Breaking Down Order to Keep Cells Tidy

Christine Slingsby1,* and Alice R. Clark1
1Department of Biological Sciences, Crystallography, Institute of Structural and Molecular Biology, Birkbeck College, London WC1E 7HX, UK
*Correspondence: c.slingsby@mail.cryst.bbk.ac.uk
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Small heat shock proteins form large assemblies that protect cytoplasmic components when stressed. In this issue of Chemistry & Biology, Stengel et al. show that disturbing oligomer symmetry allows weak interfaces to catch intact substrate dimers.

Heat shock proteins provide quality control by recognizing and dealing with errors in the expressed proteome. This vital function contributes to cellular robustness conferring tolerance to the stresses of life by preventing cells, organelles, and tissues from becoming clogged with aggregated protein junk (Tyedmers et al., 2010). Heat shock proteins usually perform this function by acting as molecular chaperones, binding to nonnative polypeptide chains and unfolding/refolding the chain using energy stored in ATP. Members of the “small heat shock proteins” family have a short polypeptide sequence (molecular mass ~20 kDa), but they assemble into very large but polydisperse oligomers that undergo rapid subunit exchange and equilibrium dissociation. Although polydispersity has hindered the structural biology of small heat shock proteins, it is likely key to their function.

Stengel et al. (2010) have previously employed a two-stage nanoelectrospray mass spectrometry technique (nanoES MS) whereby a spectrum of distributions of macromolecular assemblies within a polydisperse ensemble is measured first, followed by precise but arduous stoichiometric measurements of components of individual assemblies. In the new work published here they replace the second experimental step with modeling from the one dimensional data using newly developed algorithms, rendering the methodology suitable for high throughput applications.

NanoES MS can measure the mass of single assemblies in the gas phase under conditions that simulate stress, such as heat. Using this new technique, the small heat shock protein under study by Stengel et al. (2012) in this issue of Chemistry & Biology is from peas. Plants must withstand a wide range of temperatures, and it has been shown that at laboratory ambient temperature, the pea small heat shock protein is a dodecamer. The crystal structure of a closely related dodecameric small heat shock protein from wheat showed it was symmetrically built from six dimers (van Montfort et al., 2001). Previously, nanoES MS techniques revealed that the pea dodecamer rearranged to a polydisperse distribution of higher-order oligomers, with a preference for an even number of monomers, coincident with it becoming an active chaperone binding thermally unstable protein substrate (Stengel et al., 2010). In agreement with the two-dimensional experimental approach, the new method described here (Stengel et al., 2012) showed that for a given ratio of chaperone to a monomeric substrate (luciferase), the most highly populated complex assembly comprised 18 chains of small heat shock protein to 1 chain of luciferase.

Symmetric protein assemblies require strict geometric constraints between complementary, often interwoven, interfaces, and they can be visualized using PDBePISA (http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html) in all their variety (Krissinel and Henrick, 2007). Changing from a regular oligomer into a polydisperse ensemble is expected to lead to an increase in exposure of interface regions, and these rearrangements likely result in the presentation of protein surfaces ready to engage with destabilized substrates. However, most small heat shock proteins across all kingdoms of life are innately polydisperse. In fact, for the first described member, α-crystallin from the eye lens, assembly polydispersity confers solubility and transparency (Clark et al., 2012) and requires some level of activation to increase its affinity for denatured substrates. It is likely, then, that regulated exposure of chaperone binding regions for misfolded protein substrates is linked to conformational change in both substrate and chaperone. In the ATP-driven machines such as HSP60 chaperone, conformational change is driven by ATP-binding and hydrolysis, leading to substrate binding and release (Clare et al., 2012). The ability of small heat shock proteins to manage without ATP binding is in keeping with the energetically cheap exposure of binding sites in these flexible oligomeric assemblies. The new nanoES MS work now embellishes this view with the finding...
that when challenged with two destabi-
lized substrate dimers, the higher-order
small heat shock oligomers preferentially
bind them as dimers.

The crystal structure of the wheat small
heat shock protein dodecamer showed
how edge β strands in the α-crystallin
β-sandwich domain, common to all small
heat shock proteins, act as surfaces for
building the assembly by interacting with
hydrophobic flanking sequence exten-
sions coming from partner subunits
(Figure 1A). One view is that these exten-
sions mimic the binding of unfolded
substrate to binding sites formed by the
α-crystallin domain, and that by distur-
bating the symmetry of the assembly (by heat, for
example), binding sites are unmasked.
Alternatively, the released hydrophobic
extensions themselves may be the
substrate binding sites. In terms of the
conformational state of the substrate, it
is unclear whether the parts that interact
with the chaperone are partially unfolded
protein regions representing kinetically
stable (un)folding intermediates or are
highly hydrophobic segments buried
deep within the native protein fold, hence
requiring major unfolding of the substrate
protein before being bound to the
chaperone. The new work described by
Stengel et al. (2012) favors an early unfold-
ing state for two dimeric substrates
tested. For example, the citrate synthase
dimer, the first enzyme of the Krebs
cycle, forms a closed state when two
molecules of acetyl coenzyme A bind to
the dimer interface, relaxing to an open state
on coenzyme release (Figure 1A) (Reming-
ton et al., 1982). The open state is likely to
be the starting model for unfolding, with
the part of the chain that changes on
binding coenzyme a hot candidate for
early melting (Figure 1B). The idea is that
under heat stress, the partially unfolded
yet largely dimeric enzyme would bind
to disordered interface regions of the
higher-order plant chaperone (Figure 1B).

These new results are important,
because details of how a chaperone
assembly interacts with a misfolded
substrate at an atomic level are difficult
to determine but are central to our under-
standing of the protective mechanism. In
terms of human health, the family of small
heat shock proteins appears to have
acquired broad protective roles, particu-
larly in long-lived cells such as eye
lens, myofibrils, and neuroglia. Members
that are upregulated during stress are
biomarkers for a range of muscular,
vascular, and neurodegenerative dis-
eases. The challenge is to boost levels
and activity of small heat shock proteins
in long-lived cells and tissues stressed
by disease, but to block performance
in cancer cells, especially after toxic
therapy.

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