Electronic Supplementary Material (ESI) for Chemical Communications
This journal is © The Royal Society of Chemistry 2013

Supporting information

DNA sequences (Integrated DNA Technologies, Inc.)

Design E-dithiolated hairpin:
5'-thiol-TTT TTT TTT TTT TTT ACC TGG—Cy3—ACC TTC CTC CGC AAT ACT CCC CCA GGT TTT TTT TTT TTT TTT-thiol-3'

Design L-dithiolated hairpin:
5'-thiol-TTT TTT TTT TTT TTT ACC TGG ACC TTC CTC C—Cy3—GC AAT ACT CCC CCA GGT TTT TTT TTT TTT TTT-thiol-3'

Design HP-dithiolated hairpin:
5'-thiol-TTT TTT TTT TTT TTT ACC—Cy3—TGG ACC TTC CTC CGC AAT ACT CCC CCA GGT TTT TTT TTT TTT TTT-thiol-3'

thiolated polyT_{15}:
5'-thiol- TTT TTT TTT TTT TTT-3'

ATP aptamer:
5'-ACC TGG GGG AGT ATT GCG GAG GAA GGT-3'

ssDNA samples were stored at ~100-200uM concentrations in DI water at 4˚C.
Positioning of the Cy3 dye is on the phosphate backbone of the ssDNA strand.

This journal is © The Royal Society of Chemistry 2013

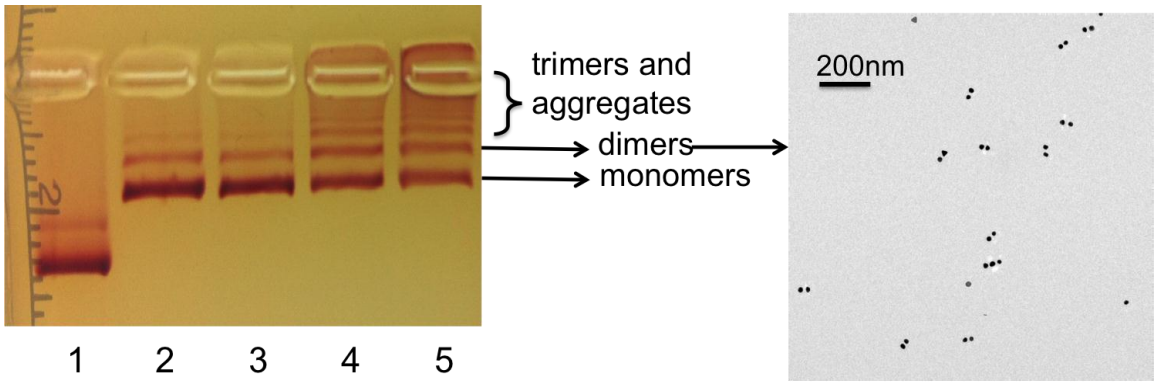

Figure S1. Image of 2.5% agarose gel electrophoresis of 20nm AuNP conjugated with dithiolated hairpin ssDNA and TEM of extracted dimer band. Lane 1 is 20nm AuNP only. Lane 2, 3, 4, 5 is 10nm AuNP conjugated to dithiolated hairpin ssDNA at 1:10, 1:20, 1:50, 1:100 AuNP:ssDNA, respectively. Monomer band fades and dimer and aggregate bands darkens as ratio of ssDNA is increased. Dimer band is gel extracted at conjugation ratio to maximize dimer band, at 1:50 AuNP:ssDNA. Relative yields at optimal ratio is 71% AuNP dimers, 13% monomers, and 16% trimers or higher aggregates.

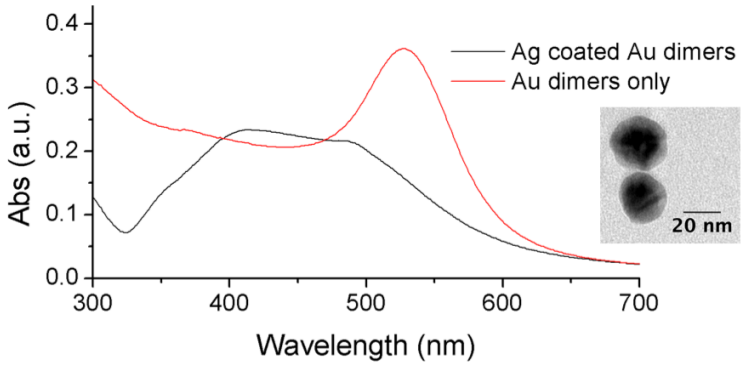

Figure S2. UV-Vis spectra for Au NDB dimers only and Au@Ag core-shell NDB dimers. Clear shift in main Au peak is seen after Ag shell coating from 527nm to 494nm, with additional peak at 415nm corresponding to the Ag shell. Slight peak at ~350nm could be due to small nucleations of Ag nanoparticles. TEM analysis shows shell coating to be ~5nm in thickness.

This journal is © The Royal Society of Chemistry 2013

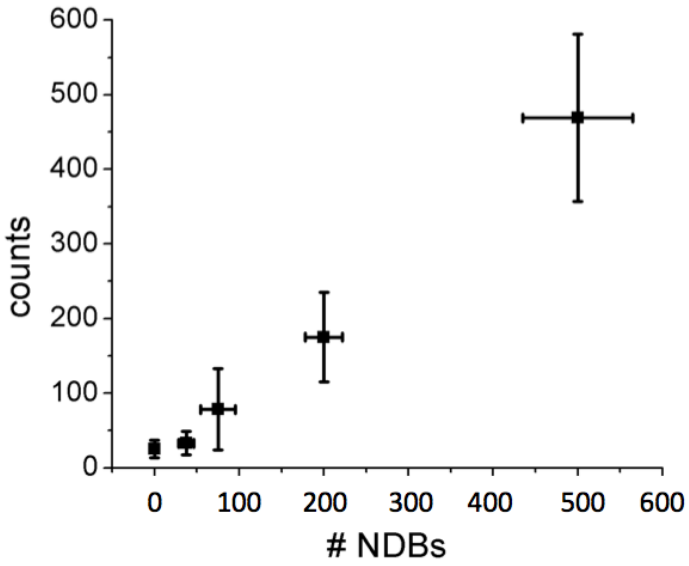

Figure S3. SERS intensity ranges for NDBs measuredon multiple pillarsbefore ATP aptamer and ATP addition. Y error bars show standard deviation of intensity measured. X error bars show standard deviation of NDBs per pillar measured. Although sensitivity of SERS signal can be seen with only 50-100 NDBs per pillar, the variation in signal is large and overlaps with signal seen with no NDBs on pillar. Therefore, pillars with 150-250 NDBs were used for SERS measurements in the rest of this study.

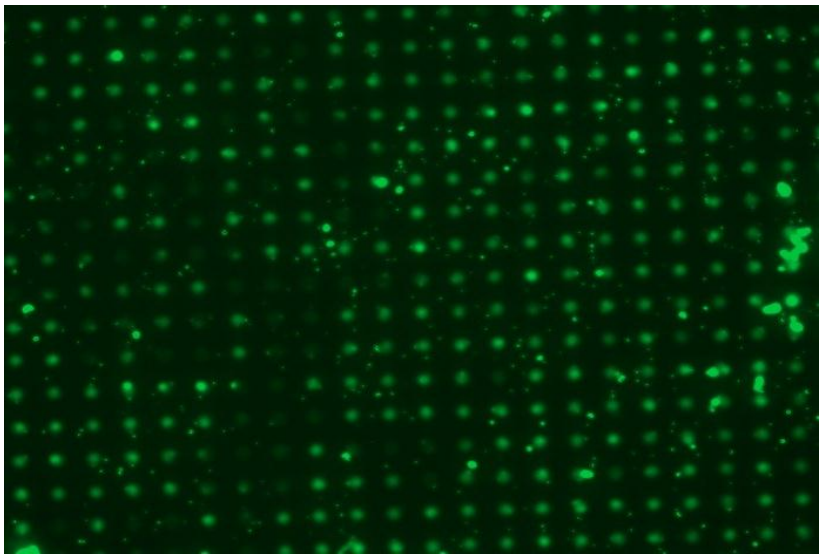

Figure S4. Fluorescently labeled DNA patterned preferentially to top of 3 micron Si pillars.

This journal is © The Royal Society of Chemistry 2013

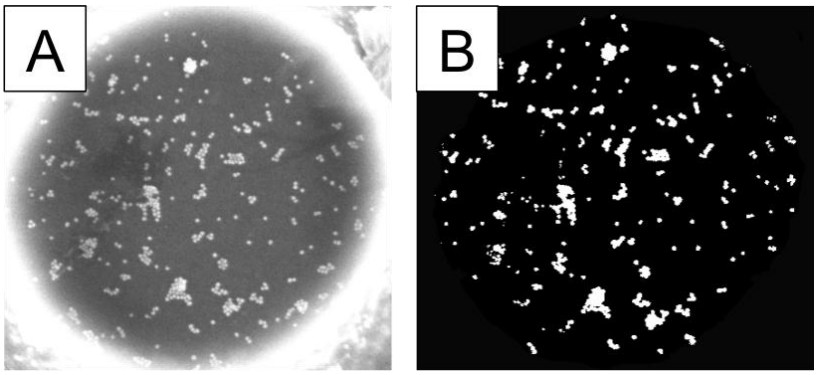

Figure S5. a) Representative SEM images of 3um Si pillar with adsorbed NDB. b) ImageJ analysis of a) used for particle counting. Pillars with 300-500 particles were sampled and measured. NDB counts were taken as half of the total number of particles, assuming all particles are NDBs. Although there is some aggregation seen on the pillars, the effect of this on SERS is not assumed to be slight. If neighboring NDBs had a large effect on the SERS enhancement, then the SERS signal in the open NDB state would also show large increases in enhancement.

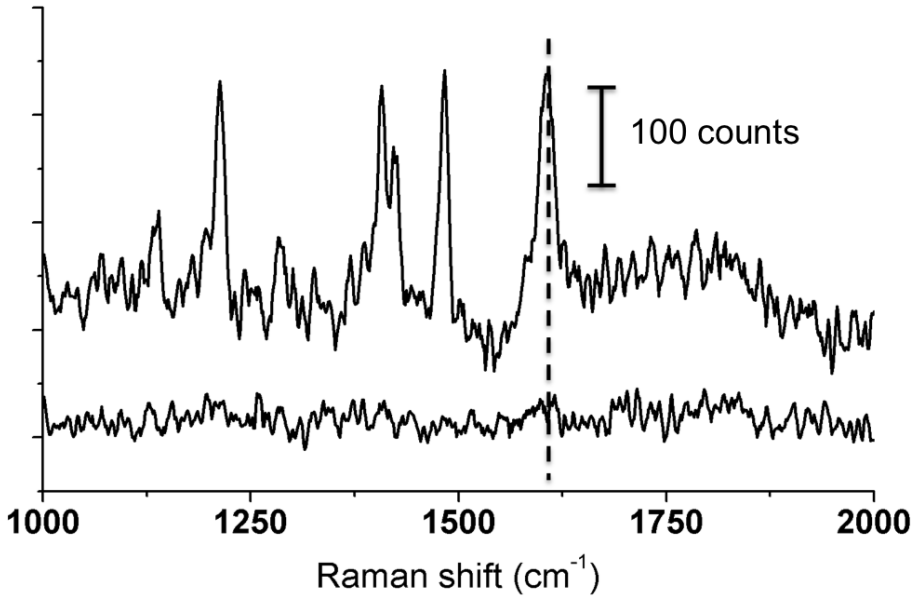

Figure S6. Representative Raman spectra of "closed" (upper spectrum) and "open" (lower spectrum) NDBs. Intensity counts were taken at Cy3 fingerprint peak at 1580cm^{-1}. Average intensity/NDB values were taken as the intensity at 1580cm^{-1} (dotted line) divided by the number of NDB on each particular sampled Si pillar.

This journal is © The Royal Society of Chemistry 2013

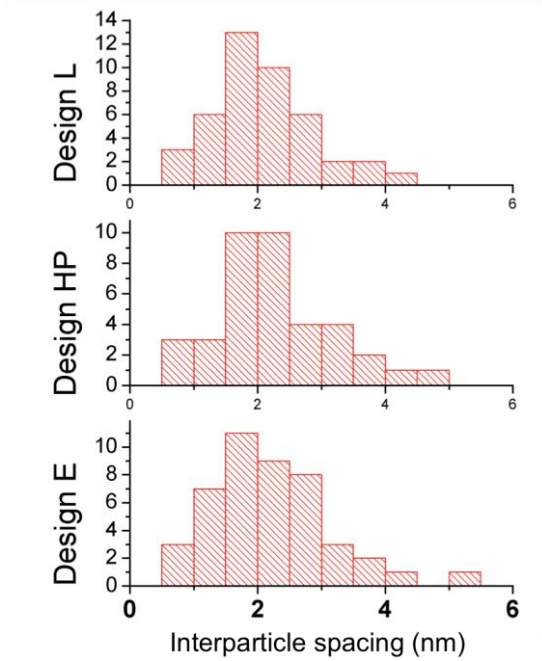

Figure S7. Histogram of as-made NDB in closed form for all three NDB designs (different position of Cy3 dye within dithiolated DNA). Similarities in average interparticle distance of all three designs show that position of Cy3 dye does not affect interparticle distance of NDB in closed state.

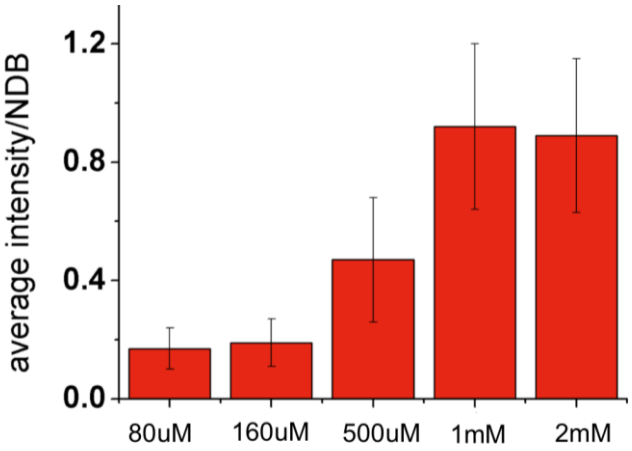

Figure S8. Average intensity per NDB after addition of different concentrations of ATP. Quantitative estimations of ATP can be obtained from average intensity/NDB data. However, standard deviations at different ATP concentrations overlap each other, which may produce quantitative values that are off from actual concentrations. Though estimations of ATP concentration can only be estimated below saturation of closed NDBs, which is at 1mM ATP.

This journal is © The Royal Society of Chemistry 2013

SERS Enhancement Factor (EF) calculations

To calculate the SERS EF, a value for the intensity of the free Cy3 dye must be determined. For this measurement, a wavelength longer than the Cy3 excitation wavelength of 550nm must be used. Thus, a 785nm was used to measure the free Cy3 dye dissolved in DMSO. The measurement conditions (100uW power, 20s accumulation) were consistent with those used for the NDB samples.

Calculations of SERS EF was determined by the following equation (S1):

$$EF = \frac{I_{NDB} \times N_{bulk}}{I_{bulk} \times N_{NDB}} \times \frac{f_{bulk}^4}{f_{NDB}^4}$$

I_{NDB} = Raman intensity of Cy3 in NDB sample
I_{bulk} = Raman intensity of free Cy3 in solution
N_{NDB} = Number of Cy3 measured in NDB sample
N_{bulk} = Number of Cy3 measured in solution
f_{NDB} = Frequency of laser used to measure NDB sample (532nm wavelength)
f_{bulk} = Frequency of laser used to measure free Cy3 in solution (785nm wavelength)

To calculate N_{bulk}, the height h of the excitation volume must first be calculated using the following equation (S1):

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

where η is the refractive index of the solution medium, DMSO (value taken from reference S2), r is the radius of the laser beam, and NA is the numerical aperture of the objective lens. The excitation volume was assumed as a cylinder.

This journal is © The Royal Society of Chemistry 2013

Experimental Methods

AuNP purification: Gold nanoparticles (AuNP) were phosphine coated and purified before use by adding bis(p-sulfonatophenyl)phenylphosphine dehydrate dipotassium salt (3mg, Strem Chemicals, Inc.) to 20nm gold colloid solution (10mL, Ted Pella, Inc.) with gentle stirring overnight. NaCl was then added to the solution up to 1-2M concentration and vortexed so that the solution turned purple. The purple solution of AuNP was then centrifuged for 15min at 15000rpm to precipitate the AuNP, and the supernatant was discarded. Deionized (DI) water was added to the AuNP precipitant and the resulting solution was filtered through a 30K MWCO Nanosep centrifuge filter (Pall Corp.) 3 times with water at 7000rpm for 10min each time. DI water was then added to the AuNP to a final AuNP concentration of 30-40nM. Purified AuNP solution was stored at 4˚C.

AuNP-ssDNA conjugation: Starting with purified AuNP (50-100uL), a 1:50:1000 ratio of AuNP:dithiolated hairpin ssDNA:polyT_{15} ssDNA was allowed to react in 0.3M NaCl solution at room temperature overnight. The solution turned from blue to red after conjugation. The solution was then used for gel extraction.

Nanodumbbell dimer purification by gel extraction: DNA-conjugated AuNPs were loaded into a 2.5% agarose gel made in 1x TAE buffer and run for ~40min at 140V (Figure S1). The dimer bandwas cut out from the gel and run through a Mini GeBAflex-tube (12K MWCO, Gene Bio-Application, Ltd.), also in 1x TAE buffer. The extracted nanodumbbell dimers were then stored in 4˚C until further use. Final concentrations of extracted nanodumbbell dimers ranged from ~0.3-0.4nM. Note: It is possible that single dimers could be formed by multiple dithiolated ssDNA, but the expected number of each of these on each pillar is the same. Since we are comparing pillars with a similar number of NDBs, then there should also be similar number of each of these compared across samples.

Silver shell coating of NDB dimers: As-extracted NDB dimers (100uL) were coated with silver by addition of 1%PVP (10uL, 40K, Sigma-Aldrich), 0.1M L-sodium ascorbate (5uL Sigma-Aldrich), and 10mM silver nitrate (4uL, Fluka Analytical) in 0.3M PBS. The solution was allowed to react with gentle shaking for 3 h and then filtered through a 30K MWCO Nanosep centrifuge filter (Pall Corp.) 2x with water at 6000rpm for 8-10min each time and then extracted using water back up to the original volume. Presence of Ag shell coating was checked by UV-Vis and TEM (Figure S2).The Ag shell was expected to grow in a shell around the AuNP because of the poly-T15 coating covering the surface of the AuNP. The Ag nucleates evenly with the presence of DNA on the surface and grows along the poly-T15 to become a spherical shell structure around the AuNP. This method for Ag shell coating was adapted from S1.

The NDBs were made by connecting AuNP first and then coating with Ag because of the more stable conjugation between the AuNP with the thiolated DNA. The conjugation of Ag surface to thiolated DNA is not as stable. Also, by coating the Ag shell after conjugation of AuNP to the dithiolated DNA further stabilizes the connection by locking in a portion of the dithiolated DNA within the shell.

This journal is © The Royal Society of Chemistry 2013

Addition of ATP aptamer and ATP to purified nanodumbbells: The ATP aptamers were added to the purified nanodumbbell dimers in 0.1M NaCl and annealed from 95˚C down to room temperature in a water bath. This created the extended structure of nanodumbbells. The solution was then immediately stored in 4˚C until further use to prevent dehybridization.

ATP (Cell Signaling Technology, Inc.) was added in 6000x excess to the extended nanodumbbell solution in 25mM tris-buffer, 0.3M NaCl at pH 8.2. Final concentration of ATP is 1mM. The solution was allowed to incubate at ~35-40˚C for ~1 h and then put immediately into ice water until further use.

TEM imaging and analysis: Carbon-coated copper mesh TEM grids (Electron Microscopy Sciences, Inc.) were glow discharged for 90s at 20mA and then 1uL of sample were dropped promptly on the grid. The water was wicked away from the grids and allowed to air-dry before TEM (FEI Tecnai G2 Sphera) imaging. Interparticle distances of nanodumbbells were measured manually using ImageJ, from surface-surface of Au@Ag particles. Interparticle distances of ~50 nanodumbbell dimers were measured per sample from microscope images.

Adsorption of sample to ssDNA modified pillars and SEM analysis: Si pillars of 3um diameter and 1um height were created by photolithography and polyA_{15} ssDNA were covalently conjugated to the Si pillars (**Figure S4**). 5uL of 0.5 nM NDB solution was adsorbed to each Si pillar array in 0.3M NaCl in a humid chamber for 2 h. The sample was then rinsed briefly in 0.3M NaCl solution, then briefly in 50% v/v ethanol/water solution, and then finally for 30min in 90/10% v/v ethanol/water solution. The sample was then allowed to air dry at room temperature. Specific pillars corresponding to those that were measured by Raman were SEM imaged, and the particles on each pillar were counted using ImageJ (**Figure S5**).

Raman measurement and analysis: Samples were measured using a Renishaw InVia Raman microscope with a 40x objective lens at 100uW laser power using a 532nm wavelength laser with 20s accumulation time. The microscope was aligned to each pillar and then each pillar was individually measured. The resulting spectra were background subtracted before comparison across samples.

COMSOL Multiphysics simulations: Simulations of electric field around the NDB system was made using COMSOL Multiphysics 4.2 software, using the EM-field model. Refractive indices of Ag and Au were from Johnson and Christy.(S4) Model consists of two Au@Ag nanoparticles with a 20nm Au core and 5nm Ag shell, with either a 2nm or 7nm gap distance. The simulations were calculated at a wavelength of 532nm. The resulting E-field was converted to SERS enhancement using the $|E|^4$ approximation.(S5) Theoretical positions of Cy3 were determined by the lengths of DNA between the Cy3 and the silver shell surface. Experimental positions of Cy3 were determined by corresponding experimental EF values to the COMSOL simulated values ($|E^4|$ approximation).

This journal is © The Royal Society of Chemistry 2013

References:

S1. Lim, D., Jeon, K., Kim, H.M., Nam, J., Suh, Y.D. Nat. Mater. (2010)**9**, 60.

S2. LeBel, R.G., Goring, D.A.I. Chem. Eng. Data (1962)**7**, 100.

S3. Lee, J.H.; Domailles, D.W.; Oh, T.; Noh, H.; Choi, C.; Jin, S.; Cha, J.N. High-Yielding, Generalized Procedure for Bioconjugation to Patterned Surface, *in prep*

S4. Johnson, P.B.; Christy, R.W. Optical Constants of the Noble Metals, *Phys. Rev. B*1972, **6**, 4370.

S5. Kneipp, K.; Kneipp, H.; Itzkan, I.; Dasari, R.R.; Feld, M.S. *J. Phys.: Condens. Matter*2002, **14**, R597.