Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Info

DNA-mediated hierarchical organization of gold nanoprisms into 3D aggregates and their application in surface-enhanced Raman scattering

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Materials

Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot 3H_2O$) and Methylene blue (MB), high purity biological stain ($C_{16}H_{16}ClN_3S.\ xH_2O$) were purchased from Alfa Aesar (Ward Hill, MA). Sodium thiosulfate pentahydrate, phosphate buffer solution, dithiothreitol (DTT) at 1 M concentration in H_2O, and Cellulose Acetate Dialysis tubing (43 mm wide; 12 kDa molecular weight cutoff) were purchased from Sigma-Aldrich (St. Louis, MO). Ethanol 200 proof (100%) was purchased from Decon labs, Inc (King of Prussia, PA). Oligonucleotides sequences (thiolated) 5'-ATA-ACC-ATT-GTA-AAT-TAA-TTA-3' (DNA-**A**) and complementary 3'-TAT-TGG-TAA-CAT-TTA-ATT-AAT-5' (thiolated) (DNA-**A′**) were purchased from Integrated DNA

Technologies (Coralville, IA). Microscope slides (3″×1″×1 mm) were purchased from VWR International, LLC (Rednor, PA). Illustra NAP-25 columns were purchased from General Electric Healthcare (Buckinghamshire, United Kingdom). NANOpure ultrapure water (Barnstead, resistivity 18.2 MΩ-cm) was used for all aqueous solutions unless stated otherwise.

List of oligonucleotide linkers used in this study

Table S1. List of oligonucleotide linkers

DNA-**A**	Adenine rich oligonucleotide	(thiolated) 5'-ATA-ACC-ATT-GTA-AAT-TAATTA-3'
DNA-**A′**	Thymine rich oligonucleotide	3'-TAT-TGG-TAA-CAT-TTA-ATT-AAT-5'-(thiolated)

Instrumentation

UV absorption spectra were obtained with an Agilent Varian Cary 50 BIO UV Visible Spectrometer (Agilent Technologies, Santa Clara, CA) using either a plastic cuvette with 1mL sample volume or a quartz cuvette with 400 µL sample volume. Melting analysis was performed on an Agilent Cary UV-Vis 100 spectrometer (Agilent Technologies, Santa Clara, CA) equipped with a Perkin-Elmer PTP-1 Peltier Temperature Programmer.

Dark-field microscopy imaging was performed on a research grade optical microscope (Olympus BX43) equipped with an integrated hyperspectral analysis system (CytoViva Inc., Auburn, AL, USA). This system uses a halogen lamp light source and a darkfield oil condenser (NA 1.20–1.40) for sample illumination. High-resolution darkfield images were obtained using a Dage camera (model XLMCT, Dage-MTI, Michigan City, IN, USA).

Scanning electron microscopy (SEM) were performed using a Carl Zeiss SMT AG SUPRA (Carl Zeiss AG, Oberkochen, Germany) 35VP field emission scanning electron microscope (FESEM) operating at an accelerating voltage of 3-15 kV for SEM using SE2, or Inlens.

Raman analysis were carried out using a Renishaw InVia micro-Raman/PL system (Renishaw, Wotton-Under-Edge, U.K.) equipped with a 633 nm HeNe laser with an operational range 100-2000 cm^{-1} for Raman

Chemical synthesis and Purification of Gold Triangular nanoprisms

Gold nanoprisms were synthesized using the Diasynth process following literature Precedent.[1-2] This method involves the reaction between sodium thiosulfate ($Na_2S_2O_3$) with a gold salt, tetrachloroauric acid ($HAuCl_4$) in a traditional one-step or two-step process without the need of additional templates, capping reagents, or seeds. Briefly, 32.6 mL of 1.76 mM $HAuCl_4$ in water was added to a 12 cm section of dialysis tubing followed by quickly adding 7.4 mL of 3 mM aqueous sodium thiosulfate solution with agitation for 5 seconds. The membrane was then clipped and submerged in a circulated bath of DI water (≥25 L) maintained at 27 ºC by a Thermo (Waltham, MA) NESLAB RTE-221 Circulator. After reacting for 1 hour the membrane was removed and the solution was emptied into a 40 mL plastic tube. The poly-disperse particle solution was a mixture of nanoprisms and pseudo-spheroid particles, with the latter contributing almost 99% of particles. Nanoprisms were separated from pseudo-spheroid particles through 2 x 30 mins long centrifugations at a speed of 180-200 g. The plasmon resonance band for pseudo-spheroid particles at 540 nm is completely diminished after centrifugal separation as the pseudo-spheroids were removed from the particle mixture and a high purity nanoprism solution was obtained.

Functionalization of nanoprisms with oligonucleotide

Prior to the functionalization of Au nanoprisms, thiolated oligonucleotide was treated with 0.1 M dithiothreitol (DTT) in disulfide cleaving buffer (0.17 M, pH 8) to remove the thiol protecting group. Then, the oligonucleotide solution was purified using a desalting column (Nap-25, DNA grade, GE Healthcare). The purified nanoprisms were functionalized by using modifications of literature procedures.[3] Briefly, 1.87 OD_{260} (2.5 µM) of purified thiolated DNA was added to 1.0 OD of Au nanoprisms and allowed to react while shaking (1100 rpm, 22 ºC) for 30 min to 1 hour using an Eppendorf Thermomixer. The particle and DNA mixture were then brought to 0.01% SDS (sodium Dodecyl sulfate) and 0.01M sodium phosphate buffer. Then, particle solutions were slowly treated with NaCl and sequentially brought to 0.05 M, 0.1 M, 0.2 M, and 0.3 M NaCl with 30 min between each addition. After the final salt aliquot addition, the particle solution was allowed to sit overnight. The next day, the particle solution was centrifuged (2000 rpm, 10 mins) 3-4 times to remove the unreacted oligonucleotide and the pellet was resuspended in 0.01 M PBS or nanopure water.

Face-to-face stacking of nanoprisms

200 µL of 2.4 OD DNA-**A′** functionalized nanoprisms were mixed to 200 µL of 2.4 OD DNA-**A** coated nanoprisms in a DNA hybridization buffer (0.01M Phosphate buffer, pH 7.4 + 0.3 M NaCl). The mixture was then heated to 60-65ºC, followed by slow cooling to room temperature for efficient DNA-mediated interactions between nanoprisms, leading to the formation of 1D face-to-face assemblies of nanoprisms.

Melting analysis of 1D nanoprisms stacks

The melting analysis of 1D nanoprism stacks was monitored with an Agilent Cary 100 UV-Vis spectrometer (Agilent Technologies, Santa Clara, CA) equipped with a Perkin-Elmer PTP-1 Peltier Temperature Programmer, following literature precedent.[4] A melting profile was obtained by monitoring the change in absorbance of the predominant surface plasmon resonance (SPR) band of the gold nanoprisms with the change in temperature. Briefly, a 400 µL dispersion of 1D nanoprism stacks was diluted into 1.2 mL solution with the DNA hybridization buffer and loaded in a capped cuvette with a stir bar. The solution was then heated from 25 ºC to 80 ºC under continuous stirring at a ramp rate of 0.25ºC/min. The melting temperature of the conjugates was determined by taking the first derivative of the temperature curve. Melting analysis of a control experiment was carried out by heating uncoated nanoprisms in the DNA hybridization buffer under similar conditions.

Formation of 3D nanoprism aggregates

In order to assemble 1D nanoprism stacks into 3D aggregates, the solution of 1D nanoprisms stack was spun down and resuspended in 250-300 µL phosphate buffer to allow for a concentrated solution of 1D nanoprism stacks. The concentrated dispersion of 1D nanoprisms stack was then annealed at a temperature 1-3 ºC below the melting temperature of 1D stacks for 1-3 hrs. Briefly the solution was heated to and annealed at 65 ºC, 66 ºC and 67.5 ºC for several hrs.

Dark-field microscopy was used to monitor the crystal growth at each temperature. While annealing, 5 µL of solution was quickly taken from the capped cuvette and drop casted on a glass slide before placing a coverslip over the sample for dark-field microscopy imaging.

Surface Enhanced Raman Scattering Experiments

All the Raman spectra were recorded under the same ambient conditions: 50x microscope objective with a laser spot size of 1μm, 10 s data acquisition time and 150 mW laser power. To evaluate the SERS performance of the 3D nanoprism aggregates, 10.0 μL of probe molecule, MB of different concentrations (ranging from 10^{-3} M to 10^{-10} M) was drop casted on 3D nanoprism aggregates on a glass slide and kept in a fume hood until dry. Raman spectra were then collected from at least 6 different locations on the sample area.

The SERS enhancement of MB on the 3D nanoprism aggregates was assessed by calculating the enhancement factor (EF) using the following equation.[5]

$$EF = \frac{I_{SERS}}{I_{Raman}} \frac{C_{Raman}}{C_{SERS}}$$

where I_{SERS} and I_{Raman} refer to the peak intensity at 1621 cm^{-1} in the SERS spectrum of 10^{-7} M MB on the 3D SERS substrate and in the Raman spectrum of 10^{-3} M MB on the reference glass substrate, respectively. C_{SERS} and C_{Raman} refers to the concentration of MB in the SERS and Raman experiment, respectively.

The SERS signal intensity at 1621 cm^{-1} for 10^{-9} M MB, I_{SERS} = 67.7 cps, and normal Raman signal intensity at 1621 cm^{-1} for 10^{-3} M MB, I_{Raman} = 22.3 cps.

$$EF = \frac{67.7}{22.3} \frac{10^{-3}}{10^{-9}} = 2.91 \times 10^{6}$$

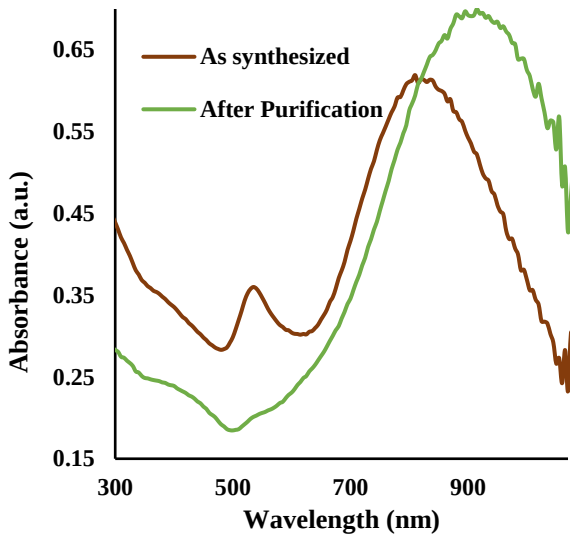

Figure S1: UV-Vis spectra of as synthesized gold nanoprisms (brown) and purified nanoprisms (Green)

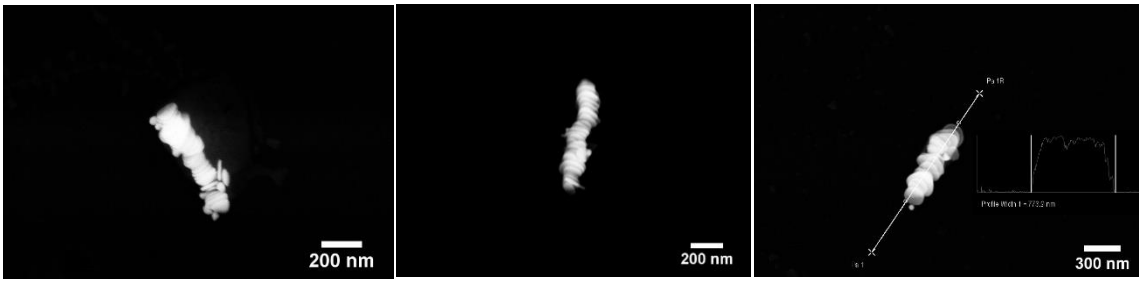

Figure S2: Representative SEM images of 1D face-to-face arrangement of gold nanoprisms.

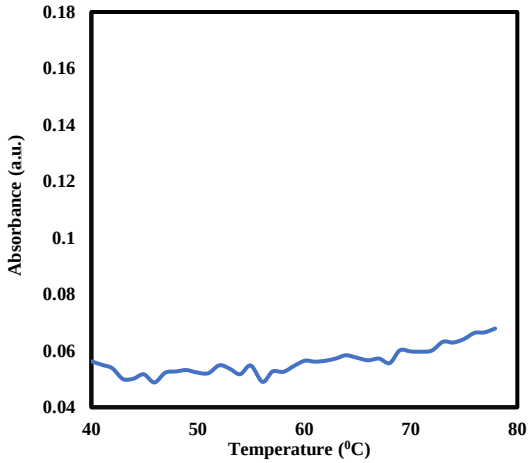

Figure S3: Melting profile of control: uncoated nanoprisms in a DNA hybridization buffer.

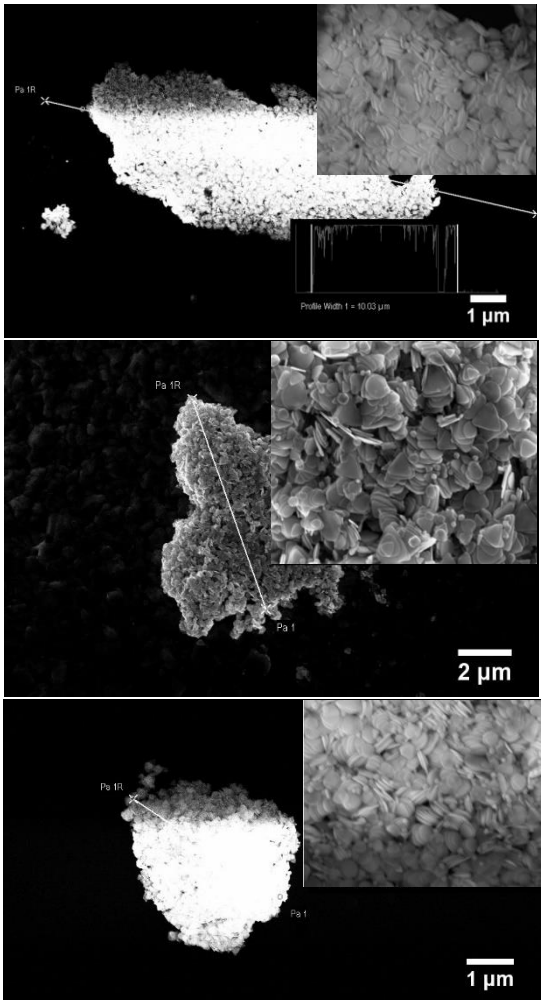

Figure S4: Representative SEM images of 3D hierarchical organization of gold nanoprisms. Insets shows magnification of the corresponding SEM images.

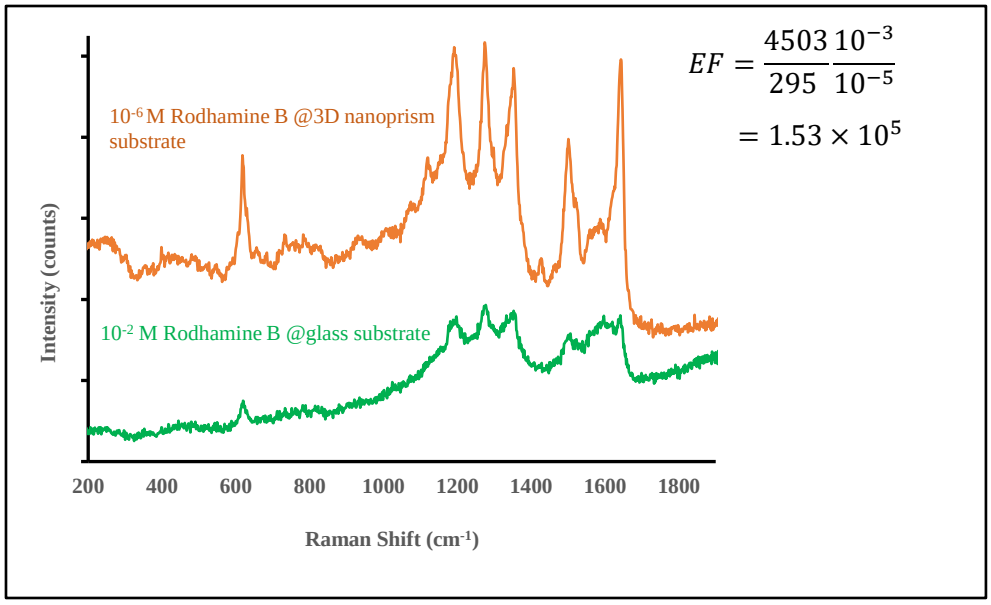

Figure S5: SERS enhancement of rhodamine B using 3D nanoprism aggregates.

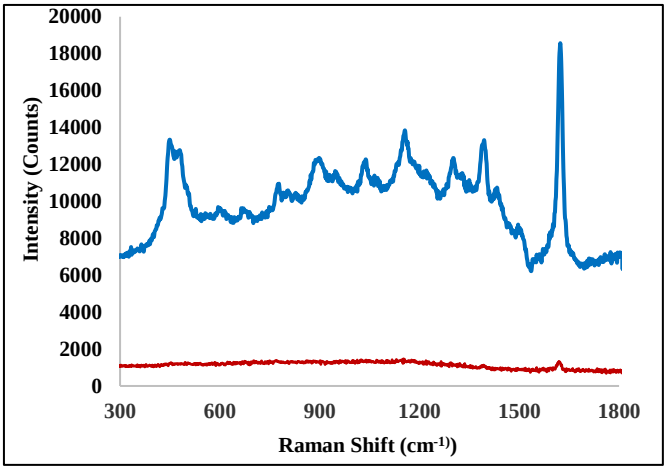

Figure S6: SERS spectra of 10^{-5} M MB on 3D nanoprism aggregates (Blue) and 1D nanoprism stacks (Dark red).

References

1) James, K. T.; O'Toole, M. G.; Patel, D. N.; Zhang, G.; Gobin, A. M.; Keynton, R. S. *RSC Adv.* **2015,** *5* (17), 12498-12505.
2) Chowdhury, E.; Grapperhaus, C. A.; O'Toole, M. G. *Journal of Nanoparticle Research* **2020,** *22* (6), 142.
3) Jones, M. R.; Macfarlane, R. J.; Lee, B.; Zhang, J.; Young, K. L.; Senesi, A. J.; Mirkin, C. A. *Nat Mater* **2010,** *9* (11), 913-7.
4) Millstone, J. E.; Georganopoulou, D. G.; Xu, X.; Wei, W.; Li, S.; Mirkin, C. A. *Small* **2008,** *4* (12), 2176-80.
5) Scarabelli, L.; Coronado-Puchau, M.; Giner-Casares, J. J.; Langer, J.; Liz-Marzán, L. M. *ACS Nano* **2014,** *8* (6), 5833-5842.