
Article:
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Supplementary information for:

Polymer Hydrogels for Glutathione-Mediated Protein Release

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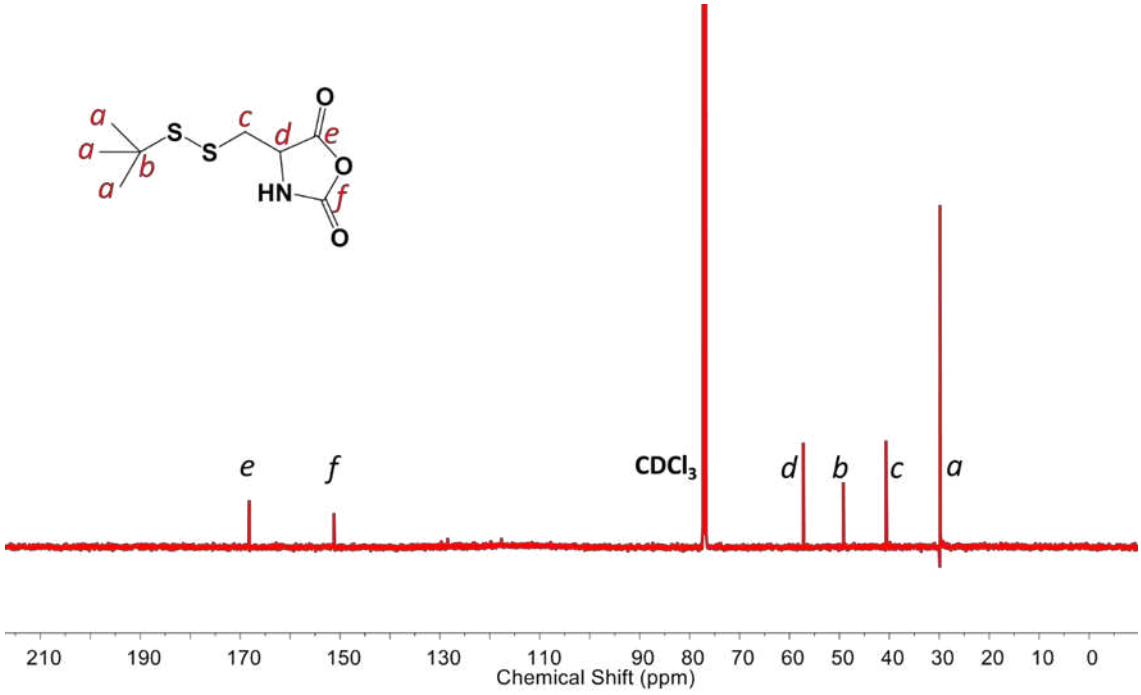

Figure S1. ^{13}C NMR spectrum of STMB-L-cysteine NCA.

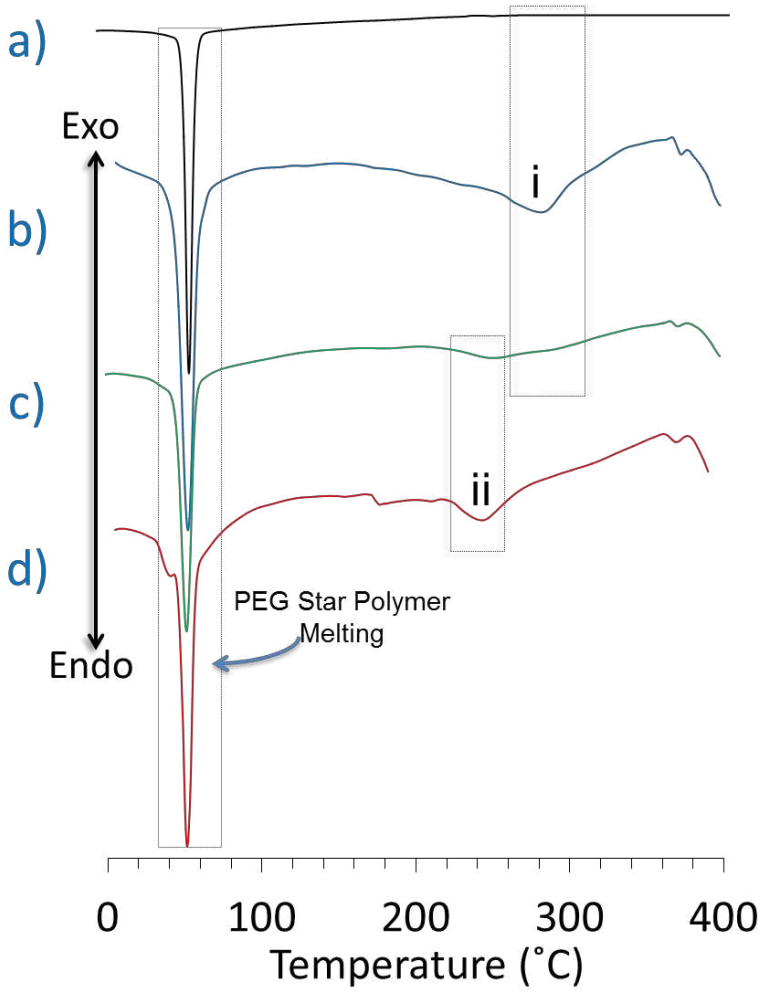

Figure S2. Differential scanning calorimetry thermograms corresponding to the PEG star polymer initiator (a), poly[(STMB-L-cysteine$_{10}$)$_4$-*b*-(StarPEG$_{10k}$)] (b) poly[(L-cysteine$_{10}$)$_4$-*b*-(StarPEG$_{10k}$)] (c) and the crosslinked xerogel (d). Successful NCA ROP is evidenced by the emergence of PEG star polymer initiator's melting point endotherm and the endotherm corresponding to STMB (b, i). Cysteine deprotection to remove the STMB group results in the disappearance of the endotherm trough (c). Successful generation of disulfide-crosslinked hydrogel scaffolds is evidenced by the emergence of an endotherm trough that is characteristic of the thermal energy required to rupture the disulfide bridges (d, ii).

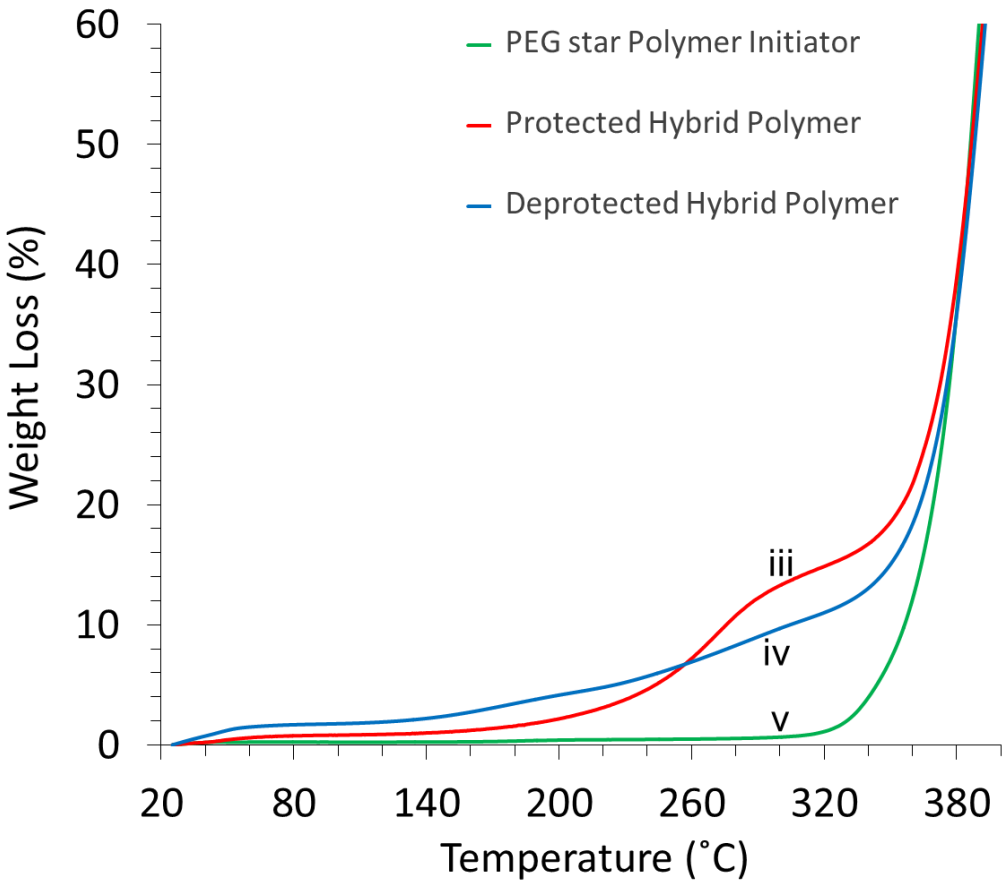

Figure S3. Thermogravimetric analysis thermograms corresponding to the PEG star polymer initiator, poly[(STBM-L-cysteine$_{10}$)$_4$-*b*-(StarPEG)] and poly[(L-cysteine$_{10}$)$_4$-*b*-(StarPEG)]. The TGA thermogram of the STBM-protected polymer (*red*) reveals a greater weight-loss shoulder (iii) at 235 °C - 300 °C due to loss of the protecting groups. Consequently, this weight-loss shoulder is decreased (iv) after polymer deprotection and is minimal (v) in the trace that corresponds to the PEG star polymer initiator.

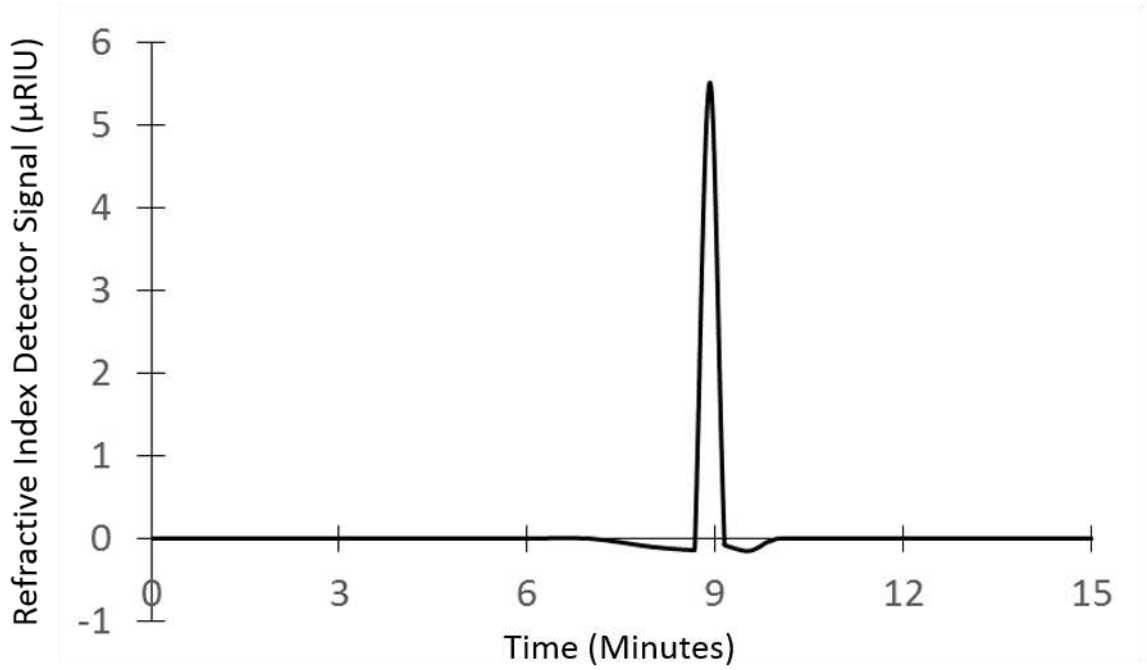

Figure S4. APC analysis of poly[(STBM-L-cysteine$_{10}$)$_4$-*b*-(StarPEG)] reveals a monomodal polymer distribution.

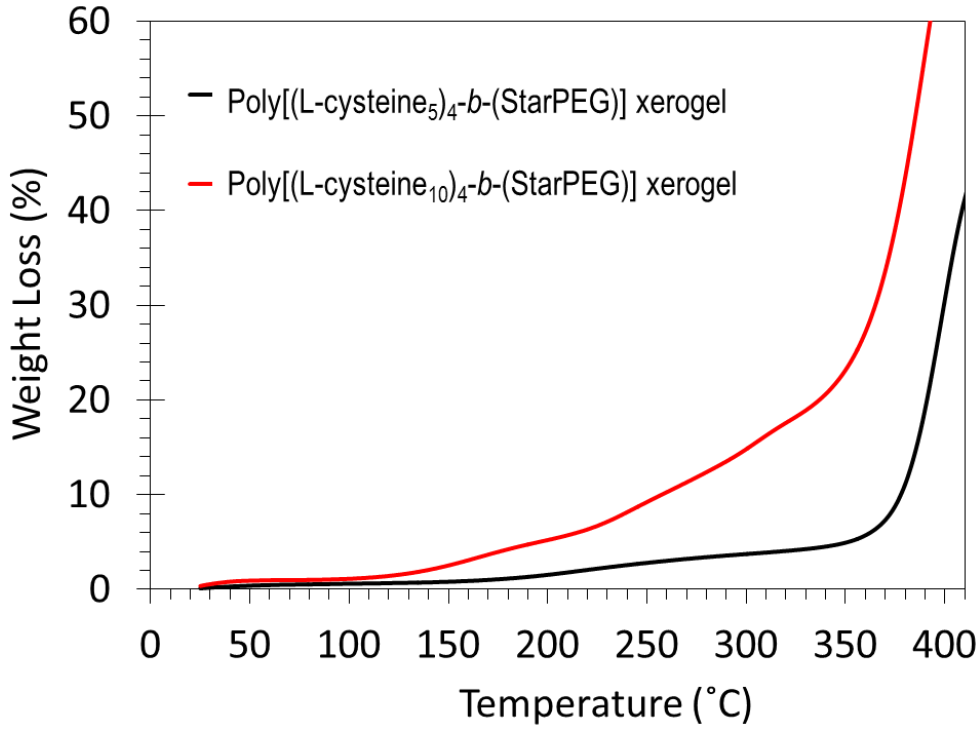

Figure S5. Comparison of thermal stability of the poly[(L-cysteine$_5$)$_4$-*b*-(StarPEG$_{10k}$)] hydrogel (i.e., 20 cysteine monomer units per polymer backbone) and poly[(L-cysteine$_{10}$)$_4$-*b*-(StarPEG$_{10k}$)] hydrogel (i.e., 40 cysteine monomer units per polymer backbone). Increasing the peptide component, results in increased weight loss upon combustion of the non-PEG content.

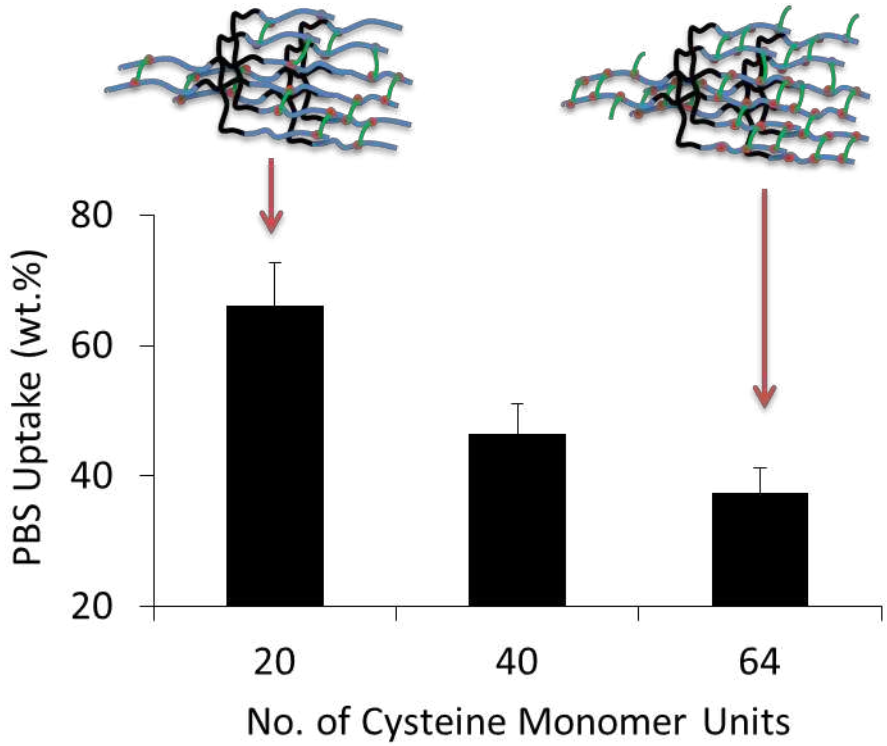

Figure S6. Variation of the amount of PBS solution absorbed by hydrogel scaffolds in relation to the number of cysteine repeat units grafted to the PEG star polymer macroinitiator.

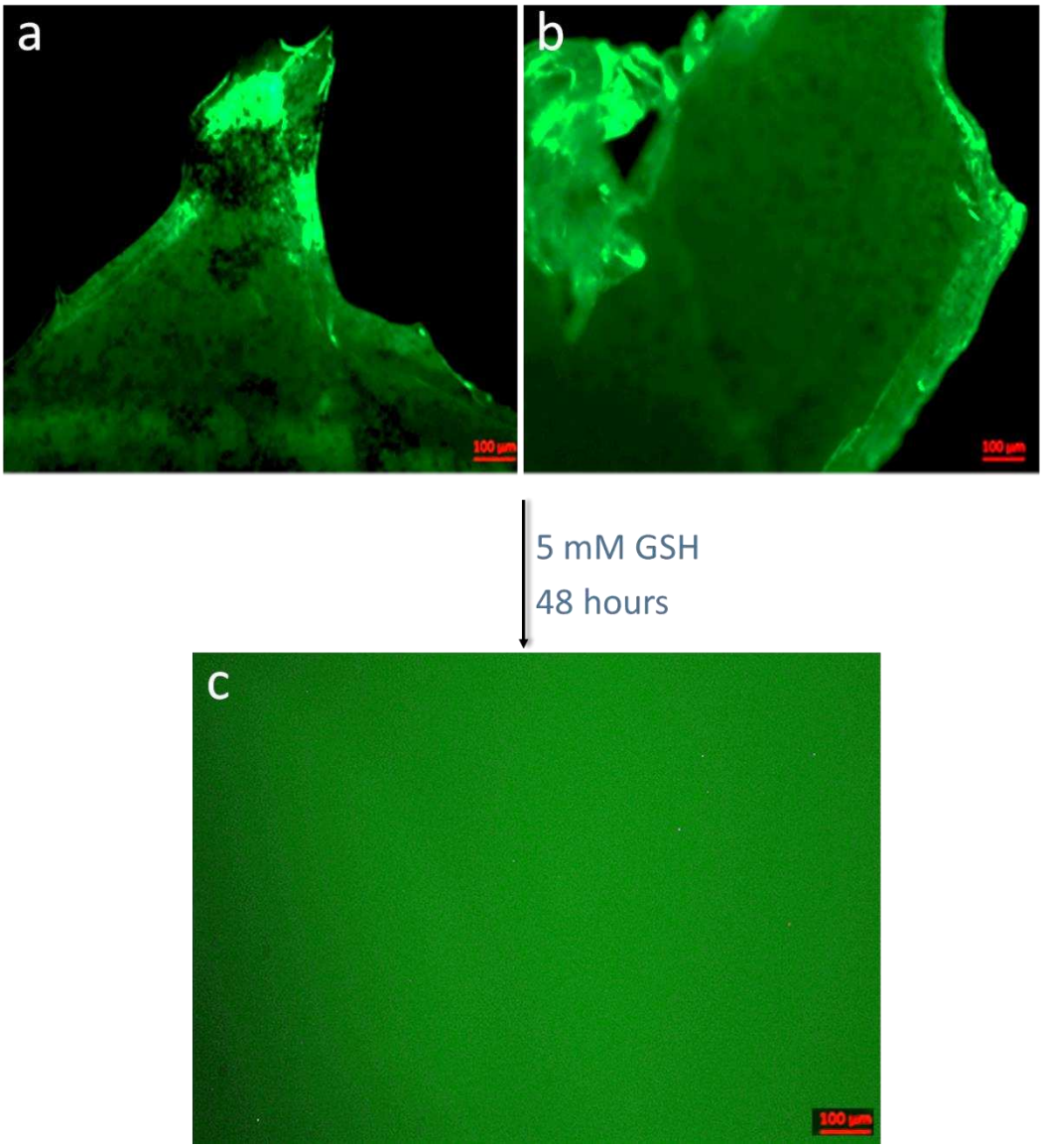

Figure S7. Fluorescence microscopy photographs obtained from the bovine serum albumin-loaded poly[(L-cysteine$_5$)$_4$-*b*-(StarPEG$_{10k}$)] hydrogel (a) and poly[(L-cysteine$_{10}$)$_4$-*b*-(StarPEG$_{10k}$)] hydrogel (b) and a representative microphotograph depicting the complete breakdown of the hydrogel structures to release the encapsulated molecular cargo in response to incubation in glutathione solutions (c).

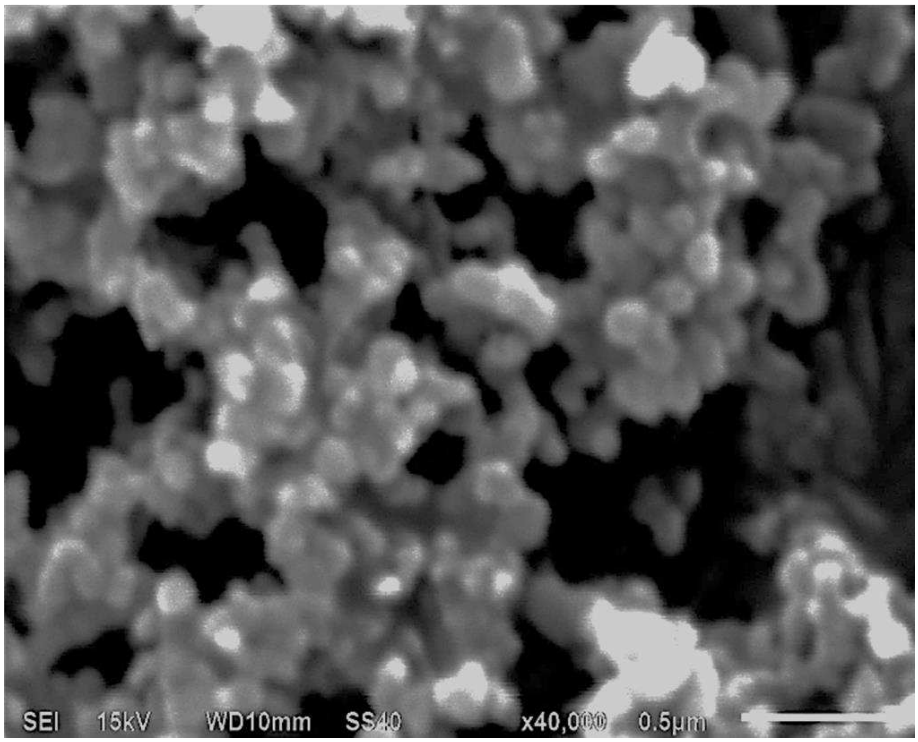

Figure S8. Scanning electron microphotograph of the poly[(L-cysteine$_5$)$_4$-*b*-(StarPEG$_{10k}$)] xerogel.

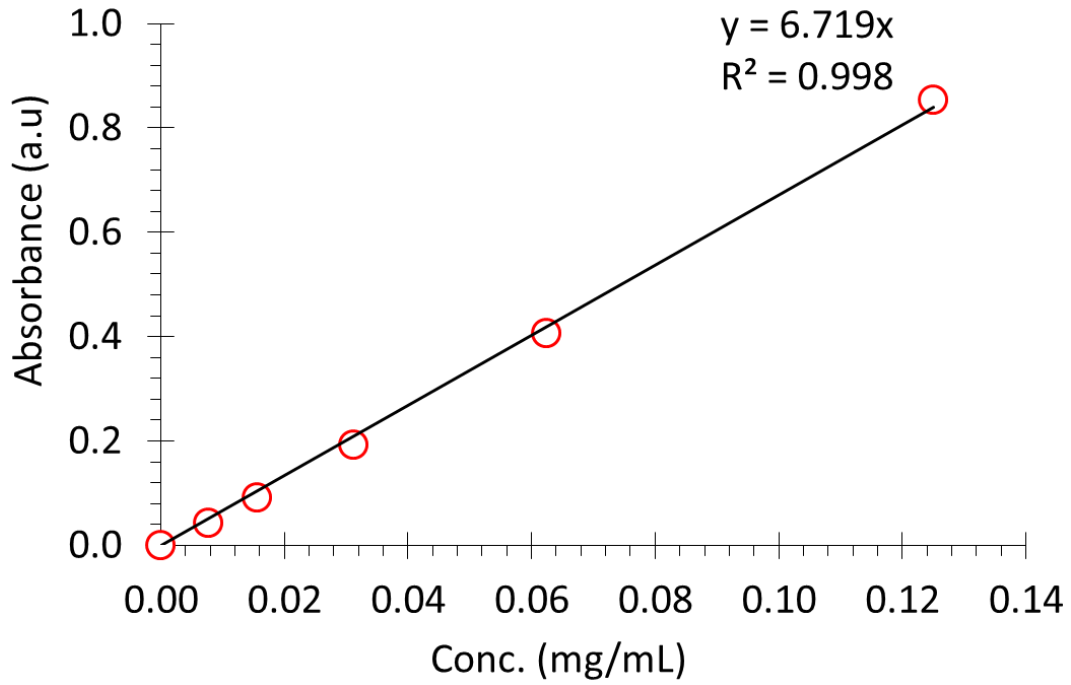

Figure S9. The calibration curve utilised to quantify the release of FITC-albumin from the hydrogels.

Disulfide Reduction

Concentration of GSH used in experiments = 5 mM

Moles of GSH available in 10 mL GSH release media = 0.05 mmol

1. **Poly[(L-cysteine5)4-b-(StarPEG10k)] hydrogel** = 66.1% water and 33.9% polymer

Mass of hydrogel used = 29.5 mg

Mass of polymer within the hydrogel = 0.339*29.5 mg = 10 mg

Molecular weight of peptide blocks grafted from the 4 arms of StarPEG = 20 x 103.15 = 2063 g.mol1

Molecular weight of poly[(L-cysteine5)4-b-(StarPEG10k)] = 12063 g.mol-1

As such, [2063/12063*100] = 17.1% of total polymer is the cross-linkable (peptide component)

Mass of cysteine within the copolymer = 17.1% x 10 mg = 0.17 x 0.01 g = 0.00171 g

Moles of cysteine within the copolymer = 0.00171 / 2063 = 8.3 x 10-7 moles = 8.3 x 10-4 mmol

Multiply by 20 to take into account 20 cysteine repeat units = 8.3 x 10-4 mmol x 20 = 0.0166 mmol

Two cysteine units form a single disulphide bond, therefore 0.0166 / 2 mmol of glutathione are required to break all the crosslinks = 0.0083 mmol.

As such, the amount of GSH provided is in excess and thus sufficient to cleave all the cross links in the hydrogel.

2. **Poly[(L-cysteine10)4-b-(StarPEG10k)] hydrogel** = 46.5% water and 53.5% polymer

Mass of hydrogel used = 18.7 mg

Mass of polymer within the hydrogel = 0.465*18.7 = 10 mg

Molecular weight of peptide blocks grafted from the 4 arms of StarPEG = 40 x 103.15 = 4126 g.mol1

Molecular weight of poly[(L-cysteine5)4-b-(StarPEG10k)] = 14126 g.mol-1

As such, [4126/14126*100] = 29.2% of total polymer is the cross-linkable (peptide component)

Mass of cysteine within the copolymer = 29.2% x 10 mg = 0.292 x 0.01 g = 0.00292 g

Moles of cysteine within the copolymer = 0.00292 / 4126 = 7.08 x 10-7 moles = 7.08 x 10-4 mmol

Multiply by 40 to take into account 40 cysteine repeat units = 7.08 x 10-4 mmol x 40 = 0.0283 mmol

Two cysteine units form a single disulphide bond, therefore 0.0283 / 2 mmol of glutathione are required to break all the crosslinks = 0.0142 mmol.

As such, the amount of GSH provided is in excess and thus sufficient to cleave all the cross links in the hydrogel.