

Electronic supplementary information

Tripodal tris-disulfides as capping agents for a controlled mixed functionalization of gold nanoparticles

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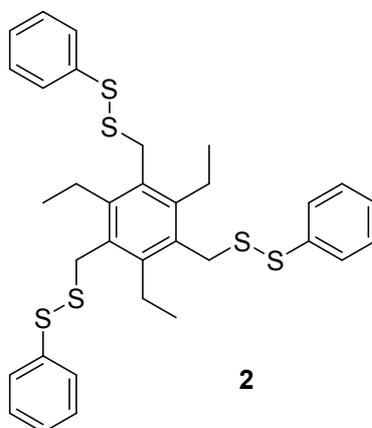
Experimental Section

General Experimental Methods and Materials. All solvents were reagent grade, available from commercial suppliers, and were purified according to standard procedures. All reactions were monitored by TLC on commercially available pre-coated plates (silica gel 60 F254), and the products were visualized with vanillin [1 g dissolved in MeOH (60 mL) and conc. H₂SO₄ (0.6 mL)]. Silica gel 60 was used for column chromatography. Microanalyses were performed at the Analytical Services Unit. ¹H and ¹³C NMR spectra were recorded in CDCl₃ solutions, at 500 and 125 MHz, respectively; coupling constants (J) are given in hertz, and the attributions are supported by heteronuclear single-quantum coherence (HSQC) and correlation spectroscopy (COSY) experiments. UV/Vis electronic spectra were obtained on a Hewlett-Packard HP8453 diode array spectrophotometer. Transmission Electron Microscopy (TEM) analyses were carried out by a JEOL JEM 2010, operating at 200 kV and equipped with a Gatan 794 MultiScan CCD camera.

General procedure for trisulfoxide, **1**, thermolysis in the presence of thiols, for the obtaining of tripodal disulfides **2**, **3**, **4**.

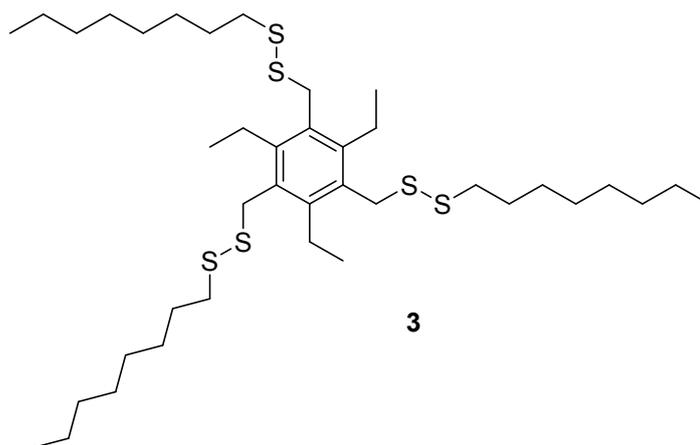
To a solution of 0.40g of **1** (0.66 mm) in dioxane (13mL), 9 equivalents (5.94mmoli) of the generic thiol were added. The reaction was kept under stirring at the 1,4-dioxane boiling temperature (101°C) until the complete disappearance of sulfoxide **1** and of intermediate thermolysis products by TLC. The reaction solvent was evaporated at reduced pressure and the obtained reaction crude was purified by column chromatography (gG/gSiO₂=1/20, eluant from Hexane to hexane/EtOAc 80/20).

2,4,6-Triethyl-1,3,5-[(1-phenyldithio)methyl]benzene (**2**).



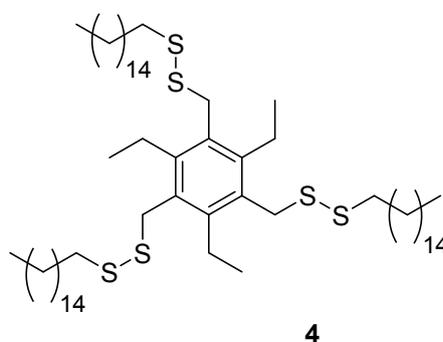
After column chromatography, precipitation of diphenyldisulfide from EtOAc left **2** in the mother liquors in pure form. Yield 50%; TLC: R_f 0,50 (Aceton/Hexane 30/70); Low melting point solid. ^1H NMR (500MHz, CDCl_3): δ 7.55-7.25 (m, 15H, H-arom), 3.99 (s, 6H, $3 \times \text{ArCH}_2\text{S}$), 2.72 (q, 6H, J_{vic} 7.5, $3 \times \text{CH}_2\text{CH}_3$), 1.06 (t, 9H, $3 \times \text{CH}_2\text{CH}_3$). ^{13}C NMR (125MHz, CDCl_3): δ 144.2, 137.1, 129.9, 129.0, 128.6, 127.3 (C-Arom), 38.5 ($3 \times \text{ArCH}_2\text{S}$), 23.1 ($3 \times \text{CH}_2\text{CH}_3$), 15.8 ($3 \times \text{CH}_2\text{CH}_3$). Anal. Calcd for $\text{C}_{33}\text{H}_{36}\text{S}_6$ (625.03): C, 63.41; H, 5.81 Found: C, 63.56; H, 5.82.

2,4,6-Triethyl-1,3,5-[(1-*n*-octyldithio)methyl]benzene (**3**)



Yield 75 %; TLC: R_f 0,40 (Aceton/Hexane 30/70); Low melting solid. ^1H NMR (500MHz, CDCl_3): δ 4.03 (s, 6H, $3 \times \text{ArCH}_2\text{S}$), 2.95 (q, 6H, J_{vic} 7.4, $3 \times \text{CH}_2\text{CH}_3$), 2.62 (t, 6H, J_{vic} 7.3, $3 \times \text{H}_2-1'$), 1.67 (p, 6H, J_{vic} 7.6, $3 \times \text{H}_2-2'$), 1.39- 1.25 (m, 39H, $3 \times \text{H}_2-3'-7'$, $3 \times \text{CH}_2\text{CH}_3$), 0.89 (t, J_{vic} 7.3, 9H, $3 \times \text{H}_3-8'$). ^{13}C NMR (125MHz, CDCl_3): δ 143.6, and 130.6 (C-Arom), 39.0 ($3 \times \text{C}-1'$), 38.5 ($3 \times \text{ArCH}_2\text{S}$), 31.8, 29.3, 29.2, 28.6 ($3 \times \text{C}2'-6'$), 23.3 ($3 \times \text{ArCH}_2\text{CH}_3$), 22.6 ($3 \times \text{C}-7'$), 15.9 ($3 \times \text{CH}_2\text{CH}_3$), 14.1 ($3 \times \text{C}-8'$). Anal. Calcd for $\text{C}_{39}\text{H}_{72}\text{S}_6$ (733,38): C, 63.87; H, 9.90 Found: C, 63.85; H, 9.91.

2,4,6-Triethyl-1,3,5-[(1-*n*-hexadecyldithio)methyl]benzene (**4**)



Yield 80 %; TLC: R_f 0,42 (Aceton/Hexane 30/70); Low melting solid. ^1H NMR (500MHz, CDCl_3): δ 4.03 (s, 6H, $3 \times \text{ArCH}_2\text{S}$), 2.95 (q, 6H, J_{vic} 7.7, $3 \times \text{CH}_2\text{CH}_3$), 2.62 (t, 6H, J_{vic} 7.3, $3 \times \text{H}_2-1'$), 1.66 (p, 6H,

J_{vic} 7.4, 3xH₂-2'), 1.38- 1.24 (m, 87H, 3xH₂-3'-15', 3xCH₂CH₃), 0.88 (t, J_{vic} 7.0, 9H, 3xH₃-16'). ¹³C NMR (125MHz, CDCl₃): δ143.6 and 130.6 (C-Arom), 38.9 (3xC-1'), 38.5 (3xArCH₂S), 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 28.6, 28.4 (3xC2'-14'), 23.3 (3xArCH₂CH₃), 22.7 (3xC-15'), 15.9 (3xCH₂CH₃), 14.1 (3xC-16'). Anal. Calcd for C₆₃H₁₂₀S₆ (1070,02): C, 70.72; H, 11.30 Found: C, 70.90; H, 11.30.

General synthetic procedure of AuNP functionalization. Amine-protected AuNP were synthesized according to a literature procedure.¹ (i) 6mL of a 30mM solution of TOAB (tetraoctylammoniumbromide) in toluene, (ii) 6mL solution of *n*-octadecylamine (180 mM) in toluene and (iii) 6mL of an aqueous solution of NaBH₄ (180mm) were subsequently added to 6mL of an aqueous solution of [AuCl₄]⁻ (12mM). The obtained solutions is left under continuous stirring at room temperature for 24h. The organic phase, containing the gold nanoparticles, were then separated and the AuNP were purified from the free amine excess by three cycles of precipitation and resolubilisation in methanol and toluene, respectively. The obtained amine-protected AuNP were dissolved in 6mL of toluene and left to react with approximately 2 mg of the chosen *tris*-disulfide (1:3=[S-S]:[Au]) under magnetic stirring for 24 h at room temperature. The AuNP@S-SR nanoparticles were finally purified by precipitation and resolubilisation in ethanol and toluene, respectively.

TEM Particle analysis.

The sizes of the nanoparticles were obtained by image analysis of the TEM micrographs recorded for each sample, by means of the automatic particle counting routine of ImageJ.²

In case of non-uniform gray values for the background, the micrographs were pre-processed with Gwyddion³ for a background levelling, to allow a better image segmentation.

Particle analysis was preceded by a series of image processing steps (thresholding, erode/dilate cycle, watershed segmentation).

After particle counting, the values of the particles diameters were calculated from the measured areas.

Besides the data set containing all measured particles for each sample (Fig. S7-S10, black columns), the data were filtered and two additional subsets were obtained for each sample,

1 Leff, D. V.; Brandt, L.; Heath, J. R. *Langmuir* 12, 4723–4730 (1996).

2 Schindelin, J.; Rueden, C. T. & Hiner, M. C. et al. (2015), "The ImageJ ecosystem: An open platform for biomedical image analysis", *Molecular Reproduction and Development*, PMID 26153368

3 David Nečas, Petr Klapetek, Gwyddion: an open-source software for SPM data analysis, *Cent. Eur. J. Phys.* 10(1) (2012) 181-188

consisting only of the objects with particle circularity⁴ higher than 0.8 and 0.9 (Fig. S7-S10, red and green columns, respectively).

The binned data were fitted to normal distributions (Fig. S7-S10, solid lines), and the mean and standard deviation values for the three data sets of each sample showed that no relevant changes occur to the distributions upon limiting the statistics to rounder objects.

Thermogravimetric analysis.

TGA was carried out by an Uginge-Eyraud Model B60 Setaram thermobalance. The measuring cell is suspended to the arm of the microbalance, with a standard measurement uncertainty of 0.01 mg, and is placed inside a quartz tube (measuring chamber) externally heated by a graphite resistance. The measuring chamber is connected to a high vacuum system. The temperature was measured using a type K thermocouple placed at less than 1 mm from the bottom of the measurement cell. The temperature standard measurements uncertainty is estimated to be less than 2 °C. The measuring cell is machined of aluminium and it has an inner diameter of 8 mm and a height of 15 mm. In order to remove the solvent, the cell is filled with the solution and it has been kept in air for some hours at about 40 ° C, then the cell was loaded into the thermobalance and the vacuum was made. After few hours (to be sure that solvent was evaporated), the sample was heated at 5 °C/min. For AuNPs capped by octadecylamine a sample of about 16 mg (after toluene removal) was charged, for other tripodal disulfides derivative about 2 mg of samples were treated.

XPS analysis.

XPS spectra were collected by means of a VG Microtech ESCA3000 Multilab spectrometer, using a standard Al K α excitation source ($h\nu=1486.6$ eV), a five channeltrons detection system and a base pressure in the main chamber lower than 1×10^{-6} Pa during data collection (ultra-high vacuum conditions). A constant pass energy of 20 eV was used for the hemispherical analyzer (CAE mode). The binding energy (BE) scale was calibrated by using the C 1s peak from the adventitious carbon at BE = 285.1 eV. The accuracy of the energy measure is ± 0.1 eV. Photoemission data analysis was performed by using the VGX900 software. A nonlinear least square curve-fitting procedure with a properly weighted sum of Lorentzian and Gaussian component curves was used, after background subtraction according to Sherwood.⁵ Relative surface atomic concentrations were calculated by a standard quantification routine, including Wagner's energy dependence of attenuation length⁶ and a standard set of VG Escalab sensitivity

4 Circularity = $4p$ (area / perimeter²)

5 P.M.A. Sherwood, Data Analysis in X-ray Photoelectron Spectroscopy in Practical Surface Analysis by Auger and X-ray Photoelectron Spectroscopy, in: D. Briggs, M.P. Seah (Eds.), Wiley, New York, 1990, pp. 1.

factors, with about $\pm 10\%$ uncertainty on the atomic quantitative analysis. To perform the XPS analyses, the samples with AuNPs were investigated on a fresh surface of graphite by ESCALAB MkII (VG Scientific – UK) equipped with a standard Al K α excitation source and a 5-channeltron detection system. The binding energy scale was calibrated positioning the C 1s peak of C – C bond at BE = 284.6 eV, characteristic for C in graphite reference sample. The spectra were acquired at pass energy of 20 eV and were processed by using the software Advantage v.5.52.

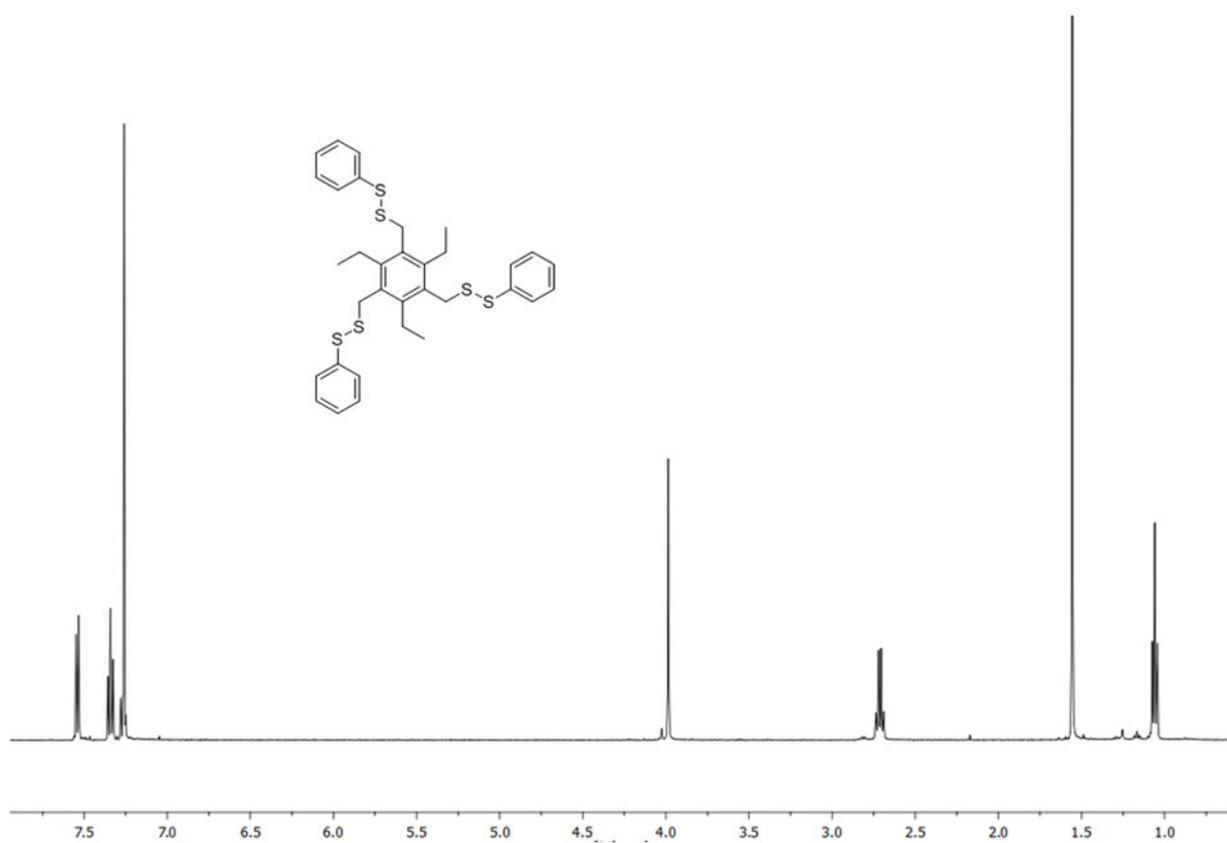


Figure S1. ^1H NMR spectrum of **2**.

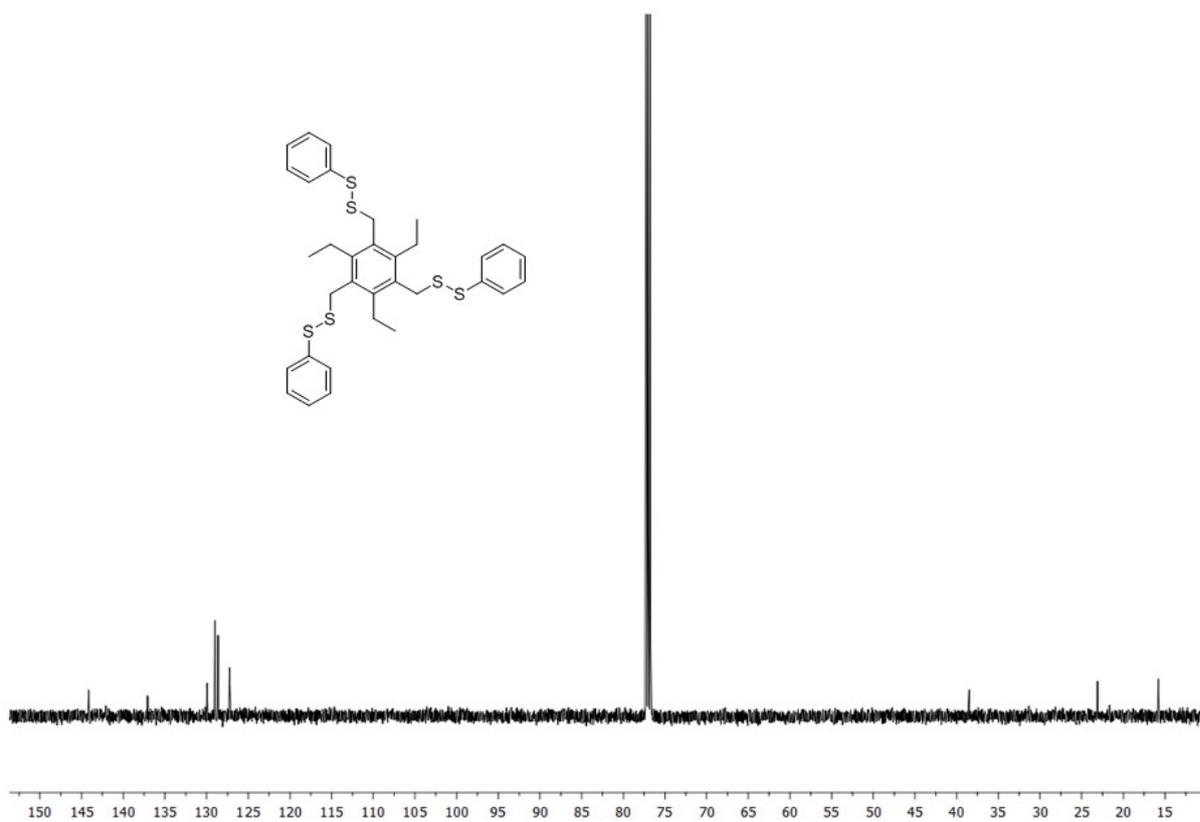


Figure S2. $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **2**.

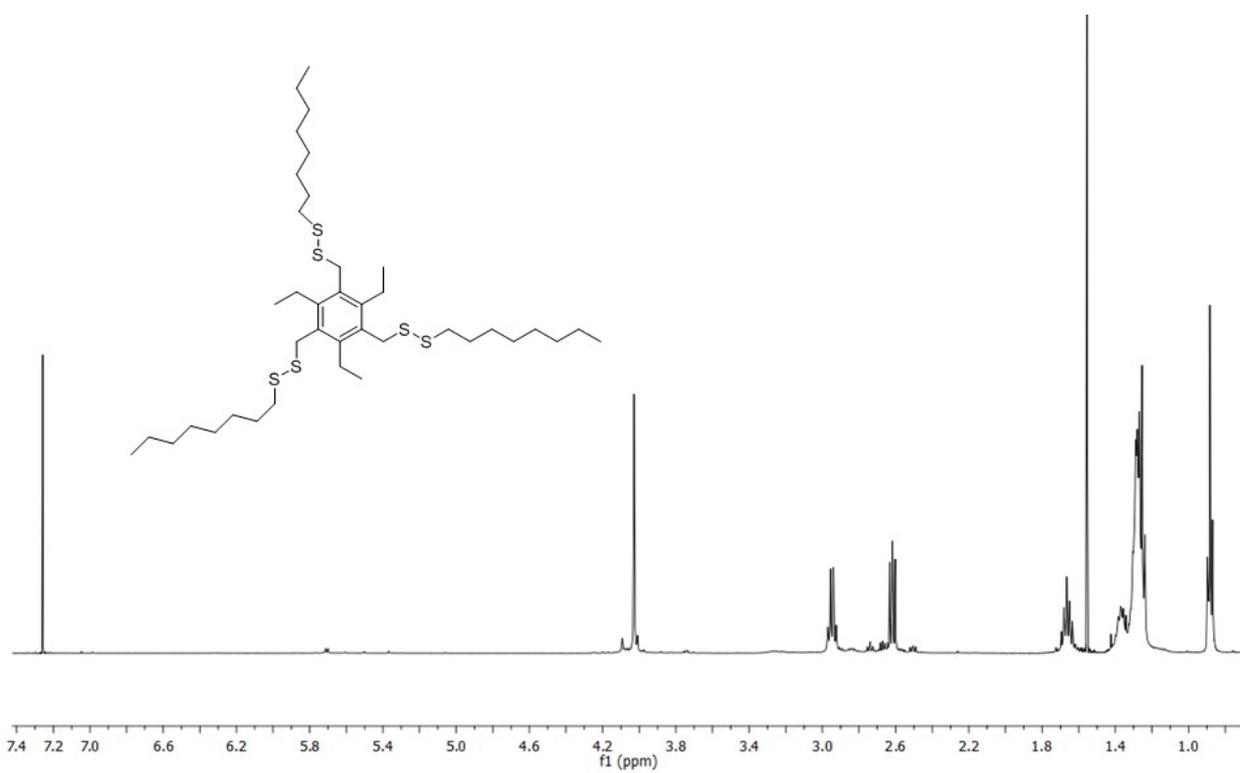


Figure S3. ¹H NMR spectrum of **3**.

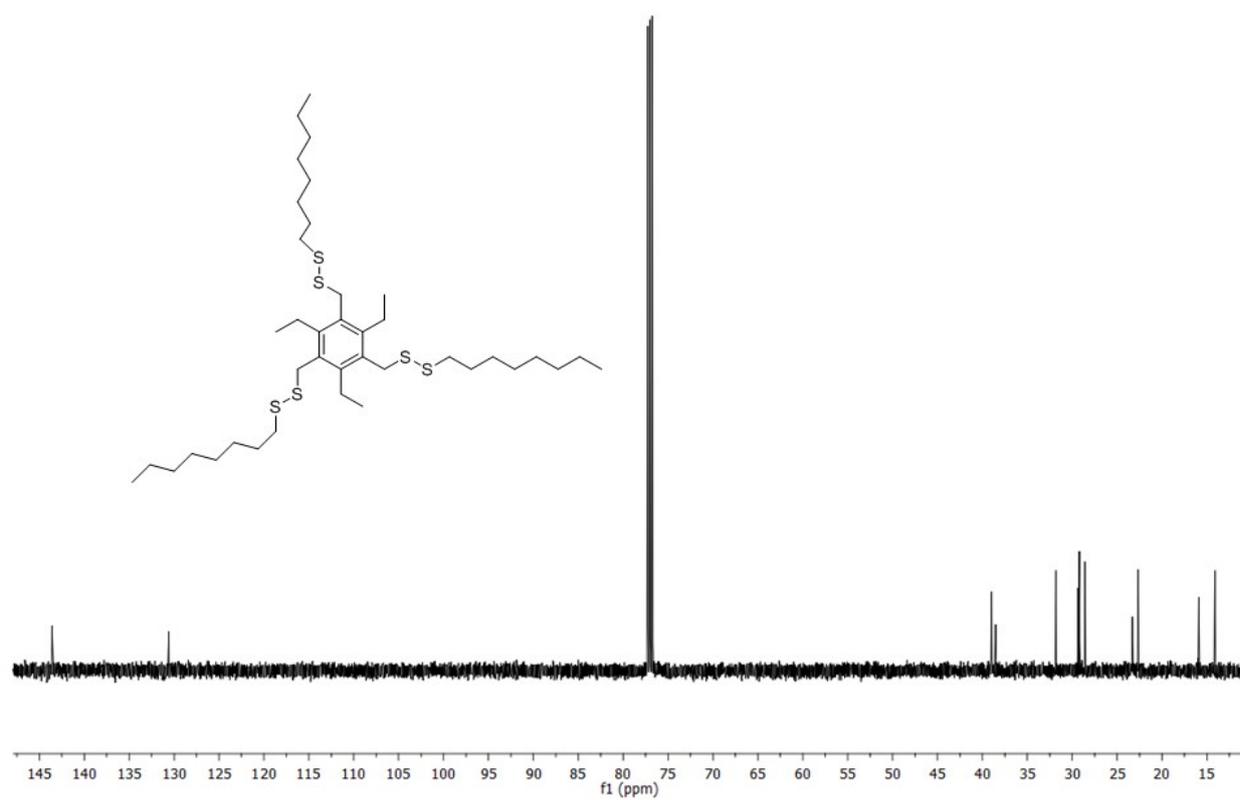


Figure S4. ¹³C{¹H} NMR spectrum of **3**.

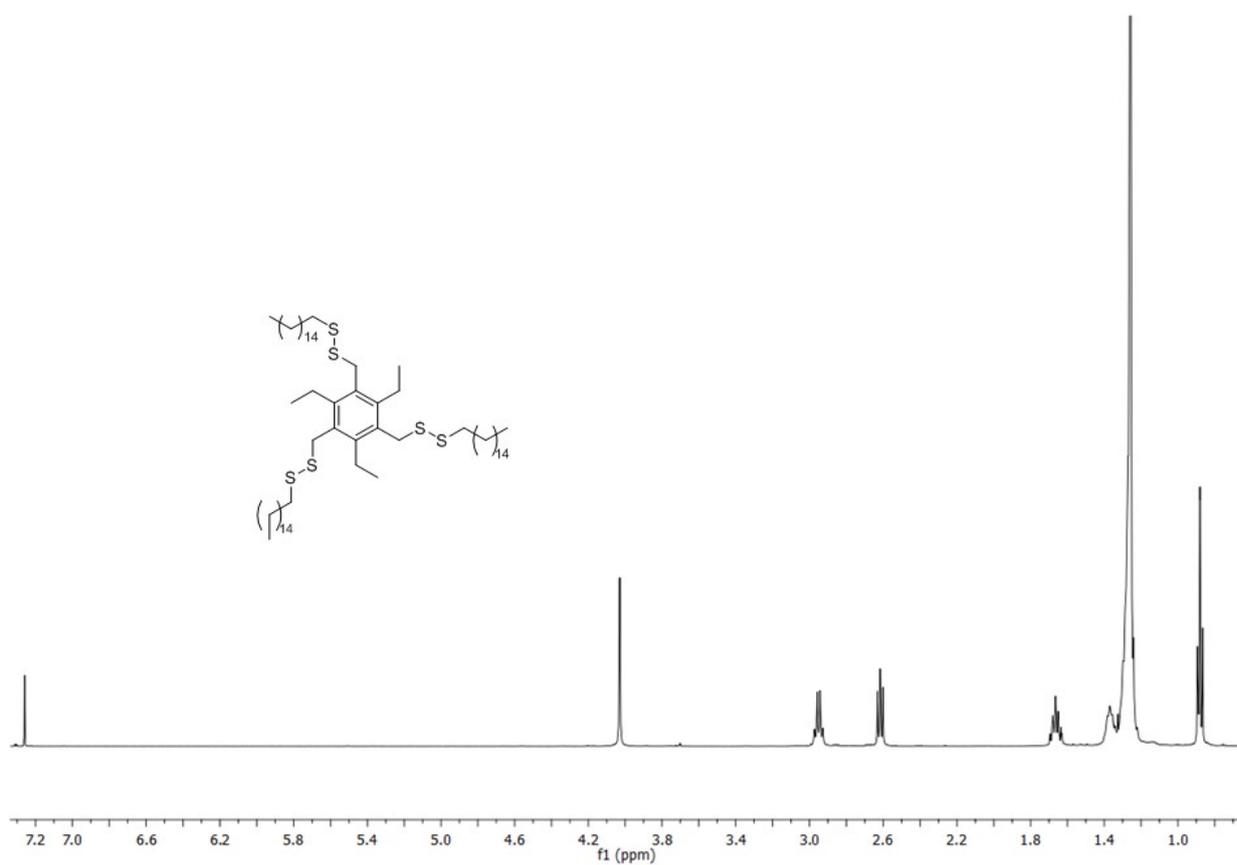


Figure S5. ^1H NMR spectrum of 4.

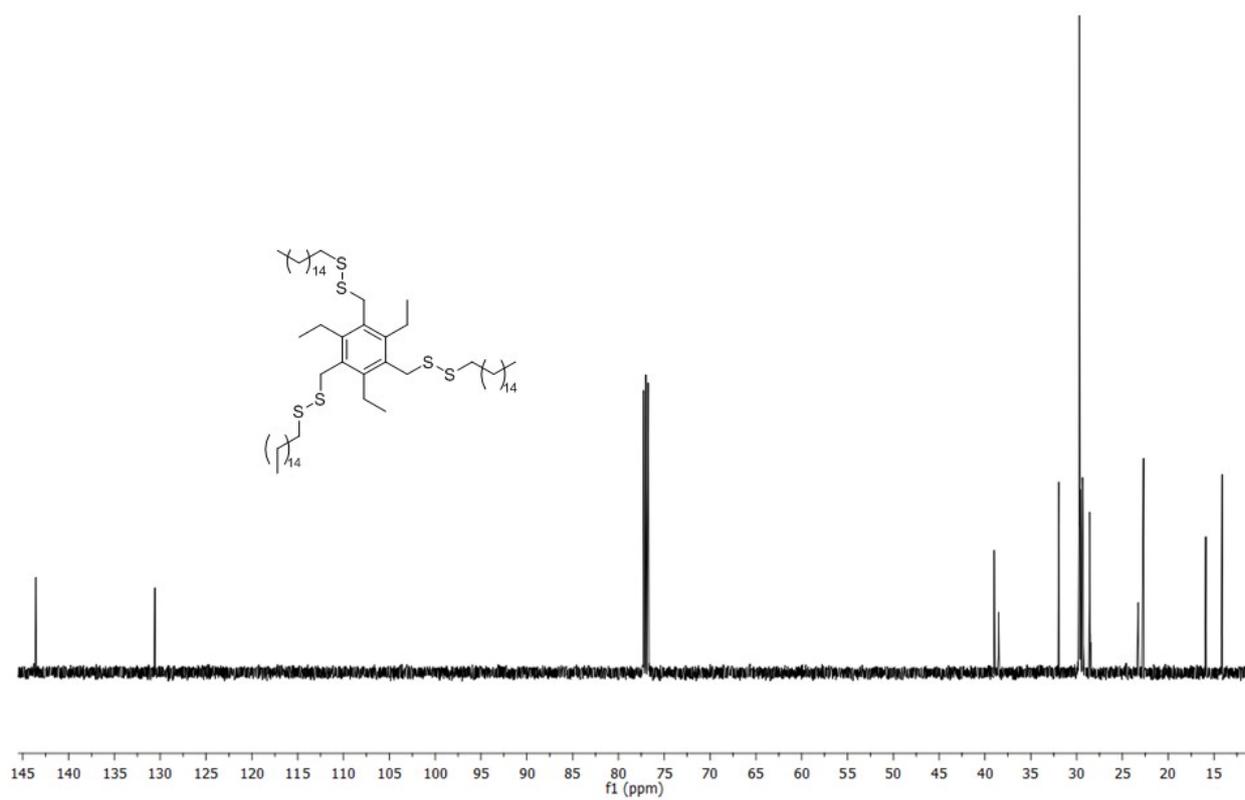


Figure S6. $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of 4.

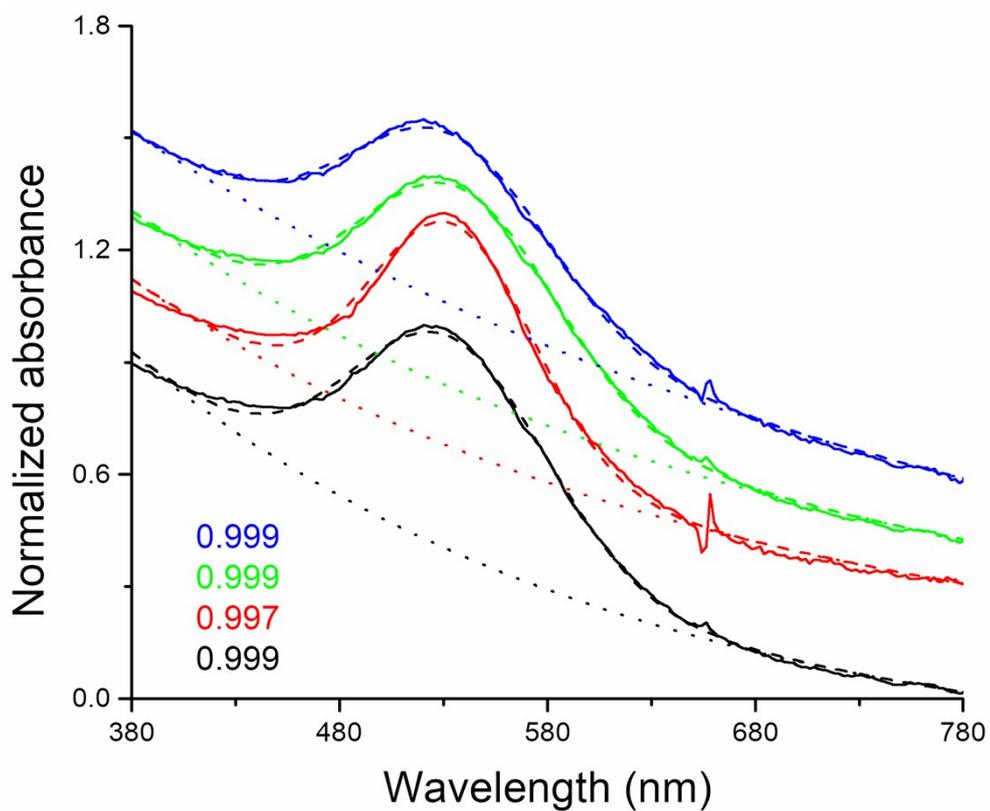


Figure S7. Deconvolution analysis of the normalized UV/vis spectra of AuNPs capped by $C_{18}H_{21}NH_2$ (black, AuNP@ $C_{18}H_{37}NH_2$), or by the tripodal disulfides **2-4** (AuNP@**2-4**, red, green and blue, respectively), as toluene solutions: experimental spectrum (solid), baseline (dotted; exponential function), baseline plus one Gaussian peak (dashed). The numbers represent the R^2 values for the various deconvolutions.

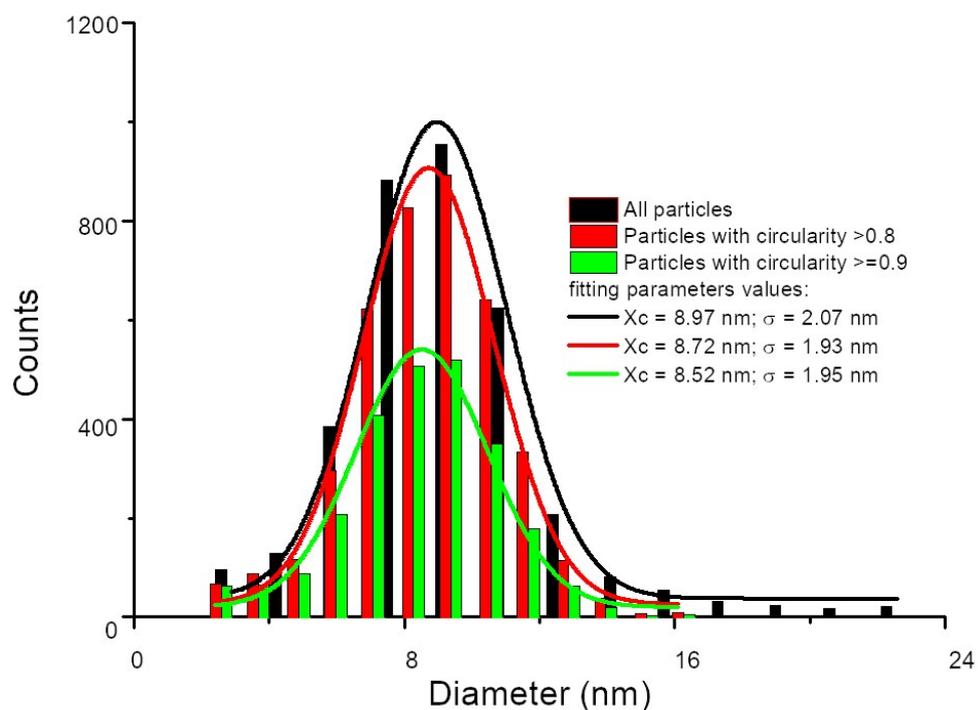


Figure S8. Distribution of TEM diameters size of AuNP@C₁₈H₃₇NH₂, calculated from measured areas.

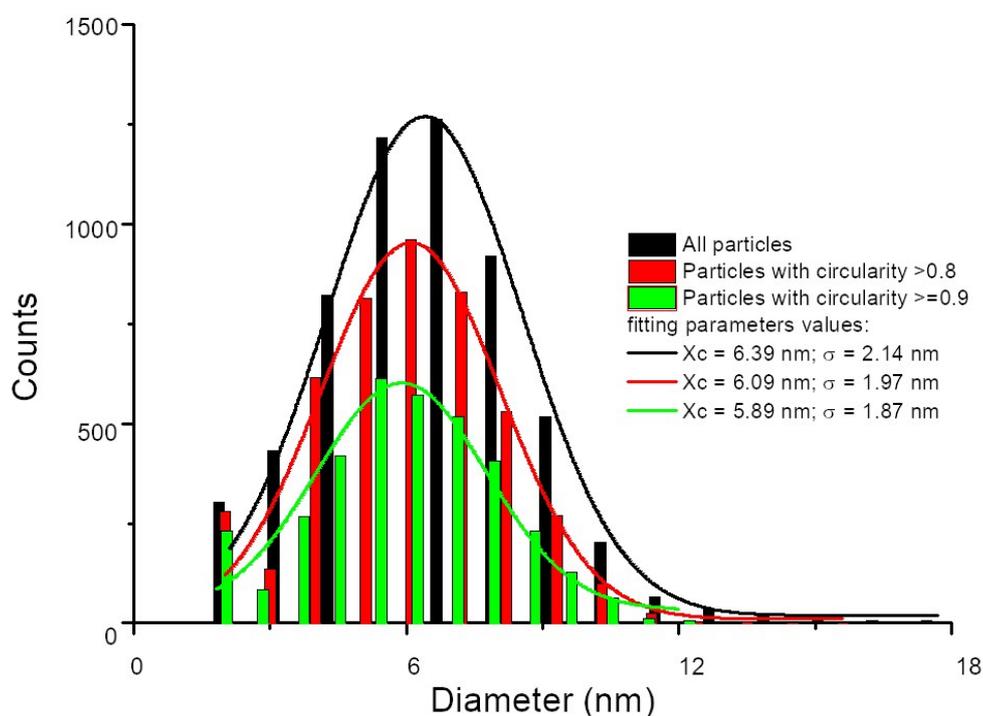


Figure S9. Distribution of TEM diameters size of AuNP@2, calculated from measured areas.

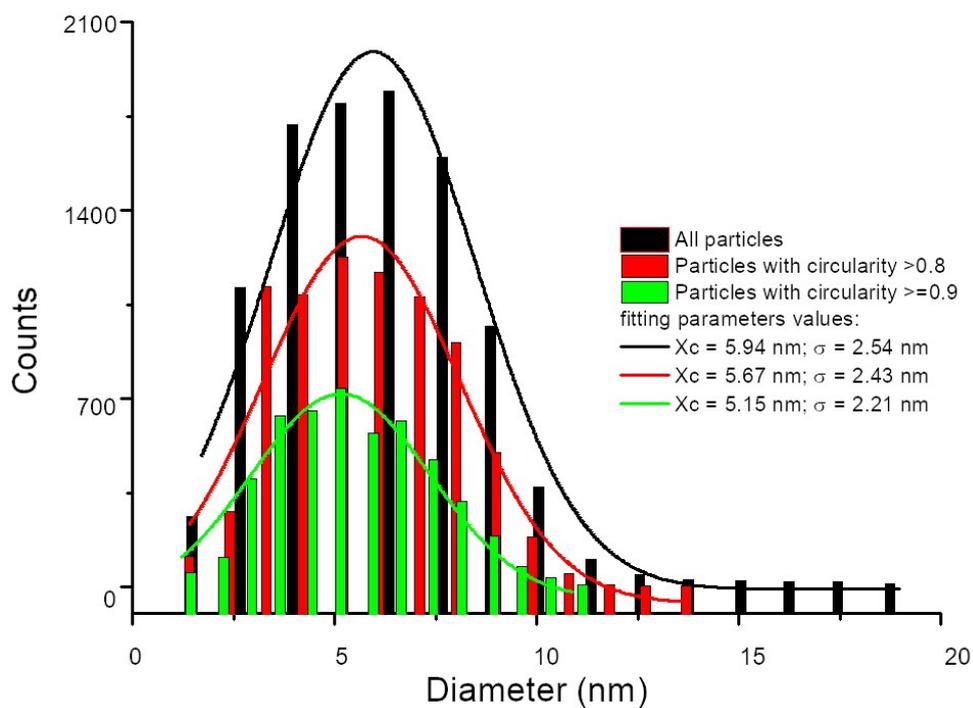


Figure S10. Distribution of TEM diameters size of AuNP@3, calculated from measured areas.

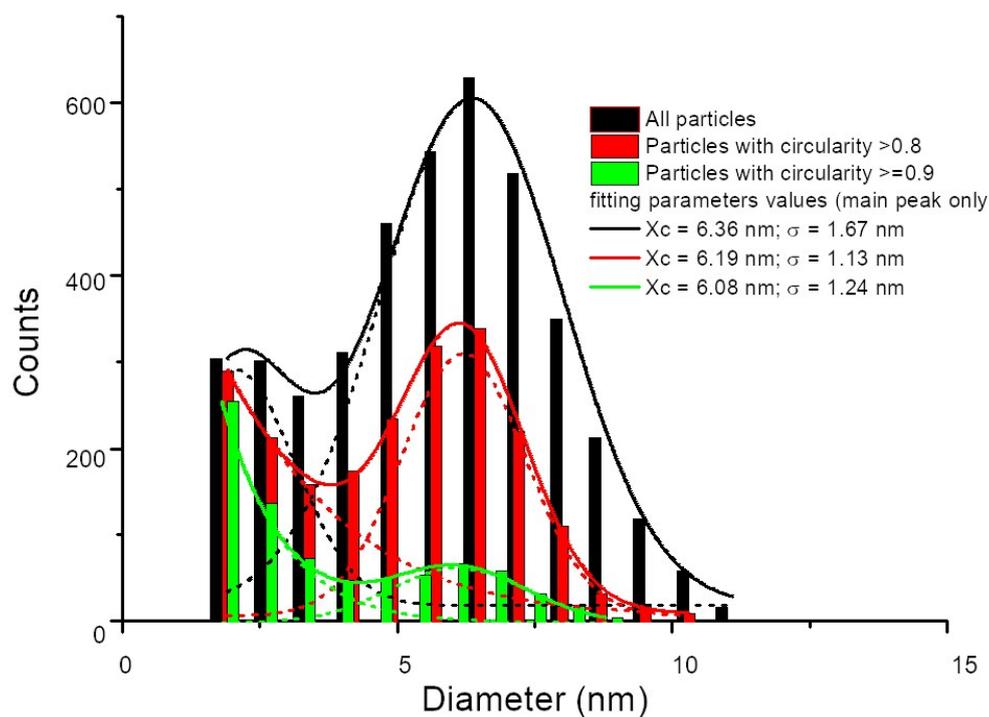
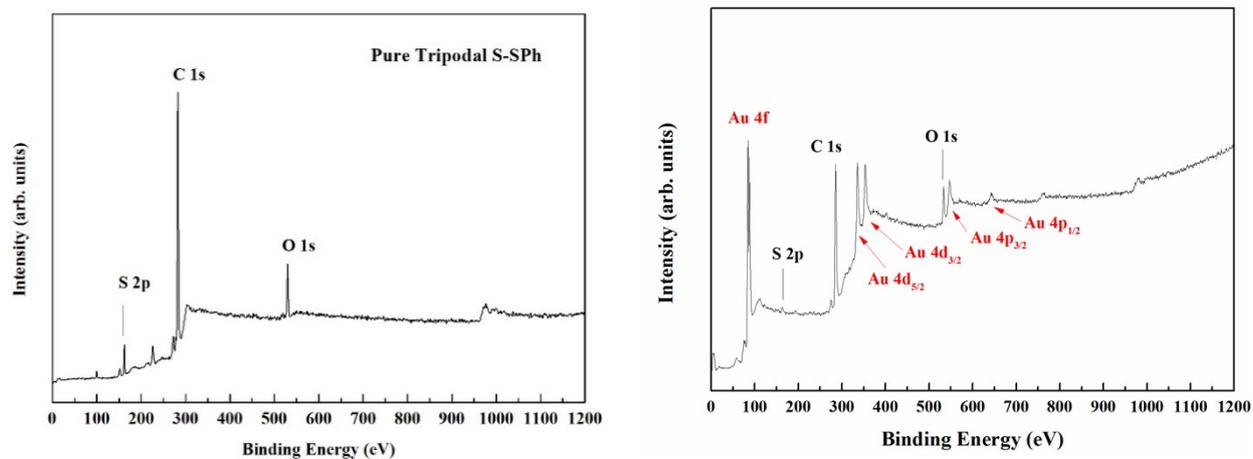


Figure S11. Distribution of TEM diameters size of AuNP@4, calculated from measured areas.



a) b)

Figure S12. XPS survey spectra of (a) pure tripodal ligand **2** and (b) AuNP@**2** samples.

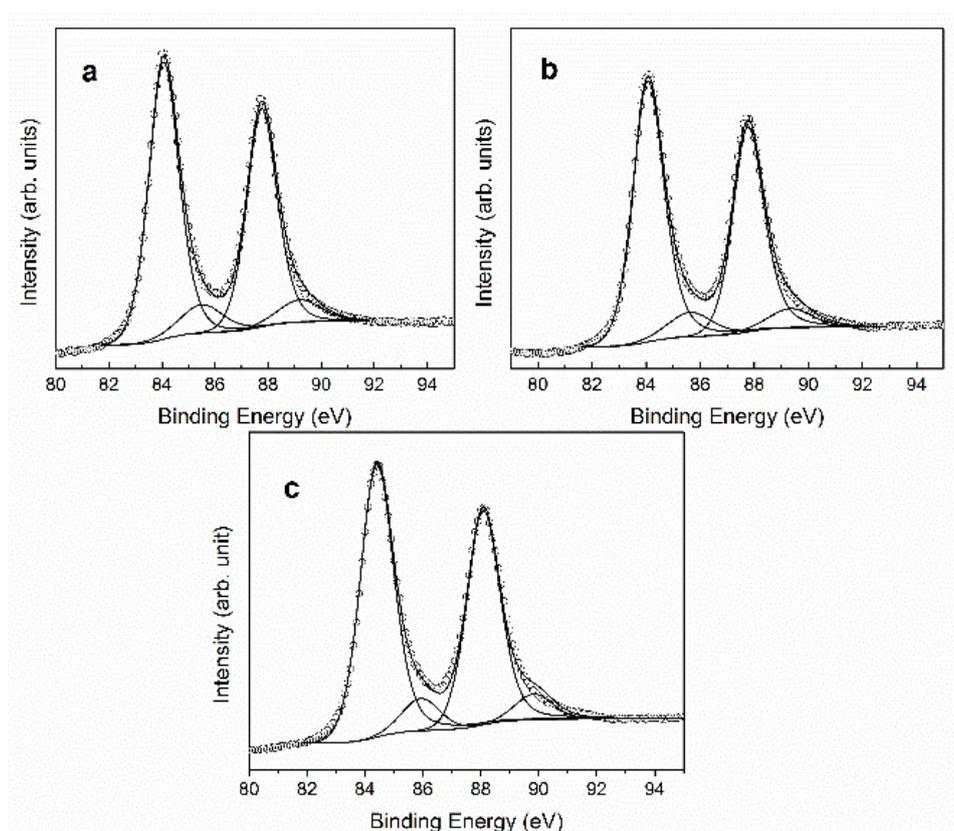


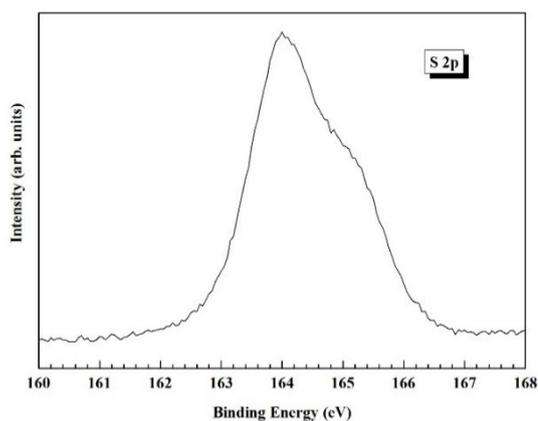
Fig. S13. XPS curve fitting of Au 4f spectra in (a) AuNP@**2**, (b) AuNP@**3** and (c) AuNP@**4** samples.

Table S1. XPS surface chemical composition of the investigated samples (at. %).

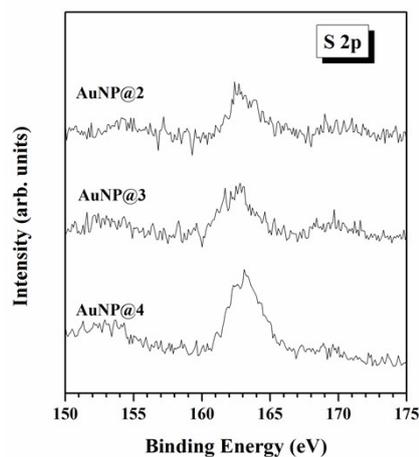
SAMPLE	C 1s	O 1s	S 2p	Au 4f
2	83.0	9.9	7.0	
AuNP@ 2	83.0	6.6	1.9	8.5
3	78.3	14.3	7.4	
AuNP@ 3	80.9	3.7	2.6	12.9
4	82.6	11.9	5.4	
AuNP@ 4	87.2	2.1	2.8	8.0

Table S2. XPS results of the curve fitting of Au 4f photoelectron signals (total peak area = 100%).

SAMPLES	BE		Peak area %	BE		Peak area %
	Au 4f _{7/2}	Au 4f _{5/2}		Au 4f _{7/2}	Au 4f _{5/2}	
AuNP@ 2	84.1	87.8	87.5	85.6	89.3	12.5
AuNP@ 3	84.1	87.8	88.7	85.6	89.3	11.3
AuNP@ 4	84.1	87.8	88.7	85.6	89.3	11.3



a)



b)

Figure S14. XPS S 2p spectra in (a) the pure tripodal **4** and (b) AuNP@**2**, AuNP@**3** and AuNP@**4** samples.

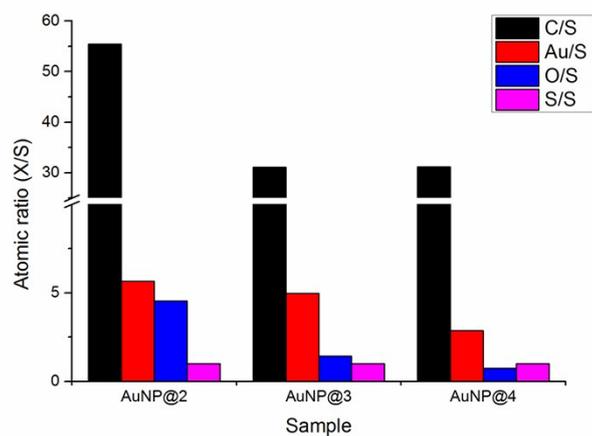


Figure S15. XPS quantitative analysis shown as atomic ratio X/S for AuNP@2, AuNP@3 and AuNP@4 samples.

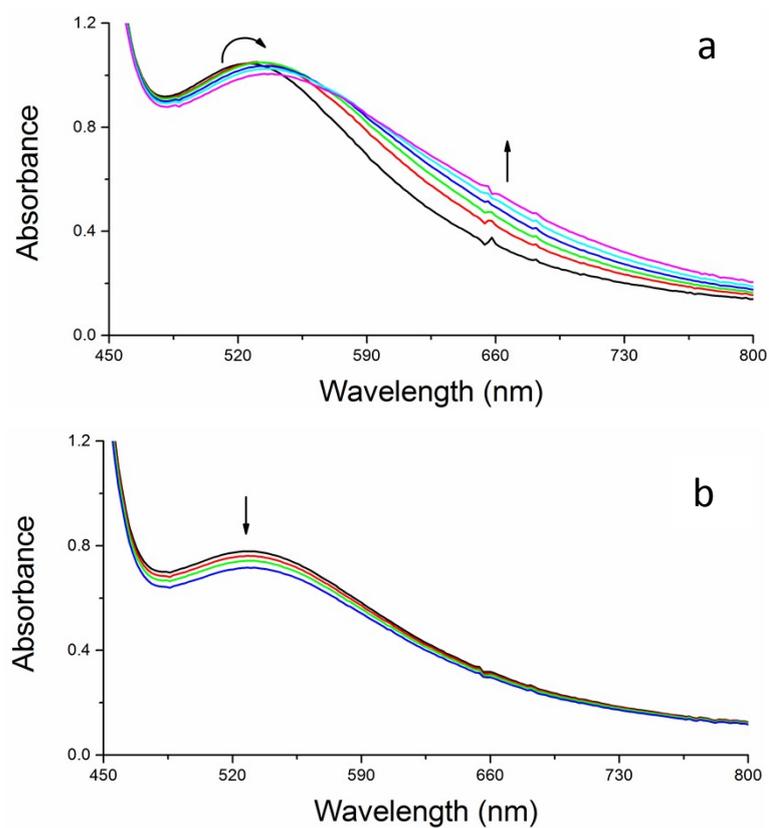


Figure S16. UV/Vis spectral changes relative to the addition of ammonium picrate to (a) AuNP@3 and (b) AuNP@4 in a toluene/acetone (1/1=v/v) solution with $[\text{NH}_4\text{C}_6\text{H}_2\text{N}_3\text{O}_7]=1$ mM, observed during 90 minutes following the addition of the salt ($t_s=10$ minutes).