Interactions between Amyloid-β and Tau Fragments Promote Aberrant Aggregates: Implications for Amyloid Toxicity

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SUPPORTING INFORMATION

S1. Representative ATDs of pure Ac-Aβ(25-35)-NH_2

Representative ATDs are shown are obtained at the highest drift voltage (~3500V). Each feature is annotated with oligomer size to charge (*n*/*z*) ratios and experimental cross section (σ, $Å^2$). These experimental cross sections are used to generated Figure 2A shown in the main text.

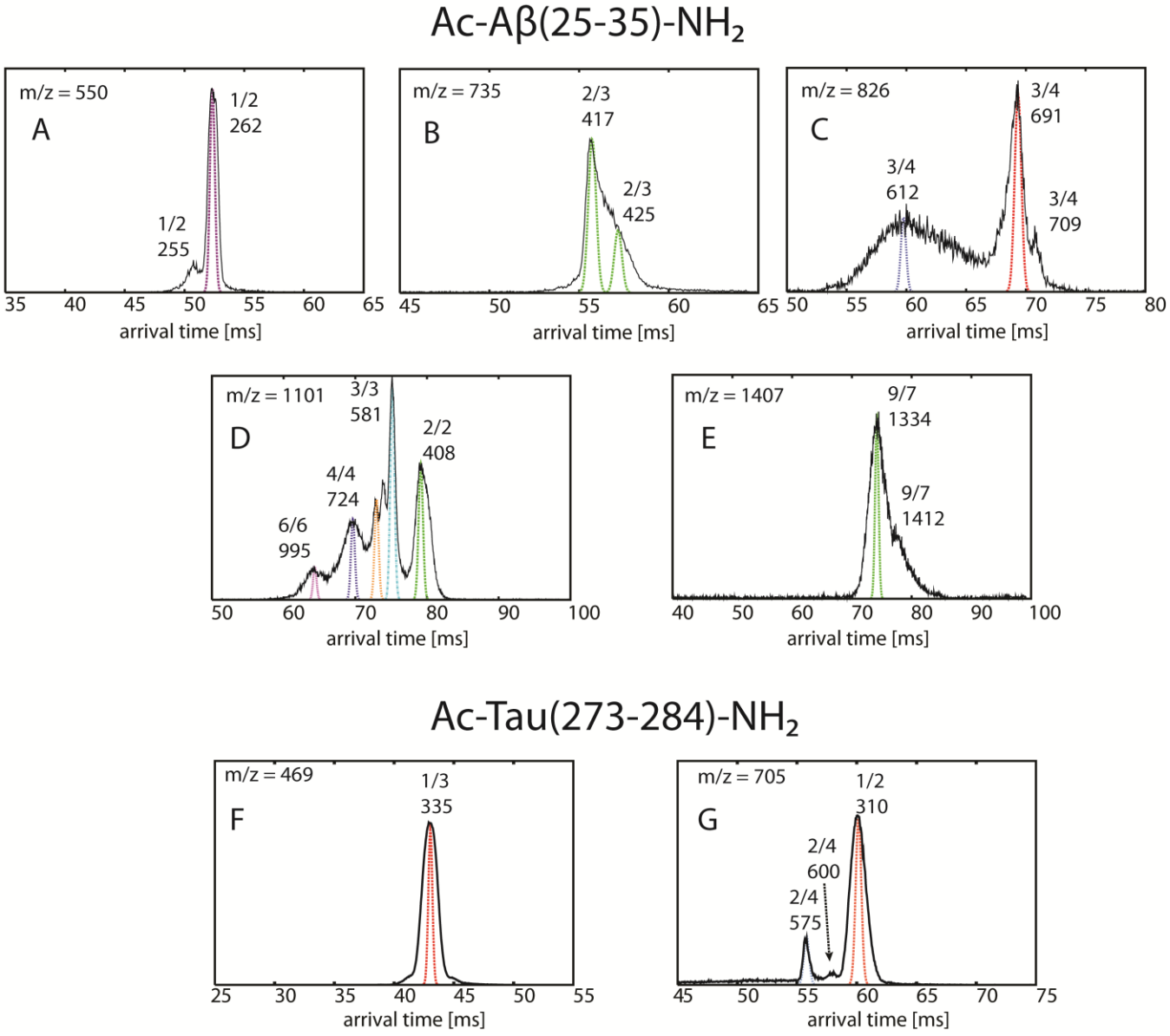

Figure S1. Representative ATDs of pure Aβ(25-35) peaks obtained from ion-mobility experiments at V ≈ 3350V, P ≈ 12-13 torr.

S2.1 Isotropic and Correlated Cross Section Calculation

The isotropic cross sections of Aβ(25-35) and the isotropic cross sections of heteroligomers are calculated as:

$$\sigma_{iso}(n) = \sigma_{\text{Aβ(25-35),mon}} \cdot n^{2/3}$$

$$\sigma_{iso}(n,k) = \left[n \cdot {\sigma_{\text{Aβ(25-35),mon}}}^{3/2} + k \cdot {\sigma_{\text{Tau(273-284),mon}}}^{3/2}\right]^{2/3}$$

where n is the number of Aβ(25–35) chains, and m is the number of Tau(273-284) chains within the heteroligomers and $\sigma_{\text{Aβ(25-35),mon}}$(262 Å^2) and $\sigma_{\text{Tau(273-284),mon}}$(310 Å^2) are the collision cross sections of the compact monomers of Aβ(25-35) and Tau(273-284), respectively. From here, $\varepsilon(n,k) = {\sigma_{\text{exp}}(n,k)}/{\sigma_{\text{iso}}(n,k)}$ is defined as the ratio of experimental to isotropic heteroligomers cross sections, and can be used to calculate the correlated cross section as

$$\sigma_{\text{corr}}(n) = \sigma_{iso}(n) \cdot \varepsilon(n,k) = \sigma_{iso}(n) \cdot {\sigma_{\text{exp}}(n,k)}/{\sigma_{\text{iso}}(n,k)}$$

The values of $\sigma_{\text{corr}}(n)$ and the isotropic cross section $\sigma_{\text{iso}}(n,k)$ are used to generate Figure 3B in the main text.

Table S1. Collision cross sections for oligomers of Aβ(25-35) incubated with Tau(273-284).

n	k	z	m/z	$\sigma_{\text{exp}}(n,k)$	$\sigma_{\text{iso}}(n,k)$	$\varepsilon(n,k)$	$\sigma_{\text{corr}}(n)$
1	1	3	837	492.	455.76	1.080058	280.81
1	1	4	628	559	455.76	1.226514	318.89
2	3	5	1280	979	854.93	1.145742	472.88
2	1	4	903	673	578.73	1.16279	479.91
2	1	4	903	706	578.73	1.22034	503.66
3	2	6	1016	1018	823.98	1.23521	668.03
3	2	6	1016	1074	823.98	1.303382	704.90
1	2	4	979	718	615.43	1.167354	303.51
1	2	4	979	769	615.43	1.249536	324.88
3	3	7	1076	1123	942.86	1.191188	644.22
3	3	7	1076	1225	942.86	1.298796	702.42
3	2	7	875	1036	820.28	1.263061	683.09
3	2	7	875	1052	820.28	1.282364	693.53
3	2	7	875	1081	820.28	1.31746	712.51
3	2	7	875	1102	820.28	1.343381	726.53
3	2	7	875	1111	820.28	1.35482	732.72
3	2	7	875	1147	820.28	1.398245	756.20

S2.2. Dissociation constant K_d estimation for the formation of heterodimers.

We can estimate the dissociation constant of heterodimers using the intensities of the spectral peaks corresponding to the monomers of each peptide and the heterodimers, with an assumption that the concentrations and intensities of the species listed in Equation 1 dominate all other species (see main text Figure 2C-D). In detail, the K_d is computed as shown below.

$$K_d = \frac{\left[M_{\text{Aβ}(25-35)}\right]\left[M_{\text{Tau}(273-284)}\right]}{D_{\text{Aβ}(25-35)+\text{Tau}(273-284)}} = \text{R}\frac{\left[I_{\text{Aβ}}^{z=+2} + I_{\text{Aβ}+Na}^{z=+2}\right]\left[I_{\text{Tau}}^{z=+2} + I_{\text{Tau}}^{z=+3}\right]}{\left(I_{\text{Aβ+Tau}}^{z=+3} + I_{\text{Aβ+Tau}}^{z=+4}\right)}$$

Here M and D denote monomers and dimers, respectively. R is response factor that correlates the spectral peak intensity with the solution concentration (here for simplicity we assume that R is the same for all species listed in the above equation). $I_{\text{Aβ}}^{z=+2}$is the intensity of the spectral peak at 550 *m/z* (doubly charged Aβ(25-35) monomer), $I_{\text{Aβ}+Na}^{z=+2}$ is of the spectral peak at 561 *m/z* (sodium

adduct monomer); these are the two most intense peaks of Aβ(25-35) monomers. $I_{\mathrm{Tau}}^{z=+2}$ and $I_{\mathrm{Tau}}^{z=+3}$ are the intensities of the spectral peaks at 705 *m/z* and 470 *m/z* (doubly and triply charged Tau monomers). $I_{\mathrm{A\beta+Tau}}^{z=+3}$ and $I_{\mathrm{A\beta+Tau}}^{z=+4}$ are the intensities of the heterodimers with *z* = +3 and +4 at 837 *m/z* and 628 *m/z*. To obtain *R*, we use *R* = [total concentration]/[total intensities] = 0.000911. Solving for K_d from the spectral intensities of three different mass spectra yields 154 ± 8 μM. The micromolar K_d range suggests a weakly binding when putting in the context of protein-ligand complexes. However in our approximation, we ignore the contributions of heteroligomers larger than dimer and Aβ(25-35) oligomers (i.e. the aggregation kinetics of the two individual peptides) and the difference in response factors. Hence, the exact K_d can be smaller.

S3. Theoretical Modeling

Temperature-based replica exchange molecular dynamics

Explicit solvent simulations for heterodimer of Aβ(25-35) and Tau(273-284) were performed using the GROMACS 4.6.3 package[1-2] and all-atom Optimized Potentials for Liquid Simulations (OPLS-AA) force field[3] in TIP3P water with periodic condition boundary condition. The initial structure was chosen from previous simulations of Ac-Aβ(25-35)-NH_2 (unpublished) and Ac-Tau(273-284)-NH_2,[4] followed by a solvent, the addition of three Cl- ions to neutralize the charges, and then volume equilibration simulation in NPT ensemble (T = 300K and P = 1 bar) to optimize the box size. The number of water molecules in each system is approximately 7066 molecules. The box size after equilibration was 6.018, 6.018 and 6.018 nm. Another equilibration run was performed in NVT ensemble for 6 ns after the optimized volume is obtained. Initial guess for temperature in T-REMD simulations with 32 replicas was taken from Patriksson and Spoel's temperature predictor[5] and then adjusted to obtain the exchange rate of approximately 25-30%. The set of temperatures used is 283.9, 286.2, 288.5, 290.8, 293.1, 295.4, 297.7, 300, 302.5, 305, 307.5, 310, 312.5, 314.5, 317, 319.5, 322, 324.7, 327.4, 330.1, 332.8, 335.5, 338.2, 341.2, 344.2, 347.2, 350.2, 353.2 , 356.2, 359.2, 362.2, 365.2 in Kelvin. Each replica is equilibrated at the desired temperature for 6 ns before the production run for T-REMD was begun. Exchanges between replicas were attempted at every 3 ps. The production run is 300-ns long per replica, but only the last 150-ns data were subjected to analysis. The LINCS algorithm[6] was employed to constrain bonds between heavy atoms and hydrogens, and the SETTLE algorithm[7] was used for water molecules. These constraints allow an integration time step of 2.0 fs. Electrostatic and dispersion forces were computed with a real space cutoff of 1.2 nm and the particle mesh Ewald method[8] was used to treat long-range electrostatics. All simulations were performed at neutral pH in which the temperature was maintained by the Nose-Hoover thermostat.[9] The temperature and pressure coupling constants were 0.1 ps and 1.0 ps, respectively. The equations of motion were integrated accordingly to the leap-frog algorithm.

For hetero-Aβ trimer and hetero-Tau trimer, the simulation parameters are similar, with the only differences are box sizes, lists of temperatures and simulation time. Each side of the cubic water box after equilibration was 4.995 for hetero-Aβ trimer and 6.015 for hetero-Tau trimer. Each replica was simulated for 390 ns, and the total number of replicas used in each system was 48. The temperature values are: 282.00, 284.67, 287.35, 290.06, 292.78, 295.53, 298.29, 301.09, 303.89, 306.71, 309.55, 312.41, 315.29, 318.20, 321.12, 324.06, 327.03, 330.02, 333.03, 336.06, 339.11, 342.19, 345.28, 348.40, 351.54, 354.70, 357.89, 361.10, 364.33, 367.58, 370.86, 374.16, 377.49, 380.84, 384.21, 387.61, 391.04, 394.49, 397.96, 401.46 for hetero-Aβ trimer and 287.00, 289.12, 291.26, 293.41, 295.56, 297.73, 299.92, 302.11, 304.32, 306.55, 308.78, 311.03, 313.28, 315.55, 317.82, 320.11, 322.42, 324.74, 327.07, 329.41, 331.77, 334.14, 336.52, 338.92, 341.33, 343.76, 346.16, 348.61, 351.07, 353.55, 356.04, 358.55, 361.07, 363.60, 366.15, 368.68, 371.26, 373.85, 376.45, 379.07, 381.71, 384.35, 387.01, 389.69, 392.39, 395.10, 397.82, 400.56 for hetero-Tau trimer, all temperatures are in Kelvin. The production run is 395 ns per replica for hetero-Aβ trimer and 403 ns per replica for hetero-Tau trimer. The first 100ns per replica is not analyzed.

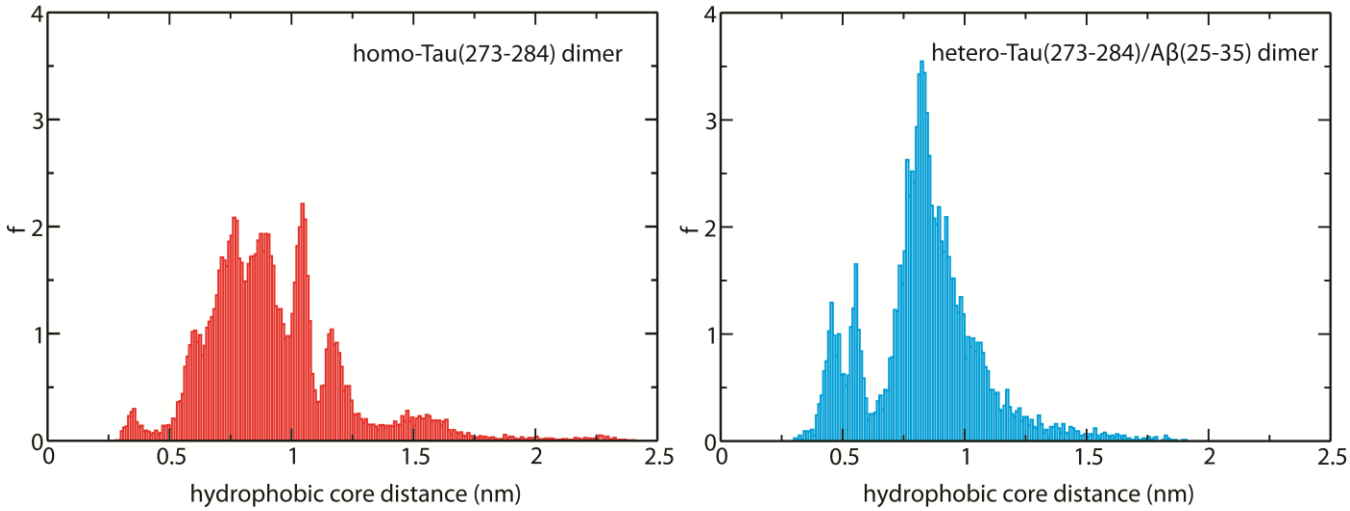

Figure S2. Normalized distributions of center of mass distances between hydrophobic cores in (left) homo-Tau(273-284) dimer and (right) hetero-Tau(273-284)/Aβ(25-35) dimer. For the homo-Tau dimer, the two hydrophobic cores are both PHF6* (VQIINK). For the heterodimer, the hydrophobic cores are PHF6* of Tau(273-284) and GAIIGL of Aβ(25-35). The center of mass of each hydrophobic core consists of backbone atoms only (N, C, CA atom types). The homo-Tau(273-284) dimer data are obtained from Larini et al.[4] A distance of 4.8-5.0 Å is typical for two adjacent chains within a β-sheet. From this Figure, there is a significant population within this distance range in the distribution of heterodimer, but not homo-Tau dimer.

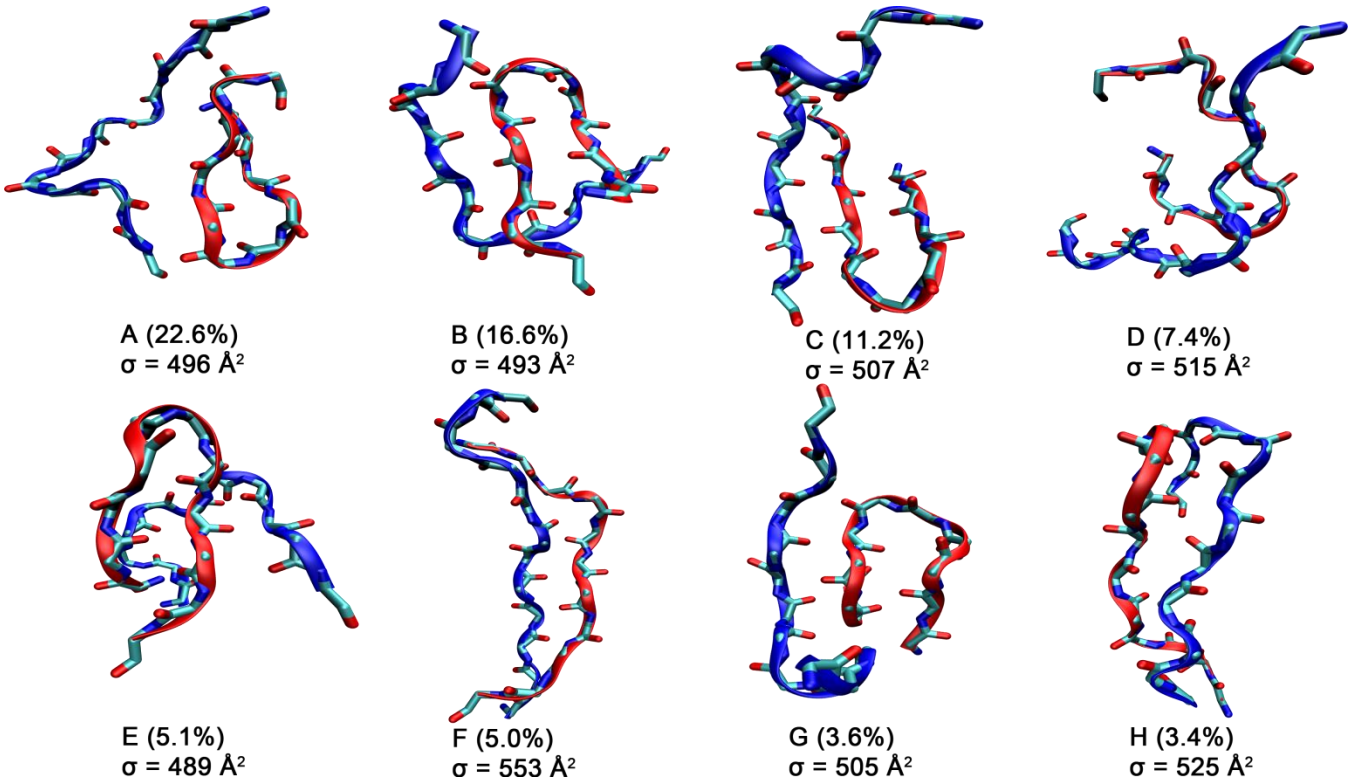

Figure S3. Representative clusters of probed by T-REMD simulations. Ac-Aβ(25-35)-NH_2 is shown in red, and Ac-Tau(273-284)-NH_2 is shown in blue. Backbone atoms are shown in licorice.

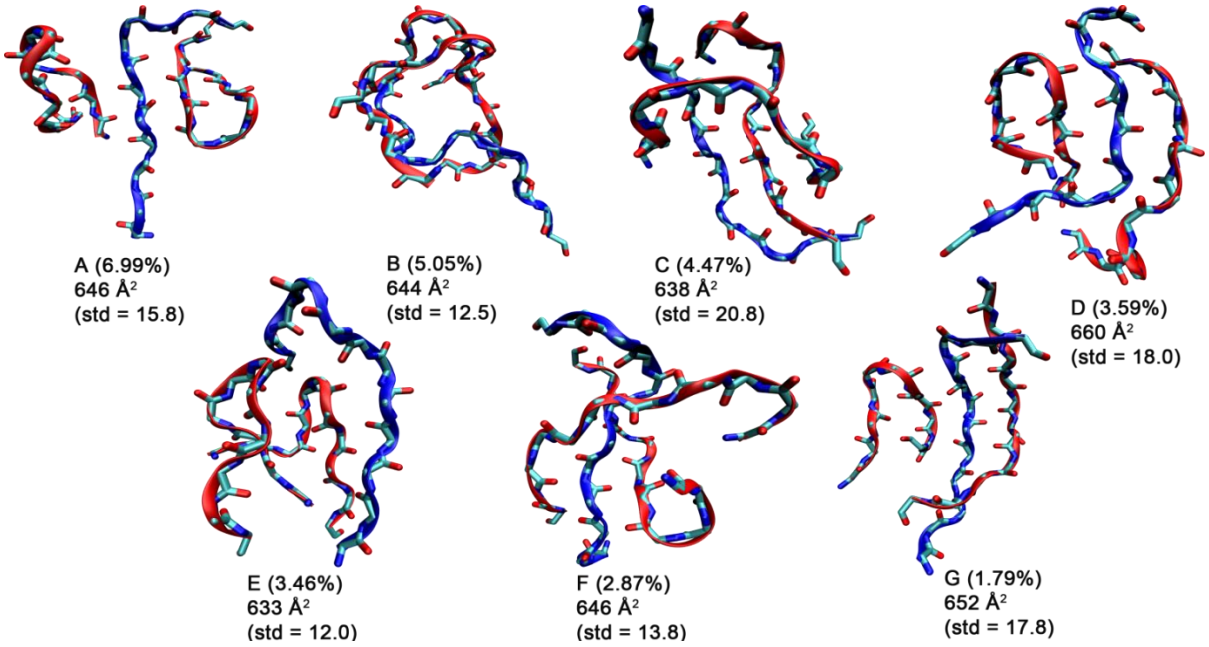

Figure S4. Representative structures of populated clusters obtained from T-REMD simulation of hetero-Aβ trimer [2+1] and their cross sections. Ac-Aβ(25-35)-NH_2 is shown in red, and Ac-Tau(273-284)-NH_2 is shown in blue. Backbone atoms are shown in licorice. In clusters A, D, F and G, the Tau(273-284) monomer is intercalating between two Aβ(25-35) monomers. There is no significant contact between the two Aβ(25-35). In other clusters, there are interactions among all three peptide chains. Theoretical cross section is reported as an average of 50 structures chosen randomly from each cluster.

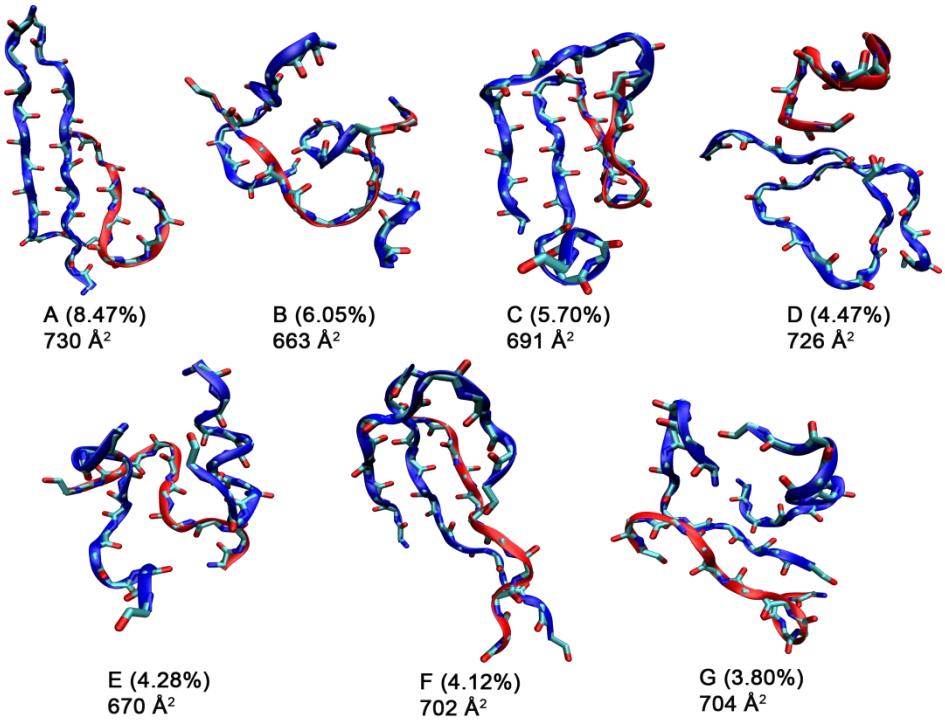

Figure S5. Representative structures of populated clusters obtained from T-REMD simulation of hetero-tau trimer [1+2]. Ac-Aβ(25-35)-NH_2 is shown in red, and Ac-Tau(273-284)-NH_2 is shown in blue. Backbone atoms are shown in licorice. Clusters A, C and F shows the strong hydrogen bond network between Tau peptide chains, and the structures are relatively extended. The remaining clusters are disordered and more compact.

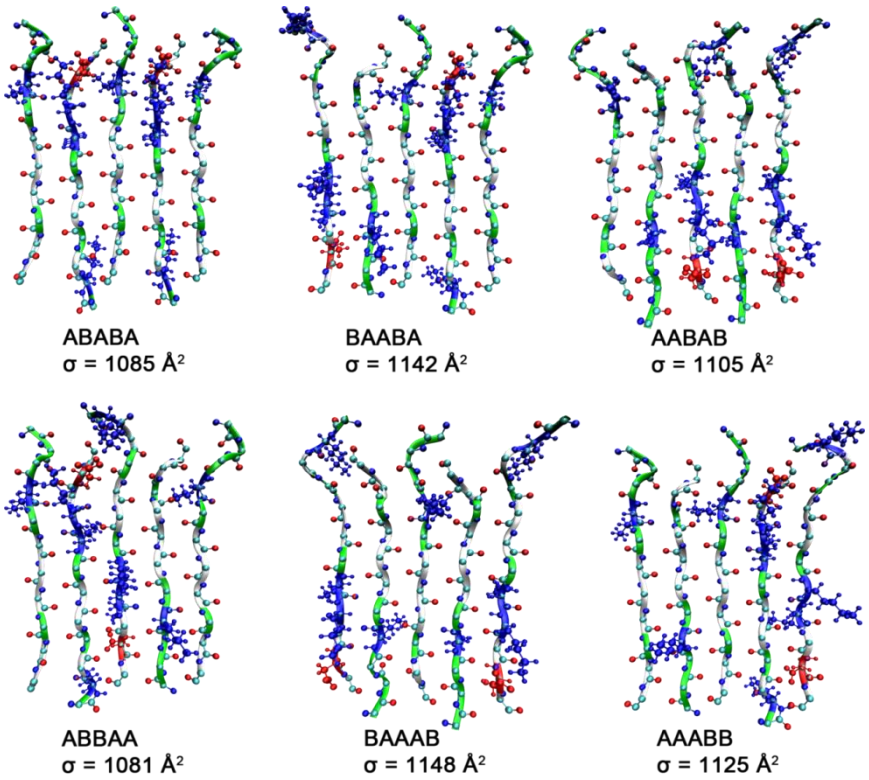

Figure S6. The β-sheet model structures of hetero-Aβ pentamers. Each chain is colored based on residue types (white = non-polar, green = polar, blue = basic, red = acidic). The side chains of Asp and Lys residues are shown explicitly. The structures were minimized in the gas phase before cross section calculation. Each structure is annotated with A = Aβ(25-35) and B = Tau(273-284). For visualization purpose, the Asp residues (shown in red) indicate the Tau(273-284) peptide chains.

S4. Atomic Force Microscopy

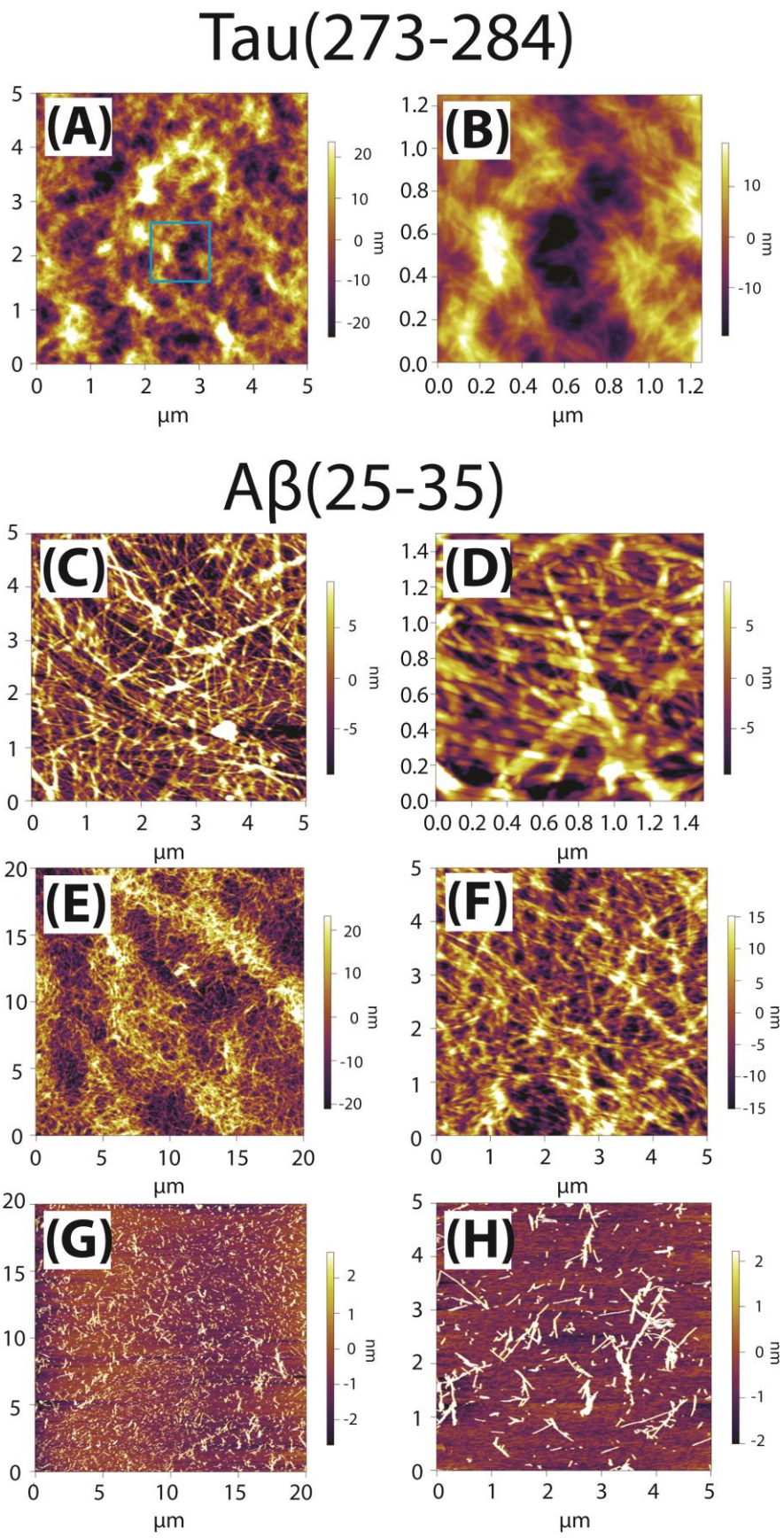

Figure S7. Representative AFM images of Tau(273-284) and Aβ(25-35) at 200 µM in water. (A,B) 1-hour incubated Tau(273-284) with 11kDa heparin showing both individual fibrils and aggregate clusters, (C,D) 1-hour incubated Aβ(25-35) showing extensive fibril formation, (E,F) 2-day incubated Aβ(25-35) imaged at the center of the AFM slide showing a uniform distribution of some individual fibrils and many clusters of fibrils, (G,H) At the edges of the AFM slide, individual and small aggregates can also be imaged.

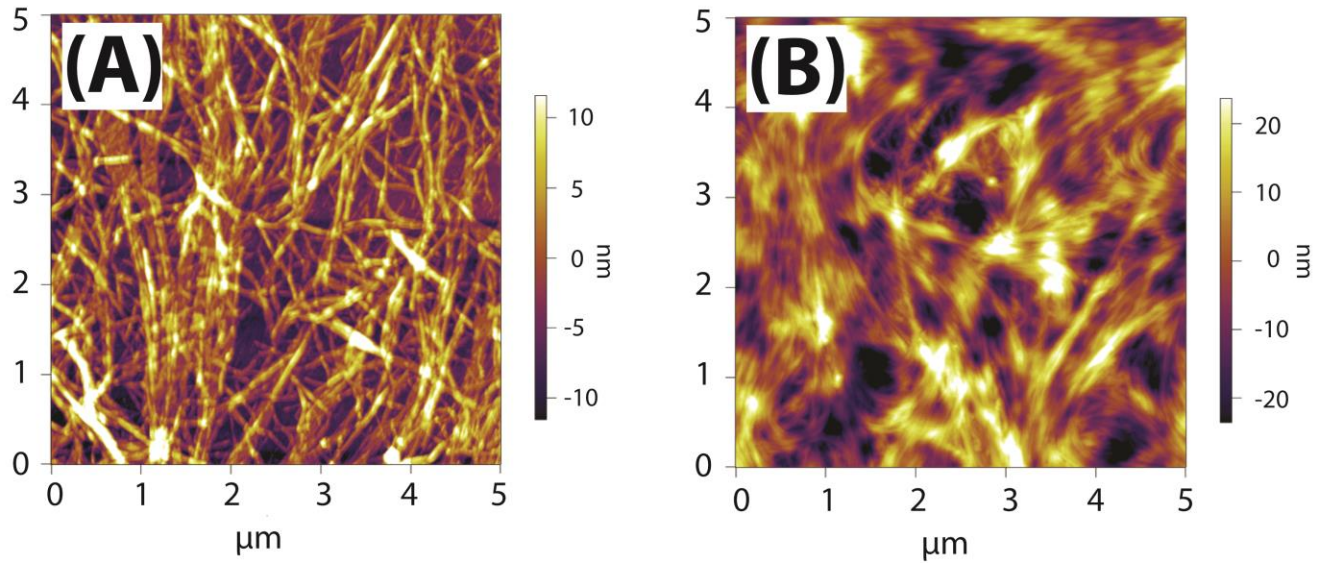

Figure S8. Representative AFM images of (A) 1-week incubated Aβ(25-35) at 100 µM in water, and (B) 1:1 ratio of Tau(273-284):Aβ(25-35) at 100 µM in water in which fresh tau was added to 1-week incubated Aβ(25-35) and incubated for additional two days. There is a small change in aggregate morphology but no decrease in aggregate abundance.

References

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