

Supplementary Information

Facile Gold Nanorod Purification by Fractionated Precipitation

T. Thai,^{a,c} Y. Zheng,^{b,c} SH. Ng,^{a,c} H. Ohshima,^d M. Altissimo^c and U. Bach^{a, b, c}

^a Department of Materials Engineering, Monash University Wellington Road, Clayton, Victoria 3800, Australia

^b Materials Science and Engineering, Commonwealth Scientific and Industrial Research Organisation Bayview Avenue, Clayton, Victoria 3168, Australia

^c Melbourne Centre for Nanofabrication 151 Wellington Road, Clayton, Victoria 3168, Australia

^d Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan

I. Materials and chemicals

4-inch p-type silicon wafers were purchased from Atecom Technology (Taipei, Taiwan) with a [100] crystal orientation. The second type of 4-inch <100> p-type silicon wafers coated with a 110 nm layer of SiO₂ were purchased from UniversityWafer.com (Boston, USA). Titanium (Ti, 99.995% purity) and gold (Au, 99.99% purity) pellets for electron beam evaporation were acquired from Ezzi Vision (Scoresby, Australia). The gold (III) chloride trihydrate (HAuCl₄), sodium borohydride (NaBH₄), L-ascorbic acid, (3-aminopropyl)triethoxysilane (APTES) and bis(p-sulfonatephenyl)phenylphosphine dehydrate dipotassium salt (BSPP) were purchased from Sigma-Aldrich. The surfactant cetyltrimethylammonium bromide (CTAB) was obtained from GFS Chemicals. The silver nitrate (AgNO₃) was purchased from Fluka, the 2-[methoxy(polyethyleneoxy)propyl]trimethoxysilane (PEG-silane) from Gelest, the thiol-PEG-carboxyl (SH-C₁₁H₂₂-(OCH₂CH₂)₆-OCH₂COOH) from Prochimia and the poly(methyl methacrylate) PMMA used for the electron beam lithography (EBL) from MicroChem Corp.

II. Methods

Chemical synthesis of gold nanorods (GNRs)

The well-known seed-mediated synthesis¹ was scaled up to syntheses up to 200 ml to work with highly concentrated GNR solutions (see below).² The protocol was carried out in water baths at 30 °C. All stock solutions were prepared on the same day with ultrapure water. The CTAB stock solution was prepared by stirring the solution at 600 rpm during 30 min at 30 °C for complete dissolution. For the preparation of the NaBH₄ solution, the desired amount of ultrapure water was kept in a beaker filled with ice. The following amount of AgNO₃ corresponds to GNRs with an aspect ratio of 3. Firstly, a seed solution was prepared by

adding an ice-cold NaBH_4 solution (600 μl , 0.01 M) into a mixture of HAuCl_4 (25 μl , 0.1 M) and CTAB (10 ml, 0.1 M), which was then vigorously stirred for 3 minutes at 1,000 rpm. A growth solution was made by mixing HAuCl_4 (1000 μl , 0.1 M), AgNO_3 (160 μl , 0.1 M) and CTAB (200 ml, 0.1 M) followed by the addition of ascorbic acid (1260 μl , 0.1 M). Finally the seed solution (200 μl) was added to the growth solution and aged for 2 hours. The aged solution was transferred into 50 ml Falcon tubes. Each tube was filled with 33 ml of solution and centrifuged at 5,000 rcf for 3.5 hours. The supernatant was discarded and replaced by ultrapure water. The process was repeated a second time and concentrated to 100 ml.

Purification of GNR solution by fractionated precipitation

As-synthesized GNR solutions are washed with ultrapure water by centrifugation and concentrated to 100 μl with a final concentration of 33.5 nM (3 mg/ml).² GNRs are then functionalized with thiol-PEG-carboxyl. In this procedure, 5 μl of 2% Tween 20 is added to the GNR solution followed by 30 μl of 0.1 M phosphate buffer, 5 μl of 100 mM BSPP, 5 μl of 1.6 mM thiol-PEG-carboxyl and 50 μl of ultrapure water. The mixture is left in a vortexer at 300 rpm for 4 hours. Then, 300 μl of NaCl is added for the first purification step. The NaCl concentration is adjusted to identify the final NaCl concentration at which the precipitation of spherical byproducts is maximized. 1 M has shown to be an adequate final NaCl concentration for these experimental conditions. The solution is vortexed for 2 hours at 300 rpm. The precipitated spherical nanoparticles are centrifuged at 1,000 rpm for 1 min to facilitate the supernatant extraction. Once the supernatant is pipetted out, the nanoparticulated byproducts are redispersed in a solution of 0.05% Tween 20 and 20 mM phosphate buffer. For the second purification step, 100 μl ethanol is added to the supernatant. The mixture is left for 30 min in a vortexer at 300 rpm. The time and the volume of ethanol are tuned to precipitate the maximum amount of GNRs while leaving other nanoparticulate byproducts in the supernatant. The latter is pipetted out into another Eppendorf microcentrifuge tube. The desired GNR precipitates are redispersed in a solution of 0.05% Tween 20 and 20 mM phosphate buffer. UV-Vis spectra were collected with Cary 60 spectrometer.

APTES surface treatment

To confer positive charges to the surface of non-patterned silicon substrates, they were modified with a layer of APTES. 4 μl of APTES were mixed in an Eppendorf microcentrifuge tube with 190 μl of ethanol and 6 μl of ultra pure water. The substrate was immersed for 1 hour and then washed with ethanol 3 times. Each wash consists of pipetting out the supernatant, adding fresh ethanol and vortexing the microcentrifuge tube. The substrate is dried under a stream of nitrogen and finally baked at 110 °C for 10 min.

Template fabrication

A 4-inch silica-coated silicon wafer was immersed in piranha solution (3:1 by volume mixture of concentrated H_2SO_4 and 30% H_2O_2) for 15 minutes. It was then transferred to ultrapure water for another 10 minutes. The water was replaced 3 times before the wafer was rinsed thoroughly with ultrapure water to remove any residue. After cleaning, the wafer was immediately transferred to a Laurell WS-650MZ-23NPP/Lite spin coater. 1.5 ml of 950PMMA-A4 (950 kD MW, 4% in anisole) was extracted from its bottle using a plastic bulb pipette. The PMMA was applied in a continuous stream, spiraling from the center to form a pool of PMMA roughly 4 cm in diameter. Great care was taken to prevent the production of

air bubbles and to that effect, some PMMA was left in the pipette. The wafer was spun at 500 rpm for 30 seconds, then 2,000 rpm for 60 seconds. Immediately after spin coating, it was transferred to a preheated hot plate and baked at 170 °C for 1 and a half minutes. From AFM measurements conducted with a Dimension Icon by Veeco, this resulted in a 350 nm thick PMMA layer. The resist-coated wafer was mounted onto a 4-inch wafer holder then transferred into the electron beam lithography instrument. Then the aperture of the instrument was set to 200 μm using the command line and manually on the hardware. This was aligned manually using feedback from the software. The pattern was written using the following parameters: an accelerating voltage of 100 kV, a dose of 1200 $\mu\text{C}/\text{cm}^2$, beam current and step size of 10 nA and 25 nm respectively. After writing, the wafer was immersed for 1 minute in a developer solution consisting of a 1:3 volume ratio of methyl isobutyl ketone to isopropyl alcohol, then transferred to (IPA) for another minute. It was subsequently rinsed with IPA and dried with nitrogen. An optical microscope was used to check that that sufficient development had occurred. To etch the structures into the SiO₂ layer, the wafer was transferred to an inductively coupled plasma reactive ion etching (ICP-RIE) instrument. Two different gasses were used for etching, SF₆ was set with a flow rate of 7 sccm and CHF₃ was set for 50 sccm. The machine ICP and RIE power was set to 2500 W and 50 W respectively. The gas pressure was set to 7 mT and the He float to 10 T. The wafer was etched for 15 seconds, which, from prior AFM measurements resulted in a SiO₂ etch depth of 55 nm. 3 nm of Ti were deposited by electron beam evaporation followed by 23 nm of Au.

Surface passivation with PEG-silane

To passivate silica surfaces on patterned substrates, a solution of PEG-silane was prepared by mixing 2.5 μl of PEG-silane and 1.25 ml of toluene. After vortexing the solution, 1 μl of HCl was added and left to react for 1 min. The supernatant is then transferred into other microcentrifuge tubes (200 μl per tube) where substrates are left to react for 50 min. The substrates are washed 3 times with toluene and then with ethanol. Finally, the substrates are dried under a stream of nitrogen.

Electrostatic immobilization of GNRs onto APTES modified Si substrates

The assembly of thiol-PEG-carboxyl functionalized GNRs onto APTES-modified silicon substrates were done in a hybridization chamber from Corning Incorporated. Droplets of ultrapure water were deposited in the humidification wells to maintain a constant humidity during the immobilization. 6 μl of functionalized GNRs in ultrapure water was placed onto the positively charged substrates and left to incubate for 4 hours. The substrates were thereafter dipped in and out three times in ultrapure water to remove non-specific adsorbed nanoparticles and dried under a stream of nitrogen. Samples were analyzed with a Helios NanoLab focused-ion beam SEM from FEI.

For the statistical analysis, the data corresponding to the “1st step” was obtained by analyzing GNRs after undergoing a salt-induced precipitation at 1M. For the “2nd Step”, the analysis was performed on GNRs after an ethanol-induced precipitation for 30 min at a volume concentration of 20%.

Gel electrophoresis

1% agarose solution was prepared by adding 3 g of agarose and 300 ml of 0.05x Tris-borate-EDTA (TBE) buffer into a 500 ml conical flask. The conical flask was then put into a 60 °C

water bath for about 2 hours to dissolve the agarose. Subsequently, the hot agarose solution was poured into a casting tray in a gel box sideways. All bubbles were pushed away to the side using a disposable tip. The wells of the gel were made by inserting a comb into the slots in the tray. The gel was left to cool down to room temperature prior to the transfer to an electrophoresis cell. 50 μ l of gold nanoparticle solutions obtained at different stages of the separation process was added into the wells and 0.05x TBE was used as a running buffer. A voltage of 25 V was applied to the electrophoresis cell for about 8 hours.

Fabrication of Standing Arrays of Gold Nanorods

Firstly, the functionalized GNRs solution was adjusted to 9 nM in an Eppendorf tube containing 120 μ l in a buffer of 0.05% Tween 20, 20 mM phosphate buffer and 0.5 M NaCl. The substrate was immersed in the GNR solution and placed in the center of the Eppendorf tube. It was slightly pushed downward so that it could stand vertically without any tilted angle. The microcentrifuge tube that contained the substrate and the GNR solution was placed into a thermoblock system that ensures a precise fit for the Eppendorf tube. The thermoblock was inserted into the Eppendorf Thermomixer, heated at 45 $^{\circ}$ C and left to dry for approximately 12 hours. Before the complete evaporation of the solvent, the substrate was transferred from the microcentrifuge tube to a Kimwipe tissue for a gentle drying at room temperature. Samples were kept in a desiccator to avoid any water condensation on the substrate surface.

Estimation of the fraction of spherical nanoparticles and nanoplates after each purification step

Spherical nanoparticles

Statistical analysis displayed in Fig. 3a was conducted based on the precipitation of spherical byproducts at a salt concentration of 1M. After the first purification step at 1M, the fraction of spherical nanoparticles in the supernatant decreased from 2.6% to 0.2%. The absorption of spherical byproducts that have precipitated at a salt concentration of 1M can be found in Fig. 2a.

The fraction of spherical byproducts ($F_s(c)$) in supernatant after the first purification step at a salt concentration c can be determined according to the following equation:

$$F_s(c) = \left[2.6 - \frac{A_c}{A_{c=1M}} \times 2.4 \right] \%$$

with A_c the absorption of the spherical byproducts in the redispersed precipitate after the first purification step at a salt concentration c and $A_{c=1M}$ the corresponding absorption following precipitation at salt concentration of 1M.

Nanoplates

The nanoplates from the supernatant after the second purification step exhibit a shoulder peak at 555 nm (Fig. 2b). The absorption at 555 nm in Fig. 2b (A_{555}) can be defined as the sum of the absorption of GNR ($A_{555(\text{GNR})}$) and nanoplates ($A_{555(\text{NP})}$) (Eq. 1):

$$A_{555} = A_{555(\text{GNR})} + A_{555(\text{NP})} \quad (1)$$

We define k as the ratio between the absorption at the transverse ($A_{555(\text{GNR})}$) and longitudinal ($A_{\text{long}(\text{GNR})}$) peaks for a purified GNR solution that can be determined based the absorption in Fig. 2b (purified GNR solution, dotted line):

$$k = \frac{A_{555(GNR)}}{A_{long(GNR)}} \quad (2)$$

We assume that k does not vary considerably when the GNR longitudinal peak undergoes a 50 nm shift. Thus, the absorption of nanoplates can be estimated integrating Eq.(2) into Eq.(1) :

$$\begin{aligned} A_{555} &= A_{555(GNR)} + A_{555(NP)} \\ A_{555} &= k.A_{long(GNR)} + A_{555(NP)} \\ A_{555(NP)} &= A_{555} - k.A_{long(GNR)} \end{aligned}$$

Following the same approach described for the spherical byproducts, the fraction of nanoplates in the purified GNR solution after the second step can be estimated by the following equation:

$$F_{NP}(c) = \left[9.4 - \frac{A_{555(NP)}}{A_{opt}} \times 7.5 \right] \%$$

III. Electrostatic interaction energy in a medium with a decreasing dielectric constant

1. Effective surface potential ψ_{eff} of a sphere of radius a

The potential around a sphere of radius a having surface potential ψ_0 at a position r far from the sphere in a symmetrical electrolyte solution of bulk concentration n and valence z can be expressed by

$$\psi(r) = \psi_{eff} \frac{a}{r} e^{-\kappa(r-a)} \quad (1)$$

or in its scaled form

$$y(r) = \frac{ze}{kT} \psi(r) = Y \frac{a}{r} e^{-\kappa(r-a)} \quad (2)$$

where r is the radial distance measured from the sphere center, ψ_{eff} is the effective surface potential, κ is the Debye-Hückel parameter, and $Y = ze\psi_{eff}/kT$ is the scaled effective surface potential of a sphere.

The effective surface potential ψ_{eff} is related to the surface potential ψ_0 by

$$\psi_{eff} = \frac{kT}{ze} \cdot \frac{8 \tanh\left(\frac{ze\psi_0}{4kT}\right)}{1 + \left\{ 1 - \frac{2\kappa a + 1}{(\kappa a + 1)^2} \tanh^2\left(\frac{ze\psi_0}{4kT}\right) \right\}^{1/2}} \quad (3)$$

or

$$Y = \frac{8 \tanh\left(\frac{y_0}{4}\right)}{1 + \left\{1 - \frac{2\kappa a + 1}{(\kappa a + 1)^2} \tanh^2\left(\frac{y_0}{4}\right)\right\}^{1/2}} \quad (4)$$

where κ is the Debye-Hückel parameter given by

$$\kappa = \left(\frac{2z^2 e^2 n}{\varepsilon_r \varepsilon_0 k T}\right)^{1/2} \quad (5)$$

where ε_r is the relative permittivity of the medium, ε_0 the permittivity of vacuum (8.854×10^{-12} F/m), k the Boltzmann constant (1.381×10^{-23} J/K), T the absolute temperature, e the elementary unit charge (1.602×10^{-19} C), and N_A the Avogadro number (6.022×10^{23} mol⁻¹).

2. The surface charge density/surface potential relationship

Further, the surface potential ψ_0 of a sphere of radius a is related to its surface charge density σ by: ³

$$\sigma = \frac{2\varepsilon_r \varepsilon_0 \kappa k T}{ze} \sinh\left(\frac{ze\psi_0}{2kT}\right) \times \left[1 + \frac{1}{\kappa a} \frac{2}{\cosh^2(ze\psi_0/4kT)} + \frac{1}{(\kappa a)^2} \frac{8 \ln[\cosh(ze\psi_0/4kT)]}{\sinh^2(ze\psi_0/2kT)}\right]^{1/2} \quad (6)$$

3. Electrostatic interaction energy between two identical spheres

The electrostatic interaction energy $V(H)$ between two spheres of radius a separated by a distance H is given by

$$V(H) = 4\pi\varepsilon_r \varepsilon_0 a^2 \left(\frac{kT}{ze}\right)^2 Y^2 \frac{e^{-\kappa H}}{2a + H} \quad (7)$$

4. The dependence of $V(H)$ on the dielectric permittivity ε_r

For the limiting case of large $\kappa a \rightarrow \infty$ and low ψ_0 , it can easily be found this dependence. In this limiting case, from Eq. (6) we have

$$\psi_0 = \frac{\sigma}{\varepsilon_r \varepsilon_0 \kappa} = \frac{\sigma}{\varepsilon_r \varepsilon_0} \cdot \sqrt{\frac{\varepsilon_r \varepsilon_0 kT}{2nz^2 e^2}} = \sigma \cdot \sqrt{\frac{kT}{2nz^2 e^2 \varepsilon_r \varepsilon_0}} \quad (8)$$

which gives

$$Y = y_0 = \frac{ze}{kT} \cdot \sigma \cdot \sqrt{\frac{kT}{2nz^2 e^2 \varepsilon_r \varepsilon_0}} = \sigma \cdot \frac{1}{\sqrt{2n\varepsilon_r \varepsilon_0 kT}} \quad (9)$$

Now there are two cases, that is, (i) constant surface potential case and (ii) constant surface charge density case. In case (i), ψ_0 remains constant (and thus σ changes) during interaction independent of H , while in case (ii) σ remains constant (and thus ψ_0 changes) during interaction independent of H .⁴

Thus the ε_r dependence of $V(H)$ can be given as follows.

Case (i)

In this case Eq. (7) reduces to

$$V(H) = 4\pi\varepsilon_r \varepsilon_0 a^2 \psi_0^2 \frac{e^{-\kappa H}}{2a + H} \quad (10)$$

which decreases as ε_r decreases.

Case (ii)

By substituting Eq. (9) into Eq. (7), we obtain

$$V(H) = \frac{2\pi a^2 \sigma^2 kT}{(ze)^2 n} \cdot \frac{e^{-\kappa H}}{2a + H} \quad (11)$$

In this case $V(H)$ depends on ε_r only through κ . But, κ increases with decreasing ε_r so that $V(H)$ decreases just as in case (i).

Both in cases (i) and (ii), $V(H)$ decrease as ε_r decreases.

5. Effective dielectric permittivity of a mixed solution

If a medium 1 dielectric permittivity ε_1 contains small amount of medium 2 of dielectric permittivity ε_2 , then the effective dielectric permittivity ε_r of a mixed solution of medium 1 and 2 is most simply given by

$$\varepsilon_r = (1 - \varphi)\varepsilon_1 + \varphi\varepsilon_2 \quad (12)$$

where ϕ is the volume fraction of medium 2.

IV. Experimental details

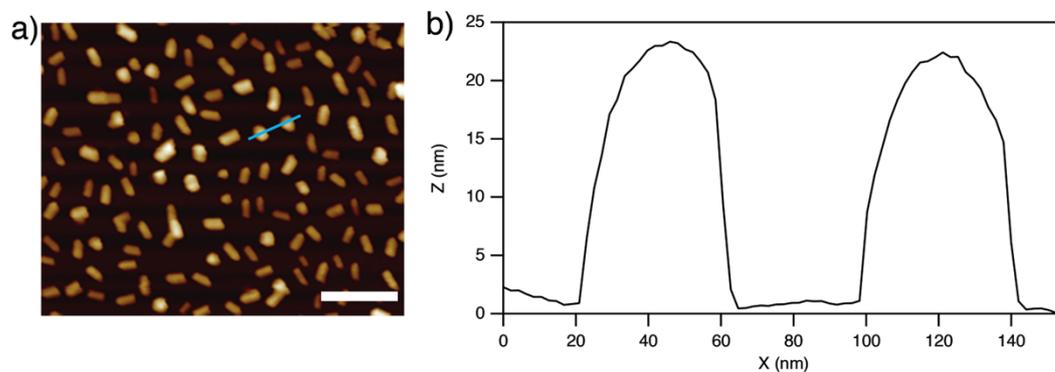


Figure S1. (a) AFM image of gold nanoparticles immobilized on a Si substrate after the first nanoseparation. The scale bar corresponds to 200 nm. (b) Cross-section analysis of two nanoplates (blue line on the AFM image). The height of nanoplates is approximately equal to 25 nm.

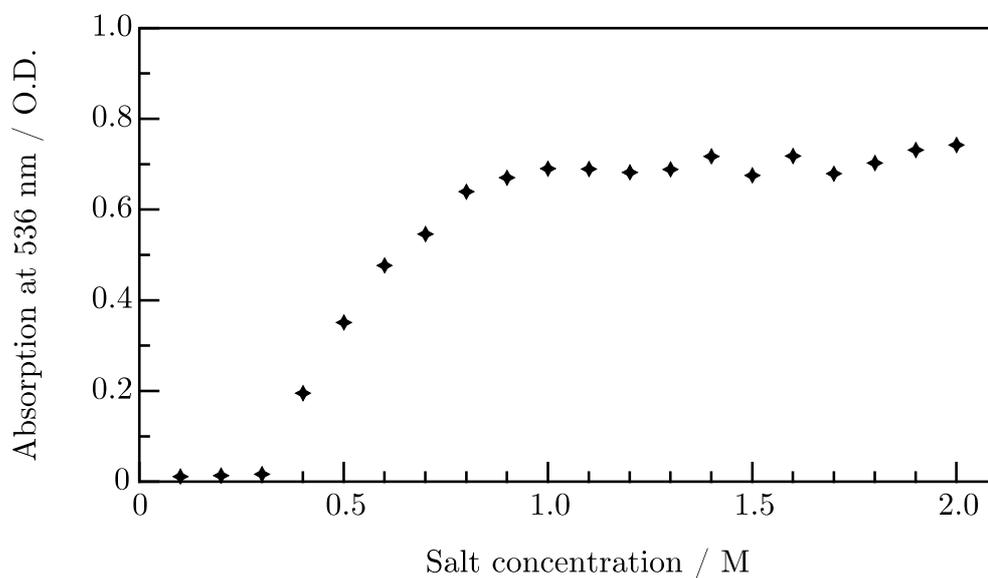


Figure S2. Spectral absorptions at 536 nm of re-dispersed large spherical nanoparticles after the first nanoseparation. The 536 nm wavelength corresponds to the absorption maximum of the spherical nanoparticulate impurities that precipitates during the first purification step. To evaluate the optimal conditions in which a maximum amount of spherical nanoparticles precipitate, the UV-Vis spectra of solutions containing re-dispersed precipitates obtained in NaCl solutions with a concentration range from 0.1 to 2M and in increments of 0.1 M were measured. This graphs plots the corresponding absorption at 536 nm on the x-axis and the associated NaCl concentration on the y-axis.

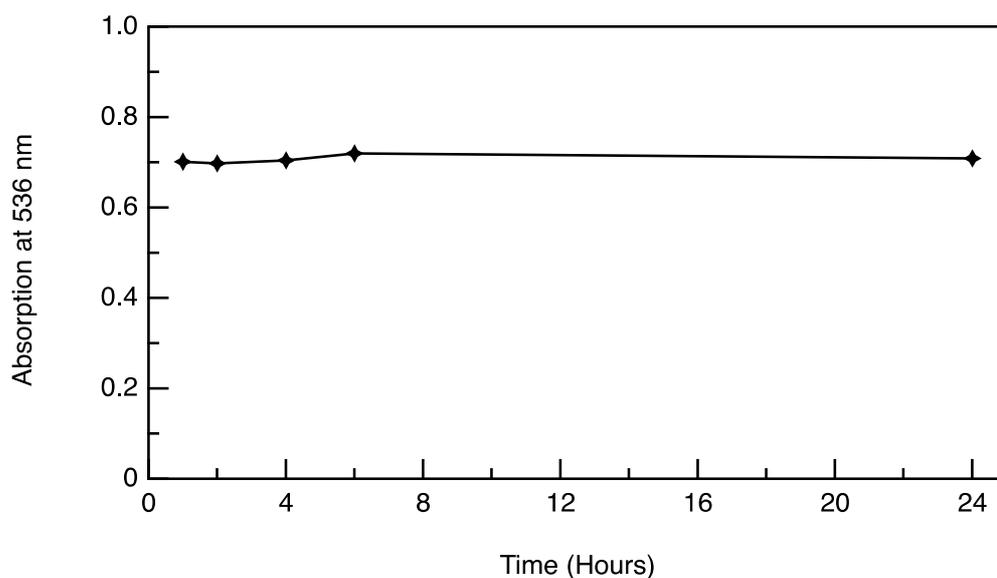


Figure S3. Effect of the precipitation time on the quantity of precipitated large spherical nanoparticles during the first nanoseparation. The amount of precipitate is measured through the spectral absorption of the re-dispersed spherical nanoparticle solution at 536 nm. The increase in the precipitation time appeared not to drastically influence the amount of spherical impurities, showing that the fractionated precipitation protocol is a convenient method that does not require a long reaction time.

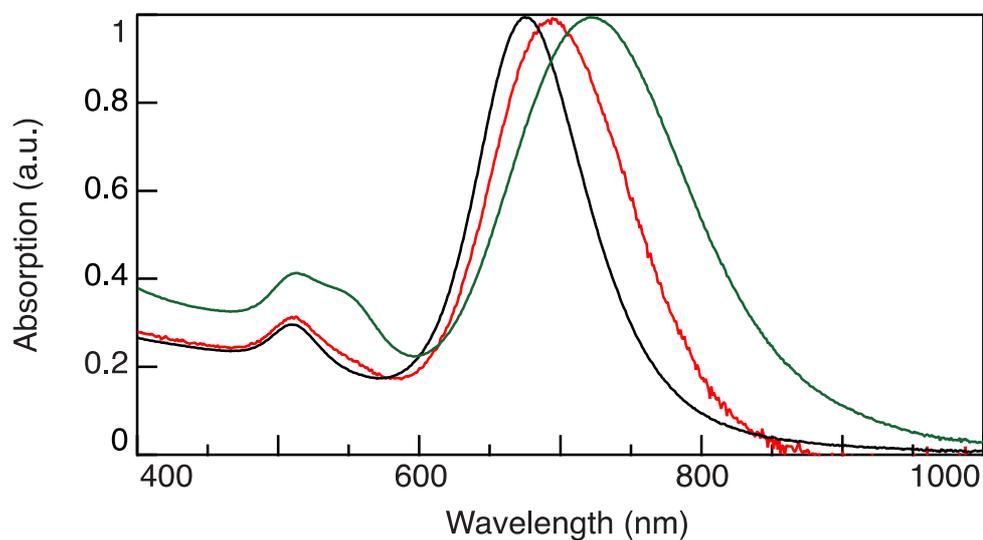


Figure S4. Difference spectrum (red) obtained by subtracting the spectrum of the supernatant after exposure to 20 vol % ethanol for 30 minutes (green) from the 60-minute spectrum. The purified GNR solution (after the two precipitation steps) is represented in black.

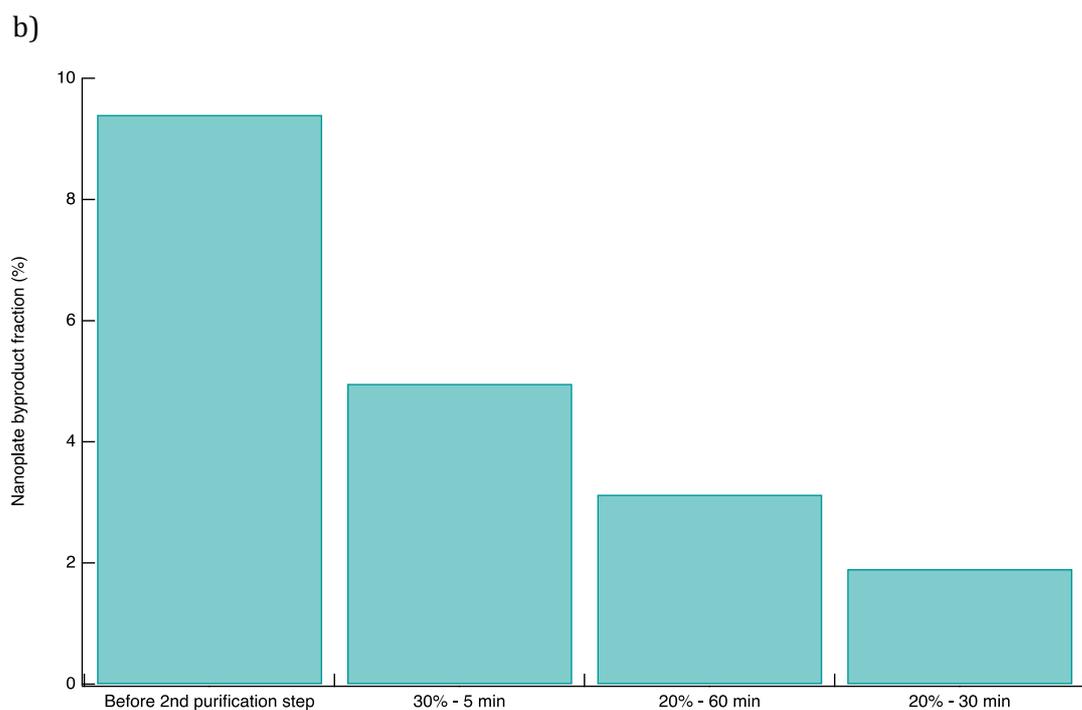
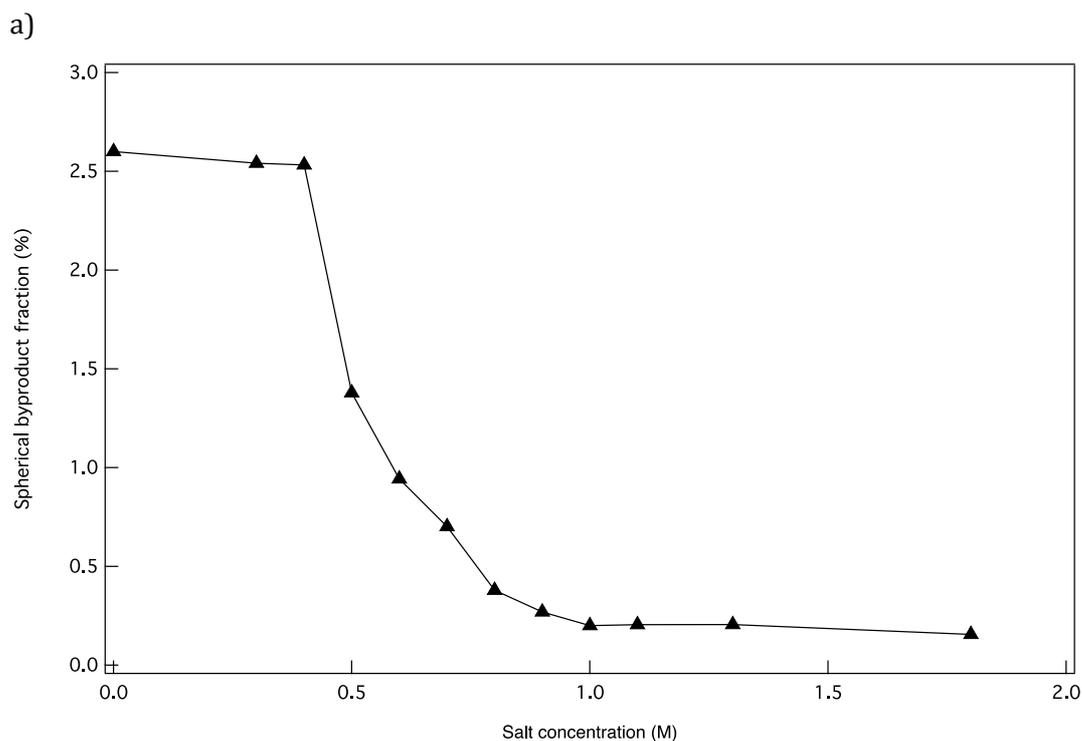


Figure S5. Estimated fraction of particle byproducts after each precipitation step as a function of the precipitation conditions shown in Figure 2. a) Estimate of the percentage of remaining large spheres in the supernatant (particle count) as a function of the salt concentration used during the first precipitation step as outlined in Figure 2a. b) Estimate of the percentage of nanoplates (particle counts) which co-precipitate with the nanorods during the second precipitation process under the three different precipitation conditions outlined in Figure 2b (ethanol vol. % and precipitation time).

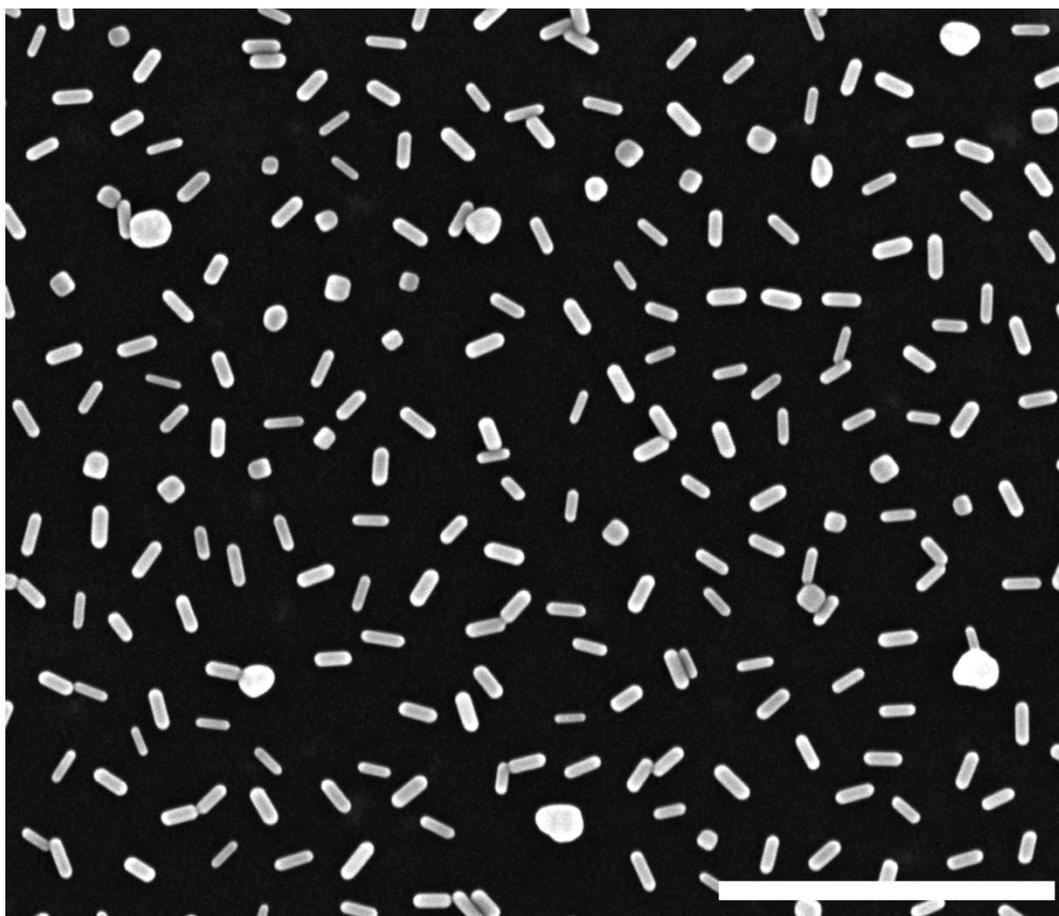


Figure S6. Low magnification SEM image of as-synthesized GNRs immobilized on a Si substrate. The scale bar corresponds to 400 nm.

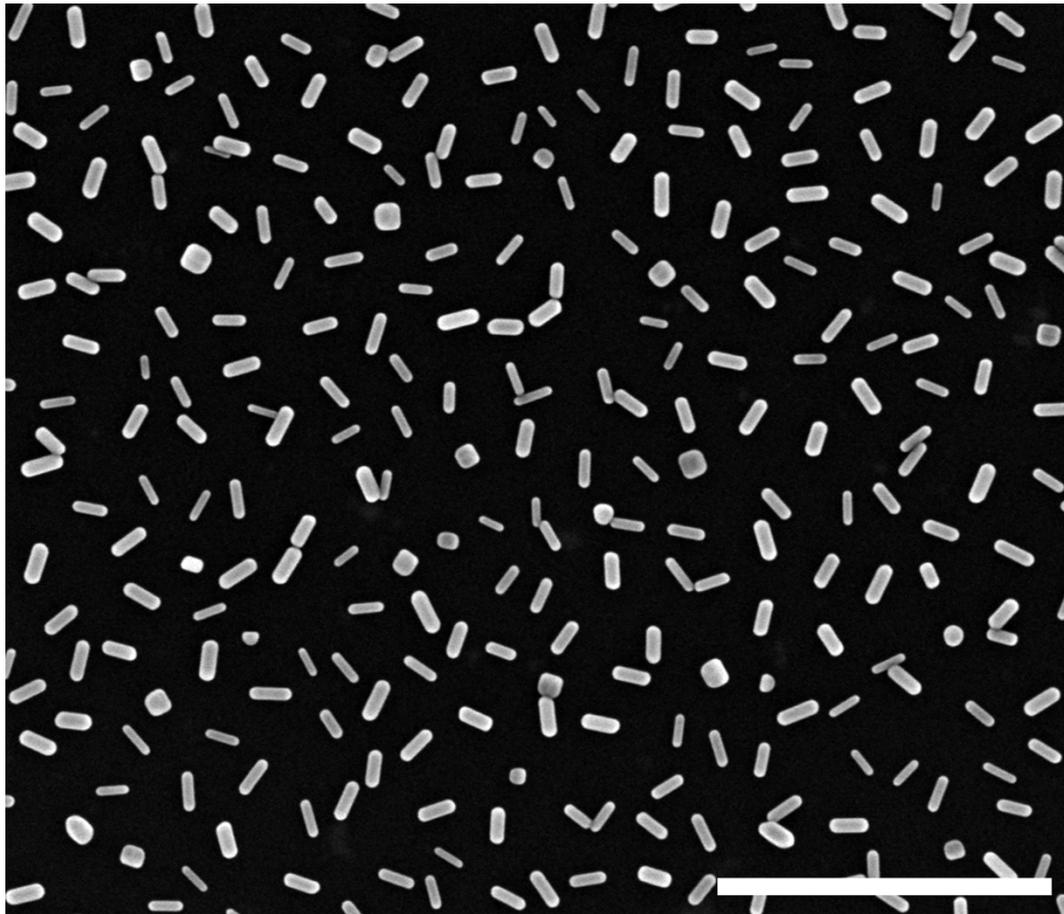


Figure S7. Low magnification SEM image of GNRs immobilized on a Si substrate after the first purification step. The scale bar corresponds to 400 nm.

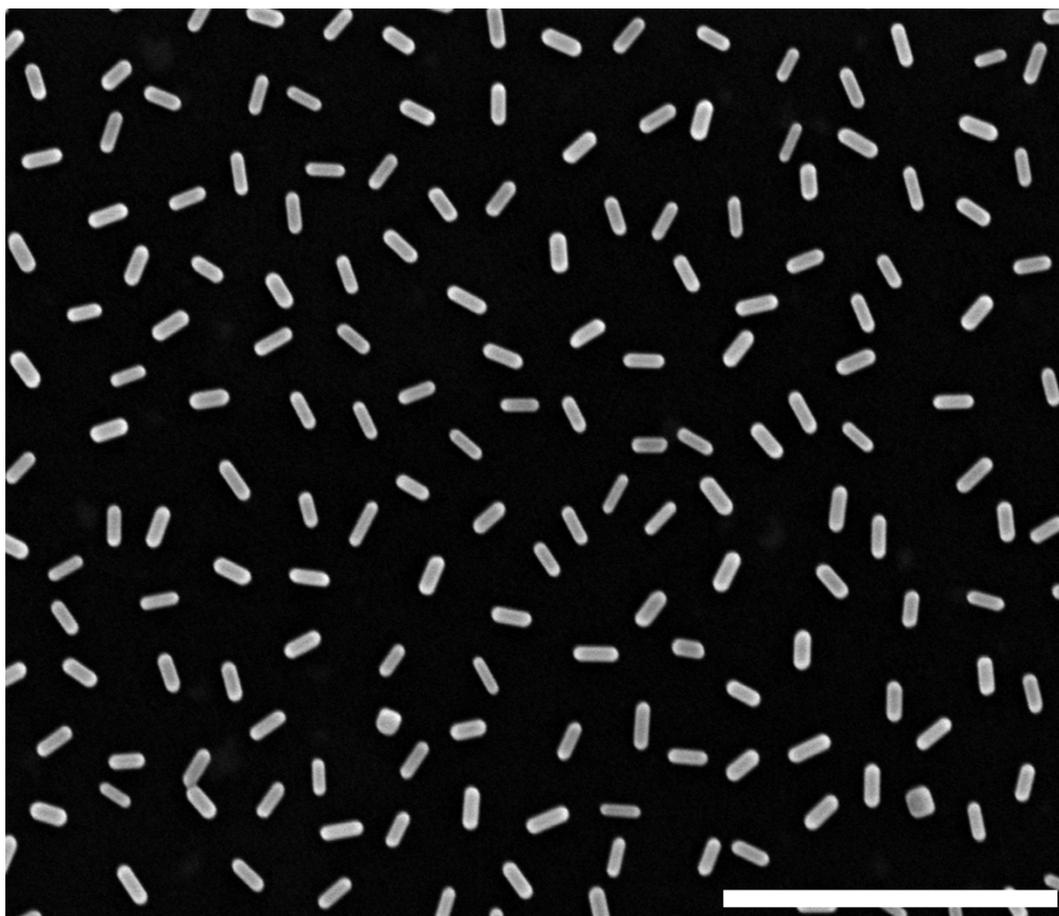


Figure S8. Low magnification SEM image of GNRs immobilized on a Si substrate after the second nanoseparation step. The scale bar corresponds to 400 nm.

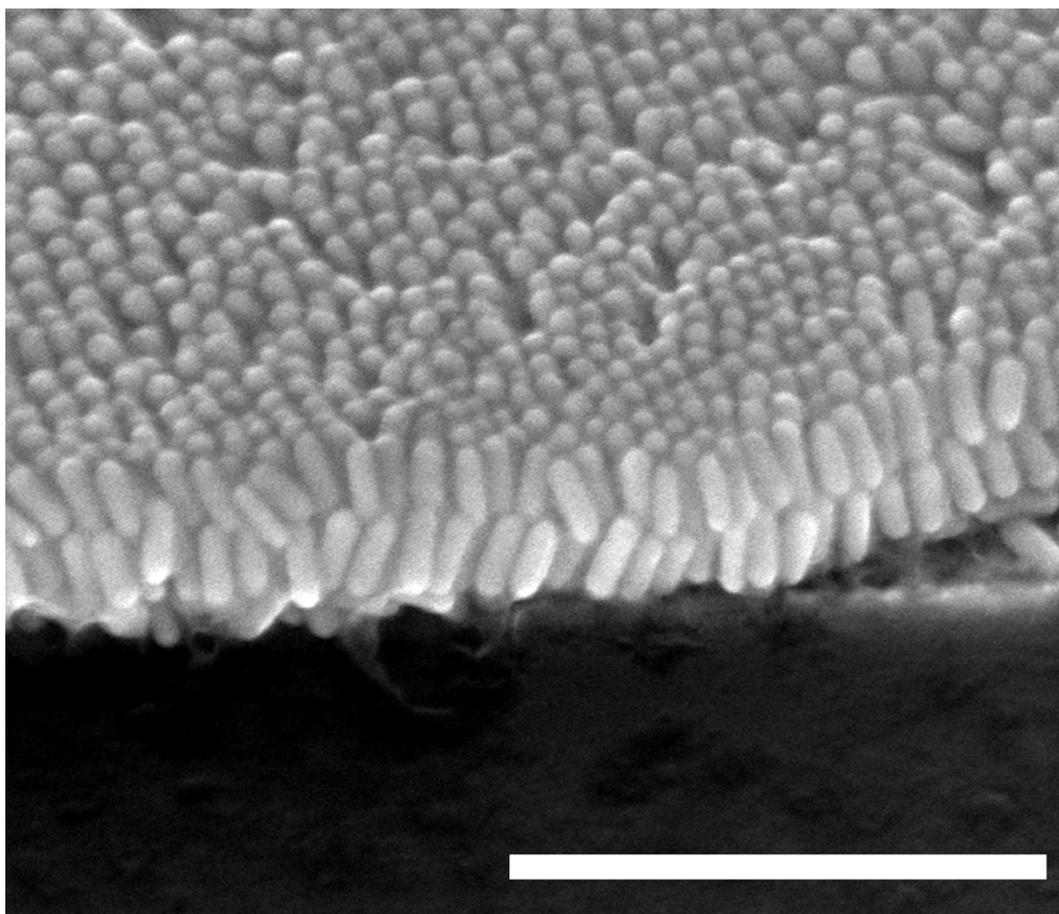


Figure S9. Tilted SEM image of one vertically aligned GNR array self-assembled with purified GNRs as building blocks. The image clearly displays the nanorod layers structured in standing arrays in a close-packed arrangement. The scale bar corresponds to 300 nm.

References

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