

Controlled Assembly of Eccentrically Encapsulated Gold Nanoparticles

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Interpretations of the TEM Images.

(NH₄)₆Mo₇O₂₄ was used as stain (3.4 mM) to improve the contrast in all TEM images reported in our study. The salty stain solution is expected to cause some aggregation, particularly when a sample solution becomes concentrated when dried on a TEM grid. However, with proper control of the amount of stain used, we were able to avoid aggregation of the sample on most part of the TEM grids. Our observations have indicated that the stain often formed a thin layer with thickness close to the diameter of AuNP@polymer (5-50 nm) on the TEM grids. It is expected for the thickness of the stain layer to vary from sample to sample as it is highly dependent on sample preparation. For a AuNP@polymer embedded in the stain layer (Figure S1a), electron beam transmitted through the polymer were less scattered as compared to the beams transmitted through the stain layer. Therefore, the polymer shell normally appears white in contrast to the stain. The AuNPs deflect electrons most effectively, resulting in a deep black color. In some cases, AuNP@polymer were not embedded in the stain layer (Figure S1b, and lower panels), causing the polymer shell to appear as light grey in contrast to the stain as more scattering of the electron beam occurred when it passes through the polymer shell.

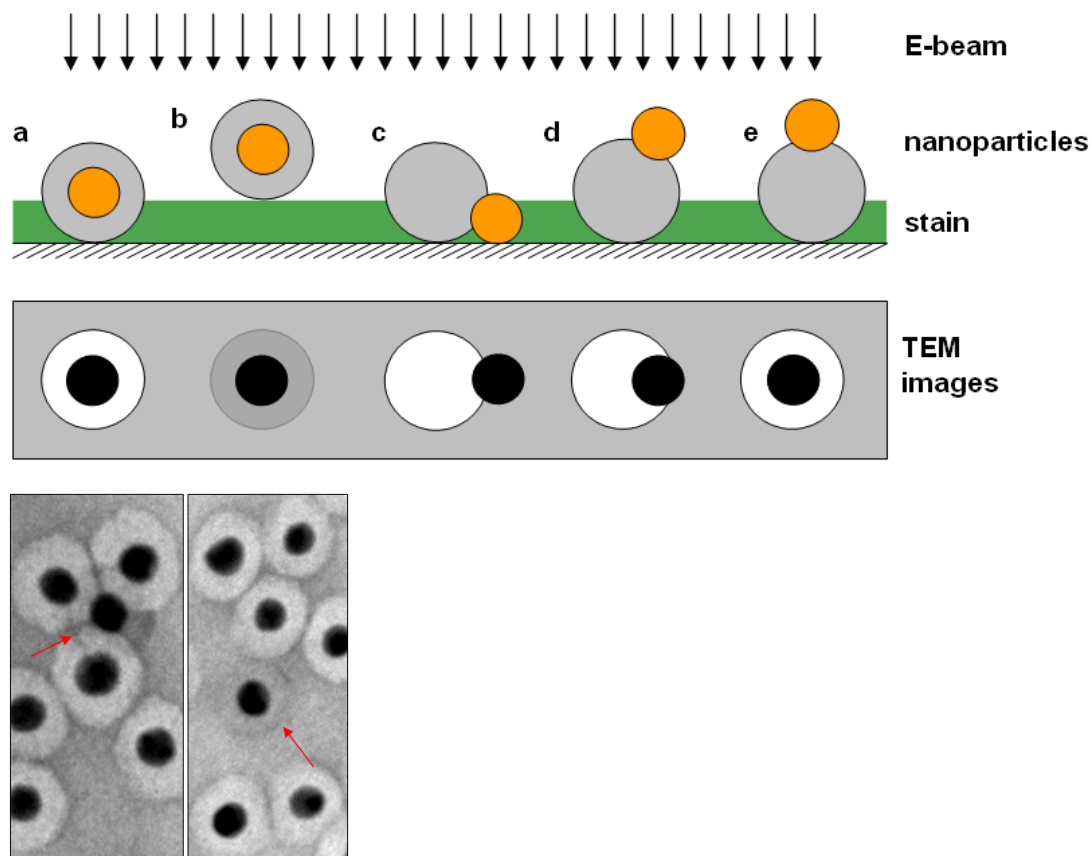


Figure S1. Possible interpretations of AuNP@polymer in TEM images. AuNP@polymer could be buried inside the stain layer (a), or in rare cases, sit on top of it (b). Most ecc-[AuNP@polymer] would likely maximize their contact with surface (c) whereas some NPs could align tilted (d) or perpendicular to the stain layer (e), which may lead to misinterpretations.

While symmetrically encapsulated AuNPs (homo-[AuNP@polymer]) appear the same regardless of their orientation on the TEM grid surface, the ecc-[AuNP@polymer] may position on the surface with different orientations (Figure S1c-e). The observation by TEM, which gives a planar view, could result in completely different interpretations of the core/shell arrangements for these different orientations. At nanoscale, gravity is unlikely the major determining factor for the orientation, but one would expect the AuNP@polymer with hydrophilic PAA corona layer to attach in an orientation that maximizes its

interaction with the hydrophilic grid surface. This argument is consistent with the fact that most of the ecc-[AuNP@polymer] observed had uniform core/shell arrangements (Figure 1, S3 and S4). However, it is possible that the orientation of some ecc-[AuNP@polymer] could deviate from the most stable mode. The stain layer that buries the ecc-[AuNP@polymer] could encourage such unstable modes by providing extra interactions with the NPs. In such cases, a homo-[AuNP@polymer] (Figure S1a) and an ecc-[AuNP@polymer] aligned parallel to the electron beam (Figure S1e) both give the same TEM planar view and are difficult to be distinguished. Most of the ecc-[AuNP@polymer] as shown in Figure 1, S3 and S4 are quite uniform in the polymer coverage ratio, despite a few exceptions. It is not clear whether these NPs were truly homo-[AuNP@polymer] resulting from complete L_A coverage, or misinterpretations due to the parallel orientation of the ecc-[AuNP@polymer] relative to the electron beam (Figure S1e). Neither interpretation could be ruled out at this moment.

Materials

All chemical reagents were used without further purification. Hydrogentetrachloroaurate(III) hydrate, 99.9% (metal basis Au 49%) was purchased from Alfa Aesar; amphiphilic diblock copolymer polystyrene-block-poly(acrylic acid) (PS₁₅₄-*b*-PAA₆₀, $M_n = 16000$ for the polystyrene block and $M_n = 4300$ for the poly(acrylic acid) block, $M_w/M_n = 1.15$) was purchased from Polymer Source, Inc.; 2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol (sodium salt), **1**, was purchased from Avanti Polar Lipids; 200 mesh copper specimen grids with formvar/carbon support film (referred to as TEM grids in text) was purchased from Electron Microscopy Sciences; AuNPs of different sizes were prepared following literature procedures by sodium citrate reduction of HAuCl₄.¹ Concentrations of AuNPs were estimated from the total amount of Au used during the synthesis, the density of Au, and the average size of AuNPs.

The average size of AuNPs was measured using ImageJ (<http://rsb.info.nih.gov/ij/>). All other chemicals were purchased from Aldrich.

Methods and Characterization

TEM images were collected from an FEI EM208S Transmission Electron Microscopy (Philips) operated at 100 kV. $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ was used as stain (3.4 mM) in all TEM images reported in this study. Raman spectra were collected from colloidal NP samples in a cuvette (path length = 1.00 cm) on an R-3000HR spectrometer (Raman Systems, Inc, R-3000 series) using Red LED laser ($\lambda = 785 \text{ nm}$).

Preparation of TEM Samples. TEM grids were treated with oxygen plasma in a Harrick plasma cleaner/sterilizer for 1 min to render their surfaces hydrophilic. The hydrophilic face of the TEM grid was then placed in contact with the stained sample laid on a plastic Petri dish. A filter paper was used to wick off the excess liquid on the TEM grid, which was then left to dry in air for 5 min.

Homocentric Encapsulation of Gold Nanoparticles. The method used here was modified from our previous report on symmetric single-encapsulation of gold nanoparticles.² Two 1.5 mL tubes of AuNPs solution ($d_{\text{av}} = 15 \text{ nm}$, 4.75 nM) were concentrated to $\sim 20 \mu\text{L}$ by centrifugation at 16000 g for 15 min. The deep red solution collected at the bottom of the tubes was diluted with trisodium citrate solution (180 μL , 1.162 mM, same as the citrate concentration in the as-synthesized AuNP solution). Then the mixture was added to 800 μL of polymer solution which was prepared by mixing 720 μL DMF with $\text{PS}_{154}\text{-}b\text{-PAA}_{60}$ in DMF (80 μL , 8 mg/mL). Finally, ligand **1** in EtOH (80 μL , 2 mg/mL) was added to the mixture. The mixture was heated at 110 $^\circ\text{C}$ for 2 hrs and then allowed to slowly cool down till room temperature. The

color of the mixture remains red before and after heating. The solution is stable under room temperature for months.

Eccentric Encapsulation of AuNPs without Pre-incubation. Procedures for eccentric encapsulation were analogous to the homocentric encapsulation except that an extra hydrophilic ligand was added in the reaction mixture. Firstly, two 1.5 mL tubes of AuNPs solution ($d_{av} = 15$ nm, 4.75 nM) was concentrated to ~ 20 μ L by centrifugation at 16000 g for 15 min. The deep red solution collected at the bottom of the tube was diluted by trisodium citrate solution (180 μ L, 1.162 mM). The mixture was then added to 800 μ L of polymer solution which was prepared by mixing 720 μ L DMF with PS₁₅₄-*b*-PAA₆₀ in DMF (80 μ L, 8 mg/mL). Neat diethylamine (0.5-3 μ L) was then added to the mixture, followed by addition of **1** in EtOH (80 μ L, 2 mg/mL). Finally, the mixture was heated at 110 °C for 2hrs, and then cooled down till room temperature.

Eccentric Encapsulation of AuNPs with Pre-incubation. Multiple polymer micelle attachment on AuNPs ($d = 15$ and 40 nm) was achieved with the introduction of a pre-incubation step that conduces the close-packing of L_B on AuNP surface. In detail, two 1.5 mL tubes of AuNPs solution ($d_{av} = 40$ nm, 0.25 nM) was concentrated to ~ 20 μ L by centrifugation at 5000 g for 10 min. The brown solution collected at the bottom of the tube was diluted with trisodium citrate solution (180 μ L, 1.162 mM). Then the mixture was added into 800 μ L polymer solution which was prepared by mixing 720 μ L DMF with PS₁₅₄-*b*-PAA₆₀ in DMF (80 μ L, 8 mg/mL). Neat **2** (5 μ L) or **3** in EtOH (40 μ L, 10 M) was then added to the mixture, which was then incubated for 2 hrs at room temperature. After incubation, **1** in EtOH (80 μ L, 2 mg/mL) was added. Finally, the mixture was heated at 110 °C for 2 hrs, followed by gradual cooling till room temperature.

To test the ligand exchangeability of **2** on ecc-[AuNP@polymer] ($d_{\text{AuNP}} = 40 \text{ nm}$), 200 μL of the ecc-[AuNP@polymer] encapsulated using ligand **1** and **2** was first purified twice with water, concentrated to $\sim 20 \mu\text{L}$, and then incubated in **3** (100 μL , 2 mg/mL) overnight. After incubation, the sample was purified again to remove the freestanding **3**. The purified concentrated sample was then diluted with 1.2 mL of DI water for surface-enhanced Raman scattering characterization.

Purification of Polymer-Encapsulated AuNPs. Both homocentrally and eccentrically encapsulated AuNPs were readily purified by centrifugation with the following procedures. The as-synthesized mixtures ($\text{DMF:H}_2\text{O} = 4:1$) was first diluted with water (15 times in volume) to trap the AuNP@polymer in a kinetically stable state. The solutions were then centrifuged at various speeds to separate the AuNP@polymer from the supernatant. The concentrated AuNP was re-diluted with water and purified again by centrifugation. The final solution contains the desired AuNP@polymer, with little DMF, free ligands and empty micelles.

Dimerization of Eccentrically Encapsulated AuNPs. 300 μL eccentrically encapsulated AuNPs ($d_{\text{av}} = 15 \text{ nm}$) was synthesized with $[\mathbf{1}]:[\mathbf{2}] = 1:132$; the product was purified twice by centrifugation and concentrated till $\sim 20 \mu\text{L}$. This concentrated solution was then added to 80 μL aqueous solution containing 0.1 M NaCl and 0.04 M NaOH. The mixture was incubated overnight. TEM images of the sample before and after further purification to remove NaCl and NaOH showed no observable differences in the degree of aggregation or in the morphology of polymer shell.

References:

- (1) Frens, G. *Nature Physical Science* **1973**, 241, 20-22.
- (2) Chen, H. Y.; Abraham, S.; Mendenhall, J.; Delamarre, S. C.; Smith, K.; Kim, I.; Batt, C. A. *ChemPhysChem* **2008**, 9, 388-392.

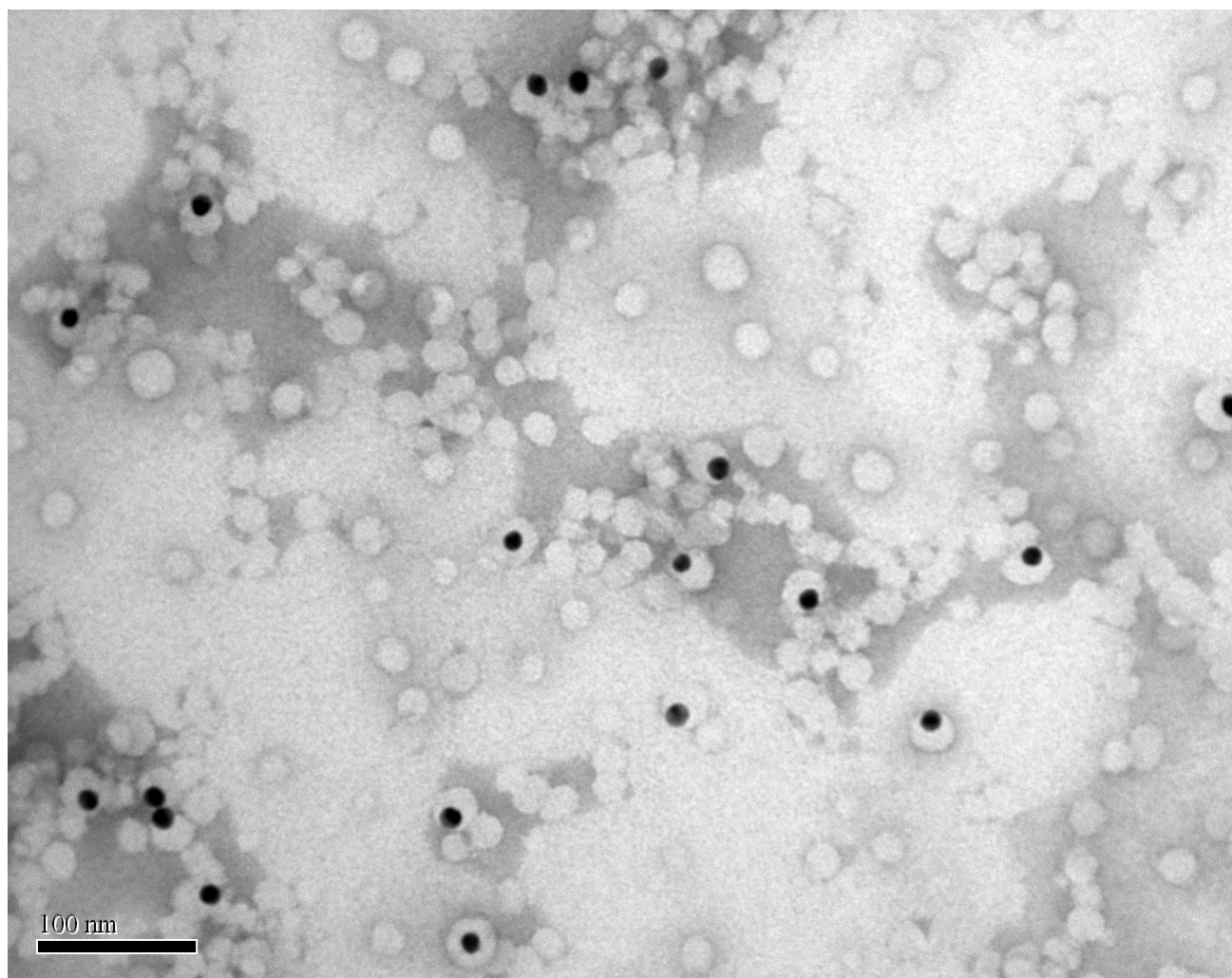


Figure S2. A typical TEM image of eccentrically encapsulated AuNPs ($d_{av} = 15$ nm, $[1]:[2] = 1:22$) before purification, showing both ecc-[AuNP@polymer] and empty micelles.

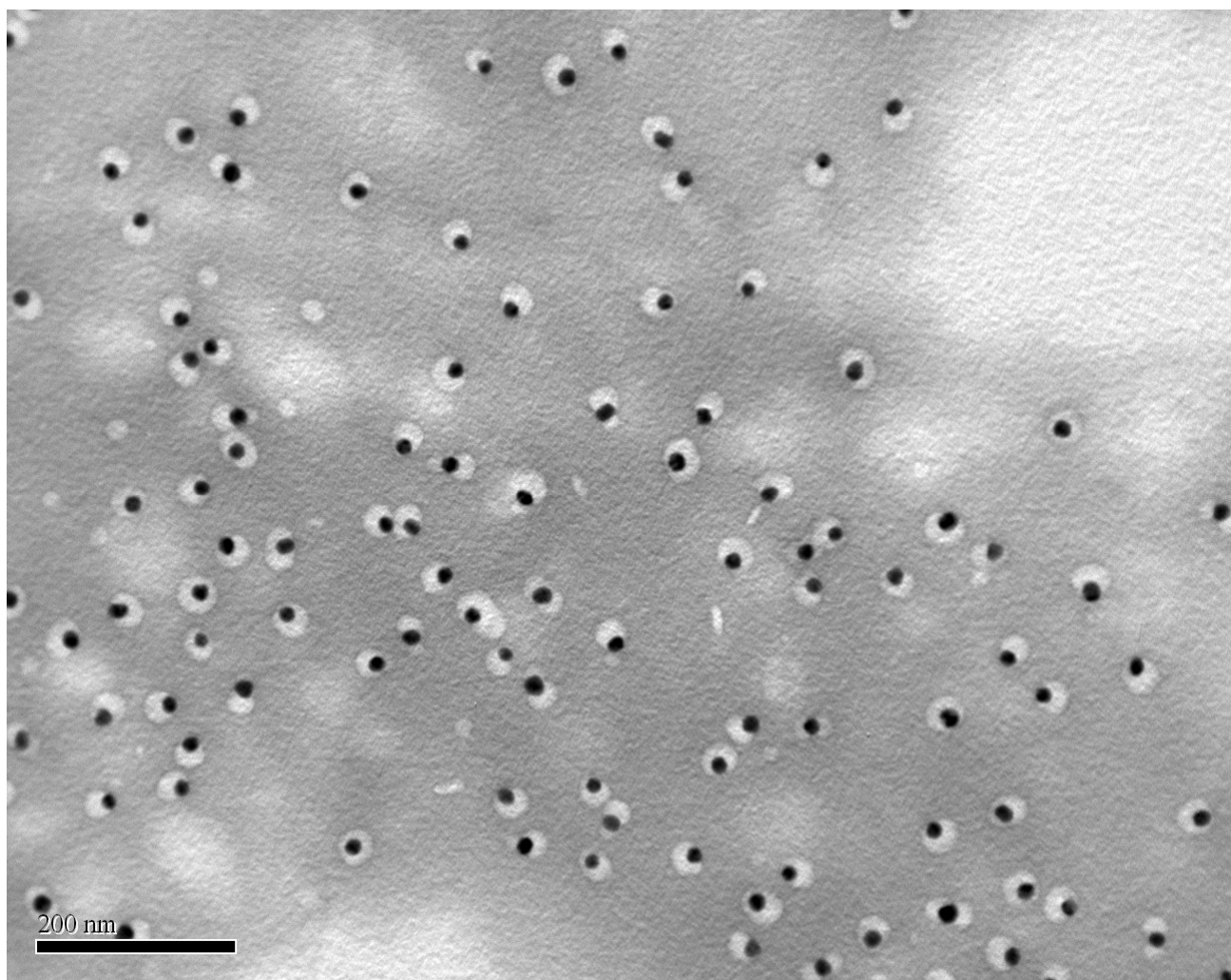


Figure S3. A typical TEM image of eccentrically encapsulated AuNPs ($d_{av} = 15$ nm, [1]:[2] = 1:22) after purification, showing ecc-[AuNP@polymer] with few empty micelles.

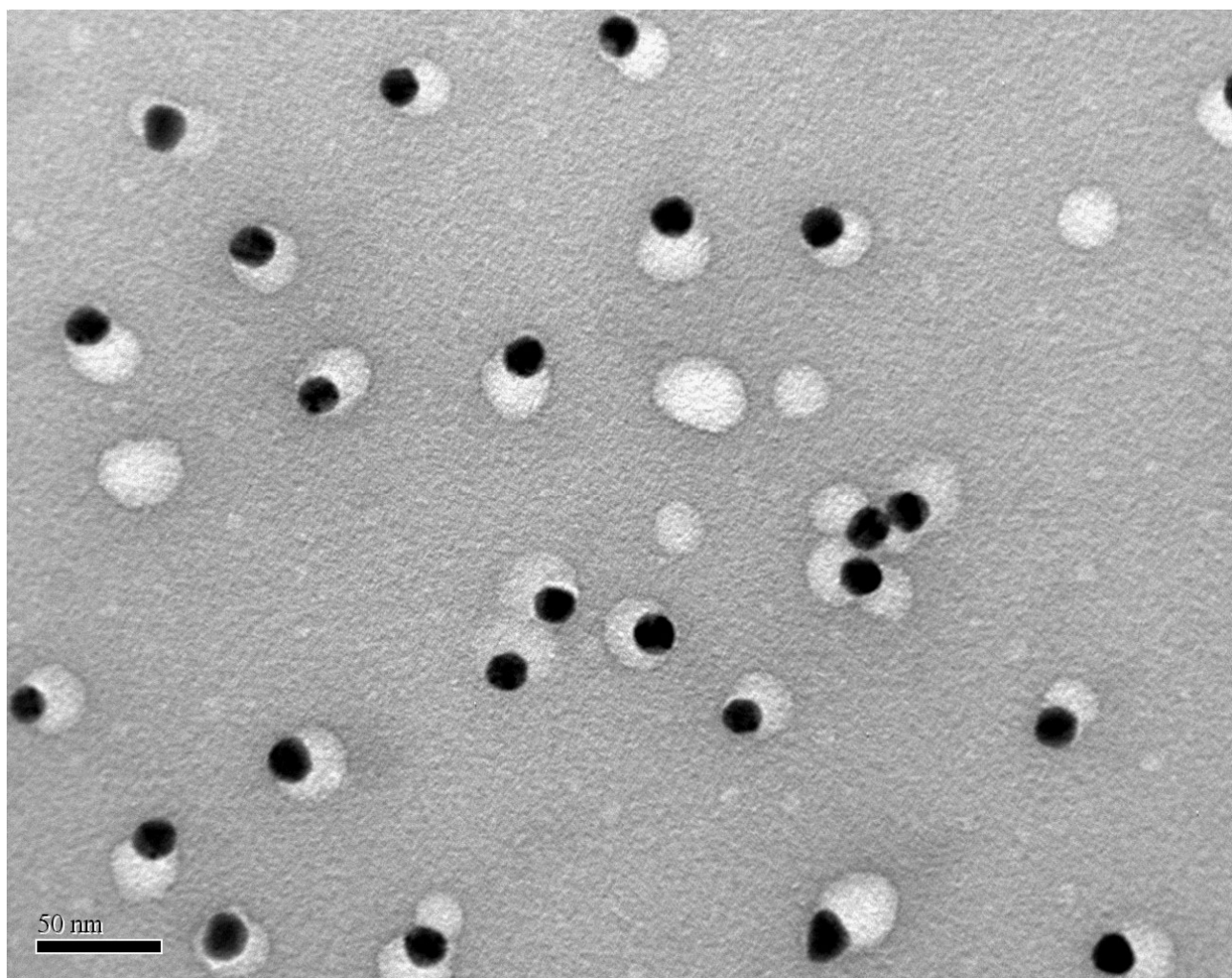


Figure S4. A typical TEM image of eccentrically encapsulated AuNPs ($d_{av} = 15$ nm, $[1]:[2] = 1:132$) after purification, showing ecc-[AuNP@polymer] with larger “exposed” area on AuNPs, compared to that of Figure S3.

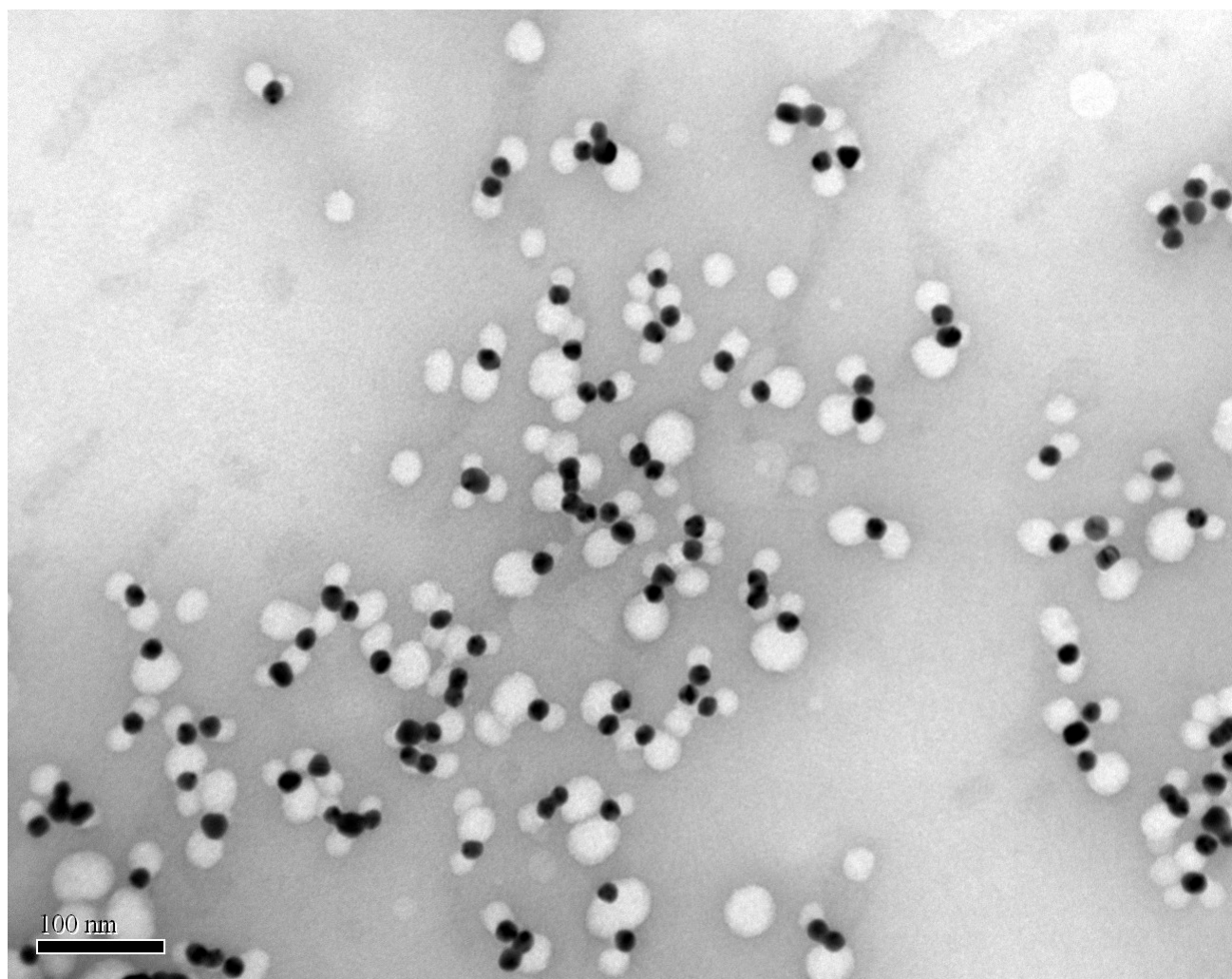


Figure S5. A typical TEM image of eccentrically encapsulated AuNPs ($d_{av} = 15$ nm, $[1]:[2] = 1:220$) pre-incubated with **2**, after purification, showing multiple polymer attachments on the AuNPs.

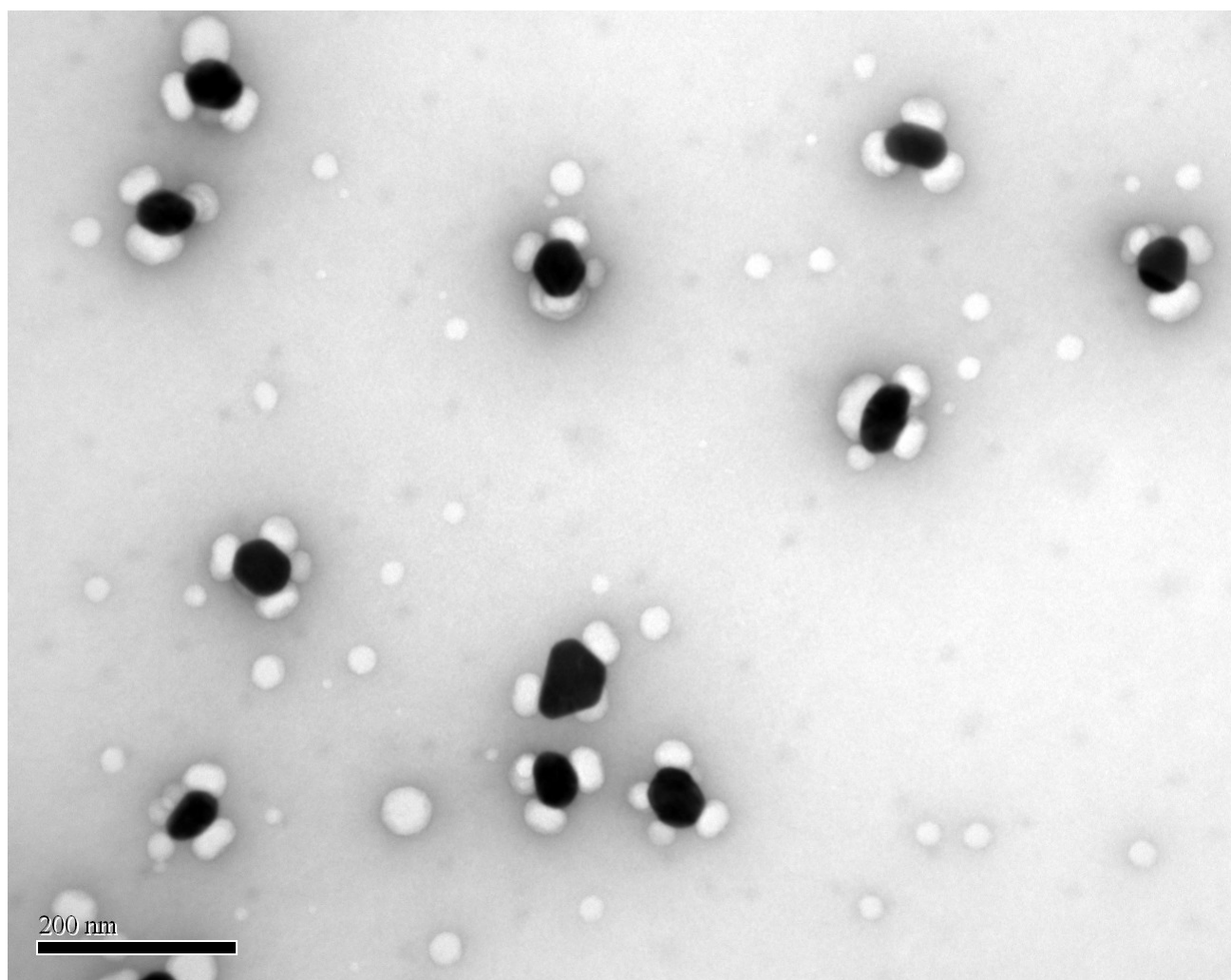


Figure S6. A typical TEM image of eccentrically encapsulated AuNPs ($d_{av} = 40$ nm, $[1]:[3] = 1:12$) pre-incubated with **3**, after purification, showing multiple polymer attachments on the AuNPs.

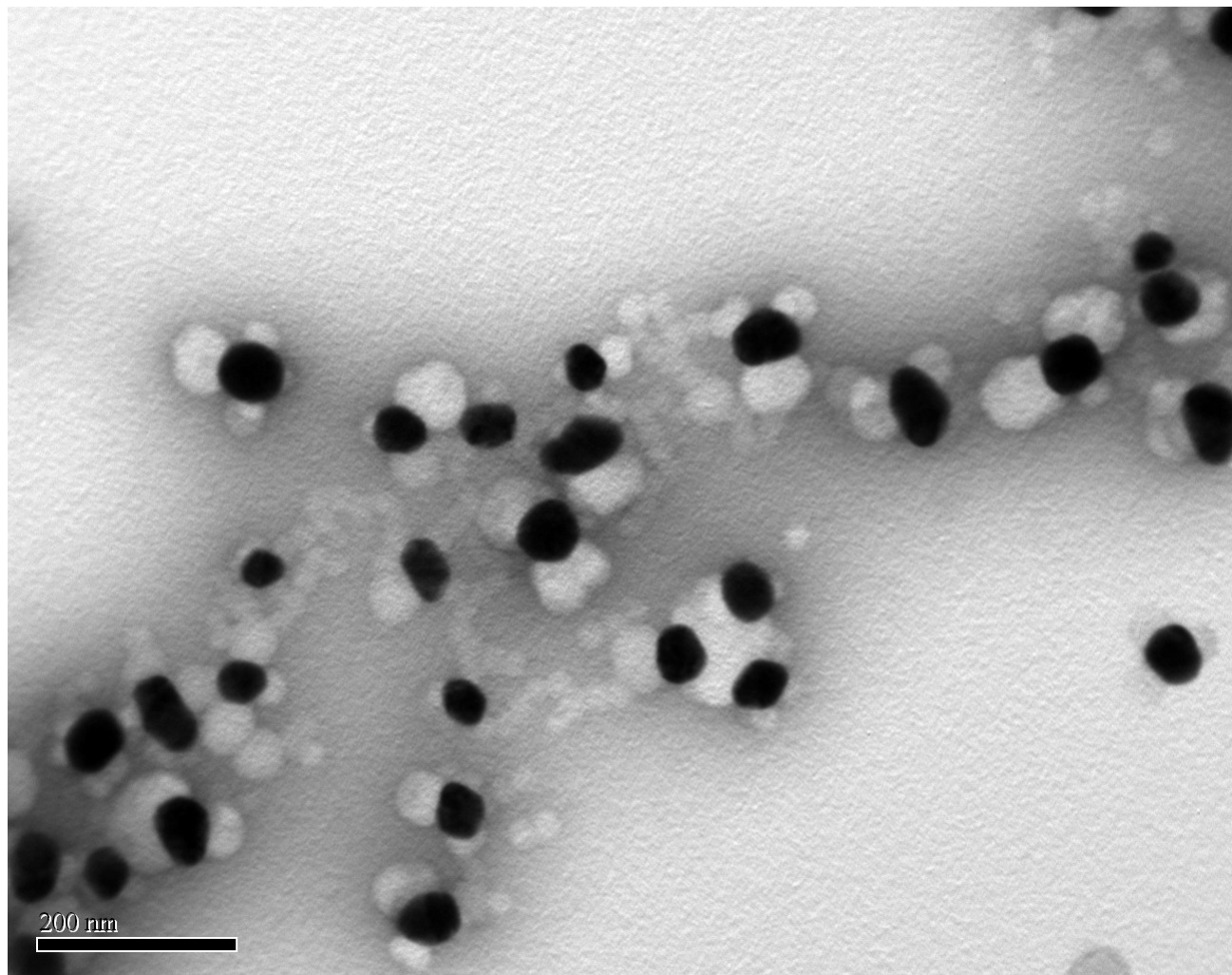


Figure S7. A typical TEM image of eccentrically encapsulated AuNPs ($d_{av} = 40$ nm, $[1]:[2] = 1:220$) pre-incubated with **2**, after purification, showing multiple polymer attachments on the AuNPs.

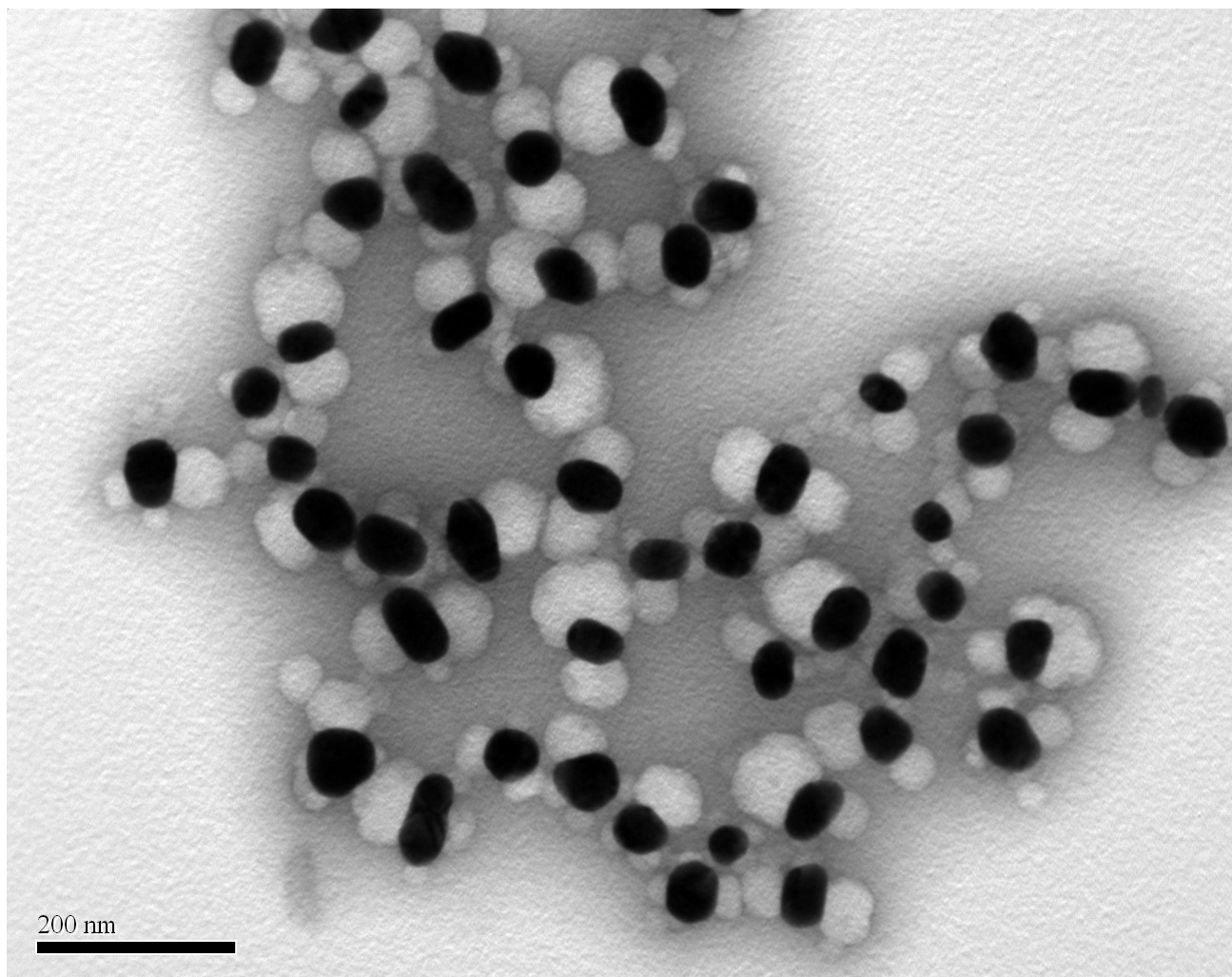


Figure S8. A typical TEM image of the sample in Figure S7, after incubation with **3** at room temperature overnight.

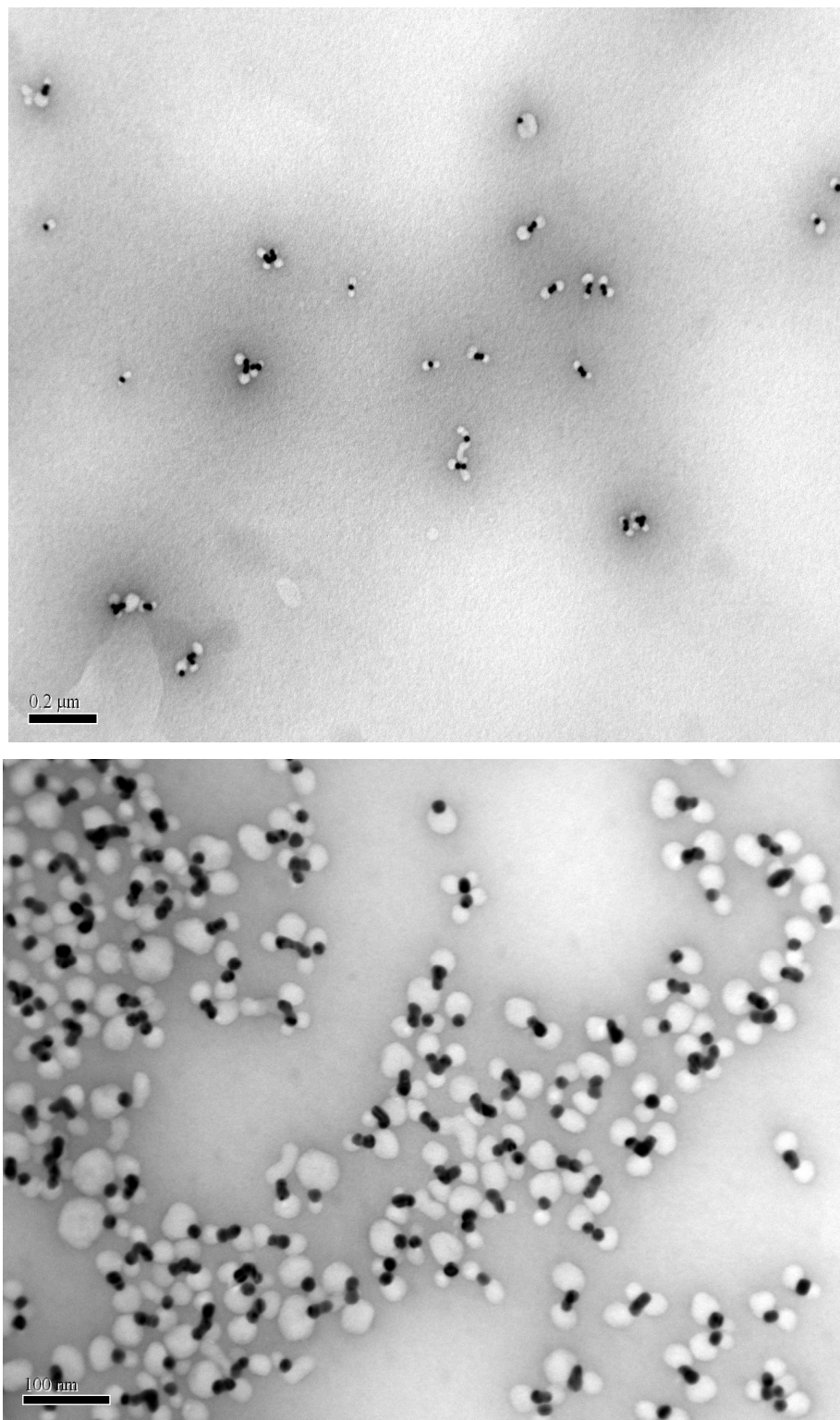


Figure S9. Typical TEM images of dimers resulted from incubation of purified ecc-[AuNP@polymer] in a basic NaCl solution (0.1 M NaCl and 0.04 M NaOH), showing two different regions on the TEM grid.