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**Selective sensing of adenosine monophosphate (AMP) over adenosine diphosphate (ADP),
adenosine triphosphate (ATP), and inorganic phosphates with zinc(II)-dipicolylamine-
containing gold nanoparticles**

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Nanoparticle Synthesis and Characterisation

General details. Analyses were carried out as follows: analytical precision balance, Kern ABT 100-5M; size exclusion chromatography, Sephadex[®] G-10 GE Healthcare; membrane centrifugation; ¹H NMR spectroscopy, Bruker Avance[™] III 400 spectrometer, spectra were recorded at 22 °C, CD₃OD and D₂O were used as purchased, the spectra were referenced to the residual solvent signal of MeOD-*d*₄ ($\delta^H = 3.31$ ppm); UV-vis spectroscopy, Varian Cary 100 spectrometer, cuvettes: semi-micro PMMA disposable cuvettes, spectra were recorded between 350 and 800 nm at 22 °C, water/methanol, 1:2 (v/v) was used in the reference cell; zetasizer, Malvern Zetasizer Nano ZS by using disposable cuvettes of the type Folded Capillary Zeta Cell DTS1070, the measurements were performed at 22 °C; transmission electron microscopy, JEOL JEM-2100 LaB6 Transmission Electron Microscope (TEM) equipped with a Gatan Orius SC1000 CCD camera for bright-field imaging at 200 kV accelerating voltage, the images had a size of 1024×1024 pixels (acquisition time 0.5 s), the measurements were performed by placing a droplet of an aqueous AuNP solution on a holey carbon grid (Plano S147-4) followed by drying under ambient conditions, the average diameters of the AuNPs were determined by processing the images with ImageJ followed by statistical analysis with MS Excel.

Starting materials and reagents were commercially available and were used without further purification. The syntheses of ligands **1** and **2** are described elsewhere.¹ All qualitative and quantitative binding studies were performed by using HPLC grade solvents. The nanoparticles and salts were weighed by using an analytical precision balance.

Syntheses

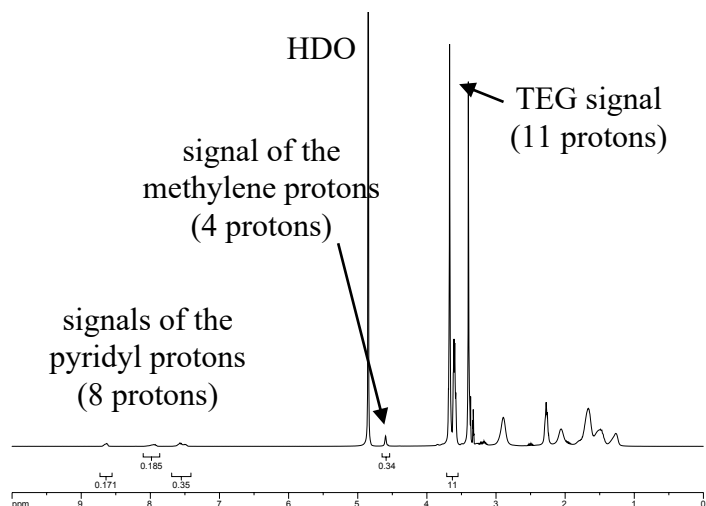
Citrate-stabilised AuNPs (NP^{cit}).² Trisodium citrate dihydrate (484 mg, 1.65 mmol) was dissolved in water (250 mL) and the resulting solution was refluxed for 15 min. Meanwhile, a solution of HAuCl₄ (45 mg, 132 μ mol) in water (1 mL) was also heated to 100 °C and then added quickly to the refluxing citrate solution. The reaction mixture was refluxed for additional 20 min and then allowed to cool to 25 °C. Prior to functionalisation, an aliquot of the thus obtained nanoparticles solution was dialysed against water for 24 h at 25 °C.

Synthesis of the Mixed Monolayer-Protected Gold Nanoparticles. The pH of the dialysed stock solution of NP^{cit} (40 mL) was adjusted to 3 by adding aqueous citric acid (10 mL, 0.1 M). A ligand mixture was prepared by mixing stock solutions of **1** and **2** in methanol (0.1 M) and adding citric acid (2 mL, 0.1 M). The amounts of stock solutions used for the preparation of NP^8 and NP^{30} are specified in Table S1. The ligand solution was added to the reaction mixture, which was then stirred at room temperature for 16 h. The solvent was removed *in vacuo* and the residue was re-dissolved in water/methanol, 1:2 (v/v) (1.00 mL) and acidified with 0.1 M HNO_3 to pH 3. The nanoparticles were purified by size exclusion chromatography by using the same solvent mixture as eluent in which the nanoparticles were dissolved. Subsequently, the obtained nanoparticles were further purified by using centrifugal concentrators. For this, they were dissolved in the solvent mixture (10 mL) also used for the size exclusion chromatography. The solution was subjected to concentrators with a MWCO membrane (5000 Da) and the solvent was removed by centrifugation (3000 rpm, 12 °C). This step was repeated three times. The resulting AuNPs were collected and dried *in vacuo*. Selected analytical results for the two types of nanoparticle thus prepared are collected in Figure S1 and Figure S2. These figures also provide information about the determination of the surface-bound ligand ratio from the corresponding ^1H NMR spectra. Details about the characterisation of the nanoparticles and determination of their composition are described elsewhere.¹

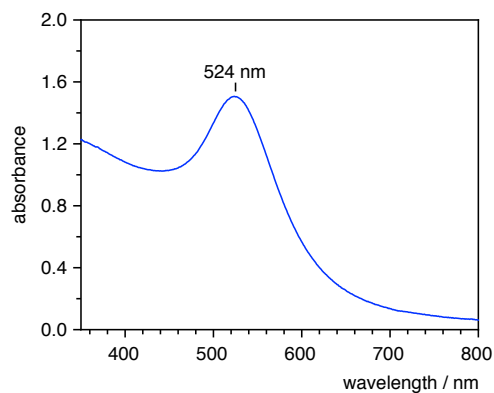
Table S1: Amounts of stock solutions of ligands **1** and **2** in methanol (0.1 M) used for the preparation of NP^8 and NP^{30} .

	$V(\mathbf{1})$ / mL	$V(\mathbf{2})$ / mL	$V(\mathbf{1})/V(\mathbf{2})$ during reaction	$V(\mathbf{1})/V(\mathbf{2})$ in product	Yield / mg
NP^8	1.60	0.40	80:20	92:8	6.6
NP^{30}	1.00	1.00	50:50	70:30	7.3

(a)



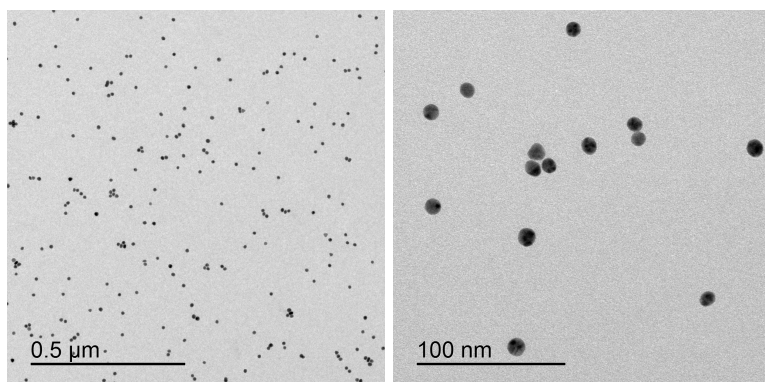
(b)



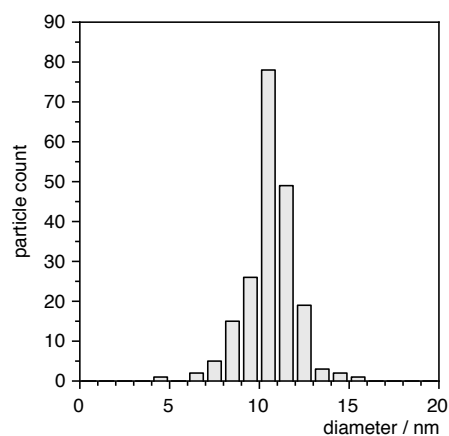
relative content of the functionalised ligand:

$$\text{Content (\%)} = \frac{1.046/12}{(1.046/12 + 11/11)} \times 100 = 8.0\%$$

(c)



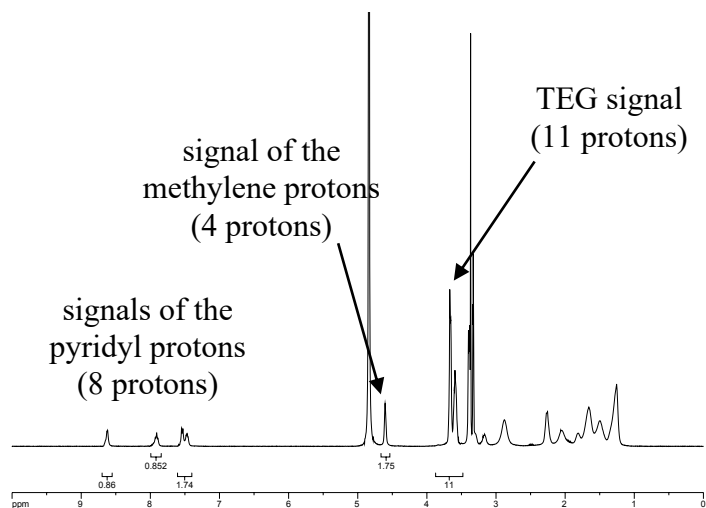
(d)



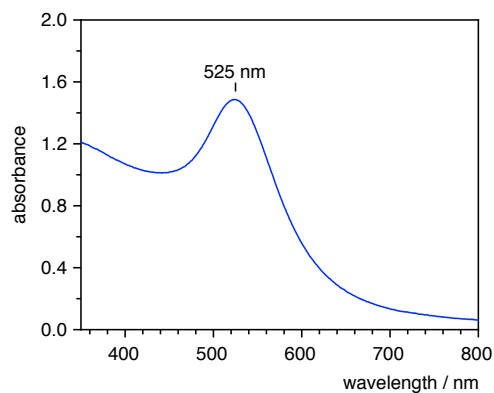
average diameter: 10.6 ± 1.4 nm

Figure S1: ^1H NMR spectrum in $\text{D}_2\text{O}/\text{CD}_3\text{OD}$, 1:2 (v/v) (a) and UV-vis spectrum (b) in water/methanol, 1:2 (v/v) of **NP⁸**, and TEM image (c) together with the histogram illustrating the size distribution of the nanoparticles (d) that was derived from the TEM image (d).

(a)



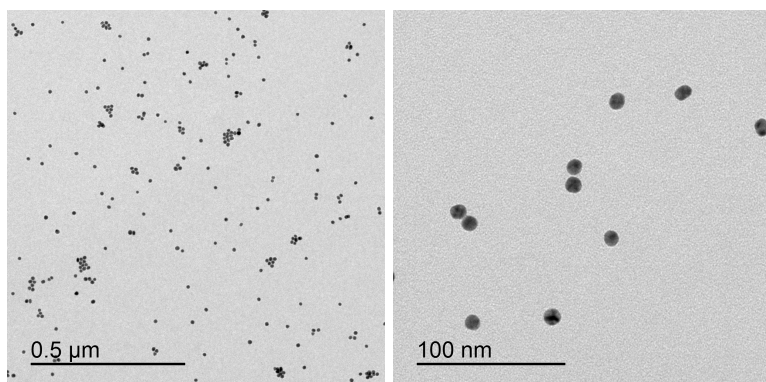
(b)



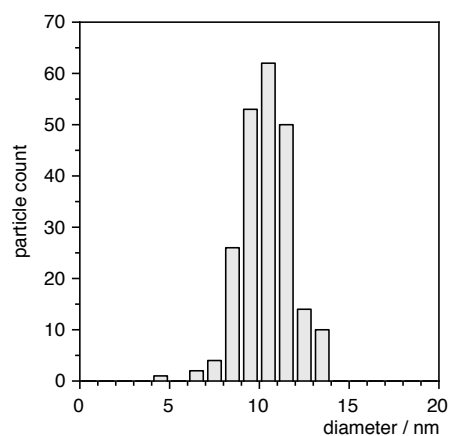
relative content of the functionalised ligand:

$$\text{Content (\%)} = \frac{5.202/12}{(2.202/12 + 11/11)} \times 100 = 30.2\%$$

(c)



(d)



average diameter: 10.4 ± 1.4 nm

Figure S2: ^1H NMR spectrum in $\text{D}_2\text{O}/\text{CD}_3\text{OD}$, 1:2 (v/v) (a) and UV-vis spectrum (b) in water/methanol, 1:2 (v/v) of NP^{30} , and TEM image (c) together with the histogram illustrating the size distribution of the nanoparticles (d) that was derived from the TEM image (d).

Binding Studies

Preparation of the Nanoparticle Stock Solutions. The isolated nanoparticles were dissolved in water/methanol, 1:2 (v/v) (12 mL). Prior to the preparation of the stock solutions, the degrees to which these solutions had to be diluted to reach an extinction of 1.5 at 525 nm were determined by UV/vis spectroscopy. Based on the obtained dilution factor, the volumes were calculated with which these solutions had to be diluted with water/methanol, 1:2 (v/v) and a Zn(NO₃)₂ stock solution (10 mM in water/methanol, 1:2 (v/v)) to obtain solutions with zinc(II) concentrations of 5, 50, or 500 μM. These volumes are summarised in Table S2. Since the starting concentrations of NP⁸ and NP³⁰ differed, the conditions differed for the two types of nanoparticles.

Table S2: Volumes of the initial nanoparticle solutions, of pure solvent, and of the Zn(NO₃)₂ stock solution used to prepare the nanoparticle stock solutions for the binding studies.

nanoparticle	$V(\text{nanoparticle solution}) / \mu\text{L}$	$V(\text{water/methanol, 2:1 (v/v)}) / \mu\text{L}$	$V(\text{Zn(NO}_3)_2 \text{ solution}) / \mu\text{L}$	$c(\text{Zn(NO}_3)_2) / \mu\text{M}$
NP ⁸	1000	1000	0	0
	1000	999	1	5
	1000	990	10	50
	1000	900	100	500
NP ³⁰	667	1323	10	50
	667	1233	100	500

Qualitative Binding Study. The nanoparticle stock solutions were prepared as described above. In addition, a series of stock solutions were prepared of the salts that were used as analytes (1 mM in H₂O). As analytes, adenosine 5'-triphosphate disodium salt hydrate (ATP), adenosine 5'-diphosphate disodium salt hydrate (ADP), adenosine 5'-monophosphate disodium salt hydrate (AMP), adenosine 3',5'-cyclic monophosphate sodium salt (cAMP), adenosine, guanosine 5'-triphosphate disodium salt (GTP), D-ribose-5-phosphate disodium salt hydrate, Na₂HPO₄, Na₄P₂O₇ (PP_i), and Na₅P₃O₁₀ (TP) were used.

Eleven vials were prepared, each containing 200 μL of a nanoparticle stock solution (NP⁸ in the absence of Zn(NO₃)₂ or in the presence of 5, 50, or 500 μM of Zn(NO₃)₂), and NP³⁰ in the presence

of 50 or 500 μM of $\text{Zn}(\text{NO}_3)_2$). One vial was used as blank while each of the other vials was treated with a specific analyte stock solution. Initially, 1 μL of the respective analyte solution was added to each vial. The subsequent additions comprised 4 μL , 5 μL , 10 μL , and 20 μL . The salt concentrations thus increased from 5 μM after the first addition over 24 μM , 48 μM , 91 μM , to 167 μM after the last one. Photographs of the vials were taken 10 min after each addition. The results obtained for **NP⁸** at a 50 μM $\text{Zn}(\text{NO}_3)_2$ concentration are shown in Figure 2 of the main article and those at 5 μM and 500 μM of $\text{Zn}(\text{NO}_3)_2$ in Figure S3 and Figure S5, respectively. Figure S6 shows an analogous binding study for **NP⁸** in the absence of $\text{Zn}(\text{NO}_3)_2$, and Figure S7 and Figure S8 show binding studies for **NP³⁰** in the presence of 50 μM and 500 μM of $\text{Zn}(\text{NO}_3)_2$, respectively.

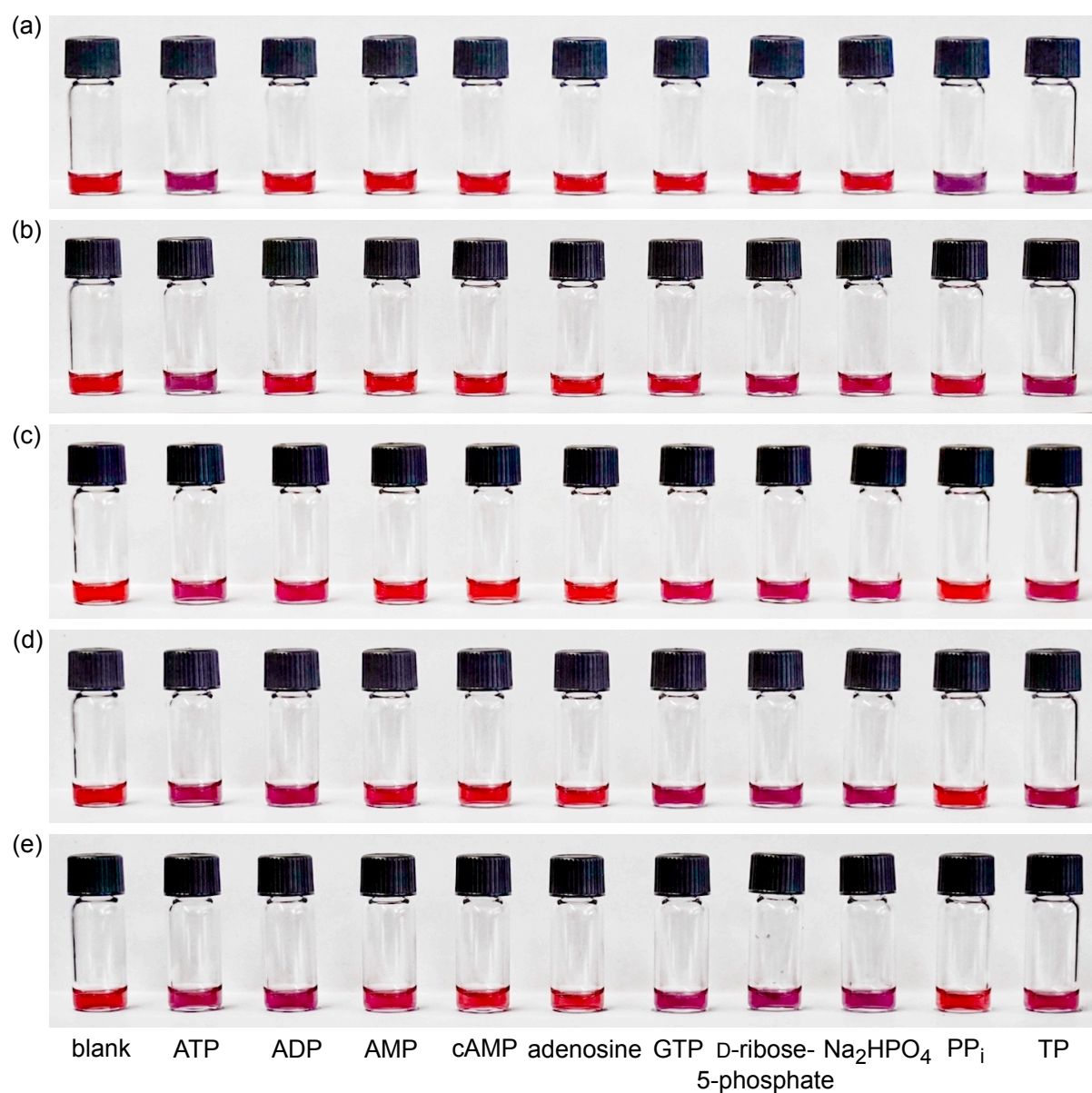


Figure S3: Images of solutions of NP⁸ in water/methanol, 1:2 (v/v) containing 5 μM of Zn(NO₃)₂ after the addition of the analytes specified in the bottom row at concentrations of 5 μM (a), 24 μM (b), 48 μM (c), 91 μM (d), and 167 μM (e).

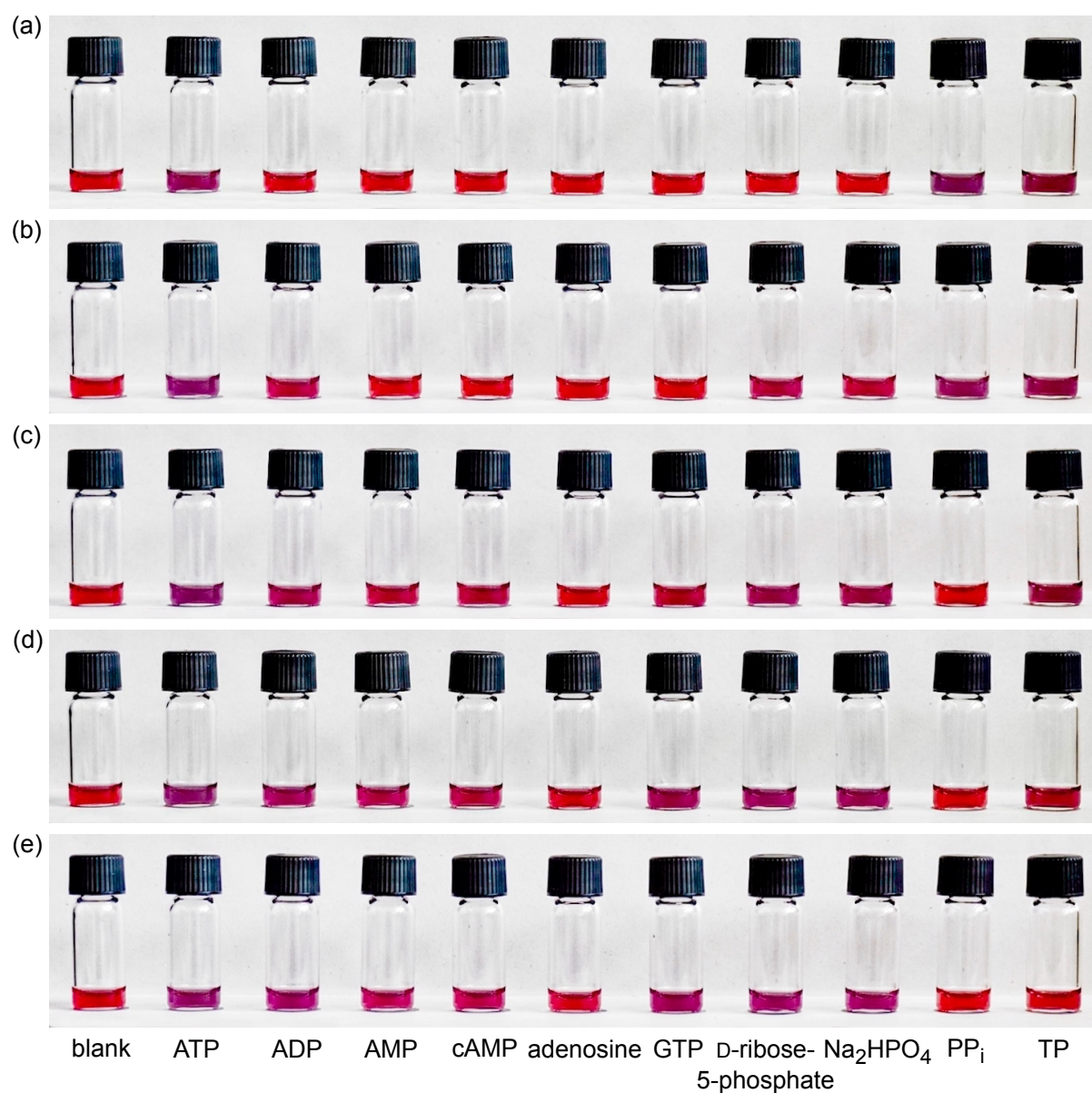


Figure S4: Images of solutions of NP⁸ in water/methanol, 1:2 (v/v) containing 50 μM of Zn(NO₃)₂ after the addition of the analytes specified in the bottom row at concentrations of 5 μM (a), 24 μM (b), 48 μM (c), 91 μM (d), and 167 μM (e).

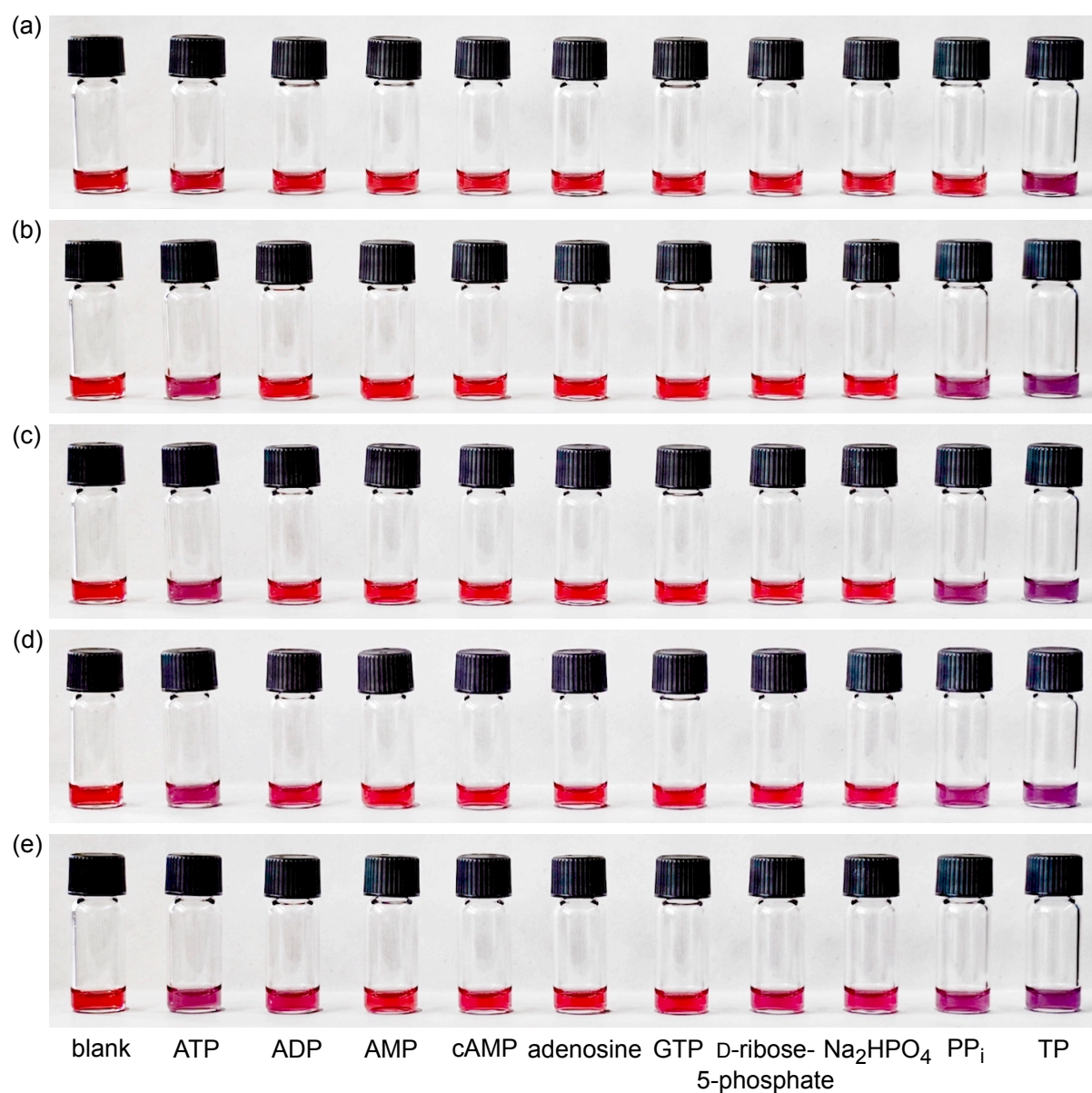


Figure S5: Images of solutions of NP⁸ in water/methanol, 1:2 (v/v) containing 500 μM of Zn(NO₃)₂ after the addition of the analytes specified in the bottom row at concentrations of 5 μM (a), 24 μM (b), 48 μM (c), 91 μM (d), and 167 μM (e).

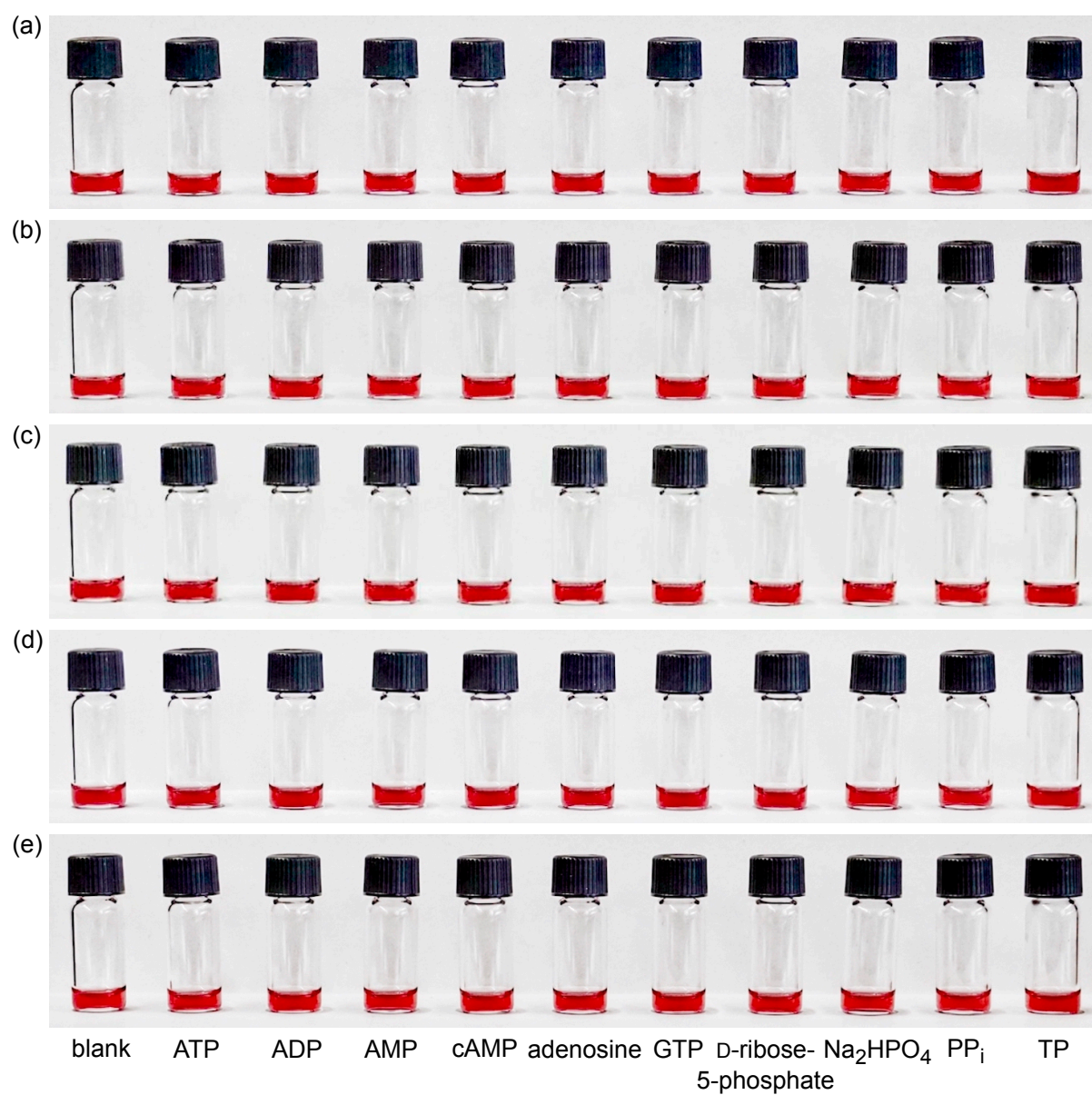


Figure S6: Images of solutions of NP^8 in water/methanol, 1:2 (v/v) in the absence of $\text{Zn}(\text{NO}_3)_2$ after the addition of the analytes specified in the bottom row at concentrations of $5\ \mu\text{M}$ (a), $24\ \mu\text{M}$ (b), $48\ \mu\text{M}$ (c), $91\ \mu\text{M}$ (d), and $167\ \mu\text{M}$ (e).

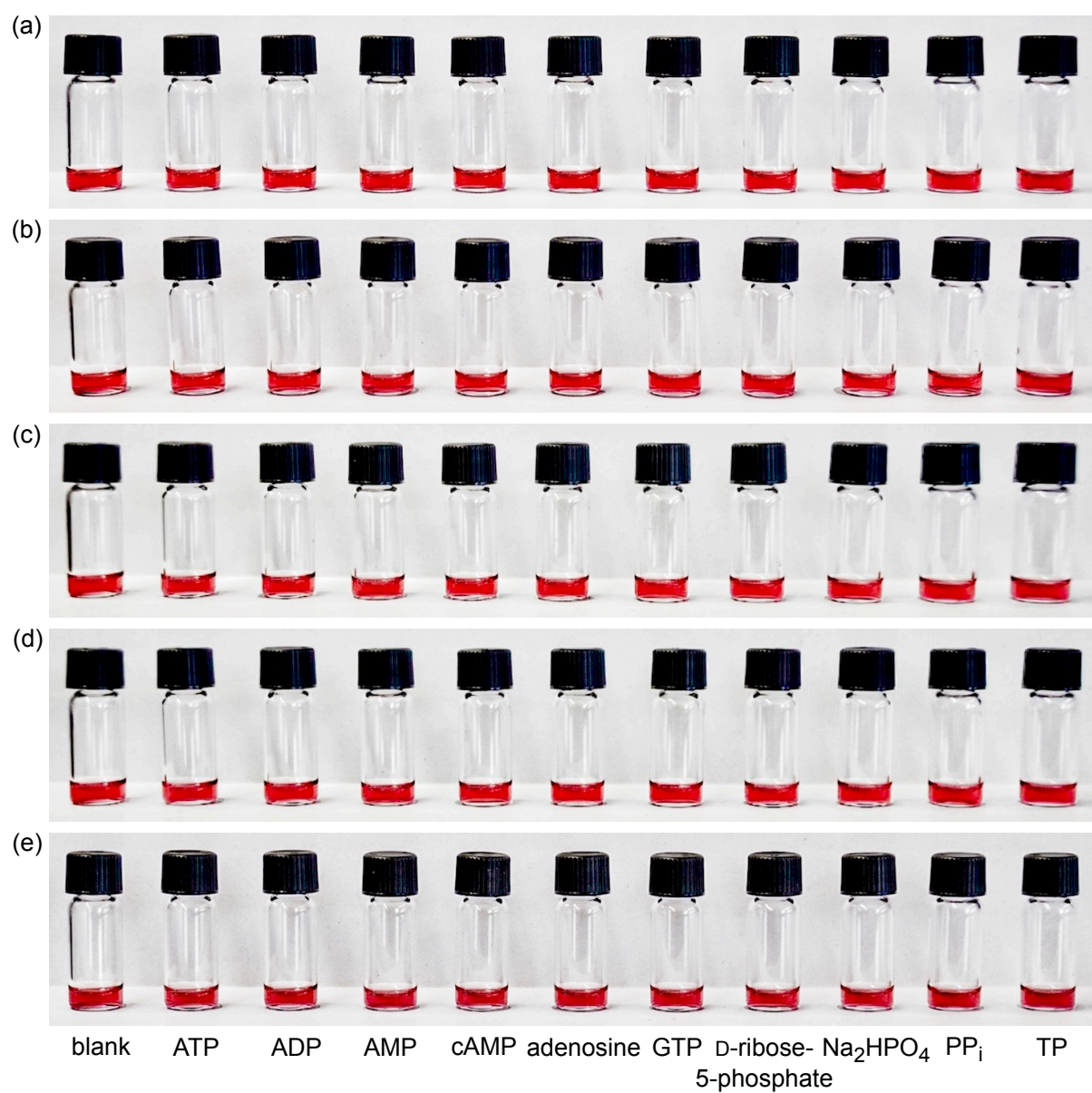


Figure S7: Images of solutions of NP³⁰ in water/methanol, 1:2 (v/v) containing 50 μM of Zn(NO₃)₂ after the addition of the analytes specified in the bottom row at concentrations of 5 μM (a), 24 μM (b), 48 μM (c), 91 μM (d), and 167 μM (e).

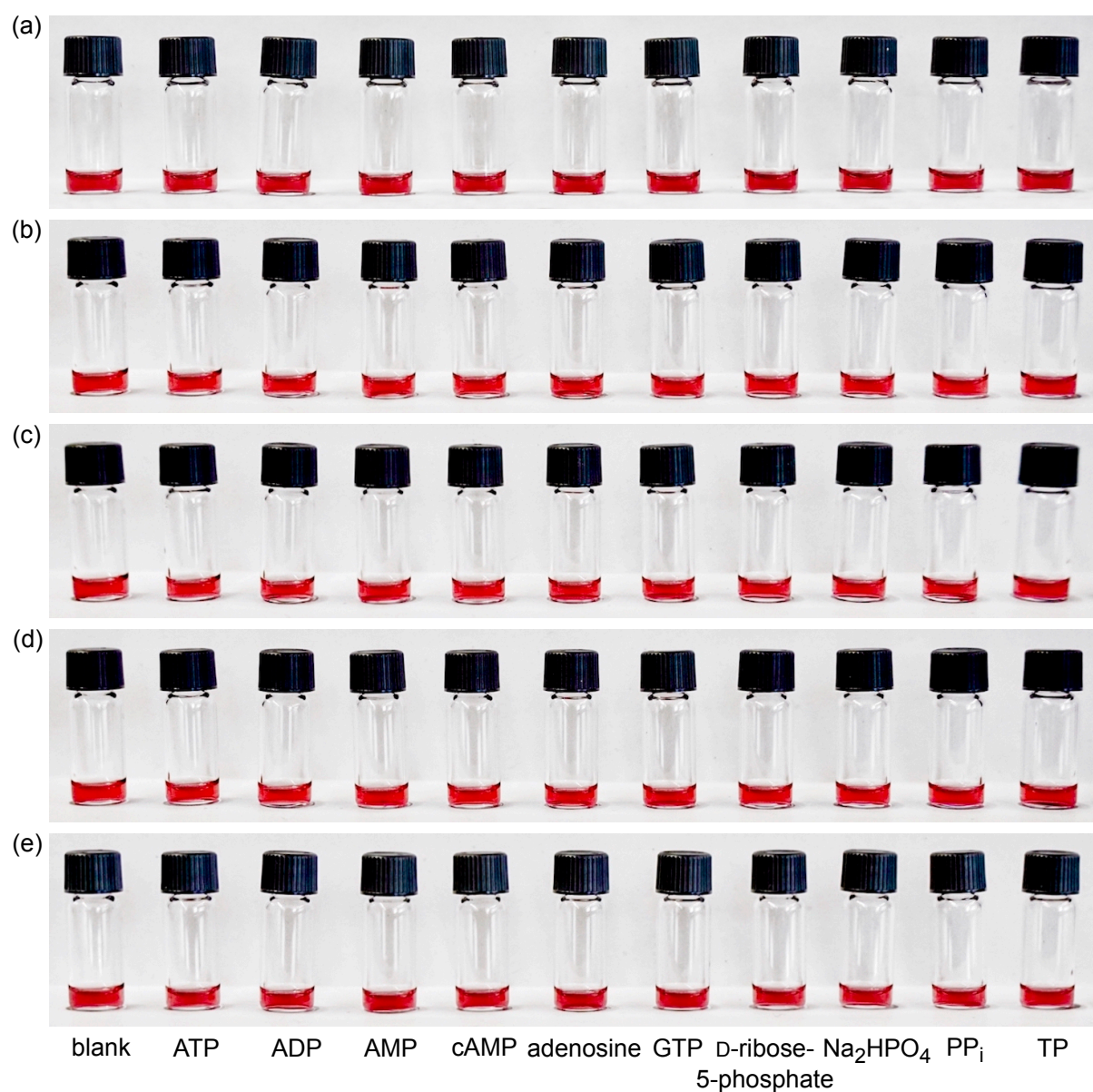


Figure S8: Images of solutions of **NP³⁰** in water/methanol, 1:2 (v/v) containing 500 μM of $\text{Zn}(\text{NO}_3)_2$ after the addition of the analytes specified in the bottom row at concentrations of 5 μM (a), 24 μM (b), 48 μM (c), 91 μM (d), and 167 μM (e).

UV-vis Spectroscopic Binding Study. The nanoparticle stock solutions were prepared as described on page 6. For each analyte, four stock solutions with concentrations of 0.1 mM (salt solution #1), 1 mM (salt solution #2), 10 mM (salt solution #3), and 100 mM (salt solution #4) were prepared in water. Each series of measurements was performed in one cuvette. The first measurement involved adding a nanoparticle solution (1 mL) to the cuvette and recording the UV-vis spectrum between 350 and 800 nm. The following measurements involved adding defined volumes of the stock solutions of an analyte in a given sequence. After each addition, the cuvette was shaken and the UV-vis spectrum recorded after 10 min. The exact amounts of the salt solutions and the sequence of their addition are summarised in Table S3. The obtained spectra are shown in Figure S9 and Figure S10.

Table S3: Amounts of salt solutions and sequence of addition to the nanoparticle solution in water/methanol, 1:2 (v/v) and concentrations resulting after each addition.

entry	# stock solution	$c(\text{stock solution}) / \text{mM}$	$V(\text{salt solution}) / \mu\text{L}$	$c(\text{salt}) \text{ in sample} / \mu\text{M}$
1	-	-	0	0
2	1	0.1	1	0.1
3	2	1	1	1
4	2	1	4	5
5	2	1	5	10
6	2	1	5	15
7	2	1	5	20
8	2	1	5	24
9	3	10	2.5	49
10	3	10	2.5	73
11	3	10	2.5	97
12	4	100	1	193
13	4	100	1	290
14	4	100	1	386
15	4	100	1	482
16	4	100	5	959

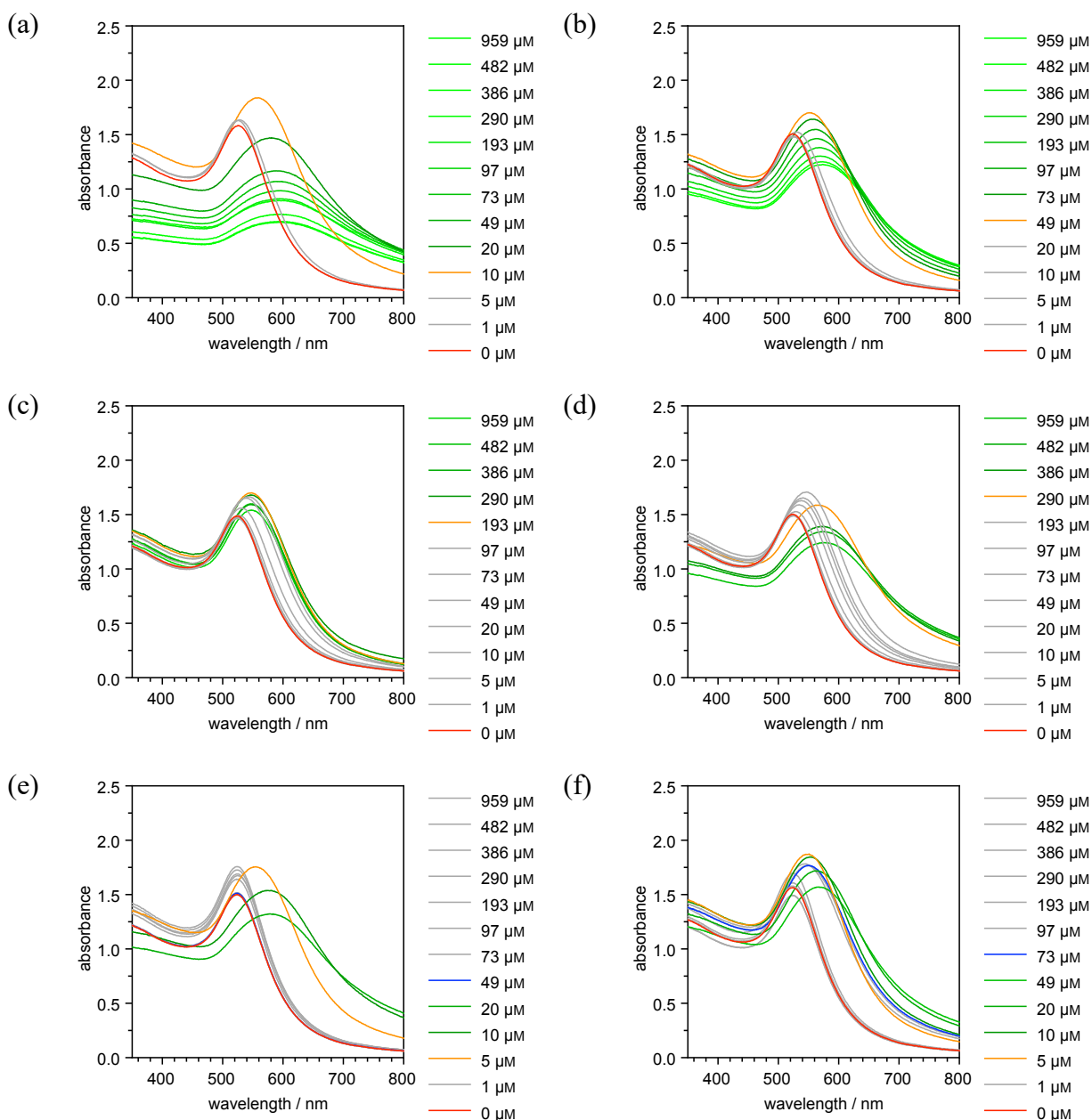


Figure S9: Selection of UV-vis spectra of **NP⁸** in water/methanol, 1:2 (v/v) containing 5 μM of $\text{Zn}(\text{NO}_3)_2$ (in the initial solution) and between 0 and 959 μM of ATP (a), ADP (b), AMP (c), GTP (d), $\text{Na}_4\text{P}_2\text{O}_7$ (PP_i) (e), and $\text{Na}_5\text{P}_3\text{O}_{10}$ (TP) (f). The first spectrum is shown in red and the spectrum corresponding to the concentration at which a pronounced red shift and intensity increase of the SPR band occurred in orange. In the spectra of the titrations with the inorganic anions, the spectrum relating to the analyte concentration at which redissolution of the nanoparticles occurred is shown in blue.

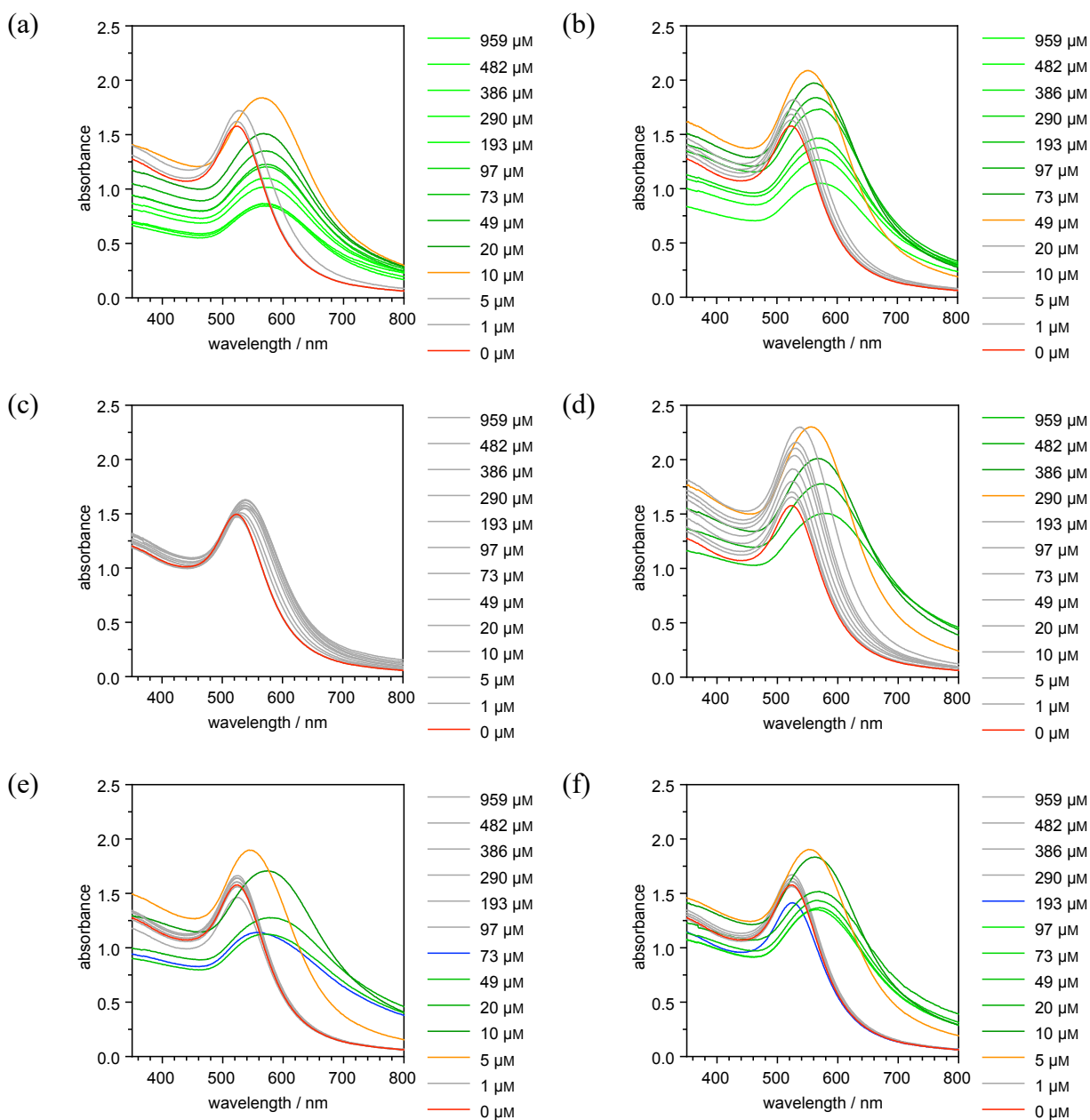


Figure S10: Selection of UV-vis spectra of NP^8 in water/methanol, 1:2 (v/v) containing $50 \mu\text{M}$ of $\text{Zn}(\text{NO}_3)_2$ (in the initial solution) and between 0 and $959 \mu\text{M}$ of ATP (a), ADP (b), AMP (c), GTP (d), $\text{Na}_4\text{P}_2\text{O}_7$ (PP_i) (e), and $\text{Na}_5\text{P}_3\text{O}_{10}$ (TP) (f). The first spectrum is shown in red and the spectrum corresponding to the concentration at which a pronounced red shift and intensity increase of the SPR band occurred in orange. In the spectra of the titrations with the inorganic anions, the spectrum relating to the analyte concentration at which redissolution of the nanoparticles occurred is shown in blue.

Time-Dependent UV-Vis Spectroscopic Measurement. The **NP⁸** stock solution with a 50 μM $\text{Zn}(\text{NO}_3)_2$ concentration (1 mL) (see page 6) was treated with an aqueous ATP stock solution (25 μL , 1 mM) and the UV/vis spectra of the resulting mixture were recorded between 350 and 800 nm after 5, 15, 30, 45, 60, 120, 180, and 240 min. The same experiment was repeated by using an aqueous $\text{Na}_4\text{P}_2\text{O}_7$ (25 μL , 1 mM) instead of the ATP solution. The obtained spectra are shown in Figure S11.

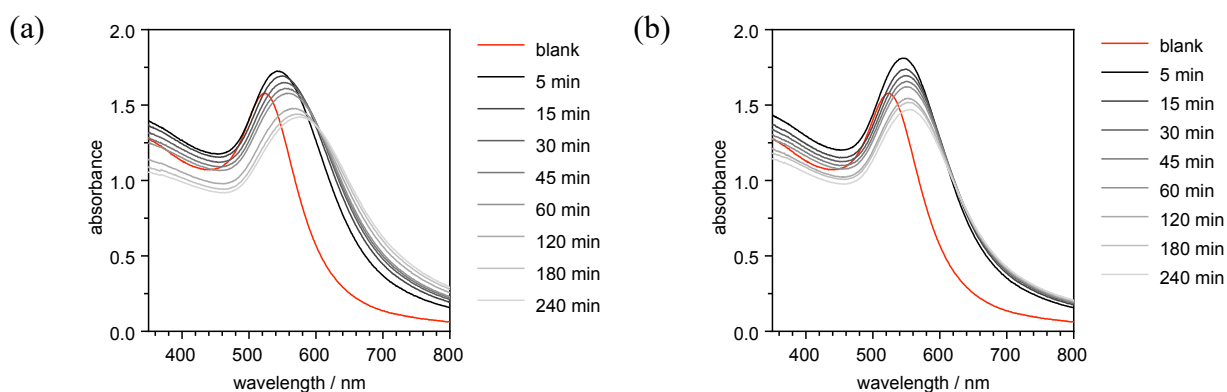


Figure S11: UV-vis spectra of **NP⁸** in water/methanol, 1:2 (*v/v*) containing 50 μM of $\text{Zn}(\text{NO}_3)_2$ (in the initial solution) and 24 μM of ATP (a) or $\text{Na}_4\text{P}_2\text{O}_7$ (b), recorded at the times specified in the legends. The red spectrum depicts the spectrum of the nanoparticle prior to the analyte addition.

NMR Spectroscopic Characterisation of Zinc Complexation. For this experiment, **NP³⁰** was used because of the higher content of surface-bound DPA units in comparison to **NP⁸**, which made it easier to follow the effects of zinc complexation and of the addition of PPI on the NMR spectrum. **NP³⁰** (7.3 mg) was dissolved in $\text{D}_2\text{O}/\text{CD}_3\text{OD}$, 1:2 (*v/v*) (600 μL) and the ^1H NMR spectrum of the resulting solution was recorded. Subsequently, a solution of $\text{Zn}(\text{NO}_3)_2$ (132 μL , 0.1 M in $\text{D}_2\text{O}/\text{CD}_3\text{OD}$, 1:2 (*v/v*)) was added, the NMR tube thoroughly shaken, and another ^1H NMR spectrum recorded after 30 min. Finally, a solution of $\text{Na}_4\text{P}_2\text{O}_7$ (264 μL , 0.1 M in D_2O) was added, the sample again shaken, and a third NMR spectrum recorded.

The three spectra are shown in Figure S12. The first spectrum contains a singlet at ca. 4.7 ppm, which belongs to the methylene protons of the uncomplexed surface-bound DPA units. In addition, the signals of the pyridyl units are visible in the aromatic region of the spectrum. The addition of $\text{Zn}(\text{NO}_3)_2$ caused changes in the aromatic region of the NMR spectrum. More importantly, the singlet of the methylene protons is no longer visible in this spectrum and replaced by two doublets at slightly

higher field. This spectral change is a clear indication for the complete conversion of the DPA units into the respective zinc complex. The associated rigidification of the DPA units rendered the CH₂ protons diastereotopic, explaining the change of the signal pattern. The addition of the diphosphate salt reduced the quality of the spectrum, likely because solvent composition also changed, causing the HDO peak to become much larger. However, no singlet is visible in the spectral region in which free CH₂ protons of free DPA units absorb. By contrast, the two doublets of the zinc(II)-DPA complex are still observable, indicating that surface-bound zinc(II)-DPA units were still present even in the presence of an excess of the PP_i salt.

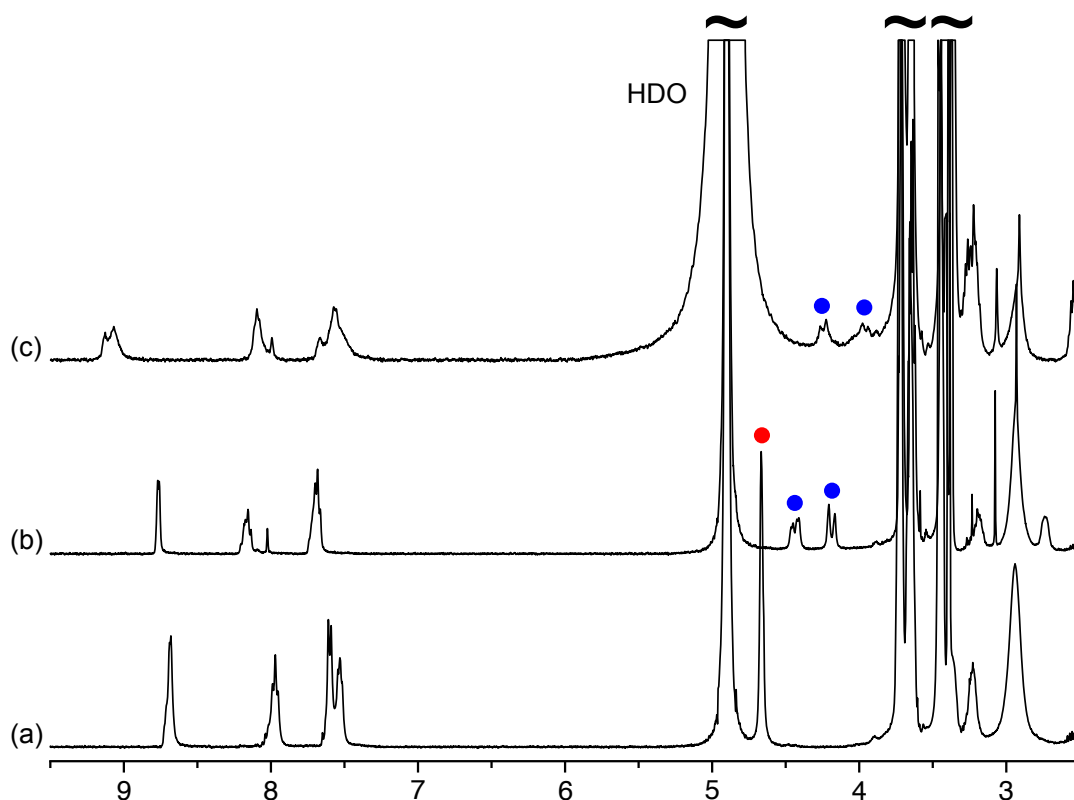


Figure S12: ¹H NMR spectra of NP³⁰ in D₂O/CD₃OD, 1:2 (v/v) (a), of NP³⁰ after the addition of an excess of Zn(NO₃)₂ to give a 18 mM concentration (b), and after further adding Na₄P₂O₇ to give a concentration of 27 mM (c). The signals of the CH₂ groups of free and of complexed DPA units are marked with red and blue dots, respectively.

Qualitative Binding Study Using Redissolved Nanoparticles. The solutions for the experiments involving redissolved nanoparticles were prepared as follows. Stock solutions of NP⁸ were prepared containing 5 or 50 μM Zn(NO₃)₂ as described on page 6. These stock solutions (2 mL) were treated with an aqueous solution of either Na₄P₂O₇ or Na₅P₃O₁₀ (6 μL, 0.1 M) to obtain solutions that were 5

or 50 μM in $\text{Zn}(\text{NO}_3)_2$ and 299 μM in the respective inorganic salt. In addition, one solution was prepared from a **NP**⁸ stock solution with a 5 μM $\text{Zn}(\text{NO}_3)_2$ concentration by adding only 1 μL of the 0.1 M aqueous $\text{Na}_4\text{P}_2\text{O}_7$ solution to afford a PPi concentration of 50 μM . The analytes [adenosine 5'-triphosphate disodium salt hydrate (ATP), adenosine 5'-diphosphate disodium salt hydrate (ADP), adenosine 5'-monophosphate disodium salt hydrate (AMP), adenosine 3',5'-cyclic monophosphate sodium salt (cAMP), guanosine 5'-triphosphate disodium salt (GTP), and guanosine 5'-monophosphate monosodium salt (GMP), and (for one experiment) adenosine] were used as 1 mM stock solutions in H_2O .

Seven vials were prepared, each containing 200 μL of a nanoparticle stock solution (**NP**⁸ in the presence of 5 or 50 μM of $\text{Zn}(\text{NO}_3)_2$ and 299 μM of $\text{Na}_4\text{P}_2\text{O}_7$ or $\text{Na}_5\text{P}_3\text{O}_{10}$, and **NP**⁸ in the presence of 5 μM of $\text{Zn}(\text{NO}_3)_2$ and 50 μM of $\text{Na}_4\text{P}_2\text{O}_7$). One vial was used as blank while each of the other vials was treated with a specific stock solution of the analytes. Initially, 1 μL of the respective analyte solution was added to each vial. The subsequent additions comprised 4 μL , 5 μL , 10 μL , and 80 μL . The salt concentrations thus increased from 5 μM after the first addition over 24 μM , 48 μM , 91 μM , to 333 μM after the last one. Photographs of the vials were taken 10 min after each addition. The pictures obtained for a 50 μM $\text{Zn}(\text{NO}_3)_2$ concentration and a 299 μM $\text{Na}_5\text{P}_3\text{O}_{10}$ concentration are shown in Figure 5 of the main article. Figure S13 shows the pictures of the corresponding measurement at 299 μM $\text{Na}_4\text{P}_2\text{O}_7$. The results of the measurements obtained for 5 μM $\text{Zn}(\text{NO}_3)_2$ and $\text{Na}_4\text{P}_2\text{O}_7$ (50 μM and 299 μM) and $\text{Na}_5\text{P}_3\text{O}_{10}$ (299 μM) are shown in Figure S14–Figure S16.

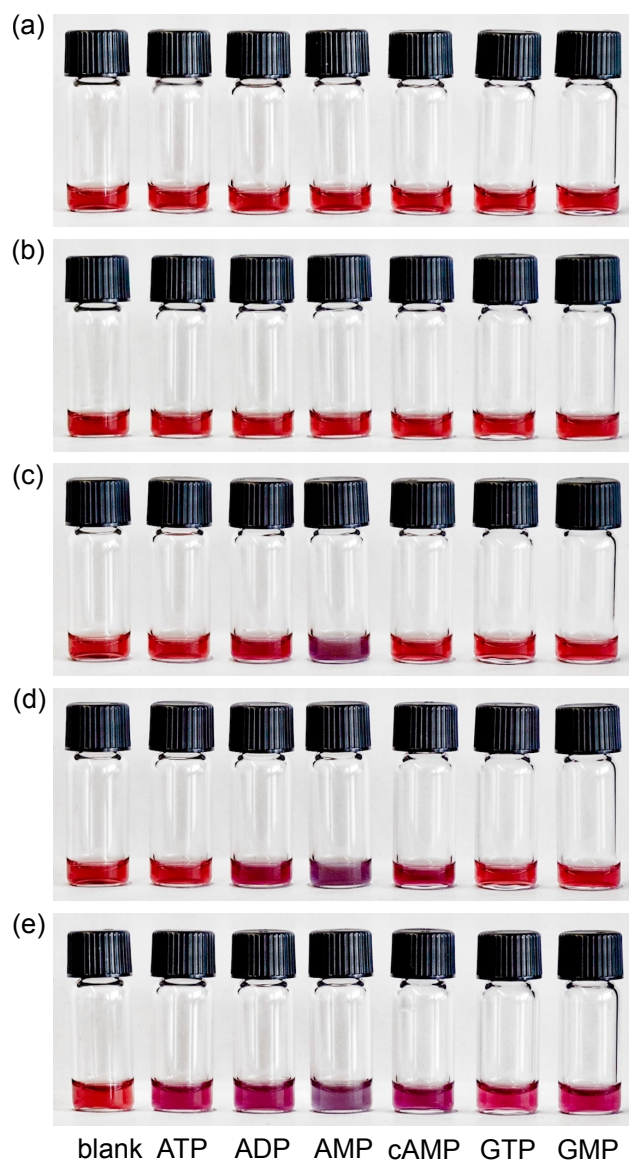


Figure S13: Images of solutions of **NP⁸** in water/methanol, 1:2 (v/v) containing 50 μM of $\text{Zn}(\text{NO}_3)_2$ and 299 μM of $\text{Na}_4\text{P}_2\text{O}_7$ after the addition of the analytes specified in the bottom row at concentrations of 5 μM (a), 24 μM (b), 48 μM (c), 91 μM (d), and 333 μM (e).

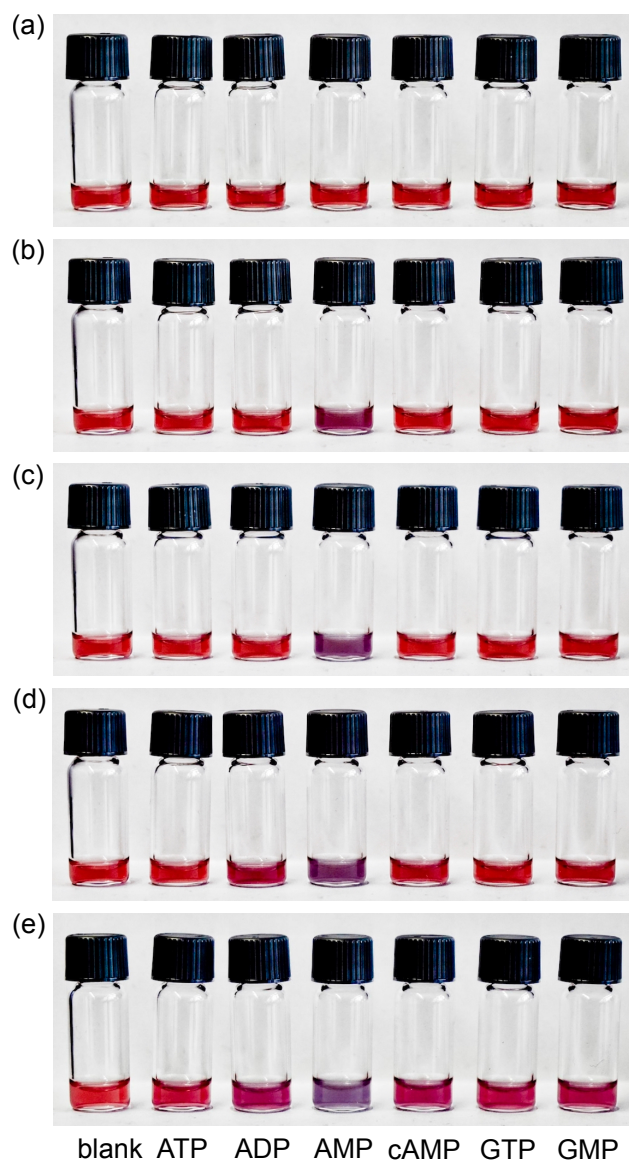


Figure S14: Images of solutions of **NP⁸** in water/methanol, 1:2 (v/v) containing 5 μM of $\text{Zn}(\text{NO}_3)_2$ and 50 μM of $\text{Na}_4\text{P}_2\text{O}_7$ after the addition of the analytes specified in the bottom row at concentrations of 5 μM (a), 24 μM (b), 48 μM (c), 91 μM (d), and 333 μM (e).

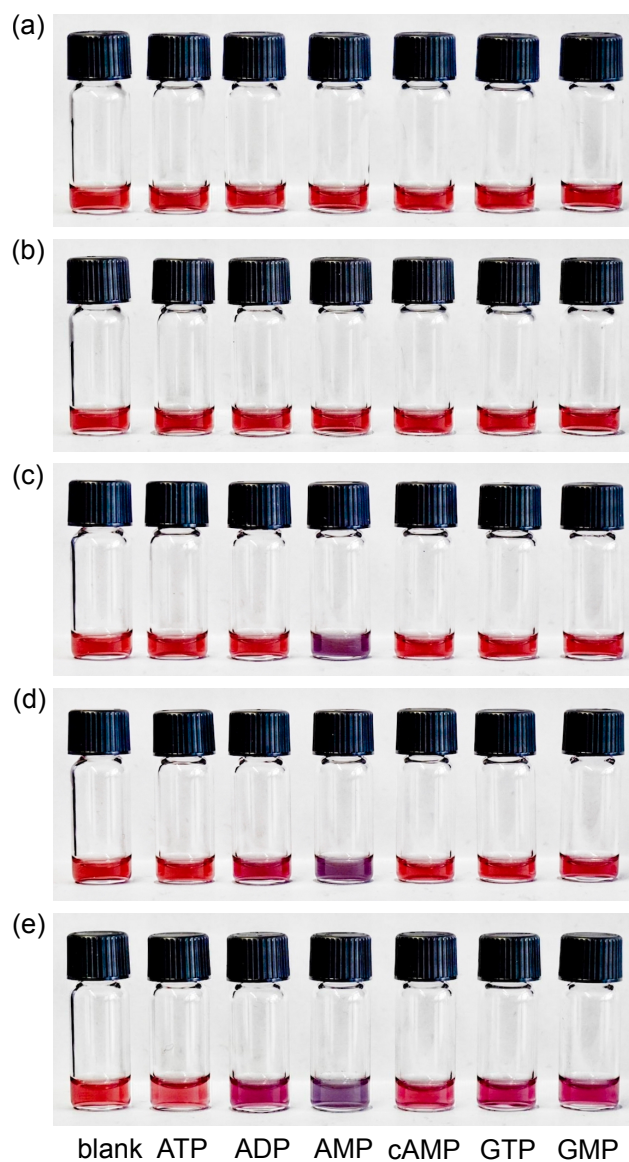


Figure S15: Images of solutions of NP⁸ in water/methanol, 1:2 (v/v) containing 5 μM of $\text{Zn}(\text{NO}_3)_2$ and 299 μM of $\text{Na}_4\text{P}_2\text{O}_7$ after the addition of the analytes specified in the bottom row at concentrations of 5 μM (a), 24 μM (b), 48 μM (c), 91 μM (d), and 333 μM (e).

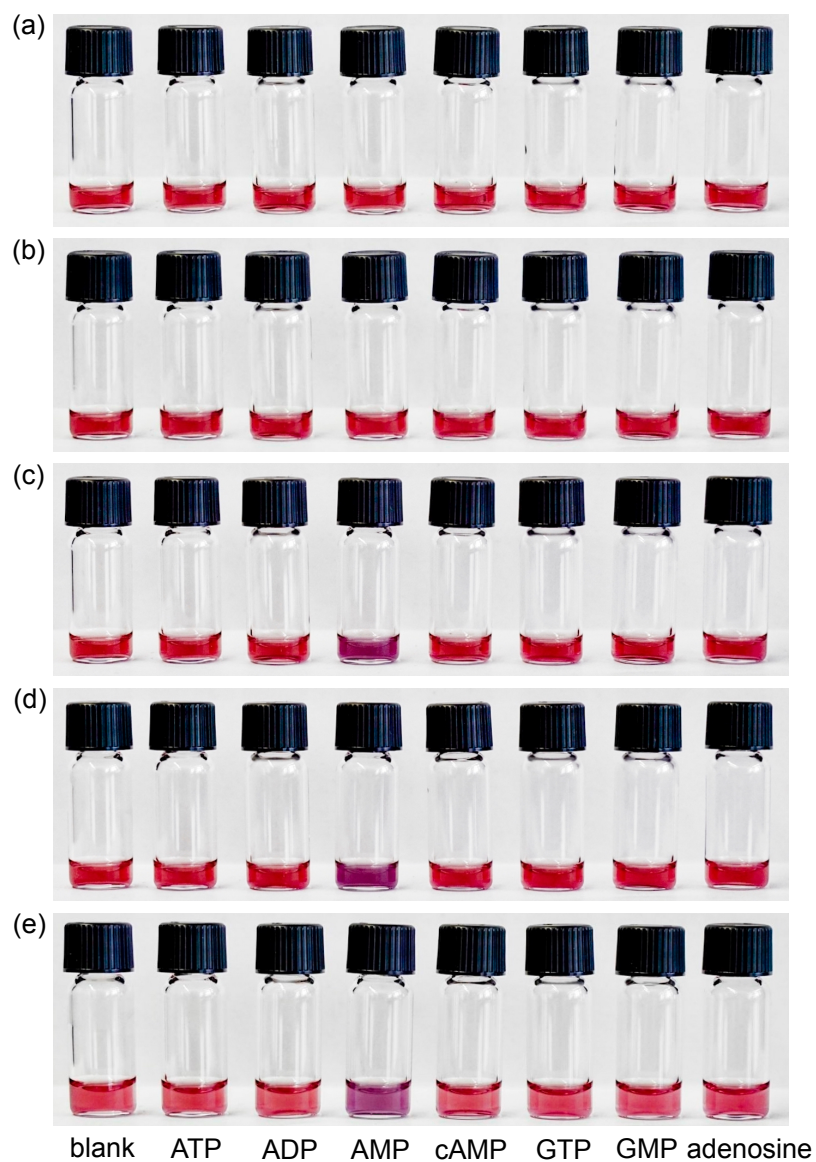


Figure S16: Images of solutions of **NP⁸** in water/methanol, 1:2 (v/v) containing 50 μM of $\text{Zn}(\text{NO}_3)_2$ and 299 μM of $\text{Na}_5\text{P}_3\text{O}_{10}$ after the addition of the analytes specified in the bottom row at concentrations of 5 μM (a), 24 μM (b), 48 μM (c), 91 μM (d), and 333 μM (e).

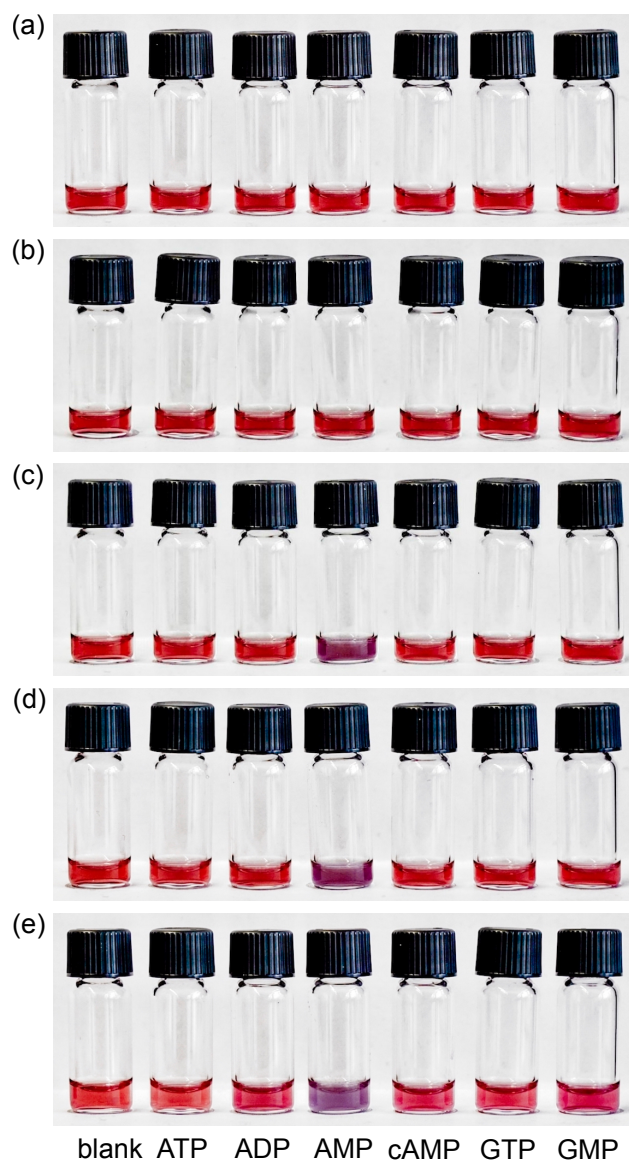


Figure S17: Images of solutions of NP^8 in water/methanol, 1:2 (v/v) containing 5 μM of $\text{Zn}(\text{NO}_3)_2$ and 299 μM of $\text{Na}_5\text{P}_3\text{O}_{10}$ after the addition of the analytes specified in the bottom row at concentrations of 5 μM (a), 24 μM (b), 48 μM (c), 91 μM (d), and 333 μM (e).

UV-Vis Spectroscopic Binding Study Using Redissolved Nanoparticles. The nanoparticle stock solutions were prepared as described above for the qualitative binding studies. For each analyte, three stock solutions with concentrations of 1 mM (salt solution #1), 10 mM (salt solution #2), and 100 mM (salt solution #3) were prepared in water. Each series of measurements was performed in one cuvette. The first measurement involved adding a nanoparticle solution (1 mL) to the cuvette and recording the UV-vis spectrum between 350 and 800 nm. The following measurements involved adding defined volumes of the stock solutions of an analyte in a given sequence. After each addition, the cuvette was shaken and the UV-vis spectrum recorded after 10 min. The exact amounts of the salt solutions and the sequence of their addition are summarised in Table S4. The obtained spectra are shown in Figure S18–Figure S22.

Table S4: Amounts of salt solutions and sequence of addition to the nanoparticle solution in water/methanol, 1:2 (v/v) and concentrations resulting after each addition.

entry	# stock solution	$c(\text{stock solution}) / \text{mM}$	$V(\text{salt solution}) / \mu\text{L}$	$c(\text{salt}) \text{ in sample} / \mu\text{M}$
1	-	-	0	0
2	1	1	1	1
3	1	1	4	5
4	1	1	5	10
5	2	10	1.5	25
6	2	10	2.5	49
7	2	10	2.5	74
8	2	10	2.5	98
9	3	100	1	196
10	3	100	1	294
11	3	100	1	391
12	3	100	1	489
13	3	100	5	973

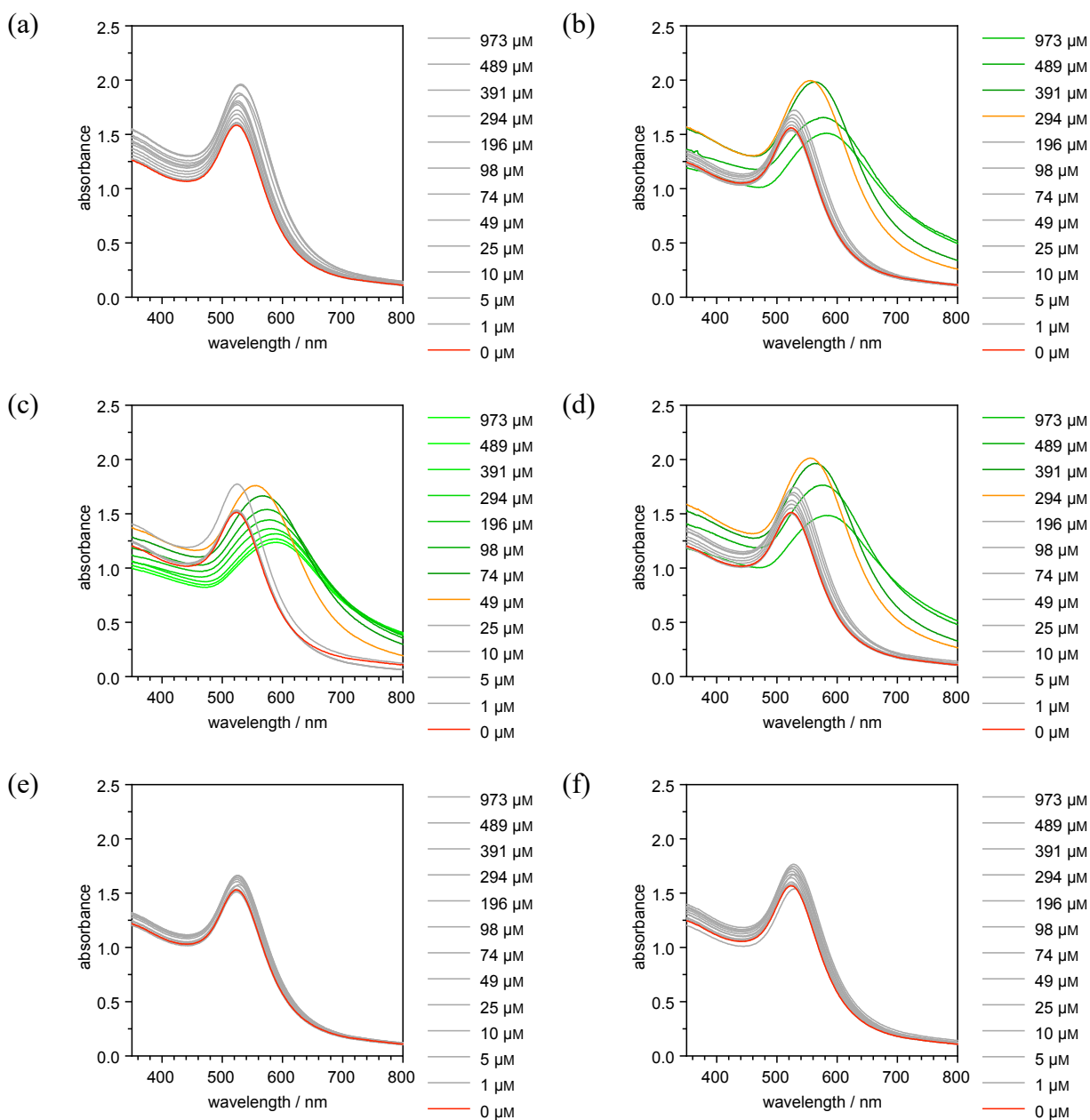


Figure S18: UV-vis spectra of NP⁸ in water/methanol, 1:2 (v/v) containing 5 μM of Zn(NO₃)₂ and 299 μM of Na₅P₃O₁₀ (in the initial solution) and between 0 and 977 μM of ATP (a), ADP (b), AMP (c), cAMP (d), GTP (e), and GMP (f). The first spectrum is shown in red and the spectrum corresponding to the concentration at which a pronounced red shift and intensity increase of the SPR band occurred in orange.

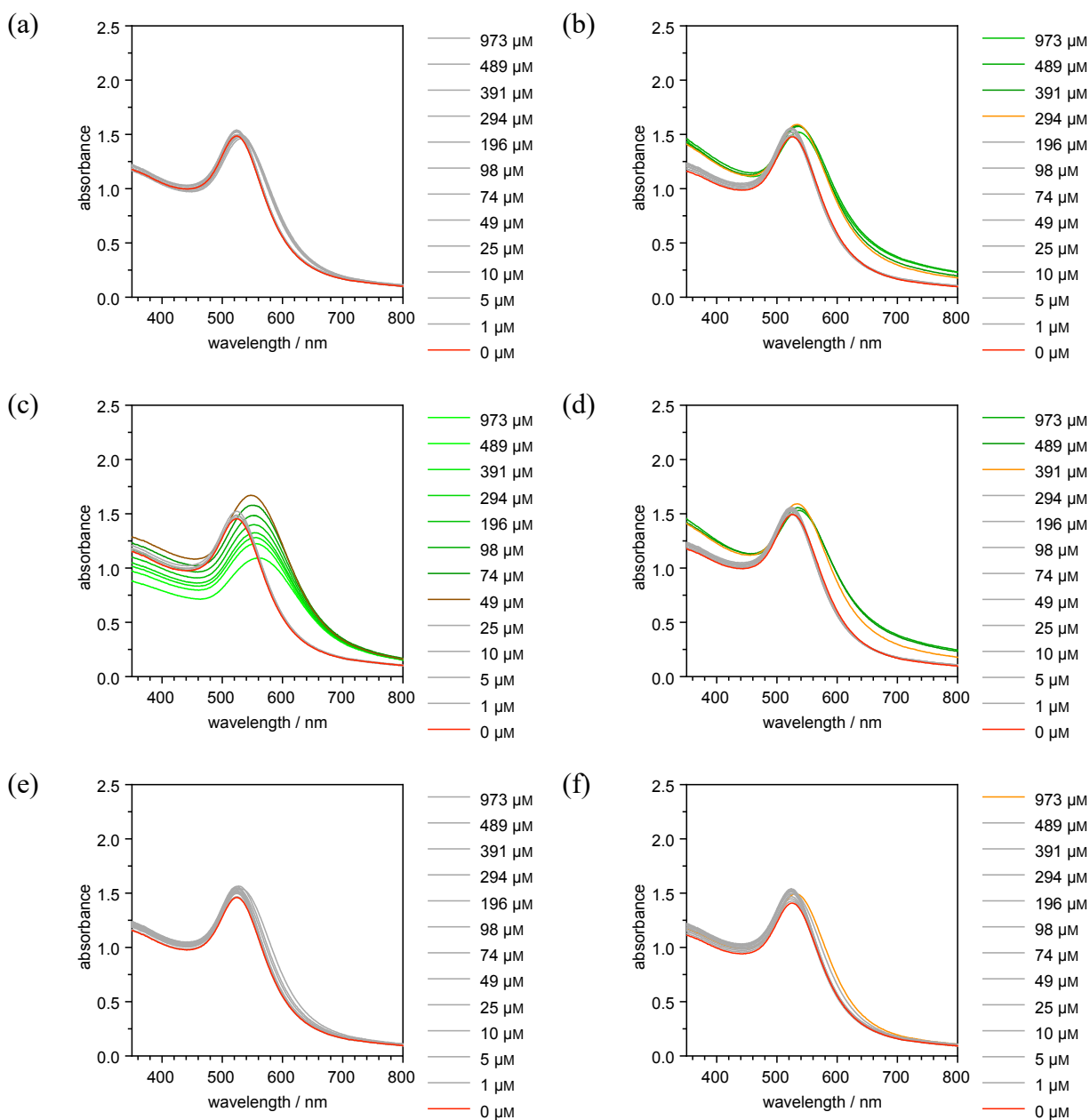


Figure S19: UV-vis spectra of NP⁸ in water/methanol, 1:2 (v/v) containing 50 μM of Zn(NO₃)₂ and 299 μM of Na₅P₃O₁₀ (in the initial solution) and between 0 and 977 μM of ATP (a), ADP (b), AMP (c), cAMP (d), GTP (e), and GMP (f). The first spectrum is shown in red and the spectrum corresponding to the concentration at which a pronounced red shift and intensity increase of the SPR band occurred in orange.

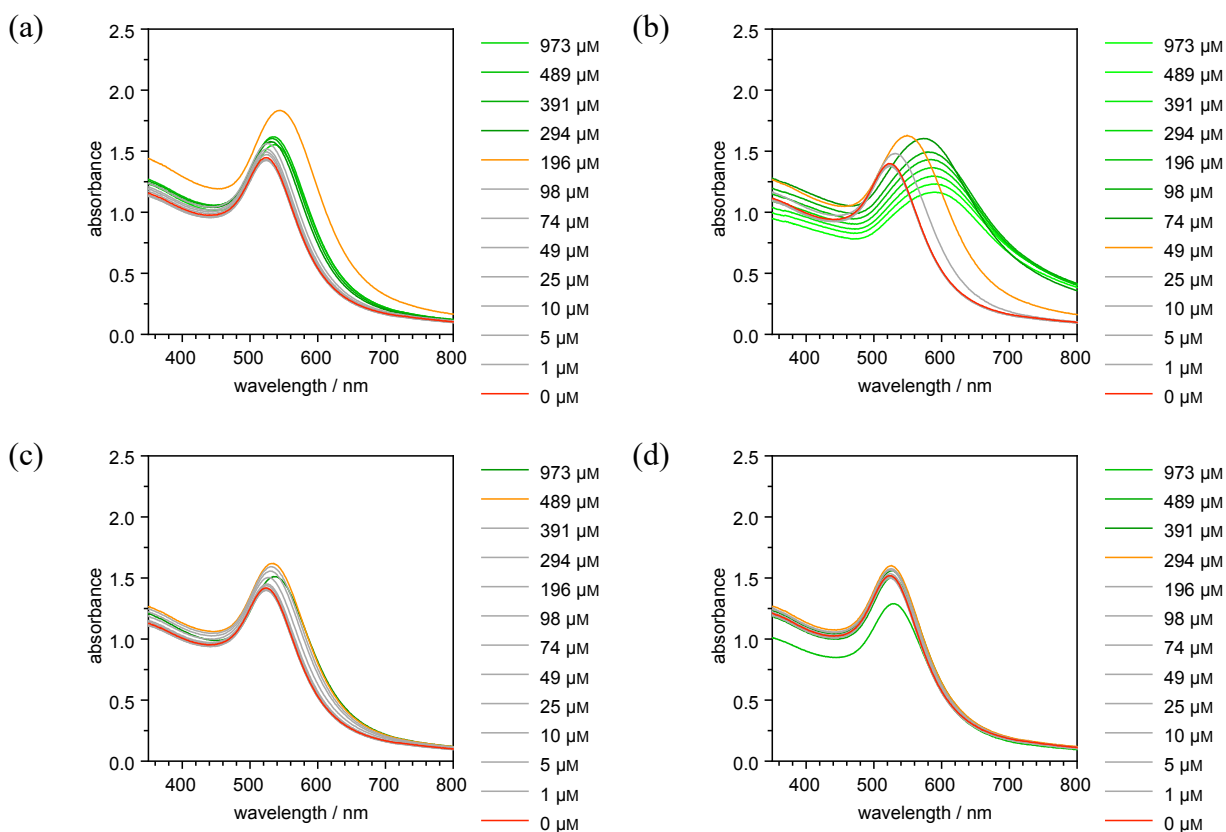


Figure S20: UV-vis spectra of NP^8 in water/methanol, 1:2 (v/v) containing $5 \mu\text{M}$ of $\text{Zn}(\text{NO}_3)_2$ and $299 \mu\text{M}$ of $\text{Na}_4\text{P}_2\text{O}_7$ (in the initial solution) and between 0 and $977 \mu\text{M}$ of ADP (a), AMP (b), cAMP (c), and GMP (d). The first spectrum is shown in red and the spectrum corresponding to the concentration at which a pronounced red shift and intensity increase of the SPR band occurred in orange.

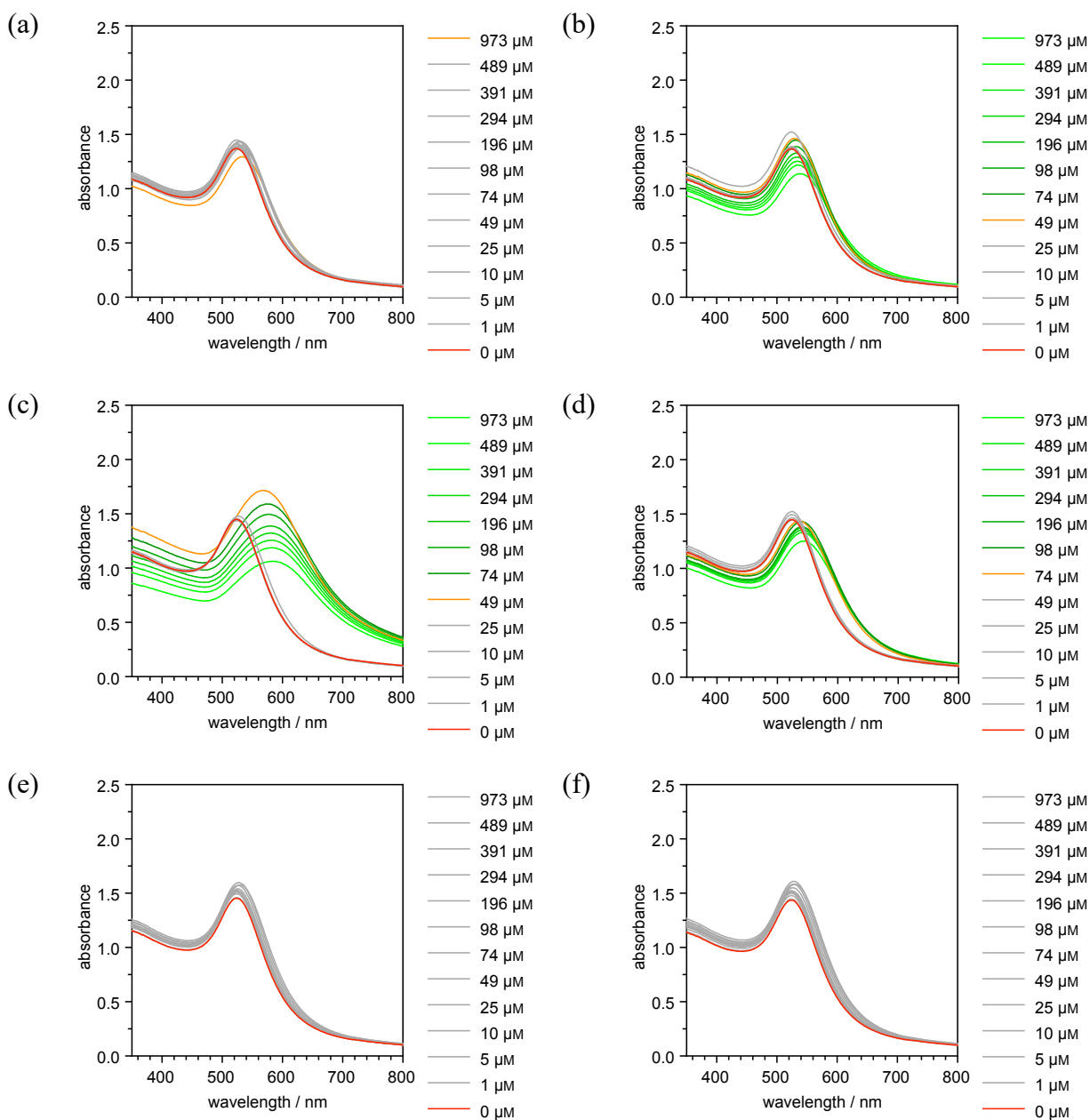


Figure S21: UV-vis spectra of NP⁸ in water/methanol, 1:2 (v/v) containing 50 μM of Zn(NO₃)₂ and 299 μM of Na₄P₂O₇ (in the initial solution) and between 0 and 977 μM of ATP (a), ADP (b), AMP (c), cAMP (d), GTP (e), and GMP (f). The first spectrum is shown in red and the spectrum corresponding to the concentration at which a pronounced red shift and intensity increase of the SPR band occurred in orange.

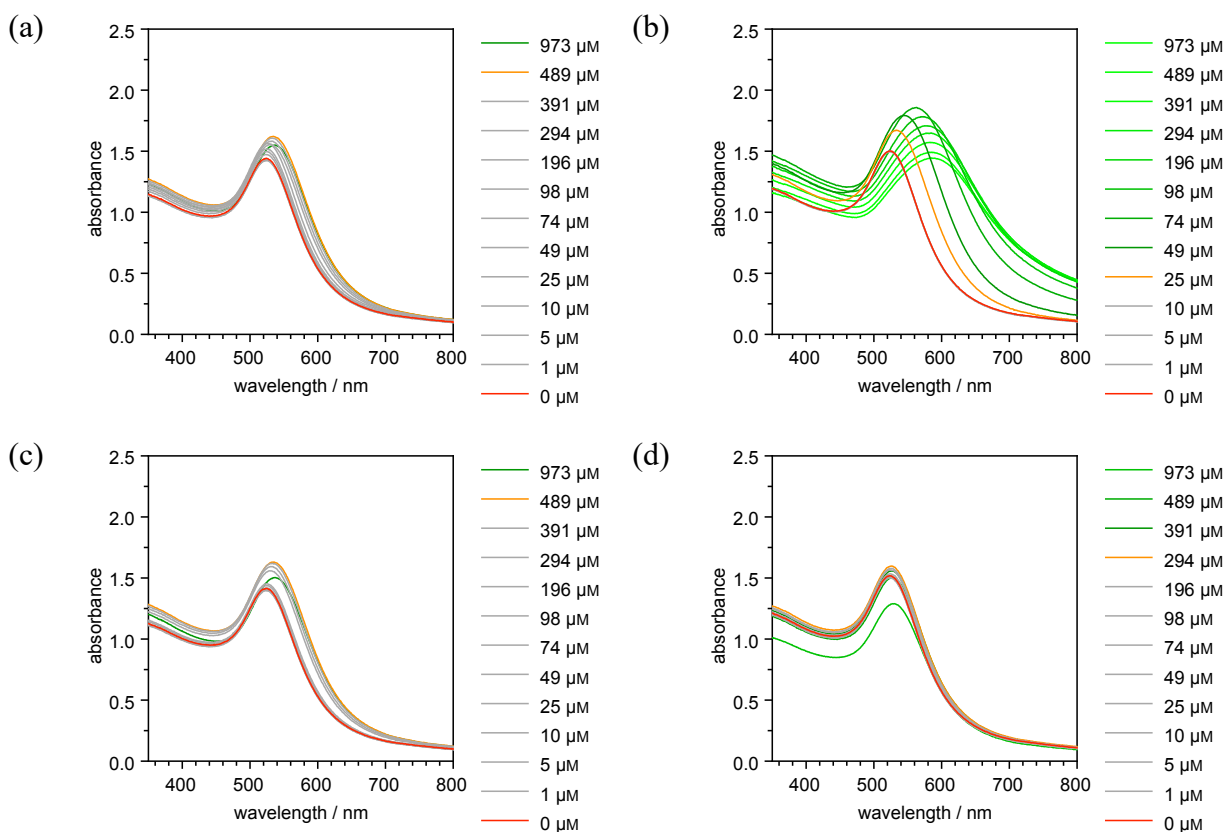


Figure S22: UV-vis spectra of **NP⁸** in water/methanol, 1:2 (v/v) containing 5 μM of $\text{Zn}(\text{NO}_3)_2$ and 50 μM of $\text{Na}_4\text{P}_2\text{O}_7$ (in the initial solution) and between 0 and 977 μM of ADP (a), AMP (b), cAMP (c), and GMP (d). The first spectrum is shown in red and the spectrum corresponding to the concentration at which a pronounced red shift and intensity increase of the SPR band occurred in orange.

Competitive Binding Studies. A stock solution of **NP⁸** that was 50 μM in $\text{Zn}(\text{NO}_3)_2$ and 299 μM in $\text{Na}_5\text{P}_3\text{O}_{10}$ was prepared as described above for the qualitative binding studies. In addition, aqueous stock solutions of NaCl (10 mM), Na_2SO_4 (10 mM), Na_2HPO_4 (10 mM), Na_2CO_3 (10 mM), sodium acetate (10 mM), sodium citrate (10 mM), ATP (10 mM), GTP (10 mM) and AMP (10 mM) were prepared. Three vials were set up, each containing the nanoparticle stock solution (200 μL). The first solution was kept as blank. This solution was diluted with water (9 μL). To the other two vials, all but the AMP stock solutions were added (1 μL each). Finally, water was added to the second vial (1 μL) and the AMP stock solution to the third vial (1 μL) to reach the concentrations specified in entry 1 of Table S5. The concentrations of the competing analytes were subsequently increased twice by adding more of the respective stock solutions to the latter two vials as specified in entry 2 and 3 of Table S5. During these two rounds of additions, the corresponding amount of water was added to the

blank solution to ensure that the total volume was the same in all vials. Pictures were taken of the three vials 10 min after each round of addition was complete. The obtained pictures are shown in Figure S23.

Table S5: Concentrations in the vial containing all analytes after each round of addition.

Entry	added stock solution per competing analyte / μL	V_{total} / μL	concentration of each competing analyte / μM	AMP concentration / μM
1	1	209	48	48
2	4	241	207	41
3	5	281	356	36

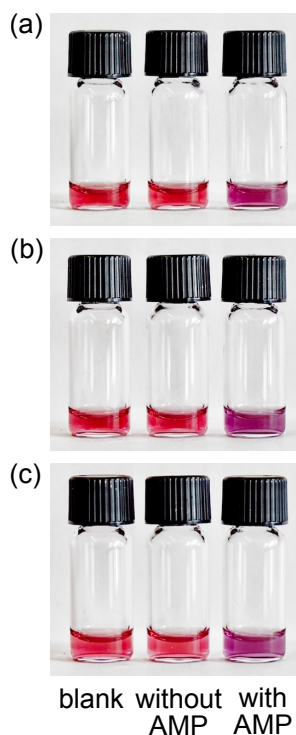
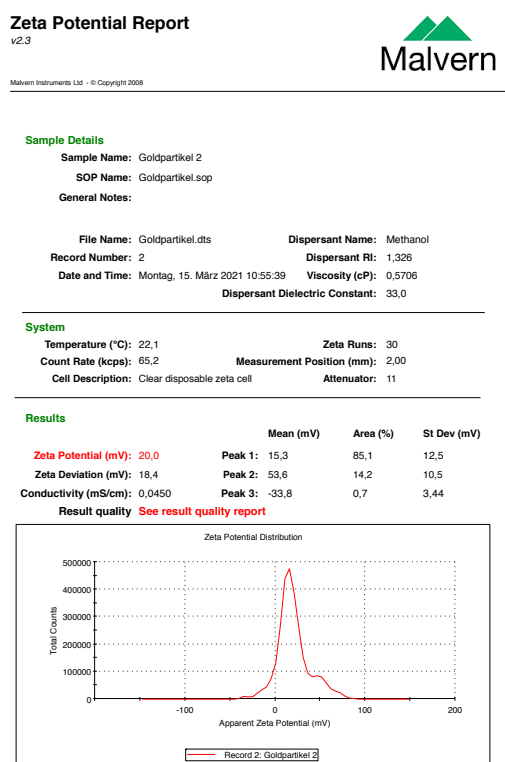


Figure S23: Images of solutions of NP^8 in water/methanol, 1:2 (v/v) containing $\text{Zn}(\text{NO}_3)_2$ ($50 \mu\text{M}$), $\text{Na}_5\text{P}_3\text{O}_{10}$ ($299 \mu\text{M}$), and NaCl , Na_2SO_4 , Na_2HPO_4 , Na_2CO_3 , sodium acetate, sodium citrate, ATP, and GTP [$48 \mu\text{M}$ each in (a), $207 \mu\text{M}$ each in (b), $356 \mu\text{M}$ each in (c)] in the centre and the right vial. The right vial additionally contains AMP [$48 \mu\text{M}$ in (a), $41 \mu\text{M}$ in (b), $36 \mu\text{M}$ in (c)]. The photos were taken 10 min after the analyte addition.

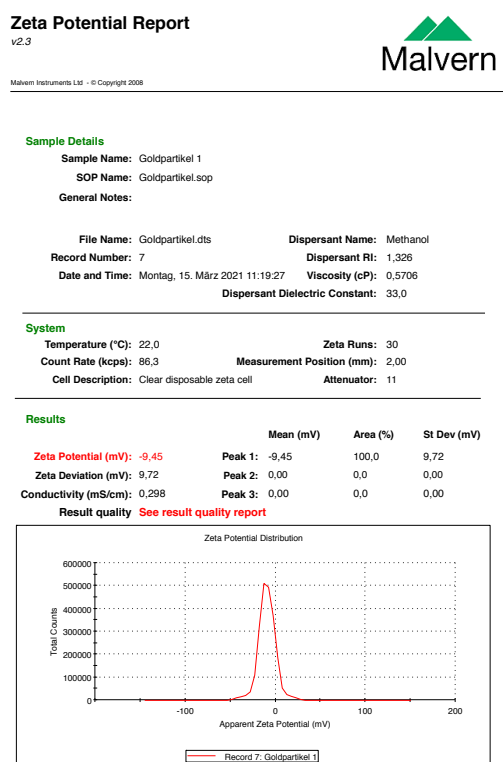
ζ-Potentials

Measurement of the ζ-Potentials. The nanoparticle concentration for these measurements was twice the concentration used for the binding studies. Accordingly, the initially obtained solution of NP⁸ in water/methanol, 1:2 (v/v) was diluted such that it had an absorbance of 3 at 525 nm. This solution (12 mL) was then treated with an aqueous Zn(NO₃)₂ stock (6 μL, 100 mM in water/methanol, 1:2 (v/v)) to obtain a zinc(II) concentration of 50 μM. The obtained solution (825 μL in a respective cuvette) was used to determine the ζ-potential of the nanoparticles after zinc(II) complexation. The nanoparticle samples were prepared by adding a stock solution of either Na₄P₂O₇ or Na₅P₃O₁₀ (25 μL, 10 mM in water) to the solution of NP⁸ containing Zn(NO₃)₂ (800 μL) to obtain salt concentrations of 303 μM. Each measurement was repeated three times, with each cycle comprising 30 subruns. Representative outputs of the measurements are shown in Figure S24 and the results are summarized in Table S6.

(a)



(b)



Transmission Electron Microscopy

ATP precipitation. The TEM images of the solutions in the absence of ATP were taken from the stock solutions of NP⁸ in water/methanol, 1:2 (v/v) that were 5 μM or 50 μM in $\text{Zn}(\text{NO}_3)_2$. Nanoparticle aggregation was induced by adding an aqueous ATP stock solution (1 mM, 1 μL) to both stock solutions (100 μL), leading to ATP concentrations of 10 μM . TEM images were recorded 5 min after the addition.

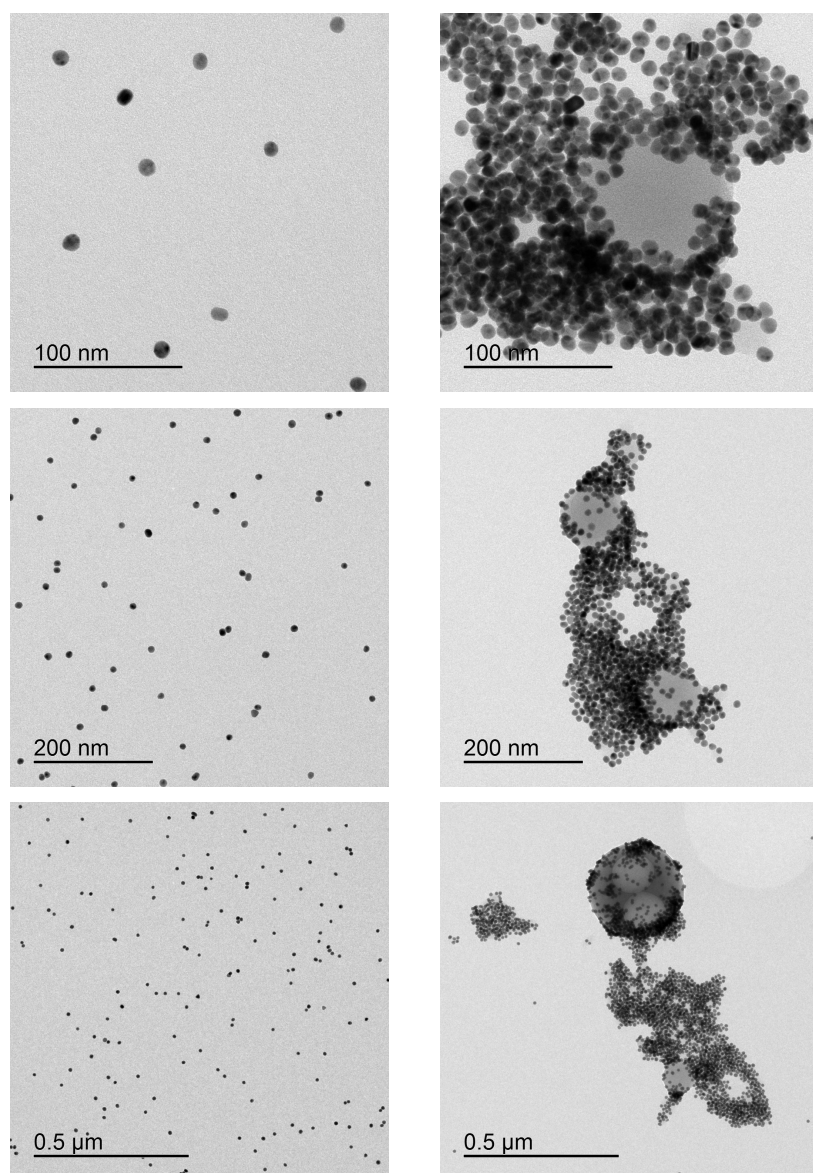


Figure S25: TEM images of NP⁸ in water/methanol, 1:2 (v/v) with a $\text{Zn}(\text{NO}_3)_2$ concentration of 5 μM in the absence (left) and the presence (right) of ATP (10 μM).

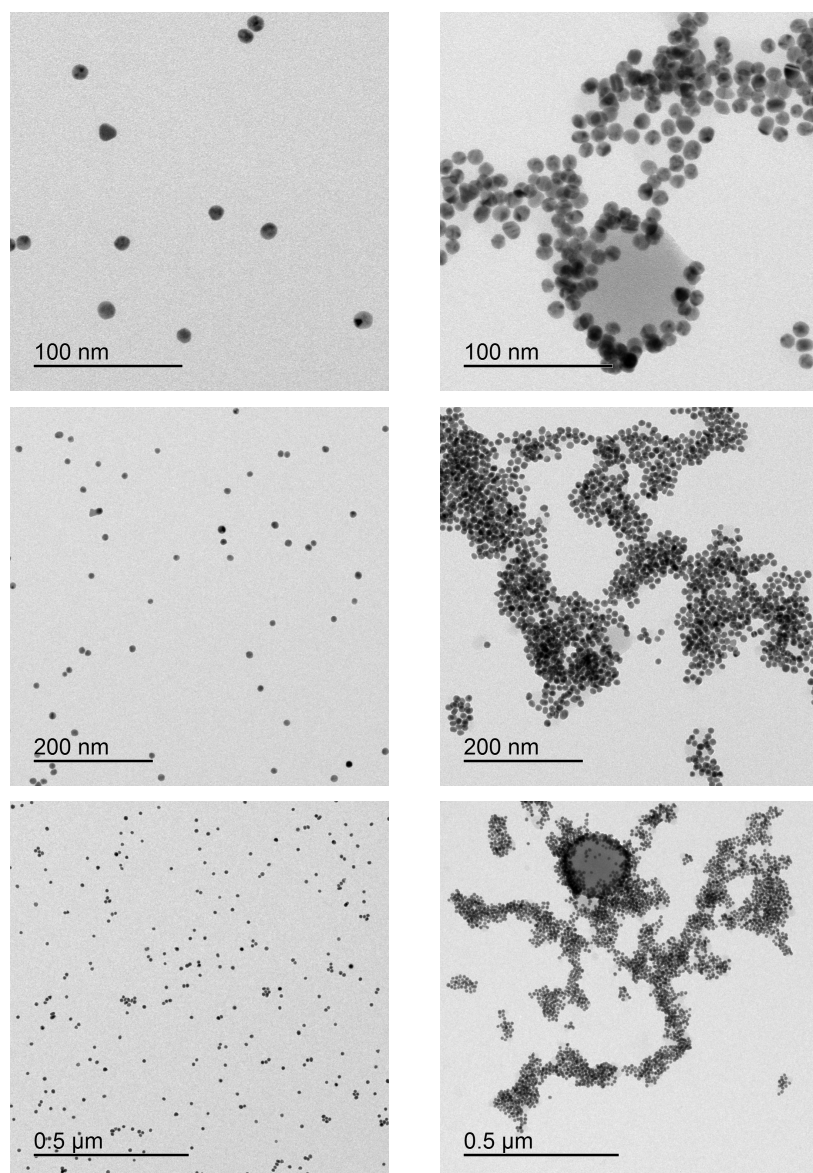


Figure S26: TEM images of **NP⁸** in water/methanol, 1:2 (v/v) with a $\text{Zn}(\text{NO}_3)_2$ concentration of 50 μM in the absence (left) and the presence (right) of ATP (10 μM).

AMP precipitation. Stock solutions of **NP⁸** in water/methanol, 1:2 (v/v) that were 5 μM or 50 μM in $\text{Zn}(\text{NO}_3)_2$ (2 mL) were treated with an aqueous solution of $\text{Na}_5\text{P}_3\text{O}_{10}$ (0.1 M, 6 μL), affording a $\text{Na}_5\text{P}_3\text{O}_{10}$ concentration of 299 μM in each solution. These solutions were used to record the TEM images prior to the AMP addition. Aliquots of the two solutions (200 μL) were then treated with an aqueous AMP solution (10 mM, 1 μL) so that each solution had an AMP concentration of 50 μM . The TEM images were recorded 5 min after the addition.

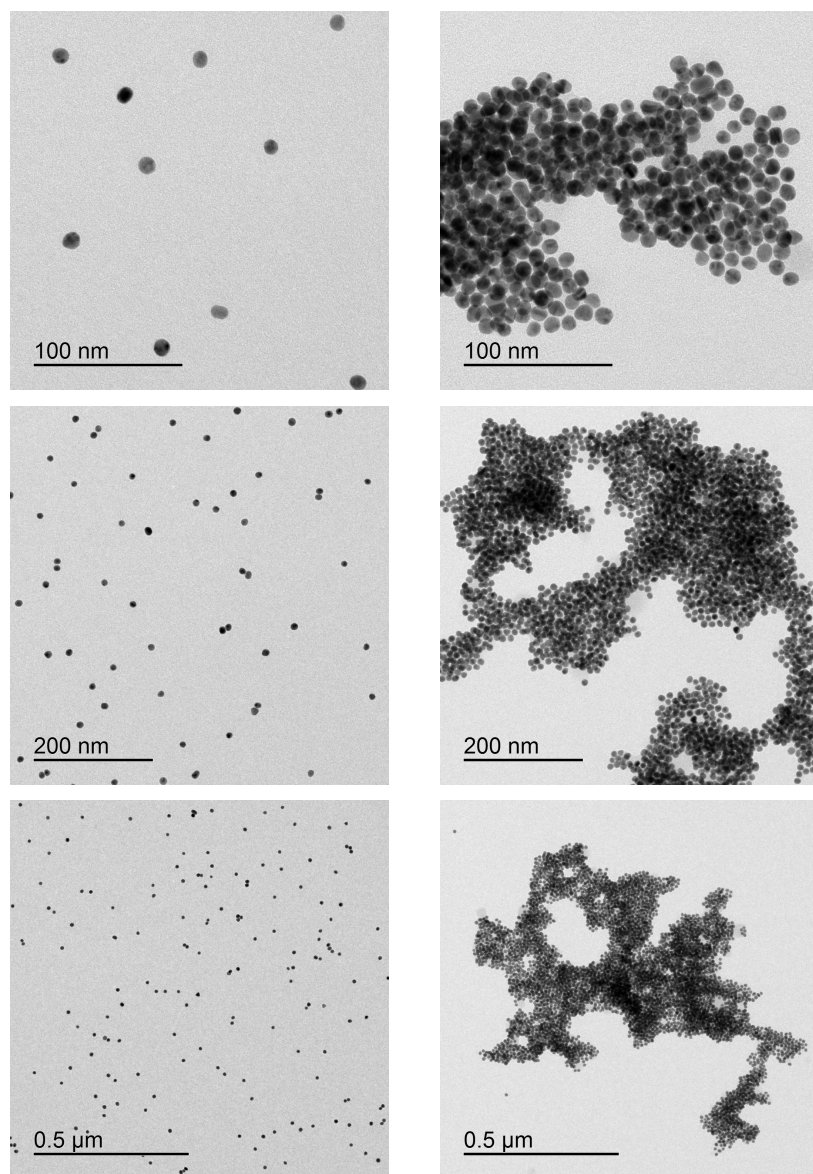


Figure S27: TEM images of **NP⁸** in water/methanol, 1:2 (v/v) with a $\text{Zn}(\text{NO}_3)_2$ concentration of $5 \mu\text{M}$ and a $\text{Na}_5\text{P}_3\text{O}_{10}$ concentration of $299 \mu\text{M}$ in the absence (left) and the presence (right) of AMP ($50 \mu\text{M}$).

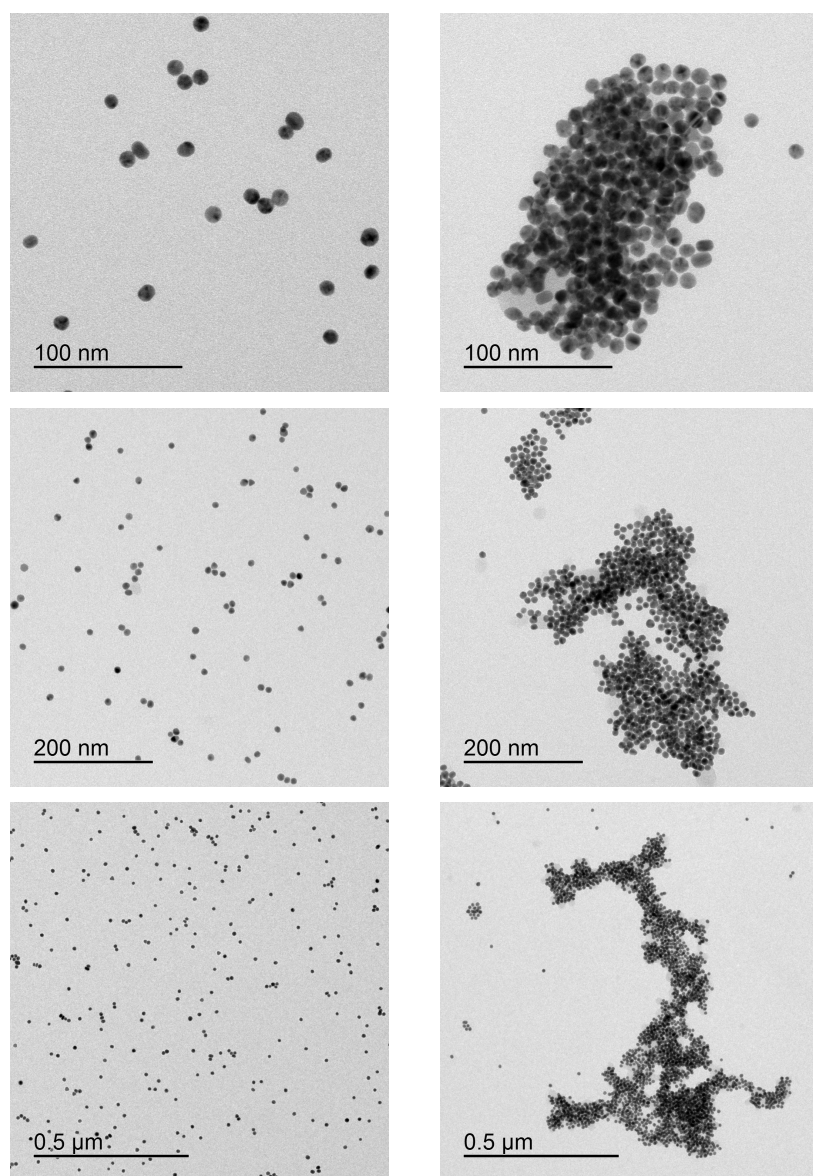


Figure S28: TEM images of NP^8 in water/methanol, 1:2 (v/v) with a $\text{Zn}(\text{NO}_3)_2$ concentration of 50 μM and a $\text{Na}_5\text{P}_3\text{O}_{10}$ concentration of 299 μM in the absence (left) and the presence (right) of AMP (50 μM).

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1. L. Reinke, J. Bartl, M. Koch and S. Kubik, *Beilstein J. Org. Chem.*, 2020, **16**, 2687–2700.
2. I. Ojea-Jiménez, N. G. Bastús and V. Puntes, *J. Phys. Chem. C*, 2011, **115**, 15752–15757.