Supporting Information

Experimental Section

Au seeds were prepared according to reported procedure with slight modifications.[19] Briefly, 100 mL water in a 250 mL round-bottom flask was heated to boil and 1 ml 4% HAuCl4 solution was added. Then, rapid addition of 12 mL of sodium citrate (38.8 mM) to the boiling solution resulted in a color change from pale yellow to burgundy. After for further 10 min, the heating mantle was then removed. The resulting colloidal solution was characterized using Transmission Electron Microscopy (TEM), indicating that the prepared gold particles have a size of 15 nm ± 1.1 nm (70 particles sampled).

Large Au particles were prepared by using “seeding” approach. To a boiling aqueous solution of 0.01% HAuCl4, 1 mL gold seed solution was added coincidentally with the addition of 38.8 mM sodium citrate. This resulting mixture was heated for further 15 min under stirring. After cooling, the resulting solution is light pink and consists of gold particles with a mean equivalent diameter of 80 nm.

1:1 inclusion complex of ferroence with CB[7] was prepared as follows and denoted as Fc@CB[7]. Briefly, 100 μL of 0.1 M Fc in CH_3CN was added into10mL aqueous solution of CB[7] (1 mM), and the mixed solution was sonicated until the Fc covered the solution surface disappeared and the solution become dark cyan.

To prepare reproducible SERS substrate, the Au nanoparticles were assembled on amino-functionalized glass substrate in a three-step process: firstly, amino-functionalized glass was inserted in Au nanoparticles solution for 4 h; secondly, linker molecules (CB[7] or Fc@CB[7]) were attached to the Au nanoparticles by immersing the glass into a 1 mM solution of CB[7] or Fc@CB[7] for 2 h; finally, the substrate was exposed to Au nanoparticles for 4 h to allow additional nanoparticles to bind to CB[7] or Fc@CB[7] already present on the surfaces of the Au nanoparticles.

FDTD simulations were performed using FDTD Solutions 6.5 (Lumerical Solutions, Inc.). An electromagnetic pulse fixed at 785 nm was launched into a box containing the target Au nanostructure to simulate a propagating plane wave interacting with the nanostructure. The Au nanostructure and its surrounding space were divided into 2 nm meshes and the gap region between the Au particles was divided into 0.2 nm meshes. The refractive index of the surrounding medium was taken to be 1.0. The Au particles were modeled as 80 nm-diameter spheres. These sizes were approximate to the mean equivalent diameter of the particles sample. For the CB-bridged dimer, the spacing between the nanoparticles at the closest point was set at 1.0 nm.

Normal Raman and SERS spectra were collected using a micro-Raman LabRAM HR800 spectrometer (HORIBA Jobin Yvon) and excited by 785 nm laser light focused to 2 μm spot. 0.9 mW of excitation laser, an acquisition time of 1s and a 50X microscope objective were used to collect the SERS data. 0.3474mW of excitation laser, an acquisition time of 30s, acquisition four times and a 50X microscope objective were used to collect the Normal Raman data of Fc and CB crystal. After performing the SERS measurements, the sample was examined with SEM (LEO-1503, Germany). To facilitate identifying nanoparticle structures that give rise to SERS hot spots, we labeled the substrate associated with a copper grid with nummeriert 100 μm×100 μm squares. The TEM images were obtained with JEOL TEM-1200(Japan). Extinction spectral measurements were performed on a Perkin Elmer UV/Vis/NIR Lambda 35 spectrophotometer.

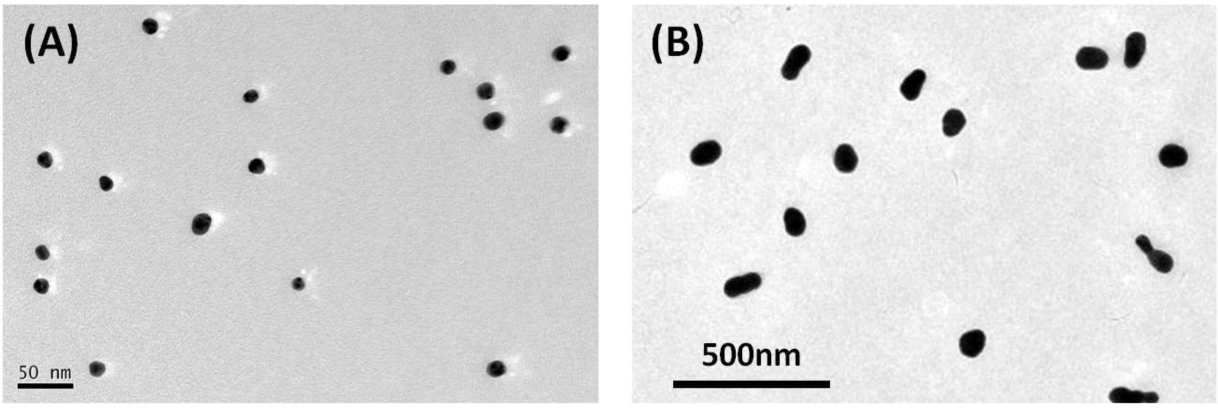

Figure S1 TEM images of (A) Au seed particles and (B) large Au particles

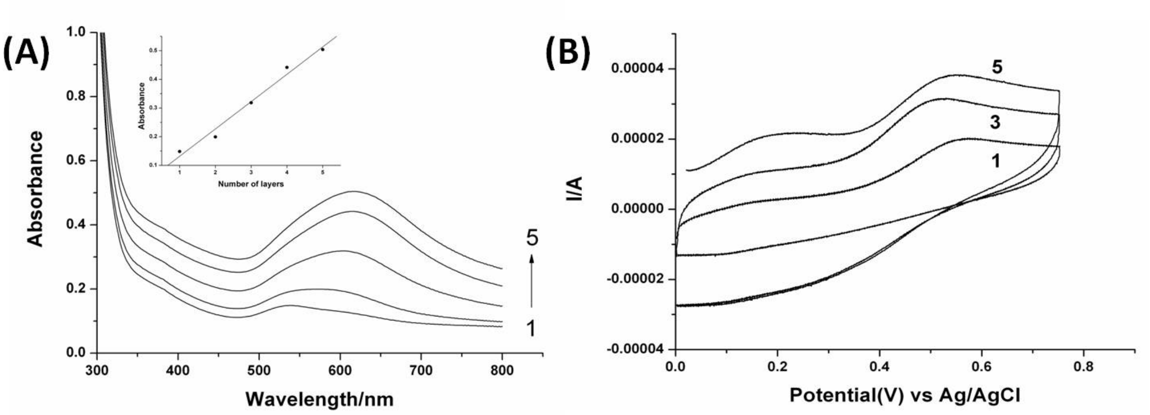

Figure S2. (A) UV/Vis spectra of each bilayer of Au/Fc@CB[7]. Inset: Absorbance increases linearly with the layer number (1~5). (B) CV curves of different number Au/Fc@CB[7] bilayers.

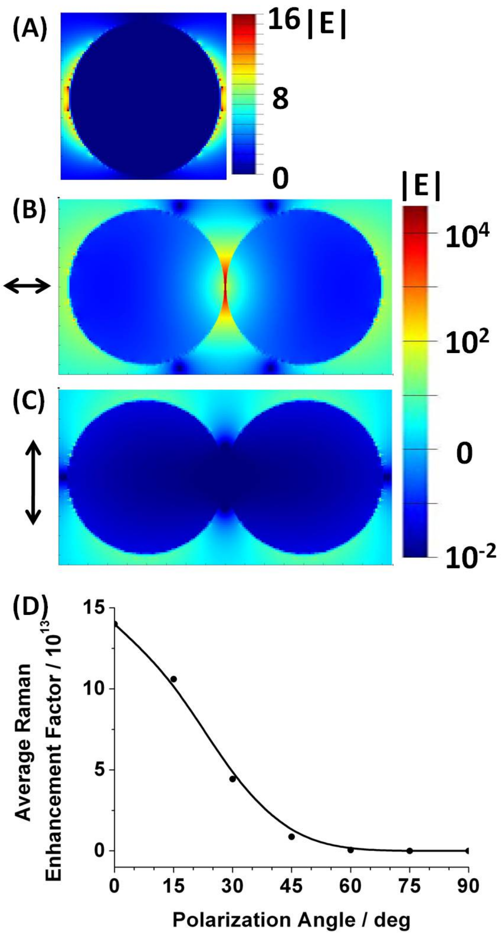

Figure S3. Electromagnetic near field enhancement at excitation laser's wavelength (785 nm) for (A) an isolated 80 nm Au nanosphere, (B) and (C) an adjacent nanosphere pair with an axis parallel and perpendicular to the incident polarization, (D) Raman enhancement factor as a function of incident polarization for two 80 nm gold spheres separated by 1 nm and illuminated with light of wavelength λ=785 nm. The refractive index for the surrounding medium in all calculations is set at n=1.

Table S1. Comparison of Local Electromagetic Field Magnitude within 1 nm of the Surface for Nanostructures of the Same Dimensions*.

	FDTD simulation				
Particle type	Max $\|E\|$	Max $\|E\|^4$	$\int\|E\|\,ds$ (nm^2)	$\int\|E\|^4\,ds$ (nm^2)	EF
Isolated nanosphere (40nm radius)	16.0	6.61×10^4	3.16×10^4	1.30×10^7	1.31×10^3
Nanosphere dimer (⊥)	24.0	3.33×10^5	6.30×10^4	3.53×10^7	1.65×10^3
Nanosphere dimer (∥)	2.92×10^4	7.29×10^{17}	1.18×10^6	3.00×10^{18}	1.40×10^{14}

All fields are dimensionless, normalized to the amplitude of the input wave incident on the nanostructure. Field values for the nanostructure pairs were calculated assuming a 1nm interparticle distance.

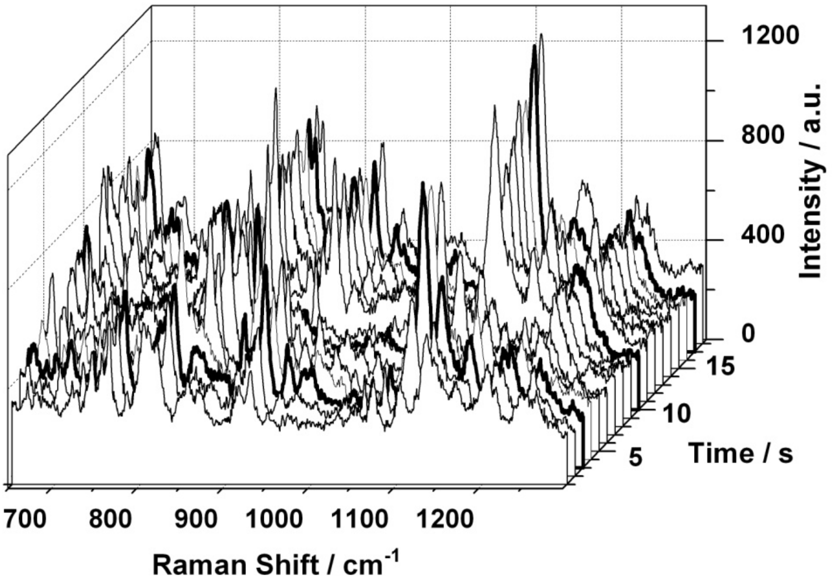

Figure S4. Time-evolution of SERS spectra of Fc@CB-bridged Au aggregates deposited substrate, showing the characteristic single molecule blinking.

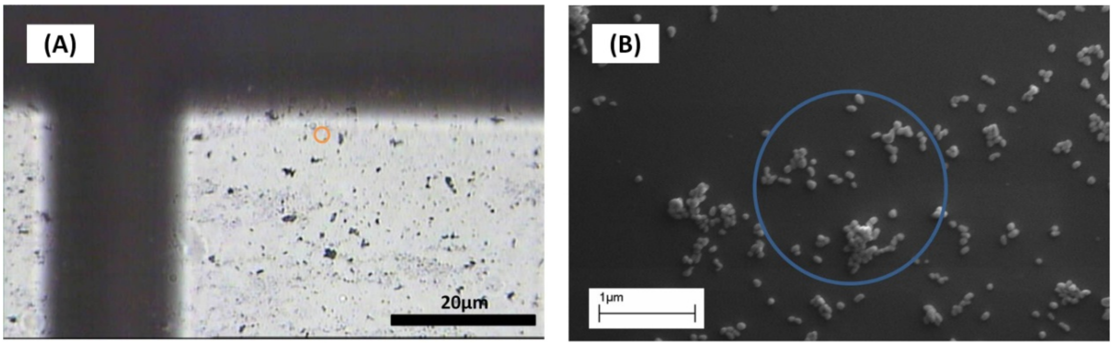

Figure S5. (A) Optical photograph of the SERS substrate. The orange circle indicates the position of laser spot, (B) SEM image of surface of the SERS substrate. The blue circle indicates the position of laser spot.

Details of the Calculation of Enhancement Factor:

To estimate the SERS enhancement factor (EF) of the guest molecule in the hot spot container, we employed the characteristic peak at 1091cm^{-1} and calculated EF by using the following equation:

$$EF = (I_{SERS} \times N_{normal}) / (I_{normal} \times N_{SERS})$$

where I_{SERS} and I_{normal} are the intensities of the same band for the SERS and normal Raman spectra, N_{normal} is the number of molecules probed for a normal Raman setting, and N_{SERS} is the number of molecules probed in SERS. The area of the 1091cm^{-1} band was used as the intensity of I_{SERS} or I_{normal}. We chose this band because it is the strongest band in the spectra. I_{normal} originates from the Fc crystal (Figure 2D). For determination of parameter N_{SERS}, we assumed that the Fc molecules were located just within the gap region formed by CB linker molecules. This assumption is based on the fact that most of the SERS signal comes from the hot spots. The parameter N_{normal} was obtained using the following equation:

$$N_{normal} = \pi r^2 h \times \rho_{Fc} \times N_A / M_{Fc}$$

where $\pi r^2 h$ is the optical excitation volume; ρ_{Fc} is the density of Fc (1.49 g/cm^3) in the bulk crystal; N_A is the Avogadro number, and M_{Fc} is the molecular weight of Fc molecule.

The volumn of the focusd laser spot was estimated as the product of the laser spot (πr2, r=0.61×λ/N.A.) and the depth of focus $h=2\lambda/(N.A.)^2$, where λ=785nm is the excitation wavelength and N.A.=0.5 is the numerical aperture of the objective, thus yielding h≈6.3μm.[1]

$$\begin{aligned} V_{bulk} &= \pi r^2 \bullet h \\ &= 3.14 \times (0.9577\mu m)^2 \times 6.28\mu m \\ &= 18.89\mu m^3 \end{aligned}$$

The number of CB[7] contained in laser volumn:

$$V_{CB[7]} = (1.6nm)^2 \times 0.91nm = 2.33nm^3$$

$$N_{CB[7]} = \frac{V_{bulk}}{V_{CB[7]}} = \frac{18.89\mu m^3}{2.33nm^3} = 8.11 \times 10^9$$

The number of Fc contained in laser volumn:

$$\begin{aligned} N_{Fc} &= \frac{V_{bulk} \bullet \rho_{Fc}}{M} \bullet N_A \\ &= \frac{18.89\mu m^3 \times 1.490g/cm^3}{186.04g/mol} \times 6.02 \times 10^{23}/mol \\ &= 9.11 \times 10^{10} \end{aligned}$$

Based on the equation:

$$EF = \frac{I_{SERS}/N_{Surf}}{I_{RS}/N_{vol}}$$

The enhancement factor based on the 1100cm-1 mode:

$$EF_{Fc} = \frac{I_{SERS,Fc}/N_{Surf,Fc}}{I_{RS,Fc}/N_{vol,Fc}} = \frac{\dfrac{12168}{0.9mW \times 1s \times 1}/56}{\dfrac{526520}{0.3474mW \times 30s \times 4}/9.11 \times 10^{10}} = 1.7 \times 10^9$$

The enhancement factor based on the 800 cm-1 mode:

$$EF_{CB} = \frac{I_{SERS,CB} / N_{Surf,CB}}{I_{RS,CB} / N_{vol,CB}} = \frac{\dfrac{4513}{0.9mW \times 1s \times 1} / 56}{\dfrac{99010}{0.3474mW \times 30s \times 4} / 8.11 \times 10^{9}} = 3.0 \times 10^{8}$$

The number of Fc and CB[7] in the SERS sample was counted from the SEM image in Figure S5, assuming that only one CB molecule at each hot spot.

[1] A. Gopinath, S. V. Boriskina, B. M. Reinhard and L. Dal Negro, *Opt. Express* 2009, ***17***, 3741-3753.