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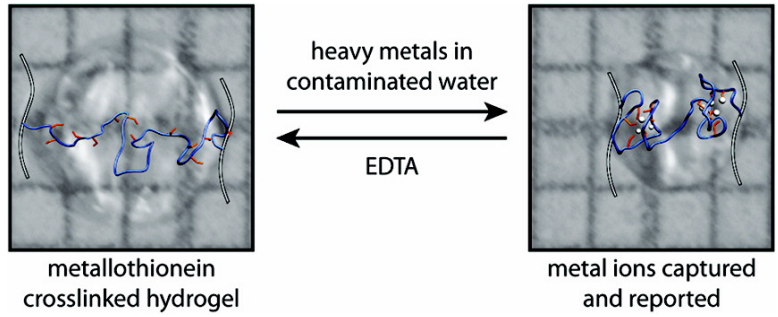

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Metallothionein-Cross-Linked Hydrogels for the Selective Removal of Heavy Metals from Water

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Proteins have evolved to meet a diverse set of materials needs in living systems. In several cases, the unique binding, sensing, catalytic, and structural abilities of these biomolecules have been integrated with nonbiological components, producing polymers and surfaces with properties that are difficult to replicate using more traditional building blocks. Examples of this concept include plastics with enzymatic activity,[1a] thermo- and antigen-responsive materials,[1b,c] supports for tissue engineering and drug release,[1d,e] and peptide- and ion-switchable materials.[1f,g] As new techniques are developed to wed proteins with synthetic components, a growing collection of materials will be available for biomedical, environmental, and energy-related applications.

As a particularly fitting example of this potential, nature offers a series of proteins that are able to sequester heavy metal contaminants that can cause risk to municipal water supplies.[2] These metallothioneins[3] have evolved across several biological kingdoms to bind copper, zinc, cadmium, mercury, chromium, and arsenic ions with dissociation constants as low as 1×10^{-12} M by forming a series of cysteine-lined binding pockets.[4] A particularly important feature of these proteins is their ability to bind submicromolar concentrations of heavy metal ions in the presence of the vast excesses of sodium, calcium, and magnesium ions that typically accompany them. The selectivity and facile production of these proteins could thus render them uniquely effective components of water treatment materials, but the high frequency of cysteines (typically 12−16) in their primary sequence[3] presents a significant challenge for incorporating them into supports and devices in a well-defined manner.

Recently we reported an efficient chemical strategy for the cross-linking of polymer chains through the N- and C-termini of proteins, producing hybrid hydrogels with structural and mechanical properties that are intimately related to the folding state of the biomolecules.[5] As this approach is largely independent of the protein's sequence, it can in principle be used to incorporate almost any polypeptide into a polymer through uniform linkages. In this report, we have exploited the site specificity and cysteine tolerance of this strategy to convert metallothioneins into a recyclable polymer gel that can remove toxic metal ions from environmental water samples, report their binding through changes in the swelling volume, and actuate valves that could result in sample diversion or the addition of treatment additives, Figure 1a. This material also retains the key ability of the protein to bind trace amounts of heavy metals in the presence of innocuous ions, as demonstrated from the removal of cadmium ions from a contaminated sample of slough water.

The basis of our protein incorporation strategy is the introduction of uniquely reactive ketone groups at the N- and C-termini of the peptide chains. This provides chemical handles that can be used to attach the protein to appropriately functionalized polymer chains through oxime formation. To do this, a 75 residue segment of a metallothionien derived from pea plants (*Pisum sativum*)[6,7] was expressed in *E. coli* fused to an intein segment and a chitin binding domain. In addition to facilitating its purification, this construct was used to install a ketone group at the C-terminus of the protein through a native chemical ligation[8] with an appropriately functionalized cysteine, Figure 1b. Analysis of the sample by mass spectrometry indicated virtually complete installation of the new ketone group during this step. Next, we installed another ketone at the N-terminus by exposing the protein to pyridoxal 5′-phosphate, which has been shown previously to effect site-selective transamination under mild conditions.[9] In a recent report,[10] we have shown that this reaction proceeds best with sterically unencumbered N-terminal residues, such as A, D, N, G, and E. In anticipation of this behavior, an N-terminal Ala-Gly sequence had been installed on the protein during expression. At this stage, mass spectrometry indicated that ~38% of the protein had been

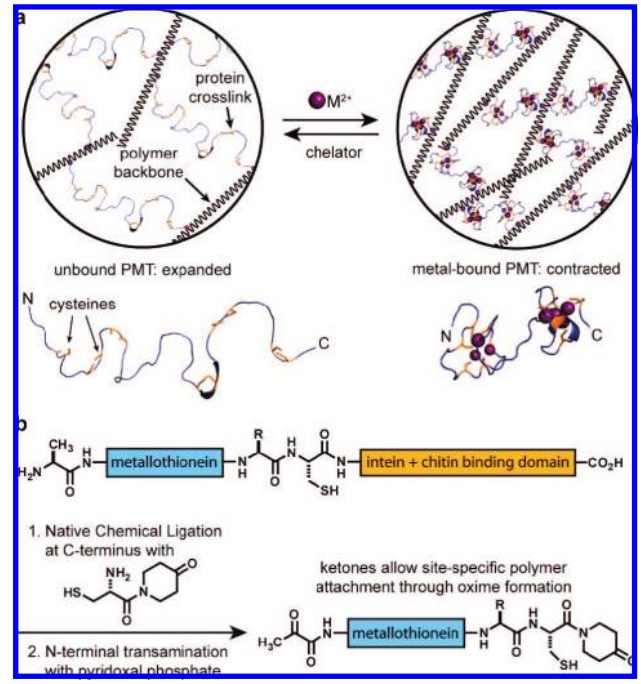

Figure 1. Protein-cross-linked hydrogels for the detection and sequestration of heavy metal ions. (a) Pea metallothioneins (PMTs) can capture toxic metal ions by condensing to form binding pockets. When these proteins are incorporated as the sole cross-links of a polymeric gel, this conformational change results in a bulk contraction of the material. Following isolation, the bound metal ions can be removed by chelators to allow reuse of the polymer. (b) The synthetic route to access these materials involves the introduction of ketones on both protein termini. These groups are then used to cross-link alkoxyamine-substituted polymers through oxime formation.

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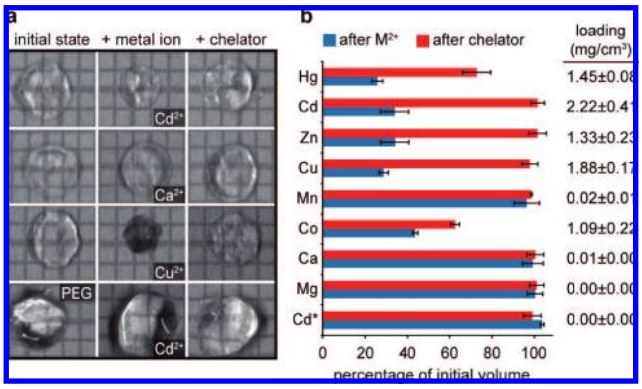

Figure 2. Response of PMT-cross-linked polymers to aqueous solutions of metal ions. (a) Images of gel samples show changes in size upon exposure to 100 mM solutions of toxic metal ions. Cd^{2+} and Cu^{2+} ions lead to significant contraction upon binding, while Ca^{2+} does not. A control polymer that is linked with polyethylene glycol (PEG) chains instead of PMTs does not undergo changes upon exposure to Cd^{2+} ions. (b) The volume of the polymer changes in response to many metal ions (100 mM each, blue bars), represented as a percentage of the starting hydrogel volume. The red bars indicate the volume recovery upon recycling with metal chelators. The quantities of metal retained per unit volume of polymer are listed to the right of each bar, as determined by inductively coupled plasma optical emission spectroscopy (ICP-OES). The Cd* entry was carried out using the PEG-cross-linked control polymer.

converted to the doubly modified sample, with the remaining sample being modified only at the C-terminus (see the Supporting Information, Figure S1). Although both of these components can be incorporated into polymers and can contribute to metal ion binding, only the doubly functionalized material can serve to cross-link the gel. This simple procedure has been used in our laboratory to prepare the modified protein on a 100 mg scale; using larger fermentation facilities, it could potentially be scaled to yield much larger quantities at relatively low cost.

The polymer backbone used in these experiments was synthesized through the radical polymerization of an alkoxyamino-substituted acrylamide monomer, as previously reported.[5] The resulting material had an average molecular weight of 132 kDa and a polydispersity of 1.75, and was highly water soluble. The ability of the polymer chains to undergo conjugation to the ketone-functionalized metallothioneins was verified by exposing them to fluorescently labeled proteins bearing a single ketone at either the N- or the C-terminus, and then quantifying the retention of the protein by ultrafiltration membranes (see the Supporting Information, Figure S3). Upon exposure to doubly functionalized protein, a hydrogel was formed with the protein distributed and retained throughout the material,[11] as evidenced by fluorescence imaging (see the Supporting Information, Figure S5). Considering that the concentration of the protein cross-links will determine the ultimate swelling properties of the material, three different ratios of protein to polymer were explored (see the Supporting Information, Figure S4).

We next characterized the dynamic volume change of the hybrid hydrogel in the presence of a series of metal ions. A 3 mm biopsy punch was used to cut uniform pieces from thin sheets of the polymeric material swollen in phosphate buffered saline (PBS). Digital calipers were used to measure the cut sample's height and width independently. Upon exposure to 10 mM solutions of Hg^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, and Co^{2+} ions the material underwent large reductions in volume, as would be expected from the condensation of the protein cross-links around the metal ions (Figure 2a) We found that there was an increase in volume change and metal binding with the increase of protein concentration (see the Supporting Information, Figure S6 and Table S2), with a 1:2 polymer:

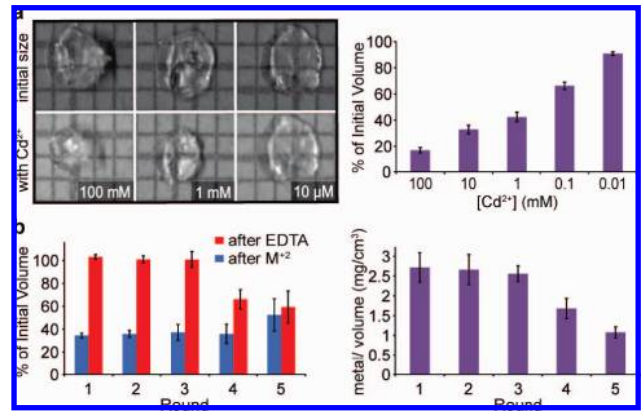

Figure 3. Change in hydrogel size in response to metal ion concentration. (a) Comparison of the gel size before and after exposure to Cd^{2+} ions can be used to estimate metal ion concentration, and can indicate when regeneration of the material is necessary. (b) Through the use of inexpensive chelators (such as EDTA), the gel can be subjected to multiple rounds of reuse.

protein mass ratio providing the highest capacity. All of the materials underwent minimal contraction in the presence of Ca^{2+}, Mg^{2+}, and Mn^{2+}, which are all common background elements in water samples.[12] To confirm that the binding selectivity was solely due to the protein component, we created an equivalently cross-linked material with poly(ethylene glycol) (PEG)-dialdehyde,[13] which serves as a nonfunctional protein substitute. Although a hydrogel was formed, PEG-linked material did not produce an appreciable size change in response to any ion.

After isolation of the gels from the test solutions, it was found that the change in volume could be reversed by the addition of a chelator (such as EDTA)[14] to remove the bound ions in all but the Co^{2+} case. Analysis of the resulting solutions using inductively coupled plasma optical emission spectroscopy (ICP-OES) provided the quantity of metal ions that had been sequestered by the gel (Figure 2b). We found a clear correlation between the amount of metal ions that were bound and the degree of mechanical response that was measured. The material was found to bind Cd^{2+} in the largest amount, followed by Cu^{2+} and Hg^{2+}. In the case of Cd^{2+}, the polymer was found to bind up to 4.5% of its dry weight in metal ions (assuming a density of 1 g/mL and a swelling ratio of 21, see the Supporting Information, Table S2). The PEG material only retained minute levels of the ions, with the interesting exception of Mn^{2+} (see the Supporting Information, Table S2).

Two general types of materials are traditionally involved in treating water contamination: those that actively remove contaminants[15] and others that serve to report their presence.[16] Because the metallothionein hydrogels mechanically couple the protein conformational changes to bulk material properties, they have the useful ability to do both.[17] Samples of the gel were exposed to Cd^{2+} solutions ranging in concentration from 10 μM to 100 mM, and the changes in gel volume were quantified (Figure 3a). Gels contracted their volume in direct relation to the concentration of Cd^{2+}, with a modest volume change of 10% at 10 μM and a nearly 90% change for 100 mM. This feature provides an estimate of the metal ion concentration through simple visual inspection, and can report when the gel is in need of recycling.

Recycling of the gel was examined by a repeated exposure of the material to 10 mM Cd^{2+}, followed by an EDTA solution (100 mM). Samples retained good dynamic range of volume change through the third round of recycling, at which point they exhibited significantly reduced changes in size (Figure 3b). This correlated well with ICP-OES measurements of the EDTA solution, which

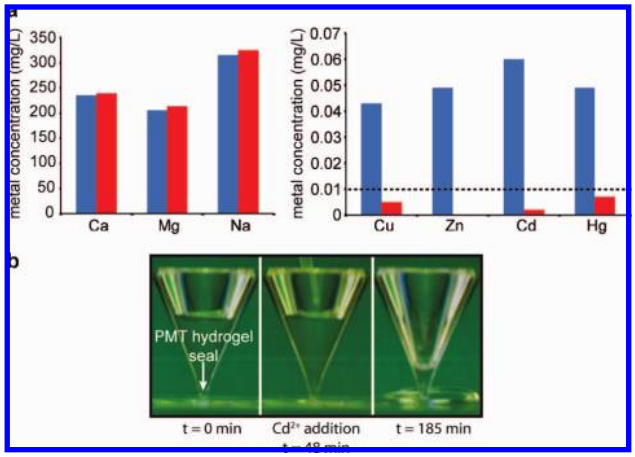

Figure 4. Removal of heavy metal ions from contaminated slough water. (a) The concentrations of metal ions in a 3 mL sample were determined before (blue bars) and after (red bars) exposure to 8 mg of PMT hydrogel. Contaminating quantities of the heavy metals were removed without interference from high concentrations of nontoxic ions found in the water sample. All measurements were taken from a single sample, but are plotted on separate graphs because of the large differences in the *y*-axis values. The dotted line represents limit of reproducible ICP-OES values. (b) 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 that was sealed with the PMT hydrogel, and 1 mL of slough water lacking heavy metals was added. Upon addition of Cd^{2+} to 0.1 mM, the polymer contracted to open the valve and allow the water to escape. No such change occurred upon the addition of 0.1 mM Ca^{2+}.

also indicated a reduction in Cd^{2+} binding after the third round. A possible explanation for this behavior is the oxidation of the cysteines to disulfides, resulting in a loss in volume change and metal binding ability. Nevertheless, these results suggest the material can be used for multiple cycles of water purification.

Unlike many chelators, such as EDTA, metallothioneins have evolved to have little binding affinity for Ca^{2+}, Mg^{2+}, and Mn^{2+} ions. This selectivity makes them more appropriate for the removal of very small quantities of heavy metal contaminants from saline water. To demonstrate this, water was collected from the Damon slough at a point where it enters Arrowhead Marsh in Oakland, CA, to provide a realistic background of these ions. Samples of this water were then contaminated with a mixture of Cu^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+} ions (~50 ppb each), which is above the EPA recommended limits for cadmium and mercury. When placed in this solution for 16 h, the PMT-hydrogel reduced the concentrations of all four metal ions to levels below the limits of reproducible ICP-OES detection. In contrast, the concentrations of the background ions remained unchanged (Figure 4a).

The conformational change of the polymer provides a low cost method for daily water quality assessment that could be used by those who have limited access to expensive analysis technology. To demonstrate this, we adapted previous designs[1g] to construct a simple acrylic funnel with a 2 mm hole at the bottom. To seal the opening it was brought into contact with a 3 mm hydrogel disk on the surface of another acrylic piece (Figure 4b). Damon slough water was placed in the funnel and was retained for 45 min. At this time the solution was contaminated with 100 μM Cd^{2+}, which triggered contraction of the polymer and draining of the sample from the funnel. In contrast, the addition of 100 μM Ca^{2+} to an identical experimental setup caused no sample loss. Thus, this type of device could serve as an inexpensive water tester or flow control valve that has no need for electronic components.

These studies highlight the advantages of combining the function of proteins with the bulk properties of synthetic materials. Our metallothionein-based system is capable of selectively sensing and removing heavy metal contaminants in the presence of large concentrations of background ions, and it can be recycled using solutions of inexpensive chemical chelators. It removes ions from river water samples and can easily be integrated into low cost, practical tools for contaminant sensing. Most importantly, this material was synthesized using chemistry that is independent of the protein sequence, and thus it can be readily applied to the generation of hybrid materials with diverse binding capabilities. The expansion of this strategy to incorporate proteins known to bind PCBs,[18a] dioxins,[18b] estradiol,[18c] and other water contaminants[18d] is currently underway.

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Supporting Information Available: Figures S1−S7, Tables S1−S2, and all experimental details (PDF); Movies S1−S2 (.avi). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Wang, P.; Sergeeva, M. V.; Lim, L.; Dordick, J. S. *Nat. Biotechnol.* **1997**, *15*, 789–93. (b) Wang, C.; Stewart, R. J.; Kopecek, J. *Nature* **1999**, *397*, 417–420. (c) Miyata, T.; Asami, N.; Uragami, T. *Nature* **1999**, *399*, 766–9. (d) Rizzi, S. C.; Ehrbar, M.; Halstenberg, S.; Raeber, G. P.; Schmoekel, H. G.; Hagenmuller, H.; Muller, R.; Weber, F. E.; Hubbell, J. A. *Biomacromolecules* **2006**, *7*, 3019–29. (e) Petka, W. A.; Harden, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A. *Science* **1998**, *281*, 389–92. (f) Sui, Z. J.; King, W. J.; Murphy, W. L. *Adv. Mater.* **2007**, *19*, 3377–3385. (g) Ehrick, J. D.; Deo, S. K.; Browning, T. W.; Bachas, L. G.; Madou, M. J.; Daunert, S. *Nat. Mater.* **2005**, *4*, 298–302.

(2) Schwarzenbach, R. P.; Escher, B. I.; Fenner, K.; Hofstetter, T. B.; Johnson, C. A.; von Gunten, U.; Wehrli, B. *Science* **2006**, *313*, 1072–7.

(3) Coyle, P.; Philcox, J. C.; Carey, L. C.; Rofe, A. M. *Cell. Mol. Life Sci.* **2002**, *59*, 627–47.

(4) Maret, W. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 237–241.

(5) Esser-Kahn, A. P.; Francis, M. B. *Angew. Chem., Int. Ed.* **2008**, *47*, 3751–3754.

(6) Tommey, A. M.; Shi, J.; Lindsay, W. P.; Urwin, P. E.; Robinson, N. J. *FEBS Lett.* **1991**, *292*, 48–52.

(7) Robinson, N. J.; Tommey, A. M.; Kuske, C.; Jackson, P. J. *Biochem. J.* **1993**, *295* (Pt 1), 1–10.

(8) Muir, T. W.; Sondhi, D.; Cole, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6705–6710.

(9) Gilmore, J. M.; Scheck, R. A.; Esser-Kahn, A. P.; Joshi, N. S.; Francis, M. B. *Angew. Chem., Int. Ed.* **2006**, *45*, 5307–5311.

(10) Scheck, R. A.; Dedeo, M. T; Iavarone, A. T; Francis, M. B. *J. Am. Chem. Soc.* **2008**, *130*, 11762–11770.

(11) Sui, Z.; King, W. J.; Murphy, W. L. *Adv. Funct. Mater.* **2008**, *18*, 1824–1831.

(12) Sodium was excluded because of its presence in the PBS buffer.

(13) Rathna, G. V. N. *J. Appl. Polym. Sci.* **2004**, *91*, 1059–1067.

(14) Hong, S. H.; Maret, W. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 2255–60.

(15) (a) Xu, Z.; Bae, W.; Mulchandani, A.; Mehra, R. K.; Chen, W. *Biomacromolecules* **2002**, *3*, 462–5. (b) Terashima, M.; Oka, N.; Sei, T.; Yoshida, H. *Biotechnol. Prog.* **2002**, *18*, 1318–23. (c) Wildgoose, G. G.; Leventis, H. C.; Simm, A. O.; Jones, J. H.; Compton, R. G. *Chem. Commun.* **2005**, *n/a*, 3694–3696. (d) Bell, C. A.; Smith, S. V.; Whittaker, M. R.; Whittaker, A. K.; Gahan, L. R.; Monteiro, M. J. *Adv. Mater.* **2006**, *18*, 582–585.

(16) (a) Lee, J. S.; Han, M. S.; Mirkin, C. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 4093–4096. (b) Yoon, S.; Miller, E. W.; He, Q.; Do, P. H.; Chang, C. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 6658–6661.

(17) For interesting crown ether-based materials that can both bind and detect Pd^{2+}, see (a) Holtz, J. H.; Asher, S. A. *Nature* **1997**, *389*, 829–832. (b) Reese, C.; Asher, S. *Anal. Chem.* **2003**, *75*, 3915–3918. (c) Asher, S.; Peteu, S.; Reese, C.; Lin, M.; Finegold, D. *Anal. Bioanal. Chem.* **2002**, *373*, 632–638.

(18) (a) Denison, M. S.; Heath-Pagliuso, S. *Bull. Environ. Contam. Toxicol.* **1998**, *61*, 557–68. (b) Pandini, A.; Denison, M. S.; Song, Y.; Soshilov, A. A.; Bonati, L. *Biochemistry* **2007**, *46*, 696–708. (c) Buckman, J.; Miller, S. M. *Biochemistry* **1998**, *37*, 14326–36. (d) Jiang, G.; Gong, Z.; Li, X. F.; Cullen, W. R.; Le, X. C. *Chem. Res. Toxicol.* **2003**, *16*, 873–80.

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