Use of Fluorescent DNA-Templated Gold/Silver Nanoclusters for the Detection of Sulfide Ions

ARTICLE *in* ANALYTICAL CHEMISTRY · DECEMBER 2011

Impact Factor: 5.64 · DOI: 10.1021/ac202162u · Source: PubMed

3 AUTHORS, INCLUDING:

Huan-Tsung Chang
National Taiwan University
298 PUBLICATIONS **10,220** CITATIONS

SEE PROFILE

analytical chemistry

ARTICLE

pubs.acs.org/ac

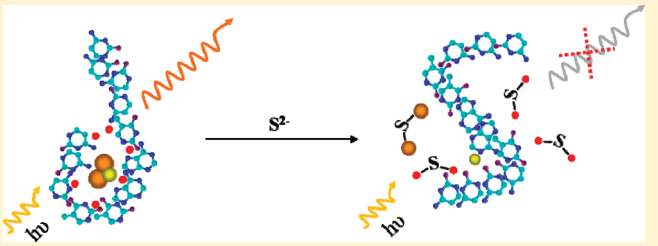
Use of Fluorescent DNA-Templated Gold/Silver Nanoclusters for the Detection of Sulfide Ions

Wei-Yu Chen, Guo-Yu Lan, and Huan-Tsung Chang*

Department of Chemistry, National Taiwan University, No. 1, Section 4, Roosevelt Road, Taipei 10617, Taiwan

Supporting Information

ABSTRACT: We have developed a one-pot approach to prepare fluorescent DNA-templated gold/silver nanoclusters (DNA-Au/Ag NCs) from Au^{3+}, Ag^{+}, and DNA (5′-CCCT-TAATCCCC-3′) in the presence of $NaBH_4$ in order to detect sulfide (S^{2-}) ions on the basis of fluorescence quenching. The as-prepared DNA-Au/Ag NCs have been characterized by UV−vis absorption, fluorescence, circular dichroism, X-ray photoelectron spectroscopy, and electrospray ionization-mass spectrometry measurements. Relative to DNA-Ag NCs, DNA-Au/Ag NCs are much more stable in high ionic strength media (e.g., 200 mM NaCl). The quantum yield of the as-prepared DNA-Au/Ag NCs is 4.5%. We have demonstrated that the fluorescence of DNA-Au/Ag NCs is quenched by S^{2-} ions through the interaction between sulfide ions and gold/silver atoms/ions, a result which leads to changes in the conformation of the templated DNA from packed hairpin to random coil structures. These changes in fluorescence intensity allow sensitive detection of S^{2-} ions at concentrations as low as 0.83 nM. To minimize interference from I^- ions for the detection of S^{2-} ions using the DNA-Au/Ag NCs, the addition of sodium peroxydisulfate to the solution is essential. We have validated the practicality of this probe for the detection of S^{2-} ions in hot spring and seawater samples, demonstrating its advantages of simplicity, sensitivity, selectivity, and low cost.



Sulfide (S^{2-}) ions are widely distributed in natural water and wastewater, and the concentration of S^{2-} ions is an important environmental index.[1] Several methods, including electrochemistry, chromatography, spectroscopy, titration, and gravimetric analysis, have been applied to the detection of S^{2-} ions.[2−6] However, these methods often require time-consuming analysis, complicated procedures, large sample volumes, and/or specialized skills. Alternatively, various probe systems for the detection of S^{2-} ions have been developed on the basis of analyte-induced signal changes through their interactions with metal ions such as Cu^{2+} and Pb^{2+} ions.[7,8] Although these probe systems are selective, poor sensitivity and high costs are still problematic. Thus, there is a need for sensitive and selective probes for the detection of S^{2-} ions.

Fluorescent gold (Au) and silver (Ag) nanodots (NDs)/nanoclusters (NCs) have been the subject of great attention in the sensing of analytes of interest such as DNA, metal ions, proteins, hydrogen peroxide, thiols, and toxic anions,[9−17] mainly because of their advantages such as ease in preparation and conjugation, biocompatibility, stability, and large Stokes shifts.[9−11,18−20] Au and Ag NDs/NCs, typically consisting of several to several tens of atoms (under 2 nm in size), possess molecular-like optical properties that are size, oxidation state, and surface property dependent. Advances in the template-based synthetic strategies have led to preparations of fluorescent and water-soluble Au and Ag NDs/NCs from their corresponding salts in the presence of reducing agents such as $NaBH_4$ and templates such as poly(amidoamine) (PAMAM) dendrimers, polyglycerol-*block*-poly(acrylic acid) copolymers, proteins, and DNA.[13h,i,20−25] In addition, etching of Au nanoparticles (diameter < 3 nm) by thiol compounds under alkaline conditions has been demonstrated as a method of preparation for Au NDs.[13a]

Relative to Au NDs, DNA-templated Ag NCs usually have greater quantum yields (>16%).[25d−f] DNA-Ag NCs exhibit discrete, size-tunable electronic transitions and strong fluorescence emissions as a result of strong quantum-confinement effects.[10,26] The fluorescence properties of DNA-Ag NCs can be easily tuned by varying the length or sequence of DNA templates.[21,25c−f] One disadvantage of preparing DNA-Ag NCs is the need for long reaction times (usually 3−5 days).[13d,e,14c] Recently, a fast (90 min) and simple approach has been demonstrated for the preparation of highly fluorescent DNA-Ag/Cu NCs (quantum yield up to 51.2%) from DNA-Ag NCs and copper (Cu) ions.[13d,e,14c] Although DNA-Ag and DNA-Cu/Ag NCs have been applied to the detection of various analytes, such as metal ions, DNA, thiols, and proteins,[12,13c−e,14b,14c,16] their applications are usually limited to low ionic strength samples. This is mainly because of salt induced instability and

Received: August 16, 2011
Accepted: October 27, 2011
Published: October 27, 2011

quenching of the NCs. Thus, preparing NCs that are highly stable in high ionic strength media remains a challenge.

We present one-pot synthesis of fluorescent DNA-Au/Ag NCs from $HAuCl_4$, $AgNO_3$, and $NaBH_4$ (reducing agent) in the presence of 5′-CCCTTAATCCCC-3′ as a template. We carefully investigated the relative molar ratio of Au^{3+} to Ag^+ and the concentration of $NaBH_4$ to prepare stable and fluorescent DNA-Au/Ag NCs. We then employed the as-prepared DNA-Au/Ag NCs to selectively detect sulfide (S^{2-}) ions on the basis of their specific interactions with Au^+/Ag^+ ions and/or Au/Ag atoms. The effects of masking agents on the sensitivity and selectivity of the DNA-Au/Ag NCs toward S^{2-} ions were evaluated. We further validated the practicality of this probe through the detection of sulfide ions in natural water samples.

EXPERIMENTAL SECTION

Chemicals. All metal salts, hydrogen tetrachloroaurate trihydrate, isopropyl alcohol, and sodium borohydride (powder, 98%) were purchased from Sigma-Aldrich (Milwaukee, WI). The sodium citrate dehydrate, sodium citrate tribasic, sodium phosphate dibasic anhydrous, and sodium phosphate monobasic monohydrate used to prepare citrate (100 mM, pH 5.0) and phosphate (200 mM, pH 5.0) buffers, respectively, were obtained from J. T. Baker (Phillipsburg, NJ). Silver(I) nitrate (99+%, ACS reagent) was obtained from Acros (Morris Plains, NJ). TOTO-3 was from Molecular Probes (Portland, OR). The DNAs (5′-CCCTTAATCCCC-3′, 5′-CCCCCCCCCCCC-3′, and 5′-CCCTCTTAACCC-3′) were purchased from Integrated DNA Technology (Coralville, IA) and were used without further purification. Milli-Q ultrapure water was used in all experiments.

Synthesis of DNA-Ag and DNA-Au/Ag NCs. For the preparation of DNA-Ag NCs, $AgNO_3$ solution (1 mM, 30 μL) was added to aliquots of DNA (200 μM, 25 μL) solution containing 40 mM citrate (pH 5.0) to provide a Ag^+-to-DNA molar ratio of 6:1.[13d] We noted that the ratio allowed preparation of stable and strongly fluorescent DNA-Au/Ag NCs. After 15 min under ice bath, this mixture was reduced by adding $NaBH_4$ (2 mM, 15 μL) and reacted for another 15 min. For the preparation of DNA-Au/Ag NCs, $AgNO_3$ and $HAuCl_4$ were added to provide a Ag^+-to-Au^{3+}-to-DNA molar ratio of 6:6:1; after 15 min under ice bath, this mixture was reduced by adding $NaBH_4$ (1 mM, 15 μL). Although the reaction could be completed within 1 h, the reaction in the dark was kept for 3 h to obtain reproducible results. For simplicity, the concentrations of the as-prepared DNA-Ag NCs and DNA-Au/Ag NCs are all designated as 1×. The DNA-Ag and DNA-Au/Ag NCs were stable in the dark at 4 °C for more than 2 weeks and 2 months, respectively.

Characterization of DNA-Ag and DNA-Au/Ag NCs. Prior to conducting electrospray ionization-mass spectrometry (ESI-MS), inductively coupled plasma mass spectrometry (ICP-MS), and X-ray photoelectron spectroscopy (XPS) analyses, we separately purified the fluorescent DNA-Ag and DNA-Au/Ag NCs (500 μL) by conducting centrifugal filtration (13 500 g) for 40 min through a filter having a cutoff of 3 kDa and by washing the pellets with ultrapure water (3 × 450 μL) 3 times. The fluorescence and absorption spectra of the as-prepared DNA-Ag and DNA-Au/Ag NCs were recorded using a spectrofluorometer (Varian, Walnut Creek, CA) and a double beam UV−vis spectrophotometer (Cintra 10e, GBC, Victoria, Australia). For the determination of quantum yield (Φ_f) values of the NCs, the diluted solutions of DNA-Ag or DNA-Au/Ag NCs were placed in 1 cm quartz cuvette. The Φ_f values for the NCs were calculated using fluorescein in 0.1 N NaOH (Φ_f = 0.95) as a reference chromophore.[27] Circular dichroism (CD) spectra of the DNA, the as-prepared DNA-Ag NCs, and DNA-Au/Ag NCs solutions were recorded using a JASCO 720 instrument (Easton, MD).

XPS was performed using an ES-CALAB 250 spectrometer (VG Scientific, East Grinstead, U.K.) with Al Kα X-ray radiation as the X-ray source for excitation. The molecular mass of the DNA strand and number of Ag and Au atoms bound to the DNA were determined using an LTQ mass spectrometer from Thermal Fisher (San Jose, CA). For ESI-MS measurements, samples were prepared by separately mixing solutions of the DNA strand, the DNA-Ag NCs, or DNA-Au/Ag NCs (50 μL) with isopropyl alcohol (50 μL) to enhance the ionization efficiency.[28] Direct ESI-MS infusion was performed using a capillary column (375 μm o.d., 50 μm i.d.) tapered to 20 μm i.d. The flow rate was set at 250 nL/min using a NE-1000 syringe pump (Wantagh, NY), and an ESI voltage of 1.8 kV was applied to the syringe for ESI infusion. The concentrations of metal species in the DNA-Ag and DNA-Au/Ag NCs were determined by inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7500a system (Santa Clara, CA). We further conducted capillary electrophoresis and gel electrophoresis for the separations of DNA in the absence and presence of Ag^+ ions, and, for confirming the purity of the DNA-Ag and DNA-Au/Ag NCs, separately.[14c] TOTO-3 was used as an intercalator for the analysis of free DNA and its complexes with Ag^+ and/or Au^+ ions. A 5.0 mW solid-state laser with 475 nm output and a UV-light at 365 nm were used in the capillary electrophoresis and gel electrophoresis systems for excitation, respectively.

DNA-Au/Ag NCs Probe for S^{2-} Ions. To phosphate solutions (10 mM, 240 μL, pH 5.0) containing S^{2-} (0−9 μM) and $Na_2S_2O_8$ (0.5 mM), aliquots of DNA-Au/Ag NCs (0.2×, 60 μL) were added separately. The mixtures were incubated at 27 °C for 15 min and then subjected to fluorescence measurement. CD spectra of the free DNA (5 μM), DNA-Au/Ag NCs (0.1×), were recorded in the absence and presence of S^{2-} (50 μM). Three different batches of the mixtures were prepared for the fluorescence and CD measurements.

Real Samples. Hot spring and seawater samples were collected from the Beitou district of Taipei and the ocean near National Taiwan Ocean University, respectively. The hot spring water contained abundant minerals (including SO_4^{2-}, Cl^-, Ca^{2+}, and Na^+).[1] The pH value of the sample was 3.9. The natural water samples were filtered through 0.2 μm membranes prior to use. The pretreated samples were then subjected to the determination of the concentrations of S^{2-} ions using the DNA-Au/Ag NC probe; procedures were the same for the standard sample solutions. The concentrations of S^{2-} ions in the samples were also determined by a standard approach using methyl blue (MB), which was carried out by the Asia Environmental Technical Corporation and authorized by the Taiwanese Environmental Protection Agency (EPA).

RESULTS AND DISCUSSION

Characterization of the DNA-Au/Ag NCs. The fluorescence of DNA-Au/Ag NCs is highly dependent on the length and sequence of DNA.[25a,d] Figure S1 displays that 5′-CCCTTAATCCCC-3′ over other DNAs (e.g., 5′-CCCCCCCCCCCC-3′ and 5′-CCCTCTTAACCC-3′) allowed one to prepare DNA-Au/Ag NCs possessing stronger fluorescence. Both the gel

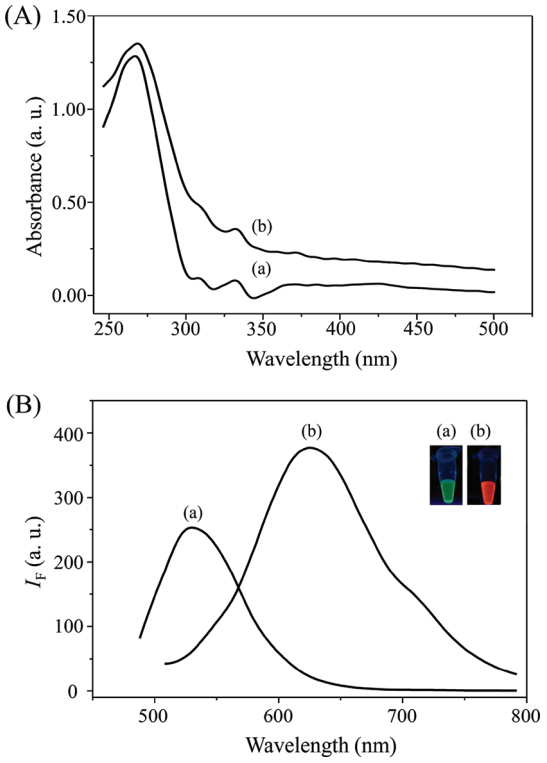
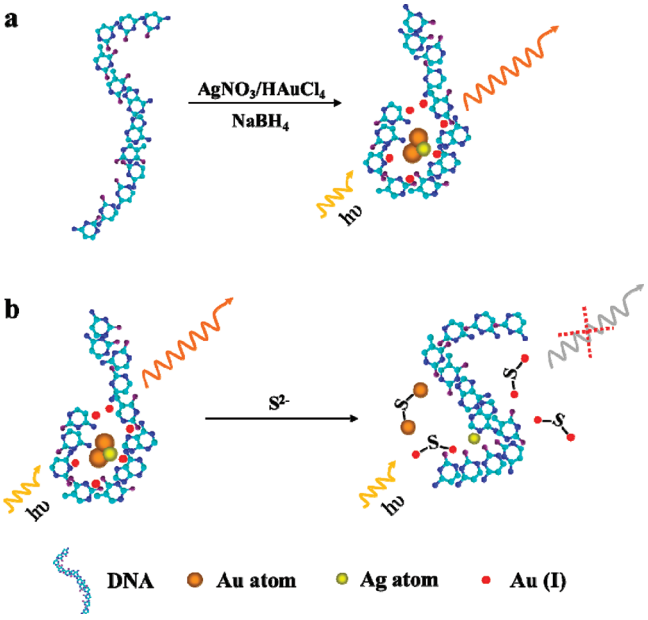


Figure 1. (A) UV−vis and (B) fluorescence spectra of the DNA-Ag NCs (0.02×, a) and DNA-Au/Ag NCs (0.02×, b) in phosphate solutions (10 mM, pH 5.0). Inset shows photograph of the fluorescence of solutions (a and b) upon excitation under a hand-held UV lamp (365 nm). The DNA-Ag and DNA-Au/Ag NCs were excited at 440 and 460 nm, respectively.

electrophoresis images of the DNA-Ag NCs and DNA-Au/Ag NCs show each having one single band (not shown), revealing their high purity.[14c] The absorption spectra depicted in Figure 1A display that the DNA-Ag and DNA-Au/Ag NCs NCs have similar absorption properties, while the fluorescence spectra (Figure 1B) show that the latter has a longer emission wavelength (630 vs 530 nm) and stronger fluorescence intensity (quantum yields 4.5% vs 3.9%). It has been reported that organic-soluble tiopronin-coated Ag clusters (1.6 nm) reacted with Au(I)[$SCH_2(C_6H_4)C(CH_3)_3$] to form Ag/Au bimetallic clusters that have a longer luminescence wavelength relative to that of the Ag clusters.[29] Noble metal nanoclusters (NCs) typically consist of few atoms,[12b,24a] and thus, it is impossible to obtain TEM images of the DNA-Ag and DNA-Au/Ag NCs. To further examine the compositions of the DNA-Ag and DNA-Au/Ag NCs, we conducted ESI-MS measurements.[25a,b,f] The most dominant peaks for the DNA-Ag and DNA-Au/Ag NCs occurred at m/z 971.6 and 823.6 Da, which are assigned to the $[DNA - 7H + 3Na + 3Ag]^{4-}$ and $[DNA - 10H + 5Na + Ag + 2Au]^{5-}$ anions, respectively. The results reveal three Ag atoms, and two Au and one Ag atoms per DNA strand in the DNA-Ag and DNA-Au/Ag NCs, respectively. Our ICP-MS results revealed a Au/Ag molar ratio of 5/3 in the DNA-Au/Ag NCs, which is close to that obtained by the ESI-MS. The XPS spectra (Figure S2 in the Supporting Information) reveal the presence of Au and Ag atoms in the DNA-Au/Ag NCs. The broad peak (Au $4f_{7/2}$) at 84.9 eV was deconvoluted into two distinct components (dot curves) centered at the binding energies of 83.6 and 85.0 eV,

Scheme 1. Schematic Representation of the Preparation and the Operation of the DNA-Au/Ag NCs Probe for the Detection of S^{2-} Ions: (a) One-Pot Synthesis of Fluorescent DNA-Au/Ag NCs and (b) S^{2-} Ions Induced Fluorescence Quenching of the DNA-Au/Ag NCs



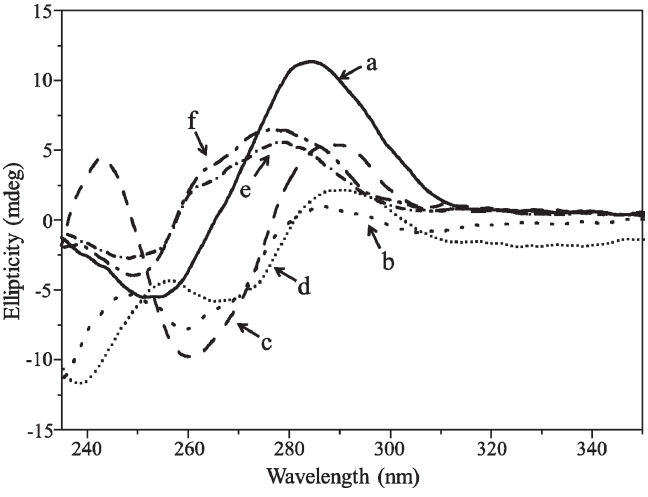


Figure 2. CD spectra of phosphate solutions (10 mM, pH 5.0) containing 0.5 mM $Na_2S_2O_8$ and (a) DNA (5 μM), (b) DNA-Au/Ag NCs (0.1×), (c) DNA-Ag NCs (0.1×), (d) Ag^+-to-Au^{3+}-to-DNA molar ratio of 6:6:1 (30 μM, 30 μM and 5 μM in solution containing 40 mM citrate, pH 5), (e) DNA-Au/Ag NCs (0.1×) and S^{2-} ions (50 μM), and (f) DNA-Ag NCs (0.1×) and S^{2-} ions (50 μM). Other conditions are the same as those described in Figure 1.

which were assigned to Au(0) and Au(I), respectively (Figure S2A).[17,24] The peak for Ag $3d_{5/2}$ in the DNA-Au/Ag NCs occurred at 368.7 eV (Figure S2B), revealing the existence of Ag^+ ions besides Ag atoms in the DNA scaffold.[13d,e,16b]

Detection of S^{2-} Ions Using DNA-Au/Ag NCs. On the basis of the ESI-MS data, XPS data, and our previous study,[13d,e] we proposed the formation of DNA-Au/Ag NCs from the reaction

of $HAuCl_4$, $AgNO_3$, and $NaBH_4$ in the presence of 5′-CCCTTAATCCCC-3′ (Scheme 1A). The Au^{3+} and Ag^+ ions interact with cytosine preferably,[25,30] which induced the conformation change of the DNA scaffold from random coiled to folded structures. Then, some of the Au^{3+} and Ag^+ ions were reduced to form Au and Ag atoms by $NaBH_4$. As a result of the strong quantum-confinement effect, the DNA-Au/Ag NCs fluoresced. In the presence of S^{2-} ions, the fluorescence of the DNA-Au/Ag NCs was quenched (Scheme 1B), mainly because of the interaction of the Au/Ag atoms/ions with S^{2-} ions. We note that the solubility product (K_{sp}) values of Au_2S and Ag_2S are 1.58×10^{-73} and 8.0×10^{-51} M^2, respectively.[31,32] As a result of the formation of Au_2S and Ag_2S, the DNA-Au/Ag NCs dissociated and the DNA scaffolds reverted to random coiled structures.

To support our reasoning for S^{2-} ions induced fluorescence quenching, we separately conducted fluorescence measurements of the DNA-Ag and DNA-Au/Ag NCs in the presence of S^{2-} ions (5 μM). Their fluorescence at 530 and 630 nm decreased about 67% and 58%, respectively. Figure S3 in the Supporting Information reveals that the reaction reached completion within 5 min. We further conducted CD spectroscopy to separately investigate the interactions of S^{2-} ions with the DNA-Ag and DNA-Au/Ag NCs. CD is a common tool for the study of conformational changes of DNA;[25a] the ellipticity at 284 nm of ds-DNA is typically smaller than that of ss-DNA.[33] Figure 2 displays the CD spectra of the DNA, DNA-Ag, and DNA-Au/Ag NCs under various conditions. We pointed out that there were only a few free DNA molecules existent in the solutions, mainly because of strong interactions of the DNA with Ag^+ ions through C—M—C bonding and efficient reducing capability of $NaBH_4$.[25,30] Increases in the electrophoretic mobility of the DNA in the presence of Ag^+ ions reveals that the DNA changed from a random to a folded structure due to its strong interactions with Ag^+ ions.[14c] The electropherograms of the DNA-Ag and DNA-Au/Ag NCs obtained by CE-LIF reveal that there were no detected free DNA and their complexes with the two metal ions. Our ICP-MS data revealed that 95% and 71% of Au^{3+} and Ag^+ ions were reduced by $NaBH_4$ to form the DNA-Au/Ag NCs, respectively. Thus, the effect of free DNA and its complexes on the CD spectra of the DNA-Ag and DNA-Au/Ag NCs could be ignored. The ellipticity at 284 nm of the DNA-Au/Ag NCs (curve b) is less positive than those of the free DNA (curve a), the DNA-Ag NCs (curve c), and free DNA with Ag^+/Au^{3+} (curve d), revealing that a more tightly folded DNA structure was formed in the DNA-Au/Ag NCs as a result of stronger interaction of the DNA with Au/Ag atoms and ions. The 12-mer was unlikely to form a secondary structure, mainly because of its thermal stability.[34] In the presence of S^{2-} ions (50 μM), the CD spectrum (curves e) of the DNA-Au/Ag NCs is similar to that of the free DNA, supporting the idea that S^{2-} interacted with Au/Ag atoms/ions to induce the conformation change in the DNA (Scheme 1B). In the presence of S^{2-} ions (50 μM), the CD spectrum (curves f) of the DNA-Ag NCs solutions is similar to that of the free DNA (curves a), lending further support for a strong interaction occurring between Ag atoms/ions and S^{2-} ions. We also found that the ellipticity of DNA-Au/Ag NCs solutions underwent a red shift upon increasing the S^{2-} ions concentration from 0 to 250 μM (data not shown). On the basis of the fluorescence, CD, ESI-MS, and ICP-MS data, we proposed a sensing mechanism depicted in Scheme 1.

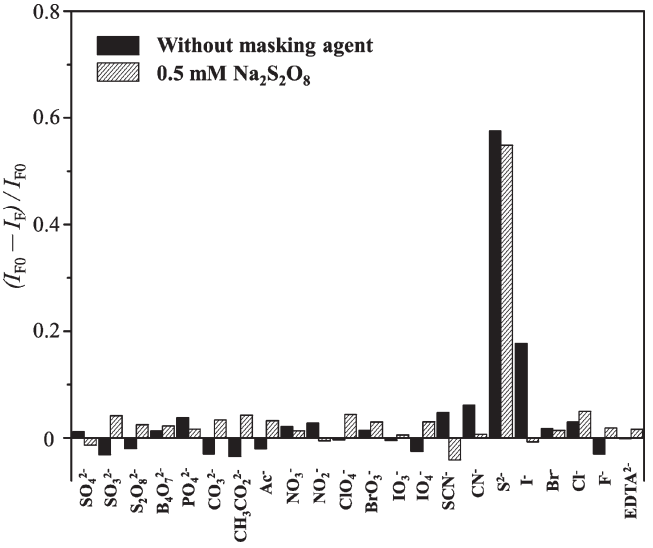


Figure 3. Relative fluorescence intensity $[(I_{F0} - I_F)/I_{F0}]$ of DNA-Au/Ag NCs probe in phosphate solution (10 mM, pH 5.0) in the presence of anions with and without the addition of a masking reagent. The concentrations of S^{2-} ions and each of the other anions were 5 and 25 μM, respectively. I_F and I_{F0} represent the fluorescence intensities of the DNA-Au/Ag NCs at 630 nm in the presence and absence of anions, respectively. Other conditions are the same as those described in Figure 1.

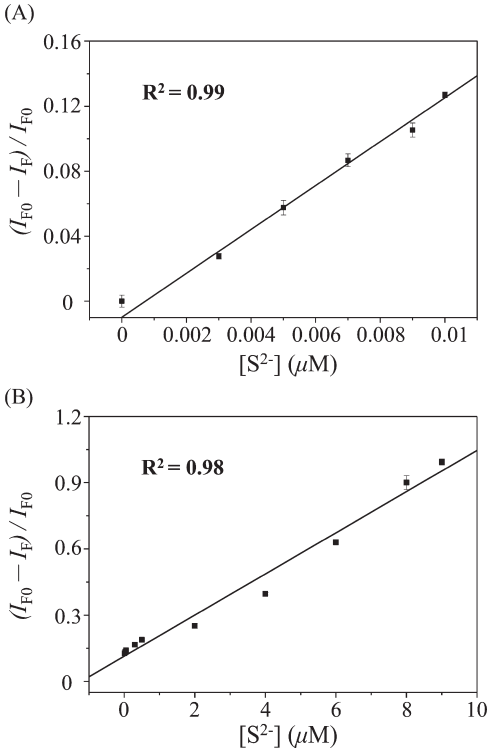


Figure 4. Relative fluorescence intensity $[(I_{F0} - I_F)/I_{F0}]$ of DNA-Au/Ag NCs in the presence of S^{2-} ions (0—9 μM) and NaS_2O_8 (0.5 mM). Plots of the values of $[(I_{F0} - I_F)/I_{F0}]$ at 630 nm versus the concentrations of S^{2-} ions over (A) 0—0.01 μM and (B) 0.01—9 μM. The error bars represent standard deviations based on three independent measurements. Other conditions are the same as those described in Figure 1.

dx.doi.org/10.1021/ac202162u |*Anal. Chem.* 2011, 83, 9450–9455

Selectivity and Sensitivity. Under the optimal conditions [phosphate buffer (10 mM, pH 5.0)], we tested the selectivity of the DNA-Au/Ag NCs (0.02×) probe toward S^{2-} ions (5 μM) against one of the following ions and their corresponding species (for simplicity, CN^-, SCN^-, $B_4O_7^{2-}$, acetate (Ac^-), PO_4^{3-}, NO_3^-, NO_2^-, CO_3^{2-}, F^-, Cl^-, Br^-, I^-, ClO_4^-, BrO_3^-, IO_3^-, IO_4^-, SO_3^{2-}, SO_4^{2-}, $S_2O_8^{2-}$, $EDTA^{2-}$, and citrate were denoted; each 25 μM). We investigated the role that pH played in determining the selectivity of the DNA-Au/Ag NCs toward S^{2-} ions. Over the pH range from 3.0 to 11.0, we found that pH 5.0 provided the greatest fluorescence signal (Figure S4A in the Supporting Information). We tested that the probe responded toward S^{2-} ions (Figure 3 and Figure S4B in the Supporting Information) of the solutions at pH 5.0, 7.0, and 9.0, respectively. Figure 3 and Figure S4B display that pH 5.0 provided the greater selectivity and lower interference toward S^{2-} ions. The fluorescence quenching efficiencies in the presence of 5 μM S^{2-} and 25 μM CN^- were 58% and 5%, respectively. We note that the solubility product (K_{sp}) values for AuI and AgI are 1.6×10^{-23} and 8.3×10^{-17} M^2, respectively.[35] To minimize the interference from I^- ions, $S_2O_8^{2-}$ ions (0.5 mM) were added to the phosphate buffer as a masking agent; I^- reacted with $S_2O_8^{2-}$ to form I_2 and SO_4^{2-}.[36] As expected, the interference from I^- ions for the DNA-Au/Ag NCs probe toward S^{2-} ions is negligible in the presence of $S_2O_8^{2-}$.

The fluorescence at 630 nm of the DNA-Au/Ag NCs decreased upon increasing the concentration of S^{2-} ions from 0 to 9 μM. Two linear regions were obtained in the plots of the value of $(I_{F0} - I_F)/I_{F0}$ versus the concentrations of S^{2-} ions; 0−0.01 μM and 0.01−9 μM (Figure 4). The fluorescence intensities of the DNA-Au/Ag NCs in the absence and presence of S^{2-} ions are denoted by I_{F0} and I_F, respectively. The correlation coefficients (R^2) of the two linear plots were 0.99 and 0.98, respectively. Two linear regions are likely due to differential interactions of S^{2-} ions with Au/Ag atoms/ions.[31,32] The solubility products of Au_2S and Ag_2S were 1.58×10^{-73} and 8.0×10^{-51} M^2, respectively.[31,32] In addition, formation of sparingly soluble salts may be another contributor. Figure S5 reveals that fluorescent DNA-Ag NCs was quenched by S^{2-} ions linearly over the concentration region 0.5−2 μM. The LODs of DNA-Au/Ag NCs and DNA-Ag NCs at a signal-to-noise ratio of 3 for S^{2-} were 0.83 nM and 110 nM, respectively. The DNA-Au/Ag NCs relative to the DNA-Ag NCs provided higher sensitivity for S^{2-}, mainly because they fluoresced more strongly and were stable in the high-ionic strength media. In addition, Au−S over Ag−S bonding is stronger. We note that the maximum level of H_2S in drinking water permitted by the World Health Organization (WHO) is 15 μM (500 ppb).[37]

Detection of S^{2-} Ions in Natural Water Samples. To further test the possibility of the DNA-Au/Ag NCs probe for the S^{2-} ions in real samples, we investigated the effects of NaCl on their fluorescence. Figure S6 in the Supporting Information displays that the DNA-Au/Ag NCs probe is highly stable in high-salt media; only 5% fluorescence quenching took place in the presence of 200 mM NaCl. As a control, we also investigated the stability of DNA-Ag NCs in the presence of NaCl; about 99% fluorescence quench occurred in 200 mM NaCl.[12c,25] The as-prepared DNA-Au/Ag NCs relative to the reported DNA-templated Ag NCs/NDs are much more stable in high ionic strength media.[12,13c−e,14b,14c,16,25f] Our results suggest that the DNA-Au/Ag NCs probe can be applied to determine the concentration of S^{2-} ions in complicated samples.

We then applied the DNA-Au/Ag NCs probe to determine the concentrations of S^{2-} ions in hot spring and seawater samples. We chose the hot spring sample, mainly because it contained high concentrations of sulfide species. There are more than 1 500 000 tourists visiting Beitou district of Taipei in the winter. We detected the concentration of S^{2-} ions in a hot spring water sample to be 330 ± 2 μM. On the basis of a *t*-test (95% confidence level, four degrees of freedom) and an F-test (95% confidence level), the result obtained from our method was in good agreement with those obtained using a conventional methylene blue (MB) method (360 ± 3 μM). The recovery percentage was 101% for a 100-times diluted hot spring sample that was spiked with S^{2-} (final concentration 5 μM). Neither our probe nor the MB method detected the existence of S^{2-} ions in the seawater sample. The recovery percentage was 91% for the seawater sample that was spiked with S^{2-} (final concentration 50 nM). The results reveal that the DNA-Au/Ag NCs probe provides advantages of simplicity, precision, and speed for determining the concentration of S^{2-} ions in complex samples.

CONCLUSIONS

We have developed a straightforward one-pot approach to synthesize fluorescent DNA-Au/Ag NCs that allow sensitive (LOD: 0.83 nM) and selective detection of S^{2-} ions. Although the quantum yield of the DNA-Au/Ag NCs is not as high as some DNA-Ag NCs and our previously prepared DNA-Cu/Ag NCs, they are much more stable in high ionic strength media. The DNA-Au/Ag NCs probe allows detection of S^{2-} ions in a complex environment without needing to conduct the tedious sample pretreatment processes. To minimize the interference from I^- ions, the addition of $S_2O_8^{2-}$ ions prior to analysis using the DNA-Au/Ag NCs is required. Because the DNA-Au/Ag NCs are highly stable in 200 mM NaCl, it is possible to use different DNA templates to prepare functional DNA-Au/Ag NCs for detecting proteins, DNA, and metal ions in biological media such as cells.

ASSOCIATED CONTENT

Supporting Information. Figures S1−S6 as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone and fax: 011-886-2-33661171. E-mail: changht@ntu.edu.tw.

ACKNOWLEDGMENT

This study was supported by the National Science Council of Taiwan under Contract NSC 98-2113M-002-011-MY3 and 99-2627-M-002-016, and by the National Health Research Institutes, Taiwan, under Contract NHRI-EX100-10047NI.

REFERENCES

(1) (a) Morse, J. W.; Millero, F. J.; Cornwell, J. C.; Rickard, D. *Earth Sci. Rev.* **1987**, *24*, 1–42. (b) Bagarinao, T. *Aquat. Toxicol.* **1992**, *24*, 21–62.

(2) Lawrence, N. S.; Davis, J.; Compton, R. G. *Talanta* **2000**, *52*, 771–784.

(3) Antonisse, M. M. G.; Reinhoudt, D. N. *Electroanalysis* **1999**, *11*, 1035–1048.
(4) Pawlak, Z.; Pawlak, A. S. *Talanta* **1999**, *48*, 347–353.
(5) Bérubé, P. R.; Parkinson, P. D.; Hall, E. R. *J. Chromatogr., A* **1999**, *830*, 485–489.
(6) Spaziani, M. A.; Davis, J. L.; Tinani, M.; Carroll, M. K. *Analyst* **1997**, *122*, 1555–1557.
(7) Choi, M. G.; Cha, S.; Lee, H.; Jeon, H. L.; Chang, S.-K. *Chem. Commun.* **2009**, 7390–7392.
(8) Zhou, T.; Wang, N.; Li, C.; Yuan, H.; Xiao, D. *Anal. Chem.* **2010**, *82*, 1705–1711.
(9) Shang, L.; Dong, S.; Nienhaus, G. U. *Nano Today* **2011**, *6*, 401–418.
(10) Díez, I.; Ras, R. H. A. *Nanoscale* **2011**, *3*, 1963–1970.
(11) Guo, S.; Wang, E. *Nano Today* **2011**, *6*, 240–264.
(12) (a) Yeh, H.-C.; Sharma, J.; Han, J. J.; Martinez, J. S.; Werner, J. H. *Nano Lett.* **2010**, *10*, 3106–3110. (b) Guo, W.; Yuan, J.; Dong, Q.; Wang, E. *J. Am. Chem. Soc.* **2010**, *132*, 932–934. (c) Lan, G.-Y.; Chen, W.-Y.; Chang, H.-T. *Biosens. Bioelectron.* **2011**, *26*, 2431–2435.
(13) (a) Huang, C.-C.; Yang, Z.; Lee, K.-H.; Chang, H.-T. *Angew. Chem., Int. Ed.* **2007**, *46*, 6824–6828. (b) Shang, L.; Dong, S. *J. Mater. Chem.* **2008**, *18*, 4636–4640. (c) Guo, W.; Yuan, J.; Wang, E. *Chem. Commun.* **2009**, 3395–3397. (d) Lan, G.-Y.; Huang, C.-C.; Chang, H.-T. *Chem. Commun.* **2010**, *46*, 1257–1259. (e) Su, Y.-T.; Lan, G.-Y.; Chen, W.-Y.; Chang, H.-T. *Anal. Chem.* **2010**, *82*, 8566–8572. (f) Xie, J.; Zheng, Y.; Ying, J. Y. *Chem. Commun.* **2010**, *46*, 961–963. (g) Lin, Y.-H.; Tseng, W.-L. *Anal. Chem.* **2010**, *82*, 9194–9200. (h) Adhikari, B.; Banerjee, A. *Chem. Mater.* **2010**, *22*, 4364–4371. (i) Guo, C.; Irudayaraj, J. *Anal. Chem.* **2011**, *83*, 2883–2889.
(14) (a) Huang, C.-C.; Chiang, C.-K.; Lin, Z.-H.; Lee, K.-H.; Chang, H.-T. *Anal. Chem.* **2008**, *80*, 1497–1504. (b) Sharma, J.; Yeh, H.-C.; Yoo, H.; Werner, J. H.; Martinez, J. S. *Chem. Commun.* **2011**, *47*, 2294–2296. (c) Lan, G.-Y.; Chen, W.-Y.; Chang, H.-T. *Analyst* **2011**, *136*, 3323–3328.
(15) (a) Shiang, Y.-C.; Huang, C.-C.; Chang, H.-T. *Chem. Commun.* **2009**, 3437–3439. (b) Jin, L.; Shang, L.; Guo, S.; Fang, Y.; Wen, D.; Wang, L.; Yin, J.; Dong, S. *Biosens. Bioelectron.* **2011**, *26*, 1965–1969. (c) Wen, F.; Dong, Y.; Feng, L.; Wang, S.; Zhang, S.; Zhang, X. *Anal. Chem.* **2011**, *83*, 1193–1196.
(16) (a) Han, B.; Wang, E. *Biosens. Bioelectron.* **2011**, *26*, 2585–2589. (b) Huang, Z.; Pu, F.; Lin, Y.; Ren, J.; Qu, X. *Chem. Commun.* **2011**, *47*, 3487–3489.
(17) Liu, Y.; Ai, K.; Cheng, X.; Huo, L.; Lu, L. *Adv. Funct. Mater.* **2010**, *20*, 951–956.
(18) Zheng, J.; Nicovich, P. R.; Dickson, R. M. *Annu. Rev. Phys. Chem.* **2007**, *58*, 409–431.
(19) (a) Vosch, T.; Antoku, Y.; Hsiang, J.-C.; Richards, C. I.; Gonzalez, J. I.; Dickson, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 12616–12621. (b) Yu, J.; Patel, S. A.; Dickson, R. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 2028–2030. (c) Yu, J.; Choi, S.; Dickson, R. M. *Angew. Chem., Int. Ed.* **2009**, *48*, 318–320. (d) Lin, C.-A. J.; Yang, T.-Y.; Lee, C.-H.; Huang, S. H.; Sperling, R. A.; Zanella, M.; Li, K.; Shen, J.-L.; Wang, H.-H.; Yeh, H.-I.; Park, W. J.; Chang, W. H. *ACS Nano* **2009**, *3*, 395–401.
(20) Xu, H.; Suslick, K. S. *Adv. Mater.* **2010**, *22*, 1078–1082.
(21) O'Neill, P. R.; Velazquez, L. R.; Dunn, D. G.; Gwinn, E. G.; Fygenson, D. K. *J. Phys. Chem. C* **2009**, *113*, 4229–4233.
(22) (a) Zheng, J.; Dickson, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 13982–13983. (b) Zheng, J.; Petty, J. T.; Dickson, R. M. *J. Am. Chem. Soc.* **2003**, *125*, 7780–7781.
(23) Zhang, J.; Xu, S.; Kumacheva, E. *Adv. Mater.* **2005**, *17*, 2336–2340.
(24) (a) Xie, J.; Zheng, Y.; Ying, J. Y. *J. Am. Chem. Soc.* **2009**, *131*, 888–889. (b) Guével, X. L.; Hötzer, B.; Jung, G.; Hollemeyer, K.; Trouillet, V.; Schneider, M. *J. Phys. Chem. C* **2011**, *115*, 10955–10963.
(25) (a) Petty, J. T.; Zheng, J.; Hud, N. V.; Dickson, R. M. *J. Am. Chem. Soc.* **2004**, *126*, 5207–5212. (b) Ritchie, C. M.; Johnsen, K. R.; Kiser, J. R.; Antoku, Y.; Dickson, R. M.; Petty, J. T. *J. Phys. Chem. C* **2007**, *111*, 175–181. (c) Gwinn, E. G; O'Neill, P. R.; Guerrero, A. J.; Bouwmeester, D.; Fygenson, D. K. *Adv. Mater.* **2008**, *20*, 279–283. (d) Richards, C. I.; Choi, S.; Hsiang, J.-C.; Antoku, Y.; Vosch, T.; Bongiorno, A.; Tzeng, Y.-L.; Dickson, R. M. *J. Am. Chem. Soc.* **2008**, *130*, 5038–5039. (e) Sharma, J.; Yeh, H.-C.; Yoo, H.; Werner, J. H.; Martinez, J. S. *Chem. Commun.* **2010**, *46*, 3280–3282. (f) Lan, G.-Y.; Chen, W.-Y.; Chang, H.-T. *RSC Adv.* **2011**, *1*, 802–807.
(26) (a) Patel, S. A.; Cozzuol, M.; Hales, J. M.; Richards, C. I.; Sartin, M.; Hsiang, J.-C.; Vosch, T.; Perry, J. W.; Dickson, R. M. *J. Phys. Chem. C* **2009**, *113*, 20264–20270. (b) Petty, J. T.; Fan, C.; Story, S. P.; Sengupta, B.; Iyer, A. St. J.; Prudowsky, Z.; Dickson, R. M. *J. Phys. Chem. Lett.* **2010**, *1*, 2524–2529.
(27) Lakowicz, J.; Masters, B. *J. Biomed. Opt.* **2008**, *13*, 029901–029902.
(28) Brown, T. L.; Rice, J. A. *Anal. Chem.* **2000**, *72*, 384–390.
(29) Huang, T.; Murray, R. W. *J. Phys. Chem. B* **2003**, *107*, 7434–7440.
(30) (a) Wilkins, R. J. *Nucleic Acids Res.* **1978**, *5*, 3731–3742. (b) Šponer, J.; Sabat, M.; Burda, J. V.; Leszczynski, J.; Hobza, P.; Lippert, B. *J. Biol. Inorg. Chem.* **1999**, *4*, 537–545. (c) Berti, L.; Burley, G. A. *Nat. Nanotechnol.* **2008**, *3*, 81–87.
(31) Morris, T.; Copeland, H.; Szulczewski, G. *Langmuir* **2002**, *18*, 535–539.
(32) Goia, D. V. *J. Mater. Chem.* **2004**, *14*, 451–458.
(33) Kuruvilla, E.; Ramaiah, D. *J. Phys. Chem. B* **2007**, *111*, 6549–6556.
(34) Bishop, G. R.; Ren, J.; Polander, B. C.; Jeanfreau, B. D.; Tren, J. O.; Chaires, J. B. *Biophys. Chem.* **2007**, *126*, 165–175.
(35) Whitten, K. W.; Davis, R. E.; Peck, M. L.; Stanley, G. G. *Chemistry*, 9th ed.; Brooks/Cole-Thompson Learning: Belmont, 2009.
(36) Harris, D. C. *Exploring Chemical Analysis*, 3rd ed.; W. H. Freeman and Company: New York, 2005.
(37) *Guidelines for Drinking-Water Quality*, 3rd ed.; World Health Organization: Geneva, 2004. Also available at http://www.who.int/water sanitation_health/dwq/guidelines/en/.