Simulating the conformational transitions in Mm-cpn with Steered Molecular Dynamics

Technical report by G. Pintilie – last updated March 2008

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Abstract

Allosteric conformational changes in proteins play an important role in how they perform their biological functions. Chaperonins are multisubunit assemblies that aid proper folding of various proteins through a mechanism that involves an ATP-driven conformational change. The nature of the conformational change has been elucidated in crystal structures and Cryo-EM maps which capture the conformation of the subunits in different states. These results show starting and ending conformations, but not the actual dynamical transition from one form to the other. In this report we study the dynamical nature of the conformational change by simulating the transition using steered molecular dynamics. The simulations show subdomains within each subunit which remain relatively rigid. The rigid motions are characterized by aligning rigid subdomain structures at sequential points throughout the dynamical transition. A complete cycle of the entire chaperone is also simulated.

1. Introduction

Chaperonins faciliate succesfull folding of various proteins through a process that involves ATP-hydrolysis. They are classified into groups I and II. Group I chaperonins are found in bacteria, mitochondria and chloroplasts. Examples are, respectively, GroEL[1], Hsp60[2] and Rubisco-subunit-binding protein[3]. Group II chaperonins are found in archaea and the cytosol of eukaryotes. Examples are thermosomes[4], TF55[5], and CCT[6].

The mechanism through which protein folding is assisted has been most widely studied for GroEL[7]. Aside from extensive kinetic experiments, structures of GroEL elucidating the corresponding conformational changes have been obtained with X-ray crystallography techniques[8,9] and Cryo-electron microscopy[10]. The dynamical mechanism for GroEL has been studied using normal mode analysis of the structure in light of a molecular mechanics energy function[11], and also using an elastic-polymer model[12].

In contrast there is less known about the group II chaperonins which include the thermosome[13], TF55[14], Mm-cpn[15] and CCT[16]. The crystal structure of the thermosome has been solved[17,18]. It is shown to be a heteromer with two different types of subunits, α and β, arranged in two rings stacked on top of one another. Each ring contains 8 units in total, 4 α and 4 β, which alternate circularly to form a ring. Unlike GroEL, which

requires the lid-complex GroES to completely enclose a protein substrate, the thermosome subunits include a flexible apical domain that completely closes the cavity. It is only this closed state that has been crystallized (Figure 1). In the open state, or the state that is able to specifically bind certain misfolded proteins, the apical domains are thought to be disordered. There are currently no known crystal structures of the thermosome in an open state.

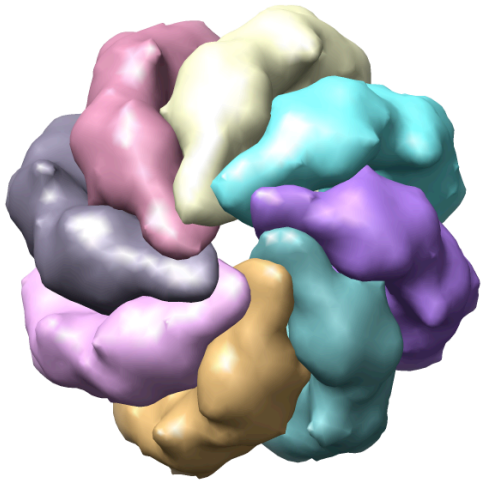


Figure 1: Thermosome structure from PDB. Each protein subunit is shown as an approximate surface. The surface was obtained using the multiscale feature in Chimera[19]. The view looks down an axis perpendicular to the plane of one of the two rings formed by 8 subunits.

The actual mechanism through which misfolded proteins are assisted by chaperonins to achieve the native state is not well known for the group II chaperonins. It is suggestive that in the open state, misfolded proteins are somehow specifically bind to the inside of the open cavity of the chaperone. Once this binding occurs, the equatorial domains of the subunits become more susceptible to binding ATP. Once ATP is bound, a conformational change drives the open cavity to close. The actual purpose of this seclusion of the misfolded protein has been proposed to potentially serve the purpose of isolating it from other misfolded proteins and allow it to fold to the native state. The closed chaperone then eventually is driven to the open state, though the exact mechanism is yet unclear.

Crystal structures of Mm-cpn and TRiC, homologs of the thermosome, have not yet been obtained. However models of both have been build using single-particle reconstruction methods applied to cryo-EM data[20]. The models aquired for Mm-cpn show the chaperone in closed, atp-bound open, and atp-free open conformations[21]. These conformations are illustrated below. Structures of individual Mm-cpn subunits have been obtained using homology-based structure prediction methods. These structures have been aligned to the density maps of Mm-cpn in closed form, with very good fits found for all the subunits (Figure 2).

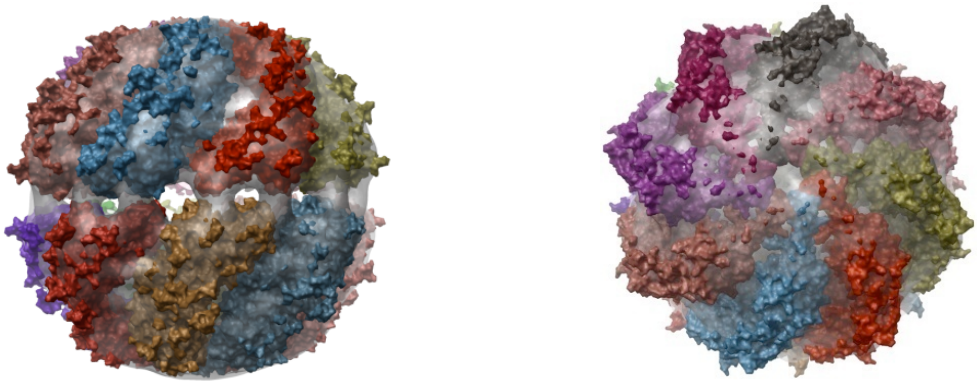


Figure 2. Mm-cpn density maps (grey transparent surface), with protein structures aligned inside it (each protein subunit is shown as an approximate surface with a random color). (a) Side view, (b) top view.

In the open states, the subunits have different conformations. To obtain these conformations, the structure of a subunit was divided into three domains: equatorial, middle, and apical. Each domain was separately aligned to the two maps corresponding to the atp-bound and atp-free open states. To divide the structure into three domains, the sequence of amino acids was split at two different spatial locations. At each location, the backbone was separated in two different locations in the sequence (the chain runs through the equatorial domain into the middle domain, then into the apical domain, returns to the middle domain and ends back in the equatorial domain). The alignment of each domain was such that the points where the sequence was separated remained in close vicinity. The backbone was then joined into a single chain again, and NAMD[22] was used to minimize the structure, removing any high-energy bonds and interactions.

The structures of all three states were equilibrated in vacuum at a temperature of 310K. Although vacuum is not the natural environment of the protein, this setup greatly reduces the computation of this and subsequent simulations. During equilibration, no major changes in the protein structure were observed, the RMSD change stayed at or close to 1 between initial and minimized structures. Figure 3 shows a plot of the energies of the three structures during minimization and subsequent brief equilibration. The energy decreases drastically for all three structures, converging to similar low values.

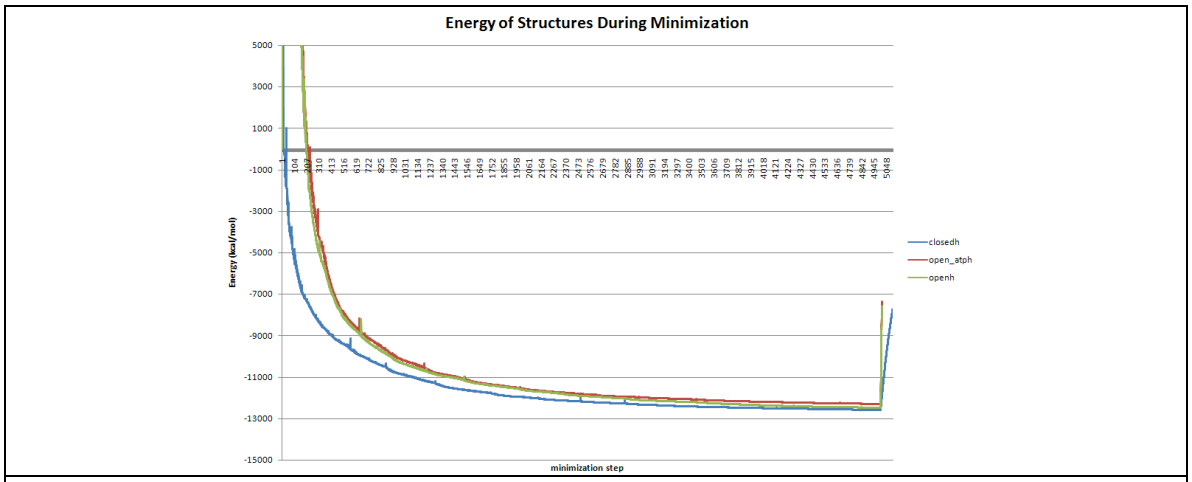
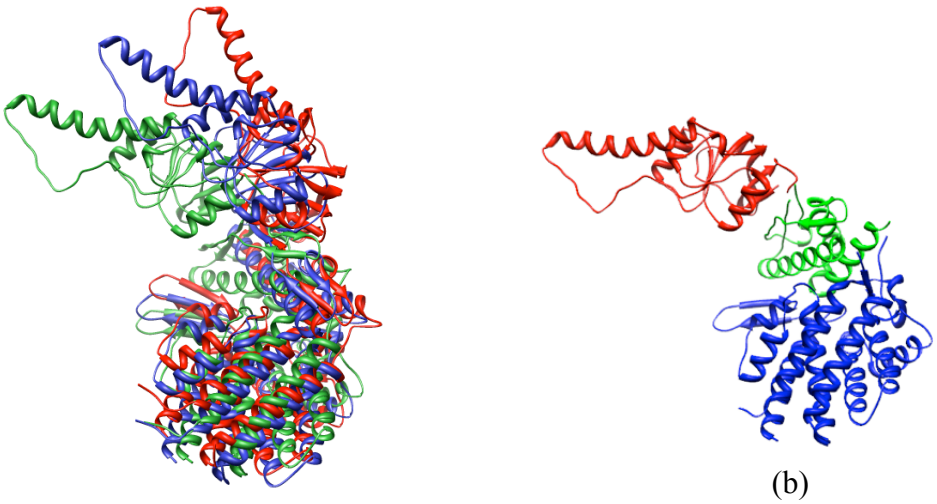


Figure 3. Plot of energies of the structures in all three states during minimization and subsequent short equilibration.

2. Steered Molecular Dynamics of Mm-cpn

The structure of a protein subunit in the three different states, after equilibration, is shown in Figure 4. Each unit is shown using the ribbon representation, with a different color for each state: green – closed, blue – open with atp bound, red – open with no atp. The image on the right shows the three domains within each subunit: blue – equatorial, green – middle, red – apical.



(a) (b)

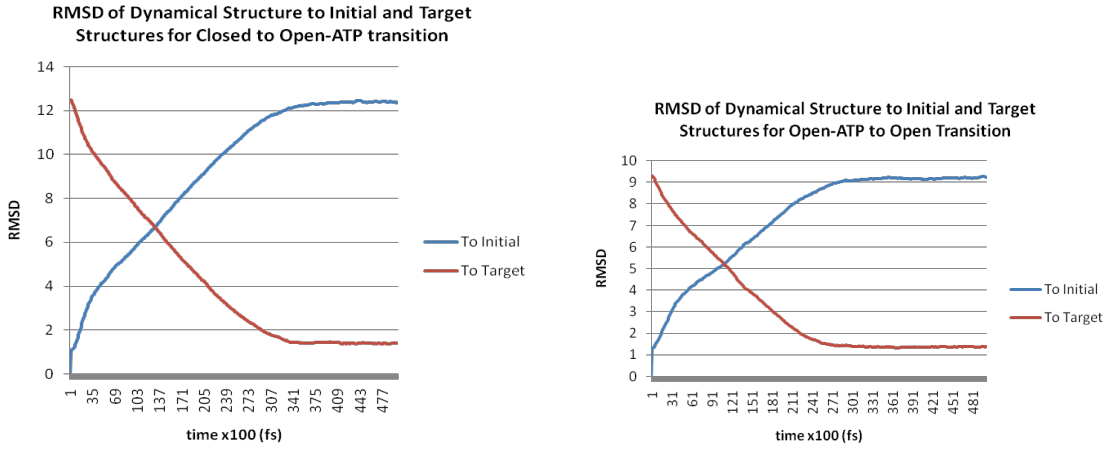
Figure 4. Ribbon representations of a single unit in different states (a), and decomposed into three separate domains (b).

The density maps that the structures were aligned to were registered with respect to one another, hence any movement in the equatorial domain should correspond to the movement of the domain in the conformational transition. In the picture on the left, it can be seen that the equatorial domains are the closest in alignment of all the domains. The

middle domain differs in position and orientation, and the apical domain seems to rotate and shift greatly during the transition.

The transition from the closed state to the open state was simulated using NAMD and the CHARMM[23] force field, and a steered molecular dynamics technique.

Two different simulations were run, one driving the closed conformation to the atp-bound open conformation, and a second one driving the atp-bound conformation toward the atp-free open conformation. The trajectory of every simulation was saved to a file, which was then used for further analysis. One metric showing the transition from initial to target states in each simulation is the root mean square deviation or RMSD between the actual structure being simulated and both intial and target conformations (Figure 5).



(a) (b)

Figure 5. Plots of RMSD differences between the structure and the initial and target states for (a) closed to open-atp and (b) open-atp to open transitions.

Both graphs show that the transition happens very gradually. For the first simulation, the initial RMSD to the target is about 12A, and it decreases to below 2 during the simulation. For the second simulation, the initial RMSD to the target is about 9A, and that also decreased to below 2.

The two simulations on the single subunit, sequentially, give the complete transition from closed to open states. A movie of the simulation can be accessed using the following link: http://people.csail.mit.edu/gdp/tr/mmcpn_smd/closed_2_open.mov. In the movie, the grey structure is the structure being simulated, the green structure is the atp-bound open state and the red structure is the atp-free open state.

In the simulation of the complete transition, it is apparent that the three domains remain intact, and no drastic loss of secondary structure can be observed. The rigidity of the domains during the simulation was further tested. The structure of each domain in the initial structure was compared to the structure of the same domain during the simulation.

The comparison was done by doing a least-squared alignment using the back-bone αC of the initial domain structure and the structure of the domain at evenly dispersed steps during the simulation. This alignment of the rigid domains to the structure during the simulation can be seen in the following movies:

http://people.csail.mit.edu/gdp/tr/mmcpn_smd/closed_2_open_aligned.mov
http://people.csail.mit.edu/gdp/tr/mmcpn_smd/closed_2_open_aligned_side.mov

The following movie illustrates the same conformational transition, however showing only the rigid domains as they are aligned to the structure during the simulation
http://people.csail.mit.edu/gdp/tr/mmcpn_smd/closed_2_open_domains.mov

In the movies, the domains are represented using blue, green and red ribbon structures, and the actual structure during the simulation is shown in gray. The RMSD scores of the least-squares alignment of each domain are shown in Figure 6.

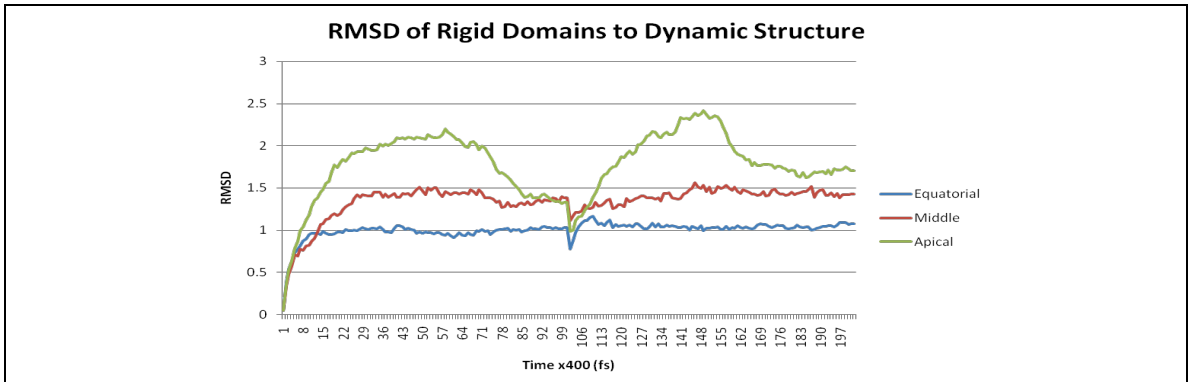
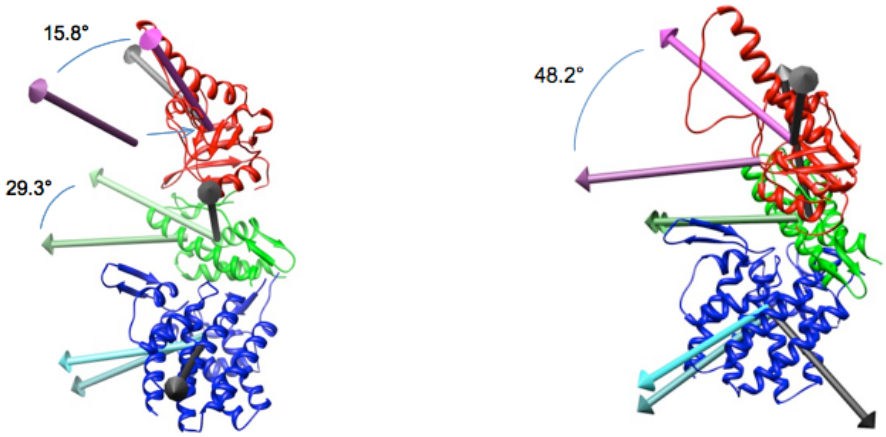


Figure 6. RMSD of rigid domains structures and the structure being simulated.

The plot in Figure 6 shows that the equatorial domain (blue curve) stays the most rigid of the three, with the RMSD score staying very close to 1A during the entire transition. The middle domain varies slightly more, with a value that is at about 1.5A. The apical domain on the other hand changes the most, the RMSD rising to over 2.0A.

The least-squares alignment of the domains between the initial and target structure allows us to calculate a rotation and displacement of the domains as if they were rigid bodies. These rotations and displacements are illustrated in Figure 7.



(a) (b)

Figure 7. Rigid body movement measurements for the three domains in (a) closed to open-atp and (b) open-atp to open transitions.

Figure 7a shows the transition from closed to atp-bound open states, and Figure 7b shows the atp-bound open to the atp-free open states. For each domain, there are three arrows which illustrate the rigid body movement. The gray arrows show the axis of rotation during the transition. The cyan, green, and purple arrows show an arbitrary direction in the domain, starting at the domain's center of mass, which is in the same direction relative to the domain. The darker version of the arrow is for the initial domain structure, and the lighter version is for the target state.

The exact angles and displacements corresponding to the two transitions for each domain are tabulated below:

Closed to Open-ATP Transition	Open-ATP to Open Transition
Apical domain - angle: 15.811816 - displacement: 19.604586 Middle domain - angle: 29.340817 - displacement: 7.977318 Equatorial domain - angle: 13.634379 - displacement: 2.332830	Apical domain - angle: 48.227076 - displacement: 11.193612 Middle domain - angle: 2.586134 - displacement: 3.872926 Equatorial Domain - angle: 13.052389 - displacement: 2.049189

From the data above, we can see that in the first and second transitions, the equatorial domain undergoes relatively small displacements of about 2A, but significant rotations of about 13°. The middle domain undergoes an even larger displacement of ~8A and rotation of ~30° in the first transition, but relatively low displacements and rotations in the second transition. The apical domain undergoes a very large displacement in the first transition with a moderate rotation of ~16°, and a moderate further displacement of ~11A along with a very large rotation of almost 50° in the second transition.

To get an idea of the conformational mechanism as it applies in the context of the whole chaperonin structure, the same steered molecular dynamics process was applied to every subunit in the structure simultaneously. A complete cycle was simulated: closed → atp-bound open → atp-free open → atp-bound open → closed. Figure 8 shows the RMSD between initial and target structures throughout the entire cycle:

The resulting simulations are shown in the following two movies:

http://people.csail.mit.edu/gdp/tr/mmcpn_smd/mmcpn_ring1_cycle.mov
http://people.csail.mit.edu/gdp/tr/mmcpn_smd/mmcpn_ring1_cycle_side.mov

In the above two movies, to avoid clutter usually seen in ribbon-representations of multiple proteins at the same time, the subunits are shown as an approximate surface, thus giving a better idea as to what their shape looks like during the conformational transition.

Figure 8. RMSD deviations of structure to initial and target states during a simulation of a complete closed to open to closed cycle.

3. Conclusions and future work

The conformational transition of Mm-cpn was simulated using steered molecular dynamics. The transition was very gradual, and showed that the three domains within each subunit remains rigid. The rigid body motions were measured from least-squares alignments of the rigid domains between the initial (closed) and target (open) states. The same simulation method was extended to every subunit in the complete chaperone to simulate a complete cycle of opening and closing.

The simulation system was rather inaccurate, since no explicit nor implicit solvent models were used. However this made the simulation feasible – adding solvent would involve a greater requirement of computational power; this is something that should be pursued in the future.

Many questions remain about the mechanism through which the chaperone works. Is the binding specific? What is it about the binding and subsequent conformational change that persuades a misfolded protein to find its native state? What is the role of ATP? Is it involved only in the transition from closed to open states, or only in the open to closed transitions? Future work might explore extending the analysis of the simulation so as to try to try to answer these and other questions. Some possibilities would be to include a misfolded-protein substrate and/or ATP molecules that catalyze the conformational transition.

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