Supplementary Information to

Controlled Functionalisation of Gold Nanoparticles with Mixtures of Calix[4]arenes revealed by Infra-red Spectroscopy

Hennie Valkenier,[a] Volodymyr Malytskyi,[b] Pascale Blond,[b] Maurice Retout,[a] Alice Mattiuzzi,[b,e] Jonathan Goole,[c] Vincent Raussens,[d] Ivan Jabin,[b] Gilles Bruylants[a]

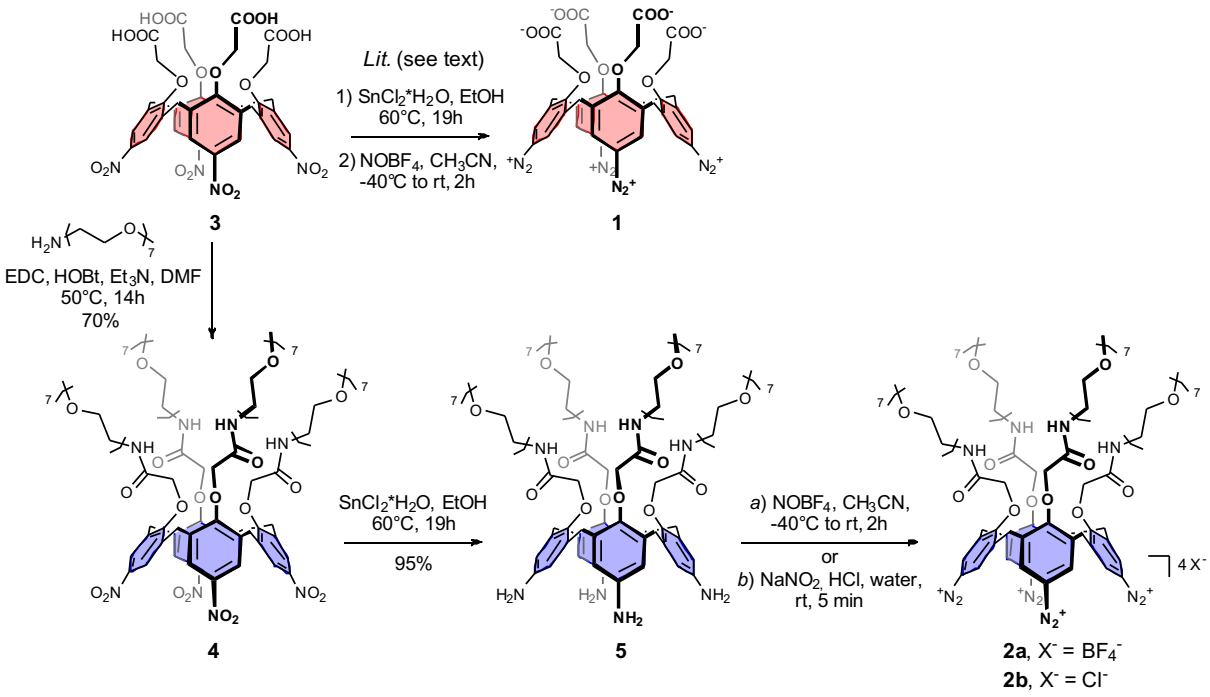
a. *Engineering of Molecular NanoSystems, Ecole Polytechnique de Bruxelles, Université Libre de Bruxelles (ULB), Avenue F.D. Roosevelt 50, CP165/64, B-1050 Brussels, Belgium.*
b. *Laboratoire de Chimie Organique, Université Libre de Bruxelles (ULB), Avenue F.D. Roosevelt 50, CP 160/06, B-1050 Brussels, Belgium.*
c. *Laboratory of Pharmaceutics and Biopharmaceutics, Faculty of Pharmacy, Université libre de Bruxelles (ULB), Brussels, Belgium*
d. *Laboratory for the Structure and Function of Biological Membranes, Center for Structural Biology and Bioinformatics, Université Libre de Bruxelles (ULB), B-1050 Bruxelles, Belgium.*
e. *X4C, Rue Chêne Bonnet 128, 6110 Montigny-le-Tilleul, Belgium.*

Table of Contents

1. Synthesis and characterisation of 2

Instrumentation

NMR spectra were recorded on a Bruker Avance 300 (7.0 T) spectrometer. Traces of residual solvents were used as internal standards for ^{1}H (7.26 ppm for $CHCl_3$, 4.79 ppm for HDO) and ^{13}C (77.16 ppm for $CDCl_3$, the spectrum of **2b** in D_2O was referenced against added CH_3OH at 49.50 ppm) chemical shift referencing. Abbreviations: s: singlet, d: doublet, t: triplet, br: broad, m: multiplet. The high-resolution mass spectra were recorded with an Agilent QTOF 6520 spectrometer. ATR-FTIR spectra for the characterisation of organic compounds were recorded on a Bruker ALPHA FTIR spectrophotometer at room temperature.



Caution! *Although we have not encountered any problem, it is noted that diazonium salts derivatives are potentially explosive and should be handled with appropriate precautions.*

Compounds **3** and **1** were prepared accordingly to previously described procedures.[2]

[2] (a) Mattiuzzi, A.; Jabin, I.; Mangeney, C.; Roux, C.; Reinaud, O.; Santos, L.; Bergamini, J.-F.; Hapiot, P.; Lagrost, C. *Nat Commun* **2012**, *3*, 1130; (b) Troian-Gautier, L.; Valkenier, H.; Mattiuzzi, A.; Jabin, I.; Van den Brande, N.; Van Mele, B.; Hubert, J.; Reniers, F.; Bruylants, G.; Lagrost, C.; Leroux, Y. *Chem. Commun.* **2016**, *52*, 10493–10496.

2,5,8,11,14,17,20-heptaoxadocosan-22-amine ($NH_2(CH_2CH_2O)_7Me$) was purchased from BioMatrik and used as received. Other reagents and solvents for the syntheses were at least of reagent grade quality and were used without purification.

Calix[4]arene tetra-oEG$_7$Me-tetra-NO$_2$ (4)

Calix[4]arene tetra-acid-tetra-NO_2 **3** (0.300 g, 0.36 mmol, 1 equiv.) and HOBt (0.243 g, 1.8 mmol, 5 equiv.) were dissolved in 6 mL of anhydrous DMF under inert atmosphere. 2,5,8,11,14,17,20-heptaoxadocosan-22-amine ($NH_2(CH_2CH_2O)_7Me$) (0.611 g, 1.8 mmol, 5 equiv.) was dissolved in 6 mL of anhydrous DMF under inert atmosphere and added to the calix[4]arene solution. EDC·HCl (0.345 g, 1.8 mmol, 5 equiv.) and NEt_3 (0.50 mL, 3.6 mmol, 10 equiv.) were added and the mixture was stirred at 50°C overnight. The reaction mixture was concentrated at reduced pressure, the residue dissolved in 30 mL of DCM, and washed 2 times with 40 mL of demineralized water. The aqueous phase was extracted once with 20 mL of DCM, organic phases were combined and evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel using a mixture of DCM with 4% of MeOH as eluent to yield compound **4** as a yellow oil-like liquid (0.536 g, 0.25 mmol, 70%). FTIR, ν (cm^{-1}): 3077, 2917, 2871, 1674, 1531, 1346, 1099; ^{1}H NMR (300 MHz, $CDCl_3$, 298K), δ (ppm): 3.36 (s, 12H, -OC*H*$_3$), 3.47-3.63 (m, 116H, -OC*H*$_2$CH$_2$- + -NC*H*$_2$- + ArC*H*$_{2eq}$Ar), 4.65 (s, 8H, -OC*H*$_2$CONH-), 4.84 (d, ^2J = 14.4 Hz, 4H, ArC*H*$_{2ax}$Ar), 7.59 (s, 8H, Ar*H*), 8.54 (t, ^3J = 5.4 Hz, 4H, -CON*H*-); ^{13}C NMR (75 MHz, $CDCl_3$, 298K), δ (ppm): 31.3, 39.3, 59.2, 69.7, 70.3, 70.6 – 70.7 (m), 72.1, 74.5, 124.6, 135.2, 143.6, 161.1, 168.3; HRMS: calcd. for $C_{96}H_{152}N_8O_{44}$ $(M+2H)^{2+}$ 1062.0040, found 1062.0047.

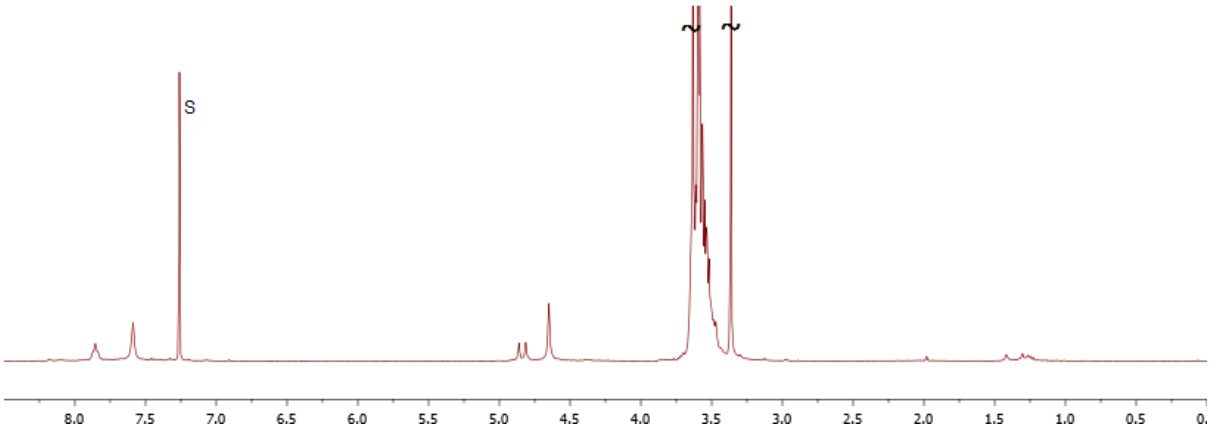


Figure S1. ^{1}H NMR spectrum of compound **4** (300 MHz, $CDCl_3$, 298K). S = solvent.

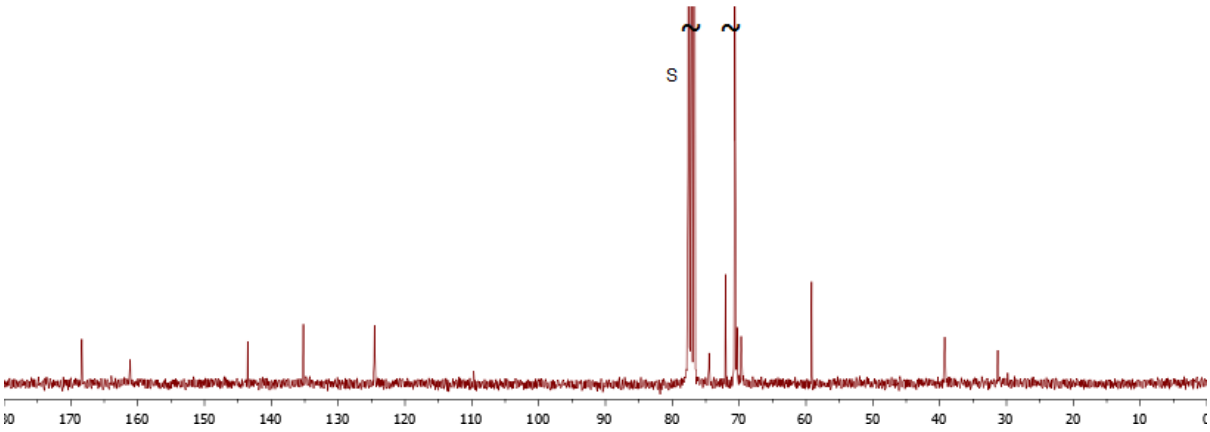


Figure S2. ^{13}C NMR spectrum of compound **4** (75 MHz, $CDCl_3$, 298K). S = solvent.

Calix[4]arene tetra-oEG$_7$Me-tetra-NH$_2$ (5)

Calix[4]arene tetra-oEG$_7$Me-tetra-NO$_2$ **4** (0.400 g, 0.19 mmol, 1 equiv.) and $SnCl_2 \cdot 2H_2O$ (0.852 g, 3.77 mmol, 20 equiv.) were suspended in absolute EtOH (20 mL) in a sealed flask. The reaction mixture was stirred at 60°C overnight. After being cooled to r.t. the mixture was basified by addition of 20 mL of 0.5 M sodium hydroxide aqueous solution (pH > 10), transferred to an extraction funnel and diluted with 50 mL of CH_2Cl_2. The organic phase was separated and the aqueous phase was extracted once more with 50 mL of CH_2Cl_2. Organic phases were combined and evaporated affording compound **5** as dark-yellow oil (0.358 g, 0.18 mmol, 95%). FTIR, ν (cm^{-1}): 3067, 2870, 1668, 1474, 1103; ^{1}H NMR (300 MHz, $CDCl_3$, 298K), δ (ppm): 3.01 (d, ^2J = 13.8 Hz, 4H, ArCH_{2eq}Ar) 3.37 (s, 12H, -OCH_3), 3.47-3.65 (m, 112H, -OCH_2CH$_2$- + -NCH_2-), 4.41 (s, 8H, -OCH_2CONH-), 4.46 (d, ^2J = 13.8 Hz, 4H, ArCH_{2ax}Ar), 6.04 (s, 8H, Ar*H*), 7.75 (br t, 4H, -CON*H*-); ^{13}C NMR (75 MHz, $CDCl_3$, 298K), δ (ppm): 31.4, 39.0, 59.2, 69.9, 70.2, 70.6 – 70.7 (m), 72.1, 74.5, 116.5, 134.8, 141.3, 150.0,[3] 170.2; HRMS: calcd. for $C_{96}H_{160}N_8O_{36}$ $(M+2H)^{2+}$ 1002.0557, found 1002.0562.

[3] determined from HMBC

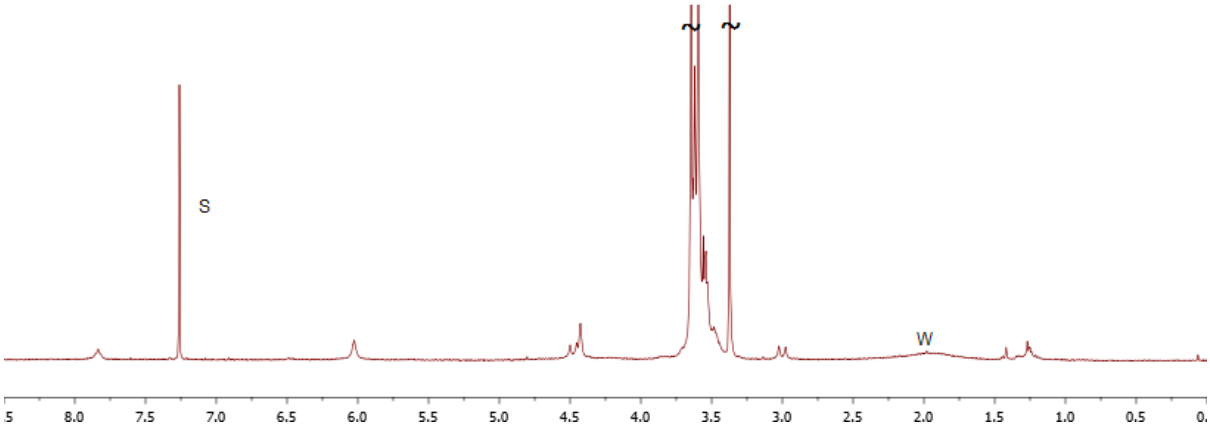


Figure S3. ^{1}H NMR spectrum of compound **5** (300 MHz, $CDCl_3$, 298K). S = solvent, W = water.

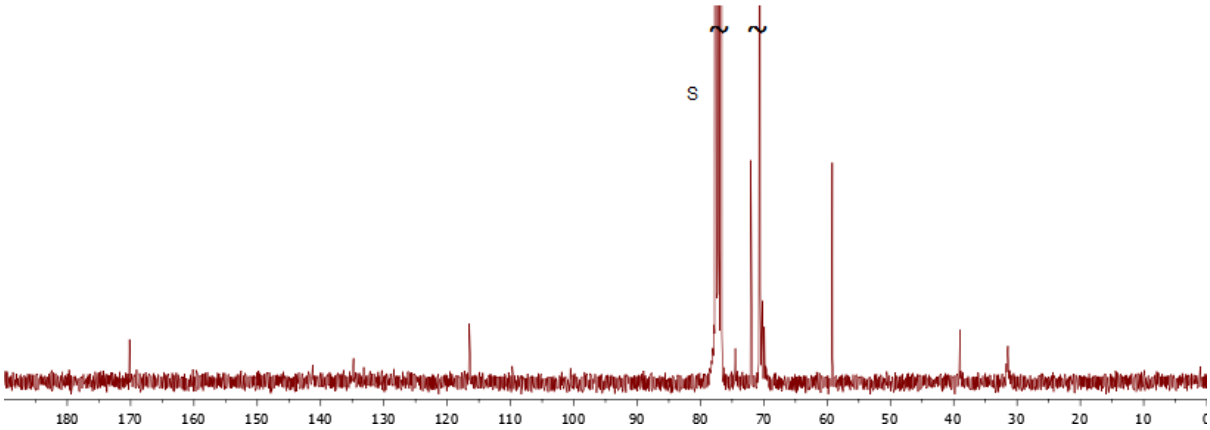


Figure S4. ^{13}C NMR spectrum of compound **5** (75 MHz, $CDCl_3$, 298K). S = solvent.

Calix[4]arene tetra-oEG$_7$Me-tetra-N$_2^+$ (2)

Method a) Calix[4]arene tetra-oEG$_7$Me-tetra-NH$_2$ **5** (0.033 g, 0.016 mmol, 1 equiv.) was solubilized in anhydrous acetonitrile (1 mL) and $NOBF_4$ (0.0154 g, 0.132 mmol, 8 equiv.) was added at -40°C under Ar. After 30 minutes, the mixture was warmed slowly to room temperature and stirred 1.5 h. The solution was concentrated under reduced pressure and the residue washed twice with 1 mL of Et_2O to yield compound **2a** as a yellow oil (0.044 g, quant.).

Method b) Calix[4]arene tetra-oEG$_7$Me-tetra-NH$_2$ **5** (0.1076 g, 0.054 mmol, 1 equiv.) was dissolved in D_2O (4.86 mL) in a round-bottom flask. The solution was acidified with deuterium chloride (85 µL, 20% w/w solution in D_2O) and a sodium nitrite solution (430 µL of 1 M solution in D_2O, 8 equiv.) was added. The mixture was stirred for 5 minutes at r.t. NMR analysis of an aliquot has proved complete formation of the desired diazonium salt. The product **2b** has not been isolated and the diazonium-containing solution was directly used for AuNPs modification.

FTIR, ν (cm^{-1}): 2924, 2882, 2274, 1668, 1449, 1081; ^{1}H NMR (300 MHz, D_2O, 298K), δ (ppm): 3.3 (s, 12H, -OCH_3), 3.40 (br t, 8H, -NCH_2-), 3.50-3.75 (m, 104H, -OCH_2CH_2-), 3.93 (d, ^{2}J = 15.6 Hz, 4H, ArCH_{2eq}Ar), 4.81(d, ^{2}J = 15.6 Hz, 4H, ArCH_{2ax}Ar), 4.93 (s, 8H, -OCH_2CONH-), 8.29 (s, 8H, Ar*H*); ^{13}C NMR (75 MHz, $CDCl_3$, 298K), δ (ppm): 30.9, 39.6, 58.7, 69.3, 70.1, 70.2 (m), 71.6, 74.9, 108.9, 134.8, 138.2, 166.9, 169.4. HRMS analysis was not performed due to thermal instability of the compound **2**.

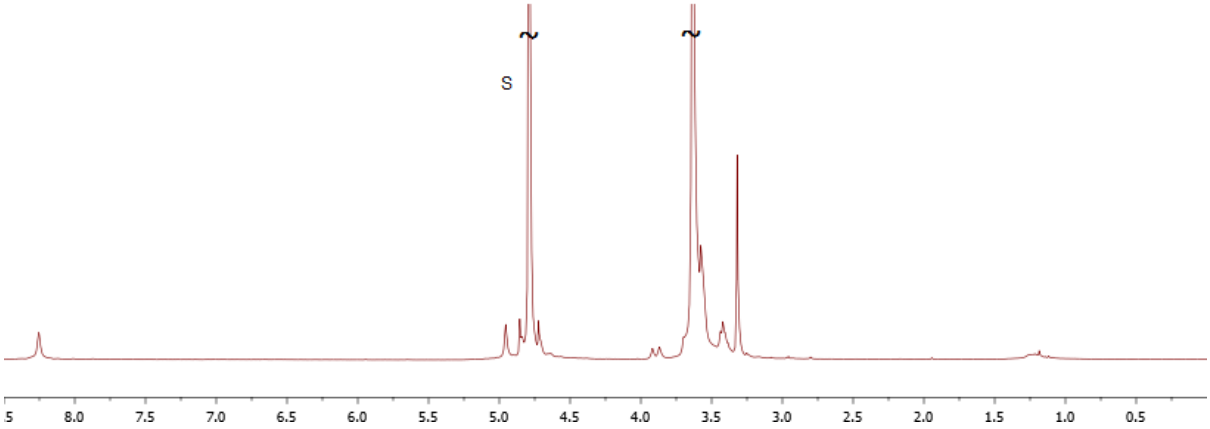


Figure S5. ^{1}H NMR spectrum of compound **2b** (300 MHz, D_2O, 298K). S = solvent.

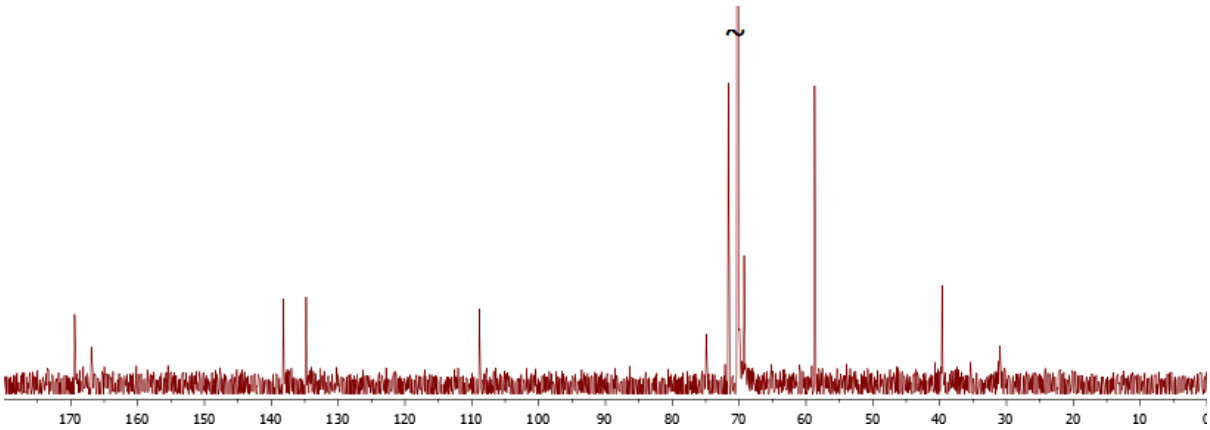


Figure S6. ^{13}C NMR spectrum of compound **2b** (75 MHz, D_2O, 298K).

2. Characterisation of AuNPs-calix(oEG)$_4$ with TEM

TEM images were obtained with a Philips CM20-UltraTWIN microscope equipped with a lanthanum hexaboride (LaB6) crystal at 200 kV accelerating voltage.

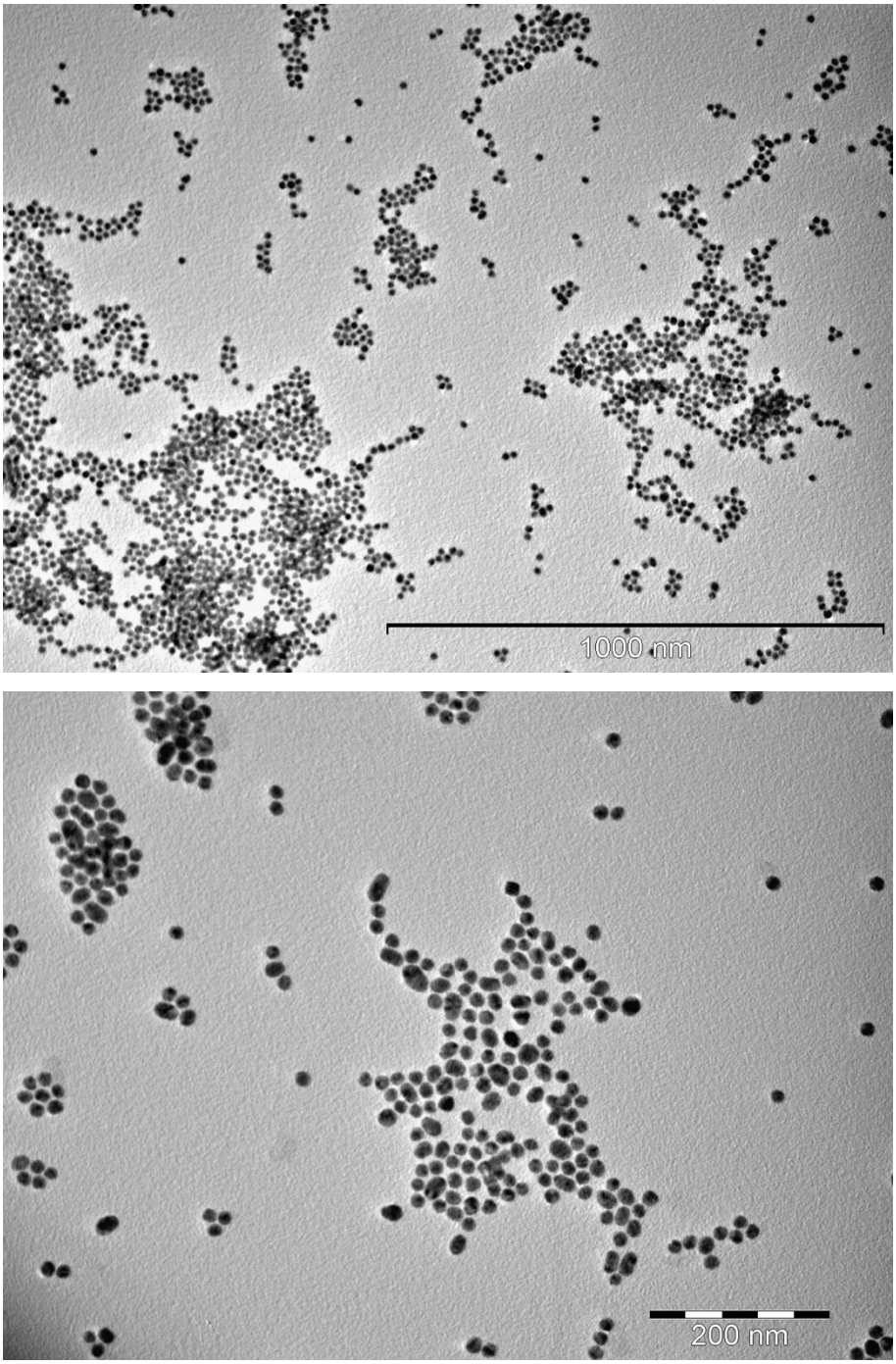


Figure S7. TEM micrographs of two different batches of AuNPs-calixPEG, magnified 53000x (top) or 88000x (bottom).

3. Characterisation of AuNPs- calix(oEG)$_4$ by TGA

Grafting of AuNPs-calix(oEG)$_4$ on large scale for TGA.

To the gold nanoparticles (17 nm, 21.5 mL, 21 nM, 0.45 nmol) in a glass vial was added a solution of $NaBH_4$ (90 µL, 0.3 M, 0.027 mmol) in water upon magnetic stirring. The acidic solution of calix(oEG)$_4$(N_2^+)$_4$ **2b** (5.4 mL, 10 mM, 0.054 mmol) was neutralised by addition of phosphate buffer (6 mL, 0.2 M, pH 6.7) to obtain a final pH of 6.4. The calixarene solution was added dropwise to the AuNPs, resulting in effervescence of the red colloidal suspension. The reaction mixture was stirred overnight (20 h), followed by the centrifugation of the mixture for 30 minutes at 16500 G, after which the supernatant was removed. The functionalised AuNPs were washed by 8 cycles of resuspension in NaOH (15 mL, 1 mM), subsequent centrifugation (30 min at 16500 G), and removal of the supernatant. For the TGA experiments, the AuNPs were washed an additional 4 times with water (1.5 mL). The functionalised AuNPs were stored in water at room temperature and further concentrated to ~40 µL prior to the TGA measurements.

TGA measurements

The thermogravimetric analyses (TGA) were performed on a TA Instruments Q5000 TGA with air as purge gas (25 mL/min). A suspension of AuNPs in 40 µL water was placed in a DSC crucible (Tzero) and the water was evaporated in a vacuum oven by slowly decreasing the pressure and increasing the temperature. When the dark red liquid had turned into a gold colored film, the crucible was further dried for 30 minutes at 10 mbar and 75 °C. The crucible was loaded in the TGA instrument and a 2 h isotherm was measured at 60 °C, followed by a linear ramp up to 600 °C at a rate of 10°C/min, a 60 min isotherm at 600 °C, cooling, and another 2 h isotherm at 60 °C. The loss of organic material was obtained from the difference in weight at the plateaus of the 60 °C isotherms (average of last 20 minutes) before and after heating.

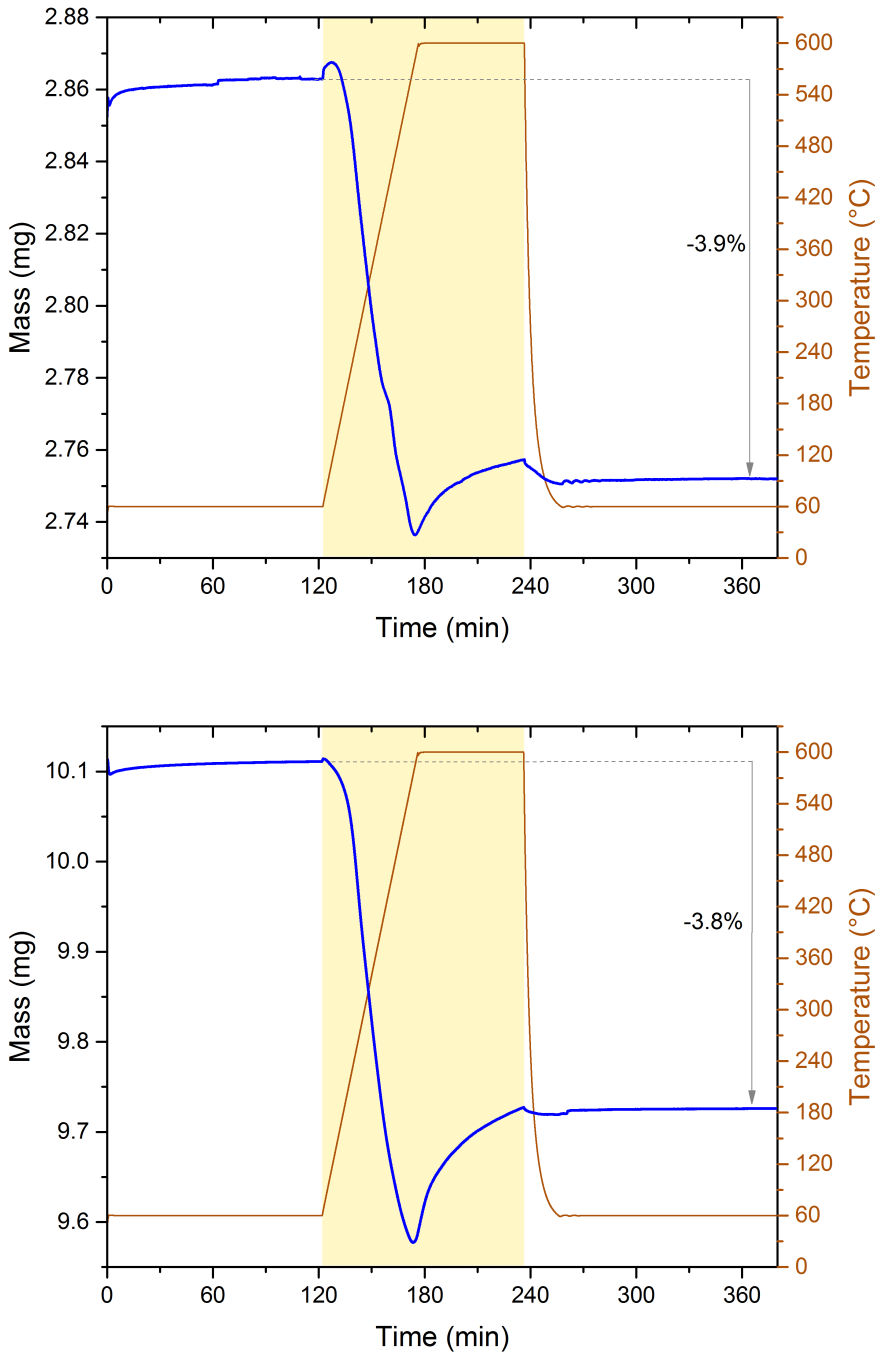


Figure S8. Thermogravimetric analysis of two batches of AuNPs-calix(oEG)$_4$.

The TGA data were used to determine the grafting density of the calixarenes on the surface of the AuNPs. The average diameter of the AuNPs was determined from TEM images, after which the molar mass of the AuNPs (MW_{AuNP}) was calculated as follows:

$$MW_{AuNP} = \frac{4}{3}\pi r^3 \cdot D \cdot N_A \qquad (1)$$

where r is the average radius of the AuNPs, D is the density of gold (19.3 g/cm^3), and N_A is Avogadro's number. Knowing the molar weight of the calix[4]arene(oEG)$_4$ (here assumed to be 1940.3 g/mol when bound to a gold surface), it is thus possible to determine the number of calixarenes per AuNP when we assume that the mass lost during the TGA experiment is corresponding to the mass of the calixarene and the mass remaining is corresponding to the total mass of the AuNPs:

$$Calixarenes\ per\ AuNP = \frac{Mass_{lost}/MW_{calixarene}}{Mass_{remaining}/MW_{AuNP}} \qquad (2)$$

And from that, the density of calixarenes on the surface of the AuNPs can be readily calculated:

$$Grafting\ density = \frac{Calixarenes\ per\ AuNP}{A} \qquad (3)$$

where A is the surface area per AuNP ($A = 4\pi r^2$).

Table S1. Structural parameters of AuNPs-calix(oEG)$_4$, determined from TEM and TGA analyses.

Calixarene	Diameter [nm][a]	Au [%]	Calix / AuNP	Calix / nm^2
Calix(oEG)$_4$	17.3 ± 1.4	96.1	650 ± 50	0.7
Calix(oEG)$_4$	16.9 ± 2.2	96.2	600 ± 20	0.7

[a] The errors are obtained from the comparison of the TGA curve for coated AuNPs to the curve obtained on an empty crucible.

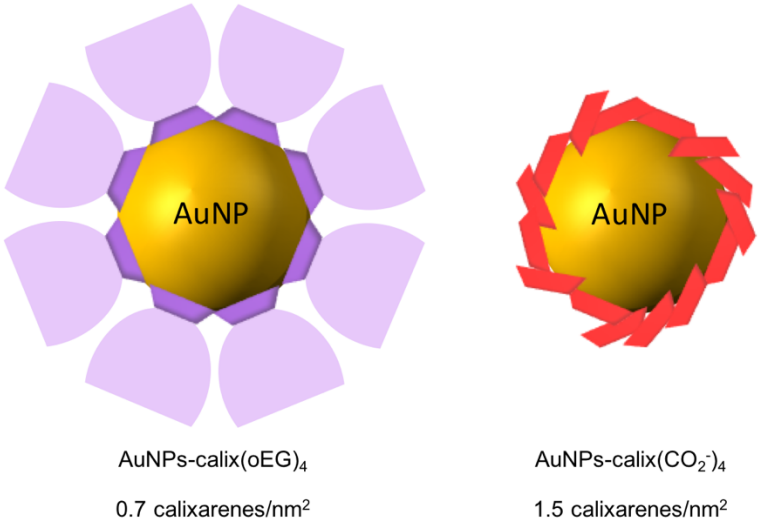


Figure S9. Cartoons showing the different grafting densities of calix(oEG)$_4$ and calix(CO_2^-)$_4$.

4. Stability of AuNPs-calix(oEG)$_4$

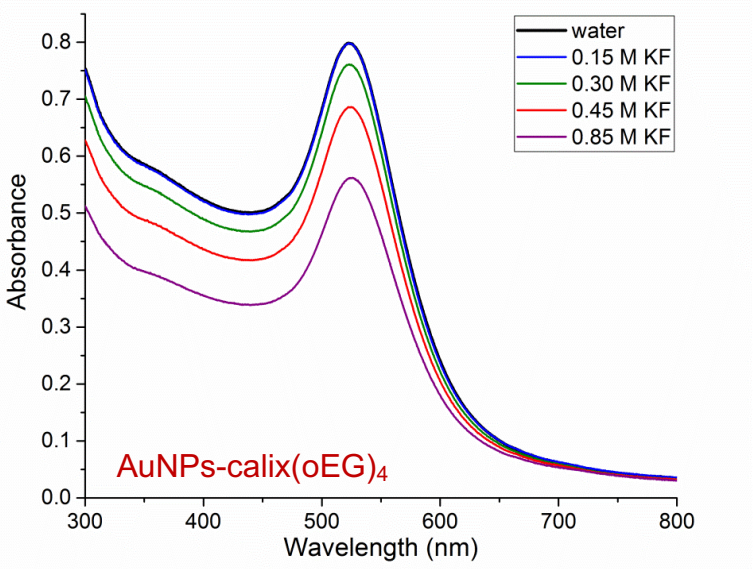


Figure S10. UV-Visible absorbance spectra of AuNPs-calix(oEG)$_4$ suspended in water (black) and upon addition of a solution of KF up to 0.85 M concentration, showing stable AuNPs dispersions. The decrease in intensity upon increasing concentrations of KF is attributed to the adsorption of AuNPs to the walls of the cuvette.

For comparison, AuNPs in citrate were functionalised with $HS(CH_2CH_2O)_7CH_3$ upon grafting of the thiol overnight (1.5 mL, [AuNPs] = 17 nM, [thiol] = 0.68 mM) and four washing cycles with water. KF was added to these AuNPs (Figure S11).

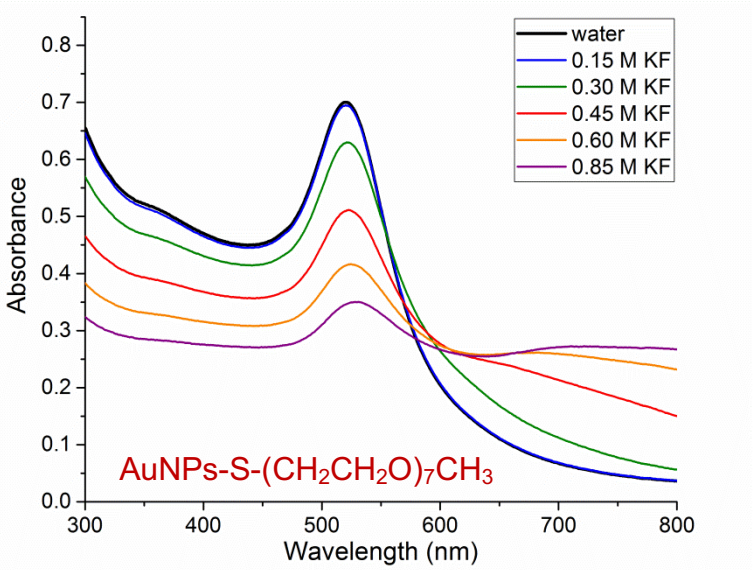


Figure S11. UV-Visible absorbance spectra of AuNPs with oEG-thiols suspended in water (black) and upon addition of a solution of KF up to 0.85 M concentration, showing aggregation at fluoride concentrations of 0.3 M and above.

Drying of the AuNPs into a film and resuspension

Similar to the experiment reported previously to AuNPs-calix(CO_2^-)$_4$,[1b] we have allowed a dispersion of AuNPs-calix(oEG)$_4$ to dry inside a cuvette. Subsequent addition of a solution of NaOH (0.05 M) and sonication led to the recovery of well-dispersed AuNPs (Figure S12).

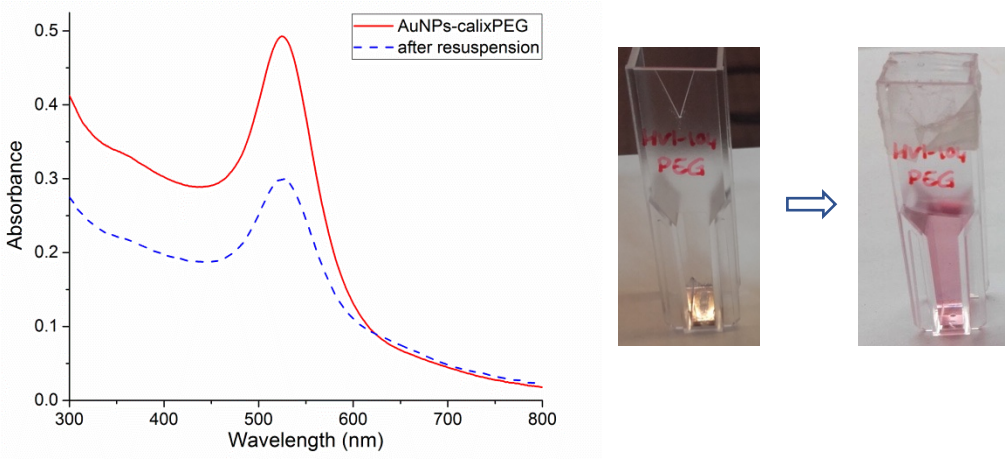


Figure S12. UV-Visible absorbance spectra of AuNPs- calix(oEG)$_4$ suspended in water (red) and after drying + resuspension in 0.05 M NaOH (blue dashed). Photos of AuNPs- calix(oEG)$_4$ allowed to dry into a gold-coloured film (left), and after resuspension in 0.05 M NaOH (right).

5. UV-Vis absorbance spectra of AuNPs with mixtures of calixarenes

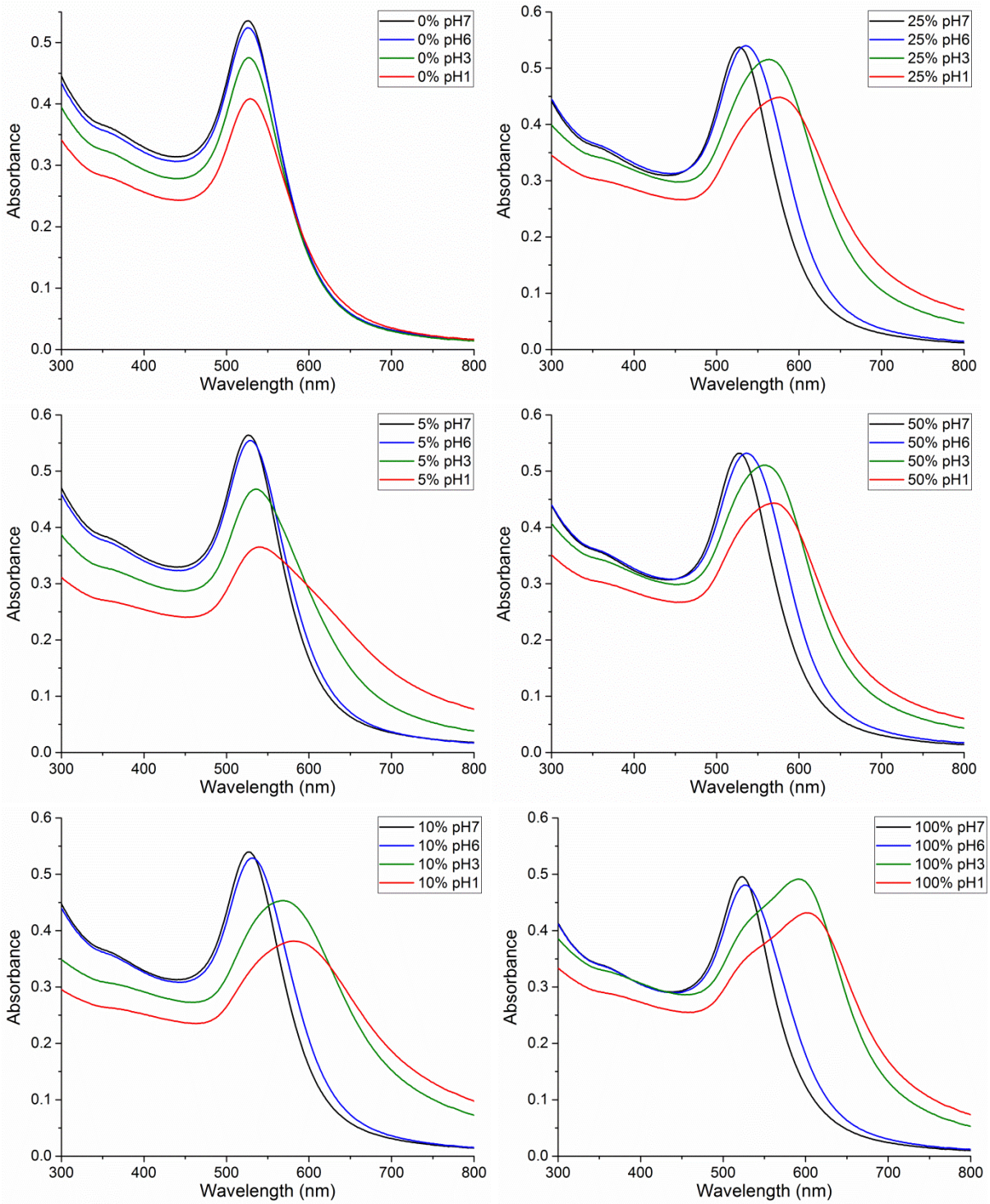


Figure S13. UV-Visible absorbance spectra of AuNPs with mixtures 0% calix$(CO_2^-)_4$ (thus calix$(oEG)_4$ only) to 100% (calix$(CO_2^-)_4$ suspended in water at neutral pH (black) and under acidic conditions obtained by the addition of HCl to obtain pH 6 (blue), pH 3 (green), and pH 1 (red). The spectra were recorded 8 minutes after the adjustment of the pH, which was measured with pH indicator strips (for pH 0-6).

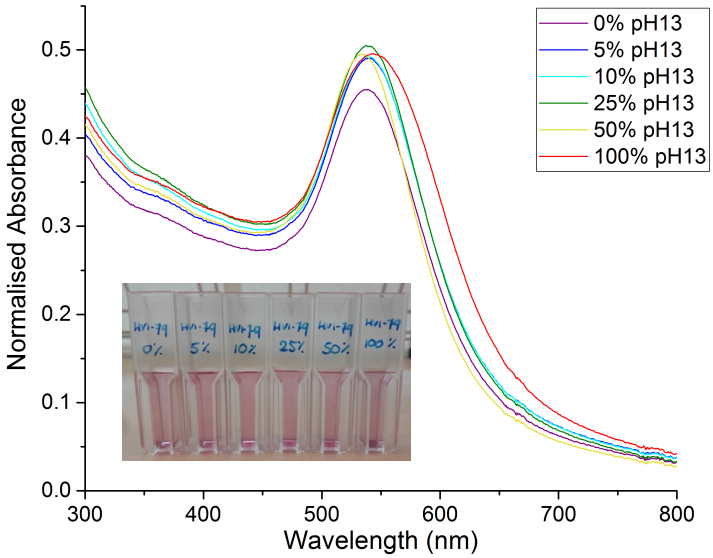


Figure S14. UV-Visible absorbance spectra of AuNPs with mixtures 0% calix(CO_2^-)$_4$ to 100% (calix(CO_2^-)$_4$ after firstly decreasing their pH to 1 by addition of HCl and subsequently increasing the pH to 13 by addition of NaOH. The inset shows an image of the cuvettes with AuNPs dispersions after the addition of NaOH, indicating that the aggregation of the AuNPs is reversible.

6. Infra-Red measurements

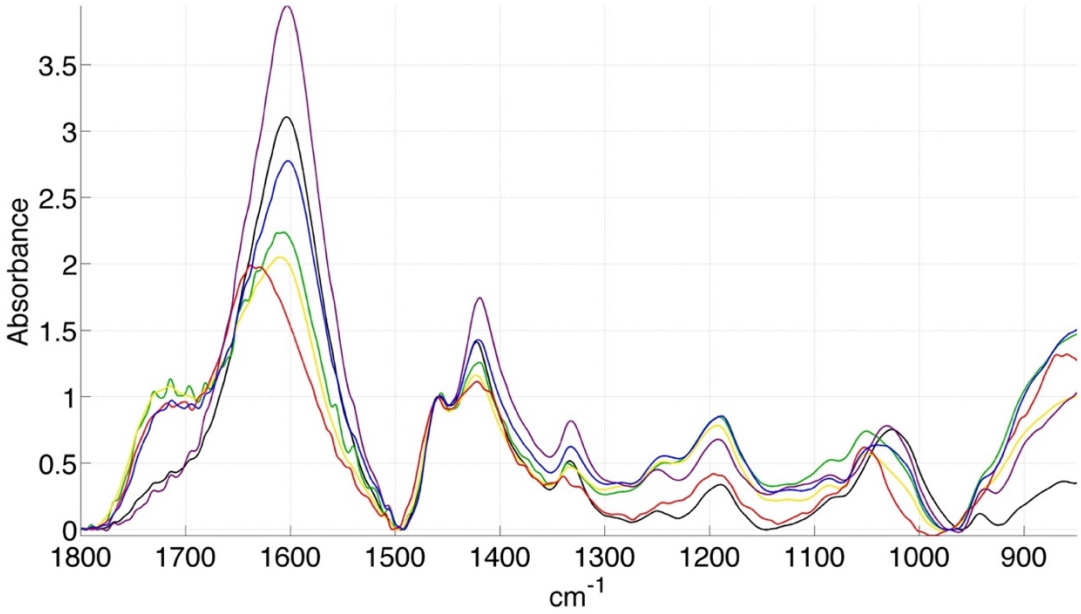


Figure S15. FTIR absorbance spectra of AuNPs-calix(CO_2^-)$_4$ deposited from solutions of which the pH was adjusted by addition of HCl or NaOH, to obain pH 2.7 (red), 6.7 (yellow), 7.7 (green), 8.9 (blue) and 9.7 (purple), or from a solution in D_2O (black, pD 8)

Table S1. Quantification of the IR absorbances of AuNPs with mixtures of calix(CO_2^-)$_4$ and calix(oEG)$_4$ and the calculated ratio of these calixarenes from the average of the four quantified signals.

calix(CO_2^-)$_4$ / calix(oEG)$_4$	**COC_{oEG} ν_{asym}** (1105 cm^{-1})		**Amide I** (1669 cm^{-1})		**$CO_2^-\nu_{asym}$** (1604 cm^{-1})		**$CO_2^-\nu_{sym}$** (1420 cm^{-1})		Average ratio of calixarenes < CO_2^->	<oEG$^-$>
0/100	1.83	0/100	1.87	0/100	0.88	0/100	0.73	0/100	0%	100%
5/95	1.67	10/90	1.69	14/86	1.07	13/87	0.83	13/87	12 ± 2%	88 ± 2%
10/90	1.50	19/81	1.70	14/86	1.19	21/79	0.94	26/74	20 ± 5%	80 ± 5%
25/75	1.30	31/69	1.55	25/75	1.33	31/69	0.97	30/70	30 ± 3%	70 ± 3%
50/50	1.10	43/57	1.35	41/59	1.64	53/47	1.10	46/54	46 ± 5%	54 ± 5%
100/0	0.14	100/0	0.60	0%	2.32	100/0	1.54	100/0	100%	0%

Table S2. Assignments of the IR bands

cm^{-1}\ **Calix**	$(CO_2^-)_4$	$(CO_2H)_4$	$(oEG)_4$	Assignment	Why
2919	X	X	X	CH_2 ν_{asym}	Reference 4, 5
2852	X	X	X	CH_2 ν_{sym}	Reference 4, 5
1730			X	?	Present on AuNPs with oEGs, but not on calix-oEG diazonium salt **2a**
1720		X		C=O ν from COOH	Present at low pH, not in D_2O
1669			X	Amide I	Relates to amount of amide, Reference 5
1640-1610	X	X	X	C_{Ar}-C_{Ar} ν	Reference 6, 7
1604	X			CO_2^- ν_{asym}	pH dependent; disappears in amide, Reference 5
1540			X	Amide II	Relates to amount of amide, Reference 5
1459	X	X	X	C_{Ar}-C_{Ar} ring stretch	Reported for other tetra-subsituted aromatics (like 2,4,6-tribromophenol in SDBS spectral database), Reference 6
1420	X			CO_2^- ν_{sym}	pH dependent; disappears in amide, Reference 5
1350			X	OCH_2 $_{wagging}$ of oEG	Reference 4
1330	X			CO_2^-	pH dependent; weak in amide
1289	X	X	X	CC ν + $C_{Ar}C_{Ar}H$ δ	Reference 6
1247	X	X	X	$C_{Ar}C_{Ar}H$ δ	Reference 6
1188-1196	1188	1188	1196	COC_{Ar} ν_{asym}	Reference 5
1105			X	COC ν_{asym} of oEG	Reference 4
1039-1020	1020	1050	1038	COC_{Ar} ν_{sym}	Reference 5

[4] Devouge, S.; Conti, J.; Goldsztein, A.; Gosselin, E.; Brans, A.; Voué, M.; De Coninck, J.; Homblé, F.; Goormaghtigh, E.; Marchand-Brynaert, J. Surface Functionalization of Germanium ATR Devices for Use in FTIR-Biosensors. *J. Colloid Interface Sci.***2009**, *332*, 408–415.

[5] Silverstein, R. M.; Webster, F. X.; Kiemle, D. *Spectrometric Identification of Organic Compounds, 7th Edition*; Wiley, **2005**.

[6] Furer, V. L.; Borisoglebskaya, E. I.; Zverev, V. V.; Kovalenko, V. I. The Hydrogen Bonding and Conformations of P-Tert-Butylcalix[4]Arene as Studied by IR Spectroscopy and by DFT Calculations. *Spectrochim. Acta Part A* **2005**, *62*, 483–493.

[7] Collins, G.; Fleming, P.; O'Dwyer, C.; Morris, M. A.; Holmes, J. D. Organic Functionalization of Germanium Nanowires Using Arenediazonium Salts. *Chem. Mater.* **2011**, *23*, 1883–1891.