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Supporting Information

The role of surface functionality in nanoparticle exocytosis

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Flow system design:

Total AuNP amount in the media containing the AuNPs and PBS was added to final AuNP amount to compare the efficiency between closed and flow systems.

To compensate the subtle changes of the flow rate, an internal standard, Rh, was employed. The cell viability studies confirmed that the 0.3 and 0.5 ppb concentrations of Rh in media are biocompatible to use with MCF-7 cells (see **Figure S1** and **S2**). Each flow replicate had another well of AuNPs treated cells without using the flow system (a closed system). The exocytosis amount of NPs in the closed system can be found in **Figure S3**.

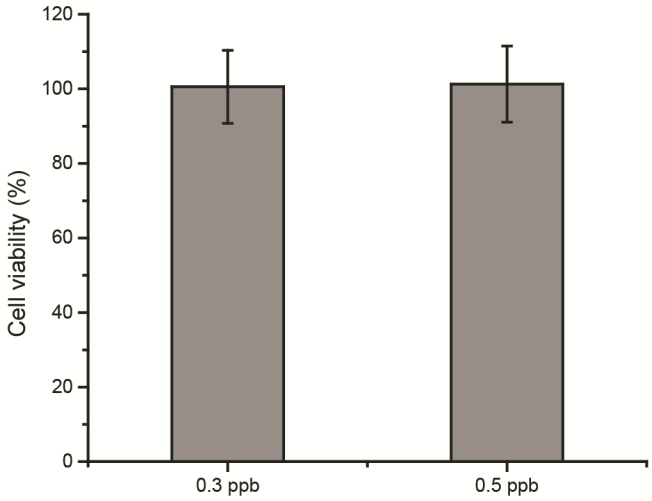

Figure S1. Cell Viability studies with different concentration of Rh.

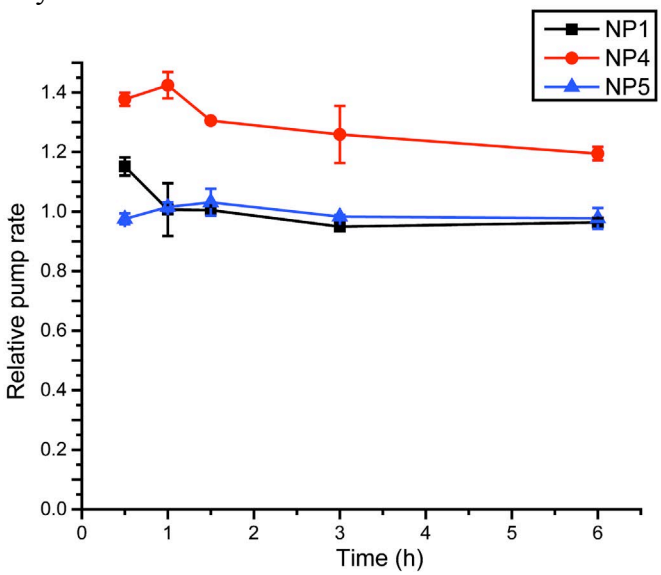

Figure S2. Pump rate control by monitoring internal standard (Rh) concentration using ICP-MS

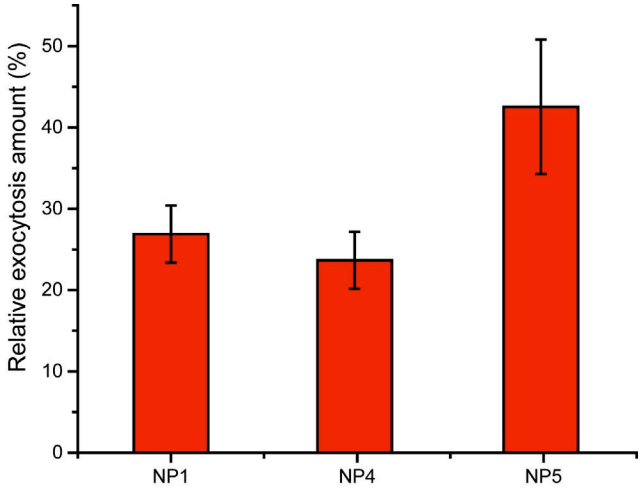

Figure S3. Relative exocytosis amount of NP **1**, **4**, and **5** in the closed system.

AuNP stability measurements using laser desorption ionization mass spectrometry (LDI-MS)

The external calibration curve was generated with different analyte AuNP to internal standard (AuNP I.S., see **Figure S4**) ratios (0.1, 0.3, 0.5, 0.8, 1.0 and 2.0). These samples were prepared in the same manner as the experimental samples. The molecular ion (MH^+) intensity ratios of analyte AuNP to AuNP I.S. were plotted against the amount ratios of analyte AuNP to AuNP I.S. to generate the calibration curves.[1] Thus, the analyte AuNP amount could be calculated from the known internal standard AuNP amount based on the external calibration curves. All the LDI-MS experiments were done on a Bruker Autoflex III MALDI-TOF mass spectrometer (**Figure S4**). A reflection mode and an average of 200 shots under 100 Hz frequency were applied. Data was analyzed by the FlexAnalysis Version 3.3.

Figure S4. Internal standard AuNPs (AuNP I.S.)

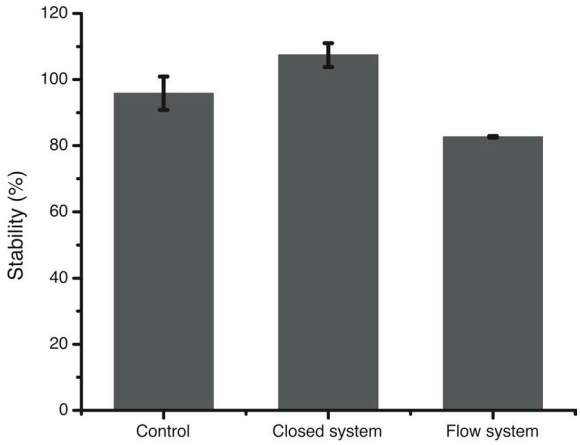

Figure S5. Ligand stability studies of NP **4** in the cells. The amounts of surface ligands detected by LDI-MS in the 6 h incubation samples in closed system and flow system were

compared with the amount of surface ligands detected immediately after the NPs mixed with cell culture media.

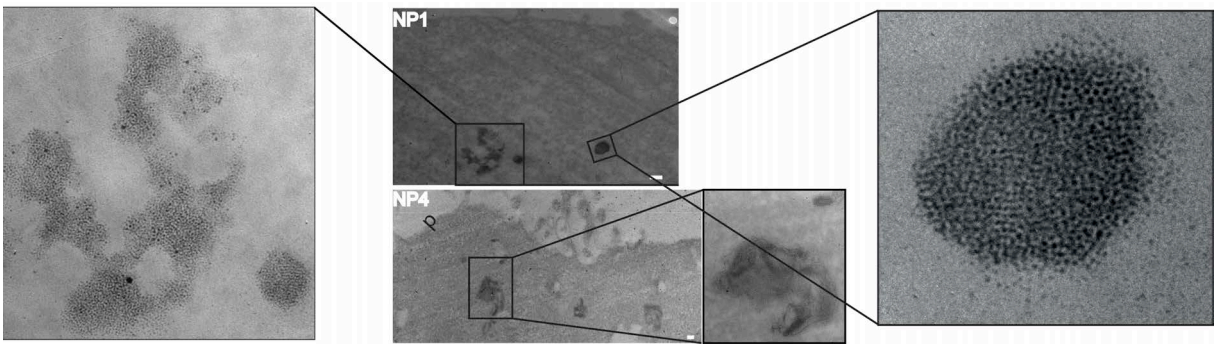

Figure S6. Cell TEM images from the closed system. Scale bar: 100 nm.

Au amount changes with different washing steps.

To establish whether non-specific adsorption of particles occurred to the plate or cells, we washed the cells after incubation with NP5 using cell culture media for 3, 6, and 9 times, respectively. We then measured the total gold amount in the washing solutions using ICP-MS as shown in **Figure S7**. Gold amounts in the same volume media after 3 times of washing and 6 times of washing are 37.34 ng and 48.43 ng, respectively. The 11.09 ng of gold is minimal relative to the values observed in **Figure 2c**, in which the total amount of gold is around 100 - 200 ng.

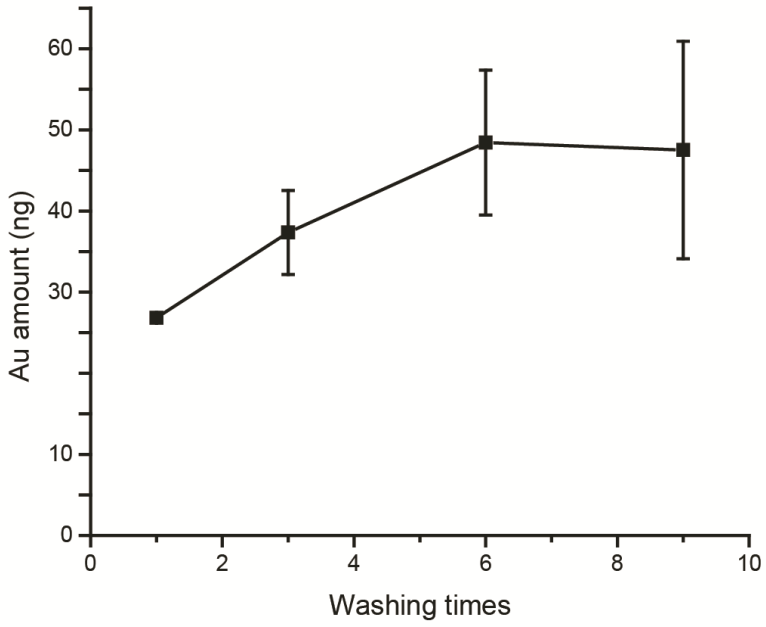

Figure S7. Au amount changes with different washing steps.

	Hydrodynamic diameter (nm)	Zeta potential (mV)
NP1	10.29 ± 2.4	21.1 ± 5.9
NP2	11.03 ± 2.6	19.2 ± 4.0
NP3	11.43 ± 3.4	22.2 ± 5.3
NP4	11.55 ± 3.0	17.1 ± 7.2
NP5	11.23 ± 3.4	21.4 ± 5.6

Table S1. Characterization of nanoparticles including sizes and zeta potentials. One milliliter of NP solution (0.5 μM) in 5 mM PB was prepared for the characterization. The size (diameter) of NPs was measured by DLS from three independent experiments. Zeta potential of NPs was measured also by DLS. The overall charge of NPs was measured from three independent replicates.

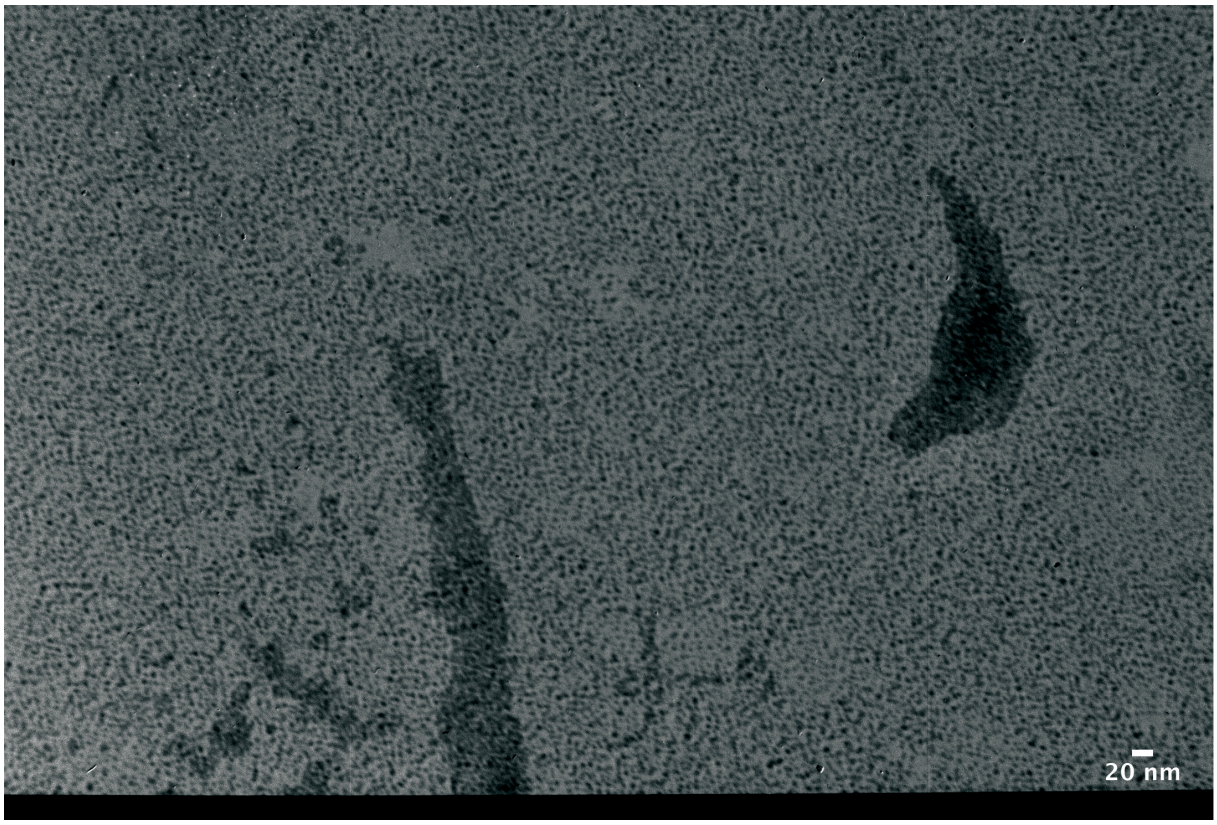

Figure S8. TEM image of pentanethiol-coated AuNPs showing homogeneous size distribution of Au NPs (2 nm).

Synthesis of Ligands 1-5

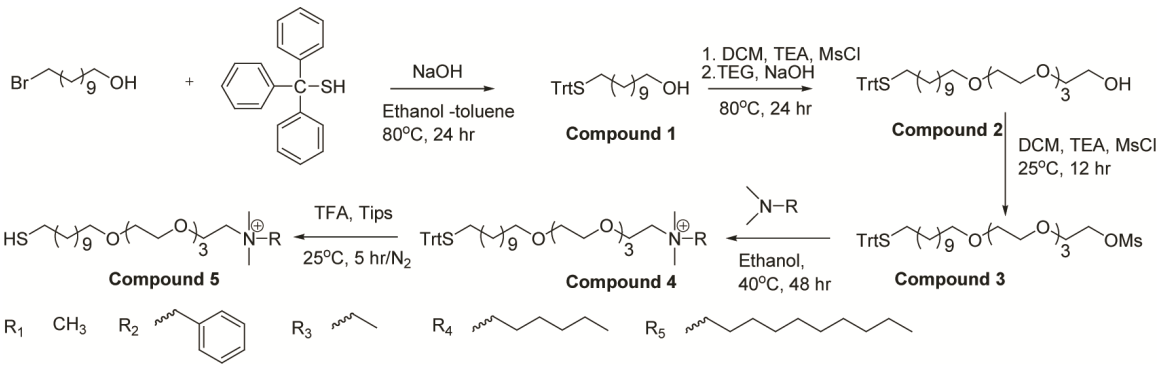

Figure S9. Synthetic scheme of ligands 1-5

General procedure:

Compound 1: 11-bromo-1-undecanol (8.22 g, 32.74 mmol) was dissolved in a solution of ethanol/toluene (1:1, 100 mL). Then, triphenylmethanethiol (10.86 g, 39.29 mmol) was also dissolved in a solution of ethanol/toluene (1:1, 50 mL) and added to the 11-bromo-1-undecanol mixture. NaOH (1.96 g, 49.11 mmol) in 3 mL of H_2O was also added into the mixture. The reaction mixture was stirred for 24 hours at 80°C. Once the reaction was completed (checked by TLC), the reaction mixture was extracted with a saturated solution of $NaHCO_3$ twice. The organic layer was separated and extracted with a saturated NaCl solution twice. Afterward the organic layer was separated, dried over Na_2SO_4, and concentrated using a rotavapor. The crude product was purified by column chromatography over silica gel using hexane/ethyl acetate (1:1, v/v) as an eluent.

The solvent was removed in vacuum to obtain compound **1** as a colorless oil (Yield 13.88 g, 95 %).

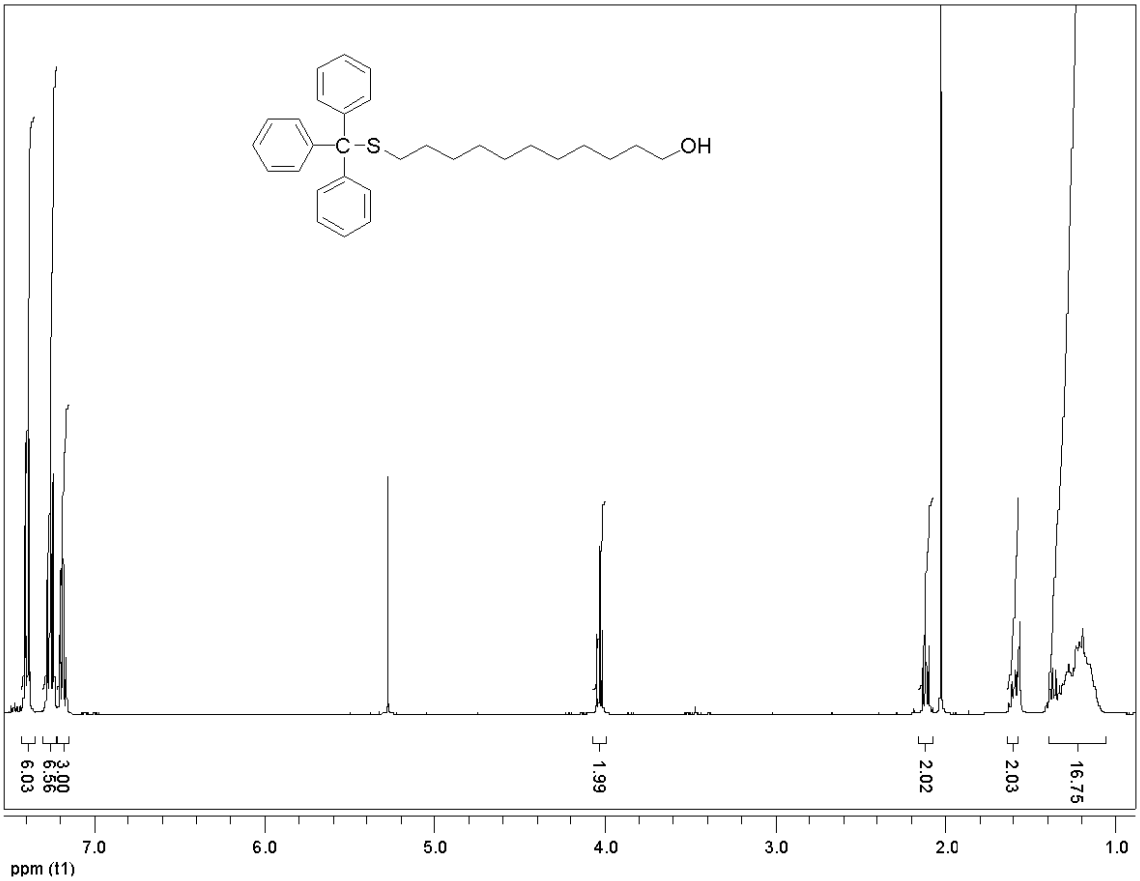

Figure S10. 400 MHz ^{1}H NMR spectrum of compound 1 in chloroform-D (D, 99.8%).

Compound 2: Compound 1(13.88 g, 31.1 mmol) was dissolved in dry DCM. Then, triethylamine (4.72g, 6.48 mL, 46.65 mmol) was added into this solution at 4 ^{o}C. Methanesulfonyl chloride (3.92 g, 2.65mL, 34.21 mmol) was added dropwise to the solution kept in ice bath. After 30 minutes the reaction mixture was warmed up to room temperature and stirred overnight. After the reaction was completed (according to TLC), solvent was evaporated. The compound was again diluted with DCM and extracted with 0.1 M solution of HCl twice. Organic layer was collected and treated with a saturated solution of NaHCO3 and washed three times. The organic layer was separated and added into saturated NaCl solution and extracted three times. After extraction, organic layer was separated, dried over Na_2SO_4 and concentrated at reduced pressure. The crude product was purified by column chromatography over silica gel using hexane/ethyl acetate (1:1, v/v) as an eluent. Solvent was

removed in vacuum to obtain mesylated compound as light yellow oil (Yield 15 g, 92 %). To synthesize compound 2, NaOH (1.37 g, 34.3 mmol) dissolved in 1 mL of H_2O was added to 99.24 mL of tetraethyleneglycol (111.15 g, 57.22 mmol) and stirred for 2 h at 80 °C. To this reaction mixture, 15 g of 11 (tritylthio)undecyl methanesulfonate was added and stirred for 48 h at 80 °C. The reaction mixture was extracted by washing with a solution of hexane/ethyl acetate (4:1, v/v) six times. Then, the organic layer was separated and concentrated at reduced pressure. The crude product was purified by column chromatography over silica gel using ethyl acetate as an eluent. The solvent was removed in vacuum to obtain compound **2** as light yellow oil (Yield 15.28 g, 68%).

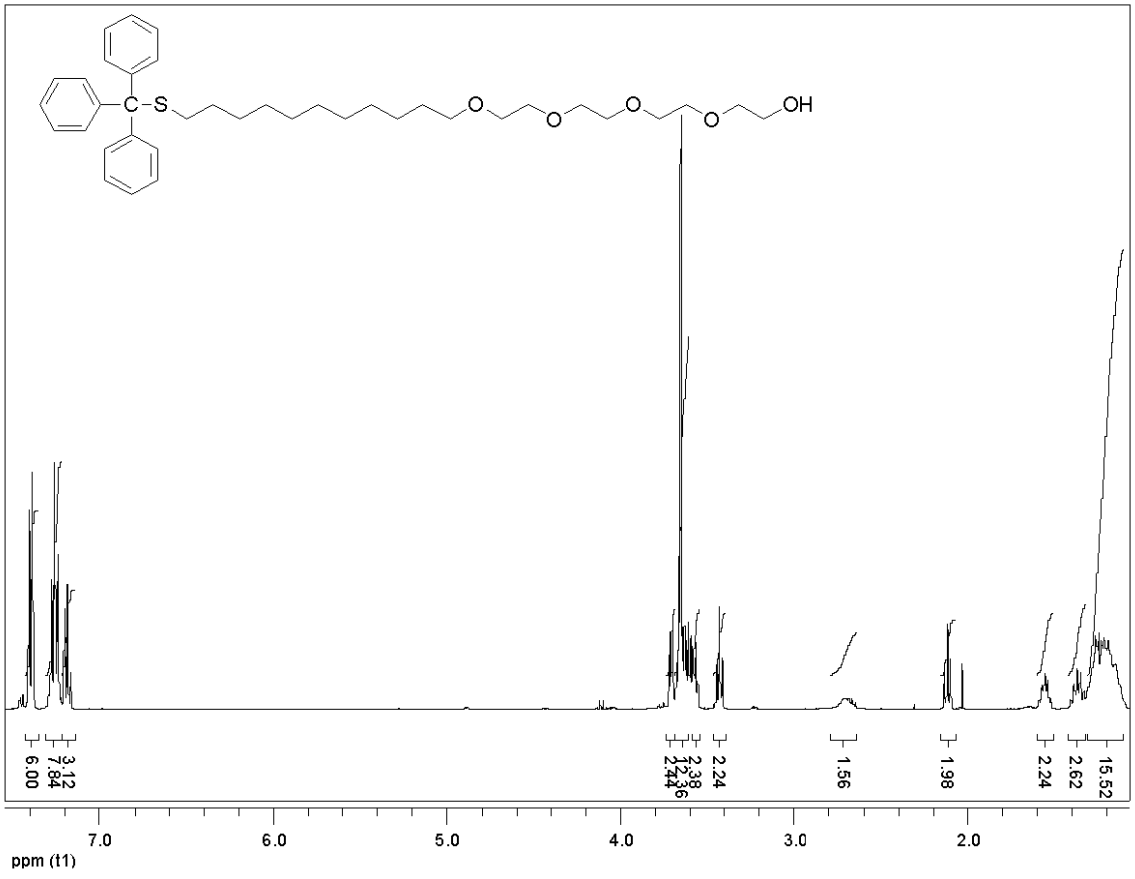

Figure S11. 400 MHz ^{1}H NMR spectrum of compound 2 in chloroform-D (D, 99.8%).

Compound 3: Compound **2** (10 g, 16.1 mmol) was dissolved in dry DCM at 4 °C. Triethylamine (3.26g, 4.49 mL, 32.2 mmol) was added into the solution. Methanesulfonyl chloride (2.77 g, 1.87 mL, 24.1 mmol) was added drop by drop to the reaction mixture that

was kept in ice-bath. After 30 minutes the reaction mixture was warmed up to room temperature and stirred overnight. Afterward, DCM solvent was evaporated under reduced pressure. The viscous compound was again diluted with DCM and was extracted with 0.1 M solution of HCl twice. The organic layer was poured into a saturate solution of $NaHCO_3$ and washed three times. Organic layer was separated and added into saturated NaCl solution and also washed for three times. Then, the organic layer was separated, dried over Na_2SO_4 and concentrated at reduced pressure. The crude product was purified by column chromatography over silica gel using ethyl acetate as an eluent. Solvent was removed in vacuum to obtain compound **3** as a light yellow oil (Yield 10.7 g, 95 %).

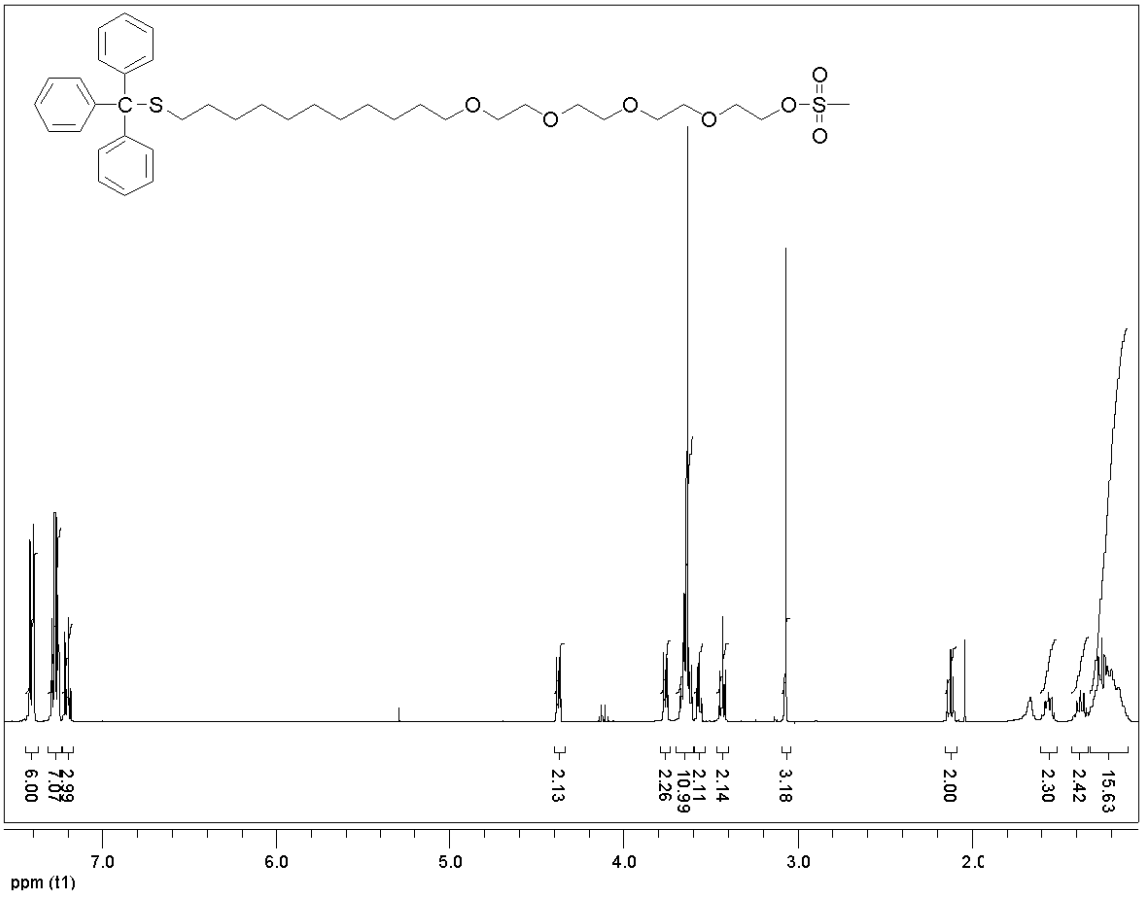

Figure S12. 400 MHz 1H NMR spectrum of compound 3 in chloroform-D (D, 99.8%).

Compound 4 (Trt L): Compound **3** (1 g, 1.43 mmol) was added to an available library of dimethylamine solutions (28 mmol for Trt L1 and 7.15 mmol for TrtL2-5) in ethanol. The

reaction mixtures were stirred at 40 ^{o}C for 48 hours. Crude product was checked by TLC and ethanol was eliminated at reduced pressure. The light yellow residue was purified by successive hexane washings with support of sonication and then dried in a high vacuum system. The product formation was quantitative and their structure was confirmed by NMR.

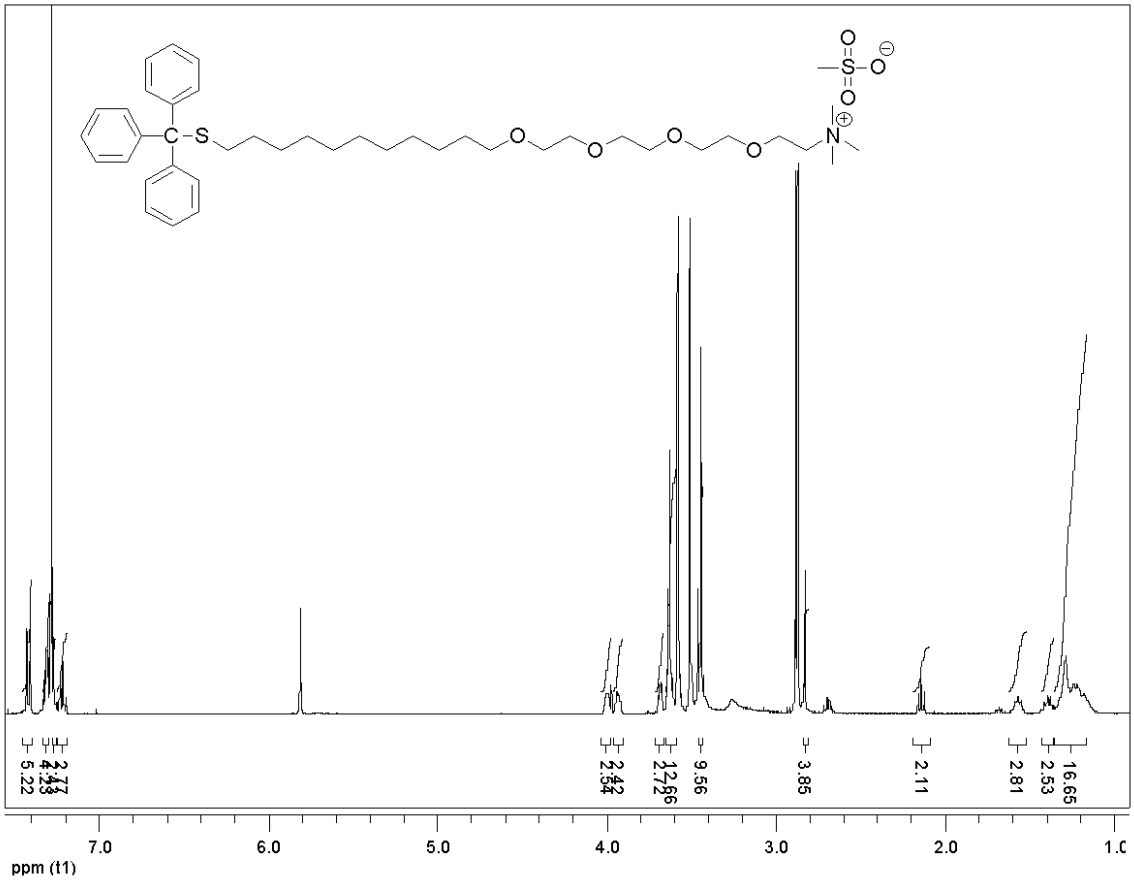

Figure S13. 400 MHz ^{1}H NMR spectrum of TrtL2 in chloroform-D (D, 99.8%).

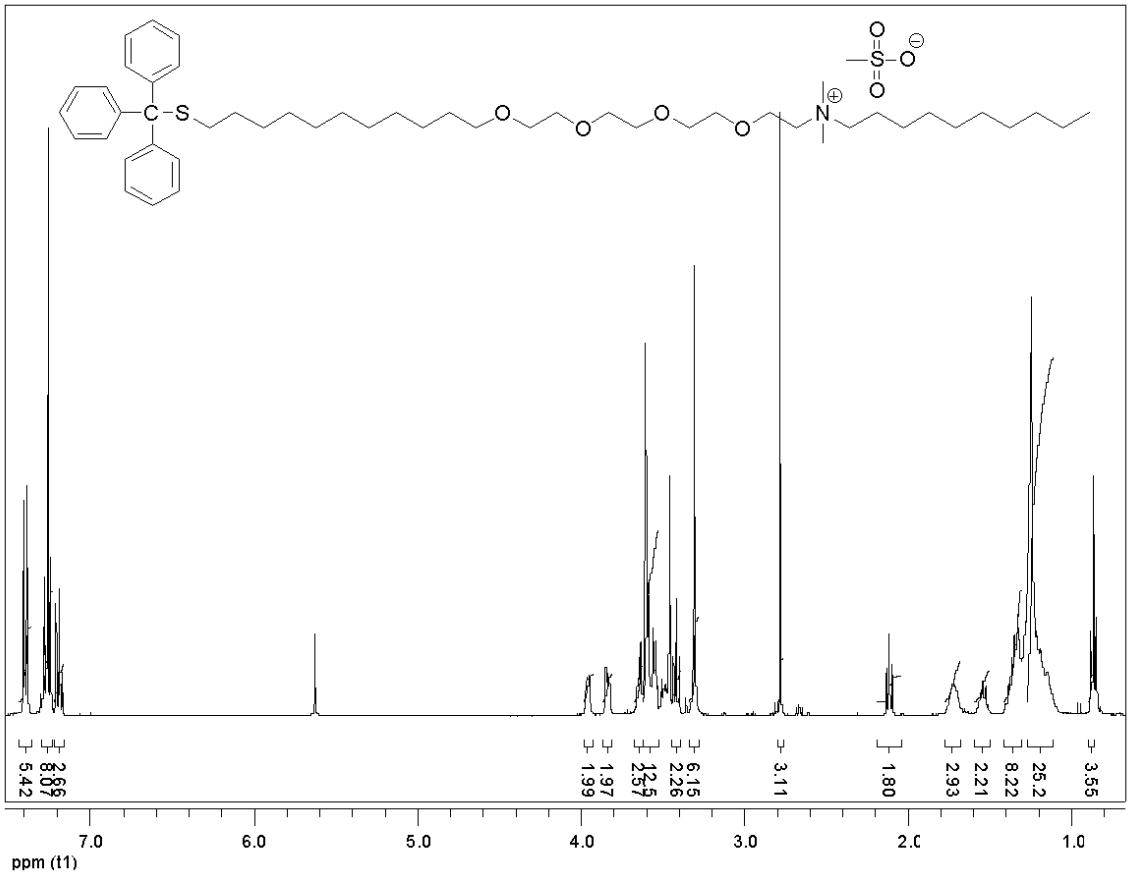

Figure S14. 400 MHz ^{1}H NMR spectrum of TrtL2 in chloroform-D (D, 99.8%).

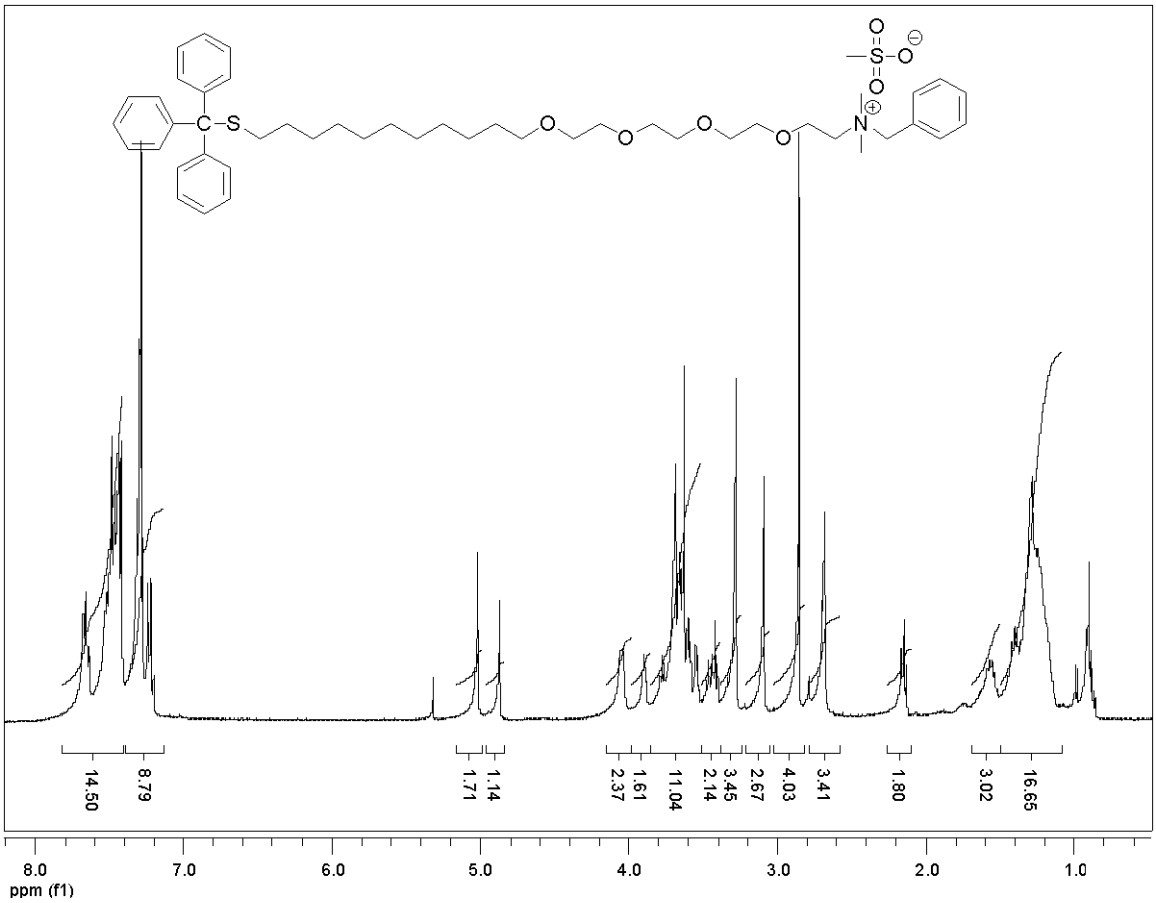

Figure S15. 400 MHz ^{1}H NMR spectrum of TrtL3 in chloroform-D (D, 99.8%).

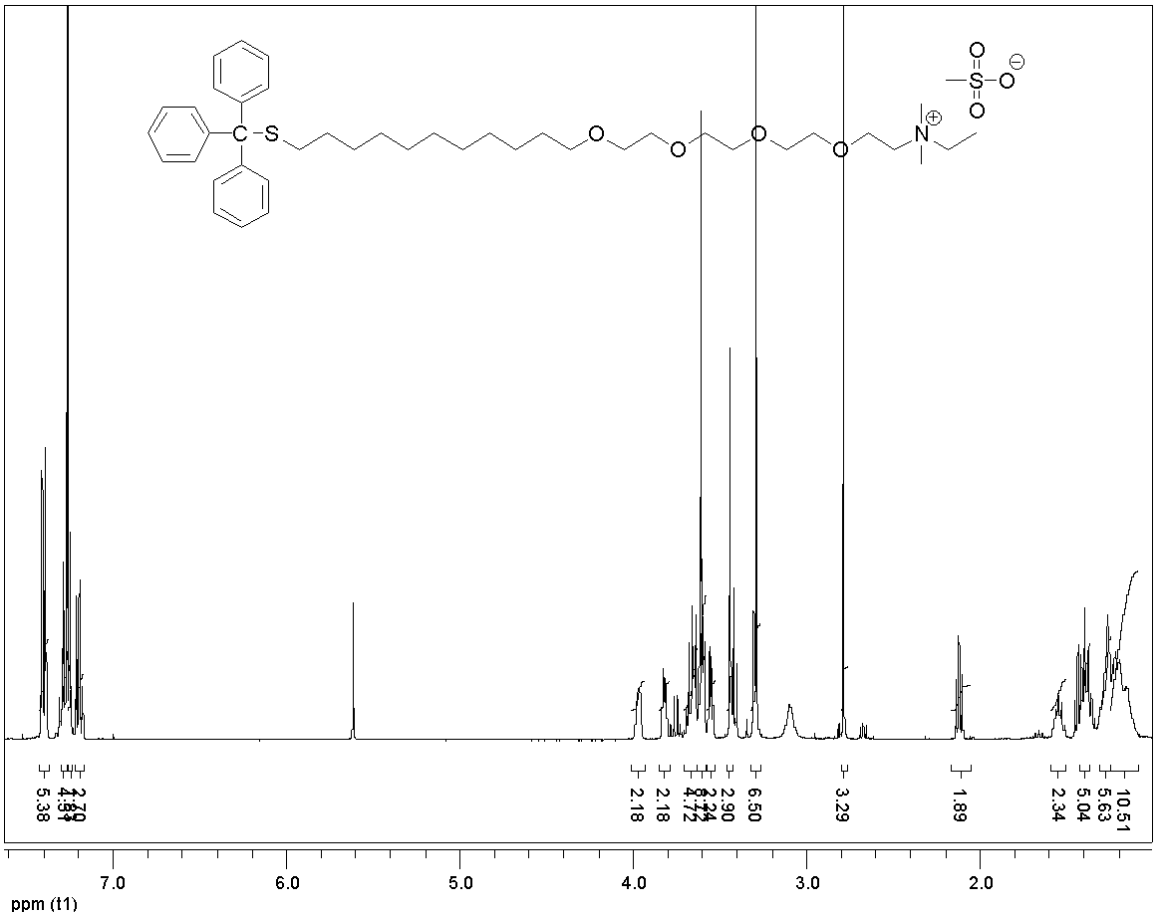

Figure S16. 400 MHz ^{1}H NMR spectrum of TrtL4 in chloroform-D (D, 99.8%).

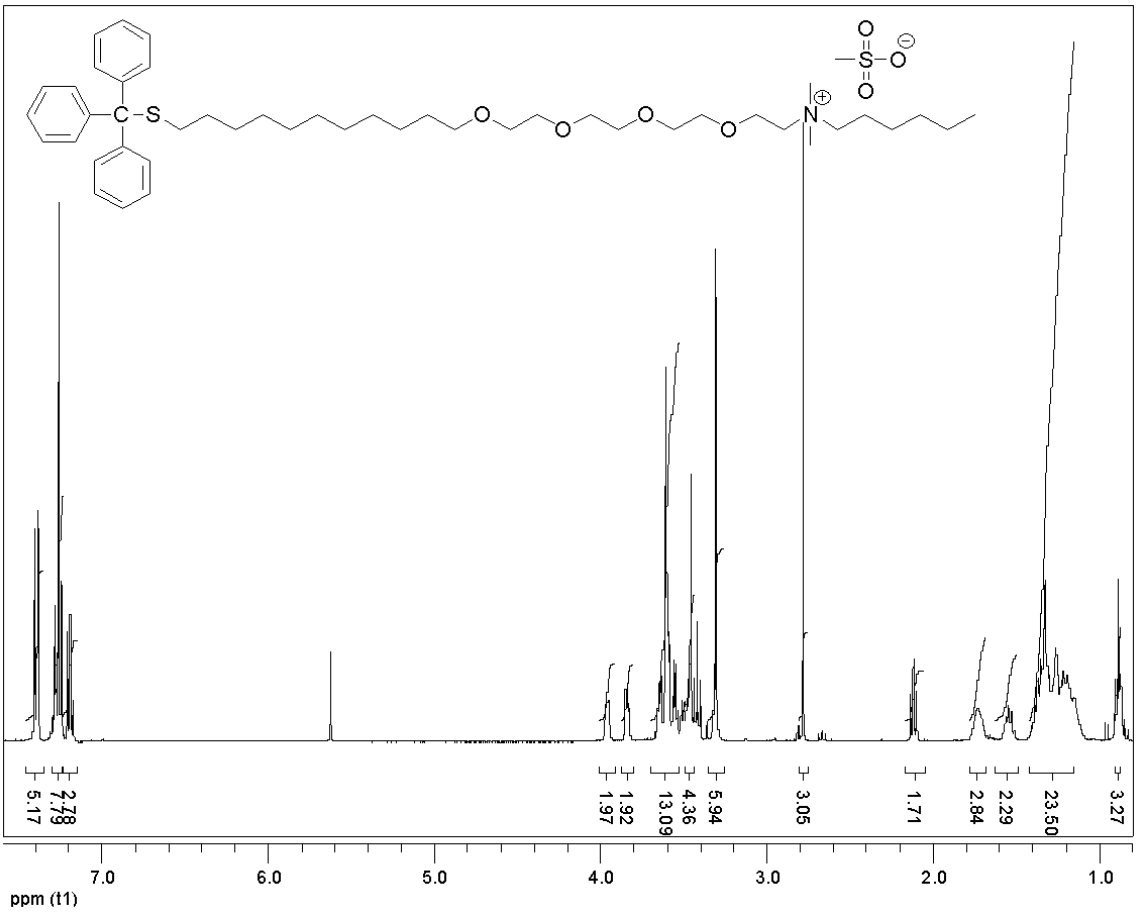

Figure S17. 400 MHz ^{1}H NMR spectrum of TrtL5 in chloroform-D (D, 99.8%).

Compound 5: Compound **4** was dissolved in dry dichloromethane (DCM) and an excess of trifluoroacetic acid (TFA, 20 equivalents) was added. The color of the solution was turned to yellow upon addition of TFA. Then, triisopropylsilane (TIPS, 1.3 equivalents) was added to the reaction mixture. The reaction mixture was stirred overnight under N_2 at room temperature. The solvent and most TFA and TIPS were evaporated under reduced pressure. The yellow residue was purified by hexane washings and dried in a high vacuum system. The product (**L**) formation was quantitative and their structure was confirmed by NMR.

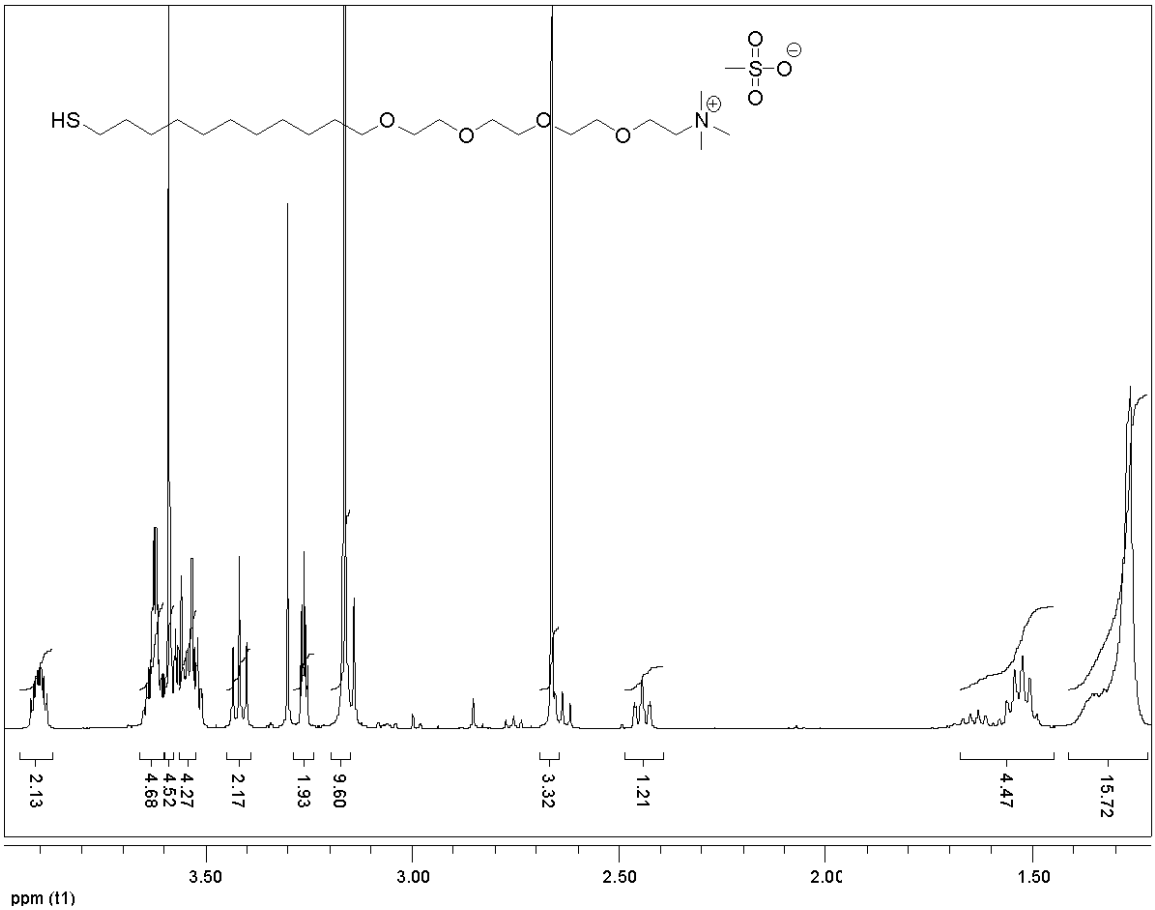

Figure S18. 400 MHz ^{1}H NMR spectrum of **L1** in chloroform-D (D, 99.8%).

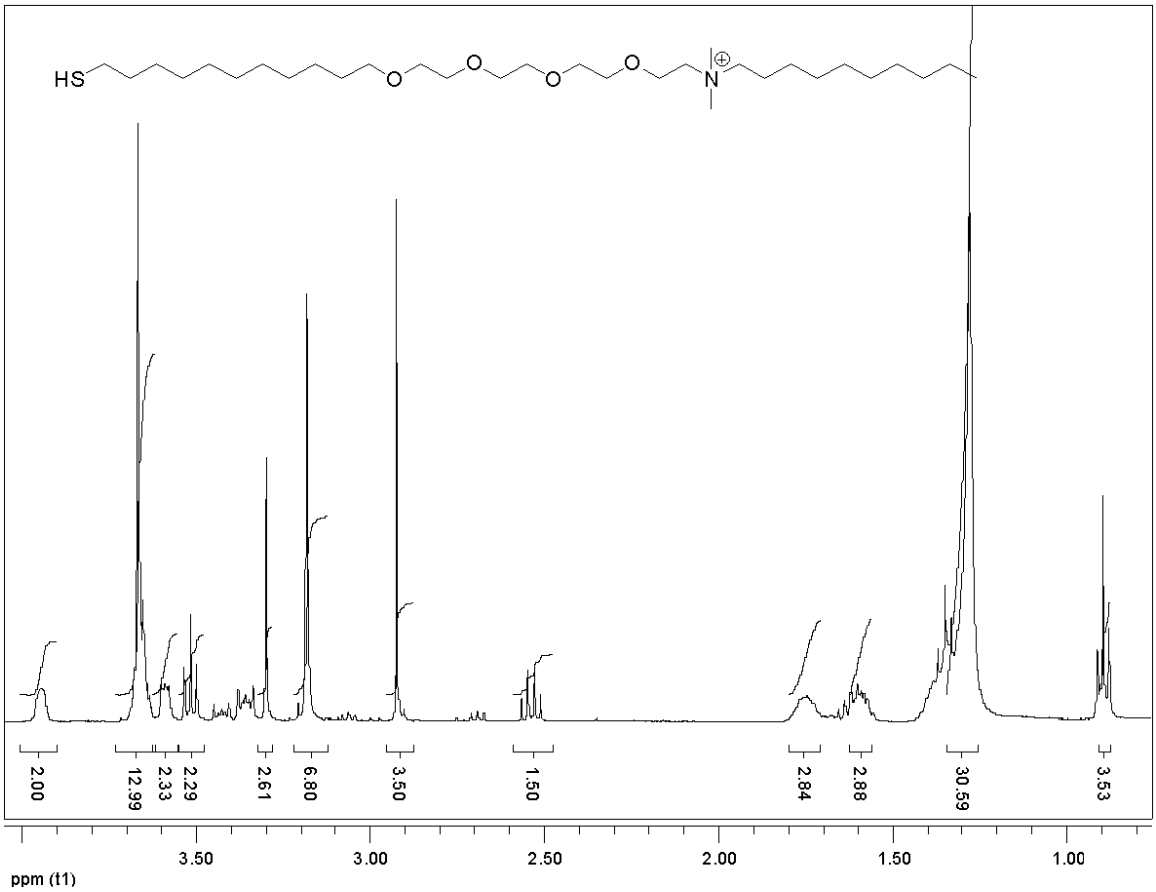

Figure S19. 400 MHz ^{1}H NMR spectrum of **L2** in chloroform-D (D, 99.8%).

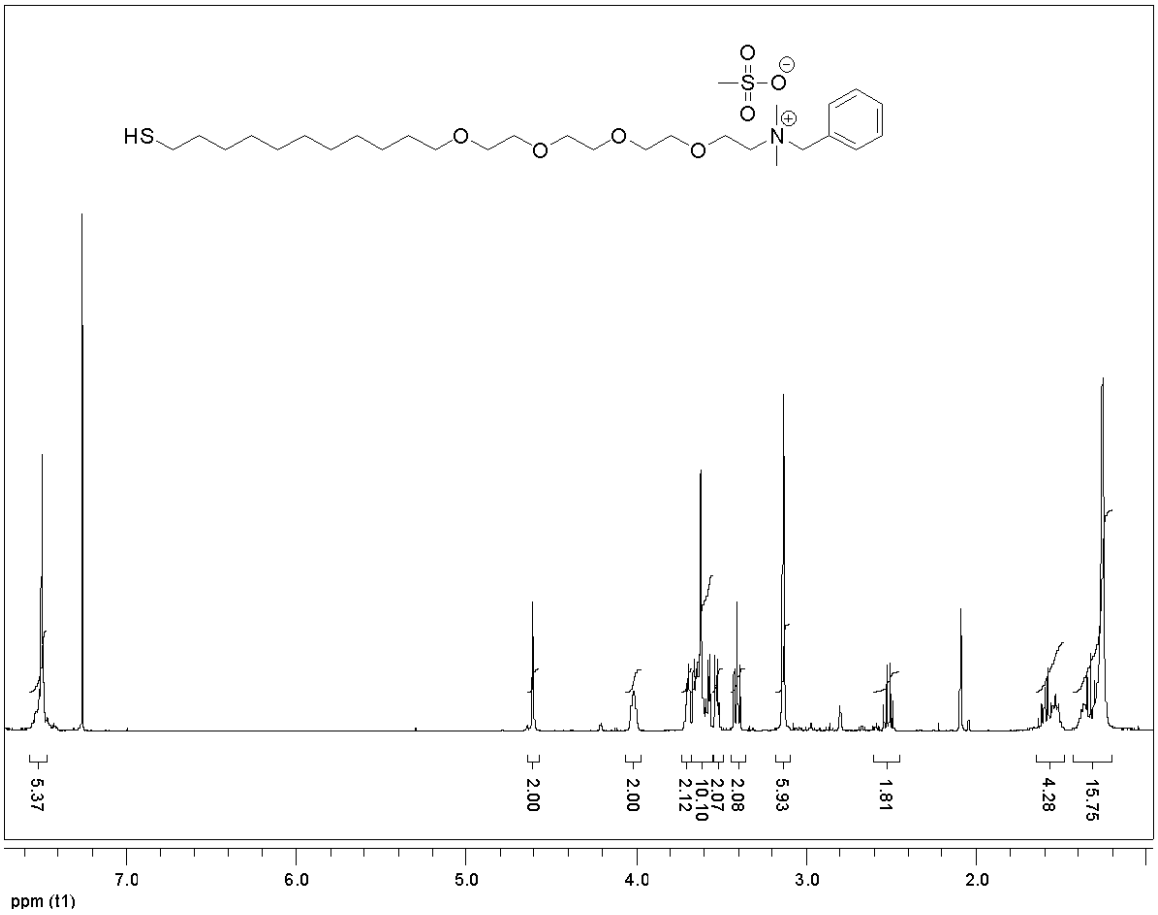

Figure S20. 400 MHz ^{1}H NMR spectrum of **L3** in chloroform-D (D, 99.8%).

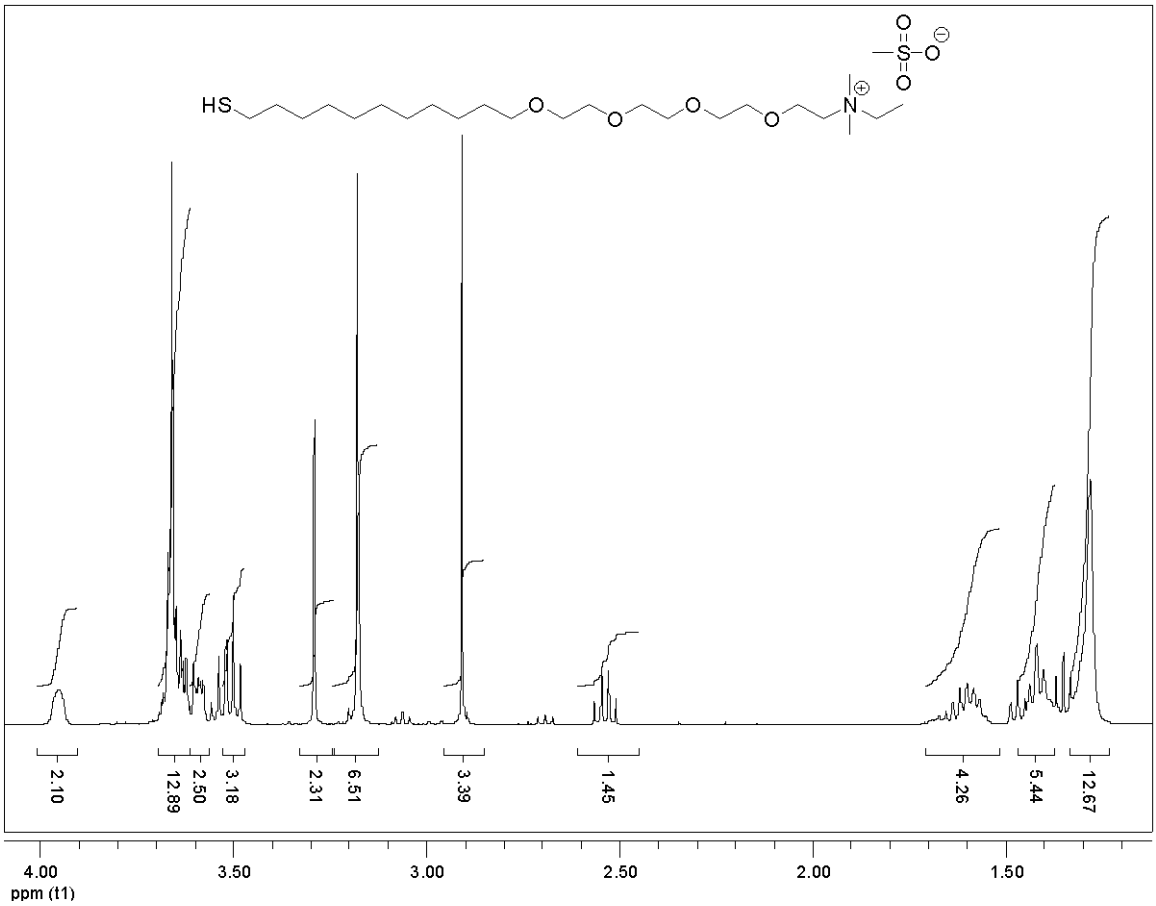

Figure S21. 400 MHz 1H NMR spectrum of **L4** in chloroform-D (D, 99.8%).

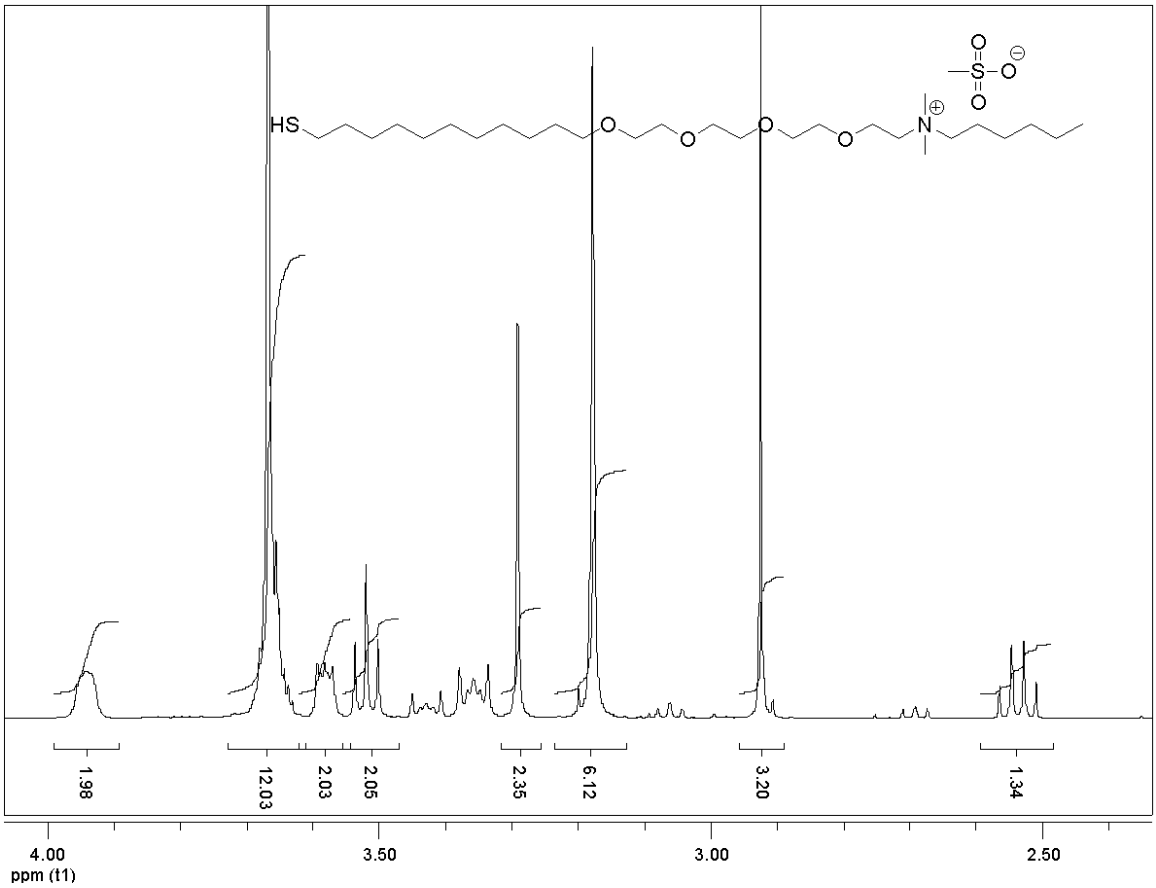

Figure S22. 400 MHz ^{1}H NMR spectrum of **L5** in chloroform-D (D, 99.8%).

Synthesis of NP 1-NP 5

Brust-Schiffrin two-phase synthesis method was used to synthesize pentanethiol-coated AuNPs with core diameter ~2 nm.[2] Murray place-exchange method[3] was followed to obtain the quaternary ammonium functionalized AuNPs **1-5.** Pentanethiol conjugated AuNPs (30 mg) and thiol ligand (90 mg) was dissolved in dry DCM and stirred under N_2 atmosphere for 4 days at room temperature. The DCM was removed under reduced pressure and the resulting precipitate was washed with hexane/DCM (1:1, v/v) six times. Then the precipitate was dissolved in distilled water and dialyzed for 4 days (membrane molecular cut-off =10000) to remove excess ligands and pentanethiol, acetic acid and other salts present in the nanoparticle solution. After dialysis, the particle was lyophilized to yield a solid brownish product. The

particles are then redispersed in deionized water. ^{1}H NMR-spectrum in D_2O showed substantial broadening of the proton peaks with no sign of free ligands.

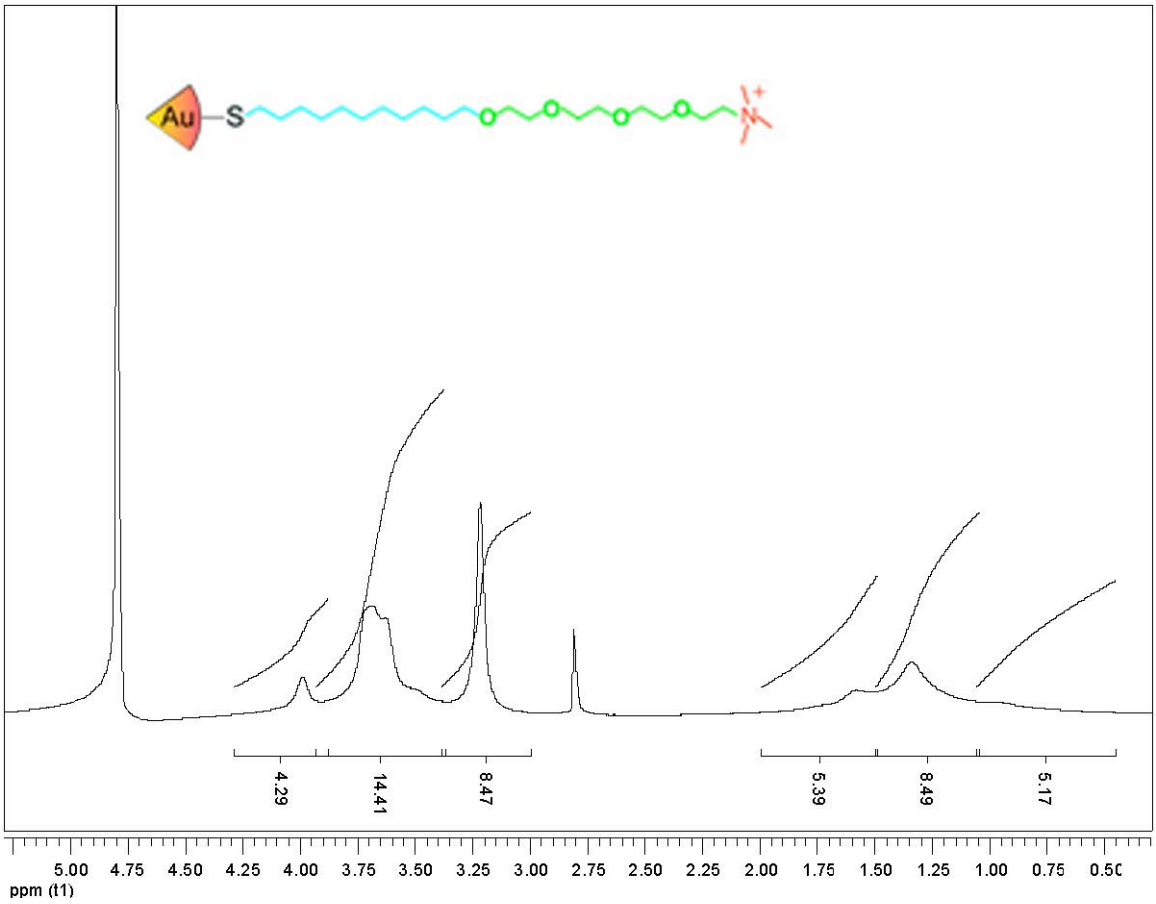

Figure S23. 400 MHz ^{1}H NMR spectra of NP **1** in water-D (D, 99.8%).

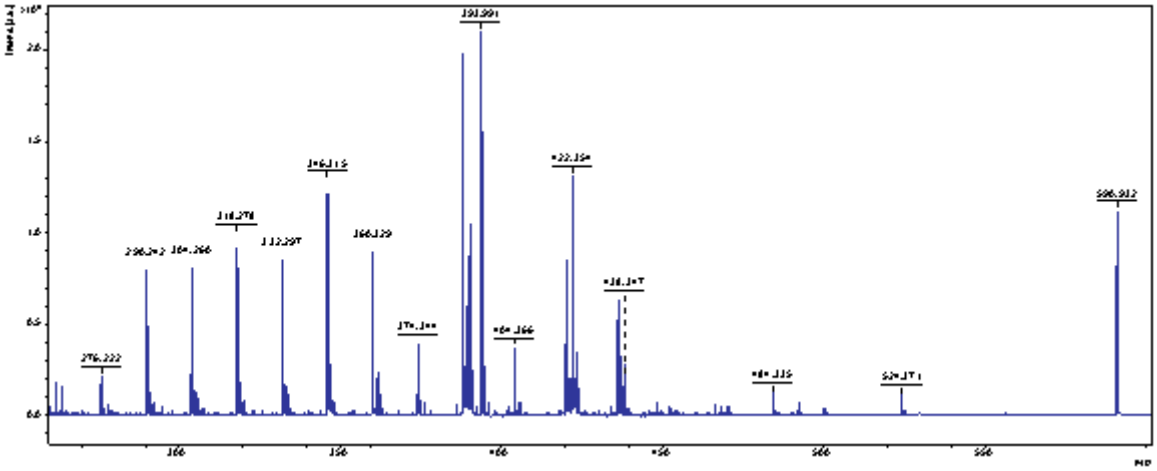

Figure S24. Laser desorption/ionization mass spectroscopy (LDI-MS) analysis of NP **1**. All the NPs have been characterized by LDI-MS and the surface functionalities have been confirmed following the reported method.[4]

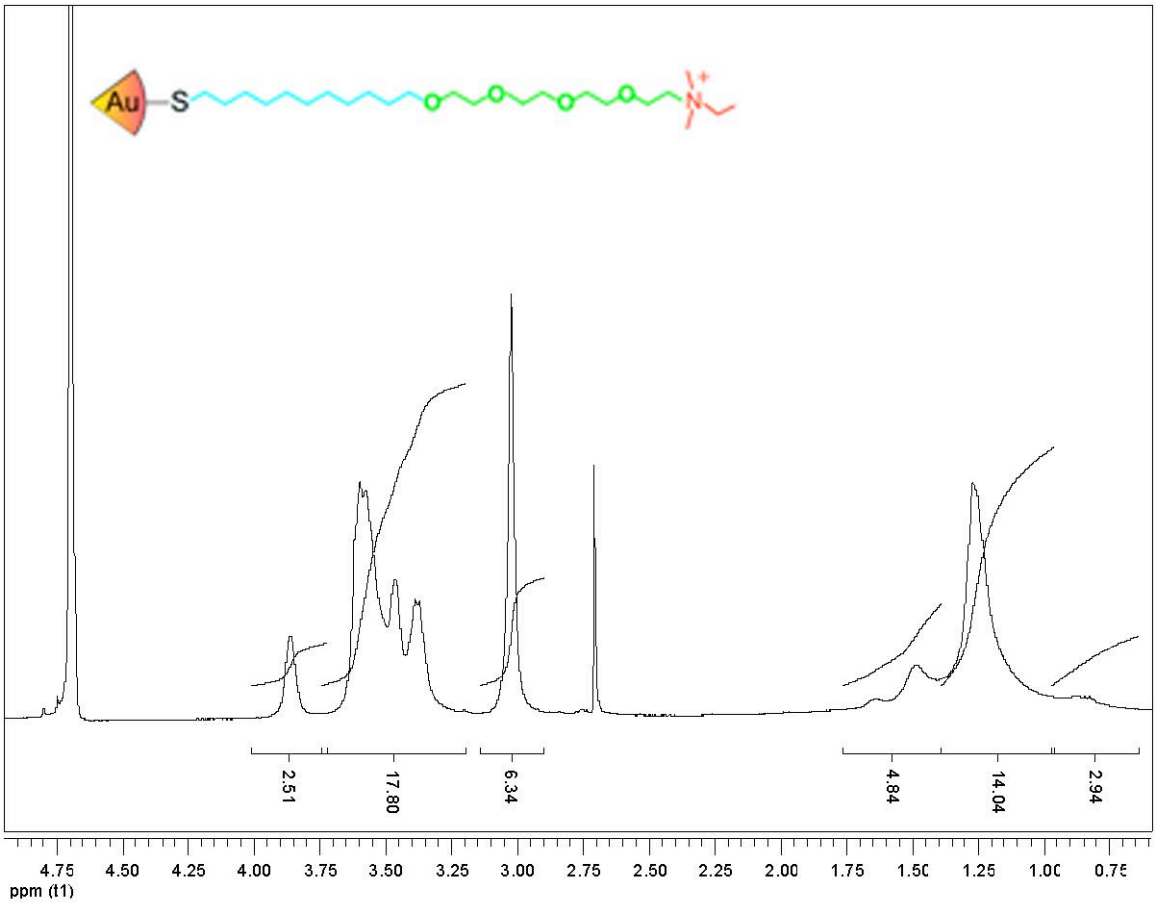

Figure S25. 400 MHz ^{1}H NMR spectrum of NP **2** in water-D (D, 99.8%).

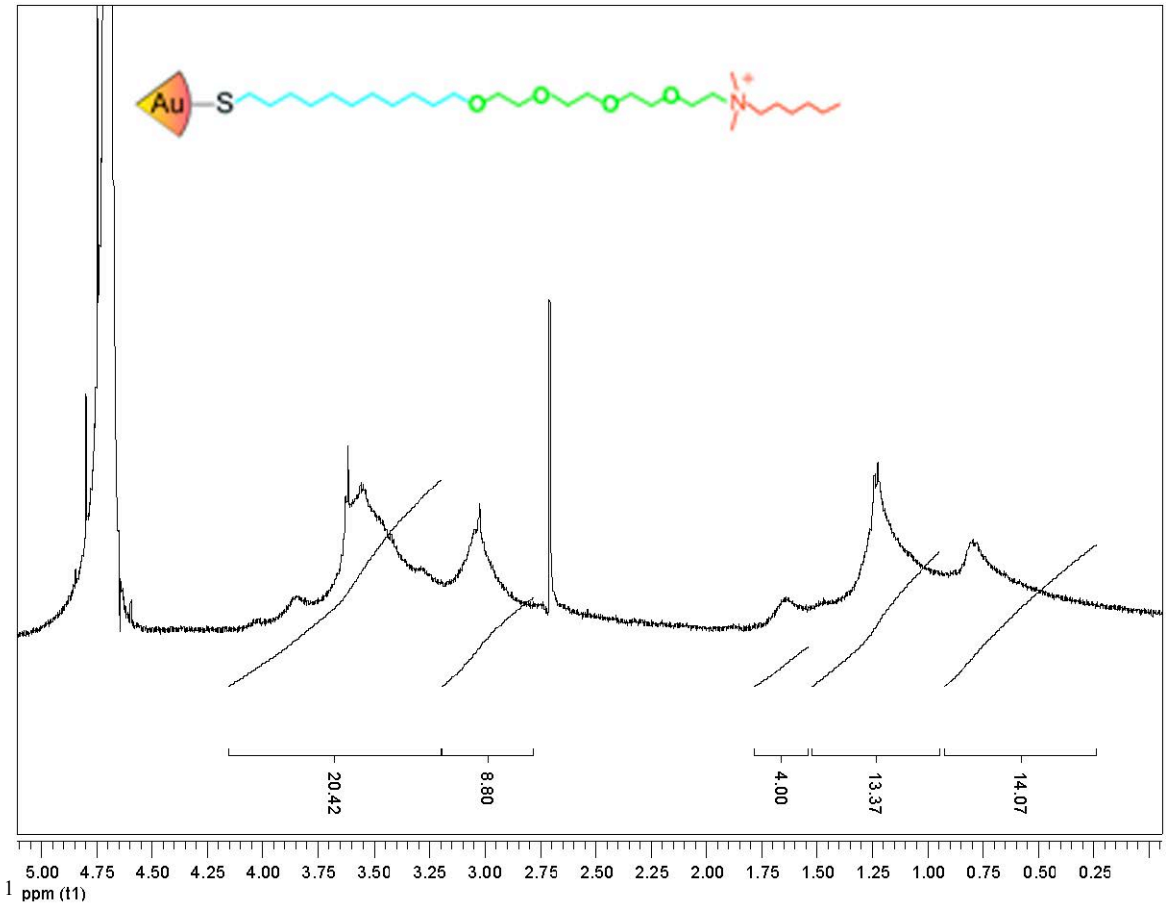

Figure S26. 400 MHz ^{1}H NMR spectrum of NP **3** in water-D (D, 99.8%).

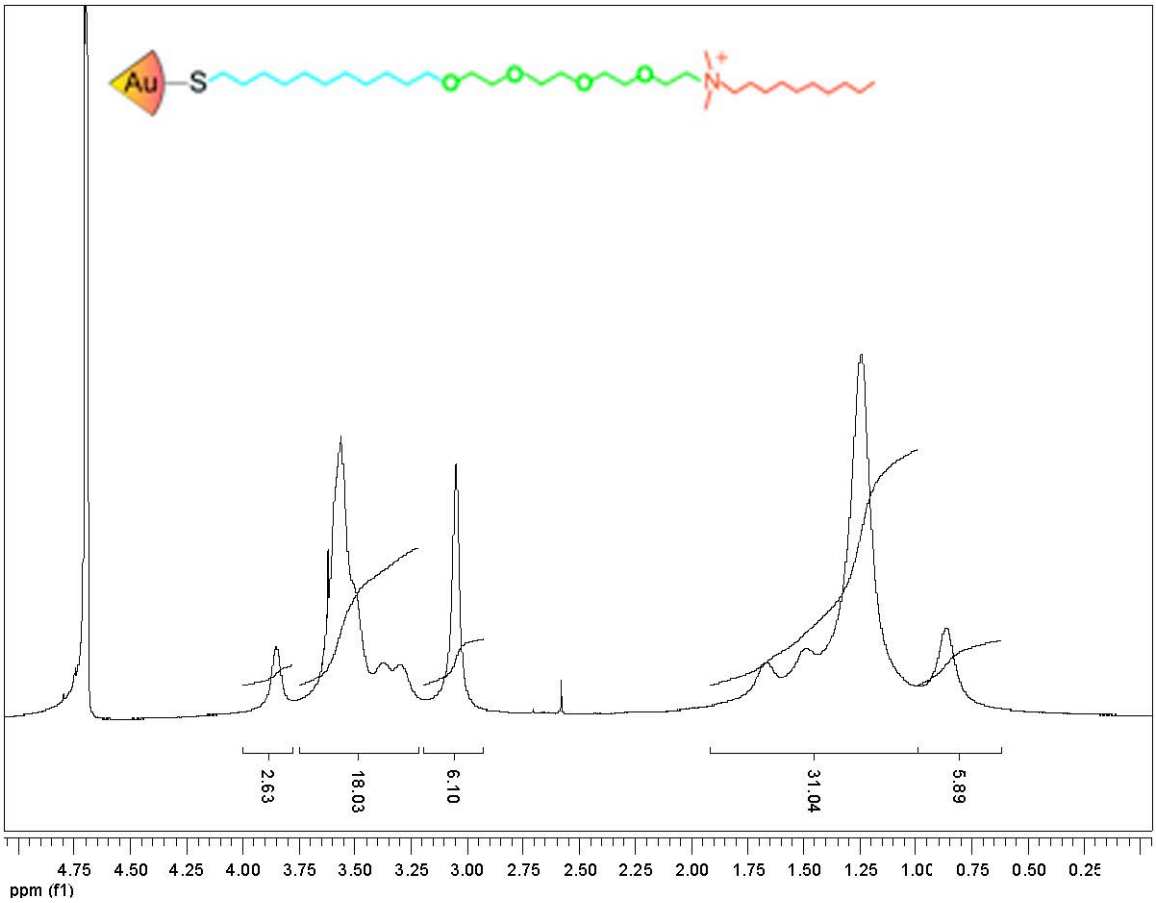

Figure S27. 400 MHz ^{1}H NMR spectrum of NP **4** in water-D (D, 99.8%).

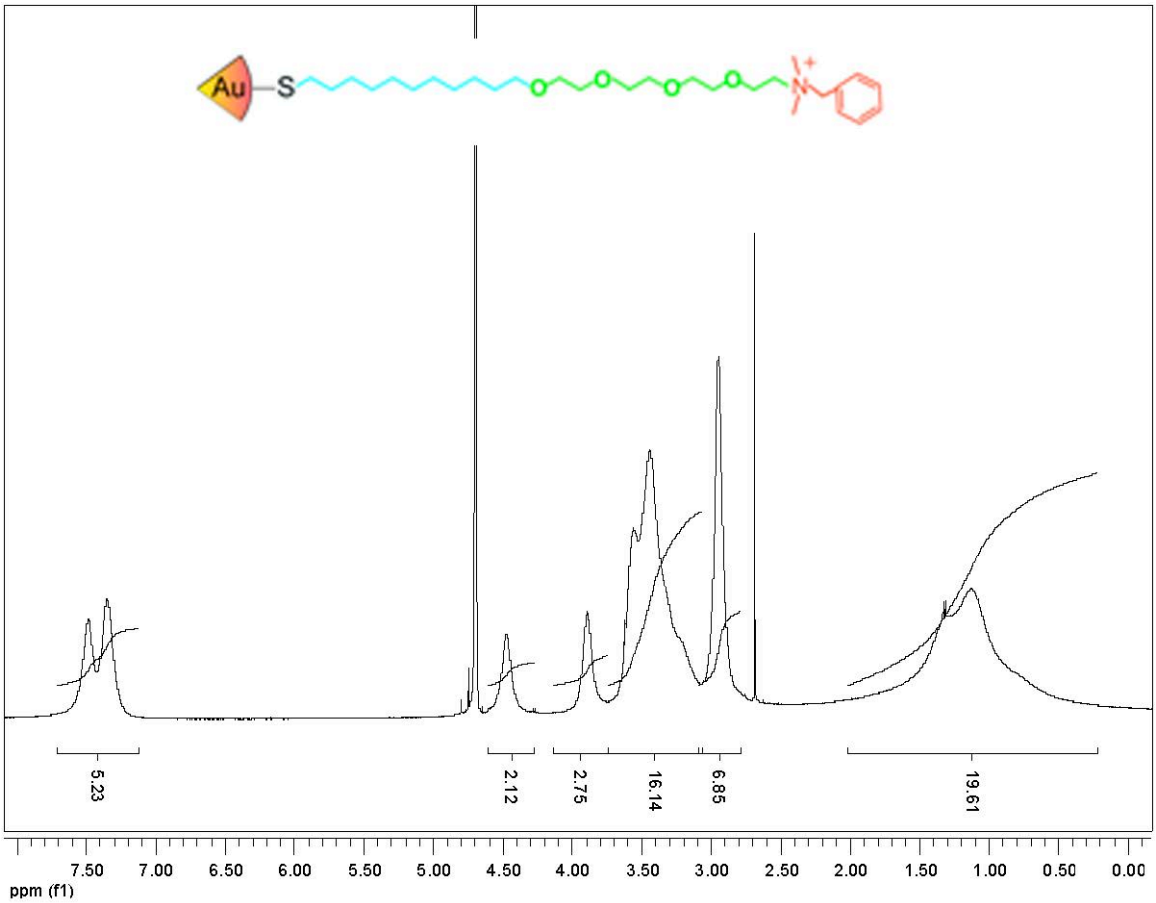

Figure S28. 400 MHz ^{1}H NMR spectrum of NP **5** in water-D (D, 99.8%).

References

[1] a) Z.-J. Zhu, Y. C. Yeh, R. Tang, B. Yan, J. Tamayo, R. W. Vachet, V. M. Rotello, *Nat. Chem.* **2011,** *3*, 963. b) Z.-J. Zhu, R. Tang, Y.-C.Yeh, O. R. Miranda, V. M. Rotello, R. W. Vachet, *Anal. Chem.* **2012,** *84*, 4321.
[2] a) A. G. Kanaras, F. S. Kamounah, K. Schaumburg, C. J. Kiely, M. Brust, *Chem Commun.* **2002**, 2294. b) M. Brust, M. Walker, D. Bethell, D. J. Schiffrin, R. Whyman, *J. Chem. Soc. Chem. Commun.* **1994**, 801.
[3] A. C. Templeton, W. P. Wuelfing, R. W. Murray, *Acc. Chem. Res.* **2000**, *33*, 27.
[4] B. Yan, Z.-J. Zhu, O.R. Miranda, A. Chompooser, V.M. Rotello, R. W. Vachet, *Anal. Bioanal. Chem.* **2010**, *396*, 1025.

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