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Supplemental Data

Species-Dependent Ensembles of Conserved Conformational States Define the Hsp90 Chaperone ATPase Cycle

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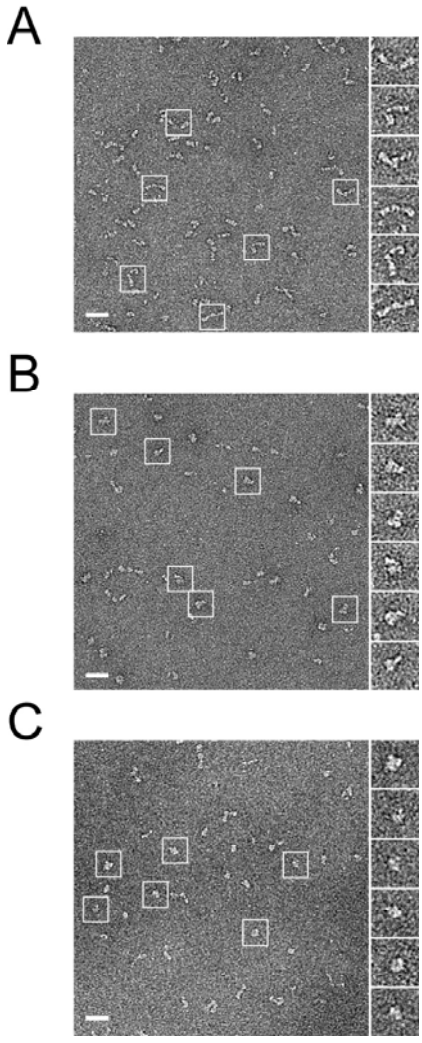

Figure S1. Three Distinct Nucleotide-Dependent Conformational States in HtpG. In (**A**), the micrograph and single particles highlight the extended and open conformation observed in apo HtpG. In (**B**) a closed ATP conformation is seen when HtpG is incubated with 5mM AMPPNP. In (**C**) a compact conformation predominates when HtpG is incubated with 2mM ADP. The scale bar equals 250 Å and the box sizes are 330 Å.

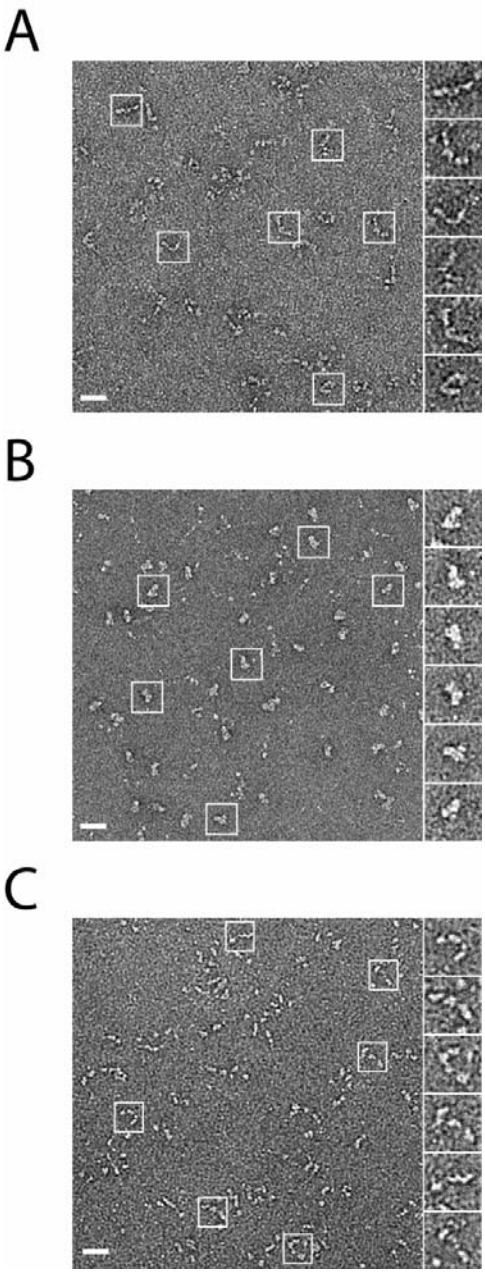

Figure S2. Apo and ATP Conformations Observed in Yeast Hsc82. Shown are typical micrographs and single particles of yeast Hsc82 incubated in the absence (**A**) or presence of 5mM AMPPNP (**B**) and 2mM ADP (**C**). Scale bar is equal to 250 Å and the box sizes are 330 Å.

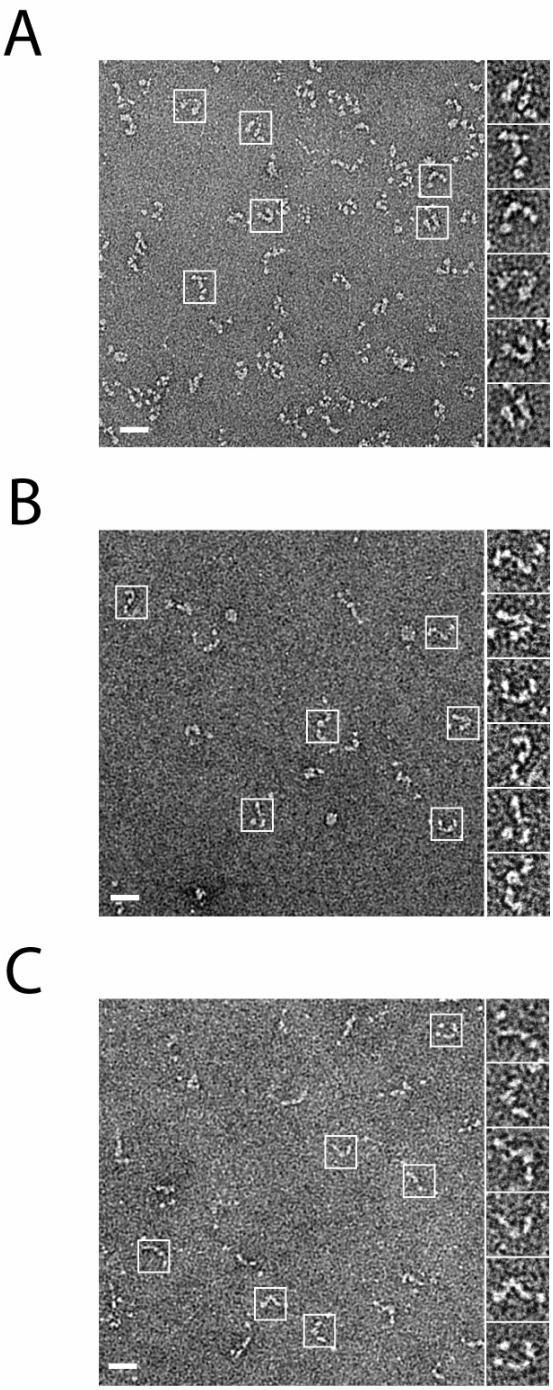

Figure S3. No Significant Nucleotide-Dependent Conformational Changes in Human Hsp90α. Shown are typical micrographs and single particles of apo human Hsp90α (**A**), Hsp90α incubated with 5 mM AMPPNP (**B**) and 2 mM ADP (**C**). The scale bar is equal to 250 Å and the box sizes are 330 Å.

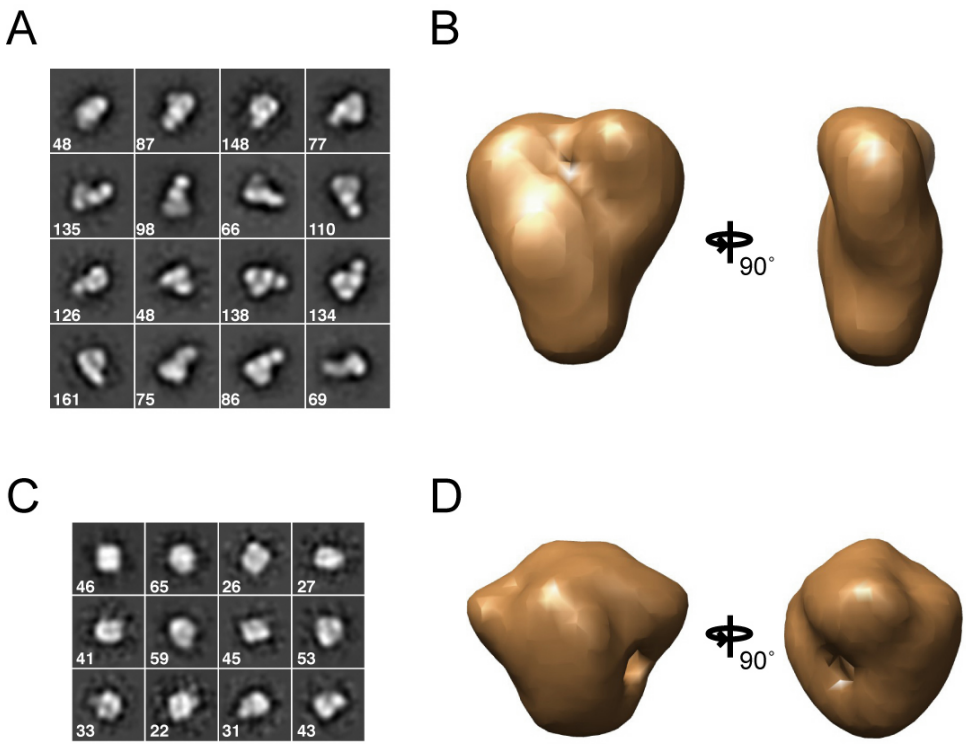

Figure S4. Three-Dimensional Common-Lines Initial Models Used for the Reconstructions.
In (**A**) are the HtpG:AMPPNP reference-free class averages used for generating the initial model (**B**), with the number of particles listed for each average. This model was used for both the HtpG:AMPPNP and Hsc82:AMPPNP reconstructions. In (**C**) are the reference-free class averages used for generating the HtpG:ADP initial model (**D**). The averages were aligned using the cross-common lines method in *EMAN* with two-fold symmetry imposed. The box sizes are 250 and 210 Å, respectively.

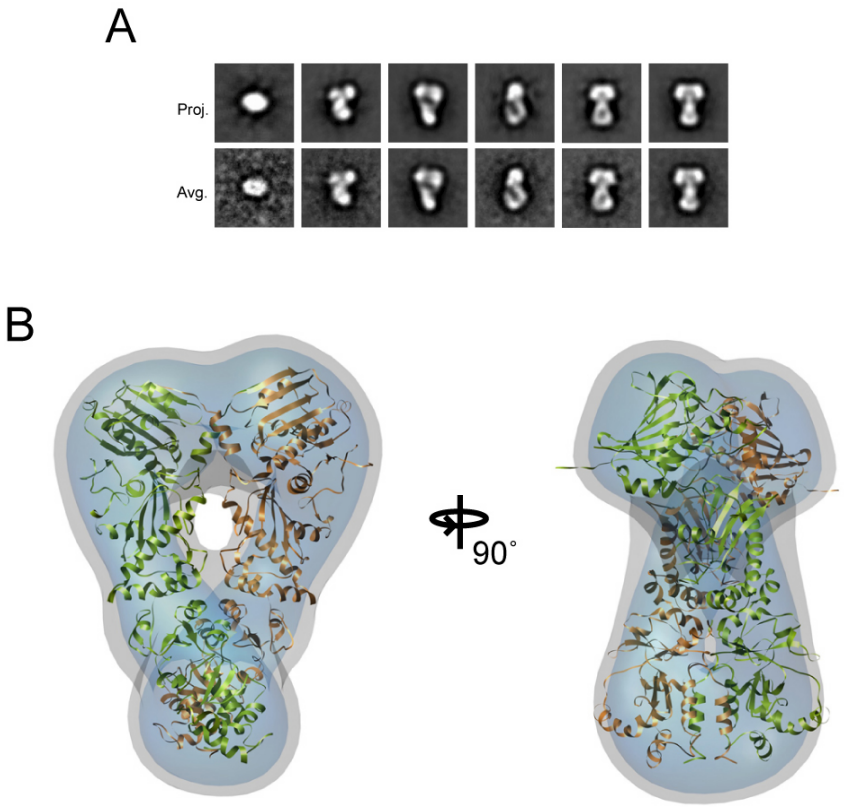

Figure S5. Yeast Hsc82:AMPPNP Class Averages and Rigid Body Domain Fitting of the Crystal Structure.
In (**A**) are example single particle averages and projections for the final refinement round of the Hsc82:AMPPNP reconstruction. The box size equals 250 Å. In (**B**) is an improved, rigid body fit of the yeast Hsp90:AMPPNP crystal structure aligned to the reconstruction at two threshold contours. The blue volume is equivalent to the approximate molecular weight of yeast Hsp90, while the grey volume is contoured slightly lower, revealing that remaining crystal structure density between the domains and in the CTD fits in the EM volume.

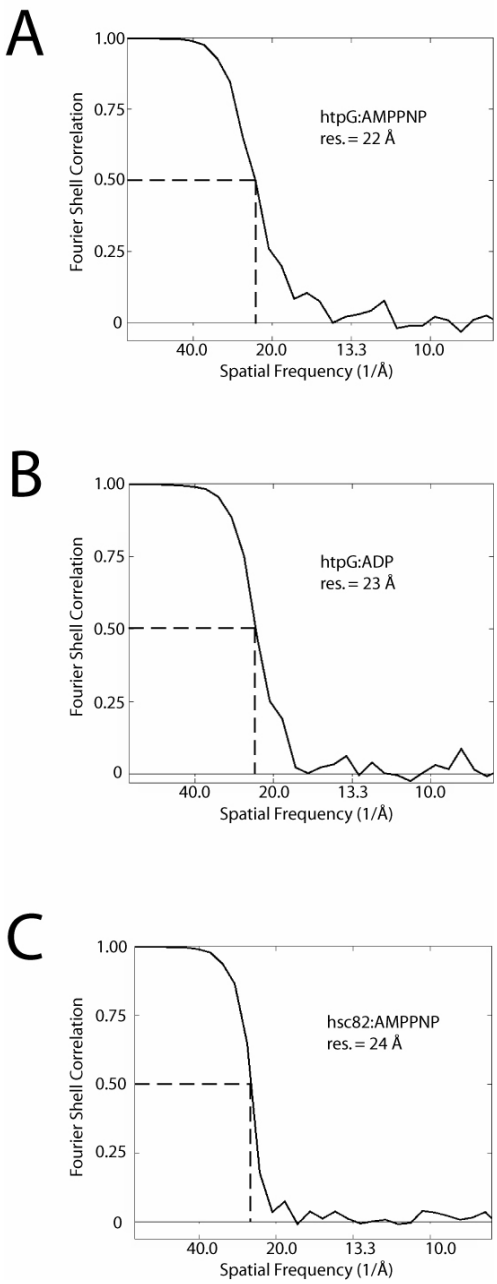

Figure S6.) Estimation of Model Resolution.
The fourier shell correlation (FSC) method was used by comparing two models derived from half of the data set (even odd test in *EMAN*). The model resolution of 22 Å for HtpG:AMPPNP (**A**), 23 Å for HtpG:ADP (**B**) and 24 Å Hsc82:AMPPNP (**C**) was estimated as the spatial frequency where the FSC drops below 0.5. In (D) are example single particle averages and projections for the final refinement round of the Hsc82:AMPPNP reconstruction.

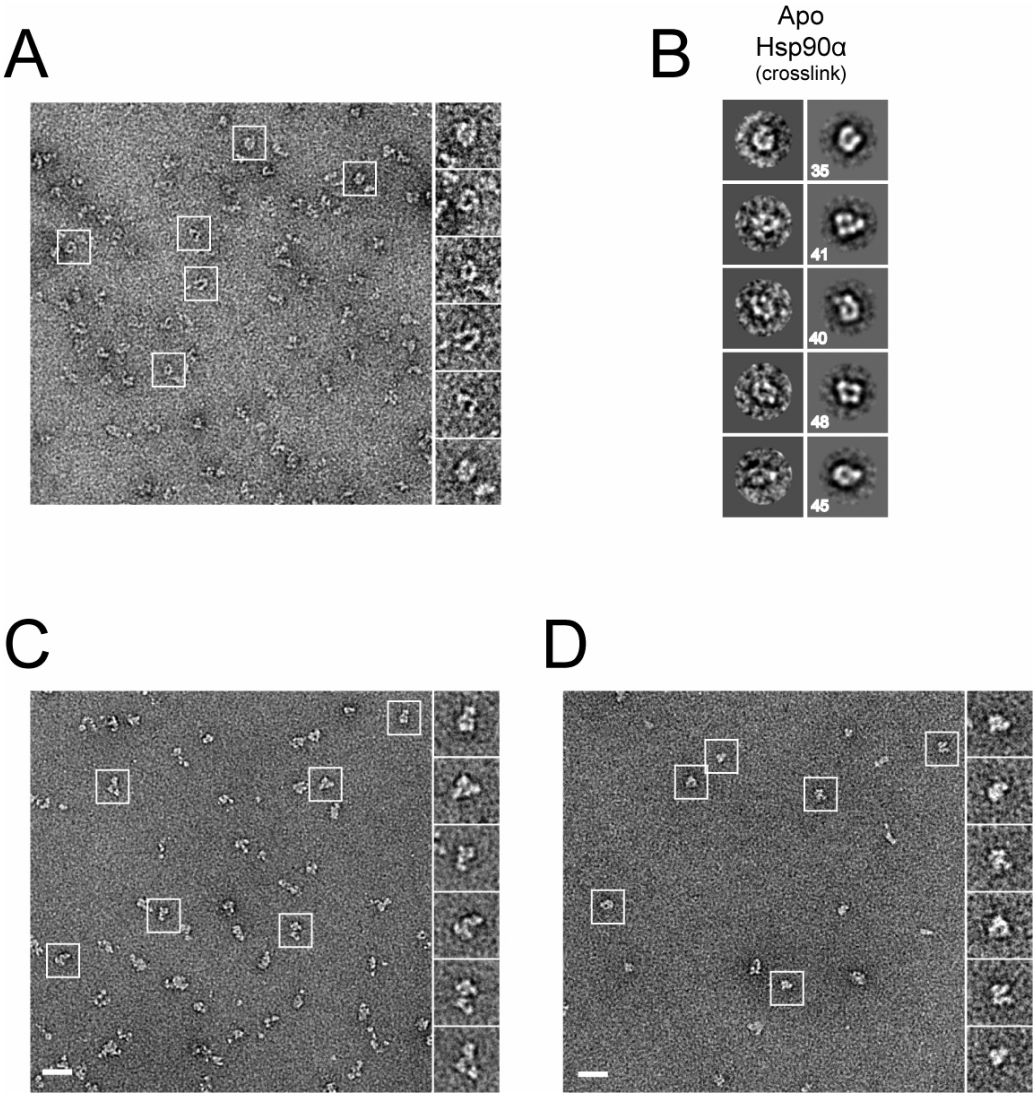

Figure S7. Nucleotide-Dependent Closed (ATP) and Compact (ADP) Conformations of Human Hsp90α Revealed Following Crosslinking.
(**A**) negative-stain micrograph and single particles of apo-Hsp90α incubated with 0.005% glutaraldehyde. In (**B**) are aligned particles and reference-free class averages of crosslinked apo Hsp90α showing a more circular conformation that is different than the ATP or ADP states but resembles the low population of 'semi open' particles of mammalian apo Hsp90 observed previously by cryo-EM (Bron et al., 2008). Typical micrographs and single particles of crosslinked Hsp90α incubated with AMPPNP (**C**) and ADP (**D**) are shown. Scale bar equals 250 Å and box sizes are 260 Å.

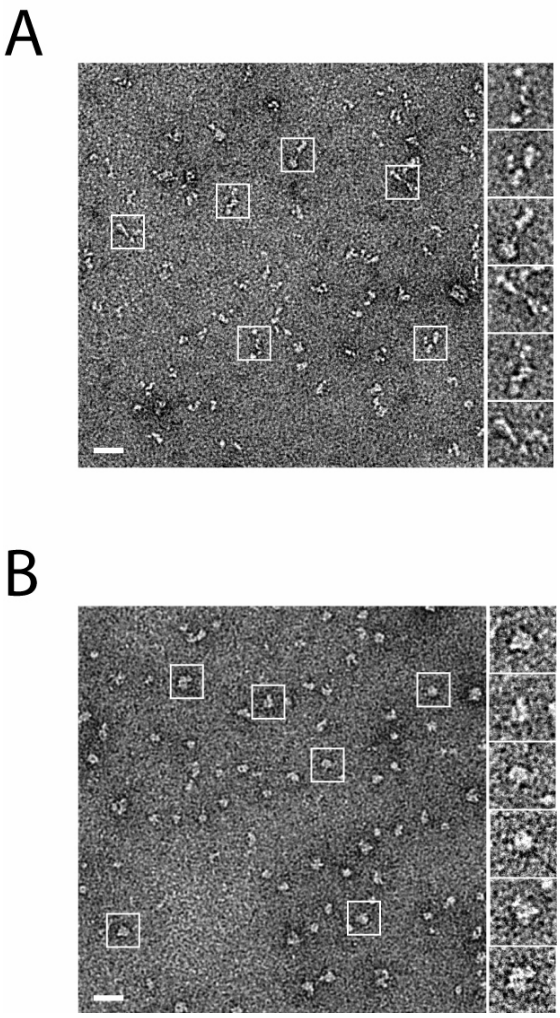

Figure S8. Compact ADP Conformation in Yeast Hsp82:ADP Revealed Following Crosslinking. Hsc82 was crosslinked with 0.005% glutaraldehyde in the absence of nucleotide (**A**) or in the presence of 2 mM ADP (C). Scale marker is equal to 250 Å and the box sizes are 330 Å (A) and 260 Å (**B**)