Supporting information

Protein-Cross-Linked Hydrogels with Tailored Swelling and Bioactivity Performance: A Comparative Study

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Biocompatibility of the Protein-Based Hydrogels. In vitro cytotoxicity of thiolated proteins, 4-arm PEG-acrylate and the extracts of the protein-cross-linked hydrogels were evaluated by MTT assay against L929 mouse fibroblasts. Thiolated Hb was prepared at a 2-IT/Hb molar feeding ratio of 50, and the Hb hydrogels were prepared in a 24-well culture plate and extracted by Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), supplemented with 50 IU/mL streptomycin and 50 IU/mL penicillin at 37 ^{o}C for 72 h. L929 cells were seeded in a 96-well plate at a density of 1×10^{4} per well and incubated at 37 ^{o}C and in 5% CO_2 atmosphere for 24 h. The cells were then incubated in fresh media containing thiolated Hb or 4-arm PEG-acrylate at different concentrations, or the extraction media of Hb hydrogels (100% extract) for another 24 h. All the sample solutions were freshly prepared and sterilized by filtration before introduction into the cell medium. Subsequently, the cell medium was exchanged with fresh medium, and 20 μL of MTT (5 mg mL^{-1}) was added to each well and incubated at 37 ^{o}C for 4 h. The precipitated formazan was dissolved in DMSO (150 μL per well) and the absorbance value at 490 nm was measured on a Bio-Rad 808 microplate reader. Cell viability (%) was calculated from the followed equation: cell viability (%) = $(A_{sample}/A_{control}) \times 100\%$, where A_{sample} and $A_{control}$ are the absorbance of the sample and control wells, respectively. The measurements were performed in triplicate.

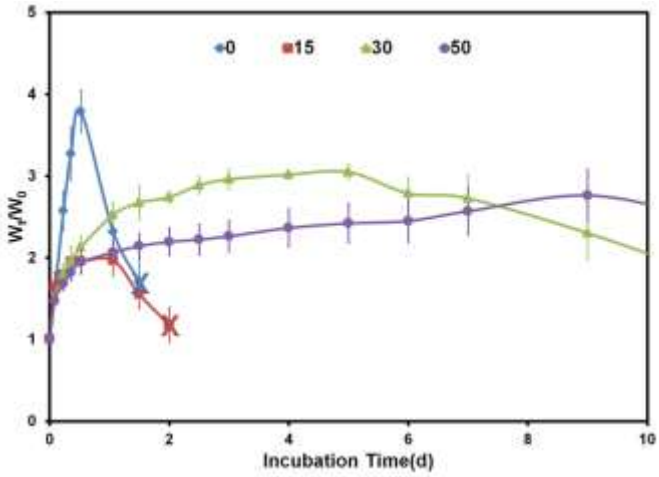

Figure S1. The gel swelling stability of 4-arm PEG-acrylate cross-linked with thiolated Hb at different 2-IT/Hb molar feeding ratio. The cross symbols indicate the gels disintegrated during the experimental period.

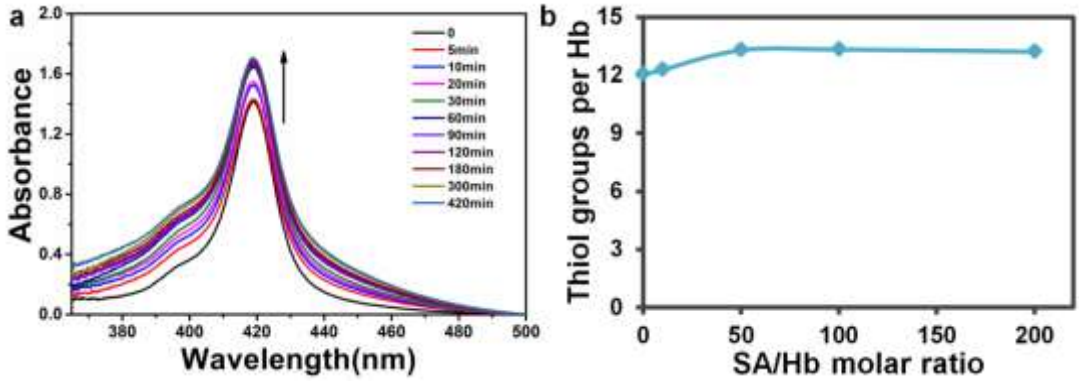

Figure S2. (a) Kinetic UV-vis spectra change of thiolation reaction of Hb assayed by Ellman's reagent. (b) The plot of thiol production versus sodium ascorbate (SA)/Hb molar feeding ratio ([2-IT]/ [Hb] = 50).

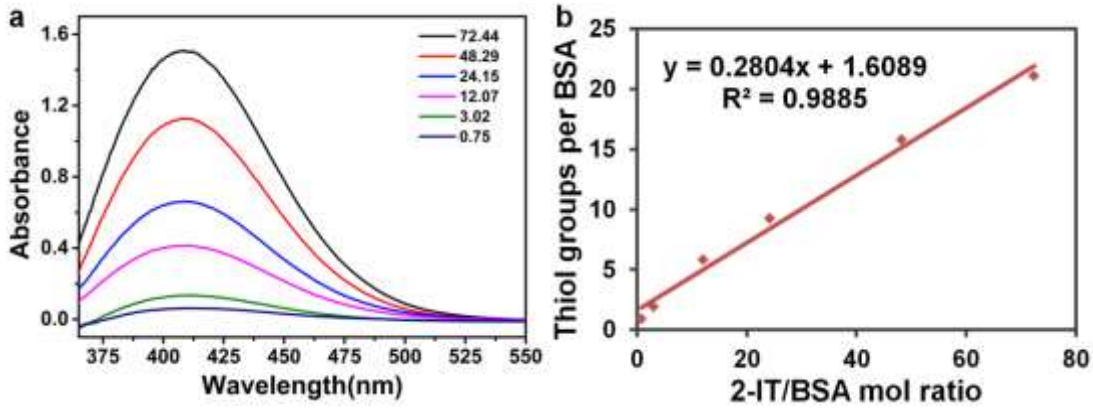

Figure S3. (a) UV spectra of Ellman's assay for thiolated BSA at different 2-IT/BSA molar feeding ratio. (b) The plot of thiol groups per BSA versus 2-IT/BSA molar feeding ratio. The data were fitted as a straight line with R^2= 0.9885, and the thiol number could be predicted by using the fitting formula y = 0.2804x + 1.6089.

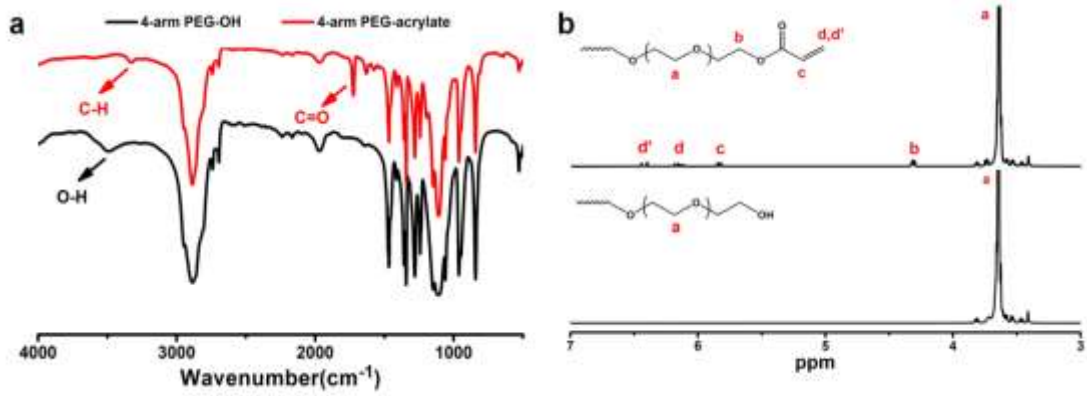

Figure S4. (a) FTIR spectra and (b) ^{1}H NMR spectra (400 MHz, $CDCl_3$) of 4-arm PEG and 4-arm PEG-acrylate.

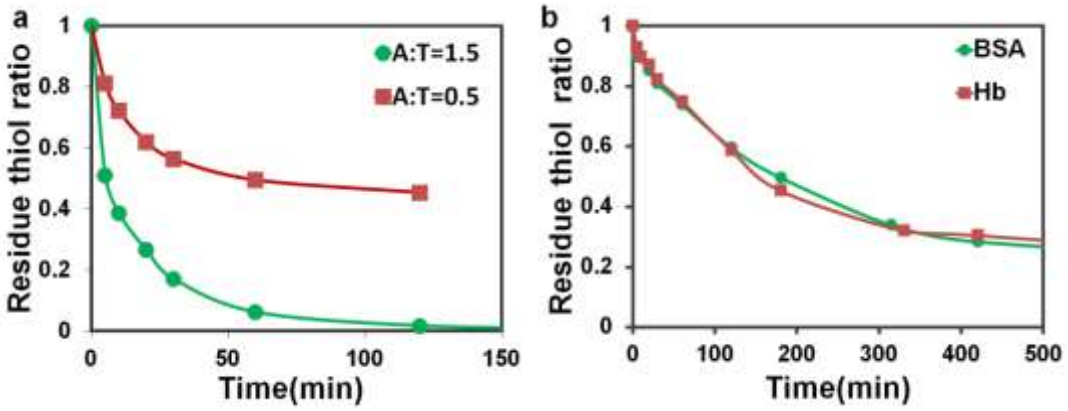

Figure S5. (a) Thiol conversion kinetics of Michael addition reaction between PEG diacrylate and DTT at different acrylate/thiol (A: T) feeding ratio. (b) Thiol conversion kinetics of thiolated BSA and thiolated Hb at the acrylate/thiol feeding ratio of 1.5.

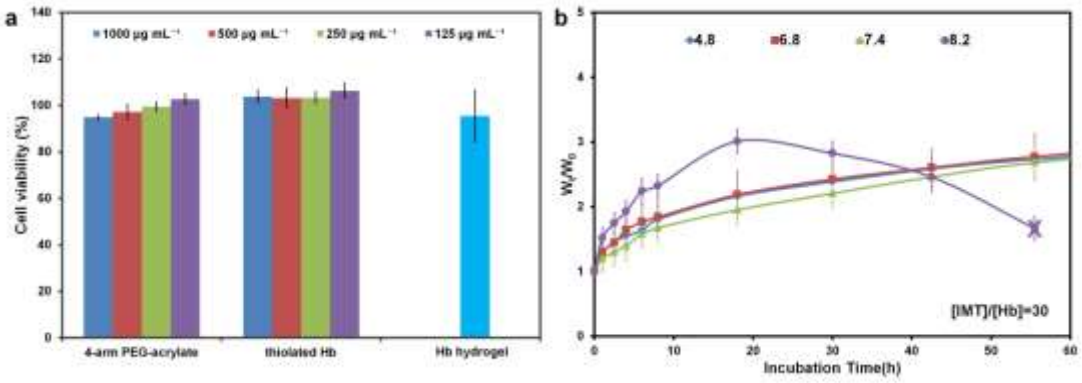

Figure S6. (a) In vitro cytocompatibility of protein-based hydrogels against L929 cells after incubation for 24 h as measured via MTT assay (n=3). Both the prepolymer solutions with different concentrations and the extraction medium of Hb hydrogels were tested. (b) Swelling behaviors of Hb hydrogels at different pH. The gel was made of 4-arm PEG-acrylate and thiolated Hb at a fixed 2-IT/Hb molar feeding ratio of 30. The cross symbol indicates the gel disintegrated during the experimental period.

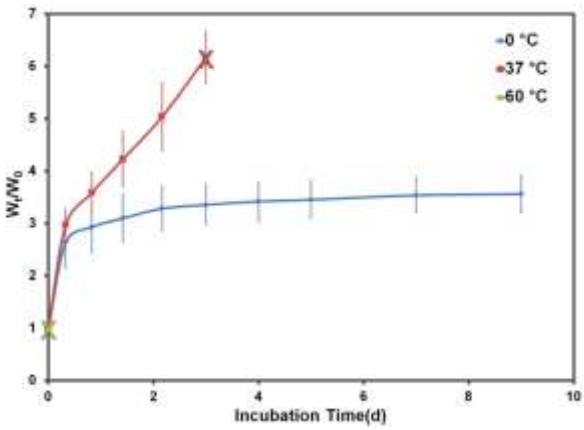

Figure S7. Swelling behavior of DTT cross-linked hydrogel at varied temperatures. The cross symbols indicate the gels disintegrated during the experimental period.

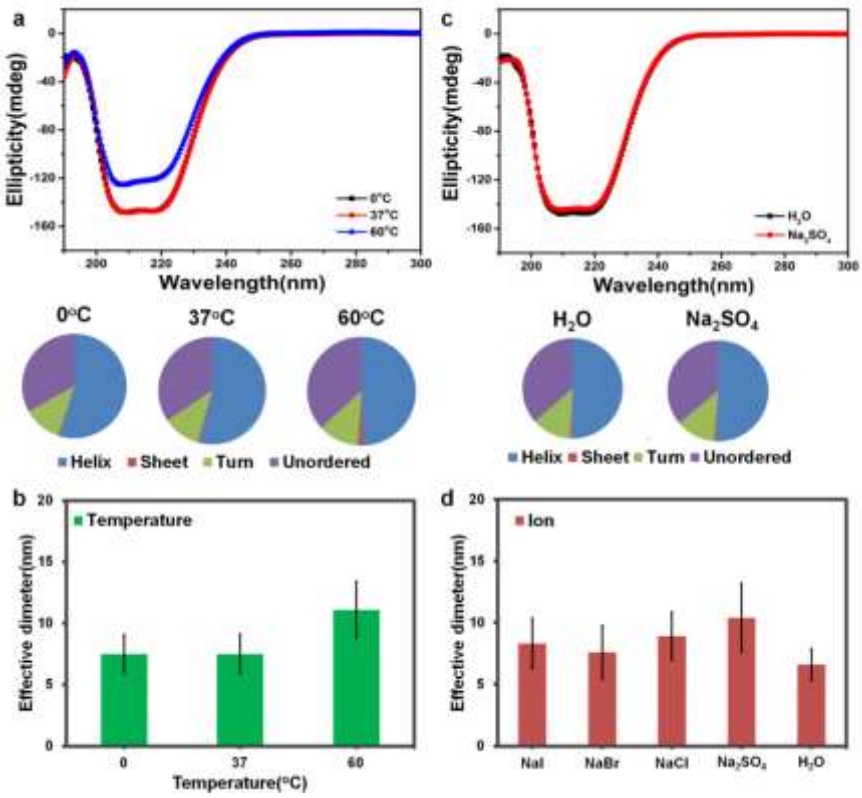

Figure S8. CD spectra of BSA solution incubated at different (a) temperature and (c) ion conditions. The pie chart below shows the calculated proportions of various secondary structures using the CONTINLL algorithms. DLS results for effective diameter of BSA with varied (b) temperatures and (d) ion types.