Electronic Supplementary Information

Synthesis of Thermo-responsive Polymer-Protein Conjugates through Disulfide Bonding

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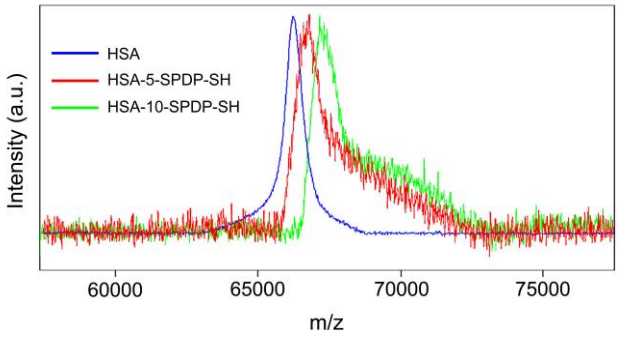

Figure S1. MALDI-TOF/MS spectra for HSA, HSA-5-SPDP-SH, and HSA-10-SPDP-SH; HSA-5-SPDP-SH and HSA-10-SPDP-SH were comprised of HSA coupled to 5 and 10 SPDPs (feed ratio) and reduced by TCEP. Analyses of HSA prior to and after functionalization with SPDP were performed on an Ultraflex III MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a nitrogen laser operating at 337 nm with a 3 ns pulse width, a repetition rate of 100 Hz, and operated in linear positive mode with flexControl software (version 3.3.108.0, Bruker). Protein solutions (10 mg mL^{-1}) were dissolved in 0.1% (v/v) trifluoroacetic acid and mixed in a 10:1 ratio of a matrix solution of 2,5-dihydroxybenzoic acid dissolved in the same solvent at a concentration of 20 mg mL^{-1}. The mixture (1 μL) was then spotted onto a stainless steel target and allowed to air-dry. The equipment was calibrated with protein standard II (Bruker) prepared in the same matrix as recommended by the manufacturer. Each spectrum represents accumulation of 6,500 shots. Spectral analysis was performed using flex Analysis 2.4 software.

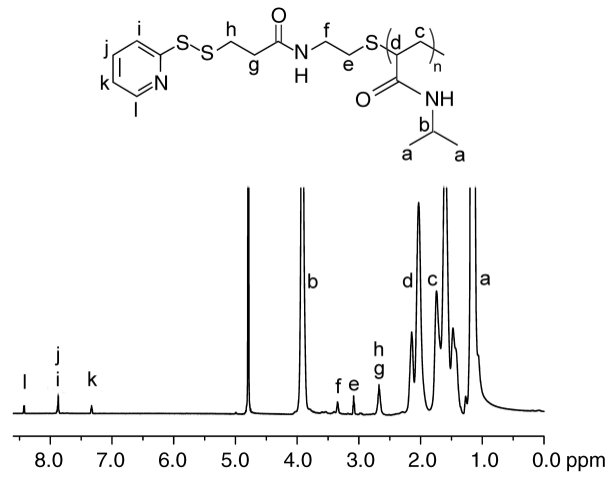

Figure S2. ^{1}H NMR spectrum of SPDP capped PNIPAM in D_2O. The PNIPAM-NH_2/SPDP feed ratio was 1:2; dialysis was performed against Milli-Q water using a membrane with a molecular weight cut-off of 14 kDa, at room temperature for 48 h with six changes. The dialysate was centrifuged to remove any precipitate, and then freeze-dried.

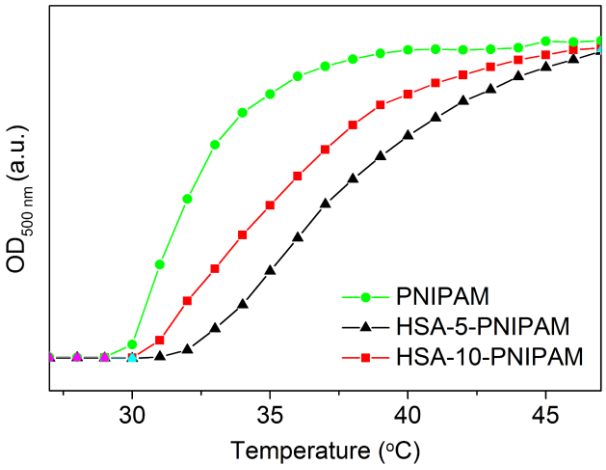

Figure S3. Relative absorbance at 500 nm versus temperature. The HSA-PNIPAM conjugates and PNIPAM-NH_2 were dissolved in Milli-Q water (0.2 mg mL^{-1}), then transferred to a quartz cuvette and warmed from 24 ℃ to 48 ℃ over 5 min until the test temperature was attained, and absorbance at 500 nm was measured on a multi-mode microplate reader (SpectraMax M5 Multi-Mode Microplate Reader, Molecular Devices).

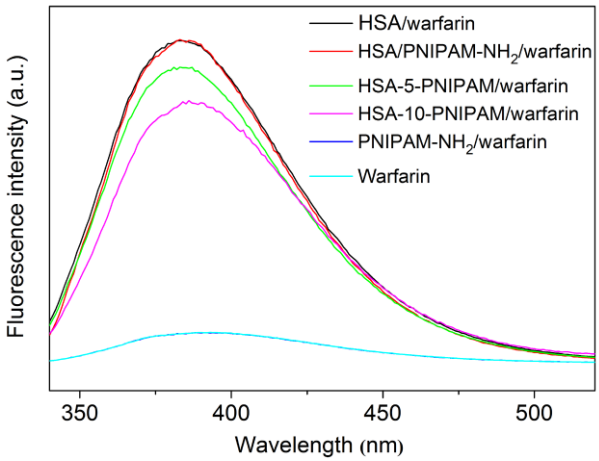

Figure S4. Fluorescence spectra were evaluated at 25 ℃ on a fluorescence spectrophotometer (Cary Eclipse) with excitation at 330 nm. HSA/PNIPAM-NH_2 indicates a mixture of HSA and PNIPAM-NH_2 (w/w = 1). The procedure was similar to the binding affinity experiment, with the following exceptions. HSA or conjugate solution (1 mL, HSA concentration: 0.105 mM) or PNIPAM-NH_2 solution (1 mL, concentration: 1.27 mM) and a solution of warfarin (1.5 ml, 0.091 mM) in PBS (0.01 M, pH 7.4) were immediately mixed and vortexed for 60 s, incubated for 15 min, and then tested; the concentration of warfarin or HSA was equal in each sample.

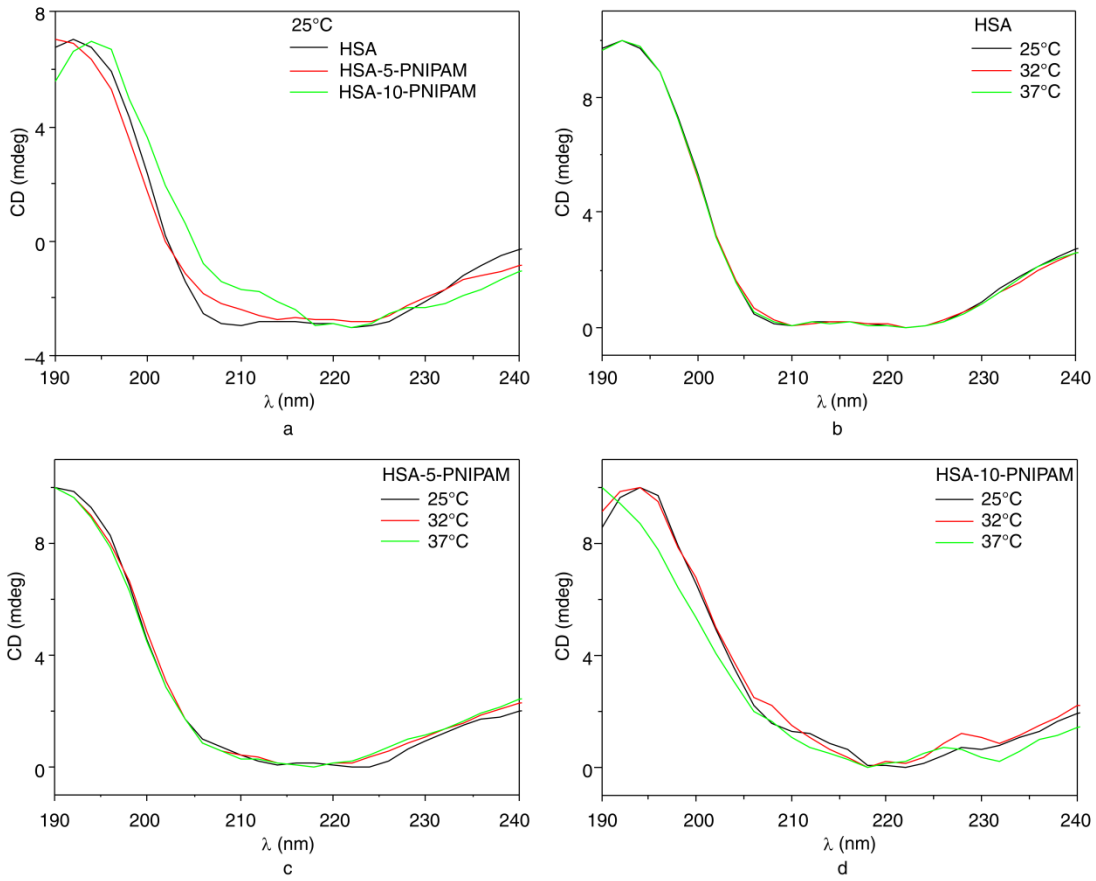

Figure S5. Circular dichroism (CD) was measured to ascertain the influence of PNIPAM conjugates on the secondary structure of HSA. CD spectra were obtained at 25 ℃, 32 ℃, and 37 ℃ with a Jasco-815 spectropolarimeter (Jasco, Tokyo, Japan) in sodium phosphate buffer (0.01 M, pH 7.4) and a cell with a 0.1-cm path length (bandwidth = 1 nm; step resolution = 0.1 nm; scan speed = 50 nm/min).

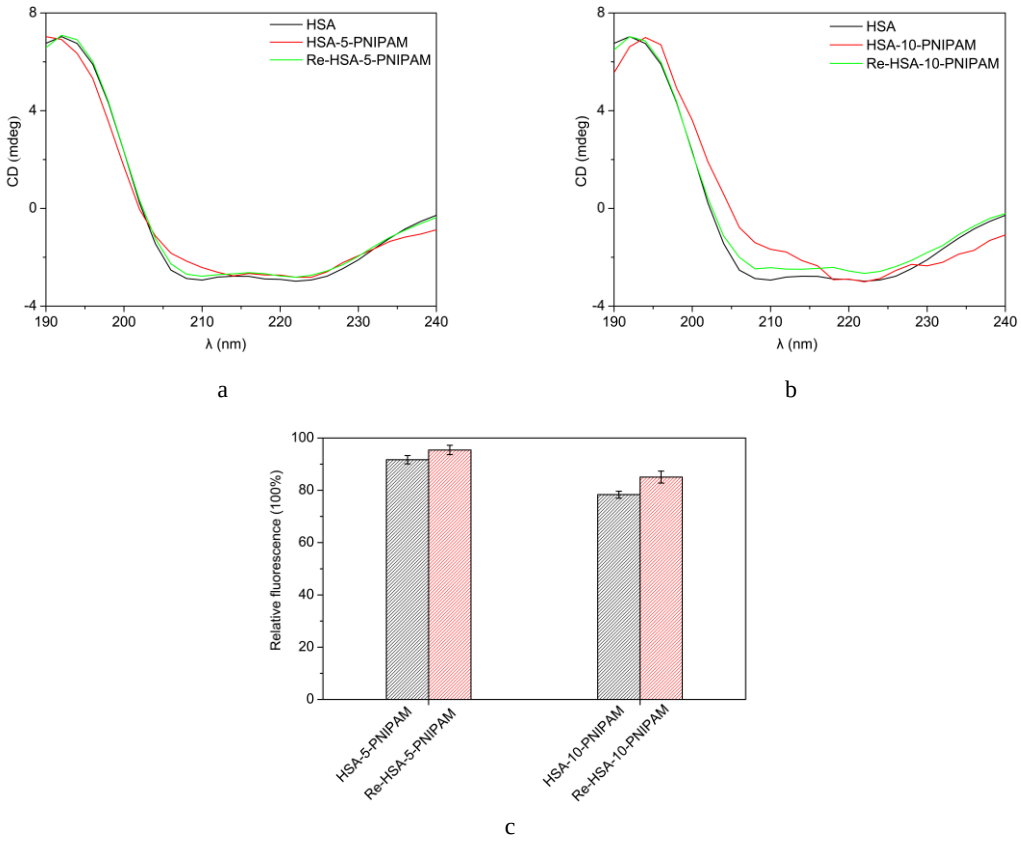

Figure S6. Comparison of the CD diagrams (a, b) and warfarin binding affinity (c) between HSA conjugate and recovered HSA. HSA was recovered by incubating the HSA-PNIPAM conjugates with TCEP at the room temperature for 2 hour. The warfarin binding affinity of HSA was defined as 100%.

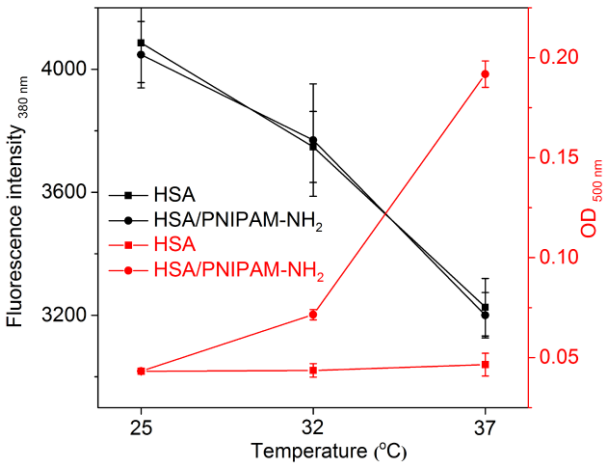

Figure S7. The fluorescence intensity after warfarin binding and absorbance of HSA and HSA/PNIPAM-NH_2 (w/w = 1:1) at 25 ℃, 32 ℃, and 37 ℃ in a procedure similar to the binding affinity experiment, in which the amount of warfarin or HSA is equal in each sample. There was no significant difference in fluorescence intensity between HSA and HSA/PNIPAM-NH_2 after warfarin binding as the temperature increased from below to above the LCST, though absorbance significantly differed under the same experimental conditions. Student's t-test, significance at $p < 0.0001$; non-significance at $p > 0.05$.