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Supporting Material

Molecular dynamics simulations of anti-aggregation effect of ibuprofen

Wenling E. Chang, Takako Takeda, E. Prabhu Raman, and Dmitri Klimov

Supplemental Materials

Implicit solvent model: The solvent accessible surface area (SASA) model has been developed by Caflisch and coworkers (see ref. [40] in the paper). In this model, solvent is treated implicitly and hydrophobic effect is accounted by solvation free energy, which scales linearly with accessible surface area of atoms. The combination of SASA model and CHARMM19 united atom force field has been used to fold α-helices and β-sheet polypeptides to their native states [1,2] and for studying aggregation of amyloidogenic peptides (ref. [23] in the paper). In our previous studies, CHARMM19+SASA model was applied to probe the elongation thermodynamics of Aβ fibrils and provided the results consistent with experimental data, which include the value of dissociation temperature and the deposition mechanism (ref. [26] in the paper). Due to reduced number of degrees of freedom CHARMM19+SASA computations are several orders of magnitude faster than those employing explicit solvent. Consequently, with CHARMM19+SASA model one can obtain exhaustive sampling of conformational space that cannot be usually achieved with explicit solvent models.

Description of simulation system: Solid-state NMR studies have shown that the two-fold symmetry fibril structures of Aβ1-40 and Aβ10-40 peptides are similar ([3] and ref. [10] in the paper). Similarities in oligomerization pathways of Aβ1-40 and Aβ10-40 were reported experimentally [4] and computationally [5]. Consequently, we use Aβ10-40 as a model of the full-length Aβ1-40 peptide.

The structure of the Aβ10-40 fibril fragment (Fig. 1c) is modeled using the coordinates of backbone atoms determined from the solid-state NMR measurements (ref. [10] in the paper). To emulate the stability of large fibril sample, the backbones of fibril peptides were constrained to their experimental positions using soft harmonic potentials with the constant k_c=0.6 kcal/(mol$Å^2$) (ref. [26] in the paper). The constraints limit the amplitude of backbone fluctuations to about 0.6 Å at 360K, which are comparable with the fluctuations of atoms on the surface of folded proteins [6]. Constraints were not applied to the side chains of fibril peptides or to incoming peptides. The constraints capture the rigidity of amyloid fibril and eliminate the necessity to simulate large fibril systems to maintain their thermodynamic stability. Aβ10-40 peptides and ibuprofen molecules were subject to spherical boundary condition with the radius R_s=90 Å and the force constant k_s=10 kcal/(mol$Å^2$). The concentrations of Aβ peptides and ibuprofen are ≈3 and 30 mM, respectively.

It is important to point out that CHARMM19 is an united atom force field, in which non-polar hydrogens are not explicitly considered As a result, this force field does not distinguish ibuprofen isomers, such as S- and R-ibuprofens, which are known from the experiments to have different binding affinities with respect to Aβ fibrils (ref. [20] in the paper).

Computation of peptide-fibril interactions: A peptide-fibril parallel HB (pHB) is formed between the residues *i* and *j*, if at least one other hydrogen bond (HB) is also present between *i*+2 and *j* or *j*+2 (or between *i*-2 and *j* or *j*-2). An antiparallel HB (aHB)

is formed between the residues i and j, if at least one other HB is also formed between either i+2 and j-2 or between i-2 and j+2. For any HB a registry offset $R=|j-i|$ is defined, where j and i are the indexes of the residues in the incoming and fibril peptides linked by HB. In general, pHB may have arbitrary R. In-registry parallel alignment of peptides in the wild-type Aβ fibril displayed in Fig. 1c corresponds to R=1.

Computation of contacts formed by ibuprofen: Ibuprofen molecule contains three structural groups (Fig. 1b). The groups G1 and G2 are hydrophobic and G3 is hydrophilic. A contact with Aβ side chain occurs, if the distance between the centers of mass of side chain and one of the ibuprofen groups is less than 6.5 Å. If the contact involves G1 or G2 and hydrophobic side chain, then it is assumed hydrophobic. A contact between two ibuprofen molecules is formed, if any of the G1-G3 centers of mass from different molecules are within the 6.5 Å distance. Ibuprofen is considered bound, if it forms at least one contact with Aβ side chain.

Convergence of REMD simulations: To probe the convergence of REMD sampling of Aβ conformations we considered the number $N_{s,p}$ of the unique states (E_{eff},N_{hb}) sampled in the course of simulations at least once. Each state (E_{eff},N_{hb}) is defined by the effective energy E_{eff} (a sum of potential and solvation energies) and the number of HBs between incoming peptide and the fibril N_{hb}. Fig. S1 shows $N_{s,p}$ as a function of the cumulative equilibrium simulation time τ_{sim}. The convergence of sampling of Aβ-ibuprofen interactions was tested by computing the number $N_{s,l}$ of the unique states (E_{eff},L), where L is the number of ligands bound to Aβ. The behavior of $N_{s,l}$ was found to be very similar to $N_{s,p}$. Because both counters of unique states start to level off at $\tau_{sim} > 10$ μs, REMD appears to exhaust Aβ and ibuprofen conformational space. Additional check of REMD convergence was performed by dividing simulation trajectories into two equal subsets and analyzing them separately. The thermodynamic quantities probing binding of incoming peptides to the fibril obtained from the two subsets generally differed by < 7%. The exception is the computations of the free energy gap between the locked and docked states, $\Delta F_{L\text{-}D}$, for which the error was 15%. The errors in the thermodynamic quantities describing ibuprofen binding were less than 1%.

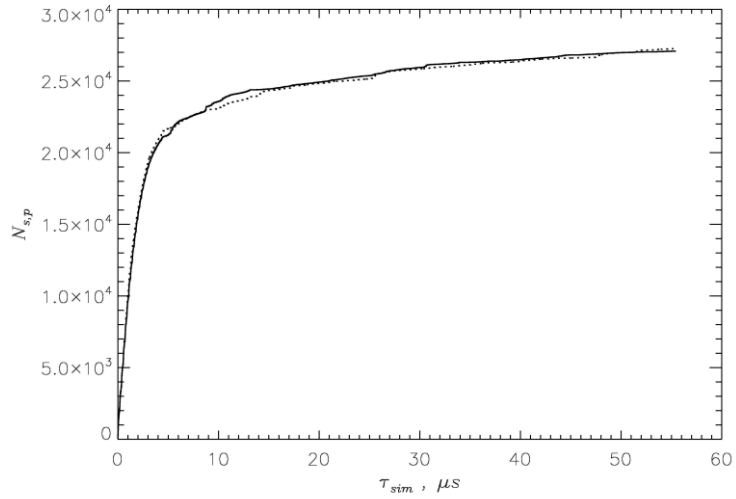

Fig. S1 The number of unique states $N_{s,p}$ sampled in the course of REMD simulations as a function of cumulative equilibrium simulation time τ_{sim}. The solid and dashed lines correspond to $N_{s,p}$ computed separately for two incoming peptides. Almost identical behavior of $N_{s,p}$ for both peptides suggests that they sample similar conformational ensembles.

Impact of ibuprofen on the affinities of fibril edges: Fig. S2 compares the affinities of fibril edges with respect to binding incoming Aβ peptides in water and ibuprofen solution.

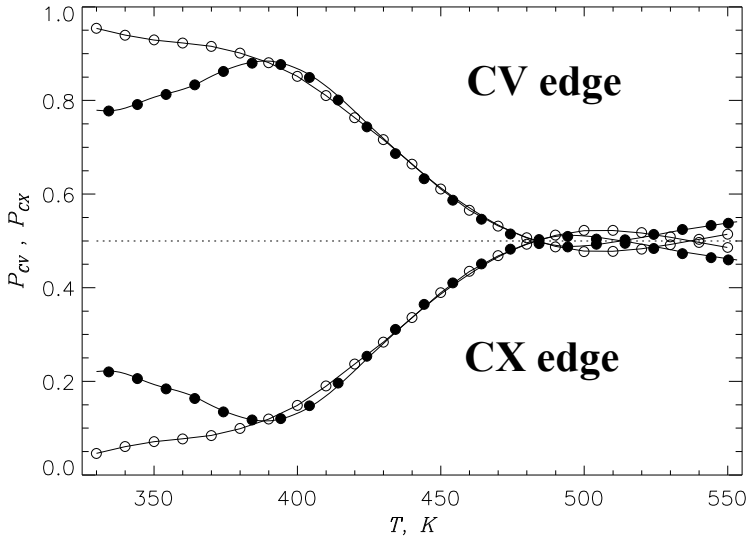

Fig. S2 Probabilities of concave (CV) and convex (CX) edge Aβ binding as a function of temperature, $P_{CV}(T)$ and $P_{CX}(T)$, in ibuprofen (filled circles) and in water (open circles). Due to small size of fibril fragment used in the study the probability P_{CV} can be obtained, if one assumes that a peptide is bound to the CV edge, when the z-component of its center of mass is positive (Fig. 1c). Then, P_{CX}=1-P_{CV}. The decrease in $P_{CV}(T)$ at the temperatures below the ibuprofen binding midpoint T_b≈376K implies that ibuprofen reduces the difference in edge affinities with respect to incoming Aβ peptides.

Thickness of the layer formed by bound Aβ peptides: The thickness D of the layer formed by bound Aβ peptide on the fibril edge (Fig. 1c) was estimated using the following procedure. From REMD simulations the probability distribution $P(z)$ for the position of incoming peptide center of mass along z-axis can be computed. At the temperatures below the midpoint of peptide binding (~500K) $P(z)$ displays two well-defined peaks reflecting the binding of Aβ to the CV and CX fibril edges (Fig. 1c). The thickness D is then defined as the width of the peaks in $P(z)$ at the level of one-third of the maximum.

The temperature dependencies $D(T)$ computed for ibuprofen solution and water are shown in Fig. S3. The dependencies $D(T)$ are fitted with the inverse temperature functions $D_0/(T_u-T)$ containing two adjustable parameters D_0 and T_u. In water, a single fitting function with D_0=711 ÅK and T_u=585K can be used. In contrast, two fitting functions (D_0=647ÅK, T_u=564K and D_0=2437ÅK, T_u=902K) must be used to approximate $D(T)$ in ibuprofen solution. The onset of ibuprofen binding swells the peptide layer making necessary to apply two fitting functions.

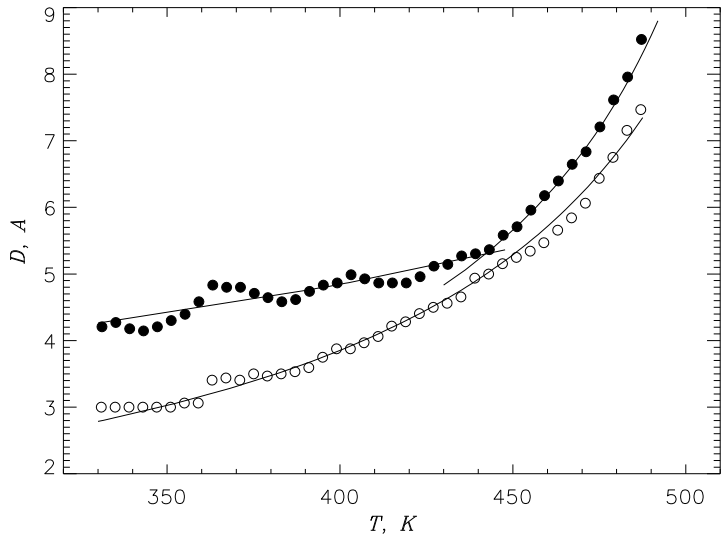

Fig. S3 The thickness D of the layer formed by bound Aβ peptide on the fibril edge as a function of temperature T: ibuprofen solution (filled circles), water (open circles). The thickness D is averaged over the bound layers formed on the CV and CX edges. The fits are shown by continuous lines.

References

[1] Ferrara, P, Caflisch, A (2000) Folding simulations of a three-stranded antiparallel beta-sheet peptide. Proc. Natl. Acad. Sci. USA 97:10780-10785.

[2] Hiltpold, A, Ferrara, P, Gsponer, J, Caflisch, A (2000) Free energy surface of the helical Peptide Y(MEARA)6. J. Phys. Chem. B 104:10080-10086.

[3] Paravastu, AK, Petkova, AT, Tycko, R (2006) Polymorphic fibril formation by residues 10-40 of the Alzheimer's beta-Amyloid Peptide. Biophys. J. 90:4618-4629.
[4] Bitan, G, Vollers, SS, Teplow, DB (2003) Elucidation of primary structure elements controlling early amyloid beta-protein oligomerization. J. Biol. Chem. 278:34882-34889.
[5] Takeda, T, Klimov, DK (2009) Probing the effect of amino-terminal truncation for Abeta1-40 Peptides. J. Phys. Chem. B 113: 6692-6702
[6] Zhou, Y, Vitkup, D, Karplus, M (1999) Native proteins are surface-molten solids: Application of the Lindemann criterion for the solid versus liquid state. J. Mol. Biol. 285:1371-1375.