Controllable “Click-to-Assembled” Plasmonic Core–Satellite Nanostructures and Its Surface-Enhanced Fluorescence in Living Cells

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Supporting Information

ABSTRACT: The assembly of noble-metal core–satellite (CS) nanostructures is an appealing means to control their plasmonic properties for their applications such as surface-enhanced fluorescence or Raman scattering. However, till now there is a lack of some rapid or convenient methods to construct stable CS nanostructures. Here, we proposed a “clicked-to-assembly” strategy based on the fast and specific “click chemistry” reaction between trans-cyclooctene (TCO) and 1,2,4,5-tetrazine (Tz). The CS nanostructures were constructed within 8 min by simple mixing of TCO- or Tz-modified nanoparticles (TCO-NPs or Tz-NPs) without any catalysts or heating required. Transmission electron microscopy experiments show that the constructed CS nanostructures are uniform, and particularly the number of “satellite” nanoparticles in the core surface is controllable by simply adjusting the feeding ratio of TCO-NPs or Tz-NPs in the reaction. The strong surface plasmon coupling effect (SPCE) was observed in these CS nanostructures, which was dependent on the coverage degree, size and composition of the satellite, and core NPs. The nanostructures with tuned surface plasmon resonance (SPR) effect were tried for the surface-enhanced fluorescence in living cells. Such well-defined CS nanostructures could potentially serve as efficient SPR-enhanced fluorescent probes as diagnostics or biomedical imaging agents in nanomedicine.

INTRODUCTION

Plasmonic core–satellite (CS) nanostructure constructed with subwavelength noble-metal nanoparticles has been attracting great interest due to their unique optoelectronic characteristics.1 The CS nanostructure may induce strong localized surface plasmon resonance (LSPR) different to individual nanoparticles originating from the collective oscillation of free conduction electrons between core and satellite nanoparticles.2 The LSPR properties have stimulated such a rapid advance in CS nanostructure and its applications in such as resonant plasmonic-enhanced optical signal.3

The enhancement of optical signal highly depends on the resonant plasmonic effect from individual nanoparticles (NPs) or their assemblies, which is influenced by size, shape, materials of particles, etc.4 Meanwhile, it is observed that their enhanced fluorescence effect decreases sharply when their SPR wavelength moves away from the excitation wavelength of the fluorophore or when the SPR has only a partial overlap with the emission spectrum of the fluorophore.4b Herein, the regulation of its SPR wavelength of nanoparticles or their assemblies is essential for resonant plasmonic enhancement.5

To tune the SPR in a wide range of wavelengths, various methods including top-down and bottom-up methods have been developed to construct these nanoassemblies or nanostructures. The top-down method uses lithography to build a special nanostructure on a substrate. Generally, it is expensive and difficult for scale production.6 Now, the bottom-up approaches are the predominant strategy to construct these CS nanostructures.7 In these approaches, the surface of the core or satellite nanoparticles is modified with certain molecules. Then CS nanostructures are self-assembled utilizing the electrostatic interaction, van der Waals force, affinity reaction between these molecules, etc. The used molecules include DNA,5c,8 proteins,9 polymers,10 small molecules,2,11 ions,12 etc. Despite the success, rapid, convenient, or controllable methods are still expected with a shorter reaction time or more mild reaction conditions.

Recently, click chemical reactions have demonstrated their importance in material assembly as linking tools.13 In the CS nano-assemblies, Gandra and Singamaneni used a Huisgen 1,3-dipolar cycloaddition reaction between azide and alkyne to assemble gold CS nanostructures.14 The assembly rate was relatively slow although catalyst was used in the reaction. On comparison, the cycloaddition reaction between trans-cyclooctene (TCO) and 1,2,4,5-tetrazine (Tz) is more highly efficient.15 Here, based on the click reaction between TCO and Tz, we developed a clicked-to-assembly method to construct stable and controllable CS nanostructures using noble-metal

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membrane. The assembly can be completed within 8 min. The different CS nanostructures with various ratios of satellite to core NPs were constructed by simply adjusting their feeding ratio. The assembled nanostructures are uniform, stable, and show high tolerance to salt similar to the TCO-NPs or Tz-NPs, which demonstrates their potential applications in biological system. The surface plasmon coupling effect (SPCE) between satellites and core NPs was investigated in detail. It was observed that SPCE was depended on the number of satellites (i.e., surface coverage) and the size and composition of the building nanoparticles. As model, the constructed CS nanostructures were applied for the surface-enhanced fluorescence of fluorophores in the membrane of living cells.

RESULTS AND DISCUSSION

The overall approach to assemble the CS nanostructures is shown in Scheme 1. Gold nanoparticles with TCO and Tz (Scheme 1a) and their fluorescence enhancement on the fluorophores in the cell membrane (Scheme 1b) were prepared by modifying GNPs with PEG-TCO or PEG-Tz via the Au–S bond, respectively. PEG-TCO and PEG-Tz was prepared by the fast reaction between the NHS ester of PEG and the amine of TCO or Tz. The PEG was used as a bridge molecule to modify GNPs, which may improve the chemical stability of GNPs in the buffer containing high concentration of NaCl. Then, the CS nanostructures with different number ratio of satellite to core GNPs (S/C ratio) were assembled via direct mixing and fast reaction at room temperature (RT) by controlling the relative feeding ratio of two GNPs (Scheme 1a). Then these CS nanostructures were used for surface-enhanced fluorescence experiments of fluorophore in the cell membrane (Scheme 1b).

To understand the assembly strategy of CS nanostructures, two GNPs with different diameters (70 and 30 nm) were prepared as core or satellite NPs, respectively. The synthesis procedures of different sizes GNPs are described in the Supporting Information. Their Transmission electron microscopy (TEM) photographs are shown in Figure 1A,1B. Their average diameters are 69.3 ± 6.2 and 32.0 ± 3.1 nm, respectively. The visible absorption spectra of Tz-GNPs and TCO-GNPs are shown in Figure S3. The minor absorption peak shifts observed indicate their good monodispersity in the buffer after the modification and purification steps. Also, these GNPs have their SPR maximum peak at 543 and 525 nm, which are typical for their sizes. Meanwhile, to finely control the feeding ratio of core and satellite GNPs in the assembly step, the particle’s mole concentration of their concentrated samples were measured with resonance light scattering correlation spectroscopy (RLSCS) method. Its working principle is based on the fact that the amplitude of correlation curve is equal to the reciprocal of the particle number in the detection volume. Figure 1E,F is the typical single-particle RLSCS (upper) and intensity trace (lower) curves of TCO-GNPs and Tz-GNPs, respectively. Meanwhile, their uniform scattering intensity traces also indicated that no NP aggregates exist in the prepared GNP solutions.

The prepared TCO- or Tz-GNPs showed good stability. Figure S4 shows the change of visible absorption spectra of TCO- or Tz-GNPs within days. It demonstrates that the SPR peaks keep constant within one week. Figure S5 shows the change of the visible absorption spectra of TCO- or Tz-GNPs in different concentrations of NaCl solution (from 0 to 1 M). Both GNPs demonstrate a high resistance to salt, which is related with the choosing of PEG as a linker. The good monodispersity and stability of TCO- or Tz-GNPs can help to construct the “perfect” CS nanostructure and for its bioapplications as nanoprobes.

Different CS nanostructures (Figure 2) were assembled with the click reaction between TCO and Tz. Figure 2A(i–vi) shows typical TEM micrographs of CS nanostructures prepared with different satellite to core (S/C) feeding ratios from 1 to 8. The concentration of Tz-GNPs as core NPs was fixed, but the mole concentration of TCO-GNPs as satellite NPs changed. TEM results demonstrated that CS nanostructures with different number of satellite NPs in the surface can be prepared. Also, when the S/C feeding ratio is close to 8, the surface of core NPs was completely covered with satellite NPs.
like a sunflower. Figure 2B shows the excellent linear relationship between the numbers of satellite NPs in the core surface with the S/C feeding ratio, which were extracted from no less than 200 core–satellite nanostructures for different ratios. The linear slope is close to 1. It suggests that two NPs reacted together with high efficiency according to their relative concentration within the S–C ratio range of 1 and 8. It should be related with the high efficiency of the click reaction between TCO and Tz. This reported reaction rate constant is far larger than those of other bioorthogonal reactions without requiring a catalyst.15

Besides, the gel electrophoresis experiments of these CS nanostructures also proved their uniformity and controllability. As shown in Figure 2D, CS nanostructure with the least satellite GNPs bound (channel i) showed the fastest mobility rate. When the number of satellite NPs increased, the electrophoretic mobility gradually decreased, which should be attributed to the influence of the increased sizes of CS nanostructures on mobility rates. It also proved that these CS nanostructures had good stability and robustness under harsh electrophoretic conditions. Meanwhile, the clear but color change of the CS nanostructures with the S/C feeding ratio was observed as shown in Figure S6. Also, no obvious red shift was observed in the absorption peak when the NaCl concentration was increased (Figure S7). These results indicated that the chemically assembled CS nanostructures were stable and did not agglomerate even in these NaCl solutions.

The assembling dynamics of CS nanostructures was investigated by recording the change of SPR wavelength with time.
time as shown in Figure 3A. The SPR maximum absorption peaks were 572, 579, 585, and 589 nm with the reaction time interval of 2 min, respectively. It is revealed in Figure 3B that the SPR maximum absorption peak of the mixture reached the max value of 589 nm at 8 min and reached an equilibrium, indicating that the assembly was completed within 8 min. This result suggests that the clicked-to-assembly strategy based on the click reaction between TCO and Tz is far more efficient than the reported assembly procedure based on azide-alkyne Huisgen 1,3-dipolar cycloaddition reaction.14 The simple but highly efficient assembly strategy is useful for the further applications of CS nanostructures in nanophotonics.

Surface Plasmon Coupling Effect. The influence of the number of satellites (i.e., surface coverage), size, and composition of the building blocks on strong SPCE was investigated. It was observed that the number of satellites greatly affects the SPCE in the CS nanostructures. The red and black lines are the absorption spectrum of core Tz-GNPs and satellite TCO-GNPs, and their maximum SPR is at 541 or 523 nm, respectively. Figure 2C demonstrates that the SPR shifts to the red with the increased S/C feeding ratio. Also, the red shift of SPR shows the linear dependence on the satellite number per core NPs (Figure S8). These results were consistent with the simulations reported by Ross and co-workers and the experiments by Yoon and co-workers.18

The influence of satellite size on the SPCE was also investigated. As shown in Figure 4, the clicked-to-assembly strategy was extended to the construct CS nanostructures with the same size of core NPs but with different sizes of satellite NPs. The S/C feeding ratio was adjusted to make the entire core NPs completely covered with satellite NPs. For 30, 20, and 5 nm satellite NPs, the SPR peaks of the constructed CS nanostructures are 589, 556, and 546 nm, respectively. The SPR peaks red-shifted to long wavelength with the increased size of satellite NPs due to the SPCE effect. It indicates that the size of satellite NPs remarkably influences the SPR coupling effect between nanoparticles.

Besides, the SPR coupling effect was observed in the compositionally heterogeneous CS nanostructures where silver nanoparticles (AgNPs) instead of GNPs were used as satellite (Figures 4C,D). It was observed that the SPR peaks at 408 and 568 nm in the extinction spectrum of CS nanostructure were red-shifted from the SPR peak of AgNPs (404 nm) and GNPs (541 nm), indicating the resonance coupling between the plasmas of GNPs and AgNPs.

The influence of core sizes of NPs on the surface plasmon coupling effect was further investigated. Compared with Figure 4A,B, 70 nm core GNPs were replaced with 50 nm GNPs to construct the CS nanostructure. It was found that the red-shift of SPR peak decreased with the decreased size, which also happened between the AgNP and GNPs (Figure 4C,D).

The above results suggest that the SPCE happened in these CS nanostructures and the SPR red-shift can be tuned by controlling their size and composition of satellite and core NPs, besides adjusting the coverage degree of satellite NPs in the core.

SPR-Enhanced Fluorescence in Living Cells. To enhance the fluorescence of Cy3 as much as possible, a CS nanostructure with the number ratio of satellite to core NPs of 5 was constructed. The measured SPR wavelength of this CS nanostructure perfectly overlapped with the wavelength of excitation lamp and absorption spectrum of Cy3, which agrees with the requirements reported by Khatua et al.4b In this designed aptamer, Cy3 was labeled in the 3 terminal of the aptamer with the thiol group in the terminal was used as a linker to bind CS nanostructures with cell via the Au−S bond as shown in Scheme 1. The dark field images of the cells incubated with CS nanostructures proved the function of the aptamer. Compared with Figure S9A, Figure S9B shows that the Hela cells appeared to have a higher scattering intensity where they were incubated with 100 nM aptamer in the binding with nanostructures. After this interaction, Cy3 due to its flexible arm of DNA was pulled to the surface of CS nanostructures, which may provides Cy3 a chance to enter into the SPR hot spots of nanostructures for fluorescence enhancement.3c

Figure 4. Surface plasmon coupling effect between Au–Au (A, B) and Au–Ag CS nanostructures (C, D). The effect was influenced by sizes or composites of core or satellite NPs. The sizes of GNPs as satellite are 20 nm (A) and 5 nm (B) when using about 70 nm GNPs as core. Accordingly, the size of AgNPs as satellite is about 15 nm when using 70 nm (C) and 50 nm (D) GNPs as core.
The SPR enhancement effect on Cy3 is remarkable as shown in Figure 5. As shown in Figure 5A, the fluorescence intensity of cells were weak when no nanostructures were added. However, a strongly enhanced fluorescence is observed in Figure 5B where the cells were incubated with CS nanostructures. The transmission images of HeLa cells can be found in Figure S10A,B. Also, the influence of CS nanostructure concentration on enhancement was studied as shown in Figure S11. Also, it was found that 50 pM nanostructures had the optimal concentration to enhance fluorescence. This shows that the average fluorescent intensity of cells increased from 1475 to 4525 compared with those of the cells without CS nanostructure. Figure 5C,D expresses the PL decay curves of Cy3 in the cells incubated without or with 50 pM CS nanostructures, respectively. They are well-fitted by a single exponential. Figure S12 shows the instrument response function of time-resolved fluorescence lifetime measurement system. It was observed that the lifetime of Cy3 decreased from 4.10 to 3.43 ns when cells were incubated with nanostructure. This suggests that the shorter fluorescence lifetimes are associated with the enhancements.4b

■ CONCLUSIONS

We have developed a facile and controllable clicked-to-assembly strategy to prepare CS nanostructures based on the click reaction between TCO and Tz. The time to assemble CS nanostructures is remarkably shortened compared with that in the present methods. The constructed nanostructures show excellent uniformity and a high salt tolerance. The surface plasmon coupling effect occurred in the core, and satellite NPs resulted in a red-shift effect of the SPR wavelength, which verified that it may be controlled by number, size, and composition of satellite and core NPs in these core–satellite nanostructures. The surface plasmon coupling effect of CS nanostructure is important to construct the enhancement matrix for fluorescence signal. We believe that this new method will provide a new avenue for the assembly of core–satellite nanostructures.

■ EXPERIMENTAL METHODS

Chemicals. Sulfuric acid, nitric acid, sodium hydroxide, chloroauric acid, trisodium citrate, and silver nitrate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). SH-PEG-NH₂ (Mw = 5000) and SH-PEG-COOH (Mw = 5000) were purchased from Creative PEG Works. 4,7,10,13,16,19,22,25,32,35,38,41, 44,47,50,53-hexadecaoxa-28,29-dithiahexapentacontanedioic acid di-N-succinimidyl ester (PEG-NHS ester) (Catalog No: 671630), (4-(1,2,4,5-tetrazin-3-yl)phenyl)methanamine hydrochloride (Tz-NH₂) (Catalog No: 761591, 95%), (E)-cyclooct-4-enyl 2,5-dioxo-1-pyrrolidinyl carbonate (TCO-NHS) (Catalog No:764523, 95%), methyl viologen dichloride hydrate (MV) (Catalog No: 865177, 98%) were products of Sigma-Aldrich (St. Louis, MO). All solutions were prepared with ultrapure water purified on a Millipore Simplicity apparatus (Millipore, Billerica, MA). All the aptamers were synthesized and purified through high-performance liquid chromatography (HPLC) by Shanghai Sangon Biotechnology (China). The aptamer sequences targeted to Her2 receptors were as follows:19 5′-GCAGCGGTGTGGGGGCAGCGGTGTGGGGGCAGCGGTGTGGGG-3′. The 5′ terminal of aptamer is modified with thiol group and the 3′ terminal is modified with Cy3 dye.19

Apparatus. UV–visible spectrophotometer (UV-3502) and fluorescence spectrophotometer (F-380) were purchased from Gangdong Technology Development Co. Ltd. (Tianjin, China).

Figure 5. Surface-enhanced fluorescence on the fluorophore in the cell membrane induced by CS nanostructures. Fluorescence microscopic images of HeLa cells without CS nanostructures (A) or with 50 pM CS nanostructures with 5 of the ratios of satellite to core NPs (B). Scale bar is 10 μm. PL decay profiles of cells without CS nanostructures (C) or with CS nanostructures (D).
PEG-NHS with Tz-NH₂. In the second step, the TCO- or Tz-HS-PEG-Tz (about 10 μM) were mixed with the same volume of HS-PEG-TCO or HS-PEG-NHS and amine. The same volume of 20 μM HS-PEG-NH₂ and TCO-NHS were mixed in ultrapure water at room temperature for 4 h to obtain HS-PEG-TCO. Similarly, HS-PEG-Tz was obtained by reacting same volume of 20 μM PEG-NHS with Tz-NH₂. In the second step, the TCO- or Tz-modified nanoparticles were prepared via the bonding of Au-S or Ag-S. The prepared Au or silver nanoparticles (about 1 mM) were mixed with the same volume of HS-PEG-TCO or HS-PEG-Tz (about 10 μM) and reacted for 12 h in a refrigerator at 4 °C. Later, to ensure the stability of the modified nanoparticles, 20 μM HS-PEG-COOH was added and continued to react for 4 h. In the third step, the centrifugation was used to remove free HS-PEG-COOH, HS-PEG-Tz, and HS-PEG-TCO and the precipitate was redispersed in the phosphate-buffered saline (PBS) solution. The molar concentration of the prepared TCO- and Tz-NPs was determined based on their absorbance at the SPR peak or concentration (Figures S1 and S2).

**Preparation of TCO- or Tz-Modified NPs.** The TCO- or Tz-NPs were prepared in three steps. In the first step, TCO or Tz was reacted and linked with PEG based on the active group NHS and amine. The same volume of 20 μM HS-PEG-NH₂ and TCO-NHS were mixed in ultrapure water and reacted at room temperature for 4 h to obtain HS-PEG-TCO. Similarly, HS-PEG-Tz was obtained by reacting same volume of 20 μM PEG-NHS with Tz-NH₂. In the second step, the TCO- or Tz-modified nanoparticles were prepared via the bonding of Au-S or Ag-S. The prepared Au or silver nanoparticles (about 1 mM) were mixed with the same volume of HS-PEG-TCO or HS-PEG-Tz (about 10 μM) and reacted for 12 h in a refrigerator at 4 °C. Later, to ensure the stability of the modified nanoparticles, 20 μM HS-PEG-COOH was added and continued to react for 4 h. In the third step, the centrifugation was used to remove free HS-PEG-COOH, HS-PEG-Tz, and HS-PEG-TCO and the precipitate was redispersed in the phosphate-buffered saline (PBS) solution. The molar concentration of the prepared TCO- and Tz-NPs was determined based on their absorbance at the SPR peak or measured with our developed RLSCS method.

**Assembly of CS Nanostructures.** Different CS nanostructures were constructed by reacting Tz-NPs as the core with TCO-NPs as the satellite particles at room temperature for about 8 min. Their feeding ratio was adjusted according to the expected satellite number in the surface of core GNP. The product was centrifuged at 2000 rpm for 20 min, and the precipitate was redispersed in PBS solution.

**Gel Electrophoresis.** A 0.5% agarose gel was used and 10 μL of the sample mixed with the 6 × loading buffer was added to the bottom of the sample well. The samples were subjected to gel electrophoresis separation with a voltage of 120 V. After the electrophoresis is completed, the experimental results are photographed and collected.

**Fluorescent Labeling of Her2 Receptor in Living Cells.** HeLa cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% penicillin/ streptomycin (Invitrogen). Cells were grown in a humidified air atmosphere with 5% CO₂ at 37 °C. After reaching 70% confluence in a 35 mm culture dish, the cells were rinsed with PBS (10 mM, pH 7.4). The cells were co-incubated with different concentrations of Cy3-modified aptamers to bind with Her2 receptor for 4 h. Then, the cells were rinsed with PBS buffer for three times to remove the free aptamers.

**Fluorescence Quenching of Cy3 with MV.** Ten millimolar of MV in PBS buffer was added into the HeLa cell labeled with Cy3 probe to quench the fluorescence of Cy3. The incubation time is 15 min. Then, the cells were rinsed with PBS solution for three times to remove free MV. The obtained cell images were used for further surface-enhanced fluorescence experiments. Cellular images before fluorescence enhancement were collected using an inverted fluorescence microscope (IX71, Olympus, Japan) with a 40× objective and an EMCCD (Evolve S12, Photometrics). The fluorescence intensity of the cells was extracted with micromanager software (NIH).

**CS Nanostructure-Induced Surface-Enhanced Fluorescence.** CS nanostructures of different concentrations were incubated with the above cells. The incubation time is 2 h. Then, the cells were rinsed with PBS solution for three times to remove the unbound CS nanostructures. Then, fluorescent images of the cells were collected, and the extracted fluorescence intensity was compared with the cells without CS nanostructures.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b02581.

**Synthesis procedure, their TEM photograph and UV-vis absorption spectra of GNP and AgNP of different sizes (Figure S1, S2); UV-vis absorption spectra of TCO-GNP and Tz-GNP (Figure S3); their spectra change within stock days (Figure S4); and their stability in different NaCl solution (Figure S5); the photograph of CS nanostructures in solution assembled with different S/C feeding ratios (Figure S6), and their stability in NaCl solution (Figure S7); the effect of satellites number on SPR (Figure S8); dark field images (Figure S9); and transmission images of cells incubated with CS nanostructures (Figure S10). (PDF)

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**Notes**

The authors declare no competing financial interest.

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