Supplementary material (ESI) for Journal of Materials Chemistry

Supporting information for

Structural control of the monolayer stability of water-soluble gold nanoparticles

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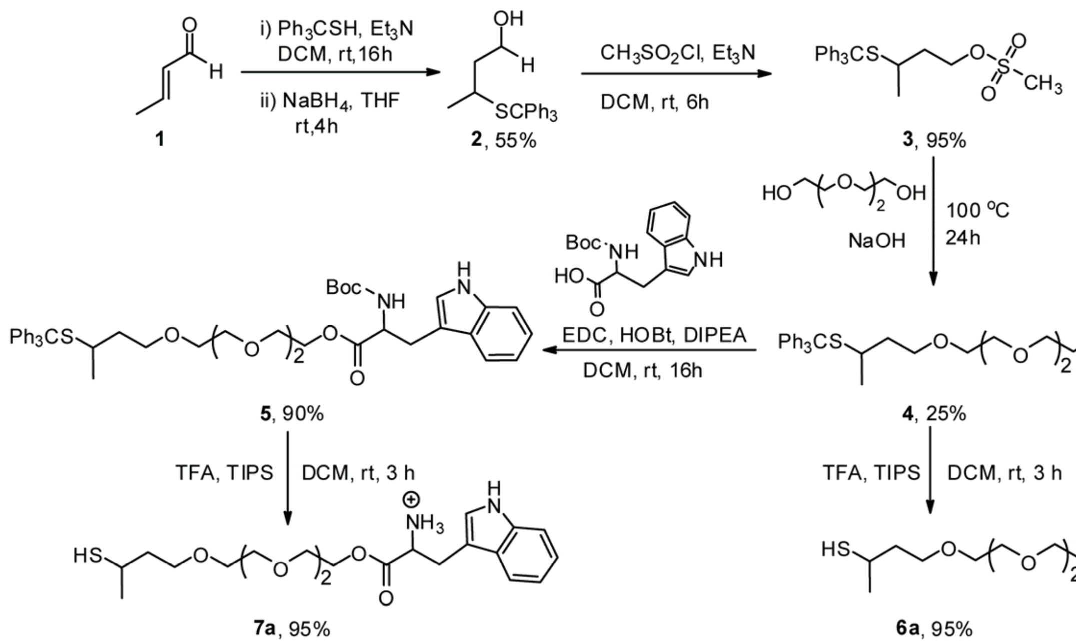
Section I. Materials



Scheme S1. Synthesis of *Sec* thiol ligands**.**

Synthesis of compound 2. The mixture of compound **1** (0.98 g, 14 mmol), triethylamine (1.4 g, 14mmol), and triphenylmethyl mercaptan (2.76 g, 10 mmol) in DCM (50 mL) was stirred at room temperature for 16 h. After removal of the solvent, the crude product was dissolved in THF (75 mL), to which was added an alcoholic solution of $NaBH_4$ (0.794 g, 21 mmol). The reaction mixture was stirred at room temperature for 4 h. Subsequently, the solution was neutralized to pH ~ 7 with 2M HCl (aq). After evaporation of the solvent, the residue was dissolved in ethyl acetate (200 mL), which was washed successively with water and brine and dried over anhydrous sodium sulfate. After removal of the solvent under reduced pressure, the residue was charged on a SiO_2 column for purification (eluent: 25% ethyl acetate in hexane). Compound **2** was obtained as a viscous liquid. Yield 2.7 mg (55%). ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 7.52 (m, 6H, H_{Ar}), 7.27 (m, 6H, H_{Ar}), 7.19 (m, 3H, H_{Ar}), 3.53 (m, 1H, $–CH_2O–$), 3.40 (m, 1H, $–CH_2O–$), 2.39 (m, 1H, >CHS–), 1.44 (m, 2H, $–CH_2–$), 1.06 (d, ^3J = 6.8 Hz, 3H, $–CH_3$).

Synthesis of compound 3. Compound **2** (2g, 5.7 mmol) was dissolved in DCM (30 mL) and triethylamine (1.2 g, 11.4mmol) was added dropwise into the solution. The solution was chilled to 0 °C with an ice bath. Subsequently, methane sulfonylchloride (1.0 g, 8.6 mmol) was added dropwise under stirring and the temperature was maintained at 0 °C. After addition, the reaction was stirred below 5 °C for another 30 min. Then the reaction mixture was allowed to reach room temperature automatically and the stirring continued for another 5 h. TLC was used to monitor the reaction. After reaction completion, the solution was diluted with DCM (300 mL). The organic layer was washed successively with 5% HCl (×2), saturated aqueous sodium bicarbonate (×1), water (×1), and brine (×1) and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded compound **3** pure enough for the next step of reaction. Yield 2.3 mg (95%). ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 7.52 (m, 6H, H_{Ar}), 7.27 (m, 6H, H_{Ar}), 7.19(m, 3H, H_{Ar}), 4.06 (m, 2H, $–CH_2O–$), 2.88 (s, 3H, $–CH_3$), 2.39 (m, 1H, >CHS–), 1.65 (m, 1H, $–CH_2–$), 1.47 (m, 1H, $–CH_2–$), 1.09 (d, ^3J = 7.1 Hz, 3H, $–CH_3$).

Synthesis of compound 4. Sodium hydroxide (0.2 g, 4.6 mmol) was dissolved in 4 mL of water in a round bottom flask. Subsequently, tri (ethylene glycol) (7.04 g, 46 mmol) was added and the resulting mixture was heated to 100 °C under stirring. Then, compound **3** (2.0 g, 4.6 mmol) was added and the reaction mixture was stirred at 100 °C for 24 h. After cooling to room temperature, the solution was extracted with hexane for 5 times. The hexane layers were combined. After evaporation of the solvent under reduced pressure, the residue was charged on a SiO_2 column for purification (eluent: 50% ethyl acetate in hexane). Compound **4** was obtained as a viscous pale yellow liquid), yield 0.56 mg (25%). ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 7.49 (m, 6H, H_{Ar}), 7.27 (m, 6H, H_{Ar}), 7.17(m, 3H, H_{Ar}), 3.65-3.55 (m, 10H, $-CH_2O-$), 3.45 (t, ^3J = 9.6 Hz, 2H, $-OCH_2-$), 3.34 (t, ^3J = 6.8 Hz, 2H, $-CH_2O-$), 2.37 (m, 1H, >CHS–), 1.64 (m, 1H, $-CH_2-$), 1.50 (m, 1H, $-CH_2-$), 0.94 (d, ^3J = 6.8 Hz, 3H, $-CH_3$).

Synthesis of compound 5. *tert*-Butoxycarbonyl protected L-tryptophan (0.222 g, 0.73 mmol) was dissolved in 10 mL of dry DCM, which was cooled to 0 °C with an ice bath. Then HOBt·H_2O (0.1 g, 0.73 mmol), DIPEA (0.1 g, 0.73 mmol) and EDC (0.157 g, 0.91 mmol) were added to the solution and the mixture was stirred at 0 °C for 5 min. Subsequently, compound **4** (0.350g, 0.73 mmol) was added to the reaction mixture. The reaction mixture was allowed to rise automatically to room temperature and stirred for 16 h. Then, the reaction mixture was poured into water (300 mL). The aqueous solution was extracted with ethyl acetate for four times. The organic layers was combined and dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was charged on a SiO_2 column for purification (eluent: 50% ethyl acetate in hexane). Compound **5** was obtained as a viscous liquid), yield 0.5 mg (90%). ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 8.73 (s, 1H, –NH–), 7.56 (d, ^3J = 7.8 Hz, 1H, –NH–), 7.49 (m, 6H, $TrtH_{Ar}$), 7.28-7.07

(m, 14H, $TrtH_{Ar}$ + $TrpH_{Ar}$), 5.1 (m, 1H, –NCHCO–), 4.69 (m, 1H, $–CH_2–$indole ring), 4.38 (m, 1H, $–CH_2–$indole ring), 3.64-3.47 (m, 10H, $–CH_2O–$), 3.37 (t, ^3J = 9.6 Hz, 2H, $–OCH_2–$), 3.32 (t, ^3J = 5.8 Hz, 2H, $-CH_2-O$), 2.36 (m, 1H, >CHS–), 1.66 (m, 1H, $–CH_2–$), 1.50 (m, 1H, $–CH_2–$), 1.44 (s, 9H, $–C(CH_3)_3$), 0.92 (d, ^3J = 6.8, 3H, $–CH_3$).

General procedure for the synthesis of compounds 6a and 7a. The trityl protected thiol ligand was dissolved in dry DCM and an excess of trifluoroacetic acid (TFA, ~ 10 equivalent) was added. The colour of the solution was turned to yellow immediately. Subsequently, triisopropylsilane (TIPS, ~ 1.5 equivalent) was added to the reaction mixture. The reaction mixture was stirred at room temperature for 3 h. The solvent and most TFA and TIPS were distilled off under reduced pressure. The pale yellow residue was further dried in high vacuum. The product formation was quantitative and their structure was confirmed by NMR.

Compound **6a**: ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 4.5 (m, 2H, $–CH_2O–$), 3.8 (m, 2H, $–OCH_2–$), 3.68-3.58 (m, 10H, $–CH_2O–$), 3.1 (m, 1H, >CHS–), 2.1 (br s, 1H, –OH), 1.91 (m, 1H, $–CH_2–$), 1.70 (m, 1H, $–CH_2–$), 1.52 (d, ^3J = 6.5 Hz, 1H, –SH), 1.36 (d, ^3J = 6.8 Hz, 3H, $–CH_3$). *m/z* (EI) 239.0 ($[M+1]^+$, $C_{10}H_{23}O_4S$ requires 239.1).

Compound **7a**: ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 8.80 (s, 1H, –NH–), 7.64 (br, 3H, $–NH_3^+$), δ 7.50-7.08 (m, 5H, $TrtH_{Ar}$), δ 4.3-4.19 (m, 3H, N-CH-CO + $-CH_2$-indole ring), δ 3.64-3.37 (m, 14H, $-CH_2-O$), δ 2.94 (m, 1H, >CH-S), δ 1.82 (m, 1H, $-CH_2-$), δ 1.63 (m, 1H, $-CH_2-$), δ 1.28 (d, ^3J=6.8, 3H, $-CH_3$). *m/z* (ESI) 425.2 (M^+, $C_{21}H_{33}N_2O_5S^+$ requires 425.2).

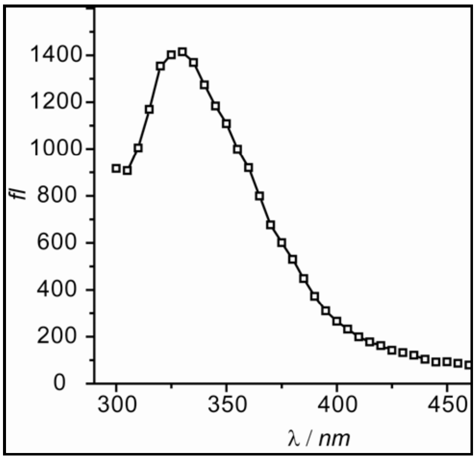
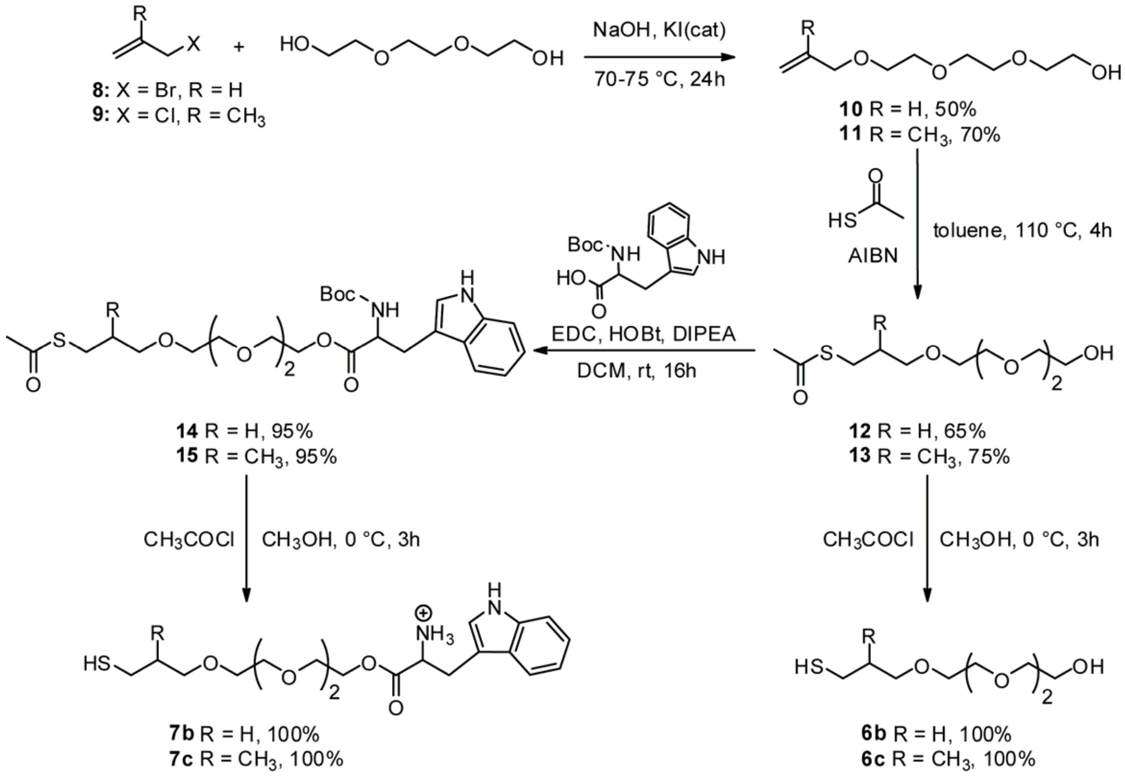


Figure S1. Fluorescence spectra of compound **7a** in toluene (λ_{ex}=295).



Scheme S1. Synthesis of *Nor* and *Iso* thiol ligands**.**

General procedure for the synthesis of compounds 10 and 11. Sodium hydroxide (2.64 g, 66 mmol) was dissolved in 4 mL of water in a round bottom flask. Subsequently, tri(ethylene glycol)

(8 mL, 660 mmol) was added and the resulting mixture was heated to 70-75 °C under stirring. Compound **8** (5.7 mL, 66 mmol) or **9** (6.5 mL, 66 mmol) together with the catalyst KI (2.192 g, 13.2 mmol) was added to the reaction mixture. The reaction mixture was stirred at 70-75 °C for 24 h. After cooling to room temperature, the solution was poured into water (300 mL) and dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was charged on a SiO_2 column for purification (eluent: ethyl acetate).

Compound **10**: Yield = 50%; ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 5.92 (m, 1H, =CH–), 5.25 (m, 1H, HC=), 5.16 (m, 1H, HC=), 4.03 (m, 2H, $–CH_2O–$), 3.74-3.60 (m, 12H, $–OCH_2–$).

Compound **11**: Yield = 70%; ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 4.93 (m, 1H, HC=), 5.87 (m, 1H, HC=), 3.90 (s, 2H, $–CH_2O–$), 3.71-3.53 (m, 12H, $–OCH_2–$), 2.64 (br s, 1H, –OH), 1.71 (s, 3H, $–CH_3$).

General procedure for the synthesis of compounds 12 and 13. Compound **10** (3.8 g, 20 mmol) or **11** (4 g, 20 mmol) was dissolved in 20 mL of dry toluene. Then, AIBN was added under an argon atmosphere. The reaction mixture was stirred for 10 min and thioacetic acid (4.32 ml, 60 mmol) was added. The reaction mixture was heated to reflux and stirred under argon for 12 h. After cooling to room temperature, the reaction mixture was concentrated and charged on a SiO_2 column for purification.

Compound **12**: Yield = 65%; ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 3.71-3.57 (m, 12H, $–OCH_2–$), 3.49 (t, ^3J = 6.2 Hz, 2H, $–CH_2O–$), δ 2.93 (t, ^3J = 7.1 Hz, 2H, $–CH_2S–$), 2.70 (br s, 1H, –OH), 2.30 (s, 3H, $CH_3CO–$), 1.86 (m, 2H, $–CH_2–$).

Compound **13**: Yield = 75%; ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 3.68-3.56 (m, 12H, $-OCH_2-$), 3.34 (d, ^{3}J = 6.1 Hz, 2H, $-CH_2O-$), 3.03 (m, 1H, $-CH_2S-$), 2.81 (m, 1H, $-CH_2S-$), 2.56 (br s, 1H, −OH), 2.33 (s, 3H, CH_3CO-), 1.99 (m, 1H, >CH−), 0.96 (d, ^{3}J = 6.8 Hz, 3H, $-CH_3$).

General procedure for the synthesis of compounds 14 and 15. The synthesis of compounds **14** and **15** followed the procedure as described for compound **5**. Purification was achieved on a SiO_2 column with 50% ethyl acetate in hexane as eluent.

Compound **14**: Yield = 95%; ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 8.7 (s, 1H, −NH−), 7.57 (d, ^{3}J = 7.8 Hz, 1H, −NH−), 7.36-7.07 (m, 5H, H_{Ar}), 5.13 (m, 1H, −NCHCO−), 4.69 (m, 1H, $-CH_2-$indole), 4.34 (m, 1H, $-CH_2-$indole), 3.65- 3.52 (m, 12H, $-CH_2O-$), 3.32 (m, 2H, $-CH_2O-$), 2.92 (t, ^{3}J = 7.1 Hz, 2H, $-SCH_2-$), 2.30 (s, 3H, $-CH_3CO-$), 1.86 (m, 2H, $-CH_2-$), 1.46 (s, 9H, $-C(CH_3)_3$).

Compound **15**: Yield = 95%; ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 8.6 (s, 1H, −NH−), 7.56 (d, ^{3}J = 7.8 Hz, 1H, −NH−), 7.34-7.07 (m, 5H, H_{Ar}), 5.12 (m, 1H, −NCHCO−), 4.67 (m, 1H, $-CH_2-$indole), 4.33 (m, 1H, $-CH_2-$indole), 3.66-3.52 (m, 10H, $-CH_2O-$), 3.32 (m, 4H, $-CH_2O-$), 3.00 (m, 1H, $-SCH_2-$), 2.80 (m, 1H, $-SCH_2-$), 2.30 (s, 3H, CH_3CO-), 1.99 (m, 1H, >CH−), 1.43 (s, 9H, $-C(CH_3)_3$), 0.93 (m, 3H, $-CH_3$).

General procedure for the synthesis of 6b and 6c. Thioester **12** or **13** (1.9 mmol) was dissolved in 25 mL of ethanol, which was purged with argon for 5 min. Then, 0.6 mL of concentrated HCl was added to the reaction mixture. The solution was stirred at room temperature for 3 h. Then, methanolic ammonium hydroxide (5%) was used to adjust the pH value ca. 5. The solvent was evaporated under reduced pressure and 15 mL of water was added to dissolve the salt formed. The aqueous solution was extracted with ethyl acetate for five times. The organic layers were combined and washed successively with water (×2) and brine (×2) and dried over anhydrous sodium sulfate. After removal of the solvent, spectroscopically pure product was obtained in quantitative yields.

Compound **6b**: ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 3.73-3.56 (m, 14H, $-OCH_2-$), 2.63 (q, 2H, $-CH_2S-$), 1.87 (m, 2H, $-CH_2-$), 1.4 (t, ^3J = 8.1 Hz, 1H, –SH).). *m/z* (EI) 225.1 ($[M+1]^+$, $C_9H_{21}O_4S$ requires 225.1).

Compound **6c**: ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 3.74-3.59 (m, 12H, $-OCH_2-$), 3.39 (d, ^3J = 6.3 Hz, 2H, $-CH_2O-$), 2.65 (m, 1H, $-CH_2S-$), 2.50 (m, 1H, $-CH_2S-$), 1.96 (m, 1H, >CH–), 1.33 (t, 1H, –SH), 0.97 (d, ^3J = 6.8 Hz, 3H, $-CH_3$). *m/z* (EI) 239.0 ($[M+1]^+$, $C_{10}H_{23}O_4S$ requires 239.1).

General procedure for the Synthesis of 7b and 7c. Compound **14** or **15** (0.3 mmol) was dissolved in methanol (15mL). The solution was cooled to 0 °C with an ice bath. Excess amount of acetyl chloride (4.5 mmol) was added slowly under an argon atmosphere. The reaction mixture was allowed to rise automatically to room temperature and stirred for 3 h. The solvent was evaporated under reduced pressure. The residue was dried in high vacuum to get compounds **7b** and **7c**.

Compound **7b**: ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 9.44 (s, 1H, –NH–), 8.3 (br, 3H, $-NH_3^+$), 7.52-6.99 (m, 5H, H_{Ar}), 4.13 (m, 3H, –NCHCO– + $-CH_2-$indole ring), 3.73- 3.43 (m, 12H,

–CH_2O–), 2.94 (t, ^3J = 7.32 Hz, 2H, –CH_2O–), 2.61 (q, 2H, –SCH_2–), 1.87 (m, 2H, –CH_2–), 1.39 (t, 1H, –SH). *m/z* (ESI) 411.1 (M^+, $C_{20}H_{31}N_2O_5S^+$ requires 411.1).

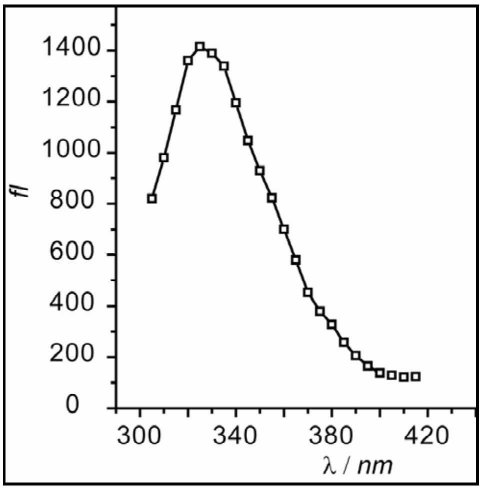


Figure S2. Fluorescence spectra of compound **7b** in toluene (λ_{ex}=295).

Compound **7c**: ^{1}H NMR (400MHz, $CDCl_3$, TMS): δ 9.3 (s, 1H, –NH–), 7.51-6.99 (m, 5H, H_{Ar}), δ 4.09 (m, 3H, –NCHCO– + –CH_2–indole ring), 3.72-3.3 (m, 14H, –CH_2O–), 2.63 (m, 1H, –SCH_2–), 2.47 (m, 1H, –SCH_2–),1.96 (m, 1H, >CH–),1.32 (t, 1H, –SH), 0.96 (d, ^3J = 6.8 Hz, 3H, –CH_3). *m/z* (ESI) 425.1 (M^+, $C_{21}H_{33}N_2O_5S^+$ requires 425.2).

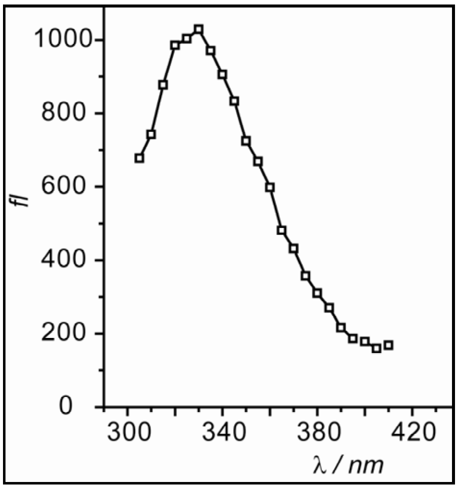
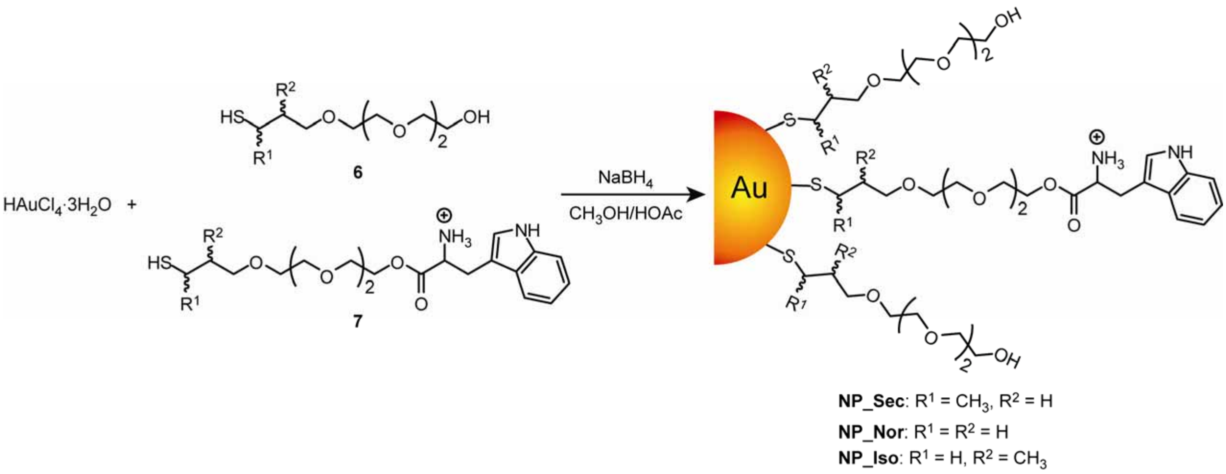


Figure S3. Fluorescence spectra of compound **7c** in toluene (λ_{ex}=295).



Scheme S3. Reaction scheme for the preparation of gold nanoparicles.

General procedure for preparation of gold nanoparticles. Gold nanoparticles were prepared through the single phase reduction of chloroauric acid by sodium borohydride in presence of corresponding thiol ligands according to a procedure reported by Huang *et al.* (Scheme S3).[1] In a typical reaction, chloroauric acid ($HAuCl_4 \cdot 3H_2O$, 0.675 mmol) was dissolved in a mixture of 75 mL of methanol and 10 mL of glacial acetic acid. Subsequently, ligands **6** (0.225 mmol) and **7**

(0.075 mmol) were added to the solution and stirred for 5 h. Then, to the solution was added $NaBH_4$ (6 mmol in 10 mL of water) dropwise. The solution was turned from bright yellow to dark brown immediately with the addition of the first drop of $NaBH_4$, indicating the formation of gold nanoparticles. After addition, the solution was stirred at room temperature for 3 h. Then, methanol was evaporated under reduced pressure. The residue was dissolved in a small amount of water and dialyzed (membrane MWCO = 12,000-14,000) to remove excess lignds, acetic acid and the other salts present with the nanoparticles. After dialysis, the particles were lyophilized to afford a brownish solid. The particles are redispersed in water and PBS buffer solution. ^{1}H NMR spectra in D_2O showed substantial broadening of the proton signals and no free ligands were observed.

Section II. Methods

Transmission Electron Microscopy (TEM). A drop of aqueous solution of the nanoparticle was placed on a 300 mesh carbon-coated copper grid. The grid was dried at room temperature for overnight to evaporate the water. The TEM images were taken on a JEOL 2000fx instrument operated at 200 keV. TEM images of the particles coupled with their respective size distributions are shown in Figure S1.

Thermogravimetric analysis (TGA). TGA was performed on a TGA 2950 high-resolution thermogravimetric analyzer (TA Instruments, Inc., New Castle, DE), which was equipped with an open platinum pan and an automatically programmed temperature controller. The nanoparticles were placed in the TGA pan and heated from room temperature (ca. 25 °C) to 500

°C at a rate of 10 °C min^{-1} under a nitrogen atmosphere. The weight loss was recorded as a function of temperature to create the TGA curves.

Kinetics of cyanide induced nanoparticle decomposition. Nanoparticle decomposition was conducted in phosphate buffered saline (PBS) solution at 37 °C. In all cases, the gold clusters (0.2 µmol dm^{-3}, [Au] ~ 0.21 mmol dm^{-3}) were combined with a KCN solution (3 mmol dm^{-3}) and the UV/vis absorbance at 520 nm was recorded every 15 s on a Molecular Devices SpectraMax M5 spectrophotometer. The absorption data (A) were introduced to the following first-order reaction function for curve-fitting analysis.[2]

$$A = A_S + A_1 \exp(-k_I \cdot t)$$

where A_S denotes the absorbance due to the light scattering by finely suspended reaction by-products, which is assumed to be constant in each data set; k_I is the pseudo first-order rate constant.

Determination of L-tryptophan ligand number per particle. The gold core of a known concentration of cluster was decomposed using excess amount of cyanide solution. Among the resultant products only L-tryptophan shows substantial absorption at 280 nm. The L-tryptophan concentration was deduced by Beer-Lambert Law where the molar extinction coefficient of L-tryptophan was 5690 dm^3 mol^{-1} cm^{-1} at 280 nm.[3] The number of L-tryptophan ligand per nanoparticle was then determined by the ratio of L-tryptophan to cluster concentrations. For a 3 nm particle, the total number of surface ligands was estimated as 187,[4] which was used to calculate the percentage of L-tryptophan ligand per particle.

Kinetics of in situ ligand-exchange. A PBS solution (500 µL) containing Au-MPCs (0.2 µmol dm^{-3}) and external thiol ligands ([DDT] = [DHLA] = 1 mmol dm^{-3}, [GSH] = 5 mmol dm^{-3}) was placed in a conventional quartz fluorescence cuvette (10 × 10 × 40 mm). Then, toluene (1500 µL) was added on the top of the aqueous phase to form a two-phase system. The cell was covered with a cap and placed in a Molecular Devices SpectraMax M5 fluorimeter. The fluorescence intensity of the toluene phase at 350 nm (λ_{ex} = 295 nm) was recorded overtime at 37 °C. As there are excess amount of external ligands which can be treated as a constant, a pseudo first-order kinetics was assumed. The parameter $\ln[(I_f - I)/(I_f - I_0)]$ was plotted as a function of time to generate a straight line.[5] In the expression, I_f stands for the fluorescence intensity after completion of the ligand exchange reaction, I denotes the instantaneous fluorescence intensity during the course of ligand-exchange, and I_0 refers to the initial fluorescence intensity from the solution. The pseudo first-order rate constants were obtained through a regression analysis on the $\ln[(I_f - I)/(I_f - I_0)] \sim t$ plots using Origin 7.0 program (OriginLab Co., Northampton, USA).

1 M. Zheng, F. Davidson and X.-Y. Huang, *J. Am. Chem. Soc.*, 2003, **125**, 7790-7791.

2 A. C. Templeton, M. J. Hostetler, C. T. Kraft and R. W. Murray, *J. Am. Chem. Soc.*, 1998, **120**, 1906-1911.

3 S. C. Gill and P. H. von Hippel, *Anal. Biochem.*, 1989, **182**, 319-326.

4 M. J. Hostetler, J. E. Wingate, C.-J. Zhong, J. E. Harris, R. W. Vachet, M. R. Clark, J. D. Londono, S. J. Green, J. J. Stokes, G. D. Wignall, G. L. Glish, M. D. Porter, N. D. Evans and R. W. Murray, *Langmuir*, 1998, **14**, 17-30.

5 (a) M. Montalti, L. Prodi, N. Zaccheroni, R. Baxter, G. Teobaldi and F. Zerbetto, *Langmuir*, 2003, **19**, 5172-5174; (b) M. J. Hostetler, A. C. Templeton and R. W. Murray, *Langmuir*, 1999, **15**, 3782-3789.

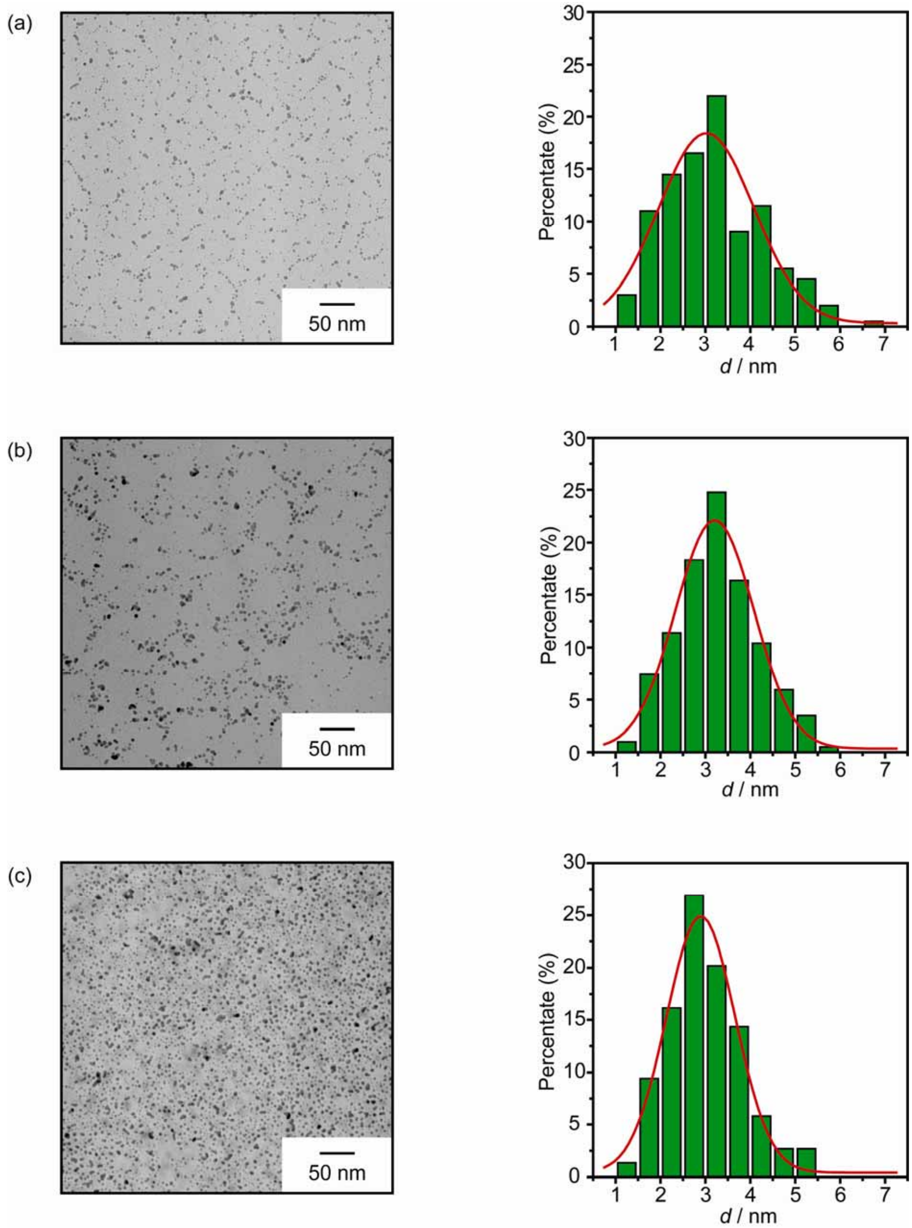


Figure S4. Transmission electron microscopic (TEM) images and histograms of size distribution of (a) **NP_Sec** (d = 3.2±1.1 nm), (b) **NP_Nor** (d = 3.3±0.9 nm), and (c) **NP_Iso** (d = 3.0±0.8 nm).