Synthesis of Monofunctionalized Gold Nanocrystals by Fmoc Solid-Phase Reactions

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General Information. All reactions were carried in an anaerobic atmosphere using a standard Schlenk techniques unless otherwise stated. Water was purified (18.2 Ω) by a Milli-Q system. Dry solvents were purchased and used without further purifications. Ethylene diamine was distilled over $CaSO_4$ prior to use. Resin beads for solid phase synthesis, Fmoc-Lys(Dde)-Wang resin (substitution level 0.54 mmol/g, 1% divynylbenezene crosslinkage, 100-200 mesh) and HMPA-PEGA resin (substitution level 0.06 mmol/g, methanol-swollen, 50-100 mesh) were obtained from SynPep and Novabiochem, respectively. Other reagents were commercial samples and used as received. Octanethiolate monolayer-protected gold nanocrystals **(1)** and mercaptoundecanoic acid and octanethiolate mixed-monolayer-protected gold nanocrystals (**2**) were prepared by modified literature methods.[1,2]

Preparation of Compounds. Fmoc-Lys-Wang resin. Protected polystyrene lysine-beads, Fmoc-Lys(Dde)-Wang resin (0.67 g) were placed on a fritted reactor, suspended, and swollen in DMF (10 mL). Then the beads were washed with DMF (3 x 1 mL) and the solvent was drained as much as possible by suction. A deprotection reagent (2% v/v hydrazine, 1 mL) was added and agitated for 5 min and then the excess reagent was removed by suction. This process was repeated 4 times. The Dde-deprotected beads were washed with diisopropylethylamine (10% in DMF, 3 x1 mL). The beads were subsequently washed with DMF (10 x 1 mL), dichloromethane (5

x 1 ml), and shrunk with ethylether (5 x 1 ml). The beads were then dried in vacuo for 2 h. Qualitative Dde deprotection was confirmed by a ninhydrin test (such as a Kaiser test)[3] and the final loading level of the lysine group measured was 0.5 ± 0.1 mmol/g by Fmoc concentration determination.[4]

Fmoc-Lys(Dde)-HMPA-PEGA resin. HMPA-PEGA resin (2.5 g, swollen in MeOH) was placed in a dry reaction vessel, and was washed and swollen in DMF (4 mL). To this suspension was added Fmoc-Lys(Dde)-OH (0.40 g) and pyridine (0.10 mL), and this mixture was gently agitated until the amino acid was dissolved completely. Then 2,6-dichlorobenzyl chloride (DBC, 0.11 mL) was added to the mixture, which was agitated further for 18 h at room temperature. After the reaction, the product resin was isolated by filtration, washed thoroughly with DMF (10 x 3 mL) and kept at 0 ºC. Loading level of Lys was measured by Fmoc determination and was 0.02 mmol/g (swollen resin in DMF). Deprotection reaction of Dde was carried out with 1.0 resin scale in similar way to that of Fmoc-Lys(Dde)-Wang resin as described above.

Loading of gold nanocrystals on Fmoc-Lys-Wang resin beads. The Dde-deprotected Fmoc-Lys-Wang resin (0.10 g) was placed on a frit-tube reactor, washed with DMF (5 x 2 mL), and swollen in DMF (1 mL) for 1 h. A solution of gold nanocrystals **2** (45 mg) in dry THF/DMF (1:1 v/v, 2 ml) was added to the swollen beads under dinitrogen and mixed well for 2 min. Then 500 μL of 1,3-diisopropylcarbodiimide (DIC) was added to the mixture followed by immediate shaking of the mixture. The mixture was further agitated for 24 h at room temperature. The beads became dark during the reaction indicating nanocrystal-loading reaction. The remaining solution was drained and the dark brown beads were isolated by filtration and washed with DMF (5 x 2 mL), dichloromethane (5 x 2 ml), and diethyl ether (3 x 2 ml) to afford 0.114 g of **3a**. The positive Kaiser test for the gold nanocrystal-loaded beads **3a** indicated partial loading level. On the basis of the weight gain of the resin, approximately 30 % of gold nanocrystals were loaded on the beads. The gold-nanocrystal-loaded beads **3a** were also characterized by SEM and EDX (Figure S1a).

A resin salt [(Fmoc-Lys-Wang)$^{n+}$·**2**$^{n-}$] was prepared separately to examine charging effects. To a suspended Fmoc-Lys-Wang resin (32 mg) beads in 1 mL of DMF

was added a solution of **2** (excess) and stirred for 8 h at 80 ºC. The dark beads were isolated by filtration, washed thoroughly with DMF and diethylether, and dried in vacuo. An SEM image of the resin salt is shown in Figure S1b.

Loading of gold nanocrystals on Fmoc-Lys-HMPA-PEGA resin beads. Fmoc-Lys(Dde)-HMPA-PEGA resin (53 mg) was placed in a fritted reactor and was washed and swollen in DMF (1.5 mL). To the light yellow suspension of the resin was added a dark brown solution of gold nanocrystal **2** (44 mg) followed by immediate addition of DIC (50 μL). The mixture was agitated for 48 h at room temperature. The resulting black resin was isolated by filtration and washed thoroughly with DMF and dichloromethane to yield 79 mg of **3b**. Further inactivation of carboxyl groups of **3b** was performed by the reaction with $MeNH_2$ (2.0 M in THF, 1.0 mL) and DIC (20 μL) in dichloromethane for 18 h at room temperature. The resin was washed thoroughly with dichloromethane/THF/DMF to afford **4b** in nearly quantitative yield.

Inactivation of carboxyl groups. The gold nanocrystal-loaded Wang-resin beads **3a** (0.56 g) were swollen in DMF (2 mL). To this suspension was added a solution of $MeNH_2$ in THF (2 M, 500 μL) and DIC (100 μL). After 12 h agitation, the supernatant was drained, the beads were thoroughly washed with DMF and dichloromethane , and then dried in a vacuo for 10 h to produce beads loaded with amide-protected gold nanocrystals (**4a**). Similar procedure was applied for generation of HMPA-PEGA beads **4b**.

Monofunctionalized Fmoc-Lys-gold nanocrystals (5). The dry gold nanocrystal-loaded beads **4a** (0.3 g) were placed into a glass tube with a 60 % trifluoroacetic acid (TFA) cleavage cocktail (TFA:water:triisopropylsilane:DMF = 60:2.5:2.5:35, v:v:v, 2 mL). The suspension was agitated for 24 h. During the reaction, the solution color slowly changed to brown from colorless. The supernatant were collected by filtration and all volatiles were removed in vacuo to produce 3.8 mg of black solid, which was washed with dichloromethane (5 x 3 mL) and ethyl ether (10 x 3 mL). The same procedure was applied for the gold nanocrystal-loaded HMPA-PEGA resin beads **4b** and the yield of **5** improved to 25% from **4b**. FT-IR (KBr, cm^{-1}): 3270 (w, br), 2920 (vs), 2850 (s), 1700 (m), 1650 (s). HRTEM: 2.2 ± 0.3 nm.

Gold nanocrystal dimers (6). Due to the difficulty in the detection of a single lysine on a 2 nm sized nanosphere, direct microscopic evidence of monofunctionalization was provided by dimerization reaction of the Fmoc-lysine nanocrystals. Fmoc-Lys-gold nanocrystals **5** (2.5 mg) were treated with a bridging reagent ethylenediamine (excess, 5 μL, diluted in 1 mL of DMF, dropwise addition over 30 min) in the presence of DIC (5 μL) in DMF (2 ml). After 6 h, diethyl ether (10 ml) was added resulting in precipitation of black powdery solid, which was isolated by filtration. The isolated solid was washed with THF and ether and dried in air. Characterization of the gold nanocrystal dimers was carried out using TEM. By counting the number of **6**, it is possible to assay the monofunctionalized nanocrystals in **5**. Aprroximately 300 nanocrystals were surveyed for the statistic analysis and we found that 55-60% of nanocrystals from the TEM sample were dimeric species (Figure S3).

Physical Measurements. Absorption spectra were obtained on a Hewlett Packard 8452A diode array spectrophotometer. FTIR spectra were measured in KBr pellets with a Digilab Excalibur Series FTS 3000 instrument. Centrifugation was carried out with a Hermle Z200A benchtop centrifuge working at 6000 rpm (4185 g) maximum speed. Transmission electron microscopy (TEM) was performed and phase contrast images of nanocrystals were obtained using with a JEOL 2010 or a JEOL 200 CX microscopes at an accelerating voltage of 200 kV. Samples for TEM analysis were prepared by depositing a drop of ~10 nM solution of the nanocrystals in DMF/THF (1:3 v/v) onto carbon films supported on a standard copper TEM grid.

References

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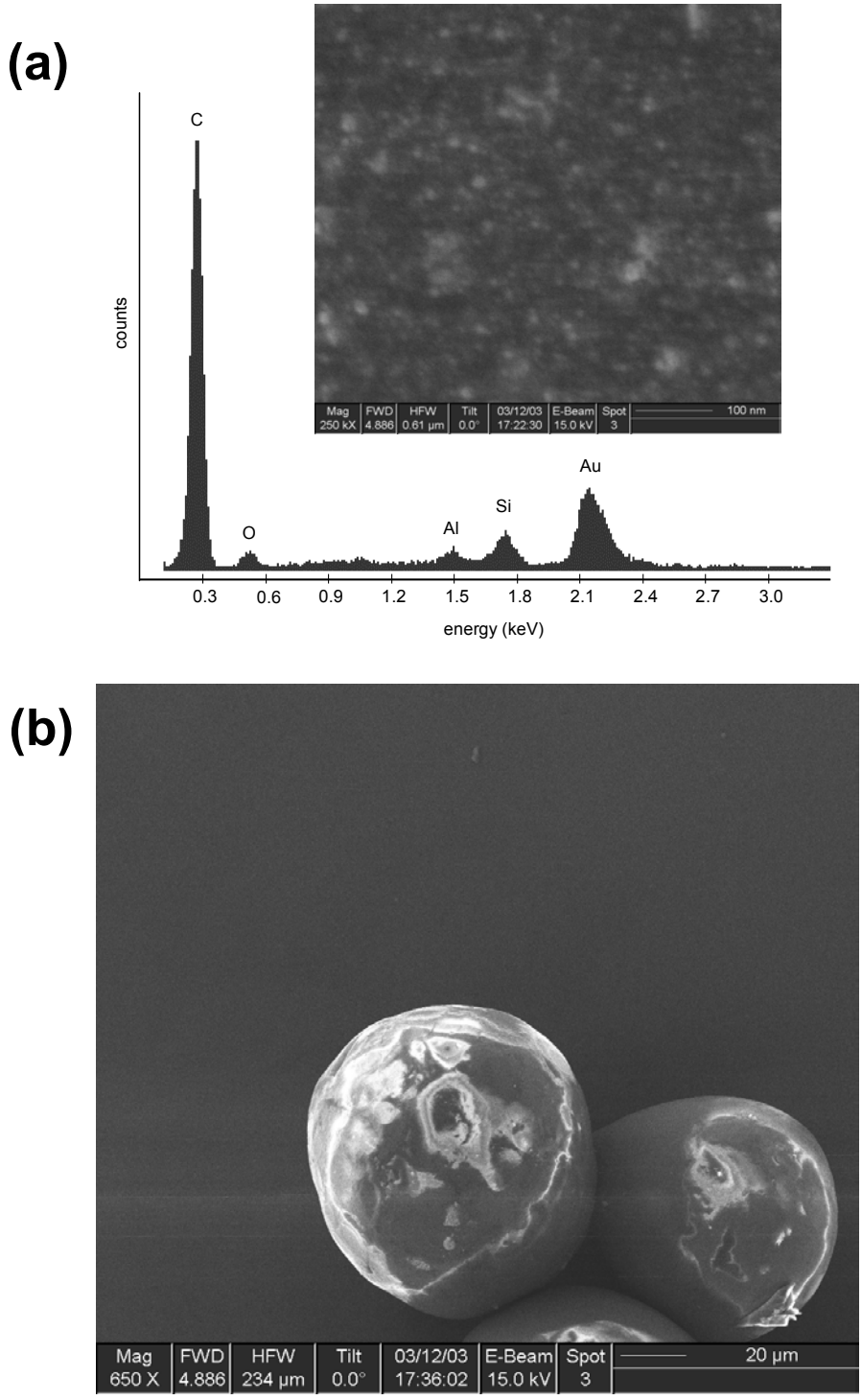

Figure S1. (a) SEM image (x 250 k magnification) of the surface of a gold nanocrystal-loaded resin bead **3a** showing deposited gold nanocrystals in resin pores (inset) and a spot-profile of EDX spectrum, and (b) SEM image (x 650 magnification) of charged beads, [(Fmoc-Lys-Wang)$^{n+}$·**2**$^{n-}$] salt.

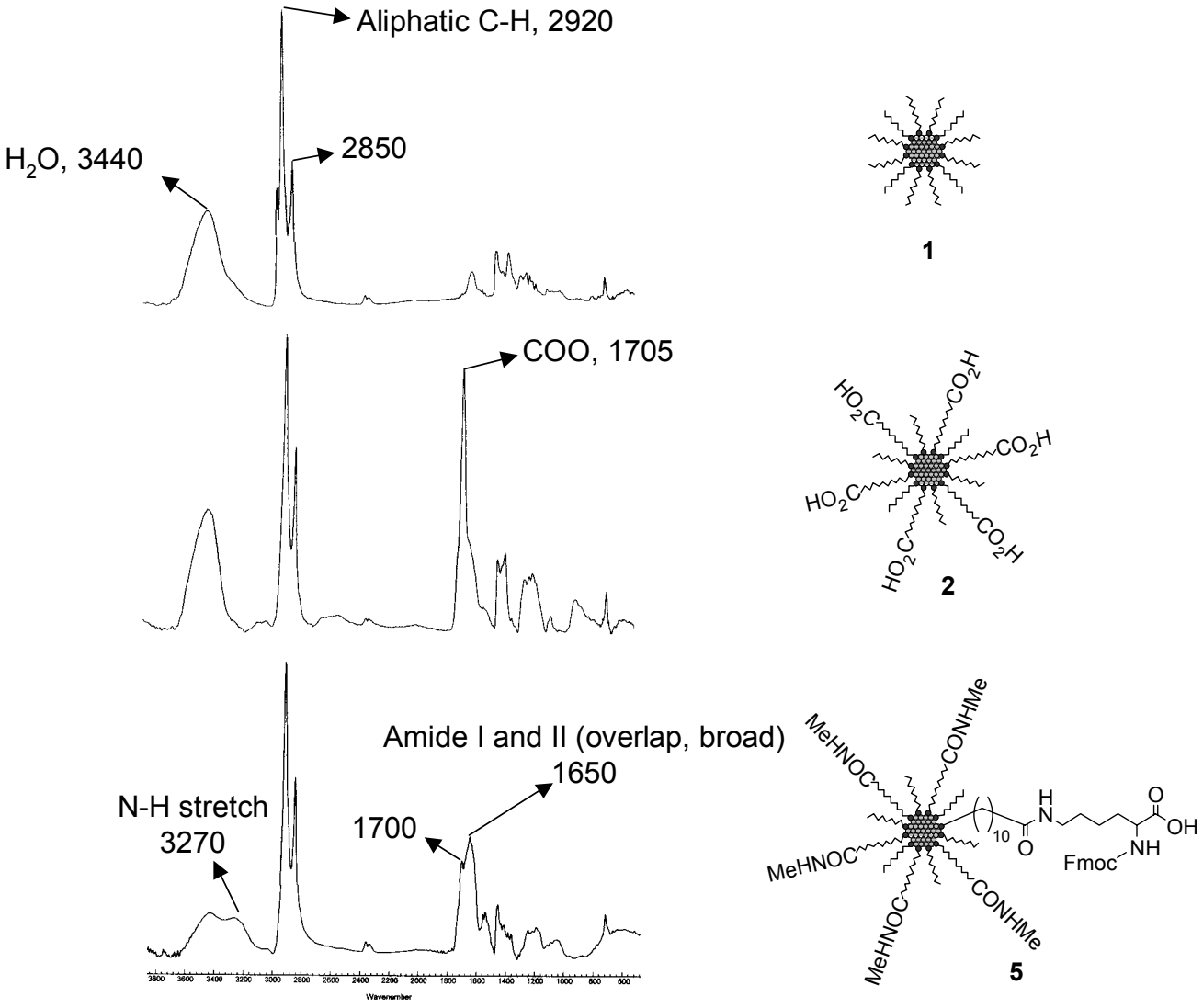

Figure S2. IR spectra for gold nanocrystals **1**, **2**, and **5**.

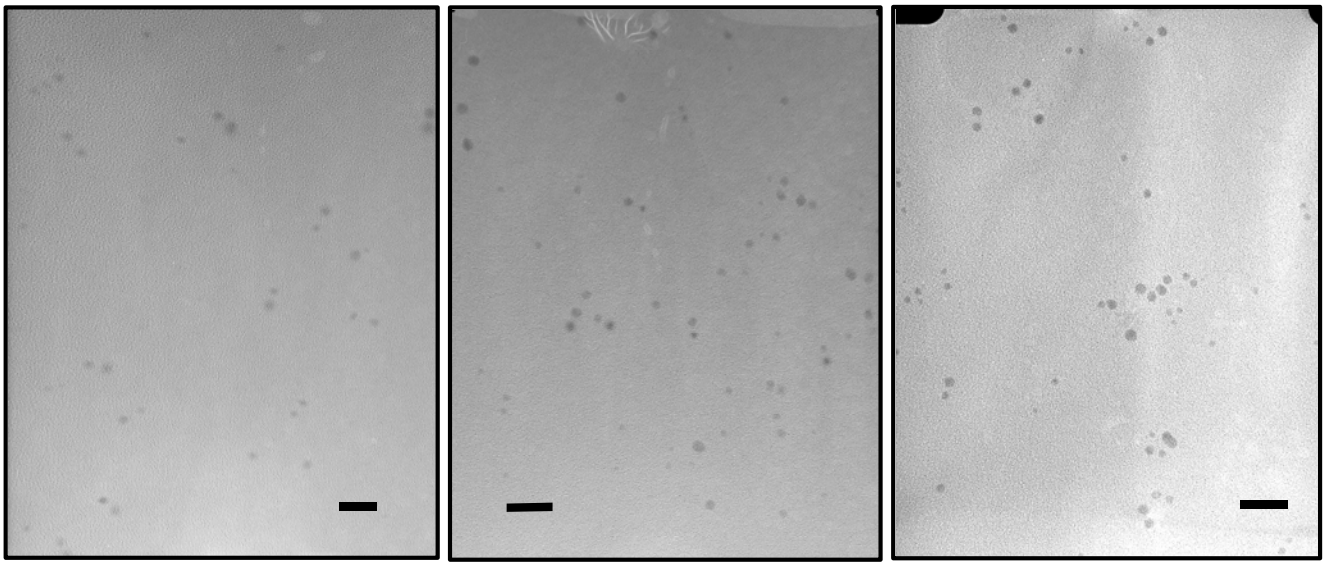

Figure S3. TEM images of gold nanocrystal dimers (**6**).