Near-Infrared-Activated Fluorescence Resonance Energy Transfer-Based Nanocomposite to Sense MMP2-Overexpressing Oral Cancer Cells

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ABSTRACT: The matrix metalloproteinases (MMPs) are well-known mediators that are activated in tumor progression. MMP2 is a kind of gelatinase in extracellular matrix remodeling and cancer metastasis processes. MMP2 secretion increased in many types of cancer diseases, and its abnormal expression is associated with a poor prognosis. We fabricated a nanocomposite that sensed MMP2 expression by a red and blue light change. This nanocomposite consisted of an upconversion nanoparticle (UCNP), MMP2-sensitive peptide, and CuInS2/ZnS quantum dot (CIS/ZnS QD). An UCNP is composed of NaYF4:Tm/Yb@NaYF4:Nd/Yb, which has multiple emissions at UV/blue-visible wavelengths under 808 nm laser excitation. The conjugated CIS/ZnS QD showed the red-visible fluorescence through the FRET process. The two fluorophores were connected by a MMP2-sensitive peptide to form a novel MMP2 biosensor, named UCNP@p-QD. UCNP@p-QD was highly biocompatible according to cell viability assay. The FRET-based biosensor was employed in the MMP2 determination in vitro and in vivo. Furthermore, it was administrated into the tumor-bearing mouse to check MMP2 expression. UCNP@p-QD could be a promising tool for biological study and biomedical application. In this study, we demonstrated that the CIS/ZnS QD improved the upconversion intensity through a near-infrared-induced FRET process. This nanocomposite has the advantage of light penetration, excellent biocompatibility, and high sensitivity to sense MMP2. The near-infrared-induced composites are a potential inspiration for use in biomedical applications.

INTRODUCTION

Cancer metastasis is one of the deadliest symptoms in cancer-related diseases. The extracellular matrix (ECM) remodeling occurs during cancer metastasis, which assists tumor growth and cancer cell dissemination.1-2 The matrix metalloproteinase-2 (MMP2) family plays the pivotal role in ECM remodeling, leading to cancer cell invasion.1 In general, the active MMP2 is responsible for degrading type IV collagen. However, in tumorigenesis, abnormal MMP2 expression is associated with cancer cell motility and angiogenesis.1-6 MMP2 overexpression significantly promoted the lymphatic metastasis in oral cancer patients.7 Administration of MMP2 microRNA could be an adjuvant to inhibit mouse glioma in radiotherapy.8 An MMP2-sensitive paclitaxel prodrug could maintain anticancer activity by increasing permeability, cancer cell targeting, and retention effect.9 A self-assembly nanodrug that conjugated an amphiphilic molecule and antitumor drug by an MMP2-sensitive peptide was suggested to have the potential in clinical translation to increase the biocompatibility and reduce the side effects of drug accumulation.10 A photosensitizer therapeutic agent was conjugated with MMP2-cleavable peptide and then modified with PEG to form a self-assembled and redox-responsive system for fluorescent monitoring and photodynamic therapy.11 The pterostilbene compound that blocked the MMP2-induced signal pathway suppressed the oral cancer cell invasion.12 The MMP2 antibody labeled with the Cy5 probe could be used in in vivo animal imaging.13 Thus, an MMP2-sensitive probe has potential in cancer metastasis theranostics.
Lanthanide-doped upconversion nanoparticles (UCNPs) have been extensively investigated according to its unique photochemistry.\textsuperscript{14} By NIR excitation, UCNP emits an antistoke type and split emissions at UV/visible spectra. Upconversion luminescence is triggered by sensitizer (Yb\textsuperscript{3+}) and emitters (Er\textsuperscript{3+} and Tm\textsuperscript{3+}) under 980 nm irradiation.\textsuperscript{15} Moreover, a core/shell UCNP emits upconversion luminescence by an 808 nm laser.\textsuperscript{16} After PEG coating, UCNP-based nanomaterial can catch protein for bioanalytical applications.\textsuperscript{17} The core/shell UCNP coated with iron and organic compounds shows a tumor-specific targeting ability.\textsuperscript{18} Yao et al. showed that an UCNP-assisted liposome drug delivery system could overcome drug resistance and release drug by a NIR trigger.\textsuperscript{19} A CdTeS QD has been shown to have potential for biosensor detection of MMP2 secretion in vitro and in vivo.\textsuperscript{20} The copper indium sulfide (CIS) QD is more biocompatible than other QDs.\textsuperscript{21} CIS QD is a 1−III−V ternary semiconductor.\textsuperscript{22} CIS QD has the dramatic advantages in tunable photoluminescence and high quantum yield. However, the hydrophobic property limited its applications. The cytotoxicity of CIS/ZnS QD with chitosan coating has been determined by long-term incubation with C. elegans.\textsuperscript{23} The silica-encapsulated CIS/ZnS QD remains an excellent optical property with hydrophilic ability.\textsuperscript{24}

Optical imaging is a considerable strategy in medical diagnostics because of its noninvasiveness, sensitivity, and convenience. However, the tissue penetration is an Achilles’ heel for the optical diagnostic tool. The penetration depth increased depending on increasing wavelength, except the 900−1000 nm.\textsuperscript{25} The penetration of laser light at 808 and 980 nm reached 3.4 and 2.2 cm, respectively.\textsuperscript{26} The decrease of penetration depth at 900−1000 nm was due to water absorption, suggesting that the 808 nm irradiation has potential in biomedical application.\textsuperscript{27} This study demonstrated a fluorescence resonance energy transfer-based (FRET) nanocomposite consisting of UCNP, MMP2-sensitive peptide, and CIS/ZnS QD, named UCNP@p-QD. The core/shell UCNP contributed NIR-induced upconversion energy to CIS/ZnS QD with red-visible emission. We showed that UCNP@p-QD was a biocompatible nanocomposite, and it responded to the MMP2 protein in oral cancer cells and the animal tumor model.

**RESULTS AND DISCUSSION**

In this study, we developed a FRET-based nanocomposite that detected MMP2 expression in vitro. This nanocomposite consists of UCNP and CIS/ZnS QDs, which were linked by a MMP2-sensitive peptide (Figure 1a). UCNP@p-QD has FRET-induced red fluorescence by 808 nm irradiation. This nanocomposite revealed the blue fluorescence in the presence of MMP2. At first, we fabricated a core/shell type NaYF\textsubscript{4}:Yb/Tm@NaYF\textsubscript{4}:Yb/Nd UCNP. The core was NaYF\textsubscript{4}:Yb\textsuperscript{3+}/Tm\textsuperscript{3+} that emitted multifluorescence under NIR irradiation. The data of the hexagonal β-NaYF\textsubscript{4} database (JCPDS 16-0334) were compared with NaYF\textsubscript{4}:Yb,Tm and NaYF\textsubscript{4}:Yb,Tm. The diffraction peaks were similar to those of the standard peaks (Figure 1b). UCNPs were uniform in aqueous solution of which the diameter was approximately 20 nm (Figure 1c). Doped with Yb\textsuperscript{3+} and Nd\textsuperscript{3+}, the core/shell type, which formed a hexagonal structure, was in response to 808 nm excitation. In order to graft the MMP2-sensitive peptide, UCNP was modified with primary amine on the SiO\textsubscript{2} layer. The SiO\textsubscript{2} absorption was revealed at 1100 cm\textsuperscript{-1} compared to ligand-free UCNP (Figure S1a, Supporting Information). The absorption of UCNP@p at the Si−O−Si (1100 cm\textsuperscript{-1}), N−C═O (1660 cm\textsuperscript{-1}), and C═O (1100−1350 cm\textsuperscript{-1}) corresponded with the standard curve of UCNP and peptide. The SiO\textsubscript{2}-coated UCNP showed the size of 50 nm ±1 nm (Figure 1d). The N-terminal part from UCNP embedded a cysteine residue, to which the QD conjugated through disulfide linkage.\textsuperscript{28} X-ray diffraction (XRD) of the CIS/ZnS QD showed the major peaks corresponding to the standard diffraction patterns of CIS crystal (JCPDS No.
85-1175) and ZnS crystal (JCPDS No. 77-2100) at 27°, 47°, and 55° (Figure S1b, Supporting Information). The CIS/ZnS QD is a stable and biocompatible nanoparticle that is appropriate for bioapplication. The distance between QD and UCNP was close to 10 nm that facilitated FRET between donor and acceptor. UCNP@p-QD was approximately 30 nm in size (Figure 1e). CIS/ZnS QD with a size of 3.5 nm ± 0.2 nm was surrounded around the UCNP@p (inset, Figure 1e). The EDS analysis indicated that QDs were bound tightly with UCNP (Figure S1c, Supporting Information), in which the elements of QDs, such as In, S, and Cu, were located next to the elemental cluster within Nd and Yb. Moreover, the elements of UCNP and CIS/ZnS QD were identified from the conjugated structure using elemental mapping (Figure 1f). According to the elemental distribution, the fluorine (F), yttrium (Y), and neodymium (Nd) elements of UCNP appeared in the core, while the indium (In) of CIS/ZnS QD was distributed in the periphery (Figure 1g).

UCNP showed an antistoke shift fluorescence under 808 nm laser irradiation (Figure 2a). The Nd³⁺ ion was a sensitizer, in which the electron was excited from the ground state to the excited state. Meanwhile, a nonradiative energy transfer to the excited level ³F₅/₂ of Yb³⁺. The excited state of Tm³⁺ has four energy relaxations at wavelengths of 340, 360, 450, and 475 nm. 340 and 360 nm were due to the electronic transition ¹I₆ → ³F₄ and ¹D₂ → ⁵H₆. The blue-visible light was contributed by transitions of ¹D₂ → ³F₄ and ¹G₄ → ⁵H₆. QD has a broad absorption below 500 nm and has red fluorescence emission at 625 nm (Figure 2b). QD showed an orange emission under UV irradiation (inset, Figure 2b). To develop not only high penetration of excitation source but also a red shift, the fluorophore is desired for clinical use. UCNP acted as a donor that was excited by 808 nm irradiation. The acceptor CIS/ZnS QD that was excited by the upconversion fluorescence was a red fluorophore. The FRET process was manifested by the fact that the fluorescence intensity of the UV and visible-light spectrum showed a gradual decrease depending on increasing QD concentration (Figure 2c). To delineate the FRET dynamic change, we focused on the wavelengths at 475 and 600 nm, to which UCNP and QD contributed, respectively (Figure S2a, Supporting Information). The red fluorescence revealed at 25 mg/mL of QD conjugation implied a threshold of FRET-induced red fluorescence. Furthermore, the zeta-potential analysis indicated that the positive charge of UCNP@p decreased by the increasing QDs (Figure S2b, Supporting Information). Since the CIS/ZnS QD was hydrophobic, it dissolved in organic solvent rather than in H₂O. We then modified the UCNP@p-QD synthesis process by PEGylation onto the surface of the QD. PEGylation was carried out by methyl-PEG₄-thiol and carboxy-PEG₁₂-thiol, which increased the hydrophilicity and minimized nonspecific binding (Figure S2c, Supporting Information). The 2853 and 2925 cm⁻¹ were from the methylene-stretching group of PEG. The emission of UCNP@p-QD with 808 nm irradiation is similar to QD under

![Figure 2](image-url)
UV excitation. The PEGylated UCNP@p-QD has orange fluorescence at wavelength of 550 nm (Figure 2d). Obviously, there is about a 100 nm blue shift after PEGylation. Park et al. suggested that cation exchange could promote a blue shift in emission.33 Ryu et al. suggested that the addition of surfactant could contribute to the blue shift.34 In our experimental result, the surface reconstruction of UCNP@p-QD led to an apparent blue-shift emission. The blue-shift mechanism should be further demonstrated.

We soaked 10 μg of UCNP@QD into 1 mL of PBS at different pH values of 2, 4, 7, 9, and 12, respectively (Figure S3a, Supporting Information). Dynamic light scattering (DLS) showed that the hydration radii between pH 4 and pH 9 were not obviously changed, implying that UCNP@p-QD was stable in biological solution. UCNP@p-QD (0.2 mg/mL) was incubated with different concentration of MMP2 (Figure S3b, Supporting Information). The 600 and 475 nm emissions delineated a FRET change that originated from QD and UCNP, respectively. The 600 and 475 nm emissions showed blue upconversion fluorescence at wavelength of 550 nm (Figure 2d). Obviously, there is about a 100 nm blue shift after PEGylation. Park et al. suggested that cation exchange could promote a blue shift in emission.33 Ryu et al. suggested that the addition of surfactant could contribute to the blue shift.34 In our experimental result, the surface reconstruction of UCNP@p-QD led to an apparent blue-shift emission. The blue-shift mechanism should be further demonstrated.

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The cell viability analysis showed that UCNP@p-QD could be biocompatible to oral cancer cells (Figure S4a, Supporting Information). The high dose of UCNP@p-QD reduced 5% viabilities of FADU and OEC-M1. This implied that 83 μg/mL of dosage was safe to FADU and OEC-M1 cells rather than 250 μg/mL. The 808 nm laser showed a little phototoxicity (Figure S4b, Supporting Information). Moreover, a high dose of UCNP@p-QD showed little toxicity to Cal27 cells (Figure S4c, Supporting Information). To our knowledge, CIS/ZnS QD was safe for C. elegans.35 The cover of the QD might reduce certain cytotoxicity from UCNP@p because the increasing UCNP@p revealed toxicity (Figure S4d, Supporting Information). Thus, UCNP@p-QD was biocompatible to cells.

The extracellular UCNP@p-QD showed red emission under 808 nm irradiation. After MMP2 digestion, the UCNP@p showed blue upconversion fluorescence (Figure 4a). To detect the MMP2 in the cell model, constituted MMP2-overexpressing cells (Cal27/MMP2) and their cognate cells (Cal27/VC) were cultured for UCNP@p-QD detection. Cal27/MMP2 overexpressed the MMP2 protein not only in cytoplasm but also in the culture medium.35 The FRET-induced red fluorescence showed in Cal27/VC but also in Cal27/MMP2 (Figure 4b). The UCNP@p-QD accumulated around Cal27/VC cells, indicating there was no MMP2 expression; oppositely, the FRET-induced fluorescence was significantly reduced in Cal27/MMP2. Moreover, UCNP@p-QD showed the same consequence in OEC-M1 and FADU cells (Figure S5, Supporting Information), which were MMP2-null and MMP2-overexpressing cells, respectively.35 This result indicated that UCNP@p-QD could be detected in extracellular MMP2. The effective penetration is usually an obstacle for scientists to develop the photodependent biosensors, so the high penetrating infrared has attracted the attention of investigators.

In order to take an in vivo image, we refined an optical imaging system to employ the upconversion luminescence detection (Figure S6a, Supporting Information). Under 808 nm irradiation, the FRET-induced fluorescence was detected on the...
separated dorsal side within 1 h (Figure 4c; column 1H). Distribution of UCNP@p-QD was visualized uniformly in tumor and its adjacent part. Additionally, the RFP-harbor Cal27/VC cell could be specifically examined on the left dorsal side (Figure S6b, Supporting Information). The FRET-induced fluorescence significantly decreased over time in Cal27/MMP2-induced tumors (1H to 48H). Finally, the FRET-induced fluorescence almost disappeared from the Cal27/MMP2-induced tumor part (Figure 4c; column 48H). Although there is a little FRET-induced fluorescence from Cal27/MMP2-induced tumor (Figure 4c; NO.3), most UCNP@p-QDs were significantly degraded by MMP2. The harvested tumors showed the similar size and pathological feature between Cal27/VC- and Cal27/MMP2-induced tumors (Figures S6c and S6d, Supporting Information). Because the blue-visible filter is not equipped in an in vivo imaging system, the tumor sections were examined using a multiphoton microscope (Figure 4d). The UCNP@p-QDs were shown ubiquitously in the Cal27/VC tumor tissue. Oppositely, the upconversion fluorescence was detected in the Cal27/MMP2 tumor. Hence, UCNP@p-QD detected not only rhMMP2 protein in vitro but also MMP2 expression in the cell model and animal tumor imaging.

**CONCLUSIONS**

In summary, we fabricated a FRET-based nanocomposite comprising an 808 nm-induced upconversion nanoparticle, the core/shell type of CIS/ZnS quantum dot, and the MMP2-sensitive peptide, named UCNP@p-QD. Upconversion fluorescence from UCNP was excited by 808 nm laser irradiation which is known for low phototoxicity and high penetration. Consequently, the upconversion fluorescence transferred to FRET-induced fluorescence though an antistokes shift and then a stokes shift process. This improved the poor upconversion fluorescence of UCNP by using CIS/ZnS QDs. The in vitro experiment characterized that UCNP@p-QD was susceptible to rhMMP2 proteinase with high sensitivity and selectivity. Because the FRET-based UCNP@p-QD was biocompatible in cell viability assay, we employed UCNP@p-QD in determining MMP2 expression in the cell model. Furthermore, the
biosensor was used to monitor the MMP2-induced tumor in an in vivo mouse tumor model. The FRET-based nanocomposite would be worthy to further investigate by replacing other specific enzyme-sensitive peptides in clinical diagnostics.

## MATERIALS AND METHODS

**Chemicals and Media.** All chemicals were purchased from commercial suppliers without further purification. Y- (CH3CO2)3·H2O, Yb(CH3CO2)3·4H2O, Tm(CH3CO2)3·H2O, Nd(CH3CO2)3·H2O, octadecene (ODE, 90%), oleic acid (OA, 90%), ammonium fluoride (NH4F), sodium hydroxide (NaOH, 98%), hydrochloric acid (HCl, 37%), ethanol (C2H5OH, 99%), urea powder (NH2CONH2, 98%), 3-triethoxysilylpropylamine (APTE, 98%), folic acid 1-dodecanethiol (DDT; ≥98%), N-hydroxysuccinimide (NHS), ethyl(dimethylaminopropyl) carbodiimide (EDC), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. Trisodium citrate (Na3C6H5O7·H2O, 99%), was purchased from Hopax Chemicals (Taiwan Hopax Chemicals. Mfg. Co., Ltd.). Cyclohexane (C6H12, 99%) was purchased from J. T. Baker. Poly(ethylene glycol) monomethyl ether thiol (methyl-PEG-tiol) and poly(ethylene glycol) carboxyl ether thiol (carboxy-PEG12-thiol) were purchased from Thermo scientific. DMEM medium, MEM medium, and RPMI medium were purchased from Invitrogen. Human recombinant MMP2 (rhMMP2) protein was purchased from Biotools (Taiwan). CIS/ZnS QD was purchased from Hopax Chemicals (Taiwan Hopax Chemicals. Mfg. Co., Ltd.).

### Preparation of NaYF4:Yb/Tm@NaYF4:Yb/Nd. The core nanoparticles, NaYF4:Yb/Tm, were synthesized by the coprecipitation method under high-temperature reaction. Amounts of 0.4 mL of Tm(CH3CO2)3·H2O, Yb(CH3CO2)3·4H2O, and Y- (CH3CO2)3·H2O were dissolved in a mixture of OA (6 mL) and ODE (14 mL) at 120 °C for 30 min. The dissolved salts were removed by cooling to room temperature, and methanol solution with NH4F (0.15 g) and NaOH (0.1 g) was added with vigorous stirring. The reaction mixture was heated to 290 °C for 2 h and was returned to room temperature. Nanoparticle precipitates were washed with cyclohexane by centrifuging at 5000 rpm for 5 min and were resuspended with methanol. The shell precursors were synthetized by Nd(CH3CO2)3 (0.48 mmol), Yb(CH3CO2)3 (0.48 mmol), and Y(CH3CO2)3 (1.28 mmol) as well as the process of NaYF4:Yb/Tm synthesis. The core nanoparticles were embedded in NaYF4:Yb/Nd nanoparticles in methanol with NH4F (0.15 g) and NaOH (0.1 g). The reaction was performed at 290 °C for 2 h, and the core–shell upconversion nanoparticles were obtained in cyclohexane after ethanol washing.

### Preparation of UCNP@p-QD. The core–shell type of UCNP@p-QD was synthesized using the modified coprecipitation method. The UCNP@p-QD were then coated with a silica shed and surface modified with an amino group. The MMP2-sensitive peptide was conjugated to UCNP through the amino group. The core/shell quantum dot that consists of CuInS2 (core) and ZnS (shell) was prepared by a modified thermal method. The UCNP@p was conjugated with the CIS/ZnS QD in DMSO/chloroform solution (1:5) for 24 h conjugation because of disulfide bonding. The folic acid (10 nM) was further conjugated onto UCNP@p-QD using the NHS/EDC method. The upconversion nanoparticles were coated with silica shed using a modified microemulsion method. The water-soluble NaYF4:Yb/Tm@NaYF4:Yb/Nd particles were reconstituted by alcohol-diluted HCl washing that removed the OA and ODE ligands and were dissolved in the mixture of IGEPAL CO-520 (1 mL) and cyclohexane (10 mL) with vigorous stirring for 4 h. Subsequently, the pH value was adjusted by NH4OH (0.1 mL) solution, and the reaction was stirred for an additional hour. Finally, the tetraethyl orthosilicate (TEOS, 98%, 30 μL) was slowly added into the reaction mixture by the speed of 40 μL per hour for further 24 h stirring. The silica-coated nanoparticles were modified with an amino group by adding APTES (0.1 mL). The nanoparticles were collected by methanol precipitation using centrifuging at 8000 rpm for 20 min. The MMP2-sensitive peptide with a sequence of CSGAVRWLLTA was activated for 2 h by a mass proportion of peptide, NHS, and EDC of 5:1:5:1, respectively, in DMSO. Active peptides were grafted onto the amino group of silica-coated nanoparticles by the NHS/EDC method to form the peptide-grafted upconversion nanoparticle (UCNP@p). The UCNP@p was dissolved in DMSO. To manifest the FRET process, UCNP@p (0.5 mg/mL) was conjugated with a different concentration of QD in DMSO/chloroform solution (5:1).

### Surface Modification of UCNP@p-QD. CIS QD was synthesized using the DDT, on which the ZnS was coated to form a core/shell type nanoparticle. Since the DDT was hydrophobic, the CIS/ZnS QDs were dissolved in chloroform before use. UCNP@p-QD (100 ng) was PEGylated using methyl-PEG-tiol (100 ng) and carboxy-PEG12-thiol (500 ng) in DMSO/chloroform solution (1:10) for 30 min. The UCNP@p-QD was then washed by chloroform DMSO washing and was dissolved in ddH2O. The photoluminescence of nanoparticles was detected by Fluoromax 3. The morphology was determined using the JEM-2100F (TEM, Japan) and JEOL transmission electron microscope (HRTEM, Japan). The electron gun of TEM and HRTEM worked at 80 and 200 keV, respectively. Zeta potential of conjugated nanocomposite was characterized at room temperature (RT) by a Malvern Zetasizer 3000.

### Cell Culture.** Oral cancer cell lines were cultured in a growth medium with 10% FBS (Invitrogen, USA) and 1% penicillin–streptomycin–glutamine. The cell lines were cultured according to ATCC instruction. The cells were incubated in a CO2 incubator containing 5% CO2 at 37 °C. Cal27 and FADU were purchased from the ATCC cell line bank. The control vector (VC), pLAS3w.RFP-C.Ppuro, was purchased from RNAi core facility (Academia Sinica, Taiwan). Cal27/ MMP2 overexpressed MMP2 due to the lentiviral infection, at which the viruses were prepared by pLAS3w:MMP2.puro. Cal27/VC was introduced to the similar lentivirus DNA backbone that replaced the MMP2 reading frame with the frame of the red fluorescent protein (pLAS3w.RFP-C.Ppuro). The opposite Cal27/VC stable cell line expressed the RFP fluorescent protein (pLAS3w.RFP-C.Ppuro).

### Cytoxicity Assay of UCNP@p-QD. The stable cell lines of Cal27/VC and Cal27/MMP2 were performed in MMP2 sensing. Cytotoxicity was evaluated by an alamar-blue assay using SpectraMax M2. An aliquot of 2000 cells was added in a 96-well plate. An aliquot of UCNP@p-QD was added with serial dilution (250, 83, 27, 9, 3, and 1 μg/mL) for 72 h of incubation. The cytotoxic data were assessed from six independent tests with the standard deviation.

### In Vitro and in Vivo MMP2 Detection.** To perform the sensing ability of UCNP@p-QD, rhMMP2 was employed in...
dose- and time-dependent verification at 37 °C incubation. The aliquot of rhMMP2 was added in 0.2 mg/mL of UCNP. In dose-dependent experiment, the rhMMP2 was serially diluted with 1, 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, and 10⁻⁶ pg/mL. The 10⁻³ pg/mL of rhMMP2 was employed in a time-lapse FRET change. The time course of the FRET change was carried out with 0.2 mg/mL of UCNP@p-QD with 1 pg/mL of rhMMP2. To depict the FRET change, we focused on the fluorescence emission at 475 and 600 nm, which were donated from UCNP and UCNP@p-QD, respectively. The data of three individual tests showed the means with standard derivation.

To explore in vivo MMP2 sensitivity of UCNP@p-QD, an MMP2-overexpressing stable cell line was employed compared to its cognate cell. Aliquot 20000 cells were seeded onto the coverslips for overnight incubation, and the UCNP@p-QD (10 µg/mL) was added for a further 12 h incubation. The cell and tissue images were obtained using a Leica TCS SPS confocal microscope with multiphoton laser supplement.

**Detection of MMP2 in the Mouse Xenograft Tumor Model.** The animal experiments was approved by Academia Sinica Institutional Animal Care and Utilization Committee. The 5 × 10⁶ aliquot of Cal27/VC and Cal27/MMP2 cells was subcutaneously injected on both opposite dorsal sides of the NSG mouse at 6-weeks old. After 4 weeks of tumor growth (62.5 mm³ of tumor volume), UCNP@p-QD (20 mg/mL) was administrated on both tumors by intratumoral injection. The real-time images were obtained using In-Vivo Xtreme (Bruker, Germany), with an additional 808 nm infrared diode laser module (1.50 W). The RFP acquisition was detected at 600 nm using 540 nm excitation. Xenograft tumors were harvested using a formalin-fixed and paraffin-embedded method. The tumor section was stained with propidium iodide (1 µg/mL) according to instruction. The images were obtained by confocal microscopy.

**ASSOCIATED CONTENT**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01494.

The characterizations of UCNP@p, UCNP@p-QD, conjugation of UCNP@p and Cis/ZnS QD, and FRET process are shown. The cell sensing and animal study are also disclosed (PDF)

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**Author Contributions**
Yung-Chieh Chan and Michael Hsiao designed and performed the study and cowrote the manuscript. Chieh-Wei Chen, Ming-Hsien Chan, Ru-Shi Liu, and Din Ping Tsai provided technical support and materials. Michael Hsiao and Din Ping Tsai discussed the data and developed the theoretical aspect and wrote the manuscript. All authors commented on the manuscript.

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The authors acknowledge financial support from Ministry of Science and Technology, Taiwan (Grant Nos. MOST 106-2745-M-002-003-ASP and MOST 104-2113-M-002-012-MY3) and Academia Sinica (Grant No. AS-103-TP-A06). They are also grateful to National Center for Theoretical Sciences, NEMS Research Center of National Taiwan University, National Center for High-Performance Computing, Taiwan, and Research Center for Applied Sciences, Academia Sinica, Taiwan, for their support. This research was also supported by Academia Sinica and Ministry of Science and Technology (MOST 106-0210-15-02, MOST 107-0210-19-01).

**ABBREVIATIONS**

MMP, matrix metalloproteinase; UCNP, upconversion nanoparticle; CIS/ZnS QD, CunS₅/ZnS quantum dot; ECM, extracellular matrix; FRET, fluorescence resonance energy transfer; TEM, transmission electron microscope; EDS, energy-dispersive spectrometer; HNSCC, head-and-neck squamous-cell carcinoma

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