

Pattern-based sensing of nucleotides with functionalized gold nanoparticles

Cristian Pezzato^a, Boram Lee^b, Kay Severin^b and Leonard J. Prins^{a,*}

Electronic Supplementary Information

^a Department of Chemical Sciences, University of Padova, via Marzolo 1, I-35131 Padova, Italy;

^b Institut des Sciences et Ingenierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland.

E-mail: leonard.prins@unipd.it

Tel: +39 049 8275256, +41 (0)21 6939302

Table of contents

1. Instrumentation	3
2. Synthesis and characterization of indicator B	4
3. Optical properties of the fluorescent indicators	8
4. Determination of the surface saturation concentrations (SSCs)	9
5. Dynamic behavior of the multi-indicator surfaces	10
5.1 Loading and kinetic stability of the signals	10
5.2 Displacement experiments	11
5.2.1 Response patterns and PCA plots at 5, 50 and 100 μM analyte concentrations (Au MPC 1 • Zn^{2+})	13
5.2.2 Response patterns at 5, 50 and 100 μM analyte concentrations (Au MPC 1 • Cu^{2+})	14
6. Statistical analyses	15

1. Instrumentation

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

^1H and ^1H - ^1H COSY NMR spectra were recorded using a Bruker AV500 operating at 500 MHz for ^1H . ^{13}C NMR spectra, proton decoupled, were recorded using a Bruker AV300 operating at 75.5 MHz for ^{13}C . Chemical shifts are reported in ppm using CD_3CN residual solvent value as internal reference (ppm: 1.94, ^1H ; 1.39/118.69, ^{13}C).

ESI-MS measurements were performed on an Agilent Technologies 1100 Series LC/MSD Trap-SL spectrometer equipped with an ESI source, hexapole filter and ionic trap.

UV-Visible spectra were recorded on a Varian Cary50 Biospectrophotometer equipped with thermostatted multiple cell holders. Fluorescence measurements were recorded on a Varian Cary Eclipse Fluorescence spectrophotometer equipped with a thermostatted cell holder and on a TECAN M1000 PRO micro-plate reader using Microtiter Plates, polystyrene 96-WELL*F (Greiner bio-one).

The buffer, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma) was used without further purification. The probes **A** (2-aminopurine riboside-5'-O-triphosphate) and **C** (2'-deoxy-3'-O-(N'-methylantraniloyl)-adenosine-5'-diphosphate) were obtained from BioLog Life Science Institute and used as received. Analytes (ATP, ADP, GTP, GDP, CTP, CDP, TTP and TDP, Sigma) were used as received. All concentrations in the stock solution were determined by UV spectroscopy using the following molar extinction coefficients: $\epsilon_{243}(\text{ATP}) = 8000 \text{ M}^{-1}\text{cm}^{-1}$, $\epsilon_{355}(\text{MANT-ADP}) = 5800 \text{ M}^{-1}\text{cm}^{-1}$; $\epsilon_{259}(\text{ATP, ADP}) = 15400 \text{ M}^{-1}\text{cm}^{-1}$, $\epsilon_{253}(\text{GTP, GDP}) = 13700 \text{ M}^{-1}\text{cm}^{-1}$, $\epsilon_{272}(\text{CTP, CDP}) = 9100 \text{ M}^{-1}\text{cm}^{-1}$ and $\epsilon_{268}(\text{TTP, TDP}) = 9600 \text{ M}^{-1}\text{cm}^{-1}$ at pH 7.0). $^{\text{S1}} \text{Zn}(\text{NO}_3)_2$ and $\text{Cu}(\text{NO}_3)_2$ were analytical grade products. Metal ion stock solutions were titrated against EDTA following standard procedures.

^{S1} Z. Yao, X. Feng, W. Hong, C. Li, G. Shi, *Chem. Commun.* **2009**, 4696-4698

2. Synthesis and characterization of indicator B

The probe **B** was synthesized on solid support using a Fmoc-based peptide chemistry standard procedure. After the cleavage of the Fmoc group of the Gly residue, coupling reaction with C343-COOH was performed, leaving the suspension shaken overnight. After the washing procedure the resin was cleaved with a TFA/H₂O/TIPS : 96/2/2 mixture (2 hours). The suspension was filtered and the final solution was evaporated under reduced pressure. The resulting solid residue was purified by preparative HPLC to give the desired peptide as a dark yellow solid (10 mg).

HPLC (Column: Phenomenex RP Jupiter 4U Proteo 90 Å. Gradient : from 90:10 to 10:90 H₂O:ACN (0.1% TFA) in 35 minutes, 450 nm): 18.55 min (purified compound).

¹H-NMR (500 MHz, D₂O/ACN-d₃ 3:7, 300 K) δ ppm: 8.43 (s, 1H), 7.09 (s, 1H), 4.71 (t, *J* = 6.4 Hz, 1H), 4.61 (t, *J* = 6.0 Hz, 1H), 4.02 (d, *J* = 3.7 Hz, 2H), 3.40 – 3.20 (m, 4H), 2.92 – 2.58 (m, 8H), 1.92 – 1.76 (m, 4H).

¹³C-NMR (300 MHz, D₂O/ACN-d₃ 3:7, 300 K) δ ppm: 174.1, 173.9, 171.6, 171.0, 165.7, 163.7, 153.2, 149.6, 149.0, 148.9, 127.9, 121.1, 108.4, 106.8, 105.9, 96.0, 95.9, 50.4, 50.1, 49.9, 43.4, 36.5, 36.0, 27.4, 21.0, 20.0.

MS (ESI+, ACN+0.1% HCOOH) *m/z*: 573.1 ([M+H⁺], 100, calcd 573.5), 595.0 ([M+Na⁺], 33, Calcd 595.5).

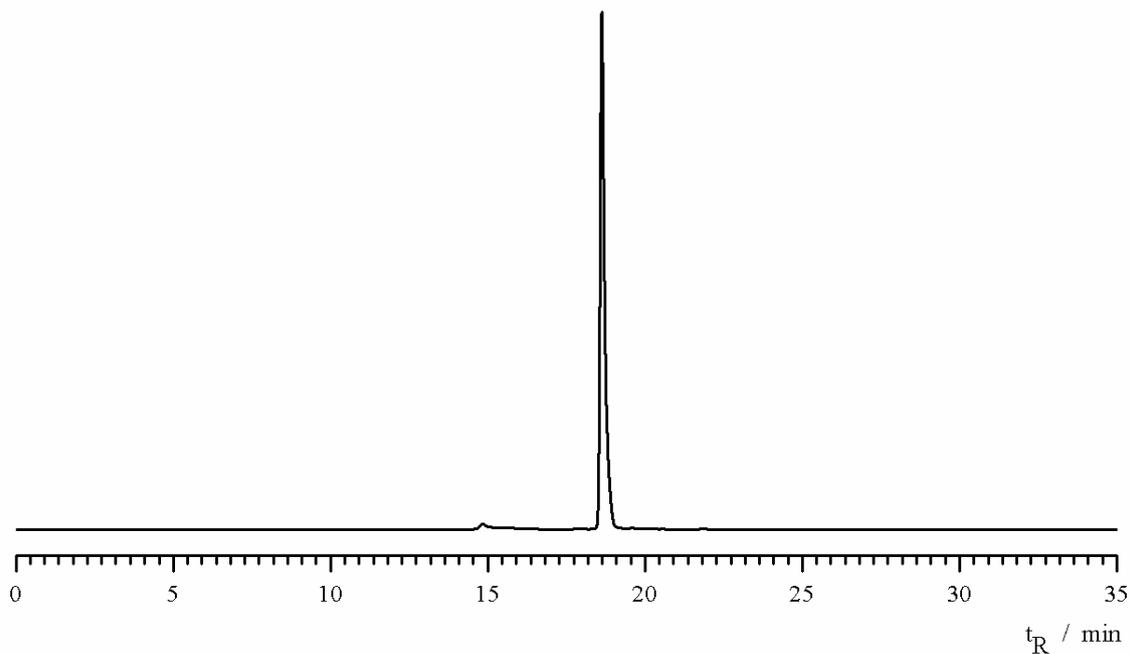


Fig. S1. Analytical HPLC chromatogram (normalized) of purified **B**.

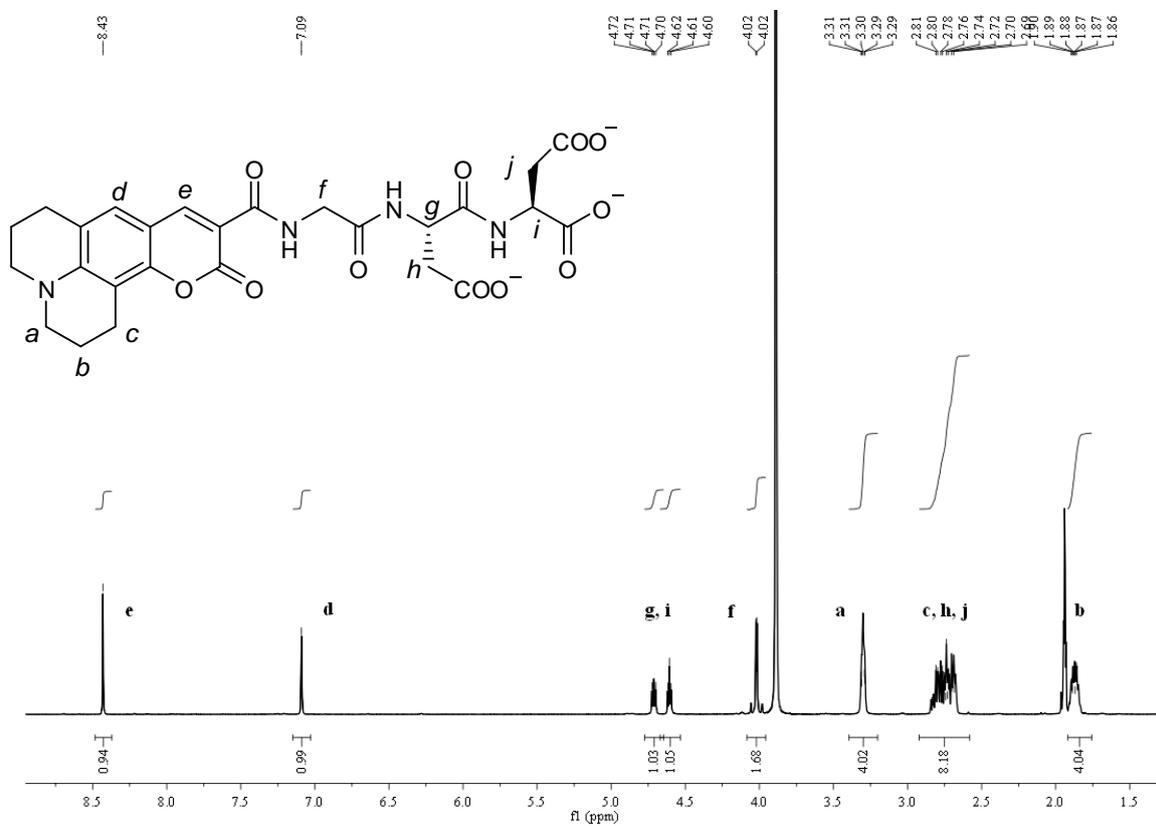


Fig. S2. $^1\text{H-NMR}$ (500 MHz, $\text{D}_2\text{O}/\text{ACN-d}_3$ 3:7, 300 K).

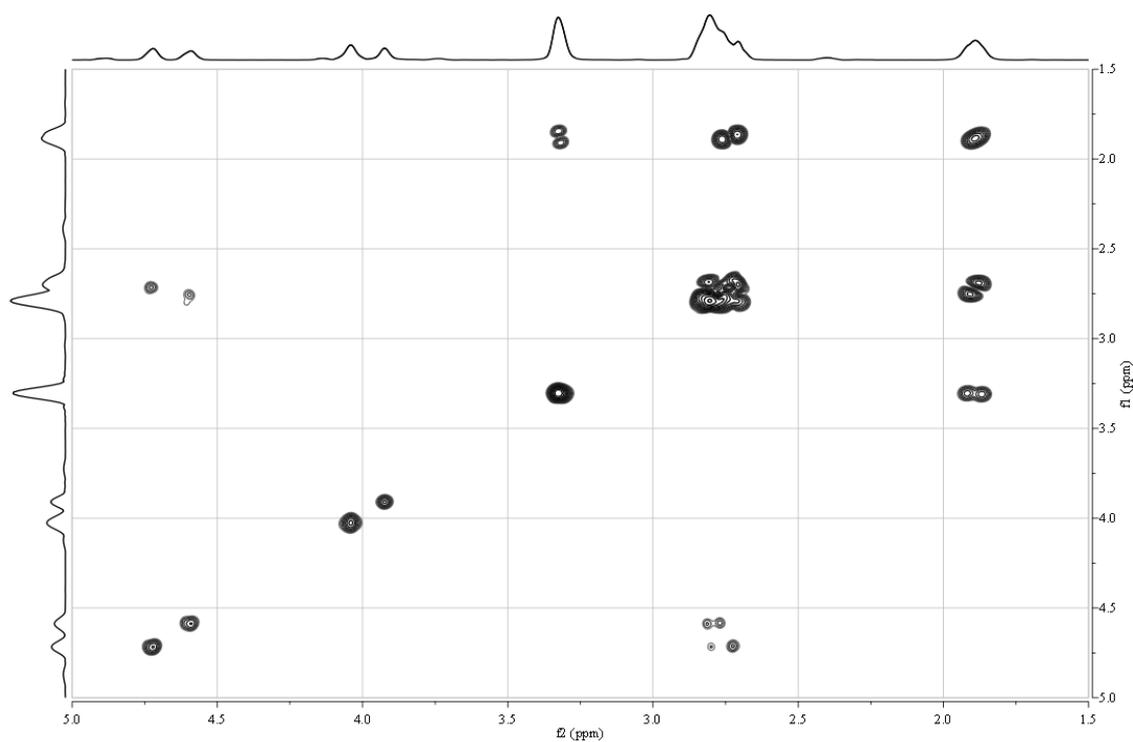


Fig. S3. ^1H - ^1H COSY-NMR (500 MHz, $\text{D}_2\text{O}/\text{ACN-d}_3$ 3:7, 300 K, zoom from 5.0 to 1.5 ppm).

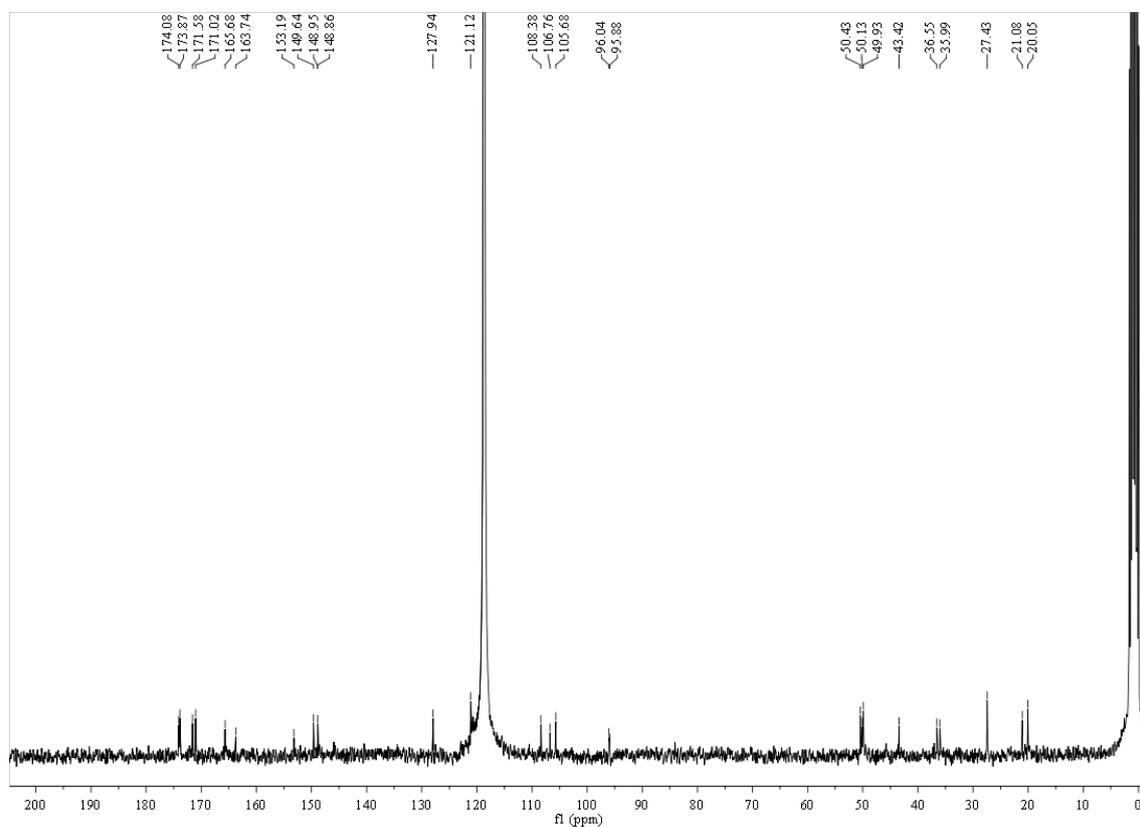


Fig. S4. ^{13}C -NMR (300 MHz, $\text{D}_2\text{O}/\text{ACN-d}_3$ 3:7, 300 K)

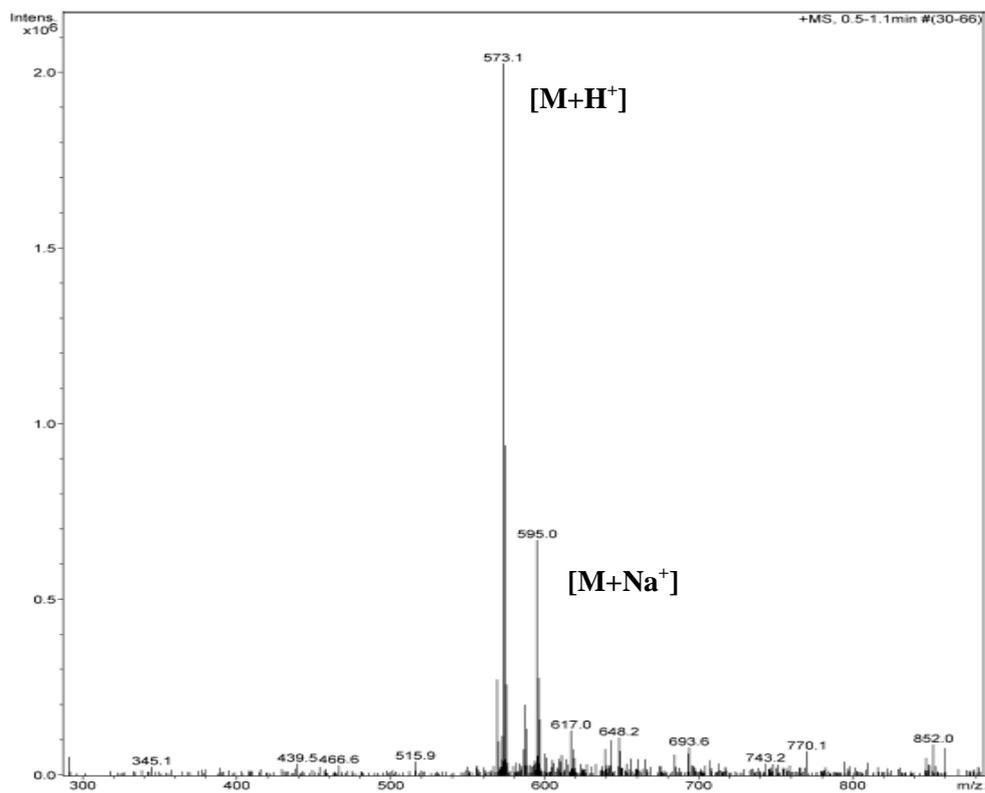


Fig. S5. MS (ESI+, ACN+0.1% HCOOH).

3. Optical properties of the fluorescent indicators

2.2 mg of **B** were solubilized with ACN/mQ water 7:3 and kept to 1.00 mL in a graduated flask (3.9 mM). As the other probes, was then prepared a 0.2 mM solution and its concentration was determined by UV spectroscopy (assuming the extinction coefficient of Coumarin 343 is unaltered : $\epsilon = 45000 \text{ M}^{-1} \text{ cm}^{-1}$, pH 7.0).^{S2}

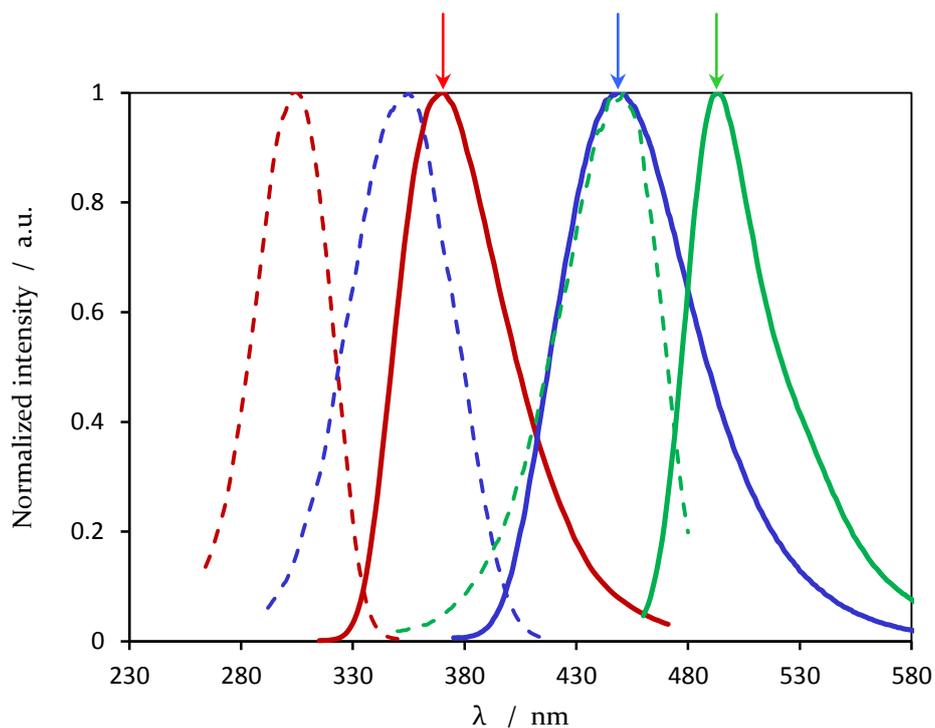


Fig. S6. Normalized excitation (dashed lines) and emission spectrum (solid lines) of **A** (red), **B** (green), and **C** (blue). [probe] = 2 μM , [HEPES] = 10 mM, pH 7.0, 25 °C.

Fluorimeter parameters (Eclipse):

$\lambda_{\text{ex}} = 305 \text{ nm}$; slit 5/5 (A);

$\lambda_{\text{ex}} = 445 \text{ nm}$; slit 2,5/5 (B);

$\lambda_{\text{ex}} = 355 \text{ nm}$; slit 10/10 (C).

^{S2} M. R. Webb, J. E. T. Corrie, *Biophys. J.* **2001**, *81*, 1562-1569;

4. Determination of the Surface Saturation Concentrations (SSCs)

The SSCs of each probe were calculated as we described previously, using the same parameters used to recorder the excitation and emission spectra. The additions of **C** and **B** (Fig. S7) was followed by kinetic, in order to allow the complete organization of the fluorophore on the surface (see below). All values listed in Tab. S1 were determined via fitting of the curve to a 1:1 binding model.^{S3}

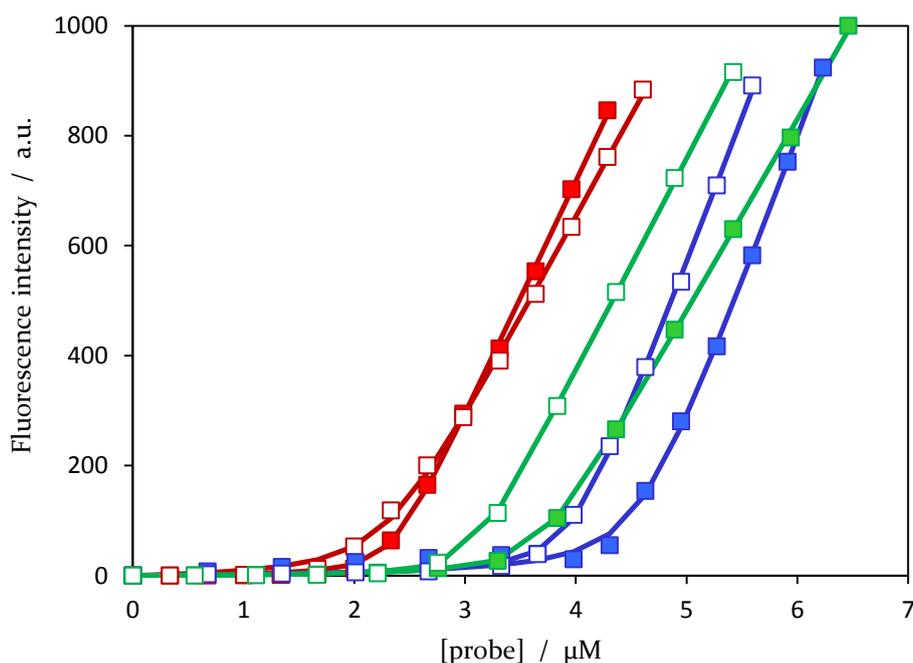


Fig. S7. Fluorescence intensity as a function of the amount of **A** (red, 370 nm), **B** (green, 493 nm) or **C** (blue, 450 nm) added to a solution of Au MPC **1**•Zn²⁺ (■) or Au MPC **1**•Cu²⁺ (□). [Au MPCs] = 10 μM, [HEPES] = 10 mM, pH 7.0, 25 °C.

Tab. S1. SSC values of the three probes with Au MPC **1**•Zn²⁺ and Au MPC **1**•Cu²⁺.

Probe	Au MPC 1 •Zn ²⁺ / μM	Au MPC 1 •Cu ²⁺ / μM
A	2.5 ± 0.2	2.2 ± 0.2
B	3.7 ± 0.2	3.1 ± 0.2
C	4.3 ± 0.2	4.0 ± 0.2

^{S3} G. Pieters, A. Cazzolaro, R. Bonomi, L. J. Prins, *Chem. Commun.* **2012**, 48, 1916-1918.

5. Dynamic behavior of the multi-indicator surfaces

5.1 Loading and kinetic stability of the signals

In the presence of 10 μM concentration of headgroups, a 1:1:1 mixed surfaces on Au MPC $\mathbf{1}\cdot\text{Zn}^{2+}$ and Au MPC $\mathbf{1}\cdot\text{Cu}^{2+}$ were typically realized by adding the three probes at a concentration equal to $(0.7\times\text{SSC})/3$. The kinetics of the displacement experiments were followed and after 2000 seconds changes in fluorescence were no longer observed, because equilibrium was established. In all cases, in the absence of competitor (analyte) the signals are less than 3% of the corresponding maximum fluorescence signals, indicating that the loaded probes are quantitatively bound to the surface (Fig. S8).

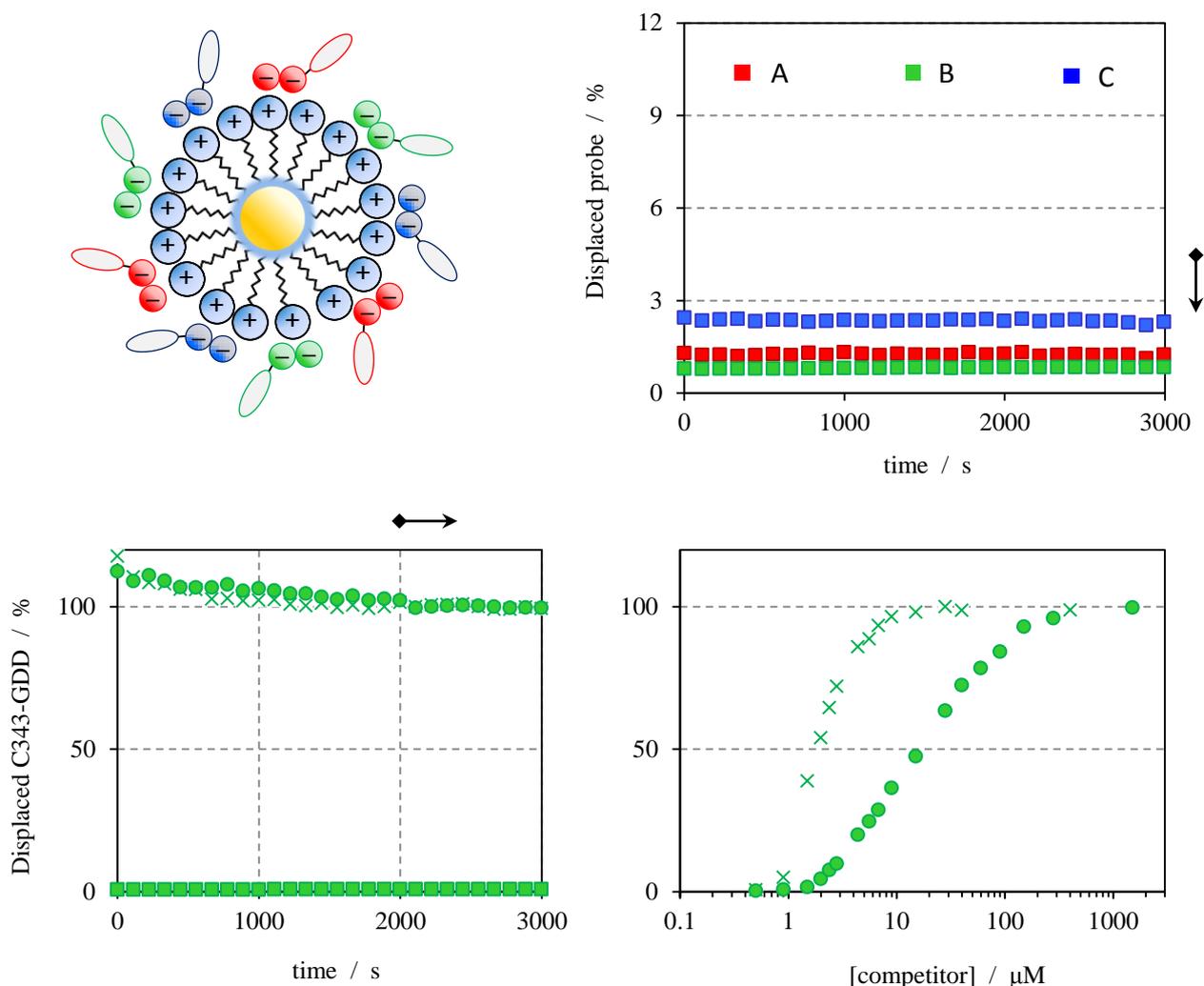


Fig. S8 Top: Stability of a 1:1:1 heteromeric surface on Au MPC $\mathbf{1}\cdot\text{Zn}^{2+}$; in the absence of analyte no release of probe is observed. Bottom: time course of the fluorescence emission at 493 nm upon addition of ATP (400 μM , \times) or ADP (1.5 mM, \bullet) (sx). Overall displacement curves of indicator **B** from the three components surface (dx, see below the complete representations).

5.2 Displacements experiments

The displacement experiments were performed on a TECAN M1000 PRO plate reader by adding 40 μL of a fresh solution of 1:1:1 probes-coated Au MPC **1**• Zn^{2+} (or Au MPC **1**• Cu^{2+}) in HEPES buffer (10 mM, pH 7.0) to the plate-wells, each of them containing 310 μL of a solution of analyte at the desired concentration and buffered in the same manner. All volumes added were calculated in order to obtain the right concentrations in the final volume (350 μL) and all measurements were performed at 25 °C.

Probe parameters: ^{S4}

$\lambda_{\text{ex/em}} = 305/370 \text{ nm}$; slit 5/5; gain = 135; z-position = 23078 μm (A);

$\lambda_{\text{ex/em}} = 445/493 \text{ nm}$; slit 5/5, gain = 80; z-position = 23392 μm (B);

$\lambda_{\text{ex/em}} = 355/450 \text{ nm}$; slit 10/10, gain = 121; z-position = 23392 μm (C).

Instrument settings:

Shaking: double orbital for 40 s

Mode: top fluorescence reading;

Number of flashes = 50

Integration time = 20 μs

Lag time = 0 μs

Settle time = 75 ms

^{S4} For the analysis of various nucleotide concentrations the gain parameters have been optimized on the 100 μM series.

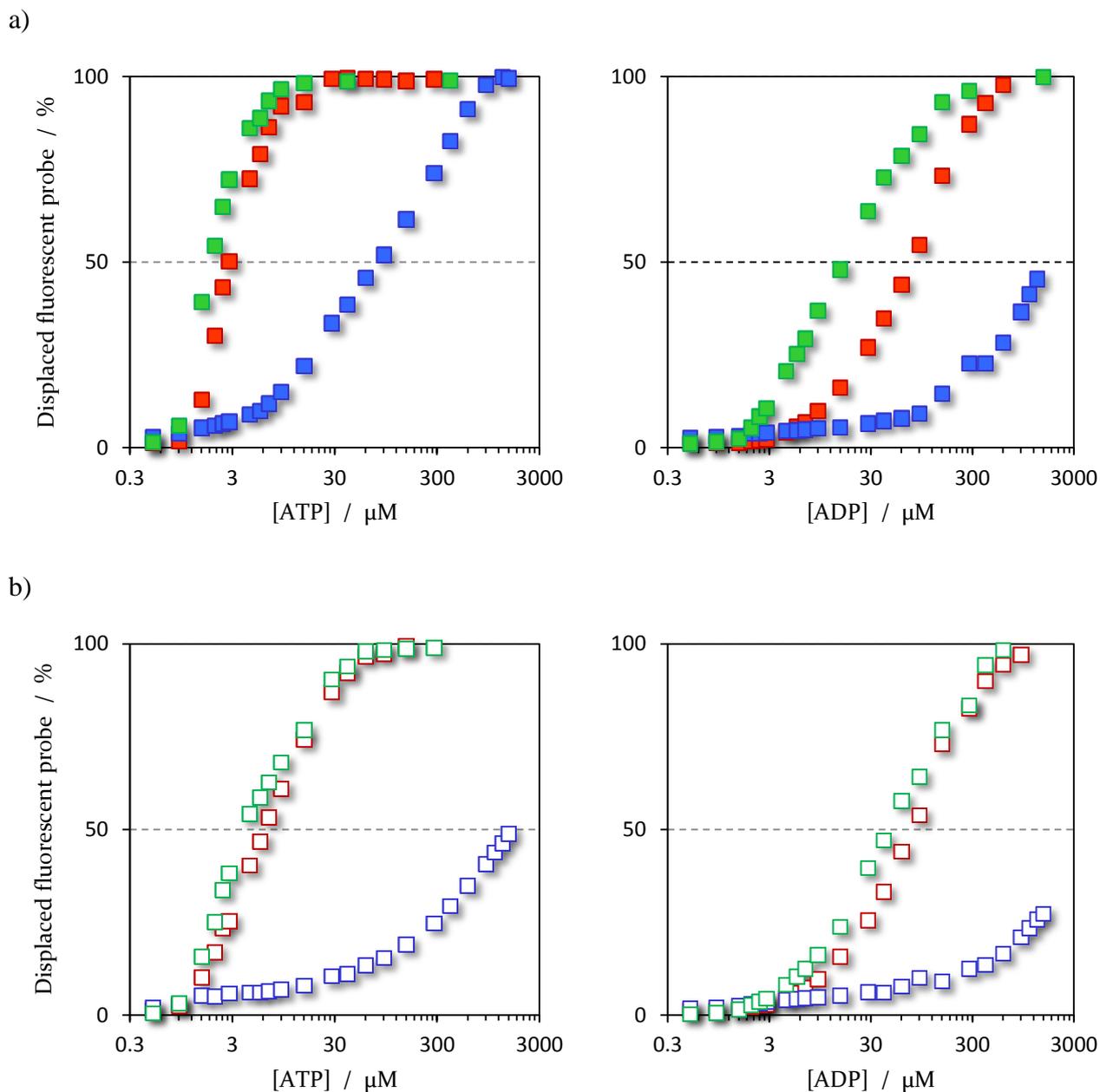


Fig. S9 Displacements of the loaded probes **A** (red), **B** (green), and **C** (blue) from the surface of Au MPC **1•Zn²⁺** (a) and Au MPC **1•Cu²⁺** (b) as a function of concentration of ATP and ADP. [probes] = 0.7•SSC/3, [TACN•Zn(II)] = 10 μM, [HEPES] = 10 mM, pH = 7, 25 °C.

Tab. S2. Concentration of competitor to displace 50% of the probe in the 1:1:1 covered surface.

probe	Au MPC 1•Zn²⁺		Au MPC 1•Cu²⁺	
	ATP / μM	ADP / μM	ATP / μM	ADP / μM
B	1.9	17	4.5	46
A	3.0	71	6.3	75
C	75	>1000	>1000	>>1000

5.2.1 Response patterns and PCA plots at 5, 50 and 100 μM analyte concentrations (Au MPC $1 \cdot \text{Zn}^{2+}$)

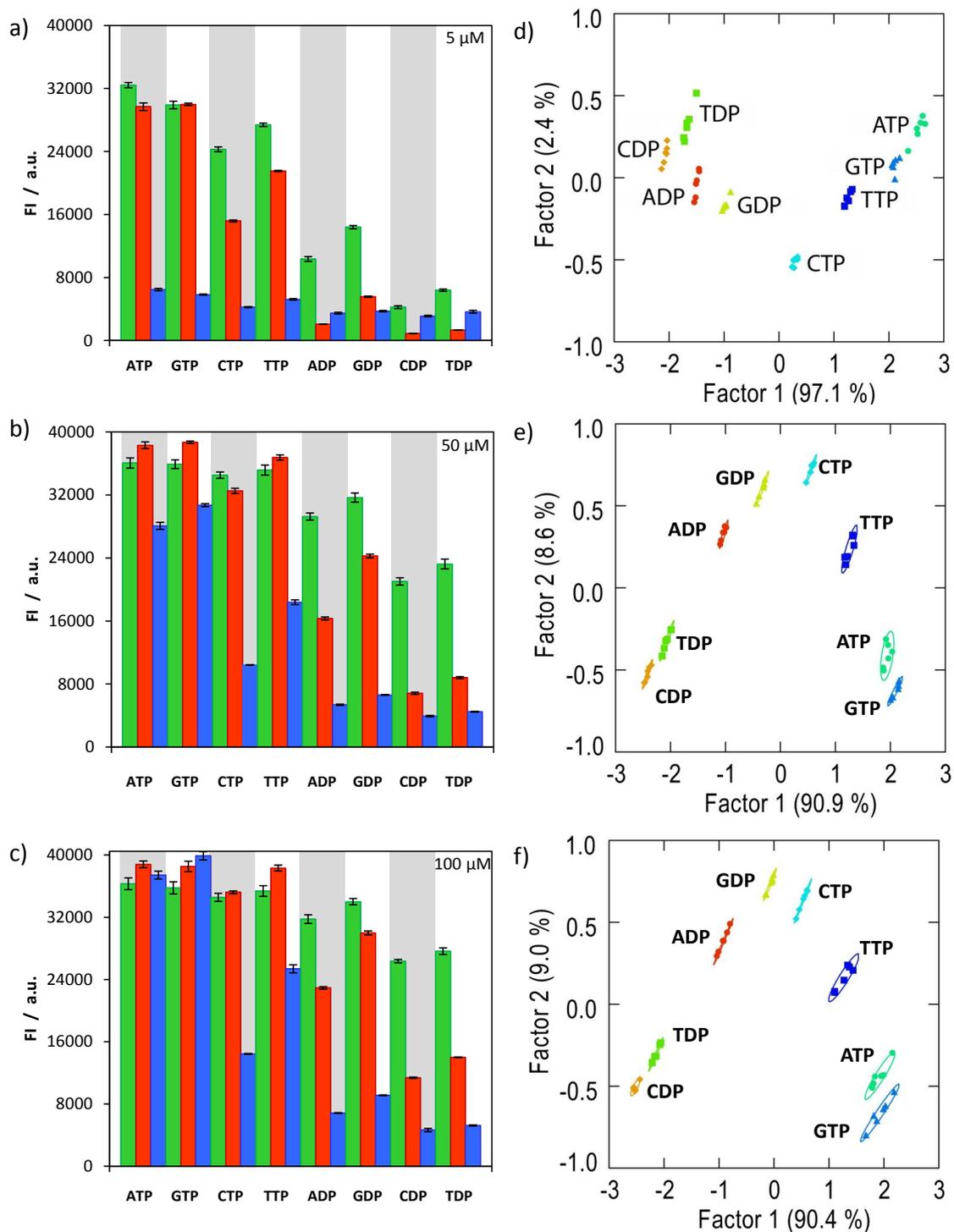


Fig. S10 Response patterns at 5 μM (a), 50 μM (b) and 100 μM (c) analyte concentration (each barrel represents the average of six independent repetitions) and the corresponding PCA score plots (d-f). The data were obtained by displacement assays using Au MPC $1 \cdot \text{Zn}^{2+}$ and the indicators **A** (red), **B** (green), and **C** (blue) as described in the main text.

5.2.2 Response patterns at 5, 50 and 100 μM analyte concentrations ($\text{Au MPC 1}\cdot\text{Cu}^{2+}$)

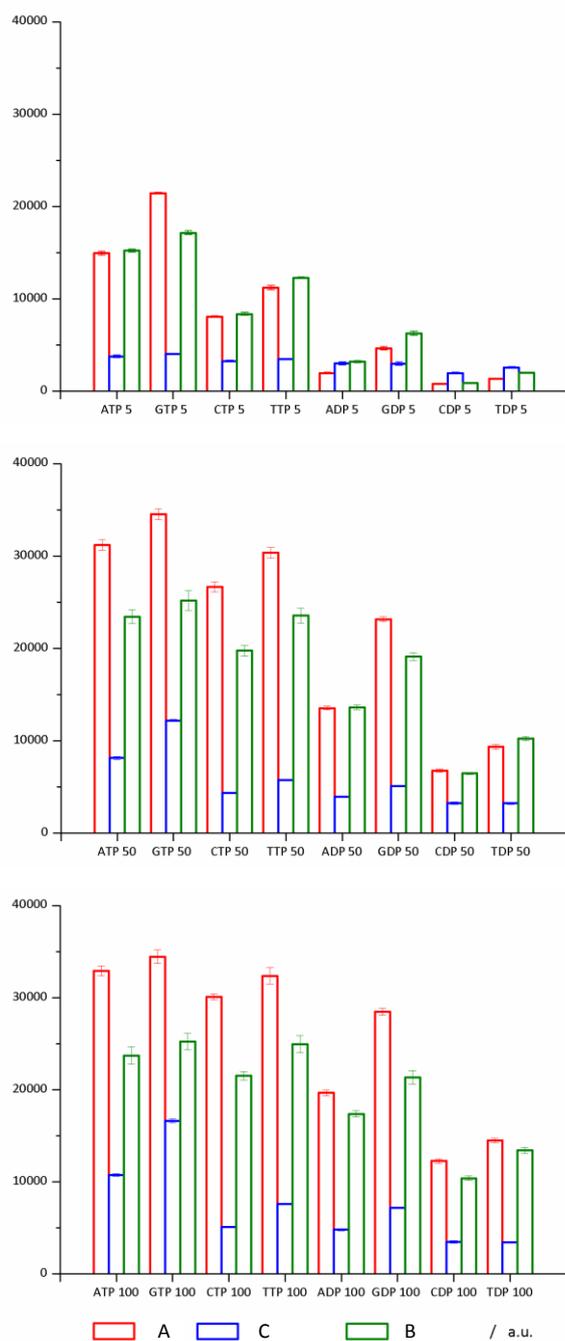


Fig. S11 Response patterns at 5 μM (top), 50 μM (middle) and 100 μM (bottom) analyte concentration (each barrel represents the average of six independent repetitions). The data were obtained by displacement assays using $\text{Au MPC 1}\cdot\text{Cu}^{2+}$ and the indicators **A**, **B**, and **C**.

7. Statistical analyses

The multivariate analyses (LDA and PCA) were performed as implemented in the SYSTAT software package (version 11, Systat Software Inc., Chicago, Illinois, USA).

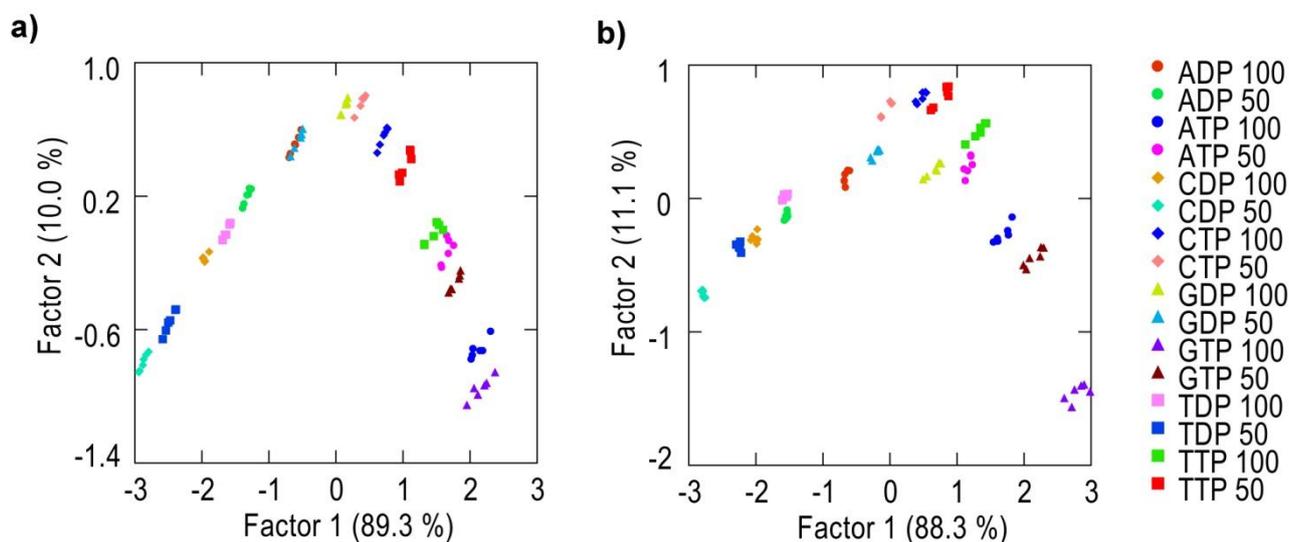


Fig. S12. PCA score plots for the analysis of eight nucleotides at two different concentrations (50 and 100 μM). The data were obtained by displacement assays using Au MPC $\mathbf{1}\cdot\text{Zn}^{2+}$ (a) or Au MPC $\mathbf{1}\cdot\text{Cu}^{2+}$ (b) and the indicators **A**, **B**, and **C**. For the Zn^{2+} -based assay, some overlap of data clusters is observed. The best resolution is obtained when the two data sets are combined (see Figure 4 in the main text).

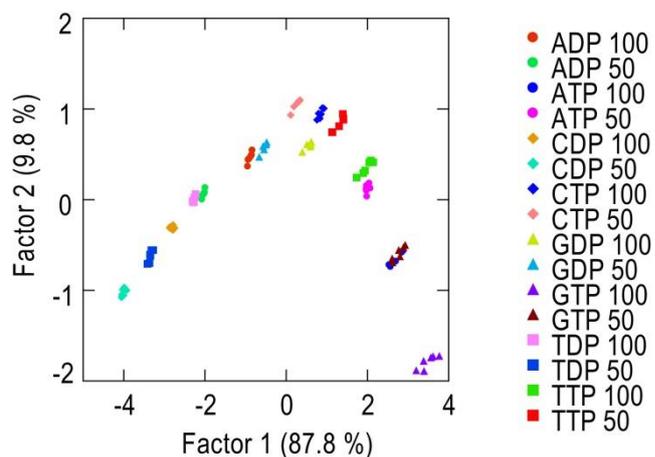


Fig. S13 Two dimensional version of Figure 4 of the manuscript showing the overlap of the GTP50 and ATP100 clusters.

Cross validation: The data from the displacement assays using Au MPC $\mathbf{1}\cdot\text{Zn}^{2+}$ and Au MPC $\mathbf{1}\cdot\text{Cu}^{2+}$ at two different concentrations (50 and 100 μM) were used. 20% of the data were taken out randomly and reclassified. This process was repeated five times. In all cases, a 100% correct classifications observed.