Targeted surface-functionalized gold nanoclusters for mitochondrial imaging

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Due to mitochondria involved in both apoptotic and necrotic cell death, labeling and imaging mitochondria has attracted considerable interest. However, conventional organic dyes used for mitochondrial imaging are limited because of their poor photostability. Considering that gold nanoclusters (AuNCs) possess some advantages over considerable interest, such as excellent photostability and strong fluorescence emission, we herein prepared a mitochondria-targeted fluorescent probe, AuNCs@CS-TPP, based on a covalent link between triphenylphosphonium (TPP) cations and chitosan-coated AuNCs (AuNCs@CS). The as-prepared AuNCs@CS-TPP exhibited a bluish fluorescence emission at 440 nm with a quantum yield of 8.5%. Meanwhile, the fluorescence intensity of AuNCs@CS-TPP labeled HeLa cells did not show apparent decrease after 8 min irradiation. Cytotoxicity assay showed that AuNCs@CS-TPP did not display any appreciable cytotoxicity on cells even at a concentration of 60 μg mL⁻¹. In addition, the result of fluorescence co-localization imaging in vitro indicated that AuNCs@CS-TPP could selectively accumulate into mitochondria of HeLa cells and HepG2 cells. These findings demonstrated that AuNCs@CS-TPP possessed superior photostability, low cytotoxicity, high sensitivity and target-specificity to mitochondria, allowing labeling and imaging of the mitochondria in living cells.

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1. Introduction

Mitochondria play a central role in cellular energy metabolism (Green and Reed, 1998; Hoppins and Nunnari, 2012). Recent discoveries have showed that mitochondrial dysfunctions severely affected human health and led to many diseases (Vafai and Mootha, 2012; Wallace, 1999). Therefore, the fundamental understanding of mitochondrial behavior in living cells is of great importance for clear explanation of biological processes in mitochondria, and advancing early diagnosis and drug development in biomedicine (Smith et al., 2011).

Fluorescent labeling and imaging mitochondria in living cells is very likely to be a powerful tool for understanding of mitochondrial function and changes. To observe the biochemical events in living cells for long time, the fluorescent probe must be photostable under the continual irradiation (Leung et al., 2013). As is well known, conventional organic dyes have been developed in mitochondrial imaging, but their poor photostability often limits their applications in living cells studies, leaving much to be desired (Lee and Chen, 2011; Derfus et al., 2004). To overcome this limitation, recent advances in the development of fluorescent quantum dots (QDs) have emerged as an alternative method for live-cell imaging with strong photoluminescence and excellent photostability (Biju et al., 2010; Kuo et al., 2011; Hsieh et al., 2011). Nevertheless, their intrinsic toxicity (the release of heavy metals like cadmium, selenium and lead) of II-VI QDs is the main concerns for their applicability in live-cell studies (Chan et al., 2006; Lee et al., 2009; Li et al., 2011).

In contrast, gold nanoclusters (AuNCs) have recently attracted enormous interests, because they possess extraordinary advantages over QDs and conventional fluorescence dyes such as low toxicity, robust resistance to photobleaching and facile synthesis (Chen et al., 2012; Palmal et al., 2013; Wang et al., 2011). However, it has been reported that bare gold nanomaterials could accumulate in the liver and spleen at high level, and these accumulations can cause potential cytotoxicity (Zhang et al., 2012; Du et al., 2012; Jia et al., 2009). To reduce the potential cytotoxicity of AuNCs, many biocompatible materials such as poly(lactic-co-glycolic acid (PLGA), chitosan (CS), bovine albumin (BSA), and glutathione (GSH) were used as protection ligands coated onto the surface of the AuNCs (Geng et al., 2012; Chen et al., 2012; Xie et al., 2009; Zhou et al., 2011). Among these various coatings, CS has attracted much attention due to its low-toxicity, good biocompatibility, biodegradability and non immunogenicity.
1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) hydrogen tetrachloroaurate (III) trihydrate (HAuCl₄·3H₂O), 1-undecanethiol and N,N-dimethylformamide (DMF) were purchased from Sigma-Aldrich (St Louis, MO). (4-Carboxybutyl)triphenylphosphonium bromide was obtained from J&K Chemical Ltd. (Beijing, China). Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum, penicillin, streptomycin, MitoTracker® Red CMXRos (Mito Tracker) and phosphate buffered saline (PBS) were purchased from Invitrogen Co. (Beijing, China). Ultrapure water (Milli-Q plus, Millipore Inc., Bedford, MA) was used throughout. Unless specified, all other chemicals were of analytical reagent grade and were used without further treatment.

2.1. Reagents and chemicals

CS (MW = 80 kDa, degree of deacetylation = 85%), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) hydrogen tetrachloroaurate (III) trihydrate (HAuCl₄·3H₂O), 1-undecanethiol and N,N-dimethylformamide (DMF) were purchased from Sigma-Aldrich (St Louis, MO). (4-Carboxybutyl)triphenylphosphonium bromide was obtained from J&K Chemical Ltd. (Beijing, China). Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum, penicillin, streptomycin, MitoTracker® Red CMXRos (Mito Tracker) and phosphate buffered saline (PBS) were purchased from Invitrogen Co. (Beijing, China). Ultrapure water (Milli-Q plus, Millipore Inc., Bedford, MA) was used throughout. Unless specified, all other chemicals were of analytical reagent grade and were used without further treatment.

2.2. Instrumentation

UV–visible absorption spectra were recorded in a conventional quartz cell (light path 10 mm) by using a Hitachi UV–3310 spectrophotometer (Japan). Fluorescence measurements were performed on a Hitachi F–2500 fluorescence spectrophotometer (Tokyo, Japan). FT-IR scans were collected on a Bruker Tensor-27 Fourier-transform infrared spectrometer (Bruker, Germany). The fluorescence lifetimes were measured with a compact fluorescence lifetime spectrometer CL1367 (Hamamatsu, Japan). High resolution transmission electron microscopy (HRTEM) was determined on a JEM-2100F (JEOL) instrument by placing colloidal solutions on the carbon-coated copper grid and then drying at room temperature. SEM and EDS measurements were made on a 4800 scanning electron microscope (Hitachi, Japan) at an accelerating voltage of 30 kV. The samples for SEM and EDS characterization were prepared by dropping the colloidal solutions onto silicon slice, and then spraying platinum onto its surface. DLS measurements were performed using a Zetasizer Nano ZS (ZEN3600) instrument (Malvern, England). XPS measurements were recorded with an ESCALab220i-XL (VG, England). The absorbance for MTT analysis was recorded on a microplate reader (BIO-TEK Synergy HT, USA) at 490 nm. All measurements were herein performed at room temperature.

2.3. Synthesis of AuNCs

DMF-protected AuNCs were prepared according to the previously reported method (Kawasaki et al., 2010). Aqueous HAuCl₄ solution (150 μL, 0.1 M) was added to 15 mL DMF at 140 °C, and the DMF solution was refluxed by simply heating with an oil bath (140 °C for 6 h) with vigorous stirring. After evaporating the excess solvent under a vacuum, the residue was redissolved in methanol or water.

The hydrophilic DMF-protected AuNCs were then ligand exchanged with 1-undecanethiol to make AuNCs hydrophobic. To a stirred methanol solution of AuNCs (3 mL) was added 100 μL of 1-undecanethiol solution in methanol (5 × 10⁻² M) dropwise. The mixture was stirred and maintained in the dark for more than 24 h. After removal of the solvent, the residue (1-undecanethiol-protected AuNCs) was redissolved in methanol.

2.4. Synthesis of AuNCs@CS and AuNCs@CS–TPP

Stock solutions of 1-undecanethiol-protected AuNCs (2 mg mL⁻¹) and CS (10 mg mL⁻¹) were prepared by dissolving the solid in chloroform and methanol, respectively. Then, the solutions of the AuNCs (250 μL) and CS (200 μL) were successively added to a 10 mL water (50 °C), and mixed thoroughly with vigorous stirring. After 5 min, the chloroform and methanol were removed under vacuum. Finally, 3 μL glutaraldehyde (25%) was added and stirred for 2 h at 40 °C. The product, AuNCs@CS, was then freeze-dried.

TPP was conjugated to amino groups on the surface of AuNCs@CS to form AuNCs@CS–TPP conjugates. The AuNCs@CS–TPP was synthesized by following several steps: First, (4-carboxybutyl)
triphenylphosphonium bromide (1 g) was dissolved in 20 mL of dimethyl sulfoxide. NHS (0.5 g) and EDC (0.9 g) were then added successively to the resulting solution, and the mixture solution was stirred overnight at room temperature. Second, 80 µL of the activated (4-carboxybutyl)triphenylphosphonium bromide solution was added to 2 mL of 2.5 mg mL⁻¹ AuNCs@CS in 10 mM PBS solution (pH 8.0). After stirring overnight at room temperature, the mixture was dialyzed against water for 3 h to remove excessive TPP.

2.5. Cell culture

The human cell lines HeLa (cervical cancer) and HepG2 (liver cancer) were purchased from the American Type Culture Collection (ATCC). The cells were cultured in DMEM medium with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin–streptomycin at 37 °C in a 5% CO₂ incubator, as reported previously (Song et al., 2012).

2.6. Cytotoxicity assay

In order to evaluate the cytotoxicity of DMF-protected AuNCs and AuNCs@CS-TPP, a standard MTT assay was carried out using two different cell lines (HeLa and HepG2). Cells were seeded at a density of 1 × 10⁴ cells per well in 96-well U-bottom plates, and then incubated with DMF-protected AuNCs and AuNCs@CS-TPP at a concentration range from 0 to 60 μg mL⁻¹. Each concentration was tested in 6 wells. After 24 h, the culture media were discarded, the cells were washed with PBS (pH 7.4), and 0.1 mL of MTT solution (0.5 mg mL⁻¹ in DMEM) was added to each well. After incubation for another 4 h, the medium containing MTT was carefully removed from each well and 150 µL of dimethyl sulfoxide was added into each well. After shaking the plates for 10 min, absorbance values of the wells were recorded with a microplate reader at 490 nm. The cell viability rate was estimated by the following equation:

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\text{Viable cells (\%) =} \frac{\text{OD}_{\text{treated}}}{\text{OD}_{\text{control}}} \times 100\%, \quad \text{where OD}_{\text{treated}} \text{ denotes the absorbance recorded in the presence of AuNCs@CS-TPP or DMF-protected AuNCs, and OD}_{\text{control}} \text{ represents the absorbance of the control group recorded in the incubation medium.}
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2.7. Cell uptake of AuNCs@CS-TPP

To investigate the cellular uptake of AuNCs@CS-TPP by means of FV 1000-IX81 confocal laser scanning microscope for excitation at 405 nm. Briefly, HeLa cells were plated with 1 × 10⁵ cells/dishes in a glass-bottom plate and incubated overnight at 37 °C. The cells were washed with PBS (pH 7.4) and suspended in 1 mL of fresh DMEM. Then, 0.1 mL of AuNCs@CS-TPP (20 μg mL⁻¹) was added and fluorescence imaging was performed after different incubation time (0.5 h, 1 h, 2 h and 4 h).

2.8. The photostability of AuNCs@CS-TPP

To test photostability of AuNCs@CS-TPP and Mito Tracker, the HeLa cells were imaged by FV 1000-IX81 confocal microscope. AuNCs@CS-TPP was excited at 405 nm (6% laser power) and the fluorescence was collected at 420–520 nm. Mito Tracker was excited at 559 nm (18% laser power) and fluorescence was collected at 580–680 nm.

2.9. Mitochondria-targeted imaging

For mitochondrial imaging of cancer cells, the HeLa and HepG2 cells grown on glass-bottom culture dishes containing 1 mL of DMEM were first incubated with DMF-protected AuNCs (20 μg mL⁻¹) or AuNCs@CS-TPP (20 μg mL⁻¹) for 2 h at 37 °C, and washed with PBS (pH 7.4). In addition, to illustrate the targeted imaging, the mitochondria were further stained by Mito Tracker. A volume of 500 μL of 20 nM Mito Tracker solution was incubated with the cells for 30 min. After washing with PBS, the solution was kept in PBS for imaging. The cells were imaged under an FV 1000-IX81 confocal laser scanning microscope, using different excitation for each dye: for DMF-protected AuNCs and AuNCs@CS-TPP, excitation wavelength: 405 nm; for Mito Tracker, excitation wavelength: 559 nm.

3. Results and discussion

3.1. Synthesis and characterization

In this work, DMF-protected AuNCs were first synthesized following the previously reported procedure (Kawasaki et al., 2010), and then they were ligand exchanged with 1-undecanethiol (Liu et al., 2008). Next, the amphiphilic CS was assembled on the surface of the resulting AuNCs through hydrophobic interaction to form water-soluble AuNCs@CS. Finally, the AuNCs@CS was functionalized with the mitochondria-targeted molecule, TPP, to produce the fluorescent probe, AuNCs@CS-TPP.

The as-prepared DMF-protected AuNCs and AuNCs@CS-TPP were first characterized by UV–vis spectroscopy and fluorescence spectroscopy, as shown in Fig. 1. As can be noticed in Fig. 1A, the absorption spectra of DMF-protected AuNCs and AuNCs@CS-TPP exhibited no characteristic broad surface plasmon resonance (SPR) band of gold nanoparticles (~520 nm), implying that the core diameters of both AuNCs were less than 2 nm (Kawasaki et al., 2010; Wang et al., 2010). Concomitantly, it can be clearly seen that compared with DMF-protected AuNCs, the AuNCs@CS-TPP displayed a characteristic absorption peak of TPP at 265 nm, denoting the successful conjugation of TPP with the surface of the AuNCs@CS. In addition, Fig. 1A clearly showed that DMF-protected AuNCs had an emission spectral band with a characteristic peak wavelength of 437 nm. After the modification of 1-undecanethiol, CS and TPP to the AuNCs, the fluorescence intensity of AuNCs@CS-TPP increased, accompanied by a red shift of the fluorescence peak to 440 nm. The variations in fluorescence emission spectra are very likely to be mainly due to the alterations in gross electrostatic/polar environment of AuNCs after conjugated with CS (Dejneka et al., 2003). And fluorescence quantum yields (QY) of DMF-protected AuNCs and AuNCs@CS-TPP (Table S1) were estimated to be 5.6% and 8.5% using Rhodamine B as a standard (65%) (Kubin and Fletcher, 1982). It was worth noting that the fluorescence QY obtained in this work were comparable with or higher than that of the previously reported AuNCs (Xie et al., 2009; Kawasaki et al., 2011). Correspondingly, the optical imaging of DMF-protected AuNCs and AuNCs@CS-TPP was taken under white light and UV irradiation conditions (Fig. 1B). As can be seen from the photomages in Fig. 1B, both of DMF-protected AuNCs and AuNCs@CS-TPP showed transparent, and light blue fluorescence under white light and 365 nm UV irradiation, respectively.

The successful synthesis of DMF-protected AuNCs and AuNCs@CS-TPP is further confirmed by various analytical techniques, such as HRTEM, SEM, DLS, XPS, EDS and FT-IR. The HRTEM image of DMF-protected AuNCs (Fig. S1A) showed uniformly dispersed particles with an average diameter of 0.72 ± 0.16 nm (note that the size of AuNCs is obtained based on more than 200 NCS in the HRTEM images). A representative SEM image of AuNCs@CS-TPP in Fig. S1B showed that AuNCs@CS-TPP are spherical in shape and nearly monodispersed with a diameter of 8.07 ± 1.77 nm (note that the size of AuNCs@CS-TPP is estimated based on more than 200 NCS in the SEM images), suggesting the
Data obtained from DLS experiments (Fig. 1C and D) displayed that the hydrodynamic diameter of DMF-protected AuNCs and AuNCs@CS-TPP were 2.34 \pm 0.26 nm and 9.41 \pm 0.35 nm, respectively. The increase in size suggested that CS and TPP were very likely to be attached to AuNCs surface. In addition, the zeta potential of AuNCs@CS-TPP was found to be 10.68 \pm 0.42 mV (Fig. S1D), demonstrating the presence of positively charged TPP. The elemental analysis of AuNCs@CS-TPP given by EDS (Fig. S1C) displayed that the sample was composed of gold (Au), sulfur (S), phosphorus (P), nitrogen (N), carbon (C) and oxygen (O), confirming the tight attachment of CS and TPP to AuNCs surface. The XPS Au 4f7/2 obtained from DMF-protected AuNCs and 1-undecanethiol-protected AuNCs (Fig. S2A) indicated that the main peak was located at 85.47 eV and 85.52 eV. Moreover, the XPS N1s and S2p obtained from DMF-protected AuNCs and 1-undecanethiol-protected AuNCs were 400.37 eV and 168.2 eV, respectively (Fig. S2B and C). These results were consistent with those previously reported (Kawasaki et al., 2010), suggesting the DMF-protected AuNCs and the 1-undecanethiol-protected AuNCs were successfully prepared. Data from FT-IR spectra (Fig. S3) showed that DMF-protected AuNCs had an IR spectrum with a band around 1651 cm\(^{-1}\) and a band around 1385 cm\(^{-1}\), which were assigned to the C=O stretching and C–N stretching, respectively. After ligand exchanged with 1-undecanethiol, the resulting AuNCs displayed a band around 2925 cm\(^{-1}\) and a band around 1654 cm\(^{-1}\). Both bands originated from –CH\(_2\)– stretching and C= S stretching, respectively, confirming the successful coating of 1-undecanethiol on the surface of AuNCs to form 1-undecanethiol-protected AuNCs. After the addition of CS to 1-undecanethiol-protected AuNCs, the C–O stretching (1060 cm\(^{-1}\)), N–H bending (1560 cm\(^{-1}\)), O–H bending (3410 cm\(^{-1}\)) and C= S stretching (1651 cm\(^{-1}\)) were detected, indicating the conjugation of CS with 1-undecanethiol-protected AuNCs. Finally, the addition of TPP to AuNCs@CS gave C–C= O stretching (531 cm\(^{-1}\)), C= C stretching (1438 cm\(^{-1}\), 1543 cm\(^{-1}\) and 1600 cm\(^{-1}\)) and C–O stretching (1074 cm\(^{-1}\)), suggesting that TPP was attached to the AuNCs@CS.

Fluorescence lifetime and fluorescence photostability of DMF-protected AuNCs and AuNCs@CS-TPP were further performed to evaluate the performance and characteristics of the fluorescent probes. Fig. 2A showed the fluorescence lifetimes of AuNCs@CS-TPP was about 4.79 ns, close to that (5.06 ns) of DMF-protected AuNCs, suggesting that AuNCs retained almost all of its original features after functionalized with CS and TPP. The results of fluorescence photostability experiment (Fig. 2B) clearly showed that after the UV irradiation for 30 min, AuNCs@CS-TPP and DMF-protected AuNCs kept more than 95% and 80% of their original fluorescence, respectively, while the standard mitochondria-targeted fluorescent probe (Mito Tracker) only retained 20% of its original fluorescence. All the results suggested that AuNCs can resist photobleaching well, and after coated with the biocompatible CS, the AuNCs possessed almost complete resistance to photobleaching. The fluorescence stability of AuNCs@CS-TPP was assessed against both pH and storage time. It was found in Fig. S4A that there was almost no changes in fluorescence intensity when the pH of the solution was between 2.0 and 12.0, suggesting the superior resistance of AuNCs@CS-TPP to the solution pH. And Fig. S4B showed that the fluorescence intensity of AuNCs@CS-TPP in PBS solution (10 mM, pH 7.4) did not show any significantly decrease during one month storage at ambient temperature and in room light, suggesting the superior photostability of AuNCs@CS-TPP (Zhang et al., 2013).
Fig. 2. (A) Fluorescence decay curves of DMF-protected AuNCs and AuNCs@CS-TPP. (B) Photostability experiments of DMF-protected AuNCs, AuNCs@CS-TPP and Mito Tracker in PBS (pH 7.4) with a 150 W xenon lamp excitation source.

Fig. 3. In vitro cytotoxicity studies of DMF-protected AuNCs and AuNCs@CS-TPP in HeLa cells (A) and HepG2 cells (B) using the standard MTT assay. The error bars represent the standard deviation of three measurements.

Fig. 4. Fluorescent images of living HeLa cells stained with AuNCs@CS-TPP (20 μg mL⁻¹) with increasing scanning time (0–8 min). Excitation wavelength: 405 nm.
3.2. In vitro cytotoxicity

Cytotoxicity of AuNCs is one of the major concerns when applied for live-cell imaging. The cytotoxicity of the two fluorescent probes, DMF-protected AuNCs and AuNCs@CS-TPP, was evaluated using a standard MTT assay. Fig. 3A and B displayed that almost no significant toxicity was observed in HeLa cells and HepG2 cells after incubation with up to 60 μg mL−1 of AuNCs@CS-TPP for 24 h, while DMF-protected AuNCs started to cause cytotoxicity at 20 μg mL−1, with a corresponding cell viability of ~84% and ~80%, and the cytotoxicity enhanced with the increase of AuNCs concentration. As reported previously, the toxicity of AuNCs can be reduced through coating the biocompatible materials like BSA and GSH (Xie et al., 2009; Zhou et al., 2011). Therefore, we suppose that our results are very likely to follow the similar reason: the biocompatible CS, as a surface coating material, provided AuNCs@CS-TPP with much less cytotoxicity than DMF-protected AuNCs. The results indicated that AuNCs@CS-TPP may be far more suited for live-cell imaging as a fluorescent probe than DMF-protected AuNCs because of its low cytotoxicity.

3.3. Cell uptake of AuNCs@CS-TPP

To investigate the temporal evolutions of cellular uptake of the AuNCs@CS-TPP, we herein used HeLa cells as a model. The HeLa cells were incubated with AuNCs@CS-TPP for different time periods. As shown in Fig. 5A, there was no auto-fluorescence from the cells themselves. After incubation for 0.5 h, the cells produced weak fluorescence, and seemed heterogeneous. After 1 h of incubation, it showed bright fluorescence, indicating that AuNCs@CS-TPP entered the cells. The fluorescence intensity was strengthened with prolonged incubation time, and it almost no
changed when the time reached 2 h (Fig. S5B), suggesting the prepared AuNCs@CS-TPP could be located in mitochondria in a short time. It is worth noting that there were no variations in cell morphology after incubation with AuNCs@CS-TPP (Fig. S5A), implying that AuNCs@CS-TPP did not damage the cells. The results were in good accordance with those of the MIT experiments, clearly demonstrating that AuNCs@CS-TPP had almost no cytotoxicity. Moreover, it was reported that, the nanoparticles of size less than 50 nm entered cells mainly by endocytosis through a clathrin-mediated process, and intracellular trafficking through the endosomal pathway (Shang and Nienhaus, 2013; Jiang, et al., 2010). We therefore speculate that AuNCs@CS-TPP may also enter cells through clathrin-mediated endocytosis. Subsequently, they reside in early endosomes and lysosomes, and then release back into the cytoplasm and eventual located in mitochondria. Certainly, further study on the mechanism of the entrance of AuNCs@CS-TPP into cells is needed.

### 3.4. The photostability of AuNCs@CS-TPP

Photostability is one of the important factors for the development of fluorescent probes. To estimate the photostability of AuNCs@CS-TPP under irradiation with laser confocal microscope, the HeLa cells were incubated with AuNCs@CS-TPP for 2 h. After washing, the HeLa cells were irradiated by laser confocal microscope with excitation at 405 nm. As shown in Fig. 4, there was almost no significant difference of the fluorescence intensity in the HeLa cells treated by AuNCs@CS-TPP after irradiation 8 min. Moreover, we performed the photostability experiment of the Mito Tracker. It was found in Fig. S6 that the fluorescence of the Mito Tracker disappeared completely after irradiation for 60 s, suggesting that the conventional commercially available Mito Tracker suffered from poor photostability. The result agreed with those reported previously (Derfus, et al., 2004; Leung, et al., 2013).

### 3.5. Mitochondria-targeted imaging

To test the feasibility of AuNCs@CS-TPP to target mitochondria, we took the confocal fluorescence images of HeLa cells and HepG2 cells incubated with the fluorescence probe. AuNCs@CS-TPP and the control probe, DMF-protected AuNCs. Fig. 5A and Fig. S7A showed that the subcellular localization of DMF-protected AuNCs was inconsistent with Mito Tracker, a commercially available mitochondria-targeted fluorescent dye, suggesting that the DMF-protected AuNCs had no mitochondrial targeting ability. The colocalization of AuNCs@CS-TPP and Mito Tracker indicated that AuNCs@CS-TPP can selectively accumulate into cellular mitochondria of living cells (Fig. 5B, C and Fig. S7B). The selectivity of the AuNCs@CS-TPP targeting mitochondria very possibly originated from the specific conjugation of TBP with mitochondria (Zhou, et al., 2013). In general, the fluorescent probe, AuNCs@CS-TPP, is an ideal fluorescent materials for targeting mitochondria in living cells.

### 4. Conclusion

In conclusion, a new fluorescent probe (AuNCs@CS-TPP) was successfully synthesized and utilized for mitochondrial imaging of living cells. The probe is demonstrated to possess many advantages in mitochondrial imaging, such as superior photostability, low cytotoxicity, high sensitivity and target-specificity to mitochondria. Perhaps more importantly, our study provides a new strategy for the design and fabrication of high-efficient mitochondrial fluorescent probe, as well as exhibits their potential applications in biology and medicine. Further researches are required to verify how we fulfill the fluorescence imaging of different organelle via adjusting the surface functional group of AuNCs.