Study of amyloid β-peptide (Aβ12-28-Cys) interactions with Congo red and β-sheet breaker peptides using electrochemical impedance spectroscopy

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Key words: Electrochemical Impedance Spectroscopy, amyloid β-peptide, Congo red, β-sheet breaker.

Supporting information (revised)

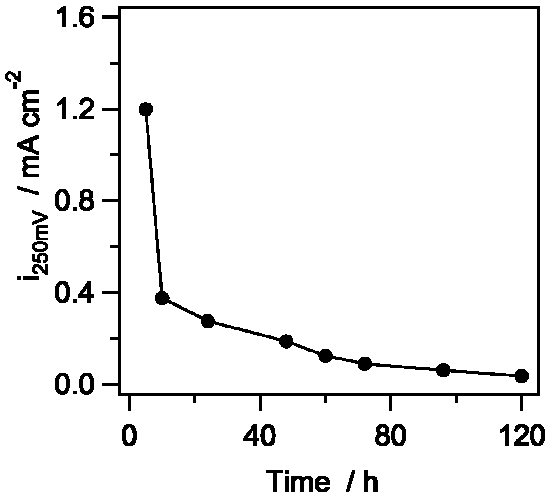


Figure S1. The influence of incubation time on cyclic voltammograms (CV) of a gold electrode incubated with 50 μM Aβ12-28-Cys in a 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) solution prepared in phosphate buffer (50 mM Na_2HPO_4, 50 mM KH_2PO_4, pH 7.4) at scan rate of $0.1Vs^{-1}$. All potentials are given vs. Ag/AgCl reference electrode. The voltage range was −0.1 to 0.6V.

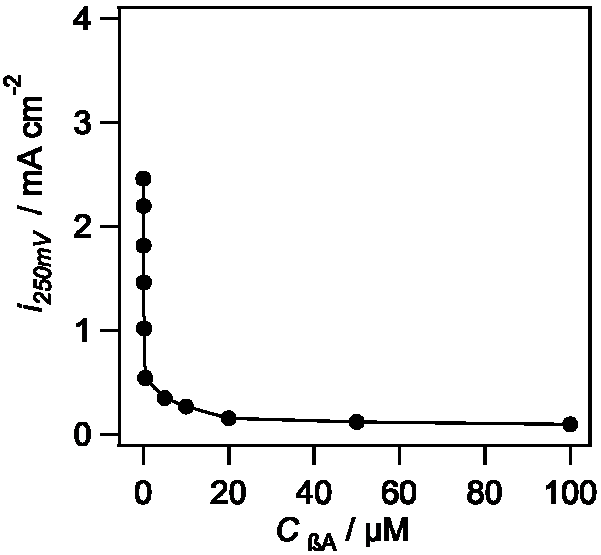


Figure S2. The influence of Aβ12-28-Cys concentration on cyclic voltammogram (CV) responses after 72 h incubation. CV runs in a 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) solution prepared in phosphate buffer (50 mM Na_2HPO_4, 50 mM KH_2PO_4, pH 7.4) at scan rate of $0.1Vs^{-1}$. All potentials are given vs. Ag/AgCl reference electrode. The voltage range was −0.1 to 0.6V.

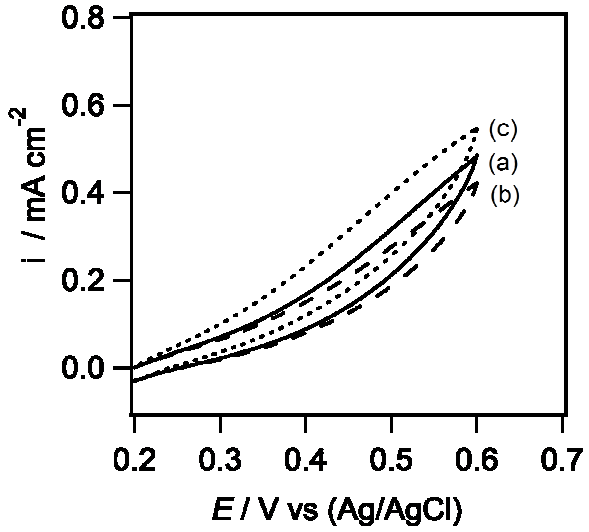


Figure S3. Cyclic voltammograms of Aβ12-28-Cys peptide film alone (a) after 30 min interaction with; (c) 5mM Congo red (CR) and (b) 5mM be β-sheet breaker (BSB). The solution composition was 5.0 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) in phosphate buffer (50 mM Na_2HPO_4, 50 mM KH_2PO_4, pH 7.4) at scan rate of $0.1Vs^{-1}$. All potentials are given vs. Ag/AgCl reference electrode. The voltage range was –0.1 to 0.6V.

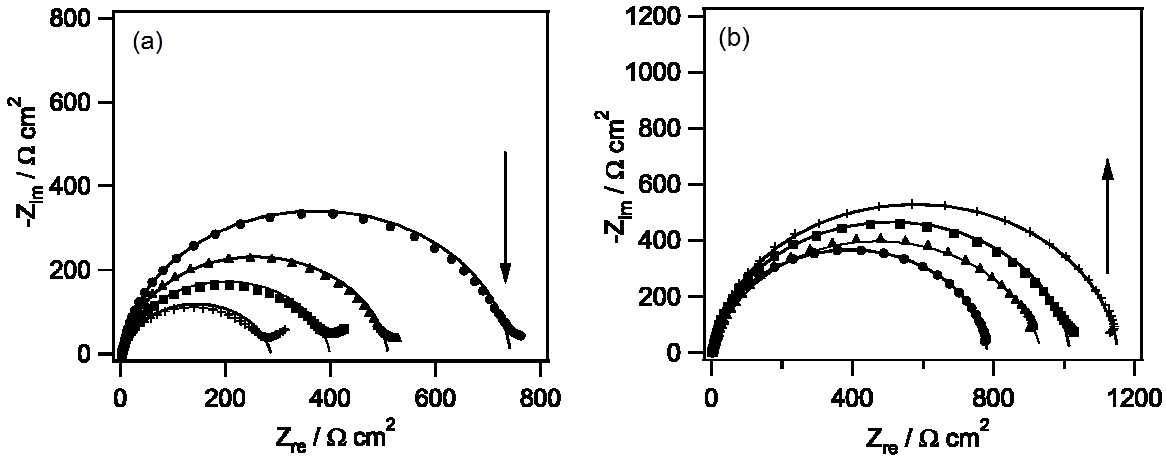


Figure S4. Electrochemical impedance spectra of Aβ12-28-Cys peptide film in 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) in phosphate buffer (50 mM Na_2HPO_4, 50 mM KH_2PO_4, pH 7.4) after 1 h treatment with (a) (●) 1 (▲)100 (■) 5000 (+) 10000 μM of Congo Red (CR). (b) (●) 100 (▲)1000 (■) 5000 (+) 10000 μM of β-sheet breaker (BSB). Measured data are shown as symbols with calculated fit to the equivalent circuit (Figure 5b) as solid lines. Impedance spectra obtained

in phosphate buffer (50 mM Na_2HPO_4, 50 mM KH_2PO_4, pH 7.4), containing 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) as a redox probe, at a formal potential of 250 mV vs Ag/AgCl, frequency range from 100 kHz to 0.1 Hz, and AC amplitude of 10 mV.

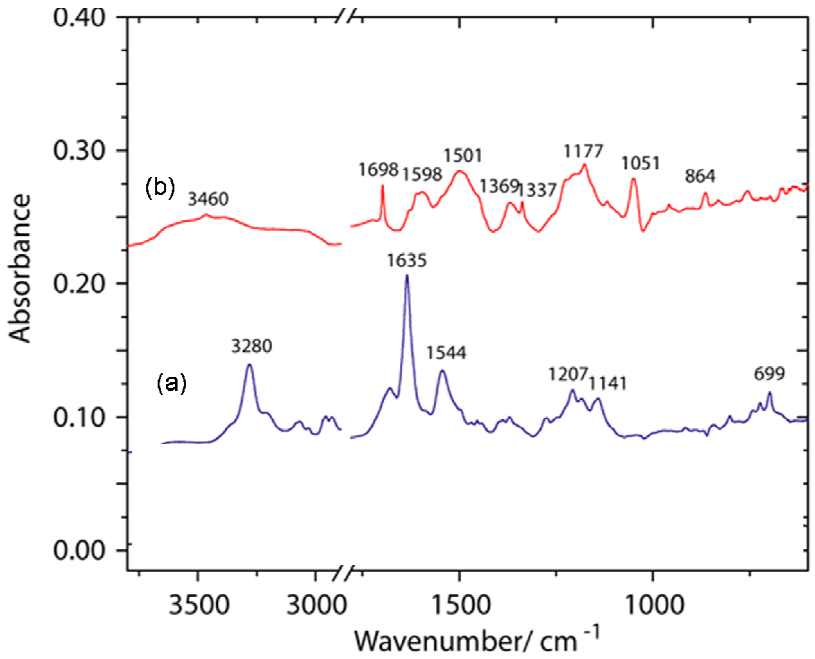


Figure S5. FT-RAIRS of (a) 50 µM BSB and (b) CR, separately for 120 in phosphate buffer (50 mM Na_2HPO_4, 50 mM KH_2PO_4, pH 7.4).

Ellipsometry measurement:

For ellipsometry measurement the gold electrode was prepared, under the same protocol employed for the FT-RAIRS experiment. Ellipsometric thickness measurement was performed using a J. A. Wollom. Co, Inc ellipsometer. The angle of incidence was set 45-70°. The thickness was measured using V.A.S.E software. Data were fitted by regression analysis to a film-substrate model as described by their thickness and their complex refractive indices.

Ellipsometry was used to characterize the thickness of the peptide film. The estimated layer thickness for the monolayer peptide film was 32.7˚A which is in good agreement with theory.

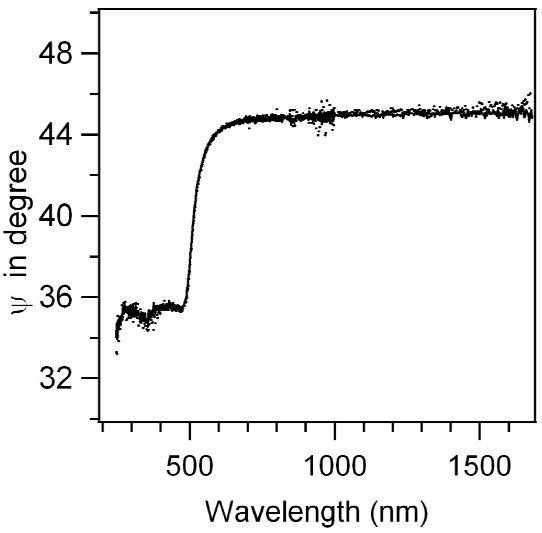


Figure S6. The spectrum for of Aβ12-28-Cys peptide film**,** showing the ellipsometric data (dots) and the fit to the data (line).

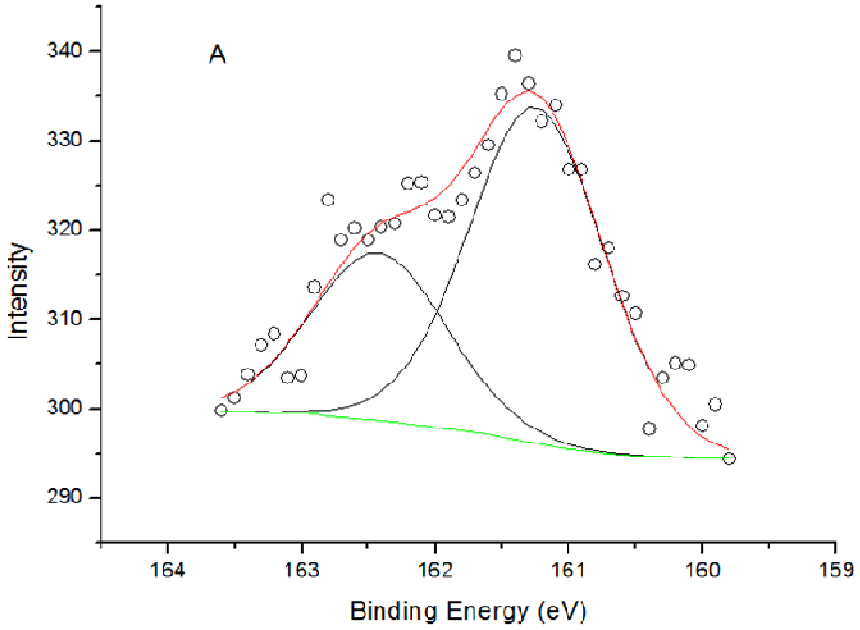
X-ray Photoelectron Spectroscopy Analyses:

The XPS analyses were carried out with a Kratos AXIS Nova spectrometer using a monochromatic Al K(alpha) source (15mA, 14kV). XPS can detect all elements except hydrogen and helium, probes the surface of the sample to a depth of 5-7 nanometres, and has detection limits ranging from 0.1 to 0.5 atomic percent depending on the element. The instrument work function was calibrated to give a binding energy (BE) of 83.96 eV for the Au 4f7/2 line for metallic gold. The Kratos charge neutralizer system was used on all specimens. Survey scan analyses were carried out with an analysis area of 300 x 700 microns and pass energy of 160 eV. High resolution analyses were carried out with an analysis area of 300 x 700 microns and a pass energy of 20 eV. Spectra were analysed using CasaXPS software (version 2.3.14). The peptide film preparation was similar to those for FT-RAIRS.

X-ray photoelectron spectroscopy (XPS) was used to obtain detailed information about the chemical composition of Aβ12-28-Cys peptide film and its interaction with BSB and CR.

The XPS peaks of S2p for A): Aβ12-28-Cys peptide; (B): Aβ12-28-Cys peptide with Congo red (CR); (C): Aβ12-28-Cys peptide with β-sheet breaker (BSB) were fitted and deconvoluted to give the chemical shift data of the components within the coated molecules, respectively. As shown in Figure S7 (A), two dominant peaks located at ~161.5 and ~162.9 eV with an area ratio

of 2:1 and a peak separation of ~1.4 eV were observed in the S_{2p} spectra, which could be assigned to the S atom bound on the gold surface.[7] Moreover, two additional peaks are observed at lower binding energies in Figure S7 (B), that is, at 167.4 and 168.7 eV for the S2$p_{3/2}$ and S2$p_{1/2}$ components, respectively. These peaks can be ascribed to the sulfonic acid groups (-SO_3-) compensated by the Congo red. This confirmed the Aβ12-28-Cys peptide anchors at the gold surface. Figure S7 (C) is similar to Figure S7 (A), because there is no sulfur atom from β-sheet breaker.



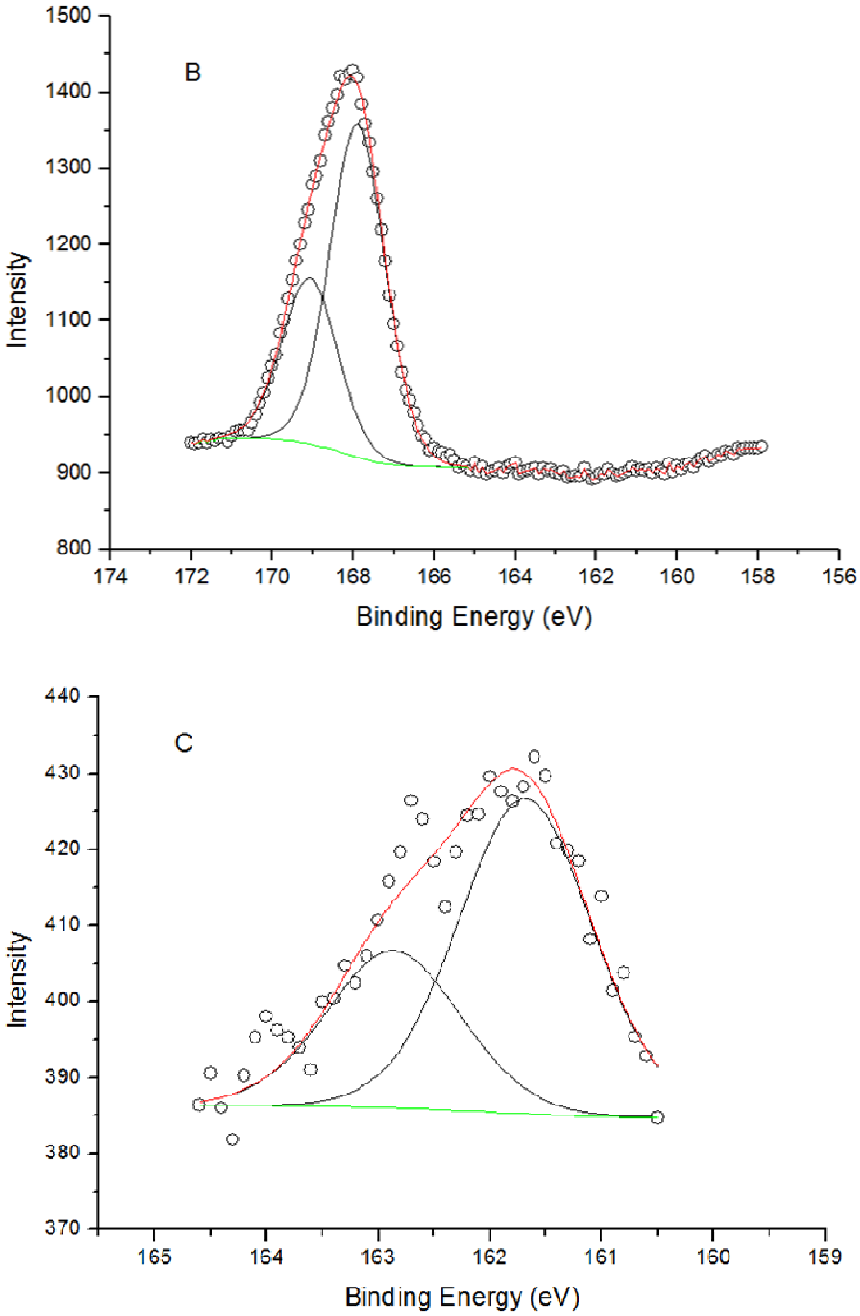
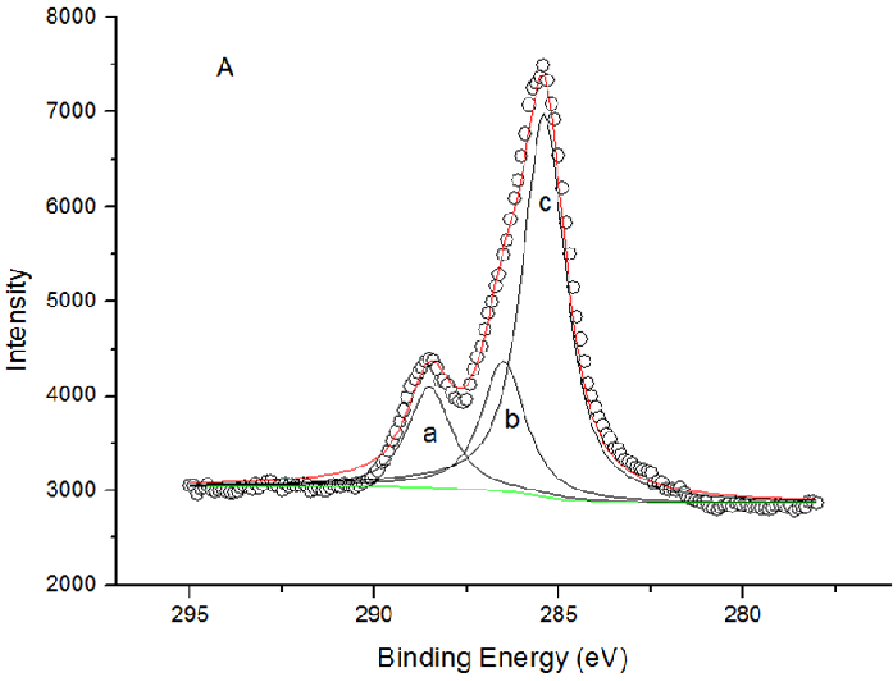


Figure S7. High-resolution XPS spectra of S_{2p} for (A): Aβ12-28-Cys peptide; (B): Aβ12-28-Cys peptide with Congo red (CR); (C): Aβ12-28-Cys peptide with β-sheet breaker (BSB). Open circles stand for experimental raw data, red solid lines are for the total fits, black lines are for the component-fitted peaks, and green lines are for the baselines. The data of C_{1s} are fit with three components: aliphatic C – C carbons (a); C – N carbons of the peptide backbone (b); highly antiscreened peptide N – C=O carbons and carboxyl groups of amino acid (c).

Figure S8 shows the XPS spectra of C_{1s} for (A): Aβ12-28-Cys peptide; (B): Aβ12-28-Cys peptide with Congo red (CR); (C): Aβ12-28-Cys peptide with β-sheet breaker (BSB). We observed two peaks in the C1s spectra at binding energies of ~285.0 and ~288.5 eV. The C1s peak was divided into three peaks: highly antiscreened peptide N-C=O carbons and carboxyl groups of amino acid (a); C-N carbons of the peptide backbone (b); aliphatic C-C carbons (c). Following the condensation reaction between β-Amyloid and CR, the XPS spectra (Figure S8(B)) of the lower binding energy at 284.5eV for aliphatic C-C carbons increased markedly after CR immobilization. Meanwhile, for Aβ12-28-Cys peptide with β-sheet breaker system, we found the area of the higher binding energy at 288.0 eV for carboxyl groups become larger than Aβ12-28-Cys peptide after BSB immobilization.



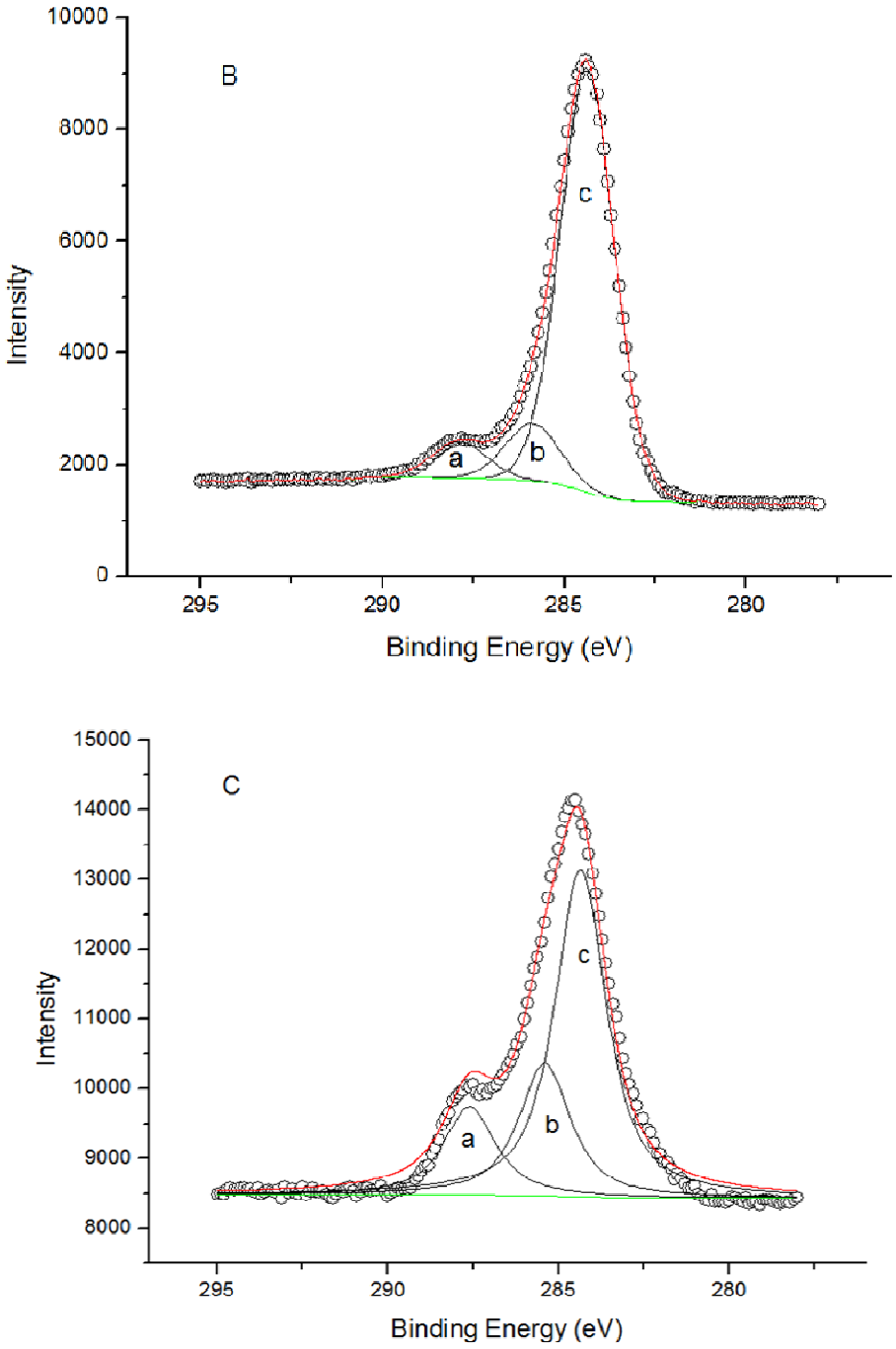
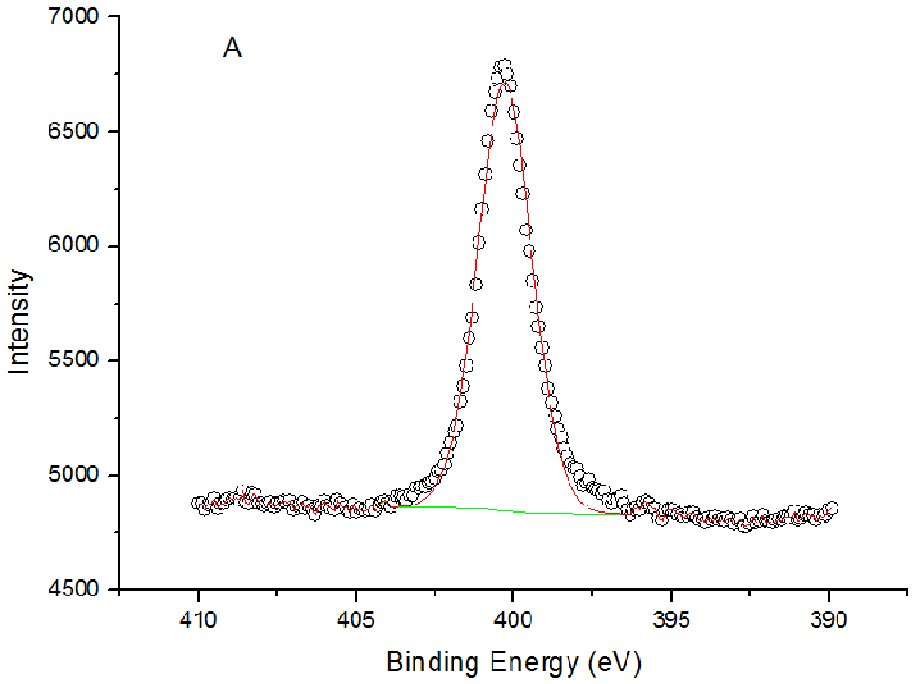


Figure S8. High-resolution XPS spectra of C_{1s} for (A): Aβ12-28-Cys peptide; (B): Aβ12-28-Cys peptide with Congo red (CR); (C):Aβ12-28-Cys peptide with β-sheet breaker (BSB). Open circles stand for experimental raw data, red solid lines are for the total fits, black lines are for the component-fitted peaks, and green lines are for the baselines. The data of C_{1s} are fit with three

components: highly antiscreened peptide N-C=O carbons and carboxyl groups of amino acid (a); C-N carbons of the peptide backbone (b); aliphatic C-C carbons (c).

The XPS spectra for N_{1s} are not good to explain the change after CR/BSB modification because there are N element in three compounds and no characteristic N_{1s}. The similar data happened to the XPS spectra for $O_{1s.}$



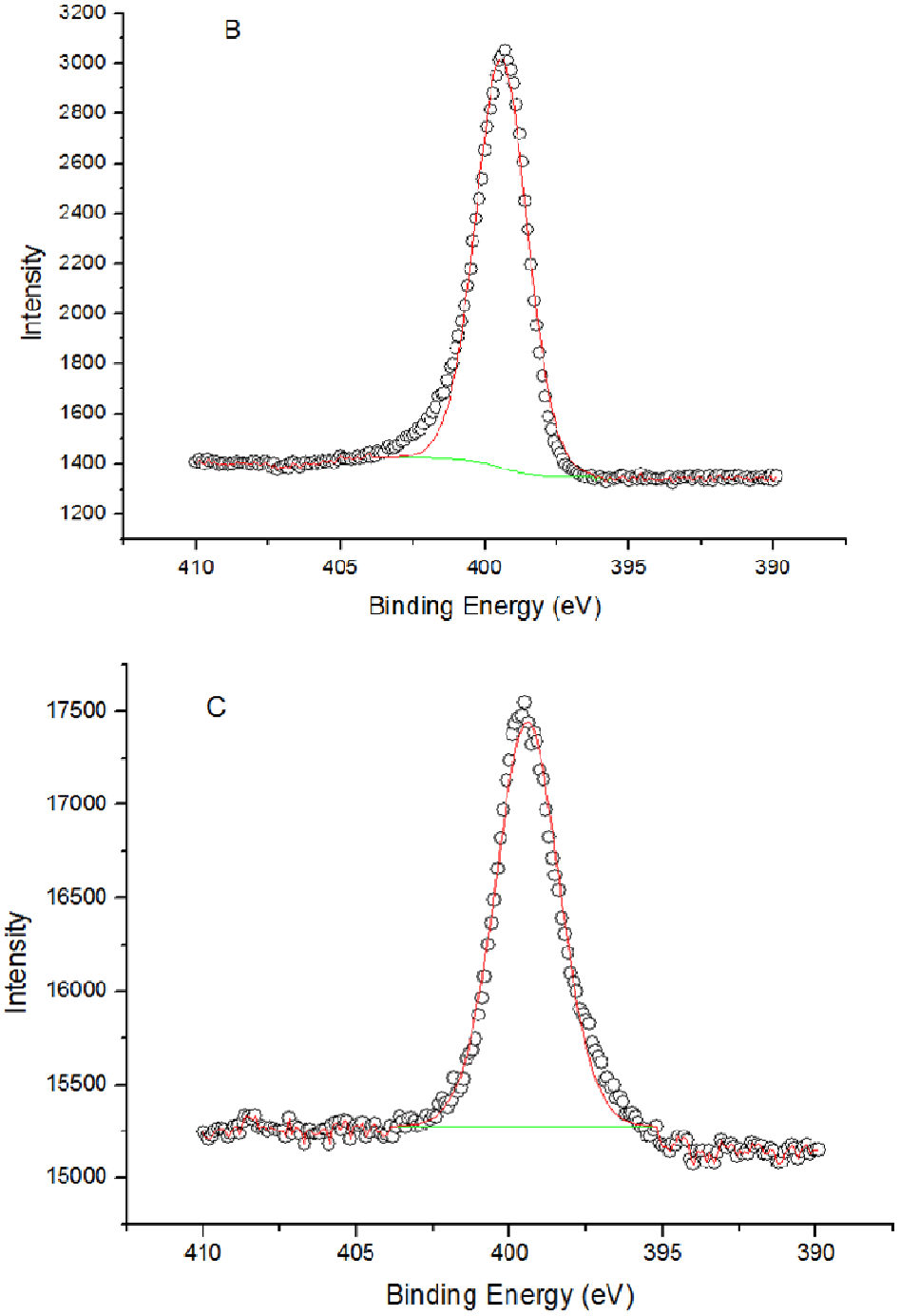


Figure S9. High-resolution XPS spectra of N_{1s} for (A): Aβ12-28-Cys; (B): Aβ12-28-Cys peptide with Congo red (CR); (C): Aβ12-28-Cys peptide with β-sheet breaker (BSB). Open circles stand for experimental raw data, red solid lines are for the total fits, and green lines are for the baselines.

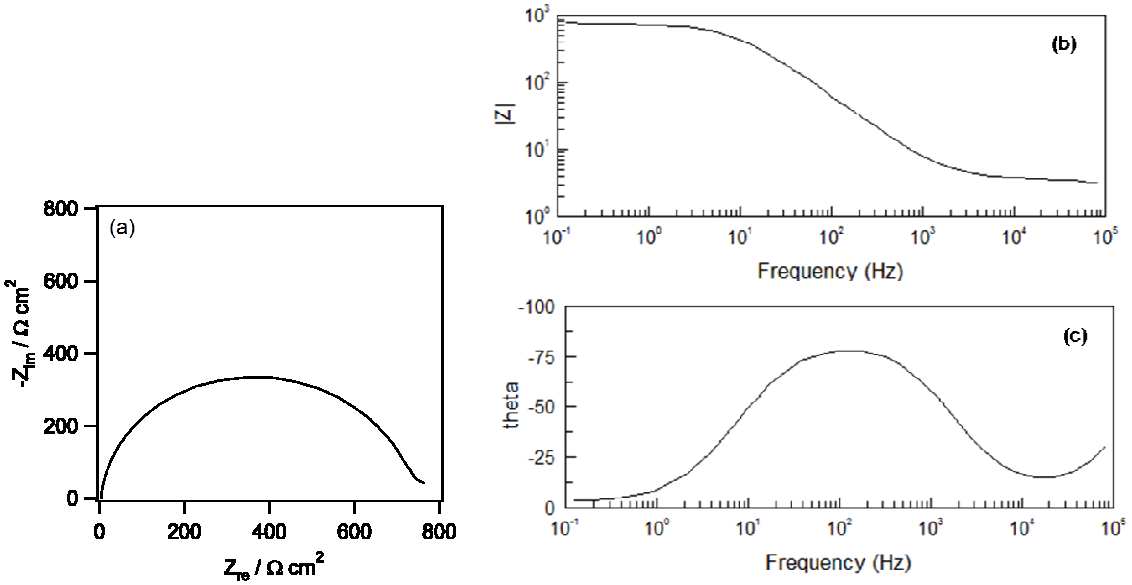


Figure S10. (a) Nyquist and (b)-(c)Both Bode plots of Aβ12-28-Cys peptide film in 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) in phosphate buffer (50 mM Na_2HPO_4, 50 mM KH_2PO_4, pH 7.4). Impedance spectra obtained in phosphate buffer (50 mM Na_2HPO_4, 50 mM KH_2PO_4, pH 7.4), containing 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) as a redox probe, at a formal potential of 250 mV vs Ag/AgCl, frequency range from 100 kHz to 0.1 Hz, and AC amplitude of 10 mV.

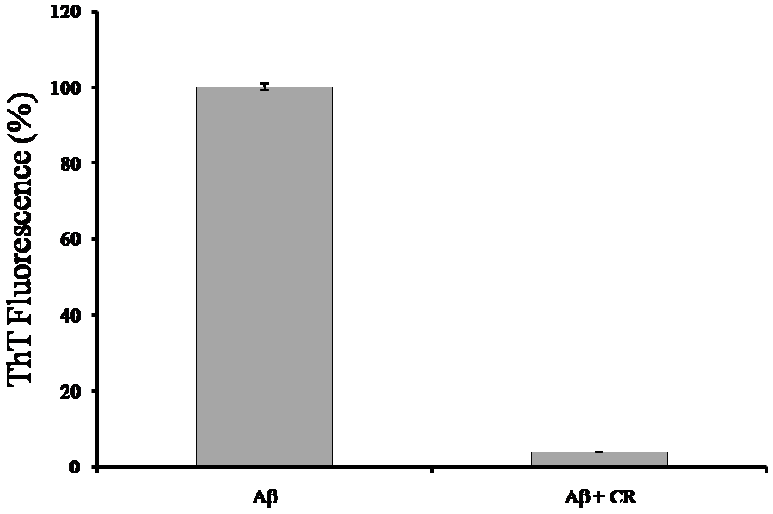


Figure S11. ThT fluorescence of preformed fibrils (4 days) (Aβ, 50 μM) and Aβ mixed with CR (1:1 molar ratio) immediately after addition of CR. Values represent means± standard deviation (n = 3).

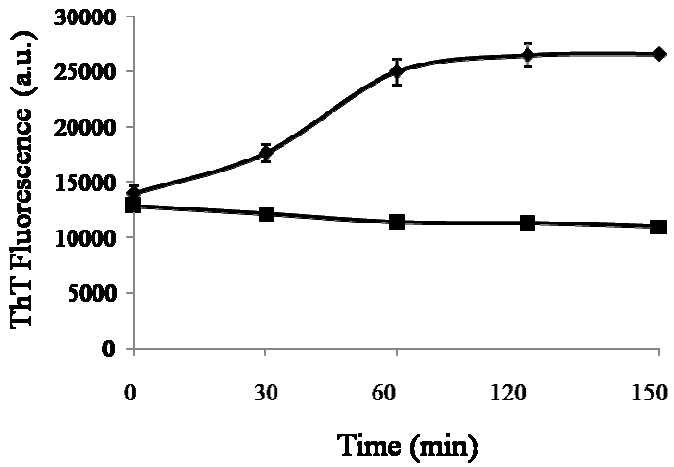


Figure S12. ThT fluorescence of preformed fibrils (4 days) (Aβ, 50 μM) (square line) and Aβ mixed with BSB (1:1 molar ratio) (circle line). Values represent means± standard deviation (n = 3).