Metallothionein-Crosslinked Hydrogels for the Selective Removal of Heavy Metals from Water

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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-F_{254} plates with visualization by ultraviolet (UV) irradiation at 254 nm, vanillin or potassium permanganate stain. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) was performed on an Optima 300 DV (Perkin Elmer). 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 Varian Cary 300 WinUV spectrophotometer (J & M, Germany). Fluorescence measurements were obtained on a Fluoromax-2 spectrofluorometer (ISA Instruments). Centrifugations were conducted with a Sorvall RC 5C plus (Sorvall, USA) for samples greater than 50 mL, a Sorvall LEGEND mach 1.6R for samples between 1 and 50 mL, and an Eppendorf Mini Spin plus for samples less than 1 mL (Eppendorf, USA). Samples were lyophilized using a LAB CONCO Freezone 4.5 (Lab Conco). All images were captured with an Epichemi3 Darkrooom Bioimager (UVP Bioimaging). Large scale production of proteins was performed at the UC Berkeley Fermentation Facility (Berkeley, CA).

Instrumentation and Sample Analysis

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.

ICP-OES. All inductively coupled plasma optical emission spectroscopy (ICP-OES) was performed on an Optima 300 DV (Perkin Elmer). Solutions were digested overnight with nitric acid. Analyzed samples were all run in 5% nitric acid. The instrument was calibrated for all metals with EPA

Method Standard Quality Control Solution 1 (VHG Labs). All dilutions were made using volumetric glassware. All samples were stored in high density polyethylene tubes (HPDE), except for mercury containing samples that were stored in glass. All ICP-OES data were collected as the average of triplicate measurements.

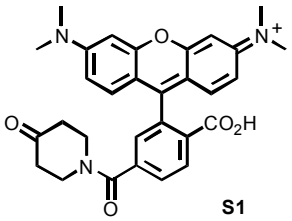
Gel Analyses. For protein analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on a Mini-Protean apparatus from Bio-Rad (Hercules, CA), following the general protocol of Laemmli.[1] All protein electrophoresis samples were heated for 10 minutes at 100 °C in the 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).

Liquid Chromatography. LC-MS analyses were performed using a Waters nanoACQUITY UPLC™ (Milford, MA) liquid chromatograph equipped with C18 trapping (180 µm × 20 mm) and analytical (100 µm × 100 mm) columns and a 10 µL sample loop. Solvent A was 0.1% v/v formic acid/99.9% v/v water and solvent B was 0.1% v/v formic acid/99.9% v/v acetonitrile. Trapping was performed for 5 min with 100% A at a flow rate of 3 µL/min. The elution program consisted of a linear gradient from 15% to 40% B over 90 min, a linear gradient to 95% B over 0.33 min, isocratic conditions at 95% B for 2.67 min, a linear gradient to 1% B over 0.33 min, and then isocratic conditions at 1% B for 8.67 min, at a flow rate of 500 nL/min. The analytical column and sample compartment were maintained at 35 ºC and 8 ºC, respectively.

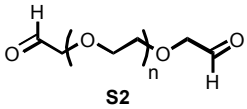
Mass Spectrometry. The LC was connected on-line to a quadrupole time-of-flight (Q-Tof) mass spectrometer equipped with a Z-spray electrospray ionization (ESI) source (Q-Tof Premier™, Waters) and operated in the positive ion mode. The ion source parameters were as follows: ESI capillary voltage 2.2 kV, nebulizing gas (nitrogen) flow rate 800 L/hr, sample cone voltage 30 V, extraction cone voltage 3 V, ion guide voltage 2 V, source block temperature 80 ºC, and nebulizing gas temperature 200 ºC. No cone gas was used. For the analysis of intact, non-digested proteins, sample solutions were withdrawn into a 250 µL Gastight® syringe (Hamilton, Reno, NV) and infused into the ESI probe at a flow rate of 5 µL/min using a syringe pump. The Tof analyzer was operated in "V" mode. Under these conditions, a mass resolving power, R, of 1.0×10^4 was routinely achieved, where $R = m/\Delta m_{50\%}$, m is the mass-to-charge ratio of an ion, and $\Delta m_{50\%}$ is the full width of the mass spectral peak at half-maximum height.[2] External mass calibration was performed immediately prior to measuring samples, using solutions of sodium formate. Survey scans were acquired over the range *m*/*z* 300-1500 using a 1.0 s scan integration and a 0.05 s interscan delay. In the data-dependent mode, up to 8 precursor ions exceeding an intensity threshold of 80 counts/second (cps) were selected from each survey scan for tandem mass spectrometry (MS/MS) analysis. Deisotoping and charge state recognition were used to select 2+, 3+, 4+, and 5+ charge state precursor ions for MS/MS. Collision energies for collisionally activated dissociation (CAD) were automatically selected based on the mass and charge state of a given precursor ion. MS/MS spectra were acquired over the range *m*/*z* 50-2000 using a 1.5 s scan integration and a 0.05 s interscan delay. Ions were fragmented to achieve a minimum total ion current (TIC) of 12,000 cps in the cumulative MS/MS spectrum for a maximum of 3 s. To avoid the occurrence of redundant MS/MS measurements, real time exclusion was used to preclude re-selection of previously analyzed precursor ions over an exclusion width of ±1 *m*/*z* unit for a period of 45 s. Data were processed using MassLynx software (version 4.1, Waters). MS/MS spectra of multiply charged precursor ions were deconvoluted using the MaxEnt-3 algorithm of MassLynx, which transforms multiply charged fragment ions onto a singly charged x-axis, to simplify

spectral interpretation. Theoretical masses of tryptic peptides were calculated using the PeptideMass tool on the ExPASy server.[3]

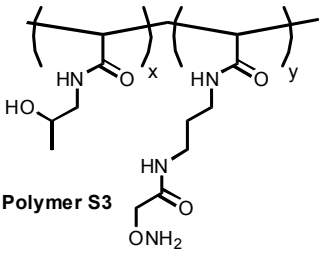
Synthetic Procedures



Tetramethylrhodamine ketone S1. To 30 µL of DMF was added (3 mg, 5.5 µmol) of the *N*-hydroxysuccinimide ester of 5- and 6-carboxytetramethylrhodamine (TAMRA-NHS, mixed isomers, Invitrogen), followed by 4-piperidone hydrochloride monohydrate (2.6 mg, 16.5 µmol). The solution was stirred until both additives had dissolved, and then *N*,*N*-diisopropylethylamine (DIPEA, 5 µL) was added. After an additional 3 h of stirring, DMF and excess DIPEA were removed by rotary evaporation. The resulting deep purple material was then purified using a Strata-X-CW chromatography column (Strata Inc, USA) eluting with Methanol. Monoisotopic molecular mass calculated for $C_{30}H_{30}N_3O_5$ ([M^+]): 512.2185 Da; measured (by FAB-HRMS): 512.2181 (± 0.0005) Da.



Poly(ethylene glycol) dialdehyde S2. This compound was prepared based on a previous literature procedure.[4] Briefly, to 1.9 mL (20 mmol) of acetic anhydride, was added 2.0 g (333 µmol) of polyetheylene oxide 6,000 (PEG 6K) dissolved in 10 mL of dry dimethyl sulfoxide, and the mixture was stirred at room temperature for 2 h. The PEG product was then precipitated by the slow addition of 100 mL of ethyl ether. The precipitate was redissolved in the minimum amount of methylene chloride and was again precipitated with 100 mL of ethyl ether, collected via filtration, and vacuum-dried. ^{1}H NMR (400 MHz, $CDCl_3$): δ, 3.62 (m, 1100H), 4.66 (s, 4H), 9.75 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$), δ, 70.5, 75.2, 200.0.



Alkoxyamine-substituted HPMA polymer S3. The synthesis of this polymer was performed using AIBN as a radical initiator, as described previously.[5] The material used for these experiments had an x:y ratio of 1.0:0.55, as measured by ^{1}H NMR. GPC analysis using PEG standards indicated that Mn=75,600, Mw=132,000, and PDI=1.75.

Construction of PMT-S2AG-pTYB1 plasmid. The Pea-Metallothionein gene was amplified out of pGPMT[6] using primers 5'-GGTGGTCATATGGGATGTGGTTGTGG-3' and 5'-GGTGGTTGCTCTTCCGCATTTGCAGTTGCAAGGGTC-3', placing a Sap I restriction site at the C-terminus and removing the N-terminal Ser. The PCR product was digested with Nde I and Sap I and placed into the pTYB1 vector (NEB, USA), which contains an ampicillin resistance gene. The identity of the ligation product was verified by sequencing. Quick Change Mutagenesis (Stratagene, CA) was performed using primers 5'-GAAGGAGATATACATATGGCTGGCGGATGTGGTTGTGGAAGCAG–3' and 5'-CTGCTTCCACAACCACATCCGCCAGCCATATGTATATCTCCTTC-3' in order to place an AG sequence at the N-terminus. The mutation was confirmed by sequencing.

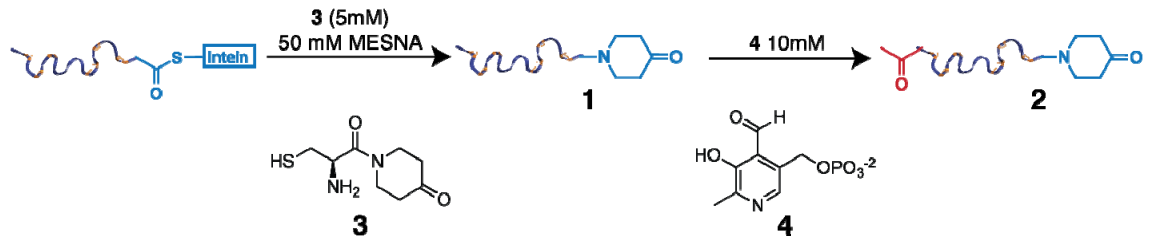
Small Scale Expression of PMT. PMT-S2AG-pTYB1 was transformed into T7 Express Competent *E. coli* (NEB) via electroporation utilizing a Micro-Pulser (Biorad, CA) and plated on LB

Agar Plates (ampicillin at 100 μg/mL). Cells were grown in 500 mL of Luria Broth (LB) containing ampicillin at 100 μg/ml at 37 °C until an optical density (OD) of 0.5 was observed at 600 nm. PMT expression was induced by the addition of 1 mL of 0.3 mM isopropyl-ß-D-thiogalactopyranoside (IPTG). Cultures were grown for an additional 5 h at 30 °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 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 15 min to give a murky brown solution.

Large Scale Expression of PMT. PMT-S2AG-pTYB1 was transformed into T7 Express Competent *E. coli* via electroporation utilizing a Micro-Pulser (Biorad, CA) and plated on LB Agar Plates (ampicillin at 100 μg/mL). Cells were grown overnight in 4 L of Luria Broth containing ampicillin at 100 μg/mL. This 4L was then introduced into 120 L of Luria Broth containing ampicillin at 100 μg/mL in a BioFlo Pro Industrial Fermentor 150 Liter (New Brunswick Scientific Co. Inc). Cells were grown at 37 °C until an OD_{600} of 0.5 was reached, at which point IPTG was added to a final concentration of 300 μM. The temperature was then lowered to 30 °C. Cells were allowed to grow for 5 additional hours and then collected in a Sharples Super-Centrifuge over the period of one hour. The cells were scraped from the sides of the centrifuge and heat sealed in plastic envelopes. Cells were then frozen between two blocks of dry ice. The cells were thawed and lysed by homogenization using a Emulsiflex-C3 Homogenizer (Avestin Inc, Canada) for 3 rounds. Debris was removed by centrifugation at 8,000×*g* for 15 min to give a murky brown solution. Typical expression yielded 400 g of wet cell mass per 120 L of broth.

General Procedure for the Purification of PMT and the Modification of C-terminus (Scheme S1). The production of chemically modified PMT was based on a literature protocol.[7] Before use, all buffers were sparged with nitrogen for at least 1 h to remove dissolved oxygen. PMT-containing lysate was washed over 50 mL of chitin resin (NEB, USA) in a 500 mL centrifuge flask for 1 h, and then removed by centrifugation at 4 °C. The resulting resin-bound protein was washed with 1 L of Wash Buffer (0.02 M Tris, 0.5 M NaCl, 1 mM EDTA, pH 7.5) that was cooled to 4 °C. Binding to the column was confirmed by SDS-PAGE analysis of the eluent. A 150 mL solution of 50 mM MESNA and 5 mM cysteine-ketone **3**[5] in Wash Buffer (pH adjusted to 7.5) was flowed over the resin bound protein using suction. The column bed was then allowed to stand in a minimal amount of this solution at RT for 16 h with protection from light. The protein was eluted from the column with the addition of 50 mL of Wash Buffer. Purified protein was then buffer exchanged into 25 mM phosphate buffer (pH 6.5) (PB), using Amicon Ultra 15 mL 3,000 MWCO (Millipore) centrifugal ultrafiltration membranes. This purification typically yielded 1 mg/L of purified, modified protein.

General Procedure for Modification of N-terminus of PMT (Scheme S1). This procedure is a modification of a previously reported[8] method for the modification of protein N-termini. Briefly, PMT samples with concentrations ranging from 10-50 μM in 25 mM phosphate buffer, pH 6.5 (PB), were mixed 1:1 with a solution of 20 mM pyridoxal-5'-phosphate (PLP) in PB. Samples were reacted at 37 °C overnight (16 h), at which time the excess PLP was removed by 4 rounds of ultrafiltration (3,000 MWCO 15 mL, Millipore)[9].



Scheme S1. Double activation of PMT termini for polymer attachment. PMT was expressed as an Intein-Chitin fusion, purified by chitin affinity chromatography, and reacted with 5 mM **3** and 50 mM MESNA to yield **1** with 95% conversion. To activate the N-terminus, PMT **1** was exposed to 10 mM PLP (**4**) for 18 h, yielding **2** with 40% conversion. Conversions were determined using LC-MS analysis after reaction of the carbonyl groups with *O*-Benzylhydroxylamine ($BnONH_2$) (see Figure S1). PLP = pyridoxal-5'-phosphate, MESNA = mercaptoethane sulfonate, sodium salt.

General Procedure for preparation of Mass Spectrometry Samples (Figures S1 and S2, Table S1). All protein samples were first placed into PB and then reacted with 0.01 M *O*-Benzylhydroxylamine ($BnONH_2$) for 1 h. Samples were desalted and purified using the OMIX C-18 (Varian Inc USA) following their outlined procedure:
http://www.varianinc.com/image/vimage/docs/products/biosolutions/omix/shared/pdfs/OMIXug.pdf.

General Procedure for preparation of Edman Sequencing Samples. 100 µL of PMT samples (50 µM) in Tris Buffer (pH 8) were reduced by the addition of 10 µL of 1 M dithiothreitol (DTT). The solution was also brought to 1% SDS by addition from a 20% stock solution. The resulting solution was heated at 70 °C for 30 mins. The solution was then cooled to RT and 50 µL of 6 M acrylamide was added to bring the solution to 2 M in acrylamide. This solution was place under a nitrogen atmosphere, shielded from light and heated at 37 °C for 1 h. The resulting solution was seperated using SDS-PAGE with the addition of 50 µM thioglycolic acid (TGA) to the running buffer. Proteins were then blotted on a Sequi-Blot PVDF Membrane (Bio-rad, CA). Samples were analyzed directly from the Coomasie Brilliant Blue R-250 stained membrane. Edman sequencing was conducted at the The Division of Biological Sciences Protein Sequencing Facility at the University of California, San Diego, on an Applied Biosystems PROCISE 494HT Protein Sequenator.

Procedures for Polymer Attachment and Gel Analysis

Verification of Protein Reactivity with Polymer S3. Samples that were 20 µM in PMT were reacted with 100 µM BODIPY® FL *N*-(2-aminoethyl)maleimide (Invitrogen, USA) in 0.1 M sodium phosphate, pH 8.0, containing 1 mM EDTA. The reaction proceeded for 5 minutes and was quenched by elution through a NAP-5 Column (GE Healthcare, USA) to remove unreacted BODIPY. The eluted protein (in 25 mM phosphate buffer, pH 6.5) was then reacted with polymer **S3** for 16 h, and a fluorescence emission spectrum was obtained from 505 nm to 700 nm (the integration over this wavelength range was recorded as **I**). Samples were then subjected to 3 rounds of ultrafiltration (100K MWCO, Millipore). The retentate was collected, brought to a volume of 500 µL with PB, and the same fluorescence measurement was performed (yielding an integrated spectral value of **R**). The percent retention was calculated by the equation (**I-R**)/**I** x 100 and graphed in **Figure S3**. The error bars represent ±1 standard deviation from the mean for three independent measurements.

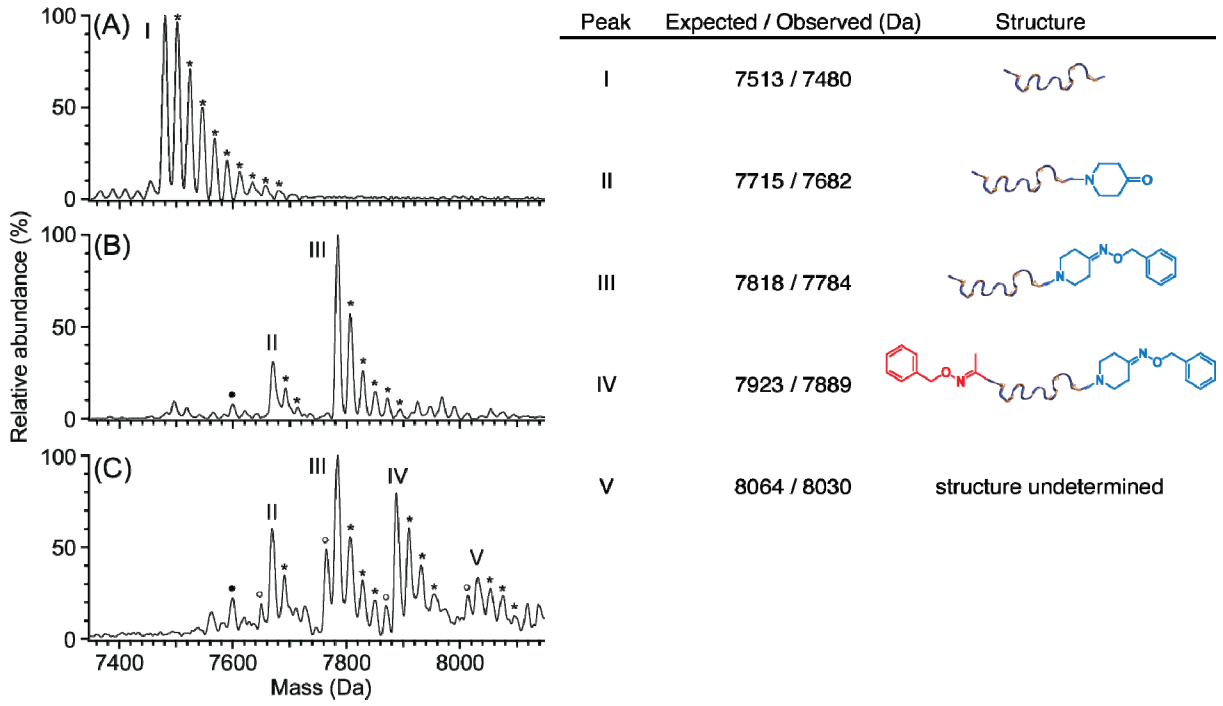


Figure S1. Deconvoluted mass spectra of (A) intact, unmodified PMT, (B) PMT bearing a C-terminal ketone (**1**) after derivatization with $BnONH_2$, and (C) doubly-activated PMT **2**, after derivatization with $BnONH_2$. Peaks are labeled as follows: I: unmodified PMT; II: **1** ; III: oxime resulting from **1** + $BnONH_2$; IV: double oxime from **2** + $BnONH_2$; V: C-terminal oxime of **2** after addition of $BnONH_2$, with an additional PLP adduct at the N-terminus[9]. The open circles denote losses of 18 Da, presumably through dehydration. The asterisks denote additions of 22 Da, which are due to sodium impurities. The closed circle denotes an unknown species that corresponds to unmodified PMT + 120 Da. Conversions were obtained by calculating the total area under each curve.

General Procedure for Gel Formation. The various formulations of gels that were made and tested are denoted 2:1, 1:1 and 1:2. The numbers indicate the mass/mass ratio of polymer **S3**:PMT. To prepare them, samples of PMT **2** (25-40 μM) were concentrated by ultrafiltration (3,000 MWCO) to a minimum volume (~200 μL) and PB was added to bring the PMT final concentration to 50 mg/ml. This solution was then divided into 200 μL aliquots. A 400 mg/mL stock solution of polymer **S3** was then prepared and added in the appropriate amounts to the PMT samples (50 μL for 2:1, 25 μL for 1:1, and 12.5 μL for 1:2). The resulting solutions were vigorously mixed using a pipet, resulting in rapid gel formation. Reactions were allowed to proceed for 16 hrs while ensuring that the mixture did not dry out at any point. After this time, 200 μL of PB were added to each sample, and the mixture was allowed to stand for 2 h to dissolve any unreacted protein. Unreacted protein was then removed by ultrafiltration (100 kD MWCO), repeating the wash process 3 times in total. The mixture was then concentrated to a minimal volume and the retenate was removed. To cast the gels, the mixture was carefully pipetted unto a Teflon sheet (United States Plastic Corporation, Lima, Ohio; usplastics.com) using PFA PureTip (Elemental Scientific, USA), avoiding air bubbles. Any remaining bubbles were carefully removed using a 1 μL micropipet tip. The gel was allowed to dry under a stream of N_2 in a glove bag at RT for 16 h.

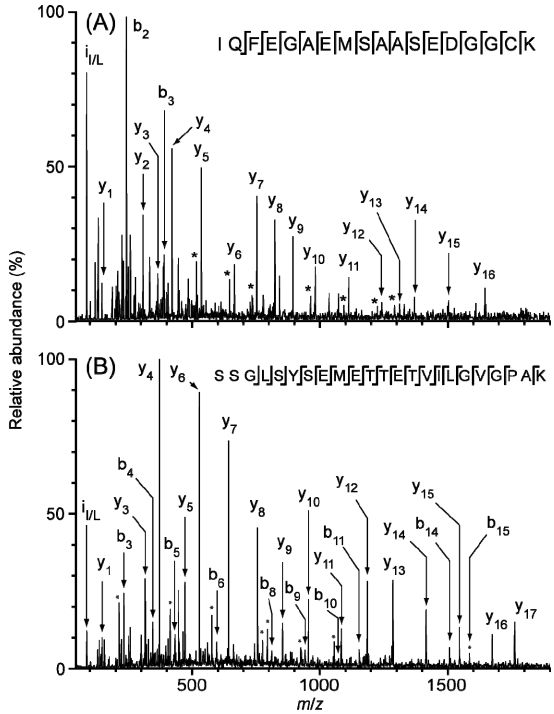


Figure S2. Example MS/MS spectra and corresponding cleavage diagrams resulting from collisionally activated dissociation of the $(M + 2H)^{2+}$ ions of PMT tryptic peptides (A) IQFEGAEMSAASEDGGCK (position 45-62, *m/z* 943.9) and (B) SSGLSYSEMETTETVILGVG PAK (position 22-44, *m/z* 1178.6). The label, "$i_{I/L}$", denotes the singly charged immonium ion corresponding to leucine/isoleucine, at *m/z* 86.1. The asterisks denote neutral losses of 18 Da, presumably due to the elimination of water molecules. All fragment ions are displayed on singly charged x-axes to simplify spectral interpretation.

Table S1

Position	Sequence	Monoisotopic mass (Da)
1-17	AGGCGCGSSCNCGD SC*K	N/A
18-21	CNKR	576.28
22-44	SSGLSYSEMETTETVI LGVGP AK	2355.15
45-62	IQFEGAEMSAASEDG GCK	1885.78
63-75	CGDNCTCDPCNCK	1659.52

Table S1. Sequences of peptides resulting from a tryptic digest of PMT. Positions 18-75 were confirmed by LC/MS and MS/MS analysis. The remainder of the sequence was confirmed by Edman N-terminal sequencing. A "blank" signal was obtained in place of C16, possibly indicating the presence of a chemically modified amino acid residue. This difference corresponds to the overall discrepancy (32 Da) between the theoretical and measured total protein mass.

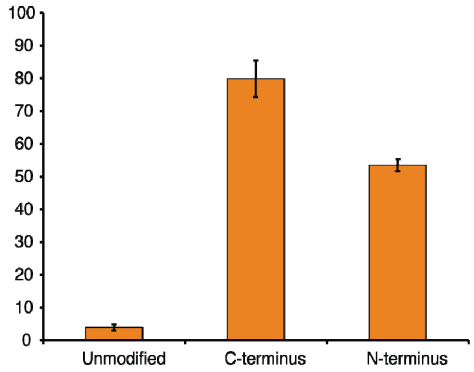


Figure S3. Verification of PMT reactivity with polymer S3. Samples of unmodified PMT or PMT bearing ketones at either the C-terminus or the N-terminus were labeled with BODIPY® FL *N*-(2-aminoethyl)maleimide and then exposed to polymer **S3** for 16 hrs. The total integrated fluorescence of each sample was collected. Free PMT was then removed by ultracentrifugation (100K MWCO) and total integrated fluorescence was measured again. The percentage of protein retained (and thus attached to the polymer) was then calculated. Error bars represent standard deviations from the mean for 3 separate measurements.

Ellman Assay for Cysteine quantification. PMT fractions in the resulting polymers were determined by the Ellman Assay. Immediately prior to each analysis, a solution was prepared by combining 100 µL of Ellman's Reagent Solution (4 mg DTNB in 1 mL Reaction Buffer) and 2.5 mL of Reaction Buffer (0.1 M sodium phosphate, pH 8.0, containing 1 mM EDTA). To this solution were added varying amounts of protein sample, typically in volumes of 10 µL. The solution was sonicated for 30 mins, at which time the absorption at 412 nm was measured. Protein concentration was determined by dividing the absorption value by the molar extinction coefficient (14,150 M^{-1} cm^{-1}) and the number of cysteines that were present in each peptide chain (13). Sample dilutions were chosen such that the measured absorbance values were less than 1. These data are listed in **Figure S4A**.

Swelling of Gels. After storage under N_2, the gels were placed in Gibco PBS pH 7.4 (PBS) and allowed to swell to equilibrium over a period of 24 h. Both dried and swollen gels were weighed with a XP26 DeltaRange Microbalance (Mettler Toledo, USA). The ratios of these values are graphed in **Figure S4B**.

Verification of Crosslinking. PMT samples that were 100 µM were reacted with 50 µM BODIPY® FL *N*-(2-aminoethyl)maleimide (Invitrogen) in 0.1 M sodium phosphate, pH 8.0, containing 1 mM EDTA. The reaction proceeded for 5 minutes and was quenched by elution through a NAP-5 Column (GE Healthcare, USA) to remove unreacted BODIPY. The eluted protein (in 25 mM Phosphate Buffer, pH 6.5) was then concentrated by ultra-centrifugation and subjected to standard gel forming conditions. The resulting gel had an orange tint and was swollen in PBS buffer. Samples were swollen for 24 h, after which fluorescence images were taken to verify that the protein remained bound in the gel (**Figure S5**).

As a control experiment, a gel was also assembled using a 1:1 ratio of polymer **S3** to a sample of PMT that had only been reacted at the C-terminus (**1**). These gels were then allowed to stand for 24 hrs in PBS buffer, during which time complete dissolution occurred.

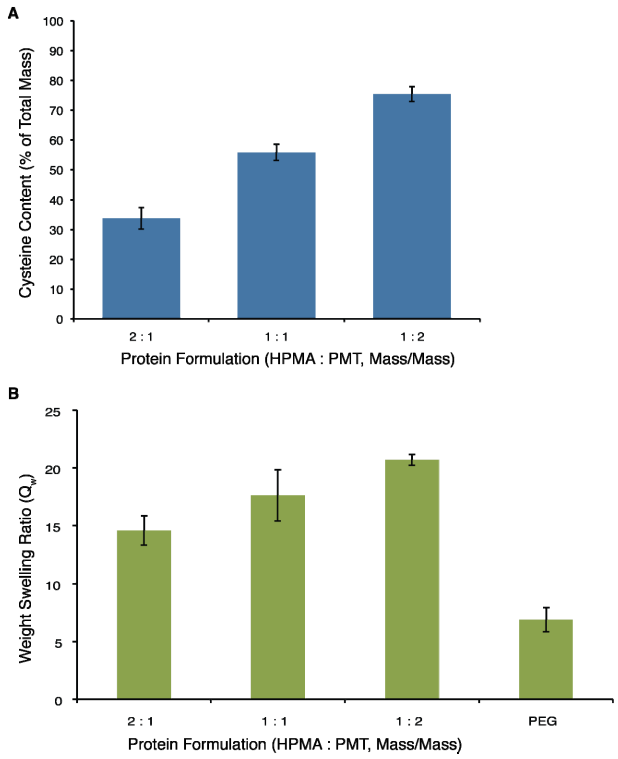


Figure S4. Cysteine content & swelling of PMT-polymer hydrogels. (A) The cysteine content of dried, weighed gel samples (DRY) was assessed by adding gel pieces to Ellman Reagent in buffer. The solutions were sonicated for 30 mins to promote full cysteine reactivity. The quantity of PMT was then calculated (PMT) using the resulting absorbance and the number of cysteine residues in the sequence. This value was divided by the dry weight to obtain percentage of total mass (PMT/DRY x 100). (B) To determine the weight swelling ration, dried samples were weighed (DRY) and then allowed to swell for 24 hrs in PBS. The wet mass was then measured (WET) and the swelling ratio was calculated (Q_w = WET/DRY). Increasing the PMT content of the gel corresponded to a higher swelling ratio, presumably because PMT requires more water to be hydrated than the polymer. HPMA = Polymer **S3**.

General Procedure for Dynamic Volume Change of Material. Swollen gel segments were uniformly cut using a 3 mm biopsy punch (Miltex, USA). A simple twisting motion was required to release the samples. Samples were then placed on microscope cover glass (Fisher Scientific, USA) and their height and width independently measured with Absolute Digimatic Calipers (Mitutoyo, USA). Samples were then placed on graph paper and imaged. The samples were then placed in micro petri dishes (Millipore USA) and covered with 3 mL of a metal solution. Solutions of various metals were prepared by dissolving the corresponding salt, MCl_2, at 10 mM in non-degassed 50 mM Tris buffer, pH 7.5 (the only exception being Co^{2+}, which was prepared from $CoSO_4$). When necessary, the samples were adjusted to pH 7.5 with 5 M NaOH. The samples were exposed to a given metal solution for 5 h with constant shaking on a DS 500 Orbital Shaker (VWR USA) in order to ensure maximum volume changes for all metals, though most changes were seen after 15 min. Samples were then carefully removed from the solution and the excess liquid was removed by blotting with a Kimwipe. Samples were again measured with Digital Calipers and imaged on graph paper. Samples were then quickly transferred back into a solution of 0.1 M EDTA (or for Cu^{2+}, 0.1 DMSA) in non-degassed 50 mM Tris buffer, pH 7.5. Samples

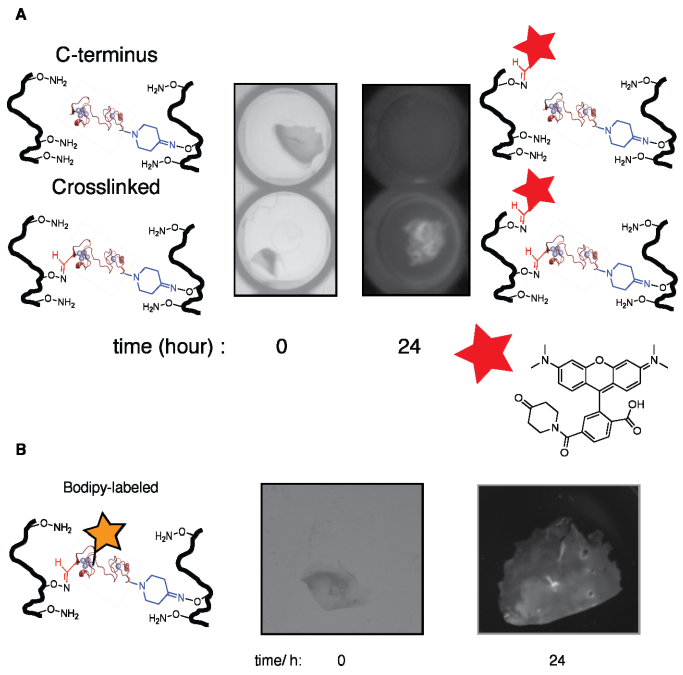


Figure S5. Verification of polymer crosslinking by ketone-modified PMTs. (A) PMT samples bearing ketones at the C-terminus (**1**) or at both termini (**2**) were subjected to gel forming conditions using a 1:1 ratio of polymer **S3** to protein. The gels were then swollen for 24 hrs. In order to assist visualization, the polymer backbones were labeled with fluorescent compound **S1**, which was injected at 50 µM and allowed to react for 8 hrs. Only the use of doubly-modified PMT **2** produced a gel that retained its shape; the polymer prepared with only the C-terminally activated material (**1**) dissolved. (B) PMT samples activated at both termini were labeled with BODIPY® FL *N*-(2-aminoethyl)-maleimide and subjected to gel forming conditions of formulation 1:1. The resulting gel fragment was swollen in PBS for 24 hrs and visualized. It can be seen that the protein is evenly distributed throughout the gel and is retained for 24 hrs.

were allowed to shake for 16 hrs. Finally, the samples were again removed from solution and the excess liquid was removed. The samples were then measured and imaged. Gels subjected to multiple rounds of binding were placed in 50 mM Tris buffer, pH 7.5, for 1 h before the process was repeated. Volumes were calculated by taking the independent measurements of height (h) and radius (r). Approximating the samples as cylinders, the volume, V, was calculated as $V = \pi r^2 h$, and changes in volume were calculated as $r_c^2 h_c / r_o^2 h_o$ (subscript "c" denotes the changed value and subscript "o" denotes the original value). These values are reported for a series of metal ions and polymer formulations in **Figure S6**.

Water Analysis Using the Funnel-Based Actuator. The funnel design was based on a previous report by Daunert.[10] The funnel in this experiment consisted of two pieces of solid acrylic that were held together by two screws. The upper block contained a funnel that spanned 1 inch at the top and held ~2 mL. The opening in the bottom of the funnel was exactly 2 mm in diameter. The screws were threaded at the top and springs were placed along them in between the two acrylic pieces. This allowed for gradual lowering of the upper block onto a swollen, cut, gel segment (1 mm in height) that was placed on the lower block. The screws were tightened until the gel sealed the hole in the funnel, which was tested by the addition of a minimal amount of Tris buffer. A minimal amount of 50 mM Tris buffer, pH 7.5, was placed

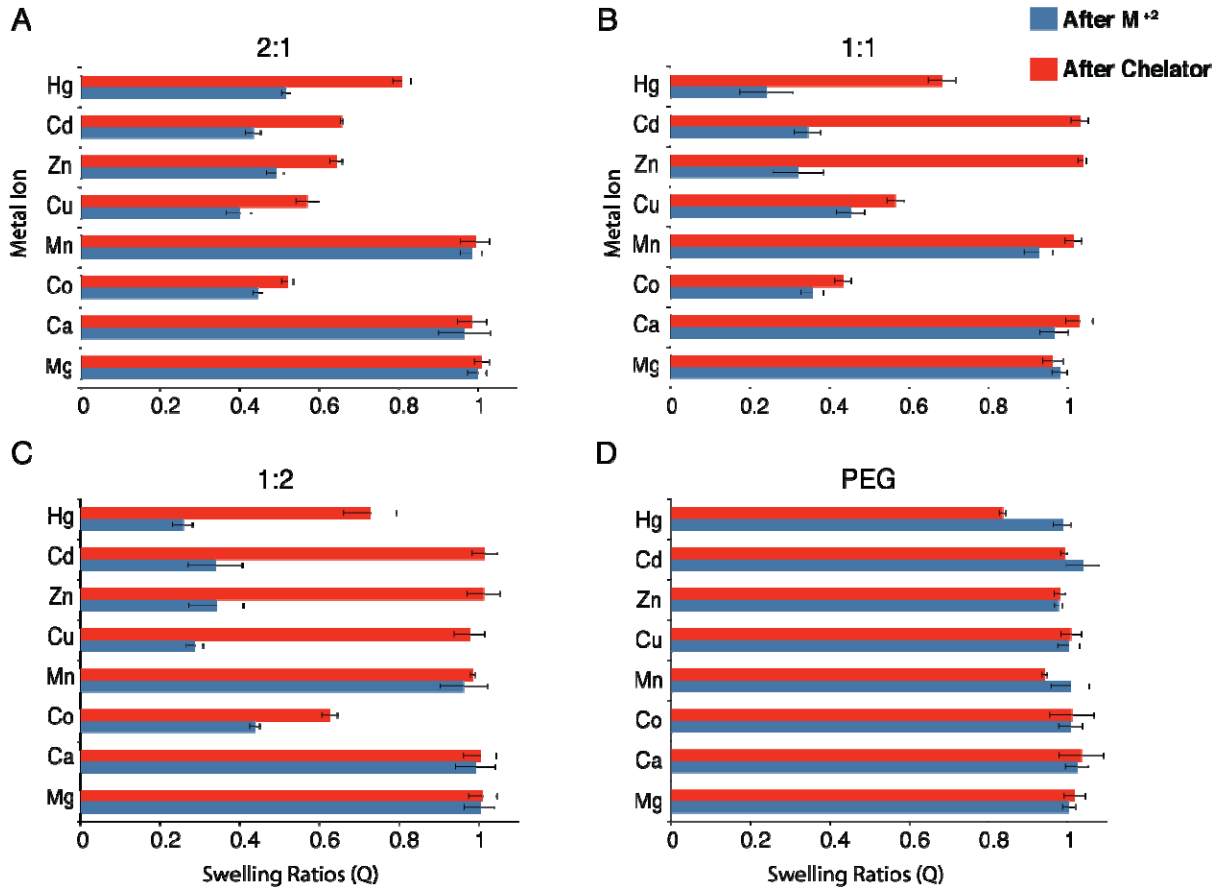


Figure S6. Dynamic volume change of PMT hydrogels in the presence of various metal ions. Swelling Ratios (Q) were calculated from the independently measured heights and radii of the gels, which were approximated as cylinders. The blue bars indicate Q after exposure to the metal ion indicated, and the red bars indicate Q after the removal of the metal with EDTA. Gel formulations are listed as polymer **S3**:PMT **2** ratios (mass:mass), and correspond to (A) 2:1, (B) 1:1, and (C) 1:2. For the samples exposed to Cu^{2+} ions in data set C, DMSA was used in place of EDTA in order to obtain full size recovery. In data set (D), a gel formulated 1:1 with aldehyde PEG 6K (**S2**) was used as a control material.

around the gel to prevent it from drying out and prematurely shrinking. A 1 mL sample of Damon Slough water was then placed on the gel sample and allowed to incubate for 45 minutes. This time point marks the beginning of the supplementary time lapse movies. A 10 µL aliquot of either 10 mM Cd^{+2} or 10 mM Ca^{+2} was slowly injected over a period of 1 minute, after which the gel responded by either allowing the liquid to drain or by retaining the uncontaminated water. Time lapse movies were made using BTV Pro Carbon 5.4.1 (BTV, bensosoftware.com). One frame was recorded every 2 seconds during the experiment and the video is shown at a rate of 30 frames per second (fps); thus, 1 second of the movie = 1 minute of the experiment. The graph of water loss through the funnel was generated by loading the movie into ImageJ (NIH freeware) and evaluating the area of a triangle drawn over the funnel. Data points were collected every 3 minutes and the initial area was set to 1 mL.

Supplementary Movie S1. Time lapse movie of addition of 10 mM Cd^{+2}. Time lapse counter is showing lapse in minutes. First 45 minutes are the Damon Slough water resting on the gel. The yellow tip lowering down is the addition of 10 µL of 10 mM Cd^{+2} for a total concentration of 100 µM.

Supplementary Movie S2**.** Time lapse movie of addition of 10 mM Ca^{+2}. Time lapse counter is showing lapse in minutes. First 45 minutes are the Damon Slough water resting on the gel. The yellow tip lowering down is the addition of 10 µL of 10 mM Ca^{+2} for a total concentration of 100 µM.

Analysis of Damon Slough Water Samples (Oakland, CA). Water samples were collected from Damon Slough (Latitude: 37.75289/Longitude: -122.210599) following EPA Method 1669. *The authors would like to make clear that they had no previous training in water collection and sampling; therefore these values should only be viewed as relative and not absolute levels of metals in the Damon Slough.*

For analysis purposes, aliquots of Damon Slough water were filtered (0.45 µm) and adulterated with ~50 µg/L Zn^{2+}, Cd^{2+}, Cu^{2+} and Hg^{2+} ions using 1 mg/L solutions of the chloride salts. The levels of metal were then determined by ICP-OES. Levels of Ca^{2+}, Mg^{2+} and Na^{+} were measured after a 100-fold dilution. A swollen PMT-hydrogel (7.94 mg dry weight) of formulation 1:2 was placed into 3 mL of spiked Damon Slough water and allowed to absorb metal ions for 16 hrs. Ca^{2+}, Mg^{2+} and Na^{+} were then measured from a 1000-fold dilution of this sample. Zn^{2+}, Cd^{2+}, Cu^{2+} and Hg^{2+} were measured directly from the remainder of the sample. Each value reported is the average of triplicate ICP-OES measurements.

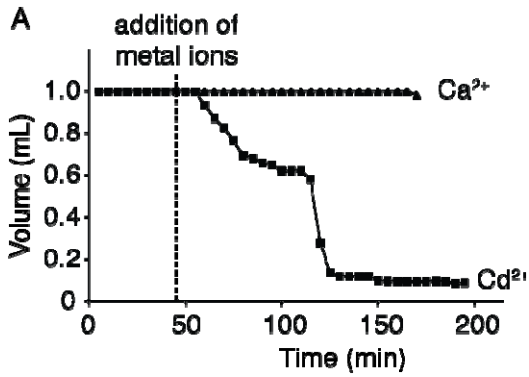


Figure S7. The shape change of the polymer can serve as a valve that responds to the presence of heavy metal ions. A conical vial was constructed with an opening at the bottom which was sealed with the PMT hydrogel, and 1 mL of slough water lacking heavy metal ions was added. Upon addition to 0.1 mM of Cd2+, the resulting contraction of the polymer opened the valve and allowed the water to escape. The time course of the experiment is shown in the graph on the right. No such change occurred upon the addition of 0.1 mM Ca2+. Such a device could serve as a low-cost test kit for the detection of heavy metal ions, or it could be used to

Table S2

	2 : 1		1 : 1		1 : 2		PEG	
Metal	Loading (mg/cm^3)	Std Dev	Loading (mg/cm^3)	Std Dev	Loading (mg/cm^3)	Std Dev	Loading (mg/cm^3)	Std Dev
Mg	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.00
Ca	0.03	0.01	0.05	0.00	0.01	0.00	0.01	0.01
Co	0.48	0.10	1.03	0.46	1.09	0.22	0.05	0.00
Mn	0.03	0.01	0.02	0.00	0.02	0.01	0.10	0.00
Cu	0.78	0.10	1.12	0.15	1.88	0.17	0.01	0.01
Zn	0.55	0.23	0.78	0.03	1.33	0.23	0.01	0.00
Cd	0.81	0.21	1.36	0.40	2.22	0.41	0.02	0.00
Hg	0.49	0.14	0.51	0.17	1.45	0.08	0.03	0.01

Table S2. ICP-OES quantification of loading values. A table detailing the loading of gel samples by metal for all the different formulations. These data can be compared to the data in Figure S6. In general, gels that contained more PMT led to higher levels of metal binding. Loading was calculated by obtaining the metal concentration (in mg/L) from ICP-OES and multiplying by the volume of EDTA solution (2 mL) to obtain total metal mass. This number was then divided by the measured gel volume ($V = \pi r^2 h$) to obtain the loading. Standard deviations were calculated from 3 independent samples. Each reported ICP-OES value is the average of triplicate measurements.

References

1. U. K. Laemmli, *Nature* **227**, 680 (1970).
2. A. G. Marshall, C. L. Hendrickson, G. S. Jackson, *Mass Spectrometry Reviews* **17**, 1 (1998).
3. http://ca.expasy.org/tools/peptide-mass.html, accessed May 23, 2008.
4. G. V. N. Rathna, *Journal of Applied Polymer Science* **91**, 1059 (2004).
5. A. P. Esser-Kahn, M. B. Francis, *Angewandte Chemie-International Edition* **47**, 3751 (2008).
6. X. Deng, D. B. Wilson, *Applied Microbiology and Biotechnology* **56**, 276 (2001).
7. J. M. Hooker, A. P. Esser-Kahn, M. B. Francis, *Journal of the American Chemical Society* **128**, 15558 (2006).
8. J. M. Gilmore, R. A. Scheck, A. P. Esser-Kahn, N. S. Joshi, M. B. Francis, *Angewandte Chemie-International Edition* **45**, 5307 (2006).
9. R. A. Scheck, M. B. Francis, *Acs Chemical Biology* **2**, 247 (2007).
10. J. D. Ehrick *et al.*, *Nature Materials* **4**, 298 (2005).