Supplementary information

A Bumpy Gold Nanostructure exhibiting DNA-Engineered Stimuli-Responsive SERS signals

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Materials and Methods

Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot 3H_2O$), Hexadecyltrimethyl ammonium bromide (CTAB), Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), L-ascorbic acid (AA), Sodium dodecyl sulfate (SDS), Polyvinylpyrrolidone (PVP) and Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich. Kryptofix [2.2.2] (KP) was purchased from TCI (Shanghai). All chemicals were used as received without further purification. Oligonucleotides with no modification were purchased from Invitrogen. Cy3 modified oligonucleotides and thiolated oligonucleotides were purchased from Sangon Biotech (Shanghai) Co. Ltd.

Synthesis of gold nanorod

The synthesis of gold core was conducted using seeded-growth method with minor adjustment.

First, 50 μL of 50 mM $HAuCl_4$ was added into 10ml of 0.1 M CTAB aqueous solution and vortexed vigorously. Then, 0.6 ml 10mM fresh prepared ice cold $NaBH_4$ was added to the mixture and the stirring speed was increased. The solution color changed to yellowish brown immediately. Then the solution was left still for 1 hour before use. The resulting solution consisted of gold seeds, which acted as seeds for AuNR nucleation and growth.

600μL of 50mM $HAuCl_4$ solution was added into 100mL of 0.1M CTAB solution. Then 500μL of 10mM $AgNO_3$ was added. The resulting solution was stirred for 1min for better mixing. Afterwards, 500μL of 100mM ascorbic acid solution was added. The solution color turned from light yellow to transparent immediately. After another 1min vigorous stirring, 48μL previously prepared AuNP seed solution was added. The mixture was incubated in 30℃ water overnight. The solution color turned from transparent to purple. The gold core was separated and purified by centrifugation (8min, 8000rpm) for 3 times.

Then, the gold core was redispersed in MilliQ water.

Modification of the gold nanorod with DNA

DNA modified gold nanorod core was synthesized using the previous protocol published by our group (D. W. Shi, C. Song, Q. Jiang, Z. G. Wang and B. Q. Ding, **Chem Commun**, 2013, **49**, 2533-2535.).

Firstly, 3mL 1×TBE, 400μL 5M NaCl and 200μL 0.2% SDS were mixed together and mildly shaken. HCl was used to adjust mixture pH to 3.0. Then TCEP reduced thiolated oligos were added into the solution, followed by the addition of s-synthesized gold nanorod core and incubation at 30℃ overnight. DNA modified gold core was collected by centrifugation (8000rpm, 8min) and washed with MilliQ deionized water 3 times. The product was redispersed in deionized water for further use.

Bumpy nanostructure formation

100μL 2nM gold core, 5μL 10% PVP, and 10μL 15mM $HAuCl_4$ were mixed together in 50mM PB solution (pH 7.0). Then 10μL 100mM AA was added, followed by incubation for 1 min. The bumpy nanostructures were purified by centrifugation (5500rpm, 5min) and washed by deionized water. The product was redispersed in deionized water for further use.

Characterization

TEM images were obtained with a T-20 transmission electron microscope by FEI cooperation. HR-TEM images were obtained with a F-20 transmission electron microscope by FEI cooperation.

SERS measurement

Raman and SERS spectra were obtained at room temperature with a Raman spectrometer by Renishaw cooperation equipped with a He/Ne laser (633nm) and a CCD detector. An integration time of 50s was used for all Raman spectra. In a typical experiment, 20μL bumpy nanostructure colloid was loaded in a capillary with a diameter of 200μm and the capillary was laid on the spectrometer stage. Then the laser was focused in the center of the capillary and SERS signals were collected.

Calculation of EF

The analytical enhancement factor can be estimated from the experiments as follows:

$$AEF = (I_{SERS}/I_{Raman})\ (N_{bulk}/N_{structure})$$

Where I_{SERS} and I_{Raman} are the Raman intensity of the same Raman peak under SERS and normal Raman conditions respectively. And N_{bulk} is the number of free Cy3 labeled DNA strand in the focused volume. and $N_{structure}$ is the number of Cy3 labeled DNA located on the surface of bumpy nanostructure. The peak area at 1585 cm^{-1} (aromatic ring vibration)[1] was used to represent the signal intensity (I_{SERS} and I_{Raman}). Because the

laser focus volume was the same for both Raman signal and SERS signal, the N_{bulk} and $N_{structure}$ can be replaced by C_{bulk} and $C_{structure}$. A critical part in the calculation of AEF is to determine the number of Cy3 labeled DNA on a single bumpy structure[2]. We used a DNA strand that was fully complementary to strand 1 to displace the Cy3 labeled DNA from gold nanorod prior to the formation of the bumpy nanostructures. After removal of the nanorod, the fluorescence spectra of the solution were collected and the concentration of the displaced Cy3 labeled DNA was calculated according to a plotted standard curve (fluorescence intensity vs. DNA concentration). The number of Cy3 labeled DNA on a single bumpy structure was determined to be ~28. Thus, the analyte (Cy3) concentration on the bumpy nanostructure colloid was determined to be 56nM. The 1585 cm^{-1} SERS peak intensity of the bumpy nanostructure was calculated as 3869. While the 1585 cm^{-1} Raman peak intensity of 10uM pure Cy3 molecules was 44. The resultant analytical enhancement factor was calculated to be $1.56 \bullet 10^4$.

To calculate the analytical enhancement factor of the gold nanorod, the intensity of the same peak (1585cm^{-1}) was used in the calculation process. The number of Cy3 labeled DNA on a single nanorod was determined to be ~28. Thus, the analyte (Cy3) concentration in the gold nanorod was determined to be 56nM. And the resultant analytical enhancement factor was calculated to be $1.36 \bullet 10^2$.

FDTD simulation

Full-field electromagnetic wave calculations were performed using a commercially available FDTD simulation software package (Lumerical FDTD Solutions). Three-dimensional simulation scenario was used with PML absorbing boundary conditions. Electric and magnetic fields are detected by frequency profile monitors. Dielectric constants of ITO and Gold are extracted from previous reports (S. Linden, J. Kuhl, H. Giessen. Phys Rev Lett 2001, 86, 4688; D. Beaglehole, M. De Crescenzi, M. L. Theye, G. Vuye. Phys Rev B, 1979, 19, 6303.)

Signal switch by K^+/KP

First, 100-fold excess DNA strand **4** and 100μL 2nM DNA modified gold core were mixed together in 50mM PB buffer. Then the mixture was annealed from 45℃ to 25℃ slowly for two cycles. After washed and purification by centrifugation for 2 times, the bumpy gold layer was synthesized using the method described above. Then the bumpy nanostructure was washed and purified by centrifugation for 2 times. After the structure redispersed in 50mM PB buffer, 100-fold excess DNA strand **3** was added to the solution and strand **4** and strand **3** were left still for hybridization for 2h, and the excess strand **3** was removed. Raman spectra were collected from the bumpy nanostructure solution. At 2 hours after 10mM K^+ was added, Raman spectra were collected. Following this step, 10mM KP was added, Raman spectra were collected after 2 hours. The addition of K^+ and KP was repeated for 3 times.

Signal switch by H^+/OH^-

First, 100-fold excess DNA strand **5** and DNA strand **6** and 100μL 2nM DNA modified gold core were mixed together in 50mM PB buffer. Then the mixture annealed from 45℃ to 25℃ slowly for two cycles. After washed and purification by centrifugation for 2 times, the bumpy gold layer was synthesized using the method described above. The pH was tuned to 7.8, and Raman spectra were collected from the bumpy nanostructure solution. After that the pH of the solution was tuned to 5.2 using HAc. Raman spectra were collected. Then the pH is neutralized to 7.8 using NaOH. Raman spectra were collected. The addition of HAc and NaOH was repeated for 3 times.

DNA design and sequences

Table S1 DNA sequences used in the experiments

Number	Sequences (5'-3')
1	TTTATAACTATTCCTATGCGTCTGAAAAAAAAAAAAAAA-SH
2	ATAGTTATAAACGCATAGGAATAGTTATAA-Cy3
3	GGGTTAGGGTTAGGGTTAGGG-Cy3
4	AGGAATAGTTATAAATTTTTTTCCCTAACCCTAACCC
5	TTTTTCAGACGCATAGGAATAGTTATAAACGCATAAAACCCTAACCCTAACCCT AACCC-Cy3
6	GTTAGTGTTAGTGTTAG

Supporting figures

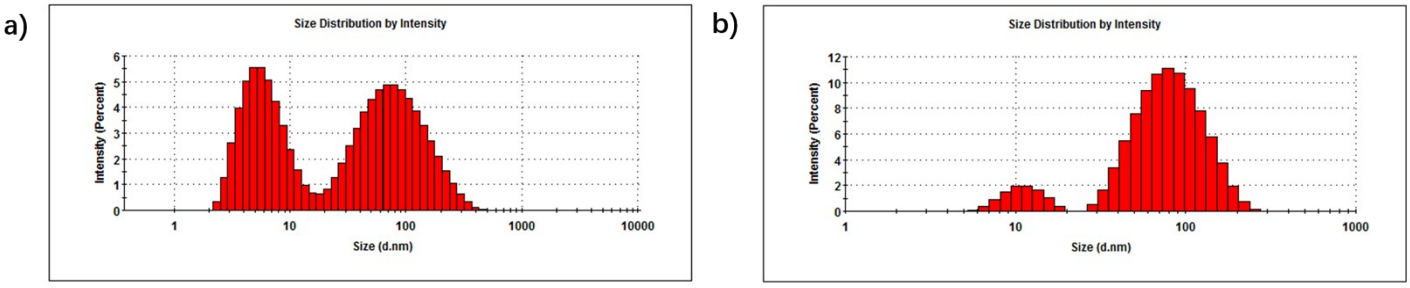

Figure S1 Dynamic light scattering indicates the different particle size between gold core and bumpy nanostructure. a) DLS histogram of gold core; b) DLS histogram of the bumpy nanostructure.

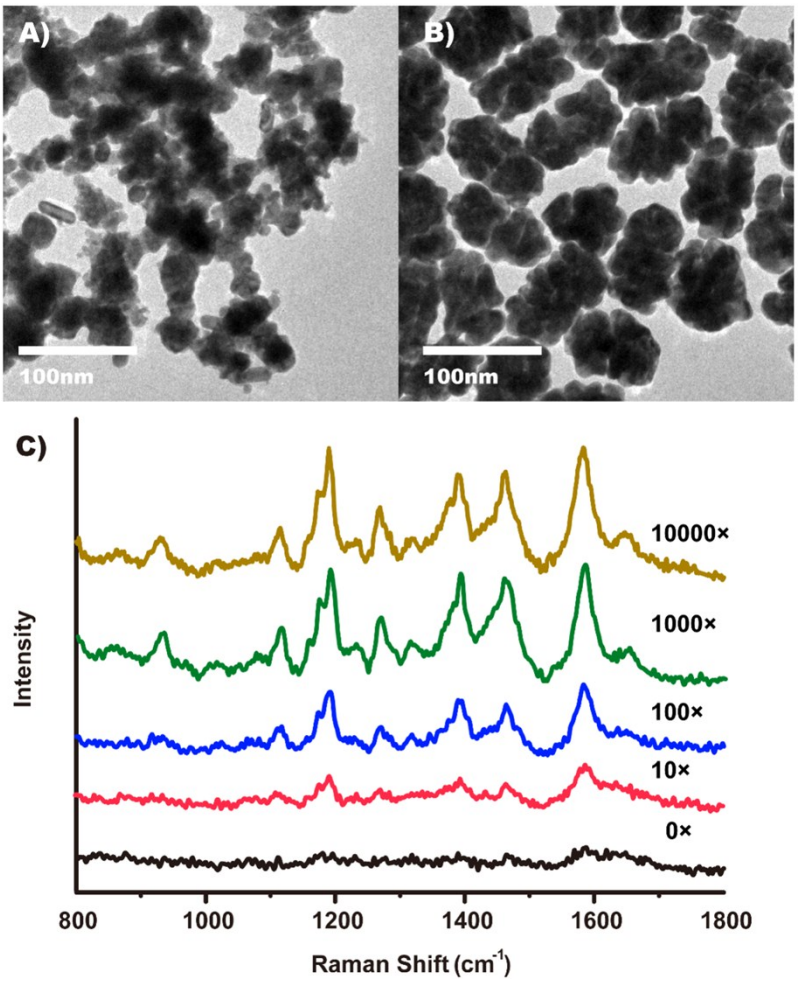

Figure S2 TEM images of the bumpy structures synthesized without (a) and with PVP (b). (c) Raman spectra indicates that when [PVP]/[Gold nanorod] ratio reaches 1000/1, the strongest SERS signals were obtained.

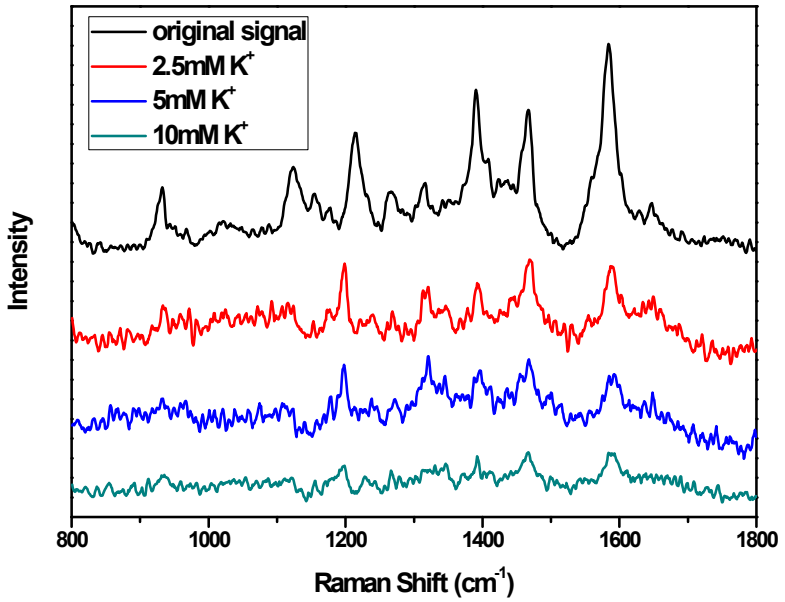

Figure S3 SERS spectra observed from the bumpy particles decorated by Cy3 modified G-quadruplex before and after treatment with K^+ of different concentration.

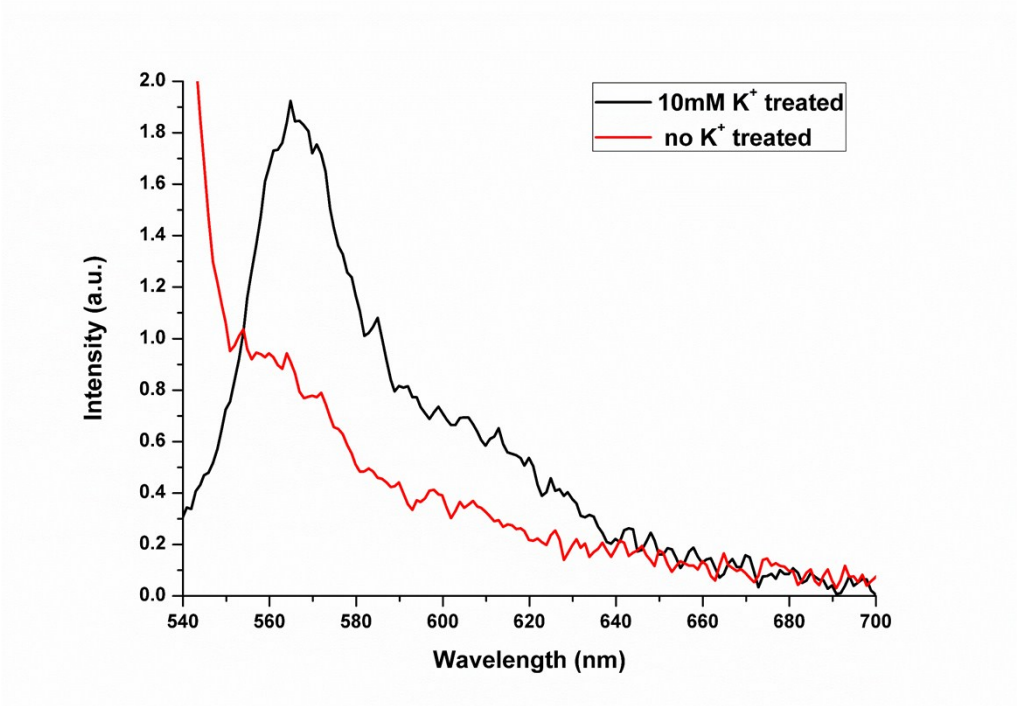

Figure S4 Fluorescence spectra of the supernatant of the dye labeled bumpy nanostructure solution before and after 10mM K^+ added for 2h. (Excited wavelength: 530nm)

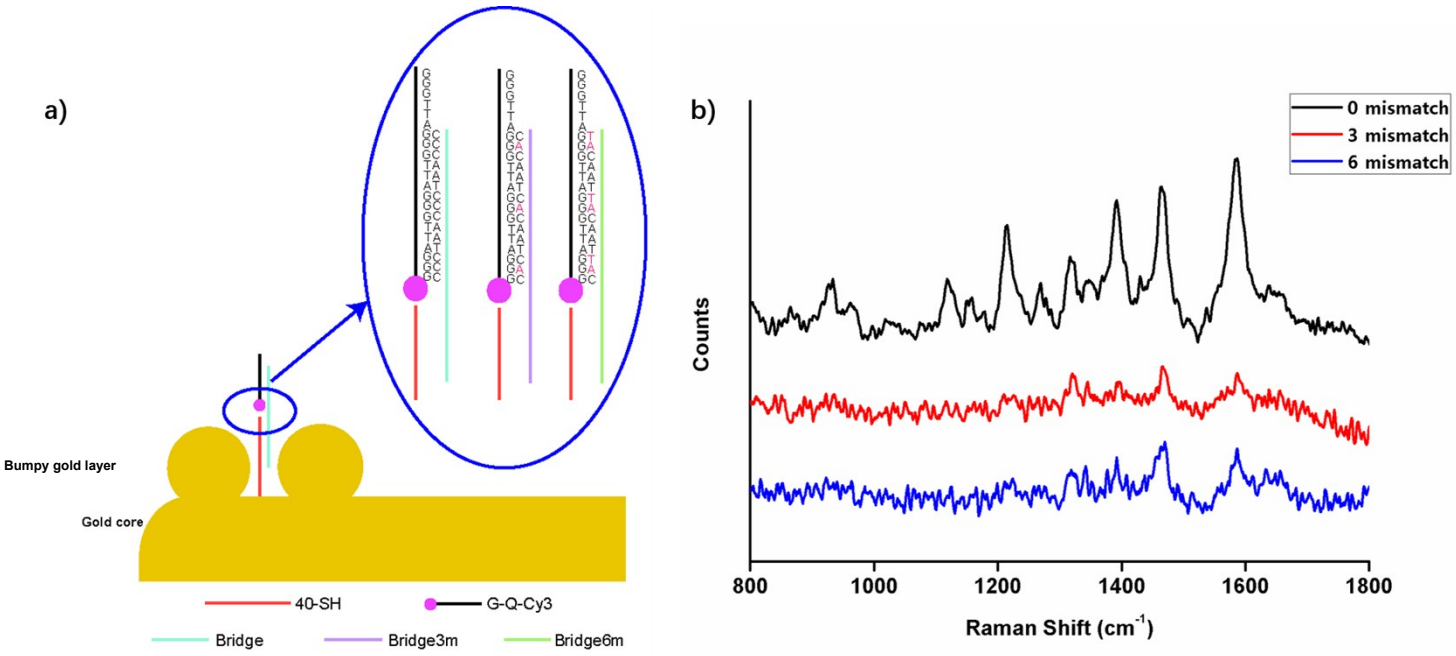

Figure S5 a) Schematic illustration for introducing the mismatches into the complementary region between the bridge strand and the G-quadruplex strand. b) SERS spectra observed from the bumpy gold nanostructures decorated with bridge strands/G-quadruplex duplex containing different base-pair mismatches.

Table S2 DNA sequences demonstrated in Figure S5

Oligo	Sequence (5'-3')
40-SH	TTTATAACTATTCCTATGCGTCTGAAAAAAAAAAAAAAAA-SH
Bridge (**Strand 4**)	AGGAATAGTTATAAATTTTTTTCCCTAACCCTAACCC
Bridge3m	AGGAATAGTTATAAATTTTTTTCACTAACACTAACAC
Bridge6m	AGGAATAGTTATAAATTTTTTTCATTAACATTAACAT
G-Q-Cy3	GGGTTAGGGTTAGGGTTAGGG-Cy3

To confirm the hybridization specificity of strand 4 to dye-labeled G-quadruplex, we engineered the complementary region between these two strands with different mismatches. The fully complementary hybridization resulted in strongest SERS signals, while the 3 or 6 mismatches in the hybridization region led to much weaker SERS signals (Figure S5). This indicates that the mismatches led to the inefficient adsorption of dye-labeled G-quadruplex to the SERS nanoparticles, due to instable hybridization. The results, together with Figure 4, demonstrated the capability of the designed additional

strands to hybridize with the dye-labeled G-quadruplex after the formation of the gold satellites.

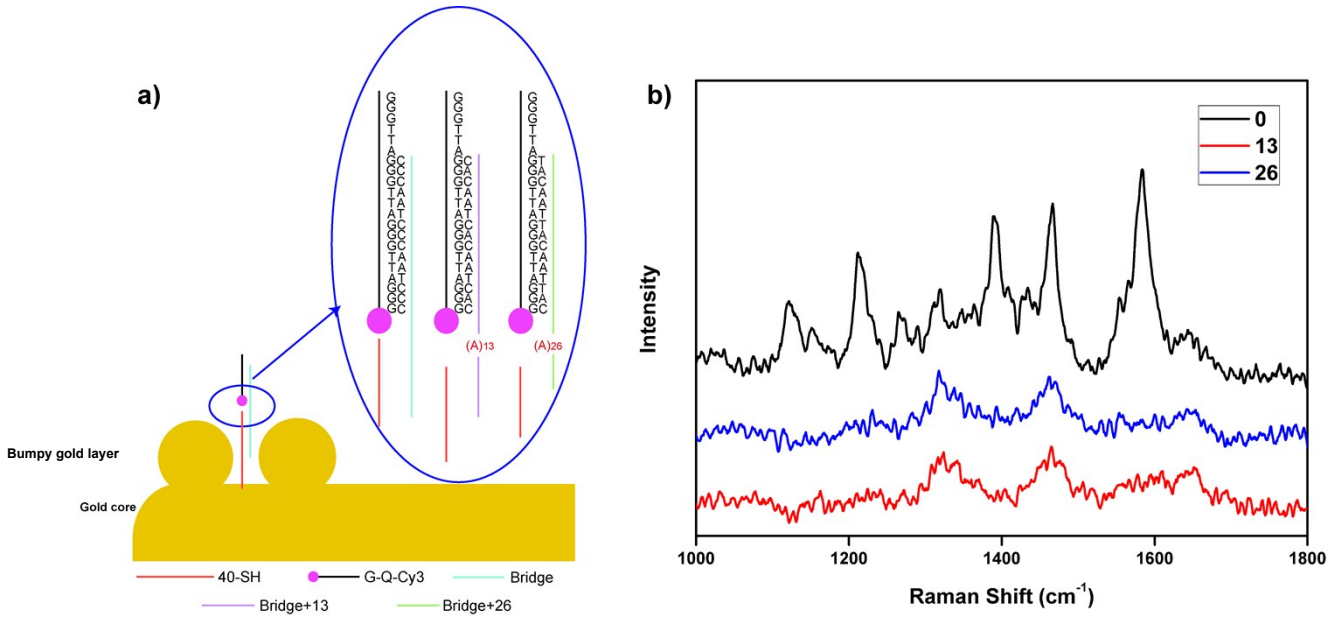

Figure S6 a) Schematic illustration of strand designing to introduce spacer bases into the bridge strand (additional strand) b) SERS spectra observed from the bumpy gold nanostructures decorated with bridge strands-G-quadruplex duplex containing different spacer bases.

Table S3 DNA sequences demonstrated in figure S6

Oligo	Sequence (5'-3')
40-SH	TTTATAACTATTCCTATGCGTCTGAAAAAAAAAAAAAAAA-SH
Bridge (**Strand 4**)	AGGAATAGTTATAAATTTTTTTCCCTAACCCTAACCC
G-Q-Cy3	GGGTTAGGGTTAGGGTTAGGG-Cy3
Bridge+13	AGGAATAGTTATAAATTTTTTTAAAAAAAAAAAAACCCTAACCCT
C13	TTTTTTTTTTTTTAAAAAAA
Bridge+26	AGGAATAGTTATAAATTTTTTTAAAAAAAAAAAAAAAAAAAAAAAAACC CTAACCCT
C26	TTTTTTTTTTTTTTTTTTTTTTTTTAAAAAAA

We changed the length of the bridge strand **4** to control the distance between the dye and the SERS substrate surface. It is observed that the larger distance significantly reduced SERS signals. This again demonstrated the availability of strand **4** to hybridize with dye-labeled G-quadruplex after the growth of the gold satellites. (Figure S6)

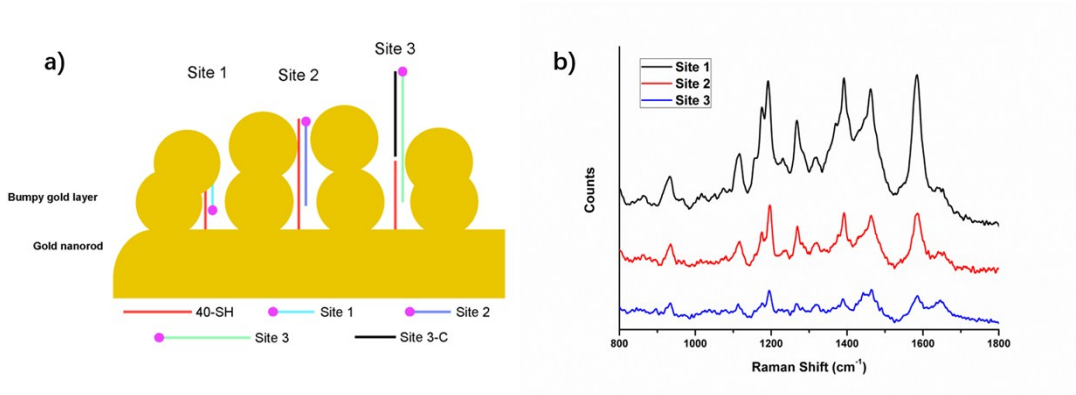

Figure S7 a) Schematic illustration for regulating the location of Cy3 dye molecules relative to the interstices by rational designing the DNA sequence and b) the relevant SERS spectra observed from the particles with different dye location.

Table S4 DNA sequences used in Figure S7

Oligo	Sequence (5'-3')
40-SH	TTTATAACTATTCCTATGCGTCTGAAAAAAAAAAAAAAAA-SH
Site 1	Cy3-TTTTTCAGACGCATAGGAATAGTTAT
Site 2	TTTTTCAGACGCATAGGAATAGTTAT-Cy3
Site 3	ACGCATAGGAATAGTTATAACCGTAACGCTAACACTAAGCC-Cy3
Site 3-C	GGC TTA GTG TTA GCG TTA CGG

We modulated the location of the Raman dyes by controlling the DNA hybridization (Figure S7) to investigate the origin of the SERS signals in the bumpy nanostructure. We observed stronger SERS signals of the dyes at location 1 than those at the surface of the bumpy nanostructures (the dyes were between the overgrown particles, location 2). This indicated higher density of the hotspots nearby the gold nanorod than on the surface of the bumpy nanostructures.

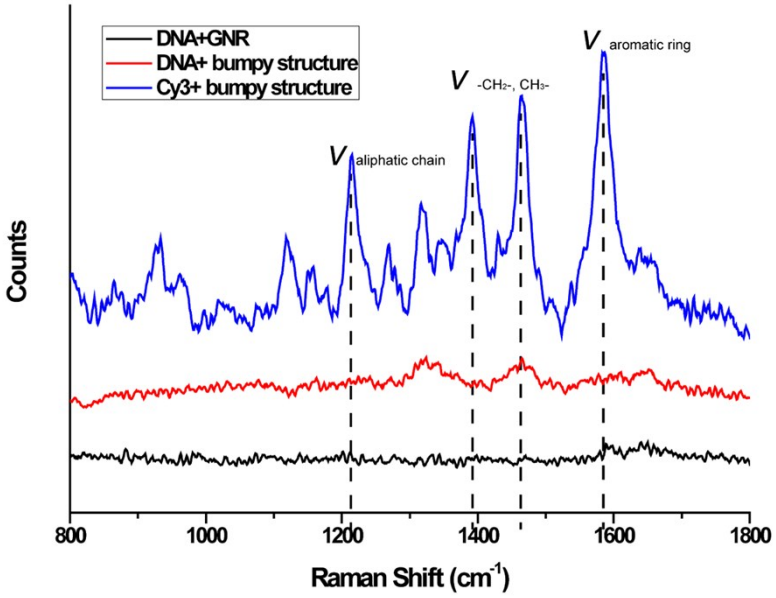

Figure S8 SERS spectra of DNA decorated gold nanorod, DNA decorated bumpy gold nanostructure and Cy3 decorated bumpy gold nanostructure. The aromatic ring vibration peak at 1585cm^{-1} was used to illustrate the SERS signal switch.

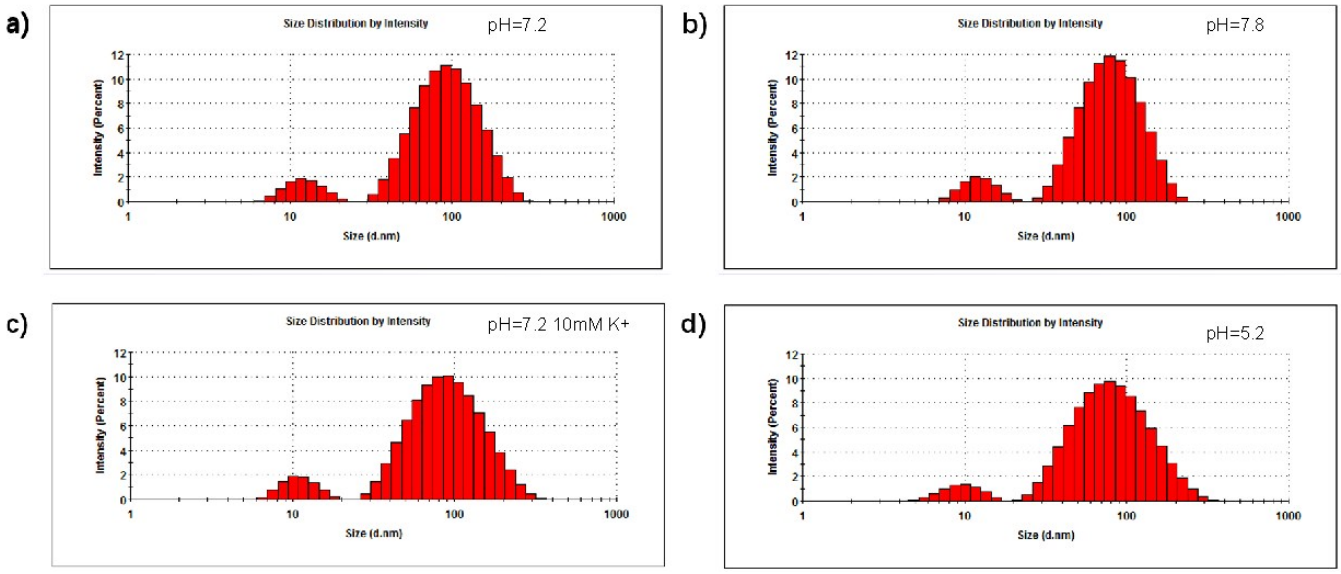

Figure S9 DLS characterization of the bumpy gold nanostructure under different solution environment a) pH=7.2, b) pH=7.8, c) pH= 7.2 & 10mM K^+, d) pH=5.2

1 K. Gracie, E. Correa, S. Mabbott, J. A. Dougan, D. Graham, R. Goodacre and K. Faulds, *Chemical Science*, 2014, **5**, 1030-1040.

2 S. J. Hurst, A. K. R. Lytton-Jean and C. A. Mirkin, *Anal. Chem.*, 2006, **78**, 8313-8318.