Multivalent Interactions Regulate Signal Transduction in a Self-Assembled Hg$^{2+}$ Sensor

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Supporting Information

**Abstract:** A self-assembled sensing system able to detect Hg$^{2+}$ at low nanomolar concentrations is reported that operates through a signal transduction pathway involving multivalent interactions. The analyte causes dimerization of low-affinity ligands, resulting in a complex with a high affinity for a multivalent monolayer-protected gold nanoparticle (AuNP). This complex displaces a quenched fluorescent reporter from the AuNP, resulting in a turn ON of fluorescence. It is shown that the strength of the output signal can be regulated by tuning the multivalent interactions between the complex and the NP. Finally, it is shown that multivalent interactions drive the self-selection of a high-affinity complex from a mixture of low-affinity ligands.

Protein dimerization constitutes a key step in many natural signal transduction pathways. Examples include ligand-induced dimerization of cell surface receptors (e.g., TGF-$\beta$ or EGF receptor) to initiate intracellular signaling pathways and dimerization of transcription factors (e.g., nuclear hormone receptors) to initiate gene expression. Protein dimerization is a powerful regulatory mechanism because it affects various physical and physiological parameters, such as the proximity and orientation of the proteins, specificity, surface area, etc. The installment of similar mechanisms in synthetic systems is of interest not only for developing innovative sensors and responsive materials, but also for controlling dynamic networks in the emerging area of systems chemistry. Various supramolecular sensing systems have been developed in which the target analyte induces dimerization of two components, which results in signal generation, typically by activating a catalytic or an energy-transfer process. Here, we present a sensing system in which a signal is generated in an alternative way. The presence of analyte induces dimerization of low-affinity ligands into a ternary complex with a high affinity for a multivalent monolayer-protected gold nanoparticle (AuNP). This complex then displaces a (quenched) fluorescent probe from the surface, resulting in a turn ON of fluorescence (Figure 1). The innovative feature of this system is that just forming the ternary complex by itself is not sufficient for signal generation. Rather, the efficacy of signal generation is determined by the strength of the interaction between the ternary complex and the NP. It will be shown that this peculiar feature permits a straightforward regulation of the signal strength for a given analyte concentration and results in nonlinear response curves when mixtures of receptors are present. Finally, it is shown that multivalent interactions drive the self-selection of the high-affinity complex from a mixture of low-affinity ligands.

The key component of the system is AuNP 1, a gold nanoparticle ($d = 1.8 \pm 0.4$ nm) covered with hydrophobic C9-thiols terminating with a 1,4,7-triazacyclononane (TACN)-Zn$^{2+}$ headgroup (Figure 1). Previously, we have shown that small oligoanions, such as di- or trinucleotides and Asp/Glu-rich peptides, have a high affinity for AuNP 1 and bind to the monolayer surface under saturation conditions, even at low $\mu$M concentrations in aqueous buffer. Although binding is mainly driven by electrostatic interactions, it is also aided by the development of hydrophobic interactions between apolar parts of the molecules and the monolayer. An attractive feature of the multivalent nature of AuNP 1 is that the binding affinity is correlated to the number of negative charges present in the oligoanions. On the basis of this observation, we hypothesized that such multivalent interactions could be at the basis of a sensing system, as described above (Figure 1).

Search for a suitable system to test this hypothesis, we were intrigued by the pioneering work of Ono and Togashi, which had demonstrated that Hg$^{2+}$ highly selectively binds to the DNA nucleobase thymine (T), forming a T-Hg$^{2+}$-T sandwich complex (Figure 1). The subsequent development of numerous assays based on this recognition motif confirmed the robustness and

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high fidelity of this recognition process.\textsuperscript{14} For our purposes this recognition motif was highly attractive because the availability of a series of thymine nucleotides (T XP) with a varying number of phosphate groups would enable us to study the role of the multivalent interactions in the signal transduction pathway.

Our initial studies focused on TDP in order to determine the responsiveness of the system against Hg\textsuperscript{2+}. Fluorescence displacement studies were performed to determine the affinity of TDP for the surface of AuNP 1 compared to the fluorescent probe A. Probe A was selected for the following reasons: (i) previous studies had revealed that carboxylate probe A has a high affinity for AuNP 1 but can be readily displaced by phosphate competitors,\textsuperscript{11b} (ii) the excellent fluorescence properties of coumarin 343 permit a high sensitivity,\textsuperscript{15} and (iii) the fluorescence properties of probe A are not altered by Hg\textsuperscript{2+} (see SI). The displacement study was performed at a constant TACN-headgroup concentration of 20 \(\mu\)M and a probe A concentration of 7.3 \(\mu\)M, corresponding to 90% of the surface saturation concentration (Figure 2a, see SI for determination of the SSC). It was observed that adding 35 \(\mu\)M TDP displaced 50% of A from the surface. Next, we tested the sensing ability of the system by titrating increasing amounts of an aqueous solution of Hg(NO\textsubscript{3})\textsubscript{2} to a solution containing AuNP 1 (20 \(\pm\) 1 \(\mu\)M headgroup), A (7.3 \(\mu\)M), and TDP (16 \(\mu\)M) in the presence of HEPES (10 mM) at pH 7.0 and \(T = 37^\circ\)C. The concentration of TDP was chosen as a compromise: as high as possible to favor formation of the ternary TDP-Hg\textsuperscript{2+}-TDP complex, and as low as possible to avoid displacement of probe A by TDP itself. At the selected concentration of 16 \(\mu\)M TDP, \(\sim 30\%\) of A is displaced in the absence of Hg\textsuperscript{2+}. We were very pleased to observe a strong increase in fluorescence intensity (FI) already upon addition of Hg\textsuperscript{2+} at sub-\(\mu\)M concentrations (Figure 2b). This supported our hypothesis that the ternary complex TDP-Hg\textsuperscript{2+}-TDP is a more effective competitor for A than TDP alone. A plateau level was reached after addition of \(\sim 7.0\mu\)M Hg\textsuperscript{2+}, corresponding to a displacement of \(\sim 75\%\) of A (\(\Delta F_{493} = 245\) a.u.). The fact that adding 7.0 \(\mu\)M Hg\textsuperscript{2+} to the system in the absence of TDP resulted in a fluorescence increase of just 15 a.u. indicated that formation of the ternary complex is indeed required for signal generation (see SI). When the instrument settings were optimized, the system was able to generate a linear response curve in the low nM concentration regime (5–50 nM, Figure 2b inset). A response to [Hg\textsuperscript{2+}] in the nM regime could also be measured in real water samples (tap and rain water), illustrating the sensing system’s tolerance to the presence of other components (see SI). The selectivity of the sensing system emerged clearly from a comparative study among a series of 12 metal ions (including Pb\textsuperscript{2+}, Cd\textsuperscript{2+}, Pd\textsuperscript{2+}, Ag\textsuperscript{+}, and As\textsuperscript{3+}) at a constant concentration of 1 \(\mu\)M (Figure 2c). Only for Hg\textsuperscript{2+} a significant increase in FI was observed, indicative of the inability of the other metal ions to form a ternary complex with TDP. The increase in FI induced by 1 \(\mu\)M Hg\textsuperscript{2+} was hardly affected by the presence or absence of a mixture of all other metal ions (1 \(\mu\)M each; final column, Figure 2c). The sensing system’s tolerance of anions depends mainly on the anion charge (SI). Singly charged anions (chloride, acetate, nitrate) do not displace A up to mM concentrations, whereas doubly charged anions (carbonate, phosphate) interfere at high \(\mu\)M concentrations (100–500 \(\mu\)M). Higher charged anions (pyrophosphate) are not tolerated, as they cause a displacement of probe A already at low \(\mu\)M concentrations. In any case, a linear response curve for Hg\textsuperscript{2+} could be obtained at 50–800 nM in the presence of the above-mentioned anions (apart from pyrophosphate) at 100 \(\mu\)M each (SI).

The excellent performance of this sensing system in terms of selectivity and sensitivity reflects the reliability of the T-Hg\textsuperscript{2+}-T recognition motif and is in line with that observed for other Hg\textsuperscript{2+}-sensing systems based on the same motif.\textsuperscript{12} However, apart from the fact that our system does not require chemical modification of the nucleotide, the most intriguing feature of the system presented here is that formation of the ternary complex between thymine and Hg\textsuperscript{2+} leads only indirectly to an output signal. Signal generation requires displacement of probe A from AuNP 1 through competitive binding of the ternary complex. It is the strength of this interaction that determines the efficacy at which ternary complex formation is transduced into signal. As such, the system bears a close resemblance to natural signal transduction pathways. Likewise, this implies that it should, in principle, be possible to modulate the strength of the output signal simply by changing the number of phosphate groups attached to the recognition unit. Such a control mechanism is not possible in regular supramolecular sensors that rely on dimerization.\textsuperscript{8} This would provide for a straightforward means to regulate the response of the system without altering the recognition (thymine) or reporter unit (probe A). This possibility was investigated by comparing the response of the system to 0.5–5 \(\mu\)M Hg\textsuperscript{2+} using TMP and cTMP instead of TDP. Initial displacement studies of probe A by TMP and cTMP in the absence of Hg\textsuperscript{2+} revealed that, as expected, the affinity of the nucleotides for AuNP 1 diminished strongly with a decrease in the number of negative charges (Figure 2a). Indeed, addition of...

Figure 2. (a) Fluorescence intensity (a.u.) at 493 nm as a function of the concentration of competitor (TDP, TMP, or cTMP) added to a solution containing AuNP 1 and probe A. The dashed gray line indicates the concentration of 16 \(\mu\)M used for panels b and c. (b) Fluorescence intensity (a.u.) at 493 nm as a function of [Hg\textsuperscript{2+}] added to a solution containing AuNP 1, probe A, and TDP. The inset shows the response curve at [Hg\textsuperscript{2+}] = 5–50 nM, obtained with optimized instrument settings. (c) Changes in the fluorescence intensity at 493 nm upon addition of 1 \(\mu\)M concentrations of a series of metal ions to a solution containing AuNP 1, probe A, and TDP.
1.5 mM TMP led to only a minor displacement of A (17%), whereas cTMP was unable to displace A from the surface of AuNP I over the entire concentration range studied. Next, Hg$^{2+}$ response curves were measured at constant concentrations of nucleotide ([TDP] = [TMP] = [cTMP] = 16 μM) and probe A (7.3 μM) in order to have the same concentrations of recognition (thymine) and reporter units. A plot of ΔFI (compared to the FI measured for [Hg$^{2+}$] = 0.5 μM) as a function of [Hg$^{2+}$] clearly showed that the strength of the output signal diminished as the number of negative charges present in the thymine nucleotide decreased (Figure 3). A quantitative analysis, comparing the slopes of the response curves, revealed that the signal change induced by the same amount of Hg$^{2+}$ is 5 times stronger when TDP is present compared to cTMP (ΔFI$_{493nm}$/Δ[Hg$^{2+}$]$_{TDP}$ = 39.8; ΔFI$_{493nm}$/Δ[Hg$^{2+}$]$_{cTMP}$ = 7.8). Thus, the strength of the multivalent interaction between the ternary complex TXP-Hg$^{2+}$, TDP and AuNP I acts as a regulatory element that determines the effectiveness at which the presence of Hg$^{2+}$ is transduced into signal.

Many natural ligand—receptor systems involved in signal transduction are composed of multiple receptors and/or ligands. It has been observed that the systems’ response can be dictated by the thermodynamic equilibrium distribution of the ligand in different receptors. We were interested in finding out how the Hg$^{2+}$-sensing system discussed here would respond if different ligands for Hg$^{2+}$ were present (Figure 4). We chose to use TMP and cTMP for these studies, as these probes by themselves do not displace probe A from AuNP I (Figure 2a) below 100 μM. The interaction of TMP and cTMP with Hg$^{2+}$ (0–100 μM) was first studied in the absence of AuNP I to determine the intrinsic behavior of the two receptors. Separate UV/vis titrations showed that the complex cTMP-Hg$^{2+}$-cTMP has a slightly higher association constant (log β$_{12}$ = 8.14) than TMP-Hg$^{2+}$-TMP (log β$_{12}$ = 7.53), presumably owing to the lower number of negative charges present in cTMP (SI). Next, Hg$^{2+}$ was titrated to mixtures containing both TMP and cTMP at different ratios, but at a constant overall concentration of 100 μM. In this case, in addition to the homomeric complexes, formation of the heteromeric complex TMP-Hg$^{2+}$-cTMP is also possible. A plot of log β$_{12}$ as a function of the ratio TMP:cTMP showed a gradual increase in log β$_{12}$ from 7.53 to 8.14, indicating that the heteromeric complex has an intermediate association constant (SI). Thus, in the absence of AuNP I, adding Hg$^{2+}$ to a mixture of TMP and cTMP results in the formation of all three complexes, with a distribution slightly shifted toward cTMP-Hg$^{2+}$-cTMP.

A completely different scenario was observed when Hg$^{2+}$ titrations were performed in the presence of AuNP I. Response curves in the presence of AuNP I were obtained by measuring the amount of probe A displaced upon addition of increasing amounts of Hg$^{2+}$ to a solution containing AuNP I, probe A, and one of the thymidine probes TDP, TMP, or cTMP.

![Figure 3](image_url)  
**Figure 3.** Increase in the fluorescence intensity at 493 nm (with respect to the FI at [Hg$^{2+}$] = 0.5 μM) upon addition of Hg$^{2+}$ to a solution containing AuNP I, probe A, and one of the thymidine probes TDP, TMP, or cTMP.
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quantitative response. Interestingly, in the presence of 5 μM Hg²⁺, a significant drop in [TMP] was observed (2.3 μM), whereas [cTMP] remained virtually unchanged (73.1 μM). This confirms the hypothesis that adding small amounts of Hg²⁺ results in self-selection of the TMP-Hg²⁺-TMP complex on the surface of AuNP 1. From the fluorescence displacement experiments, it was observed that, at [Hg²⁺] = 10 μM, the curve for the 20:80 mixture started to deviate from the TMP curve, indicating that, at this concentration, cTMP is also displaced.

Figure 5. (a) Amount of displaced A as a function of [Hg²⁺] for a 20:80 mixture of TMP:cTMP (100 μM), 20 μM TMP, or 80 μM cTMP. (b) Amount of displaced A as a function of the ratio TMP:cTMP in the presence of 5 μM Hg²⁺. The black dots represent the values obtained when only TMP was present at the same concentration. (c) Amount of TMP (top) or cTMP (bottom) present in the dialysate after ultrafiltration of a solution containing AuNP 1 ([TACN-Zn²⁺] = 30 μM), probe A (11.7 μM), TMP (20 μM) and cTMP (80 μM), and different amounts of Hg²⁺ (0, 5, 10, or 20 μM).

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References


Associated Content

Supporting Information
Materials and methods, experimental conditions, SSC determination, experiments with rain and tap water, fluorescence and UV/vis titrations, and UPLC analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
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