

Materials and Experiments

Hydrogen tetrachloroaurate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), sodium borohydrate, cetyl trimethylammonium bromide, tri-sodium citrate, cystamine dihydrochloride, were purchased from Sigma-Aldrich and used without further purification. Methicillin-resistant staphylococcus aureus (MRSA) (ATCC 33592) & ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline antibiotics drug resistant *Salmonella typhimurium* DT104 and *E. coli* bacteria were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Aptamers were purchased from Midland Certified Reagent. Growth media for bacteria were obtained from the American Type Culture Collection.

Synthesis of Oval Shape Gold Nanoparticle: Oval shape gold nanoparticles with aspect ratio 1.3, as shown in Figure 1, were synthesized using seed-mediated growth procedure in the presence of CTAB, as we reported before^{7,23}. At first, very small, reasonably uniform, spherical seed particles are generated using tri-sodium citrate as the stabilizer and sodium borohydride as the strong nucleating agent. In the second step, we have used ascorbic acid as a weak reductant as well as CTAB as a shape templating surfactant so that the seeds can grow into larger particles of particular morphology we desired. The ascorbic anions transfer electrons to the seed particles, which will reduce gold ions to form gold shell, which grows into different shapes in presence of CTAB. Detailed synthesis have reported before^{7,23}. JEM-2100F transmission electron microscope (TEM) and UV-visible absorption spectroscopy were used to characterize the nanoparticles. TEM image shows (as shown in Figure 1A) that the aspect ratio of these oval shape nanoparticle is 1.1-1.2. As shown in Figure 1C, oval shape gold nanoparticle has only one plasmon bond at 550 nm like spherical gold nanoparticle, but their λ_{max} shifted about 35 nm, in comparison to the spherical gold nanoparticle of the same size. Oval shape gold nanoparticle

concentration was measured using plasmon absorption peak at 550 nm given the oval shape nanoparticle extinction coefficient is $2.3 \times 10^9 \text{ M}^{-1} \text{ cm}^{-1}$. Extinction coefficient was measured by using ICP analysis to quantitatively determine the gold concentration in nanoparticle solution and the nanoparticle volume was measured by TEM. This experiment was performed 5-6 times and the average values are reported in this manuscript.

Synthesis of single-stranded DNA (ssDNA) attached Oval Shape Gold Nanoparticle: Since oval shape gold nanoparticles were synthesized using seed-mediated growth procedure in the presence of CTAB and CTAB is known to be cytotoxic, it will not be ideal for in vivo diagnosis. CTAB is positively charged at physiological pH and as a result, it will be able to attract negatively charged proteins easily. To overcome this problem, the CTAB surfactant on the surface of oval shape gold nanoparticle was replaced by mercaptohexanoic acid using round-trip phase transfer ligand exchange method as reported recently by Wijaya et. al ¹⁷. After that, SH modified single-stranded extended DNA aptamers were gradually exposed to oval shape gold nanoparticles in the presence of sodium dodecyl sulfate (SDS), sodium chloride and PBS buffer for about 10-12 hours. To remove the unbound single-strand DNA strand, we centrifuged the solution at 6,000 rpm for 20 minutes and the precipitate was redispersed in 2 mL of the buffer solution. To measure the number of 5'Rh6G modified ss-DNA strand molecules attached to each oval shape gold nanoparticle, after conjugation, we have treated the Rh-6G modified DNA conjugated oval shape gold nanoparticle with 10 μM potassium cyanide to oxidize the oval shape gold nanoparticle. After that, the solution containing the released Rh6G-6G labeled DNA was collected for the fluorescence spectroscopy analyses. By dividing the total number of Rh-6G-labeled DNAs by the total number of oval shape gold nanoparticles, we estimated that there were

about 100-120 DNAs per oval shape gold nanoparticle. This experiment was performed 4-5 times and the average values are reported in this manuscript.

Design of Distance Dependent SERS Assay

For the selective imaging and killing of MRSA, we have used aptamer APT^{SEB1} extended sequences. Recently it has been reported that ²⁴ aptamer APT^{SEB1} can be used for very selective detection of *staphylococcal enterotoxin B*. The sequence of the DNA aptamer used is as follows, where the underlined sequence was the extended part: 5'-GGTATT GAG GGT CGC ATC CAC TGG TCG TTG TTG TCT GTT GTC TGT TAT GTT GTT TCG TGA TGG CTC TAA CTC TCC TCT GA ATT AAA TGC CCG CCA TGA CCA G-C₆-SH-3'. For the design of SERS assay, at first we have attached -SH modified aptamer APT^{SEB1} extended sequence with popcorn shape gold nanoparticle, as shown in Scheme 1. For the covalent attachment of -SH modified DNA aptamer with oval shape gold nanoparticle, the CTAB surfactant on the nano-popcorn surface was replaced by mercaptohexanoic acid (MHA) by reported method ¹⁷. In the next step, we hybridized the extended part of aptamer with capture Rh-6G modified DNA (5'-Rh6G-CTG GTC ATG GCG GGC ATT TAA TTC-3') which is reverse complement to the aptamer extension sequence. Since after hybridization, Rh-6G dye is placed very near to the nanoparticle surface, we observed a very good SERS signal.

For the selective killing of MDRB *Salmonella DT104*, we have used aptamer 45 extended sequence, which is known to be very selective for the detection of *Salmonella typhimurium*²⁵. The sequence of the DNA aptamer used was as follows, where the underlined sequence was the extended part which is exact same we have used for MRSA:

3'GAGGAAAGTCTATAGCAGAGGAGATGTGTGAACCGAGTAA GA ATT AAA TGC CCG
CCA TGA CCA G-SH. Similarly, for the hybridization, we have used exactly the same Rh-6G modified complementary DNA for the extended sequence, as we have used for *MRSA*.

Bacteria sample preparation and Understanding Bacteria-Nanoparticle Intercation

Multidrug resistance (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline antibiotics drug resistant) *Salmonella typhimurium* DT104 & methicillin-resistant staphylococcus aureus (MRSA) & *E. coli* bacteria were obtained from the ATCC. To culture MRSA and Salmonella DT104 bacteria, we followed ATCC protocol as instructed. At first, the supplied pellet was rehydrated on 5 to 6 ml of Bacto tryptic soy broth (BD) and incubated at 37⁰C for 24 hours. After that, from the growth culture, a loop full of bacteria was streaked on tryptic agar plate and then incubated for 24 hours at 37⁰C. At the end, a single colony from tryptic agar plate was inoculated into 10 ml of Tryptic Soy Broth and then it was incubated at 37⁰C for 16 hours in a shaker at 150 rpm which has an inoculum of 10⁸ CFU/ml. All the growth medium and agar were autoclaved at 121⁰C for 15 min at high pressure (0.1MPa) before the experiment.

After that, different concentrations (measured in colony-forming units (cfu) per mL) of bacteria were added to 1 mL solutions of aptamer-conjugated oval-shaped nanoparticles for 10–15 minutes. One drop of sample mixture was then placed on a mesh for TEM experiments. TEM experiments were performed by using a JEM-2100F advanced field emission electron microscope operating at 100–200 KV.

Photothermal Antibacterial Activity and % of live bacteria determination

For photothermal therapy, we have used a portable continuous wavelength OEM laser operating at 670 nm, with 0.2W/cm² power as an excitation light source. After that, the bacteria were

transferred to tryptic agar plate which is incubated for 24 h at 37°C and colony number for each countable plate was counted with a colony counter.