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Small heat-shock proteins: important players in regulating cellular proteostasis

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Abstract

Small heat-shock proteins (sHsps) are a diverse family of intra-cellular molecular chaperone proteins that play a critical role in mitigating and preventing protein aggregation under stress conditions such as elevated temperature, oxidation and infection. In doing so, they assist in the maintenance of protein homeostasis (proteostasis) thereby avoiding the deleterious effects that result from loss of protein function and/or protein aggregation. The chaperone properties of sHsps are therefore employed extensively in many tissues to prevent the development of diseases associated with protein aggregation. Significant progress has been made of late in understanding the structure and chaperone mechanism of sHsps. In this review, we discuss some of these advances, with a focus on mammalian sHsp hetero-oligomerisation, the mechanism by which sHsps act as molecular chaperones to prevent both amorphous and fibrillar protein aggregation, and the role of post-translational modifications in sHsp chaperone function, particularly in the context of disease.

Keywords

small heat-shock protein, protein aggregation, molecular chaperone, proteostasis, cataract, neurodegenerative disease

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Small heat-shock proteins: important players in regulating cellular proteostasis

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Abbreviations

Aβ: amyloid β
αAc: αA-crystallin
αBc: αB-crystallin
αc: α-crystallin
ACD: α-crystallin domain
AD: Alzheimer's disease
ALS: amylotrophic lateral sclerosis
α-syn: α-synuclein
CJD: Creutzfeldt-Jakob disease
CMT: Charcot-Marie-Tooth
cryo-EM: cryo-electron microscopy
DRM: desmin-related myopathy
GAFP: glial acidic fibrillary protein
HMN: hereditary motor neuropathy
Hsp: heat-shock protein
MS: Multiple sclerosis
PD: Parkinson's disease
PrP^{sc}: prion protein
SAXS: small-angle X-ray scattering
sHsp: small heat-shock protein
TEM: Transmission Electron Microscopy

Keywords

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Running Title

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Abstract (word count = 159)

Small heat-shock proteins (sHsps) are a diverse family of intracellular molecular chaperone proteins that play a critical role in preventing protein unfolding, misfolding and aggregation, particularly under stress conditions such as elevated temperature, oxidation and infection. In doing so, they assist in the maintenance of protein homeostasis (proteostasis) and thereby avoid the deleterious effects that result from loss of protein function and/or protein aggregation. The chaperone properties of sHsps are therefore employed extensively in many tissues to prevent the development of diseases associated with protein aggregation. There has been much research into the structure and mechanism of chaperone action of sHsps over approximately the past 30 years, and significant progress has been made of late, however, there are still many unanswered questions relating to these aspects of sHsps. In this review, we outline some of the recent advances in understanding the structure and function of mammalian sHsps, particularly in the context of their many and varied roles in disease.

Introduction

The proteome is inherently meta-stable [1,2]. Arguably, the most important means nature uses to maintain the integrity of the proteome (i.e. proteostasis) is via network of molecular chaperone proteins, which are present both intra- and extracellularly. The abundant and ubiquitous small heat-shock proteins (sHsps) are a family of intracellular molecular chaperones that interact with unfolding target proteins to stabilise them and prevent their aggregation and precipitation. They do so under constitutive conditions but are particularly prevalent under stress conditions when their expression is significantly up-regulated. Many sHsps have a wide tissue distribution and as a result are associated with a plethora of disease states, particularly those related to compromised proteostasis (e.g. involving defective protein folding). In the eye lens, for example, the high concentration of the two major sHsps, αA- and αB-crystallin, prevents protein aggregation, in addition to ensuring proper lens transparency. In this review, we summarise recent research into sHsp structure, function and interactions. Following a brief introduction to the structure and chaperone action of sHsps and the effects of sHsp post-translational modification, specifically phosphorylation, we discuss the involvement of sHsps in diseases, particularly neurodegenerative ones. Related to this, we examine the effect of sHsp mutations on chaperone function and hence disease manifestation, particularly cataract.

SECTION I: The structure and function of sHsps

Each sHsp is comprised of three regions: N- and C-terminal regions which are separated by a highly conserved α-crystallin domain (ACD) of approximately 80 amino acids in length that is a defining characteristic of all sHsps. By contrast, the N- and C-terminal flanking regions are variable in length between the sHsps and lack significant sequence similarity. The structures of mammalian sHsps have proved difficult to decipher. Of late, a battery of powerful and complementary techniques, i.e. X-ray crystallography, solid-state and solution NMR spectroscopy, small-angle X-ray scattering (SAXS), mass spectrometry and cryo- and transmission electron microscopy (cryo-EM and TEM, respectively) have been utilised to gain unprecedented insight into the structure of sHsps. We shall only discuss the salient structural features that have become apparent from these studies as more in-depth discussion has been presented in recent reviews of sHsp structure [3-5].

The subunit monomeric mass of sHsps ranges from 15 to 40 kDa, however their classification (and nomenclature) as sHsps are somewhat of a misnomer as they typically exist as large, spherical, oligomeric

species in solution. In the case of αBc, these oligomers are highly heterogeneous with a mass range under physiological conditions, as determined by light scattering, of 420 – 980 kDa [6] and an average mass of approximately 650 kDa [7]. Moreover, sHsps are highly dynamic species with extensive subunit exchange occurring which may be important in their chaperone function [8]. In addition, the dynamic nature of sHsps is also due to large portions, particularly within the N- and C-terminal regions, having little well-defined secondary structure and exhibiting flexibility. The extreme C-terminus is very much so with solution-phase NMR spectroscopy revealing that the last 10 and 12 amino acids in αAc and αBc respectively are unstructured C-terminal extensions that have mobility comparable to isolated peptides of the same length [9-12]. This heterogeneity, dynamism and flexibility have inhibited crystallisation attempts of the full-length proteins, however X-ray crystal structures are available for some non-mammalian sHsps which lack the flexible C-terminal extensions and form well-ordered oligomers, e.g. of Hsp16.5, a 24-mer from a hyperthermophilic archeon *Methanococcus jannaschii* [13] (Fig. 1A) and Hsp16.9, a 12-mer from wheat [14].

More recently, crystal structures of the isolated ACD from αBc [15], Hsp27 [16] and Hsp20 [15] have confirmed the predicted features of this domain, i.e. that it is highly β-sheet in character and arranged in an immunoglobulin-like fold. Structures of the ACD of αAc and αBc that also contained the majority of the C-terminal region, but not the C-terminal extension, were recently solved [17]. These structures demonstrated that two β-strands (numbers 6 and 7) from each monomer are arranged in an anti-parallel manner and provide the dimer interface that mediates higher-order assembly [17] (Fig. 1B). Furthermore, the conserved I-X-I motif (I159-P160-I161 in αBc) in the C-terminal region of one subunit can bind in a groove formed between the β4 and β8 strands on a nearby subunit [18] (Fig. 1B). The I-X-I motif is the central part of a nine amino acid palindromic sequence that enables equivalent interactions in both directions of the polypeptide chain with other subunits. As a result, αBc polydisperse oligomerisation is facilitated which prevents its crystallisation. Crystal formation is deleterious to lens transparency and this palindromic sequence may be the crucial factor in inhibiting crystallisation and in enabling dynamic interactions both with itself and its target proteins during chaperone action.

There is much evidence, e.g. from the above-mentioned X-ray crystallographic studies and mass spectrometry [19], that the building block of the sHsp oligomer is a dimer. In agreement with the X-ray crystallographic studies, a solution and solid-state NMR and SAXS investigation of full-length αBc concluded that the ACD adopts an immunoglobulin-like β-sheet structure [20]. Subsequent solid-state NMR studies concluded that the

N-terminal flanking region has two short helices and a small stretch of anti-parallel β-sheet near the boundary with the ACD [21]. The heterogeneity of NMR resonances, however, implied that significant conformational mobility exists in the N-terminal region. From these data, in combination with TEM and SAXS measurements, a model for the quaternary arrangement of αBc was constructed [21].

The quaternary structure of αBc has been studied for many years, yet there is no agreement as to the precise quaternary arrangement of mammalian sHsp oligomers with various models proposed (summarised in [22]). TEM and cryo-EM studies under a variety of solution conditions and temperatures have provided insight into the quaternary arrangement of the αBc oligomer. Under physiological conditions, human αBc forms large roughly spherical assemblies of 8-18 nm in diameter [7,23]. Under partially denaturing conditions and elevated temperature, αBc also assembles into amyloid fibrils as revealed by TEM and atomic force microscopy [24,25]. Cryo-EM reconstructions applied to αBc suggest a dynamic and variable asymmetric quaternary structure, with a roughly spherical protein shell of average diameter ~15 nm which surrounds a large central cavity of ~8 nm in diameter [7,23,26]. By contrast, for particular purifications of αBc, TEM studies reveal a homogeneous population of defined oligomers, comprising 24 subunits [27]. The 3D reconstructions from these images show 13.5 nm diameter sphere-like structures, with an 8.5 nm diameter cavity, and a symmetrical shell of thickness measuring 2.5 to 4.0 nm [27], with large openings in the shell leading to the interior [27] (Fig. 1C). A large central cavity is characteristic of sHsp oligomers (Fig. 1C), as was predicted from simple consideration of polypeptide occupancy within such oligomers [28].

α-Crystallin in the lens

In humans, there are ten sHsps (HSPB1-10), the predominant being αA-crystallin (αAc, HSPB4), αB-crystallin (αBc, HSPB5) and Hsp27 (HSPB1). α-Crystallin (αc), the complex between αAc and αBc subunits (at a 3:1 molar ratio of αAc to αBc in humans [29]) that is present in the eye lens, along with the other crystallins (β and γ), forms a concentrated, closely associated and ordered array that enables proper refraction of light through the lens and its focusing onto the retina. αC is the principal lens protein and, like its individual subunits, αc forms large, heterogeneous, oligomeric and dynamic complexes, in this case of ~160 to 1,000 kDa in mass [19,23,30]. X-ray and neutron solution scattering experiments have shown that αc increases in size significantly at higher temperature [31-35] such that the radius of gyration changes from 69 ± 3 Å to 81 ± 5 Å following incubation at

65°C [33]. This change is not reversed by cooling, in contrast to the behaviour of αc at high pressure where reversibility is observed [35]. Neutron scattering experiments of complexes formed between αc and γE-crystallin at high temperature (65°C) demonstrated that the latter binds inside the central cavity of αc [33] most likely becoming more surface-exposed at higher temperature due to major structural rearrangements (including partial unfolding) that occur to αc at these temperatures leading to exposure of the core of the oligomer. Access to the core would be facilitated by the porous nature of the αc oligomer [27], i.e. the presence on its surface of openings or 'fenestrations' [32]. Interestingly, under milder stress conditions, target proteins are probably located in the 'fenestrations' rather than inside the oligomer cavity [32,36] implying that αc has a variety of modes of interaction with target proteins, depending on the stress conditions.

For many years, αc was regarded as simply a 'filler' protein that was only found in the lens in high concentrations to ensure proper lens transparency. This situation is certainly not true nowadays. Major research interest in αc (and sHsps in general) was sparked by two seminal observations: (1) Bhat and Nagineni's finding [37] that αBc is present in many non-lens tissues and (2) Horwitz's identification of the molecular chaperone activity of αc whereby it prevents the aggregation and precipitation of a diversity of proteins under stress conditions, e.g. elevated temperature [38]. Horwitz's observation was an experimental verification of Ingolia and Craig's earlier classification of αc as a member of the sHsp molecular chaperone family [39]. In the lens, the chaperone ability of αc is of paramount importance in that it prevents the aggregation and precipitation of the crystallin proteins and hence delays the onset of age-related cataract. In this section we will discuss the role of αc in the lens.

αC possesses two interlinking characteristics within the eye lens that are crucial to maintaining lens transparency and hence visual accuity, namely its structural organisation and its chaperone activity. Firstly, however, it is helpful to describe the unusual environment of the lens to gain a further appreciation of the importance of αc's biological role in this tissue. The avascular eye lens, surrounded by a collagen rich capsule, is composed of fibre cells, which develop from the single layer of epithelial cells on the anterior surface of the lens [40]. The lens fibre cells undergo a differentiation process involving cell elongation, expression of the predominant lens proteins, the crystallins [41], and degradation of cellular organelles, including the removal of nuclei [40,42-44]. This loss of cellular organelles is thought to aid lens transparency, by removing potentially

light scattering structures from the light path [43]. Lens transparency is limited by absorption and scattering of visible light. Visible light absorption is usually negligible for eye lenses since the electronic energy level separations of proteins in general, and thus including the crystallins, are too great to enable molecules to be promoted to the excited state upon irradiation by visible light. Lens transparency is thus determined by scattering of light by the lens cytoplasm protein solution [45].

Crystallin protein concentration has been estimated at approximately 200 mg/mL in the outer lens cortex, and up to 460 mg/mL in the central lens nucleus [46,47]. Despite this extremely high protein concentration and high degree of organisation in the lens, the crystallin proteins do not crystallise, and are free to move [45]. The crystallins are organised in a highly stable supramolecular β-sheet structure within the lens [48-50]. Lens transparency is thought to be maintained by a liquid-like, short-range order in the highly concentrated crystallin solutions, as found in dense liquids or glasses [51]. αC comprises 30-40% w:w of the lens soluble protein [26,52], and is thus a key player in this highly organised structural array.

The loss of cellular organelles in the lens renders the lens fibre cells incapable of protein synthesis, hence there is almost no protein turnover in the lens. Consequently, the crystallin proteins must be maintained in a stable state throughout the lifespan of the organism. The chaperone action of αc in preventing the aggregation of itself and β- and γ-crystallins [38,53-55] is therefore especially important in the lens given the absence of cellular organelles and lack of new protein production. Indeed, it has been proposed that the reason for the predominance of αAc over αBc in the lens is that αAc acts as a chaperone for αBc, which is intrinsically less stable than αAc [53,56].

Expression and function of sHsps extra-lenticularly

Bhat [37] and Horwitz's [38] discoveries are highly interconnected: the extensive extra-lenticular expression of αBc and other sHsps unquestionably arises from their fundamental chaperone activity, i.e. nature has utilised their activity in many and varied roles in cells in order to maintain proteostasis. In addition to the lens, αBc is also located in many parts of the body and is found at high levels in the retina, heart, skeletal muscle, skin, brain, spinal cord, kidneys, lungs [37,57,58], the cochleae of the mammalian ear [59], and the lacrimal gland duct and tears [60]. αAc is present to a much lesser extent, for example in the spleen and thymus [61]. Outside

of the lens, αBc has a wide variety of metabolic and regulatory functions [62,63]; expression is induced by stress, for example by pH extremes, elevated temperature, chemical or heavy metal exposure, or hypertonic stress [64-66] and this confers both thermotolerance [67] and protection against oxidative stress to cells [68,69].

Many of the other members of the sHsp family exhibit wide extra-lenticular tissue distribution with Hsp20, for example, being constitutively and highly expressed in all types of muscle [70]. Hsp22, Hsp27 and αBc are also present in muscle cells, along with being expressed in many other cell types including neurons [70]. The extra-lenticular expression of the major sHsps is summarized in Table 1.

Outside the lens, the sHsps play a vital role in maintaining cell viability under stress conditions: they can reach levels of up to 1% of the total cellular protein pool following stress and they are the most acutely induced of the heat-shock proteins (Hsps). Moreover, the sHsps are an integral component of the proteostasis network in cells and their chaperone (mal)function and/or over-expression is associated with many diseases, e.g. neurodegenerative diseases, various cancers, cataract, cardiomyopathies and multiple sclerosis [71,72] (see Section III). The significance of sHsp malfunction in the context of disease is exemplified by the development of therapeutics that target expression of one sHsp, Hsp27, for the treatment of bladder, prostate and pancreatic cancers [73]. The common theme from this work is that failure of sHsps to act, through their chaperone activity, to stabilise target proteins and prevent their improper interactions in cells can cause disease.

Hetero-oligomerisation of sHsps

Whilst much work has focussed on the structure and function of homo-oligomeric forms of sHsps (see discussion above), *in vivo* sHsps most likely exist predominately as hetero-oligomers. For example, both Hsp27 and αBc are expressed in the kidneys, bladder, lungs, stomach, cardiac and skeletal muscle [66,74-76] and co-localise in both normal and pathological tissues of neurodegenerative patients [74,77,78]. Moreover, they associate, to a partial degree, with each other in these tissues [79] and in stably transfected HeLa cells expressing wild type or the disease-causing mutant R120G αBc [80]. Mixing of subunits is facilitated by the dynamic subunit exchange of mammalian sHsps [8,81,82] and is proposed to play a functional role in chaperone action (see next section). We have recently used mass spectrometry to quantify the rate of subunit exchange from Hsp27 and αBc homo-oligomers to form hetero-oligomers, and characterise the end products [83]. The rate of subunit exchange between Hsp27 and αBc was more rapid than that reported for Hsp27 and αAc, and αAc

and αBc [81,83]. The hetero-oligomers had masses and thermo-stabilities intermediate of the homo-oligomers and their chaperone ability was equivalent to that of αBc (and better than Hsp27).

To-date, there is also evidence for the formation of hetero-oligomeric complexes between αA- and αBc [37,54], αAc and Hsp27 [81], HspB2 and HspB3 [84,85], αBc and Hsp20 [86] and Hsp27 and Hsp20 [82]. However, caution is required in interpreting work that has involved adding a bulky tag directly to the sHsps to measure sub-unit exchange (e.g. adding green fluorescent protein or one of its derivatives for fluorescence energy transfer studies) as these can directly interfere with the dynamism of the sHsps, their ability to form oligomers and thus their chaperone function [87,88]. The formation of hetero-oligomers between sHsps is temperature dependent [8,81,82,89] and, upon heat shock, the hetero-oligomeric assemblies of Hsp27 and αBc in cells dissociate and reform once the cell has recovered [79]. This raises questions as to whether homo- and hetero-oligomeric forms of sHsps play different functional roles in the cells. For example, variation in the subunit composition of hetero-oligomers (as opposed to homo-oligomers) may influence the interaction with and binding of target proteins and therefore facilitate interactions with some cellular components in preference to others.

The chaperone mechanism of sHsps

The sHsps are typically classified as 'holdase' chaperones, however, this description does not fully describe the multi-faceted nature by which they can interact with target proteins to prevent their aggregation. Whilst the sHsps can bind tightly to, and form high-molecular-mass complexes with, destabilised protein intermediates to maintain them in a refolding competent state (e.g. so that they are amenable to refolding by ATP-dependent chaperones such as Hsp 70, when cellular conditions permit [90]), they can also interact with target proteins in a weak and transient manner, briefly stabilising them in order to facilitate their intrinsic ability to refold back to their native (functional) state. For example, αBc forms stable high-molecular-mass complexes with disordered, precipitation-bound intermediates of the target protein α-lactalbumin that expose significant amounts of hydrophobicity to solution but also interacts through weak, transient interactions to suppress the aggregation of relatively stable α-lactalbumin intermediates that expose less hydrophobicity to solution [36,91-94]. The weak transient interactions with target proteins is the mechanism that most likely predominates in cells that are not subjected to stress since these conditions are not conducive to large-scale protein destabilisation. Thus, it is envisaged that sHsps only mediate stable high-molecular-mass complex formation with protein intermediates

(i.e. act as 'holdases') when binding is more energetically favourable than refolding. As such, their broad mechanism of action indicates that a more apt description of sHsp chaperones is as 'stabilisers' rather than 'holdases'.

Currently, no well-defined target protein binding site(s) has been identified for mammalian sHsps. Various studies have suggested that target protein binding is mediated by the N-terminal domain [95-98] or the ACD [99-101]. Indeed, with regards to the latter, we [18] and others [17,20,102] have shown that isolated ACDs exhibit chaperone function. Moreover, binding may also be regulated by multiple regions of the protein. For example, it has been recently suggested that target protein access to a binding site formed between β-sheets 4 and 8 in αBc's ACD is regulated by auto-inhibitory docking of the C-terminal I-X-I hinge region into this groove [22,101]. In this case, hydrophobic target proteins compete with this C-terminal region for binding at this site on αBc. The overall emerging picture is that there is no single target protein binding site on sHsps, rather binding is mediated by hydrophobic sites on the surface of the sHsp and these sites of interaction vary depending on the sHsp and target protein in question.

The remarkable ability of sHsps to distinguish disordered protein intermediates from stable (native-like) intermediates is proposed to be based upon the intermediate's free energy of unfolding [103], the lifetime of the intermediate and its relative degree of exposed hydrophobicity [91]. To-date, the most widely accepted model of sHsp chaperone action is that dissociated species (normally depicted as dimers) are the more chaperone-active species and large oligomers are 'reservoirs' of these species; the dissociated species associate with target proteins and then can re-associate with the large oligomers to form high-molecular-mass sHsp-target protein complexes. This model is favoured because (i) the dissociated species would expose more surface hydrophobicity to solution and therefore be more capable of binding to destabilised target proteins and, (ii) the chaperone activity of sHsps is dependent on subunit exchange. Recent work, including our own, has shed further light on the multi-faceted manner by which sHsps interact with aggregation-prone proteins, particularly those aggregating to form amyloid fibrils (reviewed in [30]). In summary, this work has demonstrated that sHsps can interact with monomeric, oligomeric, prefibrillar and fibrillar forms of target protein in order to prevent their aggregation, i.e. at each stage of the amyloid fibril-forming pathway. Based on this work we propose an expanded model of the chaperone action of sHsps (Fig. 2).

Most studies that have tested the *in vitro* chaperone action of sHsps have involved addition of the chaperone prior to aggregation commencing. Thus, the design of such studies does not address the effect(s) sHsps have on the latter stages of aggregation, which is as important to consider since, *in vivo*, levels of sHsps in the cell increase after aggregation has commenced as a result of the activation of the stress response [104,105]. Apart from interacting with monomeric species, sHsps also bind to species formed further along the aggregation pathway, including mature amyloid fibrils [106-109]. For example, when introduced during the elongation phase of α-synuclein (α-syn) or amyloid-β peptide (Aβ) aggregation, αBc prevents further fibril growth by binding along the length of mature fibrils, preventing secondary nucleation events that facilitate further fibril growth [106-108]. Our recent work, using apolipoprotein C-II (apoC-II) as a model fibril-forming protein, has shown that, by binding to fibrils, αBc stabilises them, preventing their (dilution-induced) fragmentation, and causes them to associate (tangle) into larger species reminiscent of protein inclusions [109]. Both fibril fragmentation and secondary nucleation can be the main sources of small oligomers thought to be responsible for the toxicity associated with the aggregation process [110]. Thus, the fibril-binding activity of sHsps helps to rationalise why sHsps (and indeed other Hsps) are found in protein deposits associated with disease, i.e. by binding to fibrils they facilitate their packing into inclusions, thereby limiting fibril fragmentation and secondary nucleation and providing an alternative protective mechanism to the cell. Recently αBc has also been shown to promote the dissociation of potentially toxic β_2-microglobulin oligomers into monomers, highlighting another role these chaperones may have in cells to protect them from the adverse effects of protein aggregation [111].

Despite our growing appreciation of the mechanisms by which sHsps interact with aggregating proteins there are still critical aspects of the chaperone model of sHsps that require further clarification: (1) There is no definitive evidence to-date that dissociated (dimeric) species are the sole chaperone active species (indeed for some sHsps such as αBc there is no direct evidence for the presence of dissociated species in solution) and some reports suggest that the large oligomers are also chaperone active [112]. For instance, some studies have indicated that there is no correlation between the concentration of sub-oligomeric species (or subunit exchange rate) and apparent chaperone activity [113]. Moreover, glutaraldehyde cross-linked α-crystallin, which is incapable of subunit exchange, retains significant chaperone activity *in vitro* [112]. (2) Little is known about the stoichiometry of mammalian sHsp-target protein complexes. To-date, efforts to study, in precise detail, the manner by which sHsps bind to target proteins to form complexes have been hampered by the large, polydisperse and dynamic nature of sHsp oligomers and the low abundance of individual species in these

heterogeneous samples. This problem is further confounded by the use of 'bulk' averaging techniques that can mask the presence of rare species formed in such dynamic systems. (3) The model is predominately based on *in vitro* studies of sHsp chaperone action; little is known about how this model relates to the chaperone activity of sHsps inside the crowded environment of the cell. For example, nothing is known about the precise concentrations of sHsps inside a cell (let alone cellular compartments such as the nucleus versus the cytoplasm) and since the oligomeric state of sHsps is concentration dependent (see discussion for Hsp27 below) it therefore remains to be elucidated which oligomeric forms are relevant in a cellular context.

Defining the precise molecular mechanisms that underpin the chaperone activity of sHsps is essential for understanding their role in the suppression of aggregation in cells. Such knowledge will in turn inform efforts aimed at exploiting the biological activity of sHsps in the treatment of diseases.

SECTION II: Role of post-translational modification in the structure/function of sHsps

sHsps undergo extensive post-translational modification. In the lens, αAc and αBc are subject to significant post-translational modification from early on in the organism's lifespan [114]. The modifications include phosphorylation, truncation (particularly within the unstructured C-terminus), deamidation and glycation. In general, the mechanisms and functional roles of these alterations are unclear.

Phosphorylation

Both intra- and extra-lenticularly, the major post-translational sHsp modification is phosphorylation, the levels of which generally increase with age and under stress conditions [115-119]. For example, αBc is phosphorylated at three serine residues, Ser19, Ser45 and Ser59 [118,120,121]; phosphorylation at Ser45 is mediated by p44/p42 MAPK, at Ser59 by MAPKAPK-2 [118,122], whilst the kinase responsible for phosphorylation at Ser19 remains to be identified. Similarly, Hsp27 has three serine residues (Ser15, Ser78 and Ser82) that undergo phosphorylation [123,124]. Mitogen activated protein kinase activated protein (MAPKAP) kinase-2 is responsible for Hsp27 phosphorylation at all three sites [125,126]. In addition to αAc, αBc and Hsp27, phosphorylation is also common to other sHsps such as Hsp20 [127,128] and occurs readily in all tissues. Phosphorylation of Hsp20 occurs at Ser16 and is mediated by cyclic nucleotide-dependent protein kinases [129]. There have been other reports of sHsp serine phosphorylation (e.g. Hsp22 at Ser24 and Ser57

[130] and HSPB10 at Ser193 [131]), however the kinases responsible for these phosphorylation events *in vivo* remain to be definitively established.

The introduction of a strong negative charge via phosphorylation at serine residues in the N-terminal region of αBc and Hsp27 alters their oligomerisation state. Phosphorylation reduces the average oligomer size, and increases oligomeric polydispersity and rate of subunit exchange of αBc [98,132-134], whereas it leads to a dramatic decrease in the size of Hsp27 oligomers, such that the triply phosphorylated isoform is predominately dimeric in solution [135,136]. Thus, under stress conditions, Hsp27 is phosphorylated, triggering dissociation of the high molecular mass Hsp27 oligomers [117,135,137,138] and an increase in the amount of exposed hydrophobicity on the newly formed Hsp27 dimers [139]. Phosphorylation also affects the cellular distribution of some sHsps. For example, following stress, phosphorylation of αBc and Hsp27 causes them to be translocated into the nucleus [140-144], presumably to protect nuclear proteins important to cell survival. Thus, phosphorylation of sHsps functions as a 'molecular switch' by regulating their structure and cellular localisation during periods of cellular stress.

While purified αBc from bovine lens has been used to investigate of the effects of phosphorylation on αBc structure and function (as it is extensively phosphorylated with age [114,120,121,145,146]), such sources of sHsps do not afford homogeneous phosphorylated isoform. Instead these contain a mixture of non-phosphorylated, mono-, di- and/or tri-phosphorylated forms in the one oligomer. Denaturation and subsequent ion-exchange high performance liquid chromatography can be used to separate these phosphorylated forms [147,148], however denaturation modifies the oligomeric state and activity of αBc once it is refolded [149]. As an alternative, phosphomimics are useful tools to investigate the effects of phosphorylation upon sHsp structure and function. Phosphomimics are created by replacing phosphorylatable serines with a negatively charged amino acid, such as aspartic acid or glutamic acid, to mimic the negative charge introduced by the addition of the phosphate group. The main advantage of phosphomimicking forms is that they afford a single homogeneous isoform of the protein. Significantly, phosphomimics of αBc and Hsp27 have similar attributes to the phosphorylated forms of the protein with regards to their oligomeric distribution, chaperone activity, subcellular localisation and cellular trafficking [136,143,150,151]. For example, the translocation of αBc to the nucleus and its association with nuclear speckles during mitosis is phosphorylation-dependent and this is replicated by the S19D/S45D/S59D αBc phosphomimic [143] and both *in vitro* phosphorylated Hsp27 and the S15D/S78D/S82D

Hsp27 phosphomimic have comparable abilities to prevent the aggregation of target proteins *in vitro* [136]. Both S59 phosphorylated and S59E phosphomimicking forms of αBc bind to the anti-apoptotic regulator Bcl-2, promoting apoptosis of breast cancer MCF7 cells and therefore making them more susceptible to chemotherapeutic agents [152].

The precise effect that phosphorylation has on the chaperone function of sHsps remains controversial. For example, some studies have concluded that Hsp27 phosphorylation increases chaperone activity (e.g. [136,138]), others have reported no difference (e.g. [153]) whilst others have reported that phosphorylation decreases activity (e.g. [135]). Similar discrepancies exist in the literature with regards to the effect of phosphorylation on αBc's chaperone activity [133,134,148,150]. Various factors may account for these apparent differences including the target protein used to assess the chaperone activity, the final concentration of sHsp in the assays (wild-type Hsp27 dissociates at low concentrations and therefore may be predominately dimeric, like the phosphorylated form, in some assays [136,138]), and buffer and temperature conditions of the assay, all of which have been shown to influence sHsp chaperone activity [133]. Overall, the consensus emerging from work with αBc and Hsp27 concerning the impact of phosphorylation on sHsp chaperone function is that phosphorylation boosts chaperone activity by (i) promoting dissociation of large oligomers into smaller species [98,132,133,135,136] and (ii) enhancing their affinity to bind destabilised target proteins [103,138]. Based on structural modelling of a full-length αBc into a 24-mer oligomer [154]. Multiple molecular architectures of the eye lens chaperone αB-crystallin elucidated by a triple hybrid approach), recent work has concluded that the negative charges introduced into the N-terminal domain of αBc by phosphorylation at S19, S45 and S59 result in oligomer destabilisation and dissociation due to the close proximity of adjacent N-terminal domains in the higher-order oligomeric structure [98]. Moreover, phosphorylation increases the rate of subunit exchange between oligomers and the flexibility of the N-terminal domain, leading to an increase in chaperone activity [98].

With regards to the overall impact sHsp phosphorylation has a cellular context, it is envisaged that under normal conditions sHsps exists at relatively low levels in most cells and in non-phosphorylated forms. The activity of sHsps under these conditions is sufficient to maintain cellular proteostasis. Upon stress, the sHsps are rapidly phosphorylated, which modifies their oligomeric state and facilitates their translocation into the nucleus where they interact with and bind to destabilised proteins in danger of aggregating and precipitating. Prolonged or

chronic cell stress (e.g. that which occurs during some diseases) leads to an increase in sHsp levels in the cell (due to their up-regulation as part of the heat shock response pathway). This increase in expression facilitates the formation of a heterogeneous pool of sHsp oligomers (including phosphorylated and non-phosphorylated forms), which maximises the possible binding interactions of the sHsps with various intracellular target proteins.

Other post-translational modifications

In contrast to phosphorylation, other post-translational modifications, i.e. truncation (particularly from the C-terminus), deamidation and glycation appear to be largely confined to the long-lived lens sHsps, αAc and αBc. Thus, they occur to a significant extent to these sHsps with age and are associated with aggregation of the crystallin proteins, and hence cataract formation. *In vitro*, it is well known that C-terminal truncation within the exposed C-terminal extension decreases the solubility of both αAc and αBc [6]. Deamidation occurs extensively to αAc and to αBc with age in the human lens [155] and leads to potential destabilising structural changes due to the introduction of an additional negative charge from the resultant aspartic or glutamic acid sidechains [156]. Glycation is a common post-translational modification of lens αAc and αBc and is particularly associated with diabetes where there are increased levels of blood glucose [157,158]. Glycation leads to the formation of covalent cross-links, aggregation and compromised chaperone activity of αc [159] with obvious implications for the development of diabetic cataract.

SECTION III: sHsps and their roles in disease

In extra-lenticular tissues, the roles played by sHsps may vary according to specific cell and tissue types. Further complicating the picture *in vivo* is that the entire range of interactions of sHsps with target proteins that may occur in the crowded environment of the cell are not easily replicated by *in vitro* experiments [70]. Just as the precise mechanism(s) of action of sHsps in protein stabilisation remain to be elucidated *in vivo*, the precise roles of sHsps in the cellular pathologies of many diseases also remain largely unclear. As a result of their increased expression under a variety of cellular stresses, particularly those present in pathological states (i.e. heat (inflammation), oxidation, ischaemia), it is perhaps not surprising that sHsps and their (mal)function has been associated with a plethora of diseases including neurodegenerative diseases, multiple sclerosis and cancers [160,161].

Elucidating the precise role(s) played by sHsps in the context of disease is complex as they may be present as a consequence or cause of the disease. Moreover, as a result of their ubiquitous role in interacting with and stabilising a wide range of partially unfolded proteins against aggregation, sHsps are involved in many processes central to disease development and manifestation. These include cellular growth and differentiation [84,162,163]; interaction with cytoskeletal components such as actin and intermediate filaments [70,164,165]; and apoptosis [166]. Several of the sHsp family members (Hsp27, HSPB2, Hsp27, Hsp20 and αBc) have been attributed a protective role in the development of neurodegenerative and neuromuscular diseases, roles thought to be related to their abilities to both stabilise target proteins and to interact with cytoskeletal elements as the key features of these diseases are protein aggregation and defective axonal transport [70].

The role of sHsps in neurodegenerative disease

αBc is expressed at high levels in the neurons and glial cells of patients with neurodegenerative diseases such as Alzheimer's (AD), Huntington's, Alexander, Neuman-Pick, Creutzfeldt-Jakob (CJD) and amylotrophic lateral sclerosis (ALS) (summarised in [70]). Furthermore, αBc inhibits the aggregation of glial acidic fibrillary protein (GAFP) and α-syn which are associated with Alexander and Parkinson's disease (PD) pathologies, respectively [167,168]. Hsp20 and Hsp22 are also present at increased levels following neuronal stress associated with AD, PD, and ALS [169-173]. Hsp27 is also upregulated in AD and Neuman-Pick diseases [70]. αBc and/or Hsp27 have also been identified in the protein deposits characteristic of several neurodegenerative and neuromuscular diseases (see below), co-depositing with the protein or peptide identified as being responsible for the pathological features of the disease [78].

The Rosenthal fibres characteristic of Alexander disease constitute abnormal inclusions containing αBc (some of which is ubiquitinated) [174], Hsp27 and GAFP [175,176]. The involvement of Hsp27 and αBc in the formation of Rosenthal fibres within astrocytes is believed to be a response to an as-yet unknown stress caused by the disease [77]. The close association of sHsps with intermediate filaments in these pathologies [175] indicates that sHsps may play a role in their structural rearrangement [177]. Rosenthal fibres accumulate as a result of insufficient protein degradation in the cell. αBc's lysine residues, particularly the three in the solvent-exposed and highly flexible C-terminal extension (K166, K175 and K174) [9] serve as potential sites for the binding of ubiquitin in order to target the protein for degradation [178-180]. The presence of αBc-ubiquitin conjugates in such pathological inclusions implies a breakdown of the cell's protein degradation system.

The pathology of AD is characterised by extracellular accumulation of amyloid plaques and intracellular neurofibrillary tangles [181]. Amyloid plaques are composed primarily of Aβ peptides which are derived from the abnormal processing of α-amyloid precursor protein by β-secretase. In patients with familial AD, overproduction of Aβ leads to early onset AD [182-185]. The level of αBc expression in brains of patients suffering AD is markedly increased compared to that in the normal human brain [105,186] presumably due to the cellular stress caused by disease. We and others have shown that aggregation of Aβ and its associated cellular toxicity is prevented by αBc [187] or its isolated ACD [18] and thus sHsp overexpression in the context of AD may be as a protective mechanism. Fändrich *et al*. have shown that there is an intracellular component to the aggregation and pathogenic nature of Aβ which may explain the presence of αBc in the extracellular plaques of AD [188]. The intracellular neurofibrillary tangles characteristic of AD (and other 'tauopathies') contains hyperphosphorylated tau protein [189]. Both αBc and Hsp27 are also found in these tangles, however, their contributions to the formation of these plaques and tangles, and hence the pathology of AD, remains unclear [190].

The presence of αBc, Hsp27 and ubiquitin in CJD is thought to be related to the degenerative processes that neurons undergo as the disease manifests [179]. CJD is a transmissible spongiform encephalopathy resulting from the intracellular deposition of misfolded prion protein (PrP^{sc}). The bovine precursor of this misfolded protein associates with αBc both *in vitro* and *in vivo* [191]. However, it remains unclear as to whether αBc acts to inhibit or enable the conversion of correctly folded prion to its toxic PrP^{sc} form. Ubiquitinated αBc has also been identified by immunochemical analysis in cytoplasmic inclusions in brains of patients with multiple system atrophy [192]. More recently, it has been shown that the aggregation of SOD1, a major protein involved in the pathogenesis of ALS, is prevented by both αBc and Hsp27 [193,194] and that Hsp22, in concert with other chaperones, promotes autophagic removal of misfolded proteins in ALS [171].

PD is characterised by a gradual loss of dopaminergic neurons in the brain. The precise role of αBc in the development and progression of this disease is still unknown, despite the findings that the disease is characterised by 'ballooned' neurons [195] which express αBc [105]. Both PD and Huntington's disease are intracellular protein aggregation diseases, like CJD. We have previously demonstrated that αBc prevents the aggregation of α-syn, the principal protein in Lewy body deposits, and that αBc also binds to intact α-syn fibrils

to prevent their further growth [106,107,167]. Furthermore, several sHsps (Hsp20, Hsp22, HspB7 and HspB9) inhibit the aggregation of the polyQ huntingtin protein responsible for Huntington disease and also protect against cell death triggered by the deposition of the aberrant protein [173,196]. Our subsequent studies have shown, however, that polyQ aggregation occurs in stages, and while αBc potently inhibits the first stage of fibril formation by the polyQ protein, ataxin (responsible for spinocerebellar ataxia), through interaction with its Josephin domain, the second stage of polyQ aggregation can still proceed [197]. The potency of sHsps in individual disease states therefore appears to be very much dependent on target protein aggregation models and specific sequences can modulate this effect.

The role of sHsps in multiple sclerosis

Multiple Sclerosis (MS) is a relatively common demyelinating neurodegenerative disease. The exact mechanism of demyelination is unknown but appears to be an autoimmune disorder, triggered by environmental factors (e.g. viral infection). Patients with MS have numerous demyelinated plaques in the brain and spinal cord and these lesions have varying histopathological profiles depending on the stage of disease (i.e. acute or chronic). Inflammation plays a central role in disease progression as macrophages initially phagocytose the degraded myelin to form an acute plaque (with other immune cells such as T-lymphocytes and plasma cells) before a reactive gliosis is established, leading to chronic inflammation and plaque formation. It has been shown that αBc is the most prominent protein present in plaques of MS patients [198] and the CRYAB gene is the most highly induced in plaque tissue compared to normal tissue [199]. The increased expression of αBc, along with other Hsps, is proposed to be part of the oligodendrocyte's survival response [180]. Initial studies identifying αBc bound to antibodies in the sera of affected patients suggested αBc as an auto-antigen to human T-cells in MS [173,200,201]. Subsequent studies showed that most of the sHsps (HspB1-8) were capable of binding immunoglobulin molecules with varying affinities and through multiple binding sites [201]. The association of these proteins with antibodies in the context of MS therefore appears to be related to their intrinsic ability to bind immunoglobulins rather than any inherent immunogenic property of the sHsp itself. As proposed by Clark and Muchowski [160], the up-regulation of sHsps in diseases characterised by hyper- or autoimmune responses such as MS may lead to stabilisation of antigenic components by the chaperones which then act to exacerbate an immune response.

The role of sHsps in cancer

Several sHsps, particularly Hsp27 and αBc, have been identified as playing potential roles in the pathogenesis of cancer. Both αAc and αBc have been found in a range of tumours [78] and especially in high-grade tumours [202]. Hsp27 has been identified at high levels in metastatic tissues compared to non-metastatic tissues, indicating a role in the metastasis of cancer [203,204]. Indeed, the ability to regulate apoptosis and thus prolong cell life, which in some cases is tumorigenic, is a feature consistent with the finding that Hsp27 and αBc expression is associated with increased cellular resistance to cytostatic agents [205]. Interestingly, the metastatic properties of breast cancer cells can be inhibited by phosphorylation of αBc. Phosphorylation at S59 specifically reduces the anti-apoptotic action of the protein, by affecting oligomer formation [204,206].

In terms of the mechanisms by which sHsps promote tumorigenesis, many pathways have been identified by which sHsps regulate both the intrinsic and extrinsic pathways of apoptosis. For example, Chen *et al.* showed that both αAc and αBc are able to bind to the pro-apoptotic Bcl-2 proteins Bax and Bcl-X_s, inhibiting their mitochondrial translocation and thereby switching off the intrinsic cell death pathway [202]. Specific naturally-occurring mutations in the α-crystallins (R116C αAc and R120G αBc) impair the ability of these sHsps to sequester Bax and Bcl-X_s [207]. Previous work has also shown that Hsp27 interacts directly with Daxx to prevent Fas-mediated apoptosis [208], a cell death pathway that has previously been shown to involve Hsp27 [209]. Future therapies may therefore include those which aim to suppress the action of sHsps in such situations [78]. For example, a recent clinical trial of an antisense oligonucleotide (ASO; OGX-427) designed to specifically inhibit the expression of Hsp27 for the treatment of prostate, bladder, breast and lung cancer has shown promising results [73].

The role of sHsps in cataract and myopathies

Several naturally-occurring mutations in sHsps have been identified as being responsible for various forms of human cataract and myopathy. These will be discussed further in the following section describing the occurrence and phenotypic effects of specific mutations in sHsps.

SECTION IV: Naturally-occurring mutations in sHsps – implications for altered function and disease

Our understanding of the roles played by sHsps in disease aetiology has been increasingly enhanced by the identification of sHsp mutations directly associated with specific pathologies (Table 2). Typically, these mutations lead to altered chaperone function and therefore provide a clear link between molecular chaperone action and disease manifestation.

Mutations in the α-crystallin domain

A naturally-occurring missense mutation (R120G) in the conserved ACD of αBc was first identified as being responsible for a form of desmin-related myopathy (DRM) [210]. R120G αBc is associated with aggregation of the intermediate filament protein, desmin, in the cytoplasm of myofibrils [211]. Desmin is essential for tensile strength in myofibrils of striated muscle and as a result DRM manifests with progressive muscle weakness. Moreover, gene mutations producing DRM are clinically heterogeneous with affected individuals often also exhibiting cardiomyopathy and cataract [210,212]. The formation of desmin aggregates was initially attributed to loss of chaperone function in αBc as a result of the R120G mutation, a hypothesis supported by the finding that formation of desmin aggregates could be delayed or prevented *in vivo* by the addition of wild-type αBc or other molecular chaperones [213]. Subsequent studies by our group and others have shown that the R120G mutation causes significant structural changes in αBc compared to the wild-type protein, leading to a loss of chaperone activity *in vitro* [214] and an inherent instability in the mutated protein [215]. Similarly, D140N αBc, a mutant identified in relation to congenital lamellar cataract, exhibits abnormal oligomerisation and impaired chaperone activity [216,217]. Recent biophysical studies by Hilton *et al.* [218] support the rationale that mutations in the ACD of αBc interfere with important transient interactions between subunits and therefore impact on the association and dissociation properties of the oligomeric form. The locations of naturally-occurring, disease-associated mutations in a model of an αBc monomer is shown in Fig. 3.

Mutation in αAc at the equivalent residue to R120G in αBc (i.e. R116C) results in hereditary cataract [219] and similar mutations in the gene coding for αAc have profound effects on lens opacity [220]. Mutation of R116 in the ACD of αAc causes a decrease in chaperone activity and subsequent aggregation of the natural target proteins of αAc in the lens (the β- and γ-crystallins) [160]. Altered chaperone activity in R116C αAc (as with R120G αBc) results from secondary and tertiary structural changes and increased oligomeric size as a result of

the mutation [221-223]. The substitution of an arginine for a cysteine at this position exposes a previously buried residue which most likely disrupts salt bridges and potentially leads to the formation of non-native, inter-subunit disulphide bonds [224]. It is highly likely therefore that perturbation at this site is the reason for the abnormal oligomeric assembly of R116C αAc. This same residue is affected in the R116H mutant of αAc which also causes cataract [225]. As discussed in Section I, the interaction between αA- and αBc is crucial to the maintenance of lens transparency. The R116C mutation in αAc also leads to a reduction in its protective ability against epithelial cell apoptosis in the lens [226]. A number of other site-specific mutations involving residues within the ACD have been found to be responsible for the development of either whole (lenticular) or nuclear cataract in humans (R49C and G98R αAc) [227], or in mice (R54C/Y118D αAc) [228].

Six naturally-occurring mutations in Hsp27 have been identified that are associated with Charcot-Marie-Tooth (CMT) disease, an inherited motor and sensory neuropathy, and distal hereditary motor neuropathy (HMN). Three of these mutations, R127W, S135F (one causing CMT and the other HMN) and R136W, are located in the ACD [229]. Neuronal cells transfected with the mutant Hsp27s are less viable than those expressing wild-type Hsp27 and mutation also leads to altered neurofilament assembly within cells [229]. Similar mutations in the ACD of Hsp22, (i.e. K141E [230], K141T [231] and K141N [230,232,233]), also give rise to CMT disease indicating a critical role for sHsps in motor and sensory neurons.

Mutations in the C-terminal region

Outside of the ACD, naturally-occurring mutations in the C-terminal regions of both αAc and αBc are associated with disease. Mutation of conserved residues in αBc, R157H and G154S, lead to dilated cardiomyopathies but not cataract [234,235]. Another form of DRM arises from a deletion mutation (Q151X) in αBc [236]. Similarly, several other naturally-occurring mutations in the C-terminal region of Hsp27; P182L [229], P182S [237], R140G [238] and K141Q [239] cause distal HMN. Other rarer forms of fibrillar neuropathy have been identified, with deletions in the gene encoding αBc (CRYAB) resulting in the absence of the protein from affected muscle fibres [240]. A naturally-occurring deletion of a single nucleotide base in the CRYAB gene has been described and leads to a frameshift mutation in the C-terminal region of the resulting αBc protein [216]. Replacement of the wild-type sequence after residue 149 with 35 novel residues (450delA) results in posterior polar cataract [216]. Similarly, deletion of two bases in CRYAB leads to truncation at position 464 (del464CT), giving a myofibrillar myopathy phenotype [236]. These provide further evidence for the

importance of the C-terminal region in the functioning of sHsps. The complex phenotypical profiles associated with naturally-occurring mutations of αBc and Hsp27 are a consequence of their widespread tissue distributions (which largely overlap) and their wide array of cellular functions.

Mutations in the N-terminal region

In the N-terminal region, there are small regions of high (≥ 85%) sequence identity between members of the sHsp family. Thus, residues 48-55 in αBc contain the RFLDQxFG motif, which is also present in Hsp27 and Hsp20 [241]. Several naturally-occurring mutations in the N-terminal region of Hsp27 have been shown to exist in individuals affected by distal HMN (Table 2; [238]).

The P20S mutant of αBc is the only cataract-causing mutation to be identified outside the ACD, but it affects a highly conserved N-terminal residue that also impairs the chaperone activity of αAc [242]. The naturally-occurring mutation W9X in αAc has also been identified in individuals with cataract [243,244], as have mutations involving R21 (Table 1; [245-247]). Similarly, R49C [243] and R54H αAc [228] cause cataract, with R49C αAc exhibiting impaired chaperone function *in vivo*, resulting in greater perturbation of oligomer distribution and higher levels of cell death than even R116C αAc [243]. Furthermore, the same mutation induces apoptosis in human lens epithelial cells, an effect thought to be related to aberrant phosphorylation at S19, S45 and S59 of αBc, leading to its nuclear import and accumulation which results in subsequent cell death [242].

Future perspectives

From the above, it is apparent that there is still a great deal to learn about sHsps – they are very much an enigmatic class of molecular chaperone proteins. They appear to have many roles intra-cellularly that are based around their ability to stabilise proteins, thereby preventing their unfolding and potential aggregation under both normal (constitutive) and abnormal (stress) conditions in the cell. Their ability to interact with amyloid fibril-forming proteins at the various stages of their aggregation pathway also imparts a multi-functional dimension to sHsp chaperone activity.

To rationalise sHsp functions will require a continued concerted effort on various fronts to:

1. Understand fully the mechanism and role of sHsp subunit exchange (if any) in chaperone action.
2. Understand the functional role of each of the three sHsp structural regions.
3. Determine an accurate quaternary structure for αB-crystallin and other sHsps (which, most likely, will provide major insight into points 1 and 2).
4. Understand, and explain, the role of post-translational modification, particularly phosphorylation, in sHsp chaperone action.
5. Understand why nature provided so many distinct sHsps – a feature indicating a need for diversity and/or compartmentalisation of sHsp functionalities.

Clearly, insight into each of these areas will provide us with a much greater understanding of the links between sHsp function and disease, including the effect of sHsp mutations on disease manifestation and progression. Furthermore, these advances will lead to the potential targeting of specific sHsps in therapeutic treatments of the wide range of diseases associated with protein aggregation, many of which (e.g. AD, PD and cataract) will become much more prevalent in our increasingly ageing population.

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Tables

Table 1 Tissue distribution of the major sHsps

sHsp	Alternative name	Tissue distribution
αAc	HSPB4	Eye lens, spleen, thymus [248]
αBc	HSPB5	Eye lens, retina, heart, skeletal muscle, skin, brain, spinal cord, kidneys, lungs [37,57,58], cochlea [59], lacrimal gland duct & tears [61], sciatic nerve [70]
Hsp27	HSPB1	Skeletal, cardiac and smooth muscle, brain and spinal cord (summarised in [70])
Hsp22	HSPB8	Muscle, brain, spinal cord (summarised in [70])
Hsp20	HSPB6	Skeletal, cardiac and smooth muscle , brain, spinal cord, sciatic nerve (summarised in [70])

Table 2 Disease-causing sHsp mutations in humans

sHsp	Mutant	Disease
αAc	W9X, R12C, R12W, R21L, R49C, R54H, G98R, R116C*, R116H*	Cataract [219,225,227,228,243,245-247,249]
αBc	R120G*	Desmin-related myopathy, cataract [210]
αBc	P20S, R56W, D140N, 450delA	Cataract [216,217,242,250]
αBc	R157H, G154S	Dilated cardiomyopathy [234,235]
αBc	Q151X (STOP)	Desmin-related myopathy [236]
αBc	454delCT *CRYAB gene*	Desmin-related myopathy [236]
αBc	450delA *CRYAB gene*	Cataract [216]
Hsp27	R127W, S135F, R136W, T151L, P182L	Distal hereditary motor neuropathy, motor and sensory neuropathy, Charcot-Marie-Tooth disease [229]
Hsp27	P182S	Distal hereditary motor neuropathy [237]
Hsp22	K141N*, K141E*	Distal hereditary motor neuropathy [230,232]
Hsp22	K141N*	Charcot-Marie-Tooth disease [233]
Hsp22	K141T*	Charcot-Marie-Tooth disease [231]

*Residue 116, 120 and 141 in αA-, αBc and Hsp22, respectively, are equivalent.

Figures

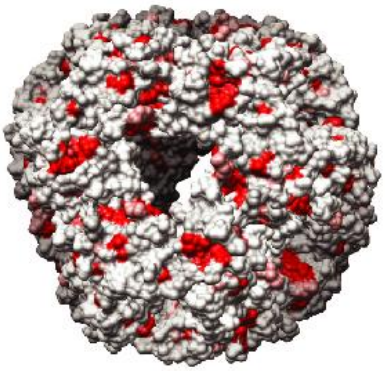


Fig. 1A

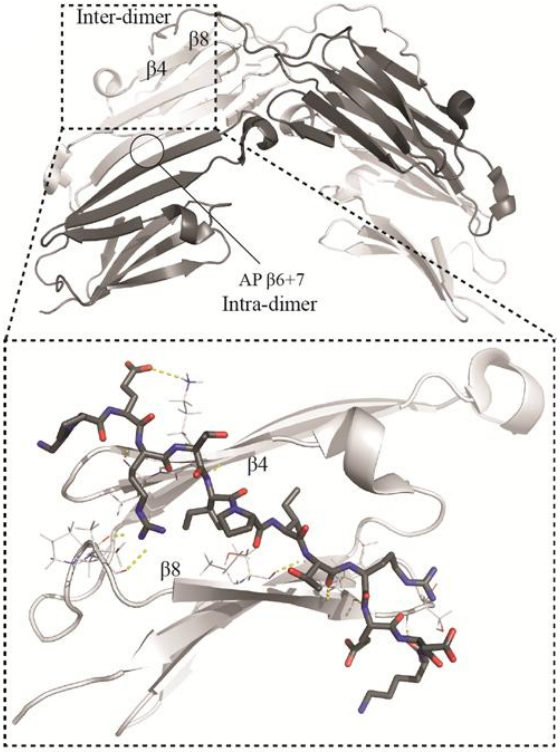


Fig. 1B

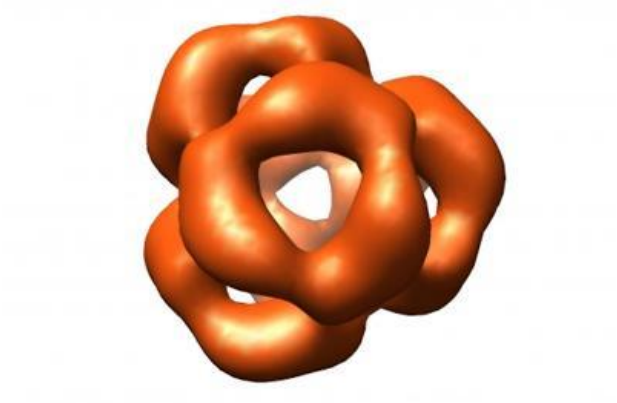
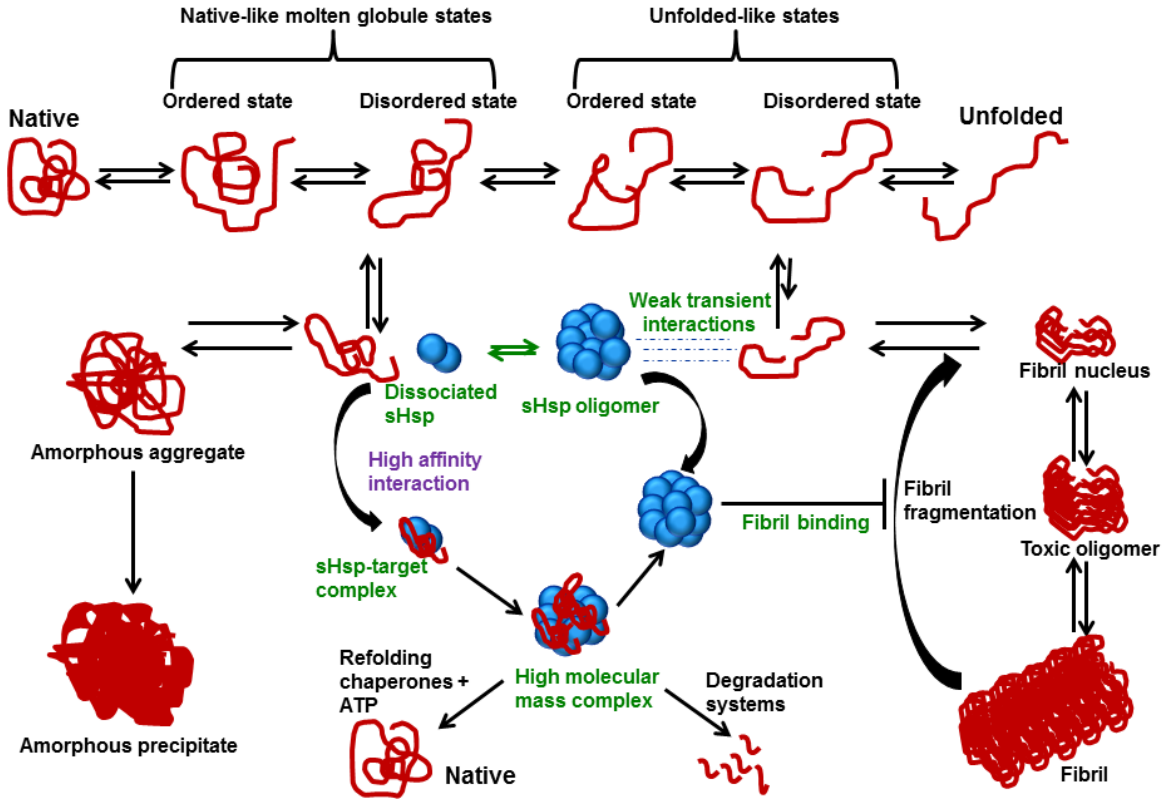


Fig. 1C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sHsp oligomer
High affinity interaction
Fibril binding
Fibril fragmentation
Toxic oligomer
sHsp-target complex
Refolding chaperones + ATP
High molecular mass complex
Degradation systems
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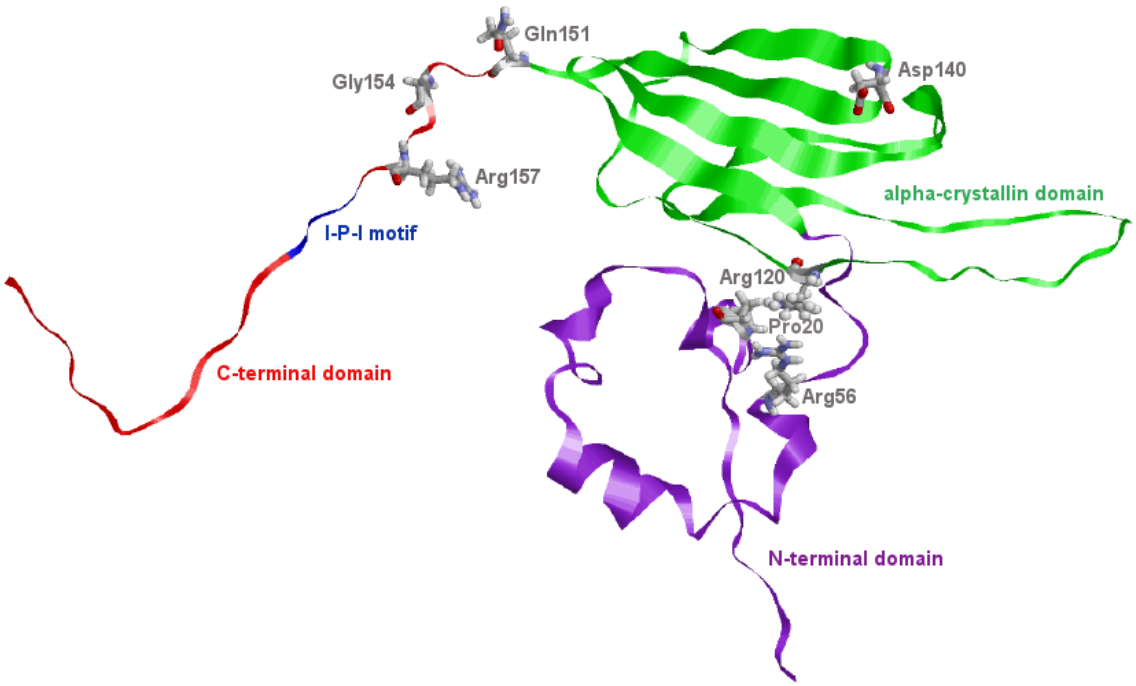
Gln151
Gly154
Asp140
Arg157
alpha-crystallin domain
I-P-I motif
Arg120
Pro20
Arg56
C-terminal domain
N-terminal domain

Figure Legends

Fig. 1A Crystal structure of the *Methanococcus jannaschii* Hsp16.5 24-mer oligomer showing its large central cavity [13]. Reprinted with permission from [13].

Fig. 1B X-ray crystal structure of the ACD and C-terminal region of αBc without its flexible C-terminal extension [218]. At the top is shown the arrangement of the αBc dimer in which the six β-strands of each ACD are arranged in an immunoglobulin-like fold. The intra-dimer contacts arise between β-strands 6 and 7 of each subunit. The expanded section (below) shows in detail the inter-dimer interaction of an αBc peptide encompassing a palindromic nine amino acid sequence (residues 156 to 164), including the 'IXI' sequence (IPI in αBc; residues 159 to 161), with the fourth and eighth β-strands of the adjacent αBc subunit. Reprinted with permission from [218].

Fig. 1C Model of the αB-crystallin oligomer as determined from reconstruction of TEM images [27]. The porous nature of the oligomer is readily apparent along with the presence of a large central cavity. Reprinted with permission from [27].

Fig. 2 The chaperone mechanism of sHsps

Multiple partially-folded protein intermediate states populate the folding/unfolding pathway of a protein. The mechanism by which sHsps, such as αB-c, prevent target protein aggregation (either amorphous or fibrillar) is dictated by the conformational stability and exposed hydrophobicity of the precursor protein intermediates. High affinity interactions occur with highly destabilised intermediates (which exceed the threshold of binding) and these are sequestered into stable high molecular mass complexes. Target proteins in these complexes can be re-folded through the action of other ATP-dependent chaperones or shuttled for degradation or via chaperone-mediated autophagy. Alternatively, weak, transient interactions occur with more stable protein intermediates, which re-direct them back to the folding pathway so as to facilitate their re-folding. sHsps can also interact with pre-fibrillar and fibrillar aggregates formed by target proteins. By binding to these species sHsps stabilise them preventing their further elongation and fibril fragmentation and secondary nucleation events, which can be the main source of toxic oligomeric species formed during amyloid fibril-type aggregation. (Adapted from [95]).

Fig. 3. Homology model of human αB-crystallin monomer showing approximate locations of naturally-occurring disease causing mutations. Adapted and reused with permission from [251]. Copyright (2014) American Chemical Society.

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Figure 1A

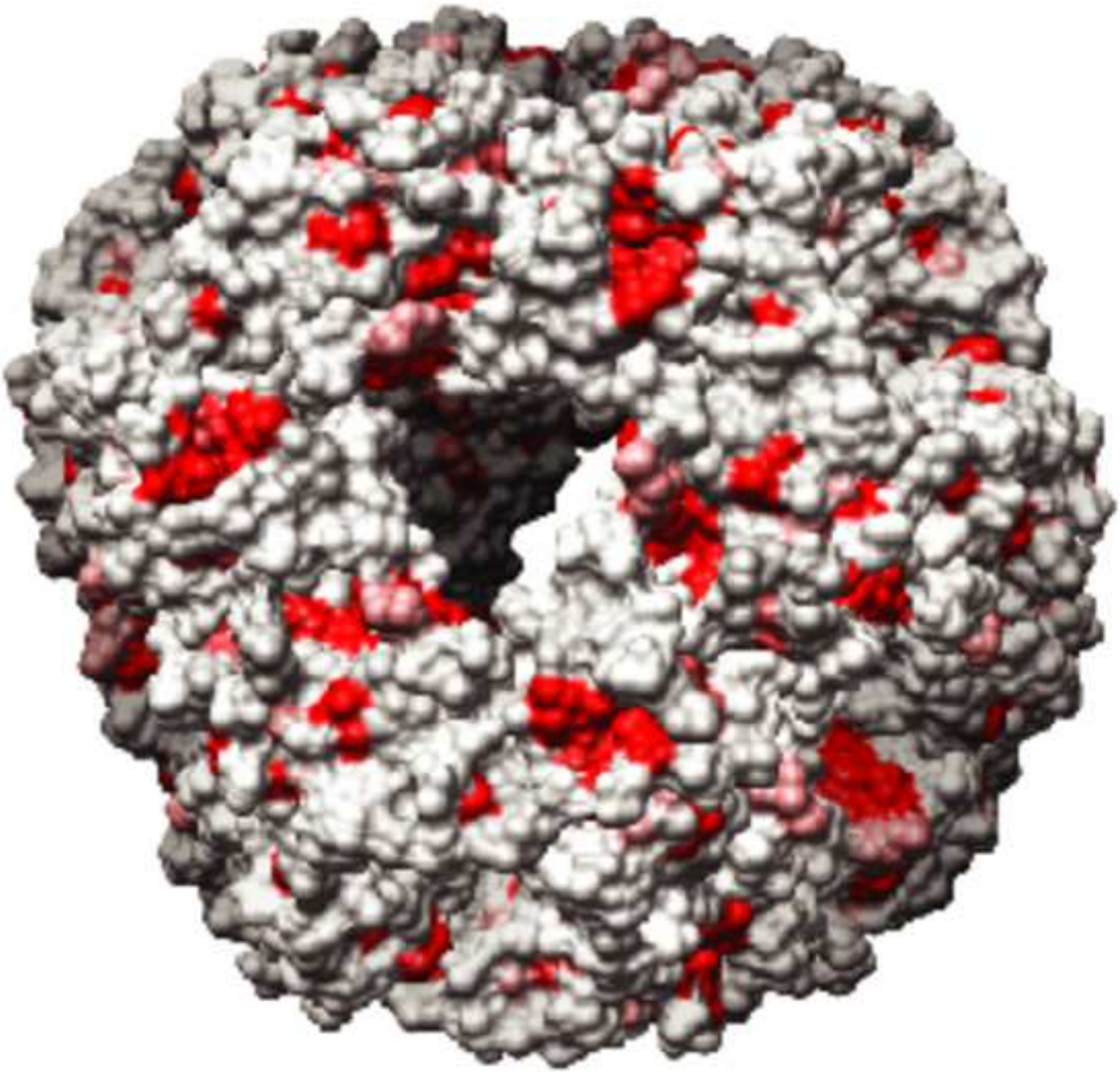
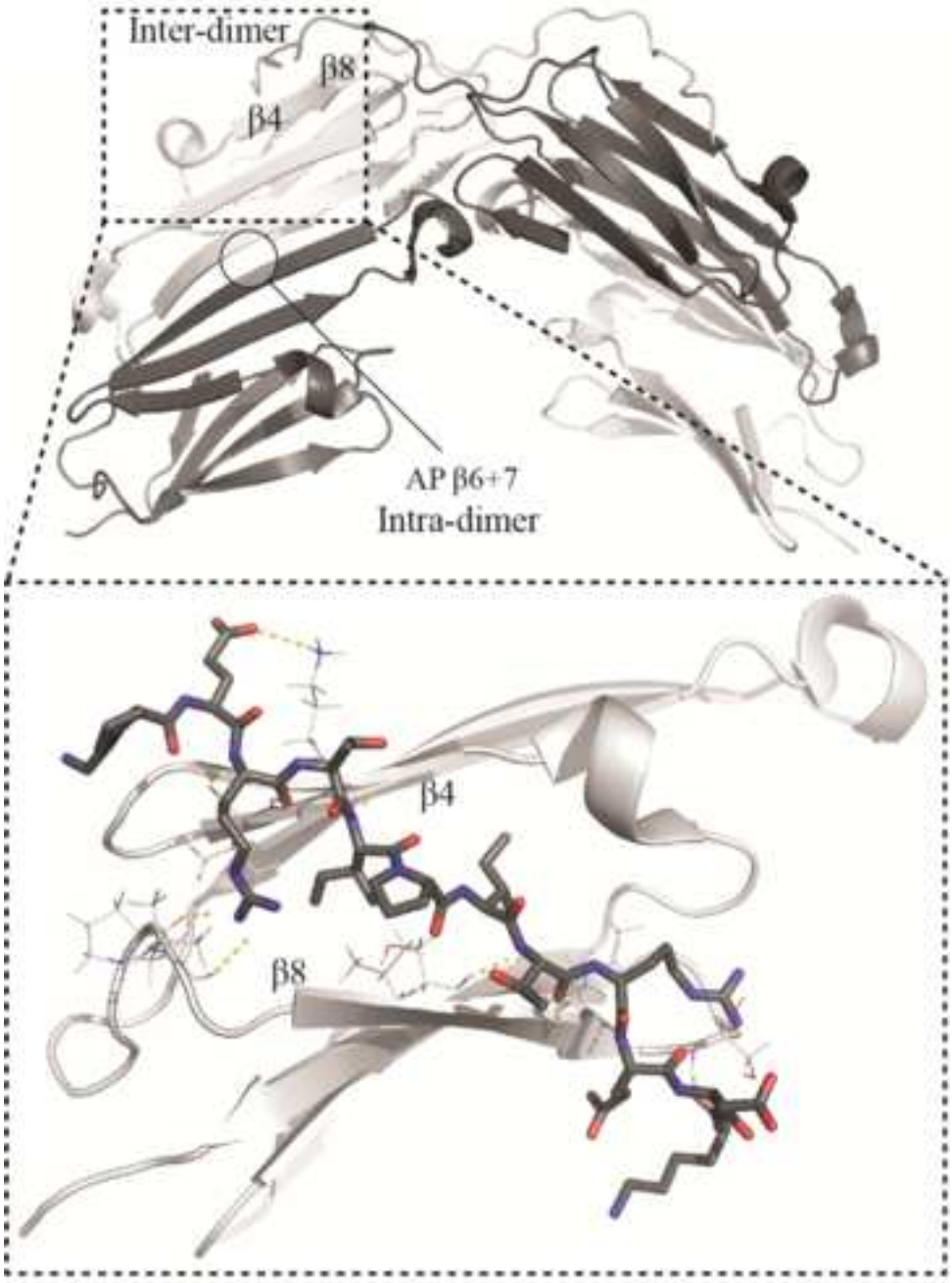


Figure 1B



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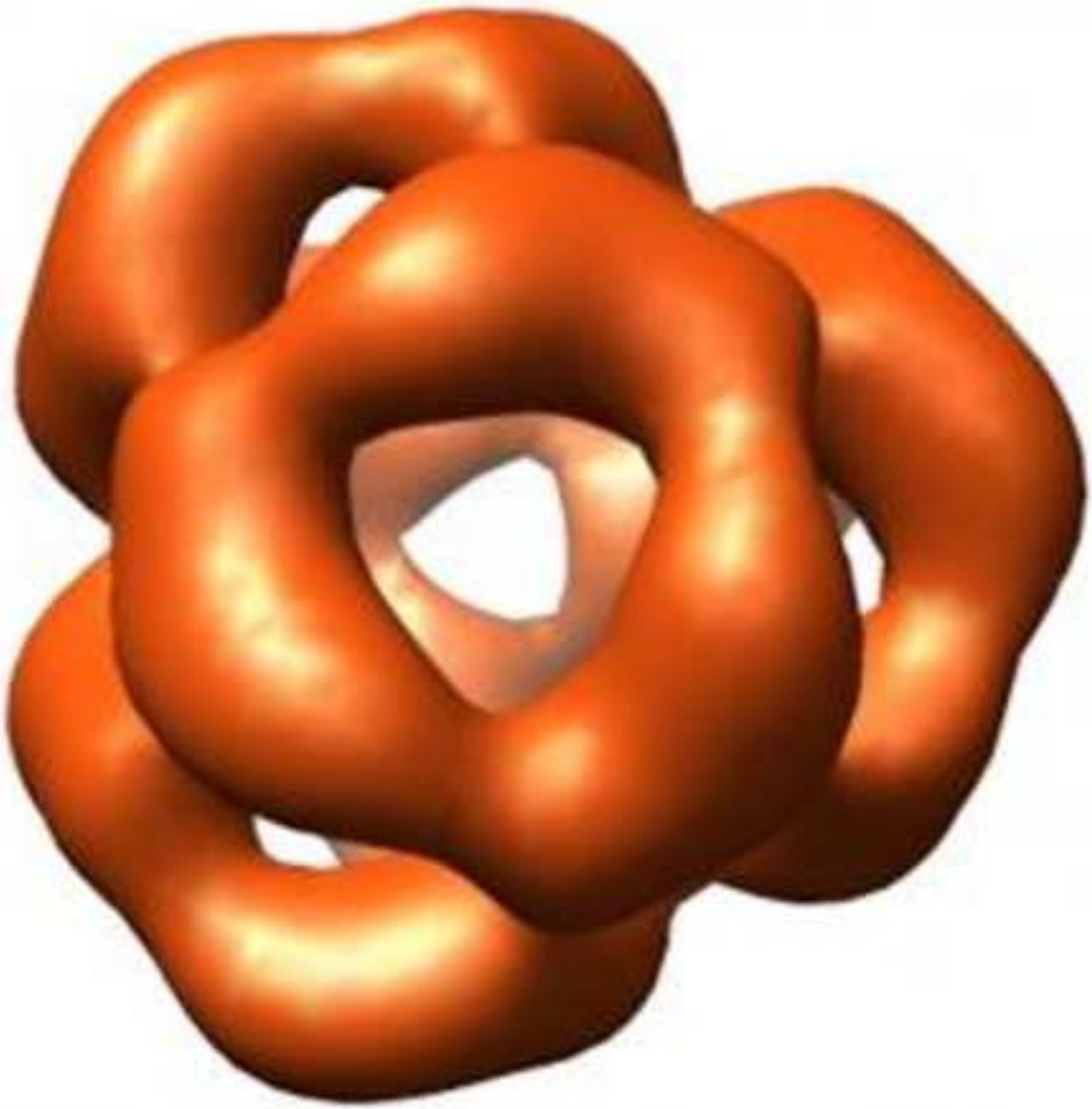
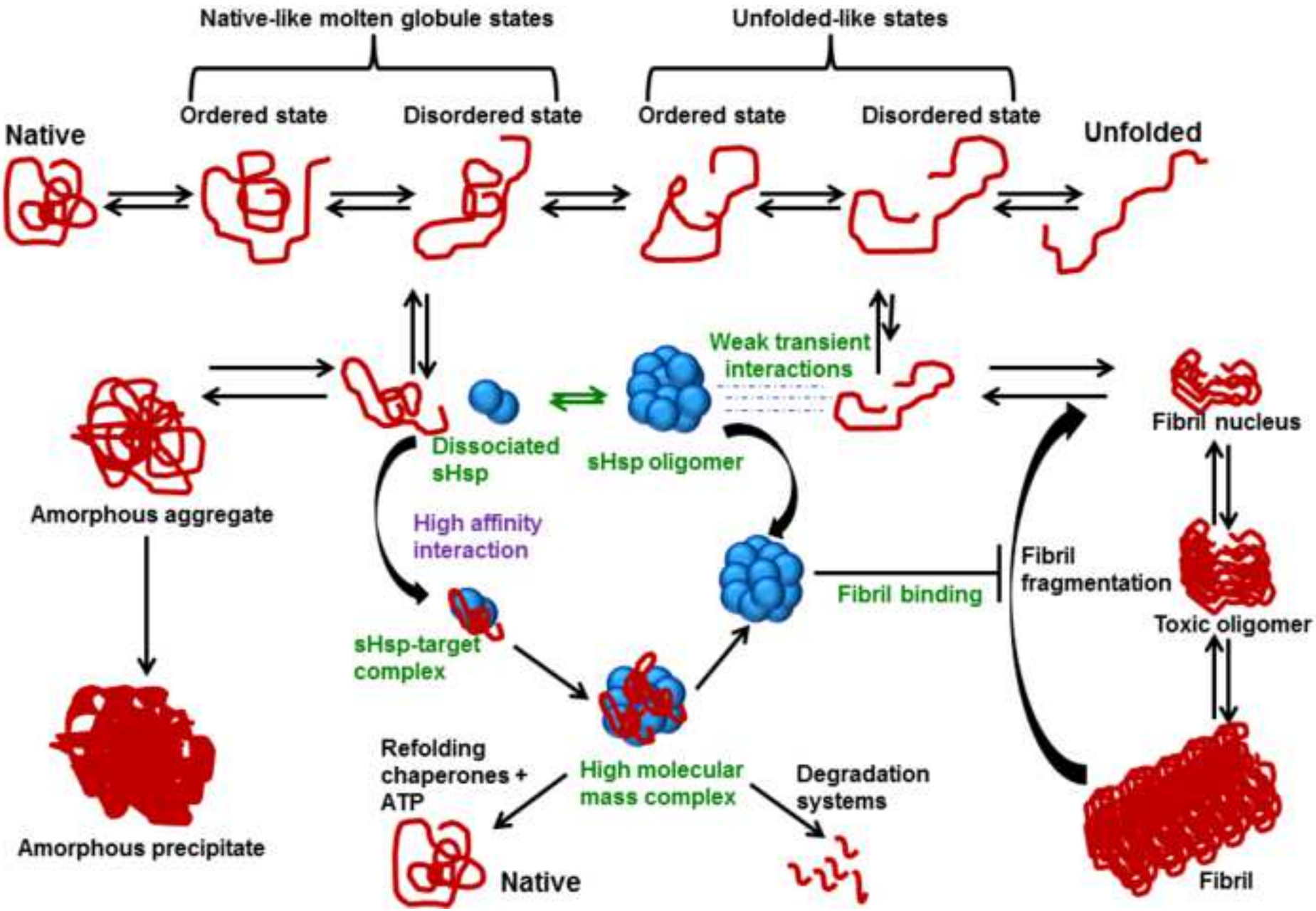


Figure 2


Native-like molten globule states
Unfolded-like states
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Disordered state
Ordered state
Disordered state
Native
Unfolded
Weak transient interactions
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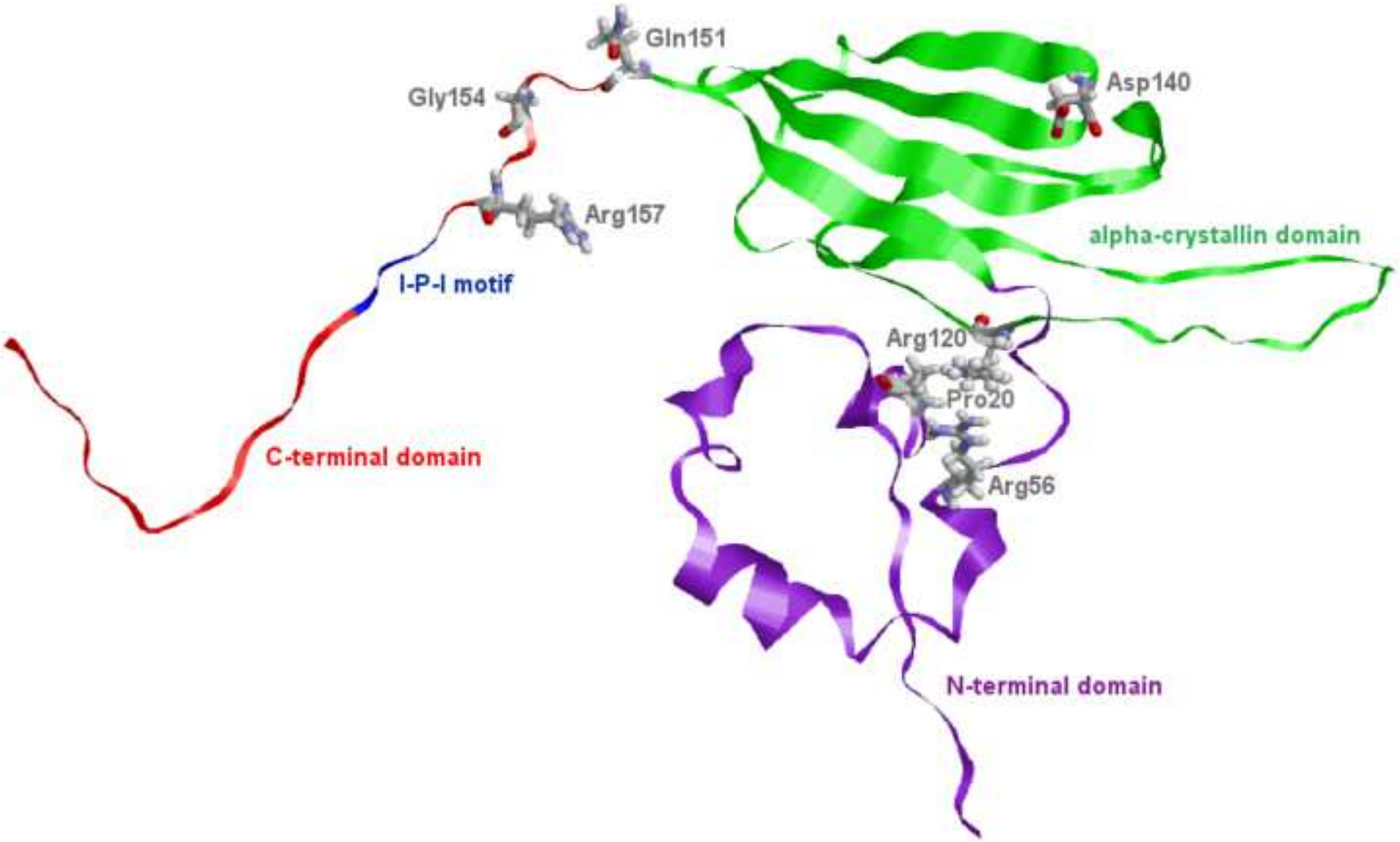
sHsp oligomer
High affinity interaction
Fibril binding
Fibril fragmentation
Toxic oligomer
sHsp-target complex
Refolding chaperones + ATP
High molecular mass complex
Degradation systems
Amorphous precipitate
Native
Fibril

Figure 3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