Controlling supramolecular complex formation on the surface of a monolayer protected gold nanoparticle in water

Grégory Pieters, Cristian Pezzato and Leonard J. Prins*

Supporting Information

Dr. G. Pieters, Cristian Pezzato, Prof. Dr. L. J. Prins
Department of Chemical Sciences, University of Padova, via Marzolo 1,
I-35131 Padova, Italy.
E-mail: leonard.prins@unipd.it
Tel: +39 049 8275256
# Table of Contents

1. General information and instrumentation ................................................................. 3
2. Surface saturation concentrations of $d\text{AXP}_{\text{MANT}}$ .......................................................... 4
3. Fluorescent titrations of Au MPC $1\cdot Zn^{2+}$ with $d\text{AXP}_{\text{MANT}}$ at pH 7.0 ....................... 5
4. Fluorescent titrations of Au MPC $1$ (without $Zn^{2+}$) with $d\text{ATP}_{\text{MANT}}$ at pH 7.0 ............... 8
5. Fluorescent titrations of Au MPC $1\cdot Zn^{2+}$ with $d\text{ATP}_{\text{MANT}}$ at pH 8.0 ........................... 9
6. Fluorescent titrations of Au MPC $1$ (without $Zn^{2+}$) with $d\text{ATP}_{\text{MANT}}$ at pH 8.0 ................... 10
7. Displacement of $d\text{AXP}_{\text{MANT}}$ from the surface of Au MPC $1\cdot Zn^{2+}$ with ATP ..................... 11
8. Verification of the thermodynamic equilibrium .......................................................... 12
9. Formation of heteromeric surfaces on Au MPC $1\cdot Zn^{2+}$ composed of $\text{WDDD}$ and $d\text{ATP}_{\text{MANT}}$ 13
10. Selective displacement on a heteromeric surface composed of $\text{WDDD}$ and $d\text{ATP}_{\text{MANT}}$ ....... 14
11. General procedure for the selective ‘catch-and-release’ of $\text{WDDD}$ ...................................... 15
12. Catalytic inhibition experiments with $d\text{ATP}_{\text{MANT}}$ and $d\text{AMP}_{\text{MANT}}$ ............................. 16
1. General information and instrumentation

Solvents were purified by standard methods. All commercially available reagents and substrates were used as received.

4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma) was used without further purification. dATP\textsubscript{MANT} (2'-deoxy-3'O-(N'-methylanthraniloyl)adenosine-5'O-triphosphate), dADP\textsubscript{MANT} (2'-deoxy-3'O-(N'-methylanthraniloyl)adenosine-5'O-diphosphate) and dAMP\textsubscript{MANT} (2'-deoxy-3'O-(N'-methylanthraniloyl)adenosine-5'O-monophosphate) were obtained from BioLog Life Science Institute and used as received. The concentration of dAXP\textsubscript{MANT} in the stock solutions were determined by UV spectroscopy (pH 7, $\varepsilon_{355} = 5800$ l.mol$^{-1}$.cm$^{-1}$). The synthesis and characterization of WDDD has been described elsewhere.\textsuperscript{S1} TPEN (N,N,N,N-Tetrakis(2-pyridylmethyl) ethylenediamine was purchased from Sigma-Aldrich and used as received. Zn(NO$_3$)$_2$ was analytical grade products.

The synthesis and characterization of Au MPC 1 has been described elsewhere. Stock solutions of Au MPC 1 were conserved at 4°C in mQ water.\textsuperscript{S2}

Fluorescence spectra were recorded on a Varian Cary Eclipse Fluorescence spectrophotometer equipped with a thermostatted cell holder.


2. Surface saturation concentrations of AXP<sub>MANT</sub>

Fluorescence titrations (see sections 3-6 for the spectra) were performed by adding consecutive amounts of a stock solution of fluorescent probes dAXP<sub>MANT</sub> (dAMP<sub>MANT</sub> (0.22 mM in mQ water), dADP<sub>MANT</sub> (0.18 mM in mQ water), dATP<sub>MANT</sub> (0.21 mM in mQ water)) to a 3 mL aqueous solution ([HEPES] = 10 mM) of Au MPC<sub>1•Zn</sub><sup>2+</sup> (10 μM) at 25°C. The emission spectra were recorded 10 min after each addition (slits 10/5).

For each fluorescence titration, surface saturation concentrations were determined both via extrapolation of the linear part of the titration curve and via fitting of the curve to a 1:1 binding model as described before.<sup>52</sup>

The calculated dAXP<sub>MANT</sub> saturation concentrations on Au MPC<sub>1•Zn</sub><sup>2+</sup> are given in Table S-1 at pH = 7.0.

<table>
<thead>
<tr>
<th>dAXP&lt;sub&gt;MANT&lt;/sub&gt;</th>
<th>extrapolation</th>
<th>curve fitting</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>dATP</td>
<td>3.2</td>
<td>3.0</td>
<td>3.1</td>
</tr>
<tr>
<td>dADP</td>
<td>4.2</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td>dAMP</td>
<td>4.7</td>
<td>4.9</td>
<td>4.8</td>
</tr>
</tbody>
</table>
3. Fluorescent titrations of Au MPC 1•Zn$^{2+}$ with dAXP$\text{MANT}$ at pH 7.0

Figure S-1. a) Fluorescence intensities upon the addition of increasing amounts of dATP$\text{MANT}$ to a solution of Au MPC 1•Zn$^{2+}$ ([TACN•Zn$^{2+}$] = 10 µM; [HEPES] = 10 mM; pH = 7.0; T = 25 °C). On the right a zoom is depicted to show the emission spectrum after the first additions; b) Maximum of the MANT-emission wavelength as a function of the concentration of dATP$\text{MANT}$ (red) superimposed on...
the fluorescence intensity at 448 nm (blue) as a function of the concentration of \textit{dATPMANT}; c) Emission spectra after the first additions corrected for the contribution from Raman scattering.

\textbf{Figure S-2.} a) Fluorescence intensities upon the addition of increasing amounts of \textit{dADPMANT} to a solution of Au MPC 1•Zn\textsuperscript{2+} ([TACN•Zn\textsuperscript{2+}] = 10 µM; [HEPES] = 10 mM; pH = 7.0; T = 25 °C). On the right a zoom is depicted to show the emission spectrum after the first additions; b) Maximum of the MANT-emission wavelength as a function of the concentration of \textit{dADPMANT} (red) superimposed on the fluorescence intensity at 448 nm (blue) as a function of the concentration of \textit{dADP} c) Emission spectra after the first additions corrected for the contribution from Raman scattering.
Figure S-3. a) Fluorescence intensities upon the addition of increasing amounts of dAMP$_{\text{MANT}}$ to a solution of Au MPC 1•Zn$^{2+}$ ([TACN•Zn$^{2+}$] = 10 µM; [HEPES] = 10 mM; pH = 7.0; T = 25 °C). On the right a zoom is depicted to show the emission spectrum after the first additions; b) Maximum of the MANT-emission wavelength as a function of the concentration of dAMP$_{\text{MANT}}$ (red) superimposed on the fluorescence intensity at 448 nm (blue) as a function of the concentration of dAMP$_{\text{MANT}}$; c) Emission spectra after the first additions corrected for the contribution from Raman scattering.
4. Fluorescent titrations of Au MPC 1 (without Zn^{2+}) with dATP\textsubscript{MANT} at pH 7.0

Figure S-4. a) Fluorescence intensities upon the addition of increasing amounts of dATP\textsubscript{MANT} to a solution of Au MPC 1•Zn^{2+} ([TACN•Zn^{2+}] = 10 µM; [HEPES] = 10 mM; pH = 7.0; T = 25 °C). On the right a zoom is depicted to show the emission spectrum after the first additions; b) Maximum of the MANT-emission wavelength as a function of the concentration of dATP\textsubscript{MANT} (red) superimposed on the fluorescence intensity at 448 nm (blue) as a function of the concentration of dATP\textsubscript{MANT}; c) Emission spectra after the first additions corrected for the contribution from Raman scattering.
5. Fluorescent titrations of Au MPC $1\cdot$Zn$^{2+}$ with dATP$_{\text{MANT}}$ at pH 8.0

**Figure S-5.** a) Fluorescence intensities upon the addition of increasing amounts of dATP$_{\text{MANT}}$ to a solution of Au MPC $1\cdot$Zn$^{2+}$ ([TACN$\cdot$Zn$^{2+}$] = 10 µM; [HEPES] = 10 mM; pH = 7.0; T = 25 °C). On the right a zoom is depicted to show the emission spectrum after the first additions; b) Maximum of the MANT-emission wavelength as a function of the concentration of dATP$_{\text{MANT}}$ (red) superimposed on the fluorescence intensity at 448 nm (blue) as a function of the concentration of dATP$_{\text{MANT}}$; c) Emission spectra after the first additions corrected for the contribution from Raman scattering.
6. Fluorescent titrations of Au MPC 1 (without Zn\(^{2+}\)) with dATP\(_{\text{MANT}}\) at pH 8.0

![Diagram of dATP\(_{\text{MANT}}\)]

**Figure S-6.** a) Fluorescence intensities upon the addition of increasing amounts of dATP\(_{\text{MANT}}\) to a solution of Au MPC 1•Zn\(^{2+}\) ([TACN•Zn\(^{2+}\]) = 10 µM; [HEPES] = 10 mM; pH = 7.0; T = 25 °C). On the right a zoom is depicted to show the emission spectrum after the first additions; b) Maximum of the MANT-emission wavelength as a function of the concentration of dATP\(_{\text{MANT}}\) (red) superimposed on the fluorescence intensity at 448 nm (blue) as a function of the concentration of dATP\(_{\text{MANT}}\); c) Emission spectra after the first additions corrected for the contribution from Raman scattering.
7. Displacement of $dAXP_{MANT}$ from the surface of Au MPC 1•Zn$^{2+}$ with ATP

The displacement experiments were performed by adding consecutive amounts of a stock solution of ATP (25 mM in mQ water) to a 3 mL aqueous solution (pH 7.0, [HEPES] = 10 mM) containing Au MPC 1•Zn$^{2+}$ ([TACN] = 10 µM) coated with the fluorescent probes ($dATP_{MANT}$: 3.0 µM, $dADP_{MANT}$: 3.5 µM, $dAMP_{MANT}$: 3.5 µM) at 25°C. The evolution of the FI after each addition was followed at 455 nm (slit 5/10).

The displacement curves were fitted to a simple model

$$NP \cdot A + B \leftrightarrow K \rightarrow NP \cdot B + A$$

in which the nanoparticle complex is reduced to a single species. Since complex formation occurs under saturation conditions it is assumed that [NP•B] equal [A]. It was noted that the model fits the initial points of the displacement curves poorly, which originates from the fact that the probes are present at a concentration below the SSC. Consequently, initial additions of ATP may result in surface binding without displacement. This is particularly evident in the $dAMP_{MANT}$-curve (Figure 4b, manuscript).

// MicroMath Scientist Model File
IndVars: B0
DepVars: FI, A, NPA, B
Params: K, X, NPA0
A=SQRT(K*NPA*B)
NPA=NPA0-A
B=B0-A
FI=X*A
//
0<NPA<NPA0
0<A<NPA0
0<B<B0
***
8. Verification of the thermodynamic equilibrium

To ensure that the exchange experiments are under thermodynamic control a control experiment was performed on the last point of the competition experiment between dATP<sub>MANT</sub> and ATP. A solution with an identical final composition ([TACN] = 10 µM; [dATP<sub>MANT</sub>] = 3 µM; [ATP] = 1 mM) was prepared either by adding ATP to a solution of Au MPC 1•Zn<sup>2+</sup> and dATP<sub>MANT</sub> or by adding dATP<sub>MANT</sub> to a solution of Au MPC 1•Zn<sup>2+</sup> and ATP. For both experiments, the fluorescence intensity rapidly converged to the same value (Figure S-7).

**Figure S-7.** Normalized fluorescence intensities at 448 nm as a function of time upon the addition (at the arrow) of dATP<sub>MANT</sub> (3 µM) to a solution of Au MPC 1•Zn<sup>2+</sup> ([TACN] = 10 µM) and ATP (1 mM) (blue line) and the addition of ATP (1 mM) to a solution of Au MPC 1•Zn<sup>2+</sup> ([TACN] = 10 µM) and ATP<sub>MANT</sub> (3 µM) (red line).
9. Formation of heteromeric surfaces on Au MPC $1\text{•Zn}^{2+}$ composed of WDDD and dATP$_{MANT}$

The determination of the surface saturation concentrations of the WDDD probe in presence of various amounts of dATP$_{MANT}$ assembled on the Au MPC $1\text{•Zn}^{2+}$ surface were performed by adding consecutive amounts of a stock solution of WDDD (0.25 mM in mQ water) to a 3 mL aqueous solution (pH 7.0, [HEPES] = 10 mM) containing Au MPCs $1\text{•Zn}^{2+}$ coated with various amounts of dATP$_{MANT}$ (from 0.5, 1.0, 1.5, 2.0 µM) at 25°C. The evolution of the FI at 360 nm (slit 10/10) was followed for 10 min after each addition.

![Graph a) Fluorescence intensity at 360 nm (a.u.) as a function of the amount of WDDD added to Au MPCs $1\text{•Zn}^{2+}$ in the presence of different amounts of dATP$_{MANT}$ loaded on the surface. b) Surface composition as a function of the amount of dATP$_{MANT}$ present.](image)

**Table S-2.** Surface composition as a function of the amount of dATP$_{MANT}$ present.

<table>
<thead>
<tr>
<th>dATP$_{MANT}$ loaded (in µM)</th>
<th>Surface coverage of dATP$_{MANT}$ in % of SSC</th>
<th>Surface saturation concentration of WDDD (in µM)</th>
<th>Total probe concentration on the surface (in µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>0.5</td>
<td>15</td>
<td>2.2</td>
<td>2.7</td>
</tr>
<tr>
<td>1.0</td>
<td>30</td>
<td>1.7</td>
<td>2.7</td>
</tr>
<tr>
<td>1.5</td>
<td>45</td>
<td>1.4</td>
<td>2.9</td>
</tr>
<tr>
<td>2.0</td>
<td>60</td>
<td>0.7</td>
<td>2.7</td>
</tr>
</tbody>
</table>
10. Selective displacement on a heteromeric surface composed of WDDD and dATP<sub>MANT</sub>

The displacement experiments were performed by adding consecutive amounts of a stock solution of ADP (2.5 mM in mQ water) to a 3 mL aqueous solution (pH 7.0, [HEPES] = 10 mM) containing 1•Zn<sup>2+</sup> coated with the fluorescent probes (dATP<sub>Mant</sub>: 1.0 µM, WDDD: 1.4 µM) at 25°C. The evolution of the FI was followed for 10 min after each addition at 448 nm and 360 nm (slit 10/10).

\[ \text{dATP}_{\text{MANT}} \text{ (30 µL of an 0.2 mM solution in mQ water) and WDDD (15 µM of an 0.2 mM solution in mQ water) were added to a solution of AuMPC 1 ([TACN] = 10 µM) in HEPES (10 mM) and after 5 minutes of equilibration at 37 °C the fluorescence intensities were measured every minute at 360 and 448 nm (slit 10/10). Then Zn(NO}_3\text{)_2 (15 µL of a 1.0 mM solution in mQ water) was added and the fluorescence intensity was measured. After 10 min, TPEN (15 µL of 1.0 mM solution in mQ water) was added and the FI at 360 and 448 nm was measured until the complete release of WDDD. Then the addition of Zn(NO}_3\text{)_2 (15 µL of a 1.0 mM solution in mQ water) and TPEN (15 µL of 1.0 mM solution in mQ water) were repeated.} \]
12. Catalytic inhibition experiments with $d\text{ATP}_{\text{MANT}}$ and $d\text{AMP}_{\text{MANT}}$

Previously we have shown that Au-MPC 1 highly efficiently catalyzes the transphosphorylation of HPNPP (2-hydroxypropyl-4-nitrophenyl phosphate) (Chart S-1). Catalysis is inhibited in the presence of oligoanionic species, such as $d\text{AMP}_{\text{MANT}}$ and $d\text{ATP}_{\text{MANT}}$, that act as competitive inhibitors for HPNPP. Considering that $d\text{AMP}_{\text{MANT}}$ and $d\text{ATP}_{\text{MANT}}$ bind Au-MPC 1 quantitatively at low micromolar concentrations, the catalytic inhibition experiments provide for another tool to assess the amount of probe necessary to saturate the surface.

![Chart S-1](image)

**Chart S-1.** Catalytic cleavage of HPNPP by Au MPC 1.

Inhibition experiments were performed by measuring the initial rate of reaction in the presence of different amounts of either $d\text{AMP}_{\text{MANT}}$ and $d\text{ATP}_{\text{MANT}}$ using protocols described earlier (Angew.Chem.Int.Ed. 2011, 50, 2307). Experimental conditions: [TACN] = 10 µM, [Zn(NO$_3$)$_2$] = 10 µM, [HEPES] = 10 mM, [HPNPP] = 2 mM, pH = 7.0; T = 25 °C. The measured initial rates (normalized on the initial rate in the absence of probe) for $d\text{AMP}_{\text{MANT}}$ are plotted in Figure S-8. The data from the direct fluorescent titration (Figure 4a-manuscript) has been added for comparison.

Extrapolation of the linear parts of both curves give a nearly identical intersect with the x-axis, which confirms the SSC of $d\text{AMP}_{\text{MANT}}$. The same confirmation was also obtained for $d\text{ATP}_{\text{MANT}}$ (Figure S-9).

It is noted that the inhibition experiments are competition experiments between the probe and HPNPP. This may explain the different curvature (in particular for $d\text{AMP}_{\text{MANT}}$) between the inhibition experiment and the direct fluorescence titration (in which a competitor is absent).
**Figure S-8.** Normalized initial rate for the transphosphorylation of HPNPP as a function of the concentration $\text{dAMP}_\text{MANT}$ (blue squares) and the fluorescence intensity as a function of the concentration $\text{dAMP}_\text{MANT}$ (red squares). Experimental conditions: $[\text{TACN}] = 10 \, \mu\text{M}$, $[\text{Zn(NO}_3)_2] = 10 \, \mu\text{M}$, $[\text{HEPES}] = 10 \, \text{mM}$, $[\text{HPNPP}] = 2 \, \text{mM}$, pH = 7.0; $T = 25 \, ^\circ\text{C}$.

**Figure S-9.** Normalized initial rate for the transphosphorylation of HPNPP as a function of the concentration $\text{dATP}_\text{MANT}$ (blue squares) and the fluorescence intensity as a function of the concentration $\text{dATP}_\text{MANT}$ (red squares). Experimental conditions: $[\text{TACN}] = 10 \, \mu\text{M}$, $[\text{Zn(NO}_3)_2] = 10 \, \mu\text{M}$, $[\text{HEPES}] = 10 \, \text{mM}$, $[\text{HPNPP}] = 2 \, \text{mM}$, pH = 7.0; $T = 25 \, ^\circ\text{C}$.