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

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

Authors:

Andi Mainz[1,2,3], Jirka Peschek[1,3], Maria Stavropoulou[1], Katrin C. Back[1], Benjamin Bardiaux[4], Sam Asami[1], Elke Prade[1], Carsten Peters[1], Sevil Weinkauf[1], Johannes Buchner[1], Bernd Reif[1,5]

Affiliations:

[1]Munich Center for Integrated Protein Science at Department Chemie, Technische Universitaet Muenchen, Garching, Germany.

[2]Solid-state NMR Spectroscopy, Leibniz-Institut fuer molekulare Pharmakologie, Berlin-Buch, Germany.

[4]Unité de Bioinformatique Structurale, Institut Pasteur, Paris, France.

[5]Deutsches Forschungszentrum für Gesundheit und Umwelt, Helmholtz-Zentrum Muenchen, Neuherberg, Germany.

[3]Present addresses: Technische Universitaet Berlin, Germany (A.M.) and University of California, San Fransisco, USA (J.P.)

Corresponding author:

Correspondence to: reif@tum.de

Abstract

Small heat-shock proteins, among them αB-crystallin (αB), play an important role in protein homeostasis, as their ATP-independent chaperone activity inhibits uncontrolled protein aggregation. Mechanistic details of human αB, 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 αB complex is assembled from asymmetric building blocks. Interaction studies demonstrate that the fibril-forming Alzheimer's disease $Aβ_{1-40}$ peptide preferentially binds to a hydrophobic edge of the central β-sandwich of αB. By contrast, the amorphously aggregating client lysozyme is captured by the partially-disordered N-terminal domain of αB. We suggest that αB 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 αB-crystallin (αB) 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 αB), which has been reported to interact with adjacent subunits.[5–8]

The variable stoichiometry of the αB 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 αB variants, which form only dimers in solution.[11–13] The ACD adopts a β-sandwich structure, in which two β-sheets (β3, β9, β8 and β6+7, β5, β4) pack onto each other such that strands β4 and β8 form a hydrophobic groove (**Fig. 1e**).[6,11,13] Two monomers align via the elongated strands β6+7 in an antiparallel fashion, thereby forming the dimeric building block of αB 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-

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length αB.[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 β4–β8 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 β4–β8 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 αB.[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) for residues in strand β8 and the CTD upon pH drop.[5,11] 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 either form amorphous (disordered) or amyloid (ordered) aggregates.[19–26] Accordingly, αB 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 αB 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 (β3–β4) and L131–V142 (β8) 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 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 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 αB allows adjusting to different structural motifs of the aggregating species.

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 using ^{1}H-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 αB yielded spectra that displayed extensive signal overlap due to line widths of about 60–80 Hz (^{1}H) 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 β2–β3 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 ($αB_{G64-V152}$–N146D, in the following referred to as αB10m).[11,12] A comparison between spectra of oligomeric αB 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 β4 and β8 strands showing the largest effects (**Supplementary Fig. 1**). This result is in agreement with previous NMR binding studies[7] and with the existing structural models of αB 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 β4), L94, G95, V97 and E99–H104 (loop-β5), R116 (β6+7) as well as D127, V128 and L131–T134 (loop preceding β8). As shown in **Figure 1f**, the affected residues cluster around strands β4, β5 and β8. 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 ^{1}H 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 structure[5] in precipitated αB (**Supplementary Fig. 2**). The spatial vicinity between the amide groups of S115 and H119 supported a major species adopting an AP_{II} register[6] at dimer interface I with E117 near the two-fold point-symmetric axis (**Supplementary Fig. 2**). The side-chain imidazole of H104 (β5) showed two resolved ^1H-^{15}N 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 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 αB are very dynamic and yield narrow resonances in solution-state ^1H-^{15}N 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 αB 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**). ^{15}N 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 β2–β3 (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 Aβ$_{1–40}$ (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 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, 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**). 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 αB oligomer upon binding to lysozyme (**Supplementary Fig. 5**).

The observed chemical shift changes might originate from direct binding of lysozyme to the hydrophobic β4–β8 groove of αB. 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 αB10m, which is devoid of the NTD and CTD, and monitored chemical shifts upon addition of reduced lysozyme. ^{1}H spectra clearly showed the resonances of native lysozyme rapidly decreasing (quantitative precipitation after 3 h), whilst the amount of soluble αB10m 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 αB variant missing the NTD (αB–ΔNTD) 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 ^{1}H-^{15}N correlation spectrum of perdeuterated αB 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 *αB* with the amyloid client A*β*$_{1-40}$

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

The sub-stoichiometric effects of αB on Aβ$_{1-40}$ fibril formation indicated a transient interaction between chaperone and Aβ$_{1-40}$ and/or a capping-like interaction localized at the ends of protofibrillar structures. Solution-state NMR titrations of dimeric αB10m confirmed weak binding affinity of the ACD to monomeric Aβ$_{1-40}$ (**Fig. 4d**). Small chemical shift changes occurred consistently for a cluster of αB10m residues, e.g. 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 Aβ$_{1-40}$–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 methanesulfonothioate (MTSL) and added to αB10m. The paramagnetic relaxation enhancement (PRE) induced by the MTSL-moiety of bound Aβ$_{1-40}$–S26C caused a decrease in signal intensities for residues located at the β4–β8 groove of αB10m, e.g. V91, G95, S135 and L137 (**Fig. 4d** and **Fig. 4e**).

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 Aβ$_{1-40}$.

In seeded Aβ$_{1-40}$ aggregation assays, both αB–WT and αB–ΔNTD equally well suppressed amyloid formation (**Fig. 4c**). Moreover, the variant S135Q (β8 strand), that has been reported to coordinate the C-terminal IPI-motif less efficiently to the β4–β8 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 Aβ$_{1-40}$ aggregation, whereas the β-rich ACD was sufficient to block this process. Interestingly, ^{13}C chemical shifts of monomeric Aβ$_{1-40}$ 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 Aβ$_{1-40}$ fibrils.[41–43]

Discussion

Structural heterogeneity of αB

The quaternary dynamics and large polydispersity of αB 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 αB complex are not equivalent, supporting previous structural models in which an asymmetric dimer builds up the tetrahedral αB 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 αB10m yielded a single resonance set, suggesting that structural heterogeneity is induced by the different arrangement of NTDs and CTDs in αB oligomers.

Another source of heterogeneity, and thus a possible explanation for NMR peak splitting, arises from quaternary dynamics. αB 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 ($K_d \sim 2\ \mu M$),[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 (~ 25 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 αB oligomers.[10,44]

Similar to the ACDs, the CTDs of αB 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 β4–β8 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 β4–β8 groove.[8] 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 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, as several resolved cross-peaks for residues in strands β4 and β8 (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 β4–β8 groove of the ACD represents the binding site for $A\beta_{1-40}$. Previous NMR studies have shown that αB competes for $A\beta_{1-40}$ monomer-monomer interactions by transiently binding to the hydrophobic core of $A\beta_{1-40}$ ($L^{17}VFFA^{21}$).[40] These hydrophobic residues

constitute the central β-strand core in amyloid fibrils.[41–43] Interestingly, monomeric Aβ$_{1–40}$ partially populated structural states, in which residues L[17]VFFA[21] adopted β-strand conformation under physiological conditions **(Supplementary Fig. 8)**. Hence, the hydrophobic β-strand region of Aβ$_{1–40}$ is the structural motif, which is recognized by the likewise hydrophobic β4–β8 groove of αB. This is in agreement with studies that show that αB preferentially interacts with Aβ$_{1–40}$ oligomers originating from disaggregation of Aβ$_{1–40}$ fibrils and featuring a higher content of β-strand structure than Aβ$_{1–40}$ oligomers formed during the aggregation of monomers.[49,50] Notably, αB itself is capable of forming amyloid fibrils,[51] and the segment K90–V100 (β4–β5) can accumulate into β-rich oligomers.[52] The β-sandwich of αB resembles the β-strand assembly of Aβ$_{1–40}$ 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 Aβ$_{1–42}$.[53] Likewise, αB–ΔNTD was capable to inhibit amyloid formation of Aβ$_{1–40}$ similar to αB–WT (**Fig. 4c**). This demonstrates that αB–ΔNTD, 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 β4–β8 groove.[7] In agreement with such a weakening of dimer interface II in αB–S135Q and the concomitant exposure of its β4–β8 groove, we observed enhanced inhibition of Aβ$_{1–40}$ 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 β4–β8 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 β4–β8 groove of the extended conformers is unoccupied after dissociation of the CTD, and can mediate the interaction with Aβ$_{1–40}$. As illustrated in **Figure 5**,

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the hexameric ring of αB[5] and the β-helix of Aβ$_{1-40}$ fibrils[43] have both a three-fold symmetry with similar dimensions (diameter ca. 50 Å). This showcase docking model illustrates that the β-strand regions L^{17}VFFA^{21} of Aβ$_{1-40}$ fit properly onto the β4–β8 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 αB10m and monomeric Aβ$_{1-40}$, sub-stoichiometric amounts of αB were sufficient to block Aβ$_{1-40}$ 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 Aβ$_{1-42}$ fibrils thereby affecting their elongation.[22]

In contrast to the amyloid client Aβ$_{1-40}$, we found that binding of the amorphously 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 ^{13}C-^{13}C and ^{1}H-^{15}N 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 β4–β8 groove. Importantly, αB10m and αB–ΔNTD – both harboring β4–β8 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 β4–β8 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 αB[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 αB in amorphous aggregation of lysozyme and in fibril formation of Aβ$_{1-40}$ differs fundamentally in its mechanism. These findings may not be applicable for the entire interactome of αB, 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 αB are deposited in the BMRB under accession code 26640.

Author contributions

A.M., J.P., M.S. and K.C.B. cloned, recombinantly expressed, purified and characterized human αB 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 αB-S135Q. M.S. and S.A. contributed the DOSY and PDSD NMR data. B.B. generated the docking model of αB and fibrillar Aβ$_{1-40}$. E.P. prepared recombinant Aβ$_{1-40}$ and supported the resonance assignment of Aβ$_{1-40}$ in solution. C.P. performed the EM experiments of αB 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.

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Figure Legends

Fig. 1 Protomers in the αB complex are not equivalent. (a) Domain organization of αB. 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 αB 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 αB 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 β6+7) and dimer interface II (via β4–β8) 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 αB.

Fig. 2 Structural plasticity of the αB CTD. (a) Solution-state ^1H-^{15}N heteronuclear single-quantum coherence (HSQC) spectrum of human αB 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 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 1h at 37℃. The lanes represent the soluble fraction (S) and the insoluble pellet (P). See also **Supplementary Data Set 1**. **(b)** Negative-stain EM images of αB in the absence and presence of unfolded lysozyme. Molar ratios

of αB and lysozyme are given on top. The scale bar corresponds to 0.1 μm. **(c)** Threonine (left) and isoleucine spectral region (right) of ^{13}C-^{13}C correlation MAS NMR spectra of αB 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 μM αB-WT at several time points (indicated with arrows) during ongoing aggregation of 5 μM lysozyme or prior to the addition of TCEP. Right: Lysozyme (5 μM) was pre-incubated with 5 μM of αB-WT and variants (see legend) and aggregation was initiated by adding TCEP.

Fig. 4 Interaction of αB with Aβ$_{1-40}$. **(a)** Negative-stain EM images of Aβ$_{1-40}$ incubated for 4 days at 37°C in the absence (left) and presence (right) of sub-stoichiometric amounts of αB (50:1). The scale bars correspond to 1 μm. **(b)** Aggregation of Aβ$_{1-40}$ as monitored by DLS. Incubation of Aβ$_{1-40}$ was performed for 4 d at 37°C in the absence (red) and presence (black) of sub-stoichiometric amounts of αB (50:1). The initial and final size distributions are shown on top. The light scattering intensity and the hydrodynamic diameter D_h of monomeric Aβ$_{1-40}$ (triangles) and oligomeric Aβ$_{1-40}$ (diamonds) are shown below. Error bars correspond to s.d. **(c)** Seeded aggregation of Aβ$_{1-40}$ (50 μM) is suppressed by the addition of 5 μM αB–WT and its variants (see legend). Freshly prepared fibril seeds of Aβ$_{1-40}$ were used to initiate fibril growth. **(d)** Sections of ^{1}H-^{15}N HSQC spectra of αB10m. Left: Chemical shift changes of αB10m in the absence (black) and presence of Aβ$_{1-40}$ (red). Right: PREs in the presence of paramagnetic (red) and diamagnetic Aβ$_{1-40}$–S26C–MTSL (black). **(e)** Chemical shift changes ($\Delta\delta$) and PREs are plotted versus the αB10m 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 αB-mediated inhibition of amorphous and amyloid aggregation. Destabilized proteins may self-associate due to the exposure of hydrophobic segments. The asterisk denotes that αB 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 αB, in particular 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 co-precipitate. 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 pre-fibrillar assemblies thereby impeding fibril nucleation and fibril elongation, respectively. The β-sandwich core of αB (in particular 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, e.g. to the ends of protofibrils as illustrated in the showcase docking model (top and side view). The three-fold symmetric $A\beta_{1-40}$ fibril adopts β-strand conformation for residues 11–22 (light blue), including the hydrophobic core $L^{17}VFFA^{21}$, and residues 30–37 (dark blue).[43] For simplicity, αB is shown in grey and the β4–β8 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 αB10m (residues G64–V152 with the mutation N146D)[11], αB–ΔNTD as well as αB–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 His$_6$-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 (αB$_{M1-R157}$) 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]

[2]H, ^{13}C, ^{15}N-αB was heterologously expressed in *E. coli* BL21 (DE3) (Merck, Darmstadt, Germany) growing in D$_2$O-based minimal medium containing [15]NH$_4$Cl (1 g/L) and uniformly labeled ^{2}H, ^{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% H$_2$O and 80% D$_2$O (PBS$_{20}$), 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% H$_2$O.

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 αB at 280 nm (molar extinction coefficient ε of 13,980 M^{-1} cm^{-1}). Cu(II)-EDTA (300 mM in PBS_{20}) 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 µL) or thin-walled 3.2 mm (50 µL) MAS rotors (Bruker). Protein sedimentation prior to the MAS experiment via ultracentrifugation into the MAS rotor[46] was yielding severely broadened resonances with line widths on the order of 180 Hz (^{1}H) and 120 Hz (^{15}N), 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 ^{13}C, ^{15}N-αB or ^{2}H, ^{13}C, ^{15}N-αB, respectively. Typically, the final concentration of isotopically labeled αB 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 αB and reduced lysozyme was prepared, the quantitative co-precipitate was centrifuged directly into the MAS rotor. The sample with a 7-fold molar excess of αB remained virtually devoid of precipitation. Traces of precipitate were removed by centrifugation and only the supernatant (50 µL) was filled into the MAS rotor.

Heterologously expressed Aβ$_{1-40}$ was prepared as described previously.[61] To ensure that Aβ$_{1-40}$ 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 µM. This procedure was performed on ice with pre-cooled solutions. Aβ$_{1-40}$ fibrils were prepared based on a protocol described previously.[61] Briefly, Aβ$_{1-40}$ fibrils were formed by supplementing

monomeric Aβ$_{1-40}$ at a concentration of 50 μM with 10% seeds obtained from former seeding cycles (<12 generations of seeding) followed by incubation under agitation at room temperature. Aβ$_{1-40}$ seeds were obtained by ultrasonicating preformed Aβ$_{1-40}$ fibrils for 3 x 10 s.

To produce *S*-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonothioate (MTSL)-labeled Aβ$_{1-40}$ peptides, synthetic Aβ$_{1-40}$–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). Aβ$_{1-40}$–S26C (200 μM 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. Aβ$_{1-40}$–S26C– MTSL in PBS was added to ^{13}C, ^{15}N-αB10m yielding a final concentration of approx. 100 μM 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 μM 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 μM αB–WT were added at different time points. To test the chaperone activity of αB–WT, αB–S135Q and αB–ΔNTD, 5 μM lysozyme were pre-incubated with 5 μM of αB and its variants.

The solubility of denatured lysozyme in the absence and presence of αB 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.

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 Aβ$_{1-40}$ aggregation at 37°C were performed at an initial peptide concentration of 125 μM. Co-incubation with αB was done at a chaperone concentration of 2.5 μM. Bacterial growth was inhibited by adding 0.03% NaN$_3$. 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 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 Aβ$_{1-40}$ 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 Aβ$_{1-40}$ (50 μM) to freshly prepared Aβ$_{1-40}$ fibril seeds (5%) in the absence and presence of 50 μM αB and its variants (see preparation chapter).

Electron microscopy (EM)

For the visualization of αB–lysozyme complexes by negative stain EM, αB (25 μM) 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 αB monomer concentrations of 1 μM 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 $A\beta_{1-40}$ fibrils by EM, the monomeric peptide (see preparation chapter) (125 µM 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 αB was performed at a chaperone concentration of 2.5 µM. Addition of 0.03% NaN_3 inhibited bacterial growth. After incubation, amyloid samples in the absence of αB were ultrasonicated for 2 min in order to disrupt fibril clusters. Sample volumes of 3.5 µL 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 µM 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. PDSD spectra of αB solutions as well as the αB-lysozyme co-precipitate were recorded at 12 kHz MAS. ^{1}H heteronuclear decoupling during evolution and mixing periods was achieved by applying TPPM[63] using a radio frequency (RF) field of 78 kHz. A PDSD 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.

^{1}H-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 ^{1}H T_1 of approximately 200 ms, yielding recycle delays of about 400 ms. Cross polarization (CP) from ^{1}H$_N$ to ^{15}N$_H$ was performed employing the $n = -1$ Hartmann-Hahn condition with RF field strengths in the range of 60 kHz (^{1}H) 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 ^{1}H 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}H$_N$-^{13}C CP field strengths were optimized in order to achieve selective magnetization transfer to either the C' or Cα/Cβ 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 ^{1}H, ^{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 αB and the dimeric αB10m.[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 ^{2}H, ^{13}C, ^{15}N-αB (2 mg). A 2D ^{1}H-^{15}N correlation spectrum was recorded as a reference. The 3D ^{1}H-^{15}N RFDR experiment was performed with 3 ms homonuclear ^{1}H mixing. A recycle delay of 300 ms was used. Acquisition times amounted to 50 ms, 10 ms and 5 ms for the direct ^{1}H dimension, and the indirect ^{15}N and ^{1}H 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 ^{1}H-^{15}N HSQC experiments with ^{13}C, ^{15}N-αB10m were performed at 22 °C in PBS containing 10% D_2O. The concentration of αB10m 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 Aβ$_{1–40}$. Chemical shift perturbations (CSP) for ^{1}H-^{15}N correlations were calculated from $CSP = \sqrt{(0.2\Delta\delta_{15N})^2 + (\Delta\delta_{1H})^2}$, with $\Delta\delta_{15N}$ and $\Delta\delta_{1H}$ being the absolute values of the chemical shift differences in ppm for the ^{15}N and ^{1}H dimension, respectively. The experimental error was

estimated by assuming error propagation of systematic errors in resonance line widths of 0.5 Hz (^{1}H) 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.

^{1}H-^{15}N HSQC spectra of full-length ^{2}H, ^{13}C, ^{15}N-αB (2 mM) in PBS containing 10% D$_2$O 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. ^{15}N longitudinal (T_1) and transversal (T_2) relaxation times were determined using standard pulse sequences[66] 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 ^{1}H-^{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 Δ = 1.057 s and δ = 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 Aβ$_{1-40}$ peptide was solubilized as described above. Final peptide concentrations were in the range of 200–300 μM in PBS containing 10% D$_2$O. 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: ^{1}H-^{13}C HSQC, ^{1}H-^{1}H TOCSY, HNCA, HNCACB, HN(CA)CO and HN(CA)NNH. The secondary structure propensity (SSP) of Aβ$_{1-40}$ in solution was accessed by analysing the obtained C', Cα and Cβ 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 Aβ$_{1-40}$ onto the αB hexameric ring

Rigid-body docking was performed using the ClusPro server.[69] The atomic model of the αB 24-mer[16] was used as the receptor structure. For the ligand structure, we used the solid-sate NMR structural model of Aβ$_{1-40}$ 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 β4 and β8, respectively, of the solvent exposed, extended monomers in the hexameric ring, and residues K16–A21 of Aβ$_{1-40}$ 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 Aβ$_{1-40}$/αB docking model.

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a

1　　　　　　60　　　　　　　　　　　150　　175

NTD | α-crystallin domain (ACD) | CTD

b

c

CTD_bent

△ Surface

CTD_ext

▽ Interior

d

$\delta(^{15}N)$ (p.p.m.)

G147

T132　G141

G112

Q108sc　　Q108sc

E87

S135　　D127

G95　　L131

G102

T134　　H119　S76

V142

Y122　　　　　　　　　V97

S85　　S136　K90　　　　R116

E117　E106　　　A126

I114　　S115　　　Q108　　V128

F118　　　　　　　　　　L89

N146　　　　　　　　　E88

E105　　S138

V100　　I98　　　　　　I133

H104　K92　　　　　　K103

V93　L137　I161

E99　T144　R107　　D96

H101

V145　　D129

R120　　　　I124

D109

* L143　　L94

A

B

ca. 50%　　　　I133

7　　　6

10　　9　　8　　7　　6

$\delta(^{1}H)$ (p.p.m.)

e

NTD_ext

CTD_ext

I133

Dimer interface I

β6+7

Dimer interface II

β5

β4

β8

NTD_bent

I133

β3　β9

CTD_bent

f

70　　　　　80　　　　　　90　　　　　100　　　　　110　　　　　120　　　　　130　　　　　140　　　　　150　　　　　160

LEKDRFSVNLDVKHFSPEELKVKVLGDVIEVHGKHEERQDEHGFISREFHRKYRIPADVDPLTITSSLSSDGVLTVNGPRKQVSGPERTIPITR

β3　　　　β4　　　　β5　　　　β6+7　　　　　β8　　β9

a

1   60   150   175

NTD   ACD   CTD

150   160   170

KQV SGPER TIP ITREE K PAVTAA P KK

$\delta(^{15}N)$ (p.p.m.)

110

G154   G154

T158

115   T158

T170   T170
S153   T162
V169
I161   V169
E156
E156   I161
120   I159
R157   K174   K166
E164
R157
R157   E165   K166   A172
A168   R163
A168   A172   I159
125
A171
A171
K175
K175
130

8.6   8.4   8.2   8.0   7.8

$\delta(^{1}H)$ (p.p.m.)

b

1,600

$^{15}N\ T_1$ (ms)

1,200

800

400

0
I159   I161   V169   A172

250

$^{15}N\ T_2$ (ms)

200

150

100

50

0
I159   I161   V169   A172

c

$\delta(^{15}N)$ (p.p.m.)

121   I159
122   A172
123
124
125   A172   I159
126

8.2   8.0   7.8

$\delta(^{1}H)$ (p.p.m.)

a

	1:0	2:1	1:1	1:2	0:1
	S	P	S	P	S	P	S	P	S	P

αB▸

Lyso▸

b

1:0   20:1   7:1   5:1   0:1

c

δ(¹³C) (p.p.m.)

T158   T132
T162
T144
T134

64   62   60

I161   I161
I159   I159
I124   I133
I124   I124
M68

18   16   14   12   10

δ(¹³C) (p.p.m.)

25
27
29
31

δ(¹³C) (p.p.m.)

d

relative turbidity

Reduced lysozyme

+αB-WT

Reduced lysozyme
+αB-ΔNTD

+αB-WT
+αB-S135Q
Native lysozyme

Time (min)   Time (min)

a

b

c

d

e

Amorphous
aggregate

Amyloid
aggregate

Native
client

Misfolded
client

Misfolded
client

αB*

Stable
αB-client
complex

Transient
αB-client
complex

NTD

β3/β4/β8

Co-precipitate

Protofibril capping