Flexible Nets of Malleable Guardians: Intrinsically Disordered Chaperones in Neurodegenerative Diseases

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1. Introduction

This review is dedicated to the description of intrinsically disordered chaperones and their roles in neurodegenerative diseases. Therefore, three major concepts, namely, intrinsically disordered proteins, chaperones, and neurodegeneration are briefly introduced below.

1.1. Intrinsically Disordered Proteins

1.1.1. Concept

An increasing amount of evidence suggests that many protein regions and even entire proteins lack stable tertiary and/or secondary structure in solution, existing instead as dynamic ensembles of interconverting structures. These naturally flexible proteins are known by different names, including intrinsically disordered,[1] natively denatured,[2] natively unfolded,[3,4] intrinsically unstructured,[5,6] and natively disordered proteins.[7] By "intrinsic disorder" it is meant that the protein exists as a structural ensemble, either at the

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Dr. Uversky received broad training, with an M.S. in Physics (Leningrad State University, Russia, 1986), a Ph.D. (Moscow Institute of Technical Physics, 1991), and a D.Sc. in Biophysics (Institute of Experimental and Theoretical Biophysics, Russian Academy of Sciences, 1998) and with pre- and postdoctoral research in Structural Biology, Biochemistry, and Biophysics (1991−1998, Institute of Protein Research, Russian Academy of Sciences). Dr. Uversky is using various molecular biophysics methods to study protein folding, protein misfolding/aggregation and to characterize partially folded proteins and intrinsically disordered proteins. While he continues to use biophysics, more recently Dr. Uversky has focused on the development and use of bioinformatics methods for the study of intrinsically disordered proteins. Dr. Uversky has authored over 330 scientific publications and edited several books and book series on protein structure, function, folding, and misfolding. He is also an editor of several scientific journals.

secondary or tertiary level. In other words, in contrast to ordered proteins whose 3D structure is relatively stable and Ramachandran angles vary slightly around their equilibrium positions with occasional cooperative conformational switches, IDPs or intrinsically disordered regions (IDRs) exist as dynamic ensembles in which the atom positions and backbone Ramachandran angles vary significantly over time with no specific equilibrium values, and typically undergo non-cooperative conformational changes. To some extent, the conformational behavior and structural features of IDPs and IDRs resemble those of non-native states of "normal" globular proteins, which may exist in at least four different conformations: ordered, molten globule, premolten globule, and coil-like.[8−11] Using this analogy, IDPs and IDRs might be in a molten globular form, where intrinsic disorder is present in the collapsed form, and in the form of a random coil or premolten globule, where intrinsic disorder is present in the extended form, under physiological conditions *in vitro*.[7,9,12] Here, the term IDP is used loosely to refer both to proteins that are completely intrinsically disordered and those that consist of a mixture of ordered and disordered residues.

1.1.2. Experimental Techniques for IDP Detection

The disorder in IDPs has been detected by several physicochemical methods developed to characterize protein self-organization. The list includes, but is not limited to, X-ray crystallography,[13] NMR spectroscopy,[7,14−18] near-UV circular dichroism (CD),[19] far-UV CD,[4,20−22] optical rotatory dispersion (ORD),[4,20] FTIR,[4] Raman spectroscopy and Raman optical activity,[23] different fluorescence techniques,[24,25] numerous techniques that provide information on hydrodynamic parameters (including gel-filtration, viscometry, small-angle X-ray scattering (SAXS), small angle neutron scattering (SANS), sedimentation, and dynamic and static light scattering),[24,25] rate of proteolytic degradation,[26−30] aberrant mobility in SDS-gel electrophoresis,[6,31] low conformational stability,[24,32−35] H/D exchange,[25] immunochemical methods,[36,37] interaction with molecular chaperones,[24] electron microscopy or atomic force microscopy,[25,38] and the charge state analysis of electrospray ionization mass spectrometry.[39,40] For more detailed reviews on methods used to detect intrinsic disorder see refs 7, 15, 25, 41, 42.

1.1.3. Sequence Peculiarities of IDPs and Predictors of Intrinsic Disorder

IDPs and IDRs differ from structured globular proteins and domains in many ways, such as amino acid composition, sequence complexity, hydrophobicity, charge, flexibility, and type and rate of amino acid substitutions over evolutionary time. For example, IDPs are significantly depleted in a number of so-called order-promoting residues, including bulky hydrophobic (I, L, and V) and aromatic amino acid residues (W, F, Y), which would normally form the hydrophobic core of a folded globular protein, and also possess a low amount of C and N residues. On the other hand, IDPs were shown to be substantially enriched in the so-called disorder-promoting amino acids: A, R, G, Q, S, P, E, and K.[1,43−45] Many of the mentioned differences were utilized to develop numerous disorder predictors, such as PONDR,[43,46] CH-plot,[4] NORSp,[47] GlobPlot,[48,49] FoldIndex,[50] IUPred,[51] and DisoPred,[52−54] to name a few. It is important to remember that comparing several predictors on an individual protein or protein data set of interest can provide additional insight regarding the predicted disorder, if any exists.[55−60]

1.1.4. Natural Abundance of IDPs and Their Biological Functions

The application of various disorder predictors to different proteomes has revealed that intrinsic disorder is highly abundant in nature and that the overall amount of disorder in proteins increases from bacteria to archaea to eukaryota, with over one-half of the eukaryotic proteins containing long predicted IDRs.[54,55,61] One explanation for this trend is a change in the cellular requirements for certain protein functions, particularly cellular signaling. In support of this hypothesis, an analysis of a eukaryotic signal protein database indicated that a majority of known signal transduction proteins were predicted to contain significant regions of disorder.[62]

Although IDPs fail to form unique 3D structures under physiological conditions, they are known to carry out a great number of important biological functions, a fact which was recently confirmed by several comprehensive studies.[1,4−7,9,12,18,41,62−73] Furthermore, sites of post-translational modifications (acetylation, hydroxylation, ubiquitylation, methylation, phosphorylation, etc.) and proteolytic attack are frequently associated with regions of intrinsic disorder.[72] The functional diversity provided by IDRs is suggested to complement functions of ordered protein regions.[70−72]

Another very important feature of IDPs is their unique capability to fold under a variety of conditions.[1,4−6,8,14,18,41,63,64,69,73,74] In fact, the folding of these proteins can be brought about by interaction with other proteins, nucleic acids, membranes, or small molecules. It can also be driven by changes in the protein environment. The resulting conformations could be either relatively noncompact (i.e., remain substantially disordered) or tightly folded.

In a living organism, proteins participate in complex interactions which represent the mechanistic foundation of the organism's physiology and function. Regulation, recognition, and cell signaling involve the coordinated actions of many players. To achieve this coordination, each participant must have a valid form of identification that is easily recognized by the other players. For proteins, these means of identification are often located within IDRs.[64,73] Despite (or maybe due to) their high flexibility, IDPs are involved in regulation and signaling, and control pathways in which interactions with multiple partners and high-specificity/low-affinity interactions are often requisite.[64,73]

IDPs have specific functions that can be grouped into four broad classes: (i) molecular recognition; (ii) molecular assembly; (iii) protein modification; and (iv) entropic chain activities.[63] Recently, the crucial role of intrinsic disorder in the action of RNA and protein chaperones was emphasized by showing that IDRs in these complex machines can function as molecular recognition elements that act as solubilizers by locally loosening the structure of the kinetically trapped folding intermediate.[67]

1.2. Chaperones

1.2.1. Concept

Generally, a polypeptide chain of a protein contains all the information required to achieve functional conformation in a given environment.[75,76] Although this principle is generally correct for many foldable proteins, the information contained in some proteins is not complete enough to guarantee the formation of a functionally active structure. Such proteins cannot fold spontaneously and require the help of molecular chaperones. According to Ellis, molecular chaperones represent "a class of cellular proteins whose function is to ensure that the folding of certain other polypeptide chains and their assembly into oligomeric structures occur correctly".[77] Chaperones are an important part of the cellular quality control system, maintaining an intricate balance between protein synthesis and degradation and protecting cells from the devastating consequences of uncontrolled protein aggregation. In addition to chaperones, this system includes the ubiquitin−proteasome system and the autophagy−lysosome system. Molecular chaperones protect cells from apoptosis induced by toxic oligomers. There are several mechanisms by which chaperones fight devastating consequences of misfolding and aggregation. These mechanisms can be grouped into three major classes of action: prevention, reversal, and elimination. At the prevention stage, chaperones bind to unfolded stretches in proteins and keep them in a folding-competent state while preventing aggregation. In the reversal mechanism, chaperones act as disaggregating and unfolding machines which help dissolve aggregates and give a misfolded protein a second chance for folding correctly. At the elimination step, chaperones target misfolded proteins for degradation by the ubiquitin−proteasome system and/or the autophagy−lysosome system.

1.2.2. Functional Classification of Chaperones

The principal heat-shock proteins that have chaperone activity belong to five conserved classes: Hsp33, Hsp60, Hsp70, Hsp90, Hsp100, and the small heat-shock proteins. Molecular chaperones have been divided into three functional subclasses based on their mechanism of action. "Folding" chaperones (e.g., DnaK and GroEL in prokaryotes, and Hsp60 and Hsp70 in eukaryotes) rely on ATP-dependent conformational changes to mediate the net refolding/unfolding of their substrates. "Holding" chaperones (e.g., Hsp33 and Hsp31) bind partially folded proteins and maintain these substrates on their surface to await the availability of "folding" chaperones. "Disaggregating" chaperones (e.g., ClpB in prokaryotes and Hsp104 in eukaryotes) promote the solubilization of proteins that have become aggregated as a result of stress.

Molecular chaperones are classified as either inducible or constitutively expressed according to their expression mechanisms. Both types of chaperones act by the selective binding of solvent-exposed hydrophobic segments of nonfolded polypeptides and, through multiple binding-release cycles, bring about the folding, transport, and assembly of the target polypeptides.[78−80] Some chaperones are ATPases; that is, they use free-energy from ATP binding and/or hydrolysis to perform work on their substrates.

The concentration of inducible chaperones, also known as heat shock proteins (Hsp), increases as a response to stress conditions. Some illustrative examples of inducible chaperones are small heat shock proteins (e.g., αA-crystallin (HspB4), αB-crystallin (HspB5), Hsp27 (HspB1), and Hsp22 (HspB8); the Hsp40 family; Hsp70 chaperones and their regulators-co-chaperones HDJ1, HDJ2, BAG1, HSPBP1, Hip, Hop, and CHIP; the HspC group of Hsp including Hsp90, Grp94; Hsp104; and Hsp110). These molecular chaperones prevent and reverse the misfolding and aggregation of proteins that occur as a consequence of stress.[81,82] One should keep in mind though that it is not completely correct to state that HspB4 and HspB8 belong to the group of inducible chaperones since these proteins are constitutively expressed in certain cell types and at the same time are inducible in the other cell types.[83]

On the other hand, constitutively expressed chaperones, also known as heat shock cognate proteins (Hscps), facilitate protein translation, help newly synthesized proteins fold, promote the assembly of proteins into functional complexes, and assist the translocation of proteins into cellular compartments such as mitochondria and chloroplasts.[79,84] In the Hsp70 family of proteins, in addition to the inducible Hsp70 form, there is a constitutively expressed form, the heat shock cognate protein (Hsc70), which has 85% identity with human Hsp70 and binds to nascent polypeptides to facilitate their correct folding. Hsc70 also acts as an ATPase and participates in the disassembly of clathrin-coated vesicles during the transport of membrane components through the cell.[85]

Molecular chaperones evolve to protect proteins from misfolding and aggregation regardless of their classification as inducible or constitutively expressed. One important feature of chaperones is that, although they assist the noncovalent folding/unfolding and the assembly/disassembly of other macromolecular structures, they do not occur in these structures when the latter are performing their normal biological functions. Generally, molecular chaperones have no effect on a protein's folding rate. Of course, apparent folding and assembly rates can be increased by the elimination of nonproductive oligomer/aggregate formation and by reversing misfolding. Furthermore, by binding to partially folded species and preventing their aggregation, chaperones increase the yield of functional folded/assembled proteins. However, these actions do not affect intramolecular folding

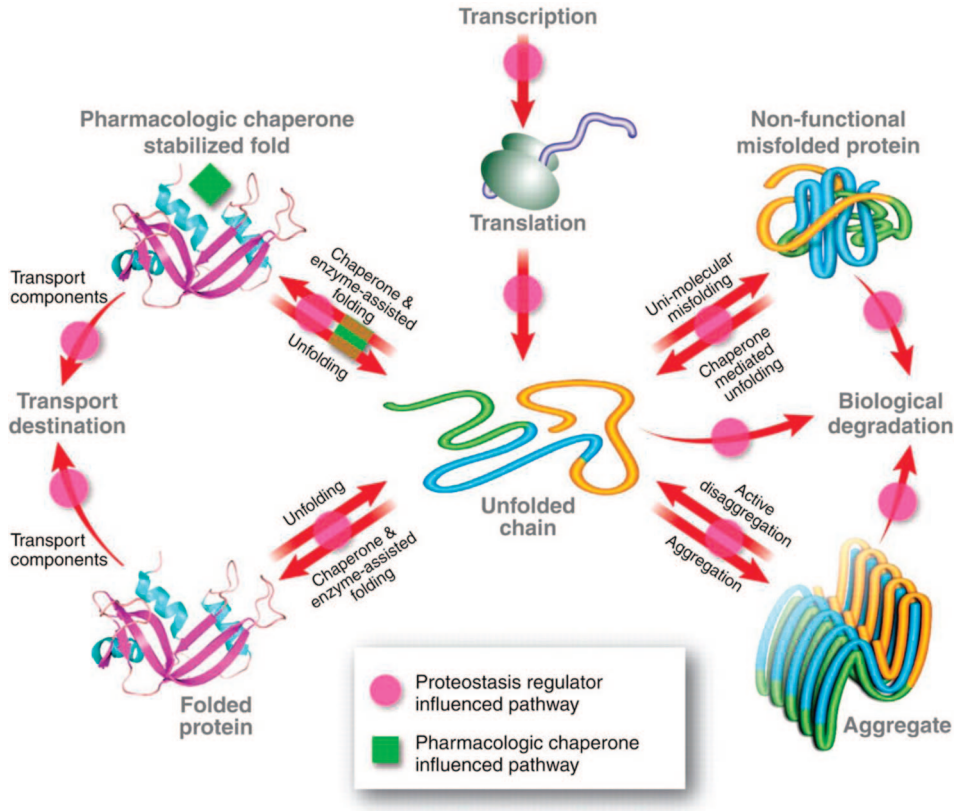

Figure 1. A model of proteostasis network. Pathways comprising this network are represented by the red arrows. Imbalances in proteostasis often lead to disease and, therefore, proteostasis regulators (magenta circles) that manipulate the proteostasis pathways/network can restore protein homeostasis and ameliorate both loss- and gain-of-function diseases. Reprinted with permission from Balch, W. E.; Morimoto, R. I.; Dillin, A.; Kelly, J. W. Adapting Proteostasis for Disease Intervention. *Science* **2008**, *319*, 916−919. 93. Copyright 2008 AAAS.

rates of globular proteins in the absence of misfolding under native conditions. On the other hand, there is a last class of proteins, helpers which assist protein folding and are not present in the final folded/assembled functional form of a protein−substrate. Therefore, these helpers, known as foldases, belong to the family of chaperones. Contrary to the typical chaperones considered so far, foldases evolved to catalyze the folding process by directly accelerating the protein folding rate-limiting steps. Well-known foldases include eukaryotic protein disulfide isomerase,[86−88] peptidyl-prolyl *cis*/*trans* isomerase,[88,89] and lipase-specific foldases, Lifs, found in the periplasm of Gram-negative bacteria.[88,90] Finally, there is a large class of so-called intramolecular chaperones, which are specific protein regions essential for protein folding but not required for protein function. Often, these N-terminal or C-terminal extensions are removed after the protein is folded by autoprocessing or by specific exogenous proteases.[91] On the basis of their roles in protein folding, intramolecular chaperones are classified into two categories. The type I category includes those intramolecular chaperones that assist tertiary structure formation and are mostly produced as the N-terminal sequence extension of the protein-carrier. The type II category contains intramolecular chaperones that are not directly involved in tertiary structure formation, but guide the assembly of the quaternary structure to form the functional protein complex and are mostly located at the C-terminus of the protein-carrier.[91]

1.2.3. Chaperones as an Important Part of the Proteostasis Network

Maintenance of proper protein homeostasis, or proteostasis, represents a vital task for any living cell.[92] Proteostasis is a complex mechanism for general control and regulation of the transcription, translation, folding, trafficking, processing, assembly/disassembly, localization, and degradation of millions of proteins inside the cell. The proteostasis, which involves controlling the conformation, concentration, binding interactions, and location of individual proteins, represents "an elaborate and integrated cellular network that governs the 'life of proteins' from conception to their demise".[92] The proteostasis concept was introduced recently to integrate a set of interacting activities that maintain the health of proteome and the organism.[93] Figure 1 represents this proteostasis network as introduced in the original paper by Balch et al.[93] and clearly shows that chaperones, being directly and indirectly involved in protein folding, trafficking, disaggregation, and degradation, are crucial players of this network. Importantly, although the capasity of proteostsis declines with age and in various metabolic, oncological, neurodegenerative, and cardiovascular disorders,[93] it is believed that declined proteostasis can be enhanced by small molecules (pharmacologic chaperones) or biological proteostasis regulators (small interfering RNA (siRNA), cDNA, or protein) that manipulate the concentration, conformation, quaternary structure, and/or the location of protein(s).[94] The restoration of normal proteostasis might help readapting the

normal biology of the cell and therefore has "the potential to ameliorate some of the most challenging diseases of our era."[93]

1.3. Neurodegeneration

1.3.1. Concept

The term neurodegeneration is derived from the Greek prefix *νευρο-*, *néuro-*, "nerval" and the Latin verb *dēgenerāre*, "to decline" or "to worsen". Therefore, neurodegenerative diseases are a large class of human maladies which includes various acquired neurological diseases with distinct phenotypic and pathologic symptoms, all characterized by the pathological conditions in which cells of the brain and/or spinal cord are lost. As the amount of dead neurons increases, affected brain regions begin to shrink: by the final stage of Alzheimer's disease (AD), damage is widespread and brain tissue has shrunk significantly; in prion diseases, the brain undergoes damage known as "spongiform change" or "spongiosis," since, when the tissue is examined under a microscope, it looks like a sponge, with many tiny holes.

Neurodegeneration is a slow process which begins long before the patient experiences any symptoms. It can take months or even years before visible outcomes of this degeneration are felt and diagnosed: in the case of AD, damage to the brain begins 10−20 years before any problems are evident. The progression through various AD stages may last from 8 to 10 years, whereas in Huntington disease (HD), death occurs approximately 18 years from the time of onset. Symptoms are usually noticed when many cells die or fail to function and a part of the brain begins to cease functioning properly. For example, the symptoms of Parkinson's disease (PD) become apparent after more than ∼70% dopaminergic neurons die in a specific area of the midbrain known as *substantia nigra*.

As neurons are not readily regenerated, their deterioration over time leads to dysfunction and disabilities. Neurodegeneration, in principle, can affect various peripheral and central areas of the nervous system resulting in a great variability of disease manifestations. Generally, neurodegenerative diseases can be divided into three groups according to their phenotypic effects: (i) conditions causing problems with movements; (ii) conditions affecting memory and leading to dementia; (iii) conditions affecting both movement and cognitive abilities; and (iv) conditions causing problems with peripheral nervous system.

Illustrative examples of movement neurodegenerative disorders include PD (characterized by symptoms originating from the neuronal loss in *substantia nigra* such as resting tremor on one (or both) side(s) of the body; generalized slowness of movement (bradykinesia); stiffness of limbs (rigidity); and gait or balance problems (postural dysfunction)); multiple system atrophy (MSA, characterized by several clinical features of PD); Kennedy disease (also known as spinal and bulbar muscular atrophy, SBMA, or X-linked spinal muscular atrophy since it affects the motor neurons of males only and characterized by muscle weakness); and various forms of spinocerebellar ataxia (characterized by a failure of muscle coordination due to pathology arising in the spinocerebellar tract of the spinal cord).

Cognitive neurodegeneration is illustrated by AD and prion diseases (Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) disease, fatal familial insomnia, and kuru). Some neurodegenerative diseases which affect movement and cognition are neurodegeneration with brain iron accumulation type 1 (NBIA1) (characterized by rigidity, dystonia, dyskinesia, and choreoathetosis,[95−98] together with dysarthria, dysphagia, ataxia, and dementia[98−100]); dementia with Lewy bodies (DLB, characterized by neuropsychiatric changes often with marked fluctuations in cognition and attention, hallucinations, and parkinsonism[101]); and HD (characterized by clinical effects on motor, cognitive, and psychological functions[102]).

Some illustrative examples of conditions with the predominant involvement of the peripheral nervous system with minimal central nervous system involvement include pure autonomic failure (also known as Bradbury-Eggleston syndrome; characterized by orthostatic hypotension leading to dizziness and fainting, visual disturbances, neck pain, chest pain, fatigue, and sexual dysfunction[103]) and Lewy body dysphagia (characterized by swallowing abnormalities caused by the localized Lewy body accumulation in both the dorsal vagal motor nucleus and the nucleus ambiguus[104]).

1.3.2. Molecular Mechanisms of Neurodegeneration

Although neurodegenerative diseases are characterized by an extremely wide range of clinical symptoms resulting from the dysfunction of different areas of the central and peripheral nervous system, the unifying mechanism of all these pathologies is the deterioration of specific regions of the nervous system caused by the highly specific and localized death of neurons. At the molecular level, many factors can induce neuronal death. Some of these factors are protein misfolding and aggregation, oxidative damage, mitochondrial dysfunction and impaired bioenergetics, the disruption of the neuronal Golgi apparatus and transport, and the failure of cell protective mechanisms including the chaperone system and protein degradation machinery (e.g., proteasomal proteolysis and autophagy−lysosome system).

1.3.2.1. Neurodegenerative Diseases as Proteinopathies and Amyloidoses. The link between AD, PD, prion diseases, HD, and several other neurodegenerative disorders has been elusive for a long time. However, recent advances in molecular biology, immunopathology, and genetics indicate that these diseases might share a common pathophysiologic mechanism, where the disarrangement of a specific protein's processing, functioning, and/or folding takes place. Therefore, neurodegenerative disorders represent a set of proteinopathies which can be classified and grouped based on their causative proteins. In fact, from this viewpoint, neurodegenerative disorders represent a subset of a broader class of human diseases known as protein conformational or protein misfolding diseases. These disorders arise from the failure of a specific peptide or protein to adopt its native functional conformational state. The obvious consequences of misfolding are protein aggregation (and/or fibril formation), loss of function, and gain of toxic function. Some proteins have an intrinsic propensity to assume a pathologic conformation, which becomes evident with aging or at persistently high concentrations. Interactions (or impaired interactions) with some endogenous factors (e.g., chaperones, intracellular or extracellular matrices, other proteins, or small molecules) can change the conformation of a pathogenic protein and increase its propensity to misfold. Misfolding can originate from point mutation(s) or result from an exposure to internal or external toxins, impaired post-translational modifications (phosphorylation, advanced glycation, deamidation, racemization, etc.), an increased probability of degradation,

impaired trafficking, lost binding partners, or oxidative damage. All these factors can act independently or in association with one another.

Many neurodegenerative diseases are, in fact, protein deposition diseases. In other words, they are associated with the formation of extracellular amyloid fibrils or intracellular inclusions with amyloid-like characteristics. Protein deposition diseases can be sporadic (idiopathic, 85%), hereditary (familial or genetically inherited, 10%), or even transmissible, as in the case of prion diseases (5%).[105] In the first case, neurodegeneration develops spontaneously, without obvious alterations in the patient's DNA, although genetic differences may act as risk factors. In the second case, neurodegeneration is caused by mutation(s) in specific gene(s). Although these diseases are very different clinically, they share similar molecular mechanisms, where a specific protein or protein fragment changes from its natural soluble form into insoluble fibrils. It has been pointed out that, prior to fibrillation, amyloidogenic polypeptides may be rich in β-sheets, α-helices, or contain both α-helices and β-sheets. They may be globular proteins with rigid 3D-structure or belong to the class of natively unfolded (or intrinsically unstructured) proteins.[106] Despite these differences, the fibrils from different pathologies display many common properties, including a core cross-β-sheet structure in which continuous β-sheets are formed, with β-strands running perpendicular to the long axis of the fibrils.[107,108] This β-pleated sheet structure of fibrils constitutes the basis of the unusual resistance of all kinds of amyloid to degradation and, therefore, the progressive deposition of the material.[109] Furthermore, all fibrils have similar twisted, rope-like structures that are typically 7−13 nm wide[107,108,110] and consist of a number of protofilaments (typically 2−6), each about 2−5 nm in diameter.[110] Alternatively, protofilaments may associate laterally to form long ribbons that are 2−5 nm thick and up to 30 nm wide.[111−113]

Although amyloid-like fibrils are frequently observed in several neurodegenerative diseases and the importance of specific amyloidogenic proteins in the etiology of corresponding diseases was established by multiple genetic and pathological studies, there is no unifying model explaining the toxicity of these deposits. In fact, several different mechanisms of toxicity have been proposed based on the monomeric/polymeric nature of the proposed toxic species. Let us consider the role of α-synuclein in the pathology of PD as an illustrative example, for which at least three different mechanisms of neurotoxicity have been discussed.[114] An increase in the intracellular abundance of monomeric α-synuclein has been considered as a potential cause of neuronal toxicity. This hypothesis is supported by the fact that a 50% or 100% increase in α-synuclein expression caused by the duplication or triplication of the α-synuclein gene is known to result in familial forms of PD or DLB.[115] Furthermore, the increased expression of α-synuclein was reported in specific brain areas or types of neurons in individuals with sporadic PD,[116] as well as in brains of model animals as a result of toxic insult.[117,118] In another model, specific oligomeric and protofibrillar forms of α-synuclein have been proposed as potent toxic species. Here, α-synuclein oligomers were proposed to form pores on intracellular membranes such as the plasma membrane, and may increase cation permeability.[119−121] Finally, it was emphasized that the fibrillation of α-synuclein and the formation of large intracytoplasmic inclusions can cause the dysfunction and the demise of neurons or oligodendrocytes.[114] These inclusions may act as "sinks," recruiting other necessary, cellular proteins from their normal cellular functions.[114] They may affect proteasome function,[122] and they can impair cellular functions by obstructing normal cellular trafficking, including disrupting the ER and Golgi apparatus, by disrupting cell morphology, by impairing axonal transport, and by trapping cellular components (e.g., mitochondria).[114] Of course, the discussed mechanisms of α-synuclein toxicity based on the different polymeric forms from small oligomers to amyloid fibrils are not necessarily mutually exclusive, since the presence of any polymeric form of α-synuclein is abnormal and may be problematic for the normal activities of cells, thereby resulting in neurodegeneration.[114]

1.3.2.2. Mitochondrial Dysfunction and Impaired Bioenergetics. Mitochondria, in addition to being a source of ATP, perform pivotal biochemical functions necessary for homeostasis and represent a convergence point for both extracellular and intracellular death signals. Mitochondrial dysfunction has been described in several neurodegenerative diseases including AD, PD, HD, and amyotrophic lateral sclerosis (ALS).[123] For example, in AD brains, the impaired activity of three tricarboxylic acid cycle proteins, pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase, has been observed[124] together with reduced respiratory chain activities in complexes I, III, and IV[125] and the presence of alterations in mitochondria morphology and distribution.[126] In PD, mitochondria have been demonstrated to be one of the direct targets of α-synuclein-triggered toxicity, which causes reduced mitochondrial complex I activity and increased production of reactive oxygen species.[127] Furthermore, in both sporadic and familial forms of PD, reported mitochondrial abnormalities include impaired functioning of the mitochondrial electron transport chain, aging-associated damage to mitochondrial DNA, impaired calcium buffering, and anomalies in mitochondrial morphology and dynamics.[128,129] Reductions in the activities of complexes II, III, and IV have been observed in the caudate and putamen of HD patients.[130] Finally, in ALS, the presence of mutant Cu/Zn superoxide dismutase (SOD1) within motor neurons has been shown to cause alterations of the mitochondrial respiratory chain,[131] increasing the mitochondrial-produced superoxide levels.[132]

1.3.2.3. Oxidative Damage. There are several factors which put the brain at risk of oxidative damage.[133] Some of these factors include high oxygen consumption (20% of the total basal O_2 consumption of the body), critically high levels of both iron and ascorbate, relatively low levels of antioxidants (e.g., catalase), a tendency to accumulate metals with age, and low regenerative capacity.[134−136] Furthermore, microglia, the resident immune cells of the brain, produce superoxide and H_2O_2 upon activation; they also produce cytokines, which can stimulate production of reactive oxygen species (ROS) and nitric oxide (NO).[135] Astrocytes also produce cytokines, through which they can be activated to generate NO from inducible nitric oxide synthase (iNOS).[135] Microglia and astrocytes are, therefore, major mediators of inflammatory processes in the brain.[137] Some cytochrome P450s are also a source of ROS in certain brain regions.[138]

Therefore, it is not surprising that, although neurodegenerative diseases differ in etiology, symptoms, and disease localization, oxidative stress is recognized as an important pathway leading to neuronal death and is implicated in many neurodegenerative diseases including AD, PD, HD, ALS, and Friedreich's ataxia.[133,134,139] In AD, the major sources of

oxidative stress and free-radical production are Aβ-bound copper and iron, and the various forms of Aβ in the AD brain are commonly found to be oxidatively modified.[134] In PD, which results from the selective degeneration of neuromelanin-containing neurons, most notably nigral dopaminergic neurons, the catechol dopamine can generate H_2O_2 and oxidative stress could come from a failure to regulate dopamine−iron biochemistry.[134] In ALS, mutations in SOD are known to lead to a gain of toxic function, promoting the pro-oxidant activity of SOD, generating ROS.[134] FA originates due to an abnormal GAA trinucleotide expansion within the gene encoding the mitochondrial protein frataxin, causing frataxin deficiency. Iron, therefore, accumulates in the mitochondria, promoting oxidative stress which leads to cardiomyopathy and neurodegeneration.[134]

1.3.2.4. Disruption of Neuronal Golgi Apparatus and Impaired Transport. The Golgi apparatus plays a central role in the transport, processing, and sorting of proteins. The complex consists of stacks of parallel cisternae and vesicles that carry molecular "cargo" from one cisterna to the next by the coordinated fission of vesicles from the lateral edge of one cisterna and fusion of to the next cisterna.[140] Interactions between amyloidogenic proteins and any of one or more proteins involved in the maintenance of the structure of the Golgi apparatus might disrupt its structure and function. Golgi apparatus fragmentation has been reported in ALS, corticobasal degeneration, AD, PD, CJD, and in spinocerebelar ataxia type 2. In a mice model of familial ALS, fragmentation of the Golgi apparatus in spinal cord motor neurons and aggregation of mutant proteins were detected months before the onset of paralysis.[141] In a cellular PD model, cells with prefibrillar α-synuclein aggregates had fragmented Golgi apparatus and showed trafficking impairment. These results strongly suggest that the fragmentation of the Golgi apparatus is an early event that occurs before the appearance of the fibrillar α-synuclein forms.[142]

1.3.2.5. Endoplasmic Reticulum Stress. The endoplasmic reticulum (ER) is a membrane-enclosed reticular network connecting the nuclear envelope to the Golgi apparatus.[143] The ER is known to be involved in a number of crucial cellular functions, such as protein folding, post-translational modification and transport to the Golgi complex, the synthesis of lipids and sterols, the maintenance of cellular calcium homeostasis, redox control, and the regulation of cellular survival by controlling a complex transducer and signaling network.[143−146] Therefore, the ER is an extremely sensitive sensor of cellular homeostasis, with various genetic and environmental insults (such as proteasomal inhibition, impaired redox regulation, or calcium balance) being able to disturb ER function, induce the loss of its integrity, and lead to ER stress. Some of the most detrimental consequences of ER stress are the accumulation of unfolded and deficiently modified proteins, disturbances in lipid metabolism, and the release of ER luminal Ca^{2+} into the cytoplasm.[144,147,148] There are three branches of stress sensors in ER—inositol-requiring protein-1 (IRE1), protein kinase RNA-like ER kinase (PERK), and activating transcription factor-6 (ATF6)—which are able to recognize the misfolding of proteins in the ER and activate a complex signaling network to generate the unfolded protein response, including signaling cascades that initiate the adaptive changes in metabolism and gene expression required to manage stress situations.[143−148] Therefore, the ER response is characterized by changes in specific proteins, causing translational attenuation, induction of ER chaperones, and degradation of misfolded proteins. The accumulation of unfolded or aggregated proteins, increased oxidative stress, and metabolic disturbances are all characteristic features of many neurodegenerative diseases, including AD[149,150] and PD.[114] Therefore, it is not surprising that ER stress has been found in several neurodegenerative diseases.[151−157] An excellent review by Lindholm, Wootz, and Korhonen[151] summarizes recent studies on ER stress and its involvement in pathogenesis of different neurodegenerative diseases, providing a useful overview of data on molecular mechanisms and cellular pathways involving the ER and discussing how various cellular events contribute to the process of different neurological disorders.

1.3.2.6. Impaired Protein Degradation Machinery. The proteasome, in collaboration with a sophisticated ubiquitin system used for marking target proteins, selectively degrades short-lived regulatory proteins as well as abnormal proteins that must be eliminated from cells. The lysosome-linked autophagy system is a bulk protein degradation system designed to eliminate cytoplasmic constituents and to play a prominent role in starvation response and quality control of organelles in cells. A majority of characteristic proteinaceous inclusions in AD, PD, ALS, and frontotemporal lobar degeneration are ubiquitin-positive.[158,159] This clearly suggests that impaired proteasomal proteolysis is the main mechanism for the accumulation of ubiquitylated proteins and inclusion body formation in many neurodegenerative diseases.[160]

Furthermore, since ubiquitylation has recently been recognized as a mechanism relevant to the autophagy/lysosome system, the fact that specific inclusions in neurodegenerative diseases are ubiquitylated may reflect the impairment of this degradation system as well. In fact, the autophagosome sequesters cytosolic material nonspecifically, and therefore, for a long time, autophagic degradation was considered a nonselective process. However, recent studies clearly show that several subcellular structures such as mitochondria and protein aggregates are degraded by selective autophagy, and that ubiquitin is involved in this process.[161,162] Later, the impairment of the autophagy system in neurons was shown to cause neurodegeneration and ubiquitin-positive inclusion formation in mice.[163,164]

1.3.2.7. Chaperone System Dysfunctions. Maintaining an appropriate intracellular complement of functional proteins depends on a robust, well-organized, and self-regulated protein quality control system that maintains a balance between protein synthesis and degradation and is able to implement a targeted response if an imbalance occurs where misfolded, aggregated, or otherwise damaged proteins accumulate.[78,165−167] This system tags misfolded and aggregated proteins either for refolding by molecular chaperones or for degradation by protein degradation machinery such as the ubiquitin-dependent proteasome system or the lysosome-linked autophagy system.[168] The first line of defense against protein misfolding and aggregation is molecular chaperones. Although, under normal conditions, any protein can spontaneously misfold and aggregate, the "non-stress" concentrations of such misfolded, aggregated, or amyloid proteins is negligible and these potentially toxic species are efficiently eliminated by the quality control system. However, several conditions are known to promote protein misfolding and aggregation. Among these conditions are classic environmental stresses such as heat and cold, heavy metals, toxic chemical compounds, UV radiation, the

synthesis of proteins with mutations, and age-related decrements in the protein quality control system itself. The increased levels of misfolding and aggregation result in the abuse and potential failure of the quality control system. In its turn, the failure of this protein quality control system to fulfill its functions or malfunction of either one or both of its components generates the potential for tissue-specific build-up of protein aggregates, termed amyloids, and is related to the development of neurodegenerative or 'conformational' diseases.[169] More details of chaperone action in neurodegeneration together with a description of the role of intrinsic disorder in their activities are given in the next part of this review.

2. Intrinsically Disordered Chaperones in Neurodegeneration

As mentioned above, molecular chaperones play a number of important roles in fighting protein misfolding and aggregation and, therefore, in protecting neurons from the cytotoxic effects of misfolded/aggregated species. This neuroprotection involves the highly coordinated and orchestrated action of multiple players. Therefore, there is an entire net of macromolecular chaperones and their helpers, co-chaperones. Earlier, it has been emphasized that the importance of intrinsic disorder for the function of chaperones can be underlined by the analysis of the abundance of predicted intrinsically disordered residues in chaperones.[67] This analysis revealed a high proportion of such regions in protein chaperones, where 36.7% of their residues fall into disordered regions and 15% fall within disordered regions longer than 30 consecutive residues.[67] The major goal of this section is to show that many neuroprotective chaperones/co-chaperones are either completely disordered or possess long disordered regions, and to emphasize that intrinsic disorder plays a crucial role in their action. Corresponding information is provided for the Hsp70 system, the Hsp90 system, several small Hsps, and members of the synuclein family.

2.1. The Hsp70 System

2.1.1. Major Players

Hsp70 is a 70 kDa molecular machine which interacts with exposed hydrophobic amino acids in various polypeptides, hydrolyzes ATP, directs its substrates into a variety of distinct fates, and therefore acts at multiple steps in a protein's life cycle, including its folding, trafficking, remodeling, and degradation.[78,170−173] Since Hsp70 is able to bind promiscuously, it is now considered a core chaperone for the proteome[174−176] and a central mediator of protein homeostasis. The activity of Hsp70 is known to be modulated by a number of co-chaperones, which bind to the core chaperone and influence its functions. Among the most important Hsp70 co-chaperones are various J-domain proteins (e.g., HDJ1 and HDJ2), a number of nucleotide exchange factors or NEFs (such as GrpE, BAG1, Hsp110, and HspBP1), and several tetratricopeptide repeat (TPR) co-chaperones (e.g., Hip). Mammalian cells contain a large net of various Hsp70s and their decorating proteins; in particular, they have ∼14 Hsp70s, >40 J-domain proteins,[177] at least 4 distinct types of NEFs, and dozens of proteins with TPR-domains. Since at any given time an individual Hsp70 molecule can only interact with a single representative of each major co-chaperone class, tens of thousands of possible chaperone−co-chaperone complexes might be formed in the cell.[173]

Finally, there are also several co-chaperones connecting Hsp70 to Hsp90 and the proteasomal degradation pathways. For example, the Hsp-organizing protein (Hop) mediates interactions between Hsp70 and Hsp90.[178] Hsp70 and Hsp90 also bind to the protein co-chaperone CHIP (C-terminus of Hsc70-interacting protein),[179,180] which is a member of the family of E3 ubiquitin ligases. CHIP ubiquitylates unfolded proteins bound to Hsp70 and Hsp90, and these tagged proteins are degraded by the proteasome. Therefore, CHIP links Hsp70 and Hsp90 chaperones to the proteasomal degradation pathway.[167]

2.1.2. Hsp70

Hsp70 is a highly abundant (∼ 1−2% of total cellular protein) and highly conserved protein, with approximately 50% sequence identity between prokaryotic and mammalian family members. Many organisms express multiple Hsp70s (e.g., 14 in humans) and members of this class of chaperones are found in all of the major subcellular compartments.[173] Hsp70 is composed of three major domains: a ∼44 kDa N-terminal nucleotide binding domain (NBD, residues 1−388), a ∼15 kDa substrate binding domain (SBD, residues 393−537), and a ∼10 kDa C-terminal α-helical, "lid" domain (residues 538−638) (see Figure 2A). All three domains are important for the function of Hsp70. NBD competitively binds ATP and ADP and can slowly hydrolyze ATP.[181] SBD binds the target peptide via the hydrophobic substrate-binding cleft. NBD and SBD are connected by a flexible linker that is crucial for the functional association of two domains: when ATP is bound to NBD, SBD and NBD exhibit coupled motion, suggesting their tight association.[182,183] In comparison with the NBD and SBD domains, the lid domain is essentially less conserved.[182] On the basis of the sequence divergence of this region, it has been proposed that the lid domain potentially acts as a regulator, providing specificity for interactions with cofactors or modulating the ATPase or peptide binding activities of Hsp70.[184] The position of the lid domain regulates the accessibility of the peptide-binding site. In the ATP-bound form, the lid domain remains open, which facilitates transient interactions with substrates. Following ATP hydrolysis, a conformational change releases the SBD, resulting in closure of the lid and a ∼10-fold increase in the affinity for substrate.[185,186] One important aspect of Hsp70's ability to bind ATP is that, although it binds ATP tightly (K_d = 1 nM), this chaperone hydrolyses it very slowly ($k_{hyd}^{app} = 3 \times 10^{-4}$ s^{-1} at 25 °C).[187] Another important feature of these chaperones is that nucleotides modulate their peptide binding and release: in the absence of co-chaperones, ADP-bound DnaK binds and releases peptides over a timescale of minutes or even hours, whereas ATP-bound DnaK binds and releases peptides over a timescale of seconds or even milliseconds.[185,186] Overall, HSP70s function in a dynamic cycle of binding and releasing polypeptide substrate, coupled to a cycle of ATP binding and hydrolysis by the intramolecular ATPase. In the ATP-bound state, HSP70s exhibit fast kinetics and low affinity for polypeptide substrates, whereas in the ADP-bound state, these chaperones exhibit slow kinetics and high affinity for polypeptide substrates. These cycling states are highly regulated by at least five different co-chaperones: Hsp40, Hip, Hop, Bag-1 (Bcl2-associated athanogene 1, or RAP46), and CHIP.[188]

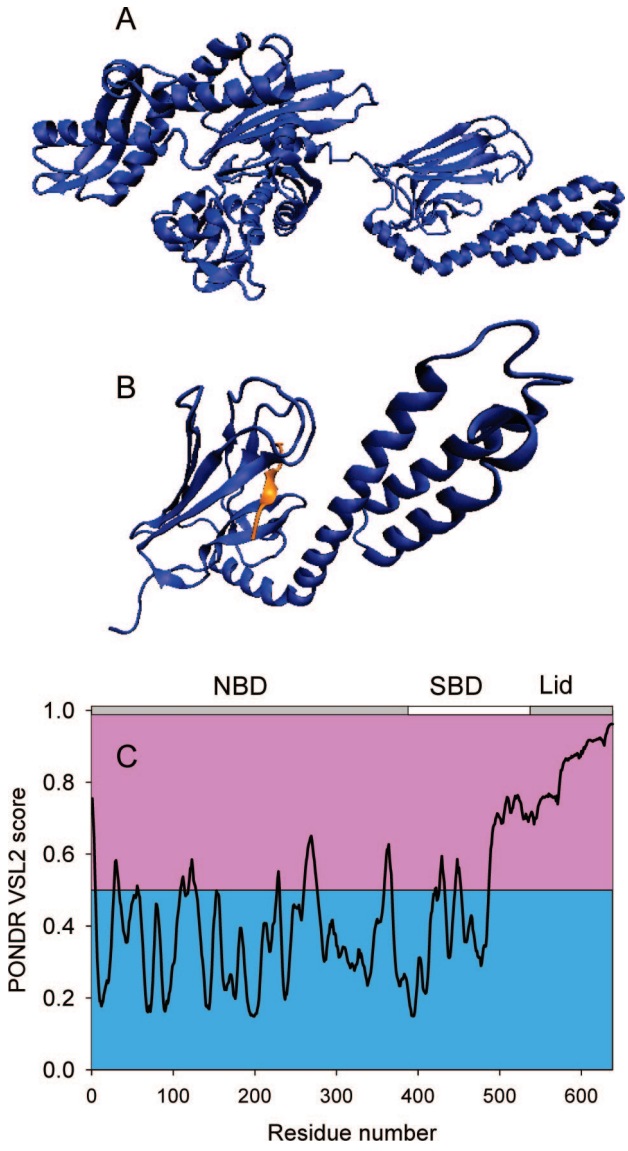

Figure 2. (A) Crystal structure of DnaK (PDB ID: 2KHO). (B) Crystal structure of the substrate binding domain of *E. coli* DnaK in complex with a long pyrrhocoricin-derived inhibitor peptide (PDB ID: 3DPP). (C) The distribution of the intrinsic disorder propensity within the DnaK sequence evaluated by PONDR VSL2. Positions of the three domains, NBD (residues 1−388), SBD (residues 389−537), and lid (538−638) are also shown. All domains contain significant amount of disorder, with the C-terminal lid domain predicted to be entirely disordered.

The substrates of Hsp70 proteins are unrelated in sequence and structure, and represent a large spectrum of folding conformers of foldable proteins. In addition, Hsp70 interacts indiscriminately with nonnative polypeptides (for instance, a broad spectrum of heat-denatured proteins).[189] The broad substrate specificity of Hsp70s implies a rather degenerative binding motif. NMR and X-ray crystallography have shown that DnaK binds its substrate in an extended conformation.[189] This is illustrated by Figure 2B, which represents the substrate binding domain of *Escherichia coli* DnaK in complex with a long pyrrhocoricin-derived inhibitor peptide.[190]

Although the classical chaperone functions associated with Hsp70 include its binding to misfolded proteins to prevent their self-aggregation (a function known as "protein stability control"), Hsp70 can also interact with exposed hydrophobic domains of native proteins, a function known as "protein activity control".[191] For example, the DnaK chaperone system formed by the Hsps DnaK, DnaJ, and GrpE negatively modulates transcription of heat shock genes. This modulation is done via inactivation and degradation of the heat shock promoter-specific σ^{32} subunit of RNA polymerase.[192−196] In fact, DnaK and DnaJ bind independently to σ^{32},[191,197,198] suggesting that their interaction with RNA polymerase (RNAP)-bound σ^{32} might strip σ^{32} from RNAP.[198] This provides a molecular mechanism for the modulation of the heat shock genes' transcription, where the σ^{32} inactivation and degradation of σ^{32} are mediated through direct association of these chaperones with the σ^{32}-dependent transcription machinery.[191]

Therefore, in a "protein activity control" function of chaperones, the chaperone−substrate complex formation can help stabilize or alter conformation and, thus, function of the substrate. This phenomenon is particularly relevant when the binding target resides within an intrinsically disordered domain. One of the illustrative examples of such "protein activity control" is Hsp70/Hsc70 binding to the intrinsically disordered N-terminal region of the p65 coactivator SIMPL that stabilizes the protein and regulates its activity.[199] In another example of the chaperone's "protein activity control" function, the formation of the human transcription elongation factor P-TEFb, which is the Cdk9/cyclin T1 heterodimer responsible for phosphorylation of the C-terminal domain of RNA polymerase II, is regulated by the Cdk9 interaction with the molecular chaperone Hsp70 or a kinase-specific chaperone complex Hsp90/Cdc37.[200] In fact, the formation of the exceptionally stable Cdk9/cyclin T1 dimer requires the sequential actions of Hsp70 and Hsp90/Cdc37 in the stabilization and folding of Cdk9, which is degraded rapidly when free and unprotected, and in subsequent assembly of an active Cdk9/cyclin T1 complex.[200] ATP-dependent high affinity interaction of the major inducible heat shock protein Hsp70 with the measles virus (MeV) nucleocapsids stimulates viral transcription and genome replication, and profoundly influences MeV virulence in mouse models of brain infection.[201] The regulation is achieved via the specific binding of this chaperone to the intrinsically disordered cytoplasmic tail of the MeV N protein (N_{TAIL}) that contains potential targets of the hsp72 interaction, three hydrophobic domains (Box-1−3).[201] Recently, it has been shown that high affinity hsp70 binding to N_{TAIL} requires an Hp40 co-chaperone, HDJ1, that interacts with the hsp70 nucleotide binding domain (NBD) and does not have significant affinity for N_{TAIL}. Importantly, the Hsp70 ATPase activity is directly enhanced by HDJ1 in an N_{TAIL}-dependent manner.[202]

The data above clearly show that the action of HSP70s involves a lot of dynamics and flexibility. To test how functional dynamics correlates with the intrinsic disorder status, a DnaK sequence (which was chosen as the illustrative member of the Hsp70 family) was analyzed by several disorder predictors. Figure 2C represents the distribution of the intrinsic disorder propensity within the DnaK sequence evaluated by PONDR VSL2, and illustrates that all three domains of the protein contain extensive amounts of intrinsic disorder. The mean disorder propensity within these domains is arranged in the following order: NBD (0.36 ± 0.13) < SBD (0.49 ± 0.19) < lid (0.84 ± 0.08), with the C-terminal lid domain predicted to be entirely disordered. This high intrinsic propensity of the protein for intrinsic disorder is crucial for its function. It is also reflected in the fact that, for a very long time, the three-dimensional structure of a

full-length Hsp70 chaperone had not been resolved, and all structural information about this important protein was derived from the analysis of its separate domains obtained by partial proteolysis.[80,186] Furthermore, even when structures comprising both the NBD and SBD became available, none of these structures were compatible with any of the others. The location where the SBD docks to the NBD differs by tens of angstroms.[182]

Recently, the solution conformations for the full-length, *E. coli* DnaK (1−638) and for a truncation (1−605) for the chaperone bound to substrate peptide (NRLLLTG) and ADP were determined by NMR techniques.[182] The analysis revealed that, although the NBD, SBD, and linker move relatively independently of each other, the motion of SBD with respect to NBD is restricted to a cone of ∼70° opening angle. However, within this cone, there is a preferred orientation of SBD with respect to NBD that can be defined with a relatively high precision.[182] Importantly, NBD−SBD linker residues (379−397) were shown to possess a large amount of flexibility, and the lack of dispersion in NMR chemical shifts suggests that this flexible linker has a random coil conformation.[182] On the other hand, SBD and lid domains were shown to move together as a single rigid unit in the ADP-peptide state, and residues 606−638 were disordered in solution.[182]

2.1.3. Hsp70 Co-chaperones

2.1.3.1. J-Domain Proteins. The members of the J-protein family, also known as the Hsp40 family (or DnaJ-related co-chaperones), are highly diverse and range in size from 116 amino acids (DnaJC19) to 2243 amino acids (DnaJC13, or Rme-8).[203] In humans, there are over 41 J-proteins, with only one common feature: a conserved ∼70 amino acid signature region, known as a J-domain.[177,204] Although J-proteins are known to modulate Hsp70 ATP catalytic activity via their conserved J-domain, all of these proteins have multiple additional domains with various functionalities, clearly reflecting the functional diversity, probably related to client protein recognition, of this family.[203] Therefore, J-proteins serve as regulators of ATPase activity and the substrate-binding specificity of Hsp70s. Hsp40 proteins are classified in three main subfamilies (*A*−*C*, also referred to as types I−III).[205−207] Subfamily *A* consists of proteins with the following four domains: the highly conserved α-helical N-terminal domain, referred to as the J-domain;[208−212] a glycine/phenylalanine-rich region that is disordered and likely to be responsible for flexibility;[208,213] the central cysteine-rich domain that includes four repeats of the motif C*XX*-C*XGXG* (where *X* is any amino acid) and folds in a zinc-dependent fashion with two repeats bound to one zinc ion;[214−216] and the C-terminal domain, which forms a *β*-sheet structure and is involved in the dimerization of Hsp40.[217] The Cys-rich and C-terminal domains are involved in substrate binding and presentation.[215,218,219] Subfamily *B* contains proteins that lack the Cys-rich domain, and subfamily *C* has only the J-domain, but it is not necessarily located at the N terminus.[205−207]

Results of the disorder prediction for illustrative members of J-proteins from classes *A* (human DnaJ homologue subfamily A member 1), *B* (human DnaJ homologue subfamily B member 1), and *C* (human DnaJ homologue subfamily C member 21) are shown in Figure 3. All three co-chaperones are predicted to be highly disordered, possessing averaged disorder scores of 0.60 ± 0.20, 0.54 ± 0.23, and 0.74 ± 0.28, respectively. The fact that all three proteins contain long disordered regions is further confirmed by the lack of the resolved 3D structures of the full-length proteins.

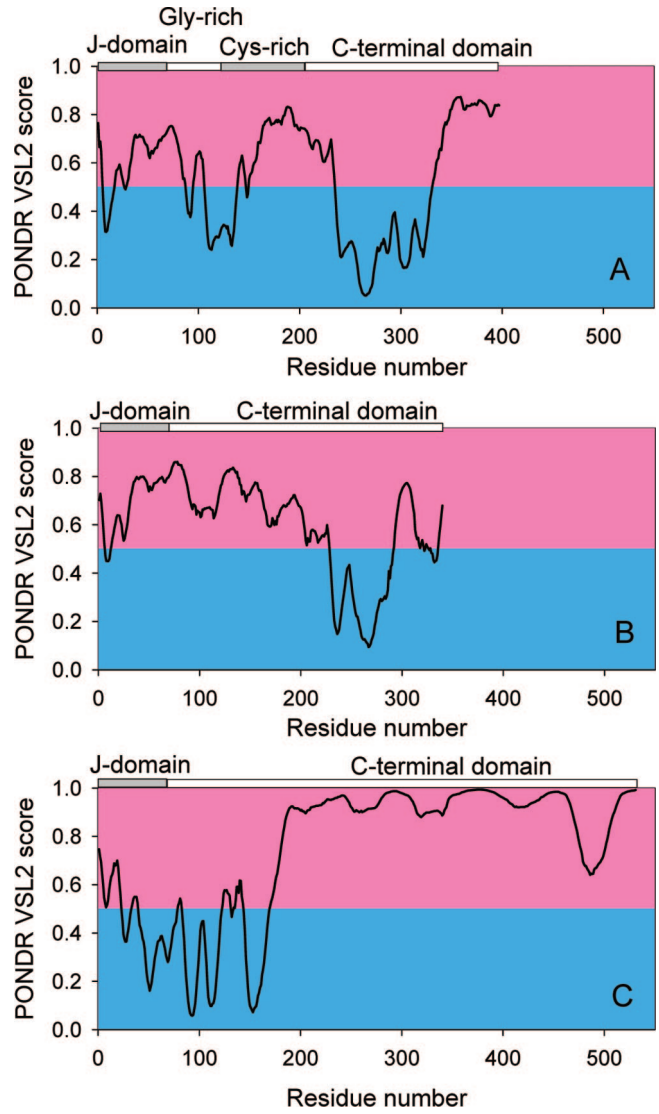

Figure 3. Intrinsic disorder in illustrative members of the J-proteins (Hsp40s) co-chaperones from the classes *A* (human DnaJ homologue subfamily A member 1, plot A), *B* (human DnaJ homologue subfamily B member 1, plot B), and *C* (human DnaJ homologue subfamily C member 21, plot C). The localizations of major domains in these Hsp40 proteins are also indicated.

2.1.3.2. TPR Co-chaperone Hip. Hip (Hsp70 interacting protein) is a 369-amino acid cytosolic protein which is composed of an N-terminal region (residues 1−100, which is responsible for protein homo-oligomerization[220]), a central TPR domain (residues 114−215), followed by a highly charged region (residues 230−272), and a C-terminal region (residues 283−369) containing GGMP repeats and a Sti1 domain (heat shock chaperonin-binding motif).[188] Hip plays a crucial role in the Hsp70 cycle. In fact, the initial Hsp70 interaction with a polypeptide substrate is achieved through chaperone cooperation with a member of the Hsp40 family, the binding of which stimulates Hsp70s ATPase activity and generates its high affinity, ADP-bound state. However, in the presence of Hsp40 alone, the ADP state of Hsp70 is unstable, and the newly formed Hsp70−substrate complex may dissociate prematurely.[221] The TPR domain of Hip binds to the Hsp70 ATPase domain and stabilizes the ADP-bound state of Hsp70, thus, stabilizing the chaperone−substrate

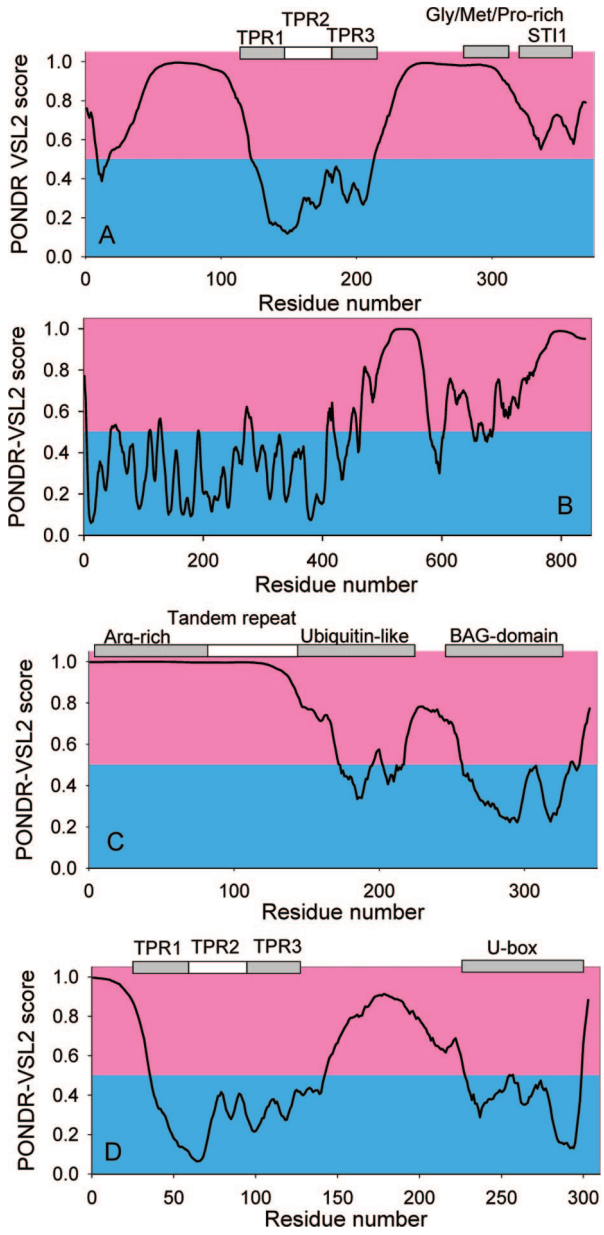

Figure 4. Predicted intrinsic disorder in the co-chaperones of the Hsp70 machinery: (A) disorder prediction for human Hip co-chaperone; (B) disorder in the NEF co-chaperone, human Hsp70-related protein APG-2 (human Hsp110); (C) intrinsic disorder prediction in the human BAG family molecular chaperone regulator 1 L (BAG-1 L); (D) predicted disorder in the human CHIP also known as the STIP1 homology and U box-containing protein 1. The localizations of major functional domains in these proteins (when known) are also indicated.

complex. A TPR domain and a flanking highly charged region are required for Hip to bind the Hsp70 ATPase domain.[221] Figure 4A represents disorder prediction for human Hip co-chaperone and shows that this protein is predicted to be intensively disordered. No crystal structure was resolved for the full-length Hip co-chaperone.

2.1.3.3. NEF Co-chaperone Hsp110. Hsp110 proteins constitute a heterogeneous family of abundant molecular chaperones which are found exclusively in the cytosol of eukaryotic organisms and are evolutionarily related to the Hsp70 family.[222] There are four members in the human Hsp100 family.[223] Hsp100 was shown to reside in a large molecular complex which includes Hsp70 and Hsp25.[224] The N-terminal ATPase domain of Hsp110 proteins possesses significant amino acid sequence homology with Hsp70 proteins, whereas the sequence homology between these proteins in their C-terminal domains is very low and hardly recognizable. The C-terminal domain of Hsp100s is considerably longer than that of classical Hsp70 proteins, partially due to a highly negatively charged insertion characteristic for Hsp110 proteins.[225] Members of the Hsp110 family are known to be efficient "holdases"; they prevent the aggregation and assist the refolding of heat-denatured model substrates in the presence of Hsp70 chaperones and their co-chaperones.[226] It has been shown recently that the Hsp110 in yeast, Sse1p, acts as an efficient NEF for yeast's cytosolic Hsp70s, Ssa1p and Ssb1p.[227] Figure 4B shows that the human Hsp70-related protein APG-2 (human Hsp110) possesses a significant amount of intrinsic disorder, especially in its C-terminal domain. In fact, the mean disorder score for this protein is 0.52 ± 0.28, whereas its last 380 residues are characterized by the disorder score of 0.76 ± 0.19. The fact that Hsp110 co-chaperones are significantly disordered is further supported by a lack of resolved 3D structures of the full-length proteins.

2.1.3.4. NEF Co-chaperone BAG1. BAG-1 is a multifunctional protein implicated in the modulation of a large variety of cellular processes, ranging from transcriptional regulation to the regulation of apoptosis and the control of cell migration.[228−230] In relation to the chaperone system, BAG-1 is intimately involved in the regulation of Hsp70 chaperone proteins in the eukaryotic cytosol and nucleus, therefore, modulating Hsp70-mediated protein folding and degradation pathways.[230] In fact, BAG-1 is known to stimulate nucleotide exchange of mammalian cytosolic Hsc70.[231] There are at least 4 isoforms of BAG-1: BAG-1L (apparent molecular mass of 52 kDa), BAG-1M (46 kDa; also termed HAP46, RAP46), BAG-1 (34 kDa), and BAG-1S (29 kDa).[232] The major difference between these isoforms is in their N-terminal domains, which differ in their inclusion of several structural elements, the presence or absence of which brings functional diversity to distinct isoforms.[230] In addition to the conserved BAG domain located at the C-terminus of BAG-1s, all isoforms contain a ubiquitin-like domain which serves as an integral sorting signal to stimulate an interaction between BAG-1 and the proteasome, and therefore provide a unique link between the Hsp70 and ubiquitin−proteasome systems.[230] Furthermore, human cells contain several BAG-1-related proteins: BAG-2, BAG-3 (CAIR-1; Bis), BAG-4 (SODD), BAG-5, and BAG-6 (Scythe, BAT3), which, in addition to the conserved BAG domain required for the binding and regulation of Hsc70, possess various functional domains that mediate their targeting to diverse partner proteins and subcellular compartments.[229] The plethora of biological functions ascribed to BAG-1 can be understood by taking into account the fact that this protein is highly disordered. Intrinsic disorder distribution within the sequence of the human BAG family molecular chaperone regulator 1L (BAG-1L) is shown in Figure 4C. Once again, there are no crystal structures for the full-length members of the BAG-1 family.

2.1.3.5. Carboxy-Terminus of Hsc70 Interacting Protein (CHIP). Similar to BAG-1, CHIP contains both a chaperone-binding site and a domain implicated in the regulation of the ubiquitin−proteasome system. CHIP interacts with binding sites for the TPR-containing co-chaperones Hsc70 and Hsp90 via a tandem of 3 TPR motifs at its amino terminus, whereas at its carboxyl terminus, this protein

contains a U-box, which is structurally related to RING finger domains found in many ubiquitin ligases.[233] CHIP, by itself, possesses ubiquitin ligase activity and, in coordination with ubiquitin-conjugating enzymes of the Ubc4/5 family, mediates ubiquitin attachment to protein substrates bound by Hsc70 and Hsp90.[179,234] Since Hsc70 contains nonoverlapping binding sites for BAG-1 and CHIP, these two factors can simultaneously associate with the chaperone.[179] Furthermore, in the ternary BAG-1/Hsc70/CHIP complex, CHIP mediates the attachment of a polyubiquitin chain to BAG-1, promoting the association of the chaperone complex with the proteasome and therefore providing the mechanism for the regulation of the chaperone-assisted degradation pathway.[235] It has been recently established that CHIP targets toxic α-synuclein oligomers for degradation, which has important applications n neurodegeneration.[236] Figure 4D represents the results of disorder prediction for human CHIP, also known as the STIP1 homology, and for U box-containing protein 1. Figure 4D shows that, although the TPR-containing domain and U-box domain are predicted to be mostly ordered, they are connected by a long, highly disordered linker. The flexibility of this linker most likely helps in decoupling CHIP interactions with BAG-1 and Hsp70.

2.1.3.6. Co-chaperone Hop. Hop (Hsp70/Hsp90-organization protein, also known as stress-induced-phosphoprotein 1, STIP1) does not act as chaperone by itself.[237] However, it is involved in the organization of the Hsp70/Hsp90 complex via its three TPR domains, which serve as nonoverlapping binding sites for both Hsp70 and Hsp90. In fact, the EEVD-containing C-termini of Hsp70 and Hsp90 bind specifically to the Hop tetratricopeptide repeat domains TPR1 and TPR2a, respectively.[178] The connection of and the interplay between the Hsp70 and Hsp90 chaperone machineries is of crucial importance for cell viability. Although originally Hop was considered to be a linker protein that brings and holds Hsp70 and Hsp90 together,[238] the functional repertoire of this co-chaperone is essentially broader, since it is also involved in the regulation of these two chaperones.[239] The chaperone activities of both Hsp70 and Hsp90 are dependent on their ability to bind and hydrolyze ATP. These two chaperones are constantly recycled between their ADP and ATP bound forms. The ATPase activity of both Hsp70 and Hsp90 can be divided mechanistically into two stages: ATP hydrolysis and ADP/ATP (nucleotide) exchange. Hsp40 enhances the binding of Hsp70 to a pre-existing Hop-Hsp90 complex by stimulating the conversion of Hsp70-ATP to Hsp70-ADP.[240] Human Hop binds to Hsp70 with low affinity, but the strength of interaction increases in the presence of Hsp90.[240] Despite noticeable sequence homology between human Hop and its yeast homologue Sti1 (37% identity), there are fundamental differences between these two proteins in the regulation of their respective systems.[241] Whereas in the mammalian system, Hop has no influence on the ATPase activity of the Hsp70 or Hsp90 component,[241] Sti1 is a noncompetitive inhibitor of yHsp90[242] and a potent activator of yHsp70.[241]

Hop exists as a dimeric molecule in solution and binds as a dimer to dimeric Hsp90.[243] Structurally, Hop is defined by the presence of nine TPR motifs (which are loosely conserved repeats of roughly 34 amino acids known to mediate protein−protein interactions) clustered into three TPR domains, each consisting of three TPR motifs. A TPR motif shows a helix-turn-helix structure and subsequent TPR motifs are ordered in antiparallel α-helices.[244] Each TPR domain is able to form a structural module that directs protein−protein interactions, and is recruited by different proteins and adapted for various different protein−protein interaction functions.[245] Because of its TPR domains, Hop participates in the formation of several Hsp70/Hsp90-unrelated complexes, for example, serving as a receptor for prion proteins.[239] The results of disorder prediction in human Hop (STIP1) are shown in Figure 5.

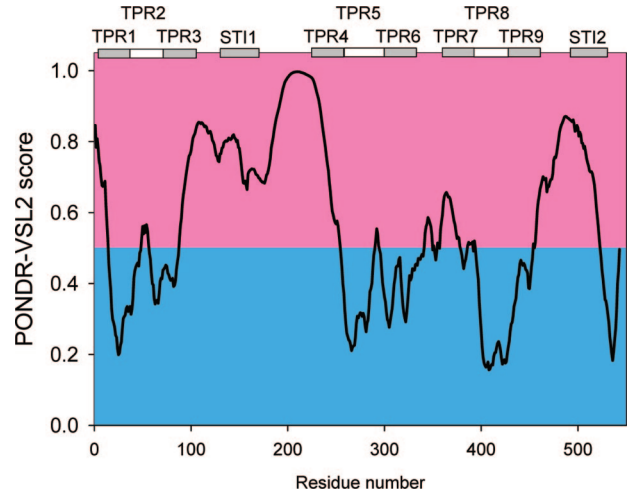

Figure 5. The distribution of the intrinsic disorder propensity within the sequence of the human co-chaperone Hop (Hsp70/Hsp90-organization protein, also known as stress-induced-phosphoprotein 1, STIP1) evaluated by PONDR VSL2. The localizations of major domains in this co-chaperone are also indicated.

2.2. The Hsp90 Chaperone System

2.2.1. Major Players

Although the Hsp70 machinery, which is responsible for the correct folding of a wide variety of protein substrates, is one of the most frequently used folding systems in the cell, some proteins are processed by Hsp70 and then transferred to the Hsp90 machinery. In this case, the scaffold protein Hop connects elements of the Hsp70 and Hsp90 machineries to form the "intermediate complex." The Hsp70 component dissociates and, at the same time, p23 and prolylisomerases enter the complex. After that, the substrate is released from this "final complex." After binding to Hop, Hsp90 is able to reenter the chaperone cycle.[189] There is some evidence that Hsp90 is also able to act independently of Hsp70. Each chaperone folding pathway can either lead to folded, functional proteins or to degradation. Neither Hsp70 nor Hsp90 acts alone. The activity of both chaperones is precisely regulated by a number of co-chaperones. Interestingly, some of the co-chaperones (e.g., Hop and CHIP) are able to interact with both Hsp70 and Hsp90. Other co-chaperones are specific for the individual chaperone machinery: Hsp70 exclusively interacts with J-proteins, Hip, Hsp110, and BAG-1, whereas co-chaperone p23, a signal transduction-related protein Cdc37/p50 (which is required for the Hsp90 substrate-specific folding activity), prolylisomerases FKPB51, FKPB52, and Cyp40 (also known as immunophilins), prolylisomerase-related protein XAP5, phosphatase PP5, and the Hsp90 ATPase regulator Aha1 are specific cofactors of Hsp90.[189] Overall, the Hsp90 system is complex machinery, and its uniqueness is defined by its close collaboration with Hsp70 and its large number of cofactors.

2.2.2. Hsp90

Hsp90 is one of the most abundant proteins in unstressed cells, accounting for 1% to 2% of total soluble cell protein.[246,247] In eukaryotes, cytoplasmic Hsp90 is essential for viability under all conditions. There are two genes encoding cytosolic Hsp90 homologues in mammalian cells. For example, the human Hsp90α shows 85% sequence identity to Hsp90β.[248] ATP hydrolysis is crucial for Hsp90 function *in vivo*. However, ATP binding to Hsp90 is generally weak, with a dissociation constant in the high micromolar range.[249] The ATPase activity of human Hsp90 is barely detectable, with a k_{cat} of 0.089 ± 0.004 min^{-1} and a K_m of 840 ± 60 μM.[250] Therefore, as in the case of Hsp70, the ATPase cycle of Hsp90 is modulated by partner proteins which act in complex with Hsp90 *in vivo*.

Hsp90 is known to regulate a number of specific targets. Among the established substrates or "client proteins" of Hsp90 are transcription factors, such as steroid hormone receptors and p53, as well as some proto-oncogenic serine/threonine and tyrosine kinases, such as Raf and Src in higher eukaryotes (for reviews see refs 251−253).

In neurodegenerative disorders associated with protein aggregation, Hsp90 is known to regulate the heat shock response.[254−256] The inhibition of Hsp90 activates heat shock factor 1 (HSF1) to induce the production of the chaperones Hsp70 and Hsp40, which promote disaggregation and protein degradation. Under nonstressed conditions, Hsp90 binds to HSF1 and maintains the transcription factor in a monomeric state.[257] Stress-induced inhibition of Hsp90 releases HSF1 from the Hsp90 complex, leading to its trimerization, activation, and translocation to the nucleus, where it initiates a heat shock response.[257]

Structurally, Hsp90 is an elongated dimer,[258,259] which, in higher eukaryotes, exists either as α−α or $\beta-\beta$ homodimers, or as $\alpha-\beta$ heterodimers.[260,261] Quaternary structure is important for ATPase activity and associated conformational changes. There are three major domains in Hsp90: a highly conserved N-terminal ATPase domain; a middle domain, which is potentially involved in binding of the substrate proteins; and a C-terminal dimerization domain, which is essential for Hsp90 function and provides the binding site for a subset of Hsp90 co-chaperones, which contain TPR domains.[249,258,262,263] In eukaryotic Hsp90s, the amino-terminal nucleotide-binding domain is connected to the remainder of the protein by a highly charged and protease-sensitive segment that is variable both in length and composition between different species and between different isoforms in the same species.[189]

For a long time, atomic resolution crystal structures were only solved for individual structural domains of Hsp90 because of the intrinsic conformational flexibility of the intact protein. Recently, a solution structure of the first nucleotide-free eukaryotic Hsp90 (apo-Hsp90) from a pig brain was analyzed using a combination of small-angle X-ray scattering and single-particle cryo-electron microscopy (cryo-EM). This analysis revealed the intrinsic flexibility of the full-length eukaryotic apo-Hsp90 and showed that apo-Hsp90 exists in a conformational equilibrium between two open states. Transitions between these states (fully open and semiopen) require large movements of the N-terminal domain and middle domain around two flexible hinge regions.[264] Figure 6A illustrates the disorder distribution within the sequence of human Hsp90α, whereas Figure 6B shows the disorder status in human Hsp90β. This analysis shows that, in agreement with structural studies, functional domains are separated by long disordered regions. These long disordered linkers likely contribute to the resistance of Hsp90 to crystallization, and no resolved 3D structures are currently available for the full-length members of this chaperone family.

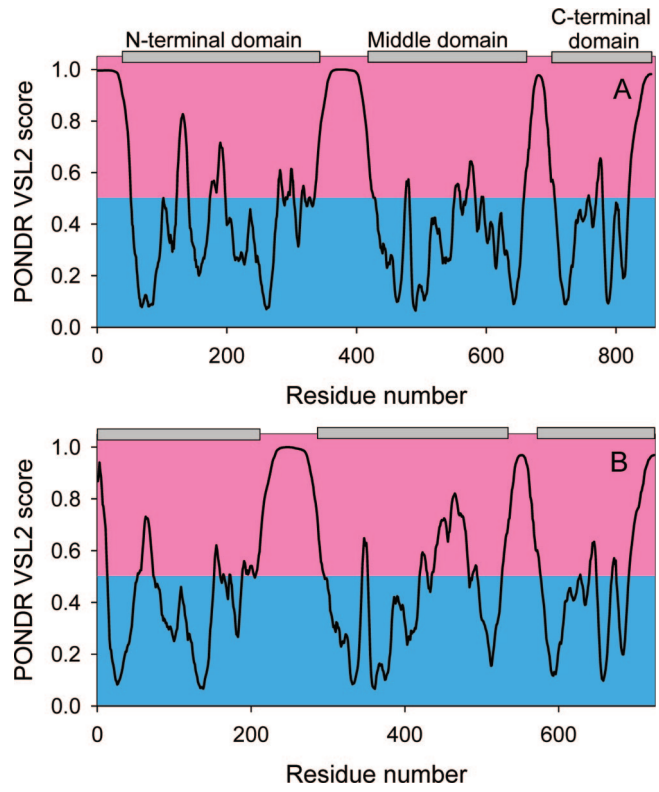

Figure 6. The disorder distribution within the sequence of human Hsp90α (plot A), and the disorder status in human Hsp90β (plot B). In agreement with structural studies, functional domains are separated by long disordered regions.

2.2.3. Co-chaperone p23

p23 (also known as prostaglandin E synthase 3, telomerase-binding protein p23, and progesterone receptor complex p23 in human or Sba1 in yeast) is a small protein with chaperone activity.[237] Similar to Hop, p23 interacts with the N-terminal ATPase domain of Hsp90.[241] This interaction is dependent on ATP binding[265−267] and inhibits the intrinsic ATPase activity of Hsp90.[268] In yeast, the amino-terminal dimerization of Hsp90 noticeably increases p23's affinity for yeast Hsp90. The p23 interaction is counteracted by Hop, which prevents amino-terminal dimerization of yeast Hsp90 and therefore the binding of p23 to yeast Hsp90.[241]

The N-terminal region of p23 contains a CS domain, which is a ~100-residue protein−protein interaction module named after CHORD-containing proteins and SGT1.[269] The CS domain has a compact antiparallel β-sandwich fold consisting of seven β-strands.[270] In the crystal structure of human p23,[271] the C-terminal tail (residues 91−160) is unresolved, which implies that it may be unstructured. This disordered tail occupies almost one-half of the protein and is highly enriched in Asp and Glu residues. Although this flexible tail is not needed for the binding of p23 to Hsp90, it is necessary for the optimum active chaperoning activity of p23 in assays measuring the inhibition of heat-induced protein aggregation.[271,272] In agreement with this structural analysis, Figure 7A shows a very high level of predicted intrinsic disorder

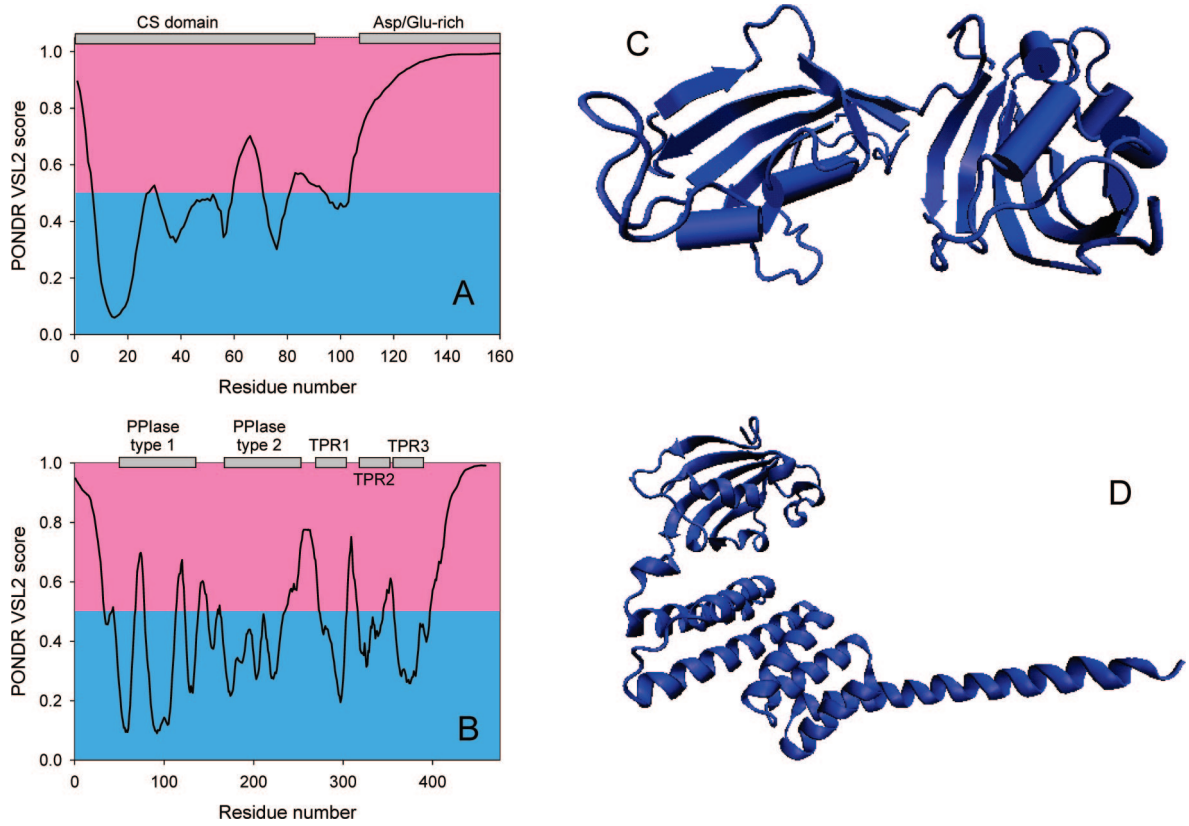

Figure 7. Predicted intrinsic disorder in Hsp90 co-chaperones and accessory proteins. Disorder distribution in human p23 co-chaperone (A) and in human FKBP52, also known as FK506-binding protein 4, HSP-binding immunophilin (HBI), 52 kDa FK506-binding protein, FKBP59, and p59 protein (B). Crystal structures of the N- (C, PDB ID: 1Q1C) and C-terminal fragments of human FKBP52 (D, PDB ID: 1P5Q).

in the C-terminal half of human p23. In agreement with this prediction, the available crystal structure of human p23 does not include the last 35 residues.[271]

2.2.4. Prolylisomerases/Immunophilins

Immunophilins are ubiquitous and conserved proteins that have peptidylprolyl isomerase (PPIase) activity, suggesting that they may play a role in protein folding in the cell.[273] Functionally, immunophilins are divided into two classes: cyclophilins (CsA-binding proteins) and FKBP (FK506/rapamycin-binding proteins).[274] High molecular mass immunophilins possess several TPR domains and a calmodulin-binding domain in their C-terminal half.[275] The binding of immunophilins to Hsp90 via TPR domains is conserved both in plants and in the animal kingdom,[276] suggesting that this is a basic function of the high molecular weight immunophilins. In addition to binding to chaperones, immunophilins interact with substrate proteins, which are either partially folded species of ordered proteins or intrinsically disordered proteins. For example, a recent study indicated that cyclophilins A and B (CypA and CypB) interact with the intrinsically disordered domains of the hepatitis C virus (HCV) nonstructural protein 5A (NS5A),[277,278] which is a large phosphoprotein (49 kDa), indispensable for HCV replication and particle assembly. Interactions of NS5A with CypA and CypB modulate the affinity of NS5A for RNA, and therefore, these cyclophilins may serve as key regulators in HCV replication.[277−279]

High molecular mass prolylisomerases/immunophilins are crucial for the effective action of the Hsp90 machinery, where, together with p23, they are involved in the release of the substrate protein from the Hsp70−Hop complex, and contribute to the formation of the "final complex". In this mechanism, the substrate protein bound to Hsp70 is brought into contact with Hsp90 via Hop. Hop and Hsp70, parts of the "intermediate complex", are exchanged for a prolylisomerase/immunophilin and p23 to yield the "final complex". Upon maturation from the "intermediate complex" to the "final complex", the substrate is transferred from Hsp70 to Hsp90.[241] In addition to this crucial role in the Hsp70−Hsp90 chaperone cycle, immunophilins are involved in a number of very important biological processes, for example, in hormonal activation. For instance, in the absence of a particular hormone, the glucocorticoid receptor (GR), which is a hormone-activated transcription factor that requires hormonally driven movement to its site of action within the nucleus, is found in the cytosolic fraction of cells as a mixture of complexes. The common feature of all of these complexes is that they all contain GR and Hsp90. However, each of these heterogeneous complexes contains only one molecule of either FKBP52, FKBP51, Cyp40, or PP5, which are all TPR domain-containing members of the prolylisomerase/immunophilin family.[280]

In FKBP52, there are three globular domains followed by a C-terminal portion containing a predicted calmodulin-binding domain.[281] The N-terminal domain possesses the highest homology (49%) with the well-characterized low molecular weight prolylisomerase FKBP12.[281] This domain has PPIase activity *in vitro*[282] and possesses the dimerization site.[283] Domain II, which has virtually no PPIase activity,[282] is less homologous to FKBP12 (28%) and contains a consensus nucleotide-binding sequence.[281] Domain III com-

prises three TPR domains and its deletion abrogates FKBP52 binding to Hsp90.[284] The functionality of the predicted calmodulin-binding domain at the C-terminus of FKBP52 was supported by specific retention of this protein by calmodulin−Sepharose in the presence of calcium.[285]

Figure 7B shows that human FKBP52 (also known as FK506-binding protein 4, HSP-binding immunophilin (HBI), 52 kDa FK506-binding protein, FKBP59, and p59 protein) contains noticeable amount of disorder. In fact, both ends of the protein are highly disordered. Furthermore, although FKBP52 contains several structured domains (PPIase type 1, PPIase type 2, and TPR-domain), all of them are separated by highly flexible linkers, which provide the protein's unique functional plasticity, allowing each domain to act independently from its neighbors. This is further illustrated by Figure 7, panels C and D, which represent crystal structures of the N- and C-terminal fragments of human FKBP52, respectively. Figure 7C shows that the N-terminal PPIase type 1 and PPIase type 2 domains are independently folded and connected by a flexible hinge (residues 139−148). Note that the first 20 residues are missing in this structure.[286] Figure 7D represents a complementary structure of the C-terminal half of the protein that includes PPIase type 2 domain and the TPR domain.[286] Once again, the PPIase domain, being independently folded, is separated from the TPR domain by a flexible linker. The TPR domain is entirely helical and consists of three units of a consensus 34-aa motif. Each single unit consists of two consecutive α-helices containing 12−15 residues (except α1 and α3, which contain 21 and 23 residues, respectively) that cross at an angle of ≈20° to each other. A long helical protrusion in the C-terminus beyond the final TPR motif contains the calmodulin-binding site.[286] This additional helix is likely to be stabilized by crystal contacts with neighboring monomers.

2.3. Small Heat Shock Proteins

Small heat shock proteins, sHsps, constitute a structurally divergent family of stress proteins characterized by the presence of the α-crystallin domain, a conserved sequence of 80−100 amino acid residues.[287−291] The sHsps are molecular chaperones, storing aggregation-prone proteins as folding competent intermediates and conferring enhanced stress resistance on cells by suppressing aggregation of denatured or nonfolded proteins.

The core of sHsps is the conserved α-crystallin domain, which is typically located in the middle of sHsps, being flanked by two extensions. The α-crystallin domains of sHsps in all members of this family share a common structure consisting of a seven-, eight-, or even nine-stranded, IgG-like β-sandwich with topology identical to p23.[290−293] The poorly conserved N-terminal region varies in sequence and length and influences oligomer construction and chaperone activity. The highly flexible and variable C-terminal extension stabilizes quaternary structure and enhances protein−substrate complex solubility.[288] In human sHsps, the α-crystallin domain modulates both structural integrity and function. This domain is similar to a major lens protein, α-crystallin, that is composed of two similar subunits, αA- and αB-crystallins. α-Crystallin is highly abundant in lens cells, where it comprises as much as 40% of the cytoplasmic protein and is typically assembled into a heterogeneous mixture of large complexes.[289] Electron microscopy analysis of a 32-subunit complex of αB-crystallin reveals a micelle-like hollow globular structure with outside dimensions of approximately 190 Å and inside dimensions of approximately 100 Å.[294]

The molecular mass of various sHsps in different species ranges from 12 to 43 kDa; even within a single species, most organisms express multiple sHsps in a cell-specific and developmentally regulated pattern.[295] For example, the number of sHsp genes in the known eukaryotic genomes ranges from 2 in yeast to 12 in *Drosophila melanogaster*,[296] 16 in *Caenorhabditis elegans*,[297] and 19 in *Arabidopsis thaliana*.[298] Furthermore, the *A. thaliana* genome also codes for 25 distantly related proteins that contain one or more α-crystallin domains.[298] In humans, there are 10 sHsps, many of which are constitutively present at high levels and are implicated in various diseases.[295,299] These 10 human sHsps are Hsp27/HspB1, HspB2, HspB3, αA-crystallin/HspB4, αB-crystallin/HspB5, Hsp20/HspB6, cvHsp/HspB7, H11/HspB8, HspB9, and a sperm tail protein known as outer dense fiber protein 1 (ODF1).[295] Their genes are dispersed over 9 chromosomes, suggesting their ancient origin.[295]

The sHsps occur as homo- or heteromeric complexes, comprising 2 to about 40 subunits. These globular complexes are often polydisperse and dynamic, readily exchanging subunits, and bind a wide range of cellular substrates. sHsps serve mostly as "holdases", preventing the *in vitro* aggregation of unfolding proteins, which can be transferred to ATP-dependent chaperones, such as Hsp70, and refolded.[300] sHsp substrate binding capacity is known to be enhanced by structural changes that expose hydrophobic surfaces which are normally occluded in the native sHsp oligomeric structure.[292] Putative substrate binding sites may become available through the dissociation of sHsp oligomers to dimers as a result of the dynamic equilibrium of sHsp subunits between oligomeric and suboligomeric species, or through more subtle environmentally induced (e.g., high temperature) changes in sHsp tertiary structure.[292,301] Therefore, one proposed mechanism of action of sHsps involves breaking down the large oligomer into smaller subunits, exposing hydrophobic surfaces in the α-crystallin domain, which enables binding of the unfolded substrate, followed by reassembly into large soluble complexes aided by sequence extensions.[293] sHsps protect against several cellular stressors,[302,303] and therefore, their expression can be up-regulated by various forms of stress.[296,304] For example, increased levels of several human sHsps have been found in patients with neurodegenerative disorders[305] and with certain tumors.[306]

Many sHsps are multifunctional proteins. For example, in addition to serving as an ATP-independent chaperone involved in protein folding, human Hsp27 (also known as HspB1) is involved in interaction with various cell structures and is implicated in creating the architecture of the cytoskeleton, cell migration, metabolism, cell survival, growth/differentiation, mRNA stabilization, and tumor progression.[307] Furthermore, a variety of stimuli induce phosphorylation of this protein at serine residues 15, 78, and 82, which is crucial for its subsequent activity.[307] This functional diversity of sHsps is translated into their exceptional structural plasticity and flexibility. For example, human HspB8 (also known as Hsp22), which has been shown to decrease or prevent aggregation of Huntington fragments and Aβ_{1-40} of the Dutch type, is a highly flexible protein belonging to the group of IDPs.[308] Recently, a comprehensive search for the specific positions of a sHsp that interact directly with partially denatured substrates revealed that, although all three domains of the chaperone—the N-terminal arm, the α-crystallin

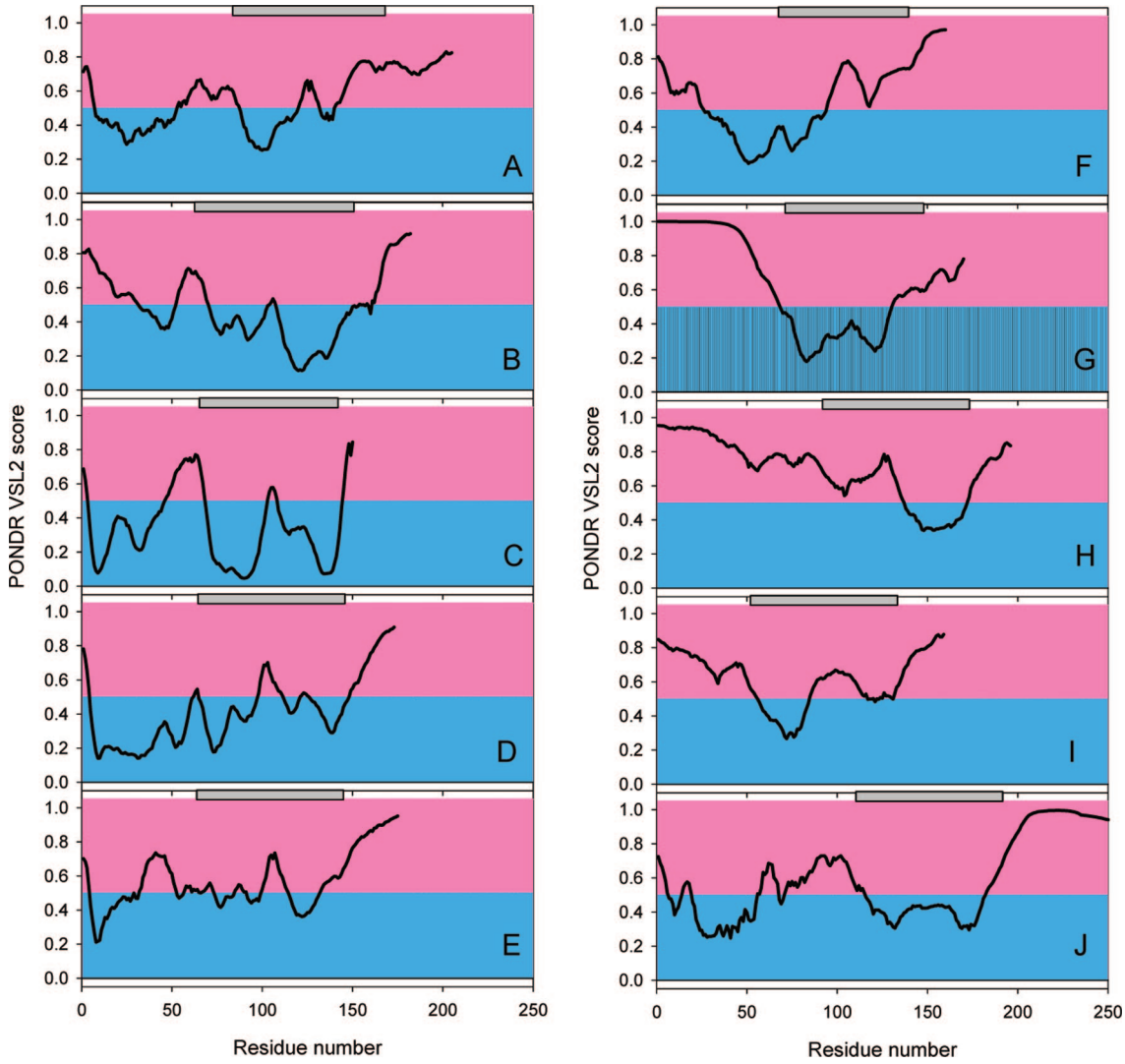

Figure 8. Distribution of the PONDR VSL2 predicted intrinsic disorder in sequences of human small heat shock proteins, sHsps: Hsp27/HspB1 (A), HspB2 (B), HspB3 (C), αA-crystallin/HspB4 (D), αB-crystallin/HspB5 (E), Hsp20/HspB6 (F), cvHsp/HspB7 (G), H11/HspB8 (H), HspB9 (I), and outer dense fiber protein 1 (ODF1) (J). In each plot, the localization of the α-crystallin domain is indicated as gray area.

domain, and the C-terminal arm—are able to interact with the substrate, the N-terminal extension plays the most important role in binding the substrate. Several substrates have been shown to form strong contacts with multiple residues of this region, as its intrinsically disordered nature helps in adopting the diverse geometries of interaction sites necessary for interaction with different substrate proteins. This property of the N-terminal arm is critical for the ability of sHsps to efficiently protect many different substrates.[309] Figure 8 reports on the disorder status of human sHsps, and shows that many of these chaperones are highly disordered. Figure 8 shows also that α-crystallin domains are typically predicted to have noticeable amount of order, often being the most ordered part of a given sHsp. In agreement with these disorder predictions, none of the full-length sHsps produce a crystal structure as of yet. However, a central region of human αB-crystallin containing the α-crystallin domain (residues 67−157) was crystallized.[310]

2.4. Synucleins as Chaperones and Anti-Chaperones

Synucleins belong to a family of closely related presynaptic proteins that arise from three distinct genes, described currently only in vertebrates.[311] This family includes: α-synuclein, which also known as the nonamyloid component precursor protein, NACP, or synelfin;[312−314] β-synuclein, also referred to as phosphoneuro-protein 14 or PNP14;[314−316] and γ-synuclein, also known as breast cancer-specific gene 1 or BCSG1 and persyn.[317−321] All three proteins belong to the family of intrinsically disordered proteins,[322,323] with α-synuclein being one of the most well-characterized IDPs.[324−329]

α-Synuclein is an abundant presynaptic brain protein, and its misfolding, aggregation, and fibrillation are all implicated as critical factors in several neurodegenerative diseases. α-Synucleins from different organisms possess a high degree of sequence conservation. For example, mouse and rat α-synucleins are identical throughout the first 93 residues, whereas human and canary proteins differ from them by only two residues.[330] At least three α-synuclein isoforms are produced in humans by alternative splicing.[331] The best known isoform is α-synuclein-140, which is the whole and the major transcript of the protein. Two other isoforms, α-synuclein-126 and α-synuclein-112, are produced by AS as a result of the in-frame deletion of exons 3 and 5, respectively. Exon 3 localizes at the N-terminal of the protein and codes for amino acid residues 41−54, whereas exon 5

is located at the C-terminal domain of the protein, coding for residues 103−130. The whole transcript of human α-synuclein, a protein composed of 140 amino acid residues, can be divided into three regions:

(1) Residues 1−60 form the N-terminal region. This region includes the sites of three familial PD mutations and contains four 11-amino acid imperfect repeats with a highly conservative hexameric motif (KTKEGV). The N-terminal region is predicted to form amphipathic α-helices, typical of the lipid-binding domain of apolipoproteins.[330,332]

(2) Residues 61−95 constitute the central region and comprise the highly amyloidogenic NAC sequence (NAC stays for Non-Aβ Component of Alzheimer's disease amyloid).[312,333] NAC contains three additional KTKEGV repeats, and represents a second major intrinsic constituent of Alzheimer's plaques, amounting to about 10% of these inclusions.[312] An 11-amino-acid segment within the central part of the NAC domain (corresponding to residues 73−83 of α-synuclein) is missing in β-synuclein.

(3) The highly charged C-terminal region consists of residues 96−140. This part of α-synuclein is highly enriched in acidic residues and prolines, suggesting that it adopts a disordered conformation. Three highly conserved tyrosine residues, which are considered a family signature of α- and β-synucleins, are located in this region. This region is mostly missing in γ-synuclein.

α-Synuclein exhibits a 40% similarity with members of the 14-3-3 protein family.[334] However, there are some contradictions on exact location of the sites which are probably homologous in the structure of 14-3-3 and synuclein.[334,335] The 14-3-3 proteins constitute a family of protein chaperones that are particularly abundant in the brain. The 14-3-3 family of proteins consists of seven different isoforms that share extensive sequence homology, both among the different isoforms and between similar isoforms in different species.[336,337] 14-3-3 proteins appear to be involved in diverse cellular functions, mostly via the regulation of protein kinases.[338,339] They often bind to ligands at sites containing phospho-serine residues. The binding of 14-3-3 to phosphorylated Raf-1 stabilizes it in an active conformation.[340] 14-3-3 binds to a phosphorylated epitope of protein kinase Cε (PKCε) and stabilizes PKCε in an inactive conformation that is unable to translocate to the membrane.[341] In general, interaction of 14-3-3 with protein kinase C strongly depends on the nature of protein kinase C isoform and can be accompanied either by inhibition or by activation of different isoforms of protein kinase C.[342−344]

14-3-3 also binds to phosphorylated death agonist BAD, a very distant BCL2 family member and a pro-apoptotic oncogene that remains inactive when sequestered in the cytosol. The interaction of 14-3-3 with BAD was shown to stabilize maintenance of BAD in a cytoplasmic localization.[345] However, 14-3-3 interacts with phosphorylated target proteins predominantly, but not exclusively. There are many examples of 14-3-3 binding to nonphosphorylated proteins.

The deposition of α-synuclein has been implicated in the pathogenesis of several neurodegenerative disorders, known as synucleinopathies. Synucleinopathies share common pathologic proteinaceous lesions, which are composed of aggregated α-synuclein and are deposited in selectively vulnerable populations of neurons and glia.[346−349] The term synucleinopathies was introduced in 1998 (i.e., just one year after the discovery of α-synuclein deposition in PD), when it was recognized that filamentous α-synuclein deposits might represent a common hallmark linking MSA with PD and DLB.[350] In addition to these three diseases, the current list of synucleinopathies includes (but is not limited to) neurodegeneration with brain iron accumulation, type I (also known as adult neuroaxonal dystrophy or Hallervorden-Spatz diseases, HSD); pure autonomic failure and several Lewy body disorders; diffuse Lewy body disease (DLBD); and the Lewy body variant of Alzheimer's disease (LBVAD).[350−360] Furthermore, even before α-synuclein was determined to be the major LB component in PD, the peptide derived from the central hydrophobic region of this protein (residues 61−95), known as NAC, was found to represent a second major intrinsic constituent of AD senile plaques.[312,333] Intriguingly, subsequent work failed to confirm the presence of NAC in amyloid plaques.[361] Growing evidence associates the onset and progression of clinical symptoms, as well as the degeneration of affected brain regions in these neurodegenerative disorders, with the formation of abnormal filamentous aggregates containing α-synuclein. Therefore, it has been concluded that all aforementioned disorders are brain amyloidoses unified by pathological intracellular inclusions of aggregates which have the α-synuclein protein as a key component.[346−350,352,360,362] It is believed that understanding why α-synuclein pathology develops in these apparently unrelated conditions may shed light on the mechanisms operating in different synucleinopathies.[363] Recently, it has been shown that α-synuclein was highly expressed in both malignant and benign melanocytic lesions, such as melanomas and nevi, whereas the protein was undetectable in the nonmelanoma cancer cell lines tested.[364] This is in agreement with recent epidemiological studies which have reported co-occurrence of melanoma and PD, suggesting that these two diseases could share common genetic components.[365−367]

It has recently been established that, in addition to the traditional α-synuclein-containing Lewy bodies (LBs) and Lewy neurites (LNs), the development of PD and DLB is accompanied by appearance of novel α-, β-, and γ-synuclein-positive lesions at the axon terminals of the hippocampus.[368] These pathological, vesicular-like lesions located at the presynaptic axon terminals in the hippocampal dentate, hilar, and CA2/3 regions have been co-stained by antibodies to α- and β-synucleins, whereas antibodies to γ-synuclein detect previously unrecognized axonal spheroid-like inclusions in the hippocampal dentate molecular layer.[368] This broadens the concept of neurodegenerative "synucleinopathies" by implicating β- and γ-synucleins, in addition to α-synuclein, in the onset/progression of these two diseases. Additionally, the abnormal expression of γ-synuclein has recently been reported in some breast tumors.[318] Using Northern blots and *in situ* hybridization, it has been shown that a high percentage of malignant breast tumors, but not benign breast tumors or normal breast tissue, express γ-synuclein mRNA.[318] In addition, a direct link between γ-synuclein overexpression and the increased invasiveness of breast tumor cells has been demonstrated.[369]

Human β-synuclein is a 134-aa neuronal protein that shows 78% identity to α-synuclein. The α- and β-synucleins share a conserved C-terminus with three identically placed tyrosine residues. However, β-synuclein is missing 11 residues within the specific NAC region.[330,357] β-synuclein was shown to be phosphorylated *in vitro* and *in vivo*, and was therefore designated as phosphoneuroprotein 14 (PNP 14).[315] This protein, like α-synuclein, is expressed predominantly in the brain; however, compared to α-synuclein, β-synuclein is

distributed more uniformly throughout the brain.[370,371] β-Synuclein was found not only in the central nervous system, but also in Sertoli cells of the testis,[372,373] whereas α-synuclein was found in platelets.[374]

The third member of the human synuclein family is the 127-aa γ-synuclein, which shares 60% similarity with α-synuclein at the amino acid sequence level.[330,357] This protein specifically lacks the tyrosine rich C-terminal signature of α- and β-synucleins.[330] γ-Synuclein is abundant in the spinal cord and sensory ganglia.[319,320] Interestingly, this protein is more widely distributed within the neuronal cytoplasm than α- and β-synucleins, as it is present throughout the cell body and axons.[319] It has also been found in metastatic breast cancer tissue[318] and epidermis.[369]

Despite the facts that α-synuclein is estimated to account for as much as 1% of the total protein in soluble cytosolic brain fractions[375] and that it is assumed to play a crucial role in the pathogenesis of several neurodegenerative disorders, the precise function of this protein remains mainly elusive. α-Synuclein is expressed in a number of neuronal and non-neuronal cell types including cortical neurons, dopaminergic neurons, noradrenergic neurons, endothelial cells, and platelets.[374,376−378] Interestingly, torpedo synuclein was reported to localize within the nucleus and presynaptic nerve terminals;[313] however, most subsequent studies have shown α-synuclein localization only within nerve terminals in the central nervous system.[311,330,379] Although the precise function of α-synuclein remains unknown, this localization, in addition to the close association of this protein with vesicular structures, has led to the hypothesis that it may regulate vesicular release and/or turnover and synaptic function in the central nervous system.[311,312,330,379,380] In agreement with this hypothesis, mice lacking α-synuclein, although superficially normal, exhibited alterations in transmitter release from dopaminergic terminals in striatum following paired electrical stimulation and in locomotor responses after amphetamine administration.[376] Additional observations suggest that α-synuclein may play a role in neuronal plasticity responses, because its avian homologue, synelphin, is up-regulated in zebra finch brains at a critical period of song learning,[332] and rat synuclein-1 is up-regulated during brain development[381,382] and in cultured neonatal sympathetic neurons after nerve growth factor treatment.[383] α-Synuclein has been shown to act as a high affinity inhibitor of phospholipase D2, which hydrolyzes phosphatidylcholine to phosphatidic acid and may be involved in vesicle trafficking in the secretory pathway.[384,385] Overall, the functions ascribed to α-synuclein include binding fatty acids and the physiological regulation of certain enzymes, transporters, and neurotransmitter vesicles, as well as roles in neuronal survival.[386]

It has been shown that α-synuclein can act as a molecular chaperone,[387] as its cellular levels in both substantia nigra and frontal cortex have been shown to be significantly increased as a response to toxic insult.[118] These toxicant-induced changes in the expression of α-synuclein have been characterized by a very peculiar time course, where levels of the protein in the mice brain were consistently enhanced at 2 days after paraquat administrations and returned to basal control values within 7 days post-treatment.[118] A similar time course of α-synuclein up-regulation has been also reported for mice treated with the parkinsonism-inducing neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).[388] These findings suggested that α-synuclein plays a role in overcoming the consequences of toxic insult. Furthermore, the overexpression of α-synuclein has been shown to actually delay cell death caused by toxic agents and protect cells against apoptotic stimuli.[389,390]

Methionine oxidation has been proposed to play a role in α-synuclein's potential function as a chaperone. In fact, since the addition of methionine-oxidized α-synuclein inhibited the fibrillation of the nonoxidized form, it has been suggested that the methionine residues in α-synuclein may be used by the cells as a natural scavenger of reactive oxygen species, and that, therefore, α-synuclein can serve as a redox chaperone.[391] This hypothesis is based on the facts that (i) methionine can react with essentially all of the known oxidants found in normal and pathological tissues; (ii) α-synuclein is a very abundant brain protein; (iii) the concentration of α-synuclein can increase significantly as a result of neuronal response to toxic insult;[118] and (iv) methionine sulfoxide residues in proteins can be cycled back to their native methionines by methionine sulfoxide reductase,[392] a process that might protect other functionally essential residues from oxidative damage.[393] However, the antifibrillation role of the methionine-oxidized α-synuclein is strongly compromised in the presence of certain heavy metals, such as lead, aluminum, zinc, titanium, and others.[394] Therefore, in the presence of enhanced concentrations of such industrial pollutants, toxic insult-induced up-regulation of α-synuclein may no longer play a protective role; rather, it may represent a risk factor, leading to metal-triggered fibrillation of the methionine-oxidized protein.[394]

Further support for chaperone activity of α-synuclein comes from the recent analysis of the presynaptic SNARE-complex assembly disassembly cycles, which are related to the repeated release of neurotransmitters by nerve terminal. These cycles were shown to be regulated via the nonclassical chaperone activity of synuclein, where α-synuclein directly binds to the SNARE-protein synaptobrevin-2/VAMP2 and promotes SNARE-complex assembly.[395] Finally, α-synuclein was shown to be protective against neurodegeneration caused by deletion of the mice presynaptic Hsc70 co-chaperone, cysteine-string protein-alpha.[396] All this shows that, depending on the conditions, α-synuclein can be protective or pathogenic, possessing both chaperone and anti-chaperone activities.[396]

Similarly, both β- and γ-synucleins, being much less amyloidogenic than α-synuclein, and being able to effectively inhibit α-synuclein fibrillation *in vitro*, can serve as molecular chaperones.[322] Furthermore, β-synuclein ameliorates α-synuclein neuropathology in mice.[397] On the other hand, β- and γ-synucleins can also act as anti-chaperones, being involved in various pathologies. In fact, these two proteins were found in specific lesions at the axon terminals of the hippocampus of some PD and DLB patients.[368] γ-Synuclein was found in metastatic breast cancer,[318] whereas missense mutations in β-synuclein were found in familial and sporadic forms of DLBD.[396] These observations suggested that synuclein family members might possess opposite functional properties, serving as normal chaperones or anti-chaperones.[396] This condition-dependent dual personality of synucleins was proposed to serve as a driving force for adaptive synaptic evolution, where the anti-chaperone synucleins initiate stress-induced responses, whereas the chaperone synucleins regulate these responses resulting in generation of nonlethal phenotypic variations in synapses.[396] Any misregulation of this chaperone and anti-chaperone duality

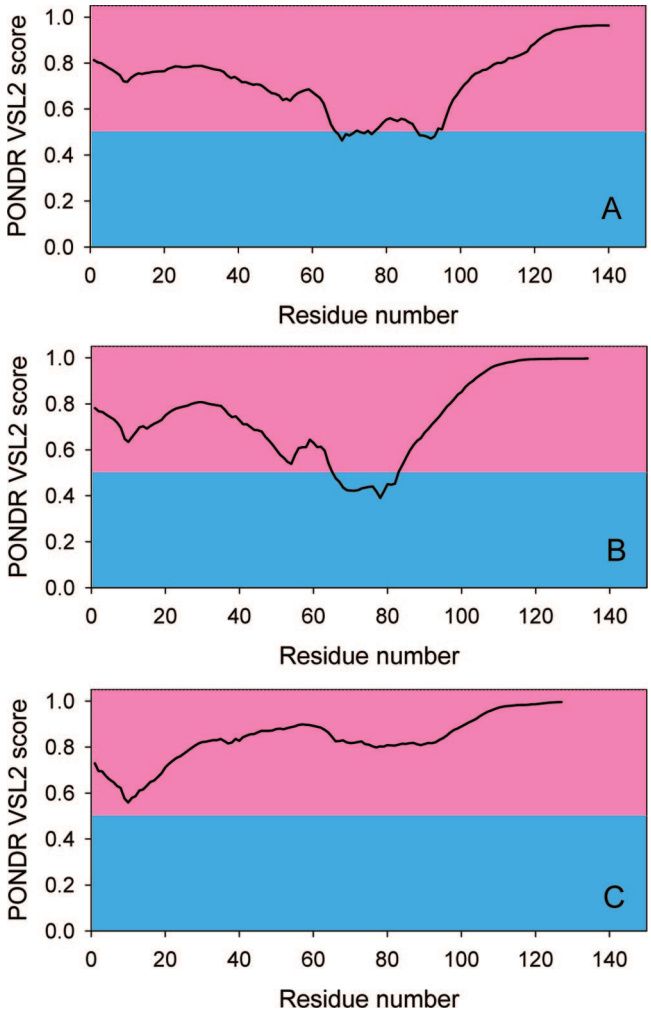

Figure 9. Intrinsic disorder in human α- (A), β- (B), and γ-synucleins (C) as evaluated by PONDR VSL2.

(e.g., via mutations or environmental risk factors) may lead to the imperfect adaptability against stresses, resulting in the development of neurodegenerative disorders.[396]

Comprehensive structural analysis of human α-, β-, and γ-synucleins revealed that these three proteins are almost completely unfolded under the physiological conditions *in vitro*.[322] Figure 9 supports this experimental data-based conclusion and shows that all three human synucleins are predicted to be completely disordered.

2.5. Prefoldin

Although the major roles ascribed to molecular chaperones are selective recognition and binding to the exposed hydrophobic surfaces of non-native proteins for the prevention of their aggregation, and for the subsequent facilitation of the correct folding of these non-native proteins *in vivo*, several chaperones are also known to be involved in the formation of toxic oligomeric species. One such promoter of toxic oligomer formation is the chaperone prefoldin (PFD).[398] PFD plays a general role in *de novo* protein folding in archaea, and is known to assist in the biogenesis of actins, tubulins, and several other proteins in the cytosol of eukaryotes.[79] Eukaryotic and archaeal PFDs are characterized by a similar jellyfish-like structure consisting of a double β-barrel assembly and a body with six long and protruding coiled coils resembling "tentacles" (see Figure 10A).[399,400] Eukaryotic PFD is a heterohexameric protein assembled from six different subunits, two of which are α-like subunits (PFD3 and PFD5) located in the center of the structure and four of which are β-like subunits (PFD1, PFD2, PFD4, and PFD6) located at the periphery.[401] In human PFD, each subunit contains central β-hairpin(s) flanked N- and C-terminally by coiled-coil helices. Structurally, coiled-coils consist of two or more parallel or antiparallel amphipathic α-helices that twist around one another to form supercoils.[402] There is no doubt that regions corresponding to the *intermolecular* coiled-coils are intrinsically disordered since they are typically highly disordered in their monomeric states but gain helical structure upon coiled-coil formation. Therefore, despite the fact that these intermolecular coiled-coils are ordered under physiological conditions, they are typical IDRs which represent a convincing illustration of the folding upon binding concept. Situation with intramolecular coiled-coils is less obvious since regions involved in the formation of such coiled-coils are located inside a single chain. However, even these intramolecular coiled-coil regions can be considered as IDRs since their helical structure is stabilized through an intramolecular association. Furthermore, since coiled-coil regions possess highly peculiar sequence properties, such as low complexity and the presence of a heptad repeat (*abcdefg*), where apolar residues are found preferentially in the first (*a*) and fourth (*d*) positions,[402] they are reliably predicted as IDRs by various disorder predictors. In agreement with described above structural organization, Figure 10B shows that the N- and C-termini of all human PFD subunits are predicted to be disordered. Therefore, intrinsic disorder most likely plays a crucial role in the formation of the PFD "tentacles".

Normally, eukaryotic PFD utilizes its "tentacles" to bind to substrate proteins that exist in an unfolded state, and transfer these unfolded substrates to cytosolic chaperonin-containing TCP-1 (CCT, also known as c-cpn or TriC) for functional folding.[401,403,404] Unfolded substrates are located along the interior of the PFD cavity, bound to the distal ends of its tentacles.[400] The number of PFD coiled coils involved in the interaction with the non-native substrate increases depending on the size of the unfolded polypeptide.

In a recent study, recombinant *Pyrococcus* PFD, which possesses high sequence identity to human PFD, was shown to produce high-molecular-mass (50−250 kDa) soluble Aβ oligomers.[398] These PFD-mediated soluble Aβ oligomers were more toxic than Aβ fibrils, and were capable of inducing apoptosis, suggesting that human PFD may be involved in the formation of toxic soluble Aβ oligomers in the cytosolic compartment *in vivo* and, therefore, can contribute to AD pathogenesis.[398]

3. The Action Mechanisms of Intrinsically Disordered Chaperones

On the basis of their molecular mechanism of action, chaperones have been divided into several functional subclasses: the "(un)folding" chaperones that utilize ATP-dependent conformational changes to promote the unfolding and subsequent refolding of their substrates; the "holding" chaperones that hold partially folded substrate in the folding-competent state, preventing their aggregation while waiting for the available "folding" chaperones; the "disaggregating" chaperones that are responsible for the solubilization of aggregated proteins; the "foldases" that catalyze the folding process by directly accelerating protein folding rate-limiting steps; and the "redox" chaperones that help prevent the

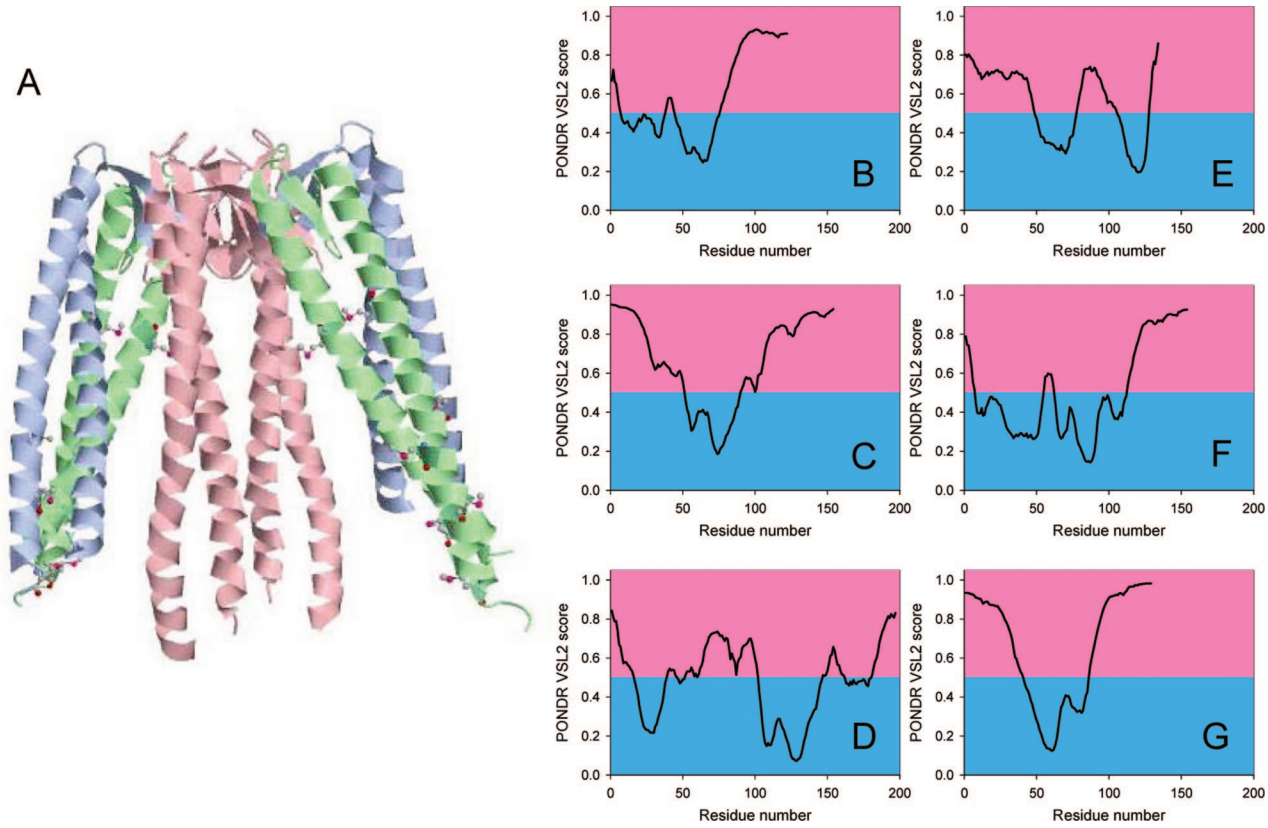

Figure 10. (A) Crystal structure of prefoldin from the archaeum *Methanobacterium thermoautotrophicum*, which possesses high identity to human PFD (PDB ID: 1FXK). Intrinsic disorder in subunits of human prefoldin: PFD1 (B), PFD2 (C), PFD3 (D), PFD4 (E), PFD5 (F), and PFD6 (G) as evaluated by PONDR VSL2.

consequences of oxidative damage. Illustrative members of all these chaperone classes were discussed in this review to show that all of them clearly belong to the family of intrinsically disordered proteins, as they are either completely disordered or contain functionally important long disordered regions. Some potential modes of involvement of intrinsic disorder in the function of some of these proteins are briefly considered below.

3.1. Intrinsically Disordered Holding Chaperones

Many proteins in their misfolded or partially (un)folded conformation(s) are sticky and, therefore, possess an increased propensity to aggregate. The efficiency of the aggregation process depends dramatically on protein concentration. Therefore, the major task of holding chaperones or holdases is to bind misfolded or partially (un)folded substrates, to decrease the pool of free molecules available for nonproductive and potentially toxic aggregation, and to hold misfolded/aggregated proteins until they can be disaggregated and refolded by the ATP-dependent chaperones. As mentioned above, sHsps, which normally exist as polydisperse oligomers that change size and organization upon exposure to stress and interaction with substrate,[405−409] serve as holding chaperones. Three possible modes of chaperone action have been suggested for sHsps:

(i) The large oligomeric form remains intact as it binds substrate on its surface;[410]

(ii) The large oligomer breaks down into smaller subunits (monomers, dimers or larger assemblies), which exposes hydrophobic surfaces, enabling the binding of unfolded substrate, and subsequently reassembles into large soluble complexes[292] that are then handled by ATP-dependent refolding machinery;[292,293,411] and

(iii) sHsp molecules are intercalated into large insoluble protein aggregates, which enables subsequent disaggregation and refolding by the ATP-dependent refolding machinery.[301,412−414]

It is also possible that all three mechanisms are realized in nature, and that the holding chaperones use different approaches to work with different targets. However, intrinsic disorder is crucial for holding chaperones regardless of their mechanism of action. Model substrates protected *in vitro*, as well as proteins found associated with sHsps *in vivo*, include proteins of a wide range of molecular weights, p*I* values, and structures, with no obvious common characteristics.[412,415] Both this astonishing promiscuity of sHsps and their ability to interact with very different targets "frozen" at different folding stages rely on the flexibility of the chaperones' binding sites. In fact, the intrinsically disordered nature of these binding sites helps them accommodate different substrate proteins via adopting structurally diverse binding platforms.[309] The fact that the surface of a holding chaperone possesses multiple substrate-binding sites explains the peculiar interaction mechanism where sHsps bind coil and secondary structural elements by wrapping them around the α-crystallin domain.[293] Although wrapping of target proteins around α-crystallin domain was revealed for an sHsp from the parasitic flatworm *Taenia saginata* Tsp36, this interaction mode is only one of many hypotheses on the potential mechanism of interaction of small heat shock proteins with their targets. The generality of this model has not been yet proved experimentally.

Furthermore, the intrinsically disordered N- and C-terminal arms of sHsps play a number of important roles in sHsp structure and function. They regulate the oligomeric state

of these chaperones, stabilize their quaternary structure, regulate chaperone activity, and enhance the solubility of the protein/substrate complex.[288] For example, the evolutionarily variable N-terminal arms of sHsp subunits, which are often unresolved in the crystal structures of sHsps[291,416] and are disordered in cryo-EM images of α-crystallins,[417] are essential for interaction with substrates, and sHsps with a naturally short or truncated (by mutagenesis) N-terminal arm most often lack chaperone function.[418−421] Similarly, the C-terminal arms of sHsps, which protrude from the domain core of the molecule, are flexible and solvent-exposed.[422,423] These highly flexible C-terminal extensions represent a conserved feature of mammalian sHsps.[424] Although they share no general sequence similarity, the C-terminal arms of mammalian sHsps have several common characteristics: polarity, a lack of ordered structure, conformational flexibility, and presence of conservative hydrophobic sequence I-X-I/V which probably plays important role in sHsp oligomerization.[291] Importantly, although these arms are typically not involved in direct binding to the substrate, this rule is violated in several instances, for example, the C-terminal extension of αB-crystallin is involved in the interaction with growth factors and insulin.[425] However, they act as polar solubilizing agents for the relatively hydrophobic sHsp proteins and sHsp−substrate complexes.[426] If the polarity and flexibility of the extensions are disrupted, as in the hydrophobic mutant of αA-crystallin, the stability and chaperone activity of the protein are reduced significantly.[427] Because of the flexibility of the C-terminal arms of mammalian sHsps, their solubilizing function has been suggested to arise from an entropic contribution to the free energy of the solution state.[426] These extensions have also been proposed to serve as entropic "spacers", which prevent sHsp−substrate complexes from aggregating.[426] In other words, disordered segments may act via the entropic exclusion effect, a long-range repulsive force which prevents molecules from approaching each other.[67] Therefore, similar to the disordered regions of microtubule-associated proteins,[428] neurofilaments,[429] and nucleoporins,[430] the highly flexible extensions of holding chaperones work as entropic brushes that, due to thermally driven motion, maintain spacing between the chaperone oligomers.

3.2. Intrinsically Disordered (Un)Folding Chaperones

Kinetically trapped misfolded substrates are stuck in a local conformational energy minimum. Chaperones have been proposed to assist folding by randomly disrupting malformed bonds via repeated cycles of binding and release, allowing the substrate to resume its search in the conformational space toward the global energy minimum.[67] ATP-dependent molecular chaperones use free energy from ATP binding and/or hydrolysis to minimize the concentrations of misfolded and nonproductively aggregated proteins in the cell. One model of such chaperone action shows that they serve as unfoldases that use free energy from ATP binding and/or hydrolysis to unfold misfolded proteins to yield productive folding intermediates.[80,431−433] These re-established productive folding intermediates can fold spontaneously to the native state. In this mechanism, ATP-dependent chaperones (e.g., Hsp70 proteins) are able to lower the activation energy barrier for the transitions from the misfolded species to the productive intermediates without changing the microscopic rate constant for the folding reaction. Acting as unfoldases, ATP-dependent chaperones reverse the nonproductive reactions leading to the misfolded species, and therefore keep the polypeptide chain in a folding-competent state.[80] The unfoldase activity was studied in detail for several members of the Hsp70 family, such as DnaK,[80] the mitochondrial heat shock protein, mtHsp70, which was shown to unfold preproteins in an ATP-dependent mechanism before their import into mitochondria,[434] and GroEL.[435] The critical prerequisites for the ATP-driven chaperone to serve as an unfoldase mediating proper unfolding/refolding of many different proteins in the cell are:[436]

(i) The propensity to specifically bind misfolded substrates with a higher affinity than their natively refolded products;

(ii) The capability to recruit the energy of ATP hydrolysis to favor unfolding of the bound misfolded substrate; and

(iii) The ability to dissociate timely from the newly unfolded product, allowing the latter to refold spontaneously into a low-affinity chaperone product.

For GroEL and several other ATP-dependent chaperones, the proposed mechanism of action involves active unfolding of misfolded proteins-substrates. Importantly, to serve as an unfoldase in protein homeostasis, these ATP-dependent chaperones must collaborate with co-chaperones: GroEL works as an unfoldase in complex with GroES, whereas Hsp70s are known to work with a J-domain co-chaperone and a nucleotide exchange factor.

There are three potential mechanisms for the unfoldase activity of chaperones: the entropic pulling model, mechanical unfolding via forcible stretching, and the entropy transfer model. The peculiarities of these mechanisms are briefly outlined below.

In Hsp70s, which consist of an N-terminal ATPase domain and a C-terminal substrate-binding domain with a lid, the hydrolysis of ATP and ADP/ATP exchange in the nucleotide-binding domain control the functional properties of the substrate-binding domain by allosteric crosstalk. Hsp70 interacts transiently with short extended peptide segments of the substrate made of about seven nonbulky hydrophobic residues, ideally flanked by positive charges.[176] ATP-liganded Hsp70 exhibits low affinity for misfolded substrates, and fast rates of substrate binding and release.[437,438] In contrast, ADP·Hsp70 is characterized by a 100-fold higher affinity for substrate, and by a very slow rate of substrate release.[439,440] During the chaperone cycle, Hsp70s alternate between the low and the high affinity state, under the control of their co-chaperones.

The binding of Hsp70 to misfolded proteins accelerates chaperones' ATP hydrolysis by up to 2 orders of magnitude.[209,441] This substrate effect is further amplified by the J-domain-containing Hsp40 co-chaperones.[209] For example, DnaJ stimulates ATP hydrolysis in substrate-bound ATP·DnaK molecules and, thus, promotes the formation of considerably more stable [ADP·DnaK·substrate] complexes. These long-lived chaperone−substrate complexes then act as entropic pulling species: the dangling bound chaperone molecule actively unfolds the misfolded regions that flank the chaperone-binding sites in the substrate.[436,442,443] GrpE accelerates the release of ADP and rebinding of ATP, triggering the "unlocking" of DnaK from its substrate. The unlocked chaperone may dissociate from an unfolded peptide loop, which may spontaneously refold into a more native structure. The final result of this cycle is the productive transient unfolding of a stably misfolded polypeptide region.

GroEL binds non-native proteins by means of a ring of hydrophobic residues that line the entrance to the central cavity of its heptameric ring.[444,445] When GroEL binds ATP and the GroES co-chaperonin, massive structural changes double the GroEL cavity volume and occlude its hydrophobic binding surface.[446,447] In fact, before binding ATP and GroES, GroEL's binding sites are located 25 Å from each other, whereas upon the addition of ATP and GroES, the apical domain of each GroEL subunit twists upward and outward so that the binding sites move apart to a position 33 Å from one another. As a result, neighboring binding sites move apart by 8 Å and non-neighboring sites by larger increments, up to 20 Å. These large-scale movements provide the means for the mechanical unfolding of the misfolded substrate protein which, as it is tethered to these sites, will be forcibly stretched and partially unfolded.[448,449] This forceful unfolding of the misfolded substrate protein relieves its misfolding and once again allows normal folding. Incompletely folded proteins undergo further iterations until they achieve their native state.[435,449]

Similar to the holdases discussed above, the intrinsic disorder and flexibility of the binding sites of ATP-dependent unfoldases add a unique versatility to the recognition process, since such regions can bind several different partners, enabling an enhanced speed of interaction and uncoupling specificity from binding strength.[67] For example, the intrinsic disorder in the C-terminal tail of GroEL has been proposed to be necessary for the ability of this chaperone to bind a wide range of unrelated, misfolded substrates.[445]

Another potential role of intrinsic disorder in the unfoldase activity of chaperones is described by a so-called entropy transfer model:[67] the ability of disordered segments of chaperone to rapidly and transiently bind the substrate and subsequently create local order may "pay" the thermodynamic cost of local substrate unfolding. In other words, the entropy transfer model implies the ordering of the chaperone with a concomitant unfolding of the substrate. Here, the binding-induced folding of a disordered, nonspecific binding segment of a chaperone may promote the local unfolding of the misfolded segment, and the energy required for the local unfolding of the substrate may be covered by the binding and folding of the chaperone.[67]

3.3. Intrinsically Disordered Disaggregating Chaperones

Misfolded and partially folded proteins are often trapped in oligomeric/aggregated forms, since these nonproductive aggregated states are energetically favorable and, therefore, half-times for the return to folding-competent intermediates are typically very long. The stability of these aggregates of misfolded polypeptides is attributed to the formation of numerous small hydrophobic surfaces, which have an increased propensity to form β-sheet-enriched structures, which tend to cooperatively associate with one another to achieve less exposure to water, thus, maintaining the tight entanglement of misfolded polypeptides.[436] This highly cooperative nature of intermolecular interactions between the misfolded polypeptide chains restrains the local random molecular motions which, if allowed, would eventually release individual misfolded polypeptides from the aggregate and thus give them a chance to spontaneously refold to their native state. Since aggregated proteins are potentially toxic and the chance of their spontaneous clearance is very low, nature has elaborated a complex protective system, a network of chaperones, which can recognize misfolded proteins, prevent their aggregation, unfold misfolded regions, and disaggregate preformed aggregates. Both holding and unfolding chaperones can disaggregate nonproductive aggregates. In the cytoplasm of animal cells, unfolding and disaggregation may be achieved by Hsp70/Hsp40/NEF alone. In lower organisms, special and very powerful disaggregating machinery has evolved. For example, yeast heat-shock protein 104 (Hsp104), plant Hsp101, and their bacterial homologue caseinolytic peptidase B (ClpB) are molecular chaperones that have the ability to solubilize almost any protein that becomes aggregated after severe stress. Since these chaperones are not found in humans, they will not be considered here.

Unfoldases can disaggregate proteins using entropic pulling, mechanical unfolding via forced stretching, and the entropy transfer mechanism. For example, as discussed in a recent review,[436] the energy of ATP hydrolysis serves to "lock" Hsp70 onto a loop at the surface of stable protein aggregates. Multiple ADP-liganded Hsp70 molecules, tightly attached to loops of the same aggregated substrate polypeptide, cooperate in applying stretching forces by entropic pulling.[442] One or more Hsp70 molecules, locked to loops in substrate polypeptide chains, can recruit random Brownian motions to pull apart aggregated proteins and thus to distend the loop segments caught up in aggregates. This mechanism is more effective with several Hsp70s. The gain in entropy resulting from the increased motility of the Hsp70•loop complexes diffusing away from the aggregate may, thus, overcome the aggregate-stabilizing energy.[431,433] In the entropy transfer mechanism, the binding and folding of a disordered chaperone fragment can induce local unfolding and disaggregation of the misfolded segment from the tightly packed aggregate. Even "passive" holdases can cause disaggregation via the entropic exclusion effects of their disordered extensions. Here, the interaction of sHsp molecules with aggregated proteins may prevent and minimize intermolecular hydrophobic interactions among the aggregating polypeptides. Then, chaperone-bound polypeptides can be "brushed away" from the aggregated species due to the entropic brush activity of the highly flexible extensions of holding chaperones, which are in constant thermally driven motion that maintains the spacing between the chaperone oligomers.

3.4. Activation by Unfolding

Holdases provide high-affinity binding platforms for unfolded proteins and prevent protein aggregation specifically during stress conditions when they are activated. The major difference between holdases and foldases is the ATP independence of the holding chaperone action. This ATP independence raises several important questions related to the mechanisms of holdase activation and regulation. A set of interesting mechanisms of holding chaperone activation, collectively known as post-translational regulation of stress-related proteins, has evolved in bacteria. The essence of these mechanisms is in the immediate response of chaperone structure to changes in its environment under stress conditions such as elevated temperature, oxidative stress, or low pH. Although these chaperones do not have mammalian analogues, the unique mechanisms of their activation and the role of intrinsic disorder in the regulation of their activity clearly deserve some attention. Therefore, several illustrative examples of such post-translationally regulated chaperones

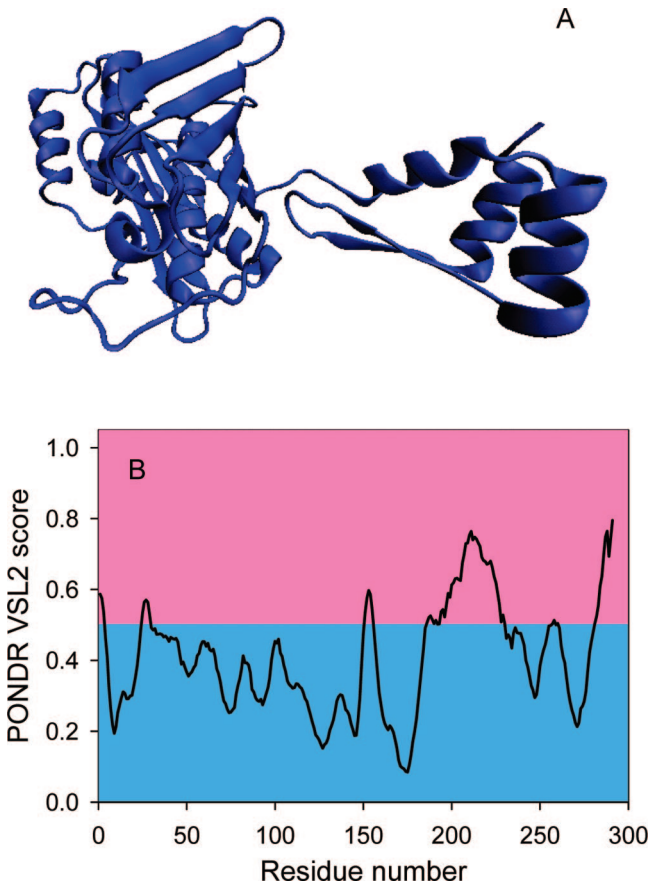

Figure 11. Crystal structure (A, PDB ID: 1VZY) and distribution of PONDR VSL2 predicted intrinsic disorder scores the sequence of the redox-regulated chaperone Hsp33 (B).

are considered below. These proteins belong to a class of stress-specific chaperones that are activated by the very stress conditions that they combat.[450] They have well-ordered crystallizable structure in the inactive form and are activated by stress-promoted partial unfolding.

3.4.1. Redox-Regulated Chaperone Hsp33

The redox-regulated chaperone Hsp33 is a well-conserved protein identified in the vast majority of prokaryotic species as well as in some unicellular eukaryotic parasites (e.g., *Trypanosomatidae*).[451] This protein is a highly specialized holdase with a unique activation mechanism that protects bacteria against oxidative stress conditions.[452] In fact, the activation of Hsp33 chaperone function requires the presence of reactive oxygen species such as H_2O_2 and hydroxyl radicals, as well as elevated temperatures.[453] Hsp33 senses oxidizing stress conditions via its C-terminal cysteine-rich redox switch domain, which consists of a zinc center that responds to the presence of oxidants and an adjacent metastable linker region, which responds to unfolding conditions. The zinc center contains four absolutely conserved cysteines arranged in a Cys^{232}-*X*-Cys^{234}—*X*—Cys^{265}-*X*-*X*-Cys^{268} motif. Under nonstress conditions, these four cysteines coordinate one Zn^{2+} ion, whose binding provides significant stability to the Hsp33 C-terminus.[454] The zinc binding domain connects to the Hsp33 N-terminal domain via a highly flexible ~52-amino acid linker region (see Figure 11A). In its inactivated form, the linker region is compactly folded and makes extensive contacts with a highly hydrophobic, four-stranded β-sheet platform of the Hsp33 N-terminal domain.[455] This tight binding of the linker region to the N-terminal domain keeps Hsp33 monomeric and functionally inactive.

One very peculiar feature of Hsp33 is that the activation of this protein requires two stimuli: the presence of a reactive oxygen species and elevated temperature. To deal with this, Hsp33 contains two interdependent stress-sensing regions located in the C-terminal redox-switch domain. These two stress-sensing regions are a zinc center, which senses peroxide stress conditions, and a linker region, which responds to unfolding conditions.[456] Since neither of these sensors works sufficiently in the absence of the other, the simultaneous presence of both stress conditions is absolutely necessary for Hsp33's full activation.[456] Under oxidative stress conditions at elevated temperatures, zinc is released and the Hsp33 chaperone function is activated via the formation of intramolecular disulfide bonds, which leads to the unfolding of the Hsp33 zinc binding domain and the linker region.[457] Therefore, upon activation, the redox-switch domain of Hsp33 adopts a natively unfolded conformation.[453] This large conformational rearrangement and partial unfolding leads to the dimerization of Hsp33 and results in the exposure of hydrophobic surface areas in the N-terminal substrate-binding domain, which can serve as binding sites for unfolded proteins.[456] In agreement with the proposed activation mechanism, where stress conditions induce the transformation of the redox-switch domain into the natively unfolded form, Figure 11B represents the distribution of predicted intrinsic disorder propensity within the sequence of *Bacillus subtilis* Hsp33 and clearly shows that the linker region is predicted to be disordered. As far as the Hsp33 zinc center is concerned, this fragment contains four cysteine residues (which are very strong order-promoting residues[1,45]) and therefore is predicted to be a mixture of short ordered and disordered segments (Figure 11B). On the other hand, this region is characterized by a relatively high net charge and low overall hydrophobicity and, therefore, is predicted to be natively unfolded by the charge-hydropathy plot analysis.[4] Taken together, these computational data suggest the C-terminal fragment of Hsp33 is mostly disordered and is held in the folded conformation, likely due to the specific coordination of a zinc ion.

3.4.2. Acid-Activated Chaperone HdeA

HdeA, one of the smallest known chaperones, has a molecular mass of only 9.7 kDa. Unlike most chaperones, HdeA functions as a monomer and is not known to require any energy factors or co-chaperones. This protein is expressed by many bacteria to combat acid-induced protein unfolding and aggregation in the periplasm. At neutral pH, HdeA exists as an inactive dimer. However, this protein specifically senses low pH conditions (pH < 3), where it partially unfolds and dissociates into chaperone-active monomers, and thus is activated by the same conditions that lead to the inactivation and aggregation of other proteins.[458] The mentioned acid-induced partial unfolding and monomerization of HdeA occur very rapidly ($k > 3.5\ s^{-1}$) and allow this chaperone to use its hydrophobic dimer interface for substrate binding.[458] Furthermore, due to its partially disordered conformation, the activated monomeric HdeA is able to adopt different conformations necessary for binding a broad range of substrate proteins. The combination of the mentioned above features results in a chaperone that is rapidly activated

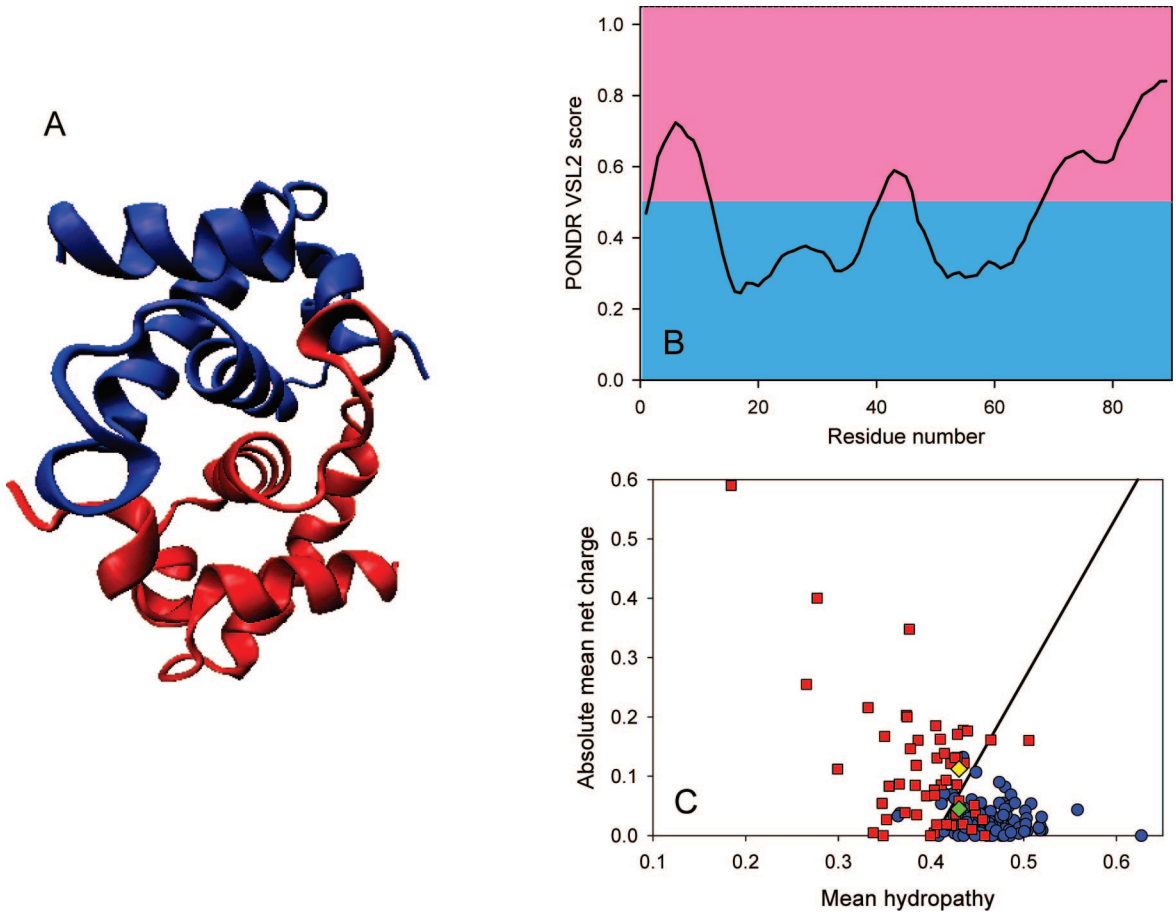

Figure 12. Analysis of structure and intrinsic disorder propensity in the acid-activated chaperone HdeA. (A) Crystal structure of HdeA (PDB ID: 1BG8); (B) intrinsic disorder in HdeA as evaluated by PONDR VSL2; (C) charge-hydropathy analysis of HdeA at neutral pH (green diamond) and at pH 3.0 (yellow diamond). The move of the spot to a position above the boundary is determined by the changes in the HdeA net charge from −4 at pH 7.5 to +10 at pH 3.0.

by acid stress and is able to efficiently suppress the aggregation of substrate proteins several times its own size.[458]

The crystal structure of the HdeA dimer is shown in Figure 12A. More than 2370 Å^2 of solvent-accessible surface area is buried at the formation of the dimer.[459] The HdeA monomer is an up-down-up-down helical bundle, consisting a short helix A (residues 18−22) and three longer helices, B (29−39), C (52−67), and D (74−83). Both termini of each molecule are disordered. The four helices are separated into two segments by the extended loop connecting helices B and C (40−51). The two segments are connected by a disulfide bond (Cys18−Cys66) located between helices A and C.[460] The dimer is stabilized mostly by hydrophobic interactions, which are formed by extensive interactions among helix B, linker BC, and the observed part of the N-terminus of each molecule. In the dimer interface, the helices B and B′ are almost parallel and unusually close to each other.[460] The observed part of the N-terminus of each monomer is likely to be stabilized by the interactions with the BC linker of its partner. Figure 12B represents the predicted disorder profile for HreA and clearly shows that both termini of the protein and its central part are predicted to be disordered. Charge-hydropathy analysis suggests that, at neutral pH, HdeA is an ordered protein, since a corresponding point is located slightly below the boundary separating ordered and natively unfolded proteins (Figure 12C). On the other hand, this protein is expected to be essentially unfolded at acidic pH (i.e., under conditions resulting in its activation), since the corresponding point in the charge-hydropathy plot moves well above the boundary, because the HdeA net charge changes from −4 at pH 7.5 to +10 at pH 3.0 (see Figure 12C). Therefore, the pH-induced conformational changes in HdeA are triggered by the "waking" of the intrinsically disordered nature of this protein.

3.4.3. Temperature-Regulated Chaperones Hsp26 and Hsp16.9

The last example of the post-translationally regulated chaperones are two small heat shock proteins, the *Saccharomyces cerevisiae* holdase Hsp26 and the wheat holdase Hsp16.9, which are both activated by heat stress conditions through heat-induced dissociation.[450] Under nonstress conditions *in vitro*, Hsp26 forms well-defined regular complexes of 24 subunits.[461] At heat shock temperatures, the Hsp26 oligomers disassemble into dimers, which possess noticeably higher chaperone activity than the 24-mers, bind the substrate proteins, and reassemble into higher oligomeric structures, which efficiently bind temperature-denatured substrates at elevated temperatures.[411]

In the Hsp26 monomer, four distinct domains are linked by flexible hinge regions: the N-terminus, middle domain, α-crystallin domain, and the C-terminal tail. The inactive Hsp26 particles are arranged as porous shells with tetrahedral symmetry.[462] In each particle, there are 24 subunits which form elongated, asymmetric dimers that are assembled into

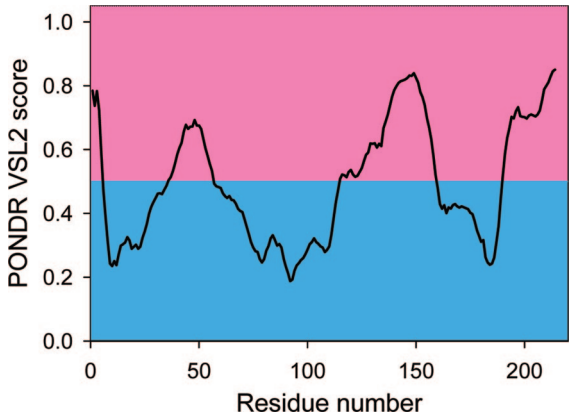

Figure 13. Intrinsic disorder in yeast Hsp26 as evaluated by PONDR VSL2.

24-mers by threefold contacts involving both termini as well as the globular middle domain.[462] Each of the 12 C-termini located in the outer shell form a contact with the globular domain of the adjacent dimer. The other 12 C-termini form the novel, trimeric insertions inside the particle.[462] Figure 13 represents the disorder profile for yeast Hsp26 and shows that all of the mentioned flexible linkers and the C-terminal tail are predicted to be disordered.

The Hsp16.9 holdase from wheat forms a dodecameric double disc with the dimensions 95 × 55 Å with a central hole 25 Å in width (see Figure 14A).[291] The dodecamer is flexible enough to allow for rapid subunit exchange with dimeric species.[450] At heat shock temperatures, the dodecamer-to-dimer equilibrium shifts toward the dimeric species and the Hsp16.9 complexes disassemble into stable dimers, which are the active species responsible for the high affinity binding of substrate proteins followed by reassembly into large oligomeric structures.[291] Each disc of the inactive Hsp16.9 holdase consists of six α-crystallin domains, which are organized as trimers of nonsymmetrical dimers. In both monomers of the dimeric building block, the α-crystallin domain and C-terminal extension are visible. However, the N-terminal arm in one monomer is fully resolved and composed of helices connected by random coil (see Figure 14B), whereas the 42 N-terminal residues in the other monomer are disordered. Figure 14C represents the Hsp16.9 dimer structure with the complete monomer in blue and the monomer with the disordered N-terminal arm in red. The completely resolved monomer is stabilized within the dodecamer at five different interfaces. The α-crystallin domain consists of a β-sandwich comprising two antiparallel β-sheets, followed by a short C-terminal extension and a long loop extending from the β-sandwich. The strongest contact in the dimer interface is made by the residues of this long loop, which is engaged in strand exchange with the partner monomer.[291] "The dimer−dimer interface is strengthened by two C-terminal extensions belonging to monomers with disordered N-terminal arms that strap across their partner dimer in the other disk. In contrast, two C-terminal extensions from monomers with ordered N-terminal arms reach out to adjacent tetramers but in this case interact with dimers in the same disk, contributing to higher order assembly... The dodecamer is further stabilized by pairwise intertwining of the six ordered N-terminal arms. Folding of a pair of N-terminal arms involves an interaction between a monomer from the top disk and a monomer in the bottom disk forming a shared helical domain located on the inside of the dodecamer."[291] As follows from this description, Hdp16.9 monomers are highly intertwined inside the dodecamer.

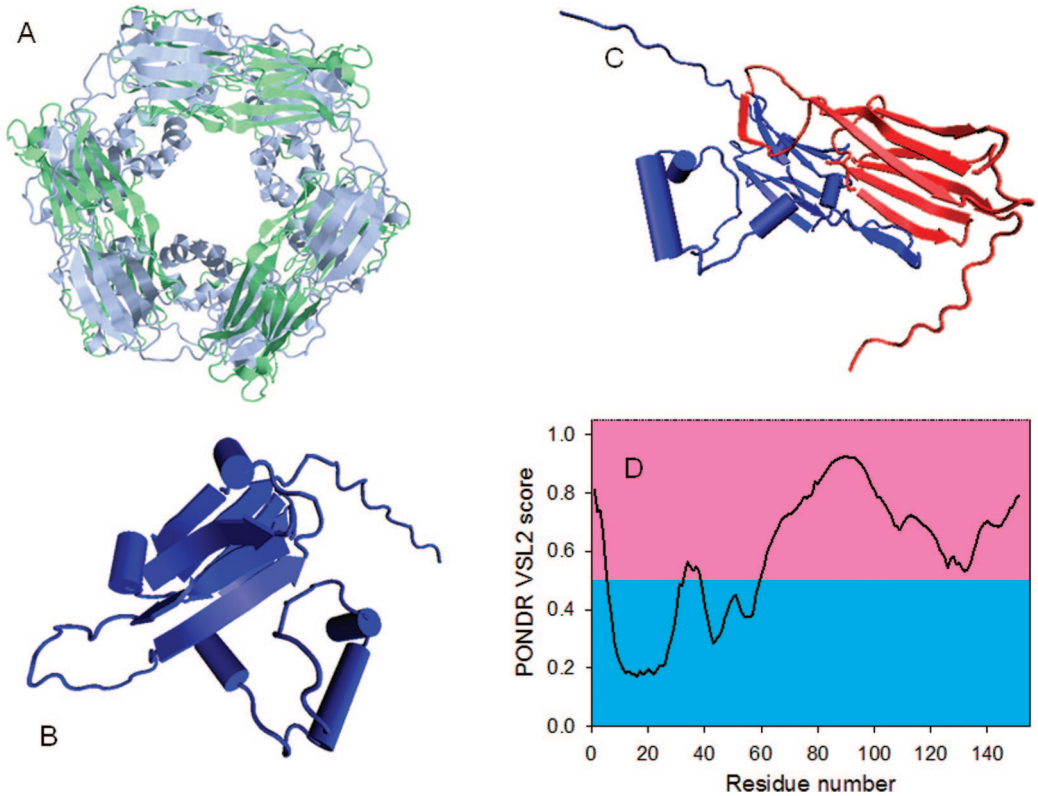

Figure 14. Structure and intrinsic disorder of the Hsp16.9 holdase from wheat. (A) Crystal structure of the dodecameric Hsp16.9 particle (PDB ID: 1GME). (B) Crystal structure of the completely resolved monomer of Hsp16.9 (PDB ID: 1GME). (C) Crystal structure of the Hsp16.9 dimer (PDB ID: 1GME). (D) Intrinsic disorder propensity of the wheat Hsp16.9 holdase as evaluated by PONDR VSL2.

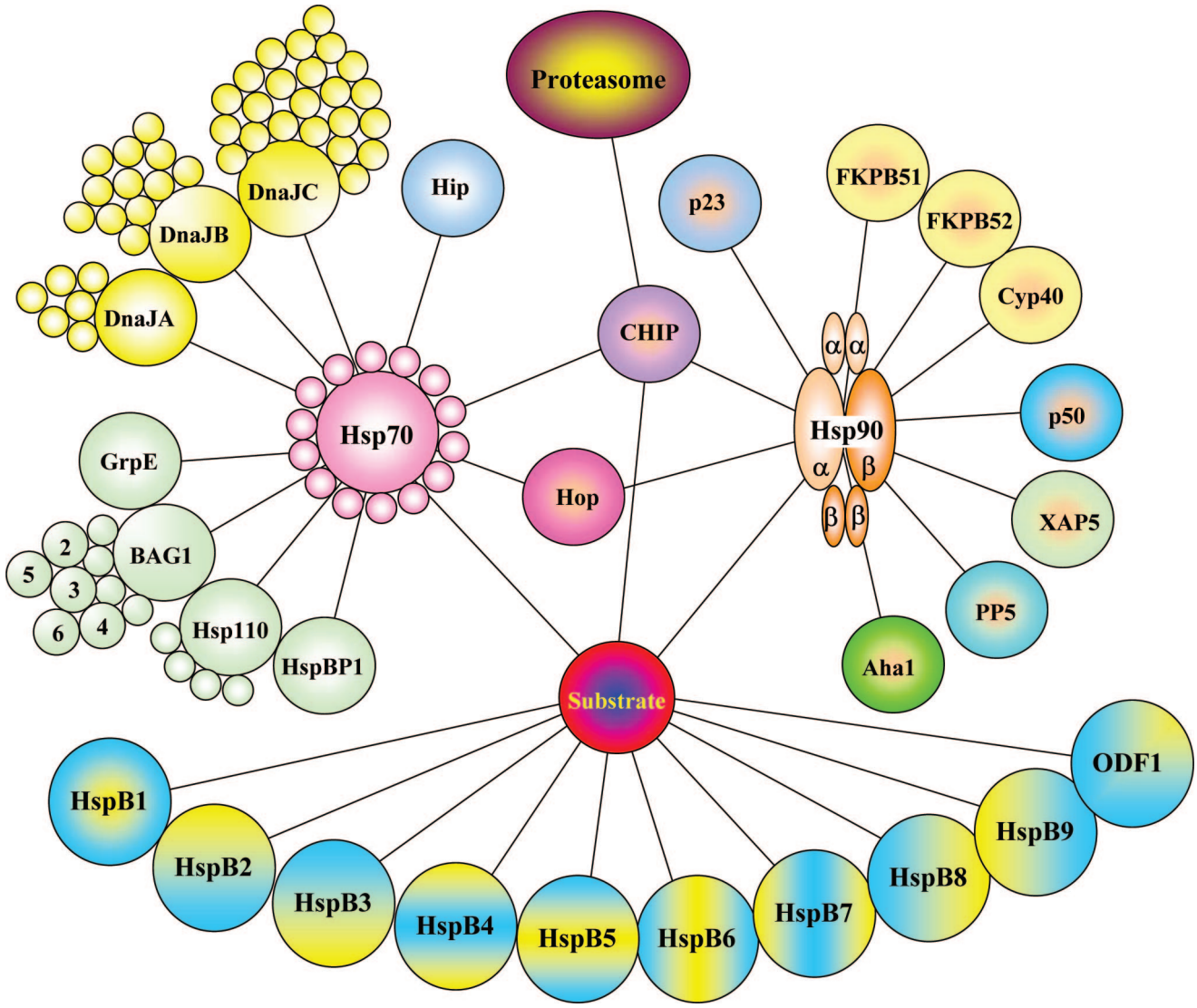

Figure 15. Flexible net of malleable guardians. An oversimplified representation of the chaperone network. Only the first shell of interactors is shown. See text for the details.

Furthermore, both N- and C-terminal fragments have almost no intermolecular contacts, as they are heavily involved in intermolecular interactions and therefore represent an illustrative example of the folding upon binding scenario for oligomer formation. In agreement with this hypothesis, Figure 14D shows that the Hsp16.9 chaperone is expected to be mostly disordered.

4. Flexible Net of Malleable Guardians

The ability of a cell to sustain and recover after various stresses depends on a well-developed and complex network of protein chaperones and co-chaperones. The unique interplay between various members of this network and the multiple roles of intrinsic disorder in the regulation of this network have already been discussed above. Figure 15 shows an oversimplified schematic representation of the human chaperone network. In reality, the network is dramatically more complex. In fact, human cells express at least 14 different Hsp70s (shown in Figure 15 as small light pink spheres around a large pink sphere marked Hsp70).[173] It was already mentioned that neither Hsp70 nor Hsp90 acts alone, and that their activity is regulated by numerous co-chaperones. In the Hsp70 corner, there are at least 41 different J-proteins (also known as Hsp40s, shown in Figure 15 as yellow spheres).[177] Although only three major subfamilies of Hsp40s are represented in Figure 15 (DnaJA, DnaJB, and DnaJC), each subfamily has multiple members. There are 6, 12, and 23 members in the DnaJA, DnaJB, and DnaJC subfamilies, respectively.[177] These are shown as small yellow spheres stacked around the corresponding large yellow sphere. HEF co-chaperones are also numerous (shown as light green spheres). The Hsp110 family has at least four members (small light green spheres around the large sphere marker Hsp110).[223] There are at least 4 isoforms of BAG-1 (shown as small light green spheres around the large sphere marker BAG-1).[232] Furthermore, human cells contain several BAG-1-related proteins: BAG-2, BAG-3, BAG-4, BAG-5, and BAG-6 (shown as numbered medium-size light green sphere).[229] To imaging the complexity of the Hsp70-related network, one could remember that an individual Hsp70 molecule can only interact with a single representative of each major co-chaperone class. Therefore, the human cell contains tens of thousands of different chaperone−co-chaperone complexes.

In Figure 15, Hsp90 is shown as an elongated dimer, which, in higher eukaryotes, exists either as $\alpha-\beta$ het-

erodimers, or as α−α or β−β homodimers. Figure 15 also represents some of the Hsp90 specific co-chaperones, co-chaperone p23, Cdc37/p50, FKPB51, FKPB52, Cyp40, XAP5, PP5, and Aha1.[189] Some co-chaperones, such as Hop and CHIP, are able to interact with both Hsp70 and Hsp90. CHIP also links both Hsp70 and Hsp90 machinery with the proteasome. Figure 15 shows that the Hsp90 chaperone system is a complex and unique machinery, which acts in close collaboration with Hsp70 and is controlled by the large number of cofactors.

An additional complexity level of the Hsp70−Hsp90 network is determined by the fact that, although co-chaperones have specific domains for interaction with their corresponding chaperones, all of them also contain multiple additional domains with various functionalities. These extra domains are not directly related to the formation of chaperone−co-chaperone complexes, but are utilized in interactions with other partner proteins and in targeting co-chaperones to diverse subcellular compartments. Finally, there are at least 10 different sHsps in humans: Hsp27/HspB1, HspB2, HspB3, αA-crystallin/HspB4, αB-crystallin/HspB5, Hsp20/HspB6, cvHsp/HspB7, H11/HspB8, HspB9, and ODF1.[295]

The data considered in this review clearly indicated that intrinsic disorder plays a number of crucial roles in the functioning of the chaperone network, since the formation of numerous chaperone complexes is often helped (if not driven) by long disordered regions found in chaperones or co-chaperones. Therefore, these complex networks of interactions helping cells recover after stress can be defined as a flexible net of malleable guardians.

5. Concluding Remarks

It is difficult to overestimate the role of protein chaperones in protein homeostasis. These guardians of the cell are intimately involved at all the stages of the protein's life, starting from its birth (chaperones are awaiting the newly synthesized polypeptides exiting the ribosome and help them avoid misfolding and aggregation before spontaneous folding into a native structure), through its maturation and productive adulthood (chaperones help in the assembly of multichain complexes and in protein translocation through membranes), in the norm and in stress (many types of stress and toxic insult can induce the transient unfolding of proteins, which then can misfold and aggregate, unless prevented by chaperones), until cell death (chaperones are involved in various proteins' clearance mechanisms). Chaperones help proteins fold, prevent them from misfolding and aggregating, and work with misfolded and aggregated species to promote their disaggregation, productive folding, or degradation. Obviously, the failure of this protective system results in the appearance of proteotoxic species which will eventually induce a strong inflammatory response, apoptosis, and tissue loss.[463]

As shown in this review, intrinsic disorder plays a number of important roles in the action of protein chaperones. Intrinsically disordered regions determine the promiscuity of chaperones, acting as pliable molecular recognition elements. They help wrap misfolded chains and participate in the disaggregation and local unfolding of aggregated and misfolded species, increasing the solubility and foldability of proteins. The abundance of intrinsically disordered regions and the versatility of their functions are crucial for the success of protein chaperones.

6. List of Abbreviations

3D	three-dimensional
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
ATF6	activating transcription factor-6
Bag-1	Bcl2-associated athanogene 1
BCSG1	breast cancer-specific gene 1
CCT	cytosolic chaperonin-containing TCP-1
CD	circular dichroism
CHIP	C-terminus of Hsc70-interacting protein
CJD	Creutzfeldt-Jakob disease
CypA	cyclophilin A
ClpB	caseinolytic peptidase B
CypB	cyclophilin B
DLB	dementia with Lewy bodies
DLBD	diffuse Lewy body disease
ER	endoplasmic reticulum
FKBP	FK506/rapamycin-binding protein
FTIR	Fourier transform infrared
GR	glucocorticoid receptor
GSS	Gerstmann-Sträussler-Scheinker disease
HCV	hepatitis C virus
HD	Huntington disease
Hop	Hsp-organizing protein
HSD	Hallervorden-Spatz disease
HSF1	heat shock factor
Hscps	heat shock cognate proteins
Hsp	heat shock protein
IDP	intrinsically disordered protein
IDR	intrinsically disordered region
iNOS	inducible nitric oxide synthase
IRE1	inositol-requiring protein-1
LB	Lewy body
LBVAD	Lewy body variant of Alzheimer's disease
LN	Lewy neurite
MeV	measles virus
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSA	multiple system atrophy
NAC	non-Aβ component of Alzheimer's disease amyloid
NACP	nonamyloid component precursor protein
NBD	nucleotide binding domain
NBIA1	neurodegeneration with brain iron accumulation type
NEF	nucleotide exchange factor
NO	nitric oxide
NS5A	nonstructural protein
ODF1	outer dense fiber protein 1
ORD	optical rotatory dispersion
PD	Parkinson's disease
PERK	protein kinase RNA-like ER kinase
PFD	prefoldin
PKCε	protein kinase Cε
RNAP	RNA polymerase
ROS	reactive oxygen species
SANS	small angle neutron scattering
SAXS	small-angle X-ray scattering
SBD	substrate binding domain
SBMA	spinal and bulbar muscular atrophy
sHsp	small heat shock protein
SOD1	Cu/Zn superoxide dismutase
TPR	tetratricopeptide repeat

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8. References

(1) Dunker, A. K.; Lawson, J. D.; Brown, C. J.; Williams, R. M.; Romero, P.; Oh, J. S.; Oldfield, C. J.; Campen, A. M.; Ratliff, C. M.; Hipps, K. W.; Ausio, J.; Nissen, M. S.; Reeves, R.; Kang, C.; Kissinger, C. R.; Bailey, R. W.; Griswold, M. D.; Chiu, W.; Garner, E. C.; Obradovic, Z. *J. Mol. Graphics Modell.* **2001**, *19*, 26.
(2) Schweers, O.; Schonbrunn-Hanebeck, E.; Marx, A.; Mandelkow, E. *J. Biol. Chem.* **1994**, *269*, 24290.
(3) Weinreb, P. H.; Zhen, W.; Poon, A. W.; Conway, K. A.; Lansbury, P. T., Jr. *Biochemistry* **1996**, *35*, 13709.
(4) Uversky, V. N.; Gillespie, J. R.; Fink, A. L. *Proteins* **2000**, *41*, 415.
(5) Wright, P. E.; Dyson, H. J. *J. Mol. Biol.* **1999**, *293*, 321.
(6) Tompa, P. *Prog. Mol. Subcell. Biol.* **2002**, *27*, 527.
(7) Daughdrill, G. W.; Pielak, G. J.; Uversky, V. N.; Cortese, M. S.; Dunker, A. K. In *Handbook of Protein Folding*; Buchner, J., Kiefhaber, T., Eds.; Wiley-VCH, Verlag GmbH & Co. KGaA: Weinheim, Germany, 2005.
(8) Fink, A. L. *Curr. Opin. Struct. Biol.* **2005**, *15*, 35.
(9) Uversky, V. N. *Cell. Mol. Life Sci.* **2003**, *60*, 1852.
(10) Uversky, V. N.; Ptitsyn, O. B. *Biochemistry* **1994**, *33*, 2782.
(11) Uversky, V. N.; Ptitsyn, O. B. *J. Mol. Biol.* **1996**, *255*, 215.
(12) Dunker, A. K.; Obradovic, Z. *Nat. Biotechnol.* **2001**, *19*, 805.
(13) Ringe, D.; Petsko, G. A. *Methods Enzymol.* **1986**, *131*, 389.
(14) Dyson, H. J.; Wright, P. E. *Adv. Protein Chem.* **2002**, *62*, 311.
(15) Bracken, C.; Iakoucheva, L. M.; Romero, P. R.; Dunker, A. K. *Curr. Opin. Struct. Biol.* **2004**, *14*, 570.
(16) Dyson, H. J.; Wright, P. E. *Chem. Rev.* **2004**, *104*, 3607.
(17) Dyson, H. J.; Wright, P. E. *Methods Enzymol.* **2005**, *394*, 299.
(18) Dyson, H. J.; Wright, P. E. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 197.
(19) Fasman, G. D. *Circular Dichroism and the Conformational Analysis of Biomolecules*; Plenem Press: New York, 1996.
(20) Adler, A. J.; Greenfield, N. J.; Fasman, G. D. *Methods Enzymol.* **1973**, *27*, 675.
(21) Provencher, S. W.; Glockner, J. *Biochemistry* **1981**, *20*, 33.
(22) Woody, R. W. *Methods Enzymol.* **1995**, *246*, 34.
(23) Smyth, E.; Syme, C. D.; Blanch, E. W.; Hecht, L.; Vasak, M.; Barron, L. D. *Biopolymers* **2001**, *58*, 138.
(24) Uversky, V. N. *Biochemistry (Moscow)* **1999**, *64*, 250.
(25) Receveur-Brechot, V.; Bourhis, J. M.; Uversky, V. N.; Canard, B.; Longhi, S. *Proteins* **2006**, *62*, 24.
(26) Markus, G. *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *54*, 253.
(27) Mikhalyi, E. *Application of Proteolytic Enzymes to Protein Structure Studies*; CRC Press: Boca Raton, FL, 1978.
(28) Hubbard, S. J.; Eisenmenger, F.; Thornton, J. M. *Protein Sci.* **1994**, *3*, 757.
(29) Fontana, A.; de Laureto, P. P.; de Filippis, V.; Scaramella, E.; Zambonin, M. *Folding Des.* **1997**, *2*, R17.
(30) Fontana, A.; de Laureto, P. P.; Spolaore, B.; Frare, E.; Picotti, P.; Zambonin, M. *Acta Biochim. Pol.* **2004**, *51*, 299.
(31) Iakoucheva, L. M.; Kimzey, A. L.; Masselon, C. D.; Smith, R. D.; Dunker, A. K.; Ackerman, E. J. *Protein Sci.* **2001**, *10*, 1353.
(32) Privalov, P. L. *Adv. Protein Chem.* **1979**, *33*, 167.
(33) Ptitsyn, O. *Adv. Protein Chem.* **1995**, *47*, 83.
(34) Ptitsyn, O. B.; Uversky, V. N. *FEBS Lett.* **1994**, *341*, 15.
(35) Uversky, V. N.; Ptitsyn, O. B. *Folding Des.* **1996**, *1*, 117.
(36) Westhof, E.; Altschuh, D.; Moras, D.; Bloomer, A. C.; Mondragon, A.; Klug, A.; Van Regenmortel, M. H. *Nature* **1984**, *311*, 123.
(37) Berzofsky, J. A. *Science* **1985**, *229*, 932.
(38) Miyagi, A.; Tsunaka, Y.; Uchihashi, T.; Mayanagi, K.; Hirose, S.; Morikawa, K.; Ando, T. *ChemPhysChem* **2008**, *9*, 1859.
(39) Kaltashov, I. A.; Mohimen, A. *Anal. Chem.* **2005**, *77*, 5370.
(40) Frimpong, A. K.; Abzalimov, R. R.; Uversky, V. N.; Kaltashov, I. A. *Proteins* **2010**, *78*, 714.
(41) Uversky, V. N. *Protein Sci.* **2002**, *11*, 739.
(42) *Instrumental Analysis of Intrinsically Disordered Proteins*; Longhi, S.; Uversky, V. N., Eds.; John Wiley & Sons, Inc: Hoboken, NJ, 2010.
(43) Romero, P.; Obradovic, Z.; Li, X.; Garner, E. C.; Brown, C. J.; Dunker, A. K. *Proteins* **2001**, *42*, 38.
(44) Williams, R. M.; Obradovic, Z.; Mathura, V.; Braun, W.; Garner, E. C.; Young, J.; Takayama, S.; Brown, C. J.; Dunker, A. K. *Pac. Symp. Biocomput.* **2001**, 89.
(45) Radivojac, P.; Iakoucheva, L. M.; Oldfield, C. J.; Obradovic, Z.; Uversky, V. N.; Dunker, A. K. *Biophys. J.* **2007**, *92*, 1439.
(46) Li, X.; Romero, P.; Rani, M.; Dunker, A. K.; Obradovic, Z. *Genome Inf. Ser.* **1999**, *10*, 30.
(47) Liu, J.; Rost, B. *Nucleic Acids Res.* **2003**, *31*, 3833.
(48) Linding, R.; Jensen, L. J.; Diella, F.; Bork, P.; Gibson, T. J.; Russell, R. B. *Structure* **2003**, *11*, 1453.
(49) Linding, R.; Russell, R. B.; Neduva, V.; Gibson, T. J. *Nucleic Acids Res.* **2003**, *31*, 3701.
(50) Prilusky, J.; Felder, C. E.; Zeev-Ben-Mordehai, T.; Rydberg, E. H.; Man, O.; Beckmann, J. S.; Silman, I.; Sussman, J. L. *Bioinformatics* **2005**, *21*, 3435.
(51) Dosztanyi, Z.; Csizmok, V.; Tompa, P.; Simon, I. *Bioinformatics* **2005**, *21*, 3433.
(52) Jones, D. T.; Ward, J. J. *Proteins* **2003**, *53* (Suppl. 6), 573.
(53) Ward, J. J.; McGuffin, L. J.; Bryson, K.; Buxton, B. F.; Jones, D. T. *Bioinformatics* **2004**, *20*, 2138.
(54) Ward, J. J.; Sodhi, J. S.; McGuffin, L. J.; Buxton, B. F.; Jones, D. T. *J. Mol. Biol.* **2004**, *337*, 635.
(55) Oldfield, C. J.; Cheng, Y.; Cortese, M. S.; Brown, C. J.; Uversky, V. N.; Dunker, A. K. *Biochemistry* **2005**, *44*, 1989.
(56) Ferron, F.; Longhi, S.; Canard, B.; Karlin, D. *Proteins* **2006**, *65*, 1.
(57) Bourhis, J. M.; Canard, B.; Longhi, S. *Curr. Protein Pept. Sci.* **2007**, *8*, 135.
(58) Dosztanyi, Z.; Sandor, M.; Tompa, P.; Simon, I. *Curr. Protein Pept. Sci.* **2007**, *8*, 161.
(59) Uversky, V. N.; Radivojac, P.; Iakoucheva, L. M.; Obradovic, Z.; Dunker, A. K. *Methods Mol. Biol.* **2007**, *408*, 69.
(60) Xue, B.; Dunbrack, R. L.; Williams, R. W.; Dunker, A. K.; Uversky, V. N. *Biochim. Biophys. Acta* **2010**, *1804*, 996.
(61) Dunker, A. K.; Obradovic, Z.; Romero, P.; Garner, E. C.; Brown, C. J. *Genome Inf. Ser.* **2000**, *11*, 161.
(62) Dunker, A. K.; Brown, C. J.; Obradovic, Z. *Adv. Protein Chem.* **2002**, *62*, 25.
(63) Dunker, A. K.; Brown, C. J.; Lawson, J. D.; Iakoucheva, L. M.; Obradovic, Z. *Biochemistry* **2002**, *41*, 6573.
(64) Dunker, A. K.; Cortese, M. S.; Romero, P.; Iakoucheva, L. M.; Uversky, V. N. *FEBS J.* **2005**, *272*, 5129.
(65) Dunker, A. K.; Garner, E.; Guilliot, S.; Romero, P.; Albrecht, K.; Hart, J.; Obradovic, Z.; Kissinger, C.; Villafranca, J. E. *Pac. Symp. Biocomput.* **1998**, 473.
(66) Tompa, P. *FEBS Lett.* **2005**, *579*, 3346.
(67) Tompa, P.; Csermely, P. *FASEB J.* **2004**, *18*, 1169.
(68) Tompa, P.; Szasz, C.; Buday, L. *Prog. Mol. Subcell. Biol.* **2005**, *30*, 484.
(69) Uversky, V. N. *Eur. J. Biochem.* **2002**, *269*, 2.
(70) Xie, H.; Vucetic, S.; Iakoucheva, L. M.; Oldfield, C. J.; Dunker, A. K.; Uversky, V. N.; Obradovic, Z. *J. Proteome Res.* **2007**, *6*, 1882.
(71) Vucetic, S.; Xie, H.; Iakoucheva, L. M.; Oldfield, C. J.; Dunker, A. K.; Obradovic, Z.; Uversky, V. N. *J. Proteome Res.* **2007**, *6*, 1899.
(72) Xie, H.; Vucetic, S.; Iakoucheva, L. M.; Oldfield, C. J.; Dunker, A. K.; Obradovic, Z.; Uversky, V. N. *J. Proteome Res.* **2007**, *6*, 1917.
(73) Uversky, V. N.; Oldfield, C. J.; Dunker, A. K. *J. Mol. Recognit.* **2005**, *18*, 343.
(74) Iakoucheva, L. M.; Brown, C. J.; Lawson, J. D.; Obradovic, Z.; Dunker, A. K. *J. Mol. Biol.* **2002**, *323*, 573.
(75) Crick, F. H. *Symp. Soc. Exp. Biol.* **1958**, *12*, 138.
(76) Anfinsen, C. B. *Science* **1973**, *181*, 223.
(77) Ellis, J. *Nature* **1987**, *328*, 378.
(78) Bukau, B.; Weissman, J.; Horwich, A. *Cell* **2006**, *125*, 443.
(79) Hartl, F. U.; Hayer-Hartl, M. *Science* **2002**, *295*, 1852.
(80) Slepenkov, S. V.; Witt, S. N. *Mol. Microbiol.* **2002**, *45*, 1197.
(81) Lindquist, S. *Annu. Rev. Biochem.* **1986**, *55*, 1151.
(82) Lindquist, S.; Craig, E. A. *Annu. Rev. Genet.* **1988**, *22*, 631.
(83) Chowdary, T. K.; Raman, B.; Ramakrishna, T.; Rao, C. M. *Biochem. J.* **2004**, *381*, 379.
(84) Young, J. C.; Agashe, V. R.; Siegers, K.; Hartl, F. U. *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 781.
(85) Goldfarb, S. B.; Kashlan, O. B.; Watkins, J. N.; Suaud, L.; Yan, W.; Kleyman, T. R.; Rubenstein, R. C. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 5817.
(86) Goldberger, R. F.; Epstein, C. J.; Anfinsen, C. B. *J. Biol. Chem.* **1963**, *238*, 628.
(87) Hatahet, F.; Ruddock, L. W.; Ahn, K.; Benham, A.; Craik, D.; Ellgaard, L.; Ferrari, D.; Ventura, S. *Antioxid. Redox Signaing.* **2009**, *11*, 2807.
(88) Nagradova, N. *Curr. Protein Pept. Sci.* **2007**, *8*, 273.
(89) Fischer, G.; Bang, H.; Mech, C. *Biomed. Biochim. Acta.* **1984**, *43*, 1101.
(90) Jorgensen, S.; Skov, K. W.; Diderichsen, B. *J. Bacteriol.* **1991**, *173*, 559.
(91) Chen, Y. J.; Inouye, M. *Curr. Opin. Struct. Biol.* **2008**, *18*, 765.
(92) Douglas, P. M.; Dillin, A. *J. Cell. Biol.* **2010**, *190*, 719.
(93) Balch, W. E.; Morimoto, R. I.; Dillin, A.; Kelly, J. W. *Science* **2008**, *319*, 916.
(94) Mu, T. W.; Ong, D. S.; Wang, Y. J.; Balch, W. E.; Yates, J. R., 3rd; Segatori, L.; Kelly, J. W. *Cell* **2008**, *134*, 769.

(95) Taylor, T. D.; Litt, M.; Kramer, P.; Pandolfo, M.; Angelini, L.; Nardocci, N.; Davis, S.; Pineda, M.; Hattori, H.; Flett, P. J.; Cilio, M. R.; Bertini, E.; Hayflick, S. J. *Nat. Genet.* **1996**, *14*, 479.
(96) Malandrini, A.; Cesaretti, S.; Mulinari, M.; Palmeri, S.; Fabrizi, G. M.; Villanova, M.; Parrotta, E.; Montagnani, A.; Montagnani, M.; Anichini, M.; Guazzi, G. C. *J. Neurol. Sci.* **1996**, *140*, 129.
(97) Sugiyama, H.; Hainfellner, J. A.; Schmid-Siegel, B.; Budka, H. *Clin. Neuropathol.* **1993**, *12*, 147.
(98) Swaiman, K. F. *Arch. Neurol.* **1991**, *48*, 1285.
(99) Dooling, E. C.; Schoene, W. C.; Richardson, E. P., Jr. *Arch. Neurol.* **1974**, *30*, 70.
(100) Jankovic, J.; Kirkpatrick, J. B.; Blomquist, K. A.; Langlais, P. J.; Bird, E. D. *Neurology* **1985**, *35*, 227.
(101) Galpern, W. R.; Lang, A. E. *Ann. Neurol.* **2006**, *59*, 449.
(102) Melone, M. A.; Jori, F. P.; Peluso, G. *Curr. Drug Targets* **2005**, *6*, 43.
(103) Hague, K.; Lento, P.; Morgello, S.; Caro, S.; Kaufmann, H. *Acta Neuropathol.* **1997**, *94*, 192.
(104) Jackson, M.; Lennox, G.; Balsitis, M.; Lowe, J. *J. Neurol. Neurosurg. Psychiatry* **1995**, *58*, 756.
(105) Chiti, F.; Dobson, C. M. *Annu. Rev. Biochem.* **2006**, *75*, 333.
(106) Uversky, V. N.; Fink, A. L. *Biochim. Biophys. Acta* **2004**, *1698*, 131.
(107) Sunde, M.; Blake, C. *Adv. Protein Chem.* **1997**, *50*, 123.
(108) Sunde, M.; Serpell, L. C.; Bartlam, M.; Fraser, P. E.; Pepys, M. B.; Blake, C. C. *J. Mol. Biol.* **1997**, *273*, 729.
(109) Westermark, P. In *Amyloid Proteins. The Beta Sheet Conformation and Disease*; Sipe, J. D. , Ed.; WILEY-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2005; Vol. 1.
(110) Serpell, L. C.; Sunde, M.; Benson, M. D.; Tennent, G. A.; Pepys, M. B.; Fraser, P. E. *J. Mol. Biol.* **2000**, *300*, 1033.
(111) Bauer, H. H.; Aebi, U.; Haner, M.; Hermann, R.; Muller, M.; Merkle, H. P. *J. Struct. Biol.* **1995**, *115*, 1.
(112) Saiki, M.; Honda, S.; Kawasaki, K.; Zhou, D.; Kaito, A.; Konakahara, T.; Morii, H. *J. Mol. Biol.* **2005**, *348*, 983.
(113) Pedersen, J. S.; Dikov, D.; Flink, J. L.; Hjuler, H. A.; Christiansen, G.; Otzen, D. E. *J. Mol. Biol.* **2006**, *355*, 501.
(114) Waxman, E. A.; Giasson, B. I. *Biochim. Biophys. Acta* **2009**, *1792*, 616.
(115) Ross, O. A.; Braithwaite, A. T.; Skipper, L. M.; Kachergus, J.; Hulihan, M. M.; Middleton, F. A.; Nishioka, K.; Fuchs, J.; Gasser, T.; Maraganore, D. M.; Adler, C. H.; Larvor, L.; Chartier-Harlin, M. C.; Nilsson, C.; Langston, J. W.; Gwinn, K.; Hattori, N.; Farrer, M. J. *Ann. Neurol.* **2008**, *63*, 743.
(116) Dachsel, J. C.; Lincoln, S. J.; Gonzalez, J.; Ross, O. A.; Dickson, D. W.; Farrer, M. J. *Mov. Disord.* **2007**, *22*, 293.
(117) Goers, J.; Manning-Bog, A. B.; McCormack, A. L.; Millett, I. S.; Doniach, S.; Di Monte, D. A.; Uversky, V. N.; Fink, A. L. *Biochemistry* **2003**, *42*, 8465.
(118) Manning-Bog, A. B.; McCormack, A. L.; Li, J.; Uversky, V. N.; Fink, A. L.; Di Monte, D. A. *J. Biol. Chem.* **2002**, *277*, 1641.
(119) Volles, M. J.; Lee, S. J.; Rochet, J. C.; Shtilerman, M. D.; Ding, T. T.; Kessler, J. C.; Lansbury, P. T., Jr. *Biochemistry* **2001**, *40*, 7812.
(120) Ding, T. T.; Lee, S. J.; Rochet, J. C.; Lansbury, P. T., Jr. *Biochemistry* **2002**, *41*, 10209.
(121) Lashuel, H. A.; Hartley, D.; Petre, B. M.; Walz, T.; Lansbury, P. T., Jr. *Nature* **2002**, *418*, 291.
(122) Lindersson, E.; Beedholm, R.; Hojrup, P.; Moos, T.; Gai, W.; Hendil, K. B.; Jensen, P. H. *J. Biol. Chem.* **2004**, *279*, 12924.
(123) Moreira, P. I.; Zhu, X.; Wang, X.; Lee, H. G.; Nunomura, A.; Petersen, R. B.; Perry, G.; Smith, M. A. *Biochim. Biophys. Acta* **2010**, *1802*, 212.
(124) Bubber, P.; Haroutunian, V.; Fisch, G.; Blass, J. P.; Gibson, G. E. *Ann. Neurol.* **2005**, *57*, 695.
(125) Valla, J.; Schneider, L.; Niedzielko, T.; Coon, K. D.; Caselli, R.; Sabbagh, M. N.; Ahern, G. L.; Baxter, L.; Alexander, G.; Walker, D. G.; Reiman, E. M. *Mitochondrion* **2006**, *6*, 323.
(126) Wang, X.; Su, B.; Fujioka, H.; Zhu, X. *Am. J. Pathol.* **2008**, *173*, 470.
(127) Devi, L.; Raghavendran, V.; Prabhu, B. M.; Avadhani, N. G.; Anandatheerthavarada, H. K. *J. Biol. Chem.* **2008**, *283*, 9089.
(128) Banerjee, R.; Starkov, A. A.; Beal, M. F.; Thomas, B. *Biochim. Biophys. Acta* **2009**, *1792*, 651.
(129) Gibson, G. E.; Starkov, A.; Blass, J. P.; Ratan, R. R.; Beal, M. F. *Biochim. Biophys. Acta* **2010**, *1802*, 122.
(130) Browne, S. E.; Bowling, A. C.; MacGarvey, U.; Baik, M. J.; Berger, S. C.; Muqit, M. M.; Bird, E. D.; Beal, M. F. *Ann. Neurol.* **1997**, *41*, 646.
(131) Dupuis, L.; Gonzalez de Aguilar, J. L.; Oudart, H.; de Tapia, M.; Barbeito, L.; Loeffler, J. P. *Neurodegener. Dis.* **2004**, *1*, 245.
(132) Zimmerman, M. C.; Oberley, L. W.; Flanagan, S. W. *J. Neurochem.* **2007**, *102*, 609.
(133) Fatokun, A. A.; Stone, T. W.; Smith, R. A. *Front. Biosci.* **2008**, *13*, 3288.
(134) Barnham, K. J.; Masters, C. L.; Bush, A. I. *Nat. Rev. Drug Discovery* **2004**, *3*, 205.
(135) Halliwell, B. *J. Neurochem.* **2006**, *97*, 1634.
(136) Gaeta, A.; Hider, R. C. *Br. J. Pharmacol.* **2005**, *146*, 1041.
(137) Duncan, A. J.; Heales, S. J. *Mol. Aspects Med.* **2005**, *26*, 67.
(138) Gonzalez, F. J. *Mutat. Res.* **2005**, *569*, 101.
(139) *Oxidative Stress and Neurodegenerative Disorders*; Qureshi, G. A.; Parvez, S. H. , Eds.; Elsevier: Amsterdam, The Netherlands, 2007.
(140) Rambourg, A.; Clermont, Y. In *The Golgi Apparatus*; Berger, E. G., Roth, J., Eds.; Birkhäuser: Basel, Switzerland, 1997.
(141) Gonatas, N. K.; Stieber, A.; Gonatas, J. O. *J. Neurol. Sci.* **2006**, *246*, 21.
(142) Gosavi, N.; Lee, H. J.; Lee, J. S.; Patel, S.; Lee, S. J. *J. Biol. Chem.* **2002**, *277*, 48984.
(143) Baumann, O.; Walz, B. *Int. Rev. Cytol.* **2001**, *205*, 149.
(144) Gorlach, A.; Klappa, P.; Kietzmann, T. *Antioxid. Redox Signaling* **2006**, *8*, 1391.
(145) Ron, D.; Walter, P. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 519.
(146) Kim, I.; Xu, W.; Reed, J. C. *Nat. Rev. Drug Discovery* **2008**, *7*, 1013.
(147) Schroder, M.; Kaufman, R. J. *Annu. Rev. Biochem.* **2005**, *74*, 739.
(148) Schroder, M.; Kaufman, R. J. *Mutat. Res.* **2005**, *569*, 29.
(149) Selkoe, D. J. *Physiol. Rev.* **2001**, *81*, 741.
(150) LaFerla, F. M.; Green, K. N.; Oddo, S. *Nat. Rev. Neurosci.* **2007**, *8*, 499.
(151) Lindholm, D.; Wootz, H.; Korhonen, L. *Cell Death. Differ.* **2006**, *13*, 385.
(152) Paschen, W.; Mengesdorf, T. *Cell Calcium* **2005**, *38*, 409.
(153) Hoozemans, J. J.; van Haastert, E. S.; Nijholt, D. A.; Rozemuller, A. J.; Eikelenboom, P.; Scheper, W. *Am. J. Pathol.* **2009**, *174*, 1241.
(154) Scheper, W.; Hoozemans, J. J. *Curr. Med. Chem.* **2009**, *16*, 615.
(155) Matus, S.; Lisbona, F.; Torres, M.; Leon, C.; Thielen, P.; Hetz, C. *Curr. Mol. Med.* **2008**, *8*, 157.
(156) Kanekura, K.; Suzuki, H.; Aiso, S.; Matsuoka, M. *Mol. Neurobiol.* **2009**, *39*, 81.
(157) Salminen, A.; Kauppinen, A.; Suuronen, T.; Kaarniranta, K.; Ojala, J. *J. Neuroinflammation* **2009**, *6*, 41.
(158) Alves-Rodrigues, A.; Gregori, L.; Figueiredo-Pereira, M. E. *Trends Neurosci.* **1998**, *21*, 516.
(159) Lim, K. L. *Expert Rev. Proteomics* **2007**, *4*, 769.
(160) Matsuda, N.; Tanaka, K. *J. Alzheimer's Dis.* **2009**, *19*, 1.
(161) Kirkin, V.; McEwan, D. G.; Novak, I.; Dikic, I. *Mol. Cell* **2009**, *34*, 259.
(162) Ishihara, N.; Mizushima, N. *Dev. Cell* **2009**, *17*, 1.
(163) Komatsu, M.; Waguri, S.; Chiba, T.; Murata, S.; Iwata, J.; Tanida, I.; Ueno, T.; Koike, M.; Uchiyama, Y.; Kominami, E.; Tanaka, K. *Nature* **2006**, *441*, 880.
(164) Hara, T.; Nakamura, K.; Matsui, M.; Yamamoto, A.; Nakahara, Y.; Suzuki-Migishima, R.; Yokoyama, M.; Mishima, K.; Saito, I.; Okano, H.; Mizushima, N. *Nature* **2006**, *441*, 885.
(165) McClellan, A. J.; Tam, S.; Kaganovich, D.; Frydman, J. *Nat. Cell Biol.* **2005**, *7*, 736.
(166) Leidhold, C.; Voos, W. *Ann. N.Y. Acad. Sci.* **2007**, *1113*, 72.
(167) Witt, S. N. *Biopolymers* **2010**, *93*, 218.
(168) Goldberg, A. L. *Nature* **2003**, *426*, 895.
(169) Gao, X.; Hu, H. *Acta. Biochim. Biophys. Sin.* **2008**, *40*, 612.
(170) Mayer, M. P.; Bukau, B. *Cell. Mol. Life Sci.* **2005**, *62*, 670.
(171) Genevaux, P.; Georgopoulos, C.; Kelley, W. L. *Mol. Microbiol.* **2007**, *66*, 840.
(172) Frydman, J. *Annu. Rev. Biochem.* **2001**, *70*, 603.
(173) Patury, S.; Miyata, Y.; Gestwicki, J. E. *Curr. Top. Med. Chem.* **2009**, *9*, 1337.
(174) Erbse, A.; Mayer, M. P.; Bukau, B. *Biochem. Soc. Trans.* **2004**, *32*, 617.
(175) Rudiger, S.; Buchberger, A.; Bukau, B. *Nat. Struct. Biol.* **1997**, *4*, 342.
(176) Rudiger, S.; Germeroth, L.; Schneider-Mergener, J.; Bukau, B. *EMBO J.* **1997**, *16*, 1501.
(177) Qiu, X. B.; Shao, Y. M.; Miao, S.; Wang, L. *Cell. Mol. Life Sci.* **2006**, *63*, 2560.
(178) Scheufler, C.; Brinker, A.; Bourenkov, G.; Pegoraro, S.; Moroder, L.; Bartunik, H.; Hartl, F. U.; Moarefi, I. *Cell* **2000**, *101*, 199.
(179) Ballinger, C. A.; Connell, P.; Wu, Y.; Hu, Z.; Thompson, L. J.; Yin, L. Y.; Patterson, C. *Mol. Cell. Biol.* **1999**, *19*, 4535.
(180) Connell, P.; Ballinger, C. A.; Jiang, J.; Wu, Y.; Thompson, L. J.; Hohfeld, J.; Patterson, C. *Nat. Cell Biol.* **2001**, *3*, 93.
(181) McCarty, J. S.; Buchberger, A.; Reinstein, J.; Bukau, B. *J. Mol. Biol.* **1995**, *249*, 126.
(182) Bertelsen, E. B.; Chang, L.; Gestwicki, J. E.; Zuiderweg, E. R. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 8471.

(183) Schuermann, J. P.; Jiang, J.; Cuellar, J.; Llorca, O.; Wang, L.; Gimenez, L. E.; Jin, S.; Taylor, A. B.; Demeler, B.; Morano, K. A.; Hart, P. J.; Valpuesta, J. M.; Lafer, E. M.; Sousa, R. *Mol. Cell* **2008**, *31*, 232.
(184) Bertelsen, E. B.; Zhou, H.; Lowry, D. F.; Flynn, G. C.; Dahlquist, F. W. *Protein Sci.* **1999**, *8*, 343.
(185) Wittung-Stafshede, P.; Guidry, J.; Horne, B. E.; Landry, S. J. *Biochemistry* **2003**, *42*, 4937.
(186) Slepenkov, S. V.; Witt, S. N. *Biochemistry* **2002**, *41*, 12224.
(187) Russell, R.; Jordan, R.; McMacken, R. *Biochemistry* **1998**, *37*, 596.
(188) Shi, Z. Z.; Zhang, J. W.; Zheng, S. *J. Zhejiang Univ., Sci., B* **2007**, *8*, 170.
(189) Wegele, H.; Muller, L.; Buchner, J. *Rev. Physiol. Biochem. Pharmacol.* **2004**, *151*, 1.
(190) Liebscher, M.; Roujeinikova, A. *J. Bacteriol.* **2009**, *191*, 1456.
(191) Gamer, J.; Multhaup, G.; Tomoyasu, T.; McCarty, J. S.; Rudiger, S.; Schonfeld, H. J.; Schirra, C.; Bujard, H.; Bukau, B. *EMBO J.* **1996**, *15*, 607.
(192) Straus, D. B.; Walter, W. A.; Gross, C. A. *Nature* **1987**, *329*, 348.
(193) Grossman, A. D.; Straus, D. B.; Walter, W. A.; Gross, C. A. *Genes Dev.* **1987**, *1*, 179.
(194) Straus, D. B.; Walter, W. A.; Gross, C. A. *Genes Dev.* **1989**, *3*, 2003.
(195) Tilly, K.; Spence, J.; Georgopoulos, C. *J. Bacteriol.* **1989**, *171*, 1585.
(196) Straus, D.; Walter, W.; Gross, C. A. *Genes Dev.* **1990**, *4*, 2202.
(197) Gamer, J.; Bujard, H.; Bukau, B. *Cell* **1992**, *69*, 833.
(198) Liberek, K.; Georgopoulos, C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11019.
(199) Haag Breese, E.; Uversky, V. N.; Georgiadis, M. M.; Harrington, M. A. *DNA Cell. Biol.* **2006**, *25*, 704.
(200) O'Keeffe, B.; Fong, Y.; Chen, D.; Zhou, S.; Zhou, Q. *J. Biol. Chem.* **2000**, *275*, 279.
(201) Zhang, X.; Bourhis, J. M.; Longhi, S.; Carsillo, T.; Buccellato, M.; Morin, B.; Canard, B.; Oglesbee, M. *Virology* **2005**, *337*, 162.
(202) Couturier, M.; Buccellato, M.; Costanzo, S.; Bourhis, J. M.; Shu, Y.; Nicaise, M.; Desmadril, M.; Flaudrops, C.; Longhi, S.; Oglesbee, M. *J. Mol. Recognit.* **2010**, *23*, 301.
(203) Gibbs, S. J.; Braun, J. E. *Neurobiol. Dis.* **2008**, *32*, 196.
(204) Zhao, X.; Braun, A. P.; Braun, J. E. *Cell. Mol. Lif. Sci.* **2008**, *65*, 2385.
(205) Mayer, M. P.; Brehmer, D.; Gassler, C. S.; Bukau, B. *Adv. Protein Chem.* **2001**, *59*, 1.
(206) Ohtsuka, K.; Hata, M. *Int. J. Hyperthermia* **2000**, *16*, 231.
(207) Cheetham, M. E.; Caplan, A. J. *Cell Stress Chaperones* **1998**, *3*, 28.
(208) Karzai, A. W.; McMacken, R. *J. Biol. Chem.* **1996**, *271*, 11236.
(209) Laufen, T.; Mayer, M. P.; Beisel, C.; Klostermeier, D.; Mogk, A.; Reinstein, J.; Bukau, B. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5452.
(210) Szabo, A.; Langer, T.; Schroder, H.; Flanagan, J.; Bukau, B.; Hartl, F. U. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 10345.
(211) Suh, W. C.; Lu, C. Z.; Gross, C. A. *J. Biol. Chem.* **1999**, *274*, 30534.
(212) Greene, M. K.; Maskos, K.; Landry, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6108.
(213) Szyperski, T.; Pellecchia, M.; Wall, D.; Georgopoulos, C.; Wuthrich, K. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11343.
(214) Szabo, A.; Korszun, R.; Hartl, F. U.; Flanagan, J. *EMBO J.* **1996**, *15*, 408.
(215) Banecki, B.; Liberek, K.; Wall, D.; Wawrzynow, A.; Georgopoulos, C.; Bertoli, E.; Tanfani, F.; Zylicz, M. *J. Biol. Chem.* **1996**, *271*, 14840.
(216) Martinez-Yamout, M.; Legge, G. B.; Zhang, O.; Wright, P. E.; Dyson, H. J. *J. Mol. Biol.* **2000**, *300*, 805.
(217) Sha, B.; Lee, S.; Cyr, D. M. *Structure* **2000**, *8*, 799.
(218) Li, J.; Qian, X.; Sha, B. *Structure* **2003**, *11*, 1475.
(219) Lu, Z.; Cyr, D. M. *J. Biol. Chem.* **1998**, *273*, 5970.
(220) Velten, M.; Villoutreix, B. O.; Ladjimi, M. M. *Biochemistry* **2000**, *39*, 307.
(221) Hohfeld, J.; Minami, Y.; Hartl, F. U. *Cell* **1995**, *83*, 589.
(222) Easton, D. P.; Kaneko, Y.; Subjeck, J. R. *Cell Stress Chaperones* **2000**, *5*, 276.
(223) Vos, M. J.; Hageman, J.; Carra, S.; Kampinga, H. H. *Biochemistry* **2008**, *47*, 7001.
(224) Wang, X. Y.; Chen, X.; Oh, H. J.; Repasky, E.; Kazim, L.; Subjeck, J. *FEBS Lett.* **2000**, *465*, 98.
(225) Oh, H. J.; Easton, D.; Murawski, M.; Kaneko, Y.; Subjeck, J. R. *J. Biol. Chem.* **1999**, *274*, 15712.
(226) Raviol, H.; Bukau, B.; Mayer, M. P. *FEBS Lett.* **2006**, *580*, 168.
(227) Raviol, H.; Sadlish, H.; Rodriguez, F.; Mayer, M. P.; Bukau, B. *EMBO J.* **2006**, *25*, 2510.
(228) Doong, H.; Vrailas, A.; Kohn, E. C. *Cancer Lett.* **2002**, *188*, 25.
(229) Takayama, S.; Reed, J. C. *Nat. Cell Biol.* **2001**, *3*, E237.
(230) Alberti, S.; Esser, C.; Hohfeld, J. *Cell Stress Chaperones* **2003**, *8*, 225.
(231) Hohfeld, J.; Jentsch, S. *EMBO J.* **1997**, *16*, 6209.
(232) Takayama, S.; Krajewski, S.; Krajewska, M.; Kitada, S.; Zapata, J. M.; Kochel, K.; Knee, D.; Scudiero, D.; Tudor, G.; Miller, G. J.; Miyashita, T.; Yamada, M.; Reed, J. C. *Cancer Res.* **1998**, *58*, 3116.
(233) Jackson, P. K.; Eldridge, A. G.; Freed, E.; Furstenthal, L.; Hsu, J. Y.; Kaiser, B. K.; Reimann, J. D. *Trends Cell Biol.* **2000**, *10*, 429.
(234) Murata, S.; Minami, Y.; Minami, M.; Chiba, T.; Tanaka, K. *EMBO Rep.* **2001**, *2*, 1133.
(235) Alberti, S.; Demand, J.; Esser, C.; Emmerich, N.; Schild, H.; Hohfeld, J. *J. Biol. Chem.* **2002**, *277*, 45920.
(236) Tetzlaff, J. E.; Putcha, P.; Outeiro, T. F.; Ivanov, A.; Berezovska, O.; Hyman, B. T.; McLean, P. J. *J. Biol. Chem.* **2008**, *283*, 17962.
(237) Bose, S.; Weikl, T.; Bugl, H.; Buchner, J. *Science* **1996**, *274*, 1715.
(238) Smith, D. F.; Sullivan, W. P.; Marion, T. N.; Zaitsu, K.; Madden, B.; McCormick, D. J.; Toft, D. O. *Mol. Cell. Biol.* **1993**, *13*, 869.
(239) Odunuga, O. O.; Longshaw, V. M.; Blatch, G. L. *BioEssays* **2004**, *26*, 1058.
(240) Hernandez, M. P.; Sullivan, W. P.; Toft, D. O. *J. Biol. Chem.* **2002**, *277*, 38294.
(241) Wegele, H.; Haslbeck, M.; Reinstein, J.; Buchner, J. *J. Biol. Chem.* **2003**, *278*, 25970.
(242) Richter, K.; Muschler, P.; Hainzl, O.; Reinstein, J.; Buchner, J. *J. Biol. Chem.* **2003**, *278*, 10328.
(243) Carrigan, P. E.; Nelson, G. M.; Roberts, P. J.; Stoffer, J.; Riggs, D. L.; Smith, D. F. *J. Biol. Chem.* **2004**, *279*, 16185.
(244) Das, A. K.; Cohen, P. W.; Barford, D. *EMBO J.* **1998**, *17*, 1192.
(245) Blatch, G. L.; Lassle, M. *BioEssays* **1999**, *21*, 932.
(246) Welch, W. J.; Feramisco, J. R. *J. Biol. Chem.* **1982**, *257*, 14949.
(247) Lai, B. T.; Chin, N. W.; Stanek, A. E.; Keh, W.; Lanks, K. W. *Mol. Cell. Biol.* **1984**, *4*, 2802.
(248) Hickey, E.; Brandon, S. E.; Smale, G.; Lloyd, D.; Weber, L. A. *Mol. Cell. Biol.* **1989**, *9*, 2615.
(249) Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *Cell* **1997**, *90*, 65.
(250) McLaughlin, S. H.; Smith, H. W.; Jackson, S. E. *J. Mol. Biol.* **2002**, *315*, 787.
(251) Buchner, J. *Trends Biochem. Sci.* **1999**, *24*, 136.
(252) Picard, D. *Cell. Mol. Life Sci.* **2002**, *59*, 1640.
(253) Pratt, W. B.; Toft, D. O. *Exp. Biol. Med. (Maywood, NJ, U.S.)* **2003**, *228*, 111.
(254) Klettner, A. *Drug News Perspect.* **2004**, *17*, 299.
(255) Barral, J. M.; Broadley, S. A.; Schaffar, G.; Hartl, F. U. *Semin. Cell Dev. Biol.* **2004**, *15*, 17.
(256) Soti, C.; Nagy, E.; Giricz, Z.; Vigh, L.; Csermely, P.; Ferdinandy, P. *Br. J. Pharmacol.* **2005**, *146*, 769.
(257) Zou, J.; Guo, Y.; Guettouche, T.; Smith, D. F.; Voellmy, R. *Cell* **1998**, *94*, 471.
(258) Maruya, M.; Sameshima, M.; Nemoto, T.; Yahara, I. *J. Mol. Biol.* **1999**, *285*, 903.
(259) Richter, K.; Muschler, P.; Hainzl, O.; Buchner, J. *J. Biol. Chem.* **2001**, *276*, 33689.
(260) Perdew, G. H.; Hord, N.; Hollenback, C. E.; Welsh, M. J. *Exp. Cell Res.* **1993**, *209*, 350.
(261) Nemoto, T.; Matsusaka, T.; Ota, M.; Takagi, T.; Collinge, D. B.; Walther-Larsen, H. *J. Biochem.* **1996**, *120*, 249.
(262) Chadli, A.; Bouhouche, I.; Sullivan, W.; Stensgard, B.; McMahon, N.; Catelli, M. G.; Toft, D. O. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12524.
(263) Chen, S.; Sullivan, W. P.; Toft, D. O.; Smith, D. F. *Cell Stress Chaperones* **1998**, *3*, 118.
(264) Bron, P.; Giudice, E.; Rolland, J. P.; Buey, R. M.; Barbier, P.; Diaz, J. F.; Peyrot, V.; Thomas, D.; Garnier, C. *Biol. Cell* **2008**, *100*, 413.
(265) Sullivan, W. P.; Owen, B. A.; Toft, D. O. *J. Biol. Chem.* **2002**, *277*, 45942.
(266) Prodromou, C.; Panaretou, B.; Chohan, S.; Siligardi, G.; O'Brien, R.; Ladbury, J. E.; Roe, S. M.; Piper, P. W.; Pearl, L. H. *EMBO J.* **2000**, *19*, 4383.
(267) Young, J. C.; Hartl, F. U. *EMBO J.* **2000**, *19*, 5930.
(268) Panaretou, B.; Siligardi, G.; Meyer, P.; Maloney, A.; Sullivan, J. K.; Singh, S.; Millson, S. H.; Clarke, P. A.; Naaby-Hansen, S.; Stein, R.; Cramer, R.; Mollapour, M.; Workman, P.; Piper, P. W.; Pearl, L. H.; Prodromou, C. *Mol. Cell* **2002**, *10*, 1307.
(269) Shirasu, K.; Lahaye, T.; Tan, M. W.; Zhou, F.; Azevedo, C.; Schulze-Lefert, P. *Cell* **1999**, *99*, 355.
(270) Garcia-Ranea, J. A.; Mirey, G.; Camonis, J.; Valencia, A. *FEBS Lett.* **2002**, *529*, 162.
(271) Weaver, A. J.; Sullivan, W. P.; Felts, S. J.; Owen, B. A.; Toft, D. O. *J. Biol. Chem.* **2000**, *275*, 23045.
(272) Weikl, T.; Abelmann, K.; Buchner, J. *J. Mol. Biol.* **1999**, *293*, 685.
(273) Schmid, F. X. *Annu. Rev. Biophys. Biomol. Struct.* **1993**, *22*, 123.
(274) Galat, A. *Eur. J. Biochem.* **1993**, *216*, 689.
(275) Pratt, W. B.; Toft, D. O. *Endocr. Rev.* **1997**, *18*, 306.
(276) Owens-Grillo, J. K.; Stancato, L. F.; Hoffmann, K.; Pratt, W. B.; Krishna, P. *Biochemistry* **1996**, *35*, 15249.

(277) Hanoulle, X.; Badillo, A.; Verdegem, D.; Penin, F.; Lippens, G. *Protein Pept. Lett.* **2010**, *17*, 1012.
(278) Hanoulle, X.; Badillo, A.; Wieruszeski, J. M.; Verdegem, D.; Landrieu, I.; Bartenschlager, R.; Penin, F.; Lippens, G. *J. Biol. Chem.* **2009**, *284*, 13589.
(279) Watashi, K.; Ishii, N.; Hijikata, M.; Inoue, D.; Murata, T.; Miyanari, Y.; Shimotohno, K. *Mol. Cell* **2005**, *19*, 111.
(280) Davies, T. H.; Ning, Y. M.; Sanchez, E. R. *J. Biol. Chem.* **2002**, *277*, 4597.
(281) Callebaut, I.; Renoir, J. M.; Lebeau, M. C.; Massol, N.; Burny, A.; Baulieu, E. E.; Mornon, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6270.
(282) Chambraud, B.; Rouviere-Fourmy, N.; Radanyi, C.; Hsiao, K.; Peattie, D. A.; Livingston, D. J.; Baulieu, E. E. *Biochem. Biophys. Res. Commun.* **1993**, *196*, 160.
(283) Wiederrecht, G.; Hung, S.; Chan, H. K.; Marcy, A.; Martin, M.; Calaycay, J.; Boulton, D.; Sigal, N.; Kincaid, R. L.; Siekierka, J. J. *J. Biol. Chem.* **1992**, *267*, 21753.
(284) Radanyi, C.; Chambraud, B.; Baulieu, E. E. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11197.
(285) Massol, N.; Lebeau, M. C.; Renoir, J. M.; Faber, L. E.; Baulieu, E. E. *Biochem. Biophys. Res. Commun.* **1992**, *187*, 1330.
(286) Wu, B.; Li, P.; Liu, Y.; Lou, Z.; Ding, Y.; Shu, C.; Ye, S.; Bartlam, M.; Shen, B.; Rao, Z. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 8348.
(287) Boelens, W. C.; Croes, Y.; de Ruwe, M.; de Reu, L.; de Jong, W. W. *J. Biol. Chem.* **1998**, *273*, 28085.
(288) Carver, J. A. *Prog. Retinal Eye Res.* **1999**, *18*, 431.
(289) Derham, B. K.; Harding, J. J. *Prog. Retinal Eye Res.* **1999**, *18*, 463.
(290) Kim, K. K.; Kim, R.; Kim, S. H. *Nature* **1998**, *394*, 595.
(291) van Montfort, R. L.; Basha, E.; Friedrich, K. L.; Slingsby, C.; Vierling, E. *Nat. Struct. Biol.* **2001**, *8*, 1025.
(292) Van Montfort, R.; Slingsby, C.; Vierling, E. *Adv. Protein Chem.* **2001**, *59*, 105.
(293) Stamler, R.; Kappe, G.; Boelens, W.; Slingsby, C. *J. Mol. Biol.* **2005**, *353*, 68.
(294) Haley, D. A.; Horwitz, J.; Stewart, P. L. *J. Mol. Biol.* **1998**, *277*, 27.
(295) Kappe, G.; Franck, E.; Verschuure, P.; Boelens, W. C.; Leunissen, J. A.; de Jong, W. W. *Cell Stress Chaperones* **2003**, *8*, 53.
(296) Michaud, S.; Morrow, G.; Marchand, J.; Tanguay, R. M. *Prog. Mol. Subcell. Biol.* **2002**, *28*, 79.
(297) Candido, E. P. *Prog. Mol. Subcell. Biol.* **2002**, *28*, 61.
(298) Scharf, K. D.; Siddique, M.; Vierling, E. *Cell Stress Chaperones* **2001**, *6*, 225.
(299) Franck, E.; Madsen, O.; van Rheede, T.; Ricard, G.; Huynen, M. A.; de Jong, W. W. *J. Mol. Evol.* **2004**, *59*, 792.
(300) Haslbeck, M.; Buchner, J. *Prog. Mol. Subcell. Biol.* **2002**, *28*, 37.
(301) Haslbeck, M.; Miess, A.; Stromer, T.; Walter, S.; Buchner, J. *J. Biol. Chem.* **2005**, *280*, 23861.
(302) Latchman, D. S. *Prog. Mol. Subcell. Biol.* **2002**, *28*, 253.
(303) Arrigo, A. P.; Paul, C.; Ducasse, C.; Sauvageot, O.; Kretz-Remy, C. *Prog. Mol. Subcell. Biol.* **2002**, *28*, 171.
(304) Davidson, S. M.; Loones, M. T.; Duverger, O.; Morange, M. *Prog. Mol. Subcell. Biol.* **2002**, *28*, 103.
(305) Krueger-Naug, A. M.; Plumier, J. C.; Hopkins, D. A.; Currie, R. W. *Prog. Mol. Subcell. Biol.* **2002**, *28*, 235.
(306) Ciocca, D. R.; Vargas-Roig, L. M. *Prog. Mol. Subcell. Biol.* **2002**, *28*, 205.
(307) Kostenko, S.; Moens, U. *Cell. Mol. Life Sci.* **2009**, *66*, 3289.
(308) Shemetov, A. A.; Seit-Nebi, A. S.; Gusev, N. B. *J. Neurosci. Res.* **2008**, *86*, 264.
(309) Jaya, N.; Garcia, V.; Vierling, E. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 15604.
(310) Bagneris, C.; Bateman, O. A.; Naylor, C. E.; Cronin, N.; Boelens, W. C.; Keep, N. H.; Slingsby, C. *J. Mol. Biol.* **2009**, *392*, 1242.
(311) Clayton, D. F.; George, J. M. *J. Neurosci. Res.* **1999**, *58*, 120.
(312) Ueda, K.; Fukushima, H.; Masliah, E.; Xia, Y.; Iwai, A.; Yoshimoto, M.; Otero, D. A.; Kondo, J.; Ihara, Y.; Saitoh, T. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11282.
(313) Maroteaux, L.; Campanelli, J. T.; Scheller, R. H. *J. Neurosci.* **1988**, *8*, 2804.
(314) Jakes, R.; Spillantini, M. G.; Goedert, M. *FEBS Lett.* **1994**, *345*, 27.
(315) Nakajo, S.; Tsukada, K.; Omata, K.; Nakamura, Y.; Nakaya, K. *Eur. J. Biochem.* **1993**, *217*, 1057.
(316) Tobe, T.; Nakajo, S.; Tanaka, A.; Mitoya, A.; Omata, K.; Nakaya, K.; Tomita, M.; Nakamura, Y. *J. Neurochem.* **1992**, *59*, 1624.
(317) Ji, H.; Liu, Y. E.; Jia, T.; Wang, M.; Liu, J.; Xiao, G.; Joseph, B. K.; Rosen, C.; Shi, Y. E. *Cancer Res.* **1997**, *57*, 759.
(318) Ninkina, N. N.; Alimova-Kost, M. V.; Paterson, J. W.; Delaney, L.; Cohen, B. B.; Imreh, S.; Gnuchev, N. V.; Davies, A. M.; Buchman, V. L. *Hum. Mol. Genet.* **1998**, *7*, 1417.
(319) Buchman, V. L.; Hunter, H. J.; Pinon, L. G.; Thompson, J.; Privalova, E. M.; Ninkina, N. N.; Davies, A. M. *J. Neurosci.* **1998**, *18*, 9335.
(320) Buchman, V. L.; Adu, J.; Pinon, L. G.; Ninkina, N. N.; Davies, A. M. *Nat. Neurosci.* **1998**, *1*, 101.
(321) Lavedan, C.; Leroy, E.; Dehejia, A.; Buchholtz, S.; Dutra, A.; Nussbaum, R. L.; Polymeropoulos, M. H. *Hum. Genet.* **1998**, *103*, 106.
(322) Uversky, V. N.; Li, J.; Souillac, P.; Millett, I. S.; Doniach, S.; Jakes, R.; Goedert, M.; Fink, A. L. *J. Biol. Chem.* **2002**, *277*, 11970.
(323) Yamin, G.; Munishkina, L. A.; Karymov, M. A.; Lyubchenko, Y. L.; Uversky, V. N.; Fink, A. L. *Biochemistry* **2005**, *44*, 9096.
(324) Uversky, V. N.; Li, J.; Fink, A. L. *J. Biol. Chem.* **2001**, *276*, 10737.
(325) Uversky, V. N. *J. Biomol. Struct. Dyn.* **2003**, *21*, 211.
(326) Uversky, V. N. *Curr. Protein Pept. Sci.* **2008**, *9*, 507.
(327) Uversky, V. N. *Front. Biosci.* **2009**, *14*, 5188.
(328) Uversky, V. N.; Eliezer, D. *Curr. Protein Pept. Sci.* **2009**, *10*, 483.
(329) Uversky, V. N. *J. Neurochem.* **2007**, *103*, 17.
(330) Clayton, D. F.; George, J. M. *Trends Neurosci.* **1998**, *21*, 249.
(331) Beyer, K. *Acta Neuropathol.* **2006**, *112*, 237.
(332) George, J. M.; Jin, H.; Woods, W. S.; Clayton, D. F. *Neuron* **1995**, *15*, 361.
(333) Han, H.; Weinreb, P. H.; Lansbury, P. T., Jr. *Chem. Biol.* **1995**, *2*, 163.
(334) Ostrerova, N.; Petrucelli, L.; Farrer, M.; Mehta, N.; Choi, P.; Hardy, J.; Wolozin, B. *J. Neurosci.* **1999**, *19*, 5782.
(335) Kim, T. D.; Choi, E.; Rhim, H.; Paik, S. R.; Yang, C. H. *Biochem. Biophys. Res. Commun.* **2004**, *324*, 1352.
(336) Layfield, R.; Fergusson, J.; Aitken, A.; Lowe, J.; Landon, M.; Mayer, R. J. *Neurosci. Lett.* **1996**, *209*, 57.
(337) Broadie, K.; Rushton, E.; Skoulakis, E. M.; Davis, R. L. *Neuron* **1997**, *19*, 391.
(338) Aitken, A. *Trends Biochem. Sci.* **1995**, *20*, 95.
(339) Aitken, A.; Jones, D.; Soneji, Y.; Howell, S. *Biochem. Soc. Trans.* **1995**, *23*, 605.
(340) Tzivion, G.; Luo, Z.; Avruch, J. *Nature* **1998**, *394*, 88.
(341) Meller, N.; Liu, Y. C.; Collins, T. L.; Bonnefoy-Berard, N.; Baier, G.; Isakov, N.; Altman, A. *Mol. Cell. Biol.* **1996**, *16*, 5782.
(342) Kostelecky, B.; Saurin, A. T.; Purkiss, A.; Parker, P. J.; McDonald, N. Q. *EMBO Rep.* **2009**, *10*, 983.
(343) Van Der Hoeven, P. C.; Van Der Wal, J. C.; Ruurs, P.; Van Blitterswijk, W. J. *Biochem. J.* **2000**, *347* (Pt. 3), 781.
(344) Van Der Hoeven, P. C.; Van Der Wal, J. C.; Ruurs, P.; Van Dijk, M. C.; Van Blitterswijk, J. *Biochem. J.* **2000**, *345* (Pt. 2), 297.
(345) Zha, J.; Harada, H.; Yang, E.; Jockel, J.; Korsmeyer, S. J. *Cell* **1996**, *87*, 619.
(346) Goedert, M. *Philos. Trans. R. Soc. London, Ser. B* **1999**, *354*, 1101.
(347) Spillantini, M. G.; Goedert, M. *Ann. N.Y. Acad. Sci.* **2000**, *920*, 16.
(348) Trojanowski, J. Q.; Lee, V. M. *Ann. N.Y. Acad. Sci.* **2003**, *991*, 107.
(349) Galvin, J. E.; Lee, V. M.; Trojanowski, J. Q. *Arch. Neurol.* **2001**, *58*, 186.
(350) Spillantini, M. G.; Crowther, R. A.; Jakes, R.; Hasegawa, M.; Goedert, M. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6469.
(351) Wakabayashi, K.; Yoshimoto, M.; Tsuji, S.; Takahashi, H. *Neurosci. Lett.* **1998**, *249*, 180.
(352) Spillantini, M. G.; Crowther, R. A.; Jakes, R.; Cairns, N. J.; Lantos, P. L.; Goedert, M. *Neurosci. Lett.* **1998**, *251*, 205.
(353) Gai, W. P.; Power, J. H.; Blumbergs, P. C.; Blessing, W. W. *Lancet* **1998**, *352*, 547.
(354) Okazaki, H.; Lipkin, L. E.; Aronson, S. M. *J. Neuropathol. Exp. Neurol.* **1961**, *20*, 237.
(355) Trojanowski, J. Q.; Goedert, M.; Iwatsubo, T.; Lee, V. M. *Cell Death Differ.* **1998**, *5*, 832.
(356) Takeda, A.; Mallory, M.; Sundsmo, M.; Honer, W.; Hansen, L.; Masliah, E. *Am. J. Pathol.* **1998**, *152*, 367.
(357) Lucking, C. B.; Brice, A. *Cell. Mol. Life Sci.* **2000**, *57*, 1894.
(358) Arawaka, S.; Saito, Y.; Murayama, S.; Mori, H. *Neurology* **1998**, *51*, 887.
(359) Spillantini, M. G.; Schmidt, M. L.; Lee, V. M.; Trojanowski, J. Q.; Jakes, R.; Goedert, M. *Nature* **1997**, *388*, 839.
(360) Wakabayashi, K.; Matsumoto, K.; Takayama, K.; Yoshimoto, M.; Takahashi, H. *Neurosci. Lett.* **1997**, *239*, 45.
(361) Bayer, T. A.; Jakala, P.; Hartmann, T.; Havas, L.; McLean, C.; Culvenor, J. G.; Li, Q. X.; Masters, C. L.; Falkai, P.; Beyreuther, K. *Neurosci. Lett.* **1999**, *266*, 213.
(362) Lundvig, D.; Lindersson, E.; Jensen, P. H. *Brain Res. Mol. Brain Res.* **2005**, *134*, 3.
(363) Goedert, M. *Nat. Rev. Neurosci.* **2001**, *2*, 492.
(364) Matsuo, Y.; Kamitani, T. *PLoS One* **2010**, *5*, e10481.
(365) Olsen, J. H.; Friis, S.; Frederiksen, K. *Epidemiology* **2006**, *17*, 582.
(366) Olsen, J. H.; Friis, S.; Frederiksen, K.; McLaughlin, J. K.; Mellemkjaer, L.; Moller, H. *Br. J. Cancer* **2005**, *92*, 201.
(367) Gao, X.; Simon, K. C.; Han, J.; Schwarzschild, M. A.; Ascherio, A. *Neurology* **2009**, *73*, 1286.
(368) Galvin, J. E.; Uryu, K.; Lee, V. M.; Trojanowski, J. Q. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13450.

(369) Ninkina, N. N.; Privalova, E. M.; Pinon, L. G.; Davies, A. M.; Buchman, V. L. *Exp. Cell Res.* **1999**, *246*, 308.
(370) Shibayama-Imazu, T.; Okahashi, I.; Omata, K.; Nakajo, S.; Ochiai, H.; Nakai, Y.; Hama, T.; Nakamura, Y.; Nakaya, K. *Brain Res.* **1993**, *622*, 17.
(371) Nakajo, S.; Shioda, S.; Nakai, Y.; Nakaya, K. *Brain Res. Mol. Brain Res.* **1994**, *27*, 81.
(372) Nakajo, S.; Tsukada, K.; Kameyama, H.; Furuyama, Y.; Nakaya, K. *Brain Res.* **1996**, *741*, 180.
(373) Shibayama-Imazu, T.; Ogane, K.; Hasegawa, Y.; Nakajo, S.; Shioda, S.; Ochiai, H.; Nakai, Y.; Nakaya, K. *Mol. Reprod. Dev.* **1998**, *50*, 163.
(374) Hashimoto, M.; Yoshimoto, M.; Sisk, A.; Hsu, L. J.; Sundsmo, M.; Kittel, A.; Saitoh, T.; Miller, A.; Masliah, E. *Biochem. Biophys. Res. Commun.* **1997**, *237*, 611.
(375) Iwai, A.; Masliah, E.; Yoshimoto, M.; Ge, N.; Flanagan, L.; de Silva, H. A.; Kittel, A.; Saitoh, T. *Neuron* **1995**, *14*, 467.
(376) Abeliovich, A.; Schmitz, Y.; Farinas, I.; Choi-Lundberg, D.; Ho, W. H.; Castillo, P. E.; Shinsky, N.; Verdugo, J. M.; Armanini, M.; Ryan, A.; Hynes, M.; Phillips, H.; Sulzer, D.; Rosenthal, A. *Neuron* **2000**, *25*, 239.
(377) Li, J. Y.; Henning Jensen, P.; Dahlstrom, A. *Neuroscience* **2002**, *113*, 463.
(378) Tamo, W.; Imaizumi, T.; Tanji, K.; Yoshida, H.; Mori, F.; Yoshimoto, M.; Takahashi, H.; Fukuda, I.; Wakabayashi, K.; Satoh, K. *Neurosci. Lett.* **2002**, *326*, 5.
(379) Lavedan, C. *Genome Res.* **1998**, *8*, 871.
(380) Davidson, W. S.; Jonas, A.; Clayton, D. F.; George, J. M. *J. Biol. Chem.* **1998**, *273*, 9443.
(381) Hsu, L. J.; Mallory, M.; Xia, Y.; Veinbergs, I.; Hashimoto, M.; Yoshimoto, M.; Thal, L. J.; Saitoh, T.; Masliah, E. *J. Neurochem.* **1998**, *71*, 338.
(382) Petersen, K.; Olesen, O. F.; Mikkelsen, J. D. *Neuroscience* **1999**, *91*, 651.
(383) Stefanis, L.; Kholodilov, N.; Rideout, H. J.; Burke, R. E.; Greene, L. A. *J. Neurochem.* **2001**, *76*, 1165.
(384) Jenco, J. M.; Rawlingson, A.; Daniels, B.; Morris, A. J. *Biochemistry* **1998**, *37*, 4901.
(385) Chen, Y. G.; Siddhanta, A.; Austin, C. D.; Hammond, S. M.; Sung, T. C.; Frohman, M. A.; Morris, A. J.; Shields, D. *J. Cell Biol.* **1997**, *138*, 495.
(386) Dev, K. K.; Hofele, K.; Barbieri, S.; Buchman, V. L.; van der Putten, H. *Neuropharmacology* **2003**, *45*, 14.
(387) Chandra, S.; Gallardo, G.; Fernandez-Chacon, R.; Schluter, O. M.; Sudhof, T. C. *Cell* **2005**, *123*, 383.
(388) Vila, M.; Vukosavic, S.; Jackson-Lewis, V.; Neystat, M.; Jakowec, M.; Przedborski, S. *J. Neurochem.* **2000**, *74*, 721.
(389) Lee, M.; Hyun, D.; Halliwell, B.; Jenner, P. *J. Neurochem.* **2001**, *76*, 998.
(390) da Costa, C. A.; Ancolio, K.; Checler, F. *J. Biol. Chem.* **2000**, *275*, 24065.
(391) Uversky, V. N.; Yamin, G.; Souillac, P. O.; Goers, J.; Glaser, C. B.; Fink, A. L. *FEBS Lett.* **2002**, *517*, 239.
(392) Levine, R. L.; Mosoni, L.; Berlett, B. S.; Stadtman, E. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 15036.
(393) Reddy, P. S.; Bhagyalakshmi, A. *Ecotoxicol. Environ. Saf.* **1994**, *29*, 255.
(394) Yamin, G.; Glaser, C. B.; Uversky, V. N.; Fink, A. L. *J. Biol. Chem.* **2003**, *278*, 27630.
(395) Burre, J.; Sharma, M.; Tsetsenis, T.; Buchman, V.; Etherton, M.; Sudhof, T. C. *Science* **2010**, *329*, 1663.
(396) Fujita, M.; Wei, J.; Nakai, M.; Masliah, E.; Hashimoto, M. *Neuropathology* **2006**, *26*, 383.
(397) Hashimoto, M.; Rockenstein, E.; Mante, M.; Mallory, M.; Masliah, E. *Neuron* **2001**, *32*, 213.
(398) Sakono, M.; Zako, T.; Ueda, H.; Yohda, M.; Maeda, M. *FEBS J.* **2008**, *275*, 5982.
(399) Siegert, R.; Leroux, M. R.; Scheufler, C.; Hartl, F. U.; Moarefi, I. *Cell* **2000**, *103*, 621.
(400) Martin-Benito, J.; Boskovic, J.; Gomez-Puertas, P.; Carrascosa, J. L.; Simons, C. T.; Lewis, S. A.; Bartolini, F.; Cowan, N. J.; Valpuesta, J. M. *EMBO J.* **2002**, *21*, 6377.
(401) Vainberg, I. E.; Lewis, S. A.; Rommelaere, H.; Ampe, C.; Vandekerckhove, J.; Klein, H. L.; Cowan, N. J. *Cell* **1998**, *93*, 863.
(402) Lupas, A. *Trends Biochem. Sci.* **1996**, *21*, 375.
(403) Hansen, W. J.; Cowan, N. J.; Welch, W. J. *J. Cell Biol.* **1999**, *145*, 265.
(404) Siegers, K.; Waldmann, T.; Leroux, M. R.; Grein, K.; Shevchenko, A.; Schiebel, E.; Hartl, F. U. *EMBO J.* **1999**, *18*, 75.
(405) Basha, E.; Lee, G. J.; Demeler, B.; Vierling, E. *Eur. J. Biochem.* **2004**, *271*, 1426.
(406) Lee, G. J.; Roseman, A. M.; Saibil, H. R.; Vierling, E. *EMBO J.* **1997**, *16*, 659.
(407) Horwitz, J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 10449.
(408) Ehrnsperger, M.; Graber, S.; Gaestel, M.; Buchner, J. *EMBO J.* **1997**, *16*, 221.
(409) Studer, S.; Narberhaus, F. *J. Biol. Chem.* **2000**, *275*, 37212.
(410) Kim, R.; Lai, L.; Lee, H. H.; Cheong, G. W.; Kim, K. K.; Wu, Z.; Yokota, H.; Marqusee, S.; Kim, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 8151.
(411) Haslbeck, M.; Walke, S.; Stromer, T.; Ehrnsperger, M.; White, H. E.; Chen, S.; Saibil, H. R.; Buchner, J. *EMBO J.* **1999**, *18*, 6744.
(412) Basha, E.; Lee, G. J.; Breci, L. A.; Hausrath, A. C.; Buan, N. R.; Giese, K. C.; Vierling, E. *J. Biol. Chem.* **2004**, *279*, 7566.
(413) Mogk, A.; Schlieker, C.; Friedrich, K. L.; Schonfeld, H. J.; Vierling, E.; Bukau, B. *J. Biol. Chem.* **2003**, *278*, 31033.
(414) Cashikar, A. G.; Duennwald, M.; Lindquist, S. L. *J. Biol. Chem.* **2005**, *280*, 23869.
(415) Haslbeck, M.; Braun, N.; Stromer, T.; Richter, B.; Model, N.; Weinkauf, S.; Buchner, J. *EMBO J.* **2004**, *23*, 638.
(416) Sharma, K. K.; Kaur, H.; Kester, K. *Biochem. Biophys. Res. Commun.* **1997**, *239*, 217.
(417) Haley, D. A.; Bova, M. P.; Huang, Q. L.; McHaourab, H. S.; Stewart, P. L. *J. Mol. Biol.* **2000**, *298*, 261.
(418) Leroux, M. R.; Melki, R.; Gordon, B.; Batelier, G.; Candido, E. P. *J. Biol. Chem.* **1997**, *272*, 24646.
(419) Haslbeck, M.; Ignatiou, A.; Saibil, H.; Helmich, S.; Frenzl, E.; Stromer, T.; Buchner, J. *J. Mol. Biol.* **2004**, *343*, 445.
(420) Stromer, T.; Fischer, E.; Richter, K.; Haslbeck, M.; Buchner, J. *J. Biol. Chem.* **2004**, *279*, 11222.
(421) Fu, X.; Zhang, H.; Zhang, X.; Cao, Y.; Jiao, W.; Liu, C.; Song, Y.; Abulimiti, A.; Chang, Z. *J. Biol. Chem.* **2005**, *280*, 6337.
(422) Carver, J. A.; Aquilina, J. A.; Truscott, R. J.; Ralston, G. B. *FEBS Lett.* **1992**, *311*, 143.
(423) Carver, J. A.; Esposito, G.; Schwedersky, G.; Gaestel, M. *FEBS Lett.* **1995**, *369*, 305.
(424) Caspers, G. J.; Leunissen, J. A.; de Jong, W. W. *J. Mol. Evol.* **1995**, *40*, 238.
(425) Ghosh, J. G.; Shenoy, A. K., Jr; Clark, J. I. *Biochemistry* **2007**, *46*, 6308.
(426) Lindner, R. A.; Carver, J. A.; Ehrnsperger, M.; Buchner, J.; Esposito, G.; Behlke, J.; Lutsch, G.; Kotlyarov, A.; Gaestel, M. *Eur. J. Biochem.* **2000**, *267*, 1923.
(427) Smulders, R.; Carver, J. A.; Lindner, R. A.; van Boekel, M. A.; Bloemendal, H.; de Jong, W. W. *J. Biol. Chem.* **1996**, *271*, 29060.
(428) Mukhopadhyay, R.; Hoh, J. H. *FEBS Lett.* **2001**, *505*, 374.
(429) Brown, H. G.; Hoh, J. H. *Biochemistry* **1997**, *36*, 15035.
(430) Rout, M. P.; Aitchison, J. D.; Suprapto, A.; Hjertaas, K.; Zhao, Y.; Chait, B. T. *J. Cell Biol.* **2000**, *148*, 635.
(431) Rothman, J. E. *Cell* **1989**, *59*, 591.
(432) Rothman, J. E.; Kornberg, R. D. *Nature* **1986**, *322*, 209.
(433) Hubbard, T. J.; Sander, C. *Protein Eng.* **1991**, *4*, 711.
(434) Voisine, C.; Craig, E. A.; Zufall, N.; von Ahsen, O.; Pfanner, N.; Voos, W. *Cell* **1999**, *97*, 565.
(435) Shtilerman, M.; Lorimer, G. H.; Englander, S. W. *Science* **1999**, *284*, 822.
(436) Sharma, S. K.; Christen, P.; Goloubinoff, P. *Curr. Protein Pept. Sci.* **2009**, *10*, 432.
(437) Palleros, D. R.; Reid, K. L.; Shi, L.; Welch, W. J.; Fink, A. L. *Nature* **1993**, *365*, 664.
(438) Schmid, D.; Baici, A.; Gehring, H.; Christen, P. *Science* **1994**, *263*, 971.
(439) Mayer, M. P.; Laufen, T.; Paal, K.; McCarty, J. S.; Bukau, B. *J. Mol. Biol.* **1999**, *289*, 1131.
(440) Siegenthaler, R. K.; Christen, P. *J. Biol. Chem.* **2006**, *281*, 34448.
(441) Han, W.; Christen, P. *J. Biol. Chem.* **2003**, *278*, 19038.
(442) De Los Rios, P.; Ben-Zvi, A.; Slutsky, O.; Azem, A.; Goloubinoff, P. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 6166.
(443) Goloubinoff, P.; De Los Rios, P. *Prog. Mol. Subcell. Biol.* **2007**, *32*, 372.
(444) Fenton, W. A.; Kashi, Y.; Furtak, K.; Horwich, A. L. *Nature* **1994**, *371*, 614.
(445) Braig, K.; Otwinowski, Z.; Hegde, R.; Boisvert, D. C.; Joachimiak, A.; Horwich, A. L.; Sigler, P. B. *Nature* **1994**, *371*, 578.
(446) Roseman, A. M.; Chen, S.; White, H.; Braig, K.; Saibil, H. R. *Cell* **1996**, *87*, 241.
(447) Xu, Z.; Horwich, A. L.; Sigler, P. B. *Nature* **1997**, *388*, 741.
(448) Lorimer, G. *Nature* **1997**, *388*, 720.
(449) Xu, Z.; Sigler, P. B. *J. Struct. Biol.* **1998**, *124*, 129.
(450) Winter, J.; Jakob, U. *Crit. Rev. Biochem. Mol. Biol.* **2004**, *39*, 297.
(451) Jakob, U.; Muse, W.; Eser, M.; Bardwell, J. C. *Cell* **1999**, *96*, 341.
(452) Kumsta, C.; Jakob, U. *Biochemistry* **2009**, *48*, 4666.
(453) Graumann, J.; Lilie, H.; Tang, X.; Tucker, K. A.; Hoffmann, J. H.; Vijayalakshmi, J.; Saper, M.; Bardwell, J. C.; Jakob, U. *Structure* **2001**, *9*, 377.

(454) Graf, P. C.; Martinez-Yamout, M.; VanHaerents, S.; Lilie, H.; Dyson, H. J.; Jakob, U. *J. Biol. Chem.* **2004**, *279*, 20529.
(455) Janda, I.; Devedjiev, Y.; Derewenda, U.; Dauter, Z.; Bielnicki, J.; Cooper, D. R.; Graf, P. C.; Joachimiak, A.; Jakob, U.; Derewenda, Z. S. *Structure* **2004**, *12*, 1901.
(456) Ilbert, M.; Horst, J.; Ahrens, S.; Winter, J.; Graf, P. C.; Lilie, H.; Jakob, U. *Nat. Struct. Mol. Biol.* **2007**, *14*, 556.
(457) Cremers, C. M.; Reichmann, D.; Hausmann, J.; Ilbert, M.; Jakob, U. *J. Biol. Chem.* **2010**, *285*, 11243.
(458) Hong, W.; Jiao, W.; Hu, J.; Zhang, J.; Liu, C.; Fu, X.; Shen, D.; Xia, B.; Chang, Z. *J. Biol. Chem.* **2005**, *280*, 27029.
(459) Gajiwala, K. S.; Burley, S. K. *J. Mol. Biol.* **2000**, *295*, 605.
(460) Yang, F.; Gustafson, K. R.; Boyd, M. R.; Wlodawer, A. *Nat. Struct. Biol.* **1998**, *5*, 763.
(461) Bentley, N. J.; Fitch, I. T.; Tuite, M. F. *Yeast* **1992**, *8*, 95.
(462) White, H. E.; Orlova, E. V.; Chen, S.; Wang, L.; Ignatiou, A.; Gowen, B.; Stromer, T.; Franzmann, T. M.; Haslbeck, M.; Buchner, J.; Saibil, H. R. *Structure* **2006**, *14*, 1197.
(463) Hinault, M. P.; Ben-Zvi, A.; Goloubinoff, P. *J. Mol. Neurosci.* **2006**, *30*, 249.

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