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Supporting Information

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Protein-Crosslinked Polymeric Materials Through Site-Selective Bioconjugation

Aaron P. Esser-Kahn and Matthew B. Francis*

Department of Chemistry, University of California, Berkeley, CA 94720-1460, and
Material Sciences Division, Ernest Orlando Lawrence
Berkeley National Laboratory, Berkeley, CA 94720

Supporting Information

General Procedures and Materials

Unless otherwise noted, all chemicals and solvents were of analytical grade and used as received from commercial sources. Analytical thin layer chromatography (TLC) was performed on EM Reagent 0.25 mm silica gel 60-F254 plates with visualization by ultraviolet (UV) irradiation at 254 nm, vanillin or potassium permanganate stain. Purifications by flash chromatography were performed using EM silica gel 60 (230-400 mesh). The eluting system for each purification was determined by TLC analysis. Chromatography solvents were used without distillation. All organic solvents were removed under reduced pressure using a rotary evaporator. Dichloromethane (CH_2Cl_2) was distilled under a nitrogen atmosphere from calcium hydride. Water (dd-H_2O) used in biological procedures or as the reaction solvent was deionized using a NANOpure purification system (Barnstead, USA). UV-Vis spectroscopic measurements were conducted in quartz cuvettes using a Tidas-II benchtop spectrophotometer (J & M, Germany). Fluorescence measurements were obtained on a Fluoromax-2 spectrofluorometer (ISA Instruments). Centrifugations were conducted either with a Sorvall RC 5C (Sorvall, USA) plus for samples greater than 50 mL, a Sorvall LEGEND mach 1.6R for samples between 1 and 50 mL, and a Eppendorf Mini Spin plus for samples less than 1 mL (Eppendorf, USA). Samples were lyophilized using a LAB CONCO Freezone 4.5 (Lab Conco).

Instrumentation and Sample Analysis Preparations

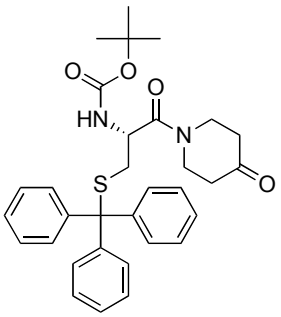
NMR. ^{1}H and ^{13}C spectra were measured with a Bruker AVQ-400 (400 MHz) spectrometer. Chemical shifts are reported as δ in units of parts per million (ppm) relative to chloroform-*d* (δ 7.26, s). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), p (pentet), m (multiplet), br (broadened), or app (apparent). Coupling constants are reported as a *J* value in Hertz (Hz). The number of protons (n) for a given resonance is indicated nH, and is based on spectral integration values.

PAGE Analyses. For protein analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-

PAGE) was carried out on a Mini-Protean apparatus from Bio-Rad (Hercules, CA), following the general protocol of Laemmli[1]. All protein electrophoresis samples were heated for 10 minutes at 100 ºC in the presence of 1,4-dithiothreitol (DTT) to ensure reduction of any disulfide bonds. Gels were run for 5 minutes at 30V and 70-90 minutes at 120V to allow good separation of bands. Commercially available markers (Bio-Rad) were applied to at least one lane of each gel for assignment of apparent molecular masses. Gel imaging was performed on an EpiChem3 Darkroom system (UVP, USA). Protein conversion was estimated from standard optical density measurements of the observed gel bands with ImageJ software (NIH, rsb.info.nih.gov/ij/) after staining with Coomassie Brilliant Blue R-250 (Bio-Rad).

Gel Permeation Chromotogaphy (GPC). GPC was performed using a Waters system including Waters 515 pump, a Waters 717 autosampler, a Waters 996 Photodiode Array detector (210-600 nm), and a Waters 2414 differential refractive index (RI) detector. GPC was performed on two SDV Linear S (5 mm) columns (Polymer Standards Service, 300 x 8 mm) at 1.0 mL/min using DMF with 0.2% LiBr as the mobile phase and linear PEO (4,200-478,000 MW) or PMMA (1,000-220,000 MW) as the calibration standards. The columns were kept at 70 °C.

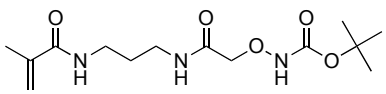
Experimental



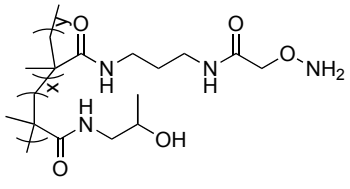
***tert*-butyl 1-oxo-1-(4-oxopiperidin-1-yl)-3-(tritylthio)propan-2-ylcarbamate (S1).** To 10 mL of DMF was added N-Boc-S-trityl cysteine (Nova BioChem) (1.0 g, 2.1 mmol) and carbonyldiimidazole (0.42 g, 2.5 mmol). The resulting solution was allowed to stir for 30 min while CO_2 evolved, at which point 4-piperidone hydrochloride monohydrate (0.43 g, 2.8 mmol) was added. After an additional 5 h of stirring, 4-piperidone hydrochloride monohydrate had completely dissolved. The reaction was seen to be complete by TLC. DMF was removed by rotary evaporation and the resulting yellow oil was dissolved in 20 mL EtOAc. The solution was washed with three 50 mL portions of water, followed by two 50 mL portions of a 0.1 M $NaHSO_4$ solution, 30 mL of saturated $NaHCO_3$ solution, and 50 mL of brine. The organic layer was dried with Na_2SO_4. Purification by flash chromatography (CH_2Cl_2: 5% MeOH) afforded 0.74 g of **S1** as a white solid (63% yield): TLC: (CH_2Cl_2: 5% MeOH) R_f = 0.72. ^{1}H NMR (400 MHz, $CDCl_3$): δ, 1.46 (s, 9H), 2.09 (m, 1H), 2.47 (m, 3H), 2.64 (dd, 1H, J = 8.0), 3.41 (m, 1H), 3.59 (m, 1H), 3.74 (m, 1H), 3.92 (m, 1H), 4.54 (d , 2H, J = 8.0), 6.96 (d , 2H, J = 8.0 Hz), 7.24-7.34 (m , 9H) 7.45 (d, 6H, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$), δ, 28.4, 34.7, 40.8, 41.2, 41.5, 44.0, 67.1, 80.1, 126.9, 128.1, 129.6, 155.0, 169.7, 206.5. HRMS (FAB) calculated for $C_{32}H_{36}N_2O_4S_1$ ([M+Li]) Expected

551.2559, Found 551.2556.

1-(2-amino-3-mercaptopropanoyl)piperidin-4-one (3): The Boc and trityl protecting groups were removed from **S1** by sequentially adding 0.9 mL of trifluoroacetic acid (TFA), 0.05 mL of H_2O and 0.05 mL of triisopropylsilane (TIPS) to an appropriate amount of solid for use in subsequent chemistry. Upon addition of TFA the solution became amber, and with addition of TIPS a white precipitate formed. The volatile components were removed by evaporation under a stream of N_2. To the dried product another 1.0 mL of TFA was added and again evaporated. The dried material was resuspended in 2.0 mL of *di* H_2O and any residual precipitate was removed by filtration (0.45 micron syringe filter). The clear solution was lyophilized to partially remove H_2O until 0.5 mL remained. This proved to be essential as complete lyophilization caused the formation of a white solid that when dissolved would precipitate out of solution if taken to a pH above 3. Presumably, polymerization occurs via imine formation upon drying and loss of TFA. In order to prevent this, a small amount of TFA needs to remain in solution.

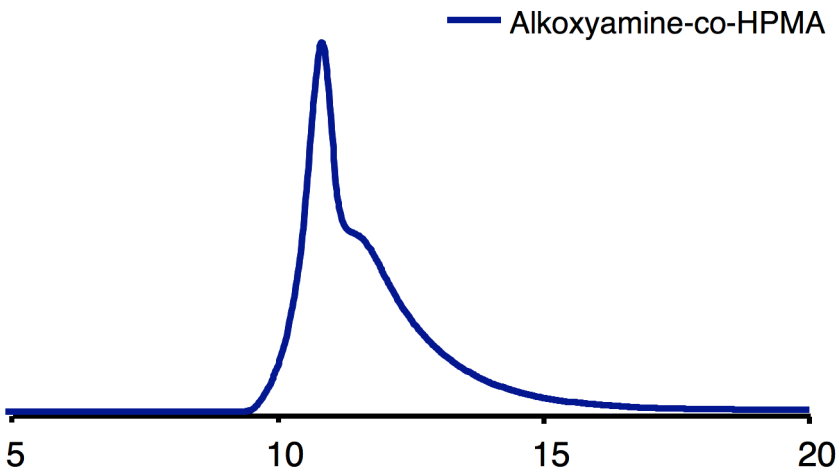


***tert*-butyl 2-(3-(2-methylprop-2-enamido)propylamino)-2-oxoethoxycarbamate (8).** A 50 mL round-bottom flask was charged with a supension of *N*-(3-aminopropyl)methacrylamide hydrochloride (0.3 g, 1.6 mmol) (Polyscience Inc, Warrington, PA, polysciences.com) and *N*-Boc-O-(carboxymethyl)-hydroxylamine NHS-ester[2] (0.55 g, 1.9 mmol) in CH_2Cl_2 (10 mL). The solution was cooled to 0 °C and *N*,*N*-diisopropylethylamine (0.55 mL, 3.3 mmol) was added dropwise. The mixture was stirred vigorously for 30 min, at which point all solids had dissolved. The solution was then allowed to warm to rt and the reaction continued for 3 h. The solution was diluted with 50 mL of CH_2Cl_2 and washed with three 20 mL portions of water followed by 30 mL of saturated $NaHCO_3$ solution and 50 mL of brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated. Purification by flash chromatography with first (EtOAc:Hxns, 1:1) followed by pure EtOAc afforded a clear, viscous oil (0.273 g, 52%). TLC: (EtOAc: Hxns , 1:1) $R_f = 0.1$. ^{1}H NMR (400 MHz, CD_3OD): δ, 1.50 (s, 9H), 1.78 (p, 2H, $J = 6$), 2.12 (s, 3H), 3.33 (q, 4H, 6.8), 4.28 (s, 2H), 5.39 (s, 1H), 5.73 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD), δ, 17.6, 27.2, 28.8, 36.2, 75.1, 81.6, 119.3, 138.9, 158.3, 169.7, 170.2. HRMS (FAB) calculated for $C_{14}H_{25}N_3O_5$ ([M+H]) Expected 316.1869, found 316.1872.



Aminooxy-methacrylamide-co-HPMA (10) . Azobisisobutylonitrile (AIBN) (Sigma-Aldrich, St. Louis, MO, sigma-aldrich.com) was recrystallized from pure EtOH before use. Polymerization was conducted using 50:50 molar ratio of **8**: *N*-

(2-hydroxypropyl)methacrylamide (HPMA) (Polysciences) and weight percent 12.5:0.6:86.9 Monomers:AIBN:CH_3OH. **8** (426 mg, 1.35 mmol), HPMA (193 mg, 1.35 mmol), and AIBN (29 mg, 0.19 mmol) were added to a clean scintillation vial. Methanol (4.31g, 134 mmol), which had been previously sparged with N_2 for 30 min, was added and the components were dissolved while a stream of N_2 was bubbled through the solution for 5 min. The vial was then sealed under N_2 and placed in a 50 °C oil bath for 24 h. The polymer was recovered from the reaction mixture by two subsequent precipitations from CH_3OH into 50 mL diethyl ether (Et_2O) followed by centrifugation. Samples were then dissolved in 4 M HCl for 6 h. Samples were purified by exhaustive dialysis in deionized water using Snake Skin Dialysis Tubing (3500 MWCO) (Pierce, Rockford, IL, piercenet.com). The material was then subjected to ultrafiltration using 100 kD MWCO membrane (Millipore) and lyophilized to afford the final polymer. (0.532g, 82%). Analysis of the ^{1}H-NMR spectrum showed the integration of peaks A:B to be 1.0 : 0.55 (Figure S2) confirming an equimolar ratio of monomers.



Standard	Mn	Mw	PDI
Poly(ethylene oxide)	56600	86600	1.53
Poly(methyl methacrylate)	75600	132000	1.75

Figure S1. GPC Analysis. GPC trace for **10** is shown in above along with relevant numbers for both Poly(ethylene oxide) and Poly(methyl methacrylate).

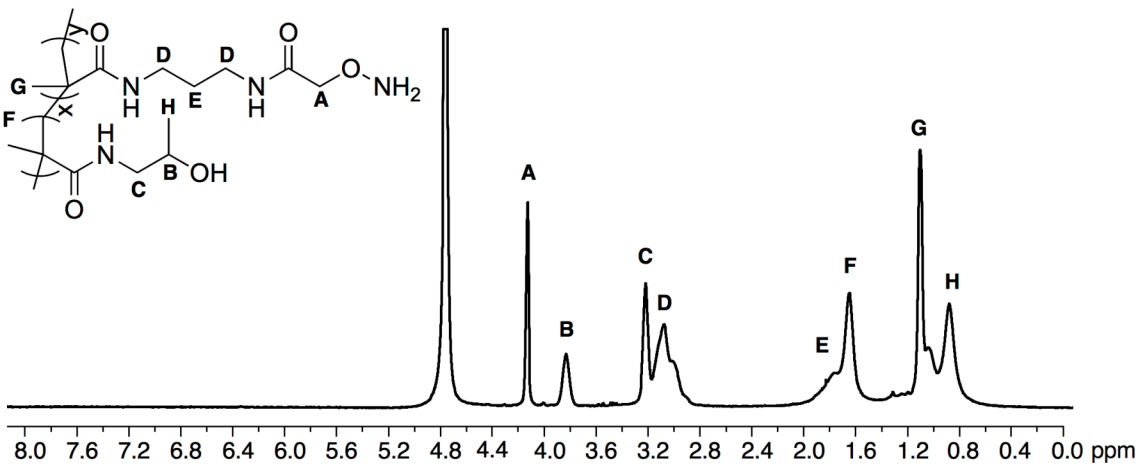


Figure S2 . NMR Analysis of **10**. ^{1}H-NMR spectra of **10** is shown with all relevant peaks assigned. Comparison of integration values of A and B provided accurate assessment of copolymer ratio.

Expression of meGFP. meGFP plasmids were transformed into *E. coli* Tuner^{TM} cells BL21 (DE3) pLysS (Novagen, CA) via electroporation utilizing a Micro-Pulser (Biorad, CA) and plated on LB Agar Plates containing ampicillin (100 μg/mL). Cells were grown in 500 mL Luria Broth (LB) containing ampicillin (100 μg/ml) at 37 °C until an optical density (OD) of 0.5 was observed at 600 nm. meGFP expression was induced by the addition of 0.1 mM isopropyl-ß-D-thiogalactopyranoside (IPTG). Cultures were grown for an additional 16 h at 25 °C. The cells were then spun down for 20 min at 7,000 rcf at 4 °C. Protein expression was verified by the presence of a green fluorescent cell pellet as well as the identity of the desired protein band by SDS-PAGE. The cells were then re-suspended in 10 mL of Lysis Buffer (0.02 M Tris, 0.15 M NaCl, 5 mM EDTA, pH 8.0) by vortexing. The cells were lysed by sonication using a Branson Digital Sonifier (VWR Scientific) for 20 min with a blunt ended tip. Debris was removed by centrifugation at 8,000 g for 1 h to give a transparent green solution.

General Procedure for Modification of C-terminus and Purification of meGFPs

The production of meGFP is a modified protocol.[3] Briefly, meGFP was purified utilizing a 25 ml fritted column loaded with 5-10 mL of Chitin Resin (New England Biolabs, neb.com). Lysate was washed over the resin under mild suction. The resulting resin-bound protein was washed with 100 mL of Wash Buffer (Tris 0.02 M, NaCl 0.5 M, EDTA 1 mM, pH 7.5). Binding to the column was confirmed by SDS-PAGE analysis of the eluent and by the green color of the resulting chitin. A 20 mL solution containing 50 mM mercaptoethane sulfonate (MESNA) (**eGFP**) or a solution of 50 mM MESNA and 5 mM **3** in Wash Buffer (pH adjusted to 7.5) was flowed over the resin bound protein using suction. The column bed was allowed to stand in this solution at RT for 16 h with protection from light. Protein was eluted from the column with addition of 20 mL of Wash Buffer. Purified protein was then buffer exchanged into 25 mM phosphate buffer (pH 6.5) (PB), using Amicon Ultra 15 mL 10,000

MWCO (Millipore) centrifugal ultrafiltration membranes. (**Note**: Due to the possibility of intermolecular thiazolidine formation, it may be possible for EPL with this particular molecule to result in the attachment of a small oligomer of the molecule at the C-terminus. This result has not been observed and exposure to an alkoxyamine gives only one modification product.)

General Procedure for Modification of N-terminus of meGFPs

This procedure is a modification of previously reported[4] method for modification of meGFP. Briefly, meGFP samples in 25 mM phosphate buffer (pH 6.5) PB with concentrations ranging from 10-50 μM were mixed 1:1 with a solution of 20 mM pyridoxal-5'-phosphate (**4**, PLP) in PB for a final concentration of 10 mM PLP and 5-25 μM meGFP. Samples were reacted overnight (16 h) and PLP was removed by 4 rounds of ultrafiltration (10,000 MWCO, 15 mL).

Procedures for Polymer Attachment

PEGylation: Samples of meGFP were buffer exchanged into PB. A stock solution of 0.1 M, 2 kD, alkoxyamine-poly(ethylene glycol)[4] was created in PB and 1 μL was added to 9 μL of meGFP (5 μM) with vigorous pipetting to ensure mixing. The reaction proceeded for 16 h and was visualized by SDS-PAGE.

Attachment of 10: *SDS-PAGE conditions:* **10 (**Alkoxyamine-co-HPMA) samples were dissolved in PB at a concentration of 80 mg/mL and mixed 1:1 with samples of meGFP (35 μM). Samples were reacted for 16 h and visualized by SDS-PAGE. The samples were stained with SYPRO Orange.

Ultrafiltration retention conditions: To samples of 500 μL of 25 μM meGFP (**2a, 2b, eGFP, 2**) was added directly 20 mg of **10** as a solid. Samples were then allowed to react for 16 h. And the absorbance at 488 nm (A_{488}) was measure to obtain initial protein concentration (**I**). Samples were then subjected to 3 rounds of ultrafiltration (100K MWCO, Millipore). The retenate was collected, diluted with 500 μL PB and the A_{488} was measured to obtain the retenate concentration (**R**). The percent retention was calculated by the equation (**I**-**R**)/**I** x 100 = percent retained. The error bars represent three independent measurements.

General Procedure for Gel formation

Samples of meGFP **2** 25-40 μM were concentrated by ultrafiltration (10,000 MWCO) to a minimum amount (~10 μL) and **PB** was added to bring meGFP final concentration to 92 mg/ml. 20 μL aliquots of this solution were prepared, and to each sample was added 10 μL of a stock solution of **10** at 370 mg/ml. The samples were mixed vigorously using pipet. Upon mixing the initially clear solution became cloudy. Reactions were allowed to

proceed for 24 h while ensuring the pre-gel mixture did not dry out at any point. After 24 h, 500 μL of **PB** were added to each aliquot and the mixture was allowed to redissolve for 2 h. Unreacted protein was then removed by ultrafiltration (100 kD MWCO) by repeating the wash process 3 times in total, though subsequent washes required only 1 min for dissolution. The solution was spun to a volume of 10 μL for every 1 mg of meGFP and the retenate was removed. The viscous solution was pipetted onto a Teflon Sheet (United States Plastic Corporation Lima, Ohio usplastics.com) so as to avoid air bubble formation. Any remaining air bubbles were meticulously removed with a 10 μL micro pipet tip. The gel was allowed to dry in air at rt for 16 h. The gel was seen to turn from a rich deep green (Lime, RBG (0, 255, 0)) to a lighter yellow/green (Electric Lime, RGB (191, 255, 0)) upon drying. Gels were then stored in a dessicator until further use.

Gel forming conditions effect on meGFP fluorescence and stability

Samples of meGFP possessing native termini, denoted (**eGFP**), were subjected to identical gel forming conditions, but were not washed 3 times. Instead samples were dried for 16 h and then redissolved in PB. The total fluorescence of these samples was compared to meGFP samples remaining in solution. The total fluorescence of samples subjected to the gel forming conditions was seen to be 90% of the total fluorescence of the solution samples. This demonstrates nearly perfect retention of fluorescence (and therefore structure) during the gel formation. Some of the loss of fluorescence can be attributed to protein loss during ultrafiltration. The Microcon Spin concentrators have a known retention of 95% for α-Chymotrypsinogen (1 mg/mL)[5]. Fluorescence spectrum of meGFP were taken (λ_{ex} = 488 nm, λ_{em} = 508-700 nm). Integration of these spectra provide the measure of total fluorescence. Integration was done using Kaleidagraph (synergy.com). Bars represent values from three separate experiments.

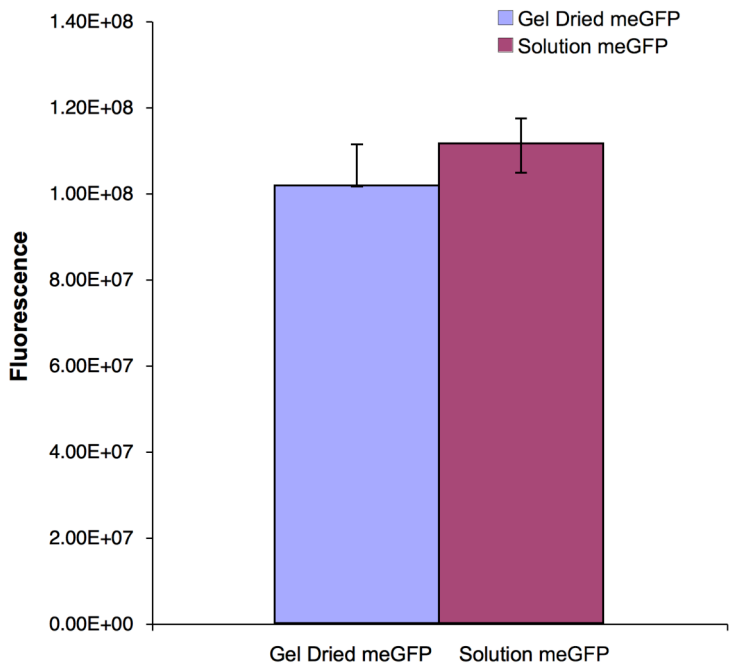


Figure S3. Assessment of meGFP stability upon drying. Total Fluorescence values were aquired by integrating the area under fluorescence spectra. Error bars represent values from 3 separate measurements.

Swelling Profile of Hydrogels

A swelling profile was created by monitoring the volume swelling ratio Q vs. time. Q is defined as V/V_0: the ratio of the swelled volume to the original dried volume. Since gels were cast as circular pieces with a small height. They were approximated as cylinders. So, $V = \pi r^2 h$ and thus the ratio of $V/V_o = r^2h/r_0^2h_0$. We measured the gel both from the top down and from the side to ensure accurate swelling measurements. We calculated Q two different ways. For "Top Down" measurements, our calculations were based on the assumption of isotropicity, and were based only on the diameter; calculating Q as r^3/r_0^3. For "Side On" measurements, we calculated based on the assumption of axiosymmetry, which is true for all but a short period at the very beginning of gel swelling. Thus, we measured both the diameter observed from the side and the height of the gel fragments and then computed Q as $r^2h/r_0^2h_0$. This comparison of measurements was performed so as to give a complete picture of both types of swelling analysis. It is clear from Figure S4 that the "Top Down" method slightly overestimates overall gel swelling. For later experiments however, it was much easier to calculate swelling based on the "Top Down" in which fluorescence was also being meaured, since most of the material is only visible from the top. This comparison however serves a conversion between the two types of measurements should anyone want to compare. Measurments of the swelling gels were conducted over a time course of 6 h. "Top Down" measurements were performed using an Epichem Darkroom 3 digital CCD camera with an exposure time of 0.214 sec for each image. For "Side On" measurements, images were collected using a USB Webcam (Sony Eye Toy), placed directly next to

the samples. Gels were placed on a fixed surface relative to both cameras and illuminated from above with UV light at 365 nm. Gels were placed on a Teflon sheet and submerged in 50 μL of Gibco PBS. Images were captured every 2 min for the first 120 min and every 60 min thereafter. Images were analyzed by application of the ruler tool in Adobe Photoshop to measure the diameter of the gel. Experiments were performed in triplicate (Figure S4). Error bars represent the standard deviation of 3 seperate measurments. Images have not been adjusted in any way.

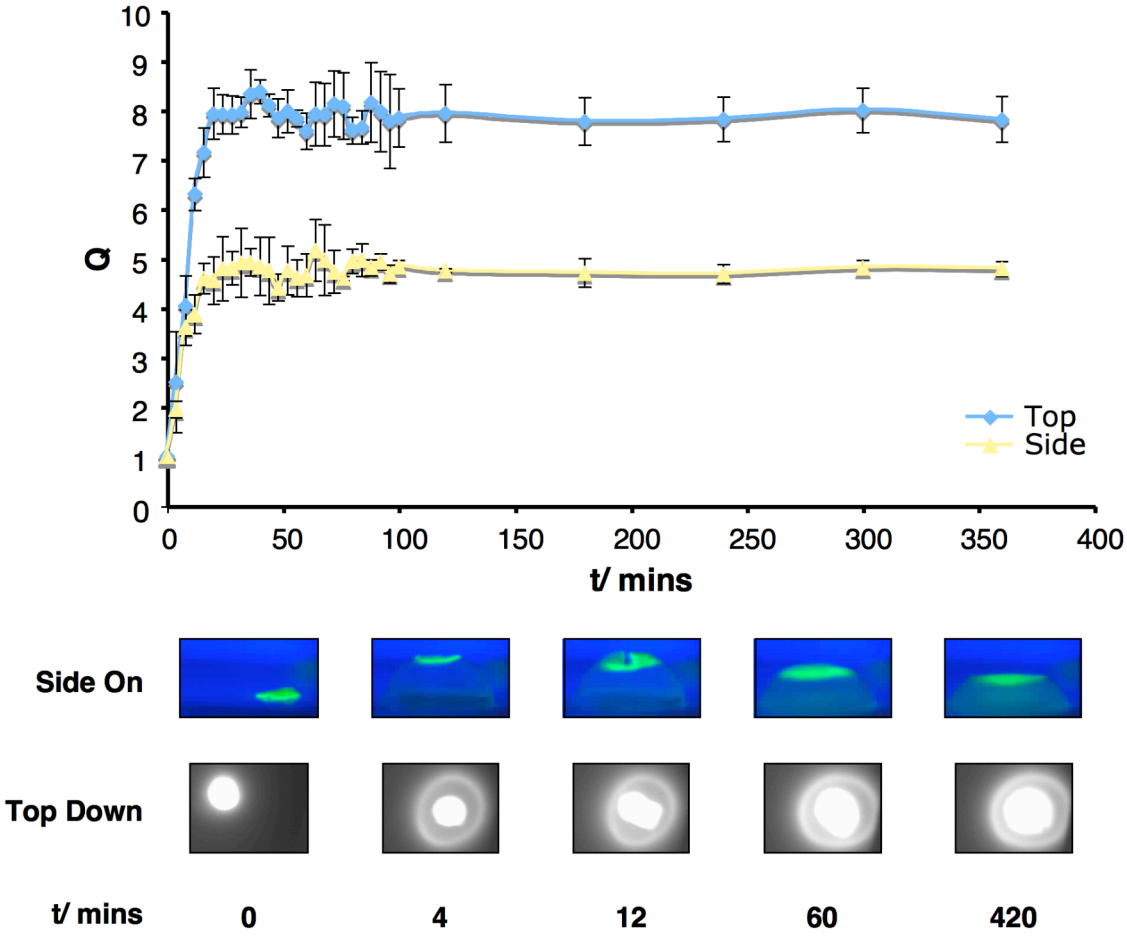


Figure S4. Swelling Profile of Hybrid Hydrogels. Error bars indicate the standard deviation derived from three measurements. Gel images are one of the three gels used in the measurements.

Gel Properties

pH sensitivity

Gels were placed in micro-petri dishes, submersed in Gibco PBS and allowed to swell to equilibrium. This PBS was then removed via micro-pipet and replaced with PBS of a different pH. The pH of PBS solutions was adjusted by the addition of 5 M HCl. Gels were allowed to equilibrate with each pH solution over a period of 60 minutes. Experiments were performed in triplicate, and two gels are shown in Figure 5. Samples were cycled through the

entire pH range twice to show the repeatability of the measurement. Fluorescence intensity measurements were performed using an Epichem Darkroom 3 digital CCD camera with an exposure time of 0.500 sec for each image. Gels were placed on a fixed surface relative to the camera and illuminated from above with UV light at 365 nm. No photobleaching was seen to occur in any of the samples or controls, this is presumably due to the low power of the UV lamps used in the experiment. Images seen in Figure 5 were only color adjusted from the monochrome image; no other adjustments or background subtractions were applied. Quantitative data were obtained using ImageJ (http://rsb.info.nih.gov/ij/). Images for quantitation were background subtracted using a rolling ball parameter of 100. Images were measured by circling each gel and using the measurement tool to obtain integrated density values. (Figure S5) These values were then converted to a percentage of the initial fluorescence at pH 6.5. One can see that fluorescence recovery is not complete, but returns to 90% of the original value. It appears the gel can be cycled at least twice and the sensing properties, though slightly diminished, still remain. Interestingly, this slight hysterisis provides a type of "memory" to the gel where by looking at the variation in fluorescence vs. pH one would know something about the previous pH state of the gel. Our guess as to the reason for this "memory" is that some of the protein becomes trapped by the gel matrix in the protonated form thus narrowing the range of sensing capacity for each cycle. Trapping might occur either by a conformational restriction of the protein or a simple restriction of solvent accessibility to a particular area of gel.

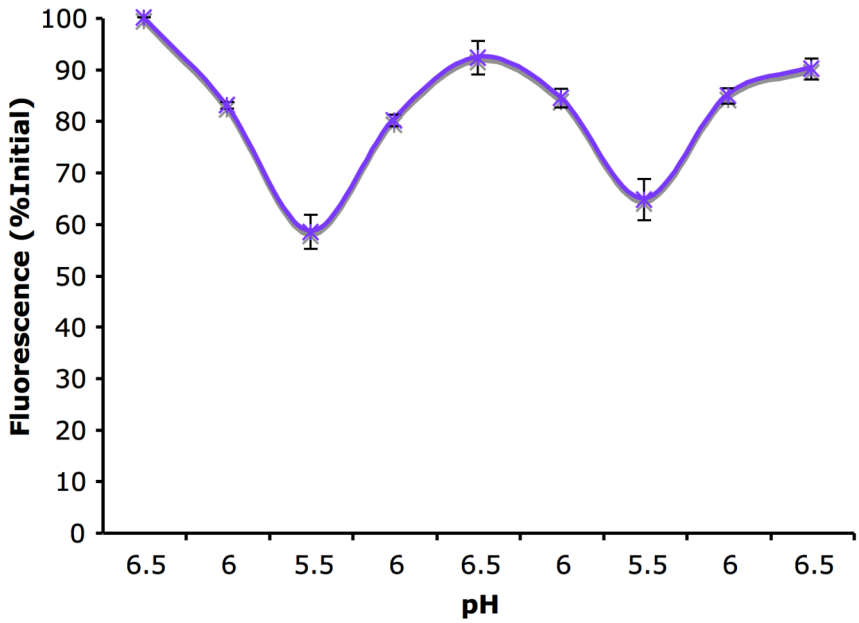


Figure S5. Fluorescence vs. pH Graph. Equilibrium-swelled gels were moved between PBS adjusted to different pHs. The fluorescence was determined by integrated density measurements.

Trypsin Digestion

Gels were placed in micro-petri dishes, submersed in Gibco PBS and allowed to swell to equilibrium. The PBS was then removed via micro-pipet and replaced with 3 mL of 50 mM $CaCl_2$ pH 8.0 or 50 mM $CaCl_2$ pH 8.0 containing 33 mg/ml trypsin which had been heat activated at 42 °C for 20 min. Initial images were captured immediately after the addition of the solutions. The samples were sealed, protected from light and allowed to shake on a rotatory shaker for 3 h. Samples were then imaged in the same manner with one of three samples being shown. (Figure 5). The gel was seen to completely dissolve with only a small fragment being left (Figure S6). To clarify this the gel is visible both by fluorescence and under ambient light. The gel was seen to dissolve completely in both cases and the fluorescence image was chosen to better illuminate the small gel fragment left. This concentration of enzyme is in far excess of what should be necessary to simply digest this amount of eGFP[26] in an attempt to fully dissociate the protein and thus the gel. While there is a decrease in fluorescence between the before & after control samples, it is minimal (<10% change) and most likely due to the vigorous shaking of the gels.

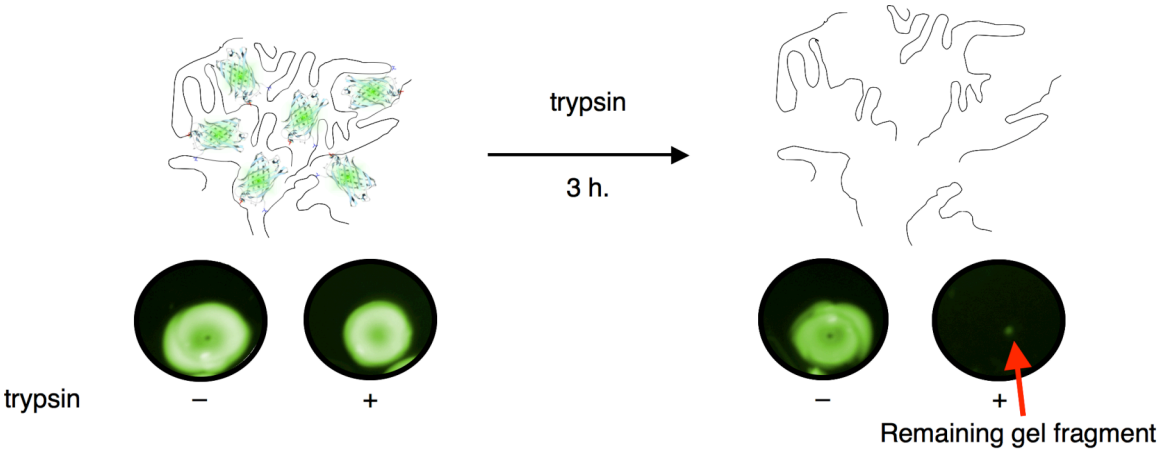


Figure S6. Copy of data presented in Figure 5 with an arrow indicating small amount of remaining gel.

Heating

Gels were placed in a 250 mL sealed centrifuge bottle (Nalgene, USA nalgenelabware.com), submersed in Gibco PBS and allowed to swell to equilibrium. After swelling was complete, the bottle was placed in an oil bath. The bath temperature was adjusted in 5 ° increments starting at 25 °C and ending at 85 °C. Each change in temperature was maintained for 15 minutes to allow the gels to reach equilibrium. Images were captured in the same manner as previously described. Experiments were performed in triplicate. A graph measuring the swelling ratio at different temperatures (Q_T/Q_0 vs T) is shown (Figure S7). In this case the Q measured refers to a "Top Down" measurement. Error bars represent the standard deviation from 3 separate measurements. Fluorescence

measurements were performed using an Epichem Darkroom 3 digital CCD camera with an exposure time of 0.487 sec for each image. Gels were illuminated from above with UV light at 365 nm. No photobleaching was seen to occur in any of the samples or controls, this is presumably due to the low power of the UV lamps used in the experiment. Images seen in Figure 5 were only color adjusted from the monochrome image, no other adjustments were applied. Quantitative data were obtained using ImageJ (http://rsb.info.nih.gov/ij/). Images for quantitation were background subtracted using a rolling ball parameter of 100. Images were measured by circling each gel and using the measurement tool to obtain integrated density values. Fluorescence is displayed as a percentage of the initial measurement at 25 °C. One can see that fluorescence loss occurs throughout heating, but the most rapid loss corresponds to a change in gel volume. It is unclear why exactly the gels shrink, but this does follow with the example shown by Kopecek et al[6] in which destabilization of the protein led to a shrinking of gels composed of HPMA.

It may be possible that oxime exchange with proteins and the polymer chain was playing some role in the size change of our gels. Oximes are dynamic covalent bonds that have been shown to exchange at 60 °C with a rate constant of 3 x 10^{-5} s^{-1} at the pH of our PBS buffer (pH 7.4) [26]. This could mean that over several hours alkoxyamines on the polymer might be exchanging with oximes on the protein, causing a change in gel shape and perhaps contributing to the results that might otherwise be attributed to the protein. Even though our heating took place for 1.5 h, we felt it prudent to insure that oxime exchange was not playing a major role. As such, we subjected our gels to similar conditions as in the heating experiment, but with the addition of 100 mM aminooxy acetic acid (the same reactive group as is on the polymer). Gels were imaged in identical manner to the previous heating experiments with exposure time of 1 sec. Experiments were performed on triplicate gels and the average and standard deviation given. If significant oxime exchange was occuring, then addition of an excess of free alkoxyamine would dissociate the protein from the gel. This would result in some change in shape, if not partial or total dissolution of the gel. After 6 h of heating at 60 °C, no significant change in volume or fluorescence was seen for the samples (Figure S9). While this does not rule out oxime exchange as a contributor to the volume shift, it does not seem to be the major contributor.

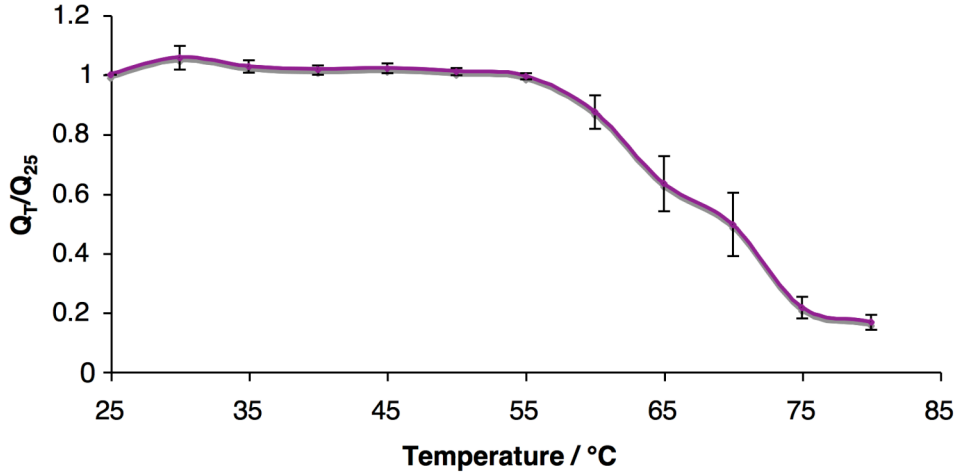


Figure S7. Swelling vs. Heating Graph. Equilibrium-swelled gels were heated in a closed container and gel size monitored by digital CCD camera. Gels begin shrinking at 60 °C and continue until 80 °C coupled with a rapid loss in fluorescence.

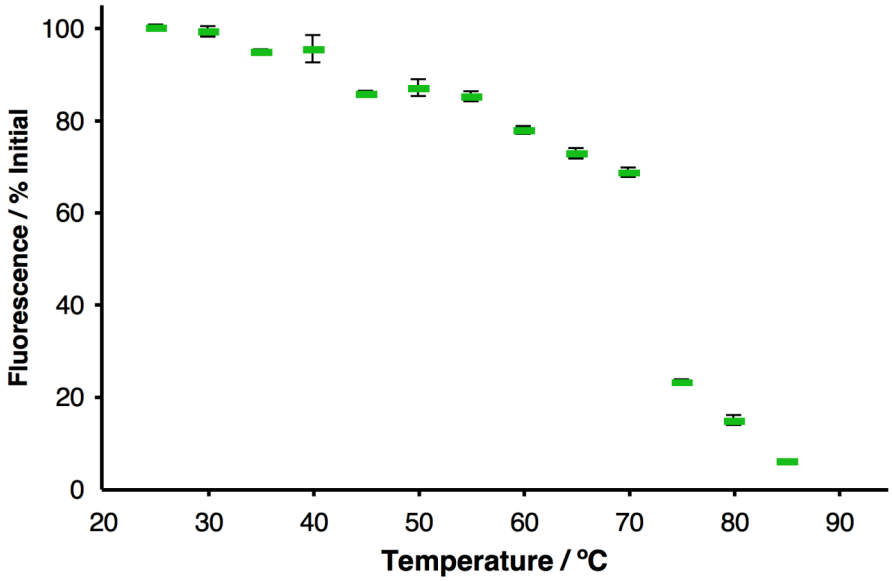
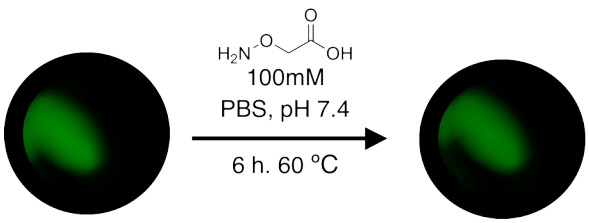


Figure S8. Fluorescence vs. Heating Graph. Equilibrium-swelled gels were heated in a closed container and gel size monitored by digital CCD camera. Gels showed a rapid loss of fluorescence begining at 65 °C



After 6hrs

Fluorescence: 85.98% Std. Dev. 1.273

Q_{6h}/Q_{start}: 1.012 Std Dev. 0.582

Figure S9. Gel denaturation by oxime exchange. The figure depicts gels placed in 100 mM aminooxy-acetic acid. Gels show little change in volume and fluorescence after 6 h of heating at 60 °C

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