Supporting Information
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Zn$^{2+}$-Regulated Self-Sorting and Mixing of Phosphates and Carboxylates on the Surface of Functionalized Gold Nanoparticles**

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Supporting Information

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1. Materials and instrumentation

The synthesis and characterization of Au MPC 1 and 2 has been described elsewhere.\textsuperscript{51} Stock solutions of Au MPC 1 and 2 were conserved at 4°C in mQ water. The concentration of TACN-head groups in Au MPC 1 was determined from kinetic titrations using either Zn(NO\textsubscript{3})\textsubscript{2} or Cu(NO\textsubscript{3})\textsubscript{2} as reported previously.\textsuperscript{52} Zn(NO\textsubscript{3})\textsubscript{2} and Cu(NO\textsubscript{3})\textsubscript{2} were analytical grade products. Metal ion stock solutions were titrated against EDTA following standard procedures. The concentration of the TMA-head groups in Au MPC 2 were estimated comparing the UV-Vis spectra of Au MPC 2 and 1.

The synthesis and characterization of probe B (C343GDD) has been described previously.\textsuperscript{53} 2’ Deoxy-3’-O-(N’-methylanthraniloyl)-adenosine-5’-diphosphate (probe A) was purchased from Biolog Life Science Institute and used as received. The buffer, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), tetraethylammonium chloride (TEACl), N,N,N’,N’-tetrakis(2-pyridylmethyl) ethylenediamine (TPEN) and adenosine 5’-triphosphate (ATP) were obtained from commercial sources and used without further purification. The stock solution concentrations were determined both by weight and UV-Vis spectroscopy using the following molar extinction coefficients: \( \varepsilon_{355} (\text{MANT}) = 5800 \, \text{M}^{-1}\text{cm}^{-1} \); \( \varepsilon_{259} (\text{ATP}) = 15400 \, \text{M}^{-1}\text{cm}^{-1} \) and \( \varepsilon_{450} (\text{C343}) = 45000 \, \text{M}^{-1}\text{cm}^{-1} \) at pH 7.0).\textsuperscript{54, 55}

UV-Vis spectra were recorded on a Varian Cary50 spectrophotometer equipped with thermostatted multiple cell holders. Fluorescence measurements were recorded on a Varian Cary Eclipse Fluorescence spectrophotometer equipped with a thermostatted cell holder.

\textsuperscript{52} R. Bonomi, A. Cazzolaro, A. Sansone, P. Scrimin, L. J. Prins \textit{Angew. Chem. Int. Ed}. \textbf{2011}, \textit{50}, 2307-2312;
2. Surface saturation concentrations (SSCs)

The SSCs of probe A (\(\lambda_{ex}=445\) nm, \(\lambda_{em}=493\) nm, slits: 2.5/5 nm) and probe B (\(\lambda_{ex}=355\) nm, \(\lambda_{em}=448\) nm, slit: 10/5 nm) on Au MPCs 1•Zn\(^{2+}\), Au MPC 1 and Au MPC 2 were determined as described previously.\(^{S1}\) After each addition, the fluorescence intensities were recorded after the signal had stabilized (typically up to 10 minutes) (Fig. S1). The values listed in Tab. S1 were determined via extrapolation of the linear part of the curves (the last 4 points) of the respective titration. The amount of Au MPC 2 was regulated to capture approximately a concentration of B equal to the SSC of B@Au MPC 1•Zn\(^{2+}\) and was estimated to be around 20 \(\mu M\) in ammonium head groups based on UV-Vis titrations.

![Fig. S1](image)

**Fig. S1** Fluorescence intensity as a function of the amount of A or B added to a solution of Au MPC 1•Zn\(^{2+}\) (■), 1 (□) or 2 (○). \([\text{Au MPC 1}] = 8 \, \mu M\), \([\text{Zn}^{2+}] = 8 \, \mu M\), \([\text{Au MPC 2}] \approx 20 \, \mu M\), \([\text{HEPES}] = 10 \, \text{mM}\), pH 7.0, 37 °C.

**Table S1.** The SSCs of probes A and B on the respective nanosystems. Errors refer to the extrapolation fitting.

<table>
<thead>
<tr>
<th>Nanosystem</th>
<th>SSC A ((\mu M))</th>
<th>SSC B ((\mu M))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au MPC 1•Zn(^{2+})</td>
<td>3.4 ± 0.2</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Au MPC 2</td>
<td>2.7 ± 0.2</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>Au MPC 1</td>
<td>1.7 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
</tbody>
</table>
3. Competition experiments between A and B

In order to follow the simultaneous emission of both probes, the instrument settings were slightly modified compared to the direct titrations (§2) in order to account for the different fluorescence properties of probes A and B. The following parameters were used:

\[
\begin{align*}
A : & \lambda_{\text{ex}} = 340 \text{ nm}, \lambda_{\text{em}} = 440 \text{ nm}, \text{ slits} = 10/5 \text{ nm}; \\
B : & \lambda_{\text{ex}} = 385 \text{ nm}, \lambda_{\text{em}} = 555 \text{ nm}, \text{ slits} = 10/5 \text{ nm}.
\end{align*}
\]

Competition experiments between A and B were performed by adding consecutive amounts of a stock solution of A (286 μM in mQ-water) to a buffered aqueous solution (HEPES 10 mM, pH = 7.0) containing Au MPC 1, 1•Zn^{2+} or 2 saturated with probe B (1.3, 2.7 and 2.7 μM respectively).

![Fig. S2. Fluorescence intensities of probes A (red squares) and B (blue squares) as a function of the amount of A added to (a) B@Au MPC 1•Zn^{2+}, (b) B@Au MPC 2 and (c) B@Au MPC 1.](image)

Conversion of the FIs into concentrations was performed using the final points of each titration. The slope of the linear part of the red curves (probe A) corresponds to \( \Delta F/I/\Delta[A]_{\text{free}} \), whereas the final part of the blue curves (probe B) gives the FI for the amount of B present in the system. Since displacement is not complete for Au MPC 2 the blue curve was extrapolated using a model. Alternatively, fitting of the curves to appropriate models describing the binding and displacement events separately (implemented in Micromath for Windows) gave virtually identical results. Table S2 lists the concentrations of all species for the concentrations used during the self-sorting experiments (addition of 3.4 μM of A to nanosystems B@Au MPC 1•Zn^{2+} and B@Au MPC 2 and addition of 1.7 μM of A to nanosystem B@Au MPC 1).
Table S2. Free and surface bound probe concentrations and surface ratio of A and B for \([A] = 3.4\) (Au MPC 1•Zn\(^{2+}\) and Au MPC 2) or \([A] = 1.7\) (Au MPC 1).

<table>
<thead>
<tr>
<th>Nanosystem</th>
<th>A(_{\text{total}}) (µM)</th>
<th>A(_{\text{free}}) (µM)</th>
<th>A(_{\text{bound}}) (µM)</th>
<th>B(_{\text{total}}) (µM)</th>
<th>B(_{\text{free}}) (µM)</th>
<th>B(_{\text{bound}}) (µM)</th>
<th>A:B surface ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au MPC 1•Zn(^{2+})</td>
<td>3.4</td>
<td>0.5</td>
<td>2.9</td>
<td>2.7</td>
<td>2.3</td>
<td>0.4</td>
<td>90:10</td>
</tr>
<tr>
<td>Au MPC 2</td>
<td>3.4</td>
<td>2.0</td>
<td>1.4</td>
<td>2.7</td>
<td>0.9</td>
<td>1.8</td>
<td>45:55</td>
</tr>
<tr>
<td>Au MPC 1</td>
<td>1.7</td>
<td>0.4</td>
<td>1.3</td>
<td>1.3</td>
<td>0.6</td>
<td>0.7</td>
<td>65:35</td>
</tr>
</tbody>
</table>
4. Displacement experiments with TEACl

The displacement experiments were performed by measuring the fluorescent intensities after adding consecutive amounts of a stock solution of tetraethylammonium chloride (TEACl, 2.05 M in mQ-water) to a 3-mL buffered aqueous solution (HEPES 10 mM, pH = 7.0) containing either Au MPC \( 1 \cdot \text{Zn}^{2+} \) or 2 coated with probe A (3.4 μM) or probe B (2.7 μM). For this experiment the instrument settings as described in §2 were used. The individual graphs for each experiment are given in Figure S3. Experiments were performed at the concentrations of A (3.4 μM) and B (2.7 μM) used in the self-sorting experiments. The maximum Fls expected for a full release were obtained by adding small additional amounts of A to Au MPC \( 1 \cdot \text{Zn}^{2+} \) and Au MPC 2 (providing \( \Delta \text{FI}/\Delta [\text{A}]_{\text{free}} \)) at the end of the TEACl titrations involving probe A (Figure S3a+b) or by adding an excess of ATP (150 μM) to Au MPC \( 1 \cdot \text{Zn}^{2+} \) and Au MPC 2 to cause a full displacement of probe B (Figure S3c+d). The obtained values were within 2% of those expected based on the SSC (see SI - §2). The intrinsic fluorescent properties of probe A are slightly affected (10%) by the presence of TEACl – a correction was performed to account for this.

Figure S3. Fluorescence intensities as a function of the amount of TEACl added to a solution of (a) A@Au MPC \( 1 \cdot \text{Zn}^{2+} \), (b) A@Au MPC 2, (c) B@Au MPC \( 1 \cdot \text{Zn}^{2+} \), (d) B@Au MPC 2. [Au MPC 1] = 8 μM, [Zn\(^{2+}\)] = 8 μM, [Au MPC 2] = 20 μM, [A] = 3.4 μM, [B] = 2.7 μM, [HEPES] = 10 mM, pH 7.0, 37 °C. The instrument settings were those of §2.
5. Probe selectivities and affinities in a complex mixture

In order to prove that the observed surface selectivities and affinities are maintained also in a complex system, titrations were performed by adding consecutive amounts of either A or B to a buffered solution (HEPES 10 mM, pH = 7.0) containing Au MPC 1•Zn²⁺, Au MPC 2, and TEACl (80 mM) in the presence of either B or A, respectively. The FIs of both probes were measured after each addition. Experiments were performed at the concentrations of A (3.4 µM) and B (2.7 µM) used in the self-sorting experiments.

![Graphs](image)

**Figure S4.** Fluorescence intensities as a function of (a) the amount of A added to a solution of B, Au MPC 1•Zn²⁺, Au MPC 2, and TEACl and (b) the amount of B added to a solution of A, Au MPC 1•Zn²⁺, Au MPC 2, and TEACl. Experimental conditions: [Au MPC 1•Zn²⁺] = 8 µM, [Au MPC 2] ≈ 20 µM, [TEACl] = 80 mM, (a) [B] = 2.7 µM, (b) [A] = 3.4 µM, [HEPES] = 10 mM, pH 7.0, 37 °C.

In paragraph 4 it was demonstrated that under these conditions probe binding to Au MPC 2 is nearly quantitatively suppressed. Probe binding under these new experimental conditions can therefore be attributed to interactions of A and B with Au MPC 1•Zn²⁺. The titrations shown in Figure S4a show that the addition of A results in a quantitative displacement of B which confirms that the selectivity of Au MPC 1•Zn²⁺ for A is maintained in the full-component mixture. Indeed, titrating B to the similar system containing already A shows no binding at all of B (Figure S4b).
6. Self-sorting experiments in the presence of Zn\(^{2+}\)

Self-sorting experiments were carried by sequentially adding to a buffered aqueous solution (HEPES 10 mM, pH = 7.0) containing probes A (3.4 μM) and B (2.7 μM):

a) Au MPC \(1\cdot\text{Zn}^{2+}\)

b) Au MPC 2

c) TEACl

d) TPEN

The FIs of both probes were simultaneously followed as a function of time analogously as described before providing the FIs as given in Figure S5.

![Self-sorting experiment in the presence of Zn\(^{2+}\).](image)

**Figure S5.** Self-sorting experiment in the presence of Zn\(^{2+}\). Final concentrations: \([\text{Au MPC } 1\cdot\text{Zn}^{2+}] = 8 \text{ μM}, [\text{Au MPC } 2] = 20 \text{ μM}, [\text{TEACl}] = 80 \text{ mM}, [\text{TPEN}] = 20 \text{ μM}, [A] = 3.4 \text{ μM}, [B] = 2.7 \text{ μM}, \text{ [HEPES]} = 10 \text{ mM, pH 7.0, 37 °C.}

Conversion of the measured FIs into concentrations of free A and B were performed by comparing the measured FIs to the expected values for free A and B in solution. These values are indicated by the dotted lines in Figure S5 and were determined as follows.
a) The expected values correspond to those from the competition experiments described in Figure S2a.

b) The expected values were obtained from the experiments described in the previous section (Figure S4a with the notion that the emission of A was corrected for the absence of TEACl (10%, - see §4).

c) The expected values were obtained from the experiments described in the previous section (Figure S4a.

d) See c). The presence of TPEN does not affect the fluorescence properties of A and B.

The concentrations of free and bound A and B at the end of each interval are given in Table S3 together with the ratio of bound A and B.

Table S3. Concentrations of free and bound A and B at the end of each time interval.

<table>
<thead>
<tr>
<th>Phase</th>
<th>A&lt;sub&gt;total&lt;/sub&gt; (µM)</th>
<th>A&lt;sub&gt;free&lt;/sub&gt; (µM)</th>
<th>A&lt;sub&gt;bound&lt;/sub&gt; (µM)</th>
<th>B&lt;sub&gt;total&lt;/sub&gt; (µM)</th>
<th>B&lt;sub&gt;free&lt;/sub&gt; (µM)</th>
<th>B&lt;sub&gt;bound&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (start)</td>
<td>3.4</td>
<td>3.4 (100)</td>
<td>-</td>
<td>2.7</td>
<td>2.7 (100)</td>
<td>-</td>
</tr>
<tr>
<td>a</td>
<td>3.4</td>
<td>0.4 (12)</td>
<td>3.0 (88)</td>
<td>2.7</td>
<td>2.5 (94)</td>
<td>0.2 (6)</td>
</tr>
<tr>
<td>b</td>
<td>3.4</td>
<td>0.2 (5)</td>
<td>3.2 (95)</td>
<td>2.7</td>
<td>0.2 (8)</td>
<td>2.5 (92)</td>
</tr>
<tr>
<td>c</td>
<td>3.4</td>
<td>0.6 (17)</td>
<td>2.8 (83)</td>
<td>2.7</td>
<td>2.6 (96)</td>
<td>0.1 (4)</td>
</tr>
<tr>
<td>d</td>
<td>3.4</td>
<td>2.4 (65)</td>
<td>1.2 (35)</td>
<td>2.7</td>
<td>2.6 (96)</td>
<td>0.1 (4)</td>
</tr>
</tbody>
</table>

Identical displacement curves were obtained in case the displacement experiments using TEACl and TPEN were performed separately on A@Au MPC 1•Zn<sup>2+</sup> and B@Au MPC 2 (Figure S6a+b). Alternatively, collocation of the probes on the opposite surfaces (i.e. B@Au MPC 1•Zn<sup>2+</sup> and A@Au MPC 2) in the self-sorting experiment would have given much different responses to the additions of TEACl and TPEN as evidenced by the profiles given in Figure S6c+d.
Figure S6. Response curves of a) $A@Au\text{MPC}_1\cdot Zn^{2+}$, b) $B@Au\text{MPC}_2$, c) $B@Au\text{MPC}_1\cdot Zn^{2+}$ and d) $B@Au\text{MPC}_2$ to the sequential addition of TEACl and TPEN. Final concentrations: $[Au\text{MPC}_1\cdot Zn^{2+}] = 8 \mu M$, $[Au\text{MPC}_2] \approx 20 \mu M$, $[TEACl] = 80 \text{mM}$, $[TPEN] = 20 \mu M$, $[A] = 3.4 \mu M$, $[B] = 2.7 \mu M$, $[HEPES] = 10 \text{mM}$, pH 7.0, 37 °C.

In the self-sorting experiment probe $B$ is almost completely displaced at $t = 60$ min (>95%), whilst probe $A$ is still bound for a 35% extent. This was confirmed by an independent titration of $A$ to $Au\text{MPC}_1$ in the presence of TEACl, which yielded an SSC of 1.1 μM (Figure S7, □). In these conditions, $A$ does not bind $Au\text{MPC}_2$ (Figure S7a, o). Similar titrations show that $B$ does not bind $Au\text{MPC}_2$ at all and $Au\text{MPC}_1$ to a very minor extent (SSC = 0.2 μM) in the presence of TEACl (Figure S7b, o and □, respectively). For completeness the effect of TEACl on the interaction between the probes and $Au\text{MPC}_1\cdot Zn^{2+}$ was also investigated (■ in Figures S7a+b). All SSCs are reported in Table S4.
Figure S7. a) Fluorescence intensities as a function of the amount of A added to a solution of Au MPC 1 (□), Au MPC 2 (o) or Au MPC 1•Zn$^{2+}$ (■) in the presence of TEACl. [Au MPC 1•Zn$^{2+}$] = [Au MPC 1] = 8 μM, [Au MPC 2] ≈ 20 μM, [TEACl] = 80 mM, [HEPES] = 10 mM, pH 7.0, 37 °C. b) Fluorescence intensities as a function of the amount of B added to a solution of Au MPC 1 (□), Au MPC 2 (o) or Au MPC 1•Zn$^{2+}$ (■) in the presence of TEACl. [Au MPC 1•Zn$^{2+}$] = [Au MPC 1] = 8 μM, [Au MPC 2] ≈ 20 μM, [TEACl] = 80 mM, [HEPES] = 10 mM, pH 7.0, 37 °C.

Table S4. SSCs for the various nanosystems in the presence of TEACl (80 mM)

<table>
<thead>
<tr>
<th>Nanosystem</th>
<th>SSC A (μM)</th>
<th>SSC B (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au MPC 1•Zn$^{2+}$</td>
<td>2.8 ± 0.2</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Au MPC 2</td>
<td>no binding</td>
<td>no binding</td>
</tr>
<tr>
<td>Au MPC 1</td>
<td>1.1 ± 0.1</td>
<td>0.2 ± 0.05</td>
</tr>
</tbody>
</table>
7. Self-sorting experiments in the absence of Zn$^{2+}$

Self-sorting experiments were carried by sequentially adding to a buffered aqueous solution (HEPES 10 mM, pH = 7.0) containing probes A (1.7 μM) and B (1.3 μM):

a) Au MPC 1
b) Au MPC 2
c) TEACl.

The FIs of both probes were simultaneously followed as a function of time analogously as described before providing the FIs as given in Figure S8.

![Figure S8](image)

**Figure S8.** Self-sorting experiment in the absence of Zn$^{2+}$. Final concentrations: [Au MPC 1] = 8 μM, [Au MPC 2] = 20 μM, [TEACl] = 80 mM, [A] = 1.7 μM, [B] = 1.3 μM, [HEPES] = 10 mM, pH 7.0, 37 °C.

Conversion of the measured FIs into concentrations of free A and B were performed by comparing the measured FIs to the expected values for free A and B in solution. These values are indicated by the dotted lines in Figure S8 and were calculated as follows.

a) The expected values correspond to those from the competition experiments described in Fig. S2c.

b) The expected values were obtained by correcting the value for the self-sorting experiment in the presence of Zn$^{2+}$ (see § 6) for the different probe concentrations. It was verified that
the presence of Zn$^{2+}$ does not affect the fluorescence intensities of either probe. Also here the emission of A was corrected for the absence of TEACl (10%, - see §4).

c) The expected values were obtained from the experiments described in §5 corrected for the different probe concentrations. The final values at t = 60 min (38% for A and 82% for B, see manuscript Figure 5) imply that 1.1 (0.62x1.7) µM of A and 0.2 (0.18x1.3) µM of B are still bound. This is fully in line with the SSCs of those probes on Au MPC 1 in the presence of TEACl (Tab. S4).

The surface ratio A:B of 66:34 (after addition of Au MPC 1 at t= 10 min) was calculated from the amount of free A and B captured upon the addition of Au MPC 1 (78% and 52%, respectively, corresponding to 1.32 and 0.68 µM)