A modular self-assembled sensing system for heavy metal ions with tunable sensitivity and selectivity

A modular self-assembled sensing system for heavy metal ions with tunable sensitivity and selectivity

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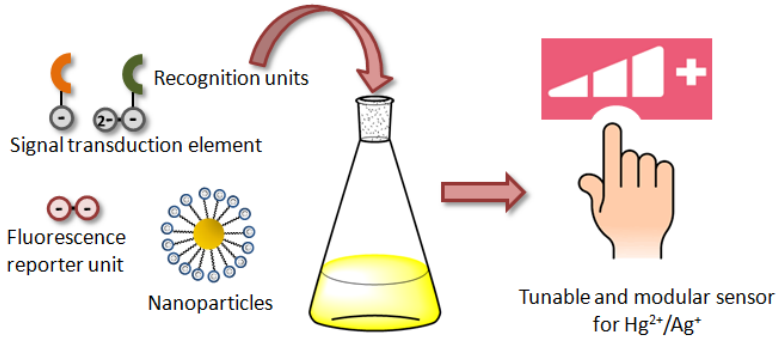
Abstract

Here we describe a self-assembled sensing system composed of three separate modules: gold nanoparticles, a reporter element, and a recognition element. The gold nanoparticles serve as a multivalent platform for the interaction with both the reporter and recognition element and the gold nucleus serves to affect the fluorescent properties of the reporter. The reporter element serves for generation of the output signal. The recognition element serves to make the assay selective. The working principle is that the interaction of the analyte with the recognition element leads to an increased affinity for the gold nanoparticle, which causes a displacement of the reporter and a turn-ON of fluorescence. It is shown that the modular nature of the system permits straightforward tuning of the dynamic detection range, the sensitivity, and the selectivity, simply by changing the recognition module. The system can detect Hg^{2+} and Ag^{+} metal ions at nanomolar concentrations in aqueous buffer.

Keywords

Gold nanoparticles, chemosensor, self-selection, self-assembly, displacement assay, heavy metal ions

Graphical abstract



1. Introduction

The development of innovative sensing systems for the detection of analytes plays a crucial role in, amongst others, molecular diagnostics, the detection of environmental pollutionand food contamination, and counter-terrorism measures.[1-6] Intensive research over the past decades has led to the development of a wide variety of chemosensors able to convert the presence of low concentrations of analytes into a detectable and easily readable output signal (optical, electrical, thermal etc.).[7-13] To favour practical applications, these systems have often been designed based on criteria of robustness and simplicity. However, there is a current interest in developing sensing systems of higher complexity, because of features that are difficult to obtain using simple molecules.[14-17] In particular, attention is being paid to the use of self-assembly as a design principle because it enables a modular approach through which the selectivity of the systems towards different analytes can be tuned in a straightforward manner simply by changing the building blocks.[18-22]

Here we report a self-assembled fluorescence turn-ON sensing system that is able to detect the heavy metal ions Hg^{2+} and Ag^{+} at nanomolar concentrations in water. Although it is worth mentioning that the detection of toxic heavy metals is extremely important from an environmental as well as a health perspective,[23-26] it is emphasized that the main focus of this work is on demonstrating the novel features offered by a self-assembly approach to chemosensor development. It will be shown that the different modules of the sensing system can be independently changed to alter the selectivity, sensitivity and dynamic detection range of the system,[27] while maintaining the same fluorescence output signal. In addition, this work provides a relevant implementation of our recently developed protocol for dynamic combinatorial chemistry (DCC) on a multivalent nanoparticle surface.[28]

2. Results and Discussion

The use of Au NP**1** for the detection of peptides, nucleotides, and small molecules has been reported by our group in recent years.[11,29-31] Au NP **1** is composed of a gold nucleus with a diameter of around 1.6 (±0.3) nm covered with a monolayer of alkyl thiols terminating with a 1,4,7-triazacyclononane (TACN)·Zn^{2+} complex (Fig. 1). Fluorescent displacement assays have been developed based on the displacement of a negatively charged fluorescent indicator from the Au NP **1** surface by analytes able to compete with binding to the surface. Recently, we have reported on a new signal transduction pathway relying on the formation of a ternary complex between two thymidine nucleotides and Hg^{2+} metal ions with an increased affinity for Au NP **1** compared to the separate nucleotides.[21, 25] An interesting feature of that study was that the multivalent NP surface drives the equilibrium towards the more stable thermodynamic complex

and self-selects the Hg^{2+} complex with the highest affinity. Indeed, it was observed that the addition of Hg^{2+} to a mixture of TMP and cTMP complex in aqueous medium in the absence of Au NP **1** led to the formation of all possible ternary complexes (TMP·Hg^{2+}·TMP, TMP·Hg^{2+}·cTMP, cTMP·Hg^{2+}·cTMP), but with an equilibrium composition shifted towards the more stable cTMP·Hg^{2+}·cTMP complex. However, the presence of Au NP **1** shifted the equilibrium entirely to the other side and exclusive formation of the TMP·Hg^{2+}·TMP complex was observed. This shift is driven by the high density of negative charges in this complex which cause a stronger interaction with the multivalent cationic surface of the Au NP **1**. This study showed that the affinity can be modulated simply by changing the signal transduction unit (the phosphates) without altering the recognition unit (the nucleobase). This stimulated us to investigate the possibility of exploiting the simultaneous use of different nucleotides to increase the dynamic detection range, which would emphasize the unique possibility offered by the signal transduction pathway that is operative in this self-assembled sensing system.

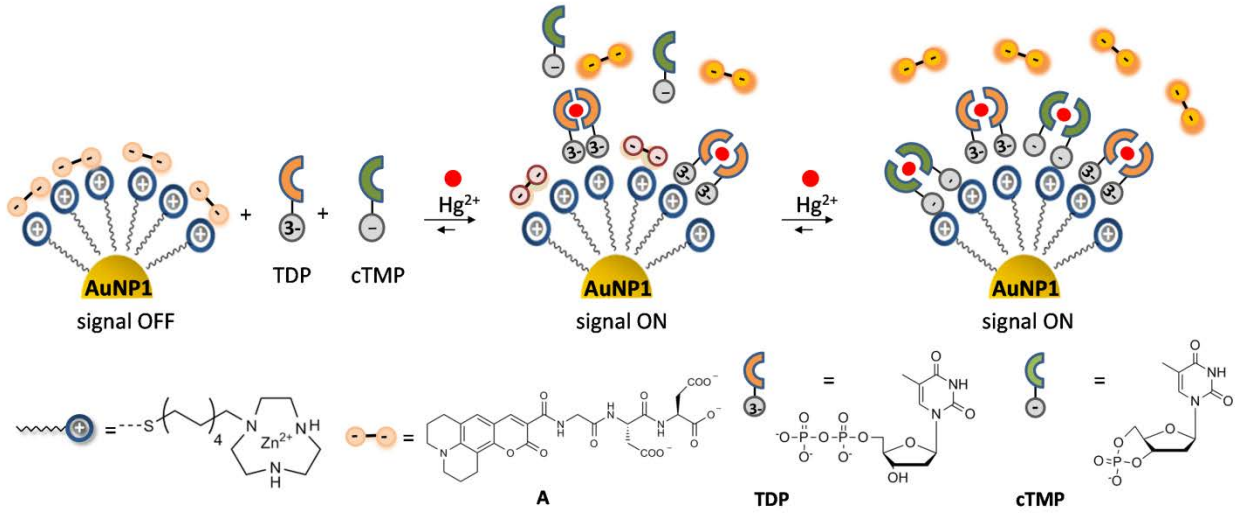


Fig. 1. Schematic representation of the signal generation process upon the addition of Hg^{2+} to the system composed of Au NP **1**, probe **A**, and both TDP and cTMP. Low concentrations of Hg^{2+} result in formation of the ternary complex TDP·Hg^{2+}·TDP. As soon as TDP is depleted, further amounts of Hg^{2+} are complexed with cTMP. This way the dynamic sensing regime of the system is enlarged covering a Hg^{2+} concentration range low nanomolar to high micromolar.

2.1. Dynamic detection range

Previous studies had shown that the sensitivity of the system is much higher when TDP is used (compared to TMP or cTMP), because of the increased number of negative charges.[21] We presumed that the combination of TDP (instead of TMP) and cTMP in the same system would

enlarge the dynamic detection range with TDP operating in the nanomolar regime and cTMP in the micromolar regime. The anionic probe **A** was selected because of the following reasons: i) the high quantum yield of coumarin343 (λ_{ex} = 445 nm; λ_{em} = 493 nm) is advantageous for creating a response even at low concentrations, ii) the carboxylate-probe is readily displaced by phosphate competitors, and iii) the fluorescence properties of the probe are not affected by the analytes.[28]

The detection range for TDP and cTMP separately was determined by measuring the fluorescence intensity after the addition of increasing amounts of Hg^{2+} to a buffered aqueous solution containing Au NP **1** ([TACN·Zn^{2+}] = 20 ± 1 μM), **A** (7.3 μM) and either TDP (16 μM) or cTMP (800 μM). A much higher concentration of cTMP was used compared to TDP, because a much higher concentration of the cTMP·Hg^{2+}·cTMP complex is required to elicit a displacement of probe **A** from Au NP **1**. For TDP the fluorescence intensity started to increase already after the addition of 20 nM of Hg^{2+} and continued until a maximum intensity was reached at around 7 μM of Hg^{2+}, which corresponds roughly to the concentration at which all free TDP has been depleted (Fig. 2a). On the other hand, when cTMP was present the increase in fluorescence intensity started at 2 μM Hg^{2+} and continued until 50 μM. These results clearly show that the negative charges present in the probes dictate the detection range. Next, the response of the system was measured in the presence of both TDP and cTMP at the same concentrations as before. We were very pleased to observe that also in this case already at low nanomolar concentrations of Hg^{2+} a fluorescence signal was generated following the response curve of only TDP. Yet, at around 5 μM of Hg^{2+} the new response curve deviated and continued at lower intensity reaching a final plateau level at around 50 μM of Hg^{2+} similar to that observed for cTMP alone. The change in response curve at around 5 μM of Hg^{2+} originates from the fact that the depletion of TDP starts to statistically favour the formation of complexes TDP·Hg^{2+}·cTMP first and cTMP·Hg^{2+}·cTMP later. The result is a significant increase in the dynamic range of the sensing system which ranges now from 20 nM to 50 μM with linearity in the 0-5 μM concentration range (Fig. 2b).

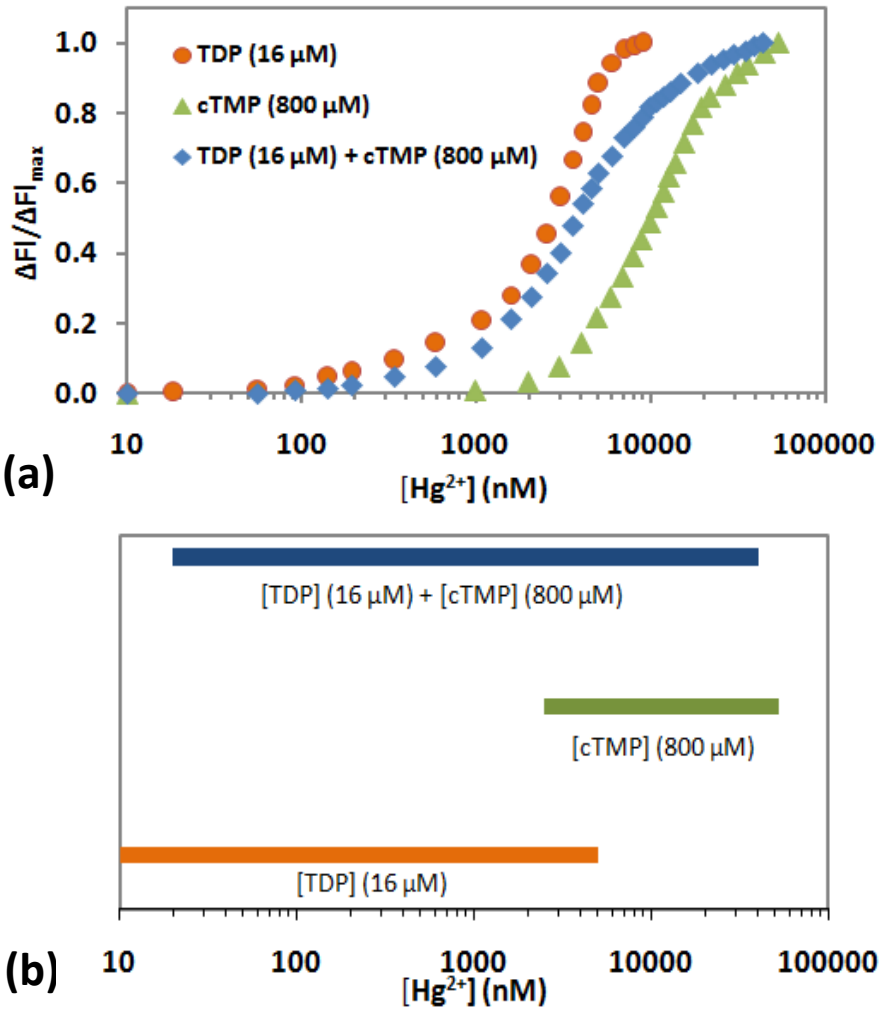


Fig. 2.(a) Normalized change in fluorescence intensity (a.u.) at 493 nm as a function of the concentration of Hg^{2+} added to a solution containing Au NP**1**, probe **A**, and TDP (orange), cTMP (green), or a mixture of TDP and cTMP (blue). Experimental conditions: [TACN·Zn^{2+}] = 20 ± 1 μM; [**A**] = 7.3 μM, [TDP] = 16 μM, [cTMP] = 800 μM, [HEPES] = 10 mM, pH 7.0, T = 37 °C, fluorescence slit width = (2.5/5) nm. (b) Hg^{2+}-detection range in the presence of TDP (orange), cTMP (green), or a mixture of TDP and cTMP (blue).

2.2. Sensitivity

Previously, we exploited a dynamic combinatorial approach for the target driven self-selection of recognition units on the surface of Au NP **1**.[28] In line with the approach described above, the ability of target metal ions to induce the clustering of receptor units led to an accumulation of only those receptor units on the monolayer surface that are able to complex the added analyte. Thus, the addition of Hg^{2+} to a mixture of 4 nucleotides (dAMP, dGMP, TMP, and dCMP) resulted in the self-selection of TMP as the optimal recognition element for Hg^{2+} metal ions. On the other hand, it was found that the addition of Ag^{+} to the same mixture of nucleotides resulted in the capturing of dGMP on Au NP **1**. As such, this was a proof-of-principle demonstration that self-selection procedures can indeed be used to affect the composition of dynamic responsive multivalent surfaces. Here, we show a logical follow-up of that study in which we exploit the information obtained from these self-selection experiments for the development of a sensing system able to selectively detect either Ag^{+} or Hg^{2+} metal ions (or both) at low nanomolar concentrations in water.

The results described in the previous section have already shown that the use of thymidine nucleotides as recognition module provides a system that produces a fluorescent signal upon the addition of Hg^{2+}. Next, we were interested whether the replacement of the TMP for GMP, while keeping all other components the same, would make the system responsive to Ag^{+} instead. Thus, titration experiments were performed by adding increasing amounts of Ag^{+} metal ions to a buffered aqueous solution containing Au NP **1** ([TACN·Zn^{2+}] = 20 ± 1 μM), **A** (7.3 μM) and dGMP (6 μM) followed by measurement of the fluorescence intensity after each addition (Fig. 3a). We were pleased to observe an increase in fluorescence as a function of Ag^{+} concentration, but regrettably with a low signal strength in the low micromolar region ($\Delta FI_{dGMP}/\Delta[Ag^{+}]$ = 1.0), indicating that the complex formed between Ag^{+} and dGMP has a poor capacity to displace probe **A** from Au NP **1**. However, the modular nature of the sensing systems permits a straightforward solution to this problem by increasing the number of negative charges in the nucleotide. Since the transduction element (the phosphates) is independent from the recognition element (the nucleobase), this alteration does not (or to a very minimal extent) affect the interaction with the analyte. Indeed, when the experiment was repeated using GDP instead of dGMP (at the same concentration) a 40-fold stronger response ($\Delta FI_{GDP}/\Delta[Ag^{+}]$ = 44.7) was measured (Fig. 3a). The use of GDP and optimized instrument settings permitted the obtainment of a linear response curve for Ag^{+} in the 100-2000 nM concentration range (Fig. 3b).

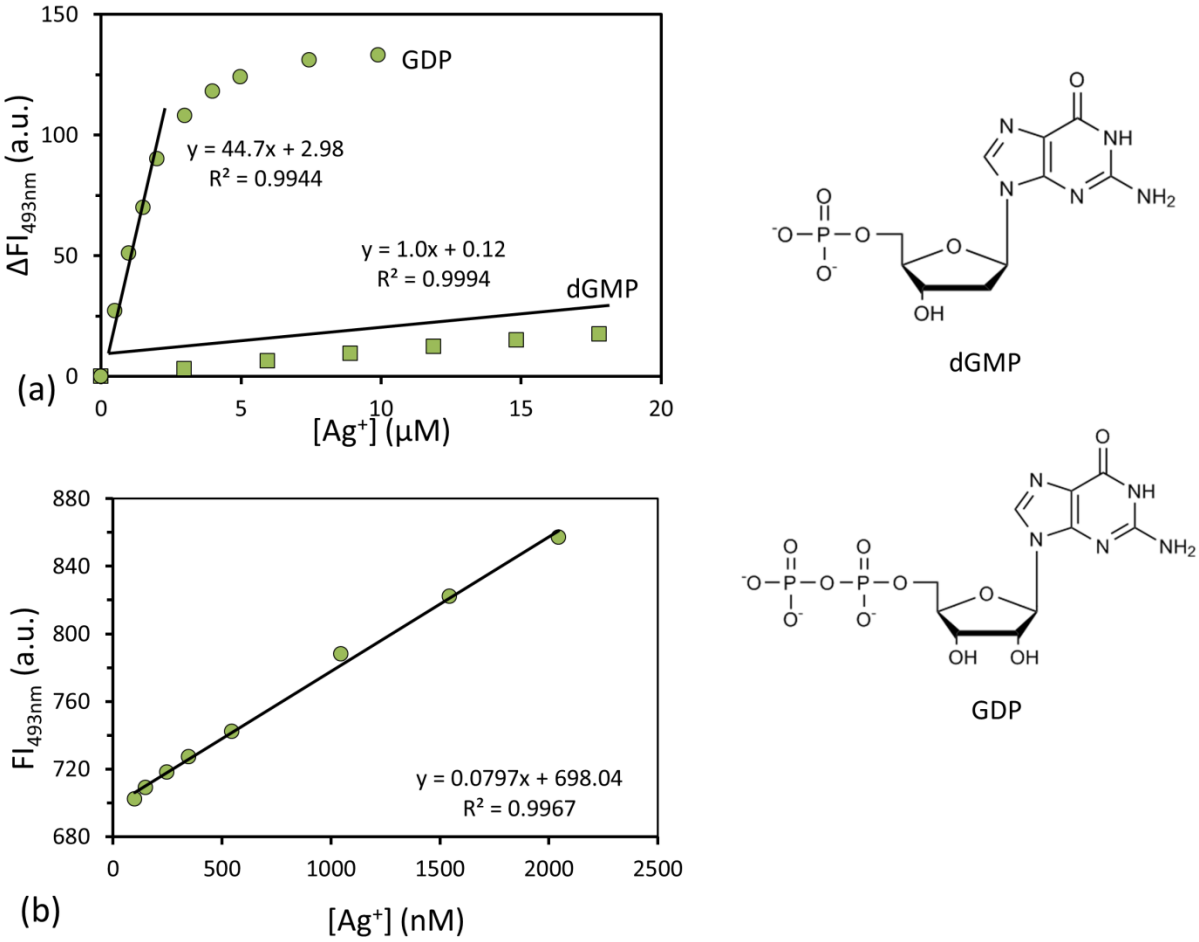


Fig. 3. (a) Change in fluorescence intensity (a.u.) at 493 nm as a function of the concentration of Ag^+ added to a solution containing Au NP **1**, probe **A** (7.3 μM) and GDP (circles) or dGMP(squares).(b) Fluorescence intensity (a.u.) at 493 nm as a function of the concentration of Ag^+ (50-2000 nM). Experimental conditions: [TACN·Zn^{2+}] = 20 ± 1μM; [**A**] = 7.3 μM, [HEPES] = 10 mM, pH 7.0, T = 37 °C, [dGMP] = [GDP] = 6 μM (for Fig. 3a); [GDP] = 6 μM (for Fig. 3b); fluorescence slit width = (2.5/5) nm (for Fig. 3a) and (5/5) nm (for Fig. 3b).

2.3. Selectivity

Finally, we were interested in the selectivity of the sensing system containing the guanine nucleobase as the recognition element. Previously we had shown that the use of the TDP recognition unit gave a highly selective sensing system for Hg^{2+}. For comparison, the already published data from a comparative study between a series of 12 metal ions (amongst which include Pb^{2+}, Cd^{2+}, Pd^{2+}, Ag^{+}, As^{5+}) at a constant concentration of 1 μM are reproduced in Fig. 4a. A repetition of the same experiment using GDP instead of TDP completely changed the selectivity of the system for Ag^{+}. Only for Ag^{+} a significant increase in fluorescence intensity was observed, indicating the inability of the other metal ions to form complexes with GDP able to compete with probe **A** for binding to Au NP **1** (Fig. 4b). Similar as observed for Hg^{2+}, the increase in fluorescence intensity induced by Ag^{+} (1 μM) was hardly affected by the presence or not of a mixture of all other metal ions (1 μM each; final column, Fig. 4b).

A peculiar feature of the sensing system presented here is that the selectivity of the system for Hg^{2+} or Ag^{+} is determined by the type of nucleobase present in the nucleotide (thymine or guanine, respectively). However, after this selective recognition event the signal transduction pathway is identical for both analytes, because the affinity of the formed complexes for Au NP **1** originates in both cases from the same phosphate groups. This gives the possibility to develop an assay that reports on the presence of either one (or both) of the analytes Hg^{2+} or Ag^{+} in a complex mixture simply by using the two recognition elements TDP and GDP contemporarily. This was demonstrated by an experiment in which both TDP (10 μM) and GDP (6 μM) were present. Their concentrations were chosen such that 1 μM of Hg^{2+} and Ag^{+} would give a comparable increase of fluorescence intensity ($\Delta FI_{Hg2+} \approx 41$a.u.; $\Delta FI_{Ag+} \approx 43$a.u.). Indeed, a screening of the set of metal ions now gave positive signals when either Hg^{2+} or Ag^{+} was added, but not for any of the other metals (Fig. 4c). As a side note, it is of interest to note that the simultaneous addition of both Hg^{2+} and Ag^{+} resulted in an increase of fluorescence intensity practically equal to the sum of the individual values (Fig. 4c). Response systems like this are attracting interest as logic gates for chemical computing.[32, 33]

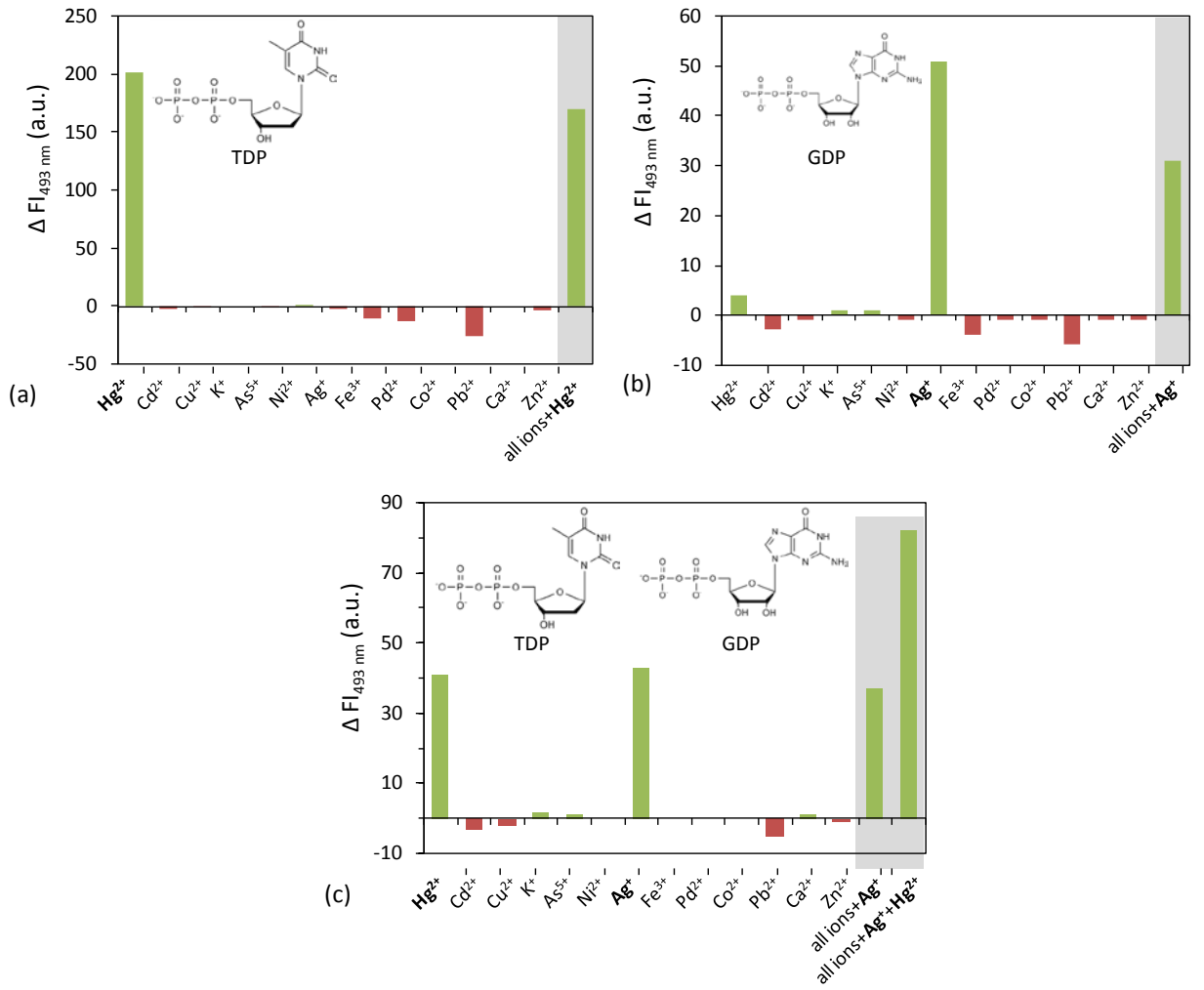


Fig. 4.(a) Changes in the fluorescence intensity at 493 nm upon the addition of 1 μM of a series of metal ions to a solution containing Au NP **1**, probe **A**, and TDP. Reproduced with permission from reference 21. (b) Changes in the fluorescence intensity at 493 nm upon the addition of 1 μM of a series of metal ions to a solution containing Au NP **1**, probe **A**, and GDP. (c) Changes in the fluorescence intensity at 493 nm upon the addition of 1 μM of a series of metal ions to a solution containing Au NP **1**, probe **A**, TDP and GDP. Experimental conditions 4a-c: [TACN·Zn^{2+}] = 20 ± 1μM; [CGDD] = 7.3 μM, [TDP] = 16 μM (for 4a) and 10 μM (for 4c), [GDP] = 6 μM (for both 4b and c), [HEPES] = 10 mM, pH 7.0, T = 37 °C, fluorescence slit width = 5/5 nm (for 4a) and 2.5/5 nm (for 4b and c).

3. Conclusions

In conclusion, we have developed a self-assembled sensing system for the selective detection of Hg^{2+} and Ag^{+} at nanomolar concentrations in water. Apart from the strong analytical performance, the novelty of the system is that it is composed of modules that can be independently changed to adapt the selectivity, sensitivity, and, in principle, also the output signal. This is a unique feature when compared to numerous other nanoparticle based sensing systems reported in the literature, that typically rely on the presence of recognition units that are covalently attached to the monolayer.[12,13] It is emphasized that all components of the system are either commercially available or synthesized in a few steps (including Au NP **1**). In addition, contrary to most other thymine and guanosine-based sensing systems for the detection of Hg^{2+} and Ag^{+}, respectively, unmodified nucleotides are used, which permits a straightforward modulation of the systems response. Currently, we are working at expanding the scope of the assay by incorporating appropriate recognition modules for the recognition of small (bio)molecules.

4. Experimental Section

The synthesis and characterization of Au NP **1** has been described elsewhere.[31] Stock solutions of Au NP **1** were preserved at 4° C in mQ water. The concentration of TACN-head groups in Au NP **1** was determined from kinetic titrations as reported previously.[30,31] $Zn(NO_3)_2$ stock solution were standardized using EDTA following standard procedures. The synthesis and characterization of the fluorescence probe (**A**) has been described before.[30]

The buffer, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was obtained from Sigma-Aldrich and used without further purification. All the nucleotides (TDP, cTMP and GDP, GMP) were obtained from Sigma-Aldrich and their stock solutions were prepared both by weight and UV-vis spectroscopy using the following molar extinction co-efficients : ε268 (TDP, cTMP) = 9600 $M^{-1}cm^{-1}$; ε272 (GDP, GMP) = 13700 $M^{-1}cm^{-1}$.[30] All metal salts [$Hg(NO_3)_2$, $AgNO_3$, $Cu(NO_3)_2$, $Fe(NO_3)_3$, $Pb(NO_3)_2$, $Ni(NO_3)_2$, $Co(NO_3)_2$, $NaAsO_4$, KNO_3, $Cd(NO_3)_2$, $Ca(NO_3)_2$, $PdCl_2$] were purchased from Sigma-Aldrich and their stock solutions were prepared by weight.

UV-Visible spectra were measured on a Varian Cary50 spectrophotometer equipped with thermostatted multiple cell holders.

Fluorescence measurements were performed on a Varian Cary Eclipse fluorescence spectrophotometer also equipped with a thermostatted cell holder.

Protocols for the displacement experiments and selectivity studies have been described before.[21, 28]

The normalization in Fig. 2a has been done by following the equation mentioned below:
Normalized value = $(F - F_{min})/(F_{max} - F_{min})$; where F = fluorescence value in presence of any concentration of added Hg^{2+} ion, F_{min} = fluorescence value when no Hg^{2+} ion was added, F_{max} = fluorescence value in presence of maximum amount of added Hg^{2+} ion. F_{min} and F_{max} values for TDP were 176 and 427; for TDP + cTMP were 181 and 419; for cTMP were 3.5 and 233, respectively under the experimental condition described in the main text.

5. Acknowledgements

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6. References

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