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α B-crystallin Chaperone Inhibits A β Aggregation by Capping the β -sheet Rich Oligomers and Fibrils

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Abstract

Inhibiting the cytotoxicity of amyloid aggregation by endogenous proteins is a promising strategy against degenerative amyloid diseases due to their intrinsically high biocompatibility and low immunogenicity. In this study, we investigated the inhibition mechanism of the structured core region of α B-crystallin (α BC) against A β fibrillization using discrete molecular dynamics simulations. Our computational results recapitulated the experimentally observed A β binding sites in α BC and suggested that α BC could bind to various A β aggregate species during the aggregation process – including monomers, dimers, and likely other high molecular weight oligomers, proto-fibrils and fibrils – by capping the exposed β -sheet elongation surfaces. Thus, the nucleation of A β oligomers into fibrils and the fibril growth could be inhibited. Mechanistic insights obtained from our systematic computational studies may aid in the development of novel therapeutic strategies to modulate the aggregation of pathological, amyloidogenic protein in degenerative diseases.

Introduction

The abnormal misfolding and aggregation of amyloid- β (A β) peptides into β -sheet enriched insoluble amyloid deposits are the major pathological hallmark of Alzheimer's disease (AD)^{1–3}. Common to most amyloid proteins (*e.g.* amylin⁴ and α -synuclein⁵), the fibrillization process⁴ usually involves oligomer formation, nucleated conformational conversion, and proto-fibril elongation before forming mature fibrils. Nearly all experimentally-determined amyloid fibril structures to date feature the in-registered cross- β core structures^{6–8} with β -strand perpendicular to the fibril axis^{9–11}. Increasing evidences reveal that the low molecule weight soluble oligomers formed during the early aggregation stage are the main cytotoxic species^{12–15}. Numerous experimental studies have demonstrated that inhibiting fibrillization is an effective approach to mitigate the

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Author Contributions

Yunxiang Sun and Feng Ding conceived and designed research; Yunxiang Sun performed the simulations and analyzed data; Yunxiang Sun and Feng Ding wrote the paper, and all authors approved the article.

Electronic supplementary information (ESI) available: Supporting Figures.

Conflicts of interest

There are no conflicts to declare.

aggregation-mediated cytotoxicity^{2, 16–19}. Therefore, the inhibition of amyloid proteins fibrillization is considered as promising strategy for future cure amyloid diseases.

To migrate the cytotoxicity induced by amyloid protein pathological aggregation, the modulation of amyloid aggregation by natural polyphenols (*e.g.* EGCG, curcumin and resveratrol)^{17, 20, 21}, nanoparticles (*e.g.*, Graphene oxide quantum dot, fullerene derivative)^{17, 18, 22–24} and small peptides^{25, 26} has been widely studied by prior experimental and computational studies. Among many anti-amyloid reagents^{2, 16–19, 27}, the endogenous α B-crystallin protein displaying amyloid-inhibiting effects against multiple amyloidogenic proteins and peptides^{28–30} is of particular interest due to its intrinsic biocompatibility, biological origin and low immunogenicity. The α B-crystallin is a small heat-shock protein (*a.k.a.* HspB5)³¹ and widely expressed in the human body, including brain, retina, heart, skeletal muscle, skin, spinal cord, kidneys, lungs and eye lens^{28, 32–35}. Hochberga *et. al.* found that the α B-crystallin could prevent A β fibrillization and reduce its toxicity in the cell assay³⁶. Shammass *et. al.* found that α B-crystallin could inhibit the seeding effect of preformed A β fibrils by stopping the elongation/growth of A β fibrils²⁸. Chemical shift perturbation analysis with NMR suggested that the hydrophobic edge of the central β -sandwich of α B-crystallin preferred to bind to the elongation surface of A β amyloid fibril, thus hindered A β fibril growth via a capping mechanism³⁰. Moreover, α B-crystallin was also observed to inhibit aggregation and mitigate aggregation-mediated cell toxicity associated for other amyloid peptides, such as α -synuclein, Tau and κ -casein^{29, 36, 37}. However, the detailed inhibition mechanism was still unclear. Better understanding the inhibition mechanism at the molecular level will be helpful for designing novel amyloid inhibitors against amyloid diseases.

To investigate the inhibition mechanism of α B-crystallin against A β fibrillization, we systematically studied the interactions between A β 42 and the structured core region of α B-crystallin (α BC) by applying discrete molecular dynamic (DMD) simulation, a rapid and predictive molecular dynamics algorithm widely used to study protein folding and misfolding by both our group^{9, 10, 27} and others^{38–42}. Our simulation results revealed that the exposed β 4& β 8 surfaces α BC dimer could bind to the β -sheet elongation surfaces of A β monomers and dimers forming parallel or anti-parallel β -sheet. Due to the fact that the elongation surfaces of A β oligomers are occupied, their growth to high molecular weight oligomers or nucleation of proto-fibrils should be inhibited. The hot-spot binding regions between A β and α BC identified from our simulation is consistent with prior A β fibril and α BC NMR chemical-shift perturbation experimental study³⁰, indicating this mechanism is also suitable for the other β -sheet abundant high molecular weight oligomers (*e.g.* proto-fibrils and fibrils). Overall, the α BC could prevent A β oligomers from nucleation into fibrils and also fibril growth by binding on the β -sheet elongation surface of A β oligomers. This study reveals a complete picture of the inhibitory mechanism of A β aggregation by the endogenous α B-crystallin protein, providing theoretical insights into the development of novel therapeutic strategies against AD.

Materials and Methods

Molecular systems.

The sequence of A β and the structured core region of α B-crystallin (α BC, residue 68–153) were shown in Fig. 1a. The initial structure of A β (PDB ID: 1zoq) was taken from the NMR structures solved in aqueous solution⁴³. The monomer and dimer structure of α BC were taken from previous experimentally determined 24-mer model structures (PDB ID: 3j07) based on solid-state NMR, small-angle X-ray scattering (SAXS), and EM data⁴⁴. Molecular systems including an A β monomer, an A β monomer with an α BC monomer or dimer, two A β monomers, two A β monomers with one α BC dimer were simulated. For each molecular system, multiple independent simulations were performed starting from different initial states (*i.e.*, coordinates, orientations, and velocities), where A β monomer, α BC monomer or dimer were randomly placed in a simulation box with randomly rigid-body rotations and any inter-molecular atomic distance was no less than 1.5 nm. Details of all the simulations were summarized in Table 1.

DMD simulations.

All simulations were performed at 300K by using the united-atom DMD algorithm with implicit solvent⁴⁵, where the continuous potential functions in classic molecular dynamics (MD) were modeled by discrete step-wise functions⁴⁶. Similar to the classic MD, both bonded interactions (*i.e.*, covalent bonds, bond angles, and dihedrals) and non-bonded interactions (*i.e.*, van der Waals, solvation, hydrogen bond, and electrostatic terms) were considered in DMD⁴⁷. The step function potentials are adapted from the Medusa forcefield⁴⁸. In Medusa forcefield the bonded interactions (including bonds, bond angles, and dihedrals) are modeled as infinite square wells, where covalent bonds and bond angles usually have a single well and dihedrals may feature multiple wells corresponding to cis- or trans-conformations. Non-bonded interactions (*i.e.*, van der Waals, solvation, hydrogen bond, and electrostatic terms) are represented as a series of discrete energetic steps, decreasing in magnitude with increasing distance until reaching zero at the cutoff distance. The van der Waals parameters are taken from the CHARMM force field⁴⁹ and the EEF1 implicit solvent model was used to model the solvation⁵⁰. A reaction-like algorithm is used to model hydrogen bond formation⁵¹. DMD software is available to academic researchers at Molecules In Action (www.moleculesinaction.com). The units of mass, time, length, and energy used in our united-atom with implicit water model were 1 Da, ~50 femtosecond, 1 Å, and 1 kcal/mol, respectively. With significantly enhanced sampling efficiency, DMD was widely used to study protein folding/aggregation^{11, 52} and protein-nanoparticle interactions^{2, 22} by both our group and others^{38, 53}. The difference between DMD and traditional MD approaches is in the form of the interaction potential functions. Interatomic interactions in DMD are approximated by step functions instead of continuous potential functions. The system's dynamics is, thus, dictated by a series of collision events at which two atoms meet at an energy step and change their velocities according to conservation laws. By iteratively updating only the two colliding atoms, predicting their new collisions with corresponding neighbors, and finding the next collision via quick sort algorithms, the sampling efficiency of DMD is significantly enhanced without frequent calculations of forces and accelerations (*e.g.*, every ~1–2 fs) in MD simulations. At an adequately small

step size, the discrete step function approaches the continuous potential function and DMD simulations become equivalent to traditional MD. Following the same physical laws, the dynamics observed in DMD are equivalent to continuous potential MD at timescales longer than picoseconds with differences mainly at short timescales within the sub-picosecond range (i.e., the average time step between two consecutive interatomic collisions where a potential energy step is encountered).

Computational Analysis.

The secondary structure was calculated using the DSSP program⁵⁴. A hydrogen bond was considered to be formed if the distance between the backbone N and O atoms was $< 3.5 \text{ \AA}$ and the angle of $\text{NH}\cdots\text{O} > 120^\circ$ ¹². Inter-chain peptide interactions were analyzed by the residue-residue contact frequencies, where two residues were in contact if they had at least one heavy atom contacts defined according to a cutoff distance of 0.65 nm.

Results and Discussions

Binding of A β monomer with α BC monomer.

To investigate the inhibition mechanism of α B-crystallin against A β fibrillization, we first studied the interaction between A β 42 (PDB: 1zoq⁴³, shown in Fig. 1a) and the structured core region of α B-crystallin (α BC, residue 68–153, PDB:3j07⁴⁴; the amino acid sequences and structures are shown in Fig. 1a). Fifty independent DMD simulations, each of which started from different initial configurations (i.e., orientations, coordinates and velocities) with minimum inter-molecule distance no less than 1.5nm and lasted 500 ns, were performed for understanding detailed interactions between monomeric α BC and A β 42. Both of the two exposed β -sheet elongation surfaces of α BC β -sandwich (i.e., β 3+ β 6–7 and β 4+ β 8 as highlighted in Fig. 1b) could bind A β peptide. The time evolution of the number of inter-molecular backbone hydrogen bonds between A β and α BC and secondary structure of each A β residue (Fig. 1b) revealed that A β could directly bind to the exposed β -strand edges of α BC β -sandwich by forming inter-chain hydrogen bonds and converting into β -sheet rich structures. The inter-chain contact frequencies between A β and α BC residues and the representative binding motifs were also analyzed (shown in Fig. 1c). Specifically, the β 6–7 strand of α BC displayed strong binding propensity with A β 1–19 and A β 29–42, forming parallel (with A β 1–12) or antiparallel (with A β 7–19 or A β 29–42) β -sheets. The β 3 strand of α BC formed antiparallel β -sheet with A β 16–22 and parallel β -sheet with A β 31–42. The β 4 strand of α BC mainly formed antiparallel β -sheets with A β 9–16 and A β 27–33. The β 8 strand of α BC was observed to form parallel β -sheet with A β 30–38 and anti-parallel β -sheet with A β 36–42. The calculation of the total number of inter-chain contacts per residue showed that α BC residues in β 6–7 had stronger binding propensities to A β than the other α BC strands, especially with the N-terminal A β residues 1–20 displaying the strongest inter-molecular binding frequencies (Fig. 1c). The inter-molecular residue-to-residue pairing analysis of these populated motifs (Fig. 1c) revealed that these β -sheets were mainly stabilized by attractive hydrophobic interactions and salt-bridges. Averaged secondary structure propensities for each A β residue revealed that those residues with high binding propensity to α BC had strong probabilities to adopt β -sheet structures (Fig. S1a). The residue-pairwise contact frequency and most populated conformation analysis demonstrated

that A β monomers were structurally dynamic with a highly tendency to adopt β -hairpin conformations (Fig. S1b&c). The observed conformational flexibility of A β monomers were consistent with FRET experiments⁵⁵. While some experiments suggested that the backbone torsion angle distributions of A β monomers derived from NMR J-couplings closely resembled those of random coils⁵⁶, other studies indicated a significant population of β -sheet conformations⁵⁷. A CD spectra experiment found that the β -sheet content of A β monomers was around 24%⁵⁸. Using experimentally determined J-coupling measurements as the benchmark, the OPLS forcefield was found as the most experimentally consistent to capture A β conformational dynamics^{59, 60} and the corresponding structural ensemble of A β 42 featured β -hairpin rich conformations as in our simulations^{59, 60}. Additionally, sequence regions with high β -sheet propensities were similar to those observed in the experimentally-determined A β amyloid fibrils⁶¹ and also in prior all-atom MD simulations^{58, 62–64}. The residue-pairwise contact map revealed that there were no major averaged conformational changes for A β 42 in the presence of α BC (Fig. S1b). Together with the representative A β - α BC heterodimer structures (Fig. S1d), we could learn that A β 42 mainly bind to the exposed β -strand edges of the α BC sandwich with un-saturated hydrogen bonding donors and acceptors (*i.e.* β 3& β 6–7 and β 4& β 8) and form inter-molecular β -sheets.

Binding of A β monomer with α BC dimer.

The α BC tends to form dimers in solution and previous X-ray crystallography and solution-state NMR spectroscopy studies showed that the β 6–7 strand of α BC is buried in the homodimer interface by forming β -sheets with each other^{30, 36, 65}. Hence, to investigate the inhibition mechanism of α BC dimer against A β amyloid aggregation in solution, we further studied the interaction between A β monomer and α BC dimer by performing fifty independent binding simulations. Initially, A β 42 monomer was randomly placed at least 1.5 nm away from the α BC dimer. Since the β 6–7 surface was buried in the α BC dimer^{19, 25, 32}, the A β 42 monomer predominantly bound to the β 4& β 8 interface in DMD simulations (Fig. 2a). Similar to our simulations with the α BC monomer, A β 42 mainly adopted β -sheet conformations after binding the α BC dimer (*e.g.* a representation trajectory in Fig. 2a). The average number of inter-molecular contacts for each α BC residue revealed that A β 42 mainly attached on β 4 and β 8 of α BC. The result agreed well with prior NMR chemical shift perturbation experiments, where residues in β 4 and β 8 were the most perturbed regions in α BC upon binding A β ^{30, 36}. The residue-wise inter-molecular contact frequency map along with the representative binding motifs in simulations demonstrated that the β 4 and β 8 strands of α BC mainly interacted with A β 14–21 and A β 28–42 forming either parallel or anti-parallel β -sheets, which were also stabilized by hydrophobic and salt-bridge interactions (Fig. 2b). Additionally, we also observed a weak binding between α BC β 3 and A β 16–26, a small NMR chemical shift exchanges for L79 in α BC β 3 was also observed in prior A β - α BC experimental study³⁰. Overall, the hydrophobic and charged residues from β 4& β 8 interface of α BC displayed significant binding propensity to A β 42. For A β 42, residues 30–42 in the C-terminus had much higher binding propensities with α BC dimer than the rest of peptide. Secondary structure analysis of A β 42 monomer before and after binding α BC confirmed that the β -sheet content of residues around A β 13–21 and A β 30–42 was enhanced due to binding with the α BC dimer (Fig. S2). Top populated complex structures of A β 42

monomer and α BC dimer from the root-mean-square deviation (RMSD)-based clustering analysis showed that A β binding extended the β -sheets of α BC (Fig. 2c). Hence, the simulation results of A β monomer binding with α BC dimer demonstrated that A β mainly bound to the β 4& β 8 interface of α BC dimer in solution.

A β dimerization in the presence of α BC dimer.

As the first step toward understanding the effect of α BC on the A β aggregation, we studied the dimerization process of A β by simulating two A β monomers in the presence of an α BC dimer. Two A β monomers and one α BC dimer were randomly placed in a 9.0 nm cubic simulation box with a periodic boundary condition and a minimum inter-molecule distance greater than 1.5 nm. In our simulations, the number of inter-chain hydrogen bonds between β 6–7 segments in the α BC dimer (Fig. 3a) was maintained around 12 without much fluctuations (less than 3), indicating the α BC dimer was stable in solution as observed in prior experimental studies^{19, 25, 32}. As shown in Fig. 3a, two A β monomers could separately bind to the two exposed β -sheet elongation surfaces of the α BC dimer and form an A β - α BC_D-A β complex. We also observed the dimerization of A β monomers before binding to the α BC dimer and forming an A β _D- α BC_D complex. These results indicated that the α BC could interact with both A β monomers and dimers (*i.e.*, the smallest oligomer), and likely other higher-molecular-weight oligomers should follow a similar manner. The average number of backbone hydrogen bonds between two A β monomers during last 100 ns simulation in each independent simulation showed that both types of complexes, A β - α BC_D-A β (with zero inter-A β hydrogen bonds formed) and A β _D- α BC_D (multiple inter-A β hydrogen bonds ranging from 5 to 21) were the stable conformations (Fig. 3b). A β could bind to the lateral surface of α BC during the early binding stage. But these states were unstable and A β spontaneously diffused to the exposed edges of the β -sheets. A β and α BC always formed backbone hydrogen bonds in all independent trajectories (Fig. 3b). The analysis of secondary structure propensities of each A β residue (Fig. S3) showed that A β mainly adopted unstructured coil and β -sheet conformations in both absence and presence of α BC dimer. The presence of α BC dimer didn't induce much secondary structure changes in terms of β -sheet content, consistent with a prior circular dichroism (CD) spectroscopy experiment demonstrating that co-incubation of A β 42 with α BC had no effect on A β 42 β -sheet formation⁶⁶. However, the time evolution of the inter-A β backbone hydrogen bonds that stabilize amyloid fibril structures^{6–8} were significantly inhibited by α BC dimer (Fig. S4). Together with the representative conformations of A β 42 with α BC complexes (Fig. 3c&d), we could learn that α BC dimer could bind A β monomers, dimers and likely other high-order oligomers along the β 4& β 8 interface forming β -sheet conformations.

The inhibition mechanism of α BC against A β fibrillization.

Prior experimental and computational simulation studies revealed that A β monomers first formed oligomers while undergo nucleated conformational conversion into β -sheet rich oligomers before forming proto-fibrils and mature fibrils^{12, 13, 63, 64, 67–72}(Fig. 4). For example, prior all-atom replica-exchange simulation studies demonstrated the A β dimer featured extend β -hairpin and β -strand abundant^{23, 73}. Our simulation results demonstrated that α BC dimer in solution could bind A β monomers, dimers, possibly other high-order oligomers by forming inter-molecular β -sheets with β 4 and β 8 strands of α BC. Different

from the most of immunoglobulin β -sandwich proteins with edge-protection⁷⁴, where the charged side-chain located in the middle of the hydrophobic side of the edge β -strand to avoid self-aggregation, the exposed $\beta 4$ & $\beta 8$ edges of αBC were favorable to interact with the β -strands they encountered. For example, numerous experimental evidences suggested the $\beta 4$ & $\beta 8$ surfaces of αBC dimeric subunit were buried in the αBC complex model^{30, 75}. While $\beta 4$ & $\beta 8$ strands of both monomers in αBC dimer can bind $A\beta$ and potentially form larger intercalated $A\beta/\alpha BC$ complexes, the highly curved β -sheet structure of the αBC dimer is not compatible with long fibers, as shown by the bended structure of the $A\beta_M$ - αBC_D - $A\beta_M$ complex in Fig. 3d and also as suggested by the formation of closed hexamers and subsequent high-order 24-mer of αBC . Therefore, the binding of αBC_D with $A\beta$ monomers and small aggregates in the early stage $A\beta$ aggregation via β -sheet pairing interrupts the continuous growth into long straight fibrils, comprised of parallel in-register β -sheets between neighboring $A\beta$ peptides. Similarly, our results also suggest that the binding of αBC_D with pre-formed or newly-formed $A\beta$ fibrils could also inhibit the further elongation into longer fibrils. Indeed, prior experimental studies revealed that αBC not only could inhibit the amyloid aggregation of $A\beta$ ^{29, 30, 36, 76}, but also prevented the growth of preformed $A\beta$ fibrils – *i.e.*, mitigating the seeding effect^{28, 36}. A similar inhibition mechanism was also reported by Hoyer et. al, where the fibrillization of $A\beta$ was inhibited when the β -hairpin structure was stabilized by affibody protein $Z_{A\beta 3}$ ⁷⁷. Although the binding of αBC with $A\beta$ fibrils was not included in the current study due to the large system size, we expect the binding of αBC with $A\beta$ fibrils because the αBC -binding hot-spot residues – e.g. $A\beta 16$ –26 and $A\beta 30$ –42 – are already in the αBC -binding compatible β -sheet conformations and solvent-exposed at the fibril elongation ends. Hence, the capping of $A\beta$ proto-fibrils and fibrils at the elongation interface by binding with $\beta 4$ and $\beta 8$ strands of αBC could inhibit the further fibril growth (Fig. 4).

Conclusion

In sum, we investigated the inhibition mechanism of endogenous αBC against $A\beta$ amyloid aggregation using united-atom DMD simulations with implicit solvent. We identified the hot-spot regions in $A\beta$ and αBC that are important for their cross-interactions in agreement with NMR chemical shift perturbation studies^{30, 36}. Upon binding αBC dimer in solution, $A\beta$ formed β -sheets with exposed $\beta 4$ and $\beta 8$ strands of αBC β -sandwich. Our results suggested that αBC could bind to various $A\beta$ aggregate species during the aggregation process - including monomers, dimers, and likely other high molecular weight oligomers, proto-fibrils and fibrils – by capping the exposed β -sheet elongation surfaces. Mechanistic insights obtained from our systematic computational studies may aid in the development of novel therapeutic strategies to modulate the aggregation of pathological, amyloidogenic protein in degenerative diseases.

Supplementary Material

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Fig. 1. Binding of A β monomer with α BC monomer.

a) The amino acid sequence and structure of A β 42 and structured core region of α B-crystallin used in our simulation. b) The time evolution of the number of inter-chain hydrogen bonds formed between A β and α BC monomers and secondary structure for each A β residue adopted in two representative trajectories, where A β 42 bound to the β 3+ β 6-7 (up) and β 4+ β 8 (down) strands of α BC. The snapshots at 50, 300 and 500 ns are shown to the right, corresponding to before and after binding. For clarity in the presentation of A β - α BC complex, the two β -sheet elongation surfaces β 3+ β 6-7 and β 4+ β 8 of α BC are colored by blue and red, respectively. The other regions of α BC are shown in cyan. A β is colored in magenta. c) The inter-molecular contact frequency map between A β and α BC were computed based on the last 200 ns trajectories of 50 independent DMD simulations after reaching steady states. For each residue in A β and α BC, its total number of inter-chain

contacting residues are also computed by integrating the corresponding pair-wise contact frequency map. The representative binding motifs of A β (colored in red) and α BC (colored in blue) segments labeled as 1–9 corresponding to the parallel or anti-parallel β -sheet patterns heighted by boxes in the contact frequency map are also presented on the right. The hydrophobic and salt bridge residue-residue pairs are highlight in bold.

Fig. 2. Binding of Aβ monomer with αBC dimer.

a) The time evolution of the number of inter-chain hydrogen bonds formed between Aβ monomer and αBC dimer, and secondary structure for each Aβ residue. The snapshots along with the simulation time are shown on the right. The representative trajectory is randomly selected out of 50 independent simulations. b) The inter-molecular contact frequency map between Aβ and αBC. For each residue in Aβ and αBC, its total number of inter-chain contacting residues are also computed by integrating the corresponding pair-wise contact frequency map. Representative binding motifs of Aβ (colored in red) and αBC (colored in blue) labeled as 1-8 are also shown. The hydrophobic and salt bridge residue-residue pairs are highlight in bold. c) The top six most populated representative complex structures are shown. For clarity, the two chains of αBC dimer are colored by orange and cyan, respectively. The hot spot binding regions β4&β8 for Aβ are shown in red. Aβ peptide is colored in magenta.

Fig. 3. $A\beta$ dimerization in the presence of αBC dimer.

a) Two representative trajectories, forming $A\beta_M-\alpha BC_D-A\beta_M$ (top) and $A\beta_D-\alpha BC_D$ (bottom) complex, are randomly selected from 40 independent DMD simulations. The time evolution of the number inter-molecular hydrogen bonds between αBC and αBC (black), $A\beta$ and $A\beta$ (orange), $A\beta$ and αBC (red) are shown on the left. The snapshots along the noted times are also shown to the right. b) The average number of hydrogen bonds between two $A\beta$ peptides (top) and between $A\beta$ and αBC (bottom) during last 100 ns of each 40 independent simulations. The representative $A\beta_M-\alpha BC_D-A\beta_M$ (c) and $A\beta_D-\alpha BC_D$ (d) structures are randomly selected from the final snapshots of independent DMD trajectories. For clarity, the two chains of αBC dimer are colored by orange and cyan, respectively. Two $A\beta$ peptides are colored in red and blue.

Fig. 4. The inhibition mechanism of α BC against $A\beta$ fibrillization.

In the absence of α BC, $A\beta$ monomers aggregated into β -sheet rich oligomers before forming proto-fibrils and mature fibrils. In the presence of α BC, the dimer (α BC)₂ in solution could directly bind the β -sheet elongation surface of $A\beta$ oligomers or proto-fibrils, and prevent them from growth and elongation into mature fibril structure via capping.

Table 1.

The details of simulation systems in our DMD simulations, including the number of α BC monomer (α BC_M), α BC dimer (α BC_D) and A β monomer molecules; the corresponding dimension of the cubic simulation box; the total number of independent DMD trajectories performed; the simulation time of each DMD trajectory.

System		Box size (nm)	DMD Run	Simulation time (ns)
Molecules	Number			
A β	1	6.0	50	500
	2	9.0	50	500
α BC _M :A β	1:1	9.0	50	500
α BC _D :A β	1:1	9.0	50	500
α BC _D :A β	1:2	9.0	40	400