Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

Active Site-Targeted Carbon Dots for the Inhibition of Human Insulin Fibrillation

Table of contents in supporting information

SI text

Synthesis of Carbon dots. Carbon dots were synthesized according to the method reported by Li groups[1] with modification. Typically, 5 g sucrose and 10 mLoleic acid were heated in a three neck flask at a temperature of 215 ˚C for 5 min under vigorously stirring. After cooling, the supernatant liquid was discarded and thesolidproduct was obtained at the bottom of flask. The precipitates were dispersed with water and then extracted with ethersix times to remove the remained oleic acid. Finally, the products were freeze-dried and obtained after dialyzed in deionized water using a 1 kDa membrane for 1 day.

Polymerization Assay. HI was dissolved in 1, 1, 1, 3, 3, 3-hexafluoroisopropanol (HFIP, Sigma- Aldrich Co.) at 25 °C, shaken at 120 rpm in an Eppendorf tube for 12 h, and then the stock solution was lyophilized to remove HFIP and stored at -20 °C prior to use. The pre-treated protein was dissolved in 6 mL 100 mM HCl-NaCl buffer (pH 2) to get a final concentration of 1 mg/mL. All reactions were incubated at 60 °C with four or more replicates. The progress of the reaction was monitored by Thioflavin T (ThT) fluorescence at room temperature: 20 μL aliquots of sample were removed at the specified times and mixed with 2 mL 50 μM ThT in PBS (pH 7.0). Emission fluorescence at 484 nm was monitored using excitation wavelength at 450 nm.

CD Spectroscopy. Far UV CD spectra of insulin before and after incubation in the absence or presence of C-Dots were measured with an Applied Photophysics (UK) spectrometer in a cell with a light path of 1 mm. Sample solutions were diluted to 0.1 mg/mL insulin in 10 mM HCl (pH 2.0) and 100 mM NaCl. CD signals between 195 and 260 nm were expressed as the value of mdeg.

As presented in Figure S2A, native HI protein shows an α-helix rich CD spectra with two negative bands centered around 208 and 222 nm. As incubation time increasing, the two negative bands shrink

to a single peak and shift to about 219 nm at 3 h, corresponding to the conformation of *β*-sheet in the partially unfolded state of HI. And the location of the single minimum band keeps moving to about 220 nm after mature fibrils formed at 6 h, which is attributed to be *β*-sheet structures in fibrils.[2,3] The influence of C-Dots with different concentrations on secondary structure of native HI monomers at the beginning was also detected by CD. Seen in Figure S3, C-Dots endow little influence on HI monomers. After incubating with C-Dots (10:3) at 60 °C, the conformation of HI has nearly no extinctive change at 3 h (Figure 3B). Interestingly, as shown in Figure 3C and D, when the mass concentration of HI/C-Dots is 1:1 or 1:2, they show the negative bands at about 218 nm which is a big span but relatively weak at its final state. FTIR was also used to ensure the structures among HI fibrillation process, which mainly gives the amide I band of the *α*-helix and *β*-sheet structures of HI monomers, oligomers and fibrils, mainly ranging from 1660 to 1620 cm^{-1}. Seen in Figure S4, native HI has a sharp IR band located at 1658 cm^{-1} before heating, after incubating at 60 °C for different time, we can observe a shift of the IR band at 1618 cm^{-1} toward low frequency, which represents the large intermolecular *β*-sheet in intermeadiates. After long incubation for 24 h, the IR spectra yield a new band at 1628 cm^{-1}, which corresponds to the intermolecular *β*-sheet in mature fibrils.

Reference

(1) Chen, B.; Li, F.; Li, S.; Weng, W.; Guo, H.; Guo, T.; Zhang, X.; Chen, Y.; Huang, T.; Hong, X., Large scale synthesis of photoluminescent carbon nanodots and their application for bioimaging. *Nanoscale* 2013**, 5**, (5), 1967-1971.

(2) Hoshino, M.; Katou, H.; Hagihara, Y.; Hasegawa, K.; Naiki, H.; Goto, Y., Mapping the core of the β2-microglobulin amyloid fibril by H/D exchange. *Nature Structural & Molecular Biology* 2002, **9**, (5), 332-336.

(3) Katou, H.; Hoshino, M.; Kamikubo, H.; Batt, C. A.; Goto, Y., Native-like β-hairpin retained in the cold-denatured state of bovine β-lactoglobulin. *Journal of Molecular Biology* 2001, **310**, (2), 471-484.

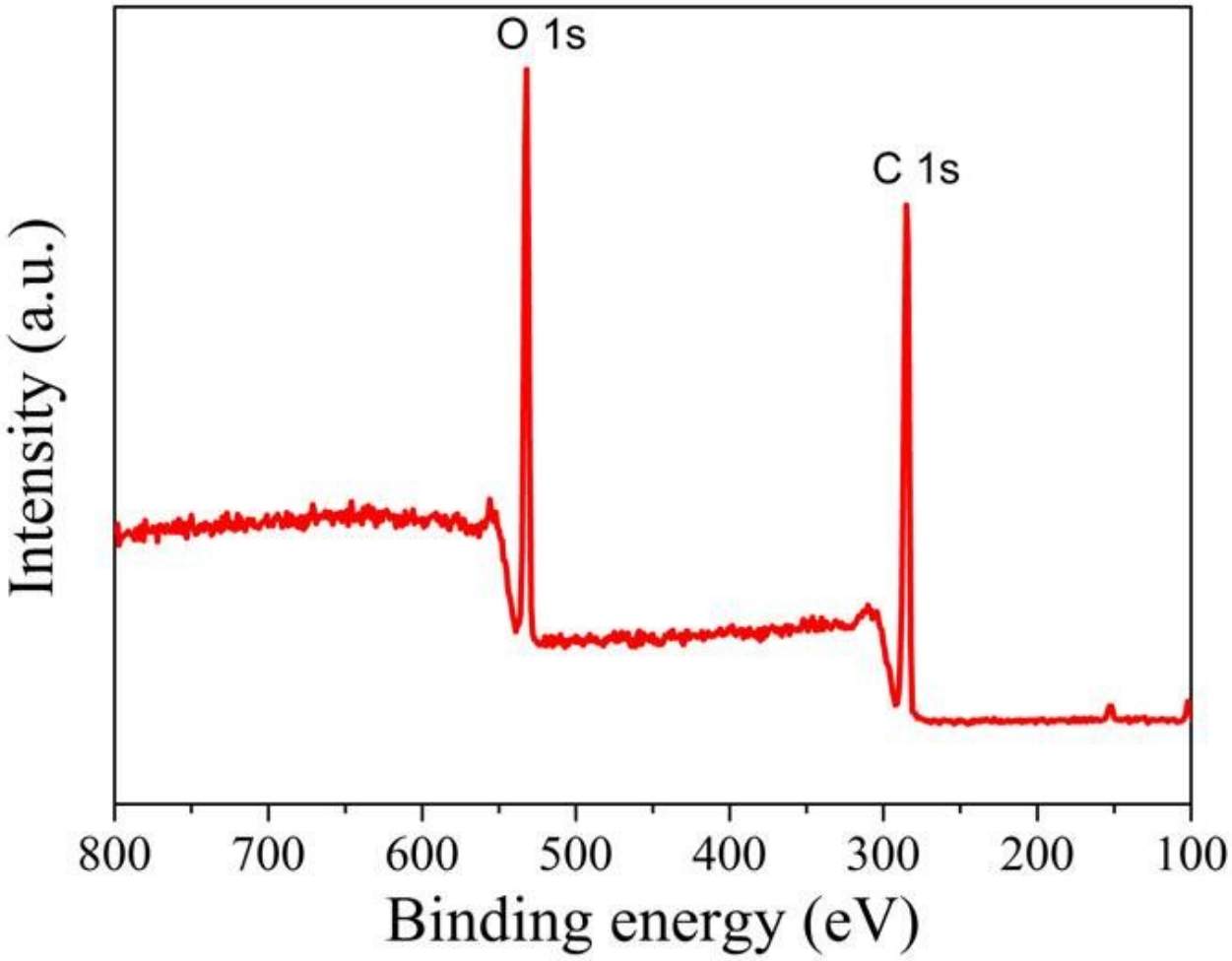

Figure S1. XPS spectra result of C-Dots.

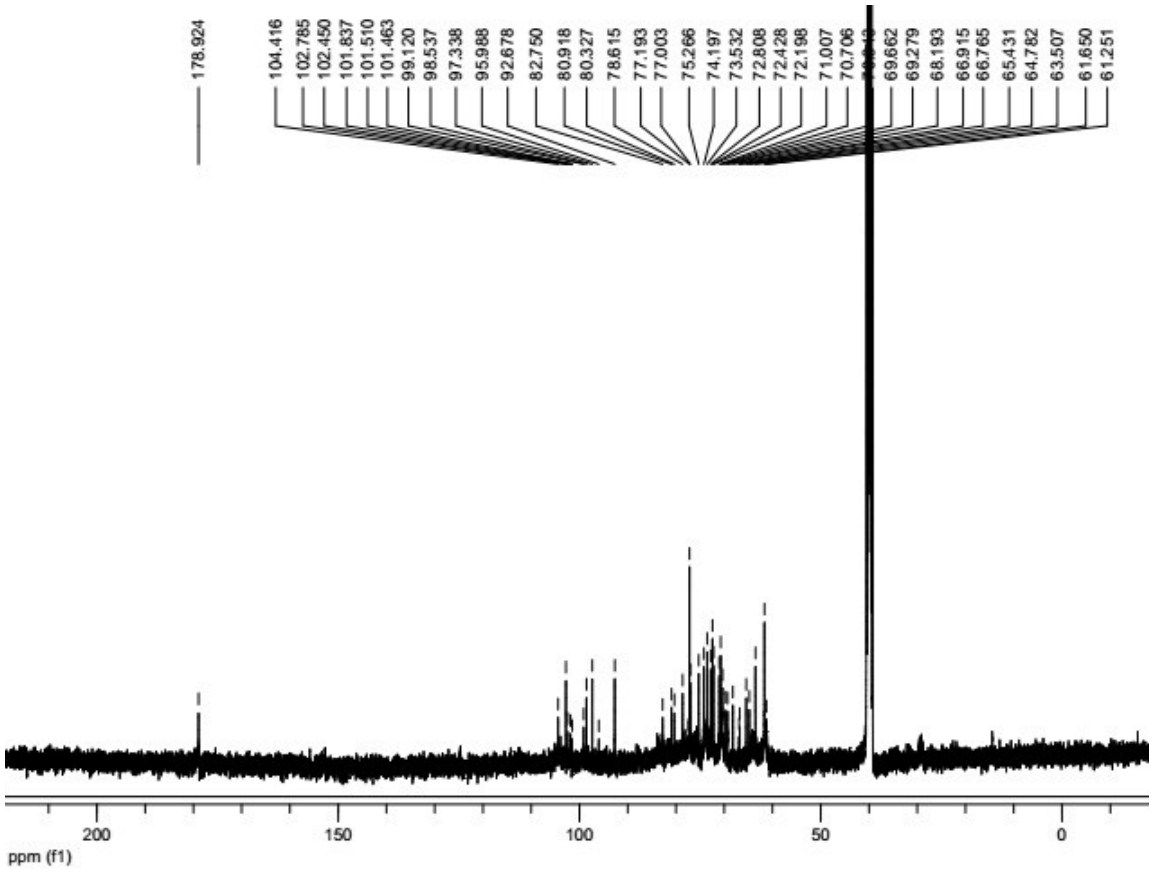

Figure S2. ^{13}C NMR spectra of the as-prepared C-Dots.

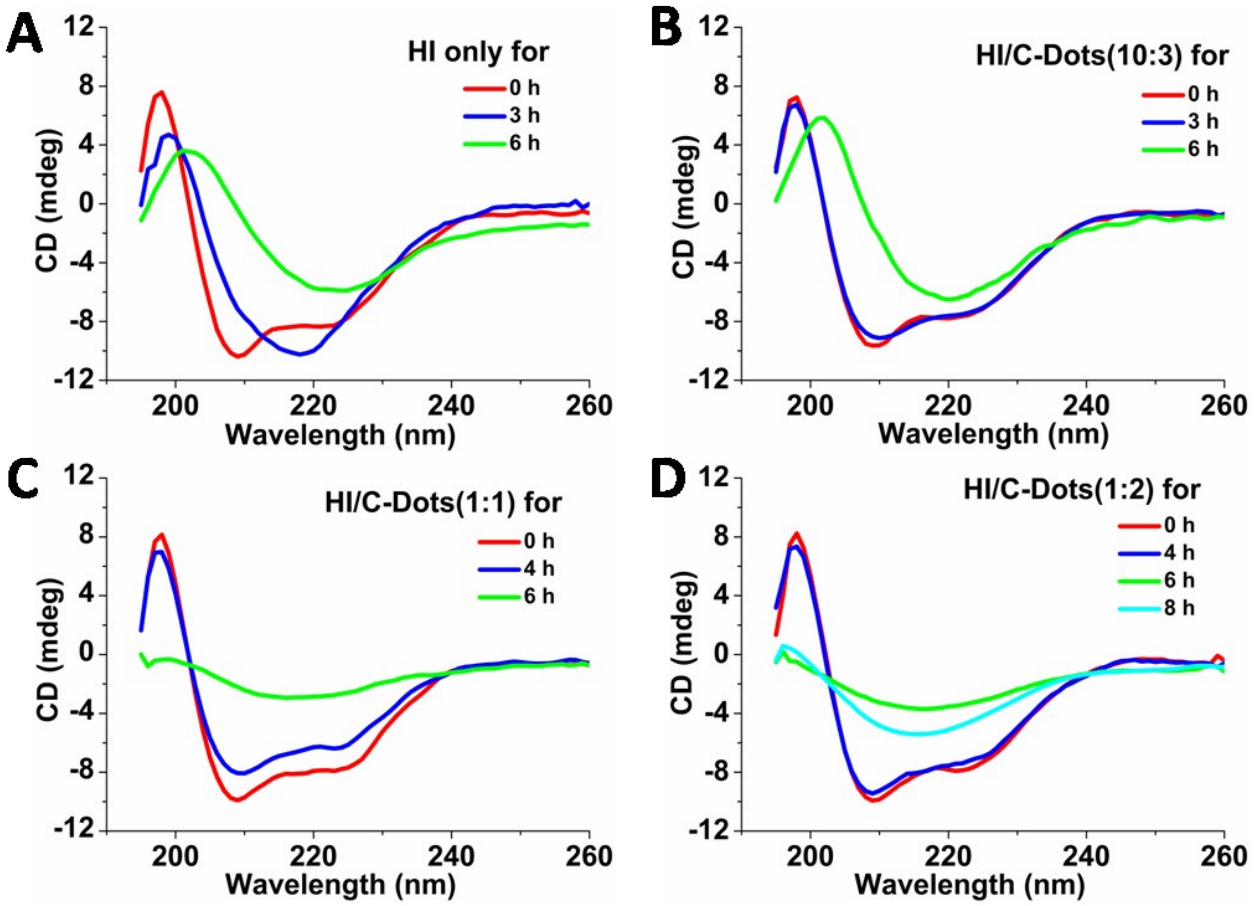

Figure S3. CD spectra of HI at different incubation time periods. The original samples contain1 mg/mL of HI (A); and inthe presence of C-Dots with mass concentration ratio of 10:3 (B), 1:1 (C), 1:2 (D).

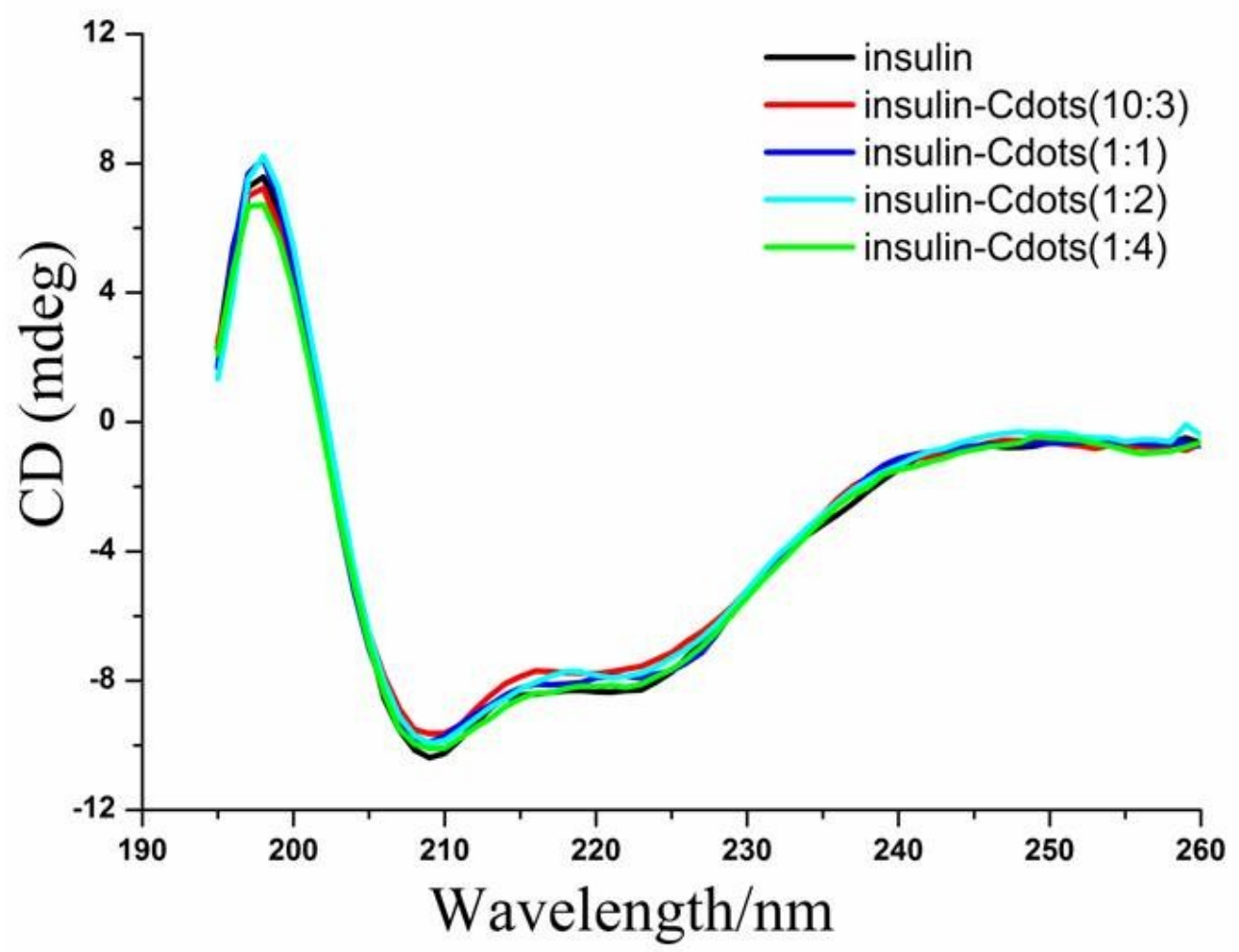

Figure S4. CD spectra results of HI or HI/C-Dots at the time of 0 h.

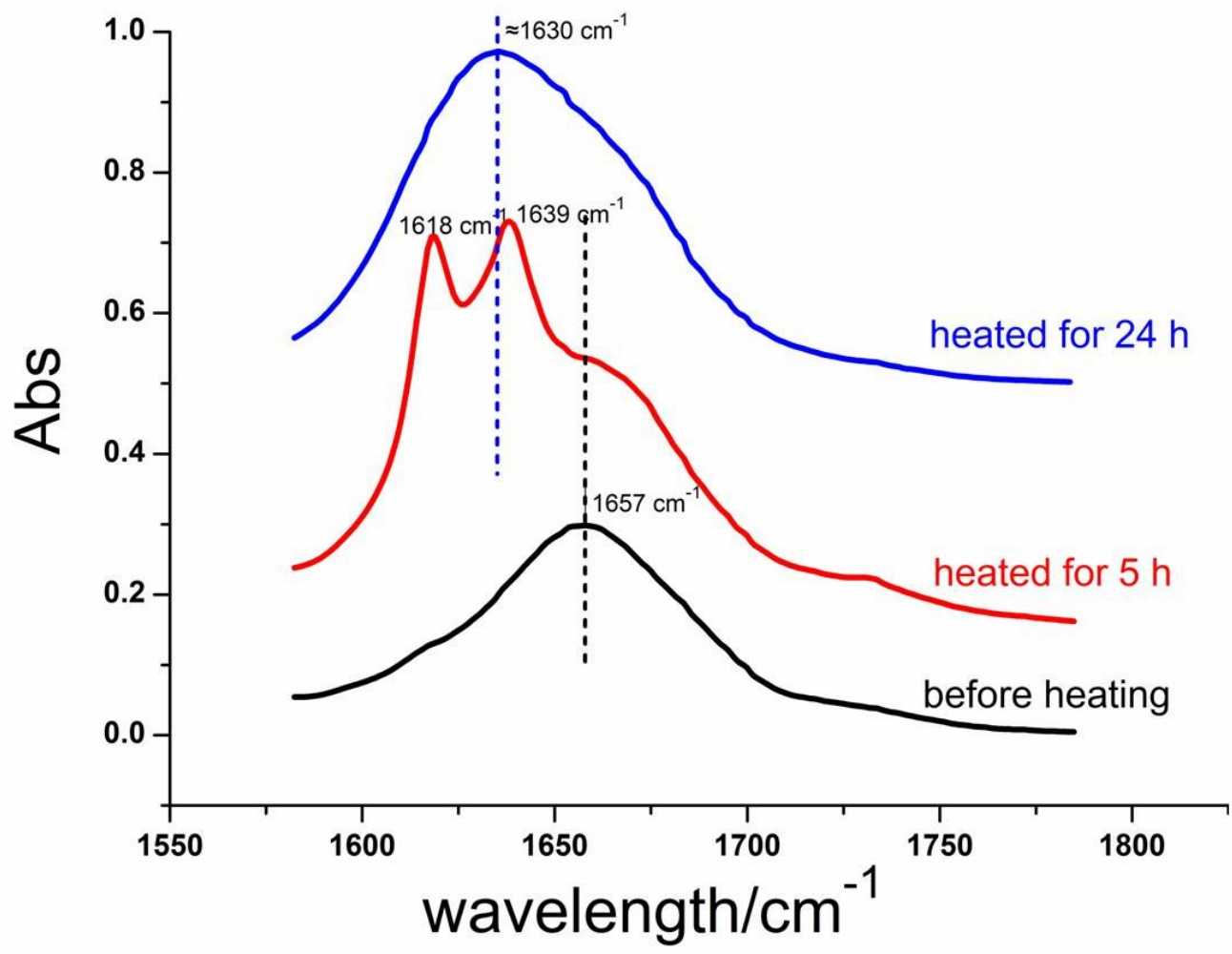

Figure S5. FTIR spectra for the secondary structure of HI before and after water bath at 60 °C. These three colored lines show the process of α-helix of HI transform to β-sheet over the incubation time.

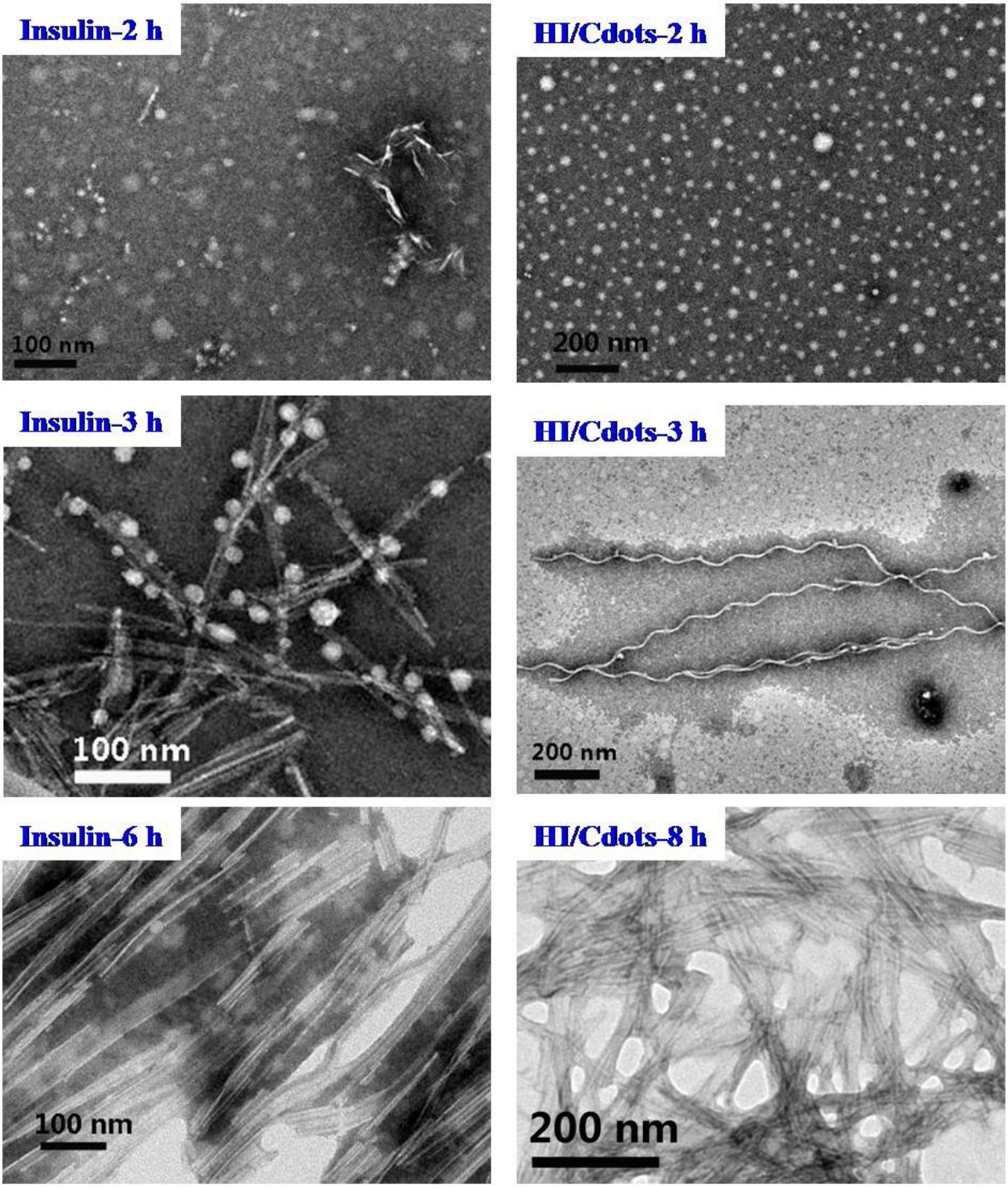

Figure S6. TEM images of HI fibrillation process with or without C-Dots. The left and right are HI panel are HI and HI/C-Dots (10:3) solutions after different incubation times relatively.

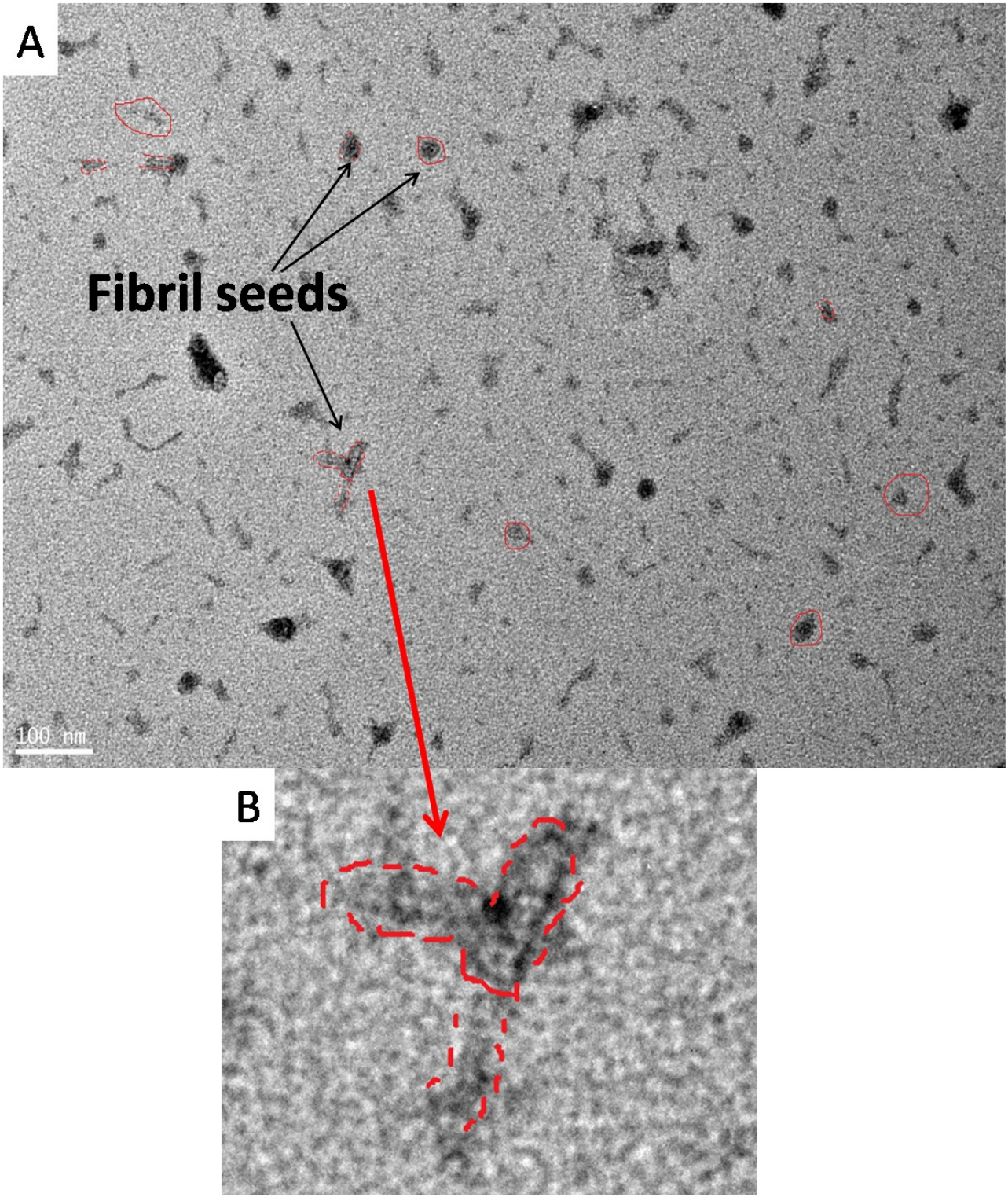

Figure S7. TEM images of fibril seeds. A) TEM image of the mixture solution of monomer HI and C-Dots incubated in HCl-NaCl buffer at 60 °C for 1 h with seed fibrils. B) High resolution TEM images of fibril seeds.

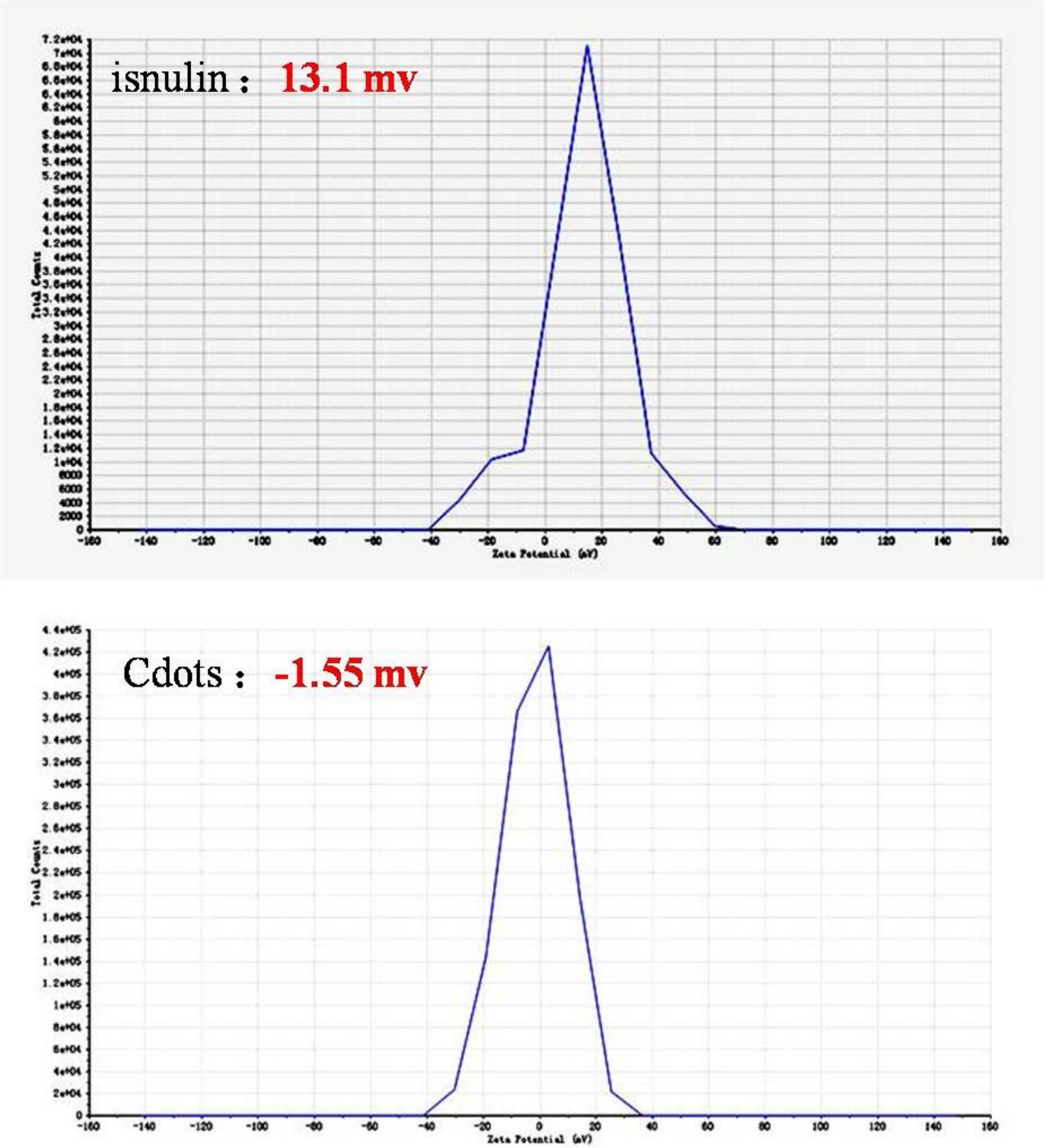

Figure S8. Zeta potential values for insulin monomers and Carbon dots in pH=2.0 HCl-NaCl solutions relatively.