Chaperone-percolator model: a possible molecular mechanism of Anfinsen-cage–type chaperones

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Summary

Although we have a rather elaborate ''working-cycle'' for the 60 kDa molecular chaperones, which possess a cavity, and are called Anfinsen-cage–type chaperones to emphasize that they provide a closed, protected environment to help the folding of their substrates, our understanding of the molecular mechanism of how these chaperones help protein folding is still incomplete. The present study adds two novel elements to the mechanism of how Anfinsen-cage–type chaperones (members of the 60 kDa chaperone family) aid protein folding. It is proposed that (1) these chaperones do not generally unfold their targets, but by a multidirectional expansion preferentially loosen the tight, inner structure of the collapsed target protein; and (2) during the expansion water molecules enter the hydrophobic core of the target, this percolation being a key step in chaperone action. This study compares this chaperone-percolator model with existing explanations and suggests further experiments to test it. *BioEssays* 1999;21:959–965.

Introduction

Folding of proteins larger than 10–20 kDa often results in a conformationally trapped intermediate after the initial hydrophobic collapse of the extended unfolded structure. Flexibility and conformational mobility of these partially folded proteins are rather restricted, when compared with the unfolded state. On one hand, the initial collapse is very helpful, because it greatly reduces the number of available conformations and, therefore, alleviates the need for a random search through the astronomical number of initially available conformational states, which would require a much greater time than the age of the Universe (the so-called "Levinthal-paradox"). Hydrophobic collapse, however, in most cases ''goes too far,'' and the composed intermediate structure (which is only 10% larger than the tightly packed native structure) makes further rearrangement of the inner hydrophobic core of the protein especially slow and difficult.[1–4] Because the correct and tight packing of the inner core is a key element of protein structure and stability,[5,6] restrictions of final folding steps of these inner segments are particularly debilitating for the development of the native structure. Amino acid side chains of protein interiors are rather flexible;[7] thus, it is not a general ''freezing,'' but the lack of extra space for inner-core backbone rearrangements, that precludes an easy transition to the native state. Thus, the maximal preservation of water structure and its hydrogen bonds is not only the major driving force of the initial hydrophobic collapse but also acts as a major restrictive force, which does not allow the intensive rearrangement of the inner, hydrophobic segments to reach the native state. The extent of hydrophobic collapse needs adjustment. Therefore, most larger proteins need help to complete the organization of their inner core, in addition to rescuing them from folding traps, arising from the improper rearrangement of some specific protein segments, and protecting them against aggregation.

All this help is provided by molecular chaperones. Chaperones bind to their target proteins by multiple bonds containing a great variety of interactions among hydrophobic, hydrophilic, and charged residues.[8–12] These bonds are usually not concentrated to a very small edge of the target protein but surround a significant portion of one or more folding domain.

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In the case of Anfinsen-cage–type chaperones, the ''encircling'' of the target is especially pronounced, where the oligomeric structure of the chaperone sequesters the target to its inner cavity and allows an undisturbed folding process preventing aggregation. At present, the 60 kDa chaperones are the only members of Anfinsen-cage–type chaperones.[8,12] The lack of three-dimensional descriptions of other multichaperone complexes, however, such as the dynamic complex of Hip/Hsp70/Hop/Hsp90 and other chaperones, makes it presently difficult to judge whether similar target ''encircling'' arrangements occur with these chaperones. The Clp-family of proteases also possess an inner cavity, but the nature and mechanism of the unfolding steps of their action are not clear yet. In this study, I propose a model for the Anfinsen-cage–type chaperones (this term referring to the 60 kDa molecular chaperones), but I will also keep in mind that future structural and functional studies might permit application of (at least some elements of) the model to other large chaperone complexes.

The present hypothesis adds two novel elements to the molecular mechanism of Anfinsen-cage–type chaperones: (1) instead of a general unfolding, these chaperones preferentially mobilize, loosen the tight, inner structure of the collapsed target protein; and (2) during the chaperone-induced initial expansion of the target, water molecules enter to the hydrophobic interior of the target, and this percolation is a key step in chaperone action. This study compares this chaperone-percolator model with existing proposals for the molecular mechanism, such as iterative annealing, lists experimental evidence supporting the model and suggests further experiments to test the idea.

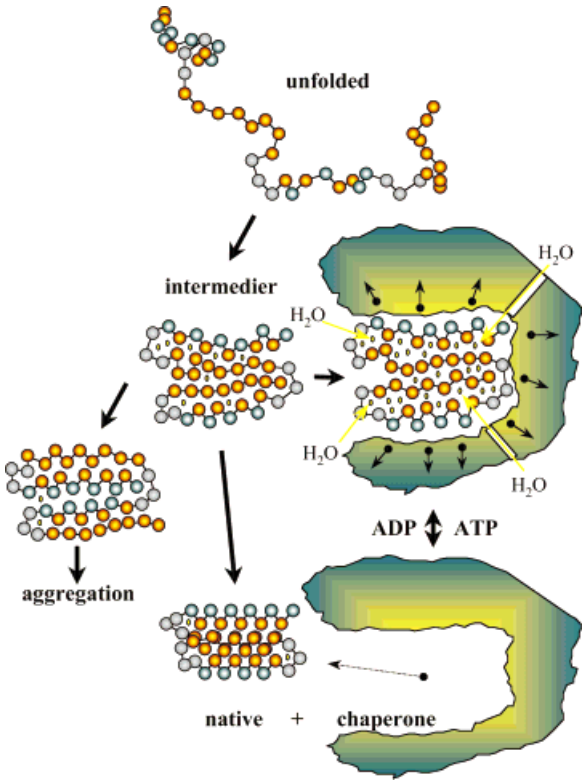

Figure 1. Schematic representation of the chaperone-percolator model. The figure describes the proposed molecular mechanism of Anfinsen-cage–type molecular chaperones. Red, blue, and white circles represent hydrophobic, charged, and hydrophilic residues of the target protein, respectively. Small yellow circles denote water molecules inside the target. Conformational states of the target protein were adapted from the work of Thirumalai and Guo.[1]

Description of the chaperone-percolator model

Previously, it was assumed that Anfinsen-cage chaperones are like passive boxes, insulating their targets from other proteins and preventing protein aggregation. Although the isolation of the target is a crucial element of their role, recent data indicate a more active role of these chaperones (members of the Hsp60 family) in target folding. Anfinsen-cage chaperones pull away a smaller or larger segment of the target protein. This stretching is helped as the nature of dominant target-binding interactions shifts from mostly hydrophobic to mostly charge-charge interactions and by geometrical changes of the chaperone-target interface (see Refs. 9–13, and the movies at the Internet address http://www.cryst.bbk.ac.uk/~ubcg16z/cpn/chaperone.html). These changes may initially loosen the structure of the target, and finally result in its release to the *cis* chamber of Anfinsen-cage–type chaperones. The bona fide folding of the target protein, i.e., the arrangement of its structure to reach the native state may happen *after* the initial stretching/pulling step. The initial expansion of the target, however, is a prerequisite for any subsequent folding process. Many times a single pulling/stretching-release folding attempt may not be successful and must be repeated. Chaperones, in general, do not accelerate folding but just make it more efficient. These elements of the molecular mechanism of Anfinsen-cage–type chaperone action have been already proven by numerous experiments and represent a consensus view.

The first major element of the present model emphasizes that instead of a general unfolding, Anfinsen-cage–type chaperones preferentially loosen the tight, inner structure of the collapsed target protein. Loosening of the hydrophobic core of the target is paralleled by a preferential mobilization of the inner amino acid side chains together with the immobilization of the external segments of the target (Fig. 1). Besides the isolation of their targets from other partially unfolded proteins (and, thus, prevention of their aggregation) Anfinsen-cage–type chaperones also isolate their targets from the bulk of the structured water outside of the chaperone machine.

Thus, according to the present model, these chaperones reverse the hydrophobic collapse by two mechanisms: (1) by a direct mechanical multidirectional expansion, and (2) by the attenuation of the hydrophobic force, i.e., the effects of the structured water to the folding target.

What happens when an Anfinsen-cage chaperone pulls away its target? To avoid creating a vacuum, something must enter the interior of the target protein. The inner space of the Anfinsen-cage–type chaperone chamber is packed with water molecules. As a second major element of the present model entry of water molecules to the interior of the folding target protein is postulated to take place (Fig. 1). Water entry is helped by two mechanisms: (1) by a specific, disordered state of certain water molecules inside the folding cavity; and (2) by a massive outflow and consecutive influx of water molecules from the cavity during a single cycle of chaperone action.

Water molecules in the cavity of the chaperone do not resemble completely the structured water outside. Although there are numerous water molecules in the chaperone cavity at each step of the chaperone cycle, water-water hydrogen bonding is limited by geometrical constraints. Water molecules finding themselves in the initially hydrophobic environment of the chaperone may adopt a rather disordered state with special thermodynamic properties.[14–17] As another important difference, the surrounding chaperone may significantly alter the dielectric constant of the ''inside water.''[18] According to the present model Anfinsen-cage–type chaperones lower the energy barriers for the entry of water molecules to the inside of folding proteins. Because of these changes, water molecules in the chaperone chamber may find an easier way to enter the inside of the loosened target protein and to fill the ''vacuum'' generated by its chaperone-mediated expansion. The energy requirement for the transition of water molecules from the outside organized water structure to the inside of the chaperone and to the inside of the target protein reaching 10 to 15 kcal/mol^{-1} [19] is most probably provided by the conformational changes of the chaperone machine that uses the energy of ATP hydrolysis.

There is a massive traffic of water molecules during chaperone action. The initial volume of the *cis* cavity (assumed to be 85,000 Å^3)[11] contains approximately 3,000 water molecules. The total volume of a large target protein (such as that of the 52-kDa RuBisCO enzyme) corresponds to 2,500 water molecules; thus, RuBisCO binding induces a large outflow of water from the chaperone machine. The amount of chaperone-contained water reaches its minimum during a single cycle (which is in between 500 and 2,000 water molecules, depending on the exact geometry of target binding) when ATP binds to the *cis* ring and induces a large conformational change increasing the cavity volume to 175,000 Å^3.[11] This enlargement is paralleled with a massive influx of water until the total number of water molecules reaches 3,500. Further geometric information on chaperone-target complexes are necessary to give a more exact estimate of the water molecules present at various steps of the chaperone cycle. (The volume of the partially unfolded RuBisCO protein has been calculated as only 110% of the native volume. A more loose initial conformation of the target would result in even larger water fluxes. On the other hand, RuBisCO probably represents the upper size limit of the proteins able to use the help of the GroE chaperone. Smaller targets induce smaller water fluxes. As a third uncertainty, the volume of water inside is unknown. Disordered water in the inside of the cavity may occupy a larger volume, which would also reduce fluxes during the cycle.) Despite the uncertainties of the numerical values, it is quite clear that chaperone cycle is paralleled with a massive outflow and influx of water molecules from the folding cavity.

The Anfinsen-cage–type chaperone machine-mediated entry of water molecules to the interior of the folding protein is not only a passive consequence of the chaperone-induced pulling/expansion of the target but may significantly promote the conformational changes required to reach the native state. At low hydration levels, the unfolding of proteins is severely hindered. Lyophilization of subtilisin or chymotrypsin and their subsequent dissolution in anhydrous solvents freezes the enzyme conformation and generates an ''enzyme memory.'' This memory can be erased by the addition of water.[20] Water induces a larger internal mobility of proteins as judged by neutron scattering, nuclear magnetic resonance (NMR), electron spin resonance (ESR), Mössbauer spectroscopy, infrared, high-frequency dielectric measurements, fluorescence, and phosphorescence lifetime analyses. These analytical methods cover an enormously large time scale of various protein movements from the picosecond scale of neutron scattering to the second range of phosphorescence lifetime measurements.[21,22] Water molecules were suggested to promote the rapid ''flickering'' between various conformational states, for which the interaction of the peptide backbone with neighboring water molecules results in various transient reverse-turn conformations forming a rather smooth path from a certain conformational state to the other.[23] Thus, Anfinsen-cage–type chaperone-induced entry of water molecules to the otherwise hidden interior of the target protein may be essential to make the necessary conformational changes possible. Water entry to the target is not only a coincident, secondary event, but may be an almost absolute necessity in assisted protein folding. The massive outflow and consecutive influx of water molecules during the chaperone cycle efficiently washes through the target with water. Therefore, I suggest calling the above hypothetical mechanism the chaperone-percolator model, referring to the process of percolation, where a solvent is passed through a permeable substance.

Whether the percolation described by the above model is accompanied by a percolation in the sense of an extension of the long-range ordered structure of water and development of long-range conductivity involving coordinated proton displacement of water molecules in the inside of the target protein[21] remains to be established. However, a — transient — connection of isolated water molecules in the inside of the target protein with molecules of the outer hydrate shell[17] may significantly increase hydrogen bond fluctuation and the subsequent catalysis of conformational rearrangement.

Limitations of the chaperone-percolator model

Limitations from the target side

The above chaperone-percolator model assumes that the target protein is relatively large, having a well-developed inner, hydrophobic core, which experiences significant difficulties in its final rearrangements. Fast-folding targets may not need the extensive help described by the present model.

In several large targets, kinetic barriers of specific final rearrangements may form the rate-limiting steps to reach the native state, and the restriction of the inner-core rearrangement may be only of secondary importance.

Limitations from the chaperone side

As noted before, the model requires an Anfinsen-cage–type chaperone structure. According to our present knowledge, this structural requirement is valid only for the 60 kDa chaperone family. Members of the 70 kDa chaperone family bind small peptide segments,[24] to which the chaperone-percolator model cannot be applied. Multichaperone complexes may use similar unfolding/percolating steps like those described in the chaperone-percolator model. However, presently, we do not have enough information to extend the model beyond the 60-kDa chaperones.

Even in Anfinsen-cage–type chaperones some chaperone-target interactions may not be strong enough, or of sufficiently large scale, to cause an increased ''breathing'' of the inner core of the target.

Relationship of the chaperone-percolator model to other chaperone mechanisms such as the iterative-annealing mechanism

Although there is a rather detailed description of the ''working-cycle'' of the 60 kDa molecular chaperones, the precise molecular description of how Anfinsen-cage–type chaperones help the refolding of their target proteins lags behind. The ''threading'' described by Hubbard and Sander[25] may be operational in chaperones, which bind their targets in an extended conformation. Until now, however, there is no direct experimental evidence to verify this model. The other possible alternative molecular mechanism of Hubbard and Sander,[25] ''plucking'' is much closer to the present model, but also involves the ''straightening out'' of the peptide backbone. The iterative annealing mechanism of Todd et al.[26] describes the 60 kDa molecular chaperones as follows: ''they repeatedly bind kinetically trapped conformers, randomly disrupt their structure, and release them in less folded states, allowing substrate proteins multiple opportunities to find pathways leading to the most thermodynamically stable state.'' As a further development, Chan and Dill[27] describe the chaperone machine as a protein ''that helps pull apart an incorrectly folded protein so it can try again to fold.'' The expression ''pulling apart'' in the study by Chan and Dill refers mostly to the disruption of hydrophobic contacts.[27] The various pulling processes described by these mechanisms is similar to the above chaperone-percolator model. However, the chaperone-percolator model makes the physical meaning of the pulling process mediated by Anfinsen-cage–type molecular chaperones clearer by emphasizing that instead of a general unfolding, Anfinsen-cage–type chaperones preferentially loosen the tight, inner structure of the collapsed target protein. The chaperone-percolator model also emphasizes the importance of intensive interactions between water molecules and the peptide backbone of the target protein. The present model provides new elements to explain the dramatic changes of the energy landscape of protein folding on chaperone action, earlier postulated by Gulukota and Wolynes[28] and describes a mechanism in which the chaperone does not act as a direct catalyst of unfolding as predicted by Schmid and coworkers.[29]

Experimental evidence supporting the chaperone-percolator model

Evidence for a multidirectional pulling process, which mobilizes the interior of the target

Alkaline phosphatase provides one of the best examples for the slow rearrangement of the inner core during refolding, where the formation of the crystalline-like inner structure may last for weeks as judged by phosphorescence analysis of an inner tryptophane residue.[30] This slow refolding emphasizes the necessity of a special mechanism able to loosen the slowly rearranging inner structure of the target folding intermediate.

Three-dimensional structures of complexes of Anfinsen-cage–type chaperones with their substrates show a rather extended conformation of the target[12,31] supporting the notion that chaperones interact with their targets by using multiple bonds surrounding the target molecule. The structure of GroEL is significantly stabilized after binding of rhodanese, which gives further proof for the multiple, large scale chaperone-target interactions.[32] However, these data do not allow us to judge whether an actual ''inflation'' of the globular target occurs.

Indirect evidence from the laboratory of Ullrich Hartl[33] suggests that GroEL ''pulls apart'' the two domains of rhodanese, exposing the hydrophobic interdomain contacts of the protein.

Direct experimental evidence supporting the chaperone-percolator model came from the studies of the group of Art Horwich.[34] Analyzing the fluorescence anisotropy of the RuBisCO tryptophanes after the addition of GroE, they found a fast drop of the anisotropy value followed by a gradual increase. The fast drop in the anisotropy corresponds to the mobilization of the inner core of the RuBisCO protein, and the gradual increase reflects the slow rearrangement and tightening of the inner structure[12] These findings are in excellent agreement with the prediction of the internal target mobilization by the chaperone-percolator model.

During the formulation of the present manuscript, a study by Persson et al.[35] was published, which analyzed the interaction between human carbonic anhydrase and GroEL by using spin-labeled variants of the target. The chaperone-percolator model would predict the mobilization of the inner segments of carbonic anhydrase with a concomitant immobilization of its peripheral portions involved in binding to GroEL. This is exactly what was observed with the great variety of spin-labeled probes used by Persson et al.,[35] and gives excellent proof for the predictive power of the model.

Evidence for water percolation

Reduction of the hydrophobic force (i.e., the sequestration of hydrophobic segments by the water structure) by detergents and other chemical chaperones may contribute to the efficiency of these small molecules in refolding of several target proteins. In addition to their increase of the ''solvophobic effect'' on the peptide backbone,[36] the disorganization of water structure induced by chemical chaperones may result in an increased ''breathing'' of the inner core of the target and a smoother entry of water molecules to the interior of the target alleviating the need for a complex chaperone structure.

The acceleration of the loss of tritium atoms from tritiated RuBisCO protein[37] upon the addition of an active GroE complex may be interpreted as a partial unfolding of the molecule but may also be the consequence of accelerated water penetration to the inner segments of the protein.

Data obtained from nuclear magnetic resonance and ESI-MS measurements show a 10^3- to 10^6-fold increased deuterium-hydrogen exchange of peptide amide groups of the chaperone-bound target protein. The degree of exchange varies from one chaperone-target pair to the other and was regarded as a measure from partial to almost complete unfolding of the target.[38–42] The varying degree of unfolding experienced in these studies may also reflect the various levels of percolation of the extended inner structure by water molecules. The dramatic increase in water-peptide bond interactions even in the inside of the target and the resemblance of the partially protected target peptide bonds to the native globular structure of the target[42] provide further strong support for the chaperone-percolator model.

The second part of the model (water entry to the interior of the target) is more hypothetical at present than the first assumption (multidirectional expansion and mobilization of the interior of the target). In the following section, I will suggest some experiments to test the validity model.

Suggested experiments to test the model

With the limitations mentioned above, let me summarize again the major consequences of the chaperone-percolator model: (1) target proteins undergo a multidirectional expansion during their interactions with Anfinsen-cage–type molecular chaperones; (2) during the interaction the hydrophobic core of the target becomes more flexible, whereas its outer, charged residues are more immobilized than in its unbound state; (3) chaperone cycle is paralleled by a massive water outflow and subsequent influx of water molecules, which contribute to the percolation of the target protein; (4) water molecules reside in a special ''high-energy'' state in the inside of the chaperone chamber and penetrate to the inside of the target protein during its expansion, significantly easing its conformational transitions.

The first of the above assumptions is difficult to test. Geometry of chaperone-target interactions may vary from one chaperone-target pair to the other. The expansion of target proteins might be too small to allow easy verification. Thus, experiments to test the chaperone-percolator model should mostly concentrate to the parallel analysis of target and water mobility in the absence and presence of chaperones. Some suggestions are as follows: (1) the lifetime of target tryptophane phosphorescence and its fluorescence anisotropy should decrease after Anfinsen-cage type chaperone binding (the first anisotropy observation has been made by the group of Art Horwich[34]); (2) ESR probes on the surface and in the interior of the target protein should report different changes during chaperone-target interactions (as seen by Persson et al. [35]); (3) the amount and distribution of nuclear magnetic relaxation rates of water protons or oxygens (similar to those described in Ref. 43) should change upon target binding to Anfinsen-cage–type chaperones because of the appearance of a significant amount of ''percolating water'' with residence times in the microsecond range; (4) Raman spectra of O–H and N–H vibrational bands should also report increasing amount of ''peculiar'' hydrogen bonding when Anfinsen-cage–type chaperones bind their target; (5) target surfaces should be less sensitive, whereas the hydrophobic cores of the target should be *more* sensitive to the decomposition by hydroxyl radicals in the presence than in the absence of Anfinsen-cage–type chaperones (common sense would dictate that Anfinsen-cage–type chaperones protect all parts of the sequestered targets equally).

If neither NMR nor Raman experiments report any unusual water molecules during the "working cycle" of Anfinsen-cage–type chaperones with appropriate (i.e., relatively large) targets, the second major assumption of the chaperone-percolator model (i.e., the penetration of water molecules to the inside of the target protein) will not hold.

Conversely, if the above experiments (and perhaps others) validate the chaperone-percolator model in the case of the 60 kDa molecular chaperones, the same experiments might be used to test the folding mechanism of target proteins with other chaperone machineries, e.g., with the Hsp90-organized foldosome (Hip/Hsp70/Hop/Hsp90 complex and its other co-chaperones). If similar results were to be obtained, this might suggest a transient Anfinsen-cage geometry of this larger chaperone complex.

Conclusions

The present study describes a novel molecular mechanism of chaperone action, the mobilization of the inner, hydrophobic core of the target protein by Anfinsen-cage–type chaperones by means of multiple interactions with the outer side chains of the target and its consequent percolation by water molecules, which allow easier transitions between the individual conformations of the folding protein (Fig. 1). This "chaperone-percolator" model is supported by several pieces of theoretical and experimental evidence and can be tested by further experiments.

Acknowledgments

The author is an International Research Scholar of the Howard Hughes Medical Institute and acknowledges the useful comments of Drs. Ken Dill (Department of Pharmaceutical Biochemistry, University of California, San Francisco CA) Peter G. Wolynes (School of Chemical Sciences, University of Illinois, Urbana IL), Csaba Sőti (Department of Medical Chemistry, Semmelweis University, Budapest, Hungary), and all the anonymous referees of the manuscript.

References

1. Thirumalai D, Guo Z. Nucleation mechanism for protein folding and theoretical predictions for hydrogen-exchange labeling experiments. Biopolymers 1994;35:137–140.
2. Bryngelson JD, Onuchic JN, Socci ND, Wolynes PG. Funnels, pathways, and the energy landscape of protein folding: a synthesis. Proteins 1995;21:167–195.
3. Dill KA, Bromberg S, Yue K, Fiebig KM, Yee DP, Thomas PD, Chan HS. Principles of protein folding: a perspective from simple exact models. Protein Science 1995;4:561–602.
4. Ptitsyn OB. Structures of folding intermediates. Curr Opin Struct Biol 1995;5:74–78.
5. Harpaz Y, Gerstein M, Chothia C. Volume changes on protein folding. Structure 1994;2:641–649.
6. Gerstein M, Chothia C. Packing at the protein-water interface. Proc Natl Acad Sci USA 1996;93:10167–10172.
7. Chan HS, Bromberg S, Dill KA. Models of cooperativity in protein folding. Philos Trans R Soc Lond B 1995;348:61–70.
8. Hartl F-U. Molecular chaperones in cellular protein folding. Nature 1996; 381:571–580.
9. Ranson NA, White HE, Saibil HR. Chaperonins. Biochem J 1998;333:233–242.
10. Xu Z, Sigler PB. GroEL/GroES: structure and function of a two-stroke folding machine. J Struct Biol 11998;24:129–141.
11. Rye HS, Roseman AM, Chen S, Furtak C, Fenton WA, Saibil, HR, Horwich AL. GroEL-GroES cycling: ATP and nonnative polypeptide direct alteration of folding-active rings. Cell 1999;97:325–338.
12. Sigler PB, Xu Z, Rye HS, Burston SG, Fenton WA, Horwich A. Structure and function in GroEL-mediated protein folding. Annu Rev Biochem 1998;67:581–608.
13. Roseman AM, Chen S, White H, Braig K, Saibil HR. The chaperonin ATPase cycle: mechanism of allosteric switching and movements of substrate-binding domains in GroEL. Cell 1996;87:241–251.
14. Wiggins PM, MacClement BAE. Two states of water found in hydrophobic clefts: their possible contribution to mechanisms of cation pumps and other enzymes. Int Rev Cytol 1987;108:249–303.
15. Ernst JA, Clubb RT, Zhou H-X, Gronenborn AM, Clore GM. Demonstration of positionally disordered water within a protein hydrophobic cavity by NMR. Science 1995;267:1813–1817.
16. Pertsemlidis A, Saxena AM, Soper AK, Head-Gordon T, Glaeser RM. Direct evidence for modified solvent structure within the hydration shell of a hydrophobic amino acid. Proc Natl Acad Sci USA 1996;93:10769–10774.
17. Yu B, Blaber M, Gronenborn AM, Clore GM, Caspar DLD. Disordered water within a hydrophobic cavity visualized by x-ray crystallography. Proc Natl Acad Sci USA 1999;96:103–108.
18. Löffler G, Schreiber H, Steinhauser O. Calculations of the dielectric properties of a protein and its solvent: theory and case study. J Mol Biol 1997;270:520–534.
19. Zhang L, Hermans J. Hydrophobicity of cavities in proteins. Proteins 1996;24:433–438.
20. Klibanov AM. What is remembered and why? Nature 1995;374:596.
21. Rupley JA, Careri G. Protein hydration and function. Adv Protein Chem 1991;41:37–172.
22. Wüthrich K. Hydration of biological macromolecules in solution: surface structure and molecular recognition. Cold Spring Harbor Symp Quant Biol 1993;58:149–157.
23. Barron LD, Hecht L, Wilson G. The lubricant of life: a proposal that solvent water promotes extremely fast conformational fluctuations in mobile heteropolypeptide structure. Biochemistry 1997;36:13143–13147.
24. Zhu X, Zhao X, Burkholder WF, Gragerov A, Ogata CM, Gottesman ME, Hendrickson WA. Structural analysis of a substrate binding by the molecular chaperone DnaK. Science 1996;272:1606–1614.
25. Hubbard TJP, Sander C. The role of heat-shock and chaperone proteins in protein folding: possible molecular mechanisms. Protein Eng 1991;4:711–717.
26. Todd MJ, Lorimer GH, Thirumalai D. Chaperonin-facilitated protein-folding: optimization of rate by an iterative annealing mechanism. Proc Natl Acad Sci USA 1996;93:4030–4035.
27. Chan HS, Dill KA. A simple model of chaperonin-mediated protein folding. Proteins 1996;24:345–351.
28. Gulukota K, Wolynes PG. Statistical mechanics of kinetic proofreading in protein folding in vivo. Proc Natl Acad Sci USA 1994;91:9292–9296.
29. Walter S, Lorimer GH, Schmid FX. A thermodynamic coupling mechanism for GroEL-mediated unfolding. Proc Natl Acad Sci USA 1996;93:9425–9430.
30. Subramaniam V, Bergenhem NCH, Gafni A, Steel DG. Phosphorescence reveals a continued slow annealing of the protein core following reactivation of *Escherichia coli* alkaline phosphatase. Biochemistry 1995;34:1133–1136.
31. Buckle AM, Zahn R, Fersht AR. A structural model for GroEL-polypeptide recognition. Proc Natl Acad Sci USA 1997;94:3571–3575.
32. Gorovits BM, Horowitz PM. The molecular chaperonin cpn60 displays local flexibility that is reduced after binding with an unfolded protein. J Biol Chem 1995;270:13057–13062.
33. Hlodan R, Tempst P, Hartl F-U. Binding of defined regions of a polypeptide to GroEL and its implications for chaperonin-mediated protein folding. Nature Struct Biol 1995;2:587–595.
34. Rye HS, Burston SG, Fenton WA, Beechem JM, Xu Z, Sigler PB, Horwich AL. Distinct actions of *cis* and *trans* ATP within the double ring of the chaperonin GroEL. Nature 1997;388:792–798.

35. Persson M, Hammarström P, Lindgren M, Jonsson B-H, Svensson M, Carlsson U. EPR mapping of interactions between spin-labeled variants of human carbonic anhydrase II and GroEL: evidence for increased flexibility of the hydrophobic core by the interaction. Biochemistry 1999;38:432–441.
36. Qu Y, Bolen CL, Bolen DW. Osmolyte-driven contraction of a random coil protein. Proc Natl Acad Sci USA 1998;95:9268–9273.
37. Shtilerman M, Lorimer GH, Englander SW. Chaperonin function: folding by forced unfolding. Science 1999;284:822–825.
38. Zahn R, Spitzfaden C, Ottiger M, Würtlich K, Plückthun A. Destabilization of the complete protein secondary structure on binding to the chaperone GroEL. Nature 1994;368:261–265.
39. Robinson CV, Gross M, Eyles SJ, Ewbank JJ, Mayhew M, Hartl FU, Dobson CM, Radford SE. Conformation of GroEL-bound α-lactalbumin probed by mass spectrometry. Nature 1994;372:646–651.
40. Gervasoni P, Staudenmann W, James P, Gehring P, Plückthun A. β-lactamase binds to GroEL in a conformation highly protected against hydrogen/deuterium exchange. Proc Natl Acad Sci USA 1996;93:12189–12194.
41. Zahn R, Perrett S, Stenberg G, Fersht AR. Catalysis of amide proton exchange by the molecular chaperones GroEL and SecB. Science 1996;271:642–645.
42. Goldberg MS, Zhang J, Sondek S, Matthews CR, Fox RO, Horwich AL. Native-like structure of a protein-folding intermediate bound to the chaperonin model. Proc Natl Acad Sci USA 1997;94:1080–1085.
43. Denisov VP, Johnsson BH, Halle B. Hydration of denatured and molten globule proteins. Nature Struct Biol 1999;6:253–260.