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SUPPLEMENTARY INFORMATION

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Nanogap Engineerable Raman-Active Nanodumbbells for Single-Molecule Detection

Dong-Kwon Lim[1,†], Ki-Seok Jeon[2,†], Hyung Min Kim[2], Jwa-Min Nam[1,*], and Yung Doug Suh[2,*]

[1] *Department of Chemistry, Seoul National University, Seoul, 151-747, South Korea,*
[2] *Laboratory for Advanced Molecular Probing (LAMP), NanoBio Fusion Research Center, Korea Research Institute of Chemical Technology, DaeJeon, 305-600, South Korea*

†These authors contributed equally to this work.

*To whom correspondance should be addressed (Email: jmnam@snu.ac.kr;

ydsuh@krict.re.kr).

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Materials and methods,
Fig. S1, Fig. S2, Fig. S3. Fig. S4, Fig. S5, Fig. S6
References

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Chemicals

Citrate-stabilized gold nanoparticles (20 nm and 30 nm) were purchased from Ted Pella, Inc. (Redding, CA, USA). All the other reagents were purchased from Sigma-Aldrich Co. (USA), and used as received without further purification. Thiolated oligonucleotides were purchased from IDT Inc. (Coralville, IA, USA) and reduced by using dithiothreitol (DTT, 0.1 M) in phosphate buffer (170 mM, pH = 8.0). The reduced oligonucleotides were then purified through a desalting NAP-5 column (Sephadex G-25 medium, DNA grade). The tosyl-activated magnetic microbeads (1.08-μm beads) were purchased from Invitrogen (California, USA) and used after washing procedures according to the manufacturer's protocol.

Determination of the number of Cy3-modified oligonucleotides per 30-nm AuNP

Photoluminescence measurements were performed on an Acton (Spectra Pro, MA. USA) spectrometer with a Xe-lamp (500 W) as an excitation source. The number of Cy3 molecules per 30-nm probe B was estimated by the fluorescence emission intensity (λ_{em} = 570 nm). The calibration curves were acquired from the oligonucleotide-Cy3 standard solutions (in triple-distilled water) for 1 nM, 2 nM and 3 nM oligonucleotide solutions, respectively. Cy3-labeled oligonucleotides (target-capture sequence for probe B) were released by DTT, and the nanoparticle solution was centrifuged to remove AuNPs. The amount of Cy3-labeled oligonucleotides in the supernatant was estimated by the emission intensity. The loading number of Cy3-modified oligonucleotides per probe B is ca. 0.75, indicating it is likely that 75% of probe B particles have ~1 Cy3 molecule per probe. Therefore, additional purification procedures were needed to eliminate probes without Cy3-modified target-capture DNA to achieve a higher synthetic yield for nanoparticle dimers (reference S1).

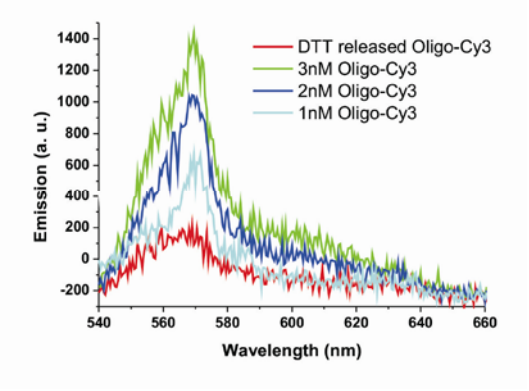

Fig. S1. The determination of the amount of Cy3-modified oligonucleotides in solutions released from 30-nm AuNPs by DTT treatment.

HR-TEM analysis of the synthesized AuNP heterodimers

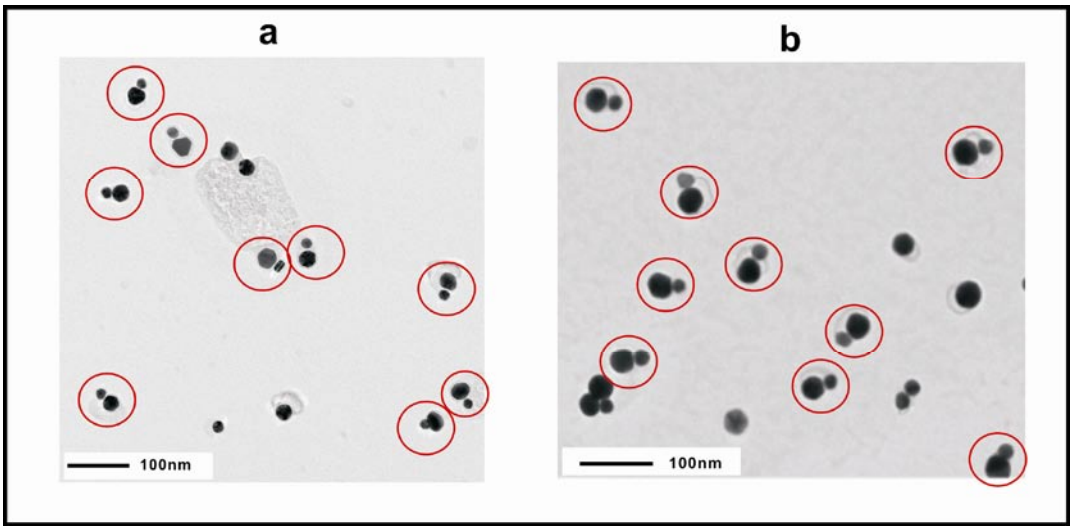

Fig. S2. The HR-TEM images of the target DNA-linked AuNP dimers prepared from magnetically-separated probes A and B. The dimeric structures are highlighted by red circles.

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The determination of the position of Cy 3 molecule in the NP heterodimer

The EELS analysis and high-resolution TEM imaging were performed using the JEOL electron microscope (JEM-2100F, Japan; 200 kV) with a point-to-point resolution of 0.23 nm. The sample solution (2 μL) was transferred onto a carbon-coated TEM grid (Ted Pella. Inc., Redding, CA, USA). The energy dispersion was set to 0.1 eV/channel. All EELS analyses were conducted in the scanning transmission (STEM) mode of the JEOL JEM-2100F microscope (reference S2). The STEM probe size was set to 0.5 nm.

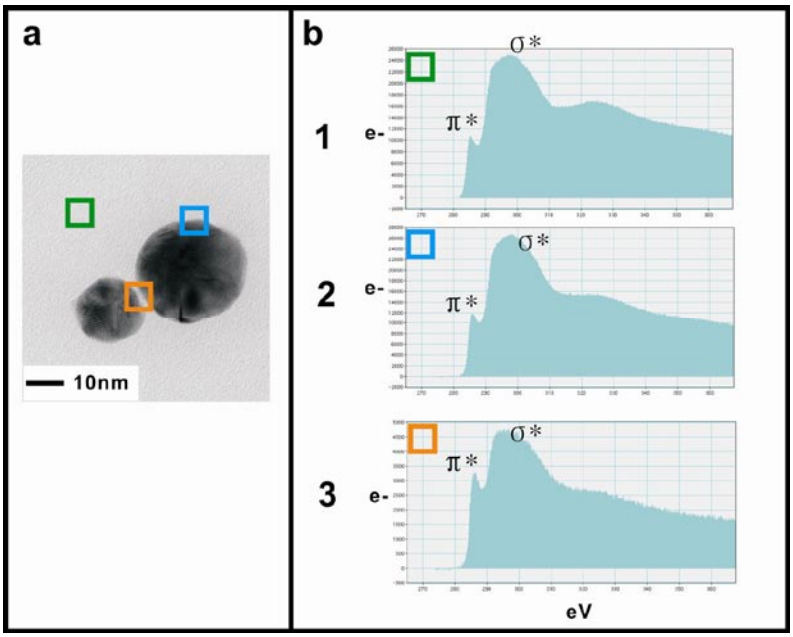

Fig. S3. HR-TEM image for AuNP dimer and corresponding EELS spectra. (a) AuNP dimer image. Colored boxes indicate the spectrum-measured locations [b-1(green), b-2(blue) and b-3(orange)]. (b) The EELS spectra. The NP junction point (orange box) shows relatively higher π* intensity than other areas (b-1: background; b-2: non-junction point).

Cy3 stability in Ag reduction conditions

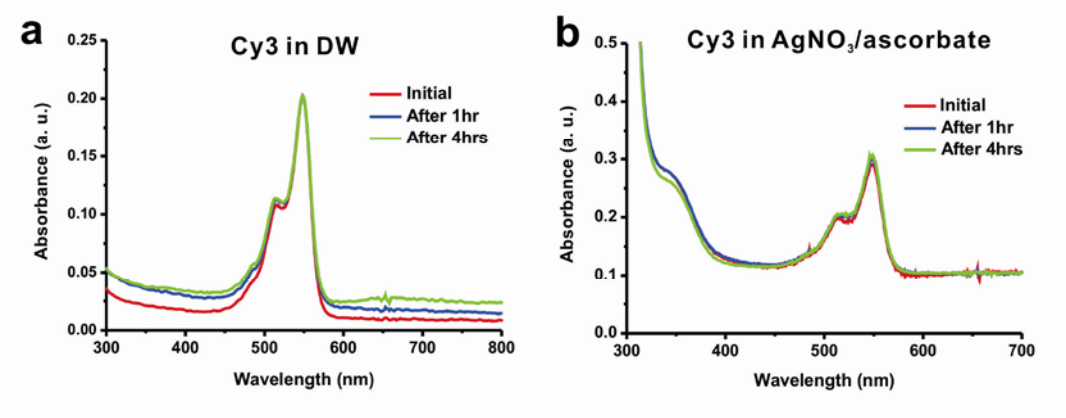

Fig. S4. (a) The time-dependent UV-spectra of Cy3 molecules (1 nM) in distilled water (DW) at room temperature, (b) the time-dependent UV-spectra of Cy3 molecules (1 nM) in a mixture of 1 % PVP(100 μL), 50 μL of $AgNO_3$(10^{-3} M), and 50 μL of sodium ascorbate (10^{-1} M) (room temperature).

SERS spectra from the GSNDs with FAM and Dabcyl-labeled oligonucleotides

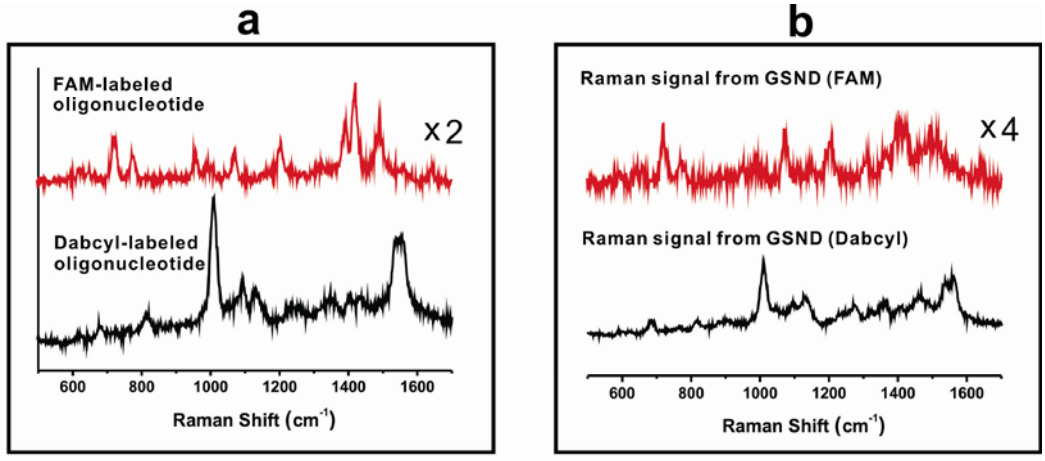

Fig. S5. (a) In-house reference Raman spectra of FAM- and Dabcyl-labeled oligonucleotides in solution, respectively (1 nM for both FAM and Dabcyl-modified oligonucleotides). (b) The acquired Raman spectra from a FAM-labeled GSND (5-nm silver shell) and a Dabcyl-labeled GSND (5-nm silver shell, 5 nm), respectively (reference S3, S4).

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The determination of the SERS enhancement factor

We compared the SERS intensity of the Cy3 in Au-Ag core-shell heterodimer, I_{SERS}, to the intensity $I_{SOLUTION}$ from pure Cy3 solution (33 mM). The enhancement factor $\overline{G}$ was calculated using the following equation (reference S5),

$$\overline{G} = \frac{I_{SERS} N_{SOLUTION}}{I_{SOLUTION} N_{DIMER}} \times \frac{\nu_{SOLUTION}^4}{\nu_{SERS}^4} \quad \text{(S5)}$$

where $N_{SOLUTION}$ and N_{DIMER} are the number densities of Cy3 in solution and in Au-Ag core-shell heterodimer, respectively, and $\nu_{SOLUTION}$ and ν_{SERS} correspond to the frequencies of Raman excitation laser.

The Raman experiment of Cy3 solution was performed with the same instrument, which was used for SMSERS experiment with some modification. 20 μl of Cy3 solution (33 mM in triple-distilled water) was transferred into the sample chamber (SecureSeal^{TM}, GRACE BIO-LABS). We employed 632.8-nm laser instead of 514.5nm laser to avoid the strong fluorescence background and focused through objective lens (50×, NA=0.5) in order to reduce instrumental noise. Integration time was 30 s. Assuming the excitation volume as a cylinder, the radius (r = 3 μm) of laser beam was measured by applying the knife-edge method (reference S6), and the height (h) was calculated by

$$\frac{h}{2r} = \frac{3.28\eta}{NA}$$

where η is the refractive index of water medium (reference S7). The enhancement factor (6.1×10^{12}) was determined, and the value was scaled using $\sim\nu_{Excitation}^4$ law for scattering intensity, because we used 632.8-nm excitation instead of 514.5-nm excitation. The corrected enhancement factor ($\overline{G}$) was 2.7×10^{12}. Generally, most errors in calculating enhancement factor arise from the uncertainty of the number of Raman-tag molecules placed in hot site. However, in our case, the number of Cy3 molecule placed in hot site (N_{DIMER}) is approximately 1. An important uncertainty in calculating enhancement factor could be minimized by this approach. There are conflicting claims in the several literatures that associate with the ability of SERS to detect a single molecule with an "enormously high enhancement factor" (on the order of $\sim 10^{14}$) or with more reasonable enhancement factor on the order of $\sim 10^7$-10^8 (reference S8).

It should be noted that there the intrinsic limitations of the off-resonance effect of Cy3 at 632.8-nm laser excitation wavelength, the different experimental procedures (I_{SERS} is based on a dry-state analysis and $I_{SOLUTION}$ is based on solution analysis), and the fluctuating SERS signal (all signals are averaged into 1s).

Our results suggest that the calculated enhancement factor (2.7×10^{12}) is sufficient to detect a single dye molecule from the gap-engineered Au-Ag core-shell heterodimer NPs, and the value could reach over ~10^{12} under a more optimized condition and if it is coupled with chemical enhancement in solution state.

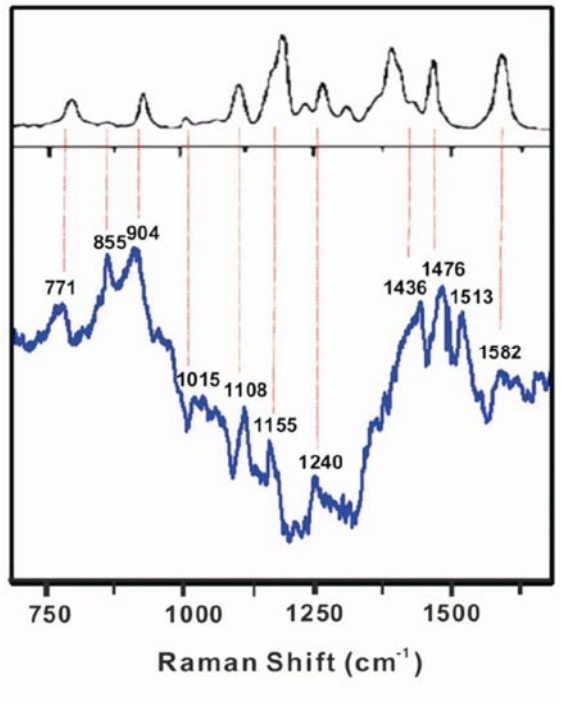

Fig. S6. The observed Raman spectra of Cy3 solution (bottom) in comparison to the SERS spectra (upper) reported by Jin et al. (reference S9). The acquired spectrum was baseline-corrected according to the spectral shape of fluorescence.

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