Supplementary Material (ESI) for Chemical Communications

Supporting Information

Double Phase Transfer of Gold Nanorods for Surface Functionalization and Entrapment into PEG-based Nanocarriers

Denis Gentili, Guido Ori, and Mauro Comes-Franchini*

Department of Organic Chemistry "A. Mangini", University of Bologna, Viale Risorgimento 4, 40136 Bologna, Italy and Department of Materials and Environmental Engineering, University of Modena e Reggio Emilia, Via Vignolese 905/A, 41100 Modena, Italy

E-mail Address: mauro.comesfranchini@unibo.it

1. Experimental Section

Material and Methods: All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used as received (CTAB H6269). Poly(D,L-lactide–co–glycolide) (50/50) with carboxylic acid end group (PLGA-COOH, inherent viscosity 0.12 dL/g, MW~7 kDa) was purchased from Lakeshore Biomaterials (Birmingham, AL, USA). Polyethylene glycol with amino and carboxylic acid end groups (NH_2-PEG-COOH, MW~3 kDa) was purchased from Rapp Polymere GmbH (Tübingen, Germany). All aqueous solutions were prepared with deionized water obtained using an ultrafiltration system (Milli-Q, Millipore) with a measured resistivity above 18 MΩ. THF was distilled from sodium/benzophenone just prior to use and stored under Ar. CH_2Cl_2 and $CHCl_3$ were passed through basic alumina prior to use. Other solvents were purified by standard procedures. Light petroleum ether refers to the fraction with bp 40-60°C. The reactions were monitored by TLC performed on silica gel plates (Baker-flex IB2-F). Column chromatography was performed with Merck silica gel 60 (70-230 mesh). Melting points were determined with a Büchi melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded using $CDCl_3$ or DMSO solutions at 300, 400 and 600 MHz for ^{1}H and 75.46,

100.6 and 150.92 MHz for ^{13}C. Chemical shifts (δ) are reported in ppm relative to $CHCl_3$ (δ = 7.26 for ^{1}H and δ = 77.0 for ^{13}C). Fourier transform infrared (FTIR) spectra were recorded on a Perkin-Elmer Spectrum 2000. UV-Vis spectra were recorded on a Perkin-Elmer Spectrometer Lambda 12. Mass spectra were obtained with an electrospray ionization source (ESIMS). All the ESIMS spectra were performed using MeOH as the solvent. Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) was performed on a Liberty 200 Varian. High Resolution Transmission Electron Microscopy (HRTEM) was conducted on a Jeol JEM 2010 at 200 keV. Sample were prepared by dipping 200 mesh carbon-Formvar coated copper grid (200C-FC) directly in GNR solutions. Oil-in-water emulsion process was performed on a microtip probe sonicator Misonix Sonicator 3000 and dispersion (when necessary) on a bath sonicator Branson 2200. DLS measurement were performed on a Malvern Zetasizer nano-S working with a 532 nm laser beam. XPS data were recorded with a double pass Perkin Elmer PHI 15-255G cylindrical-mirror electron analyzer (CMA) operated at constant pass energy and carried out with non-monochromatic Mg K_α photons (hν = 1253.6 eV) from a Vacuum Generators XR3 dual anode source operated at 15 kV, 18 mA, in ultra-high-vacuum (UHV) at a base pressure of 10^{-9} mbar. Bromine 3d (Br_{3d}) and Gold 4f (Au_{4f}) core level scans were recorded with a resolution of 0.2 eV, using pass energies of 50, 100 and 200 eV. Several drops of concentrated GNRs solution were deposited on a silicon wafer and then inserted into the XPS sample chamber.

Synthesis of water-soluble GNRs: According to El-Sayed et al,[1] gold nanorods were prepared by seed-mediated growth method, in different aspect ratio (AR). In a typical synthesis, seed solution was prepared by quick addition of 600 μL of ice-cold sodium borohydride 10 mM to 10 mL of a vigorous stirred 100 mM CTAB and 0.25 mM $HAuCl_4$ aqueous solution. Stirring, after 2 minutes, was stopped and seed solution was kept undisturbed for 10 minutes at 25 °C. Growth solution was prepared by addition of different amount of silver nitrate 4 mM (i.e. 10 mL to have about AR = 4) to 500 mL of 100 mM CTAB and 0.5 mM $HAuCl_4$ aqueous solution by stirring. Afterwards, 3.5 mL of L-ascorbic acid aqueous solution 0.0788 mM was added under vigorous stirring at 25 °C and 600 μL of seed solution was added when growth solution became colourless. Stirring, after 5 minutes, was stopped and growth solution was kept undisturbed over night at 30 °C. GNRs were centrifuged (6000 rpm, 99 min), washed one time with water and redispersed in water to have a final volume of 50 mL and about 10 mM CTAB. GNRs solution was stored at 4°C and used in the following step without further

[1] B. A. Nikoobakht, M. A. El-Sayed, M. A. *Chem. Mater.,* **2003**, *15*, 1957.

purification. During storage slight precipitation was observed, but with gentle heating GNRs were easily redispersed and used in the following steps.

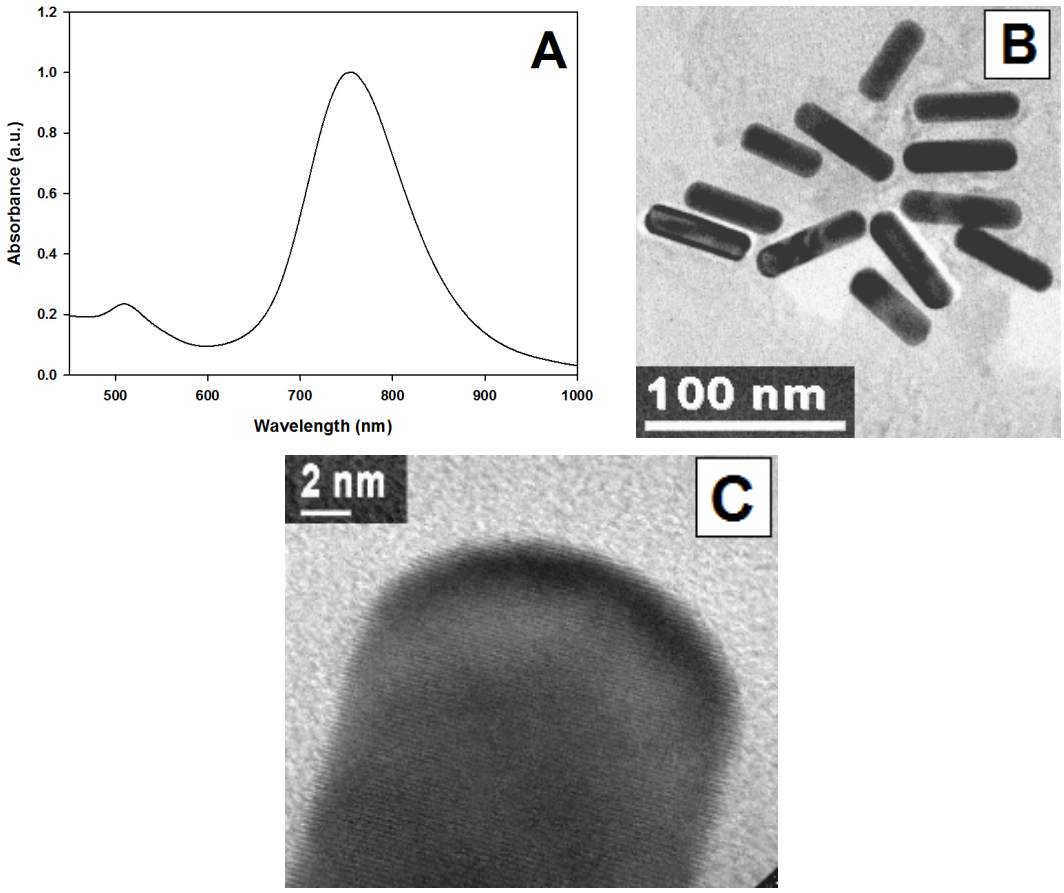
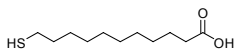
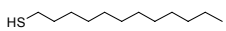
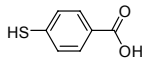
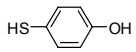
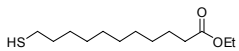
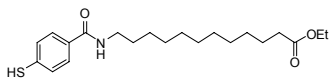
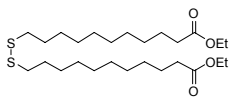
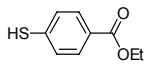


Figure S1. Representative UV-Vis spectra (A) and HRTEM (B, C) of GNRs-CTAB.

Optimization ligand exchange:

Table S1. List of ligands used.

ligand	name
	11-mercaptoundecanoic acid (**MUA**)
	dodecane-1-thiol (**DT**)
	4-mercaptobenzoic acid (**MBA**)
	4-mercaptophenol (**MP**)
	ethyl 11-mercaptoundecanoate (**1**)
	ethyl 12-(4-mercaptobenzamido)dodecanoate (**2**)
	diethyl 11,11'-disulfanediyldiundecanoate (**3**)
	ethyl 4-mercaptobenzoate (**6**)

Optimization ligand exchange procedure:

Ligand exchange was performed by adding one volume of GNRs aqueous solution to 0.5, 1 or 2 volumes of ligand (**MUA**, **DT**, **MBA**, **MP**, **1**, **3** and **6**) ethanol solution to obtain final concentration of ligand reported in Table 2. Ligands and GNRs were left to react overnight at room temperature on a rotating wheel. Excess ligand and CTAB were removed by centrifugation (6000 rpm 60 min) and washing with ethanol (3 x 2 volumes) and GNRs were redispersed in one volume of anhydrous chloroform. When possible GNRs chloroform solutions were characterized by UV-Vis (from 450 to 900 nm). Control samples were performed without ligand in the same way (Table S2 entries 43-45).

Table S2. Optimization ligand exchange results.

entry	ligand	E/W	ligand/ CTAB	$[ligand]_{final}$ (mM)	$CHCl_3$ dispersion	entry	ligand	E/W	ligand/ CTAB	$[ligand]_{final}$ (mM)	$CHCl_3$ dispersion
1	**MUA**	1	0.6	3.0	aggregate	25	**1**	1	0.6	3.0	good
2	**MUA**	0.5	0.6	4.0	aggregate	26	**1**	0.5	0.6	4.0	aggregate
3	**MUA**	2	0.6	2.0	aggregate	27	**1**	2	0.6	2.0	good
4	**MUA**	1	1	5.0	aggregate	28	**1**	1	1	5.0	good
5	**MUA**	0.5	1	6.7	aggregate	29	**1**	0.5	1	6.7	aggregate
6	**MUA**	2	1	3.3	aggregate	30	**1**	2	1	3.3	good
7	**DT**	1	0.6	3.0	aggregate	31	**3**	1	0.6	3.0	aggregate
8	**DT**	0.5	0.6	4.0	aggregate	32	**3**	0.5	0.6	4.0	aggregate
9	**DT**	2	0.6	2.0	aggregate	33	**3**	2	0.6	2.0	aggregate
10	**DT**	1	1	5.0	aggregate	34	**3**	1	1	5.0	aggregate
11	**DT**	0.5	1	6.7	aggregate	35	**3**	0.5	1	6.7	aggregate
12	**DT**	2	1	3.3	aggregate	36	**3**	2	1	3.3	aggregate
13	**MBA**	1	0.6	3.0	aggregate	37	**6**	1	0.6	3.0	aggregate
14	**MBA**	0.5	0.6	4.0	aggregate	38	**6**	0.5	0.6	4.0	aggregate
15	**MBA**	2	0.6	2.0	aggregate	39	**6**	2	0.6	2.0	aggregate
16	**MBA**	1	1	5.0	aggregate	40	**6**	1	1	5.0	aggregate
17	**MBA**	0.5	1	6.7	aggregate	41	**6**	0.5	1	6.7	aggregate
18	**MBA**	2	1	3.3	aggregate	42	**6**	2	1	3.3	aggregate
19	**MP**	1	0.6	3.0	aggregate	43	-	1			aggregate
20	**MP**	0.5	0.6	4.0	aggregate	44	-	0.5			aggregate
21	**MP**	2	0.6	2.0	aggregate	45	-	2			aggregate
22	**MP**	1	1	5.0	aggregate						
23	**MP**	0.5	1	6.7	aggregate						
24	**MP**	2	1	3.3	aggregate						

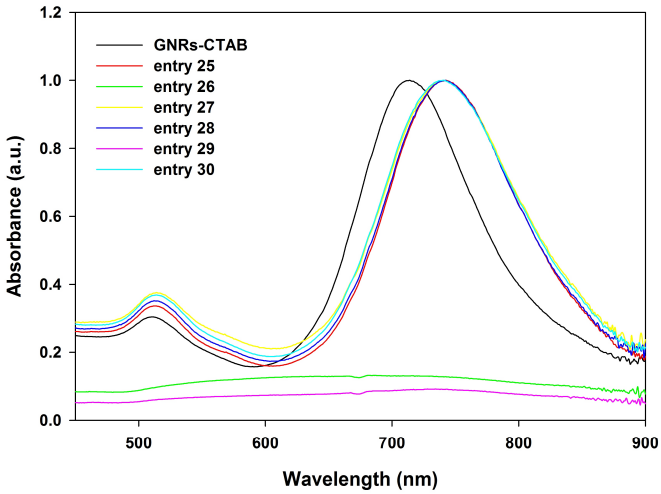


Figure S2. UV-Vis spectra of Table S2 entries 25-30 in $CHCl_3$ and GNRs-CTAB as control.

UV-Vis spectrum trend over the time

In a quartz cuvette (b=0.1 cm), one volume of GNRs-CTAB solution was added to one or half volume of **1** ethanol solution to obtain final concentrations of ligand 0.0 and 2.0 mM. GNRs solution was characterized by UV-Vis (from 450 to 1000 nm) at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 minutes and 1, 2, 3, 4, 5 and 24 hours.

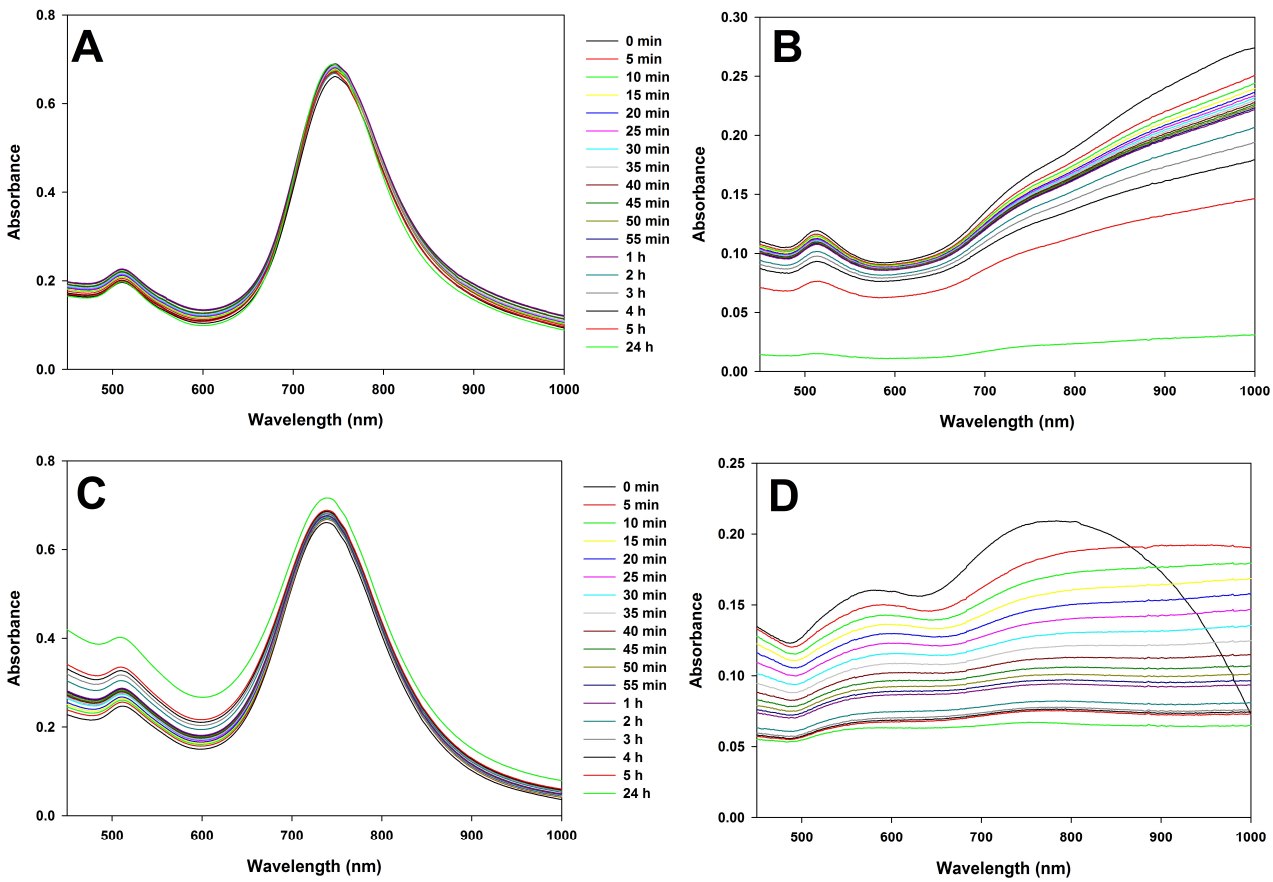


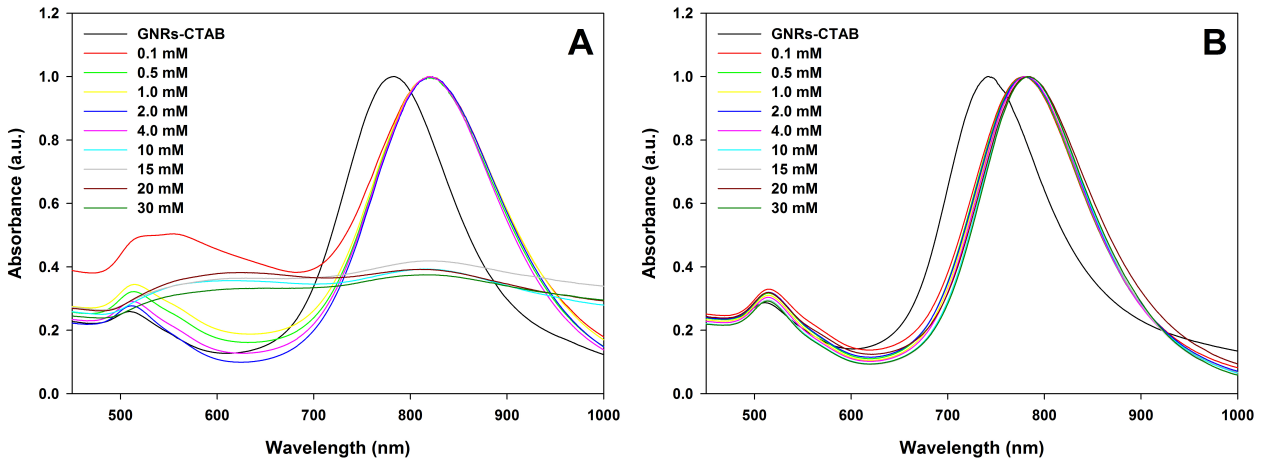
Figure S3. UV-Vis spectra of GNRs-CTAB with E/W = 0.5 and [**1**] = 0 mM (A), GNRs-CTAB with E/W = 1 and [**1**] = 0 mM (B), GNRs-CTAB with E/W = 0.5 and [**1**] = 2 mM (C) and GNRs-CTAB with E/W = 1 and [**1**] = 2 mM (D).

Ligand exchange:

Ligand exchange was performed by adding one volume of GNRs aqueous solution to one volume of ligand (**1** or **2**) ethanol solution to obtain final concentrations of ligand 0.1, 0.5, 1.0, 2.0, 4.0, 10, 15, 20 and 30 mM. Ligands and GNRs were left to react overnight at room temperature on a rotating wheel. Excess ligand and CTAB were removed by centrifugation (6000 rpm 60 min) and washing with ethanol (3 x 2 volumes) and GNRs were redispersed in one volume of anhydrous chloroform. When possible GNRs chloroform solutions were characterized by UV-Vis (from 450 to 1000 nm) and TEM.

Table S3. Ligand exchange results.

entry	ligand	E/W	$[ligand]_{final}$ (mM)	$CHCl_3$ dispersion	entry	ligand	E/W	$[ligand]_{final}$ (mM)	$CHCl_3$ dispersion
1	**1**	1	0.1	aggregate	10	**2**	1	0.1	good
2	**1**	1	0.5	good	11	**2**	1	0.5	good
3	**1**	1	1.0	good	12	**2**	1	1.0	good
4	**1**	1	2.0	good	13	**2**	1	2.0	good
5	**1**	1	4.0	good	14	**2**	1	4.0	good
6	**1**	1	10	aggregate	15	**2**	1	10	good
7	**1**	1	15	aggregate	16	**2**	1	15	good
8	**1**	1	20	aggregate	17	**2**	1	20	good
9	**1**	1	30	aggregate	18	**2**	1	30	good



Fig

Figure S4. UV-Vis spectra of Table S3 entries 1-9 (A) and entries 10-18 (B) in $CHCl_3$ and GNRs-CTAB as control.

FTIR:

GNRs-**1** and GNRs-**2** FTIR samples were prepared by $CHCl_3$ evaporation and CCl_4 dispersion by sonication for 30 min at 25 °C (bath sonicator).

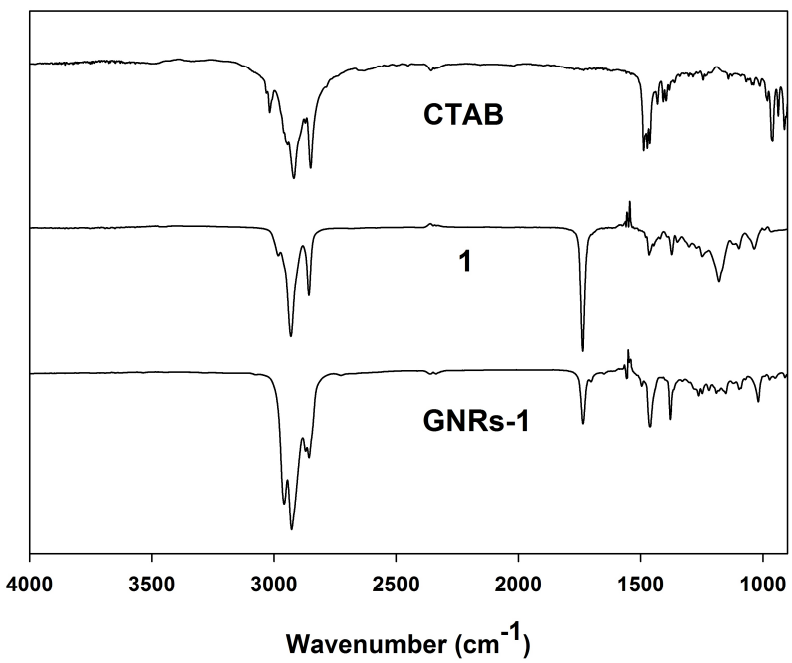


Figure S5. FTIR spectra of GNRs-**1**, neat **1** in CCl_4 and CTAB in KBr.

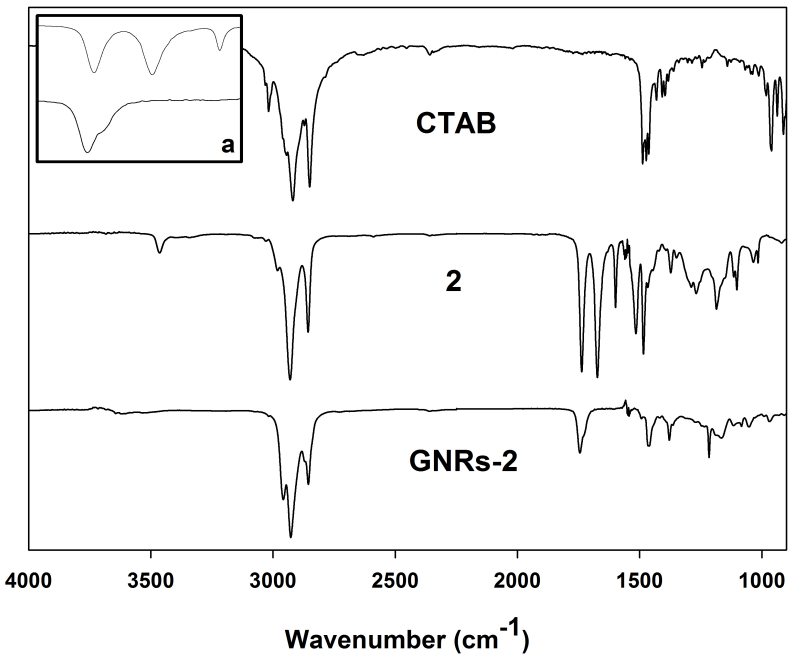


Figure S6. FTIR spectra of GNRs-**2**, neat **2** in CCl_4 and CTAB in KBr. FTIR spectra of GNRs-**2** (bottom) and neat **2** (top) in $CHCl_3$ from 1800 to 1575 cm^{-1} (inset a).

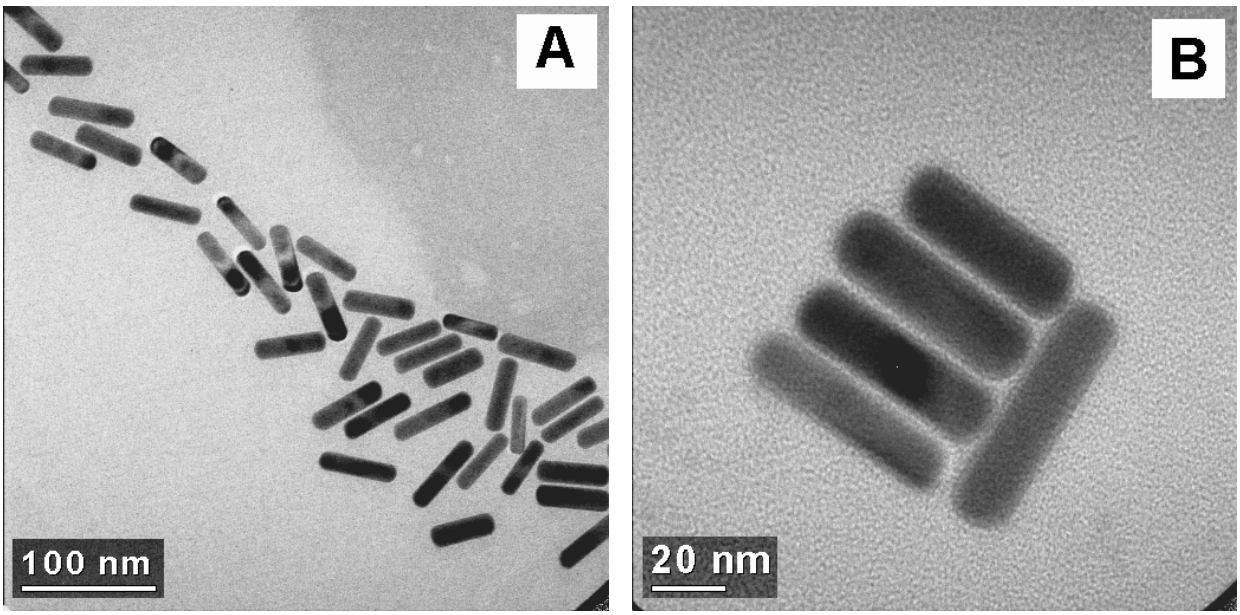


Figure S7. HRTEM (A and B) of GNRs-**2.**

Ligand exchange efficiency (ICP-AES):

The concentration of atomic gold in GNR solutions (aqueous or organic) was determined by ICP-AES titration at a λ_{Au} = 267,595 nm. Samples were prepared by addition of 2 mL of aqua regia to 0.5 mL of purified GNR solution. Table S4 shows typical concentrations before and after ligand exchange (mean values of three independent replicates with confidence intervals for 95% confidence level).

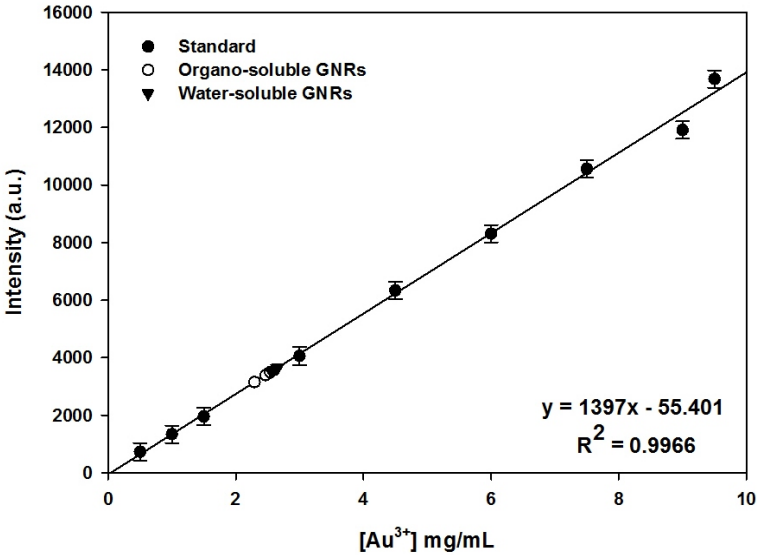


Figure S8. ICP-AES gold standard curve.

Table S4. Gold concentration

Sample	$[Au^{3+}]$ mg/mL
Water-soluble GNRs-CTAB	2.62 ± 0.06
Organo-soluble GNRs-**2**	2.49± 0.14

Refractive index sensitivity:

GNRs with an aspect ratio of 3.3 (length = 51 ± 5 nm and width = 16 ± 2 nm) have the plasmon wavelength at 746 nm in water (refractive index 1.333 at 20 °C) and 772 nm in chloroform (refractive index 1.445 at 20 °C) therefore we obtain a refractive index sensitivity of 232 nm/RIU.

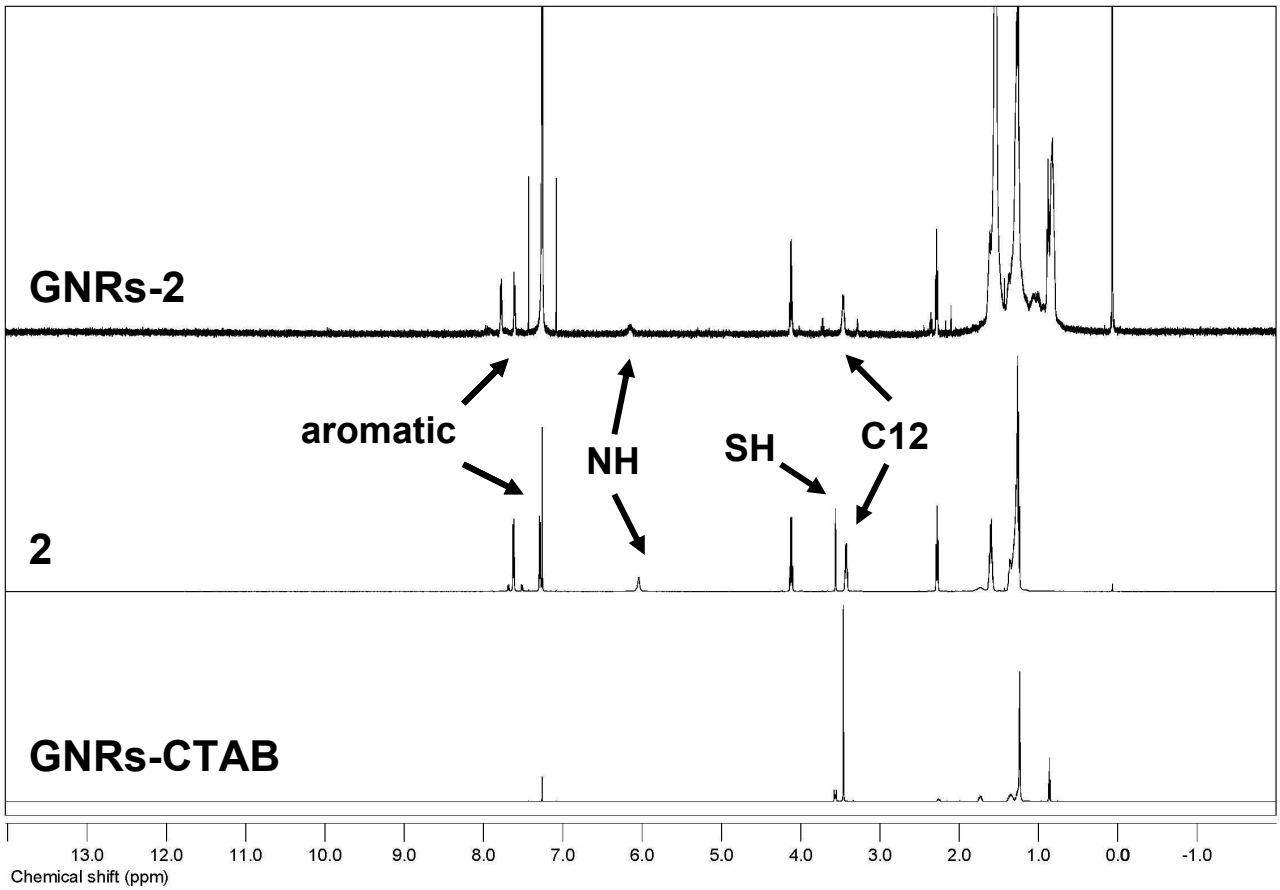


Figure S9. ^{1}H-NMR spectra of GNRs-**2**, neat **2** and CTAB in $CDCl_3$ (600 MHz).

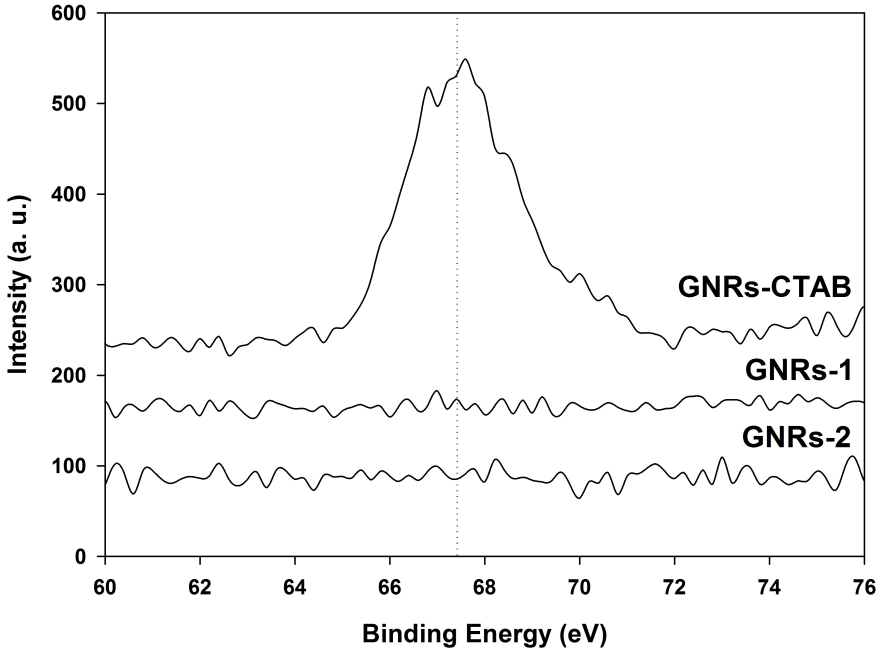


Figure S10. XPS Br 3d spectra of GNRs-CTAB, GNRs-**1** and GNRs-**2**.

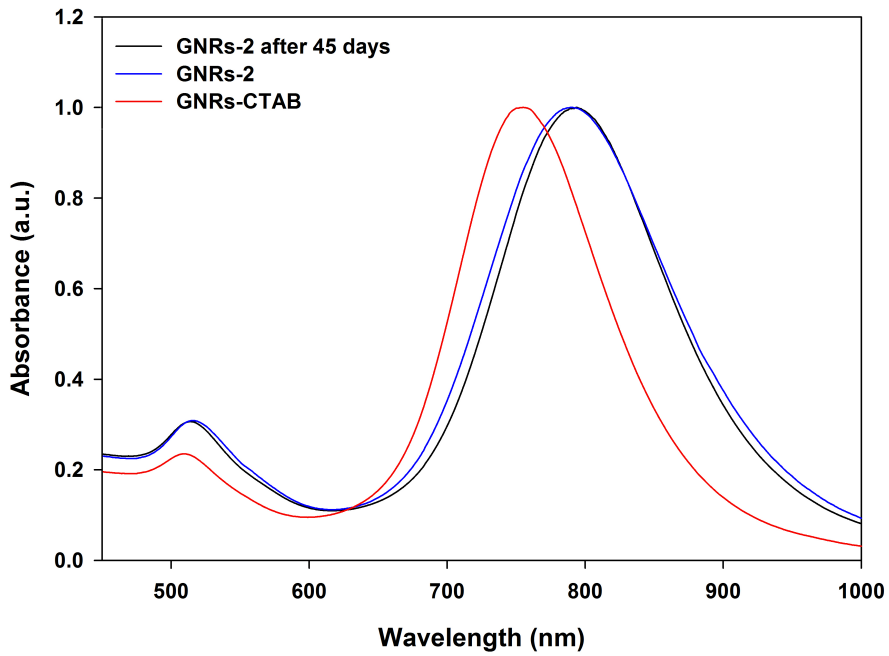


Figure S11. UV-Vis spectra of as-prepared GNRs-**2** and after 45 days.

Oil-water emulsion

0.2 mg of gold (GNRs-**2)** and 10 mg of PLGA-*b*-PEG-COOH (7 kDa-3 kDa) were dissolved in 1 mL of chloroform/dichloromethane 1:1. The organic phase was added to 10 mL of ultrapure water and after mutual saturation of the phases, the mixture was emulsified for 10 min with an tip probe sonicator operated at 600 W (input). Afterwards, residual organic solvent was evaporated under reduced pressure at 30 °C. The product was purified by filtration with regenerated cellulose membrane (0.45 μm), washed 3 times with 15 mL of ultrapure water by centrifugal filter devices (Amicon Ultra, Ultracel membrane with 100.000 NMWL, Millipore, Billerica, MA, USA) and stored at 4 °C.

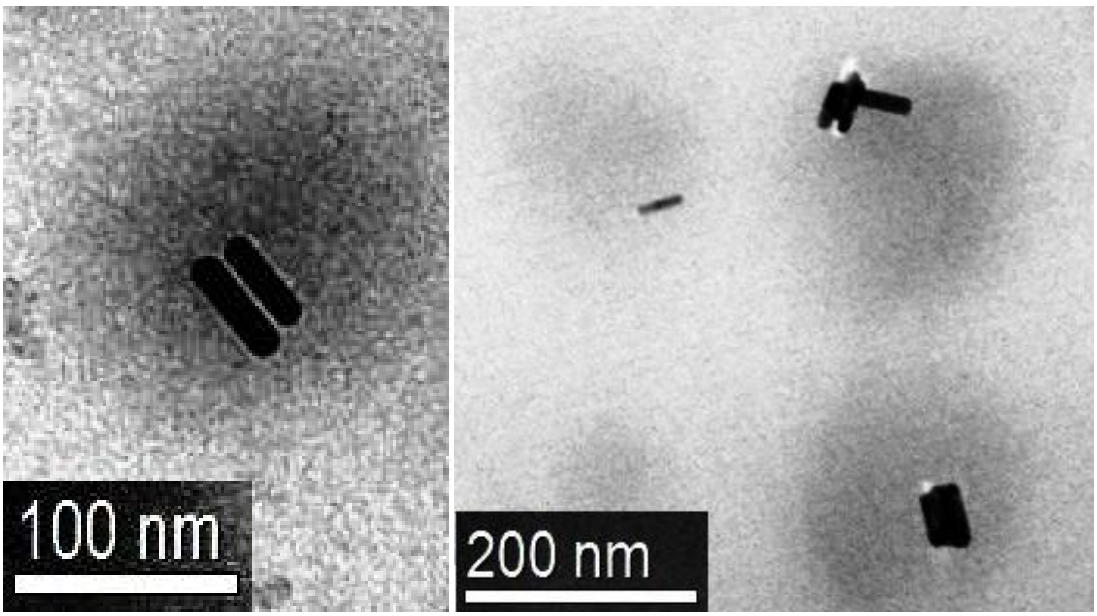
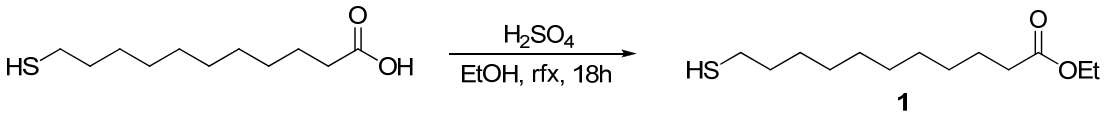
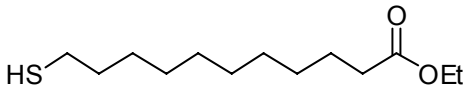


Figure S12. HRTEM of GNRs-**2**-PNPs**.**

Synthesis of compound 1:

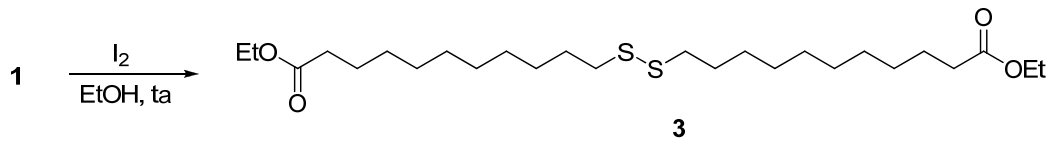


Scheme S1

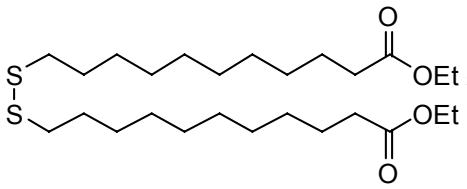


ethyl 11-mercaptoundecanoate (1): To a suspension of 11-mercaptoundecanoic acid (1.4 g, 6.4 mmol) in ethanol (99.8 %, 15 mL) was added slowly concentrate sulfuric acid (0.4 mL). The mixture was refluxed for 18 h, cooled to room temperature, and the solvent was evaporated under reduced pressure. The crude was suspended in ethyl acetate (50 mL), washed with a saturated $NaHCO_3$ aqueous solution (3 x 20 mL) and water (3 x 20 mL). The organic layer was dried over $MgSO_4$, evaporated and the crude product was purified by chromatography on silica gel (EtOAc/Light Petroleum 1/30) to yield 1.4 g (89 %) of compound **1** as a colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ= 4.10 (q, ^{3}J(H,H) = 7.0 Hz, 2H), 2.50 (q, ^{3}J(H,H) = 7.4 Hz, 2H), 2.27 (t, ^{3}J(H,H) = 7.5 Hz, 2H), 1.65-1.40 (m, 4H), 1.40-1.20 (m, 15H); ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ= 173.8, 60.1, 34.3, 33.9, 29.4, 29.3, 29.1, 29.0, 28.9, 28.3, 24.9, 24.6, 14.2; FTIR (CCl_4): ν = 2985, 2931, 2857, 1737, 1465, 1373, 1179, 1035 cm^{-1}; $[M+Na]^+$: 269; Anal. Calcd for C13H26O2S: C, 63.37; H, 10.64; S, 13.01. Found: C, 63.39; H, 10.67; S, 13.04.

Synthesis of compound 3:



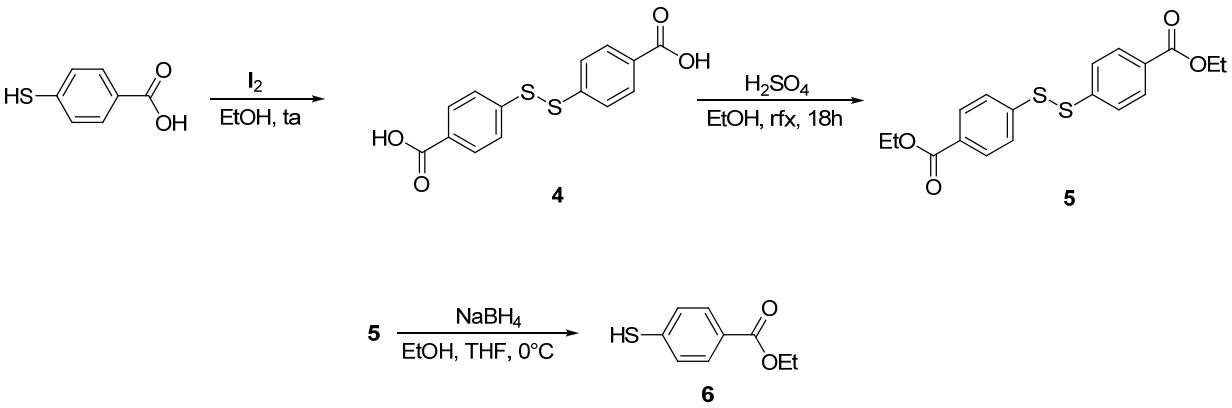
Scheme S2



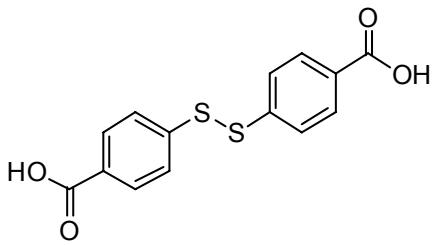
diethyl 11,11'-disulfanediyldiundecanoate (3): To a stirred solution of **1** (0.25 g, 1.0 mmol) in ethanol (95 %, 5 mL) was added slowly a saturated solution of I_2 in ethanol (95 %) until the reaction mixture becomes persistently light yellow. The solvent was evaporated under reduced pressure and the crude was suspended in chloroform (10 mL) and washed with $Na_2S_2O_3$ aqueous solution (10 %, 3 x 10 mL). The organic layer was dried over $MgSO_4$, evaporated to yield 0.23 g (94%) of compound **3** as a white solid. m.p. = 50-51 °C; ^{1}H-NMR (300

MHz, $CDCl_3$): δ= 4.12 (q, ^{3}J(H,H) = 7.6 Hz, 4H), 2.67 (t, ^{3}J(H,H) = 7.5 Hz, 4H), 2.28 (t, ^{3}J(H,H) = 7.5 Hz, 4H), 1.72-1.54 (m, 8H), 1.43-1.21 (m, 30H); ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ= 173.8 (2C), 60.1 ($2CH_2$), 39.1 ($2CH_2$), 34.3 ($2CH_2$), 29.4 ($2CH_2$), 29.3 ($2CH_2$), 29.2 ($2CH_2$), 29.1 ($2CH_2$), 29.1 ($2CH_2$), 29.0 ($2CH_2$), 28.5 ($2CH_2$), 24.9 ($2CH_2$), 14.2 ($2CH_3$); FTIR (CCl_4): ν = 2982, 2929, 2856, 1737, 1551, 1465, 1372, 1182, 1039 cm^{-1}; $[M+Na]^+$: 513; Anal. Calcd for C26H50O4S2: C, 63.63; H, 10.27; S, 13.07. Found: C, 63.65; H, 10.30; S, 13.09.

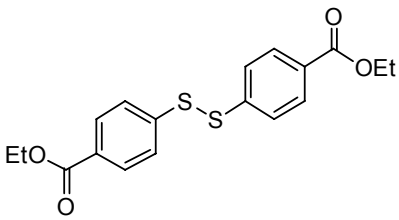
Synthesis of compound 6:



Scheme S3



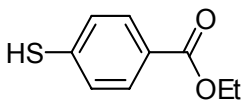
4,4'-disulfanediyldibenzoic acid (4): According to a previous report,[2] compound **4** was synthetised.



diethyl 4,4'-disulfanediyldibenzoate (5): To a suspension of **4** (0.55 g, 1.8 mmol) in ethanol (99.8 %, 15 mL) was added slowly concentrate sulfuric acid (0.1 mL). The mixture was refluxed for 18 h, cooled to room temperature, and the solvent was evaporated under reduced pressure. The crude was suspended in DCM (25 mL), washed with a saturated $NaHCO_3$ aqueous solution (3 x 20 mL) and water (3 x 20 mL). The organic layer was dried over $MgSO_4$, evaporated to yield 0.58 g (90 %) of compound **5** as a colorless oil. m.p. = 87-89 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ= 7.96 (d, ^{3}J(H,H) = 8.4 Hz, 4H), 7.51 (d, ^{3}J(H,H) = 8.4 Hz, 4H), 4.35 (q, ^{3}J(H,H) = 7.1 Hz, 4H), 1.36 (t, ^{3}J(H,H) = 7.1 Hz, 6H); ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ= 165.8 (2C), 141.9 (2C), 130.3 (4CH), 129.1 (2C), 125.6 (4CH); 61.0 ($2CH_2$), 14.2 ($2CH_3$); FTIR (CCl_4): ν = 2982, 2921, 2840, 1720, 1652, 1598, 1271,

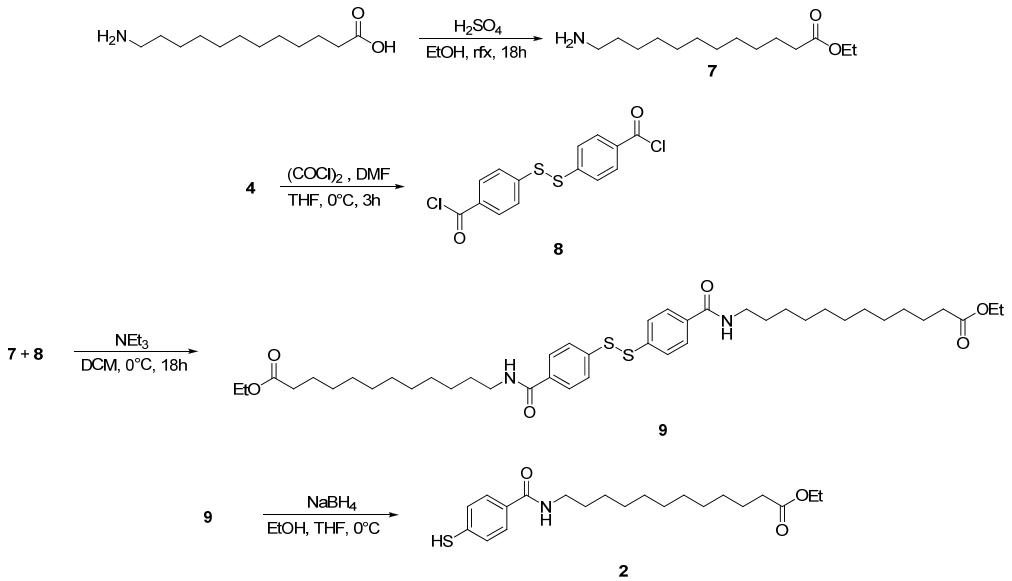
[2] R. S. Sengar, V. N. Nemykin, B. Partha, *New J. Chem,*. **2003**, *27*, 1115.

1113, 1041 cm^{-1}; $[M+Na]^+$: 385; Anal. Calcd for C18H18O4S2: C, 59.65; H, 5.01; S, 17.69. Found: C, 59.67; H, 5.03; S, 17.71.

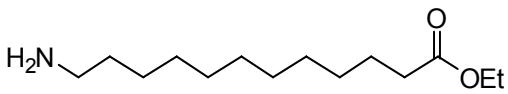


ethyl 4-mercaptobenzoate (6): After cooling at 0°C,[2] to a stirred suspension of **5** (0.58 g, 1.6 mmol) in a mixture of ethanol/THF (1:1, 20 mL) was added slowly $NaBH_4$ (0.22 g, 5.8 mmol). After complete addition, the mixture was left to warm up at room temperature and react for 4 h. Afterwards, the solvents were evaporated under reduced pressure and the crude was suspended in ethyl acetate (40 mL), washed with diluted HCl aqueous solution (0.1 M, 3 x 15 mL) and water (3 x 15 mL). The organic layer was dried over $MgSO_4$, evaporated to yield 0.55 g (95 %) of compound **6** as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ= 7.88 (d, ^{3}J(H,H) = 8.4 Hz, 2H), 7.27 (d, ^{3}J(H,H) = 8.4 Hz, 2H), 4.35 (q, ^{3}J(H,H) = 7.2 Hz, 2H), 3.60 (s, 1H), 1.37 (t, ^{3}J(H,H) = 7.2 Hz, 3H); ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ= 166.1, 138.1, 130.1 (2CH), 128.0 (2CH); 127.4, 60.9, 14.3; FTIR (CCl_4): ν = 2984, 2923, 2845, 1720, 1655, 1597, 1399, 1272, 1193, 1040 cm^{-1}; $[M-H]^-$: 181; Anal. Calcd for C9H10O2S: C, 59.32; H, 5.53; S, 17.59. Found: C, 59.34; H, 5.54; S, 17.62.

Synthesis of compound 2:

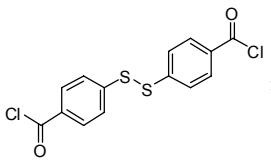


Scheme S4

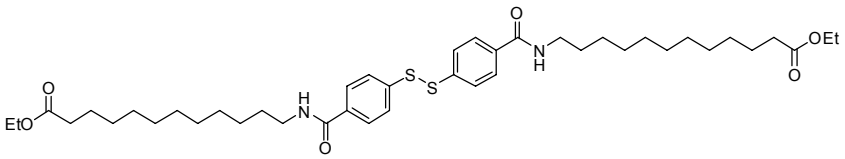


ethyl 12-aminododecanoate (7): To a suspension of 12-aminododecanoic acid (3.0 g, 13.2 mmol) in ethanol (99.8 %, 50 mL) was added slowly concentrate sulfuric acid (0.8 mL). The mixture was refluxed for 18 h, cooled to room temperature, and the solvent was evaporated under reduced pressure. The crude was suspended in ethyl acetate (200 mL), washed with 1 M ammonia aqueous solution (3 x 50 mL). The

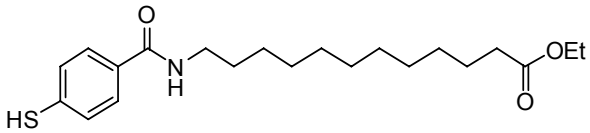
organic layer was dried over $MgSO_4$, evaporated to yield 3.2 g (98 %) of compound **7** as a white solid. m.p. = 56-58 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ= 4.10 (q, ^{3}J(H,H) = 7.2 Hz, 2H), 2.67 (t, ^{3}J(H,H) = 7.1 Hz, 2H), 2.26 (t, ^{3}J(H,H) = 7.6 Hz, 2H), 1.64-1.55 (m, 2H), 1.47-1.38 (m, 2H), 1.33-1.19 (m, 17H); ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ= 173.8, 60.1, 42.1, 34.3, 33.5, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 26.9, 24.9, 14.2; FTIR (CCl_4): ν = 3441, 2979, 2929, 2855, 1737, 1655, 1550, 1465, 1192, 1127, 1040, 1012 cm^{-1}; $[M+H]^+$: 244; Anal. Calcd for C14H29NO2: C, 69.09; H, 12.01; N, 5.75. Found: C, 69.07; H, 11.99; N, 5.76.



4,4'-disulfanediyldibenzoyl chloride (8): Compound **8** has been obtained according to a previous report.[2]



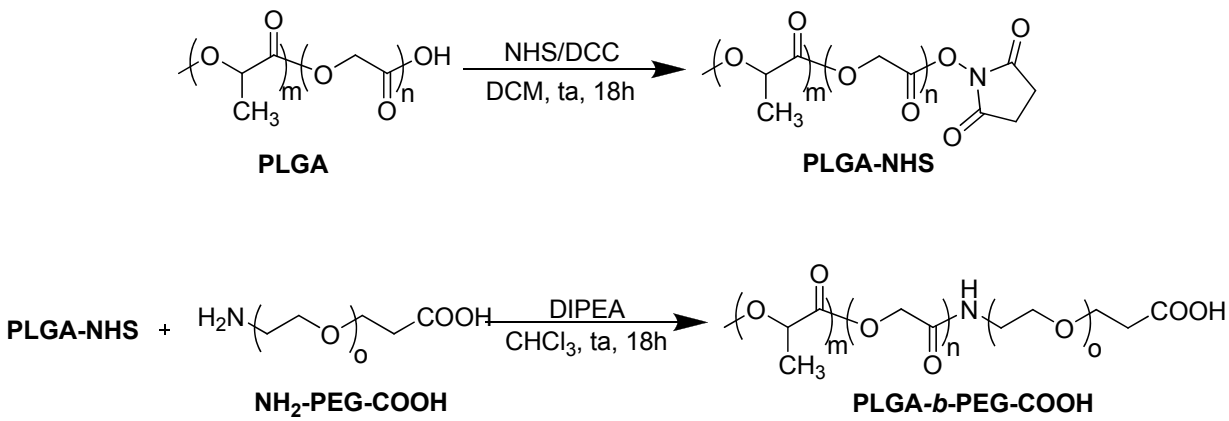
Bis- ethyl 12-(4-benzamido)dodecanoate disulfide (9): After cooling at 0°C,[2] in a flame dried flask to a stirred solution of **7** (1.8 g, 7.4 mmol) and triethylamine (NEt_3, 1.3 mL, 9.0 mmol) in DCM (20 mL) was slowly added a solution of **8** (3.7 mmol). After complete addition, the mixture was left to warm up at room temperature and react overnight. Afterwards, the solvent was evaporated under reduced pressure, crude was suspended in $CHCl_3$ (200 mL) and washed with water (4 x 50 mL). The organic layer was dried over $MgSO_4$, evaporated to yield 2.4 g (84 %) of compound **9** as a white solid. m.p. = 145-146 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ= 7.68 (d, ^{3}J(H,H) = 8.4 Hz, 4H), 7.50 (d, ^{3}J(H,H) = 8.4 Hz, 4H), 6.12 (t, ^{3}J(H,H) = 5.6 Hz, 2H), 4.11 (q, ^{3}J(H,H) = 7.1 Hz, 4H), 3.42 (q, ^{3}J(H,H) = 6.9 Hz, 4H), 2.28 (t, ^{3}J(H,H) = 7.8 Hz, 4H), 1.70-1.52 (m, 8H), 1.41-1.21 (m, 34H); ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ= 173.9 (2C), 166.6 (2C), 140.1 (2C), 133.7 (2C), 127.7 (4CH), 126.6 (4CH), 60.1 ($2CH_2$), 40.1 ($2CH_2$), 34.3 ($2CH_2$), 29.6 ($2CH_2$), 29.5 ($2CH_2$), 29.4 ($2CH_2$), 29.3 ($2CH_2$), 29.2 ($2CH_2$), 29.1 ($2CH_2$), 29.0 ($2CH_2$), 26.9 ($2CH_2$), 24.9 ($2CH_2$), 14.2 ($2CH_3$); FTIR (CCl_4): ν = 3443, 2981, 2921, 2852, 1740, 1678, 1629, 1595, 1532, 1397, 1128, 1012 cm^{-1}; $[M+Na]^+$: 779; Anal. Calcd for C42H64N2O6S2: C, 66.63; H, 8.52; N, 3.70; S, 8.47. Found: C, 66.63; H, 8.54; N, 3.72; S, 8.49.



ethyl 12-(4-mercaptobenzamido)dodecanoate (2): After cooling at 0°C,[2] to a stirred suspension of **9** (0.91 g, 1.2 mmol) in a mixture of ethanol/THF (1:1, 50 mL)

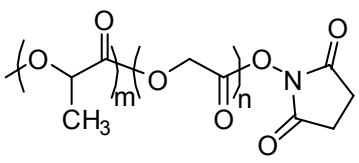
was added slowly $NaBH_4$ (0.17 g, 4.4 mmol). After complete addition, the mixture was allowed to reach room temperature and react for 4 h. Afterwards, the solvents were evaporated under reduced pressure and the crude was suspended in ethyl acetate (40 mL), washed with diluted HCl aqueous solution (0.1 M, 3 x 15 mL) and water (3 x 15 mL). The organic layer was dried over $MgSO_4$, evaporated to yield 0.79 g (87 %) of compound **2** as a white solid. m.p. = 86-87 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ= 7.62 (d, ^{3}J(H,H) = 8.4 Hz, 2H), 7.27 (d, ^{3}J(H,H) = 8.4 Hz, 2H), 6.14 (s, brs, 1H), 4.11 (q, ^{3}J(H,H) = 7.2 Hz, 2H), 3.56 (s, 1H), 3.41 (q, ^{3}J(H,H) = 6.8 Hz, 2H), 2.27 (t, ^{3}J(H,H) = 7.5 Hz, 2H), 1.64-1.53 (m, 4H), 1.39-1.21 (m, 17H); ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ= 173.9, 166.7, 135.8, 131.8, 128.5 (2CH), 127.5 (2CH), 60.1, 40.1, 34.4, 29.6, 29.4, 29.3, 29.2, 29.2, 29.1, 29.0, 26.9, 24.9, 14.2; FTIR (CCl_4): ν = 3457, 2979, 2930, 2856, 1736, 1673, 1598, 1515, 1484, 1372, 1240, 1186 cm^{-1}; $[M+Na]^+$: 402; Anal. Calcd for C21H33NO3S: C, 66.45; H, 8.76; N, 3.69; S, 8.45. Found: C, 66.46; H, 8.77; N, 3.71; S, 8.45.

Synthesis of PLGA-*b*-PEG-COOH:



Scheme S5

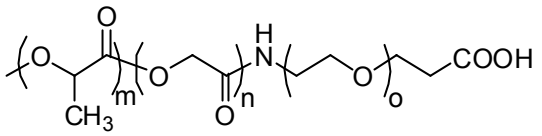
Synthesis of PLGA-*b*-PEG-COOH (7 kDa-3 kDa)



PLGA–NHS: According to a previous report,[3] poly(D,L-lactide–co–glycolide) (PLGA–COOH, 3 g, 0.43 mmol) and *N*-hydroxysuccinimide (NHS, 0.20 g, 1.7 mmol) were dissolved in anhydrous methylene chloride (15 mL). After cooling at 0°C *N*,*N*′-dicyclohexylcarbodiimide (DCC, 0.38 mg, 1.8 mmol) was

[3] O. C. Farokhzad, J. Cheng, B. A. Teply, I. Sherifi, S. Jon, P. W. Kantoff, J. P. Richie, R. Langer, *PNAS,* **2006**, *103*, 6315.

added and the mixture was left to warm up at room temperature and react for 18 h. Afterwards, dicyclohexylurea (DCU) was removed by filtration and PLGA–NHS was precipitated with cold ethyl ether (20 mL), and repeatedly washed with the same solvent (3 x 10 mL). After drying under vacuum, the resulted white solid of PLGA-NHS (2.7 g) was collected, stored at -20°C for no more than a week and used in the following step without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$): δ= 5.11-5.31 (m, $((OC\underline{H}(CH_3)C(O)OCH_2C(O))_{m+n}$–$C(O)N(C(O)CH_2)_2$, 4.63-4.92 m, $((OCH(CH_3)C(O)OC\underline{H}_2C(O))_{m+n}$–$C(O)N(C(O)CH_2)_2$, 2.8 (s, $((OCH(CH_3)C(O)OCH_2C(O))_{m+n}$–$C(O)N(C(O)C\underline{H}_2)_2$, 1.50-1.61 (m, $((OCH(C\underline{H}_3)C(O)OCH_2C(O))_{m+n}$– $C(O)N(C(O)CH_2)_2$.



PLGA-*b*-PEG-COOH: PLGA–NHS (0.63 g, 0.09 mmol) was dissolved in anhydrous chloroform (5 mL) followed by addition of NH_2-PEG-COOH (0.27 g, 0.09 mmol) and *N,N*-diisopropylethylamine (0.045 mL, 0.26 mmol). After 18 h the co-polymer was precipitated with cold ethyl ether (30 mL) and washed with the same solvent (3 x 10 mL) and cold water (3 x 20 mL) to remove unreacted NH_2-PEG-COOH. The resulting white solid of PLGA-*b*-PEG-COOH (0.55 g) was dried under vacuum, stored at -20°C and used without further treatment. ^{1}H-NMR (300 MHz, $CDCl_3$): δ= 5.11-5.31 (m, $((OC\underline{H}(CH_3)C(O)OCH_2C(O))_{m+n}$–$(CH_2CH_2O)_o$), 4.60-4.90 (m,$((OCH(CH_3)C(O)OC\underline{H}_2C(O))_{m+n}$–$(CH2CH_2O)_o$), 3.63 (s, $((OCH(CH_3)C(O)OCH_2C(O))_{m+n}$–$(C\underline{H}_2C\underline{H}_2O)_o$), 1.50-1.61 (m, $((OCH(C\underline{H}_3)C(O)OCH_2C(O))_{m+n}$–$(CH_2CH_2O)_o$).