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Protein-Protein Interactions (PPIs) in the Molecular Chaperone Network

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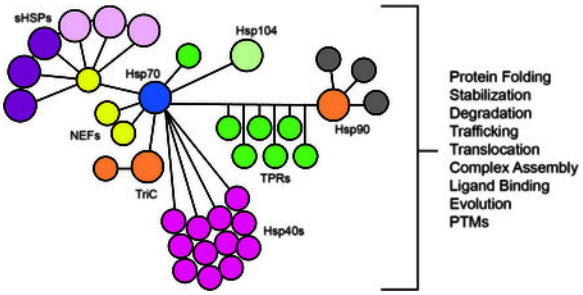
Graphical Abstract



Conspectus.

Molecular chaperones play a central role in protein homeostasis (aka proteostasis) by balancing protein folding, quality control and turnover. To perform these diverse tasks, chaperones need the malleability to bind nearly any “client” protein and the fidelity to detect when it is misfolded. Remarkably, these activities are carried out by only ~180 dedicated chaperones in humans. How do a relatively small number of chaperones maintain cellular and organismal proteostasis for an entire proteome? Further, once a chaperone binds a client, how does it “decide” what to do with it? One clue comes from observations that individual chaperones engage in protein-protein interactions (PPIs) – both with each other and with their clients. These physical links coordinate multiple chaperones into organized, functional complexes and facilitate the “hand off” of clients between them. PPIs also link chaperones and their clients to other cellular pathways, such as those that mediate trafficking (*e.g.*, cytoskeleton) and degradation (*e.g.*, proteasome). The PPIs of the chaperone network have a wide range of affinity values (nanomolar to micromolar) and involve many distinct types of domain modules, such as J domains, zinc fingers and tetratricopeptide repeats. Many of these motifs have the same binding surfaces on shared partners, such that members of one chaperone class often compete for the same interactions. Somehow, this collection of PPIs draws together chaperone families and creates multi-protein subnetworks that are able to

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make the “decisions” of protein quality control. The key to understanding chaperone-mediated proteostasis might be to understand how PPIs are regulated.

This Account will discuss the efforts of our group and others to map, measure and chemically perturb the PPIs within the molecular chaperone network. Structural biology methods, including X-ray crystallography, NMR and electron microscopy, have all played important roles in visualizing the chaperone PPIs. Guided by these efforts and –omics approaches to measuring PPIs, new advances in high throughput chemical screening, specially designed to account for the challenges of this system, have emerged. Indeed, chemical biology has played a particularly important role in this effort, as molecules that either promote or inhibit specific PPIs have proven to be invaluable research probes in cells and animals. In addition, these molecules have provided leads for the potential treatment of protein misfolding diseases. One of the major products of this research field has been the identification of putative PPI drug targets within the chaperone network, which might be used to change chaperone “decisions” and re-balance proteostasis.

1 Introduction to the Chaperone Network.

The largest class of molecular chaperones is the heat shock proteins[1], which are named for their apparent molecular mass (in kDa): Hsp90, Hsp70, Hsp60, Hsp40 and the small heat shock proteins (sHsps). The heat shock proteins are abundant and conserved through all kingdoms of life, suggesting that they are an ancient way of protecting proteomes. In their simplest form, the “job” of the chaperones is to bind clients and protect them from aggregation. Some chaperones, such as Hsp70, accomplish this task by interacting reversibly with exposed hydrophobic regions, limiting aberrant (*i.e.*, non-native) contacts. Beyond this simple anti-aggregation activity, groups of chaperones are able to carry out more sophisticated functions, such as folding or dis-aggregating proteins. These activities are typically powered by ATP hydrolysis, and they often require the coordinated efforts of multiple chaperones and cochaperones from different categories (*e.g.*, Hsp70s and Hsp40s) working together.

In this review, we focus on the PPIs between chaperones. A map of the PPIs between chaperones shows that all of the major categories are physically linked to each other, either directly or through intermediary, scaffolding factors (Figure 1). For example, PPIs link the sHsps, such as Hsp27 and Hsp22, to Hsp70 and Hsp90 through the scaffolding co-chaperones BAG3 and HOP/Sti1. Other chains of PPIs link classes of chaperones, such as prefoldin and TriC, to Hsp70 and Hsp40s. In prokaryotes and yeast, PPIs (dotted lines, Figure 1) provide additional connectivity. However, it is important to note that these PPIs are not all the same. They occur with a wide range of affinity values, from strong (e.g. Hsp70-BAG1, ~0.012 μM[2]; Hsp70-CHIP, ~0.5 μM[3]) to very weak (Hsp90-p23, ~1 μM[4]; Hsp70-Hsp40, ~110 μM[5]), and they involve contact surfaces that are either small or quite large (Hsp70-BAG1, >4000 Å^2)[6].

Our group, and many others, think that this PPI network architecture is critical to the ultimate functions of the chaperones[7]. It is known that some clients undergo “handoff” from one chaperone to another through these conduits, perhaps minimizing their exposure to

bystanders. For example, steroid hormone receptors (SHRs) are shuttled between Hsp70 and Hsp90 through the action of HOP[8] (see Figure 1). In other examples, replacing one chaperone for another has been shown to "switch" the overall function. For example, while Hsp70 and HOP are linked to folding of SHRs, a complex of Hsp70 and CHIP is linked to their degradation[8]. Together, these observations give the impression of a dynamic, interconnected web of chaperones that coordinate their functions and share molecular information to maintain proteostasis. Indeed, this concept has been mathematically modelled in the prokaryotic[9] and limited parts of the eukaryotic system[10], using inputs such as the affinity constants, protein concentrations, turnover and changes in nucleotide. This computational approach can even robustly replicate the complex folding characteristics seen in cells[11], showing the depth of knowledge in this system.

Because PPIs are so critical in dictating chaperone functionality, chemical probes that target particular interactions are extremely useful tools for understanding network function. Furthermore, it is possible that small molecules of this type could be more selective than compounds acting at conserved, active sites. The problem is that PPIs are challenging drug targets, as recently reviewed[12]. One needs high throughput and, ideally, the ability to monitor physical interactions. Chaperone networks have additional complication because many of the interactions are weak, limiting the types of discovery platforms that can be used. Towards that goal, our lab and others have used both direct and indirect high throughput screening (HTS) approach to identify small molecules that modulate chaperone PPIs. For example, we identified inhibitors of the Hsp70-BAG3 interaction using flow cytometry and isoelectric focusing capillary electrophoresis (ICE) to directly detect disruption of PPIs[13,14]. Another approach is screening against the enzymatic activity of a reconstituted multi-protein complex (RMPC), which we have used to identify inhibitors of Hsp70/J-protein/NEF interactions[15,16]. Another emerging method is differential scanning fluorimetry (DSF), which we used to identify molecules that stabilize native dimers of the small HSPs[17].

In the next sections, we briefly review the major classes of chaperones. We do not intend this discussion to be inclusive, because many reviews have covered the topics of chaperones[1], the stress response[18] and their roles in disease[19]. Rather, we focus here on the PPIs – the dynamic "glue" on which the chaperone network is assembled – and we highlight the important roles that small molecules have played.

2. Heat shock protein 70 (Hsp70).

Hsp70 is a good starting-point for a discussion of chaperone PPIs, as it makes a number of well-characterized contacts (Figure 2). Hsp70 has been shown to be critical for a wide range of activities, including protein folding, chaperone-mediated autophagy (CMA) and endocytosis. Because of its central role in delivering clients to so many different pathways, Hsp70 is often considered the "triage" chaperone. All members of the Hsp70 family are composed of a nucleotide-binding (NBD) domain, which has ATPase activity, and a substrate-binding domain (SBD) that makes contact with the client. In the ATP-bound state, Hsp70 binds weakly to its clients, while nucleotide hydrolysis slows the off-rate and increases affinity[20,21]. Through this deceptively simple system of ATP-driven "catch-and-

release", Hsp70 plays roles in folding, turnover, the assembly/disassembly of protein complexes, protein translocation and trafficking.

How can one factor be involved in so many disparate processes? By design, Hsp70 binds to clients weakly (typically micromolar) and with little sequence selectivity, making it a promiscuous chaperone. It is thought that PPIs with co-chaperones are the key to providing specificity and functionality[7,21]. For example, Hsp40s (also called J proteins) are adapters of the Hsp70 system that recruit it to specific clients; for example, auxilin recruits Hsp70 specifically to clathrin[22]. More broadly, Hsp70 binds to three major categories of co-chaperones: Hsp40s, nucleotide exchange factors (NEFs) and tetratricopeptide repeat (TPR) proteins (Figure 2). There are ~50 Hsp40/J protein genes in humans, and dozens of NEFs and TPR proteins. Moreover, each of the individual cochaperones brings its own functionality into the system, as outlined in the sections below. Thus, with the large number of Hsp70s and co-chaperones in the human genome, thousands of possible Hsp70-client-Hsp40-NEF-TPR combinations are possible.

Hsp40s/J Proteins.

The Hsp40/J proteins are a group of co-chaperones that contain a conserved J-domain[23]. J-domains make contact with Hsp70s via electrostatic interactions between the positively charged helix II of the J-domain and negatively charged region composed of lobes IA and IIA of the NBD, the inter-domain linker, and the β sandwich domain in the SBD (Figure 2B)[24–26] (Kityk et al Mol Cell 2018). This PPI surface is strikingly "spread out" and polar, as well as weak (K_D estimated ~ 10 μM), placing it in a category of PPIs that is notoriously difficult to inhibit[12]. Despite this challenge, a class of dihydropyrimidines takes advantage of a relatively deep groove between the IA and IIA lobes of Hsp70 to either disrupt or promote the J-domain contact[5]. Work by our group and others have advanced these molecules as chemical probes for a number of disease systems[27,28].

The PPI between Hsp70 and its Hsp40s/J proteins has a number of important consequences. Firstly, it stimulates Hsp70's ATPase activity[25]. The intrinsic rate of nucleotide turnover is slow, so this stimulatory activity is important for converting Hsp70 to the tight-binding conformer. Second, the Hsp40s/J proteins often include second domains that bind directly to clients and recruit them to the Hsp70 system[23]. In this role, some Hsp40s/J proteins are highly specialized; for example, auxillin (DnaJC6) recruits Hsp70 specifically to clathrin-coated vesicles[22]. However, other Hsp40s/J proteins are relatively promiscuous, recognizing a wider range of proteins[29]. Some of the Hsp40s/J proteins also have additional domains that link them to other chaperones within the network; for example, DnaJC7 also binds Hsp90[3]. Finally, many of these co-chaperones engage in PPIs with themselves as part of oligomers, which might be important for certain functions, such as disaggregation[30]. Together, these diverse PPIs make Hsp40s/J proteins central adapters of the network.

Nucleotide Exchange Factors (NEFs).

NEFs are co-chaperones that release ADP and clients from Hsp70[31]. There are four distinct structural categories of human NEFs, each of which uses a different binding mode. Although these categories of NEFs use different PPI interfaces, they all act to stabilize the "open"

form of Hsp70's NBD, accelerating release of ADP and client. The prokaryotic GrpE and its human ortholog, GrpEL1 or HMGE, accomplish this goal using a β-domain to interact with lobes IB and IIB[32]. Members of the BAG family use a 3-helix bundle to make hydrophobic and electrostatic contacts with a similar region[33]. Work from our group has indicated that some BAG family members, such as BAG1 and BAG3, make an additional, non-canonical interaction with Hsp70's SBD which is essential for release of clients[2]. Recent studies have confirmed this non-canonical interaction in other NEF systems too, such as Sil1[34], Fes1 and HspBP1[35]. Members of the Hsp110/Grp170 family use a completely different approach, making extensive contacts with lobe IIB of the NBD[36]. Finally, HspBP1/Sil1 family members use four armadillo-repeats to bind to lobe IIB, destabilizing the NBD[37]. The PPIs between Hsp70 and NEFs are relatively strong (K_D values in the low to mid nanomolar range), but the interaction surfaces have complex topology. Despite the complexity of these PPIs, inhibitors of Hsp70-NEF have been described. The best studied are the MKT-077 family of small molecules, which bind to a conserved allosteric site on Hsp70[38] and stabilize the ADP-bound state[39]. As discussed below, these molecules have been powerful probes for understanding the roles of Hsp70 and the NEFs in proteostasis.

In addition to their activity on Hsp70, some NEFs are multi-domain adapters that bind to proteins in other pathways. For example, Hsp110 binds to misfolded proteins, preferring aromatic residues and potentially expanding Hsp70's client pool[40]. Thus, NEFs are not only important for nucleotide cycling, but they also seem to be key in coordinating handoff to other chaperones and pathways.

Tetratricopeptide Repeat (TPR) Proteins.

Tetratricopeptide repeat domains are defined by three or more tandem TPR motifs that encode amphipathic helices[41,42]. There are hundreds of TPR domain-containing proteins expressed in humans, but a subset contains a conserved lysine and two asparagines, which is termed a "carboxylate clamp". These residues make hydrogen bonds with the extreme C-termini of the cytosolic Hsp70s and Hsp90s, in a shared motif defined by the amino acids EEVDCOOH[43]. While most of the binding energy for this interaction seems to come from the interaction of the EEVD with the carboxylate clamp[44], residues preceding the EEVD sequence have been shown to contribute to specificity; for example, FKBP51 and FKBP52 prefer Hsp90's MEEVD, while CHIP and DnaJC7 prefer Hsp70's IEEVD[3]. These differences provide a hierarchy of Kd values from ~10 μM to less than 1 μM. Because of the polar nature of the EEVD-TPR contact, few molecules have been reported to inhibit this PPI.

The defining feature of the TPR containing co-chaperones is that they bring specific enzymatic functions into the chaperone complexes[45]. For example, CHIP is an E3 ubiquitin ligase that acts on chaperone-bound clients, while PP5 is a protein phosphatase and FKBP51/52 are peptidyl prolyl isomerases. Thus, the identity of the bound TPR co-chaperone often seems to determine what ultimately happens to the chaperone's client.

Putting It All Together.

How do Hsp70 and its co-chaperones come together to make “decisions” about client fate? Our group has taken a chemical biology approach to this question, using small molecules to perturb individual PPIs and asking how client fate is impacted in cells and tissues. Recent reviews have described these chemical probes and their mechanisms in more detail[46,47]. Here, we focus on analogs of MKT-077, such as JG-48, which have been shown to inhibit the PPI between Hsp70-NEF[39]. Because NEFs normally promote the release of clients, JG-48 significantly stabilizes the Hsp70-client complex, as shown *in vitro* and in cells. What is the consequence of this? Strikingly, a number of groups have found that JG-48 and its analogs promote the turnover of multiple Hsp70 clients, including tau[48], polyglutamine proteins[49], Dengue viral proteins[50] and oncoproteins[51]. Thus, it seems that Hsp70 and its NEFs may normally monitor the dwell time of clients and promote their degradation if they remain “too long”[52]. Chemical inhibitors promote turnover by limiting the ability of NEFs to release client, which could be a favorable activity in models of neurodegeneration and cancer.

Other Hsp70 Interactions in Prokaryotes.

As mentioned above, Hsp70 cochaperones, such as BAG3 and HOP, play an important “adapter” role in eukaryotes. However, many of these adaptors do not exist in prokaryotes, and there is some evidence that, in those systems, the major prokaryotic chaperones coordinate directly in their absence. For example, prokaryotic DnaK/Hsp70 directly interacts with prokaryotic Hsp90 (termed HtpG) and ClpB[53,54]. Some of these direct interactions are also present in yeast orthologs, such as Hsp70 binding to Hsp104[55]. These examples suggest that PPIs have always been used to build chaperone networks and that evolution has only further elaborated that scheme.

2. Hsp90.

Hsp90 is another major “hub” of the chaperone network, interacting with a large number of co-chaperones and clients[56]. Based on pioneering work in SHRs, Hsp90 is thought to recognize clients in later stages of folding, functioning in their stability and activation[57]. Hsp90 has three domains: an amino-terminal domain (NTD) that is responsible for binding to ATP[58], a middle domain (MD) that is required for ATPase activity[59], a carboxy-terminal domain (CTD) that is responsible for homodimerization[60] and terminates in the MEEVD motif that binds TPR co-chaperones[42]. The Hsp90 homodimer undergoes dramatic conformational rearrangements over the course of its ATPase cycle, in which binding of ATP promotes a closed state where the NTDs dimerize, allowing for ATP hydrolysis. A number of inhibitors, most notably geldanamycin and radicicol, have been found to compete for binding with ATP, thus inhibiting Hsp90 ATPase activity[61,62]. There have been reports of inhibitors, such as novobiocin, that bind to the CTD, potentially acting by interfering with allostery or dimerization[63].

Hsp90 Co-Chaperones.

Hsp90 interacts with a number of co-chaperones that tune its ATPase activity and control its association with clients (Figure 3)[64]. The co-chaperone, Aha1, interacts tightly (Kd ~0.7

μM) through hydrophobic contacts with the first alpha-beta-alpha domain of the MD, as well as more widely distributed polar interactions and, by NMR, contact with the NTD[65]. Binding of Aha1 causes a conformational change in the catalytic loop of Hsp90, displacing HOP and releasing Arg 380 to access the ATP binding site in the NTD[66]. Another co-chaperone, p23, binds (Kd ~ 1 μM) as a dimer between the NTDs of two Hsp90s, inhibiting ATPase activity by stabilizing the ATP-bound, closed state[4]. Additional contacts are made between p23 and the inter-domain junction (where the MD of one protomer interacts with the NTD of the other protomer). Because this site is shared by Aha1, binding of p23 and Aha1 are mutually exclusive. Moreover, geldanamycin limits association of Hsp90 with p23[67], likely through an allosteric mechanism. Finally, the co-chaperone, Cdc37, interacts with both the NTD and MD[68]. The major feature of Cdc37 is that it is required for maturation of kinases. Indeed, the structure of a kinase/Cdc37/Hsp90 complex reveals that this interaction occurs in the closed state of Hsp90, in which Cdc37 mimics interactions between p23 and the NTD while making additional, polar contacts with the MD. This interaction partially inhibits ATPase activity by limiting lid closure. Celastrol has been reported to disrupt the interaction of Cdc37 and Hsp90 by binding to the CTD[69], although its selectivity remains uncertain.

3. Small Heat Shock Proteins.

The small heat shock proteins (sHsps) are a class of ~10 non-enzymatic chaperones that bind partially unfolded client proteins to maintain their solubility[70]. All sHsps contain a highly conserved α-crystallin domain (ACD) flanked by variable N- and C-termini (Figure 4A). A key feature is that these monomers assemble, using a series of PPIs described below, into large, polydisperse oligomers with sizes ranging from dimers to over 40 subunits[71]. Some studies suggest that smaller oligomers might be more potent chaperones, such that changes in oligomer size may be functionally important[72]. Thus, like other chaperone families, the biology of sHsps seems to be driven by dynamic PPIs, both with each other and with their clients.

Oligomeric Interactions of the sHsps.

The simplest unit of the sHsps is the dimer, which is driven by the interaction of two ACDs. Each ACD is a highly conserved β-sandwich and two of them come together into a compact structure via anti-parallel β-sheets. Although similar in overall architecture, the dimer interfaces amongst the human sHsps are not identical (Figure 4B). For example, the interface of αB crystallin's ACD dimer has many salt bridges that can act as a pH sensor[73], while that of Hsp27 contains a cysteine bridge that forms a redox sensor[74]. These observations support the idea that chaperone function is linked to regulation of this PPI. In addition, the ACD interface is the one PPI in this system that has a known chemical probe; our group discovered a class of oxysterols that stabilize this contact, with the lead molecule, C29, making contacts with both sides of the anti-parallel b-sheet[17]. Because mutations of this interface can lead to hereditary cataracts, C29 stabilizes the folding of these proteins and it is currently in pre-clinical development as a non-surgical cataract treatment.

Other PPIs within sHsp oligomers are mediated by the highly variable and flexible C-terminal extensions (CTE). Specifically, some sHsps contain a conserved IXI (or IPV) motif within their CTEs. This motif binds to a groove formed by the β4/β8 strands on the edge of the ACD[75,76]. Thus, within an oligomer, the IXI/V motif of one sHsp is proposed to "reach back" and take part in a PPI with the ACD of another sHsp (Figure 4C), stabilizing large oligomers.

The final PPI involves the N-terminal domain (NTD). Because the NTD is the least conserved region and is highly flexible, this PPI is difficult to study and remains the most enigmatic. NTDs within an oligomer seem to make contact with other NTDs (Figure 4D), though it is possible that they interact transiently with other domains as well, including the ACD (Figure 4E). Regardless, it is clear that the NTD PPIs are important for oligomer formation based on deletion studies[77]. Moreover, the NTDs of different sHsps seem to encode different oligomeric sizes and polydispersity, as transposition of the NTD from Hsp27 onto MjHsp16.5 can change the MjHsp16.5 oligomer to become more "Hsp27-like"[78].

Hetero-oligomerization of sHsps.

The sHsps are known to form hetero-oligomers. This phenomenon has been extensively observed[79], most famously in lens α-crystallin hetero-oligomers that consists of αA and αB crystallin[80]. The functions of hetero-oligomers are not always clear but their properties seem to be distinct from that of homo-oligomers[80]. Hetero-oligomers are presumably held together by the same conserved PPIs, involving the IXI/V motifs and ACDs. However, the role of the NTD is less clear because it either promotes or disfavors hetero-oligomerization through unknown mechanisms[81].

Client Interactions with sHsps.

How sHsps bind clients is not well understood, but an emerging model suggests that multiple domains are involved. Cross-linking studies in plant sHsps suggest that the NTD is the preferred client-binding site, with other regions involved in secondary interactions[82]. However, the ACD of αB crystallin, lacking an NTD, is sufficient to prevent aggregation of certain amyloid clients[83] and NMR studies indicate that the β4/β8 groove is an important binding site[84]. Thus, interactions outside the NTD might be critical for some systems. That being said, deletion of the NTD is sufficient to ablate chaperone activity for lysozyme[84]. For still other categories of clients, both the NTD and ACD have been shown to be important for chaperone activity[85]. Taken together, these data suggest that client binding can occur in multiple sites, depending on the type of client and the sHsp oligomeric state.

Connections with Other Chaperones:

Because sHsps lack enzymatic activity, they must coordinate with other chaperone families to engage in complex functions. For example, sHsps are thought to create a reservoir of partially unfolded clients that are then refolded by the Hsp70/40 system[86] and Hsp100/ClpB system[87]. In humans, BAG3 seems to be the scaffolding factor that connects the sHsps with Hsp70 via its BAG domain and IPV motifs[88]. These PPIs are especially important in autophagy[89], where the BAG3/HspB8/Hsp70 complex has been shown to be required.

Conclusions.

Protein-protein interactions are the “glue” that holds together the chaperone network (see Fig 1). However, this glue is not static; rather, dynamic changes in PPIs seem to accompany the major “decisions” of proteostasis. The dynamic nature of the system is by design, enabling the chaperone network to rapidly adapt to a number of different stresses by altering the equilibria of PPIs. For example, sudden high concentrations of a client protein can favor chaperone binding to client while disfavoring particular co-chaperone-chaperone complexes. Because of the integration of the full chaperone network through PPIs, one change in a chaperone complex might reverberate through the entire network. While fascinating, this fluidity can make it challenging to study chaperone networks in a cellular context. In the next sections, we focus conclusions on the next-generation areas that we feel deserve further development.

Linking PPI Observations Across Scales.—Although we have focused primarily on structural examinations of individual PPIs in this Account, mass spectrometry has recently been used to globally profile PPIs within cells[90]. This approach is yielding unprecedented insight into the broader interactions of chaperones, co-chaperones and clients. At the same time, advances in crystallography, NMR and cryo-EM have started to provide structures of larger and larger complexes, such as Hsp104-client[91]. These advances underscore the importance of understanding affinities of specific chaperone complexes *in vitro* and then bridging those observations across larger scales: from biochemical to cellular to organismal. Integration of this knowledge will require biophysics, mathematical modelling, biochemistry, functional genomics and other disciplines. In addition, it seems that chemical biology may play a particularly important role, because of its ability to produce molecules that operate at each scale.

Cellular Context.—While the core structure of the chaperone network seems to be maintained in different cell types and even cellular compartments, the specific cochaperones can vary significantly and the expression levels are varied. Recent work has identified a distinct chaperone complex (dubbed the epichaperome) present in many cancer cells that is absent in normal cells and other cancer lines[92], suggesting that differences in the chaperone networks can contribute to disease. Furthermore, the makeup of the chaperone network might provide a therapeutic window for targeting specific cellular types, such as cancer cells.

Chemical Biology to Drug Discovery.—Together, converging revolutions in structural biology, proteomics and other fields are creating new opportunities for chemical biology, as each of the newly characterized PPIs is a potential, new site for perturbation. We suggest that drug-like molecules targeting these PPIs will continue to be invaluable tools for understanding chaperone networks, particularly because fast chemical perturbation of these highly dynamic interactions is critically important in understanding the overall network. Moreover, such molecules might be starting points for developing next-generation therapeutics. To this end, it will be important that HTS methods and medicinal chemistry campaigns retain drug-like criteria in the selection schemes, so that these important advances can be used to go beyond chemical probes and into clinical candidates. Recent

successes in targeting PPIs in the clinic should embolden this effort and inform the specific challenges ahead.

Biographical Information

Rebecca Freilich received her B.S. in Chemistry from New York University. Her graduate work involves protein-protein interactions of the small heat shock proteins.

Taylor Arhar earned a B.S. from Loyola Marymount University. Her thesis work focuses on characterizing interactions between co-chaperones and client proteins.

Jennifer L. Abrams earned a Ph.D. from the UT Health Sciences Center in Houston. She is using genetic screens to map chaperone-client interactions.

Jason E. Gestwicki received a Ph.D. from the University of Wisconsin, Madison and completed postdoctoral studies at Stanford University. He is currently a Professor in the Department of Pharmaceutical Chemistry at the University of California, San Francisco, where his group studies protein homeostasis in disease.

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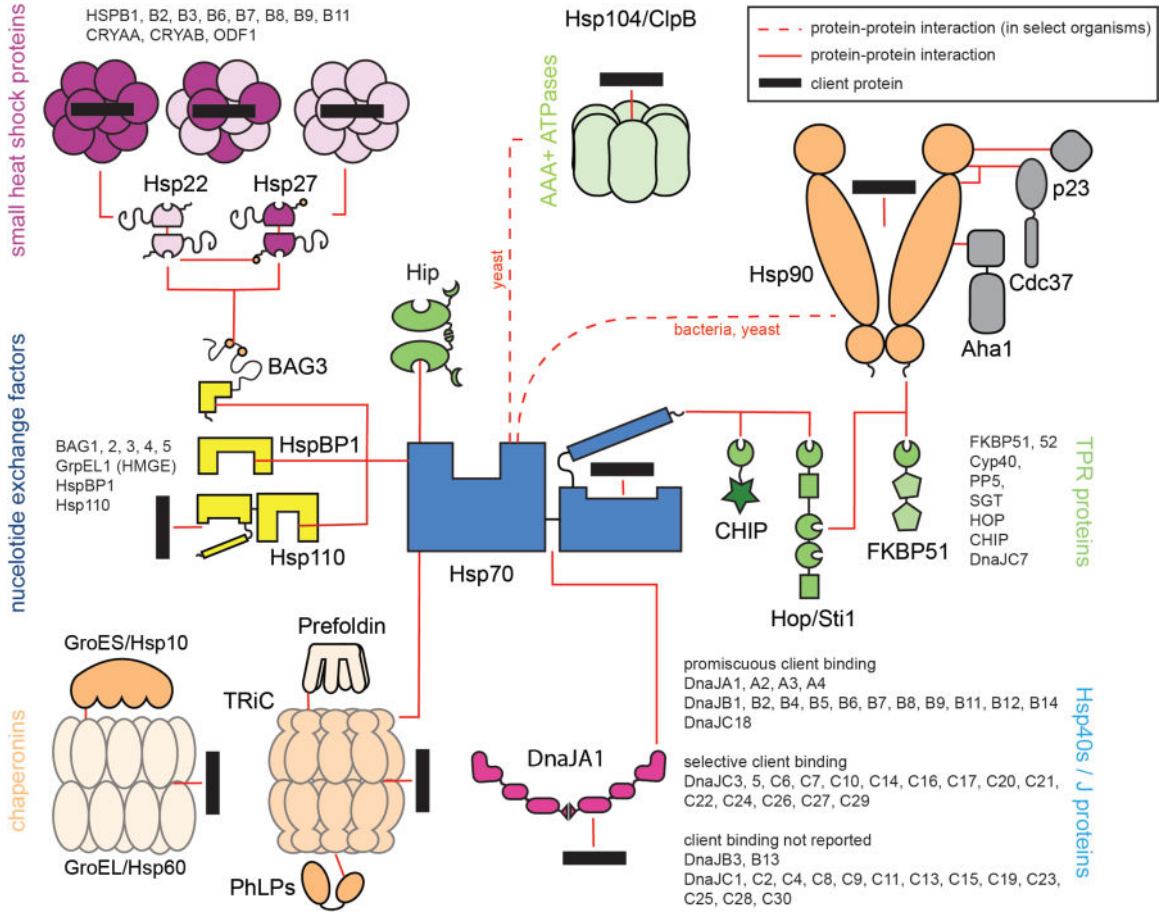
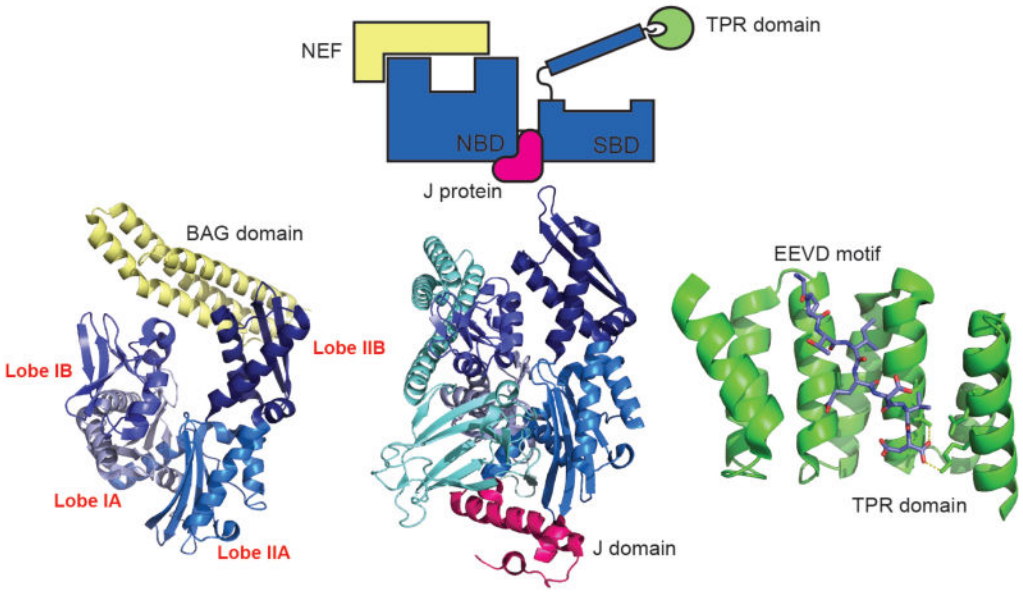


Figure 1. The physical interactions of the major chaperone families.

PPIs are shown with red lines and the approximate surfaces indicated on the cartoons. Dotted lines indicated interactions that lack a high-resolution structure.

A. Hsp70 interacts with major categories of co-chaperones through a series of PPIs



B. Reported structures of Hsp70 PPIs with co-chaperones

PDB ID [ref]	Organism	Co-chaperone type	Co-chaperone	Regions of Hsp70 contact
2QWN	Bovine	J protein	Auxilin	NBD, lobes IA and IIA
5NRO	E. Coli	J protein	DnaJ	NBD lobe IIA, interdomain linker, SBDβ
3D2F	Human	NEF	Sse1 (Hsp110)	NBD, lobes IA, IB, and IIB
1DKG	E. Coli	NEF	GrpE	NBD, lobes IA, IB, and IIB
1HX1	Human	NEF	Bag1	NBD, lobes IB and IIB
3CQX	Human	NEF	Bag2	NBD, lobes IB and IIB
38AY	Human	NEF	Bag5	NBD, lobes IB and IIB
1XQS	Human	NEF	HspBP1	NBD, lobe IIB
1ELW	Human	TPR	HOP	EEVD
4KBQ	Human	TPR	CHIP	EEVD
4J8F	Human	TPR	HIP	NBD, lobes IA, IB, and IIB

Figure 2. Hsp70's interaction with co-chaperones.

A, Categories of co-chaperone PPIs in the Hsp70 sub-network, highlighting the different regions of Hsp70 that are involved. B. Structures of Hsp70 complexes.

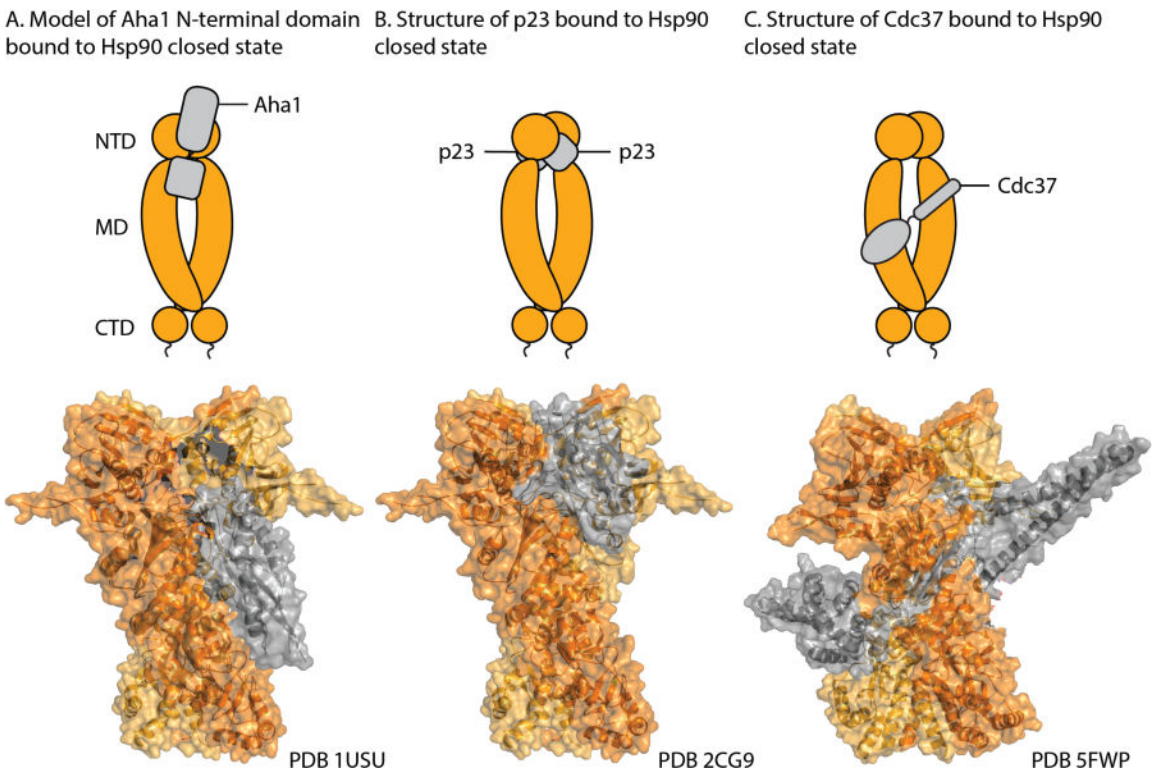


Figure 3. PPIs with Hsp90.

A) The interaction between Aha1 and Hsp90 is modeled by alignment of the co-crystal structure of the N-terminal domain of Aha1 and Hsp90 MD with the structure of full-length Hsp90 in the closed state (PDB 2CG9). The C-terminal domain of Aha1 (not shown) has been reported to interact with the Hsp90 NTD. B) Two p23 molecules bind to the Hsp90 dimer, each binding between the Hsp90 NTDs. C) Cdc37 wraps around Hsp90, splitting into two domains connected by a beta strand that packs against the Hsp90 MD.

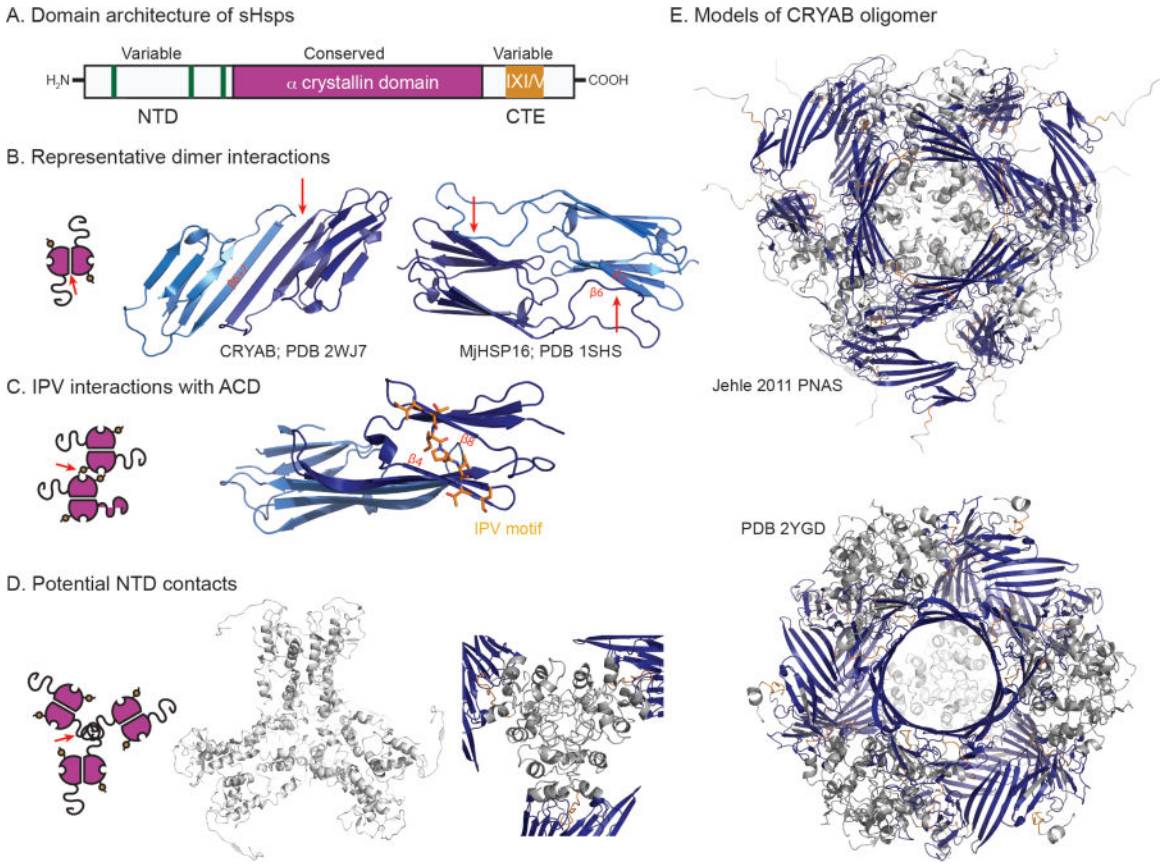


Figure 4. PPIs of the sHsps.

A, Domain architecture of sHsps. B, Examples of dimer interfaces: αB crystallin (PDB: 2WJ7) and MjHSP16 (PDB: 1SHS). Key contacts are shown (red arrows). C, Structure of an IPV peptide bound to Hsp27 β4/β8 groove (PDB: 4MJH). D, Potential NTD contacts based on EM models. E, Models of αB crystallin oligomers from electron microscopy.