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Supporting Information

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Supporting Information

A Near-Infrared Fluorescence Quenched Gold Nanoparticle Imaging Probe for Drug Screening and Protease Activity In Vivo

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1. Preparation of Cy5.5-labeled MMP substrate

Cy5.5 mono OSu: Cy5.5 mono-free acid (100 mg, 0.14 mmol) was dissolved in anhydrous methyl sulfoxide (DMSO) and N,N-dimethylformamide (DMF) (50:50, 7 ml), and di-isopropylethylamine (DIPEA) (75 μl) was added. O-(N-succinimidyl)-N,N,N'N'-bis(tetramethylene)uronium hexafluorophosphate (HSPyU, 91mg, 0.22 mmol) was added to the reaction mixture and stirred at room temperature in the dark for 30 min. After 30 minutes, samples were diluted with 0.1M acetic acid and inject immediately onto the conditioned analytical column. Separate the dye components using gradient elution: 20% to 40% acetonitrile vs. 0.1M triethylammonium acetate (TEAA) in water over 20 minutes at a flow rate of 1.0 mL/min. After complete activation, the Cy5.5 ester was precipitated by adding anhydrous ethyl acetate. The precipitate was washed with ethyl acetate and dried in vacuum. Purity (> 95%) was confirmed by analytical reversed-phase HPLC.

Cy5.5-labeled MMP substrate: Cy5.5 mono OSu was reacted with purified MMP substrate, Gly-Pro-Leu-Gly-Val-Arg-Gly-Cys, containing glycine and glycine-cysteine at both ends for conjugation. Cy5.5 mono OSu (5 mg, 4.8 μmol) was coupled to the N-terminus of the peptide (5 mg, 6.6 μmol) in anhydrous DMF (5 mL) containing DIEA at room temperature in the dark with shaking for 2 hours. The final Cy5.5-Gly-Pro-Leu-Gly-Val-Arg-Gly-Cys(amide) product as purified by semi-preparative reversed-phase HPLC; 20% to 60% acetonitrile vs. 0.1M TEAA in water over 30 minutes at a flow rate of 5.0 mL/min. Purity (> 95%) was confirmed by analytical reversed-phase HPLC. Mass: calculated/found, 1653.4/1655.5

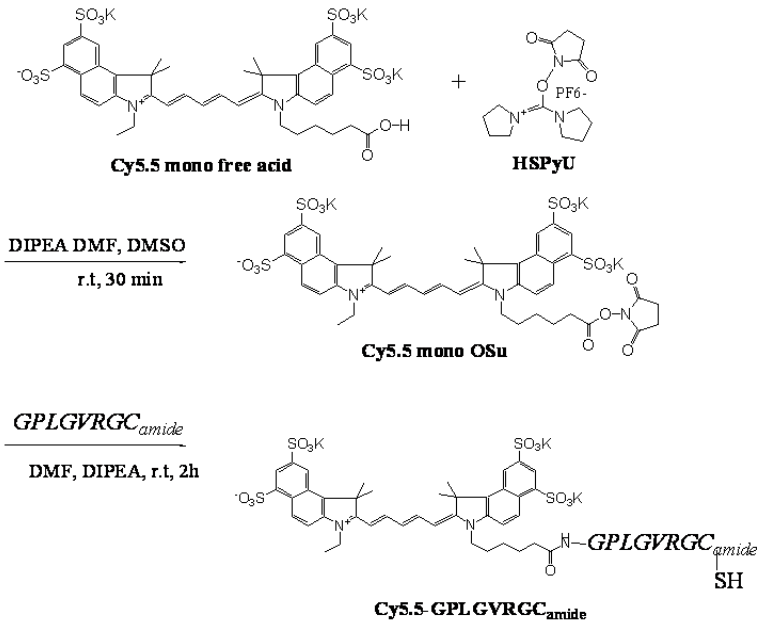

Figure S1. Synthesis of Cy5.5 mono OSu and Cy5.5-GPLGVRGC$_{amide}$

2. Preparation of AuNP probe

Functionalized AuNPs were prepared according to previous protocols [1]. Cy 5.5-substrate was co-incubated with AuNPs in 0.01 M phosphate buffer containing 0.1% SDS and 0.3 M NaCl, gently shaken for 24 h, and purified by repeated washing and centrifugation (5 times for 20 min at 13000 rpm). Finally, the AuNP probe was resuspended in PBS (10 mM, pH 7.4, 0.02% PEG), and stored at 4 °C until use

[1] Stoeva, S.I., Lee, J.S., Smith, J.E., Rosen, S.T. & Mirkin, C.A. Multiplexed detection of protein cancer markers with biobarcoded nanoparticle probes. *J Am Chem Soc* **128**, 8378-8379 (2006).

3. Characterization

TEM was performed using a JEOL 200CX microscope operating at 200 kV. Fluorescence signal intensity was detected with a Shimadzu FL-600 spectrofluorometer. The excitation wavelength was fixed at 675 nm and emission spectra recorded from 680 to 800 nm at 37°C using a 1 ml cuvette. *In vitro* optical NIRF imaging was performed using a Kodak Image Station 4000MM Digital Imaging System (Kodak, New Haven, CT) consisting of a light-tight box equipped with a 150 W halogen lamp and an excitation filter system for Cy5.5 (Omega optical, Battleboro, VT).

Enzyme specificity of the MMP-sensitive AuNP probe was examined in a 1 ml cuvette by co-incubating 27 nM AuNP probe with the appropriate concentrations of activated MMP-2, MMP-3, MMP-7, MMP-13 and MMP-2 inhibitors (1 mmol/L, 1,10 phenanthrolone). For this purpose, inactive MMPs were activated by incubation with 2.5 mmol/L of p-aminophenyl mercuric acid in TCNB buffer (100 mM Tris, 5 mM calcium chloride, 200 mM NaCl and 0.1% of Brij) for 1 h at 37°C, prior to use.

4. Biocompatibility of AuNP

Human hepatic cells (HepG2, ATCC, Rockville, MD) were cultured in MEM medium containing 10% fetal bovine serum, sodium pyrubate, penicillin, streptomycin and non-essential amino acids. Cells were seeded in a 96-well plate at a concentration of 5,000 cells/well, and incubated with various concentrations of the AuNP probe for 20 h. Next, cells were washed with media, and incubated for 2 h, followed by the MTT assay. Absorbance was recorded at 570 nm using a microplate reader, and normalized relative to the control.

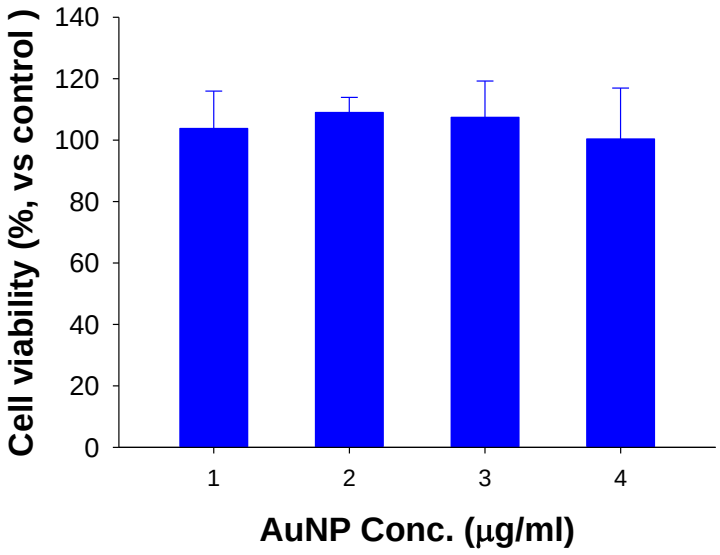

Figure S2. Cytotoxicity of MMP-sensitive AuNP estimated with the MTT assay.

5. Differential MMP-2 expression in HT1010 and SCC7 cells

MMP-2 quantification was performed by gelatin zymography. Active MMP-2 had a molecular weight of 61.5 kDa. Briefly, HT1080 and SCC7 cell-conditioned media were used to detect MMP-2 activity. Gels were incubated overnight, stained with Coomassie blue, and destained with a methanol-acetic acid solution until gelatinolytic areas appeared evident as unstained bands in a blue stained gel. Gels were acquired and processed as described previously [2].

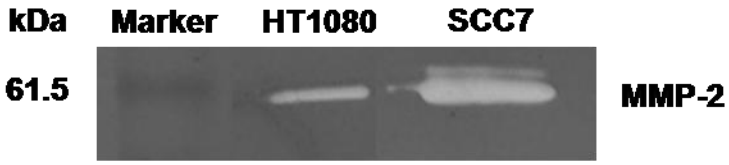

Figure S3. Cytotoxicity of MMP-2 Sensitive Gold NP.

[2] Brown, P.D., Levy, A.T., Margulies, I.M., Liotta, L.A., Stetler-Stevenson, W.G. Independent expression and cellular processing of Mr 72,000 type IV collagenase and interstitial collagenase in human tumorigenic cell lines. *Cancer Res* **50**, 6184- 6191 (1990)

6. In vivo optical imaging

SCC7 cells (1×10^6 cells/mouse) cultured in RPMI 1640 medium containing 10% fetal bovine serum were injected subcutaneously into the backs of athymic nude mice (nu/nu, 5-6 weeks old). When tumors had grown to 5 mm in size, the AuNP probe was intratumorally injected. In the MMP-2 inhibition experiment, an MMP-2 blocker was intratumorally administered into the SCC7 tumors, 30 min prior to injection of the AuNP probe. As a negative control, the AuNP probe was subcutaneously injected into the same site of normal athymic nude mice. NIRF tomographic images were obtained with an eXplore Optix system (ART Advanced Research Technologies Inc., Montreal, Canada). The imaging system uses a time-correlated single photon counting (TCSPC) technique to measure fluorescence and absorption. This technique allows the distinct separation of light scattering and light absorption in tissue, and provides accurate fluorescent lifetime imaging (FLI)-based 2D and 3D images. After intratumoral injection of MMP-sensitive AuNP, the fluorescence recovery profiles in SCC7 tumor-bearing mice were imaged by positioning mice on an animal plate heated to 36℃ in the eXplore Optix system [3]. The animal was automatically moved to the imaging chamber for scanning. Laser power and count time settings were optimized at 30 μW and 0.3 s per point, respectively. Excitation and emission spots were raster-scanned in 1 mm steps over the selected region of interest to generate emission wavelength scans. A 670 nm pulsed laser diode was applied to excite Cy5.5 molecules. NIR fluorescence emission at 700 nm was collected and detected with a fast photomultiplier tube (Hamamatsu, Japan) and time-correlated single photon counting system (Becker and Hickl GmbH, Berlin, Germany). To establish the time-dependent fluorescence recovery profile, the total NIRF intensity per selected region in the whole body was calculated as a function of time. Tumor contrast was measured by dividing the fluorescence intensities at the tumor area (*T*) and normal tissue area (*N*). All data, including whole body and 2D slice images, were calculated using the region of interest (ROS) function of Analysis Workstation software (ART Advanced Research Technologies Inc., Montreal, Canada). Values are presented as means ± SE calculated for groups of five animals.

[3] Park, K., Kim, J-H., Nam Y.S., Lee, S., Nam Y.H., Kim, K., Park, J.H., Kim, I-S., Choi, K., Kim, S.Y. & Kwon, I.C. Effect of polymer molecular weight on the tumor targeting characteristics of self-assembled glycol chitosan nanoparticles, *J Control Rel* **122**, 305-314 (2007)

7. Histological analysis

Excised tumor specimens were fixed in 10% phosphate-buffered formalin for 24 h. Specimens were embedded in paraffin, sectioned into 4-6 μm slices, and stained with hematoxylin-eosin. MMP-2 immunostaining was performed on tissue sections using the avidin-biotin peroxidase complex (ABC) technique with a primary monoclonal antibody against MMP-2. For NIRF imaging, tumor sections were viewed by fluorescence microscopy. Excitation and emission wavelengths were 650 and 700 nm, respectively.