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Electronic Supplementary Information

Dynamic combinatorial chemistry on a monolayer protected gold nanoparticle

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1. Determination of the Surface Saturation Concentrations (SSC)

The SSC of probe **A** (λ_{ex}=450 nm, λ_{em}=493 nm, slits: 2.5/5 nm) on Au NP **1** was determined as described previously (ref. 19 in the main manuscript). The fluorescence intensities generated upon subsequent additions of the probe **A** were recorded after the signal had stabilized (2-3 minutes) (Fig. S1). SSC values of probe **A** are summarized below in Table S1 at 30 μM of total headgroup concentration ([TACN•Zn^{2+}] or [TACN•Hg^{2+}] or [TACN•Zn^{2+}]+[TACN•Hg^{2+}]). The SSCs were determined via extrapolation of the linear part of the curves (the last 4 points) to the y-axis.

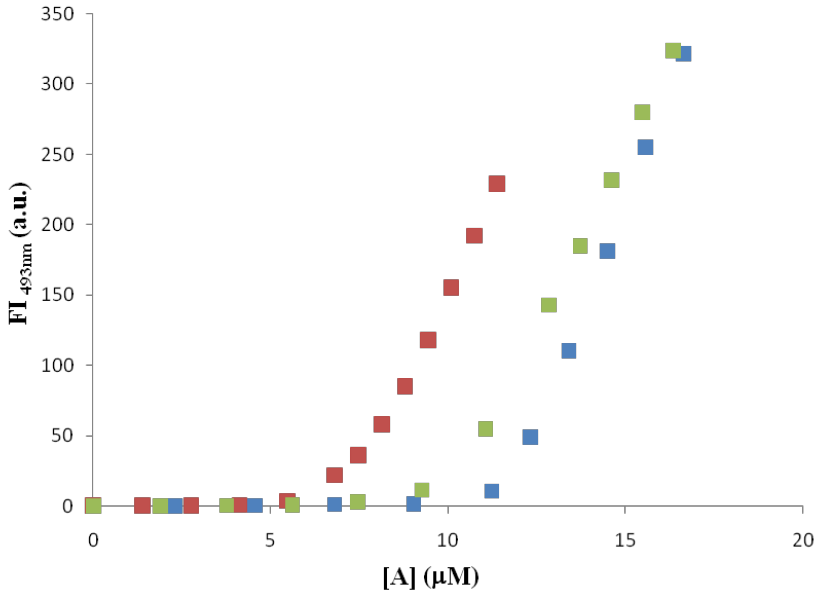

Fig. S1 Fluorescence intensity at 493 nm (a.u.) as a function of the amount of **A** added to a solution of Au NP with a headgroup concentration of 30 μM in presence of Zn^{2+} and Hg^{2+} at different concentration ratio (red square: [Hg^{2+}] = 30 μM; blue square: [Zn^{2+}] = 30 μM; green square: [Hg^{2+}] = 10 μM and [Zn^{2+}] = 20 μM). Experimental conditions: [HEPES] = 10 mM, pH 7.0, T = 25 °C, Excitation wavelength = 450 nm, Slit width (ex/em) = 2.5/5 nm.

Table S1. SSC values[a] of **A** in the presence of different concentration of Zn^{2+} and Hg^{2+}.

[Zn^{2+}] (μM)	[Hg^{2+}] (μM)	SSC (μM)
30	0	11.6 ± 0.3
20	10	10.1 ± 0.2
0	30	7.3 ± 0.2

[a][TACN] = 30 ± 2 μM,[HEPES] = 10 mM, pH 7.0, T = 25 °C, Excitation wavelength = 450 nm, Slit width (ex/em) = 2.5/5 nm.

2.Displacement of probe A by monophosphate nucleotides (*N*MP)

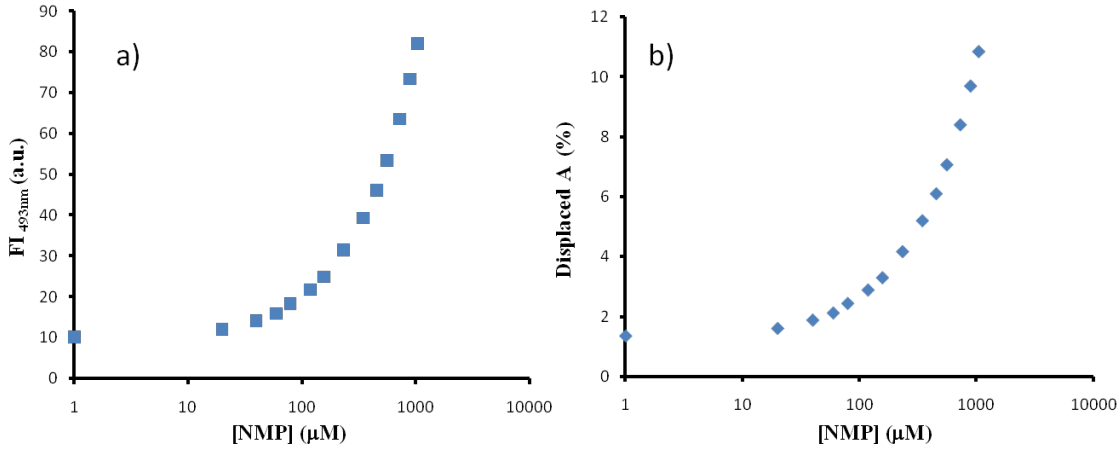

Fig. S2 a) Fluorescence intensity (a.u.) at 493 nm and b)amount of displaced **A** (in %) as a function of the concentration of all the nucleotide monophosphates *N*MP (dAMP, TMP, dGMP, dCMP in a 1:1:1:1 ratio). Experimental conditions: [TACN] = 30 ± 2µM; [Zn^{2+}] = 30 µM; [**A**] = 11.6µM, [HEPES] = 10 mM, pH 7.0, T = 37 °C, fluorescence slit width = 2.5/5 nm.

The displacement curves show that at the conditions of the ultrafiltration experiments (20 µM of each nucleotide) just 2% of probe **A** is displaced, implying that all nucleotides are free in the solution.

3. UPLC-calibration curves for the nucleotides

Calibration curves were obtained using 1:1:1:1 mixture of all nucleotides at different concentrations (10-20 μM) and measuring the corresponding SIM area (Table S2 and Fig. S3) directly (i.e. without filtration).

Table S2. SIM areas of dAMP, TMP, dGMP, dCMP at different concentration (10-20 μM). All measurements[a] were performed in triplicate (1+2+3).

Concentration (μM)		dAMP	TMP	dGMP	dCMP
10	Area[1] ($\times 10^{-6}$)	2.3	2.8	1.4	1.0
	Area[2] ($\times 10^{-6}$)	1.8	2.4	1.6	0.8
	Area[3] ($\times 10^{-6}$)	1.7	2.2	0.8	0.9
	Avg. Area ±s.d.[b] ($\times 10^{-6}$)	**1.9± 0.3**	**2.4 ± 0.3**	**1.2 ± 0.4**	**0.9 ± 0.1**
12.5	Area[1] ($\times 10^{-6}$)	2.5	3.8	2.0	1.4
	Area[2] ($\times 10^{-6}$)	2.5	3.3	1.8	1.2
	Area[3] ($\times 10^{-6}$)	2.0	2.7	1.1	0.8
	Avg. Area ±s.d.[b] ($\times 10^{-6}$)	**2.3 ± 0.3**	**3.3 ± 0.5**	**1.6 ± 0.4**	**1.1 ± 0.3**
15	Area[1] ($\times 10^{-6}$)	3.5	5.4	2.6	1.6
	Area[2] ($\times 10^{-6}$)	2.6	4.1	1.8	1.3
	Area[3] ($\times 10^{-6}$)	2.4	4.2	1.8	0.9
	Avg. Area ±s.d.[b] ($\times 10^{-6}$)	**2.8± 0.6**	**4.6 ± 0.7**	**2.1 ± 0.4**	**1.2 ± 0.4**
17.5	Area[1] ($\times 10^{-6}$)	3.8	5.5	2.4	1.6
	Area[2] ($\times 10^{-6}$)	3.7	5.0	2.1	1.5
	Area[3] ($\times 10^{-6}$)	2.7	4.9	2.2	1.0
	Avg. Area ±s.d.[b] ($\times 10^{-6}$)	**3.4 ± 0.6**	**5.1 ± 0.3**	**2.2 ± 0.1**	**1.4 ± 0.3**
20	Area[1] ($\times 10^{-6}$)	4.2	6.4	2.9	1.9
	Area[2] ($\times 10^{-6}$)	4.0	5.5	2.3	1.8
	Area[3] ($\times 10^{-6}$)	3.2	5.3	2.4	1.5
	Avg. Area ±s.d.[b] ($\times 10^{-6}$)	**3.8 ± 0.5**	**5.7 ± 0.6**	**2.5 ± 0.3**	**1.7 ± 0.2**

[a]UPLC conditions: flow rate = 0.2 ml/min, 5-95 %B (A: H_2O+0.1% HCOOH, B: ACN+0.1% HCOOH) in 5 min, SIM value selected for dAMP = 330, TMP = 321, dGMP = 346, dCMP = 306.
[b]s.d. = standard deviation.

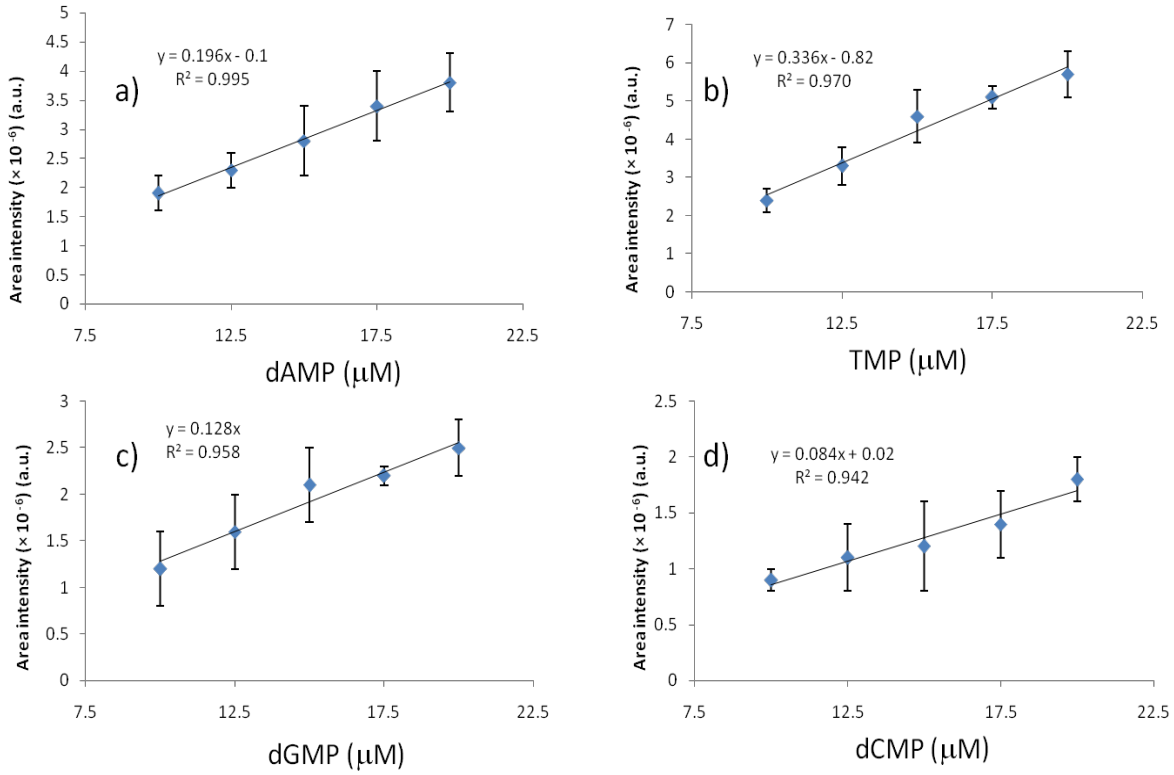

Fig. S3 Calibration curves (area intensity) of a) dAMP, b) TMP, c) dGMP and d) cCMP at different concentration (20-100 μM). Experimental condition: [HEPES] = 10 mM, pH 7.0; Selected SIM-ion value for dAMP = 330, TMP = 321, dGMP = 346, dCMP = 306

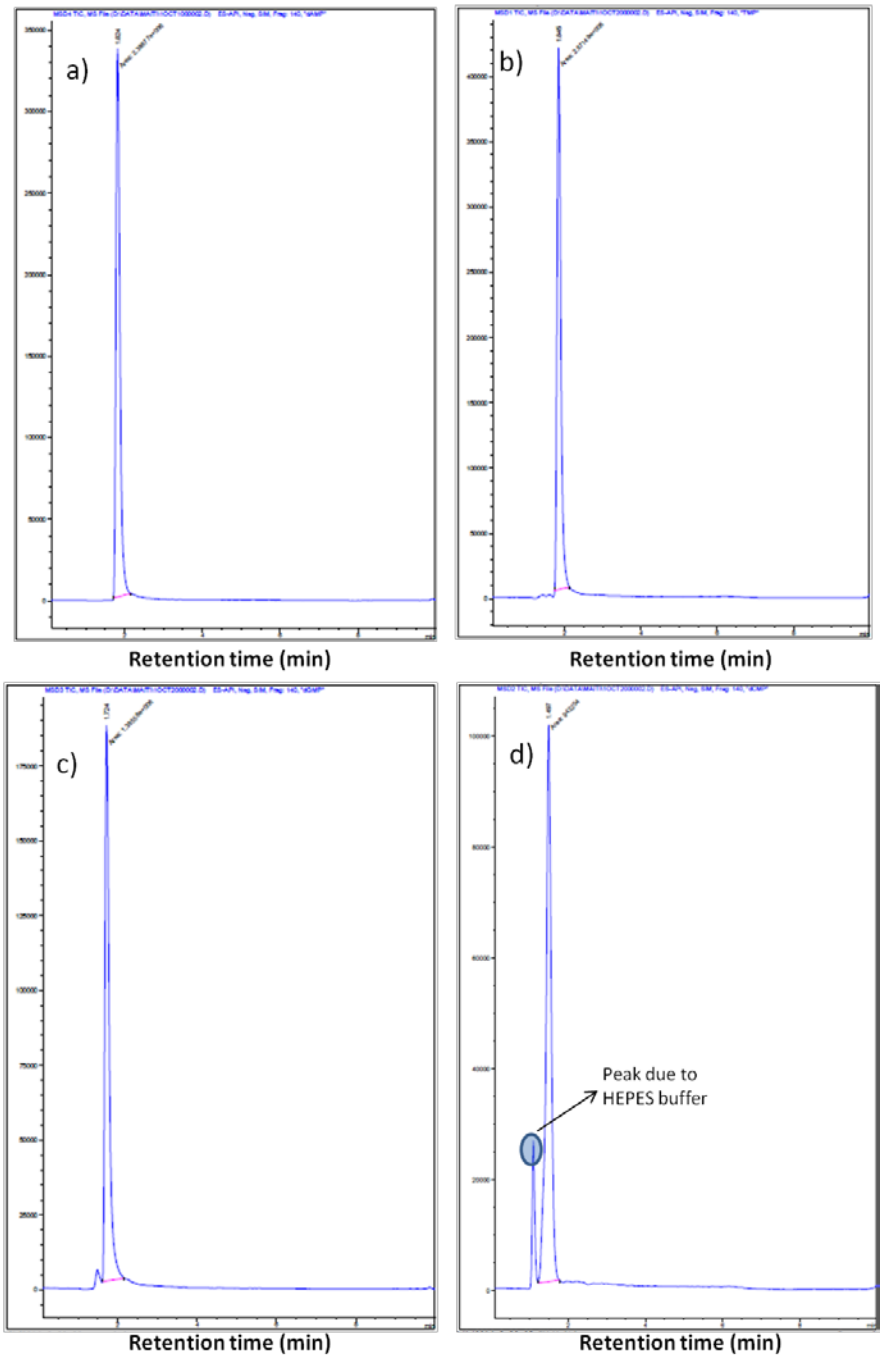

Fig. S4 Representative UPLC chromatogram ofa) dAMP, b) TMP, c) dGMP and d) dCMP at 15 μM concentration (without filtration). Experimental condition: [HEPES] = 10 mM, pH 7.0; Selected SIM Ion value for dAMP = 330, TMP = 321, dGMP = 346, dCMP = 306.

The peak marked with an asterisk in Fig. S4d) is due to the HEPES buffer (10 mM) (see also Fig. S5 and especially Fig. S5d). The intensity of this peak was constant in all measurements. The peak was not observed when injecting aqueous solution without buffer (Fig. S6).

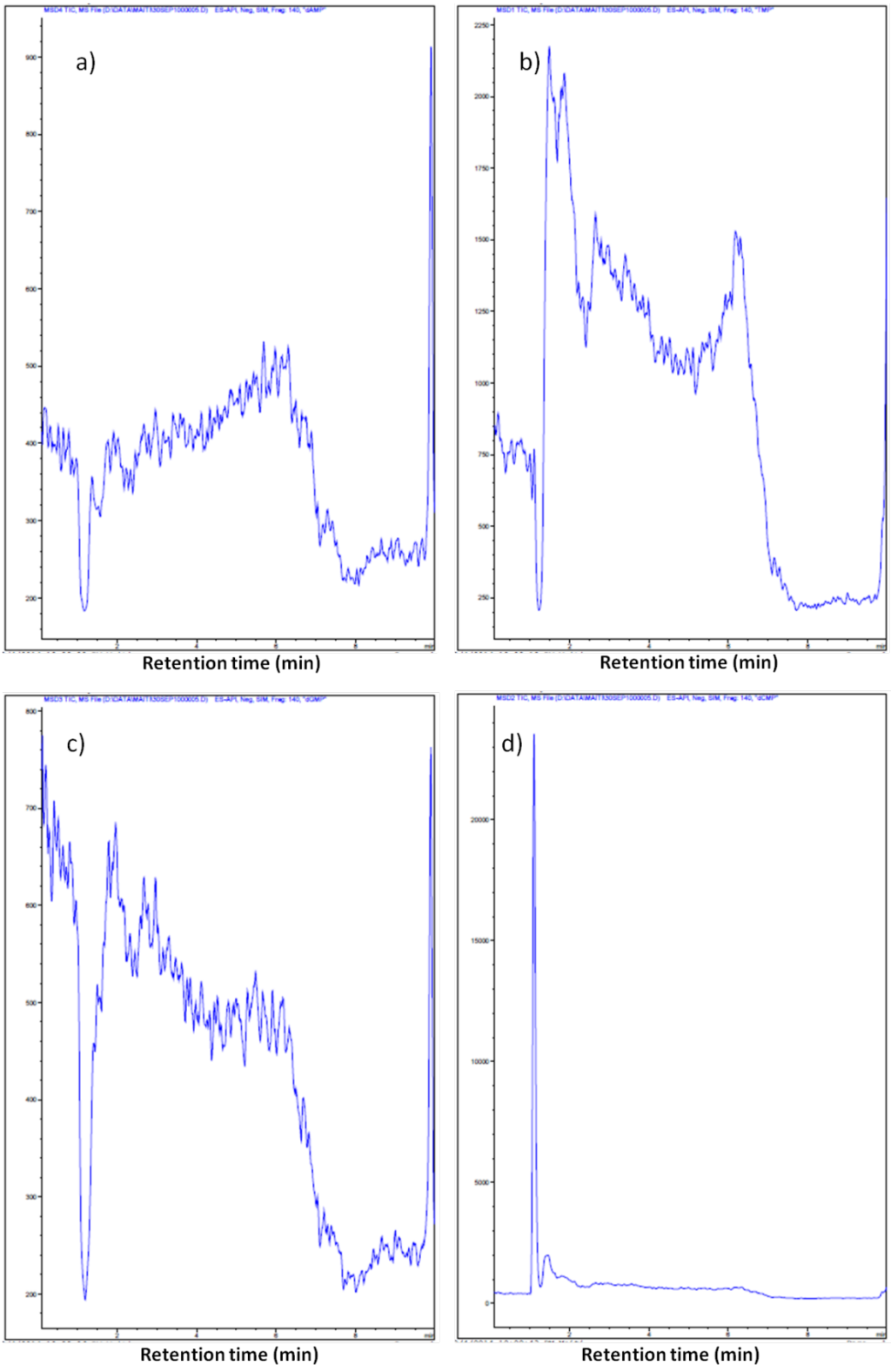

Fig. S5 UPLC chromatograms of HEPES buffer solution (pH 7.0, 10 mM) (without any nucleotide) considering the SIM values of a) dAMP, b) TMP, c) dGMP and d) dCMP. Selected SIM Ion value considered for dAMP = 330, TMP = 321, dGMP = 346, dCMP = 306.

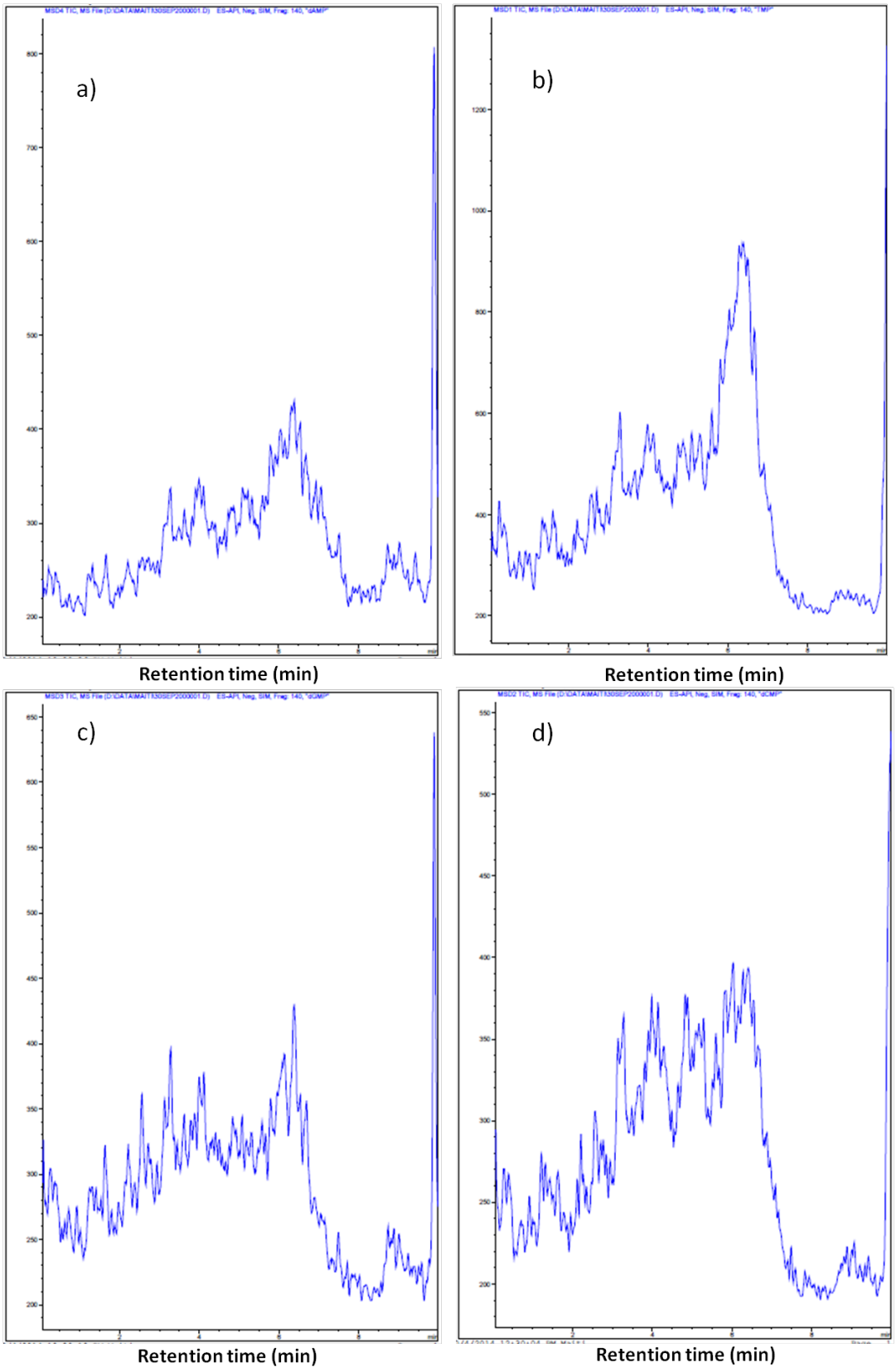

Fig. S6 UPLC chromatograms of aqueous solution without any nucleotide considering SIM ion of a) dAMP, b) TMP, c) dGMP and d) dCMP. Selected SIM Ion value considered for dAMP = 330, TMP = 321, dGMP = 346, dCMP = 306.

4. UPLC chromatogram analysis (SIM mode) of all nucleotides in the dialysate after ultrafiltration in the presence of Au NP 1 and Hg^{2+}or Ag^{+}.

Table S3. Area and corresponding concentration of dAMP, TMP, dGMP, dCMP (calculated from the calibration curve) in the dialysate[a] in the presence of Hg^{2+}or Ag^{+} (concentration 5 μM).[b] All measurements were performed in triplicate (1+2+3).

		Only NP	NP + Hg^{2+} (5 μM)	NP + Ag^{+} (5 μM)
dAMP	Area[1] (× 10^{-6})	4.5	4.3	3.8
	Area[2] (× 10^{-6})	3.7	3.4	3.3
	Area[3] (× 10^{-6})	3.6	3.3	2.9
	Area (avg.) ± s.d. (× 10^{-6})	3.93 ± 0.49	3.67 ± 0.55	3.33 ± 0.49
TMP	Area[1] (× 10^{-6})	6.2	5.0	5.4
	Area[2] (× 10^{-6})	6.0	4.5	5.5
	Area[3] (× 10^{-6})	5.7	4.0	5.0
	Area (avg.) ± s.d. (× 10^{-6})	5.97 ± 0.25	4.5 ± 0.5	5.3 ± 0.26
dGMP	Area[1] (× 10^{-6})	2.5	2.6	1.8
	Area[2] (× 10^{-6})	2.3	2.3	1.6
	Area[3] (× 10^{-6})	2.0	1.7	1.2
	Area (avg.) ± s.d. (× 10^{-6})	2.27 ± 0.25	2.2 ± 0.46	1.53 ± 0.3
dCMP	Area[1] (× 10^{-6})	2.0	2.1	2.0
	Area[2] (× 10^{-6})	2.0	2.0	1.8
	Area[3] (× 10^{-6})	1.9	1.8	1.6
	Area (avg.) ± s.d. (× 10^{-6})	1.96 ± 0.06	1.96 ± 0.15	1.8 ± 0.2

[a][TACN•Zn^{2+}]=30 μM, probe [**A**] = 11.7 μM, [dAMP] = [TMP] = [dGMP] = [dCMP] = 20 μM, [HEPES] = 10 mM, pH 7.0, centrifugation speed = 12000 rpm, centrifugation time = 15 s, initial volume = 500 μl, volume dialysate ≈ 100 μl.

[b]UPLC conditions: flow rate = 0.2 ml/min, 5-95 %B (A: H_2O+0.1% HCOOH, B: ACN+0.1% HCOOH) in 5 min,Selected SIM Ion value for dAMP = 330, TMP = 321, dGMP = 346, dCMP = 306.

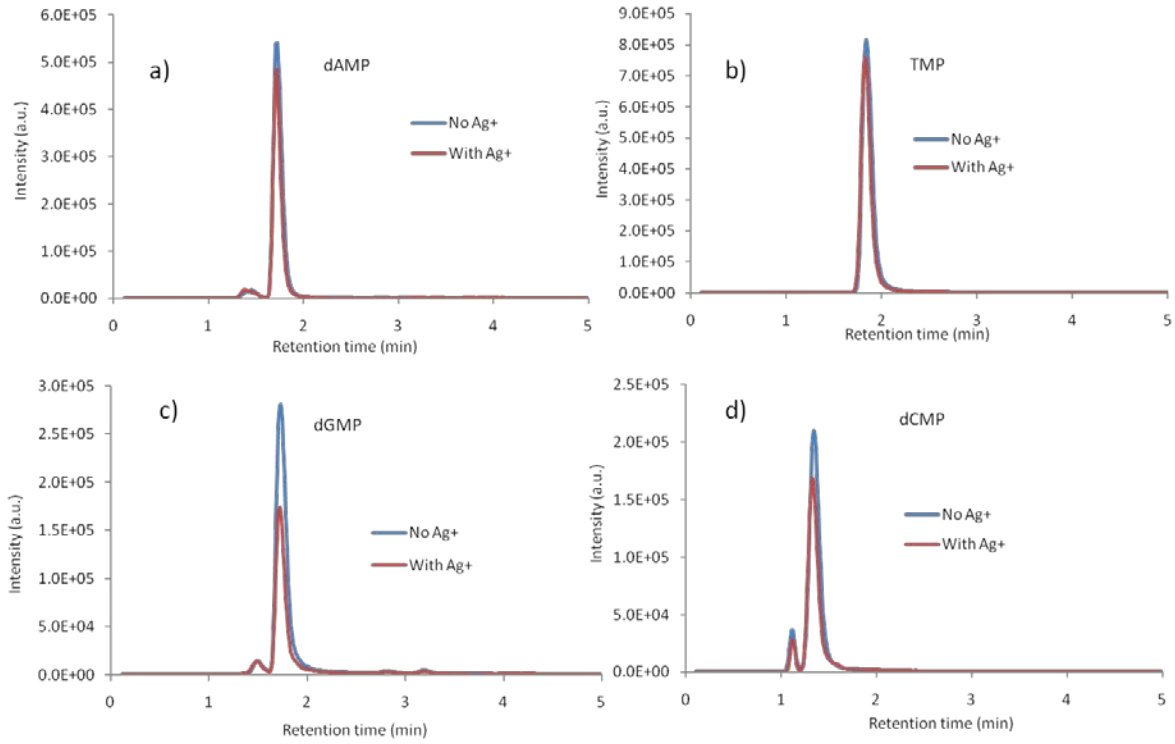

Fig. S7 Representative set of chromatogram analysis of the dialysate of a) dAMP, b) TMP, c) dGMP, d) dCMP in absence and presence of Ag^{+} (5 µM) after ultrafiltration of a solution containing Au NP **1** ([TACN•Zn^{2+}] = 30 µM), probe **A** (11.7 µM) and all the nucleotides at 20 µM. Experimental condition: [HEPES] = 10 mM, pH 7.0, Selected SIM Ion value for dAMP = 330, TMP = 321, dGMP = 346, dCMP = 306. centrifugation speed = 12000 rpm, centrifugation time = 15 s, initial volume = 500 µl, volume of the dialysate ≈ 100 µl. UPLC conditions: flow rate = 0.2 ml/min, 5-95 %B (A: H_2O+0.1% HCOOH, B: ACN+0.1% HCOOH) in 5 min

5. UPLC chromatogram analysis (SIM mode) of all nucleotides in the dialysate after ultrafiltration in the presence of Ag^+but in the absence of Au NP 1.

A slight decrease in the concentration of all nucleotides in the dialysate was observed after adding Ag^+ (5 μM) to a mixture containing Au NP **1**, prone **A**, and a 1:1:1:1 mixture of dAMP, TMP, dGMP, dCMP (see above). The intrinsic interaction between Ag^+ and the nucleotides was studied by repeating the ultrafiltration experiments in the absence of Au NP **1** and **A** (Table S3). A general decrease in the concentration of nucleotides was observed. These values were used to determine for each nucleotide the change in concentration originating from binding to Au NP **1** by subtracting the changes measured for the addition of just Au NP **1** (Table S2) and just Ag^+ (Table S3). The results are given in Table S4 and Fig. S8.

Table S4. Area and corresponding concentration of dAMP, TMP, dGMP, dCMP (calculated from the calibration curve) in the dialysate[a] in the absence and presence of Ag^+ (5 μM)[b]. All measurements were performed in triplicate (1+2+3).

		Aq. solution	Aq. solution + Ag^+ (5 μM)
dAMP	Area[1] (× 10^{-6})	3.9	4.2
	Area[2] (× 10^{-6})	3.7	2.8
	Area[3] (× 10^{-6})	2.9	2.3
	Area (avg.) ± s.d. (× 10^{-6})	3.5 ± 0.52	3.1 ± 0.98
TMP	Area[1] (× 10^{-6})	6.7	6.5
	Area[2] (× 10^{-6})	5.7	5.3
	Area[3] (× 10^{-6})	5.1	4.3
	Area (avg.) ± s.d. (× 10^{-6})	5.83 ± 0.8	5.36 ± 1.1
dGMP	Area[1] (× 10^{-6})	2.9	2.3
	Area[2] (× 10^{-6})	2.4	2.2
	Area[3] (× 10^{-6})	1.8	1.3
	Area (avg.) ± s.d. (× 10^{-6})	2.36 ± 0.55	1.93 ± 0.55
dCMP	Area[1] (× 10^{-6})	2.0	2.3
	Area[2] (× 10^{-6})	2.2	1.7
	Area[3] (× 10^{-6})	1.3	1.2
	Area (avg.) ± s.d. (× 10^{-6})	1.83 ± 0.47	1.73 ± 0.55

[a][dAMP] = [TMP] = [dGMP] = [dCMP] = 20 μM, [HEPES] = 10 mM, pH 7.0, centrifugation speed = 12000 rpm, centrifugation time = 15 s, initial volume = 500 μl, final volume = 100 μl.
[b]UPLC conditions: flow rate = 0.2 ml/min, 5-95 %B (A: H_2O+0.1% HCOOH, B: ACN+0.1% HCOOH) in 5 min, Selected SIM Ion value for dAMP = 330, TMP = 321, dGMP = 346, dCMP = 306.

Table S5. Changes in the concentration in the dialysate originating from binding to the nucleotide-Ag^+ to Au NP **1**.

	SIM area (dialysate)				
	Aq. sol	Aq. sol +Ag^+(a)	% change because of aspecific interactions	% overall change (b)	% change because of nucleotide-Ag^+ interactions with Au NP **1**
dAMP	3.5 ± 0.52	3.1 ± 0.98	11	15	4
TMP	5.83 ± 0.8	5.37 ± 1.1	8	11	3
dGMP	2.37 ± 0.55	1.93 ± 0.55	18	32	14
dCMP	1.83 ± 0.47	1.73 ± 0.55	5	8	3

a) From Table S4. b) From Table S3.

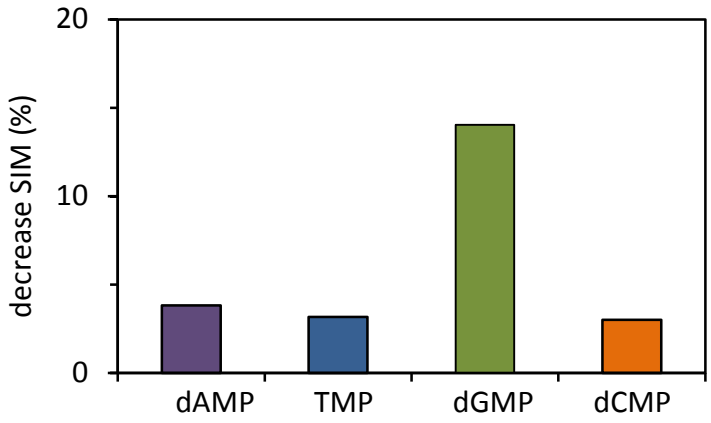

Fig. S8 Relative decrease (%) of the SIM area of each deoxynucleotide attributable to interactions between nucleotide•Ag^+ interactions with Au NP **1**.