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SERS-fluorescence bimodal nanoprobe for in vitro imaging of fatty acid responsive receptor GPR120

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Abstract

G-protein-coupled receptor 120 (GPR120), as a member of the rhodopsin family of G-protein-coupled receptors, has been shown to function as a sensor for dietary fat in the gustatory and digestive systems. Its specific role in the chemoreception of fatty acids, which is thought to be crucial in understanding the mechanism surrounding the control of fat intake and, accordingly, in the treatment of obesity, remains unclear. Here we report a novel surface-enhanced Raman spectroscopy (SERS)-fluorescence bimodal microscopic technique for detection and imaging of GPR120 in single living cells. $\text{CaMoO}_4\text{:Eu}^{3+}$ @AuNR hybrid nanoparticles are synthesized and characterized as imaging probes. Biocompatibility and imaging capability of the probes are investigated using a model HEK293 cell line with an inducible GPR120 gene transfection. Cellular distribution of GPR120 is visualized by single-cell SERS and fluorescence imaging. A dose-dependent GPR120 response to linoleic acid treatment is revealed by SERS.

Graphical Abstract

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Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary data associated with this article can be found in the online version

Keywords

GPR120; Fatty Acid; Surface-enhanced Raman Spectroscopy; Fluorescence Imaging; Bimodal Nanoprobe

Introduction

Dietary lipids (e.g. fatty acids, triglycerides) contribute up to 40% of daily caloric intake in the Western diet, which is thought to contribute greatly to the prevalence of obesity and associated diseases^{1–3}. Understanding the mechanisms underlying the perception of dietary lipids thus is important to help control fat preference and develop treatments for dietary-induced obesity. G protein-coupled receptors (GPCRs) have been shown to play important roles in cellular signaling pathways that affect human sense of taste (e.g. sweet, bitter, umami)^{4, 5}. GPR120 and GPR40, members of GPCR family, have recently been reported to mediate response to long chain fatty acids (LCFAs)⁶. Both GPR120 and GPR40 knock-out mice showed a diminished preference for linoleic acid (LA) and oleic acid, and diminished taste nerve responses. In addition, studies have shown that GPR120 and CD36, LCFA receptors identified in rodent taste bud cells, mediate differential Ca^{2+} response to fatty acids, and are differentially regulated by dietary lipids^{7, 8}. Though several lipid receptor candidates (e.g. GPR120, GPR40, and CD36) have been found in the tongue papillae, the mechanism how these receptors act in the transduction of LCFAs remains unresolved and merits further extensive studies³. It will be beneficial to develop an imaging technique that can visualize how these receptors respond when binding LCFAs in single cells, in order to understand their specific roles in fat chemoreception.

Raman spectroscopy, by measuring the energy of molecular vibrational modes, provides an intrinsic contrast mechanism for identifying molecular composition of cells^{9–12}. However, since Raman scattering is an inefficient process by nature, spontaneous Raman spectroscopy is challenged to detect specific membrane receptors in cells. Plasmonic nanoparticles have been applied to enhance Raman signals from molecules in close proximity—known as Surface-enhanced Raman spectroscopy (SERS)¹³. SERS is able to achieve 10~14 orders of magnitude enhancement of spontaneous Raman signals¹⁴, which allows detection of biomolecules with ultrahigh sensitivity^{15, 16}. To generate intense and stable SERS signals for biomedical imaging, SERS nanoprobe composed of a noble metal nanoparticle, a Raman reporter molecule, and a surface stabilizer are usually applied. Due to advantages of high stability and biocompatibility, especially the great capability of multiplex detection, SERS nanoprobe has been applied in a variety of biomedical applications such as molecular detection^{17, 18}, single living cell imaging^{19–21}, and *in vivo* biosensing^{22, 23}. In addition, SERS microscopy, by generating a pseudo-color map based on relative intensities of selected Raman bands, is able to map the distribution of biomolecules such as lipids and proteins^{24, 25}, cell surface receptors^{26–28} and even bacterial species²⁹ in single living cells. However, SERS is a low-throughput (raster scan) imaging technique and it requires a relative long acquisition time (up to seconds) due to the relatively weak signal by Raman scattering (even after surface enhancement). This limits the application of SERS in biological analyses, especially for high-throughput cell sorting and imaging³⁰.

To overcome the limitation and expand the functionality, multimodal imaging probes have been developed to integrate SERS with other imaging modalities, such as magnetic resonance imaging (MRI)³¹, X-ray computed tomography (CT)^{32, 33} and fluorescence microscopy^{34–36}. Among these modalities, fluorescence, due to its high-throughput imaging ability, is considered as a promising complementary technique to SERS. Fluorescence functions for quick recognition of the analytes in complex biosystems, while SERS is used to accurately detect multiplex targets in single cells. Recently, Choo lab reported a SERS-fluorescence bimodal imaging technique to investigate multiple cancer markers co-localized in single breast cancer cells³⁷.

Here we developed a SERS-fluorescence bimodal imaging technique for detection and imaging of GPR120 in single living cells. Europium-doped calcium molybdate nanoparticles ($\text{CaMoO}_4\cdot\text{Eu}^{3+}$) showed intense red fluorescence emission under UV light excitation³⁸. This fluorescence-active $\text{CaMoO}_4\cdot\text{Eu}^{3+}$ nanoparticle was conjugated with SERS-active gold nanorods (AuNR) encapsulated with Raman reporter molecule 4-mercaptobenzoic acid (MBA), to realize SERS-fluorescence dual functions. The composite nanoparticle was conjugated with antibody for specific targeting of GPR120 expressed in living cells. By using this bimodal nanoprobe, we successfully realize specific SERS-fluorescence imaging of GPR120 at the single cell level. In addition, for the first time, we observed a linear dose-dependence of GPR120 response following linoleic acid (LA) treatments.

Materials and Methods

Materials

4-mercaptobenzoic acid (MBA), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), doxycycline hydrochloride (DOX), LA, europium(III) nitrate hydrate ($\text{Eu}(\text{NO}_3)_3\cdot x\text{H}_2\text{O}$) were purchased from Sigma-Aldrich (St. Louis, MO). Calcium nitrate tetrahydrate, ($\text{Ca}(\text{NO}_3)_2\cdot 4\text{H}_2\text{O}$), ammonium molybdate ($\text{H}_8\text{MoN}_2\text{O}_4$), oleic acid (OA), 1-octadecene were purchased from Alfa Aesar (Ward Hill, MA). Blastidin S HCl, Hygromycin B, phosphate-buffered saline (PBS), 0.5% trypsin-EDTA, LIVE/DEAD Viability/Cytotoxicity Assay Kit were purchased from Life Technologies (Carlsbad, CA). The polyethylene glycol (PEG) products, thiol PEG acid (HS-PEG-COOH, MW 5000) and methoxyl PEG thiol (mPEG-SH, MW 5000), were purchased from Nanocs Inc. (USA). Polyclonal GPR120 antibody was purchased from Santa Cruz Biotechnology Inc. (sc-99105). Ultrapure water ($18\text{ M}\Omega\text{ cm}^{-1}$) was used in this work.

Characterization techniques

Transmission electron microscopy (TEM) images and energy-dispersive X-ray spectroscopy spectrum (EDX) were acquired using an FEI Titan 80–300 kV (S) TEM equipped with a spherical aberration (Cs) image corrector (300 kV). For the TEM measurements, the powder samples were ground and dispersed in methanol. Few drop of dispersed particles were placed on a carbon coated-copper grid and allowed to dry at room temperature. Absorption spectra were acquired by Multiskan Spectrum spectrophotometer (Thermo Scientific). FT-IR spectra were acquired by Varian 660-IR FT-IR spectrometer (Agilent Technologies). Luminescent spectra were acquired by FluoroMax-3 fluorometer (Horiba Scientific). Raman

spectra were measured by Renishaw in Via Raman spectrometer, equipped with a 785 nm near-IR laser, which was focused through a 63× water immersion lens (NA=0.90, Leica Microsystems).

Synthesis of $\text{CaMoO}_4\text{:Eu}^{3+}\text{@AuNR}$ hybrid nanoparticles

Hybrid nanoparticles were synthesized according to previously published protocol.³⁹ For typical synthesis process, (i) Europium-doped calcium molybdate ($\text{CaMoO}_4\text{:Eu}^{3+}$) nanoparticles were prepared via a simple thermolysis process at ~309 °C. The preparation procedure can be briefly described as follows: 21 mg of $\text{Eu}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$, 50 mg of NaOH, and 1.0 g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ were dissolved in 2 mL of distilled water. The mixture was treated with 2 mL of OA and 18 mL ODE and heated in a round-bottom flask at 80 °C for 1 h under continuous stirring. In another beaker, 0.423 g of $\text{H}_8\text{MoN}_2\text{O}_4$ was dissolved in 3 mL of DI water, and 0.1 g of NaOH, 2 mL of OA, and 18 mL of ODE were added and stirred the solution at 80 °C for 1 hr. The two solutions were mixed under continuous stirring and heated at 80 °C for 30 min, and then the reaction was refluxed at 309 °C for 1 hr. The resulting precipitate was collected by centrifugation at ~2500 g after washing with ethanol. The obtained precipitate was cooled at room temperature for 2 days. (ii) About 20 mg of the $\text{CaMoO}_4\text{:Eu}^{3+}$ NPs were dispersed in 5 mL of HCl (0.1 M) and sonicated for ~1 h. To this ~2 mL of diethyl ether was added and sonication was continued for further ~0.5h. Resulting solution was centrifuged at ~6000 rpm for ~15 min. Obtained precipitate was washed three times with ethanol and redispersed in ~5 mL of PBS solution. To this, 20 mg of HS-PEG-COOH was added and sonicated for ~1 h. The PEGylated capped NPs were collected by centrifugation and washed with PBS solution for three times to remove the excess of HS-PEG-COOH present in the sample. The final precipitate obtained was redispersed in a PBS solution. (iii) For the synthesis of $\text{CaMoO}_4\text{:Eu}^{3+}\text{@AuNR}$ hybrid nanoparticles, GNRs with 10 nm in diameter and 35 nm in length were purchased from Nanopartz, Loveland, CO, USA. First, 4 mL of the GNRs was centrifuged at ~13,000 rpm for 30 min and redispersed in PBS. Centrifugation was repeated for three times to reduce the excess of cetyl trimethylammonium bromide (CTAB) present on the surface of the GNRs. GNRs (4 mL) dispersed in PBS was added to ~1 mL of the PEGylated $\text{CaMoO}_4\text{:Eu}^{3+}$ NPs under continuous stirring. Resulting solution was centrifuged, and the HNPs precipitated were collected. These particles were washed with a PBS solution and redispersed in PBS.

Functionalization of SERS-fluorescence bimodal probe

1 mL of as-prepared $\text{CaMoO}_4\text{:Eu}^{3+}\text{@AuNR}$ composite nanoparticle solution was mixed with MBA solution (1mM, 10 μL) and reacted for 30 min. Solutions of HS-PEG-COOH (1 mg/mL, 10 μL) and mPEG-SH (1 mg/mL, 40 μL) were sequentially added to the nanoparticle solution and incubated for 2 hr. The resultant solution was then centrifuged (12000 g, 15 min) to remove excess PEG and MBA. Particles were resuspended in water. Freshly prepared EDC (10 mM, 10 μL) and NHS (25 mM, 10 μL) solutions were mixed with the nanoparticle solution and allowed to react for 30 min. The resulting solution was centrifuged and particles were resuspended in PBS. Finally, anti-GPR120 antibody (0.2 mg/mL, 10 μL) was added to the nanoparticle solution and incubated for 1 hr. Excess antibody was removed by centrifugation. Nanoparticles were resuspended in PBS. The

functionalized nanoprobe ($\text{CaMoO}_4\text{:Eu}^{3+}\text{@AuNR-MBA-Ab}$) was stable in solution for several days at 4 °C.

Cell culture

The human embryonic kidney cells (HEK293 and HEK293–GPR120) were kindly provided by International Flavors & Fragrances Inc. (IFF). HEK293 cell lines transfected with an inducible GPR120 gene (HEK293-GPR120) and a constitutive CD36 gene (HEK293-CD36) were used in this context. Cells were grown in DMEM-GlutaMAX media (Life Technologies, 10569-010) supplemented with 10% Tet-free fetal bovine serum (Fisher, NC0290780). Cells were cultured in a humidified atmosphere at 37 °C with 5% CO_2 , and were passaged at 80~90% confluence. Blasticidin S HCl (10 $\mu\text{g/mL}$) and Hygromycin B (100 $\mu\text{g/mL}$) were added to cell culture medium specifically for maintenance of inducible GPR120 gene. To express GPR120, HEK293-GPR120 cells were induced with DOX at 0.5 $\mu\text{g/mL}$ for 48 hr.

Cell staining and Viability test

The cell viability was analyzed using LIVE/DEAD Viability/Cytotoxicity Assay Kit (Invitrogen) according to the manufacturer's instruction. Briefly, (1) Cells were cultured in poly-D-lysine coated glass-bottom dishes (MatTek Cop. USA) for 24 hours; (2) cells were then washed with PBS twice; (3) 2 ml of mixed solution of 2 μM Calcein AM and 4 μM ethidium homodimer-1 (EthD-1) (both from Invitrogen) was added directly to cells, and incubated cells for 30 min at room temperature; (5) cells were imaged using fluorescence microscope to analyze the relative proportion of live/dead cells. A 10 $\times$ objective (0.25 NA, Olympus) was used to observe fluorescence.

Calcein AM was well retained within live cells producing green fluorescence; however, EthD-1 entered cells with damaged membrane and bonded to nucleic acids, producing a red fluorescence in dead or membrane-damaged cells. Therefore, the live/dead cells were differentiated visually.

Cellular fluorescence and SERS imaging

For fluorescence and SERS imaging experiments, cells were incubated with the functionalized nanoprobe ($\text{CaMoO}_4\text{:Eu}^{3+}\text{@AuNR-MBA-Ab}$) for 24 hr, and rinsed with PBS 3 times before imaging experiments. For LA treatments, cells were first treated with LA for 5 min at different concentrations, and then incubated with nanoprobe for 24 hr.

Cellular fluorescence imaging was performed on an Olympus IX71 inverted microscope with an external 285 nm UV lamp. A 10 $\times$ objective (0.75 NA, Olympus) was used to observe fluorescence. Images were acquired and processed using DPController software (Olympus Corporation) to maintain identical light exposure for three different cell conditions.

For SERS measurements, cells were seeded on a cleaned magnesium fluoride (MgF_2) optical window (United Crystals Co.) to minimize background signal from the substrate. A 63 $\times$ water immersion lens (NA=0.90, Leica Microsystems) was used. Raman spectra

between 600–1800 cm^{-1} were recorded under 3-mW laser exposure for 10 s acquisition time per spectrum. Hyperspectral Raman mapping generation were performed using Renishaw WiRE 3.3 software. Single peak intensity at 1078 cm^{-1} was used to generate pseudo-color SERS maps. For quantitative SERS measurements, 25 spectra from 25 cells (1 spectrum per cell) were collected on cell samples treated with LA at concentrations 0, 5, 20, 30, and 60 μM , respectively. Spectral smoothing and autofluorescence background subtraction were performed using an automated algorithm program kindly provided by BC Cancer Research Center.⁴⁰ The processed spectra were exported to OriginPro 9 software for plotting. Data were reported as mean $\pm$ SE (standard error of mean). Statistical difference was analyzed by one-way analysis of variance (ANOVA).

Results

Nanoparticle Structure characterization

The typical TEM image of the composite $\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$ NPs is shown in Fig. 1a. It confirms the formation of hybrid nanoparticles where AuNR was attached to the surface of $\text{CaMoO}_4:\text{Eu}^{3+}$ NP. The presence of each element in the particle was confirmed by EDX spectrum (Fig. 1a, **inset**). The average size of $\text{CaMoO}_4:\text{Eu}^{3+}$ nanoparticles was found to be ~ 20 nm, whereas, average lengths and widths of AuNR was found to be ~ 40 and 10 nm, respectively. Moreover, the hybrid nanoparticles show high dispersion for long times without precipitation. The crystalline nature of the $\text{CaMoO}_4:\text{Eu}^{3+}$ nanoparticles was confirmed by their X-ray diffraction pattern (data not shown). All peaks are well matched with the tetragonal structure of $\text{CaMoO}_4:\text{Eu}^{3+}$ (JCPDF#29-0351). The UV-Vis spectra of hybrid nanoparticles in the range 200–1000 nm are shown in Fig. 1b. Strong absorption bands at 512.5 and 789 nm were observed, which are assigned to transverse and longitudinal surface plasmon resonance (SPR) bands of AuNR, respectively. It was observed that the peak position of the SPR band at 789 nm was slightly red-shifted (~ 0.8 nm) and full width at half maximum (*FWHM*) increased by ~ 27 nm, whereas there was no shift for the SPR band at 512.5 nm and the *FWHM* increased by ~ 14.4 nm with antibody coating on the surface of nanoparticles. Moreover, a small peak at ~ 265 nm was observed for $\text{CaMoO}_4:\text{Eu}@\text{AuNR}$ NPs. This peak was ascribed to a charge transfer from the oxygen ligands to the central molybdenum atom within the MoO_4^{2-} cluster (also called Mo–O charge transfer band (CTB)), but no such peak was observed for the bare AuNR. It was further confirmed by excitation spectra ($\lambda_{\text{em}} = 615$ nm). Also some weak peaks in the longer wavelength region 300–500 nm were ascribed to the direct $4f^6-4f^6$ intraconfiguration transitions of Eu^{3+} ion (Fig. 1d, **inset**)⁴¹. The $\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$ NPs emits strong red fluorescence under 270, 285, 300, 395, and 464 nm excitation peaks centered at 590 ($^5\text{D}_0 \rightarrow ^7\text{F}_1$; magnetic dipole transition) and 615 nm ($^5\text{D}_0 \rightarrow ^7\text{F}_2$; electric dipole transition) (Fig. 1d). The high-energy state excited (Mo–O CTB and Eu^{3+}) electrons of Eu^{3+} are unstable and relaxed back to back to ground states of Eu^{3+} ion through photons emission in visible region. The intensity of $^5\text{D}_0 \rightarrow ^7\text{F}_2$ transition is significantly higher than other transitions of Eu^{3+} ion⁴². Strong luminescence of $\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$ NPs may be particularly useful for biological fluorescence labeling.

Functionalization and performance of SERS-fluorescence bimodal nanoprobe

As prepared the $\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$ nanocomposite was further functionalized to generate intense Raman signals and achieve specific targeting. Fig. 2a demonstrates the functionalization process containing three steps: (1) MBA, as a Raman reporter molecule providing strong chemical enhancement of the SERS signal and simple SERS spectrum, was conjugated onto the nanocomposite through covalent Au-S bonding. (2) PEG linkers were coated to improve the stability and biocompatibility of the nanoprobe, as well as to minimize the non-specific binding of the nanoprobe to cells. (3) Finally, anti-GPR120 antibodies were conjugated onto the nanocomposite to realize specific GPR120 targeting in cells. The optimal dilution ratio of GPR120 antibody was determined by immunofluorescence imaging. At a 1:250 dilution the antibody exhibited good specificity and little background labeling of GPR120 in cells (data not shown). Thus, this ratio was used in nanoprobe functionalization and cell incubation.

Fig. 1c shows the FT-IR spectra of bare $\text{CaMoO}_4:\text{Eu}^{3+}$, $\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$, and antibody-conjugated $\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$ hybrid nanoparticles in the range from 500–4000 cm^{-1} . Peaks at ~ 1650 and 3450 cm^{-1} correspond to bending and stretching vibrations of H–O–H molecules were present on the surface of the hybrid nanoparticles. The characteristic peak appeared at $\sim 802 \text{ cm}^{-1}$ was assigned to asymmetric stretching vibration of O–Mo–O vibration in the MoO_4^{2-} tetrahedron⁴³. The peaks at 2923 and 2852 cm^{-1} indicating the C–H stretching vibrations arose from OA⁴⁴. On antibody conjugation to $\text{CaMoO}_4:\text{Eu}^{3+}$ nanoparticles with AuNR and AuNR-Ab the peaks broadened. The FTIR spectrum of pure HS–PEG–COOH, mPEG–HS, and antibody are show in Fig. S1 (see ESI). It was found that pure HS–PEG–COOH has several characteristic peaks from 1000–1700 cm^{-1} , whereas some feeble peaks were observed at ~ 2050 , 1244, and 1076 cm^{-1} representing the antibody. Moreover, the conjugation of the antibody to the $\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$ nanoparticles results some small peaks from 1000–1700 cm^{-1} , which are characteristic of peaks arises from PEG and/or antibody present on the surface of the nanoparticles.

The performance of the functionalized nanoprobe ($\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$ -MBA-Ab) was assessed by SERS and fluorescence imaging. The constructed probes were incubated with cells for 24 hr at a final concentration of 100 $\mu\text{g/mL}$. Three different cell samples were used: (1) HEK293-GPR120 cells induced with DOX for 48 hr (GPR120 (+)); (2) HEK293-GPR120 cells without DOX inducing (GPR120 (–)); (3) constitutive HEK293-CD36 (CD36) cells. Representative SERS spectra of the nanoprobe-treated cells are shown in Fig. 2b. Typical spectrum of GPR120 (+) cells shows two enhanced peaks at 1078 and 1585 cm^{-1} , which are assigned to the ring breathing and axial deformation modes of MBA, respectively^{45, 46}. In contrast, spectra of GPR120 (–) and CD36 cells show nearly no Raman peaks from the reporter molecule MBA, but only regular Raman signals from cells (e.g. 1003 cm^{-1} from phenylalanine). Average peak intensity at 1078 cm^{-1} for GPR120 (+) cells is significantly higher ($P < 0.001$, $N = 25$) than GPR120 (–) and CD36 cells (Fig. 2c). This difference resulted from the specific targeting ability of the constructed nanoprobe $\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$ -MBA-Ab, which selectively binds to GPR120 (+) cells through specific antibody-antigen interactions, promoting the significantly enhanced SERS signal. This finding can also be confirmed by fluorescent images, in which the

CaMoO₄:Eu³⁺@AuNR-MBA-Ab nanoprobe-incubated GPR120 (+) cells exhibited considerable red fluorescence while the fluorescence of GPR120 (–) cells was virtually invisible (Fig. 3).

Biocompatibility of the SERS-fluorescence bimodal nanoprobe CaMoO₄:Eu³⁺@AuNR-MBA-Ab was estimated by incubating GPR120 (+) cells with the nanoprobe at different concentrations (20, 50, 80, 100, 200 µg/mL) for 24 hr, and then testing the cell viability using live/dead fluorescence imaging. As shown in Fig. S2, there were no significant decreases in cell viability when incubating cells with nanoprobe at concentration as high as 100 µg/mL. Thus, this concentration (100 µg/mL) was chosen in subsequent imaging experiments.

Cellular fluorescence and SERS microscopy

Cellular imaging capability of the bimodal nanoprobe was estimated by fluorescence and SERS measurements. Due to its high-throughput nature, fluorescence microscopy was used to quickly identify cells with high GPR120 expression levels. GPR120 (+) and GPR120 (–) HEK293 cells exhibited significant difference in fluorescence emission after incubation with the nanoprobe (Fig. 3). At single cell level, CD36 cell showed barely detectable red fluorescence at the central area of the cell, while GPR120 (+) cell had strong labeling over the entire cell (Fig. S3). Because the nanoprobe was conjugated with anti-GPR120 antibodies, they tended to bind with GPR120 but not CD36 at cell surface. Furthermore, cellular SERS mapping was performed on the nanoprobe-treated cells. SERS images were created using the Raman intensities of peak 1078 cm^{–1}, which is the most stable and reproducible characteristic peak from the reporter molecule (MBA). By collecting the peak intensity values all over the cell and transforming them into color values, GPR120 distribution was reflected by the brightness of the SERS image at the single cell or subcellular level. Fig. 4 showed the single-cell SERS mapping of GPR120. Apparently, strong SERS signals were recorded on GPR120 (+) cells (Fig. 4b), while SERS signals on the CD36 cells were nearly undetected (Fig. 4a). Additional SERS maps of GPR120 (+) cells are shown in Fig. S4. SERS maps reflected the reorganized and clustered GPR120 receptors after nanoparticle binding. It has been reported that cargo binding promotes membrane receptor clustering in several different receptors.^{47–49} Cellular imaging results demonstrated that SERS-fluorescence bimodal microscopy is able to visualize the heterogeneous distribution of GPR120 receptors in single living cells.

Detection of GPR120 under LA treatment by SERS

GPR120 responses to LA treatments were measured by SERS. GPR120 (+) cells were treated with LA at concentrations 0, 5, 20, 30, and 60 µM for 5 min, and then incubated with nanoprobe for 24 hr. After washing off the unabsorbed nanoparticles, cells were taken for Raman measurements. Fig. 5a showed the average SERS spectra (N=25) of the LA-treated cells. Two major intense peaks at 1078 and 1585 cm^{–1} were from the nanoprobe, which reflect the presence of GPR120 receptors. Peak at 1003 cm^{–1} was attributed to phenylalanine from cells. Elevated SERS signals from nanoprobe (1078 cm^{–1}) were observed in high LA concentration treatments, while signals from cells themselves (1003 cm^{–1}) were nearly unchanged (Fig. 5b, **inset**). SERS intensities (1003, 1078 cm^{–1}) vs. LA concentration were

plotted in Fig. 5b. A linear ($R^2 = 0.93$) increase in 1078 cm^{-1} intensity was observed with increased LA concentrations. This result indicates GPR120 activity is enhanced by LA treatment, which is consistent with previous reports using other methods⁶. Furthermore, for the first time, we found that there is a linear dose-dependence of GPR120 response on LA in 0~60 μM concentration range. These results demonstrated the potential of SERS to study dynamic process of biomolecules in single living cells.

Discussion

Recent studies have implicated that GPR120 and CD36 are candidate receptors that are involved in the gustatory perception of LCFA in taste bud cells³. Yet their specific roles in the detection of LCFA remain to be answered. Several hypotheses are proposed to explain the situation, but the experimental results appear to be contradictory (reviewed in Besnard *et al.*⁵⁰). An imaging technique that can visualize the behaviors of these receptors in living cells is favorable to provide direct evidences to answer these questions. Our goal in this work is to demonstrate the feasibility of the SERS-fluorescence bimodal imaging approach to achieve noninvasive live cell imaging of these fat responsive receptors. The fluorescence signal can be used as a fast detection tool for receptor recognition, while the SERS signal can be used as an accurate sensing tool for imaging localized receptor distributions³⁷. In addition, the outstanding capabilities of SERS for multiplex imaging^{51, 52} would allow for the future detection of interactions between GPR120 and CD36 in the chemoreception of fatty acids.

In summary, we developed a dual functional composite nanoprobe for SERS-fluorescence bimodal imaging of fat-responsive receptor GPR120 in single living cells. The dual functional nanoprobe was composed of europium-doped calcium molybdate and gold nanorod, and further functionalized with Raman reporter and anti-GPR120 antibody. The functionalized nanoprobe was successfully applied for both SERS and fluorescence detection of GPR120 in vitro. Cellular distribution of GPR120 was successfully detected by single-cell SERS mapping. Taking advantages of the quantification ability of SERS, we observed an up-regulation of GPR120 by LA treatment. Moreover, a linear relationship between GPR120 activity and LA concentration in 0~60 μM range was observed for the first time. These results demonstrate the potential of our SERS-fluorescence dual functional nanoprobe for multiplex detection of fat-responsive receptors (e.g. CD36 and GPR120) in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Characterization of the nanoprobe. (a) TEM image of $\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$ nanoparticle. Inset: EDX spectrum of the particle. (b) UV-Vis absorption spectra of AuNR and $\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$. (c) FT-IR spectra of bare $\text{CaMoO}_4:\text{Eu}^{3+}$, $\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$, and antibody-conjugated $\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$. (d) Luminescent properties (excitation/emission) of the $\text{CaMoO}_4:\text{Eu}^{3+}@\text{AuNR}$ nanoparticle.

Fig. 2.

(a) Schematic for functionalization process of $\text{CaMoO}_4:\text{Eu}^{3+}$ @AuNR nanocomposite. (b) Representative SERS spectra and (c) average SERS intensities at 1078 cm^{-1} (N=25) of $\text{CaMoO}_4:\text{Eu}^{3+}$ @AuNR-MBA-Ab nanoprobe-incubated cell samples: (1) HEK293-GPR120 cells induced with DOX (GPR120 (+)); (2) HEK293-GPR120 cells without DOX inducing (GPR120 (-)); (3) constitutive HEK293-CD36 (CD36) cells. **P<0.001.

Fig. 3.
Fluorescence imaging of GPR120 (+) and GPR120 (-) cells incubated with
CaMoO₄:Eu³⁺@AuNR-MBA-Ab nanoprobe for 24 hr.

Fig. 4. Raman mappings of single (a) CD36 and (b) GPR120 (+) cells incubated with CaMoO₄:Eu³⁺@AuNR-MBA-Ab nanoprobe for 24 hr. Raman mappings were generated by the selection of peak 1078 cm⁻¹. The intensities were normalized between the lowest (0) and highest (1) color values. Scale bar: 5 μm.

Fig. 5. GPR120-LA dependence measured by SERS. (a) Average Raman spectra of CaMoO₄:Eu³⁺@AuNR-MBA-Ab nanoprobe-incubated cells under 5 min LA treatment at concentrations 0, 5, 20, 30, and 60 μM. (b) Linear relationship between SERS intensities at selected peaks (1003, 1078) and LA concentration. Inset: expanded Raman spectra between 950 and 1150 cm⁻¹.