The chaperone αB-crystallin uses different interfaces to capture an amorphous and an amyloid client

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Small heat-shock proteins, including αB-crystallin (αB), play an important part in protein homeostasis, because their ATP-independent chaperone activity inhibits uncontrolled protein aggregation. Mechanistic details of human αB, particularly in its client-bound state, have been elusive so far, owing to the high molecular weight and the heterogeneity of these complexes. Here we provide structural insights into this highly dynamic assembly and show, by using state-of-the-art NMR spectroscopy, that the αB complex is assembled from asymmetric building blocks. Interaction studies demonstrated that the fibril-forming Alzheimer’s disease Aβ1–40 peptide preferentially binds to a hydrophobic edge of the central β-sandwich of αB. In contrast, the amorphously aggregating client lysozyme is captured by the partially disordered N-terminal domain of αB. We suggest that αB uses its inherent structural plasticity to expose distinct binding interfaces and thus interact with a wide range of structurally variable clients.

Proteins are the protagonists of life, and their three-dimensional folds enact a myriad of cellular functions. Stress stimuli, such as elevated temperature or pH, 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 ultimately result in protein aggregation. As a general protective mechanism in response to stress, the cell enhances its 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 aggregation1,2.

The human sHSP αB is a 175-residue protein (20 kDa) that assembles into polydisperse and highly dynamic protein complexes of high molecular weights ranging between 200 and 1,000 kDa (refs 3, 4). The monomeric subunit is organized into three regions (Fig. 1a): the central α-crystallin domain (ACD) comprising residues 60–150 and the flanking N-terminal domain (NTD) and C-terminal domain (CTD). The ACD contains a highly conserved IXI motif (residues I159, P160 and I161 in αB), which has been reported to interact with adjacent subunits5–8. The variable stoichiometry of the αB complex has been linked to dynamic subunit exchange4,8,9. These quaternary fluctuations have hindered atomistic structural investigations in the past5,10. X-ray crystallography and solution-state NMR spectroscopy have been successful in revealing the structure of the central ACD in studies using N- and C-terminally truncated αB variants, which form only dimers in solution11–13. The ACD adopts a β-sandwich structure, in which two β-sheets (formed by strands β3, β9, β8, and β6+7, β5, β4, respectively) pack onto each other, such that strands β4 and β8 form a hydrophobic groove6,11,13 (Fig. 1e). Two monomers align via the elongated strands β6+7 in an antiparallel fashion, thereby forming the dimer building block of αB oligomers. This interface has been designated dimer interface I14, for which three different registers (AP1, AP2, AP3) have been observed5,6,13,15. Structural insights into oligomer architecture have come from magic-angle spinning (MAS) solid-state NMR spectroscopy of precipitated full-length αB5,11,16. In combination with small-angle X-ray scattering (SAXS) and cryo-EM, two structural models for 24-mers have been suggested5,14. Although they differ 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 substructure is stabilized by anchoring the C-terminal IPI motif to the hydrophobic β4–β8 groove (dimer interface II) of a neighboring protomer5,14. This interaction appears to be very dynamic, and the IPI motif occurs in a free (flexible) and a bound (rigid) state17. The extent of the immobilization depends on conditions such as pH and temperature5,7,8,10. In both models, the dimer consists of structurally different protomers and thus appears asymmetric5,14. In the pseudoatomic model derived from cryo-EM, the two protomers have been designated as the extended and bent conformers18, 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 (restricted only by its interactions with neighboring β4–β8 grooves), whereas the CTD of the extended conformer (CTD_ext) is oriented toward 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

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experiments and structural homology modeling. The NMR data have indicated that these structural states are rather transiently populated and that the NTD samples multiple conformations.

A third structural model based on MS and NMR data, however, postulates a highly symmetric polyhedral complex, in which all protomers are equivalent. This structural equivalence has been deduced from solution-state NMR experiments that have revealed only single cross-peaks for the flexible IPI motif of αB (ref. 8). Single sets of resonances for the ACD and CTD of αB have also been reported in solid-state NMR studies, with the exception of (i) heterogeneous region 1 (HR1), involving residues L65–E71, and (ii) residues in strand β8 and the CTD, upon pH drop. The arrangement of protomers in the dimeric building block of αB is therefore still disputed.

Previous studies have shown that αB interacts with a wide range of client proteins that form either amorphous (disordered) or amyloid (ordered) aggregates. Accordingly, αB is involved in various human pathologies in which aggregation processes play a part, for example, cat- aract formation and neurodegenerative disorders such as Alzheimer’s disease. It has been suggested that the chaperone mechanism is distinct for amorphous and amyloid aggregation pathways. Those results have been obtained either by site-directed mutagenesis or by studying the chaperone capabilities of isolated peptides. The high structural and conformational diversity, however, have hampered further structural insights into αB–client complexes.

In this study, we aimed at understanding the heterogeneous architecture of αB and the possible role of asymmetry in its dimeric building block. We further sought to monitor interactions between the polydisperse chaperone and aggregation-prone clients by NMR spectroscopy. These complexes have molecular weights of several megadaltons and are not amenable to other high-resolution techniques. Our results integrate and confirm previous solution-state and solid-state NMR data and demonstrate that the IPI motif does exist in both a flexible and a rigid state.

RESULTS

Structural heterogeneity of αB

To test whether human wild-type (WT) αB is assembled from asymmetric dimers, we studied concentrated solutions of perdeuterated αB by 1H-detected MAS NMR spectroscopy. We have recently demonstrated that this approach enables study of large protein complexes without the need for crystallization or precipitation. The structural heterogeneity of αB yielded spectra that displayed extensive signal overlap due to line widths of about 60–80 Hz (1H) and 30–40 Hz (15N) (Fig. 1d). Overall, we were able to assign 53 amide moieties by triple-resonance experiments (Supplementary Table 1). Virtually all assigned cross-peaks originated from the ACD (Fig. 1f). We were not able to detect or to assign signals arising from the NTD or the N-terminal β2-β3 region of the ACD, including residue M68, for which at least nine different chemical environments have previously been observed. The excised ACD has recently been studied by solution-state NMR (αB64–V152 N146D, referred to herein as αB10m)11,12. A comparison between spectra of oligomeric αB and dimeric αB10m showed the characteristic signal pattern for the ACD in both proteins (Supplementary Fig. 1). The observed chemical-shift changes reported on interaction sites between neighboring dimers in the oligomer, and the β4 and β8 strands showed the largest effects (Supplementary Fig. 1). This result is in agreement with those from previous NMR binding studies and with the existing structural models of αB 24-mers (Fig. 1b,c).

We observed peak doubling or extensive peak broadening for various residues of the ACD, for example, S85, E88 (loop preceding β4), L94, G95, V97, E99–H104 (loop β5), R116 (β6+7), D127, V128 and L131–T134 (loop preceding β8), in contrast to results from previous NMR studies. The affected residues cluster around strands β4, β5 and β8 (Fig. 1f). Because of strong signal overlap, we cannot exclude that cross-peaks of other residues may exhibit a similar behavior. Notably, the inclusion of a 1H chemical-shift dimension enabled us to observe the multiple states, whereas 13C chemical shifts were mostly degenerate for affected residues (Supplementary Fig. 2).

We performed radio frequency-driven recoupling (RFDR) NMR experiments to gain information on the vicinity of exchangeable protons. The RFDR data were in agreement with the reported ACD dimer structure in precipitated αB (Supplementary Fig. 2). The vicinity of amide groups of S115 and H119 supported a major species adopting an APαI register at dimer interface 1, with E117 near the two-fold point-symmetric axis (Supplementary Fig. 2). The side chain imidazole of H104 (β5) showed two resolved 1H-15N correlations (Supplementary Fig. 2). This observation suggests that the ACD in αB oligomers exists in at least two different states that can be resolved. In its excited, dimeric form, however, the ACD did not show multiple resonance sets, for either backbone (Supplementary Fig. 1) or side chain resonances (Supplementary Fig. 2).

The C-terminal residues of αB are highly dynamic and yielded 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, in our study we assigned 11 additional residues, thus yielding assignments for 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. Our results integrate and confirm previous solution-state and solid-state NMR data and demonstrate that the IPI motif does exist in both a flexible and a rigid state.

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 αB assembly were protected from proteolytic processing at both the N terminus and the C terminus. We observed a second resonance set for most residues of the CTD (Fig. 2a), a result similar to our solid-state NMR data of the ACD. We can exclude the possibility of smaller oligomers or degradation products causing one of the two resonance sets for the CTD, because 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 (T1) and transverse relaxation times (T2) for the second set of C-terminal resonances, thus 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 1 s (Fig. 2c). Hence, our solution-state NMR data showed that the entire CTD, including its IPI motif, is highly flexible and populated 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 probably impeded the detection of strands β2–β3 (HR1). Moreover, this underlines that the extreme N terminus is fairly rigid. This contradicts results from previous NMR studies reporting that the first five N-terminal residues of αB (including I3 and I5) are highly flexible.
Interaction of \( \alpha B \) with amorphously aggregating lysozyme

To identify client-binding sites in \( \alpha B \) and to investigate its mode of action, we studied the interaction between \( \alpha B \) and two aggregation-prone clients: the amorphously aggregating model client lysozyme (14 kDa) and the amyloid \( \beta \)-peptide \( \alpha B_{1-40} \) (4 kDa).

Lysozyme readily aggregates amorphously under reducing conditions, owing to breakage of its disulfide bridges. \( \alpha B \) was capable of arresting unfolded lysozyme in a soluble \( \alpha B \)-lysozyme complex (Fig. 3a) and thereby of 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 megadaltons (Fig. 3b). Importantly, native lysozyme was stable in solution and did not interact with \( \alpha B \) (Supplementary Fig. 4).

We performed MAS solid-state NMR experiments to identify which sites of \( \alpha B \) are used to capture reduced lysozyme. At molar...
αB/lysozyme ratios of 7:1 and 1:1 (monomer concentrations), we observed consistent chemical-shift changes in spectra of αB (Fig. 3c). Affected residues included I124, T132 and I133 (loop preceding β8) as well as C-terminal residues T158, I159 and I161 of the IPI motif. Moreover, the isoleucine methyl region showed additional signals (Fig. 3c). Because all isoleucine residues in the ACD and CTD have been assigned, we attribute these additional signals to the otherwise nonobservable I3, I5 and/or I10 in the NTD. In general, the majority of signals were not affected in the presence of lysozyme, thus indicating that the ACD retained its conformation in the αB oligomer upon binding to lysozyme (Supplementary Fig. 5).

The observed chemical-shift changes might arise from direct binding of lysozyme to the hydrophobic β4-β8 groove of αB. Alternatively, the chemical-shift changes might arise 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 αB10m, which lacks 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), whereas the amount of soluble αB10m was only marginally reduced in this time course (Supplementary Fig. 6). Intriguingly, none of the ACD resonances were affected by the presence of aggregating lysozyme (Supplementary Fig. 6). The excised ACD thus seemed to be incompetent at recognizing unfolded lysozyme. Furthermore, a truncated αB variant lacking the NTD (αB ΔNTD) was incapable of inhibiting aggregation of reduced lysozyme, thus underlining the important role of the NTD in capturing unfolded lysozyme (Fig. 3d). These data demonstrate that the binding effects in the solid-state

Figure 3 Interaction of αB with unfolded lysozyme. (a) SDS-PAGE of samples containing varying molar ratios of αB and lysozyme (lyso), as indicated. Unfolding of lysozyme was induced by incubation with Tris-(2-carboxyethyl)phosphine (TCEP) for 1 h at 37 °C. The lanes represent the soluble fraction (S) and the insoluble pellet (P). Uncropped gel image is in Supplementary Data Set 1. (b) Negative-stain EM images of αB in the absence or presence of unfolded lysozyme. Molar ratios of αB and lysozyme are shown at top. Scale bar, 0.1 μm. (c) Threonine (left) and isoleucine (right) spectral region of 13C-13C correlation MAS NMR spectra of αB in the absence (red) or presence of substoichiometric (blue) and stoichiometric (black) amounts of unlabeled lysozyme unfolded after addition of TCEP. Upon titration of lysozyme, additional isoleucine signals become observable (dashed box). (d) TCEP-induced aggregation of lysozyme. Left, addition of 20 μM αB WT at several time points (indicated with arrows) during ongoing aggregation of 5 μM lysozyme or before the addition of TCEP. Right, preincubation of 5 μM lysozyme with 5 μM of αB WT and the indicated variants. Aggregation was initiated by addition of TCEP.
NMR spectra (Fig. 3c) resulted indirectly from altered subunit dynamics as a response to client binding at the NTD. In further support of this conclusion, a solid-state NMR 1H-15N correlation spectrum of perdeuterated αB cocrystallized 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 only signals arising from the rigid and well-structured ACD (Fig. 1d). Upon addition of lysozyme, the partially disordered NTD (Supplementary Fig. 7) hence seemed to become further rigidified, thus causing the additional signals in the solid state.

Interaction of αB with the amyloid client Aβ1–40

We further investigated the chaperone properties of αB toward the amyloid peptide Aβ1–40. As observed by negative-stain EM, αB was a potent inhibitor of Aβ1–40 fibril formation even at molar excess of Aβ1–40 (50:1) with respect to monomeric αB (Fig. 4a). Monitoring the aggregation of Aβ1–40 by dynamic light scattering (DLS) under the same conditions revealed that αB effectively suppressed the accumulation of high-molecular-weight species, while preserving the monomeric state of Aβ1–40 for more than 4 d at 37 °C (Fig. 4b and Supplementary Fig. 8). Nevertheless, the presence of αB resulted in the accumulation of Aβ1–40 oligomers of approximately 30 nm in hydrodynamic diameter (Dh). Soluble Aβ1–40 oligomers of similar size have been reported to be on-pathway intermediates representing the cytotoxic species.9,8

The substoichiometric effects of αB on Aβ1–40 fibril formation indicated a transient interaction between the chaperone and Aβ1–40 and/or a capping-like interaction localized at the ends of prefibrillar structures. Solution-state NMR titrations of dimeric αB10m confirmed weak binding affinity of the ACD to monomeric Aβ1–40 (Fig. 4d). Small chemical-shift changes occurred consistently for a cluster of αB10m residues, for example, V91, V93, I124, S135, S136 and L137, located at the hydrophobic β4-β8 groove (Fig. 4e). This result is supported by titration experiments in which the variant Aβ1–40 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 methanesulfonothioate (MTSL) and added to αB10m. The paramagnetic relaxation enhancement (PRE) induced by the MTSL moiety of bound Aβ1–40 S26C caused a decrease in signal intensities for residues located at the β4-β8 groove of αB10m, for example, V91, G95, S135 and L137 (Fig. 4d,e). The β4-β8 groove, which is a hydrophobic edge of the ACD (Supplementary Fig. 7) thus appeared to be a binding interface for the amyloid client Aβ1–40.

In seeded Aβ1–40 aggregation assays, αB WT and αB ΔNTD suppressed amyloid formation equally well (Fig. 4c). Moreover, the variant S135Q (β8 strand), which has been reported to coordinate the C-terminal IPI motif less efficiently to the β4-β8 groove, was more potent in suppressing amyloid formation (Fig. 4c). In contrast, the S135Q mutation did not affect αB chaperone activity toward amorphous aggregation of lysozyme (Fig. 5d). Together, the data demonstrate that the NTD played a minor part in the inhibition of Aβ1–40 aggregation, whereas the β-rich ACD was sufficient to block this process. Interestingly, 13C chemical shifts of monomeric Aβ1–40 in aqueous solution (Supplementary Table 3) indicated β-strand propensity (Supplementary Fig. 8) for regions that have been previously shown to interact with αB40 and that also adopt β-strand conformation in Aβ1–40 fibrils.41–43
**Figure 5** Model of αB-mediated inhibition of amorphous and amyloid aggregation. Destabilized proteins may self-associate, owing to the exposure of hydrophobic segments. The asterisk indicates that αB potentially undergoes subunit exchange (monomers-dimers), which has been reported to be an important property for chaperone function. The structural plasticity of αB, particularly that of its partially disordered NTD, facilitates binding of structurally undefined clients in a stable and soluble αB–client complex and thereby suppresses amorphous aggregation. Saturation of the holdase causes larger αB–client clusters that may finally coprecipitate. Amyloidogenic clients have pronounced β-strand propensity. αB targets the hydrophobic β-strand core of amyloid species by interacting transiently with protomers as well as with prefibrillar assemblies, thereby impeding fibril nucleation and fibril elongation, respectively. The β-sandwich core of αB (particularly the edge strands β3, β4 and β8) can be viewed as a structurally related unit that mimics the cross-β structure of amyloid systems and that is well suited to adhere to, for example, the ends of protofibrils, as illustrated in the showcase docking model (top and side view; coordinates of the model in Supplementary Data Set 2). The three-fold symmetric Aβ1–40 fibril adopts a β-strand conformation for residues 11–22 (light blue), including the hydrophobic core L17VFFA21, and residues 30–37 (dark blue). For simplicity, αB is shown in gray, and the β4–β8 strands of the extended states of one hexamer are highlighted in red.

**DISCUSSION**

**Structural heterogeneity of αB**

The quaternary dynamics and high polydispersity of αB are considered to be essential for its chaperone function but have hindered atomic-level structural analysis. The NMR data presented here show that monomers within the αB complex are not equivalent, supporting previous structural models in which an asymmetric dimer builds up the tetrahedral αB 24-mer. For example, peak doubling of I133 resonance for residues in strands αB raises the question of whether these observations originate from the same structural feature. We exclude binding of the IPI motif to the β4–β8 groove as a source for the observed peak doubling, because several resolved cross-peaks for residues in strands β4 and β8 (for example, S135, S136 and L137) did not display peak doubling (Supplementary Fig. 1). In the tetrahedral 24-mer model, the extreme 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. The observation of mobile and immobile states might thus reflect the variable flexibility of CTD bent segments. The asterisk indicates that αB potentially undergoes subunit exchange (monomers-dimers), which has been reported to be an important property for chaperone function. The structural plasticity of αB, particularly that of its partially disordered NTD, facilitates binding of structurally undefined clients in a stable and soluble αB–client complex and thereby suppresses amorphous aggregation. Saturation of the holdase causes larger αB–client clusters that may finally coprecipitate. Amyloidogenic clients have pronounced β-strand propensity. αB targets the hydrophobic β-strand core of amyloid species by interacting transiently with protomers as well as with prefibrillar assemblies, thereby impeding fibril nucleation and fibril elongation, respectively. The β-sandwich core of αB (particularly the edge strands β3, β4 and β8) can be viewed as a structurally related unit that mimics the cross-β structure of amyloid systems and that is well suited to adhere to, for example, the ends of protofibrils, as illustrated in the showcase docking model (top and side view; coordinates of the model in Supplementary Data Set 2). The three-fold symmetric Aβ1–40 fibril adopts a β-strand conformation for residues 11–22 (light blue), including the hydrophobic core L17VFFA21, and residues 30–37 (dark blue). For simplicity, αB is shown in gray, and the β4–β8 strands of the extended states of one hexamer are highlighted in red.

Similarly to the ACDs, the CTDs of αB appeared nonequivalent 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 and an immobile state with the IPI motif bound to the β4–β8 groove of neighboring protomers. Furthermore, a millisecond exchange process has been described for the unbound CTD undergoing a ‘flip motion’ with the IPI motif being close to, but not occupying, the β4–β8 groove. The occurrence of multiple sets of resonances for the ACD and CTD in solid-state and solution-state NMR spectra of αB raises the question of whether these observations originate from the same structural feature. We exclude binding of the IPI motif to the β4–β8 groove as a source for the observed peak doubling, because several resolved cross-peaks for residues in strands β4 and β8 (for example, S135, S136 and L137) did not display peak doubling (Supplementary Fig. 1). In the tetrahedral 24-mer model, the extreme 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. The observation of mobile and immobile states might thus reflect the variable flexibility of CTD bent and CTDext. The two flexible CTD states detected by solution-state NMR seemed to interconvert very slowly. Hence, the interconversion may be restricted by subunit exchange, which occurs on a time scale of several minutes. We cannot rule out peak doubling for the flexible CTD, owing to proline cis-trans isomerization, which occurs on a similar time scale.

**Diversity of client interactions**

Our interaction studies suggest that the hydrophobic β4–β8 groove of the ACD represents the binding site for Aβ1–40. Previous NMR studies have shown that αB competes for Aβ1–40 monomer–monomer interactions by transiently binding to the hydrophobic core of Aβ1–40 (L17VFFA21). These hydrophobic residues constitute the central β-strand core in amyloid fibrils. Interestingly, monomeric Aβ1–40 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 \( \alpha B_{1-40} \) is the structural motif that is recognized by the likewise hydrophobic β4-β8 groove of \( \alpha B \). This is in agreement with studies that show that \( \alpha B \) preferentially interacts with \( \alpha B_{1-40} \) oligomers originating from dissociation of \( \alpha B_{1-40} \) fibrils and featuring a higher content of β-strand structure than \( \alpha B_{1-40} \) oligomers formed during the aggregation of monomers.\(^{49,50} \) Notably, \( \alpha B \) itself is capable of forming amyloid fibrils,\(^{51} \) and the segment K90–V100 (β4-β5) can accumulate into β-rich oligomers.\(^{52} \) The β-sandwich of \( \alpha B \) resembles the β-strand assembly of \( \alpha B_{1-40} \) 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 \( \alpha B_{1-42} \) (ref. 53). Likewise, \( \alpha B \) ACD is capable of inhibiting amyloid formation of \( \alpha B_{1-40} \) similarly to \( \alpha B \) WT (Fig. 4c). This demonstrates that \( \alpha B \) ACD, despite its potentially altered oligomer architecture, retained chaperone function toward an amyloidogenic client, thus 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, have reported that the glutamine side chain impedes docking of the IP1 motif into the β4-β8 groove.\(^{4} \) In agreement with such a weakening of dimer interface II in \( \alpha B \) S135Q and the concomitant exposure of its β4-β8 groove, we observed enhanced inhibition of \( \alpha B_{1-40} \) fibril formation (Fig. 4c).

The asymmetric architecture of \( \alpha B \) suggests 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 β4-β8 groove of bent conformers appears to be less accessible for client interactions, owing to its orientation toward adjacent NTDs in the oligomer interior. However, the β4-β8 groove of the extended conformers is unoccupied after dissociation of the CTD and can mediate the interaction with \( \alpha B_{1-40} \). The hexameric ring of \( \alpha B \) and the β-helix of \( \alpha B_{1-40} \) fibrils both have a three-fold symmetry with similar dimensions (diameter ~50 Å) (Fig. 5). This showcase docking model illustrates that the β-strand regions L17VFFA21 of \( \alpha B_{1-40} \) fit properly onto the β4-β8 grooves of extended conformers in the \( \alpha B \) hexameric ring. However, various structural states are populated during amyloid aggregation, thus giving rise to spherical amyloid intermediates\(^{38,39} \) as well as polymorphic mixtures of fibrillar aggregates with different symmetries.\(^{42} \) Binding of the ACD to these β-rich species might be governed by the accessibility of β-strand edges in the growing aggregate, so that \( \alpha B \) can compete for fibril growth. Despite the weak interaction between \( \alpha B \) and monomeric \( \alpha B_{1-40} \) substoichiometric amounts of \( \alpha B \) were sufficient to block \( \alpha B_{1-40} \) fibril formation. Our results hint at an intervention of \( \alpha B \) in both phases of amyloidogenesis: (i) transient interaction with amyloid building blocks, which may possibly be sufficient to hinder the slow fibril nucleation process\(^{54,55} \) and (ii) inhibition of fibril elongation through binding of \( \alpha B \) to the termini of protofibrillar structures, as suggested previously\(^{32} \) (Fig. 5). At higher abundance, \( \alpha B \) also adheres to the walls of \( \alpha B_{1-42} \) fibrils, thereby affecting their elongation.\(^{52} \)

We found that, in contrast to binding of amyloid client \( \alpha B_{1-40} \), binding of the amorphously aggregating lysozyme occurred at the partially disordered NTD of \( \alpha B \). Previous studies have shown that denatured lysozyme collapses into a molten-globule state,\(^{55} \) which is trapped by \( \alpha B \).\(^{36} \) In the presence of unfolded lysozyme, additional resonances appeared in the \( ^{13}C-^{13}C \) and \( ^{1}H-^{15}N \) correlation spectra of \( \alpha B \) (Supplementary Fig. 5). These new cross-peaks must have originated from the NTD, because resonances of the ACD and CTD had been assigned. This indicates that the NTD rigidified to a higher extent upon binding to unfolded lysozyme. Because the NTD and CTD mediate subunit exchange,\(^{42} \) binding of lysozyme to the NTD would consequently alter \( \alpha B \) quaternary dynamics. This was reflected in chemical-shift changes involving the CTD and the β4-β8 groove. Importantly, \( \alpha B \) and \( \alpha B \) ACD—both containing β4-β8 grooves but lacking NTDs—were not capable of rescuing unfolded lysozyme from aggregation (Fig. 3d and Supplementary Fig. 6). This underlines the importance of structural plasticity of the NTD in lysozyme recognition and binding, consistently with previous reports on MjHSP16.5 and its sequestration of unfolded lysozyme via NTDs.\(^{57} \)

Moreover, studies on PsHSP18.1 have suggested 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 β4-β8 groove of the rather static ACD, are fairly hydrophobic (Supplementary Fig. 7) and are buried in the native oligomer through intersubunit contacts with neighboring NTDs and CTDs.\(^{5,14,16} \) (Fig. 1b,c). Structural fluctuations inherent to \( \alpha B \)\(^{8,9,17} \) 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 allows very short-lived chaperone–client complexes to compete for client self-association. Hence, intervention of \( \alpha B \) in amorphous aggregation of lysozyme and in fibril formation of \( \alpha B_{1-40} \) has fundamentally different mechanisms. These findings may not be applicable for the entire interactome of \( \alpha B \), i.e., conformational ensembles of hundreds of misfolded proteins, but they may disclose some general principles in antiaggregation 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.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Accession codes.** The chemical-shift assignments of the ACD and the CTD of \( \alpha B \) have been deposited in the Biological Magnetic Resonance Data Bank under accession code 26640.

**Note:** Any Supplementary Information and Source Data files are available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

A.M., J.P., M.S. and K.C.B. cloned, recombinantly expressed, purified and characterized human \( \alpha B \) 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 \( \alpha B \) S135Q. M.S. and S.A. contributed the diffusion-ordered spectroscopy and proton-driven spin diffusion NMR data. B.R. generated the docking model of \( \alpha B \) and fibrillar \( \alpha B_{1-40} \). E.P. prepared recombinant \( \alpha B_{1-40} \) and supported the resonance
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Sample preparation. Unlabeled as well as 13C- and 15N-labeled human full-length αβ (UniProt P02511), truncated αβ10m (residues G64–V152 with the mutation N146D)11, αβ ΔNTD and αβ S135Q were recombinantly expressed and purified according to protocols described elsewhere12,13. The entire αβ elution peak in the final size-exclusion–chromatographic step was pooled and used for further experiments. Hence, no restricted peak selection was applied to enrich a certain oligomeric state of αβ. Initial attempts to cleave off an N-terminal hexahistidine tag from recombinant αβ with the protease enterokinase (clearing 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 (αβS135B-R157) was identified via MS with an abundance of about 50% in SDS-PAGE (Supplementary Fig. 6). Longer incubation times with a high excess of enterokinase did not yield further conversion at the C-terminal nonspecific cleavage site. Owing to the low efficiency of the proteolytic cleavage, we overexpressed αβ without any affinity tag24. [3H, 13C, 15N]αβ was heterologously expressed in E. coli BL21 (DE3) (Merck) growing in D2O-based minimal medium containing [13C, 15N]NH4Cl (1 g/L) and uniformly labeled [3H, 13C]glucose (4 g/L). The purified protein was subjected 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% H2O. The protein concentration was determined by SDS-PAGE and Coomassie staining. The sediment and supernatant fraction were analyzed after centrifugation at 10,000 g for 5 min. The insoluble fraction was washed with PBS and then subjected to a second centrifugation step.

Aβ1–40 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 Aβ1–40 aggregation at 37 °C were performed at an initial peptide concentration of 125 μM. Co-incubation with αβ was performed at a chaperone concentration of 2.5 μM. Bacterial growth was inhibited by addition of 0.03% NaN3. The samples were filtered through 0.1-μm membranes (Millipore). Aliquots of 80 μL were incubated and automatically measured in sealed quartz cuvettes (Hellma) for 4 d in time intervals of 4 h. At each time point, three measurements were collected. Data accumulation encompassed over 300 runs (for 4 s for each run). Data analysis was performed with the software DTS 5.03 (Malvern Instruments). Seed Aβ1–40 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 addition of monomeric Aβ1–40 (50 μM) to freshly prepared αβ1–40 fibril seeds (5%) in the absence or presence of 50 μM αβ and its variants (preparation described above).

Electron microscopy (EM). For the visualization of αβ–lysozyme complexes by negative-stain EM, αβ (25 μM) in PBS was mixed with lysozyme in different molar ratios and incubated for 1 h at 37 °C after addition of 1 mM TCEP to induce lysozyme aggregation. The samples were diluted to αβ monomer concentrations of 1 μM and adsorbed onto EM grids with continuous carbon film, which were glow-discharged for 30 s before sample preparation. The samples were stained with 2% uranyl acetate. Images were collected on a 100-kV CM100X microscope (JEOL) on Kodak SO163 film and were digitized with a Hasselblad Flextight X5 scanner.

To visualize Aβ1–40 fibrils by EM, the monomeric peptide (preparation described above) (125 μM in 50 mM Tris-HCl, pH 7.5, and 100 mM NaCl) was incubated for 4 d at 37 °C without agitation to induce fibril formation. Co-incubation with αβ was performed at a chaperone concentration of 2.5 μM. Addition of 0.03% NaN3 inhibited bacterial growth. After incubation, amyloid samples in the absence of αβ were ultrasonicated for 2–3 s to disrupt fibril clusters. Sample volumes of 3.5 μL were pipetted onto carbon-coated nickel grids (Quantifoil Micro Tools) 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).

Analytical ultracentrifugation (AUC). Sedimentation velocity experiments were carried out with a ProteomLab XL-I (Beckman) supplied with absorbance optics. All experiments were performed with protein samples of 20 μM in PBS at 20 °C at 34,000 r.p.m. in an eight-hole Beckman-Coulter AN-50 Ti rotor. Sedimentation was monitored at 280 nm. Data analysis was carried out with Sedfit25, with a non-model based continuous Svedberg distribution method (ccdf), with time (TT) and radial (RI) invariant noise on.

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MAS solid-state NMR spectroscopy. NMR data acquisition and processing were carried out with TopSpin 2.0 (Bruker). Further data analysis and resonance assignments were performed with Sparky (https://www.cgl.ucsf.edu/home/sparky/). NMR data were 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 with Bruker wide-bore 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 -4°C. PSDS spectra of eB solutions as well as the eB-lysoyzyme coprecipitate were recorded at 12 kHz MAS. 1H heteronuclear decoupling during evolution and mixing periods was achieved by application of TPPM with a radio frequency (RF) field of 78 kHz. A PSDS 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 13C dimensions, respectively.

1H-detected NMR assignment experiments were performed at 20 kHz MAS, similarly to procedures described previously. The addition of 60 mM Cu(ii)–H2O to 15N solutions with the n = -1 Hartmann-Hahn condition with RF field strengths of approximately 60 kHz (1H) and 35 kHz (15N). A linear ramp (75–100%) on the 15N channel was used. The length of the contact pulse was set to 0.5 ms. Heteronuclear decoupling of 1H and 15N was achieved with RF field strengths of 4 kHz and 2 kHz (Waltz-16), respectively. 13C/Cα decoupling during the 15N evolution period was achieved with a composite 13C/1H/31P 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 15N magnetization. With the 3D hCXhNH experiments, the long-range 1H–13C CF CP field strengths were optimized to achieve selective magnetization transfer to either the C or the Cα/Cβ nuclei. For the long-range CP, a duration of 2.0 ms was used. 13C transmitter offsets and spectral widths were set to 174 p.p.m. and 20 p.p.m. (hCOHNH), and to 44 p.p.m. and 70 p.p.m. (hCAHNH). The acquisition times of the 3D hCOHNH spectra amounted to 80 ms, 16 ms (64 t1 increments) and 10 ms (64 t1 increments) for 1H, 15N, and 13C, respectively. The corresponding values for the 3D hCAHNH spectra were 80 ms, 16 ms (64 t1 increments) and 6.8 ms (128 t1 increments), respectively. Both spectra were recorded with 96 transits per increment and a recycle delay of 380 ms, thus giving rise to experimental times of approximately 5 d (hCAHNH) and 3 d (hCOHNH). The assignment procedure was aided by comparison to the published chemical shifts of precipitated eB and the dimeric eB10m. In particular, the assignments of S76 and I161 were obtained by comparing the chemical-shift data with those obtained by Jehle et al. with the published chemical shifts of precipitated eB (2 mg). A 2D 1H-15N correlation spectrum was recorded as a reference. The 3D 1H-15N-RF 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 15N and 13C dimensions, respectively.

Solution-state NMR spectroscopy. Solution-state NMR experiments were performed with 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-15N HSQC experiments with 13C-15N-eB10m were performed at 22°C in PBS containing 10% D2O. The concentration of eB10m was in the range of 0.05–0.1 mM (monomer concentration) to achieve equimolar ratios with respect to the less water-soluble Aβ1–40. Chemical-shift perturbations (CSPs) for 1H, 15N correlations were calculated from

\[
\text{CSP} = \sqrt{0.5}\left(\Delta\sigma_{15N}^2 + \Delta\sigma_{1H}^2\right),
\]

with \(\Delta\sigma_{15N}\) and \(\Delta\sigma_{1H}\) being the absolute values of the chemical-shift differences in p.p.m. for the 15N and 1H dimensions, 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 (15N). Paramagnetic relaxation enhancements (PREs) were calculated according to PRE = \(\frac{\Delta\sigma_{15N}}{\Delta\sigma_{1H}}\), where \(\Delta\sigma_{15N}\) and \(\Delta\sigma_{1H}\) represent the measured signal intensities for the paramagnetic (oxidized) and diamagnetic (reduced) sample. The experimental error is estimated on the basis of the corresponding signal-to-noise-ratios and error propagation. For both CSP and PRE analysis, the following residues were not included: proline and nonasigned residues, resonances showing strong signal overlap, and histidine signals to compensate for small pH changes upon titration. 1H-15N HSQC spectra of full-length [1H, 13C, 15N]eB (2 mM) in PBS containing 10% D2O were obtained at a temperature of 22°C. The resonance assignment of the CTD was achieved by recording 3D HNCO, HNCA, HN(CA)CO and HN(CO)CA experiments. 15N longitudinal (T1) and transversal (T2) relaxation times were determined with standard pulse sequences with the following relaxation delays: 17, 257, 514, 1,370, 1,712, 2,568, 3,424, 4,280, and 6,848 ms (T1 measurements) and 4, 40, 60, 80, 100, 120, 140, 160, 200, 240, and 300 ms (T2 measurements). The experimental data were fitted to a monoexponential decay with Sparky. The experimental error was estimated by the r.m.s.d. of the exponential fit. Chemical exchange between the two sets of resonances was assessed by 1H-15N HSQC–based EXSY experiments with exchange mixing times of 100, 430 and 800 ms during longitudinal 15N 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, with a 2.8-MW sample of [13C, 15N]eB. A pseudo-3D HSQC constant-time stimulated echo experiment was used with \(\Delta = 1.057\) s and \(\tau = 2.2\) ms, respectively. Theoretical attenuation profiles for different eB r-mers were generated according to \(D_{\text{theo}} = \frac{h_0^2}{7\pi\rho R_0}\), where the parameters \(h_0, T, \tau, \rho_0\) denote the Boltzmann constant, the temperature (295 K), the viscosity (1.39 cp) for a 2.8-MW solution of eB in PBS and the hydrodynamic radius (estimated from molecular weights assuming spherical particles).

Uniformly labeled [13C1, 15N]Aβ1–40 peptide was solubilized as described above. Final peptide concentrations were in the range of 200–300 μM in PBS containing 10% D2O. Samples were measured at a magnetic field strength of 14.1 T (Bruker) and at a temperature of 4°C. For backbone resonance assignment, the following experiments were performed: 1H-13C HSQC, 1H-15N TOCSY, HNCA, HN(CA)CO and HN(CO)CA. The secondary–structure propensity (SSP) of Aβ1–40, in solution was analyzed by analysis of the obtained Cα and Cβ chemical shifts according to the procedure proposed by Forman-Kay and co-workers (http://pound.med.utoronto.ca/software.html). Residue-specific SSP scores of +1.0 and −1.0 represent 100% abundance of α-helical and β-strand conformation, respectively.

Docking of Aβ1–40 onto the eB hexameric ring. Rigid-body docking was performed with the ClusPro server. The atomic model of the eB-24-mer was used as the receptor structure. For the ligand structure, we used the solid-state NMR structural model of Aβ1–40 fibrils with three-fold symmetry and negative stager (PDB 2LMQ). ClusPro allows the selection of ‘attractive’ residues in the receptor and ligand structures to guide the docking. We set the following regions as attractive: eB residues L89–L94 and 113–S138 (corresponding to strands β4 and β8, respectively, of the solvent exposed, extended monomers in the hexameric ring) and residues K16–A21 of Aβ1–40 (corresponding to strand β1 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 Aβ1–40/eB docking model.

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 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. 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 techniques. *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.* **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. *Bioinformatics* **20**, 45–50 (2004).