Supplementary Information

Dynamic colloidal nanoparticle assembly triggered by aptamer-receptor interactions on live cell membranes

Linlin Yang [a], Lingyan Meng [b], Jiaying Song [a], Yue Xiao [a], Ruowen Wang [c], Huaizhi Kang *[a,c], and Da Han *[c]

a Pen-Tung Sah Institute of Micro-Nano Science and Technology, Xiamen University, Xiamen, Fujian, 361005, China

b College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, Fujian, 361005, China

c Institute of Molecular Medicine, Renji Hospital, School of Medicine and College of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai, 200127, China

Corresponding authors

Huaizhi Kang, email: kang@xmu.edu.cn

Da Han, email: dahan@sjtu.edu.cn

Experimental Section

Reagents. All involved DNA sequences (shown in Table S1), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), 30% acrylamide/bis solution, N,N,N′,N′-tetramethylethylenediamine (TEMED), ammonium persulfate (APS), 5× TBE buffer, Tween 20 and phosphate-buffered saline (PBS, 1×, pH 7.4) were obtained from Sangon (Shanghai, China). Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 4-aminothiophenol (4-ATP) and Stains-All were obtained from Sigma-Aldrich. Dulbecco's Modified Eagle medium (DMEM), trypsin/EDTA (0.25%) and penicillin/streptomycin were purchased from Hyclone. Fetal bovine serum (FBS) was purchased from Gibco. Hydrogen tetrachloroaurate-(III) trihydrate ($HAuCl_4 \cdot 3H_2O$), trisodium citrate and other chemicals were of analytical reagent grade and obtained by Sinopharm Chemical Reagent (Shanghai, China). DI water (Milli-Q, 18.2 MΩ) was utilized in all experiments.

Instruments. UV-vis absorption spectra were detected by a UV-2550 UV-visible spectrophotometer (Shimadzu, Japan). Dynamic light scattering (DLS) was performed on a Malvern Zetasizer ZS instrument (Malvern, Britain). Transmission electron microscopy (TEM) images were conducted by a FEI Tecnai G2 F20 S-TWIN (FEI, USA). Raman spectra and Raman imaging were performed on the Nanophoton Raman-11 system equipped with a 785 nm laser and a 50×, 0.45 NA objective.

In Vitro DNA Assembly and Polyacrylamide Gel Electrophoresis (PAGE). All DNA hairpins of SS_n and H_n involved were separately annealed in the assembly buffer (1× PBS, pH 7.4; 140 mM NaCl; 5 mM KCl) by heating to 90 ℃ for 5 min and slowly cooling down to 25 ℃ at a rate of 0.1 ℃ s^{-1}. SS_n-H_n complex were formed by annealing the SS_n and H_n in the assembly buffer at a final concentration of 2 μM. The mixture of 2 μM SS_n and H_n only or the the mixture of SS_n, H_n and SA_0 (SS_n:H_n:SA_0 = 2 μM: 2 μM: 1 μM) in the assembly buffer were incubated at 37 ℃ for 3 h. Then 12% polyacrylamide gel electrophoresis was performed in 1× TBE buffer at a constant voltage of 80 V for 60 min at room temperature. After staining by Stains-All solution for 20 min, gels were photographed.

Preparation and Modification of Au Nanoparticles (AuNPs). AuNPs with average diameter of 13 and 50 nm were synthesized by classical citrate reduction of $HAuCl_4$ [1] and seeded growth method [2], respectively. The 50-nm AuNPs was modified with SS and pre-blocked SA. Before conjugation to the 50-nm AuNPs, 10 μM SA was mixed with 30 μM SYL3C aptamer (Ap) in 1× PBS (pH 7.4) at a molar ratio of 1:3 and the mixture was heated to 75 ℃ and gradually cooled to 25 ℃ at a rate of 1.2 ℃ min^{-1} to ensure the complete blocking of SA by the aptamer. Then the blocked SA and SS_1 were mixed at a molar ratio of 1:10. 60 μL of this mixture containing 1 μM blocked SA and 20 μM SS_1 were incubated with 1 mL of 50-nm Au NPs (0.4 nM) at room temperature for 16 h. Subsequently, 10 μL of 5% Tween 20 was added into the solution and then NaCl was added twice with interval of 30 min to a final

concentration of 0.05 M, followed by shaking gently at room temperature for 24 h. Finally, the resulting Au_{50}-SS_1-SA-Ap was collected by centrifugation and washed with water for three times, and resuspended in equal volume of hybridization buffer (1× PBS, pH 7.4; 0.05 M NaCl; 0.01% Tween 20) at 4 ℃ for the later use. Au_{50}-SS_1-SA was prepared by anchoring SS_1 and unblocked SA on the 50-nm AuNPs, the modifying process was the same as that of Au_{50}-SS_1-SA-Ap. For the Au_{13}-H_1, 10 μL of 100 μM 4-ATP was incubated with 1 mL of 13-nm Au NPs (11 nM) at room temperature for 24 h. Then 50 μL of 10 μM H_1 was added and anchored on the 13-nm Au NPs in same steps. After centrifugation and washing procedure with water for three times, Au_{13}-H_1 was also resuspended in equal volume of hybridization buffer and stored at 4 ℃ for later experiments.

Preparation of Core-satellite Nanostructures in Vitro. 200 μL of Au_{50}-SS_1-SA-Ap and Au_{50}-SS_1-SA were mixed with 200 μL of Au_{13}-H_1 and incubated at 37 ℃ for 12 h with gentle shaking, respectively. Then the resulting mixture was centrifuged at 2500 rpm for 20 min to remove the excess of Au_{13}-H_1, and the assembly was resuspended in hybridization buffer for the UV-vis absorption and DLS measurements, as well as TEM imaging. The Raman spectra of the resulting mixture were collected by a confocal Raman spectrometer with a 785 nm laser (0.2 mW/μm^2), a 50× objective lens with 0.45 numerical aperture (NA) and an exposure time of 10 s.

Cell Culture and Raman Imaging of Living Cells. The MCF-7 and HEK-293T cells were seeded in 35 mm^2 confocal dishes and grown in DMEM media supplemented

with 10% FBS and 1% penicillin/streptomycin, and the cells were maintained at 37 ℃ in a humidified atmosphere (95% air and 5% CO_2) for 24 h. Then both Au_{50}-SS_1-SA-Ap and Au_{13}-H_1 were added into the cell samples to final concentrations of 0.02 nM and 0.5 nM, respectively. After cultured for another 12 h at 37 ℃, the cells were washed three times with 1× PBS (pH 7.4) to remove the free probes and reduce the nonspecific binding. The Raman imaging of cells were taken by a 785 nm laser with a power of 0.1 mW/μm^2, 50× objective lens (0.45 NA) and an exposure time of 30 s.

Cellular Cytotoxicity of the Nanoprobes. The cytotoxicity of the nanoprobes to the MCF-7 and HEK-293T cells were evaluated by MTT assays. 5 × 10^3 cells /well were seeded in 96-well plates. After cultured for 24 h, varied concentrations of Au_{50}-SS_1-SA-Ap and Au_{13}-H_1 were added into the cells and incubated for 24 h, respectively. Subsequently, 10 μL of MTT solution (5 mg/mL) was added to each well and incubated for 4 h. Finally, 100 μL of DMSO was added to each well and shaken for 10 min, the absorbance at 490 nm was measured by a microplate reader. The cells incubated with DMEM media only served as the control.

Table S1 Sequences of oligonucleotides used in the work. Domains are separated by different colors.

Sample Name	Sequence and modifications (from 5′ to 3′)
Substrate strand (SS_1) for modification	SHTTTCACTACAGAGGTTGCGTCTGTCCCATGTGTACAGTCAGACGCAACCTCT
Hairpin (H_1) for modification	SHTTTTGCGTCTGACTGTACACATGGGACAGACGCAACCTCCATGTGTACAGT
Swing arm strand (SA)	SH-$(T)_{50}$CAGACGCAACCTCTGTAGTG Function domain
SYL3C aptamer (Ap)	CACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTTGGCCTG
BHQ1-Ap	BHQ1-CACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTTGGCCTG
SA_0-FAM	CAGACGCAACCTCTGTAGTG-FAM
SA_0	CAGACGCAACCTCTGTAGTG
SS_1	Domain *1* Domain *2* Domain *3* CACTACAGAGGTTGCGTCTGTCCCATGTGTACAGTCAGACGCAACCTCT Domain *2**
H_1	TGCGTCTGACTGTACACATGGGACAGACGCAACCTCCATGTGTACAGT
SS_2	CACTACAGAGGTTGCGTCTGCCCATGTGTACCAGACGCAACCTCT
H_2	TGCGTCTGGTACACATGGGCAGACGCAACCTCCCCATGTGTAC
SS_3	CACTACAGAGGTTGCGTCTGTCCCATGTGTACAACCTGACAGACGCAACCT
H_3	CGTCTGTCAGGTTGTACACATGGGACAGACGCAACCTCCATGTGTACAACCT
SS_4	CACTACAGAGGTTGCGTCTGTCCCATGTGTACAGACAGACGCAACCT
H_4	CGTCTGTCTGTACACATGGGACAGACGCAACCTCCATGTGTACA

Characterization of satellite AuNPs and core AuNPs.

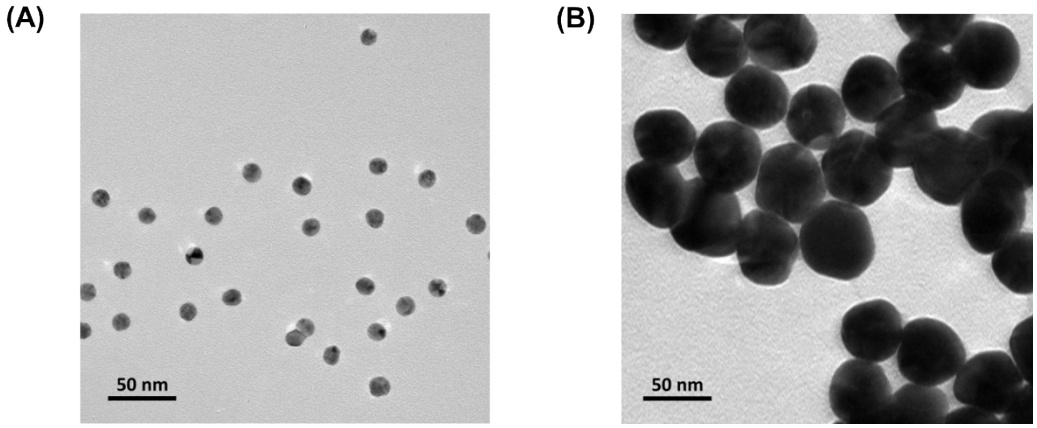

Figure S1. TEM images of (A) 13-nm AuNPs and (B) 50-nm AuNPs.

Fluorescence Spectrometry. The FRET-based SA_0-Ap duplex were prepared by annealing 1 μM BHQ1-Ap and 1 μM SA_0-FAM in Fluo buffer (1× PBS, pH 7.4; 140 mM NaCl; 5 mM $MgCl_2$). Then 0.01 μM EpCAM and 0.01 μM SA_0-Ap duplex were mixed in binding buffer (1× PBS, pH 7.4; 5 mM $MgCl_2$; 0.1 mg/mL yeast tRNA) and incubated at 37 ℃ for 30 min. The fluorescence of the resulting solution was collected from 505 to 600 nm with the excitation wavelength of 492 nm.

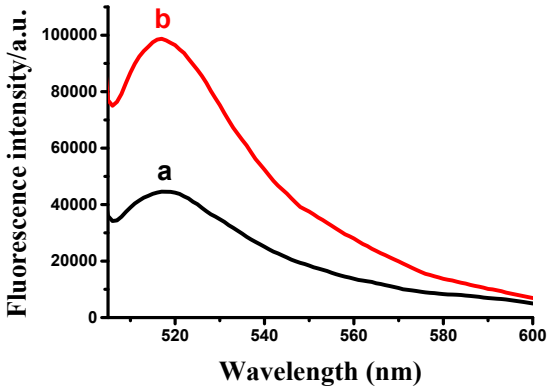

Figure S2. Fluorescence spectra of (a) 0.01 μM SA_0-Ap duplex and (b) the mixture of 0.01 μM EpCAM and 0.01 μM SA_0-Ap duplex.

Finite Difference Time Domain (FDTD) Simulation. The local electromagnetic fields of plasmonic nanostructures were theoretically simulated by FDTD method (the package of Lumerical FDTD Solutions 8.15). For the core-satellites nanostructure, the gap distance between "core" and "satellites" surfaces was estimated to be 10 nm, which was consistence with the length of DNA strands. The total-field scattered-field source with an excitation wavelength of 785 nm was employed to evaluate the local electromagnetic properties of core-satellites nanostructure. The mesh size of the simulating regions was set to be 0.5 nm.

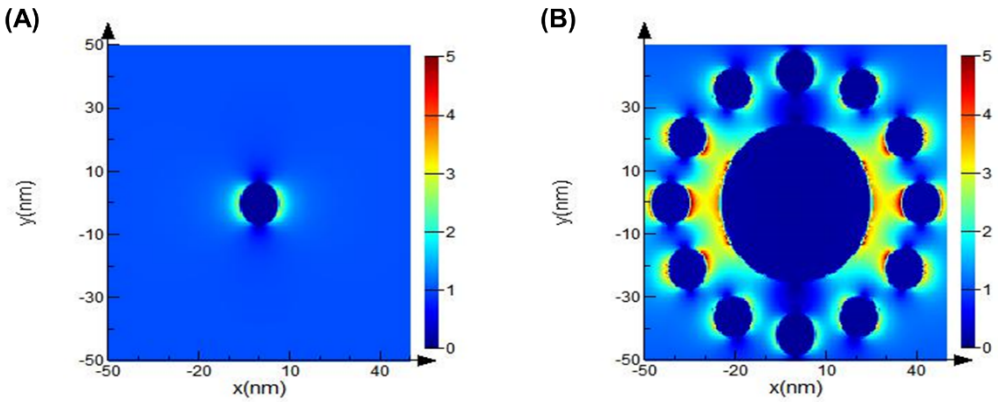

Figure S3. Electromagnetic field distribution of (A) 13-nm AuNPs, (B) core-satellites nanostructure obtained from FDTD simulations. Excitation wavelength is 785 nm, and the gap between the core and satellites is 10 nm.

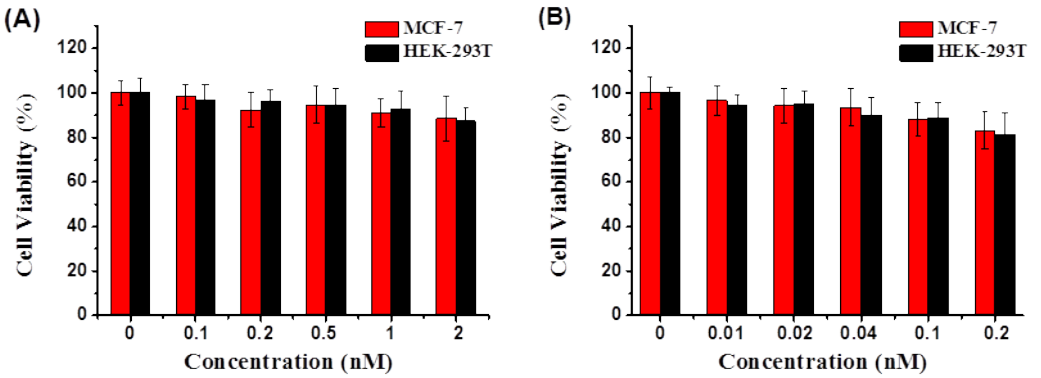

Figure S4. Viabilities of MCF-7 cells and HEK-293T cells after incubation with different concentration of (A) Au_{13}-H_1 and (B) Au_{50}-SS_1-SA-Ap, respectively.

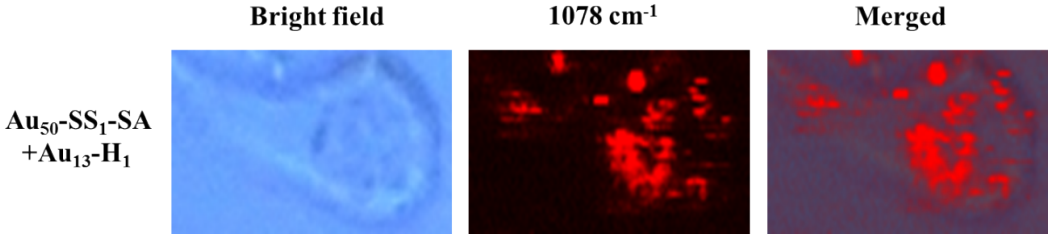

Figure S5. Bright field and Raman images of MCF-7 cells which was treated with Au_{50}-SS_1-SA (0.02 nM) and Au_{13}-H_1 (0.5 nM).

References

1. K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.*, 1995, **67**, 735-743.
2. N. G. Bastus, J. Comenge and V. Puntes, *Langmuir*, 2011, **27**, 11098-11105.