Supporting Information for: Potentiometric Biosensing of Proteins with Ultrasensitive Ion-Selective Microelectrodes and Nanoparticle Labels

Karin Y. Chumbimuni-Torres,[a] Zong Dai,[c] Nastassia Rubinova,[a] Yun Xiang,[c] Ernö Pretsch,[b] Joseph Wang,[c] Eric Bakker[a]

[a] *Department of Chemistry, Purdue University, West Lafayette, IN 47907,* [b] *Laboratorium für Organische Chemie, ETH Zürich, CH-8093 Zürich, Switzerland, and* [c] *The Biodesign Institute and Fulton School of Engineering, Arizona State University, Tempe, Arizona, 85287.*

pretsche@ethz.ch, joseph.wang@asu.edu, and bakkere@purdue.edu

Reagents. The anti-mouse IgG antibody (produced in rabbit), mouse IgG (from serum), biotinylated anti-mouse IgG antibody (produced in rabbit), streptavidin coated 10-nm dia. Au colloid, phosphate buffer, Tris-HCl, NaCl, $MgCl_2$, Tween 20, 1-ethyl-3-(3-dimethyl aminopropil)carbodiimide (EDAC), *N*-hydroxysuccinimide (NHS), ethanolamine-HCl, sodium thiosulfate pentahydrate, and silver enhancement kit were purchased from Sigma (St. Louis, MO). Hydrogen peroxide was acquired from Fisher (Pittsburgh, PA). The ionophores *o*-xylylenebis(*N,N*-diisobutyldithio-carbamate) (copper(II) ionophore (I)), 4-*tert*-butylcalix[4]arene-tetraacetic acid tetraethyl ester (Na-Ionophore X), sodium tetrakis[3,5-bis-(trifluoromethyl)phenyl]borate (Na-TFPB), lipophilic salt tetradodecylammonium tetrakis(4-chlorophenyl)-borate (ETH 500), bis(2-ethylhexyl) sebacate (DOS), poly(vinyl chloride) (PVC), and tetrahydrofuran (THF) were purchased in Selectophore® or puriss grade from Fluka (Buchs, Switzerland). The solvent methylene chloride was obtained from Fisher, poly(3-octylthiophene) (POT) was synthesized following the procedure of H. Jarvinen et. al[1] and purified according to the patent application.[2] Synthesis of methyl methacrylate decyl methacrylate (MMA-DMA) copolymer matrix was accomplished as described previously.[3] All stock and buffer solutions were prepared using autoclaved double-deionized water (18.2 MΩ cm).

Preparation of the mixed monolayer anti-mouse IgG antibody modified gold substrates

Preparation of the gold substrates. The gold substrates were obtained from Denton Vacuum LLC (Moorestown, NJ), machine cut (Advotech Company Inc., Tempe, AZ) to identical pieces (of 6 x 3 x 0.2 mm size), assuming a uniform thickness.

Preparation of the mixed monolayer. The gold substrates were cleaned first in *Piranha solution* and rinsed with water prior to use. (*Safety note*: the Piranha solution should be handled with extreme caution.) A 1:9 mixture of 11-mercaptoundecanoic acid and 6-mercaptoethanol (1 mM each in ethanol), respectively, was added to the clean/dried gold substrates and mixed overnight for the co-assembly process.

Anti-IgG antibody immobilization. This was followed by washing the gold substrates two times with ethanol. Subsequently, 1 mg of NHS and 1 mg EDAC were added in 100 μL PBS solution and mixed for 30 min, and then the supernatant was removed. 50 μL of the 1000 μg/mL Anti-IgG antibody solution was added into 1200 μL PBS (20mM, pH 8.6), then 100 μL of this solution was distributed to each vial. After 2 h of mixing, the solution was removed and 100 μL of 1 M ethanolamine solution was added to each vial and incubated for 10 min. After taking out of the solution, the gold substrates were washed three times using 100 μL of 50 mM Tris-HCl buffer (containing 100 mM NaCl, pH 7.4).

Preparation of Au colloid conjugate anti-IgG antibody. 50 µL streptavidin coated Au colloid was mixed with 15 µL of 100 µg/mL biotinylated anti-IgG antibody solution and 1200 µL of Tris-HCl buffer (50mM Tris-HCl, 100mM NaCl, pH 7.4). This was followed by shaking for 4 h before use in the sandwich assays.

Sandwich assay. The desired amount of the target IgG antigen was added to a 100 μL Tris-HCl buffer solution containing the anti-IgG antibody modified gold substrate and incubated for one hour at room temperature. The supernatant was then removed, the gold substrates were washed with 100 μL of 50 mM Tris-HCl buffer (containing 100 mM NaCl, pH 7.4). The anti-IgG antibody modified gold substrates were incubated for one hour with 100 μL of the previously prepared Au colloid conjugated anti-IgG antibody (as mentioned above). This was followed by washing once using 100 μL of 10 mM PBS (pH 7.4) + 0.1 M NaCl + 0.1% (v/v) Tween 20 + 1% BSA, twice using 100 μL of 10 mM PBS (pH 7.4) + 0.1 M NaCl + 0.1% (v/v) Tween 20, washing again once using 100 μL of 10 mM PBS (pH7.4) + 0.1 M NaCl, and finally once using 100 μL of deionized water.

Silver enhancement. The gold substrates were transferred to new vials where they were incubated with 100 μL of 1 M $Ca(NO_3)_2$ for 20 min with gentle mixing, to avoid any halide ions remaining that may otherwise interfere with our detection system. The supernatant was removed and followed by adding the mixture of silver enhancement solution with 20 times dilution using 1 M $Ca(NO_3)_2$ for 5 min. After that, 1 mL deionized water was added and the supernatant was removed quickly. Subsequently 100 μL of 2.5% $Na_2S_2O_3$ was added and left for 5 min with gentle mixing. The final gold substrates were washed two times using 100 μL of 10 mM PBS (pH 7.4) + 0.1 M NaCl + 0.1% (v/v) Tween 20, one time using 100 μL of 10 mM PBS (pH 7.4) + 0.1 M NaCl, and finally three times using 100 μL of deionized water.

Silver dissolution. Hydrogen peroxide was used since it was observed that it can efficiently oxidize silver enhanced gold particles after careful optimization of concentration and reaction time. This observation was confirmed by scanning electron microscopy of gold nanoparticles (Figure 1), gold nanoparticles + silver enhancement (Figure 2) and gold nanoparticles + silver enhancement after 2 h of reaction with 0.1 M H_2O_2 (Figure 3), in a Philips/FEI CM-100 transmission electron microscope operated at 80 kV, spot 3, 200 μm condenser aperture, 30 μm objective aperture. The magnifications were 28,500x and 39,000x.

Preliminary experiments on the dissolution of silver enhanced gold nanoparticles with 0.1 M hydrogen peroxide by potentiometric detection of the released silver showed that 98 ± 2 % of silver was fully oxidized after 30 min oxidation time for a 100 nM silver concentration. Higher concentrations gave less complete conversion in the same amount of time, and longer oxidation times were therefore chosen for the final assay.

In the final assay, dissolution of Ag was carried out by the addition of 100 μL 0.1 M H_2O_2 (in 10^{-5} $NaNO_3$) and left for two hours to ensure complete oxidation. Subsequently, 50 μL of the solution was pipetted in a 1 mL cell for final silver detection.

Potentiometric Measurements.

Membranes. The membrane for the Ag-ISE was prepared by dissolving 50 mg of components in 0.8 mL of methylene chloride: copper (II) ionophore (I) (17 mmol/kg), Na-TFPB (7 mmol/kg), ETH 500 (12 mmol/kg), and MMA-DMA. The membrane cocktail was degassed by sonication for 1 min prior to use for coating the microelectrodes.

The membrane for the Na-ISE used as a reference was prepared by dissolving 100 mg of components in 1 mL of THF: Na-ionophore (10.9 mmol/kg), Na-TFPB (5.12 mmol/kg), PVC (32.2 wt %), and DOS (66.3 wt %). The cocktail was left to evaporate for one hour and then 5 μL of the solution was guided into a 10 μL pipette tip with the help of a capillary add left to dry for at least 24 h. The resulting sodium-selective membrane was symmetrically conditioned in 10^{-3} M $NaNO_3$ overnight.

Microelectrode. The Ag-ISE solid contact was prepared by using a 2 cm long gold wire (200 μm dia.) that was soldered to a copper wire for electric contact. Before use the gold wires were thoroughly cleaned with diluted sulfuric acid, and rinsed with water, then acetone and left for 3 min in chloroform. The solution of POT was applied along the length of the gold wire at least three times until the color of the wire became black. After the gold wires were fully covered with POT they were left to dry. Then the wires were inserted into a polypropylene pipette tip so that the wires were flush with the end of the micropipette tip. The membrane cocktail was applied to the tip of the wire covered with POT for three times at 15 minute-intervals and left to dry for at least 1 hour. The resulting microelectrodes were conditioned one day in 10^{-3} M $AgNO_3$, followed by one day in a solution of 10^{-9} M $AgNO_3$ and 10^{-5} M $NaNO_3$ before use.

Measurements were performed in a 1 mL volume with addition of 50 μL of the sample.

Apparatus. Potentiometric measurements were performed with a PCI MIO16XE data acquisition board (National Instruments, Austin, TX) utilizing a four-channel high Z interface (WPI, Sarasota, FL) at room temperature (21–23 ºC).

References

1 Jarvinen, H., Lahtinen, L., Nasman, J., Hormi, O., A.-L. *Synthetic Metals* **1995**, *69*, 299-300.
2 U.S. Patent Application 20040254336.
3 Qin, Y., Peper, S., Bakker, E. *Electroanalysis* **2002**, *14*, 1375-1381.

Additional Results: The effective dissolution of silver ions by H_2O_2

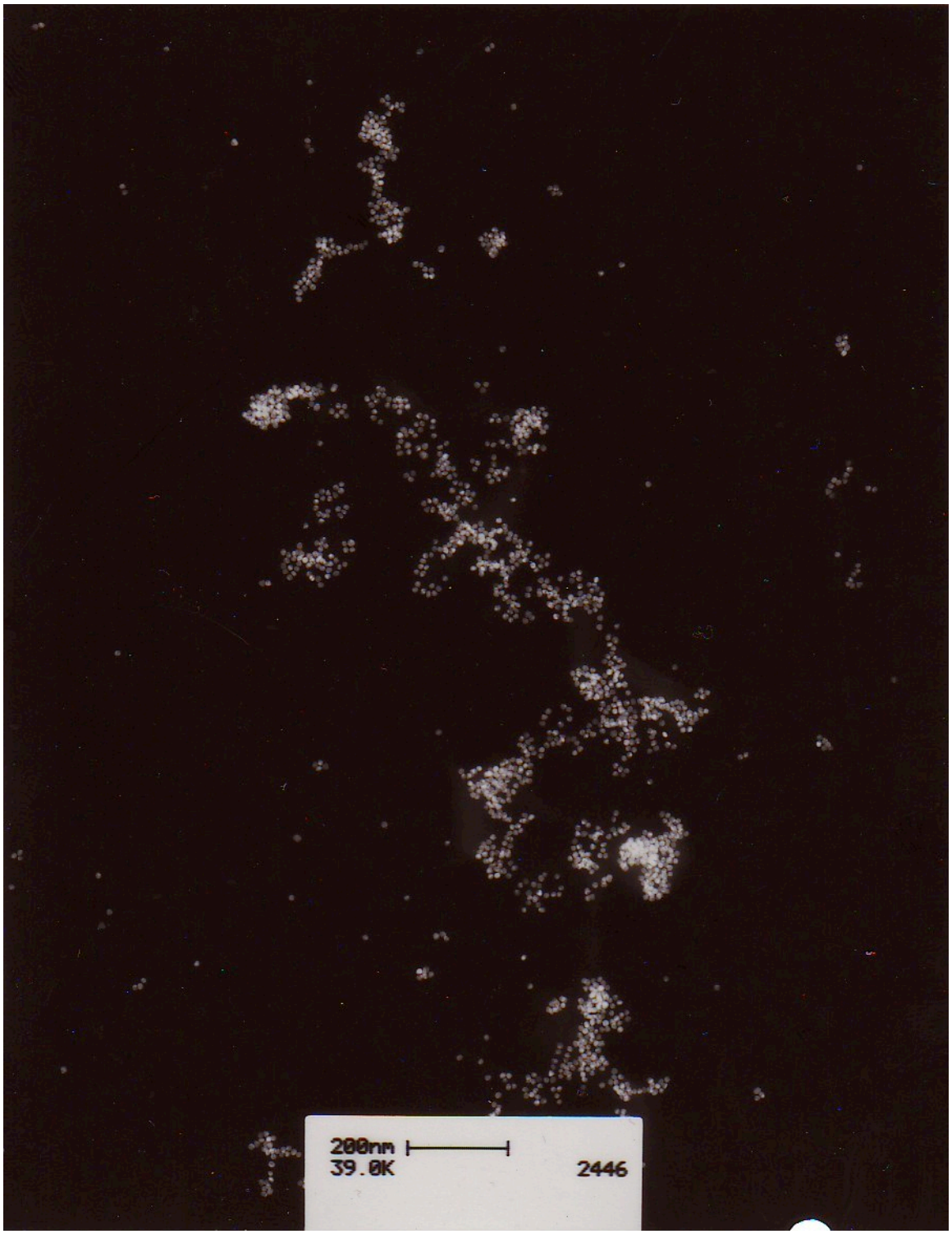

Figure 1. Transmission electron micrographs of gold nanoparticles (~13 nm dia.) before oxidation with hydrogen peroxide.

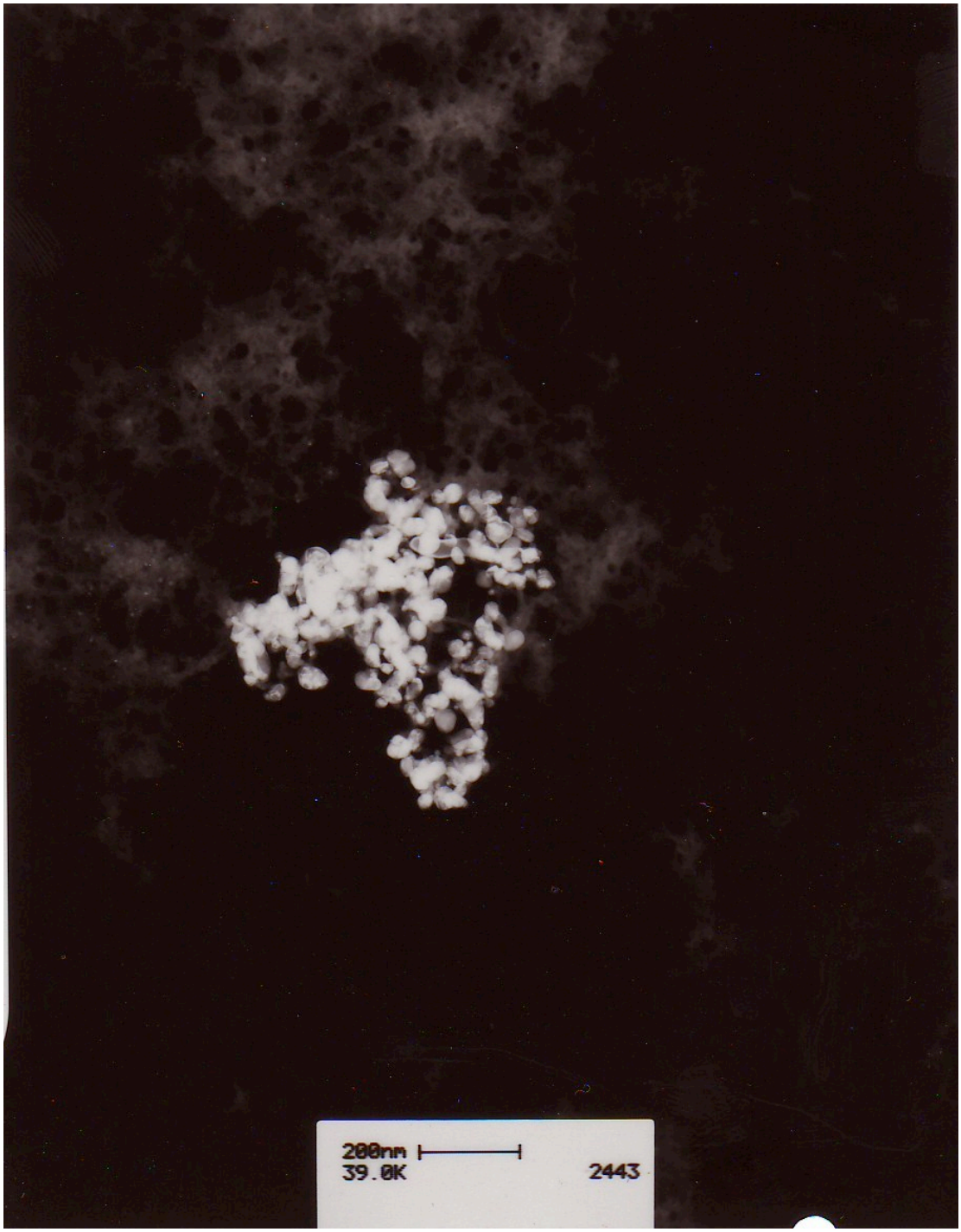

Figure 2. Transmission electron micrographs of gold nanopaticles after silver enhancement (10 min reaction time) before oxidation with hydrogen peroxide.

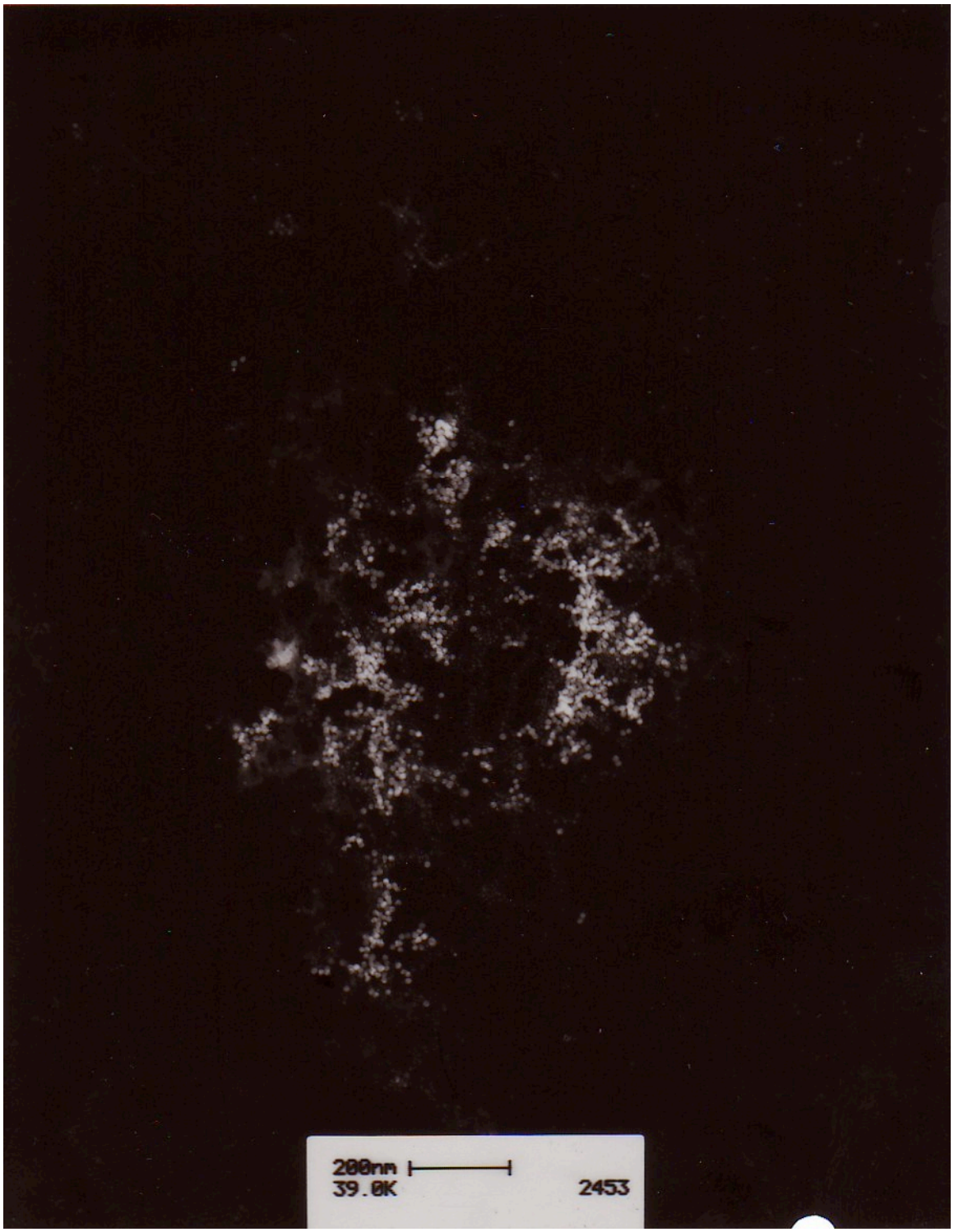

Figure 3. Transmission electron micrographs of silver enhanced gold nanoparticles after reaction with 0.1 M H_2O_2 for 2 hours.