SUPPORTING INFORMATION

A "tips 'n tricks" Practical Guide to the Synthesis of Gold Nanorods

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EXPERIMENTAL SECTION

MATERIALS:

Hexadecyltrimethylammonium bromide (CTAB, \geq 99.0 %), hexadecyltrimethylammonium chloride (CTAC, 25 % in water), 5-bromosalicylic acid (technical grade, 90 %), hydrogen tetrachloroaurate trihydrate (HAuCl₄ · 3 H₂O, \geq 99.9 %), silver nitrate (AgNO₃, \geq 99.9 %), L-ascorbic acid (AA, \geq 99 %), sodium borohydride (NaBH₄, 99 %) and trisodium citrate (\geq 98 %) were purchased from Aldrich. All chemicals were used as received. Milli-Q water (resistivity 18.2 M Ω ·cm at 25 °C) was used in all experiments. All glassware was washed with aqua regia (10 min), rinsed with water, sonicated three fold for 3 min with Milli-Q water, and dried before use.

GENERAL TIPS:

The following general practical tips can be of help in the synthesis of Au nanorods, both in terms of product quality and reproducibility of the results.

- Stock Solutions:

- ❖ AgNO₃ 10 mM (17 mg in 10 mL water) freshly prepared every week, stored in the fridge protected from sunlight; use a plastic spatula or Pasteur pipette to weigh the salt, *i.e.* avoid contact with metal surfaces.
- ❖ Ascorbic acid 100 mM (176 mg in 10 mL water) freshly prepared every week, stored in the fridge protected from sunlight.
- ❖ HAuCl₄ 50 mM stored in the fridge and protected from sunlight, stable over long periods of time.
- ❖ CTAB 0.1 M (1.82 g in 50 mL water 30 °C to dissolve) stable over long periods of time.
- ❖ HCl 1M (10 mL of HCl 37% + 110 mL of water) stable over long period of time
- ❖ NaBH₄ 10 mM (7.6 mg in 20 mL water) freshly prepared; as the powder is hygroscopic it should be weighed quickly.
- Absorbance at 400 nm: after each growth step, it is important to verify that the absorbance at 400 nm corresponds to the amount of gold precursor that has been reduced (see section 2.4); this parameter is really important since it indicates the presence of unreacted Au(I), which can compromise the stability of the rods over time.
- CTAB foam: when the solution is shaken by hand or stirred, CTAB leads to foam formation. When adding a reagent with a micropipette, dip the micropipette tip sufficiently inside the foam to add the reagent directly in the solution; anything that goes into the foam itself will not mix properly in the growth mixture, affecting the overall synthetic conditions. Hand shaking may eventually put the solution in contact with the cap, so it must be thoroughly cleaned to avoid contamination.
- Color shade and scale-up process: it is important to remember that the timing, intensity and shade of the color appearance will change if the synthesis is scaled up, since the "optical path length" is increased.

SYNTHESIS OF SEED@CTAB for the preparation of SINGLE CRYSTAL Au NANORODS:

The synthesis is performed in a water bath at 27-30 °C. After adding 25 μ L of 50 mM HAuCl₄ solution to 4.7 mL of CTAB 0.1 M, the mixture is slowly stirred for 5 min: any sign of turbidity requires further stirring (see section 2.2 and 2.3). Then, 300 μ L of a freshly prepared NaBH₄ 10 mM solution is rapidly injected under vigorous stirring (>1400 rpm). After 10-20 s the solution is mildly stirred (400 rpm) before use (light brown color, **Figure 1A**).

N.B.: The seed must be stored between 27 and 30 °C; a lower temperature will cause CTAB crystallization, whereas a higher temperature will induce further growth of the seed into bigger particles, thereby compromising seeded growth.

These seeds will be used for the synthesis of single crystal Au nanorods in the following three protocols.

SYNTHESIS OF SINGLE CRYSTAL Au NANORODS (1):

Growth (values for 10 mL):

The entire process is performed in a water bath at 30 °C. After addition of 190 μ L of HCl 1M and 100 μ L of HAuCl₄ 50 mM solution to 10 mL of CTAB 0.1 M, the mixture is gently shaken and stirred for 5 min (see section 2.2 and 2.3); the final pH should be around 1.5. Subsequently, 120 μ L of AgNO₃ 10 mM solution is added to the mixture, which is then gently shaken for few seconds. 100 μ L of ascorbic acid solution (100 mM) is then added to the growth solution and thoroughly shaken, turning colorless in few seconds. Finally, 24 μ L of seeds@CTAB is added to the mixture and the solution is vigorously shaken and then left undisturbed at 30 °C for 2 hours.

N.B.: The final product will present an absorbance maximum around 900 nm and average dimensions around 70x17 nm; the absorbance ratio between the longitudinal and transverse LSPR bands should be above 5.

SYNTHESIS OF SINGLE CRYSTAL Au NANORODS (2): Salicylic acid as additive

Gold nanorods are prepared by a modified version of the seeded growth method reported by Murray's group.¹ The process is carried out entirely at room temperature (ca. 25 °C).

Pre-reduction (values for 50 mL):

45 mg of 5-BrSalicylic acid is added to 50 mL solution of CTAB 0.05 M. Once salicylic acid is completely dissolved (sonication helps), 480 μ L of AgNO₃ 10 mM solution is added under mild stirring. After 15 min, 500 μ L of HAuCl₄ 50 mM solution is added to the mixture under mild stirring. The pre-reduction of Au(III) to Au(I) by salicylic acid starts **immediately after addition**.

N.B.: This reduction is highly sensitive to temperature: if warming up the solution was required to dissolve CTAB and 5-BrSA, wait until it cools down to room temperature before adding the gold salt (this can be a serious issue when you are dealing with large volumes).

Pre-reduction can be easily monitored by optical spectroscopy: the [AuBr₄] complex is orange, with an absorbance maximum at 396 nm, whereas [AuBr₂] is colorless; therefore a reduction in absorbance at 396 nm corresponds to more Au(III) precursor being reduced to Au(I).

Growth (values for 50 mL):

The aspect ratio of the nanorods can be controlled through pre-reduction time: the more Au(III) you reduce by 5-BrSA, the lower the final AR will be. As a reference, an absorbance at 396 nm between 0.8 and 0.85 will result in a longitudinal plasmon band centered around 800 nm.

Immediately after reaching the desired value of absorbance at 396 nm the stirring speed is increased above 1000 rpm and 130 μ L of AA 100 mM solution is added to the growth solution. The mixture turns colorless within few seconds. After stirring for 30 s 80 μ L of seed solution is finally added to start the growth. After 30 s stirring is detained and the solution is left undisturbed for 4 hours.

N.B.: The optical properties of the final product can be tuned via pre-reduction.² With an LSPR maximum around 800 nm the average dimensions are around 60x20 nm; the ratio between the longitudinal and transversal LSPR bands should be above 4.

N.B.: The absorbance at 400 nm is also dependent from the pre-reduction step. In this specific synthetic protocol, for a product with an LSPR around 800 nm, the absorbance at 400 nm is low, close to 0.4; consequently, almost 60% of the initial Au(I) is left in solution. Even in the absence of ascorbic acid, Au(I) will be reduced over time (a week or so) on the surface of your NRs, leading to changes in AR and size dispersion. To avoid that, centrifuge the NRs (9508g, 20 min) within the first 24 h. It is important to stress here that low values of absorbance at 400 nm are typical of this specific synthetic process, and not a general trend in SC Au NR synthesis: for example, in the method above, an absorbance of 1.2, corresponding to quantitative reduction, is typically achieved (see section 2.4).

SYNTHESIS OF SINGLE CRYSTAL Au NANORODS (3): small nanorods

Growth (values for 10mL):

We describe a modified version of the seeded growth method reported by Nikoobakht and El-Sayed. 3,4 A solution of HAuCl₄ (100 μ L, 50 mM) is added to CTAB (10 mL, 100 mM); the mixture is gently shaken and kept for 10 min thermostated at 27 °C in a water bath to ensure complexation between gold salt and CTAB. Afterwards, ascorbic acid solution (75 μ L, 100 mM) is added to the mixture, which is gently shaken for few seconds (the solution turns colorless). A solution of AgNO₃ (80 μ L, 5 mM) is added to the growth solution which is shaken for few seconds. Finally, the seeds solution (120 μ L) is added to the mixture and the solution is vigorously shaken for the last time and then left undisturbed at 27 °C for 30 min in a water bath.

N.B.: The final product will present an absorbance maximum around 800 nm and average dimensions around 40x10 nm; the absorbance ratio between the longitudinal and transverse LSPR bands should be above 3.

PURIFICATION OF SINGLE CRYSTAL AU NANRODS:

Purification is based on a modified version of the method reported by Jana.⁵ Typically, the solution of as-prepared nanorods is centrifuged at 8000 rpm for 30 min. The precipitate containing particles is collected and dissolved in hot (50 °C) CTAB solution (3 mL, 300 mM). The solution is then transferred into a glass test tube (10 mL), which is placed vertically. A brown precipitate at the tube bottom is observed upon cooling at room temperature (ca. 2 hours).

N.B.: The slow cooling process will avoid crystallization of CTAB due to supersaturation.

The precipitate containing nanorods is separated carefully from the supernatant using a Pasteur pipette. Nanorods are redispersed in water. The supernatant contains cubes, spheres and small nanorods (**Figure 4**).

SYNTHESIS OF PENTATWINNED Au NANORODS:

Seeds@Citrate:

At room temperature (ca. 25 °C), 100 μL HACl₄ 50 mM is added to 20.0 mL of Na₃Cit 0.25 mM. 600 μL of a freshly prepared NaBH₄ 100 mM solution is rapidly injected under vigorous stirring (>1400 rpm). After 2 min the solution is kept under mild stirring (400 rpm) for 40 min at room temperature and for 15 min at 40-45 °C before use (red color, **Figure 1A**).

N.B.: The solution must be "clean"; in case black spots are floating around repeat the preparation. The UV-vis characterization shows a peak centered at 507 nm, with an absorbance at 400 nm around 0.6.

Seeds@CTAB:

12.5 μ L of 0.05 M HAuCl₄ is added to a mixture of 3 mL of water and 2 mL of CTAB 0.1M. The solution is cooled down to 22 °C in a thermostatic bath. 12.5 μ L 0.1 M ascorbic acid is then added to the solution and shaken by hand; the mixture turns colorless in few seconds. Finally 835 μ L of the Seeds@Citrate solution is added, shaken by hand and left undisturbed for 3 hours at 22°C

N.B.: UV-vis characterization shows a peak centered at 522 nm, with an absorbance at 400 nm around 0.35.

Growth (values for 500 mL):

CTAB (40 mL 0.1M) is added to 460 mL of water. 1.25 mL of HAuCl₄ 0.05 M solution is then added, the solution gently shaken and cooled down to 20 °C in a thermostatic bath. Subsequently, 1.56 mL of 0.1 M ascorbic acid solution is added to the mixture, and the solution gently shaken until it turns completely colorless. Finally 650 μ L Seeds@CTAB is added to the growing mixture; the solution is vigorously shaken by hand and then left undisturbed overnight at 20 °C.

N.B.: Cooling down 500 mL of solution takes at least 30 minutes. The vis-NIR shows a longitudinal LSPR band around 1100 nm. The maximum achievable shape-yield is around 30%, which can be verified through the optical spectra: the ratio between the longitudinal LSPR band in the NIR and the isotropic by-product plasmon centered around 530 nm should be ca. 1 (Figure 4D red line). The absorbance at 400 nm must be around 0.3, indicating a quantitative reduction of the gold precursor (0.125 mM).

PURIFICATION OF PENTATWINNED AU NANRODS:

The solution is transferred to a test tube, so as to decrease the volume to base-surface ratio. Then, 70 mL of CTAC 25 wt % (~ 0.78 M) is added, with a final CTAC concentration around 0.1 M. The test tube is sealed and the solution is homogenized by turning the test tube upside-down several times. Precipitation of pentatwinned Au nanorods is completed within 16 hours (**Figure 4**). The supernatant is then discarded using a pipette and the purified product can be easily redispersed in 20 mL of Milli-Q water.

N.B.: Flocculation of the pentatwinned Au nanorods can be identified by vis-NIR spectroscopy after 20-30 minutes: the longitudinal band should disappear; but this does **not** mean that purification has been completed: plasmon coupling of the aggregated nanorods leads to disappearance of the band, but the deposition will still take several hours to be completed.

N.B.: The low shape-yield makes it difficult to actually see the precipitated nanoparticles. Therefore, extra care must be taken when the supernatant is discarded.

An alternative would be leaving 20-25 mL of the supernatant, redispersing the precipitated nanorods by shaking and sonication of the test tube and transferring the remaining suspension into a smaller test tube, repeating the purification process overnight. With a smaller base-surface, the precipitated pentatwinned nanorods can be clearly appreciated as a black-gold patina.

N.B.: The amount of CTAC to be added depends on the aspect ratio and dimensions of the nanorods, which influence the area in eq. [3]; in order to start the flocculation a value of |U| around 10 k_BT must be achieved: smaller NRs will thus demand a higher CTAC concentration. Calculation of the amount of CTAC to be added to achieve purification requires taking into account the presence of CTAB used in the synthesis, for which the following equation is suggested:

$$V_{x} = \frac{V_{in}(C_{fin} - C_{in})}{C_{stock} - C_{fin}}$$
 [5],

where V_x is the volume of CTAC stock solution (of concentration C_{stock}) needed to reach the desired final concentration C_{fin} from a solution of nanoparticles with an initial volume V_{in} and an initial concentration C_{in} .

References

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