Chaperone-client interactions: Non-specificity engenders multifunctionality

Published, Papers in Press, June 15, 2017 DOI 10.1074/jbc.R117.796862

Philipp Koldewey[‡1], **Scott Horowitz**[‡1], **and James C. A. Bardwell**[‡§2]

From the [‡]*Department of Molecular, Cellular, and Developmental Biology and the* [§]*Howard Hughes Medical Institute, University of Michigan, Ann Arbor, Michigan 48109*

Edited by Wolfgang Peti

Here, we provide an overview of the different mechanisms whereby three different chaperones, Spy, Hsp70, and Hsp60, interact with folding proteins, and we discuss how these chaperones may guide the folding process. Available evidence suggests that even a single chaperone can use many mechanisms to aid in protein folding, most likely due to the need for most chaperones to bind clients promiscuously. Chaperone mechanism may be better understood by always considering it in the context of the client's folding pathway and biological function.

Proteins start their lives as unfolded polypeptide chains. However, the ability of proteins to function is closely tied to their ability to fold into the correct native state conformation. The cytosol is very dense, containing up to 400 mg/ml macromolecules (1, 2). As a result, non-specific interactions that can interfere with protein folding are a constant hazard. In addition, due to the importance of conformational flexibility in generating biological activity, proteins are generally only marginally stable and are thus prone to misfolding, particularly in the presence of cellular stress (3, 4). Non-specific interactions involving misfolded states drive aggregate formation, which is often irreversible and toxic (5, 6). To ensure the integrity of its proteome, the cell thus invests in complex protein quality control machinery that includes a network of molecular chaperones. Chaperones assist in folding, ensure conformational integrity, and control aggregation under stress conditions (7). Cells respond to heat-induced folding stress by up-regulating the expression of heat shock proteins (Hsps), many of which have been found to function as molecular chaperones. Chaperones were initially named according to their monomeric molecular weights: Hsp40, Hsp60, Hsp70, Hsp90, etc. (8). In addition to being up-regulated in response to stress, many chaperones are also abundant under non-stress conditions. Furthermore, additional chaperones have been identified that are controlled by stress-response systems other than the general heat-shock response (8–10). The various classes of chaperones work together to ensure the proper folding of both newly synthesized and stress-denatured proteins (11, 12).

Although proteins can potentially fold to the native state on their own, as postulated by the Anfinsen experiment, it is now clear that in the complex, crowded environment of the cell many proteins require a network of molecular chaperones to fold effectively and on a biologically relevant time scale (13, 14). Defects in protein folding have been associated with numerous diseases, including Alzheimer's and Parkinson's (15, 16). Chaperones are also thought to be major players in the process of aging, as their levels drop dramatically during aging, likely causing the collapse of protein homeostasis (15–17). Thus, a detailed understanding of the mechanism by which chaperones assist in protein folding may eventually allow us to manipulate chaperone systems in intelligent ways to address folding diseases and aging.

Chaperones undergo complex conformational changes during their reaction cycles; these changes have been extensively studied and are the subject of recent reviews (18–21). In this review, we examine our understanding of how chaperones participate in the protein folding process, focusing on three model chaperones: Spy, Hsp60, and Hsp70. We present evidence suggesting that chaperones do not utilize a single mechanism for all clients and propose that it may be more appropriate to classify chaperone mechanisms only in the context of the client.

Spy

Spy is an ATP-independent chaperone that can aid in protein folding (10, 12, 22). This 16-kDa periplasmic protein is highly overexpressed in response to protein folding stress in a wide range of enterobacteria and protobacteria and in some cyanobacteria (23–27). Like other folding chaperones, Spy has broad client specificity; it prevents aggregation and promotes proper refolding of a diverse set of proteins (27–29). In addition to its ability to stabilize folding intermediates *in vivo*, Spy has been shown to inhibit the formation of amyloids *in vitro* and *in vivo* (30).

Spy was very recently used as a simple chaperone folding system to identify the kinetic, thermodynamic, and structural properties that allow chaperones to promote client folding and to determine how they affect the folding landscape of client proteins (31–33). The "folding-friendly" amphiphilic and flexible nature of Spy's client-binding site was found to be critical to its chaperone activity (33). The client-binding site, which

This work was supported by National Institutes of Health Grants R01-GM102829 (to J. C. A. B.) and K99-GM120388 (to S. H.). The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

[1] Both authors contributed equally to this work.

[2] Howard Hughes Medical Investigator. To whom correspondence should be addressed: Dept. of Molecular, Cellular, and Developmental Biology, University of Michigan, Rm. 4007, 830 N. University Ave., Ann Arbor, MI 48109. Tel.: 734-764-8028; Fax: 734-615-4226; E-mail: jbardwel@umich.edu.

encompasses a large part of the concave surface of Spy's cradle-shaped structure, consists of four hydrophobic patches surrounded by positively charged hydrophilic residues and flexible N-terminal helix linkers between helix one and helix two. This combination of flexibility and amphiphilic binding surface allows Spy to dynamically bind the many conformational states that occur along the folding trajectory of its client proteins and hence mediate folding while remaining continuously but loosely bound to its clients (31, 33). The attraction of the aggregation-prone unfolded client to the chaperone is driven by electrostatic forces, which are then complemented by hydrophobic interactions in the complex (32). This mixture of transient hydrophobic and hydrophilic interactions (32, 34) allows the client to explore its folding landscape while bound (33, 35). Thus, the client is bound to Spy as a conformationally heterogeneous ensemble, sampling conformations ranging from unfolded to intermediately and near-natively folded states (Fig. 1) (33). Spy binding induces a compaction of the unfolded client that favors client folding (34, 35). During folding, Spy stays bound to thermodynamically unstable areas, thereby helping the client avoid aggregation (34). Client folding results in hydrophobic core formation and thus reduces stabilizing hydrophobic contacts between chaperone and client (32, 33). This destabilizes the complex, helping to trigger client release (32). Therefore, rather than being dictated by the chaperone, client folding regulates client binding and release. Spy further enables the folding process by serving as an entropy sink, becoming more flexible as the client protein becomes more rigid in the folding process (35). Thus, Spy provides a sanctuary for folding proteins that prevents protein aggregation and misfolding, whereas the folding pathway remains dictated by the primary sequence of the client protein. The electrostatic interactions formed between Spy and the client protein are a central component of this mechanism. Not only do they enhance the client binding rate and therefore kinetically prevent protein aggregation, they also help keep the client protein bound while it folds and hence eliminate the need for pre-native client release, a requirement that was previously considered essential for the successful folding of clients by chaperones (8, 36).

Loose client binding has been postulated to be important for chaperone-mediated client folding (37, 38). The classic folding machines GroEL and TRiC are similar to Spy in that they also provide broad and heterogeneous client-binding sites consisting of small hydrophobic patches surrounded by electrostatic residues (39, 40).

One major caveat to the narrative of Spy's function is that detailed biochemical and biophysical studies on Spy have only been performed with one client, Im7. Although two separate groups have come to similar conclusions on the folding of Im7 in the presence of Spy (31, 34), there is little evidence to suggest whether or not this mechanism also applies to other client proteins. As will be discussed in the cases below for Hsp60 and Hsp70, this caveat cannot be ignored.

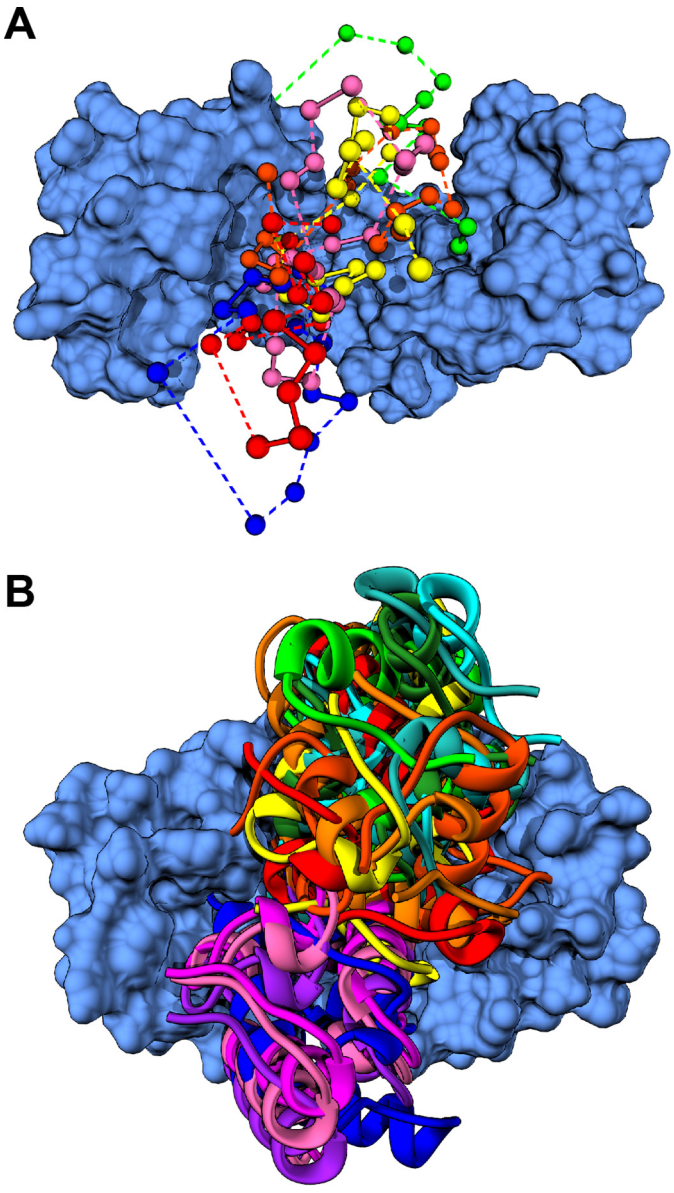

Figure 1. Binding of Im7 to Spy. *A*, residual electron and anomalous density (READ) crystallography ensemble of Im7 6–45 (multiple colored *ball* and *stick*) binding to Spy (*blue surface*) in multiple folding states, ranging from unfolded to native-like (33). *B*, NMR paramagnetic resolution enhancement-based docking of native state full-length Im7 (multiple *colored ribbons*) to Spy (*blue surface*) (34).

Hsp60

The Hsp60 family of chaperones, also called chaperonins, is found in all three branches of life (8). Hsp60s are divided into two groups based on sequence homology. Type I chaperonins, *e.g.* GroEL, are encoded in the genome of bacteria and in the endosymbiotic organelles of eukaryotes, whereas type II chaperonins, *e.g.* TRiC, facilitate protein folding in the eukaryotic cytosol and in most archaea (18, 41). Both types of chaperonins form back-to-back stacked double-ring structures that provide chambers potentially allowing client proteins to fold in isolation, thereby avoiding unwanted intermolecular interactions with the cellular proteome.

Binding of non-native client proteins to chaperonins is mediated both through electrostatic interactions and through patches of hydrophobic residues exposed in the chaperonin rings' apical domains (Fig. 2, *A* and *C*) (39, 40). After client binding, ATP binding and hydrolysis trigger conformational changes in the apical domains that lower client affinity, releas-

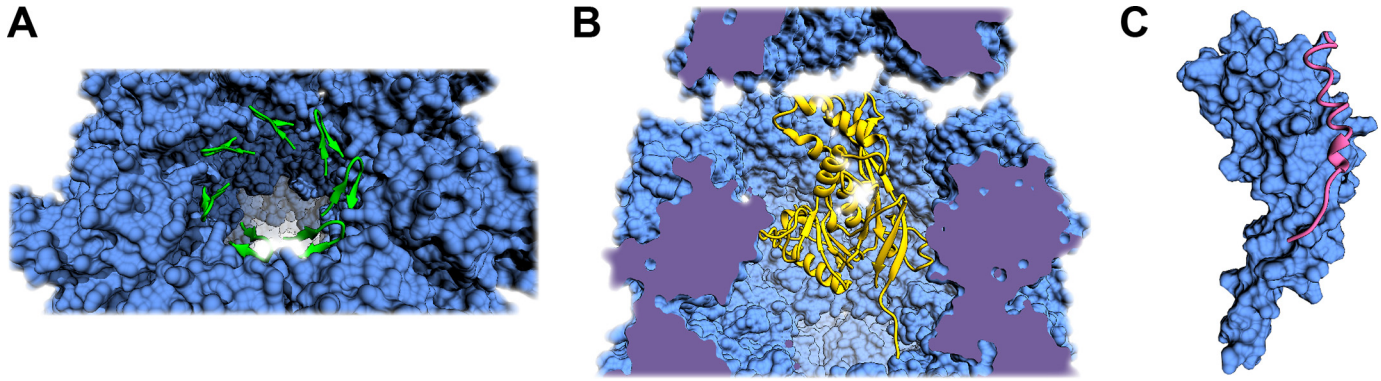

Figure 2. Binding of clients to Hsp60. *A*, crystal structure of a peptide client (*green ribbon*) binding to the apical domains of GroEL (*blue surface*) (96). *B*, cryo-EM structure of newly folded client gp23, modeled in using the structure of gp24 (*yellow ribbons*), bound within the GroEL-GroES complex (97). *C*, NMR chemical shift-Rosetta model of client p6 (*pink ribbon*) binding to the apical domain of CCT/TRiC (*blue surface*) (39).

ing the client protein into the chamber. The chamber then closes, through binding the co-chaperone GroES (in the case of GroEL) or through conformational changes (in the case of TRiC) (21). Relatively slow ATP hydrolysis provides the client protein time to fold while trapped within the chamber (Fig. 2*B*). After completion of ATP hydrolysis, the client protein is released. Client rebinding may occur if folding is incomplete (18, 39). The chamber of both chaperonin types is large enough to encapsulate client proteins up to ~60 kDa (18, 42). Larger client proteins may still use the chaperonin system by binding to the apical client-binding sites, which has been shown to facilitate folding outside the chamber (43, 44). In addition, partial encapsulation of larger multidomain proteins has been reported for TRiC, which allows isolated folding of domains separately (45, 46).

Although ATP-driven conformational cycles and mechanisms of client recognition are rather well established for chaperonins, different modes of client binding and release from the chaperonin have been reported to aid protein folding. For instance, several hydrophobic segments of the non-native client may bind to several apical domains of the chaperonin cage simultaneously. This has been reported to partially unfold the client protein, potentially by selecting out less structured states of a client's flexible ensemble (43, 47, 48). Client unfolding may be further fostered through conformational changes that are triggered by ATP binding (49). This unfolding has been postulated to pull the client protein out of kinetically trapped misfolded state(s) (43). In addition, client release into the chamber may occur bit by bit. In the case of GroEL, bound segments with increased hydrophobicity have been reported to be released later than segments with less hydrophobicity (50, 51). This sequential release of the polypeptide chain may delay hydrophobic collapse within the client and hence also delay the formation of non-native hydrophobic interactions that lead to misfolding (50, 51). Although each ring of GroEL consists of seven identical subunits, TRiC is composed of eight non-identical subunits per ring, each of which exhibits client-binding sites with distinct sets of charged and hydrophilic residues that surround the central hydrophobic binding patch. This allows for the selective binding of distinct client segments and hence a defined orientation of the bound client protein, potentially mediating sequential folding upon release for some clients (39, 52, 53). Differences in ATP binding affinity of each of the eight TRiC monomers may lead to timely delayed conformational changes and hence may facilitate an ordered release of client segments into the chamber, again potentially avoiding misfolding of topologically complex client proteins (54, 55).

Once inside the chaperonin chamber, the client can potentially interact with the cavity's inner wall and/or fold. The interior lining of the cavity of both GroEL and TRiC are hydrophilic in the closed state. The closed GroEL cavity wall exhibits an overall negative net charge, whereas the interior wall of TRiC forms a gradient of positive to negative net charge from one side of the chamber to the other (41, 56). The conformational changes that lead to the closure of the GroEL cavity through GroES binding and the ejection of the client protein into the chamber bury most of the hydrophobic residues in the apical domain involved in initial client binding (57). In contrast, the conformational changes induced by cage closure of TRiC do still allow for client binding within the cavity (58), albeit with decreased affinity, thus allowing folding (59). Experimental evidence from electron microscopic and X-ray structures, as well as single molecule spectroscopy conducted with GroEL and TRiC in the closed state, suggests that interactions do occur between the chaperonin wall and folding intermediates of certain client proteins (51, 58, 60). Although not much is known about the interactions of the encapsulated client protein with the chaperonin wall, in some cases its charged nature is thought to drive the formation of a hydrophobic core and minimize the interaction of the encapsulated polypeptide chain with the cage wall (61).

About 10% of *Escherichia coli* and mammalian proteins have been reported to use chaperonins for folding (14, 62). Although there are no clear binding motifs, chaperonins' client proteins share some very broad overall structural similarities. For instance, many of them have complex topologies that are stabilized in the native structure by long-range contacts. As a result, many chaperonin substrates have rugged folding landscapes in which kinetically trapped folding intermediates and misfolded states are frequently populated (14, 63–65). However, whether or not client encapsulation generally affects the folding landscape of chaperone clients, and in doing so enhances folding rates, is not yet clear. Three models of chaperone action have been proposed for GroEL (to date, less is known about TRiC). In

the first model, GroEL may act passively by completely isolating the folding client protein and thereby preventing aggregation (66). This model is supported by data collected for a number of client proteins whose folding kinetics are similar or slower when encapsulated than when free in solution (67–70). The broad interactions of the client protein rhodanase with the interior wall of the chaperonin GroEL-ES complex, for instance, slows down the folding kinetics but does not substantially change the folding pathway (60, 71). The second model proposes that spatial confinement and electrostatic repulsion caused by the charge present on GroEL's interior surface may actually enhance client folding rates. Experimental evidence for such rate enhancements has been obtained for several proteins, for example TIM-barrel proteins, that populate entropically stabilized (*i.e.* flexible) intermediate states with higher probability (72). In these cases, spatial confinement may reduce the entropic penalty that is associated with folding by reducing the configurational entropy of the flexible intermediates. In addition, the flexible C termini of each GroEL subunit directed toward the inside of the chamber may entropically support the folding process via the transfer of entropy from the client to the disordered tail (61, 70, 72–75). The third model for GroEL facilitated protein folding proposes that iterative binding and release of the client protein at the apical domains may help facilitate folding by, ironically, unfolding proteins. In particular, this unfolding could pull client proteins out of enthalpically stable yet misfolded states. This mechanism has the advantage that it can also apply to proteins that are too large to fit entirely within the folding chamber (43, 58, 76).

The narrative surrounding chaperonin mechanisms, primarily through studies of GroEL in past years, has centered on the debate of which of the above three mechanisms is correct. Given the considerable number of observations supporting each mechanism, it is our opinion that it might be productive to reframe the narrative to accommodate the bulk of the available evidence. The weight of this evidence leads us to the conclusion that there is no single mechanism that can adequately explain how chaperonins act on a variety of client proteins. The differing observations made on the GroEL mechanism result from experiments performed under different conditions and with many different clients. As such, the evidence suggests that depending on the conditions and the client, GroEL can use various mechanisms.

Hsp70

Hsp70 is conserved both in prokaryotes and eukaryotes (77). It is a very versatile chaperone, involved not only in protein folding and refolding of a major part of the proteome but also in cellular trafficking, protein aggregate disassembly, and protein degradation (78). Multiple paralogues of Hsp70 are commonly found. Hsp70s function in conjunction with a set of Hsp40 co-chaperones, collectively called J-proteins, because they all contain a J-domain that is required for interaction with Hsp70 (78). Hsp40s exhibit somewhat distinct client specificities; thus, Hsp70's client affinity is in part fine-tuned by its co-chaperone (79). In addition, Hsp70s function with nucleotide exchange factors, which facilitate ADP-ATP exchange. The number of Hsp70 paralogues, J-proteins, and nucleotide exchange factors increases from prokaryotes to higher eukaryotes, reflecting the corresponding increased complexity of the proteome. Human cells, for example, have 11 Hsp70 homologues and 41 J-proteins (80).

Despite the apparent diversity of Hsp70s and co-factors, all Hsp70s are structurally conserved and follow essentially the same mechanistic principles. Hsp70s consist of two domains, an ATPase domain and a substrate-binding domain. The substrate-binding domain contains a β-sheet-rich N-terminal subdomain that recognizes 5–7 amino acid-long stretches of unfolded polypeptide backbone, although neighboring regions can extend the recognition site by several more amino acids (81). These Hsp70-binding motifs are found in most proteins and are usually buried in the hydrophobic core of natively folded proteins (78). However, they are exposed during protein synthesis and under denaturing conditions such as heat or oxidative damage, and therefore, they are an indicator of protein folding stress (78). Hsp70-binding motifs are enriched in hydrophobic residues but are often flanked by basic residues, indicating that both hydrophobic and electrostatic interactions are important for client binding (81). Hsp70's affinity for these binding motifs is regulated through the opening and closing motions of the α-helical lid (20, 78, 82), located C-terminal of the β-sheet subdomain. Its opening and closing are mediated through ATP binding and hydrolysis in the ATPase domain (20). Hsp70s are thought to undergo consecutive bind and release cycles with their client proteins, in which binding and release kinetics are custom tailored to the needs of the particular folding client protein through ATP and co-factors (78, 83). The unstructured C-terminal tail of the lid subdomain may function as an additional client-binding site to keep the client in close proximity, increasing the chance of client rebinding if necessary (84). Binding of Hsp70 is thought to keep the interacting domain of the client protein in a less structured state, thereby inhibiting misfolding and allowing the client protein to explore and form native-like secondary structure before a global hydrophobic collapse, either during protein synthesis or after stress-induced denaturation (78).

More recently, the direct impact of Hsp70 on protein folding has been assessed. NMR spectroscopy studies of Hsp70 with different single-domain clients suggested that these clients associated with Hsp70 exist in a conformationally heterogeneous, but primarily unfolded, ensemble (85, 86). At least in one case, this unfolded ensemble maintained some of the local structural propensities of the folded state, regardless of whether it was free in solution or bound to the chaperone (86). Further investigation suggested that the bacterial Hsp70 homologue, DnaK, specifically disrupted tertiary contacts while enabling local structure formation (87). Similarly, Hsp70s can unfold misfolded or even folded proteins through selectively binding to conformations that transiently expose hydrophobic binding motifs, shifting the folding equilibrium to more unfolded conformations and thereby remodeling the folding energy landscape (36, 78, 88). However, biophysical studies have also suggested that the lid subdomain and its flexible tail can adopt several different conformations to accommodate bulkier folded segments of proteins, allowing Hsp70 to bind not just unfolded polypeptide stretches but also folding intermediates and even

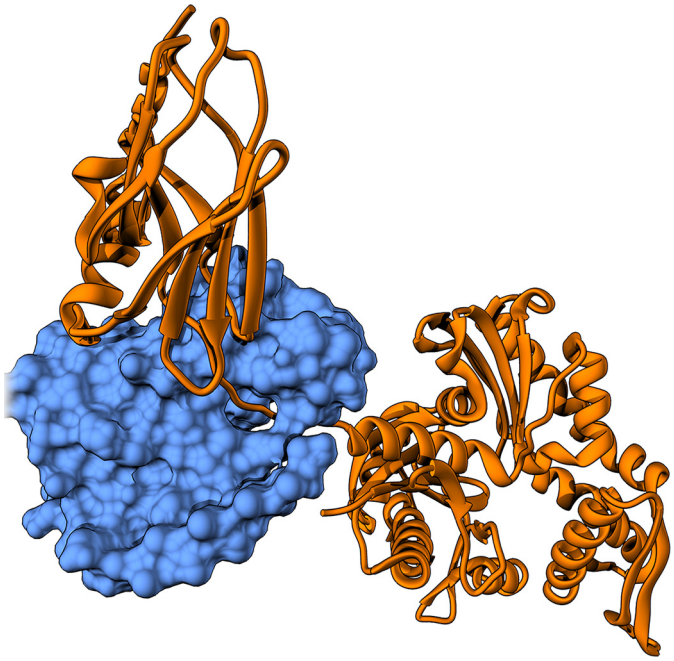

Figure 3. Crystal structure of Hsp70 dimerizing in client-binding mode. Two asymmetric units of DnaK are shown binding in chaperone configuration (*blue surface* of substrate-binding domain shown) and client configuration (*orange ribbons*) (91).

near-native conformations, potentially using other binding modes (83, 89, 90). One remarkable crystal structure even shows Hsp70 dimerizing by treating itself as a client protein, namely through binding the flexible hydrophobic linker between the ATPase domain and the substrate-binding domain, while the remainder of the protein remains folded (Fig. 3) (91). These studies suggest that Hsp70 could reshape the folding landscape both through binding to polypeptide stretches in extended conformations and by additionally binding to later stage folding intermediates in part through the α-helical lid.

Is the well-established model of Hsp70 function, in which it keeps proteins unfolded, mutually exclusive of models in which the chaperone binds to well-folded proteins? Similar to the studies of GroEL, these reports on Hsp70 function are with a variety of different client proteins, in differing conditions, and examined by methods that probe different aspects of the chaperone-client interaction. Although the number of studies finding that Hsp70 can bind to more folded clients is as of yet small, the current data suggest that like GroEL we should consider that Hsp70's effect on protein folding could be highly client-dependent (78).

Concluding comments

Understanding how chaperones work is an important question in biology. Given their function as guardians of protein folding and homeostasis, chaperones play roles in protein folding diseases such as Alzheimer's and in aging (15, 16). However, how protein folding is affected by chaperones is still a matter of debate. Although much is known about the structural, thermodynamic, and kinetic features of the sometimes complex conformational changes that drive the cycles of folding chaperones (18–20), so far less detailed information is available on the effects of chaperones on the folding energy landscape of their client proteins. In this minireview, we have explored different models of how chaperones can interact with proteins during folding to facilitate the folding process. Studies of Spy, Hsp60, and Hsp70 demonstrate that both electrostatic and hydrophobic surfaces are important for their ability to interact with their clientele and engage in protein folding, but despite intense study, the field still struggles to explain how these features of chaperones contribute to their function in a comprehensive manner.

Why has there been this continued difficulty in understanding the role of chaperones in the protein folding reaction? There are potentially many reasons. In our view, a common but understandable mistake is to use the same mental framework in thinking about chaperone action as has historically been used for enzymes, *i.e.* as performing some sort of reaction (in this case, protein folding) that is conserved and fundamental. This premise is seemingly supported by many chaperones having an ATPase activity that is coupled to conformational change. However, chaperones are much more than just ATPases. One primary difference, for instance, between an enzyme and a chaperone is the degree of specificity that is seen in substrate/client binding. Enzymes bind a particular substrate, most often in a single orientation that serves to re-organize the electrostatics/dynamics in one certain way to promote one precise reaction. For enzymes, the specificity and efficiency of these precise reactions have been honed through evolution. Chaperones, however, need to be promiscuous. They need to interact with many clients with different folding properties, and often they also need to interact with many different conformations of the same protein. This generates a distinctly different type of evolutionary pressure than that found for enzymes. Chaperones require non-specificity, whereas for enzymes, specificity is rewarded. For example, let us consider the evolutionary pressure to improve the folding of a specific protein. One result could be a mutation in a chaperone that causes it to fold that specific client more productively. Does that mutation mean that the chaperone will also fold other clients better? Our recent work with Spy suggested that this may be possible for at least a few clients by selecting for mutations that are common in evolution (28). However, a previous study with GroEL suggested that such an evolutionary pressure is often counter-balanced by a reduced overall chaperone fitness that occurs due to the increased specificity of variants selected to fold one specific protein better (92). As such, we consider it more likely that evolution pushes general chaperones to be multifunctional so that they can handle a wide variety of clients rather than acting by maximizing specificity and efficiency, as can be seen in enzyme evolution. Therefore, we propose treating chaperones as inherently multifunctional proteins at the level of basic biophysics rather than approaching their study with a more classic enzyme-based mentality. In this mindset, we think it is reasonable that principles observed for the folding of one client in the presence of a chaperone may or may not be applicable to other clients interacting with the same chaperone. Although we have here used Spy, Hsp60, and Hsp70 as examples to discuss this problem, it is likely that this issue pertains to most if not all general chaperones. The literature on how Hsp90 binds and

affects the folding of different clients is also subject to various interpretations (19, 93), perhaps in part for the same reasons as discussed here.

Further complicating matters, many chaperones cooperate in chaperone networks, in which a single client can be transferred between chaperones (11). As such, beyond the chaperones having multiple modes of action dependent on each client, these multiple modes can be increased further by stringing together the action of several chaperones that work cooperatively. As a hypothetical example, if the first and second chaperone to bind a client in its folding pathway each have four different possible mechanisms, this will yield a combination of 16 different combinations in which the two chaperones can affect the client, not counting any further modifications due to the chaperones directly modifying each other's action. Thus, even considering that chaperone mechanisms are client-dependent may still be an oversimplification, as interactions with other chaperones could further modify and diversify the effect on folding.

Contributing to this problem is that scientists are often encouraged to come to generalizable conclusions. This inclination may result in the oversimplification of the multifunctional nature of chaperones. Unlike enzymes, the inherent non-specificity and multifunctionality of chaperones may lead to heterogeneous results that may be difficult to interpret or reproduce. One potential way to increase reproducibility is to use conditions that bias the chaperone to using only one of its many possible operational modes. Using a restricted set of clients is one example of this sort of conditional bias. Although the observed function using one specific client may be valid and reproducible, it may only be able to capture a small part of the overall abilities of the chaperone.

To return to the enzyme corollary, despite a much longer history, it may come as a surprise to some that the underlying principles of how enzymes catalyze reactions are still under vigorous debate (94, 95). Regardless of this controversy, reading undergraduate biochemistry textbooks can lead to the mistaken assumption that the debate is over and that the different models discussed are all part of a "unified" theory. Perhaps finding a single unified mechanism for the action of even a single chaperone is an artificial goal. Instead, accepting the multifunctional nature of chaperones and attempting to classify the many different modes of action of chaperones may provide a more insightful direction.

Acknowledgments—We thank H. Saibil, J. Frydman, L. Joachimiak, and S. Hiller for providing structure files.

References

1. McConkey, E. H. (1982) Molecular evolution, intracellular organization, and the quinary structure of proteins. *Proc. Natl. Acad. Sci. U.S.A.* **79,** 3236–3240
2. Zimmerman, S. B., and Trach, S. O. (1991) Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of *Escherichia coli*. *J. Mol. Biol.* **222,** 599–620
3. DePristo M. A., Weinreich, D. M., and Hartl, D. L. (2005) Missense meanderings in sequence space: a biophysical view of protein evolution. *Nat. Rev. Genet.* **6,** 678–687
4. Gruebele, M. (2005) Downhill protein folding: Evolution meets physics. *C R Biol.* **328,** 701–712
5. Levy, E. D., De, S., and Teichmann, S. A. (2012) Cellular crowding imposes global constraints on the chemistry and evolution of proteomes. *Proc. Natl. Acad. Sci. U.S.A.* **109,** 20461–20466
6. Grahnen, J. A., Nandakumar, P., Kubelka, J., and Liberles, D. A. (2011) Biophysical and structural considerations for protein sequence evolution. *BMC Evol. Biol.* **11,** 361
7. Miller, S. B., Mogk, A., and Bukau, B. (2015) Spatially organized aggregation of misfolded proteins as cellular stress defense strategy. *J. Mol. Biol.* **427,** 1564–1574
8. Kim, Y. E., Hipp, M. S., Bracher, A., Hayer-Hartl, M., and Hartl, F. U. (2013) Molecular chaperone functions in protein folding and proteostasis. *Annu. Rev. Biochem.* **82,** 323–355
9. Quan, S., and Bardwell, J. C. (2012) Chaperone discovery. *BioEssays.* **34,** 973–981
10. Allen, W. J., Phan, G., and Waksman, G. (2009) Structural biology of periplasmic chaperones. *Adv. Protein Chem. Struct. Biol.* **78,** 51–97
11. Balchin, D., Hayer-Hartl, M., and Hartl, F. U. (2016) *In vivo* aspects of protein folding and quality control. *Science* **353,** aac4354
12. Goemans, C., Denoncin, K., and Collet, J. F. (2014) Folding mechanisms of periplasmic proteins. *Biochim. Biophys. Acta* **1843,** 1517–1528
13. Hartl, F. U. (1996) Molecular chaperones in cellular protein folding. *Nature* **381,** 571–579
14. Kerner, M. J., Naylor, D. J., Ishihama, Y., Maier, T., Chang, H. C., Stines, A. P., Georgopoulos, C., Frishman, D., Hayer-Hartl, M., Mann, M., and Hartl, F. U. (2005) Proteome-wide analysis of chaperonin-dependent protein folding in *Escherichia coli*. *Cell* **122,** 209–220
15. Hipp, M. S., Park, S. H., and Hartl, F. U. (2014) Proteostasis impairment in protein-misfolding and -aggregation diseases. *Trends Cell Biol.* **24,** 506–514
16. Labbadia, J., and Morimoto, R. I. (2015) The biology of proteostasis in aging and disease. *Annu. Rev. Biochem.* **84,** 435–464
17. Morawe, T., Hiebel, C., Kern, A., and Behl, C. (2012) Protein homeostasis, aging and Alzheimer's disease. *Mol. Neurobiol.* **46,** 41–54
18. Hayer-Hartl, M., Bracher, A., and Hartl, F. U. (2016) The GroEL-GroES chaperonin machine: a nano-cage for protein folding. *Trends Biochem. Sci.* **41,** 62–76
19. Mayer, M. P., and Le Breton, L. (2015) Hsp90: breaking the symmetry. *Mol. Cell* **58,** 8–20
20. Zhuravleva, A., and Gierasch, L. M. (2015) Substrate-binding domain conformational dynamics mediate Hsp70 allostery. *Proc. Natl. Acad. Sci. U.S.A.* **112,** E2865–E2873
21. Gruber, R., and Horovitz, A. (2016) Allosteric mechanisms in chaperonin machines. *Chem. Rev.* **116,** 6588–6606
22. Hagenmaier, S., Stierhof, Y., and Henning, U. L. F. (1997) A new periplasmic protein of *Escherichia coli* which is synthesized in spheroplasts but not in intact cells. *Microbiology* **179,** 2073–2076
23. Bury-Moné, S., Nomane, Y., Reymond, N., Barbet, R., Jacquet, E., Imbeaud, S., Jacq, A., and Bouloc, P. (2009) Global analysis of extracytoplasmic stress signaling in *Escherichia coli*. *PLoS Genet.* **5,** e1000651
24. Srivastava, S. K., Lambadi, P. R., Ghosh, T., Pathania, R., and Navani, N. K. (2014) Genetic regulation of spy gene expression in *Escherichia coli* in the presence of protein unfolding agent ethanol. *Gene* **548,** 142–148
25. Vogt, S. L., and Raivio, T. L. (2012) Just scratching the surface: an expanding view of the Cpx envelope stress response. *FEMS Microbiol. Lett.* **326,** 2–11
26. Fränzel, B., Penkova, M., Frese, C., Metzler-Nolte, N., and Andreas Wolters, D. (2012) *Escherichia coli* exhibits a membrane-related response to a small arginine- and tryptophan-rich antimicrobial peptide. *Proteomics* **12,** 2319–2330
27. Quan, S., Koldewey, P., Tapley, T., Kirsch, N., Ruane, K. M., Pfizenmaier, J., Shi, R., Hofmann, S., Foit, L., Ren, G., Jakob, U., Xu, Z., Cygler, M., and Bardwell, J. C. (2011) A genetic selection designed to stabilize proteins uncovers a chaperone called Spy. *Nat. Struct. Mol. Biol.* **18,** 262–269
28. Quan, S., Wang, L., Petrotchenko, E. V., Makepeace, K. A., Horowitz, S., Yang, J., Zhang, Y., Borchers, C. H., and Bardwell, J. C. (2014) Super Spy variants implicate flexibility in chaperone action. *Elife* **3,** e01584

29. Appia-Ayme, C., Hall, A., Patrick, E., Rajadurai, S., Clarke, T. A., and Rowley, G. (2012) ZraP is a periplasmic molecular chaperone and a repressor of the zinc-responsive two-component regulator ZraSR. *Biochem. J.* **442,** 85–93
30. Evans, M. L., Schmidt, J. C., Ilbert, M., Doyle, S. M., Quan, S., Bardwell, J. C., Jakob, U., Wickner, S., and Chapman, M. R. (2011) *E. coli* chaperones DnaK, Hsp33 and Spy inhibit bacterial functional amyloid assembly. *Prion* **5,** 323–334
31. Stull, F., Koldewey, P., Humes, J. R., Radford, S. E., and Bardwell, J. C. (2016) Substrate protein folds while it is bound to the ATP-independent chaperone Spy. *Nat. Struct. Mol. Biol.* **23,** 53–58
32. Koldewey, P., Stull, F., Horowitz, S., Martin, R., and Bardwell, J. C. (2016) Forces driving chaperone action. *Cell* **166,** 369–379
33. Horowitz, S., Salmon, L., Koldewey, P., Ahlstrom, L. S., Martin, R., Quan, S., Afonine, P. V., van den Bedem, H., Wang, L., Xu, Q., Trievel, R. C., Brooks, C. L., 3rd, and Bardwell, J. C. (2016) Visualizing chaperone-assisted protein folding. *Nat. Struct. Mol. Biol.* **23,** 691–697
34. He, L., Sharpe, T., Mazur, A., and Hiller, S. (2016) A molecular mechanism of chaperone-client recognition. *Sci. Adv.* **2,** e1601625
35. Salmon, L., Ahlstrom, L. S., Horowitz, S., Dickson, A., Brooks, C. L., 3rd, and Bardwell, J. C. (2016) Capturing a dynamic chaperone-substrate interaction using NMR-informed molecular modeling. *J. Am. Chem. Soc.* **138,** 9826–9839
36. Mattoo, R. U., and Goloubinoff, P. (2014) Molecular chaperones are nanomachines that catalytically unfold misfolded and alternatively folded proteins. *Cell. Mol. Life Sci.* **71,** 3311–3325
37. Jewett, A. I., and Shea, J. E. (2006) Folding on the chaperone: yield enhancement through loose binding. *J. Mol. Biol.* **363,** 945–957
38. Burmann, B. M., and Hiller, S. (2015) Chaperones and chaperone-substrate complexes: Dynamic playgrounds for NMR spectroscopists. *Prog. Nucl. Magn. Reson. Spectrosc.* **86,** 41–64
39. Joachimiak, L. A., Walzthoeni, T., Liu, C. W., Aebersold, R., and Frydman, J. (2014) The structural basis of substrate recognition by the eukaryotic chaperonin TRiC/CCT. *Cell* **159,** 1042–1055
40. Perrett, S., Zahn, R., Stenberg, G., and Fersht, A. R. (1997) Importance of electrostatic interactions in the rapid binding of polypeptides to GroEL. *J. Mol. Biol.* **269,** 892–901
41. Leitner, A., Joachimiak, L. A., Bracher, A., Mönkemeyer, L., Walzthoeni, T., Chen, B., Pechmann, S., Holmes, S., Cong, Y., Ma, B., Ludtke, S., Chiu, W., Hartl, F. U., Aebersold, R., and Frydman, J. (2012) The molecular architecture of the eukaryotic chaperonin TRiC/CCT. *Structure* **20,** 814–825
42. Lopez, T., Dalton, K., and Frydman, J. (2015) The mechanism and function of group II chaperonins. *J. Mol. Biol.* **427,** 2919–2930
43. Priya, S., Sharma, S. K., Sood, V., Mattoo, R. U., Finka, A., Azem, A., De Los Rios, P., and Goloubinoff, P. (2013) GroEL and CCT are catalytic unfoldases mediating out-of-cage polypeptide refolding without ATP. *Proc. Natl. Acad. Sci. U.S.A.* **110,** 7199–7204
44. Chaudhuri, T. K., Verma, V. K., and Maheshwari, A. (2009) GroEL assisted folding of large polypeptide substrates in *Escherichia coli*: present scenario and assignments for the future. *Prog. Biophys. Mol. Biol.* **99,** 42–50
45. Roh, S. H., Kasembeli, M., Galaz-Montoya, J. G., Trnka, M., Lau, W. C., Burlingame, A., Chiu, W., and Tweardy, D. J. (2016) Chaperonin TRiC/CCT modulates the folding and activity of leukemogenic fusion oncoprotein AML1-ETO. *J. Biol. Chem.* **291,** 4732–4741
46. Rüssmann, F., Stemp, M. J., Mönkemeyer, L., Etchells, S. A., Bracher, A., and Hartl, F. U. (2012) Folding of large multidomain proteins by partial encapsulation in the chaperonin TRiC/CCT. *Proc. Natl. Acad. Sci. U.S.A.* **109,** 21208–21215
47. Villebeck, L., Moparthi, S. B., Lindgren, M., Hammarström, P., and Jonsson, B. H. (2007) Domain-specific chaperone-induced expansion is required for β-actin folding: a comparison of β-actin conformations upon interactions with GroEL and tail-less complex polypeptide 1 ring complex (TRiC). *Biochemistry.* **46,** 12639–12647
48. Farr, G. W., Furtak, K., Rowland, M. B., Ranson, N. A., Saibil, H. R., Kirchhausen, T., and Horwich, A. L. (2000) Multivalent binding of nonnative substrate proteins by the chaperonin GroEL. *Cell* **100,** 561–573
49. Lin, Z., Madan, D., and Rye, H. S. (2008) GroEL stimulates protein folding through forced unfolding. *Nat. Struct. Mol. Biol.* **15,** 303–311
50. Motojima, F. (2015) How do chaperonins fold protein? *Biophysics* **11,** 93–102
51. Chen, D. H., Madan, D., Weaver, J., Lin, Z., Schröder, G. F., Chiu, W., and Rye, H. S. (2013) Visualizing GroEL/ES in the act of encapsulating a folding protein. *Cell* **153,** 1354–1365
52. Roh, S. H., Kasembeli, M. M., Galaz-Montoya, J. G., Chiu, W., and Tweardy, D. J. (2016) Chaperonin TRiC/CCT recognizes fusion oncoprotein AML1-ETO through subunit-specific interactions. *Biophys. J.* **110,** 2377–2385
53. Tam, S., Spiess, C., Auyeung, W., Joachimiak, L., Chen, B., Poirier, M. A., and Frydman, J. (2009) The chaperonin TRiC blocks a huntingtin sequence element that promotes the conformational switch to aggregation. *Nat. Struct. Mol. Biol.* **16,** 1279–1285
54. Reissmann, S., Joachimiak, L. A., Chen, B., Meyer, A. S., Nguyen, A., and Frydman, J. (2012) A gradient of ATP affinities generates an asymmetric power stroke driving the chaperonin TRIC/CCT folding cycle. *Cell Rep.* **2,** 866–877
55. Jiang, Y., Douglas, N. R., Conley, N. R., Miller, E. J., Frydman, J., and Moerner, W. E. (2011) Sensing cooperativity in ATP hydrolysis for single multisubunit enzymes in solution. *Proc. Natl. Acad. Sci. U.S.A.* **108,** 16962–16967
56. Chaudhry, C., Farr, G. W., Todd, M. J., Rye, H. S., Brunger, A. T., Adams, P. D., Horwich, A. L., and Sigler, P. B. (2003) Role of the γ-phosphate of ATP in triggering protein folding by GroEL $\pm$ GroES: function, structure and energetics. *EMBO J.* **22,** 4877–4887
57. Clare, D. K., Vasishtan, D., Stagg, S., Quispe, J., Farr, G. W., Topf, M., Horwich, A. L., and Saibil, H. R. (2012) ATP-triggered conformational changes delineate substrate-binding and -folding mechanics of the GroEL chaperonin. *Cell* **149,** 113–123
58. Dekker, C., Roe, S. M., McCormack, E. A., Beuron, F., Pearl, L. H., and Willison, K. R. (2011) The crystal structure of yeast CCT reveals intrinsic asymmetry of eukaryotic cytosolic chaperonins. *EMBO J.* **30,** 3078–3090
59. Douglas, N. R., Reissmann, S., Zhang, J., Chen, B., Jakana, J., Kumar, R., Chiu, W., and Frydman, J. (2011) Dual action of ATP hydrolysis couples lid closure to substrate release into the group II chaperonin chamber. *Cell* **144,** 240–252
60. Hofmann, H., Hillger, F., Pfeil, S. H., Hoffmann, A., Streich, D., Haenni, D., Nettels, D., Lipman, E. A., and Schuler, B. (2010) Single-molecule spectroscopy of protein folding in a chaperonin cage. *Proc. Natl. Acad. Sci. U.S.A.* **107,** 11793–11798
61. Gupta, A. J., Haldar, S., Miličić, G., Hartl, F. U., and Hayer-Hartl, M. (2014) Active cage mechanism of chaperonin-assisted protein folding demonstrated at single-molecule level. *J. Mol. Biol.* **426,** 2739–2754
62. Thulasiraman, V., Yang, C. F., and Frydman, J. (1999) *In vivo* newly translated polypeptides are sequestered in a protected folding environment. *EMBO J.* **18,** 85–95
63. Narayanan, A., Pullepu, D., and Kabir, M. A. (2016) The interactome of CCT complex–a computational analysis. *Comput. Biol. Chem.* **64,** 396–402
64. Yam, A. Y., Xia, Y., Lin, H.-T., Burlingame, A., Gerstein, M., and Frydman, J. (2008) Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. *Nat. Struct. Mol. Biol.* **15,** 1255–1262
65. Azia, A., Unger, R., and Horovitz, A. (2012) What distinguishes GroEL substrates from other *Escherichia coli* proteins? *FEBS J.* **279,** 543–550
66. Horwich, A. L., and Fenton, W. (2009) A Chaperonin-mediated protein folding: using a central cavity to kinetically assist polypeptide chain folding. *Q. Rev. Biophys.* **42,** 83–116
67. Horwich, A. L., Apetri, A. C., and Fenton, W. (2009) The GroEL/GroES cis cavity as a passive anti-aggregation device. *FEBS Lett.* **583,** 2654–2662
68. Apetri, A. C., and Horwich, A. L. (2008) Chaperonin chamber accelerates protein folding through passive action of preventing aggregation. *Proc. Natl. Acad. Sci. U.S.A.* **105,** 17351–17355
69. Tyagi, N. K., Fenton, W. A., Deniz, A. A., and Horwich, A. L. (2011) Double mutant MBP refolds at same rate in free solution as inside the GroEL/

GroES chaperonin chamber when aggregation in free solution is prevented. *FEBS Lett.* **585,** 1969–1972
70. Brinker, A., Pfeifer, G., Kerner, M. J., Naylor, D. J., Hartl, F. U., and Hayer-Hartl, M. (2001) Dual function of protein confinement in chaperonin-assisted protein folding. *Cell* **107,** 223–233
71. Sirur, A., and Best, R. B. (2013) Effects of interactions with the GroEL cavity on protein folding rates. *Biophys. J.* **104,** 1098–1106
72. Georgescauld, F., Popova, K., Gupta, A. J., Bracher, A., Engen, J. R., Hayer-Hartl, M., and Hartl, F. U. (2014) GroEL/ES chaperonin modulates the mechanism and accelerates the rate of TIM-barrel domain folding. *Cell* **157,** 922–934
73. Zhang, X., and Kelly, J. W. (2014) Chaperonins resculpt folding free energy landscapes to avoid kinetic traps and accelerate protein folding. *J. Mol. Biol.* **426,** 2736–2738
74. Chakraborty, K., Chatila, M., Sinha, J., Shi, Q., Poschner, B. C., Sikor, M., Jiang, G., Lamb, D. C., Hartl, F. U., and Hayer-Hartl, M. (2010) Chaperonin-catalyzed rescue of kinetically trapped states in protein folding. *Cell* **142,** 112–122
75. Weaver, J., and Rye, H. S. (2014) The C-terminal tails of the bacterial chaperonin GroEL stimulate protein folding by directly altering the conformation of a substrate protein. *J. Biol. Chem.* **289,** 23219–23232
76. Libich, D. S., Tugarinov, V., and Clore, G. M. (2015) Intrinsic unfoldase/foldase activity of the chaperonin GroEL directly demonstrated using multinuclear relaxation-based NMR. *Proc. Natl. Acad. Sci. U.S.A.* **112,** 8817–8823
77. Bardwell, J. C., and Craig, E. A. (1984) Major heat shock gene of *Drosophila* and the *Escherichia coli* heat-inducible dnaK gene are homologous. *Proc. Natl. Acad. Sci.* **81,** 848–852
78. Clerico, E. M., Tilitsky, J. M., Meng, W., and Gierasch, L. M. (2015) How Hsp70 molecular machines interact with their substrates to mediate diverse physiological functions. *J. Mol. Biol.* **427,** 1575–1588
79. Echtenkamp, F. J., and Freeman, B. C. (2012) Expanding the cellular molecular chaperone network through the ubiquitous cochaperones. *Biochim. Biophys. Acta* **1823,** 668–673
80. Kampinga, H. H., and Craig, E. A. (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat. Rev. Mol. Cell Biol.* **11,** 579–592
81. Rüdiger, S., Germeroth, L., Schneider-Mergener, J., and Bukau, B. (1997) Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries. *EMBO J.* **16,** 1501–1507
82. Mayer, M. P. (2013) Hsp70 chaperone dynamics and molecular mechanism. *Trends Biochem. Sci.* **38,** 507–514
83. Mashaghi, A., Bezrukavnikov, S., Minde, D. P., Wentink, A. S., Kityk, R., Zachmann-Brand, B., Mayer, M. P., Kramer, G., Bukau, B., and Tans, S. J. (2016) Alternative modes of client binding enable functional plasticity of Hsp70. *Nature* **539,** 448–451
84. Hu, S. M., and Wang, C. (1996) Involvement of the 10-kDa C-terminal fragment of hsc70 in complexing with unfolded protein. *Arch. Biochem. Biophys.* **332,** 163–169
85. Lee, J. H., Zhang, D., Hughes, C., Okuno, Y., Sekhar, A., and Cavagnero, S. (2015) Heterogeneous binding of the SH3 client protein to the DnaK molecular chaperone. *Proc. Natl. Acad. Sci.* **112,** E4206–E4215
86. Sekhar, A., Rosenzweig, R., Bouvignies, G., and Kay, L. E. (2015) Mapping the conformation of a client protein through the Hsp70 functional cycle. *Proc. Natl. Acad. Sci. U.S.A.* **112,** 10395–10400
87. Sekhar, A., Rosenzweig, R., Bouvignies, G., and Kay, L. E. (2016) Hsp70 biases the folding pathways of client proteins. *Proc. Natl. Acad. Sci. U.S.A.* 113, E2794–E2801
88. Marcinowski, M., Rosam, M., Seitz, C., Elferich, J., Behnke, J., Bello, C., Feige, M. J., Becker, C. F., Antes, I., and Buchner, J. (2013) Conformational selection in substrate recognition by Hsp70 chaperones. *J. Mol. Biol.* **425,** 466–474
89. Schlecht, R., Erbse, A. H., Bukau, B., and Mayer, M. P. (2011) Mechanics of Hsp70 chaperones enables differential interaction with client proteins. *Nat. Struct. Mol. Biol.* **18,** 345–351
90. Smock, R. G., Blackburn, M. E., and Gierasch, L. M. (2011) Conserved, disordered C terminus of DnaK enhances cellular survival upon stress and DnaK *in vitro* chaperone activity. *J. Biol. Chem.* **286,** 31821–31829
91. Chang, Y. W., Sun, Y. J., Wang, C., and Hsiao, C. D. (2008) Crystal structures of the 70-kDa heat shock proteins in domain disjoining conformation. *J. Biol. Chem.* **283,** 15502–15511
92. Wang, J. D., Herman, C., Tipton, K. A., Gross, C. A., and Weissman, J. S. (2002) Directed evolution of substrate-optimized GroEL/S chaperonins. *Cell* **111,** 1027–1039
93. Karagöz, G. E., and Rüdiger, S. G. (2015) Hsp90 interaction with clients. *Trends Biochem. Sci.* **40,** 117–125
94. Zhao, Q. (2017) On the indirect relationship between protein dynamics and enzyme activity. *Prog. Biophys. Mol. Biol.* **125,** 52–60
95. Warshel, A., and Bora, R. P. (2016) Perspective: defining and quantifying the role of dynamics in enzyme catalysis. *J. Chem. Phys.* **144,** 180901
96. Wang, J., and Chen, L. (2003) Domain motions in GroEL upon binding of an oligopeptide. *J. Mol. Biol.* **334,** 489–499
97. Clare, D. K., Bakkes, P. J., van Heerikhuizen, H., van der Vies, S. M, and Saibil, H. R. (2009) Chaperonin complex with a newly folded protein encapsulated in the folding chamber. *Nature* **457,** 107–110

Chaperone-client interactions: Non-specificity engenders multifunctionality

Philipp Koldewey, Scott Horowitz and James C. A. Bardwell

J. Biol. Chem. 2017, 292:12010-12017.
doi: 10.1074/jbc.R117.796862 originally published online June 15, 2017