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Gold Nanoparticle Synthesis

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TITLE:

Gold Nanoparticle Synthesis

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SUMMARY:

A protocol for synthesizing ~12 nm diameter gold nanoparticles (Au nanoparticles) in an organic solvent is presented. The gold nanoparticles are capped with oleylamine ligands to prevent agglomeration. The gold nanoparticles are soluble in organic solvents such as toluene.

ABSTRACT:

Gold nanoparticles (Au nanoparticles) that are ~12 nm in diameter were synthesized by rapidly injecting a solution of 150 mg (0.15 mmol) of tetrachloroauric acid in 3.0 g (3.7 mmol, 3.6 mL) of oleylamine (technical grade) and 3.0 mL of toluene into a boiling solution of 5.1 g (6.4 mmol, 8.7 mL) of oleylamine in 147 mL of toluene. While boiling and mixing the reaction solution for 2 hours, the color of the reaction mixture changed from clear, to light yellow, to light pink, and then slowly to dark red. The heat was then turned off, and the solution was allowed to gradually cool down to room temperature for 1 hour. The gold nanoparticles were then collected and separated from the solution using a centrifuge and washed three times; by vortexing and dispersing the gold nanoparticles in 10 mL portions of toluene, and then precipitating the gold nanoparticles by adding 40 mL portions of methanol and spinning them in a centrifuge. The solution was then decanted to remove any remaining byproducts and unreacted starting materials. Drying the gold nanoparticles in a vacuum environment produced a solid black pellet; which could be stored for long periods of time (up to one year) for later use, and then redissolved in organic solvents such as toluene.

INTRODUCTION:

Gold nanoparticles are an interesting and useful class of nanomaterials that are the subject of many research studies and applications; such as biology[1], medicine[2], nanotechnology[3], and electronic devices[4]. Scientific research on gold nanoparticles dates back to as early as 1857, when Michael Faraday performed foundational studies on the synthesis and properties of gold nanoparticles[5]. The two primary "bottom up" techniques for synthesizing gold nanoparticles are the citrate reduction method[6,7,8] and the organic two-phase synthesis method[9,10]. The "Turkevich" citrate reduction method produces fairly monodisperse gold nanoparticles under 20 nm in diameter, but the polydispersity increases for gold nanoparticles above 20 nm in diameter; whereas the "Brust-Schiffrin" two-phase method uses sulfur/thiol ligand-stabilization to produce gold nanoparticles up to ~10 nm in diameter[11]. Gold nanoparticle solutions that are pre-synthesized using these methods are commercially available. For applications where large volumes, high monodispersity, and large diameters of gold nanoparticles are not necessary, it may be sufficient to purchase and use these pre-synthesized gold nanoparticles from suppliers. However, gold nanoparticles that are stored in solution, such as many of those that are commercially available, may degrade over time as nanoparticles begin to agglomerate and form clusters. Alternatively, for large-scale applications, long-term projects in which gold nanoparticles need to be used frequently or over a long period of time, or in which there are more stringent requirements for the monodispersity and size of the gold nanoparticles, it may be desirable to perform the gold nanoparticle synthesis oneself. By performing the gold nanoparticle synthesis process, one has the opportunity to potentially control various synthesis parameters such as the amount of gold nanoparticles that are produced, the diameter of the gold nanoparticles, the monodispersity of the gold nanoparticles, and the molecules used as the capping ligands. Furthermore, such gold nanoparticles can be stored as solid pellets in a dry environment, helping to preserve the gold nanoparticles so that they can be used at a later time, up to a year later, with minimal degradation in quality. There is also the potential for cost savings and the reduction of waste by fabricating gold nanoparticles in larger volumes and then storing them in a dry state so that they last longer. Overall, synthesizing gold nanoparticles oneself provides compelling advantages that may not be feasible with commercially available gold nanoparticles.

In order to realize the many advantages that are possible with gold nanoparticle synthesis, a process is presented herein for synthesizing gold nanoparticles. The gold nanoparticle synthesis process that is described is a modified version of a process that was developed by Hiramatsu and Osterloh[12]. Gold nanoparticles are typically synthesized with a diameter of ~12 nm using this synthesis process. The primary chemical reagents that are used to perform the gold nanoparticle synthesis process are tetrachloroauric acid ($HAuCl_4$), oleylamine, and toluene. A nitrogen glovebox is used to provide an inert dry environment for the gold nanoparticle synthesis process, because tetrachloroauric acid is sensitive to water/humidity. The gold nanoparticles are encapsulated with oleylamine ligand molecules to prevent the gold nanoparticles from agglomerating in solution. At the end of the synthesis process, the gold nanoparticles are dried out in a vacuum environment so that they can be stored and preserved in a dry state for later use, up to one year later. When the gold nanoparticles are ready to be used, they can be resuspended into solution in organic solvents such as toluene.

PROTOCOL:

Chemical Amounts:

NOTE: To obtain the appropriate chemical amounts for the nanoparticle synthesis, take the initial amounts found on the "Nanoparticle Synthesis" sheet (on the 2nd page of the supporting information from the Osterloh research article[12]), and multiply the amount of all doses by 3, with some slight modifications. **Table 1** shows the chemical amounts that are needed for the injection solution, boiling solution, washing/purification solutions, and gold etchant solution.

Cleaning and Preparation for Gold Nanoparticle Synthesis Process (Day 1)

NOTE: The following steps can be completed on the first day of the synthesis process.

1. Things to Check and Ensure Before Preparing for the Gold Nanoparticle Synthesis

CAUTION: Ensure that the pre-synthesis cleaning and preparation are performed in the fume hood and acid wet bench while wearing personal protective equipment (PPE) such as nitrile gloves, safety glasses/goggles, and a lab coat while using the fume hood; and while additionally wearing chemical gloves, a chemical gown, a face shield, and goggles while using the acid wet bench.

1.1. Ensure that a nitrogen glove box is available, in which to perform the solvent/reagent preparations and synthesis/chemical reaction process.

NOTE: If a nitrogen glove box is not available, a fume hood can be used instead (possibly with a Schlenk line), although the inert atmosphere in the nitrogen glove box should produce higher quality nanoparticles by preserving the purity of the tetrachloroauric acid ($HAuCl_4$). The gold nanoparticle injection solution that contains the tetrachloroauric acid should be prepared in an inert atmosphere or nitrogen glove box if possible.

1.2. Ensure that a stand with a clamp is located in the nitrogen glove box, to hold and support the condenser tube during the gold nanoparticle synthesis process.

NOTE: This stand with clamp will also allow the condenser tube to be lifted up and suspended over the reaction vessel while the toluene, tetrachloroauric acid and oleylamine solution is injected into the reaction vessel.

1.3. Ensure that the heater with the magnetic stirrer and circular concave receptacle with a fiberglass lining (for holding and supporting the reaction vessel sphere, and for heating the reaction vessel and for rotating the magnetic stirrer bar) is located in the nitrogen glove box.

1.4. Ensure that there are two rubber hoses (for connecting the condenser tube to the water inlet/outlet ports) located inside the nitrogen glove box.

1.5. Ensure that a microbalance that is capable of milligram (mg) resolution is located in the nitrogen glove box.

1.6. Ensure that there are enough chemical reagents and solvents for the cleaning and synthesis process (see **Table 1**).

NOTE: It is best to use fresh/new high-purity (≥99.8%) toluene and methanol which have never been opened or exposed to air/water. It is also best to use fresh/new tetrachloroauric acid ($HAuCl_4$) which is stored in the fridge and never opened until it is transferred to the nitrogen glove box. The tetrachloroauric acid should not be exposed to air or water/humidity at any time, should only be opened in the nitrogen glove box, and should be stored in the nitrogen glove box after opening it in the nitrogen glove box. It is preferable to use new oleylamine, and the oleylamine should also be stored in the nitrogen glove box. Tetrachloroauric acid and oleylamine that are brand new or less than 1 year old should produce better results.

1.7. Ensure that there are plastic bags, XL nitrile gloves, cleanroom wipes, and aluminum foil in the nitrogen glove box.

2. Clean the Chemical Reaction Glassware (Before Gold Nanoparticle Synthesis)

CAUTION: Gold etchant TFA and aqua regia are corrosive. Wear the necessary personal protective equipment (PPE) such as chemical gloves, chemical gown, goggles, and face shield. Only handle the corrosive solution in an acid wet bench while wearing the necessary PPE.

2.1. In the acid wet bench, place the glass reaction vessel with the condenser tube attached to it into a 600 mL beaker for support, and rest the side of the condenser tube against the sidewall of the acid wet bench for further support.

2.2. Clean the chemical reaction glassware (condenser tube, reaction vessel, glass pipette) and magnetic stir bar by pouring ~150 mL of the gold etchant TFA solution and ~150 mL of DI water (1:1 mixture) into the condenser tube and reaction vessel glassware. Place the magnetic stir bar and long glass graduated pipette into the condenser tube and allow the gold etchant TFA bath to sit and clean the glassware for 30 minutes.

NOTE: **Supplementary Figure 1** shows the chemical reaction glassware being cleaned with gold etchant.

2.3. After 30 minutes, separate the glassware to crack the seal between the condenser tube and the reaction vessel to collect all the gold etchant solution into the reaction vessel, and pour the used gold etchant solution into a 400 mL beaker in the acid wet bench.

NOTE: The gold etchant solution will be reused later to clean the chemical reaction glassware after the synthesis process is over.

2.4. Still in the acid wet bench, wash the chemical reaction glassware and magnetic stir bar 3-4 times with DI water to flush out the remaining gold etchant solution, and then allow the chemical reaction glassware and magnetic stir bar to sit in a DI water bath for an additional 30 minutes.

2.5. After 30 minutes of sitting in a DI water bath, empty out the water and use the DI water gun to wash the water down the acid wet bench drain. Blow the glassware dry with the nitrogen gun.

2.6. In the fume hood, clean the chemical reaction glassware (condenser tube, reaction vessel, glass pipette) and magnetic stir bar by rinsing with acetone, methanol, and isopropanol; then blow dry the glassware with nitrogen. Discard the dirty solvents into a flammable waste bottle.

2.7. In the acid wet bench, clean the chemical reaction glassware and magnetic stir bar with DI water, then blow dry the glassware with nitrogen.

2.8. In the fume hood, clean the chemical reaction glassware and magnetic stir bar with toluene, then blow dry the glassware with nitrogen. Discard the dirty toluene solution into a flammable waste bottle.

2.9. Cover the chemical reaction glassware (condenser tube, reaction vessel, glass pipette) and magnetic stir bar with aluminum foil (especially the openings/ports of the glassware) to keep the glassware clean. Poke a couple small holes into the aluminum foil with tweezers, to allow for water to evaporate from the glassware.

3. Clean the Other Glassware and Synthesis Supplies

3.1. In the fume hood, clean the other glassware (e.g., 400 mL glass beaker, 5 mL small graduated glass cylinder, two non-aqueous 20 mL glass vials with PTFE-lined caps), and supplies (e.g., metal spatula/scoopula, tweezers) with acetone, methanol or isopropanol, and DI water; then blow dry the other glassware and supplies with nitrogen. Discard the dirty solvents into a flammable waste bottle.

3.2. If there is any residue visible on the glassware or supplies, wipe them down with a cleanroom wipe or wash with soap and acetone/isopropanol until the residue disappears. Then rinse them with acetone, methanol, and isopropanol solvents again, and then blow the glassware dry with nitrogen.

3.3. In the fume hood, clean the other glassware and supplies with toluene; then blow dry the other glassware and supplies with nitrogen. Discard the dirty toluene solution into a flammable waste bottle.

3.4. In the fume hood, clean the 50 mL conical centrifuge tubes with acetone, methanol or isopropanol, and toluene; then blow dry them with nitrogen.

3.5. Cover the other glassware and supplies with aluminum foil, especially the openings/ports of the glassware, to keep the glassware clean. Poke a couple small holes into the aluminum foil with tweezers, to allow for water to evaporate from the glassware. Ensure that the caps are on the 50 mL centrifuge tubes.

3.6. Clean the rubber pipette bulb with valves by wiping it with a cleanroom wipe with isopropanol, then use the valves to suck up some isopropanol (e.g., while squirting some into it from an isopropanol squeeze bottle) into the bulb and squirt out the isopropanol into a flammable waste bottle. Ensure that there is no residue on the bulb. Blow dry the bulb with nitrogen and cover it with aluminum foil.

NOTE: **Supplementary Figure 2** shows the glassware and supplies after being cleaned.

4. Transfer the Chemicals, Glassware and Supplies into the Nitrogen Glove Box

4.1. Use a fresh pair of XL nitrile gloves over the glove box gloves for handling items and chemicals inside the nitrogen glove box.

4.2. Put the new chemical bottles (toluene and methanol) into the nitrogen glove box (by transferring them into the loadlock and pumping down to remove the ambient air with the vacuum pump, then purging the loadlock with nitrogen). Ensure that there is also a flammable waste bottle for used/dirty toluene in the nitrogen glove box.

4.3. Ensure that the tetrachloroauric acid ($HAuCl_4$) and the oleylamine are also in the nitrogen glove box, where they are stored to prevent exposure to oxygen and water/humidity.

4.4. Place the chemical reaction glassware (condenser tube, reaction vessel, glass pipette), magnetic stir bar, 50 mL conical centrifuge tubes, and other glassware (e.g., 400 mL glass beaker, 5 mL small graduated glass cylinder, two non-aqueous 20 mL glass vials with PTFE-lined caps) and other supplies (e.g., micropipette, new clean micropipette tips in a plastic bag, metal spatula/scoopula, tweezers, valved pipette bulb) in the glove box loadlock. Close the loadlock door, pump down the loadlock to vacuum, leave them under vacuum for 2 minutes, purge the loadlock with nitrogen, and then transfer/place the items inside the nitrogen glove box.

NOTE: Any residual water and solvents should have evaporated in the loadlock while pumping it down to vacuum, before purging the loadlock with nitrogen.

4.5. After transferring the items inside the nitrogen glove box, use another layer of aluminum foil to cover up the items (especially the glassware) that are covered with aluminum foil with holes in the foil, to cover the holes and prevent the items from getting dirty inside the nitrogen glove box.

4.6. Leave the clean items in the nitrogen glove box overnight, with the nitrogen circulating,

to remove and filter out any residual water/moisture/humidity from within the nitrogen glove box.

Gold Nanoparticle Synthesis Process (Day 2)

NOTE: The following steps can be completed on the second day of the synthesis process.

5. Set Up and Clean the Chemical Reaction Glassware & Supplies in the Nitrogen Glove Box

5.1. Begin setting up and cleaning the chemical reaction glassware and supplies in the nitrogen glove box. Inside the nitrogen glove box, place the glass reaction vessel on top of the fiberglass mesh receptacle on top of the heater/stirrer, and place the condenser tube over the glass reaction vessel, supporting the condenser tube with the stand with the clamps.

NOTE: **Supplementary Figure 3** shows the gold nanoparticle synthesis experimental setup.

5.2. Ensure that the magnetic stir bar is inside the glass reaction vessel. Pour ~200 mL of toluene into the glass reaction vessel. Place the glass reaction vessel with ~200 mL of toluene onto the stirring heating mantle and lower the glass condenser tube into the reaction vessel.

5.3. Connect the two hoses inside the nitrogen glove box to the water inlet and outlet ports of the condenser tube.

5.4. Outside the nitrogen glove box, place the end of the water outlet drainage hose into the drainage reservoir/sink in the adjacent fume hood. Use a clamp or tape to hold the hose and keep the hose oriented down into the drain.

5.5. Connect the water supply inlet hose to the water supply line on the adjacent fume hood.

5.6. Slowly turn on and monitor the water to ensure it is gently flowing up through the outer chamber of the condenser tube. Adjust the water flow as necessary by slightly opening/closing the water valve.

5.7. Allow water to flow through the inlet port on the bottom of the condenser tube, up the condenser tube, and out the outlet port on the top of the condenser tube.

5.8. Ensure that there are no large air bubbles in the water supply and ensure that the hoses are mechanically stable.

NOTE: When boiling solutions in the chemical reaction vessel, slowly flow some water from the bottom of the condenser tube, up through the condenser tube vessel outer chamber, to the top of the condenser tube so that water slowly drains out through the drainage hose. This slow but continuous water flow will cool the condenser tube and assist with condensing and recollecting the boiled vapor.

5.9. Ensure that water is gently flowing through the condenser tube to cool it.

5.10. Continuously flow fresh nitrogen into the nitrogen glove box to purge the glove box. Continuously ventilate the nitrogen glove box by pulling a slight vacuum on the nitrogen glove box so that nitrogen and toluene vapor is pumped out of the glove box.

NOTE: Pull a slight vacuum on the nitrogen glove box by slightly opening the equalization valve between the nitrogen glove box and the loadlock while pulling vacuum on the loadlock. Do not fully open the equalization valve or the vacuum level and nitrogen flow will be too high. Flow just enough nitrogen to continuously flush out and ventilate the toluene/chemical vapor in the glove box over time. The vacuum exhaust line should be vented into a fume hood.

5.11. Start heating and stirring the toluene with the magnetic stirrer on the stirring and heating mantle. Allow the toluene to approach a gentle boil. Do not approach or exceed the flash point temperature of toluene; reduce the heat when it starts to boil.

5.12. Allow the toluene to boil and evaporate for 30 minutes with the magnetic stir bar stirring to clean the reaction glassware (reaction vessel and condenser tube).

NOTE: The evaporated toluene will cool and condense in the condenser tube, and drip back down into the reaction vessel.

5.13. After 30 minutes, turn off the heater and magnetic stirrer, and allow the toluene to cool down for several minutes, until the toluene stops evaporating and condensing inside the reaction vessel.

5.14. After the toluene cools down, carefully lift up the condenser tube and suspend it above the reaction vessel by supporting it using the stand with clamps. Make sure to tighten the clamp and support the condenser tube properly, as it could be unstable.

5.15. Pour the toluene from the reaction vessel into the 400 mL glass beaker. Be careful to not accidentally pour out the magnetic stir bar. Place the reaction vessel back on the heating and stirring mantle.

5.16. Swirl the toluene around in the 400 mL glass beaker to clean the beaker. Pour out and discard the dirty/used toluene into the flammable waste bottle. Clean the 400 mL glass beaker again with some fresh toluene, and then discard the used toluene into the flammable waste bottle.

6. Toluene & Oleylamine Boiling Solution Preparation

CAUTION: Oleylamine is toxic and corrosive, so handle it carefully. If handling oleylamine outside the nitrogen glove box, wear the necessary personal protective equipment (PPE) such as chemical gloves, chemical gown, goggles, and face shield. If handling oleylamine inside the nitrogen glove

box, make sure to cover the glove box gloves with new/clean XL nitrile gloves. Be careful to not accidentally spill the oleylamine. Some cleanroom wipes can be put down on the lab bench surface inside the glove box to help absorb any small spills.

6.1. Inside the nitrogen glove box, make a boiling solution of 147 mL (~150 mL) of toluene and 8.7 mL (~9 mL) of oleylamine in the reaction vessel.

6.1.1. Use the 400 mL glass beaker to measure the 147 mL (~150 mL) of toluene. Pour the 147 mL (~150 mL) of toluene from the glass beaker into the reaction vessel.

6.1.2. Use the 5 mL small glass graduated cylinder to carefully measure the 8.7 mL (~9 mL) of oleylamine. First carefully measure and pour 4 mL, and then 4.7 mL, of oleylamine from the small glass graduated cylinder into the reaction vessel.

6.2. Carefully lower the condenser tube down into the glass reaction vessel again.

6.3. Ensure that water is gently flowing through the outer chamber of the condenser tube to cool, condense, and collect the toluene and oleylamine vapor.

6.4. Heat and stir the oleylamine and toluene solution in the reaction vessel and allow the solution to approach a slow/gentle boil (using the stirring and heating mantle, with the magnetic stir bar rotating to mix the solution). Once the oleylamine and toluene solution reaches a gentle boil, turn the heat down a little bit so it is boiling slowly. Do not approach or exceed the flash point of toluene.

7. Tetrachloroauric Acid, Oleylamine & Toluene Injection Solution Preparation

7.1. Begin preparing the injection solution (150 mg tetrachloroauric acid, 3.6 mL oleylamine, 3.0 mL toluene).

7.2. Ensure that the tetrachloroauric acid is fresh or hasn't been exposed to air, water, moisture, or humidity. Remove the laboratory film or seal that is protecting the tetrachloroauric acid from air and moisture.

NOTE: The tetrachloroauric acid is very sensitive to water/moisture/humidity. Every effort should be made to prevent exposing the tetrachloroauric acid powder to air/water. The tetrachloroauric acid comes in a sealed pouch and new container vessels are sealed with wax to prevent water vapor from getting into new vessels. A new batch of tetrachloroauric acid costs ~$100, but it should last a year if not exposed to water vapor. Store new unopened batches of tetrachloroauric acid in the fridge. Transfer a new unopened batch of tetrachloroauric acid to the nitrogen glove box prior to opening it. Only open a new container of tetrachloroauric acid in the nitrogen glove box, when the humidity has reached an appropriately low and stable level (less than 0.8% relative humidity). Store the tetrachloroauric acid in the nitrogen glove box after opening it. After opening the tetrachloroauric acid, wrap laboratory film around the lid of the container to help with sealing

the container and to prevent water vapor and contaminants from getting into the container.

7.3. In the nitrogen glove box, place one of the two non-aqueous 20 mL glass vials with the PTFE-lined caps on the microbalance/scale and remove the PTFE-lined cap.

7.4. Make sure to “re-zero” or “tare” the microbalance with the 20 mL glass vial on the scale before beginning to weigh out the tetrachloroauric acid powder.

7.5. In the nitrogen glove box, use the small metal spatula to deposit tetrachloroauric acid powder from the container into the 20 mL glass vial on the microbalance, to a measured weight of 150 mg of tetrachloroauric acid powder.

7.6. Remove the PTFE-lined cap from the other non-aqueous 20 mL glass vial (the empty one that is not currently on the microbalance).

CAUTION: Oleylamine is toxic and corrosive, so handle it carefully.

7.7. Use the 5 mL small glass graduated cylinder to measure 3.6 mL of oleylamine. Carefully pour the 3.6 mL of oleylamine from the 5 mL small glass graduated cylinder into the 20 mL glass vial without the tetrachloroauric acid.

7.8. Carefully pour and measure 3.0 mL of toluene into the 5 mL small glass graduated cylinder. Carefully pour the 3.0 mL of toluene from the 5 mL small glass graduated cylinder into the 20 mL glass vial with the oleylamine.

NOTE: If too much toluene is poured into the graduated glass cylinder, the excess solvent can be poured into the flammable waste bottle. It is best to use the small 5 mL graduated glass cylinder for measuring the oleylamine and toluene. Be careful to not spill the oleylamine, as it is corrosive and toxic.

7.9. Screw the PTFE-lined cap back onto the 20 mL glass vial with the oleylamine and toluene inside. Shake and swirl the closed glass vial to mix the oleylamine and toluene solution together.

7.10. Open the 20 mL solution glass vial. Carefully pour the ~150 mg of tetrachloroauric acid powder into the glass vial with the oleylamine and toluene solution.

7.11. Screw the PTFE-lined caps back onto the glass vials. Shake and swirl the closed glass vial with the tetrachloroauric acid, oleylamine and toluene to mix the solution together. Keep shaking the solution, and ensure that it is mixed thoroughly.

NOTE: The tetrachloroauric acid, oleylamine and toluene injection solution should turn dark red or purple after shaking and mixing it, as shown in **Supplementary Figure 4**.

8. **Injection of the Tetrachloroauric Acid, Oleylamine & Toluene Solution into the Vessel**

8.1. Ensure that water is slowly flowing into the bottom of the condenser tube, and up out the top of the condenser tube. Adjust the water flow as necessary by carefully opening/closing the water valve.

8.2. Ensure that the oleylamine and toluene solution in the glass reaction vessel is at a gentle boil, with some toluene and oleylamine evaporating into the condenser tube. Ensure that the magnetic stirrer is on.

8.3. Raise the condenser tube above the reaction vessel, using the stand with clamps to support the glassware. Ensure that there is enough room and clearance to inject the tetrachloroauric acid, oleylamine, and toluene solution into the reaction vessel.

8.4. Remove the long graduated glass pipette from the aluminum foil (which was protecting the pipette to keep it clean) and attach the rubber bulb with valves to the pipette. Ensure familiarity with operating the rubber bulb with valves to suck up and squirt out a solution with the long graduated glass pipette before using it.

8.5. Shake the closed 20 mL non-aqueous glass vial with the PTFE-lined cap with the tetrachloroauric acid, oleylamine, and toluene injection solution and ensure it is well-mixed. Open the 20 mL glass vial with the injection solution by removing the cap.

8.6. Press on the upper valve while squeezing the rubber bulb to deflate the rubber bulb. Carefully place the tip of the long graduated glass pipette into the 20 mL glass vial with the tetrachloroauric acid, oleylamine, and toluene injection solution.

8.7. Gently press the lower valve on the rubber bulb connected to the long graduated glass pipette to slowly draw up all of the tetrachloroauric acid, oleylamine, and toluene injection solution into the glass pipette.

NOTE: **Supplementary Figure 5** shows the injection solution being drawn into the long graduated glass pipette with the rubber bulb with valves just before injecting the solution into the reaction vessel. It may be beneficial to practice operating the long graduated glass pipette with the bulb with valves (e.g., with some toluene) before actually drawing up and injecting the tetrachloroauric acid, oleylamine, and toluene solution.

8.8. Carefully place the tip of the glass pipette into the opening of the reaction vessel, and quickly inject the tetrachloroauric acid, oleylamine, and toluene injection solution into the boiling solution of oleylamine and toluene in the reaction vessel.

NOTE: The solution color should initially change from red to yellow to white within about a minute, as gold nanoparticles begin to nucleate and grow.

8.9. Use the clamp on the stand to lower the condenser tube back down into the reaction

vessel.

8.10. Heat the gold nanoparticle chemical reaction solution at a gentle boil for 2 hours.

NOTE: The toluene vapor from the boiling solution should condense in the tube and drip back down into the reaction vessel. Over several minutes, the color of the reaction mixture should then change from white to yellow to light pink and then to red as the gold nanoparticles grow larger. Over the course of 1-2 hours, the color of the reaction mixture should gradually change from light red to deep red/purple.

8.11. After 2 hours of heating the reaction solution, turn off the heater.

NOTE: At this point, the solution can either be allowed to cool down naturally to room temperature, or the solution can be immediately quenched by adding ~100 mL of methanol into the solution. The best-known practice as of now is to allow the solution to cool down naturally rather than quenching the solution right away.

8.12. Allow the solution to cool down naturally to room temperature for 1 hour (recommended); or quench the gold nanoparticle solution immediately with 100 mL of methanol (not recommended).

9. **Quenching the Reaction with Methanol After Cooling the Gold Nanoparticle Solution**

9.1. Ensure that the heater has been turned off, and the solution has cooled down.

9.2. Stop flowing water through the condenser tube. Carefully remove the water drainage hose from the sink/drain in the adjacent fume hood and connect it to the vacuum port in the fume hood.

9.3. Pull vacuum on the drainage hose to suck away the water in the condenser tube and the drainage hose. Carefully remove the condenser tube from the stand with the clamp and lay it horizontally in the nitrogen glove box.

NOTE: The vacuum that is being pulled on the glass condenser tube should evaporate the water within the condenser tube.

9.4. In the nitrogen glove box, pour ~35 mL of methanol into each of the 50 mL conical centrifuge tubes (quantity 12).

NOTE: Methanol will be used to remove unreacted reagents and byproducts from the synthesis process, in order to clean and wash the gold nanoparticles. The 50 mL centrifuge tubes should be held upright in test tube racks.

9.5. Pour the gold nanoparticle solution in equal volumes (~12 mL) into each of the 50 mL

centrifuge tubes (quantity 12) with methanol. Be careful to not accidentally pour out the magnetic stir bar while pouring the gold nanoparticle solution into each centrifuge tube.

NOTE: **Supplementary Figure 6** shows ~12 mL of gold nanoparticle solution being poured into each of the 50 mL conical centrifuge tubes with methanol. After pouring ~12 mL of gold nanoparticle solution into each of the 50 mL conical centrifuge tubes with ~35 mL of methanol, each centrifuge tube should have ~47 mL of solution (slightly below the 50 mL mark).

9.6. Distribute any remaining gold nanoparticle solution evenly between the centrifuge tubes.

9.7. Screw the caps onto the 50 mL centrifuge tubes to close them and tighten the caps.

9.8. Disconnect the inlet and outlet hoses from the glass condenser tube, connect the inlet and outlet hoses together by feeding one into the other, and then wrap the connection of the tubes with laboratory film to seal the connection. Turn off the vacuum that is being pulled on the hoses.

NOTE: The tubes are connected and sealed to prevent water or water vapor from accidentally getting into the nitrogen glove box.

9.9. Remove the 50 mL conical centrifuge tubes with the gold nanoparticle solution and methanol from the nitrogen glove box through the load lock. Also remove the methanol bottle and toluene bottle from the nitrogen glove box. Place them in the adjacent fume hood.

9.10. Also remove the glass reaction vessel, the magnetic stir bar, the glass condenser tube, the long glass graduated pipette, and the rubber bulb with valves from the nitrogen glove box through the load lock. Place them in the adjacent fume hood.

9.11. Label the top of each 50 mL centrifuge tube on the caps with a sample number (e.g., 1, 2, 3, 4, …) to keep track of the different samples.

NOTE: After removing the gold nanoparticle solution and glassware/supplies, the nitrogen glove box should continue to be ventilated for several hours or overnight by flowing fresh nitrogen into the glovebox while pulling a slight vacuum to flush out and ventilate the toluene/oleylamine vapor. The vacuum exhaust line should be vented into a fume hood. The nitrogen glovebox should also be regenerated with regeneration gas to remove moisture/solvents from the filtration system. Some nitrogen gloveboxes may also come with a solvent trap, which helps with removing solvent vapors.

10. **Washing and Purifying the Gold Nanoparticles with Toluene and Methanol**

NOTE: Each 50 mL centrifuge tube with gold nanoparticles will be washed and purified with 10 mL of toluene and 40 mL of methanol 3 times, cleaning the gold nanoparticles in batches of 6 centrifuge tubes at a time. The centrifuge tubes should have an equal amount of gold

nanoparticle solution and should be equally weighted and balanced.

10.1. Place 6 of the 50 mL centrifuge tubes with gold nanoparticle solution into the centrifuge.

10.2. Close the lid of the centrifuge, and enter the following settings for spinning the gold nanoparticles:
RPM: 2328
RCF: 1000
Time: 5 minutes

10.3. Start spinning 6 of the 12 conical centrifuge tubes with the gold nanoparticle solution and methanol in the centrifuge.

10.4. After the first 6 centrifuge tubes with gold nanoparticles are done spinning, gently remove the tubes from the centrifuge. Be careful to not disturb the gold nanoparticle pellets while placing the centrifuge tubes in the tube racks.

NOTE: **Supplementary Figure 7** shows how the gold nanoparticle solution should appear in the 50 mL conical centrifuge tubes after centrifugation. The centrifugal force will pull down the gold nanoparticles in solution and separated them from the methanol and toluene. The gold nanoparticles will precipitate into pellets at the bottom of each centrifuge tube. The supernatant methanol/toluene solution will appear to be clear/transparent above the dark gold nanoparticle pellets, indicating that centrifugation has precipitated the gold nanoparticles from solution.

10.5. Place the last 6 of the 12 conical centrifuge tubes with the gold nanoparticle solution and methanol into the centrifuge. Close the lid of the centrifuge and enter the same centrifuge settings as before. Start spinning the tubes in the centrifuge.

10.6. After the last 6 centrifuge tubes are done spinning, gently remove the tubes from the centrifuge. Be careful to not disturb the gold nanoparticle pellets while placing the centrifuge tubes in the tube racks.

10.7. Carefully carry all of the centrifuge tubes with the gold nanoparticles over to the fume hood and try not to disturb or agitate them during transport.

10.8. Slowly and gently pour out the waste methanol into a flammable waste vessel/beaker. Be careful not disturb and to not pour out or lose the black gold nanoparticle pellets at the bottom of the centrifuge tubes.

NOTE: The first methanol rinse cycle is now complete.

10.9. Begin the second methanol rinse cycle by pouring ~10 mL of fresh toluene into each of the conical centrifuge tubes with black nanoparticle pellets in the fume hood. Screw the caps back on to close the 50 mL centrifuge tubes.

10.10. Vortex each of the 50 mL centrifuge tubes until the black liquid/precipitate/gold nanoparticles are resuspended and dispersed in the 10 mL toluene solution, and the solution looks cloudy/dark. Check the bottom of each centrifuge tube to ensure that most of the black residue (gold nanoparticles) has been resuspended into solution.

NOTE: **Supplementary Figure 8** shows the centrifuge tubes with gold nanoparticle solution and toluene being vortexed and resuspended. Vortexing is much better and gentler on the gold nanoparticles than sonicating the gold nanoparticles. Do not sonicate the gold nanoparticles as sonication could strip off the oleylamine ligands from the gold nanoparticles and cause aggregation and sedimentation of the gold nanoparticles.

NOTE: **Supplementary Figure 9** shows how the gold nanoparticle solution should appear when the gold nanoparticles are resuspended into solution by vortexing each gold nanoparticle pellet with ~10 mL of toluene.

10.11. Pour ~40 mL of fresh methanol into each of the conical centrifuge tubes with toluene and nanoparticles, so that with the 10 mL of toluene that is already in each centrifuge tube, there is a total of ~50 mL of solution in each 50 mL centrifuge tube. Screw the caps back onto the 50 mL centrifuge tubes to close the tubes, and ensure that the caps are on tight.

10.12. Place the centrifuge tubes into the centrifuge. Spin the centrifuge tubes in the centrifuge to collect the gold nanoparticles into a pellet at the bottom of each tube, 6 centrifuge tubes at a time. Use the same centrifuge settings as before (RCF 1000, 5 minutes).

10.13. After the centrifuge stops, gently take out the tubes with the nanoparticles, and then carefully take them to the fume hood (try not to disturb or agitate them during transport). Carefully pour out the waste toluene and methanol into the flammable waste vessel/beaker.

NOTE: The second methanol rinse cycle is now complete.

10.14. For the third and final rinse cycle, follow the same process as before for vortexing in 10 mL of toluene, cleaning in 40 mL of methanol, centrifugation, and carefully pouring out the toluene/methanol solvent. Ensure that the gold nanoparticles in each of the 50 mL centrifuge tubes get resuspended with toluene and washed with methanol 3 times.

11. Drying the Gold Nanoparticles

NOTE: After the gold nanoparticles in the 50 mL centrifuge tube have been washed 3 separate times, and the toluene and methanol has been poured out for the last time, the gold nanoparticles need to be dried to evaporate the remaining solvent. There are two ways to dry the gold nanoparticles and evaporate the solvent:

11.1. Option 1 - Nitrogen Gun (not recommended):

11.1.1. Use a nitrogen gun or valve in the fume hood to gently blow dry the centrifuge tube containing the black pellet of gold nanoparticles at the bottom of the tube.

11.1.2. Take care to not use too much nitrogen pressure, or the fragile gold nanoparticle pellet may get dislodged.

NOTE: Drying the gold nanoparticles with the nitrogen gun is not ideal because it could cause the gold nanoparticle pellets to get damaged/lost.

11.2. Option 2 - Vacuum Drying (recommended):

11.2.1. Loosen the caps on the 50 mL centrifuge tubes with gold nanoparticle pellets so that the tubes are still covered, but solvent can evaporate and escape from inside the tubes.

11.2.2. Place the rack of tubes with gold nanoparticles inside the vacuum load lock of the nitrogen glove box. Close and seal the outer load lock door and open the valve to the vacuum pump to start pulling vacuum on the load lock.

11.2.3. Pump down to about half the gauge pressure (~-15 in.Hg) to evaporate the solvent and dry the nanoparticles.

11.2.4. Leave the gold nanoparticles in the load lock at a moderate vacuum pressure (half-gauge, ~-15 inHg) for ~5 minutes. Do not pump down to a lower pressure and do not leave in vacuum for too long, or the oleylamine ligands may get detached.

11.2.5. After the gold nanoparticles have been under vacuum for a few minutes to dry the gold nanoparticles and evaporate the remaining solvent, purge the load lock with nitrogen until the load lock reaches atmospheric pressure.

11.2.6. Remove the 50 mL centrifuge tubes with gold nanoparticles from the load lock and inspect the dryness of the gold nanoparticle pellets in the fume hood.

NOTE: **Supplementary Figure 10** shows how a dried gold nanoparticle pellet at the bottom of a 50 mL conical centrifuge tube should look after vacuum drying it. If there is still some solvent inside the 50 mL conical centrifuge tube, the gold nanoparticles need to be dried further to evaporate the remaining solvent. Vacuum drying is the preferred method for drying because it is less likely to damage or lose the gold nanoparticle pellet, compared to more aggressive methods such as nitrogen gun drying. If a vacuum load lock is not available, or if preferred, the gold nanoparticles may also be dried in a vacuum desiccator.

11.3. After the gold nanoparticle pellets are dry, screw the caps tightly back onto the centrifuge tubes.

11.4. Wrap laboratory film around the tightly closed caps to seal the centrifuge tubes with the gold nanoparticle pellets inside.

11.5. Label the 50 mL centrifuge tubes with gold nanoparticle precipitate pellets with an appropriately descriptive label, such as “Dried Au NP” and the date (e.g., 9-28-2020).

11.6. Place the sealed centrifuge tubes with dried gold nanoparticle pellets inside a 2 °C – 8 °C fridge. Use a tray or 50 mL conical centrifuge tube racks to hold the tubes upright.

NOTE: **Supplementary Figure 11** shows the centrifuge tubes capped, wrapped with laboratory film, labelled, and stored in a 2 °C – 8 °C fridge. Each centrifuge tube can be stored in the fridge until it is used to make a solution of resuspended gold nanoparticles.

12. Clean the Chemical Reaction Glassware (After Gold Nanoparticle Synthesis)

CAUTION: Gold etchant TFA and aqua regia are corrosive. Wear the necessary personal protective equipment (PPE) such as chemical gloves, chemical gown, goggles, and face shield. Only handle the corrosive solution in an acid wet bench while wearing the necessary PPE.

12.1. In the fume hood, clean the glass reaction vessel with acetone and swirl the acetone around in the glass reaction vessel to wash away the residual gold nanoparticle solution, then dump the dirty acetone into a dirty solvent collection beaker and discard the dirt solvent into a flammable waste bottle.

12.2. In the acid wet bench, place the glass reaction vessel with the condenser tube attached to it into a 600 mL beaker for support, and rest the side of the condenser tube against the sidewall of the acid wet bench for further support.

12.3. Clean the chemical reaction glassware (condenser tube, reaction vessel, glass pipette) and magnetic stir bar by pouring the used ~300 mL gold etchant TFA solution (which was saved earlier and set aside for reuse) that was mixed 1:1 with DI water into the condenser tube and reaction vessel glassware. Place the magnetic stir bar and long glass graduated pipette into the condenser tube. Fill up the condenser tube with DI water as necessary to top it off and allow the gold etchant TFA bath to sit and clean the glassware for 30 minutes.

12.4. After 30 minutes, crack the seal between the condenser tube and the reaction vessel to collect all the gold etchant solution into the reaction vessel, and pour the used gold etchant solution into the 400 mL beaker. Pour the gold etchant solution into the chemical waste bottle for used gold etchant solution.

12.5. Still in the acid wet bench, wash the chemical reaction glassware and magnetic stir bar 3-4 times with DI water to flush out the remaining gold etchant solution, and then allow the chemical reaction glassware and magnetic stir bar to sit in a DI water bath for an additional 30 minutes.

12.6. After 30 minutes of sitting in a DI water bath, empty out the water and use the DI water gun to wash the water down the acid wet bench drain. Rinse with acetone and then blow the glassware dry with the nitrogen gun.

REPRESENTATIVE RESULTS:

Figure 1 shows how the gold nanoparticle synthesis chemical reaction mixture solution (tetrachloroauric acid, oleylamine, and toluene) should gradually change color over the course of several minutes as it initially boils in the reaction vessel; from clear, to light yellow (left image), to light pink (center image), to light red (right image). The changing color of the solution is an indication of the changing size of the gold nanoparticles as they begin to nucleate and grow larger over time. In general, the gold nanoparticle solution should become darker and more red/purple over time as the gold nanoparticles nucleate and grow. **Figure 2** shows the final dark red/purple color of the gold nanoparticle synthesis chemical reaction mixture solution after 2 hours of boiling. The dark red/purple color of the gold nanoparticle solution is characteristic of a concentrated solution of gold nanoparticles that are ~12 nm in diameter. **Figure 3** shows a scanning electron microscope (SEM) image of a gold nanoparticle monolayer (after being deposited onto a silicon substrate) which is used to characterize the size and monodispersity of the gold nanoparticles. The gold nanoparticles should all appear to have roughly the same size/diameter if they are highly monodisperse. If the gold nanoparticles are polydisperse, they will have large variations in their size/diameter. For most applications, monodispersity is usually preferred rather than polydispersity. **Figure 4** shows a scanning electron microscope (SEM) image of gold nanoparticles and their diameter measurements, which indicates a diameter of ~12 nm ± 2 nm for the gold nanoparticles. These gold nanoparticles appear to be fairly monodisperse.

FIGURE AND TABLE LEGENDS:

Table 1: Chemical Amounts

This table shows the amount and type of chemicals that are needed for preparing the injection solution, boiling solution, washing/purification solution, and gold etchant solution.

Supplementary Figure 1: Cleaning Chemical Reaction Glassware with Gold Etchant TFA Solution

This figure shows the chemical reaction glassware (condenser tube, reaction vessel, glass pipette) and magnetic stir bar being cleaned with a ~300 mL mixture of ~150 mL of the gold etchant TFA solution and ~150 mL of DI water (1:1 mixture) in the condenser tube and reaction vessel glassware. The magnetic stir bar and long glass graduated pipette are placed into the condenser tube, and the gold etchant TFA bath is left to sit and clean the glassware for 30 minutes in the acid wet bench.

Supplementary Figure 2: Clean Glassware and Supplies Before Being Transferred into Nitrogen Glove Box

This figure shows the glassware and supplies after being cleaned and dried. The glassware and supplies are wrapped/covered with aluminum foil to protect them from dirt/debris before they are transferred into the nitrogen glove box.

Supplementary Figure 3: Gold Nanoparticle Synthesis Experimental Setup in Nitrogen Glove Box

This figure shows the gold nanoparticle synthesis experimental setup in the nitrogen glove box. The glass reaction vessel is resting on top of the fiberglass mesh receptacle on top of the heater/stirrer, and the condenser tube is connected on top of the glass reaction vessel. The condenser tube is mechanically supported by the stand with the clamp. There are two hoses connected to the water inlet and outlet ports of the condenser tube (with the inlet port on the bottom of the tube, and the outlet port on the top of the tube) so that water flows from the bottom of the condenser tube to the top of the condenser tube, cooling the tube off and condensing the vapor inside.

Supplementary Figure 4: Mixing Tetrachloroauric Acid, Oleylamine, and Toluene Solution Before Injection

This figure shows the tetrachloroauric acid, oleylamine, and toluene injection solution after being mixed in a non-aqueous solution 20 mL glass vial with a PTFE-lined cap. The injection solution should look dark red or purple after shaking and mixing it.

Supplementary Figure 5: Preparing to Inject Solution into Reaction Vessel Using Glass Pipette

This figure shows the tetrachloroauric acid, oleylamine, and toluene injection solution being drawn into the long graduated glass pipette with the rubber bulb with valves, just before quickly injecting the solution with one fast squirt into the boiling solution of oleylamine and toluene in the glass reaction vessel.

Supplementary Figure 6: Pouring ~12 mL of Gold Nanoparticle Solution into Each 50 mL Conical Centrifuge Tube

This figure shows ~12 mL of gold nanoparticle solution being poured evenly into each of the 50 mL conical centrifuge tubes with ~35 mL of methanol in each tube. Methanol is used to remove unreacted starting materials and byproducts, in order to clean and wash the gold nanoparticles.

Supplementary Figure 7: 50 mL Centrifuge Tubes after Centrifugation, with Gold Nanoparticle Pellets at the Bottom

This figure shows how the gold nanoparticle solution should appear in the 50 mL conical centrifuge tubes after centrifugation, with the gold nanoparticles collected into dark gold nanoparticle pellets at the bottom of each centrifuge tube. Above the dark gold nanoparticle pellets, the supernatant methanol/toluene solution appears to be clear/transparent, indicating that centrifugation has precipitated the gold nanoparticles from solution.

Supplementary Figure 8: Vortexing 50 mL Centrifuge Tubes with Au NPs After Filling with ~10 mL of Toluene

This figure shows the centrifuge tubes with gold nanoparticle solution and toluene being vortexed and resuspended. Vortexing is much better and gentler on the gold nanoparticles than sonicating the gold nanoparticles. The gold nanoparticles should not be sonicated, as sonication could strip off the oleylamine ligands from the gold nanoparticles and cause aggregation and

sedimentation of the gold nanoparticles.

Supplementary Figure 9: Vortex Until Gold Nanoparticle Pellet/Residue is Almost Completely Resuspended

This figure shows how the gold nanoparticle solution should appear when the gold nanoparticles are resuspended into solution by vortexing each gold nanoparticle pellet with ~10 mL of toluene. The 50 mL centrifuge tubes should be vortexed until the black liquid/precipitate/gold nanoparticles are resuspended and dispersed in the toluene, and the solution looks cloudy/dark. The bottom of the centrifuge tube should be checked to ensure that virtually all or most of the black nanoparticle residue has been resuspended into solution.

Supplementary Figure 10: Dried Gold Nanoparticle Pellet in 50 mL Conical Centrifuge Tube

This figure shows how a dried gold nanoparticle pellet at the bottom of a 50 mL conical centrifuge tube should look, after vacuum drying it. After the gold nanoparticles in the 50 mL centrifuge tube have been washed 3 separate times, and the toluene and methanol has been poured out for the last time, the gold nanoparticles need to be dried to evaporate the remaining solvent. Vacuum drying is the preferred method for drying because it is less likely to damage or lose the gold nanoparticle pellet, compared to more aggressive methods such as nitrogen gun drying.

Supplementary Figure 11: Cap Tubes, Wrap with Laboratory Film, Label Tubes, and Store in 2 °C – 8 °C Fridge

This figure shows the centrifuge tubes capped, wrapped with laboratory film, labelled, and stored in a 2 °C – 8 °C fridge. The 50 mL centrifuge tubes with gold nanoparticle precipitate pellets should be labelled with an appropriately descriptive label, such as the name, sample number and date. A tray or 50 mL conical centrifuge tube racks can be used to hold the tubes upright in the fridge.

Figure 1: Gold Nanoparticle Solution Changing Colors Over Several Minutes After Injection

This figure shows how the gold nanoparticle synthesis chemical reaction mixture solution (tetrachloroauric acid, oleylamine, and toluene) should gradually change color over the course of several minutes as it initially boils in the reaction vessel; from clear, to light yellow (left image), to light pink (center image), to light red (right image). The changing color of the solution is an indication of the changing size of the gold nanoparticles as they begin to nucleate and grow larger over time.

Figure 2: Gold Nanoparticle Solution is Dark Red/Purple After 2 Hours of Boiling

This figure shows the final dark red/purple color of the gold nanoparticle synthesis chemical reaction mixture solution after 2 hours of boiling in the reaction vessel. The dark red/purple color of the gold nanoparticle solution is characteristic of a concentrated solution of gold nanoparticles that are ~12 nm in diameter.

Figure 3: Scanning Electron Microscope (SEM) Image of Gold Nanoparticle Monolayer

This figure shows a scanning electron microscope (SEM) image of a gold nanoparticle monolayer (after being deposited onto a silicon substrate) which is used to characterize the size and monodispersity of the gold nanoparticles.

Figure 4: Scanning Electron Microscope (SEM) Image with Gold Nanoparticle Diameter Measurements

This figure shows a scanning electron microscope (SEM) image of gold nanoparticles and their diameter measurements, which indicates a diameter of ~12 nm +/- 2 nm for the gold nanoparticles.

DISCUSSION:

Performing the gold nanoparticle synthesis protocol as presented above should produce gold nanoparticles with ~12 nm diameter and fairly high monodispersity (± 2 nm). However, there are some critical steps and process parameters that can be adjusted to potentially change the size/diameter and monodispersity/polydispersity of the gold nanoparticles. For example, after injecting the precursor solution into the reaction vessel and allowing the tetrachloroauric acid, oleylamine, and toluene solution to boil for two hours, there is an option to either do immediate quenching of the reaction solution or to do delayed quenching and natural cooling. If immediate quenching is desired, just after the 2-hour heated reaction step is complete, 100 mL of methanol is added to the reaction vessel to precipitate the gold nanoparticles product. Immediate quenching may provide better dispersion relationships because the nucleation occurs at roughly the same time for all nanoparticles in the saturated solution; whereas the longer the solution remains unquenched, the larger but more randomized the size of the nanoparticles become. If delayed quenching and natural cooling is instead desired, then after the 2-hour heated reaction step is complete, the solution is allowed to cool down naturally to room temperature for 1 hour. Alternatively, the solution could be left to cool even longer, until the following day (e.g., wait overnight) before 100 mL of methanol is added to precipitate the gold nanoparticles product. Researchers may want to experiment with both immediate quenching and delayed quenching, and 1 hour delayed quenching vs. overnight delayed quenching to determine which method produces the best results for making large and highly monodisperse gold nanoparticles. One hour delayed quenching is the procedure that is currently recommended to produce gold nanoparticles that are highly monodisperse, but it has not yet been determined which procedure yields superior results, so some further experimental investigations may be beneficial.

Another critical step in the protocol that affects the monodispersity of the gold nanoparticles is rapid injection of the precursor, to allow the saturated solution to form as many nuclei as possible over a very short time interval. Shortly after the precursor injection, few new nuclei form, and gold atoms should only join existing nuclei. What is necessary for high monodispersity is a long, consistent growth period relative to the nucleation period. A high growth:nucleation time ratio should benefit monodispersity. On this account, injecting the precursor solution very quickly is important for high monodispersity, and waiting to quench the reaction (delayed quenching) may also be beneficial for increasing the monodispersity. However, the competing mechanism of Ostwald ripening[13] is a driving factor for polydispersity. The surface energy of gold atoms on the surface of small nanoparticles is higher than the surface energy of gold atoms on the surface of large nanoparticles. Ostwald ripening is a thermodynamic driving force for the shrinking of small nanoparticles and the growing of large ones[14]. This is a phenomenon that can happen over time in solution.

Another variable to consider is the stability of the oleylamine ligand layer on the gold nanoparticles, and how well passivated the gold nanoparticle surfaces are by the oleylamine ligands. Although there is no indicator for the progression of the surface passivation at different points in the gold nanoparticle synthesis reaction, one can imagine how the surface passivation must evolve over time. At the beginning of the reaction, there are no gold nanoparticles, and oleylamine is actually acting as a reducing agent, to free the gold from its chlorine bonds. At the end of the reaction, the gold nanoparticle surfaces should be completely passivated. Ideally, the reaction should be allowed to continue long enough to allow the surfaces of the gold nanoparticles to become completely passivated, but not so long that Ostwald ripening begins to make the gold nanoparticles polydisperse rather than monodisperse.

Overall, the things to consider when performing the quenching of the reaction are the growth:nucleation time ratio, minimizing Ostwald ripening time, and allowing sufficient time for surface passivation. It has not yet been proven whether delayed quenching or instantaneous quenching produces superior results (i.e., large, highly passivated, and highly monodisperse gold nanoparticles). However, slightly delayed quenching (e.g., allowing the solution to cool down to room temperature for 1 hour after boiling) can produce highly monodisperse gold nanoparticles, so some finite delay before quenching the reaction is acceptable. To provide more clarity as to whether immediate quenching or delayed quenching is better for producing large and highly monodisperse gold nanoparticles, a useful experiment or modification for troubleshooting of the technique would be to separate the gold nanoparticle synthesis solution into two different batches after boiling and perform the immediate post-reaction quenching in parallel with delayed quenching. The outcome of this experiment/modification may determine whether the nucleation time window is so short that the extra time (either one hour or one night/day later) for cooling is unneeded for growth, and some combination of Ostwald ripening and surface passivation is actually decreasing the monodispersity (or increasing the polydispersity) of the gold nanoparticles during the cooldown/delay before quenching.

The final consideration for this gold nanoparticle synthesis method is how the gold nanoparticles are stored and used. After the synthesis process and the cleaning process, the gold nanoparticles are dried gently, either using a nitrogen gun or under vacuum. It is highly recommended that the gold nanoparticles are dried in a vacuum environment rather than using a nitrogen gun, as the nitrogen gun could dislodge the black pellet of gold nanoparticles and cause it to become lost/contaminated/damaged. Drying the gold nanoparticles in a vacuum environment is much gentler and prevents the gold nanoparticle pellet from getting dislodged or lost. After drying, the gold nanoparticles are then stored in a clean and dry environment (e.g., in laboratory film-sealed capped conical centrifuge tubes) in a 2 °C – 8 °C refrigerator until they are ready to be used. This clean, dry, and cool environment should give the gold nanoparticles a longer shelf-life of approximately one year with minimal degradation. In order to use the gold nanoparticles, they may be resuspended into solutions of organic solvents such as toluene by vortexing the gold nanoparticles in the presence of the organic solvent. The size and concentration of the gold nanoparticles in the toluene solution can then be verified using UV-vis spectra characterization[15] and diluted further with toluene if necessary until the desired concentration of gold nanoparticles

is achieved. One limitation is that the concentration will need to be analyzed for each solution.

The gold nanoparticle synthesis protocol that is presented here is intended to enable the synthesis of gold nanoparticles by non-chemistry experts. The significance of this protocol with respect to existing methods is that it provides the opportunity to control the quantity of nanoparticles that are produced, the size of the nanoparticles, the monodispersity of the nanoparticles, and the ligands that encapsulate the gold nanoparticles. The gold nanoparticles that are synthesized using this process have been used to create nanoelectronic devices for molecular electronics experiments, such as 2D molecule-nanoparticle arrays[16]. In this example, 2D molecule-nanoparticle arrays are formed by depositing 200 µL of the diluted gold nanoparticles in toluene solution into 15 mL conical centrifuge tubes that were partially filled with deionized water. The tubes were left undisturbed for 1 - 3 hours to allow the toluene to evaporate and the gold nanoparticles to form monolayers on the surface of the water. These gold nanoparticle monolayers were then transferred to substrates such as silicon microchips using PDMS stamps, in order to form nanoelectronic devices. The oleylamine ligands on the gold nanoparticles were then exchanged with other molecules in order to change the electronic and thermoelectric properties of the gold nanoparticle-molecule monolayers[17,18]. The gold nanoparticle synthesis protocol that is presented here produces high-quality gold nanoparticles that may be useful for many other gold nanoparticle applications within industry and medicine.

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DISCLOSURES:

The authors have nothing to disclose.

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TITLE:
Gold Nanoparticle Synthesis

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Gold nanoparticle synthesis, gold nanoparticles, Au nanoparticles, chemistry, tetrachloroauric acid, $HAuCl_4$, oleylamine, toluene.

SUMMARY:
A protocol for synthesizing ~12 nm diameter gold nanoparticles (Au nanoparticles) in an organic solvent is presented. The gold nanoparticles are capped with oleylamine ligands to prevent agglomeration. The gold nanoparticles are soluble in organic solvents such as toluene.

ABSTRACT:
Gold nanoparticles (Au nanoparticles) that are ~12 nm in diameter were synthesized by rapidly injecting a solution of 150 mg (0.15 mmol) of tetrachloroauric acid in 3.0 g (3.7 mmol, 3.6 mL) of oleylamine (technical grade, Sigma-Aldrich) and 3.0 mL of toluene into a boiling solution of 5.1 g (6.4 mmol, 8.7 mL) of oleylamine in 147 mL of toluene. While boiling and mixing the reaction solution for 2 hours, the color of the reaction mixture changed from clear, to light yellow, to light pink, and then slowly to dark red. The heat was then turned off, and the solution was allowed to gradually cool down to room temperature for 1 hour. The gold nanoparticles were then collected and separated from the solution using a centrifuge and washed three times; by vortexing and dispersing the gold nanoparticles in 10 mL portions of toluene, and then precipitating the gold nanoparticles by adding 40 mL portions of methanol and spinning them in a centrifuge. The solution was then decanted to remove any remaining byproducts and unreacted starting materials. Drying the gold nanoparticles in a vacuum environment produced a solid black pellet; which could be stored for long periods of time (up to one year) for later use, and then redissolved in organic solvents such as toluene.

INTRODUCTION:
Gold nanoparticles are an interesting and useful class of nanomaterials that are the subject of many research studies and applications; such as biology[1], medicine[2], nanotechnology[3], and electronic devices[4]. Scientific research on gold nanoparticles dates back to as early as 1857, when

Michael Faraday performed foundational studies on the synthesis and properties of gold nanoparticles[5]. The two primary "bottom up" techniques for synthesizing gold nanoparticles are the citrate reduction method[6,7,8] and the organic two-phase synthesis method[9,10]. The "Turkevich" citrate reduction method produces fairly monodisperse gold nanoparticles under 20 nm in diameter, but the polydispersity increases for gold nanoparticles above 20 nm in diameter; whereas the "Brust-Schiffrin" two-phase method uses sulfur/thiol ligand-stabilization to produce gold nanoparticles up to ~10 nm in diameter[11]. Gold nanoparticle solutions that are pre-synthesized using these methods are commercially available. For applications where large volumes, high monodispersity, and large diameters of gold nanoparticles are not necessary, it may be sufficient to purchase and use these pre-synthesized gold nanoparticles from suppliers. However, gold nanoparticles that are stored in solution, such as many of those that are commercially available, may degrade over time as nanoparticles begin to agglomerate and form clusters. Alternatively, for large-scale applications, long-term projects in which gold nanoparticles need to be used frequently or over a long period of time, or in which there are more stringent requirements for the monodispersity and size of the gold nanoparticles, it may be desirable to perform the gold nanoparticle synthesis oneself. By performing the gold nanoparticle synthesis process, one has the opportunity to potentially control various synthesis parameters such as the amount of gold nanoparticles that are produced, the diameter of the gold nanoparticles, the monodispersity of the gold nanoparticles, and the molecules used as the capping ligands. Furthermore, such gold nanoparticles can be stored as solid pellets in a dry environment, helping to preserve the gold nanoparticles so that they can be used at a later time, up to a year later, with minimal degradation in quality. There is also the potential for cost savings and the reduction of waste by fabricating gold nanoparticles in larger volumes and then storing them in a dry state so that they last longer. Overall, synthesizing gold nanoparticles oneself provides compelling advantages that may not be feasible with commercially available gold nanoparticles.

In order to realize the many advantages that are possible with gold nanoparticle synthesis, a process is presented herein for synthesizing gold nanoparticles. The gold nanoparticle synthesis process that is described is a modified version of a process that was developed by Hiramatsu and Osterloh[12]. Gold nanoparticles are typically synthesized with a diameter of ~12 nm using this synthesis process. The primary chemical reagents that are used to perform the gold nanoparticle synthesis process are tetrachloroauric acid ($HAuCl_4$), oleylamine, and toluene. A nitrogen glovebox is used to provide an inert dry environment for the gold nanoparticle synthesis process, because tetrachloroauric acid is sensitive to water/humidity. The gold nanoparticles are encapsulated with oleylamine ligand molecules to prevent the gold nanoparticles from agglomerating in solution. At the end of the synthesis process, the gold nanoparticles are dried out in a vacuum environment so that they can be stored and preserved in a dry state for later use, up to one year later. When the gold nanoparticles are ready to be used, they can be resuspended into solution in organic solvents such as toluene.

PROTOCOL:

Chemical Amounts:

NOTE: To obtain the appropriate chemical amounts for the nanoparticle synthesis, take the initial amounts found on the "Nanoparticle Synthesis" sheet (on the 2nd page of the supporting information from the Osterloh research article[12]), and multiply the amount of all doses by 3, with some slight modifications. Table 1 shows the chemical amounts that are needed for the injection solution, boiling solution, washing/purification solutions, and gold etchant solution.÷

~~1. Injection Solution:~~
- ~~1. 150 mg of tetrachloroauric acid ($HAuCl_4$) (0.15 mmol)~~
- ~~2. 3.0 g (3.7 mmol, 3.6 mL) of oleylamine~~
- ~~3. 3.0 mL of toluene~~

~~2. Boiling Solution:~~
- ~~1. 5.1 g (6.4 mmol, 8.7 mL) of oleylamine~~
- ~~2. 147 mL of toluene~~

~~3. Washing/Purification Solutions:~~
- ~~1. 10 mL of toluene (x3 washes) (x12 tubes) = 360 mL of toluene~~
- ~~2. 40 mL of methanol (x3 washes) (x12 tubes) = 1.44 L of methanol~~

~~4. Gold Etchant Solution (for cleaning chemical reaction glassware/supplies):~~
- ~~1. 150 mL of gold etchant TFA [or aqua regia]~~
- ~~2. 150 mL of deionized (DI) water~~

Cleaning and Preparation for Gold Nanoparticle Synthesis Process (Day 1):

NOTE: The following steps can be completed on the first day of the synthesis process.

1. **Things to Check and Ensure Before Preparing for the Gold Nanoparticle Synthesis:**

   CAUTION: Ensure that~~Perform~~ the pre-synthesis cleaning and preparation are performed in the fume hood and acid wet bench while wearing personal protective equipment (PPE) such as nitrile gloves, safety glasses/goggles, and a lab coat while using the fume hood; and while additionally wearing chemical gloves, a chemical gown, a face shield, and goggles while using the acid wet bench.

   ~~1.1.~~

   1.1. Ensure that~~Perform~~ a nitrogen glove box is available, in which to perform the solvent/reagent preparations and synthesis/chemical reaction process~~ in the nitrogen glove box~~.

   NOTE: If a nitrogen glove box is not available, a fume hood can be used instead (possibly with a Schlenk line), although the inert atmosphere in the nitrogen glove box should produce higher quality nanoparticles by preserving the purity of the tetrachloroauric acid ($HAuCl_4$). The gold nanoparticle injection solution that contains the tetrachloroauric acid should be prepared in an inert atmosphere or nitrogen glove box if possible.

   ~~1.2.~~

   1.2. Ensure that a stand with a clamp is located in the nitrogen glove box, to hold and support

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the condenser tube during the gold nanoparticle synthesis process.

NOTE: ~~-~~This stand with clamp will also allow the condenser tube to be lifted up and suspended over the reaction vessel while the toluene, tetrachloroauric acid and oleylamine solution is injected into the reaction vessel.

~~1.3.~~

1.3. Ensure that the heater with the magnetic stirrer and circular concave receptacle with a fiberglass lining (for holding and supporting the reaction vessel sphere, and for heating the reaction vessel and for rotating the magnetic stirrer bar) is located in the nitrogen glove box.

~~1.4.~~

1.4. Ensure that there are two rubber hoses (for connecting the condenser tube to the water inlet/outlet ports) located inside the nitrogen glove box.

~~1.5.~~

1.5. Ensure that a microbalance that is capable of milligram ~~(~~mg~~)~~ resolution is located in the nitrogen glove box.

~~1.6.~~

1.6. Ensure that there are enough chemical reagents and solvents for the cleaning and synthesis process (see Table 1).

NOTE: ~~-~~It is best to use fresh/new high-purity (≥99.8%) toluene and methanol which have never been opened or exposed to air/water. It is also best to use fresh/new tetrachloroauric acid ($HAuCl_4$) which is stored in the fridge and never opened until it is transferred to the nitrogen glove box. The tetrachloroauric acid should not be exposed to air or water/humidity at any time, should only be opened in the nitrogen glove box, and should be stored in the nitrogen glove box after opening it in the nitrogen glove box. It is preferable to use new oleylamine, and the oleylamine should also be stored in the nitrogen glove box ~~as well~~. Tetrachloroauric acid and oleylamine that are~~is~~ brand new or less than 1 year old should produce better results.

~~1.7.~~

~~1.8.~~1.7. Ensure that there are plastic~~Ziplock~~ bags, XL nitrile gloves, ~~Texwipes~~cleanroom wipes, and aluminum foil in the nitrogen glove box.

2. Clean the Chemical Reaction Glassware (Before Gold Nanoparticle Synthesis):

CAUTION: Gold etchant TFA and aqua regia are corrosive. Wear the necessary personal protective equipment (PPE) such as chemical gloves, chemical gown, goggles, and face shield. Only handle the corrosive solution in an acid wet bench while wearing the necessary PPE.

~~2.1.~~

2.1. In the acid wet bench, place the glass reaction vessel with the condenser tube attached to it into a 600 mL beaker for support, and rest the side of the condenser tube against the sidewall of the acid wet bench for further support.

2.2. ~~-~~Clean the chemical reaction glassware (condenser tube, reaction vessel, glass pipette)

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and magnetic stir bar by pouring ~150 mL of the gold etchant TFA solution and ~150 mL of DI water (1:1 mixture) into the condenser tube and reaction vessel glassware. ~~Then~~ Pplace the magnetic stir bar and long glass graduated pipette into the condenser tube and allow the gold etchant TFA bath to sit and clean the glassware for 30 minutes.

NOTE: Supplementary Figure 1 shows the chemical reaction glassware being cleaned with gold etchant.

2.3. ~~-~~After 30 minutes, separate the glassware to crack the seal between the condenser tube and the reaction vessel to collect all the gold etchant solution into the reaction vessel, and pour the used gold etchant solution into a 400 mL beaker in ~~the corner of~~ the acid wet bench.

NOTE: ~~-~~The gold etchant solution will be reused later to clean the chemical reaction glassware after the synthesis process is over.

~~2.2.~~

2.4. Still in the acid wet bench, wash the chemical reaction glassware and magnetic stir bar 3-4 times with DI water to flush out the remaining gold etchant solution, and then allow the chemical reaction glassware and magnetic stir bar to sit in a DI water bath for an additional 30 minutes.

2.5. ~~-~~After 30 minutes of sitting in a DI water bath, empty out the water and use the DI water gun to wash the water down the acid wet bench drain. Blow the glassware dry with the nitrogen gun.

~~2.3.~~

2.6. In the fume hood, clean the chemical reaction glassware (condenser tube, reaction vessel, glass pipette) and magnetic stir bar by rinsing with acetone, methanol, and isopropanol; then blow dry the glassware with nitrogen. Discard the dirty solvents into a flammable~~s~~ waste bottle.

~~2.4.~~

2.7. In the acid wet bench, clean the chemical reaction glassware and magnetic stir bar with DI water, then blow dry the glassware with nitrogen.

~~2.5.~~

2.8. In the fume hood, clean the chemical reaction glassware and magnetic stir bar with toluene, then blow dry the glassware with nitrogen. Discard the dirty toluene solution into a flammable~~s~~ waste bottle.

~~2.6.~~

~~2.7.~~2.9. Cover the chemical reaction glassware (condenser tube, reaction vessel, glass pipette) and magnetic stir bar with aluminum foil~~,~~ (especially the openings/ports of the glassware)~~,~~ to keep the glassware clean. Poke a couple small holes into the aluminum foil with tweezers, to allow for water to evaporate from the glassware.

3. Clean the Other Glassware and Synthesis ~~Tools~~Supplies~~/Equipment~~:

3.1. In the fume hood, clean the other glassware (e.g., 400 mL glass beaker, 5 mL small

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graduated glass cylinder, two non-aqueous 20 mL glass vials with ~~white~~ PTFE-lined caps), and ~~tools~~ supplies (e.g., metal spatula/scoopula, tweezers) with acetone, methanol or isopropanol, and DI water; then blow dry the other glassware and ~~tools~~ supplies with nitrogen. Discard the dirty solvents into a flammable~~s~~ waste bottle.

3.2. ~~-~~If there is any residue visible on the glassware or supplie~~tools~~s, wipe them down with a cleanroom wipe~~Texwipe~~ or wash with soap and acetone/isopropanol until the residue disappears~~., and~~ T~~t~~hen rinse them with acetone, methanol, and isopropanol solvents again, and then blow the glassware dry with nitrogen.

~~3.1.~~

3.3. In the fume hood, clean the other glassware and supplie~~tools~~s with toluene; then blow dry the other glassware and supplie~~tools~~s with nitrogen. Discard the dirty toluene solution into a flammable~~s~~ waste bottle.

~~3.2.~~

3.4. In the fume hood, clean the 50 mL conical centrifuge tubes with acetone, methanol or isopropanol, and toluene; then blow dry them with nitrogen.

~~3.3.~~

3.5. Cover the other glassware and supplie~~tools~~s with aluminum foil, especially the openings/ports of the glassware, to keep the glassware clean. Poke a couple small holes into the aluminum foil with tweezers, to allow for water to evaporate from the glassware. Ensure that the caps are on the 50 mL centrifuge tubes.

~~3.4.~~

~~3.5. Also C~~c~~lean the rubber pipette bulb with valves by wiping it with a cleanroom wipe~~Texwipe~~ with isopropanol, then use the valves to suck up some isopropanol (e.g., while squirting some into it from an isopropanol squeeze bottle) into the bulb and squirt out the isopropanol into a flammable waste bottle. Ensure that there is no residue on the bulb. ~~Then B~~b~~low dry the bulb with nitrogen and cover it with aluminum foil.

3.6.

NOTE: Supplementary Figure 2 shows the glassware and supplies after being cleaned.

4. Transfer the Chemicals, Glassware and Supplie~~Tools~~s~~/Equipment~~ into the Nitrogen Glove Box:

4.1. Use a fresh pair of XL nitrile gloves over the glove box gloves for handling items and chemicals inside the nitrogen glove box.

~~4.1.~~

4.2. Put the new chemical bottles (toluene and methanol) into the nitrogen glove box (by transferring them into the loadlock and pumping down to remove the ambient air with the vacuum pump, then purging the loadlock with nitrogen). ~~There should already be~~Ensure that there is also a flammable~~s~~ waste bottle for used/dirty toluene in the nitrogen glove box.~~ If not, also place a flammables waste bottle into the nitrogen glove box.~~

~~4.2.~~

4.3. Ensure that t~~T~~he tetrachloroauric acid ($HAuCl_4$) ~~should already be stored in the nitrogen~~

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~~glove box. If not, transfer it into the nitrogen glove box. If~~and the oleylamine ~~is not already~~are also in the nitrogen glove box, ~~transfer it into~~where they ~~the nitrogen glove box as well~~ are stored to prevent exposure to oxygen and water/humidity.

~~4.3.~~

4.4. Place the chemical reaction glassware (condenser tube, reaction vessel, glass pipette), magnetic stir bar, 50 mL conical centrifuge tubes, and other glassware (e.g., 400 mL glass beaker, 5 mL small graduated glass cylinder, two non-aqueous 20 mL glass vials with ~~white~~ PTFE-lined caps) and other ~~tools/equipment/~~supplies (e.g., micropipette, new clean micropipette tips in a ~~Ziplock~~ plastic bag, metal spatula/scoopula, tweezers, valved pipette bulb) in the glove box loadlock. Close the loadlock door, pump down the loadlock to vacuum, leave them under vacuum for 2 minutes, purge the loadlock with nitrogen, and then transfer/place the items inside the nitrogen glove box.

~~4.4.~~

NOTE: Any residual water and solvents should have evaporated in the loadlock while pumping it down to vacuum, before purging the loadlock with nitrogen.

4.5. ~~-~~After transferring the items inside the nitrogen glove box, use another layer of aluminum foil to cover up the items (especially the glassware) that are covered with aluminum foil with holes in the foil, ~~can now be covered up with another layer of aluminum foil~~ to cover the holes and prevent the items~~m~~ from getting dirty inside the nitrogen glove box.

~~4.5.~~

4.6. Leave t~~T~~he clean items ~~should be left~~ in the nitrogen glove box overnight, with the nitrogen circulating, to remove and filter out any residual water/moisture/humidity from within the nitrogen glove box.

Gold Nanoparticle Synthesis Process (Day 2):

NOTE: The following steps can be completed on the second day of the synthesis process.

~~1.~~5. Set Up and Clean the Chemical Reaction Glassware & Supplies ~~Glass Condenser Tube and Glass Reaction~~ in the Nitrogen Glove Box ~~Vessel~~:

5.1. Begin setting up and cleaning the chemical reaction glassware and supplies in the nitrogen glove box. Inside the nitrogen glove box, place the glass reaction vessel on top of the fiberglass mesh receptacle on top of the heater/stirrer, and place the condenser tube over the glass reaction vessel, supporting the condenser tube with the stand with the clamps.

NOTE: Supplementary Figure 3 shows the gold nanoparticle synthesis experimental setup.

5.2. Ensure that the magnetic stir bar is inside the glass reaction vessel. Pour ~200 mL of toluene into the glass reaction vessel. Place the glass reaction vessel with ~200 mL of toluene onto the stirring heating mantle and lower the glass condenser tube into the reaction vessel.

~~1.1.~~

5.3. Connect the two hoses inside the nitrogen glove box to the water inlet and outlet ports

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of the condenser tube ~~(inlet port on the bottom of the tube, outlet port on the top of the tube) so that water flows from the bottom of the condenser tube to the top of the condenser tube, cooling it off and condensing the vapor inside.~~.

~~1.2.~~

5.4. Outside the nitrogen glove box, place the end of the water outlet drainage hose into the drainage reservoir/sink in the adjacent fume hood. ~~You can~~ Uuse a clamp or ~~beaker~~ tape to hold the hose and keep the hose oriented down into the drain.

5.5. ~~-~~Connect the water supply inlet hose to the ~~DI~~ water supply line on the adjacent fume hood.

5.6. Slowly ~~With both the inlet and outlet hoses now connected,~~ turn on and monitor the water to ensure it is gently ~~slowly fill~~ flowing up through the outer chamber of the condenser tube ~~with DI water~~. Adjust the water flow as necessary by slightly opening/closing the water valve.

5.7. Allow water to flow through the inlet port on the bottom of the condenser tube, up the condenser tube, and out the outlet port on the top of the condenser tube.

5.8. ~~-~~Ensure that t~~T~~here ~~should~~ are no~~t be any~~ large air bubbles in the water supply~~,~~ and ensure that the hoses are~~should be~~ mechanically stable. ~~Slowly fill the water until it reaches the top and drains through the drainage hose.~~

NOTE: ~~–~~When ~~performing the nanoparticle synthesis~~boiling solutions in the chemical reaction vessel, slowly ~~you should~~ flow ~~a small amount of~~some water from the bottom of the condenser tube, ~~through~~ up through the condenser tube vessel outer chamber, to the top of the condenser tube so that water slowly drains out through the drainage hose. This slow but continuous water flow will ~~to~~ cool the condenser tube and assist with condensing ~~the toluene~~and recollecting the boiled vapor.

~~1.3.~~

5.9. Ensure that water is gently flowing through the condenser tube to cool it.

5.10. ~~Ensure that some fresh nitrogen gas is C~~continuously flow fresh nitrogen~~ing from the house nitrogen supply~~ into the nitrogen glove box to purge the glove box. Continuously ventilate the nitrogen glove box by pulling~~by pulling~~ a slight vacuum ~~-~~on the nitrogen glove box ~~by slightly opening the equalization valve between the nitrogen glove box and the loadlock while pulling vacuum on the loadlock~~so that nitrogen and toluene vapor is pumped out of the glove box.

~~1.4.~~ NOTE: Pull a slight vacuum on the nitrogen glove box by slightly opening the equalization valve between the nitrogen glove box and the loadlock while pulling vacuum on the loadlock. Do not fully open the equalization valve or the vacuum level and nitrogen flow will be too high. ~~You want~~Flow just enough nitrogen ~~flowing~~ to continuously flush out and ventilate ~~some of~~ the toluene/chemical vapor in the glove box

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over time. The vacuum exhaust line should be vented into a fume hood.

~~Inside the nitrogen glove box, with the DI water gently flowing through the condenser tube to cool it, fill the glass reaction vessel with some toluene (~200 mL) and reattach the glass condenser tube to the reaction vessel.~~

5.11. Start heating and stirring the toluene with the magnetic stirrer on the stirring and heating mantle. Allow the toluene to approach a gentle boil. D~~but d~~o not approach or exceed the flash point temperature of toluene;~~. Reach a slow/gentle boil and then~~ reduce the heat when it starts to boil.

5.12. Allow the toluene to ~~b~~~~Boil the toluene for 20~~oil and evaporate for 30 minutes with the magnetic stir bar stirring to clean the reaction glassware (reaction vessel and condenser tube).~~, but do not exceed the flash point temperature. Reach a slow/gentle boil and then reduce the heat.~~

NOTE: The evaporated toluene will cool and condense in the condenser tube, and drip back down into the reaction vessel.

~~1.5.~~

5.13. After ~~20~~ 30 minutes, turn off the heater and magnetic stirrer, and allow the toluene to cool down for several minutes, until the toluene~~and~~ stops evaporating and ~~/~~condensing inside the reaction vessel.

5.14. After the toluene cools down, ~~c~~Carefully lift up the condenser tube and suspend it above the reaction vessel by supporting it ~~with~~ using the stand with~~and~~ clamps. Make sure to tighten the clamp and support the condenser tube properly, as it could be unstable.

~~1.6.~~

5.15. Pour the toluene from the reaction vessel into the 400 mL glass beaker. Be careful to not accidentally pour out the magnetic stir bar. Place the reaction vessel back on the heating and stirring mantle.

~~1.7.~~5.16. Swirl the toluene around in the 400 mL glass beaker ~~once again, and then~~ Pour out and d~~d~~iscard the dirty/used~~/dirty~~ toluene into the flammable~~s~~ waste bottle. Clean the 400 mL glass beaker again with some fresh toluene, and then discard the used toluene into the flammable waste bottle.

~~2.~~6. ~~Oleylamine &~~ Toluene & Oleylamine Boiling Solution Preparation:

CAUTION: Oleylamine is toxic and corrosive, so handle it carefully. If handling oleylamine outside the nitrogen glove box, wear the necessary personal protective equipment (PPE) such as chemical gloves, chemical gown, goggles, and face shield. If handling oleylamine inside the nitrogen glove box, make sure to cover the glove box gloves with new/clean XL nitrile gloves. Be careful to not accidentally spill the oleylamine. Some cleanroom wipes~~Texwipes~~ can be put down on the lab bench surface inside the glove box to help absorb any small spills.

~~2.1.~~

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6.1. Inside the nitrogen glove box, make a boiling solution of ~~fill the reaction vessel with the appropriate amount o~~147 mL~~f toluene~~ (~~150~~147 mL) of toluene and ~~oleylamine~~ 8.7 mL (~~9~~8.7 mL) of oleylamine in the reaction vessel~~for the boiling solution.~~.

6.1.1. ~~-Use~~using the 400 mL glass beaker to measure the 147 mL (~150 mL) of toluene. Pour the 147 mL (~150 mL) of toluene from the glass beaker into the reaction vessel.

6.1.2. ~~and~~ Use the 5 mL small glass graduated cylinder to carefully measure the 8.7 mL (~9 mL) of oleylamine. First carefully measure and pour 4 mL, and then 4.7 mL, of oleylamine from the small glass graduated cylinder into the reaction vessel. ~~Oleylamine is corrosive and toxic, so handle it carefully.~~

~~2.2.~~

6.2. Carefully lower the condenser tube ~~so that it is connected with~~down into the glass reaction vessel again. ~~and~~

6.3. ~~-E~~ensure that water is gently flowing through the outer chamber of the condenser tube to cool, ~~and~~ condense, and collect the toluene and oleylamine vapor.

~~2.3.~~

~~2.4.~~ Heat and stir~~Start bringing~~ the oleylamine and toluene ~~mixture~~ solution in the reaction vessel and allow the solution to approach a slow/gentle boil (using the stirring and heating mantle,~~heater,~~ with the magnetic stir bar rotating to mix the solution). Once the oleylamine and toluene solution~~it~~ reaches a gentle boil, turn the heat~~it~~ down a little bit so it is boiling slowly. Do not approach or exceed the flash point of toluene.

6.4.

~~3.~~7. Tetrachloroauric Acid, Oleylamine & Toluene Injection Solution Preparation~~ and Injection~~:

7.1. Begin preparing the injection solution (150 mg tetrachloroauric acid, 3.6 mL oleylamine, 3.0 mL toluene)~~The tetrachloroauric acid shouldn't be exposed to air, which contains water vapor. Every effort should be made to prevent the tetrachloroauric acid from being exposed to water or water vapor~~.

7.2. Ensure that the tetrachloroauric acid is fresh or hasn't been exposed to air, water, moisture, or humidity. Remove the laboratory film or seal that is protecting the tetrachloroauric acid from air and moisture.

~~3.1.~~

~~3.1.1.~~ ~~-~~NOTE: The tetrachloroauric acid is very sensitive to water/moisture/humidity. Every effort should be made to prevent exposing the tetrachloroauric acid powder to air/water. The tetrachloroauric acid comes in a sealed pouch and new container vessels are sealed with wax to prevent water vapor from getting into new vessels. A new batch of tetrachloroauric acid costs ~$100, but it should last a year if not exposed to water vapor. Store new unopened batches of tetrachloroauric acid in the fridge. Transfer a new unopened batch of tetrachloroauric acid to the nitrogen glove box prior to opening it.

~~3.1.2. Store new unopened batches of tetrachloroauric acid in the fridge. Transfer a new~~

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~~unopened batch of tetrachloroauric acid to the nitrogen glove box prior to opening it.~~

Only open a new container of tetrachloroauric acid in the nitrogen glove box, when the humidity has reached an appropriately low and stable level (less than 0.8% relative humidity). Store the tetrachloroauric acid in the nitrogen glove box after opening it. After opening the tetrachloroauric acid, wrap laboratory film~~Parafilm~~ around the lid of the container to help with sealing the container and to prevent water vapor and contaminants from getting into the container.

~~3.1.3.~~

7.3. In the nitrogen glove box, place one of the two non-aqueous 20 mL glass vials with the ~~white~~ PTFE-lined ~~plastic~~ caps on the microbalance/scale and remove the ~~white~~ PTFE-lined cap.

~~3.2.~~

7.4. Make sure to "re-zero" or "tare" the microbalance with the 20 mL glass vial on the scale before beginning to weigh out the tetrachloroauric acid powder.

~~3.3.~~

7.5. In the nitrogen glove box, use the small metal spatula to ~~scoop~~ deposit tetrachloroauric acid powder from the ~~small~~ container into the 20 mL glass vial on the microbalance, ~~and~~ to a measured weight ~~out the appropriate amount~~of ~~(e.g.,~~ 150 mg~~)~~ of tetrachloroauric acid powder.~~into the 20 mL glass vial using the microbalance.~~

~~3.4.~~

7.6. ~~In~~Remove the PTFE-lined cap from the other non-aqueous 20 mL glass vial ~~with the white PTFE-lined plastic cap~~ (the empty one that is not currently on the microbalance)~~, remove the white cap.~~ ~~, and~~

CAUTION: Oleylamine is toxic and corrosive, so handle it carefully.

7.7. ~~-U~~use the ~~small~~ 5 mL small glass graduated ~~glass~~ cylinder to measure ~~and then pour~~ 3.6 mL of oleylamine. Carefully pour the 3.6 mL of oleylamine from the 5 mL small glass graduated cylinder into the 20 mL glass vial without the tetrachloroauric acid.~~,~~

7.8. ~~-~~Carefully pour and ~~and then m~~measure ~~and pour~~ 3.0 mL of toluene into the 5 mL small glass graduated cylinder. Carefully pour the 3.0 mL of toluene from the 5 mL small glass graduated cylinder into the 20 mL glass vial with the oleylamine.

NOTE: ~~-~~If ~~you pour~~ too much toluene is poured into the graduated glass cylinder, ~~you can pour~~ the excess solvent can be poured into the flammable~~s~~ waste bottle. It is best to use the small 5 mL graduated glass cylinder for measuring the oleylamine and toluene. Be careful to not spill the oleylamine, as it is corrosive and toxic.

~~3.5.~~

7.9. Screw the~~Close the solution glass vial with the white~~ PTFE-lined ~~plastic~~ cap back onto the 20 mL glass vial with the oleylamine and toluene inside.~~, and~~ ~~S~~gently ~~s~~hake and swirl the closed glass vial~~it~~ to mix ~~up~~ the ~~toluene and~~ oleylamine and toluene solution together.

~~3.6.~~

7.10. Open the 20 mL solution glass vial. ~~and~~ Carefully pour ~~the contents of the glass~~

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~~vial with~~ the ~150 mg of tetrachloroauric acid powder into the ~~solution~~ glass vial with the oleylamine and ~~the~~ toluene solution.

~~3.7.~~

7.11. Screw the PTFE-lined caps back onto the glass vials.~~Close the solution glass vial with the white PTFE-lined plastic cap, and gently S~~s~~hake and swirl the closed glass vial with~~it to mix the~~ the tetrachloroauric acid, oleylamine and ~~powder with the~~ toluene to mix the solution together~~and oleylamine solution~~. Keep shaking the solution, and ~~The solution should look dark red or purple after shaking and mixing it.~~ e~~E~~nsure that ~~the tetrachloroauric acid, oleylamine, and toluene solution~~it is ~~well~~ mixed thoroughly.

NOTE: The tetrachloroauric acid, oleylamine and toluene injection solution should turn dark red or purple after shaking and mixing it, as shown in Supplementary Figure 4.

~~3.8.~~ **Injection of the Tetrachloroauric Acid, Oleylamine & Toluene Solution into the Vessel:**

~~3.9.~~8. ~~Remove the long graduated glass pipette from the aluminum foil (which was protecting the pipette to keep it clean) and attach the rubber bulb with valves to the pipette. Ensure that you know how to use the rubber bulb with valves to suck up and squirt out a solution with the long graduated glass pipette before using it.~~

8.1. ~~Ensure that the oleylamine and toluene solution in the glass reaction vessel is at a gentle boil and e~~Ensure that water is ~~gently~~ slowly flowing ~~through~~ into the bottom of the condenser tube, and up out the top of ~~outer chamber of~~ the condenser tube. Adjust the water flow as necessary by carefully opening/closing the water valve.

~~3.10.~~8.2. Ensure that the oleylamine and toluene solution in the glass reaction vessel is at a gentle boil, with some toluene and oleylamine evaporating into the condenser tube. Ensure that the magnetic stirrer is on.

~~3.11. Shake the closed 20 mL non-aqueous glass vial with the white PTFE-lined cap with the tetrachloroauric acid, oleylamine, and toluene solution and ensure it is well-mixed.~~

8.3. ~~Use the clamp and stand to position~~ Raise the condenser tube above the reaction vessel, using the stand with clamps to support the glassware. Ensure that there is~~providing~~ enough room and clearance to inject the tetrachloroauric acid, oleylamine, and toluene solution into the reaction vessel.

8.4. Remove the long graduated glass pipette from the aluminum foil (which was protecting the pipette to keep it clean) and attach the rubber bulb with valves to the pipette. Ensure familiarity with operating the rubber bulb with valves to suck up and squirt out a solution with the long graduated glass pipette before using it.

~~4.7.~~ Shake the closed 20 mL non-aqueous glass vial with the ~~white~~ PTFE-lined cap with the tetrachloroauric acid, oleylamine, and toluene injection solution and ensure it is well-mixed. Open the 20 mL glass vial with the injection solution by removing the cap.

8.5.

8.6. Press on the upper valve while squeezing the rubber bulb to deflate the rubber bulb.

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Carefully place the tip of the long graduated glass pipette into the 20 mL glass vial with the tetrachloroauric acid, oleylamine, and toluene injection solution.

~~3.12.~~

8.7. Gently press the lower valve on the ~~Use the~~ rubber bulb ~~with valves~~ connected to the long graduated glass pipette to slowly draw up all of the tetrachloroauric acid, oleylamine, and toluene injection solution into the glass pipette.~~,~~

NOTE: Supplementary Figure 5 shows the injection solution being drawn into the long graduated glass pipette with the rubber bulb with valves just before injecting the solution into the reaction vessel. It may be beneficial to practice operating the long graduated glass pipette with the bulb with valves (e.g., with some toluene) before actually drawing up and injecting the tetrachloroauric acid, oleylamine, and toluene solution.

8.8. ~~-~~Carefully place the tip of the glass pipette into the opening of the reaction vessel, and ~~and then q~~quickly inject the tetrachloroauric acid, oleylamine, and toluene injection solution into the boiling solution of oleylamine and toluene ~~with one fast squirt~~in the reaction vessel.

~~You may want to practice operating the long graduated glass pipette with the bulb with valves (e.g., with some toluene) before actually drawing up and injecting the tetrachloroauric acid, oleylamine, and toluene solution.~~

NOTE: The solution color should initially change from red to yellow to white within about a minute, as gold nanoparticles begin to nucleate and grow.

~~3.13.~~

8.9. Use the clamp on the stand to lower~~reposition~~ the condenser tube back down into the reaction vessel ~~again~~.

8.10. Heat the gold nanoparticle chemical reaction solution at a gentle boil for 2 hours.

~~3.14.~~ NOTE: ~~-~~The toluene vapor from the boiling solution should condense in the tube and drip back down into the reaction vessel. Over several minutes, the color of the reaction mixture should then change from

~~Over the course of 1-2 hours, the color of the reaction mixture should change from~~ white to yellow to light pink~~-~~ and then to red as the gold nanoparticles grow larger. Over the course of 1-2 hours, the color of the reaction mixture should gradually change from light red ~~and then gradually~~ to deep red/purple.

~~3.15.~~

8.11. After 2 hours of heating the reaction solution, turn off the heater.

~~3.16.~~

NOTE: At this point, the solution can either be allowed to cool down naturally to room temperature, or the solution can be immediately quenched by adding ~100 mL of methanol into the solution. The best-known practice as of now is to allow the solution to cool down naturally rather than quenching the solution right away.

~~3.17.~~

~~3.18.~~8.12. Allow the solution to cool down naturally to room temperature for 1 hour

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(recommended)~~;~~ or quench the gold nanoparticle solution immediately with 100 mL of methanol (not recommended).

~~4.~~ **Quenching the Reaction with Methanol After Cooling the Gold Nanoparticle Solution~~Cleaning and Drying of Gold Nanoparticles~~:**

~~4.1.~~9. ~~After allowing the gold nanoparticles to cool down for 1 hour (recommended), or after quenching the gold nanoparticle solution with 100 mL of methanol (not recommended), the following steps are performed:~~

9.1. Ensure that the heater has been turned off, and the solution has cooled down.

9.2. Stop flowing water through the condenser tube~~, and~~ C~~c~~arefully remove the water drainage hose from the sink/drain in the adjacent fume hood and connect it to the vacuum port in the fume hood.

~~4.2.~~

9.3. Pull vacuum on the drainage hose to suck away the water in the condenser tube and the drainage hose. Carefully remove the condenser tube from the stand with the clamp and lay it horizontally~~/sideways~~ in the nitrogen glove box.

NOTE: The vacuum that is being pulled on the glass condenser tube should evaporate the water within the condenser tube.

~~4.3.~~

9.4. In the nitrogen glove box, pour ~35 mL of methanol into each of the 50 mL conical centrifuge tubes (quantity 12)~~ with blue caps~~.

NOTE: ~~–~~Methanol will be used to remove unreacted ~~starting materials~~reagents and byproducts from the synthesis process, in order to clean and wash the gold nanoparticles. The 50 mL centrifuge tubes should be held upright in test tube racks~~/stands~~.

~~4.4.~~

9.5. Pour the gold nanoparticle~~s~~ solution in equal volumes (~12 mL) into each of the 50 mL centrifuge tubes (quantity 12) with ~~blue caps~~methanol. Be careful to not accidentally pour out the magnetic stir bar while pouring the gold nanoparticle solution into each centrifuge tube. ~~Screw the blue caps tightly back onto the centrifuge tubes.~~

NOTE: Supplementary Figure 6 shows ~12 mL of gold nanoparticle solution being poured into each of the 50 mL conical centrifuge tubes with methanol. After pouring ~12 mL of gold nanoparticle solution into each of the 50 mL conical centrifuge tubes with ~35 mL of methanol, each centrifuge tube should have ~47 mL of solution (slightly below the 50 mL mark).

~~4.5.~~

9.6. Distribute any remaining gold nanoparticle solution evenly between the centrifuge tubes.

9.7. Screw the caps onto the 50 mL centrifuge tubes to close them and tighten the caps.

~~4.8. You may want to label the top of each 50 mL centrifuge tube on the blue caps with a sample~~

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~~number (e.g., 1, 2, 3, 4, …) to keep track of the different samples.~~

9.8. ~~The vacuum that was being pulled on the glass condenser tube should have evaporated all/most of the water within the condenser tube.~~ Disconnect the inlet and outlet hoses from the glass condenser tube, connect the inlet and outlet hoses together by feeding one into the other, and then wrap the connection of the tubes with laboratory film~~Parafilm~~ to seal the connection. Turn off t~~T~~he vacuum that is being pulled on the hoses ~~can now be turned off~~.

NOTE: The tubes are connected and sealed to prevent water or water vapor from accidentally getting into the nitrogen glove box.

~~4.6.~~

9.9. Remove the 50 mL conical centrifuge tubes with the gold nanoparticle solution~~s~~ and methanol ~~in the test tube racks~~ from the nitrogen glove box through the load lock. Also remove~~and~~ the methanol bottle and toluene bottle from the nitrogen glove box ~~through the load lock~~. Place them in the adjacent fume hood.

~~4.7.~~

9.10. Also remove the glass reaction vessel, the magnetic stir bar, the glass condenser tube, the long glass graduated pipette, and the rubber bulb with valves from the nitrogen glove box through the load lock. Place them in the adjacent fume hood.

9.11. Label the top of each 50 mL centrifuge tube on the caps with a sample number (e.g., 1, 2, 3, 4, …) to keep track of the different samples.

NOTE: After removing the gold nanoparticle solution and glassware/supplies, the nitrogen glove box should continue to be ventilated for several hours or overnight by flowing fresh nitrogen into the glovebox while pulling a slight vacuum to flush out and ventilate the toluene/oleylamine vapor. The vacuum exhaust line should be vented into a fume hood. The nitrogen glovebox should also be regenerated with regeneration gas to remove moisture/solvents from the filtration system. Some nitrogen gloveboxes may also come with a solvent trap, which helps with removing solvent vapors.

~~4.8.~~ **Washing and Purifying the Gold Nanoparticles with Toluene and Methanol:**

10.

NOTE: Each 50 mL centrifuge tube with gold nanoparticles will be washed and purified with 10 mL of toluene and 40 mL of methanol 3 times, cleaning the gold nanoparticles in batches of 6 centrifuge tubes at a time. The centrifuge tubes should have an equal amount of gold nanoparticle solution and should be equally weighted and balanced.

~~Start spinning 6 of the 12 conical centrifuge tubes with the gold nanoparticle solution and methanol in the centrifuge.~~

10.1. Place 6 of the 50 mL centrifuge tubes with gold nanoparticle solution into the centrifuge.

~~4.9.~~

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~~4.10.~~10.2. Close the lid of the centrifuge, and enter the~~Use the~~ following settings ~~on the centrifuge~~for spinning the gold nanoparticles:

~~4.10.1.~~10.2.1. RPM: 2328

~~4.10.2.~~10.2.2. RCF: 1000

10.2.3. Time: 5 minutes

~~4.10.3.~~

~~4.11. Meanwhile, while the first 6 centrifuge tubes are spinning, begin cleaning the chemical reaction glassware:~~

~~4.12. In the fume hood, clean the glass reaction vessel with acetone and swirl the acetone around in the glass reaction vessel to wash away the residual gold nanoparticle solution, then dump the dirty acetone into a dirty solvent collection beaker and discard the dirt solvent into a flammables waste bottle.~~

~~4.13. In the acid wet bench, place the glass reaction vessel with the condenser tube attached to it into a 600 mL beaker for support, and rest the side of the condenser tube against the sidewall of the acid wet bench for further support. Clean the chemical reaction glassware (condenser tube, reaction vessel, glass pipette) and magnetic stir bar by pouring the used ~300 mL gold etchant TFA solution (which should have been saved earlier and set aside for reuse) that was mixed 1:1 with DI water into the condenser tube and reaction vessel glassware. Then place the magnetic stir bar and long glass graduated pipette into the condenser tube. Fill up the condenser tube with DI water as necessary to top it off and allow the gold etchant TFA bath to sit and clean the glassware for 30 minutes. After 30 minutes, crack the seal between the condenser tube and the reaction vessel to collect all the gold etchant solution into the reaction vessel, and pour the used gold etchant solution into the 400 mL beaker. Pour the gold etchant solution into the chemical waste bottle for used gold etchant solution, which can be found in the cabinet underneath the acid wet bench.~~

~~4.14. Still in the acid wet bench, wash the chemical reaction glassware and magnetic stir bar 3-4 times with DI water to flush out the remaining gold etchant solution, and then allow the chemical reaction glassware and magnetic stir bar to sit in a DI water bath for an additional 30 minutes. After 30 minutes of sitting in a DI water bath, empty out the water and use the DI water gun to wash the water down the acid wet bench drain. Rinse with acetone and then blow the glassware dry with the nitrogen gun.~~

10.3. Start spinning 6 of the 12 conical centrifuge tubes with the gold nanoparticle solution and methanol in the centrifuge.

10.4. After the first 6 centrifuge tubes with gold nanoparticles are done spinning~~ and the centrifuge stops (5 minutes)~~, ~~carefully take the~~gently remove the tubes from~~out of~~ the centrifuge. Be careful to not disturb the gold nanoparticle pellets while placing the centrifuge tubes in the tube racks.

~~The black gold nanoparticles should form a pellet that sticks to the bottom of the centrifuge tube.~~

NOTE: Supplementary Figure 7 shows how the gold nanoparticle solution should appear in the 50 mL conical centrifuge tubes after centrifugation. The centrifugal force will pull down the gold nanoparticles in solution and separated them from the methanol and

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toluene. The gold nanoparticles will precipitate into pellets at the bottom of each centrifuge tube. The supernatant methanol/toluene solution will appear to be clear/transparent above the dark gold nanoparticle pellets, indicating that centrifugation has precipitated the gold nanoparticles from solution.

~~4.9.~~ Place the last 6 ~~Start spinning the last 6~~ of the 12 conical centrifuge tubes with the gold nanoparticle solution and methanol into the centrifuge. Close the lid of the centrifuge and enter the same centrifuge settings as before. Start spinning the tubes in the centrifuge.

10.5. ~~=~~

10.6. After the last 6 centrifuge tubes are done spinning, gently remove the tubes from the centrifuge. Be careful to not disturb the gold nanoparticle pellets while placing the centrifuge tubes in the tube racks.

10.7. ~~-~~Carefully carry all of~~bring~~ the centrifuge tubes with the gold nanoparticles ~~back~~ over to the fume hood and try not to disturb or agitate them during transport.

~~4.15.~~10.8. ~~-~~Slowly and gently pour out the waste methanol into a flammable waste vessel/beaker~~,~~ ~~B~~be~~ing~~ careful not disturb and to not~~to~~ pour out or lose the black ~~pellet of~~ gold nanoparticle pellets at the bottom of the centrifuge tubes~~s~~.
~~Start spinning the last 6 of the 12 conical centrifuge tubes with the gold nanoparticle solution and methanol in the centrifuge.~~
NOTE: The first methanol rinse cycle is now complete.
~~4.16.~~

10.9. ~~Still in the fume hood, after pouring out the used~~Begin the second methanol rinse cycle by~~,~~ pouring ~10 mL of fresh toluene into each of the ~~6~~ conical centrifuge tubes with black nanoparticle pellets in the fume hood.~~,~~ ~~and s~~Screw the ~~blue~~ caps back on to close~~d on~~ the 50 mL centrifuge tubes.
~~4.17.~~

10.10. Vortex each of the 50 mL centrifuge tubes until the black liquid/precipitate/gold nanoparticles are resuspended and dispersed in the 10 mL toluene solution, and the solution looks cloudy/dark. Check the bottom of each~~the~~ centrifuge tube to ensure that ~~virtually all~~most ~~or most~~ of the black residue (gold nanoparticles) ~~residue~~ has been resuspended into solution.
~~4.18.~~

NOTE: Supplementary Figure 8 shows the centrifuge tubes with gold nanoparticle solution and toluene being vortexed and resuspended. Vortexing is much better and gentler on the gold nanoparticles than sonicating the gold nanoparticles. Do not sonicate the gold nanoparticles as sonication~~it~~ could strip off the oleylamine ligands from the gold nanoparticles and cause aggregation and sedimentation of the gold nanoparticles.

NOTE: Supplementary Figure 9 shows how the gold nanoparticle solution should appear when the gold nanoparticles are resuspended into solution by vortexing each gold

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nanoparticle pellet with ~10 mL of toluene.

~~4.19.~~

~~4.20.~~ Pour ~~After vortexing the centrifuge tubes with toluene and gold nanoparticles, add~~ ~40 mL of fresh methanol into each of the ~~6~~ conical centrifuge tubes with toluene and nanoparticles, so that ~~ere~~ with the 10 mL of toluene that is already in each centrifuge tube, there ~~is~~ is a total of ~50 mL of solution in each 50 mL centrifuge tube.~~,~~ ~~then~~ S~~s~~crew the ~~blue~~ caps back onto ~~closed on~~ the 50 mL centrifuge tubes to close the tubes, and ensure that the caps are on tight.

~~4.21. After the last 6 tubes are done spinning and the centrifuge stops (5 minutes), carefully take the tubes out of the centrifuge. The black gold nanoparticles should form a pellet that sticks to the bottom of the centrifuge tube. Carefully bring the centrifuge tubes with the gold nanoparticles back to the fume hood and try not to disturb or agitate them during transport. Slowly and gently pour out the waste toluene and methanol into a flammable waste vessel/beaker, being careful not to pour out or lose the black pellet of gold nanoparticles.~~

~~4.22.~~10.11. ~~Repeat this toluene and methanol wash and centrifuge process two more times, with 6 of the centrifuge tubes at a time, so that each 50 mL centrifuge tube with gold nanoparticles gets washed with toluene and methanol 3 separate times:~~

~~4.23. Pour ~10 mL of fresh toluene into each 50 mL centrifuge tube with a gold nanoparticle pellet, using the fume hood. Close the centrifuge tube cap.~~

~~4.24. Vortex the gold nanoparticle and toluene solution until it gets dark and cloudy again. Ensure that there is almost no dark powder on the bottom.~~

~~4.25. Pour in ~40 mL of fresh methanol, to bring the solution back up to the ~50 mL level, using the fume hood.~~

10.12. Place ~~6 of~~ the centrifuge tubes into the centrifuge.~~,~~ ~~and s~~Spin the centrifuge tubes in the centrifuge to collect the gold nanoparticles into a pellet at the bottom of each tube, 6 centrifuge tubes at a time. U~~u~~s~~e~~~~ing~~ the same centrifuge settings as before (RCF 1000, 5 minutes)~~.~~.

~~4.26.~~

10.13. After the centrifuge stops, gently take out the tubes with the nanoparticles, and then carefully take them to the fume hood (try not to disturb or agitate them during transport) ~~where you should pour th.~~ ~~e~~Carefully pour out the waste toluene and methanol into the flammable~~s~~ waste vessel/beaker.

NOTE: The second methanol rinse cycle is now complete.

10.14. For the third and final rinse cycle, follow the same process as before for vortexing in 10 mL of toluene, cleaning in 40 mL of methanol, centrifugation, and carefully pouring out the toluene/methanol solvent. Ensure that the gold nanoparticles in each of the 50 mL centrifuge tubes get resuspended with toluene and washed with methanol 3 times.

~~Pour ~10 mL of fresh toluene into each 50 mL centrifuge tube with a gold nanoparticle pellet, using the fume hood. Close the centrifuge tube cap.~~

~~Vortex the gold nanoparticle and toluene solution until it gets dark and cloudy again.~~

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~~Ensure that there is almost no dark powder on the bottom.~~

~~4.10. Pour in ~40 mL of fresh methanol, to bring the solution back up to the ~50 mL level, using the fume hood.~~

~~4.27.~~

~~Repeat the process as necessary, until each 50 mL centrifuge tube with gold nanoparticles has been cleaned with toluene and methanol 3 times.~~

~~4.28.~~**11. Drying the Gold Nanoparticles:**

~~4.29.~~ NOTE: After the gold nanoparticles in the 50 mL centrifuge tube have been washed 3 separate times, and the toluene and methanol has been poured out for the last time, the gold nanoparticles need to be dried to evaporate the remaining solvent.

There are two ways to dry the gold nanoparticles and evaporate the solvent:

~~4.30.~~

11.1. Option 1 - Nitrogen Gun~~:~~ (not recommended~~Not ideal because it could cause the gold nanoparticle pellet to be damaged/lost~~):

11.1.1. ~~-~~Use a nitrogen gun or valve in the fume hood to gently blow dry the centrifuge tube containing the black pellet of gold nanoparticles at the bottom of the tube.

11.1.2. ~~-~~Take care to not use too much nitrogen pressure, or ~~you may dislodge~~ the fragile gold nanoparticle~~s~~ pellet may get dislodged.

NOTE: Drying the gold nanoparticles with the nitrogen gun is not ideal because it could cause the gold nanoparticle pellets to get damaged/lost.

~~4.31.~~

11.2. Option 2 - Vacuum Drying~~:~~ (recommended~~Preferred method because it is less likely to damage or lose the gold nanoparticle pellet~~):

11.2.1. ~~-~~Loose~~nly~~ the caps on the 50 mL centrifuge tubes with gold nanoparticle pellets so that the tubes are still covered, but solvent can evaporate and escape from inside the tubes.

11.2.2. P~~and p~~lace the rack of tubes ~~in test tube racks~~with gold nanoparticles inside the vacuum load lock of the nitrogen glove box. Close and seal the outer load lock door and open the valve to the vacuum pump to start pulling vacuum on the load lock.

11.2.3. Pump down to about half the gauge pressure (~-15 in.Hg) to evaporate the solvent and dry the nanoparticles.

11.2.4. Leave the gold nanoparticles in the load lock at a moderate vacuum pressure (half-gauge, ~-15 in.Hg) for ~~over ~~5 minutes.~~,~~ Do not pump down to a lower pressure and do not leave in vacuum for too long, or the oleylamine ligands may get detached.~~longer.~~

11.2.5. After the gold nanoparticles have been under vacuum for a few minutes to dry the gold nanoparticles and evaporate the remaining solvent, purge the load lock with

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nitrogen until the load lock reaches atmospheric pressure.

11.2.6. Remove the 50 mL centrifuge tubes with gold nanoparticles from the load lock and inspect the dryness of the gold nanoparticle pellets in the fume hood.

NOTE: Supplementary Figure 10 shows how a dried gold nanoparticle pellet at the bottom of a 50 mL conical centrifuge tube should look after vacuum drying it. If there is still some solvent inside the 50 mL conical centrifuge tube, the gold nanoparticles need to be dried further to evaporate the remaining solvent. Vacuum drying is the preferred method for drying because it is less likely to damage or lose the gold nanoparticle pellet, compared to more aggressive methods such as nitrogen gun drying. If a vacuum load lock is not available, or if preferred, the gold nanoparticles may also be dried in a vacuum desiccator. ~~4.32.~~

11.3. After the gold nanoparticle pellets are dry, screw the caps tightly back onto the centrifuge tubes.

11.4. Wrap laboratory film around the tightly closed caps to seal the centrifuge tubes with the gold nanoparticle pellets inside.

11.5. Label the 50 mL centrifuge tubes with gold nanoparticle precipitate pellets with an appropriately descriptive label, such as "Dried Au NP" and the~~,~~ date (e.g., 9-28-2020)~~"~~. ~~4.33.~~

~~4.11.~~ ~~P~~Seal the caps of the tubes with Parafilm and place the sealed centrifuge tubes with dried gold nanoparticle pellets inside a 2 °C – 8 °C fridge~~.~~, U~~u~~s~~e~~ing a ~~Styrofoam~~ tray or 50 mL conical centrifuge tube racks to hold the tubes~~m~~ upright.

11.6.

NOTE: Supplementary Figure 11 shows the centrifuge tubes capped, wrapped with laboratory film, labelled, and stored in a 2 °C – 8 °C fridge. Each centrifuge tube can be stored in the fridge until it is used to make a solution of resuspended gold nanoparticles.

12. Clean the Chemical Reaction Glassware (After Gold Nanoparticle Synthesis):

CAUTION: Gold etchant TFA and aqua regia are corrosive. Wear the necessary personal protective equipment (PPE) such as chemical gloves, chemical gown, goggles, and face shield. Only handle the corrosive solution in an acid wet bench while wearing the necessary PPE.

12.1. In the fume hood, clean the glass reaction vessel with acetone and swirl the acetone around in the glass reaction vessel to wash away the residual gold nanoparticle solution, then dump the dirty acetone into a dirty solvent collection beaker and discard the dirt solvent into a flammable waste bottle.

12.2. In the acid wet bench, place the glass reaction vessel with the condenser tube attached to it into a 600 mL beaker for support, and rest the side of the condenser tube

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against the sidewall of the acid wet bench for further support.

12.3. Clean the chemical reaction glassware (condenser tube, reaction vessel, glass pipette) and magnetic stir bar by pouring the used ~300 mL gold etchant TFA solution (which was saved earlier and set aside for reuse) that was mixed 1:1 with DI water into the condenser tube and reaction vessel glassware. Place the magnetic stir bar and long glass graduated pipette into the condenser tube. Fill up the condenser tube with DI water as necessary to top it off and allow the gold etchant TFA bath to sit and clean the glassware for 30 minutes.

12.4. After 30 minutes, crack the seal between the condenser tube and the reaction vessel to collect all the gold etchant solution into the reaction vessel, and pour the used gold etchant solution into the 400 mL beaker. Pour the gold etchant solution into the chemical waste bottle for used gold etchant solution.

12.5. Still in the acid wet bench, wash the chemical reaction glassware and magnetic stir bar 3-4 times with DI water to flush out the remaining gold etchant solution, and then allow the chemical reaction glassware and magnetic stir bar to sit in a DI water bath for an additional 30 minutes.

12.6. After 30 minutes of sitting in a DI water bath, empty out the water and use the DI water gun to wash the water down the acid wet bench drain. Rinse with acetone and then blow the glassware dry with the nitrogen gun.

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REPRESENTATIVE RESULTS:

Figure 1~~8~~ shows how the gold nanoparticle synthesis chemical reaction mixture solution (tetrachloroauric acid, oleylamine, and toluene) should gradually change color over the course of several minutes as it initially boils ~~for 2 hours~~in the reaction vessel;~~,~~ from clear, to light yellow (left image), to light pink (center image), to light red (right image)~~and then slowly to dark red~~. The changing color of the solution is an indication of the changing size of the gold nanoparticles as they begin to nucleate and grow larger over time. In general, the gold nanoparticle solution should become darker and more red/purple over time as the gold nanoparticles nucleate and grow. Figure 2~~9~~ shows the final dark red/purple color of the gold nanoparticle synthesis chemical reaction mixture solution after 2 hours of boiling. The dark red/purple color of the gold nanoparticle solution is characteristic of a concentrated solution of gold nanoparticles that are ~12 nm in diameter. ~~Figure 14 shows how the gold nanoparticle solution should appear in the 50 mL conical centrifuge tubes after centrifugation, with the gold nanoparticles collected into dark gold nanoparticle pellets at the bottom of each centrifuge tube. Figure 16 shows how the gold nanoparticle solution should appear when the gold nanoparticles are resuspended into solution by vortexing each gold nanoparticle pellet with ~10 mL of toluene. Figure 17 shows how a dried gold nanoparticle pellet at the bottom of a 50 mL conical centrifuge tube should look, after vacuum drying it.~~ Figure 3~~19~~ shows a scanning electron microscope (SEM) image of a gold nanoparticle monolayer (after being deposited onto a silicon substrate) which is used to

characterize the size and monodispersity of the gold nanoparticles. The gold nanoparticles should all appear to have roughly the same size/diameter if they are highly monodisperse. If the gold nanoparticles are polydisperse, they will have large variations in their size/diameter. For most applications, monodispersity is usually preferred rather than polydispersity. Figure 4~~20~~ shows a scanning electron microscope (SEM) image of gold nanoparticles and their diameter measurements, which indicates a diameter of ~12 nm +/- 2 nm for the gold nanoparticles. These gold nanoparticles appear to be fairly monodisperse.

FIGURE AND TABLE LEGENDS:

Table 1: Chemical Amounts
This table shows the amount and type of chemicals that are needed for preparing the injection solution, boiling solution, washing/purification solution, and gold etchant solution.

Supplementary Figure 1: Cleaning Chemical Reaction Glassware with Gold Etchant TFA Solution
This figure shows the chemical reaction glassware (condenser tube, reaction vessel, glass pipette) and magnetic stir bar being cleaned with a ~300 mL mixture of ~150 mL of the gold etchant TFA solution and ~150 mL of DI water (1:1 mixture) in the condenser tube and reaction vessel glassware. The magnetic stir bar and long glass graduated pipette are placed into the condenser tube, and the gold etchant TFA bath is left to sit and clean the glassware for 30 minutes in the acid wet bench.

Supplementary Figure 2: Clean Glassware and Supplies Before Being Transferred into Nitrogen Glove Box
This figure shows the glassware and supplies after being cleaned and dried. The glassware and supplies are wrapped/covered with aluminum foil to protect them from dirt/debris before they are transferred into the nitrogen glove box.

Supplementary Figure 3: Gold Nanoparticle Synthesis Experimental Setup in Nitrogen Glove Box

This figure shows the gold nanoparticle synthesis experimental setup in the nitrogen glove box. The glass reaction vessel is resting on top of the fiberglass mesh receptacle on top of the heater/stirrer, and the condenser tube is connected on top of the glass reaction vessel. The condenser tube is mechanically supported by the stand with the clamp. There are two hoses connected to the water inlet and outlet ports of the condenser tube (with the inlet port on the bottom of the tube, and the outlet port on the top of the tube) so that water flows from the bottom of the condenser tube to the top of the condenser tube, cooling the tube off and condensing the vapor inside.
~~Figure 4: Glass Condenser Tube Suspended Above Glass Reaction Vessel~~

~~Figure 5: Taring Microbalance/Scale with Empty 20 mL Non-Aqueous Glass Vial~~

Supplementary Figure 4~~6~~: Mixing Tetrachloroauric Acid, Oleylamine, and Toluene Solution Before Injection

This figure shows the tetrachloroauric acid, oleylamine, and toluene injection solution after being mixed in a non-aqueous solution 20 mL glass vial with a PTFE-lined cap. The injection solution should look dark red or purple after shaking and mixing it.

Supplementary Figure ~~5~~7: Preparing to Inject Solution into Reaction Vessel Using Glass Pipette
This figure shows the tetrachloroauric acid, oleylamine, and toluene injection solution being drawn into the long graduated glass pipette with the rubber bulb with valves, just before quickly injecting the solution with one fast squirt into the boiling solution of oleylamine and toluene in the glass reaction vessel.

Figure 1~~8~~: Gold Nanoparticle Solution Changing Colors Over Several Minutes After Injection
This figure shows how the gold nanoparticle synthesis chemical reaction mixture solution (tetrachloroauric acid, oleylamine, and toluene) should gradually change color over the course of several minutes as it initially boils in the reaction vessel; from clear, to light yellow (left image), to light pink (center image), to light red (right image). The changing color of the solution is an indication of the changing size of the gold nanoparticles as they begin to nucleate and grow larger over time.

Figure 2~~9~~: Gold Nanoparticle Solution is Dark Red/Purple After 2 Hours of Boiling

This figure shows the final dark red/purple color of the gold nanoparticle synthesis chemical reaction mixture solution after 2 hours of boiling in the reaction vessel. The dark red/purple color of the gold nanoparticle solution is characteristic of a concentrated solution of gold nanoparticles that are ~12 nm in diameter.
~~Figure 10: Pouring ~35 mL of Methanol into Each 50 mL Conical Centrifuge Tube~~

Supplementary Figure 6~~11~~: Pouring ~12 mL of Gold Nanoparticle Solution into Each 50 mL Conical Centrifuge Tube

This figure shows ~12 mL of gold nanoparticle solution being poured evenly into each of the 50 mL conical centrifuge tubes with ~35 mL of methanol in each tube. Methanol is used to remove unreacted starting materials and byproducts, in order to clean and wash the gold nanoparticles.
~~Figure 12: 6 of the 50 mL Conical Centrifuge Tubes with Gold Nanoparticle Solution in Centrifuge~~

~~Figure 13: Centrifuge Settings (Spin at RCF 1000 for 5 Minutes)~~

Supplementary Figure 7~~14~~: 50 mL Centrifuge Tubes after Centrifugation, with Gold Nanoparticle Pellets at the Bottom
This figure shows how the gold nanoparticle solution should appear in the 50 mL conical centrifuge tubes after centrifugation, with the gold nanoparticles collected into dark gold nanoparticle pellets at the bottom of each centrifuge tube. Above the dark gold nanoparticle pellets, the supernatant methanol/toluene solution appears to be clear/transparent, indicating that centrifugation has precipitated the gold nanoparticles from solution.

Supplementary Figure 8~~15~~: Vortexing 50 mL Centrifuge Tubes with Au NPs After Filling with ~10 mL of Toluene
This figure shows the centrifuge tubes with gold nanoparticle solution and toluene being vortexed and resuspended. Vortexing is much better and gentler on the gold nanoparticles than sonicating the gold nanoparticles. The gold nanoparticles should not be sonicated, as sonication could strip off the oleylamine ligands from the gold nanoparticles and cause aggregation and sedimentation of the gold nanoparticles.

Supplementary Figure 9~~16~~: Vortex Until Gold Nanoparticle Pellet/Residue is Almost Completely Resuspended
This figure shows how the gold nanoparticle solution should appear when the gold nanoparticles are resuspended into solution by vortexing each gold nanoparticle pellet with ~10 mL of toluene. The 50 mL centrifuge tubes should be vortexed until the black liquid/precipitate/gold nanoparticles are resuspended and dispersed in the toluene, and the solution looks cloudy/dark. The bottom of the centrifuge tube should be checked to ensure that virtually all or most of the black nanoparticle residue has been resuspended into solution.

Supplementary Figure 10~~17~~: Dried Gold Nanoparticle Pellet in 50 mL Conical Centrifuge Tube
This figure shows how a dried gold nanoparticle pellet at the bottom of a 50 mL conical centrifuge tube should look, after vacuum drying it. After the gold nanoparticles in the 50 mL centrifuge tube have been washed 3 separate times, and the toluene and methanol has been poured out for the last time, the gold nanoparticles need to be dried to evaporate the remaining solvent. Vacuum drying is the preferred method for drying because it is less likely to damage or lose the gold nanoparticle pellet, compared to more aggressive methods such as nitrogen gun drying.

Supplementary Figure ~~1~~11~~8~~: Cap Tubes, Wrap with Laboratory Film~~Parafilm~~, Label Tubes, and Store in 2 °C – 8 °C Fridge
This figure shows the centrifuge tubes capped, wrapped with laboratory film, labelled, and stored in a 2 °C – 8 °C fridge. The 50 mL centrifuge tubes with gold nanoparticle precipitate pellets should be labelled with an appropriately descriptive label, such as the name, sample number and date. A tray or 50 mL conical centrifuge tube racks can be used to hold the tubes upright in the fridge.

Figure 3~~19~~: Scanning Electron Microscope (SEM) Image of Gold Nanoparticle Monolayer
This figure shows a scanning electron microscope (SEM) image of a gold nanoparticle monolayer (after being deposited onto a silicon substrate) which is used to characterize the size and monodispersity of the gold nanoparticles.

Figure 4~~20~~: Scanning Electron Microscope (SEM) Image with Gold Nanoparticle Diameter Measurements
This figure shows a scanning electron microscope (SEM) image of gold nanoparticles and their diameter measurements, which indicates a diameter of ~12 nm +/- 2 nm for the gold nanoparticles.

DISCUSSION:

Performing the gold nanoparticle synthesis protocol as presented above should produce gold nanoparticles with ~12 nm diameter and fairly high monodispersity (+/- 2 nm). However, there are some critical steps and process parameters that can be adjusted to potentially change the size/diameter and monodispersity/polydispersity of the gold nanoparticles. For example, after injecting the precursor solution into the reaction vessel and allowing the tetrachloroauric acid, oleylamine, and toluene solution to boil for two hours, there is an option to either do immediate quenching of the reaction solution or to do delayed quenching and natural cooling. If immediate quenching is desired, just after the 2-hour heated reaction step is complete, 100 mL of methanol is added to the reaction vessel to precipitate the gold nanoparticles product. Immediate quenching may provide better dispersion relationships because the nucleation occurs at roughly the same time for all nanoparticles in the saturated solution; whereas the longer the solution remains unquenched, the larger but more randomized the size of the nanoparticles become. If delayed quenching and natural cooling is instead desired, then after the 2-hour heated reaction step is complete, the solution is allowed to cool down naturally to room temperature for 1 hour. Alternatively, the solution could be left to cool even longer, until the following day (e.g., wait overnight) before 100 mL of methanol is added to precipitate the gold nanoparticles product. Researchers may want to experiment with both immediate quenching and delayed quenching, and 1 hour delayed quenching vs. overnight delayed quenching to determine which method produces the best results for making large and highly monodisperse gold nanoparticles. One hour delayed quenching is the procedure that is currently recommended to produce gold nanoparticles that are highly monodisperse, but it has not yet been determined which procedure yields superior results, so some further experimental investigations may be beneficial.

Another critical step in the protocol that affects the monodispersity of the gold nanoparticles is rapid injection of the precursor, to allow the saturated solution to form as many nuclei as possible over a very short time interval. Shortly after the precursor injection, few new nuclei form, and gold atoms should only join existing nuclei. What is necessary for high monodispersity is a long, consistent growth period relative to the nucleation period. A high growth:nucleation time ratio should benefit monodispersity. On this account, injecting the precursor solution very quickly is important for high monodispersity, and waiting to quench the reaction (delayed quenching) may also be beneficial for increasing the monodispersity. However, the competing mechanism of Ostwald ripening[13] is a driving factor for polydispersity. The surface energy of gold atoms on the surface of small nanoparticles is higher than the surface energy of gold atoms on the surface of large nanoparticles. Ostwald ripening is a thermodynamic driving force for the shrinking of small nanoparticles and the growing of large ones[14]. This is a phenomenon that can happen over time in solution.

Another variable to consider is the stability of the oleylamine ligand layer on the gold nanoparticles, and how well passivated the gold nanoparticle surfaces are by the oleylamine ligands. Although there is no indicator for the progression of the surface passivation at different points in the gold nanoparticle synthesis reaction, one can imagine how the surface passivation must evolve over time. At the beginning of the reaction, there are no gold nanoparticles, and oleylamine is actually acting as a reducing agent, to free the gold from its chlorine bonds. At the end of the reaction, the gold nanoparticle surfaces should be completely passivated. Ideally, the

reaction should be allowed to continue long enough to allow the surfaces of the gold nanoparticles to become completely passivated, but not so long that Ostwald ripening begins to make the gold nanoparticles polydisperse rather than monodisperse.

Overall, the things to consider when performing the quenching of the reaction are the growth:nucleation time ratio, minimizing Ostwald ripening time, and allowing sufficient time for surface passivation. It has not yet been proven whether delayed quenching or instantaneous quenching produces superior results (i.e., large, highly passivated, and highly monodisperse gold nanoparticles). However, slightly delayed quenching (e.g., allowing the solution to cool down to room temperature for 1 hour after boiling) can produce highly monodisperse gold nanoparticles, so some finite delay before quenching the reaction is acceptable. To provide more clarity as to whether immediate quenching or delayed quenching is better for producing large and highly monodisperse gold nanoparticles, a useful experiment or modification for troubleshooting of the technique would be to separate the gold nanoparticle synthesis solution into two different batches after boiling and perform the immediate post-reaction quenching in parallel with delayed quenching. The outcome of this experiment/modification may determine whether the nucleation time window is so short that the extra time (either one hour or one night/day later) for cooling is unneeded for growth, and some combination of Ostwald ripening and surface passivation is actually decreasing the monodispersity (or increasing the polydispersity) of the gold nanoparticles during the cooldown/delay before quenching.

The final consideration for this gold nanoparticle synthesis method is how the gold nanoparticles are stored and used. After the synthesis process and the cleaning process, the gold nanoparticles are dried gently, either using a nitrogen gun or under vacuum. It is highly recommended that the gold nanoparticles are dried in a vacuum environment rather than using a nitrogen gun, as the nitrogen gun could dislodge the black pellet of gold nanoparticles and cause it to become lost/contaminated/damaged. Drying the gold nanoparticles in a vacuum environment is much gentler and prevents the gold nanoparticle pellet from getting dislodged or lost. After drying, the gold nanoparticles are then stored in a clean and dry environment (e.g., in ~~Parafilm~~laboratory film-sealed capped conical centrifuge tubes) in a 2 °C – 8 °C refrigerator until they are ready to be used. This clean, dry, and cool environment should give the gold nanoparticles a longer shelf-life of approximately one year with minimal degradation. In order to use the gold nanoparticles, they may be resuspended into solutions of organic solvents such as toluene by vortexing the gold nanoparticles in the presence of the organic solvent. The size and concentration of the gold nanoparticles in the toluene solution can then be verified using UV-vis spectra characterization[15] and diluted further with toluene if necessary until the desired concentration of gold nanoparticles is achieved. One limitation is that the concentration will need to be analyzed for each solution.

The gold nanoparticle synthesis protocol that is presented here is intended to enable the synthesis of gold nanoparticles by non-chemistry experts. The significance of this protocol with respect to existing methods is that it provides the opportunity to control the quantity of nanoparticles that are produced, the size of the nanoparticles, the monodispersity of the nanoparticles, and the ligands that encapsulate the gold nanoparticles. The gold nanoparticles that are synthesized using this process have been ~~successfully~~ used to create nanoelectronic

devices for molecular electronics experiments, such as 2D molecule-nanoparticle arrays[16]. In this example, 2D molecule-nanoparticle arrays are formed by depositing 200 µL of the diluted gold nanoparticles in toluene solution into 15 mL conical centrifuge tubes that were partially filled with deionized water. The tubes were left undisturbed for 1 - 3 hours to allow the toluene to evaporate and the gold nanoparticles to form monolayers on the surface of the water. These gold nanoparticle monolayers were then transferred to substrates such as silicon microchips using PDMS stamps, in order to form nanoelectronic devices. The oleylamine ligands on the gold nanoparticles were then exchanged with other molecules in order to change the electronic and thermoelectric properties of the gold nanoparticle-molecule monolayers[17,18]. The gold nanoparticle synthesis protocol that is presented here produces high-quality gold nanoparticles that may be useful for many other gold nanoparticle applications within industry and medicine.

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DISCLOSURES:
The authors have nothing to disclose.

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Figure 1 - Gold Nanoparticle Solution After Injection

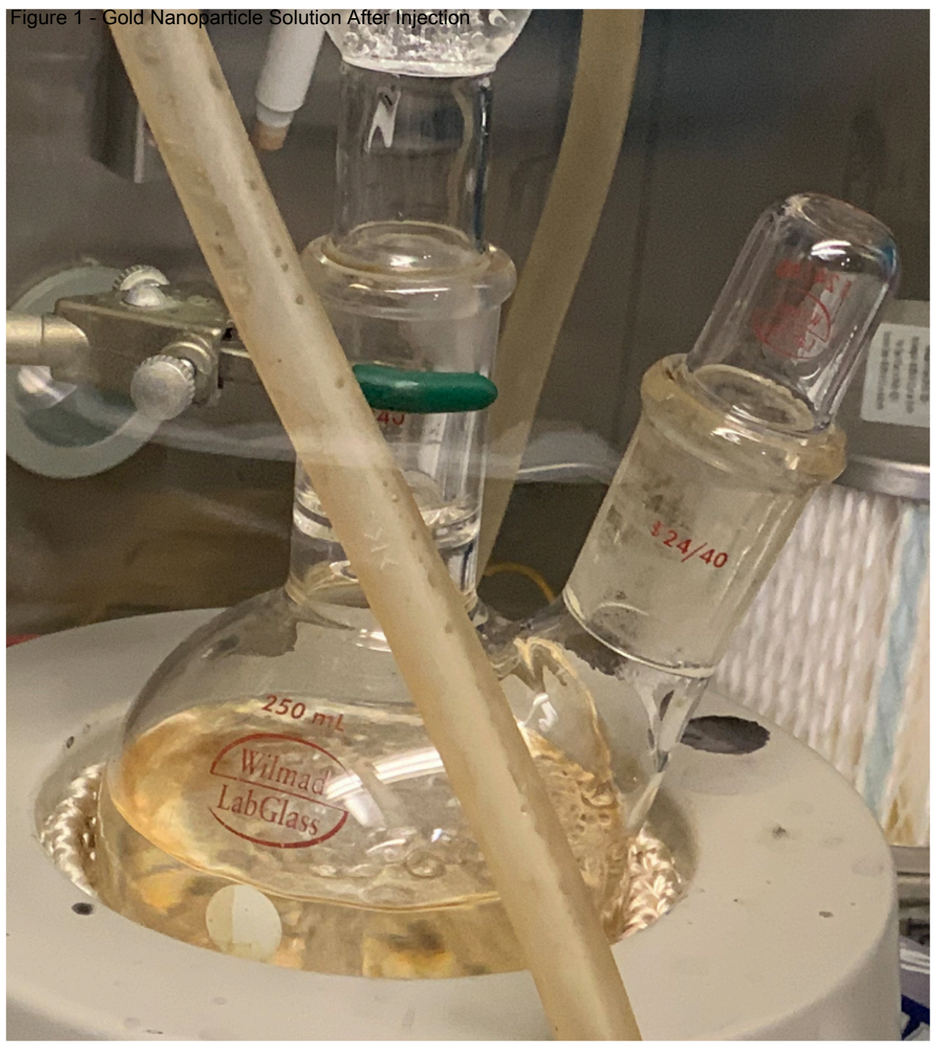


Figure 2 - Gold Nanoparticle Solution After Boiling

Click here to access/download;Figure;Figure 2 - Gold

Figure 3 - SEM Image of Gold Nanoparticle Monolayer

Click here to access/download;Figure;Figure 3 - SEM Image of Gold Nanoparticle Monolayer.pdf

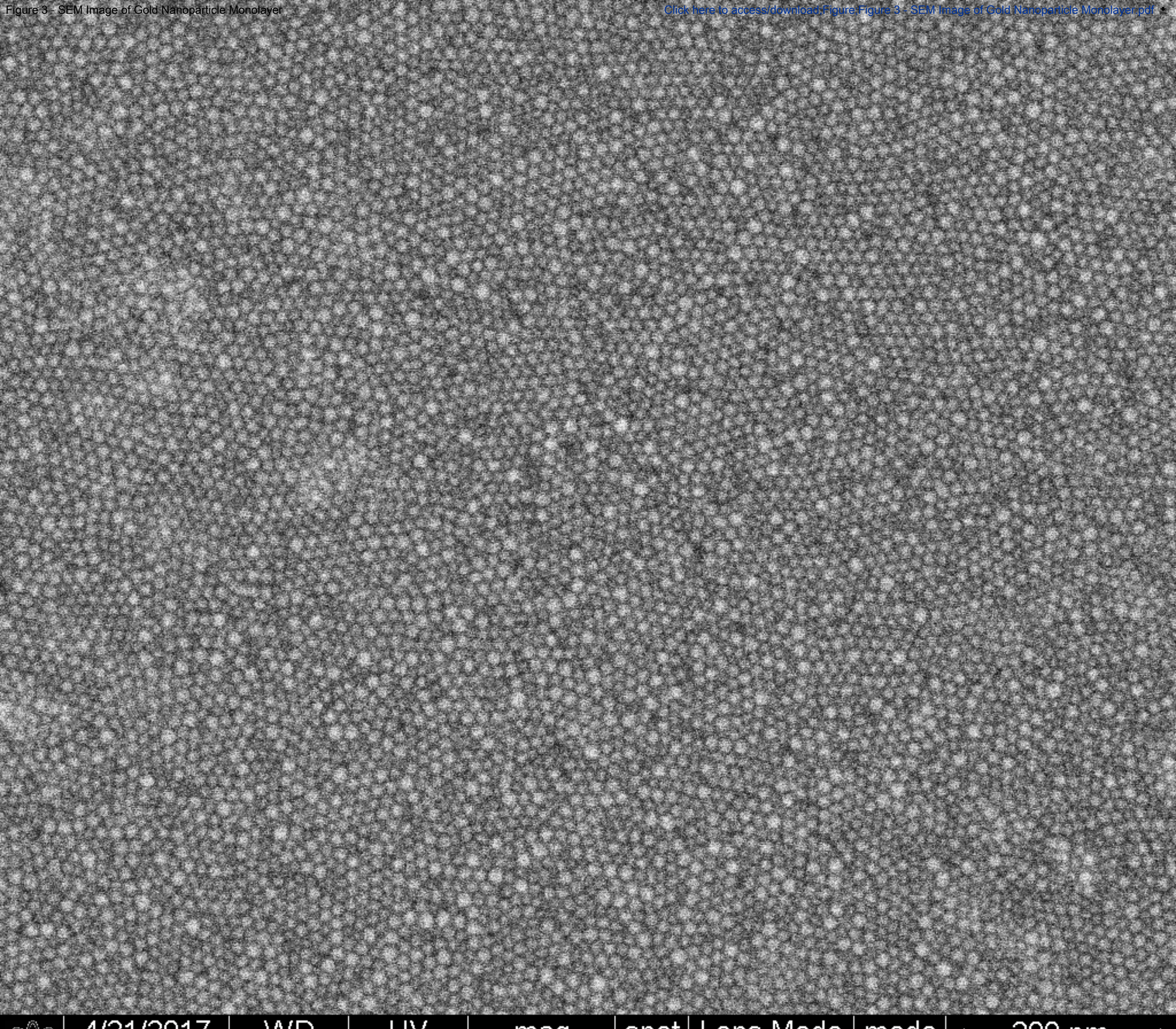
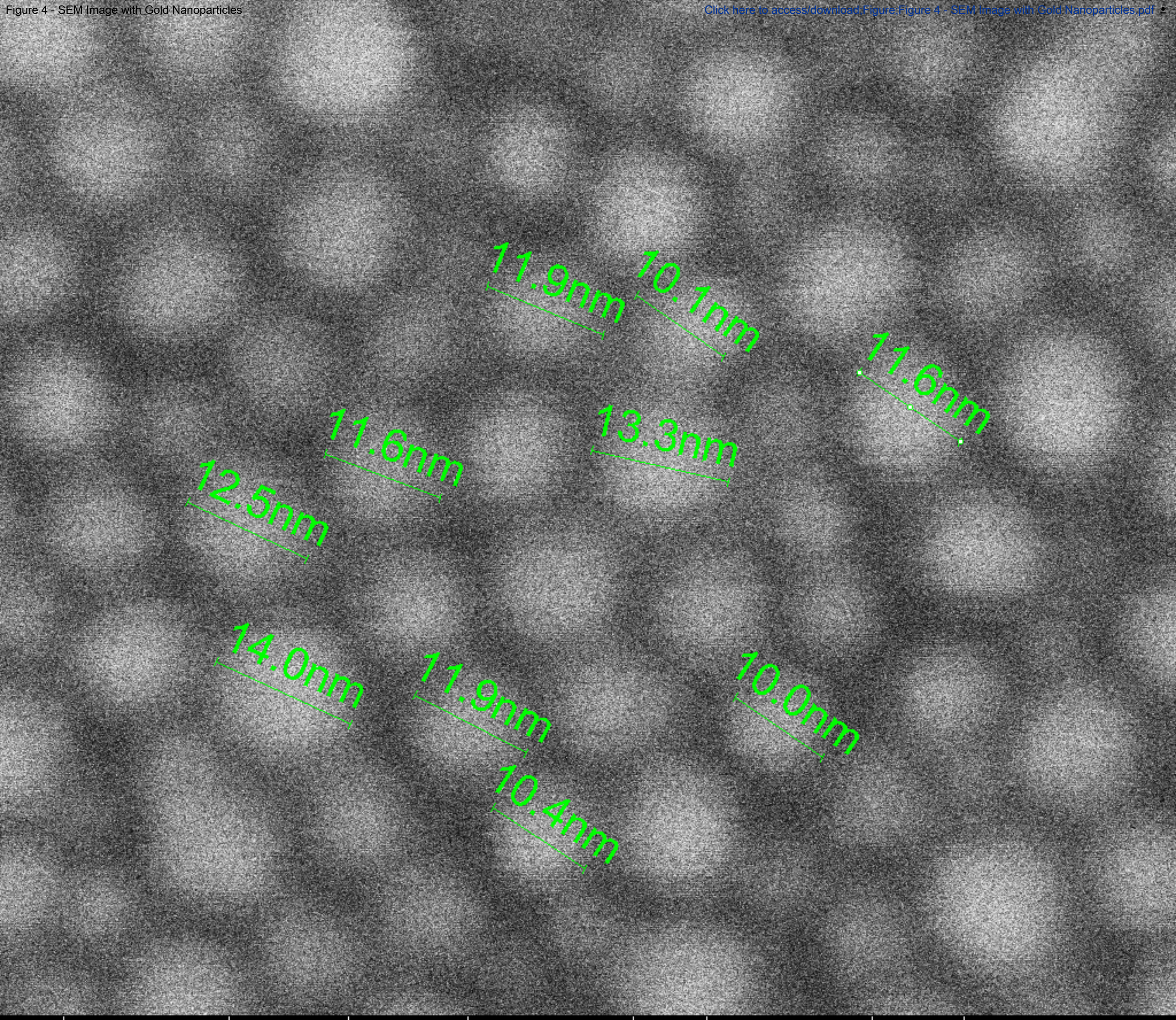


Figure 4 - SEM Image with Gold Nanoparticles

Click here to access/download;Figure;Figure 4 - SEM Image with Gold Nanoparticles.pdf



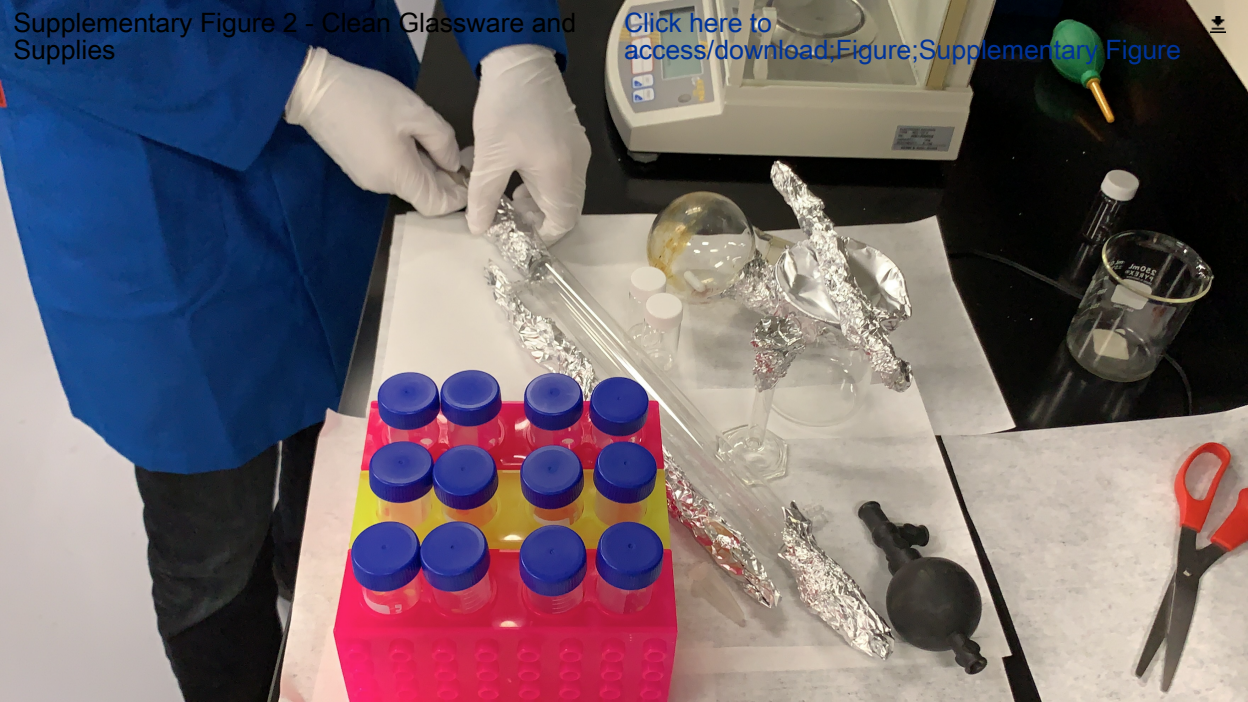
4/21/2017	WD	HV	mag	spot	Lens Mode	mode	10 nm
3:43:28 PM	5.4 mm	10.0 kV	2 677 706 x	2.0	Immersion	SE	

Supplementary Figure 1 - Cleaning Chemical Reaction Glassware

Click here to access/download;Figure;Supplementary

Supplementary Figure 2 - Clean Glassware and Supplies

Click here to access/download;Figure;Supplementary Figure



Supplementary Figure 3 - Gold Nanoparticle Synthesis Experimental Setup

Click here to access/download;Figure;Supplementary

Supplementary Figure 4 - Mixing

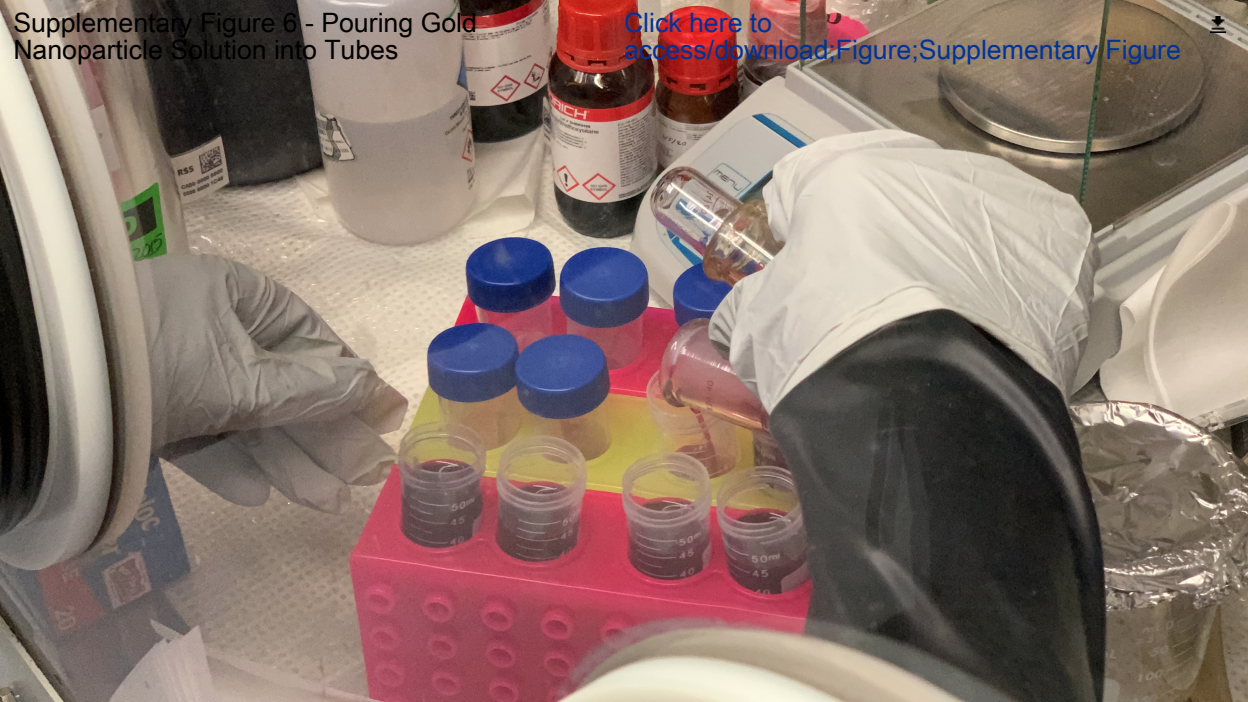
Click here to access/downlo

Supplementary Figure 5 - Preparing to Inject Solution into Reaction Vessel

Click here to access/download;Figure;Supplementary Figure

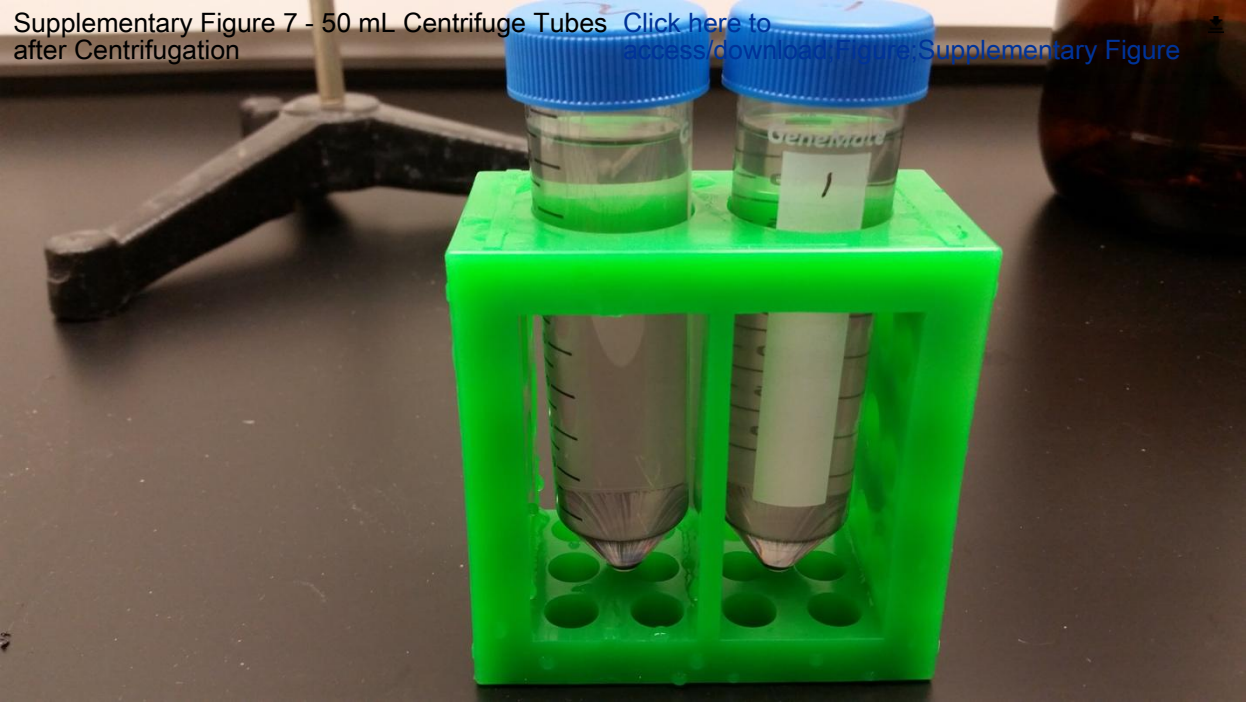
Supplementary Figure 6 - Pouring Gold Nanoparticle Solution into Tubes

Click here to access/download;Figure;Supplementary Figure



Supplementary Figure 7 - 50 mL Centrifuge Tubes after Centrifugation

Click here to access/download;Figure;Supplementary Figure

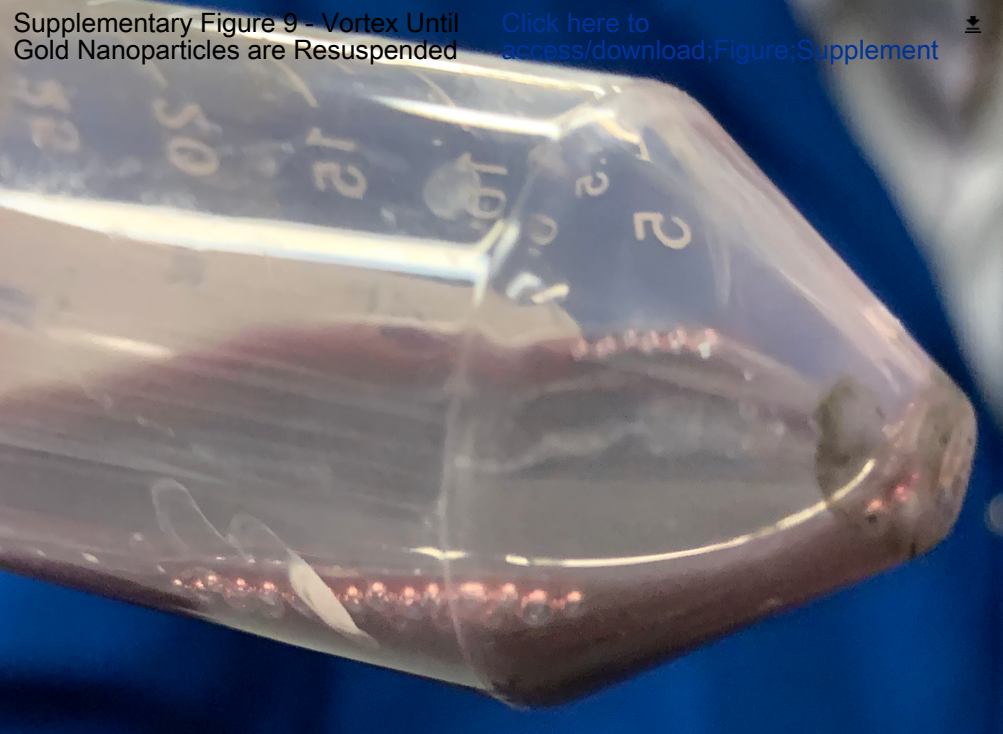


Supplementary Figure 8 - Vortexing Centrifuge Tubes with Au NPs

Click here to access/download;Figure;Supplementary

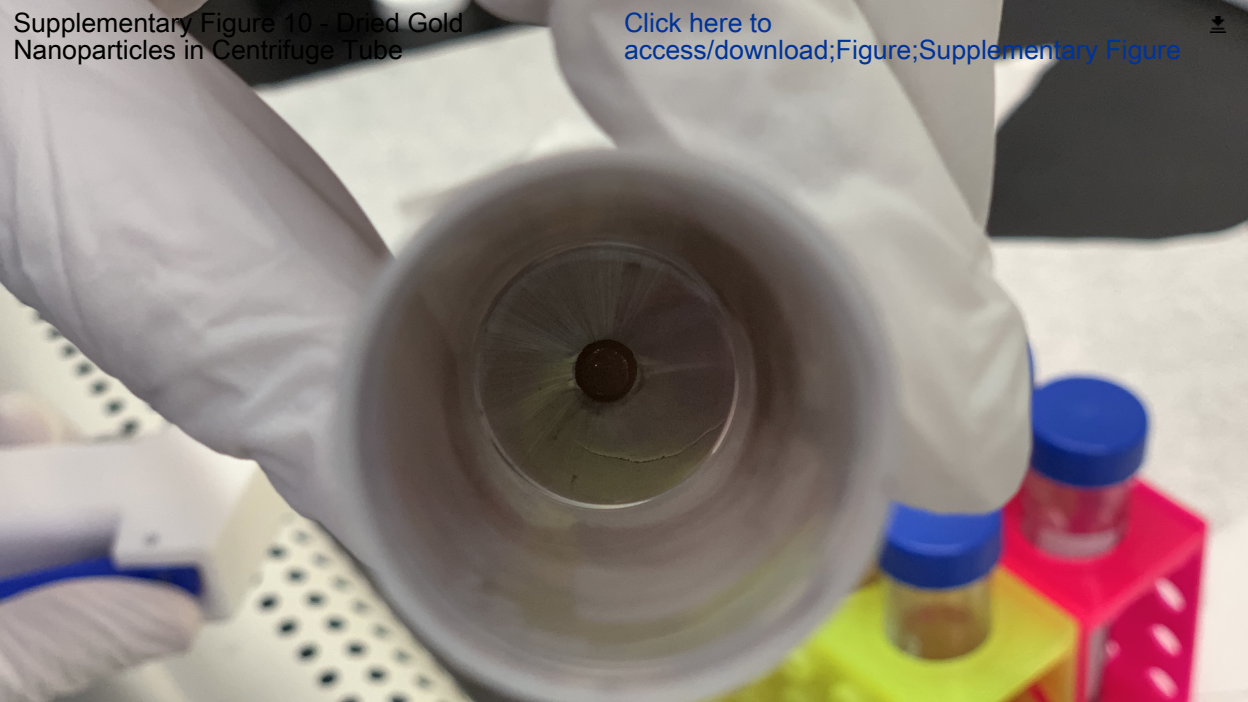
Supplementary Figure 9 - Vortex Until Gold Nanoparticles are Resuspended

Click here to access/download;Figure;Supplement



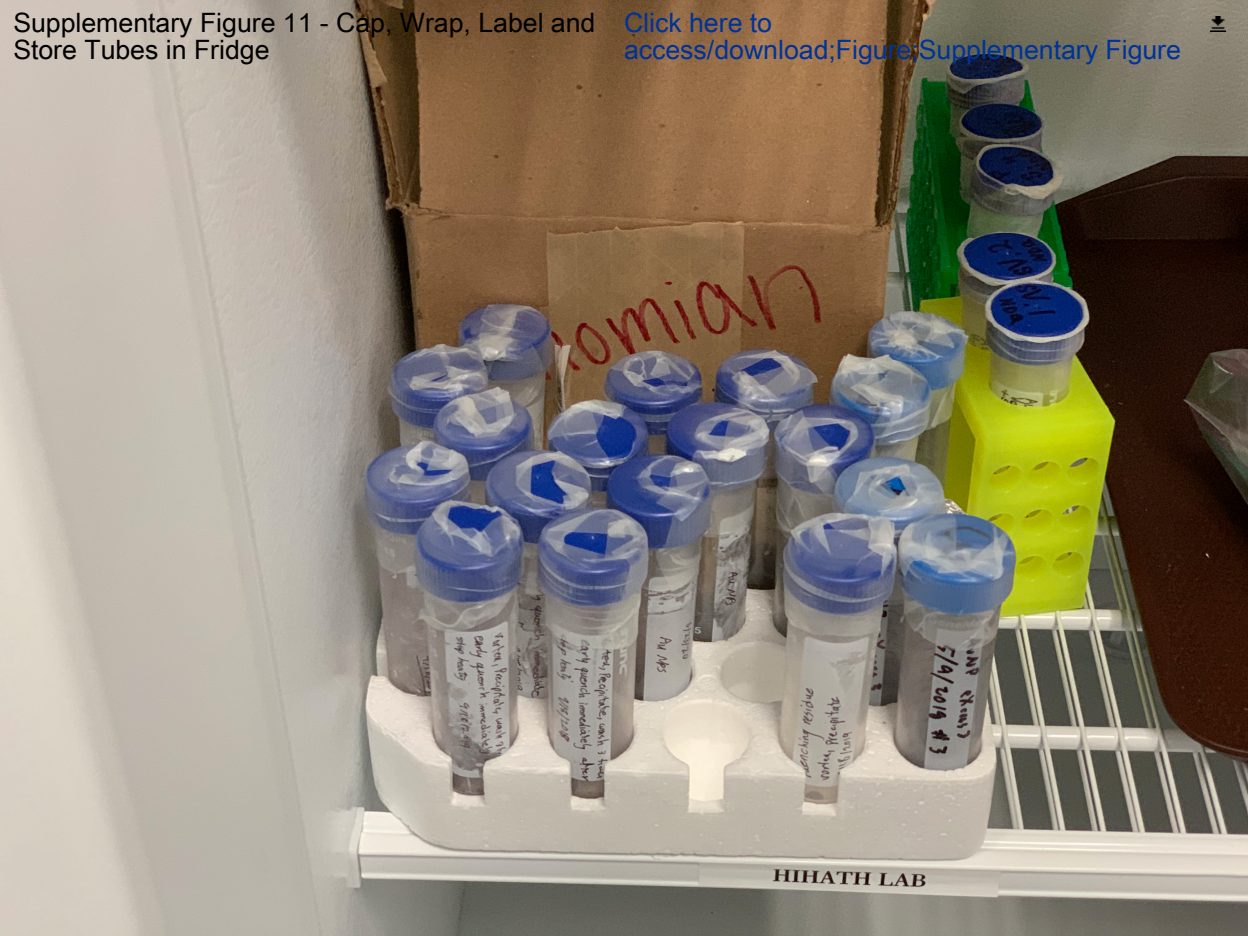
Supplementary Figure 10 - Dried Gold Nanoparticles in Centrifuge Tube

Click here to access/download;Figure;Supplementary Figure



Supplementary Figure 11 - Cap, Wrap, Label and Store Tubes in Fridge

Click here to access/download;Figure;Supplementary Figure



Solution Type	Item Number	Amount and Type of Chemical
Injection	1	150 mg of tetrachloroauric acid ($HAuCl_4$) (0.15 mmol)
	2	3.0 g (3.7 mmol, 3.6 mL) of oleylamine
	3	3.0 mL of toluene
Boiling	1	5.1 g (6.4 mmol, 8.7 mL) of oleylamine
	2	147 mL of toluene
Washing/Purification	1	10 mL of toluene (x3 washes) (x12 tubes) = 360 mL of toluene
	2	40 mL of methanol (x3 washes) (x12 tubes) = 1.44 L of methanol
Gold Etchant	1	150 mL of gold etchant TFA [or aqua regia]
	2	150 mL of deionized (DI) water

Comments/Description
for injecting into reaction vessel
for boiling in reaction vessel
for washing/purifying gold nanoparticles
for cleaning chemical reaction glassware/supplies

Gold Nanoparticle Synthesis – JoVE – Rebuttal Document

Editorial and production comments:
Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
The manuscript has been proofread for spelling and grammar issues.

2. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note."
The protocol section was revised to use the imperative tense.

3. The Protocol should contain only action items that direct the reader to do something. Please convert section 1 of the protocol as a table and reference the table here. Also please ensure that the table is uploaded as .xlsx file.
Section 1 of the protocol was converted into a .xlsx file table.

4. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, alphabets, or dashes. Please do not number "caution" and "note" but start it from a new line.
The numbering of the Protocol was adjusted to follow the JoVE Instructions for Authors.

5. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed?
Details have been added to the protocol steps.

6. Line 113: How do you perform these reactions?
Line 113 was modified to explain that a nitrogen glove box should be used to perform the chemical preparations/reactions, if possible. The detailed instructions for performing the chemical reactions are explained later in the document when the reactions are actually being performed.

7. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step.
The protocol has been simplified so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step.

8. Please revise the protocol text to avoid the use of any personal pronouns in the protocol

(e.g., "we", "you", "our" etc.).
The protocol text was revised to avoid using any personal pronouns.

9. Please include a one-line space between each step substep, note and caution statements. Please ensure that the protocol text is no more than 10 pages including the headings and spaings.
I included a one-line space between each step, substep, note and caution statement. Unfortunately, it was not possible to reduce the protocol text to no more than 10 pages due to the requested formatting/one-line spaces between steps in the text, as well as the required details and complexity of the protocol.

10. Please ensure all figures are referenced in the text in order.
All figures are now referenced in the text in order.

11. Some of the figures can be removed as there is an associated video and some of the figures can be converted to supplementary files.
Some of the figures that were featured in the associated video were removed, and some of the figures were converted to supplementary files.

12. We cannot have commercial terms in the manuscript. Please remove commercial terms and use generic terms instead. e.g., Styrofoam, Centrifuge label in Figure 13 (This figure can be removed), etc.
Commercial terms (e.g., Styrofoam, Parafilm) have been removed, and have been replaced with generic terms. Figure 13 has been removed.

13. Please ensure the results are described in the context of the presented technique. e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. Data from both successful and sub-optimal experiments can be included.
The results are described in the context of the presented technique.

14. Please expand the figure legends to include a title and a short description of the data presented in the Figure and relevant symbols.
The figure legends have been expanded as requested.

15. As we are a methods journal, please ensure that the Discussion explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique
The Discussion section now explicitly covers the above.

16. Please sort the materials table in alphabetical order.

The materials table has been sorted in alphabetical order.

Changes to be made by the Author(s) regarding the video:
1. Please increase the homogeneity between the video and the written manuscript. Ideally, all figures in the video would appear in the written manuscript and vice versa. The video and the written manuscript should be reflections of each other.
Corrected.

2. Furthermore, please revise the narration to be more homogenous with the written manuscript. Ideally, the narration is a word for word reading of the written protocol. However, not the entire protocol needs to be presented in the video. Only the protocol highlights which forms a cohesive story should be in the video and remaining text can stay in the manuscript.
Corrected.

3. Please ensure that the protocol section heading for text and video are same.
Corrected.

4. For most part of the video, hand in covering the action. Please ensure that the actual action is seen.
It's difficult to get a different visual angle on some parts of the gold nanoparticle synthesis process because it is performed in a nitrogen glove box, so there will be some shots where there are gloved hands that are covering objects/actions that might have otherwise been visible.

5. For the title card please follow our guidelines: Title followed by authors name, followed by affiliation. All should be in one card.
Corrected.

6. @ 15 minutes of duration, you are right up against the limit of JoVE video duration. Consider making some edits to remove extraneous, redundant, or prerequisite information. The aim should be to make the shortest video possible while still maintaining the integrity and core features of the protocol. Some information can be assumed, and some can be consolidated or abbreviated.
Corrected.

7. • 00:31 Remove parenthesis around two bottom sentences ("All chemicals..." and "Place these...", they are unnecessary.
Corrected.

8. Protocol section: The main feature of the video should be the picture, not the text. If you would like to convert the on-screen text to synchronized closed captions / subtitles, that would be acceptable, but the text should be in sync with the narration and not come in paragraph form as currently presented- it should be broken up into maximum two lines of text per on-screen phrase. For example: @01:06, this paragraph of text should not be displayed all at once.
Corrected.

9. • 01:19 There is an editing glitch here, as the scaling of the video image changes instantly. If this is from an in-camera zoom, start the clip after the zoom.
Corrected.

10. • 01:43 There is some background noise of machinery here. This noise should be muted and only the narration should be heard. If this noise is part of the narration track, then it may need to be re-recorded in a quieter location. This principle should be applied to all clips with background noise. Unless hearing the noise is essential to understanding the protocol, please mute the sounds from all clips except for the narration.
Corrected.

11. • 05:10 Some of the narration and instruction here regarding use of the microbalance can probably be omitted. Consider editing out some of the more specific (but assumed) instructions such as the tare operation and the other fine details about the vial and scale. The aim is to weigh out some tetrachloroauric acid, so consider skipping over the prep steps of using the microbalance.
Corrected.

12. • 06:45 Especially here, there is another person speaking while the narration continues. So do mute the background audio.
Corrected.

13. Since your location falls under our videographer network, our professional team can produce the video for the submitted manuscript. Please let me know via email. In this case, please select JoVE Produced Video in the editorial manager during submission and would require additional charges to cover up the video production cost.
No, thank you.

14. Please describe the result with respect to your experiment, you performed an experiment, how did it help you to conclude what you wanted to and how is it in line with the title. Please include all the result figures in this case. Also please ensure that for figures with microscope please include a scale bar.
Corrected.

15. Please ensure there is a title card at the end of the video as well.
Corrected.

Once done please ensure that the video is no more than 15 min in length. Please upload the revised to:
https://www.dropbox.com/request/vuSwEYVKwuJBFkIwdpLQ?oref=e

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The mansucript contributed by Marrs et al. decribes a protcol of synthesizing gold nanoparticles that is performed in climate-controlled glovebox. The as-synthesized gold nanoparticles are uniform in size around 12 nm.

Major Concerns:

1. Can this protocol be performed using Schlenk line to control the reaction environment? If ths synthesis can be carried out with Schlenk line, the synthesis protocol can be adapted widely because not all labs are equipped with the expensive glovebox.

Although it may be possible to use a Schlenk line instead of a nitrogen glove box for providing an inert atmospheric environment for the chemical reaction, there will be some degradation in the quality of the gold nanoparticles (e.g., conglomerates, polydisperse nanoparticles) if the gold nanoparticle precursor/injection solution (especially the tetrachloroauric acid) is not prepared in an inert environment such as a nitrogen glove box. Therefore, a nitrogen glovebox is strongly recommended at least for handling/storing the tetrachloroauric acid and preparing the gold nanoparticle precursor/injection solution. If a nitrogen glovebox is not available, it may be possible to use a Schlenk line instead for the chemical reaction, although some degradation in the quality of the nanoparticles should be expected if the injection solution is exposed to ambient air. Degraded gold nanoparticles that are polydisperse or non-uniform in size may be acceptable for some applications, so the decision to use a Schlenk line instead of a nitrogen glovebox will depend on the application.

2. Can the size of gold nanoparticles be tuned?

The size of the gold nanoparticles can be tuned to some extent by decreasing the reaction time (e.g., 30 minutes instead of 2 hours). The less the reaction time, the smaller the gold nanoparticles will be, as the nanoparticles grow over time due to Ostwald ripening (smaller gold nanoparticles merging with larger gold nanoparticles). However, the gold nanoparticles may be polydisperse rather than monodisperse if the reaction time is too short, so there is a tradeoff between creating very small nanoparticles and creating monodisperse nanoparticles. A longer reaction time (e.g., 4 hours instead of 2 hours) may lead to larger nanoparticles, although there is a limit to how large the nanoparticles can grow as eventually the chemical reagents get used up, and the growth due Ostwald ripening diminishes as the gold nanoparticles increase in size. There is also the possibility of tuning the size of the gold nanoparticles by changing the amount or proportion of chemical reagents/precursors, although these parameters have not been optimized yet, and the gold nanoparticles may also become more polydisperse rather than monodisperse as the amount or proportion of chemical reagents change. If ~12 nm gold nanoparticles are not desirable, then some further experimentation and optimization will be necessary to arrive at a gold nanoparticle synthesis recipe that produces the desired size of gold nanoparticles.

3. In the video, some frames are full of text that block the operation details.

The video will be edited to clarify the operation details.

Minor Concerns:
Why was DI water used for condenser?
DI water was used in the condenser tube because the wet chemical laboratory where the gold nanoparticle synthesis was performed has a readily available supply of DI water. However, tap water or some other water source would be acceptable as well, as the water doesn't come in contact with the chemical reagents. The purpose of the water flowing through the inner chamber of the condenser tube is to cool the tube and condense/collect the toluene/oleylamine vapor as it boils in the reaction vessel. However, it may be preferable to use DI water because it is cleaner than regular tap water, so using DI water should help to keep to glassware clean overall.

Reviewer #2:
Manuscript Summary:
This study focused on synthesis of AuNPs in the organic phase. The preparation procedure provided detail recipe. All steps are similar to aqueous AuNPs, only use oleylamine and toluene to replace sodium citrate and water.
The prepared AuNPs were characterized by SEM with size of 12 +/- 2 nm, indicating the excellent monodispersity. This manuscript was written well and eventually could be accept after minor correction.

Major Concerns:
none

Minor Concerns:
1. UV-Vis and XRD analysis were suggested to characterize the prepared AuNPs.
UV-Vis can be used to verify the size of the gold nanoparticles, as well as the concentration of the gold nanoparticles in solution. In some applications, knowing the concentration of gold nanoparticles is important, such as when forming monolayers of gold nanoparticles by suspending the gold nanoparticle solution on top of DI water in test tubes and allowing the toluene to evaporate. The gold nanoparticle monolayers will not form properly (e.g., they will form multi-layer clusters or have holes/gaps in the monolayer) if the concentration of gold nanoparticles is too high or too low.
The size as well as the monodispersity/polydispersity of the gold nanoparticles can be characterized by imaging these gold nanoparticles with scanning electron microscopy (SEM) after transferring the gold nanoparticle monolayers onto a substrate (e.g., silicon chip) using a PDMS stamp. Overall, the type of characterization that is performed on the gold nanoparticles depends on the application requirements of the gold nanoparticles.

2. Please indicate the advantages of the proposed preparation method comparing to that prepared in aqueous system.

One of the primary advantages of the proposed preparation method compared to the aqueous system is that the gold nanoparticles are soluble in organic solvents such as toluene rather than water. This allows the gold nanoparticles to be utilized in applications that require solutions of organic solvents rather than aqueous solutions. There are many molecules that are soluble in organic solvents such as toluene, but not soluble in water. These oleylamine-capped gold nanoparticles can be utilized in applications that required the oleylamine ligands to be exchanged with other molecules that are soluble in organic solvents. For example, we utilize these oleylamine-capped gold nanoparticles by exchanging the oleylamine ligands with organic molecules (e.g., alkanethiols). This would not be possible with water-soluble gold nanoparticles as some organic molecules (e.g., alkanes) are nonpolar and are not soluble in water.
Another advantage is that the oleylamine ligands are quite long and therefore the gold nanoparticles tend to aggregate less in solution. The gold nanoparticles can also be dried and stored for long periods of time in a fridge, giving them a long shelf life of at least 1 year. The gold nanoparticles can then be readily resuspended by vortexing them in toluene. The ability of the oleylamine-capped gold nanoparticles to aggregate less in solution, be kept for long periods of time in the dry state, and be easily resuspended back into solution are all advantages that should save money, produce less waste, require less frequent gold nanoparticle synthesis procedures, as well as providing the possibility of exchanging the oleylamine ligands with various other molecules that are soluble in organic solvents.