Supplementary Information

for

In Vivo Mapping and Assay of Matrix Metalloproteases for the Liver Tumor Diagnosis

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Materials and Methods

Preparation process and optimization of GNP-p-FITC conjugate

Preparation of GNPs. GNPs were synthesized in a single-phase system by filtering sub-boiling water through a microporous membrane with an aperture of 0.22 μm. All glassware was cleaned in a bath of freshly prepared aqua regia and rinsed thoroughly with deionized water prior to use. $HAuCl_4 \cdot 3H_2O$ (0.615 mL, 0.02 g/mL) and solid sodium citrate (50 mg, 1.3 mmol) were dissolved in 50 mL deionized H_2O. A freshly prepared and cooled aqueous solution of sodium borohydride (25

mL, 0.04 mg/mL) was added to the reaction solution, stirring for 30 min, the resulting solution appeared as a claret red colloidal dispersion of gold. The concentration of the GNPs stock solution was 1.176×10^{-4} g/mL (The concentration of GNPs was expressed in the concentration of gold.).

Optimization of GNP-p-FITC conjugate

The amount of p-FITC is constant. Various volumes of GNPs stock solution were added to different samples of p-FITC stock solution (1 μg/mL), to a volume of 180 μL. After the solutions were completely mixed, deionized H_2O was added to make the total volume 3 mL. The concentration of p-FITC was fixed at 60 ng/mL. The final concentrations of GNPs were in the range from 3.92×10^{-5} to 98.0 μg/mL. The mixtures were stirred for 30 min in darkness and the fluorescence intensity of the samples, at room temperature, were measured at $\lambda_{ex/em}$ = 494/518 nm.

The amount of GNPs is constant. Various volumes of p-FITC stock solution were added to different samples of GNPs stock solution, to a volume of 1 mL. After the solutions were completely mixed, deionized H_2O was added to make the total volume 3 mL. The concentration of GNPs was fixed at 39.20 μg/mL. The final concentrations of p-FITC were in the range from 1 to 90 ng/mL. The mixtures were stirred for 30 min in the dark and the fluorescence intensity of the samples, at room temperature, were measured at $\lambda_{ex/em}$ = 494/518 nm.

The ratio of GNPs: p-FITC is constant. Samples of different concentration of GNPs (27.44 ~ 50.96 μg/mL) were mixed with different concentration of p-FITC (35 ~ 65 ng/mL), with a fixed optimal ratio of GNPs to p-FITC (39.20 μg/mL: 50 ng/mL). Deionized H_2O was added to make the total volume 3 mL. The mixtures were stirred for 30 min in the dark and the fluorescence intensity of the samples, at room temperature, were measured at $\lambda_{ex/em}$ = 494/518 nm.

Enzymatic hydrolysis experiments

Enzymatic reaction concentration. Different samples of GNP-p-FITC/mPEG conjugate containing p-FITC 10 ng were redispersed into 3 mL PBS (0.03 M, pH=7.4). One was for direct fluorescence measurement, while the other six samples had different amounts of collagenase IV added. The final concentrations of collagenase IV were from 0 to 5.6 mg/mL. The fluorescence intensity with a fixed reaction time of 15 min, while in incubation at 37 ^{o}C, was measured.

Enzymatic reaction time. GNP-p-FITC/mPEG conjugate containing p-FITC 10 ng was redispersed into 3 mL of PBS (0.03 M, pH=7.4), followed by the addition of 7.2 mg collagenase IV. The final concentration of collagenase IV was 2.4 mg/mL. The mixture was incubated at 37 ^{o}C. The fluorescence intensity as a function of time (from 0 to 60 mins) was monitored.

Fluorescence recovery by collagenase IV versus GSH. GNP-p-FITC/mPEG conjugate samples containing p-FITC 10 ng were redispersed into 3 mL of PBS (0.03 M, pH=7.4) containing 2 μM GSH, 10 mM GSH, and 2.4 mg/mL collagenase IV, respectively. The mixture was incubated at 37 ^{o}C. The fluorescence intensity as a function of time was monitored.

Additional results and discussion:

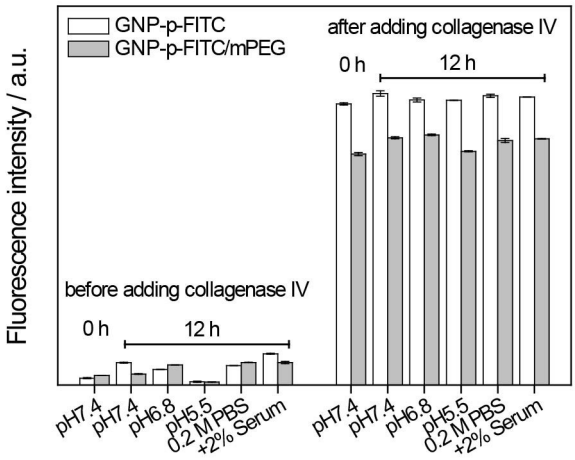

Figure S1. Stability and fluorescence recovery of GNP-p-FITC and GNP-p-FITC/mPEG conjugates after the incubation for 12 h at 37^{o}C in the following environments: 0.03 M PBS at pH 7.4, pH 6.8, pH 5.5, 0.2 M PBS at pH 7.4, and 0.03 M PBS at pH 7.4 with 2% serum. The result was compared to the freshly prepared GNP-p-FITC and GNP-p-FITC/mPEG conjugates solution in 0.03 M PBS at pH 7.4. The concentration of collagenase IV was 2.4 mg/mL and the fluorescence recovery intensity was detected after the addition of collagenase IV for 5 min.