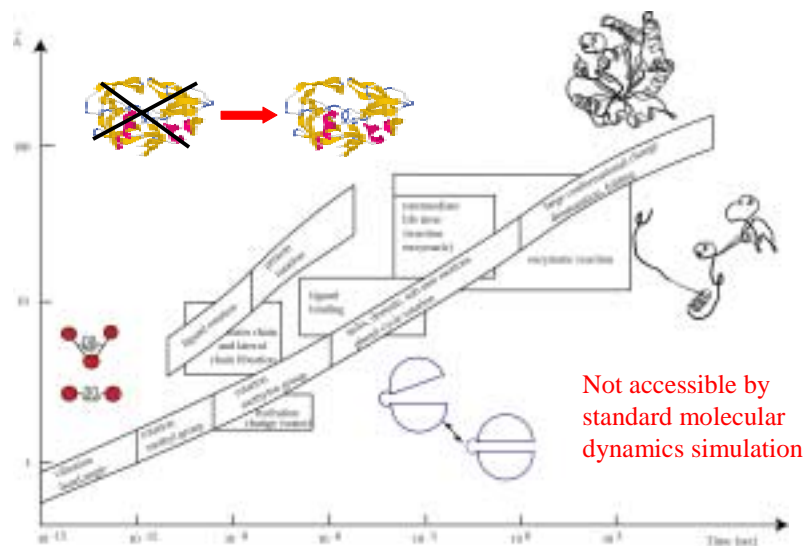


Introduction to Normal Mode Analysis (NMA)

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DYNAMICS OF BIOLOGICAL SYSTEMS



NORMAL MODE ANALYSIS: THEORY

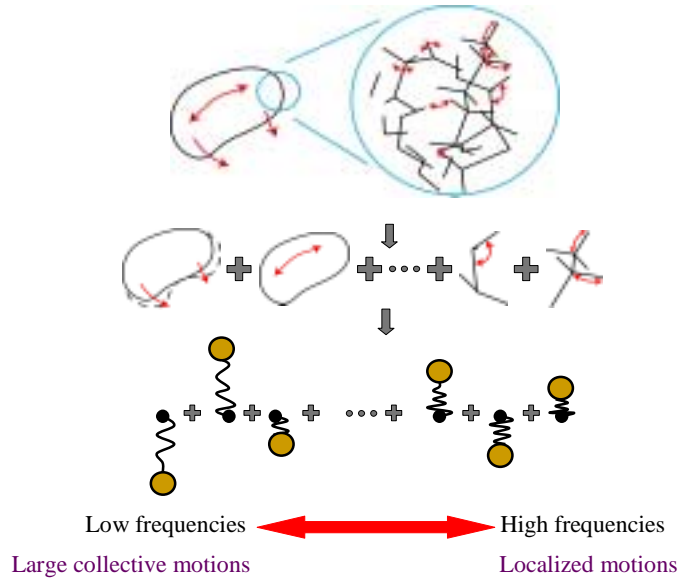
Potential energy => harmonic



Dynamic = harmonic potential
independent

$$L = \frac{1}{2} \sum_{i=1}^{3N-6} \dot{q}_i^2 - \frac{1}{2} \sum_{i=1}^{3N-6} \omega_i^2 q_i^2$$

NORMAL MODE ANALYSIS



EXAMPLES

Adenylate kinase:
Mode 1 (2.95 cm^{-1})

Collective
motion



EXAMPLES

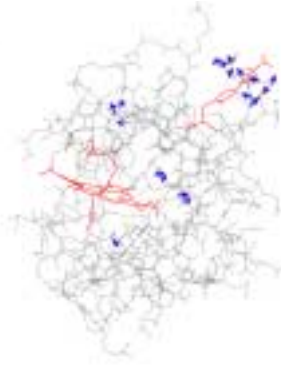
Adenylate kinase:
Mode 1 (2.95 cm^{-1})

Collective
motion



Cytochrome c:
Mode 2757 (1519 cm^{-1})

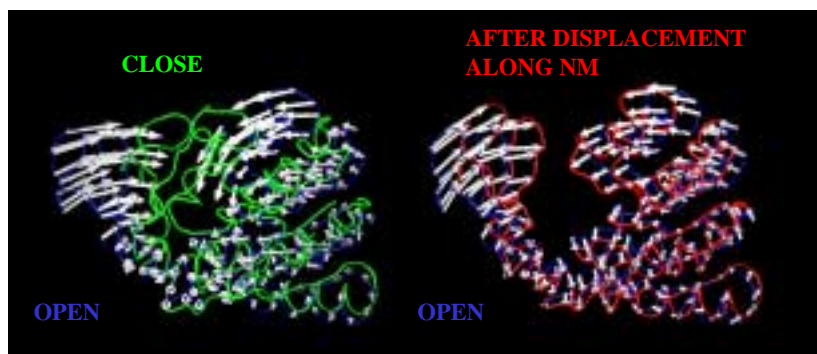
Localized
motion



NMA GIVES CONFORMATIONAL CHANGE

Adenylate Kinase =>

large conformational change upon ligand binding



1 normal mode can represent up to 80-90 % of the overall conformational change

NORMAL MODE CALCULATIONS

Requires

- minimization
- diagonalization of the
2nd derivative of the
potential energy : Hessian

NORMAL MODE CALCULATIONS

Requires



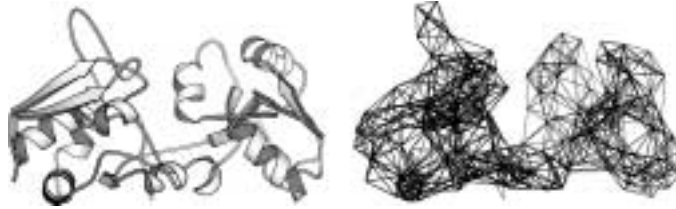
Problems for large systems

- minimization
- diagonalization of the
2nd derivative of the
potential energy : Hessian
- long and distortion of the
structure
- size of the system

TO AVOID MINIMIZATION ...

Elastic Network Model²

$$E(r_a, r_b) = \frac{C}{2} \left(|r_{a,b}| - |r_{a,b}^0| \right)^2 \quad E_p = \sum_{a,b} E(r_a, r_b)$$



Coarse grained model (up to 1 point for 40 residue)

2 - Tirion MM (1996) *Phys Rev Lett.* **77**, 1905-1908

TO DIAGONALIZE ...

DIMB => Diagonalization in Mixed Basis

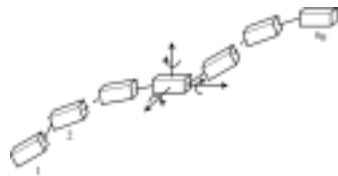
(Perahia & Mouawad, 1995, *J. Comp. Chem.* **19**, 241)

Group theory => Use symmetrical properties of viruses

(Roux & Karplus, 1988, *Biophys. J.* **53**, 297; Simonson & Perahia, 1992, *Biophys. J.*, **61**, 410; van Vlijmen & Karplus, 2001, *J. Chem. Phys.*, **115**, 691)

RTB => Rotation Translation Blocks method gives approximate low-frequency NM

(Tama et al. 2000, *Proteins: Struct. Funct. Genet.*, **41**, 1)



- block = 1 or several residues
- rotation + translation of block => new basis
- expression of Hessian in this new basis
- diagonalization of a matrix $6n_B * 6n_B$

APPLICATIONS OF NMA: VIRUSES

Cowpea chlorotic Mottle virus (CCMV) => native CCMV is stable at pH=5, at pH=7, the particle undergoes a concerted transition to a swollen form. It has been interpreted as the result of **electrostatic repulsion of charged carboxylic clusters** at the quasi 3-fold axis

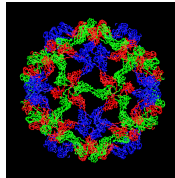
➤ NMA on native CCMV

➤ Search for a conformational pathway of the swelling process by using:

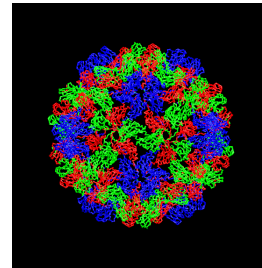
CRYO EM DATA or PSEUDO ATOMIC MODEL



Reddy V et al. *J. of Virology*
75:11943



Selection of modes contributing to the conformational change



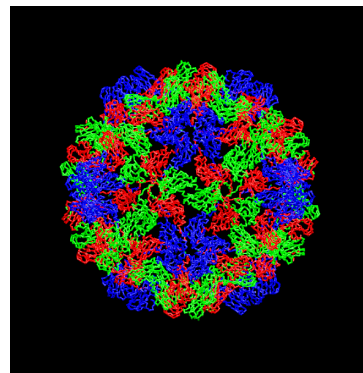
CONFORMATIONAL PATHWAY FOR THE SWELLING PROCESS

➤ Several intermediates structures (IS)

➤ Association energy at the interface

➤ IS2 => no association energy at Q3

➤ Pka calculations to determine the origin of the swelling process



ORIGIN OF THE SWELLING PROCESS

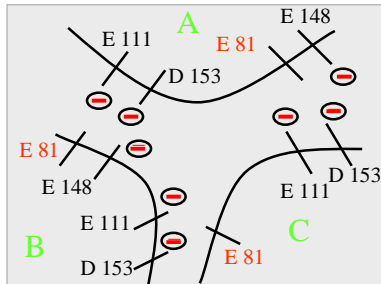
Pka calculations

	Subunit	Native	IS2
ASP 153	A	5.4	4.1
	B	5	5.5
	C	6.8	4.7
GLU 81	A	7.3	4.5
	B	8.5	4
	C	7.2	4
GLU 148	A	5.2	5.5
	B	3.6	3.5
	C	4.2	3.6
GLU 111	A	4.1	4.3
	B	3.7	4
	C	3.8	4.3

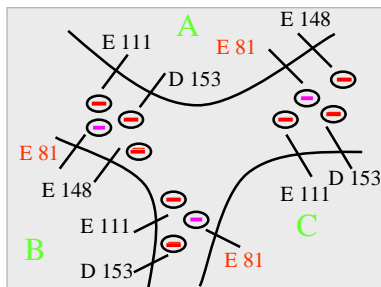
pH ↗ from 5 to 7 => deprotonation of GLU 81

Repulsion interactions with GLU 111 and ASP 153 that may trigger the swelling process

Ph < 7

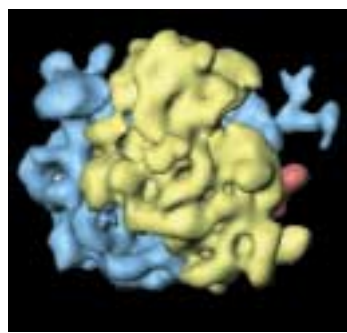
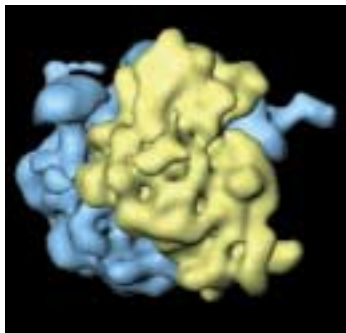


Ph > 7



APPLICATIONS OF NMA : RIBOSOME

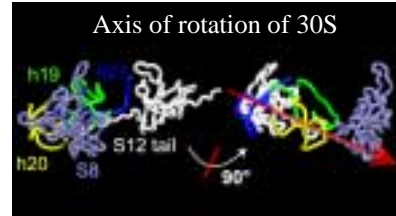
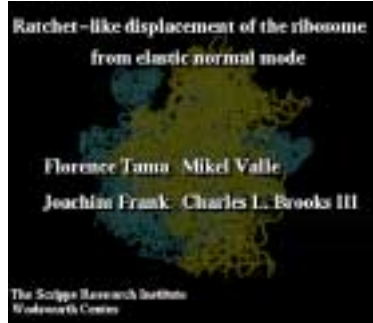
Frank J. and Agrawal RK. 2000, 318, Nature



Rotation of the 30S relative to the 50S: Ratchet-like motion

Key mechanical step in the translocation

RATCHET LIKE-MOTION



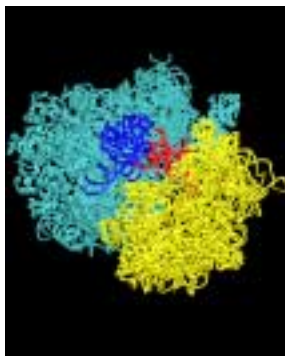
- Region of ribosome interacting with EF-G shoulder, head, stalk ⇒ large rearrangements



Mechanical coupling between rearrangements of the binding sites of EF-G and the ratchet-like motion

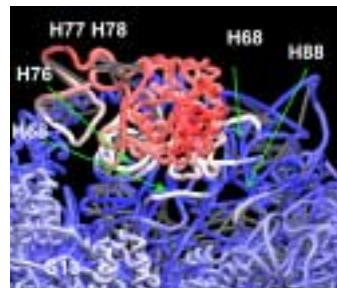
- **H27 Conformational Switch:** Promote large conformational rearrangements in the whole ribosome
- H27 is part of the axis of rotation of 30S
 - ⇒ provides possible explanation of the dramatic effects of the switching

MOTION OF THE L1 STALK



L1 stalk (protein L1 + H76-H79) maybe important for the E-site function

Rearrangements related to L1 stalk motion: head (30S), central protuberance (50S)



Small conformational change

- H88 and H68
- H69 interacts with tRNA in A, P and E sites
- H68 interacts with tRNA in E site



Pathway for motions of the L1 stalk implicates this region in the E-site function

DYNAMICS OF THE RIBOSOME

- ⇒ Motions observed by cryo-EM are reproduced by NMA
- ⇒ Atomic details (H27, bridges)
- ⇒ L1 may play a role in effecting or regulating the removal of the E-site tRNA
- ⇒ Specific mechanical interconnections between the subunits facilitate the functionally important ratchet-like motion

Properties capture by the model ⇒ SHAPE

Ribosome is designed to utilize its shape

APPLICATIONS OF NMA: EM DATA

Simulated EM map



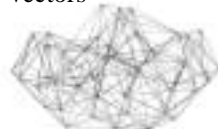
858 residues

Vector quantization

set of control points that provide information about the shape



858 codebook vectors



50 codebook vectors

NMA

reduced representation

$$E(r_a, r_b) = \frac{C}{2} (|r_{a,b}| - |r_{a,b}^0|)^2$$



Choice of the cut-off ?

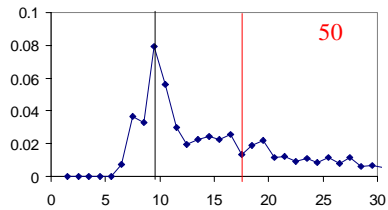
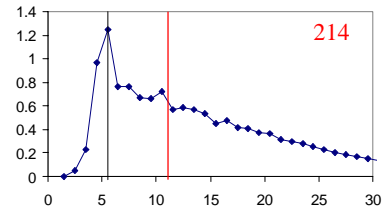
CHOICE OF CUT-OFF

1 codebook vector \approx 1 residue
 \Rightarrow 10-12 Å cut-off is enough

Reducing number of codebook vector
 \Rightarrow too sparse connectivity

Look at the distribution of codebook-vector center codebook-vector center distribution

Example: Adenylate kinase,
214 residues



Projection onto X-ray normal modes \approx 1 for the first few modes

↓

Low frequency NM from EM data are similar to X-ray NM

Models can reproduce functional rearrangements even at 30 Å resolution

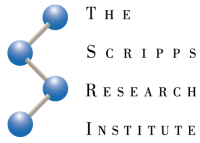
Applications to experimental EM maps

X-ray structure

214 codebook vectors

50 codebook vectors

Open-Close X-ray



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INSTITUTE

PEOPLE

NMA-RIBOSOME X-RAY STRUCTURE

Florence Tama, Charles L. Brooks III

Mikel Valle, Joachim Frank (Howard Hughes Medical Institute, Wadsworth Center, Albany)

NMA-VIRUS

Florence Tama, Charles L. Brooks III

NMA-SIMULATED EM MAPS

Florence Tama, Charles L. Brooks III, Pablo Chacon, Willy Wriggers