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Electronic Supplementary Information (ESI)

for

Flow-Controlled Synthesis of Gold Nanoparticles in a Biphasic System with Inline Liquid-Liquid Separation

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Apparatus and reagents

Analytical grade reagents of gold chloride ($HAuCl_4.3H_2O$), sodium borohydride ($NaBH_4$), tetraoctyl ammonium bromide (TOAB), 4-dimethylaminopyridine (DMAP), sodium sulfate decahydrate ($Na_2SO_4.10H_2O$), toluene and sodium hydroxide (NaOH) were purchased from Sigma Aldrich (Sydney, Australia). Ultra-pure Milli-Q water (> 18.2 MΩ.cm) was used for preparation of solutions unless otherwise specified.

A modular system (cetoni, Germany) with a BASE 600 power supply system, six neMESYS mid-pressure syringe pumps, a peRISYS-S peristaltic pump, and a QmixP pressure monitor was used. Operation of the modules was achieved via the Qmix ELEMENTS software. An additional peristaltic pump (Ismatec, Sydney, Australia) was used and operated manually. Separation of the organic and aqueous phases was achieved via SEP 10 liquid-liquid membrane separators (Zaiput, Cambridge, MA) equipped with 0.5 μm hydrophobic polytetrafluoroethylene (PTFE) filters.

All standard and replaceable fittings were purchased from Kinesis, Australia unless otherwise specified. Filter units of three types were used. Glass fibre, and glass fibre + 0.2 μm PTFE were purchased from Millipore, Australia, and Whatman™ Anotop 0.02 μm was purchased from GE Healthcare, Australia.

Glass syringes, 50 mL in volume (ILS, Germany) were used to deliver solutions via syringes pumps to the fluidic lines connected to 1/16 inch PTFE tubing (1.0 mm internal diameter) or Teflon® AF 2400 gas permeable tubing (0.81 mm internal diameter). Alternatively, solutions were delivered via the use of peristaltic pumps using Tygon 2-stop peristaltic tubing (SC011 with internal diameter 1.02 mm for the Perisys pump and SC077 with internal diameter 0.76 mm for the Ismatec pump) and connected downstream to 1/16 inch PTFE tubing (1.0 mm internal diameter).

Contact of two liquid streams was achieved via a simple T-piece, a static T-piece mixer with 0.5 μm polyether ether ketone (PEEK) frit, or with a KombiMix microfluidic chip (cetoni, Germany).

Flow chemistry synthesis of gold nanoparticles

Stage 1 – Synthesis of TOAB-stabilised gold nanoparticles

The flow-controlled synthesis of DMAP-Au nanoparticles was split into three separate stages to reduce significant backpressure in the lines that would occur from one long continuous stage. The first stage comprises the production of TOAB-Au nanoparticles in toluene, the second stage the washing of TOAB-Au nanoparticles, and the final stage the DMAP transfer of TOAB-Au nanoparticles. Solutions were collected in vials at each stage once steady-state concentrations had been reached which was verified by a SPECTROstar Nano UV-Vis spectrometer (BMG LABTECH, Australia).

Four syringe pumps were used with 50 mL glass syringes which were loaded with 25 mM (1% w/v) aqueous gold chloride (syringe 1), 150 mM TOAB in toluene (syringes 2 and 3) and 400 mM sodium borohydride dissolved in 100 mM NaOH (syringe 4). The basic sodium borohydride solution was prepared just prior to use and was glass fibre filtered prior to loading in the syringe. A stream of gold chloride was pumped at 0.5 mL/min into a simple T-piece to contact a stream of TOAB in toluene at a flow rate of 0.5 mL/min. The two solutions were allowed to come together in a 2 m long 1/16 inch PTFE mixing coil (~1.5 min residence time) prior to separation of the organic and aqueous streams with a Zaiput liquid-liquid separator. The aqueous stream was sent to waste and the organic stream retained for downstream processes in a further 0.5 m of tubing. Concurrently, a stream of $NaBH_4$ in NaOH was pumped at 0.5 mL/min into a simple T-piece to contact a stream of TOAB in toluene at a flow rate of 0.5 mL/min. The two solutions were allowed to come together in a 2 m long 1/16 inch PTFE mixing coil (~1.5 min residence time) prior to separation of the organic and aqueous streams with a Zaiput liquid-liquid separator. The aqueous stream was sent to waste and the organic stream retained for downstream processes in a further 0.5 m of tubing.

The two separated organic streams were then allowed to come together at 1.0 mL/min via a static T-piece mixer with a 0.5 μm PEEK frit to produce the TOAB-stabilised gold nanoparticles. The outlet tubing was a 10 m long gas permeable PTFE line (~5.5 min residence time) which allowed the evolved hydrogen gas to diffuse out. At the end of the mixing coil, a glass fibre with 0.2 μm PTFE membrane filter was attached via luer fittings and the TOAB-stabilised gold nanoparticles in toluene were collected into a glass vial.

The pressure upstream of the static T-piece mixer with PEEK frit was monitored using a pressure sensor configured to stop pump operation if the pressure exceeded 4 bar if blockage of the PEEK frit was to occur due to nanoparticle aggregation. The pressure reading was logged throughout the TOAB-Au nanoparticles synthesis stage.

Stage 2 – Washing of TOAB-stabilised gold nanoparticles

The TOAB-stabilised gold nanoparticles in toluene were washed three times with 20% v/v sodium sulfate decahydrate solution. Three lines of sodium sulfate were delivered to various points of the stream of TOAB gold nanoparticles via a peristaltic pump operating at approximately 1 mL/min. The stream of TOAB gold nanoparticles in toluene was operated via a separate peristaltic pump at 1 mL/min. The mixing coils (1/16 inch PTFE tubing) were 2 m long for each washing stage to provide a residence time of ~1 min for each stage. At the end of each washing stage a Zaiput liquid-liquid separator was used to separate the organic and aqueous streams. The aqueous outlet tubing after passing through the first liquid-liquid separator was 4 m long, and after the second liquid-liquid separator was 2 m long in order to balance the pressure on the toluene and aqueous sides for efficient separation. The aqueous streams were sent to waste and the organic streams were

retained for subsequent washing stages or collected in a glass vial after the final washing step. A 20 nm Anotop filter was attached to the end of the line just prior to sample collection.

Stage 3 - Transfer of TOAB-stabilised gold nanoparticles to the aqueous phase using DMAP

Two syringe pumps were used with 50 mL glass syringes containing TOAB-Au nanoparticles in toluene (syringe 5) and aqueous 100 mM DMAP (syringe 6). In one particular process, a stream of TOAB-Au nanoparticles was pumped at 0.15 mL/min and a stream of DMAP was pumped at 0.15 mL/min in which they were mixed in a 13 μL split-and-recombine cetoni KombiMix microfluidic chip (with a channel width of 300 μm, depth of 100 μm). Micromixers based on the concept of splitting and recombination[60] have been successfully used for liquid-liquid extraction to give an effective combination of diffusive and advective mixing. The micromixer splits the fluid into different streams and then recombines them further downstream. At a total flow rate of 0.3 mL/min, rapid and uniform mixing was observed as the micromixer took different layers of the fluid, separated them, and then combined them back together. The design of the micromixer reduced the laminarity of the flow, allowing chaotic advection to take over and reduce the mixing length. A 5 m long 1/16 inch PTFE tubing (~13 min residence time) was connected to the outlet of the microfluidic chip to allow transfer of the gold nanoparticles from the toluene to the aqueous phase to come to completion. A liquid-liquid separator was employed in the final stage to separate the aqueous DMAP-Au nanoparticles from the toluene solution. To investigate the DMAP transfer efficiency, the ratio of TOAB-Au in toluene to aqueous DMAP was varied from 1:1 to 2:1 to 4:1 while maintaining the total flow rate at 0.3 mL/min.

Batch synthesis of gold nanoparticles

DMAP-stabilised gold nanoparticles were synthesised under similar reaction conditions to the flow chemistry process at a smaller scale.

Stage 1 – Synthesis of TOAB-stabilised gold nanoparticles

In a 20 mL glass vial, 8 mL of 25 mM $HAuCl_4$ in water was added to 8 mL of 150 mM TOAB in toluene. After mixing for 5 min on a shaker platform, the organic layer was isolated and labelled as $HAuCl_4$/TOAB. In another 20 mL glass vial, 8 mL of 400 mM $NaBH_4$ in 100 mM NaOH was added to 8 mL of 150 mM TOAB in toluene. After mixing for 5 min on a shaker platform, the organic layer was isolated and labelled as $NaBH_4$/TOAB. Subsequently, equal volumes of the two organic layers were mixed by either (i) adding $HAuCl_4$/TOAB to $NaBH_4$/TOAB, (ii) adding $NaBH_4$/TOAB to $HAuCl_4$/TOAB or (iii) simultaneously adding $HAuCl_4$/TOAB and $NaBH_4$/TOAB into a glass vial. The organic nanoparticle solution was mixed on a shaker platform for 10 min.

Stage 2 – Washing of TOAB-stabilised gold nanoparticles

The TOAB-stabilised gold nanoparticles were filtered using a glass fibre + 0.2 μm PTFE membrane. Then 6 mL of the nanoparticle solution was pipetted into a glass vial. Subsequently, 6 mL of 20% w/v sodium sulfate decahydrate solution was added to the nanoparticle solution and the vial was capped and the solution was shaken. The cap was loosened to release any evolved gas before the shaking was continued for a further one minute. The solution was then allowed to stand for separation of the layers. The aqueous layer was removed with a pipette and discarded. The washing procedure proceeded a further two times. Following the last washing step, the organic nanoparticle layer was carefully isolated and filtered using a 20 nm Anotop filter.

Stage 3 - Transfer of TOAB-stabilised gold nanoparticles to the aqueous phase using DMAP

The washed TOAB-stabilised gold nanoparticles was then mixed with a half volume equivalent of 100 mM DMAP. The two solutions were added simultaneously into a glass vial and placed on a shaker platform for 15 min. Following mixing, the DMAP-stabilised gold nanoparticles in aqueous solution were isolated.

Determination of DMAP concentration by UV-Vis spectroscopy

The total concentration of DMAP in the DMAP-Au nanoparticle aqueous solution (nanoparticle-bound and free) was determined by UV-Vis. First, DMAP-Au nanoparticles (30 μL) were cross-linked with 10 mM cysteamine (30 μL) in 1440 μL water to release the bound DMAP and to precipitate the nanoparticles. After shaking for 10 min on a Thermo Comfort Mixer (350 rpm) the solution was centrifuged at 14 krpm for 2 min and the supernatant was collected.

For UV-Vis measurements, the supernatant was diluted 10-fold in a 1 cm path length quartz cuvette (Hellma™ from Sigma Aldrich, Australia) and the sample was scanned between 220 nm – 900 nm. The OD at the DMAP absorption maximum of 281 nm was recorded. The concentration of DMAP in the original DMAP-AuNP solution was determined from a calibration curve of known DMAP concentrations.

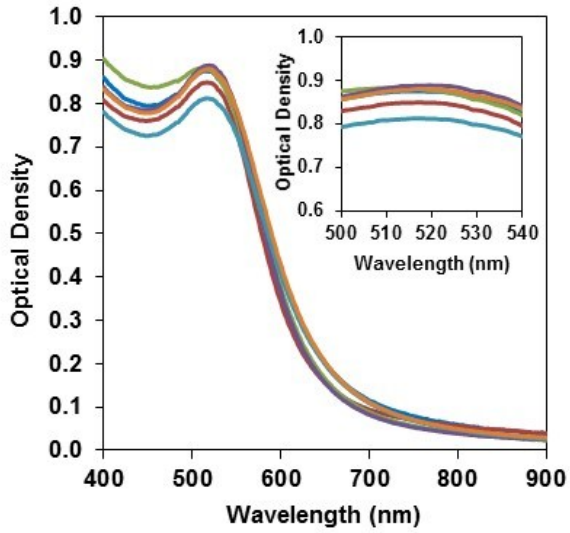

Figure S1. UV-Vis spectroscopy of replicate batches of TOAB-stabilised gold nanoparticles synthesised via the flow chemistry method. The average surface plasmon resonance band was centred at 517 nm ± 2 nm with optical density of 0.86 ± 0.03.

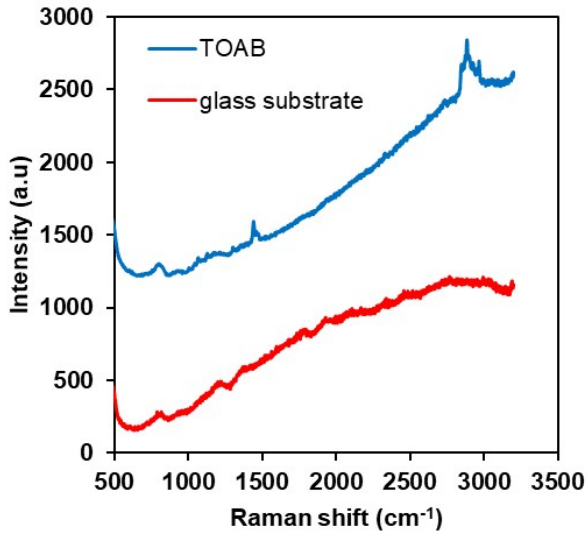

Figure S2. Raman spectra of TOAB and of the glass substrate upon which the gold nanoparticles are deposited on. The glass substrate is coated with bovine serum albumin.

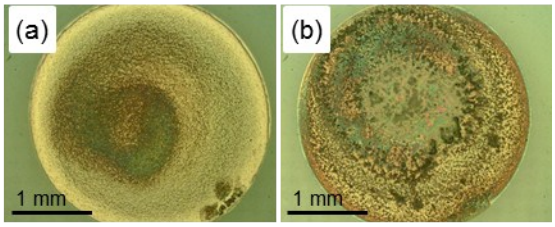

Figure S3. Images (Leica digital microscope) of the gold nanoparticle films (DMAP-Au) deposited on a glass substrate (a) with and (b) without washing using Na_2SO_4 solution.

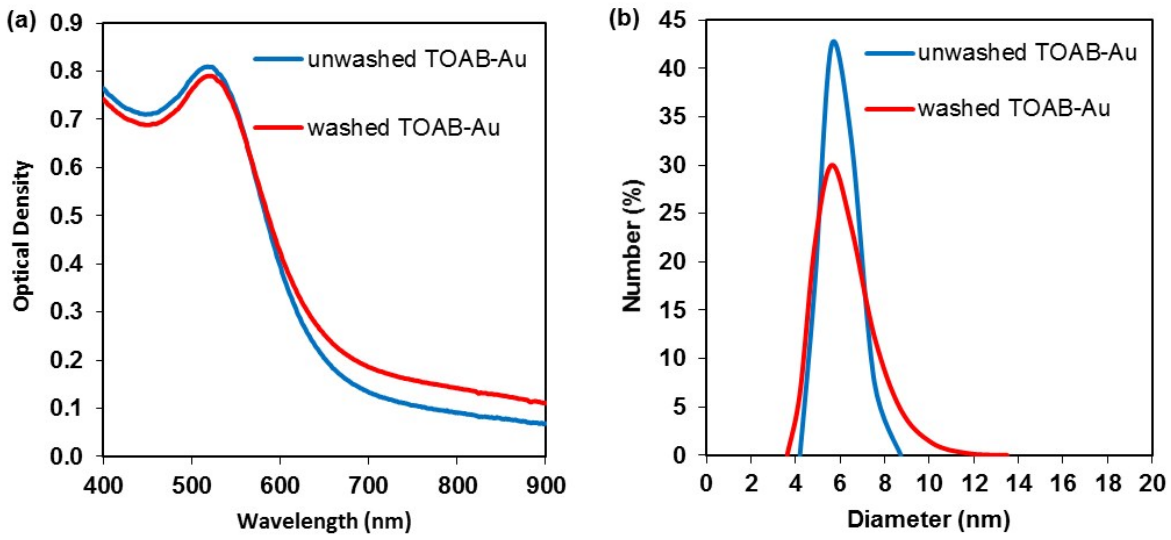

Figure S4. Comparison of unwashed and washed TOAB-stabilised gold nanoparticles synthesised via the flow chemistry method as determined by (a) UV-Vis spectroscopy and (b) dynamic light scattering.

Table S1. Calculated distribution of 100 mM DMAP between toluene and water (pH 10.5)[1]

Parts of toluene (TOAB-AuNP) to water (100 mM DMAP)	**1:1**	**2:1**	**4:1**
Ratio of water: toluene	0.43	0.21	0.11
Distribution of DMAP in toluene – theoretical (mM)	70	82	90
Distribution of DMAP in water – theoretical (mM)	30	18	10

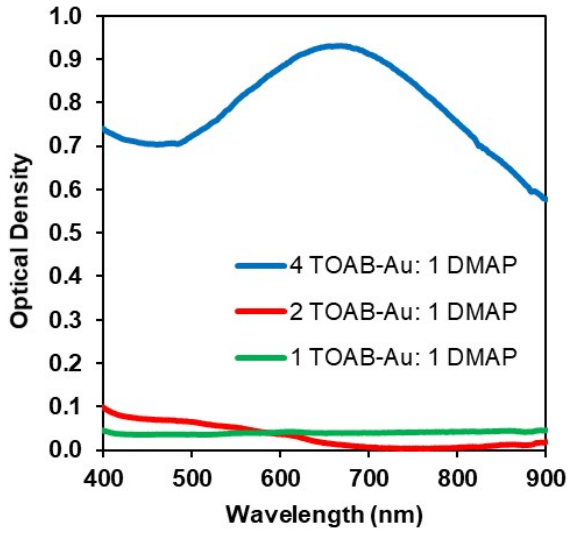

Figure S5. UV-Vis spectra (neat solution) of toluene layer after the transfer of Au nanoparticles. Transfer occurred from a toluene: water ratio of 1:1, 2:1 and 4:1 resulting in DMAP-Au nanoparticles of concentration 0.25% w/v, 0.5% w/v and 1.0% w/v, respectively.

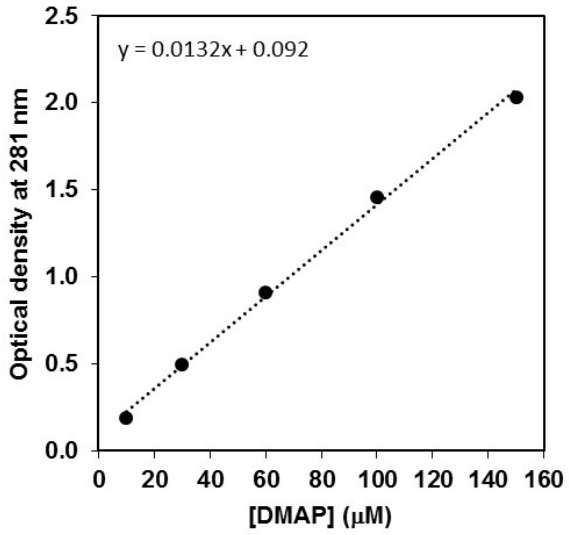

Figure S6. Calibration curve for DMAP in water. The original concentration of DMAP was determined from the best fit equation (y = 0.0132x + 0.092), and by taking into account the dilution factor in the cuvette and during the sample preparation (overall 500 times dilution).

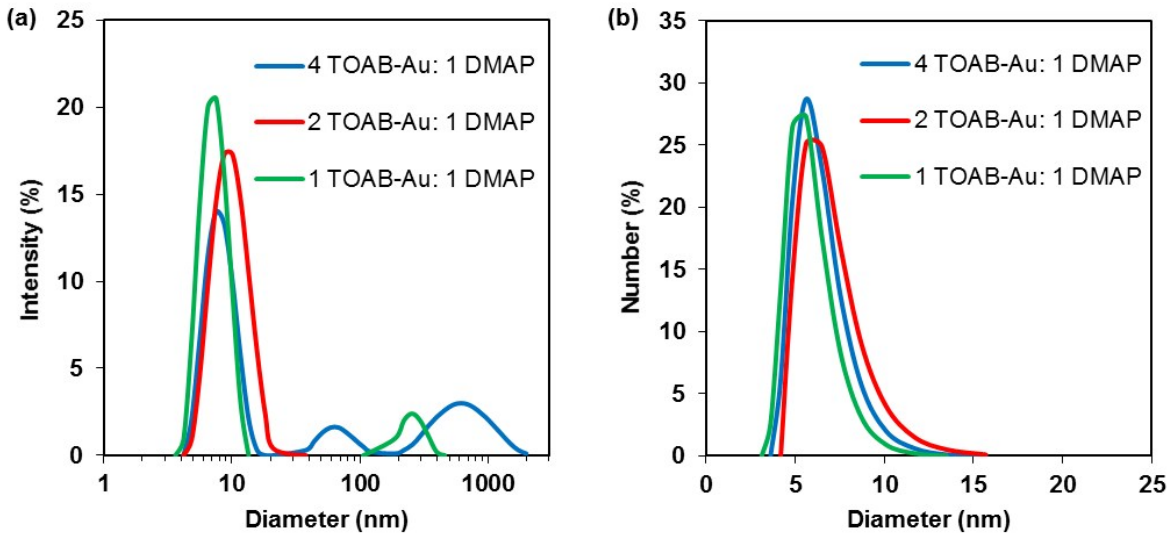

Figure S7. Particle size distribution of DMAP-stabilised gold nanoparticles as determined by dynamic light scattering. The gold nanoparticles were formed by transferring TOAB-stabilised gold nanoparticles to 100 mM DMAP at different ratios. (a) Intensity distribution (raw data) and (b) number distribution (calculated from HPPS software).

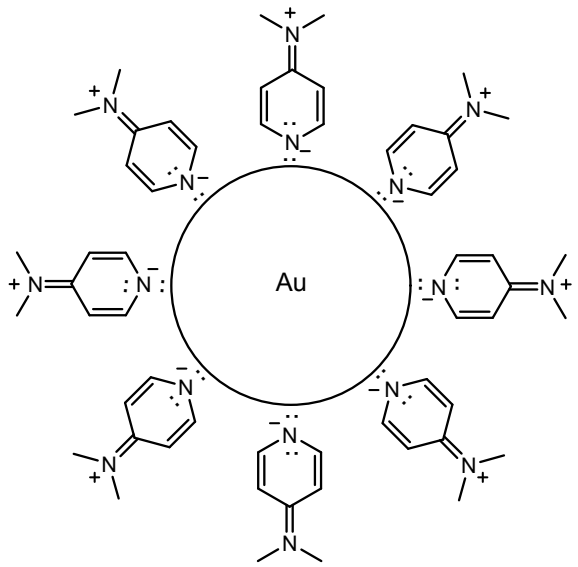

Figure S8. DMAP adopting a vertical orientation on a gold nanoparticle surface. DMAP is adsorbed onto gold *via* the endocyclic nitrogen.

Discussion on yield of gold nanoparticles, automation, scale-up

Gold nanoparticles can be synthesised in high yield and minimal nanoparticle aggregation using flow-controlled methods. Any loss in gold nanoparticles is due to achieving steady-state conditions and residual gold nanoparticles left in the lines when the syringe pumps come to a stop.

Once-steady state concentrations of the gold nanoparticles have been reached, the yield of gold nanoparticles is over 99% as determined by evaporating the solvent and weighing the resulting gold nanoparticle powder. The main limitation to achieving steady state concentrations rapidly is the 400 μL dead volume in the organic side of the Zaiput liquid-liquid separators. The dead volume holds the organic solvent, toluene. The residence time is approximately three times the dead volume, so at a flow rate of 0.5 mL/min, steady-state concentrations can be achieved after 3.75 min. Thus, upon commencing stage one of the synthesis, the first appearance of TOAB-Au nanoparticles at the end of the lines was after 8 minutes, and steady-state concentrations were not achieved until after 12 min (as verified by UV-Vis). The time to reach steady-state concentration can be reduced by using an alternate Zaiput liquid-liquid separator with a smaller dead volume. Alternatively, the separators and tubing can be purged with air prior to beginning the synthesis.

Syringe pumps were used to drive the fluid delivery for stages 1 and 2. When the solutions were exhausted, the pumps were stopped, and the gold nanoparticles remaining in the lines were cleaned out and discarded. The gold nanoparticles in the lines could be recovered, however stopping the pumps and refilling with solvent disrupts the flow in the line. Thus, for consistency reasons, the gold nanoparticles were discarded. An alternative is to use two syringe pumps per reagent which are programmed (Qmix ELEMENTS, cetoni) to provide continuous flow without significant pressure drop between switching syringes. The second syringe can be loaded with cleaning solvent that is programmed to dispense just as the first syringe is completely emptied. The syringes used can hold up to 50 mL of solution, and thus with an initial concentration of 1% w/v $HAuCl_4.3H_2O$ (0.5% w/v Au), one expects up to 50 mL of ~0.5% w/v DMAP-Au nanoparticles after a 1:1 mixture with $NaBH_4$/TOAB in toluene and a 2:1 toluene/water transfer to the aqueous phase. In reality, the volume obtained is much lower due to the reasons discussed above.

The synthesis of gold nanoparticles can be scaled up to much larger quantities by implementing dual syringes for continuous flow (refilling and dispensing multiple times) and starting subsequent stages as nanoparticle solutions are being formed in the previous stage.

To achieve gold nanoparticles with high volume yield in an automated manner is possible by implementing dual syringe pumps for continuous flow, and programming selection valves to load solutions to the next stage while sending non-steady state solutions to waste. All these extra modules will contribute to additional cost of the system but have the potential for complex, multi-stage processes to be carried out with minimal user-intervention.

Zhang and Xia[2] provide a more detailed discussion on the high-volume production of nanoparticles which is an important step towards manufacturing.

Discussion on cost

The overall cost in producing gold nanoparticles, in terms of reagents, equipment, time and labour are all important considerations. Although liquid-liquid membrane separators are not low cost, they provide an efficient, automated and controlled means for washing, extraction and phase transfer of chemicals. This ultimately leads to long term cost savings, especially for larger scale productions where processes are well-defined and other factors such as batch-to-batch reproducibility and yield become more important. For the synthesis of new materials, where experimental conditions are being defined and optimised, it is recommended that small-scale batch processes are tested and evaluated.

Precision pumps and in-line monitors (pressure, cameras, UV-Vis etc.) are also costly and the benefits of each need to be carefully weighed. In some situations, precise control of flow rate is not critical, thus the use of peristaltic pumps can be substituted.

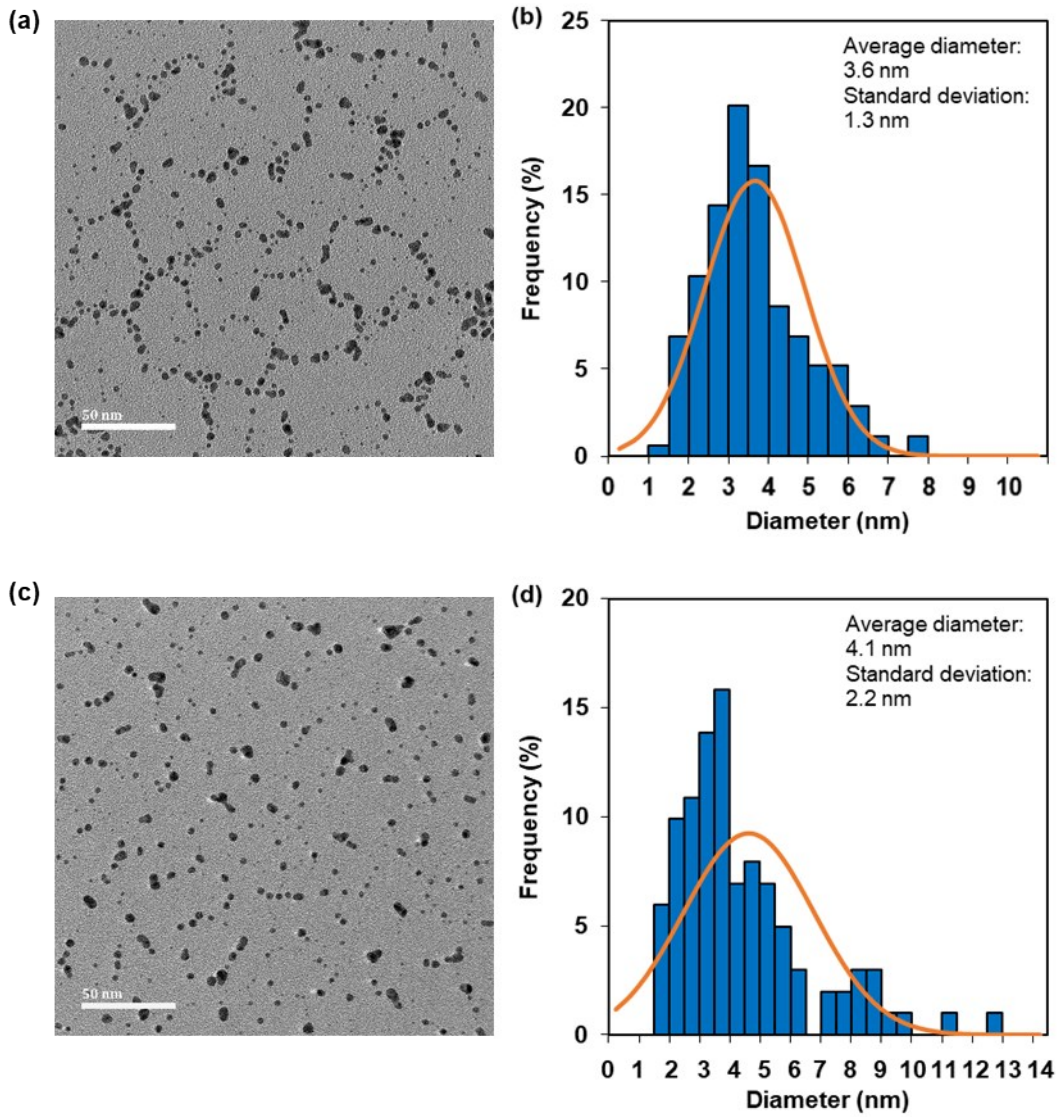

Figure S9. DMAP-stabilised gold nanoparticles prepared from a toluene to water ratio of 2:1. (a,b) In the first stage, $NaBH_4$/TOAB in toluene was added to $HAuCl_4$/TOAB in toluene. (a) TEM image of DMAP-stabilised gold nanoparticles. (b) Particle size distribution of the gold nanoparticles. (c, d) In the first stage, $HAuCl_4$/TOAB in toluene was added to $NaBH_4$/TOAB in toluene. (c) TEM image of DMAP-stabilised gold nanoparticles. (d) Particle size distribution of the gold nanoparticles.

References

[1] V. J. Gandubert and R. B. Lennox, *Langmuir*, 2005, **21**, 6532-6539.
[2] L. Zhang and Y. N. Xia, *Adv Mater*, 2014, **26**, 2600-2606.