for a wide range of circumstances, various parameters may be specified for TREF, PATT or TEXP and further instructions may be included between UNIT and HKLF for ‘fine tuning’ in the case of difficult structures. The parameter summary printed out after the data reduction in every job should be consulted before this is attempted, since the default settings for parameters that are not specified depend on the space group, the size of the structure, and the parameters that are actually specified (this is sometimes referred to as ‘artificial intelligence’!).

All instructions commence with a four (or less) letter word (which may be an atom name); numbers and other information follow in free format, separated by one or more spaces. Upper and lower case input may be freely mixed; with the exception of the text strings input using TITL it is all converted to upper case for internal use in XS. The TITL, CELL, ZERR, LATT, SYMM, SFAC and UNIT instructions must be given in that order; all remaining instructions, atoms, etc. should come between UNIT and the last instruction, which is almost always HKLF (to read in reflection data).

Defaults are given in square brackets in this documentation; '#' indicates that the program will generate a suitable default value based on the rest of the available information. Continuation lines are flagged by '=' at the end of a line, the instruction being continued on the next line which must start with at least one space. Other lines beginning with one or more spaces are treated as comments, so blank lines may be added to improve readability. All characters following '!' or '=' in an instruction line are ignored, except after TITL or SYMM (for which continuation lines are not allowed). AFIX, BASF, EQIV, EXTI, HFIX, RESI, SIZE, TEMP, WGHT and most other XL instructions may be present in the .ins file but are ignored.

5.3 Instructions Common to all Modes of Structure Solution

5.3.1 TITL [ ]

Title of up to 76 characters, to appear at suitable places in the output. The characters '!' and '=' may form part of the title. The title can include a chemical formula and/or space group, but one must be careful to update these if the UNIT or SYMM instructions are later changed!

5.3.2 CELL lambda a b c α β γ

Wavelength and unit-cell dimensions in Angstroms and degrees.

5.3.3 ZERR Z esd(a) esd(b) esd(c) esd(α) esd(β) esd(γ)

Z value (number of formula units per cell) followed by the estimated errors in the unit-cell dimensions. This information is not actually required by XS but is allowed for compatibility with XL.

5.3.4 LATT N [1]

Lattice type: 1=P, 2=I, 3=rhombohedral obverse on hexagonal axes, 4=F, 5=A, 6=B, 7=C. N must be made negative if the structure is non-centrosymmetric.

5.3.5 SYMM symmetry operation

Symmetry operators, i.e., coordinates of the general positions as given in International Tables. The operator X, Y, Z is always assumed, so may NOT be input. If the structure is centrosymmetric, the origin MUST lie on a center of symmetry. Lattice centering should be indicated by LATT, not SYMM. The symmetry operators may be specified using decimal or fractional numbers, e.g., 0.5-x, 0.5+y, -z or Y-X, -X, Z+1/6; the three components are separated by commas. At least one SYMM instruction must be present unless the structure is triclinic.

5.3.6 SFAC elements

These element symbols define the order of scattering factors to be employed by the program. The first 94 elements of the periodic system are recognized. The element name may be preceded by '$' but this is not obligatory (the '$' character is allowed for logical consistency with certain XL instructions but is ignored). The program uses absorption coefficients from International Tables for Crystallography (1991), Volume C. For organic structures the first two SFAC types should be C and H, in that order; the E-Fourier recycling generally assigns the first SFAC type (i.e., C) to peaks (unless they are obviously heavy atoms).

5.3.7 SFAC a1 b1 a2 b2 a3 b3 a4 b4 c df' df" mu r wt

Scattering factor in the form of an exponential series, followed by real and imaginary corrections, linear absorption coefficient, covalent radius and atomic weight. In addition, a 'label' consisting of up to 4 characters beginning with a letter (e.g., Ca2+) may be included before a1 (the first character may be a '$', but this is not obligatory). The two SFAC formats may be used in the same .ins file; the order of the SFAC instructions (and the order of element names in the first type of SFAC instruction) define the scattering factor numbers which are referenced by atom instructions. Not all numbers on this instruction are actually used by XS, but the full data must be given for compatibility with XL. For neutron data, c should be the scattering length (which may be negative) and a1..b4 will usually all be zero.

5.3.8 UNIT n1 n2 ...

Number of atoms of each type in the cell, in SFAC order.

5.3.9 REM

Followed by a comment on the same line. This comment is ignored by the program but is copied to the results file (.res). Note that comments beginning with one or more blanks are only copied to the .res file if the line is completely blank; REM comments are always copied.

5.3.10 MORE verbosity [1]

More sets the amount of (printer) output; verbosity takes a value in the range 0 (least) to 3 (most verbose).

5.3.11 TIME t [#]

If the time t (measured in seconds from the start of the job) is exceeded, XS performs no further blocks of phase permutations (direct methods), but goes on to the final E-map recycling etc. In the case of Patterson interpretation no further vector superpositions are performed after this time has expired. The default value of t is installation dependent, and is usually set to a little less than the maximum time allocation for a particular class of job. Usually t is 'CPU time', but on PCs the elapsed time has to be used instead.

5.3.12 OMIT s[4] 2theta(lim)[180]

Thresholds for flagging reflections as 'unobserved'. Note that if no OMIT instruction is given, ALL reflections are treated as 'observed'. Internally in the program s is halved and applied to F^2, so the test is roughly equivalent to suppressing all reflections with $F < s \bullet \sigma(F)$, as required for consistency with XS and XLS in version 4 of SHELXTL. Note that s may be set to 0 (to suppress reflections with negative F^2) or even to a negative threshold (to suppress very negative F^2) which has no equivalent in XLS. If 2theta(lim) is POSITIVE, it specifies a 2θ value ABOVE which the data are treated as 'unobserved'; if it is NEGATIVE, the absolute value is used as a LOWER 2θ cutoff.

5.3.13 OMIT h k l

The reflection h k l is flagged as 'unobserved' in the list of merged reflections after data reduction. It will not be used directly in phase refinement or Fourier calculations, but is retained for statistical purposes and as a possible cross-term in a negative quartet. Thus if it is known that a strong reflection has been included accidentally in the .hkl file with a very small intensity (e.g., because it was cut off by the beam stop), it is advisable to delete it from the .hkl file rather than using OMIT (which is intended for imprecisely measured data rather than blunders).

5.3.14 ESEL Emin[1.2] Emax[5] dU[.005] renorm[.7] axis[0]

Emin sets the minimum E-value for the list of largest E-values which the program normally retains in memory; it should be set so as to give more than enough reflections for TREF etc. It is also the threshold used for tangent expansion and 'peak-list optimization'. It is advisable to reduce Emin to about 1.0 for triclinic structures and pseudosymmetry problems. If Emin is negative, acentric triclinic data are generated for use in ALL calculations. The other parameters control the normalization of the E-values.

$$new(E) = old(E) \bullet \exp[8 \bullet dU \bullet (\pi \infty \sin(\theta)/\lambda)^2] / [old(E)^{-4} + Emax^{-4}]^{0.25}$$

renorm is a factor to control the parity group renormalization; 0.0 implies no renormalization, 1.0 sets full renormalization, i.e., the mean value of E^2 becomes unity for each parity group.

If axis is 1, 2 or 3, an additional similar renormalization is applied for groups defined by the absolute value of the h, k or l index respectively. If axis is set to zero, no such additional renormalization is applied.

5.3.15 EGEN d(min) d(max)

All missing reflections in the resolution range d(min) to d(max) Angstroms (the order of d(min) and d(max) is unimportant) are generated on a statistical basis, assuming that they were skipped during the data collection because a prescan indicated that they were weak. These reflections will then be flagged as 'unobserved', but improve the estimation of the remaining E-values and enable an increased number of negative quartets to be identified. d(min) should be safely inside the resolution limit of the data and d(max) should be set so that there is no danger of regenerating strong reflections (as weak) which were cut off by the beam stop etc.

5.3.16 LIST m [0]

m = 1 and m = 2 write h, k, l, A and B lists to the name.res file, where A and B are the real and imaginary parts of a point atom structure factor respectively. If m = 1 the list corresponds to the phased E-values for the 'best' direct methods solution, before partial structure expansion (if any). If m = 2 the list is produced after the final cycle of partial structure expansion, and corresponds to weighted E-values used for the final Fourier synthesis. These options enable other Fourier programs to be used, e.g., for graphical display of 3D-Fouriers for data which do not give atomic resolution.

After data reduction and merging equivalent reflections, a list of h, k, l, F and $\sigma(F)$ (for m = 3) or h, k, l, F^2 and $\sigma(F^2)$ (for m = 4) is written to the name.res file. This provides a useful input file for programs such as DIRDIF and MULTAN, which do not include sort/merge and rejection of systematic absences etc. XS always averages Friedel opposites.

In all four cases the output format is (3I4,2F8.2), and the list is terminated by a dummy reflection 0,0,0 or by the end of the file.

5.3.17 FMAP code[#] axis[#] nl[#]

The unique unit of the cell for performing the Fourier calculation is set up automatically unless specified by the user using FMAP and GRID. The program chooses a 53 × 53 × nl or 103 × 103 × nl grid depending the resolution of the data (provided sufficient memory is available).

code = 1 (F^2 Patterson), 3 (Patterson with coefficients input using HKLF 7; negative coefficients are allowed. 4 (E-map without peak-list optimization, e.g., because the peaks correspond to unequal atoms), 5 (Fourier with A and B coefficients input using HKLF 3), 6 (E•F Patterson), code > 6 (E-map followed by [code - 6] cycles peak-list optimization). Note that the peak-list optimization assigns all peaks to scattering factor type 1, so for many structures this should be specified as carbon on a SFAC instruction. FMAP 4 may be used with atoms but without TEXP etc. for an E-map based on calculated phases.

5.3.18 GRID sl[#] sa[#] sd[#] dl[#] da[#] dd[#]

Fourier grid, when not set automatically. Starting points and increments are multiplied by 100. s means starting value, d increment, l is the direction perpendicular to the layers, a is across the paper from left to right, and d is down the paper from top to bottom. Note that the grid is 53 x 53 x nl points, i.e., twice as large as in XLS in version 4 of SHELXTL, and that sl and dl need not be integral. The 103 x 103 x nl grid is only available when it is set automatically by the program (see above).

5.3.19 PLAN npeaks[#] d1[0.5] d2[1.5]

If npeaks is positive it is the number of highest unique Fourier peaks which are written to the .res and .lst files; the remaining parameters are ignored.

If npeaks is given as negative, the program attempts to arrange the peaks into unique molecules taking the space group symmetry into account, and to ‘plot’ a projection of each such molecule on the printer (i.e., the .lst file). Distances involving peaks which are less than r1+r2+d1 (the covalent radii r are defined via SFAC; 1 and 2 refer to the two atoms concerned) are considered to be 'bonds' for purposes of the molecule assembly and tables. Distances involving atoms and/or peaks which are less than r1+r2+d2 are considered to be ‘non-bonded interactions’. Such interactions are ignored when defining molecules, but the corresponding atoms and distances are included in the line-printer output. Thus an atom may appear in more than one map, or more than once on the same map. Negative d2 includes hydrogen atoms in these non-bonds, otherwise they are ignored (the absolute value of d2 is used in the test). Peaks are always assigned the radius of SFAC type 1, which is usually set to carbon. Peaks appear on the printout as numbers, but in the .res file they are given names beginning with ‘Q’ and followed by the same numbers.

To simplify interpretation of the lineprinter plots, extra symmetry-generated atoms are added, so that atoms or peaks may appear more than once. A table of the appropriate coordinates and symmetry transformations appears at the end of the output. See also MOLE for forcing molecules (and their environments) to be printed separately.

5.3.20 MOLE n[#]

Forces the following atoms, and atoms or peaks that are bonded to them, into molecule n of the PLAN output. n may not be greater than 99. MOLE is only appropriate for partial structure expansion.

5.3.21 HKLF n[0] s[1] r11...r33[1 0 0 0 1 0 0 0 1] wt[1] m[0]

Before running XS, a reflection data file name.hkl must usually be prepared. The HKLF command tells the program which format has been chosen for this file, and allows the indices to be reoriented using a 3x3 matrix r11..r33 (which should have a positive determinant). n is negative if reflection data follow, otherwise they are read from the .hkl file. The data are read in fixed format 3I4,2F8.2 (except for n = 1) subject to FORTRAN-77 conventions. The data are terminated by a record with h, k and l all zero (except n=1, which contains a terminator and checksum). If batch numbers, direction cosines or wavelengths are present in the .hkl file (e.g., for absorption corrections using the SHELXTL program XPREP) they will be ignored. The multiplicative scale s multiplies both F^2 and $\sigma(F^2)$ (or F and $\sigma(F)$ for n = 1 or 3). The multiplicative weight wt multiplies all $1/\sigma^2$ values and m is an integer 'offset' needed to read ‘condensed data’ (HKLF 1); both are included only for compatibility with SHELX-76. Usually simply 'HKLF 4' is all that will be required.

n = 1: SHELX-76 condensed data. Although now obsolete this format is both ASCII and compact, and contains a checksum, so is sometimes used for network transmission and testing purposes.

n = 3: h k l F $\sigma(F)$ or h k l A B depending on FMAP setting. In the first case the sign of F is ignored (for use with macromolecular ΔF data). This format should NOT be used for routine structure determination purposes because the approximation(s) required for the derivation of F and $\sigma(F)$ severely degrade the quality of the data.

n = 4: h k l F^2 $\sigma(F^2)$. THIS IS THE CORRECT FORMAT for all normal purposes (except macromolecular isomorphous or anomalous ΔF's).

n = 7: h k l E or h k l P (Patterson coefficient) depending on FMAP.

There may only be one HKLF instruction and it must come last!

5.3.22 END

This is the last instruction in the rare cases when the .ins file is not terminated by the HKLF instruction.

5.4 Structure Solution by Direct Methods

Usually direct methods will be initiated with the single command TREF; for especially easy structures one can save time with (say) TREF 20, or for large structures brute force (e.g., TREF 2000) may prove necessary. In fact there are a large number of parameters which can be varied, though the program is based on experience of many thousands of structures and can usually be relied upon to choose sensible default values. A summary of these parameters appears after the data reduction output, and should be consulted before attempting any direct methods options other than 'TREF n'.

The phase refinement of multiple random starting phase sets takes place in three stages, controlled by the INIT, PHAN and TREF instructions. The 'best' solution is then expanded further by tangent expansion and E-Fourier recycling (see the section on partial structure expansion).

5.4.1 INIT nn[#] nf[#] s+[0.8] s-[0.2] wr[0.2]

The first stage involves five cycles of weighted tangent formula refinement (based on triplet phase relations only) starting from nn reflections with random phases and weights of 1. Single phase seminvariants which have sigma-1 formula P+ values less that s- or greater than s+ are included with their predicted phases and unit weights. All these reflections are held fixed during the INIT stage but refined freely in the subsequent stages. The remaining reflections also start from random phases with initial weights wr, but both the phases and the weights are allowed to vary.

If nf is non-zero, the nf 'best' (based on the negative quartet and triplet consistency) phase sets are retained and the process repeated for (npp-nf) parallel phase sets, where npp is the previous number of phase sets processed in parallel (often 128). This is repeated for nf fewer phase sets each time until only a quarter of the original number are processed in parallel. This rather involved algorithm is required to make efficient use of available computer memory. Typically nf should be 8 or 16 for 128 parallel permutations.

The purpose of the INIT stage is to feed the phase annealing stage with relatively self-consistent phase sets, which turns out to be more efficient than starting the phase annealing from purely random phases. If TREF 0 is used to generate partial structure phases for all reflections, the INIT stage is skipped. To save time, only ns reflections and the strongest mtpr triplets for each reflection (or less, if not so many can be found) are used in the INIT stage; these numbers are given on the PHAN instruction.

5.4.2 PHAN steps[10] cool[0.9] Boltz[#] ns[#] mtpr[40] mnqr[10]

The second stage of phase refinement is based on 'phase annealing' [Acta Cryst., A46 (1990) 467-473]. This has proved to be an efficient search method for large structures, and possesses a number of beneficial side-effects. It is based on steps cycles of tangent formula refinement (one cycle is a pass through all ns phases), in which a correction is applied to the tangent formula phase. The phase annealing algorithm gives the magnitude of the correction (it is larger when the 'temperature' is higher; this corresponds to a larger value of Boltz), and the sign is chosen to give the best agreement with the negative quartets (if there are no negative quartets involving the reflection in question, a random sign is used instead). After each cycle through all ns phases, a new value for Boltz is obtained by multiplying the old value by cool; this corresponds to a reduction in the 'temperature'. To save time, only ns reflections are refined using the strongest mtpr triplets and mnqr quartets for each reflection (or less, if not so many phase relations can be found). The phase annealing parameters chosen by the program will rarely need to be altered; however if poor convergence is observed, the Boltz value should be reduced; it should usually be in the range 0.2 to 0.5. When the 'TEXP 0 / TREF' method of multisolution partial structure refinement is employed, Boltz should be set at a somewhat higher value (0.4 to 0.7) so that not too many solutions are duplicated.

5.4.3 TREF np[100] nE[#] kapscal[#] ntan[#] wn[#]

np is the number of direct methods attempts; if negative, only the solution with code number |np| is generated (the code number is in fact a random number seed). Since the random number generation is very machine dependent, this can only be relied upon to generate the same results when run on the same model of computer. This facility is used to generate E-maps for solutions which do not have the 'best' combined figure of merit. No other parameter may be changed if it is desired to repeat a solution in this way. For difficult structures, it may well be necessary to increase np (e.g., TREF 2000) and of course the computer time allocated for the job.

nE reflections are employed in the full tangent formula phase refinement. Values of nE that give fewer than 20 unique phase relations per reflection for the full phase refinement are not recommended.

kapscal multiplies the products of the three E-values used in triplet phase relations; it may be regarded as a fudge factor to allow for experimental errors and also to discourage overconsistent (uranium atom) solutions in symorphic space groups. If it is negative the cross-term criteria for the negative quartets are relaxed (but all three cross-term reflections must still be measured), and more negative quartets are used in the phase refinement, which is also useful for symorphic space groups.

ntan is the number of cycles of full tangent formula refinement, which follows the phase annealing stage and involves all nE reflections; it may be increased (at the cost of CPU time) if there is evidence that the refinement is not converging well. The tangent formula is modified to avoid overconsistency by applying a correction to the resulting phase of cos-1(<alpha>/alpha) when <alpha> is less than alpha; the sign of the correction is chosen to give the best agreement with the negative quartets (a random sign is used if there are no negative quartets involving the phase in question). This tends to drive the figures of merit R(alpha) and Nqual simultaneously to desirable values. If ntan is negative, a penalty function of (<sigma1> - sigma1)² is added to CFOM (see below) if and only if sigma-1 is less than its estimated value <sigma1>. sigma1 is a weighted sum of the products of the expected and observed signs of one-phase seminvariants, normalized so that it must lie in the range -1 to +1. This is useful (i.e., better than nothing) if no negative quartets have been found or if they are unreliable, e.g., when macromolecular ΔF data are employed (see below).

wn is a parameter used in calculating the combined figure of merit CFOM: CFOM = R(alpha) (NQUAL < wn) or R(alpha) + (wn-NQUAL)^2 (NQUAL >= wn); wn should be about 0.1 more negative than the anticipated value of NQUAL. If it is known that the measurements of the weak reflections are unreliable (i.e., have high standard deviations), e.g., because data were collected using the default options on a CAD-4 diffractometer, then the NQUAL figure of merit is less reliable. If the space group does not possess translation symmetry, it is essential to obtain good negative quartets, i.e., to measure ALL reflections for an adequate length of time.

Only the TREF instruction is essential to specify direct methods; appropriate INIT, PHAN, FMAP, GRID and PLAN instructions are then generated automatically if not given.

5.5 Direct Methods Example (log.ins)

A direct methods test example is provided in the form of the file log.ins; this file could have been prepared by the program XPREP (Chapter 4). The test job can be started on all systems with:

```
xs log
```

Extracts from the log.lst output file are given below. The instructions TITL..UNIT define the space group to be $P2_12_12_1$ with 68 C, 104 H and 40 O atoms per cell. The mean (non-hydrogen) atomic volume and density are typical for a sugar derivative. TREF invokes direct methods with default options. The reflection data are also included in the .ins file in the obsolete 'condensed data' format; this is convenient for setting up compact self-contained test jobs, but normally a .hkl file would be read instead (with HKLF 4). On generating a unique (Friedel-averaged) data set two reflections are commented (but retained in the data set) because the equivalents are inconsistent; however the values for R(int) and R(sigma) indicate that the data are of respectable quality.

```
++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
+  XS -  CRYSTAL STRUCTURE SOLUTION  - Bruker  SHELXTL - Ver. 5.10  +
+  Copyright(c) 1997 Bruker  Analytical Xray - All Rights Reserved  +
+  log                          started at 08:46:55 on 26 May 1997  +
++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

TITL LOGANIN
CELL 0.71069 8.187 14.277 15.693 90 90 90
LATT -1
SYMM 0.5-X,-Y,0.5+Z
SYMM 0.5+X,0.5-Y,-Z
SYMM -X,0.5+Y,0.5-Z
SFAC C H O
UNIT 68 104 40

V = 1834.29     At vol = 17.0     F(000) = 832.0     mu = 0.12 mm-1

Max single Patterson vector = 12.5    cell wt = 1561.51    rho = 1.414

TREF
HKLF -1 1  1 0 0  0 1 0  0 0 1  1       0

Checksum O.K.

   3498  Reflections read, of which     0  rejected

   Maximum h, k, l and 2-Theta =   10.   18.   20.   56.95


INCONSISTENT EQUIVALENTS

  h   k   l       F*F    Sigma(F*F)  Esd of mean(F*F)

  3   3   6     3114.04     20.83    116.66
  2   9  17       25.47      2.50     12.61

   2428  Unique reflections, of which   2177  observed

   R(int) = 0.0203     R(sigma) = 0.0216      Friedel opposites merged
```

```
NUMBER OF UNIQUE DATA AS A FUNCTION OF RESOLUTION IN ANGSTROMS

Resolution Inf 5.0 3.50 2.50 2.00 1.70 1.50 1.40 1.30 1.20 1.10 1.00 0.90 0.80

N(observed)  12.  23.  51.  72.  93. 103.  72. 106. 123. 194. 255. 348. 490.

N(measured)  12.  23.  51.  72.  96. 106.  73. 107. 129. 199. 272. 391. 581.

N(theory)    13.  23.  51.  72.  96. 106.  73. 107. 129. 199. 273. 393. 621.

Two-theta  0.0 8.2 11.7 16.3 20.5 24.1 27.4 29.4 31.7 34.4 37.7 41.6 46.5 52.7

Highest memory for sort/merge =  4530 / 12140


Observed E .GT. 1.200 1.300 1.400 1.500 1.600 1.700 1.800 1.900 2.000 2.100

Number           694   575   478   399   316   260   205   161   130   104


                 Centric Acentric    0kl      h0l      hk0      Rest

Mean Abs(E*E-1)    0.968    0.736    0.978    0.936    0.910    0.762

      4.0 seconds elapsed time


   SUMMARY OF PARAMETERS FOR  LOGANIN

ESEL  Emin  1.200    Emax  5.000    DelU 0.005    renorm 0.700    axis 0
OMIT  s  4.00    2theta(lim)  180.0
INIT  nn   11    nf   16    s+  0.800    s-  0.200    wr  0.200
PHAN  steps   10   cool 0.900   Boltz 0.300   ns  139   mtpr   40   mnqr  10
TREF  np       64.    nE   219    kapscal  0.850    ntan   3    wn -0.560
FMAP  code  8
PLAN  npeaks   -37    del1 0.500    del2 1.500
MORE  verbosity  1
TIME  t    9999999.


    139 Reflections and   1206. unique TPR for phase annealing

    219 Phases refined using    4592. unique TPR
    296 Reflections and    8211. unique TPR for R(alpha)

      5.5 seconds elapsed time

   1252 Unique negative quartets found,  1080 used for phase refinement

      5.9 seconds elapsed time

Highest memory used to derive phase relations =    4508 /   21539
```

Since 194 out of a possible 199 reflections are observed in the critical 1.1 to 1.2 Å resolution range, direct methods should perform well. The E-statistics show that the three projections are centrosymmetric but that the remaining data are non-centrosymmetric, as expected for this space group. The parameter summary includes the value set by the program; this information may prove useful if the automatic direct methods prove unsuccessful. Further details of the direct methods follow, but are too extensive to give here; the critical output is the table of figures of merit and seminvariant phases for the refined phase sets, and the distribution of the combined figure of merit:

```
  Try    Ralpha Nqual Sigma-1 M(abs) CFOM   Seminvariants

 745727. 0.163 -0.229  0.582  0.898  0.273  +--++ +--++ +--+- -++-+ -+-++
1631483. 0.071 -0.705  0.776  1.003  0.071  +-+++ +--+- -+--+ ++--+ -+++-
1865959. 0.164 -0.093  0.580  0.878  0.382  +-+++ +--+- ++-+- +++++ -++--
 941187. 0.224 -0.041  0.122  0.819  0.494  +---+ +---- +---- ++++- +---+
 511631. 0.232  0.012  0.166  0.780  0.560  +--++ -+-++ -+--- -+-+- -++-+
 461003. 0.210 -0.090  0.154  0.812  0.431  +--++ --+++ +--++ ++--- -+-++
 207863. 0.203 -0.150  0.381  0.861  0.371  +--+- +--++ ---+- -++++ -+-+-
1039315. 0.271 -0.288  0.135  0.769  0.345  ---++ ++--+ ++-+- ++-++ -+-++
1002271. 0.177 -0.135  0.556  0.884  0.357  +--++ +---+ +--+- -++-+ -+-++
 817051. 0.159 -0.286  0.746  0.881  0.234  +-+++ +---+ -+--- +++-+ -++--
1988103. 0.219  0.052  0.335  0.789  0.595  +-+++ +--++ ---++ ++-+- +-+++
1551907. 0.378 -0.194  0.525  0.665  0.512  +-+++ +-+-+ -+--+ -+--- --+--
1468079. 0.178 -0.005  0.561  0.883  0.487  +-+++ +--++ +--++ -+-+- +++--
1048939. 0.154  0.168  0.426  0.911  0.684  +--++ +--+- +---- ++++- ++--+
1050391. 0.093 -0.563  0.776  0.952  0.093  +-+++ +--+- -+--+ ++--+ -+++-
1057651. 0.259 -0.278  0.645  0.786  0.338  +--++ ++--+ ++--- +-+-+ ++--+
 953859. 0.234 -0.153  0.281  0.799  0.400  +-++- +-+++ -+-+- --++- -++--
1684763. 0.143 -0.371  0.242  0.902  0.179  +-++- --++- -+--- +---+ --++-
 709243. 0.249  0.141  0.011  0.823  0.740  +---+ +-++- -+-+- ++++- ++-++
1791863. 0.229  0.143  0.163  0.854  0.724  +--++ -+-++ ---++ ++--- ---++
 777803. 0.157 -0.038  0.469  0.923  0.429  +--++ ++--+ +--+- -++-+ ---++
 756383. 0.244 -0.138  0.584  0.792  0.422  +-++- +--+- -+--+ ++--+ --++-
1340015. 0.206 -0.129  0.527  0.869  0.392  +--+- +--+- +---- ++--+ -+-+-
 408619. 0.241 -0.289  0.461  0.769  0.315  +-+++ +--+- ---++ ++++- ++-++
 774735. 0.180  0.054  0.550  0.873  0.558  +-+++ +--++ +---+ -+-+- +++-+
 372883. 0.199 -0.503  0.367  0.806  0.202  +--+- +--++ +--+- -++-- -+-++
1776523. 0.165  0.103  0.182  0.919  0.605  +-++- +-+++ -+-+- ---+- +++-+
 494007. 0.114 -0.458  0.423  0.950  0.124  +-++- +-++- -+--- +---+ --++-
 206571. 0.240 -0.442  0.547  0.783  0.255  +-+++ ++-++ ----- -++++ -++-+
  35207. 0.234  0.057  0.401  0.785  0.615  +-+++ +--++ ---++ ++-+- +++++
 574991. 0.140 -0.303  0.657  0.918  0.206  +-+++ +--+- ++--- +++++ -++--
1826867. 0.170 -0.190  0.490  0.874  0.307  +--++ +---+ +--+- -++-+ ---++
1826751. 0.207  0.063  0.528  0.836  0.596  +-+++ +-+++ -+--- +-+-- +++-+
 932187. 0.159  0.096  0.518  0.906  0.590  +-+++ +--+- ++--- ++++- +++-+
 413395. 0.069 -0.700  0.776  1.001  0.069  +-+++ +--+- -+--+ ++--+ -+++-
 322067. 0.130 -0.622  0.789  0.911  0.130  +-+++ +--+- --+-+ ++--+ -+++-
2064259. 0.208 -0.243  0.693  0.809  0.309  +-+++ +---+ ++--- --+++ -++--
1717875. 0.193  0.303  0.283  0.878  0.939  +-++- +-+++ ---+- -+--- +++-+
  68715. 0.305 -0.512  0.525  0.708  0.307  +-+-+ +--++ ----+ -+--+ --+--
1742135. 0.244 -0.235  0.350  0.765  0.350  +-+-+ +-+++ ---+- ----+ -+++-
1342727. 0.172 -0.479  0.506  0.818  0.178  +-++- +-+++ -+-+- --+-+ -+++-
1760219. 0.161  0.108  0.214  0.888  0.608  +---+ +--+- +---- ++++- ++--+
 701235. 0.214  0.041  0.539  0.850  0.576  +-++- +--+- ----+ +++-- +++-+
1631123. 0.147  0.104  0.381  0.952  0.589  +-++- +--++ +---- ---+- +++++
```

```
 753659. 0.169  0.000  0.442  0.895  0.483  +-+++ +-+++ +--++ -+-+- +++--
  82679. 0.064 -0.688  0.776  1.008  0.064  +-+++ +--+- -+--+ ++--+ -+++-
1851459. 0.166  0.160  0.214  0.885  0.685  +---+ +--+- +---- ++++- ++--+
1229227. 0.142 -0.301  0.592  0.910  0.209  +-+++ +--+- ++--- +++++ --+--
1626667. 0.169  0.112  0.214  0.881  0.620  +---+ +--+- +---- ++++- ++--+
1032659. 0.238 -0.169  0.083  0.778  0.392  +--++ -++++ -+--- -+-+- -----
1339787. 0.174 -0.410  0.728  0.817  0.196  +-+++ +--++ -+-++ -++-+ --++-
1586211. 0.169 -0.148  0.657  0.879  0.339  +-+++ +--+- ++--- +++++ -++--
 400611. 0.195 -0.330  0.582  0.827  0.247  --+++ +--++ -+++- -++-+ -+++-
1952075. 0.176  0.427  0.404  0.914  1.152  +--+- +--++ ---++ ----- ++--+
 142479. 0.143 -0.253  0.744  0.909  0.237  +-+++ +---- -+--- +++-+ -++--
 968991. 0.062 -0.703  0.776  1.009  0.062* +-+++ +--+- -+--+ ++--+ -+++-
2037395. 0.179 -0.090  0.556  0.895  0.400  +--++ +---+ +--+- -++-+ -+-++
 603227. 0.150  0.358  0.388  0.944  0.994  +--+- +--+- +---- ++++- ++---
1798367. 0.185 -0.427  0.508  0.816  0.203  +-++- +-+++ -+-+- --+-+ -++--
1945679. 0.063 -0.687  0.776  1.012  0.063  +-+++ +--+- -+--+ ++--+ -+++-
1156103. 0.143 -0.232  0.770  0.904  0.251  +-+++ +--+- -+--- +++-+ -++--
 546523. 0.145 -0.271  0.632  0.908  0.229  +-+++ +--+- -+--- +++++ -++--
 650651. 0.146 -0.361  0.657  0.892  0.185  +-+++ +--+- ++--- +++++ -++--
1079703. 0.067 -0.707  0.776  0.999  0.067  +-+++ +--+- -+--+ ++--+ -+++-
```

CFOM Range	Frequency
0.000 - 0.020	0
0.020 - 0.040	0
0.040 - 0.060	0
0.060 - 0.080	6
0.080 - 0.100	1
0.100 - 0.120	0
0.120 - 0.140	2
0.140 - 0.160	0
0.160 - 0.180	2
0.180 - 0.200	2
0.200 - 0.220	4
0.220 - 0.240	3
0.240 - 0.260	3
0.260 - 0.280	1
0.280 - 0.300	0
0.300 - 0.320	4
0.320 - 0.340	2
0.340 - 0.360	3
0.360 - 0.380	1
0.380 - 0.400	4
0.400 - 0.420	1
0.420 - 0.440	3
0.440 - 0.460	0
0.460 - 0.480	0
0.480 - 0.500	3
0.500 - 0.520	1
0.520 - 0.540	0
0.540 - 0.560	2
0.560 - 0.580	1
0.580 - 0.600	4

```
0.600 - 9.999     11

      64. Phase sets refined - best is code   968991.  with CFOM =  0.0620


     54.5 seconds elapsed time


Tangent expanded to  694  out of  694  E greater than  1.200
Highest memory used =  2917 /  7312

      3.8 seconds elapsed time


FMAP and GRID set by program

FMAP   8   3  19
GRID    -1.563  -2  -2     1.563   2   2


E-Fourier for  LOGANIN

Maximum =  197.76,  minimum =  -53.94       highest memory used =  8873 / 11662

      3.3 seconds elapsed time


Peak list optimization
RE = 0.185 for  27 surviving atoms and  694 E-values    Highest memory used =  1688 /
6246

      2.9 seconds elapsed time


E-Fourier for  LOGANIN

Maximum =  174.46,  minimum =  -57.70       highest memory used =  8873 / 11662

      3.2 seconds elapsed time


Peak list optimization
RE = 0.176 for  27 surviving atoms and  694 E-values    Highest memory used =  1688 /
6246
```

It will be seen that there are 6 or 7 good solutions out of 64 tries; the best is marked with an asterisk in the table. The figures of merit R(alpha) = 0.062 and NQUAL = -0.703 are decisive. It will also be seen that the good solutions all have the same list of plus and minus signs for the seminvariant phases; if this were not so, it might mean that there are more than one solution with equally good figures of merit, and it might be necessary to generate further E-maps using TREF -n, where n is the random number seed given in the first column. Because the random numbers are system-dependent, this output may look different on a different computer, but the number of correct solutions and their figures of merit should be similar to those shown above. Two cycles of E-Fourier recycling give RE (R factor based on the agreement between E_o and E_c) of 0.176 for the full 27 non-hydrogen atoms in this structure.

5.6 What To Do When Direct Methods Fail

If direct methods fail to give a clearly correct answer, the diagnostic information printed out during the data reduction at the start of the name.lst file should first be carefully reexamined.

After reading the SFAC and UNIT instructions the program uses the unit-cell contents and volume to calculate the volume per non-hydrogen atom, which is usually about 18 for typical organic structures. Condensed aromatic systems can reduce this value (to about 13 in extreme cases) and higher values (20-30) are observed for structures containing heavier elements. The estimated maximum single weight Patterson vector may be useful (in comparison with the Patterson peak-list) in deciding whether the expected heavy atoms are in fact present. However in general the program is rather insensitive to the given unit-cell contents; the assignment of atom types in the E-Fourier recycling (after direct methods when heavier atoms are present) and in the Patterson interpretation do however assume that the elements actually present are those named on the SFAC instructions.

Particularly useful checks are the values of 2-theta(max) and the maximum values of the (unsigned) reflection indices h, k and l; for typical small-molecule data the latter should be a little greater than the corresponding unit-cell dimensions. If not, or if 2-theta(max) does not correspond to the value used in the data collection, there must be an error in the CELL or HKLF instructions, or possibly in the reflection data.

The R(int) value may be used as a test of the Laue group provided that appropriate equivalent reflections have been measured. Generally R(int) should be below 0.1 for the correct assignment. R(sigma) is simply the sum of $\sigma(F^2)$ divided by the sum of F^2; a value above 0.1 indicates the data are very weak or that they have been incorrectly processed.

The mean values of $|E^2-1|$ show whether the E-value distribution for the full data and for the 0kl, h0l and hk0 projections are centric or acentric; this provides a check on the space group assignment, but such statistics may be unreliable if heavy atoms are present (especially when they lie on special positions) or if there are very few reflections in one of these three projections. Twinned structures may give an acentric distribution even when the true space group is centrosymmetric. These numbers may also show up typing errors in the LATT and SYMM instructions; although the program checks the LATT and SYMM instructions for internal consistency, it is not possible to detect all possible errors in this way.

Direct methods is based on the assumption of ‘equal resolved atoms’. If the data do not suffice to ‘resolve’ the atoms from each other, direct methods are doomed to failure. A good empirical test of resolution is to compare the number of reflections ‘observed’ in the 1.1 to 1.2 Å range with the number theoretically possible (assuming that OMIT is at its default setting of 4) as printed out by the program. If this ratio is less than one half, it is unlikely that the structure will be ever be solved by direct methods. This criterion may be relaxed somewhat for centrosymmetric structures and those containing heavy atoms. It also does not apply to the location of heavy atoms from macromolecular ΔF data because the distances between the ‘atoms’ are much larger. If the required resolution has not been reached, there is little point in pursuing direct methods further; the only hope is to recollect the data with a larger crystal, stronger radiation source, longer measurement times, area detector, real-time profile fitting and lower temperature, or at least as many of these as are simultaneously practicable.

If the data reduction diagnostics give no grounds for suspicion and no direct methods solution gives good figures of merit, a brute force approach should be applied. This takes the form of TREF followed by a large number (e.g., TREF 10000); it may also be necessary to set a larger value for TIME. If either of the methods for interrupting a running job are available (see above), an effectively infinite value may be used (TREF 999999). Any change in this number of phase permutations will also change the random number sequence employed for the starting phases.

If more than one solution has good R(alpha) and NQUAL values, it is possible that the structure has been solved but the program has chosen the wrong solution. The list of one-phase seminvariant signs printed by the program can be used to decide whether two solutions are equivalent or not. In such a case other solutions can be regenerated without repeating the complete job by means of 'TREF -n', where n is a solution code number (in fact the random number seed). Because of the effect of small rounding errors the 'TREF -n' job must be performed on the same computer as the original run. No other parameters should be changed when this option is used.

In cases of pseudosymmetry it may be necessary to modify the E-value normalization (i.e., by increasing the renorm parameter on the ESEL instruction to 0.9, or by setting a non-zero value of axis on the same instruction). E(min) should be set to 1.0 or a little lower in such cases.

If there is evidence of pseudosymmetry (or just more than one chemically identical molecule in the asymmetric unit), or if many solutions have similar figures of merit, the discrimination of the figures of merit can usually be improved by reducing the E-threshold (e.g., ESEL 1.0) and increasing the number of phases refined (the second TREF parameter).

When direct methods only reveal a fragment of the structure, it may well be correctly oriented but incorrectly translated relative to the origin. In such cases a non-centrosymmetric triclinic expansion with 'ESEL -1' may enable the symmetry elements and hence the correct translation (and perhaps the correct space group) to be identified.

Finally, if any heavier (than Na) elements are present, automatic Patterson interpretation should be tried...

6 Automated Patterson Interpretation and Partial Structure Expansion

The algorithm used to interpret the Patterson to find the heavier atoms in XS is totally different to that used in version 4 of SHELXTL or in SHELXS-86; it may be summarized as follows:

1. One peak is selected from the sharpened Patterson (or input by means of a VECT instruction) to be used as a superposition vector. This must correspond to a correct heavy-atom to heavy-atom vector otherwise the method will fail. The entire procedure may be repeated any number of times with different superposition vectors by specifying 'PATT nv', with |nv| > 1, or by including more than one VECT instruction in the same job.

2. The Patterson function is calculated twice, displaced from the origin by +U/2 and –U/2, where U is the superposition vector. At each grid point the lower of the two values is taken, and the resulting 'superposition minimum function' is interpolated to find the peak positions. This is a much cleaner map than the original Patterson and contains only 2**N** (or 4**N** etc. if the superposition vector was multiple) peaks rather than $\mathbf{N}^2$. The superposition map should ideally consist of one image of the structure and its inverse; it has an effective 'space group' of P-1 (or C-1 for a centred lattice etc.).

3. Possible origin shifts are found which place one of the images correctly with respect to the cell origin, i.e., most of the symmetry equivalents can be found in the peak-list. The SYMFOM figure of merit (normalized so that the largest value for a given superposition vector is 99.9) indicates how well the space group symmetry is satisfied for this image.

4. For each acceptable origin shift, atomic numbers are assigned to the potential atoms based on average peak heights, and a 'crossword table' is generated. This gives the minimum distance and Patterson minimum function for each possible pair of unique atoms, taking symmetry into account. This table should be interpreted by hand to find a subset of the atoms making chemically sensible minimum interatomic distances linked by consistently large Patterson minimum function values. The PATFOM figure of merit measures the internal consistency of these minimum function values and is also normalised to a maximum of 99.9 for a given superposition vector. The Patterson values are recalculated from the original F(obs) data, not from the peak-list. For high symmetry space groups the minimum function is calculated as an average of the two (or more) smallest Patterson densities.

5. For each set of potential atoms a 'correlation coefficient' (Fujinaga and Read, *J. Appl. Cryst.,* **20** (1987) 517-521) is calculated as a measure of the agreement between E(obs) and E(calc), and expressed as a percentage. This figure of merit may be used to compare solutions from different superposition vectors.

6.1 CUMOS2 Test Job Output

The CUMOS2 test may be run by:

```
xs cumos2
```

Extracts from the output are discussed below; the numerical results should agree within the usual rounding errors; the output has been compressed to fit it onto the page. The logo is followed by an echo of the .ins file (excluding the reflection data which is contained in the .ins file in the form of 'condensed data'. This now obsolete format is convenient for setting up very compact test jobs, but for new structures a .hkl file should be used.

```
++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++
+  XS -  CRYSTAL STRUCTURE SOLUTION  - Bruker  SHELXTL - Ver. 5.10  +
+  Copyright(c) 1997 Bruker  Analytical Xray - All Rights Reserved  +
+  cumos2                       started at 08:52:08 on 26 May 1997  +
++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

TITL [PR4N]2 [PHSCUS2MOS2CUSPH]
CELL .71069 17.064 15.646 18.448 90 110.47 90
LATT 7
SYMM -X, Y, .5-Z
SFAC C H N S CU MO
UNIT 144 264 8 24 8 4

V = 4614.30     At vol = 24.5     F(000) = 1968.0   mu = 1.48 mm-1

Max single Patterson vector = 68.4    cell wt = 3769.15    rho = 1.356

PATT
HKLF -1 1  1 0 0  0 1 0  0 0 1  1       0.

Checksum O.K.


   4059  Reflections read, of which     0  rejected

   Maximum h, k, l and 2-Theta =   20.   18.   21.   50.01

   4059  Unique reflections, of which   2404  observed

   R(int) = 0.0000     R(sigma) = 0.0615      Friedel opposites merged
```

```
NUMBER OF UNIQUE DATA AS A FUNCTION OF RESOLUTION IN ANGSTROMS

Resolution Inf 5.0 3.50 2.50 2.00 1.70 1.50 1.40 1.30 1.20 1.10 1.00 0.90 0.80

N(observed)    8.  39.  85. 138. 162. 183. 134. 161. 209. 298. 343. 404. 240.

N(measured)    9.  40.  95. 154. 184. 223. 170. 215. 298. 427. 602. 893. 749.

N(theory)     19.  40.  95. 154. 184. 223. 170. 215. 298. 427. 602. 893. 1414.

Two-theta 0.0  8.2 11.7 16.3 20.5 24.1 27.4 29.4 31.7 34.4 37.7 41.6 46.5 52.7


Highest memory for sort/merge = 16446 / 20295



Observed E .GT. 1.200 1.300 1.400 1.500 1.600 1.700 1.800 1.900 2.000 2.100

Number          1042   915   801   685   586   509   423   349   296   231


                  Centric Acentric    0kl      h0l      hk0      Rest

Mean Abs(E*E-1)    0.968    0.736    0.986    1.038    1.021    1.022


      4.8 seconds elapsed time
```

From the reflection statistics it is clear that the resolution of the data will be adequate for structure solution, and that the structure is very likely to be in the centrosymmetric space group C2/c rather than the non-centrosymmetric Cc. A summary of the parameters (including those set by the program) now appears; this could be useful if it were necessary to fine-tune and reinput these values to solve a difficult structure.

```
   SUMMARY OF PARAMETERS FOR  [PR4N]2 [PHSCUS2MOS2CUSPH]

ESEL  Emin  1.200    Emax  5.000    DelU 0.005    renorm 0.700    axis 0
OMIT  s  4.00    2theta(lim)  180.0
PATT  nv   1   dmin   1.80   resl  0.84   Nsup  252   Zmin  7.00   maxat 11
FMAP  code  6
PLAN  npeaks    80    del1 0.500    del2 1.500
MORE  verbosity  1
TIME  t    9999999.
FMAP and GRID set by program

FMAP   6   2  36
GRID    -1.515  -1  -1     1.515   1   1
```

Now the list of unique Patterson peaks is printed:

```
Super-sharp Patterson for  [PR4N]2 [PHSCUS2MOS2CUSPH]

Maximum =  999.10,  minimum =  -81.19       highest memory used =  9228 / 47670

     10.9 seconds elapsed time

         X       Y       Z     Weight  Peak  Length

1     0.0000  0.0000  0.0000     4.    999.    0.00
2     0.1549  0.0000  0.0899     2.    399.    2.58
3     0.0000  0.1219  0.5000     2.    315.    9.42
4     0.8449  0.8784  0.4068     1.    230.    8.99
5     0.3035  0.0000  0.1755     2.    171.    5.06
6     0.8993  0.9203  0.0105     1.    133.    2.19
7     0.0558  0.0804  0.1035     1.    129.    2.21
8     0.2058  0.3663  0.3298     1.     95.    8.20
9     0.1021  0.2015  0.4889     1.     85.    9.13
10    0.4451  0.0000  0.2592     2.     76.    7.43
11    0.9443  0.9607  0.3961     1.     72.    7.72
12    0.9426  0.7996  0.3946     1.     58.    8.29
13    0.2407  0.2991  0.4179     1.     54.    8.72
14    0.2102  0.0813  0.1941     1.     53.    4.28
15    0.7833  0.9644  0.3072     1.     53.    7.79
16    0.0536  0.3639  0.2403     1.     52.    7.08
17    0.2574  0.0818  0.0822     1.     52.    4.31
18    0.1001  0.0403  0.4905     1.     46.    8.63
19    0.0624  0.0000  0.0603     2.     41.    1.24
20    0.0778  0.0000  0.0263     2.     37.    1.24
```

... etc. ...

The program then selects the best vector for Patterson superposition; PATT followed by a number greater than one could be used to select a larger number of superposition vectors in the case of a difficult structure.

```
Vectors selected for Patterson superposition:

Vector   X       Y       Z     Weight  Peak  Length
1     0.8449  0.8784  0.4068     1.    230.    8.99

FMAP and GRID set by program

FMAP   6   2  69
GRID    -1.515  -1  -1     1.515   1   1

Patterson vector superposition minimum function for [PR4N]2 [PHSCUS2MOS2CUSPH]

Patt. sup. on vector 1     0.8449  0.8784  0.4068   Height 230.   Length  8.99

Maximum = 296.93,  minimum = -83.05       highest memory used = 13405 /180882

     38.1 seconds elapsed time
   38 Superposition peaks employed, maximum height  47.7  and minimum height   2.8  on
atomic number scale
```

This superposition function is now interpreted. In this case there are two different plausible origin shifts, and indeed these correspond to the two plausible solutions one would obtain trying to solve the Patterson by hand. The program is able to select the correct solution (printed first) on the basis of its combined figure of merit, and only this solution gives the correct number of molybdenum and copper atoms per cell. Usually the correlation coefficient is the most reliable figure of merit, especially when the heavy atoms lie on general positions and contribute less to the total scattering power. As it happens, the element types have also been assigned correctly (on the basis of atomic numbers derive from the peak heights in the superposition map) in the correct solution, though it is better to consider the chemical significance of the interatomic distances before accepting the element types suggested by the program.

The results are presented in the form of a 'crossword table' in which the shortest distance between two atoms (taking all symmetry equivalents into account) is the top entry and the bottom entry is the minimum sharpened Patterson height at all symmetry generated vectors between the two atoms in question. These Patterson heights are calculated from the data, not from the Patterson peaklist. For example in the first table the minimum Cu..Mo distance is 2.55 Å and the minimum Cu..Cu distance is 5.09 Å, from which it can be seen that the Cu..Mo..Cu arrangement is linear (bridged by sulfur); the molybdenum atom lies on a special position on the twofold axis. S4 and S5 are bonded to both Mo and Cu with S-Mo 2.21 Å and S-Cu 2.16 Å. The Patterson heights correspond roughly to the products of the atomic numbers, but of course the S..S vector heights are very small and close to the noise level of the Patterson, which explains why one of them is printed as zero (negative heights are rounded up to zero).

```
Heavy-Atom Location for  [PR4N]2 [PHSCUS2MOS2CUSPH]
  2404 reflections used for structure factor sums

Solution 1   CFOM = 53.25   PATFOM = 99.9   Corr. Coeff. = 81.5  SYMFOM = 80.1
Shift to be added to superposition coordinates:  0.5754  0.5004  0.5439

Name  At.No.   x       y       z    s.o.f.  Min. dist. / PATSMF (self first)
MO1   44.9  0.5000  0.5611  0.2500  0.5000    9.42
                                             391.5
CU2   23.4  0.6525  0.5619  0.3391  1.0000    5.09    2.55
                                             120.3   299.1
S3    12.5  0.7960  0.5673  0.4213  1.0000    6.84    4.93  2.39
                                               0.0   128.1  76.5
S4    10.3  0.5573  0.4798  0.3531  1.0000    3.62    2.21  2.16  4.06
                                               5.4    87.8  55.5  10.7
S5     9.8  0.6012  0.6411  0.2375  1.0000    3.64    2.21  2.16  4.00  3.55
                                              18.6   104.2  63.0  17.9  17.3


Solution 2   CFOM = 36.66  PATFOM = 51.9  Corr. Coeff. = 84.1  SYMFOM = 99.9
Shift to be added to superposition coordinates:  0.5000  0.5000  0.5000

Name  At.No.   x       y       z    s.o.f.  Min. dist. / PATSMF (self first)
MO1   44.9  0.5791  0.4390  0.7967  1.0000    2.65
                                             300.2
S2    17.2  0.7229  0.4356  0.8793  1.0000    7.18    2.39
                                              57.7   179.3
S3    15.2  0.4776  0.3597  0.8084  1.0000    2.52    2.20  4.10
                                               0.0    88.1  30.7
S4    13.6  0.4788  0.5192  0.8066  1.0000    2.44    2.18  4.12  2.50
                                               0.0    67.2  26.9   7.9
S5    13.0  0.6363  0.5216  0.9000  1.0000    5.85    2.22  2.13  3.66  2.64
                                               0.0    68.8  12.3   0.0   5.5
 ... etc. ...
```

6.2 Instructions for Patterson Interpretation

6.2.1 PATT nv[#] dmin[#] resl[#] Nsup[#] Zmin[#] maxat[#]

nv is the number of superposition vectors to be tried; if it is negative the search for possible origin shifts is made more exhaustive by relaxing various tolerances etc.

dmin is the minimum allowed length for a heavy-atom to heavy-atom vector; it affects ONLY the choice of superposition vector. If it is negative, the program does not generate any atoms on special positions in stage 4 (useful for some macromolecular problems).

resl is the effective resolution in Angstroms as deduced from the reflection data, and is used for setting various tolerances. If the data extend further than the crystal actually diffracted, or if the outer data are incomplete, it may well be worth increasing this number. This parameter can be relatively critical for macromolecular structures.

Nsup is the number of unique peaks to be found by searching the superposition function.

Zmin is the minimum atomic number to be included as an atom in the crossword table etc. (if this is set too low, the calculation can take appreciably longer).

maxat is the maximum number of potential atoms to be included in the crossword table, and can also appreciably affect the time required for PATT.

6.2.2 VECT X Y Z

A superposition vector (with coordinates taken from the Patterson peak-list) may be input by hand by a VECT instruction, in which case the first two numbers on the PATT instruction are ignored (except for their signs!), and a PATT instruction will be automatically generated if not present in the .ins file. There may be any number of VECT instructions.

6.3 Difficult Structures

In the unlikely event of a routine PATT run failing to give an acceptable solution, the best approach—after checking the data reduction diagnostics carefully as explained above—is to select several potential heavy-atom to heavy-atom vectors by hand from the Patterson peak-list and specify them on VECT instructions (either in the same job or different jobs according to local circumstances) for use as superposition vectors. The exhaustiveness of the search can also be increased - at a significant cost in computer time - by making the first PATT parameter negative and/or by increasing the value of resl a little. The sign of the second PATT parameter (a negative sign excludes atoms on special positions) and the list of elements which might be present (SFAC/UNIT) should perhaps also be reconsidered. The value of the second PATT parameter - the minimum vector length for superposition - can also usefully be increased in the light of chemical knowledge. For large structures and not very heavy ‘heavy atoms’ (e.g., P or S in an organic structure) the correct solution is very often the one with the highest correlation coefficient.

6.4 Instructions for Partial Structure Expansion

6.4.1 TEXP na[#] nH[0] Ek[1.5]

na PHAS reflections with E(obs) > Ek and the largest values of E(calc)/E(obs) are generated for use in partial structure expansion or direct methods. The first nH atoms (heavy atoms) in the atom list are retained during partial structure expansion, the rest are thrown away after calculating phases. At least one atom MUST be given! TEXP automatically generates appropriate FMAP, GRID and PLAN instructions.

TEXP (and/or PHAS) may be used in conjunction with TREF to generate fixed phases for use in direct methods; the special TEXP option na = 0 provides point atom phases for ALL reflections, which are then refined during the phase annealing and tangent expansion stages of direct methods (as specified on the PHAN and TREF instructions). It is not necessary to use different starting phases for the different phase sets, because the phase annealing stage itself introduces (statistically distributed) random phase shifts! This is a powerful method of partial structure expansion for cases when the phasing power of the partial structure is not quite adequate, e.g., when it consists of only one atom (say P or S in a large organic structure). If at least 5 atoms have been correctly located then TEXP alone should suffice.

When TEXP is used without TREF a tangent formula expansion (to all reflections with E > Emin as specified on the ESEL instruction) is first performed, followed by several cycles (see FMAP) of E-Fouriers and peak-list optimization. TEXP is particularly useful for cases in which several not very heavy atoms (e.g., P, S) have been located by PATT followed by hand interpretation of the resulting 'crossword table'. In such cases nH should be set to the number of such atoms and na to about half the number of reflections with E > 1.5 (see the first page of the XS output).

6.4.2 PHAS h k l phi

A fixed phase for structure expansion or direct methods. PHAS may be used to fix single phase seminvariants that have been obtained from other programs or derived by examination of the best TREF solutions. The phase angle phi must be present, and should be given in degrees.

6.4.3 atomname SFAC x y z sof[1] U (or U11 U22 U33 U23 U13 U12)

Atom instructions begin with an atom name (up to 4 characters which do not correspond to any of the SHELXS command names, and terminated by at least one blank) followed by a scattering factor number (which refers to the list defined by the SFAC instruction(s)), x, y, and z in fractional coordinates, and (optionally) a site occupation factor (s.o.f.) and an isotropic U or six anisotropic Uij components (both in $Å^{-2}$). The U or Uij values are ignored by XS but may be included for compatibility with XL.

When XS writes the .res output file, a dummy U value is followed by a peak height (unless an atom type has been assigned by the program before the E-Fourier recycling). Both the dummy U and the peak height are ignored if the atom is read back into XS (e.g., for partial structure expansion) or XL.

In contrast to XLS etc. it is not necessary to pad out the atom name to 4 characters with blanks, but it should be followed by at least one blank. References to 'free variables' and fixing of atom parameters by adding 10 as in XL etc. will be interpreted correctly. The site occupation factor for an atom on a special position should be divided by the number of atoms in the general position which have coalesced to give the special position. It may also be found by dividing the multiplicity of the special position (as given in International Tables) by the multiplicity of the general position. Thus an atom on a fourfold axis will usually have s.o.f. = 10.25 (i.e., 0.25, fixed by adding 10).

6.4.4 MOVE dx[0] dy[0] dz[0] sign[1]

The coordinates of the following atoms are changed to: x = dx + sign • x, y = dy + sign • y, z = dz + sign • z (after applying FRAG and SPIN - if present - according to XPS conventions); MOVE applies to all following atoms until superseded by a further MOVE. MOVE is normally used in conjunction with SPIN and FRAG (see below) but is also useful on its own for applying origin shifts.

TEXP may be used in conjunction with ESEL -1 for a partial structure expansion in the effective space group P1 (C1 etc. if the lattice is centered). This can be very effective if it is suspected that a fragment is correctly ,oriented but translated from its real position, or if the space group cannot be unambiguously assigned. Hand interpretation of the resulting E-map is then however necessary to locate the positions of the crystallographic symmetry elements.

6.5 Commands for Communication with the Program XPS

6.5.1 SPIN phi1[0] phi2[0] phi3[0]

The following fragment (which should begin with a FRAG instruction) is rotated by the specified angles (in radians). This instruction is used to reinput angles from Patterson search programs (in particular XPS).

6.5.2 FRAG code[#] a[1] b[1] c[1] α[90] β[90] γ[90]

FRAG enables the XPS search fragment to be read in using the original cell or orthogonal coordinates. This instruction will usually be preceded by SPIN and MOVE commands to give the rotation angles and translation (same conventions as for XPS), and followed by a list of atoms. FRAG, SPIN and MOVE instructions remain in force until superseded by another instruction of the same type. Code is ignored by XS but is included for compatibility with XPS and XL (where it is used for different purposes).

6.5.3 PSEE m[200] 2theta(max)[#]

The largest |m| E-values and the complete Patterson map are dumped into the name.res file in fixed format for use by Patterson search programs (in particular XPS) etc. 2theta(max) should be used to limit the resolution of the E-values generated; the default value uses $\sin(\theta) = \lambda/2$. The 2theta(max) value is also written to the .res file, so it is possible to restrict the resolution of the E-values actually used by XPS to a lower 2theta(max) by editing this file without rerunning XS; of course the E-values with higher 2θ than the value used in XS were not written to the .res file and so cannot be recovered in this way. When m is negative a 'super-sharp' Patterson with coefficients Sqrt(E^3F) is used; if m is positive a standard sharpened Patterson with coefficients (EF) is employed. The resulting name.res file must be renamed name.inp (or name.pat if the search fragment and encoded Patterson are to be read from separate files) for use by XPS. After a PSEE instruction, UNIT is followed by the strongest E-values and the full Patterson map in this output file (which may be rather long!).

6.6 Location of Heavy Atoms from Protein ΔF Data

In principle both the Patterson interpretation and direct methods are suitable for the location of heavy atoms from protein isomorphous or anomalous ΔF data-sets. In both cases the user must prepare a file name.hkl containing h, k, l, ΔF and $\sigma(\Delta F)$ in the usual format (3I4,2F8.2), terminated by the dummy reflection with h = k = l = 0. The sign of ΔF is ignored. Careful scaling of the derivative and native data, pruning of statistically unreasonable ΔF's, and good estimated standard deviations are essential to the success of this approach. It should be emphasised that treating ΔF as if it were F involves an approximation which, at best, will add appreciable ‘noise’.

XS will usually recognize that it has been given macromolecular ΔF data (from the cell volume and contents) and will then set appropriate defaults, so as with small molecules the .ins file will often simply consist of TITL..UNIT, then TREF (for direct methods) or PATT (Patterson interpretation) and finally HKLF 3 (because the .hkl file contains ΔF not F-squared). The UNIT instruction should contain the correct number of heavy atoms and the SQUARE ROOT of the number of light atoms in the cell; they may conveniently be assumed to be nitrogen. The mean atomic volume and density printed by the program should of course be ignored. It is strongly recommended that these standard TREF and PATT jobs are tried first before any parmaters are varied.

Unfortunately, there are two fundamental difficulties with the application of direct methods to ΔF data. The first is that the negative quartets are meaningless, because the ΔF values represent lower bounds on their true values, and so are unsuitable for identifying the very small E-values which are required for the cross-terms of the negative quartets. On the other hand the ΔF values do correctly identify the LARGEST E-values, and so the old triplet formula works well. The second problem is that the estimation of probabilities for the triplet formula for the use in figures of merit: what should replace the 1/N term (where N is the number of atoms per cell) when ΔF data are used? Most of the recent advances in direct methods exploit either the weak reflections or more sophisticated formulae for probability distributions, so are wasted on ΔF data. Nevertheless, direct methods will tend to perform better in space groups with (a) translation symmetry (not counting lattice centering), (b) a fixed rather than a floating origin and (c) no special positions; thus $P2_12_12_1$ (the only space group to fulfill all three criteria ?!) is good but P1, C2, R3 and I4 are unsuitable.

If the standard direct methods run fails to find convincing heavy-atom sites, it should first be checked that the program has put out a comment that it has set the defaults for macromolecular data. The number of phase permutations may have to be increased (the first TREF parameter) or the number of large E-values for phase refinement may have to be changed (one should aim for at least 20 triplets per refined phase), but if too many phases are refined the performance is degraded because the ΔF values only identify the strongest E-values reliably. The probability estimates may be changed by modifying the UNIT instruction, or more simply by changing the third TREF parameter, which multiplies the products of the three E-values in the triplet probability formula; for small molecules a value in the range 0.75 to 0.95 gives the best probability estimates.

For location of the heavy-atom site by Patterson interpretation of ΔF data it may well be necessary to increase the number of superposition vectors to be tried (the first parameter on the PATT instruction), since the heavy-atom to heavy-atom vectors may be well down the Patterson peak-list. This number can be made negative to increase the ‘depth of search’ at the cost of a significant increase in computer time. The second number (the minimum vector length for the superposition vector) should be set to at least 8 Å (and to a larger value if the cell is large), and it can usually be made negative to indicate that special positions are not to be considered as possible heavy atom sites. An advantage of Patterson as opposed to direct methods is that such false solutions can be eliminated at a much earlier stage.

The third PATT parameter is also fairly critical for macromolecular ΔF data; it is the apparent resolution, and is used to set the tolerances for deconvoluting the superposition map. If - as can easily happen with area detector data - a few ΔF values are at appreciably higher resolution than the rest of the data, this may fool the program into setting too high an effective resolution. In such cases it is worth experimenting with several different values, e.g., 2.5 Å instead of 2.0 etc. The only other parameter which may need to be altered is maxat, if more than 8 sites are expected

A typical ΔF PATT run (e.g., PATT 10 -12 2.5) will produce a relatively large number of possible solutions, some of which may be equivalent. The ‘correlation coefficient’ (which is defined in the same way as in most molecular replacement programs) is the only useful figure of merit for comparison purposes. Hand interpretation of the 'crossword table' is not as easy as for small molecules, because the minimum interatomic distances are not so useful; it is however still necessary to find a set of atoms for which the Patterson minimum function values are consistently high for at least most of the pairs of sites involved. This information tends to be more decisive for the higher symmetry space groups, because when there are more vectors between symmetry equivalents, it is unlikely that all will be associated with large Patterson values simultaneously by accident.

7 XPS - Fragment Search

XPS is based on the public-domain program PATSEE for location of a fragment of known geometry by integrated Patterson, packing, and direct methods; PATSEE was written by Ernst Egert of the University of Frankfurt, West Germany.

This program attempts to combine the merits of both Patterson and direct methods in order to position a fragment of known geometry in the unit cell. It is valid for all space groups in all settings.

The rotation search can find the orientation of a search model of any size and allows one torsional degree of freedom. (Searching for two independent fragments simultaneously would be very time consuming because a large number of combinations of orientations would have to be considered.) The translation search may locate up to two independent fragments of any size (including single atoms) taking into account known atoms at fixed positions, if any.

XPS expects to read an input file “name.inp” which contains TITL ... UNIT (as for XS) etc. followed by further instructions, a search fragment, and possibly the list of E-values and tabulated asymmetric unit of the sharpened Patterson function (created using the PSEE instruction in XS). Alternatively the E-values and Patterson may be read from a separate file (name.pat). The “best” final coordinates are written to a file (name.rep) ready for partial structure extension using XS (edit or renamed to name.ins for this purpose).

Execute XPS by:

```
XPS name
```

A summary of the progress appear on the console, and the results are sent to name.lst (printer listing, may be printed with SPRINT on a PC) and name.rep (new atom coordinates, etc.).

7.1 XPS Strategy

A structure solution with XPS normally consists of three stages:

1. The data is processed with XS using the PSEE command to calculate the sharpened Patterson function and the largest n E-values. This instruction creates an output file (name.inp) to be read by XPS. Normally, 200 E-values are sufficient.

2. A fragment search is performed with XPS. The normal procedure will be a rotation search (ROTS) followed by a translation search (TRAN) within the same run. The input must contain:
   - The usual XS sequence: TITL ...UNIT.
   - ROTS and/or TRAN.
   - Atomic coordinates of the search model(s); at least one atom belonging to FRAG 1 (default) must be present.
   - E-magnitudes and Patterson grid values, which must form the end of the input stream (conveniently appended via END n and read from name.pat).

   All other input instructions are optional but - like the various parameters in the ROTS and TRAN commands - could prove very useful when applied by an expert.

3. The fragment undergoes partial structure expansion with another run of XS. XPS writes a file with suitable instructions for the expansion of the best solution starting from the first (best) orientation (which, however, may not be the fragment position with the highest overall CFOM). Note that the last line (HKLF 4) may have to be changed according to your data type and format. For the tangent expansion and E-Fourier recycling, positions of atoms heavier than Al are automatically kept fixed provided that they come first in the atom list.

Any other solution can also be tested conveniently by specifying SPIN (with the appropriate rotation angles phi1, phi2, and phi3) and MOVE (with the translational shifts X1, Y1, and Z1, and possibly X2, Y2, and Z2) followed by FRAG (if not Cartesian) and the original input atomic coordinates which produced the particular XPS solution; suspicious atoms may be omitted, of course.

In order to demonstrate how a crystal structure is solved by Patterson search using a suitable model, the test structure LAC1 (which used to be difficult for direct methods) serves as an excellent example. It is recommended that you study the test output carefully before using XPS with your own data.

7.2 XPS Instructions

All input commands are read in free format with parameters separated by one or more blanks. The input stream must begin with the sequence TITL ... UNIT and end with END n or READ; all other commands may be given in any order (for exceptions, see below). Continuation lines are allowed for all instructions except SFAC with element symbols and SYMM.

Characters following "=", "/" or four blank spaces in columns 1-4 are treated as comment and thus only printed (but otherwise ignored).

In the following list of instructions, most parameters have default values which are given in brackets ("#" means that it is problem dependent). All distances are in Angstroms.

7.2.1 ROTS nt[#] ns[5] lmax[6] dmin[#]

This initiates the rotation search for fragment 1.

|**nt**| Random orientations are tested. If |**nt**| < 1, a suitable number (10000 - 60000 depending on the Laue group) will be calculated. Negative **nt** produces a list of bond lengths and angles for the search model; this is useful for detecting possible errors in the atomic coordinates.

The |**ns**| best rotation solutions are kept and transferred to the subsequent translation search and up to |**ns**| + 3 solutions are printed out. Negative **ns** indicates that the search model has a mirror plane or an inversion center, which makes a more extensive equivalence test necessary. For the rotation search, only vectors shorter than |**lmax**| are used. |**lmax**| may be increased for very accurate models and should be reduced for unreliable ones. Vectors shorter than 2 Angstroms are only used if **lmax** < 0; this is recommended only for short vectors with high weights. Two solutions are regarded as similar if all equivalent atoms are closer than |**dmin**|, whose optimum value depends on the size of the model. Negative **dmin** retains low weight vectors (which are normally ignored in order to save time).

If the SPIN command is present, the specified orientation is refined by a restricted rotation search (this could improve the chances of the subsequent translation search); in that case, however, ROTS must occur before the first FRAG 1 atom!

The rotation search starts with phi 1 = phi 2 = phi 3 = 0; thus the combination of ROTS -1 with TRAN gives bond lengths and angles followed by a translation search.

7.2.2 RFOM fr[0.3] nv[100]

A fraction |**fr**| of worst-fitting intramolecular vectors (but not more than **nv**) are used for calculating the RFOM figure of merit, which resembles either the sum (|**fr**| = 1) or the minimum function (**nv** = 1). The number of vectors incorporated into RFOM should be increased for bad models so that single (strong) vectors get less weight, and may be reduced for models consisting of a few "heavy" atoms. If **fr** < 0, a sorted (according to the weight) list of intramolecular vectors used for the rotation search is printed together with the corresponding lengths; if this list contains vectors with high weights, a comparison with the most prominent peaks in the Patterson function (printed by the XS command PSEE) might be useful.

7.2.3 TRAN nt[#] ns[#] dmin1[1.8] dmin2[2.4] nE[100] ntpr[100]

This initiates the translation search for fragments 1 and 2 (if present). |**nt**| random positions are refined for each orientation; if |**nt**| < 1, a suitable number will be calculated from the number of search parameters and their range. Negative **nt** produces a sorted list of E-magnitudes linked by strong three-phase structure invariants. If it is found that a few E-values participate in a large number (e.g., more than ten) of triple-phase relations, they should be omitted (see the READ instruction). The |**ns**| best solutions are retained, but only the atomic coordinates for the first are normally printed (give **ns** a negative value in order to get them all).Only fragment positions without intermolecular contacts shorter than |**dmin1**| (preliminary distance test) and |**dmin2**| (final distance test), respectively, are assumed to be physically reasonable. If |**dmin**| < 0.1, the preliminary distance test is skipped (this may save time if the fragment occupies only a small portion of the asymmetric unit). If **dmin1** < 0, a list of atoms used for the first test is printed. |**nE**| and |**ntpr**| are the maximum numbers of E-magnitudes and triple-phase relations used for the translation search. If **ntpr** < 0, the TPRSUM rejection criterion is reduced from 0.5 to 0.3; this is recommended for very small or inaccurate models. The default TPRSUM rejection criterion is reduced by 0.1 for non-centrosymmetric structures.

The random numbers may be influenced by the user. If the first parameter **nt** on the ROTS and TRAN commands is given as “m.n”, n determines the random number seed (however “m.5” has the same effect as “m.0”). So when a rotation search is to be repeated with 60,000 instead of 40,000 tries, this should be done with ROTS 60000 n; otherwise a large proportion of the search will just be a repetition of the previous runs. If **nE** is made negative, 3 cycles of translation refinement are applied instead of the usual 2. If **dmin2** < 0, a different formula is used for CFOM.

7.2.4 TFOM fr[0.2] nv[250]

The meaning of **fr** and **nv** is similar to that of the parameters in the RFOM instruction, but for TFOM intermolecular vectors are used. If **fr** < 0, CFOM will be calculated ignoring the TPRSUM value; this gives the Patterson criterion TFOM more weight and is advisable for the location of single heavy atoms. A special option for very large structures (possibly at low resolution) is invoked if **nv** < 1: a very rapid translation search without using the Patterson function (TFOM is arbitrarily set to 1.0 and the final distance test restricted to a small number of atoms representing the search model). Note that **fr** and **nv** must not both be negative!

7.2.5 SPIN phi1[0] phi2[0] phi3[0]

The following atoms are rotated by the specified angles (all in radians) about the x, y and z axis of a Cartesian coordinate system. This instruction defines the orientation of a fragment for a subsequent translation search or a restricted rotation search (see the ROTS command).

7.2.6 MOVE delx[0] dely[0] delz[0] sign[1]

The coordinates of the following atoms are changed to:

$$x' = delx + sign \cdot x$$

$$y' = dely + sign \cdot y$$

$$z' = delz + sign \cdot z$$

The three shifts are ignored for atoms belonging to FRAG 1 or 2, which may only be inverted (**sign** = -1).

7.2.7 FRAG code[1] a[1] b[1] c[1] α[90] β[90] γ[90]

This instruction defines the coordinate system to which all following atomic positions (until the next FRAG instruction) are referred and thus enables the convenient input of search models from published structures (default: Cartesian coordinates). The six cell parameters in Angstroms must be either specified completely or omitted. The default search fragment (code = 1) must be present. **code** = 2 indicates a fragment with known orientation (defined by SPIN) but unknown position, for which a translation search is to be performed (simultaneously with fragment 1). **code** < 0 indicates a fragment with known orientation and position (from a previous XPS run) fixed by SPIN and MOVE. A special option (**code** = 0) is provided for fragments whose true crystal coordinates are already known (e.g., heavy atoms from a Patterson interpretation via the XS command PATT); for such fragments the cell parameters must not be specified.

7.2.8 name SFAC x y z sof[11] U (or U11 to U12)

This defines the name, type, coordinates and site occupation factor for an atom belonging to one of the fragments (see the ATOM description in Section 11.5); temperature factors are ignored. On reading such an atom instruction, FRAG, SPIN and MOVE are applied in that order. The site occupation factor can be used to reduce the weight of possibly doubtful, but not definitely wrong atoms. Negative **sfac** indicates that the atom may lie on a special position or take part in short intermolecular interactions so that the packing criteria defined by ROTS and TRAN should not be applied to it (see TWIS for the use of dummy atoms). This is much more economic than softer global criteria in the ROTS and TRAN instructions.

7.2.9 TWIS w0 Dw we [w0+355] rtest[2]

The vector between the atoms immediately preceding and following this instruction is treated as a bond (irrespective of its length) connecting two rigid groups with a variable torsion angle. The number and range of torsion angles to be tested is defined by a minimum ($\mathbf{w_0}$) and a maximum value ($\mathbf{w_e}$) and an increment (**Dw**) given in degrees. It is important to note that the input geometry is arbitrarily defined as w = 0.

If only $\mathbf{w_0}$ is given, a single search with the corresponding torsion angle will be performed. The distance |**rtest**| serves as a criterion: 1) for establishing the connectivity and thus for deciding which atoms belong to which part of the flexible fragment (if **rtest** < 0 it is assumed that TWIS separates the two parts) and 2) for detecting short contacts upon rotation (ignored if |**rtest**| < 0.2), in which case the next geometry is immediately generated.

TWIS is only allowed for fragment 1. If, as in ferrocene-type complexes, the rotation axis does not correspond to a bond between two atoms, dummy atoms with negative sfac and sof < 1 can be introduced.

7.2.10 CODE lv1 lv2 lv3 lv4 lv5 lv6 lv7

(All seven parameters must be specified in ascending order.)

CODE lv7 incr [lvn=lv7-(7-n).incr]

The CODE instruction, which can take the above two forms, over-writes the automatic encoding of Patterson grid values (0 ... 7) according to seven test levels. This is sometimes necessary if the Patterson distribution is not smooth enough; the warning printed by the program (see test output) can often be ignored since the criterion for this is rather strict. Normally the count for the first (negative Patterson grid values) and possibly the last (origin of Patterson function) test level yields considerably larger numbers than for the others. Of course, the distribution among the test levels could be forced to be regular, but then two levels could be too close (which would disturb RFOM and TFOM) and “features” of the data or structure would be hidden.

7.2.11 END n

This is normally the last input line and indicates that subsequent instructions (generated by XS) are to be read from unit **n**; they will not be interpreted or printed until a READ instruction is found.

7.2.12 READ nE 2h(max)

This must be the last instruction if the whole input (including the Patterson function) resides in one file; in that case, however, the TITL ... UNIT sequence preceding READ in the output file generated by XS has to be removed. The characters between the parameters **nE** (number of E-values in the subsequent list) and 2h (max) (limit applied to E-values, not to Patterson function) are not interpreted. The E-list contains four reflections per line (h, k, l, and E for each). Since negative E-values are ignored, unsuitable reflections may thus be suppressed (see TRAN), but be careful not to change the format!

8 Examples of Small Molecule Refinements with XL

Two test structures supplied with the XL are intended to provide a good illustration of routine small moiety structure refinement. The output discussed here should not differ significantly from that of the test jobs, except that it has been abbreviated and there may be differences in the last decimal place caused by rounding errors.

8.1 First example (ags4)

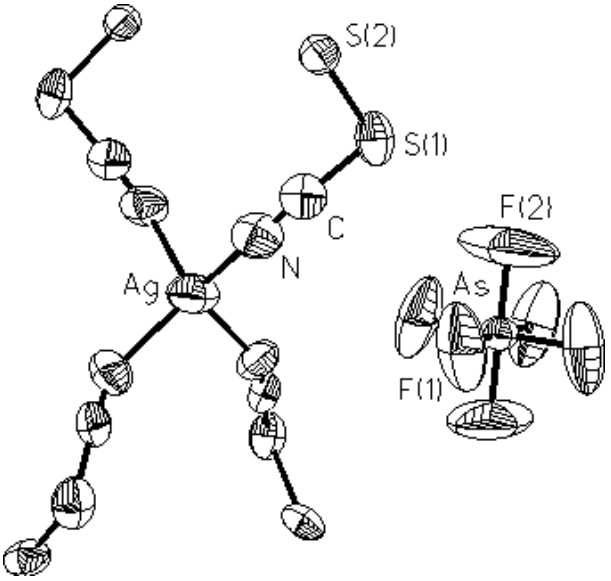

Figure 8.1 – ags4

The first example (provided as the files *ags4.ins* and *ags4.hkl*) is the final refinement job for the polymeric inorganic structure $Ag(NCSSSSCN)_2$ AsF_6, determined by Roesky, Gries, Schimkowiak & Jones (1986). Each ligand bridges two Ag^+ ions so each silver is tetrahedrally coordinated by four nitrogen atoms. The silver, arsenic and one of the fluorine atoms lie on special positions. Normally the four unique heavy atoms (from Patterson interpretation using XS) would have been refined isotropically first and the remaining atoms found in a difference synthesis, and possibly an intermediate job would have been performed with the heavy atoms anisotropic and the light atoms isotropic. For test purposes we shall simply input the atomic coordinates which assumes isotropic U's of 0.05 for all atoms. In this job all atoms are to be made anisotropic (ANIS). We shall further assume that a previous job has recommended the weighting scheme used here (WGHT) and shown that one reflection is to be suppressed in the refinement because it is clearly erroneous (OMIT).

The first 9 instructions (TITL...UNIT) are the same for any XS and XL job for this structure and define the cell dimensions, symmetry and contents. The SHELXTL program XPREP can be used to generate these instructions automatically for any space group etc. XL knows the scattering factors for the first 94 neutral atoms in the Periodic Table. Ten least-squares cycles are to be performed, and the ACTA instruction ensures that the CIF files *ags4.cif* and *ags4.fcf* will be written for archiving and publication purposes. ACTA also sets up the calculation of bond lengths and angles (BOND) and a final difference electron density synthesis (FMAP 2) with peak search (PLAN 20). The HKLF 4 instruction terminates the file and initiates the reading of the *ags4.hkl* intensity data file.

It is possible to set up special position constraints on the x,y,z-coordinates, occupation factors, and U_{ij} components by hand. However this is totally unnecessary because the program will do this automatically for any special position in any space group, conventional or otherwise. Similarly the program recognizes polar space groups ($P\bar{4}$ is non-polar) and applies appropriate restraints (Flack & Schwarzenbach, 1988), so it is no longer necessary to worry about fixing one or more coordinates to prevent the structure drifting along polar axes. It is not necessary to set the overall scale factor using an FVAR instruction for this initial job, because the program will itself estimate a suitable starting value. Comments may be included in the .ins file either as REM instructions or as the rest of a line following '!'; this latter facility has been used to annotate this example.

```
TITL AGS4 in P-4                          ! title of up to 76 characters
CELL 0.71073 8.381 8.381 6.661 90 90 90   ! wavelength and unit-cell
ZERR 1 .002 .002 .001 0 0 0               ! Z (formula-units/cell), cell esd's
LATT -1                                 ! non-centrosymmetric primitive lattice
SYMM -X, -Y, Z
SYMM Y, -X, -Z              ! symmetry operators (x,y,z must be left out)
SYMM -Y, X, -Z
SFAC C AG AS F N S          ! define scattering factor numbers
UNIT 4 1 1 6 4 8            ! unit cell contents in same order
L.S. 10                     ! 10 cycles full-matrix least-squares
ACTA                        ! CIF-output, bonds, Fourier, peak search
OMIT -2 3 1                 ! suppress bad reflection
ANIS                        ! convert all (non-H) atoms to anisotropic
WGHT 0.037 0.31             ! weighting scheme
AG  2  .000  .000  .000
AS  3  .500  .500  .000
S1  6  .368  .206  .517     ! atom name, SFAC number, x, y, z (usually
S2  6  .386  .034  .736     ! followed by sof and U(iso) or Uij); the
C   1  .278  .095  .337     ! program automatically generates special
N   5  .211  .030  .214     ! position constraints
F1  4  .596  .325 -.007
F2  4  .500  .500  .246
HKLF 4                      ! read h,k,l,Fo^2,sigma(Fo^2) from 'ags4.hkl'
```

The *.lst* listing file starts with a header followed by an echo of the above *.ins* file. After reading TITL...UNIT the program calculates the cell volume, F(000), absorption coefficient, cell weight and density. If the density is unreasonable, perhaps the unit-cell contents have been given incorrectly. The next items in the *.lst* file are the connectivity table and the symmetry operations used to include a shell of symmetry equivalent atoms (so that all unique bond lengths and angles can be found):

```
Covalent radii and connectivity table for AGS4 in P-4

C    0.770
AG   1.440
AS   1.210
F    0.640
N    0.700
S    1.030

Ag - N N_$3 N_$4 N_$2
As - F2 F2_$6 F1_$7 F1_$6 F1_$5 F1
S1 - C S2
S2 - S2_$1 S1
C - N S1
N - C Ag
F1 - As
F2 - As

Operators for generating equivalent atoms:

$1   -x+1, -y+1, z
$2   -x, -y, z
$3   y, -x, -z
$4   -y, x, -z
$5   -x+1, -y+1, z
$6   y, -x+1, -z
$7   -y+1, x, -z
```

Note that in addition to symmetry operations generated by the program, one can also define operations with the EQIV instruction and then refer to the corresponding atoms with _$n in the same way. Thus:

```
EQIV $1 1-x, -y, z
CONF S1 S2 S2_$1 S1_$1
```

could have been included in *ags4.ins* to calculate the S-S-S-S torsion angle. If EQIV instructions are used, the program renumbers the other symmetry operators accordingly.

The next part of the output is concerned with the data reduction:

```
 1475  Reflections read, of which     1  rejected

0 =< h =< 10,     -9 =< k =< 10,      0 =< l =<  8,   Max. 2-theta =   55.00

    0  Systematic absence violations

Inconsistent equivalents etc.

 h   k   l       Fo^2    Sigma(Fo^2)  Esd of mean(Fo^2)

 3   4   0      387.25       8.54       47.78

     1  Inconsistent equivalents
   903  Unique reflections, of which      0  suppressed

R(int) = 0.0165     R(sigma) = 0.0202      Friedel opposites not merged
```

Special position constraints are then generated and the statistics from the first least-squares cycle are listed (the output has been compacted to fit the page). The maximum vector length refers to the number of reflections processed simultaneously in the rate-determining calculations; usually the program utilizes all available memory to make this as large as possible, subject to a maximum of 511. This maximum may be reduced (but not increased) by means of the fourth parameter on the L.S. (or CGLS) instruction; this may be required to prevent unnecessary disk transfers when large structures are refined on virtual memory systems with limited physical memory. The number of parameters refined in the current cycle is followed by the total number of refinable parameters (here both are 55).

```
Special position constraints for Ag
x =  0.0000       y =  0.0000         z =  0.0000         U22 = 1.0 * U11
U23 = 0           U13 = 0             U12 = 0             sof = 0.25000

Special position constraints for As
x =  0.5000       y =  0.5000         z =  0.0000         U22 = 1.0 * U11
U23 = 0           U13 = 0             U12 = 0             sof = 0.25000

Special position constraints for F2
x =  0.5000       y =  0.5000         U23 = 0             U13 = 0
sof = 0.50000


Least-squares cycle 1   Maximum vector length=511  Memory required=1092/82899

wR2 =  0.5042 before cycle   1 for    903 data and   55 /   55 parameters
```

```
GooF = S =  8.127;     Restrained GooF =      8.127  for      0 restraints

Weight = 1/[sigma^2(Fo^2)+(0.0370*P)^2+0.31*P] where P=(Max(Fo^2,0)+2*Fc^2)/3

** Shifts scaled down to reduce maximum shift/esd from   17.64  to   15.00 **

  N     value       esd        shift/esd  parameter

  1     2.31065     0.04324     9.042     OSF
  2     0.07314     0.00206    11.250     U11 Ag
 11     0.07309     0.00669     3.453     U33 S1
 47     0.11304     0.01391     4.533     U33 F1

Mean shift/esd =   1.238    Maximum =  11.250 for  OSF

Max. shift = 0.045 A for C      Max. dU = 0.033 for F2
```

Only the largest shift/esd's are printed. More output could have been obtained using 'MORE 2' or 'MORE 3'. The largest correlation matrix elements are printed after the last cycle, in which the mean and maximum shift/esd have been reduced to 0.003 and 0.017 respectively. This is followed by the full table of refined coordinates and U_{ij}'s with esd's and by a final structure factor calculation:

```
Final Structure Factor Calculation for  AGS4 in P-4

Total number of l.s. parameters = 55  Maximum vector length = 511
wR2 =  0.0780 before cycle  11 for    903 data and    2 /   55 parameters

GooF = S =     1.063;     Restrained GooF =      1.063  for      0 restraints
Weight = 1/[sigma^2(Fo^2)+(0.0370*P)^2+0.31*P] where P=(Max(Fo^2,0)+2*Fc^2)/3
R1 =  0.0322 for    818 Fo > 4.sigma(Fo)  and  0.0367 for all    903 data
wR2 =  0.0780,  GooF = S =   1.063,  Restrained GooF =    1.063  for all data

Flack x parameter = 0.0224   with esd  0.0260   (expected values are 0
(within 3 esd's) for correct and +1 for inverted absolute structure)
```

There are some important points to note here. The weighted *R*-index based on F_o^2 is (for compelling statistical reasons) much higher than the conventional *R*-index based on F_o with a threshold of say $F_o > 4\sigma(F_o)$. For comparison with structures refined against *F* the latter is therefore printed as well (as *R*1). Despite the fact that *wR*2 and not *R*1 is the quantity minimized, *R*1 has the advantage that it is relatively insensitive to the weighting scheme, and so is more difficult to manipulate.

Since the structure is non-centrosymmetric, the program has automatically estimated the Flack absolute structure parameter x in the final structure factor summation. In this example x is within one esd of zero, and its esd is also relatively small. This provides strong evidence that the absolute structure has been assigned correctly, so that no further action is required. The program would have printed a warning here if it would have been necessary to 'invert' the structure or to refine it as a racemic twin.

This is followed by a list of principal mean square displacements U for all anisotropic atoms. It will be seen that none of the smallest components (in the third column) are in danger of going negative [which would make the atom 'non positive definite' (NPD)] but that the motion of the two unique fluorine atoms is highly anisotropic (not unusual for an AsF_6 anion). The program suggests that the fluorine motion is so extended in one direction that it would be possible to represent each of the two fluorine atoms as disordered over two sites, for which x, y and z coordinates are given; this may safely be ignored here (although there may well be some truth in it). The two suggested new positions for each 'split' atom are placed equidistant from the current position along the direction (and reverse direction) corresponding to the largest eigenvalue of the anisotropic displacement tensor.

This list is followed by the analysis of variance (reproduced here in squashed form), recommended weighting scheme (to give a flat analysis of variance in terms of F_c^2), and a list of the most disagreeable reflections. For a discussion of the analysis of variance see the second example.

```
Principal mean square atomic displacements U

    0.1067    0.1067    0.0561    Ag
    0.0577    0.0577    0.0386    As
    0.1038    0.0659    0.0440    S1
    0.0986    0.0515    0.0391    S2
    0.0779    0.0729    0.0391    C
    0.1004    0.0852    0.0474    N
    0.3029    0.0954    0.0473    F1
   may be split into  0.5965  0.3173  0.0288  and 0.5946  0.3324 -0.0369
    0.4778    0.1671    0.0457    F2
   may be split into  0.5320  0.5089  0.2462  and 0.4680  0.4911  0.2462


Analysis of variance for reflections employed in refinement
K = Mean[Fo^2] / Mean[Fc^2]  for group

Fc/Fc(max)     0.000 0.026 0.039 0.051 0.063 0.082 0.103 0.147 0.202 0.306 1.0
Number in group    94.   89.   90.   91.   89.   91.   89.   91.   88.   91.
GooF         1.096 1.101 0.997 1.078 1.187 1.069 1.173 0.922 1.019 0.966
K           1.560 1.053 1.010 1.004 1.007 1.021 1.026 1.002 0.997 0.984

Resolution(A)  0.77  0.81  0.85  0.90  0.95  1.02  1.10  1.22  1.40  1.74  inf
Number in group    97.   84.   92.   91.   89.   90.   89.   90.   93.   88.
GooF         1.067 0.959 0.935 0.895 1.035 1.040 1.115 1.149 1.161 1.228
K            1.047 1.010 1.009 0.991 1.004 0.996 0.989 1.012 0.997 0.982
R1           0.166 0.100 0.069 0.059 0.051 0.036 0.033 0.027 0.020 0.020

Recommended weighting scheme:  WGHT   0.0314 0.3674


Most Disagreeable Reflections (* if suppressed or used for Rfree)

    h   k   l       Fo^2      Fc^2    Delta(F^2)/esd  Fc/F(max)  Resolution(A)
    4   4   4       18.32    33.30      3.62          0.062       1.11
   -4   1   3       15.79     4.17      3.50          0.022       1.50
    0   2   2       41.60    57.32      3.26          0.082       2.61

etc.
```

After the table of bond lengths and angles (BOND was implied by the ACTA instruction), the data are merged (again) for the Fourier calculation after correcting for dispersion (because the electron density is real). In contrast to the initial data reduction, Friedel's law is assumed here; the aim is to set up a unique reflection list so that the (difference) electron density can be calculated on an absolute scale.

The algorithm for generating the 'asymmetric unit' for the Fourier calculations is general for all space groups, in conventional settings or otherwise. The rms electron density (averaged over all grid points) is printed as well as the maximum and minimum values so that the significance of the latter can be assessed. Since PLAN 20 was assumed, only a peak list is printed (and written to the *.res* file), followed by a list of shortest distances between peaks (not shown below); PLAN -20 would have produced a more detailed analysis with 'printer plots' of the structure. The last 40 peaks and some of the interatomic distances have been deleted here to save space. In this table, 'distances to nearest atoms' takes symmetry equivalents into account.

```
Bond lengths and angles        [severely squashed to fit page!]

Ag - Distance     Angles
N    2.2788(0.0058)
N_$2 2.2788(0.0058) 113.08(0.15)
N_$4 2.2788(0.0058) 113.08(0.15) 102.47(0.29)
N_$3 2.2788(0.0058) 102.47(0.29) 113.08(0.15) 113.08(0.15)
      Ag -          N            N_$3         N_$4

As - Distance     Angles
F2    1.6399(0.007)
F2_$6 1.6399(0.007)180.00(0.00)
F1_$7 1.6724(0.0037) 89.08(0.41) 90.92(0.41)
F1_$6 1.6724(0.0037) 89.08(0.41) 90.92(0.41)178.15(0.82)
F1_$5 1.6724(0.0037) 90.92(0.41) 89.08(0.41) 90.01(0.01) 90.01(0.01)
F1    1.6724(0.0037) 90.92(0.41) 89.08(0.41) 90.01(0.01) 90.01(0.01)178.15(0.82)
       As -         F2          F2_$6       F1_$7       F1_$6       F1_$5

S1 - Distance     Angles
C     1.6819(0.0069)
S2    2.0633(0.0025)  98.61(0.20)
         S1 -        C

S2 - Distance  Angles
S2_$1 2.0114(0.0028)
S1    2.0633(0.0025) 105.37(0.07)
         S2 -        S2_$1

C -  Distance  Angles
N   1.1472(0.0074)
S1  1.6819(0.0069) 175.67(0.49)
       C -         N

N -  Distance  Angles
C   1.1472(0.0074)
Ag  2.2788(0.0058) 152.38(0.45)
       N -         C
F1 - Distance  Angles
As  1.6724(0.0037)
       F1 -
```

```
F2 - Distance  Angles
As  1.6399(0.0075)
       F2 -


FMAP and GRID set by program

FMAP 2   3  18
GRID  -3.333  -2  -1     3.333   2   1

R1 = 0.0370 for 590 unique reflections after merging for Fourier


Electron density synthesis with coefficients Fo-Fc

Highest peak   0.32  at  0.0000  0.0000  0.5000  [2.60 A from N]
Deepest hole  -0.36  at  0.5000  0.5000  0.1863  [0.40 A from F2]
Mean = 0.00, Rms deviation from mean = 0.07 e/A^3 Highest memory used 1133/13851

Fourier peaks appended to .res file

         x       y       z       sof      U   Peak  Dist to nearest atoms
Q1  1  0.0000  0.0000  0.5000  0.25000  0.05  0.32  2.60 N  2.69 C  3.33 AG
Q2  1  0.5690  0.3728  0.1623  1.00000  0.05  0.27  1.20 F1 1.34 F2 1.62 AS
Q3  1  0.5685  0.3851 -0.1621  1.00000  0.05  0.24  1.19 F1 1.25 F2 1.56 AS
Q4  1  0.4075  0.4717  0.2378  1.00000  0.05  0.23  0.81 F2 1.78 AS 1.79 F1
Q5  1  0.5848  0.2667  0.0312  1.00000  0.05  0.23  0.55 F1 2.09 AS 2.47 F1
Q6  1  0.5495  0.3425 -0.1122  1.00000  0.05  0.21  0.83 F1 1.57 AS 1.65 F2
Q7  1  0.2617 -0.1441  0.1446  1.00000  0.05  0.20  1.59 N  2.17 F1 2.40 C
Q8  1  0.7221  0.1898  0.0030  1.00000  0.05  0.20  1.55 F1 2.39 N  2.54 N
Q9  1  0.1997  0.0293  0.1024  1.00000  0.05  0.19  0.75 N  1.79 C  1.82 AG
Q10 1  0.4606 -0.0113  0.8165  1.00000  0.05  0.19  0.91 S2 1.41 S2 2.82 S1
```

8.2 Second example (sigi)

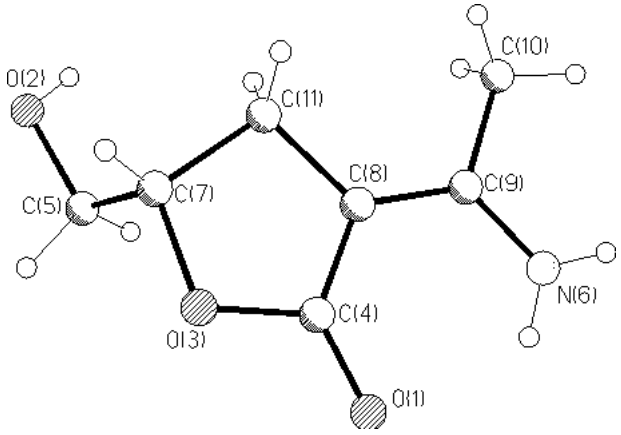

Figure 8.2 – sigi

In the second example (provided as the files *sigi.ins* and *sigi.hkl*), a small organic structure is refined in the space group $P\bar{1}$. Only the features that are different from the ags4 refinement will be discussed in detail. The structure consists of a five-membered lactone [-C7-C11-C8-C4(O1)-O3-] with a -CH2-OH group [-C5-O2] attached to C7 and a $=C(CH_3)(NH_2)$ unit [=C9(C10)N6] double-bonded to C8.

Of particular interest here is the placing and refinement of the 11 hydrogen atoms via HFIX instructions. The two $-CH_2-$ groups (C5 and C11) and one tertiary CH (C7) can be placed geometrically by standard methods; the algorithms have been improved relative to those used in version 4 of SHELXTL, and the hydrogen atoms are now idealized before each refinement cycle (and after the last). Since N6 is attached to a conjugated system, it is reasonable to assume that the $-NH_2$ group is coplanar with the C8=C9(C10)-N6 unit, which enables these two hydrogens to be placed as ethylenic hydrogens, requiring HFIX (or AFIX) 9n; the program takes into account that they are bonded to nitrogen in setting the default bond lengths. All these hydrogens are to be refined using a ‘riding model’ (HFIX or AFIX m3) for x, y and z.

The -OH and $-CH_3$ groups are trickier, in the latter case because C9 is sp^2-hybridized, so the potential barrier to rotation is low and there is no fully staggered conformation available as the obvious choice. Since the data are reasonable, the initial torsion angles for these two groups can be found by means of difference electron density syntheses calculated around the circles which represent the loci of all possible hydrogen atom positions. The torsion angles are then refined during the least-squares refinement. Note that in subsequent cycles (and jobs) these groups will be re-idealized geometrically with retention of the current torsion angle; the circular Fourier calculation is performed only once. Two 'free variables' (2 and 3 yes, they still exist!) have been assigned to refine common isotropic displacement parameters for the ‘rigid’ and ‘rotating’ hydrogens respectively. If these had not been specified, the default action would have been to hold the hydrogen U values at 1.2 times the equivalent isotropic U of the atoms to which they are attached (1.5 for the -OH and methyl groups).

The *sigi.ins* file (which is provided as a test job) is as follows. Note that for instructions with both numerical parameters and atom names such as HFIX and MPLA, it does not matter whether numbers or atoms come first, but the order of the numerical parameters themselves (and in some cases the order of the atoms) is important.

```
TITL SIGI in P-1
CELL 0.71073 6.652 7.758 8.147 73.09 75.99 68.40
ZERR 2 .002 .002 .002 .03 .03 .03
SFAC C H N O
UNIT 14 22 2 6        ! no LATT and SYMM needed for space group P-1

L.S. 4
EXTI 0.001            ! refine an isotropic extinction parameter
WGHT .060 0.15        ! (suggested by program in last job);  WGHT
OMIT 2 8 0            ! and OMIT are also based on previous output
BOND $H               ! include H in bond lengths / angles table
CONF                  ! all torsion angles except involving hydrogen
HTAB                  ! analyse all hydrogen bonds
FMAP 2                ! Fo-Fc Fourier
PLAN -20              ! printer plots and full analysis of peak list

HFIX 147 31 O2        ! initial location of -OH and -CH3 hydrogens from
HFIX 137 31 C10       ! circular Fourier, then refine torsion, U(H)=fv(3)

HFIX 93 21 N6         ! -NH2 in plane, xyz ride on N, U(H)=fv(2)
HFIX 23 21 C5 C11     ! two -CH2- groups, xyz ride on C, U(H)=fv(2)
HFIX 13 21 C7         ! tertiary CH, xyz ride on C, U(H)=fv(2)

EQIV $1 X-1, Y, Z     ! define symmetry operations for H-bonds
EQIV $2 X+1, Y, Z-1
HTAB N6 O1            ! outputs H-bonds D-H...A with esds
HTAB O2 O1_$1         ! _$1 and _$2 refer to symmetry equivalents
HTAB N6 O2_$2
                                  ! l.s. planes through 5-ring and through
MPLA 5 C7 C11 C8 C4 O3 O1 N6 C9 C10 ! CNC=CCC moiety, then find deviations
MPLA 6 C10 N6 C9 C8 C11 C4 O1 O3 C7 ! of last 4 and 3 named atoms resp. too

FVAR 1 .06 .07                    ! overall scale and free variables for U(H)

REM name sfac# x y z sof(+10 to fix it) U11 U22 U33 U23 U13 U12 follow

O1  4   0.30280   0.17175   0.68006   11.00000   0.02309   0.04802 =
    0.02540  -0.00301  -0.00597  -0.01547
O2  4  -0.56871   0.23631   0.96089   11.00000   0.02632   0.04923 =
    0.02191  -0.00958  0.00050  -0.02065
O3  4  -0.02274   0.28312 0.83591 11.00000 0.02678 0.04990 =
    0.01752  -0.00941  -0.00047  -0.02109
C4  1   0.10358   0.23458 0.68664 11.00000 0.02228 0.02952 =
    0.01954  -0.00265  -0.00173  -0.01474
C5  1  -0.33881   0.18268 0.94464 11.00000 0.02618 0.03480 =
    0.01926  -0.00311  -0.00414  -0.01624
N6  3  0.26405    0.17085 0.33925 11.00000 0.03003 0.04232 =
    0.02620  -0.01312  0.00048  -0.01086
C7  1  -0.25299   0.33872 0.82228 11.00000 0.02437 0.03111 =
    0.01918  -0.00828  -0.00051  -0.01299
C8  1  -0.03073   0.27219 0.55976 11.00000 0.02166 0.02647 =
    0.01918  -0.00365  -0.00321  -0.01184
C9  1  0.05119    0.24371 0.39501 11.00000 0.02616 0.02399 =
    0.02250  -0.00536  -0.00311  -0.01185
C10 1  -0.10011   0.29447 0.26687 11.00000 0.03877 0.04903 =

    0.02076  -0.01022  -0.00611  -0.01800
C11 1  -0.26553   0.36133 0.63125 11.00000 0.02313 0.03520 =
    0.01862  -0.00372  -0.00330  -0.01185

HKLF 4   ! read intensity data from 'sigi.hkl'; terminates '.ins' file
END
```

The data reduction reports 1904 reflections read (one of which was rejected by OMIT) with indices $-7 \leq h \leq 7$, $-8 \leq k \leq 9$ and $-9 \leq l \leq 9$. Note that these are the limiting index values; in fact only about 1.5 times the unique volume of reciprocal space was measured. The maximum 2θ was 50.00, and there were no systematic absence violations, 34 (not seriously) inconsistent equivalents, and 1296 unique data. R(int) was 0.0196 and R(sigma) 0.0151.

The program uses different default distances to hydrogen for different bonding situations; these may be overridden by the user if desired. These defaults depend on the temperature (set using TEMP) in order to allow for librational effects. The list of default X-H distances is followed by the (squashed) circular difference electron density syntheses to determine the C-OH and C-CH_3 initial torsion angles:

```
Default effective X-H distances for T =   20.0 C

AFIX m =    1    2    3     4   4[N]  3[N]  15[B]  8[O]   9   9[N]   16
d(X-H) =  0.98 0.97 0.96  0.93  0.86  0.89  1.10  0.82  0.93  0.86  0.93

Difference electron density (eA^-3x100) at 15 degree intervals for AFIX 147 group
attached to O2. The center of the range is eclipsed (cis) to C7 and rotation is
clockwise looking down C5 to O2
  2 -2 -6 -9 -8 -5 -1  0  0  0  1  0 -2 -2  0  9 23 39 48 42 29 16  9  5

Difference electron density (eA^-3x100) at 15 degree intervals for AFIX 137 group
attached to C10. The center of the range is eclipsed (cis) to N6 and rotation is
clockwise looking down C9 to C10
  50 47 39 28 19 15 20 30 38 41 39 37 34 29 25 27 33 35 29 19 12 15 29 43

After local symmetry averaging:   40  41  36  28  21  20  24  33
```

It will be seen that the hydroxyl hydrogen is very clearly defined, but that the methyl group is rotating fairly freely (low potential barrier). After three-fold averaging, however, there is a single difference electron density maximum. The (squashed) least-squares refinement output follows:

```
Least-squares cycle 1   Maximum vector length=511  Memory required=1836/136080

wR2 =  0.1130 before cycle   1 for   1296 data and  105 /  105 parameters

GooF = S =     1.140;     Restrained GooF =      1.140  for      0 restraints

Weight = 1/[sigma^2(Fo^2)+(0.0600*P)^2+0.15*P] where P=(Max(Fo^2,0)+2*Fc^2)/3

   N     value      esd      shift/esd  parameter

   1    0.97891    0.00384   -10.702     OSF
   2    0.04044    0.00261    -7.494    FVAR  2
   3    0.07317    0.00394     0.805    FVAR  3
   4    0.01781    0.00946     1.777    EXTI

Mean shift/esd =   0.747    Maximum = -10.702 for FVAR  2

Max. shift = 0.028 A for H10A     Max. dU =-0.020 for H5A

  .......... etc (cycles 2 and 3 omitted) .........

Least-squares cycle 4  Maximum vector length = 511 Memory required =1836/136080

wR2 =  0.1035 before cycle   4 for   1296 data and  105 /  105 parameters

GooF = S =     1.016;     Restrained GooF =      1.016  for      0 restraints

Weight = 1/[sigma^2(Fo^2)+(0.0600*P)^2+0.15*P] where P=(Max(Fo^2,0)+2*Fc^2)/3

   N     value       esd     shift/esd  parameter
   1    0.97902    0.00358    -0.003     OSF
   2    0.03605    0.00176     0.012    FVAR  2
   3    0.07345    0.00376    -0.031    FVAR  3
   4    0.02502    0.01081    -0.010    EXTI

Mean shift/esd =   0.008    Maximum =  -0.244 for tors H10A

Max. shift = 0.004 A for H10A       Max. dU = 0.000 for H2


Largest correlation matrix elements

 0.509 U12 O2 / U22 O2    0.507 U12 O3 / U11 O3
 0.509 U12 O2 / U11 O2    0.500 U12 O3 / U22 O3


Idealized hydrogen atom generation before cycle   5

Name     x       y       z     AFIX  d(X-H)  shift  Bonded   Conformation
                                                    to      determined by
H2   -0.6017  0.2095  0.8832  147   0.820   0.000   O2        C5  H2
H5A  -0.2721  0.0676  0.9001   23   0.970   0.000   C5        O2  C7
H5B  -0.2964  0.1554  1.0576   23   0.970   0.000   C5        O2  C7
H6A   0.3572  0.1389  0.4085   93   0.860   0.000   N6        C9  C8
H6B   0.3073  0.1559  0.2347   93   0.860   0.000   N6        C9  C8
H7   -0.3331  0.4598  0.8575   13   0.980   0.000   C7        O3  C5  C11
H10A -0.0176  0.2947  0.1525  137   0.960   0.000   C10       C9  H10A
H10B -0.2042  0.4192  0.2692  137   0.960   0.000   C10       C9  H10A
H10C -0.1764  0.2036  0.2964  137   0.960   0.000   C10       C9  H10A
H11A -0.3575  0.2948  0.6198   23   0.970   0.000   C11       C8  C7
H11B -0.3198  0.4943  0.5737   23   0.970   0.000   C11       C8  C7
```

The final structure factor calculation, analysis of variance etc. produces the following edited output:

```
Final Structure Factor Calculation for  SIGI in P-1
Total number of l.s. parameters = 105    Maximum vector length =  511

wR2 =  0.1035 before cycle 5 for 1296 data and    0 /  105 parameters

GooF = S =   1.016;     Restrained GooF =      1.016  for    0 restraints

Weight = 1/[sigma^2(Fo^2)+(0.0600*P)^2+0.15*P] where P=(Max(Fo^2,0)+2*Fc^2)/3
R1 = 0.0364 for   1189 Fo > 4.sigma(Fo)  and  0.0397 for all   1296 data
wR2 =  0.1035,  GooF = S = 1.016,  Restrained GooF =    1.016  for all data

Occupancy sum of asymmetric unit = 11.00 for non-hydrogen and 11.00 for
hydrogen atoms.


Principal mean square atomic displacements U

   0.0504   0.0254   0.0188   O1
   0.0492   0.0229   0.0189   O2
   0.0513   0.0194   0.0165   O3
   0.0326   0.0208   0.0159   C4
   0.0376   0.0204   0.0190   C5
   0.0439   0.0319   0.0214   N6
   0.0329   0.0201   0.0185   C7
   0.0276   0.0190   0.0181   C8
   0.0289   0.0220   0.0191   C9
   0.0493   0.0352   0.0181   C10
   0.0353   0.0215   0.0183   C11

Analysis of variance for reflections employed in refinement
K = Mean[Fo^2] / Mean[Fc^2]  for group

Fc/Fc(max)    0.000 0.009 0.017 0.027 0.038 0.049 0.065 0.084 0.110 0.156 1.0

Number in group   135.  125.  131.  139.  119.  132.  131.  128.  131.  126.

           GooF  1.034 1.000 1.085 1.046 1.093 0.999 0.937 0.995 1.027 0.931

           K     1.567 1.127 0.964 1.023 1.008 0.992 0.997 0.998 1.008 1.010


Resolution(A)  0.84  0.88  0.90  0.95  0.99  1.06 1.14  1.25  1.44  1.79  inf

Number in group   136.  127.  128.  128.  136.  124.  128.  130.  130.  129.

           GooF  0.978 0.881 0.854 0.850 0.850 0.921 0.874 1.088 1.242 1.434

           K     1.024 1.013 1.017 0.990 0.991 0.989 1.013 0.995 1.037 1.004

           R1    0.061 0.049 0.050 0.046 0.034 0.034 0.031 0.039 0.038 0.037

Recommended weighting scheme:  WGHT   0.0545   0.1549
```

The analysis of variance should be examined carefully for indications of systematic errors. If the *Goodness of Fit* (GooF) is significantly higher than unity and the scale factor K is appreciably lower than unity in the extreme right columns in terms of both *F* and resolution, then an extinction parameter should be refined (the program prints a warning in such a case). This does not show here because an extinction parameter is already being refined. The scale factor is a little high for the weakest reflections in this example; this may well be a statistical artifact and may be ignored (selecting the groups on F_c will tend to make F_o^2 greater than F_c^2 for this range). The increase in the GooF at low resolution (the 1.79 to infinity range) is caused in part by systematic errors in the model such as the use of scattering factors based on spherical atoms which ignore bonding effects, and is normal for purely light-atom structures (this interpretation is confirmed by the fact that difference electron density peaks are found in the middle of bonds). In extreme cases the lowest or highest resolution ranges can be conveniently suppressed by means of the SHEL instruction; this is normal practice in macromolecular refinements, but refining a diffuse solvent model with SWAT may be better, inadequate solvent modeling for macromolecules produces similar symptoms to lack of extinction refinement for small molecules.

The weighting scheme suggested by the program is designed to produce a flat analysis of variance in terms of F_c, but makes no attempt to fit the resolution dependence of the GooF. It is also written to the end of the *.res* file, so that it is easy to update it before the next job. In the early stages of refinement it is better to retain the default scheme of WGHT 0.1; the updated parameters should not be incorporated in the next *.ins* file until all atoms have been found and at least the heavier atoms refined anisotropically.

The list of most disagreeable reflections and tables of bond lengths and angles (BOND $H - omitted here) and torsion angles (CONF) are followed by the HTAB (hydrogen bonds) and MPLA (least-squares planes) tables:

```
 Selected torsion angles

  -175.08 ( 0.12)  C7 - O3 - C4 - O1
     5.73 ( 0.15)  C7 - O3 - C4 - C8
   109.69 ( 0.12)  C4 - O3 - C7 - C5
   -11.65 ( 0.15)  C4 - O3 - C7 - C11
   171.12 ( 0.10)  O2 - C5 - C7 - O3
   -72.04 ( 0.15)  O2 - C5 - C7 - C11
    -1.46 ( 0.24)  O1 - C4 - C8 - C9
   177.61 ( 0.12)  O3 - C4 - C8 - C9
  -176.27 ( 0.14)  O1 - C4 - C8 - C11
     2.80 ( 0.16)  O3 - C4 - C8 - C11
     3.08 ( 0.22)  C4 - C8 - C9 - N6
   176.93 ( 0.13)  C11 - C8 - C9 - N6
  -177.23 ( 0.13)  C4 - C8 - C9 - C10
    -3.39 ( 0.22)  C11 - C8 - C9 - C10
   176.05 ( 0.13)  C9 - C8 - C11 - C7
    -9.39 ( 0.14)  C4 - C8 - C11 - C7
    12.37 ( 0.14)  O3 - C7 - C11 - C8
  -104.74 ( 0.13)  C5 - C7 - C11 - C8


 Specified hydrogen bonds (with esds except fixed and riding H)

  D-H          H...A        D...A        <(DHA)
  0.86         2.23         2.8486(18)   129.3        N6-H6A...O1
  0.82         2.04         2.8578(16)   174.0        O2-H2...O1_$1
  0.86         2.17         2.9741(19)   155.1        N6-H6B...O2_$2


Least-squares planes (x,y,z in crystal coordinates) and deviations from them
 (* indicates atom used to define plane)

   2.3443 (0.0044) x + 7.4105 (0.0042) y - 0.0155 (0.0053) z = 1.9777 (0.0044)

 *   -0.0743 (0.0008)  C7
 *    0.0684 (0.0008)  C11
 *   -0.0418 (0.0009)  C8
 *   -0.0062 (0.0008)  C4
 *    0.0538 (0.0008)  O3
     -0.0061 (0.0020)  O1
     -0.0980 (0.0028)  N6
     -0.0562 (0.0023)  C9
     -0.0314 (0.0030)  C10

 Rms deviation of fitted atoms =   0.0546


   2.5438 (0.0040) x + 7.3488 (0.0040) y - 0.1657 (0.0042) z = 1.8626 (0.0026)

 Angle to previous plane (with approximate esd) =  2.45 ( 0.07 )

 *    0.0054 (0.0008)  C10
 *    0.0082 (0.0008)  N6
 *   -0.0052 (0.0012)  C9
```

```
*   -0.0337 (0.0012)  C8
*    0.0135 (0.0008)  C11
*    0.0118 (0.0009)  C4
     0.0568 (0.0019)  O1
     0.0214 (0.0018)  O3
    -0.1542 (0.0020)  C7

Rms deviation of fitted atoms =   0.0162


Hydrogen bonds with  H..A < r(A) + 2.000 Angstroms  and  <DHA > 110 deg.

D-H           d(D-H)   d(H..A)   <DHA    d(D..A)   A
O2-H2          0.820    2.041   174.05    2.858    O1 [ x-1, y, z ]
N6-H6A         0.860    2.225   129.29    2.849    O1
N6-H6B         0.860    2.172   155.06    2.974    O2 [ x+1, y, z-1 ]
```

All esds printed by the program are calculated rigorously from the full covariance matrix, except for the esd in the angle between two least-squares planes, which involves some approximations. The contributions to the esds in bond lengths, angles and torsion angles also take the errors in the unit-cell parameters (as input on the ZERR instruction) rigorously into account; an approximate treatment is used to obtain the (rather small) contributions of the cell errors to the esds involving least-squares planes.

There follows the difference electron density synthesis and line printer 'plot' of the structure and peaks. The highest and lowest features are 0.27 and -0.17 eA_{-3} respectively, and the rms difference electron density is 0.04. These values confirm that the treatment of the hydrogen atoms was adequate, and are indeed typical for routine structure analysis of small organic molecules. This output is too voluminous to give here, and indeed users of the Bruker SHELXTL molecular graphics program XP will almost always suppress it by use of the default option of a positive number on the PLAN instruction, and employ interactive graphics instead for analysis of the peak list.

9 Constraints and Hydrogen Atoms

9.1 Constraints Versus Restraints

In crystal structure refinement, there is an important distinction between a *constraint* and a *restraint*. A *constraint* is an exact mathematical condition that enables one or more least-squares variables to be expressed exactly in terms of other variables or constants, and hence eliminated. An example is the fixing of the x, y and z coordinates of an atom on an inversion center. A *restraint* takes the form of additional information that is not exact but is subject to a probability distribution; for example two chemically but not crystallographically equivalent bonds could be restrained to be approximately equal. A restraint is treated as an extra experimental observation, with an appropriate esd that determines its weight relative to the X-ray data. An excellent account of the use of constraints and restraints to control the refinement of difficult structures has been given by Watkin (1994).

Often there is a choice between constraints and restraints. For example, in a triphenylphosphine complex of a heavy element, the light atoms will be less well determined from the X-ray data than the heavy atoms. In version 4 of SHELXTL a rigid group *constraint* was often applied to the phenyl groups in such cases: the phenyl groups were treated as rigid hexagons with C-C bond lengths of 1.39 Å. This introduces a slight bias (e.g., in the P-C bond length), because the *ipso*-angle should be a little smaller than 120°. In XL such rigid group constraints may still be used, but it is more realistic to apply FLAT and SADI (or SAME) *restraints* so that the phenyl groups are planar and have mm2 (C_{2v}) symmetry, subject to suitable esds. In addition, the phenyl groups may be restrained to have similar geometries to one another.

9.2 Free Variables, Occupancy and Isotropic U-constraints

XL employs the concept of *free variables*. A free variable is a refinable parameter that can be used to impose a variety of additional linear constraints, e.g., to atomic coordinates, occupancies or displacement parameters. Starting values for all free variables are supplied on the FVAR instruction. Since the first FVAR parameter is the (*F*-relative) overall scale factor, there is no free variable 1. If an atom parameter is given a value greater than 15 or less than -15, it is interpreted as a reference to a free variable. A positive value ($10k+p$) is decoded as p times free variable number k [$fv(k)$], and a negative value (i.e., k and p both negative) is decoded as p times [$fv(-k)-1$]. This appears more complicated than it is in practice: for example to assign a common occupancy parameter to describe a two component disorder, the occupancies of all atoms of one component can be replaced by 21, and the occupancies of all atoms of the second component by -21, where the starting value for the occupancy is the second FVAR parameter. A further disorder, not correlated with the first, would then use free variable number 3 and codes 31 and -31 etc. If there are more than two components of a disordered atom or group, it is necessary to apply a restraint (SUMP) to the free variables used to represent the occupancies.

Free variables may be used to constrain the isotropic U-values of chemically similar hydrogen atoms to be the same; for example one could use the fourth FVAR parameter and code 41 for all methyl hydrogens (which tend to have larger U-values), and the fifth FVAR parameter and code 51 for the rest. An alternative way to constrain hydrogen isotropic displacement parameters is to replace the U-value on the atom instruction by a code *q* between -0.5 and -5; the U-value is then calculated as |*q*| times the (equivalent) isotropic U of the last atom not treated in this way (usually the carbon or other atom on which the hydrogen rides). Typical q values are -1.5 for methyl and hydroxyl hydrogens and -1.2 for others.

9.3 Special Position Constraints

Constraints for the coordinates and anisotropic displacement parameters for atoms on special positions are generated automatically by the program for ALL special positions in ALL space groups, in conventional settings or otherwise. For upwards compatibility with version 4 of SHELXTL, free variables may still be used for this, but it is better to leave it to the program. If the occupancy is not input, the program will fix it at the appropriate value for a special position. If the user applies (correct or incorrect) special position constraints using free variables etc., the program assumes this has been done with intent and reports but does not apply the correct constraints; accidental application of wrong special position constraints is one of the easiest ways to cause a refinement to ‘blow up’!

9.4 Atoms on the Same Site

For two or more atoms sharing the same site, the xyz and U_{ij} parameters may be equated using the EXYZ and EADP constraints respectively (or by using ‘free variables’). The occupation factors may be expressed in terms of a ‘free variable’ so that their sum is constrained to be constant (e.g., 1.0). If more than two different chemical species share a site, a *linear free variable restraint* (SUMP) is required to restrain the sum of occupation factors.

9.5 Rigid Group and Riding Model Constraints; Fitting of Standard Fragments

The generation of idealized coordinates and geometrical constraints in the refinement are defined in XL by the two-part AFIX code number (*mn*). This notation is perhaps a little too concise, but has been retained for upwards compatibility with version 4 of SHELXTL, although several of the options are new. The last digit, *n*, describes the constraints to be used in the refinement, and the one or two-digit component *m* defines the starting geometry. For example AFIX 95 followed by five carbon atoms (possibly with intervening hydrogens) and then AFIX 0 means that a regular pentagon (*n*=5) should be fitted (to at least three atoms with non-zero coordinates), and then refined as a rigid group with variable overall scale (*m*=9). This could be used to refine a cyclopentadienyl ligand. Similarly AFIX 106 would be used for an idealized pentamethyl-cyclopentadienyl ligand refined as a rigid group with fixed interatomic distances. Note that riding (or restrained) hydrogens may be included in such rigid groups, and are ignored when fitting the idealized group (in contrast to version 4 of SHELXTL).

A rigid group involves 6 refinable parameters: three rotation angles and three coordinates. The first atom in the group is the pivot atom about which the other atoms rotate; this is useful when it is necessary to fix its coordinates (by adding 10 in the usual way). In a variable metric rigid group (m=9) a seventh parameter is refined; this is a scale factor that multiplies all distances within the group. Any of the atoms in a rigid group may be subject to restraints, e.g., to restrain their distances to atoms not in the same rigid group (this was not allowed in version 4 of SHELXTL).

A particularly useful constraint for the refinement of hydrogen atoms is the *riding model* (*n*=3):

$$\mathbf{x}(\mathrm{H}) = \mathbf{x}(\mathrm{C}) + \mathbf{d}$$

where **d** is a constant vector. Both atoms contribute to the derivative calculation and the same shifts are applied to both; the hydrogen atoms are re-idealized after each cycle (although this is scarcely necessary). The riding model constraint costs no extra parameters, and improves convergence of the refinement. XL provides several variations of this riding model; for example the C-H distances (but not the XCH angles) may be allowed to refine (*n*=4; one extra parameter per group), the torsion angle of a methyl or hydroxyl group may be refined (*n*=7), or these two options may be combined (*n*=8).

Fragments of known geometry may be fitted to target atoms (e.g., from a previous Fourier peak search), and the coordinates generated for any missing atoms. Four standard groups are available: regular pentagon (*m*=5), regular hexagon (*m*=6), naphthalene (*m*=11) and pentamethylcyclopentadienyl (*m*=10); any other group may be used simply by specifying orthogonal or fractional coordinates in a given cell (AFIX *mn* with *m*>16 and FRAG...FEND). This is usually, but not always, followed by rigid group refinement.

9.6 Hydrogen Atom Generation and Refinement

It is difficult to locate hydrogen atoms accurately using X-ray data because of their low scattering power, and because the corresponding electron density is smeared out, asymmetrical, and is not centered at the position of the nucleus. In addition, hydrogen atoms tend to have larger librational amplitudes than other atoms. For most purposes, it is preferable to calculate the hydrogen positions according to well-established geometrical criteria and then to adopt a refinement procedure which ensures that a sensible geometry is retained. The following table summarizes the options for generating hydrogen atoms; the hydrogen coordinates are re-idealized before each cycle. The distances given in this table are the values for room temperature, they are increased by 0.01 or 0.02 Å for low temperatures (specified by the TEMP instruction) to allow for the smaller librational correction at low temperature.

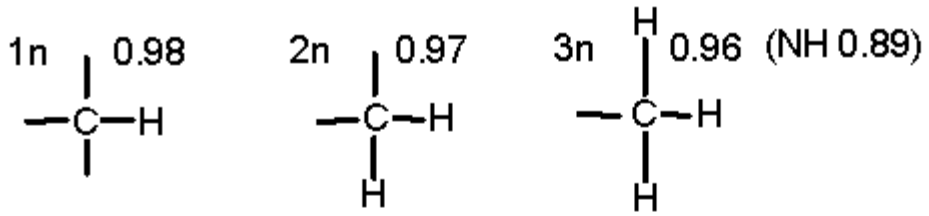

Figure 9.1 – Hydrogen atom generation and refinement

9.7 Special Facilities for -CH_3 and -OH groups

Methyl and hydroxyl groups are difficult to position accurately (unless neutron data are available!). If good (low-temperature) x-ray data are available, the method of choice is HFIX 137 for -CH_3 and HFIX 147 for -OH groups; in this approach, a difference electron density synthesis is calculated around the circle which represents the locus of possible hydrogen positions (for a fixed X-H distance and Y-X-H angle). The maximum electron density (in the case of a methyl group after local threefold averaging) is then taken as the starting position for the hydrogen atom(s). In subsequent refinement cycles (and in further least-squares jobs) the hydrogens are re-idealized at the start of each cycle, but the current torsion angle is retained; the torsion angles are allowed to refine whilst keeping the X-H distance and Y-X-H angle fixed (*n*=7). If unusually high quality data are available, AFIX 138 would allow the refinement of a common C-H distance for a methyl group but not allow the group to tilt; a variable metric rigid group refinement (AFIX 9 for the carbon followed by AFIX 135 before the first hydrogen) would allow it to tilt as well, but still retain tetrahedral H-C-H angles and equal C-H distances within the group.

If the data quality is less good, then the refinement of torsion angles may not converge very well. In such cases the hydrogens can be positioned geometrically and refined using a riding model by HFIX 33 for methyl and HFIX 83 for hydroxyl groups. This staggers the methyl groups, and -OH groups attached to saturated carbons, as well as possible; -OH groups attached to aromatic rings are tested in one of the two positions with one hydrogen in the plane. In both cases the choice of hydrogen position is then determined by best hydrogen bond (to an N, O, Cl or F atom) that can be created. For disordered methyl groups (with two sites rotated by 60 degrees from one another) HFIX 123 is recommended, possibly with refinement of the corresponding site occupation factors via a 'free variable' so that their sum is unity (e.g., 21 and -21).

The choice of a suitable (default) O-H distance is very difficult. O-H internuclear distances for isolated molecules in the gas phase are about 0.96 Å (cf. 1.10 for C-H), but the appropriate distance to use for X-ray diffraction must be appreciably shorter to allow for the displacement of the center of gravity of the electron distribution towards the oxygen atom, and also for librational effects. Although the (temperature dependent) value assumed by the program fits reasonably well for O-H groups in predominantly organic molecules, appreciably longer O-H distances are appropriate for low-temperature studies of strongly (cooperatively) hydrogen-bonded systems; short H...O distances are always associated with long O-H distances. If there are many such O-H groups and good quality data are available, HFIX 88 (or 148) plus SADI restraints to make all the O-H distances approximately equal (with an esd of say 0.02) is a good approach.

9.8 Further Peculiarities Involving Hydrogen Atoms

Hydrogen atoms are identified as such by their scattering factor numbers, which must correspond to a SFAC name H (or $H). The special treatment of hydrogens does not apply if they reference a different SFAC name (e.g., D!). Other elements that need to be specifically identified (e.g., so that HFIX 43 can use different default C-H and N-H distances) are defined similarly. However for the output of the PLAN instruction, hydrogen atoms are identified as those atoms with a radius of less the 0.4 Å. This is not as illogical as it may sound; the PLAN output is concerned with potential hydrogen bonds etc., not with the scattering power of an atom, and XL has to handle neutron as well as X-ray data.

Hydrogen atoms may also ‘ride’ on atoms in rigid groups (unlike version 4 of SHELXTL); for example HFIX 43 could reference carbon atoms in a rigid phenyl ring. In such a case further geometrical restraints (SADI, SAME, DFIX, FLAT) are not permitted on the hydrogen atoms; this is the only exception to the general rule that any number of restraints may be applied to any atom, whatever constraints are also being applied to it.

OMIT $H (or OMIT_* $H if residues are employed) combined with L.S. 0, FMAP 2 and PLAN -100 enables an ‘omit map’ to be calculated, in which the hydrogen atoms are retained but do not contribute to F_c. If a non-zero electron density appears in the ‘Peak’ column for a hydrogen atom in the Fourier output, then there was an actual peak in the difference electron density synthesis within 0.31 Å of the expected hydrogen position.

Sometimes it is known that the crystal contains a deuterated solvent molecule (e.g., $CDCl_3$) because it was crystallized in an n.m.r. tube. In such a case, an element ‘D’ may be added after ‘H’ on the SFAC instruction, and the appropriate numbers of H and D in the cell specified on the UNIT instruction. This enables the formula weight and density to be calculated correctly. The H and D atoms that follow in the *.ins* file should both be given the SFAC number corresponding to H, so that they are both treated as ‘hydrogens’ for all other purposes.

10 Restraints and Disorder

A *restraint* is incorporated in the least-squares refinement as if it were an additional experimental observation; $w(yt–y)^2$ is added to the quantity $\Sigma w(F_o^{2-}F_c^2)^2$ to be minimized, where a quantity y (which is a function of the least-squares parameters) is to be restrained to a target value yt, and the weight w (for either a restraint or a reflection) is $1/\sigma^2$. In the case of a reflection, σ^2 is estimated using a weighting scheme; for a restraint σ is simply the effective standard deviation. In XL the restraint weights are multiplied by the mean value of $w(F_o^{2-}F_c^2)^2$ for the reflection data, which allows for the possibility that the reflection weights may be relative rather than absolute, and also gives the restraints more influence in the early stages of refinement (when the Goodness of Fit is invariably much greater than unity), which improves convergence. It is possible to use Brünger's R_{free} test (Brünger, 1992) to fine-tune the restraint esds. In practice the optimal restraint esds vary little with the quality and resolution of the data, and the standard values (assumed by the program if no other value is specified) are entirely adequate for routine refinements. Default values for the various classes of restraint may be also set with DEFS instructions; there may be several DEFS instructions in the same .ins file: each applies to all restraints encountered before the next DEFS instruction (or the end of the file).

10.1 Floating Origin Restraints

Floating origin restraints are generated automatically by the program as and when required by the method of Flack & Schwarzenbach (1988), so the user should not attempt to fix the origin in such cases by fixing the coordinates of a heavy atom. These floating origin restraints effectively fix the X-ray 'center of gravity' of the structure in the polar axis direction(s), and lead to smaller correlations than fixing a single atom in structures with no dominant heavy atom. Floating origin restraints are not required (and will not be generated by the program) when CGLS refinement is performed.

10.2 Geometrical Restraints

A particularly useful restraint is to make chemically but not crystallographically equivalent distances equal (subject to a given or assumed esd) without having to invent a value for this distance (SADI). The SAME instruction can generate SADI restraints automatically, e.g., when chemically identical molecules or residues are present. This has the same effect as making equivalent bond lengths and angles but not torsion angles equal (see also section 10.5).

The FLAT instruction restrains a group of atoms to lie in a plane (but the plane is free to move and rotate); the program achieves this by treating the restraint as a sum of chiral volume restraints with zero target volumes. Thus the restraint esd has units of $Å^3$. For comparison with other methods, the r.m.s. deviation of the atoms from their restraint planes is also calculated.

DFIX and DANG restrain distances to target values. DANG was introduced so that the default sigma for 1,3-distances could be made twice that for 1,2-distances (the first DEFS parameter). The DANG restraints are applied in exactly the same way as DFIX, but are also listed separately in the restraints summary tables.

CHIV restrains the *chiral volume* of an atom that makes three bonds; the chiral volume is the volume of the 'unit-cell' (i.e., parallelepiped) whose axes are represented by these three bonds. In the version 5.1 of SHELXTL, the sign of the chiral volume is determined by the alphabetical (ASCII) order of the atoms, rather than the order in the connectivity list (which caused some confusion in version 5.0 of SHELXTL).

When 'free variables' are used as the target values for DFIX, DANG and CHIV restraints, it is possible to restrain different distances etc. to be equal and to refine their mean value (for which an esd is thus obtained). ALL types of geometrical restraint may involve ANY atom, even if it is part of a rigid group or a symmetry equivalent generated using EQIV $n and referenced by _$n, except for hydrogen atoms which ride on rigid group atoms.

10.3 Anti-bumping Restraints

Anti-bumping restraints are usually only necessary for lower resolution structures, e.g., of macromolecules. They may be applied individually, by means of DFIX distance restraints with the distance given as a negative number, or generated automatically by means of the BUMP instruction. In combination with the SWAT instruction for diffuse solvent, BUMP provides a very effective way of handling solvent water in macromolecules, and is also useful in preventing unreasonably close contacts between protein molecules.

DFIX restraints with negative distance d are ignored if the two atoms are further from one another than |d| in the current refinement cycle; if they are closer than |d|, a restraint is applied to increase the distance to |d| with the given (or assumed) esd. The automatic generation of anti-bumping restraints includes all possible symmetry equivalents, and has been substantially enhanced since version 5.0 of SHELXTL. PART numbers are taken into account, and anti-bumping restraints are not applied if the sum of the occupancies of the two atoms is less than 1.1.

BUMP applies to all pairs of non-hydrogen atoms, provided that they are not linked by three or fewer bonds in the connectivity array. In addition, anti-bumping restraints are generated for all pairs of unreasonably close hydrogen atoms that are not bonded to the same atom. This discourages energetically unfavorable side-chain rotamers. If the BUMP esd is given as negative, the symmetry equivalents of bonds in the connectivity array are taken into account in applying the above rules, otherwise all short distances to symmetry generated atoms are potentially repulsive. The (default) positive esd action is usually the appropriate action for macromolecules, and prevents symmetry equivalents of one side-chain wandering too close to another, irrespective of whether spurious bonds between them have been (automatically) generated in the connectivity array. In contrast to version 5.0 of SHELXTL, the anti-bumping restraints are now regenerated each cycle.

The BUMP instruction also outputs a list of bonds and 1,3-distances in the connectivity array that have not been restrained in any way; this is a good way to detect spurious bonds and errors and omissions in the restraints. In some cases the lack of restraints is of course intentional, in which case the warnings can be ignored (e.g., for bonds involving metal atoms in a protein).

10.4 Restraints on Anisotropic Displacement Parameters

Three different types of restraint may be applied to U_{ij} values. DELU applies a *rigid-bond* restraint to U_{ij}-valus of two bonded (or 1,3-) atoms; the anisotropic displacement components of the two atoms along the line joining them are restrained to be equal. This restraint was suggested by Rollett (1970), and corresponds to the rigid-bond criterion for testing whether anisotropic displacement parameters are physically reasonable (Hirshfeld, 1976; Trueblood & Dunitz, 1983). Didisheim & Schwarzenbach (1987) have shown that in many but not all cases, rigid-bond restraints are equivalent to the TLS description of rigid body motion in the limit of zero esds; however this requires that (almost) all atom pairs are restrained in this way, which for molecules with conformational flexibility is unlikely to be appropriate. An extensive study (Irmer, 1990) has shown that the rigid bond condition is fulfilled within the experimental error for routine X-ray studies of bonds and 1,3-distances between two first-row elements (B to F inclusive), and so may be applied as a ‘hard’ restraint (low esd). A rigid bond restraint is not suitable for systems with unresolved disorder, e.g., AsF_{6-} anions and dynamic Jahn-Teller effects, although its failure may be useful in detecting such effects.

Isolated (e.g., solvent water) atoms may be restrained to be approximately isotropic, e.g., to prevent them going ‘non-positive-definite’; this is a rough approximation and so should be applied as a 'soft' restraint with a large esd (ISOR). Similarly the assumption of ‘similar’ U_{ij} values for spatially adjacent atoms (SIMU) causes the thermal ellipsoids to increase and change direction gradually going along a side-chain in a polypeptide, but this treatment is approximate and thus also appropriate only for a soft restraint; it is also useful for partially overlapping atoms of disordered groups. A simple way to apply SIMU to all such overlapping atoms (but not to others) is to give a SIMU instruction with no atoms (i.e., all atoms implied) and the third number set to a distance less than the shortest bond; additional SIMU restraints may be included in the same job. The default SIMU esd of 0.04 $Å^2$ is intended for anisotropic displacement parameters; SIMU may also be used for isotropic parameters (e.g., for refinement of a protein against 2 Å data) but in that slightly larger esd's, say 0.1 $Å^2$, might be more appropriate.

XL does not permit DELU, SIMU and ISOR restraints to reference symmetry-generated atoms, although this is allowed for all geometrical restraints. To permit such references for displacement parameter restraints as well would considerably complicate the program, and is rarely required in practice.

10.5 Non-crystallographic Symmetry Restraints

The new NCSY instruction provides a way of imposing *local non-crystallographic symmetry*. This is a very powerful restraint that holds remarkably well for many macromolecules, and it should be used whenever possible, especially when the resolution is not very high. The use of such restraints is slower than using NCS constraints (which involve performing a structure factor summation over just part of the structure, extending it to the whole structure by matrix operations) but has the advantage that no transformation matrix or real-space mask is required. The restraints make equivalent 1,4-distances (defined using the connectivity array) equal, and the isotropic *U*-values of equivalent atoms equal. Either of these restraints may be switched off, and any number of NCS domains may be defined. 1,2- and 1,3-distances are usually restrained using DFIX, DANG, SADI or SAME, so NCSY does not apply to them. The atoms to which NCS is applied are defined in a simple and flexible manner, so it is possible for example to leave out side-chains that deviate from NCS because they are involved in interaction with other (non-NCS related) molecules.

10.6 Shift Limiting Restraints

Shift limiting restraints (Watkin, 1994) may be applied in XL by the Marquardt (1963) algorithm. Terms proportional to a ‘damping factor’ (the first parameter on the DAMP instruction) are added to the least-squares matrix before inversion. Shift limiting restraints are particularly useful in the refinement of structures with a poor data to parameter ratio, and for pseudosymmetric problems. The 'damping factor' should be reduced towards the end of the refinement, otherwise the least-squares estimates of the esds in the less well determined parameters will be too low (the program does however make a first order correction to the esds for this effect). The shifts are also scaled down if the maximum shift/esd exceeds the second DAMP parameter. In addition, if the actual and target values for a particular restraint differ by more than 100 times the given esd, the program will temporarily increase the esd to limit the influence of this restraint to that produced by a discrepancy of 100 times the esd. This helps to prevent a bad initial model and tight restraints from causing dangerously large shifts in the first cycle.

10.7 Restraints on Linear Combinations of Free Variables

Constraints may be applied to atom coordinates, occupation and displacement parameters, and to restrained distances (DFIX) and chiral volumes (CHIV), by the use of free variables. Linear combinations of free variables may in turn be restrained (SUMP). This provides a way of restraining the sum of the occupancies of a multi-component disorder to be (say) unity and of restraining the occupancies to fit the charge balance and chemical analysis of a mineral with several sites occupied by a mixture of cations. In the latter case, the atoms occupying the same site will also usually be constrained (using EXYZ and EADP) to have the same positional and displacement parameters.

10.8 Examples of Restraints and Constraints

A major advantage of applying chemically reasonable restraints is that a subsequent difference electron density synthesis is often more revealing, because the parameters were not allowed to ‘mop up’ any residual effects. The refinement of pseudosymmetric structures, where the X-ray data may not be able to determine all of the parameters, is also considerably facilitated, at the cost of making it much easier to refine a structure in a space group of unnecessarily low symmetry!

By way of example, assume that the structure contains a cyclopentadienyl (Cp) ring π-bonded to a metal atom, and that as a result of the high thermal motion of the ring only three of the atoms could be located in a difference electron density map. We wish to fit a regular pentagon (default C-C 1.42 Å) in order to place the remaining two atoms, which are input as dummy atoms with zero coordinates. Since the C-C distance is uncertain (there may well be an appreciable librational shortening in such a case) we refine the C_5-ring as a *variable metric rigid group*, i.e., it remains a regular pentagon but the C-C distance is free to vary. In XL this may all be achieved by inserting one instruction (AFIX 59) before the five carbons and one (AFIX 0) after them:

```
AFIX 59                  ! AFIX mn with m = 5 to fit pentagon (default C-C
C1 1 .6755 .2289 .0763   ! 1.42 A) and n = 9 for v-m rigid-group refinement
C2 1 .7004 .2544 .0161
C3 1 0 0 0               ! the coordinates for C3 and C4 are obtained by the
C4 1 0 0 0               ! fit of the other 3 atoms to a regular pentagon
C5 1 .6788 .1610 .0766
AFIX 0                   ! terminates rigid group
```

Since U_{ij} values were not specified, the atoms would refine isotropically starting from U = 0.05. To refine with anisotropic displacement parameters in the same or a subsequent job, the instruction:

```
ANIS C1 > C5
```

should be inserted anywhere before C1 in the '.ins' file. The SIMU and ISOR restraints on the U_{ij} would be inappropriate for such a group, but:

```
DELU C1 > C5
```

could be applied if the anisotropic refinement proved unstable. The five hydrogen atoms could be added and refined with the 'riding model' by means of:

```
HFIX 43 C1 > C5
```

anywhere before C1 in the input file. For good data, in view of possible librational effects, a suitable alternative would be:

```
HFIX 44 C1 > C5
SADI 0.02 C1 H1 C2 H2 C3 H3 C4 H4 C5 H5
```

which retains a riding model but allows the C-H bond lengths to refine, subject to the restraint that they should be equal within about 0.02 Å.

In analogous manner, it is possible to generate missing atoms and perform rigid group refinements for phenyl rings (AFIX 66) and Cp* groups (AFIX 109). Very often it is possible and desirable to remove the rigid group constraints (by simply deleting the AFIX instructions) in the final stages of refinement; there is good experimental evidence that the *ipso*-angles of phenyl rings differ systematically from 120° (Jones, 1988; Maetzke & Seebach, 1989; Domenicano, 1992).

As a second example, assume that the structure contains two molecules of poorly defined THF solvent, and that we have managed to identify the oxygen atoms. A rigid pentagon would clearly be inappropriate here, except possibly for placing missing atoms, since THF molecules are not planar. However we can *restrain* the 1,2- and the 1,3-distances in the two molecules to be similar by means of a 'similarity restraint' (SAME). Assume that the molecules are numbered O11 C12 ... C15 and O21 C22 ... C25, and that the atoms are given in this order in the atom list. Then we can either insert the instruction:

```
SAME O21 > C25
```

before the first molecule, or:

```
SAME O11 > C15
```

before the second. These SAME instructions define a group of five atoms that are considered to be the same as the five (non-hydrogen) atoms which immediately follow the SAME instruction. The entries in the connectivity table for the latter are used to define the 1,2- and 1,3-distances, so the SAME instruction should be inserted before the group with the best geometry. This one SAME instruction restrains five pairs of 1,2- and five pairs of 1,3-distances to be nearly equal, i.e.,

```
d(O11-C12) = d(O21-C22),  d(C12-C13) = d(C22-C23),  d(C13-C14) = d(C23-C24),
d(C14-C15) = d(C24-C25),  d(C15-O11) = d(C25-O21),  d(O11-C13) = d(O21-C23),
d(C12-C14) = d(C22-C24),  d(C13-C15) = d(C23-C25),  d(C14-O11) = d(C24-O21),
and  d(C15-C12) = d(C25-C22).
```

In addition, it would also be reasonable to restrain the distances on opposite sides of the same ring to be equal. This can be achieved with one further SAME instruction in which we count the other way around the ring. For example we could insert:

```
SAME O11 C15 < C12
```

before the first ring. The symbol '<' indicates that one must count up the atom list instead of down. The above instruction is exactly equivalent to:

```
SAME O11 C15 C14 C13 C12
```

This generates 10 further restraints, but two of them [d(C13-C14) = d(C14-C13) and d(C12-C15) = d(C15-C12)] are identities and each of the others appears twice, so only four are independent and the rest are ignored. It is not necessary to add a similar instruction before the second ring, because the program also automatically generates all 'implied' restraints, i.e., restraints that can be derived by combining two existing distance restraints that refer to the same atom pair.

In contrast to other restraint instructions, the SAME instructions must be inserted at the correct positions in the atom list. These similarity restraints provide a very general and powerful way of exploiting non-crystallographic symmetry; in this example two instructions suffice to restrain the THF molecules so that they have (within an assumed standard deviation) twofold symmetry and are the same as each other. However we have not imposed planarity on the rings nor restricted any of the torsion angles.

To complicate matters, let us assume that the two molecules are two alternative conformations of a THF molecule disordered on a single site. We must then ensure that the site occupation factors of the two molecules add to unity, and that no spurious bonds linking them are added to the connectivity table. The former is achieved by employing site occupation factors of 21 (i.e., 1 times free-variable 2) for the first molecule and -21 {i.e., 1 times [1-fv(2)] } for the five atoms of the second molecule. Free variable 2 is then the occupation factor of the first molecule; its starting value must be specified on the FVAR instruction. The possibility of spurious bonds is eliminated by inserting 'PART 1' before the first molecule, 'PART 2' before the second, and 'PART 0' after it. Hydrogen atoms can be inserted in the usual way using the HFIX instruction since the connectivity table is 'correct'; they will automatically be assigned the site occupation factors of the atoms to which they are bonded.

Finally we would like to refine with anisotropic displacement parameters because the thermal motion of such solvent molecules is certainly not isotropic, but the refinement will be unstable unless we restrain the anisotropic displacement parameters to behave 'reasonably' by means of rigid bond restraints (DELU) and 'similar U_{ij}' restraints (SIMU); fortunately the program can set up these restraints automatically. DELU restrains the differences in the components of the displacement parameters of two atoms to zero along the 1,2- and 1,3-vector directions; these restraints are derived automatically with the help of the connectivity table. Since the SIMU restraints are much more approximate, we restrict them here to atoms which, because of the disorder, are almost overlapping (i.e., are within 0.7 Å of each other). Note that the SIMU restraints ignore the connectivity table and are based directly on a distance criterion specifically because the connectivity table does not link the disordered atoms. In order to specify a non-standard distance cut-off which is the third SIMU parameter, we must also give the first two parameters, which are the restraint esds for distances involving non-terminal atoms (0.02) and at least one terminal atom (0.04) respectively.

The *.ins* file now contains:

```
HFIX 23 C12 > C15 C22 > C25
ANIS O11 > C25
DELU O11 > C25
SIMU O11 > C25 0.04 0.08 0.7
FVAR ..... 0.75
....
PART 1
SAME O21 > C25
SAME O11 C15 < C12
O11 4 ..... ..... ..... 21
C12 1 ..... ..... ..... 21
C13 1 ..... ..... ..... 21
C14 1 ..... ..... ..... 21
C15 1 ..... ..... ..... 21
PART 2
O21 4 ..... ..... ..... -21
C22 1 ..... ..... ..... -21
C23 1 ..... ..... ..... -21
C24 1 ..... ..... ..... -21
C25 1 ..... ..... ..... -21
PART 0
```

An alternative type of disorder common for THF molecules and proline residues in proteins is when one atom (say C14) can flip between two positions (i.e., it is the flap of an envelope conformation). If we assign C14 to PART 1, C14' to PART 2, and the remaining ring atoms to PART 0, then the program will be able to generate the correct connectivity, and so we can also generate hydrogen atoms for both disordered components (with AFIX, not HFIX):

```
SIMU C14 C14'
ANIS O11 > C14'
FVAR ..... 0.7
....
SAME O11 C12 C13 C14' C15
O11 4 ..... ..... .....
C12 1 ..... ..... .....
AFIX 23
H12A 2 ..... ..... .....
H12B 2 ..... ..... .....
AFIX 0
C13 1 ..... ..... .....
PART 1
AFIX 23
H13A 2 ..... ..... ..... 21
H13B 2 ..... ..... ..... 21
PART 2
AFIX 23
H13C 2 ..... ..... ..... -21
H13D 2 ..... ..... ..... -21
AFIX 0
PART 1
C14 1 ..... ..... ..... 21
AFIX 23
H14A 2 ..... ..... ..... 21
H14B 2 ..... ..... ..... 21
AFIX 0
PART 0
C15 1 ..... ..... .....
PART 1
AFIX 23
H15A 2 ..... ..... ..... 21
H15B 2 ..... ..... ..... 21
PART 2
AFIX 23
H15C 2 ..... ..... ..... -21
H15D 2 ..... ..... ..... -21
AFIX 0
C14' 1 ..... ..... ..... -21
AFIX 23
H14C 2 ..... ..... ..... -21
H14D 2 ..... ..... ..... -21
AFIX 0
PART 0
```

It will be seen that six hydrogens belong to one conformation, six to the other, and two are common to both. The generation of the idealized hydrogen positions is based on the connectivity table but also takes the PART numbers into account. These procedures should be able to set up the correct hydrogen atoms for all cases of two overlapping disordered groups. In cases of more than two overlapping groups the program will usually still be able to generate the hydrogen atoms correctly by making reasonable assumptions when it finds that an atom is 'bonded' to atoms with different PART numbers, but it is possible that there are rare examples of very complex disorder which can only be handled by using dummy atoms constrained (EXYZ and EADP) to have the same positional and displacement parameters as atoms with different PART numbers (in practice it may be easier - and quite adequate - to ignore hydrogens except on the two components with the highest occupancies!).

When the site symmetry is high, it may be simpler to apply similarity restraints using SADI or DFIX rather than SAME. For example the following three instruction sets would all restrain a perchlorate ion (CL,O1,O2,O3,O4) to be a regular tetrahedron:

```
SAME CL O2 O3 O4 O1
SADI  O1 O2  O1 O3
```

followed immediately by the atoms CL, O1... O4; the SAME restraint makes all the Cl-O bonds equal but introduces only FOUR independent restraints involving the O...O distances, which allows the tetrahedron to distort retaining only one $\bar{4}$ axis, so one further restraint must be added using SADI.

or:

```
SADI  CL O1  CL O2  CL O3  CL O4
SADI  O1 O2  O1 O3  O1 O4  O2 O3  O2 O4  O3 O4
```

or:

```
DFIX 31  CL O1  CL O2  CL O3  CL O4
DFIX 31.6330  O1 O2  O1 O3  O1 O4  O2 O3  O2 O4  O3 O4
```

In the case of DFIX, one extra least-squares variable (free variable 3) is needed, but it is the mean Cl-O bond length and refining it directly means that its esd is also obtained. If the perchlorate ion lies on a three-fold axis through CL and O1, the SADI method would require the use of symmetry equivalent atoms (EQIV $1 y, z, x and O2_$1 etc. for R3 on rhombohedral axes) so DFIX would be simpler (same DFIX instructions as above with distances involving O3 and O4 deleted) [the number 1.6330 in the above example is of course twice the sine of half the tetrahedral angle].

If you wish to test whether you have understood the full implications of these restraints, try the following problems:

(a) A C-O-H group is being refined with AFIX 87 so that the torsion angle about the C-O bond is free. How can we restrain it to make the 'best' hydrogen-bond to a specific Cl- ion, so that the H...Cl distance is minimized and the O-H...Cl angle maximized, using only one restraint instruction (it may be assumed that the initial geometry is reasonably good)?

(b) Restrain a C_6 ring to an ideal chair conformation using one SAME and one SADI instruction. Hint: all 1-2, 1-3 and 1-4 distances are respectively equal for a chair conformation, which also includes a regular planar hexagon as a special case. A non-planar boat conformation does not have equal 1-4 distances. To force the ring to be non-planar, the ratio of the 1-2 and 1-3 distances would have to be restrained using DFIX and a free variable.

11 Refinement of Twinned Structures; Absolute Structure

A typical definition of a twinned crystal is the following: “Twins are regular aggregates consisting of crystals of the same species joined together in some definite mutual orientation” (Giacovazzo,1992). So for the description of a twin two things are necessary: a description of the orientation of the different species relative to each other (twin law) and the fractional contribution of each component. The *twin law* can be expressed as a matrix that transforms the *hkl* indices of one species into the other.

11.1 Twin Refinement Method

In XL the twin refinement method of Pratt, Coyle & Ibers (1971) and Jameson, Schneider, Dubler & Oswald (1982) has been implemented. F_c^2 values are calculated by:

$$(F_c^2)^* = \mathrm{osf}^2 \sum_{m=1}^{n} k_m F_{c_m}^2$$

where osf is the overall scale factor, k_m is the fractional contribution of twin domain m and F_{cm} is the calculated structure factor of twin domain m. The sum of the fractional contributions k_m must be unity, so (n-1) of them can be refined and k_1 is calculated by:

$$k_1 = 1 - \sum_{m=2}^{n} k_m$$

In XL two kinds of twins are distinguished:

(a) For twins in which the reciprocal lattices exactly coincide (twinning by merohedry or pseudo-merohedry), the procedure is relatively simple. The command TWIN r_{11} r_{12} r_{13} r_{21} r_{22} r_{23} r_{31} r_{32} r_{33} n defines the twin law. **R** as the matrix that transforms the hkl indices of one component into the other and n is the number of twin domains. **R** is applied (n-1) times; the default value of n is 2.

(b) In cases where only some reflections have contributions from more than one domain (non-merohedral twins or twinning by reticular merohedry) the *.hkl* file must be edited and the index transformations applied to individual contributors, which are also assigned component numbers. The code HKLF 5 is used to read in this file; no TWIN command should be used.

In both cases, starting values of the fractional contributions are input with the instruction BASF k_2 ... k_n; the k_m values will be refined. Note that (in the new version of XL) linear restraints may be applied to these k values by means of SUMP instructions; this can be very useful to prevent instabilities in the early stages of refinement. For this purpose k_2...k_n are assigned parameter numbers immediately following the free variables.

11.2 Absolute Structure

Even if determination of absolute configuration is not one of the aims of the structure determination, it is important to refine **every** non-centrosymmetric structure as the correct *absolute structure* in order to avoid introducing systematic errors into the bond lengths etc. In some cases the absolute structure will be known with certainty (e.g., proteins), but in others it has to be deduced from the X-ray data. Generally speaking, a single phosphorus or heavier atom suffices to determine an absolute structure using Cu-Kα radiation, and with accurate high-resolution low-temperature data including Friedel opposites such an atom may even suffice for Mo-Kα.

In the course of the final structure factor calculation, the program estimates the absolute structure parameter *x* (Flack, 1983) and its esd. *x* is the fractional contribution of the inverted component of a 'racemic twin'; it should be zero if the absolute structure is correct, unity if it has to be inverted, and somewhere between 0 and 1 if racemic twinning is really present. Thus the above formulas apply with n=2 and **R** = (-1 0 0, 0 -1 0, 0 0 -1).

It is a bonus of the refinement against F^2 that this calculation is a 'hole in one' and does not require expensive iteration. A comparison of *x* with its esd provides an indication as to whether the refined absolute structure is correct or whether it has to be 'inverted'; the program prints a suitable warning should this be necessary. This attempt to refine *x* 'on the cheap' is reliable when the true value of *x* is close to zero, but may produce a (possibly severe) underestimate of *x* for structures which have to be inverted, because *x* is correlated with positional and other parameters which have not been allowed to vary. Effectively these parameters have adapted themselves to compensate for the wrong (zero) value of *x* in the course of the refinement, and need to be refined with *x* to eliminate the effects of correlation. These effects will tend to be greater when the correlation terms are greater, e.g., for polar space groups and for poor data to parameter ratios (say less than 8:1). *x* can be refined at the same time as all the other parameters using the TWIN instruction with the default matrix **R** = (-1 0 0, 0 -1 0, 0 0 -1) and BASF with one parameter (*x*); this implies racemic twinning and so is refined exactly as for other simple cases of twinning. Refinement of racemic twinning should normally only be attempted towards the end of the refinement after all non-hydrogen atoms have been located. If racemic twinning is refined in this way, the automatic calculation of the Flack x parameter in the final structure factor cycle is suppressed, since the BASF parameter is x.

For most space groups 'inversion' of the structure simply involves inserting an instruction 'MOVE 1 1 1 -1' before the first atom. Where the space group is one of the 11 enantiomorphous pairs [e.g., $P3_1$ and $P3_2$] the translation parts of the symmetry operators need to be inverted as well to generate the other member of the pair. There are seven cases for which, if the standard setting of the International Tables for Crystallography has been used, inversion in the origin does **not** lead to the inverted absolute structure. This problem was probably first described in print by Parthe & Gelato (1984) and Bernardinelli & Flack (1985), but had been investigated previously by D. Rogers (personal communication to GMS, ca. 1980).

The offending space groups and corresponding correct MOVE instructions are:

Fdd2	`MOVE .25 .25 1 -1`
$I4_1$	`MOVE 1 .5 1 -1`
$I4_122$	`MOVE 1 .5 .25 -1`
$I4_1md$	`MOVE 1 .5 1 -1`
$I4_1cd$	`MOVE 1 .5 1 -1`
$I\bar{4}_{2d}$	`MOVE 1 .5 .25 -1`
$F4_132$	`MOVE .25 .25 .25 -1`

11.3 Refinement Against Powder Data

Refinement of twinned crystals and refinement against F^2-values derived from powder data are similar in that several reflections with different indices may contribute to a single F^2 observation. For powder data this requires some small adjustments to the format of the *.hkl* file; the batch number becomes the multiplicity m, and where several reflections contribute to the same observation the multiplicity is made positive for the last reflection in the group and negative for the rest.

Although XL may be useful for some high symmetry and hence reasonably well resolved powder and fibre diffraction patterns - the various restraints and constraints should be exploited in full to make up for the poor data/parameter ratio - for normal powder data a Rietveld refinement program would be much more appropriate.

For powder data the least-squares refinement fits the overall scale factor (osf^2 where osf is given on the FVAR instruction) times the multiplicity weighted sum of calculated intensities to F_o^2:

$$(F_c^2)^* = osf^2 [m_1 F_{c1}^2 + m_2 F_{c2}^2 + m_3 F_{c3}^2 + ...]$$

where the multiplicities of the contributors are given in the place of the batch numbers in the *.hkl* file. Since it is then not possible to define batch numbers as well, BASF cannot be used with powder data.

11.4 Frequently Encountered Twin Laws

The following cases are relatively common:

(a) Twinning by merohedry. The lower symmetry trigonal, tetragonal, hexagonal or cubic Laue groups may be twinned so that they look (more) like the corresponding higher symmetry Laue groups (assuming the c-axis unique except for cubic):

```
TWIN  0 1 0  1 0 0  0 0 -1
```

plus one BASF parameter if the twin components are not equal in scattering power. If they are equal, i.e., the twinning is perfect, as indicated by the R_{int} for the higher symmetry Laue group, then the BASF instruction can be omitted and k_1 and k_2 are fixed at 0.5.

(b) Orthorhombic with **a** and **b** approximately equal in length may emulate tetragonal:

```
TWIN  0 1 0  1 0 0  0 0 -1
```

plus one BASF parameter for unequal components.

(c) Monoclinic with beta approximately 90° may emulate orthorhombic:

```
TWIN  1 0 0  0 -1 0  0 0 -1
```

plus one BASF parameter for unequal components.

(d) Monoclinic with **a** and **c** approximately equal and beta approximately 120 degrees may emulate hexagonal [$P2_1/c$ would give absences and possibly also intensity statistics corresponding to $P6_3$]. There are three components, so n must be specified on the TWIN instruction and the matrix is applied once to generate the indices of the second component and twice for the third component. In German this is called a ‘Drilling’ as opposed to a ‘Zwilling’ (with two components):

```
TWIN  0 0 1  0 1 0  -1 0 -1  3
```

plus TWO BASF parameters for unequal components. If the data were collected using an hexagonal cell, then an HKLF matrix would also be required to transform them to a setting with b unique:

```
HKLF  4  1  1 0 0  0 0 1  0 -1 0
```

(e) Rhombohedral obverse/reverse twinning on hexagonal axes.

```
TWIN -1 0 0  0 -1 0  0 0 1
```

11.5 Combined General and Racemic Twinning

If general and racemic twinning are to be refined simultaneously, n (the last parameter on the TWIN instruction) should be doubled and given a negative sign, and there should be |n|-1 BASF twin component factors (or none, in the unlikely event that all are to be fixed as equal). The inverted components follow those generated using the TWIN matrix, in the same order. Sometimes it is necessary to use this approach to distinguish between possible twin laws for non-centrosymmetric structures, when they differ only in an inversion operator In a typical example (an organocesium compound), when the TWIN instruction was input as:

```
TWIN 0 1 0  1 0 0  0 0 -1  -4
```

The BASF parameters refined to:

```
BASF 0.33607 0.00001 0.00455
```

Which means that the last two components (the ones involving inversion) can be ignored, and the final refinement performed with the '-4' deleted from the end of the TWIN instruction, and a single BASF parameter. The introduction of twinning reduced the *R*1-value from 18% to 1.8% in this example. Note that the program does not allow the BASF parameters to become negative, since this would be physically meaningless (this explains the 0.00001 above).

11.6 Processing of Twinned and Powder Data

The HKLF 5 and 6 instructions force MERG 0, i.e., neither a transformation of reflection indices into a standard form nor a sort-merge is performed before refinement. If twinning is specified using the TWIN instruction, any MERG instruction may be used and the default remains MERG 2. Although this is always safe for racemic twinning, there may be other forms of twinning for which it is not permissible to sort-merge first. Whether or not MERG is used, the program ignores all systematically absent contributions, with the result that a reflection is excluded from the data if it is systematically absent for all components.

For both powder (HKLF 6) and twinned data (HKLF 5 or TWIN with HKLF 4), the reflection data are reduced to the 'prime' component, by multiplying F_o^2 by the ratio of the F_c^2 for the prime reflection divided by the total F_c^2, before performing the analysis of variance and the Fourier calculations. Similarly 'OMIT h k l' refers to the indices of the prime component. The prime component is the one for which the indices have not been transformed by the TWIN instruction (i.e., m = 1), or in the case of HKLF 5 or HKLF 6 the component given with positive m (i.e., the last contributor to a given intensity measurement, not necessarily the one with |m| = 1).

11.7 The Warning Signs for Twinning

Experience shows that there are a number of characteristic warning signs for twinning. Of course not all of them can be present in any particular example, but if one finds several of them the possibility of twinning should be given serious consideration.

(a) The metric symmetry is higher than the Laue symmetry.

(b) The R_{int}-value for the higher symmetry Laue group is only slightly higher than for the lower symmetry Laue group.

(c) The mean value for $|E^2-1|$ is much lower than the expected value of 0.736 for the non-centrosymmetric case. If we have two twin domains and every reflection has contributions from both, it is unlikely that both contributions will have very high or that both will have very low intensities, so the intensities will be distributed so that there are fewer extreme values.

(d) The space group appears to be trigonal or hexagonal.

(e) There are impossible or unusual systematic absences.

(f) Although the data appear to be in order, the structure cannot be solved.

(g) The Patterson function is physically impossible.

The following points are typical for non-merohedral twins, where the reciprocal lattices do not overlap exactly and only some of the reflections are affected by the twinning:

(h) There appear to be one or more unusually long axes, but also many absent reflections.

(i) There are problems with the cell refinement.

(j) Some reflections are sharp, others split.

(k) K = mean(F_o^2) / mean(F_c^2) is systematically high for the reflections with low intensity.

(l) For all of the 'most disagreeable' reflections, F_o is much greater than F_c.

11.8 Conclusions

Twinning usually arises for good structural reasons. When the heavy atom positions correspond to a higher symmetry space group, it may be difficult or impossible to distinguish between twinning and disorder of the light atoms; see Hoenle & von Schnering (1988). Since refinement as a twin usually requires only two extra instructions and one extra parameter, in such cases it should be attempted first, before investing many hours in a detailed interpretation of the ‘disorder’! Indeed, it has been suggested by G.B. Jameson that all structures (including proteins) that are solved in space groups (such as $P3_1$) that could be merohedrally twinned without changing the systematic absences should be tested for such twinning (possible only present to a minor extent) by:

```
TWIN 0 1 0  1 0 0  0 0 -1
BASF 0.1
```

Refinement of twinned crystals often requires the full arsenal of constraints and restraints, since the refinements tend to be less stable, and the effective data to parameter ratio may well be low. In the last analysis chemical and crystallographic intuition may be required to distinguish between the various twinned and disordered models, and it is not easy to be sure that all possible interpretations of the data have been considered.

We would like to thank Regine Herbst-Irmer, who wrote most of this chapter.

12 XL Instruction Summary

This chapter lists the instructions that may be used in the .ins file for XL. Defaults are given in square brackets; '#' indicates that the program will generate a suitable default value based on the rest of the available information. Continuation lines are flagged by '=' at the end of a line, the instruction being continued on the next line which must start with one or more spaces. Other lines beginning with spaces are treated as comments, so blank lines may be added to improve readability. All characters following '!' or '=' in an instruction line are ignored.

The .ins file may include an instruction of the form: +filename (the '+' character MUST be in column 1). This causes further input to be taken from the named file until an END instruction is encountered in that file, whereupon the file is closed and instructions are taken from the next line of the .ins file. The input instructions from such an 'include' file are not echoed to the .lst and .res file, and may NOT contain FVAR, BASF, EXTI or SWAT instructions or atoms (except inside a FRAG...FEND section) since this would prevent the .res file from being used unchanged for the next refinement job (after renaming as .ins).

The '+filename' facility enables standard fragment coordinates or long lists of restraints etc. to be read from the same files for each refinement job, and for different structures to access the same fragment or restraint files. One could also for example store the LATT and SYMM instructions for different space groups, or neutron scattering factors for particular elements, or LAUE instructions followed by wavelength-dependent scattering factors, in suitably named files. Since these 'include' files are not echoed, it is a good idea to test them as part of an *.ins* file first, to check for possible syntax errors. Such 'include' files may be nested; the maximum allowed depth depends upon the operating system and compiler used.

12.1 Crystal Data and General Instructions

12.1.1 TITL [ ]

Title of up to 76 characters, to appear at suitable places in the output. The characters '!' and '=', if present, are part of the title and are not specially interpreted.

12.1.2 CELL λ a b c α β γ

Wavelength and unit-cell dimensions in Å and degrees.

12.1.3 ZERR Z esd(a) esd(b) esd(c) esd(α) esd(β) esd(γ)

Z value (number of formula units per cell) followed by the estimated standard deviations in the unit-cell dimensions. Z is only required for the CIF output; the cell esds contribute to the estimated esds in bond lengths etc. after full-matrix refinement.

12.1.4 LATT N[1]

Lattice type: 1=P, 2=I, 3=rhombohedral obverse on hexagonal axes, 4=F, 5=A, 6=B, 7=C. N must be made negative if the structure is non-centrosymmetric.

12.1.5 SYMM symmetry operation

Symmetry operators, i.e., coordinates of the general positions as given in International Tables. The operator x, y, z is always assumed, so MUST NOT be input. If the structure is centrosymmetric, the origin MUST lie on a center of symmetry. Lattice centering and the presence of an inversion center should be indicated by LATT, not SYMM. The symmetry operators may be specified using decimal or fractional numbers, e.g., 0.5-x, 0.5+y, -z or Y-X, -X, Z+1/6; the three components are separated by commas.

12.1.6 SFAC elements

Element symbols which define the order of scattering factors to be employed by the program. The first 94 elements of the periodic system are recognized. The element name may be preceded by '$' but this is not obligatory (the '$' character is allowed for logical consistency but is ignored). The program uses the neutral atom scattering factors, f', f" and absorption coefficients from International Tables for Crystallography, Volume C (1992), Ed. A.J.C. Wilson, Kluwer Academic Publishers, Dordrecht: Tables 6.1.1.4(pp. 500-502), 4.2.6.8 (pp. 219-222) and 4.2.4.2 (pp. 193-199) respectively. The covalent radii stored in the program are based on experience rather than taken from a specific source, and are deliberately overestimated for elements which tend to have variable coordination numbers so that 'bonds' are not missed, at the cost of generating the occasional 'non-bond'. The default radii (not those set for individual atoms by CONN) are printed before the connectivity table.

12.1.7 SFAC label a1 b1 a2 b2 a3 b3 a4 b4 c f' f" mu r wt

Scattering factor in the form of an exponential series, followed by real and imaginary dispersion terms, linear absorption coefficient, covalent radius and atomic weight. Except for the 'label' and atomic weight the format is the same as that used in version 4 of SHELXTL. label consists of up to 4 characters beginning with a letter (e.g., Ca2+) and should be included before a1; for consistency the first label character may be a '$', but this is ignored (note however that the '$', if used, counts as one of the four characters, leaving only three for the rest of the label). The two SFAC formats may be used in the same *.ins* file; the order of the SFAC instructions (and the order of element names in the first type of SFAC instruction) define the scattering factor numbers which are referenced by atom instructions. The units of mu should be barns/atom, as in Table 4.2.4.2 of International Tables, Volume C (see above). For neutrons this format should be used, with a1...b4 set to zero.

Hydrogen atoms are treated specially by XL; they are recognized by having the scattering factor number that corresponds to 'H' on the SFAC instruction. For X-ray structures that contain both D and H, e.g., because the crystals were grown from a deuterated solvent in an n.m.r tube (a common source of good crystals!), both H and D should be included on the SFAC and UNIT instructions, but all the H and D atoms should employ the 'H' scattering factor number. In this way the density will be calculated correctly, but the D atoms may be idealized using HFIX etc.

12.1.8 DISP E f' f" [#] mu [#]

The DISP instruction allows the dispersion and (optionally) the absorption coefficient of a particular element (the name may be optionally prefaced by '$') to be read in without having to use the full form of the SFAC instruction. It will typically be used for synchrotron data where the wavelength does not correspond to the values (for Cu, Mo and Ag radiation) for which these terms are stored in the program. All other terms on the SFAC instruction are independent of the wavelength, so its short form may then be used. DISP instructions, if present, MUST come between the last SFAC and the UNIT instruction.

12.1.9 UNIT n1 n2 ...

Number of atoms of each type in the unit-cell, in SFAC order.

12.1.10 LAUE E

Wavelength-dependent values of f' and f" may be defined for an element E by means of the LAUE instruction, which is used in conjunction with the HKLF 2 reflection data format (in which the wavelength is given separately for each reflection). This is primarily intended for refinement of structures against Laue data collected using synchrotron radiation, but could also be used for refinement of a structure using data collected at different wavelengths for which some of the dispersion terms are significant (e.g., MAD data for macromolecules). There is no provision for handling overlapping reflection orders, and scaling for the source intensity distribution and Lp, absorption corrections etc. must have been performed before using XL. A dummy wavelength of say 0.7 Å should be given on the CELL instruction, and the absorption coefficient estimated by the program should be ignored.

The element symbol may be preceded by '$' but this is optional; it must be followed by at least one blank or the end of the line. Any remaining information on the LAUE instruction line is ignored. The line immediately following the LAUE instruction is always ignored, and so may be used for headings. The following lines contain values of wavelength (in Å), f' and f" in FORMAT(F7.3,2F8.3); further information (e.g., the absorption coefficient μ) may follow on the same line but will be ignored. The wavelength values must be in ascending order and will be linearly interpolated; the wavelength intervals do not need to be equal (but it is more efficient if most of them are) and should indeed be smaller in the region of an absorption edge. This list is terminated by a record in which all three values are given as zero. There should only be one LAUE instruction for each element type; if a reflection wavelength is outside the range specified, the constant f' and f" values defined by the corresponding SFAC instruction are used instead.

A LAUE instruction must be preceded by (normal) SFAC and UNIT instructions referencing the elements in question, and by all atoms. Thus the LAUE instruction(s) are usually the last instructions before HKLF 2 (or -2) at the end of the *.ins* file (which facilitates editing). The +filename construction may conveniently be used to read long LAUE tables from 'include' files without echoing them.

12.1.11 REM

Followed by a comment on the same line. This comment is copied to the results file (*.res*). A line beginning with at least one blank may also be used as a comment, but such comments are only copied to the *.res* file if the line is completely blank; REM comments are always copied. Comments may also be included on the same line as any instruction following the character '!', and are copied to the *.res* file (except in the case of atoms and FVAR, EXTI, SWAT and BASF instructions).

12.1.12 MORE m [1]

MORE sets the amount of (printer) output; m takes a value in the range 0 (least) to 3 (most verbose). MORE 0 also suppresses the echoing to the *.lst* file of any instructions or atoms which follow it (until the next MORE instruction).

12.1.13 TIME t [#]

If the time t (measured in seconds from the start of the job) is exceeded, XL performs no further least-squares cycles, but goes on to the final structure factor calculation followed by bond lengths, Fourier calculations etc. The default value of t is installation dependent, and is either set to 'infinity' or to a little less than the maximum time allocation for a particular class of job. Usually t is 'CPU time', but some some operating systems (e.g., MS-DOS) the elapsed time may have to be used instead.

12.1.14 END

END is used to terminate an 'include' file, and may also be included after HKLF in the *.ins* file (for compatibility with version 4 of SHELXTL).

12.2 Reflection Data Input

Before running XL, a reflection data file *name.hkl* must have been prepared. The HKLF command tells the program which format has been chosen for this file, and allows the indices to be transformed using the 3×3 matrix $r_{11}...r_{33}$, so that the new *h* is $r_{11}*h + r_{12}*k + r_{13}*l$ etc. The program will not accept matrices with negative or zero determinants. It is essential that the cell, symmetry and atom coordinates in the *.ins* file correspond to the indices AFTER transformation using this matrix.

12.2.1 HKLF n[0] s[1] r11...r33[1 0 0 0 1 0 0 0 1] wt[1] m[0]

n is negative if reflection data follow, otherwise they are read from the *.hkl* file. The data are read in FORMAT(3I4,2F8.2,I4) (except for |n| < 3) subject to FORTRAN-77 conventions. The data are terminated by a record with *h*, *k* and *l* all zero (except |n| = 1, which contains a terminator and a checksum). In the reflection formats given below, BN stands for batch number. If BN is greater than one, F_c is multiplied by the (BN-1)'th coefficient specified by means of BASF instructions (see below). If BN is zero or absent, it is reset to one. The multiplicative scale s multiplies both F_o^2 and $\sigma(F_o^2)$ (or F_o and $\sigma(F_o)$ for n = 1 or 3). The multiplicative weight wt multiplies all $1/\sigma^2$ values and m is an integer 'offset' needed to read 'condensed data' (HKLF 1); both are included for compatibility with version 4 of SHELXTL. Negative n is also only retained for upwards compatibility; it is much better to keep the reflection data in the *name.hkl* file, otherwise the data can easily get lost when editing *name.res* to *name.ins* for the next job.

n = 1: SHELX-76 condensed data (BN is set to one). 'Condensed data' impose unnecessary index restrictions and can introduce rounding errors; although they still have their uses (email!), XL cannot generate condensed data and their use is discouraged.

n = 2: *h k l* F_o^2 $\sigma(F_o^2)$ BN [1] λ [#] in FORMAT(3I4,2F8.2,I4,F8.4) for refinement based on singlet reflections from Laue photographs. The data are assumed to be scaled for source intensity distribution and geometric factors and (if necessary) corrected for absorption. If λ is zero or absent the value from the CELL instruction is used. n = 2 switches off the merging of equivalent reflections BEFORE l.s. refinement (i.e., sets MERG 0); equivalents and measurements of the same reflections at different wavelengths are merged after least-squares refinement and the subsequent application of a dispersion correction, but before Fourier calculations.

The remaining options (n > 2) all require FORMAT(3I4,2F8.2,I4); other compatible formats (e.g., F8.0 or even I8) may be used for the floating point numbers provided that eight columns are used in all and a decimal point is present.

n = 3: *h k l* F_o $\sigma(F_o)$ BN [1] (if BN is absent or zero it is set to 1).The use of data corresponding to this format is allowed but is NOT RECOMMENDED, since the generation of F_o and $\sigma(F_o)$ from F_o^2 and $\sigma(F_o^2)$ is a tricky statistical problem and could introduce bias.

n = 4: *h k l* F_o^2 $\sigma(F_o^2)$ BN [1] is the standard reflection data file. Since F_o^2 is obtained as the difference of the experimental peak and background counts, it may be positive or slightly negative. BN may be made negative (e.g., by XPRO) to flag a reflection for inclusion in the R_{free} reference set (see CLGS and L.S. with a second parameter of -1).

n = 5: *h k l* F_o^2 $\sigma(F_o^2)$ m where m is the twin component number. Each measured F_o^2 value is fitted to the sum of $k_{|m|}F_{c|m|}^2$ over all contributing components, multiplied by the overall scale factor. m should be given as positive for the last contributing component and negative for the remaining ones (if any). The values of F_o^2 and $\sigma(F_o^2)$ are taken from the last ('prime') reflection in a group, and may simply be set equal for each component, but the indices *h,k,l* will in general take on different values for each component. The starting values of the twin factors $k_2..k_{max(m)}$ are specified on BASF instruction(s); k_1 is given by one minus the sum of the other twin factors. Note that many simple forms of twinning can also be handled with HKLF 4 and a TWIN instruction to generate the indices of the remaining twin component(s); HKLF 5 is required if the reciprocal space lattices of the components cannot be superimposed exactly. HKLF 5 sets MERG 0, and may not be used with TWIN.

n = 6: *h k l* F_o^2 $\sigma(F_o^2)$ m as for n = 5, there may be one or more sets of reflection indices corresponding to a single F_o^2 value. The last reflection in a group has a positive m value and the previous members of the group have negative m. The values of F_o^2 and $\sigma(F_o^2)$ are taken from the last ('prime') reflection in a group, and may simply be set to the same values for the others. m is here the reflection MULTIPLICITY, and is defined as the number of equivalent permutations of the given *h, k* and *l* values, not counting Friedel opposites. This is intended for fitting resolved powder data for high symmetry crystal systems. For example, in a powder diagram of a crystal in the higher cubic Laue class (m3m) the reflections 3 0 0 (with multiplicity 3) and 2 2 1 (multiplicity 12) would contribute to the same measured F_o^2. HKLF 6 sets MERG 0. HKLF 6 may not be used with BASF or TWIN.

NOTE: THERE MAY ONLY BE ONE HKLF INSTRUCTION AND IT MUST COME LAST, except when HKLF -n is followed by reflection data in the *.ins* file, in which case the file is terminated by the end of the reflection data. Negative n is retained for compatibility with SHELX-76 but is not recommended!

12.2.2 OMIT s[-2] 2θ(lim)[180]

If s is given as negative, all reflections with $F_o^2 < 0.5s\sigma(F_o^2)$ are replaced by $0.5s\sigma(F_o^2)$; thus if no OMIT instruction is given the default action is to replace all F_o^2 values less than $-\sigma(F_o^2)$ by $-\sigma(F_o^2)$. If s is positive it is interpretated as a threshold for flagging reflections as 'unobserved'. Unobserved data are not used for least-squares refinement or Fourier calculations, but are retained for the calculation of *R*-indices based on all data, and may also appear (flagged with an asterisk) in the list of reflections for which F_o^2 and F_c^2 disagree significantly. Internally in the program s is halved and applied to F_o^2, so for positive F_o^2 the test is roughly equivalent to suppressing all reflections with $F_o < s\ \sigma(F_o)$, as required for consistency with SHELX-76. Note that s may be set to 0 or (as in the default setting) to a negative threshold (to modify very negative F_o^2). An OMIT instruction with a positive s value is NOT ALLOWED in combination with ACTA, because it may introduce a bias in the final refined parameters; individual aberrant reflections may still be suppressed using OMIT *h k l*, even when ACTA is used.

2θ(lim) defines a limiting 2θ above which reflections are totally ignored; they are rejected immediately on reading in. This facility may be used to save computer time in the early stages of structure refinement, and is also sometimes useful for macromolecules. The SHEL command may also be used to ignore reflections above or below particular limiting resolution values.

OMIT followed by atom names but no numbers may be used to calculate an 'omit map' and is described in the section 'Atom Lists ...'.

12.2.3 OMIT *h k l*

The reflection *h,k,l* (the indices refer to the standard setting after data reduction, and correspond to those in the list of ‘disagreeable reflections’ after refinement) is ignored completely. Since there may be perfectly justified reasons for ignoring individual reflections (e.g., when a reflection is truncated by the beam stop) this form of OMIT is allowed with ACTA; however it should not be used indiscriminately. If MERG N with non-zero N is employed (or the (default) MERG 2 is assumed), all reflections which would generate the final indices *h,k,l* are ignored; if MERG 0 is specified, the indices must match those in the input *.hkl* file exactly.

12.2.4 SHEL lowres[infinite] highres[0]

Reflections outside the specified resolution range in Å are ignored completely. This instruction may be useful for macromolecules.

12.2.5 BASF scale factors

Relative batch scale factors are included in the least-squares refinement based on the batch numbers in the *.hkl* file. For batch number BN, the F_c^2 value is multiplied by the (BN-1)'th scale factor from the BASF instruction, as well as by the overall scale factor. For batch number one (or zero), F_c is multiplied by the overall scale factor, but not by a batch scale factor. The least-squares matrix will be singular if there are no reflections with BN=1 (or zero), so the program considers this to be an error. Note that BASF scale factors, unlike the overall scale factor (see FVAR) are relative to F^2, not *F*. For twinned crystals, i.e., when either TWIN or HKLF 5 are employed, BASF specifies the fractional contributions of the various twin components. BASF parameters may also be used by the HOPE instruction. Except when they are used by HOPE, the program does not allow BASF parameters to become negative.

12.2.6 TWIN 3x3 matrix [-1 0 0 0 -1 0 0 0 -1] n[2]

n is the number of twin components (2 or greater) and the matrix is applied (iteratively if |n| > 2) to generate the indices of the twin components from the input reflection indices, which apply to the first (prime) component. If a transformation matrix is also given on the HKLF instruction, it is applied first before the (iterative) application of the TWIN matrix. This method of defining twinning allows the standard HKLF 4 format to be used for the *.hkl* file, but can only be used when the reciprocal lattices for all twinned components are metrically superimposable. In other cases HKLF 5 format must be used. The F_o^2 values are fitted to the sum of $k_{m*}F_{cm}^2$ multiplied by the overall scale factor, where k_1 is one minus the sum of k_2, k_3, .. and the starting values for the remaining twin fractions k_2, k_3, .. are specified on a BASF instruction. Only one TWIN instruction is allowed. If BASF is omitted the TWIN factors are all assumed to be equal (i.e., 'perfect' twinning).

If the racemic twinning is present at the same time as normal twinning, n should be doubled (because there are twice as many components as before) and given a negative sign (to indicate to the program that the inversion operator is to be applied multiplicatively with the specified TWIN matrix). The number of BASF parameters, if any, should be increased from m-1 to 2m-1 where m is the original number of components (equal to the new |n| divided by 2). The TWIN matrix is applied m-1 times to generate components 2 ... m from the prime reflection (component 1); components m+1 ... 2m are then generated as the Friedel opposites of components 1 ... m.

12.2.7 EXTI x[0]

An extinction parameter x is refined, where F_c is multiplied by:

$$k\,[\,1\,+\,0.001\,\times\,F_c^2\,\lambda^3\,/\,\sin(2\theta)\,]^{-1/4}$$

where k is the overall scale factor. Note that it has been necessary to change this expression from SHELX-76 (which used an even cruder approximation) and XLS in version 4 of SHELXTL version 4 (which used 0.002 instead of $0.001\lambda^3$). The wavelength dependence is needed for HKLF 2 (Laue) data. The program will print a warning if extinction (or SWAT - see below) may be worth refining, but it is not normally advisable to introduce it until all the non-hydrogen atoms have been found. For twinned and powder data, the F_c^2 value used in the above expression is based on the total calculated intensity summed over all components rather than the individual contributions, which would be easier to justify theoretically (but makes little difference in practice). For the analysis of variance and *.fcf* output file, the F_o^2 values are brought onto the absolute scale of F_c^2 by dividing them by the scale factor(s) and the extinction factor. The above expression for the extinction is empirical and represents a compromise to cover both primary and secondary extinction; it has been shown to work well in practice but does not appear to correspond exactly to any of the expressions discussed in the literature. The article by Larson (1970) comes closest and should be consulted for further information.

12.2.8 SWAT g[0] U[2]

The SWAT option allows two variables g and U to be refined in order to model diffuse solvent using Babinet's principle (Moews & Kretsinger, 1975; the same formula is employed in the program TNT, but the implementation is somewhat different). The calculated intensity is modified as follows:

$$F_c^2(\text{new}) = F_c^2(\text{old}) \,.\, (1 - g \,.\, \exp\,[\,-8\pi^2 U(\sin\theta\,/\,\lambda)^2\,]\,)$$

A large value of U ensures that only the low theta F_c^2 values are affected. Subtracting the term in g in this way from the occupied regions of the structure is equivalent to adding a corresponding diffuse scattering term in the (empty) solvent regions in its effect on all calculated F_c^2 values except F(000). For proteins g usually refines to a value between 0.7 and unity, and U usually refines to a value between 2 and 5; for small molecules without significant diffuse solvent regions g should refine to zero. Since g and U are correlated, it is better to start the diffuse solvent refinement by giving SWAT with no parameters; the program will then invent suitable starting values. Note that a different formula was employed in version 5.0 of SHELXTL, and so parameter values from verion 5.0 may well be unsuitable starting values for the new version.

Since both extinction and diffraction from diffuse solvent tend to affect primarily the strong reflections at low diffraction angle, they tend to show the same symptoms in the analysis of variance, and so a combined warning message is printed. It will however be obvious from the type of structural problem which of the two should be applied. The program does not permit the simultaneous refinement of SWAT and EXTI.

12.2.9 HOPE nh [1]

Refines 12 anisotropic scaling parameter as suggested by Parkin, Moezzi & Hope (1995). nh points to the BASF parameter that stores the value of the first HOPE parameter; if nb is negative the 12 parameters are fixed at their current values. These parameters are highly correlated with the individual atomic anisotropic displacement parameters, and so are only useful for structures that are refined isotropically , e.g., macromolecules at moderate resolution. To some extent they can also model absorption errors. If HOPE is given without any parameters and there are no BASF instructions, the program will generate appropriate starting values. If BASF parameters are needed for twin refinement or as scale factors for different batches of data, nh should be given an absolute value greater than one.

12.2.10 MERG n[2]

If n is equal to 2 the reflections are sorted and merged before refinement; if the structure is non-centrosymmetric the Friedel opposites are not combined before refinement (necessary distinction from XS). If n is 1 the indices are converted to a 'standard setting' in which *l* is maximized first, followed by *k*, and then *h*; if n is zero, the data are neither sorted nor converted to a standard setting. n = 3 is the same as n = 2 except that Friedel opposites are also merged (this introduces small systematic errors and should only be used for good reason, e.g., to speed up the early stages of a refinement of a light atom structure before performing the final stages with MERG 2). Note that the reflections are always merged, and Friedel opposites combined, before performing Fourier calculations in XL so that the (difference) electron density is real and correctly scaled. Even with n = 0 the program will

change the reflection order within each data block to optimize the vectorization of the structure factor calculations (it is shuffled back into the MERG order for LIST 4 output). Note that MERG may not be used in conjunction with TWIN or HKLF 5 or 6. In version 4 of SHELXTL, MERG 3 had a totally different meaning, namely the determination of inter-batch scale factors; in XL, these may be included in the refinement using the BASF instruction.

MERG 4 averages all equivalents, including Friedel opposites, and sets all δf" values to zero; it is often used in refinement of macromolecules.

12.3 Atom Lists and Least-squares Constraints

Atom instructions begin with an atom name (up to 4 characters that do not correspond to any of the XL command names, and terminated by at least one blank) followed by a scattering factor number (which refers to the list defined by the SFAC instruction(s)), x, y, and z in fractional coordinates, and (optionally) a site occupation factor (s.o.f.) and an isotropic U or six anisotropic U_{ij} components (both in $Å^2$). Note that different program systems may differ in their order of U_{ij} components; XL uses the same order as SHELX-76. The exponential factor takes the form $\exp(-8\pi^2 U[\sin(\theta)/\lambda]^2)$ for an isotropic displacement parameter U and:

$$\exp (-2\pi^2 [h^2(a^*)^2U_{11} + k^2(b^*)^2U_{22} + ... + 2hka^*b^*U_{12}])$$

for anisotropic U_{ij}. An atom is specified as follows in the *.ins* file:

`atomname sfac x y z sof [11] U [0.05] or` U_{11} U_{22} U_{33} U_{23} U_{13} U_{12}

The atom name must be unique, except that atoms in different residues - see RESI - may have the same names; in contrast to SHELX-76 it is not necessary to pad out the atom name to 4 characters with blanks. To fix any atom parameter, add 10. Thus the site occupation factor is normally given as 11 (i.e., fixed at 1). The site occupation factor for an atom in a special position should be multiplied by the multiplicity of that position (as given in International Tables, Volume A) and divided by the multiplicity of the general position for that space group. This is the same definition as in SHELX-76 and is retained for upwards compatibility; it might have been less confusing to keep the multiplicity and occupation factor separate. An atom on a fourfold axis for example will usually have s.o.f. = 10.25.

If any atom parameter is given as (10*m+p), where abs(p) is less than 5 and m is an integer, it is interpreted as $p \bullet fv_m$, where fv_m is the mth 'free variable' (see FVAR). Note that there is no fv_1, since this position on an FVAR instruction is occupied by the overall scale factor, and m=1 corresponds to fixing an atom by adding 10. If m is negative, the parameter is interpreted as $p \bullet (fv_{-m}-1)$. Thus to constrain two occupation factors to add up to 0.25 (for two elements occupying the same fourfold special position) they could be given as 20.25 and -20.25, i.e., $0.25 \bullet fv_2$ and $0.25 \bullet (1-fv_2)$, which correspond to p=0.25, m=2 and p=-0.25, m=-2 respectively.

In SHELX-76, it was necessary to use free variables and coordinate fixing in this way to set up the appropriate constraints for refinement of atoms on special positions. In XL, this is allowed (for upwards compatibility) but is NOT NECESSARY: the program will automatically work out and apply the appropriate positional, s.o.f. and U_{ij} constraints for any special position in any space group, in a conventional setting or otherwise. If the user applies (correct or incorrect) special position constraints using free variables etc., the program assumes that this has been done with intent, and reports but does not apply the correct constraints. Thus the accidental application of a free variable to a U_{ij} term of an atom on a special position can lead to the refinement 'blowing up'! All that is necessary is to specify atomname, sfac, x, y and z, and leave the rest to the program; when the atom is (later) made anisotropic using the ANIS command, the appropriate U_{ij} constraints will be added by the program. For a well-behaved structure, the list of atom coordinates (from direct methods and/or difference electron density syntheses) suffices. If the multiplicity factor (s.o.f.) is left out, it will be fixed at the appropriate value of 1 for a general position and less than 1 for a special position. Since XL automatically generates origin restraints for polar space groups, no atom coordinates should be fixed by the user for this purpose (in contrast to version 4 of SHELXTL).

It may still be necessary to apply constraints by hand to handle disorder; a common case is when there are two possible positions for a group of atoms, in which the first set should all have s.o.f.'s of (say) 21, and the second set -21, with the result that the sum of the two occupation factors is fixed at 1, but the individual values may refine as fv_2 and $1\text{-}fv_2$. Similarly if a special position with 2/m symmetry is occupied by Ca^{2+} and Ba^{2+}, the two ions could be given the s.o.f.'s 30.25 and -30.25 respectively. In this case it would be desirable to use the EADP instruction to equate the Ca^{2+} and Ba^{2+} (anisotropic) displacement parameters.

If U is given as -T, where T is in the range $0.5 < T < 5$, it is fixed at T times the U_{eq} of the previous atom not constrained in this way. The resulting value is not refined independently but is updated after every least-squares cycle.

12.3.1 SPEC del[0.2]

All following atoms (until the next SPEC instruction) are considered to lie on special positions (for the purpose of automatic constraint generation) if they lie within del (Å) of a special position. The coordinates of such an atom are also adjusted so that it lies exactly on the special position.

12.3.2 RESI class[ ] number[0] alias

Until the next RESI instruction, all atoms are considered to be in the specified 'residue', which may be defined by a class (up to four characters, beginning with a letter) or number (up to four digits) or both. The same atom names may be employed in different residues, enabling them to be referenced globally or selectively. The residue number should be unique to a particular residue, but the class may be used to refer to a class of similar residues, e.g., a particular type of amino acid in a polypeptide.

Residues may be referenced by any instruction that allows atom names; the reference takes the form of the character '_' followed by either the residue class or number without intervening spaces. If an instruction codeword is followed immediately by a residue number, all atom names referred to in the instruction are assumed to belong to that residue unless they are themselves immediately followed by '_' and a residue number, which is then used instead. Thus:

```
RTAB_4 Ang N H0 O_11
```

would cause the calculation of an angle N_4 - H0_4 - O_11, where the first two atoms are in residue 4 and the third is in residue 11.

If the instruction codeword is followed immediately by a residue class, the instruction is effectively duplicated for all residues of that class. '_* ' may be used to match all residue classes; this includes the default class ' ' (residue number 0) which applies until the first RESI instruction is encountered. Thus:

```
MPLA_phe CB > CZ
```

would calculate least-squares planes through atoms CB to CZ inclusive of all residues of class 'phe' (phenylalanine). In the special case of HFIX, only the FIRST instruction which applies to a given atom is applied. Thus:

```
HFIX_1 33 N
HFIX_* 43 N
```

would add hydrogens to the N-terminal nitrogen (residue 1) of a polypeptide to generate a (protonated) $-NH_3^+$ group, but all other (amide) nitrogens would become -NH-.

Individual atom names in an instruction may be followed by '_' and a residue number, but not by '_*' or '_' and a residue class. If an atom name is not followed by a residue number, the current residue is assumed (unless overridden by a global residue number or class appended to the instruction codeword). The symbols '_+' meaning 'the next residue' and '_-' meaning 'the preceding residue'(i.e., residues number n+1 and n-1 if the current residue number is n) may be appended to atom names but not to instruction codenames. Thus the instruction:

```
RTAB_* Omeg CA_+ N_+ C CA
```

could be used to calculate all the peptide ω torsion angles in a protein or polypeptide. If (as at the C-terminus in this example) some or all of the named atoms cannot be found for a particular residue, the instruction is simply ignored for that residue.

'_$n' does not refer to a residue; it uses the symmetry operation $n defined by a preceding 'EQIV $n' instruction to generate an equivalent of the named atom (see EQIV). alias specifies an alternative value of the residue number so that cyclic chains of residues may be created; for a cyclic pentapeptide (residue numbers 2,3,..6) it could be set to 1 for residue 6 and to 7 for residue 2. If more than one RESI instruction refers to the same number, alias only needs to be specified once. alias is referenced only by the _+ and _- operations (see above), and a value used for alias may not be used as a residue number on a RESI instruction. Note that if there is more than one cyclic peptide in the asymmetric unit, it is a good idea to leave a gap of TWO residue numbers between them. e.g., a cyclic pentapeptide with two molecules in the asymmetric unit would be numbered 2 to 6 and 9 to 13, with aliases 7 on RESI 2, 1 on RESI 6, 14 on RESI 9 and 8 on RESI 13. It will generally be found convenient for applying restraints etc. to use the same names for atoms in identical residues. Since XL does not recognize chain ID's (used in PDB format) it is normal to add a constant to the residue numbers to denote a different chain (e.g., chain A could be 1001 to 1234 and chain B 2001 to 2234). The auxiliary program XPRO provides extensive facilities for handling residues.

12.3.3 MOVE dx[0] dy[0] dz[0] sign[1]

The coordinates of the atoms that follow this instruction are changed to: x = dx + sign * x, y = dy + sign * y, z = dz + sign * z until superseded by a further MOVE. MOVE should not be used at the same time as the specification of zero coordinates to indicate that an atom should not be used in fitting a fragment of known geometry (e.g., AFIX 66), because after the move the coordinates will no longer be zero!

12.3.4 ANIS n

The next n isotropic non-hydrogen atoms are made anisotropic, generating appropriate special position constraints for the U_{ij} if required. Intervening atoms which are already anisotropic are not counted. A negative n has the same effect.

12.3.5 ANIS names

The named atoms are made anisotropic (if not already), generating the appropriate constraints for special positions. Note that names may include '$' followed by a scattering factor name (see SFAC); 'ANIS $CL' would make all chlorine atoms anisotropic. Since ANIS, like other instructions, applies to the current residue unless otherwise specified, ANIS_* $S would be required to make the sulfur atoms in all residues anisotropic (for example). ANIS MUST precede the atoms to which it is to be applied. ANIS on its own, with neither a number nor names as parameters, makes all FOLLOWING non-hydrogen atoms (in all residues) anisotropic. The L.S. and CGLS instructions provide the option of delaying the conversion to anisotropic of all atoms specified by ANIS until a given number of least-squares cycles has been performed.

12.3.6 AFIX mn d[#] sof[11] U[10.08]

AFIX applies constraints and/or generates idealized coordinates for all atoms until the next AFIX instruction is read. The digits mn of the AFIX code control two logically quite separate operations. Although this is confusing for new users, it has been retained for upwards compatibility with SHELX-76, and because it provides a very concise notation. m refers to geometrical operations which are performed before the first refinement cycle (hydrogen atoms are idealized before every cycle), and n sets up constraints which are applied throughout the least-squares refinement. n is always a single digit; m may be two, one or zero digits (the last corresponds to m = 0).

The options for idealizing hydrogen atom positions depend on the connectivity table that is set up using CONN, BIND, FREE and PART; with experience, this can also be used to generate hydrogen atoms attached to disordered groups and to atoms on special positions. d determines the bond lengths in the idealized groups, and sof and U OVERRIDE the values in the atom list for all atoms until the next AFIX instruction. U is not applied if the atom is already anisotropic, but is used if an isotropic atom is to be made anisotropic using ANIS. Any legal U value may be used, e.g., 31 (a free variable reference) or -1.2 (1.2 times Ueq of the preceding normal atom). Each AFIX instruction must be followed by the required number of hydrogen or other atoms. The individual AFIX options are as follows; the default X-H distances depend on both the chemical environment and the temperature (to allow for librational effects) which is specified by means of the TEMP instruction.

m = 0 No action.

m = 1 Idealized tertiary C-H with all X-C-H angles equal. There must be three and only three other bonds in the connectivity table to the immediately preceding atom, which is assumed to be carbon. m = 1 is often combined with a riding model refinement (n = 3).

m = 2 Idealized secondary CH_2 with all X-C-H and Y-C-H angles equal, and H-C-H determined by X-C-Y (i.e., approximately tetrahedral, but widened if X-C-Y is much less than tetrahedral). This option is also suitable for riding refinement (n = 3).

m = 3 Idealized CH_3 group with tetrahedral angles. The group is staggered with respect to the shortest other bond to the atom to which the -CH_3 is attached. If there is no such bond (e.g., an acetonitrile solvent molecule) this method cannot be used (but m = 13 is still viable).

m = 4 Aromatic C-H or amide N-H with the hydrogen atom on the external bisector of the X-C-Y or X-N-Y angle. m = 4 is suitable for a riding model refinement, i.e., AFIX 43 before the H atom.

m = 5 Next five non-hydrogen atoms are fitted to a regular pentagon, default d = 1.42 Å.

m = 6 Next six non-hydrogen atoms are fitted to a regular hexagon, default d = 1.39 Å.

m = 7 Identical to m = 6 (included for upwards compatibility from SHELX-76). In SHELX-76 only the first, third and fifth atoms of the six-membered ring were used as target atoms; in XL this will still be the case if the other three are given zero coordinates, but the procedure is more general because any one, two or three atoms may be left out by giving them zero coordinates.

m = 8 Idealized OH group, with X-O-H angle tetrahedral. If the oxygen is attached to a saturated carbon, all three staggered positions are considered for the hydrogen. If it is attached to an aromatic ring, both positions in the plane are considered. The final choice is based on forming the 'best' hydrogen bond to a nitrogen, oxygen, chlorine or fluorine atom. The algorithm involves generating a potential position for such an atom by extrapolating the O-H vector, then finding the nearest N, O, F or Cl atom to this position, taking symmetry equivalents into account. If another atom that (according to the connectivity table) is bonded to the N, O, F or Cl atom, is nearer to the ideal position, the N, O, F or Cl atom is not considered. Note that m = 8 had a different effect in SHELX-76 (but was rarely employed).

m = 9 Idealized terminal $X=CH_2$ or $X=NH_2^+$ with the hydrogen atoms in the plane of the nearest substituent on the atom X. Suitable for riding model refinement (AFIX 93 before the two H atoms).

m = 10 Idealized pentamethylcyclopentadienyl (Cp*). This AFIX must be followed by the 5 ring carbons and then the 5 methyl carbons in cyclic order, so that the first methyl group (atom 6) is attached to the first carbon (atom 1). The default d is 1.42 Å, with the $C-CH_3$ distance set to 1.063d. A variable-metric rigid group refinement (AFIX 109) would be appropriate, and would allow for librational shortening of the bonds. Hydrogen atoms (e.g., with AFIX 37 or 127) may be included after the corresponding carbon atoms, in which case AFIX 0 or 5 (in the case of a rigid group refinement) must be inserted before the next carbon atom.

m = 11 Idealized naphthalene group with equal bonds (default d = 1.39 A). The atoms should be numbered as a symmetrical figure of eight, starting with the alpha C and followed by the beta, so that the first six atoms (and also the last six) describe a hexagon in cyclic order. m = 11 is also appropriate for rigid group refinement (AFIX 116).

m = 12 Idealized disordered methyl group; as m = 3 but with two positions rotated from each other by 60 degrees. The corresponding occupation factors should normally be set to add up to one, e.g., by giving them as 21 (i.e., 1*fv(2)) and -21 (1*(1-fv(2))). If HFIX is used to generate an AFIX instruction with m=12, the occupation factors are fixed at 0.5. AFIX 12n is suitable for a *para* methyl on a phenyl group with no *meta* substituents, and should be followed by 6 half hydrogen atoms (first the three belonging to one $-CH_3$ component, then the three belonging to the other, so that hydrogens n and n+3 are opposite one another). The six hydrogens should have the same PART number as the carbon to which they are attached (e.g., PART 0).

m = 13 Idealized CH_3 group with tetrahedral angles. If the coordinates of the first hydrogen atom are non-zero, they define the torsion angle of the methyl group. Otherwise (or if the AFIX instruction is being generated via HFIX) a structure-factor calculation is performed (of course only once, even if many hydrogens are involved) and the torsion angle is set that maximizes the sum of the electron density at the three calculated hydrogen positions. Since even this is not an infallible method of getting the correct torsion angle, it should normally be combined with a rigid or rotating group refinement for the methyl group (e.g., mn = 137 before the first H). In subsequent least-squares cycles the group is re-idealized retaining the current torsion angle

m = 14 Idealized OH group, with X-O-H angle tetrahedral. If the coordinates of the hydrogen atom are non-zero, they are used to define the torsion angle. Otherwise (or if HFIX was used to set up the AFIX instruction) the torsion angle is chosen which maximizes the electron density (see m = 13). Since this torsion angle is unlikely to be very accurate, the use of a rotating group refinement is recommended (i.e., AFIX 147 before the H atom).

m = 15 BH group in which the boron atom is bonded to either four or five other atoms as part of an polyhedral fragment. The hydrogen atom is placed on the vector that represents the negative sum of the unit vectors along the four or five other bonds to the boron atom.

m = 16 Acetylenic C-H, with X-C-H linear. Usually refined with the riding model, i.e., AFIX 163.

m > 16 A group defined in a FRAG...FEND section with code = m is fitted, usually as a preliminary to rigid group refinement. The FRAG...FEND section MUST precede the corresponding AFIX instruction in the '*.ins*' file, but there may be any number of AFIX instructions with the same m corresponding to a single FRAG...FEND section.

When a group is fitted (m = 5, 6, 10 or 11, or m > 16), atoms with non-zero coordinates are used as target atoms with equal weight. Atoms with all three coordinates zero are ignored. Any three or more non-colinear atoms may be used as target atoms.

'Riding' (n = 3, 4) and 'rotating' (n = 7, 8) hydrogen atoms, but not other idealized groups, are re-idealized (if m is 1, 2, 3, 4, 8, 9, 12, 13, 14, 15 or 16) before each refinement cycle (after the first cycle, the coordinates of the first hydrogen of a group are always non-zero, so the torsion angle is retained on re-idealizing). For n = 4 and 8, the angles are re-idealized but the (refined) X-H bond length is retained, unless the hydrogen coordinates are all zero, in which case d (on the AFIX instruction) or (if d is not given) a standard value which depends on the chemical environment and temperature (TEMP) is used instead.

n = 0 No action.

n = 1 The coordinates, s.o.f. and U or U_{ij} are fixed.

n = 2 The s.o.f. and U (or U_{ij}) are fixed, but the coordinates are free to refine.

n = 3 The coordinates, but not the s.o.f. or U (or U_{ij}) 'ride' on the coordinates of the previous atom with n not equal to 3. The same shifts are applied to the coordinates of both atoms, and both contribute to the derivative calculation. The atom on which riding is performed may not itself be a riding atom, but it may be in a rigid group (m = 5, 6 or 9).

n = 4 This constraint is the same as n = 3 except that the X-H distance is free to refine. The X-H vector direction does not change. This constraint requires better quality reflection data than n = 3, but allows for variations in apparent X-H distances caused by libration and bonding effects. If there is more than one equivalent hydrogen, the same shift is applied to each equivalent X-H distance (e.g., to all three C-H bonds in a methyl group). n = 4 may be combined with DFIX or SADI restraints (to restrain chemically equivalent X-H distances to be equal) or embedded inside a rigid (n = 6) group, in which case the next atom (if any) in the same rigid group must follow an explicit AFIX instruction with n = 5. Note that n = 4 had a different effect in SHELX-76.

n = 5 The next atom(s) are 'dependent' atoms in a rigid group. Note that this is automatically generated for the atoms following an n = 6 or n = 9 atom, so does not need to be included specifically unless m has to be changed (e.g., AFIX 35 before the first hydrogen of a rigid methyl group with AFIX 6 or 9 before the preceding carbon).

n = 6 The next atom is the 'pivot atom' of a NEW rigid group, i.e., the other atoms in the rigid group rotate about this atom, and the same translational shifts are applied to all atoms in the rigid group.

n = 7 The following (usually hydrogen) atoms (until the next AFIX with n not equal to 7) are allowed to ride on the immediately preceding atom X and rotate about the Y-X bond; X must be bonded to one and only one atom Y in the connectivity list, ignoring the n = 7 atoms (which, if they are F rather than H, may be present in the connectivity list). The motion of the atoms of this 'rotating group' is a combination of riding motion (c.f. n = 3) on the atom X plus a tangential component perpendicular to the Y-X and X-H bonds, so that the X-H distances, Y-X-H and H-X-H angles remain unchanged. This constraint is intended for -OH, $-CH_3$ and possibly $-CF_3$ groups. X may be part of a rigid group, which may be resumed with an AFIX n = 5 following the n = 7 atoms.

n = 8 This constraint is similar to n = 7 except that the X-H distances may also vary, the same shifts being applied along all the X-H bonds. Thus only the Y-X-H and H-X-H angles are held constant; the relationship of n = 8 to n = 7 corresponds to that of n = 4 to n = 3. DFIX and SADI restraints may be useful for the X-H distances. This constraint is useful for $-CF_3$ groups or for $-CH_3$ groups with good data.

n = 9 The first (pivot) atom of a new 'variable metric' rigid group. Such a group retains its 'shape' but may shrink or expand uniformly. It is useful for C_5H_5 and BF_4 groups, which may show appreciable librational shortening of the bond lengths. Subsequent atoms of this type of rigid group should have n = 5, which is generated automatically by the program if no other AFIX instruction is inserted between the atoms. Riding atoms are not permitted inside this type of rigid group. Only the pivot atom coordinates may be fixed (by adding 10) or tied to free variables, and only the pivot atom may lie on a special position (for the automatic generation of special position constraints).

Although there are many possible combinations of m and n, in practice only a small number is used extensively, as discussed in the section on hydrogen atoms. Rigid group fitting and refinement (e.g., AFIX 66 followed by six atoms of a phenyl ring or AFIX 109 in front of a Cp* group) is particularly useful in the initial stages of refinement; atoms not found in the structure solution may be given zero coordinates, in which case they will be generated from the rigid group fit.

A rigid group or set of dependent hydrogens must ALWAYS be followed by 'AFIX 0' (or another AFIX instruction). Leaving out 'AFIX 0' by mistake is a common cause of error; the program is able to detect and correct some obvious cases, but in many cases this is not logically possible.

12.3.7 HFIX mn U[#] d[#] atomnames

HFIX generates AFIX instructions and dummy hydrogen atoms bonded to the named atoms, the AFIX parameters being as specified on the HFIX instruction. This is exactly equivalent to the corresponding editing of the atom list. The atom names may reference residues (by appending '_n' to the name, where n is the residue number), or SFAC names (preceded by a '$' sign). U may be any legal value for the isotropic temperature factor, e.g., 21 to tie a group of hydrogen U value to free variable 2, or -1.5 to fix U at 1.5 times U(eq) of the preceding normal atom. HFIX MUST precede the atoms to which it is to be applied. If more than one HFIX instruction references a given atom, only the FIRST is applied. 'HFIX 0' is legal, and may be used to switch off following HFIX instructions for a given atom (which is useful if they involve '_*' or a global reference to a residue class).

12.3.8 FRAG code[17] a[1] b[1] c[1] α[90] β[90] γ[90]

Enables a fragment to be input using a cell and coordinates taken from the literature. Orthogonal coordinates may also be input in this way. Such a fragment may be fitted to the set of atoms following an AFIX instruction with m = code (code must be greater than 16); there must be the same number of atoms in this set as there are following FRAG, and they must be in the same order. Only the coordinates of the FRAG fragment are actually used; atom names, sfac numbers, sof and U_{ij} are IGNORED. A FRAG fragment may be given anywhere between UNIT and HKLF or END, and must be terminated by a FEND instruction, but must precede any AFIX instruction which refers to it. This 'rigid fit' is often a preliminary to a rigid group refinement (AFIX with n = 6 or 9).

12.3.9 FEND

This must immediately follow the last atom of a FRAG fragment.

12.3.10 EXYZ atomnames

The same x, y and z parameters are used for all the named atoms. This is useful when atoms of different elements share the same site, e.g., in minerals (in which case EADP will probably be used as well). The coordinates (and possibly free variable references) are taken from the named atom which precedes the others in the atom list, and the actual values, free variable references etc. given for the x, y and z of the other atoms are ignored. An atom should not appear in more than one EXYZ instruction.

12.3.11 EADP atomnames

The same isotropic or anisotropic displacement parameters are used for all the named atoms. The displacement parameters (and possibly free variable references) are taken from the named atom which precedes the others in the atom list, and the actual values, free variable references etc. given for the U_{ij} of the other atoms are ignored. The atoms involved must either be all isotropic or all anisotropic. An atom should not appear in more than one EADP instruction. 'Opposite' fluorines of PF_6 or disordered -CF_3 groups are good candidates for EADP, e.g.

```
EADP F11 F14
EADP F12 F15
EADP F13 F16
C1 .......
PART 1
F11 ...... 21 ......
```

```
F12 ...... 21 ......
F13 ...... 21 ......
PART 2
F14 ...... -21 ......
F15 ...... -21 ......
F16 ...... -21 ......
PART 0
```

EADP applies an (exact) *constraint*. The SIMU instruction *restrains* the Uij components of neighboring atoms to be approximately equal with an appropriate (usually fairly large) esd.

12.3.12 EQIV $n symmetry operation

Defines symmetry operation $n for referencing symmetry equivalent atoms on any instruction which allows atom names, by appending '_$n' (where n is an integer between 1 and 511 inclusive) to the atom name. Such a symmetry operation must be defined beforeit is used; it does not have to be an allowed operation of the space group, but the same notation is used as on the SYMM instruction. The same $n may not appear on two separate EQIV instructions. Thus:

```
EQIV $2 1-x, y, 1-z
CONF C1 C2 C2_$2 C1_$2
```

could be used to calculate a torsion angle across a crystallographic twofold axis (note that this may be required because CONF with no atom names only generates torsion angles automatically that involve the unique atom list and a one atom deep shell of symmetry equivalents). If the instruction codeword refers to a residue, this is applied to the named atoms before any symmetry operation specified with '_$n'. Thus:

```
RTAB_23 O..O OG_12 O_$3
```

would calculate the (hydrogen bond) distance between OG_12 and (O_23)_$3, i.e., between OG in residue 12 and the equivalent obtained by applying the symmetry operation defined by EQIV $3 to the atom O in residue 23.

12.3.13 OMIT atomnames

The named atoms are retained in the atom list but ignored in the structure factor calculation and least-squares refinement. This instruction may be used, together with L.S. 0 and FMAP 2, to create an 'OMIT map' to get a clearer picture of disordered regions of the structure; this concept will be familiar to macromolecular crystallographers. In particular, 'OMIT $H' can be used to check the hydrogen atom assignment of -OH groups etc. If an actual peak is present within 0.31 A of the calculated hydrogen atom position, the electron density appears in the 'Peak' column of the output created by PLAN with a negative first parameter. OMIT_* $H must be used for this if residues are employed.

12.4 The Connectivity List

The connectivity list is a list of 'bonds' that is set up automatically, and may be edited using BIND and FREE. It is used to define idealized hydrogen atom positions, for the BOND and PLAN output of bond lengths and angles, and by the instructions DELU, CHIV, SAME and SIMU. Hydrogen atoms are excluded from the connectivity list (except when introduced by hand using BIND).

12.4.1 CONN bmax[12] r[#] atomnames or CONN bmax[12]

The CONN instruction fine-tunes the generation of the connectivity table and is particularly useful when π-bonded ligands or metal ions are present in the structure. For the purposes of the connectivity table (which is always generated), bonds are all distances between non-hydrogen atoms less than r1 + r2 + 0.5 Å, where r1 and r2 are the covalent radii of the atoms in question (taking PART into consideration as explained below). A shell of symmetry equivalent atoms is also generated, so that all unique bonds are represented at least once in the list. All bonds, including those to symmetry equivalent atoms, may be deleted or added using the FREE or BIND instructions.

Default values of r (identified by the scattering factor type) are stored in the program. These defaults may be changed (for both the connectivity table AND the PLAN -n output) by using the full form of the SFAC instruction. Alternatively the defaults may be overridden for the named atoms by specifying r on a CONN instruction, in which case r is used in the generation of the connectivity list but not by the PLAN instruction. '$' followed by an element name (the same as on a SFAC instruction) may also be employed on a CONN instruction (and also does not apply to PLAN). The second form of the CONN instruction may be used to change the maximum coordination number bmax for all atoms (which defaults to 12 if there is no CONN instruction).

If, after generating bonds as above and editing with FREE and BIND, there are more than bmax bonds to a given atom, the list is pruned so that only the bmax shortest are retained. A harmless side-effect of this pruning of the connectivity list is that symmetry operations may be stored and printed that are never actually used. Note that this option only removes one entry for a bond from the connectivity list, not both, except in the case of 'CONN 0' which ensures that there are no bonds to or from the named atoms. 'CONN 0' is frequently used to prevent the solvent water in macromolecular structures from making additional 'bonds' to the macromolecule which confuse the generation of idealized hydrogen atoms etc. In some cases it will be necessary to use FREE to remove a 'bond' from a light atom to an alkali metal atom (for example) in order to generate hydrogen atoms correctly. Refinements of macromolecules will often include BUMP and 'CONN 0 O_200 > LAST' (where the water happens to begin with residue 200). 'LAST' is used to indicate the last atom in the file, which saves trouble when adding extra waters.

The CONN instruction, like ANIS and HFIX, MUST precede the atoms to which it is to be applied. Repeated CONN instructions are allowed; the LAST relevant CONN preceding a particular atom is the one which is actually applied. CONN without atom names changes the default value of bmax for all following atoms. The following example illustrates the use of CONN:

```
CONN Fe 0
MPLA 5 C11 > C15 Fe
MPLA 5 C21 > C25 Fe
Fe  .....
C11 .....
.........
C25 .....
```

which would prevent bonds being generated from the iron atom to all 10 carbons in ferrocene. In this example, the distances of the iron atom from the two ring planes would be calculated instead.

12.4.2 PART n sof

The following atoms belong to PART n of a disordered group. The automatic bond generation ignores bonds between atoms with different PART numbers, unless one of them is zero (the value before the first PART instruction). If a site occupation factor (sof) is specified on the PART instruction, it overrides the value on the following atom instructions (even if set via an AFIX instruction) until a further PART instruction, e.g., 'PART 0', is encountered).

If n is negative, the generation of special position constraints is suppressed and bonds to symmetry generated atoms with the same or a different non-zero PART number are excluded; this is suitable for a solvent molecule disordered on a special position of higher symmetry than the molecule can take (e.g., a toluene molecule on an inversion center). A PART instruction remains in force until a further PART instruction is read; 'PART 0' should be used to continue with the non-disordered part of the structure.

Some care is necessary in generating hydrogen atoms where disordered groups are involved. If the hydrogen atoms are assigned a PART number, then even if the atom to which they are attached has no part number (i.e., PART 0) the above rules may be used by the program to work out the correct connectivity for calculating the hydrogen atom positions. HFIX hydrogens are assigned the PART number of the atom to which they are attached. If the hydrogens and the atom to which they are attached belong to PART zero but the latter is bonded to atoms with non-zero PART, the LOWEST of these non-zero PART numbers is assumed to be the major component and is used to calculate the hydrogen positions. In general, if the same residue numbers and names and the same atom names but different PART numbers are used for different disorder components in a macromolecule, HFIX will generate hydrogen atoms correctly without any special action being required. For example the use of HFIX with the following disordered serine residue:

```
HFIX_Ser 33 N
HFIX_Ser 13 CA
HFIX_Ser 23 CB
HFIX_Ser 83 CG
 :
RESI 32 Ser
N.....
CA.....
C.....
O.....
PART 1
CB   1  ...  ...  ...  21  ...
OG   4  ...  ...  ...  21  ...
PART 2
CB   1  ...  ...  ... -21  ...
OG   4  ...  ...  ... -21  ...
PART 0
```

would set up the AFIX hydrogens as if the following had been input. Note that only one, fully occupied, hydrogen is attached to CA; for this reason, and also to prevent small inconsistencies in the DFIX and DANG restraints, the disorder should be traced back one more atom than can be resolved (i.e., CB should be split even if it does not look as though this would be necessary in an electron density map):

```
RESI 32 Ser
N.....
AFIX 43
H0   2  ...  ...  ...  11  -1.2
AFIX 0
CA.....
AFIX 13
HA   2  ...  ...  ...  11  -1.2
AFIX 0
C.....
O.....
PART 1
CB   1  ...  ...  ...  21  ...
AFIX 23
HB1  2  ...  ...  ...  21  -1.2
HB2  2  ...  ...  ...  21  -1.2
AFIX 0
OG   4  ...  ...  ...  21  ...
AFIX 83
HG   2  ...  ...  ...  21  -1.5
AFIX 0
PART 2
CB   1  ...  ...  ... -21  ...
AFIX 13
HB1  2  ...  ...  ... -21  -1.2
HB2  2  ...  ...  ... -21  -1.2
AFIX 0
OG   4  ...  ...  ... -21  ...
AFIX 83
HG   2  ...  ...  ... -21  -1.5
AFIX 0
PART 0
```

where free variable 2 is the occupation factor for PART 1 (say 0.7) and the occupation factor of the second component is tied to 1-fv(2) (i.e., 0.3). The value for this free variable is set on the FVAR instruction and is free to refine. If there were more than two components, a linear free variable restraint (SUMP) could be used to restrain the sum of occupation factors to unity. The addition of disorder components after including hydrogen atoms will require some hand editing and so is less efficient, but the auxiliary program XPRO can be persuaded to do most of the work

12.4.3 BIND atom1 atom2

The specified 'bond' (which may be of any length) is added to the connectivity list if it is not there already. Only one of the two atoms may be an equivalent atom (i.e., have the extension _$n).

12.4.4 FREE atom1 atom2

The specified 'bond' is deleted from the connectivity list (if present). Only one of the two atoms may be an equivalent atom (i.e., have the extension _$n).

12.5 Least-squares Restraints

12.5.1 DFIX d s[0.02] atom pairs

The distance between the first and second named atom, the third and fourth, fifth and sixth etc. (if present) is restrained to a target value d with an estimated standard deviation s. d may refer to a 'free variable', otherwise it is considered to be fixed. Fixing d by adding 10 is not allowed, so the value may lie between 0 and 15.

If d is given a negative sign, the restraint is applied ONLY if the current distance between the two atoms is LESS than |d|. This is an 'anti-bumping' restraint, and may be used to prevent solvent (water) molecules from approaching too close to one another or to a macromolecule. Antibumping restraints may also be generated automatically using the BUMP instruction (see below). The default value of s is 0.02. The default s may be changed by means of a preceding DEFS instruction (see below).

12.5.2 DANG d s[0.04] atom pairs

This instruction is interpreted in exactly the same way as DFIX, but the default value of s is twice the value of the first DEFS parameter (i.e., 0.04 if no DEFS instruction is used). The DFIX and DANG instructions appear separately in the table of restraint statistics. DANG is usually used for 1,3 or ‘angle distances’, i.e., distances between two atoms that are both bonded to the same atom. The distance between the first and second named atom, the third and fourth, fifth and sixth etc. (if present) is restrained to a target value d with an estimated standard deviation s. d may refer to a 'free variable', otherwise it is considered to be fixed. Fixing d by adding 10 is not allowed, so the value may lie between 0 and 15.

12.5.3 BUMP s [0.02]

'Anti-bumping' restraints are generated automatically for all distances involving two non-bonded C, N, O and S atoms (based on the SFAC type) that are shorter than the expected shortest non-bonded distances, allowing for the possibility of hydrogen bonds. All pairs of atoms that are not connected by one, two or three bonds in the connectivity table are considered to be non-bonded for this purpose. Anti-bumping restraints are also generated for short contacts between hydrogen atoms (if present) provided that the two hydrogen atoms are not bonded to the same atom; this should help to avoid energetically unfavorable side-chain conformations. If the sum of occupancies of the two atoms is less than 1.1, no restraint is generated; also if the atoms have different PART numbers and neither of them is zero no restraint is generated.

The default esd s is the first DEFS parameter (0.02 if there is no DEFS instruction). If s is given a negative sign, the absolute value is used as an esd, and symmetry equivalent atoms in the connectivity array are considered too in deciding which atoms are connected and so should not have anti-bumping restraints applied. Thus when s is positive (the default action if s is not specified on the BUMP instruction) short contacts between appropriate atoms in different asymmetric units ALWAYS result in anti-bumping restraints. This will be the normal procedure for macromolecular refinements (where it helps to eliminate accidental contacts between molecules in low-resolution refinements), but in the (unusual) case of a crystallographic twofold axis running through (say) a disulfide bond it will be necessary to make s negative to prevent the generation of anti-bumping restraints that would break the bond. Refinement with anti-bumping restraints provides a solvent model with acceptable hydrogen bonding distances that is consistent with the diffraction data. The anti-bumping restraints are regenerated before each refinement cycle. Anti-bumping restraints can also be added by hand using DFIX instructions with negative distances d.

12.5.4 SAME s1[0.02] s2[0.02] atomnames

The list of atoms (which may include the symbol '>' meaning all intervening non-hydrogen atoms in a forward direction, or '<' meaning all intervening non-hydrogen atoms in a backward direction) is compared with the same number of atoms which follow the SAME instruction. All bonds in the connectivity list for which both atoms are present in the SAME list are restrained to be the same length as those between the corresponding following atoms (with an effective standard deviation s1). The same applies to 1,3 distances (defined by two bonds in the connectivity list which share a common atom), with standard deviation s2. The default value of s1 is taken from the first DEFS parameter; the default value of s2 is twice this. s1 or s2 may be set to zero to switch off the corresponding restraints. The program automatically sets up the n*(n-1)/2 restraint equations required when n interatomic distances should be equal. This ensures optimum efficiency and avoids arbitrary unequal weights. Only the minimum set of restraints needs to be specified in the *.ins* file; redundant restraints are ignored by the program, provided that they have the same sigma values as the unique set of restraints. See also SADI and NCSY for closely related restraints.

The position of a SAME instruction in the input file is critical. This creates problems for programs such as XPRO that provide a user interface to XL, and for protein refinements SADI is to be preferred (e.g., to apply 4m local symmetry to a heme group); normally for proteins most of the 1,2- and 1,3-distances will be restrained to target values using DFIX and DANG respectivelly anyway. However SAME provides an elegant way of specifying that chemically identical but crystallographically independent molecules have the same 1,2 and 1,3 distances, e.g.

```
C1A
:
C19A
SAME C1A > C19A
C1B
:
C19B
SAME C1A > C19A
C1C
:
C19C
```

etc. This requires just n-1 SAME instructions for n equivalent molecules. In a more complicated example, assume that a structure contains several toluene solvent molecules that have been assigned the same atom names (in the same order!) and the same residue name (Tol) but different residue numbers, then one SAME instruction suffices:

```
SAME_Tol C1 > C7
```

This instruction may be inserted anywhere except after the last Tol residue; the program applies it as if it were inserted before the next atom that matches C1_Tol . This is convenient for proteins with repeated non-standard residues, since one command suffices to apply suitable restraints, and no target values are needed, for compatibility with XPRO this SAME instruction has to be placed before the FVAR instruction. This is an exception to the usual rule that the action of a SAME instruction is position dependent; but it might be best to put it before a toluene residue with good geometry, since the connectivity table for this residue will be used to define the 1,2- and 1,3-distances. In this case it would also be reasonable to impose local two-fold symmetry for each phenyl ring, so a further SAME instruction could be added immediately before one toluene residue (the ring is assumed to be labeled cyclicly C1 .. C6 followed by the methyl group C7 which is attached to C1):

```
SAME C1 C6 < C2 C7
```

which is equivalent to:

```
SAME C1 C6 C5 C4 C3 C2 C7
```

Note that these two SAME restraints are all that is required, however many PHE residues are present; the program will generate all indirectly implied 1,2 and 1,3 equal-distance restraints! In this case it would also be sensible to restrain the atoms of each tolune molecule to be coplanar by a FLAT restraint:

```
FLAT_Tol C1 > C7
```

12.5.5 SADI s[0.02] atom pairs

The distances between the first and second named atoms, the third and fourth, fifth and sixth etc. (if present) are restrained to be equal with an effective standard deviation s. The SAME and SADI restraints are analyzed together by the program to find redundant and implied restraints. The same effect as is obtained using SADI can also be produced by using DFIX with d tied to a free variable, but the latter costs one more least-squares parameter (but in turn produces a value and esd for this parameter). The default effective standard deviations for SADI may be changed by means of a DEFS instruction before the instruction in question.

12.5.6 CHIV V[0] s[0.1] atomnames

The chiral volumes of the named atoms are restrained to the value V (in $Å^3$) with standard deviation s. The chiral volume is defined as the volume of the tetrahedron formed by the three bonds to each named atom, which must be bonded to three and only three non-hydrogen atoms in the connectivity list; the (ASCII) alphabetical order of the atoms making these three bonds defines the sign of the chiral volume. Note that RTAB may be used to list chiral volumes defined in the same way but without restraining them. The chiral volume is positive for the alpha-carbon (CA) of an L-amino-acid if the usual names (N, CB and C) are used for the three non-hydrogen atoms bonded to it. It is also possible to define a chiral volume when two substituents are chemically eqivalent but have different names; this may be useful to ensure that CB of a valine retains a pyramidal geometry with the conventional labeling of CG1 and CG2. Note that 'CHIV 0' (or just CHIV since the default V is zero) may be used to impose a planarity restraint on an atom which is bonded to three other non-hydrogen atoms, by making its chiral volume zero. CHIV restraints with zero and non-zero target values are listed separately in the restraints summary printer out after each refinement cycle.

12.5.7 FLAT s[0.1] four or more atoms

The named atoms are restrained to lie a common plane. This restraint is actually applied by restraining a sufficient number of tetrahedra involving the atoms in question to have (chiral) volumes of zero, using the same algorithm as CHIV. This way of applying a planarity restraint has good convergence properties because it does not fix the orientation of the plane in its current position. s should be given in $Å^3$ as for CHIV, but for comparison with other methods the r.m.s. deviation from the plane is also printed. The default values of s is set by the second DEFS parameter.

12.5.8 DELU s1[0.01] s2[0.01] atomnames

All bonds in the connectivity list connecting atoms on the same DELU instruction are subject to a 'rigid bond' restraint, i.e., the components of the (anisotropic) displacement parameters in the direction of the bond are restrained to be equal within an effective standard deviation s1. The same type of restraint is applied to 1,3-distances as defined by the connectivity list (atoms 1, 2 and 3 must all be defined on the same DELU instruction). If s2 is omitted it is given the same value as s1. A zero value for s1 or s2 switches off the corresponding restraint. If no atoms are specified, all non-hydrogen atoms are assumed. DELU is ignored if (in the refinement cycle in question) one or both of the atoms concerned is isotropic; in this case a 'hard' restraint is inappropriate, but SIMU may be used in the usual way as a 'soft' restraint. DELU without atom names applies to all non-hydrogen atoms (in the current residue); DELU_* without atoms applies to all non-hydrogen atoms in all residues. SFAC element names may also be referenced, preceded by the symbol '$'. The default values of s1 and s2 may be changed by means of a preceding DEFS instruction.

12.5.9 SIMU s[0.04] st[0.08] dmax[1.7] atomnames

Atoms closer than dmax are *restrained* with effective standard deviation s to have the same U_{ij} components. If (according to the connectivity table, i.e., ignoring attached hydrogens) one or both of the two atoms involved is terminal (or not bonded at all), st is used instead as the esd. If s but not st is specified, st is set to twice s. If no atoms are given, all non-hydrogen atoms are understood. SIMU_* with no atoms applies to all non-hydrogen atoms in all residues. SFAC element names may also be referenced, preceded by '$'. The interatomic distance for testing against dmax is calculated from the atom coordinates without using the connectivity table (though the latter is used for deciding if an atom is terminal or makes no bonds).

Note that SIMU should in general be given a much larger esd (and hence lower weight) than DELU; whereas there is good evidence that DELU restraints should hold accurately for most covalently bonded systems, SIMU (and ISOR) are only rough approximations to reality. s or st may be set to zero to switch off the appropriate restraints.

SIMU is intended for use for larger structures with poorer resolution and data to parameter ratios than are required for full unrestrained anisotropic refinement. It is based on the observation that the U_{ij} values on neighboring atoms in larger molecules tend to be both similar and (when the resolution is poor) significantly correlated with one another. By applying a very weak restraint of this type, we allow a gradual increase and change in direction of the anisotropic displacement parameters as we go out along a side-chain, and we restrain the motion of atoms perpendicular to a planar group (which DELU cannot influence). The use of a distance criterion directly rather than via the connectivity table enables the restraints to be applied automatically to partially overlapping disordered atoms, for which it is an excellent approach. dmax can be set so that coordination distances to metal ions etc. are excluded. Terminal atoms tend to show the largest deviations from equal U_{ij}'s and so st should be set higher than s (or made equal to zero to switch off the restraints altogether). SIMU restraints are NOT recommended for SMALL molecules and ions, especially if free rotation or torsion is possible (e.g., C_5H_5-groups, AsF_6- ions). For larger molecular fragments, the effective rotation angles are smaller, and the assumption of equal U_{ij} for neighboring atoms is more appropriate: both translation and libration of a large fragment will result in relatively similar U_{ij} components on adjacent atoms. SIMU may be combined with ISOR, which applies a further soft but quite different restraint on the U_{ij} components. SIMU may also be used when one or both of the atoms concerned is isotropic, in which case experience indicates that a larger esd (say 0.1 $Å^2$) is appropriate. The default value of s may be changed by a preceding DEFS instruction (st is then set to twice s).

12.5.10 DEFS sd[0.02] sf[0.1] su[0.01] ss[0.04] maxsof[1]

DEFS may be used to change the default effective standard deviations for the following DFIX, SAME, SADI, CHIV, FLAT, DELU and SIMU restraints, and is useful when these are to be varied systematically to establish the optimum values for a large structure (e.g., using R_{free}). sd is the default for s in the SADI and DFIX instructions, and also for s1 and s2 in the SAME instruction. sf is the default effective standard deviation for CHIV and FLAT, su is the default for both s1 and s2 in DELU, and ss is the default s for SIMU. The default st for SIMU is set to twice the default s.

maxsof is the maximum allowed value that an occupation factor can refine to; occupation factors that are fixed or tied to free variables are not restricted. It is possible to change this parameter (to say 1.1 to allow for hydrogen atoms) when refining both occupation factors and U's for solvent water in proteins (a popular but suspect way of improving the R factor).

12.5.11 ISOR s[0.1] st[0.2] atomnames

The named atoms are *restrained* with effective standard deviation s so that their U_{ij} components approximate to isotropic behavior; however the corresponding isotropic U is free to vary. ISOR is often applied, perhaps together with SIMU, to allow anisotropic refinement of large organic molecules when the data are not adequate for unrestrained refinement of all the U_{ij}; in particular ISOR can be applied to solvent water for which DELU and SIMU are inappropriate. ISOR should in general be applied as a weak restraint, i.e., with relatively large sigmas, for the reasons discussed above (see SIMU); however it is also useful for preventing individual atoms from becoming 'non-positive-definite'. However it should not be used indiscriminately for this purpose without investigating whether there are reasons (e.g., disorder, wrong scattering factor type etc.) for the atom going n.p.d. If (according to the connectivity table, i.e., ignoring attached hydrogens) the atom is terminal (or makes no bonds), st is used instead as the esd. If s but not st is specified, st is set to twice s. If no atoms are given, all non-hydrogen atoms are understood. SFAC element names may also be referenced, preceded by '$'. s or st may be set to zero to switch offthe appropriate restraints. ISOR without atom names (or ISOR_* if residues are used) applies this restraint to all non-hydrogen atoms. Note also the use of the keyword 'LAST' to indicate the last atom in the .ins file; an anisotropic refinement of a macromolecule will often include:

```
ISOR 0.1 O_201 > LAST
```

assuming that the solvent water starts with O_201 and continues until the end of the atom list. ISOR should in general be given a much larger esd (and hence lower weight) than DELU; whereas there is good evidence that DELU restraints should hold accurately for most covalently bonded systems, ISOR (and SIMU) are only rough approximations to reality.

12.5.12 NCSY DN sd[0.1] su[0.05] atoms

The NCSY instruction applies local non-crystallographic symmetry restraints. In contrast to the widely used global NCS constraints, these do not save any CPU time but do not require the definition (and refinement) of a matrix transformation and mask. They are also very flexible, and can accommodate rotation of the molecule about hinges etc. Since for macromolecules at modest resolution the 1,2- and 1,3-distances are normally restrained to fixed target values by DFIX and DANG restraints, the NCS restraints are generated for equivalent 1,4-distances (if sd is non-zero or absent) and equivalent isotropic U-values (if su is non-zero or absent). The default sd is set to five times the first DEFS parameter, and the default su is equal to the fourth DEFS parameter.

For each atom the program attempts to find an ‘equivalent’ atom with the same name but with a residue number DN greater than the residue number of the named atom. If sd is greater than zero, the connectivity array is used to find 1,4-distances for which both atoms are specified in the same NCSY instruction; a SADI restraint is then created to make the distance equivalent to the same distance between the equivalent atoms. This is not quite the same as restraining torsion angles to be the same, because + and – gauche would have the same distance; however it is chemically plausible that equivalent side-chain conformations could differ in this way. If su is greater than zero (or absent), a SIMU restraint is generated to make the U-values approximately equal for each pair of ‘equivalent’ atoms, provided that both are isotropic. NCS restraints should be used whenever possible for isotropic (protein) refinement at modest resolution, since they increase the effective data to parameter ratio and so have a similar effect to that of increasing the resolution of the data. They are also very easy to set up; for example, to apply three-fold NCS restraints to a protein structure containing three equivalent chains numbered 1001-1109, 2001-2109 and 3001-3109, the following two instructions are all that is required:

```
NCSY 1000 N_1001 > OT2_1109
NCSY 2000 N_1001 > OT2_1109
```

The atom list may easily be modified to leave out particular loops, residues or side-chains. This is not only easier than specifying a transformation matrix and mask: it also will correspond more closely to reality, because the restraints are more flexible than constraints and also act *locally* rather than *globally*.

12.5.13 SUMP c sigma c1 m1 c2 m2 ...

The linear restraint: c = c1*fv(m1) + c2*fv(m2) + ... is applied to the specified free variables. This enables more than two atoms to be assigned to a particular site, with the sum of site occupation factors restrained to be a constant. It also enables linear relations to be imposed between distances used on DFIX restraints, for example to restrain a group of atoms to be collinear. sigma is the effective standard deviation. By way of example, assume that a special position on a four-fold axis is occupied by a mixture of sodium, calcium, aluminium and potassium cations so that the average charge is +2 and the site is fully occupied. The necessary restraints and constraints could be set up as follows (the program will take care of the special position constraints on the coordinates and U_{ij} of course):

```
SUMP 1.0 0.01 1.0 2 1.0 3 1.0 4 1.0 5   ! site fully occupied
SUMP 2.0 0.01 1.0 2 2.0 3 3.0 4 1.0 5   ! mean charge = +2
EXYZ Na1 Ca1 Al1 K1                     ! common x, y and z coordinates
EADP Na1 Ca1 Al1 K1                     ! common U or Uij
FVAR ... 0.20 0.30 0.35 0.15     ! starting values for free variables 2..5
...
Na1 ... ... ... ... 20.25 ...    ! 0.25 * fv(2)  [the 0.25 is required for
Ca1 ... ... ... ... 30.25 ...    ! 0.25 * fv(3)  a special position on a
Al1 ... ... ... ... 40.25 ...    ! 0.25 * fv(4)  four-fold axis, i.e., site
K1  ... ... ... ... 50.25 ...    ! 0.25 * fv(5)  symmetry 4]
```

This particular refinement would probably still be rather unstable, but the situation could be improved considerably by adding weak SUMP restraints for the elemental analysis. Such SUMP restraints may be used when elements are distributed over several sites in minerals so that the elemental composition corresponds (within suitable standard deviations) to an experimental chemical analysis.

SUMP may also be applied to BASF, EXTI and BASF parameters, including parameters used to describe twinning (TWIN) and anisotropic scaling (HOPE). The parameters are counted in the order overall scale and free variables, EXTI, then BASF.

12.6 Least-Squares Organization

12.6.1 L.S. nls[0] nrf[0] nextra[0] maxvec[511]

nls cycles of full-matrix least-squares refinement are performed, followed by a structure factor calculation. When L.S. (or CGLS) is combined with BLOC, each cycle involves refinement of a block of parameters which may be set up differently in different cycles. If no L.S. or CGLS instruction is given, 'L.S. 0' is assumed.

If nrf is positive, it is the number of these cycles that should be performed before applying ANIS. This two-stage refinement is particularly suitable for the early stages of least-squares refinement; experience indicates that it is not advisable to let everything go at once!

Negative nrf indicates which reflections should be ignored during the refinement but used instead for the calculation of free *R*-factors in the final structure factor summation; for example L.S. 4 –10 would ignore every 10th reflection for refinement purposes. It is desirable to use the same negative value of nrf throughout, so that the values of '*R*1(free)' and 'w*R*2(free)' are not biased by the 'memory' of the contribution of these reflections to earlier refinements. These independent *R*-factors (Brünger, 1992) may be used to calibrate the sigmas for the various classes of restraint, and provide a check as to whether the data are being 'over-refined' (primarily a problem for macromolecules with a poor data to parameter ratio). In XL, these ignored reflections are not used for Fourier calculations.

nrf=–1 selects the R_{free} reference set that is flagged (with negative batch numbers) in the *.hkl* file (XPRO may be used to do this). The division of the data into reference and working set is then independent of the space group and the MERG, OMIT and SHEL settings. However on merging reflections, to play safe a reflection is retained in the reference set only if all equivalents have the R_{free} flag set. Thus if equivalents are present, it is a good idea to use the XPRO option to set the R_{free} flag in thin shells, so that all equivalents of a particular unique reflection are either all in the reference set or all in the working set. nrf=–1 is the recommended way of applying the R_{free} test in XL.

nextra is the number of additional parameters which were derived from the data when performing empirical absorption corrections etc. It should be set to 44 for DIFABS [or 34 without the theta correction; Walker & D. Stuart (1983)]. It ensures that the standard deviations and GooF are estimated correctly; they would be underestimated if the number of extra parameters is not specified. nextra is zero (and so can be omitted) if extra information in the form of indexed crystal faces or psi-scan data was used to apply an absorption correction.

maxvec refers to the maximum number of reflections processed simultaneously in the rate-determining calculations. Usually the program utilizes all available memory to process as many reflections as possible simultaneously, subject to a maximum of maxvec, which may not be larger than 511. For complicated reasons involving the handling of suppressed and 'R_{free}' reflections and input/output buffering, some blocks may be smaller than the maximum, especially if the facilities for refinement against twinned or powder data are being used. It may be desirable to set maxvec to a smaller number than 511 to prevent unnecessary disk transfers when large structures are refined on virtual memory systems with limited physical memory.

12.6.2 CGLS nls[0] nrf[0] nextra[0] maxvec[511]

As L.S., but the Konnert-Hendrickson conjugate-gradient algorithm is employed instead of the full-matrix approach. Although BLOC may be used with CGLS, in practice it is much better to refine all parameters at once. CGLS is much faster than L.S. for a large number of parameters, and so will be the method of choice for most macromolecular refinements. The convergence properties of CGLS are good in the early stages (especially if there are many restraints), but cannot compete with L.S. in the final stages for structures which are small enough for full-matrix refinement. The major disadvantage of CGLS is that it does not provide estimated standard deviations, so that when a large structure has been refined to convergence using CGLS it may beworth performing a blocked full-matrix refinement (L.S./ BLOC) to obtain the standard deviations in quantities of interest (e.g., torsion angles, in which case only xyz blocks would be required).The other parameters have the same meaning as with L.S.; CGLS is entirely suitable for R_{free} tests (negative nrf), and since it requires much less memory than L.S. there will rarely be any reason to change maxvec from its default value.

The CGLS algorithm is based closely on the procedure described by Hendrickson & Konnert (1980). The structure-factor derivatives contribute only to the diagonal elements of the least-squares matrix, but all 'additional observational equations' (restraints) contribute in full to diagonal and off-diagonal terms, although neither the l.s. matrix A nor the Jacobean J are ever generated. The preconditioning recommended by Hendrickson & Konnert is used to speed up the convergence of the internal conjugate gradient iterations, and has the additional advantage of preventing the excessive damping of poorly determined parameters characteristic of other conjugate gradient algorithms (Tronrud,1992).

A further refinement in the CGLS approach is to save the parameter shifts from the previous CGLS cycle, and to use them to improve the estimated parameter shifts in the current cycle. Since this is only possible in the second and subsequent cycles, an initial shift multiplier of 0.7 is assumed in the first cycle. If the refinement proves to be unstable, this starting value can be reset using the first DAMP parameter.

In addition to this optimization of the CGLS shift multiplication factor, the individual parameter shifts are monitored each L.S. or CGLS cycle, and the shift multiplication factors are reduced (to a value between 0.5 and 1) for parameters that tend to oscillate. This applies only to refinements in which BLOC is not used. This produces an additional improvement in the convergence of the least-squares refinement, but (unlike Marquardt damping) has no effect on esds.

12.6.3 BLOC n1 n2 atomnames

If n1 or n2 are positive, the x, y and z parameters of the named atoms are refined in cycle |n1| or |n2| respectively.. If n1 or n2 are negative, the occupation and displacement parameters are refined in the cycle. Not more than two such cycle numbers may be specified on a single BLOC instruction, but the same atoms may be mentioned in any number of BLOC instructions. To refine both x, y and z as well as displacement parameters for an atom in the same block, n1 and n2 should specify the same cycle number, but with opposite signs. A BLOC instruction with no atom names refines all atoms (in residue 0) in the specified cycles. The pattern of blocks is repeated after the maximum block number has been reached if the number of L.S. refinement cycles is larger than the maximum BLOC |n1| or |n2|. If a cycle number less than the maximum |n1| or |n2| is not mentioned in any BLOC instruction, it is treated as full-matrix. The overall scale, batch/twin scale factors, extinction coefficient, SWAT g parameter, HOPE parameters and free variables (if present) are refined in every block. Riding (hydrogen) atoms and atoms in rigid groups are included in the same blocks as the atoms on which they ride.

For example, a polypeptide consisting of 30 residues (residue numbers 1..30 set by RESI instructions) could be refined efficiently as follows (all non-hydrogen atoms assumed anisotropic):

```
BLOC 1
```

```
BLOC -2 N_1 > O_16
BLOC -3 N_14 > O_30
```

which would ensure 3 roughly equally sized blocks of about 800 parameters each and some overlap between the two anisotropic blocks to avoid problems where they join. The geometric parameters would refine in cycles 1,4,7 .. and the anisotropic displacement parameters in the remaining cycles. In this example it is assumed that the first atom in each residue is N and the last is O. An alternative good blocking strategy would be to divide the structure into three overlapping blocks of xyz and U_{ij} parameters, and to add a fourth cycle in which all xyz but no U_{ij} values are refined (these four blocks would then also each contain about 800 parameters), i.e.,

```
BLOC 1 -1 N_1 > O_11
BLOC 2 -2 N_10 > O_21
BLOC 3 -3 N_20 > O_30
BLOC 4
```

A BLOC instruction with no parameters fixes all atomic parameters (xyz, sof and U or U_{ij}). Such a BLOC instruction takes priority over all other BLOC instructions, irrespective of their order in the *.ins* file.

12.6.4 DAMP damp[0.7] limse[15]

The DAMP parameters take different meanings for L.S. and CGLS refinements. For L.S., damp is usually left at the default value unless there is severe correlation, e.g., when trying to refine a pseudo-centrosymmetric structure, or refining with few data per parameter (e.g., from powder data). A value in the range 1-10000 might then be appropriate. The diagonal elements of the least-squares matrix are multiplied by (1+damp/1000) before inversion; this is a version of the Marquardt (1963) algorithm. A side-effect of damping is that the standard deviations of poorly determined parameters will be artificially reduced; it is recommended that a final least-squares cycle be performed with little or no damping in order to improve these estimated standard deviations. Theoretically, damping only serves to improve the convergence properties of the refinement, and can be gradually reduced as the refinement converges; it should not influence the final parameter values. However in practice damping also deals effectively with rounding error problems in the (single-precision) least-squares matrix algebra, which can present problems when the number of parameters is large and/or restraints are used (especially when the latter have small esd's), and so it may not prove possible to lift the damping entirely even for a well converged refinement.

Note the use of 'DAMP 0 0' to estimate esds but not apply shifts, e.g., when a final L.S. 1 job is performed after CGLS refinement.

For CGLS refinements, damp is the multiplicative shift factor applied in the first cycle. In subsequent CGLS cycles it is modified based on the experience in the previous cycles. If a refinement proves unstable in the first cycle, damp should be reduced from its default value of 0.7.

If the maximum shift/esd for a L.S. refinement (excluding the overall scale factor) is greater than limse, all the shifts are scaled down by the same numerical factor so that the maximum is equal to limse. If the maximum shift/esd is smaller than limse no action is taken. This helps to prevent excessive shifts in the early stages of refinement. limse is ignored in CGLS refinements.

12.6.5 STIR sres step[0.01]

The STIR instruction allows a stepwise improvement in the resolution. In the first refinement cycle, the high-resolution limit (i.e., lowest d) is set at sres, in the next cycle to (sres–step), in the next (sres–2•step) etc. This continues until the limit of the data or the SHEL limit is reached, after which any remaining cycles to complete the number specified by CGLS or L.S. are completed with a constant resolution range. By starting at lower resolution and then gradually improving it, the radius of convergence for models with significant coordinate errors should be increased. This may be regarded as a primitive form of 'simulated annealing'; it could be useful in the early stages of refinement of molecular replacement solutions, or for getting rid of bias for R_{free} tests (in cases where the solution of the struture was - possibly of ncessity - based on all the data).

12.6.6 WGHT a[0.1] b[0] c[0] d[0] e[0] f[.33333]

The weighting scheme is defined as follows:

$$w = q / [\sigma^2(F_o^2) + (a*P)^2 + b*P + d + e*\sin(\theta)]$$

where P = [f * Maximum of (0 or F_o^2) + (1-f) * F_c^2]. It is possible for the experimental F_o^2 value to be negative because the background is higher than the peak; such negative values are replaced by 0 to avoid possibly dividing by a very small or even negative number in the expression for w. For twinned and powder data, the F_c^2 value used in the expression for P is the total calculated intensity obtained as a sum over all components. q is 1 when c is zero, $\exp[c*(\sin(\theta)\lambda)^2]$ when c is positive, and 1 - $\exp[c*(\sin(\theta)/\lambda)^2]$ when c is negative.

The use of P rather than (say) F_o^2 reduces statistical bias (Wilson 1976). The weighting scheme is NOT refined if a is negative (contrast SHELX-76). The parameters can be set by trial and error so that the variance shows no marked systematic trends with the magnitude of F_c^2 or of resolution; the program suggests a suitable WGHT instruction after the analysis of variance. This scheme is chosen to give a flat analysis of variance in terms of F_c^2, but does not take the resolution dependence into account. It is usually advisable to retain default weights (WGHT 0.1) until all atoms have been found and the refinement is essentially complete, when the scheme suggested by the program can be used for the next refinement job by replacing the WGHT instruction (if any) by the one output by the program towards the end of the *.res* file. This procedure is adequate for most routine refinements.

It may be desirable to use a scheme which does not give a flat analysis of variance to emphasize particular features in the refinement; for example c = +10 or -10 would weight up data at higher 2θ, e.g., to perform a 'high-angle' refinement (uncontaminated by hydrogen atoms which contribute little at higher diffraction angle) prior to a difference electron density synthesis (FMAP 2) to locate the hydrogens. The exponential weights which are obtained when c is positive were advocated by Dunitz & Seiler (1973). Weighting up the high angle reflections will in general give X-ray atomic coordinates which are closer to those from neutron diffraction.

Refinement against F^2 requires different weights to refinement against F; in particular, making all the weights equal ('unit weights'), although useful in the initial stages of refinement against F, is NEVER a sensible option for F^2. If the program suspects that an unsuitable WGHT instruction has been accidentally retained for a structure which had been refined previously with SHELX-76 or the XLS program in version 4 of the SHELXTL system, it will output a warning message.

12.6.7 FVAR osf[1] free variables

The overall scale factor is followed by the values of the 'free variables' fv(2) ... The overall scale factor is given throughout as the square root of the scale factor which multiplies F_c^2 in

the least-squares refinement [to make it similar to the scale factor in SHELX-76 which multiplied F_c], i.e., osf$^2 F_c^2$ is fitted to F_o^2.

XL goes to some trouble to ensure that the initial value of the scale factor has very little influence. Firstly, if the initial scale is exactly 1.0, a quick structure factor summation with a small fraction of the total number of reflections is performed to estimate a new scale factor. If the values differ substantially then the new value is used. Secondly the scale factor is factored out of the least-squares algebra so that, although it is still refined, the only influence the previous value has is an indirect one via the weighting scheme and extinction correction.

Before calculating electron density maps and the analysis of variance, and writing the structure factor file (*name.fcf*), the observed F^2 values and esds are brought onto an absolute scale by dividing by the scale factor.

The free variables allow extra constraints to be applied to the atoms, e.g., for common site occupation factors or isotropic displacement parameters, and may be used in conjunction with the SUMP, DFIX and CHIV restraints. If there is more than one FVAR instruction, they are concatenated; they may appear anywhere between UNIT and HKLF (or END).

12.7 Lists and Tables

The esds in bond lengths, angles and torsion angles, chiral volumes, Ueq, and coefficients of least-squares planes and deviation of atoms from them, are estimated rigorously from the full correlation matrix (an approximate treatment is used for the angles between least-squares planes). The errors in the unit-cell dimensions (specified on the ZERR instruction) are taken into account exactly in estimating the esds in bond lengths, bond angles, torsion angles and chiral volumes. Correlation coefficients between the unit-cell dimensions are ignored except when determined by crystal symmetry (so that for a cubic crystal the cell esds contribute to errors in bond lengths and chiral volumes but not to the errors in bond angles or torsion angles). The (rather small) contributions of the unit-cell errors to the esds of quantities involving least-squares planes are estimated using an isotropic approximation.

For full-matrix refinement, the esds are calculated after the final refinement cycle. In the case of BLOC'ed refinement, the esds are calculated after every cycle (except that esds in geometric parameters are not calculated after pure Uij/sof cycles etc.), and the maximum estimate of each esd is printed in the final tables. This prevents some esds being underestimated because not all of the relevant atoms were refined in the last cycle, but at the cost of overestimating all the esds if the R-factor drops appreciably during the refinement. Thus large structures should first be refined almost to convergence (either by CGLS or L.S./BLOC), and then a separate final blocked refinement job performed to obtain the final parameters and their esds. It is important that there is sufficient overlap between the blocks to enable every esd to be estimated with all contributing atoms refining in at least one of the refinement cycles.

12.7.1 BOND atomnames

BOND outputs bond lengths for all bonds (defined in the connectivity list) that involve two atoms named on the same BOND instruction. Angles are output for all pairs of such bonds involving a common atom. Numerical parameters on a BOND instruction are ignored, but not treated as errors (for compatibility with SHELX-76). A BOND instruction with no parameters outputs bond lengths (and the corresponding angles) for ALL bonds in the connectivity table, and 'BOND $H' on its own includes all bonds to hydrogens as well (but since the hydrogens are not included in the connectivity table, bonds involving symmetry equivalent hydrogens are not included). Other element names may also be referenced globally by preceding them with a '$' on a BOND instruction. BOND is set automatically by ACTA, and the bond lengths and angles are written to the *.cif* file. Note that the best way to calculate B-H-B angles is with RTAB!

12.7.2 CONF atomnames

The named atoms define a chain of at least four atoms. CONF generates a list of torsion angles with esd's for all torsion angles defined by this chain. CONF is often used to specify an n-membered ring, in which case the first three atoms must be named twice (n+3 names in all). If no atoms are specified, all possible torsion angles not involving hydrogen are generated from the connectivity array. The torsion angles generated by CONF are also written to the .cif file if an ACTA instruction is present. All torsion angles calculated by XL follow the conventions defined by Allen & Rogers (1969).

12.7.3 MPLA na atomnames

A least-squares plane is calculated through the first na of the named atoms, and the equation of the plane and the deviations of all the named atoms from the plane are listed with estimated standard deviations (from the full covariance matrix). The angle to the previous least-squares plane (if any) is also calculated, but some approximations are involved in estimating its esd. na must be at least 3. If na is omitted the plane is fitted to all the atoms specified.

12.7.4 RTAB codename atomnames

Chiral volumes (one atomname), bonds (two), angles (three) and torsion angles (four atomnames) are tabulated compactly against residue name and number. codename is used to identify the quantity being printed; it must begin with a letter and not be longer than 4 characters (e.g., 'Psi' or 'omeg'). There may not be more than 4 atom names. It is assumed that the atoms have the same names in all the required residues. For chiral volumes only, the necessary bonds must be present in the connectivity list (the same conventions are employed as for CHIV). Since the atoms do not themselves have to be in the same residue (it is sufficient that the names match), the residue name (if any) is printed as that of the first named atom for distances, the second for angles, and the third in the case of torsion angles. The latter should be consistent with generally accepted conventions for proteins. A typical application of RTAB for small-molecule structures is the tabulation of hydrogen-bonded distances and angles (with esd's) since these will not usually appear in the tables created automatically by BOND. For an example of this see the 'sigi' test job in chapter 3.

If RTAB refers to more than one residue (e.g., RTAB_*), it is ignored for those residues in which not all the required atoms can be found (e.g., some of the main chain torsional angles for the terminal residues in a protein).

12.7.5 HTAB dh[2.0]

The new HTAB instruction provides an analysis of the hydrogen bonds. A search is made over all polar hydrogens (i.e., hydrogen bonded to electronegative elements) present in the structure, and hydrogen bonds printed for which: **H•••A < r(A)+dh** and **<DHA > 110**°. If it appears likely that the hydrogens have been assigned wrongly (e.g., two -OH groups have been assigned to the same O•••O vector) a suitable warning message appears. This output should be checked carefully, since the algorithms used by HFIX/ AFIX to place hydrogens are by no means infallible! To obtain esd's on the distances and angles involved in the hydrogen bond, the second form of the HTAB instruction (and if necessary EQIV) should be used (see below); HTAB without atom names is used first to find the necessary symmetry transformations for EQIV..

12.7.6 HTAB donor-atom acceptor-atom

The second form of the HTAB instruction is required to generate the esds and the CIF output records. The donor atom D and acceptor A should be specified; the program decides which of the hydrogen atoms (if any) makes the most suitable hydrogen bond linking them. Only the acceptor atom may specify a symmetry operation (_$n) because this standard CIF entry for publication in Acta Crystallographica requires this.

12.7.7 LIST m[#] mult[1]

m = 0: No action.

m = 1: Write *h,k,l,* F_o, F_c and phase (in degrees) to .fcf in X-PLOR format. Only unique reflections after removing systematic absences, scaling [to an absolute scale of F(calc)], applying dispersion and extinction or SWAT corrections (if any), and merging equivalents including Friedel opposites are included. If F_o^2 was negative, F_o is set to zero. Reflections suppressed by OMIT or SHEL [or reserved for R(free)] are not included.

m = 2: List *h,k,l,* F_o, $\sigma(F_o)$ and phase angle in degrees in FORMAT(3I4,2F8.2,I4) for the reflection list as defined for m = 1.

m = 3: List *h,k,l*, F_o, $\sigma(F_o)$, A(real) and B(imag) in FORMAT(3I4,4F8.2), the reflections being processed exactly as for m = 2.

m = 4: List *h,k,l*, F_c^2, F_o^2, $\sigma(F_o^2)$ and a one-character status flag. F_o^2 are scaled to F_c^2 and possibly corrected for extinction, but no corrections have been made for dispersion and no further merging has been performed. FORMAT (3I4,2F12.2,F10.2,1X,A1) is employed. The status flag is 'o' (observed), 'x' [observed but suppressed using 'OMIT *h k l*', SHEL or reserved for R(free)], or '<' (F_o^2 is less than t.$\sigma(F_o^2)$, where t is one half of the *F*-threshold s specified on an OMIT instruction).

m = 5: Write *h,k,l*, F_o, F_c, and ϕ (phase angle in degrees) in FORMAT(3I4,2F10.2,F7.2) for the reflection list as defined for m = 1. Like the m = 1 option, this is intended for input to somestandard macromolecular FFT programs (such as W. Furey's PHASES program), thereby providing a possible route to a graphical display of the electron density.

m = 6: Write a free-format CIF file containing *h,k,l*, F_o^2, $\sigma(F_o^2)$, F_c and ϕ (phase angle in degrees) for the reflection list as defined for m = 1. This is the recommended format for the deposition of reflection data with the PDB, and is also the format required for the generation of refinement statistics and electron density maps using XPRO.

For m = 4 only, mult is a constant multiplicative factor applied to all the quantities output (except the reflection indices!), and may be used if there are scaling problems. For other m options mult is ignored. For m = 2,3 or 4 only a blank line is included at the end of the file as a terminator. The reflection list is written to the file *name.fcf*, which is in CIF format for n = 3, 4 or 6; however the actual reflections are always in fixed format except for n = 1 or 6. The program CIFTAB can - amongst other options - read the m = 4 output and print $F_o/F_c/\sigma(F)$ tables in compact form on an HP-compatible laser printer. n = 4 is the standard archive format for small-molecule structures, n = 6 for macromolecules (with Friedel opposites averaged). Since the final refinement is normally performed on all data (including the R_{free} reference set) the LIST 6 output is not able to flag the R_{free} reflections.

12.7.8 ACTA 2thetafull[#]

A 'Crystallographic Information File' file *name.cif* is created in self-defining STAR format. This ASCII file is suitable for data archiving, network transmission, and (with suitable additions) for direct submission for publication. ACTA automatically sets the BOND, FMAP 2, PLAN and LIST 4 instructions, and may not be used with other FMAP or LIST instructions or with a positive OMIT s threshold. A warning message appears if the cell contents on the UNIT instruction are not consistent with the atom list, because they are used to calculate the density etc. which appears in the *.cif* output file.

2thetafull is used to specify the value of 2θ for which the program calculates the completeness of the data for the CIF output file as required by Acta Crystallographica. If no value is given, the program uses the maximum value of 2θ for the reflection data. If the data were collected to a specific limiting 2θ, or if a limit was imposed using SHEL, this would be a good choice. Otherwise the choice of 2thetafull is a difficult compromise; if it is too low, the paper will be rejected because the resolution of the data is not good enough; if it is higher, the lower completeness might lead to rejection by the automatic Acta rejection software! XL calculates the completeness by counting reflections after merging Friedel opposites and eliminating systematic absences (and the reflection 0,0,0).

12.7.9 SIZE dx dy dz

dx, dy and dz are the three principal dimensions of the crystal in mm, as usually quoted in publications. This information is written to the *.cif* file. If a SIZE instruction is present in the *.ins* file, XL uses it to write the estimated minimum and maximum transmission to the *.cif* file. This should give order of magnitude estimates that should be replaced by the values from the actual absorption correction if these were applied. The empirical XL estimates take into account that most of the diffraction from strongly absorbing crystals takes place at the edges and corners; these estimates of the actual absorption of the crystal may be a little smaller than those from psi-scan and other semi-empirical routines that include absorption by the mounting fiber and glue or oil.

12.7.10 TEMP T[20]

Sets the temperature T of the data collection in degrees Celsius. This is reported to the *.cif* file and used to set the default isotropic U values for all atoms. TEMP must come before all atoms in the *.ins* file. TEMP also sets the default X-H bond lengths (see AFIX) which depend slightly on the temperature because of librational effects. The default C-H bond lengths and default U-values are rounded to two decimal places so that they may be quoted more easily.

12.7.11 WPDB n[1]

Writes the refined coordinates to a *.pdb* file. If n is positive hydrogen atoms are omitted; if |n| is 1 all atoms are converted to isotropic and ATOM statements generated, and if |n| is 2 ANISOU statements are also generated (but the equivalent B value is still used on the ATOM statement). The atom names and residue classes and numbers should conform to PDB conventions. This provides a direct link to X-PLOR and other programs which use (more of less) the official (Brookhaven) dialect of the PDB format. Note that XPRO can be used to extend the PDB output file to include refinement details etc. (from the .lst file) for deposition with the PDB, and also to modify disordered residues so that they can be interpreted by programs such as O that cannot read the full standard PDB format.

12.8 Fouriers, Peak Search and Line Printer Plots

12.8.1 FMAP code[2] axis[#] nl[53]

The unique unit of the cell for performing the Fourier calculation is set up automatically unless specified by the user using FMAP and GRID; the value of axis must be non-zero to suppress the automatic selection. The program chooses a 53 × 53 × nl or 103 × 103 × nl grid depending on the resolution of the data. axis is1, 2 or 3 to define the direction perpendicular to the layers. Dispersion corrections are applied (so that the resulting electron density is real) and Friedel opposites are merged after the least-squares refinement and analysis of variance but before calculating the Fourier synthesis. This will improve the map (and bring the maximum and minimum residual density closer to zero) compared with SHELX-76. In addition, since usually all the data are employed, reflections with $\sigma(F)$ relatively large compared with F_c are weighted down. This should be better than the use of an arbitrary cutoff on $F_o/\sigma(F)$. The rms fluctuation of the map relative to the mean density is also calculated; in the case of a difference map this gives an estimate of the 'noise level' and so may be used to decide whether individual peaks are significant. Usually FMAP 2 is employed to find missing atoms, but if a significant part of the structure is missing, FMAP 5 or 6 may be better. ACTA requires FMAP 2 so that the difference density is on an absolute scale.

If code is made negative, both positive and negative peaks are included in the list, sorted on the absolute value of the peak height. This is intended to be useful for neutron diffraction data.

code = 2: Difference electron density synthesis with coefficients (F_o–F_c) and phases ϕ(calc).

code = 3: Electron density synthesis with coefficients F_o and phases ϕ(calc).

code = 4: Electron density synthesis with coefficients ($2F_o$–F_c) and phases ϕ(calc). F(000) is included in the Fourier summations for code = 3 and 4.

code = 5: Sim-weighted ($2mF_o$-F_c) Fourier (Giacovazzo, 1992).

code = 6: Sim-weighted ($2mF_o$-F_c) Fourier with coefficients sharpened by multplying with $\sqrt{E / F}$.

12.8.2 GRID sl[#] sa[#] sd[#] dl[#] da[#] dd[#]

Fourier grid, when not set automatically. Starting points and increments multiplied by 100. s means starting value, d increment, l is the direction perpendicular to the layers, a is across the paper from left to right, and d is down the paper from top to bottom. Note that the grid is 53 x 53 x nl points, i.e., e.g., twice as large as in SHELX-76, and that sl and dl need not be integral. The 103 x 103 x nl grid is only available when it is set automatically by the program (see above).

12.8.3 PLAN npeaks[20] d1[#] d2[#]

If npeaks is positive a Fourier peak list is printed and written to the *.res* file; if it is negative molecule assembly and line printer plots are also performed. Distances involving peaks which are less than r1+r2+d1 (the covalent radii r are defined via SFAC; 1 and 2 refer to the two atoms concerned) are printed and used to define 'molecules' for the line printer plots. Distances involving atoms and/or peaks which are less than r1+r2+|d2| are considered to be 'non-bonded interactions'; however distances in which both atoms are hydrogen or at least one is carbon (recognised by SFAC label 'C') are ignored. These non-bonded interactions are ignored when defining molecules, but the corresponding atoms and distances are included in the line printer output. Thus an atom or peak may appear in more than one map, or more than once on the same map. A table of the appropriate coordinates and symmetry transformations appears at the end of each molecule.

Negative d2 includes hydrogen atoms in the line printer plots, otherwise they are left out (but included in the distance tables). For the purposes of the PLAN instruction, a hydrogen atom is one with a radius of less than 0.4 Å. Peaks are assigned the radius of SFAC type 1, which is usually set to carbon. Peaks appear on the printout as numbers, but in the .res file they are given names beginning with 'Q' and followed by the same numbers. Peak heights are also written to the .res file (after the sof and dummy U values) in electrons $Å^{-3}$. See also MOLE for forcing molecules (and their environments) to be printed separately.

A default npeaks of +20 is set by FMAP; to obtain line printer plots, an explicit PLAN instruction with negative npeaks is required. If npeaks is positive the nearest unique atoms to each peak are tabulated, together with the corresponding distances. A table of shortest distances between peaks is also produced. For macromolecules and for users of the Bruker' SHELXTL system npeaks will almost always be positive! If npeaks is positive d1 and d2 have a different meaning. The default of d1 is then -1 and causes the full peaklist to appear in the .res file. If it is positive (say 2.3) then the full peaklist is still printed in the *.lst* file, but only suitable candidates for (full occupancy) water molecules appear in the *.res* file (with SFAC 4 and U set to 0.75). The water molecules must be less than 4 Å from an atom which begins with 'O', 'N' or 'W', and may not be nearer than d2 (default 3.0) from any atom which does not begin with 'O', 'N', 'W' or 'H', and may not be nearer than d1 to any 'O', 'N' or 'W' atom or to other potential waters which have larger peak heights. This facility is intended for extending the water structure of proteins in connection with BUMP and SWAT. To include the waters in the next refinement job, their names need to be changed and they need to be moved to before the HKLF instruction at the end of the atom list in the new *.ins* file. This can be performed automatically using XPRO. It is recommended that the last water be called 'LAST' on the ISOR and CONN instructions so that its name does not need to be updated each job.

The heights and positions of the highest (difference) electron density maximum and the deepest minimum are output irrespective of the PLAN parameters.

12.8.4 MOLE n

Forces the following atoms, and atoms or peaks that are bonded to them, into molecule n of the PLAN output. n may not be greater than 99. n = 99 has a special meaning: the ‘line printer plot’ is suppressed for the following atoms, but the table of distances is still printed. This is sometimes useful for saving paper.

13 Strategies for Macromolecular Refinement

XL is designed to be easy to use and general for all space groups and uses a conventional structure-factor calculation rather than a FFT summation; the latter would be faster, but in practice involves some small approximations and is not very suitable for the treatment of dispersion or anisotropic thermal motion. The price to pay for the extra generality and precision is that XL is much slower than programs written specifically for macromolecules, but this is to some extent compensated for by the better convergence properties, reducing the amount of manual intervention required (and also the *R*-factor).

Recent advances in cryogenic techniques, area detectors, and the use of synchrotron radiation enable macromolecular data to be collected to higher resolution than was previously possible. In practice this tends to complicate the refinement because it is possible to resolve finer details of the structure; it is often necessary to model alternative conformations, and in a few cases even anisotropic refinement is justified. Although XL provides a number of other features not found in many macromolecular refinement programs, it is probably the flexible treatment of disorder and the facilities for restrained anisotropic refinement that are most likely to be of immediate interest to macromolecular crystallographers.

An auxiliary program XPRO (Chapter 14) is provided as an interface to other macromolecular programs. XPRO is able to generate an *.ins* file from a PDB format file, including the appropriate restraints etc. XPRO can also generate map files for the program O and can display the refinement results in the form of Postscript plots, as well as including the updated coordinates in the *.ins* file for the next refinement. XL produces PDB and CIF format files that can be read by XPRO and used for archiving.

13.1 The Radius of Convergence

A crucial aspect of any macromolecular refinement program is the radius of convergence. A larger radius of convergence reduces the amount of time-consuming manual intervention using interactive graphics. Many claims that XL gives *R*-factors one or two percent lower than other programs have been tracked down either to subtle differences in the model or to not getting trapped in local minima. The differences in the model include the treatment of diffuse solvent and hydrogen atoms, and the ability to refine common occupancies for disordered groups. The inclusion of dispersion terms and the use of a conventional rather than a FFT structure factor summation are also more precise; the approximations in the FFT summation may become significant for high resolution data and atoms with small displacement parameters. There are probably a number of contributing factors to the good convergence typically observed for XL, e.g., the refinement against ALL data, the inclusion of important off-diagonal terms in the least-squares algebra, the ability to refine all parameters at once (i.e., coordinates and displacement parameters in the same cycle), and the restriction to unimodal restraint functions; multimodal restraint functions such as torsion angles or hydrogen bonds tend to increase the number of spurious local minima. It is much better to reserve the multimodal chemical information such as torsion angles for verifying the structure with an independent program such as PROCHECK (Laskowski, MacArthur, Moss & Thornton, 1993), and to use the unimodal information as restraints. The errors in the FFT calculation of derivatives are larger that those in the structure factors (for the same grid intervals); this would also impede convergence.

13.2 Residues

Macromolecular structures are conventionally divided up into *residues*, for example individual amino-acids. In XL residues may be referenced either individually, by '_' followed by the appropriate residue number, or as all residues of a particular class, by '_' followed by the class. For example 'DFIX 2.031 SG_9 SG_31' could be used to restrain a disulfide distance between two cystine residues, whereas 'FLAT_PHE CB > CZ' would apply planarity restraints to all atoms between CB and CZ inclusive in all PHE (phenylalanine) residues. Plus and minus signs refer to the next and previous residue numbers respectively, so 'DFIX_∗ 1.329 C_– N' applies a bond length restraint to all peptide bonds ('_∗' after the command name applies it to all residues). This way of referring to atoms and residues is in no way restricted to proteins; it is equally suitable for oligonucleotides, polysaccharides, or to structures containing a mixture of all three. It enables the necessary restraints and other instructions to be input in a concise and relatively self-explanatory manner. These instructions are checked by the program for consistency and appropriate warnings are printed.

13.3 Constraints and Restraints for Macromolecules

The lower data to parameter ratio for macromolecules makes the use of constraints and especially restraints essential. Rigid group constraints enable a structure to be refined with very few parameters, especially when the (thermal) displacement parameters are held fixed (BLOC 1). After a structure has been solved by molecular replacement using a rather approximate model for the whole protein or oligonucleotide, it may well be advisable to divide the structure up into relatively rigid domains (using a few AFIX 6 and AFIX 0 instructions) and to refine these as rigid groups, initially for a limited resolution shell (e.g., SHEL 8 3), then stepwise extending the resolution, e.g., using the STIR instruction. Restraints may still be required to define flexible hinges and prevent the units from flying apart. In view of the small number of parameters and the high correlations introduced by rigid group refinement, L.S. (full-matrix refinement) should be used for this stage (but CGLS will be necessary for the subsequent refinement). After this initial step which exploits the large convergence radius of rigid group refinement, in general the more flexible restraints will be used in preference to constraints for the rest of the refinement.

XL provides distance, planarity and chiral volume restraints, but not torsion angle restraints or specific hydrogen bond restraints. For oligonucleotides, good distance restraints are available for the bases (Taylor & Kennard, 1982), but for the sugars and phosphates it is probably better to assume that chemically equivalent 1,2- and 1,3-distances are equal (using the SAME and SADI restraints) without the need to specify target values. In this way the effect of the pH on the protonation state of the phosphates and hence the P-O distances does not need to be predicted, but it is assumed the whole crystal is at the same pH. For proteins, since some amino-acid residues occur only a small number of times in a given protein, it is probably better to use 1,2- and 1,3-target distances based on the study of Engh and Huber (1991); these are employed in the restraints added by XPRO to the *.ins* file.

The three bonds to a carbonyl carbon atom can be restrained to lie in the same plane by means of a *chiral volume restraint* (Hendrickson & Konnert, 1980) with a target volume of zero (e.g., 'CHIV_GLU 0 C CD' to restrain the carbonyl and carboxyl carbons in all glutamate residues to have planar environments). The planarity restraint (FLAT) restrains the chiral volumes of a sufficient number of atomic tetrahedra to zero; in addition the r.m.s. deviation of the atoms from the best planes is calculated. Chiral volume restraints with non-zero targets are useful to prevent the inversion of α-carbon atoms and the β-carbons of Ile and Thr, e.g., 'CHIV_ILE 2.5 CA CB'. It is also useful to apply chiral volume restraints to non-chiral atoms such as CB of valine and CG of leucine in order to ensure conformity with conventional atom-labeling schemes (from the point of view of the atom names, these atoms could be considered to be chiral!).

Anti-bumping restraints are distance restraints that are only applied if the two atoms are closer to each other than the target distance. They can be generated automatically by XL, taking all symmetry equivalent atoms into account. Since this step is relatively time-consuming, in the 1993 release it was performed only before the first refinement cycle, and the anti-bumping restraints were generated automatically only for the solvent (water) atoms (however they could be inserted by hand for any pairs of atoms). In practice this proved to be too limited, so in later releases the automatic generation of anti-bumping restraints was extended to all C, N, O and S atoms (with an option to include H•••H interactions) and was performed each refinement cycle.

Anti-bumping restraints are not generated automatically for

(a) atoms connected by a chain of three bonds or less in the connectivity array (unless separated by more than a specified number of residues),

(b) atoms with different non-zero PART numbers, and

(c) pairs of atoms for which the sum of occupancies is less than 1.1.

The target distances for the O...O and N...O distances are less than for the other atom pairs to allow for possible hydrogen bonds.

13.4 Restrained Anisotropic Refinement

There is no doubt that macromolecules are better described in terms of anisotropic displacements, but the data to parameter ratio is very rarely adequate for a free anisotropic refinement. Such a refinement often results in 'non-positive definite' (NPD) displacement tensors, and at the best will give probability ellipsoids that do not conform to the expected dynamical behavior of the molecule. Clearly constraints or restraints must be applied to obtain a chemically sensible model. It is possible to divide a macromolecule up into relatively rigid domains, and to refine the 20 TLS parameters of rigid body motion for each domain (Driessen, Haneef, Harris, Howlin, Khan & Moss, 1989). This is a good model for the bases in oligonucleotides and for the four aromatic side-chains in proteins, but otherwise macromolecules are probably not sufficiently rigid for the application of TLS constraints, or they would have to be divided up into such small units that too many parameters would be required. As with the refinement of atomic positions, restraints offer a more flexible approach.

The *rigid bond restraint* (DELU) assumes that the components of the anisotropic displacement parameters (ADPs) along bonded (1,2-) or 1,3-directions are zero within a given esd. This restraint should be applied with a low esd, i.e., as a 'hard' restraint. Didisheim & Schwarzenbach (1987) showed that for many non-planar groups of atoms, rigid bond restraints effectively impose TLS conditions of rigid body motion. Although rigid-bond restraints involving 1,2- and 1,3-distances reduce the effective number of free ADPs per atom from 6 to less than 4 for typical organic structures, further restraints are often required for the successful anisotropic refinement of macromolecules.

The *similar ADP restraint* (SIMU) restrains the corresponding U_{ij}-components to be approximately equal for atoms which are spatially close (but not necessarily bonded because they may be in different components of a disordered group). The isotropic version of this restraint has been employed frequently in protein refinements. This restraint is consistent with the characteristic patterns of thermal ellipsoids in many organic molecules; on moving out along side-chains, the ellipsoids become more extended and also change direction gradually.

Neither of these restraints are suitable for isolated solvent (water) molecules. A linear restraint (ISOR) restrains the ADP's to be *approximately isotropic*, but without specifying the magnitude of the corresponding equivalent isotropic displacement parameter. Both SIMU and ISOR restraints are clearly only approximations to the truth, and so should be applied as 'soft' restraints with high esds. When all three restraints are applied, structures may be refined anisotropically with a much smaller data to parameter ratio, and still produce chemically sensible ADP's. Even when more data are available, these restraints are invaluable for handling disordered regions of the structure.

Constraints and restraints greatly increase the radius and rate of convergence of crystallographic refinements, so they should be employed in the early stages of refinement wherever feasible. The difference electron density syntheses calculated after such restrained refinements are often more revealing than those from free refinements. In large small-molecule structures with poor data to parameter ratios, the last few atoms can often not be located in a difference map until an anisotropic refinement has been performed with geometrical and ADP restraints. Atoms with low displacement parameters that are well determined by the X-ray data will be relatively little affected by the restraints, but the latter may well be essential for the successful refinement of poorly defined regions of the structure. Premature removal or softening the restraints (to improve the *R*-value!) is probably the most common cause of unstable macromolecular refinements with XL.

13.5 The free *R*-factor

The questions of whether the restraints can be removed in the final refinement, or what the best values are for the corresponding esds, can be resolved elegantly by the use of R_{free} (Brünger, 1992). To apply this test, the data are divided into a working set (about 95-90% of the reflections) and a reference set (about 5-10%). The reference set is only used for the purpose of calculating a conventional *R*-factor that is called R_{free}. It is very important that the structural model is not in any way based on the reference set of reflections, so these are left out of ALL refinement and Fourier map calculations. If the original model was in any way derived from the same data, then many refinement cycles are required to eliminate memory effects. This ensures that the *R*-factor for the reference set provides an objective guide as to whether the introduction of additional parameters or the weakening of restraints has actually improved the model, and not just reduced the *R*-factor for the data employed in the refinement ('*R*-factor cosmetics'). The best way to set up the R_{free} test is to use XPRO to flag reflections in the *.hkl* file for use in the reference set, and to set the second CGLS parameter to '-1'. If NCS or twinning is anticipated, it is advisable to use the 'thin shells' method of flagging the reflections for R_{free} in XPRO.

R_{free} is invaluable in deciding whether a restrained anisotropic refinement is significantly better than an isotropic refinement. Experience indicates that both the resolution and the quality of the data are important factors, but that restrained anisotropic refinement is unlikely to be justified for crystals that do not diffract to better than 1.5 Å. An ensemble distribution created by molecular dynamics is an alternative to the harmonic description of anisotropic motion (Gros, van Gunsteren & Hol, 1990; Clarage & Phillips, 1994), and may be more appropriate for structures with severe conformational disorder that do not diffract to high resolution.

Despite the overwhelming arguments for using R_{free} to monitor macromolecular refinements, it is only a single number, and is itself subject to statistical uncertainty because it is based on a limited number of reflections. Thus R_{free} may be insensitive to small structural changes, and small differences in R_{free} should not be taken as the last word; one should always consider whether the resulting geometrical and displacement parameters are *chemically reasonable*. The final refinement and maps should always be calculated with the ***full*** data, but without introducing additional parameters or changing the weights of the restraints. R_{free} is most useful for establishing refinement protocols; for a series of closely similar refinements (e.g., for mutants to similar resolution) the R_{free} tests only need to be applied to the first.

13.6 Disorder in Macromolecules

To obtain a chemically sensible refinement of a disordered group, we will probably need to constrain or restrain a sum of occupation factors to be unity, to restrain equivalent interatomic distances to be equal to each other or to standard values (or alternatively apply rigid group constraints), and to restrain the displacement parameters of overlapping atoms. In the case of a tight unimodal distribution of conformations, restrained anisotropic refinement may provide as good a description as a detailed manual interpretation of the disorder in terms of two or more components, and is much simpler to perform. With high-resolution data it is advisable to make the atoms anisotropic BEFORE attempting to interpret borderline cases of side-chain disorder; it may well be found that no further interpretation is needed, and in any case the improved phases from the anisotropic refinement will enable higher quality difference maps to be examined.

Typical warning signs for disorder are large (and pronounced anisotropic) apparent thermal motion (in such cases the program may suggest that an atom should be split and estimate the coordinates for the two new atoms), residual features in the difference electron density and violations of the restraints on the geometrical and displacement parameters. This information in summarized by the program on a residue by residue basis, separately for main-chain, side-chain and solvent atoms. In the case of two or more discrete conformations, it is usually necessary to model the disorder at least one atom further back than the maps indicate, in order that the restraints on the interatomic distances are fulfilled. The different conformations should be assigned different PART numbers so that the connectivity array is set up correctly by the program; this enables the correct rigid bond restraints on the anisotropic displacement parameters and idealized hydrogen atoms to be generated automatically even for disordered regions (it is advisable to model the disorder before adding the hydrogens).

Several strategies are possible for modeling disorder with XL, but for macromolecules the simplest is to include all components of the disorder in the same residues and use the same atom names, the atoms belonging to different components being distinguished only by their different PART numbers. This procedure enables the standard restraints etc. to be used unchanged, because the same atom and residue names are used. No special action is needed to add the disordered hydrogen atoms, provided that the disorder is traced back one atom further than it is visible (so that the hydrogen atoms on the PART 0 atoms bonded to the disordered components are also correct). Note that this very simple and effective treatment of disorder was not available in the original 1993 release of XL.

13.7 Automatic Water Divining

It is relatively common practice in the refinement of macromolecular structures to insert water molecules with partial occupancies at the positions of difference electron density map peaks in order to reduce the *R*-factor (another example of '*R*-factor cosmetics'). Usually when two different determinations of the same protein structure are compared, only the most tightly bound waters, which usually have full occupancies and smaller displacement parameters, are the same in each structure. The refinement of partial occupancy factors for the solvent atoms (in addition to their displacement parameters) is rarely justified by R_{free}, but sometimes the best R_{free} value is obtained for a model involving some water occupancies fixed at 1.0 and some at 0.5.

Regions of diffuse solvent may be modeled using *Babinet's principle* (Moews & Kretsinger, 1975); the same formula is employed in the program TNT, but the implementation is somewhat different. In XL it is implemented as the SWAT instruction and usually produces a significant but not dramatic improvement in the agreement of the very low angle data. Anti-bumping restraints may be input by hand or generated automatically by the program, taking symmetry equivalents into account. After each refinement job, the displacement parameters of the water molecules should be examined, and waters with very high values (say U greater than 0.8 Å^2, corresponding to a B of 63) eliminated. The F_o-F_c map is then analyzed automatically to find the highest peaks which involve no bad contacts and make at least one geometrically plausible hydrogen bond to an electronegative atom. These peaks are then included with full occupancies and oxygen scattering factors in the next refinement job. This procedure is repeated several times; in general R_{free} rapidly reaches its minimum value, although the conventional R-index continues to fall as further waters are added. It should be noted that the automatic generation of anti-bumping restraints is less effective when the water occupancies are allowed to have values other than 1.0 or 0.5. This approach provides an efficient way of building up a chemically reasonable (but not necessarily unique) network of waters that are prevented from diffusing into the protein, thus facilitating remodeling of disordered side-chains etc. The occupancies of specific waters may also be tied (using free variables) to the occupancies of particular components of disordered side-chains where this makes chemical sense. This procedure may be facilitated by using XPRO to convert the *.res* output file from one refinement job to the *.ins* file for the next, or fully automated using the program XWAT that calls XL repeatedly. A similar but much more sophisticated approach (ARP) described by Lamzin & Wilson (1993) may also be used in conjunction with XL.

13.8 Refinement of Structures at Modest Resolution

Although the unique features of XL are primarily useful for refinement against very high resolution data, tests indicated that only small changes would be required to the original release to extend its applicability to medium resolution data (say 2.5Å). The most important of these changes were improved diagnostics and more sophisticated anti-bumping restraints (see above), and the addition of non-crystallographic symmetry (NCS) restraints. The use of NCS restraints considerably improves the effective data to parameter ratio, and the resulting Fourier maps often look as though they were calculated with higher resolution data than were actually used (because the phases are more accurate). Two types of NCS restraint may be generated automatically with the help of the NCSY instruction. The first type uses the connectivity table to define equivalent 1,4-distances, which are then restrained to be equal. The second restrains the isotropic U-values of equivalent atoms to be equal. It is not normally necessary to restrain equivalent 1,2- and 1,3-distances to be equal because the DFIX and DANG restraints will have this effect anyway; but SAME may be used to add such restraints in the absence of DFIX and DANG. The use of *restraints* rather than applying NCS as an exact constraint (e.g., in the structure factor calculation) is more flexible (but slower) and does not require the specification of transformation matrices and real-space masks. Experience indicates that NCS restraints should be used wherever possible; it is not difficult to relax them later (e.g., for specific side-chains involved in interactions with other non-NCS related molecules) should this prove to be necessary.

13.9 A Typical XL refinement using High-Resolution Data

An example of a typical XL refinement (kindly provided by Thomas R. Schneider) against high resolution data, for an inhibited form of serine protease, is summarized in Table 5.2. Data were collected at 120 K on a synchrotron to 0.96 Å resolution with an overall mean I/ of 15.2 and a R_{merge} (based on intensities) of 3.7%. Molecular dynamics refinement using X-PLOR (Brünger, Kuriyan & Karplus, 1987) from initial *R*-values of 42.5% produced the results shown as Job 1. A reference set consisting of 10% of the reflections and a working set of the remaining 90% were used throughout the X-PLOR and XL refinement. The final X-PLOR and initial XL refinements were performed with the resolution range restricted to 1.1 to 8 Å (48495 working set reflections) to save computer time. 10 conjugate gradient cycles were performed in each of the XL refinement jobs; where new atoms were introduced they were always refined isotropically for 2 cycles before making them anisotropic. The CPU times (150 MHz Silicon Graphics R4400 processor) varied from 6.1 hours for job 2 to 21.7 for job 13. The weighting scheme was fixed at 'WGHT 0.2' until jobs 12 and 13, where the two-parameter scheme with values suggested by the program was employed. The restraints (DEFS 0.015 0.2 0.01 0.025) were made tighter than usual to make the refinements more comparable with X-PLOR; the mean distance deviation was 0.009 Å for X-PLOR and 0.014 Å for the final XL job.

Job	Action Taken	NP	NH	NW/NW½/NX	N_{par}	R_1	R_{free}
1	Final X-PLOR, 1.1-8Å	1337	0	176 / 0 / 19	6129	19.47	21.14
2	Same atoms, XL	1337	0	176 / 0 / 19	6129	17.15	18.96
3	SWAT added	1337	0	176 / 0 / 19	6130	17.07	18.95
4	All atoms anisotropic	1337	0	176 / 0 / 19	13790	12.96	16.10
5	Disorder, added solvent	1376	0	207 / 0 / 34	14565	11.46	14.20
6	More disorder and solvent	1422	0	214 / 2 / 39	14831	11.35	14.22
7	Disorder, half occ. waters	1447	0	213 / 20 / 37	15478	11.13	14.10
8	Resolution: 0.96Å-Inf.	1447	0	218 / 28 / 37	15595	10.75	12.95
9	Riding Hydrogens added	1451	1088	220 / 38 / 40	15769	9.58	11.56
10	Minor adjustments	1477	1052	222 / 48 / 40	16114	9.15	11.19
11	Minor adjustments	1491	1042	211 / 64 / 48	16173	9.29	11.31
12	Weighting changed	1491	1029	222 / 84 / 38	16357	8.74	10.85
13	Further refinement	1499	1025	212 / 96 / 38	16353	8.76	10.79

NP = Number of protein atoms (including partially occupied atoms)

NH = Number of hydrogens (all fully occupied)

NW = Number of fully occupied waters

NW½ = Number of half occupied waters

NX = Number of other atoms (inhibitor, formate, glycerol, some of them partially occupied)

Npar = Number of least-squares parameters

Table 13.1 – XL refinement of a serine protease (188 residues)

Introduction of the diffuse solvent parameter in job 3 (which started from the same parameters as job 2) was not significant, although usually it reduces R_{free} by about 0.5%; probably this was a consequence of leaving out the low angle data at this stage. Making all atoms anisotropic resulted in almost a 3% drop in R_{free}, but from experience of similar structures we believe that the drop would have been larger if all the data had been used at this stage. This helps to explain the further drop in R_{free} on using all the reflection data (job 8), and the fact that the difference between R_1 and R_{free} was about 3% for jobs 4 to 7 and about 2% for the remaining jobs. Particularly noteworthy is the drop in the *R*-factors on introducing hydrogens (no extra parameters); a parallel job using exactly the same model but excluding hydrogens showed that 1.25% of the drop in R_{free} was contributed by the hydrogens. On the other hand the drop in job 12 is caused almost entirely by the improvements to the model; the same job with the original weights gave an R_{free} of 10.90%. After using R_{free} to monitor the refinement as discussed here, a final refinement was performed against all 80102 unique reflections without any further changes to the model; this converged to R_1 = 8.77%, essentially identical to the final R_1 for the working set.

The more important keywords for macromolecular refinement are summarized in the following table (* indicates significant changes from version 5.0 of SHELXTL):

Keyword	Function
DEFS	Set global restraint esd defaults.
DFIX	Restrain 1,2-distance to target (which may be a free variable).
DANG*	Restrain 1,3-distance to target (which may be a free variable).
SADI	Restrain distances to be equal without specifying target.
SAME	Generate SADI automatically for 1,2- and 1,3-distances using connectivity.
CHIV	Restrain chiral volume to target (default zero; may be a free variable).
FLAT*	Planarity restraint.
DELU	Generate rigid bond U_{ij} restraints automatically using connectivity.
SIMU	Generate similar U (or U_{ij}) restraints automatically using distances.
ISOR	'Approximately isotropic' restraints.
BUMP*	Generate anti-bumping restraints automatically (incl. symm. equivalents).
NCSY*	Generate non-crystallographic symmetry restraints.
FVAR	Starting values for overall scale factor and free variables.
SUMP	Restrain linear combination of free variables.
PART	Atoms with different non-zero PART numbers not connected by program.
AFIX	Riding H, rigid groups and other constraints on individual atoms.
HFIX	Generate hydrogens and suitable AFIX instructions for their refinement.
MERG	'MERG 4' averages equiv. reflections., incl. Friedel opposites., sets all f " to 0.
SHEL	Set maximum and minimum resolution (data ignored outside range).
STIR*	Stepwise increase of resolution.
SWAT	Refine diffuse solvent parameter (Babinet's principle).
WGHT	Weighting scheme, probably best left at default 'WGHT 0.1' throughout.
CGLS	No. of cycles conjugate gradient least-squares, select R_{free} reflections.
BLOC, L.S.	Blocked-matrix least-squares (for esds).
RTAB, MPLA, HTAB*	Tables of bonds, angles, torsions, planes, H-bonds etc.
WPDB*, ACTA, LIST*	Output PDB and CIF files for archiving and data transfer.

Table 13.2 – Useful XL Keywords for macromolecules

14 XPRO: Protein Interface for SHELXTL

A new program XPRO has been added as an interactive user interface between XL and other programs often used by protein crystallographers. It is designed to be self-explanatory so that it can be used without constant reference to a manual. It is started by:

```
xpro name
```

When started, XPRO creates a log file *name.pro* and a Postscript output file *name.ps*. These may be printed after exiting from XPRO and provide text and graphical summaries of the operations performed. Many options in XPRO expect that the files *name.lst*, *name.fcf*, *name.pdb*, *name.res* etc. have been generated in a XL job using the LIST 6 and WPDB instructions. A menu of possible options is displayed by XPRO; choosing a particular option by typing the appropriate letter (upper or lower case) produces a detailed description of that option, after which the user has the choice of typing [Enter] to continue or N[Enter] to return to the menu. The menu consists of:

```
[F] New output filename                  [V] R(free) files
[A] Anisotropic scaling (Hope & Parkin)  [I] .ins from PDB file
[P] Progress of LS refinement diagram    [L] Luzzati plot
[T] Thermal displacement analysis        [E] Esd analysis
[U] Update .res (and .pdb) to .ins file  [N] NCS analysis
[R] Ramachandran Phi-Psi plot            [K] Kleywegt NCS plot
[M] Map file for O from .fcf             [O] PDB file for O
[H] .hkl file from other data formats    [B] PDB deposition
[D] Convert DENZO/SCALEPACK .sca to .hkl [C] Color plots (now on)
[X] Write XTALVIEW map coefficients      [W] Write Turbo-Frodo map
[S] Reflection statistics from .fcf      [Z] Least-squares fit
[G] Generate PDB file from .res or .pdb  [Q] Quit

Enter option:
```

The various options will now be discussed individually. Several of them add Postscript plots to the file *name.ps*. In these plots, the main-chain atoms are often color-coded according to the secondary structure, which the user is prompted for (blue for alpha-helix, green for beta-sheet and red for others). The side-chains are often color-coded according to residue characteristics:

Yellow	= Cys, Met
Green	= Phe, Tyr, Trp, His
Cyan	= Gly, Ala, Leu, Ile, Val, Pro
Red	= Glu, Asp
Blue	= Arg, Lys
Purple	= Gln, Asn
Gray	= Ser, Thr

14.1 Outline of the Available Features

The options provided by XPRO can be divided into three general groups.

14.1.1 Files and Communication with Other Protein Programs

[H] *.hkl* file from other data formats. This provides general interactive reformatting of reflection data files, avoiding the need to write a FORTRAN program or UNIX shell-script each time it is necessary to reformat reflection data.

[D] Convert DENZO/SCALEPACK *.sca* to *.hkl*. This is often the safest and quickest way of generating the *.hkl* reflection data file for XL, XS etc.

[V] R(free) files. This adds an R_{free} flag to selected reflections in an *.hkl* file; they may be chosen at random or in thin shells. This is the preferred method of calculating a *free R-factor* using XL, and requires the XL instructions CGLS n –1 or L.S. n –1.

[I] *.ins* from PDB file. This will normally be used when a structure is transferred from another program to XL for the first time. It generates most of the restraints and other extra instructions automatically as well as converting the atoms to fractional coordinates in SHELXTL format. For editing and updating between XL refinement cycles the following [U] option should be used instead.

[U] Update *.res* (and *.pdb*) to *.ins* file. This should be used to read the *.res* output file from a XL refinement job and update it to create the *.ins* input file for the next job. Alterations such as extra residues or disorder components may be added from a PDB format file written by a graphics program such as O or XtalView.

[G] Generate PDB file from *.res* or *.pdb*. Although XL can write a PDB format file directly, this option provides for more user interaction, e.g., for setting up a PDB format file containing symmetry equivalents or modified temperature factors for use with molecular replacement programs such as AMoRe.

[B] PDB deposition. Collects the information needed for PDB deposition from the *.lst* and *.pdb* files written by XL and creates a file according to the current specifications for deposition with the Brookhaven PDB. The resulting file contains all the compulsory records, but still requires some hand editing e.g., to include information about the data collection.

[F] New output filename. New *.ps* and *.pro* files are started and the previous *.ps* and *.pro* files closed. This enables the Postscript plots to be viewed in another window without leaving XPRO etc.

[C] Color plots (now on). This option toggles color on or off in the Postscript output files. For some journals it may be necessary to produce black and white diagrams rather than color.

[Q] Quit. Terminates XPRO and returns to the command line prompt.

14.1.2 Creation of Map (and pdb) Files for Various Graphics Packages

[M] Map file for O from *.fcf*. This creates a map file that can be read by O and some versions of FRODO. A variety of maps may be created, including Sigma-A maps. XPRO reads the *.fcf* file written by XL (it contains calculated structure factors and phases) and the *.pdb* file (in order to work out the extent of the map).

[W] Write Turbo-Frodo map. Very similar to the corresponding option for O.

[O] PDB file for O. The otherwise exemplary program O is unfortunately not able to read standard PDB format files (as written by e.g., XL) when they contain disordered groups. This option provides a (not very elegant) work-around.

[X] Write XtalView map coefficients. Writes a *.phs* file with coefficients for various types of map including Sigma-A maps for input to XtalView. XtalView should be instructed to calculate an F_o-map whatever type of map is actually required! This produces MUCH better maps than inputting the atoms from XL as a *.pdb* file into XtalView and repeating the structure factor calculation in XtalView (because of various incompatibilities such as the solvent model, anisotropic temperature factors, complex scattering factors as well as approximations made by XtalView in the structure factor calculation).

14.1.3 Analysis of a Structure after Refinement with XL

[P] Progress of LS refinement diagram. Produces a diagram of the *R*-factor as a function of the refinement cycle, with special action for automated water divining (XWAT). The *R*-factors are extracted from the REM instructions in the current *.res* file, which are accumulated there when the U option in XPRO is used to update the *.res* file written by one refinement job to create the *.ins* file for the next.

[T] Thermal displacement analysis. Creates bar-plots to show the variation of B-value (and anisotropy) with residue number for main-chain and side-chain atoms.

[R] Ramachandran Phi-Psi plot. A Ramachandran plot is created and the outliers listed. Reads the *.lst* file that must contain the necessary torsion angles calculated in XL using RTAB instructions.

[K] Kleywegt NCS plot. A Kleywegt plot is a Ramachandran plot with NCS-related residues joined by straight lines. The lines cross the edges of the plot and reappear at the other side if necessary. If the plot is too hairy you may be in trouble.

[N] NCS analysis. Creates bar-plots of differences in B-values and various torsion angles between NCS related monomers. These are read from the *.lst* file so the torsion angles should have been calculated using RTAB instructions in XL.

[S] Reflection statistics from *.fcf*. *R*-factors, data completeness, mean(I/sigma) etc. may be calculated for user-specified resolution ranges.

[L] Luzzati plot. Similar to [S] but the resolution ranges are fixed by the program and a Luzzati plot of *R*-factor against resolution is created as well as the statistics.

[E] Esd analysis. Graphical analysis of the esds estimated by a (blocked) full-matrix refinement using XL.

[Z] Least-squares fit. Allows parts of one or more structures to be fitted to each other and r.m.s. deviations calculated. The deviations may be plotted against residue number as bar plots and superimposed structures may be output in suitable format for preparing diagrams with MOLSCRIPT or the XP program in XTL.

[A] Anisotropic scaling (Hope & Parkin). Applies an anisotropic scaling analysis to the .fcf file output from XL using LIST 6. It is similar to the action of the HOPE instruction in XL, but is much faster. This instruction may be used as a quick check to see whether the introduction of the HOPE instruction would be justified.

14.2 Communication with Other Programs

The various options will now be described in more detail. Much of this information is provided by the program when an option is chosen. This section contains useful information on the best ways of using XL for protein refinements.

[H] .hkl file from other data formats

The program can read a variety of reflection data file formats and write a *.hkl* file in SHELXTL *.hkl* format. If the original file contained *F*-values, the *.hkl* file should be read into XL with HKLF 3; if the original file contained intensities, HKLF 4 is appropriate. The input file should contain one reflection per line, but lines may be stripped from the beginning and end, e.g., to process data transferred by email. On reading the file, the first line is displayed. To skip this line and move to the next, hit the [Enter] key. To read *h,k,l, F* (or F^2) and $\sigma(F)$ [or $\sigma(F^2)$] from this and subsequent lines in free format, enter the character * followed by [Enter>; to read in fixed format, fill the positions under these quantities with H,K,L,F or S. Thus to read a correctly formatted *.hkl* file, enter the line:

HHHHKKKKLLLLFFFFFFFFSSSSSSSS

For technical reasons, the following option [D] should always be used instead of [H] to read files produced by SCALEPACK.

[D] Convert DENZO/SCALEPACK .sca to .hkl

The SCALEPACK *.sca* and XL *.hkl* formats look very similar, but there are some subtle differences. The .sca file has three lines of header information but *.hkl* has no header. The *.hkl* file may be terminated by a line with all items zero that is not present in the .sca file; however both are also terminated by the end of the file. Unlike *.hkl*, the *.sca* file may contain floating-point numbers in 'I8' format. If the 'anomalous' flag was applied, the .sca file may contain reflections $\mathbf{h}_+$ and **h–** on the same line, with dummy values if not measured. The [D] option handles these differences and may also be used to extract anomalous ΔF values (with esds) for heavy-atom location using Patterson or direct methods in XS.

[V] R(free) files

This command is used to flag say 5 or 10% of the reflections in the *.hkl* file for use as a reference set in calculating free *R*-values (Brünger, 1992). As a rule of thumb, at least 500 reflections or 5% of the total number should be flagged, whichever is larger. It is difficult to obtain statistically meaningful free *R*-values for datasets containing a total of less than 5000 reflections before division into reference and working sets. The flag is applied by making the 'batch number' at the end of each line in the *.hkl* file negative. The unflagged reflections constitute the working set. The *.hkl* file is read into XL in the normal way using HKLF 4 (or 3), and the flags are ignored (i.e., all reflections are used for refinement and no free *R* is calculated) unless the second number on the CGLS (or L.S.) instruction is –1, in which case only the working set is used for the refinement, and only the reference set is used to calculate the free *R*-values. It is customary to perform the final refinement using all the data, but not increasing the number of independent parameters or reducing the weights of the restraints. This may be done by simply deleting the second number on the CGLS or L.S. instruction.

The reference set may either be chosen at random or in thin shells. The latter option is strongly recommended if a twinned structure is being refined or if NCS restraints are applied, because otherwise the reference and working sets will not be independent. When the reflections are averaged in XL, they are included in the final reference set only if all contributors have the R_{free} flag set, otherwise they are used in the working set. In such a case it is advisable to use thin shells rather than flagging the reflections at random, otherwise there will not be many reflections left in the reference set after averaging!

Note that if the second CGLS (or L.S.) parameter is negative (–N) with N not equal to 1, XL will generate its own reference set consisting of every Nth reflection (after merging) irrespective of the flags in the *.hkl* file. This possibility is retained for upwards compatibility with version 5.0 of SHELXTL, but is NOT RECOMMENDED, because the reference set may possibly change if a different space group. resolution range, merging procedure or a different version of XL is used, and because it is inappropriate for problems involving NCS or twinning.

[I] .ins from PDB file

Usually, when XL is used for a high-resolution refinement, a low-resolution or preliminary refinement will already have been performed with another program, or a model will be available from molecular replacement or map interpretation in the form of a PDB file. XPRO can read PDB files taken from the Brookhaven database as well as files written by X-PLOR and other widely used protein programs. The [I] option incorporates standard Engh & Huber (1991) restraints, and other instructions needed for a refinement job, into the .ins file. The program applies some consistency checks and searches for disulfide bridges, generating the necessary restraints automatically. The user may renumber the residues and must specify the residue numbers for N- and C-termini so that appropriate action can be taken. Since XL does not recognize chains, these must be flagged by adding e.g., 1000, 2000, ... to the residue numbers (note that the [B] and [G] options in XPRO provide the reverse transformation). It is advisable to ignore hydrogen atoms in the input PDB file because it is better to regenerate and refine them using the riding model in XL.

It is almost inevitable that some hand editing of the resulting *.ins* file will still be necessary. For example, XPRO is not able to define restraints, torsion angles and hydrogen atoms for residues that it doesn't recognize. Bad initial geometry may require the addition of FREE or BIND instructions so that the connectivity array is generated correctly by XL, and chain breaks, ligands or solvent molecules other than water may require special action. The [I] option, followed by any necessary hand editing, should be used once per structure before the first XL refinement. Thereafter it is much more convenient to use the [U] option in XPRO to update the *.res* file from one refinement job to produce the *.ins* file for the next., because special restraints and other instructions are retained, and because there are extra facilities for defining and checking disorder, solvent molecules, etc. The restraints incorporated into the *.ins* file are stored internally in XPRO, so no dictionary file is required (in contrast to the now obsolete program XPDB supplied with version 5.0 of SHELXTL, which used a dictionary file *xl.dic*).

[U] Update .res (and .pdb) to .ins file

This option converts a XL .res file to a new *.ins* file by including new or changed atoms from PDB format files such as those written by the graphics programs O, Turbo-Frodo and XtalView. All other XL commands are retained unchanged. This option also provides for setting up disorder refinement and updating the list of solvent molecules. The .res file should not contain instructions other than RESI, AFIX, PART and atoms between FVAR and HKLF, and both FVAR and HKLF must be present. Note that although it is possible to set up threefold or multiple disorders in this way, the necessary SUMP restraints must be edited into the .ins file later by hand; no extra editing is needed for twofold disorders. The [U] option may also be used without a *.pdb* file to update the *.res* file to *.ins* and apply various checks. It is recommended that the *.res* file is always updated to .ins in this way rather than by using an editor, so that the REM records that contain a summary of the course of the refinement are accumulated correctly; if necessary the resulting *.ins* file can then be edited further with a text editor before rerunning XL.

[G] Generate PDB file from .res or .pdb

The WPDB instruction in XL is normally used to write PDB format files, but the [G] option in XPRO provides additional editing facilities that are particularly useful for the creation of PDB format files for use as molecular replacement search models, and are also sometimes useful before calculating least-squares fits, etc. An *.ins*, *.res* or PDB format file serves as input. B-values may be reset automatically to typical values, disordered atoms, solvent molecules and H-atoms may be removed, chain ID's (not recognized by XL) may be (re)inserted, and multiple copies of chains may be generated using (non-)crystallographic symmetry. In the resulting PDB file all atoms are isotropic.

[B] PDB deposition

The [B] option reads files *.pdb* and *.lst* files written by the 'final' XL refinement job and creates a file with the default extension *.ent* in PDB format suitable for deposition in Brookhaven.

Some of this file is in the form of a template suitable for hand editing, e.g., to include literature references, experimental details, special features of the structure and refinement, etc. The user is prompted for details of chains and possible renumbering of the residues; except for structures consisting of a single chain, chain ID's should be (re)inserted in this way before deposition. The resulting file should contain all the compulsory records, but some of them will need completion by subsequent hand editing. The following notation is used to redefine residue numbers and chains. When prompted by the program, the new chain ID letter (the character '$' should be used if a blank chain ID is required) is followed by the first and last old residue numbers and the first new residue number. One chain should be specified per input line, and the list of chains is terminated by a blank line. Thus if there were two chains numbered 1001-1189 and 2001-2189, followed by waters with residue numbers 1-111, the following three lines should be entered:

```
A 1001 1189 1
B 2001 2189 1
$ 1 111 201
```

For example, residue 1001 in this example would become chain A residue 1. Similarly, residue 2189 becomes chain B residue 189. The solvent water that used to start at residue 1 now starts at residue 201.

For the deposition of reflection data, the CIF format *.fcf* file written by XL may be used directly.

14.3 Creation of Map (and pdb) Files for Various Graphics Packages

In a computer utopia, interactive graphics packages would all read the CIF format *.fcf* file written by XL directly; this contains all the information necessary for generating maps. For the couple of years before this comes to pass, XPRO provides the necessary generation of maps or (in the case of XtalView) coefficients. For the programs O and Turbo-Frodo, it is also necessary to define the region of space for which the map is calculated; XPRO does this by scanning a PDB file to find the maximum and minimum atomic coordinates in each direction. Furthermore, O is liable to be confused by disordered residues even if these are specified exactly according to the PDB rules (as XL does), so it is also necessary for XPRO (option [O]) to be able to modify the PDB file so that all disorder components are given separate residue numbers. Note that the option [U] provides the reverse procedure, i.e., separate residues obtained using O may be recombined as different disorder components of the same residue for refinement using XL. XPRO does not make the changes that may be required to the *all.dat* connectivity file read by O.

The [M], [O], [W] and [X] options should be self-explanatory. The following questions are asked by the program; usually the answers suggested by the program are suitable, so most of the questions are answered by [Enter].

```
Name of .fcf file created using XL and LIST 6 [name.fcf]:
Enter name of PDB file [name.pdb]:
Include all waters in the volume covered by map? [Y]:
Number of grid points per cell in x, y and z (the first two MUST be powers of 2, and
the last MUST be a multiple of 8) [64 64 88]:
Origin of map along x, y and z (grid points) [-32 -24 24] (must all be multiples of
8):
Extent of map along x, y and z (grid points) [128 136 88] (must all be multiples of
8):
Fourier type (-3=mFo-DFc (Sigma-A difference map), -2=2mFo-DFc  (Sigma-A map), -1=Fo-
Fc, 0=Fc, 1=Fo, 2=2Fo-Fc, n=nFo-(n-1)Fc [-2]:
Enter reference/working set Sigma-A ratio from XL [0.97]:
Apply sharpening (Y or N) ? [N]:
Enter name of map file [sigmaa.map]:
```

For XtalView, the questions about the grid are skipped. Note that there is a choice of maps. Thus the input '3' for the Fourier type generates a $3F_o$-$2F_c$ map; '4' gives a $4F_o$-$3F_c$ map, etc. The sigma-A ratio is calculated in each XL job that uses the free *R*-factor; it is designed to correct the sigma-A weight for overfitting. For refinement at low resolution this might be about 0.8, for medium resolution 0.9; the default is appropriate for structures with a high ratio of data to parameters. If the free *R*-factor was not used in the refinement, a estimated value should be input. 'Sharpening' multiplies the coefficients by $<F^2>^{¼}$, where $<F^2>$ is the mean reflection intensity in the appropriate resolution shell (this factor is used in preference to the almost identical factor $\sqrt{(E/F)}$ because the latter involves a statistical factor for certain reflections that is inappropriate for this application). Finally, the program outputs the maximum and minimum electron density (in sigma units as well as -electrons per cubic Å) and electron density histogram.

Note that XtalView MUST be told to do an F_o synthesis, whatever type of map the coefficients actually represent!

14.4 Analysis of the Refined Structure

The *.lst* file produced by XL contains a great deal of important information, but for proteins (in contrast to small molecules) it is not very economical to print it out and read it after every job. Many of the following options are designed to summarize the essential information in more digestible form, e.g., as Postscript plots. Usually the *.lst* and/or *.fcf* and sometimes the *.res* or *.pdb* files are required from a XL refinement job in which the LIST 6, FMAP 2 and WPDB instructions were employed.

[P] Progress of LS refinement diagram

At the end of each refinement job, and after each XL stage in the XWAT water divining procedure, XL outputs three lines of remarks to the *.res* file containing current R-values etc. If the *.res* file is edited to the next *.ins* file in such a way as to retain these remarks, they provide a convenient summary of the course of the refinement. The remarks are written after the HKLF instruction so they must be moved ahead of this instruction in order to be preserved; if the [U] option is used to update from *.res* to *.ins* this happens automatically. The [P] option extracts the *R*-factors from these remarks and prepares a Postscript plot of *R*-factor against refinement job number. Points that were part of the XWAT water divining procedure are plotted with a smaller horizontal gap between them. This plot provides a convenient summary of the course of refinement; it can be seen at a glance which stage produced the biggest drop in free *R*-factor, and whether *R* continues to fall but the free *R*-factor rises again, indicating over-refinement.

[T] Thermal displacement analysis

This reads a XL *.lst* file from an isotropic or anisotropic refinement and prepares Postscript bar plots of the mean (equivalent) B and (optionally) anisotropy (minimum eigenvalue divided by maximum eigenvalue) against residue number. The refinement should have been performed with FMAP 2, so that the residue diagnostics table is present in the *.lst* file. Unless black and white Postscript output is set, the main-chain plots are color coded according to secondary structure (it is useful to run PROCHECK first to obtain this information) and the side-chain plots by residue type. The color schemes are defined in the .pro output file.

Alpha-helices and beta-strands are entered one per line with 'A n1 n2' or 'B n1 n2' respectively, where n1 and n2 are the first and last residues of the helix or strand. The letters may be upper or lower case. The list is terminated with a blank line. Thus:

```
a 21 45
b 48 55
a 67 108
```

would define two alpha-helices (residues 21 to 45 and 67 to 108 resp.) and one beta-strand (48 to 55). The alpha-helix regions are colored blue, the beta-strands green, and the rest red. There may be up to four diagrams on one page, starting at the top. Each should be defined by entering three characters: a symbol to label the diagram, then either B (B-values) or A (anisotropy), followed by M (main-chain) or S (side-chain) and then the numbers of the first and last residues. END terminates the list. The program will suggest suitable parameters. A typical sequence, selecting these defaults by [Enter] each time, would be:

```
Next diagram [aBM 1 204]:
Maximum value and step for vertical scale [50 10]:
Next diagram [bAM 1 204]:
Next diagram [cBS 1 204]:
Maximum value and step for vertical scale [60 10]:
Next diagram [dAS 1 204]:
```

Note that no scale needs to be specified for the anisotropy, because the range is always from 0 to 1.

[R] Ramachandran Phi-Psi plot

The [R] option reads the XL .lst output file and extracts the psi and phi torsion angles to make Ramachandran plots. If the main-chain is disordered, only the PART 1 (and of course PART 0) atoms are used. Glycines are included optionally as open squares; prolines are treated as normal residues. A list of outliers appears on the screen and in the *.pro* file. Residues are color-coded according to residue type unless black and white Postscript has been specified (option [C] in the main menu). The refinement should have been performed with appropriate RTAB instructions for the phi and psi torsion angles and with FMAP 2, so that the residue diagnostics table is present in the .lst file. See Kleywegt & Jones (1996), who kindly provided the distribution table used in XPRO.

[K] Kleywegt NCS plot

This is the same as the normal Ramachandran plot (option [R] above) except that the phi/psi dots for each residue are smaller and residues related by non-crystallographic symmetry (NCS) are joined by lines (Kleywegt, 1996). The lines may cross the edges of the plot and reappear at the other side if this makes the differences between the angles smaller. Ramachandran outliers (as defined by Kleywegt and Jones) are also reported. This plot gives an immediate indication of how well NCS is obeyed for the main-chain atoms, and is also a good indicator of the overall quality of the structure. If the main-chain is disordered, only PART 0 and PART 1 atoms are considered. Glycines are optionally included as open squares; prolines are treated as normal residues. Unless color has been switched off (option [C]) the dots and lines are color-coded according to residue type. The refinement should have been performed with FMAP 2 and the RTAB instructions needed to calculate the phi and psi torsion angles in XL.

[N] NCS analysis

This option provides a detailed analysis of deviations from non-crystallographic symmetry (NCS). The Kleywegt plot [K] can also be used to provide an overall picture of how well NCS is obeyed by the main-chain torsion angles. Before using these options, a XL refinement should be performed in which RTAB is used to calculate the phi, psi, omega and chi1...chi4 torsion angles. The instruction FMAP 2 is also required so that the *.lst* file contains the residue diagnostics table. It is also useful to have secondary structure assignments to hand for color coding of the NCS bar plots; many standard protein programs such as PROCHECK are able to supply this information.

Differences (2 NCS related components) and maximum deviations and r.m.s. deviations (if there are more than two components) are plotted and tabulated as a function of the base residue number (i.e., the residue number minus the offset such as 1000, 2000 ... that XL uses instead of a chain ID). Because of the large number of factors involved this option requires some attention to detail.

Alpha-helices and beta-strands are entered one per line as 'A n1 n2' or 'B n1 n2' where n1 and n2 are the first and last residues of the helix or strand. Base residue numbers should be used and the list is terminated with a blank line. Then the numbers that have to be added to the base residue numbers to generate the NCS related units are defined in answer to a prompt by the program. For fourfold NCS the usual XL convention of numbering equivalent chains 1001..., 2001... etc. would require the input '1000 2000 3000 4000' here. The program then requests the minimum deviations in angles (deg.) and B for output to .pro file; 0 would print all and 999 would not print any.:

There may be up to four diagrams on one page, starting at the top. Each should be defined by entering three characters: a symbol to label the diagram, then either D (absolute difference [rms absolute difference from mean if more than 2 components]), M (maximum absolute deviation [from mean if more than 2]) or A (average), followed by the letter H (phi), Y (psi), P (phi and psi), O (omega), C (chi1), T (all chi), M (main-chain B) or S (side-chain B) and then the numbers of the first and last base residues. Note that A is only allowed with S or M and that P or T must be preceded by M. END terminates the list.

The default diagrams are:

aMH (diagram a; maximum absolute deviation of phi angles)

bMY (diagram b; maximum absolute deviation of psi angles)

cMO (diagram c; maximum absolute deviation of omega angles)

dMT (diagram d; maximum absolute deviation of all chi angles)

eMM (diagram e; maximum absolute deviation of main-chain B)

fMS (diagram f; maximum absolute deviation of side-chain B)

gAM (diagram g; average main-chain B)

hAS (diagram h; average side-chain B)

[S] Reflection statistics from .fcf

This option creates reflection statistics from a *.fcf* file written by XL in response to a LIST 6 instruction. The user must specify the resolution ranges, e.g., to be the same as those used for data reduction. A table of data completeness, *R*-factors etc. is written to the console and to the .pro output file.

[L] Luzzati plot

This plots the resolution vs. *R*1. The *.fcf* file must have been created using LIST 6 in XL. XPRO outputs a Postscript Luzzati (1952) plot, which gives estimates of the average errors in atomic coordinates for an incompletely refined structure assuming perfect data, NOT (as widely assumed by people who have not read this paper which happens to be in French) estimates of the esds in the atomic positions. For small proteins and high resolution data, esds in individual bond lengths and atomic positions may be estimated rigorously using XL (see the [E] option in XPRO described below). Nevertheless, a plot of *R*-factor against resolution is always entertaining.

[E] Esd analysis

This option reads XL *.lst* file and prepares Postscript scatter-plots of esds in atom positions and bond lengths against (equivalent) B values. The refinement should normally have been performed with the XL instructions L.S. 1, DAMP 0 0, BLOC 1 and BOND. If geometrical restraints were used in the refinement the bond length esds will be very low, but high resolution data are required to perform such a refinement without restraints. Similarly the damping has to be switched off because this can also lead to underestimated esds. Disordered atoms, atoms on special positions, and atoms other than C, N and O are not included in the diagrams. Such atoms are recognized by the first letter of their names in the atom coordinate table, so it may be necessary to remove calcium and other atoms that might be mistakenly identified from this table by editing the *.lst* file before running XPRO.

A quadratic may be fitted to the atom radial esds, which enables the results to be compared with the formula suggested by Cruickshank (1996). Note that this formula predicts positional esds in one direction, which should be a factor of $\sqrt{3}$ smaller than the radial esds output by XL.

[Z] Least-squares fit

The [Z] option may be used to perform a least-squares fit of two molecules, taken from the same or different structures. The iterative quaternion method is employed. This option is of necessity rather complex, and it is important to read each request for information by the program carefully because the default action (<Enter>) may well not be suitable and an incorrect answer can lead to complications.

It is necessary first to define the first molecule (called 'current structure'), which is extracted from a PDB format file. The a second molecule ('model') is obtained from another (or possibly the same) PDB file. Both PDB files may be as output by XL or may be taken directly from the PDB databank, so 'chains' may be present. Since the residues may be numbered differently in the two molecules, it is necessary to convert the residue numbers in both molecules to a matching set of residue numbers referred to as XPRO residue numbers. These numbers are also used to annotate the plots etc. The set of residues used for fitting is in general a subset of those used for the plots and calculation of r.m.s. esds.

After performing the fit for specified atoms in each of the specified residues, the program prints the r.m.s. deviation of the atoms fitted and the largest individual deviations (greater than 2σ). Then appears the question:

New current structure (C), new model (M), Repeat fit (R), write PDB file (P), XP file (X), Postscript bar plot of differences (D) or exit (E) [E]:

'R' repeats the fit (possibly using different residues and atoms) of the 'model' (second molecule) to the 'current structure' (first molecule). 'M' replaces the 'model' but keeps the 'current structure'. 'C' starts again with a new 'current structure'. 'P' writes a new PDB format file that contains the two molecules as two separate chains with the XPRO residue numbers; this can be used as input to the program MOLSCRIPT. 'X' writes an orthogonal coordinate file that can be read by the Bruker SHELXTL program XP and used to make a (stereo) Cα-trace of the superposition. 'D' prepares a Postscript bar plot of the differences between the two molecules, using all stored residues, not just those that were fitted.

[A] Anisotropic scaling (Hope & Parkin)

This option reads an *.fcf* file created using the LIST 6 instruction in XL, and writes a NEW *.hkl* file after application of anisotropic scaling by the method of Parkin, Moezzi & Hope (1995). The modification of the observed structure factors in this way is scientifically suspect and is intended for testing purposes only. It is much better to use the HOPE instruction in XL so that parameter correlations are taken into account and the observed data are not modified. The XPRO correction provides a quick test as to whether HOPE in XL will result in a significant improvement; in this case the question about the filename for corrected data should be answered with [Enter]. A 'local' R_{free} test is applied to establish how many parameters [none(!), 12, 18 or 24] may justifiably be fitted. A significant improvement is not to be expected if anisotropic refinement has been performed or if a large number of symmetry equivalents were merged in the data reduction.

15 XWAT: Automated Water Divining

A simple program XWAT has been added that iteratively recycles XL to provide automatic water divining. This may be regarded as a cheap and inadequate imitation of ARP (V. Lamzin & K.S. Wilson, *Acta Cryst.* **D49** (1993) 129-147), but is relatively easy to use and useful if you intend to take a holiday. XWAT is started by means of a command line with OPTIONAL UNIX-type switches (the filename must come last):

```
xwat name
```

or, e.g.,

```
xwat -n10 -s4 -u0.6 -r0.8 -m50 -f name
```

These are the default settings for the switches -n (number of overall cycles), -s (scattering factor number for oxygen), -u (starting isotropic U for new waters), -r (water rejected if U refines to greater than this value), -m (maximum number of waters to be added in one cycle) and -h (half/full occupancies) or -f (full occupancies only). All switches present must come before 'name'.

Standard XL files *name.ins* and name.hkl are required; the *.ins* file should contain 'CGLS 3 -20', 'FMAP 2', 'PLAN 200 2.4' or 'PLAN 200 -2.4' (half occupancies allowed), 'CONN 0 O_501 > LAST', 'BUMP' or similar instructions (the free R test is not obligatory) and MUST include at least one water at the end of the atom list. The waters will then be assigned dynamical residue numbers starting with the residue number of this water (501 in the above example) and should all have residue class 'HOH' and atom name 'O' with one atom per residue and no PART numbers. On starting, XWAT makes a backup copy (*name.bak*) of the *.ins* file, since the *.ins* file is repeatedly overwritten during the recycling. The recycling may be terminated tidily before the preset number of iterations has been performed by creating a file *name.end* in the same directory; this operates like the *name.fin* file for XL, but is 'deleted' by XWAT once per iteration.

XWAT calls XL once each cycle, then edits the resulting *.res* file to prepare the *.ins* file for the next cycle. The $R1$ (and $R1_{free}$, if present) indices are extracted from the *.lst* file and included in the *.res* files as remarks; these and other remarks (REM) provide a protocol of the refinement, and may be converted to a Postscript plot using the "P" option in XPRO. Note that the XPRO option "U" provides the facilities necessary to update that solvent etc. interactively, in much the same way that XWAT does automatically.

By changing the PLAN instruction to (say) 'PLAN 200 1 1' and leaving out the BUMP instruction it might be possible to emulate ARP in its structure extension mode; this has yet to be tested, but might be useful for completing high-resolution (better than 2Å) structures.

16 Examples of Macromolecular Refinement

The following extracts from the file *6rxn.ins* (provided together with *6rxn.hkl* as an example) illustrate a number of points. The structure was determined by Stenkamp, Sieker & Jensen, (1990) who have kindly given permission for it to be used in this way. As usual in *.ins* files, comments may be included as REM instructions or after exclamation marks. The resolution of 1.5Å does not quite justify refinement of all non-hydrogen atoms anisotropically ('ANIS' before the first atom would specify this), but the iron and sulfur atoms should be made anisotropic as shown below. Note that it would be better to flag the R_{free} reflections randomly using XPRO rather than just using every twelfth reflection.

```
TITL Rubredoxin in P1 (from 6RXN in PDB)
CELL 1.54178 24.920 17.790 19.720 101.00 83.40 104.50  ! Lambda & cell
ZERR       1  0.025  0.018  0.020   0.05  0.05   0.05  ! Z & cell esds
LATT -1                       ! Space group P1
SFAC  C   H   N  O   S  FE    ! Scattering factor types and
UNIT  224 498 55 136 6  1     ! unit-cell contents

DEFS 0.02 0.2 0.01 0.05       ! Global default restraint esds

CGLS 10 -12    ! 10 Conjugate gradient cycles, every 12th reflection
SHEL 999 0.1   ! for R(free), all other data used for refinement
FMAP 2         ! Difference Fourier
PLAN 200 2.3   ! Peaksearch and identification of potential waters

LIST 6    ! Output phased reflection file to generate maps etc.
WPDB      ! Write PDB output file
HTAB      ! Output analysis of hydrogen bonds (requires H-atoms !)

DELU $C_* $N_* $O_* $S_*    ! Rigid bond retraints - ignored for iso.

SIMU 0.1 $C_* $N_* $O_* $S_*  ! Similar U restraints - iso. or anis.
    ! Esd should be changed to ca. 0.05 if whole structure is anis.

ISOR 0.1 O_201 > LAST   ! Approximate isotropic restraints for waters;
                        ! ignored for isotropic

ANIS_* FE SD SG         ! Make iron and all sulfur atoms anisotropic

CONN 0 O_201 > LAST    ! Don't include water in connectivity array and
BUMP                   ! generate antibumping restraints automatically

SWAT                   ! Diffuse water model

REM HOPE               ! Anisotropic scaling not included

MERG 4 ! Remove MERG 4 if Friedel opposites should not be merged

MORE 1 ! MORE 0 for minimum, 2 or 3 for more output for diagnostics

REM Special restraints etc. specific to this structure follow:

REM HFIX 43 C1_1       !
DFIX C1_1 N_1 1.329    ! O=C(H)- (formyl) on N-terminus
DFIX C1_1 O1_1 1.231   ! incorporated into residue 1
DANG N_1 O1_1 2.250    !
DANG C1_1 CA_1 2.435   !

DFIX_52 C OT1 C OT2 1.249     !
DANG_52 CA OT1 CA OT2 2.379   ! Ionized carboxyl at C-terminus
DANG_52 OT1 OT2 2.194         !

SADI_54 0.04 FE SG_6 FE SG_9 FE SG_39 FE SG_42 ! Equal but unknown Fe-S
SADI_54 0.08 FE CB_6 FE CB_9 FE CB_39 FE CB_42 ! distances around Fe
```

```
REM HFIX 83 SG_38 SG_138  ! -SH for remaining cysteine (disordered)

DFIX C_18 N_26 1.329            ! Patch break in numbering - residues
DANG O_18 N_26 2.250            ! 18 and 26 are bonded but there is a
DANG CA_18 N_26 2.425           ! gap in numbering for compatibility
DANG C_18 CA_26 2.435           ! with other rubredoxins that have an
FLAT 0.3 O_18 CA_18 N_26 C_18 CA_26  ! extra loop
RTAB Omeg CA_18 C_18 N_26 CA_26      !
RTAB Phi C_18 N_26 CA_26 C_26        !
RTAB Psi N_18 CA_18 C_18 N_26        !

REM DFIX from CSD and R.A.Engh & R.Huber, Acta Cryst. A47 (1991) 392.
REM Remove 'REM ' before HFIX to activate H-atom generation

REM HFIX_ALA 43 N
REM HFIX_ALA 13 CA
REM HFIX_ALA 33 CB

REM HFIX_ASN 43 N
REM HFIX_ASN 13 CA
REM HFIX_ASN 23 CB
REM HFIX_ASN 93 ND2

REM HFIX_ASP 43 N
REM HFIX_ASP 13 CA
REM HFIX_ASP 23 CB
```

... etc ...

```
REM HFIX_VAL 43 N
REM HFIX_VAL 13 CA CB
REM HFIX_VAL 33 CG1 CG2

REM Peptide standard torsion angles and restraints

RTAB_* Omeg CA C N_+ CA_+
RTAB_* Phi C_- N CA C
RTAB_* Psi N CA C N_+
RTAB_* Cvol CA

DFIX_* 1.329 C_- N
DANG_* 2.425 CA_- N
DANG_* 2.250 O_- N
DANG_* 2.435 C_- CA

FLAT_* 0.3 O_- CA_- N C_- CA

REM Standard amino-acid restraints etc.

CHIV_ALA C
CHIV_ALA 2.477 CA
```

```
DFIX_ALA 1.231 C O
DFIX_ALA 1.525 C CA
DFIX_ALA 1.521 CA CB
DFIX_ALA 1.458 N CA
DANG_ALA 2.462 C N
DANG_ALA 2.401 O CA
DANG_ALA 2.503 C CB
DANG_ALA 2.446 CB N

RTAB_ASN Chi N CA CB CG

CHIV_ASN C CG
CHIV_ASN 2.503 CA

DFIX_ASN 1.231 C O  CG OD1
DFIX_ASN 1.525 C CA
DFIX_ASN 1.458 N CA
DFIX_ASN 1.530 CA CB
DFIX_ASN 1.516 CB CG
DFIX_ASN 1.328 CG ND2
DANG_ASN 2.401 O CA
DANG_ASN 2.462 C N
DANG_ASN 2.455 CB N
DANG_ASN 2.504 C CB
DANG_ASN 2.534 CA CG
DANG_ASN 2.393 CB OD1
DANG_ASN 2.419 CB ND2
DANG_ASN 2.245 OD1 ND2

RTAB_ASP Chi N CA CB CG

CHIV_ASP C CG
CHIV_ASP 2.503 CA

DFIX_ASP 1.231 C O
DFIX_ASP 1.525 C CA
DFIX_ASP 1.530 CA CB
DFIX_ASP 1.516 CB CG
DFIX_ASP 1.458 CA N
DFIX_ASP 1.249 CG OD1 CG OD2
DANG_ASP 2.401 O CA
DANG_ASP 2.462 C N
DANG_ASP 2.455 CB N
DANG_ASP 2.504 C CB
DANG_ASP 2.534 CA CG
DANG_ASP 2.379 CB OD1 CB OD2
DANG_ASP 2.194 OD1 OD2

RTAB_CYS Chi N CA CB SG

CHIV_CYS C
CHIV_CYS 2.503 CA

DFIX_CYS 1.231 C O
```

```
DFIX_CYS 1.525 C CA
DFIX_CYS 1.458 N CA
DFIX_CYS 1.530 CA CB
DFIX_CYS 1.808 CB SG
DANG_CYS 2.401 O CA
DANG_CYS 2.504 C CB
DANG_CYS 2.455 CB N
DANG_CYS 2.462 C N
DANG_CYS 2.810 CA SG
```

... etc ...

```
RTAB_VAL Chi N CA CB CG1
RTAB_VAL Chi N CA CB CG2

CHIV_VAL C
CHIV_VAL 2.516 CA

DFIX_VAL 1.231 C O
DFIX_VAL 1.458 N CA
DFIX_VAL 1.525 C CA
DFIX_VAL 1.540 CA CB
DFIX_VAL 1.521 CB CG2 CB CG1
DANG_VAL 2.401 O CA
DANG_VAL 2.462 C N
DANG_VAL 2.497 C CB
DANG_VAL 2.515 CA CG1 CA CG2
DANG_VAL 2.479 N CB
DANG_VAL 2.504 CG1 CG2

WGHT    0.100000
FVAR       1.00000   0.5   0.5   0.5   0.5

RESI   1  MET
C1      1  -0.01633   0.35547   0.44703  11.00000   0.11817
O1      4   0.01012   0.32681   0.48491  11.00000   0.17896

N       3   0.00712   0.35446   0.37983  11.00000   0.11863
CA      1   0.05947   0.33273   0.35391  11.00000   0.06229
CB      1   0.07411   0.33732   0.27909  11.00000   0.15678
CG      1   0.03196   0.28864   0.22872  11.00000   0.14569
SD      5   0.04907   0.31846   0.14359  11.00000   0.23570
CE      1   0.11380   0.29170   0.12261  11.00000   0.21476
C       1   0.10634   0.38738   0.39766  11.00000   0.09178
O       4   0.10329   0.45513   0.41972  11.00000   0.16480
RESI   2  GLN
N       3   0.14741   0.35678   0.40741  11.00000   0.08599
CA      1   0.18940   0.39931   0.45565  11.00000   0.09291
CB      1   0.22933   0.34643   0.45886  11.00000   0.13253
CG      1   0.27354   0.38674   0.51173  11.00000   0.09866
CD      1   0.24547   0.38838   0.58387  11.00000   0.05748
OE1     4   0.22482   0.32772   0.60689  11.00000   0.16301
```

```
NE2      3   0.24704   0.46053   0.62045  11.00000   0.10164
C        1   0.22198   0.47895   0.43826  11.00000   0.08193
O        4   0.25019   0.48377   0.38408  11.00000   0.10402

RESI   3  LYS
N        3   0.21781   0.54034   0.48673  11.00000   0.07413
CA       1   0.25088   0.62006   0.47934  11.00000   0.05181
CB       1   0.21991   0.68311   0.51795  11.00000   0.09646
CG       1   0.16130   0.66288   0.49255  11.00000   0.10455
CD       1   0.12843   0.72146   0.52924  11.00000   0.22324
CE       1   0.10532   0.70085   0.60053  11.00000   0.26354
NZ       3   0.05943   0.74195   0.62796  11.00000   0.40338
C        1   0.30678   0.63497   0.50917  11.00000   0.05714
O        4   0.31462   0.59598   0.55179  11.00000   0.07986
```

etc ...

```
RESI  12  GLU
N        3   0.41413   1.09215   0.48246  11.00000   0.06790
CA       1   0.37955   1.01183   0.48195  11.00000   0.05761
PART   1
CB       1   0.32666   1.01321   0.52971  21.00000   0.12219
CG       1   0.29679   0.93111   0.54638  21.00000   0.15333
CD       1   0.25357   0.93709   0.60700  21.00000   0.20272
OE1      4   0.24346   1.00278   0.63210  21.00000   0.26315
OE2      4   0.23012   0.87537   0.63031  21.00000   0.21375
PART   2
CB       1   0.32549   1.01718   0.52772 -21.00000   0.12065
CG       1   0.27756   0.94582   0.50954 -21.00000   0.15928
CD       1   0.22547   0.95184   0.55635 -21.00000   0.20457
OE1      4   0.20774   0.90241   0.59575 -21.00000   0.22329
OE2      4   0.20259   1.00588   0.55325 -21.00000   0.31441
PART   0
C        1   0.36477   0.97439   0.40859  11.00000   0.04768
O        4   0.34317   1.00861   0.37369  11.00000   0.06890
```

etc ...

```
RESI  38  CYS
N        3   0.77141   0.92674   0.00625  11.00000   0.10936
CA       1   0.78873   0.97402   0.07449  11.00000   0.13706
PART   1
CB       1   0.83868   1.04271   0.05517  41.00000   0.11889
SG       5   0.89948   1.00271   0.02305  41.00000   0.18205
PART   2
CB       1   0.84149   1.03666   0.06538 -41.00000   0.14933
SG       5   0.83686   1.10360   0.01026 -41.00000   0.17328
PART   0
C        1   0.74143   1.01670   0.10383  11.00000   0.08401
O        4   0.70724   1.02319   0.06903  11.00000   0.10188
```

```
RESI  39  CYS
N       3   0.74699   1.04547   0.17051  11.00000   0.08888
CA      1   0.70682   1.09027   0.20876  11.00000   0.06869
CB      1   0.72588   1.11964   0.28230  11.00000   0.04269
SG      5   0.67932   1.17560   0.33481  11.00000   0.08016
C       1   0.70922   1.16093   0.17333  11.00000   0.06208
O       4   0.75427   1.20325   0.15858  11.00000   0.07437
```

etc ...

```
RESI  52  ALA
N       3   0.33596   0.63469   0.69557  11.00000   0.04662
CA      1   0.30961   0.68882   0.74487  11.00000   0.08939
CB      1   0.34040   0.77357   0.74194  11.00000   0.13277
C       1   0.24852   0.67507   0.73435  11.00000   0.09032
OT1     4   0.22236   0.72170   0.77321  11.00000   0.11368
OT2     4   0.22682   0.61667   0.69191  11.00000   0.08341

RESI  54  FE
FE      6   0.72017   1.22290   0.43784  11.00000   0.07929

REM Only the waters with high occupancies and low U's have been
REM retained, and all the occupancies have been reset to 1, with
REM a view to running the automatic water divining.  Water
REM residue numbers have been changed to start at 201.

RESI 201  HOH
O       4   0.13450   0.53192   0.60802  11.00000   0.13132
RESI 202  HOH
O       4   0.84795   0.53873   0.69488  11.00000   0.15273
RESI 203  HOH
O       4   0.27771   0.95750   0.25086  11.00000   0.11315
RESI 204  HOH
O       4   0.37066   0.71872   0.90376  11.00000   0.10854
```

etc ...

```
RESI 233  HOH
O       4   0.27813   1.38725   0.25914  11.00000   0.10698

HKLF 3
```

17 CIF, XCIF and Electronic Publication

17.1 CIF Archive Format

The *CIF* format represents a major step forward in the archiving, publication and communication of crystallographic data. At last it is possible to publish crystal structures and incorporate structural data into the crystallographic databases without the expensive and error-prone retyping of tables by hand. CIF format also provides a convenient method of transferring data from one program system to another. The ACTA instruction instructs XL to write two CIF-format files: *name.fcf* contains the reflection data and ‘name.cif’ all other data. These files contain all the items needed for archiving the structure; those answers not known to XL (e.g., the color of the crystal) are left as a question mark. In general the final 'name.cif' file should be edited using XCIF or any text editor to replace most of these question marks. The file is then suitable for deposition in the CSD (organic) and ICSD (inorganic crystal structure) databases.

For publication of a routine structure determination via electronic mail it will normally be necessary to add the authors’ names, title, text etc., which may also be done in CIF-format; this is followed by the edited contents of one or more *.cif* files each describing one structure (or possibly the same structure at different temperatures etc.). In general XL provides all the CIF identifiers required by Acta Cryst. except those that begin with ‘_publ’. Further details are given below, and an example of a paper submitted to Acta Cryst. in this way may be found in the file *example.cif* (it has been brought up to date for the 1997 requirements for authors; whether it would pass the new stricter quality controls is another matter!). XL users are strongly recommended to familiarize themselves with the definitive paper by the I.U.Cr. Commission on Crystallographic Data by Hall, Allen & Brown (1991), and with the current Acta Crystallographica Instructions for Authors.

Since the archiving of macromolecular data in CIF format is still being debated, XL only creates a standard ‘small molecule’ CIF file, suitable for Acta Cryst. etc.; a macromolecular CIF file is likely to contain much more information. However the LIST 6 instruction in the new version of XL does produce a CIF format reflection data file suitable for archiving with the PDB. This file also contains all the information necessary for the calculation of electron density maps, though as yet it appears that no standard macromolecular graphics package is able to read CIF format. Macromolecular coordinates etc. should be deposited in PDB format; XPRO provides the necessary facilities for extending the *.pdb* file produced by XL so that it can be used as a template for deposition.

17.2 The Auxiliary Program XCIF

XCIF is a simple program that reads CIF files and convert them into tables. It may prove useful for padding out Ph.D. theses and for submission of table to old-fashioned journals. It is also intended as an example of how to read CIF files, and it is hoped that X users will be able to modify it for their own purposes. XCIF is started by the command:

```
xcif name
```

where `name` is the first component of the filenames for the structure in question. XCIF enables tables to be produced from the *.cif* or *.fcf* files written by XL and provides the following facilities, which may be selected from a simple menu.

Tables of crystal data, atom parameters, bond lengths and angles, anisotropic displacement parameters and hydrogen atom coordinates may be produced in a format specified in a file *XCIF.???* (where *???* is any three letter combination). A standard ASCII file *XCIF.def* is provided; users may use it as a model for preparing standard ASCII tables files for input to word processors etc.

The format file is simply copied to the output file, except that directives (lines beginning with '?' or '$') have a special meaning, '\n\' (where n is a number) is replaced by the ASCII character n (e.g., \12\ starts a new page), and CIF identifiers (which begin with the character '_') are replaced by the appropriate number or string from the CIF file. CIF identifiers may optionally be followed (without an intervening space) by one or more of: '<n', '>n', ':n' and '=n' where n is an integer; the CIF identifier (including qualifier) must be terminated by one space that is not copied to the output file. '<n' left justifies the CIF item so that it starts in column n, and is usually used for strings. '>n' right justifies a string or justifies a number so that the figure immediately to the left of the decimal point appears in column n; if there is no decimal point then the last digit appears in column n. In either case the standard deviation (if any) extends to the right with brackets but without intervening spaces. If '<n' and '>n' are both absent, the CIF item is inserted at the current position. If ':n' is absent the item is treated as a string (see above), otherwise it is treated as a number; n is the power of 10 with which the CIF item should be multiplied, and is useful for converting Å to pm or printing coordinates as integers; n may be negative, zero or positive. '=n' rounds the CIF item (after application of ':n') so that there are not more than n figures after the decimal point; n must be zero or positive.

A line beginning with 'loop_' is repeated until the corresponding loop in the CIF file is exhausted; all the CIF items in the line must be in the same loop in the CIF input file

A line containing at least 4 consecutive underscores is copied to the output file unchanged, and may be used for drawing a horizontal line. There are also two pseudo-CIF-identifiers: '_tabno' is the number of the table, and '_comno' is a number or text string to identify the compound. Both may be set via the XCIF menu. '_tabno' but not '_comno' is incremented each time it is used.

An underscore '_' followed by a space may be used to continue on the next line without creating a new line in the output file. Lines beginning with question marks are output to the console (without the leading question mark) as questions; if the answer to the question is not 'Y' or 'y', everything in the format file is skipped until the next line which begins with a question mark. Lines beginning with a dollar '$' are not interpreted as text, but are scanned for the following strings (upper or lower case, quotes not essential):

'xtext': output should be formatted for the Bruker SHELXTL XCIF program (which now incorporates XTEXT, which was a separate program in version 4 of SHELXTL).

'xtext,deutsch': as above, but translated into German.

The above directive, if present, should be the first line of the format file.

The directive $symops:n, where n is an integer, prints the symmetry operations used to generate equivalent atoms, starting each line of text in column n. These operators are referenced by '#m' (where m is an integer) after the atom name. The line beginning '$symops:n' usually follows the tables of selected bond lengths and angles, torsion angles and hydrogen bonds.

The remaining directives may appear at any point in the format file except immediately after a continuation line marker, but always on a line beginning with '$'.

'h=none': leave out all hydrogen atoms.

'h=only': leave out all non-hydrogen atoms.

'h=free': leave out riding or rigid group hydrogens but include the rest.

'h=all': include all hydrogen and all other atoms.

The hydrogen atom directives apply only to tables of coordinates; hydrogen atoms are recognized by the type symbol 'H'. A common user error on writing format files is to forget that 'h=only' etc. applies until it is replaced by another 'h=...' directive! The publication flags can be used to control which hydrogen atoms appear in tables of bond lengths, angles etc.

'brack': Atom names should include brackets (if present in the CIF file).

'nobrack': Brackets are deleted from the atom names.

'flag': Only output items for which the publication flag is 'Y' or 'y'.

'noflag': Output all items, ignoring the publication flag.

The default settings are '$h=none,brack,flag'. The standard tables file *XCIF.def* illustrates the use of most of these facilities. XCIF extends some of the standard CIF codes to make them more suitable for tables, and also takes special action when items such as _refine_ls_extinction_coef are missing or undefined.

The above description refers to the version of XCIF distributed with XL. The simplest method of altering the contents and format of results tables is to create a different XCIF.??? format file (or a collection of such files for various purposes), using the standard file XCIF.def as a starting model. Thus the output can be tailored to different journals, doctoral theses, reports, etc.

XCIF includes a procedure for replacing undefined data items by values taken from one or more other files conforming to CIF rules. Thus items such as diffractometer or area detector operating parameters, details of absorption corrections, and crystal color, which are unknown to XL, can be incorporated from separate files. This is more reliable than using a text editor.

17.3 Using XL CIF files for Publication in Acta Crystallographica

The process of converting a virgin XL CIF output file into an electronic manuscript submission for Acta Cryst. Section C may seem at first rather complex and daunting, but the journal's Instructions for Authors are very detailed, and much of the conversion is routine and can be semi-automated; it can soon become an accustomed habit!

The important first step is to be properly informed of what is involved. The I.U.Cr. makes a variety of useful information available, and it can conveniently be accessed in its most up-to-date form at the World Wide Web location http://www.iucr.ac.uk/welcome.html by any standard Web browser. Printed Instructions for Authors can be found each year in the journal itself, and copies are available on request from The Managing Editor, I.U.Cr., 5 Abbey Square, Chester CH1 2HU, England. The Chester office can also supply copies of a technical account of how a CIF becomes a printed paper (reprinted from McMahon, 1993), and of 'A Guide to CIF for Authors' (published in 1995).

For a manuscript describing a single structure, the XL CIF output needs only the addition of a well-defined set of publication information (the items that begin with '_publ'), itself in correct CIF format. A template for this can be obtained by ftp from I.U.Cr., and the XL CIF output is attached to the end of it. Into the template are inserted (by any standard text editor) items such as manuscript title, authors' names and addresses, descriptive text, some extra experimental details as necessary (such as chemical synthesis and crystallization details, and a description of hydrogen atom refinement procedures), literature references, acknowledgments, and figure captions. There is also a place for inserting a formal submission letter. Some parts of the XL output need changing; in particular, bond lengths and angles to be printed in the journal must be identified by changing their publication flag from '.?' to 'yes'.

When the CIF appears to be ready for submission, its completeness and validity can be checked anonymously by emailing it to the address checkcif@iucr.ac.uk; a report will be automatically generated and returned by email listing and CIF syntax errors and any unrecognized data items. If there are no errors, the file is also checked for completeness and for some aspects of self-consistency (geometry is checked against coordinates, and possible higher symmetry is searched for). Any errors or omissions should be corrected and the checkcif procedure repeated, until everything is correct.

Beware of adding anything to emailed CIF submissions which does not accord with the syntax rules. In particular, there must be no non-CIF lines at the beginning or end of the message, and this includes automatically appended email signatures! These should be disabled or, safer, set up such that every line begins with the # character, which signals a CIF comment line to be ignored.

There is also a facility for previewing a manuscript in the form which will be produced from the CIF. Sending the CIF by email to printcif@iucr.ac.uk will produce, as a reply message, a PostScript file of the manuscript; this can be printed or viewed by appropriate software. A useful feature is the highlighting (in bold) of any items which may subsequently be queried by editorial staff, and it may be possible to deal with these potential problems now, before final submission.

When everything is ready and checked, the CIF is emailed to med@iucr.ac.uk; after automatic checking is complete, a reply will list any problems requiring attention, will give a Co-Editor reference, and will ask for further material to be sent. This includes structure factor data, figures (diagrams), a copyright transfer form, and a formal signed letter of submission. The last two must still be sent by normal mail, but the others can be transferred electronically (ftp), using the method specified in the Instructions for Authors and the submission acknowledgment. None of these items should be sent until the acknowledgment and reference code arrive.

If these instructions are followed carefully, the editorial process should proceed smoothly! We are grateful to Bill Clegg for writing much of this chapter.

18 XP: Molecular Graphics

XP provides interactive molecular geometry calculations, thermal motion analysis, least-squares planes, symmetry transformations and generation of symmetry-equivalent atoms, librational analysis, contoured electron density and a wide variety of options for the display of crystal structures on the workstation. Hidden lines are removed automatically in all plots. Atoms may be labeled interactively in the mono and stereo molecule plots, as may the cell axes in packing plots. XP may be used as an interactive graphical editor to read the .res files written by the SHELXTL refinement program XL and prepare the .ins files for input to the next refinement job.

XP may be started by typing the command `XP` at the system prompt (or `XP` followed by the first part of the .res filename). XP is driven by a command line interface, but many of the individual commands enter a menu-driven mode. In the command line interface, local recall and editing of the command line may be performed using the cursor, [Ins], [Del] or [BS] keys (as in the DOSKEY facility under MS-DOS); the [PgUp] and [PgDn] keys may be used to browse through recent text output. Verbose output may be truncated with [Esc] (without impairing the browse facility). Many graphical operations may also be interrupted with [Esc]. The [F10] key toggles a molecular diagram on and off in the top right-hand corner of the screen; the DIAG instruction is used to create this diagram.

Examples of plots which may be created using XP may be obtained by typing `DEMO` in response to the "#" prompt. DEMO (and indeed any XP command) may be interrupted by pressing the [Esc] key which returns to the "#" prompt. To return to DOS, type `EXIT` or `QUIT`.

When generating structure diagrams, note that MPLN/N almost always produces a convenient orientation for plotting a molecule (the molecule is oriented according to its inertial axes) and that FMOL followed by GROW (usually with one atom specified) is a good way of isolating a single molecule or ion, including symmetry generated atoms, if it lies on a crystallographic symmetry element.

Bond types and the connectivity table may be edited by JOIN, LINK, PRUN and UNDO, and parameters for plotting atoms by ATYP and ARAD. However, very often the default settings will produce sensible plots, e.g., TELP or PERS for a ball-and-stick plot (TELP 3 -50 for stereo thermal ellipsoids) or SFIL or SPIX for a space filling model.

PROJ provides a quick view of the molecule with menu-driven options to change the orientation, display atom names, or real-time rotation of a wire-frame model.

PICK, possibly followed by SORT, may be used to edit a peak list from direct methods or a difference Fourier, with deletion and renaming of the peaks/atoms. ENVI and or SRCH may be used to search for symmetry related atoms, H-bonds etc. Such symmetry generated atoms may be included in the atom lists by SGEN followed by FMOL. The least-squares plane (MPLN), ROTA, MATR, and PROJ instructions enable a convenient orientation to be selected. Ball and stick, thermal ellipsoid, and space filling plots may then be generated (PERS, TELP, SFIL, SPIX); in the case of SPIX, a hardcopy may be generated on a color printer by pressing [Ctrl-P] whilst the plot is on the screen. The MGEN, PGEN and PACK instructions provide a variety of automatic and interactive options for packing plots, and POLP and POLY provide general polyhedral displays of inorganic or mineral structures.

There are routines for thermal motion analysis (RIDE, LIBR), model fitting (OFIT), and poster preparation (POST). POST provides facilities for drawing chemical formulas, combining plot files, and adding formulas and text to plot files. Plots may be displayed (VIEW), plotted on an HPGL pen-plotter (DRAW), or plotted using the HP LaserJet or Deskjet (RAST). HPGL and Postscript files may be generated (DRAW) for plotting on other devices.

The instructions usually require an atom list to be specified, possibly using keywords, e.g., “C1 TO C6 O1 O2” or “ALL LESS $H.” If no atom list is specified, “ALL” is assumed. Both the local atoms list (i.e., for the current instruction) and the atom list defined by the last FMOL (or GROW or UNIQ) instruction have special significance; the local list is interpreted as a subset of the current FMOL list.

If the atoms in the .res file were assigned to residues (by means of RESI instructions in the .res file) they may be referenced by e.g., CB_21 (meaning atom CB in residue number 21). Otherwise CB would refer to all atoms named CB in all residues. This convention is (for good reasons) slightly different to the use of atom names without residue numbers in XL. Residue numbers and classes may be changed by the use of the RESI/S instruction.

XP also recognizes the PART numbers used in XL to describe disorder. The FMOL instruction sets different bond types for different parts. The instruction:

```
FMOL LESS PART2
```

provides a convenient way of selecting the dominant conformation (i.e., PARTs 0 and 1) of a structure.

```
# READ name
```

typed on the keyboard will cause data (and possibly instructions) to be read from the file name.res, or if it is not found, the file name.ins. Cell dimensions, symmetry, atom coordinates, etc., are extracted and saved. The following instructions could then be typed in; the “#” prompt is output by the computer, and each instruction is completed by [Enter]:

```
# FMOL LESS $H
# BANG/L
# MPLN/N
# PROJ
# PERS
# SPIX
# TELP 3 -50
```

This sets up a connectivity table for all atoms except hydrogen, prints the resulting bond lengths and angles, finds (but does not list) the best least-squares plane through all atoms, displays a line diagram of the structure on the screen with a view direction perpendicular to the plane (the molecule may then be rotated etc. by using the mouse or cursor keys), displays a ball and stick model on the screen with perspective (PERS), displays a space filling model (SPIX), and then outputs a stereo thermal ellipsoid molecule plot in the same orientation to the plot file. After this plot file is displayed on the screen, the interactive atom labeling routine is entered.

XP is compatible with any mouse which is “Microsoft compatible.” If no mouse is available, the cursor keys may be used to imitate mouse motion, and the [F6], [F7] and [F8] keys instead of the left, center and right mouse keys.

18.1 Structural Data

The following XS or XL instructions are interpreted by XP. They may be input at the console or read directly from a .res or .ins file: TITL, CELL, LATT, SYMM, SFAC, END, MOVE, and atom instructions. Atom colors, radii, and the type of shading to be used by TELP are determined by the scattering factor number and the element symbol in the corresponding position on the SFAC instruction; the standard values may subsequently be altered if necessary by ATYP and ARAD. If atoms are renamed by NAME or PICK the program tries to deduce the atom type from the new name; this can lead to problems, e.g., when a carbon atom is named ‘CA’. Other XS and XL instructions are completely ignored. MOVE is applied immediately to each subsequent atom read; PUSH may be used to move atoms in the current (FMOL) list. Atoms are always stored in the order in which they are read, unless sorted (SORT), but further atoms may be added at any time. All information (except the title which is reset by a new TITL instruction) is reinitialized by the CELL instruction. Continuation lines are allowed for all instructions (as in XS and XL). Orthogonal coordinates may also be input, for example, to display the results of theoretical calculations.

18.2 Orientation Matrix

The program employs orthogonal coordinates XO, YO, and ZO so that the XO axis is parallel to $\mathbf{a}^*$, YO to $\mathbf{c}\times\mathbf{a}^*$, and ZO to $\mathbf{c}^*$. The coordinates X', Y' and Z' for plots on the computer screen are defined so that the axis X' runs from top to bottom, Y' runs across the screen from left to right, and Z' comes out of the screen towards the viewer. The orientation matrix P gives plot coordinates in terms of orthogonal coordinates:

$X' = P_{11}.XO + P_{12}.YO + P_{13}.ZO$ etc.

This matrix is initialized (as a unit matrix) by CELL, and may be changed by the MPLN, MATR, PROJ, and ROTA instructions. The PREV instruction resets the orientation matrix to the settings immediately preceding the last change.

18.3 Symcodes

XP employs symmetry codes for referencing symmetry operations similar, but not identical, to “ORTEP codes” (it was necessary to change the definition around to allow space groups with more than 100 operations including lattice centering). The code N555 refers to the initial coordinates transformed by symmetry operator N; to add 1.0 to the resulting x coordinate, the 555 is replaced by 655 etc. Thus the XP instruction:

```
SGEN C17 12564
```

adds a new atom with a unique name assigned by the program (e.g., C17A) to the atom list: the coordinates are generated from those of C17 by applying symmetry operator 12 and then adding 1.0 to y and -1.0 to z. New atoms generated in this way are automatically included in the current “FMOL list.” A list of symcodes and the appropriate symmetry operations may be obtained by typing “SYMM” on its own. The PGEN and MGEN instructions output the symcodes for the symmetry equivalent polyhedra and molecules which they generate, enabling these to be used with SGEN if more user control is desired.

18.4 Keywords

XP instructions may be followed by various keywords, separated by one or more spaces. The allowed keywords are: atom names, TYPEn, ALL, TO, LESS and LPT. The “?” character may be used as a “wild card” in atom names; it matches all characters, including a blank. Thus S?? would find sulfur atoms with two-digit numbers and silicon atoms with one-digit numbers, but $S would only find only sulfur (see $E below).

ALL	Means all atoms in the list.
TO	Defines a string of consecutive atoms.
LESS	Is used to omit atoms from the list.
TYPEn (n=1,2,...9)	Means all atoms of SFAC type n.
PARTn (n=0,1...9)	All atoms with PART number n.
$E	All atoms of element E (a one or two character element symbol).
LPT	Sends output to the printer (file) for the current instruction.
/L	The switch /L has the same effect as the keyword LPT.

A variety of other switches are specific to individual commands, e.g., FMOL/N.

For example:

```
# MPLN/L LESS $H
```

generates a least-squares plane through all non-hydrogen atoms, with printer output. If no atom names or keywords that could generate atom names are given, “ALL” is assumed. Similarly if “LESS” appears before any atom name, “ALL LESS” is understood. Thus “PROJ LESS $H” displays all non-hydrogen atoms. The FMOL instruction sets up a connectivity table for a subset of the input atom list. This table is used by subsequent instructions, which also use keywords to define local subsets of the current FMOL atom list. On the FMOL and KILL instructions, an additional keyword REST is permitted; it means all atoms not in the current FMOL list, and should not be used in combination with other keywords or atom names.

18.5 Atoms

Atoms are input exactly as for the program XL. An atom name consists of up to four characters, terminated by one or more spaces. Atom names are recognized simply by first checking whether they are legal XS, XPS, XL or XP instructions; thus occasionally a mistyped instruction may be mistaken for an atom (it can be deleted by KILL in XP).

If an atom with the same name is already in the XP list, BOTH atoms are retained. It is advisable to avoid this situation, because all subsequent references apply to all atoms with the same name. If you do find that by accident two atoms have identical names, you can use PICK followed by the name to rename one or both of them.

On an atom instruction, the name is followed by SFAC type, x, y, z, site occupation factor and either U (isotropic) or U_{11}, U_{22}, U_{33}, U_{23}, U_{13} and U_{12} in order (anisotropic). Alternatively the site occupation factor and U may be omitted (the default values are 1.0 and 0.04). The SFAC type may be any non-zero integer; if it is negative, the absolute value is used. The SFAC TYPE is only used for the keyword “TYPEn” and output of atom lists to file (ORTH and FILE); the atom NAME is employed by the program to set up initial radii and colors. The usual XL conventions for fixing parameters and tying parameters to “free variables” apply, but are decoded as soon as the atom is read into XP. Similarly, any MOVE instruction is applied ONLY to subsequent atoms read (the default “no action” setting may be restored by: MOVE 0 0 0 1). AFIX instructions are saved for use by FILE when writing a new .ins file, but are not otherwise used in XP. HADD may be used to generate idealized hydrogen atoms.

Note the use of the SRCH, MGEN, PGEN, ENVI, UNIQ, GROW and SGEN facilities for finding and/or generating symmetry equivalent atoms and NAME or PICK for changing atom names.

18.6 Plot Files

A number of instructions -- TELP, SFIL, PACK, POST, EDEN, OFIT, POLP and DIAG -- create plot files. These contain all necessary plotting instructions encoded in the form of two fundamental operations (change color, and plot a string of straight line segments, possibly with retracing). A technical specification of plot file format may be found in Appendix E. Because this format is so simple, it is easy to integrate other programs and graphics devices into the system. For example letters and numbers (in atom labels) are also encoded in this way; hardware generated characters are not used. The XP instructions VIEW and DRAW output plots to the graphics screen and HP (or compatible) plotters respectively; VIEW/W plots against a white background. The DRAW instruction may be used to plot a color plot file as black on white by answering the question about the number of pens with '1'. DRAW may also be used to convert plot files to HPGL or Postscript format. The RAST command in XP produces raster plots directly on HP LaserJet or Deskjet printers.

Before the XP instructions TELP, PACK, SFIL, EDEN, OFIT, PACK or POLP create a plot, the user is asked to provide a name for it; the plot is then displayed on the screen as it is being created. In the case of POST, the plot file name may be specified on the command line. If plot generation is interrupted by [Esc] the plot file is deleted. Atom labels (SFIL, TELP, EDEN or OFIT) or axis labels (TELP CELL) may be added interactively (type HELP LABELS for details). An “H” or “Q” instruction during interactive labeling also deletes the plot file. The POST instruction provides facilities for annotating and combining plot files.

The realistic space-filling models generated by SPIX are bitmaps and so are device-dependent; the resolution and number of colors that may be used is different for different devices. They may be output directly to a color HP Deskjet printer by hitting [Ctrl-P] whilst the diagram is displayed on the screen, but may not be stored as plot files. The hardcopy is produced at the resolution of the printer (300 dpi) and is independent of the resolution of the computer monitor.

18.7 Colors

The following table shows the color employed by XP and their standard assignments:

Code	Monitor	Deskjet	Pen-Plotter	Suggested Use
0	brown	black	black	bonds
1	green	green	green	F, Al, Ni, Fourier peaks ($Q)
2	dark red	ed	ed	Pt, Fe, Br
3	dark blue	blue	blue	Co
4	yellow	orange	orange	S, Na
5	purple	magenta	purple	P, K, Mn
6	white	light green	green*	H (*turquoise for 8-pen plotters)
7	gray†	gray	black	C (†black for PERS and SPIX)
8	blue	cyan	blue	D, N, Ru, Ag
9	dark green	green	green*	Cl, others (*dark green for 8-pen)
10	lilac	purple	purple	Li, Cr, I
11	orange	orange	orange	B, Sn, Au
12	turquoise	light cyan	blue*	Cu, Si, Os (*turquoise for 8-pen)
13	brown	brown	orange	Se, Sb, Mo
14	dark gray	gray	black	As, Hg
15	red	red	red	O

Table 18.1 – XP color assignments

Plotter pens should be inserted in the following order (note that this is different to the order in version 4 of SHELXTL, to conform with HP and MICROSOFT): Black, red, green, orange, blue, purple, (turquoise), (dark green). Colors are specified by means of ATYP instructions, and are ignored when producing red-green stereo plots. The program uses the above list to set default colors on reading in the atoms (the atom name is used, not the SFAC type). Color 0 is used for bonds (TELP, DIAG) and the cell outline (PACK). Atom names (PROJ, DOTS, DIAG, TELP) are given the same color as the corresponding atom. VIEW/W displays a plot file against a white background, plotting color 0 as black, 4 as orange and 6 as light blue.

18.8 Labels (Atoms, Names, and Axes)

The EDEN, SFIL, TELP, DIAG, DOTS, OFIT, and PACK commands enable labels to be added interactively to plots. This option is available for mono and stereo plots. After creating the plot the program temporarily places the atom name (exactly as it will appear on the plot) in the bottom right hand corner of the screen, and then sets a box-cursor on the first atom (or cell corner) to be labeled. The cursor may be moved using the mouse or cursor keys. To accept a label at the current position, click the left mouse button or hit the [space] bar; to leave a label out, type [Enter]. To abort the plot, type Q (or H) and to indicate that no further atoms need to be labeled, type E (or B). To go back to the PREVIOUS label drawn, type [BS]. This deletes the previous attempt from the plot file and screen. When all atoms have been labeled, the computer bleeps; [Enter] should then be typed to delete the plot from the screen (but keep the plot file) and return to the “#” prompt. Any characters other than those defined above are ignored during the labeling.

18.9 Molecular Plots: Examples

XP is designed to provide acceptable molecular diagrams with a minimum of user input, while retaining many flexible options for the more ambitious. To illustrate the standard options first, consider the following command sequence for the YLID test crystal. The program XP must first be started by entering “XP” at the DOS prompt, and then each command is input when the “#” prompt appears. Before running these demonstrations, you will need to copy all the files from the examples directory into your own directory.

```
> XP
# READ YLID.ATS
# FMOL
# MPLN/N

# PERS
# SPIX
# TELP 0 -50
# SFIL
```

The first instruction causes the file ylid.res (and failing that, ylid.ins) to be read. It is then necessary to set up a “current atom list” and generate a connectivity table using FMOL. The MPLN/N instructions calculates the best plane through all the atoms (but doesn't print it); then provides a good viewing orientation for the subsequent plots. PERS produces a quick “ball and stick” plot on the screen, and SPIX a “space filling model” with highlights. In both cases [Enter] is entered to clear the screen and return to the “#” prompt. Note in the above example that if no atoms are specified, “ALL” is implied. The last two instructions create plot files for subsequent plotting on a pen-plotter (DRAW) or laser-printer (RAST). XP prompts for the name of the plot file; for the purposes of this demonstration, “TEMP” may be given as an answer each time, creating a plot file temp.plt. TELP 0 -50 creates a mono plot with ellipsoids for the non-hydrogen atoms (like an “ORTEP” plot); TELP with no numbers would create a mono ball and stick plot (like “PLUTO”). SFIL creates a space-filling plot file (also like “PLUTO”) as shown in Figure 18.1.

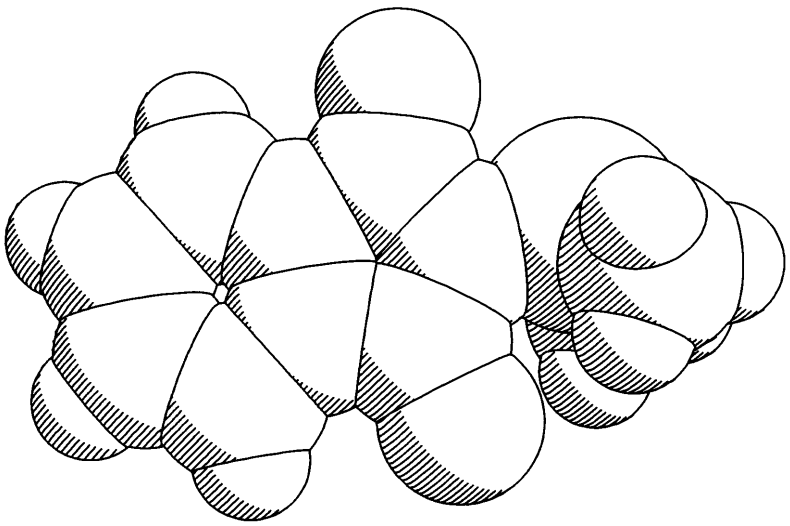

Figure 18.1 – Space-filling plot

Both SFIL and TELP enter the automatic atom labeling routine. In the second example, the bond types and atom shading are also changed. [Enter] follows each command:

```
# READ BORSON
# FMOL
# MPLN/N (select an initial viewing direction)
# DIAG(display diagram in top right corner)
# RING 1 (type 1 [filled] for all cyclic bonds)]
# JOIN 2(make all bonds type 2 [open])
# JOIN 5 $H (single line bonds to hydrogen)
# JOIN 4 B1 S11 (dotted open bond for dative S11!B1)
# ATYP 9 LESS $H (dotted representation for non-H atoms)
# ATYP 8 $N
# ATYP 7 $B $S
# TELP
```

This is followed by automatic atom labeling of the plot (which resembles a “SCHAKAL” plot with dotted highlighted atoms) shown in Figure 18.2:

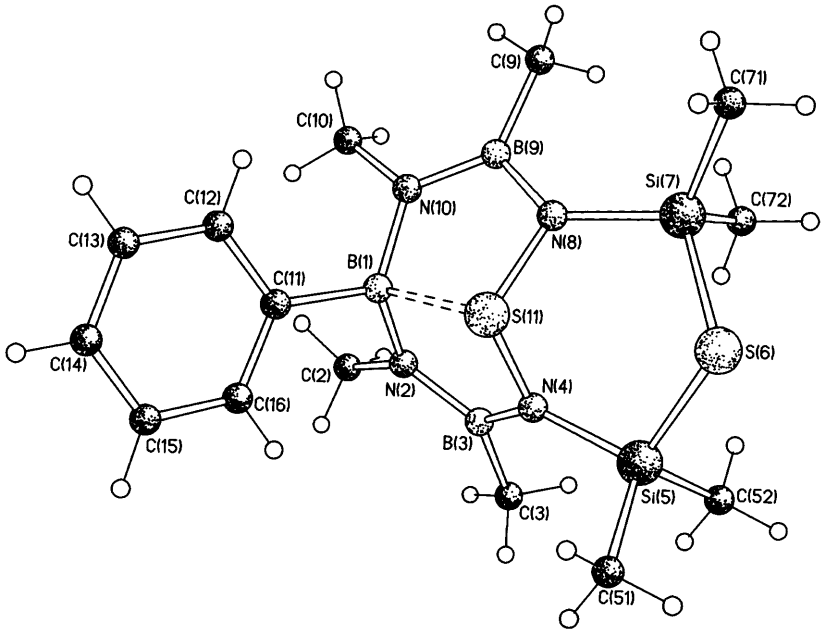

Figure 18.2 – Bond types and atom shading

A final molecule example involves the generation of symmetry equivalent atoms (GROW) and illustrates a useful technique for C_5H_5 rings: a dummy atom (which does not appear in the final plot) is generated at the middle of each ring, and is connected to the titanium atom with a dotted bond. The Ti-C “bonds” are removed by PRUN Ti 2, the two (shorter) Ti-N bonds being retained. PROJ followed by rotations (using the menu) selects a suitable orientation for the stereo molecular plot (TELP 3).

```
# READ PLINIUS
# FMOL
# GROW
# PROJ
# CENT/X C1 C2 C3 C4 C5
# CENT/X C1A C2A C3A C4A C5A
# PRUN TI 2
# LINK 6 TI X1A
# LINK 6 TI X1B
# PROJ LESS $H
# RING
# LABL 2 400
# TELP 3
```

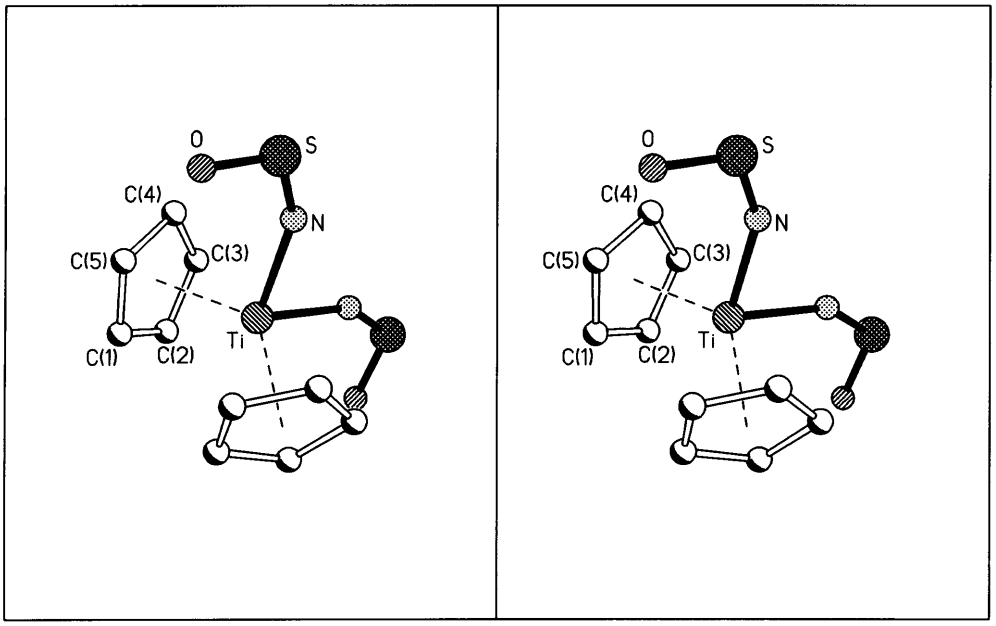

Figure 18.3 – Stereo view including symmetry equivalent atoms

18.10 Packing Plots: Examples

The general philosophy of the routine PACK is to generate automatically a little more of the structure than is required, then to prune it down interactively to produce an artistically effective and chemically meaningful plot. In the first example (a cyclopropane dicarboxylic acid), the molecule (which lies on a twofold axis) is completed using GROW, a good view direction is selected along the shortest (b) cell axis with MATR 2, and a plot box of size 15 Å (screen width) by 6 Å (depth) is defined.

```
# READ CARB2T
# FMOL LESS $H
# GROW
# MATR 2
# PBOX 15 6
# PACK
```

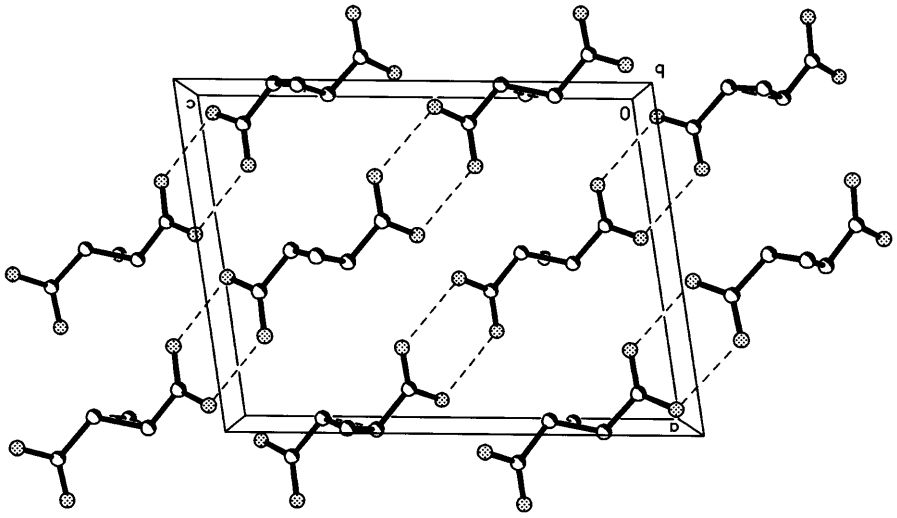

Figure 18.4 – Packing plot including hydrogen bonds

PACK takes a little time to “fill” the box defined by PBOX. After the contents are displayed, they may be selectively edited using the menu options “Scan mols” and “Scan non-bonds” to remove superfluous molecules (in this example, one above and one below the plot) and the three short O...O distances which are not hydrogen bonds. Then there are three menu options for creating simplified plots, plus the option “SGEN/FMOL” which sets up new lists containing all the atoms and bonds currently displayed (including symmetry transformations to anisotropic temperature factors, etc. as appropriate). After exiting from PACK with this option, “TELP CELL” can be used to generate a more detailed packing plot (with atom labeling and a choice of ways to display atoms and bonds, Figure 18.4). The MGEN instruction finds all molecules for which the specified atoms or their symmetry equivalents lie within a given box in crystal coordinates, and so provides a non-interactive alternative to PACK. Unlike PACK, MGEN does not generate additional H-bonds, etc.

In the next example, an inorganic structure containing Sb, S, and O (H atoms were not located) is used to demonstrate the polyhedral representation:

```
# READ MERCIER
# FMOL
# ENVI $SB
# SGEN O11 4544 (it is necessary to find and then
# SGEN O33 4545complete the environment of each
# SGEN O41 3545antimony; the sulfate groups are
# SGEN O23 2555already complete)
# MATR 3
# ATYP SB1 1 12(different colors are used for the
# ATYP SB2 4 5two different Sb-polyhedron types
# PGEN $SB $S 01 02 1 1.3
```

The atom list is now complete, and a quick polyhedral plot may be produced on the screen by entering:

```
# POLY $SB $S
```

In this case just “POLY” is sufficient. POLY produces a display only, not a plot file. The alternative instruction:

```
# POLP $SB $S
```

(or just “POLP”) is slower, but writes a plot file too, so that hard-copy output may be produced (Fig. 18.5). In both plots the sulphate groups appear as yellow tetrahedra, and the irregular antimony polyhedra are purple and light blue. Note that if we had forgotten to set the two different colors and styles for the antimony polyhedra before using PGEN, we could still have set them between the PGEN and POLP (or POLY) instructions by using “wild” characters, e.g.,

```
# ATYP SB1 SB1? 1 12
```

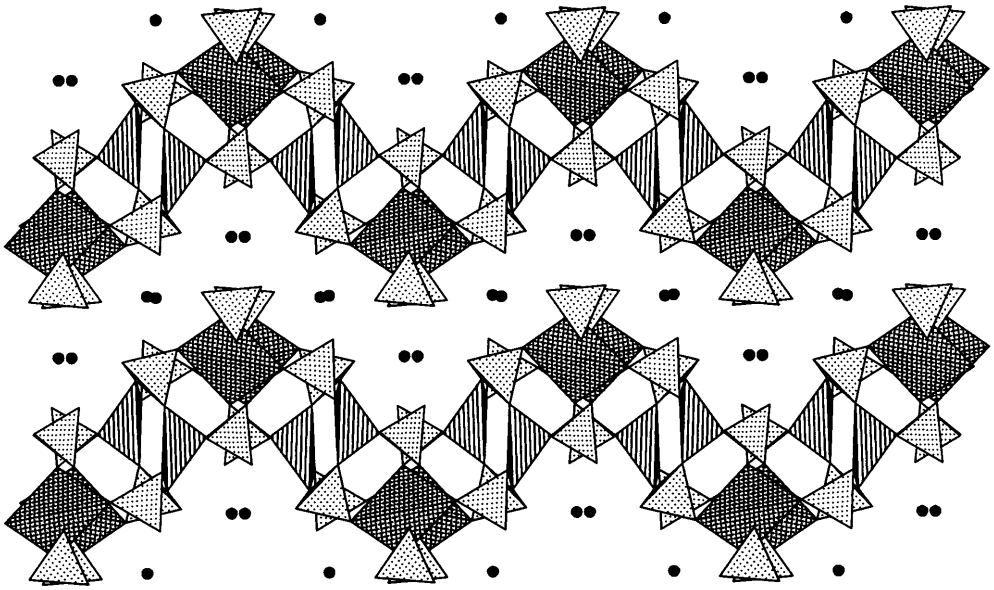

Figure 18.5 – Very discerning viewers may notice that POLP has accidentally created an M. C. Escher-type picture, and also find out why!

Polymeric structures may also be conveniently processed by PACK; GROW only generates atoms less than one cell apart.

```
# READ AGS4
# FMOL
# GROW AG AS
# PERS
# ATYP AG 4 5
# MATR 3
# PBOX 25 3 .5 .5 0 (new center because Ag and As have z=0)

# PACK .5 .5        (to avoid superfluous "non bonds")
```

PACK is followed by the "**`Plot +3`**" menu option (Figure 18.6) and "SGEN/FMOL" menu options. Then PERS or TELP CELL produces an excellent plot of a layer of the polymeric structure (Figure 18.7). Alternatively MGEN may be employed instead of PBOX and PACK:

```
# MGEN 1.6 2.6 .5 .5 .5 0 AG AS
```

It is necessary to follow MGEN with FMOL, since MGEN, in contrast to PACK, doesn't generate the S-S bonds to complete the polymeric network. On the other hand, MGEN is faster.

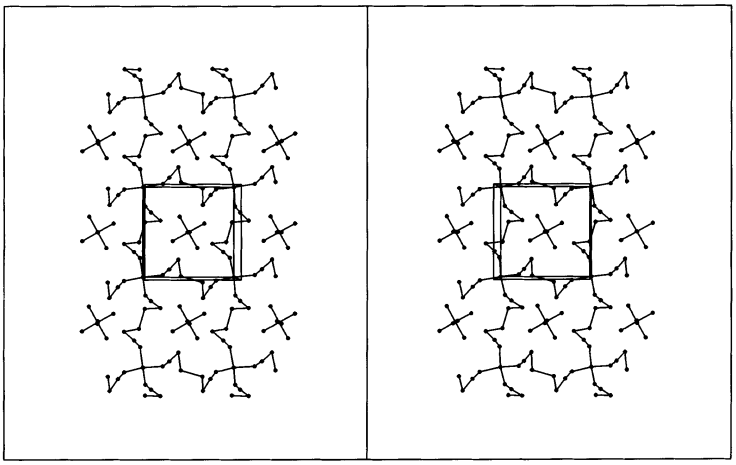

Figure 18.6 – Plot of polymeric structure generated using PACK

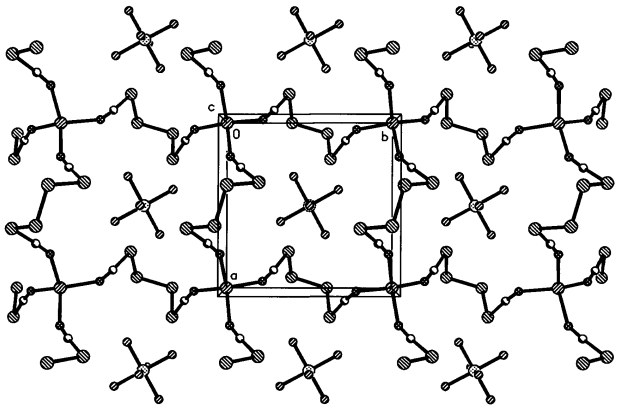

Figure 18.7 – Layer of polymeric structure

18.11 Molecule Fitting: Examples

Data are provided for two steroid isomers, which differ only in the configuration of an $HCCH_3$ unit in the five membered ring.

```
# READ AZIDEX
# FMOL
# MPLN/N
# OFIT C1 TO C10

Orthogonal coordinates from file: AZIBET
Invert model (Y or N): N
Enter model atoms to be fitted to.....
C1 = =
...
```

In this case the numbering of the two isomers is the same, so answer '=' followed by [Enter] for each C and just [Enter] to each H atom. A list of deviations is followed by a weighted R.M.S. deviation (here, 0.0597 Å) and idealized model crystal coordinates. The fit may be displayed as a plot file with the "fitted" atoms labeled (via the automatic atom labeling routine), as shown in Figure 18.8.

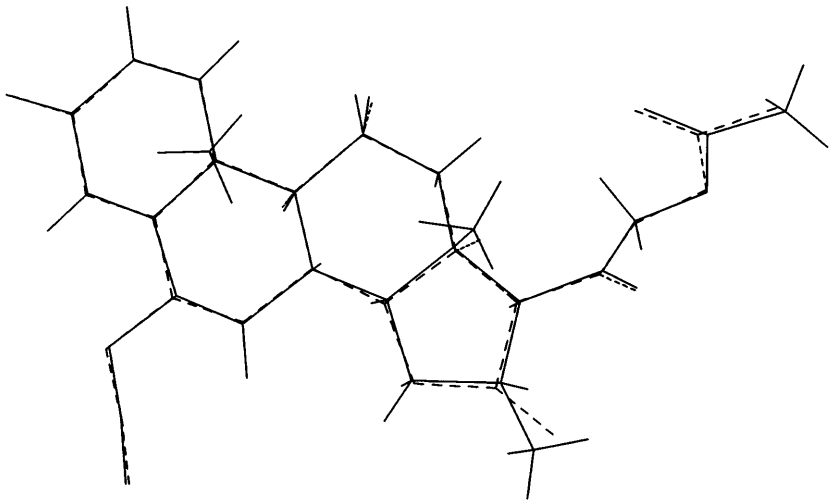

Figure 18.8 – Model fitting plot

18.12 Librational Analysis: Examples

Two XP instructions provide librational analysis: LIBR uses the full Schomaker-Trueblood tensor analysis, and is suitable for a general rigid molecule; and RIDE may be used for a “riding model” (i.e., ligands attached to a heavy atom). Both can be illustrated with the YLID data:

```
# READ YLID.ATS
# FMOL LESS $H
# LIBR
# RIDE S1
```

Both methods calculate librationally-corrected bond lengths, but subject to different assumptions. LIBR describes the motion of the group, which is assumed to be “rigid,” in terms of libration, translation, and libration-translation correlation tensors. RIDE calculates the parallel and perpendicular amplitudes of the atoms along the bonds, and deduces lower limit (LL), riding (2/1) and upper limit (UL) corrected bond lengths:

```
RMS AMPLITUDES IN ANGSTROMS PARALLEL AND PERPENDICULAR TO BONDS UN = UNCORR. BOND, LL
= LOWER, UL = UPPER LIM., 2/1 = ATOM 2 RIDES ON 1BOND 1-2

  U1(PAR)  U2(PAR)  U1(PER)  U2(PER)   R(UN)    R(LL)   R(2/1)   R(UL)
S1 C10   0.212    0.214   0.175    0.251    1.791    1.794   1.809    1.886
S1 C2   0.172    0.170    0.196    0.188   1.708    1.708   1.708    1.794
```

It will be seen that the riding model is justified for C10 and C11 (the two methyl carbons) on S1, because U2 (perpendicular) is significantly larger than the other three amplitudes, i.e., the R(2/1) value may be used as the corrected bond length. For C2 on S1, this model is (quite reasonably) not justified by the amplitude components.

18.13 Electron Density Contouring: Examples

The EDEN instruction in XP provides facilities for calculating slant Fouriers in a plane section defined by the user, and contouring the Fourier interactively. By way of example, a structure (HOF) is provided in which a difference electron density synthesis is performed to locate the hydrogen atoms; from chemical evidence it was not clear whether a >C=N-H unit was *cis* or *trans*. Further details of this structure, including the hydrogen atom location, may be found in Chemische Berichte 117 (1984) 2681-2685.

Before starting XP and setting up electron density contouring with, for example, READ, FMOL, and MPLN/N, it is necessary to run XL with LIST 3 for the refined structure (the XL job will often include several cycles of least-squares refinement) so that F_o, F_c etc. are written to the .fcf file. This .fcf file is then read by EDEN in XP. This XL job has been run for HOF and the resulting files hof.res and hof.fcf may be used for this test (hof.hkl is not required for the test, but is provided for users who wish to repeat the XL job with different parameters). Note that, for the purpose of determining electron density or finding hydrogen atoms, it is a good strategy to weight up the high angle data (i.e., perform a “high angle refinement” in the XL job, so that the parameters of the other atoms are not biased by the “missing” electron density. This is achieved by a non-zero number (say -10) as the third parameter on the WGHT instruction for XL (see WGHT in Chapter 12).

The plane for the electron density calculation is defined by the current orientation as understood by XP, the EDEN parameters which are established by question and answer, and the atom list specified by keywords. The plane passes through the weighted (see WAIT) centroid of the molecule and is perpendicular to the current view direction. The scale is chosen so that the molecule can be projected onto the plane with a margin specified between each edge and the atom nearest to it. The plane may then be shifted so that the electron density (and molecule projection) is moved by delX, delY, and delZ in unit Angstrom coordinates; this is most useful for contouring slices with different delZ values for a non-planar structure. The named atoms are represented as dots, and bonds between them defined by the current connectivity table are added to the plot as lines (bond types 1-4) or dashed lines (bond types 5-7).

After copying the file hof.res and hof.fcf to your own directory, XP may be started and an atom list, connectivity table, and orientation found. The electron density calculation is then started by means of the EDEN instruction:

```
# READ HOF
# FMOL
# MPLN/N
# EDEN
```

The user is first asked whether he wishes to define the plane himself by entering the position in crystal coordinates corresponding to the center of the region plotted, and the length of the top edge in Angstroms, or whether the plane for the Fourier calculation should pass through the weighted (see WAIT) centroid of the molecule and be scaled so as to accommodate all the named atoms in projection. In the latter case a “margin” (typically 1 Å) will also be requested so that no atom is too close to the edge, and the crystal coordinates of the center and the scale are output for future use. In either case the orientation is taken to be perpendicular to the current view direction.

The program asks whether an F_o, $2F_o$-F_c, or F_o-F_c Fourier should be calculate; the first of these gives the electron density, and the last the difference electron density. In this case a difference electron density is required, so the answer is D (or [Enter], since D is the default setting). Similarly, the question as to whether the atoms and bonds should be superimposed onto the plot may be answered with Y (or [Enter]). The resolution in Angstroms is then requested; this has the effect of excluding reflections outside the specified sphere in reciprocal space. A value of 0 uses ALL reflections, which takes longer (the Fourier calculation is rather slow because the usual tricks cannot be used for a general slant Fourier) since the electron density associated with hydrogen atoms are relatively diffuse, a value of 1.2 may be given here. A value in this range will also reduce noise and result in smoother contours -- the hydrogen atoms contribute very little to the high angle data. The program then requests the value of F(000) or difference F(000). For an F_o or $2F_o$-F_c Fourier this should be the value of F(000) as printed out by XL or XS (with correct SFAC and UNIT instructions); for a difference Fourier, it is the number of electrons in the cell not accounted for in the XL structure Factor calculation. In searching for hydrogen atoms, this will simply be the number of hydrogens per CELL not yet included; in this case the answer is 16. Finally the user is prompted for the names of the phased reflection file from the XL LIST 3 job (the extension .fcf is assumed if not given) and the name of the plot file to be created (the extension .plt is added if not specified). Both questions may thus be answered with HOF. At this point the frame (with an Angstrom scale at the bottom) and projection of the atoms and bonds appears on the screen. The slant Fourier is calculated, which usually takes several minutes (be patient!) and when it is finished, the program displays the minimum, mean, and maximum (difference) electron densities at the top of the screen. The user must then input the zero level (usually 0 - contours below this level will be plotted dashed) and then each contour level in turn. Here increments of 0.05 electrons per $Å^3$ with one negative (dashed) contour are recommended. A new value may be input when the box at the top right of the screen is empty. A useful strategy is to give the zero level as -0.001 so that the 0.00 contour is drawn as a full line. The contours plotted so far are listed at the bottom of the screen. Finally, the [Enter] key terminates contouring and enters the usual atom labeling procedure (the B or E key may be used to skip labeling if it is not required). As usual, the [Esc] key may be used to abort the calculation at any stage and return to the XP “#” prompt. The full XP dialog and resulting contour map are given below:

```
# READ HOF
# FMOL
# MPLN/N
# EDEN

Scale to fit atoms[<CR>]or input center and scale[I]:

Enter margin around molecule in Angstroms: 1.5
Center at x=0.1110 y=0.6341 z=0.1151
Longest edge = 11.19 Angstroms

Enter shift dx, dy, dz in Angstroms: 0 0 0
Fo [E], 2Fo-Fc [M] or Fo-Fc [D=<CR>] Fourier: D
Display bonds and atoms [YES (=<CR>) or NO]: Y
F(000) or difference F(000) = 16
File created by LIST 3 in XL (.fcf assumed if no extension): HOF
Plotfile: HOF
```

At this point, XP plots a box containing a molecular diagram. The slant Fourier is then calculated and when it is complete the following appears at the top of the display:

```
Min:-0.30   Mean: 0.00   Max: 0.67 electrons per cubic Angstrom
Zero level (dashed below):
```

You should then input the zero level (-0.01). The program then adds to the second line of the display:

```
Next contour (<CR> if no more):
```

and you may input the first contour (0.6). After plotting each contour, the space after “Next contour...:” is cleared indicating that another contour height (0.5...-0.1) may be input. Finally, when all required contours have been plotted, a [Enter] is input. The program then enters the automatic labeling routine (as after TELP etc.); to skip this, but retain the plot, hit the E key. The plot file (here called hof.plt) may then be viewed on the screen with:

```
# VIEW HOF
```

or plotted with DRAW or RAST. An example is shown in Figure 18.9, (of course, the screen contours are colored); it may be annotated using POST:

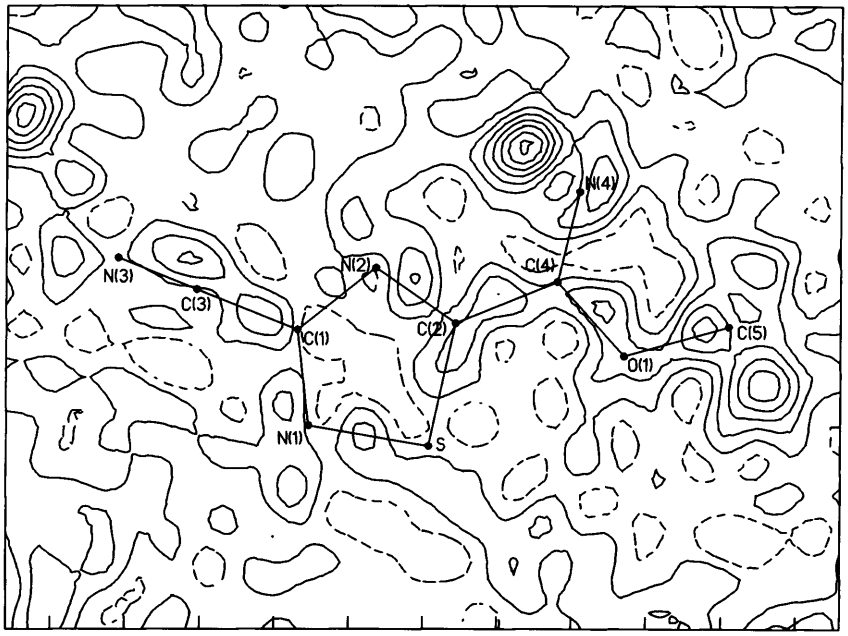

Figure 18.9 – Difference electron density plot

19 Alphabetical List of XP Instructions

ARAD ar br sr keywords

Defines atomic radii for the specified atoms (that must be in the current FMOL list). 'ar' is the radius in Angstroms used for representing the atom as a (possibly shaded) circle in TELP, PERS, POLP or POLY plots. 'br' is the radius used to define bonds and is employed by the FMOL, SRCH, ENVI, PACK, GROW and UNIQ instructions; these instructions automatically generate a bond between two atoms when it is shorter that br(1) + br(2) + delta, where the parameter delta may be specified (the default value is 0.5). The resulting connectivity table may be edited using JOIN, LINK, PRUN or UNDO. 'sr' is the radius to be used in space-filling models (SPIX and SFIL).

The (new) atom name (i.e., element symbol) determines the default values of ar, br and sr on reading in the atoms or renaming them by means of PICK or NAME; on generating symmetry equivalent atoms the current radii are copied. The INFO instruction lists atom radii etc.

ATYP type color keywords

Sets the mode of representation of the specified atoms (that must be present in the current FMOL list) in TELP and POLP plots, and the color to be used in all plots (except red-green stereo). For the colors available and their default assignments on reading in the atoms see Chapter 18. On reading in the atoms, 'type' is set to the SFAC number (that immediately follows the atom name). The atom 'type' has the following effect for TELP plots (see POLP for a description of polyhedral shading):

-4: Dotted ellipse (thermal ellipsoid boundary ellipse)

-3: Thermal ellipsoid boundary ellipse

-2: Thermal ellipsoid boundary ellipse and principal ellipses

-1: Full thermal ellipsoid with shaded segments

0: Nothing (useful for dummy atoms, e.g., in the middle of a C_5H_5 ring)

1: Shaded circle with highlight

2: Open circle

3: Circle with regular dot pattern

4: Circle shaded bottom left to top right

5: Cross-hatched circle

6: Circle shaded bottom right to top left

7: Circle with light random dot pattern and highlight

8: Circle with medium random dot pattern and highlight

9: Circle with heavy random dot pattern and highlight

10: Dotted circle (useful for minor component of a disordered atom)

The separation of the shading and dots for types 1 and 3-9 may in addition be varied by changing the second parameter on the GAPS instruction. On reading in the atoms they are assigned types in the range 1-9 based on the atom name; on generating symmetry equivalent atoms the current type is copied. It is not necessary to use ATYP to reset all atom types in order to obtain a standard thermal ellipsoid plot, since making the second parameter on the TELP instruction negative (e.g., 'TELP 0 -50') forces all atoms except H to be plotted using type -1. The current atom types, colors etc. may be examined using INFO.

BACK col int

Background color (an XP color index in the range 0-15 inclusive) and intensity in the range 0 (darkest) to 3 (lightest) for SPIX space filling models. The default values will usually be set by a BACK instruction in the initialization file sxtl.ini, but may be changed at any time with a new BACK instruction.

BANG keywords

BANG prints all interatomic distances specified in the current (FMOL) connectivity table for each atom defined by the (BANG) keywords, plus all angles involving two such bonds with an atom in common. The type of bond (as set by LINK or JOIN) makes no difference.

CELL λ, a, b, c, α, β, γ

CELL (usually read from the .res or .ins file by READ) followed by seven numbers inputs the crystallographic unit-cell and reinitializes all structural parameters (except TITL).

CELL followed only by lambda (that may be given the arbitrary value 1) signals that all following atomic coordinates refer to a unit orthogonal system; free variables etc. may not then be used (but MOVE is allowed), and instructions implying crystallographic symmetry or crystal coordinates (e.g., LATT, SYMM, FILE, SRCH, ENVI, SGEN, UNIQ, GROW, POLP, POLY and PACK) are not permitted.

CELL with no arguments causes the current cell to be printed.

CENT keywords

The x, y and z coordinates of the (unweighted) centroid of the specified atoms are calculated. This is sometimes useful in conjunction with the OFIT instruction, e.g., to fit the centers of two aromatic rings.

If CENT is followed immediately by /X (without an intervening space), an atom of ATYP 0 with the calculated coordinates is added to the FMOL list, but no bonds are added to the connectivity table. This is useful for π-bonded organometallic complexes; all that remains is to bond the (dummy) atom to the metal atom (JOIN 3 or 4) and then PERS or TELP may be used immediately. In such a case FMOL should be avoided, because it will generate too many 'bonds'.

DEMO

The DEMO instruction enters an infinite loop of graphical examples that are intended to illustrate the capabilities of XP. After each plot, the program pauses for 10 seconds before starting the next (2 seconds for PROJ displays). The demonstration may be aborted by hitting [Esc], which returns to the XP prompt '#'. The demonstration loop may also be started by giving the codeword 'demo' on the XP command line. The EGSD instruction defines the subdirectory containing the demo master file (called 'demo') and the data files that it uses.

DEMO followed by a filename may be used to run your own (infinite loop) demonstration. The file should contain a list of XP instructions. The DEMO command itself may not be included, and the instructions TELP, SFIL, OFIT, EDEN, HELP, PACK and POST wait for user input and so are not suitable. On reaching the end of the master file the demo loop is repeated.

DIAG keywords

DIAG draws a labeled diagram that is saved as the plotfile diag.plt and also displayed in the top right hand corner of the screen. This display may be toggled off and on with the [F10] key. Since the [F10] key always displays the contents of the file diag.plt, it may be used to display a file created in a previous XP run, or a plotfile created with (say) TELP and given the name diag.plt.

DIAG/B uses the first two PBOX parameters to define the width and depth of the limiting box; atoms and bonds outside this box are clipped. The centroid of the named (or implied) atoms becomes the center of the region displayed.

DRAW plotfile

The DRAW instruction may be used to convert a SHELXTL plotfile to HPGL format (either to file or for immediate output to a pen-plotter) or to Adobe Postscript format. The program action is determined by the answers to questions about output type and device, paper size, number of pens, etc.

See Chapter 18 for the order in which pens should be fitted in the case of a pen-plotter. The program also requests the size of paper, so that the plot is scaled automatically to US or European paper, and to larger paper (if the plotter can take it). It is also necessary to press the 'size' key on the plotter when changing the paper size. Before issuing a DRAW instruction, make sure that the plotter is ready to go (i.e., that pens and paper are present)! For overhead foils the S (slow) option should be chosen.

The DRAW instruction also contains an option for plotting directly on an HP-Laserjet III (or later model) via HPGL instructions. A memory extension of at least 1MB is required for the Laserprinter, that must also be configured using the front panel so that one full page is reserved for graphics.

When HPGL output is written to file, the order of colors is the same as the HP and Microsoft WinWord defaults. Retrace instructions are ignored when generating HPGL plotfiles, to keep them as short as possible. Several word processors allow such HPGL files to be incorporated into manuscripts etc. Alternatively an Adobe Postscript file may be created. Both HPGL and Postscript files are ASCII and so are convenient for network transmission etc.; they are however larger than the corresponding SHELXTL (binary) plotfiles.

ECHO text

The text on the same line as ECHO is displayed on the screen. This is primarily intended for demonstrations and for echoing important remarks (e.g., local variations in the parameter defaults) from the file sxtl.ini that is read when XP is started. The ECHO instruction may however be included in any file that XP reads using DEMO, READ or REAP.

EDEN keywords

Electron density calculation and contouring for a plane that is defined by the answers to questions set by the program (see below). The named atoms are represented as dots, and bonds between them defined by the current connectivity table are added to the plot as lines (types 1-4) or dashed lines (bond types 5-7).

Before starting XP and setting up electron density contouring with (e.g.) READ, FMOL and MPLN/N, it is necessary to run XL with LIST 3 for the refined structure (the XL job will often include several cycles of least-squares refinement) so that F_o, F_c etc. are written to the .fcf file. It is also possible to input 'LIST 3' output from the version 4 SHELXTL refinement program XLS (by specifying the name of the resulting .res file including the .res extension).

The user is first asked whether he wishes to define the plane himself by entering the position in crystal coordinates corresponding to the center of the region plotted, and the length of the top edge in Angstroms, or whether the plane for the Fourier calculation should pass through the weighted (see WAIT) centroid of the molecule and be scaled so as to accommodate all the named atoms in projection. In the latter case a 'margin' (typically 1 Å) will also be requested so that no atom is too close to the edge, and the crystal coordinates of the center and the scale are output for future use. In either case the orientation is taken to be perpendicular to the current view direction, and the plane may then be shifted so that the electron density (and molecule projection) is moved by delX, delY and delZ in unit Angstrom view coordinates; this is useful for contouring slices with different delZ values for a non-planar structure.

The program asks whether an F_o, $2F_o$-F_c or F_o-F_c Fourier should be calculated - the first of these gives the electron density, and the last the difference electron density - and whether the atoms and bonds should be superimposed onto the plot. The resolution in Angstroms is then requested - this has the effect of excluding reflections outside then specified sphere in reciprocal space. A value of 0 uses ALL reflections, which takes longer - the Fourier calculation is rather slow because the usual tricks cannot be used for a general slant Fourier - and for exploration a value of 1.0 or even 1.2 can be given to speed up the calculation. For the location of hydrogen atoms from difference Fouriers, a value in this range will also reduce noise and result in smoother contours - the hydrogen atoms contribute very little to the high angle data. The program then requests the value of F(000) or difference F(000). For a F_o or $2F_o$-F_c Fourier this should be the value of F(000) as printed out by XL or XS (with correct SFAC and UNIT instructions); for a difference Fourier, it is the number of electrons in the cell not accounted for in the XL structure Factor calculation (in searching for hydrogen atoms, this will simply be the number of hydrogens per CELL not yet included). Finally the user is prompted for the names of the .fcf file from the XL LIST 3 job (the extension .fcf is assumed if not given) and the name of the plotfile to be created (the extension .plt is added if not specified).

At this point the frame (with an Angstrom scale at the bottom) and projection of the atoms and bonds appears on the screen. The slant Fourier is calculated, which usually takes several minutes (be patient!) and when it is finished, the program displays the minimum, mean and maximum (difference) electron densities at the top of the screen. The user must then input the zero level (usually 0 - contours below this level will be plotted dashed) and then each contour level in turn. A new value may be input when the box at the top right of the screen is empty. The contours plotted so far are listed at the bottom of the screen. Finally the [CR] key terminates contouring and enters the usual atom labeling procedure (the B or E key may be used to skip labeling if it is not required). As usual, the [Esc] key may be used to abort the calculation; if it is used whilst drawing contours, the user is prompted as to whether he wishes to redraw the contours without recalculating the slant Fourier or exit to the '#' prompt.

EGSD path

EGSD sets the path for SHELXTL examples files. Under MS-DOS it is usually C:\SAXI\SXTL\EGS, under UNIX /usr/saxi/sxtl/egs.

END

In XL, XS etc. the END instruction terminates the .ins file; all subsequent data (if any) are ignored. In XP, END returns command to the console. If issued from the console it has no effect. Note that the REAP instruction can read through one END before returning command to the console; this enables Fourier peaks (Q...) to be added to the atom list if desired.

ENVI del keywords

ENVI prints the environments of the specified atoms, i.e., all unique ligand distances less than r1+r2+del and all angles between them, plus the symcodes used to generate the ligand atoms. The default value of del is 0.5 Å. The connectivity table is not used by this instruction. ENVI is particularly useful for exploring the coordination polyhedra of atoms in complex inorganic structures, and may be followed by adding specific symmetry related atoms using SGEN and the symcodes printed out by ENVI. This is a useful preliminary to PGEN followed by POLY or POLP for polyhedral packing plots. Note that 'ENVI/L' is able to output more information to the printer than would fit on the screen.

EXAM or EXAM template

The EXAM instruction has a similar function to MS-DOS 'DIR' or UNIX 'ls', listing filenames in a convenient format. The template may specify a drive and/or subdirectory, otherwise the current 'USER' is assumed. The '*' and '?' wildcards may be used (e.g., EXAM *.res) if they can be understood by the operating system.

EXIT or EXIT filename

EXIT terminates XP and returns to the operating system. Any 'printer' output created during the XP session is spooled to the printer (defined by the SLXP instruction). QUIT also terminates XP, but deletes rather than prints the accumulated 'printer' output. EXIT followed by a filename sends the 'printer' output to file rather than printing it.

EYES n

EYES 1 sets the default setting for PROJ, PICK and PACK to mono; EYES 2 sets it to stereo.

FILE filetitle

The atoms in the current FMOL list are written to the specified file, that will often be an .ins file for the next refinement stage. The instructions TITL...UNIT etc. are also copied to the file (if XP can find them, i.e., if a .res or .ins file exists in the current directory with the same first filename component). This facility is useful for creating the .ins file for the initial refinement from the peaklist in the .res file from structure solution using XS, but may also be used later in the refinement so that XP can apply symmetry transformations to atom coordinates and U_{ij}, sort the atoms into a more suitable order, etc.

Some instructions such as SAME, MOLE and LAUE may need to be inserted into the resulting .ins file by hand. as will some constraints involving free variables (e.g., occupancies of disordered groups). Warning messages will also appear if equivalent atoms are included by accident (e.g., after using GROW). Despite these checks, it is strongly recommended that the FILE output is checked by hand before submitting a refinement job.

FMOL keywords, delta

This instruction defines the 'FMOL atom list', and is normally the first XP instruction after the atoms have been read in. It sets up and displays a connectivity table that is used in many subsequent calculations. ONLY the atoms defined by the FMOL keywords can be used for subsequent calculations, until the next call of FMOL, that may select a different set of atoms from the original list. Keywords after other instructions define a SUBSET of the FMOL list. Two atoms are considered to be bonded if they are closer than delta plus the sum of the two covalent radii (that are set to appropriate default values on reading in the atoms, and may be changed by the ARAD instruction). The default value of delta is 0.5 Å. The FMOL connectivity table may, if necessary, be edited by JOIN, LINK, PRUN and UNDO. The special instruction: **FMOL REST** Means that all atoms NOT in the current FMOL atom list should form the new FMOL atom list.

FMOL/N suppresses all output - this is faster than using the [Esc] key for proteins!

FUSE delta

FUSE 'fuses' together all atoms within del (default 0.5) Å of each other. This will usually be used after PUSH (and SAVE!) instructions to establish the correct position of the origin, and reduce the atoms to the unique set. FUSE almost always needs to be followed by UNIQ and one or more atom names to gather the 'fused' atoms into the smallest number of unique molecules. The number of atoms that coalesce is printed.

GAPS ovlp shade

The GAPS instruction may be used to change the overlap gap and shading of atoms in TELP and PACK plots, and the shading in POLP plots. The 'ovlp' parameter defines the gap to be left when a bond or atom obscures another; the units are plot units, that correspond approximately to 100 plot units = 1 mm on A or A4 size plots. It may be advisable to increase the value of ovlp if it is known that the diagram will be substantially reduced in size before eventual publication. 'shade' is proportional to the gap between shading lines for atom types 1, 4, 5 and 6, and the average distance between dots for atom types 3, 7, 8 and 9. The optimal defaults for GAPS tend to be hardware dependent, and so are often included in the initialization file sxtl.ini that is read on starting XP.

GROW delta keywords

GROW uses all atoms in the current FMOL list and all their symmetry equivalents to assemble molecule(s) in which each atom is bonded (possibly through several other atoms) to one of the specified atoms. GROW provides a convenient way of constructing isolated molecules or ions; if they lie on special positions all necessary atoms are generated automatically. UNIQ provides a similar function but uses each atom (or equivalent) once. GROW redefines the FMOL list. For the purposes of building the molecules and setting up a new connectivity table, two atoms are considered to be bonded if their distance apart is less that br(1)+br(2)+delta, where the bond radius br is set on reading in the atoms and may be changed by means of the ARAD instruction. The default value of delta is 0.5 Å. Normally GROW will be used with a single atom specified, but it is also possible to name more than one atom in order to build several molecules or ions simultaneously. For polymeric structures, GROW never adds atoms that are a whole number of unit-cell displacements from existing atoms. In order to extend polymeric structures, GROW may conveniently be followed by SGEN.

GROW copies atom parameters such as radii, color, atom type etc. to the newly generated atoms, and applies the appropriate symmetry transformation to anisotropic temperature factors. New unique atom names are generated automatically; if necessary they may be changed using NAME or PICK.

HADD type distance U keywords

Hydrogen atoms are generated in idealized positions attached to the named C, N and O atoms. If other elements are referenced (e.g., by an implied 'ALL') they are ignored. If 'distance' is not specified it is assumed to be 0.96 A for C-H, 0.90 for N-H and 0.85 for O-H. If the 'type' is given as zero or omitted, the program tries to make an intelligent guess (but will never spot a terminal $=CH_2$ or $-NH_3^+$ of its own accord, and will often fail to protonate a carboxyl group especially if libration is significant). Thus, provided that the molecule is complete (GROW!) except for H, has unexceptional geometry and reasonably well refined bond lengths and angles, the instruction 'HADD' without any parameters stands a sporting chance of assigning all hydrogen atoms correctly; however it should always be checked with PROJ etc.

In cases of disorder HADD takes PART numbers into account (these may be set or changed by PART/S). In practice a large structure could be processed automatically using HADD, followed by removal of any erroneous hydrogens (KILL) and regeneration by 'HADD type'.

type=1: tertiary C-H with all X-C-H angles equal.

type=2: secondary CH_2 with all X-C-H angles equal and H-C-H dependent on the X-C-X angle.

type=3: methyl (could also be used for $-CF_3$ if the 'distance' parameter is set). One hydrogen atom is set trans to the longest bond to the adjacent atom; all H-C-H and X-C-H are given tetrahedral values.

type=4: 'aromatic' C-H (or amide N-H) with the hydrogen atom on the external bisector of the X-C-X angle.

type=8: O-H trans to the longest bond to the adjacent atom: may well deviate considerably from the correct position (possible hydrogen bonding is not considered).

type=9: terminal $=CH_2$ or $=NH_2^+$ with the hydrogen atoms in the plane of the more distant (if more than one) substituent on the adjacent atom.

In each case, other hydrogen atoms are ignored since they might introduce errors. A unique name is assigned to each new hydrogen, that is added to the current FMOL list with isotropic U's as specified on the HADD instruction (default 0.08) and site occupation factors of unity.

These hydrogen atoms are intended primarily as a rough approximation for improved visualization of molecules (e.g., using SPIX). The extensive facilities provided in the least-squares refinement program XL are in every respect superior, so HFIX (in the .ins file for XL) should be used in preference to HADD followed by FILE.

HELP

The XP instruction "HELP keyword" provides information about all XP commands and facilities. For a full list of keywords allowed with HELP, use HELP without a keyword.

HIMP distance keywords

The named (hydrogen) atoms are moved along the bond to the nearest atom so that the distance acquires the desired value. This may be used to improve hydrogen positions (e.g., for hydroxyl hydrogens) from difference electron density maps, and also to reset C-H distances to (say) 1.08 Å for a calculation of 'interproton' non-bonded distances (e.g., for estimating n.m.r. nuclear Overhauser effects). The default 'distance' is 0.96 for C, 0.90 for N and 0.85 for O; hydrogens bonded to other atoms are only moved if 'distance' is set explicitly.

HLPD path

HLPD sets the path for SHELXTL help files. Under MS-DOS it is usually C:\SAXI\SXTL\HELP, under UNIX /usr/saxi/sxtl/help.

INFO keywords

A summary of atom parameters is displayed on the console; INFO/L sends a more extensive list to the lineprinter. In both cases the program also outputs the numbers of atoms and bonds in the current lists.

INVT Xi Yi Zi keywords

The specified atoms are inverted through the point with crystal coordinates Xi, Yi, Zi (default 0, 0, 0). Atoms not referenced or not in the current FMOL list are NOT INVERTED. This facility may be used to produce the mirror image of a TELP or other structural plot.

ISOT keywords

Anisotropic atoms are converted to isotropic with U=Ueq; atoms which are already isotropic are not affected.

JOIN bondtype keywords

If precisely TWO atoms are specified, a bond of type 'bondtype' joining the two atoms is added to the connectivity table. If the bond was already present in the connectivity table, the type is altered as required. JOIN followed by any number of atoms other than two turns all existing bonds to those atoms into bonds of type bondtype. Allowed values for bondtype are:

1: solid (TELP and PERS), full line (DIAG and PROJ)

2: open (TELP and PERS), full line (DIAG and PROJ)

3: dashed solid (TELP and PERS), dashes (DIAG, PROJ)

4: dashed open (TELP and PERS), dashes (DIAG, PROJ)

5: full line (DIAG, TELP, POLP and PERS), dashes (PROJ)

6: dashes (all)

7: dashes (PROJ), dots (DIAG, TELP, PERS and POLP)

The default setting of bondtype is 1 (this is the only difference from the LINK instruction).

KILL keywords

The specified atoms (a subset of the FMOL list) are completely eradicated and the atom list pushed up to make room for new atoms. Entries in the connectivity table (if any) for the atoms in question are also expurgated. Note the use of UNIQ followed by 'KILL ALL' to remove a complete molecule or ion. The special option 'KILL REST' may be used to eradicate all atoms NOT in the current FMOL list.

LABELS (Atom names and axes)

The LABL instruction in conjunction with the DIAG, SFIL, TELP, EDEN and OFIT commands enables labels to be added (interactively except for DIAG in which the labels are positioned automatically) to mono or stereo plots. The size and type of label may be set using the LABL instruction. After creating the plot the program temporarily places the atom name (exactly as it will appear on the plot) in the bottom right hand corner of the screen, and then sets a rectangular cursor on the first atom (or cell corner) to be labeled; the rectangle is sized so that the proportionally spaced atom name exactly fits inside it. The cursor may be moved using the mouse or cursor keys. When a suitably uncluttered position is found for the label, the [Space] key adds the label to the plot. Alternatively the [Enter] key skips the current label or [Backspace] goes back to the previous label. E or B may be used to signal that no further labels are to be added and the plot should be retained in its current state; Q or H abort the plot. When all atoms have been labeled, the computer bleeps twice (one bleep indicates that it is ready to label the next atom); [Space] should then be typed to delete the plot from the screen (but keep the plotfile) and return to the "#" prompt. [Backspace] may still be typed here if the last name plotted is bad. Any characters other than those defined above are ignored (with a bleep) during the labeling, as are attempts to place labels off the edges of the plotting area.

LABL code size

LABL defines the type of atom labels to be used with TELP, SFIL, EDEN, OFIT and DIAG.

code = 0 no labeling, 1 (labels without H and without brackets), 2 (without H but with brackets - default), 3 (with H but without brackets) and 4 (with both).

size (default 600) is the vertical dimension of the labels in plot units.

The program automatically converts letters after the first character to lower case, and puts an opening bracket (if required by code) before the first digit (if any). If an opening bracket has been inserted in this way, then a closing bracket is added at the end of the atom name.

Labeling is also performed for stereo plots; labels positioned on the left hand plot are automatically added to the right. For further details about interactive labeling, see 'HELP LABELS'.

LIBR keywords

Librational analysis of rigid body motion is performed using the method of Schomaker and Trueblood [Acta Cryst., B24 (1968) 63]. The output conforms to the conventions given in that paper, and includes the generalized R index for the agreement of observed and calculated orthogonalized U_{ij}, using the orthogonal axes XO (see Chapter 18), and the librationally corrected bond lengths for unique bonds for which both atoms are specified by the LIBR keywords.

```
LINE two atoms
```

Calculates the equation of the line joining the atoms in question in orthogonal and in crystal coordinates, and outputs the angles to previously calculated lines and planes (MPLN).

The /N switch may be used to suppress all output, e.g., when LINE is used purely to obtain a good orientation for plotting.

LINK bondtype keywords

If precisely TWO atoms are specified, a bond of type 'bondtype' joining the two atoms is added to the connectivity table. If the bond was already present in the connectivity table, the type is altered as required. LINK followed by any number of atoms other than two turns all existing bonds to those atoms into bonds of type bondtype. Allowed values for bondtype are:

1: solid (TELP and PERS), full line (DIAG and PROJ)

2: open (TELP and PERS), full line (DIAG and PROJ)

3: dashed solid (TELP and PERS), dashes (DIAG, PROJ)

4: dashed open (TELP and PERS), dashes (DIAG, PROJ)

5: full line (DIAG, TELP, POLP and PERS), dashes (PROJ)

6: dashes (all)

7: dashes (PROJ), dots (DIAG, TELP, PERS and POLP)

The default setting of bondtype is 6 (this is the only difference from the JOIN instruction).

LITE I dif spe phong

Intensity of soft direct light (usually in the range 2-10), diffuse and specular reflection coefficients (between 0 and 1) and Phong shading model parameter (typically in the range 4-20) for the SPIX space filling models. The default values for LITE will usually be set in the initialization file sxtl.ini, but may be changed at any time with a new LITE instruction.

LOSE filename

The corresponding file is deleted. Wildcards such as '*' and '?' may be recognized, depending on the operating system. Note that the drive and subdirectory may be set by the USER instruction. If no '.' appears in the filename, the extension '.plt' is assumed.

MATR p11 p12 p13 p21 p22 p23 p31 p32 p33

MATR followed by 9 numbers resets the current orientation matrix P.

'MATR n' sets a special matrix: n=1 sets the matrix for a projection down the real crystal x axis, n=2 down y, and n=3 down z.

MATR followed by three numbers h, k and l defines an orientation with the crystal planes with Miller indices (h,k,l) perpendicular to the view direction (Z').

MATR with no arguments causes the current matrix to be printed.

MGEN keywords delX delY delZ Xc Yc Zc

MGEN generates symmetry equivalent molecules (and isolated atoms) to fill the volume defined by the crystal coordinates x = (Xc-delX) to (Xc+delX), y = (Yc-delY) to (Yc+delY), z = (Zc-delZ) to (Zc+delZ). Before starting the search, all atoms not in the current FMOL list are eradicated irrevocably. Symmetry equivalents are then generated for which the equivalents of the named atoms lie within the specified volume. The molecules are constructed by examining the current FMOL list for atoms bonded by bonds of type 1 and 2; the same symmetry transformations are then applied to these bonds (but not to other types of bond). After generating the required equivalents, the molecules not inside the specified volume are removed from the current FMOL list but not deleted. The default values of delX to Zc inclusive are all 0.5, i.e., the default volume is the unit cell.

The primary purpose of MGEN is to prepare the atom and connectivity lists for packing plots (TELP, SPIX or PERS) of molecular structures. MGEN also prints the "symcodes" required for the transformations (for use by SGEN). It is important to set up complete molecules first (one complete example of each unique molecule suffices) before multiplying them with MGEN. If a molecule lies on a special position it should be completed by including symmetry equivalents before using MGEN; the GROW instruction is very useful for this purpose. MGEN can take an appreciable amount of computer time; it is most efficient when only one atom (usually a relatively central atom) is specified on the MGEN instruction for each unique molecule. If no atom names are specified, the default assumption of "ALL" will ensure the (slow!) generation of all molecules with at least one atom within the specified volume. MGEN does not generate extra interactions between the generated molecules; to generate hydrogen bonded patterns etc. the interactive PACK facility should be used.

MGEN is so constructed that it may be used repeatedly if the first attempt is not quite right; however it may be necessary to use FMOL between MGEN calls to avoid accidental deletion of all examples of a particular unique molecule or ion. It is a very good idea to use the "SAVE filename" instruction to save a snapshot of the structure immediately before MGEN is invoked; one can always return to this position later with "NEXT filename".

If all generated atoms or bonds cannot be stored, MGEN exits without generating any symmetry equivalents; it may also be interrupted with [Esc] at any stage with similar effect.

MODL keywords scale deltar

Prints drilling instructions for Supper models for the specified atoms. The angles are rho (to the initial hole) and phi (about the initial hole) as defined by Supper. 'scale' should be in cm/Å (default 3.0) and deltar is the radius in cm (default 0.25) of the 'unbored' atom core.

MOVE dx dy dz sign

The coordinate shift dx, dy, dz is applied to all atoms SUBSEQUENTLY read in, after resolving free variable references etc. The coordinates are first multiplied by sign, that must be plus or minus one. The MOVE instruction in XP operates in the same way as the MOVE instruction for the programs XL and XS, so that reading a .ins file containing MOVE generates the same coordinates. The CELL instruction resets MOVE. In practice PUSH, that acts on all atoms in the current FMOL list, is more useful for applying an origin shift and/or inverting a structure within XP (see also INVT).

MPLN keywords

The best least-squares plane through the specified atoms is calculated. The orientation matrix is reset so that Z' is parallel to the normal to the plane (or to the interatomic vector). The orientation of the molecule with respect to the remaining axes is also determined by the inertial axes as found in the MPLN calculation. All angles between the current normal and previous normals (or vectors found by LINE) are printed.

The /N switch may be used to suppress all output, e.g., when MPLN is being used purely to obtain a suitable orientation for plotting.

NAME oldname newname sfaccode

The NAME instruction is used to rename atoms changing color, ATYP code, and radii to the default values for the new element symbol (deduced from 'newname'). It may be used for example if the automatically generated names created by SGEN, PACK or GROW prove unsuitable. An attempt to assign a name that already exists is regarded as an error. The new scattering factor number (if not the same as the old) may also be specified (sfaccode). Both atom names may contain the wild character "?". In the case of "oldname" this means all matching names (where "?" can stand for any character except blank), and in the case of "newname" it means that "?" is replaced by the corresponding character from the old filename. e.g., the command:

```
NAME Q?? C??
```

would change all peaks from Q10 to Q99 inclusive to carbon atoms with the same numbers, but leave Q9, Q100 etc. (if present) unchanged.

RESI/S should be used to change residue numbers and classes; NAME leaves them unchanged.

NEWM two atoms

The necessary information for constructing the Newman projection along the bond in question is printed. The first named atom becomes the 'top' atom, the second the 'bottom' atom. Positive angles correspond to clockwise rotations viewed from the 'top'.

NEXT filename

The specified XP 'save' file (that must have been written by XP using the SAVE instruction) is read into memory, overwriting all current structural parameters. This restores a 'snapshot' of atoms and parameters previously created by XP. If the filename does not contain '.', the suffix '.sav' is assumed. NEXT should always be able to read a 'save' file created by the same or by an earlier version of XP, but not necessarily a more recent version, because new information may have to be stored. NEXT is specific to a particular computer system, i.e., the PC version of XP cannot read files written using SAVE on a Silicon Graphics Iris, but version 5 of XP on a PC can read SAVE files written by version 4.

NOPL

The NOPL instruction deletes any currently stored lines and planes, so that they are not included when calculating angles involving subsequently calculated lines and planes.

OFIT keywords

The specified atoms (or at least some of them) are fitted by a model that is read from file. The user is prompted for the name of the file, that will normally have been prepared using the ORTH instruction (or is in ORTH format). It helps to have a diagram (e.g., prepared using DIAG) of the model to hand, because the user is then asked to name the model atom to be fitted to each atom specified in the OFIT atom list. At this point [Enter] leaves the atom out, and '=' sets the name of the model atom to be the same as that of the atom to be fitted. After listing the deviations of the atoms fitted and the weighted (WAIT) r.m.s. deviation, as well as the angle of rotation required (that is only meaningful if the coordinate system is the same and the model was not rotated before the ORTH instruction was used, e.g., when fitting two chemically similar but crystallographically independent molecules in the asymmetric unit), the program waits until a key is hit before proceeding so that the results can be inspected on the screen. The idealized CRYSTAL coordinates and nearest atom (from the FMOL list, ignoring symmetry operations) are then output for each model atom. These coordinates may conveniently be used as starting coordinates for rigid group refinement. The program then pauses again; when a key is hit a diagram of the fit is plotted on the screen (with the model dotted). The plot is based on the current FMOL list and connectivity table; in defining bonds for the model covalent radii are set that assume the same scattering factor order for both structures, and distances less than $r_1+r_2+0.5$Å are treated as bonds. At this stage the atom labeling routine is entered (as set by LABL) exactly as in the TELP instruction; only the fitted atoms are labeled. If no plotfile is required, just type [Enter] instead of the name of the plotfile.

The program also asks whether the model atoms should be added to the current FMOL list (with ATYP 10, i.e., dotted circles); this enables the TELP instruction to be used to plot the results of the fit. If the model atoms (and bonds) are retained in this way, it is not advisable to use FMOL, UNIQ or GROW, because additional 'bonds' will be created between model atoms and the fitted structure.

ORTH filename

The current FMOL list of atom names and unit orthogonal coordinates (in the current orientation, with the first atom at the origin) is written to the specified file. The coordinates are in the current orientation. This file may be read by the model fitting instruction OFIT, and provides a convenient method of saving the current XP information in the form of a relatively short ASCII file. For this purpose the ATYP numbers are given after the atom names (they are usually the same as the SFAC types) and the connectivity table is output (using LINK instructions) after an intervening 'END'. Thus READ will reinput just the atoms (and must be followed by FMOL etc.) whereas REAP will also recover the connectivity and bond types. This format is also recommended for transfer of structural data between XP and other programs, e.g., molecular mechanics programs. If the filename does not contain the character '.', the extension '.ort' is assumed.

ORTH/F includes the crystallographic unit-cell, the symmetry operators, and the information needed to transform the orthogonal coordinates back to crystal coordinates in the form of additional 'comments' (lines that begin with four spaces). This may be useful as input to user-written programs.

PACK del2 del1 d keywords

PACK provides interactive selection of the contents of a packing plot. A simple packing plot (using only lines and dots) may then be produced directly, or a new atom list and connectivity table may be set up so that the very flexible TELP instruction may then be used to create the plot. TELP is most suitable for packing plots of relatively small structures, since otherwise it is hardly possible to recognize all the details. PACK starts by using the connectivity table to break up the atom list defined by the PACK keywords into 'molecules'. Symmetry equivalents are then generated so that all 'molecules' with a least one atom inside the box defined by PBOX and the current orientation are included. When 'molecules' lie on special positions, it may be worth first completing them with GROW, so that only complete molecules are considered by PACK. PACK does not generate atoms with duplicate coordinates. After creating a PACK atom list in this way, the connectivity is regenerated so that polymeric structures are handled correctly: distances less than del1+r_1+r_2 are considered to be 'bonds' (bond type 1), and the remaining distances less than del2+r_1+r_2 are considered to be 'non-bonds' (bond type 6). Additional 'non-bonds' are added from the original connectivity table.

Atom types 1 and 2 are assumed to be C and H respectively; it is assumed that C does not make 'non-bonds', and that H does not make 'non-bonds' to itself or to C. This facility may be switched off by making del2 negative (e.g., for inorganic structures).

The atoms found by the above search are sorted into new 'molecules' (the cell is treated as molecule 0) and the structure is displayed with blue depth cueing. The various options may then be selected from a menu using the mouse, [F6], [F7], [F8] or [Enter] keys. If the 'scan mols' option is selected, each 'molecule' is blinked in turn. It may be deleted with [Enter], retained with [Space], the bond color (for direct PACK plots only) may be changed by hitting a digit, mistakes may be corrected using [Backspace], and the scan may be interrupted (returning to the menu) with [F6] etc. 'Scan atoms' and 'scan bonds' perform similar scans for the current molecule only (that must first be selected using 'scan mols' followed by [F6] etc. at the appropriate point), and 'scan non-bonds' allows superfluous non-bonds to be eliminated. Since information about the interactions is displayed on the bottom line of the screen, this facility also provides interactive facilities for analyzing the packing using a stereo display. This procedure is continued, if necessary utilizing the fact the molecule scan recyles if required, until only those atoms, bonds and non-bonds remain that are desired to be included in the packing plot.

Three menu options provide for the generation of plotfiles containing line/dot packing plots without leaving PACK; in addition to a normal exit (reinstating the original FMOL list and connectivity table) it is also possible to exit from PACK with the creation of a new FMOL list and connectivity table based on the displayed atoms, bonds and non-bonds. The program refuses this option if it would result in the creation of too many

atoms or bonds. After setting up new lists in this way, all other XP instructions (e.g., PROJ, ATYP, JOIN, PERS etc.) may be used to improve further the composition of the full packing plot before it is created using TELP (usually with the CELL option). In this way, all 15 ways of displaying atoms and 7 ways of displaying bonds may be used in the final plot.

Note that the MGEN instruction (for molecular structures) and PGEN (for coordination polyhedra) provide alternative ways of preparing packing plots.

PAGE

A form-feed (new page) is sent to the file that is later spooled to the printer. This is useful for separating unrelated outputs.

PART n

The following atoms are assigned to component n of a disordered group. The default value of n (before a PART instruction is read) is 0. When bonds are generated automatically by FMOL etc., no bonds are formed between atoms with different PART numbers unless one of the numbers is zero. When GROW is applied to atoms with negative PART numbers, no symmetry equivalents are generated. The structure refinement program XL interprets PART similarly. FMOL C11] N2 less PART2 would generate all atoms between C11 and N2 inclusively, but leaving out the disorder component PART 2.

PART/S n atomnames

PART/S changes the PART number of the specified atoms to n (default 0). A new FMOL is required to revise the connectivity array to reflect the new PART assignments.

PAWS

PAWS causes the program to wait until a key is hit. It is primarily intended for demonstration and tutorial purposes, but may in principle be included in any file that XP reads using READ or REAP.

PBOX width depth xc yc zc

Defines the box used by PACK for selecting fragments for plotting. All fragments containing at least one atom inside the box are generated. Width is the width of the box (across the screen) in Angstroms; the distance from the top to the bottom of the screen is always 0.75 times width. The default setting of width is 20 Å. depth is the depth of the box in Angstroms perpendicular to the screen; the default is 8 Å. xc, yc and zc define the center of the box in CRYSTAL coordinates (default 0.5, 0.5, 0.5). Note that changing the orientation will also affect the selection of fragments by PACK.

PBOX without any parameters prints the current settings. The first two PBOX parameters are also used by DIAG/B.

PERS bondrad keywords

The PERS instruction displays a perspective ball and stick plot on the screen using 'polygon fill' techniques for solid atoms (with highlights) and various types of bond (see LINK or JOIN). Hidden line removal is achieved by the simple strategy of plotting the furthest atom first and moving towards the viewer. No plotfile is created.

The keyword CELL may be used to add the unit-cell to the diagram.

'bondrad' (default 0.05 Å) is the radius of bonds of types 1-4.

PGEN keywords delX delY delZ Xc Yc Zc

Generates symmetry equivalent polyhedra (and isolated atoms) to fill the volume defined by the crystal coordinates x = (Xc-delX) to (Xc+delX), y = (Yc-delY) to (Yc+delY), z = (Zc-delZ) to (Zc+delZ). The keywords specify the atoms at the centers of the polyhedra to be transformed, plus the isolated atoms to be transformed (note that the latter are not specified on the POLP and POLY instructions). Before starting the search, all atoms not in the current FMOL list are eradicated irrevocably. After generating the required equivalents, polyhedra or isolated atoms not inside the specified volume are removed from the current FMOL list but not deleted. The default values of delX to Zc inclusive are all 0.5, i.e., the default volume is the unit cell.

The primary purpose of PGEN is to prepare the atom and connectivity lists for the POLY and POLP polyhedral plots of inorganic structures. PGEN also prints the "symcodes" used for the transformations (for use with SGEN). It is important to set up complete polyhedra first (one complete example of each unique polyhedron suffices) before multiplying them with PGEN. PGEN can take an appreciable amount of computer time; for efficiency only one example of each unique central or isolated atom should be specified on the PGEN instruction. If the central atom lies on a special position the polyhedron must be completed by including symmetry equivalents before using PGEN.

All equivalents of the named atoms that lie within the specified volume are generated, plus the corresponding equivalents of the atoms bonded to them with bonds of type 1 or 2 that are not themselves named. The symmetry equivalents of all such bonds (but not of other bonds) are also generated by PGEN. If all generated atoms or bonds cannot be stored, PGEN exits without generating any symmetry equivalents; it may also be interrupted with [Esc] at any stage (say 1 Å); all atoms within r_1+r_2+delta of the metal atom are then listed with the appropriate symmetry operations. The ligands that do not have the identity operator (1555) must then be generated using SGEN (note that more than one atom and/or symmetry operator may be used per SGEN instruction to save time). After this has been repeated for all metal atoms it may be necessary to add bonds to complete some of the coordination polyhedra, and to delete other bonds (e.g., between metal atoms) that are not required. A quick method is FMOL with a larger than usual delta (say 1 Å) followed by "PRUN n" (possibly separately for individual atoms or atom types - e.g., "PRUN 4 $SI") to eliminate the longer bonds. In special cases JOIN may be used to add individual bonds and UNDO to delete them, or the bond radii can be altered (ARAD) and FMOL used again. The PICK instruction is useful to check the results and possibly to delete atoms that are not required. It saves time if the shading patterns and colors (ATYP) of the central atoms of the polyhedra, as well as the display radii of isolated atoms (ARAD), are also set BEFORE using PGEN.

When a correct atom and connectivity list defining the unique polyhedra (plus any isolated atoms such as H_3O^+ ions) has been prepared in this way it should be saved on disk by means of "SAVE filename". One can then come back to this point later by means of "NEXT filename" in the same or a subsequent XP run. Only then should PGEN followed by the names of central and isolated atoms be used to fill a specified volume in crystal space. The POLY instruction then provides a quick check before making a plotfile with POLP. PGEN is so constructed that it may be used repeatedly to add or delete polyhedra if the first attempt is not quite right. Note that it starts by

deleting all atoms not in the current FMOL list, and finishes by relegating all atoms outside the given volume from this list, so it is important to retain at least one example of each unique polyhedron needed in the current list; sometimes it may be necessary to go back to the SAVE file.

PICK keywords

PICK displays a labeled line diagram of all atoms in the current FMOL list. If EYES was set to 2 the diagram is in red/blue stereo, otherwise it is mono with depth cueing. Each atom in the local (PICK) atom list, and all bonds to it, then blink in turn. The user may hit the [Enter] key to delete the atom, the space bar to retain it with the same name, or a new name (followed by [Enter]) to rename the atom. The color, radii, ATYP code etc. are deduced by the program from the new name. Note the use of PICK $Q, SORT and then FILE to insert selected Fourier peaks into the atom list for the next refinement job. The [Backspace] key steps back to the previous atom processed, reinstating it if it had been deleted; it may be used to step back any number of atoms. Thus mistakes may easily be corrected. When an atom is deleted, the bonds in which it takes part are deleted from the screen; they are reinstated by [Backspace]. [Esc] provides an emergency exit, all atoms removed by the current PICK instruction being reinstated. However [BS] and [Esc] do NOT change atom NAMES and other parameters back to their original values. The '/' key may be used to exit from PICK before the scan of the specified atoms is completed, but applying the changes made so far.

The [Tab] key may be used to "zoom", i.e., enlarge the region around the current atom. A further [Tab] undoes the zoom. A list of bond lengths and current atom (with peak heights if appropriate) appears on the lower status line.

PICK/H labels all atoms, including hydrogen; otherwise the hydrogen atom names are omitted for clarity (but the bonds involving hydrogen are always drawn). All bond types are drawn as full lines.

POLP keywords

The POLP instruction is used to display a structure using the polyhedral representation popular for minerals and inorganic structures; in contrast to POLY a plotfile is created. The keywords (e.g., $AL $SI for an aluminosilicate) define the atoms at the centers of the polyhedra. The current FMOL list is searched with the aid of the connectivity table (all bond types) to find the atoms that form the vertices of each polyhedron. For all polyhedra with four or more such vertices, all visible faces are shaded with the color assigned to the central atom. Atoms that are neither central atoms nor vertices are displayed as shaded circles in the appropriate atom color. The unit-cell edges are superposed on the diagram if the keyword CELL is included. POLP/L provides in addition a detailed analysis of the diagram on the lineprinter; in complicated cases it makes the interpretation much easier.

Nine different types of shading pattern may be used, and are set by the ATYP numbers of the atoms at the centers of the polyhedra (and of the isolated atoms). The shading patterns are as follows:

1: Herringbone pattern of dashes.

2: Blank.

3: Regular dot pattern.

4: Parallel lines.

5: Hatching (two sets of parallel lines perpendicular to each other).

6: Crosses.

7: Dashes along parallel lines, dashes "in phase" on adjacent lines.

8: Dashes along parallel lines, alternate lines 180 degrees out of phase.

9: Random dot pattern (very effective, but lethal to plotter pens).

These patterns are generated BEFORE the three-dimensional coordinates are projected, so a surface viewed edge-on will appear denser. In practice this provides useful contrast variation. Some of these patterns produce a lot of detail and so create relatively large plotfiles. The spacings used in the patterns are proportional to the second parameter of the GAPS instruction, and so may be set before calling POLP; GAPS without any parameters prints the current settings.

The best way to set up the atom list and connectivity table for POLP is by the use of the ENVI, SGEN and PGEN instructions, though PACK is also useful in some cases. It is important to set up complete polyhedra first (one example of each unique polyhedron suffices) before multiplying them with PGEN or PACK.

The recommended procedure is as follows. After reading in the atoms and preparing a current atom list with FMOL, the instruction ENVI should be used specifying one metal atom and possibly a larger delta than usual (say 1 Å); all atoms within r1+r2+delta of the metal atom are then listed with the appropriate symmetry operations. The ligands that do not have the identity operator (1555) must then be generated using SGEN (note that more than one atom and/or symmetry operator may be used per SGEN instruction to save time). After this has been repeated for all metal atoms it may be necessary to add bonds to complete some of the coordination polyhedra, and to delete other bonds (e.g., between metal atoms) that are not required. A quick method is FMOL with a larger than usual delta (say 1 Å) followed by "PRUN n" (possibly separately for individual atoms or atom types - e.g., "PRUN 4 $SI") to eliminate the longer bonds. In special cases JOIN may be used to add individual bonds and UNDO to delete them, or the bond radii can be altered (ARAD) and FMOL used again. The PICK instruction is useful to check the results and possibly to delete atoms that are not required. It saves time if the shading patterns and colors (ATYP) of the central atoms of the polyhedra, as well as the display radii of isolated atoms (ARAD), are also set at this stage.

When a correct atom and connectivity list defining the unique polyhedra (plus any isolated atoms such as H_3O^+ ions) has been prepared in this way it should be saved on disk by means of "SAVE filename". One can then come back to this point later by means of "NEXT filename" in the same or a subsequent XP run. Only then should PGEN followed by the names of central and isolated atoms be used to fill a specified volume in crystal space. The POLY instruction then provides a quick check before making a plotfile with POLP. PGEN is so constructed that it may be used repeatedly to add or delete polyhedra if the first attempt is not quite right. Note that it starts by deleting all atoms not in the current FMOL list, and finishes by relegating all atoms outside the given volume from this list, so it is important to retain at least one example of each unique polyhedron needed in the current list; sometimes it may be necessary to go back to the SAVE file. PGEN works faster if only one example of each crystallographically independent atom is named. Any of the instructions that affect the view direction may be employed at any stage; e.g., MATR 2 to look down the Y axis, ROTA for small adjustments, PROJ to rotate interactively.

The vertices of the polyhedra are assumed to be bonded to the central atoms by bonds of type 1 or 2. Bonds of type 5, 6 and 7 appear on POLP (but not POLY) plots as single, dashed or dotted lines respectively, and can be used to indicate additional interactions, e.g., between atoms that represent vertices of the polyhedra and isolated atoms. These bonds are not multiplied by PGEN and so must be added after PGEN has been used. Alternatively in some cases (e.g., hydrogen bonds) they may be generated by using the interactive facility PACK (and the "Scan Non-Bonds" option) instead of PGEN.

POLY keywords

The POLY instruction is used to display a structure using the polyhedral representation popular for minerals and inorganic structures. No plotfile is created because it would not be compatible with the 'solid fill' procedure employed (use POLP instead). The keywords (e.g., $AL $SI for an aluminosilicate) define the atoms at the center of the polyhedra. The current FMOL list is searched with the aid of the connectivity table (all bond types) to find the atoms that form the vertices of each polyhedron. For all polyhedra with three or more such vertices, all visible faces are filled with the color assigned to the central atom. Atoms that are neither central atoms nor vertices are displayed as filled circles in the appropriate atom color. Since the furthest polyhedra and isolated atoms are plotted first, they will (usually) be correctly overlapped by the nearer polyhedra and atoms. Finally the unit-cell edges are superposed on the diagram if the keyword CELL is included.

POLY/L provides in addition a detailed analysis of the diagram on the lineprinter; in complicated cases it makes the interpretation much easier!

The best way to set up the atom list and connectivity table for POLY is by the use of the ENVI, SGEN and PGEN instructions, though PACK is also useful in some cases. It is important to set up complete polyhedra first (ENVI/SGEN or GROW) before multiplying them with PGEN or PACK. Further details on setting up the polyhedra may be found under POLP.

POST plotfile

The POST instruction enables text and straight line segments to be combined in the form of a plotfile (that must be named on the POST instruction), and also allows existing plotfiles (that might contain chemical formulae previously created using POST etc.) to be incorporated. POST may thus be used to scale and annotate existing plotfiles and prepare posters for conferences etc. POST starts by drawing a blue grid to aid user input; the grid is NOT incorporated into the plotfile. The grid lines are spaced to facilitate text input and the drawing of benzene rings. A main menu appears on the screen at the right hand side; it is used to select the following options using the

mouse and/or cursor keys, followed by a mouse key or [F6].

'Cursor': the cursor may be moved using the mouse or cursor keys.

'Text mode': Until text mode is terminated by a mouse key or [F6], input is assumed to be text. Each character is plotted with proportional spacing on the screen. The cursor is displayed throughout, and may be moved in the usual way. If you make a typing error recovery is possible using the rubout key (if necessary stepwise). It is only possible to delete characters entered since leaving the main menu. Unknown characters and characters that would (partly) fall outside the screen are ignored (with a bleep). The current cursor position forms the upper left hand corner of the character. [Enter] starts a new line of text, suitably placed below the 'current text origin', that is the cursor position at the start of the previous line of text. The [PgUp] key shifts up about a third of a line (for superscripts) and [PgDn] shifts down by the corresponding amount (to finish superscripts or start subscripts).

'Draw mode': After entering draw mode, the cursor may be moved in the usual way using the mouse and/or cursor keys. Pressing the space bar defines a point in a vector chain, and causes a line to be drawn from the previous point (if any) to the current point. The rubout key may be used to delete line segments back to the point at which draw mode was entered. After a chain or vectors has been 'drawn', the left-hand mouse key (or [F6]) may be used to return to the main menu. The middle mouse key (or [F7]) replaces the chain of straight line segments by a smooth curve passing through all the vertices. Similarly the right-hand mouse key (or [F8]) replaces the vectors by a closed curve, such that the next point is the same as the starting point (that should NOT be repeated!). Thus any three points define a circle. For both 'smooth curve' options at least three points (2 vectors) must have been input.

'Enlarge character': The character size for text mode is increased by a factor of the square root of two.

'Reduce character': The character size is reduced by a factor of the square root of two. Character size operations are cumulative, so may be repeated (simply hit the mouse key or [F6] several times) for larger effects; similarly 'enlarge' and 'reduce' operations cancel each other out.

'Change color': The current color for text and draw operations may be selected from a sub-menu using the mouse, cursor keys, or digit keys, followed in each case by a mouse key or [F6].

'Add form': A plotfile is copied onto the screen, with an origin defined by the cursor position when this mode is entered. A prompt appears for the name of the plotfile; the suffix .plt is assumed if none is specified. Replying to the prompt with [Enter] returns control to the main menu. POST is aborted with an error message if any part of the plot overlaps the edges of the screen. This facility is usually used to insert a small plotfile such as a chemical formula, without any rescaling, but may also be used immediately after starting POST (and before moving the cursor) to input a plot that will then be annotated.

'Add plot': As above, but the plot stored on the plotfile may be rotated and/or reduced in size. The cursor position on entering is taken to define the top left-hand corner of the new plot, and the top right hand corner must then be defined by moving the cursor and hitting any key. The area to be occupied by the plot is outlined by a box whilst the cursor is being moved. This facility is particularly useful for annotating and combining plotfiles produced using the XP instructions TELP, OFIT, SFIL, DIAG and PACK.

'Erase Area': This option enables a selected rectangular area (with edges parallel to the edges of the screen) to be erased from the plot (and plotfile). The current cursor position defines one corner of the box, and the user defines the opposite corner of the erase box using the cursor keys or mouse. An outline of the box appears on the screen whilst this is being done. After hitting any other key, the existing plot is then replotted, leaving out all vector chains that have at least one vertex inside the erase box.

'Finish': returns to the main menu, with the contents of the screen (except the blue grid and menus) saved as the plotfile named on the POST instruction line. The resulting plotfile may be re-edited with POST, displayed on the screen with VIEW, or plotted with DRAW. To abort a particular operation and return to the POST menu, the [Esc] key may be used.

PREV

The orientation immediately before the previous instruction that changed it (e.g., ROTA, MATR, MPLN, PROJ) is reinstated. This 'previous orientation' information is NOT retained in a file created by SAVE.

PRINT

PRINT (the first four letters suffice) spools the accumulated 'printer' output immediately to the printer (defined by SLXP).

PROJ deg keywords

PROJ displays a line drawing of the molecule in mono or red/blue stereo, and at the right hand side a menu from which options may be chosen using the mouse and/or cursor keys, followed by a mouse key (or [F6] or [Enter]). The initial mono/stereo setting is determined by the EYES instruction, but may be changed using the corresponding menu option. The mono plots use intensity depth cueing. The options include real-time rotations about the three plot axes in either direction. Hitting the space bar alternatively speeds up and slows down the rotation (by a factor of 5). The number of degrees per step for a fast rotation is set by deg (the default is hardware dependent). In addition it is possible to display still mono or red/blue stereo diagrams with no labels, with just non-H atoms labeled, or with all atoms labeled. The keyword CELL may be used to display the unit-cell as well.

PROJ/P is designed for alpha-carbon traces of proteins; the /P switch adds bonds linking CA atoms in numerically adjacent residues, and uses residue numbers instead of atoms names in the menu option 'No H etc.'. Thus to display a C-alpha trace and the heme unit of myoglobin, the instruction would be:

```
PROJ/P CA_* *_HEME
```

PRUN nb keywords or PRUN d1 d2 keywords

The connectivity table is 'pruned' so that none of the atoms specified by the keywords make more than nb bonds, the shortest bonds being retained. The default setting of nb is zero, i.e., all bonds to the named atoms are deleted. If there are exactly two numerical parameters, the second form of the PRUN instruction is assumed; all bonds involving at least one of the specified atoms that do not lie inside the range d1 to d2 (in Å) are deleted.

PUSH dx dy dz sign

The coordinate shift dx, dy, dz is applied to all atoms IN THE CURRENT (FMOL) atom list. The coordinates are first multiplied by sign, that must be plus or minus one. Note the distinction to MOVE, that acts on atoms subsequently read in (for compatibility with XL and XS). PUSH is in general more useful than MOVE for applying an origin shift and/or inverting a structure within XP (see also INVT).

QUIT

The QUIT instruction is used to terminate XP and return to the operating system, any accumulated 'printer' output being deleted (unlike EXIT, this output is NOT spooled to the printer).

RASD code

The RASD instruction defines the type of device for raster plots (RAST and [Ctrl-P] during SPIX). The allowed codes are:

L - Monochrome plotting on a HP Laserjet I or II (no tiff encoding).

F - Fast monochrome plotting using tiff encoding on a Laserjet III etc.

C -Color plotting on a Deskjet 550C or compatible printer (tiff encoding).

D Color but using the black cartridge for true blacks on the Deskjet 560C

(automatic with option C for the Deskjet 1200C).

The RASD instruction will normally be placed in the initialization file sxtl.ini that is read on starting XP, but may also be input from the console if it is necessary to change the setting temporarily, e.g., for black and white plots on a color printer. RAST/X and SPIX/X (X=L,F,C or D as above) override the current RASD setting.

RAST filename

The plotfile is output to the laser or inkjet printer (defined by the SLPR instruction) in the form of a raster plot. The RASD instruction must have been used to set the type of printer, but may be overruled by e.g., RAST/F (or RAST/L for older Laserjets) to produce a black and white plot on a color printer. The SLPR and RASD instructions will usually be included in the file sxtl.ini that is automatically read each time XP is started.

RAST is the recommended method of obtaining hard-copy output of SHELXTL plotfiles.

READ filetitle

READ reads atoms, XP instructions etc. from the named file; if no filename extension is specified, ‘.res’ is assumed. If the ‘.ins’ file is not found, '.ins' is tried instead. The file read by READ may not include DEMO, READ or REAP instructions. Note the use of REAP to read through one END instruction.

REAP filetitle

REAP causes further XP instructions to be read from the specified file until END is encountered a second time (or the end of file is reached). This enables Fourier peaks to be read and interpreted as well as atoms; they take names beginning with 'Q' and are plotted green, but otherwise are treated as carbon. If the filetitle does not include a '.', the suffix '.res' will be assumed. If this file is not found, '.ins' is tried instead.

RESI n class

The following atoms (until the next RESI instruction) are assigned to the specified residue, exactly as in the structure refinement program XL.

Residue numbers (but not classes) may also be used in setting up lists of atoms; FMOL *_2 *_3 *_4 N_5 LESS $H would prepare a current atom list of all atoms in residues 2, 3 and 4, plus N in residue 5, but leaving out hydrogen atoms.

RESI/S n class atomnames

RESI/S changes the residue numbers and classes of the specified atoms. Note that 'class' must be given and must precede all atom names, otherwise an atom name will be used as the class! XP does not check whether different classes are used for atoms with the same residue number (but this would upset XL).

RIDE keywords

Parallel and perpendicular r.m.s. amplitudes are calculated for all atoms bonded to the named atoms, and are used to estimate lower limit, riding model, and upper limit bond length corrections. The method of Johnson, Crystallographic Computing, Munksgaard, 1970, pp. 220-226 is employed. The riding (2/1) correction is only appropriate if the parallel amplitudes of the two atoms are approximately equal, and the perpendicular amplitude of atom-2 is significantly greater than that of atom-1 and also significantly greater than the parallel amplitudes.

RING code keywords

The RING instruction finds which bonds are members of rings (as defined by the current connectivity table) and assigns bond type 'code' to them (see 'JOIN' for the specification of code). The default value of 'code' is 2. All seven bond types are permitted in defining the connectivity, so care is needed if hydrogen bonds have been added (as dotted lines) etc. Since all atoms in the ring must be defined by the keywords in order for the ring to be recognized, the ring(s) containing (say) C11 are not considered if the keywords include 'LESS C11' etc.

ROTA n1,phi1; n2,phi2; ...

Modifies the current orientation matrix. n=1, 2 or 3 for rotations about X', Y' and Z'. Positive phi (in degrees) rotates the structure anticlockwise when viewed looking towards the origin from the positive end of the axis. This convention is the same as that adopted for rotations in ORTEP.

ROTA two atoms

Rotates the plot about Z' (the view direction) to make the vector in question horizontal (i.e., parallel to Y') with the first named atom on the left hand side.

SAVE filename

A snapshot of the current structural parameters is written to the file. To re-enter XP at precisely the "SAVEd" point, use the NEXT instruction. Any number of such SAVE files (with different filetitles) may be created during an XP run. This provides a convenient method of saving different views for subsequent plotting etc. If the filename does not contain a '.', the suffix '.sav' is assumed. Such 'save' files are intended for local rather than long-term archiving; they are generally upwards (but not downwards) compatible within the same operating system, but are not transferable between different platforms, e.g., PC's and SG Irises. Addition of extra facilities to future versions of XP could well lead to specific compatibility problems (an example was the introduction of PART numbers for disordered groups in version 5). The .res files produced by XL and the orthogonal coordinate files written by the ORTH instruction in XP are ASCII, and so much more suitable for network transfer and archiving.

SFIL s d keywords

A space-filling molecular model plot is displayed on the screen and written to a plotfile. XP prompts for the name of the plotfile (unless SFIL is called from a DEMO loop, in which case the name temp.plt is used). The extension .plt is assumed if the filename does not contain a '.' character. s is the stereo view angle for each eye, and should typically be 3 for left-right stereo and -3 for red-green stereo. The default value is zero (mono). d is the distance of the eye from the screen or paper in cm; it is used to

calculate perspective; it may usually be left at the default value of 50. The space filling atomic radii may be changed using ARAD and the atom colors may be changed with ATYP.

After creating the plotfile and displaying it on the screen, the interactive atom labeling routine is entered (unless SFIL is called from a DEMO file); mono and stereo plots may be labeled. Stereo labels are always placed at the same height as the center of the atom, so may conveniently be placed INSIDE the atoms. The shading lies on the surface of the spheres, creating a realistic three-dimensional effect in stereo plots.

SGEN symcodes keywords

New atoms (with unique names assigned by the program) are generated for all permutations of the specified atoms and symcodes. Atoms are NOT generated if the operation produces an atomic position that is already occupied, even if the corresponding atom is not in the current FMOL list. For information about symcodes see 'SYMCODES', 'SYMM' and 'SRCH'. The appropriate transformations are applied to anisotropic temperature factors; atom radii, colors, types etc. are simply copied. To change atom names, use NAME or PICK.

A negative symcode transforms the named atoms rather than creating new atoms; in this case only one symcode is allowed.

SGEN/N suppresses output of new coordinates etc.

If the program is not able to assign unique atoms names by varying the last character of the atom name, the new atom(s) are still generated, but with the last character set to 'Z'.

SLPF instructions

SLPF defines how XCIF and SPRINT output is spooled to a printer and then deleted. It is not used by XP, but is normally present in the sxtl.ini file that XP reads when it is started. Examples:

`SLPF copy/A ? lpt1; del ? or SLPF >lpt1` for MS-DOS, and

`SLPF lp ?; rm ?` for UNIX systems.

In these operating system instructions, ? is replaced by the name of the scratch file used for spooling, and the '>' symbol may be used to output directly to the device without spooling (PC's only).

SLPR instructions

SLPR defines how XP and XPREP spool (binary) raster output to a printer, and then delete the corresponding scratch file. SLPR is usually present in the sxtl.ini file that XP reads when it is started. Examples:

`SLPR copy/B ? lpt1; del ? or SLPR >lpt1` for MS-DOS, and

`SLPR lp -oraw ?; rm ?` for SGI systems.

In these operating system instructions, ? is replaced by the name of the scratch file used for spooling, and the '>' symbol may be used to output directly to the device without spooling (PC's only). The '>' shortcut may only be used when 'raster' (SLPR) and 'printer' (SLXP) output is sent to different devices.

SLXP instructions

SLXP defines how XP spools 'printer output' to a printer, and then deletes the corresponding scratch file. SLXP is usually present in the sxtl.ini file that XP reads when it is started. Examples:

`SLXP copy/B ? lpt1; del ? or SLXP >lpt1` for MS-DOS, and

`SLXP lp ?; rm ?` for SGI systems.

In these operating system instructions, ? is replaced by the name of the scratch file used for spooling, and the '>' symbol may be used to output directly to the device without spooling (PC's only). The '>' shortcut may only be used when 'raster' (SLPR) and 'printer' (SLXP) output is sent to different devices.

SORT atom1 atom2 ... atomn

SORT followed by precisely one atom name moves that atom to the beginning of the atom list. SORT followed by more than one atom name inserts all atoms except atom1 at the position immediately following atom1 in the list. In either case hydrogen atoms are included immediately after the carbon (or other) atom to which they are attached. The current connectivity table is used to decide which atoms the hydrogens are bonded to; if a hydrogen appears to be bonded to more than one atom (e.g., because an H-bond has been added to the connectivity table) the shortest distance decides. SORT is useful for sorting atom lists that are then written to an .ins file by FILE for further structure refinement etc.

After SORT/H, hydrogens are treated in the same way as other atoms, i.e., do not move with the atoms to which they are bonded.

SORT/N sorts the specified atoms according to the numerical order of their atom names, ignoring any alphabetic or other non-numerical characters. The atoms are placed starting at the position corresponding to the highest of the specified atoms in the previous current atom list. Very often this form of the SORT instruction will be applied several times to suitable subsets of the full atom list, possibly in combination with calls to SORT without /N.

SORT/R sorts atoms in numerical residue order, and SORT/P sorts in residue order and within residues into standard PDB order (assuming relatively standard PDB atom names).

SPIX keywords

The SPIX instruction displays 'solid' space filling models with specular reflections and diffuse lighting from a spotlight that can be positioned, as well as diffuse direct lighting. The resulting pictures take a couple of minutes to calculate but are particularly realistic. No plotfile is generated.

The space filling radii and colors are determined by the SFAC numbers on reading in the atoms; they may be changed by ARAD and ATYP respectively. The background color for a SPIX diagram is set using BACK; medium blue (BACK 3 3) is a good choice. The intensity of the soft direct light, diffuse and specular reflection coefficients, and Phong model parameter are set with the LITE instruction, and the spotlight intensity and position are set using SPOT. These instructions with suitable default values will usually be present in the file sxtl.ini that XP reads on starting. To switch off the specular reflection (that appears as a small white circle of light) the specular reflection coefficient should be set to zero. The ZOOM instruction may be used to generate a close-up view of a region of interest.

If [p] or [Ctrl-P] is hit whilst a SPIX diagram is displayed on the screen, a copy is output to a color raster printer (defined by RASD and SLPR). This uses the (higher) resolution of the printer, but at the cost of fewer colors (an inkjet printer can effectively only use 7 different colors for a pixel), which in turn means that 'dithering' has to be used to simulate a greater range of color. The process of generating a hard-copy is slow, so be patient. Several copies may be made by hitting [Ctrl-P] the required number of times. To obtain a hardcopy with a white background, use W or [Ctrl-W] instead; P and [Ctrl-P] always use a blue background, independent of the background used for the screen display.

SPLT instructions

SPLT defines how XP spools HPGL format output to a pen-plotter, then deletes the corresponding scratch file. SPLT is usually present in the sxtl.ini file that XP reads when it is started. Examples:

`SPLT copy/A ? com2; del ?` or `SPLT >com2` for MS-DOS

In these operating system instructions, ? is replaced by the name of the scratch file used for spooling, and the '>' symbol may be used to output directly to the device without spooling (PCs only). The '>' shortcut may only be used when 'raster' (SLPR) and 'printer' (SLXP) output not sent to the device used by SPLT.

SPOT I mu phi

Spotlight intensity I (typically in the range 4-20), azimuthal angle mu in degrees (i.e., angle from the normal to the screen, typically 60) and compass angle phi in degrees (0=N, 45=NE, 90=E, 180=S etc.) for use with the SPIX space filling models. The default values will usually be set by a SPOT command in the initialization file sxtl.ini, but may be changed at any time with a new SPOT instruction.

SRCH keywords delta

All possible symmetry transformed positions are generated for the specified atoms. Whenever the resulting position is less than 'delta' plus the sum of the two covalent radii from one of the untransformed atoms in the full current atom list, the program prints both atom names, interatomic distance, and the SYMCODE necessary for the transformation of the atom named on the SRCH command. This procedure may be used to generate atoms from the unique set in a molecule containing crystallographic symmetry, also to search for short intermolecular contacts and possible hydrogen bonds. The default value of 'delta' is 0.5 Å. The symcodes may be used to add the symmetry transformed atoms to the atom list (see 'symcodes' and SGEN). Alternatively the GROW instruction may be used to add bonded symmetry equivalent atoms automatically.

SYMM symmetry operator

The SYMM instruction is used to read in symmetry operators in standard SHELX format, e.g., the space group $P3_112$ would be defined as follows:

```
LATT -1
SYMM -Y, X-Y, .333333+Z
SYMM -X+Y, -X, .666667+Z
SYMM -Y, -X, .666667-Z
SYMM -X+Y, Y, .333333-Z
SYMM X, X-Y, -Z
```

Lower case letters and fractions are also allowed, e.g., **`SYMM -y, x-y, z+1/3`** is an allowed alternative to the first SYMM instruction above.

These operators correspond to the coordinates of the general position as given in International Tables, except that the operator X, Y, Z is always omitted (since it must be present), and the effect of an inversion center or lattice centering is conveyed by the LATT instruction, and should not be repeated using SYMM instructions.

SYMM without any operator produces a list of currently stored operators and their codes, including operators generated by combining LATT and SYMM input. See 'symcodes' for further details.

SYSD path

SYSD sets the path for SHELXTL program and system files. Under MS-DOS it is usually C:\SAXI\SXTL, under UNIX /usr/saxi/sxtl.

TELP s p b d keywords

TELP creates a molecule plot with bonds defined by FMOL, LINK, JOIN and UNDO. The atoms are plotted as ellipsoids (and the principal amplitudes and corresponding eigenvectors in the XO coordinate system printed) if the atom type is negative (see 'ATYP'), otherwise as (possibly shaded) spheres. The random dot atom display (types 7-9) and the principal ellipses of the thermal ellipsoids take perspective and stereo effects into account, and so give a particularly effective 3-dimensional effect in stereo plots.

s is the stereo view angle in degrees (i.e., the angle between each eye and the vertical; actually the stereo effect is generated by translation, but the equivalent rotation is easier to remember). Typical values are 3 (for 'normal' stereo using a double frame), and -3 (red/green stereo). The default setting of 0 specifies a mono plot. To suppress the double frame around a stereo plot, add 100.

p is the percentage probability for the thermal ellipsoid; default 50. If p is negative, thermal ellipsoids (atom type -1) are plotted for all non-hydrogen atoms, overwriting the set atom types, so both ball and stick and thermal ellipsoid plots may be obtained without changing the default settings.

b is the bond radius, default 0.05 Å. A negative value causes bond types 1 and 3 to be plotted as 2 and 4 respectively (see JOIN or LINK for bond type definitions); this improves red/green stereo plots.

d is the view distance in cm (default 50). A zero value produces a plot in parallel projection rather than perspective; for logical reasons this is not permitted for stereo diagrams (that require perspective). A negative value of d is taken to be the scale of the final plot in 'plot units' per Angstrom. The conversion factor from plot units to inches (or cm) depends on the type of plotter etc. but is about 100 plot units per mm for A or A4 size plots. In this case a parallel projection is assumed (such a scale would be meaningless for a perspective plot), and there is no automatic check on whether the plot will fit on the paper or not. All plots are generated in the 'current orientation' and centered on the paper (or screen). Double stereo plots are always rotated 90 degrees about Z'.

The program prompts for the name of the plotfile; the extension '.plt' is assumed if the filename do not contain the character '.'. If TELP is called from a DEMO file, a dummy file 'temp.plt' is created.

After completing the plot the interactive atom labeling routine is entered unless TELP was called from a DEMO file (see LABL and LABELS); mono and both types of stereo plots may be labeled, and in stereo plots the labels are always placed at the same height as the atom centroids.

The special keyword 'CELL' adds the unit cell to the plot, and allows the axes to be labeled. This may be used to prepare packing plots, that may incorporate the full flexibility of the other TELP features. However the user is responsible for generating the symmetry equivalent molecules, e.g., by using PACK.

TELP/P is designed for alpha-carbon traces of proteins; the /P switch adds bonds linking CA atoms in numerically adjacent residues, and uses residue numbers instead of atoms names. N and C terminii are also labeled, and every 10th CA atom is drawn as a small ball (with radius p); the other CA atoms have zero radius. The default setting is double-frame stereo (s=3); otherwise all the usual TELP facilities are available unchanged. This instruction produces diagrams as specified for the journal 'Structure'. To display a Cα trace and the heme unit of myoglobin, the comand would be:

```
TELP/P CA_* *_HEME
```

TITL text

A title of up to 76 characters may be read in to identify or annotate a structure.

TORS keywords

TORS calculates torsion angles in which all atoms are specified by the TORS keywords, by making a search of the connectivity table. The torsion angle W(IJKL) (type omega) is defined as the angle between the vector JI and the vector KL when viewed down JK. The sign of W is positive if JI is to be rotated clockwise into KL and negative if anticlockwise. For further details of the calculation see F.H. Allen and D. Rogers, Acta Cryst., (1969) B25 1326. Torsion angles of type delta and tau as defined by Allen and Rogers are more conveniently obtained via the NEWM (Newman projection) instruction.

UNDO keywords

Deletes all bonds from the connectivity table that involve two atoms included in the list defined by the keywords. To delete e.g., all bonds to one specific atom or type of atom, use PRUN.

UNIQ keywords

UNIQ makes an iterative search to assemble 'molecules' starting from the named atom(s). Atoms in the current FMOL list but not in the UNIQ list are symmetry transformed to find bonds shorter than br1+br2+delta to the atoms in the UNIQ list. The appropriate U_{ij} transformations are also applied. When no further bonds can be found the current UNIQ list becomes the FMOL list, and a connectivity table is calculated in the usual way. This command results in atoms being removed from the FMOL list if they are not connected to those in the UNIQ list. It therefore provides a very simple method of setting up a FMOL list containing one molecule or ion in a structure containing several molecules or ions in the asymmetric unit. Note that if the molecule or ion lies on a crystallographic symmetry element, UNIQ only uses the unique atoms, whereas GROW will also generate symmetry equivalents. When all the necessary calculations have been made with the part of the structure in question, the next fragment may be selected by e.g., 'FMOL REST' followed by UNIQ.

USER path

Sets the default path for all file operations. 'path' is added to the beginning of all subsequent filenames (until the next USER instruction); the exact specification depends on the operating system employed.

USER without parameters displays the current default.

VIEW plotfile

The specified plotfile is displayed on the screen. If the plotfile name does not contain the character '.', the extension '.plt' is assumed. VIEW/W may be used to display the plot against a white background (with color 6 - usually white - changed to turquoise to aid visibility). VIEW/S can be used to display TELP, PACK or SFIL red/green stereo plots with green displayed as blue and color addition rather than overwriting (this optimizes the stereo effect). To return to command mode after displaying the plot, hit any key.

WAIT wt keywords

Atoms may be assigned weights for use in subsequent MPLN, EDEN, OFIT and LIBR calculations. The default setting for all atoms is unit weight. For many calculations atomic numbers are suitable weights.

WIPE

The WIPE instruction may be used to clear the screen.

ZOOM scal

ZOOM sets a scaling factor for close-up views of space filling models using SPIX. The default scal value of 1.0 corresponds to the normal magnification. It is sometimes useful to use a value such as 0.9 when photographing from the monitor.

Appendix A: Compatibility Considerations

In general, all ASCII files read or written by SHELXTL are compatible over different operating systems, e.g., on a NFS shared network. In general ALL SHELXTL programs on both MS-DOS and UNIX systems can read either DOS or UNIX format ASCII files (or even mixed format!). In some cases file protection problems may make it necessary to copy or chmod a file before it can be accessed by another computer on a shared network.

Although SHELXTL plotfiles are binary (which makes them much more compact) they can be read and written by XP (and written by XPREP) on all platforms supported by SHELXTL. The necessary record delimeters, byte reversal etc. are handled internally by the program and require no special action by the user. Thus for example the PC, SGI and RS/6000 versions of SHELXTL can all access the same plotfiles on a disk shared using NFS. Note that in version 5 of SHELXTL the format is the same for all platforms, but that this was not true for previous releases. The common format adopted for version 5 is the same as the PC format for previous versions.

SAVE files written by XP are intended for local rather than long-term archiving; they are generally upwards (but not downwards) compatible within the same operating system, but are not transferrable between different platforms, e.g., PCs and SG Irises. Addition of extra facilities to future versions of XP could well lead to specific compatibility problems (an example was the introduction of PART numbers for disordered groups in version 5). The .hkl and .pcf files written by XPREP, the .res, .cif and .fcf files produced by XL, and the orthogonal coordinate .ort files written by the ORTH instruction in XP are ASCII, and so much more suitable for network transfer and archiving.

The .hkl, .ins, .res, .cif and .fcf files used by SHELXTL are completely compatible with the files with the same extensions used by the public domain programs SHELXS-86, SHELXL-93 and CIFTAB, and can also be read by many other program systems.

Appendix B: SHELXTL Plot File Format

The SHELXTL programs XPREP and XP are able to create plot files. These contain all necessary plotting instructions encoded in the form of two fundamental operations (change color, and plot a string of straight line segments, possibly with retracing). Letters and numbers (in atom labels) are also encoded in this way; hardware generated characters are not used. The simplicity of this format makes it easy to incorporate new graphics devices into the system.

Although SHELXTL plot files are binary (which makes them much more compact) they can be read and written by XP (and written by XPREP) on all platforms supported by SHELXTL. The necessary record delimiters, byte reversal etc. are handled internally by the program and require no special action by the user. Thus for example the PC, SGI and RS/6000 versions of SHELXTL can all access the same plot files on a disk shared using NFS. Note that in version 5 of SHELXTL the format is the same for all platforms, but that this was not true for previous releases. The common format adopted for version 5 is the same as the PC format for previous versions.

The following notes are provided for users who wish to read or write SHELXTL plot files using their own programs. For historical reasons involving the RM-FORTRAN compiler that was used for SHELXTL-PC up to version 4 inclusive, SHELXTL plot files consist of 1032-byte blocks, in which the first 4 and the last four bytes are record delimiters that can be ignored on reading (they each contain 1032 expressed as integer*4). The middle 1024 bytes should be interpreted as 512 16-bit signed integers (MS-DOS byte order). The following subroutine GPLF can be used to read the plotting instructions from such a file, assuming that it has been opened on unit LP as a byte-stream, i.e., with no automatic interpretation of record delimiters (e.g., with ACCESS='TRANSPARENT' for the Lahey PC compilers). NPL should be set to at least 512 before calling GPLF for the first time; after that, NPL and IP should not be changed between calls.

```
      SUBROUTINE GPLF(LP,NPL,IP,IS,N)
      INTEGER*4 I,M,N,LP,NPL,IS(N)
      INTEGER*2 IP(512)
        DO 2 I=1,N
        IF(NPL.LT.512)GOTO 1
        READ(LP)M,IP,M
        NPL=0
    1   NPL=NPL+1
        IS(I)=IP(NPL)
    2   CONTINUE
      RETURN

      END
```

On computer systems with the other byte order, it would be necessary to swap the order of bytes for each element of IP; many compilers provide a subroutine for doing this. The following general strategy is used to interpret the file:

```
      INTEGER*4 L,N,LP,NPL,IW(1024)
      INTEGER*2 IP(512)
      ...
      Open plotfile on unit LP (readonly, shared, byte-stream)
      NPL=512
      ...
1     CALL GPLF(LP,NPL,IP,IW,1)  ! get command word
      N=IW(1)
      IF(N.EQ.1)GOTO ...         ! end of plot has been reached
      IF(N.GT.0)GOTO 2
```

The color code is now set to -N. Values of N less than -15 are not currently defined, but are reserved for future use in SHELXTL. Thus currently 16 colors (0 to 15 inclusive) are supported; they are defined in the manual (see also HELP color). N should also not be greater than 510; it is likely that values above 16384 will be used to indicate that (N-16384) coordinate pairs follow, to be plotted as dots.

```
      GOTO 1

2     L=2*N+1
      CALL GPLF(LP,NPL,IP,IW,L)
```

IW(1) now contains the 'retrace count', i.e., the number of times a line should be drawn. This is only of interest to pen-plotters, and is often 1. Values outside the range 1 to 15 inclusive are reserved for future use in SHELXTL; it is likely that -15 to 0 will indicate the fill color for a convex polygon defined by the list of points that follow (the edge color being set as for lines).

IW(2),IW(3); IW(4),IW(5); ... IW(N-1),IW(L) now contain the DOWN and ACROSS (left to right) coordinates of points that define a connected chain of line segments. The coordinate system is defined so that the origin (0,0) is at the top left hand corner of the page; X runs DOWN the page to a maximum allowed value of 24000, Y runs across the page to a maximum allowed value of 32000. There must be at least two points in this chain (i.e., L must be at least 5), and it is permitted that two points have the same coordinates (indeed, this is the only way to make a dot). SHELXTL plots are all scaled to make effective use of the full (0-24000,0-32000) plotting area.

```
      GOTO 1
```

That's all! Programmers familiar with the complexities and idiosyncrasies of HPGL or Postscript should now take a break to recover from the shock. SHELXTL plot files contain ONLY color changes and chains of line segments; characters are converted to this format by the use of a “stroked” font when XP creates the plot file.

Appendix C: Examples of SHELXTL Initialization Files: sxtl.ini

The sxtl.ini file contains the information SHELXTL programs need to define the types of hard-copy device available, and how to access these devices (possibly over a network). The file also contains various installation dependent default settings, e.g., the parameters for making the realistic SPIX space-filling models in XP; the optimum values depend to some extent on the monitor characteristics, and are also a matter of taste. On all systems an environment variable SXTL is used to point to the full path and filename of this file; if this variable is not set, then the programs search for sxtl.ini in a standard directory (specified below).

sxtl.ini is the only initialization file required by SHELXTL, and is used by all the SHELXTL programs. XP reads sxtl.ini in the same way as any other file (e.g., after the XP instruction READ), so sxtl.ini may contain any legal XP instructions (except those requiring user interaction); sxtl.ini is read before the file specified on the command line when XP is invoked.

Often the XP instruction ECHO is used to include information about recent changes to XP, so that these are displayed on the screen each time XP is started; to save space these instructions have been omitted in the examples given below.

Lines beginning with '#' are treated as comments; if you customize your version of sxtl.ini you should explain this (and give details of any instructions that have been replaced) in the form of such comments.

C.1 MS-DOS systems (Default: c:\saxi\sxtl\sxtl.ini)

```
# SXTL.INI for MSDOS.  The environment variable SXTL points to this file.
# Comments begin with '#', defaults in square brackets, '?' = dummy filename.
#
# Define directory containing executable and system files [SYSD c:\exe\]
SYSD c:\saxi\sxtl\
#
# Define directory containing examples files [EGSD c:\egs\]
EGSD c:\saxi\egs\
#
# Define directory containing help files [HLPD c:\hlp\]
HLPD c:\saxi\help\
#
# Type of raster device (L=Laserjet-2, F=Laserjet-3, C=Deskjet-550C) [RASD L]
RASD L
#
# Spool raster file to device, then delete [SLPR copy/B ? lpt1; del ?];
# >lpt1  etc. may be used to write directly to the device (MSDOS only).
SLPR >lpt1
#
# Spool XCIF and XPRINT output, then delete it [SLPF copy/B ? lpt1; del ?]

# This may use the same device as SLPR, and may use >lpt1  etc. to write
# directly to the device (MSDOS only).
SLPF >lpt1
#
# Spool XP printer output, then delete it.  The >lpt1 (etc.) facility may
# only be used under MSDOS and when SLPR and SPLT use different devices.
# [SLXP copy/B ? lpt1; del ?]
SLXP copy/B ? lpt1; del ?
#
# Spool HPGL output to pen plotter, then delete [SPLT copy/A ? com2; del ?]
# >com2  etc. may be used to write directly to device (MSDOS only) only if
# not used by SLPR or SLXP to access the same physical device.
SPLT copy/A ? com2; del ?
#
# Spotlight intensity, azimuthal and compass angle for space filling (SPIX)
# plots [SPOT 12 60 35]
SPOT 12 60 35
#
# Intensity of soft direct light, diffuse and specular reflection coefficients
# and Phong shading model parameter for SPIX [LITE 4 0.3 0.05 12]
LITE 4 0.3 0.05 12
#
# Background color (in XP color table) and intensity for SPIX [BACK 8 3]
BACK 8 3
#
# Overlap and shading gaps for TELP and polp PLOTS [GAPS 80 80]
GAPS 80 80
#
# Start in mono (1) or stereo (2) mode for PROJ, PICK and MAPS [EYES 1]
EYES 1
#
```

C.2 UNIX Version for Silicon Graphics

```
# SXTL.INI for IRIX.  The environment variable SXTL points to this file.
# Comments begin with '#', defaults in square brackets, '?' = dummy filename.
#
# Define directory containing executable and system files [SYSD c:\exe\]
SYSD /usr/saxi/sxtl/
#
# Define directory containing examples files [EGSD c:\egs\]
EGSD /usr/saxi/sxtl/egs/
#
# Define directory containing help files [HLPD c:\hlp\]
HLPD /usr/saxi/sxtl/help/
#
# Type of raster device (L=Laserjet-2, F=Laserjet-3, C=Deskjet-550C) [RASD L]
RASD C

#
# Spool raster file to device, then delete [SLPR copy/B ? lpt1; del ?];
# >lpt1  etc. may be used to write directly to the device (MSDOS only).
SLPR lp -c -oraw ?; rm ?
#
# Spool XCIF and XPRINT output, then delete it [SLPF copy/B ? lpt1; del ?]
# This may use the same device as SLPR, and may use >lpt1  etc. to write
# directly to the device (MSDOS only).
SLPF lp -c -oraw ?; rm ?
#
# Spool XP printer output, then delete it.  The >lpt1 (etc.) facility may
# only be used under MSDOS and when SLPR and SPLT use different devices.
# [SLXP copy/B ? lpt1; del ?]
SLXP lp -c ?; rm ?
#
# Spool HPGL output to pen plotter, then delete [SPLT copy/A ? com2; del ?]
# >com2  etc. may be used to write directly to device (MSDOS only) only if
# not used by SLPR or SLXP to access the same physical device.
SPLT rm ?
#
# Spotlight intensity, azimuthal and compass angle for space filling (SPIX)
# plots [SPOT 12 60 35]
SPOT 12 60 35
#
# Intensity of soft direct light, diffuse and specular reflection coefficients
# and Phong shading model parameter for SPIX [LITE 4 0.3 0 12]
LITE 4 0.3 0 12
#
# Background color (in XP color table) and intensity for SPIX [BACK 3 3]
BACK 3 3
#
# Overlap and shading gaps for TELP and polp PLOTS [GAPS 50 50]
GAPS 80 80
#
# Start in mono (1) or stereo (2) mode for PROJ, PICK and MAPS [EYES 1]
EYES 2
#
```

C.3 UNIX Version for IBM RS/6000 Series

```
# SXTL.INI for RS/6000.  The environment variable SXTL points to this file.
# Comments begin with '#', defaults in square brackets, '?' = dummy filename.
#
# Define directory containing executable and system files [SYSD c:\exe\]
SYSD /usr/saxi/sxtl/
#
# Define directory containing examples files [EGSD c:\egs\]
EGSD /usr/saxi/sxtl/egs/

#
# Define directory containing help files [HLPD c:\hlp\]
HLPD /usr/saxi/sxtl/help/
#
# Type of raster device (L=Laserjet-2, F=Laserjet-3, C=Deskjet-550C) [RASD L]
RASD L
#
# Spool raster file to device, then delete [SLPR copy/B ? lpt1; del ?];
# >lpt1  etc. may be used to write directly to the device (MSDOS only).
SLPR lp -c -o -dp ? ; rm ?
#
# Spool XCIF and XPRINT output, then delete it [SLPF copy/B ? lpt1; del ?]
# This may use the same device as SLPR, and may use >lpt1  etc. to write
# directly to the device (MSDOS only).
SLPF lp -c -o -dp ? ; rm ?
#
# Spool XP printer output, then delete it.  The >lpt1 (etc.) facility may
# only be used under MSDOS and when SLPR and SPLT use different devices.
# [SLXP copy/B ? lpt1; del ?]
SLXP lp -c ? ; rm ?
#
# Spool HPGL output to pen plotter, then delete [SPLT copy/A ? com2; del ?]
# >com2  etc. may be used to write directly to device (MSDOS only) only if
# not used by SLPR or SLXP to access the same physical device.
SPLT rm ?
#
# Spotlight intensity, azimuthal and compass angle for space filling (SPIX)
# plots [SPOT 12 60 35]
SPOT 12 60 35
#
# Intensity of soft direct light, diffuse and specular reflection coefficients
# and Phong shading model parameter for SPIX [LITE 4 0.3 0 12]
LITE 4 0.3 0 12
#
# Background color (in XP color table) and intensity for SPIX [BACK 3 3]
BACK 3 3
#
# Overlap and shading gaps for TELP and polp PLOTS [GAPS 50 50]
GAPS 80 80
#
# Start in mono (1) or stereo (2) mode for PROJ, PICK and CMAP [EYES 1]
EYES 2
#
```

Appendix D: Atomic Radii

The following atomic radii are assumed by XS, XL and XP when the atom name is given on the SFAC instruction. They are based on single bond covalent radii where available, and in the case of metals, on the shortest distances in the metal. This proves to be more useful in practice than using ionic radii, especially for organometallic compounds and others containing metal-metal bonds. The sum of two of these radii is usually not far from the sum of the corresponding ionic radii.

In general, two atoms are considered to be “bonded” if they are closer than r1 + r2 + **delta**, where **delta** is given on the CONN instruction in XL or the FMOL instruction in XP.

H	0.32	HE	1.50	LI	1.52	BE	1.11
B	0.82	C	0.77	N	0.70	O	0.66
F	0.64	NE	1.50	NA	1.86	MG	1.60
AL	1.25	SI	1.17	P	1.10	S	1.03
CL	0.99	AR	1.50	K	2.27	CA	1.97
SC	1.61	TI	1.45	V	1.31	CR	1.24
MN	1.37	FE	1.24	CO	1.25	NI	1.25
CU	1.28	ZN	1.33	GA	1.26	GE	1.22
AS	1.21	SE	1.17	BR	1.14	KR	1.50
RB	2.48	SR	2.15	Y	1.78	ZR	1.59
NB	1.43	MO	1.36	TC	1.35	RU	1.33
RH	1.35	PD	1.38	AG	1.44	CD	1.49
IN	1.44	SN	1.40	SB	1.41	TE	1.37
I	1.33	XE	1.50	CS	2.65	BA	2.17
LA	1.87	CE	1.83	PR	1.82	ND	1.81
PR	1.81	SM	1.80	EU	2.00	GD	1.79
TB	1.76	DY	1.75	HO	1.74	ER	1.73
TM	1.72	YB	1.94	LU	1.72	HF	1.56
TA	1.43	W	1.37	RE	1.37	OS	1.34
IR	1.36	PT	1.37	AU	1.44	HG	1.50
TL	1.64	PB	1.60	BI	1.60	PO	1.60
AT	1.60	RN	1.80	FR	2.80	RA	2.20
AC	1.90	TH	1.85	PA	1.80	U	1.80
NP	1.80	PU	1.80				

Appendix E: References

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