Supporting Material

Effects of Hydroxylated Carbon Nanotubes on the Aggregation of Aβ(16-22) Peptides: A Combined Simulation and Experimental Study

Luogang Xie,[†,§] Dongdong Lin,[†,§] Yin Luo,[†] Huiyu Li,[‡] Xinju Yang[†] and Guanghong Wei[†,*]

[†]State Key Laboratory of Surface Physics, Key Laboratory for Computational Physical Sciences (Ministry of Education), and Department of Physics, Fudan University, 220 Handan Road, Shanghai, 200433, China

[‡]Department of Mathematics and Physics, Shanghai University of Electric Power, 2588 Changyang Road, Shanghai, 200090, China

This material includes the descriptions of REMD simulations, analysis parameters, AFM images and ThT measurements of Aβ(16-22) aggregation in the absence/presence of SWCNTs (pristine and hydroxylated), connectivity length analysis, and five figures.

Description of REMD simulations and analysis parameters

Our REMD simulations include 40 replicas, and the temperatures were varied from 310 K to 420 K (temperature list: 310.00, 312.42, 314.87, 317.33, 319.81, 322.31, 324.83, 327.37, 329.93, 332.50, 335.10, 337.72, 340.36, 343.02, 345.71, 348.41, 351.13, 353.88, 356.64, 359.43, 362.24, 365.07, 367.93, 370.80, 373.70, 376.62, 379.57, 382.53, 385.52, 388.54, 391.57, 394.63, 397.72, 400.83, 403.96, 407.12, 410.30, 413.51, 416.74, 420.00 K). Exchanges between two neighboring replicas were tried every 2 ps. The acceptance ratios for isolated Aβ and Aβ+SWCNT$_{216}$(OH)$_{30}$ systems are ~ 17% and 13%, respectively. Constraints were applied to bond lengths using the SETTLE algorithm for water(1) and the LINCS method for the peptides(2), allowing an integration time step of 2 fs. Non-bonded pair lists were updated every 5 integration time steps. A cutoff of 1.4 nm was used for the non-bonded interactions and a reaction-field correction with dielectric permittivity of $\varepsilon = 78$ was employed to calculate the long-range electrostatics interactions. The temperature and the pressure (1 bar) were maintained by weakly coupled to temperature and pressure baths.(3) The solute and solvent were separately coupled to external temperature and pressure baths. The temperature- and pressure-coupling constants were 0.1 ps and 1.0 ps, respectively.

The free energy surfaces (or potential of mean force, PMF) were constructed using the relation $-RT\ln H(x, y)$, where $H(x, y)$ is the histogram of two selected reaction coordinates, x and y. In this study, the x coordinate is the number of inter-peptide hydrogen bonds (H-bonds) and the y coordinate is the radius of gyration of the Aβ(16-22) octamers. One H-bond is taken

as formed if the N…O distance is less than 0.35 nm and the N-H…O angle is greater than 150°. The Daura cluster analysis method(4) was used to cluster the conformations sampled in the REMD simulations with a C_α-root mean square deviation (C_α-RMSD) cutoff of 0.3 nm using residues L17-A21, with K16 and E22 excluded because of their high flexibilities. The chain-independent C_α-RMSD is calculated by completely neglecting the chain identifier in the coordinate file of Aβ(16-22) octamers to obtain the smallest RMSD as the eight chains are topologically identical. The inter-peptide interactions were estimated by the probability of residue-residue contacts. A contact is defined when the aliphatic carbon atoms of two non-sequential main chains (or side chains) come within 0.54 nm or any other atoms of two non-sequential main chains (or side chains) lie within 0.46 nm.

AFM images and ThT fluorescence intensity of Aβ(16-22) samples in the absence/presence of SWCNTs

The morphology of samples at different incubation periods was measured by utilizing commercial SPM equipment (Multimode V, Bruker Nano Surface, USA). The topography images of all dried samples were obtained by AFM in tapping mode with Si tips at a scanning rate of 1 Hz. All images, shown without any image processing except the flattening action by its own software, were collected under ambient conditions at 25 °C and room humidity < 35 %. To prepare the samples for AFM imaging, about 10 μL Aβ(16-22) solution was dropped on a freshly cleaved mica surface. The droplet was left on the substrate for 30 s covered with parafilm, and then dried under a gentle stream of nitrogen.

The thioflavin T (ThT) dye fluorescence measurements were performed with F-2500 FL Spectrophotometer (Hitachi). ThT is a dye known to preferentially bind amyloid fibrils. 1 mM ThT solution was prepared by dissolving 31.9 mg ThT powder in 100 ml PBS solution. The stock solution was stored at 4 °C covered with foil for further use. The samples for ThT fluorescence measurements were prepared by fully mixing 100 μL Aβ solution or Aβ+SWCNTs (pristine and hydroxylated) dispersion with 10 μL ThT (1 mM) and 890 μL PBS solutions in a quartz cuvette. The control ThT sample was prepared by mixing 10 μL ThT (1 mM) solution with 990 μL PBS solution. The emission intensities were measured at 485 nm with excitation at 440 nm. Measurements were performed at room temperature with excitation and emission slit widths of 5 nm.

Analysis of the connectivity length of Aβ octamers in isolated Aβ and Aβ+SWCNT$_{216}$(OH)$_{30}$ complex systems

To characterize the configurations of Aβ aggregates in more details, we calculate the connectivity length (CL) of Aβ octamers in isolated Aβ and Aβ+SWCNT$_{216}$(OH)$_{30}$ complex.

The percentage of CL is presented in Fig. S3. To have an intitutive idea about the configurations of Aβ(16-22) octamers, we also label in Fig. S3 the configuration types (CTs) for the most populated configurations. It can be seen that the CL probability distributions of Aβ octamers in these two systems are significantly different. For Aβ(16-22) octamers in the isolated Aβ system, their CLs are mainly varied from 2.828 (CT = 8) to 6.242 (CT = 3+2+1(3), i.e. 3+2+1+1+1), while the CLs are mostly in the range of 6.732 (CT = 3+1(5)) to 8 (CT = 1(8)) in Aβ+SWCNT$_{216}$(OH)$_{30}$ system. The four most-populated aggregates of Aβ(16-22) octamers in the isolated Aβ system show higher ordered CLs, consisting of bilayer β-sheets (CL = 4.732, CT = 4+3+1, 13.7%), β-barrels (CL = 2.828, CT = 8, 7.4%) and aggregates with a mixture of random chains, and two- and three- or four-stranded β-sheets (CL = 5.414, CT = 4+2+1(2), 8.9 % and CL = 6.146, CT = 3+2+1(3), 8.8 %). In contrast, in the presence of SWCNT$_{216}$(OH)$_{30}$, Aβ aggregates display mainly disordered configurations, mostly populated by eight disordered chains (CL = 8, CT = 1(8), 16.8 %), random chains with two-stranded β-sheets (CL = 7.414, CT = 2+1(6), 42.1 % and CL = 6.828, CT = 2(2)+1(4), 22.4 %) and three-stranded β-sheet (CL = 6.732, CT = 3+1(5), 11.6 %).
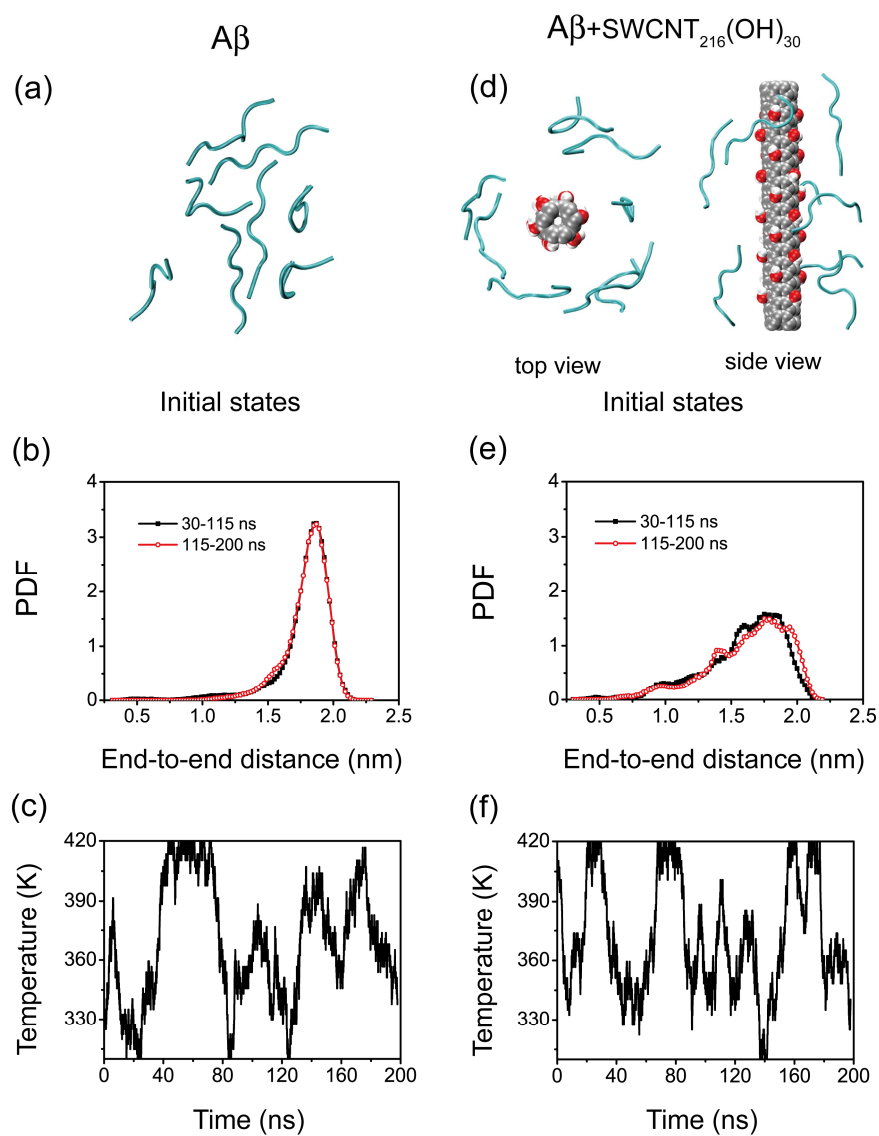
Supplemental figures:

FIGURE S1 The initial random states and convergence check for the REMD runs of isolated Aβ and Aβ+SWCNT$_{216}$(OH)$_{30}$ complex at 310 K. (a, d) Initial random states. (b, e) Probability density distribution (PDF) of end-to-end distance (the C$_\alpha$-C$_\alpha$ distance between K16 and E22) of Aβ(16-22) octamer within the time intervals 30-115 ns and 115-200 ns. (c, f) The time evolution of temperature swapping of one representative replica in temperature space (from 310 to 420 K).
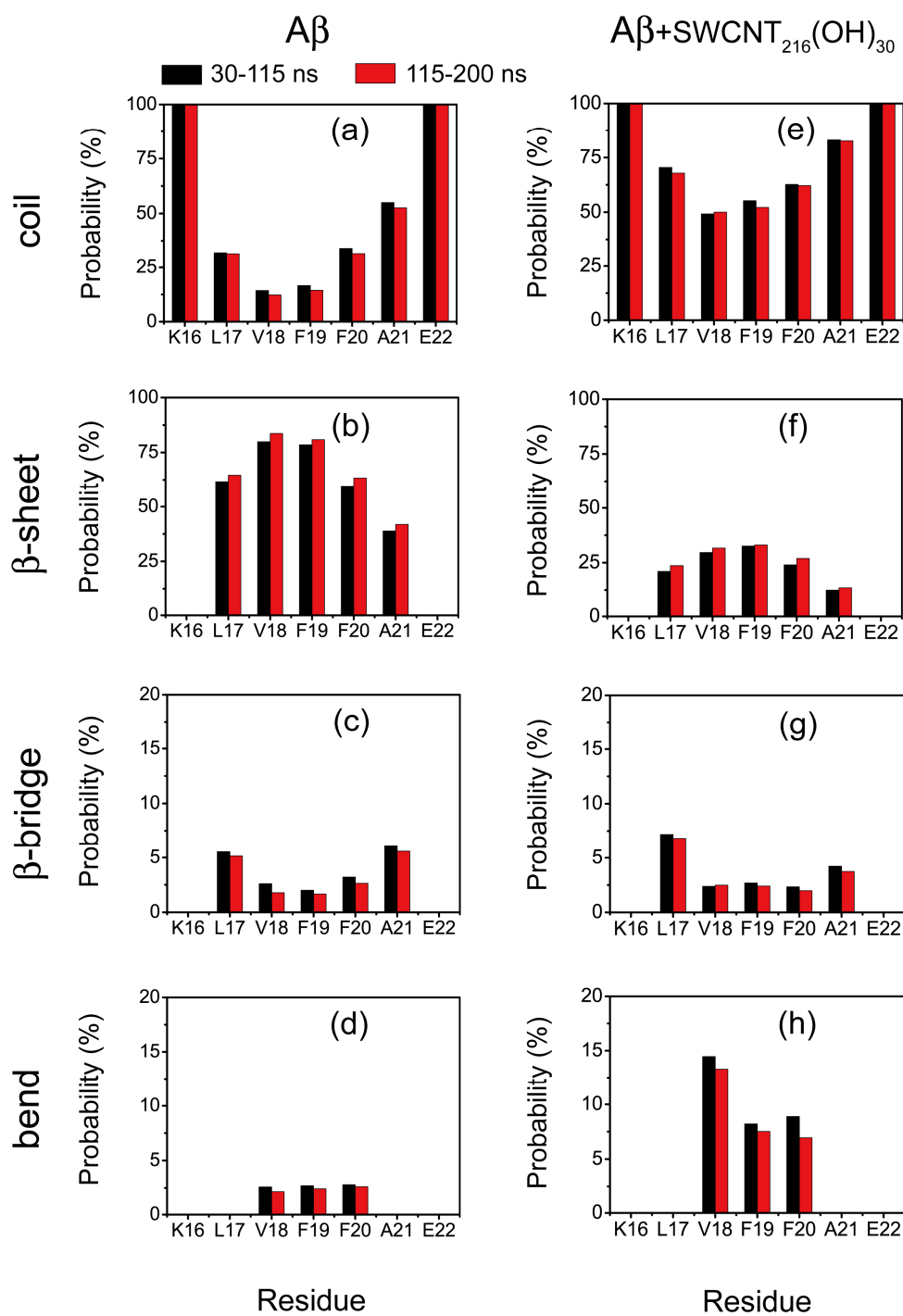
FIGURE S2   The calculated secondary structure probability of each residue in the REMD runs within the time intervals of 30-115 ns and 115-200 ns for Aβ(16-22) octamers in the absence and presence of SWCNT$_{216}$(OH)$_{30}$ at 310 K for (a, e) coil, (b, f) β-sheet, (c, g) β-bridge, and (d, h) bend structures.
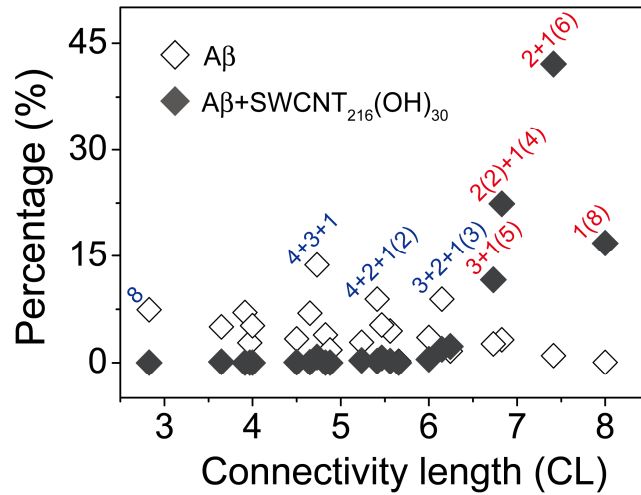
FIGURE S3 Probability distribution of connectivity length (CL) of Aβ(16-22) octamers in isolated Aβ (□) and in Aβ+SWCNT$_{216}$(OH)$_{30}$ complex (■) at 310 K. To have an intitutive idea about the configurations of Aβ(16-22) octamers, we label the configuration types (CTs) for the most populated configurations. Noting that for the CTs, we use 4+2+1(2) to denote 4+2+1+1, the same to others.
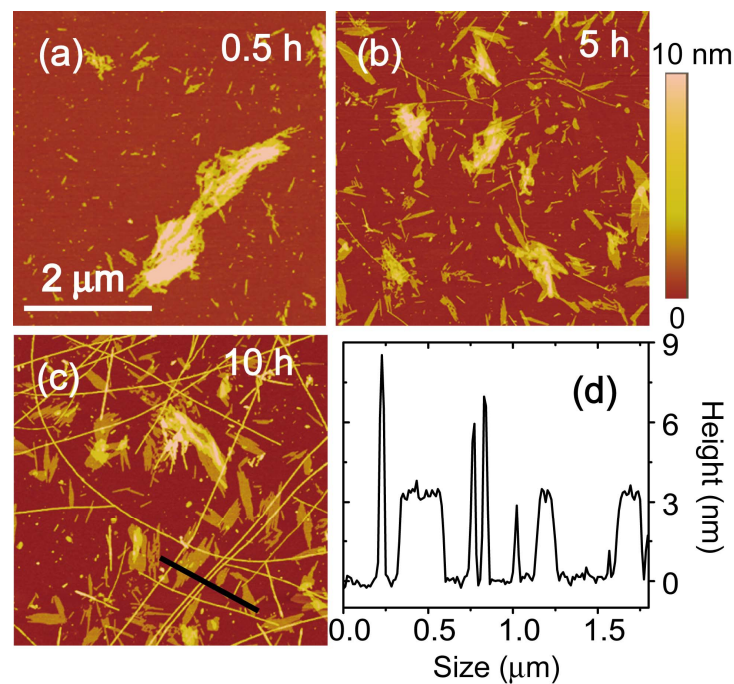

FIGURE S4 AFM images of Aβ(16-22) samples with pristine SWCNTs at three different co-incubation time: (a) 0.5 h, (b) 5 h and (c) 10 h. The section analysis of Aβ(16-22) aggregates incubated at 10 h is given in (d).
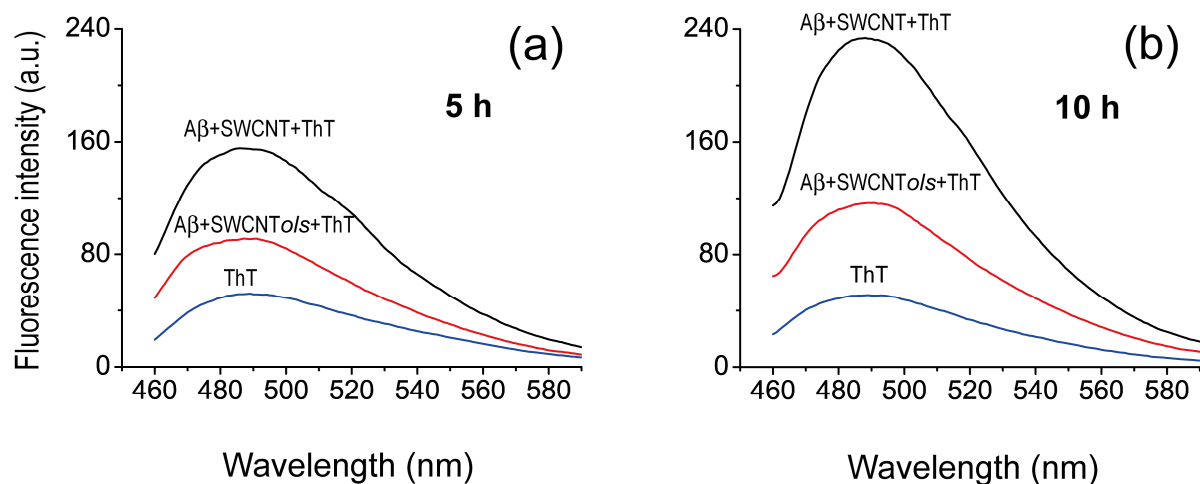
FIGURE S5   The emission spectra of ThT bound to Aβ(16-22) samples in the presence of pristine SWCNTs (Aβ+SWCNT) and hydroxylated SWCNTs (Aβ+SWCNT$_{216}$(OH)$_7$) at an incubation time of 5 h (a) and 10 h (b). The background fluorescence of ThT is also shown.

SUPPORTING REFERENCES

1   Miyamoto, S.; P. A. Kollman. 1992. SETTLE: an analytical version of the SHAKE and RATTLE algorithm for rigid water models. *J. Comput. Chem.* 13: 952-962.

2   Hess, B.; H. Bekker; H. J. Berendsen; J. G. Fraaije. 1997. LINCS: a linear constraint solver for molecular simulations. *J. Comput. Chem.* 18: 1463-1472.

3   Berendsen, H. J.; J. P. M. Postma; W. F. van Gunsteren; A. DiNola; J. Haak. 1984. Molecular dynamics with coupling to an external bath. *J. Chem. Phys.* 81: 3684-3690.

4   Daura, X.; K. Gademann; B. Jaun; D. Seebach; W. F. van Gunsteren; A. E. Mark. 1999. Peptide folding: when simulation meets experiment. *Angew. Chem., Int. Ed.* 38: 236-240.