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Supplementary Information

Improving brightness and photostability of NIR fluorescent silica nanoparticles through rational fine-tuning of the covalent encapsulation methods

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Experimental section

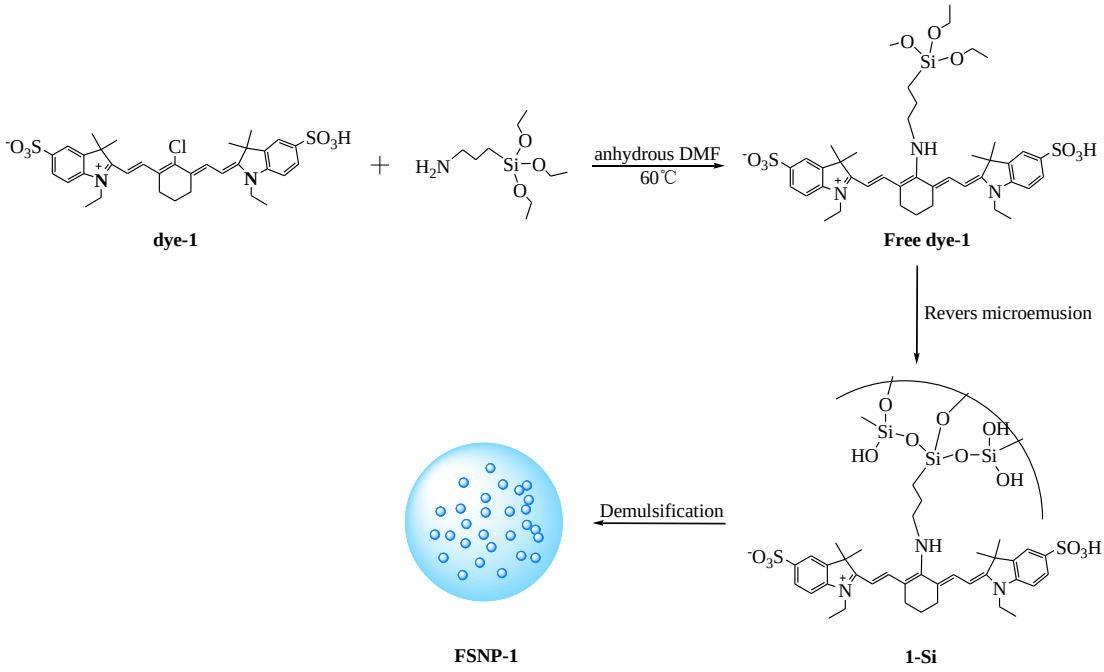
***Synthesis of Free dye-2, Free dye-3 and Free dye-4*:** Respectively, heptamethine 5-sulfo-3H-indocyanine **dye-2** (0.15 mmol), **3** (0.15 mmol) or **4** (0.15 mmol) and 6-aminocaproic acid (0.6 mmol), n-Propylaminwe or 6-aminocaproic acid (0.6 mmol), were mixed in anhydrous DMF (15 mL) under nitrogen atmosphere, and kept stirring at 60 ℃, 45 ℃, 45 ℃ for 4 h, 4 h, 12 h, respectively. The reaction product was precipitated with anhydrous ether. Then, the crude products were obtained by filtration, and purified by HPLC using methanol, deionized water as the mobile phase.

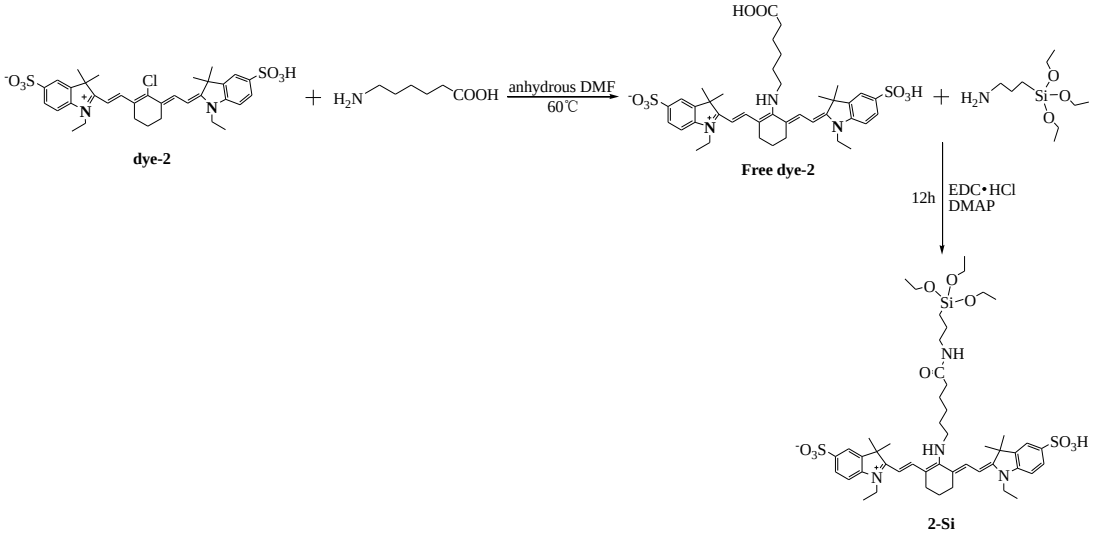
***Synthesis of cyanine-APTES derivative (1-Si, 2-Si, 3-Si or 4-Si)*:** Respectively, **Free dye-2**(10 μmol), **Free dye-3**(10 μmol) or **Free dye-4**(10 μmol) and EDC·HCl(0.1 mmol), DMAP(0.04 mmol) were stirred in anhydrous DMF (10 mL) under nitrogen atmosphere at room temperature for 1 h. Then APTES(40 μL) was mixed to continue reacting for 12 h.

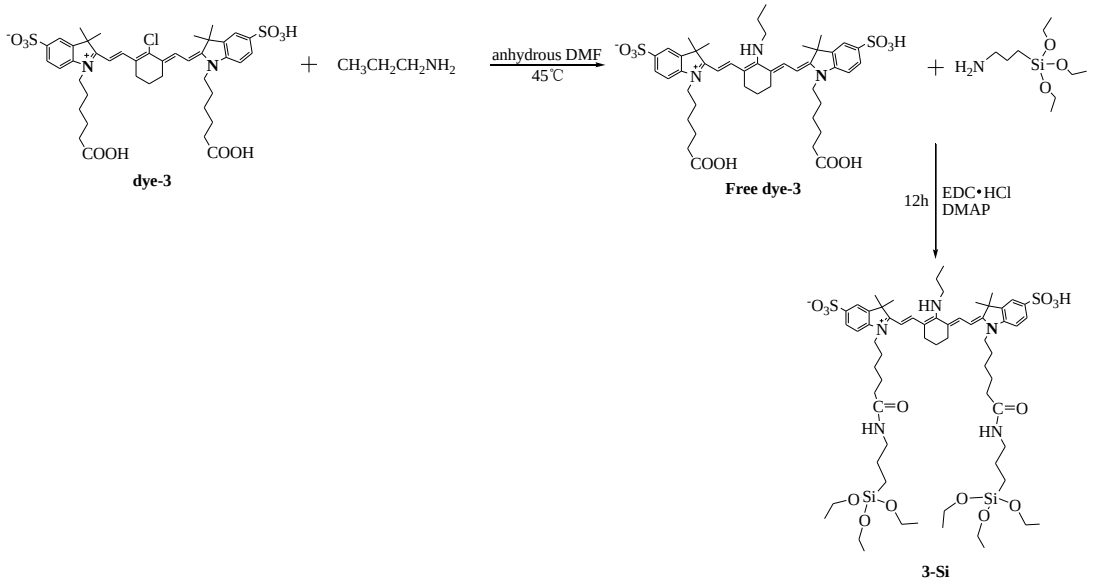
The big difference was the synthesis of **1-Si**. **1-Si** were obtained from **dye-1**(0.15 mmol) by a direct nucleophilic substitution reaction with APTES(40 μL) in anhydrous DMF (10 mL) at 60 ℃ for 4 h. The reaction product was precipitated with anhydrous ether and filtered.

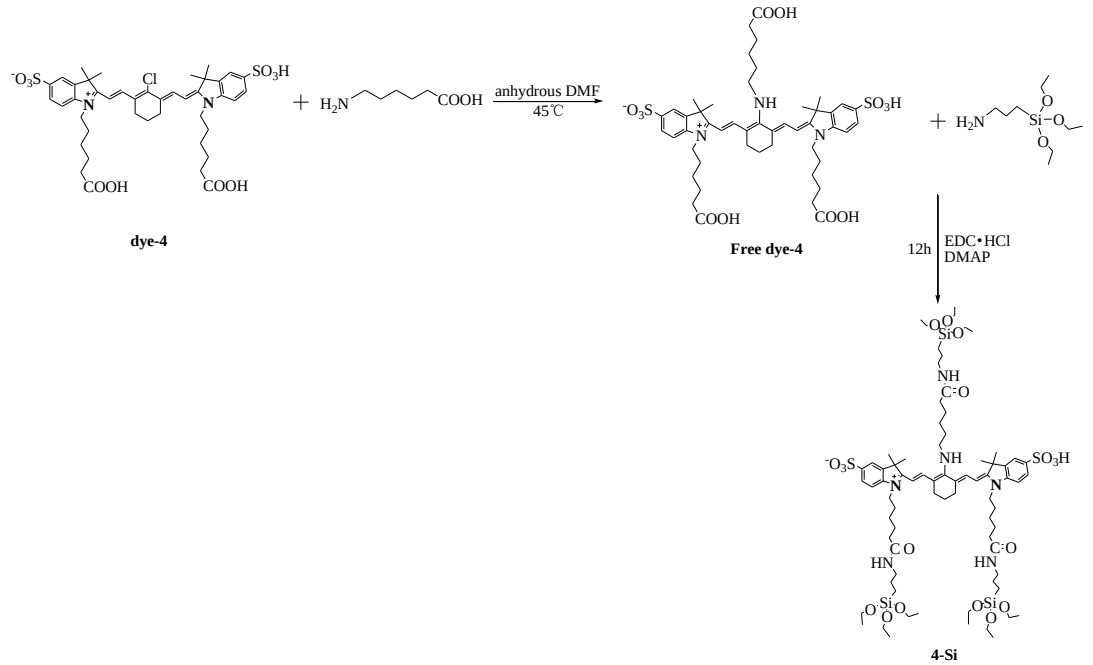
***Synthesis of FSNPs*:** The synthesis of **FSNPs** was carried out by microemulsion method. The microemulsion system was prepared by mixing 3.5 g Triton X-100, 4.4 mL n-octanol and 20 mL cyclohexan and 1 mL deionized water to stir for 30 min. Then, the unpurified product **(1-Si, 2-Si, 3-Si or 4-Si)** in the previous step was added. After stirring at 350 rpm for 30 min at room temperature, TEOS (4.5 mmol, 1 mL) and NH_4OH (28−30%, 5.3 mmol, 0.7 mL) were added to the microemulsion system for 24 h at room temperature. Then, the microemulsion system was interrupted by the addition of acetone (25 mL). **FSNPs** were collected by centrifugation and washed with absolute acetone twice, and with ethanol three times to remove any unreacted chemicals and free dye molecules. At last, **FSNPs** were vacuum dried, and re-dispersed in deionized water at 4 ℃ for cryopreservation.

Scheme S1. Synthetic routes of the **FSNPs**.




dye-2
anhydrous DMF
60℃
Free dye-2
12h
EDC•HCl
DMAP
2-Si


dye-3
anhydrous DMF
45℃
Free dye-3
12h
EDC•HCl
DMAP
3-Si


dye-4
anhydrous DMF
45℃
Free dye-4
12h
EDC•HCl
DMAP
4-Si

Hydrodynamic diameter distribution of FSNPs

Dynamic light scattering (DLS) Nanozs90 was used to measure the size distribution. The samples for DLS were tested at 25 ℃.

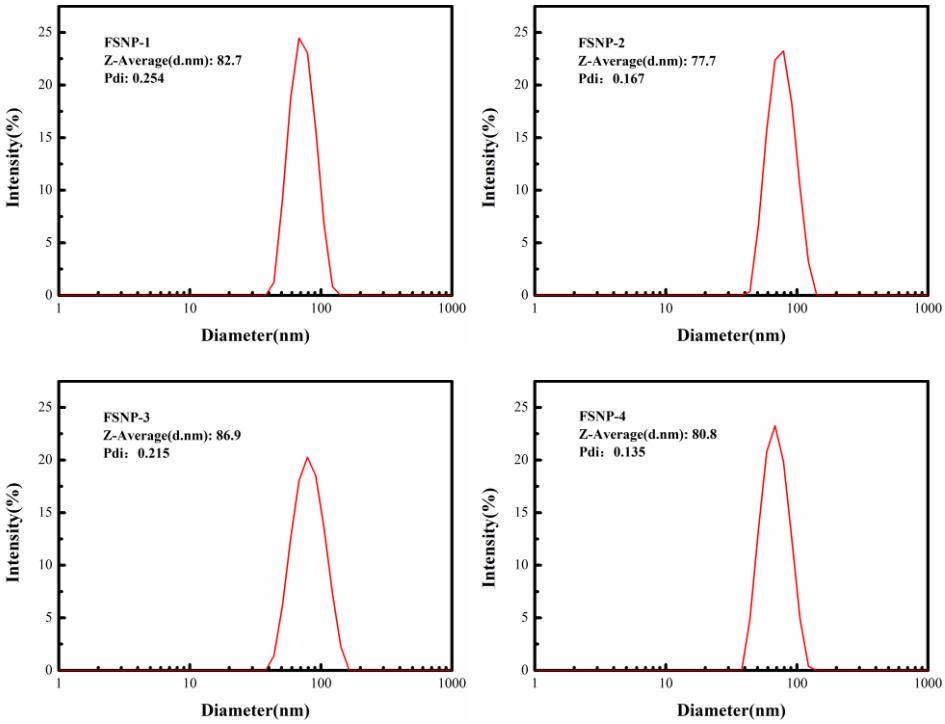
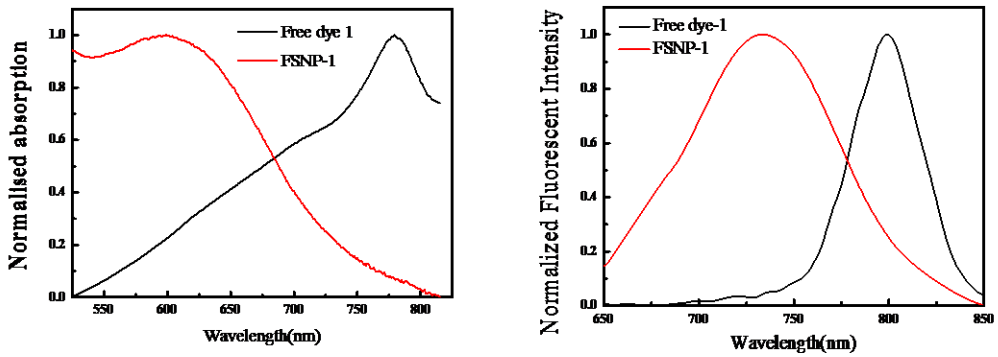


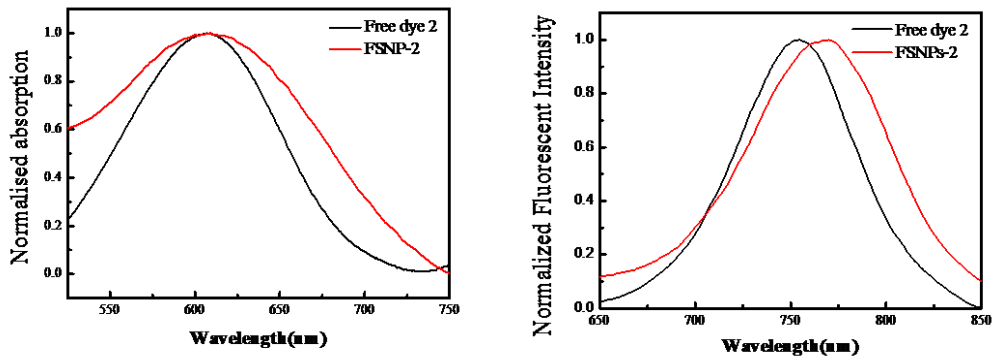
Figure S1. Size distribution of **FSNPs** using dynamic light scattering in neutral PBS buffer. C_{FSNPs}=5 μg mL^{-1}.

Absorption and fluorescence spectra of free dye and FSNPs

(a)



(b)



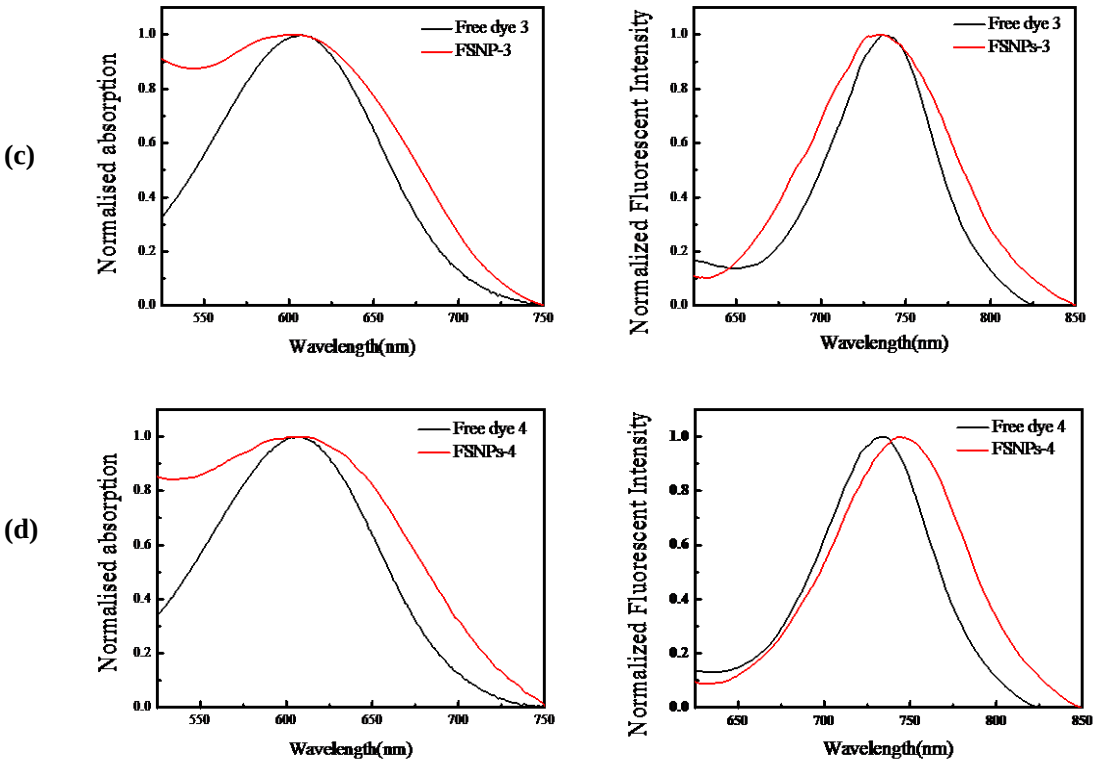


Figure S2. Normalised absorption(left) and fluorescent(right) spectra of (a)**Free dye-1** and **FSNP-1**, (b)**Free dye-2** and **FSNP-2**,(c) **Free dye-3** and **FSNP-3**, (d)**Free dye-4** and **FSNP-4** in PBS. $C_{\text{Free dye}}$=5 μmol, C_{FSNPs}=0.5 mg mL^{-1}. λ_{em1}=608 nm and 780 nm, λ_{em2}=608 nm, λ_{em3}=606 nm, λ_{em4}=605 nm.

Leakage experiments of FSNPs

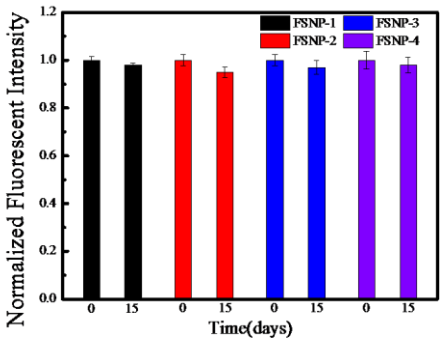


Figure S3. Normalized fluorescent intensity of **FSNPs-1~4**. C_{FSNPs}=0.5 mg mL^{-1}. λ_{em}=608 nm.

pH stability experiment

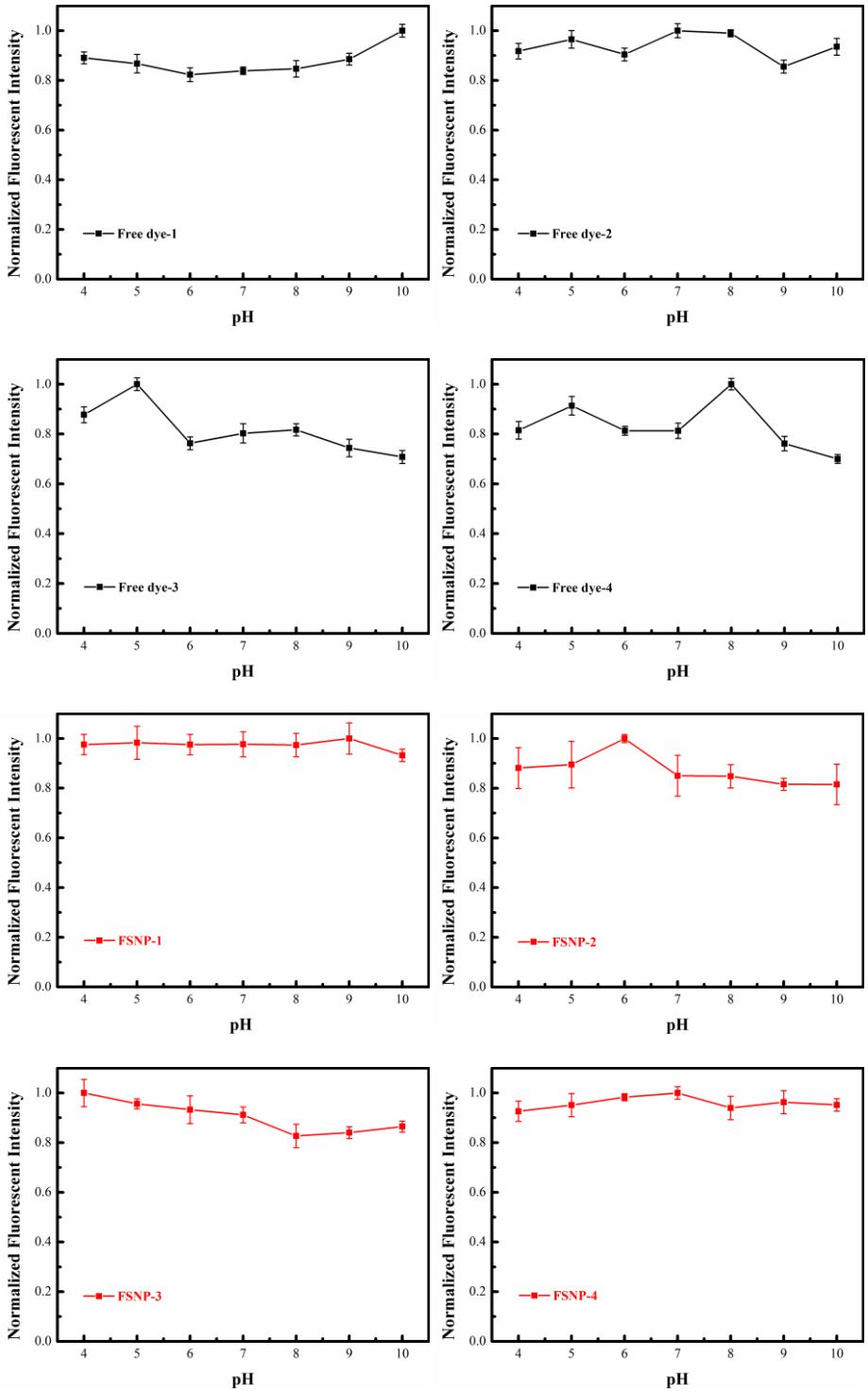


Figure S4. The pH-dependent curve of Free dyes and FSNPs' normalised fluorescent intensity. $C_{\text{Free dye}}$=5 μmol, C_{FSNPs}=0.5 mg mL^{-1}.

Fluorescence intensity curve of diffirent Free dye-2 concentrations in FSNP-2

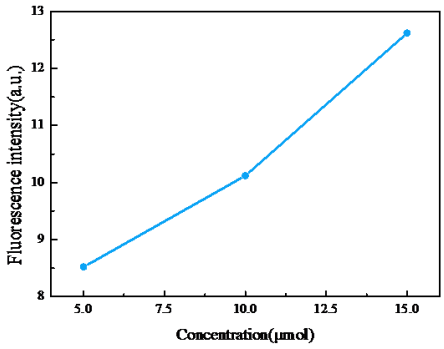


Figure S5. The fluorescence intensity curve of diffirent Free dye-2 concentrations in FSNP-2. All samples were dispersed in ethanol to a final concentration with almost the same absorbance (0.01). The starting reactant concentrations of Free dye-2 are 5 μmol, 10 μmol and 15 μmol, respectively.

Results of elemental analysis

Only Free dye molecules containing sulfur element in the microemulsion system, however, carbon, nitrogen and other elements in the Free dyes and microemulsion systems are all contained, so the elemental analysis test is to sulfur model prevail.

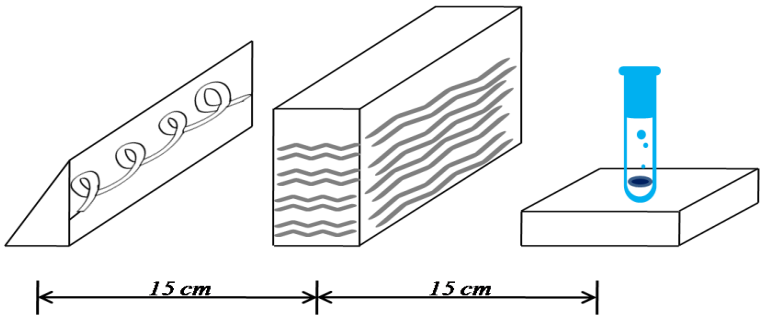
Table S1. The results of Elemental Analysis.

Sample	Weight（mg）	Content of sulphur（*wt*.%）	Content of **Free dyes** in **FSNPs**（μmol mg^{-1}）
FSNP-1	1.8850	S：1.255	0.1957
FSNP-2	1.6470	S：1.684	0.2626
FSNP-3	1.6020	S：1.236	0.1927
FSNP-4	1.8910	S：1.222	0.1905

Photostability test in vitro

Free dyes and FSNPs were dissolved in 6 mL ethanol, accompanied by magnetic stirring to avoid precipitate of FSNPs. Sample tubes were exposed to light under a W-Halogen lamp (500 W), the distance between lamp and sample tubes is 30 cm. A cold trap equipped with aqueous solution of sodium nitrite at 50 g L^{-1} was placed in the middle of lamp and sample tubes. Fluorescence spectra of samples were taken after every 10 min.

(a)



(b)

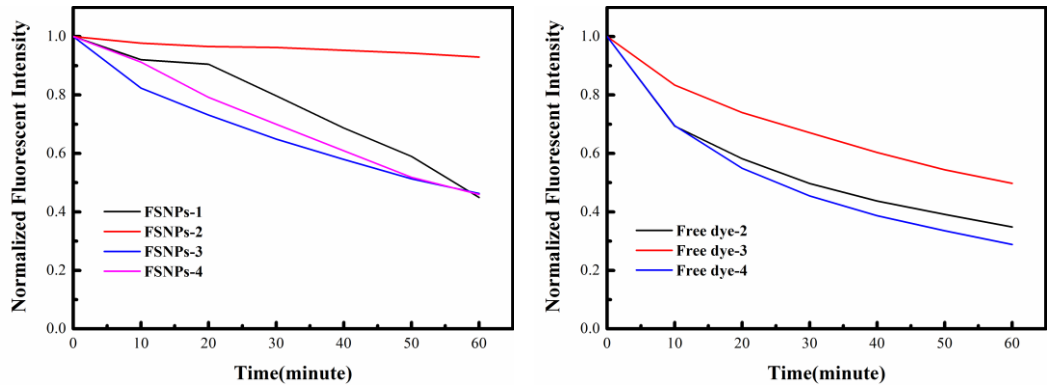


Figure S6. (a) The schematic diagram of the photostability testing device, (b) The results of photostability evaluation. C_{FSNPs}=0.5 mg mL^{-1}. λ_{em}=608 nm. Free dyes and FSNPs were dissolved in ethanol, to ensure each group of Free dyes and FSNPs has almost the same fluorescence intensity by adjusting concentration.

Determination of IC_{50}

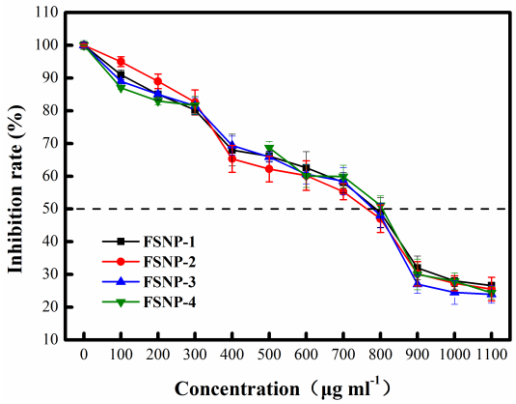
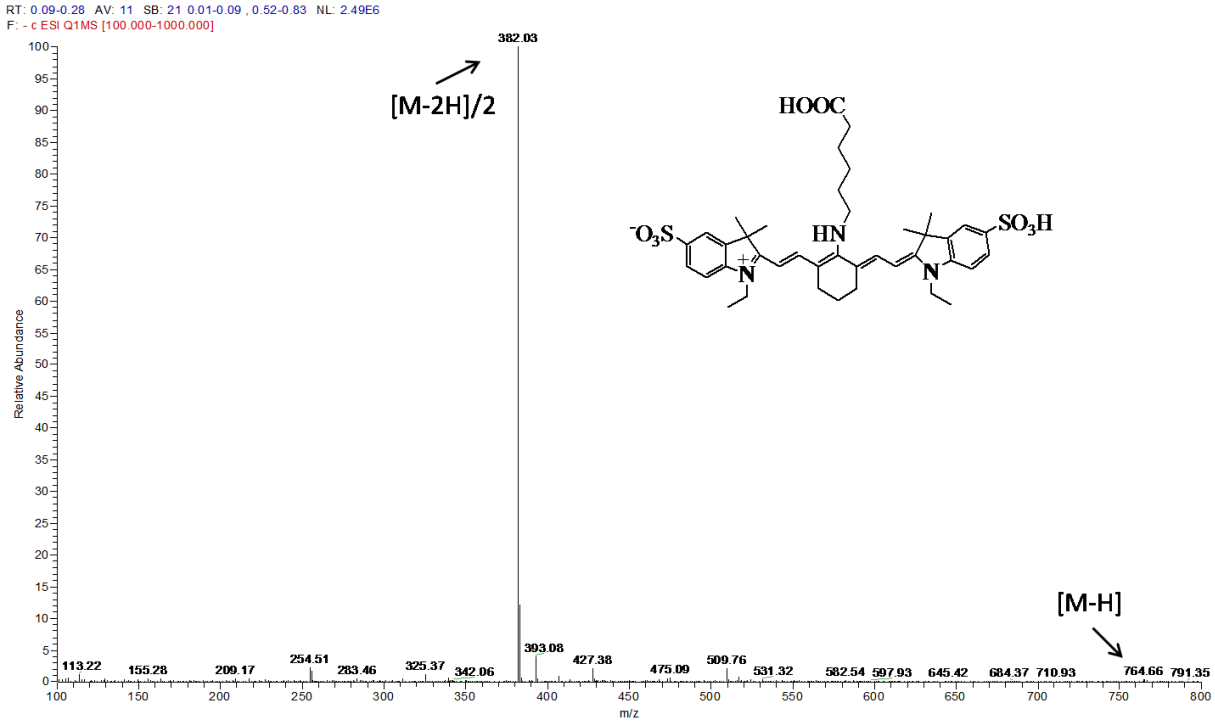


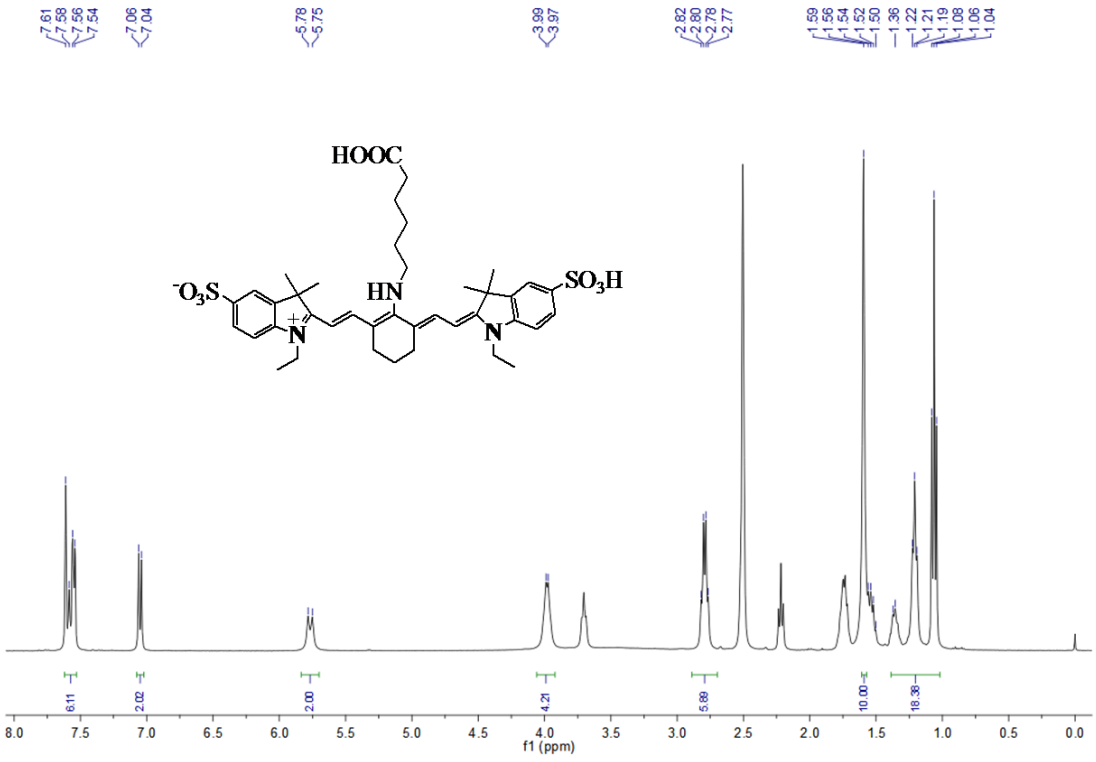
Figure S7. The inhibition rate curve of raw264.7 macrophages cells incubated with different concentrations of FSNPs from 0 to 1100 μg mL^{-1}.

Mass and NMR spectra of compounds

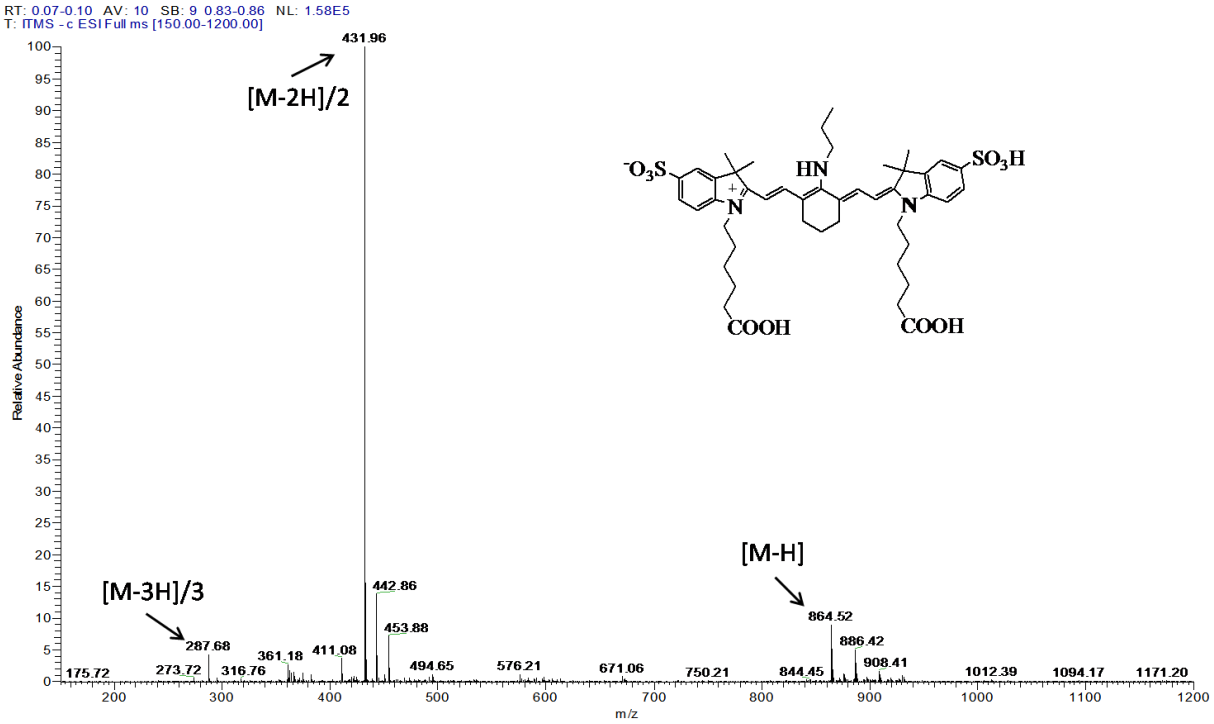
MS of **Free dye-2**.



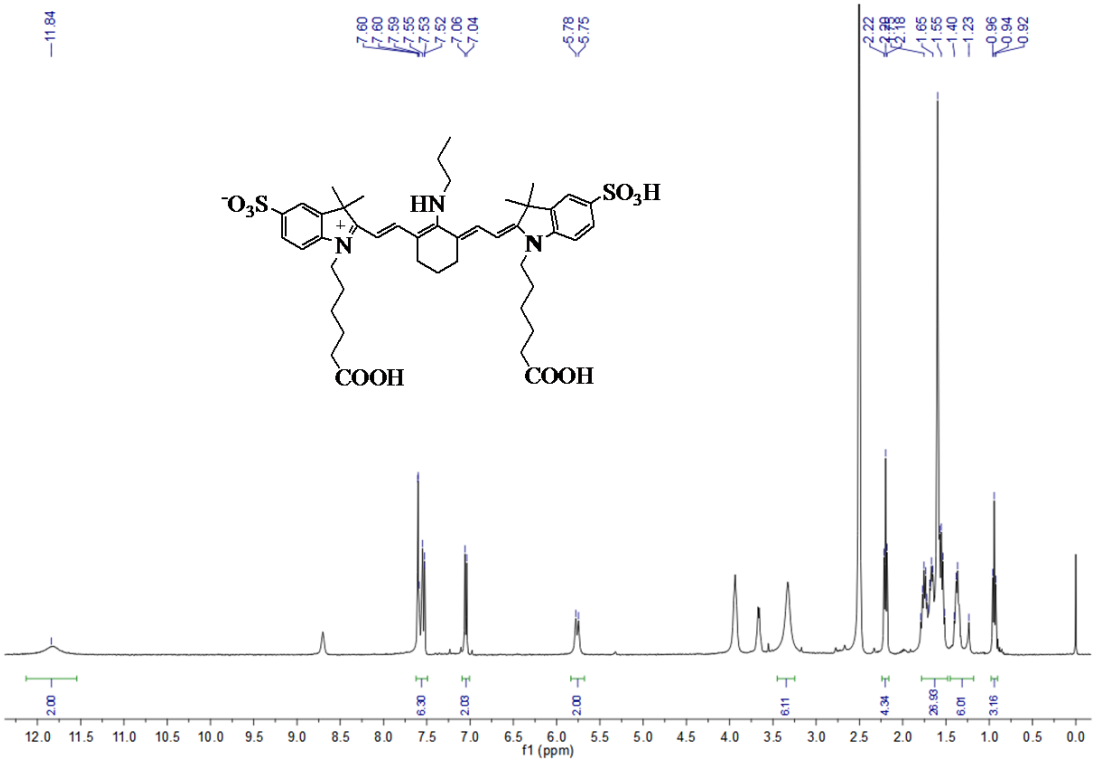
^{1}H-NMR spectrum of **Free dye-2** in $(CD_3)_2SO$.



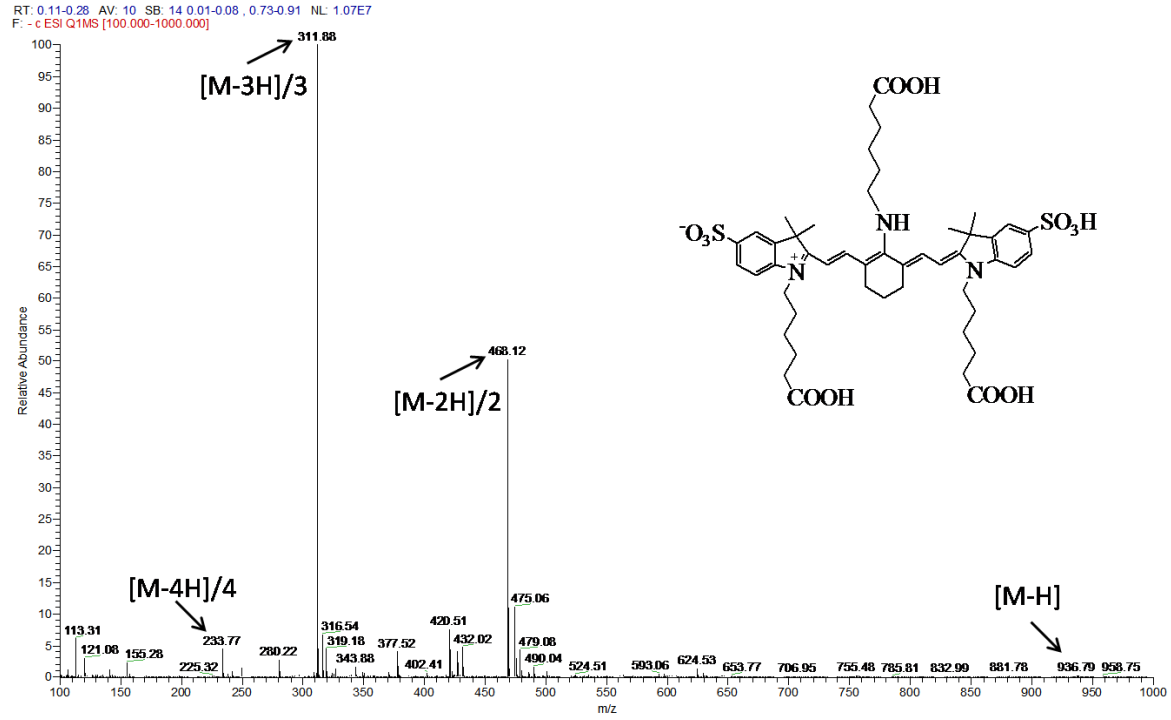
MS of **Free dye-2**.



^{1}H-NMR spectrum of **Free dye-3** in $(CD_3)_2SO$.



MS of **Free dye-4**.



^{1}H-NMR spectrum of **Free dye-4** in $(CD_3)_2SO$.