Supplementary Information for

Symmetry-Controllable Assembly of Au Nanooctahedron Superlattice Membranes for SERS

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Experimental Section

Materials

The following chemicals were purchased from Aladin: gold chloride trihydrate (HAuCl$_4$•3H$_2$O, 99%), sodium borohydride (NaBH$_4$, 98%), toluene (anhydrous, 99.8%), azobisisobutyronitrile (AIBN, 98%), ethylene glycol (EG, 98%). The following chemicals were purchased from Sigma-Aldrich: hexadecyltrimethylammonium bromide (CTAB, 99%), cetylpyridinium chloride monohydrate (CPC, 99%), L-ascorbic acid (AA, 99%), ethyl α-bromoisobutyrate (EBIB, 98%), copper(II) bromide (CuBr$_2$, 99%), tin(II) 2-ethylhexanoate (Sn(EH)$_2$, 95%), pentaethylene-hexamine (PEHA, technical grade), tris[2-(dimethylamino)ethyl]amine (Me$_6$TREN, 98%), 4-aminothiophenol (4-ATP, 97%). Hexadecyltrimethylammonium chloride (CTAC, ≥95%) , and 4-mercaptobenzoic acid (4-MBA, ≥95%) were purchased from TCI. Tetrahydrofuran (THF, ≥99.0%) was purchased from Sinopharm Chemical Reagent Co., Ltd. Ultrapure water with a resistivity of 18.2 MΩ•cm at 25 °C was used in all experiments.

Synthesis of Au nanooctahedra

The synthesis of 10 nm Au nanospheres involved two steps.[1] First, for the initial CTAB-stabilized seeds, a solution of NaBH$_4$ (600 μL, 10 mM) was quickly injected into a mixture of CTAB (9.9 ml, 100 mM) and HAuCl$_4$ (100 μL, 25 mM) under rapid stirring, and the reaction mixture was left for 3 h without agitation. After that, 500 μL of these CTAB-seeds were mixed with AA (15 ml, 100 mM) and CTAC (20 mL, 200 mM),

followed by a one-shot injection of $HAuCl_4$ (20 mL, 0.5 mM). After 15 min of stirring

(300 rpm), the Au nanospheres were purified by centrifugation (20,000 rpm, 20 min)

and the pellet were redispersed in CTAC (10 mL, 20 mM). The 30 nm Au nanospheres

were synthesized using 10 nm Au nanospheres as seeds. CTAC (10 mL, 100 mM) and

10 nm Au nanosphere seeds (2 mL, in 20 mM CTAC) were mixed under sonication for

10 minutes. Subsequently, the mixture was stirred in a water bath at 30 °C, and AA (530

µL, 100 mM) was added. After one minute, the addition of $HAuCl_4$ (5 mL, 5 mM) was

initiated using a syringe pump at a rate of 5 mL per hour. Once the addition was

completed, the mixture was stirred for an additional 15 minutes at 30 °C, then

centrifuged (11000 rpm, 15 min), and the 30 nm Au nanosphere pellet was redispersed

in 10 mL of water.

The obtained 30 nm Au nanospheres need to have their CTAC ligands exchanged

for CPC before they can be used for the synthesis of Au nanooctahedra. In a typical

synthesis, CPC (50 mL, 100 mM), $HAuCl_4$ (1 mL, 10 mM), AA (130 µL, 100 mM), and

30 nm Au nanospheres (1 mL) were mixed and allowed to react for 1 hour at 30 °C.

Afterward, the mixture was centrifuged (8000 rpm, 10 min), and the Au nanooctahedra

pellet was redispersed in 10 mL of water.

Synthesis of PS-PEHA

PS-PEHA was synthesized by a two-step reaction, based on a modification of a

previously reported method.[2] In the first step, bromine-terminated PS (PS-Br) was

synthesized using atom transfer radical polymerization (ATRP). In the second step, the

PS-Br obtained from the first step was converted to PS-PEHA, as illustrated in Scheme S1. In a typical reaction for PS-Br with a molecular weight of 3 kDa, styrene (50 eq, 43.6 mmol), anhydrous toluene, EBIB (1 eq), $CuBr_2$ (0.01 eq), and Me_6TREN (0.1 eq) were added to a Schlenk flask at room temperature. After oxygen was removed from the Schlenk flask by three freeze-evacuate-thaw cycles, $Sn(EH)_2$ (0.1 eq) was injected into the reactor. All reactants were stirred at 90 °C for 20 h. The reaction was stopped by rapidly cooling the Schlenk flask in an ice water bath and exposing the reactants to air. The resulting products were diluted with THF and precipitated into methanol. Unreacted monomers and impurities were removed by three repeated counter precipitations. PS-Br was collected by filtration and dried in a vacuum oven for 12 h. To convert of PS-Br to PS-PEHA, PS-Br (2 g, 1 eq), PEHA (75 eq) and triethylamine (100 eq) were added to THF. The mixture was stirred at room temperature for at least 72 h. The impurities were removed by three repeated counter precipitations. After collected by filtration, PS-PEHA was dried in a vacuum oven for 12 h. The ^{1}H-NMR spectrum of PS-PEHA was shown in Figure S4.
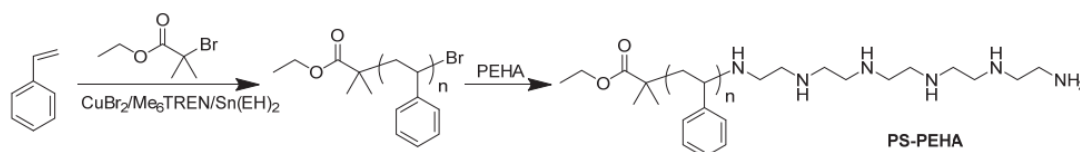
Scheme S1. Synthesis of PS-PEHA.

Surface modification of Au nanooctahedra and Au nanospheres

Typically, 1 mL of the previously prepared Au nanooctahedra solution was used. To reduce the concentration of CPC in the aqueous solution of Au nanooctahedra,

centrifugation was performed. The highly concentrated Au nanooctahedra solution with a low CPC content was combined with a prepared THF solution of PS-PEHA (20 mL, 1 mg/mL). After sonication for 30 min, the mixture was left undisturbed for a minimum of 24 h. The PS-PEHA-grafted Au nanooctahedra were purified through three repeated centrifugations at 8000 rpm and then dispersed in 20 mL of toluene. To enhance the grafting densities of PS-PEHA, 5 mL of ultrapure water was added to the toluene phase containing the PS-PEHA-grafted Au nanooctahedra. The mixture was vigorously stirred for 3 min and then left undisturbed for 2 h. Finally, the PS-PEHA-grafted Au nanooctahedra were dispersed in 500 μL of toluene after undergoing three rounds of water washing. The surface modification of Au nanospheres was similar to that of Au nanooctahedra.

Self-assembly of 2D SL membranes

The SL membranes were fabricated by a liquid-air interfacial self-assembly approach. Typically, 30 μL of toluene solution of Au nanooctahedra (or Au nanospheres) at approximately 10 mg/mL was spread onto the EG surface within a Teflon well. The evaporation of toluene occurred through the gaps between the Teflon surface and a glass slide that covered the well. The rate of evaporation was controlled by adjusting the size of the gaps. As toluene completely evaporated, a solid membrane was formed. To collect the membrane, a substrate, such as TEM grids and Si wafers, was positioned beneath the floating membrane and then gently lifted. Finally, the membrane was dried in a vacuum oven to remove any remaining traces of EG.

To add free PS-PEHA ligands, typically, 1 μL of toluene solution of PS-PEHA at a 1 mg/ml was added into 30 μL of toluene solution of Au nanooctahedra at approximately 10 mg/mL. After thorough mixing, the resulting solution was spread onto the EG surface within a Teflon well. The evaporation of toluene occurred through the gaps between the Teflon surface and a glass slide that covered the well. The rate of evaporation was controlled by adjusting the size of the gaps. As toluene completely evaporated, a solid membrane was formed. To collect the membrane, a substrate, such as TEM grids and Si wafers, was positioned beneath the floating membrane and then gently lifted. Finally, the membrane was dried in a vacuum oven to remove any remaining traces of EG.

The disordered samples of Au nanooctahedra before and after ligand exchange were prepared by the spin coating method. Typically, 30 μL of the previously prepared Au nanooctahedra solution (or the toluene solution containing PS-PEHA-grafted Au nanooctahedra) was dropped onto a Si wafer and spin-coated at 2000 rpm for 1 min, followed by drying under ambient conditions.

SERS measurements of the 2D SLs of Au nanooctahedra

For the Raman spectroscopy measurements, 4-ATP and 4-MBA were selected as the Raman probes. The 2D SLs were transferred onto a Si wafer, which served as the SERS substrate. The substrate was then immersed in 1 mL of an ethanol solution of 4-ATP (or 4-MBA, 10^{-5} M). It was left in contact with the 4-ATP (or 4-MBA) solution for 24 h to allow for adsorption of the Raman probes onto the surface. After immersion, all the

SERS substrates were rinsed with ethanol to remove any unbound 4-ATP (or 4-MBA). For the Raman spectra collection, a laser with a wavelength of 785 nm was used. The acquisition time for each spectrum was typically set to 10 seconds, and the laser power was generally 0.1 mW. The laser spot size used for the measurements was 1.06 μm.

Calculations of θ

The actual length (L) of Au nanooctahedra was determined before self-assembly. The length of the 2D projection of Au nanooctahedra was measured through HRTEM images of the 2D SLs, denoted as L'.

The angle θ between the diagonal of the tilted Au nanooctahedra and the horizontal plane was calculated according to the following formula:

$$\theta = arccos(L'/L)$$

Calculations of enhancement factors (EFs and EFs')

The EFs were calculated according to the following formula:

$$EF = (I_{SERS}N_{bulk}/I_{bulk}N_{SERS})$$

where I_{SERS} is the intensity of the characteristic peak (1078 cm^{-1}) of 4-ATP absorbed on the 2D SLs, I_{bulk} is the intensity of the characteristic peak of bulk 4-ATP, N_{SERS} is the number of 4-ATP molecules absorbed on the 2D SLs within the excitation volume, and N_{bulk} is the number of 4-ATP molecules in a bulk sample within the excitation volume. The N_{SERS} was estimated by the following formula:

$$N_{SERS} = A_N / \sigma$$

where A_N is the surface area of the monolayer membrane on the beam, and σ is the

footprint size of 4-ATP molecule or 4-MBA molecule, which is approximately 0.20 nm^2 and 0.33 nm^2, respectively. [3, 4]

The N_{bulk} was estimated by the following formula:

$$N_{bulk} = (\rho V / M) N_A$$

$$V = \pi r^2 h$$

where r is the radius of the beam size, h is the effective depth of the beam focus, ρ is the density of 4-ATP (1.17 g/cm^3) or the density of 4-MBA (1.30 g/cm^3), M is the molar mass of 4-ATP (125.19 g/mol) or the molar mass of 4-MBA (154.19 g/mol), and N_A is the Avogadro constant (6.02 × 10^{23} mol^{-1}).

The calculation of EF when considering only the hotspots was similar to EF. The hotspot area of the Au nanooctahedra was estimated based on the simulated results shown in Figure 4.

Parameter settings of finite element method (FEM)

FEM was utilized to simulate the electromagnetic field distribution of the two 2D SLs under laser irradiation. In the simulation, the shape parameters of Au nanooctahedra were taken from Figure S1 and the structure parameters of the two 2D SLs were taken from Figure 2. The permittivity of Au was obtained from the literature.[5] The 2D SLs were assumed to be located on Si wafers. The excitation wavelength of the incident light, which is circularly polarized, was selected as 785 nm.

Characterization

Transmission electron microscopy (TEM) was performed using a Hitachi HT7700

TEM operating at 100 kV, while scanning electron microscopy (SEM) was carried out using a Zeiss Gemini SEM500 FESEM at 3 kV. The optical properties of Au nanooctahedra were analyzed using a UV/VIS/NIR spectrophotometer (Perkin-Elmer, Lambda1050+). The average molecular weight and molecular structure of PS-PEHA were determined using gel permeation chromatography (GPC) with an Agilent 1260 Infinity II HT GPC system. Proton nuclear magnetic resonance spectroscopy (^{1}H NMR) was conducted on a Bruker AVANCE III HD 400 MHz FT-NMR spectrometer. The functional groups present in PS-PEHA-grafted Au nanooctahedra were identified by Fourier transform infrared spectroscopy (FTIR) using a Thermofisher Nicolet 6700 spectrometer. Raman spectroscopy analysis was performed using the Renishaw InVia Qontor micro-Raman spectroscopy system, with an excitation laser wavelength of 785 nm and a corresponding laser spot size of 1.06 μm.
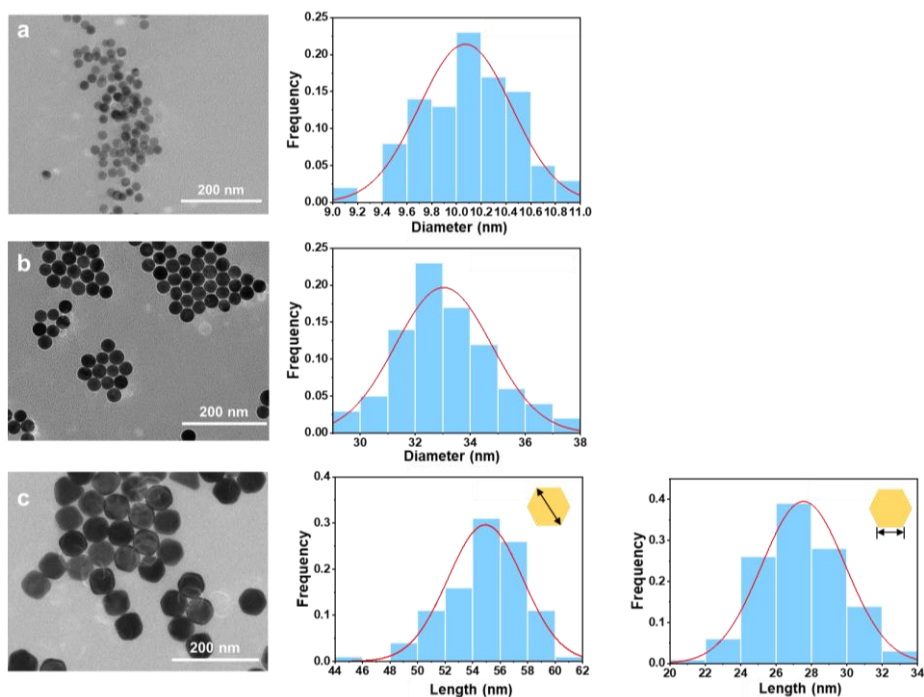
Figure S1. TEM images and corresponding statistical analyses of different Au NCs: (a) 10 nm Au nanospheres, (b) 30 nm Au nanospheres, (c) Au nanooctahedra. Statistical analyses from left to right in (c) correspond to the diagonal and side length of the 2D projected hexagons of Au nanooctahedra, respectively.
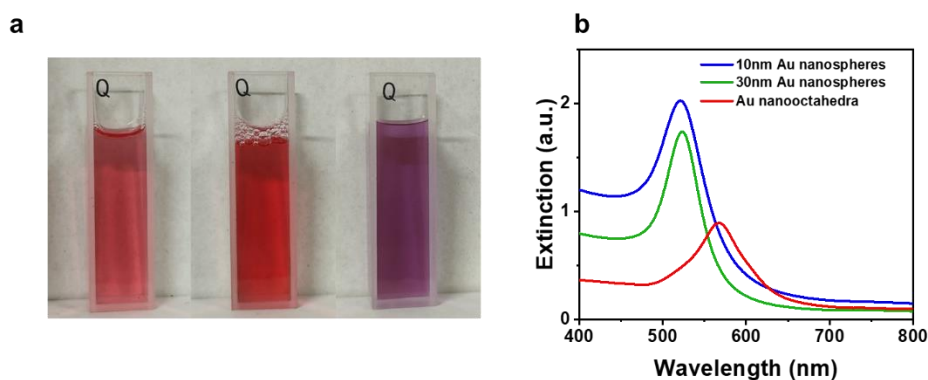
Figure S2. (a) From left to right: photographs of colloidal solutions of 10 nm Au nanospheres, 30 nm Au nanospheres, and Au nanooctahedra, respectively. (b) UV-vis extinction spectra of 10 nm Au nanospheres, 30 nm Au nanospheres, and Au nanooctahedra, respectively.
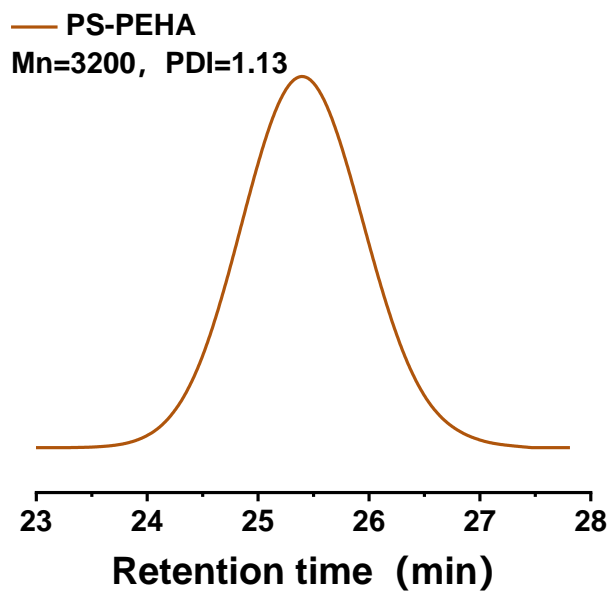
Figure S3. GPC traces of PS-PEHA.
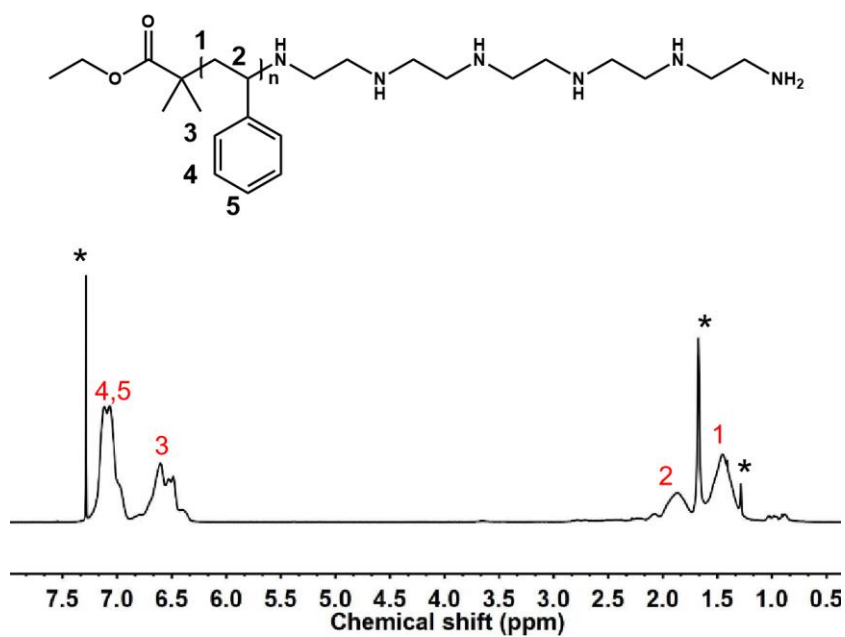
Figure S4. ^{1}H NMR spectrum of PS-PEHA in CDCl$_3$. The asterisks indicate the solvent peaks.
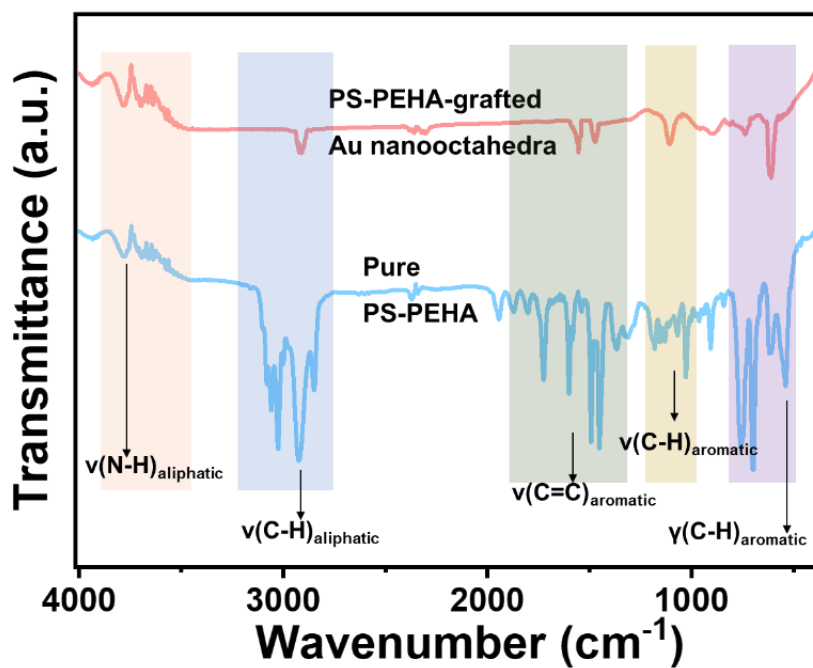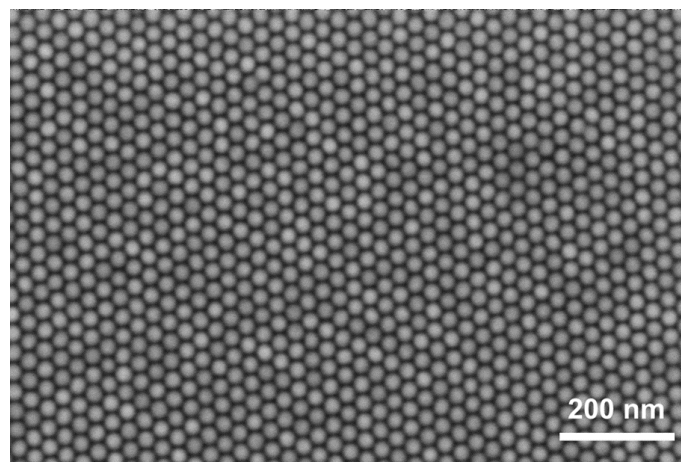
Figure S5. FTIR spectra of PS-PEHA-grafted Au nanooctahedra and PS-PEHA, respectively. The N-H stretching, C=C aromatic stretching, and C-H aromatic stretching occurs at 3500 cm^{-1}, 1600 cm^{-1}, and 1000 cm^{-1}, respectively.
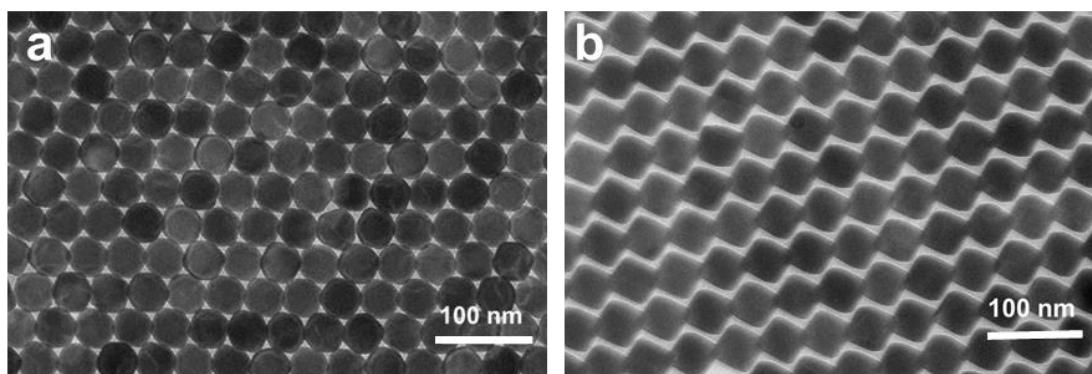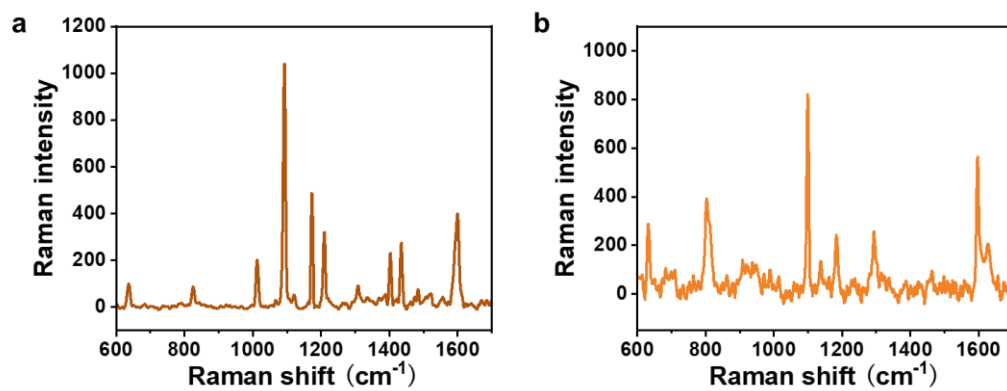

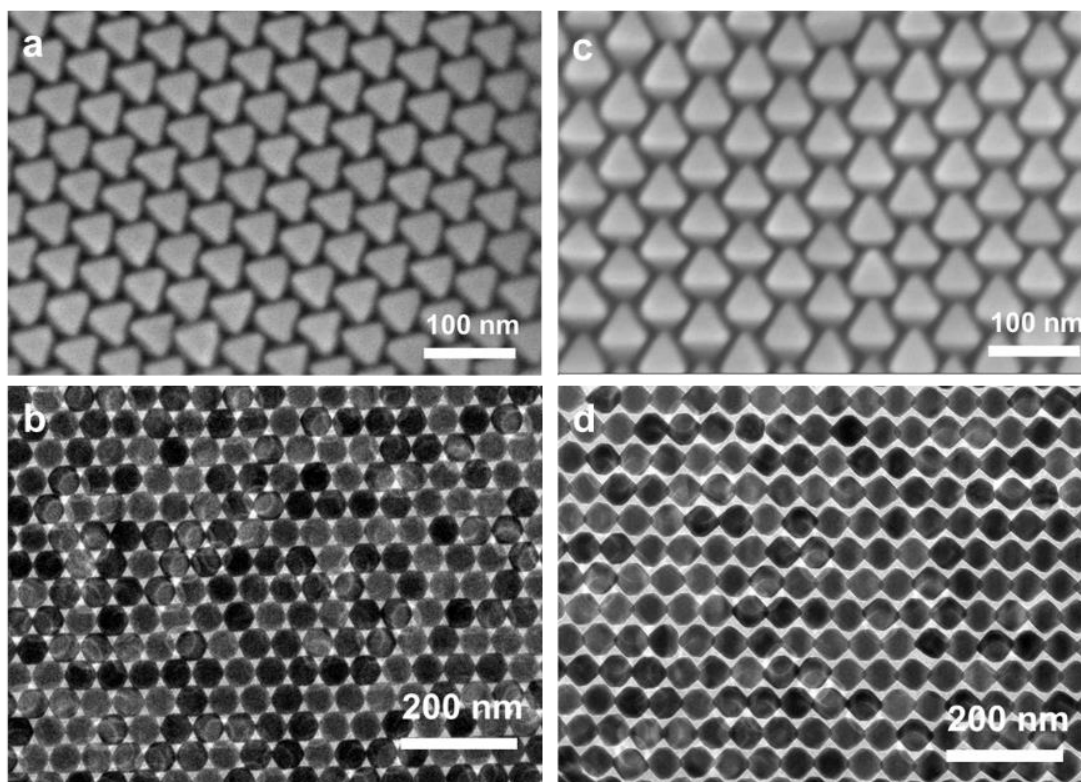**Figure S6**. TEM images of (a) *p31m* SLs and (b) *cm* SLs.
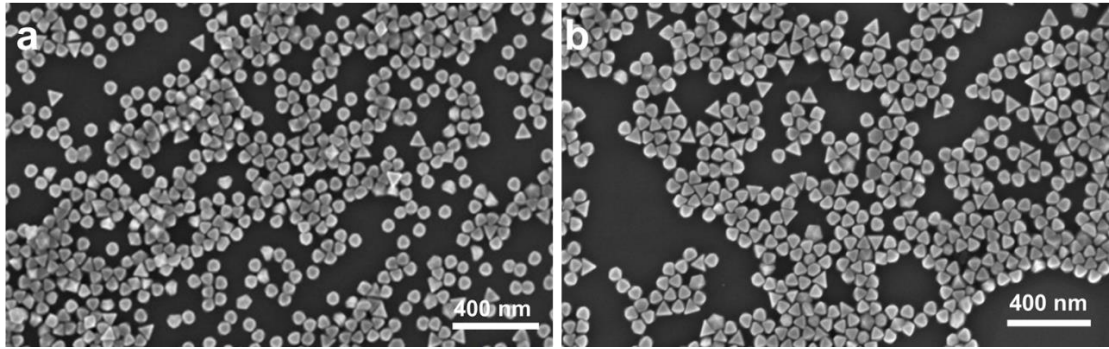
Figure S7. SEM of disordered assemblies of (a) Au nanooctahedra@PS-PEHA and (b) Au nanooctahedra@CPC.
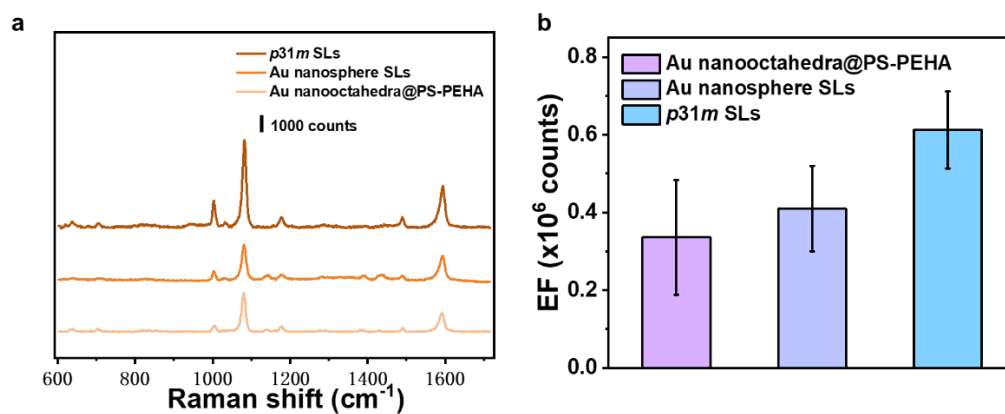

**Figure S8.** SEM image of 2D SLs self-assembled from 30 nm Au nanospheres.

Figure S9. Raman spectra of (a) 4-ATP molecules and (b) 4-MBA molecules.

Figure S10. SEM (a, c) and TEM images (b, d) of various SLs after immersion in the 4-ATP solution for 24 h: (a, b) *p*31*m* SLs and (c, d) *cm* SLs.

Figure S11. (a) SERS spectra and (b) corresponding EFs of 4-ATP on *p*31*m* SLs, Au nanosphere SLs, and Au nanooctahedra@PS-PEHA.
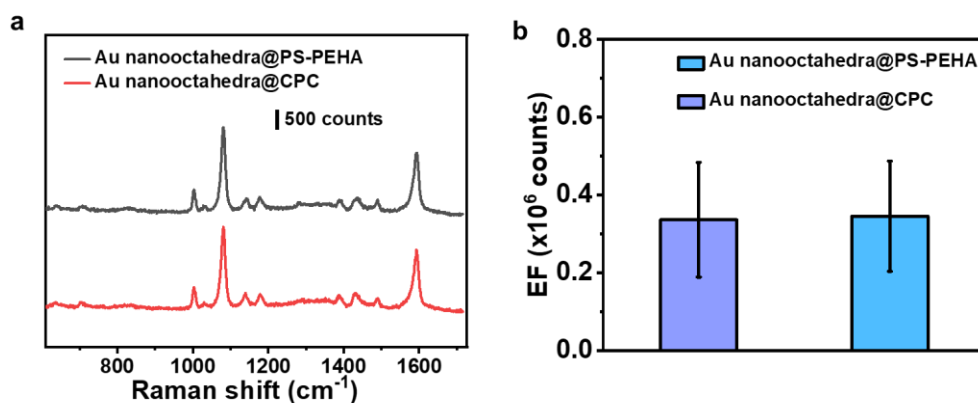
Figure S12. (a) SERS spectra and (b) corresponding EFs of 4-ATP on disordered assemblies of Au nanooctahedra before and after ligand exchange with PS-PEHA.
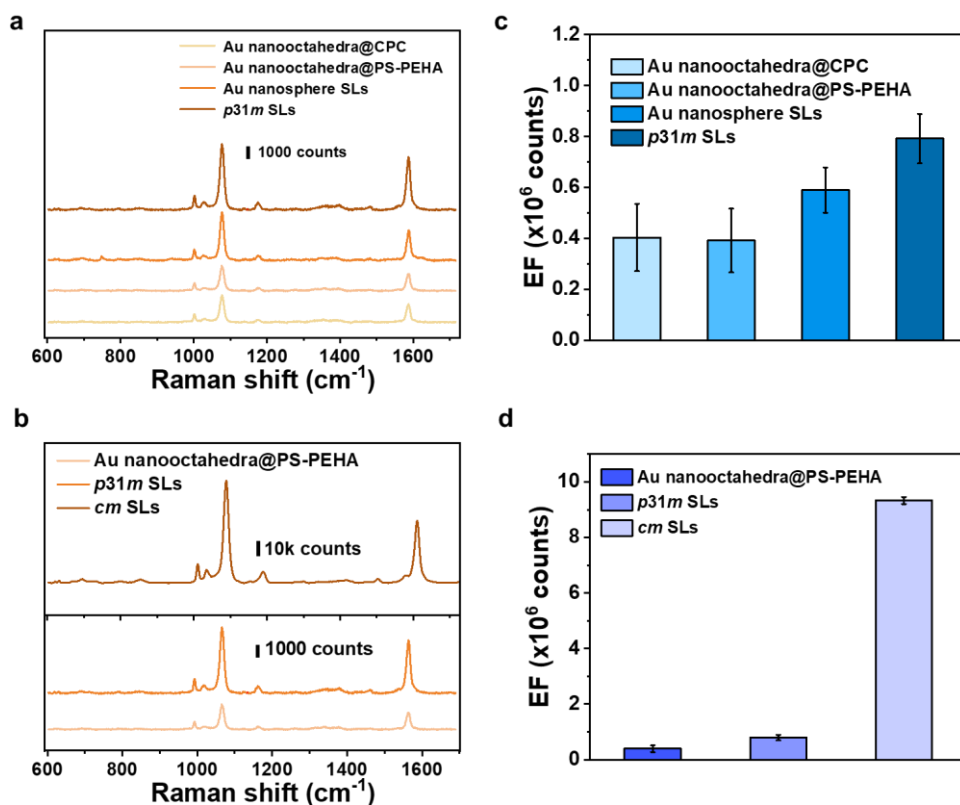
Figure S13. (a, b) SERS spectra and (c, d) corresponding EFs (1078 cm^{-1}) of 4-MBA on various SERS substrates.

References

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5. P. B. Johnson and R. W. Christy, *Physical Review B*, 1972, **6**, 4370-4379.