Gd₂O₃-doped silica @ Au Nanoparticles for *In Vitro* Imaging Cancer Biomarkers Using Surface-Enhanced Raman scattering

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ABSTRACT

There has been an interest in developing multimodal approaches to combine the advantages of individual imaging modalities, as well as to compensate for respective weaknesses. We previously reported a composite nano-system composed of gadolinium-doped mesoporous silica nanoparticle and gold nanoparticle (Gd-Au NPs) as an efficient MRI contrast agent for *in vivo* cancer imaging. However, the MRI lacks sensitivity and is unsuitable for *in vitro* cancer detection. Thus, here we performed a study to use the Gd-Au NPs for detection and imaging of a widely recognized human cancer biomarker, epidermal growth factor receptor (EGFR), in individual human cancer cells with surface-enhanced Raman scattering (SERS). The Gd-Au NPs were sequentially conjugated with a monoclonal antibody recognizing EGFR and a Raman reporter molecule, 4-mercaptobenzoic acid (MBA), to generate characteristic SERS signal at 1075 cm^{-1} . By spatially mapping the SERS intensity at 1075 cm^{-1} , cellular distribution of EGFR and relocation on the plasma membrane were measured *in situ*. In addition, the EGFR expression level in three human cancer cell lines (S18, A431 and A549) was measured using this SERS probe, which was consistent with the comparable measurements using immunoblotting and immunofluorescence. Our SERS results show that functionalized Gd-Au NPs successfully targeted EGFR molecules in three human cancer cell lines and monitored changes in single cell EGFR distribution *in situ*, demonstrating its potential to study cell activity under physiological conditions. This SERS study, combined with our previous MRI study, suggests the Gd-Au nanocomposite is a promising candidate contrast agent for multimodal cancer imaging.

Keywords: gadolinium-gold nanoparticles, surface-enhanced Raman scattering, epidermal growth factor receptor, human cancer cells

INTRODUCTION

Noninvasive cancer imaging to exhibit tumor anatomical structure and to investigate its metabolism plays an important role in early cancer detection and localization [1]. Currently, there are several imaging modalities widely applied in cancer research, such as magnetic resonance imaging (MRI) [2-5], photoacoustic (PA) imaging [6-8], surface-enhanced Raman scattering (SERS) [9-11] and optical fluorescent imaging [12-15]. Although each individual modality has specific advantages in cancer imaging, none of them is able to support comprehensive structural and functional studies of tumors independently. Thus, there has been an interest in developing multimodal approaches to combine the advantages of these individual imaging modalities, as well as to compensate for **their individual weaknesses**. Recently, several composite nanoparticles have been successfully synthesized and applied to multimodal imaging [16-21]. **These composite particles are usually constructed by combining nanostructures with different imaging modalities.** For example, Gao and co-workers [16] synthesized an iron oxide and gold-coupled core-shell nanoparticles to integrate both MRI and PA imaging, which provided remarkable contrast enhancement in bioimaging. Despite quickly growing interest in designing multifunctional imaging contrast agents, the challenge remains to combine different imaging modalities while preserving the controlled particle size.

Among varied imaging modalities, MRI has the advantages of high spatial and temporal resolution and unlimited tissue penetration, which make it a great technique for clinical diagnostics. But it also suffers the limitation of insufficient sensitivity and it is unable to detect the subcellular distribution of the nanoscale contrast agent in living cancer cells. In recent years, SERS has become an emerging non-invasive imaging tool in detection of cancer cells and cancer biomarkers, due to its ultrahigh sensitivity and the ability to reflect subtle changes in chemical composition and molecular structure of living cells [22-24]. To date, only a few studies have been

published integrating MRI and SERS for multimodal bioimaging [17,18], even though this combination can achieve both high resolution and high sensitivity. We previously reported a gadolinium-doped mesoporous silica nanoparticle ($\text{Gd}_2\text{O}_3@\text{MCM-41}$) as an efficient MRI contrast agent for cancer imaging [25,26]. In addition, we incorporated the gadolinium-doped mesoporous silica nanoparticle and gold nanoparticle (AuNP) into one single nano-system to form a composite Gd-Au nanostructure ($\text{Gd}_2\text{O}_3@\text{MCM-41}@\text{Au}$) [27]. The Gd-Au nanocomposite was an efficient amplification strategy to increase MRI signals for *in vivo* cancer imaging. MRI alone did not show enough sensitivity to study the relocation and endocytosis of surface markers at (sub)cellular level.

Epidermal growth factor receptor (EGFR) is a cell surface receptor tyrosine kinase that, when activated by binding its specific ligands (e.g. EGF, TGF- α), triggers cell signaling pathways that result in cell proliferation, inhibition of apoptosis, angiogenesis, cell migration, adhesion and invasion [28,29]. EGFR over-expression and up-regulation have been reported in variety of cancers, including breast cancer [30], lung cancer [31], and esophageal cancer [32]. As a result, EGFR is the target in an expanding class of anticancer therapies [31,33].

Here we report synthesis and characterization of a Gd-Au nanocomposite ($\text{Gd}_2\text{O}_3@\text{MCM-41}@\text{Au}$) capable for SERS detection of EGFR and validation of function in human nasopharyngeal carcinoma (S18), epidermoid carcinoma (A431), and lung adenocarcinoma (A549) cells. Importantly, SERS mapping demonstrate capability for noninvasive monitoring of subcellular distribution of EGFR on individual cancer cells in real time.

EXPERIMENTAL METHODS

Materials

4-mercaptobenzoic acid (MBA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), Tris-HCl, β -glycerol phosphate, EDTA, glycerol, triton X-100, sodium orthovanadate, benzamidine, aprotinin, leupeptin, phenylmethylsulfonyl fluoride, tween 20, phosphate buffer saline (PBS) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). All solutions were prepared using deionized water ($18 \text{ M}\Omega \text{ cm}^{-1}$). Monoclonal anti-EGFR antibody was purchased from Life Technology (Carlsbad, CA). The polyethylene glycol (PEG) products, Thiol-PEG-COOH and mPEG-Thiol, were purchased from Nanocs Inc. (New York, NY).

Preparation of the Gd-Au nanoprobe

The Gd-Au nanocomposite composed of Gd_2O_3 -MCM41 silica nanoparticle and gold (Au) nanoparticle were synthesized as previously described [25,27]. A typical one-step synthesis process of Gd_2O_3 -MCM41 nanoparticles is described as follows: 0.2 g of cetyltrimethylammonium bromide (C_{16}TAB) as surfactant was dissolved in 100 mL of distilled water. 2 mL of 25% $\text{NH}_3\cdot\text{H}_2\text{O}$ was then added to the vigorously stirred solution at room temperature, followed by the addition of 4.49 mmol of tetraethoxysilane (TEOS). After stirring for 15 min, 0.27 mmol of $\text{GdCl}_3\cdot 6\text{H}_2\text{O}$ was added to the solution. After another hour of stirring, gadolinium hydroxide was derived from gadolinium chloride in alkaline solution, and the fine particle precipitate was centrifuged and dried in a freeze dryer for 15 h. Samples were calcined at 500°C for 5 h to remove the templates and harvest Gd_2O_3 -MCM41 nanoparticle. For further optimization, the dispersion solution of Gd_2O_3 -MCM41 colloids was treated with polyethylenimine (PEI). Finally, the Gd-Au nanocomposite was completed via self-assembly of colloidal AuNP. The synthesized Gd-Au nanocomposite was imaged by high-angle annular dark-field scanning electron microscopy (HAADF-STEM,

University of Oregon). The size distribution of the Gd-Au nanocomposite was measured by dynamic light scattering (DynaPro NanoStar, Wyatt Technology, Santa Barbara, CA).

The Gd-Au nanocomposite was sequentially coated with Raman reporter molecule 4-mercaptobenzoic acid (MBA), polyethylene glycol (PEG), and anti-EGFR antibody (Life Technology, AHR5062) to achieve SERS capability, biocompatibility and specific targeting as detailed below. Gd-Au (8.1 mg) was dissolved in phosphate-buffered saline (10 mL) followed by addition of MBA solution (100 μ L in 0.03 M in EtOH) before stirring for 30 min. Then Thiol-PEG-COOH (1 mL, 0.24 mM, MW 5000) was introduced to react for 30 min before 2.8 mL of mPEG-Thiol (0.42 mM, MW 5000) was added to stir for another 3 hrs. The resulting mixture solution was centrifuged (9700 g, 10 min) twice to remove excess PEG molecules. The conjugation of monoclonal antibody was achieved by using EDC/NHS method [34,35] to activate the Gd-Au nanoparticle. PEGylated Gd-Au nanocomposite was resuspended in water. EDC (10 μ L, 10 mM) and 10 μ L of NHS solution (25 mM) were added to 1 mL of Gd-Au suspension (0.8 mg/mL) and incubated for 30 min at room temperature. Activated Gd-Au suspension was then incubated with 20 μ L of mAb EGFR (0.21 mg/mL) for 1 hr at room temperature. Excess antibody was removed by centrifugation (9700 g, 10 min). The antibody functionalized Gd-Au bioprobes were stored at 4 °C before measurements. The nanoparticles were stable for several days.

Cell culture, viability and sample preparation

All cells were purchased from ATCC and were determined to be free of *Mycoplasma* contamination. Cells were grown at 37°C with 5% CO₂ in a humidified atmosphere. The human epidermoid carcinoma cell line A431 was cultured in Dulbecco's-modified eagle's medium (DMEM) and Ham's F-12 medium (1:1) with 10% fetal bovine serum (FBS). The human lung carcinoma cell line A549 was cultured in F-12k medium with 5% FBS and 1% penicillin-

148 streptomycin. The human nasopharyngeal carcinoma cell line S18 was cultured in DMEM with
149 10% FBS.

150 Cells were passaged at 80-90% confluence and seeded at a density of 10^5 cells per 2 mL of
151 media. Cells were let grow for 24 hr after seeding before imaging measurements. For Raman
152 measurements, cells were seeded on magnesium fluoride (MgF_2) substrates to minimize the
153 background noise. Cells were seeded on glass bottom Petri dishes for other optical imaging
154 measurements.

155 The cell viability was analyzed using LIVE/DEAD Viability/Cytotoxicity Assay Kit
156 (Invitrogen) according to manufacturer's instructions.

157 **Cellular SERS measurements**

158 Raman measurements were performed by a Renishaw inVia Raman system (Renishaw,
159 UK) coupled with a Leica DMLM microscope (Leica microsystems, USA) equipped with a 785
160 nm near-IR laser. A 63X water immersion objective ($\text{NA} = 0.90$) was used to image the cells in
161 culture media and a temperature control unit (ALA Scientific Instruments, USA) was used to keep
162 cells under physiological temperature (37.5°C). The Raman instrument was calibrated with silicon
163 at 520.5 cm^{-1} .

164 Prior to Raman measurements, all three cell lines (A431, A549 and S18) were incubated
165 with anti-EGFR-conjugated Gd-Au nanoprobe for 1h and rinsed with PBS to remove unbound
166 nanoparticles. Raman spectra ($600\sim 1800\text{ cm}^{-1}$) and Raman streamline mapping (at 1075 cm^{-1}) of
167 the nanoprobe-treated cells were recorded under a laser intensity of 3 mW. Spectra smoothing,
168 baseline subtraction and mapping generation were performed by Renishaw WiRE 3.3 software.

169 Processed data were exported to Origin Pro 9 software (OriginLab Corp., USA) for statistical
170 analysis.

171 **Cellular optical imaging**

172 Fluorescence images of the nanoprobe-treated cells were collected by an Olympus IX71
173 inverted fluorescence microscope equipped with an Olympus DP30BW CCD camera. Dark field
174 images were obtained by using an oil-immersed dark field condenser (NA=1.5). Images were
175 acquired with 40x objectives using DPController software (Olympus).

176 **Western blot**

177 Cells were rinsed twice with ice-cold PBS and lysed in a buffer containing 25 mM Tris-
178 HCl (pH 7.4), β -glycerol phosphate (50 mM), EDTA (0.5 mM), glycerol (5%), triton X-100 (0.1%),
179 sodium orthovanadate (1 mM), benzamidine (1 mM), and a protease inhibitor cocktail containing
180 aprotinin, leupeptin, and phenylmethylsulfonyl fluoride. Protein concentration was determined
181 using a bicinchoninic acid (BCA) assay (Pierce, Rockford, IL). Protein was denatured with
182 Laemmli's buffer at 95°C for 5 min and lysate (50 μ g) was loaded to each well. Proteins were
183 separated using 10% SDS-PAGE gel electrophoresis and resolved proteins were transferred to
184 PVDF before incubating in Tris-buffered saline containing Tween-20 (0.05%) and fat-free dry
185 milk (5%) for 1 hr at room temperature. Membranes were incubated with primary antibodies to
186 EGFR and β -Actin overnight at 4°C and subsequently with HRP-conjugated secondary antibody
187 at room temperature for 1 hr. Signals were visualized using ECL (Pierce, Rockford, IL) following
188 manufacturer's instructions.

189 **Immunofluorescence imaging**

S18, A431 and A549 cells grown on coverslips for 24 hours were fixed using 4% paraformaldehyde for 20 minutes and permeabilized using 0.1% triton X-100 for 10 minutes. After blocking with 5% BSA in PBS, cells were incubated with anti-EGFR antibody conjugated with Alexa Fluor 555 at 1:50 dilution in 5% BSA solution overnight at 4°C. After washing the cells thrice with PBS, the cover slips were mounted using Vectashield mounting solution containing the nuclear counter-stain 4', 6-diamidino-2-phenylindole (DAPI). Images were collected under a Nikon inverted epifluorescence microscope. Representative images were combined and processed using ImageJ software.

RESULTS AND DISCUSSION

Characterization of the Gd-Au nanocomposite

The synthesized Gd-Au nanoparticle has a composite structure illustrated in Fig. 1a. Gadolinium oxide (Gd_2O_3) nanoparticles were embedded in the mesoporous silica structure (MCM-41). Gd_2O_3 -MCM41 is conjugated with gold (Au) nanoparticles through a polymer linker polyethylenimine (PEI). The structure of the Gd-Au nanocomposite can be visualized by HAADF-STEM imaging (Fig. 1b). Energy dispersive X-ray (EDX) spectrum (supporting information, Fig.S1) and the elemental mapping (Fig. 1c) have presented the existence of gadolinium and gold, indicating the successful assembly of the core-shell like structure of the Gd-Au nanocomposite. A line profile of integrated intensity plot shows the elemental distribution across the nanocomposite (Fig.S2). Diameter of AuNPs in the synthesized Gd-Au nanocomposite is around 30 nm, as determined from the HAADF-STEM images (Figs 1b and S2). The hydraulic diameter is measured to be 208.05 ± 5.98 nm using dynamic light scattering (DLS) (Fig. 1d).

Functionalization of the Gd-Au nanocomposite

To make the Gd-Au nanocomposite a specific SERS probe for EGFR sensing and imaging, step-by-step functionalization was conducted. As presented in Fig. 2a, Gd-Au nanoparticles were firstly conjugated with 4-mercaptopbenzoic acid (MBA) through Au-S covalent bond as a Raman reporter molecule. The reasons to use MBA were: (1) MBA can produce high SERS effect when binding with gold nanoparticle due to its high binding affinity with gold and relatively large cross section area for Raman scattering [36]; (2) MBA has simple SERS spectrum in the fingerprint region (600~1800 cm^{-1}) with two intensive characteristic peaks around 1075 and 1587 cm^{-1} , which could be assigned to the ring breathing and axial deformation modes of MBA, respectively [11,36]. SERS spectra of Gd₂O₃@MCM-41@Au-MBA and AuNP-MBA displayed both characteristic peaks of MBA, though the peak positions shifted a little ($\sim 2 \text{ cm}^{-1}$) due to the differences in the nanostructures (Fig.S3). The presence of both characteristic peaks on spectra of nanoparticle-treated S18 cells (Fig.2b) confirmed the successful adsorption of MBA on the Gd-Au nanocomposite. Then, thiolated PEG linkers were also coated onto Au surfaces (PEG-NP) to eliminate the non-specific particle-cell binding as well as to improve the biocompatibility of the Gd-Au nanoprobes when incubated with cells *in vitro*. Lastly, to realize specific EGFR targeting, monoclonal antibody to EGFR was introduced and conjugated with the PEGylated Gd-Au nanocomposite using EDC/NHS method. The antibody-targeted Gd-Au nanoprobe (mAb-NP) was then used for *in vitro* SERS detection of EGFR and SERS imaging of cellular EGFR distribution at single cell level. The stability of the functionalized Gd-Au nanoprobe was proved by consistent SERS intensities at 0, 24, 48 and 72 hr after functionalization (Fig.S4).

Specificity of EGFR targeting

In order to study how anti-EGFR functionalized Gd-Au nanoprobes specifically bind to EGFR and are subsequently internalized via receptor-mediated endocytosis, control experiments

are performed under different conditions. S18 cells (EGFR-positive) were incubated with Gd-Au nanoprobe under three conditions: (1) PEG-NP for 1 hr; (2) mAb-NP for 1 hr; and, (3) preblocked with free anti-EGFR antibody for 1 hr, followed by incubation with mAb-NP for 1 hr (**mAb_Block**). Cells were rinsed with PBS thrice to remove unbound nanoprobe, before Raman measurements.

Typical SERS spectra are shown in Fig. 2b. Cells with PEG-NP incubation typically present a much lower SERS signal compared to cells incubated with mAb-NP, while cells in the **mAb_Block** group show almost non-observable SERS spectra (Fig. 2c). Moreover, as shown in Fig.2c, the average intensities at 1075 cm^{-1} calculated from 50 spectra (1 cell/spectra) for each condition confirm the same finding. S18 cells alone (CTRL), cells treated with PEG-NP, and cells treated with mAb-NP were also visualized using dark field imaging (Fig. 2d). PEG-NP treated cells exhibit minimal light scattering, similar to CTRL, while cells incubated with mAb-NP have many more bright spots, indicative of nanoparticles binding to the cells.

Thus, both SERS and dark field imaging indicate that the Gd-Au nanoprobe binds to the S18 cells through a selective antibody-antigen recognition. High SERS signal was only observed in cells incubated with mAb-NP, indicating that the Gd-Au nanoprobe is capable of specific SERS detection of EGFR.

To investigate the biocompatibility of the Gd-Au nanoprobe, viability of S18 cells was measured following incubation with mAb-NPs at different concentrations (8, 20, 28, 40 and 48 $\mu\text{g/ml}$) for 24 hours. The cell viability remains >90% even at the highest concentrations (Fig.S5). For further SERS detection studies, a dose of 40 $\mu\text{g/ml}$ was chosen.

SERS mapping of cellular EGFR distribution

SERS mapping is generated by firstly taking spectral acquisition (2s exposure) centered at 1100 cm^{-1} (400 cm^{-1} bandwidth) at each point over the 2D area of a single cell with 0.8 μm spacing. Then, color mapping with the peak intensity at 1075 cm^{-1} is plotted to depict the intensity variation over the area, which reflects the cellular EGFR distribution via the binding of Gd-Au nanoprobe.

Changes of cellular EGFR distribution were measured (Fig. 3). A single S18 cell after 1hr incubation with mAb-NPs is presented in the bright field image (Fig. 3a). *In situ* SERS mapping is performed over the area of cell body (dotted area in Fig. 3a) at 60, 90 and 120 min. As shown in Fig. 3b-d, the bright spots, which represent EGFR molecules, are heterogeneously distributed and are relocating in the cell. Fig. 3e-g show the SERS spectra at the same position (green crosses) in the mappings at different times (Fig. 3b-d). The time-dependent changes indicate the reorganization of cell membrane after nanoparticle binding. The membrane reorganization is most likely due to internalization of nanoprobe after binding through a receptor-mediated endocytosis, as suggested by a decreased mapping intensity with longer incubation (Fig.S6). As is reported in previous studies, nanoparticle conjugation may distinctly alter antibody-induced receptor endocytosis[37,38]. Yet SERS-active nanoprobe have been constructed for real-time monitoring of molecular events in living cells[39-41]. Physical and chemical properties of particles, including size, shape, modulus, surface charge and surface chemistry, play important roles in particle-cell interactions [42]. Though it is not the focus of this work, effects of nanoconjugation on cellular activities merits further study, as it helps facilitate the design and nanofabrication of imaging nanoprobe for effective diagnosis and therapy. The results of *in situ* SERS mapping here have demonstrated the feasibility of the methodology to detect and monitor dynamic cellular processes, although the mechanisms underlying the cellular relocation will require additional studies.

EGFR detection in three human cancer cell lines (S18, A431 and A549)

The proof-of-concept studies with S18 were validated using two unrelated human cancer cell lines. SERS mapping shows EGFR in discrete domains on each cell, but at different intensities, suggesting that the EGFR expression varies between the cell lines (Fig. 4). To verify the EGFR levels, average Raman peak intensity at 1075 cm^{-1} was calculated using data from 50 randomly selected cells (1 spectrum/cell) in each cell line. A431 cell had significantly (** $P < 0.01$, one-way ANOVA) higher EGFR level than S18 or A549 cells (Fig. 5a). These observations were consistent with the western blot (i.e., EGFR band is darker in A431 than S18 and A549, Fig. 5b). EGFR expression levels in the three cancer cell lines were also confirmed by immunofluorescence imaging (Fig. 6). Comparing SERS with fluorescence, the most widely used imaging technique, SERS is not so competitive for analyzing samples with large sizes due to its low-throughput nature and relatively low imaging speed. However, SERS has other important advantages. First, SERS is a non-invasive and non-photobleaching imaging technique that enables long term *in situ* monitoring of the dynamic cellular process, which normal fluorescence imaging technique does not offer. In addition, due to its narrow spectral bands, SERS represents a better candidate for multiplex imaging in complex biosystems [43-46].

CONCLUSIONS

In conclusion, we have synthesized a gadolinium-gold composite nanoparticle and functionalized this nanoparticle for specific *in vitro* detection and imaging of EGFR using surface-enhanced Raman scattering. The functionalized Gd-Au nanoprobe successfully targeted EGFR molecules for SERS detection in three human cancer cell lines. In addition, changes in EGFR distribution at single cell level could be monitored *in situ*, demonstrating the potential of SERS to study cell activity under physiological conditions. SERS results were also compared with immunoblotting and immunofluorescence. The advantages of SERS in non-invasive and dynamic

detection was discussed. Our current work exhibited the capability of the Gd-Au nanoparticles as an effective imaging agent for SERS detection of cancer biomarker (EGFR) at (sub)cellular level. Together with previously reported MRI study [27], we demonstrated the potential of this Gd-Au nanocomposite as a noninvasive multifunctional nanoprobe not only for the *in vitro* early detection and localization of cancer biomarker, but also for the excellent MRI contrast agent for *in vivo* imaging tumor.

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FIGURE CAPTIONS

Fig. 1. (a) Schematic illustration of the Gd-Au nanocomposite. (b) HAADF-STEM image of the Gd-Au nanocomposite. (c) EDX elemental mapping of oxygen (O), silicon (Si), gadolinium (Gd) and gold (Au) within the area labeled with an orange square in (b). (d) Hydraulic diameter of the nanocomposite determined by DLS.

Fig. 2. (a) Schematic of the functionalization of Gd-Au nanocomposite. (b) Typical normalized SERS spectra ($600\sim 1800\text{ cm}^{-1}$) and (c) average SERS intensity ($N=25$) at 1075 cm^{-1} of S18 cells under the treatments of PEG-NP, mAb-NP, and first free anti-EGFR molecules then mAb-NP (**mAb_Block**). Error bar represents standard error of mean (SEM). (d) Dark field images of S18 cells alone (CTRL), cells incubated with PEG-NP, and cells incubated with mAb-NP.

Fig. 3. (a) Bright field image of a single S18 cell with 1-h incubation with mAb-NP and the selected area for SERS mapping. Scale bar: $10\text{ }\mu\text{m}$. (b-d) SERS images of the selected area after 60, 90 and 120 min incubation, respectively. The color scale is generated using peak intensity at 1075 cm^{-1} . (e-g) Extracted SERS spectra at positions indicated by blue crosses in (b-d).

Fig. 4 Representative SERS images of single S18, A431 and A549 cells incubated with Gd-Au nanoprobe (mAb-NPs). The first row shows bright field image and the selected area, and second row shows the corresponding SERS images. The color scale is generated with peak intensity at 1075 cm^{-1} and is kept constant with Fig. 3.

Fig. 5. (a) Average peak intensity at 1075 cm^{-1} for S18, A431 and A549 cells with Gd-Au nanoprobe incubation. Data are collected from 50 spectra for each sample. $^{**}P<0.01$. (b) Western blot result showing EGFR expression levels in S18, A431 and A549 cells.

Fig. 6. Immunofluorescence images showing the EGFR expression in S18, A431 and A549 cells. Scale bar: $50\text{ }\mu\text{m}$.

Fig. 1

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