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Multiplexed Analysis of Hg^{2+} and Ag^{+} Ions by Nucleic Acid Functionalized CdSe/ZnS Quantum Dots and Their Use for Logic Gate Operations**

*Ronit Freeman, Tali Finder, and Itamar Willner**

Semiconductor quantum dots (QDs) attract extensive research interest as optical labels for sensing events. The unique photophysical properties of QDs, such as high fluorescence quantum yields, narrow emission bands, high Stokes shifts, and stability against photobleaching, make them a superior sensing material. Specifically, the size-controlled luminescence features of QDs should enable the multiplexed detection of different analytes in solution or on-chip formats.[1] Indeed, substantial progress was accomplished in the past few years in the application of QDs as optical labels for sensing and biosensing.[2] Various QD-based biosensors were developed, including sensors that follow the activities of enzymes or their substrates,[3] immunosensors,[4] and sensors that follow DNA hybridizations[5] or the formation of aptamer–substrate complexes.[6] The development of multiplexed analytical assays is a major goal in sensing.[7] The size-controlled emission properties of QDs enable the multiplexed analysis of different targets by QDs exhibiting controlled sizes. Indeed, different-sized QDs were used as optical labels for the multiplexed labeling of different immunocomplexes on surfaces.[8] Whereas the use of QDs for biosensing is well advanced, the use of QDs for chemical sensing is scarce. The sensing of pH values by QDs[9] or the detection of alkali metal ions by crown-ether-modified QDs[10] has been reported. Recently, modified QDs were used to detect the RDX explosive,[11] and boronic acid functionalized QDs were used for the competitive luminescence detection of saccharides and neurotransmitters.[12] Also, β-cyclodextrin-modified QDs were applied to sense different substrates that bind to the cyclodextrin cavity using a competitive FRET assay.[13]

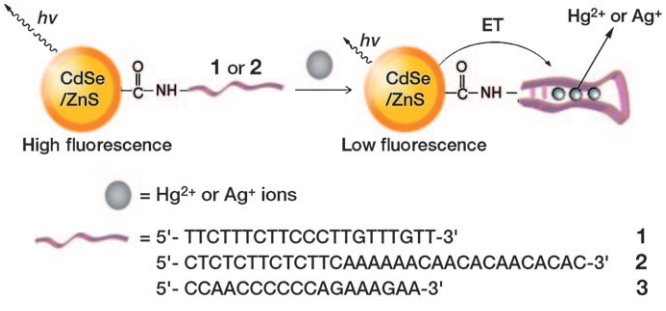
Metal ions, such as Hg^{2+} and Ag^{+}, act as severe environmental pollutants and have serious medical effects on human health. Thus, the rapid and sensitive analysis of different ions in water or food resources is important. Various procedures for the analysis of Hg^{2+} have been developed, including electrochemical[14] and optical[15] methods. Various ions form stable complexes by bridging specific nucleotide bases. For example, Hg^{2+} ions bridge thymine bases, and Ag^{+} ions specifically bridge cytosine bases.[16] These features were recently applied to develop oligo-T- or oligo-C-modified Au-NPs for the optical detection of Hg^{2+} or Ag^{+} through the ion-induced aggregation of the NPs.[17]

Herein we report on the use of T-rich- or C-rich-modified QDs for the selective analysis of Hg^{2+} or Ag^{+} ions using an electron-transfer-quenching path. Specifically, we demonstrate the multiplexed analysis of Hg^{2+} and Ag^{+} by different-sized QDs. Furthermore, the specific quenching of the QDs by the ions enabled us to use Hg^{2+} and Ag^{+} ions as inputs that activate logic gates, and to implement QDs as optical readout signals for logic gate operations.

CdSe/ZnS QDs ($d = 3.8$ nm, $\lambda_{em} = 560$ nm, QD^{560}) were modified with the thymine-rich nucleic acid **1** using bis(sulfosuccinimidyl)suberate (BS^3) as a bifunctional coupling reagent. The average loading of the QD^{560}s with **1** was estimated to be three units per QD. Similarly, CdSe/ZnS QDs ($d = 5.8$ nm, $\lambda_{em} = 620$ nm, QD^{620}) were modified with the cytosine-rich nucleic acid **2**. The average loading of QD^{620} with **2** corresponded to five units per QD. Scheme 1 outlines the principle for the selective analysis of Hg^{2+} or Ag^{+} ions by **1**-QD^{560} or **2**-QD^{620}, respectively. A rigid hairpin structure is formed in the presence of Hg^{2+} or Ag^{+} ions, in which the T or C residues of the spatially separated nucleotides are linked by the ions.

As the Hg^{2+}–thymine or Ag^{+}–cytosine complexes lack any color that would enable energy transfer from the QDs, the decrease in their luminescence is attributed to electron-transfer quenching of the QDs by the ions bound to the thymine or cytosine bases.

Figure 1 A depicts the time-dependent luminescence changes upon interaction of **1**-QD^{560} with 1×10^{-4} M Hg^{2+}. The luminescence of the QDs decreased and reached (after 24 min) a steady-state value of 86% quenching of the initial luminescence of the QDs. The inset in Figure 1 A shows the



Scheme 1. Optical analysis of Hg^{2+}/Ag^{+} ions by nucleic acid modified QDs.

[*] R. Freeman, T. Finder, Prof. I. Willner
Institute of Chemistry and Center for Nanoscience and Nanotechnology
The Hebrew University of Jerusalem
Jerusalem 91904 (Israel)
Fax: (+972) 2-652-7715
E-mail: willnea@vms.huji.ac.il
Homepage: http://chem.ch.huji.ac.il/willner

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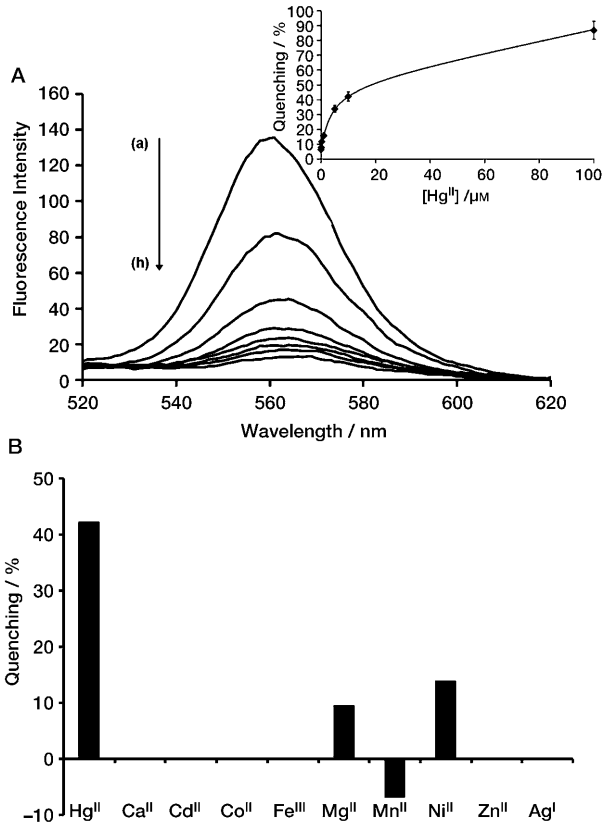


Figure 1. A) Time-dependent luminescence spectra of **1**-QD^{560}: a) in the absence of Hg^{2+}; b)–h) upon interaction with 1×10^{-4} M Hg^{2+}. Spectra were recorded at time intervals of 3 min. Inset: Calibration curve for various concentrations of Hg^{2+} in the presence of **1**-QD^{560} (1.25×10^{-7} M) after a fixed time interval of 24 min. B) Selectivity of the analysis of Hg^{2+} by **1**-QD^{560}. The concentration of all metal ions was 10 μM.

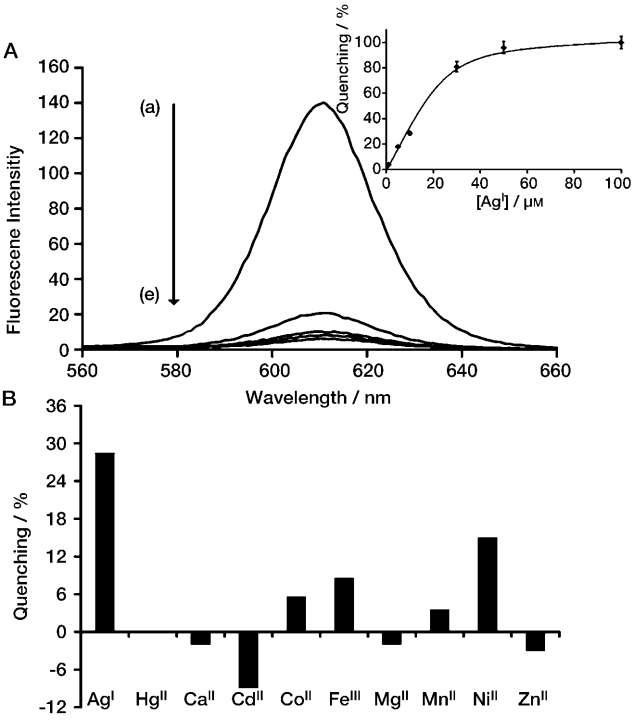


Figure 2. A) Time-dependent luminescence spectra of **2**-QD^{620}: a) in the absence of Ag^{+}; b)–e) upon interaction with 5×10^{-5} M Ag^{+}. Spectra were recorded at time intervals of 2 min. Inset: Calibration curve for various concentrations of Ag^{+} in the presence of **2**-QD^{620} (1.25×10^{-7} M) after a fixed time interval of 30 min. B) Selectivity of the analysis of Ag^{+} by **2**-QD^{620}. The concentration of all metal ions was 10 μM.

derived calibration curve corresponding to the degree of quenching after 24 min of the luminescence of QD^{560} for various concentrations of Hg^{2+}. As the concentration of Hg^{2+} increases, the degree of the luminescence quenching is enhanced. The detection limit for analyzing Hg^{2+} corresponded to 10 nM (2 ppb). Control experiments revealed that the C-rich **2**-QD^{620} were not quenched by Hg^{2+}, which is consistent with the specific formation of a T–Hg^{2+}–T complex on the QDs. Also, the luminescence of QD^{560} modified with a DNA sequence that lacks T residues (**3**) was not affected by the interaction with Hg^{2+} ions. Figure 1 B shows the luminescence features of **1**-QD^{560} in the presence of other metal ions.

Similarly, **2**-QD^{620} was used to detect Ag^{+} ions. Figure 2 A depicts the time-dependent luminescence changes of **2**-QD^{620} upon interaction with 5×10^{-5} M Ag^{+}. At this concentration of Ag^{+}, approximately 95% of the luminescence of the QDs is quenched. The inset in Figure 2 A shows the calibration curve for the quenching of the luminescence of **2**-QD^{620} after 30 min in the presence of various concentrations of Ag^{+}. The results indicate that Ag^{+} can be analyzed to a detection limit of 1 μM (200 ppb). **2**-QD^{620} showed selectivity for Ag^{+} ions (Figure 2 B). It should be noted that **1**-QDs^{560} as well as **2**-QDs^{620} are effectively quenched by Pb^{2+}, presumably because of the tight binding of these ions to the phosphate residues. The addition of 2,6-pyridinedicarboxylic acid[18] to complex Pb^{2+} and to prevent its interference was unsuccessful. One further aspect relates to the reversible activation of nucleic acid functionalized QDs for the re-useable sensing. Accordingly, **1**- and **2**-QDs were used for a first sensing cycle of Hg^{2+} and Ag^{+}, respectively. Subsequently, the QDs were heated to 45 °C for 20 min to unfold the metal ion cross-linked nucleic acids and precipitated by centrifugation. The resuspended **1**- or **2**-QDs retained 70% and 65%, respectively, of the sensing activity of the QDs. This degradation might be attributed to the partial thermal desorption of **1** or **2** from the QDs or to incomplete separation of the ions from the respective cross-linked structures.

The successful selective analysis of Hg^{2+} and Ag^{+} enabled, then, the multiplexed analysis of the two ions by the two-sized nucleic acids-functionalized QDs (Figure 3).

Besides using the QDs as optical transducers for sensing of Hg^{2+} and Ag^{+}, we implemented them as optical labels to follow logic gate operations using Hg^{2+} and Ag^{+} as inputs. The information encoded in nucleic acids or in aptamer structures was recently utilized to develop different logic gate systems by using fluorescent dyes or DNAzymes as transducers.[19] To the best of our knowledge, the specific interactions of ions with nucleic acids, and the application of QDs as readout labels for logic gate operations, have not been reported.

Accordingly, **1**-QDs^{560} and **2**-QDs^{620} were used to develop logic gates. The mixture of the two QDs yielded an "AND" gate upon interaction with Ag^{+} and Hg^{2+} ions as inputs (Scheme 2). Fluorescence quenching of the luminescence of the system at either $\lambda = 560$ nm or $\lambda = 620$ nm is defined as a

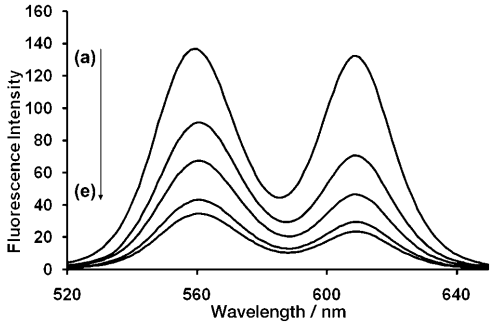
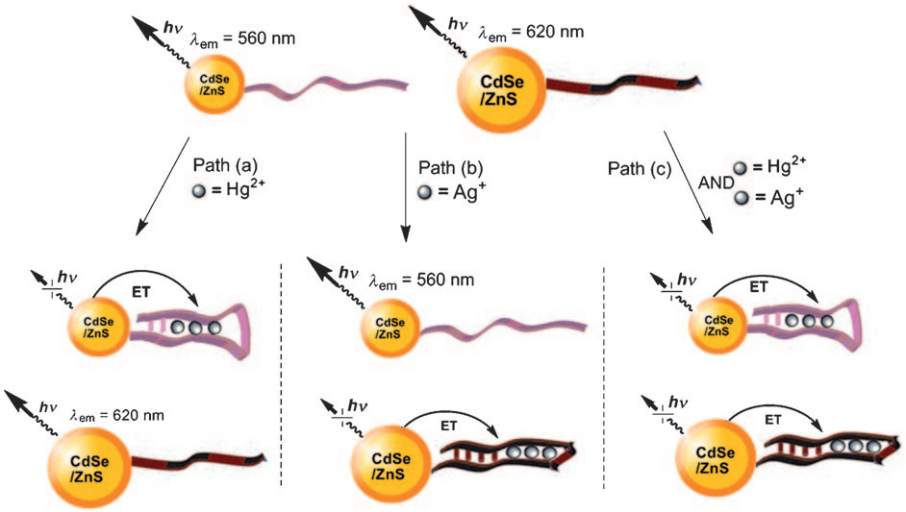


Figure 3. Time-dependent luminescence spectra corresponding to the multiplexed analysis of Hg^{2+} and Ag^{+} by **1**-QD^{560} and **2**-QD^{620}: a) in the absence of Hg^{2+} and Ag^{+}; b)–e) upon interaction with Hg^{2+} and Ag^{+}, 10, 20, 30, and 50 μM, respectively. Spectra recorded after a fixed time interval of 30 min.

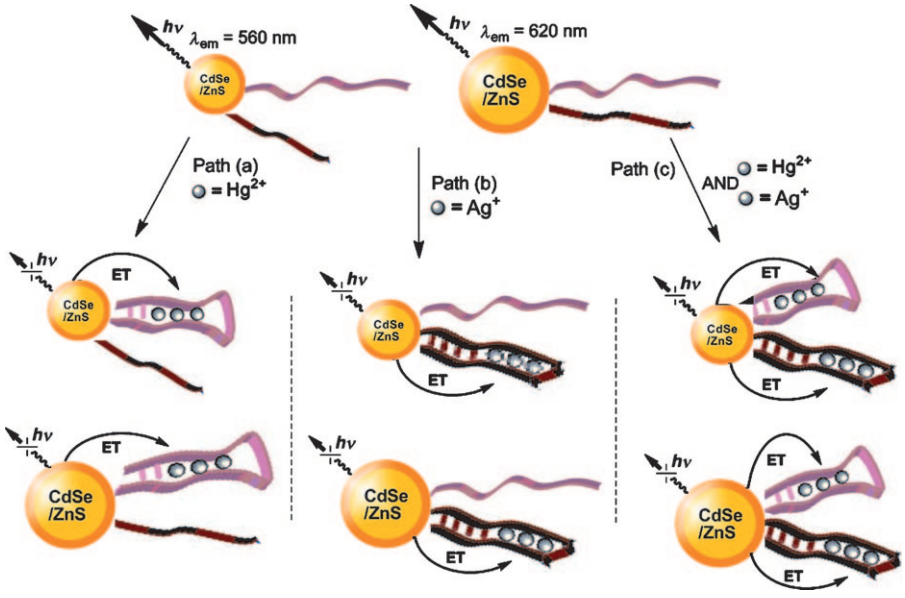
"False" output, or "0". Only in the presence of the two inputs, Ag^{+} and Hg^{2+} ions, are both emissions quenched ("True" output, or "1"). Figure 4 shows the luminescence features of the QDs in the presence of the different inputs. The "AND" logic gate activity of the system is summarized in the form of a truth table.

To design the "OR" gate (Scheme 3), each of the QDs systems QDs^{560} and QDs^{620} were functionalized with both nucleic acids, **1** and **2**. The luminescence of the QDs was quenched by either of the inputs Hg^{2+} or Ag^{+}, giving rise to the "OR" logic gate (Figure 5).

To conclude, the present study has introduced nucleic acid functionalized QDs for the multiplexed optical analysis of ions (Hg^{2+}, Ag^{+}). Besides the sensing features of the modified QDs, their use as labels to follow logic gate operations was demonstrated.



Scheme 2. The "AND" logic gate system based on electron-transfer quenching of **1**- and **2**-QDs by Hg^{2+} and Ag^{+} inputs, respectively.



Scheme 3. The "OR" logic gate system based on the electron-transfer quenching of **1**- and **2**-QDs by Hg^{2+} and Ag^{+} inputs.

Experimental Section

Materials: Ultrapure water from NANOpure Diamond (Barnstead Int., Dubuque, IA) source was used throughout the experiments. Hops Yellow Core–Shell EviDots, CdSe/ZnS quantum dots in toluene were purchased from Evident Technologies. BS^3 was purchased from Pierce Biotechnologies. All other chemicals and DNA were purchased from Sigma.

Preparation of glutathione(GSH)-capped QDs: QDs were precipitated from toluene (0.5 mL) by addition of methanol (2 mL), followed by centrifugation for 5 min at 3000 rpm. The resulting precipitate was dissolved in chloroform (1 mL), to which was added GSH solution (200 μL from a stock solution containing 0.142 g GSH and 40 mg KOH in 2 mL methanol), and the resulting mixture was shaken. NaOH solution in water (1.5 mL, 1 mM) was added to transfer all particles to the water phase. The QDs solution was separated from the chloroform layer by centrifugation for 1 min. Excess GSH was removed by two successive precipitation steps using NaCl and methanol followed by centrifugation. The resulting QDs were dissolved in 10 mM HEPES buffer (200 μL, pH 7.4).

Preparation of DNA-capped QDs: To the GSH-capped QDs (1 nmol) in HEPES buffer was added a BS^3 stock solution (100 μL, 1 mg mL^{-1}), and the mixture was shaken for 15 min. The QDs were purified by precipitation and dissolved in 10 mM HEPES buffer (pH 7.4). DNA stock solution (10^{-4} M, 1 mg mL^{-1}) was added, and then the resulting solution was shaken for 1.5 h. Finally, the excess DNA was removed by precipitation, and the purified particles were dissolved in the appropriate buffer solution (100 μL):[16b,20] MOPS (10 mM, pH 7.0), NaCl (25 mM), $NaNO_3$

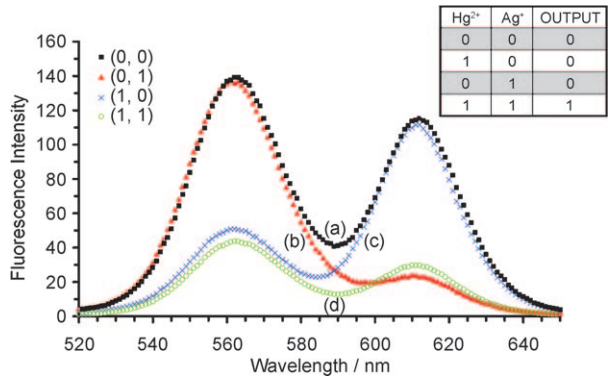


Figure 4. Time-dependent fluorescence changes of the "AND" logic gate system depicted in Scheme 2 and activated by the following inputs: a) no Hg^{2+}, no Ag^{+} (0, 0); b) no Hg^{2+}, 30 μM Ag^{+} (0, 1); c) no Ag^{+}, 30 μM Hg^{2+} (1, 0); d) 30 μM Hg^{2+}, 30 μM Ag^{+} (1, 1). Inset: Truth table of the "AND" gate logic system.

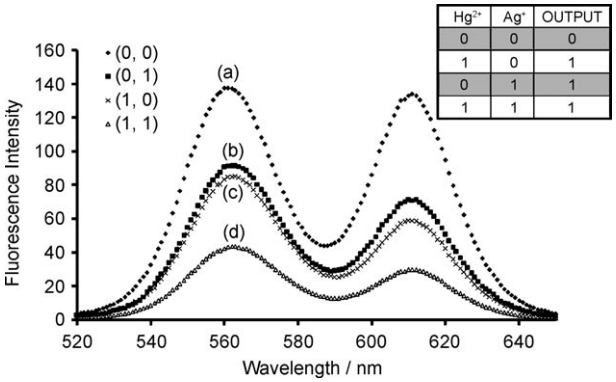


Figure 5. Time-dependent fluorescence changes of the "OR" logic gate system depicted in Scheme 3 and activated by the following inputs: a) no Hg^{2+}, no Ag^{+} (0, 0); b) no Hg^{2+}, 30 μM Ag^{+} (0, 1); c) no Ag^{+}, 30 μM Hg^{2+} (1, 0); d) 30 μM Hg^{2+}, 30 μM Ag^{+} (1,1). Inset: Truth table of the "OR" logic-gate system.

(500 mM), ethylenediamine (0.1 mM) for **1**-QD^{560} or MOPS (10 mM, pH 7.0) and $NaNO_3$ (50 mM) for **2**-QD^{620}. For the analysis of Ag+ and Hg+ ions, a buffer solution of MOPS (10 mM, pH 7.0) and $NaNO_3$ (50 mM) was used.

Determination of the loading of **1** and **2** on the particles: The loading of T-rich **1** or C-rich **2** on the QDs was determined spectroscopically. The spectra of the QDs were recorded before and after modification with the different modifiers. From the difference spectrum and the extinction coefficient of the residues (**1**: 171400 $M^{-1}cm^{-1}$ at $\lambda = 260$ nm, **2**: 301700 $M^{-1}cm^{-1}$ at $\lambda = 260$ nm), the concentrations of the different units were determined. Knowing the QD concentration and its extinction coefficient (QD^{560}: 100000 $M^{-1}cm^{-1}$ at $\lambda = 540$ nm, QD^{620}: 300000 $M^{-1}cm^{-1}$ at $\lambda = 600$ nm) allowed calculation of the average loading of units per particle.

Instrumentation: Real-time fluorescence measurements were carried out using a fluorescence spectrophotometer (Cary Eclipse, Varian Inc).

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