Regulating Exocytosis of Nanoparticles via Host-Guest Chemistry

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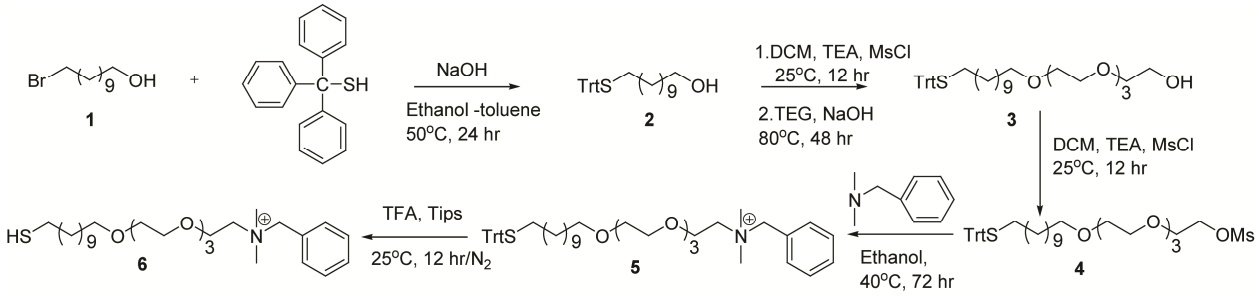
The ligands were synthesized according to literature procedure.[1 2]



Supporting Scheme 1. The scheme followed for the synthesis of benzyl-ligand **6** for functionalizing **AuNP-TBen**.

Compound 2: 11-bromo-1-undecanol (8.22 g, 32.74 mmol) was dissolved in 80 mL 1:1 ethanol/toluene mixture. Triphenylmethanethiol (10.86 g, 39.29 mmol) dissolved in 80 mL 1:1 ethanol/toluene was added to 11-bromo-1-undecanol in solution. Then, sodium hydroxide (1.96 g, 49.11 mmol) was dissolved in 2 mL water and added to the mixture. The reaction mixture was stirred for 24 hours at 50°C. Upon completion, the reaction mixture was extracted twice with a satrated solution of sodium bicarbonate ($NaHCO_3$) The organic layer was extracted, dried over sodium sulfate (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 the eluent. The solvent was removed in vacuum to obtain compound **2** as colorless oil (yield: 13.88 g, 95%).

^{1}H NMR (400 MHz, $CDCl_3$, TMS) of Compound 2 : δ 7.48-7.40 (m, 6H, *HAr*-), 7.37-7.27 (m, 6H, *HAr*-), 7.26-7.18 (m, 3H, *HAr*-), 3.65 (t, *J* = 6.7Hz, 2H,$\boldsymbol{CH_2}$OH), 2.16 (t, *J* = 7.2Hz, 2H,-***CH2***-), 1.66-1.52 (m, 2H, -$SCH_2\boldsymbol{CH_2}$) , 1.44-1.12 (m, 16H, -$\boldsymbol{CH_2}CH_2OH$ + -$\boldsymbol{(CH_2)_8}$ CH_2OH).

Compound 3: Compound **2** (13.88 g, 31.1 mmol) in 150 mL dry dichloromethane (DCM) was mixed with triethylamine (TEA) (4.72g, 6.48 mL, 46.65 mmol), followed by dropwise addition of methanesulfonyl chloride (3.92 g, 2.65mL, 34.21 mmol) in ice bath. After 30 minutes the reaction mixture was warmed to room temperature and stirred for 12 hr. After the reaction was completed (by TLC), solvent was evaporated. The compound was diluted again with 100 mL DCM and extracted with

100 mL 0.1 M HCl twice. The organic layer was collected, neutralized with a saturated $NaHCO_3$ solution, and washed with water three times. Following extraction, the organic layer was 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 the eluent. Solvent was removed in vacuum to obtain the mesylated compound as light yellow oil (yield: 15 g, 92%).

^{1}H NMR (400 MHz, $CDCl_3$, TMS) of intermediate msylation product: δ 7.48-7.40 (m, 6H, *HAr*-), 7.34-7.27 (m, 6H, *HAr*-), 7.26-7.19 (m, 3H, *HAr*-), 4.24 (t, *J* = 6.8Hz, 2H, -***CH2***SO_3CH_3), 3.01 (s, 3H, -SO_3***CH_3***), 2.16 (t, *J* = 7.6Hz, -S***CH_2***-), 1.76 (p, *J* = 6.8Hz, 2H, -***CH2***$CH2SO_3CH_3$), 1.41 (p, *J* = 7.2Hz, 4H, -SCH2***CH_2***- + -SCH2CH_2***CH_2***-), 1.35-1.1 (m, 12H, -***$(CH_2)_6$***$CH_2CH_2SO_3CH_3$).

To synthesize compound **3**, NaOH (1.37 g, 34.3 mmol) solution (1 mL) was added to 99.24 mL of tetraethyleneglycol (TEG) (111.15 g, 57.22 mmol) and stirred for 2 hr at 80 °C. To this reaction mixture, 15 g of 11-(tritylthio)undecyl methanesulfonate was added and stirred for 48 hr at 100 °C. The product was extracted in hexane/ethyl acetate (4:1, v/v) six times. Then, the organic layer was concentrated at reduced pressure and the crude product was purified by column chromatography over silica gel using ethyl acetate as the eluent. The solvent was removed in vacuum to obtain compound **3** as light yellow oil (yield: 15.28 g, 68%).

^{1}H NMR (400 MHz, $CDCl_3$, TMS) of Compound 3: δ 7.47-7.40 (m, 6H, *HAr*-), 7.34-7.26 (m, 6H, *HAr*-), 7.25-7.19 (m, 3H, *HAr*-), 3.77-3.57 (m,16H, -CH_2-***$(OCH_2CH_2)_4$***-OH), 3.46 (t, *J* = 6.8 Hz, 2H, -***CH_2***-$(OCH_2CH_2)_4$-OH), 2.95 (br, s, 1H, -TEG-O**H**), 2.15 (t, *J* = 7.2Hz, -S***CH_2***-), 1.59 (p, *J* = 7.2Hz, 2H, -***CH_2***CH_2TEG-OH), 1.4 (p, *J* = 7.6Hz, 2H, -SCH2***CH_2***-), 1.35-1.13(m, 14H, -***$(CH_2)_7$***CH_2CH_2TEG-OH).

Compound 4: Triethylamine (3.26g, 4.49 mL, 32.2 mmol) was added to compound **3** (10 g, 16.1 mmol) in 100 mL dry DCM in an ice bath. Methanesulfonyl chloride (2.77 g, 1.87 mL, 24.1 mmol) was added dropwise to the reaction mixture in ice-bath. After 30 minutes the reaction mixture was warmed up to room temperature and stirred overnight. The reaction mixture was worked up and the organic layer was extracted. The extracted DCM layer was 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 the eluent. Solvent was removed in vacuum to obtain compound **4** as light yellow oil (yield 10.7 g, 95 %).

^{1}H NMR (400 MHz, $CDCl_3$, TMS) of Compound 4: δ 7.44-7.37 (m, 6H, *HAr*-), 7.31-7.23 (m, 6H, *HAr*-), 7.22-7.16 (m, 3H, *HAr*-), 4.40-4.34 (m, 2H, -***CH_2***OSO_3CH_3), 3.78-3.54 (m, 14H, CH_2-***$(OCH_2CH_2)_3$***-***CH_2***$CH_2OSO_3CH_3$), 3.44 (t, *J* = 6.8Hz, 2H, CH_2-***CH_2***-$(OCH_2CH_2)_3$-), 3.07 (s, 3H, -OSO_3***CH_3***), 2.12 (t, *J* = 7.2Hz, 2H, -S***CH_2***-), 1.56 (p, *J* = 7.2Hz, 2H, -***CH_2***CH_2TEG-N$(CH_3)_2$), 1.38 (p, *J*=7.6Hz, 2H, -SCH2***CH_2***-), 1.32-1.11 (m, 14H, -***$(CH_2)_7$***CH_2CH_2TEGOSO_3CH_3).

Compound 5: Compound **4** (1.075 g, 1.53 mmol) was added to dimethylbenzylamine (0.62 g, 0.7 ml, 4.6 mmol) in 10 mL ethanol. The reaction mixture was stirred at 40 ^oC for 72 hr. After evaporating ethanol at reduced pressure, the light yellow residue was purified by successive washings with hexane (10 mL, 4 times) and hexane/diethylether (1:1 v/v, 10 mL, 6 times) and then dried in high vacuum. The product formation was quantitative and was confirmed by NMR.

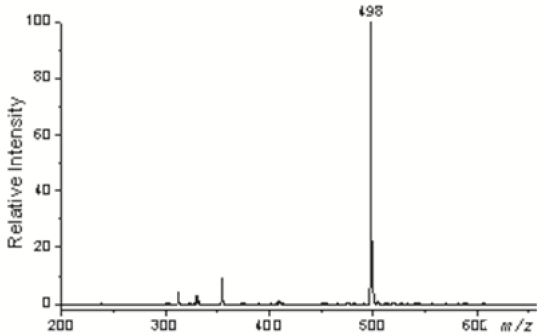
^{1}H NMR (400MHz, $CDCl_3$, TMS) of Compound 5: δ 7.64-7.58 (m, 2H, *HAr*-), 7.38-7.32 (m, 9H, *HAr*-), 7.24-7.17 (m, 6H, *HAr*-), 7.16-7.09 (m, 3H, *HAr*-), 4.9 (s, 2H, -***CH_2***-C_6H_5), 3.94 (s, br, 2H, -O***CH_2***$CH_2N(CH_3)_2$-), 3.8 (s, br, 2H, -OCH_2***CH_2***$N(CH_3)_2$-), 3.77-3.22 (m, 12H, -***$(OCH_2CH_2)_3$***-$CH_2CH_2N(CH_3)_2$-), 3.33 (t, *J* = 6.8Hz, 2H, -CH_2***CH_2***O-), 3.23 (s, 6H, -N***$(CH_3)_2$***-), 2.06 (t, *J* = 7.2Hz, 2H,

-S***CH₂***-), 1.51-1.42 (p, *J* = 6.8Hz, 2H, -***CH₂***CH_2O-), 1.36-1.28 (p, *J* = 7.6Hz, 2H, -SCH2***CH₂***-) 1.24-1.08 (m, 14H, -***(CH₂)₇*** CH_2CH_2O-).

Compound 6: An excess of trifluoroacetic acid (TFA, 20 equivalents, 3.69 g, 2.5 mL, 32.4 mmol) was added to compound **5** (1.2 g, 1.62 mmol) in 10 mL dry DCM. The color of the solution turned yellow upon addition of TFA. Then, triisopropylsilane (TIPS, 3 equivalents, 0.77g, 1 mL, 4.86 mmol) was added to the reaction mixture. The reaction mixture was stirred for 12 hr under N_2 at room temperature. The solvent, most of TFA, and TIPS were evaporated under reduced pressure. The yellow residue was purified by repeated washing with hexane (10 mL, 4 times) and dried in high vacuum. The final product formation was quantitative and was confirmed by NMR spectroscopy.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) of Compound 6: δ 7.57-7.47 (m, 5H), 4.61 (s, 2H, -***CH₂***-C_6H_5), 4.01 (s, br, 2H, -O***CH₂***$CH_2N(CH_3)_2$-), 3.74-3.48 (m, 14H, -***(OCH₂CH₂)₃***-CH_2**CH_2**$N(CH_3)_2$-), 3.41 (t, *J* = 6.8Hz, 2H, -CH_2***CH₂***O-), 3.14 (s, 6H, -N***(CH₃)₂***-), 2.52 (q, *J* = 7.2Hz, HS***CH₂***-), 1.65-1.48 (m, 4H, -***CH₂***CH_2O-,+ HSCH2***CH₂***-), 1.43-1.20 (m, 15H, -***(CH₂)₇*** CH_2CH_2O- + ***H***S-).

^{13}C NMR(400 MHz, CDCl3, TMS) of Compound 6: δ 132.92, 131.11, 129.39, 126.65, 116.69, 114.10, 71.51, 70.31, 70.21, 70.03, 69.92, 69.78, 64.59, 63.34, 50.69, 34.01, 29.49, 29.45, 29.37, 29.34, 29.18, 29.02, 28.31, 25.91, 24.60.

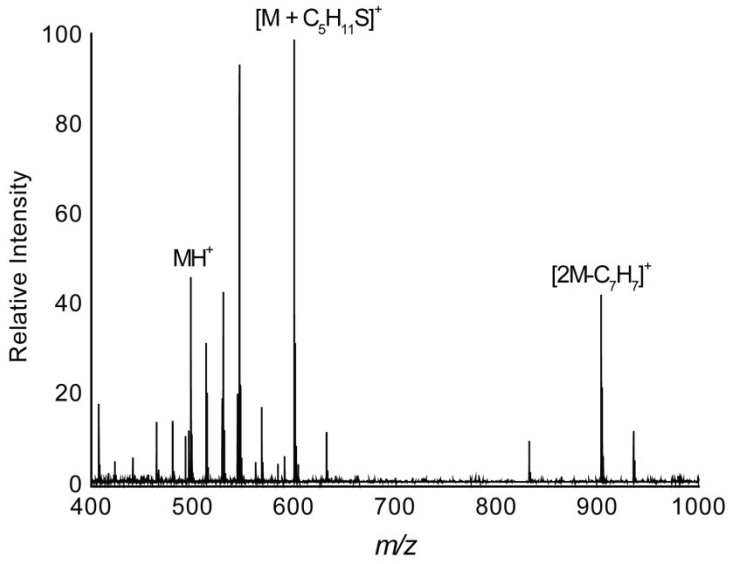


Supporting Figure 1. MALDI-MS spectrum of AuNP-TBenz. The molecular ion (MH^+) was detected at *m/z* =498.

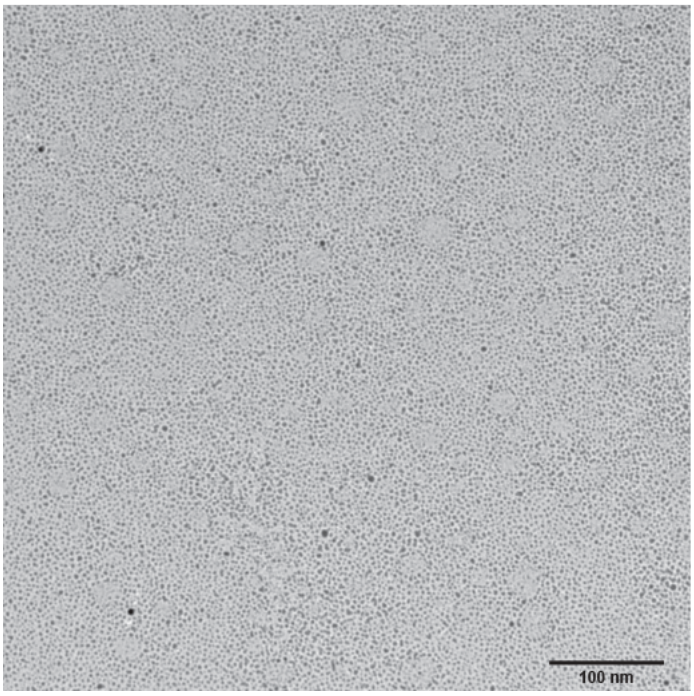
Synthesis of benzyl-ligand protected gold nanoparticle (AuNP-TBenz)

The gold salt was purchased from Strem Chemicals Inc. We followed two-step method for synthesizing **AuNP-TBen**, where a gold nanoparticle core was synthesized followed by place-exchange with the ligand of interest. First, pentanethiol-coated AuNPs with core diameter ~2 nm were synthesized using the Brust-Schiffrin two-phase synthesis protocol[3,4]. Subsequently, Murray place-exchange method[5] was followed to obtain the benzyl-ligand protected AuNPs. Pentanethiol conjugated AuNPs (10 mg) and compound **6** (27 mg) was dissolved in a mixture of 5 mL dry DCM, and 1 mL methanol and stirred under N_2 atmosphere for 72 hr at room temperature. Then, solvents were removed under reduced pressure and the resulting precipitate was washed with hexane (10 mL) three times and with DCM (10 mL) twice. Then the precipitate was dissolved in distilled water and dialyzed for 72 hr (membrane molecular weight cut-off =10,000) to remove excess ligands, pentanethiol, acetic acid, and other salts present in the nanoparticle solution. After dialysis, the particle was lyophilized to yield a solid brownish product. The

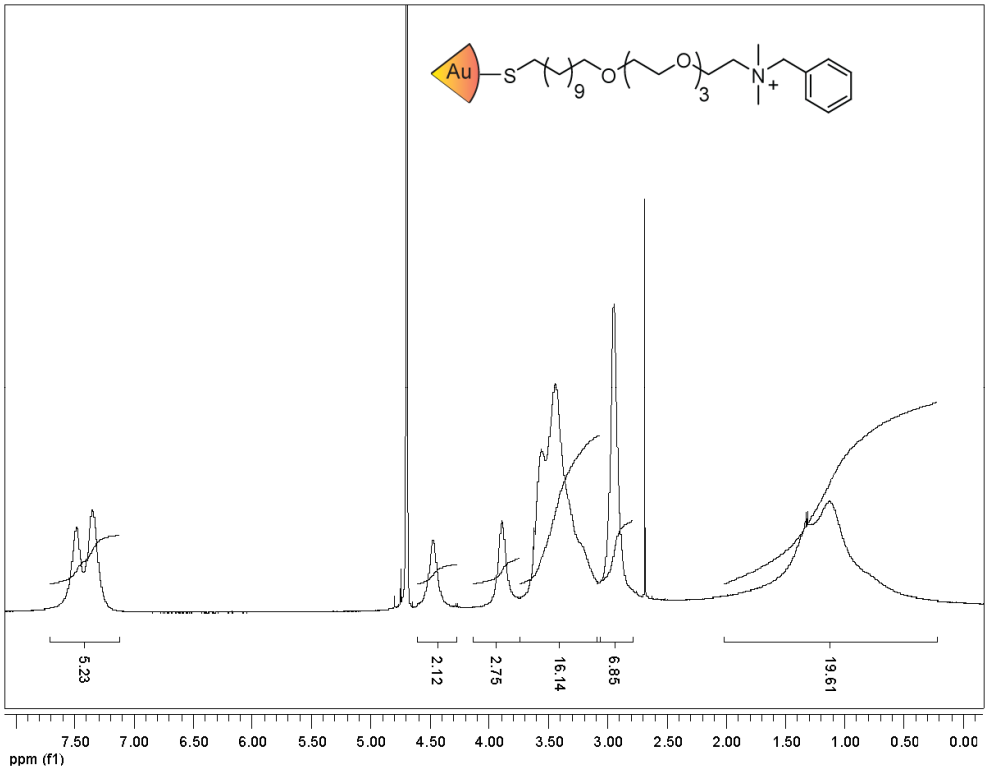
particles were then redispersed in deionized water. The presence of ligands on AuNP was also confirmed by mass spectrometry (Supporting Fig. 2). ^{1}H NMR-spectra in D_2O showed substantial broadening of the proton peaks with no sign of free ligands (Supporting Fig. 4).



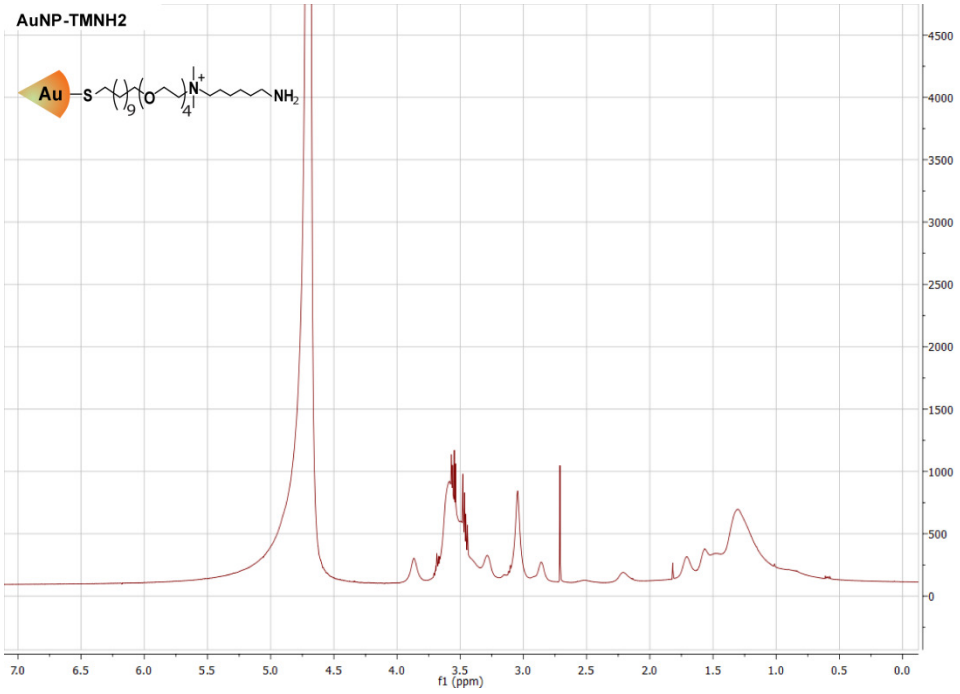
Supporting Figure 2. MALDI-MS spectrum of AuNP-TBenz. The molecular ion (MH^+, *m/z* =498) was detected, and the disulfide ion formed by the benzyl ligand and the original pentanethiol was also detected at *m/z* 600.



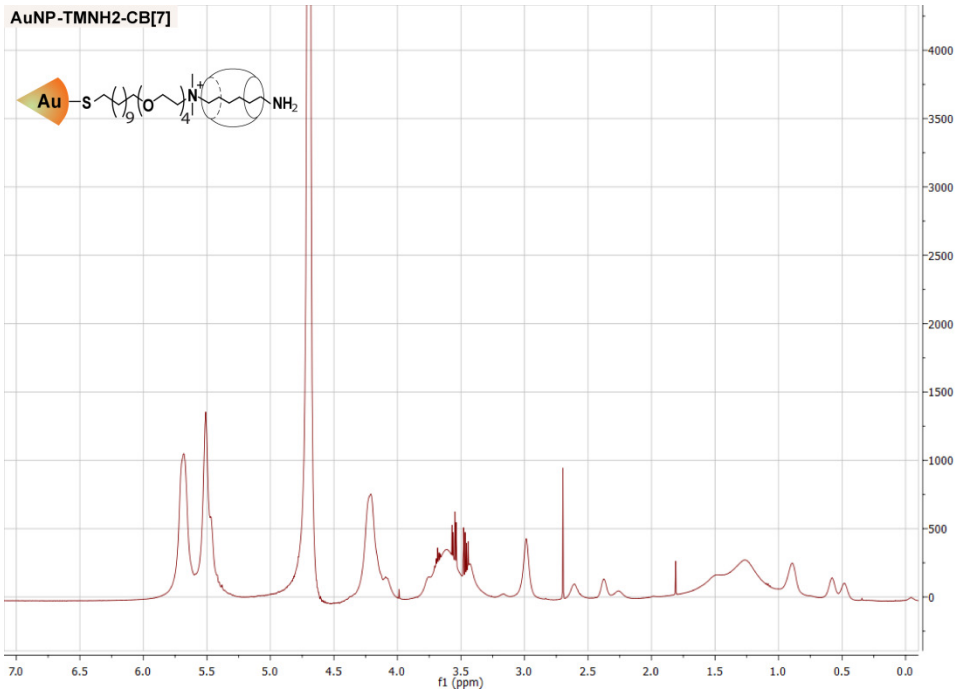
Supporting Figure 3. Transmission electron micrograph of AuNP-TBen.



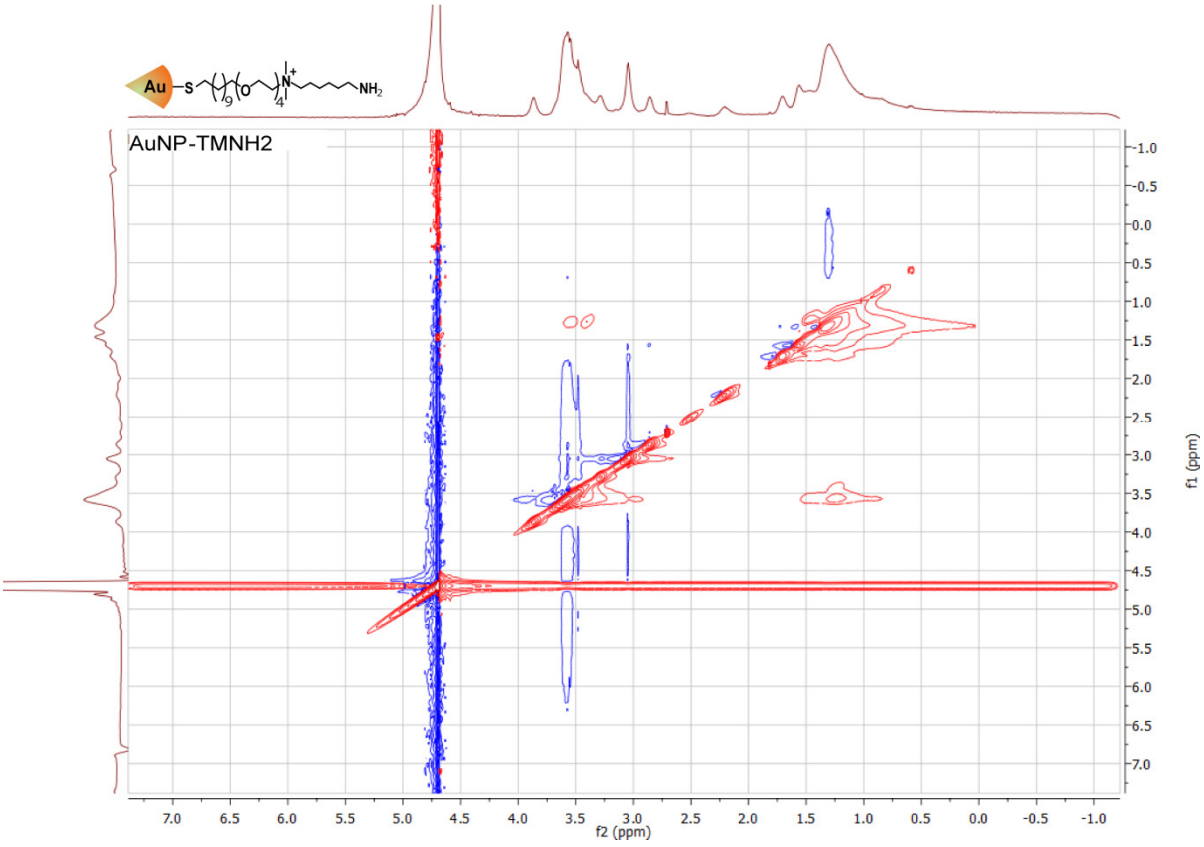
Supporting Figure 4. ^{1}H-NMR of AuNP-TBen showing the ligand attachment on AuNP surface.



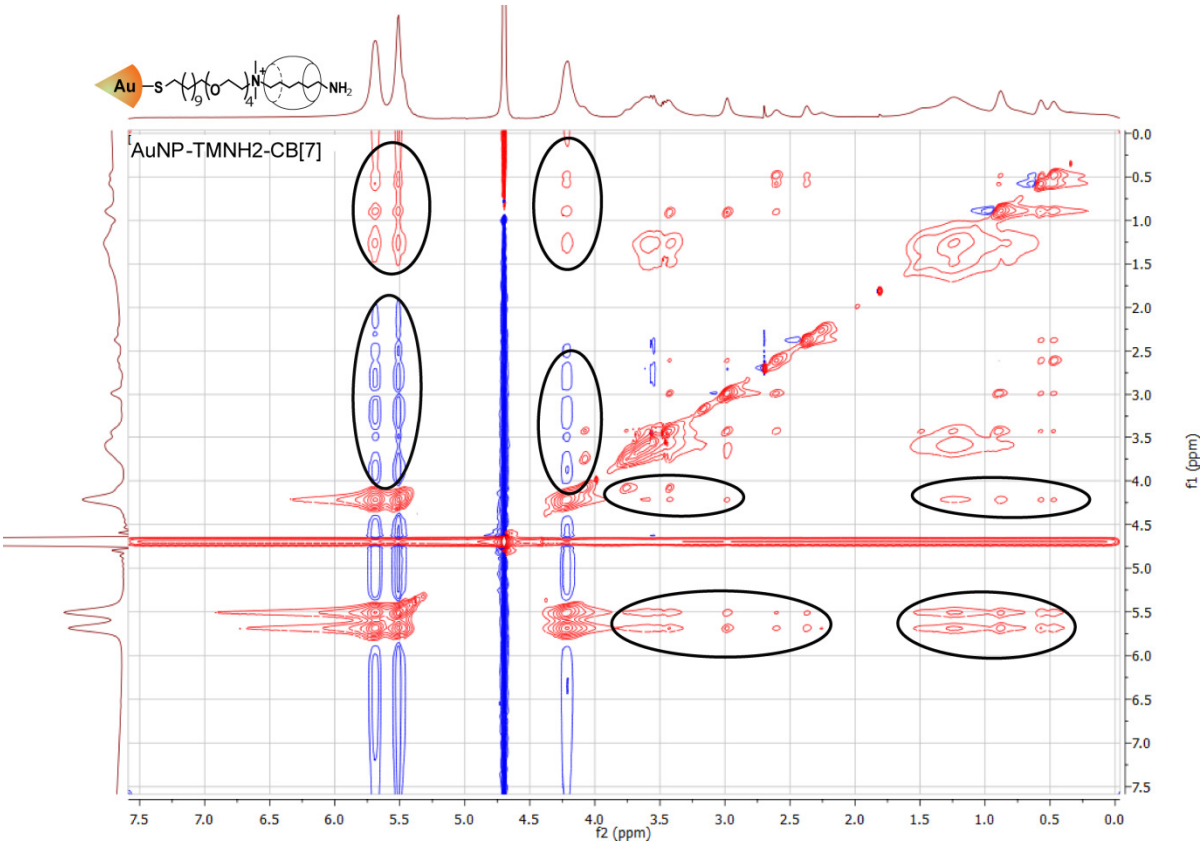
Supporting Figure 5. ^{1}H-NMR of AuNP-TMNH2 in D_2O.



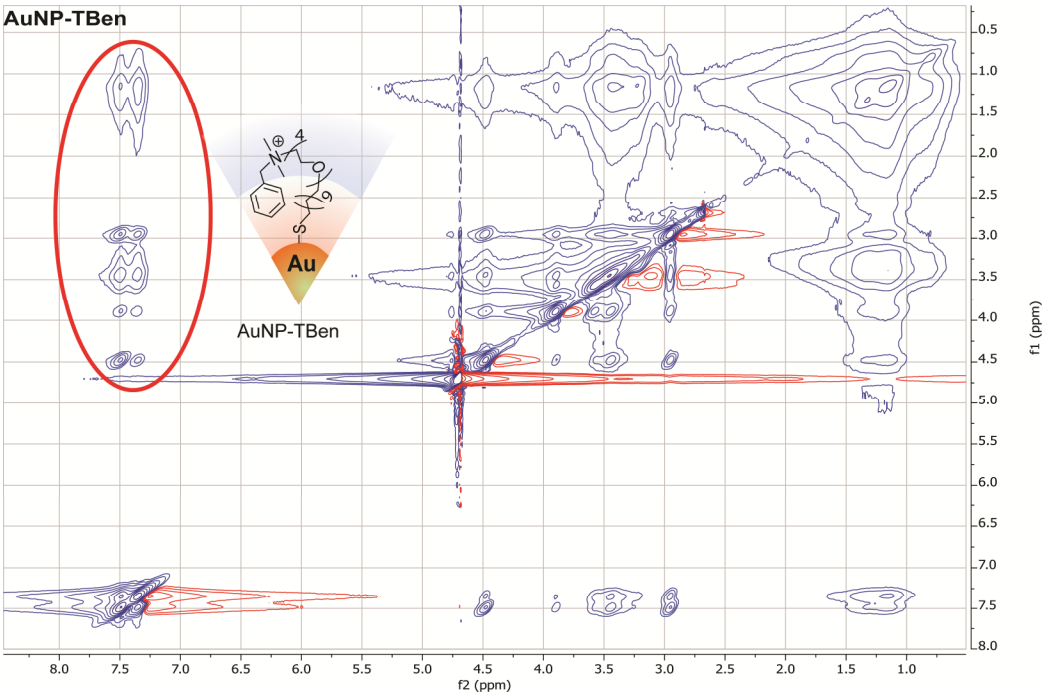
Supporting Figure 6. ^{1}H-NMR of AuNP-TMNH2-CB[7] in D_2O.



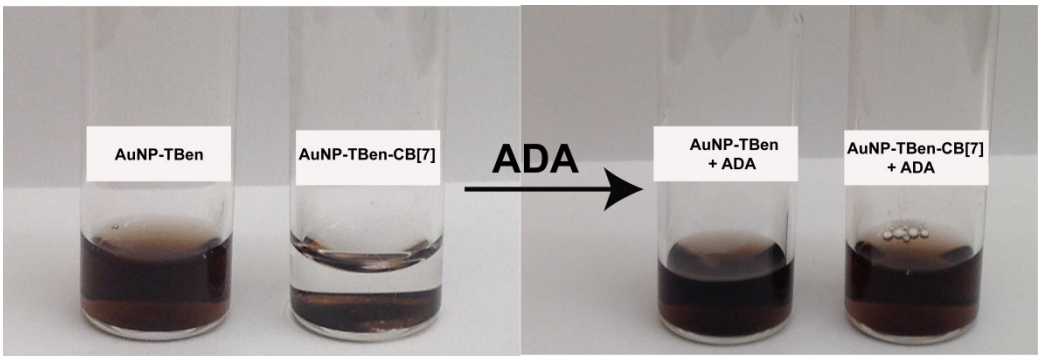
Supporting Figure 7. Noesy 2D NMR of AuNP-TMNH2.



Supporting Figure 8. Noesy 2D NMR of AuNP-TMNH2-CB[7] showing the host-guest interactions between CB[7] and AuNP-TMNH2 (black circles).



Supporting Figure 9. Noesy 2D NMR shows the interaction of benzyl moiety with TEG and C11 units, indicating head group is bending in towards the monolayer (red circle).



Supporting Figure 10. CB[7] treatment induced the formation of large assemblies. Addition of ADA triggered the particle assemblies to disassemble, making the particles soluble back into the PBS.

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