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Rattling the cage: computational models of chaperonin-mediated protein folding

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Chaperonins are known to maintain the stability of the proteome by facilitating the productive folding of numerous misfolded or aggregation-prone proteins and are thus essential for cell viability. Despite their established importance, the mechanism by which chaperonins facilitate protein folding remains unknown. Computer simulation techniques are now being employed to complement experimental ones in order to shed light on this mystery. Here we review previous computational models of chaperonin-mediated protein folding in the context of the two main hypotheses for chaperonin function: iterative annealing and landscape modulation. We then discuss new results pointing to the importance of solvent (a previously neglected factor) in chaperonin activity. We conclude with our views on the future role of simulation in studying chaperonin activity as well as protein folding in other biologically relevant confined contexts.

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Introduction

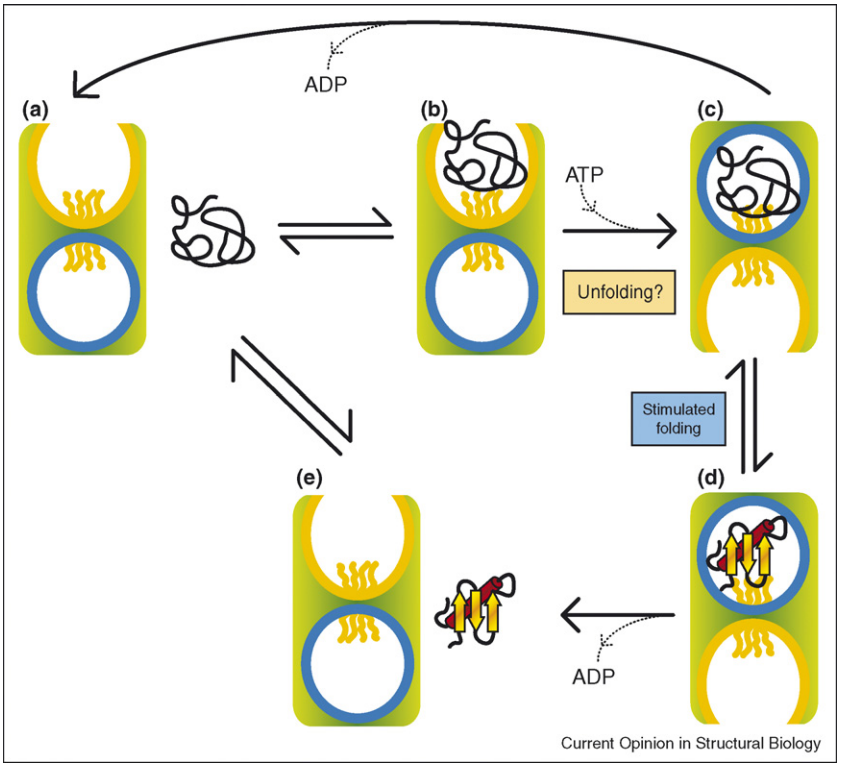
The native folding of cellular proteins is essential to the survival of all living things, yet protein folding is sometimes impossible without the help of the chaperonins, a class of large mega-Dalton protein complexes found ubiquitously in all forms of life (GroEL in prokaryotes, TRiC in eukaryotes, and the thermosome in archaea). Chaperonins are responsible for binding and encapsulating non-native proteins, thereby helping them to reach their functional conformations. It is estimated that 10% of cytosolic proteins interact with chaperonins [1••,2•], and in prokaryotes, at least 13 of these are essential proteins that cannot reach the native state without chaperonin assistance [3]. It follows that chaperonin function is an absolute requirement for cell viability.

The chaperonin structure and reaction cycle have been well documented in previous reviews [1••,2•,4••,5•,6]. The complex comprises two barrel-shaped, multimeric rings with lids that open and close as they bind and hydrolyze ATP. Evidence from the crystal structure of the *Escherichia coli* chaperonin GroEL suggests that closing of the cavity may be accompanied by a pronounced conformational change, whereby the inner surface of each ring goes from displaying many hydrophobic residues to being more charged and hydrophilic in character (Figure 1). In this way, the open cavity is able to bind non-native proteins with exposed hydrophobic moieties for subsequent encapsulation following closure of the barrel.

The question of how it is that these steps of binding and encapsulation facilitate folding, however, still remains unresolved. Unfortunately, it is very difficult to observe the dynamics of the unfolded/misfolded peptide bound to (or enclosed inside) the chaperonin with sufficient temporal and spatial resolution using current experimental techniques. This makes the study of chaperonin mechanism a fertile ground for the application of computer simulation methods. Using simulations, it is generally possible to view dynamic information with the spatial resolution of angstroms and the temporal resolution of femtoseconds. The most effective use of simulation techniques involves making predictions that can be verified by experiment (calculating rates of folding, suggesting the effects of mutations, etc.), but then going further by using the great resolution of these methods to understand molecular elements of the biological system that are difficult to directly observe experimentally.

Computer simulation techniques do have difficulty dealing with systems composed of very large numbers of atoms (millions or more) and very long timescales (milliseconds to seconds), the regimes in which experimental techniques shine. Thus, simulation techniques are very much complementary to experimental techniques and a concerted use of both methods should be the most effective strategy for the study of chaperonin mechanism. Both simulation and experiment, however, can still greatly benefit from the use of simpler analytical models both to explain phenomena observed, suggest ways of analyzing empirical data, and to inform the design of new experiments/simulations. In the end, all models must leave some details out, both for tractability and their utility, so the relevant question is which details are the most important and which can be ignored?

Figure 1



Chaperonin reaction cycle. **(a)** The two homoheptameric subunits of the GroEL double ring (green) operate with negative allostery such that while one chamber is open, the other is closed. While open, the chaperonin cavity is hydrophobic (yellow), but once closed it becomes more charged and hydrophilic (blue). **(b)** The hydrophobic surface of the open cavity binds to a non-native substrate, and may play a role in unfolding it, either before or after the binding of ATP. **(c)** Upon binding of ATP, the cavity closes, releasing the substrate from the barrel wall, and allowing it to fold. **(d)** Following release of ADP **(e)**, the cavity opens and may either relinquish or rebind the substrate. The hydrophobic GGM tails protrude into the cavity have unknown structure and function.

Iterative annealing models

Experiments have shown that chaperonins can bind and unfold substrate proteins and also that many cycles of chaperonin activity are sometimes needed to sufficiently populate the native state [7,8•,9•]. These observations gave rise to the idea of iterative annealing, whereby a chaperonin facilitates repeated attempts at folding by unfolding substrates stuck in non-native kinetic traps. Still, it is not well understood how exactly chaperonins might mediate unfolding, and also how, in quantitative detail, unfolding can lead to sufficient population of the native state. Various chaperonin simulations have aimed to address these questions.

The first theoretical model for chaperonin mechanism, proposed by Gulukota and Wolynes in 1994, was referred to as kinetic proofreading [10]. It relies on the assumption that the unfolded state created by chaperonin-assisted unfolding is different from the unfolded state commonly found post-translation. Thus the chaperonin-unfolded conformation would most probably be in a different position on the energy landscape and therefore have a different probability of correctly reaching the native state.

This model was extended two years later by Todd *et al.* and was dubbed iterative annealing [9•] (but has also been referred to as kinetic partitioning [11], or transient binding and release [12,13]). The underlying assumption behind this model is that the rate of chaperonin-assisted unfolding is greater than the rate of folding to the native state from a misfolded intermediate. With this assumption, if even a small fraction of protein molecules are able to reach the native state directly from an unfolded state, repeated cycles of chaperonin binding, unfolding, and release will eventually facilitate productive folding.

These mechanisms were first tested *in silico* using simple Monte-Carlo lattice models (models in which proteins are represented as a 2D or 3D polymer that can only occupy discrete, evenly spaced nodes on a grid) [11,14,15]. All of these early simulations seem to agree that proteins can be unfolded by the use of an effective force between a surface and hydrophobic residues of the model peptide

and that this could lead to an enhancement of the overall folding rate.

Since then, other groups have employed more sophisticated models to examine variants of iterative annealing in chaperonins [12,13,16]. Using an off-lattice model and Langevin dynamics, Jewett and Shea were able to demonstrate that repeated binding and unbinding of a 27-residue alpha/beta protein to the interior surface of a noncycling simple model of the chaperonin (a moderately hydrophobic sphere) effectively iteratively annealed the peptide to its native state via a new unfolded intermediate [16]. They argue that weak binding by the chaperonin is essential to elicit the conformational changes needed to follow this alternate fast-folding pathway.

The use of an off-lattice model in this study represents an important step forward in terms of simulation complexity. In this type of model, residues are represented as spheres and the energy function is constructed such that each sphere can be classified as hydrophobic, hydrophilic, or neutral. The use of this model allows one to see a more realistic picture of protein structure and dynamics than is available in a lattice framework, and the Langevin dynamics employed similarly provide a more realistic depiction of kinetics than fitting Monte-Carlo acceptance rates to kinetic models. Unfortunately, because of the coarse-grained nature of the energy function it is difficult to use such a model to make quantitative predictions that can be experimentally validated.

Landscape modulation models

Although substrate unfolding has been shown to be potentially important to chaperonin function, it is by no means the whole story. For some substrates, a single round of encapsulation can stimulate folding [7], and conventional annealing models cannot explain this fact. Experimental evidence points to numerous structural features of the chaperonin cage that may be able to modulate the folding landscape experienced by substrate proteins [17]. It is, however very difficult to spectroscopically 'see' in detail how the folding process is affected by confinement inside the closed chaperonin cavity. In a study by Lin and Rye, for example, some light was shed on the folding pathway of GroEL-encapsulated rubisco by labeling the substrate with fluorophores that undergo fluorescence resonance energy transfer (FRET) [18], but the level of detail provided by the experimental method was necessarily limited to what can be gleaned from relatively large changes in distances between fixed pairs of points on the folding protein. Simulations and analytical theories therefore stand to play an important role by picking up where experiments leave off in the pursuit of an explanation for chaperonin function.

It has been proposed that the act of confining a non-native peptide to a small volume can have large effects on its stability. Basically, if an unfolded protein is encapsulated inside a cavity that imposes limitations on its conformational flexibility, conformations with a large radius of gyration are no longer permitted. This effectively reduces the entropy of the unfolded ensemble and thus stabilizes the native state. Furthermore, if the transition state is minimally perturbed by confinement, the destabilization of the unfolded state should yield a faster rate of folding (although this assumes that diffusion within the unfolded state is unaffected, which may not be the case).

Zhou and Dill formulated an elegant analytical model for this effect in 2001 [19•]. If one assumes that the chaperonin can be modeled as an inert sphere of a given radius, and the unfolded state can be treated as a Gaussian chain, then one can solve a classical diffusion equation subject to the spherical boundary condition. Using this solution, one can predict the $\Delta\Delta G$ (change in free energy of folding) for confinement of a protein of a certain length confined to a cavity of fixed size. This model predicts that a large stabilization of the folded state is expected to occur when the size of the confining volume is only slightly larger than the native state. Beyond this, the native state will not fit inside the cavity and the free energy goes to infinity. As the confining volume increases, the confinement-induced stabilization approaches zero.

Recent experiments from Tang *et al.* are believed to support this model [17]. In these experiments, the length and sequence of unstructured C-terminal GGM repeats (which are presumed to protrude into the chaperonin cavity) were changed, which Tang *et al.* argue allowed them to modulate the volume of the chaperonin cavity. They observed an enhancement of folding qualitatively consistent with Zhou and Dill's model. Notwithstanding the cleverness of this method, the true effects of the GGM repeats on the volume of the chaperonin cavity, the ATPase activity of the enzyme [20], and their possible interaction with substrate peptide needs to be explored in more detail.

In addition to Zhou and Dill's analytical theory, computer simulation methods have also been employed to investigate the possibility of confinement-induced protein folding [21–23]. Most of these simulations utilized off-lattice models with Langevin dynamics as described above, but included an extra term in their energy function known as a Go potential (a term that increases the energetic contribution of native contacts over non-native contacts). Although usage of a Go potential can enhance the sampling of folding transitions, the assumption that non-native interactions are unimportant to the underlying folding dynamics may not be valid for confined systems.

Despite the obvious applicability of the simple polymer entropy description for stimulated folding, it is believed that this is only one piece of the puzzle. During its reaction cycle, the chaperonin cavity changes from mostly hydrophobic to mostly hydrophilic (see Figure 1). Recently, Tang *et al.* have shown that mutations that alter the hydrophilicity of the cavity have large effects on foldase activity [17]. Numerous simulations have also suggested the importance of surface hydrophobicity. Early lattice model simulations from Betancourt and Thirumalai [11], as well as more recent off-lattice simulations from Jewett *et al.* [12] and Cheung *et al.* [24] have shown that the hydrophobicity of the confining wall affects both the rate of folding (or unfolding) as well as the types of states that appear during the course of the simulation.

All of these simulations model the effect of hydrophobicity as a tunable potential between hydrophobic amino acids and the cavity wall. These simulations seem to agree that a hydrophobic surface is needed for unfolding, but are unable to explain why a hydrophilic surface seems to be required for stimulated folding. A full understanding of how the chaperonin cavity may modulate folding would most probably need to take this into account.

The role of water

Although some proposed mechanisms for landscape modulation focus on direct interactions between the chaperonin and its substrate, others suggest a role for the aqueous solvent that surrounds the substrate as it folds. Water is an essential participant in the folding reaction, and solvation forces such as the hydrophobic effect are crucial determinants of protein structure in most cases [25–27]. When it comes to chaperonins, the effect of the solvent on folding arguably warrants especially close attention because of the high degree of confinement the water inside the barrel may experience. Studies in both simulation and experiment have documented marked changes in the structure and properties of water when it becomes confined on the nanoscale [28,29•,30], and size comparisons between substrates of the *E. coli* chaperonin GroEL and the GroEL cavity suggest that the layer of water between the cavity wall and the substrate may, in some cases, be less than a nanometer thick [4••].

The question of what happens to water in such exotic circumstances is potentially a subtle one. Water is atypical among molecular liquids because it is permeated by a relatively strong, extensive network of hydrogen bonds. The tendency of water molecules to lower either their density or their entropy in order to avoid breaking these bonds in the vicinity of a hydrophobic solute leads to the solvent-mediated attraction between nonpolar surfaces known as the hydrophobic effect [31••]; by reducing the amount of hydrophobic surface area exposed to the solvent, the system minimizes the free energy gained from solvating the surface.

At the same time, water molecules possess electric dipole and higher multipole moments that experience torques and forces in the presence of an electrostatic field. Thus, charged surfaces can also bring about a reorganization of the aqueous solvent around them, often pulling surrounding water molecules into enthalpically favored solvation shells that have lower entropy and higher density than the bulk liquid [32].

Consequently, water that finds itself tightly confined between hydrophobic and charged surfaces faces a frustrating choice between at least two different modes of organization, and one might therefore expect the solvent to mediate a repulsive force between the surfaces when they come too close together. This expectation is borne out by various all-atom simulations. Molecular dynamics studies by both Bulone *et al.* [33,34] and Dzubiella and Hansen [32,35] have demonstrated a solvation repulsion between charged and hydrophobic groups in a water bath. More recently, Vaitheeswaran and Thirumalai [36] performed simulations of a methane molecule dissolved in a water nanodroplet and found that the initial hydrophobic attraction between the methane and the nonpolar surface of the droplet was converted to a repulsion once the methane became sufficiently charged. There is good reason to suspect that solvation forces under confinement deserve careful consideration as part of a complete account of chaperonin action because all proteins, including chaperonins, are amphipathic molecules comprising both charged and hydrophobic species.

Several studies of folding under confinement have provided further justification for this line of thinking. Sorin and Pande [37•] performed molecular dynamics simulations of a peptide trapped inside carbon nanotubes of various radii, and calculated helical propensity as a function of the tightness of confinement. Contrary to what one would expect from a polymeric entropy argument (which predicts that increased confinement favors lower entropy helical conformations over the more disordered coil ensemble), the study demonstrated that smaller nanotube radii actually lead to a decreased tendency toward helix formation. The authors were able to explain this surprising result by paying attention to the entropy of the solvent confined between the peptide and the tube wall. As the confinement became tighter, the translational entropy loss associated with formation of a hydrogen bond between a water molecule and the protein backbone decreased, thus increasing the relative stability of solvated, nonhelical peptide conformations. Thus, the free energy of the trapped solvent played a crucial role in shaping the folding landscape of a confined protein.

Subsequently, Lucent *et al.* [38•] have more systematically assayed the effect of confining solvent on folding for the case of the globular protein villin inside a spherical hydrophobic cavity. Performing a test only possible in simulation, they compared the probabilities of folding before unfolding, or p-folds, of different villin conformations solvated by water that either did or did not interact repulsively with the confining hydrophobic surface. Thus, it was possible to distinguish between the effect on folding of confining only the polypeptide, and the effect of confining both polypeptide and solvent. Consistent both with polymeric arguments and with the earlier helix work, their p-fold comparisons demonstrated that while confinement of only the protein accelerates folding, confining the solvent in addition produces a drive to unfolding, in this case because of an adsorption of the polymer on the hydrophobic cavity surface driven by its solvent-mediated attraction to nonpolar amino acid side chains. This adsorption constituted a decisive remodeling of the folding transition, providing the first evidence from all-atom simulation of modulation of the folding free energy landscape through confinement for a protein with tertiary structure.

Although both of these studies have improved our understanding of the solvation physics of polypeptides under confinement in ways that are certainly relevant to *in vivo* folding, the scenarios they describe do not resemble chaperonin action in at least two important respects. First, from the crystal structures of both the thermosome and GroEL, it is clear that chaperonins are porous, and therefore able to exchange water with the surrounding bath. Physically, this means that while the above simulations took place at constant particle number, folding inside a chaperonin arguably takes place at constant chemical potential, which could theoretically lead to a qualitatively different folding landscape depending on how important depletion or electrostriction are to adjusting the number of water molecules inside the cavity as a substrate folds. Second, the interior wall of a chaperonin is not a purely hydrophobic surface, but rather displays heterogeneous chemical functionality that shifts from being more hydrophobic to being more charged and hydrophilic upon closure of the cavity (Figure 1) [4••]. It seems probable that work seeking to shed light on chaperonin function must attempt to describe not only how tightly a folding protein is confined but also the physical properties of the surface that confines it because it has already been established experimentally that charged residues on the interior of GroEL are important to its foldase activity [17,39].

In a recent attempt to address both of these concerns, England and Pande developed an analytical model for solvent at constant chemical potential confined between surfaces of varying charge and hydrophobicity [40]. By abstracting the state of the solvent into two, spatially varying order parameters describing the particle density and hydrogen bond order as functions of space, they were able to calculate free energies for the solvent confined between a chaperonin (represented as a spherical cavity) and its substrate (represented as a concentric spherical surface of smaller radius). The model predicted that while the chaperonin cavity surface is hydrophobic, as when the complex is in its open conformation, confinement of a substrate should produce a thermodynamic drive toward unfolding because of the attraction between the cavity and the unfolded conformations of the protein with more exposed hydrophobic surface area. Once the cavity closes, however, and the confining surface becomes much more charged and hydrophilic, the opposite effect obtains: confinement of the solvent stabilizes the folded state because of the solvent-mediated repulsion between the cavity wall and exposed hydrophobic residues in the unfolded substrate that are more packed together in the native state. Thus, by modeling the effect that the chaperonin reaction cycle has on solvation forces during folding, the authors were able to suggest a possible function for the chaperonin as a chamber where the hydrophobic effect that normally helps to drive folding is enhanced above normal levels. Although this result comes from theoretical arguments, and obviously demands testing through detailed simulation and experiment, it nevertheless strengthens the argument that understanding the effect of confinement on solvation forces may be one of the keys to explaining chaperonin function.

Conclusions

Perhaps the most important thing to remember in solving the puzzle of how chaperonins work is that many of the answers that have been suggested are not mutually exclusive. Nothing prevents evolution from realizing multiple solutions to the same problem in its development of a single enzyme, and chaperonins may well offer an excellent illustration of this fact. For example, there is no reason that a substrate of GroEL could not benefit both from having its kinetically trapped intermediates unfolded, and from being immersed in a solvent environment that more strongly drives acquisition of the native state. Moreover, even if some of the proposed mechanisms for chaperonin action turn out to not be utilized by nature, the success they have already had in a theoretical setting suggests they might still be worth incorporating into the design of human-engineered tools for facilitating protein folding.

That being said, much work still remains in determining which factors are most effective in facilitating folding, whether *in vivo* or *ex vivo*. In the case of chaperonins, all-atom simulations of water and proteins in open and closed complexes will be necessary to more precisely

gauge the relative importance of direct protein–chaperonin interactions versus solvent effects, and to clarify a role, if any exists, for the unstructured GGM tails that project into the barrel's interior. At the same time, the use of theory and simulation to do comparative analyses that examine differences and similarities between the chaperonin and other biologically relevant folding/unfolding cavities (e.g. trans-membrane channels, the proteasome, or the ribosome exit tunnel) may be very informative. Such studies may set the stage for a new view of protein folding, as a process not accomplished by the polypeptide alone, but rather through the complexly regulated interplay of the chain, its solvent, and the surface that confines them.

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