Supporting Information

Method for Separating PEGylated Au Nanoparticle Ensembles as a Function of Grafting Density and Core Size

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EXPERIMENTAL DETAILS

Chemicals and Instrumentation. $HAuCl_4$ solution (30% in HCl solution), 0.05 M Iodine solution, $NaBH_4$, tetra-n-octylammonium bromide (TOAB), dodecylamine (DDA), sodium chloride (NaCl), silica gel (200-400 mesh, 60Å), methanol and toluene were purchased from Sigma Aldrich. Chloroform was purchased from Fisher Scientific. Monofunctional methoxy polyethylene glycol thiol (mPEG-SH) of different lengths were purchased from Laysan Bio Inc. All the chemicals were of analytical grade and used as received without further purification.

UV-vis spectra were obtained by using a Cary 50 Bio spectrophotometer. Hydrodynamic diameters were measured through dynamic light scattering (DLS) on a 90 Plus Particle Size Analyzer (Brookhaven Instruments Corp.). The wavelength of the laser was set at 660 nm and the detection angel was 90°. Transmission electron microscopy (TEM) was performed using a JEOL JEM 1200EX electron microscope. Samples were prepared on 400 mesh carbon film grids and the TEM instrument was operated at an acceleration voltage of 80 kV.

Preparation of PEGylated Au NPs. A modified Brust-Schiffrin method[1] was used to synthesize Au NPs, as described earlier.[2] Briefly, 0.25 mmol of TOAB, 0.6 mmol of DDA and 0.53 mmol of $HAuCl_4$ solution were added to 5 ml of toluene in

sequence. Then, 2 mmol $NaBH_4$ in cold water was added into the above solution and stirred vigorously for 2 h. The DDA stabilized Au NPs were then precipitated in ethanol and re-dispersed in 5 mL chloroform. The PEGylation of the Au NPs was conducted in chloroform by mixing DDA-coated Au NPs with different molecular weight mPEG-SH ligands at room temperature. The mixture was stirred for two days and then stored in the refrigerator.

The concentration of Au NPs in chloroform was estimated based on Lambert-Beer's law (extinction coefficient of 1.5×10^7 M^{-1} cm^{-1} used for as-prepared Au NPs).[2] All the Au NPs were dispersed in chloroform and adjusted to the same concentration (2 μM), based on absorption spectra, before analysis and separation. During all tests, PEGylated Au NPs with a PEG to NP ratio of 500:1 were used if not mentioned otherwise.

General Procedure for Liquid Chromatography of Au NPs. Analytical thin layer chromatography (TLC) was carried out on Whatman Aluminum-backed silica gel plates (thickness: 250 μm). Typically, sample amounts and concentrations were used as follows: 1 μL Au NPs (DDA capped or PEGylated, 2 μM) and 2 μL mPEG-SH (1mg/mL) in $CHCl_3$. Different ratios of methanol in chloroform were used as the mobile phase. Migration of free mPEG-SH on TLC plates was monitored by staining with a 0.05 M iodine solution for several minutes and subsequent rinsing with water.[3] In the case of symmetrical spots, the distance Au NPs traveled on the plate was measured from the center of the spot to the starting line; with spots that included a pronounced trailing band, the farthest point was measured instead of the center.

Chromatography behaviors of Au NPs capped with PEG of different MWs were studied in a LC column (inner diameter: 10 mm) with silica gel as stationary phase. The column was packed with 5 g of silica gel by the slurry method resulting in a column length of around 14.5 cm. Then, 0.4 nmol of prepared PEGylated Au NPs in 300 μL eluent were loaded onto the column and eluted. Fractions (0.5 mL) were collected and UV-vis spectra of each fraction were recorded after separation. The amount of Au NPs in each fraction was estimated by the intensity of its surface plasmon resonance (SPR) absorption peak around 520 nm. The trapped Au NPs were

further eluted with pure methanol (in case of 14.3% methanol eluted column) or water (in case of 25% methanol eluted column). When eluted with water, a short column (4 cm) was used to collect the fraction). 14.3% methanol was utilized as a standard eluent, except when the examination of a trapped fractions was involved.

For the measurement of UV-vis spectra of dried Au NP film, a small volume of Au NPs solution (approximate 30 μL) in $CHCl_3$ or $CHCl_3$/methanol mixture was dropped on a transparent glass slide to dry. After the solvent had evaporated the spectra was measured in transmission mode.

Table S1. DLS results of the original Au NPs and the fractions eluted from column chromatography.

Sample ID	Multimodal Size Distribution (nm)[a]	Log-normal Size Distribution (nm)[b]	Polydispersity Index[c]
5 kDa Original NPs	35.7 120	66.4 ± 3.4 (in 14.3% methanol)	0.164 ± 0.055
	39.3 83.2	60.3 ± 0.3 (in 25% methanol)	0.118 ± 0.010
5 kDa Fraction 1 (14.3% methanol elution)	36.1	36.7 ± 0.7	0.034 ± 0.025
5 kDa Fraction 1 (25% methanol elution)	38.5	38.2 ± 0.6	0.036 ± 0.017
Au NPs (5 kDa PEG to Au NP ratio = 50:1)[d]	25.7	25.4 ± 0.2	0.014 ± 0.002
1 kDa PEG-Au NPs[d]	17.5	18.8 ± 0.4	0.017 ± 0.012
2 kDa PEG-Au NPs[d]	25.0	25.3± 0.2	0.023 ± 0.010
10 kDa PEG-Au NPs[d]	54.5	54.8± 0.2	0.020 ± 0.009

[a]Multimodal size distribution was provided by the instrument[e] automatically after combination of 3 runs. [b]Lognormal size distribution provides the average size of the sample. [c]Polydispersity index was obtained from the log-normal size distribution model, here the low polydispersity index (<0.04) indicates the monodispersity of the mobile fraction. [d]Measured after the Au NPs passed through the column with 25% methanol elution.[e]Measured with a 90 Plus Particle Size Analyzer (Brookhaven Instruments Corp.).

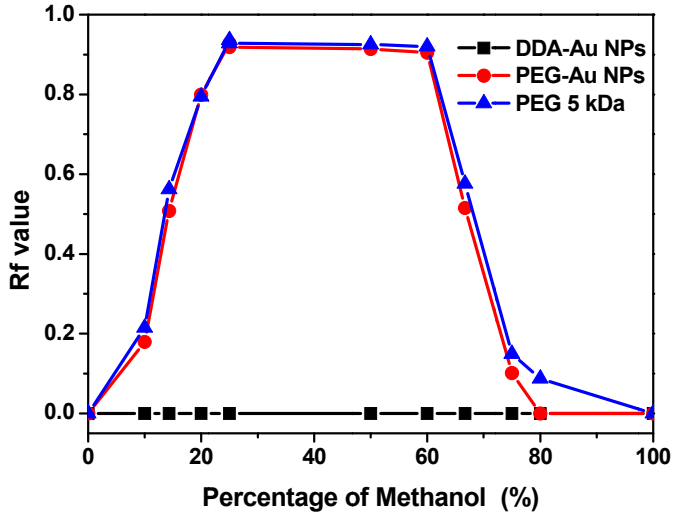

Figure S1. (A) TLC results for DDA and 5 kDa PEG capped Au NPs and free PEG run with a co-solvent mixture of methanol and chloroform.

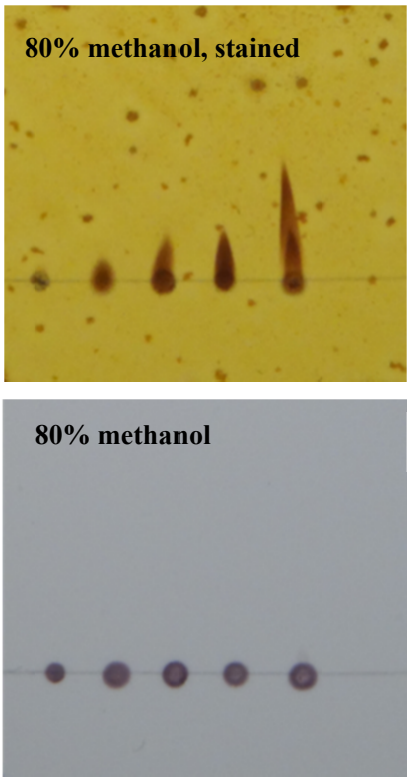

Figure S2. Photographs of TLC plates analyzed in 80% methanol (20% chloroform). The 5 kDa PEG to Au NP ratios were 50:1, 200:1, 350:1, 500:1 and 650:1 from left to right respectively. The upper plate was iodine stained to reveal the band of free PEG, as evidence of a PEG saturated Au NP surface.

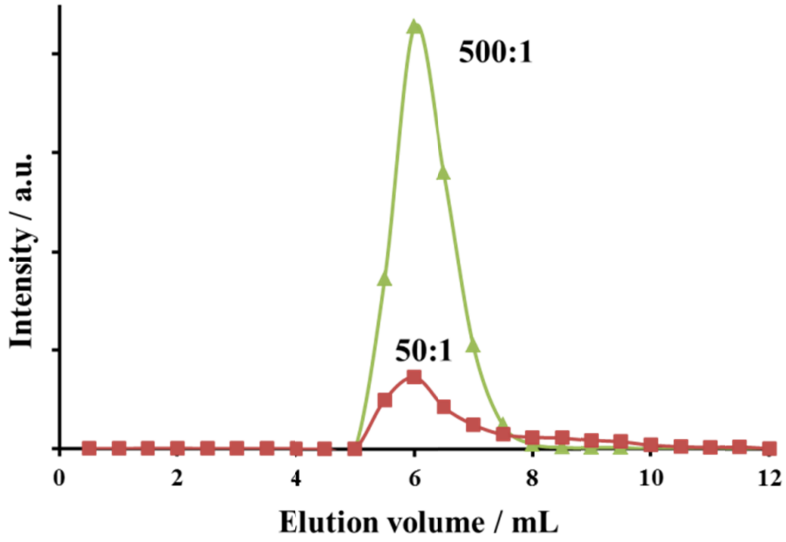

Figure S3. Chromatography profiles of 5 kDa PEGylated Au NPs with different solvents and different PEG to Au NP ratios (50:1 and 500:1).

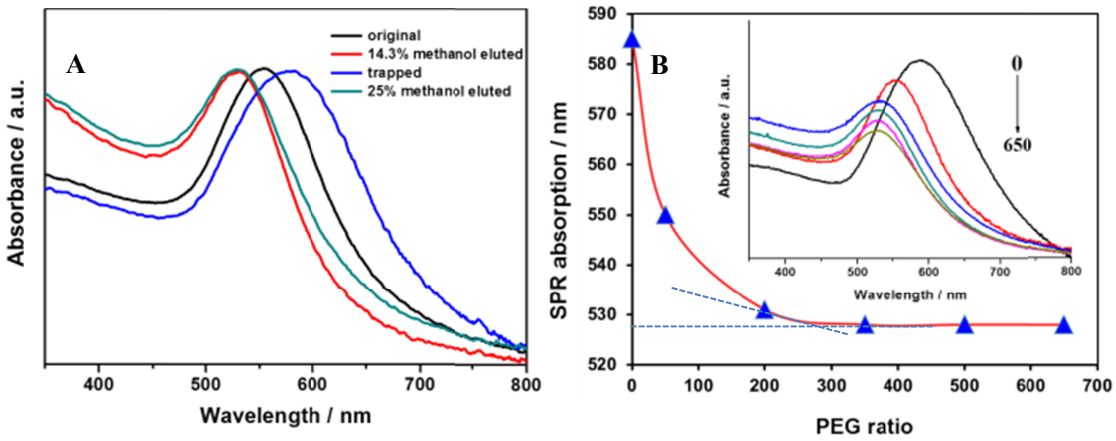

Figure S4. (A) UV-vis spectra of Au NPs with a PEG (5 kDa) to Au NP ratio 50:1 before and after column separation. And the trapped portion under 14.3% methanol elution. (B) SPR absorption band position and spectra (inset) of Au NPs capped with DDA and different PEG (5 kDa) ratios, suggesting a PEG-saturation behavior at around 300:1 PEG : Au NP ratio.

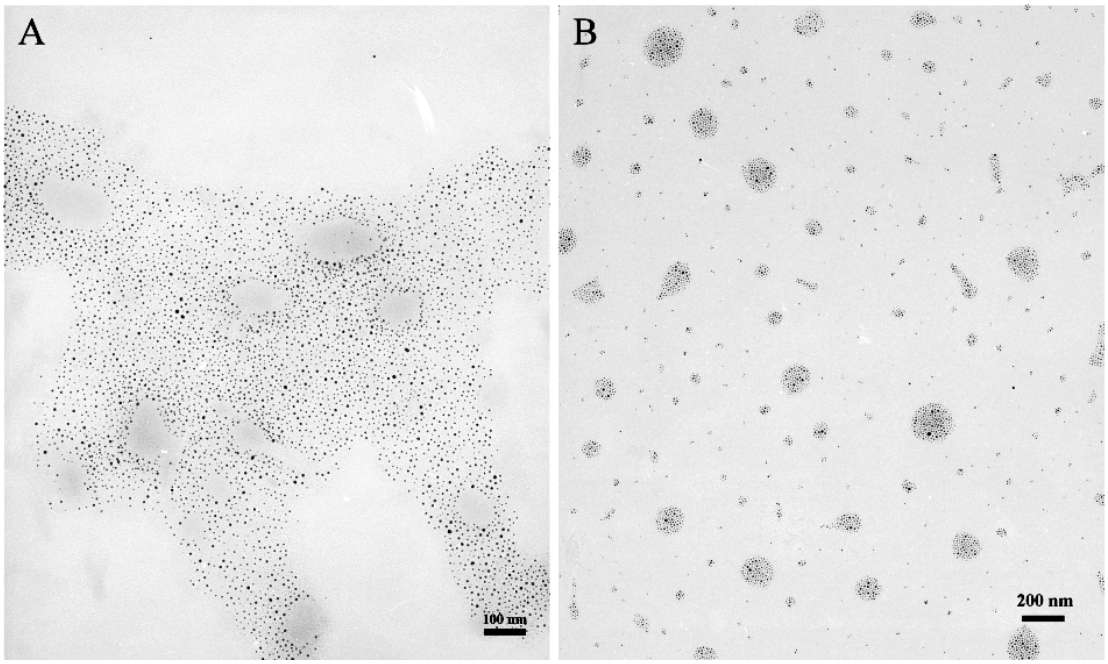

Figure S5. TEM images of Au NPs that (A) migrated through the column, F1, and (B) Au NPs that were retained on the column, F2, after they were redispersed in DI water, then imaged via TEM.

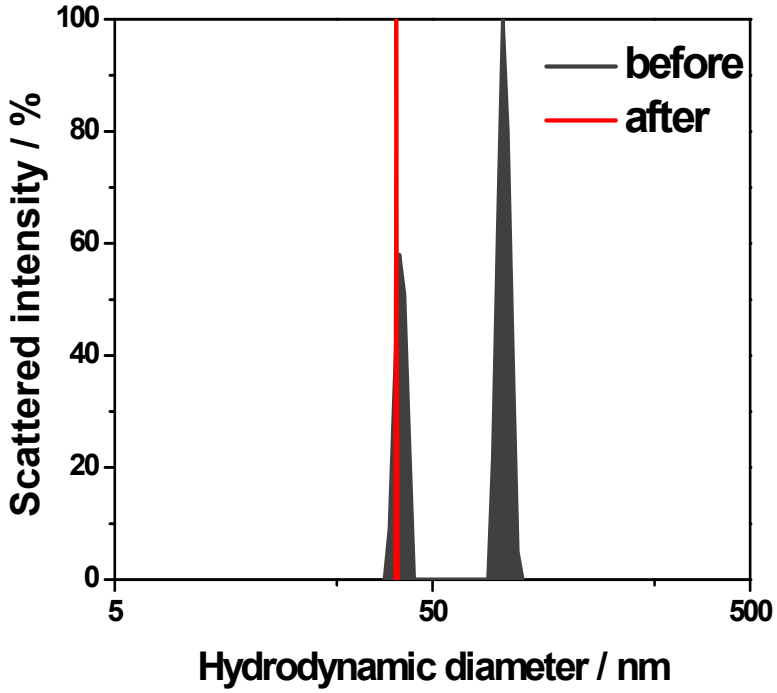

Figure S6. DLS multimodal size distribution (MSD) of 5 kDa PEGylated Au NPs before and after column separation.

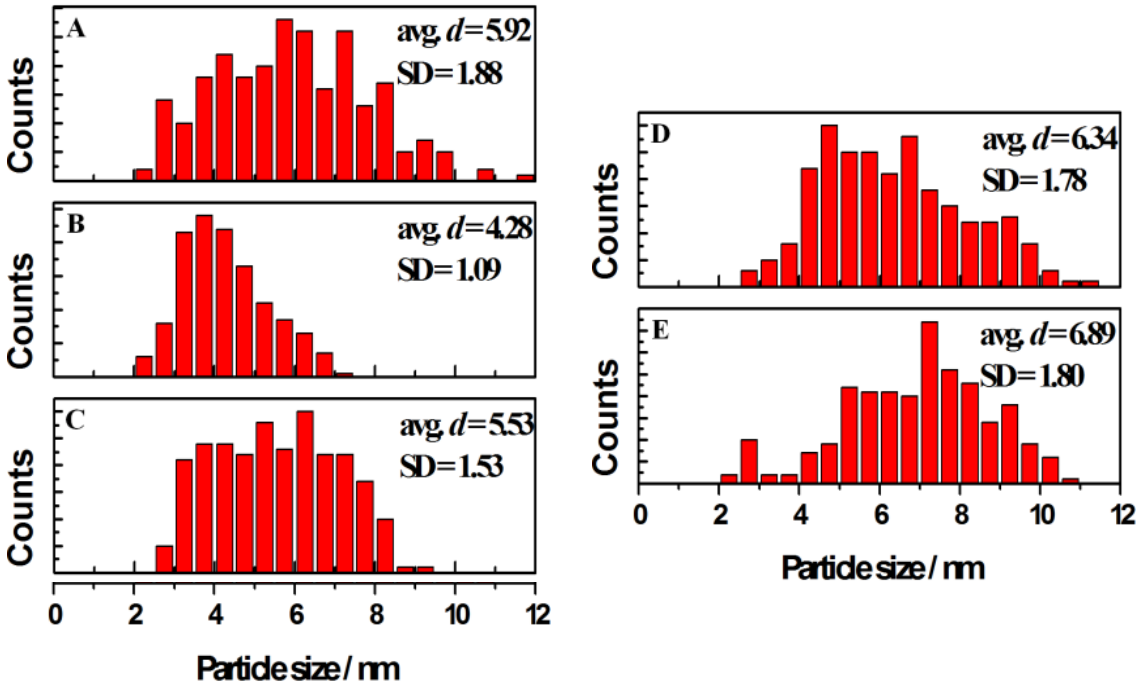

Figure S7. Size histograms of 5 kDa PEGylated Au NPs separated by column. (A) Au NPs before separation, (B) elution volume 5.0-5.5 mL, (C) elution volume 5.5-6.0 mL, (D) elution volume 7-7.5 mL and (E) trapped part.

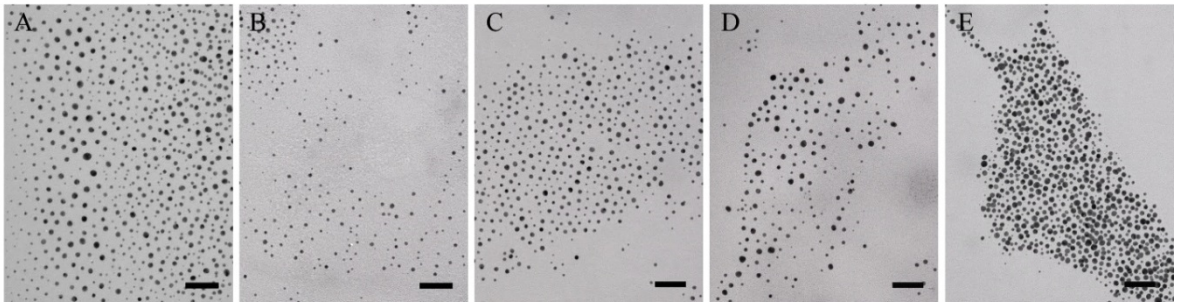

Figure S8. TEM images of 5 kDa PEGylated Au NPs separated by column chromatography. (A) Au NPs before separation, (B) elution volume 5.5 mL, (C) elution volume 6.0 mL, (D) elution volume 7.5 mL and (E) the trapped fraction. Scale bar is 50 nm.

Table S2. Experimental versus calculated PEG Shell Thickness on Au NPs

PEG MW (kDa)	Shell thickness (nm)[a]	Calculated (nm)[b]
1	5.8	6
2	9.6	9.2
5	16.3	16
10	24.3	24.4
5 (ratio: 50:1)	9.9	-

[a] Based on the calculation $L_{shell} = {}^{1}/_{2}\ (d_{hydro} - d_{Au})$, where d_{Au} = 5.9 nm, distribution peak in the first column of **Table 1** was used as d_{hydro}.

[b] Based on the calculation $L_{shell} \approx 6.0\ M_{\mathrm{PEG}}^{0.61}$.[2]

Van der Waals free energy calculation:

Treating the silica chromatography beads as spherical particles, the van der Waals interaction free energy U_{vdW} can be described as follows: [4]

$$U_{vdW} = -\frac{A}{6}\left[\frac{2a_1a_2}{r^2-(a_1+a_2)^2} + \frac{2a_1a_2}{r^2-(a_1-a_2)^2} + \ln\left(\frac{r^2-(a_1+a_2)^2}{r^2-(a_1-a_2)^2}\right)\right] \quad (1)$$

$$r = a_1 + a_2 + L_{shell} \quad (2)$$

Here, A is the Hamaker coefficient (17.2 $\times 10^{-20}$ J for gold–silica system),[5] a_1 and a_2 are radii of the silica beads (55 μm) and gold cores, and r is the center-to-center distance (Figure S9, inset). L_{shell} is the thickness of the PEG corona, 5.8 nm, 9.6 nm, 16 nm and 24.3 nm for PEG MWs of 1, 2, 5 and 10 kDa, respectively, based on DLS measurements which are consistent with the calculated results (Table S2).

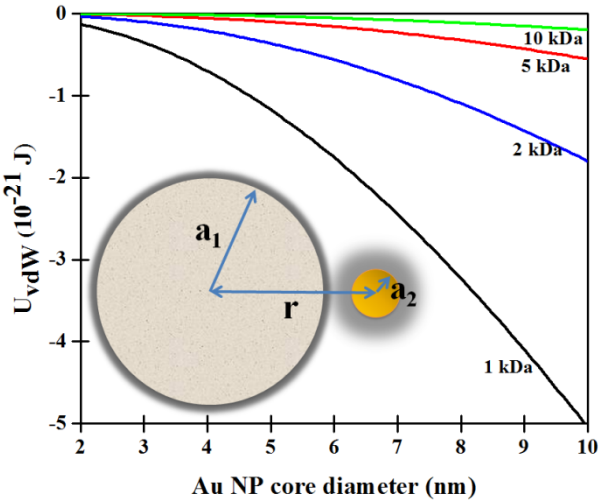

Figure S9. VdW interaction between silica gel and gold core, which shows that for PEG-coated Au NPs (MW_{PEG}< 5 kDa) a size-dependent NP separation is indeed realistic.

Reference:

[1] M. Brust, M. Walker, D. Bethell, D. J. Schiffrin, R. Whyman, *Journal of the Chemical Society-Chemical Communications* **1994**, 801-802.

[2] T. L. Doane, Y. Cheng, A. Babar, R. J. Hill, C. Burda, *J. Am. Chem. Soc.* **2010**, *132*, 15624-15631.

[3] W. Sun, P. B. Davis, *J. Controlled Release* **2010**, *146*, 118-127.

[4] (a) T. Cosgrove, *Colloid Science: Principles, Methods and Applications,* 2nd ed.; A John Wiley and Sons Ltd. Publication, **2010**. (b) K.J. Bishop, C.E. Wilmer, S. Soh, B.A. Grzybowski, *Small* **2009**, 5, 1600-1630.

[5] M. Gotzinger, W. Peukert, *Langmuir* **2004**, *20*, 5298-5303.