Supporting Information

DNA Assembly and Enzymatic Cutting in Solutions: A Gold Nanoparticle Based SERS Detection Strategy

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Synthesis of Au nanoparticles: Two types of aqueous gold nanoparticles were used for these experiments, citrate-capped (I) and acrylate-capped (II) AuNPs. Typical synthesis procedures are detailed, as follows.

(I) The citrate-capped gold nanoparticles $(11.7 \pm 1.6 \text{ nm})$ were synthesized using the reported procedure¹. Briefly, 5 mL freshly prepared sodium citrate (38.8 mM) is added to 45 mL boiling solution containing 1 mM of HAuCl₄. Upon the addition of sodium citrate, the color of the solution turned ruby-red, which is an indication for the formation of the gold nanoparticles. The solution is heated for an additional 30 min, then the heating mantle is removed and the nanoparticle solution is allowed to cool to room temperature with continuous stirring. The final solution is about 0.02% Au by weight.

(II) The acrylate-capped gold nanoparticles $(39.7 \pm 1.8 \text{ nm} \text{ and } 62.4 \pm 2.0 \text{ nm})$ were synthesized following a seeded growth procedure, previously reported^{2, 3}. Briefly, seeds were synthesized by combining an aqueous solution of HAuCl₄ (0.5 mM) with acrylate (1 mM) and refluxed for 30 min. The seeds then underwent a seeded growth reaction in the presence of HAuCl₄ under controlled concentrations of the reducing and capping agents to form the larger AuNPs. The actual sizes of the resulting nanoparticles depend on the seed sizes and precursor concentrations^{2, 3}. The final solutions are about 0.004% Au by weight.

Instrumentation and measurements. UV-Visible (UV-Vis) spectra were acquired with a Hewlett Packard 8453 spectrophotometer. The spectra were collected over the range of 200-1100 nm.

Detailed Experimental procedures. The general procedures for the assembly and the restriction enzyme cutting of the ds-DNA/AuNPs are similar to those reported previously⁴ but with some important modifications. Two different oligonucleotides, (bottom-DNA: 5'-/5ThioMC6-D/AGGCCAGACCTGCCCGGGCAAGCCTTGGCA-3' and top-DNA: 5'-/5ThioMC6-D/TGCCAAGGCTTGCCCGGGCAAGGTCTGGCCT-3') were used. Bottom-DNA and top-DNA were dissolved in 0.1 M phosphate buffer (pH 8) at a concentration ranging from 260 to 300 μ M. The disulfide bonds in bottom-DNA and top-DNA were cleaved using the reported procedure⁴ where dithiothreitol (DTT, 0.1 M final concentration) was added to 10 OD of the oligonucleotides in a volume of 400 μ L and reacted at room temperature for 2 hrs, then put through a NAP-5 column (G.E. Healthcare) and eluted with a 1.1 mL aliquot of 0.1 M phosphate

buffer (pH 8). The final concentration of the cleaved DNAs was 10 μ M as determined by optical density at 260 nm.

The surface of 13-nm AuNPs was functionalized with the cleaved bottom-DNA similar to the reported procedure⁴ to form bottom-DNA/AuNP. Briefly, 1.45 mL of the cleaved bottom-DNA (10 μ M) was added to 10 mL of gold nanoparticles (stock concentration 16 nM). The solution was left standing at room temperature for 16 hrs, after which a salt solution was added (final concentration of 20 mM NaCl and 10 mM phosphate buffer (pH 7)), and allowed to stand for 40 hrs at room temperature. The bottom-DNA/AuNPs were centrifuged at 14000 rpm (18620 g) for 20 min and re-dispersed in a 20 mM NaCl/ 10 mM phosphate buffer (pH 7) solution, then centrifuged again and re-dispersed in 20 mM NaCl/ 10 mM phosphate buffer (pH 7)/ 0.01% sodium azide solution and stored at room temperature. A similar procedure was used with the 39-nm AuNPs.

The MBA-labeled 13-, 39-, and 62-nm gold nanoparticles (MBA/AuNP) (which correspond to stock concentrations of 16, 0.1, and 0.028 nM, respectively) were prepared⁵ by adding 0.2 mL (13-nm) or 0.1 mL (39- and 62-nm) of 0.1 mM MBA to 10 mL of gold nanoparticles. Surface coverage of MBA (θ) for the 13-, 39-, and 62-nm AuNPs was theoretically 0.07, 0.5 and 0.7 (fraction of one full monolayer coverage), respectively. The nanoparticle solution was left standing at room temperature overnight.

Polyacrylamide gel electrophoresis was used to confirm the cutting. Briefly, solutions were treated with EDTA (0.02 M), DTT (0.4 M), and incubated overnight with constant shaking⁴. After removal from the nanoparticles, DNAs were then analyzed.



Figure S1. Top panel: TEM micrographs of gold nanoparticles of different sizes synthesized the same method used in this work: 10 nm (A); 40 nm (B); and 60 nm (C). Bottom panel: Size distributions of gold nanoparticles of different sizes, (a) 12.3 ± 1.3 nm; (b) 40.3 ± 1.7 nm; (c) 61.3 ± 1.5 nm. (The *y*-axis represents normalized frequency.)² The apparent aggregation of the AuNPs in the TEM image is due to the sample drying process, and not a representation of the particles in solution.



Scheme S1. Reaction schematic of the DNA mediated assembly, where the size of the red lightning symbol represents the expected enhancement in SERS intensity from MBA molecules in the interparticle "hot-spot".



Scheme S2. Structures of bis(p-sulfonatophenyl)phenylphosphine (BP) and 4-mercaptobenzoic acid (MBA).



Figure S2. Spectral evolution of the SP band showing the assembly of 13-nm bottom-DNA/AuNP with 39-nm acrylate-capped AuNPs in the presence of top-DNA. Concentration for various components: [bottom-DNA/AuNP(13 nm)]= 3.9 nM; [top-DNA]= 0.25 μ M; [39-nm AuNPs]= 7×10^{-2} nM. (The black line is the initial solution; red line is after assembly).



Figure S3. Dependence SERS intensity the band at 1594 cm⁻¹ vs. the top-DNA concentration. Note that the concentration of gold nanoparticles in the solution was kept constant ([bottom-DNA/AuNP(13 nm)]= 3.9×10^{12} NP/mL; [MBA/AuNP(39 nm)]= 2.5×10^{10} NP/mL).



Figure S4. Spectral evolution of the SP band showing the assembly of 13-nm bottom-DNA/AuNP with 62-nm acrylate-capped AuNPs in the presence of top-DNA. Concentration for various components: [bottom-DNA/AuNP(13 nm)]= 3.9 nM; [top-DNA]= 0.25 μ M; [62-nm AuNPs]= 2×10⁻² nM. (The black line is the initial solution; blue line is after assembly 5hrs; red line is the assembled sample after 1 day).



Figure S5. (A) SERS spectra (60 s integration time) for the assembly of 13-nm MBA/AuNP with 13-nm bottom-DNA/AuNP in the presence of top-DNA. (a) 13-nm MBA/AuNP, (b) 13-nm bottom-DNA/AuNP, (d) mixture of 13-nm bottom-DNA/AuNP and 13-nm MBA/AuNP, and (e) the assembly solution. (B) Spectral evolution of the SP band showing 13 nm bottom-DNA/AuNP, assembly with 13 nm citrate-capped AuNPs before assembly (black) and in the presence of top-DNA (red). Concentration for various components: [bottom-DNA/AuNP]= 3.3 nM; [top-DNA]= 1.0 μ M; [MBA/AuNPs]= 3.3 nM; [citrate-capped AuNP]= 3.3 nM.

Calculation of enhancement factor (EF): The enhancement factor (EF) was estimated based on the following relationship⁶:

$$EF = \frac{I_{sample}}{I_{MEA}} \times \frac{N_{MEA}}{N_{sample}} = \frac{I_{sample}}{I_{MEA}} \times \left(\frac{\frac{MDAS}{cm^3}}{\frac{NPS}{cm^3} \times \frac{MBAS}{NP} \times \theta}\right)$$

where, I_{sample} is the Raman intensity of the assembly solution (data extracted from figures 2 through 5), I_{MBA} is the Raman intensity of the standard MBA solution, N_{MBA} is the number of MBA molecules in the standard solution, N_{sample} is the number of MBA molecules in the assembly solution, $\frac{MBAs}{cm^3}$ is the number of MBA molecules dissolved per mL (1.02×10²⁰, 0.17 mM), $\frac{MPs}{cm^3}$ is the number of gold nanoparticles per mL, $\frac{MBAs}{NP}$ is the number of MBA molecules in 1 monolayer (39-nm: 1.6×10⁴, 62-nm: 3.9×10⁴), and θ is surface coverage of MBA on the nanoparticles. Note that differences in integration time were considered when performing the calculations.



Scheme S3. Reaction schematic of the restriction enzyme cutting, where the reduced size of the red lightning symbol represents the expected drop in SERS intensity from MBA molecules as a result of the removal of the interparticle "hot-spot", and (f1) and (f2) refer to the two ds-DNA fragments created by the *MspI* cleavage.



Figure S6. Spectral evolution of the SP band for the disassembly of 13-nm bottom-DNA/AuNP and 62-nm MBA/AuNPs with *MspI*, both before (black) and after (red) the enzyme cleavage procedure. (The spectra have been normalized, for comparison of peak shape.).

Additional control experiments: Control experiments showing very little change in the SP bands for the assemblies involving 13-nm bottom-DNA/AuNP and either 39-nm (A) or 62-nm (B) acrylate-capped AuNPs in the absence of top-DNA were performed (Figure S7). Since there was no mediator to form assembly, there were no changes in the spectra as expected.



Figure S7. Control experiments for 13-nm bottom-DNA/AuNP assembly with 39-nm AuNPs (A) and 13-nm bottom-DNA/AuNP assembly with 62-nm AuNPs (B) upon heating and cooling, where no top-DNA was added. Concentration for various components: [bottom-DNA/AuNP(13 nm)]= 3.9 nM; [39-nm AuNP]= $7 \times 10^{-2} \text{ nM}$; and [62-nm AuNP]= $2 \times 10^{-2} \text{ nM}$.

References:

1. S. R. Nicewarner-Pena, R. G. Freeman, B. D. Reiss, L. He, D. J. Pena, I. D. Walton, R. Cromer, C. D. Keating, M. J. Natan, *Science*, 2001, **294**, 137.

- P. N. Njoki, I-I. S. Lim, D. Mott, H. Y. Park, B. Khan, S. Mishra, R. Sujakumar, J. Luo, C. J. Zhong, J. Phys. Chem. C, 2007, 111, 14664.
- 3. P. N. Njoki, J. Luo, M. M. Kamundi, S. Lim, C. J. Zhong, Langmuir, 2010, 26, 13622.
- 4. I-I. S. Lim, U. Chandrachud, L. Wang, S. Gal, C. J. Zhong, Anal. Chem., 2008, 80, 6038.
- 5. I-I. S. Lim, P. N. Njoki, H. Y. Park, X. Wang, L. Wang, D. Mott, C. J. Zhong, *Nanotechnology*, 2008, **19**, 305102.
- 6. H. Yan, S. I. Lim, L. C. Zhang, S. C. Gao, D. Mott, Y. Le, D. L. An, C. J. Zhong, J. *Mater. Chem.*, 2011, **21**, 1890.