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Manuscript Title:

The Chaperone α B-Crystallin Deploys Different Interfaces to Capture an Amorphous and an Amyloid Client

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Abstract

Small heat-shock proteins, among them α B-crystallin (α B), play an important role in protein homeostasis, as their ATP-independent chaperone activity inhibits uncontrolled protein aggregation. Mechanistic details of human α B, in particular in its client-bound state, were elusive so far due to the high molecular weight and the heterogeneity of these complexes. Here we provide structural insights into this highly dynamic assembly and show – using state-of-the-art NMR spectroscopy – that the α B complex is assembled from asymmetric building blocks. Interaction studies demonstrate that the fibril-forming Alzheimer's disease $A\beta_{1-40}$ peptide preferentially binds to a hydrophobic edge of the central β -sandwich of α B. By contrast, the amorphously aggregating client lysozyme is captured by the partially-disordered N-terminal domain of α B. We suggest that α B utilizes its inherent structural plasticity to expose distinct binding interfaces to interact with a wide range of structurally variable clients.

Introduction

Proteins are the protagonists of life with their three-dimensional folds encoding a myriad of cellular functions. Stress stimuli like elevated temperature can compromise the structural integrity of proteins and increase the population of partially-unfolded protein states. The concomitant exposure of hydrophobic residues causes non-native interactions that finally result in protein aggregation. As a general protective mechanism, the cell enhances the expression of small heat-shock proteins (sHSPs).¹ These ATP-independent molecular chaperones, also termed 'holdases', can form soluble complexes with partially-unfolded client proteins and thereby rescue them from irreversible aggregation.^{1,2}

The human sHSP α B-crystallin (α B) is a 175-residue protein (20 kDa), which assembles into polydisperse and highly dynamic protein complexes of large molecular weights ranging between 200–1,000 kDa.^{3,4} The monomeric subunit is organized in three regions (**Fig. 1a**): the central α -crystallin domain (ACD) comprising residues 60–150, as well as the flanking N-terminal domain (NTD) and C-terminal domain (CTD). The CTD harbors a highly conserved IXI-motif (residues I159, P160 and I161 in α B), which has been reported to interact with adjacent subunits.^{5–8}

The variable stoichiometry of the α B complex has been linked to dynamic subunit exchange.^{4,8,9} These quaternary fluctuations have hindered atomistic structural investigations in the past.^{3,10} X-ray crystallography as well as solution-state nuclear magnetic resonance (NMR) spectroscopy have been successful to study the central ACD by using N- and C-terminally truncated α B variants, which form only dimers in solution.^{11–13} The ACD adopts a β -sandwich structure, in which two β -sheets (β 3, β 9, β 8 and β 6+7, β 5, β 4) pack onto each other such that strands β 4 and β 8 form a hydrophobic groove (**Fig. 1e**).^{6,11,13} Two monomers align via the elongated strands β 6+7 in an antiparallel fashion, thereby forming the dimeric building block of α B oligomers. This interface has been designated as the dimer interface I,¹⁴ for which three different registers (AP_{I–III}) have been observed.^{5,6,13,15} Structural insights into oligomer architecture came from magic-angle spinning (MAS) solid-state NMR spectroscopy employing precipitated full-

length α B.^{5,11,16} In combination with small-angle X-ray scattering (SAXS) and cryo-electron microscopy (cryo-EM), two structural models for 24-mers have been suggested.^{5,14} Although differing in their details, both 24-mer models propose a tetrahedral symmetric arrangement of four hexameric rings, each consisting of three dimeric subunits (**Fig. 1b,c**). The hexameric sub-structure is stabilized by anchoring the C-terminal IPI-motif to the hydrophobic β 4– β 8 groove (dimer interface II) of a neighboring protomer.^{5,14} This interaction appears to be very dynamic with the IPI-motif occurring in a free (flexible) and a bound (rigid) state.¹⁷ The extent of the immobilization obviously depends on conditions such as pH and temperature.^{5,7,8,10} In both models, the dimer consists of structurally different protomers and thus appears asymmetric.^{5,14} In the pseudo-atomic model derived from cryo-EM, the two protomers have been designated as the extended and bent conformers,¹⁴ which differ in the orientation of their NTDs and CTDs with respect to their ACDs (**Fig. 1c,e**). For example, the two CTDs of a dimer reside in different positions: the CTD of the bent conformer (CTD_{bent}) is fairly solvent-exposed (only restricted by its interactions with neighboring β 4– β 8 grooves), whereas the CTD of the extended conformer (CTD_{ext}) is oriented towards the interior of the complex (**Fig. 1c**). Structural models of the NTD have been obtained from sparse solid-state NMR data in combination with cross-linking experiments and structural homology modeling.^{14,16} The NMR data indicates that these structural states are rather transiently populated and that the NTD samples multiple conformations.

A third structural model based on mass spectrometry (MS) and NMR data, on the other hand, postulates a highly symmetric polyhedral complex, in which all protomers are equivalent.^{8,9,18} This structural equivalence has been deduced from solution-state NMR experiments that revealed merely single cross-peaks for the flexible IPI-motif of α B.⁸ Single sets of resonances for the ACD and CTD of α B have also been reported in solid-state NMR studies with the exception of i) heterogeneous region 1 (HR1) involving residues L65–E71 and ii) for residues in strand β 8 and the CTD upon pH drop.^{5,11} The arrangement of protomers in the dimeric building block of α B is therefore still disputed.

Previous studies have shown that α B interacts with a wide range of client proteins that either form amorphous (disordered) or amyloid (ordered) aggregates.^{19–26} Accordingly, α B is involved in various human pathologies, in which aggregation processes play a role, e.g. cataract formation and neurodegenerative disorders like Alzheimer’s disease.²⁷ It has been suggested that the chaperone mechanism is distinct for amorphous and amyloid aggregation pathways.^{25,28,29} Several regions of α B have been identified to be involved in client binding, e.g. residues W9–P20 and S43–P58 of the NTD as well as residues D73–K92 (β 3– β 4) and L131–V142 (β 8) of the ACD.^{30–33} Those results have been obtained either by site-directed mutagenesis or by studying the chaperone capabilities of isolated peptides. The large molecular weight and conformational diversity, however, have hampered further structural insights into α B–client complexes.

In this study we aimed at understanding the heterogeneous architecture of α B and the possible role of asymmetry in its dimeric building block. We further intended to monitor interactions between the polydisperse chaperone and aggregation-prone clients by NMR spectroscopy. These complexes have molecular weights of several MDa and are not amenable by other high-resolution techniques. Our results illustrate that the inherent structural plasticity of α B allows adjusting to different structural motifs of the aggregating species.

Results

Structural heterogeneity of α B

To test whether human wild-type (WT) α B is assembled from asymmetric dimers, we studied concentrated solutions of perdeuterated α B using ^1H -detected MAS NMR spectroscopy.³⁴ We recently demonstrated that this approach enables to study large protein complexes without the need for crystallization or precipitation.³⁵ The structural heterogeneity of α B yielded spectra that displayed extensive signal overlap due to line widths of about 60–80 Hz (^1H) and 30–40 Hz (^{15}N), respectively (**Fig. 1d**). Overall, we were able to assign 53 amide moieties using triple-resonance experiments (**Supplementary Table 1**). Virtually all assigned cross-peaks originate from the ACD (**Fig. 1f**). We were not able to detect or to assign signals arising from the NTD or the N-terminal β 2– β 3 region of the ACD including residue M68 for which at least nine different chemical environments have been previously observed.^{5,11} The excised ACD has recently been studied by solution-state NMR ($\alpha\text{B}_{\text{G64-V152-N146D}}$, in the following referred to as αB10m).^{11,12} A comparison between spectra of oligomeric α B and dimeric αB10m shows the characteristic signal pattern for the ACD in both proteins (**Supplementary Fig. 1**). The observed chemical shift changes report on interaction sites between neighboring dimers in the oligomer, with the β 4 and β 8 strands showing the largest effects (**Supplementary Fig. 1**). This result is in agreement with previous NMR binding studies⁷ and with the existing structural models of α B 24-mers (**Fig. 1b,c**).^{5,14}

In contrast to previous NMR studies, we observed peak doubling or extensive peak broadening for various residues of the ACD, e.g. S85, E88 (loop preceding β 4), L94, G95, V97 and E99–H104 (loop- β 5), R116 (β 6+7) as well as D127, V128 and L131–T134 (loop preceding β 8). As shown in **Figure 1f**, the affected residues cluster around strands β 4, β 5 and β 8. Due to strong signal overlap, we cannot exclude that cross-peaks of other residues may exhibit a similar behavior. It should be mentioned, that solely the inclusion of a ^1H chemical shift dimension enabled us to observe the multiple states. ^{13}C chemical shifts were mostly degenerate for affected residues (**Supplementary Fig. 2**).

We performed radio frequency-driven recoupling (RFDR) NMR experiments to gain information on spatial vicinity between exchangeable protons. The RFDR data is in agreement with the reported ACD dimer structure⁵ in precipitated α B (**Supplementary Fig. 2**). The spatial vicinity between the amide groups of S115 and H119 supported a major species adopting an AP_{II} register⁶ at dimer interface I with E117 near the two-fold point-symmetric axis (**Supplementary Fig. 2**). The side-chain imidazole of H104 (β 5) showed two resolved ¹H-¹⁵N correlations (**Supplementary Fig. 2**). This observation suggests that the ACD in α B oligomers exists in at least two different states that can be resolved. In its excised, dimeric form, however, the ACD did not show multiple resonance sets, neither for backbone (**Supplementary Fig. 1**) nor for side-chain resonances (**Supplementary Fig. 2**).

The C-terminal residues of α B are very dynamic and yield narrow resonances in solution-state ¹H-¹⁵N correlation experiments (**Fig. 2a**). In contrast to previous studies, in which only residues E164–K175 have been observed,^{36,37} we assigned 11 additional residues comprising the entire C-terminal stretch S153–K175 (**Supplementary Table 2**). Importantly, this sequence also includes the conserved IPI-motif, of which isoleucine methyl moieties have been reported to primarily populate a flexible, unbound state.^{8,17} Hence, in addition to our (**Fig. 1d**) and previous solid-state NMR results,^{5,11} which show that I161 of the IPI-motif is rigid, we also observed a flexible and disordered IPI-motif in solution (**Fig. 2a**).

Proteolytic cleavage experiments supported fractional solvent accessibility for the NTD and CTD (**Supplementary Fig. 3**). We found that approximately half of the protomers in the α B assembly were protected from proteolytic processing at both the N-terminus and the C-terminus. Similar to our solid-state NMR data of the ACD, we observed a second resonance set for most residues of the CTD (**Fig. 2a**). We can exclude the possibility of smaller oligomers or degradation products causing one of the two resonance sets for the CTD, as both conformational states possessed similar translational diffusion coefficients corresponding to a molecular mass of approximately 560 kDa (**Supplementary Fig. 3**). ¹⁵N relaxation measurements showed a general decrease in both longitudinal (T_1)

and transverse relaxation times (T_2) for the second set of C-terminal resonances suggesting differential dynamics for the minor state (**Fig. 2b**). This observation was most prominent for the amide resonances of I159 and I161 in the IPI-motif (**Fig. 2b**). Chemical exchange experiments revealed no measurable exchange between the two CTD states within a period of about one second (**Fig. 2c**). Hence, our solution-state NMR data showed that the entire CTD, including its IPI-motif, is highly flexible and populates at least two different states that undergo very slow chemical exchange.

Interestingly, amide resonances of N-terminal residues were observed neither in solution nor in the solid-state. This indicated that the NTD exists in multiple conformations, which most likely also impeded the detection of strands $\beta 2$ – $\beta 3$ (HR1). Moreover, this underlines that the very N-terminus is fairly rigid. This contradicts previous NMR studies, which reported that the first five N-terminal residues of αB (including I3 and I5) are highly flexible.^{5,36}

Interaction of αB with amorphously-aggregating lysozyme

To identify client binding sites in αB and to investigate its mode of action, we studied the interaction between αB and two aggregation-prone clients: the amorphously-aggregating model client lysozyme (14 kDa), as well as the amyloid β -peptide A β_{1-40} (4 kDa).

Lysozyme readily aggregates amorphously under reducing conditions due to breakage of its disulfide bridges. αB was capable of arresting unfolded lysozyme in a soluble αB -lysozyme complex (**Fig. 3a**) and thereby efficiently inhibiting amorphous aggregation at any time point during the aggregation kinetics (**Fig. 3d**). Negative-stain EM showed an augmentation of these complexes with increasing lysozyme concentration, corresponding to molecular masses of up to several MDa (**Fig. 3b**). Importantly, native lysozyme was stable in solution and did not interact with αB (**Supplementary Fig. 4**). We performed MAS solid-state NMR experiments to identify which sites of αB are deployed to capture reduced

lysozyme. At molar α B:lysozyme ratios of 7:1 and 1:1 (monomer concentrations), we observed consistent chemical shift changes in spectra of α B (**Fig. 3c**). Affected residues included I124, T132, I133 (loop preceding β 8) as well as C-terminal residues T158, I159 and I161 of the IPI-motif. Moreover, the isoleucine methyl region showed additional signals (**Fig. 3c**). Since all isoleucine residues in the ACD and CTD have been assigned,¹¹ we attribute these additional signals to the otherwise non-observable I3, I5 and/or I10 in the NTD. In general, the majority of signals was not affected in the presence of lysozyme indicating that the ACD retained its conformation in the α B oligomer upon binding to lysozyme (**Supplementary Fig. 5**).

The observed chemical shift changes might originate from direct binding of lysozyme to the hydrophobic β 4– β 8 groove of α B. Alternatively, the chemical shift changes can originate indirectly from global structural changes upon binding of lysozyme to the hydrophobic NTD. To exclude one of these scenarios, we recorded solution-state NMR spectra of the dimeric α B10m, which is devoid of the NTD and CTD, and monitored chemical shifts upon addition of reduced lysozyme. ^1H spectra clearly showed the resonances of native lysozyme rapidly decreasing (quantitative precipitation after 3 h), whilst the amount of soluble α B10m was only marginally reduced in this time course (**Supplementary Fig. 6**). Intriguingly, none of the ACD resonances was affected by the presence of aggregating lysozyme (**Supplementary Fig. 6**). The excised ACD seemed thus incompetent to recognize unfolded lysozyme. Furthermore, a truncated α B variant missing the NTD (α B- Δ NTD) was incapable to inhibit aggregation of reduced lysozyme, underlining the important role of the NTD in capturing unfolded lysozyme (**Fig. 3d**). These data demonstrate that the binding effects in the solid-state NMR spectra (**Fig. 3c**) resulted indirectly from altered subunit dynamics as a response to client binding at the NTD. In agreement with this, a solid-state NMR ^1H - ^{15}N correlation spectrum of perdeuterated α B co-precipitated with equimolar amounts of unfolded lysozyme was characterized by resonance broadening (**Supplementary Fig. 5**) reflecting the conformational heterogeneity of the client-bound state. Various new cross-peaks emerged in the presence of unfolded lysozyme (**Supplementary Fig. 5**). The solid-state NMR

spectrum in the absence of lysozyme revealed merely signals arising from the rigid and well-structured ACD (**Fig. 1d**). Upon addition of lysozyme, the partially-disordered NTD (**Supplementary Fig. 7**) thus seemed to become further rigidified causing the additional signals in the solid-state.

Interaction of α B with the amyloid client $A\beta_{1-40}$

We further investigated the chaperone properties of α B towards the amyloid peptide $A\beta_{1-40}$. As observed by negative-stain EM, α B was a potent inhibitor of $A\beta_{1-40}$ fibril formation even at molar excess of $A\beta_{1-40}$ (50:1) with respect to monomeric α B (**Fig. 4a**). Monitoring the aggregation of $A\beta_{1-40}$ by dynamic light scattering (DLS) under the same conditions revealed that α B effectively suppressed the accumulation of high-molecular weight species, whilst preserving the monomeric state of $A\beta_{1-40}$ for more than 4 days at 37°C (**Fig. 4b** and **Supplementary Fig. 8**). Nevertheless, the presence of α B resulted in the accumulation of $A\beta_{1-40}$ oligomers of approximately 30 nm in hydrodynamic diameter (D_h). Soluble $A\beta_{1-40}$ oligomers of similar size have been reported to be on-pathway intermediates representing the cytotoxic species.^{38,39}

The sub-stoichiometric effects of α B on $A\beta_{1-40}$ fibril formation indicated a transient interaction between chaperone and $A\beta_{1-40}$ and/or a capping-like interaction localized at the ends of protofibrillar structures. Solution-state NMR titrations of dimeric α B10m confirmed weak binding affinity of the ACD to monomeric $A\beta_{1-40}$ (**Fig. 4d**). Small chemical shift changes occurred consistently for a cluster of α B10m residues, e.g. V91, V93, I124, S135, S136 and L137, located at the hydrophobic β 4– β 8 groove (**Fig. 4e**). This result is supported by titration experiments in which the variant $A\beta_{1-40}$ –S26C was covalently linked to the paramagnetic spin label *S*-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonylthioate (MTSL) and added to α B10m. The paramagnetic relaxation enhancement (PRE) induced by the MTSL-moiety of bound $A\beta_{1-40}$ –S26C caused a decrease in signal intensities for residues located at the β 4– β 8 groove of α B10m, e.g. V91, G95, S135 and L137 (**Fig. 4d** and **Fig. 4e**).

The $\beta 4$ – $\beta 8$ groove, which is a hydrophobic edge of the ACD (**Supplementary Fig. 7**) thus appeared to be a binding interface for the amyloid client $A\beta_{1-40}$.

In seeded $A\beta_{1-40}$ aggregation assays, both αB -WT and αB - Δ NTD equally well suppressed amyloid formation (**Fig. 4c**). Moreover, the variant S135Q ($\beta 8$ strand), that has been reported to coordinate the C-terminal IPI-motif less efficiently to the $\beta 4$ – $\beta 8$ groove,⁷ was more potent in suppressing amyloid formation (**Fig. 4c**). By contrast, the S135Q mutation did not affect αB chaperone activity towards amorphous aggregation of lysozyme (**Fig. 3d**). Taken together, the data demonstrate that the NTD played a minor role in the inhibition of $A\beta_{1-40}$ aggregation, whereas the β -rich ACD was sufficient to block this process. Interestingly, ¹³C chemical shifts of monomeric $A\beta_{1-40}$ in aqueous solution (**Supplementary Table 3**) indicated β -strand propensity (**Supplementary Fig. 8**) for regions that have been previously shown to interact with αB ⁴⁰ and that also adopt β -strand conformation in $A\beta_{1-40}$ fibrils.⁴¹⁻⁴³

Discussion

Structural heterogeneity of α B

The quaternary dynamics and large polydispersity of α B are considered essential for its chaperone function,⁴⁴ but have hindered atomic-level structural analysis. The NMR data presented here show that monomers within the α B complex are not equivalent, supporting previous structural models in which an asymmetric dimer builds up the tetrahedral α B 24-mer.^{5,14} For example, peak doubling of I133 can be rationalized by its different chemical environment in the bent and extended conformer (**Fig. 1e**). Dimeric α B10m yielded a single resonance set, suggesting that structural heterogeneity is induced by the different arrangement of NTDs and CTDs in α B oligomers.

Another source of heterogeneity, and thus a possible explanation for NMR peak splitting, arises from quaternary dynamics. α B is known for its broad oligomer distribution with a major species of 560 kDa (28-mer).⁴⁴ Accommodation of further dimers into the 24-mer probably involves the groove at the two-fold symmetric axis (**Fig. 1b**).^{5,14} Such an arrangement breaks the tetrahedral symmetry of the complex. For a 28-mer, this would theoretically give rise to three sets of resonances for i) the additional dimers, ii) the subunits surrounding the additional dimers at the two-fold axis and iii) the subunits surrounding the unoccupied two-fold axis. By contrast, we detected only two sets of resonances. Likewise, even and odd numbered oligomers might be the origin of resonance doubling, since subunit exchange involves dimers and monomers.^{4,9} However, even though the dimer interface I is very weak ($K_d \sim 2 \mu\text{M}$),⁴⁵ we estimate that the fraction of monomers missing one of its direct neighbors is negligible, as the protein concentration in MAS-induced sediments is about 4 orders of magnitude higher ($\sim 25 \text{ mM}$).⁴⁶ We note that all the above-mentioned processes and multiple states contribute to broadening or disappearance of resonances, and are thus not resolved in the NMR experiments. Accordingly, the entire NTD appeared molten-globule like and underwent exchange broadening (see also **Supplementary Note 1**). Importantly, the fact that we observed two sets of resonances for various residues of the ACD does not imply a simple two-state

model, but further extends the picture of a structurally heterogeneous ensemble of α B oligomers.^{10,44}

Similar to the ACDs, the CTDs of α B appeared non-equivalent and populated a minimum of two mobile conformers as well as one immobile conformer. We were able to observe and assign the entire CTD including its IPI-motif, which has been recently shown to exchange between an unbound, flexible state^{8,17} and an immobile state with the IPI-motif bound to the β 4– β 8 groove of neighboring protomers.^{5,11} Furthermore, a millisecond exchange process has been described for the unbound CTD undergoing a “flap motion” with the IPI-motif being close to, but not occupying the β 4– β 8 groove.⁸ The occurrence of multiple sets of resonances for the ACD and CTD in solid-state and solution-state NMR spectra of α B raises the question whether these observations originate from the same structural feature. We exclude binding of the IPI-motif to the β 4– β 8 groove as a source for the observed peak doubling, as several resolved cross-peaks for residues in strands β 4 and β 8 (e.g. S135, S136, L137) did not display peak doubling (**Supplementary Fig. 1**). In the tetrahedral 24-mer model, the very N- and C-termini of the bent conformer are accessible for proteolytic degradation (**Supplementary Fig. 3**), whereas both termini of the extended conformer reside in the inner cavity of the complex.^{14,16} The observation of mobile and immobile states might thus reflect the variable flexibility of CTD_{bent} and CTD_{ext}. The two flexible CTD states detected by solution-state NMR seemed to interconvert very slowly. Hence, it is tempting to speculate that the interconversion is restricted by subunit exchange, which occurs on a time scale of several minutes.^{9,47,48} We cannot rule out peak doubling for the flexible CTD due to proline cis-trans isomerization, which occurs on a similar time scale.

Diversity of client interactions

Our interaction studies suggest that the hydrophobic β 4– β 8 groove of the ACD represents the binding site for A β _{1–40}. Previous NMR studies have shown that α B competes for A β _{1–40} monomer-monomer interactions by transiently binding to the hydrophobic core of A β _{1–40} (L¹⁷VFFA²¹).⁴⁰ These hydrophobic residues

constitute the central β -strand core in amyloid fibrils.^{41–43} Interestingly, monomeric $A\beta_{1-40}$ partially populated structural states, in which residues L¹⁷VFFA²¹ adopted β -strand conformation under physiological conditions (**Supplementary Fig. 8**). Hence, the hydrophobic β -strand region of $A\beta_{1-40}$ is the structural motif, which is recognized by the likewise hydrophobic $\beta 4$ – $\beta 8$ groove of αB . This is in agreement with studies that show that αB preferentially interacts with $A\beta_{1-40}$ oligomers originating from disaggregation of $A\beta_{1-40}$ fibrils and featuring a higher content of β -strand structure than $A\beta_{1-40}$ oligomers formed during the aggregation of monomers.^{49,50} Notably, αB itself is capable of forming amyloid fibrils,⁵¹ and the segment K90–V100 ($\beta 4$ – $\beta 5$) can accumulate into β -rich oligomers.⁵² The β -sandwich of αB resembles the β -strand assembly of $A\beta_{1-40}$ fibrils and appeared to play an important role in molecular recognition of amyloid aggregates. In fact, the excised ACD inhibits fibril formation of other amyloid clients such as κ -casein and $A\beta_{1-42}$.⁵³ Likewise, αB – Δ NTD was capable to inhibit amyloid formation of $A\beta_{1-40}$ similar to αB –WT (**Fig. 4c**). This demonstrates that αB – Δ NTD, despite its potentially altered oligomer architecture, retained chaperone function towards an amyloidogenic client, highlighting the importance of the ACD in this process. Previous studies, in which the excised ACD and its S135Q variant were titrated with CTD-derived peptides, reported that the glutamine side chain impedes docking of the IPI-motif into the $\beta 4$ – $\beta 8$ groove.⁷ In agreement with such a weakening of dimer interface II in αB –S135Q and the concomitant exposure of its $\beta 4$ – $\beta 8$ groove, we observed enhanced inhibition of $A\beta_{1-40}$ fibril formation (**Fig. 4c**).

The asymmetric architecture of αB implicates a differential involvement of the identified chaperone sites and might fulfill an important function upon client binding: while one half of the protomers maintains the structural integrity of the oligomer, the other half is vacant for client binding. The $\beta 4$ – $\beta 8$ groove of bent conformers appears to be less accessible for client interactions owing to its orientation towards adjacent NTDs in the oligomer interior. On the other hand, the $\beta 4$ – $\beta 8$ groove of the extended conformers is unoccupied after dissociation of the CTD, and can mediate the interaction with $A\beta_{1-40}$. As illustrated in **Figure 5**,

the hexameric ring of αB^5 and the β -helix of $A\beta_{1-40}$ fibrils⁴³ have both a three-fold symmetry with similar dimensions (diameter ca. 50 Å). This showcase docking model illustrates that the β -strand regions L¹⁷VFFA²¹ of $A\beta_{1-40}$ fit properly onto the $\beta 4$ – $\beta 8$ grooves of extended conformers in the αB hexameric ring. However, various structural states are populated during amyloid aggregation giving rise to spherical amyloid intermediates^{38,39} as well as polymorphic mixtures of fibrillar aggregates with different symmetries.⁴³ Binding of the ACD to these β -rich species might be governed by the accessibility of β -strand edges in the growing aggregate, such that αB can compete for fibril growth. Despite the weak interaction between αB_{10m} and monomeric $A\beta_{1-40}$, sub-stoichiometric amounts of αB were sufficient to block $A\beta_{1-40}$ fibril formation. Our results hint at an intervention of αB in both phases of amyloidogenesis: i) transient interaction with amyloid building blocks – possibly sufficient to hinder the slow fibril nucleation process,⁵⁴ and ii) as suggested previously,²² inhibition of fibril elongation through binding of αB to the termini of protofibrillar structures (**Fig. 5**). At higher abundance, αB also adheres to the walls of $A\beta_{1-42}$ fibrils thereby affecting their elongation.²²

In contrast to the amyloid client $A\beta_{1-40}$, we found that binding of the amorphaously aggregating lysozyme occurred at the partially-disordered NTD of αB . Previous studies have shown that denatured lysozyme collapses into a molten-globule state,⁵⁵ which is trapped by αB .⁵⁶ In the presence of unfolded lysozyme we found that additional resonances appeared in ¹³C-¹³C and ¹H-¹⁵N correlation spectra of αB (**Supplementary Fig. 5**). These new cross-peaks must originate from the NTD as resonances of the ACD and CTD have been assigned. This indicates that the NTD rigidified to a higher extent upon binding to unfolded lysozyme. Since the NTD and CTD mediate subunit exchange,⁴⁴ binding of lysozyme to the NTD will consequently alter αB quaternary dynamics. This was reflected in chemical shift changes involving the CTD and the $\beta 4$ – $\beta 8$ groove. Importantly, αB_{10m} and αB - Δ NTD – both harboring $\beta 4$ – $\beta 8$ grooves, but devoid of NTDs – were not capable of rescuing unfolded lysozyme from aggregation (**Supplementary Fig. 6** and **Fig. 3d**). This underlines the importance of structural plasticity of the NTD for lysozyme recognition and binding, being

consistent with previous reports on MjHSP16.5 and its sequestration of unfolded lysozyme via NTDs.⁵⁷ Moreover, studies on PsHSP18.1 suggest that structural disorder allows the NTD to adapt to a continuum of client conformations.⁵⁸ We propose that this might particularly apply to amorphously aggregating clients.

Both client binding regions, i.e. the dynamic NTD and the β 4– β 8 groove of the rather static ACD, are fairly hydrophobic (**Supplementary Fig. 7**) and buried in the native oligomer through inter-subunit contacts with neighboring NTDs and CTDs (**Fig. 1b,c**).^{5,14,16} Structural fluctuations inherent to α B^{8,9,17} cause a transient liberation of these binding sites and enable the interaction with hydrophobic patches of unfolded clients. From a structural point of view, inhibition of amyloid aggregation appears to be less demanding compared to the requirement to adapt to an entire ensemble of disordered protein states in amorphous aggregation. Likewise, the rather slow kinetics of amyloid nucleation allow very short-lived chaperone–client complexes to compete for client self-association. Hence, intervention of α B in amorphous aggregation of lysozyme and in fibril formation of A β _{1–40} differs fundamentally in its mechanism. These findings may not be applicable for the entire interactome of α B, i.e. conformational ensembles of hundreds of misfolded proteins, but may disclose some general principles in anti-aggregation strategies of sHSPs. The picture of a chaperone, which captures destabilized proteins by structurally mimicking their key conformational properties, appears to be intuitive, but needs to be validated in future experiments with a wider range of clients.

Accession codes

The chemical shift assignments of the ACD and the CTD of α B are deposited in the BMRB under accession code 26640.

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Author contributions

A.M., J.P., M.S. and K.C.B. cloned, recombinantly expressed, purified and characterized human α B and its variants. A.M. performed the NMR experiments and analyzed the data. J.P. performed the lysozyme aggregation assays. K.C.B. performed the seeded amyloid aggregation assays and characterized the variant α B-S135Q. M.S. and S.A. contributed the DOSY and PDSD NMR data. B.B. generated the docking model of α B and fibrillar $A\beta_{1-40}$. E.P. prepared recombinant $A\beta_{1-40}$ and supported the resonance assignment of $A\beta_{1-40}$ in solution. C.P. performed the EM experiments of α B and lysozyme. A.M., J.P., J.B., S.W. and B.R. designed the experiments. A.M. and B.R. wrote the manuscript, with contributions from all authors.

Competing financial interests

The authors declare no competing financial interests.

References

1. Haslbeck, M. & Vierling, E. A first line of stress defense: small heat shock proteins and their function in protein homeostasis. *J. Mol. Biol.* **427**, 1537–1548 (2015).
2. Horwitz, J. Alpha-crystallin can function as a molecular chaperone. *Proc. Natl. Acad. Sci.* **89**, 10449–10453 (1992).
3. Horwitz, J. Alpha crystallin: the quest for a homogeneous quaternary structure. *Exp. Eye Res.* **88**, 190–194 (2009).
4. Aquilina, J. A., Benesch, J. L. P., Bateman, O. A., Slingsby, C. & Robinson, C. V. Polydispersity of a mammalian chaperone: Mass spectrometry reveals the population of oligomers in α B-crystallin. *Proc. Natl. Acad. Sci.* **100**, 10611–10616 (2003).
5. Jehle, S. *et al.* Solid-state NMR and SAXS studies provide a structural basis for the activation of alphaB-crystallin oligomers. *Nat. Struct. Mol. Biol.* **17**, 1037–1042 (2010).
6. Laganowsky, A. *et al.* Crystal structures of truncated alphaA and alphaB crystallins reveal structural mechanisms of polydispersity important for eye lens function. *Protein Sci.* **19**, 1031–1043 (2010).
7. Delbecq, S. P., Jehle, S. & Klevit, R. Binding determinants of the small heat shock protein, α B-crystallin: recognition of the 'Ixl' motif. *EMBO J.* **31**, 4587–4594 (2012).
8. Baldwin, A. J. *et al.* Quaternary dynamics of α B-crystallin as a direct consequence of localised tertiary fluctuations in the C-terminus. *J. Mol. Biol.* **413**, 310–320 (2011).

9. Baldwin, A. J., Lioe, H., Robinson, C. V., Kay, L. E. & Benesch, J. L. P. α B-crystallin polydispersity is a consequence of unbiased quaternary dynamics. *J. Mol. Biol.* **413**, 297–309 (2011).
10. Delbecq, S. P. & Klevit, R. E. One size doesn't fit all: the oligomeric states of α B-crystallin. *FEBS Lett.* **587**, (2013).
11. Jehle, S. *et al.* α B-crystallin: a hybrid solid-state/solution-state NMR investigation reveals structural aspects of the heterogeneous oligomer. *J. Mol. Biol.* **385**, 1481–1497 (2009).
12. Mainz, A. *et al.* Structural and mechanistic implications of metal binding in the small heat-shock protein α B-crystallin. *J. Biol. Chem.* **287**, 1128–1138 (2012).
13. Bagn  ris, C. *et al.* Crystal structures of alpha-crystallin domain dimers of α B-crystallin and Hsp20. *J. Mol. Biol.* **392**, 1242–1252 (2009).
14. Braun, N. *et al.* Multiple molecular architectures of the eye lens chaperone α B-crystallin elucidated by a triple hybrid approach. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 20491–20496 (2011).
15. Clark, A. R., Naylor, C. E., Bagn  ris, C., Keep, N. H. & Slingsby, C. Crystal Structure of R120G Disease Mutant of Human α B-Crystallin Domain Dimer Shows Closure of a Groove. *J. Mol. Biol.* **408**, 118–134 (2011).
16. Jehle, S. *et al.* N-terminal domain of {alpha}B-crystallin provides a conformational switch for multimerization and structural heterogeneity. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 6409–6414 (2011).
17. Baldwin, A. J. *et al.* Probing Dynamic Conformations of the High-Molecular-Weight α B-Crystallin Heat Shock Protein Ensemble by NMR Spectroscopy. *J. Am. Chem. Soc.* **134**, 15343–15350 (2012).

18. Baldwin, A. J. *et al.* The polydispersity of α B-crystallin is rationalized by an interconverting polyhedral architecture. *Struct. Lond. Engl.* 1993 **19**, 1855–1863 (2011).
19. Ghosh, J. G., Shenoy, A. K., Jr & Clark, J. I. Interactions between important regulatory proteins and human α B crystallin. *Biochemistry* **46**, 6308–6317 (2007).
20. Rekas, A. *et al.* Interaction of the molecular chaperone α B-crystallin with α -synuclein: effects on amyloid fibril formation and chaperone activity. *J. Mol. Biol.* **340**, 1167–1183 (2004).
21. Waudby, C. A. *et al.* The interaction of α B-crystallin with mature α -synuclein amyloid fibrils inhibits their elongation. *Biophys. J.* **98**, 843–851 (2010).
22. Shammass, S. L. *et al.* Binding of the molecular chaperone α B-crystallin to A β amyloid fibrils inhibits fibril elongation. *Biophys. J.* **101**, 1681–1689 (2011).
23. Houck, S. A., Landsbury, A., Clark, J. I. & Quinlan, R. A. Multiple sites in α B-crystallin modulate its interactions with desmin filaments assembled in vitro. *PloS One* **6**, e25859 (2011).
24. Regini, J. W. *et al.* The interaction of unfolding α -lactalbumin and malate dehydrogenase with the molecular chaperone α B-crystallin: a light and X-ray scattering investigation. *Mol. Vis.* **16**, 2446–2456 (2010).
25. Treweek, T. M., Meehan, S., Ecroyd, H. & Carver, J. A. Small heat-shock proteins: important players in regulating cellular proteostasis. *Cell. Mol. Life Sci. CMLS* **72**, 429–451 (2015).
26. Sandilands, A. *et al.* Altered aggregation properties of mutant gamma-crystallins cause inherited cataract. *EMBO J.* **21**, 6005–6014 (2002).

27. Sun, Y. & MacRae, T. H. The small heat shock proteins and their role in human disease. *FEBS J.* **272**, 2613–2627 (2005).
28. Raman, B. *et al.* AlphaB-crystallin, a small heat-shock protein, prevents the amyloid fibril growth of an amyloid beta-peptide and beta2-microglobulin. *Biochem. J.* **392**, 573–581 (2005).
29. Kulig, M. & Ecroyd, H. The small heat-shock protein α B-crystallin uses different mechanisms of chaperone action to prevent the amorphous versus fibrillar aggregation of α -lactalbumin. *Biochem. J.* **448**, 343–352 (2012).
30. Ghosh, J. G., Estrada, M. R. & Clark, J. I. Interactive domains for chaperone activity in the small heat shock protein, human alphaB crystallin. *Biochemistry* **44**, 14854–14869 (2005).
31. Ghosh, J. G., Estrada, M. R., Houck, S. A. & Clark, J. I. The function of the beta3 interactive domain in the small heat shock protein and molecular chaperone, human alphaB crystallin. *Cell Stress Chaperones* **11**, 187–197 (2006).
32. Ghosh, J. G., Estrada, M. R. & Clark, J. I. Structure-based analysis of the beta8 interactive sequence of human alphaB crystallin. *Biochemistry* **45**, 9878–9886 (2006).
33. Bhattacharyya, J., Padmanabha Udupa, E. G., Wang, J. & Sharma, K. K. Mini-alphaB-crystallin: a functional element of alphaB-crystallin with chaperone-like activity. *Biochemistry* **45**, 3069–3076 (2006).
34. Mainz, A., Jehle, S., van Rossum, B. J., Oschkinat, H. & Reif, B. Large Protein Complexes with Extreme Rotational Correlation Times Investigated in Solution by Magic-Angle-Spinning NMR Spectroscopy. *J. Am. Chem. Soc.* **131**, 15968–15969 (2009).

35. Mainz, A. *et al.* NMR Spectroscopy of Soluble Protein Complexes at One Mega-Dalton and Beyond. *Angew. Chem. Int. Ed.* **52**, 8746–8751 (2013).
36. Carver, J. A., Aquilina, J. A., Truscott, R. J. & Ralston, G. B. Identification by ^1H NMR spectroscopy of flexible C-terminal extensions in bovine lens alpha-crystallin. *FEBS Lett.* **311**, 143–149 (1992).
37. Treweek, T. M., Rekas, A., Walker, M. J. & Carver, J. A. A quantitative NMR spectroscopic examination of the flexibility of the C-terminal extensions of the molecular chaperones, αA - and αB -crystallin. *Exp. Eye Res.* **91**, 691–699 (2010).
38. Chimon, S. *et al.* Evidence of fibril-like β -sheet structures in a neurotoxic amyloid intermediate of Alzheimer's β -amyloid. *Nat. Struct. Mol. Biol.* **14**, 1157–1164 (2007).
39. Chimon, S. & Ishii, Y. Capturing intermediate structures of Alzheimer's beta-amyloid, Abeta(1-40), by solid-state NMR spectroscopy. *J. Am. Chem. Soc.* **127**, 13472–13473 (2005).
40. Narayanan, S., Kamps, B., Boelens, W. C. & Reif, B. αB -crystallin competes with Alzheimer's disease β -amyloid peptide for peptide–peptide interactions and induces oxidation of Abeta-Met35. *FEBS Lett.* **580**, 5941–5946 (2006).
41. Schütz, A. K. *et al.* Atomic-Resolution Three-Dimensional Structure of Amyloid β Fibrils Bearing the Osaka Mutation. *Angew. Chem. Int. Ed.* **54**, 331–335 (2015).
42. Petkova, A. T. *et al.* A structural model for Alzheimer's β -amyloid fibrils based on experimental constraints from solid state NMR. *Proc. Natl. Acad. Sci.* **99**, 16742–16747 (2002).

43. Paravastu, A. K., Leapman, R. D., Yau, W.-M. & Tycko, R. Molecular structural basis for polymorphism in Alzheimer's beta-amyloid fibrils. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 18349–18354 (2008).
44. Hochberg, G. K. A. & Benesch, J. L. P. Dynamical structure of α B-crystallin. *Prog. Biophys. Mol. Biol.* **115**, 11–20 (2014).
45. Hilton, G. R. *et al.* C-terminal interactions mediate the quaternary dynamics of α B-crystallin. *Philos. Trans. R. Soc. B Biol. Sci.* **368**, 20110405 (2013).
46. Bertini, I. *et al.* Solid-state NMR of proteins sedimented by ultracentrifugation. *Proc. Natl. Acad. Sci.* **108**, 10396–10399 (2011).
47. Ahmad, M. F., Raman, B., Ramakrishna, T. & Rao, C. M. Effect of Phosphorylation on $[\alpha]$ B-crystallin: Differences in Stability, Subunit Exchange and Chaperone Activity of Homo and Mixed Oligomers of $[\alpha]$ B-Crystallin and its Phosphorylation-mimicking Mutant. *J. Mol. Biol.* **375**, 1040–1051 (2008).
48. Peschek, J. *et al.* Regulated structural transitions unleash the chaperone activity of α B-crystallin. *Proc. Natl. Acad. Sci. U. S. A.* **110**, E3780–3789 (2013).
49. Narayan, P. *et al.* Amyloid- β oligomers are sequestered by both intracellular and extracellular chaperones. *Biochemistry* **51**, 9270–9276 (2012).
50. Lee, J., Culyba, E. K., Powers, E. T. & Kelly, J. W. Amyloid- β forms fibrils by nucleated conformational conversion of oligomers. *Nat. Chem. Biol.* **7**, 602–609 (2011).
51. Meehan, S. *et al.* Amyloid fibril formation by lens crystallin proteins and its implications for cataract formation. *J. Biol. Chem.* **279**, 3413–3419 (2004).

52. Laganowsky, A. *et al.* Atomic View of a Toxic Amyloid Small Oligomer. *Science* **335**, 1228–1231 (2012).
53. Hochberg, G. K. A. *et al.* The structured core domain of α B-crystallin can prevent amyloid fibrillation and associated toxicity. *Proc. Natl. Acad. Sci.* **111**, E1562–E1570 (2014).
54. Cohen, S. I. A. *et al.* A molecular chaperone breaks the catalytic cycle that generates toxic A β oligomers. *Nat. Struct. Mol. Biol.* **22**, 207–213 (2015).
55. Klein-Seetharaman, J. *et al.* Long-range interactions within a nonnative protein. *Science* **295**, 1719–1722 (2002).
56. Raman, B., Ramakrishna, T. & Rao, C. M. Effect of the chaperone-like alpha-crystallin on the refolding of lysozyme and ribonuclease A. *FEBS Lett.* **416**, 369–372 (1997).
57. Shi, J. *et al.* Cryoelectron microscopy analysis of small heat shock protein 16.5 (Hsp16.5) complexes with T4 lysozyme reveals the structural basis of multimode binding. *J. Biol. Chem.* **288**, 4819–4830 (2013).
58. Jaya, N., Garcia, V. & Vierling, E. Substrate binding site flexibility of the small heat shock protein molecular chaperones. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 15604–15609 (2009).

Figure Legends

Fig. 1 Protomers in the α B complex are not equivalent. (a) Domain organization of α B. In the structural model, four hexameric rings assemble into a 24-mer with tetrahedral symmetry.¹⁴ (b) Views along the two three-fold point-symmetric axis centered in the hexameric ring and along the two-fold axis, which is proposed to accommodate an additional dimer in (24+n)-mers.^{14,16} The extended and bent conformers are colored in orange and purple, respectively. Schematic representations are depicted below. (c) Side view onto the hexameric ring formed by three asymmetric dimers.¹⁴ CTD_{ext} (orange) is involved in interactions with NTD_{ext}, whereas CTD_{bent} (purple) is fairly solvent-accessible. (d) Solid-state NMR ^1H - ^{15}N correlation spectrum of α B at 4 °C and resonance assignments. Multiple sets or very broad cross-peaks are highlighted with squares. 1D ^1H traces at the ^{15}N chemical shifts of the two cross-peaks of I133 are shown on the bottom. The asterisk denotes the folded cross peak of L143. The data is mapped onto the α B dimer^{5,14} in (e). The ACD consists of a 6-stranded β -sandwich (labeled for one protomer). The dimeric subunit is composed of an extended and a bent conformer. NTDs (cyan) and CTDs (magenta) of bent and extended conformers are labeled. The position of I133 is highlighted. Dimer interface I (via $\beta 6+7$) and dimer interface II (via $\beta 4-\beta 8$) are indicated with dashed lines. Assigned residues showing one and two cross-peaks in NMR spectra are colored in blue and red, respectively, and are mapped in (f) onto the primary structure of α B.

Fig. 2 Structural plasticity of the α B CTD. (a) Solution-state ^1H - ^{15}N heteronuclear single-quantum coherence (HSQC) spectrum of human α B at 22 °C. Resonance assignments for the major and the minor states are indicated in black and red, respectively. The CTD sequence is shown on top. Residues that show one and two cross-peaks are colored in blue and red, respectively. (b) ^{15}N relaxation data for the major (white bars) and minor state (red bars) of representative residues. Error bars were obtained from a mono-exponential fit of the experimental relaxation data. (c) Solution-state ^1H - ^{15}N HSQC-nuclear Overhauser Enhancement spectroscopy (NOESY) to probe chemical exchange (mixing time: 430 ms) between the two states of the CTD. Dashed circles indicate expected exchange peaks for I159 and A172, which are, however, absent in the spectrum.

Fig. 3: Interaction of α B with unfolded lysozyme. (a) SDS-PAGE of samples containing varying molar ratios of α B and lysozyme (Lyso) as indicated. Unfolding of lysozyme was induced by incubation with tris-(2-carboxyethyl)phosphine (TCEP) for 1h at 37°C. The lanes represent the soluble fraction (S) and the insoluble pellet (P). See also **Supplementary Data Set 1.** (b) Negative-stain EM images of α B in the absence and presence of unfolded lysozyme. Molar ratios

of α B and lysozyme are given on top. The scale bar corresponds to 0.1 μ m. **(c)** Threonine (left) and isoleucine spectral region (right) of ^{13}C - ^{13}C correlation MAS NMR spectra of α B in the absence (red), in the presence of sub-stoichiometric (blue) and stoichiometric (black) amounts of unlabeled lysozyme unfolded by the addition of TCEP. Upon titration of lysozyme, additional isoleucine signals become observable (dashed box). **(d)** TCEP-induced aggregation of lysozyme. Left: Addition of 20 μ M α B-WT at several time points (indicated with arrows) during ongoing aggregation of 5 μ M lysozyme or prior to the addition of TCEP. Right: Lysozyme (5 μ M) was pre-incubated with 5 μ M of α B-WT and variants (see legend) and aggregation was initiated by adding TCEP.

Fig. 4 Interaction of α B with $\text{A}\beta_{1-40}$. **(a)** Negative-stain EM images of $\text{A}\beta_{1-40}$ incubated for 4 days at 37°C in the absence (left) and presence (right) of sub-stoichiometric amounts of α B (50:1). The scale bars correspond to 1 μ m. **(b)** Aggregation of $\text{A}\beta_{1-40}$ as monitored by DLS. Incubation of $\text{A}\beta_{1-40}$ was performed for 4 d at 37°C in the absence (red) and presence (black) of sub-stoichiometric amounts of α B (50:1). The initial and final size distributions are shown on top. The light scattering intensity and the hydrodynamic diameter D_h of monomeric $\text{A}\beta_{1-40}$ (triangles) and oligomeric $\text{A}\beta_{1-40}$ (diamonds) are shown below. Error bars correspond to s.d. **(c)** Seeded aggregation of $\text{A}\beta_{1-40}$ (50 μ M) is suppressed by the addition of 5 μ M α B-WT and its variants (see legend). Freshly prepared fibril seeds of $\text{A}\beta_{1-40}$ were used to initiate fibril growth. **(d)** Sections of ^1H - ^{15}N HSQC spectra of α B10m. Left: Chemical shift changes of α B10m in the absence (black) and presence of $\text{A}\beta_{1-40}$ (red). Right: PREs in the presence of paramagnetic (red) and diamagnetic $\text{A}\beta_{1-40}$ -S26C-MTSL (black). **(e)** Chemical shift changes ($\Delta\delta$) and PREs are plotted versus the α B10m sequence. More strongly affected residues are highlighted in red. Asterisks denote residues that have been excluded from the analysis and error bars correspond to s.d. (for details see Online Methods). β -strands of the ACD are shown on top of the panel.

Fig. 5 Model of α B-mediated inhibition of amorphous and amyloid aggregation. Destabilized proteins may self-associate due to the exposure of hydrophobic segments. The asterisk denotes that α B potentially undergoes subunit exchange (monomers/dimers), which has been reported to be an important property for chaperone function.^{9,48,59,60} The structural plasticity of α B, in particular that of its partially disordered NTD, facilitates binding of structurally undefined clients in a stable and soluble α B-client complex, and thereby suppresses amorphous aggregation. Saturation of the holdase causes larger α B-client clusters that may finally co-precipitate. Amyloidogenic clients have pronounced β -strand propensity. α B targets the hydrophobic β -strand core of amyloid species by interacting transiently with protomers as well as with pre-fibrillar assemblies thereby impeding fibril nucleation and fibril elongation, respectively. The β -sandwich core of α B (in particular the edge strands β 3, β 4 and β 8) can be

viewed as a structurally related unit that mimics the cross- β structure of amyloid systems and that is well suited to adhere, e.g. to the ends of protofibrils as illustrated in the showcase docking model (top and side view). The three-fold symmetric A β_{1-40} fibril adopts β -strand conformation for residues 11–22 (light blue), including the hydrophobic core L¹⁷VFFA²¹, and residues 30–37 (dark blue).⁴³ For simplicity, α B is shown in grey and the β 4– β 8 strands of the extended conformers of one hexamer are highlighted in red.

Online Methods

Sample preparation

Unlabeled as well as ^{13}C , ^{15}N -labeled human full-length αB (UniProt accession code P02511), the truncated $\alpha\text{B}10\text{m}$ (residues G64–V152 with the mutation N146D)¹¹, $\alpha\text{B}-\Delta\text{NTD}$ as well as $\alpha\text{B}-\text{S135Q}$ were recombinantly expressed and purified according to the protocols described elsewhere.^{12,34,48} The entire αB elution peak in the final size exclusion chromatographic step has been pooled and used for further experiments. Hence, no restricted peak selection has been applied to enrich a certain oligomeric state of αB . Initial attempts to cleave-off an N-terminal His₆-tag from recombinant αB with the protease enterokinase (cleaving downstream of the lysine residue in the recognition sequence XDDDDK) revealed two findings: i) Very long incubation times and excess of the protease were required to yield quantitative cleavage of the N-terminal tag, and ii) a non-specific cleavage product ($\alpha\text{B}_{\text{M1-R157}}$) was identified via mass spectrometry with an abundance of about 50% in SDS-PAGE (**Fig. S6**). Longer incubation times with high excess of enterokinase did not yield further conversion at the C-terminal non-specific cleavage site. Owing to the low efficiency of the proteolytic cleavage, we overexpressed αB without any affinity tag.³⁴

^2H , ^{13}C , ^{15}N - αB was heterologously expressed in *E. coli* BL21 (DE3) (Merck, Darmstadt, Germany) growing in D₂O-based minimal medium containing $^{15}\text{NH}_4\text{Cl}$ (1 g/L) and uniformly labeled ^2H , ^{13}C -glucose (4 g/L). The purified protein was subject to three cycles of lyophilization with subsequent dissolution in 50 mM sodium phosphate buffer (pH 7.5), 100 mM sodium chloride (PBS) with 20% H₂O and 80% D₂O (PBS₂₀), in order to assure a homogeneous degree of protonation of exchangeable sites for the entire protein. For the RFDR experiments at 40 kHz MAS, this procedure was performed with PBS in 100% H₂O.

Solid-state NMR samples were prepared according to the FROSTY approach.³⁴ In brief, αB was concentrated using low-volume ultrafiltration devices (Millipore)

with a molecular weight cut-off of 100 kDa. The protein concentration was determined after dilution using the intrinsic absorbance of α B at 280 nm (molar extinction coefficient ϵ of 13,980 M⁻¹ cm⁻¹). Cu(II)-EDTA (300 mM in PBS₂₀) was added to the protein solution to a final concentration of 60 mM Cu(II)-EDTA in order to speed up data acquisition.³⁵ Subsequently, the viscous, but visually clear solution was filled into 1.9 mm (10 μ L) or thin-walled 3.2 mm (50 μ L) MAS rotors (Bruker). Protein sedimentation prior to the MAS experiment via ultracentrifugation into the MAS rotor⁴⁶ was yielding severely broadened resonances with line widths on the order of 180 Hz (¹H) and 120 Hz (¹⁵N), presumably due to rapid dehydration during MAS.

For interaction studies, lyophilized lysozyme from hen egg white (Sigma-Aldrich, Taufkirchen, Germany) was balanced and directly dissolved in concentrated solutions of ¹³C, ¹⁵N- α B or ²H, ¹³C, ¹⁵N- α B, respectively. Typically, the final concentration of isotopically labeled α B was in the range of 5 mM (monomer concentration), whereas the final lysozyme concentration amounted to 0.7 mM and 5 mM, respectively. Lysozyme unfolding was initiated by addition of a 10-fold molar excess of TCEP (Sigma-Aldrich, Taufkirchen, Germany) from a 0.5–1.0 M stock solution in water (pH adjusted to 7.5). The reaction mixture was subsequently incubated for 30 min at 37°C. In the case in which an equimolar mixture of α B and reduced lysozyme was prepared, the quantitative coprecipitate was centrifuged directly into the MAS rotor. The sample with a 7-fold molar excess of α B remained virtually devoid of precipitation. Traces of precipitate were removed by centrifugation and only the supernatant (50 μ L) was filled into the MAS rotor.

Heterologously expressed A β _{1–40} was prepared as described previously.⁶¹ To ensure that A β _{1–40} is monomeric, the lyophilized peptide was initially dissolved in 10 mM NaOH, ultrasonicated for 10 min and centrifuged for 10 min at 14,800 rpm. This stock solution was diluted in 2x PBS to finally yield a pH value of 7.5 in 1x PBS at a peptide concentration of <200 μ M. This procedure was performed on ice with pre-cooled solutions. A β _{1–40} fibrils were prepared based on a protocol described previously.⁶¹ Briefly, A β _{1–40} fibrils were formed by supplementing

monomeric A β ₁₋₄₀ at a concentration of 50 μ M with 10% seeds obtained from former seeding cycles (<12 generations of seeding) followed by incubation under agitation at room temperature. A β ₁₋₄₀ seeds were obtained by ultrasonication of preformed A β ₁₋₄₀ fibrils for 3 x 10 s.

To produce S-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonylthioate (MTSL)-labeled A β ₁₋₄₀ peptides, synthetic A β ₁₋₄₀-S26C (AnaSpec, USA) was reduced with a 10-fold molar excess of TCEP. The reducing agent was removed with a PD-G10 desalting column (GE Healthcare). A β ₁₋₄₀-S26C (200 μ M in PBS) was then incubated overnight at 4 °C with a 10-fold molar excess of MTSL (Toronto Research Chemicals Inc., 300 mM stock solution in acetonitrile). Subsequently, the mixture was again subjected to a PD-G10 desalting column thereby efficiently removing the unreacted label. A β ₁₋₄₀-S26C-MTSL in PBS was added to ¹³C, ¹⁵N- α B10m yielding a final concentration of approx. 100 μ M for both binding partners. Reduction of the protein-bound MTSL label to yield the diamagnetic species was achieved with a 10-fold molar excess of freshly prepared ascorbic acid in PBS.

Lysozyme aggregation assays

The aggregation of 5 μ M lysozyme in PBS buffer was initiated by 1 mM TCEP at 37°C. The aggregation process was monitored by recording absorbance changes at 350 nm in a Varian Cary 50 UV/Vis spectrophotometer (Agilent). For interrupted aggregation assays, 20 μ M α B-WT were added at different time points. To test the chaperone activity of α B-WT, α B-S135Q and α B- Δ NTD, 5 μ M lysozyme were pre-incubated with 5 μ M of α B and its variants.

The solubility of denatured lysozyme in the absence and presence of α B was determined by SDS-PAGE and Coomassie staining, analyzing the sediment and supernatant fraction after centrifugation at 10,000 g for 5 min. The insoluble fraction was washed with PBS followed by a second centrifugation step.

A β ₁₋₄₀ aggregation assays

Dynamic light scattering (DLS) experiments were performed on a ZEN3500 Zetasizer NanoZS instrument (Malvern Instruments) equipped with a 50 mW laser operating at a wavelength of 532 nm. Back-scattering was detected at an angle of 173°. Temperature control was accomplished with an in-built peltier-element. Kinetic measurements of A β ₁₋₄₀ aggregation at 37°C were performed at an initial peptide concentration of 125 μ M. Co-incubation with α B was done at a chaperone concentration of 2.5 μ M. Bacterial growth was inhibited by adding 0.03% NaN₃. The samples were filtered through 0.1 μ m membranes (Millipore). Aliquots of 80 μ L were incubated and automatically measured in sealed quartz-cuvettes (Hellma) for 4 days in time intervals of 4 h. At each time point, three measurements were performed with data accumulation over 300 runs (with 4 s for each run). Data analysis was done using the software DTS 5.03 (Malvern Instruments).

Seeded A β ₁₋₄₀ amyloid formation was carried out in PBS at 37°C and monitored at 350 nm in a Varian Cary 50 UV/Vis spectrophotometer (Agilent) equipped with a temperature adjustable cuvette holder. Fibril growth was initiated by adding monomeric A β ₁₋₄₀ (50 μ M) to freshly prepared A β ₁₋₄₀ fibril seeds (5%) in the absence and presence of 50 μ M α B and its variants (see preparation chapter).

Electron microscopy (EM)

For the visualization of α B-lysozyme complexes by negative stain EM, α B (25 μ M) in PBS was mixed with lysozyme in different molar ratios and incubated for 1 h at 37 °C after adding 1 mM TCEP to induce lysozyme aggregation. The samples were diluted to α B monomer concentrations of 1 μ M and adsorbed onto EM-grids with continuous carbon film, which were glow-discharged for 30 s prior to sample preparation. The samples were stained with 2% uranyl acetate.

Images were collected on a 100 kV CM100X microscope (Jeol, Tokyo, Japan) onto Kodak SO163 film and digitized with a Hasselblad Flextight X5 scanner.

To visualize A β ₁₋₄₀ fibrils by EM, the monomeric peptide (see preparation chapter) (125 μ M in 50 mM TrisHCl, pH7.5, 100 mM NaCl) was incubated for 4 days at 37 °C without agitation in order to provoke fibril formation. Co-incubation with α B was performed at a chaperone concentration of 2.5 μ M. Addition of 0.03% NaN₃ inhibited bacterial growth. After incubation, amyloid samples in the absence of α B were ultrasonicated for 2 min in order to disrupt fibril clusters. Sample volumes of 3.5 μ L were pipetted onto carbon-coated nickel grids (Quantifoil Micro Tools GmbH) and incubated for 45 s to achieve particle adsorption to the surface. The samples were stained with 2% uranyl acetate. Images were acquired on an EM-902 microscope (Zeiss, Jena, Germany).

Analytical ultracentrifugation (AUC)

Sedimentation velocity experiments were carried out with a ProteomLab XL-I (Beckman, Krefeld, Germany) supplied with absorbance optics. All experiments were performed using protein samples of 20 μ M in PBS at 20 °C at 34,000 rpm in an eight-hole Beckman-Coulter AN-50 Ti rotor. Sedimentation was monitored at 280 nm. Data analysis was carried out using Sedfit,⁶² using a non-model based continuous Svedberg distribution method (c(s)), with time (TI) and radial (RI) invariant noise on.

MAS solid-state NMR spectroscopy

NMR data acquisition and processing were done using TopSpin 2.0 (Bruker). Further data analysis and resonance assignments were performed using the software Sparky (Goddard T. D., Kneller D. G., University of California, San Francisco). NMR data was processed with square sine-bell apodization in direct and indirect dimensions with a shift of the sine-bell of 60°–90°, depending on

spectral resolution and sensitivity. Zero filling and linear forward prediction were applied to improve the spectral resolution when required.

Solid-state NMR experiments were performed using Bruker widebore NMR spectrometers operating at magnetic field strengths of 14.1 T and 16.4 T. Both spectrometers were equipped with standard 4 mm and 3.2 mm triple-resonance MAS probes. Spectra were recorded at an effective temperature of approx. 4 °C. PDSO spectra of α B solutions as well as the α B-lysozyme co-precipitate were recorded at 12 kHz MAS. ^1H heteronuclear decoupling during evolution and mixing periods was achieved by applying TPPM⁶³ using a radio frequency (RF) field of 78 kHz. A PDSO mixing time of 50 ms and a recycle delay of 3 s were used. The acquisition times were 10 ms and 12 ms for the indirect and the direct ^{13}C dimensions, respectively.

^1H -detected NMR assignment experiments were performed at 20 kHz MAS, similarly as described previously.³⁵ The addition of 60 mM Cu(II)-EDTA resulted in ^1H T_1 of approximately 200 ms, yielding recycle delays of about 400 ms. Cross polarization (CP) from $^1\text{H}_\text{N}$ to $^{15}\text{N}_\text{H}$ was performed employing the $n = -1$ Hartmann-Hahn condition with RF field strengths in the range of 60 kHz (^1H) and 35 kHz (^{15}N), respectively. A linear ramp (75–100%) on the ^{15}N channel was used. The length of the CP contact pulse was set to 0.5 ms. Heteronuclear decoupling of ^1H and ^{15}N was achieved with RF field strengths of 4 kHz and 2 kHz (Waltz-16), respectively. ^{13}C J -decoupling during the ^{15}N evolution period was achieved with a composite ^{13}C π -pulse. The solvent signal was suppressed by implementing a pulse train of 4×15 ms with an RF field strength of 4–10 kHz and alternating phase during longitudinal ^{15}N magnetization.^{64,65} For the 3D hCXhNH experiments, the long-range $^1\text{H}_\text{N}$ - ^{13}C CP field strengths were optimized in order to achieve selective magnetization transfer to either the C' or C α /C β nuclei. For the long-range CP, a duration of 2.0 ms was used. ^{13}C transmitter offsets and spectral widths were set to 174 ppm and 20 ppm (hCOhNH), and to 44 ppm and 70 ppm (hCAhNH), respectively. The acquisition times of the 3D hCOhNH spectrum amounted to 80 ms, 16 ms (64 t_2 increments) and 10 ms (64 t_1 increments) for ^1H , ^{15}N and ^{13}C , respectively. The corresponding values for the 3D hCAhNH spectrum were 80 ms, 16 ms (64 t_2 increments) and 6.8 ms (128 t_1

increments), respectively. Both spectra were recorded with 96 transients per increment and a recycle delay of 380 ms giving rise to experimental times of approximately 5 d (hCAhNH) and 3 d (hCOhNH), respectively. The assignment procedure was aided by comparison to the published chemical shifts of precipitated α B and the dimeric α B10m.^{11,12} In particular, the assignments of S76 and I161 were obtained by comparing the chemical shifts from our data with those obtained by Jehle et al.,¹¹ e.g. I161 shows chemical shifts and dipolar contacts to the preceeding residue in 3D hCAhNH and hCOhNH experiments, which are unique in the sequence of the ACD and CTD and fit very well with those observed by Jehle et al..

The RFDR experiments were performed at a magnetic field strength of 21.1 T using a narrow-bore 1.9 mm triple-resonance MAS probe (Bruker). The experiments were performed at 40 kHz MAS adjusting the effective sample temperature to approx. 16 °C. Spectra were recorded using a 200 mg/mL sample of ^2H , ^{13}C , ^{15}N - α B (2 mg). A 2D ^1H - ^{15}N correlation spectrum was recorded as a reference. The 3D ^1H - ^{15}N RFDR experiment was performed with 3 ms homonuclear ^1H mixing. A recycle delay of 300 ms was used. Acquisition times amounted to 50 ms, 10 ms and 5 ms for the direct ^1H dimension, and the indirect ^{15}N and ^1H dimensions, respectively.

Solution-state NMR spectroscopy

Solution-state NMR experiments were performed using Bruker NMR spectrometers operating at magnetic field strengths of 14.1 T and 17.6 T. Spectrometers were equipped with cryogenically cooled probes. Solution-state ^1H - ^{15}N HSQC experiments with ^{13}C , ^{15}N - α B10m were performed at 22 °C in PBS containing 10% D_2O . The concentration of α B10m was in the range of 0.05-0.1 mM (monomer concentration) in order to achieve equimolar ratios with respect to the less water-soluble $\text{A}\beta_{1-40}$. Chemical shift perturbations (CSP) for ^1H - ^{15}N correlations were calculated from $\text{CSP} = \sqrt{(0.2\Delta\delta_{^{15}\text{N}})^2 + (\Delta\delta_{^1\text{H}})^2}$, with $\Delta\delta_{^{15}\text{N}}$ and $\Delta\delta_{^1\text{H}}$ being the absolute values of the chemical shift differences in ppm for the ^{15}N and ^1H dimension, respectively. The experimental error was

estimated by assuming error propagation of systematic errors in resonance line widths of 0.5 Hz (^1H) and 0.8 Hz (^{15}N), respectively. Paramagnetic relaxation enhancements (PRE) were calculated according to $PRE = I_{para}/I_{dia}$, where I_{para} and I_{dia} represent the measured signal intensities for the paramagnetic (oxidized) and diamagnetic (reduced) sample. The experimental error is estimated based on the corresponding signal-to-noise ratios and error propagation. For both, CSP as well as PRE analysis, the following residues are not included: proline and non-assigned residues, resonances showing strong signal overlap as well as histidine signals to compensate for small pH changes upon titration.

^1H - ^{15}N HSQC spectra of full-length ^2H , ^{13}C , ^{15}N - αB (2 mM) in PBS containing 10% D_2O were obtained at a temperature of 22 °C. The resonance assignment of the CTD was achieved by recording 3D HNCQ, HNCA, HN(CA)CO and HN(CO)CA experiments. ^{15}N longitudinal (T_1) and transversal (T_2) relaxation times were determined using standard pulse sequences⁶⁶ with the following relaxation delays: 17, 257, 514, 1370, 1712, 2568, 3424, 4280, 6848 ms (T_1 measurements) and 4, 40, 60, 80, 100, 120, 140, 160, 200, 240, 300 ms (T_2 measurements), respectively. The experimental data was fitted to a mono-exponential decay using SPARKY. The experimental error is estimated by the r.m.s.d. of the exponential fit. Chemical exchange between the two sets of resonance was assessed by ^1H - ^{15}N HSQC-based EXSY experiments with exchange mixing times of 100, 430 and 800 ms during longitudinal ^{15}N magnetization. None of these experiments revealed exchange peaks. The pulsed-field gradient NMR experiment for determination of translational diffusion coefficients was performed at an external magnetic field of 21.1 T, employing a 2.8 mM sample of ^{13}C , ^{15}N - αB . A pseudo-3D HSQC constant-time stimulated echo experiment was used with $\Delta = 1.057$ s and $\delta = 2.2$ ms, respectively.⁶⁷ Theoretical attenuation profiles for different αB n-mers were generated according to $D_{theo} = k_b T / 6\pi\eta R_h$, where the parameters k_b , T , η and R_h denote the Boltzmann constant, the temperature (295 K), the viscosity (1.39 cP for a 2.8 mM solution of αB in PBS) and the hydrodynamic radius (estimated from molecular weights assuming spherical particles).

Uniformly ^{13}C , ^{15}N -labeled $\text{A}\beta_{1-40}$ peptide was solubilized as described above. Final peptide concentrations were in the range of 200–300 μM in PBS containing 10% D_2O . Samples were measured at a magnetic field strength of 14.1 T (Bruker) and at a temperature of 4 $^{\circ}\text{C}$. For backbone resonance assignment, the following experiments were performed: ^1H - ^{13}C HSQC, ^1H - ^1H TOCSY, HNCA, HNCACB, HN(CA)CO and HN(CA)NNH. The secondary structure propensity (SSP) of $\text{A}\beta_{1-40}$ in solution was accessed by analysing the obtained C' , $\text{C}\alpha$ and $\text{C}\beta$ chemical shifts according to the procedure proposed by Forman-Kay and co-workers (<http://pound.med.utoronto.ca/software.html>).⁶⁸ Residue-specific SSP scores of +1.0 and -1.0 represent 100% abundance of α -helical and β -strand conformation, respectively.

Docking of $\text{A}\beta_{1-40}$ onto the αB hexameric ring

Rigid-body docking was performed using the ClusPro server.⁶⁹ The atomic model of the αB 24-mer¹⁶ was used as the receptor structure. For the ligand structure, we used the solid-state NMR structural model of $\text{A}\beta_{1-40}$ fibrils with three-fold symmetry and negative stagger (PDB entry 2LMQ).⁴³ ClusPro allows the selection of “attractive” residues in the receptor and ligand structures to guide the docking. We set the following regions as “attractive”: αB residues L89–L94 and I133–S138 corresponding to strands $\beta 4$ and $\beta 8$, respectively, of the solvent exposed, extended monomers in the hexameric ring, and residues K16–A21 of $\text{A}\beta_{1-40}$ corresponding to strand $\beta 1$ of the top layer in the trimeric fibril. The lowest energy model of the cluster with the best total score was selected as the final $\text{A}\beta_{1-40}/\alpha\text{B}$ docking model.

References for Online Methods

59. Aquilina, J. A. *et al.* Subunit exchange of polydisperse proteins: mass spectrometry reveals consequences of alphaA-crystallin truncation. *J. Biol. Chem.* **280**, 14485–14491 (2005).
60. Aquilina, J. A. *et al.* Phosphorylation of alphaB-crystallin alters chaperone function through loss of dimeric substructure. *J. Biol. Chem.* **279**, 28675–28680 (2004).
61. Dasari, M. *et al.* Bacterial inclusion bodies of Alzheimer's disease β -amyloid peptides can be employed to study native-like aggregation intermediate states. *Chembiochem Eur. J. Chem. Biol.* **12**, 407–423 (2011).
62. Schuck, P. Size-distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and lamm equation modeling. *Biophys. J.* **78**, 1606–1619 (2000).
63. Bennett, A. E., Rienstra, C. M., Auger, M., Lakshmi, K. V. & Griffin, R. G. Heteronuclear decoupling in rotating solids. *J. Chem. Phys.* **103**, 6951–6958 (1995).
64. Paulson, E. K. *et al.* Sensitive high resolution inverse detection NMR spectroscopy of proteins in the solid state. *J. Am. Chem. Soc.* **125**, 15831–15836 (2003).
65. Zhou, D. H. & Rienstra, C. M. High-Performance Solvent Suppression for Proton-Detected Solid-State NMR. *J. Magn. Reson. San Diego Calif* **1997** **192**, 167–172 (2008).
66. Kay, L. E., Torchia, D. A. & Bax, A. Backbone dynamics of proteins as studied by ^{15}N inverse detected heteronuclear NMR spectroscopy: application to staphylococcal nuclease. *Biochemistry* **28**, 8972–8979 (1989).

67. Choy, W.-Y. *et al.* Distribution of molecular size within an unfolded state ensemble using small-angle X-ray scattering and pulse field gradient NMR techniques1. *J. Mol. Biol.* **316**, 101–112 (2002).
68. Marsh, J. A., Singh, V. K., Jia, Z. & Forman-Kay, J. D. Sensitivity of secondary structure propensities to sequence differences between alpha- and gamma-synuclein: implications for fibrillation. *Protein Sci. Publ. Protein Soc.* **15**, 2795–2804 (2006).
69. Comeau, S. R., Gatchell, D. W., Vajda, S. & Camacho, C. J. ClusPro: an automated docking and discrimination method for the prediction of protein complexes. *Bioinforma. Oxf. Engl.* **20**, 45–50 (2004).









