

Supporting Information

Stimuli-Responsive Plasmonic Core-Satellite Assembly: i-Motif DNA Linker Enabled Intracellular pH Sensing

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Experimental Section

Materials and Characterization.

5'-thiol modified DNA strands were purchased from Shanghai Sangon Biotechnology Incorporation (Shanghai, China). The DNA sequences are listed in Table S1. Hydroxylamine hydrochloride, sodium citrate tribasic dehydrate (99.0%), sodium acetate, potassium chloride, Poly (ethylene glycol) (Typical Mn 10,000), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), Dimethyl sulfoxide (99.9%), Sodium hydrogen carbonate, Lithium chloride and Poly-L-lysine (0.01% solution) were purchased from Sigma-Aldrich (Missouri, USA). 10×TAE buffer pH 8.0 and agarose were purchased from 1st base (Singapore). 10× TBE

buffer pH 8.3 was purchased from Promega (Madison, USA). Sodium chloride was purchased from Merck (Darmstadt, Germany). Hydrogen tetrachloroaurate (III) trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, 99.99%) was purchased from Alfa Aesar (Ward Hill, Massachusetts, USA). Hoechst 33342, DMEM (Powder, High Glucose, no sodium pyruvate, no sodium bicarbonate) and 10× PBS pH 7.2 were purchased from Life technologies (Carlsbad, USA).

Transmission Electron Microscopy (TEM) images were obtained by a Jeol JEM 2010 electron microscope operating at an acceleration voltage of 300 kV. UV-vis absorption spectra were acquired on a Thermo Evolution 500 UV-visible spectrophotometer and processed using OriginLab software. Dark field images of single particles and cells and, also, scattering spectra of single particles were conducted on an Olympus IX71 inverted microscope with an oil-immersion dark field condenser, combined with Photometrics CoolSNAP-cf cooled CCD camera and a PIXIS:100B spectroscopy CCD.

Experimental Section.

Synthesis of 14nm Gold Nanoparticles (AuNPs, Satellite Structure). Uniform 14 nm AuNPs, were prepared using the previously published method. [1] Briefly, add a sodium citrate water solution (205 mg in 4 ml DI-water) into a boiling aqueous HAuCl_4 solution (60 mg in 400 ml DI-water) under vigorous stirring. A color change from colorless to red was observed in 5 min. The resulted solution was cooled to room temperature after another 30 min boiling. Store the gold solution at 4 °C for further use.

Synthesis of 50nm AuNPs (Core Structure). 50 nm AuNPs, core structure, were prepared following the procedures mentioned in previous literature with minor modifications. [2, 3]

Typically, seed AuNPs with a diameter of 20 nm were prepared firstly. An aqueous sodium citrate solution (5 ml 38.8 mM sodium citrate solution) was rapidly injected into a boiling HAuCl₄ DI-water solution (5 mg in 50 ml DI-water) under vigorous stirring. A color change from colorless to red was observed in 5 min. Heat the solution for another 20 min and then allows the solution cool to room temperature before further use. Then, this seed AuNP solution was used to synthesize 50 nm AuNPs. Briefly, 50ml water was added into a 100 ml round-bottom flask. 2 ml of seed AuNP solution and 200 µl of 0.2 M NH₂OH·HCl were added into this flask consecutively. Afterwards, 3 ml of 0.1 % (wt) HAuCl₄ was dropwise added into the solution under vigorous stirring followed by 30 min reaction at room temperature. A gradual color change from light red to dark red was observed. Finally, adjust the concentration of sodium citrate to 1 mM. After another two hour reaction, nanoparticle solution was stored at 4 °C for further use.

DNA Functionalization of AuNPs. The functionalization of AuNPs using thiolated DNA followed the method described in the literature previously published. [4]

Activation of disulfide bond of DNA. We dissolved DNA (powder) in 0.5×TAE buffer (20 mM Tris-Acetate, 0.5 mM EDTA, pH 8.0) with 0.3 M sodium acetate. TCEP was added into DNA solution to cleave disulfide bond to activate the thiolated DNA.

Modification of thiolated DNA onto AuNPs. AuNP solution was placed into a beaker with vigorous stirring. Appropriate amount of DNA was added into AuNP solutions carefully followed by the adjustment of the concentration of PEG 10,000 to 10 % (wt). Then, LiCl solution was added dropwise into DNA solution and the salt concentration was adjusted to 80 mM for 14 nm AuNPs and 100 mM for 50 nm AuNPs. After overnight incubation, AuNPs

dispersions were centrifuged and the resulted DNA functionalized AuNPs were stored in 4 °C for further use.

Self Assembly of DNA-functionalized AuNPs. Two kinds of DNA functionalized AuNPs were mixed in a certain ratio after which the salt solution was increased to 50 mM using 0.3 M NaCl. The reaction solution was maintained at room temperature for 1.5 h. After this hybridization procedure, the assembled core-satellite nanostructures (CSNSs) were collected by centrifugation and redispersed in 0.5×TBE buffer (44.5 mM Tris-borate, 44.5 mM boric acid, 1 mM EDTA, pH=8.3) at 4 °C.

Gel Analysis of Samples. Processed samples were loaded in 1 % agarose gels using 0.5×TBE buffer as running buffer. Gel wells were sealed with 8 % agarose gels. Gels ran at 100 V, 8 V/cm for 45min, after which images were taken.

Cellular Experiments. RAW264.7 cells were cultured in DMEM medium mixed with 1.5 g/l sodium bicarbonate and 10 % fetal bovine serum (FBS), with 5 % CO₂ at 37 °C. For cell imaging experiment, cells were planted and grown on poly-L-lysine modified glass coverslips and incubated for one day. CSNSs or 50 nm AuNP/DNA in 1 ml PBS with the concentration of 10 pM was incubated with cells for 15 min. Then, cells were washed twice with PBS to remove free AuNP assemblies. Cells were maintained in fresh medium before microscopy observation at certain time intervals. Hoechst 33342 was utilized to stain cell nuclei under the instruction of commercial protocols. Typically, Hoechst 33342 was dispersed in PBS to reach a concentration of 1µg/ml and to interact with macrophages for 15 min in 37°C cell incubator. After this staining process, cell slides were washed three times using PBS and observed using fluorescence microscopy. Control experiments at 4°C were also conducted with cells on glass

coverslips. The same concentration (10 pM) of CSNSs were used and the incubation of assemblies with cells was performed at 4 °C for 1h.

Cytotoxicity Analysis. A standard Cell Counting Kit-8 (CCK-8) was utilized to analyze the cytotoxicity of CSNSs following a general protocol. Briefly, RAW 264.7 cells were seeded in a 96-well plate with the concentration of 10000 cells/well. After a 24 h incubation in the incubator at 37 °C, CSNSs with final concentrations of 5 pM, 10 pM, 20 pM and 40 pM were incubated with cells for 30 min, 60 min, 120 min, 180 min and 240 min, respectively, after which 10 µl of CCK-8 solution was added to each well of the 96-well plate to incubate for another 4 h. The amount of an orange formazan dye, produced by the reduction of WST-8 (active gradient in CCK-8) by dehydrogenases in living cells, is directly proportional to quantity of living cells in the well. Therefore, by measuring the absorbance of each well at 450 nm using a microplate reader, cell viability could be determined with the calculation of the ratio of absorbance of experimental well to that of the cell control well. All experiments were triplicated and results were averaged.

Discussions

Optimizations. It should be noticed that the main parameters influencing the fabrication of the core-satellite nanostructures included the ratio of core AuNPs to satellite AuNPs, salt concentration and also the hybridization time. Figure S3 was the UV-vis spectra of assemblies through different ratios of 50 nm AuNPs/G-rich DNA (50AuNP/G-rich) to 14 nm AuNPs/i-motif DNA (14AuNP/i-motif). It was evident that the hybridization time was also needed to optimize. So the kinetic experiment was carried out to determine the optimal hybridization time. The optical signal response was recorded, and the results were shown in

Figure S4. The LSPR wavelength red shifted significantly, and it reached a plateau at about 90 min. Therefore, 90 min was used for hybridization.

Calculation of numbers of satellite nanoparticles (NP) per core NP. Initial volume and concentration of core and satellite NPs were recorded. After hybridization process, free satellite NPs that were not attached to the core NPs were recovered by centrifugation. The number of the AuNPs linked to the cores was calculated accordingly, which led to an average number of 40 satellites per core.

Another important control experiment, K^+ responsive experiment, was carried out for the reason that the complementary strands of cytosine-rich pH-responsive strands have plenty of guanine bases and the sequence can form G-quadruplex in the presence of K^+ [5] which are widespread in PBS buffer and cytoplasm.[6] A series of concentrations up to 110 mM K^+ were used and we found that no obvious blue shift of LSPR band (Figure S5). Moreover, we also observed no obvious blue shift of LSPR band even when incubating the core-satellite nanostructures with 20 mM K^+ for 2 h (Figure S6). The results show that K^+ cannot cause the disassembly of CSNSs. It is deduced that K^+ cannot unwind such DNA duplex linked on two AuNPs to induce the formation of G-quadruplex because of the fully complementary sequence of the SH-G-rich DNA and SH-i-motif DNA. The thermodynamics stability of fully complementary DNA duplex is higher than that of K^+ induced G-quadruplex.

We also coated the SH-G-rich DNA on 14 nm AuNPs and SH-i-motif DNA on 50 nm AuNPs to form another kind of CSNSs (14nm AuNPs/G-rich DNA (14AuNP/G-rich) and 50 nm AuNPs/i-motif DNA (50AuNP/i-motif)). This kind of CSNSs is also pH-sensitive (Figure S7) and isn't K^+ -sensitive (Figure S8) in several minutes. However, we can observe a slight

blue shift of LSPR band (about 4 nm) when they were incubated with 10 mM and 20 mM K⁺ for 2 h (Figure S9). Thus, to eliminate the interference of K⁺, in our experiments, we use the core-satellite nanostructures of 50AuNP/G-rich and 14AuNP/i-motif.

Table S1. Sequence information of ssDNA used in this study.

ssDNA	Sequence
G-rich DNA	HS-5' -TTTTTTTTTTT-GGG TTA GGG TTA GGG TTA GGG-3'
i-motif DNA	HS-5' -TTTTTTTTTTT-CCC TAA CCC TAA CCC TAA CCC-3'
Control 1	HS-5' -TTTTTTTTTTT-ACA AGT TCA AGT TGC AAG TTG-3'
Control 2	HS-5' -TTTTTTTTTTT-CAA CTT GCA ACT TGA ACT TGT-3'

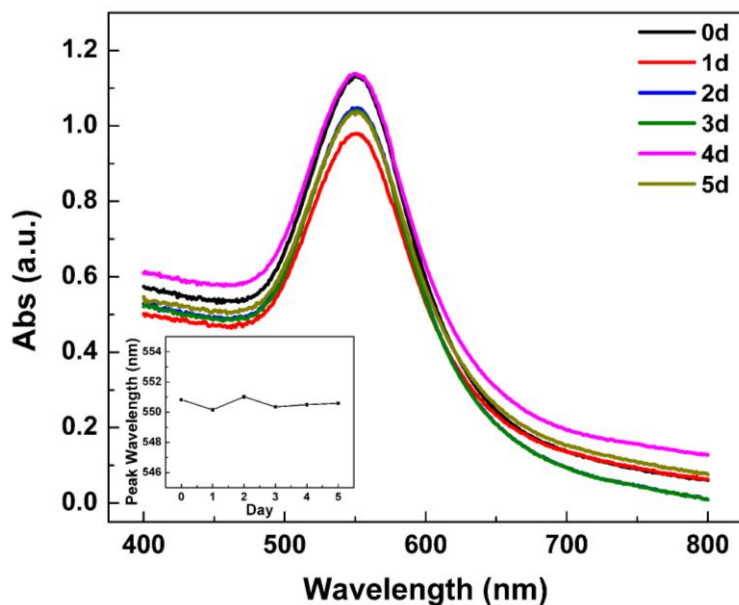


Figure S1. UV-Vis spectra of core satellite nanostructures in 0.5×TAE (pH 8.0) buffer with 40mM LiCl for five consecutive days.

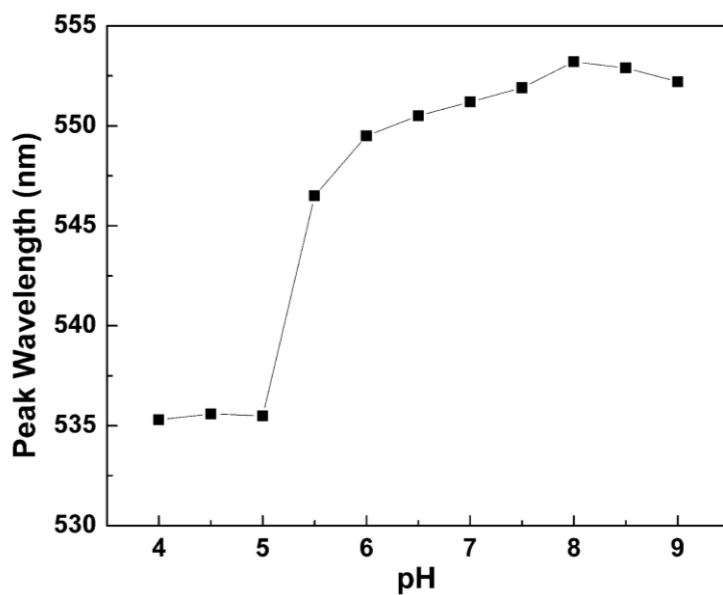


Figure S2. LSPR peak wavelength of the core-satellite nanostructures in buffers with different pHs.

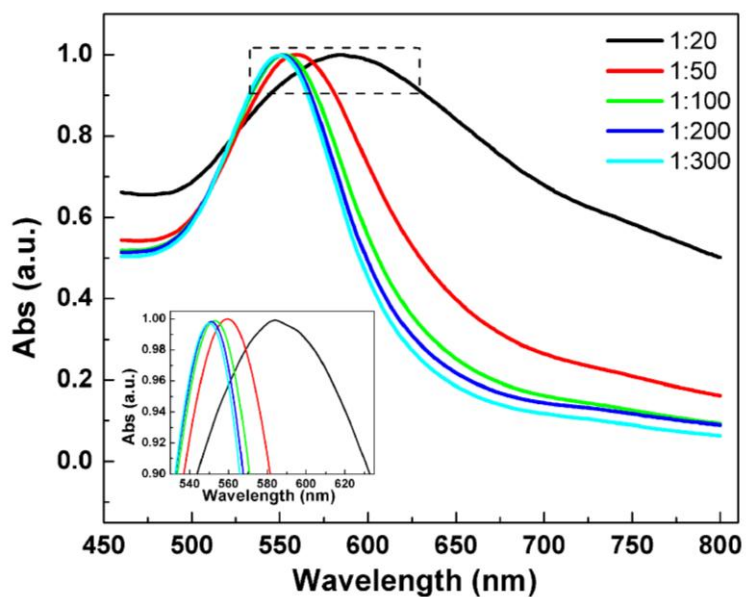


Figure S3. UV-vis spectra of the assembly of 50AuNP/G-rich to 14AuNP/i-motif at different ratios.

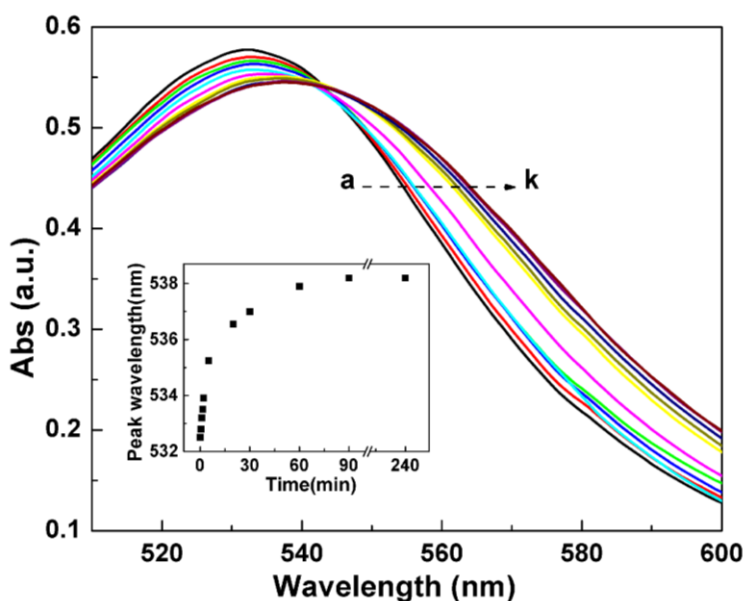


Figure S4. UV-vis spectra of time dependent hybridization of nanoparticles. A continuous red shift of hybridization solutions was observed within 90 minutes. Inset: peak wavelength of different time.

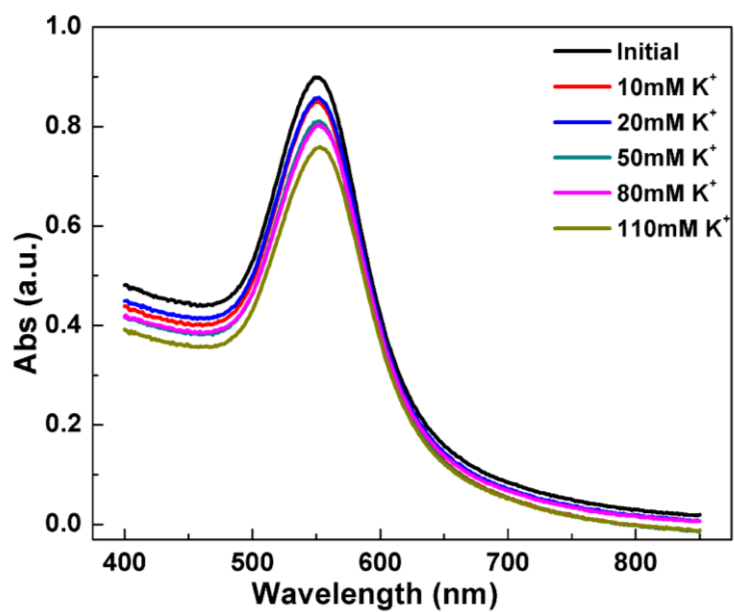


Figure S5. UV-vis spectra of core-satellite nanostructures (50Au/G-rich and 14Au/i-motif) in the presence of different concentrations of K⁺.

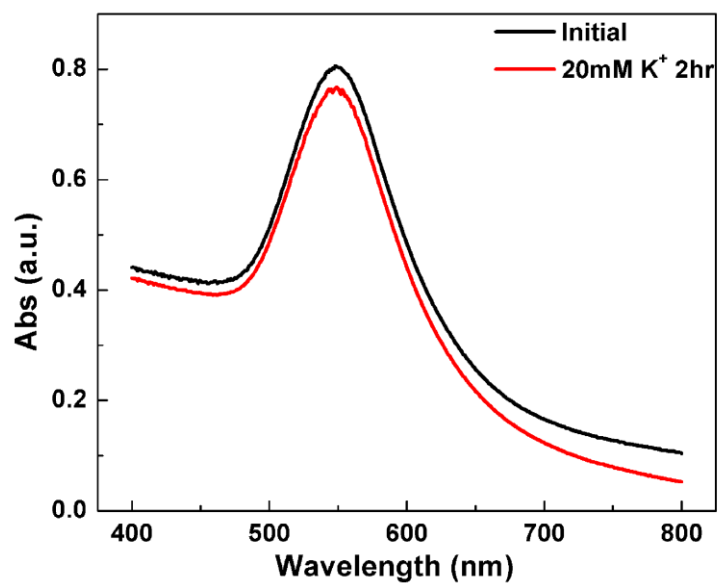


Figure S6. UV-vis spectra of core-satellite nanostructures (50Au/G-rich and 14Au/i-motif) after incubated with 20 mM K⁺ for 2 h.

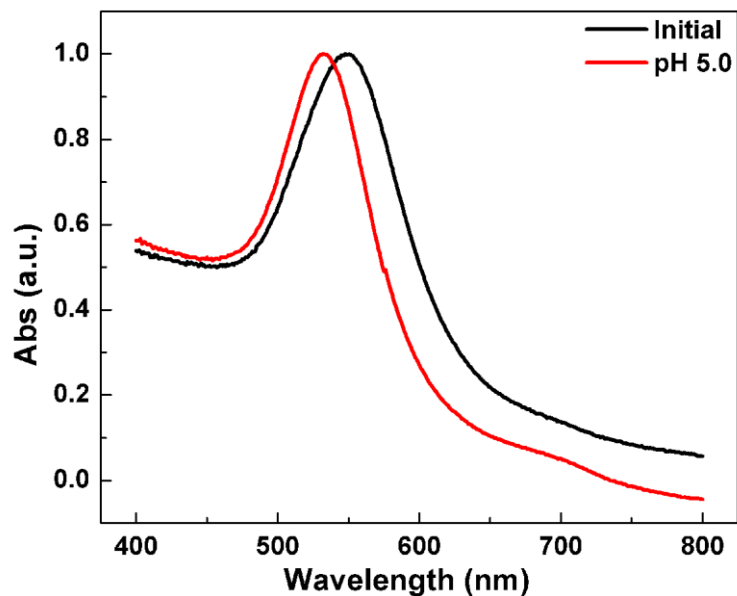


Figure S7. UV-vis spectra of core-satellite nanostructures (50Au/i-motif and 14Au/G-rich) in pH 8.3 and at pH 5.0 buffer solutions.

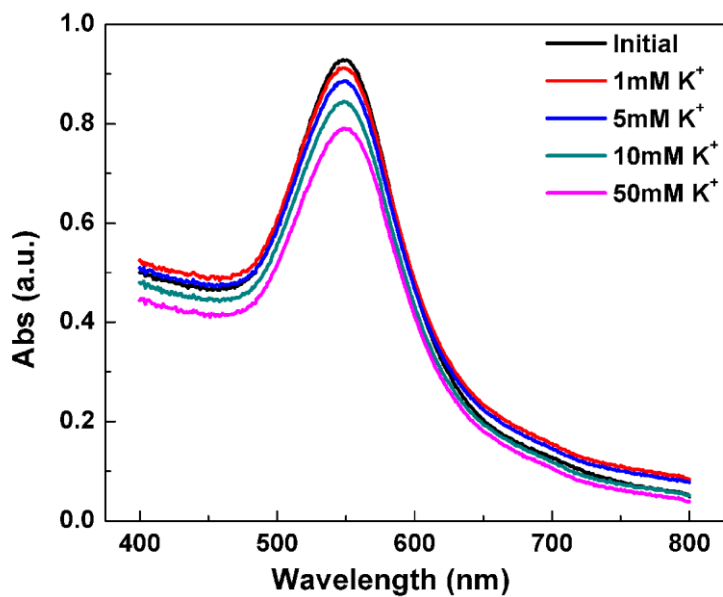


Figure S8. UV-vis spectra of core-satellite nanostructures (50Au/i-motif and 14Au/G-rich) in the presence of different concentrations of K^+ .

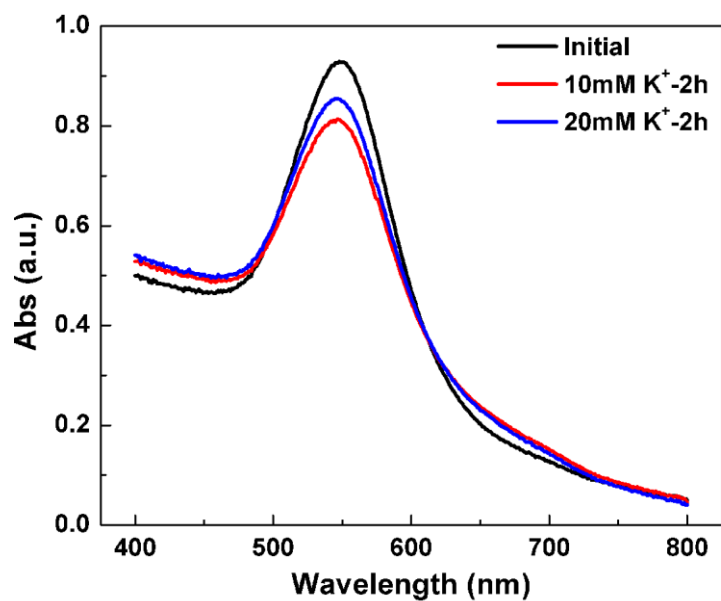


Figure S9. UV-vis spectra of core-satellite nanostructures (50Au/i-motif and 14Au/G-rich) after incubated with 10 mM and 20 mM K⁺ for 2h.

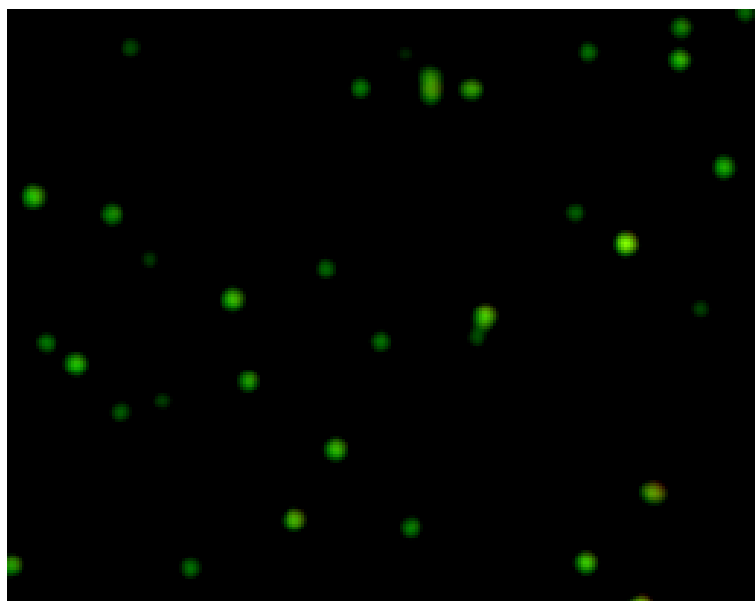


Figure S10. Dark field image of 50Au/G-rich nanoparticles.

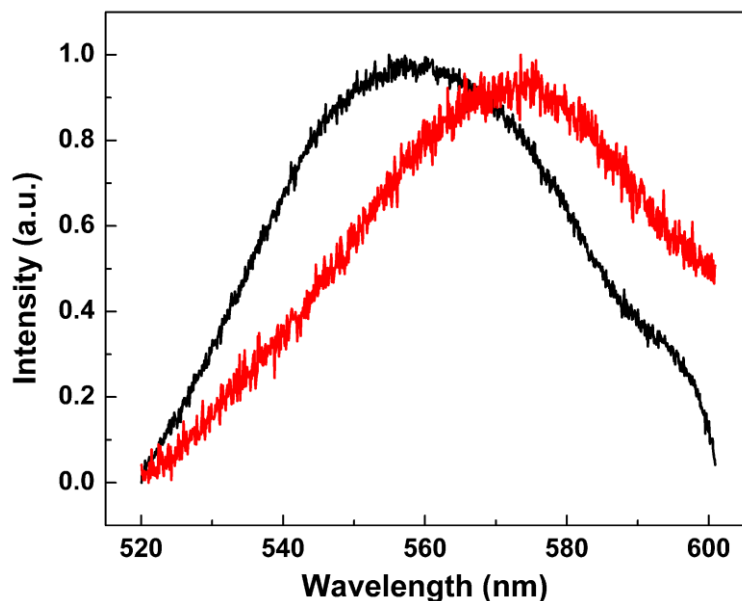


Figure S11. Representative scattering spectra of i-motif CSNSs uptake by macrophages after 15 min incubation (red line) and 30 min post incubation (black line).

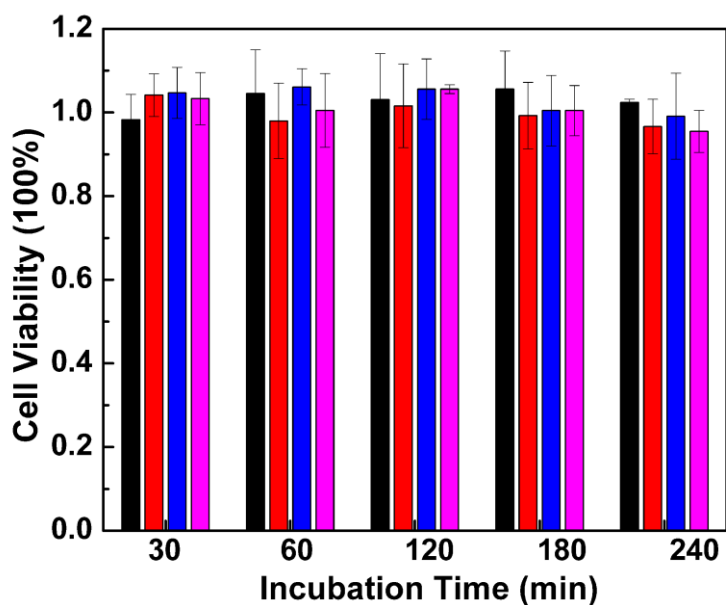


Figure S12. Cytotoxicity images of core-satellite nanostructures with the concentration of 5 pM (black), 10 pM (red), 20 pM (blue) and 40 pM (magenta) in 30 min, 60 min, 120 min, 180 min and 240 min. This indicated that the nanostructures have good biocompatibility and low cytotoxicity which make such core-satellite nanostructures suitable for living cell imaging application.

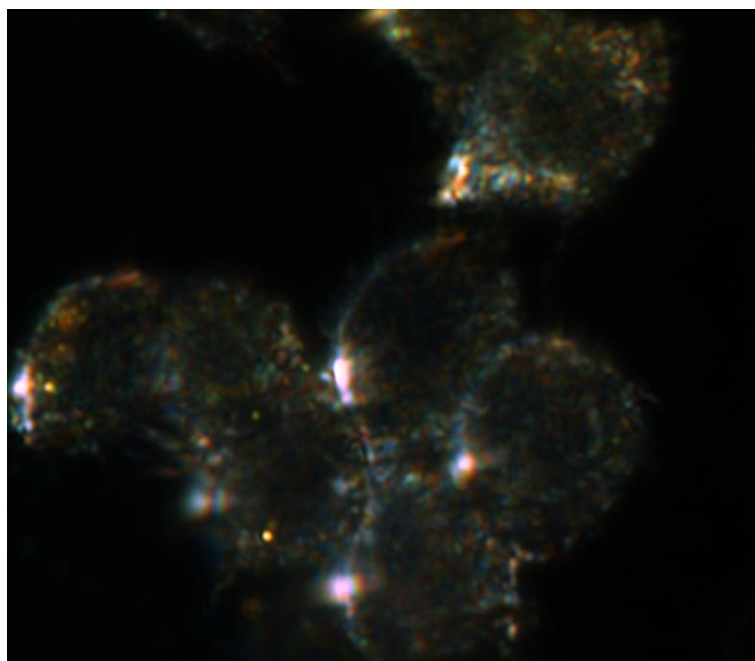


Figure S13. Dark field image of RAW 264.7 cells incubated with CSNSs at 4°C for 1h.

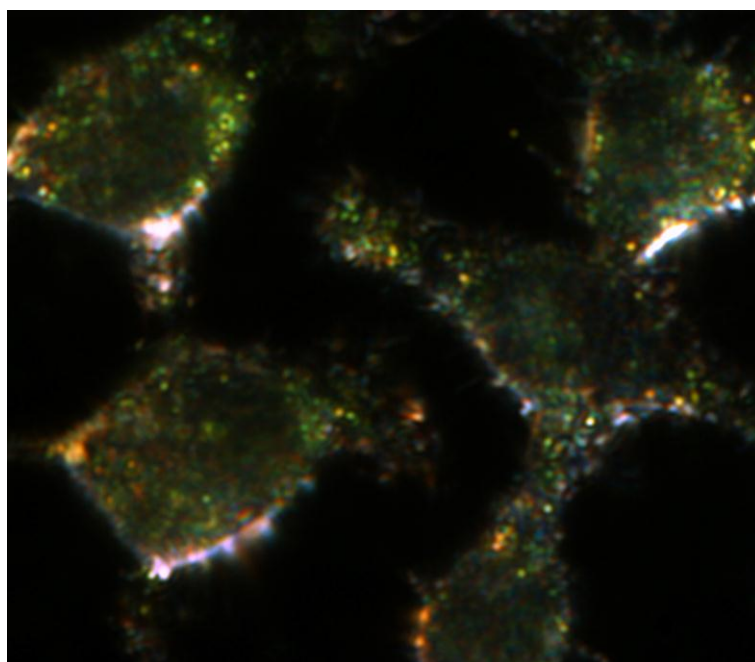


Figure S14. Dark field image of RAW 264.7 cells incubated with 50Au/G-rich.

Reference:

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