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Small Heat-Shock Proteins: Paramedics of the Cell

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Abstract The small heat-shock proteins (sHSPs) comprise a family of molecular chaperones which are widespread but poorly understood. Despite considerable effort, comparatively few high-resolution structures have been determined for the sHSPs, a likely consequence of their tendency to populate ensembles of interconverting conformational and oligomeric states at equilibrium. This dynamic structure appears to underpin the sHSPs' ability to bind and sequester target proteins rapidly, and renders them the first line of defence against protein aggregation during disease and cellular stress. Here we describe recent studies on the sHSPs, with a particular focus on those which have provided insight into the structure and dynamics of these proteins. The combined literature reveals a picture of a remarkable family of molecular chaperones whose thermodynamic and kinetic properties are exquisitely balanced to allow functional regulation by subtle changes in cellular conditions.

Keywords α-Crystallin, Molecular chaperone, Polydispersity, Protein dynamics, Small heat-shock protein (sHSP)

Contents

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Abbreviations

Af	*Archaeoglobus fulgidus*
At	*Arabidopsis thaliana*
Bt	*Bos taurus* (cow)
Dr	*Danio rerio* (zebrafish)
E. coli	*Escherichia coli*
EM	Electron microscopy
EPR	Electron paramagnetic resonance spectroscopy
Hs	*Homo sapiens* (human)
IM	Ion mobility
Mj	*Methanocaldococcus jannaschii*
MS	Mass spectrometry
Mt	*Mycobacterium tuberculosis*
nES	Nanoelectrospray
NMR	Nuclear magnetic resonance spectroscopy
Ps	*Pisum sativum* (pea)
Rn	*Rattus norvegicus* (brown rat)
Sc	*Saccharomyces cerevisiae* (bakers' yeast)
sHSP	Small heat shock protein
Ta	*Triticum aestivum* (wheat)
Tsp	*Taenia saginata* (beef tapeworm)
Xa	*Xanthomonas axoponidis*

Gillian R. Hilton, Hadi Lioe and Florian Stengel contributed equally to this work.

1 Introduction

The small heat-shock proteins (sHSPs) are a family of almost ubiquitous stress proteins [1]. Most organisms encode multiple sHSP genes, an observation particularly clear in the case of higher organisms [2], with Californian Poplar having as many as 36 [3]. In humans the sHSPs number 10 [4] and are implicated in a range of cellular processes including modulation of cytoskeletal dynamics, stabilisation of membranes and apoptosis [5]. These diverse cellular roles appear to be linked by the ability of the majority of the sHSPs to interact with non-native states of proteins [6, 7]. This property is fundamental to their general behaviour as "molecular chaperones" [8], acting to prevent improper polypeptide associations and aggregation [9].

Molecular chaperones play a vital role in protein homeostasis [10], the mechanism through which the cell maintains proper function by balancing the influence of a multitude of biochemical pathways [11]. It has recently become apparent that the native state of proteins is in general less thermodynamically favoured than the amyloid aggregates they can form [12], revealing an underlying metastability of the proteome [13]. Consequently, the breakdown of "proteostasis" can lead to a variety of diseases [14], many of which are characterised by the aggregation and deposition of misfolded proteins [15]. sHSPs represent a central node in the proteostasis "network" [11], and in the main are dramatically up-regulated under conditions of cellular stress to being among the most abundant of all proteins [16, 17]. Furthermore, they are often found associated with protein aggregates obtained post mortem from victims of protein-misfolding disorders [18]. The sHSPs' chaperone function is therefore crucial to the cell's tolerance to stress, and their malfunction is implicated in a range of human pathologies [19–21]. Together these observations suggest that the sHSPs are on the front line of defence against the deleterious consequences of protein unfolding.

Despite their obvious importance, the sHSPs remain relatively poorly characterised on the molecular level. This is largely due to their tendency to populate a range of dynamic oligomeric states at equilibrium, rendering them refractory to many structural biology approaches [22]. As a consequence, high-resolution information exists only for very few members of the family [9]. Recent years have, however, seen considerable developments in the techniques available to structural biologists, and the means to combine data from multiple sources into "hybrid" approaches [23, 24]. Concomitantly there have been significant recent advances in our understanding of the sHSPs. Here we describe the current knowledge of the structure and dynamics of these remarkable molecular chaperones and their interaction with target proteins.

2 The Dynamic Architecture of sHSPs

Proteins are inherently highly dynamic entities [25–27], and an appreciation of how their different structural forms interconvert is necessary to understand how they carry out their cellular roles [24]. These fluctuations can span picoseconds to days,

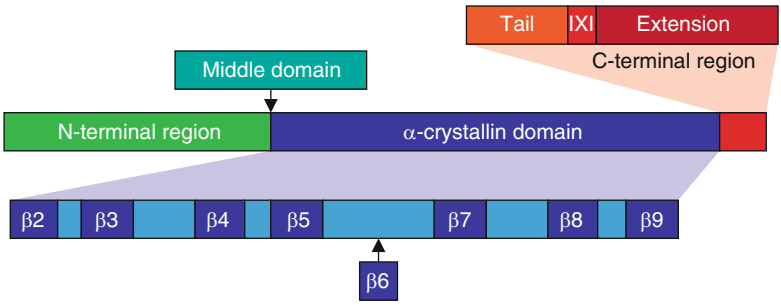

Fig. 1 Domain architecture of the sHSPs. The defining element and most highly conserved region of the sHSP sequence is the α-crystallin domain, which is flanked by the variable N- and C-terminal regions. The α-crystallin domain is composed of seven or eight β-strands, for metazoans or non-metazoans, respectively [37]. In the latter, the sequence between β5 and β7 contains an additional, and distinct, β6 strand. In the metazoans this loop region is shortened, and instead the β7 strand is elongated, into a "β6 + 7" strand. This leads to two alternative modes of dimerisation for the sHSPs (see Fig. 2). The C-terminal region is split into two parts, referred to here as the "tail" and "extension", separated by an IXI motif. The N-terminal region by contrast has no obvious sub-divisions. In HSP26, however, limited proteolysis has revealed a "middle domain" which is inserted between the N-terminal region and α-crystallin domain [35]

have diverse amplitudes and span all levels of protein organisation [28]. Furthermore, the emerging consensus is that sparsely populated "excited" states are frequently responsible for the molecular recognition events underpinning biological function [29–33]. The sHSPs represent a particularly intriguing illustration of this dynamical paradigm: although they share common features, these chaperones undergo intrinsic motions and conformational rearrangements on a wide range of both spatial and temporal scales.

2.1 sHSP Primary Structure

The sHSP family is characterised by the presence of an "α-crystallin" domain [34], derived from the eponymous mammalian sHSP. This central domain is flanked by N- and C-terminal regions (Fig. 1). As perhaps to be expected for a family as large as the sHSPs, exceptions to this basic subdivision exist, including the presence of a "middle domain" [35] or multiple α-crystallin domains [36]. A comprehensive bioinformatics analysis of more than 8,700 sHSP sequences has revealed sHSPs to be composed of on average 161 amino acids [2], corresponding to approximately 17.9 kDa. With an average length of 94 residues, the α-crystallin domain typically composes the bulk of the sequence (approximately 58%). The N-terminus has an average length of 56 residues (35%), whereas at 10 residues (6%) the C-terminus is much shorter.

As the defining element of the sHSP family, the α-crystallin domain is the most conserved region of the sHSP sequence. Interestingly, genomic data has revealed there to be an under-representation of aromatic residues, and an over-representation

of charged amino acids in this domain [2]. Additionally there are notable positions of particularly high conservation, for example the "disease arginine" (at position 120 in αB-crystallin) [37], mutation at which results in a variety of pathologies [38].

The C-terminal region is generally considered to be of two segments, termed here as the "tail" and "extension" [39], which are separated by a highly conserved IXI motif (Fig. 1). The extension appears to be present primarily in higher eukaryotes [39]. In some members of the sHSP family, e.g. human HSP20 [40] and *Taenia saginata* TSP36 [36], the entire C-terminal region is absent. The N-terminal region is however essentially omnipresent and, in the main, considerably longer. It displays almost no sequence conservation, and is responsible for the majority of the sequence variation between sHSPs in the same organism [2]. Additionally, sites available for post-translational modification appear to be found largely in this part of the protein [41]. It is quite possible that this variability of the N-terminus may have a role to play in ensuring that a cell's cohort of sHSPs can recognise a wide range of target proteins.

2.2 The Protomeric α-Crystallin Domain Dimer

High-resolution structures have been very hard to come by for the sHSPs, and the vast majority stem from isolated α-crystallin domains, truncated of the terminal regions. All of these structures, however, reveal a common basic fold of the α-crystallin domain, namely an immunoglobulin-like β-sandwich comprising up to nine β-strands (Fig. 2). The different structures align very well, and are replicated in the two structures solved for full-length, oligomeric sHSPs [42, 43]. However a significant difference can be seen between the structures from animals relative to other organisms (Fig. 2). In the structures determined for plant [43], archaeal [42, 44], and bacterial [45] sHSPs dimerisation occurs via reciprocal donation of the β6 strand, located in a loop, into the β-sandwich of a neighbouring monomer. By contrast, in the mammalian sHSPs the β6 strand has fused with β7 [46–48] into an elongated "β6 + 7" strand which had previously been suggested by spectroscopic experiments, and predicted to enable dimerisation [49–51].

Interestingly, SAXS data has indicated that this dimeric interface observed in truncated forms of the mammalian sHSPs has significant flexibility [52]. Further insight comes from X-ray crystallography which has found three distinct alternative registers formed by the paired β6 + 7 strands, causing a translation in the dimer interface, two residues at a time, spanning approximately 15 Å [46, 48, 53] (Fig. 2). These polymorphic states are termed, in order of decreasing overlap between anti-parallel β6 + 7 strands, AP_I, AP_{II} and AP_{III} [48]. Solid-state nuclear magnetic resonance spectroscopy (NMR) revealed that dimerisation of αB-crystallin mediated by β6 + 7 pertains also to full-length protein, but to date only one register, AP_{II}, has been observed [54]. While relating hydrogen/deuterium exchange rates determined for full-length αB-crystallin [55] to the structure of the truncated dimer certainly reveals the interfaces to be dynamic, it remains to be elucidated to what

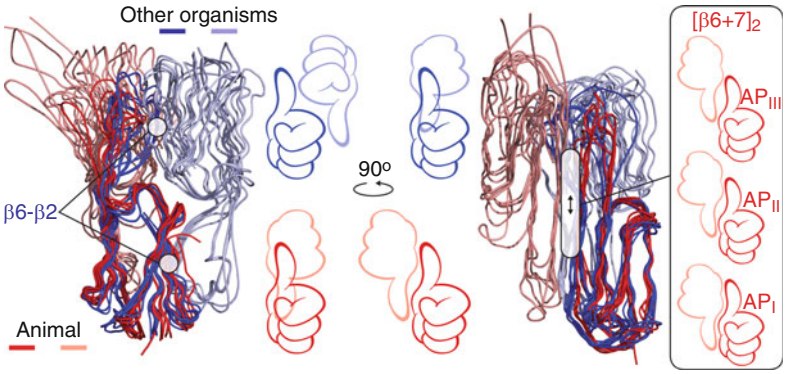

Fig. 2 Distinct dimeric α-crystallin cores. The overall fold of the α-crystallin domain is an immunoglobulin-like β-sandwich with a protruding loop, reminiscent of a "thumbs-up" hand gesture. The structures are highly conserved amongst sHSPs, and accordingly monomers from animals (*red*) and other organisms (*blue*) align very well. The corresponding dimeric partners (*light red* and *light blue*, respectively), however, are found in distinctly different locations, rotated ≈ 180° relative to each other. This is as a result of the non-metazoan proteins dimerising through reciprocal interaction between β6 and β2 strands; whereas the metazoan proteins dimerise through their extended β6 + 7 strands. The latter dimerisation form has been observed in three distinct registers, termed AP_I, AP_{II} and AP_{III}

extent multiple AP interfaces are populated in the oligomers at equilibrium in solution, and whether they interconvert. However, irrespective of these registry shifts, it is clear that, despite very similar basic monomer structures, two distinctly different modes of dimerisation have evolved across the kingdoms of life.

2.3 Heterogeneous N-Termini; Dynamic C-Termini

In contrast to the recent wealth of structural insight into the α-crystallin domain, equivalent information about the termini remains relatively limited. In the crystal structure of *Methanocaldococcus jannaschii* HSP16.5 none of the N-termini are resolved [42]; however EM data revealed additional density within the central cavity of the oligomer [56]. In HSP14.0 from *Sulfolobus tokodaii* two crystal forms were obtained, with the N-termini resolved in one but not the other [44]. Similarly, in the crystal structure of the *Triticum aestivum* HSP16.9 oligomer, half of the N-termini are unresolved; the remainder are structured and found in the centre of the oligomer [57]. Atomic models generated for the N-termini of HSP16.5 [58] and αB-crystallin [59, 60] using sparse spectroscopic restraints, and the N-termini resolved in the crystal structures of HSP16.9 [43], TSP36 [61] and HSP14.0 [44], reveal a propensity to form helical secondary structure.

In apparent contradiction with these results, in hydrogen/deuterium exchange studies of two plant sHSPs, HSP16.9 and *Pisum sativum* HSP18.1, rapid

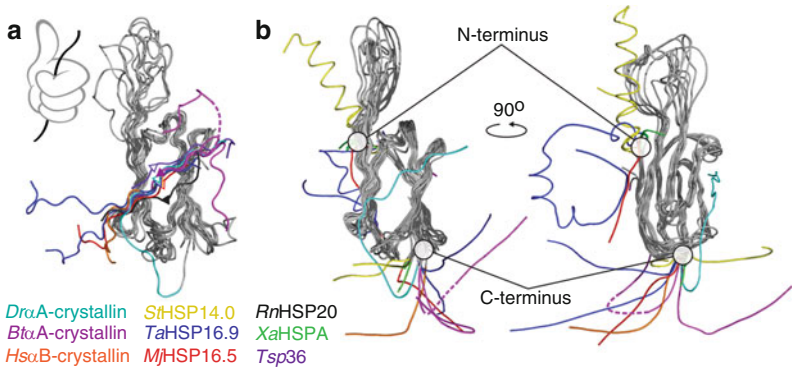

Fig. 3 Variability in the termini. C-termini make inter-dimer contacts, binding over the groove between β4 and β8 strands; by analogy with Fig. 2, the α-crystallin core "hand" grasps the C-terminal "string" from an adjacent monomer (**a**). This interaction has been observed in all crystal structures in which the C-terminal IXI motif is resolved. In other cases (*Tsp*36, *Rn*HSP20) the groove is instead occupied by residues from the N-terminus. Notably both directions of binding have been observed (direction indicated by *triangles* placed in the location of the X in the IXI, *solid arrows* bind *top-right* to *bottom left*). Note that this interaction can even be intra-molecular, as observed in the structure of *Dr*αA-crystallin (*cyan*). The angle which the C-terminus makes from the α-crystallin domain is very variable (**b**). Illustrated are all C-termini resolved in sHSP crystal structures, and it is notable that this variability in angle is found even for the same protein, either in the same oligomer (*Ta*HSP16.9) or in different crystal forms (*St*HSP14.0, *Bt*αA-crystallin). N-terminal residues are observed in fewer structures, but also reveal heterogeneity. This ability of the terminal regions to adopt different orientations is likely crucial for the sHSPs' ability to populate multiple oligomeric states

exchange of the N-terminal backbone amides was observed [62, 63]. Even those positions involved in inter-dimer contacts approached complete exchange with 100 s, and only a single exchanging population was observed [63]. This reveals that all 12 of the N-termini in these dodecameric proteins are essentially equivalent, but does not rule out that at any given moment a sub-population thereof may be structured and form relatively transient interactions. Taking these results in combination with the evidence from the crystal structures suggests that the N-termini populate multiple slowly inter-converting conformations in the centre of the oligomer, perhaps helping to maintain the integrity of the assemblies [64]. Such intrinsic heterogeneity could conceivably be important in recognising and binding variable target proteins by presenting diverse geometries for interaction [65].

In the structures of HSP16.9 and HSP16.5 the C-terminal tails were revealed to span between dimers, such that the IXI motif binds into a groove between strands β4 and β8 (Fig. 3a). Similar "cross-linking" interactions mediated by the C-termini were observed both in X-ray structures of α-crystallin constructs lacking the C-terminal extension [48] and solid-state NMR data on the full-length protein [54], both obtained at temperatures below freezing. Interestingly, however, for the α-crystallins at physiological temperature in solution it appears that the IXI motif is actually predominantly detached from the oligomer [66–68]. This apparent

contradiction can be rationalised by the strong temperature dependence of fluctuations of the tail [66], and points to a careful thermodynamic regulation of the IXI binding [69]. Unlike the tail, NMR studies have revealed that the C-terminal extension, for those sHSPs in which it is present, is intrinsically disordered and tumbles freely in solution [70, 71]. This region of sequence is primarily hydrophilic and is thought to facilitate the detachment of the remainder of the C-terminus [72]. Considering that α-crystallins with truncated extensions are associated with cataract [73], a picture emerges in which the dynamics of the C-terminus are crucial to chaperone function, potentially through regulating access to the β4/β8 groove in a form of "auto-inhibitory" regulation [43, 54].

2.4 Oligomeric Assembly Is Mediated by Flexible Terminal Interactions

While the sHSPs are prefixed by "small" due to their low monomeric molecular mass, relative to the other heat-shock proteins (HSPs), this is something of a misnomer. The sHSPs are typically oligomeric, with the majority studied so far comprising 12 or more subunits and having masses in excess of 200 kDa [9], making them among the largest of the HSPs. Though only two high-resolution structures (for HSP16.5 and HSP16.9) exist for such oligomers, in both cases they are stabilised by inter-dimer connections formed by the terminal regions of the protein as well as specific interactions between the α-crystallin core building blocks [42, 57].

In these structures the C-termini decorate the surface of the oligomer, holding it together by bridging between the α-crystallin domains of adjacent dimers [42, 57] (Fig. 3a). It is notable that the angle made between the domain and the tail is variable, even within the same oligomer [57] (Fig. 3b). Such flexibility in the tail is mediated by a "hinge-loop" just C-terminal to the core domain [48, 74] and, it is tempting to speculate, explains how the sHSPs can be found in a range of oligomeric forms [75]. Furthermore, it is notable that for the α-crystallins the area of sequence around the IXI is palindromic [46, 48], which may allow an additional degree of variability in assembly. The versatility of the C-terminus is reminiscent of that in the coat protein VP1 whose conformational flexibility mediates its variable assembly in simian virus 40 [76].

In the case of TSP36, which lacks a C-terminus, the β4/8 groove in the α-crystallin domain is instead occupied by N-terminal residues [61]. The presence of IXI motifs in both the C-terminus and extreme N-terminus of a number of sHSPs, such as the α-crystallins, raises the possibility that there may be some extent of inter-changeability between the two termini. Alternatively, the structure of HSP16.9 demonstrated the ability of the N-termini to extend across the central cavity of the oligomer, intertwined in a pairwise manner [57]. Considering HSP16.9 is a monodisperse dodecamer, this may amount to a specific interaction acting to lock the protein into a single oligomeric stoichiometry, reminiscent of the role played by the protein P30 in the bacteriophage PRD1 [77].

Conceivably, as the N-termini in the main appear heterogeneous, it is possible that they interact with each other relatively non-specifically, driven together by hydrophobic interactions [64]. Constructs of the α-crystallins, truncated of the N-terminus but retaining the C-terminus, were only able to form oligomers to very low abundance relative to sub-oligomeric species [48]. However, in the case of HSP16.5, the protein was still observed as an oligomer after removal of the N-terminus [78], but was completely disassembled after further removal of the C-terminus [79]. It appears therefore that the N-termini are not necessarily required for oligomerisation, but contribute to the thermodynamic stability of the resultant assemblies. While the importance of the N-terminus therefore appears to vary between sHSPs, it clearly has a role to play in defining oligomerisation, the details of which warrant further investigation.

2.5 sHSPs Assemble into Multiple Polyhedral Topologies

Members of the sHSP family populate a continuum of oligomeric states, from monodisperse to extremely polydisperse [56]. Notably, plant sHSPs typically exist as single oligomers, generally dodecamers [80]. Conversely, many mammalian sHSPs co-populate a wide range of oligomeric states at equilibrium; for example the α-crystallins adopt all possible stoichiometries between approximately 10 and 50 subunits [81, 82]. Between these two extremes, sHSPs have been characterised that populate an intermediate number of oligomeric states, with certain amongst them preferred, such as for example *Saccharomyces cerevisiae* HSP26 [83] and Acr2 from *Mycobacterium tuberculosis* [84]. This tendency towards polydispersity has proven to be a major hindrance in the structural characterisation of sHSPs [22].

Nonetheless, the X-ray structures determined for sHSPs at conditions in which a single oligomeric state was populated provide considerable insight. HSP16.5 crystallised as a 24mer, in which the subunits are assembled into an octahedron, with a protomeric dimer comprising each edge (Fig. 4a) [42]. Remarkably, insertion of additional residues in the N-terminus resulted in an expanded symmetric oligomer, with 24 dimers assembled into a cuboctahedron [75] (Fig. 4a). The dodecamer of HSP16.9, by contrast, assembles into a "double-ring" topology, i.e. two triangular rings stacked on top of each other (Fig. 4a) [43]. Docking of a dimer into the electron microscopy (EM) reconstruction of Acr1 from *M. tuberculosis* reveals an alternative arrangement for dodecameric sHSPs, namely a tetrahedron (Fig. 4a) [84]. This striking oligomerisation into polyhedral geometries reveals the important observation that all the α-crystallin core dimers within the oligomers are essentially equivalent structural environments, connected via terminal interactions (Fig. 4b). It is plausible that this characteristic results in there being no great energetic difference for a dimer residing in a specific oligomeric stoichiometry, and therefore enables multiple oligomeric states to be populated at equilibrium [66]. As polyhedral arrangements result in dimers being arranged symmetrically while also

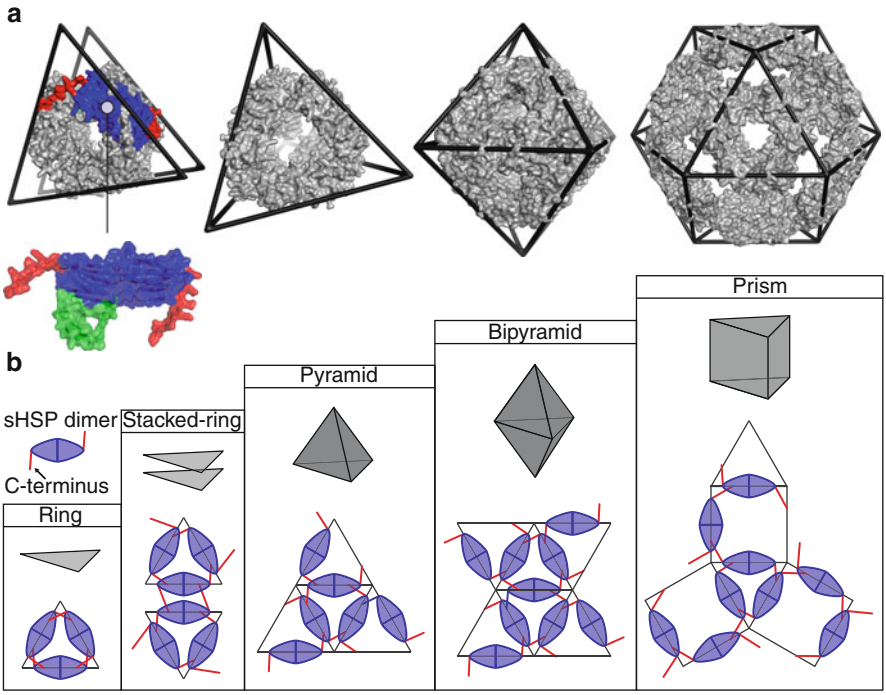

Fig. 4 Polyhedral architecture of the sHSPs. The structures, from *left to right*, of HSP16.9 (12mer), Acr1 (12mer), HSP16.5 (24mer), and a modified HSP16.5 (48mer) display striking polyhedral geometries (**a**). They assemble as a double ring, tetrahedron, octahedron and cuboctahedron, respectively (*in sequence*, *left to right*). In each case the α-crystallin domain dimers (*blue*) are collinear with the edges of the polyhedron, as illustrated for HSP16.9. The dimers are held together by extended C-termini (*red*, cf. Fig. 3), and the N-termini (*green*) are sequestered on the inside of the oligomers. This assembly of core dimers into polyhedra via C-termini can be illustrated schematically using nets of several classes of polyhedra (**b**). Nets are shown based on a triangular base-unit, but could easily be drawn for larger polygons in these classes (e.g. a square pyramid). Similar nets can be drawn for any given polyhedron such that all the C-termini are "satisfied", binding dimers together at the vertices

satisfying similar terminal interactions, it has been proposed that polydisperse sHSPs share these scaffolds [85].

2.6 *Hybrid Approaches to Determine the Structure of Polydisperse sHSPs*

The inherent polydispersity and plasticity of sHSPs are a significant impediment to their structural characterisation. These characteristics are likely however to be important to their cellular function, for example in preventing the unwanted crystallisation of the α-crystallins despite their high concentration in the eye lens [86], but

accordingly render the determination of X-ray structures of these proteins extremely challenging [22]. This has in recent years led to the application of both novel and integrated structural biology approaches to the sHSPs. The size limit of traditional solution NMR approaches has been circumvented to provide insights into the α-crystallins, either by examining truncated forms [50] or regions of marked flexibility [70, 71]. Moreover, the large oligomeric species have even be examined directly by means of solid-state NMR [54, 59], or via selective labelling of amino acids in methyl transverse relaxation optimised spectroscopy (TROSY) solution NMR [66] and electron paramagnetic resonance (EPR) approaches [51, 87, 88].

These varied strategies all provide structural information, ensemble-averaged onto the monomer level. In order to translate these insights onto the quaternary structure, studies have combined NMR data with that obtained from EM [59, 85], small-angle X-ray scattering (SAXS) [54, 59] or ion-mobility spectrometry (IM) [85], all of which report on the oligomeric form. Particular challenges are posed in the cases of polydisperse sHSPs and techniques are required which can separate, and address individually, the constituent oligomeric states. Single-particle analysis of EM data provides the opportunity to generate three-dimensional reconstructions of particles after their sorting according to size [89, 90]; currently however, the highest resolution of separation for macromolecular assemblies is afforded by MS approaches [91]. In the case of αB-crystallin, the archetypal polydisperse sHSP, early EM analysis revealed a broad range of oligomeric sizes and masses, with apparently variable symmetries [92, 93]. MS enabled the identification and relative quantification of the underlying individual oligomeric states, revealing a broad distribution of stoichiometries centred around ∼28 subunits [81, 82] (Fig. 5a).

Recently, an EM reconstruction for αB-crystallin was obtained by assuming that the principal oligomer states shared common symmetry elements [94]. This was combined with solid-state NMR [59] and cross-linking MS [60] data to generate model oligomers constructed from hexameric sub-complexes [59, 60]. An orthogonal approach, using IM–MS to discriminate between candidates based on a variable polyhedral architecture, and cross-validation with EM data, revealed alternative structures for this protein [85] (Fig. 5a). While these studies have reported plausible models for αB-crystallin, they differ in terms of symmetry and size distribution. While definitive structures therefore remain elusive, it is clear that the emergence of novel and hybrid approaches have provide new impetus to the structural study of this notorious target for structural biology.

2.7 sHSP Oligomers Can Transition Between Compact and Expanded Forms

Aside from the variability in quaternary structure afforded by polydispersity, it appears that sHSPs oligomers themselves can exist in multiple conformations (Fig. 5b). A cryoEM study of HSP26 revealed two distinct populations of 24mers, differing by approximately 5% in diameter [90, 95]. Additionally, subunit

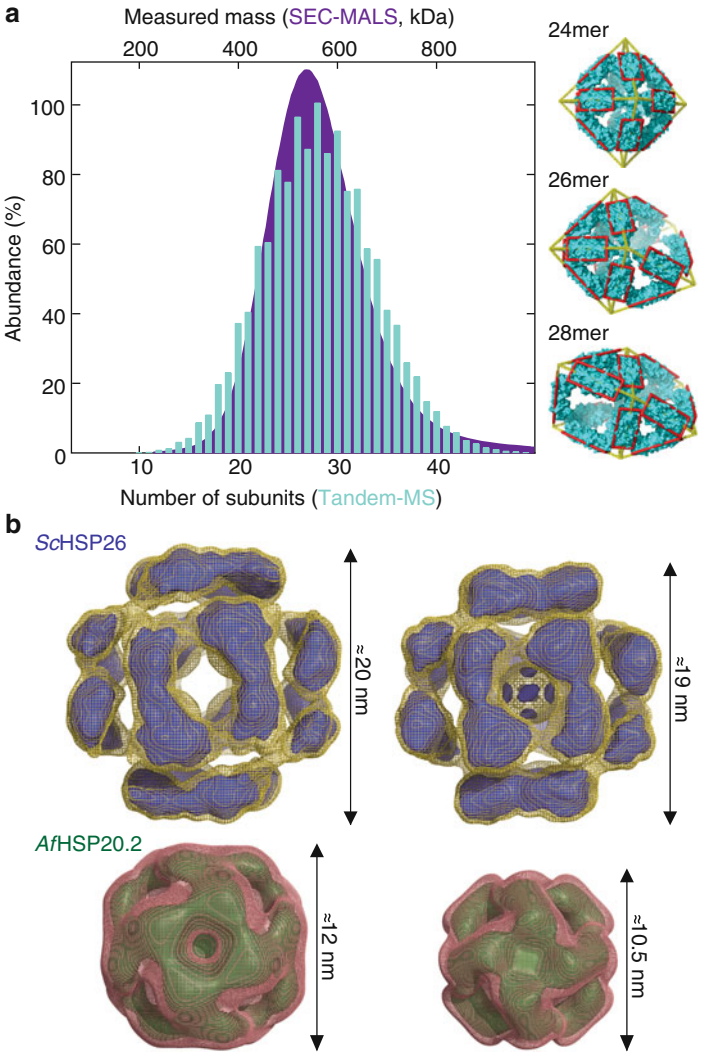

Fig. 5 Macro- and micro-heterogeneity of the sHSPs. Many sHSPs are polydisperse, populating a range of oligomeric states at equilibrium (**a**). The most famous example of this macro-heterogeneity is αB-crystallin, which forms oligomers spanning 200–1,000 kDa, as determined using size-exclusion chromatography coupled to multi-angle light scattering (SEC-MALS) (*purple*) [91]. Mass spectrometry measurements have revealed the underlying distribution of oligomers (*cyan*) to be centred on 28 subunits, with those stoichiometries composed of an even number of subunits to be preferred [82]. Ion-mobility mass spectrometry measurements allowed the filtering of structural models, based on polyhedral scaffolds (Fig. 4), to reveal the likely architecture of the 24mer, 26mer and 28mer forms [85]. These structures are based on an octahedron, augmented triangular prism and gyrobifastigium, respectively (*right*). Aside from populating multiple stoichiometries, different conformations of individual sHSP oligomeric states have been observed (**b**). Electron microscopy investigations of both *Sc*HSP26 and *Af*HSP20.2 suggested the possibility of sHSPs populating "expanded" and "compact" forms, differing in overall size but not topology. This micro-heterogeneity adds an additional level to the quaternary complexity of the sHSPs

exchange of HSP26 could only be quantitatively understood by invoking at least two separate dissociation rate constants, consistent with two types of oligomers [83]. Differences were noted between negative-stain EM reconstructions of *Arabidopsis thaliana* HSP21, in the presence or absence of cross-linker, hinting at the possibility of multiple conformations differing in terms of compactness [96]. A similar observation was also made in a negative-stain EM study of the octahedral HSP16.5 and *Archaeoglobus fulgidus* HSP20.2 24mers. For both proteins the relative proportions of the two forms varied as a function of temperature, demonstrating that the two states can interconvert [97]. Previous studies of HSP16.5 had reported some heterogeneity of the protein [56, 98, 99]. As such it appears that sHSPs not only display "macro-heterogeneity", that is populating multiple oligomeric stoichiometries, but that these individual stoichiometries can adopt multiple quaternary structures in a form of "micro-heterogeneity". Moreover, for HSP20.2 the larger form was predominant at temperatures both above and below the physiological temperature of the organism, suggesting a functional role for these quaternary conformational fluctuations.

The underlying structural origin of these expansions and contractions of the oligomers is unclear. There is evidence that they might stem from packing differences enforced by rearrangement of the inter-dimer contacts formed by the terminal regions [95]. For HSP26, they may be caused by its unique middle domain, which undergoes a conformational switch upon heat shock [100]. Alternatively, the α-crystallin domain dimeric building-block itself might fluctuate in length, for example due to changes in register at the interface [48, 53], which would propagate to have consequences on the overall size of the oligomer. Alternative registers have so far only been observed for mammalian sHSPs, which have an extremely labile dimer interface formed by the β6 + 7 strand [50, 82]. However, considering that the interface of dimers formed via the β6 loop are also easily broken [101], it is not inconceivable that a similar mechanism might also occur in sHSPs from lower organisms.

2.8 sHSPs Co-Assemble into a Recycling Oligomeric Ensemble

In addition to the complexity afforded by both macro- and micro-heterogeneity, it has long been known that members of the sHSP family co-assemble into hetero-oligomers *in vivo* [102]. Isolated sub-populations of oligomers re-equilibrate to the parent distribution [103] in a process mediated by the movement of individual subunits [104]. The combinations of sHSPs which are compatible for co-assembly are dependent on their evolutionary relationships [80]; however the subunit exchange of the individual oligomers appears to be a general property of these proteins [105–108]. As such, the sHSPs should not be considered as static homomeric proteins, but rather as a continually "recycling" ensemble of hetero-oligomers.

Subunit exchange occurs via the dissociation of the oligomer, with a rate strongly dependent on solution conditions [82, 105]. This reveals that the sHSPs are in a rapid equilibrium with a small population of sub-oligomeric forms. The

identity of the exchanging unit depends on the sHSP in question, with monomers [82], dimers [108] and mixtures thereof [101] all having been observed directly in high-resolution mass spectrometry experiments. This equilibrium between oligomers and smaller species is shifted towards dissociation at elevated temperatures, with appreciable concentrations of the latter observed under heat-shock conditions for some sHSPs [80, 83, 109], but not for others [80, 82]. Concomitant to this dissociation, high-order oligomers have also been observed, such that sHSPs which are monodisperse under ambient temperatures effectively become polydisperse at elevated temperature [110, 111]. These processes of assembly, dissociation and exchange are also affected by modifications to the sHSPs thought to regulate or compromise sHSP activity such as post-translational modification [72, 112, 113] or mutation [114–116]. Given these characteristics, it is tempting to speculate that such quaternary dynamics are important for chaperone function, presumably by exposing target-protein binding regions either on the sub-oligomeric species, or *en route* to dissociation [9, 57].

3 The Molecular Chaperone Function of sHSPs

As might be expected from their evolutionary diversity, sHSPs have been reported to be involved in a range of cellular processes. The role which is common to most members of the family is the ability to act as molecular chaperones [117, 118]. This function of the sHSPs was first demonstrated when it was found that α-crystallin could prevent the accumulation of aggregation-prone eye-lens proteins [119]. This *in vitro* observation was later confirmed *in vivo* when αA-crystallin knockout mice developed inclusion bodies rendering the eye lens opaque [120]. Furthermore, disruption of the αB-crystallin and the adjacent HSPB2 sHSP genes resulted in degeneration of some skeletal muscles [121]. Since the pioneering work on α-crystallin, many other sHSPs have been demonstrated to have molecular chaperone activity, and it is quite likely that the ability to interact with non-native states even underpins the mechanism of their other activities in the cell [6].

3.1 High-Capacity Holdase Function of sHSPs Sequesters Destabilised Targets from Aggregation

It has been established for some time that the molecular chaperone role of sHSPs is to bind target proteins whose native structure is destabilised by a range of stresses [119, 122–125]. Under such conditions, these proteins can have a tendency to form amorphous or amyloid aggregate morphologies [18]. Rather than refolding the targets, as is the case for the ATP-dependent "foldase" chaperones such as HSP60, HSP70 and HSP90 [126], the sHSPs instead act in an ATP-independent manner to trap them as they unfold [119, 122–125]. The resultant complexes formed between

the sHSP and target proteins can range in mass up to several MDa [111, 127, 128]. The binding capacities of the sHSPs vary between different members of the family, but can be very high, with the chaperones capable of protecting stoichiometric quantities of target [128, 129]. The capacity appears to be somewhat dependent on the identity of the target protein [127, 129], perhaps purely reflecting the mass of the target [7, 130]. As such, the sHSPs can be viewed as high-capacity "sponges" for non-native proteins, preventing the deleterious consequences of their aggregation [131].

The majority of assays for studying sHSP activity rely on assessing the ability of the chaperone to suppress the aggregation of model proteins, due to the difficulties in purifying inherently unstable targets [132]. It is not uncommon that the apparent efficiency of protection is dependent on the choice of model protein [133]. As such these *in vitro* assays might not be expected to capture all aspects of *in vivo* function [134]. Nevertheless, the capacity of sHSPs to interact with a range of destabilised model proteins renders it likely that they have multiple targets in the cell [9, 57]. Determining the characteristics of actual cellular substrates has however been hampered by the absence of easily assayed phenotypes associated with sHSP deletions in yeast or *Escherichia coli* [135, 136]. However, a study using *Synechocystis* sp. PCC 6803, in which only a single sHSP is encoded and the deletion of which results in lack of thermo-tolerance [137], identified interactions with numerous proteins [132]. These interactors displayed no commonality in sequence or structure, and spanned functions ranging from transcription, translation, to cell signalling, and secondary metabolism [132]. A similarly heterogeneous set of targets was also observed in yeast, corroborating the apparent broad specificity of sHSPs [138]. The general chaperone function of the sHSPs therefore appears to be to act as "holdases", sequestering target proteins and thereby impeding the deleterious consequences of their aggregation [131].

3.2 sHSP Activity Is Influenced by Environmental Conditions

Multiple different stresses have been reported to stimulate the activity of sHSPs. Primary to these is heat shock, with sHSPs generally thought to be more effective chaperones at elevated temperature. For example, HSP26 has been demonstrated to undergo significant structural and dynamical changes around 40°C, consistent with a thermal activation of the protein [83, 100, 109]. While HSP26 is unusual in containing a middle domain, HSP18.1 undergoes a similar dynamic transition in oligomerisation from an inactive "storage form" into a functional chaperone with temperature [111]. In fact chaperone functions at temperatures far in excess of those normally termed "heat shock" have been reported [139]. On the other hand, some sHSPs have been shown to retain chaperone activity below heat shock temperatures [140], demonstrating that thermal activation is not a universal requirement for sHSP function.

Solution pH is also known to affect the molecular properties of the sHSPs. Studies of αB-crystallin have revealed dramatic changes in the thermodynamics and kinetics of the inter-subunit interfaces [50, 54, 66, 82]. This is reflected in pH-dependent changes in chaperone function *in vitro* [141–143], and a role for αB-crystallin in responding to cellular acidification [144–146]. sHSPs have also been demonstrated to provide protection against toxicity from metal ions *in vivo* [147]. The α-crystallins have been shown to bind metal ions directly [48], potentially silencing any tendency to oxidise [148, 149], and resulting in modulation of their chaperone function [150, 151]. This behaviour is interesting in light of the role of the redoxins, which switch from their enzymatic function to become molecular chaperones upon oxidative stress [152, 153].

While it has been generally accepted that the function of sHSPs is as ATP-independent molecular chaperones, there have been reports suggesting that the sHSPs can bind nucleotides [154, 155]. This finding is supported by the observation of sulphate ions accumulated at the dimer interface in recent crystal structures [48, 156]. Mapping changes in residue-specific protease susceptibility of αB-crystallin upon the addition of nucleotide [157] on the structure of the core domain suggest that the location of the sulphate may represent ATP-binding sites [53]. This is in line with the notion that nucleotide binding (rather than hydrolysis) might regulate activity [154], a mode of action that contrasts with the canonical chaperones, in which the turnover of ATP drives their action [158].

Members of the sHSPs can become post-translationally modified upon stress, with phosphorylation in particular implicated in affecting their function [159–161]. Accordingly, profound effects of phosphorylation on the chaperone activity have been observed *in vitro* [112, 162–164]. However, the identity of the target protein and solution conditions both strongly influence whether the post-translational modification leads to an increase or decrease in chaperone efficacy [133]. As such, while the evidence clearly points to phosphorylation regulating the function of sHSPs, a simple description of the mechanism appears unlikely [165]. Overall, from the differences in activity observed for the sHSPs as a result of multiple and varied stimuli, a picture emerges of a family of molecular chaperones which are exquisitely and directly controlled by the insult responsible for a particular stress condition.

3.3 sHSPs Co-Operate with the Cellular Machinery to Allow Reactivation or Degradation of Targets

While the sHSPs are active under stress conditions and act to bind non-native target proteins, they do not themselves appear to enable their refolding. Instead the target protein is subsequently retrieved from the sHSP:target complex and refolded upon interaction with ATP-dependent chaperones [166–169]. This renaturation pathway involves the HSP70/HSP40 system (DnaK/J in prokaryotes), a nucleotide exchange factor, and HSP100 working in concert with the sHSPs to liberate and refold unfolded substrate proteins [170].

While it has been demonstrated that the sHSPs facilitate the disaggregation of insoluble protein deposits [135, 170–174], the mechanism by which this is achieved is presently unclear. Recent studies suggest that different sHSPs may play varying roles in the resolubilisation process [175]. It is likely that they act to compete kinetically for the inter-molecular interactions that would otherwise ultimately lead to the formation of stable aggregates, instead holding proteins in a conformation more amenable to subsequent refolding [170].

While the link between the sHSPs and ATP-dependent foldases is clear, recent evidence also points to their interaction with the protein degradation machinery [176]. A number of studies have linked sHSPs with the proteasome/ubiquitin pathway [177–180], and the *E. coli* sHSPs IbpA and IbpB have been shown to be substrates of the Lon protease [181]. Though this field warrants considerably more attention, the involvement of sHSPs in both the refolding and degradation pathways reveals them as crucial switching points in determining the fate of unfolded proteins [182].

3.4 sHSPs Possess Multiple Sites that Become Exposed to Bind Targets

Considerable effort has been expended in an attempt to elucidate the region of the sHSPs which interact directly with the target proteins. Different studies have implicated the N-terminal region [65, 134, 183, 184], the C-terminus [185, 186] and the α-crystallin core [149, 187, 188]. Indeed, isolated α-crystallin domains from some sHSPs have been shown to have a certain amount of molecular chaperone activity [48, 52]. Taking these results together implies that there is no single binding site within the oligomer, but rather that these are dependent on the sHSP or target protein in question [6] (Fig. 6).

A commonality observed in the majority of putative interacting regions which have been elucidated is that they are hydrophobic in nature [6]. This is unsurprising, considering that it is the exposure of complementary hydrophobic surfaces on target proteins which renders them aggregation prone. It has been shown that the number of accessible hydrophobic sites on the sHSP increase upon heat shock [80, 167, 189], prompting the question whether this is a consequence of structural rearrangements of the sHSP oligomer.

A popular hypothesis, based on cumulative evidence from studies of several different members of the family which demonstrated oligomeric dissociation at heat-shock temperatures, is that a sub-oligomeric species form of the sHSP initially binds the target [109, 137, 190]. This mechanism does not appear to be universal, however, as a number of examples have emerged showing that dissociation, or its corollary, the rate of subunit exchange, is not necessarily correlated with chaperone activity [72, 80, 82, 191, 192]. In this regard it is informative to consider the case of HSP26. An early study of this protein demonstrated it to undergo dissociation into sub-oligomeric species at heat shock temperatures, suggesting this event to predicate chaperone activity [109]. However, subsequent studies revealed that chaperone

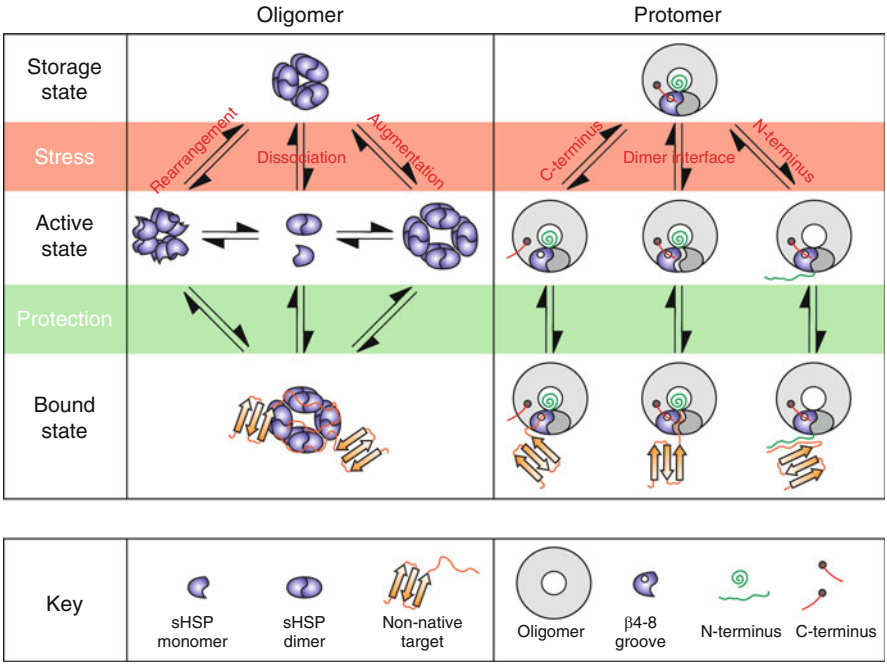

Fig. 6 Molecular basis for activation of binding of target by the sHSPs. A range of structural and dynamical changes in the sHSPs have been observed which have been ascribed to switching the chaperone into an "active" state. These are separated here according to effects on the oligomer (*left*) or protomer (*right*) level. The former include dissociation of the oligomer; a conformational rearrangement of a domain within the oligomers; or a change in oligomeric distribution. At present no universal pathway has emerged, and activation is likely to be sHSP dependent. Ultimately, and common to all sHSPs studied, large and heterogeneous sHSP:target complexes are formed. On the protomer level evidence for the exposure of the β4–β8 groove by detachment of the C-terminus, exposure of the dimer interface and unfurling the N-terminus has been proposed. The diversity in mechanism is likely to reflect the evolutionary diversity of both the sHSPs and their targets

activity was unaffected if the oligomer was cross-linked such that it could not dissociate [191]. Instead, a thermally regulated conformational change in the middle domain [100], which in the unrestrained protein is concomitant to changes in oligomerisation [83], has been implicated as underlying activation of this sHSP. This example illustrates that even if a protein undergoes dissociation into sub-oligomeric species at heat-shock temperatures, this does not necessarily imply that the sub-oligomeric species is the active target-binding form.

An alternative model is motivated by a recent cross-linking study between HSP18.1 and malate dehydrogenase which suggested that the N-terminal region was primarily responsible for binding [65]. However, examination of the structure of its homologue HSP16.9 reveals at least some of the N-termini to be sequestered within the centre of the oligomer [43], a location structurally incompatible with the high binding capacity of the chaperone. It is possible therefore that the N-terminal

arm unfurls from the central cavity of the protein to become exposed at heat-shock temperatures in an intrinsically disordered state which can present diverse geometries of interaction sites for binding [65]. One can even envisage a situation whereby the N-terminus acts to modulate the surface of the oligomer, similar to the way in which the specificity of protein phosphatase 1 is governed by the binding of its unstructured regulator proteins such as spinophilin [193]. Such a release of the N-termini would necessitate the loss of the directional inter-dimer constraints they form in the crystal structure of HSP16.9. Circumstantial evidence for this mechanism therefore comes from that fact that at heat-shock temperatures the oligomeric form of this protein is no longer confined to a dodecamer [110].

This change in protein partitioning, with most of the protein being re-allocated from a monodisperse oligomer at ambient temperatures into a polydisperse ensemble, arises purely from thermal motions that transfer subunits between oligomers of varying relative stability. This is particularly pronounced for HSP18.1 [111] where, interestingly, the resultant distribution of higher oligomers that was observed in this study was very similar to that populated by the α-crystallins at ambient temperatures [82, 162], conditions under which they remain chaperone-active. This raises the possibility that a polydisperse ensemble of oligomers may not only be a direct consequence of sHSP activation, but may even itself be of direct functional benefit.

In combination these studies suggest that there are not only multiple binding sites on the oligomer but also different mechanisms for their exposure to target proteins (Fig. 6). A common thread appears to be that the native oligomer represents a chaperone-inactive "storage form", which undergoes a transition to a chaperone-active form. Such a change may exist purely to prevent unwanted, retarding associations with non-native states during non-stress conditions. Alternatively it may signal a switch between different cellular functions, analogous to redoxins which have been observed to become chaperone active upon a stress-induced change in the oligomeric state [152, 153]. Overall, however, it is clear that there are considerable mechanistic variations in the chaperone function of different sHSPs, emphasising the broad specificity of these chaperones in protecting the proteome.

3.5 sHSP:Target Complexes are Plastic and Polydisperse

The complexes formed when sHSPs are incubated with target proteins are very large and extremely heterogeneous [127, 128]. This is in stark contrast to the defined stoichiometries of interaction observed for foldase chaperones and their targets [158]. Such heterogeneity severely hampers structural interrogations, but can be overcome using the high resolution of separation afforded by MS approaches [91]. Employing a tandem-MS strategy [81], the different complexes formed between HSP18.1 and luciferase were identified and quantified [111]. Despite these experiments being performed with an excess of chaperone, remarkably over 300 stoichiometries of interaction were observed, variable in both the number of

sHSP and target subunits [111]. In light of the observation that sHSP:target complexes scale with the amount of target protein [127], a glimpse of the bewildering polydispersity of these complexes is obtained.

The complexes are not static entities; indeed they can continue to incorporate ever-increasing amounts of substrate [111, 127, 128]. Moreover, the sHSP subunits continue to exchange with free sHSP oligomers and other complexes [127]. By contrast, target proteins appear unable to transfer from one complex to another [127]. Hydrogen/deuterium exchange experiments revealed no difference in backbone amide protection between the sHSP free in solution or in complex [62], and α-lactalbumin associated with α-crystallin is still visible in proton NMR spectra, implying that it must spend a significant proportion of its time tumbling independently in solution [194]. These data therefore reveal that the interaction between sHSP and target protein is, at least in some cases, only transient, presumably to facilitate subsequent transfer to the refolding machinery.

In addition to these inherent dynamics and variability, it also appears that sHSP: target complexes can adopt different morphologies [128, 130]. Interestingly it appears that this is target-protein dependent: the same sHSPs formed different morphologies with different targets [128, 130] but complexes of different sHSPs and the same target appear similar [128]. This can be rationalised by the observation that the sHSPs bind target proteins early during the unfolding process, when their structure is largely preserved [62, 130]. In summary, the complexes formed between sHSPs and targets are extremely polydisperse and dynamic, which renders their structural characterisation very challenging, but is likely integral to their cellular function.

4 Paramedics Within the Proteostasis Network

Molecular chaperones are crucial for the maintenance of cellular protein homeostasis [10]. sHSPs are an important part of this network, being dramatically upregulated and activated during cellular stress, and sequestering destabilised targets from aggregation. In this way, sHSPs can be regarded as the paramedics of the cell [83], rapidly stabilising the targets prior to their attention by the refolding or degradation machinery. Aside from this important role in overcoming the kinetics of aggregation, the sHSPs also act to increase the proteostasis capacity of the cell. By temporarily storing the aggregation-prone proteins, awaiting refolding or degradation, they act as a vital buffer between protein unfolding and its potentially deleterious consequences.

While the sHSPs form a crucial part of the proteostasis network [11], they themselves can be thought of as a highly dynamic sub-network. As discussed above, many studies have shown that sHSPs can adopt a range of plastic oligomeric states, containing chains from multiple different sHSPs. As many organisms express multiple sHSPs in the same cellular compartment, this will lead to their

coassembly upon synthesis. If all particular subunit arrangements are allowed the number possible of oligomers N_{Oli} is given by

$$N_{\mathrm{Oli}} = \sum_{i}^{n} \frac{[N_{\mathrm{sHSP}} + P_i - 1]!}{P_i![N_{\mathrm{sHSP}} - 1]!}$$

where N_{sHSP} is the number of sHSPs capable of coassembly and i the number of sHSP subunits in a particular oligomeric stoichiometry P.

As an example, for monodisperse hetero-dodecamers ($P_i = 12$), if there are six compatible sHSPs ($N_{\mathrm{sHSP}} = 6$), as is the case for class I sHSPs in the Arabidopsis cytosol [3], the number of potential oligomers is 6,188. In the analogous calculation for class I cytosolic sHSPs in Californian poplar ($N_{\mathrm{sHSP}} = 18$) [3], almost 52,000,000 combinations are possible. While what proportion of these potential hetero-oligomers are formed *in vivo* will be influenced by factors such as tissue specificity and relative expression rates and levels, the number of possibilities is nonetheless remarkable. Combined with the observation that the oligomers rapidly exchange subunits, this suggests that sHSPs should not be regarded as individual oligomers but rather an extensive and interconverting ensemble.

The very large numbers of compatible sHSPs in plants are not replicated in mammals but instead it appears that a different mechanism to achieve the same effect might be at play. In the case of α-crystallin in the eye lens, where both isoforms A and B are expressed and populate oligomeric states between 10 and 50mers [66], 1,271 combinations are possible. Additionally, αB-crystallin is found outside the lens, and is one of seven sHSPs abundant in muscle, leading to potentially over 264,000,000 different complexes. Although, specificities of interaction between sHSP pairs [195] will act to reduce this number in the cell, the polydispersity of many members of the family can act as a means to magnify the diversity of hetero-oligomers.

Indeed, in the case of plant sHSPs it has been shown that a mono- to polydisperse transition occurs under heat-shock conditions, and therefore both the large number of sHSPs and polydispersity can combine to create astonishingly large possible numbers [111]. It remains to be proven to what extent this diversity exists *in vivo*, and how it acts to regulate molecular chaperone function. It is however enlightening to consider the parallels with the immune system in higher eukaryotes, whereby a relatively small number of genes (~300) can give rise to over 10^8 different antibodies [196], allowing the recognition of the diverse structures of antigens. It is tempting to speculate that evolution of such an extensive sHSP ensemble, within the context of the wider chaperone network [197], allows organisms to protect themselves against the diversity of unfolding client proteins and thereby maintain proteostasis.

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References

1. Narberhaus F (2002) Alpha-crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network. Microbiol Mol Biol Rev 66(1):64–93 (table of contents)
2. Kriehuber T et al (2010) Independent evolution of the core domain and its flanking sequences in small heat shock proteins. FASEB J 24(10):3633–3642
3. Waters ER, Aevermann BD, Sanders-Reed Z (2008) Comparative analysis of the small heat shock proteins in three angiosperm genomes identifies new subfamilies and reveals diverse evolutionary patterns. Cell Stress Chaperones 13(2):127–142
4. Kappe G et al (2003) The human genome encodes 10 alpha-crystallin-related small heat shock proteins: HspB1-10. Cell Stress Chaperones 8(1):53–61
5. Nakamoto H, Vigh L (2007) The small heat shock proteins and their clients. Cell Mol Life Sci 64(3):294–306
6. Haslbeck M et al (2005) Some like it hot: the structure and function of small heat-shock proteins. Nat Struct Mol Biol 12(10):842–846
7. Basha E, O'Neill H, Vierling E (2012) Small heat shock proteins and alpha-crystallins: dynamic proteins with flexible functions. Trends Biochem Sci 37(3):106–117
8. Ellis RJ, van der Vies SM (1991) Molecular chaperones. Annu Rev Biochem 60:321–347
9. McHaourab HS, Godar JA, Stewart PL (2009) Structure and mechanism of protein stability sensors: chaperone activity of small heat shock proteins. Biochemistry 48(18):3828–3837
10. Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. Nature 475(7356):324–332
11. Balch WE et al (2008) Adapting proteostasis for disease intervention. Science 319 (5865):916–919
12. Baldwin AJ et al (2011) Metastability of native proteins and the phenomenon of amyloid formation. J Am Chem Soc 144(36):14160–14163
13. Olzscha H et al (2011) Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. Cell 144(1):67–78
14. Powers ET et al (2009) Biological and chemical approaches to diseases of proteostasis deficiency. Annu Rev Biochem 78:959–991
15. Chiti F, Dobson CM (2006) Protein misfolding, functional amyloid, and human disease. Annu Rev Biochem 75:333–366
16. Beck M et al (2009) Visual proteomics of the human pathogen Leptospira interrogans. Nat Methods 6(11):817–823
17. Malmstrom J et al (2009) Proteome-wide cellular protein concentrations of the human pathogen Leptospira interrogans. Nature 460(7256):762–765
18. Ecroyd H, Carver JA (2009) Crystallin proteins and amyloid fibrils. Cell Mol Life Sci 66 (1):62–81
19. Arrigo AP et al (2007) Hsp27 (HspB1) and alphaB-crystallin (HspB5) as therapeutic targets. FEBS Lett 581(19):3665–3674
20. Sun Y, MacRae TH (2005) The small heat shock proteins and their role in human disease. FEBS J 272(11):2613–2627
21. Carra S et al (2011) Alteration of protein folding and degradation in motor neuron diseases: implications and protective functions of small heat shock proteins. Prog Neurobiol (in press). doi:10.1016/j.bbr.2011.03.031
22. Horwitz J (2009) Alpha crystallin: the quest for a homogeneous quaternary structure. Exp Eye Res 88(2):190–194
23. Cowieson NP, Kobe B, Martin JL (2008) United we stand: combining structural methods. Curr Opin Struct Biol 18(5):617–622
24. Robinson CV, Sali A, Baumeister W (2007) The molecular sociology of the cell. Nature 450 (7172):973–982

25. Frauenfelder H, Sligar SG, Wolynes PG (1991) The energy landscapes and motions of proteins. Science 254(5038):1598–1603
26. Karplus M, McCammon JA (1983) Dynamics of proteins: elements and function. Annu Rev Biochem 52:263–300
27. Wüthrich K, Wagner G (1978) Internal motion in globular proteins. Trends Biol Sci 3(4):227–230
28. Russel D et al (2009) The structural dynamics of macromolecular processes. Curr Opin Cell Biol 21(1):97–108
29. Boehr DD, Nussinov R, Wright PE (2009) The role of dynamic conformational ensembles in biomolecular recognition. Nat Chem Biol 5(11):789–796
30. Henzler-Wildman K, Kern D (2007) Dynamic personalities of proteins. Nature 450 (7172):964–972
31. Karplus M, Kuriyan J (2005) Molecular dynamics and protein function. Proc Natl Acad Sci USA 102(19):6679–6685
32. Smock RG, Gierasch LM (2009) Sending signals dynamically. Science 324(5924):198–203
33. Baldwin AJ, Kay LE (2009) NMR spectroscopy brings invisible protein states into focus. Nat Chem Biol 5(11):808–814
34. Kappe G, Boelens WC, de Jong WW (2010) Why proteins without an alpha-crystallin domain should not be included in the human small heat shock protein family HSPB. Cell Stress Chaperones 15(4):457–461
35. Haslbeck M et al (2004) A domain in the N-terminal part of Hsp26 is essential for chaperone function and oligomerization. J Mol Biol 343(2):445–455
36. Kappe G et al (2004) Tsp36, a tapeworm small heat-shock protein with a duplicated alpha-crystallin domain, forms dimers and tetramers with good chaperone-like activity. Proteins 57(1):109–117
37. Poulain P, Gelly JC, Flatters D (2010) Detection and architecture of small heat shock protein monomers. PLoS One 5(4):e9990
38. Benndorf R, Welsh MJ (2004) Shocking degeneration. Nat Genet 36(6):547–548
39. Carver JA (1999) Probing the structure and interactions of crystallin proteins by NMR spectroscopy. Prog Retin Eye Res 18(4):431–462
40. Chernik IS et al (2007) Small heat shock protein Hsp20 (HspB6) as a partner of 14-3-3gamma. Mol Cell Biochem 295(1–2):9–17
41. MacCoss MJ et al (2002) Shotgun identification of protein modifications from protein complexes and lens tissue. Proc Natl Acad Sci USA 99(12):7900–7905
42. Kim KK, Kim R, Kim SH (1998) Crystal structure of a small heat-shock protein. Nature 394 (6693):595–599
43. van Montfort RL et al (2001) Crystal structure and assembly of a eukaryotic small heat shock protein. Nat Struct Biol 8(12):1025–1030
44. Takeda K et al (2011) Dimer structure and conformational variability in the N-terminal region of an archaeal small heat shock protein, StHsp14.0. J Struct Biol 174(1):92–99
45. Hilario E et al (2011) Crystal structures of Xanthomonas small heat shock protein provide a structural basis for an active molecular chaperone oligomer. J Mol Biol 408(1):74–86
46. Bagnéris C et al (2009) Crystal structures of alpha-crystallin domain dimers of alphaB-crystallin and Hsp20. J Mol Biol 392(5):1242–1252
47. Baranova EV et al (2011) Three-dimensional structure of alpha-crystallin domain dimers of human small heat shock proteins HSPB1 and HSPB6. J Mol Biol 411(1):110–122
48. Laganowsky A et al (2010) Crystal structures of truncated alphaA and alphaB crystallins reveal structural mechanisms of polydispersity important for eye lens function. Protein Sci 19 (5):1031–1043
49. Berengian AR, Parfenova M, McHaourab HS (1999) Site-directed spin labeling study of subunit interactions in the alpha-crystallin domain of small heat-shock protein – comparison of the oligomer symmetry in alpha A-crystallin, HSP 27, and HSP 16.3. J Biol Chem 274(10):6305–6314

50. Jehle S et al (2009) [alpha]B-crystallin: a hybrid solid-state/solution-state NMR investigation reveals structural aspects of the heterogeneous oligomer. J Mol Biol 385(5):1481–1497
51. Koteiche HA, McHaourab HS (1999) Folding pattern of the alpha-crystallin domain in alpha A-crystallin determined by site-directed spin labeling. J Mol Biol 294(2):561–577
52. Feil IK et al (2001) A novel quaternary structure of the dimeric alpha-crystallin domain with chaperone-like activity. J Biol Chem 276(15):12024–12029
53. Clark AR et al (2011) Crystal structure of R120G disease mutant of human alphaB-crystallin domain dimer shows closure of a groove. J Mol Biol 408(1):118–134
54. Jehle S et al (2010) Solid-state NMR and SAXS studies provide a structural basis for the activation of alphaB-crystallin oligomers. Nat Struct Mol Biol 17(9):1037–1042
55. Hasan A et al (2004) Thermal stability of human alpha-crystallins sensed by amide hydrogen exchange. Protein Sci 13(2):332–341
56. Haley DA et al (2000) Small heat-shock protein structures reveal a continuum from symmetric to variable assemblies. J Mol Biol 298(2):261–272
57. van Montfort R, Slingsby C, Vierling E (2001) Structure and function of the small heat shock protein/alpha-crystallin family of molecular chaperones. Adv Protein Chem 59:105–156
58. Koteiche HA et al (2005) Atomic models by cryo-EM and site-directed spin labeling: application to the N-terminal region of Hsp16.5. Structure 13(8):1165–1171
59. Jehle S et al (2011) N-terminal domain of alphaB-crystallin provides a conformational switch for multimerization and structural heterogeneity. Proc Natl Acad Sci USA 108(16):6409–6414
60. Braun N et al (2011) Multiple molecular architectures of the eye lens chaperone alphaB-crystallin elucidated by a triple hybrid approach. Proc Natl Acad Sci USA 108 (51):20491–20496
61. Stamler R et al (2005) Wrapping the alpha-crystallin domain fold in a chaperone assembly. J Mol Biol 353(1):68–79
62. Cheng GL et al (2008) Insights into small heat shock protein and substrate structure during chaperone action derived from hydrogen/deuterium exchange and mass spectrometry. J Biol Chem 283(39):26634–26642
63. Wintrode PL et al (2003) Solution structure and dynamics of a heat shock protein assembly probed by hydrogen exchange and mass spectrometry. Biochemistry 42(36):10667–10673
64. Kim R et al (2003) On the mechanism of chaperone activity of the small heat-shock protein of Methanococcus jannaschii. Proc Natl Acad Sci USA 100(14):8151–8155
65. Jaya N, Garcia V, Vierling E (2009) Substrate binding site flexibility of the small heat shock protein molecular chaperones. Proc Natl Acad Sci USA 106(37):15604–15609
66. Baldwin AJ et al (2011) Quaternary dynamics of aB-crystallin as a direct consequence of localised tertiary fluctuations in the C-terminus. J Mol Biol 413(2):310–320
67. Treweek TM et al (2010) A quantitative NMR spectroscopic examination of the flexibility of the C-terminal extensions of the molecular chaperones, [alpha]A- and [alpha]B-crystallin. Exp Eye Res 91(5):691–699
68. Ghahghaei A et al (2009) Structure/function studies of dogfish alpha-crystallin, comparison with bovine alpha-crystallin. Mol Vis 15:2411–2420
69. Hilton GR et al (2012) A labile C-terminal interaction mediates the quaternary dynamics of αB crystallin. Phil Trans R Soc B (under consideration)
70. Carver JA et al (1992) Identification by H-1-NMR spectroscopy of flexible C-terminal extensions in bovine lens alpha-crystallin. FEBS Lett 311(2):143–149
71. Carver JA, Lindner RA (1998) NMR spectroscopy of alpha-crystallin. Insights into the structure, interactions and chaperone action of small heat-shock proteins. Int J Biol Macromol 22(3–4):197–209
72. Aquilina JA et al (2005) Subunit exchange of polydisperse proteins: mass spectrometry reveals consequences of alphaA-crystallin truncation. J Biol Chem 280(15):14485–14491
73. Takemoto LJ (1997) Changes in the C-terminal region of alpha-A crystallin during human cataractogenesis. Int J Biochem Cell Biol 29(2):311–315

74. Laganowsky A, Eisenberg D (2010) Non-3D domain swapped crystal structure of truncated zebrafish alphaA crystallin. Protein Sci 19(10):1978–1984
75. Shi J et al (2006) Cryoelectron microscopy and EPR analysis of engineered symmetric and polydisperse Hsp16.5 assemblies reveals determinants of polydispersity and substrate binding. J Biol Chem 281(52):40420–40428
76. Liddington RC et al (1991) Structure of simian virus 40 at 3.8-A resolution. Nature 354 (6351):278–284
77. Abrescia NG et al (2004) Insights into assembly from structural analysis of bacteriophage PRD1. Nature 432(7013):68–74
78. Koteiche HA, McHaourab HS (2002) The determinants of the oligomeric structure in Hsp16.5 are encoded in the alpha-crystallin domain. FEBS Lett 519(1–3):16–22
79. Bertz M et al (2010) Structural and mechanical hierarchies in the alpha-crystallin domain dimer of the hyperthermophilic small heat shock protein Hsp16.5. J Mol Biol 400 (5):1046–1056
80. Basha E et al (2010) Mechanistic differences between two conserved classes of small heat shock proteins found in the plant cytosol. J Biol Chem 285(15):11489–11497
81. Aquilina JA et al (2003) Polydispersity of a mammalian chaperone: mass spectrometry reveals the population of oligomers in alphaB-crystallin. Proc Natl Acad Sci USA 100 (19):10611–10616
82. Baldwin AJ et al (2011) αB-crystallin polydispersity is a consequence of unbiased quaternary dynamics. J Mol Biol 413(2):297–309
83. Benesch JLP et al (2010) The quaternary organization and dynamics of the molecular chaperone HSP26 are thermally regulated. Chem Biol 17(9):1008–1017
84. Kennaway CK et al (2005) Dodecameric structure of the small heat shock protein Acr1 from Mycobacterium tuberculosis. J Biol Chem 280(39):33419–33425
85. Baldwin AJ et al (2011) The polydispersity of αB-crystallin is rationalised by an interconverting polyhedral architecture. Structure 19(12):1855–1863
86. Tardieu A (1988) Eye lens proteins and transparency: from light transmission theory to solution X-ray structural analysis. Annu Rev Biophys Biophys Chem 17:47–70
87. Alexander N et al (2008) De novo high-resolution protein structure determination from sparse spin-labeling EPR data. Structure 16(2):181–195
88. McHaourab HS, Berengian AR, Koteiche HA (1997) Site-directed spin-labeling study of the structure and subunit interactions along a conserved sequence in the alpha-crystallin domain of heat-shock protein. 27. Evidence of a conserved subunit interface. Biochemistry 36 (48):14627–14634
89. Scheres SH et al (2007) Disentangling conformational states of macromolecules in 3D-EM through likelihood optimization. Nat Methods 4(1):27–29
90. White HE et al (2004) Recognition and separation of single particles with size variation by statistical analysis of their images. J Mol Biol 336(2):453–460
91. Benesch JLP, Ruotolo BT (2011) Mass spectrometry: an approach come-of-age for structural and dynamical biology. Curr Opin Struct Biol 21(5):641–649
92. Haley DA, Horwitz J, Stewart PL (1998) The small heat-shock protein, alphaB-crystallin, has a variable quaternary structure. J Mol Biol 277(1):27–35
93. Haley DA, Horwitz J, Stewart PL (1999) Image restrained modeling of alpha B-crystallin. Exp Eye Res 68(1):133–136
94. Peschek J et al (2009) The eye lens chaperone alpha-crystallin forms defined globular assemblies. Proc Natl Acad Sci USA 106(32):13272–13277
95. White HE et al (2006) Multiple distinct assemblies reveal conformational flexibility in the small heat shock protein Hsp26. Structure 14(7):1197–1204
96. Lambert W et al (2011) Subunit arrangement in the dodecameric chloroplast small heat shock protein Hsp21. Protein Sci: Publ Protein Soc 20(2):291–301
97. Haslbeck M et al (2008) Structural dynamics of archaeal small heat shock proteins. J Mol Biol 378(2):362–374

98. Cao A et al (2008) Preheating induced homogeneity of the small heat shock protein from Methanococcus jannaschii. Biochim Biophys Acta Proteins Proteomics 1784(3):489–495
99. Kim DR et al (2003) Activation mechanism of HSP16.5 from Methanococcus jannaschii. Biochem Biophys Res Commun 307(4):991–998
100. Franzmann TM et al (2008) Activation of the chaperone Hsp26 is controlled by the rearrangement of its thermosensor domain. Mol Cell 29(2):207–216
101. Sobott F et al (2002) Subunit exchange of multimeric protein complexes. Real-time monitoring of subunit exchange between small heat shock proteins by using electrospray mass spectrometry. J Biol Chem 277(41):38921–38929
102. Siezen RJ, Bindels JG, Hoenders HJ (1978) The quaternary structure of bovine alpha-crystallin. Size and charge microheterogeneity: more than 1000 different hybrids? Eur J Biochem 91(2):387–396
103. Siezen RJ, Owen EA (1983) Physicochemical characterization of high-molecular-weight alpha-crystallin subpopulations from the calf lens nucleus. Biochim Biophys Acta 749 (3):227–237
104. van den Oetelaar PJ et al (1990) A dynamic quaternary structure of bovine alpha-crystallin as indicated from intermolecular exchange of subunits. Biochemistry 29(14):3488–3493
105. Bova MP et al (1997) Subunit exchange of alpha A-crystallin. J Biol Chem 272 (47):29511–29517
106. Bova MP et al (2000) Subunit exchange of small heat shock proteins – analysis of oligomer formation of alpha A-crystallin and Hsp27 by fluorescence resonance energy transfer and site-directed truncations. J Biol Chem 275(2):1035–1042
107. Guan YH et al (2006) Subunit exchange of MjHsp16.5 studied by single-molecule imaging and fluorescence resonance energy transfer. J Am Chem Soc 128(22):7203–7208
108. Painter AJ et al (2008) Real-time monitoring of protein complexes reveals their quaternary organization and dynamics. Chem Biol 15(3):246–253
109. Haslbeck M et al (1999) Hsp26: a temperature-regulated chaperone. EMBO J 18 (23):6744–6751
110. Benesch JLP, Sobott F, Robinson CV (2003) Thermal dissociation of multimeric protein complexes by using nanoelectrospray mass spectrometry. Anal Chem 75(10):2208–2214
111. Stengel F et al (2010) Quaternary dynamics and plasticity underlie small heat shock protein chaperone function. Proc Natl Acad Sci USA 107(5):2007–2012
112. Ito H et al (2001) Phosphorylation-induced change of the oligomerization state of alpha B-crystallin. J Biol Chem 276(7):5346–5352
113. Kato K et al (1994) Dissociation as a result of phosphorylation of an aggregated form of the small stress protein, hsp27. J Biol Chem 269(15):11274–11278
114. Michiel M et al (2009) Abnormal assemblies and subunit exchange of alpha B-crystallin R120 mutants could be associated with destabilization of the dimeric substructure. Biochemistry 48(2):442–453
115. Treweek TM et al (2007) Site-directed mutations in the C-terminal extension of human alphaB-crystallin affect chaperone function and block amyloid fibril formation. PLoS One 2 (10):e1046
116. Hayes VH, Devlin G, Quinlan RA (2008) Truncation of alphaB-crystallin by the myopathy-causing Q151X mutation significantly destabilizes the protein leading to aggregate formation in transfected cells. J Biol Chem 283(16):10500–10512
117. Ke L et al (2011) HSPB1, HSPB6, HSPB7 and HSPB8 protect against RhoA GTPase-induced remodeling in tachypaced atrial myocytes. PLoS One 6(6):e20395
118. Vos MJ et al (2010) HSPB7 is the most potent polyQ aggregation suppressor within the HSPB family of molecular chaperones. Hum Mol Genet 19(23):4677–4693
119. Horwitz J (1992) Alpha-crystallin can function as a molecular chaperone. Proc Natl Acad Sci USA 89(21):10449–10453

120. Brady JP et al (1997) Targeted disruption of the mouse alpha A-crystallin gene induces cataract and cytoplasmic inclusion bodies containing the small heat shock protein alpha B-crystallin. Proc Natl Acad Sci USA 94(3):884–889
121. Brady JP et al (2001) AlphaB-crystallin in lens development and muscle integrity: a gene knockout approach. Invest Ophthalmol Vis Sci 42(12):2924–2934
122. Jakob U et al (1993) Small heat-shock proteins are molecular chaperones. J Biol Chem 268 (3):1517–1520
123. Lee GJ, Pokala N, Vierling E (1995) Structure and in vitro molecular chaperone activity of cytosolic small heat shock proteins from pea. J Biol Chem 270(18):10432–10438
124. Merck KB et al (1993) Structural and functional similarities of bovine alpha-crystallin and mouse small heat-shock protein – a family of chaperones. J Biol Chem 268(2):1046–1052
125. Chang Z et al (1996) Mycobacterium tuberculosis 16-kDa antigen (Hsp16.3) functions as an oligomeric structure in vitro to suppress thermal aggregation. J Biol Chem 271 (12):7218–7223
126. Richter K, Haslbeck M, Buchner J (2010) The heat shock response: life on the verge of death. Mol Cell 40(2):253–266
127. Friedrich KL et al (2004) Interactions between small heat shock protein subunits and substrate in small heat shock protein-substrate complexes. J Biol Chem 279(2):1080–1089
128. Stromer T et al (2003) Analysis of the interaction of small heat shock proteins with unfolding proteins. J Biol Chem 278(20):18015–18021
129. Basha E, Friedrich KL, Vierling E (2006) The N-terminal arm of small heat shock proteins is important for both chaperone activity and substrate specificity. J Biol Chem 281 (52):39943–39952
130. Stengel F et al. Dissecting heterogeneous molecular chaperone complexes using a mass spectrum deconvolution approach. Chem Biol (in press)
131. Eyles SJ, Gierasch LM (2010) Nature's molecular sponges: small heat shock proteins grow into their chaperone roles. Proc Natl Acad Sci USA 107(7):2727–2728
132. Basha E et al (2004) The identity of proteins associated with a small heat shock protein during heat stress in vivo indicates that these chaperones protect a wide range of cellular functions. J Biol Chem 279(9):7566–7575
133. Ecroyd H et al (2007) Mimicking phosphorylation of alphaB-crystallin affects its chaperone activity. Biochem J 401(1):129–141
134. Giese KC et al (2005) Evidence for an essential function of the N terminus of a small heat shock protein in vivo, independent of in vitro chaperone activity. Proc Natl Acad Sci USA 102(52):18896–18901
135. Mogk A et al (2003) Small heat shock proteins, ClpB and the DnaK system form a functional triade in reversing protein aggregation. Mol Microbiol 50(2):585–595
136. Petko L, Lindquist S (1986) Hsp26 is not required for growth at high temperatures, nor for thermotolerance, spore development, or germination. Cell 45(6):885–894
137. Giese KC, Vierling E (2002) Changes in oligomerization are essential for the chaperone activity of a small heat shock protein in vivo and in vitro. J Biol Chem 277(48):46310–46318
138. Haslbeck M et al (2004) Hsp42 is the general small heat shock protein in the cytosol of Saccharomyces cerevisiae. EMBO J 23(3):638–649
139. Ehrnsperger M et al (1999) The dynamics of Hsp25 quaternary structure. Structure and function of different oligomeric species. J Biol Chem 274(21):14867–14874
140. Farahbakhsh ZT et al (1995) Interaction of alpha-crystallin with spin-labeled peptides. Biochemistry 34(2):509–516
141. Bennardini F, Wrzosek A, Chiesi M (1992) Alpha B-crystallin in cardiac tissue. Association with actin and desmin filaments. Circ Res 71(2):288–294
142. Koretz JF, Doss EW, LaButti JN (1998) Environmental factors influencing the chaperone-like activity of alpha-crystallin. Int J Biol Macromol 22(3–4):283–294
143. Poon S et al (2002) Mildly acidic pH activates the extracellular molecular chaperone clusterin. J Biol Chem 277(42):39532–39540

144. Bassnett S, Duncan G (1986) Variation of pH with depth in the rat lens measured by double-barrelled ion-sensitive microelectrodes. In: Duncan G (ed) The lens: transparency and cataract. Proceedings of the EURAGE/BBS symposium, Eurage, Rijswijk, pp. 77–85
145. Mathias RT, Riquelme G, Rae JL (1991) Cell to cell communication and pH in the frog lens. J Gen Physiol 98(6):1085–1103
146. Poole-Wilson PA (1978) Measurement of myocardial intracellular pH in pathological states. J Mol Cell Cardiol 10(6):511–526
147. Matuszewska E et al (2008) Escherichia coli heat-shock proteins IbpA/B are involved in resistance to oxidative stress induced by copper. Microbiology 154(Pt 6):1739–1747
148. Ahmad MF et al (2008) Selective Cu^{2+} binding, redox silencing, and cytoprotective effects of the small heat shock proteins alphaA- and alphaB-crystallin. J Mol Biol 382(3):812–824
149. Narayanan S et al (2006) alphaB-crystallin competes with Alzheimer's disease beta-amyloid peptide for peptide-peptide interactions and induces oxidation of Abeta-Met35. FEBS Lett 580(25):5941–5946
150. Biswas A, Das KP (2008) Zn^{2+} enhances the molecular chaperone function and stability of alpha-crystallin. Biochemistry 47(2):804–816
151. Ganadu ML et al (2004) Effects of divalent metal ions on the alphaB-crystallin chaperone-like activity: spectroscopic evidence for a complex between copper(II) and protein. J Inorg Biochem 98(6):1103–1109
152. Jang HH et al (2004) Two enzymes in one; two yeast peroxiredoxins display oxidative stress-dependent switching from a peroxidase to a molecular chaperone function. Cell 117 (5):625–635
153. Lee JR et al (2009) Heat-shock dependent oligomeric status alters the function of a plant-specific thioredoxin-like protein, AtTDX. Proc Natl Acad Sci USA 106(14):5978–5983
154. Biswas A, Das KP (2004) Role of ATP on the interaction of alpha-crystallin with its substrates and its implications for the molecular chaperone function. J Biol Chem 279 (41):42648–42657
155. Muchowski PJ, Clark JI (1998) ATP-enhanced molecular chaperone functions of the small heat shock protein human alphaB crystallin. Proc Natl Acad Sci USA 95(3):1004–1009
156. Hilario E et al (2006) Crystallization and preliminary X-ray diffraction analysis of XAC1151, a small heat-shock protein from Xanthomonas axonopodis pv. citri belonging to the alpha-crystallin family. Acta Crystallogr Sect F Struct Biol Cryst Commun 62(Pt 5):446–448
157. Muchowski PJ et al (1999) ATP and the core: "alpha-crystallin" domain of the small heat-shock protein alphaB-crystallin. J Biol Chem 274(42):30190–30195
158. Saibil HR (2008) Chaperone machines in action. Curr Opin Struct Biol 18(1):35–42
159. Ito H et al (1997) Phosphorylation of alphaB-crystallin in response to various types of stress. J Biol Chem 272(47):29934–29941
160. Wang K, Gawinowicz MA, Spector A (2000) The effect of stress on the pattern of phosphorylation of alphaA and alphaB crystallin in the rat lens. Exp Eye Res 71(4):385–393
161. Marin R, Landry J, Tanguay RM (1996) Tissue-specific posttranslational modification of the small heat shock protein HSP27 in Drosophila. Exp Cell Res 223(1):1–8
162. Aquilina JA et al (2004) Phosphorylation of alpha B-crystallin alters chaperone function through loss of dimeric substructure. J Biol Chem 279(27):28675–28680
163. Koteiche HA, McHaourab HS (2002) Effect of phosphorylation on the chaperone function of alpha B crystallin. Invest Ophthalmol Vis Sci 43:3567
164. Koteiche HA, McHaourab HS (2003) Mechanism of chaperone function in small heat-shock proteins – phosphorylation-induced activation of two-mode binding in alpha B-crystallin. J Biol Chem 278(12):10361–10367
165. Gaestel M (2002) sHsp-phosphorylation: enzymes, signaling pathways and functional implications. Prog Mol Subcell Biol 28:151–169
166. Ehrnsperger M et al (1997) Binding of non-native protein to Hsp25 during heat shock creates a reservoir of folding intermediates for reactivation. EMBO J 16(2):221–229

167. Lee GJ et al (1997) A small heat shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state. EMBO J 16(3):659–671
168. Lee GJ, Vierling E (2000) A small heat shock protein cooperates with heat shock protein 70 systems to reactivate a heat-denatured protein. Plant Physiol 122(1):189–198
169. Veinger L et al (1998) The small heat-shock protein IbpB from Escherichia coli stabilizes stress-denatured proteins for subsequent refolding by a multichaperone network. J Biol Chem 273(18):11032–11037
170. Liberek K, Lewandowska A, Zietkiewicz S (2008) Chaperones in control of protein disaggregation. EMBO J 27(2):328–335
171. Matuszewska M et al (2005) The small heat shock protein IbpA of Escherichia coli cooperates with IbpB in stabilization of thermally aggregated proteins in a disaggregation competent state. J Biol Chem 280(13):12292–12298
172. Mogk A et al (2003) Refolding of substrates bound to small Hsps relies on a disaggregation reaction mediated most efficiently by ClpB/DnaK. J Biol Chem 278(33):31033–31042
173. Haslbeck M et al (2005) Disassembling protein aggregates in the yeast cytosol. The cooperation of Hsp26 with Ssa1 and Hsp104. J Biol Chem 280(25):23861–23868
174. Cashikar AG, Duennwald M, Lindquist SL (2005) A chaperone pathway in protein disaggregation. Hsp26 alters the nature of protein aggregates to facilitate reactivation by Hsp104. J Biol Chem 280(25):23869–23875
175. Ratajczak E, Zietkiewicz S, Liberek K (2009) Distinct activities of Escherichia coli small heat shock proteins IbpA and IbpB promote efficient protein disaggregation. J Mol Biol 386 (1):178–189
176. Vos MJ et al (2011) Small heat shock proteins, protein degradation and protein aggregation diseases. Autophagy 7(1):101–103
177. den Engelsman J et al (2003) The small heat-shock protein alpha B-crystallin promotes FBX4-dependent ubiquitination. J Biol Chem 278(7):4699–4704
178. Parcellier A et al (2003) HSP27 is a ubiquitin-binding protein involved in I-kappaBalpha proteasomal degradation. Mol Cell Biol 23(16):5790–5802
179. Lin DI et al (2006) Phosphorylation-dependent ubiquitination of cyclin D1 by the SCF (FBX4-alphaB crystallin) complex. Mol Cell 24(3):355–366
180. Ahner A et al (2007) Small heat-shock proteins select deltaF508-CFTR for endoplasmic reticulum-associated degradation. Mol Biol Cell 18(3):806–814
181. Bissonnette SA et al (2010) The IbpA and IbpB small heat-shock proteins are substrates of the AAA+ Lon protease. Mol Microbiol 75(6):1539–1549
182. Meyer AS, Baker TA (2011) Proteolysis in the Escherichia coli heat shock response: a player at many levels. Curr Opin Microbiol 14(2):194–199
183. Stromer T et al (2004) Analysis of the regulation of the molecular chaperone Hsp26 by temperature-induced dissociation: the N-terminal domain is important for oligomer assembly and the binding of unfolding proteins. J Biol Chem 279(12):11222–11228
184. Aquilina JA, Watt SJ (2007) The N-terminal domain of alphaB-crystallin is protected from proteolysis by bound substrate. Biochem Biophys Res Commun 353(4):1115–1120
185. Lindner RA et al (2000) Mouse Hsp25, a small heat shock protein – the role of its C-terminal extension in oligomerization and chaperone action. Eur J Biochem 267(7):1923–1932
186. Smulders R et al (1996) Immobilization of the C-terminal extension of bovine alphaA-crystallin reduces chaperone-like activity. J Biol Chem 271(46):29060–29066
187. Bhattacharyya J et al (2006) Mini-alphaB-crystallin: a functional element of alphaB-crystallin with chaperone-like activity. Biochemistry 45(9):3069–3076
188. Sharma KK, Kaur H, Kester K (1997) Functional elements in molecular chaperone alpha-crystallin: identification of binding sites in alpha B-crystallin. Biochem Biophys Res Commun 239(1):217–222
189. Sharma KK et al (1998) Interaction of 1,1′-bi(4-anilino)naphthalene-5,5′-disulfonic acid with alpha-crystallin. J Biol Chem 273(15):8965–8970

190. Shashidharamurthy R et al (2005) Mechanism of chaperone function in small heat shock proteins: dissociation of the HSP27 oligomer is required for recognition and binding of destabilized T4 lysozyme. J Biol Chem 280(7):5281–5289
191. Franzmann TM et al (2005) The activation mechanism of Hsp26 does not require dissociation of the oligomer. J Mol Biol 350(5):1083–1093
192. Augusteyn RC (2004) Dissociation is not required for alpha-crystallin's chaperone function. Exp Eye Res 79(6):781–784
193. Ragusa MJ et al (2010) Spinophilin directs protein phosphatase 1 specificity by blocking substrate binding sites. Nat Struct Mol Biol 17(4):459–464
194. Carver JA et al (2002) The interaction of the molecular chaperone alpha-crystallin with unfolding alpha-lactalbumin: a structural and kinetic spectroscopic study. J Mol Biol 318 (3):815–827
195. Fontaine JM et al (2005) Interactions of HSP22 (HSPB8) with HSP20, alphaB-crystallin, and HSPB3. Biochem Biophys Res Commun 337(3):1006–1011
196. Tonegawa S (1983) Somatic generation of antibody diversity. Nature 302(5909):575–581
197. Gong Y, Kakihara Y, Krogan N, Greenblatt J, Emili A, Zhang Z, Houry WA (2009) An atlas of chaperone-protein interactions in Saccharomyces cerevisiae: implications to protein folding pathways in the cell. Mol Syst Biol 5:275, Epub June 16, 2009