Supplementary File 1

Synthetic procedures were as follow：

1. The modification of IR780.

1.1 IR780p: Piperazine (50 mg) was added to a degassed solution of the commercial IR780 probe (IR780, 45 mg) in anhydrous DMF (5 mL). The mixture was heated at 80 ^{o}C under magnetic stirring and protection from light under an argon atmosphere for 6 h. The crude product was purified by HPLC and then lyophilized to obtain a solid product (IR780p, 70% yield, Mw=589.43, Figure S1).The purification condition of HPLC was as followed: V_{MeOH} : V_{water}= 50: 50, 1 mL/min, Thermo C18 HPLC (250 mm x 4.6 mm, 5 um).

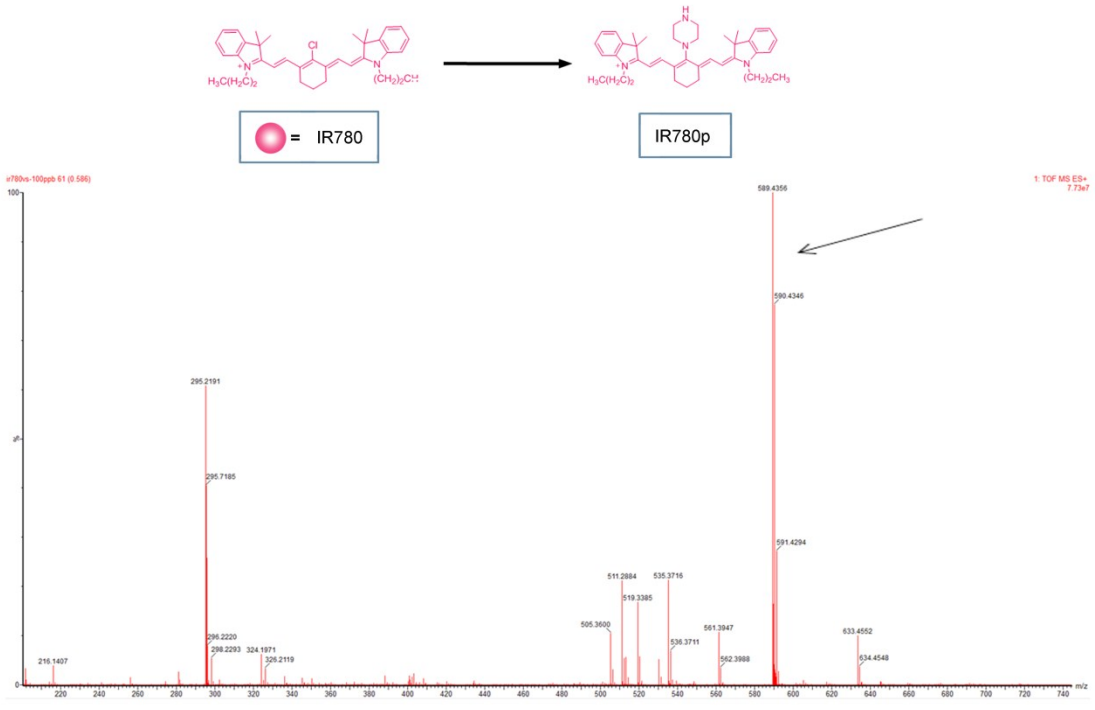

Figure S1. Mass Spectrometry (MS) of IR780p

1.2 IR780pVS: DVS (50 mg) was added to a solution of IR780p (35 mg) and Et3N (0.01 mL) in CH_2Cl_2-MeOH (2:1, 5 mL). The crude product was purified by HPLC and then lyophilized to obtain a solid product (named **IR780pvs**, 42.3% yield, Mw=785.41, Figure S2A). The purification condition was the same as above.

^{1}H NMR (600 MHz, $CDCl_3$) (Figure S2B): δ 7.65 (d, J = 13.2 Hz, 1H), 7.35 (brd, J = 7.8 Hz, 2H), 7.32 (brd, J = 7.8, 2H), 7.20 (d, J = 2.4 Hz, 1H), 7.16 (d, J = 2.4 Hz, 1H), 7.12 (brs, 1H), 7.14 (d, J = 7.8Hz, 2H), 6.96 (d, J = 7.8Hz, 2H), 5,77 (d, J = 13.2 Hz, 1H), 5.15 (m, 2H), 4.00 (m, 2H), 3.89 (m, 2H), 3.27 (m, 2H), 2.93 (m, 2H), 2.70-2.50 (m,4H), 2.48 (m, 4H), 2.06 (m, 2H), 1.87 (m, 4H), 1.68 (s, 6H), 1.42 (s, 6H), 1.07 (t, J = 7.2 Hz, 6H)

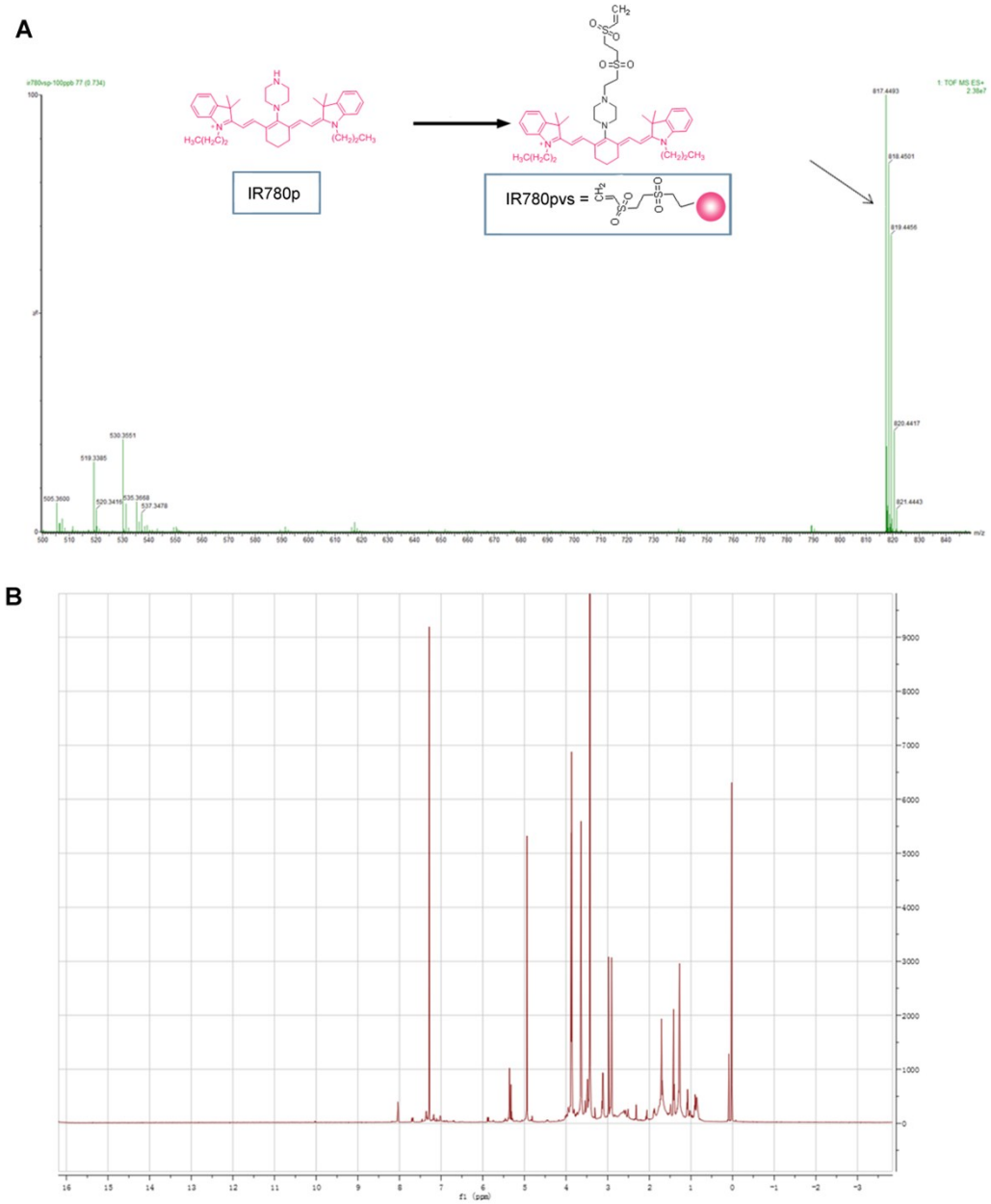

Figure S2. Mass Spectrometry (MS) (A) and ^{1}H NMR (B) of IR780pVS

2. **Synthesis of $I_{780}P$.** 5 mg of protective peptide GPLGVRGKGG (**P**) was dissolved in anhydrous DMF, and then 7 mg of IR780pvs ($\mathbf{I_{780}}$) was added, the mixture was stirred under protection from light for 16 h at room temperature. The synthesized peptide was purified by reversed-phase high-performance liquid chromatography (RP-HPLC) on a18 semipreparative column using a 10 to 100% linear gradient of ancetonitrile/water mixture (0.1% trifluoroacetic acid) for 30 min at a low rate of 1 mL/min and lyophilized. The freeze-dried production (Mw=2037.11, 20.14% yield, Figure S3A) was then deprotected by reaction in 90% TFA dichlorochloride for 1 h and was precipitated by adding ice ethylether. After that, the production peptide-IR780 ($\mathbf{I_{780}P}$, Mw=1682.96, Figure S3B) was steamed by rotary evaporator and dried by vacuum drying oven.

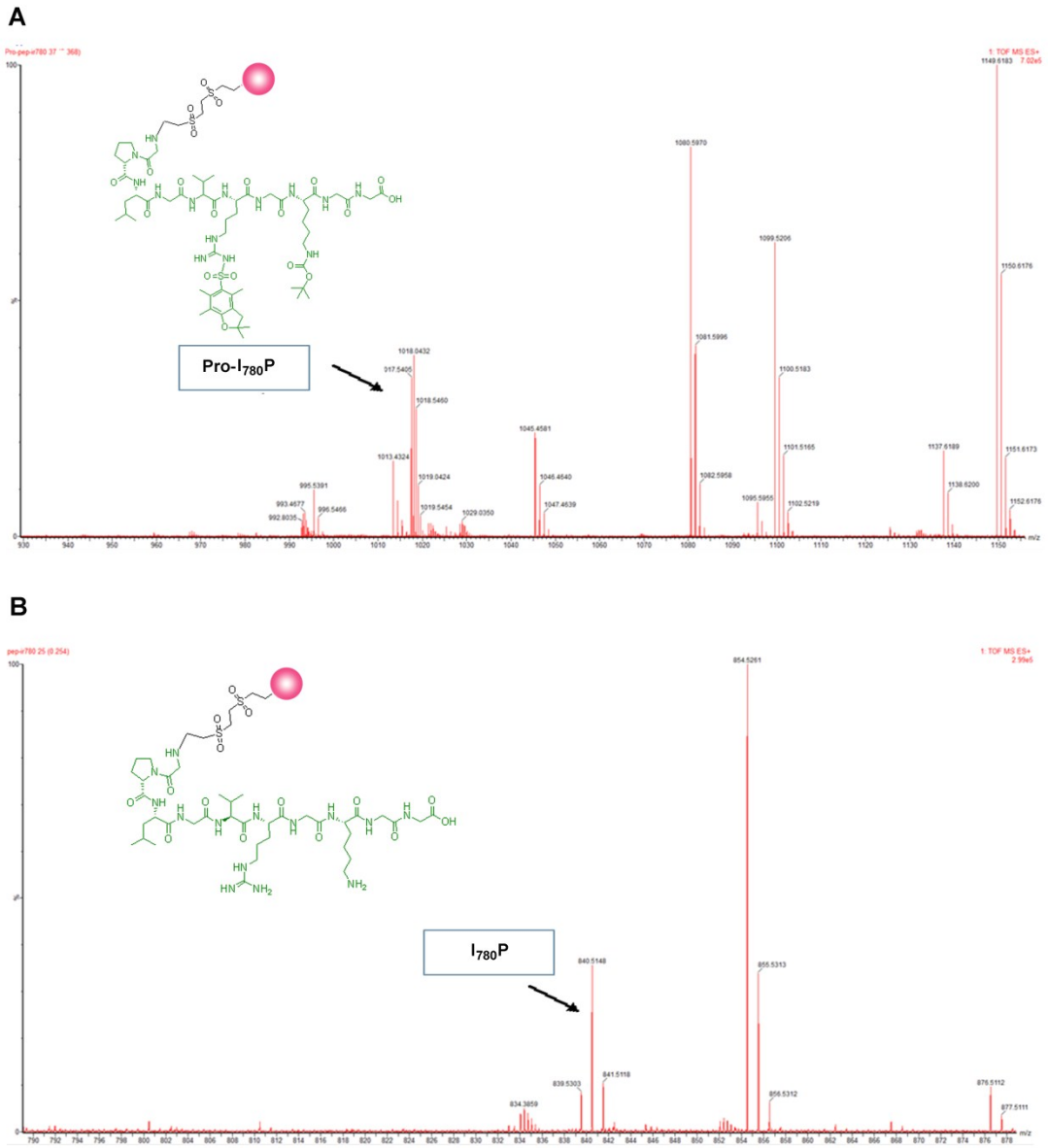

Figure S3. Mass Spectrometry (MS) of Pro-I_{780}P (A) and I_{780}P (B)

3 **Synthesis of I_{780}BP.** Peptide-IR780 (**I_{780}P**, 5 mg) and BHQ-3 (1.5 mg) were respectively dissolved in 200 μl of anhydrous DMF, and then respectively were added 12 μl of DIPEA and mixed for 10 minutes at room temperature. At last, the mixture containing BHQ-3 solution was added into Peptide-IR780 solution and protected from light for 4 h, and the production **I_{780}BP** (Mw=2213.24, 17.23% yield, Figure S4) purified by HPLC. The purification conditions were the same with the peptide.

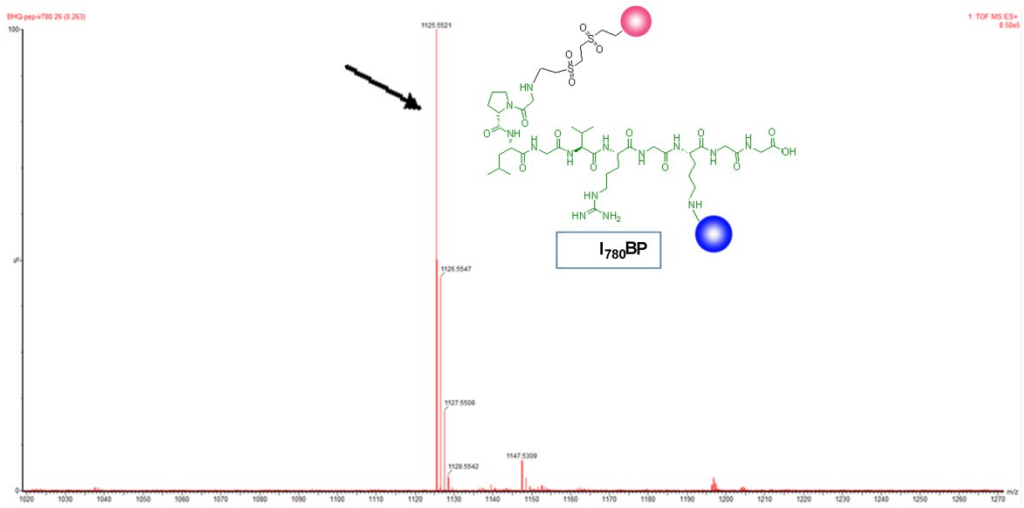

Figure S4. Mass Spectrometry (MS) of I_{780}BP

4 **Synthesis of I_{780}BP-PEG12.** BHQ-3-Peptide-IR780 (**I_{780}BP**, 3 mg) was dissolved in 400 μl of DMF/water (v:v=1:1). Then EDC (0.26mg) and NHS (0.16mg) were added into the mixture and activated for 30 minutes, and then 2 mg of NH2-PEG12-NH2 was added in reaction system, then reacted under protection from light for 24 h at room temperature and the production **I_{780}BP-PEG12** (Mw=2827.42, Figure S5) was purified by HPLC. The purification conditions were the same with the peptide.

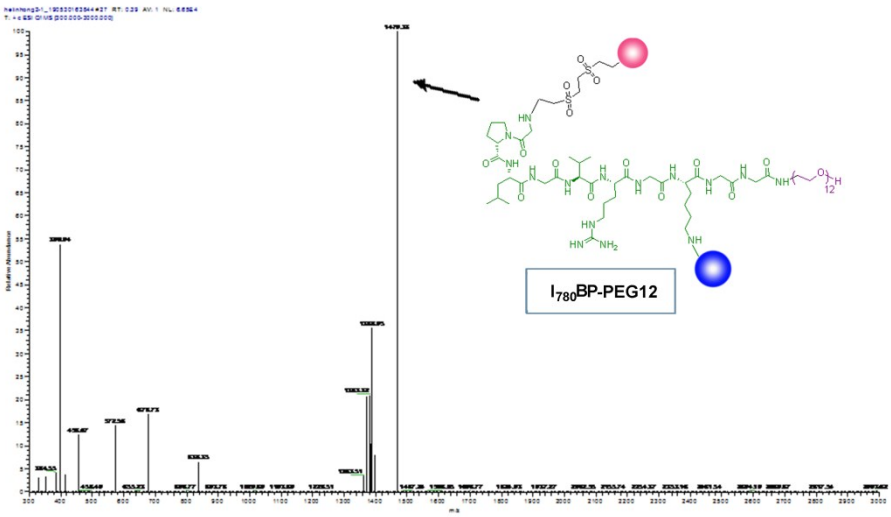

Figure S5 Mass Spectrometry (MS) of I_{780}BP-PEG12