Dynamical structure of αB-crystallin

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Abstract

The human small heat-shock protein αB-crystallin is an extremely difficult molecule to study, with its inherent structural dynamics posing unique challenges to all biophysical and structural biology techniques. Here we highlight how the polydispersity and quaternary dynamics of αB-crystallin are intricately inter-twined, and how this can impact on measurements of the oligomeric distribution. We show that, in spite of these difficulties, considerable understanding of the varied fluctuations αB-crystallin undergoes at equilibrium has emerged in the last few years. By reporting on data obtained from a variety of biophysical techniques, we demonstrate how the αB-crystallin solution ensemble is governed by molecular motions of varying amplitude and time-scales spanning several orders of magnitude. We describe how these diverse measurements are being used to construct an integrated view of the dynamical structure of αB-crystallin, and highlight areas that require further interrogation. With its study motivating the refinement of experimental techniques, and the development of new approaches to combine the hybrid datasets, we conclude that αB-crystallin continues to represent a paradigm for dynamical biology.

1. αB-crystallin: a refractory refractor

αB-crystallin is a member of the small heat-shock protein (sHSP) family of molecular chaperones (Horwitz, 1992; Jakob et al., 1993; Muchowski et al., 1997). It is expressed in particularly high abundance in the eye lens, but is found throughout the human body (Lowe et al., 1992). In line with the canonical chaperone function of the sHSPs, αB-crystallin can bind partially unfolded proteins and prevent their accumulation into irreversible and potentially toxic aggregates (Basha et al., 2012; Hilton et al., 2013b; McLaourab et al., 2009). In vitro, this activity strongly inhibits the formation of both amorphous and fibrillar aggregates of a variety of model targets (Ecroyd and Carver, 2009). Consequently, αB-crystallin is associated with insoluble protein deposits in several important amyloid diseases, including Alexander’s, Alzheimer’s and Parkinson’s, as well as variants leading to myofibrillar myopathy, desmin-related cardiomyopathy, and dilated cardiomyopathy (Boncoraglio et al., 2012; Ecroyd and Carver, 2009). These observations indicate that αB-crystallin is an important member of the human protein homeostasis network (Balch et al., 2008), where it acts not to refold misfolded proteins nor disaggregate protein deposits, but as an ATP-independent overflow reservoir that sequesters destabilized proteins in situations that might otherwise overwhelm the ATP-dependent chaperone machinery (Hartl et al., 2011; Richter et al., 2010).

In the human eye lens, αB-crystallin remains soluble over the organism’s lifespan at very high concentrations (>300 mg/mL of total lens protein) (Tardieu, 1988), providing a refractive index sufficient for vision, while concomitantly inhibiting the formation of protein aggregates that would otherwise scatter light (Bloemendal et al., 2004; Horwitz, 1993). A number of miss-sense mutations in αB-crystallin lead to cataract, revealing its importance for eye-lens transparency (Clark et al., 2012), although a homozygous knock-out in mice (Brady et al., 2001) and a severe truncation in humans (Forrest et al., 2011) do not lead to cataract formation, possibly because the more abundant αA-crystallin can compensate for the loss of αB-crystallin in the eye lens. This suggests that congenital cataract formation represents a deleterious gain of function by αB-crystallin. Accordingly, it evolved by acquiring properties in order to remain soluble while performing its functions in the eye lens (Platigorsky and Wistow, 1991), with the corollary that its does not crystallize, even at extremely high concentrations and over long time frames (Bloemendal et al., 2004; Horwitz, 1993). Chief amongst these characteristics is that αB-crystallin does not have a single defined quaternary structure, but rather populates a wide range of stoichiometries at equilibrium. This polydispersity is also apparent for mammalian sHSPs not present in the eye-lens (and may prove to be the rule rather than

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the exception across the whole sHSP family), and has inevitably frustrated attempts at structure determination of αB-crystallin oligomers by crystallographic means. Similarly, other structural biology techniques such as nuclear magnetic resonance spectroscopy (NMR), electron microscopy (EM), or small angle X-ray scattering (SAXS), either do not directly report on quaternary architecture, or require knowledge of oligomeric populations for accurate model building. The question of quaternary assembly therefore lies at the heart of any discussion on the solution structure of αB-crystallin, and a clear understanding of its stoichiometry at equilibrium is a prerequisite for the construction of candidate structures. Although details have begun to emerge over the past decade, the complete structure of αB-crystallin remains elusive and one of the most coveted targets in structural biology.

2. Coupled quaternary dynamics and polydispersity of αB-crystallin

An understanding of the quaternary assembly of αB-crystallin rests on two key observations: the protein exists as an ensemble of oligomeric states, and these states are capable of inter-conversion. An accurate biophysical description of αB-crystallin therefore requires quantitative knowledge of two fundamental properties: the oligomeric distribution populated by αB-crystallin at equilibrium, and the rate of its “recycling” via subunit exchange. As we demonstrate below, these quantities are intrinsically inter-twined, with the consequence that subunit exchange can profoundly influence measurements of αB-crystallin stoichiometries.

2.1. The extent of polydispersity

αB-crystallin’s polydispersity was noted almost fifty years ago in analytical ultracentrifugation (AUC) experiments (Spector, 1965), nearly three decades earlier than the first unequivocal observations of subunit exchange (Thomson and Augusteyn, 1989; van den Oetelaar et al., 1990). Much of the research in intervening years had been taken to suggest that the population of αB-crystallin oligomers does not represent a chemical equilibrium. The reason for this deduction, which we now know to be inaccurate, can be readily understood in terms of the experimental time-scale and the rate of subunit exchange. For instance, different average molecular weights were obtained when AUC was performed on aliquots from either the front or the tail of a size-exclusion chromatography (SEC) peak (Spector et al., 1971). However, the SEC separation and AUC analysis was performed at 4 °C, at which temperature it actually takes weeks to re-establish equilibrium after fractionation. This phenomenon has been exploited to isolate oligomeric sub-populations of αB-crystallin by using SEC at low temperature, and submit them to downstream analysis. In this way EM data were obtained that revealed substantially different sizes of spherical particles for different fractions (Siezen et al., 1978). Two decades later, cryo-EM studies with subsequent single-particle analysis resulted in poorly defined globular shapes for the oligomers, further confirming αB-crystallin’s heterogeneity (Haley et al., 1998), but stopped short of detailed reconstructions due to the need for classification strategies that can accurately separate individual stoichiometries (Haley et al., 1999).

The advent of nanoelectrospray mass spectrometry (MS) techniques that allow the interrogation of intact protein complexes in the gas-phase (Hilton and Benesch, 2012) enabled the first measurements of αB-crystallin that resolved individual stoichiometries (Aquilina et al., 2003). The resulting distributions revealed an ensemble of oligomers containing between 10 and 50 subunits, centred approximately on a 28 mer (Fig. 1A).

Interestingly, the ability to resolve individual stoichiometries demonstrated that both oligomers with odd and even numbers of subunits are present, with the latter slightly more abundant (Aquilina et al., 2004). The presence of odd-numbered oligomers was unexpected as it revealed that monomers play an important role in the assembly of αB-crystallin, while sHSPs had in general been thought to be constructed exclusively from dimeric building blocks (van Montfort et al., 2001). Notably, though displaying a much higher resolution of separation, the MS data are in good agreement with multi-angle laser light scattering (MALS) and EM measurements, when effort is made to size-sort the oligomers, respectively by means of SEC or in silico (Fig. 1A). However, using similar solution-based techniques, other researchers have arrived at apparently more homogeneous distributions, even leading to calls in recent years to question whether αB-crystallin is poly-disperse at all. This led to the unsatisfying situation that the technique and experimental conditions used appeared to strongly influence the measurement αB-crystallin’s quaternary assembly.
2.2. Quantifying the rate at which αB-crystallin exchanges subunits

It is our contention that subunit exchange lies at the heart of many of the inconsistencies in estimates of the oligomeric distribution populated by αB-crystallin and, conversely, can therefore be used to resolve them. Such quaternary dynamics had been hypothesised in the late 1970s (Siezen et al., 1978), but were unverified experimentally only much later by using isoelectric focussing. Through exploiting the different charges of αA- and αB-crystallin in solution, the hetero-oligomerisation of the two pure isofoms was observed (Thomson and Augusteijn, 1989), and the evolving mixture monitored (van den Oetelaar et al., 1990). This discovery provided an immediate explanation for several previously puzzling observations: the re-equilibration of the oligomeric distribution after fractionation (Siezen and Owen, 1983), the “charge micro-heterogeneity” of α-crystallin (Siezen et al., 1978), and gradual shifts in its molecular weight during ageing (Bessemens et al., 1983). Importantly, the fact that αB-crystallin exchanges subunits and is co-expressed with αA-crystallin in the eye lens, and with other sHSPs in non-lenticular tissues, means that oligomers consisting solely of αB-crystallin subunits probably do not exist at significant abundances in vivo (Mynrikov et al., 2012). The rate of subunit exchange revealed more precisely a few years later by using more sensitive fluorescence techniques, in which homo-oligomers of αA-crystallin labelled with either a fluorescence donor or acceptor were found to reach an equilibrium of hetero-oligomers within an hour at 37 °C (Bova et al., 1997). Further refinement in the ability to monitor subunit exchange came from anion exchange chromatography (Michiel et al., 2009), and MS experiments (Aquilina et al., 2005), both of which have the advantage of not requiring a potentially disruptive bound label. The attraction of the MS experiment is that, by combining iso-structural samples (differentiated through metabolic labelling), the precise change in mass can be monitored, without being obscured by potential conformational effects. Measured in this way, the subunit exchange rate of αB-crystallin, in ammonium acetate buffer over the pH range 5–9 and at temperatures between 24 and 50 °C, was found to vary by four orders of magnitude (Baldwin et al., 2011b).

2.3. Fast subunit exchange can compromise measurements of polydispersity

The rate of subunit exchange is a crucial parameter for αB-crystallin oligomer distributions, because it can indirectly affect measurements of the size of αB-crystallin oligomers. As described in Section 2.1, early AUC data were erroneously interpreted to conclude that the different oligomeric states of αB-crystallin are not at equilibrium, because the timescale of the experiments was far shorter than the rate of oligomeric inter-conversion. Accordingly, the converse situation must also hold true: if the rate of subunit exchange is faster than the timescale of the experiment, then unanticipated effects may occur.

To illustrate this effect we have obtained SEC traces for a modified version of αB-crystallin (αB3D), in which three serines have been mutated to aspartic acids to mimic phosphorylation (Ito et al., 2001). This protein populates a polydispersely ensemble centred on a 28 mer that is broadly similar to the wild type but with a reduced "preference" for even-numbered oligomers (Ecroyd et al., 2007), and exchanges subunits an order of magnitude faster than the wild-type (Peschek et al., 2013). At 4 °C αB3D elutes as a single broad but symmetric peak corresponding to a mass of approximately 600 kDa on a calibrated SEC column (Fig. 1B, red trace), consistent with published SEC-MALS data (Ecroyd et al., 2007). Performing the experiment at 27 °C results in an appreciable change in the SEC trace, with the peak having shifted to longer elution times and being notably asymmetric (Fig. 1B, blue trace). This change in elution time translates into an apparent mass decrease in excess of 150 kDa (>25%), yet mass spectra obtained after SEC at both temperatures are identical. This seeming conflict is readily resolved by noting that during SEC at 27 °C subunit exchange is much more rapid that at 4 °C. At this higher temperature, the dissociation rate of subunits from the αB3D oligomers is large enough that they undergo a significant number of dissociation and re-association steps over the 25 min SEC run, each of which acts to retard them. Cumulatively this acts to causes a displacement, broadening, and skewing of the elution profile, which could easily be misinterpreted as a shift in oligomeric state to smaller stoichiometries (Cann et al., 1988; Gilbert and Jenkins, 1956; Stevens, 1989; Winzor and Scheraga, 1963).

In general, therefore, techniques based on mass transport, such as SEC, AUC (both sedimentation velocity and sedimentation equilibrium experiments) and natal gels, will give potentially misleading results if the rate of oligomer dissociation is comparable to or faster than the timescale of the measurement. The biophysical concept and mathematical formulation of this phenomenon is well established, even if not very accessible (Cann and Goad, 1970; Nichol and Winzor, 1972). However, together with the sensitivity of the αB-crystallin subunit exchange rate to solution conditions (Aquilina et al., 2013; Baldwin et al., 2011b) and mutation (Hilton et al., 2013a; Michiel et al., 2009; Peschek et al., 2013), neglect of this effect provides an explanation for at least some of the discrepancies in oligomeric state in the literature.

2.4. Fast kinetics require faster measurements of the oligomeric distribution

Nanoelectrospray MS, while also a mass-transport approach, can be thought of as a “desolvation-jump” experiment: proteins are delivered from solution into the vacuum of the instrument in a rapid process during which individual protein molecules are isolated from each other on the order of milliseconds (Ruo et al., 2006). The speed at which protein molecules are separated from one another effectively “freezes” the solution-phase oligomeric distribution, and allows for accurate quantification of the stoichiometries that are populated at equilibrium (Benesch and Ruo et al., 2011). In this way, the effect on stoichiometry caused by perturbations known to dramatically increase the subunit exchange rate of αB-crystallin can be readily assessed. We have collated here a series of oligomeric distributions for αB-crystallin, including the wild-type protein in different solution conditions (Baldwin et al., 2011b), and a range of point mutants (Aquilina et al., 2004; Hilton et al., 2013a). It is apparent that in all these cases the average number of subunits comprising the oligomeric ensemble remains approximately constant (Fig. 1C). Functionally, this might be important to maintain oligomeric integrity during stress conditions, or to ensure that post-translational modifications accumulated during ageing do not lead to aggregation and, as a result, opacity of the eye lens.

This data set reveals one parameter that does change significantly however: the ratio of even- to odd-numbered oligomers. For instance, at pH 7 this [Even]/[Odd] ratio is 1.3, but at pH 5 is decreased by 22% (Fig. 1C). This reveals the variable dimeric “substructure” of the oligomers (Aquilina et al., 2004), and can be interpreted in terms of a modulation of the strength of the dimer interface (Baldwin et al., 2011b). It also provides a rationale post hoc as to why αB-crystallin is isolated from eye lenses at low temperatures (Aquilina et al., 2003) does not display the same preference for even-numbered oligomers as the recombinant protein (Aquilina et al., 2004). Namely, notwithstanding any covalent modifications they may cause (Volk et al., 1997), the high concentrations of
denaturant that are typically used in the chromatographic separation of αβ- from αA-crystallin and post-translationally modified forms stimulate complete dissociation of the oligomers and unfolding of the constituent monomers (van den Oetelaar and Hoenders, 1987). If equilibrium is not re-established thereafter, for instance if refolding and reassembly is carried out at low temperature, altered chaperone activity can be observed (Benesch et al., 2008). This experiment demonstrates how it is necessary to not only consider the dissociation and association kinetics relative to the timescale of the experiment, but also whether the distribution is already at equilibrium, or if it may be kinetically trapped. Despite these caveats, it is reassuring to note that when measurements of stoichiometry and subunit exchange rate are combined, many of the apparent conflicts in the literature can be resolved. This has led to an emerging consensus of αβ-crystallin populating a range of stoichiometries between 10 and 50 subunits, with subunit exchange kinetics that are highly dependent on solution conditions and modification of the protein.

3. Kinetic and allosteric determinants of the αβ-crystallin ensemble

Ever since the polydispersity of αβ-crystallin was established, many qualitative models have been put forward in an attempt to rationalise its size distribution. The majority of these models have sought explanations from a structural standpoint: for instance, a “micellar” quaternary architecture for αβ-crystallin oligomers, because micelles themselves exhibit size heterogeneity (Thomson and Augusteyn, 1989; Walsh et al., 1991). Although such structural hypotheses may provide a rationale as to why αβ-crystallin is polydisperse, they make few predictions as to the nature of the distribution and its response to physical perturbation. As our knowledge of the equilibrium properties of αβ-crystallin has evolved, and in particular our understanding that polydispersity and quaternary dynamics are linked, more predictive models can be constructed. In this way, a quantitative biophysical model was formulated to estimate the shape of the distribution from purely kinetic considerations, with minimal assumptions as to the proposed structures of the oligomers (Baldwin et al., 2011b).

3.1. The exchange model for the origin of αβ-crystallin polydispersity

The “exchange model” is based on the fundamental hypothesis put forward in the 1970s that “existing aggregates [oligomers] could be in continuous equilibrium with their monomeric subunits allowing random exchange of polypeptide chains between aggregates [oligomers]” (Siezen et al., 1978). In this way the distribution can be described as an equilibrium of many coupled exchange reactions, each consisting of oligomers of a particular stoichiometry gaining or losing monomeric subunits. The biophysical framework of the model essentially relies on three simple premises (Baldwin et al., 2011b): 1) the association (“on”) rate constant for a monomer binding to an oligomer is invariant with stoichiometry. In other words, a free monomer is equally likely to associate with oligomers of all sizes in the distribution, in a manner that depends solely on the concentrations of the two partners. 2) The dissociation (“off”) rate of each monomer in any oligomer is directly proportional to the stoichiometry of the oligomer. In other words, all the monomers in the polydisperse ensemble have the same probability of dissociating, but larger oligomers are more likely to lose a monomer at any given time simply because they comprise more. These assumptions (1 and 2) combine, when the on-rate is faster than the off-rate, to make both very small and very large oligomers short lived, naturally leading to a bell-shaped distribution with defined maximum.

This is in accordance with low-resolution measurements of the polydisperse ensemble that provide a mean stoichiometry and associated width of the distribution (Fig 1A). However, as described in Section 2.4, this bell-curve is not necessarily smooth: at many conditions (including the wild-type protein at pH 7) oligomers containing an even number of subunits are more abundant than their “odd” counterparts (Fig. 1C). This observation led to the introduction of an additional assumption into the exchange model: 3) monomers make two sets of contacts, one to form a dimer (i.e. intra-molecular to the dimer, ascribed the free energy ΔG_D) and another between dimers, such as they build the oligomers (inter-molecular to the dimer, ΔG_D). The oligomers are consequently assembled such that all monomers make the maximum number of contacts to minimize both free energies (Fig. 2A).

The exchange model allows the shape of the distribution of αβ-crystallin ensembles to be fully described in terms of just these two quantities. Physically, this requires that the conformation of a monomer must be similar across all different sizes of oligomers, a proposition that is validated by both solid- and solution-state NMR data that find single peaks for most residues despite the heterogeneous ensemble (Baldwin et al., 2011a; Jehle et al., 2010, 2009). Another prediction of the model is that the average oligomer size should be approximately constant over a wide concentration range, an observation that has been made by means of AUC (Loutas et al., 1996; Peschek et al., 2009) and SEC (Horwitz, 2009) in the range 10^{-6}–10^{0} g/mL. Importantly, the exchange model does not assign the interactions to particular regions of the protein, but relies solely on conceptual definitions. In general the model, despite its simplicity, reproduces the available data extremely well, and provides an important biophysical link between the quaternary dynamics and polydispersity of αβ-crystallin.

3.2. Allosteric communication between αβ-crystallin interfaces

The practical utility of the exchange model lies in its ability to quantify changes in the thermodynamics and kinetics of αβ-crystallin upon perturbation, for instance through change in solution conditions, post-translation modification, or mutation. Here we have interrogated our set of oligomeric distributions of αβ-crystallin (Fig. 1C) to extract the free energies of the inter- and intra-dimer interfaces, and compared this to the wild-type protein at standard conditions (Fig. 2B). Changes significantly greater than the thermal energy in the strength of these interfaces are observed. Most strikingly, however, is that there is a clear negative linear correlation between ΔΔG_F and ΔΔG_D. In other words, weakening of one interface strengthens the other, and vice versa. This is a remarkable phenomenon, as it demonstrates that despite their separation, there is a flow of energy, i.e. allostery, between these two sites. The shift in the relative contributions of ΔG_D and ΔG_F to the overall stability of the oligomers then produces changes in the ratio of even- to odd-numbered oligomers, with larger contributions of ΔG_D leading to a stronger bias towards the former. A notable consequence of this allosteric communication is that the sum of the two free energies (ΔG_F + ΔG_D) remains approximately constant, with the corollary that the overall stoichiometry does not change significantly upon perturbation of the interfaces that are responsible for oligomerization (Fig. 1C).

4. Dynamical structure of the αβ-crystallin interfaces

One difficulty in delineating the mechanism of allostery is that it is not fully understood how precisely the αβ-crystallin dimers are held together in the oligomers (i.e. what defines ΔG_F structurally).
Indeed, it appears that there are contributions from at least both the N- and C-terminal regions of β-crystallin. While it has only been possible to get relatively sparse direct structural information about the former due to its conformational heterogeneity (Jehle et al., 2010, 2011), considerable insight has been obtained as to the structure and dynamics of the latter.

4.1. The C-terminal tail cross-links dimers into oligomers

The crystal structures of a construct of β-crystallin truncated of its N-terminal region revealed the C-terminal tail to bind onto an adjacent monomer, effectively amounting to cross-links between dimers (Laganowsky et al., 2010). This inter-monomer interaction was validated in the full-length protein by means of solid-state NMR experiments (Jehle et al., 2010), and has been replicated through the development of a system composed of the β-crystallin core domain (ACD) dimer together with a peptide mimicking its palindromic C-terminal region. This reductionist system has proven very useful, both as a means for improving crystallographic resolution (Hochberg et al., 2014), and assaying the affinity of the C-terminal interaction (Delbecq and Klevit, 2012; Hilton et al., 2013a).

The details of the contacts made at this interface have proven extremely interesting. The structural data demonstrate that the tail can bind diagonally across one side of the β-sandwich, forming hydrogen bonds with the β4 and β6-strands, with a conserved IXI motif docking into a hydrophobic groove between them. Interestingly, solution state NMR experiments showed that, within the oligomers at physiological temperature, the IXI motif is bound in this way with a population of only about 2%. The remainder of the time it is detached, but largely remains localised to the vicinity of the groove by the upstream interactions the tail makes with the β-strands (Baldwin et al., 2011a). Interestingly, the proportion of bound versus unbound states is strongly temperature dependent (Baldwin et al., 2012), and can be correlated very well with the rate of monomer dissociation from the oligomer (Baldwin et al., 2011a), suggesting a regulatory function (Jehle et al., 2010).

4.2. C-terminal transitions mediate oligomer disassembly

As such, somewhat counter-intuitively, binding of the IXI is linked to dissociation of a monomer (Baldwin et al., 2012). Reconciliation of this apparent paradox comes from experiments in which the binding of a palindromic peptide, containing the IXI from the C-terminus (Fig. 3A), to the ACD was quantified. The ACD populates an equilibrium of monomers and dimers at low micromolar concentrations (Fig. 3B), so the advantage of a MS-based experiment over more traditional titration approaches is that dissociation constants can be obtained for each co-populated binding stoichiometry (Hilton et al., 2013a). In this manner we found that the ACD dimer binds a first peptide equivalent with a dissociation constant ($K_D$) of approximately 70 μM, and a second at a substantially higher value (Fig. 3B, upper panel). The average of these $K_D$s is in close accord with the ensemble measurement obtained by means of NMR (Delbecq and Klevit, 2012). The discrepancy in affinity for binding the first and second peptide equivalent is much increased if the peptide incorporates the “extension” (residues 165–175) of the C-terminal tail (Fig. 3A), which is known to be highly dynamic (Carver et al., 1992), yielding a $K_D$ that is four

![Fig. 2. Allosteric interaction between two interfaces in β-crystallin.](image)
which binding of the palindromic peptide induces chemical shift perturbations in spectra of the ACD (Delbecq and Klevit, 2012). Even though these perturbations are relatively small, they propagate from the peptide-binding site all the way to the dimer interface (Fig. 3C). This could conceivably hint at the allosteric pathway through which energy is transferred to the dimer interface upon binding of the C-terminal tail.

4.3. Variable binding of the C-terminal tail

The β-crystallin C-terminus has the capacity for considerable variation in its interaction with the ACD. Notably, two different inter-molecular orientations across the β-sandwich have been observed (Fig. 4A), running either parallel or antiparallel to the β8 strand, for both βB- and αA-crystallin (Hochberg et al., 2014; Laganowsky et al., 2010) (Fig. 4B). This bi-directional binding has also been observed in solution, with NMR measuring the three possible combinations of two peptides binding to a dimer approximately at the 1:2:1 ratio (both parallel: one parallel and one antiparallel: both antiparallel) that would be expected statistically if both binding modes were equally favourable (Delbecq and Klevit, 2012).

Notwithstanding amino-acid chirality, this phenomenon can be explained by the palindromic sequence of the peptide leading to pseudo-equivalent interactions in either direction. However, it is unlikely that the two orientations are equally favourable in full-length βB-crystallin, due to conformational restrictions on the tail made by the hinge-loop that connects it to the ACD (Treweek et al., 2010). This has been demonstrated by alanine scanning of the palindromic sequence, which revealed that mutation of 156ERT158 to three alanines had a five-fold larger destabilisation effect on the interface than equivalent mutation of 162TRE164 (Hilton et al., 2013a). Another intriguing hypothesis as to conformations adopted by the C-terminus comes from a structure of zebrafish αA-crystallin, in which the tail binds to the same site, but intra-molecularly, by looping back onto itself (Laganowsky and Eisenberg, 2010) (Fig. 4C). This binding mode has not yet been observed directly for βB-crystallin but, even when noting that the zebrafish αA-crystallin has a hinge loop three amino acids longer than human βB-crystallin, is geometrically feasible.

In order to relate these crystallographic data to our understanding of full-length βB-crystallin dynamics we can consider spectroscopic data that indicate that the most populated ("ground") arrangement of the C-terminal tail is inter-molecular (Baldwin et al., 2011a; Jehle et al., 2010; Pasta et al., 2004). Oligomer dissociation then necessitates the breaking of two C-terminal interactions, as any given monomer donates one tail and accommodates another. Accordingly, subunit exchange rates are predicted very well by coincident transitions of two C-terminals from their ground state to an excited one (Baldwin et al., 2011a). While it is clear that in this excited state the IXI is bound, whether this is inter- or intra-molecular is not known (Baldwin et al., 2012).

As a means to reconcile the currently available data, we put forward the following hypothesis. In its ground state the C-terminal tail makes an inter-molecular contact with the IXI localized over, but unbound to, a binding pocket on an adjacent monomer. This state is in equilibrium with two lowly populated ones, in both of which the IXI is bound to the hydrophobic groove, but one is inter-molecular, and the other intra-molecular (Fig. 5, middle panel). In the event of two tails on neighbouring monomers transitioning to the intra-molecular configuration a monomer is released, catalysed by the allosteric communication between the two interfaces. An alternative rationale could be that two coincident transitions from the ground state to the inter-molecular IXI bound state could also lead to dissociation of the monomer if they caused a
conformational distortion that compromised other inter-dimer contributions to ΔG‡ made, for instance, by the N-terminus (Baldwin et al., 2012). In either case, the C-terminal dynamics would effectively rate-limit the subunit exchange of oligomers.

4.4. Polymorphic dimeric interfaces

The dynamic nature of αB-crystallin appears not to be confined to transitions of its terminal regions, but also to its protomeric building block, the ACD dimer. When, after decades of frustrating crystallographers, the structure of the ACD was finally solved it presented the community with several surprises that had been hinted at previously (Feil et al., 2001; Jehle et al., 2009; Koteiche and McHaourab, 1999). Specifically, the dimeric interface of αB-crystallin (and other mammalian sHSPs) is markedly different to that from lower organisms, in that its β6 and β7 strands combine into one long “β6−7” strand that pairs in an antiparallel orientation (Bagnéris et al., 2009). Notably there is substantial variation of angle between the two β6−7 strands of dimers in the different atomic-resolution structures of αB-crystallin (Clark et al., 2011; Hochberg et al., 2014) (Fig. 4D). Furthermore, the registration state of this dimer interface varies between different structures, with three different registers having been observed for αB-crystallin (Bagnéris et al., 2009; Hochberg et al., 2014; Laganowsky et al., 2010). These are shifted relative to one another by two residues in the β-sheet and are termed AP1-AP10 (Laganowsky et al., 2010), from the most compact to the most extended, respectively (Fig. 4E).

Whether all three registration states are populated in solution, or are merely crystallographic artefacts, is an intriguing question. Solid state NMR experiments of αB-crystallin oligomers only gave evidence for the AP1 state (Jehle et al., 2010), while both NMR (Delbecq and Klevirt, 2012; Jehle et al., 2009) and ion mobility MS (Hochberg et al., 2014) investigations also failed to detect alternative registers. Nonetheless, the possibility of lowly populated, rapidly exchanging registration states cannot be ruled out, especially in the context of the oligomer. What is clear however is that the dimer interface is weak, with a kD on the order of a few micromolar (Fig. 3B), and that the ACD dimer exchanges subunits extremely rapidly, with an off-rate >10^3 s^-1 at 4 °C (Hochberg et al., 2014). This suggests that no large rearrangements are necessary for two monomers to assemble into a dimer, and paint the picture of a dynamic and labile dimer interface. Indirect evidence that such flexibility may be important comes from studying the interaction of αB-crystallin with aggregating target proteins. We recently found that, by using an engineered disulphide bond (Hochberg et al., 2014), the ACD of αB-crystallin inhibits the aggregation of the amyloidogenic peptide Aβ1-42 somewhat more effectively if it is covalently locked into AP1, suggesting that the exact conformation of the dimer interface may yet prove to be relevant to chaperone function.

5. Integrative biology of αB-crystallin

In this review we have attempted to paint the dynamical landscape of αB-crystallin. A picture has emerged in which we have to consider fluctuations varying in both timescale and amplitude. These include, but are unlikely to be limited to: the subunit exchange of oligomers mediated by their dissociation (rate constants at physiological pH and temperature ranges: 10^-1<k<10^-2 s^-1); transitions of the C-terminal tails (10^2 > k > 10^1 s^-1); and the breaking of the dimer interface in oligomers (k > 10^3 s^-1 at 4 °C) (Fig. 5). All these dynamics appear to be key features of αB-crystallin, but do they, together with our knowledge of the distribution of stoichiometries, and atomic structures of the ACD dimer, allow us to construct useful models of the assembled protein?

5.1. Forging consensus structures of αB-crystallin oligomers

Fundamentally, any structural model should satisfy as many experimental observations as possible, but ideally also provide a rationale for previously unexplained aspects of the protein under investigation. Similarly, it is of course desirable that the model provides hypotheses for further testing. Over the last decades many
Fig. 5. Dynamic movements of αB-crystallin in solution. A model of how the dynamic motions of αB-crystallin at three different time scales are inter-related. The C-terminus is localized to an adjacent dimer with the IXI unbound for the majority of time, but converts on the millisecond time scale into a bound conformation that can be either inter- or intra-molecular (middle panel). This tail-binding may induce distortions in the dimer interface that lead to rearrangements including breaking of the dimer interface or registration shifts (lower panel). Together these two effects determine the dimer interface that lead to rearrangements including breaking of the dimer interface or registration shifts. For clarity, only pseudo-atomic models of even-numbered oligomers are shown, although subunit exchange proceeds via the dissociation of monomers.

5.2. αB-crystallin and the dynamics-function paradigm

Over the past few years significant progress has been made in describing the structure and dynamics of the αB-crystallin ensemble, stemming directly from exciting technological advances in the bioanalytical approaches used. While all the ensuing data have not yet been reconciled into a fully coherent picture, we hope to have shown here how further progress crucially depends on a clear understanding of αB-crystallin’s complex dynamics that, if neglected, can lead to measurements that do not reflect the equilibrium state of the polydisperse ensemble. This principle will certainly be equally important to other areas of structural biology, as increasingly complex and dynamic protein assemblies are investigated.

The emerging view of a dynamic ensemble of αB-crystallin oligomers poses important questions about its functional significance. Subunit exchange has been characterized in other proteins, including the β-crystallins of the eye lens (Hejtmancik et al., 1997; Srivastava et al., 2009), but in much less detail than in the sHSPs. Similarly, polydisperse yet globular quaternary architectures are not well documented outside of the sHSP family, so analogies to better-understood protein families cannot be easily drawn. It has long been assumed that these properties must be integral to αB-crystallin chaperone function, but recently some evidence is emerging that this relationship may not be straightforward. For instance, αB-crystallin can function efficiently as a chaperone in forms that either cannot oligomerise beyond dimers (Hochberg et al., 2014), or cross-linked into large oligomers that cannot dissociate (Augusteyn, 2004). The relationship between the characteristic quaternary dynamics and structure of αB-crystallin and its cellular functions represent the next frontier of research in the field, where a particular challenge will be to relate the in vitro data to the situation in the cell. The data that have recently emerged on αB-crystallin, and we have described here, will provide excellent springboard for these investigations, and as a means to probe the emerging dynamics-function paradigm in structural biology.

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