

Supporting Information for:

**Exploring Amphiphilicity of PEGylated Gold Nanorods:
Mechanical Phase Transfer and Self-Assembly**

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Materials and Methods

Hexadecyltrimethylammonium bromide (CTAB, >98%), L-ascorbic acid (99+%), gold (III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), sodium borohydride (NaBH_4 , 98.0%) were purchased from Sigma Aldrich. Silver nitrate (AgNO_3 , 99+% metal basis) was obtained from Alfa Aesar. Methylate-polyethylene glycol-thiol (M.W. 2000, thiol-PEG) was from Laysan Bio, Inc. All chemicals were used without further purification. Centrifugation was carried out on a VWR Galaxy 14D. UV-Vis absorbance spectra were obtained on a Shimadzu UV-3600 spectrometer. ^1H NMR of PEG-GNR in CDCl_3 was performed on a Varian 500M spectrometer. TGA was studied on a NETZSCH TG 209 F1. TEM Grids (Carbon Type B) were purchased from Ted Pella Inc.

Synthesis of CTAB-Coated GNR¹

The gold seeds were synthesized by reducing gold salt in the presence of CTAB as a capping agent. This method has been previously described by Nikoobakht *et al.*¹ Briefly, 10 ml of 0.1 M CTAB solution was mixed with 200 μl of 25 mM HAuCl_4 . Then, 1 ml of ice-cold 0.01 M NaBH_4 was quickly added, with vigorous stirring for 2 min. A light brown solution (seed solution) was formed, which was then kept in a water bath at 33 °C for further use. The average size of these Au seeds was 3–5 nm. Meanwhile, to synthesize Au NRs, 10 ml of 0.1 M CTAB was mixed with 200 μl of 25 mM HAuCl_4 in a separate vial, in the presence of a small amount of Ag^+ ion (8×10^{-5} M). Then, a mild reducing agent, 100 μl of 0.1 M ascorbic acid, was added at room temperature, resulting in the formation of a colorless solution. This mixture is the growth solution. Finally, the growth solution was heated to 33 °C in a water bath and 12 μl of seed solution was gently added to it. The rod formation was permitted undisturbed at 33 °C for at least 3 h. Then, to remove the excess CTAB from the prepared gold nanorods, the bilayer CTAB-

coated Au NRs were centrifuged at 6.6KG for 15 min. The colorless supernatant solution was gently removed.

(b) PEG exchange^{2,3}

The pellet at the bottom of the centrifuge tube was re-dispersed by adding 12 ml of 12.5 mM mPEG-SH (~2000 M.W.) and left for 24 h with magnetic stirring, which results in PEG-coated gold nanorods. Afterward, the PEGylated gold nanorods were again centrifuged to remove any unreacted mPEG-SH, and re-dispersed by adding water.

Centrifugation-Based Phase Transfer

The relative centrifugal force (RCF) expressed in units of gravity (times gravity or $\times G$) is used in the article, because different centrifuge rotors will have different rotor radii, resulting in different centrifugal force (G) for the same rpm. The rpm and RCF (expressed as G) are related by the following formula:

$$G = (1.118 \times 10^{-5}) \times R \times \text{rpm}^2$$

Where G or times G , means times gravity (KG means times 1000G); R is the rotor radius and rpm is the rotor angular velocity in revolutions per minute.

1. Centrifugal concentration and re-dispersion procedure: 1 mL PEG-GNR solution with O.D. around 7 was placed into a 1.5 mL centrifuge tube. Centrifugation at 12-14KG was used to condense the PEG-GNR in a pellet at the bottom of the tube, followed by addition of chloroform and vigorous shaking.

2. Two-phase direct phase transfer procedure: 0.5 mL of PEG-GNR solution with different O.D. and 1 mL chloroform were placed in a 1.5 mL centrifuge tube. Centrifugation was then carried out at different speeds as described in the main manuscript. It is difficult to determine the exact speed at which PEG-GNR with different concentration started crossing the interface. Generally, the higher the O.D., the lower the required speed to observe pellet at the bottom of centrifuge tube. The transfer efficiency of PEG-GNR with different O.D. was carried out at 14KG, which is the highest speed. Average residual O.D. at this speed was around 1.26. For salt-promoted phase transfer, 50-100 mg NaCl was added to 0.5 mL of GNR dispersion. The amount could be adjusted to avoid aggregation through the 'salting out' effect. For alcohol promoted phase transfer, 100-300 μ L of ethanol was added to 0.5 mL of GNR dispersion. The amount could be adjusted to avoid mixing of the water and chloroform phases.

Manual Phase Transfer

1 mL of aqueous PEG-GNR solution (O.D. 4.8-7.0) and 2 mL chloroform were added to a vial. Alcohols or salts were slowly added, with shaking after each addition. The efficiency of transfer was measured by comparing the longitudinal plasmonic resonance absorbance peak intensity in the water phase before and after phase transfer.

Rings of Nanorod

Basically, PEG-GNR in either chloroform or dichloromethane was dropped once on a carbon-coated TEM grid.

Table S1. Transfer efficiency (E_T) and Residual O.D. versus initial O.D. before transfer.

O.D.	TRANSFER EFFICIENCY (E_T)%	RESIDUAL O.D. IN WATER
8.46	89.13	1.18
7.0	83.0	1.19
4.23	62.65	1.39
3.4	56.18	1.36
2.12	37.74	1.31
1.28	21.88	1.02

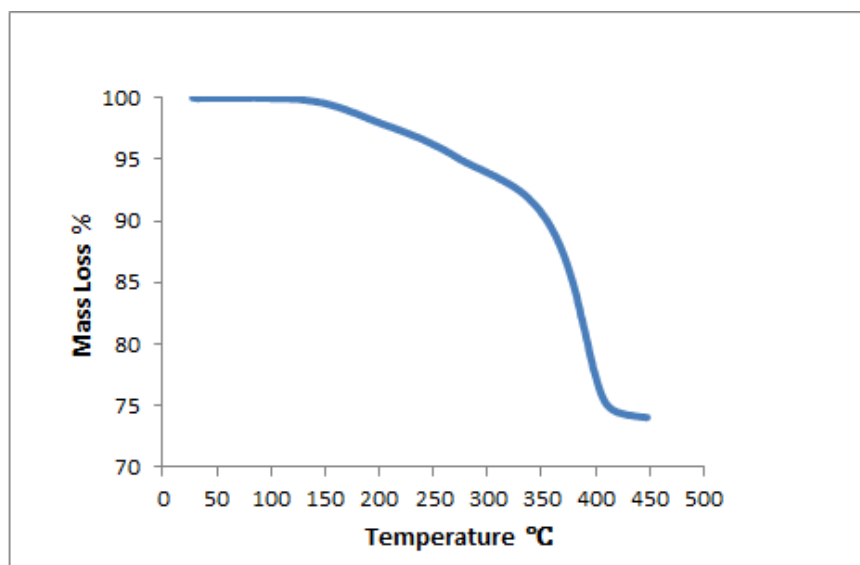


Figure S1. TGA data of transferred PEG-GNR.

The sample used for TGA test was centrifuged and redispersed twice to remove any free PEG and CTAB. Below 150 °C, there is no loss of mass, indicating total de-hydration after transfer. The mass ratio between pure GNR and PEG ligands is about 3:1. The TGA curve resembles that of pure PEG reported previously.⁴

¹HNMR Data

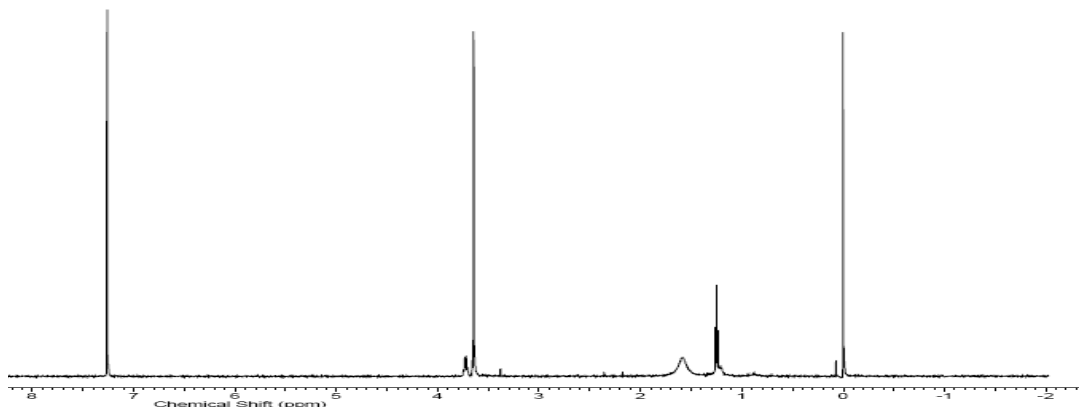


Figure S2. ¹HNMR data detected immediately after transfer.

¹HNMR was taken immediately after transfer and simple concentration. The sample was prepared by transferring PEG-GNR from water to CDCl₃. The ¹HNMR data indicates the presence of PEG on nanoparticles based on the strong proton signal at 3.64 ppm from methylene groups in PEG. Other peaks arise from trace water in the CDCl₃ (1.56 ppm), trace CHCl₃ in CDCl₃ (7.26 ppm), and small amounts of residual CTAB. The fact that PEG-GNR can be transferred to the organic phase, while CTAB-GNR cannot, combined with both TGA and ¹HNMR results confirms that mPEG-SH has displaced CTAB on the GNR surface.

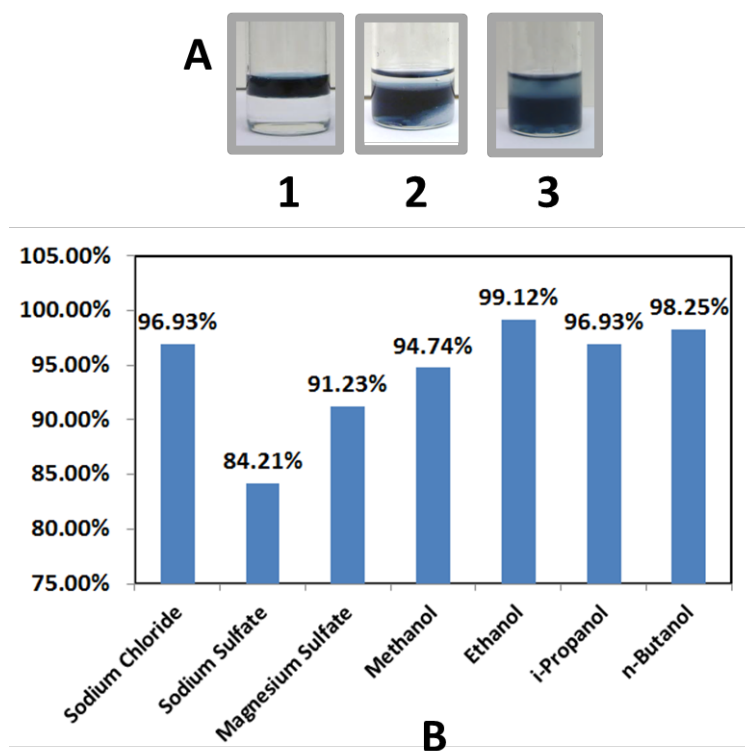


Figure S3. (A) Manual phase transfer induced by addition of NaCl or ethanol. (A1). No reagents added. Addition of (A2) NaCl and (A3) Ethanol. (B) Manual phase transfer efficiency with commonly used salts and alcohol. The GNR were well dispersed in the water phase after salt or alcohol addition, and addition of alcoholic reagent induced no mixing between water and chloroform phases.

When enough NaCl is added, phase transfer can be easily accomplished through shaking the sample by hand. Similarly, gentle shaking was capable of performing phase transfer upon the addition of appropriate amount of various alcohols to the aqueous PEG-GNR solutions, as shown in Figure S3. Different salts and alcohols have also been tested. The efficiencies (91% - 99%) varied slightly except for Na₂SO₄ as shown in Figure S3B. The reason for lower efficiency using Na₂SO₄ is still under investigation.

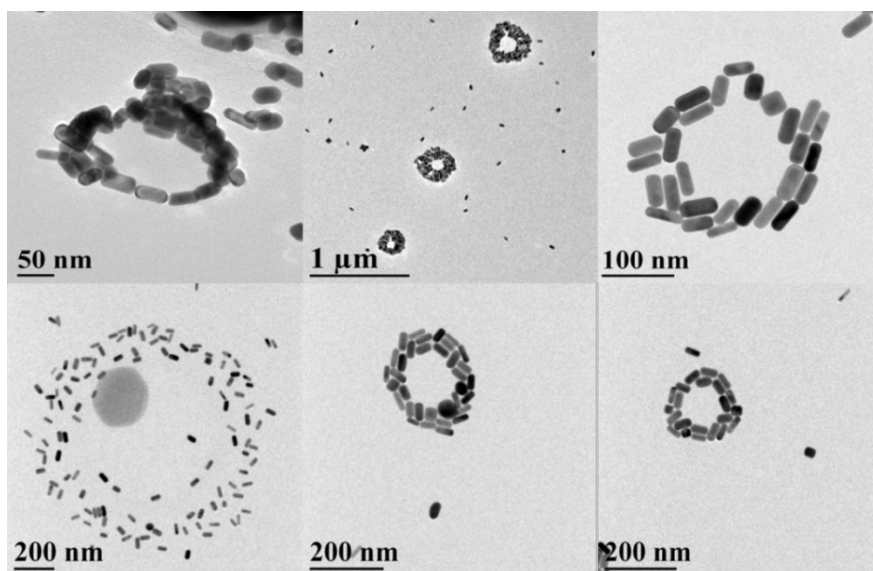


Figure S4. Closed Rings from Chloroform Evaporation

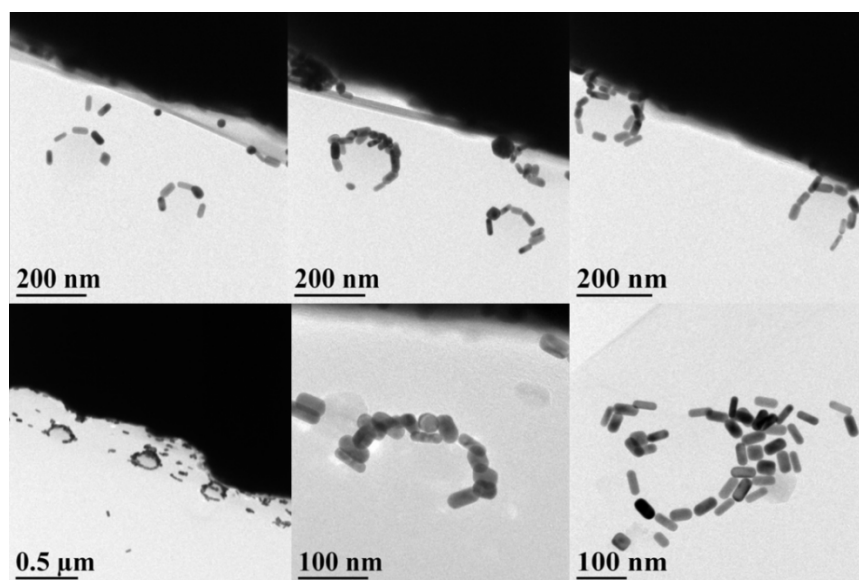


Figure S5. Open Rings from Chloroform Evaporation

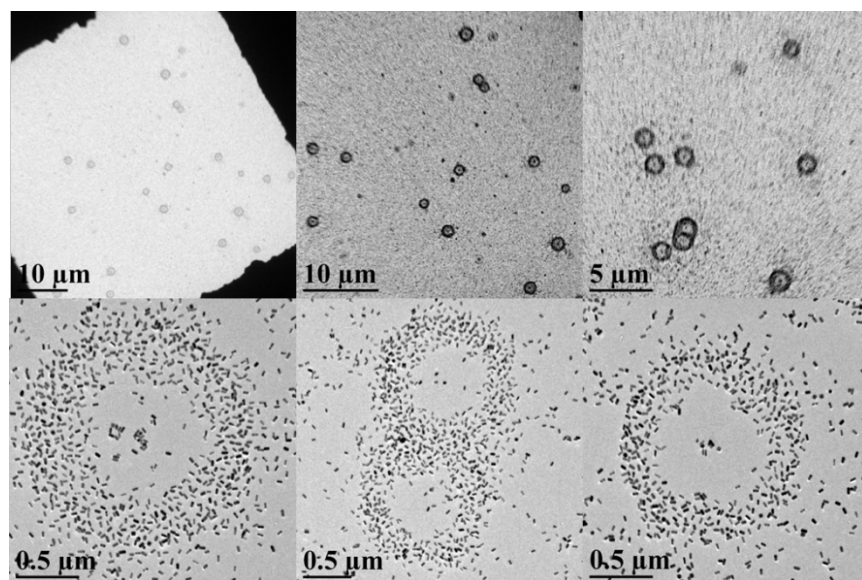


Figure S6. Rings from Dichloromethane Evaporation

Supplementary References:

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4. D. L. Han and L. F. Yan, *Carbohydr Polym*, 2010, **79**, 614-619.