



# Small heat shock proteins: multifaceted proteins with important implications for life

Serena Carra<sup>1</sup> · Simon Alberti<sup>2,3</sup> · Justin L. P. Benesch<sup>4</sup> · Wilbert Boelens<sup>5</sup> · Johannes Buchner<sup>6</sup> · John A. Carver<sup>7</sup> ·  
Ciro Cecconi<sup>8,9</sup> · Heath Ecroyd<sup>10,11</sup> · Nikolai Gusev<sup>12</sup> · Lawrence E. Hightower<sup>13</sup> · Rachel E. Klevit<sup>14</sup> · Hyun O. Lee<sup>15</sup> ·  
Krzysztof Liberek<sup>16</sup> · Brent Lockwood<sup>17</sup> · Angelo Poletti<sup>18</sup> · Vincent Timmerman<sup>19</sup> · Melinda E. Toth<sup>20</sup> ·  
Elizabeth Vierling<sup>21</sup> · Tangchun Wu<sup>22</sup> · Robert M. Tanguay<sup>23</sup>

Accepted: 1 February 2019  
© Cell Stress Society International 2019

## Abstract

Small Heat Shock Proteins (sHSPs) evolved early in the history of life; they are present in archaea, bacteria, and eukaryota. sHSPs belong to the superfamily of molecular chaperones: they are components of the cellular protein quality control machinery and are thought to act as the first line of defense against conditions that endanger the cellular proteome. In plants, sHSPs protect cells against abiotic stresses, providing innovative targets for sustainable agricultural production. In humans, sHSPs (also known as HSPBs) are associated with the development of several neurological diseases. Thus, manipulation of sHSP expression may represent an attractive therapeutic strategy for disease treatment. Experimental evidence demonstrates that enhancing the chaperone function of sHSPs protects against age-related protein conformation diseases, which are characterized by protein aggregation. Moreover, sHSPs can promote longevity and healthy aging *in vivo*. In addition, sHSPs have been implicated in the prognosis of several types of cancer. Here, sHSP upregulation, by enhancing cellular health, could promote cancer development; on the other hand, their downregulation, by sensitizing cells to external stressors and chemotherapeutics, may have beneficial outcomes. The complexity and diversity of sHSP function and properties and the need to identify their specific clients, as well as their implication in human disease, have been discussed by many of the world's experts in the sHSP field during a dedicated workshop in Québec City, Canada, on 26–29 August 2018.

**Keywords** Small heat shock proteins · Protein quality control · Human diseases · Plant biology

## Introduction

Small Heat Shock Proteins (sHSPs) are ATP-independent molecular chaperones conserved across species, and expressed throughout the kingdoms of life from archaea to humans (Bult et al. 1996; Caspers et al. 1995; Eyles and Gierasch 2010; Richter et al. 2010). sHSPs form dynamic oligomeric complexes that can exchange subunits and can dissociate into dimers and monomers; the latter can be influenced by post-translational modifications, including phosphorylation which tends to promote oligomer dissociation (Candido 2002;

Hilton et al. 2012; Kim et al. 1998; McDonald et al. 2012; Van Montfort et al. 2001a). The flexibility of sHSP assembly is thought to be important in regulating their binding affinity for non-native and misfolded proteins, preventing irreversible protein aggregation. Once complexed with sHSPs, these non-native and aggregated proteins can be recovered and refolded with the assistance of ATP-dependent chaperones such as the HSP70 system. Thus, sHSPs can be considered as sponges that neutralize non-native and aggregate-prone intermediates, safeguarding the cellular proteome in response to stress. Importantly, sHSPs can be themselves part of proteinaceous inclusions; here, their role is to facilitate disaggregation (Mogk and Bukau 2017; Mogk et al. 2018). Based on these findings, sHSPs act not only as “holdase” chaperones, as was thought for a long time, but they actively regulate the aggregation process, improving the ability of the cells to recover protein homeostasis following exposure to different stress conditions, such as temperature upshift or oxidative stress.

✉ Serena Carra  
serena.carra@unimore.it

✉ Robert M. Tanguay  
Robert.tanguay@fmed.ulaval.ca

Extended author information available on the last page of the article

sHSPs are not only important in regulating the cell stress response and responding to changes in cellular conditions. Many sHSPs are expressed in normally growing cells, where they can regulate the interactions between native proteins, shaping protein-protein interactions; this, in turn, influences disparate cellular functions, ranging from cell signaling to differentiation and apoptosis. Understanding how sHSP oligomerization and sHSP-substrate binding are regulated, how sHSP structure-function is influenced by post-translational modifications, and identifying selective and cell-specific sHSP targets will shed light on their roles in thermotolerance, cell differentiation, development, and cell death (Arrigo 2000; Arrigo and Ducasse 2002; Arrigo and Gibert 2014; Balogi et al. 2008; Benjamin et al. 1997; Bruey et al. 2000; Haslbeck et al. 2016; Hong and Vierling 2000; Kamradt et al. 2002; Kamradt et al. 2005; Lavoie et al. 1993; Lavoie et al. 1995; Litt et al. 1998; Nicholl and Quinlan 1994; Parcellier et al. 2006; Park et al. 2016; Perng et al. 1999a; Perng et al. 1999b; Qian et al. 2009; Quinlan and Van Den Ijssel 1999; Takayama et al. 2003; Tanguay and Hightower 2015; Webster 2003).

Due to their ability to interact promiscuously with a large variety of substrates and to their dynamic oligomerization, it is not surprising that deregulated expression of sHSPs and genetic mutations in several human sHSP/HSPB genes have been linked to different pathological conditions, including congenital cataracts and neuromuscular and age-related protein conformation diseases, as well as cancer (Evgrafov et al. 2004; Ghaoui et al. 2016; Irobi et al. 2004; Kolb et al. 2010; Morelli et al. 2017; Nam et al. 2018; Perng et al. 1999b; Vicart et al. 1998). Unraveling how disease-linked mutations in human HSPB genes affect their properties and functions will highlight potential drug targets for future therapeutic approaches.

sHSPs are also implicated in plant stress resistance and fungal and microbial infections (Haslbeck and Vierling 2015; Mayer et al. 2012). Thus, dissecting the relationship between sHSP structure-function will open new avenues for biotechnological interventions aimed at improving plant stress tolerance to abiotic insults, with the ultimate goal of ameliorating seed maturation and crop production, and developing pharmacological approaches for the treatment of human infectious diseases.

Here, we summarize recent advances in the field of sHSPs that were discussed by international experts during the Third Cell Stress Society International (CSSI) Workshop on sHSPs that was held in Québec, Canada (26–29 August 2018). This meeting followed on the Second International Workshop, organized in 2016 in Bertinoro, Italy, by Serena Carra and Robert M. Tanguay (Carra et al. 2017), and further highlighted the need for building an interdisciplinary worldwide community for the advanced study of sHSPs.

## sHSP structure: order among disorder

sHSPs belong to the superfamily of proteins with disordered domains, which represent up to 40% of the eukaryotic proteome and are present in all three kingdoms of life (Oates et al. 2013; Potenza et al. 2015; Ward et al. 2004). Disordered segments have been considered for a long time as protein fragments without any specific function, which are mainly required to link protein domains with well-defined three-dimensional structures. However, recent studies have demonstrated the biological importance of disordered sequences, adding the disorder-function paradigm to the existing structure-function paradigm (Dyson and Wright 2005). Intrinsically disordered regions (IDRs) or low complexity domains are polypeptide segments enriched for polar or charged amino acids, with low/no hydrophobic amino acids and that lack a defined three-dimensional structure in their native state. IDRs can adopt extended or compact conformations and offer several advantages to proteins. First, by exposing short linear motifs, disordered proteins have the ability to act as scaffolds and promiscuously interact with a large variety of substrates. This, in turn, enables the formation of dynamic macromolecular assemblies. Second, disordered proteins can acquire different conformations when interacting with different substrates, thereby functioning as signaling and regulatory proteins. Third, by being post-translationally modified, IDRs can regulate protein functionality. In agreement, many signaling molecules, kinases, splicing factors, and transcription factors are disordered proteins (Babu 2016; Jana et al. 2001).

From the structural point of view, sHSPs are composed of three domains: the N-terminal domain (NTD), the middle alpha-crystallin domain (ACD), and the C-terminal domain (CTD) (Van Montfort et al. 2001a). The ACD contains several  $\beta$ -strands that form two  $\beta$ -sheets arranged similarly to immunoglobulin, while the C- and N-terminal domains are intrinsically disordered (Sudnitsyna et al. 2011). In contrast to the ACD, which is conserved and represents the signature of the sHSP family, the C- and N-termini are only marginally conserved among the various sHSPs and across species. Together, these three domains contribute to regulate the dynamic association of sHSP monomers, which have low molecular masses (13–43 kDa), into large oligomers, which can contain at the top range 24–40 subunits (van Montfort et al. 2001a, b).

The role of hetero-oligomerization on sHSP structure and function remains enigmatic. This aspect has been addressed by Dr. Buchner (Germany). He reported the structure of the eye lens-specific sHsp alpha-A crystallin (HSPB4) solved by cryo-electron microscopy, together with Sevil Weinkauf's group (Germany). Besides identifying the principles of assembly, the structure(s) also reveal the basis for the heterogeneity of the oligomeric ensemble.

Ten different sHsps are encoded in the human genome. They differ in their oligomeric states and chaperone activities

(Mymrikov et al. 2017). Some of them are expressed simultaneously in cells and are known to form mixed complexes (Haslbeck et al. 2018). This phenomenon can be considered as a means to regulate sHsps function. However, the impact of hetero-oligomers on the mechanism of sHsps remained enigmatic. Dr. Buchner and colleagues analyzed hetero-oligomer formation *in vitro* and in cells. They report that hetero-oligomerization affects the size distribution of the oligomers and also their chaperone activities.

sHSP oligomerization is a dynamic process that can be influenced by changes in pH (Fleckenstein et al. 2015) and post-translational modifications, such as phosphorylation (Aquilina et al. 2004; Arrigo and Gibert 2012; Benn et al. 2002; Maitre et al. 2012). The phosphorylation sites are often located within the disordered N-terminal or C-terminal domain of many sHSPs. Phosphorylation induces conformational changes and influences sHSP hydrophobicity and binding affinity to other proteins. A direct link has been demonstrated between phosphorylation of specific sites located in disordered regions of sHSPs. Changes in oligomerization and chaperone activity have been shown using human HSPB1 and HSPB5 phosphomimicking mutants and non-phosphorylatable mutants, as well as deletion mutants or chimeric sHSP mutants. Together, these data point to the importance of disordered domains for the modulation of sHSP chaperone-like function (Delbecq and Klevit 2013; Delbecq et al. 2015; Ecroyd et al. 2007; Giese et al. 2005; Giese and Vierling 2002; Hilton et al. 2012; McDonald et al. 2012; McHaourab et al. 2002; Peschek et al. 2013; Stromer et al. 2004; van Montfort et al. 2001b). This is further supported by studies using yeast Hsp42. The N-terminal domain of Hsp42 contains a prion-like domain (PrLD) and a canonical IDR that act in a coordinated manner to promote formation of stress-induced macromolecular assemblies by recruiting misfolded proteins and regulating their aggregation. The ability of Hsp42 to sequester misfolded proteins into large proteinaceous aggregates is important to protect cells from proteotoxic insults and for cell fitness (Grousl et al. 2018).

However, the scenario for sHSPs is more complex, since also the disordered CTD and the folded ACD regulate sHSP oligomerization and, consequently, affinity for a given substrate, which can then vary based on the binding interfaces that are exposed by sHSP. In fact, recent studies from Dr. Reif (Germany) and Buchner, using state-of-the-art NMR spectroscopy, showed that while the ACD of HSPB5 preferentially binds to amyloid-like fibrils, such as Alzheimer disease-linked A $\beta$ 1–40, the disordered N-terminal domain of HSPB5 preferentially captures amorphously aggregating substrates, such as lysozyme. The intrinsic structural plasticity of sHSP, which can expose different binding interfaces, confers on them the ability to bind to a wide range of structurally heterogeneous clients. This inherent structural plasticity represents a powerful way of regulating promiscuous interaction

with a large variety of substrates, and timely regulating transient interaction with a given substrate. Indeed, the oligomerization state of sHSPs is influenced by reversible and transient post-translational modifications, enabling the same sHSP to display differential affinity for a given substrate (Grousl et al. 2018; Mainz et al. 2015; McDonald et al. 2012; Sudnitsyna et al. 2011; Van Montfort et al. 2001a). Thus, the common theme that is emerging from several studies performed using sHSP from various species and by mutating specific sites is that protein disorder contributes to regulate the dynamic assembly and disassembly of oligomers of variable size. This dynamic assembly, in turn, is a prerequisite for sHSP functionality, since it confers transient and variable affinity for a heterogeneous class of proteins in response to external stimuli, stressors, and also developmental and pro-differentiation stimuli.

Another example in support of sHSP “disorder” as an important feature regulating their functionality is a recent work discussed during this meeting by Dr. Carver (Australia). Together with his colleagues, Dr. Carver investigated the structure and molecular chaperone action of  $\alpha$ B-crystallin (HSPB5) under *in vitro* macromolecular crowding conditions induced by the inert polysaccharide, Ficoll 400. HSPB5 and its partner small heat shock protein  $\alpha$ A-crystallin (HSPB4) are the predominant eye lens proteins that, with the unrelated  $\beta$ - and  $\gamma$ -crystallins, arrange themselves in an ordered supramolecular array that enables proper refraction and focussing of light onto the retina. The crystallins are present at very high concentrations in the eye lens (up to 400 mg/mL in the center of aged lenses). Highly crowded conditions cause excluded volume effects that can alter protein structure, for example, leading to unfolding and aggregation. In contrast to HSPB4, HSPB5 is present extensively outside the lens where it has a molecular chaperone role to stabilize proteins to prevent their unfolding, for example under conditions of cellular stress. Performing small-angle neutron scattering (SANS) of deuterated HSPB5 and protonated Ficoll 400 solutions, Dr. Carver and colleagues studied the conformation of HSPB5 under crowded conditions comparable to those in the lens (and in many other cells). Under these conditions at physiological temperatures over 30 h, HSPB5’s central, conserved  $\beta$ -sheet region (the ACD) unfolded, which was accompanied by co-association of the protein to form amorphous and subsequently fibrillar, possibly amyloid-like, aggregates. Despite these structural alterations, HSPB5 retained its chaperone ability to prevent the aggregation of destabilized proteins, implying that the unfolded state is the chaperone functional form of HSPB5 in intra- and extra-lenticular environments. This conclusion is consistent with many other studies showing that HSPB5 functions effectively as a chaperone under destabilized conditions (for example low pH, high temperature, and in the presence of denaturant) and when fragmented into its peptide components. Of note, changes in pH and high

temperature regulate the dynamic assembly and liquid-demixing of many disordered proteins, with important biological implications (Alberti 2017; Riback et al. 2017). This remarkable finding opens the question: To what extent, under these unfolded state conditions, do the intrinsically disordered terminal regions of HSPB5 contribute to regulating its chaperone activity? Thus, HSPB5 is a highly malleable protein that exhibits characteristics of an intrinsically disordered protein, including during its chaperone action.

Experimental evidence in support of the importance of disordered regions as regulators of chaperone function exists not only for sHSPs. We cite as examples Hsp33 and San1. Hsp33 possesses a redox-sensor domain that unfolds upon oxidative stress. Importantly, Hsp33 can bind to misfolded proteins only in this unfolded state, thereby inducing folding and stabilizing folding intermediates that can then be fully refolded by ATP-dependent foldases (Reichmann et al. 2012). San1 is a yeast nuclear E3 ubiquitin ligase that uses its disordered N- and C-terminal domains to recognize misfolded proteins and target them for proteasome-mediated destruction (Rosenbaum et al. 2011). Collectively, these findings support the conclusion that IDRs are important functional regions for chaperones, including sHSPs. Identifying the molecular grammar that regulates sHSP oligomer dissociation and specific sites that may lock a given sHSP into a conformationally active or inactive state will advance our understanding of how sHSP recognize and bind specific substrates, regulating heterogeneous biological processes. We anticipate that such molecular grammar may differ from sHSP to sHSP, pointing to the need for careful study of each sHSP using interdisciplinary *in vitro* and *in vivo* approaches. This, in turn, highlights the need to develop new strategies to study sHSP structure/function relationships.

Aware of this complexity, Dr. Klevit (USA) presented a talk entitled “sHSPs: the more we know, the more we don’t understand” in which she presented new structural and biochemical data on human HSPB1. Although ubiquitously expressed and implicated in several serious neuropathies, structural information on HSPB1 is lacking. Substitution of three serine residues in the disordered N-terminal domain (NTD) known to be phosphorylation sites under stress to aspartic acid and mutation of the IxI motif in the C-terminal region yield a fully dispersed dimeric form of HSPB1 that is an effective chaperone that delays aggregation of Tau (Baughman et al. 2018). This form, called HSPB1 dimer, is presumed to mimic a stress-activated HSPB1. Application of solution NMR and hydrogen-deuterium exchange/mass spectrometry (HDXMS) to HSPB1 dimer and to wild-type HSPB1 oligomers offered a first structural glimpse at the disordered N-terminal domain (NTD). Although the NTD is dynamic and heterogeneous, the NMR results show that it is in contact with the structured alpha-crystallin domain that defines the dimer. In the phosphomimic HSPB1 dimer, the phosphorylation sites

are highly exposed, indicating that they are available for protein-protein interactions. Curiously, two missense mutations implicated in neuropathies, G34R and P39L, have distinct effects on both the local and global structure and dynamics of HSPB1 despite being only five residues apart in a disordered region of the NTD. These studies represent the first atomic-level information regarding the NTD of HSPB1, where a majority of disease-associated mutations are harbored and demonstrate the ability of hydrogen:deuterium exchange mass spectrometry (HDXMS) to provide novel structural and dynamic insights into sHSPs.

The complexity of structure/function relationships and identification of key molecular grammars are further increased when considering that different sHSPs can interact with each other forming hetero-oligomers. Dr. Boelens (The Netherlands) presented a recent study of HSPB2 and HSPB3 structure. These two vertebrate sHSPs interact in neuronal and muscular cells. They are components of skeletal and cardiac muscle and are upregulated during differentiation of myoblasts. Although their exact functions within the neuromuscular system are still enigmatic, variants and mutations of HSPB3 (R7S, R116P, A33fsX50, and Y118H) are associated with neuromuscular diseases, including axonal Charcot-Marie-Tooth disease (Kolb et al. 2010; Morelli et al. 2017; Nam et al. 2018). These findings strongly suggest that HSPB3, and its partner HSPB2, play important roles for the functionality of neuronal and muscular cells. HSPB2 and HSPB3 co-assemble into a tetramer with a 3:1 ratio, a unique composition within the sHsp family. Although this tetramer forms the building block for higher oligomer assemblies, consisting of 8–24 subunits, it is potentially a more tractable target for structural study than other human sHsps. By assessing the assembly and flexibility of HSPB2 and HSPB3 from both rat and human by means of size-exclusion chromatography, native mass spectrometry, and nuclear magnetic resonance (NMR) spectroscopy, a human HSPB3 mutant was engineered that suppresses the formation of higher assemblies of HSPB2/B3. The resulting monodisperse hetero-tetramer was crystallized and solved at a resolution of 3.9 Å (Clark et al. 2018). In the HSPB2-HSPB3 tetramer, the four  $\alpha$ -crystallin domains (ACDs) assemble to form a flattened tetrahedron that is pierced by two non-intersecting approximate dyads and portions of the unstructured N-terminus bind to the ACD grooves. Detailed description of the structure of HSPB2-HSPB3 assemblies can be found elsewhere (Clark et al. 2018). Future studies should aim at understanding how the heterogeneous interactions and plasticity of HSPB2-HSPB3 heteromers regulate their physiological functions.

Another typical feature of IDR-containing proteins is their ability to undergo liquid-liquid phase separation (LLPS) and form membraneless organelles or biomolecular condensates that have heterogeneous composition (Banani et al. 2017). Examples of membraneless organelles are cytoplasmic stress

granules and P-bodies, and nuclear speckles, paraspeckles, PML bodies, and Cajal bodies, as well as DNA damage foci (Banani et al. 2017). Although sHSPs possess IDRs, only limited evidence exists in support of their ability to undergo LLPS. For example, while the role of Hsp42-IDR in modulating its chaperone activity has been experimentally demonstrated, evidence for Hsp42 undergoing LLPS is still lacking (Grousl et al. 2018). Instead, Dr. Carra (Italy) presented recent evidence demonstrating that human HSPB2 undergoes concentration-dependent LLPS in mammalian cells. When the local concentration of HSPB2 reaches a critical threshold, HSPB2 forms nuclear membraneless compartments that sequester nuclear lamin-A, affecting its dynamics and function; this has functional consequences on chromatin organization and gene expression in mammalian cells, including HeLa and muscle cells (Morelli et al. 2017). HSPB2 phase separation is negatively regulated by its stoichiometric partner HSPB3, but not by two HSPB3 mutants linked to congenital myopathy. Based on these findings, it was suggested that deregulation of HSPB2 compartmentalization, due to decreased HSPB3 expression or HSPB3 mutations, could contribute to muscle aging and disease by affecting nuclear lamin distribution/function. Whether HSPB2 phase separation is required for its chaperone function is yet unclear. Whether lamin-A is a specific substrate of HSPB2 that is sequestered into the condensates observed in mammalian cells is not yet clear. Moreover, considering the regulatory role of HSPB3 on HSPB2 phase separation, it will be important to further understand how these proteins interact and what regulates their dynamic assembly/disassembly. Detailed understanding of HSPB2/B3 co-assembly (Clark et al. 2018) could be exploited in the future to understand how these heterogeneous interactions influence HSPB2 LLPS, with potential functional implication on nuclear lamins.

Although direct demonstration of sHSP undergoing LLPS is currently limited, several independent experimental findings suggest a strong link between sHSP, membraneless compartments that originate via LLPS, stress response, and disease. For example, human HSPB1 and HSPB8, as well as plant Hsp23, have been localized to stress granules (Arrigo et al. 1988; Ganassi et al. 2016; Jain et al. 2016; Kederasha et al. 1999; Mateju et al. 2017; Nover et al. 1989), while human HSPB1 and HSPB5 have been localized to nuclear splicing speckles (Adhikari et al. 2004; den Engelsman et al. 2005; van den et al. 2003; van Rijk et al. 2003). Recruitment of sHSPs inside these compartments seems to be regulated in response to stress conditions and has been proposed to enable the cell to adapt and respond to external stimuli. For example, HSPB1 and HSPB5 are recruited inside splicing speckles upon heat shock (Adhikari et al. 2004; den Engelsman et al. 2005). Of note, HSPB1 and HSPB5 recruitment is regulated by their phosphorylation and is impaired in disease-linked mutants, at least in the case of HSPB5 (Adhikari et al. 2004;

den Engelsman et al. 2005). This finding opens the possibility that deregulated function of HSPB5 at the level of nuclear splicing speckles, due to the R120G mutation, might lead to HSPB5-related myopathies. One possible function of disordered regions could be that they enable sHSPs to partition into these membraneless organelles and regulate the solubility of their binding partners in these subcellular compartments. Future studies should address this aspect.

Concerning stress granules, it has been recently shown that they can accumulate misfolded proteins. When this occurs, stress granules acquire aberrant properties and tend to convert from dynamic liquid-like compartments into solid-like aggregates (Ganassi et al. 2016; Mateju et al. 2017). The recruitment of sHSPs inside stress granules has been linked to the presence of misfolded proteins. Once recruited inside stress granules, sHSPs, along with other chaperones and co-chaperones such as HSP70, BAG3, and valosine containing protein (VCP), exert chaperone functions by inhibiting the irreversible aggregation of stress granules and promoting their disassembly (Alberti et al. 2017; Buchan et al. 2013; Seguin et al. 2014). This has important implications for human health, since accumulation of persisting stress granules seems to lie at the heart of several age-related neurodegenerative diseases (Kim et al. 2013; Taylor et al. 2016).

The ability of sHSPs to exert a chaperone function at the level of stress granules was further discussed during the meeting by Dr. Alberti (Germany), who presented unpublished data demonstrating the interaction of two sHSPs, HSPB8 and HSPB1, with the disordered protein Fused In Sarcoma (FUS), a component of stress granules. In vitro, FUS forms liquid droplets that undergo a transition into solid fibrils. This process of molecular aging is accelerated by mutations in FUS that have been linked to amyotrophic lateral sclerosis (ALS) (Patel et al. 2015). Drs. Alberti and Boczek used purified full-length proteins to reconstitute the quality control machinery of stress granules (SGs) in vitro. They find that HSPB8 plays a central role in maintaining the liquid properties of FUS droplets. In contrast, HSPB1 preferentially targets misfolded proteins that accumulate in the liquid droplet phase of SGs. These data are intriguing because they not only reinforce the link between sHSPs and membraneless compartments, but they seem to suggest that different sHSPs may exert distinct functions once recruited inside these compartments. Future studies are needed to understand the molecular events that lead to the enrichment of sHSPs inside these condensates and their exact function. As observed for splicing speckles, stress-induced post-translational modifications of sHSPs, by regulating their oligomerization, may control sHSP recruitment inside stress granules that acquire aberrant properties. The identification of the residues and motifs required for chaperone recruitment inside membraneless organelles such as speckles and aberrant stress granules may offer new therapeutic avenues in both age-related neurodegenerative diseases and HSPB-linked diseases.

## sHSP and human disease

The implication of sHSPs/HSPBs in human diseases represented a strong theme throughout the workshop, with several groups focusing on the understanding of how mutations in HSPB genes affect their structure and function, in vitro and in cells, and how to exploit HSPB chaperone activity for the treatment of age-related neurodegenerative diseases. Genetic analysis to identify either mutations or variants, as well as deregulated expression of HSPBs, is also being undertaken to expand the spectrum of diseases that are directly or indirectly associated with HSPB “malfunction.”

The role of sHSPs in opposing toxic protein aggregation in cells was discussed by Dr. Lee (Canada), who presented unpublished data on cellular mechanisms that maintain the solubility of FUS, a stress granule protein that aggregates in ALS. Using Fluorescence Recovery after photobleaching, Dr. Lee and colleagues demonstrated that depletion of HSPB1 and HSPB8, which have been implicated in stress granule dynamics, by RNAi alone or together does not affect the solubility of FUS in cells. However, their results indicate that HSPB8 does appear to function synergistically with ATP-dependent chaperones and aggregate clearance mechanisms to regulate FUS solubility in cells. Interestingly, they report that the effect of HSPB8 is particularly pronounced in human neurons differentiated from induced pluripotent stem cells (iPSCs). Neurons exhibit significantly higher HSPB8 expression compared to iPSCs and neural progenitors. CRISPR/Cas9-mediated knockout of HSPB8 leads to increased protein aggregation in neurons as measured by Filtration Retardation Assay and defects that promoted neurodegeneration. These results raise the intriguing possibility that mutations or defects in sHSP expression and decline in protein quality control machinery with age accelerate protein aggregation and drive degeneration of neurons in disease.

In line with these data, Dr. Poletti (Italy) discussed the possibility of exploiting pharmacological or genetic induction of HSPB8 expression to combat motor neuron diseases such as ALS and Kennedy’s disease (or Spinal and Bulbar Muscular Atrophy/SBMA). HSPB8 associates with BAG3 HSP70 and CHIP (an ubiquitinating enzyme) to deliver misfolded protein to autophagosomes (Carra et al. 2008; Crippa et al. 2010; Gamerding et al. 2011). This specific form of autophagy is called Chaperone-assisted selective autophagy (CASA); therefore, the HSPB8-BAG3-HSP70 complex is also referred to as CASA complex (Arndt et al. 2010). HSPB8 is induced in response to several neuronal stresses such as proteotoxic and oxidative stresses (Crippa et al. 2010). Dr. Poletti reported that HSPB8 is highly induced in the two main targets of misfolded protein toxicity in transgenic mouse models of SBMA and ALS, the motoneurons and muscle. The pharmacological or genetic induction of HSPB8 expression is protective in motor neuron diseases, while its

silencing has opposite effects. Therefore, pharmacological approaches that potentiate the HSPB8-BAG3 autophagic pathway could contribute to maintain proteostasis in motoneuron and muscle cells, with therapeutic implication in motor neuron diseases.

Both metabolic and neurodegenerative diseases are characterized by mitochondrial dysfunction (Mattson et al. 2008; Schon and Przedborski 2011; Schrepfer and Scorrano 2016). Several reports support the implication of sHSPs in mitochondria function. Examples include *Drosophila melanogaster* Hsp22, which is localized in mitochondria and whose overexpression in fruit flies extends life span by increasing resistance to oxidative stress (Morrow et al. 2000; Morrow et al. 2004) and HSPB2, whose knockout in mice reduces mitochondrial energetics following pressure overload, by as yet unclear mechanisms (Grose et al. 2015; Ishiwata et al. 2012). Dr. Timmerman and colleagues (Belgium) studied the role of human HSPBs in mitochondria. Given the mitochondrial transport defects in a mouse model of Charcot-Marie-Tooth (CMT) disease type 2F due to the HSPB1 mutations (S135F and P182L) (d’Ydewalle et al. 2011), Dr. Timmerman and colleagues investigated whether HSPB1 may participate in mitochondrial homeostasis and whether this role is altered by HSPB1-disease causing mutations. Mr. Adriaenssens from Dr. Timmerman’s lab reported that a fraction of several HSPBs can be imported into mitochondria. Importantly, this process was disturbed by the C-terminal HSPB1-P182L mutation, likely due to the propensity of this mutant to form larger oligomeric complexes, compared to WT HSPB1. By contrast, mutations in the ACD of HSPB1 seemed to cause the opposite phenotype, as they were detected in higher amounts in mitochondrial fractions. Interestingly, none of these processes seemed to depend on the phosphorylation status of HSPB1. These preliminary studies highlight a potential link between yet unidentified mitochondrial functions of HSPB1 and CMT disease. Future studies are required to understand to what extent mitochondrial dysfunctions are directly or indirectly affected by HSPB1 mutations.

Next, Mrs. Vendredy from Dr. Timmerman’s lab reported the generation and characterization of a mouse model to study HSPB8 implication in neuromuscular diseases. Dr. Timmerman previously identified mutations in the HSPB8 gene as one of the underlying genetic causes of autosomal dominant distal hereditary motor neuropathy (dHMN) which leads to progressive motor impairments. Interestingly, most of the identified mutations target the same amino acid residue (Lys141) in the HSPB8 protein. More recently, distal myopathy was also found to be associated with mutations in HSPB8. To delineate the molecular deficits and functional consequences of HSPB8 mutations, they generated a knock-in (KI) mouse model for the K141N missense mutation mimicking the human neuropathy genotype. They observed that homozygous mutant mice (Hspb8K141N/K141N) develop a

progressive axonopathy, with decreased Compound Muscle Action Potential (CMAP) amplitudes, and loss of large and medium myelinated axons. This results in locomotor deficits with an impaired performance in the Rotarod test. At the ultrastructural level, mice accumulate mutant HSPB8 protein and display degenerative patterns similar to dHMN patients with the K141N mutation. Interestingly, these animals also develop a progressive myofibrillar myopathy (MFM) as observed in some patients with HSPB8 mutations (Bouhy et al. 2018). Additionally, Dr. Timmerman's group generated HSPB8 knockout (KO) mice using the same targeting vector. Strikingly, the homozygous HSPB8-KO animals do not show any sign of axonopathy and display a much milder myopathy than the HSPB8-KI animals (Bouhy et al. 2018). Dr. Timmerman's team is currently investigating whether modifying the expression levels of HSPB8 can be exploited as a therapeutic strategy in motor neuron and muscle disease.

Dr. Tóth in collaboration with Dr. Miklós Sántha (Hungary) presented the hypothesis that increasing HSP expression and/or augmented stress response could be involved in the protective mechanisms of physical activity. Therefore, they studied the functional, morphological, and gene expression changes in transgenic mice in response to acute and regular exercise trainings, comparing normal and overweight animals. They observed differential changes in the expression of HSP genes in the two mice populations. Following acute exercise, Hspa1a expression showed a 4-fold increase in normal weight and a 9-fold increase in the overweight mice in skeletal muscles (m. quadriceps femoris). HSPB1 and HSPB5 were only slightly induced in the normal group; by contrast, their expression showed 12–13-fold increase in overweight mice after training. In addition, HSPB2 was induced only in the trained overweight group. These significant changes in gene expression were confirmed using Western blot analysis and immunohistochemistry. Together, these results show that moderate exercise training induces the expression of HSPBs in the skeletal muscle and this effect strongly depends on the body weight of the animals (unpublished data). Physical exercise has been suggested as a preventive or disease-modifying treatment of age-related diseases such as dementia and brain aging (Ahlskog et al. 2011). Whether part of the beneficial effects of physical exercise on cognition and neuronal cell survival also depends on the upregulation of HSPBs is still an open question.

Finally, Dr. Wu (Wuhan, China) discussed the implication of HSPB1 in cardiopulmonary diseases, which are the leading causes of morbidity and mortality worldwide and are caused by environments, genes, and their interactions. Dr. Wu and colleagues reported that the functional HSPB1 promoter 1271G\_C variant affected lung cancer susceptibility and survival by modulating endogenous HSPB1 synthesis (Guo et al. 2010). Dr. Wu further reported the association of the DNA methylation network with the risk of acute coronary syndrome

(Li et al. 2017). Finally, he suggested that the results obtained are promising, but need to be confirmed by prospective cohorts such as the Dongfeng-Tongji Cohort (Wang et al. 2013).

### Ongoing studies, new hypotheses, and future perspectives

Although the majority of the communications were focusing on human HSPBs, due to their expression among all kingdoms of life and their implications in the biology of many different organisms, the workshop also included selected reports on sHSP of bacterial, fruit fly, and plant origins. Dr. Liberek (Poland) analyzed specific functional interplay between two *Escherichia coli* sHsps, IbpA and IbpB, in directing the protein aggregation process towards assembly formation. Dr. Liberek's results suggest that after an IbpA gene duplication event at the base of Enterobacteriales functional diversification, post-duplication caused IbpA to specialize in efficient substrate binding upon aggregation while the second post-duplication sHsp (IbpB) became crucial for sHsp release from assemblies at the disaggregation step. In other bacteria possessing only one sHsp gene, these functions are fulfilled by a single IbpA-like protein (*Vibrio harveyi*, *Erwinia amylovora*). Dr. Liberek inferred that the chaperone systems with two sHSPs, in contrast to a single sHsp, allow for substantially easier release of sHsps from assemblies without compromising assembly formation; this, in turn, would ensure lower demand for Hsp70 in disaggregation and refolding.

Next, Afroz Dabbaghizadeh from Dr. Tanguay's laboratory (Canada) reported on the organization of *Drosophila melanogaster* Hsp22 (DmHsp22), focusing on the role of the ACD in oligomer assembly. In size-exclusion chromatography, DmHsp22 forms a single symmetric peak with an apparent molecular weight of approximately 820 kDa. Dr. Tanguay and colleagues also examined the influence of arginine to glycine mutations in the conserved ACD region on the structure and function of DmHsp22. Mutation in R109G did not result in structural disruption of the oligomeric structure. By contrast, mutation of R110 induces the dissociation of DmHsp22 to smaller oligomers. While all mutants demonstrate the same efficiency as wild-type in a DTT-induced insulin aggregation assay, they all are more efficient chaperones in preventing aggregation of malate dehydrogenase (Dabbaghizadeh et al. 2017). Thus, dynamic oligomerization differentially affects the affinity and chaperone activity of this sHSP. Then, they identified the proteins that interact with DmHSP22, using immunoaffinity conjugation (IAC) with mass spectroscopy analysis. Since DmHsp22 is found in mitochondria (Morrow et al. 2000), the analysis was performed using mitochondria from HeLa cells transfected with DmHsp22. In two assays, 139 and 72 proteins were found to be associated with various functional classifications. Most of the proteins interacting with DmHsp22 are transporters

localized in the inner mitochondrial membrane. Among these, several ATP synthase subunits were found. Moreover, they reported that expression of DmHsp22 in transiently transfected HeLa cells increased mitochondrial oxygen consumption and ATP content, providing a mechanistic link between DmHsp22 and mitochondrial functions. Thus, DmHsp22 may be involved as a chaperone in assembly of complex V. Among the DmHsp22 interacting proteins, ATP synthase subunits alpha, beta, and gamma were the most abundant peptides detected. DmHsp22 significantly increases oxidative capacity of the electron transport system. Mitochondrial O<sub>2</sub> consumption rate was also somewhat increased in cells transfected with DmHsp22. Moreover, mitochondrial ATP levels increased upon expression of DmHsp22. Dr. Tanguay concluded that DmHsp22 could be involved in the modulation of ATP synthase (Dabbaghizadeh et al. 2018). Together with the current report from Dr. Timmerman's group, who identified several human HSPBs in mitochondrial extracts, these data suggest potential unexplored functions of sHSP in mitochondrial homeostasis, which may be partly conserved throughout species, from fruit flies to humans.

Dr. Lockwood (USA) reported on the role of sHSPs in the physiological responses to sudden changes in temperature that allow organisms to cope with thermal variability in their natural environments (Lockwood et al. 2015). Dr. Lockwood showed examples in which the expression of sHSP genes causes large changes in whole-organism thermal tolerance, in both marine and terrestrial invertebrates. His work suggests that because sHSP genes are loci of potentially large phenotypic effect (Lockwood et al. 2017; Lockwood et al. 2010), these genes are likely to be targets of natural selection (Dilly et al. 2012; Healy et al. 2010; Lockwood et al. 2010) and may facilitate evolutionary responses to a warming world.

Plants express a unique set of sHSPs that have evolved independently from metazoan and bacterial sHSPs. They comprise nuclear genes encoding proteins targeted to every membrane-bound cellular compartment, the cytoplasm, nucleus, endoplasmic reticulum, peroxisomes, mitochondria, and chloroplasts. This diversity likely arose from new stresses encountered by plants on their movement to land. Studies of cytosolic plant sHSPs have been critical to developing the current model of sHSP chaperone activity, as well as important to defining conserved structural features of sHSPs (Basha et al. 2012). The crystal structure of the cytoplasmic, dodecameric Hsp16.9 from wheat (PDB:1GME) has also provided an excellent platform for modeling homologous plant sHSPs and for defining how sHSPs interact with denaturing substrates. Utilizing an extensive foundation of biochemical studies of plant cytosolic sHSPs, Dr. Vierling (USA) reported on the *in vitro* interaction of purified sHSPs from pea, Arabidopsis, and wheat with heat-denaturing porcine malate dehydrogenase as substrate. Contacts between sHSPs and substrate were detected using an amine-amine crosslinker

followed by mass spectrometry. The results with all three sHSPs support and extend previous work indicating that the substrate is only partially unfolded (Cheng et al. 2008) and that the N-terminal domain is involved in multiple substrate contacts (Jaya et al. 2009). To determine whether the interactions observed in this heterologous sHSP-substrate system reflect interactions in a homologous system, additional *in vitro* experiments were described with an Arabidopsis sHSP and the Arabidopsis enzyme fructose-bisphosphate aldolase (FBA). FBA was found associated with Arabidopsis sHSP during heat stress *in vivo* (McLoughlin et al. 2016) and may reflect an important sHSP-substrate. Crosslinking and mass spectrometry data with these proteins support the conclusion that similar interactions occur with native substrates as observed with model substrates.

Several other aspects of sHSP biology and the development of new techniques to study sHSP structure and functions were discussed during this meeting. Dr. Benesch (UK) presented unpublished data obtained from advanced biophysical and structural biology methods that challenge the canonical view that sHSPs act as generalist interceptors of protein aggregation stemming from interaction with non-native states. Based on his recent findings, Dr. Benesch proposed the hypothesis that sHSPs also interact with mechanosensitive proteins to regulate physiological extension and contraction cycles. He also reported on another important type of native state interaction made by sHSPs: their interaction with each other. In collaboration with Dr. Vierling, Dr. Benesch uncovered the balance co-assembly and selective self-assembly of these proteins that is a key step in evolving new sHSP functions. He discussed how this work represents a paradigm for understanding the biophysical basis for protein evolution (Hochberg et al. 2018). Building on this work, and capitalizing on his group's recent advances in mass spectrometry and the development of mass imaging in solution (Young et al. 2018), Dr. Benesch and colleagues were able to quantify how sHSPs co-assemble to form a bewildering array of polydisperse hetero-oligomers, allowing speculation on their functional significance.

Next, the use of optical tweezers to study sHSP chaperone function was discussed by Dr. Cecconi (Italy), who investigated *in vitro* the effect of HSPB8 on the folding and aggregation processes of maltose binding protein (MBP), a previously published substrate (Mashaghi et al. 2013; Ungelenk et al. 2016). Optical tweezers stretch and relax polypeptides thereby enabling the study of rare and transient intermediate unfolding or refolding states of the substrate protein. Dr. Cecconi and colleagues mechanically denatured homotetramers of MBP and analyzed their folding and aggregation processes in the presence or absence of HSPB8. In line with the well-established role of sHSPs as chaperone holdases, their results reveal a strong holdase activity of HSPB8, which either prevents completely the aggregation of denatured MBP molecules or allows the substrate to form only small and

mechanically weak aggregates. Importantly, a careful analysis of the data also discloses an unexpected foldase activity of HSPB8, which guides the folding process of denatured MBP domains into their native states. Their findings highlight new mechanisms of interaction between HSPB8 and its substrates and suggest a more complex physiological role for this chaperone than previously assumed, in line with data presented by Dr. Benesch using advanced biophysical and structural biology methods. Further information on the description of single-molecule approaches and their importance to study chaperone activity is summarized elsewhere (Avellaneda et al. 2017; Choudhary et al. 2019; Johnston et al. 2018).

Besides *in vitro* single-molecule approaches, new methodologies to study sHSP chaperone and anti-aggregation activity in living cells were also discussed. Dr. Ecroyd (Australia) presented a new technique developed in collaboration with his colleagues called FloIT (Flow cytometric analysis of Inclusions and Trafficking). FloIT is a simple and rapid new flow cytometry-based method that enumerates, characterizes and, if desired, physically recovers protein inclusions from cells (Whiten et al. 2016). Dr. Ecroyd used this technique to compare the ability of all ten human HSPBs to inhibit the intracellular aggregation of the model protein firefly luciferase (Fluc). Their unpublished work shows that HSPB4 and HSPB6 are the most potent suppressors of Fluc aggregation, whereas HSPB2 and HSPB3 enhance inclusion formation by Fluc. These tools are therefore providing the scientific community with new mechanistic and functional insights into the molecular chaperone action of sHSPs in cells.

Other aspects and technical issues were addressed during the workshop. For example, the importance of identifying and developing specific antibodies that efficiently and specifically detect post-translational modifications of sHSPs was discussed. In particular, Dr. Gusev (Russia) put forward the need to develop reliable antibodies that detect methylglyoxal modification. Methylglyoxal (MGO) is a highly reactive dicarbonyl formed in the course of glucose metabolism. MGO-modification results in the formation of a number of different products such as hydroimidazolones, argpyrimidines, carboxymethyllysines, and different cross-linked products. MGO levels are increased under certain pathological conditions, such as diabetes or carcinogenesis and MGO-modification of HSPB1 was previously reported and supposed to be important for HSPB1 function (Oya-Ito et al. 2011; Sakamoto et al. 2002). Interestingly, HSPB1 has been implicated in the etiology of metabolic diseases and its expression levels are reduced in skeletal muscle of aged-insulin resistant animals (Gupte et al. 2008). Based on these findings, it will be important to develop specific tools to study HSPB1 (and other HSPB) MGO-modifications to then evaluate their impact (if any) on HSPB function and implication in metabolic diseases and cancer.

To accommodate the increased research efforts on sHSP, which have increasing implications in biology and human disease, as briefly summarized here, the fourth in this meeting series is planned for the year 2020.

**Acknowledgements** We are grateful to the Cell Stress Society International (CSSI) for its financial support of the workshop. We thank the Fund for Scientific Research in Flanders-Belgium (FWO) for supporting the attendance at the workshop of PhD students, Elias Adriaenssens and Leen Vendredy.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## References

- Adhikari AS, Sridhar Rao K, Rangaraj N, Parnaik VK, Mohan Rao C (2004) Heat stress-induced localization of small heat shock proteins in mouse myoblasts: intranuclear lamin A/C speckles as target for alphaB-crystallin and Hsp25. *Exp Cell Res* 299:393–403
- Ahlskog JE, Geda YE, Graff-Radford NR, Petersen RC (2011) Physical exercise as a preventive or disease-modifying treatment of dementia and brain aging. *Mayo Clin Proc* 86:876–884
- Alberti S (2017) Phase separation in biology. *Curr Biol* 27:R1097–R1102
- Alberti S, Mateju D, Mediani L, Carra S (2017) Granulostasis: protein quality control of RNP granules. *Front Mol Neurosci* 10:84
- Aquilina JA, Benesch JL, Ding LL, Yaron O, Horwitz J, Robinson CV (2004) Phosphorylation of alphaB-crystallin alters chaperone function through loss of dimeric substructure. *J Biol Chem* 279:28675–28680
- Arndt V, Dick N, Tawo R, Dreiseidler M, Wenzel D, Hesse M, Furst DO, Saftig P, Saint R, Fleischmann BK et al (2010) Chaperone-assisted selective autophagy is essential for muscle maintenance. *Curr Biol* 20:143–148
- Arrigo AP (2000) sHsp as novel regulators of programmed cell death and tumorigenicity. *Pathologie-biologie* 48:280–288
- Arrigo AP, Ducasse C (2002) Expression of the anti-apoptotic protein Hsp27 during both the keratinocyte differentiation and dedifferentiation of HaCat cells: expression linked to changes in intracellular protein organization? *Exp Gerontol* 37:1247–1255
- Arrigo AP, Gibert B (2012) HspB1 dynamic phospho-oligomeric structure dependent interactome as cancer therapeutic target. *Curr Mol Med* 12:1151–1163
- Arrigo AP, Gibert B (2014) HspB1, HspB5 and HspB4 in human cancers: potent oncogenic role of some of their client proteins. *Cancers* 6: 333–365
- Arrigo AP, Suhan JP, Welch WJ (1988) Dynamic changes in the structure and intracellular locale of the mammalian low-molecular-weight heat shock protein. *Mol Cell Biol* 8:5059–5071
- Avellaneda MJ, Koers EJ, Naqvi MM, Tans SJ (2017) The chaperone toolbox at the single-molecule level: from clamping to confining. *Protein Sci* 26:1291–1302
- Babu MM (2016) The contribution of intrinsically disordered regions to protein function, cellular complexity, and human disease. *Biochem Soc Trans* 44:1185–1200
- Balogi Z, Cheregi O, Giese KC, Juhasz K, Vierling E, Vass I, Vigh L, Horvath I (2008) A mutant small heat shock protein with increased thylakoid association provides an elevated resistance against UV-B damage in *synechocystis* 6803. *J Biol Chem* 283:22983–22991
- Banani SF, Lee HO, Hyman AA, Rosen MK (2017) Biomolecular condensates: organizers of cellular biochemistry. *Nat Rev Mol Cell Biol* 18:285–298

- Basha E, O'Neill H, Vierling E (2012) Small heat shock proteins and alpha-crystallins: dynamic proteins with flexible functions. *Trends Biochem Sci* 37:106–117
- Baughman HER, Clouser AF, Klevit RE, Nath A (2018) HspB1 and Hsc70 chaperones engage distinct tau species and have different inhibitory effects on amyloid formation. *J Biol Chem* 293:2687–2700
- Benjamin IJ, Shelton J, Garry DJ, Richardson JA (1997) Temporospatial expression of the small HSP/alpha B-crystallin in cardiac and skeletal muscle during mouse development. *Dev Dyn* 208:75–84
- Benn SC, Perrelet D, Kato AC, Scholz J, Decosterd I, Mannion RJ, Bakowska JC, Woolf CJ (2002) Hsp27 upregulation and phosphorylation is required for injured sensory and motor neuron survival. *Neuron* 36:45–56
- Bouhy D, Juneja M, Katona I, Holmgren A, Asselbergh B, De Winter V, Hocheplid T, Goossens S, Haigh JJ, Libert C et al (2018) A knock-in/knock-out mouse model of HSPB8-associated distal hereditary motor neuropathy and myopathy reveals toxic gain-of-function of mutant HspB8. *Acta Neuropathol* 135:131–148
- Bruey JM, Paul C, Fromentin A, Hilpert S, Arrigo AP, Solary E, Garrido C (2000) Differential regulation of HSP27 oligomerization in tumor cells grown in vitro and in vivo. *Oncogene* 19:4855–4863
- Buchan JR, Kolaitis RM, Taylor JP, Parker R (2013) Eukaryotic stress granules are cleared by autophagy and Cdc48/VCP function. *Cell* 153:1461–1474
- Bult CJ, White O, Olsen GJ, Zhou L, Fleischmann RD, Sutton GG, Blake JA, FitzGerald LM, Clayton RA, Gocayne JD, Kerlavage AR, Dougherty BA, Tomb JF, Adams MD, Reich CI, Overbeek R, Kirkness EF, Weinstock KG, Merrick JM, Glodek A, Scott JL, Geoghagen NSM, Weidman JF, Fuhrmann JL, Nguyen D, Utterback TR, Kelley JM, Peterson JD, Sadow PW, Hanna MC, Cotton MD, Roberts KM, Hurst MA, Kaine BP, Borodovsky M, Klenk HP, Fraser CM, Smith HO, Woese CR, Venter JC (1996) Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. *Science (New York, NY)* 273:1058–1073
- Candido EP (2002) The small heat shock proteins of the nematode *Caenorhabditis elegans*: structure, regulation and biology. *Prog Mol Subcell Biol* 28:61–78
- Carra S, Seguin SJ, Lambert H, Landry J (2008) HspB8 chaperone activity toward poly(Q)-containing proteins depends on its association with Bag3, a stimulator of macroautophagy. *J Biol Chem* 283:1437–1444
- Carra S, Alberti S, Arrigo PA, Benesch JL, Benjamin IJ, Boelens W, Bartelt-Kirbach B, Brundel B, Buchner J, Bukau B et al (2017) The growing world of small heat shock proteins: from structure to functions. *Cell Stress Chaperones* 22:601–611
- Caspers GJ, Leunissen JA, de Jong WW (1995) The expanding small heat-shock protein family, and structure predictions of the conserved “alpha-crystallin domain”. *J Mol Evol* 40:238–248
- Cheng G, Basha E, Wysocki VH, Vierling E (2008) Insights into small heat shock protein and substrate structure during chaperone action derived from hydrogen/deuterium exchange and mass spectrometry. *J Biol Chem* 283:26634–26642
- Choudhary, D, Mossa, A, Jadhav, M, Cecconi C (2019). Bio-Molecular Applications of Recent Developments in Optical Tweezers. *Biomolecules* 9(1). <https://doi.org/10.3390/biom9010023>
- Clark AR, Vree Egberts W, Kondrat FDL, Hilton GR, Ray NJ, Cole AR, Carver JA, Benesch JLP, Keep NH, Boelens WC, Slingsby C (2018) Terminal regions confer plasticity to the tetrameric assembly of human HspB2 and HspB3. *J Mol Biol* 430:3297–3310
- Crippa V, Sau D, Rusmini P, Boncoraglio A, Onesto E, Bolzoni E, Galbiati M, Fontana E, Marino M, Carra S, Bendotti C, de Biasi S, Poletti A (2010) The small heat shock protein B8 (HspB8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (ALS). *Hum Mol Genet* 19:3440–3456
- d'Ydewalle C, Krishnan J, Chiheb DM, Van Damme P, Irobi J, Kozikowski AP, Vanden Berghe P, Timmerman V, Robberecht W, Van Den Bosch L (2011) HDAC6 inhibitors reverse axonal loss in a mouse model of mutant HSPB1-induced Charcot-Marie-Tooth disease. *Nat Med* 17:968–974
- Dabbaghizadeh A, Finet S, Morrow G, Moutaoufik MT, Tanguay RM (2017) Oligomeric structure and chaperone-like activity of *Drosophila melanogaster* mitochondrial small heat shock protein Hsp22 and arginine mutants in the alpha-crystallin domain. *Cell Stress Chaperones* 22:577–588
- Dabbaghizadeh A, Morrow G, Amer YO, Chatelain EH, Pichaud N, Tanguay RM (2018) Identification of proteins interacting with the mitochondrial small heat shock protein Hsp22 of *Drosophila melanogaster*: implication in mitochondrial homeostasis. *PLoS One* 13:e0193771
- Delbecq SP, Klevit RE (2013) One size does not fit all: the oligomeric states of alphaB crystallin. *FEBS Lett* 587:1073–1080
- Delbecq SP, Rosenbaum JC, Klevit RE (2015) A mechanism of subunit recruitment in human small heat shock protein oligomers. *Biochemistry* 54:4276–4284
- den Engelsman J, Gerrits D, de Jong WW, Robbins J, Kato K, Boelens WC (2005) Nuclear import of {alpha}B-crystallin is phosphorylation-dependent and hampered by hyperphosphorylation of the myopathy-related mutant R120G. *J Biol Chem* 280:37139–37148
- Dilly GF, Young CR, Lane WS, Pangilinan J, Girguis PR (2012) Exploring the limit of metazoan thermal tolerance via comparative proteomics: thermally induced changes in protein abundance by two hydrothermal vent polychaetes. *Proc Biol Sci* 279:3347–3356
- Dyson HJ, Wright PE (2005) Intrinsically unstructured proteins and their functions. *Nat Rev Mol Cell Biol* 6:197–208
- Ecroyd H, Meehan S, Horwitz J, Aquilina JA, Benesch JL, Robinson CV, Macphree CE, Carver JA (2007) Mimicking phosphorylation of alphaB-crystallin affects its chaperone activity. *Biochem J* 401:129–141
- Evgrafov OV, Mersyanova I, Irobi J, Van Den Bosch L, Dierick I, Leung CL, Schagina O, Verpoorten N, Van Impe K, Fedotov V et al (2004) Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. *Nat Genet* 36:602–606
- Eyles SJ, Gierasch LM (2010) Nature's molecular sponges: small heat shock proteins grow into their chaperone roles. *Proc Natl Acad Sci U S A* 107:2727–2728
- Fleckenstein T, Kastenmuller A, Stein ML, Peters C, Daake M, Krause M, Weinfurter D, Haslbeck M, Weinkauff S, Groll M et al (2015) The chaperone activity of the developmental small heat shock protein Sip1 is regulated by pH-dependent conformational changes. *Mol Cell* 58:1067–1078
- Gamerding M, Kaya AM, Wolftrum U, Clement AM, Behl C (2011) BAG3 mediates chaperone-based aggresome-targeting and selective autophagy of misfolded proteins. *EMBO Rep* 12:149–156
- Ganassi M, Mateju D, Bigi I, Mediani L, Poser I, Lee HO, Seguin SJ, Morelli FF, Vinet J, Leo G, Pansarasa O, Cereda C, Poletti A, Alberti S, Carra S (2016) A surveillance function of the HSPB8-BAG3-HSP70 chaperone complex ensures stress granule integrity and dynamism. *Mol Cell* 63:796–810
- Ghaoui R, Palmio J, Brewer J, Lek M, Needham M, Evila A, Hackman P, Jonson PH, Penttila S, Vihola A et al (2016) Mutations in HSPB8 causing a new phenotype of distal myopathy and motor neuropathy. *Neurology* 86:391–398
- 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* 277:46310–46318
- Giese KC, Basha E, Catague BY, Vierling E (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 U S A* 102:18896–18901
- Grose JH, Langston K, Wang X, Squires S, Mustafi SB, Hayes W, Neubert J, Fischer SK, Fasano M, Saunders GM, Dai Q, Christians E, Lewandowski ED, Ping P, Benjamin IJ (2015) Characterization of the cardiac overexpression of HSPB2 reveals mitochondrial and myogenic roles supported by a cardiac HspB2 interactome. *PLoS One* 10:e0133994
- Grousl T, Ungelenk S, Miller S, Ho CT, Khokhrina M, Mayer MP, Bukau B, Mogk A (2018) A prion-like domain in Hsp42 drives chaperone-facilitated aggregation of misfolded proteins. *J Cell Biol* 217:1269–1285
- Guo H, Bai Y, Xu P, Hu Z, Liu L, Wang F, Jin G, Wang F, Deng Q, Tu Y, Feng M, Lu D, Shen H, Wu T (2010) Functional promoter -1271G>C variant of HSPB1 predicts lung cancer risk and survival. *J Clin Oncol* 28:1928–1935
- Gupte AA, Bomhoff GL, Geiger PC (2008) Age-related differences in skeletal muscle insulin signaling: the role of stress kinases and heat shock proteins. *J Appl Physiol* (1985) 105:839–848
- Haslbeck M, Vierling E (2015) A first line of stress defense: small heat shock proteins and their function in protein homeostasis. *J Mol Biol* 427:1537–1548
- Haslbeck M, Peschek J, Buchner J, Weinkauff S (2016) Structure and function of alpha-crystallins: traversing from in vitro to in vivo. *Biochim Biophys Acta* 1860:149–166
- Haslbeck M, Weinkauff S, Buchner J (2018) Small heat shock proteins: Simplicity meets complexity. *J Biol Chem* <https://doi.org/10.1074/jbc.REV118.002809>
- Healy TM, Tymchuk WE, Osborne EJ, Schulte PM (2010) Heat shock response of killifish (*Fundulus heteroclitus*): candidate gene and heterologous microarray approaches. *Physiol Genomics* 41:171–184
- Hilton GR, Lioe H, Stengel F, Baldwin AJ, Benesch JL (2012) Small heat-shock proteins: paramedics of the cell. *Top Curr Chem* 328:69–98
- Hochberg GKA, Shepherd DA, Marklund EG, Santhanagopalan I, Degiacomi MT, Laganowsky A, Allison TM, Basha E, Marty MT, Galpin MR, Struwe WB, Baldwin AJ, Vierling E, Benesch JLP (2018) Structural principles that enable oligomeric small heat-shock protein paralogs to evolve distinct functions. *Science (New York, NY)* 359:930–935
- Hong SW, Vierling E (2000) Mutants of *Arabidopsis thaliana* defective in the acquisition of tolerance to high temperature stress. *Proc Natl Acad Sci U S A* 97:4392–4397
- Irobi J, Van Impe K, Seeman P, Jordanova A, Dierick I, Verpoorten N, Michalik A, De Vriendt E, Jacobs A, Van Gerwen V et al (2004) Hot-spot residue in small heat-shock protein 22 causes distal motor neuropathy. *Nat Genet* 36:597–601
- Ishiwata T, Orosz A, Wang X, Mustafi SB, Pratt GW, Christians ES, Boudina S, Abel ED, Benjamin IJ (2012) HSPB2 is dispensable for the cardiac hypertrophic response but reduces mitochondrial energetics following pressure overload in mice. *PLoS One* 7:e42118
- Jain S, Wheeler JR, Walters RW, Agrawal A, Barsic A, Parker R (2016) ATPase-modulated stress granules contain a diverse proteome and substructure. *Cell* 164:487–498
- Jana NR, Zemskov EA, Wang G, Nukina N (2001) Altered proteasomal function due to the expression of polyglutamine-expanded truncated N-terminal huntingtin induces apoptosis by caspase activation through mitochondrial cytochrome c release. *Hum Mol Genet* 10:1049–1059
- Jaya N, Garcia V, Vierling E (2009) Substrate binding site flexibility of the small heat shock protein molecular chaperones. *Proc Natl Acad Sci U S A* 106:15604–15609
- Johnston CL, Marzano NR, van Oijen AM, Ecroyd H (2018) Using single-molecule approaches to understand the molecular mechanisms of heat-shock protein chaperone function. *J Mol Biol* 430:4525–4546
- Kamradt MC, Chen F, Sam S, Cryns VL (2002) The small heat shock protein alpha B-crystallin negatively regulates apoptosis during myogenic differentiation by inhibiting caspase-3 activation. *J Biol Chem* 277:38731–38736
- Kamradt MC, Lu M, Werner ME, Kwan T, Chen F, Strohecker A, Oshita S, Wilkinson JC, Yu C, Oliver PG, Duckett CS, Buchsbaum DJ, LoBuglio AF, Jordan VC, Cryns VL (2005) The small heat shock protein alpha B-crystallin is a novel inhibitor of TRAIL-induced apoptosis that suppresses the activation of caspase-3. *J Biol Chem* 280:11059–11066
- Kedersha NL, Gupta M, Li W, Miller I, Anderson P (1999) RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2 alpha to the assembly of mammalian stress granules. *J Cell Biol* 147:1431–1442
- Kim KK, Kim R, Kim SH (1998) Crystal structure of a small heat-shock protein. *Nature* 394:595–599
- Kim HJ, Kim NC, Wang YD, Scarborough EA, Moore J, Diaz Z, MacLea KS, Freibaum B, Li S, Molliex A, Kanagaraj AP, Carter R, Boylan KB, Wojtas AM, Rademakers R, Pinkus JL, Greenberg SA, Trojanowski JQ, Traynor BJ, Smith BN, Topp S, Gkazi AS, Miller J, Shaw CE, Kottlors M, Kirschner J, Pestronk A, Li YR, Ford AF, Gitler AD, Benatar M, King OD, Kimonis VE, Ross ED, Weihl CC, Shorter J, Taylor JP (2013) Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. *Nature* 495:467–473
- Kolb SJ, Snyder PJ, Poi EJ, Renard EA, Bartlett A, Gu S, Sutton S, Arnold WD, Freimer ML, Lawson VH, Kissel JT, Prior TW (2010) Mutant small heat shock protein B3 causes motor neuropathy: utility of a candidate gene approach. *Neurology* 74:502–506
- Lavoie JN, Gingras-Breton G, Tanguay RM, Landry J (1993) Induction of Chinese hamster HSP27 gene expression in mouse cells confers resistance to heat shock. HSP27 stabilization of the microfilament organization. *J Biol Chem* 268:3420–3429
- Lavoie JN, Lambert H, Hickey E, Weber LA, Landry J (1995) Modulation of cellular thermoresistance and actin filament stability accompanies phosphorylation-induced changes in the oligomeric structure of heat shock protein 27. *Mol Cell Biol* 15:505–516
- Li J, Zhu X, Yu K, Jiang H, Zhang Y, Deng S, Cheng L, Liu X, Zhong J, Zhang X, He M, Chen W, Yuan J, Gao M, Bai Y, Han X, Liu B, Luo X, Mei W, He X, Sun S, Zhang L, Zeng H, Sun H, Liu C, Guo Y, Zhang B, Zhang Z, Huang J, Pan A, Yuan Y, Angileri F, Ming B, Zheng F, Zeng Q, Mao X, Peng Y, Mao Y, He P, Wang QK, Qi L, Hu FB, Liang L, Wu T (2017) Genome-wide analysis of DNA methylation and acute coronary syndrome. *Circ Res* 120:1754–1767
- Litt M, Kramer P, LaMorticella DM, Murphey W, Lovrien EW, Weleber RG (1998) Autosomal dominant congenital cataract associated with a missense mutation in the human alpha crystallin gene CRYAA. *Hum Mol Genet* 7:471–474
- Lockwood BL, Sanders JG, Somero GN (2010) Transcriptomic responses to heat stress in invasive and native blue mussels (genus *Mytilus*): molecular correlates of invasive success. *J Exp Biol* 213:3548–3558
- Lockwood BL, Connor KM, Gracey AY (2015) The environmentally tuned transcriptomes of *Mytilus* mussels. *J Exp Biol* 218:1822–1833
- Lockwood BL, Julick CR, Montooth KL (2017) Maternal loading of a small heat shock protein increases embryo thermal tolerance in *Drosophila melanogaster*. *J Exp Biol* 220:4492–4501
- Mainz A, Peschek J, Stavropoulou M, Back KC, Bardiaux B, Asami S, Prade E, Peters C, Weinkauff S, Buchner J, Reif B (2015) The chaperone alphaB-crystallin uses different interfaces to capture an amorphous and an amyloid client. *Nat Struct Mol Biol* 22:898–905

- Maitre M, Weidmann S, Rieu A, Fenel D, Schoehn G, Ebel C, Coves J, Guzzo J (2012) The oligomer plasticity of the small heat-shock protein Lo18 from *Oenococcus oeni* influences its role in both membrane stabilization and protein protection. *The Biochemical journal* 444:97–104
- Mashaghi A, Kramer G, Bechtluft P, Zachmann-Brand B, Driessen AJ, Bukau B, Tans SJ (2013) Reshaping of the conformational search of a protein by the chaperone trigger factor. *Nature* 500:98–101
- Mateju D, Franzmann TM, Patel A, Kopach A, Boczek EE, Maharana S, Lee HO, Carra S, Hyman AA, Alberti S (2017) An aberrant phase transition of stress granules triggered by misfolded protein and prevented by chaperone function. *EMBO J* 36:1669–1687
- Mattson MP, Gleichmann M, Cheng A (2008) Mitochondria in neuroplasticity and neurological disorders. *Neuron* 60:748–766
- Mayer FL, Wilson D, Jacobsen ID, Miramon P, Slesiona S, Bohovych IM, Brown AJ, Hube B (2012) Small but crucial: the novel small heat shock protein Hsp21 mediates stress adaptation and virulence in *Candida albicans*. *PLoS One* 7:e38584
- McDonald ET, Bortolus M, Koteiche HA, McHaourab HS (2012) Sequence, structure, and dynamic determinants of Hsp27 (HspB1) equilibrium dissociation are encoded by the N-terminal domain. *Biochemistry* 51:1257–1268
- McHaourab HS, Dodson EK, Koteiche HA (2002) Mechanism of chaperone function in small heat shock proteins. Two-mode binding of the excited states of T4 lysozyme mutants by alphaA-crystallin. *J Biol Chem* 277:40557–40566
- McLoughlin F, Basha E, Fowler ME, Kim M, Bordowitz J, Katiyar-Agarwal S, Vierling E (2016) Class I and II small heat shock proteins together with HSP101 protect protein translation factors during heat stress. *Plant Physiol* 172:1221–1236
- Mogk A, Bukau B (2017) Role of sHsps in organizing cytosolic protein aggregation and disaggregation. *Cell Stress Chaperones* 22:493–502
- Mogk A, Bukau B, Kampinga HH (2018) Cellular handling of protein aggregates by disaggregation machines. *Mol Cell* 69:214–226
- Morelli FF, Verbeek DS, Bertacchini J, Vinet J, Mediani L, Marmiroli S, Cenacchi G, Nasi M, De Biasi S, Brunsting JF et al (2017) Aberrant compartment formation by HSPB2 mislocalizes lamin A and compromises nuclear integrity and function. *Cell Rep* 20:2100–2115
- Morrow G, Inaguma Y, Kato K, Tanguay RM (2000) The small heat shock protein Hsp22 of *Drosophila melanogaster* is a mitochondrial protein displaying oligomeric organization. *J Biol Chem* 275:31204–31210
- Morrow G, Samson M, Michaud S, Tanguay RM (2004) Overexpression of the small mitochondrial Hsp22 extends *Drosophila* life span and increases resistance to oxidative stress. *FASEB J* 18:598–599
- Mymrikov EV, Daake M, Richter B, Haslbeck M, Buchner J (2017) The chaperone activity and substrate spectrum of human small heat shock proteins. *J Biol Chem* 292:672–684
- Nam DE, Nam SH, Lee AJ, Hong YB, Choi BO, Chung KW (2018) Small heat shock protein B3 (HSPB3) mutation in an axonal Charcot-Marie-Tooth disease family. *J Peripher Nerv Syst* 23:60–66
- Nicholl ID, Quinlan RA (1994) Chaperone activity of alpha-crystallins modulates intermediate filament assembly. *EMBO J* 13:945–953
- Nover L, Scharf KD, Neumann D (1989) Cytoplasmic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. *Mol Cell Biol* 9:1298–1308
- Oates ME, Romero P, Ishida T, Ghalwash M, Mizianty MJ, Xue B, Dosztanyi Z, Uversky VN, Obradovic Z, Kurgan L et al (2013) D(2)P(2): database of disordered protein predictions. *Nucleic Acids Res* 41:D508–D516
- Oya-Ito T, Naito Y, Takagi T, Handa O, Matsui H, Yamada M, Shima K, Yoshikawa T (2011) Heat-shock protein 27 (Hsp27) as a target of methylglyoxal in gastrointestinal cancer. *Biochim Biophys Acta* 1812:769–781
- Parcellier A, Brunet M, Schmitt E, Col E, Didelot C, Hammann A, Nakayama K, Nakayama KI, Khochbin S, Solary E, Garrido C (2006) HSP27 favors ubiquitination and proteasomal degradation of p27Kip1 and helps S-phase re-entry in stressed cells. *FASEB J* 20:1179–1181
- Park AM, Kanai K, Itoh T, Sato T, Tsukui T, Inagaki Y, Selman M, Matsushima K, Yoshie O (2016) Heat shock protein 27 plays a pivotal role in myofibroblast differentiation and in the development of bleomycin-induced pulmonary fibrosis. *PLoS One* 11:e0148998
- Patel A, Lee HO, Jawerth L, Maharana S, Jahnel M, Hein MY, Stoyanov S, Mahamid J, Saha S, Franzmann TM, Pozniakovski A, Poser I, Maghelli N, Royer LA, Weigert M, Myers EW, Grill S, Drechsel D, Hyman AA, Alberti S (2015) A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. *Cell* 162:1066–1077
- Perng MD, Cairns L, van den IP, Prescott A, Hutcheson AM, Quinlan RA (1999a) Intermediate filament interactions can be altered by HSP27 and alphaB-crystallin. *J Cell Sci* 112(Pt 13):2099–2112
- Perng MD, Muchowski PJ, van Den IP, Wu GJ, Hutcheson AM, Clark JI, Quinlan RA (1999b) The cardiomyopathy and lens cataract mutation in alphaB-crystallin alters its protein structure, chaperone activity, and interaction with intermediate filaments in vitro. *J Biol Chem* 274:33235–33243
- Peschek J, Braun N, Rohrberg J, Back KC, Kriehuber T, Kastenmuller A, Weinkauff S, Buchner J (2013) Regulated structural transitions unleash the chaperone activity of alphaB-crystallin. *Proc Natl Acad Sci U S A* 110:E3780–E3789
- Potenza E, Di Domenico T, Walsh I, Tosatto SC (2015) MobiDB 2.0: an improved database of intrinsically disordered and mobile proteins. *Nucleic Acids Res* 43:D315–D320
- Qian J, Ren X, Wang X, Zhang P, Jones WK, Molkentin JD, Fan GC, Kranias EG (2009) Blockade of Hsp20 phosphorylation exacerbates cardiac ischemia/reperfusion injury by suppressing autophagy and increased cell death. *Circ Res* 105:1223–1231
- Quinlan R, Van Den Ijssel P (1999) Fatal attraction: when chaperone turns harlot. *Nat Med* 5:25–26
- Reichmann D, Xu Y, Cremers CM, Ilbert M, Mittelman R, Fitzgerald MC, Jakob U (2012) Order out of disorder: working cycle of an intrinsically unfolded chaperone. *Cell* 148:947–957
- Riback JA, Katanski CD, Kear-Scott JL, Pilipenko EV, Rojek AE, Sosnick TR, Drummond DA (2017) Stress-triggered phase separation is an adaptive, evolutionarily tuned response. *Cell* 168:1028–1040 e1019
- Richter K, Haslbeck M, Buchner J (2010) The heat shock response: life on the verge of death. *Mol Cell* 40:253–266
- Rosenbaum JC, Fredrickson EK, Oeser ML, Garrett-Engele CM, Locke MN, Richardson LA, Nelson ZW, Hetrick ED, Milac TI, Gottschling DE, Gardner RG (2011) Disorder targets misorder in nuclear quality control degradation: a disordered ubiquitin ligase directly recognizes its misfolded substrates. *Mol Cell* 41:93–106
- Sakamoto H, Mashima T, Yamamoto K, Tsuruo T (2002) Modulation of heat-shock protein 27 (Hsp27) anti-apoptotic activity by methylglyoxal modification. *J Biol Chem* 277:45770–45775
- Schon EA, Przedborski S (2011) Mitochondria: the next (neurode)generation. *Neuron* 70:1033–1053
- Schrepfer E, Scorrano L (2016) Mitofusins, from mitochondria to metabolism. *Mol Cell* 61:683–694
- Seguin SJ, Morelli FF, Vinet J, Amore D, De Biasi S, Poletti A, Rubinsztein DC, Carra S (2014) Inhibition of autophagy, lysosome and VCP function impairs stress granule assembly. *Cell Death Differ* 21:1838–1851
- Stromer T, Fischer E, Richter K, Haslbeck M, Buchner J (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* 279:11222–11228

- Sudnitsyna MV, Mymrikov EV, Seit-Nebi AS, Gusev NB (2011) The role of intrinsically disordered regions in the structure and functioning of small heat shock proteins. *Curr Protein Pept Sci* 13:76–85
- Takayama S, Reed JC, Homma S (2003) Heat-shock proteins as regulators of apoptosis. *Oncogene* 22:9041–9047
- Tanguay RM, Hightower LE (2015) In: Asea AAA, Calderwood SK (eds) *The big book on small heat shock proteins*, vol 8. Springer, Berlin
- Taylor JP, Brown RH Jr, Cleveland DW (2016) Decoding ALS: from genes to mechanism. *Nature* 539:197–206
- Ungelenk S, Moayed F, Ho CT, Grousl T, Scharf A, Mashaghi A, Tans S, Mayer MP, Mogk A, Bukau B (2016) Small heat shock proteins sequester misfolding proteins in near-native conformation for cellular protection and efficient refolding. *Nat Commun* 7:13673
- van den IP, Wheelock R, Prescott A, Russell P, Quinlan RA (2003) Nuclear speckle localisation of the small heat shock protein alpha B-crystallin and its inhibition by the R120G cardiomyopathy-linked mutation. *Exp Cell Res* 287:249–261
- Van Montfort R, Slingsby C, Vierling E (2001a) Structure and function of the small heat shock protein/alpha-crystallin family of molecular chaperones. *Adv Protein Chem* 59:105–156
- van Montfort RL, Basha E, Friedrich KL, Slingsby C, Vierling E (2001b) Crystal structure and assembly of a eukaryotic small heat shock protein. *Nat Struct Biol* 8:1025–1030
- van Rijk AE, Stege GJ, Bennink EJ, May A, Bloemendal H (2003) Nuclear staining for the small heat shock protein alphaB-crystallin colocalizes with splicing factor SC35. *Eur J Cell Biol* 82:361–368
- Vicart P, Caron A, Guicheney P, Li Z, Prevost MC, Faure A, Chateau D, Chapon F, Tome F, Dupret JM et al (1998) A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. *Nat Genet* 20:92–95
- Wang F, Zhu J, Yao P, Li X, He M, Liu Y, Yuan J, Chen W, Zhou L, Min X, Fang W, Liang Y, Wang Y, Wei S, Liu J, Miao X, Lang M, Jiang X, Zhang P, Li D, Lu C, Wang X, Shi W, Zheng J, Guo H, Zhang X, Yang H, Hu FB, Wu T (2013) Cohort profile: the Dongfeng-Tongji cohort study of retired workers. *Int J Epidemiol* 42:731–740
- Ward JJ, Sodhi JS, McGuffin LJ, Buxton BF, Jones DT (2004) Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. *J Mol Biol* 337:635–645
- Webster KA (2003) Serine phosphorylation and suppression of apoptosis by the small heat shock protein alphaB-crystallin. *Circ Res* 92:130–132
- Whiten DR, San Gil R, McAlary L, Yerbury JJ, Ecroyd H, Wilson MR (2016) Rapid flow cytometric measurement of protein inclusions and nuclear trafficking. *Sci Rep* 6:31138
- Young G, Hundt N, Cole D, Fineberg A, Andrecka J, Tyler A, Olerinyova A, Ansari A, Marklund EG, Collier MP, Chandler SA, Tkachenko O, Allen J, Crispin M, Billington N, Takagi Y, Sellers JR, Eichmann C, Selenko P, Frey L, Riek R, Galpin MR, Struwe WB, Benesch JLP, Kukura P (2018) Quantitative mass imaging of single biological macromolecules. *Science (New York, NY)* 360:423–427

## Affiliations

Serena Carra<sup>1</sup> · Simon Alberti<sup>2,3</sup> · Justin L. P. Benesch<sup>4</sup> · Wilbert Boelens<sup>5</sup> · Johannes Buchner<sup>6</sup> · John A. Carver<sup>7</sup> ·  
Ciro Cecconi<sup>8,9</sup> · Heath Ecroyd<sup>10,11</sup> · Nikolai Gusev<sup>12</sup> · Lawrence E. Hightower<sup>13</sup> · Rachel E. Klevit<sup>14</sup> · Hyun O. Lee<sup>15</sup> ·  
Krzysztof Liberek<sup>16</sup> · Brent Lockwood<sup>17</sup> · Angelo Poletti<sup>18</sup> · Vincent Timmerman<sup>19</sup> · Melinda E. Toth<sup>20</sup> ·  
Elizabeth Vierling<sup>21</sup> · Tangchun Wu<sup>22</sup> · Robert M. Tanguay<sup>23</sup>

<sup>1</sup> Department of Biomedical, Metabolic and Neural Sciences, and Centre for Neuroscience and Nanotechnology, University of Modena and Reggio Emilia, via G. Campi 287, 41125 Modena, Italy

<sup>2</sup> Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany

<sup>3</sup> Center for Molecular and Cellular Bioengineering (CMCB), Biotechnology Center (BIOTEC), Technische Universität Dresden, Tatzberg 47/49, 01307 Dresden, Germany

<sup>4</sup> Department of Chemistry, Physical and Theoretical Chemistry, Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford OX1 3TA, UK

<sup>5</sup> Department of Biomolecular Chemistry, Institute of Molecules and Materials, Radboud University, NL-6500 Nijmegen, The Netherlands

<sup>6</sup> Center for Integrated Protein Science Munich (CIPSM) and Department Chemie, Technische Universität München, D-85748 Garching, Germany

<sup>7</sup> Research School of Chemistry, The Australian National University, Acton, ACT 2601, Australia

<sup>8</sup> Department of Physics, Informatics and Mathematics, University of Modena and Reggio Emilia, 41125 Modena, Italy

<sup>9</sup> Center S3, CNR Institute Nanoscience, Via Campi 213/A, 41125 Modena, Italy

<sup>10</sup> School of Chemistry and Molecular Bioscience, University of Wollongong, Wollongong, NSW, Australia

<sup>11</sup> Illawarra Health and Medical Research Institute, Wollongong, NSW, Australia

<sup>12</sup> Department of Biochemistry, School of Biology, Moscow State University, Moscow, Russian Federation 117234

<sup>13</sup> Department of Molecular and Cell Biology, University of Connecticut, 91 North Eagleville Road, Storrs, CT 06269-3125, USA

<sup>14</sup> Department of Biochemistry, University of Washington, Seattle, WA, USA

<sup>15</sup> Department of Biochemistry, Faculty of Medicine, University of Toronto, Toronto, ON, Canada

<sup>16</sup> Department of Molecular and Cellular Biology, Intercollegiate Faculty of Biotechnology UG-MUG, University of Gdansk, Abrahama 58, 80-307 Gdansk, Poland

- <sup>17</sup> Department of Biology, University of Vermont, Burlington, VT 05405, USA
- <sup>18</sup> Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Centro di Eccellenza sulle Malattie Neurodegenerative, Università degli Studi di Milano, Milan, Italy
- <sup>19</sup> Peripheral Neuropathy Research Group, Department of Biomedical Sciences, University of Antwerp, 2610 Antwerp, Belgium
- <sup>20</sup> Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary
- <sup>21</sup> Department of Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst, MA 01003, USA
- <sup>22</sup> MOE Key Lab of Environment and Health, Tongji School of Public Health, Huazhong University of Science and Technology, 13 Hangkong Rd, Wuhan 430030, Hubei, China
- <sup>23</sup> Laboratory of Cell and Developmental Genetics, IBIS, and Department of Molecular Biology, Medical Biochemistry and Pathology, Medical School, Université Laval, QC, Québec G1V 0A6, Canada