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Regenerable DNA-Functionalized Hydrogels for Ultrasensitive, Instrument-Free Mercury(II) Detection and Removal in Water

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Abstract: Mercury is a highly toxic environmental pollutant with bioaccumulative properties. Therefore, new materials are required to not only detect but also effectively remove mercury from environmental sources such as water. We herein describe a polyacrylamide hydrogel-based sensor functionalized with a thymine-rich DNA that can simultaneously detect and remove mercury from water. Detection is achieved by selective binding of Hg^{2+} between two thymine bases, inducing a hairpin structure where, upon addition of SYBR Green I dye, green fluorescence is observed. In the absence of Hg^{2+}, however, addition of the dye results in yellow fluorescence. Using the naked eye, the detection limit in a 50 mL water sample is 10 nM Hg^{2+}. This sensor can be regenerated using a simple acid treatment and can remove Hg^{2+} from water at a rate of $\sim$1 h^{-1}. This sensor was also used to detect and remove Hg^{2+} from samples of Lake Ontario water spiked with mercury. In addition, these hydrogel-based sensors are resistant to nuclease and can be rehydrated from dried gels for storage and DNA protection. Similar methods can be used to functionalize hydrogels with other nucleic acids, proteins, and small molecules for environmental and biomedical applications.

Introduction

Mercury is a bioaccumulative and highly toxic heavy metal that causes serious human health problems even at low concentrations.[1,2] Severe damage to the nervous system, kidneys, and other organs has been reported after mercury exposure.[3,4] Mercury is released into the environment as a result of both natural processes and human activities. One of the major concerns is the contamination of drinking water and other natural water resources.[5] To deal with mercury contamination, new materials are required to not only selectively detect Hg^{2+} but also effectively remove it.

To date, the majority of Hg^{2+} detection and removal tasks have been performed separately. For example, many sensors can effectively detect Hg^{2+} with a fluorescence or color change.[1,6] However, immobilization of these sensors at a high concentration has not been demonstrated in most cases, making it difficult to effectively remove mercury at the same time. One of the recent advances in Hg^{2+} detection is the discovery of Hg^{2+}-mediated T-T DNA base-pairing.[7,8] The stability of this T-Hg^{2+}-T base pair (Figure 1C) is higher than that of a T-A Watson−Crick pair.[9] In addition, this interaction is highly specific, and only Hg^{2+} can stabilize the T-T base pair. This discovery has led to a large number of fluorescent,[7,10] colorimetric,[11] and electrochemical sensors.[12] In some cases, detection limits in the lower nanomolar range have allowed such sensors to be used for Hg^{2+} detection in drinking water (toxic level =

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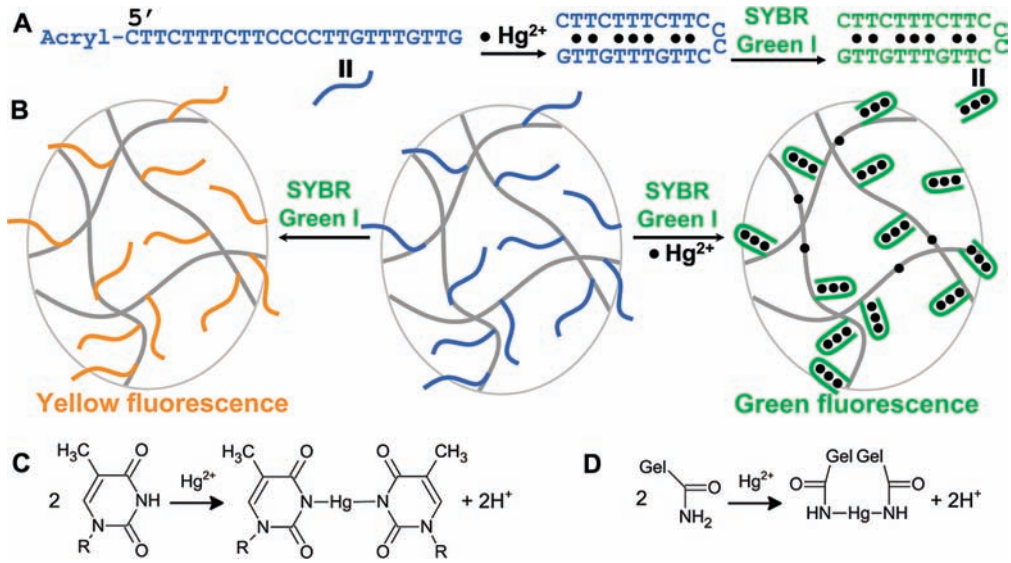

Figure 1. (A) DNA sequence of acrydite-Hg-DNA and fluorescence signal generation for Hg^{2+} detection. The 5′-end is modified with an acrydite group for hydrogel attachment. (B) Covalent DNA immobilization within a polyacrylamide hydrogel and interaction with Hg^{2+} and SYBR Green I produces a visual fluorescence signal. (C,D) Chemical reaction schemes of Hg^{2+} binding with thymine base pairs (C) and polyacrylamide in hydrogel (D), where "Gel" in the molecular formula denotes the hydrogel matrix.

10 nM mercury or 2 parts-per-billion as reported by the U.S. Environmental Protection Agency (EPA)).[13]

In addition to the research efforts devoted to mercury detection, many materials such as porous silica,[14] hydrogels,[15] magnetic beads, and polymers[16] have been used to remove mercury and other toxic metals. All of these materials have in common a large surface area, allowing chemical functional groups such as thiol and amine to selectively bind Hg^{2+}. However, the simultaneous generation of a visual signal indicating the presence of mercury is still quite challenging.[17−19] Hydrogels are ideal for immobilization of biomolecules.[20−25] With the majority of the volume of hydrogels being water, proteins and DNA can maintain their native structure and function. Acrydite-modified DNA can be conveniently incorporated during gel formation. By attaching thymine-rich DNA to the backbone of a mercury-binding hydrogel, it is possible to achieve simultaneous detection and removal of Hg^{2+} from water.

Herein, we report the preparation, characterization, and application of a thymine-rich DNA-functionalized polyacrylamide hydrogel that allows sensitive and selective detection of Hg^{2+} via a visual fluorescence change. Since this DNA is immobilized within the hydrogel, active adsorption of Hg^{2+} occurs not only by the DNA but also by the polyacrylamide matrix. A sample-volume-dependent sensitivity has been demonstrated for ultrasensitive mercury detection. In addition, most of the DNA within the gel remained intact even after nuclease treatment. This hydrogel-based sensor can be regenerated after Hg^{2+} exposure, dried for storage, and then rehydrated. Finally, this sensor was used to detect and remove Hg^{2+} from spiked Lake Ontario water samples.

Experimental Section

Materials. All DNA samples were purchased from Integrated DNA Technologies (Coralville, IA) and purified by standard desalting. Acrydite-Hg-DNA (acrydite-5′-CTTCTTTCTTCCCCTTGTTTGTTG) has a 5′-acrydite modification; Hg-DNA has the same sequence as acrydite-Hg-DNA but without the acrydite modification; C-rich DNA (acrydite-5′-CCCCCCCCCCCCCCCGCCCGCC) serves as a control where the thymines have been changed to cytidines. Acrylamide/bis-acrylamide 29:1 40% gel stock solution, bromophenol blue, ammonium persulfate (APS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), and DNase 1 were purchased from VWR (Mississauga, Ontario, Canada). Mercury perchloride, copper sulfate, zinc chloride, manganese chloride, cobalt chloride, lead acetate, magnesium chloride, and calcium chloride were obtained from Sigma-Aldrich (St. Louis, MO). Sodium nitrate and tris(hydroxymethyl)aminomethane (Tris) were purchased from Mandel Scientific (Guelph, Ontario, Canada), and

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10000× SYBR Green I in dimethyl sulfoxide (DMSO) was purchased from Invitrogen (Carlsbad, CA).

Synthesis of DNA-Functionalized Hydrogels. To prepare the hydrogel, 40% gel solution (29:1), $NaNO_3$ (2 M), Tris nitrate (pH 8.0, 0.5 M), acrydite-Hg-DNA (0.5 mM), and water were mixed. This mixture contained a final gel percentage of 4%, 100 mM $NaNO_3$, 50 mM Tris nitrate, and 10 μM DNA. To initiate polymerization, a fresh initiator solution was made by dissolving 50 mg of APS in 500 μL of water and 25 μL of TEMED. The volume of the initiator was kept at 5% of the final mixture. A 96-well plate was used for gel preparation. To each well was added 75 μL of the gel solution. The gels were polymerized for 1 h at room temperature and then soaked in buffer A (20 mM $NaNO_3$, 8 mM Tris nitrate, pH 8.0) three times (each soaking for at least 3 h) to remove free monomers, initiator, and unincorporated DNA. For some experiments, different gel percentages or DNA were used. The final concentration of DNA within the gel was determined using a SYBR Green I-based assay (see Supporting Information).

Hg^{2+} Detection. In a typical experiment, each gel was soaked in 1 mL of buffer A containing Hg^{2+} or other metal ions. To this was added 2 μL of 250× concentrated (500 μM) SYBR Green I immediately. The gel was soaked in this mixture for 1 h at room temperature on a shaker, excited with a hand-held UV lamp at 365 nm at a distance ~10 cm from the gel, and imaged using a digital camera (Canon PowerShot SD 1200 IS). The images were then processed using Photoshop. UV protection goggles were used for visual observation. To detect Hg^{2+} in 15 or 50 mL samples, the gels were transferred into appropriate conical tubes containing varying concentrations of Hg^{2+}. After soaking overnight to allow Hg^{2+} binding, the gels were then transferred to 1.5 mL tubes, and SYBR Green I was added. For quantitative analysis, the gels were imaged with a gel documentation system (Alpha Innotech FluorChem FC2). The excitation wavelength was set at 365 nm, and the emission was collected using a green filter and a cooled CCD camera.

Fluorometric Analysis. For the fluorescence spectra shown in Figure 2, 15 nM or 1 μM Hg-DNA was dissolved in 400 μL of buffer A. The molar ratio of SYBR Green I and DNA was maintained at 6:1. Spectra in the absence and in the presence of Hg^{2+} were collected using a PTI spectrofluorometer with excitation at 485 nm at room temperature.

Quantification of Hg^{2+} in the Supernatant. To quantify Hg^{2+} removal, a sensor solution containing Hg-DNA and SYBR Green I was prepared. The sensor solution contained final concentrations of 30 nM Hg-DNA and 200 nM SYBR Green I in buffer A and had a linear response for the Hg^{2+} concentration from 10 to 100 nM. To determine Hg^{2+} concentrations lower than 100 nM, a 10× sensor solution was prepared containing 300 nM Hg-DNA and 2 μM SYBR Green I. To determine the kinetics of Hg^{2+} removal, hydrogels were soaked in 1 mL of buffer A containing 1 μM Hg^{2+}. Three calibration solutions containing 1 μM, 100 nM, or 10 nM Hg^{2+} in buffer A were also prepared at the same time. At designated time points, 10-μL aliquots of supernatant solution or calibration solution were transferred to a 96-well plate and 90 μL of the sensor solution was added. When the Hg^{2+} concentration in the hydrogel soaking solution dropped below 100 nM, 90 μL of the soaking solution and 10 μL of the 10× sensor solution were mixed so that the final Hg^{2+} concentration was still within the 10−100 nM range. Calibration was performed at each time point. The fluorescence was measured using a plate reader (SpectraMax M5) with 485 nm excitation. The remaining supernatant solutions after the last time point were diluted with 1% HNO_3 to a volume of 10 mL and analyzed by ICP-MS.

Hydrogel Regeneration. After incubation with SYBR Green I and Hg^{2+}, the hydrogels show green fluorescence. To regenerate hydrogel, the gel was soaked in 1 mL of 1% HCl for 3 min. The HCl solution was discarded, and the gel was washed with 10 mL of water and then soaked in 10 mL of buffer A for 20 min. The gels were again soaked in 1 mL of 1% HCl, and this process was repeated five times. After the last soaking in buffer A, an additional soaking in 10 mL of buffer A was performed for 1 h. After that, the gels were imaged to ensure no fluorescence was observed, and these gels were used for Hg^{2+} detection.

DNase 1 Assays. DNase 1 was dissolved at a concentration of 10 mg/mL in 50% glycerol, 20 mM Tris-HCl, pH 7.5, and 1 mM $MgCl_2$. A 2.5 μM concentration of Hg-DNA was dissolved in the DNase reaction buffer (20 mM NaCl, 25 mM HEPES, pH 7.6, with 10 mM $MgCl_2$ and 10 mM $CaCl_2$). To 500 μL of this solution was added 0.5 μL of the 10 mg/mL DNase 1, and the solution was incubated at 37 °C in a dry bath. After 20 min, 50 μL of each sample was transferred into a new microcentrifuge tube to which 2 μL of 250× SYBR Green I dye and 4 μM Hg^{2+} were added, and the mixture was immediately imaged. To test the hydrogels, the gels were soaked in 1 mL of the DNase 1 reaction buffer. One microliter of the 10 mg/mL DNase 1 was added, and the gel was incubated at 37 °C for 1 h. After soaking, the gels were washed with buffer A three times before being used for Hg^{2+} detection.

Hydrogel Drying and Rehydration. To dry the DNA-functionalized hydrogels, the gels were soaked in 1 mL of water for 1 h, two times. The gels were then transferred onto a plastic weighing boat and dried in air overnight. The mass of the gel before drying was ~80 mg. After drying, the mass was reduced to 3−4 mg. For rehydration, the dried gels were soaked in buffer A for 3 h at room temperature. The gel mass recovered to the original value, and the gels were ready for Hg^{2+} detection.

Detection and Removal of Hg^{2+} from Lake Ontario Water Samples. Lake Ontario water samples were collected from Colonel Samuel Smith Park in Toronto, Ontario, Canada. ICP-MS analysis showed no detectable mercury. Therefore, $Hg(ClO_4)_2$ was added to simulate contaminated water. For Hg^{2+} detection and removal, the water samples were transferred into conical tubes (15 mL each). Some of the tubes were spiked with varying amounts of Hg^{2+}, and then the hydrogel-based sensors were added and soaked for 1 day. After soaking, the supernatant solutions were collected and acidified to contain 1% HNO_3 for ICP-MS analysis (performed by the Microanalysis Lab of the University of Illinois at Urbana−Champaign). For Hg^{2+} detection, gels previously soaked were transferred into 1.5 mL microcentrifuge tubes, and 1 mL of buffer A containing 1 μM SYBR Green I was added. After 1 h, these gels were imaged.

Results and Discussion

Visual Fluorescence Hg^{2+} Detection with DNA. In this study, we employed a thymine-rich DNA (referred to as acrydite-Hg-DNA) containing a 5′-acrydite for attachment to the hydrogel matrix (Figure 1A).[10c] In the absence of Hg^{2+}, the DNA adopts a random coil structure to which the addition of SYBR Green I gives a weak fluorescence (Figure 2A, yellow line). In the presence of Hg^{2+}, the DNA forms a hairpin structure to which SYBR Green I binds, increasing the emission by ~9-fold (Figure 2A, green line). These spectra were collected for a DNA concentration of 15 nM. Even with the 9-fold fluorescence increase, the intensity was still too low to be observed with the naked eye. To design a visual fluorescent sensor, a higher concentration of the DNA is required. Interestingly, instead of a dark background, a yellow fluorescence was observed in the absence of Hg^{2+} with 1 μM DNA (inset of Figure 2B), while in the presence of Hg^{2+}, a strong green fluorescence was observed. This suggests a blue shift of the emission peak upon Hg^{2+} binding. To quantitatively study this shift, fluorescence spectra of 1 μM DNA with 6 μM SYBR Green I in the presence and absence of 4 μM Hg^{2+} were collected (Figure 2B). By increasing the DNA concentration, background fluorescence was increased significantly, and less than 2-fold enhancement was observed upon Hg^{2+} addition. A 5 nm blue shift (from 526 to 521 nm) of the emission peak explains the yellow-to-green

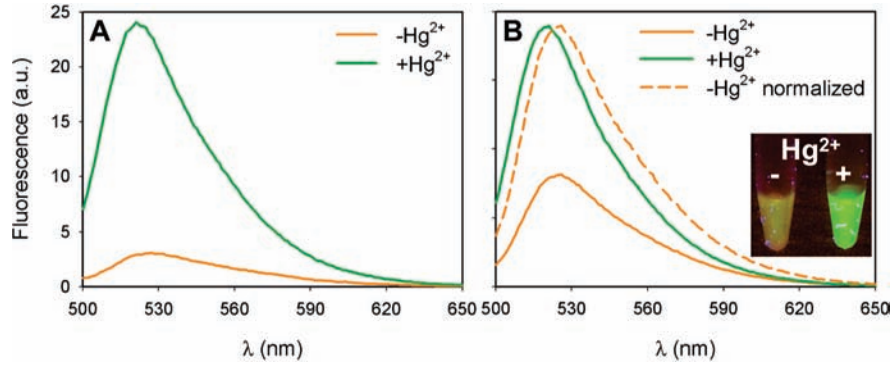

Figure 2. Fluorescence spectra of SYBR Green I and Hg-DNA when the Hg-DNA concentration is 15 nM (A) and 1 μM (B). The SYBR Green I dye and DNA ratio is maintained at 6:1 for both cases. The Hg^{2+} concentration is 90 nM in (A) and 4 μM in (B), where the inset shows a photograph for 1 μM DNA with and without 4 μM Hg^{2+} excited at 365 nm using a hand-held UV lamp. The normalized curve in (B) is obtained by multiplying the yellow curve by a factor so that it has the same peak intensity as the green curve.

transition (see the normalized curve in Figure 2B), and such an emission color change can be readily detected by the human eye.

Hydrogel Design and Preparation. The hydrogel used in this work serves as a substrate not only for DNA immobilization but also for mercury removal. Among the various types of hydrogel materials, we chose to use polyacrylamide since it is nontoxic, cost-effective, and stable because of covalent cross-linking. At the same time, acrylamide is known to selectively bind Hg^{2+} via the amide nitrogen (Figure 1D).[15c] Even though each acrydite-Hg-DNA can bind seven Hg^{2+}, if the removal of mercury has to completely rely on the DNA, the cost for high-capacity mercury removal would be very high. Within the polyacrylamide gel, the acrylamide concentration is more than 10 000 times higher than the DNA concentration. Therefore, the hydrogel can remove a significant amount of Hg^{2+} at a much lower cost, and the main purpose of the immobilized DNA is for detection.

There are several reports in the literature regarding DNA-functionalized hydrogels;[26] most employed DNA as a reversible cross-linker to observe stimuli-responsive sol−gel transitions or gel volume change. While these gels have unique physical properties, very high DNA concentrations (~1 mM) are required to cross-link the gels. In our study, we chose to use bis-acrylamide as a cross-linker, and the DNA concentration was reduced to 10 μM. Each monolithic gel was made to be 75 μL. Free monomers, unattached DNA, and initiator were washed away by repeatedly soaking the gels in buffer A (20 mM $NaNO_3$ and 8 mM Tris nitrate, pH 8.0). To determine the amount of incorporated DNA, the DNA concentration in the soaking solution was measured, and we have estimated that about half of the 10 μM initial acrydite-Hg-DNA was attached to the gel.

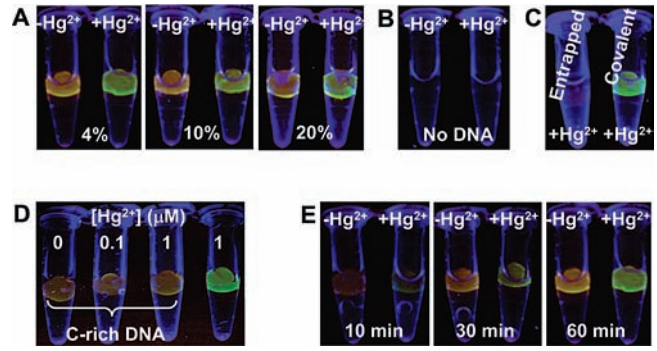

Figure 3. (A) Hg^{2+} detection as a function of gel percentage. (B) Control experiments with gels containing no DNA. (C) The gel on the left was prepared with Hg-DNA (no acrydite modification), and a very low fluorescence was observed. (D) Hydrogels functionalized with the cytidine-rich DNA showed only yellow fluorescence in the presence of varying concentrations of Hg^{2+}. The gel on the right was functionalized with the thymine-rich DNA. (E) Gel fluorescence change as a function of time. All of the experiments were performed in buffer A (20 mM $NaNO_3$, 8 mM Tris nitrate, pH 8.0). SYBR Green I was added to all of the gels.

Optimization of Gel Formulation and Detection Conditions. To optimize the gel formulation, we first varied the gel percentage. High-percentage gels (e.g., 10−20%) were very brittle and easily broken during harvesting. If the percentage was too low (e.g., <3%), the gels were too soft and also difficult to handle. To test the sensor response, gels of 4, 10, and 20% were prepared and soaked in 1 mL of buffer A containing 1 μM SYBR Green I with 0 or 1 μM Hg^{2+}. An hour later, the gels were excited with a hand-held UV lamp at 365 nm. The fluorescence can be easily observed by the naked eye and was imaged using a digital camera. As shown in Figure 3A, green and yellow fluorescence was respectively observed for samples with and without Hg^{2+}, consistent with non-immobilized DNA results. Hg^{2+} can be detected for all of the hydrogels. The gels made with a lower percentage appeared to have more homogeneous fluorescence. In the absence of the DNA, the gels were transparent even after the addition of SYBR Green I (Figure 3B), suggesting the yellow fluorescence in Figure 3A must be due to interactions between the DNA and the dye. For subsequent experiments, we chose 4% gels to achieve a uniform

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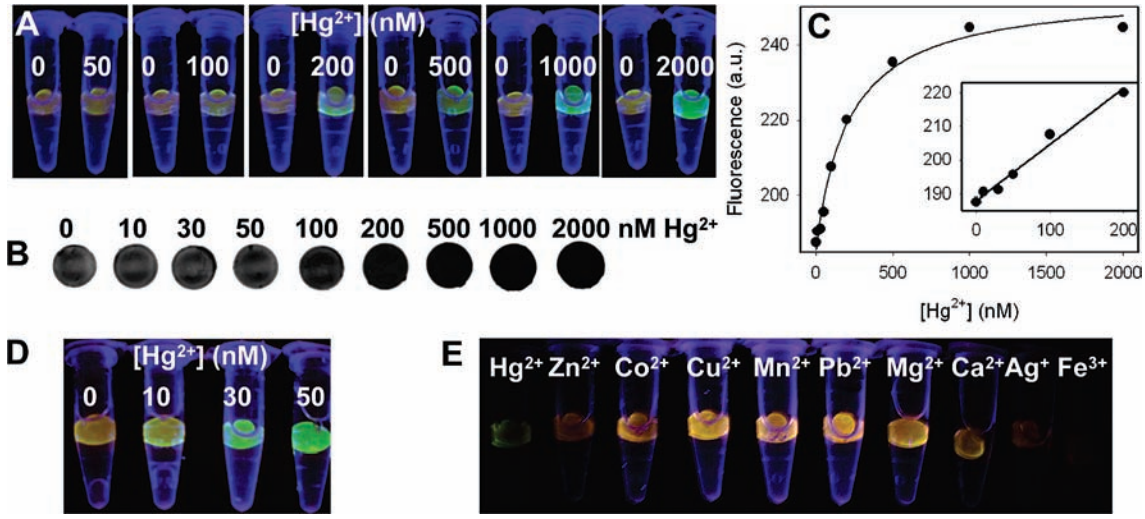

Figure 4. Sensor sensitivity detected using a digital camera (A) and a fluorescence gel documentation system (B) and its quantification (C). (D) The gel sensitivity using 50 mL samples (previously in 1 mL). (E) Selectivity test with 1 μM concentrations of various metal ions in 1 mL samples.

fluorescence and ease of handling. To test the importance of covalent DNA attachment, the same DNA sequence without the acrydite modification was used to make the gel. Addition of Hg^{2+} and SYBR Green I to this gel resulted in a low fluorescence (Figure 3C), suggesting that few DNA strands were left within the gel, making covalent linkage extremely important for the function of an effective Hg^{2+} sensor. Quantitative analysis indicated that ~84% of the non-acrydite DNA was lost in the first wash (see Supporting Information).

To confirm whether the Hg^{2+}-induced fluorescence enhancement was due to selective binding of Hg^{2+} with thymine bases, hydrogels functionalized with an acrydite DNA containing cytidines (C-rich DNA) instead of thymines were tested. As shown in Figure 3D, only yellow fluorescence was observed with this C-rich DNA in the presence of varying concentrations of Hg^{2+}, while the original thymine-rich DNA showed a bright green fluorescence (the tube on the right in Figure 3D). This control experiment suggests that Hg^{2+}-induced green fluorescence is indeed due to the specific interaction of Hg^{2+} with thymine bases, as drawn in Figure 1A.

To determine the optimal time for detection, we next studied the kinetics of fluorescence change. As shown in Figure 3E, after 10 min the difference between the samples with and without 1 μM Hg^{2+} can be observed, although the intensities are quite weak. The fluorescence increased significantly over the course of 1 h, which was chosen for most subsequent experiments.

Mercury Detection Sensitivity and Selectivity. To evaluate the sensitivity of our hydrogel-based sensor, the gels were soaked in varying concentrations of Hg^{2+}. As shown in Figure 4A, at least 200 nM Hg^{2+} was required for visual detection. For quantitative analysis, a gel documentation system was used. The gels were excited at 365 nm, and the emission was collected using a CCD camera through a green filter (Figure 4B). The plot of fluorescence intensity versus Hg^{2+} concentration is shown in Figure 4C. The intensity initially increased linearly with [Hg^{2+}] (inset) and saturated at ~1 μM Hg^{2+}. The detection limit was determined to be 75 nM on the basis of the Hg^{2+} concentration required to generate a signal greater than 3 times the standard deviation of the noise. The overall quantified intensity increase was relatively small compared to that of the solution-based assay, where a low DNA concentration was used (Figure 2A). This is because the high DNA concentration (~5 μM) within the gel caused an intense yellow background fluorescence (Figure 2B).

An important property of this acrylamide hydrogel is its ability to actively adsorb Hg^{2+}. Therefore, unlike most sensors whose responses are limited by the target concentration, the sensitivity of our sensor should be increased by simply increasing the sample volume. To test this hypothesis, gels were soaked in 50 mL of buffer A (previously in 1 mL). As shown in Figure 4D, even 10 nM Hg^{2+} (the toxic level in drinking water) showed an easily visible green fluorescence, and the sample containing 30 nM Hg^{2+} was highly fluorescent green. This sensitivity is among the highest of all the reported Hg^{2+} sensors where no analytical instruments or signal amplification methods were used for detection purposes.[11] The selectivity was also tested by incubating the gels with various metal ions, and only Hg^{2+} produced a green fluorescence (Figure 4E), suggesting that the high selectivity of the DNA is still maintained within the hydrogel matrix.

Mercury Removal. The unique volume-dependent sensitivity of our hydrogels confirms that the gel can actively adsorb and remove Hg^{2+} from water. To study the kinetics, the supernatant Hg^{2+} concentration was monitored after the hydrogel treatment. Starting with 1 μM Hg^{2+}, the concentration decreased to ~30 nM in 6 h at a rate of ~1 h^{-1} (Figure 5A, red line), representing a >30-fold decrease in Hg^{2+}. Interestingly, for hydrogels prepared without the DNA, similar kinetics of Hg^{2+} removal was also observed (black line), which can be explained by the ability of polyacrylamide to bind Hg^{2+} via the amide nitrogen (Figure 1D).[15c] Since a 4% acrylamide gel has a monomer concentration of ~500 mM, while the Hg^{2+} binding site in DNA is less than 0.05 mM, this concentration difference may explain why DNA did not significantly increase the kinetics of Hg^{2+} removal in our system. The supernatant solutions after hydrogel treatment were acidified and analyzed by ICP-MS as an independent verification, and a mercury concentration of lower than 10 nM was obtained. This confirms that the hydrogels were effective in removing Hg^{2+} from water. The fact that Hg^{2+} removal is almost independent of the DNA while the gel can still detect down to 10 nM Hg^{2+} suggests that the acrylamide gel matrix has a high Hg^{2+} adsorption capacity while the DNA has a much higher Hg^{2+} binding affinity. Such a combination

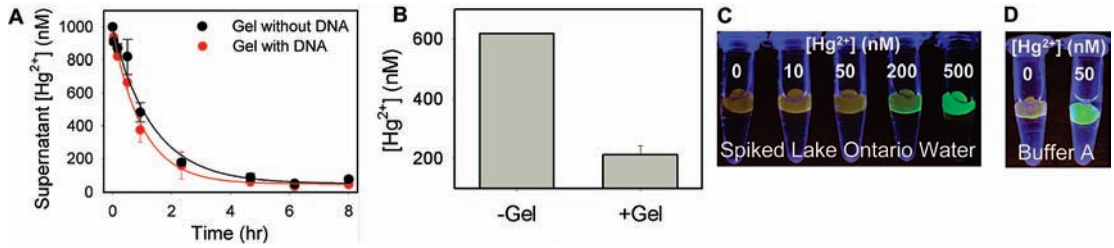

Figure 5. (A) Kinetics of Hg^{2+} removal in buffer A after gel treatment. (B) Mercury concentrations in spiked Lake Ontario water samples before and after gel treatment. Detection of Hg^{2+} in spiked Lake Ontario water samples (C) and in buffer A (D) with a sample volume of 15 mL. The sensitivity in the lake water is slightly lower than that in buffer A.

offers a high sensitivity for the detection and at the same time makes high capacity Hg^{2+} removal cost-effective.

Detection and Removal of Hg^{2+} from Lake Ontario Water. To evaluate whether the hydrogel-based sensor was capable of detecting and removing Hg^{2+} from environmental water samples, samples from Lake Ontario were tested. Since these water samples did not contain Hg^{2+} as determined by ICP-MS, Hg^{2+} was deliberately added to simulate contaminated water. Each gel was soaked in a volume of 15 mL in a conical tube with no additional salt or buffer. After gel treatment, the supernatant solutions were collected, acidified, and analyzed using ICP-MS for mercury. As shown in Figure 5B, the Hg^{2+} concentration decreased from 620 to 210 nM after the gel treatment, suggesting that the gels were capable of Hg^{2+} removal from natural water sources. Interestingly, the amount of Hg^{2+} removed exceeded the capacity of DNA within the gels by ~100%. Therefore, at least half of the Hg^{2+} was adsorbed by the gel matrix, confirming that the Hg^{2+} removal capacity is not limited to the DNA concentration.

To detect Hg^{2+} in Lake Ontario water, the soaked hydrogels described above were transferred to 1 mL of buffer A with 1 μM SYBR Green I. After 1 h, the gels were imaged. As shown in Figure 5C, a weak green fluorescence was observed for 50 nM Hg^{2+}, and an intense green fluorescence was observed for 200 nM Hg^{2+}. This sensitivity is slightly lower in comparison to that obtained in buffer A, where 50 nM Hg^{2+} was easily detected under the same conditions (15 mL sample volume, Figure 5D). This may be attributed to the fact that anions such as Cl^- and SO_4^{2-} in the lake water can also bind Hg^{2+} to decrease its effective concentration.[27] These results clearly demonstrate that our hydrogel is capable of detecting and removing Hg^{2+} from environmental water samples.

Hydrogel Regeneration, Nuclease Resistance, Drying, and Rehydration. Immobilized sensors may allow regeneration. To test this, Hg^{2+} and SYBR Green I-treated gels (Figure 6A) were incubated with 1% HCl for 3 min and then soaked in buffer A for 20 min, five times. As shown in Figure 6B, the hydrogels were nonfluorescent after regeneration. However, addition of Hg^{2+} and SYBR Green I to the same gels regained the sensor response (Figure 6C). Next, we tested whether the DNA within the gels can be protected from nucleases. After the hydrogels were treated with DNase 1 for 1 h, Hg^{2+}-induced green fluorescence could still be observed, although with a slightly lower intensity (Figure 6D). In comparison, no fluorescence was observed if free DNA in buffer was treated with DNase 1 for only 20 min (Figure 6E), suggesting that the gel matrix effectively decreased enzymatic DNA degradation, possibly by reducing the DNase diffusion kinetics inside the gel. Finally, the effect of drying was studied. Drying provides a convenient means for gel storage and DNA protection. The gels can be dried such that the dry mass is ~4% of the fully hydrated gel mass. The dried gels can be easily rehydrated by soaking in buffer A to the original volume (Figure 6F). These rehydrated gels can still effectively detect Hg^{2+} (Figure 6G).

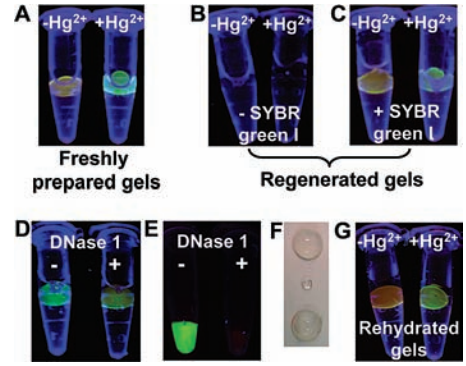

Figure 6. Test of freshly prepared (A) and regenerated (B−C) hydrogels and response of the gels (D) and free DNA (E) to Hg^{2+} after DNase 1 treatment. (F) Photograph of freshly prepared (top), dried (middle), and rehydrated (bottom) gels. (G) Detection of Hg^{2+} with rehydrated hydrogel sensor.

In summary, we have prepared and characterized a DNA-functionalized polyacrylamide hydrogel that can effectively detect and remove Hg^{2+} both in buffers and in environmental water samples. The ability to increase sensitivity by using a larger sample volume distinguishes this gel-based sensor from others. The immobilization method is applicable to other nucleic acids, aptamers, proteins, and small molecules for environmental and biomedical applications.

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Supporting Information Available: Methods for DNA quantification and additional data on diffusion kinetics within hydrogels. This material is available free of charge via the Internet at http://pubs.acs.org.

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