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Chemosensing

Orthogonal Sensing of Small Molecules Using a Modular Nanoparticle-Based Assay

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Abstract: Herein, we present a modular indicator-displacement assay able to selectively recognize small molecules with biological relevance under competitive conditions. The assay relies on the change in affinity of macrocyclic receptors, such as cavitands, cyclodextrins or calixarenes, for monolayer-protected gold nanoparticles upon complexation of the respective target analyte. This change affects the equilibrium between the nanoparticles and a fluorescent reporter leading to a change in intensity of the fluorescent output signal. The recognition modules can be changed in order to tune the selectivity of the assay without affecting the nature of the output signal. The combined use of recognition modules results in an assay able to detect multiple analytes simultaneously with high selectivity.

The ability to detect specific analytes, such as biomarkers, food contaminants, and pollutants, in complex mixtures is of crucial importance in many fields. Over the years, chemists have developed elaborate molecular structures specifically designed to signal the presence of target analytes with high selectivity.[1] However, the excellent performances of such chemosensors are counterbalanced by their tedious synthesis and the limitation to detect only the analyte it was designed for. This has led to a strong interest in chemosensors that rely on the self-assembly of recognition and signaling units to form the functional system.[2] In particular, indicator-displacement assays (IDAs) have emerged as a very powerful class of chemosensing systems.[3] In IDAs, the analyte displaces an indicator from a recognition site causing a detectable change in its properties. Although the self-assembly approach has significantly facilitated synthetic access to chemosensors, the selectivity of these systems is still determined by a given recognition site. This implies that adaptation of the assay to different analytes still requires a modification of the recognition site and, consequently, also the indicator (which alters the output signal). Here, we report a fully modular sensing assay for the selective detection of small organic molecules that overcomes this limitation. The innovative feature of this assay is that the selectivity can be tuned simply by changing the recognition modules, which are easily accessible macrocyclic receptors such as cavitands, calixarenes or cyclodextrins. The simultaneous use of different receptors permits the detection of multiple analytes with high selectivity.

Previously, we have shown that small anionic biomolecules, such as peptides and nucleotides, have a high affinity for Au NP **1**, which are gold nanoparticles ($d = 1.8 \pm 0.4$ nm) covered with C_9-thiols terminating with a 1,4,7-triazacyclononane (TACN)·Zn^{2+} head group (Figure 1).[4] We have recently exploited these interactions for the development of an assay able to selectively detect metal ions.[5] This assay relied on the selective interaction of Hg^{2+} or Ag^{+} metal ions with nucleotides (TMP or dGMP, respectively), leading to complexes with a high affinity for Au NP **1**. This caused the displacement of a fluorescent indicator from the surface of Au NP **1** and, consequently, a turn ON of fluorescence intensity. The challenge in applying a similar approach for the selective detection of small organic molecules lies in the identification of suitable recognition modules. The key criterion is that the affinity of the recognition unit for Au NP **1** has to change upon complex formation with the analyte. Several indicators pointed towards the use of macrocyclic receptors, such as calixarenes, cyclodextrins, cavitands, etc. as potentially suitable units.[6] The interaction of this kind of receptors with a large variety of analytes is well documented and the selectivity of these interactions has been at the basis of numerous sensing assays.[7] This class of receptors are synthetically easily accessible or even commercially available (Supporting Information). Finally, and of particular importance for our purposes, the presence of multiple anionic functional groups on the receptor guarantees both water-solubility and a high affinity for Au NP **1**. Based on these evaluations we selected tetraphosphonate cavitand **A**, tetrasulfonate calix[4]arene **B** and heptakis (6-*O*-sulfo)-β-cyclodextrin **C** for our initial studies. To emphasize the possibility to tune the selectivity of the assay by changing the receptor, we choose a series of structurally very similar analytes **I**–**VII**. These include the biologically relevant molecules acetylcholine (**I**), which is a neurotransmitter,[8] and sarcosine (**V**), which is a biomarker for prostate cancer present in urine.[9] Analyte **VII** is a close analog of

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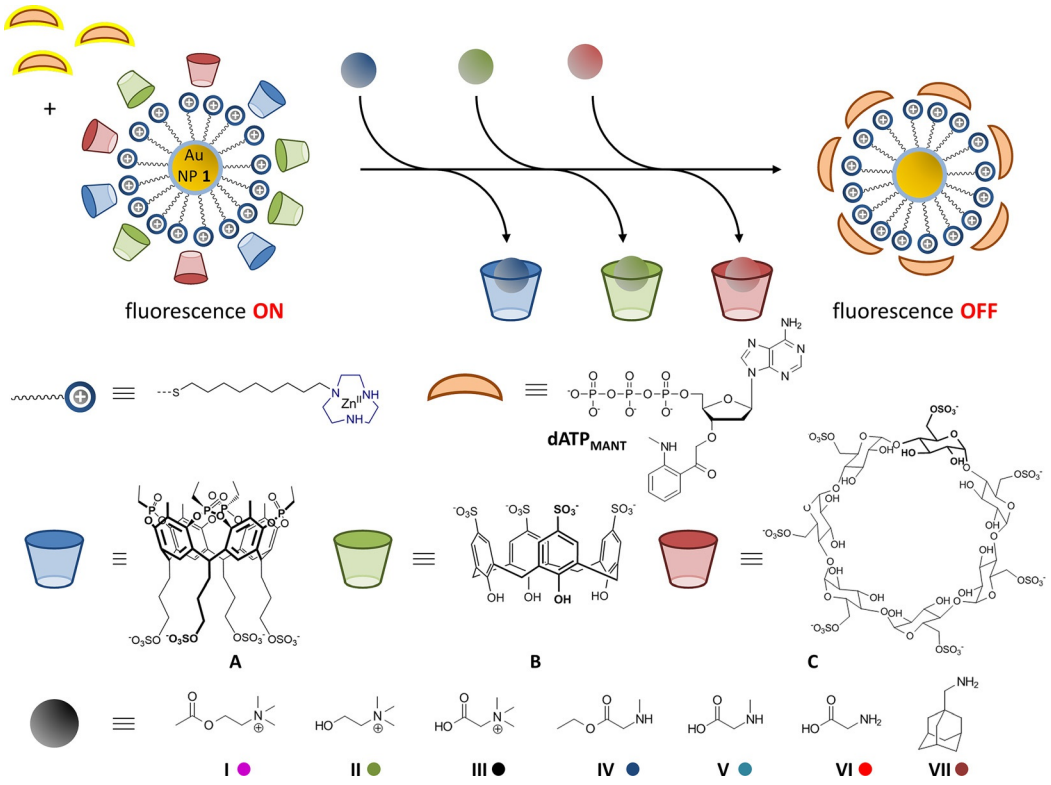

Figure 1. Working principle of the assay and assay components.

rimantadine, which is an antiviral drug used to treat influenza A infection.[10] The complexation of some of these analytes by receptors **A**–**C** or analogues has been reported before, but always in a separate manner.[11] The combined use of the receptors in a single sensing system would serve to emphasize the opportunities and strengths of a modular approach. The fluorescent reporter **dATP$_{MANT}$** (λ_{ex} = 355 nm, λ_{em} = 448 nm) was chosen because prior studies had shown the high affinity of this probe for Au NP **1**, which is essential to effectively compete with the anionic receptors **A**–**C** for binding to Au NP **1**.[12]

First, the competitiveness between the receptors **A**–**C** and fluorophore **dATP$_{MANT}$** was measured by means of competition experiments (Supporting Information). Increasing amounts of **A**–**C** were added to buffered aqueous solutions of Au NP **1** (20 μM) and **dATP$_{MANT}$** (5 μM) causing a displacement of the fluorophore **dATP$_{MANT}$** from the surface of Au NP **1**. The displacement is accompanied by an increase in fluorescence intensity, because the fluorescence of displaced **dATP$_{MANT}$** is no longer quenched by the gold nucleus. The high affinity of all receptors was evidenced by the fact that the addition of low micromolar concentrations of either one of receptors **A**–**C** was sufficient to displace significant amounts of **dATP$_{MANT}$**. The working principle of the assay was first validated by monitoring the fluorescence response of a system composed of Au NP **1**, **dATP$_{MANT}$** and receptor **A** to the addition of guest molecule **IV**, which has been reported to form a complex with **A** (Figure 2a).[11b] The concentration of receptor **A** was chosen such to have a sufficient amount of **A** complexed on Au NP **1** without having a large excess of free receptor in solution. The fluorescence intensity after the successive additions of receptor **A** (5.8 μM) and analyte **IV** (100 μM) to a solution of Au NP **1** (20 μM) and **dATP$_{MANT}$** (5 μM) was monitored as a function of time in order to ensure that equilibrium was reached after each addition (Figure 2b). The addition of **IV** resulted in an immediate decrease in the intensity of the fluorescent signal, which stabilized after around 5 min. This observation is of key importance, as it shows that the equilibria in the system are affected by the presence of **IV**. The decrease in signal intensity

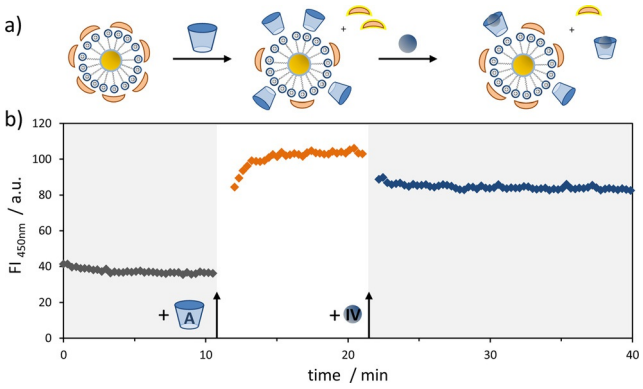

Figure 2. a) Schematic representation of the experiments used to determine the selectivity of the assay for each combination of receptor **A**–**C** and analyte **I**–**VII** (a specific example using receptor **A** and analyte **IV** is shown). b) Fluorescence intensity at 450 nm as a function of time upon the successive additions of receptor **A** and analyte **IV** to a solution containing Au NP **1** and **dATP$_{MANT}$**. See Supporting Information for details.

indicates that the complex **A**·**IV** has a lower affinity for Au NP **1** compared to **A**, which most likely is the result of partial charge neutralization in the complex by the (protonated) secondary amine in **IV**. The addition of **IV** to the system in the absence of receptor **A** did not cause any change in fluorescent intensity (Supporting Information). The hypothesis that complex formation between **A** and **IV** is at the origin of the change is supported by the lack of any response upon addition of any of the other analytes (**I**–**III**, **V**–**VII**) (Figure 3 a). In addition, a dose-response curve revealed a clear correlation between the amount of **IV** added (10–2000 μM) and the magnitude of the signal (Figure 3 d). This curve also showed that an analyte concentration of around 50 μM was sufficient to generate a detectable signal. These results illustrate that the selectivity of the assay is determined by the selective interaction between receptor **A** and **IV**. This implies that the assay selectivity can be tuned simply by substituting **A** for the other receptors **B** or **C**, leaving all other assay components unchanged, that is, Au NP **1** and **dATP**$_{MANT}$. This was verified by monitoring the assay response to analytes **I**–**VII** in the presence of either **B** or **C** (Figure 3 b + c). In the presence of receptor **B** the assay now responded to analytes **I**, **II**, and **VII**, whereas the use of receptor **C** resulted in an exclusive response to analyte **VII**. Dose-response curves confirmed that the observed changes in output signal were caused by complex formation between the receptors **B** and **C** and the respective analytes (Figure 3 e + f). These results show that the selectivity of the assay is determined by the selectivity of the interactions between the receptor units and the analytes. Thus, the apparently low selectivity of the assay featuring receptor **B** reflects the lower selectivity of receptor **B** compared to **A** or **C**. Indeed, previous studies by Nau et al. had shown that receptor **B** is unable to discriminate between analytes **I** and **II**.[11a]

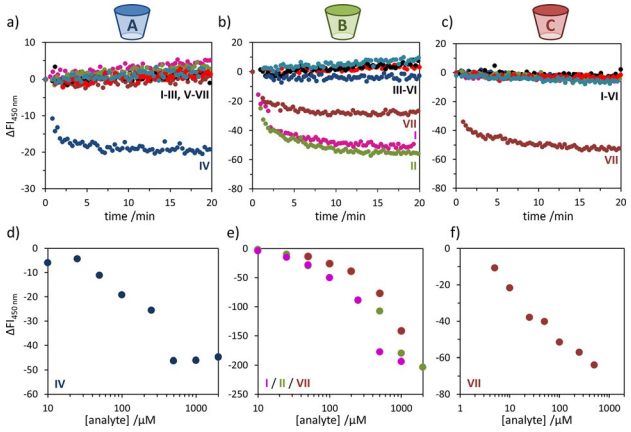

Figure 3. a)–c) Changes in the fluorescence intensity (450 nm) as a function of time upon the addition of analytes **I**–**VII** (100 μM) to solutions containing Au NP **1**, **dATP**$_{MANT}$ and either a) **A**, b) **B**, or c) **C**. d)–e) Changes in the fluorescence intensity (450 nm) measured 20 min after the addition of increasing amounts of analytes to solutions containing Au NP **1**, **dATP**$_{MANT}$ and either d) **A**, e) **B**, or f) **C**. See Supporting Information for details.

of salts and small molecules. To explore the tolerance of the assay to the presence of such potentially interfering compounds, we performed a series of experiments in synthetic urine (SURINE^{TM}), which contains all major components of human urine and is used as a negative control in urine analysis (pH 7.7). In particular we focused our studies on the assay containing receptor **A**, because of its reported ability to detect sarcosine (**V**) in acidified human urine.[11b,c] Our previous studies in HEPES-buffer at pH 7.0 (Figure 3 a) had shown that the assay containing **A** was able to detect sarcosine ethyl ester (**IV**), but not sarcosine. We were very pleased to observe that also the addition of **IV** to an equilibrated solution of Au NP **1**, **dATP**$_{MANT}$, and **A** in synthetic urine caused a significant decrease in fluorescence intensity, albeit lower in intensity compared to the studies performed in buffer (Figure 4 a). The dose-response curve confirmed that the decrease resulted indeed from complex formation between **A** and **IV** (Figure 4 b). A repetition of the experiment using the other analytes showed that the selectivity of the assay was maintained (Figure 4 a). However, to our surprise, the addition of sarcosine (**V**) at 200 μM seemed to cause a small decrease in fluorescence intensity. This prompted us to measure the assay response at higher sarcosine concentrations (up to 1.5 mM) which confirmed the assay response to sarcosine. This enhanced response, as compared to aqueous buffer, is not entirely clear and is currently under investigation.

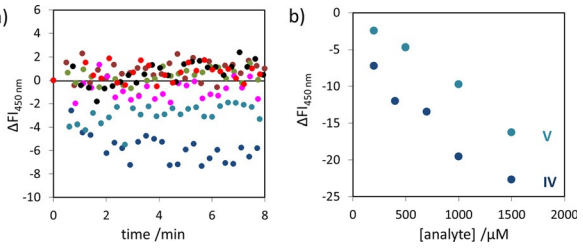

Figure 4. a) Changes in the fluorescence intensity (450 nm) as a function of time upon the addition of analytes **I**–**VII** to a solution of synthetic urine containing Au NP **1**, **dATP**$_{MANT}$ and **A**. b) Changes in the fluorescence intensity (450 nm) measured 10 min after the addition of increasing amounts of analytes **IV** and **V** to a solution of synthetic urine containing Au NP **1**, **dATP**$_{MANT}$ and **A**. See Supporting Information for details.

The modular nature of the assay provides controlled access to sensing systems of higher complexity that go beyond those developed to detect a single analyte. We argued that the simultaneous use of receptors **A**–**C** in combination with Au NP **1** and **dATP**$_{MANT}$ would lead to a single system able to detect multiple analytes, but, as opposed to common differential sensing assays,[13] with a high selectivity. Thus, we prepared an equilibrated solution containing all components and measured the response of the system to the subsequent additions of analytes **II**, **IV**, and **VII**, which were shown to interact with receptors **B**, **A**, and **C**, respectively. Concentrations were chosen such that the formation of each analyte-receptor complex would cause a comparable change in fluorescence intensity. After each addition, the output signal (FI at 450 nm) was measured in time in order to ensure that the system had reached equilib-

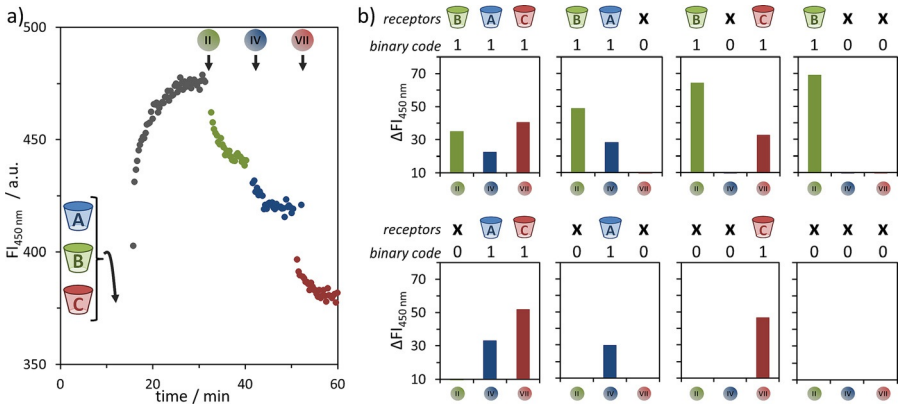

Figure 5. a) Fluorescence intensity (450 nm) as a function of time upon the successive additions of analytes **II** (200 μM), **IV** (200 μM) and **VII** (100 μM) to a solution containing Au NP **1**, **dATP$_{MANT}$** and receptors **A**–**C**. b) Assay response as a function of the presence/absence of receptors **A**–**C**. See Supporting Information for details.

rium before the subsequent analyte was added. We were pleased to observe that each analyte caused a significant change in the output signal, which indeed demonstrated the capacity of the sensing system to respond to multiple analytes (Figure 5 a). However, in order to demonstrate that the response induced by each analyte resulted from a selective interaction with the respective receptor, we proceeded with a study of the systems' response in case one or more receptors **A**–**C** were systematically removed. The use of three receptors implies that eight different combinations can be created, including the system in which none of the receptors is present (Figure 5 b). Two important conclusions could be drawn from the obtained results. First, a neat correlation was observed between the presence of a given receptor (**B**, **A**, or **C**) and the ability of the analyte (**II**, **IV**, and **VII**, respectively) to create a positive response. It is noted that the addition of analyte **VII** to the system containing just receptor **B** did not provide a positive response, contrary to what observed during the individual studies (Figure 3 b). This originates from the fact that analyte **VII** was added after analyte **II** and at lower concentrations (to tune the response of the system). Given the higher affinity of **II** for receptor **B** compared to **VII** (Figure 3 e), this makes that the formation of the complex between **B** and **II** suppresses the interaction between **B** and **VII**. The second feature of the system is the observed orthogonality of the interactions between receptors **A**–**C** and their respective analytes. Clearly, the magnitude of signal change induced by the analytes depends on the number of receptors present, since all of them compete with **dATP$_{MANT}$** for binding to Au NP **1**. However, the individual interactions between the receptors and their respective analytes were found to be independent of the number and type of receptors present.

In conclusion, we have developed a modular sensing system that can be tuned to selectively detect the presence of one or more analytes under competitive conditions. Considering the availability of a large number of macrocyclic receptors similar to **A**–**C** (for example, α-, β-, and γ-cyclodextrins and calix[*n*]arenes with $n = 4$–8) each with different selectivity, we are convinced that this system can be easily extended to the detection of other analytes.

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