Electronic Supplementary Information

Highly sensitive and selective detection of silver ion and silver nanoparticles in aqueous solution using an oligonucleotide-based fluorogenic probe

Yen-Hsiu, Lin¹ and Wei-Lung Tseng¹, ²*

1. Department of Chemistry, National Sun Yat-sen University, Taiwan

2. National Sun Yat-sen University-Kaohsiung Medical University Joint Research Center, Kaohsiung, Taiwan

Correspondence: Dr. Wei-Lung Tseng, Department of Chemistry, National Sun Yat-sen University, 70, Lien-hai Road, Kaohsiung, Taiwan 804.

E-mail: tsengwl@mail.nsysu.edu.tw

Fax: 011-886-7-3684046.
Experimental section

**Chemicals.** HNO₃, H₃PO₄, dimethyl sulfoxide, ethanol and H₂O₂ were purchased from Riedel-deHaën (Seelze, Germany). Tris, Na₂CO₃, NaH₂PO₄, Na₂HPO₄, NaNO₃, NaBH₄, AgNO₃, and 3-morpholinopropanesulfonic acid (MOPS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All of metal ions were ordered from Acros. A DNA sample was synthesized from Integrated DNA Technology (Coralville, IA). SYBR Green I (SG) was purchased from Molecular Probe Inc. (Portland, OR). Except for the determination of Ag⁺ in drinking water, Milli-Q ultrapure water (Milli-pore, Hamburg, Germany) was used in all of the experiments.

**Apparatus.** The fluorescence spectra of SG were measured using a Hitachi F-4500 fluorometer (Hitachi, Tokyo, Japan) while the excitation wavelength was set at 494 nm.

**Synthesis of silver nanoparticles (AgNPs).** We prepared citrate-capped AgNPs by means of the chemical reduction of a metal salt precursor (AgNO₃) in the liquid phase.¹ To achieve this, we rapidly added 1% w/v trisodium citrate (2 mL) to a solution of 1-mM AgNO₃ (100 mL) that was heated under reflux. This heating continued for an additional 30 min. TEM images (not shown) confirmed that the diameter of the AgNPs (density 10.49 g/cm³) is 50 ± 8 nm, with a standard deviation
of 8.0 nm. To estimate the concentration of AgNPs, we assumed that the reduction from Ag(I) to Ag atoms was 100% complete. The concentration of the original AgNPs is calculated to be 0.42 nM.

**Sample preparation.** A solution of SG (10000×) was diluted to 250× with dimethyl sulfoxide. The resulting solution was diluted to 6.25× with ultrapure water in order to make a stock solution. Using Beer’s law, the concentration of a solution of 6.25× SG is calculated to be $1.23 \times 10^{-5}$ M. Note that the molar absorption coefficient of SG at the optical wavelength of 494 nm is approximately 73000 M$^{-1}$ cm$^{-1}$. We added metal ions (0.2–200 μM, 500 μL) to 500 μL of 40 mM MOPS solution (pH 5.5–8.0) containing 100 mM NaNO$_3$, 320 nM SG, and 100 nM C$_{20}$. To investigate the effect of the DNA length and sequence on our analytical system, we replaced C$_{20}$ with C$_{5}$, C$_{10}$, C$_{15}$, A$_{20}$, T$_{20}$, or 5′-GGG TTA GGG TTA GGG TTA GGG-3′, one at a time. We equilibrated the resulting solutions for the optimum incubation time, and then recorded the fluorescence spectra of the solutions.

**Analysis of Real Samples.** We collected drinking water from the National Sun Yat-sen University campus. A series of samples were prepared by “spiking” them with standard solutions of Ag$^+$. We then added these spiked samples (500 μL) to a solution (500 μL) containing 40 mM MOPS, 100 mM NaNO$_3$, 320 nM SG, and 100 nM C$_{20}$, at pH 7.0. We incubated the resulting solutions for 10 min before measuring
their fluorescence spectra.

This proposed method was utilized to detect 50-nm AgNPs. Different concentrations of AgNPs (128.4–482 nM) were oxidized to Ag⁺ with a solution of 1 mM H₂O₂ and 1 μM H₃PO₄. After 20 min, the resulting solution (500 μL) was added to a solution (500 μL) containing 40 mM MOPS, 100 mM NaNO₃, 320 nM SG, and 100 nM C₂₀, at pH 7.0. The fluorescence spectra of the resulting solutions were recorded after 10-min incubation.

References

**Fig. S1.** Relative fluorescence increases \[
\frac{(F - F_0)}{F_0}
\] at 521 nm of a solution of SG and DNA samples—including A$_{20}$, T$_{20}$, C$_{20}$, and G-containing oligonucleotide (5’-GGG TTA GGG TTA GGG TTA GGG-3’)—on the addition of 1 μM Ag$^+$. A mixture of 160 nM SG and 50 nM DNA samples was prepared in 20 mM MOPS and 50 mM NaNO$_3$ at pH 7.0. The incubation time is 10 min.
**Fig. S2.** Fluorescence spectra of a solution containing 160 nM SG, 20 mM MOPS, 50 mM NaNO₃, and different concentrations of Cₙ in the presence of 1 μM Ag⁺: (a) 200 nM C₅, (b) 100 nM C₁₀, (c) 67 nM C₁₅, (d) 50 nM C₂₀. The incubation time is 10 min.
Fig. S3. Effect of the pH on the value of \((F - F_0)/F_0\) at 521 nm of a solution containing 160 nM SG, 20 mM MOPS, 50 mM NaNO₃ in the presence of (a) 1 μM Ag⁺ and 50 nM C₂₀, (b) 50 nM A₂₀ and 50 nM T₂₀. The incubation time is 10 min. (b) We added A₂₀ (200 nM, 250 μL) to 500 μL of 40 mM MOPS solution (pH 5.5–8.0) containing 100 mM NaNO₃ and 320 nM SG. After 10 min, the resulting solutions were mixed with 250 μL of 200 nM T₂₀. We equilibrated these solutions for 10 min and then recorded the fluorescence spectra of the solutions.
Fig. S4. Fluorescence spectra of a solution containing 160 nM SG, 20 mM MOPS, and 50 nM C\textsubscript{20} upon the addition of (a) 16 types of metal ions and (b) 16 types of metal ions and Ag\textsuperscript{+}. 16 types of metal ions include Li\textsuperscript{+}, K\textsuperscript{+}, Mg\textsuperscript{2+}, Ca\textsuperscript{2+}, Ba\textsuperscript{2+}, Fe\textsuperscript{2+}, Fe\textsuperscript{3+}, Co\textsuperscript{2+}, Ni\textsuperscript{2+}, Cu\textsuperscript{2+}, Zn\textsuperscript{2+}, Au\textsuperscript{3+}, Cd\textsuperscript{2+}, Hg\textsuperscript{2+}, Pb\textsuperscript{2+}, and Cr\textsuperscript{3+}. The concentration of each metal ion is 1 μM. The incubation time is 10 min.
**Fig. S5.** A plot of the logarithm of \((F - F_0)/(F_s - F)\) at 521 nm versus the logarithm of the Ag\(^+\) concentration. \(F_0\) and \(F\) represent the fluorescence intensity of the SG and \(C_{20}\) solution before and after the addition of Ag\(^+\), respectively. \(F_s\) represents the fluorescence intensity of the solution of SG and \(C_{20}\) saturated with Ag\(^+\).
Fig. S6. Change in the value of \((F - F_0)/F_0\) at 521 nm of a solution (160 nM SG, 50 nM C\textsubscript{20}, 20 mM MOPS and 50 mM NaNO\textsubscript{3}) upon the addition of H\textsubscript{2}O\textsubscript{2}-treated AgNPs. A solution of 420 nM AgNPs (50 ± 8 nm) was added to a solution of 1 mM H\textsubscript{2}O\textsubscript{2} and 1 \(\mu\text{M}\) H\textsubscript{3}PO\textsubscript{4}. 