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Dynamic combinatorial chemistry on a monolayer protected gold nanoparticle†

Subhabrata Maiti and Leonard J. Prins*

Here, we show that the addition of Hg^{2+} or Ag^{+} metal ions to a dynamic system composed of monolayer protected gold nanoparticles (Au NPs) and a mixture of four nucleotides (dGMP, dAMP, TMP, and dCMP) leads to the self-selection of TMP or dGMP, respectively, on the monolayer surface.

Dynamic combinatorial chemistry (DCC) relies on the target-induced change in the composition of a dynamic combinatorial library, *i.e.* a library of which the components are held together either by noncovalent bonds or reversible covalent bonds, in favor of the library member that interacts most strongly with the target.[1–3] Over the years, DCC has emerged as a powerful tool for the development of molecular receptors,[4] sensors,[5] catalysts,[6] and materials.[7] The self-selection procedure occasionally has led to the identification of complex structures that would have been hard to come by in any other way, which illustrates the attractiveness of this approach.[8,9] However, despite the enormous activity in this research area, DCC on nanoparticles has so far remained relatively unexplored with limited examples dedicated to surfactant-based systems.[10–13] Yet, DCC at surfaces gives an unprecedented opportunity for developing self-selected nanosystems for applications in sensing and (bio)-recognition or as responsive materials.[14] In particular the use of gold nanoparticles as scaffolds is very appealing, because of their attractive properties related to stability, (bio)compatibility, and intrinsic photophysical properties, which have led to numerous applications in diagnostics and nanomedicine.[15,16] Here, we apply DCC on a monolayer protected gold nanoparticle (Au NP) and show that the surface composition spontaneously adapts to the added target (Hg^{2+} or Ag^{+}).[17]

In the past years we have exploited the interaction between Au NP **1** and oligoanions for the development of sensing and catalytic systems.[18,19] Au NP **1** are gold nanoparticles ($d = 1.8 \pm 0.4$ nm) covered with hydrophobic C9-thiols terminating with a 1,4,7-triazacyclononane (TACN)·Zn^{2+} head group (Fig. 1).[20] Oligoanions such as Asp-rich peptides and nucleotides were found to bind to Au NP **1** under saturation conditions even at low micromolar concentrations in aqueous buffer as a combination of electrostatic and hydrophobic interactions.[21] The number of negative charges present in the oligoanions strongly affects the affinity for Au NP **1**. Recently, we have exploited this property for the development of a sensing system able to detect Hg^{2+} at low nanomolar concentrations in water.[22] In that system, Hg^{2+} induced the dimerization of TDP leading to the formation of a ternary TDP·Hg^{2+}·TDP complex which has a much higher affinity for Au NP **1** than TDP. The ternary complex displaced (quenched) fluorescent probe **A** from the surface of Au NP **1** resulting in a turn-ON of the fluorescence. The selective interaction between Hg^{2+} and thymidine is at the basis of the selective response of that sensing system to Hg^{2+}. It was also shown that the system self-selected TMP over cTMP for complex formation with Hg^{2+}, driven by the high affinity of the TMP·Hg^{2+}·TMP complex for Au NP **1**.

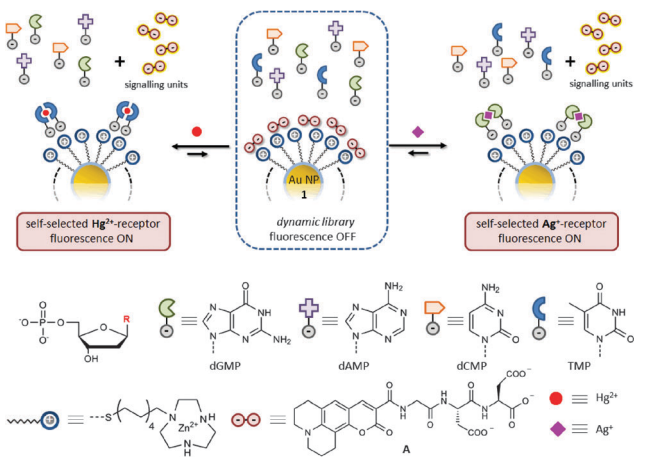

Fig. 1 Schematic representation of the self-selection. Complex formation between two recognition units and the analyte results in the formation of a ternary complex with a high affinity for Au NP **1**, which displaces the quenched fluorescent probe **A** from the surface.

Department of Chemical Sciences, University of Padova, Via Marzolo 1, 35131 Padova, Italy. E-mail: leonard.prins@unipd.it; Fax: +39 049 8275050

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These results made us wonder whether it would be possible to perform DCC on a monolayer surface, *i.e.* would Hg^{2+} be able to self-select the thymidine recognition unit from a mixture containing also guanine, adenine, and cytosine nucleotides (Fig. 1)? In order to test this possibility we decided to use solutions containing Au NP **1** ([TACN·Zn^{2+}] = 30 μM), probe **A** (11.6 μM) and the monophosphate nucleotides dGMP, dAMP, dCMP, and TMP (20 μM each). The presence of probe **A** is important for two reasons. First, its higher affinity for Au NP **1** compared to the monophosphate nucleotides ensures that the surface of Au NP **1** is covered with **A** (which is present at its surface saturation concentration) and that all nucleotides are free in solution (see ESI†). This is important because previous studies had shown that purine nucleotides have an intrinsically higher affinity for Au NP **1** compared to pyrimidine nucleotides.[19] Thus, the interaction of nucleotides with Au NP **1** in the absence of **A** would lead towards a bias in the surface composition making it more complicated to observe amplification induced by Hg^{2+}. The second reason for using probe **A** is that its fluorescence is a handle to confirm the results of the self-selection experiments by means of fluorescence displacement experiments. Previously, we have shown that Au NP **1** and molecules that are non-covalently bound to the monolayer do not permeate PES-membranes with a 10 kDa MW cut-off upon centrifugation (12 000 rpm), whereas unbound molecules do.[21] Thus, analysis of the dialysate by LC/MS (in SIM detection mode) is a straightforward manner to determine which nucleotides (if any) are bound to Au NP **1** (Fig. 2a). Samples were centrifuged only for a limited time (typically 15 s) to avoid large changes in the volume of the nanoparticle solution (max 20%), because large changes in concentration could affect the composition at thermodynamic equilibrium. All measurements were performed in triplicate (see Fig. 2b for a representative set of chromatograms, see ESI† for all data). Using this procedure we analyzed the concentration of the 4 nucleotides in the dialysate in the absence and presence of 5 μM of Hg^{2+} (Fig. 2c). We were pleased to observe that the addition of Hg^{2+} caused a significant decrease of only the TMP concentration (25%) in the dialysate, indicating that the presence of Hg^{2+} resulted in the near exclusive capture of TMP on the surface of Au NP **1** (Fig. 2d). Comparison of the SIM area with calibration curves indicated that around 4.3 μM of TMP was captured on Au NP **1** (see ESI†).

However, based on our previous results[22] and the high fidelity of the T·Hg^{2+}·T recognition motif the selection of TMP did not come entirely as a surprise. This changed when we used Ag^{+} as the target metal ion. Our choice to target Ag^{+} was motivated by the fact that Ag^{+} had been reported to form a ternary complex with cytosine (C·Ag^{+}·C) very similar to the T·Hg^{2+}·T complex.[23] Therefore, we anticipated that the addition of Ag^{+} would result in the self-selection of dCMP. This would then show the ability of the same dynamic combinatorial library to evolve in different directions depending on the added target, which is one of the most appealing features of dynamic combinatorial chemistry. However, after repeating the ultrafiltration experiments in the presence of Ag^{+} (5 μM) we were surprised to find out that the largest change in concentration was observed for dGMP (32%) rather than dCMP (just 8%, Fig. 2c and e). In this case, all nucleotides showed a

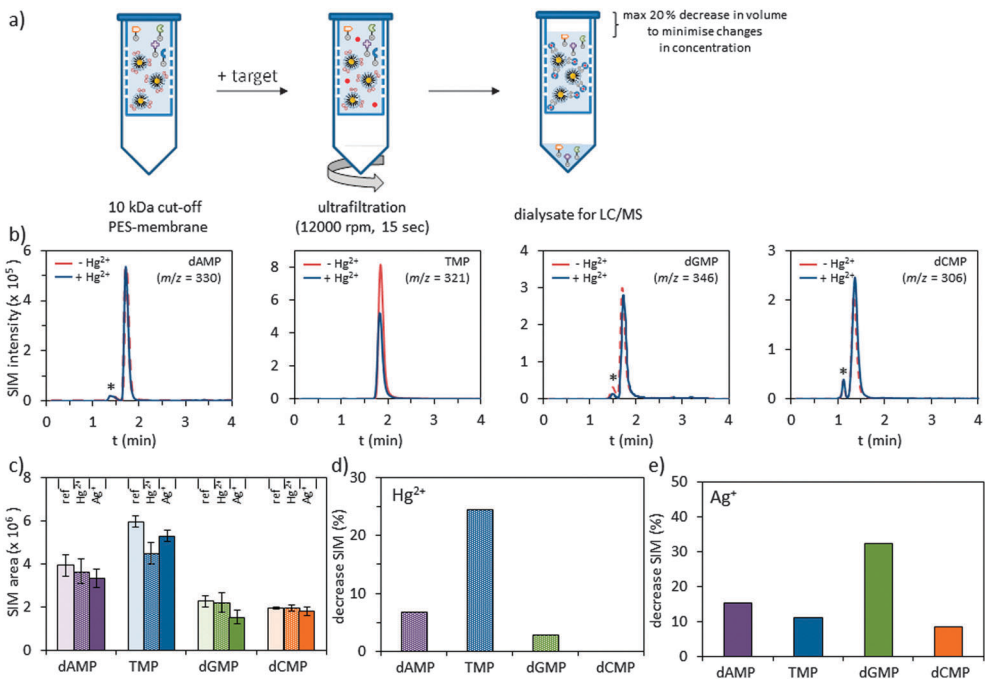

Fig. 2 (a) Schematic representation of the ultrafiltration experiments. (b) Representative chromatograms of each nucleotide in the presence and absence of Hg^{2+}. All measurements were performed in triplicate. The peaks marked with an asterisk originate from the buffer. (c) SIM areas of the deoxynucleotides (dAMP, TMP, dGMP, dCMP) in the dialysate before and after the addition of Hg^{2+} (5 μM) or Ag^{+} (5 μM). (d) Relative decrease (%) of the SIM area of each deoxynucleotide before and after addition of Hg^{2+}. (e) Relative decrease (%) of the SIM area of each deoxynucleotide before and after addition of Ag^{+}. Experimental conditions: [TACN] = 30 ± 2 μM; [Zn^{2+}] = 30 μM; [**A**] = 11.6 μM, [dAMP] = [TMP] = [dGMP] = [dCMP] = 20 μM, [Hg^{2+}] = [Ag^{+}] = 5 μM; [HEPES] = 10 mM, pH 7.0. Ultrafiltration procedure: PES membrane with 10 kDa MW cut-off, centrifugation at 12 000 rpm for 15 s, starting volume (before filtration) = 500 μl, dialysate volume (after filtration) ≈100 μl.

modest decrease in the dialysate-concentration upon the addition of Ag^+, but control filtration experiments performed in the absence of Au NP **1** showed that changes up to 15% could be accounted for by aspecific effects of Ag^+ (see ESI†). Indeed, after correction for this effect, only a significant selection of dGMP was observed (14%, see ESI†). The selection of dGMP instead of the anticipated dCMP indicates that Ag^+ is more promiscuous in its interaction with nucleobases compared to Hg^{2+}. Indeed, apart from cytosine, several types of complexes have been proposed for the interaction between Ag^+ and guanine.[24,25] It should be pointed out, though, that the self-selection in our system is determined by the affinity of the formed Ag^+–nucleotide complex for Au NP **1**, which does not necessarily mean that this is the most stable complex in the absence of Au NP **1**.

We next proceeded with a series of fluorescence studies to confirm the results of self-selection experiments. As pointed out above, displacement of probe **A** from Au NP **1** results in a turn-ON of fluorescence. This general property of fluorophore–gold nanoparticle complexes has been the basis of numerous sensing assays.[26] In a first set of experiments we titrated Hg^{2+} to a solution containing Au NP **1** ([TACN·Zn^{2+}] = 30 μM), probe **A** (11.6 μM) and either one of the nucleotides dGMP, dAMP, dCMP, and TMP (20 μM) and measured the increase of fluorescence at 493 nm caused by the displacement of probe **A** from Au NP **1** (Fig. 3a). As confirmation of the self-selection experiments TMP was indeed most effective in generating an output signal. Nevertheless, a general increase in fluorescence intensity was also observed for the other nucleotides. However, this turned out to be actually unrelated to the nucleotides, because the same behaviour was also observed in the absence of any nucleotides. The reason for the generic release of **A** upon adding Hg^{2+} is the exchange between Hg^{2+} and the Zn^{2+}-metal ions complexed in the monolayer by TACN (log $K_{TACN\cdot Zn(II)}$ = 11.5 and log $K_{TACN\cdot Hg(II)}$ = 12.5).[27] This process is accompanied by a decrease in the surface saturation concentration of **A** (see ESI†). Repetition of fluorescence titrations using Ag^+ instead of Hg^{2+} gave a strong increase in signal intensity only when dGMP was present (Fig. 3b). In this case, there was no intrinsic effect of Ag^+ over the concentration range studied (0–100 μM). These results are in full agreement with the self-selection results.

In conclusion, we have applied dynamic combinatorial chemistry on gold nanoparticles for the identification of the best recognition units for Hg^{2+} and Ag^+ metal ions. The driving force for the self-selection process is the target-induced clustering of negative charges leading towards a high affinity binder for the multivalent monolayer surface. This approach led to the unexpected identification of the guanine nucleotide as the best recognition unit for the detection of Ag^+, which underlines the advantage of dynamic combinatorial chemistry compared to rational design. The ability to apply dynamic combinatorial chemistry on a nanoparticle offers an enormous potential for the development of multivalent receptor systems of high complexity.

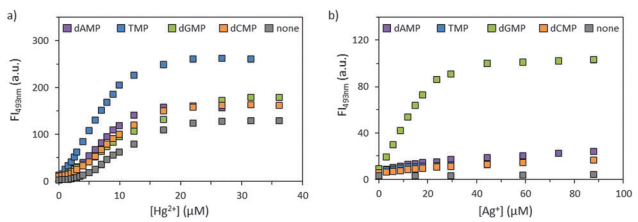

Fig. 3 Fluorescence intensity (a.u.) at 493 nm as a function of the concentration of (a) Hg^{2+} and (b) Ag^+ added to a solution containing Au NP **1** (30 μM), probe **A** (11.6 μM) and either dAMP, TMP, dGMP or dCMP (20 μM). Experimental conditions: [TACN] = 30 ± 2 μM; [Zn^{2+}] = 30 μM; [**A**] = 11.6 μM, [HEPES] = 10 mM, pH 7.0, T = 37 °C; [dAMP] = [TMP] = [dGMP] = [dCMP] = 20 μM. Fluorescence slit width = (2.5/5) nm.

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