Supporting Information for "Molecular and thermodynamic insights into the conformational transitions of Hsp90"

*Mijo Simunovic and Gregory A. Voth**

Department of Chemistry, Institute for Biophysical Dynamics, James Franck Institute, and Computation Institute, University of Chicago, Chicago, Illinois

*corresponding author

Simulation systems and the terminology of their conformations:

1) apo: original X-ray determined structure, **open** conformation ($\vartheta = 80°$) – final conformations: **stretched** ($\vartheta > 120°$) and **compact** ($\vartheta < 60°$);
2) ADP-bound: original X-ray determined structure with ADP bound, **closed extended** conformation
3) ADP-removed: ADP removed from the closed extended conformation
4) ADP-inserted: ADP inserted into the apo system (in the original open conformation)
5) ATP-inserted: ATP inserted into the apo system (in the original open conformation)

Table S1: Molecular dynamics simulation systems and their simulation time.

Systems:	apo	ADP-bound	ADP-removed	ADP-inserted	ATP-inserted
Trajectory 1	100 ns	150 ns	100 ns	150 ns	100 ns
Trajectory 2	100 ns	100 ns	100 ns	100 ns	100 ns
Trajectory 3	150 ns	100 ns			
Trajectory 4	100 ns	100 ns			
Trajectory 5	100 ns	100 ns			

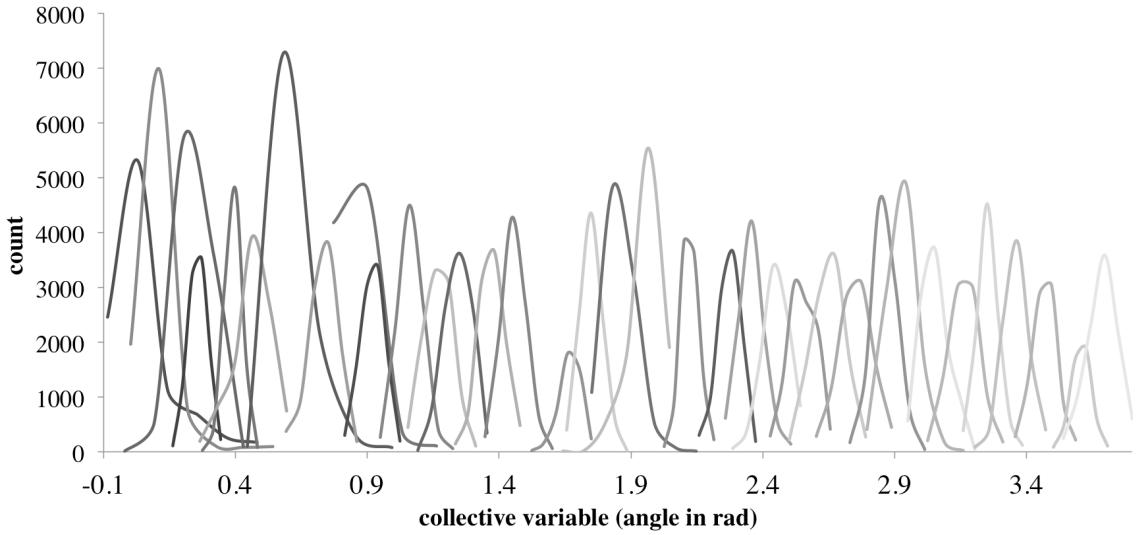

Figure S1: Histogram of the free-energy simulations (see Methods).

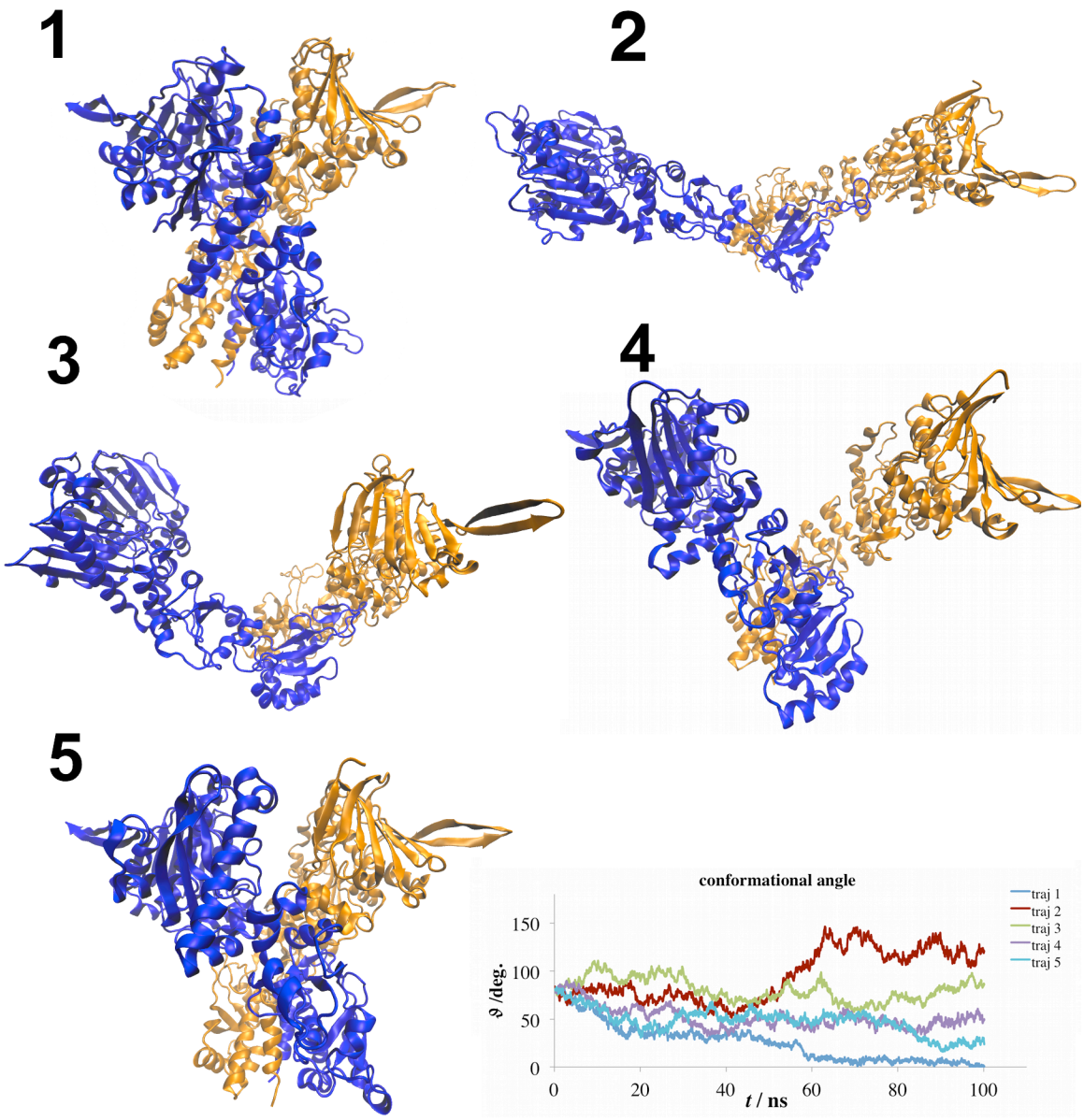

Figure S2: Simulations of apo Hsp90. Shown are final configurations of each of the five independent trajectories (trajectory numbers are consistent with the main article). The graph shows conformational angle traced in time for each trajectory. In trajectories 1, 2, and 5 there is a large-scale structural rearrangement, whereas trajectories 3 and 4 show moderate change (opening and closing, respectively), further confirmed with other measurements, e.g. inter-domain distance and RMSD (see main text).

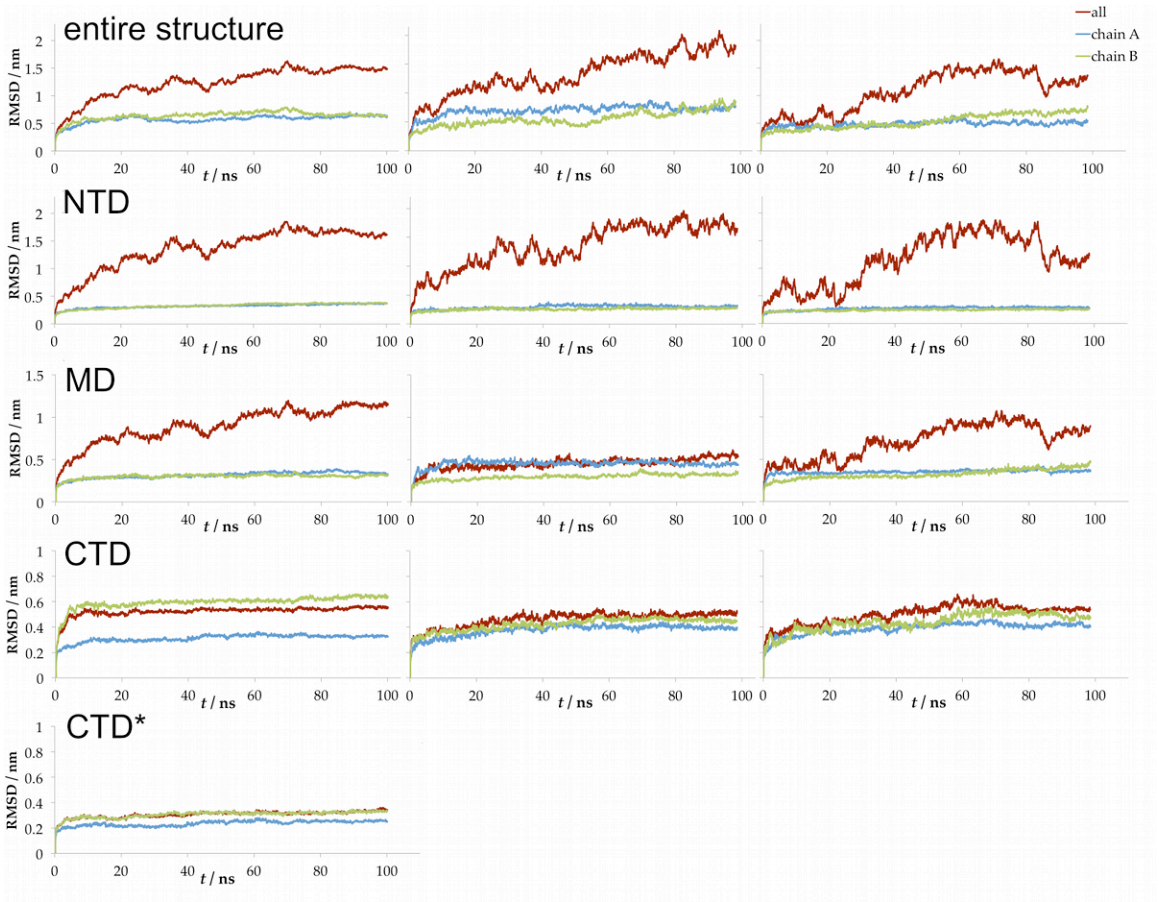

Figure S3: Root mean square analysis of apo Hsp90 simulations, square averaged over all independent trajectories. Left: apo Hsp90; middle: ADP-inserted simulation; right: ATP-inserted simulation. In CTD* H21 region was omitted from calculations.

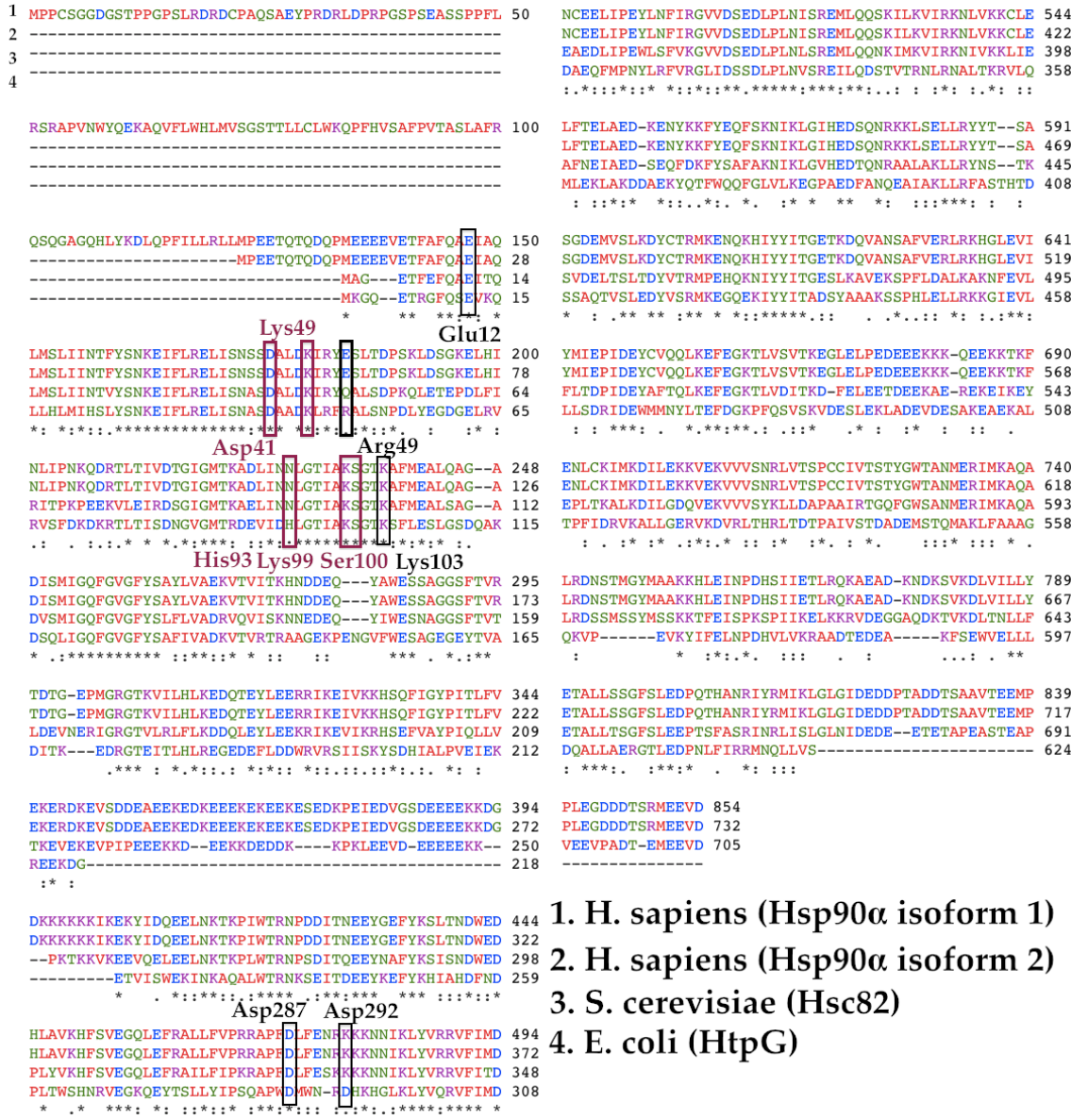

Figure S4: Sequence alignment of four different homologs of Hsp90 (two human, yeast and bacterial), highlighting the residues involved in the conformational change (boxed black) and coordinating the nucleotide in the binding pocket (boxed maroon). All residues are conserved, but Asp-292, which is involved in the transition into the compact form from both open and ligand-free closed extended state and also involved in the MD-MD interactions in the ADP-bound closed extended state. Arg-49 is not conserved either, although its involvement was limited and accompanied by interactions between other residues.

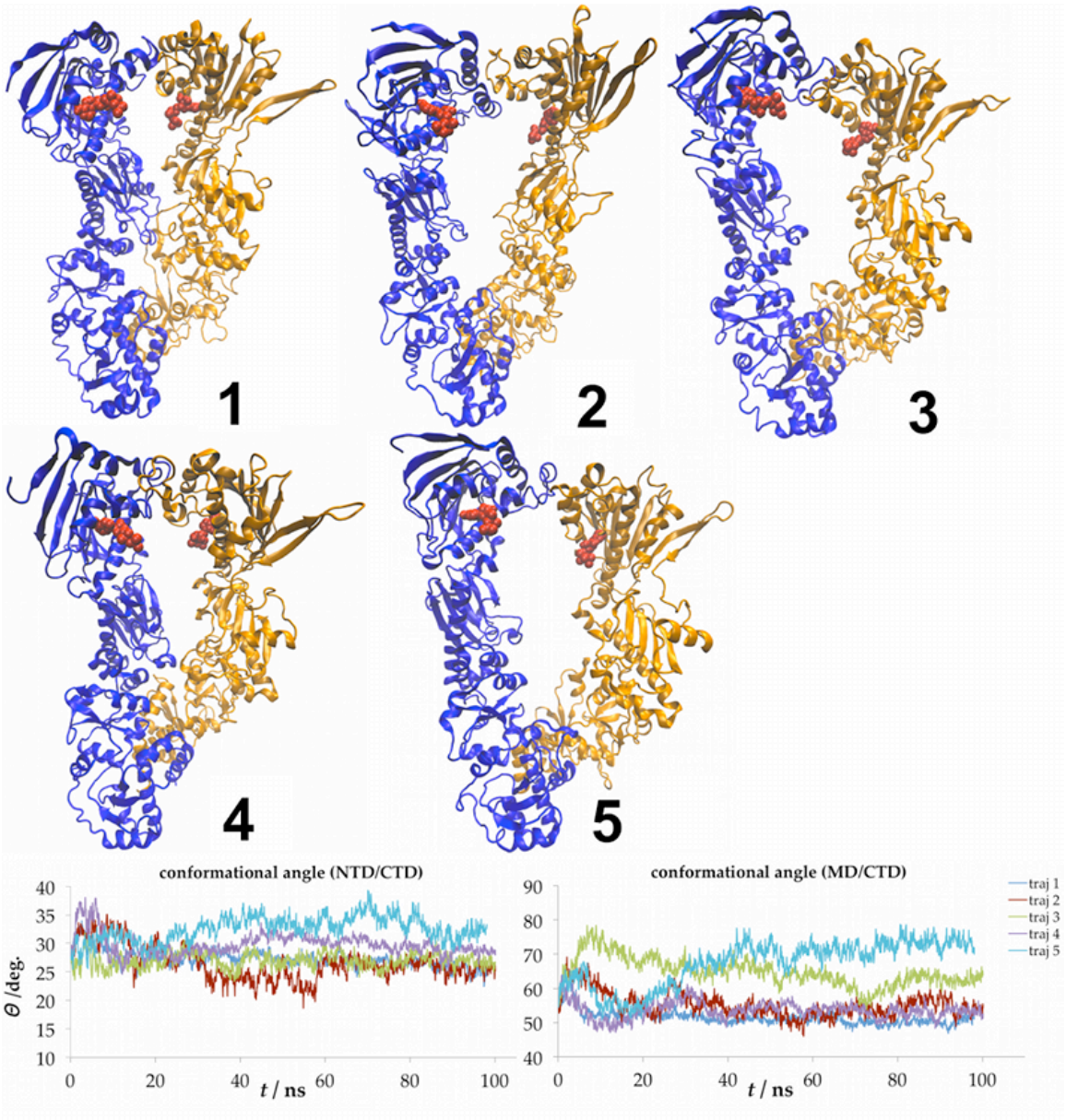

Figure S5: Simulations of the ADP-bound (extended) state. Shown are final configurations of the five independent trajectories. ADP molecules are colored red. Graphs show the conformational angle change, measured between planes passing through the NTDs (bottom left) or through the CTDs (bottom right).

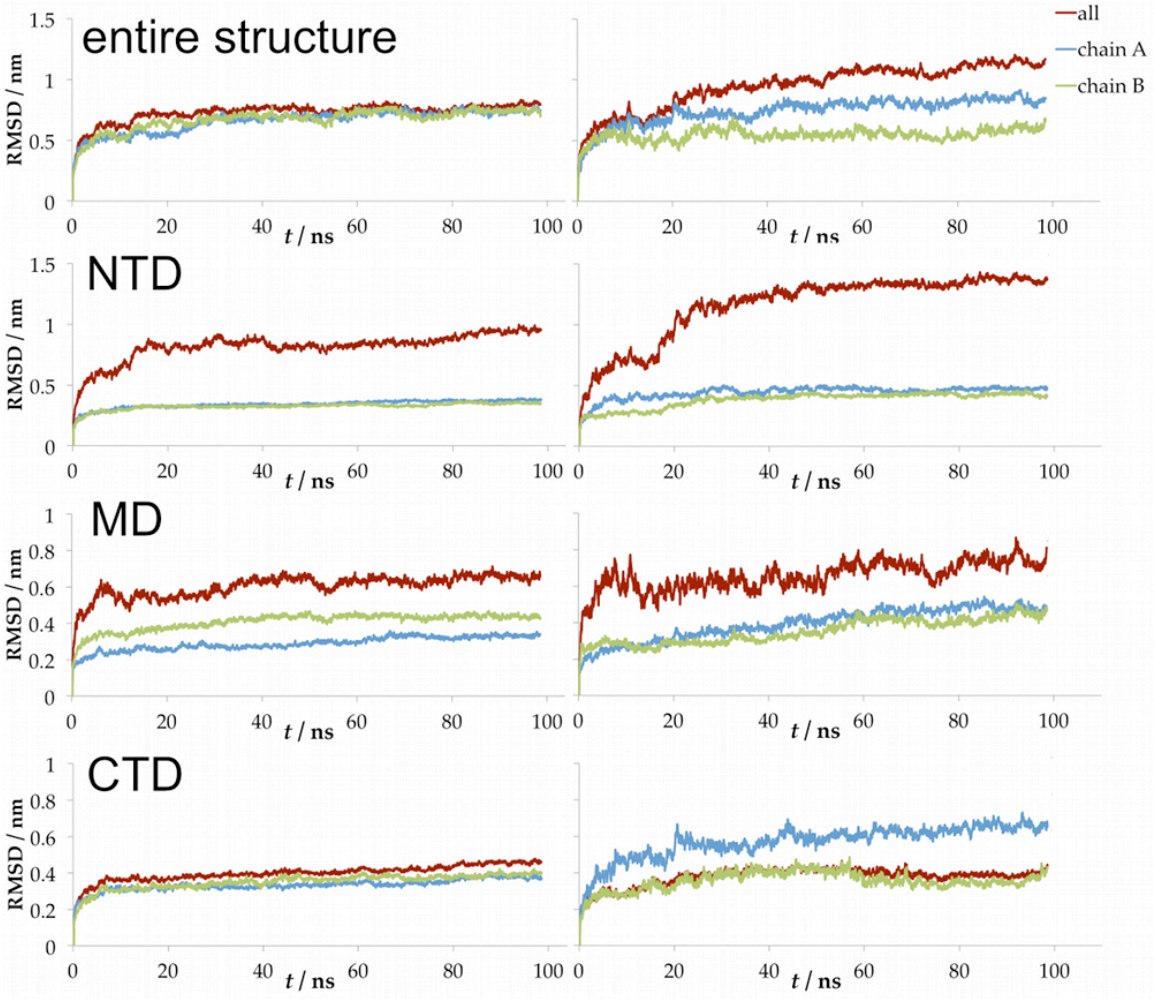

Figure S6: Root mean square analysis of the extended state of Hsp90 with ADP bound (left) and ADP removed from the binding site (right). The values are square averaged over all independent trajectories. Nucleotide binding, regardless of the initial state, seems to stabilize the H21 region.

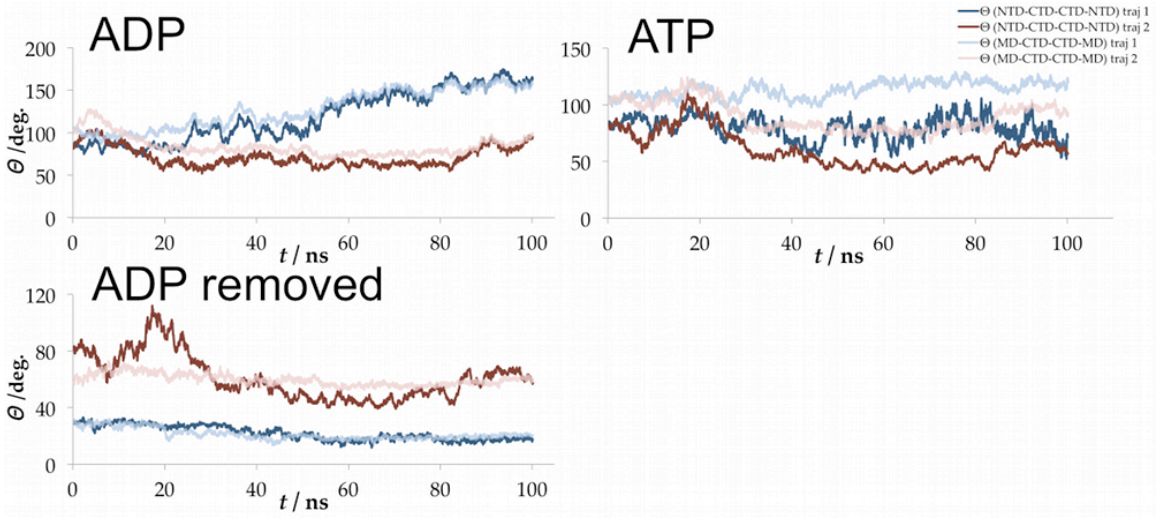

Figure S7: Tracing the conformational angle in simulations with ADP (left) and ATP (right) inserted into the open state and in simulations with ADP removed (bottom) from the extended closed state. Dark lines show the angle as described in the main text. Light lines represent the alternative conformational angle, in which the planes are defined going through the middle domains, instead of the N-terminal domains.