Supporting Information for

Aβ Aggregation Behavior at Interfaces with Switchable Wettability: A Bioinspired Perspective to Understand Amyloid Formation

Yijia Guan,[a,b] Dongqin Yu,[a,c] Hanjun Sun,[a] Jinsong Ren,[a,c] and Xiaogang Qu*[a,c]

[a] Laboratory of Chemical Biology and State Key Laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China.
[b] Henan Key Laboratory of Materials on Deep-Earth Engineering, School of Materials Science and Engineering, Henan Polytechnic University, Jiaozuo 454003, Henan, China.
[c] University of Science and Technology of China, Hefei, Anhui, 230029, P. R. China.

Materials and Method

1. Materials

Pyrrole (Py) and taurocholic acid (TCA) were purchased from Sigma-Aldrich. Aβ1-40 (Aβ40) was purchased from American Peptide. Aβ1-28 and Aβ25-35 were purchased from Sigma-Aldrich. And all chemicals were used as received from the suppliers without further purification. In all experiments, we used ultrapure water (18.2 MΩ; Millpore Co., USA).

2. Methods

2.1 Fabrication of PPy/TCA films

PPy/TCA films on the indium tin oxide (ITO) were formed by template-free electrochemical method. A small electrochemical cell consist of an electrolyte, an ITO film (effective area of 10 mm × 10 mm) as a working electrode, platinum electrode as a counter electrode, and Ag/AgCl as a reference electrode. Firstly, prenucleated PPy films (PNPF) were formed on the ITO films. Briefly, the ITO film (as a working electrode) was put into an electrolyte (including 0.1 M Py and 0.2 M KCl). Then, electrochemical station run at 0.8 V (vs SCE) for 20 seconds at room temperature. Secondly, the PNPF was washed with ultrapure water for three times and dried naturely. In order to fabricate the PPy/TCA film, a phosphate buffer solution (PBS, 0.1 M, pH 7.4) including 0.2 M Py and 0.07 M TCA was used as an electrolyte. PPy/TCA was formed on the PNPF-coated ITO film (as working electrode) galvanostatically at 70 μA for 800 seconds. Finally, the PPy/TCA films were washed with ultrapure water for three times, and dried.

2.2 Potential-induced reversible switch in wettability

To determine the redox potentials of PPy/TCA films, cyclic voltammetry (CV) was monitored in an electrochemical system made of an electrolyte (PBS, pH 7.4, containing 0.07 M TCA), PPy/TCA as a working electrode, Ag/AgCl as a reference electrode, and platinum electrode as a counter electrode. The scanning potential ranges from 1 V to -1 V with a scan rate of 0.02 V/s. Based on these resultant CV curves of PPy/TCA, the oxidation and reduction potential of PPy/TCA films were +0.15 V and -0.26 V, respectively. Then we used the electrochemical station to apply

-0.55 V (as reduction potential) to trigger reduction reactions of the PPy/TCA coated ITO (working electrode) for 10 min. The PPy/TCA coated ITO was then taken out of the station and dried in vacuum, followed by the measurement of static contact angle (SCA) of a 1μL water droplet on PPy/TCA by surface contact angle analyzer at ambient temperature. Then the PPy/TCA coated ITO was placed into the station again and an oxidation potential (+0.75 V) was applied to trigger the oxidation reactions of the PPy/TCA coated titanium (working electrode) for 10 min. The PPy/TCA coated ITO was dried in vacuum again and the SCA of 1 μL water droplet on PPy/TCA by surface was measured. The average and standard deviation were calculated according to at least five measurements for each specimen. The reduction and oxidation potential were repeatedly applied to the PPy/TCA coated ITO in sequence along with the subsequent SCA measurements after applying each potential in order to characterize the potential-induced reversible wettability.

2.3 Aβ stock solution preparation

Aβ40, Aβ1-28 and Aβ25-35 peptides were prepared as previously reports.[S1] Firstly, we injected 1.0 mL 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) into a bottle with the powdered Aβ40 (Aβ1-28 or Aβ25-35) peptide. For further dissolution, the solution in a sealed vial (2 mL) was shaken at 4 °C for 4 hours. Then the stock solutions were stored at -20 °C. Before experiments, the polar solvent HFIP was removed under a gentle stream of nitrogen. Then Aβ40 (Aβ1-28 or Aβ25-35) peptide was dissolved in PBS buffer (10 mM, pH 7.4).

2.4 Electrochemical impedance spectroscopy measurements

Using a CHI 660B Electrochemistry Workstation, electrochemical impedance spectroscopy measurements were performed in PBS (100 mM, pH 7.4) including a 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ mixture with 1 M KCl (1:1) as a supporting electrolyte. Within the frequency ranges of 10^{-2}–10^5 Hz, the impedance spectra were recorded with a conventional three-electrode system (PPy/TCA as the working electrode, Ag/AgCl as the reference electrode, and a platinum eletrode as the auxiliary electrode). The amplitude of the applied sine wave potential was 5 mV in each case.

2.5 Potential-induced reversible switch in protein adsorption

Before incubating with Aβ40, the PPy/TCA specimens in hydrophilic or hydrophobic states were immersed in PBS (pH 7.4) for 30 min. The isoelectric point of Aβ40 is approximately 5.2 (acidic). To study Aβ40 adsorption, a PPy/TCA film with a diameter of 5 mm was exposed to 100 μL Aβ40 peptide solution (10 μM) and incubated at 37°C for 12 hours. Then the Aβ40 solution was removed and the films were washed with ultrapure water for twice. Subsequently, the PPy/TCA films were shaken in 0.2 mL of sodium dodecylsulfate (SDS, 1 wt %) to elute the adsorbed Aβ40 peptide. The amount of the adsorbed protein on PPy/TCA films was evaluated by bicinchoninic acid (BCA) assay. The absorbance of the eluent was measured at 562 nm by a microplate reader with at least three repetitions for each group.

2.6 Atomic Force Microscopy (AFM) Studies

10 μM Aβ peptides (Aβ40, Aβ1-28 or Aβ25-35) with the incubation of PPy/TCA films were placed at 37 °C for 7 days. Then the film was removed from protein solution, followed by washing the PPy/TCA with deionized water for three times gently. After drying naturely prior to AFM studies, the images were obtained under ambient conditions, tapping mode.

For AFM studies of supernatant, 50 μL of supernatant was dropped on newly prepared mica substrates. After incubating for 30 minutes, the substrates were washed with ultrapure water for twice and dried prior to AFM studies. The images were obtained under ambient conditions, tapping mode.

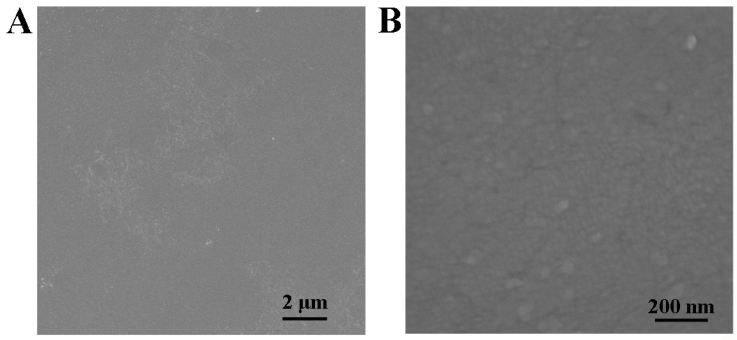

Figure S1 The morphology of PPy/TCA is imaged by SEM at different magnifications.

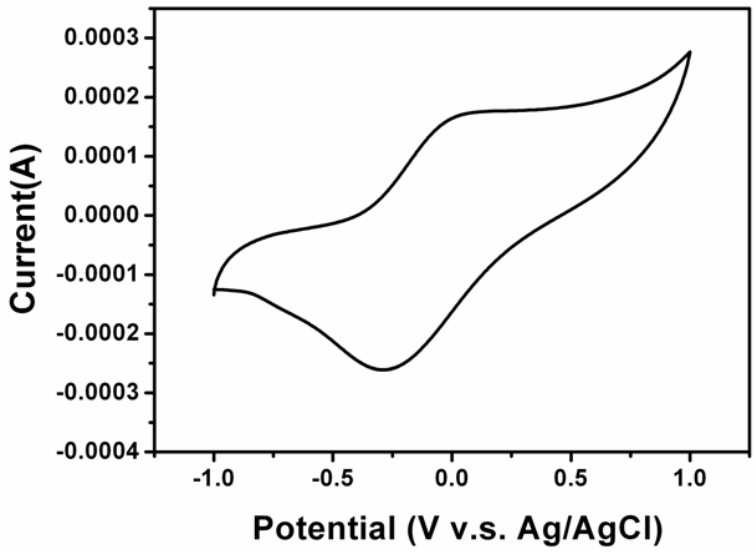

Figure S2 Cyclic voltammogram of PPy/TCA.

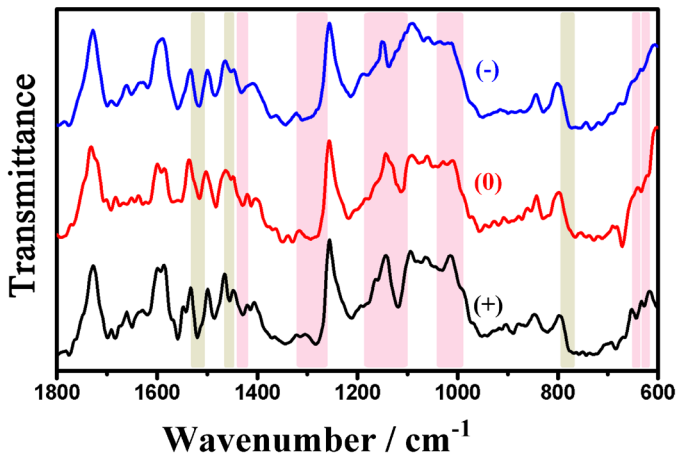

Figure S3 Attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectra of PPy/TCA in original state (0), hydrophobic state (+, α-face, oxidation state) and hydrophilic state (-, β-face, reduction state). The yellow regions represent the peaks of PPy, and pink regions represent the peaks of TCA. Three peaks at 1520, 1451 and 777 cm^{-1} appeared in all spectra and could be assigned to the C=C stretching vibration, C–N stretching vibration, C-H out-plane ring deformation of PPy,[S2] respectively. The peaks at 1426, 647 and 621 cm^{-1} corresponded to $–CH_3$ deformation vibration, –OH out-plane deformation vibration and O=C–NH deformation vibration of TCA, respectively. The peak regions of 1320–1250 cm^{-1}, 1180–1100 cm^{-1} and 1040–990 cm^{-1} were attributed to the –OH stretching vibration, asymmetric and symmetric O=S=O stretching vibration of TCA, respectively.[S3] It was obvious that, compared with original state and hydrophobic state, the three peaks in Figure S3 (hydrophilic state) exhibited a blue shift in those peak regions, implying that the chemical environment of TCA molecule in hydrophilic state was different from that in other states. In hydrophilic state, the decreased ratio in the intensity of peaks at 647 cm^{-1} (–OH) and 621 cm^{-1} (O=C–NH) confirmed the deprotonation of a part of –C–OH in forming reduction state of TCA.

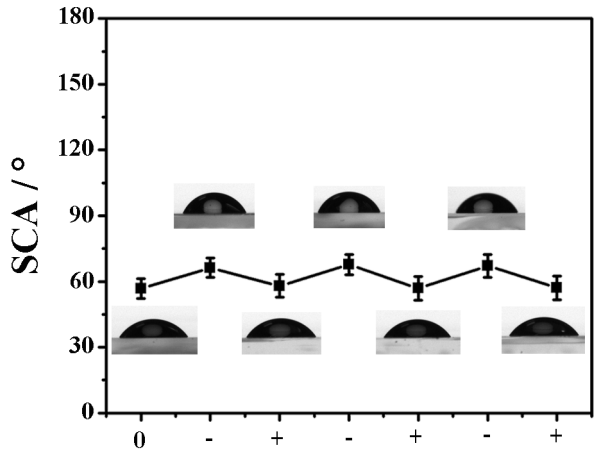

Figure S4 Static contact angle (SCA) of PPy/Cl^- versus negative potentials (-0.55 V; *vs* Ag/AgCl electrode) and positive potentials (+0.75 V; *vs* Ag/AgCl electrode) in situ. Inset: SCA images. This result showed that when the dopant of PPy/TCA was changed from TCA to Cl^-, the reversible switch in wettability becomes trivial.

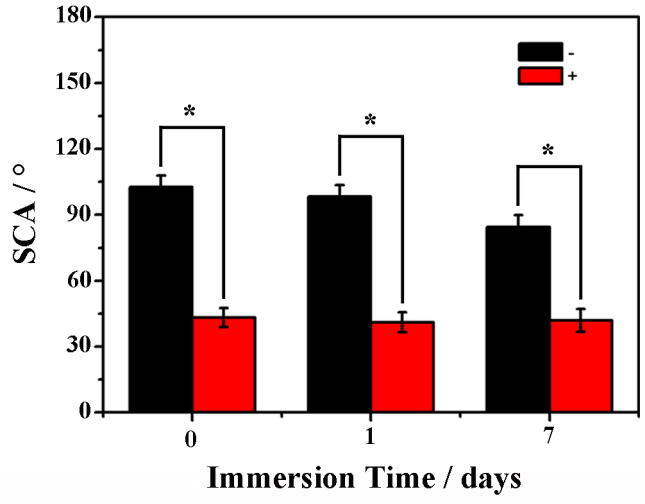

Figure S5 The plots of SCA of PPy/TCA in hydrophobic state (-) and hydrophilic state (+) versus the time of immersion in PBS. The * indicated significant difference ($p<0.05$). This data showed that regardless of the time of immersion in PBS, both the hydrophobic state and hydrophilic state of specimens exhibited a relatively stable static contact angle. Therefore, the PPy/TCA was stable in physiological condition.

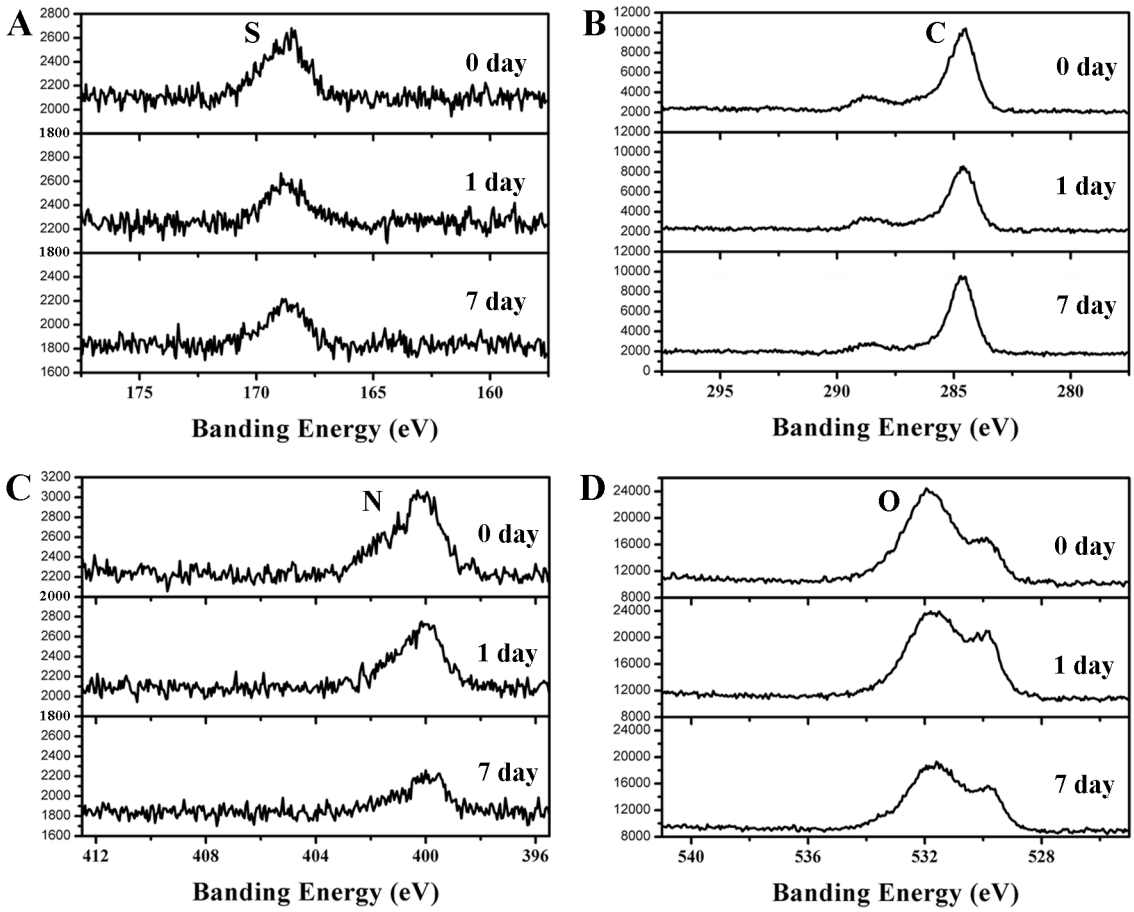

Figure S6 X-ray photoelectron spectroscopy (XPS) analysis of PPy/TCA after immersion in PBS for 0, 1 and 7 days. To find out whether TCA would be released from PPy/TCA in physiological medium, the specimens were immersed in PBS (pH 7.4) for various times. After the PPy/TCA was washed with deionized water, XPS was employed to analyze the TCA amount in specimens. XPS analysis showed that no visible change in the peak intensity of S (sulphur, presence in TCA molecule) could be observed for up to 7 days (A), implying that almost no TCA was released from PPy/TCA in physiological medium. In addition, the intensities of other peaks also remained nearly unchanged (B, C, D), further suggesting chemical stability of the PPy/TCA films in physiological conditions.

Table S1 The amount of adsorbed Aβ on PPy/TCA in origin state, versus negative potentials (-0.55 V *vs* Ag/AgCl electrode) and positive potentials (+0.75 V *vs* Ag/AgCl electrode).

	+	0	−
Protein adsorption[a] ($\mu g/cm^2$)	1. 01	0. 83	0. 12

[a]The amount of absorbed protein was measured by bicinchoninic acid (BCA) assay. The values are the average of three independent measurements.

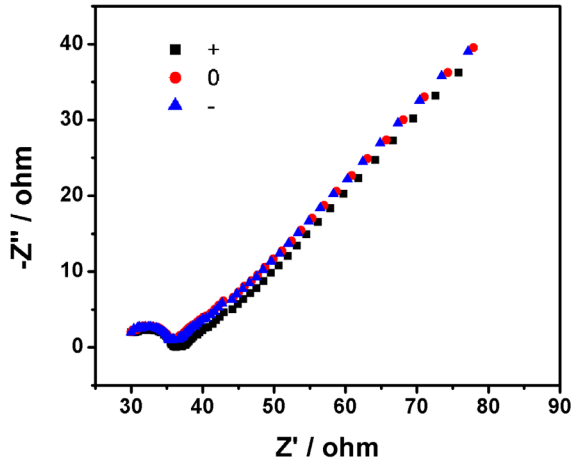

Figure S7 Nyquist diagrams of electrochemical impedance spectra of PPy/TCA in origin state, versus negative potentials (-0.55 V *vs* Ag/AgCl electrode) and positive potentials (+0.75 V *vs* Ag/AgCl electrode) without the incubation of Aβ, respectively.

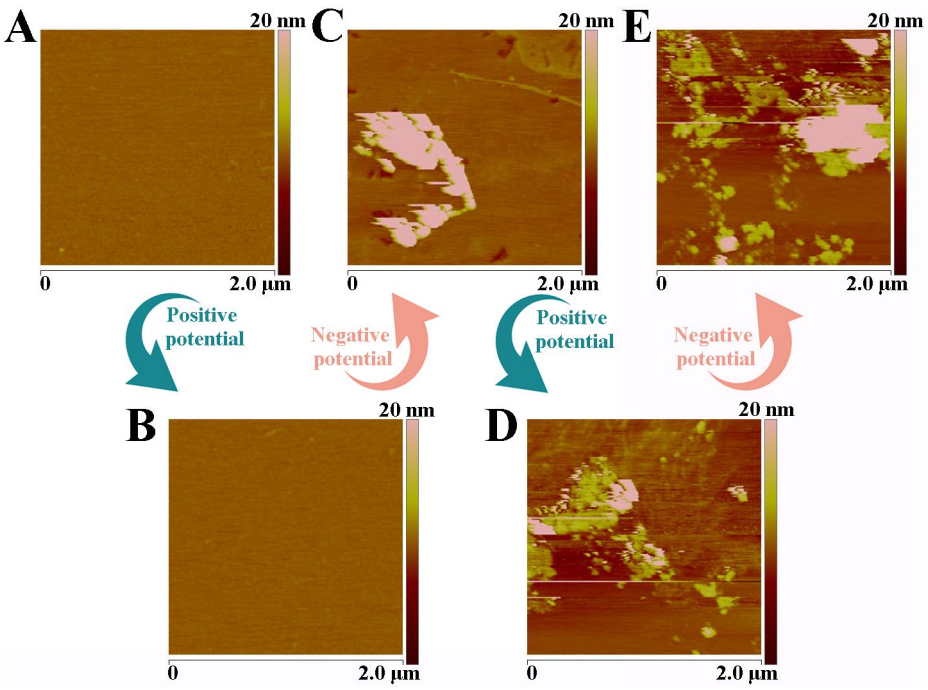

Figure S8 AFM image of Aβ in supernatant after incubation with (A) initial surface, (B, D) hydrophobic surface, (C, E) hydrophilic surface.

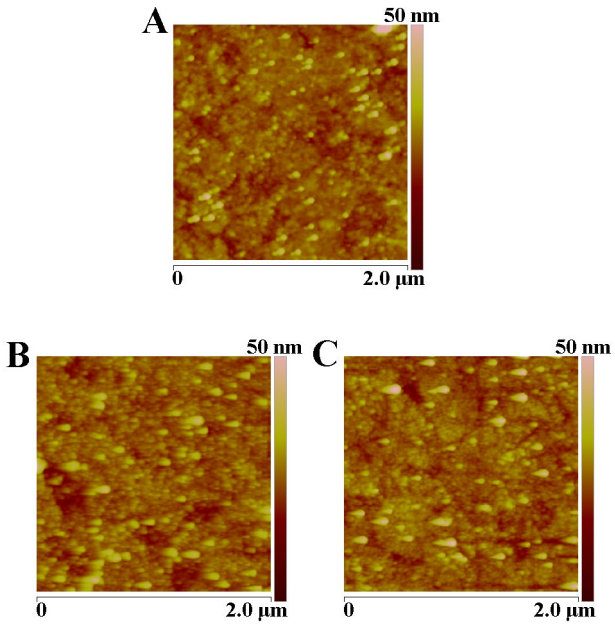

Figure S9 (A) AFM image of the initial state of PPy/TCA film. AFM image of (B) hydrophobic and (C) hydrophilic PPy/TCA film.

In order to rule out that surface roughness may have an effect on Aβ adsorption and aggregation, we characterize the root-mean-square (RMS) surface roughness by AFM.

The RMS surface roughnesses of PPy/TCA in original, hydrophobic and hydrophilic state are 3.75 nm, 3.70 nm and 3.91 nm, which indicated that these films share a similar RMS surface roughness and RMS surface roughness is independent on the switching state/applied potential. Therefore, in this study, surface roughness is not a main factor on Aβ adsorption and aggregation.

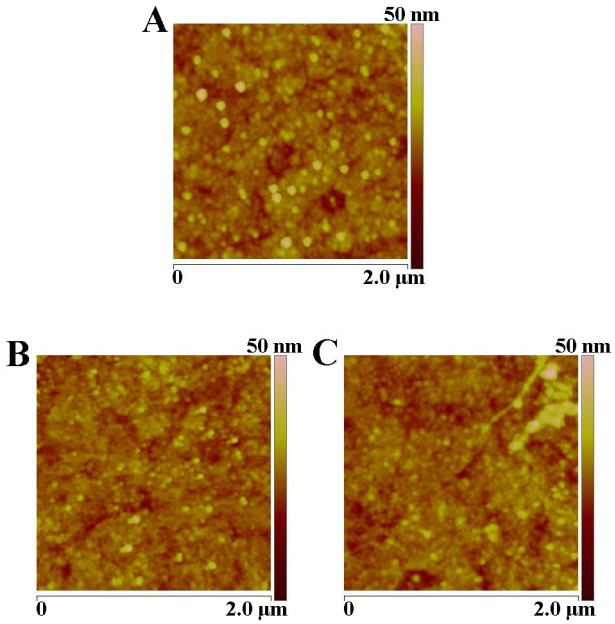

Figure S10 (A) AFM image of hydrophobic PPy/TCA film. AFM image of hydrophobic PPy/TCA film with the incubation of (B) Aβ1-28 and (C) Aβ25-35.

References

[S1] Y. Guan, Z. Du, N. Gao, Y. Cao, X. Wang, P. Scott, H. Song, J. Ren, X. Qu, Stereochemistry and amyloid inhibition: Asymmetric triplex metallohelices enantioselectively bind to A beta peptide. *Sci. Adv.* 2018, **4**, eaao6718.

[S2] G. Lu, C. Li, G. Shi, Polypyrrole micro- and nanowires synthesized by electrochemical polymerization of pyrrole in the aqueous solutions of pyrenesulfonic acid, Polymer 2006, 47, 1778.

[S3] Y. Zhu, D. Hu, M. Wan, L. Jiang, Y. Wei, Conducting and superhydrophobic

rambutan-like hollow spheres of polyaniline, Adv. Mater. 2007, 19, 2092