

Supporting Information

Arresting the Time-Dependent H₂O₂ Mediated Synthesis of Gold Nanoparticles for Analytical Detection and Preparative Chemistry

Pierangelo Gobbo,^{‡a} Mia J. Biondi^{‡a,b}, Jordan J. Feld^b and Mark S. Workentin^{*a}

^a *The University of Western Ontario and the Centre for Materials and Biomaterials Research,
Richmond Street, London, Ontario, Canada. E-mail: mworkentin@uwo.ca; Tel: +1 519-661-2111 extn
86319*

^b *Toronto Western Hospital, University Health Network, McLaughlin-Rotman Centre for Global Health,
University of Toronto.*

[‡] *These authors contributed equally.*

Materials and Methods

All chemicals were used as received. Hydrogen peroxide (30%) was purchased from Caledon, gold (III) chloride trihydrate ($\geq 99.9\%$), reduced L-glutathione ($\geq 98.0\%$), and 2-(N-morpholino)ethanesulfonic acid (MES) (biotechnology performance certified, $\geq 99.5\%$) were purchased from Sigma Aldrich. Centrifugal tubes with a 50 kDa membrane (Amicon® Ultra-4 centrifugal filter units) were purchased from Millipore.

Dilute solutions of hydrogen peroxide were always freshly prepared, while the solutions of MES and $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ were prepared weekly and stored at 4°C . UV-Vis spectra were recorded using a Tecan Infinite® M1000 PRO plate reader spectrometer in 96-well 300 μL Immulon® 2 HB Flat Bottom MicroTiter® plates (Thermo Fisher Scientific).

HRTEM images were recorded using a FEI Tecnai G2 F20 microscope. TEM grids (Electron Microscopy Sciences, Formvar carbon film on 400 mesh copper grids) were prepared by putting directly on them a drop of dilute AuNS or AuNP solution. The drop was gently removed after ~ 15 minutes using a soft paper towel, and the TEM grids were left to dry for at least 4 hours before recording the images.

Plate Based Assays

In order to kinetically monitor the absorbance at 550 nm or to record the absorbance spectra of the samples, each reaction mixture was added to a single well of the plate. Final concentrations of H_2O_2 ranging from 0-140 μM were added to individual wells containing 1 mM MES buffer (pH 6.5). To initiate the reaction, a final concentration of 1 mM $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ was added to each well, and mixed manually. The absorbance at 550 nm was immediately recorded by the plate reader each minute following the addition of the gold (III) salt for 60 minutes, followed by every 15 minutes up to 360 minutes at room temperature. Alternatively, a spectra from 400-900 nm was recorded every 5 minutes. During both sets of measurements, each cycle was initiated by an orbital shake for 15 seconds, followed by a 15 second wait period. Photographs were taken at specified times in a parallel experiment.

So as to block the slow disaggregation of AuNS, 20 μM of L-glutathione was added 10 minutes after the addition of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (time-point determined quantitatively). Single measurements at 550 nm and absorbance spectra from 400-900 nm were recorded at room temperature as described above.

To mimic the conditions of the large-scale reactions, a final concentration of 1 mM of H_2O_2 was added to individual wells containing 10 mM MES buffer (pH 6.5). Each reaction was initiated by a final concentration of 10 mM prepared gold (III) chloride trihydrate, and mixed manually. An absorbance spectra was measured every 30 seconds for 5 minutes. Thereafter each cycle was initiated by an orbital shake for 15 seconds; followed by a 15 second wait period, every minute up to 60 minutes; followed by every 15 minutes until measurements were terminated at 360 minutes.

Large-Scale Synthesis of AuNS and AuNP

In a little vial were insert 394 μl of a 76 mM solution of MES buffer pH 6.5, 30 μl of H_2O_2 100 mM and 2.566 ml of nanopure water. The solution stirred manually and 10 μl of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ 300 mM were added. The reaction mixture was quenched adding 0.20 eq of L-glutathione with respect to the Au(III) at the different times to obtain large AuNS (minutes) and AuNP (hours). To purify the AuNS or the AuNP the solution was added to a centrifugal filter unit (MWCO 50 kDa) and centrifuged at 4000 rpm for 5 minutes until ~ 500 μl of solution remained. Approximately 2.5 mL of nanopure water was added, and then centrifuged again. This was repeated four times, where the final 500 μl was rediluted to 3 mL and stored at 4°C.

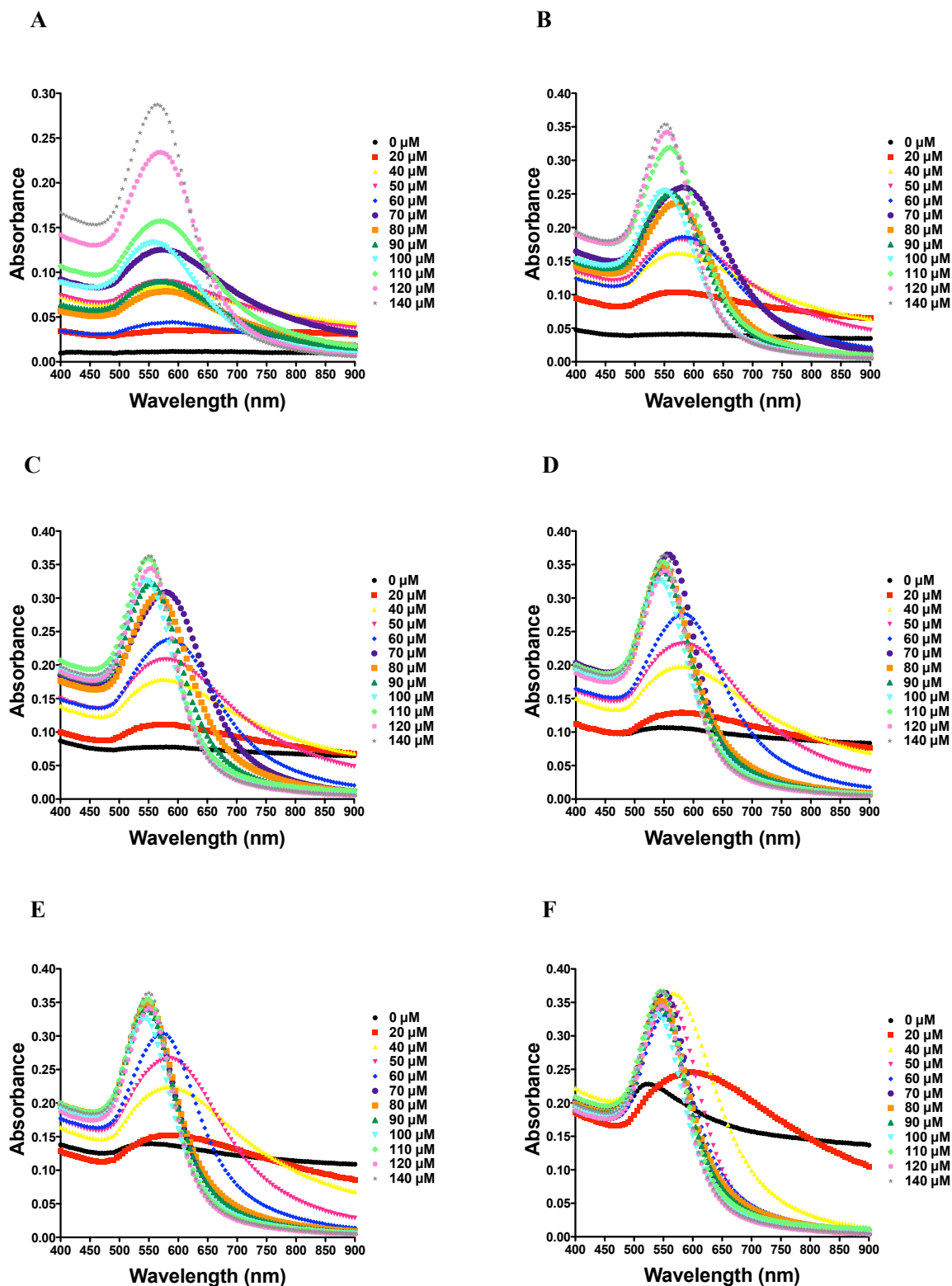


Fig. S1 UV-Vis absorbance spectra demonstrating the slow disaggregation process of AuNSs. Readings were taken following the addition of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ in the presence of increasing concentrations of H_2O_2 after A) 5, B) 10, C) 15, D) 30, E) 60, and F) 300 minutes.

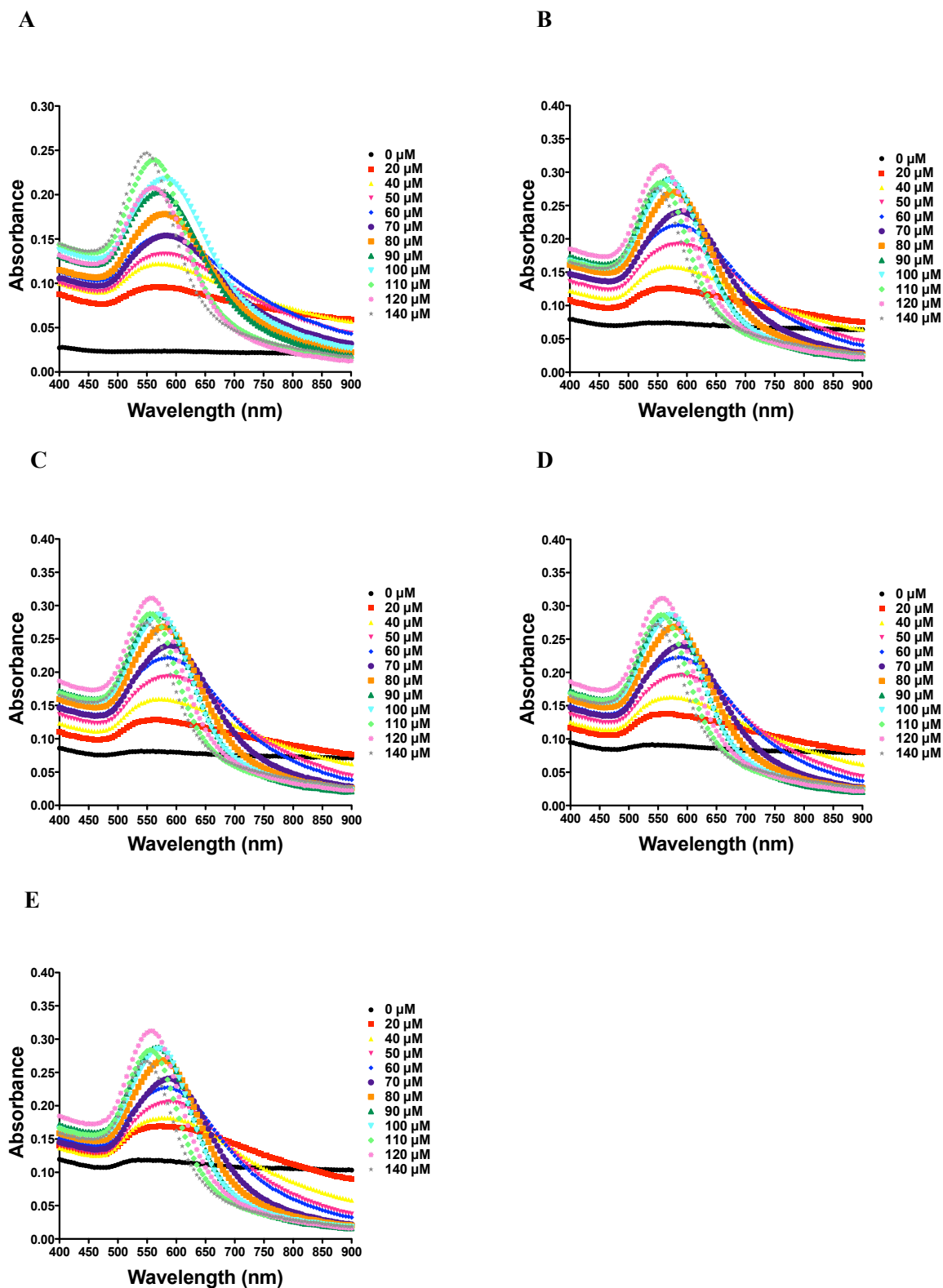


Fig. S2 Relative UV-Vis absorbance measurements showing the stabilization of the reaction mixtures attributed to the addition of glutathione. A reading was taken after the addition of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ in the presence of increasing concentrations of H_2O_2 at A) 5 minutes. L-glutathione was then added and spectra were recorded at B) 15, C) 30, D) 60, and E) 300 minutes with respect to the addition of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$.

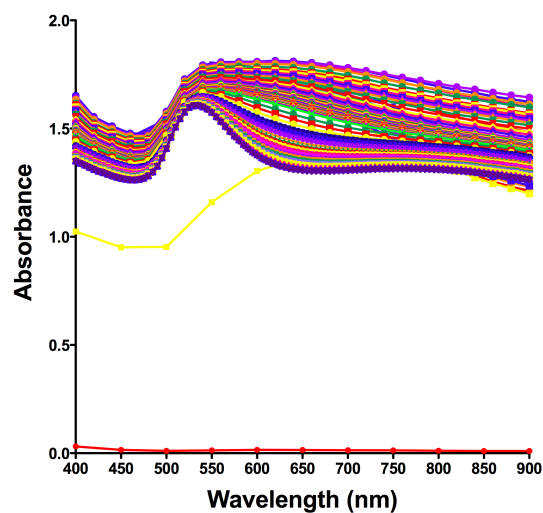


Fig. S3 Relative UV-Vis absorbance measurements for the large-scale experiment. Reaction concentrations were increased 10-fold (see *Materials and Methods*), and spectra were recorded after the addition of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ every 30 seconds for the first 5 minutes, followed by every minute up to 60 minutes, and every 15 minutes thereafter up to 360 minutes.

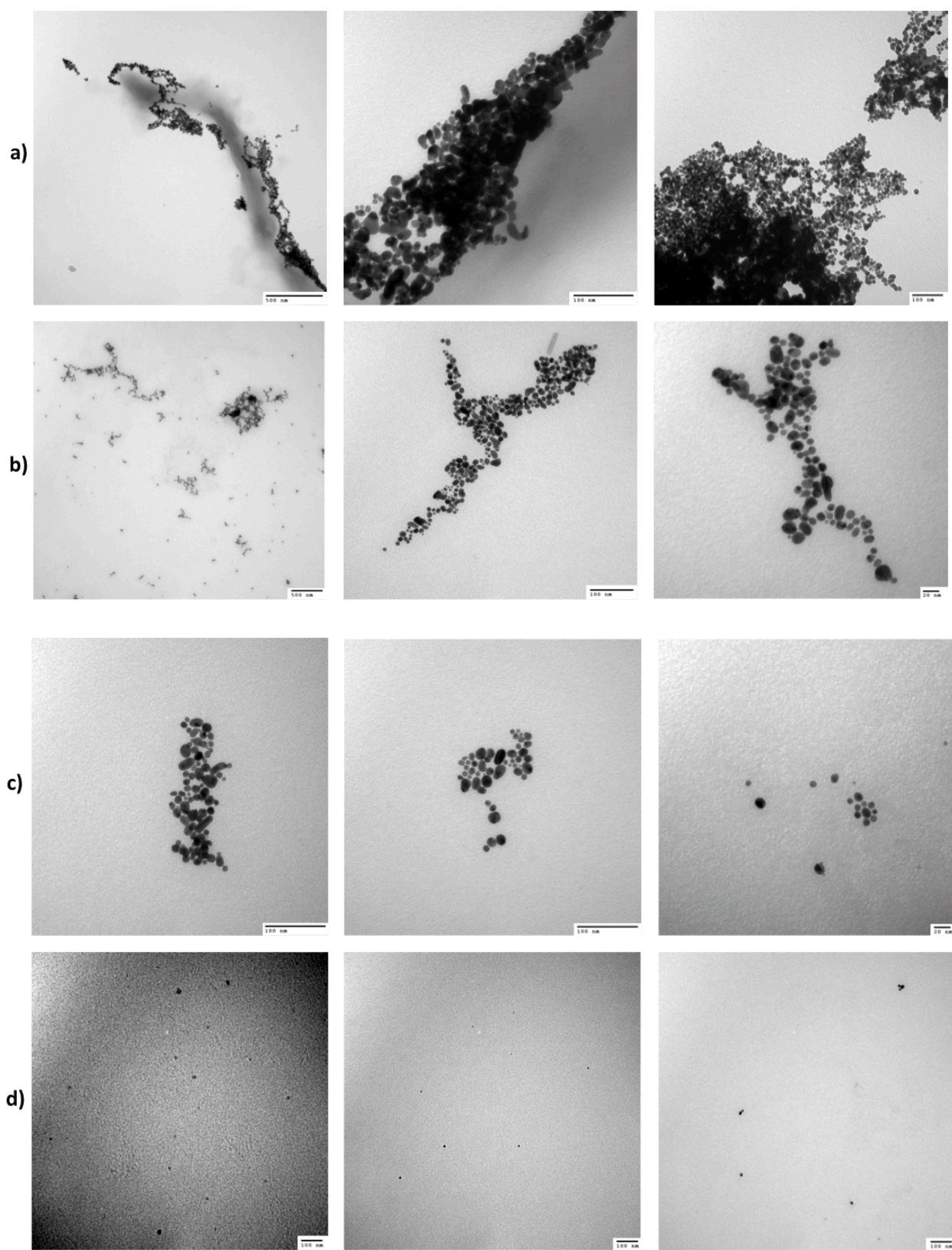


Fig. S4 Low magnification HRTEM images of the large-scale synthesis of AuNS and AuNP recorded after purification. Reactions were quenched with glutathione after A) 5 minutes, B) 1, C) 3 and D) 6 hours.

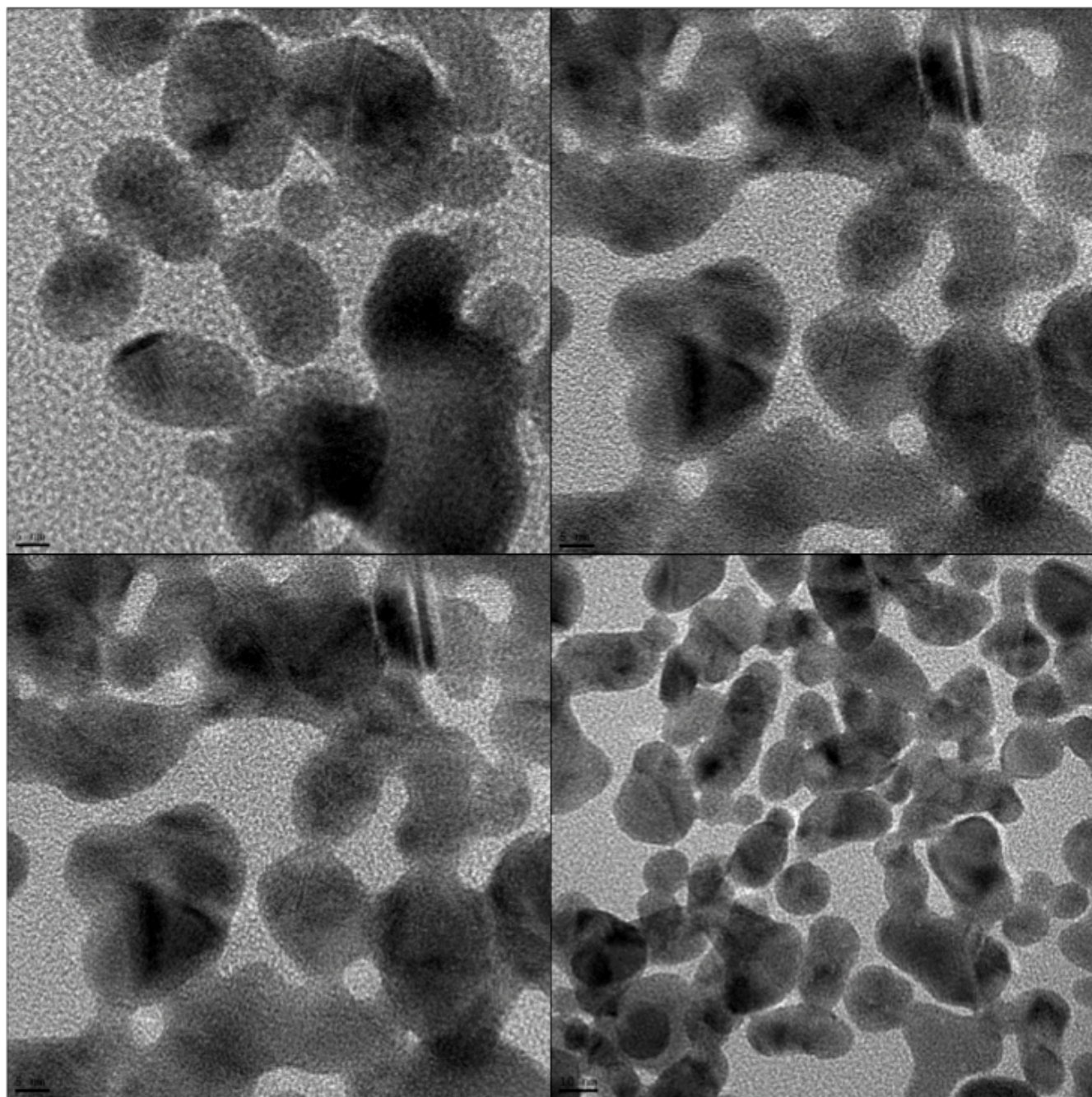


Fig. S5 High magnification HRTEM images of the large-scale synthesis of AuNS and AuNP recorded after purification. Reactions were quenched with glutathione after 5 minutes.

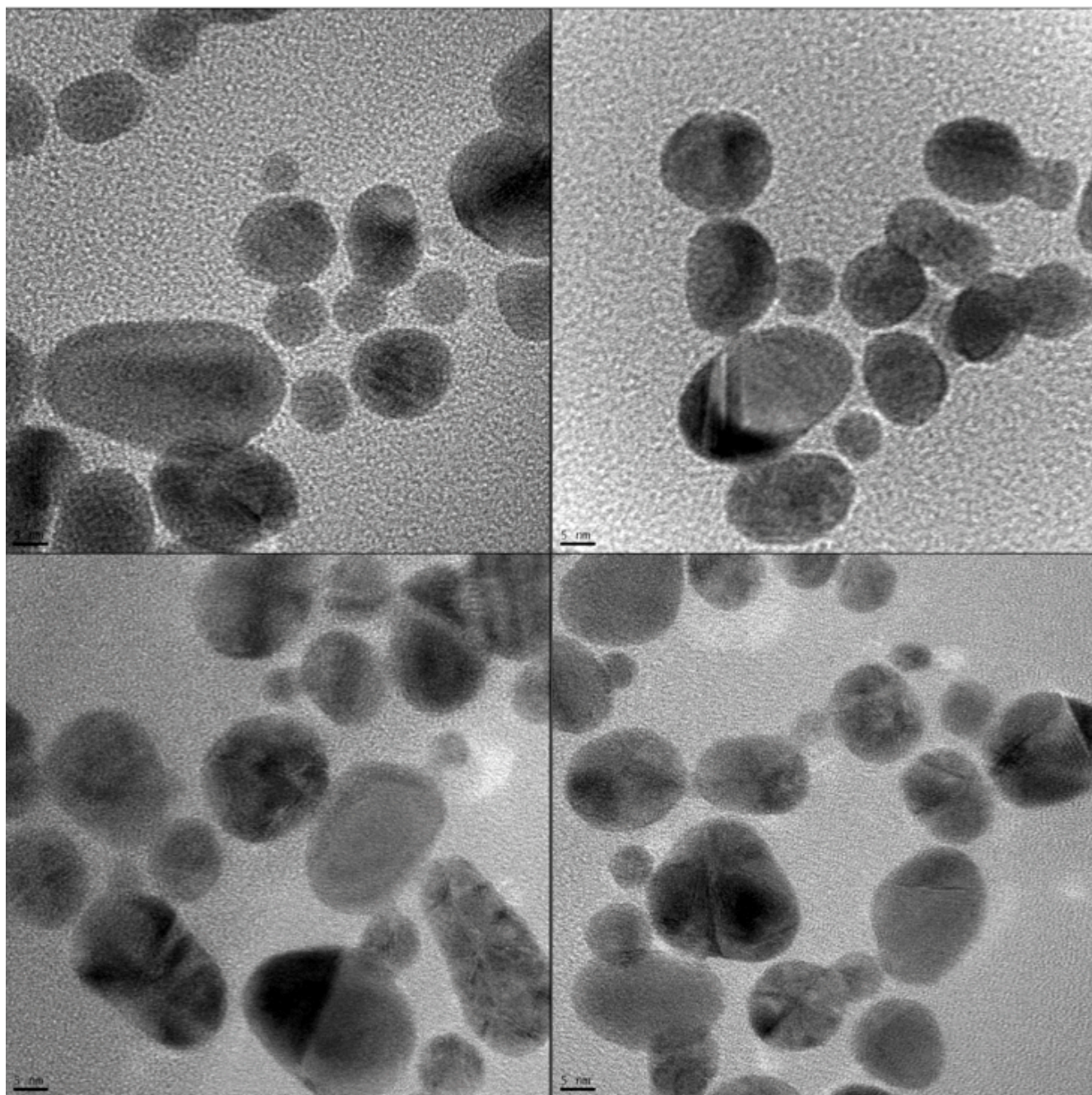


Fig. S6 High magnification HRTEM images of the large-scale synthesis of AuNS and AuNP recorded after purification. Reactions were quenched with glutathione after 1 hour.

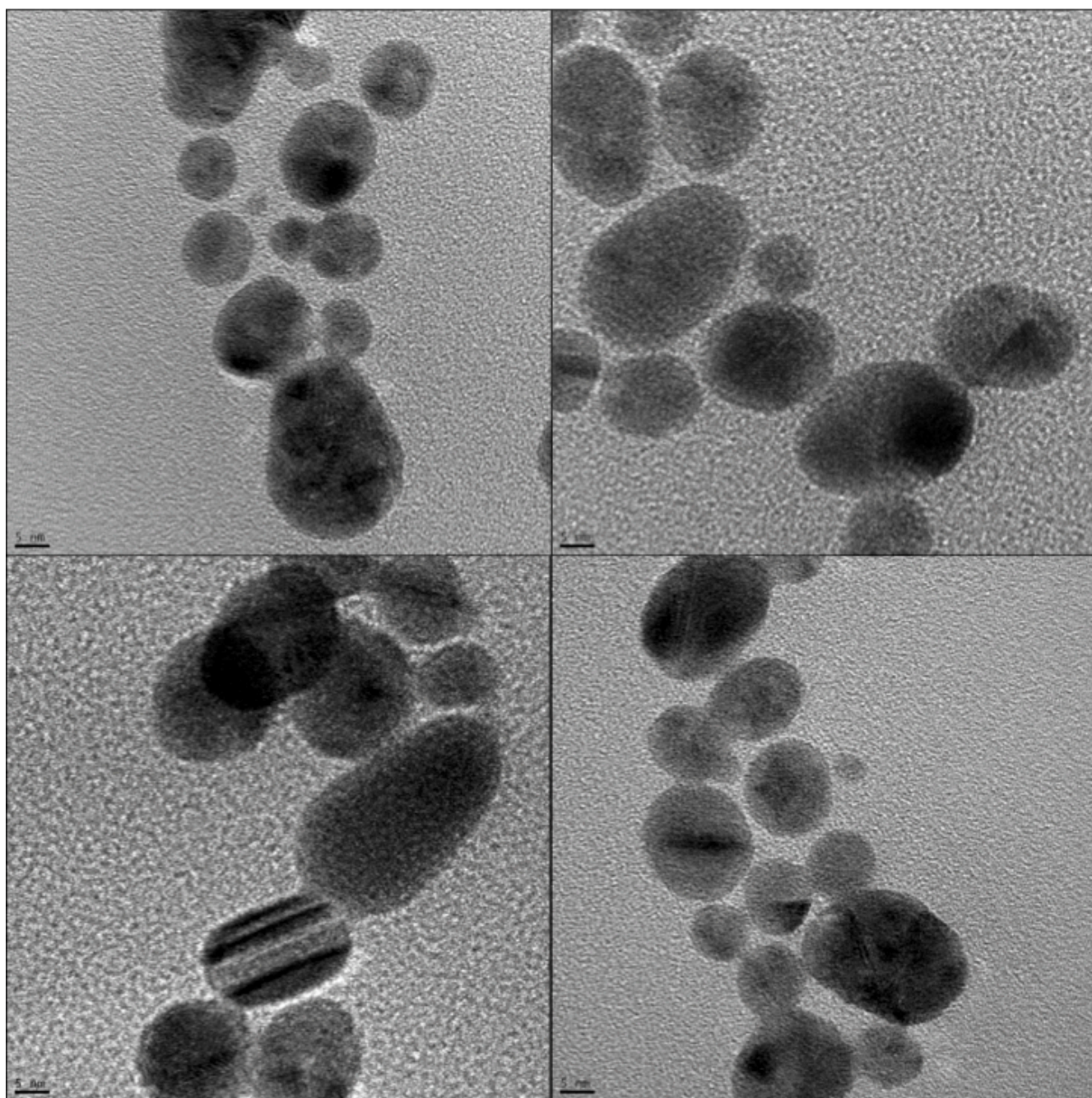


Fig. S7 High magnification HRTEM images of the large-scale synthesis of AuNS and AuNP recorded after purification. Reactions were quenched with glutathione after 3 hours.

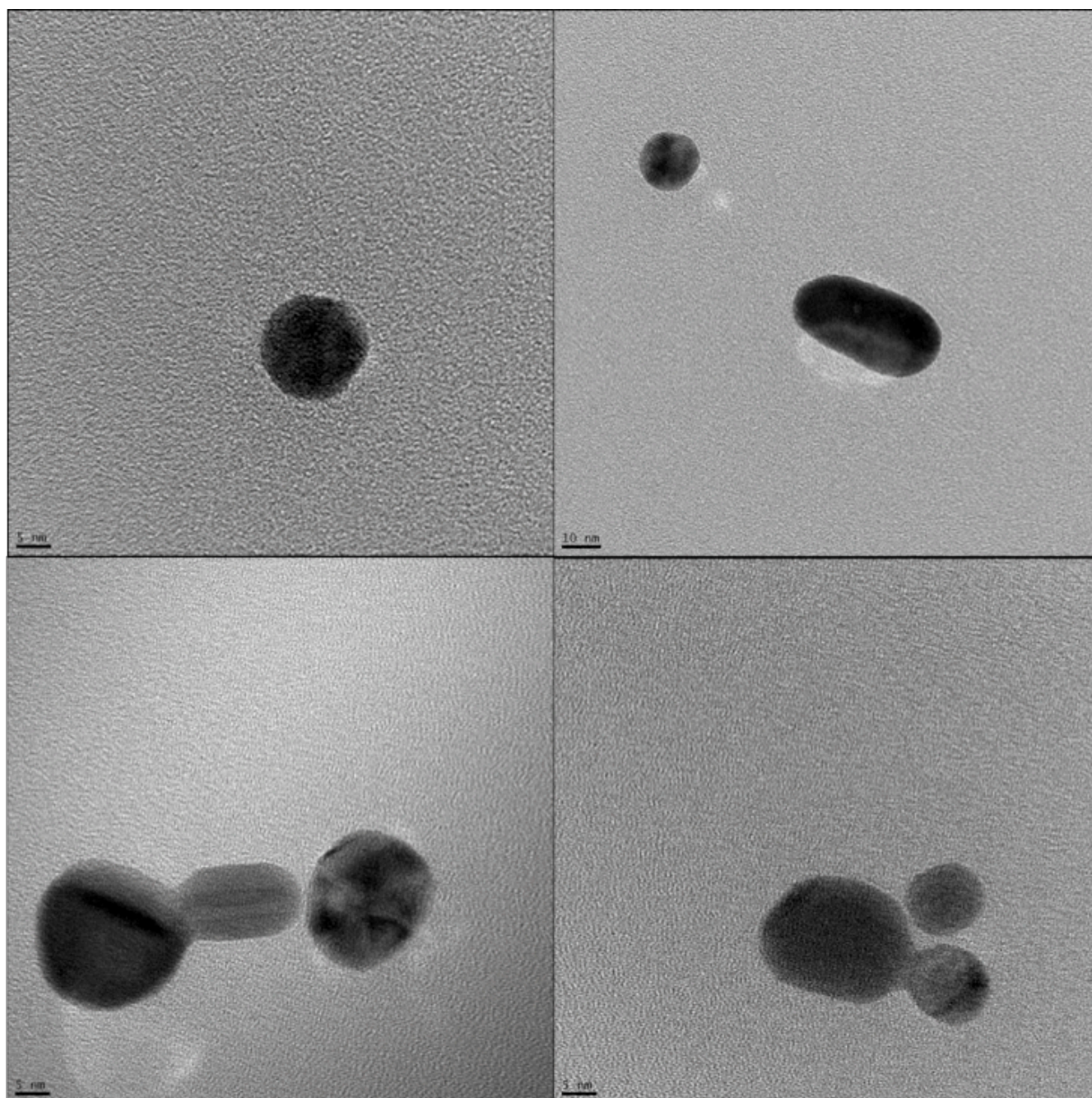


Fig. S8 High magnification HRTEM images of the large-scale synthesis of AuNS and AuNP recorded after purification. Reactions were quenched with glutathione after 6 hours.