Self-assembly and selective exchange of oligoanions on the surface of monolayer protected Au nanoparticles in water

Grégory Pieters, Alessandro Cazzolaro, Renato Bonomi, Leonard J. Prins*

Electronic Supplementary Information

Dr. G. Pieters, A. Cazzolaro, Dr. R. Bonomi, 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;
Fax: +39 049 8275829
Tel: +39 049 8275256

Table of content

1. Instrumentation

Solvents were purified by standard methods. All commercially available reagents and substrates were used as received. TLC analyses were performed using Merck 60 F254 precoated silica gel glass plates. Column chromatography was carried out on Macherey-Nagel silica gel 60 (70-230 mesh).

NMR spectra were recorded using a Bruker AC250F spectrometer operating at 250 MHz for 1H and 62.9 MHz for 13C or a Bruker AV300 operating at 300 MHz for 1H. Chemical shifts are reported in ppm using residual solvent $CDCl_3$ (7.26 ppm), CD_3OD (3.31 ppm) for calibration. ^{13}C NMR spectra, proton decoupled, were recorded at 62.5 MHz using solvent as internal reference $CDCl_3$ (77.00 ppm) or CD_3OD (49.00 ppm).

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.

TEM images were recorded on a Jeol 300 PX electron microscope.

Dynamic light scattering was performed on a Malvern Zetasizer Nano-S instrument.

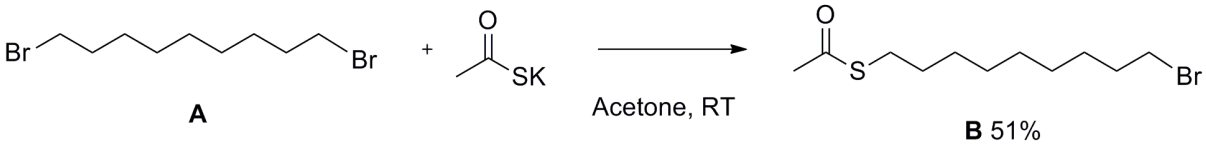
UV-Visible spectra were recorded on a Varian Cary50 Biospectrophotometer equipped with thermostatted multiple cell holders.

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

For the buffers, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Sigma) was used without further purification. **ATP_F** (2-aminopurine riboside-5-O-triphosphate) was obtained from BioLog Life Science Institute and used as received. Its concentration in the stock solution was determined by UV spectroscopy (pH 7). $Zn(NO_3)_2$ was an analytical grade product. Metal ion stock solutions were titrated against EDTA following standard procedures.

2. Synthesis and characterization of thiol **5**

S-(9-bromononyl) ethanethioate (B)

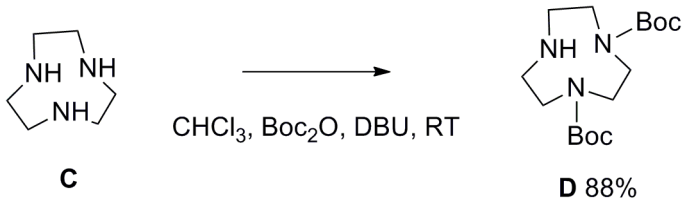


1,8-Dibromononane **A** (5.60 g, 19.6 mmol) was dissolved in acetone (50 mL). Potassium thioacetate was added (2.23 g, 19.6 mmol) and the resulting mixture was kept at room temperature under nitrogen overnight. The resulting suspension was then filtred and after solvent evaporation, the crude product was purified by flash chromatography (silica gel, eluent: EP/CH_2Cl_2: 60/40). 2.79 g (51%) of **B** were obtained as a colorless oil.

^{1}H NMR: (δ ppm, $CDCl_3$, 300K, 200 MHz): 3.38 (t, J = 6.8 Hz, 2H), 2.84 (t, J = 7.2 Hz, 2H), 2.30 (s, 3H), 1.95 – 1.69 (m, 2H), 1.56 (m, 2H), 1.53 – 1.07 (m, 10H).

^{13}C NMR: (δ ppm, $CDCl_3$, 300K, 75 MHz): 195.8, 33.8, 32.7, 30.5, 29.4, 29.1, 29.0, 28.9, 28.7, 28.6, 28.0.

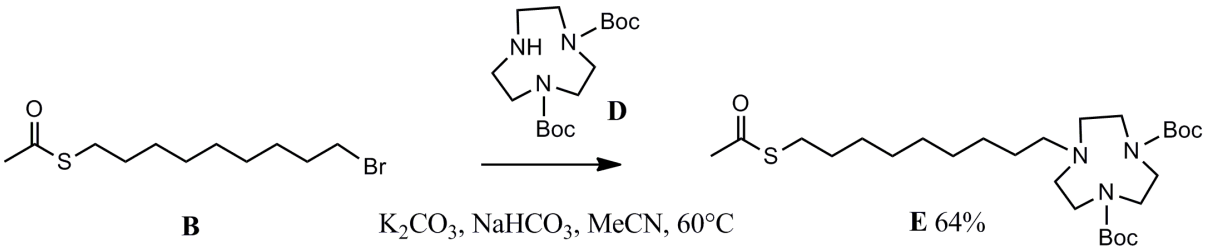
Di-tert-butyl 1,4,7-triazanonane-1,4-dicarboxylate (D)



Boc_2O (0.989 g, 4.53 mmol) was solubilized in anhydrous chloroform (10 mL) and a solution of TACN **C** (0.60 g, 2.52 mmol) and DBU (1.88 g, 12.3 mmol) in chloroform (12 mL) was slowly added with a syringe pump (0.5 mL/hour) at room temperature under nitrogen. The resulting solution was stirred for 18 hours then chloroform was added (125 mL) and the organic layer was washed with $NaHCO_3$ sat. (3x25mL). After solvent evaporation, the crude product was purified by flash chromatography (silica gel, eluent: CH_2Cl/CH_3OH: 94/6). 0.722 g (88%) of **D** were obtained as a viscous yellow oil.

^{1}H NMR: (δ ppm, $CDCl_3$, 300K, 250 MHz): 3.48 (m, 4H), 3.29 (m, 4H), 2.97 (s_{br}, 4H), 2.11 (s_{br}, 1H), 1.50 (s, 18H).
Data in accordance with the literature [*J. Med. Chem.* 2008, ***51,*** 118]

Di-tert-butyl 7-(9-(acetylthio)nonyl)-1,4,7-triazanonane-1,4-dicarboxylate (E)



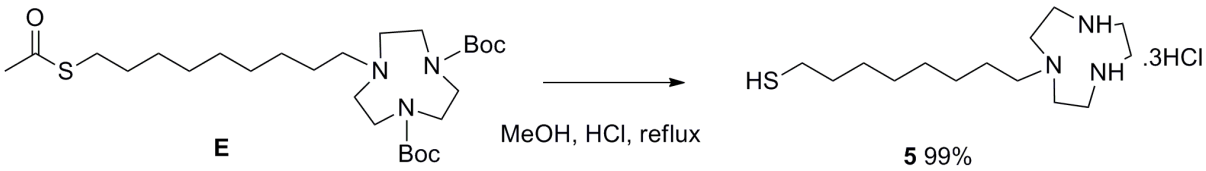
Compound **B** (0.344g, 1.224 mmol) and **D** (0.335g, 1.02 mmol) were added to a suspension of K_2CO_3 (0.418g, 3.03 mmol) and $NaHCO_3$ (0.255g, 3.03 mmol) in MeCN (10 mL). The suspension was stirred at 60°C for 3 hours the suspension was filtred under a gooch filter. After evaporation under reduced pressure the crude product was purified by flash chromatography (silica gel, eluent: CH_2Cl_2/MeOH: 97/3). 0.345 g (64%) of **E** was obtained as a slightly yellow oil.

^{1}H NMR: (δ ppm, $CDCl_3$, 300K, 250 MHz): 3.43 (m, 4H), 3.22 (m_{br}, 4H), 2.81 (t, *J = 7.2 Hz* , 2H), 2.59 (m, 4H), 2.44 (m, 2H), 2.29 (s, 3H), 1.57 (m, 2H), 1.44 (s, 18H), 1.24 (s_{br}, 12H).

^{13}C NMR: (δ ppm, $CDCl_3$, 300K, 75 MHz): 195.8, 155.6, 155.5, 155.3, 79.2, 77.2, 56.9, 56.7, 53.9, 53.5, 50.5, 50.3, 50.1, 49.9, 49.6, 30.5, 29.5, 29.4, 29.1, 29.0, 28.7, 28.5, 27.9, 27.8, 27.6, 27.4, 27.3.

ESI+MS : m/z = 530.4 [MH^+], 552.3 [MNa^+].

9-(1,4,7-triazanonan-1-yl)nonane-1-thiol (5)



Compound **E** (24 mg, 0.045 mmol) was solubilized in MeOH (2 mL) and HCl 6M (2 mL) was added. The resulting solution was stirred for 4 hours at 60°C and after evaporation of the solvant under reduced pressure 17 mg of the thiol **5** (99%) was obtained as a white solid.

^{1}H NMR: (δ ppm, $CDCl_3$, 300K, 250 MHz): 3.43 (s, 4H), 3.25 (m, 4H), 3.10 (m, 4H), 2.75 (m, 2H), 2.29 (t, *J = 7 Hz*, 2H), 1.43 (m, 4H), 1.34 (s_{br}, 12H).

^{13}C NMR: (δ ppm, $CDCl_3$, 300K, 75 MHz): 59.6, 56.6, 51.5, 46.2, 45.4, 37.1, 32.4, 32.3, 32.0, 31.3, 30.1, 27.4, 26.9.

ESI+MS : m/z = 288.3 [MH^+].

3. Characterization Au MPCs 2

3.1. NMR

Diffusion-ordered ^{1}H NMR spectra were recorded using the low eddy currents distortion bipolar gradients (LEDbp) sequence [D. H. Wu, A. D. Chen, C. S. Johnson, *J. Magn. Res. Series A* 1995, *115*, 260-264] which allows differentiation of molecules based on their diffusion coefficients. Not only does this provide unequivocal confirmation that the thiols are bound to the Au NP surface, but also provides a way to assess the purity of the samples. The obtained NMR spectra with and without the diffusion filter showed that only minimal amounts of unbound additives were present in the purified sample.

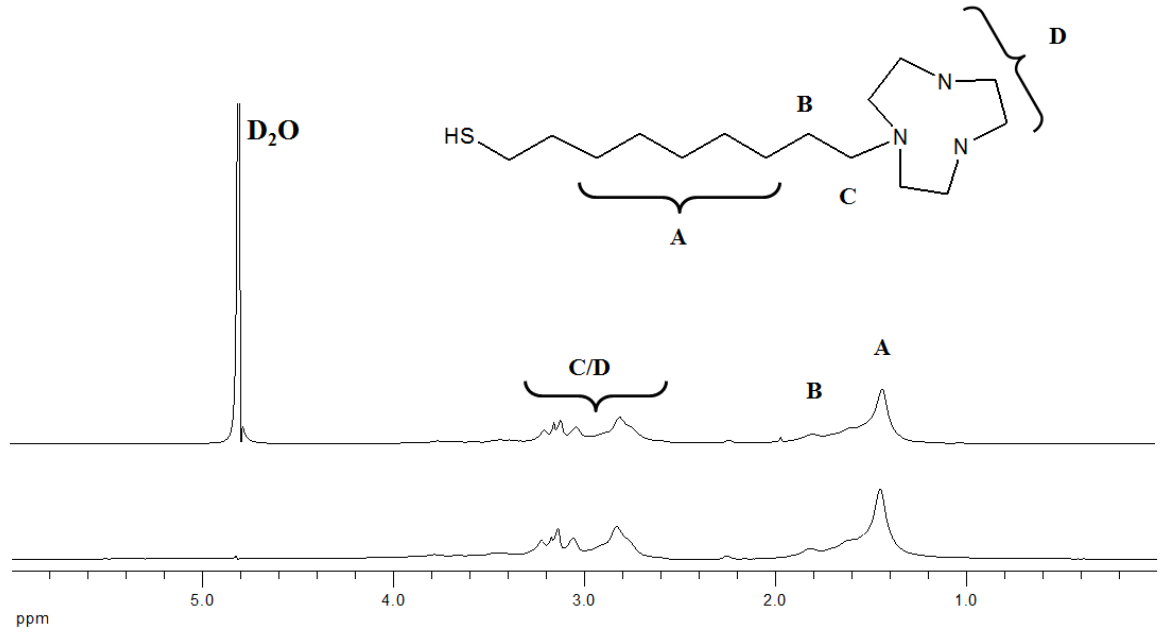


Fig. S1: ^{1}H-NMR spectra (300 MHz) of Au MPC **2**, without (above) and with (below) diffusion filter in D_2O.

3.2. TEM

The diameter of the gold nuclei in Au MPCs **2** was determined as 1.8 ± 0.5 nm by HR-TEM.

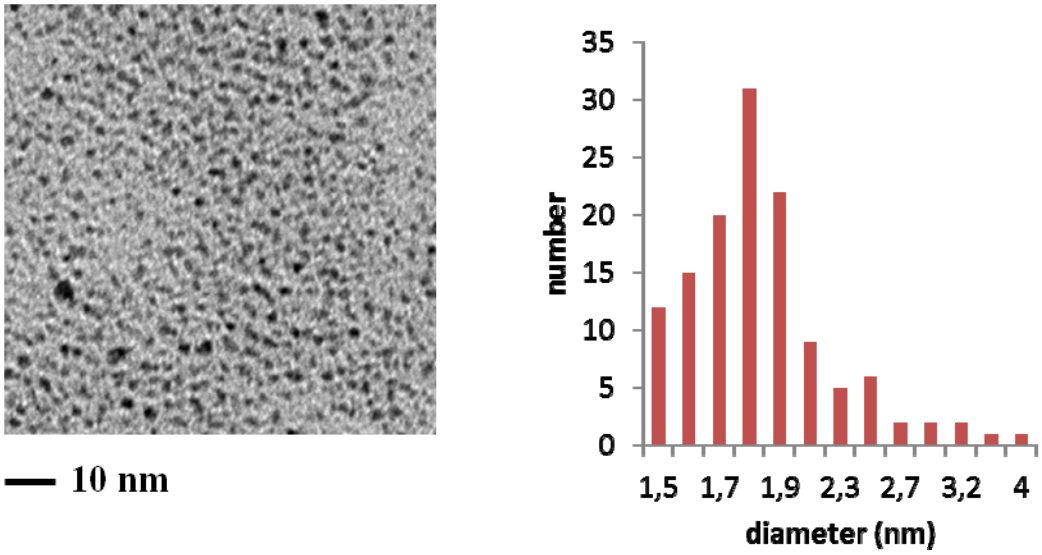


Fig. S2 and S3: TEM analysis and size distribution for Au MPC **2**.

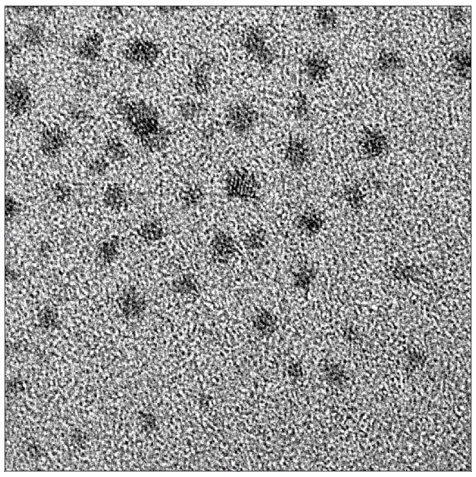


Fig. S4 HR-TEM analysis (area size is 28.21 nm x28.21 nm)

3.3. UV-Vis

The absence of the surface plasmon resonance band at 520 nm in the UV/Vis spectrum gives additional proof for the presence of sub 3 nm sized Au NPs.

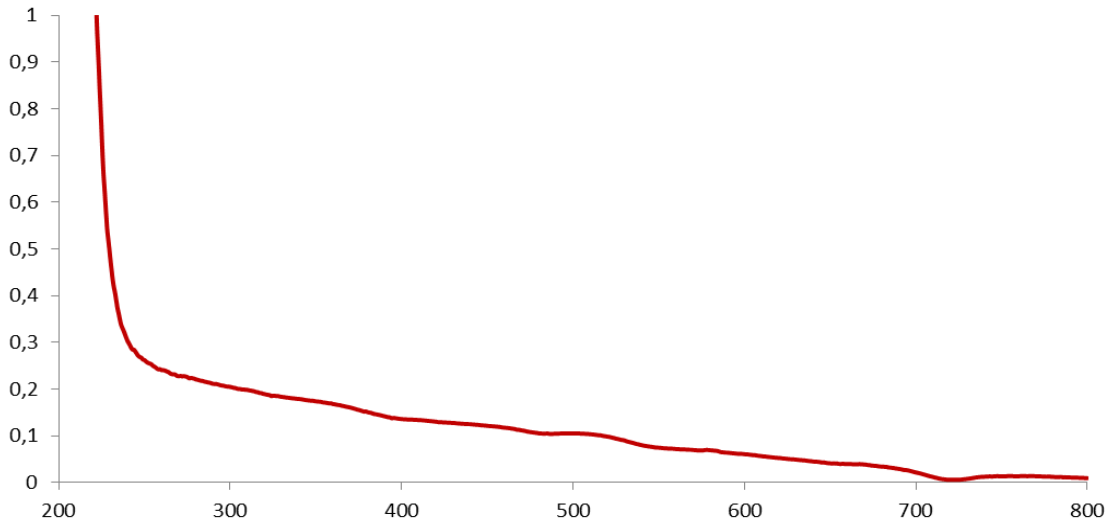


Fig. S5: UV-Vis absorption spectrum of Au MPC **2**, [TACN]=20µM, [HEPES]=10mM pH 7.

3.4. DLS

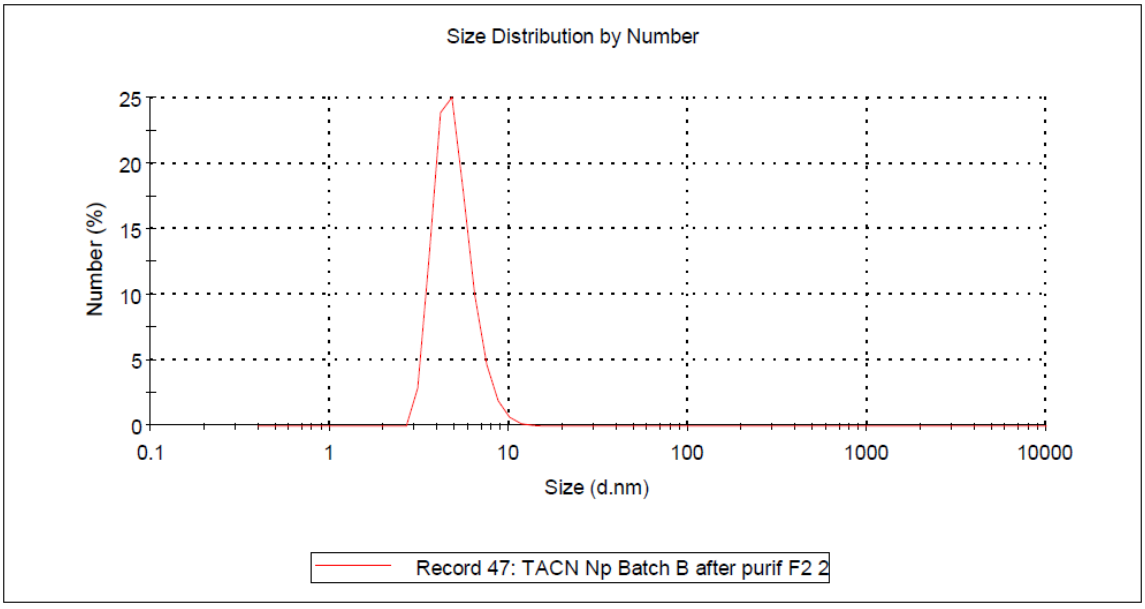


Fig. S6: DLS of nanoparticles **2** in water.

3.5. TGA

Thermogravimetric analysis provides the ratio of organic to inorganic material. For Au MPC 2, residual weight at 1000°C corresponded to 68 %. Considering the atomic weight of Au

(196.97 g/mol) and the molecular weight of thiol **5** (287.5 g/mol) this corresponds to a molar ratio Au:**5** of 0.35:0.11.

Applying an ideal truncoctahedron as model, Au nanoparticles with a 1.8 nm diameter (as determined from TEM) contain 201 Au atoms and 71 surface thiols [M. J. Hostetler, J. E. Wingate, C. J. Zhong, J. E. Harris, R. W. Vachet, M. R. Clark, J. D. Londono, S. J. Green, J. J. Stokes, G. D. Wignall, G. L. Glish, M. D. Porter, N. D. Evans, and R. W. Murray, *Langmuir*, 1998, **14**, 17-30.] For this ratio the calculated percentage of inorganic material amounts to 66%, which is in close agreement to that measured.

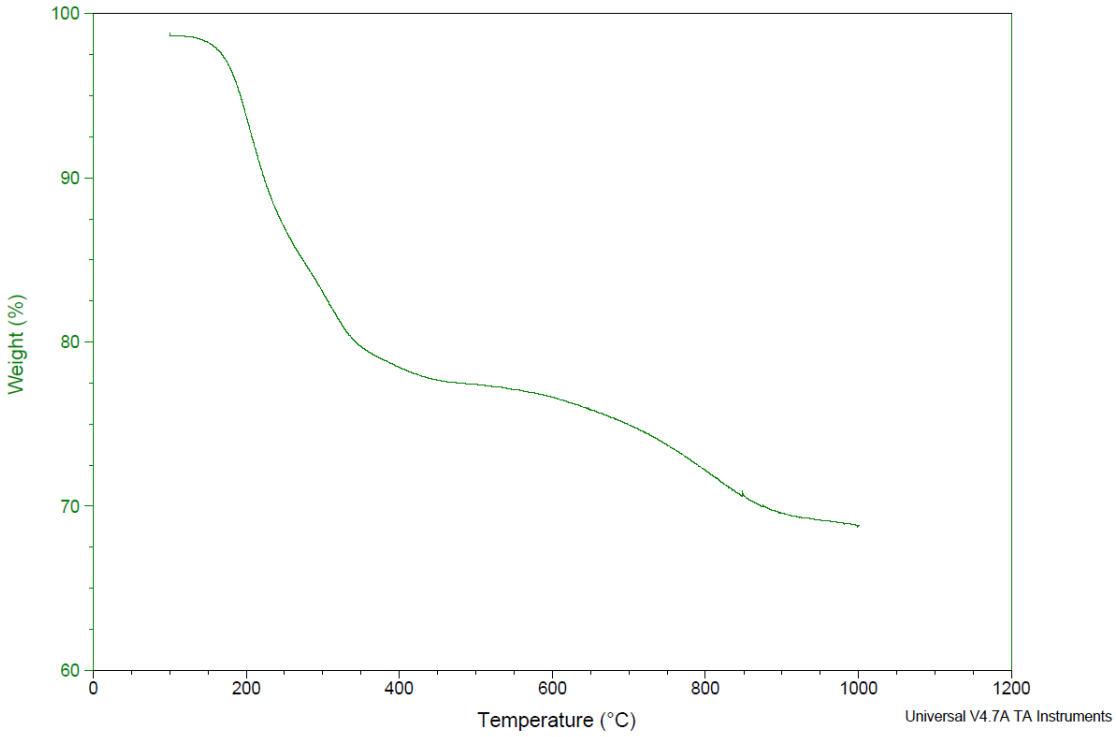


Fig. S7: TGA of Au MPCs **2**

4. Characterization Au MPCs **4**

4.1. NMR

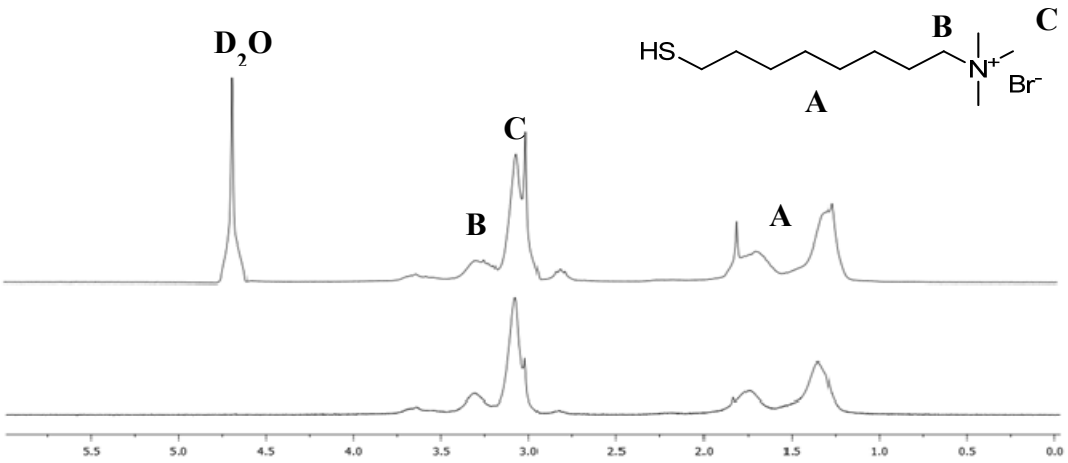
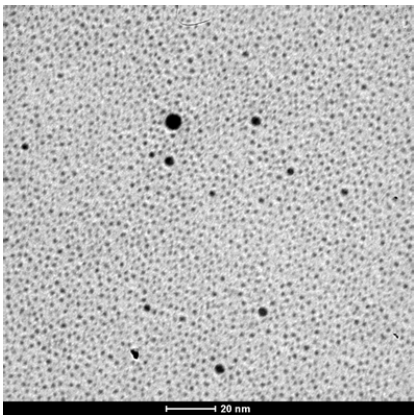


Fig. S8: ^{1}H-NMR spectra (300 MHz) of Au MPC **4**, without (above) and with (below) diffusion filter in D_2O.

4.2. TEM

The diameter of the gold nuclei in Au MPCs **4** was determined as 1.8 ± 0.4 nm by HR-TEM.



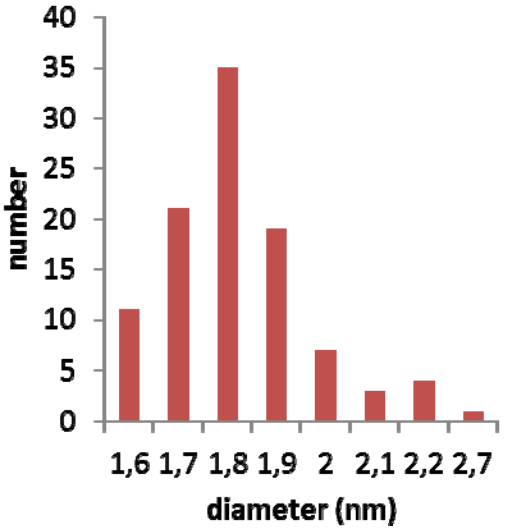


Fig S9: TEM analysis and size distribution for Au MPC **4.**

4.3. UV/Vis

The absence of the surface plasmon resonance band at 520 nm in the UV/Vis spectrum gives additional proof for the presence of sub 3 nm sized Au NPs.

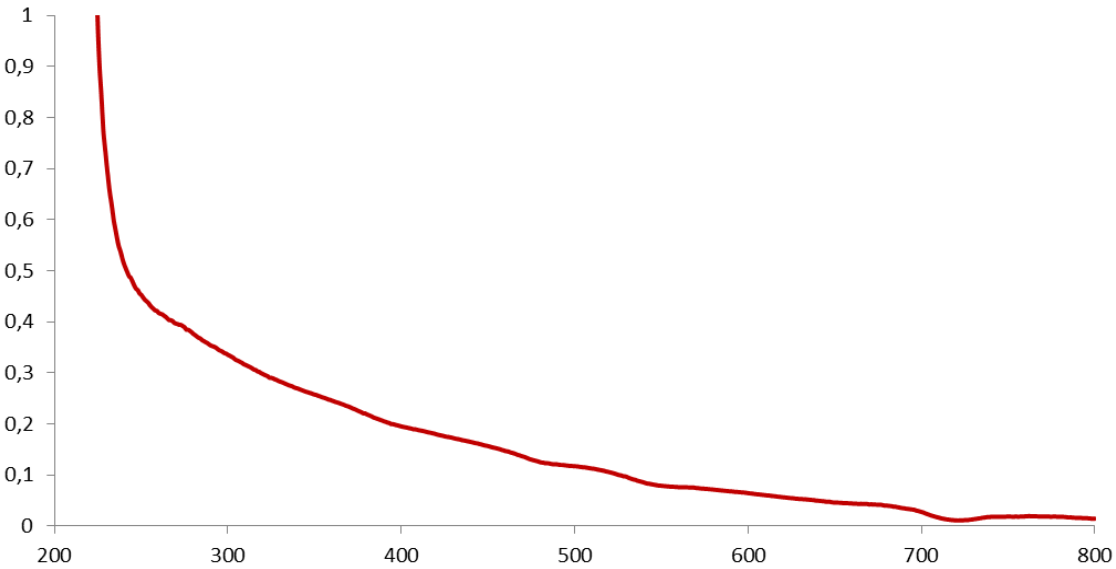


Fig. S10: UV-Vis absorption of Au MPC **4,** [headgroup]=20µM, [HEPES]=10mM pH = 7.

4.4. DLS

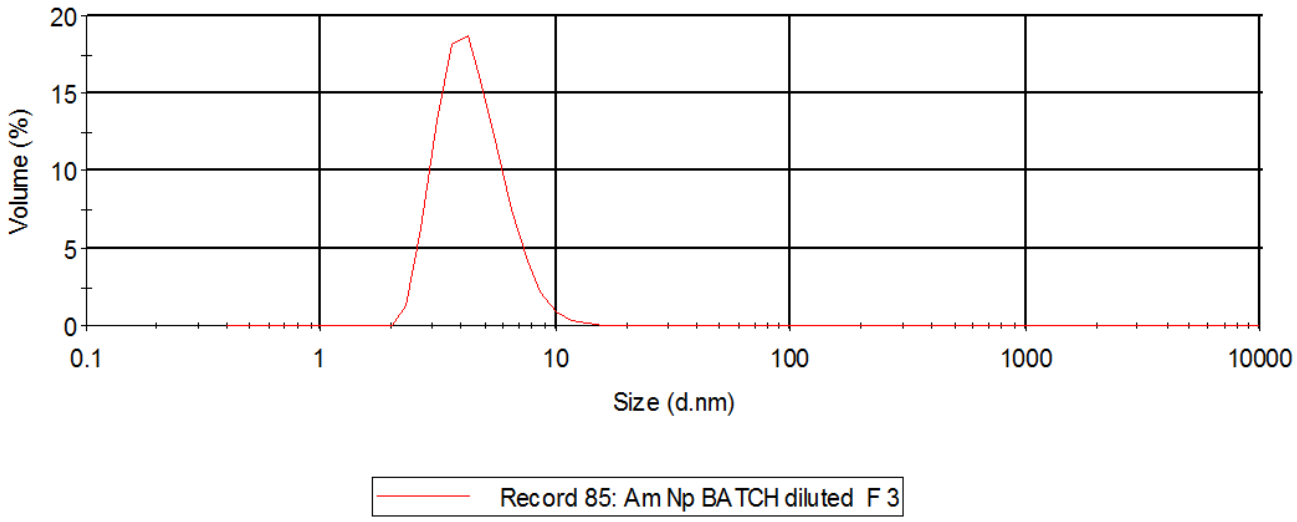
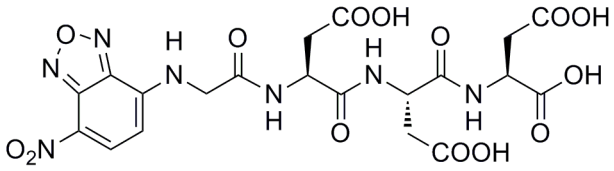


Fig. S11: DLS of nanoparticles **4** in water.

5. Synthesis and characterization of NBD-GDDD

The NBD-GDDD was synthesized on solid support using Fmoc-based peptide chemistry using a standard procedure. After the cleavage of the Fmoc group of the glycine residu, DMF, NBD-Cl (2 eq.) and DIPEA (2 eq) was added to the resine and the suspension was shaked overnight. After the washing procedure the resine was cleaved with a mixture TFA/H_2O/TMS-Cl : 94/3/3 (1 hour). The resulting suspension was filtred and the filtrate was then pourred in cold ether (20 mL) where a yellow precipitate was form. After centrifugation this solid was purified by preparative HPLC to give the desired peptide as a yellow solid (20 mg).



^{1}H NMR: (δ ppm, CD_3OD, 300K, 300 MHz): 8.55 (d, *J* = 8.7 Hz, 1H), 6.30 (d, *J* = 8.7 Hz, 1H), 4.79 – 4.59 (m, 4H), 4.30 (s, 2H), 2.96 – 2.53 (m, 6H).

^{13}C NMR: (δ ppm, CD_3OD, 300K, 75 MHz): 174.2, 174.1, 174.0, 173.7, 172.6, 172.4, 170.3, 146.3, 146.0, 145.4, 138.2, 124.8, 101.2, 51.6, 51.2, 50.4, 47.1, 36.7, 36.5, 36.4.

ESI+MS: m/z = 584.1[MH^+]

6. Determination of the surface saturation concentration

The fluorescence titrations were performed by adding consecutive amounts of a stocksolution of 2-aminopurine riboside-5'-O-triphosphate **ATP_F** (0.2 mM) in mQ water to a 3-mL aqueous solution (pH 7.0, HEPES = 10 mM) containing the Au MPCs at the temperature of 25°C.

Fluorimeter parameters: λex = 305 nm; slit 5/5 (**ATP_F**); λex = 484 nm; slit 10/5 (**NBD-GDDD** probe)

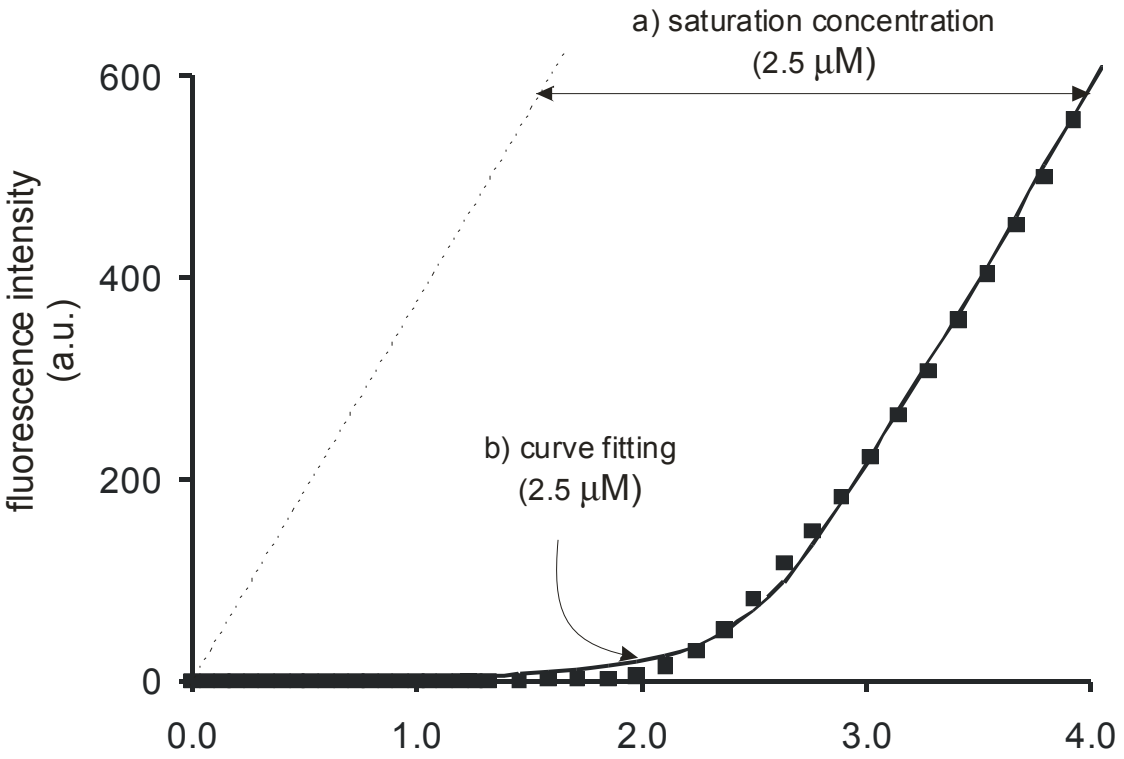


Figure S12. Determination of the saturation concentration of **ATP_F** on Au MPCs **1** ([TACN] = 10 µM) via extrapolation (a) and curve fitting (b).

For each fluorescence titration surface saturation concentrations were determined in two ways: a) via extrapolation of the linear part of the titration curve and b) via fitting of the curve to a 1:1 binding model (Figure S1). Both methods gave essentially the same values.

Both methods are exemplified using the fluorescence titration of **ATP_F** to Au MPCs **2** with a TACN•Zn(II) headgroup concentration equal to 10 µM.

a) extrapolation

After having reached the saturation concentration, the additional amount of ATP_F remains free in solution and, consequently, in that concentration regime the fluorescence intensity

increases linearly as a function of the amount of **ATP_F** added (y = *a*x+b, with *a* = 396 and *b* = -995). Since in the absence of binding the hypothetical increase in fluorescence is given by y = *a*x, it follows that the amount of **ATP_F** bound to the surface corresponds to -*b*/*a* (*i.e.* 2.5 µM).

b) curve fitting

The experimental points were fitted to a 1:1 binding model using Micromath Scientist for Windows, version 2.01.

```
// MicroMath Scientist Model File
IndVars: ATPF0
DepVars:  FI, ATPF, NP, NPATPF
Params: x, K, NP0
NPATPF=K*NP*ATPF
ATPF=ATPF0-NPATPF
NP=NP0-NPATPF
FI=x*ATPF
// constraints
0<ATPF<ATPF0
0<NP<NP0
0<NPATPF<NP0
***
```

The model assumes a 1:1 complex formation between **ATP_F** and a fictitious binding site NP. The parameter NP0 gives the maximum number of binding sites for ATP_F (*i.e.* the surface saturation concentration). This gives a saturation concentration of 2.5 µM (NP0).

The calculated **ATP_F** saturation concentrations as a function of the concentration of TACN-headgroups are given in Table S1.

Table S1: ATP_F and NBDP saturation concentrations as a function of the concentration of headgroups obtained via both extrapolation and curve fitting.

	ATP_F saturation concentrations (μM)		
[TACN•Zn] (μM)	**extrapolation**	**curve fitting**	**average**
0.1[a]	N/A	N/A	N/A
0.5	0.05	0.06	0.06
1	0.1	0.1	0.1
5	1.1	1.0	1.1
10	2.5	2.5	2.5

a) no binding could be detected.

	ATP_F saturation concentrations (μM)		
[Amonium] (μM)	**extrapolation**	**curve fitting**	**average**
10	1.6	1.7	1.7

	ATP_F saturation concentrations (μM)		
[TACN] (μM)	**extrapolation**	**curve fitting**	**average**
10	0.6	0.5	0.6

	NBD-GDDD saturation concentrations (μM)		
[TACN•Zn] (μM)	**extrapolation**	**curve fitting**	**average**
10	2.6	2.7	2.7

7. Fluorescence displacement experiments

The displacement essays were performed by adding consecutive amounts of a stocksolution of analyte (**ATP** 5 mM, **ADP** 25 mM, **Ac-DDD** 50mM) in mQ water to a 3-mL aqueous solution (pH 7.0, HEPES = 10 mM) containing the Au MPCs **1** or **4** coated with the fluorescent probe at 80% of the saturation concentration at 25°C.

Fluorimeter parameters: λex = 305 nm/λem = 370 nm; slit 5/5.

Table S2: Concentrations of competitors necessary to displace 50% of the fluorescent probe loaded on the Au MPCs.

	concentration of competitor to displace 50% of the **ATP_F** loaded (in µM)		
Au MPC [10 µM]	**ATP**	**ADP**	**Ac-DDD**
1	3.3	120	580
4	7	44	10

	concentration of competitor to diplace 50% of the **NBD-GDDD** probe loaded (in µM)	
Au MPC [10 µM]	**ATP**	**ADP**
1	1.6	15

8. Mixed surface compositions

The determination of the saturation concentration of the **NBD-GDDD** probe in presence of various amounts of **ATP$_F$** coated on the Au MPC **1** were performed by adding consecutive amounts of a stocksolution of **NBD-GDDD** probe (0.2 mM) in mQ water to a 3-mL aqueous solution (pH 7.0, HEPES = 10 mM) containing the Au MPCs **1** coated with various amount of **ATP$_F$** (from 0.33 to 2µM) at 25°C.

Fluorimeter parameters: λex = 305 nm/λem = 370 nm; slit 5/5 (to control the absence of free **ATP$_F$** in solution); λex = 484 nm/λem = 545; slit 10/5 (to evaluate the presence of free **NBD-GDDD** probe in solution).

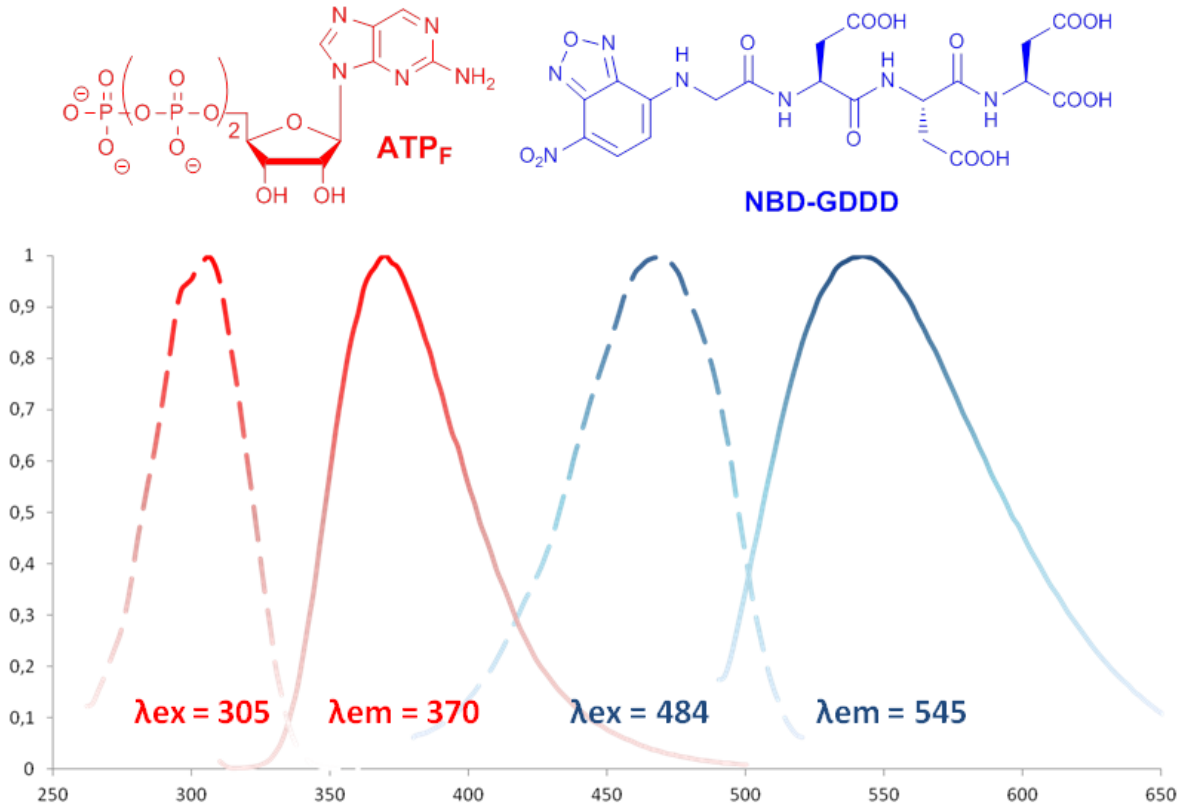


Fig. S13: Normalized emission and excitation spectra of the two probes (C = 2 µM, [HEPES] = 10 mM, pH 7.0)

Table S3: Saturation concentration of **NBD-GDDD** probe in function of the **ATP_F** preloaded and total concentration of the both probes on the AuMPC **1**.

ATP_F loaded (in µM)	% of the saturation concentration of **1** with **ATP_F**	Saturation concentration of **1** with **NBDP**	Total probe concentration on the surface
0	0	2.72	2.72
0.33	13	2.37	2.71
0.66	26	1.92	2.58
0.8	32	1.74	2.54
1.33	53	1.54	2.87
1.66	66	1.27	2.93
2.0	80	0.69	2.69
2.5	100	0	2.50

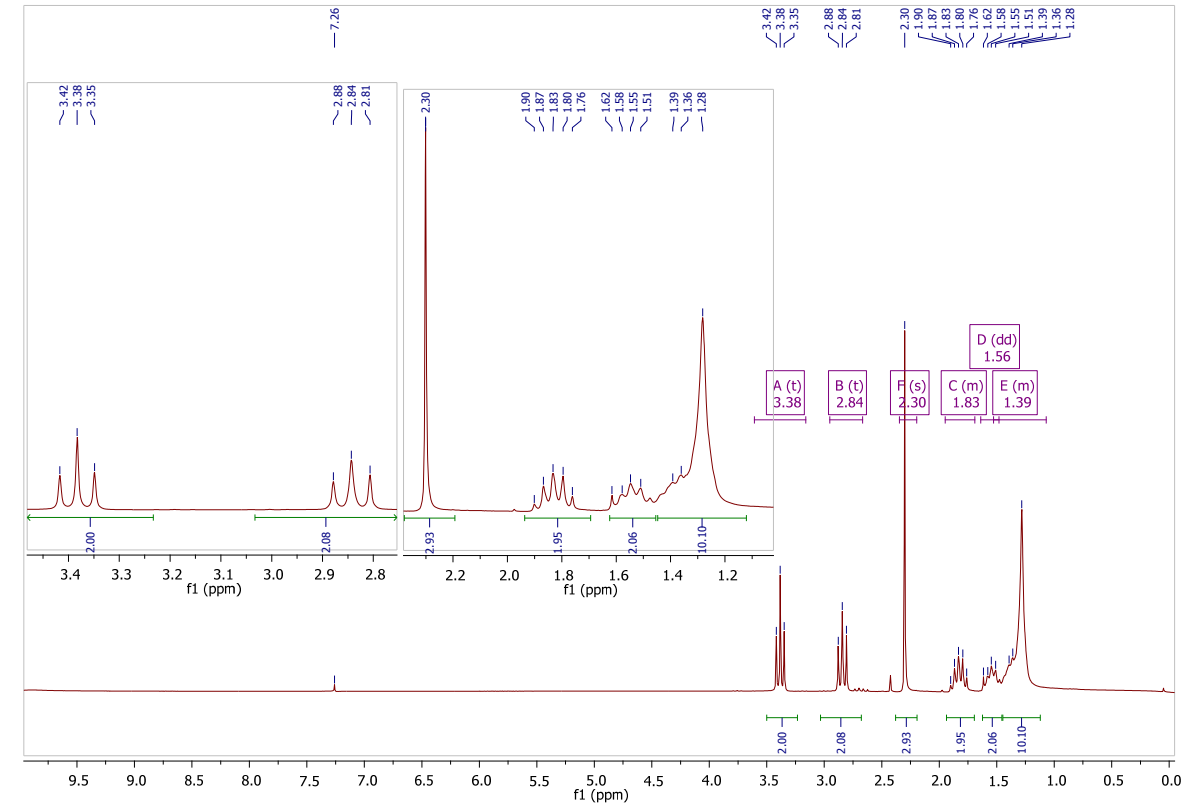
9. Selective displacements

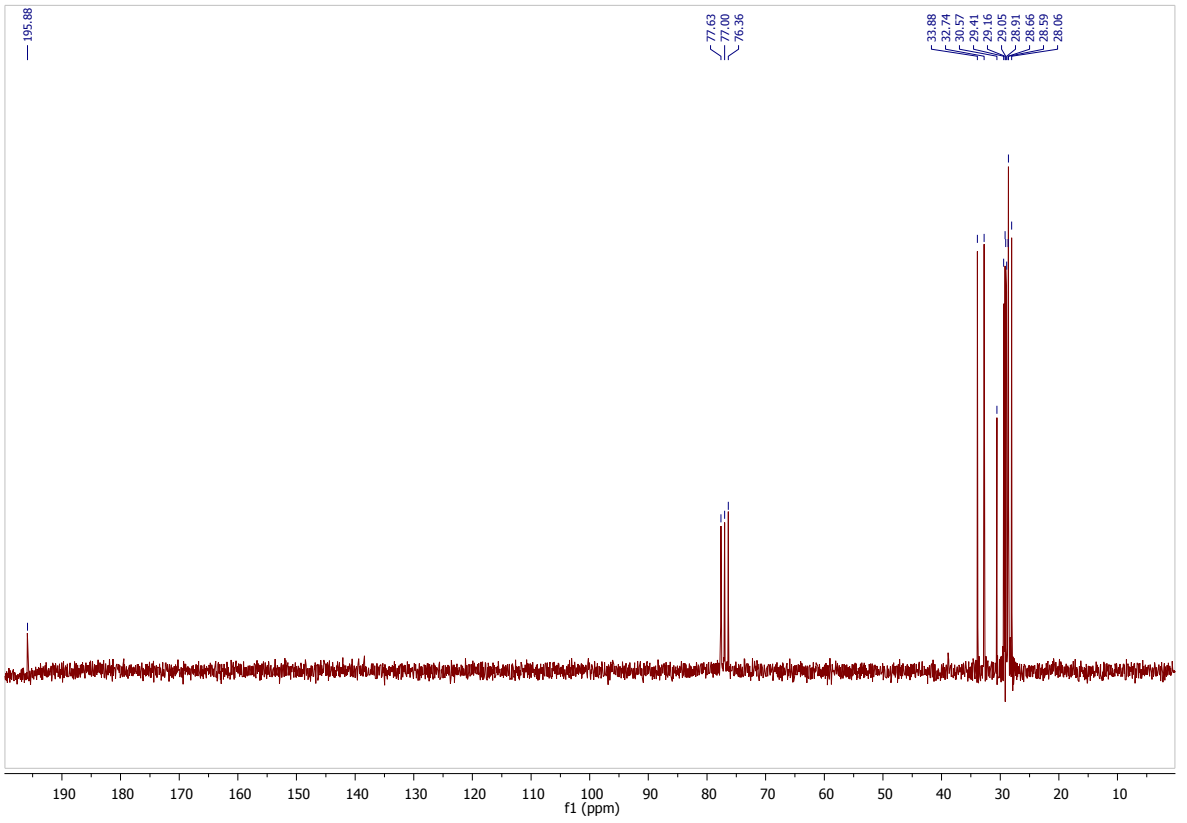
The displacement essays were performed at 25 °C by adding consecutive amounts of a stocksolution of analyte (**ADP** 25 mM) in mQ water to a 3-mL aqueous solution (pH 7.0, HEPES = 10 mM) containing Au MPCs **1** (C = 10µM**)** loaded with **ATP_F** (1.0 µM) and **NBD-GDDD** (1.0 µM).

Fluorimeter parameters: λex = 305 nm; slit 10/5 (to follow the release of **ATP_F**); λex = 484 nm; slit 10/10 (to follow the release of the **NBD-GDDD** probe).

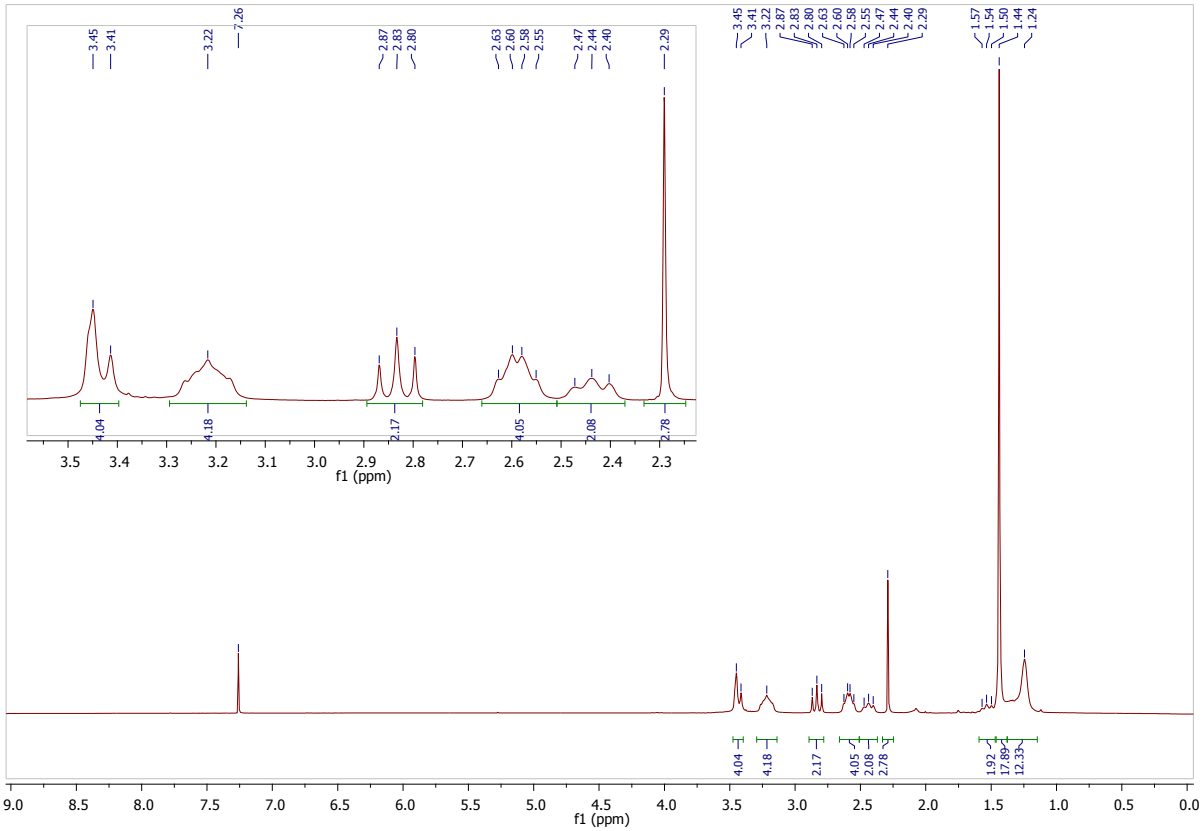
10. NMR Spectra

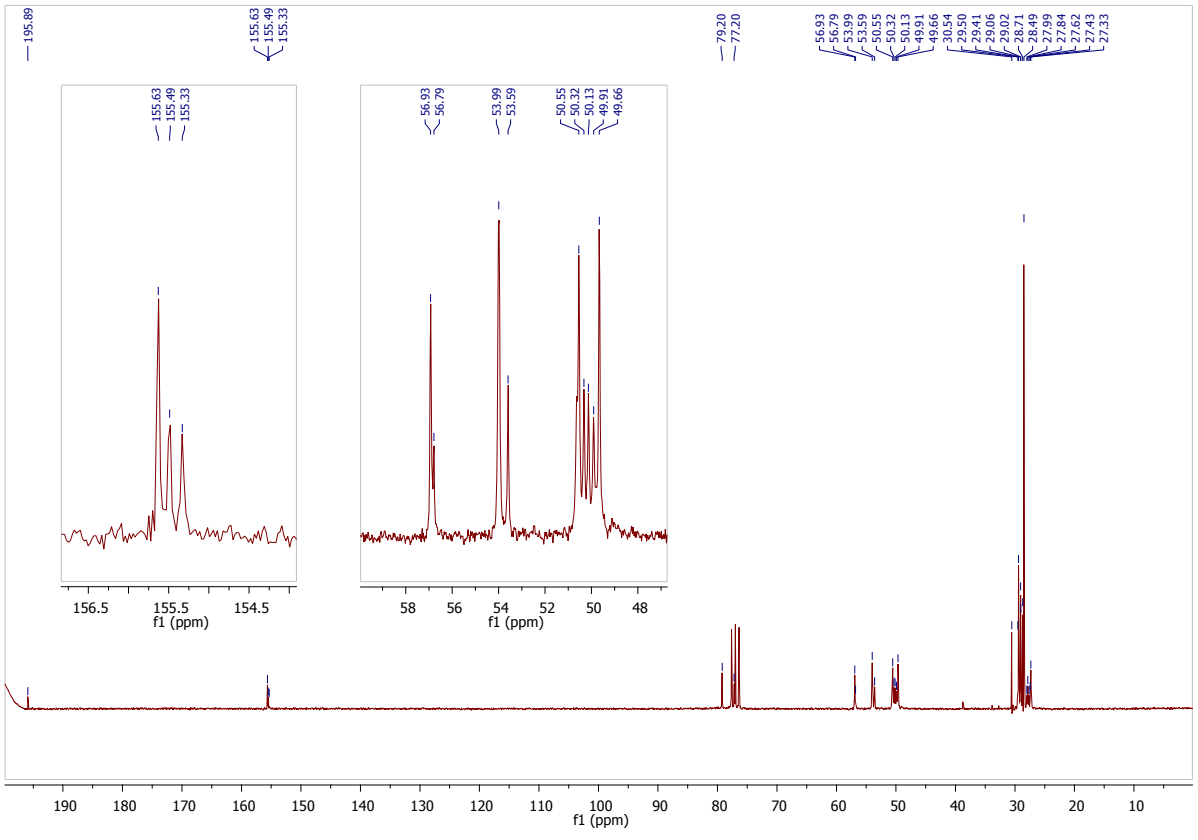
Compound B



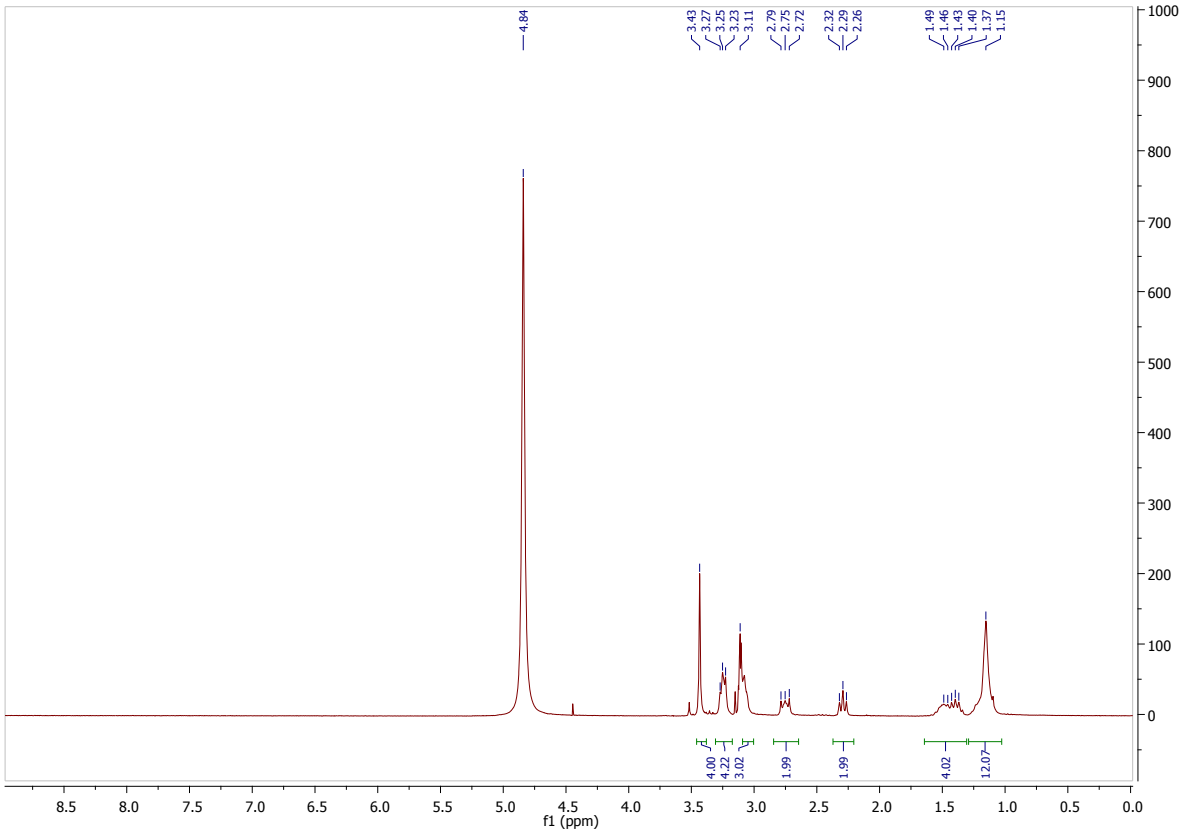


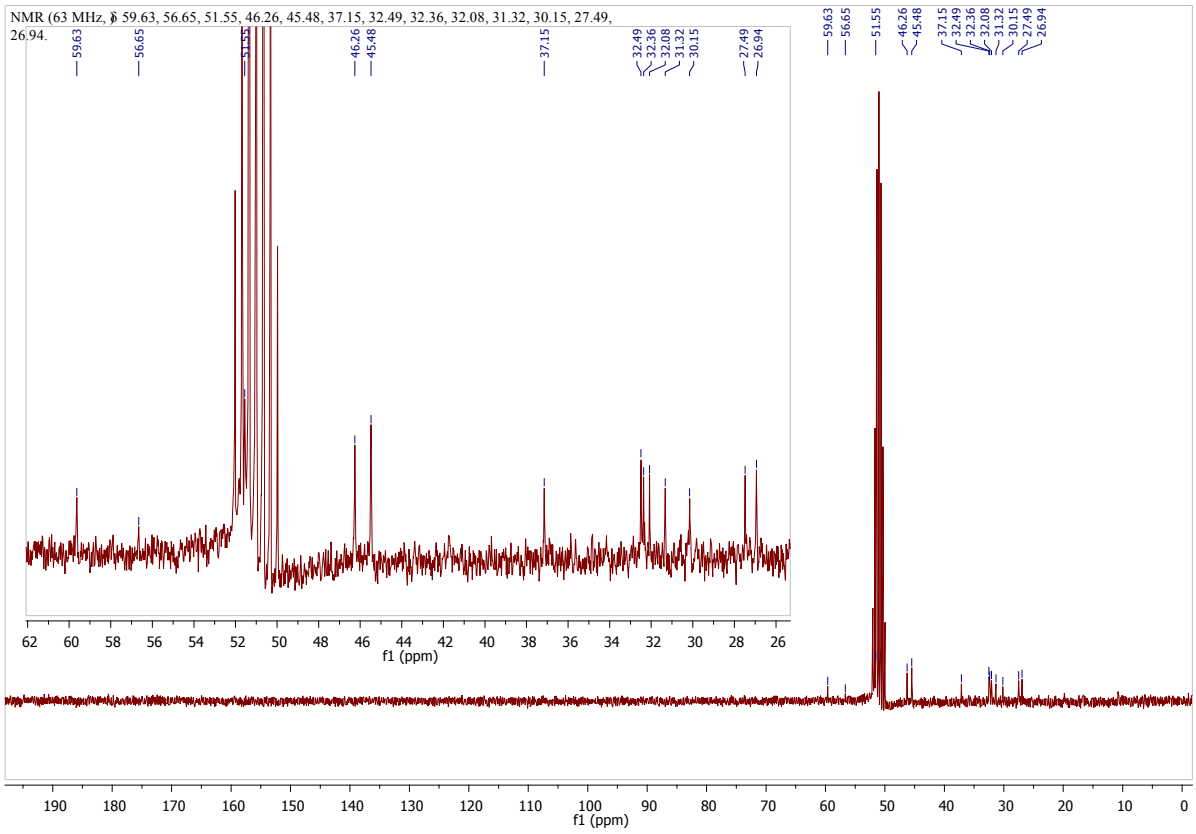
Compound E





Compound 5


4.84
3.43
3.27
3.25
3.23
3.11
2.79
2.75
2.72
2.32
2.29
2.26
1.49
1.46
1.43
1.40
1.37
1.15
4.00
4.22
3.02
1.99
1.99
4.02
12.07
f1 (ppm)


NMR (63 MHz,) δ 59.63, 56.65, 51.55, 46.26, 45.48, 37.15, 32.49, 32.36, 32.08, 31.32, 30.15, 27.49, 26.94.
59.63
56.65
51.55
46.26
45.48
37.15
32.49
32.36
32.08
31.32
30.15
27.49
26.94
f1 (ppm)

NBD-GDDD probe