Review

CellPress

Hsp90 interaction with clients

G. Elif Karagöz[1] and Stefan G.D. Rüdiger[2]

[1] Howard Hughes Medical Institute and Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94158, USA
[2] Cellular Protein Chemistry, Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

The conserved Hsp90 chaperone is an ATP-controlled machine that assists the folding and controls the stability of select proteins. Emerging data explain how Hsp90 achieves client specificity and its role in the cellular chaperone cascade. Interestingly, Hsp90 has an extended substrate binding interface that crosses domain boundaries, exhibiting specificity for proteins with hydrophobic residues spread over a large area regardless of whether they are disordered, partly folded, or even folded. This specificity principle ensures that clients preferentially bind to Hsp70 early on in the folding path, but downstream folding intermediates bind Hsp90. Discussed here, the emerging model is that the Hsp90 ATPase does not modulate client affinity but instead controls substrate influx from Hsp70.

Hsp90 as a major chaperone: what does it do, and how?

Protein folding is a fundamental process that is essential for life. Proteins fold by embarking on folding pathways in which the protein adopts a 3D structure by nucleating around a hydrophobic core [1]. In the cell, this vital process is guarded by the proteostasis network, which controls protein fate at all stages and thereby prevents toxic side reactions (see Glossary) [2–4]. Of particular importance is the shielding of hydrophobic residues, which are temporally exposed during initial folding but also upon damaging of existing proteins [2–4]. Uncontrolled exposure of hydrophobic stretches leads to protein aggregation and has fatal consequences. Therefore, the proteostasis network controls protein fate at any time, which includes supporting initial protein folding, repairing damaged proteins, and initiating degradation on demand. Major players in the proteostasis network are conserved families of molecular chaperones [2–4]. Key chaperones in the cytoplasm are the ATP-driven Hsp70 and Hsp90 chaperone families [5–8]. Originally their genes were discovered to be upregulated upon heat stress, thus they were named heat shock proteins (Hsps) [9]. By now, however, we know that most family members are rather constitutively expressed and involved in maintaining proteostasis at any time.

Molecular chaperone families differ in architecture and mechanism, but also in their substrate pool [5–7]. There is no obligate, stringently required order of chaperone action that would be essential for all proteins, and in particular small single-domain proteins may fold without any chaperone assistance. However, specificity defines some order in chaperone action. Hsp70s recognise short and highly hydrophobic stretches, which often are integral components of the hydrophobic core of the protein [10,11]. Hsp70s thus act early on the folding path, and they have the potential to interact with most proteins in the cell when they are unfolded. Hsp90 typically interacts with intermediates at later folding stages than Hsp70 [8]. Proteins requiring Hsp90 assistance are a select pool of proteins, highly enriched in signalling proteins or factors destabilised in protein folding-related diseases [8,12]. These Hsp90 substrates are often referred,

Glossary

Aha1: the Hsp90 co-chaperone that stimulates the ATP hydrolysis rate. Aha1 binds to Hsp90-N and Hsp90-M and competes with p23.
Cdc37: a kinase-specific substrate targeting factor of Hsp90. The human homologue is known as p50.
Cdk4: cyclin-dependent kinase 4 requires Hsp90 to reach the active state, like many other kinases.
CHIP: an E3 ubiquitin ligase, specifically interacts with both Hsp70 and Hsp90. It specifically targets the TPR motifs at the C terminus of the chaperone by its TPR domain and ubiquitylates chaperone-bound clients.
Client: Hsp90 substrates are also known as clients. In this review, we use both terms synonymously.
EM: single-particle electron microscopy provides structural information on protein complexes by constructing 3D models of biomolecules from 2D electron micrographs. In cryo-EM, particles are studied at cryogenic temperatures in their native states, whereas negative staining techniques involve the use of heavy metal salts, which interact with the electron beam and produce phase contrast.
Hsp90: 'Hsp' stands for heat shock protein, '90' for an apparent molecular weight of 90 kDa. The predominant homologue in the human cytosol is the constitutive Hsp90β, while Hsp90α is heat inducible. Thus, Hsp90β is not a heat shock protein, and it is 83 kDa.
NMR: nuclear magnetic resonance provides a dynamic picture of molecules and protein complexes in solution, based on signals of nuclei that have a particular label.
p23: the Hsp90 co-chaperone that slows down ATP hydrolysis. p23 binds to both N-terminal domains and Hsp90-M and competes with Aha1. p23 binding induces N-terminal dimerisation of Hsp90.
Proteostasis network: 'proteostasis' is an acronym for protein homeostasis. It involves all maintenance processes from protein synthesis to protein degradation. In particular, molecular chaperones and proteases control proteostasis; together they form the proteostasis network.
SAXS: small angle X-ray scattering, a solution method that provides a distance distribution curve between the atoms of a molecule. The distance distribution allows one to calculate structural models in solutions if further data are available, e.g., from crystallographic or NMR studies.
TPR motif: a tetratricopeptide motif is a specific recognition site at the C terminus of a protein. Recognition typically involves the carboxy group of the last residue, thus several TPR binding proteins need to compete for the same site.

Corresponding author: Rüdiger, S.G.D. (s.g.d.rudiger@uu.nl).
Keywords: molecular chaperones; protein folding; heat shock proteins; protein–protein interactions; Alzheimer disease; intrinsically disordered proteins.

0968-0004/
 http://dx.doi.org/10.1016/j.tibs.2014.12.002

somewhat anthropomorphically, as 'clients' [12]. The function of Hsp90 in the folding path of its clients, however, remained largely elusive because it was unclear how Hsp90 recognised its clients [13]. Recent progress now offers answers.

Here, we provide a synthesis of the concepts of Hsp90-mediated chaperoning based on the recent advances in understanding how it recognises clients. We describe the nature of the Hsp90 substrate binding site, discuss regulation of Hsp90–substrate interaction, and walk step-by-step along the Hsp90 interaction path with client proteins.

The nature of the Hsp90 substrate binding site

The current understanding of the nature of the Hsp90 substrate binding site is based on our structural understanding of Hsp90 as obtained by a range of biophysical techniques, and interaction studies with a range of natural clients, in particular kinases, steroid receptors, and the disordered protein Tau. We will elucidate the shape of the Hsp90 dimer and compare the regions to which the various studies mapped substrate binding.

Hsp90 is a challenging target for structural analysis

The Hsp90 family is conserved from bacteria to man, implying that it is part of a fundamental process in biology [12]. Hsp90 chaperones consist of three domains, the N-terminal, middle, and C-terminal domains (Hsp90-N, -M, and -C) (Figure 1A). A four-helix bundle in Hsp90-C ensures that Hsp90 is a homo-dimer under physiological conditions [14]. The domain interfaces between Hsp90-N and Hsp90-M and between Hsp90-M and Hsp90-C are dynamic, resulting in an ensemble of conformations in which the tips of the N-terminal domains of the Hsp90 dimer span an approximately 250-Å range according to small angle X-ray scattering (SAXS) experiments (Figure 1B) [14–16]. Remarkably, this elongated surface does not have any pocket suitable to enclose protein substrates. Because Hsp90 lacked an obvious location for client binding, controversial discussions around the mechanism of Hsp90 function raged for years [13].

The difficulty in identifying the client binding site is linked to the technically challenging nature of the Hsp90 system for structural biology methods. Earlier structural work with isolated domains proposed substrate binding sites in each domain, but a decisive structural picture remained elusive [14,17,18]. When finally full-length structures of Hsp90 homologues appeared, they did not show an obvious binding site as they lacked a bound client [16,19–21]. Crystal structures of Hsp90–substrate complexes do not exist to date, as the dynamic nature of such a complex has so far frustrated any crystallisation attempts. Hsp90, however, is also a challenging object to study by other structural biology methods; the size of Hsp90–substrate complexes (around 200 kDa) makes them large for nuclear

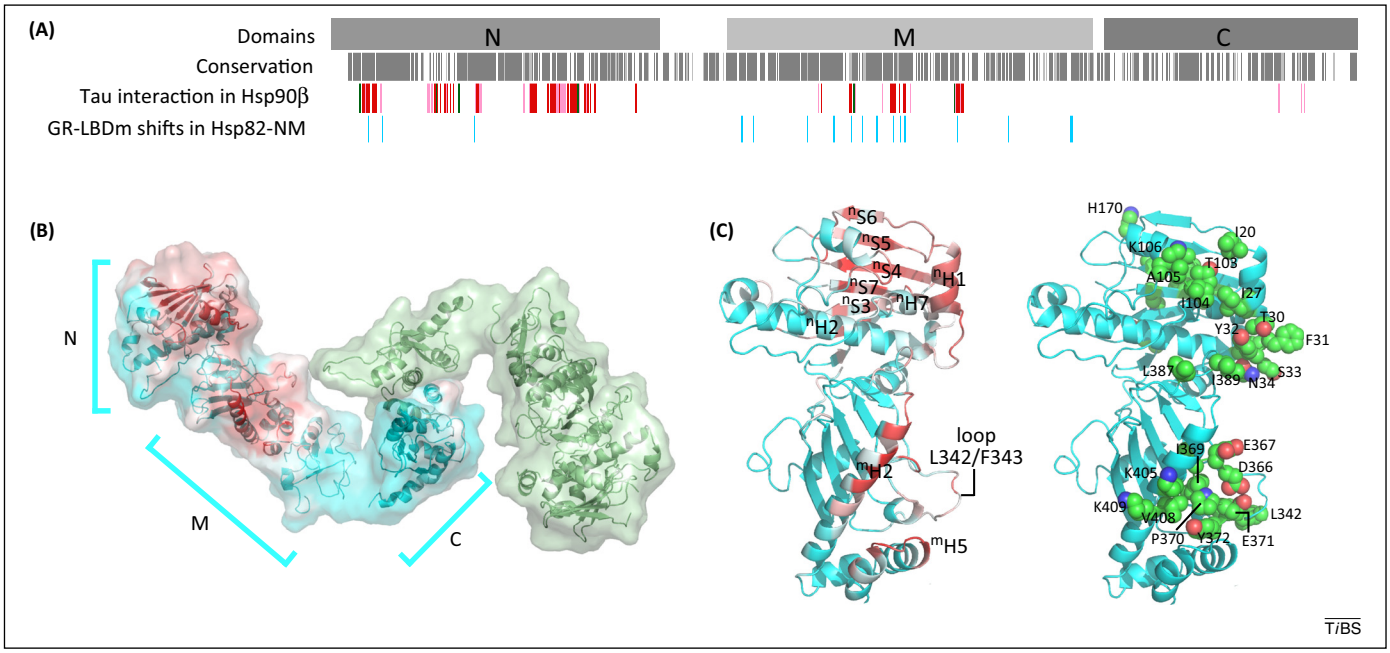

Figure 1. Substrate binding in Hsp90. **(A)** The first row shows the domain boundaries of Hsp90-N, -M, -C, indicated in shades of grey. The second row shows that human Hsp90β and yeast Hsp82 are highly conserved (dark grey, identical; light grey, conserved). The third row indicates the Tau binding site in human Hsp90β (Tau contact residues, increasing red indicates increasing closeness to Tau; isoleucine shifts in NMR experiments, dark green [15]). The fourth row shows residues shifting upon binding of a stabilised ligand binding domain of glucocorticoid receptor (GR-LBDm) to the yeast Hsp82–NM fragment (cyan), which was key to mapping the complex [32]. **(B)** The Tau binding site on Hsp90 dimer. The structural model of Hsp90 bound to Tau obtained by nuclear magnetic resonance (NMR) and small angle X-ray scattering (SAXS) is shown as ribbon and surface (80% transparent) representation. Shown is the Hsp90 dimer (Tau-binding Hsp90 protomer, cyan; other Hsp90 protomer, green; domains are indicated [15]; Tau binding on the Hsp90 surface is indicated in red). **(C)** A zoomed-in view of the Tau binding site. On the left, Hsp90 is shown in ribbon and coloured as in (B). On the right, residues involved in Tau interaction are depicted as spheres. Hsp90 is shown in cyan, interacting residues are coloured by atom (green, carbon; blue, nitrogen; red, oxygen). In Hsp90-N, hydrophobic contacts are spread over the β-sheet nS2–nS7, the helices nH1, nH7, and nH8, and the loop segments G102–K106 and T30–K35, where a small hydrophobic patch is formed on the β-sheet nS2–nS7 by the methyl groups from Thr84 (Cα, Cγ2), Lys179 (Cγ), Val143 (Cγ1, Cγ2), Ile181 (Cδ1, Cα, Cγ2), and Ile145 (Cδ1). Three aromatic residues, Tyr215, His170, and His183, are scattered around the hydrophobic patch as well as several charged residues with no distinct charge pattern. Closer to the N–M interface, residues Leu23, Leu26, Phe31, and Ile104 provide exposed hydrophobic sites that are involved in an interaction with Leu387 and Ile389 on the other Hsp90 protomer when Hsp90 is in a closed state, yet are fully exposed in an open dimer. In Hsp90-M, the Tau binding interface consists of the helices mH1, mH2, mH5, mH4, and mH9 and the loop N terminus of mH2. Pro370, Val408, Lys405, and Lys409 provide methyl groups to a small hydrophobic site. The binding site is on one side bordered by the long helix mH2 and on the other side by the loops around Leu342/Phe343 and Asp366-Tyr372.