Online Supporting Document

Deposition of CTAB terminated nanorods on bacteria to form highly conducting hybrid system

Vikas Berry, Anand Gole, Subrata Kundu, Catherine J. Murphy and Ravi F. Saraf*

Bacteria Substrate Preparation

Bacillus cereus (ATCC 21634), growing on nutrient agar plates, is inoculated in nutrient broth (8 g/l) (Difco) and incubated in a shake flask at 30^{o}C for 14 hrs. The bacteria thus grown are washed four times by centrifugation at 4000 rpm for 15 min each. The bacteria are then suspended in sterile water. A silica substrate is cleaned by exposing it to oxygen plasma (600 mTorr, 100 W) for 120 s. The cleaning process also leads to formation of a highly negatively charged silica surface. This silica substrate is then put in poly-L-lysine solution (164000 Daltons, 2.5 mg/ml) for 2 hrs to form a Lysine monolayer. Poly-L-lysine is well known agent for deposition of bacteria on surfaces. Bacteria are then deposited on this substrate by exposing the substrate to the bacterial solution for 10 min.

Nanorod Preparation

Synthesis of gold nanoparticle seeds.

Gold seeds were synthesized by the method described by us previously.[1] Specifically, to a 10 ml 0.1 M aqueous solution of CTAB was added 250 µl of 0.01M $HAuCl_4$ and kept under stirring conditions. To this stirred solution was added 0.60 ml of

0.01 M sodium borohydride, which resulted in the formation of a brown-yellow solution. Vigorous stirring of this seed solution, which was kept at 25 °C, was continued for 2 min. This seed solution was further used for the synthesis of gold nanorods after 30 min of its synthesis. The size of these seed particles was less than 4 nm.

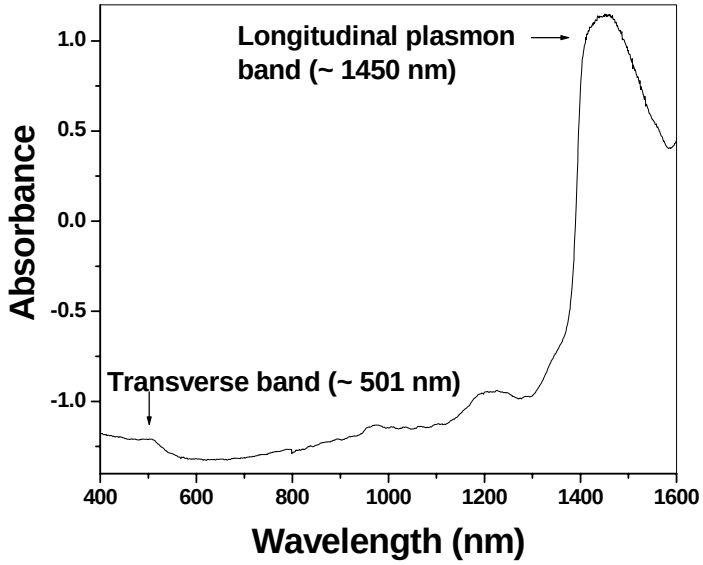
Synthesis of gold nanorods by seed mediated approach [(1)].

Gold nanorods were synthesized by the three-step seeding protocol as described previously.[1] Specifically, two 20 ml conical flasks and one 100 ml conical flask (labeled A, B, and C, respectively) were taken. To these flasks were added 9 ml (in flasks A and B) and 45 ml (in flask C) of growth solution containing a mixture of 2.5 x 10^{-4} M $HAuCl_4$ and 0.1 M CTAB solution. To these solutions were added 50 µl (flasks A and B) and 250 µl (flask C) of 0.1 M freshly prepared ascorbic acid, and the resulting solutions were stirred gently. The orange color of the gold salt in the CTAB solution disappeared when ascorbic acid was added. We have attributed this color change to the reduction of Au^{3+} to Au^{+}. However, the reduction of Au^{+} to Au^{0} does not occur, and we do not observe the gold plasmon band indicative of Au^{0} nanoparticles even after 24 h. This indicates that ascorbic acid is too weak to reduce Au^{+} under our experimental conditions. However, further reduction of Au^{+} to Au^{0} occurs when 1.0 ml of the seed solution is mixed with sample A (step 1). This is evidenced by a rapid development of red color to the solution in sample A, which earlier was colorless, thus indicating the formation of gold nanoparticles. After 15 s, 1.0 ml of sample A was mixed with sample B (step 2). This leads to a color change in sample B, indicating the generation of gold nanoparticles. The reduction in this case is slower compared to that in step 1. A 5.0 ml portion of sample B was further added to sample C after 30 s (step 3). The color of this solution slowly

changed to purple. In all cases, each flask was gently stirred to homogenize the solutions. The solution in flask C was kept at 25 °C for a period of 16 h. After the solution was stored for 16 h, purification was necessary to obtain gold nanorods. All the top red-brown solution (which contains mostly spheres) was slowly removed by suction. A faint brown tinge can be observed at the bottom of the flask. A 5.0 ml sample of deionized water was flushed into the beaker, and the contents were agitated for some time. A greenish-brown color developed in the deionized water and intensified upon repeated agitation. This solution contains a high percentage of gold nanorods, though other shapes (triangles, hexagons, and small rods) are also present in small amounts. The excess CTAB was removed by centrifugation twice (at 7000 rpm) and washing with deionized water.

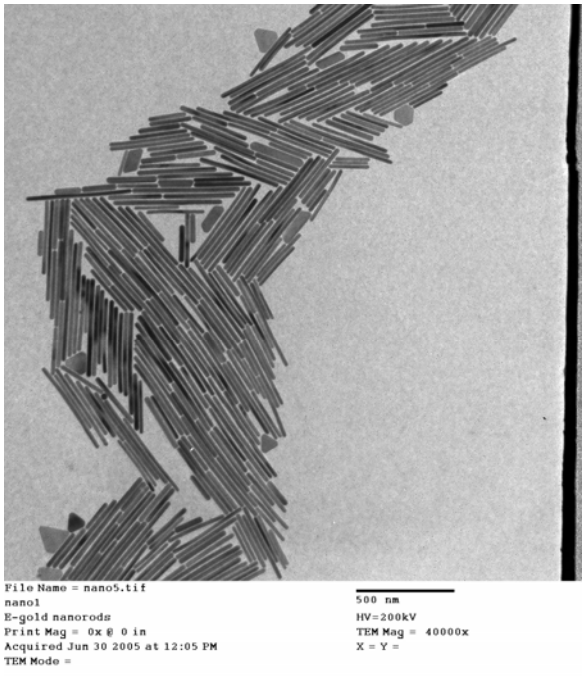
Characterization:

UV-vis Spectra:



The UV-vis measurements were carried out by dispersing the nanorod solution in D_2O. The transverse (~ 500 nm) and longitudinal plasmon band (~ 1450 nm) can be clearly seen in the figure.

TEM:



Size ~ 400 nm length and thickness ~ 25-30 nm

Nanosphere Preparation [1]

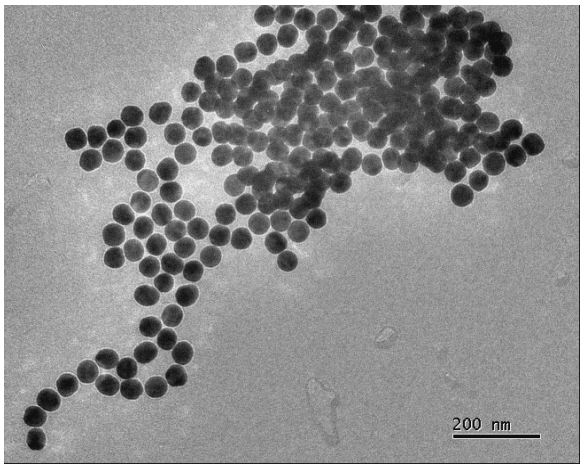
For the preparation of large size gold nanospheres, gold nanoparticle seeds are prepared by reducing 1 ml of 10 mM $HAuCl_4$ with 1 ml of 100 mM $NaBH_4$ in the presence of 1 ml of 10 mM sodium citrate and 36 ml of fresh deionized water (DI) water. The resulting mixture is aged for 2-4 hours in order to allow the hydrolysis of unreacted $NaBH_4$. These gold nanoparticle seeds exhibit a plasmon resonance peak at 500 nm, and have an average diameter of 5.2 ± 0.6 nm.

Three growth solutions are then prepared for seed-mediated growth step. The first two solutions (1 and 2) contained 0.25 ml of 10 mM $HAuCl_4$, 0.05 mL of 100 mM NaOH, 0.05 ml of 100 mM ascorbic acid, and 9 ml of a 7.5 x 10^{-2} M CTAB solution. The third solution (3), contained 2.5 ml of 10 mM $HAuCl_4$, 0.50 ml of 100 mM NaOH, 0.50 ml of 100 mM ascorbic acid, and 9 ml of CTAB solution.

Nanosphere formation with large diameter was initiated by adding 1 ml of seed solution to growth solution 1. After 5 mins, one ml of resultant solution 1 was then added to 2, and then again after 5 min all of the resulting growth solution in 2 was added to 3. After the addition, the color of 3 changed from clear to deep magenta-purple over a period of 30 minutes.

Solution exhibited a plasmon resonance peak at 535-540 nm, and had nanospheres with average diameter of 40-45 nm. This solution is then centrifuged at 8000 rpm for 20 mins to remove the excess CTAB and the precipitated gold nanospheres is redispersed in DI water.

TEM image of gold nanospheres:



Average particle size ~ 40-45 nm.

EDAX and FTIR studies and the zeta potential measurements revealed that surfactant CTAB molecules, adsorb onto surfaces of the gold nanocrystals and play major roles in the direction-specific self-assembly of the coated nanocrystals via inter-digitation of the tails forming a bilayer. The cationic head groups face the solvent. The interparticle spacings were similar irrespective of the size and shape of the gold nanocrystals. The zeta potential of the nanorod and nanospheres are in the range of +48 mV to +71 mV, which suggests that they are positively charged. The deposition on nanorods on positively charged physical surface showed no deposition and on negatively charged surface showed deposition, indicating that the deposition is electrostatic [(2)].

Nanoparticle Deposition

The bacteria substrate is exposed to each nanoparticle solution for 15 min to form percolating channels of nanoparticles.

Reference:

1. Gole, A. and Murphy, C.J. *"Seed-mediated synthesis of gold nanorods: Role of size and nature of seed." Chem. Mater.* **2004**, *16*, 3633-3640.

2. Sau, T. K.; Murphy, C. J. *"Self-Assembly Patterns Formed Upon Solvent Evaporation of Aqueous Cetyltrimethylammonium Bromide-Coated Gold Nanoparticles of Various Shapes,"* Langmuir **2005**, *21*, 2923-2929.).