

# NANO LETTERS

## Letters

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### Gold Nanoparticle-Based Sensing of "Spectroscopically Silent" Heavy Metal Ions

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#### ABSTRACT

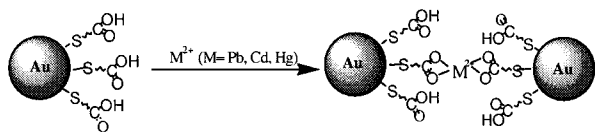
A simple colorimetric technique for the detection of small concentrations of aqueous heavy metal ions, including toxic metals such as lead, cadmium, and mercury, is described. Functionalized gold nanoparticles are aggregated in solution in the presence of divalent metal ions by an ion-templated chelation process; this causes an easily measurable change in the absorption spectrum of the particles. The aggregation also enhances the hyper-Rayleigh scattering (HRS) response from the nanoparticle solutions, providing an inherently more sensitive method of detection. The chelation/aggregation process is reversible via addition of a strong metal ion chelator such as EDTA. Suggestions for improving the sensitivity and selectivity of the technique are given.

Divalent lead, mercury, and cadmium ions pose significant public health hazards when present in drinking water at parts per million concentrations or higher.<sup>1</sup> For absolute identifica-

tion and total concentration assessment, the method of choice is ion-coupled-plasma spectroscopy. Clearly desirable, however, would be simpler qualitative or semiquantitative identification methods. Ideally, these would be field-portable, inexpensive, rapid, reliable, and usable without specialized

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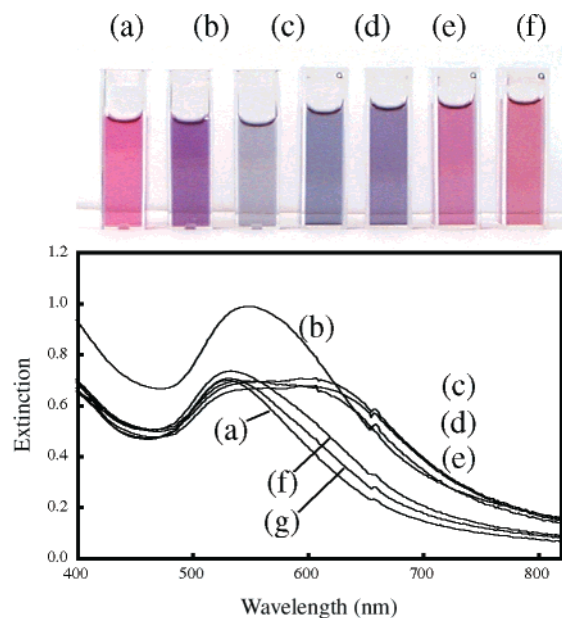
Scheme 1



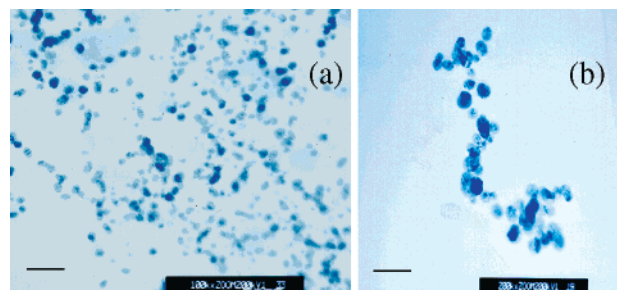
technical training. In principle, colorimetric methods satisfy these requirements and could be particularly appropriate for these otherwise largely spectroscopically silent species. The challenge in devising colorimetric methods for trace  $\text{Pb}^{2+}$ ,  $\text{Hg}^{2+}$ , and  $\text{Cd}^{2+}$  is to identify appropriately strongly binding leuco dyes capable of yielding sufficiently intensely absorbing metal/dye complexes. Even the most intensely colored conventional molecular dyes (porphyrins, azo dyes, etc.) rarely exhibit extinction coefficients greater than  $10^5 \text{ M}^{-1} \text{ cm}^{-1}$ , thereby limiting noninstrumental colorimetric detection to ca.  $0.5 \mu\text{M}$ . (An intriguing hybrid approach to ultrasensitive detection has been described by Alivisatos and co-workers.<sup>2</sup> They retain the fluorescence-reporting scheme, but employ emissive nanoparticles (semiconductors rather than metals) featuring higher extinction (i.e. light-collecting ability) than molecular dyes.) Certain nonmolecular chromophores such as free-electron metal (e.g., gold, silver, and copper) nanoparticles, however, can display visible-region extinction coefficients that are up to several orders of magnitude higher. We reasoned that by functionalizing metal nanoparticles with appropriate heavy-metal ion receptors, the particles might be coaxed to function as high-intensity colorimetric reporters for the otherwise spectrally silent ions. We describe below a series of proof-of-concept sensing experiments based on linear extinction as well as related experiments involving aggregation-sensitive nonlinear scattering (hyper-Rayleigh scattering (HRS)).<sup>3</sup>

Employed as chromophores were  $13.6 \pm 0.4 \text{ nm}$  diameter gold particles capped with 11-mercaptoundecanoic acid (MUA).<sup>4,5</sup> Aqueous suspensions of the functionalized gold particles display an intense plasmon absorption ( $\lambda_{\text{max}} = 526 \text{ nm}$ ,  $\epsilon = 6 \times 10^8 \text{ M}^{-1} \text{ cm}^{-1}$  per particle) that renders the suspensions red.<sup>6</sup> Aggregation of the particles (e.g., by addition of a salt solution) yields both a shift in plasmon band energy and a substantial increase in long-wavelength Rayleigh scattering; these, in turn, result in a red-to-blue color change.<sup>7</sup> If the aggregation had been driven by heavy-metal ion recognition and binding (Scheme 1), the color change could be employed for visual sensing of the ions. In fact, aggregation of DNA-functionalized metal nanoparticles has been used for sequence-specific detection of oligonucleotides.<sup>8</sup> In addition, metal cation-templated aggregation of ligand-functionalized nanoparticles has been used by Murray and co-workers to initialize formation of metal films and solution-phase aggregates.<sup>9</sup> (These reports concern smaller particles having 100 to 1000 times less extinction than the ones used here.)

Figure 1 shows the colorimetric response to  $\text{Pb}^{2+}$  of a 2.4 nM suspension of functionalized Au particles in water containing 1.0% poly(vinyl alcohol) (PVA) as a stabilizer.<sup>10</sup> As shown, the red-to-blue color change can be reversed by addition of EDTA, a good  $\text{Pb}^{2+}$  extracting agent. Similar



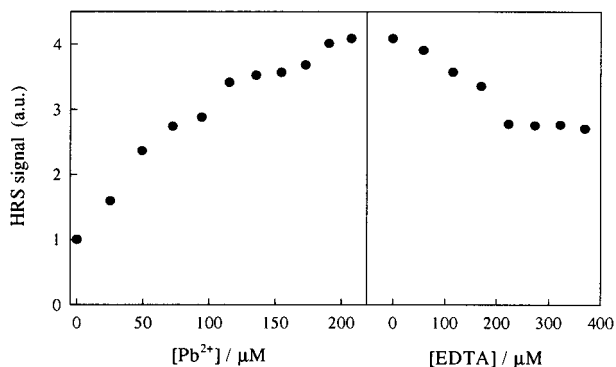
**Figure 1.** Colorimetric responses (top panel) and corresponding spectral traces (bottom panel) from: (a) Au–MUA, (b) Au–MUA/ $\text{Pb}^{2+}$ , and (c)–(g) Au–MUA/ $\text{Pb}^{2+}$  and increasing amounts of EDTA.  $\text{Pb}^{2+}$  concentration in sample (b) is 0.67 mM; EDTA concentrations in samples (c)–(g) are 0.191, 0.284, 0.376, 0.467, and 0.556 mM.



**Figure 2.** TEM images of (a) Au–MUA nanoparticles; (b) Au–MUA nanoparticles that have been aggregated by adding  $\text{Pb}^{2+}$  to an aqueous colloid solution containing 1.0% PVA. Scale bars correspond to (a) 100 nm and (b) 50 nm.

responses were obtained with  $\text{Hg}^{2+}$ - and  $\text{Cd}^{2+}$ -containing solutions, but not from  $\text{Zn}^{2+}$ , consistent with the much lower affinity of simple carboxylic acids for aqueous zinc.<sup>11</sup> We attribute the response in Figure 1 to reversible aggregation induced by MUA coordination of  $\text{Pb}^{2+}$ . The interpretation is supported by TEM measurements indicating aggregate formation of Au-MUA colloid containing lead (Figure 2).

Despite receptor-functionalized nanoparticle concentrations in the nanomolar range, the colorimetric detection limit for  $\text{Pb}^{2+}$  (operationally defined as a 0.1 au change in long-wavelength extinction over a 1 cm sample path length) is much higher, ca.  $400 \mu\text{M}$ . The discrepancy is attributed in part to redundancy in the metal-ion-based particle linking process and in part to the limited affinity of MUA for  $\text{Pb}^{2+}$ . The detection limit, in principle, could be very substantially improved by decreasing the number of receptor groups per nanoparticle by increasing the nanoparticle size (and, by extension, the extinction coefficient) and by replacing the carboxylic acid functionality with a superior  $\text{Pb}^{2+}$  bind-



**Figure 3.** Effect of successive  $\text{Pb}^{2+}$  and EDTA additions upon HRS signal intensities. The scattering scales are arbitrary.

ing agent, in addition to implementing trivial remedies such as preconcentrating the sample or increasing the path length.

Although these strategies merit investigation, we have also examined the ability of HRS to report on heavy-metal ion contamination. We reasoned that higher sensitivity might be attained here because of the known responsiveness of HRS to the formation of aggregates<sup>12</sup> containing as few as three nanoparticles;<sup>13</sup> in contrast, substantive changes in the linear spectroscopic response appear to require aggregation of as many as a few hundred to a few thousand nanoparticles.<sup>14</sup> Figure 3 shows the response of a 2.4 nM Au–MUA particle suspension to the sequential addition of  $\text{Pb}^{2+}$  followed by EDTA.<sup>15</sup> Notably, an increase in HRS intensity is readily evident with the addition of as little as 25  $\mu\text{M}$   $\text{Pb}^{2+}$ , a concentration too low to yield a visible color change. From previous work, the increase appears to be due both to symmetry lowering and to an increase in the effective size of the chromophoric entity responsible for plasmon-enhanced HRS.<sup>16</sup> As with the colorimetric experiments, addition of EDTA partially reverses the response, as expected if  $\text{Pb}^{2+}$  chelation is responsible for particle aggregation. That the response is not fully reversible suggests that small aggregates persist. Presumably, once formed these are held together by forces in addition to ligand-based metal ion coordination.

To summarize, appropriately functionalized gold nanoparticles can be used as exceptionally high extinction dyes for colorimetric sensing of otherwise spectroscopically silent heavy-metal ion contaminants in water via an ion-chelation-induced aggregation process. The ion-driven aggregation also elicits enhanced hyper-Rayleigh scattering from the nanoparticles. Current efforts are directed toward improving the modest colorimetric detection limit, as outlined above, and to enhancing chemical selectivity by altering the receptor–ligand composition.

**Acknowledgment.** We gratefully acknowledge support from the Army Research Office through the DOD-MURI program.

**Supporting Information Available:** Preparative procedure, power dependences of HRS responses,  $^1\text{H}$  and  $^{13}\text{C}$  NMR

data, and elemental analysis data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) According to the EPA guidelines (<http://oaspub.epa.gov>), tolerance limits for lead, mercury, and cadmium in drinking water are no greater than 0.015 mg/L, 0.002 mg/L, and 0.005 mg/L, respectively.
- (2) For example: Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013–2016.
- (3) For reviews of HRS techniques and applications, see: (a) Clays, K.; Persoons, A. *Phys. Rev. Lett.* **1991**, 2980–2983. (b) Clays, K.; Persoons, A. *Rev. Sci. Instrum.* **1994**, *5*, 2190–2194. (c) Johnson, R. C.; Hupp, J. T. *The Spectrum*, Bowling Green State University, **2000**, *13*, 1–8.
- (4) The capping procedure, which is detailed further in the Supporting Information, is a modification of literature methods (Turkevich, J.; Stevenson, P. C.; Hillier, J. *Discuss. Faraday Soc.* **1951**, *11*, 55–75 and Templeton, A. C.; Hostetler, M. J.; Warmoth, E. K.; Chen, S. W.; Hartshorn, C. M.; Krishnamurthy, V. M.; Forbes, M. D. E.; Murray, R. W. *J. Am. Chem. Soc.* **1998**, *120*, 4845–4849). Capping was confirmed by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and elemental analysis (see Supporting Information).
- (5) The particle size and degree of dispersion were determined by HRTEM (Hitachi HR 2000, 200 kV accelerating voltage).
- (6) Interestingly, the plasmon absorption line shape and wavelength maximum remain essentially unchanged with increasing particle size, up to particle diameters of ca. 50 nm. The particle extinction coefficient, on the other hand, increases in proportion to the number of atoms per particle, up to particle diameters of ca. 50 nm. Thus, the extinction coefficient of the largest gold particles featuring the same red hue as 13.6 nm particle is ca.  $8 \times 10^9 \text{ M}^{-1} \text{ cm}^{-1}$ .
- (7) See, for example: Quinten, M.; Kreibitz, U. *Surf. Sci.* **1986**, *172*, 557–577.
- (8) (a) Storhoff, J. J.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 1959–1964. (b) Mucic, R. C.; Storhoff, J. J.; Mirkin, C. A. *Nature* **1996**, *382*, 607–609.
- (9) (a) Zamborini, F. P.; Hicks, J. F.; Murray, R. W. *J. Am. Chem. Soc.* **2000**, *122*, 4514–4515. (b) Templeton, A. C.; Zamborini, F. P.; Wuelfling, W. P.; Murray, R. W. *Langmuir*, **2000**, *16*, 6682–6686. (c) Wuelfling, W. P.; Zamborini, F. P.; Templeton, A. C.; Wen, X.; Yoon, H.; Murray, R. W. *Chem. Mater.* **2001**, *13*, 87–95.
- (10) Without the stabilizer, the metal-ion specificity of the aggregation is lost, at least at millimolar and high micromolar concentrations, an effect likely caused by collapse of the suspension-stabilizing diffuse double layer upon increasing the ionic strength. If large amounts of salt are added, even with PVA, indiscriminate irreversible aggregation and precipitation occur.
- (11) For acetate, for example, the stability constants are  $\log K(\text{Pb}) = 4.1$ ,  $\log K(\text{Hg}) = 10.1$ ,  $\log K(\text{Cd}) = 3.2$ , and  $\log K(\text{Zn}) = 1.8$ . Morel, F. M. M. *Principles of Aquatic Chemistry*, Wiley-Interscience: New York, 1983; pp 237–310.
- (12) (a) Clays, K.; Hendrickx, E.; Triest, M.; Persoons, A. *J. Mol. Liq.* **1995**, *67*, 133–155. (b) Vance, F. W.; Lemon, B. I.; Hupp, J. T. *J. Phys. Chem. B* **1998**, *102*, 10091–10093. (c) Johnson, R. C.; Hupp, J. T. In *Metal Nanoparticles: Synthesis, Characterization, and Applications*; Feldheim, D. L.; Foss, C., Eds.; Marcel-Dekker: New York, in press. (d) Galletto, P.; Brevet, P. F.; Girault, H. H.; Antoine, R.; Broyer, M. *J. Phys. Chem. B* **1999**, *103*, 8706–8710.
- (13) Novak, J. P.; Brousseau, L. C.; Vance, F. W.; Johnson, R. C.; Lemon, B. I.; Hupp, J. T.; Feldheim, D. L. *J. Am. Chem. Soc.* **2000**, *122*, 12029–12030.
- (14) (a) Lazarides, A. A.; Schatz, G. C. *J. Phys. Chem. B* **2000**, *104*, 460–467. (b) Jensen, T.; Kelly, L.; Lazarides, A. A.; Schatz, G. C. *J. Clust. Sci.* **1999**, *10*, 295–317.
- (15) Measurements were made by using a mode-locked Ti:sapphire laser (820 nm) with lock-in detection at 410 nm, as previously described (ref 12b). Variable wavelength detection experiments established that the observed signals were due to HRS rather than two-photon-induced fluorescence. The absorption spectrum of the colloid is not affected by prolonged laser excitation at 820 nm.
- (16) Agarwal, G. S.; Jha, S. S. *Solid State Commun.* **1982**, *41*, 499–501.

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