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Dextran based pH-sensitive near-infrared nanoprobe for *in vivo* differential-absorption dual-wavelength photoacoutic imaging of tumor

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Supporting Information

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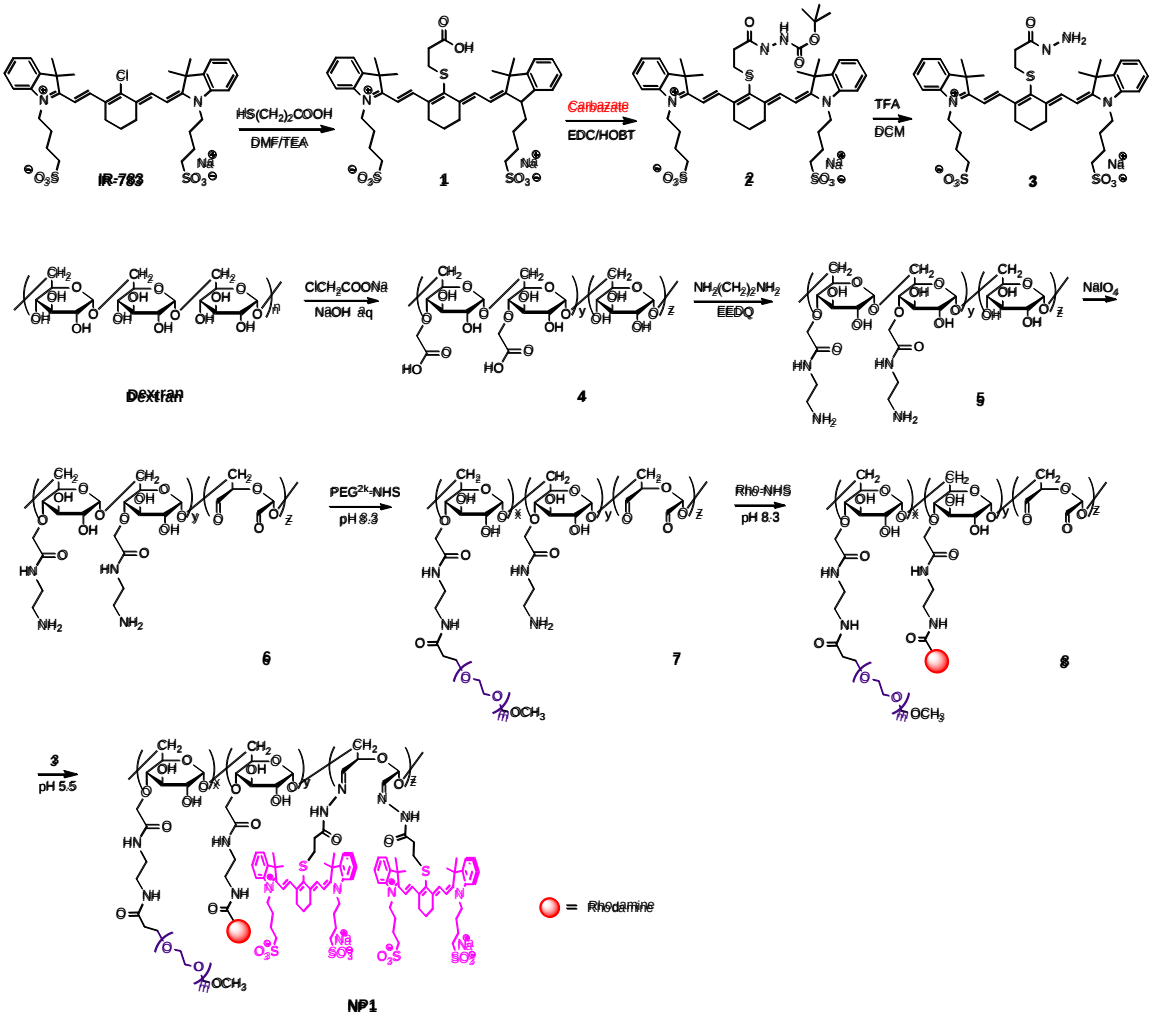
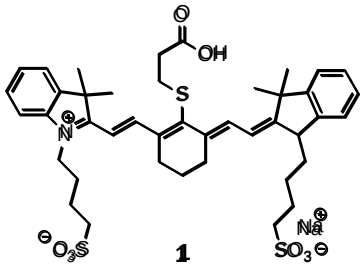
Detailed Synthesis of NP1:

Nanoprobe **NP1** was prepared according to below synthetic procedure.



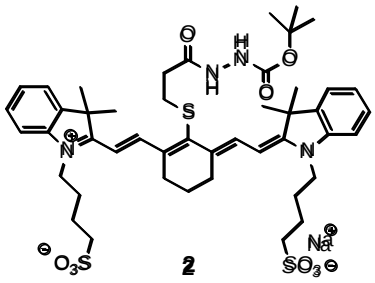
Scheme S1 The detailed synthesis procedure of **NP1**.

Preparation of compound **1.** To a solution of IR783 (100 mg, 1.34×10^{-4} mol) in 2 mL anhydrous DMF was added 3-mercaptopropionic acid (14 μL, 17 mg, 1.6×10^{-4} mol, 1.2 equiv.) and triethylamine (22 μL, 16 mg, 1.6×10^{-4} mol). The green color solution was allowed to stir in the dark at r.t. for 15 h. The crude product was precipitated by addition of excess diethyl ether



and further purified through gravity chromatography (silica gel, $CH_3CN:H_2O$ = 100:10, V:V). The purified product dissolved in water was lyophilized as a deep green powder (yield: 82%, 1.1×10^{-4} mol, 90 mg). ^{1}H NMR (400 MHz, CD_3OD): δ 8.70 (d, 2H, *J* = 14.0 Hz), 7.33 (d, 2H, *J* = 7.6 Hz), 7.27-7.23 (t, 2H, *J* = 7.2 Hz), 7.19 (d, 2H, *J* = 8.0 Hz), 7.11-7.07 (t, 2H, *J* = 7.2 Hz), 6.17 (d, 2H, *J* = 13.6 Hz), 4.05-4.02 (t, 4H, *J* = 6.4 Hz), 2.92-2.88 (t, 2H, *J* = 6.8 Hz), 2.77-2.73 (t, 4H, *J* = 6.8 Hz), 2.55-2.52 (t, 4H, *J* = 6.4 Hz), 2.44-2.41 (t, 2H, *J* = 7.2 Hz), 1.83-1.74 (m, 10H), 1.58 (s, 12H); ^{13}C NMR (100 MHz, CD_3OD): 175.68 (C), 173.88 (2 × C), 157.91 (C), 147.23 (2 × C), 143.81 (2 × CH), 142.52 (2 × CH), 135.14 (2 × CH), 129.99 (2 × C), 126.32 (2 × C), 123.57 (2 × CH), 112.24 (2 × CH), 102.46 (2 × CH), 51.95 (2 × CH_2), 50.57 (2 × CH_2), 47.87 (2 × C), 36.19 (CH_2), 34.20 (CH_2), 28.54 (4 × CH_3), 27.40 (2 × CH_2), 27.34 (2 × CH_2), 23.72 (2 × CH_2), 22.30 (CH_2); HRFAB-MS: $C_{41}H_{51}O_8N_2S_3Na$ $[M+H]^+$, found 819.2794 (44.7%), calculated 819.2784.

Preparation of compound **2**. Compound **1** (90 mg, 1.1×10^{-4} mol) was dissolved in dry DMF (0.5 mL) and cooled to 0 °C. EDC (25.3 mg，1.32×10^{-4} mol), and HOBt（17.84 mg，1.32×10^{-4} mol） were added to above solution as condensation agents. Tert-butyl carbazate （17.5 mg，1.32×10^{-4} mol） was added and left to stir for 9 hours. The crude product was precipitated by addition of excess diethyl ether and purified through gravity chromatography (silica gel, $CH_2Cl_2:CH_3OH$ = 100:15~100:40 (V:V). The purified product dissolved in water was lyophilized as a deep green powder (yield: 70%, 67.5 mg). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.89 (d, J=14.2, 2H), 7.49 (d, J=7.4, 2H), 7.41 (t, J=7.3, 2H), 7.37 – 7.28 (m, 2H), 7.22 (dd, J=33.6, 26.3, 2H), 6.34 (d, J=14.2, 2H), 4.20 (t, J=6.9, 4H), 3.14 – 2.97 (m, 2H), 2.88 (dd, J=15.9, 9.0, 4H), 2.71 (s, 4H), 2.53 (t, J=7.5, 2H), 2.03 – 1.90 (m, 10H), 1.81 – 1.71 (m, 12H), 1.47 – 1.36 (m, 9H). 13C NMR (101 MHz, MeOD) δ 172.585（C）, 171.355（C）, 159.653（C）, 156.178（C）, 156.178（2×C），145.863（2×CH）,142.365(C),141.083(2×CH),140.68 （2×CH）,133.771(2×C),128.451(2×C),124.805(2×CH) ,122.025(2×CH), 110.728(2×CH2), 100.994(2×CH2),50.433(2×CH2), 49.143(C), 43.548(2×C),33.846(CH2), 32.402（CH2）, 27.227(3×CH3), 27.128(4×CH3), 27.015(2×CH2), 25.898(2×CH2), 22.208(2



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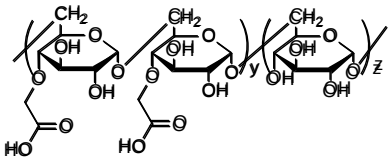
×CH2), 20.804(CH2). FAB-MS: $C_{46}H_6O_9N_4S_3Na$ $[M+Na]^+$, found 955.3, calculated 955.3.

Preparation of compound **3**. Compound **2 (**33.7 mg, 0.39×10^{-4} mol**)** was dissolved in a mixture of DCM and TFA (1:1, V:V) and stirred at room temperature for 1 h. The solvent was removed under a vaccum and the residue was redissolved in distilled water. This product was used directly in the next step without furher purification. FAB-MS: $C_{41}H_{53}O_7N_4S_3Na$ $[M-Na+CF_3COOH]^+$, found 925.8, calculated 925.4.

Preparation of compound **4**. Dextran (20 kDa, 400 mg, 2.0×10^{-5} mol) was dissolved in 8.25 mL of



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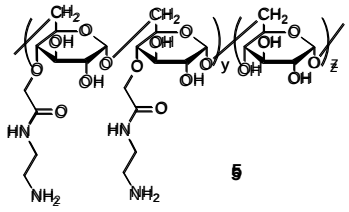
6 M NaOH that was pre-cooled by an ice bath. Then 816 mg (7.0×10^{-3} mol) chloroacetic acid sodium salt was added. Above mixture was stirred at 60 ^oC for 50 min and then cooled to room temperature. Then the polymer was precipitated by adding a large amount of ice-cold methanol. The product was filtered and dried under a vacuum to obtain a white color powder. (yield: 97%, 1.90×10^{-5} mol, 400 mg, MW is considered as 21 kDa).

Preparation of compound **5**. 0.4 g compound **4** (3.1×10^{-4} mol) was dissolved in 80 mL of water and



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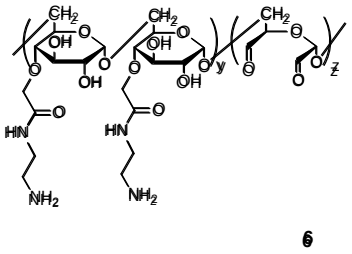
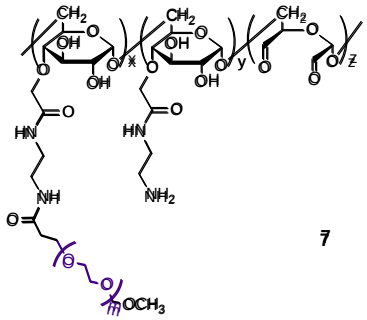
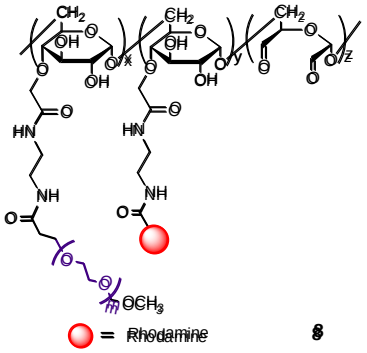
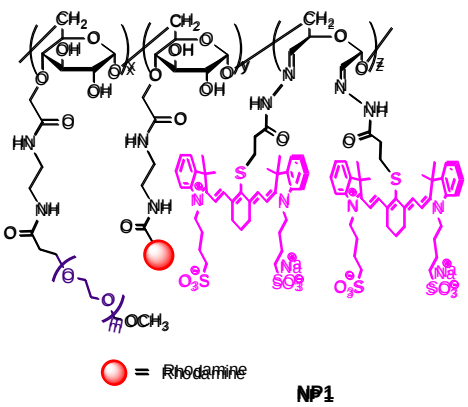
the pH was adjusted to 3 with 1.0 M HCl. Then an EEDQ solution prepared with 616 mg (2.49×10^{-3} mol) of EEDQ and 2 mL DMF was added dropwise. The resulting solution was added with 0.83 mL (3.33×10^{-3} mol) ethylenediamine under stirring. After 4 h reaction，the dextran derivative was precipitated by adding a large amount of methanol. The product was dried under vacuum as a white powder. (1.71×10^{-5} mol, 373 mg, MW is considered as 21.8 kDa yield: 90%).

Preparation of compound **6**.  Compound **5** (21.8 kDa, 350 mg, 1.61×10^{-5} mol) dissolved in 20 mL pure water and $NaIO_4$ (121 mg, 5.64×10^{-4} mol, 35 equiv.) was added as solid form. The mixture was stirred at r.t. for 24 h, and diethyene glycol (59.1 mg, 5.64×10^{-4} mol, 35 equiv.) was added to stop this reaction at the end of reaction. The product was purified in 3 kDa filter tube by washing with pure water for 3 times. The purified product was lyophilized as a white solid (233 mg, 1.11×10^{-5} mol, MW is considered as 21 kDa, yield: 69%).

Preparation of compound **7**.  Compound **6** (21 kDa, 200 mg, 9.5×10^{-6} mol) was dissolved in 4 mL 0.1 M HEPES pH 8.3 and PEG^{2k}-NHS (4.75×10^{-5} mol, 95 mg, 5 equiv.) in 200 µL DMF was added. After reaction for 1.0 h, the product was purified in 3 kDa filter tube by 3 × pure water. The product was lyophilized as a cotton-like white solid (206.15 mg, 6.65×10^{-6} mol, MW is considered as 31 kDa, yield: 70%). The molar ratio between PEG and Dextran moieties in Dex-PEG conjugate was determined by quantifying the integrated proton number of CH_2 in the PEG δ (3.67 ppm) and the integrated proton number of dextran at C1 position δ (4.95 ppm) in the ^1H NMR spectrum.

Preparation of compound **8**.  Compound **7** (31 kDa, 100.0 mg, 3.23×10^{-6} mol) was dissolved in 4 mL 0.1 M HEPES pH 8.3 and Rhodamine-NHS ester (0.645×10^{-5} mol, 3.395 mg, 2 equiv) in 200 µL DMF was added. After reaction for 1.0 h, the product was purified in 3 kDa filter tube by 3 × pure water. The product was lyophilized as a deep red color powder (31 kDa, 90.0 mg, 2.9×10^{-6} mol, yield: 90%).

Preparation of nanoprobe **NP1**. Compound **3** (33.7 mg, 3.9×10^{-5} mol) in 1.0 mL DMF was added to compound **8** (50 mg, 0.162×10^{-7} mol) dissolved in 5.0 mL 0.1 M MES buffer solution (pH 4.7). After stirring at r.t. for 16 h, the product was purified in 10 kDa centrifugal filtration tube and lyophilized as a deep green color powder (50 mg, 0.125×10^{-7} mol, MW is considered as 40 kDa, yield: 77%). The fluorophore labeling efficacy in **NP1** was calculated as 35% (35% of aldehyde moieties were labeled with NIR dye) by measuring the absorbance of IR783 (ε_{783} = 180,000 $M^{-1}cm^{-1}$).



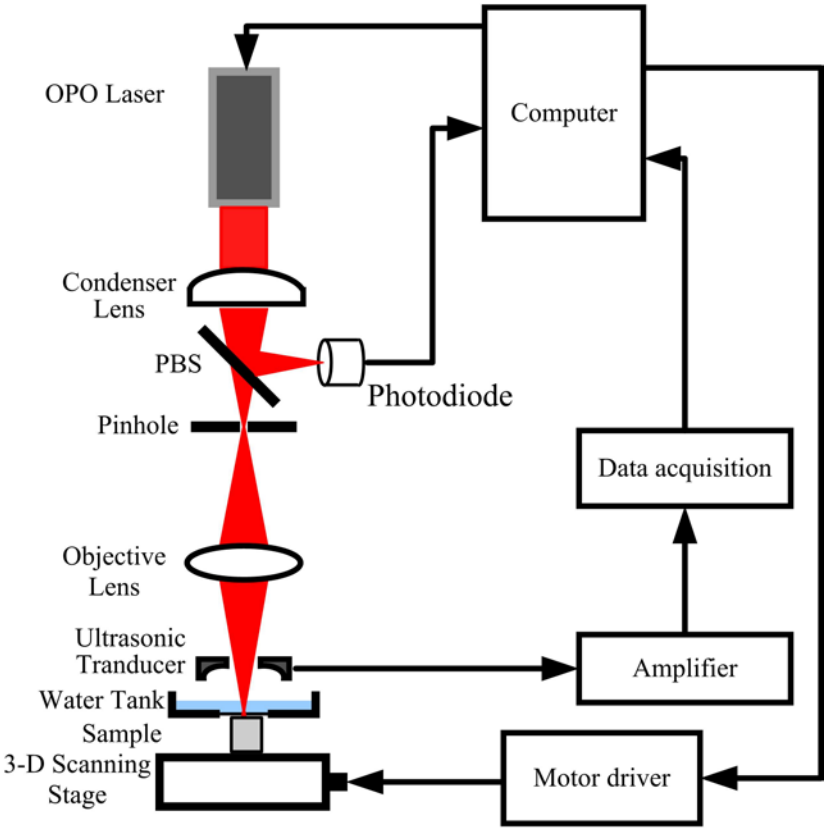


Fig. S1 Schematic of the PAM system

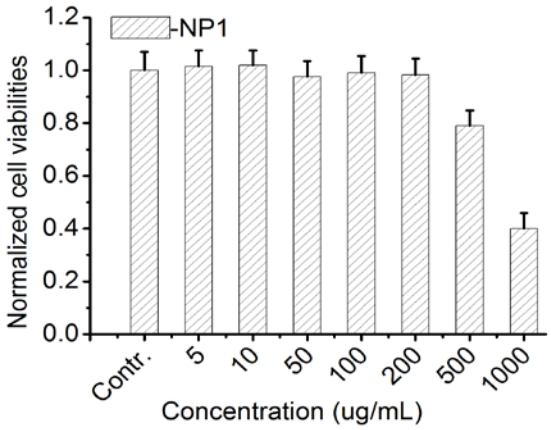


Fig. S2 NP1 demonstrated low cytotoxicity in EMT-6 cancer cells.

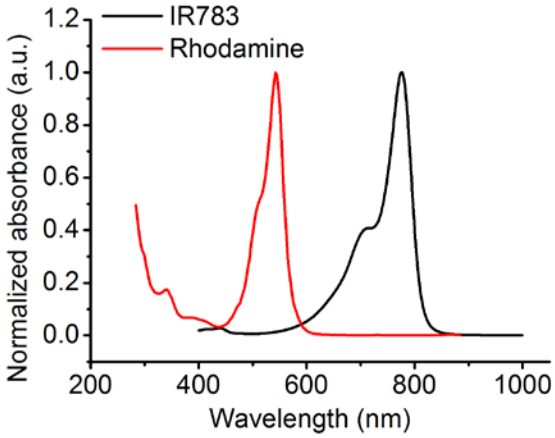


Fig. S3 The absorbance of IR783 (black) and rhodamine (red).

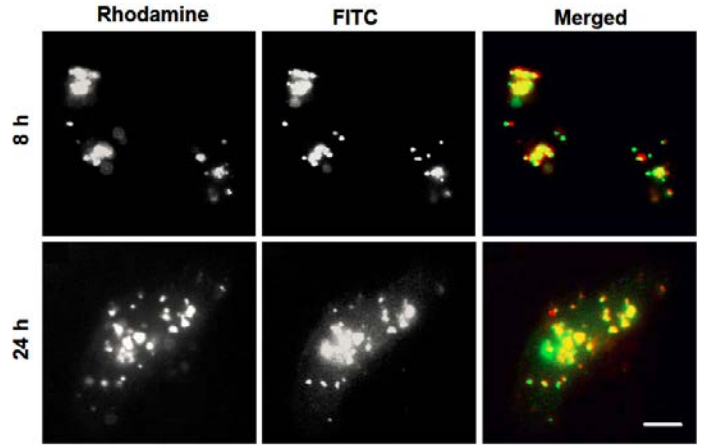


Fig. S4 The lysosomal delivery of **NP1** in live U87MG cancer cells. Scale bar, 20 μm.

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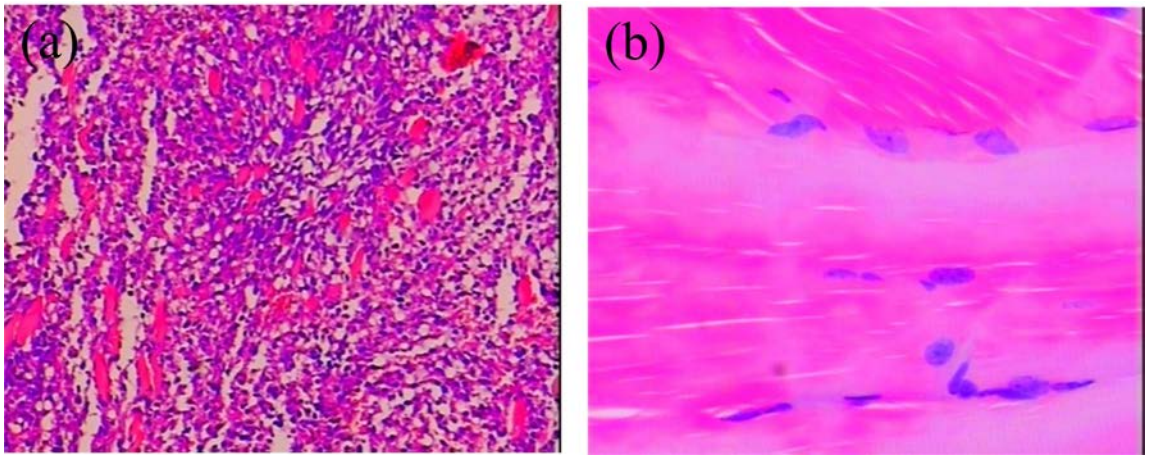

Fig. S5 H&E stained histological section of tumor (a) and normal muscle tissue (b) as the circles indicated in Fig. 7(a).

Spectra

^{1}H, ^{13}C NMR spectra were recorded at 400 MHz on Varian Mercury400 (Varian Inc. Palo Alto, CA, USA), and chemical shifts were reported in ppm relative to tetramethylsilane. Fast Atom Bombardment (FAB) mass spectra and High Resolution (HR) FAB mass spectra were obtained on a double sector JEOL JMS-A X505HA mass spectrometer (Peabody, MA, USA).