Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Assessment of the morphology of mixed SAMs on Au nanoparticles using a fluorescent probe

Renato Bonomi, Alessandro Cazzolaro, Leonard J. Prins*

Department of Chemical Sciences, University of Padova, Via Marzolo 1, 35131 Padova, Italy

fax: (+39) 049 8275239; e-mail: leonard.prins@unipd.it

Table of Contents

1. Experimental Procedures

General

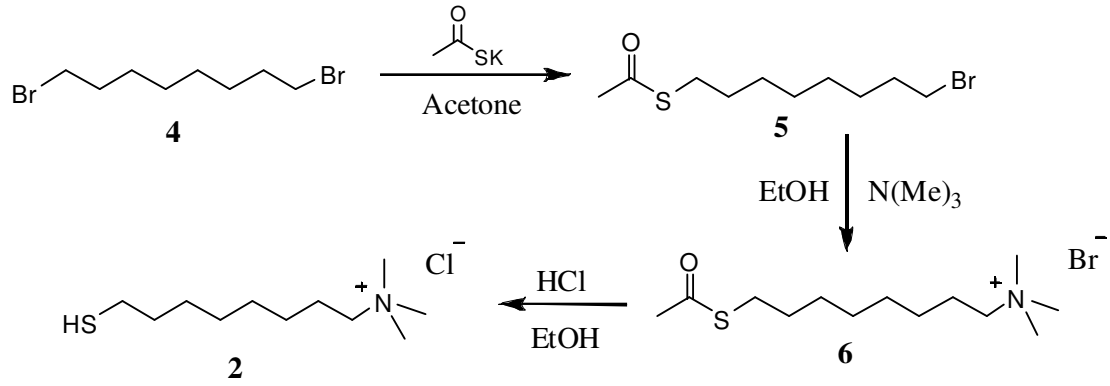
Solvents were purified by standard methods. All commercially available reagents and substrates were used as received. TLC analyses were performed using Merck 60 F_{254} precoated silica gel glass plates. Column chromatography was carried out on Macherey-Nagel silica gel 60 (70-230 mesh). NMR spectra were recorded using a Bruker AC250F spectrometer operating at 250 MHz for ^{1}H and 62.9 MHz for ^{13}C and a Bruker AV300 operating at 300 MHz for ^{1}H. Chemical shifts are reported relative to internal Me_4Si. Multiplicity is given as follow: s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, br = broad peak. ESI-MS mass spectra were obtained with an Agilent Technologies LC/MSD Trap SL mass spectrometer. 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 Bio spectrophotometer 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 (λ_{max} 243nm, pH 7, ε = 8000 $M^{-1}cm^{-1}$). $Zn(NO_3)_2$ was an analytical grade product. Metal ion stock solutions were titrated against EDTA following standard procedures.

Fluorescence Measurements

All Au MPCs **I** - **X** were fully soluble in water and stable for several weeks in this solvent. The fluorescence titrations were performed by adding consecutive amounts of a stocksolution of 2-aminopurine riboside-5'-O-triphosphate ($1.0 \cdot 10^{-2}$ M) in H_2O buffered at pH 7.0 with HEPES ($1x10^{-2}$ M) to a 3-mL aqueous solution (pH 7.0, HEPES = $1x10^{-2}$ M) containing the Au MPCs at the temperature of 25°C. The concentration of ammonium groups in the stock solution of Au MPCs **VII –X** was determined by dissolving a sample into milliQ water: an aliquot of that solution was dried and dissolved in D_2O containing nitromethane as an internal standard. The concentration of ammonium groups was determined by comparison of the integrals of the broad signals of selected regions of the spectrum with the singlet of nitromethane. The reliability of the measurements was assessed by decomposition of a nanoparticle solution with I_2 and analysis of the integrals of the much sharper NMR spectrum in CD_3OD after centrifugation of the surnatant.

2. Synthesis of 8-trimethylammonium-1-octylthiol

Thiol **3** was prepared according to the following scheme:



2.1 Synthesis of 8-thioacetyl-octyl bromide (5)

1,8-dibromoctane **4** (4.350 g, 15.99 mmol) was dissolved in acetone (40 mL). Potassium thioacetate was added (1.826 g, 15.99 mmol) and the resulting mixture was kept at room temperature under nitrogen overnight. After evaporation of the solvent, the yellowish solid was dissolved with CH_2Cl_2 and extracted with water (3 x 40 mL). The organic phase was dried with Na_2SO_4. After solvent evaporation, the crude product was purified by flash chromatography (silica gel, eluent: CH_2Cl_2, rf= 0.71). 1.750 g (41%) of **5** were obtained as a yellowish oil.

^{1}H-NMR ($CDCl_3$, 250 MHz), □: 3.32 (t, 2H, 8Hz), 2.78 (t, 2H, 8Hz), 2.24 (s, 3H), 1.77 (qn, 2H, 8 Hz), 1.48 (qn, 2H, 8Hz), 1.24 (br, 8H).

2.2 Synthesis of 8-thioacetyl-1-trimethylammonium-octane (6)

Compound **5** (0.500 g, 1.87mmol) was dissolved in AcN (10 mL). Trimethylammine ethanol solution (30% w/w) was added (0.35 g, 1.78 mmol) and the reaction was performed in a sealed tube at 82°C overnight. After solvent evaporation, the crude product was purified by flash chromatography (silica gel, eluent: CH_2Cl_2, and then MeOH). 0.490 g (81%) of **3** were obtained as a white solid.

^{1}H-NMR (CD_3OD, 250 MHz), □: 3.57 (t, 2H, 8Hz), 3.37 (s, 9H), 3.05 (t, 2H, 8Hz), 2.52 (s, 3H), 1.97 (br, 2H), 1.77 (qn, 2H, 8Hz), 1.58 (br, 8H).

^{13}C-NMR (CH_3OD, 62.9 MHz, ^{1}H decoupled), □: 194.8, 67.8, 53.5, 30.7, 30.6, 29.9, 29.8, 29.7, 29.6, 27.2, 23.9.

ESI-MS (m/z): 246.3 [100%, M^+].

2.3 Synthesis of 8-trimethylammonium octylthiol (2)

Compound **6** (0.200 g, 0.62 mmol) was dissolved in EtOH (2 mL). HCl 6M (2 ml) was added and the reaction was performed under nitrogen at 78°C for 3 hours. After solvent evaporation 0.145 g (98%) of **2** were obtained as a white solid.

^{1}H-NMR (CD_3OD, 250 MHz), □: 3.39 (m, 2H), 3.17 (s, 9H), 2.50 (t, 2H, 8Hz), 1.82 (br, 2H), 1.61 (qn, 2H, 8Hz), 1.40 (m, 8H).

^{13}C-NMR (CD_3OD, 62.9 MHz, ^{1}H decoupled), □: 67.9, 53.8, 35.2, 30.1, 30.0, 29.3, 27.3, 25.1, 24.0.

ESI-MS (m/z): 204.2 [100%, M^+].

3. Synthesis and characterisation of Au MPCs VII-X

All the glassware used in the MPC preparation was washed with aqua regia and rinsed with distilled water. $HAuCl_4$ is strongly hygroscopic and was weighted within a dry-box.

A solution of $HAuCl_4 \cdot H_2O$ (100 mg, 0.281 mmol) in water (7 mL) was extracted with a solution of tetraoctylammonium bromide (2.74 g, 5.01 mmol) in N_2 purged toluene (125 mL divided in 3 portions). To the resulting reddish-orange organic solution, a second solution of tetraoctylammonium bromide (2.74 g, 5.01 mmol) and dioctylamine (3.36 g, 13.9 mmol) is added (the amount of dioctylamine was calculated in order to obtain 2 nm nanoparticles[1]). The mixture is vigorously stirred under N_2 for 30 min. During this period of time the colour of the mixture fades. A solution of $NaBH_4$ (93.0 mg, 2.46 mmol) in H_2O (4.20) is then rapidly added. The colour of the solution turns rapidly to black due to nanoparticles formation. After 5 hours of stirring, the aqueous layer is removed. To a suitable portion of the above nanoparticle solution, different amounts of solutions of **2** and **3** in isopropanol were rapidly added in the desired ratios.

The precipitation of MPCs was immediately observed. After addition of 5 ml of water, the aqueous layer was extracted several times with toluene, diethyl ether and ethyl acetate and finally evaporated. Finally the samples were purified by Sephadex G-25 resin (eluent: water).

TEM analysis (Figure S1) yields an average diameter for the inorganic core of the Au MPCs of 2.6 ± 0.8 nm. The size of the nanoparticles was confirmed by dynamic light scattering. NMR analysis (Figure S2) indicate monolayer formation (broadening of all bands), as confirmed by diffusion filtered experiments (Figure S3).

Integration of signals *Amm-SH* and *Teg-SH* in the ^{1}H-NMR spectrum of the Au MPCs, which can be attributed respectively to thiols **2** and **3**, provides the monolayer composition**.**

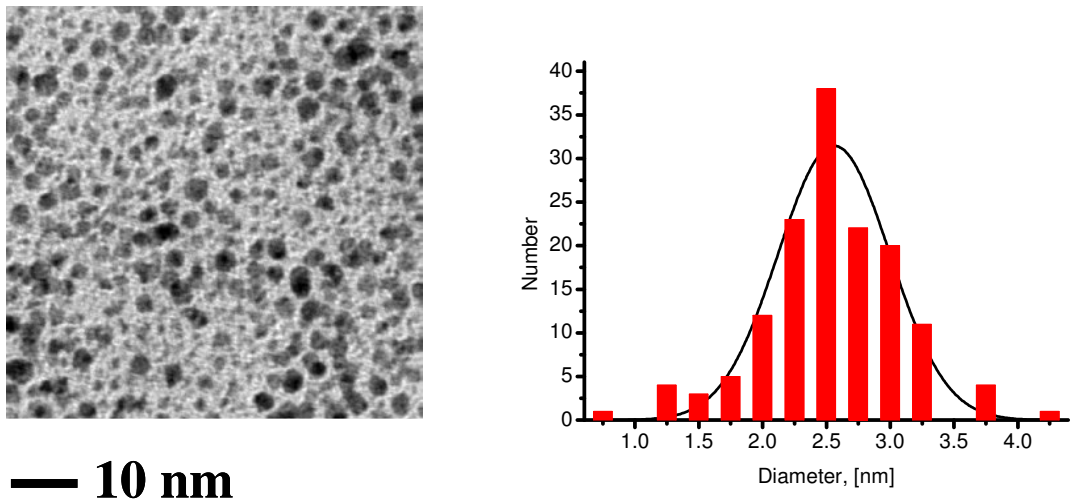


Figure S1: TEM image of Au MPC **IX** and size distribution: average diameter = 2.6 nm (= 0.8 nm).

Au MPCs **VII**
Measured diameter (DLS) = 5.5 ± 1.3 nm
Calculated diameter (gold core + 2*thiol **3**) = 5.3 nm (assuming an extended conformation of **3**)

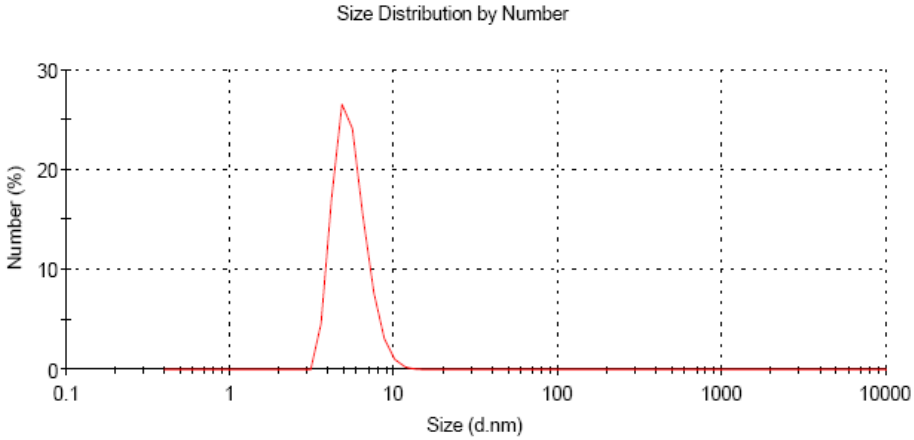


Figure S2: Size distribution of Au MPCs **VII** from dynamic light scattering.

Au MPCs **X**
Measured diameter (DLS) = 8.4 ± 2.5 nm
Calculated diameter (gold core + 2*thiol **2**) = 7.7 nm (assuming an extended conformation of **2**)

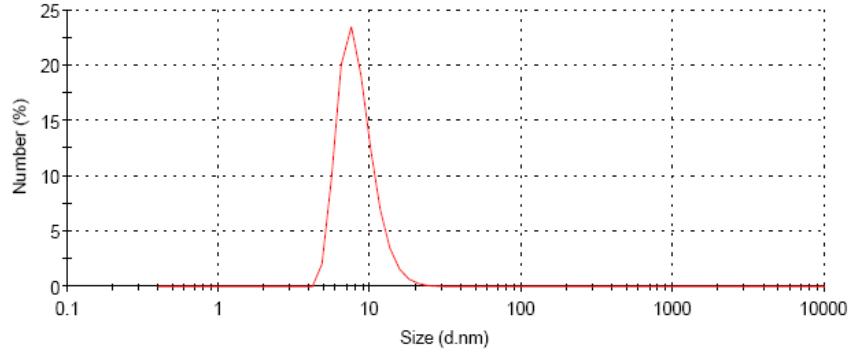


Figure S3: Size distribution of Au MPCs **X** from dynamic light scattering.

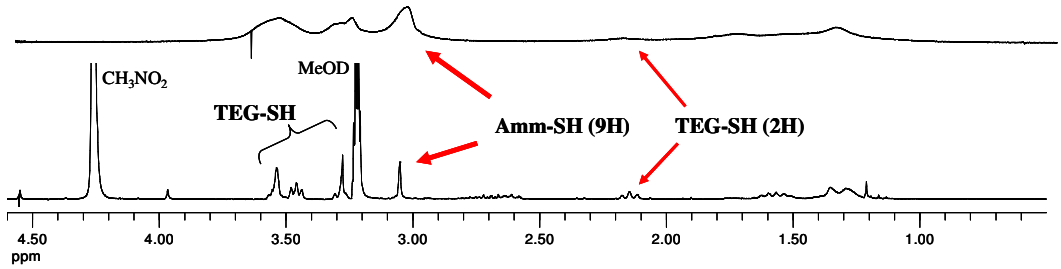


Figure S4: ^{1}H-NMR (300 MHz) spectrum of Au MPC **IX** in CD_3OD before (top) and after (bottom) treatment with I_2. Peaks used to determine the monolayer composition are indicated with red arrows. CH_3NO_2 is used as an internal standard for the determination of absolute concentration.

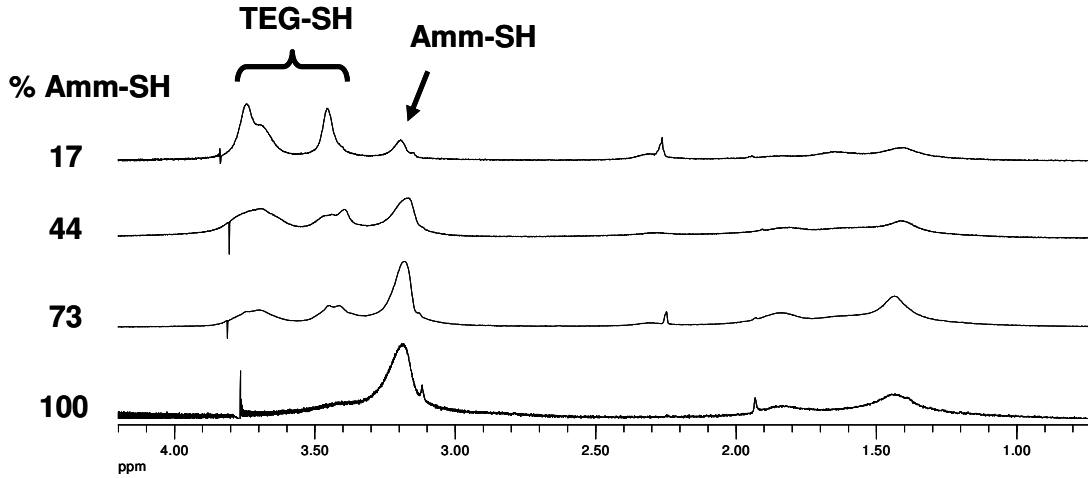


Figure S5: ^{1}H-NMR (300 MHz) spectrum of Au MPCs **VII - X** in D_2O.

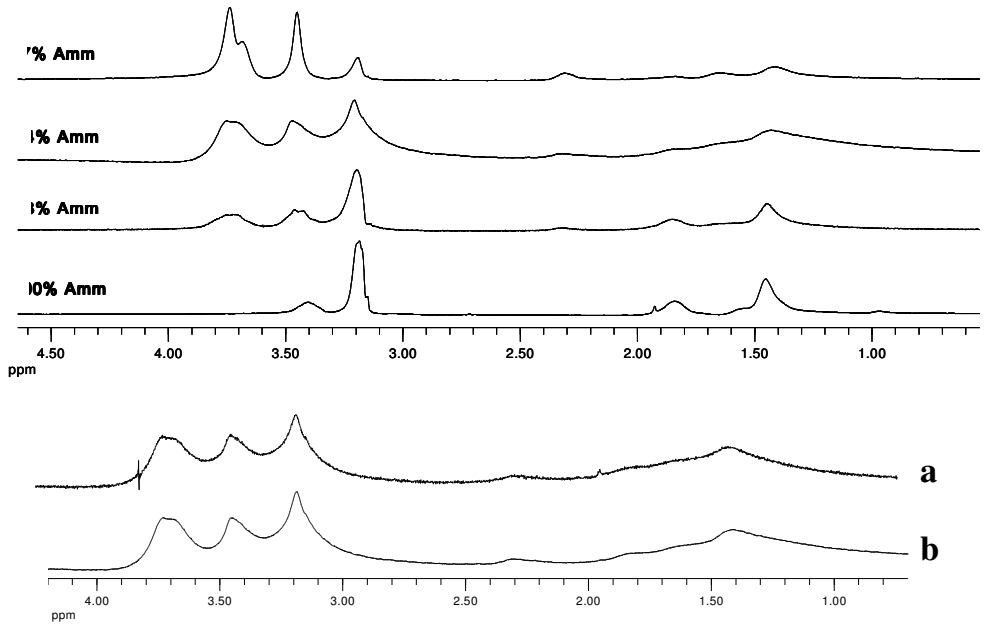


Figure S6: ^{1}H-NMR (300 MHz) spectrum of Au MPCs **VII** - **X** in D_2O (using diffusion filtered experiment) and the spectra of Au MPC **IX** with (b) and without (a) diffusion filter.

5. ATP_F fluorescence titrations

Au MPCs I - V

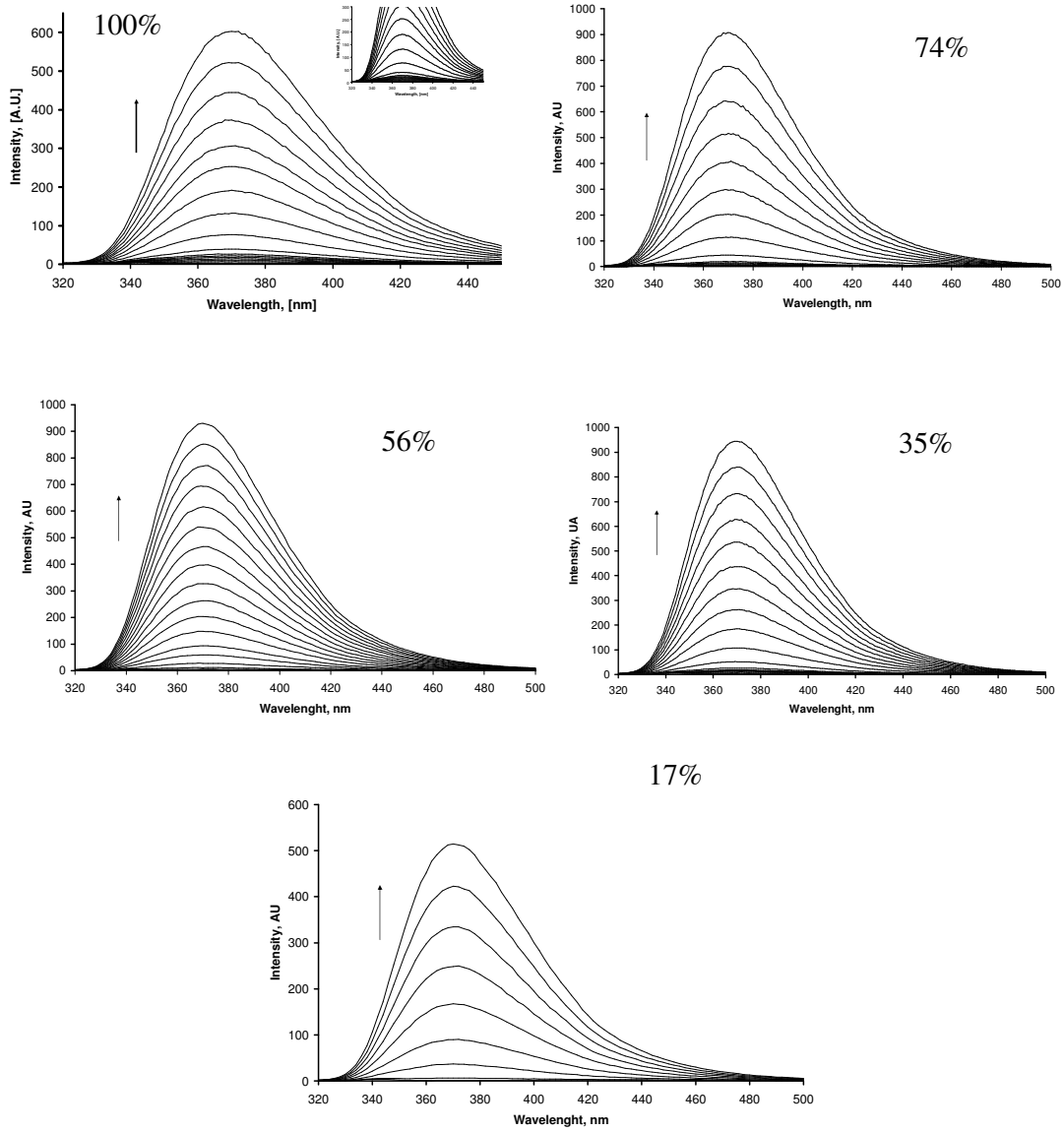


Figure S7. Fluorescence spectra upon the addition of increasing amounts of ATP_F to solutions of Au MPCs **I** - **V**. [TACN·Zn(II)] = $5.0 \cdot 10^{-6}$M, pH 7.0, [HEPES] = $1.0 \cdot 10^{-2}$M, 25°C.

Au MPCs VII - X

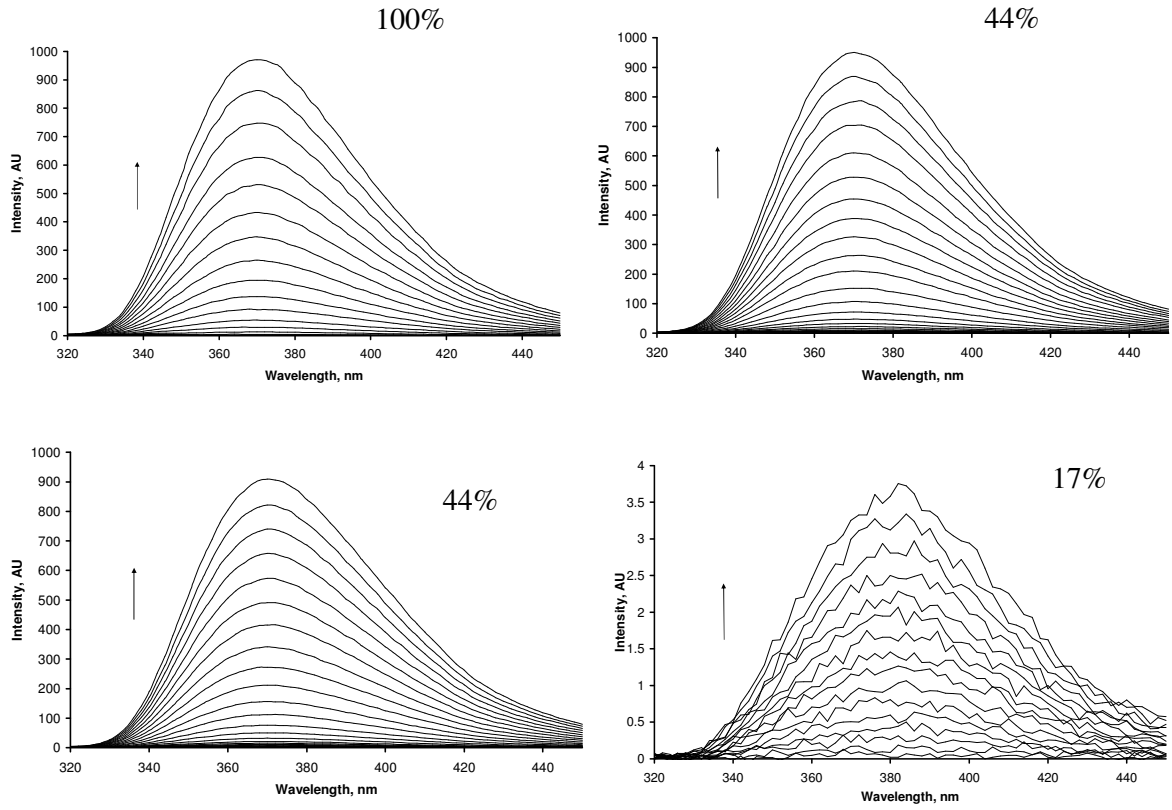


Figure S8. Fluorescence spectra upon the addition of increasing amounts of ATP_F to solutions of Au MPCs **VII** - **X**.

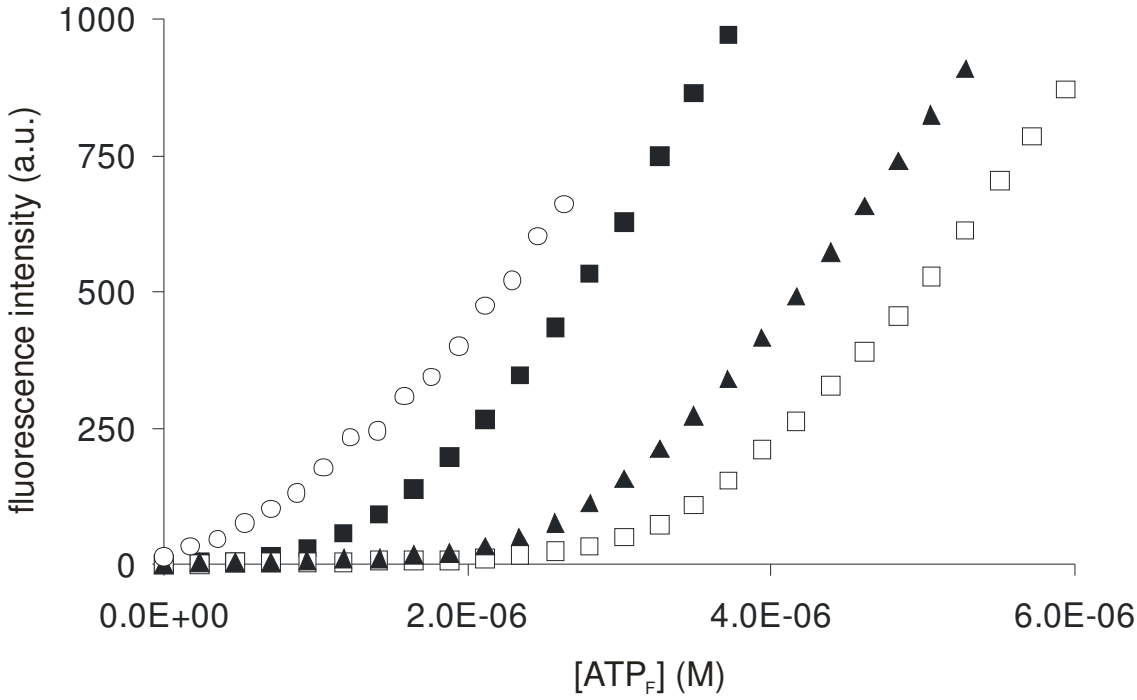


Figure S9. Fluorescence titrations of ATP_F to Au MPCs **VII** (■), **VIII** (□) **IX** (▲), and **X** (○).The trace for **X** is multiplied by 200. Experimental conditions: $[TACN{\cdot}Zn^{II}] = 5 \times 10^{-6}$ M, [HEPES] = 1×10^{-2} M, pH = 7.0, T = 25 ºC.

1) Manea, F.; Bindoli, C.; Polizzi, S.; Lay, L.; Scrimin, P. *Langmuir,* **2008***, 24,* 4120-4124.