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Supporting information

Mechanoresponsive Diselenide-Crosslinked Microgels with Programmed Ultrasound-Triggered Degradation and Radical Scavenging Ability for Protein Protection

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Experimental Section/Methods

Materials: Selenium powder (Se; 99.5%), sodium borohydride ($NaBH_4$; 98%), 11-Bromoundecanol (97%), triethylamine (TEA; 99%), acryloyl chloride (AC; 96%), dimethyl sulfoxide (DMSO; 99%) tetrahydrofuran (anhydrous THF; 99.8%), silica gel 60 (215-400 mesh) were purchased from Alfa Aesar. *N*-vinylcaprolactam (VCL; 98%), and 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH; 97%), cytochrome C (95%), myoglobin (90%), α-chymotrypsin (40 units/mg protein) and albumin (98%) were purchased from Merck. VCL was purified by vacuum distillation prior usage and other materials were used without further purification.

Synthesis of diselenide (SeSe) crosslinker. The crosslinker was prepared as described previously.[1] Briefly, disodium diselenide was synthesized from selenium powder and sodium borohydride in water followed by the addition of 11-bromoundecanol in THF to produce bis(11-hydroxyundecyl) diselenide. Subsequently, acryloylation of bis(11-hydroxyundecyl) diselenide was performed using acryloyl chloride to conjugate polymerizable vinyl groups onto the diselenide molecule to produce bis(11-acryloyloxyundecyl) diselenide.

Synthesis of disodium diselenide (Na_2Se_2). The synthesis of Na_2Se_2 was performed according to the reported procedure.[2] Sodium borohydride (0.96 g, 25.3 mmol) in 7 mL water was added with magnetic stirring to (1.00 g, 12.6 mmol) of selenium powder suspended in 13 mL water at room temperature under N_2 environment. After the vigorous reaction had subsided (15 min), additional equivalent of selenium (1.00 g, 12.6 mmol) was added. The reaction mixture was heated to 50 °C to ensure the dissolution of selenium powder. The brownish red aqueous solution of Na_2Se_2 was then ready for further use.

Synthesis of Bis(11-hydroxyundecyl) diselenide ($HOC_{11}SeSeC_{11}OH$). The Synthesis of diselenide crosslinker's precursor ($HOC_{11}SeSeC_{11}OH$) was performed based on the protocol reported.[3] To the Na_2Se_2 solution prepared above, 11-bromoundecanol (6.36 g, 25.3 mmol) dissolved in 40 mL anhydrous THF was added. The reaction was stirred overnight at 50 °C under N_2 environment. The solution was filtered to remove solid residue and vacuum dried to obtain yellowish powder. The product was purified by column chromatography in two steps manner. The first solvent flushing with C_6H_6:THF 1:1 followed by second solvent flushing C_6H_6:THF 7:1

and the resulting purified yellowish powder obtained with a yield of (87%). ^{1}H NMR ($CDCl_3$, 400 MHz) (**Figure S9**): δ 3.57 (t, 4H, $HOCH_2$), 2.84 (t, 4H, CH_2Se), 1.65 (q, 4H, $(CH_2)_7CH_2CH_2Se$), 1.50 (q, 4H, $HOCH_2CH_2(CH_2)_7$), 1.34–1.11 (br, 28H, $CH_2(CH_2)_7CH_2$).

Synthesis of Bis(11-acryloyloxyundecyl) diselenide [C=CC(=O)OC$_{11}$SeSeC$_{11}$OC(=O)C=C]. The diselenide crosslinker was synthesized by acrylation of the precursor. Bis(11- hydroxyundecyl) diselenide (5.00 g, 10 mmol) was dissolved in 200 mL anhydrous THF. Triethylamine (2.43 g, 24 mmol) was added into the mixture and was cooled in an ice bath. Acryloyl chloride (2.17 g, 24 mmol) in 10 mL anhydrous THF was added dropwise. The mixture was stirred in the ice bath for 1 hour. Then ice bath was removed and reaction was allowed to run overnight at room temperature. The reaction mixture was filtered to remove solid. Purification of the crosslinker was performed by two steps solvent flushing. The first flushing was done in C_6H_6:$C_4H_8O_2$ 40:1 followed by C_6H_6:$C_4H_8O_2$ 1:1. The purified product obtained was yellowish viscous liquid with yield of (50%). ^{1}H NMR ($CDCl_3$, 400 MHz) (**Figure S9**): δ 6.35-6.30 (dd, 2H, cis-HHC=CHC(=O)), 6.09-6.02 (dd, 2H, H_2C=CHC(=O)), 5.76-5.73 (dd, 2H, trans-HHC=CHC(=O)), 4.08 (t, 4H, $OCH_2CH_2(CH_2)_7$), 2.84 (t, 4H, $(CH_2)_7CH_2CH_2Se$), 1.65 (m, 4H, $(CH_2)_7CH_2CH_2Se$), 1.60 (m, 4H, $OCH_2CH_2(CH_2)_7$), 1.34–1.11 (br, 28H, $CH_2(CH_2)_7CH_2$).

Synthesis of diselenide-crosslinked microgels (SeμG). Series of SeμG with different content of diselenide crosslinks and sizes were synthesized through precipitation polymerization in water/DMSO mixture. In a 250 mL round bottom flask, VCL (1500 mg, 10.78 mmol) was dissolved in 133 mL ultrapure water. Bis(11-acryloyloxyundecyl) diselenide (98.4 mg, 0.16 mmol) in 15 mL DMSO was added to the VCL and the solution was stirred at 70 °C under N_2 purging for 1 h. The polymerization was initiated by the addition of AAPH (36.15 mg, 0.13 mmol) in 2 mL ultrapure water, and the reaction was carried out for 6 h. SeμGs were purified by dialysis against deionized water for one week with water exchanged twice per day.

Ultrasonication experiments. The sonication experiments were executed on a Vibra-Cell VCX 500 (Sonics & Materials) operating at 500 W and 20 kHz in a Suslick vessel under an inert atmosphere using a 13 mm full wave immersion probe. SeμGs were dispersed in ultrapure water to a concentration of 2 mg mL^{-1} with 10 mL of each microgel solution sonicated up to maximum of 10 min with 1 min per step with the sonication pulse mode (1 s On and 2 s Off).

During the ultrasonic experiment, solution temperature was kept close to 0 °C with an ice bath and 20 °C, 37 °C, and 50 °C using a thermostat. The range of amplitude in sonication experiments was set to 20, 30, and 40%.

FTIR spectroscopy. IR spectra were recorded on a Bruker Alpha II equipped with an ATR platinum diamond measuring cell. All measurements were performed within a range from 4000 cm^{-1} to 400 cm^{-1} with a spectral resolution of 4 cm^{-1} and a sample scan of 250 scans at temperature between 28-29 °C. The data analysis was carried out with the software OPUS 8.2.28.

Raman spectroscopy. Raman spectra were recorded on a Bruker RFS 100/S spectrometer. The laser used was a Nd:YAG at 1064 nm wavelength at a power of 250 mW. On average, 1000 scans were taken at a resolution of 4 cm^{-1}. For sample holding aluminum pans of 2 mm before were used. The software used for data processing was OPUS 4.0.

^{1}H-NMR spectroscopy. Proton Nuclear Magnetic Resonance (1H NMR) spectra were recorded on a Bruker Avance III 400NMR spectrometer at 400 MHz. The chemical shifts reported are all relative to the residual solvent peak.

*^{77}Se-NMR spectroscopy.*All 77Se{1H} NMR spectra were obtained using a Bruker Avance Neo 600 spectrometer operating at 114.4 MHz. During acquisition, inverse gated 1H decoupling was employed. The spectra were calibrated according to the unified scale method recommended by IUPAC (10.1006/snmr.2002.0063) using the solvent peak in corresponding 1H spectra as reference.

Electron paramagnetic resonance (EPR) spectroscopy. EPR spectra were obtained at room temperature on a Miniscope MS 400 from Magnettech with amicrowave frequency of 9.4 GHz. Microgel solutions with a concentration of 30 $mg \cdot mL^{-1}$ were measured. The B_0 field was adjusted to 335 mT with a range of 100 mT (285 – 385 mT) and asweep time of 40 s. Other parameters were adjusted as follows: smooth = 0.1000 s, NOPs = 4096, gain mantissa = 7 and gain exponent = 1.

Scanning transmission electron microscopy (STEM). STEM images were captured on a Hitachi S-4800 field emission scanning electron microscope (FE-SEM). The microgel solutions (10 µL, 0.2 mg mL^{-1}) were dropped on a carbon coated copper grid (400 Mesh) and blotted by a filter paper. The images were recorded at TEM mode at a voltage of 30 kV and a current of 20 µA.

Dynamic Light Scattering (DLS). DLS data were obtained with a laser light scattering spectrometer (ALV/DLS/SLS-5000) equipped with an ALV-5000/EPP multiple digital time correlator and laser goniometry system ALV/CGS-8F S/N 025 with a helium-neon laser (Uniphase 1145P, the output power of 22 mW and wavelength of 632.8 nm) as a light source. The microgel samples were dispersed in ultrapure water and filtered through syringe filter (pore size 1.2 µm) before the measurement at scattering angle (θ = 90 °).

Electrophoretic Mobilities (EM). EM were measured with a Zetasizer Nano Series from Malvern Instruments at a temperature of 25 °C. For every sample, three measurements with 20 runs were performed. Sample preparation was analog to dynamic light scattering. A DTS1070 cuvette from Malvern Instruments was used for the measurements.

Protein Loading Experiment. 16.7 mg of PVCL microgel with 1.5 mol% of SeSe crosslinks (Se1.5) (or 1.5 mol% BIS crosslinker) was redispersed in 20 mL ultrapure water. 6 mg of protein (cytochrome C, Myoglobin, α-Chymotrypsin) was added into the microgel dispersion and stirred overnight. Excess of protein was washed out by dialysis in water.

Ultraviolet-visible Spectroscopy (UV-Vis). UV-Vis spectra were obtained on a CARY 100 Bio (Agilent Technologies, USA). Microgel dispersion in water (1 mL) was filled into a 1.5 mL UV-cuvette semimicro (1.25×1.25×4.5 cm) and measured at room temperature in the range of 200-800 nm.

Circular Dichroism Spectroscopy (CD). CD spectra were obtained on a JASCO J-1500 circular dichroism spectrophotometer. 200 µL of microgel dispersion was loaded into Hellma Macro Cell with 1mm light path. The measurements were performed at room temperature ranging between 190-250 nm.

X-Ray Photoelectron Spectroscopy (XPS). The XPS measurements were carried out in an Ultra Axis spectrometer from Kratos Analytical; the samples were irradiated with monoenergetic Al $K\alpha_{1,2}$ radiation (1486.6 eV), and the spectra were taken at a power of 144 W (12 kV × 12 mA). For XPS analysis, the stock suspensions were placed onto a silicon surface (1.5 × 1.5 cm) and dried in vacuo at 60 °C for 20 h. Directly thereafter, the XPS analysis was performed to diminish the contact with air and water adsorption. A spectral evaluation was performed using CasaXPS Processing Software.

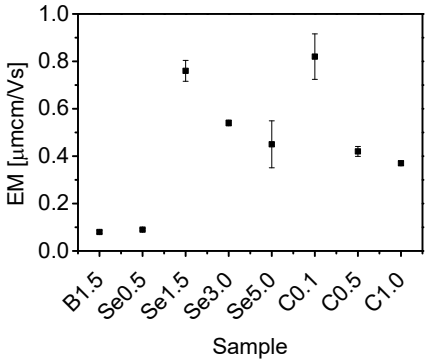

Figure S1. Electrophoretic Mobility (EM) of PVCL microgels with BIS or SeSe crosslinks. B: BIS in mol%; Se: SeSe in mol%; C: CTAB in wt%.

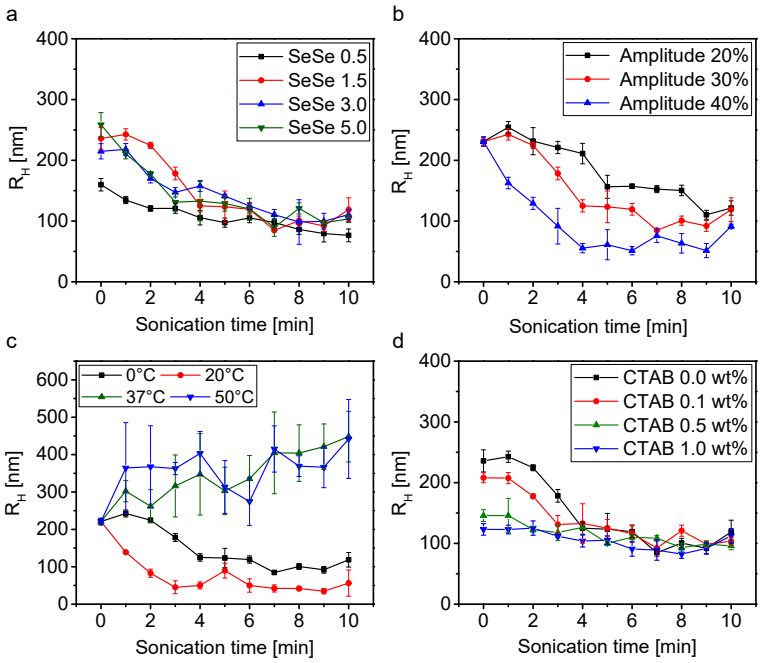

Figure S2. R_H (before dialysis) of a) SeµG with 0.5, 1.5, 3.0 and 5.0 mol% diselenide contents, b) Se 1.5 mol% with 20, 30 and 40% amplitude, c) Se 1.5 mol% at different temperature and d) Se 1.5 mol% with 0, 0.1, 0.5 and 1.0 wt% of CTAB contents. Sonication experiments of a), b) and d) were performed in an ice bath, and a), c) and d) were performed at 30% amplitude. (Power output for 30% amplitude is 1.82 W cm^{-2}).

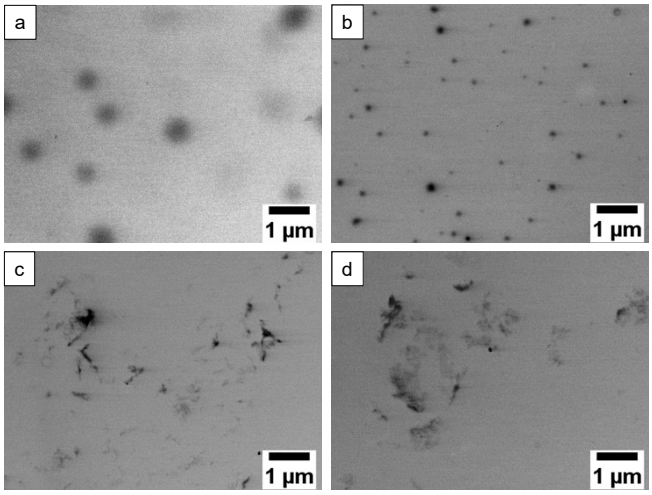

Figure S3. STEM images of Se1.5 before sonication a) and after 10 min sonication in an ice bath at b) 20%, c) 30%, and d) 40% amplitude.

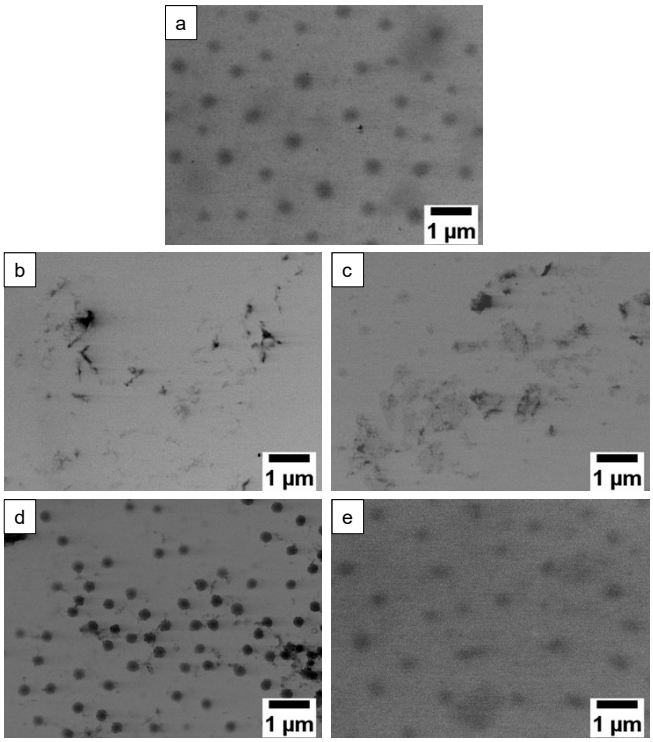

Figure S4. STEM images of Se1.5 a) before ultrasonication and after 10 min ultrasonication at 30% amplitude in an b) ice bath, at c) 20 °C, d) 37 °C, and e) 50 °C

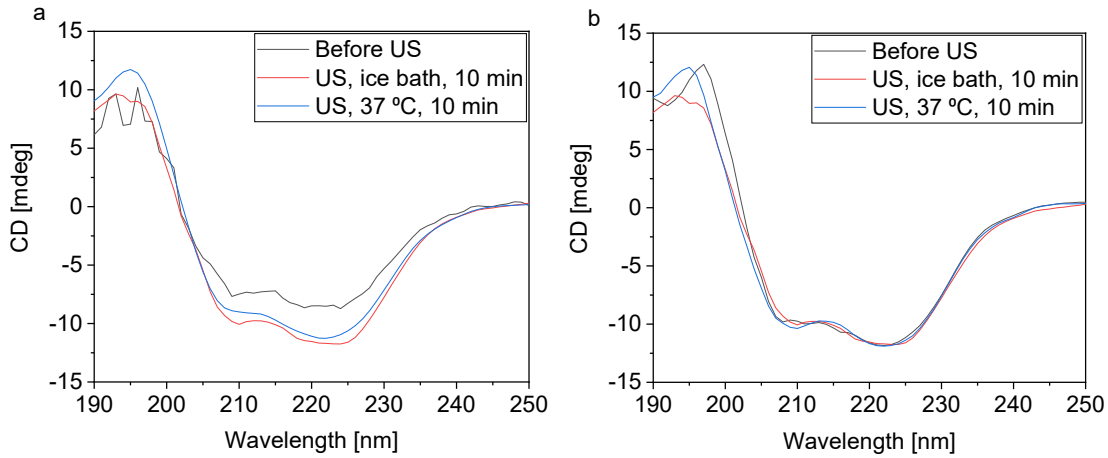

Figure S5. Circular Dichroism (CD) spectra of a) pure cytochrome C in water after 10 min ultrasonication in an ice bath and at 37 °C and b) cytochrome C loaded diselenide-crosslinked microgels before and after 10 minutes ultrasonication in an ice bath and at 37 °C.

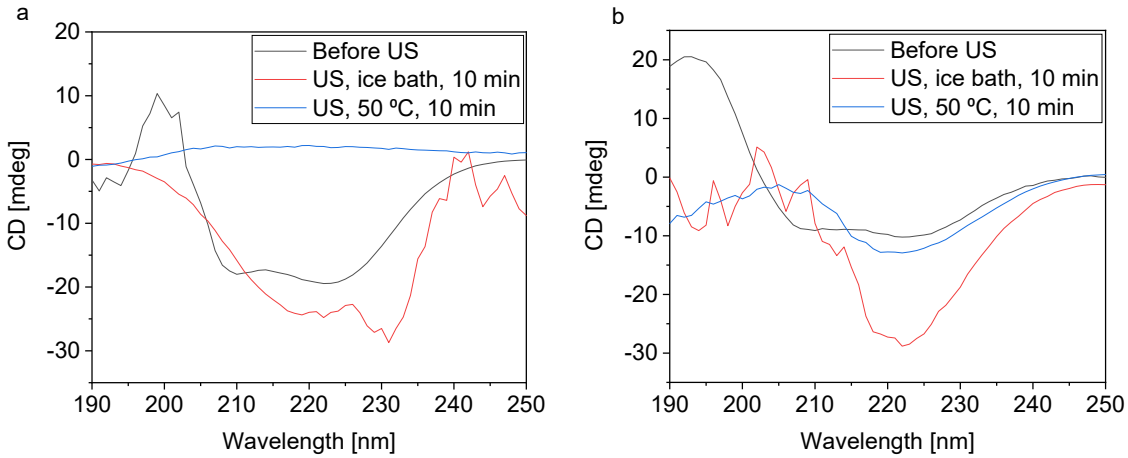

Figure S6. CD spectra of a) pure Myoglobin in water after 10 min ultrasonication in an ice bath and at 50 °C and b) Myoglobin -loaded Se1.5 before and after 10 min ultrasonication in an ice bath and at 50 °C

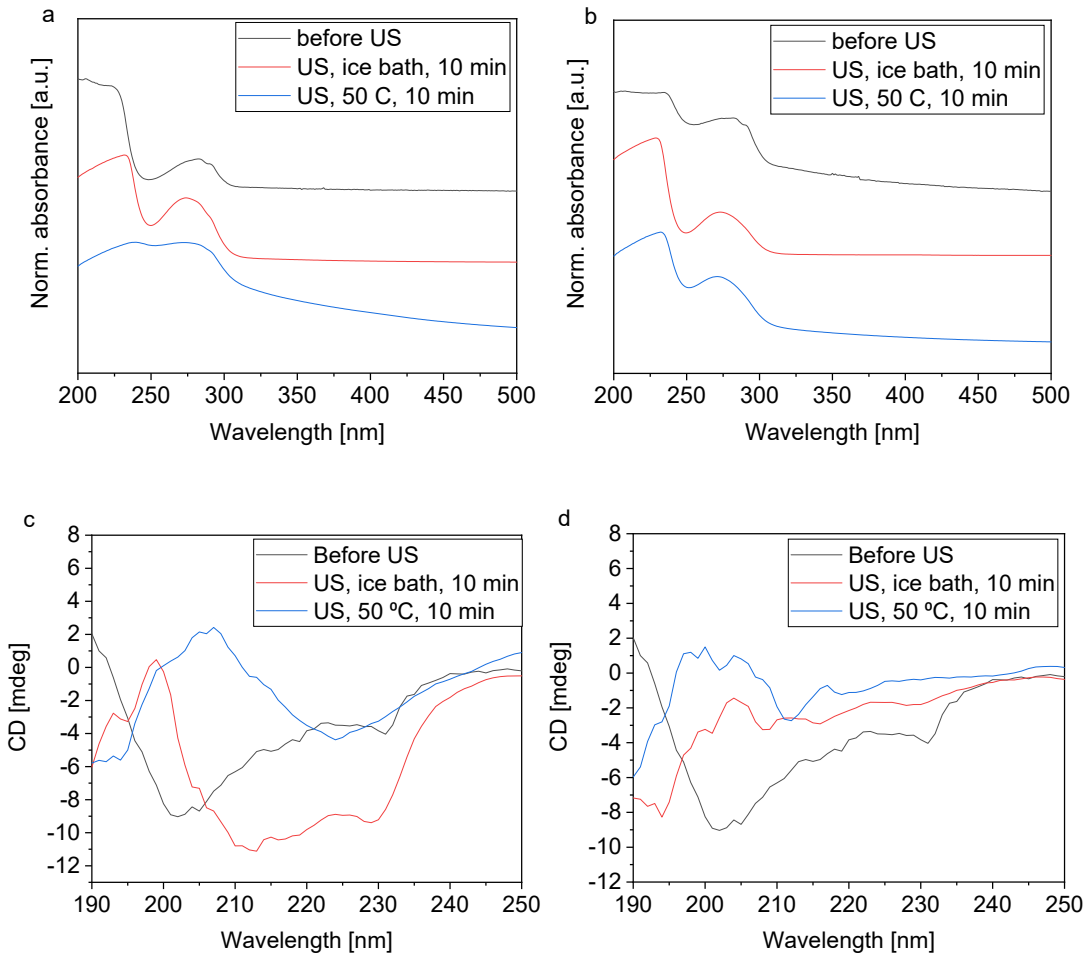

Figure S7. UV and CD spectra respectively of a, c) pure α-Chymotrypsin in water after 10 min ultrasonication in an ice bath and at 50 °C and b, d) α-Chymotrypsin -loaded Se1.5 before and after 10 min ultrasonication in an ice bath and at 50 °C.

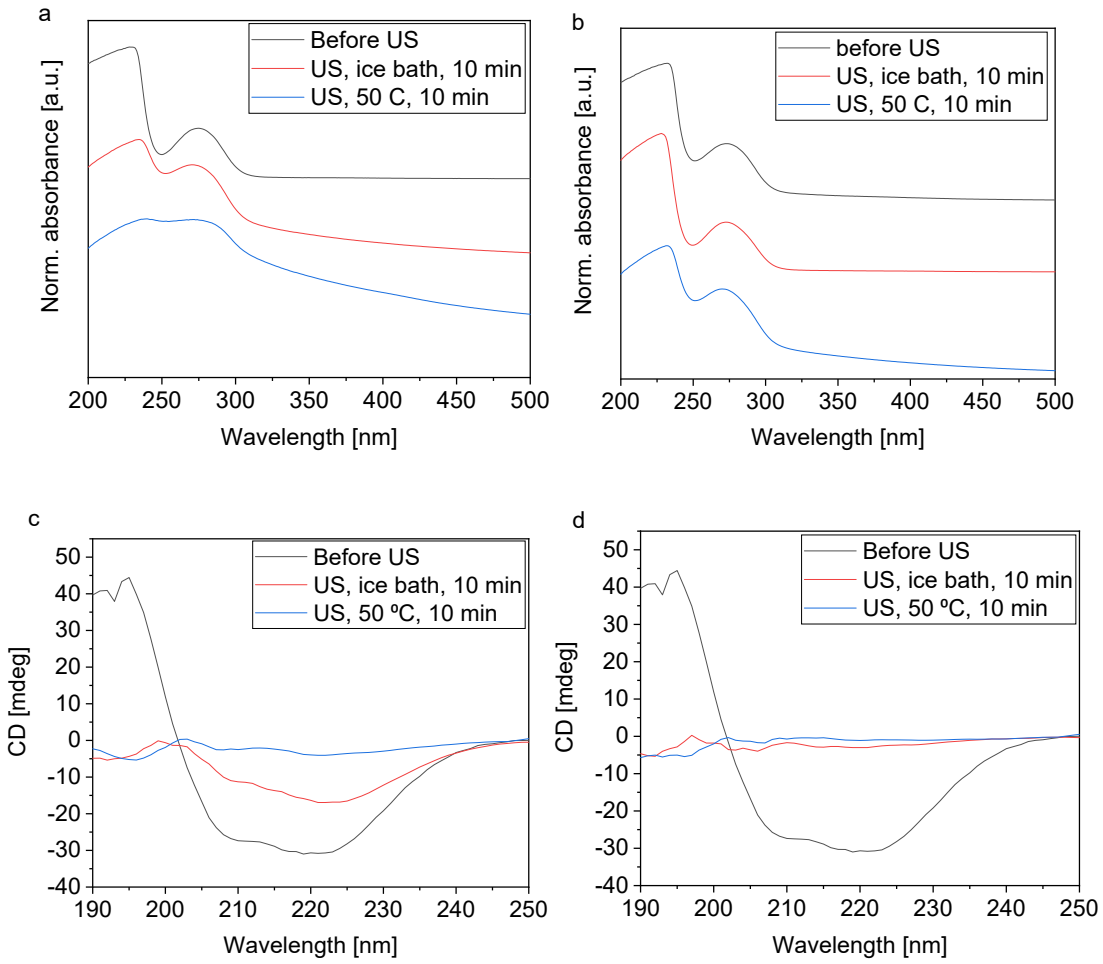

Figure S8. UV and CD spectra respectively of a, c) pure Albumin in water after 10 min ultrasonication in an ice bath and at 50 °C and b, d) Albumin -loaded Se1.5 before and after 10 min ultrasonication in an ice bath and at 50 °C.

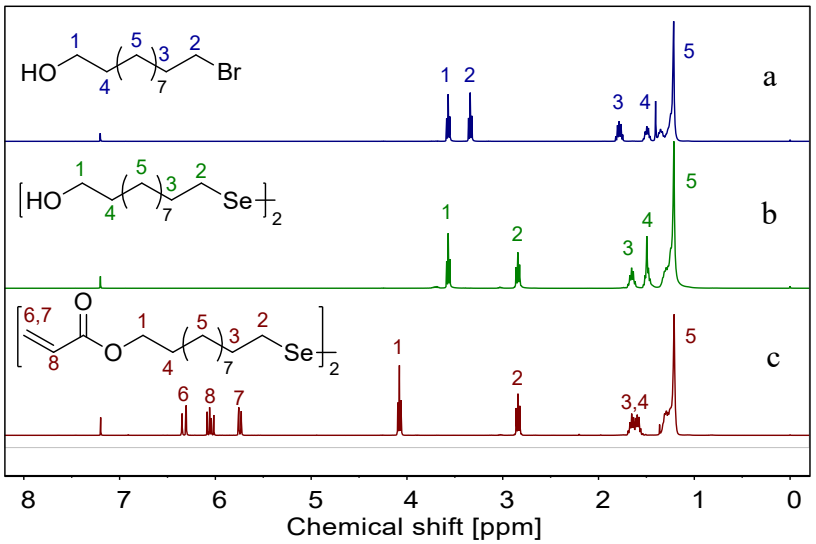

Figure S9. ^{1}H NMR of diselenide crosslinker and its precursors: a) 11-bromoundecanol, b) Bis(11-hydroxyundecyl) diselenide, c) Bis(11-acryloyloxyundecyl) diselenide.

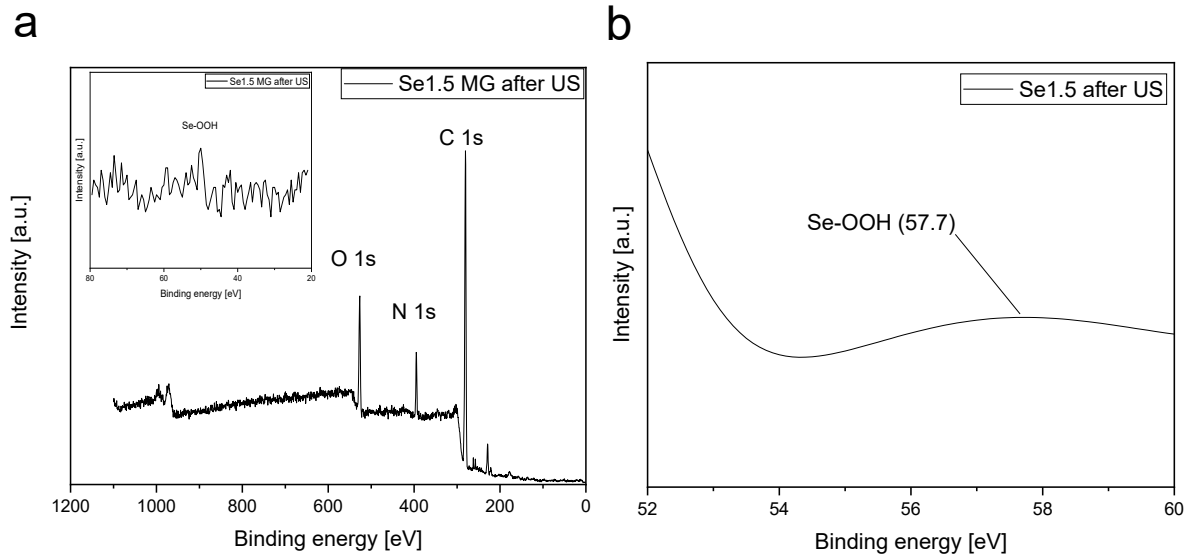

Figure S10. XPS analysis of SeμG after 10 min ultrasonication in an ice bath. (a) Full XPS spectra, (b) deconvoluted XPS peak with assignment of functional group Se-OOH. (Power output for 30% amplitude is 1.82 W·cm^{-2}).

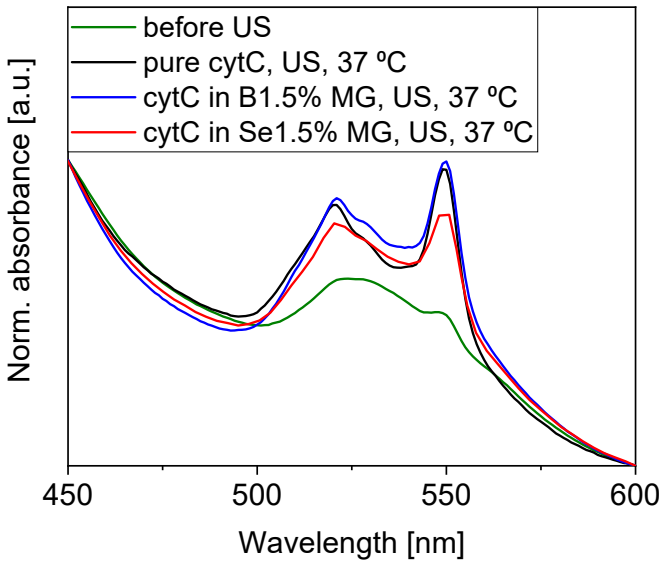

Figure S11. (a) UV-vis spectra of pure cytC, cytC loaded in B1.5%, and in Se1.5 microgels in H_2O after 10 min ultrasonication at 37 °C.

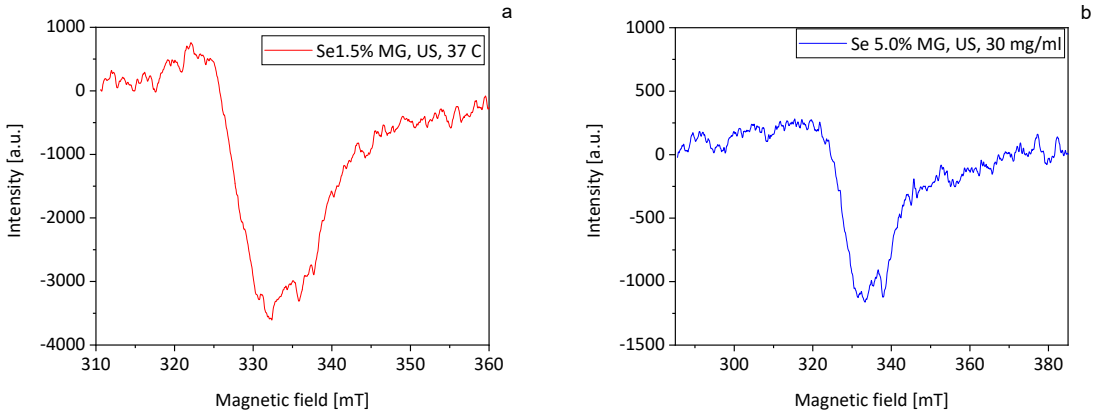

Figure S12. EPR spectra of Se-microgels after ultrasonication: a) Se1.5% microgel, concentration – 6 mg mL^{-1}; b) Se5.0% microgel, concentration – 30 mg mL^{-1}. (37 °C, 10 min sonication time, power output for 30% amplitude is 1.82 $W \cdot cm^{-2}$).

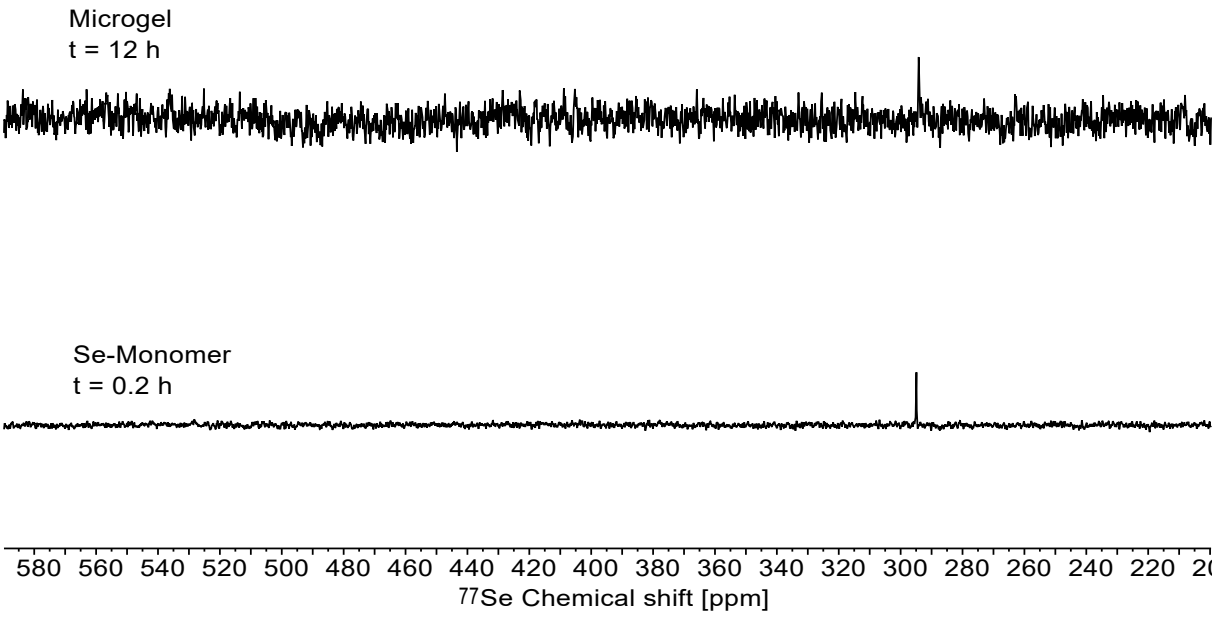

Figure S13. -^{77}Se NMR of Se-microgel and Se-monomer after sonication. Peak in area 300 ppm corresponds to -Se-Se- group. (37 °C, 10 min sonication time, power output for 30% amplitude is 1.82 $W \cdot cm^{-2}$).

Table S1. Secondary structure of cytC, cytC -loaded Se1.5 and B1.5 microgel samples before and after sonication treatment

Sample	α-Helix [%]	β-Sheet [%]	β-turn [%]	Random [%]
cytC Before US	75	14.6	10.4	0
cytC US 0 C	82.6	15.4	1.9	0
cytC US 37 C	83.5	14	2.5	0
cytC-Se1.5 MG US 0 C	75.3	18.6	0	6.1
cytC-Se1.5 MG US 37 C	80.4	8.2	0.9	10.5
cytC-B1.5 MG US 37 C	81.3	18.7	0	0

Table S2. Secondary structure of Myoglobin and Myoglobin-loaded Se1.5 microgel samples before and after sonication treatment

Sample	α-Helix [%]	β-Sheet [%]	β-turn [%]	Random [%]
Mb Before US	79.3	20.7	0	0
Mb before US at pH4	*0*	*99.8*	*0*	*0.2*
Mb US 0 C[a)]	0	56.9	0	43.2
Mb US 50 C [a)]	0.0	45.1	8.8	46.1
Mb-Se MG before US	84.7	0.6	9.1	5.6
Mb-Se MG Before US pH4 [a)]	*45.7*	*9.4*	*36.1*	*8.8*
Mb-Se MG US 0 C [a)]	54.6	17.9	27.5	0
Mb-Se MG US 50 C [a)]	24.1	28.6	12.4	34.9

[a)] experiments performed at pH 4 buffer solution

Table S3. Secondary structure of α-Chymotrypsin and α-Chymotrypsin-loaded Se1.5 microgel samples before and after sonication treatment

Sample	α-Helix [%]	β-Sheet [%]	β-turn [%]	Random [%]
α-Chy Before US	19.1	27.1	5.5	48.3
α-Chy *before US at pH4*	*33*	*37.6*	*0*	*29.4*
α-Chy US 0 C [a)]	27.6	39.9	1.9	30.6
α-Chy US 50 C [a)]	5.7	34	23.5	36.8
α-Chy-Se MG before US	26.6	39.7	8.8	25
α-Chy-*Se MG Before US pH4* [a)]	*27.4*	*40.2*	*4.1*	*28.4*
α-Chy-Se MG US 0 C [a)]	14.4	32.7	10.5	42.4
α-Chy-Se MG US 50 C [a)]	9.2	27.4	5.5	57.8

[a)] experiments performed at pH 4 buffer solution

Table S4. Secondary structure of Albumin and Albumin -loaded Se1.5 microgel samples before and after sonication treatment

Sample	α-Helix [%]	β-Sheet [%]	β-turn [%]	Random [%]
Alb Before US	64.8	32.2	3	0
Alb before US at pH4	*0*	*69.3*	*1.4*	*29.3*
Alb US 0 C [a)]	41.9	33.4	9.5	15.2
Alb US 50 C [a)]	0	49.1	23.6	27.3
Alb-Se MG before US	51	33.5	3.8	11.7
Alb-*Se MG Before US pH4* [a)]	*11.6*	*50.4*	*0*	*38*
Alb-Se MG US 0 C [a)]	11.9	22.1	11.9	54
Alb-Se MG US 50 C [a)]	6	27.1	24.1	42.8

[a)] experiments performed at pH 4 buffer solution

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