Supporting Information for

A novel Cys-activated NIR-II fluorescent probe for rheumatoid arthritis fluorescent imaging *in vivo*

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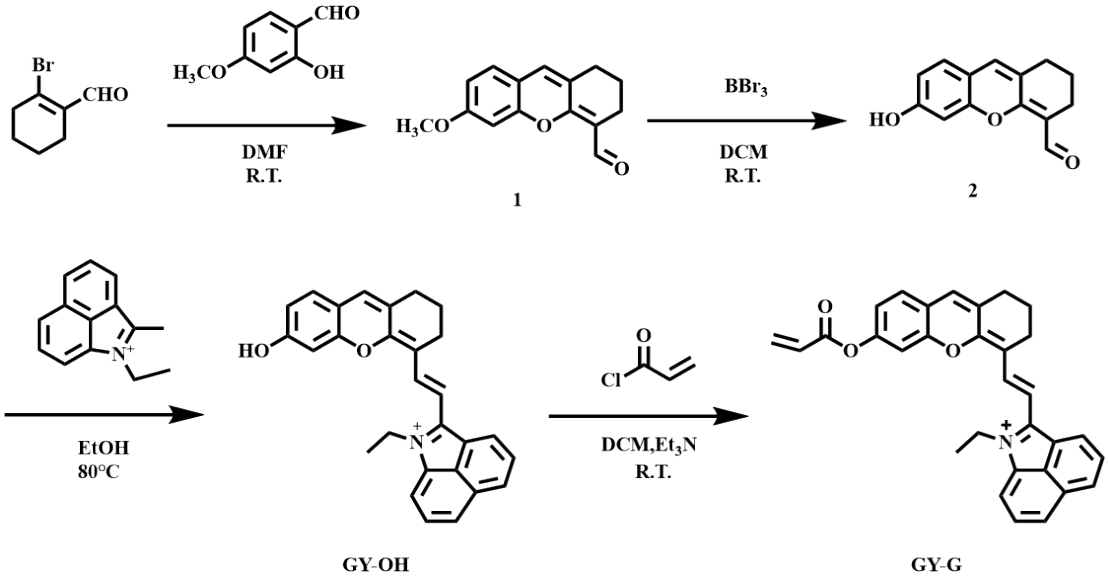
Experimental

1.Materials and Instrumentations

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All experiments used ultra-pure water. ^{1}H NMR, ^{13}C NMR spectra were measured on a Bruker AVANCE III HD 600 NMR spectrometer. Proton Chemical shifts of NMR spectra were given in ppm relative to internals reference TMS (^{1}H,0.00 ppm). HRMS spectral data were recorded on a Bruker Daltonics Bio TOF mass spectrometer. The absorbance was recorded by ultraviolet-visible absorption spectrometry (UV-2700, Shimadzu). TLC analysis carried out on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), they were purchased from the Qingdao Ocean Chemicals. All aqueous solutions were prepared with ultrapure water obtained from a Milli-Q water purification system (18.2 MΩ cm).

2. Synthesis and Characterization of Compound



Scheme S1. Synthetic route of **GY-OH** and **GY-G**.

The synthesis of compound **GY-OH** can be found in the referenced literature, quantum yield (3.8%).[1]

Add 5 ml of dry DCM to dissolve raw **GY-OH** (80 mg, 0.2 mmol) and acryloyl chloride (40 mg, 0.4 mmol), and then add 3 drops of triethylamine, and the reaction was carried out under the protection of nitrogen at room temperature for 30 min. At the end of the reaction, the solvent was removed by evaporation under reduced pressure, and then the compound was purified by silica gel column chromatography (DCM: MeOH=50:1) to obtain **GY-G**, which was a dark blue solid. The yield was 62%, quantum yield (0.0012).

3.Spectral response of GY-G toward Cys

A certain amount of **GY-G** (1 mM) was prepared by dissolving **GY-G** in DMSO for spectral test. To a PBS (pH = 7.4 contain 20% DMSO) solution (2 mL), followed **GY-G** solution (the final test concentration was 10 μM). Before the absorption spectral were measured, the solution was incubated at 37 ℃ for 10 min.

4.In *vitro* NIR-II imaging

PBS solutions (pH=7.4, contain 20% DMSO, 500 uL) were configured in each 1.5 ml centrifuge tube with a **GY-G** concentration of 10 uM and containing varying concentrations of Cys (20 uL, 0~30 uM), and then these centrifuge tubes were placed inside the NIR-II imager for imaging. (LP=880 nm, Exposure=200).

5.Animal sources

Male C57 mice (about 4 weeks aged) was purchased from Guangxi Medical University. The farming system of animals was under standard laboratory conditions. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Guangxi Medical University and approved by the Animal Ethics Committee of Guangxi Medical University (China). Mice were divided randomly to support subsequent experimental investigations. All animals fasted the day before the experiment.

6.Establishment of Arthritis model

We chose **λ**-carrageenan as a modeling drug for arthritis. C57 mice were injected with **λ**-carrageenan solution (50 uL, 1 $mg \cdot mL^{-1}$) in the right leg joint and fasted one day in advance.

7.Animal experiments

On the day prior to imaging, 50 μL of **λ**-carrageenan (1 $mg \cdot mL^{-1}$) was injected into the right hind limb of a male C57 mouse, while 50 μL of PBS solution (10 mM) was injected into the right hind limb of another male C57 mouse. After 24 h of induction, the RA model was successfully established. Subsequently, **GY-G** solution (30 μL, 50 μM) was injected into the inflamed right hind limb of the RA model mice as well as at the same location of the control mice's right hind limb. In vivo NIR-II fluorescence images were then taken by using a near-infrared second-region in vivo fluorescence lifetime imaging system (Series iii 900/1700) with an excitation laser source of 808 nm (exposure time =200 s) and a 1000 nm long-pass filter.

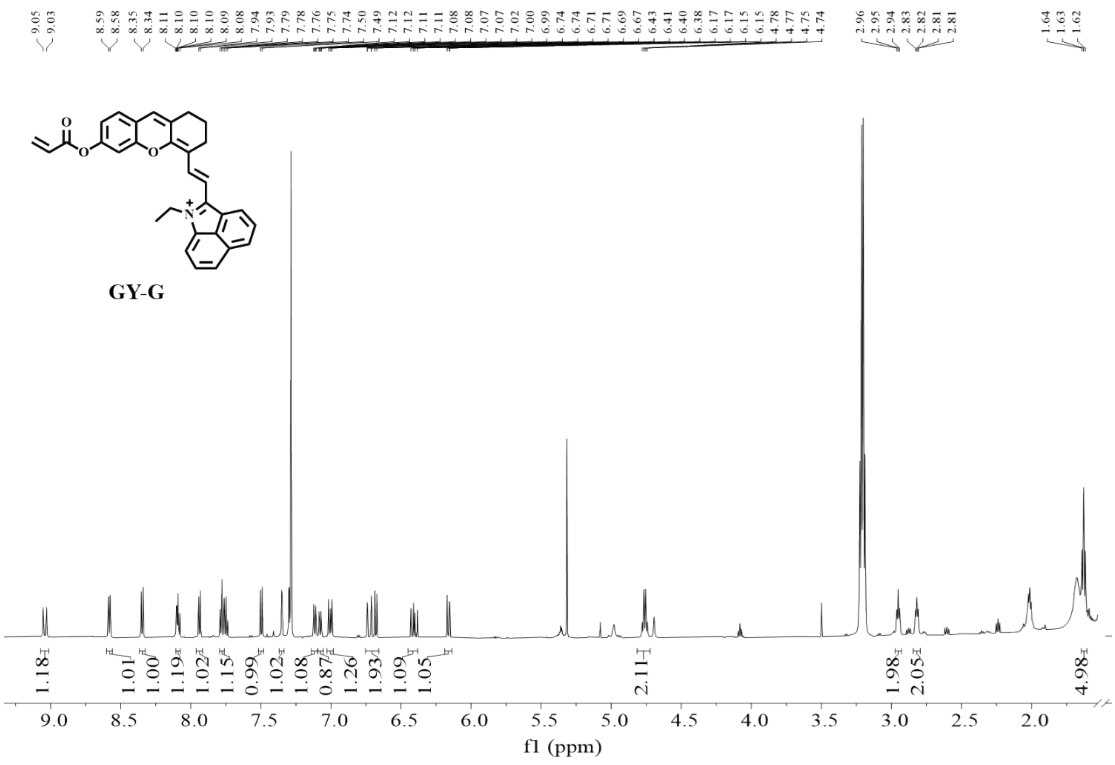


Fig. S1. ^{1}H NMR spectra of probe **GY-G** in $CDCl_3$ (contain 0.5% Et_3N).

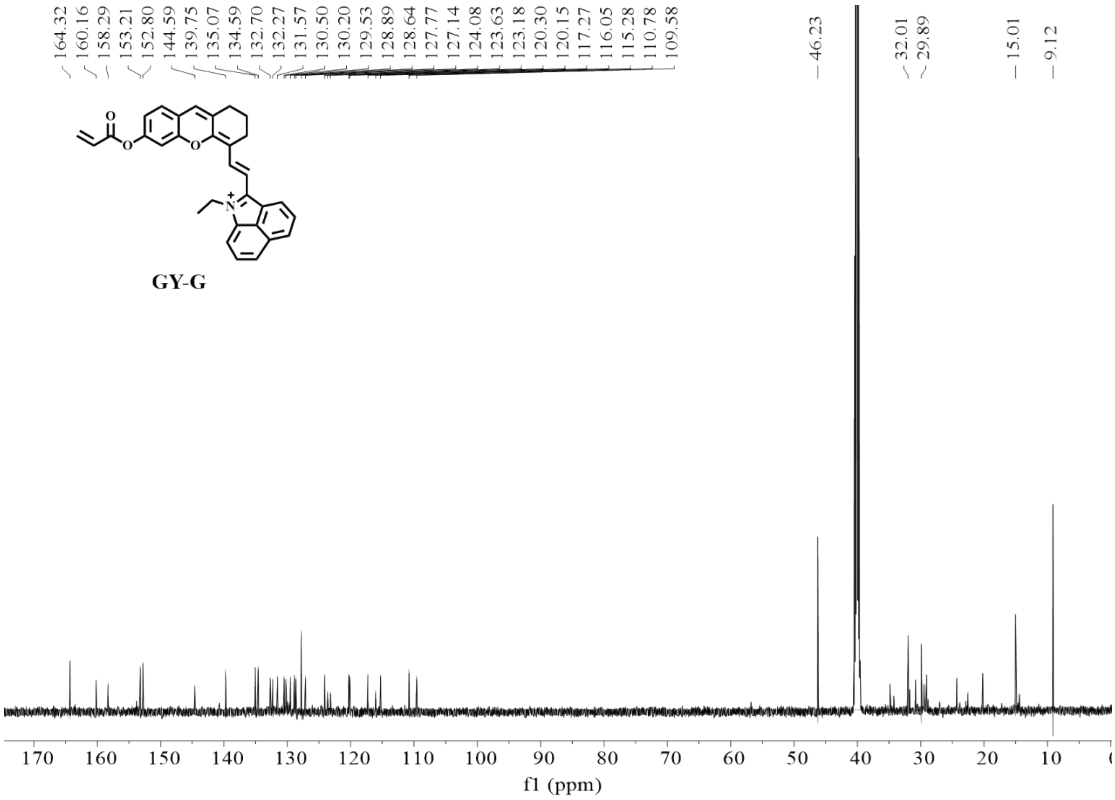


Fig. S2. ^{13}C NMR spectra of probe **GY-G** in DMSO-d_6.

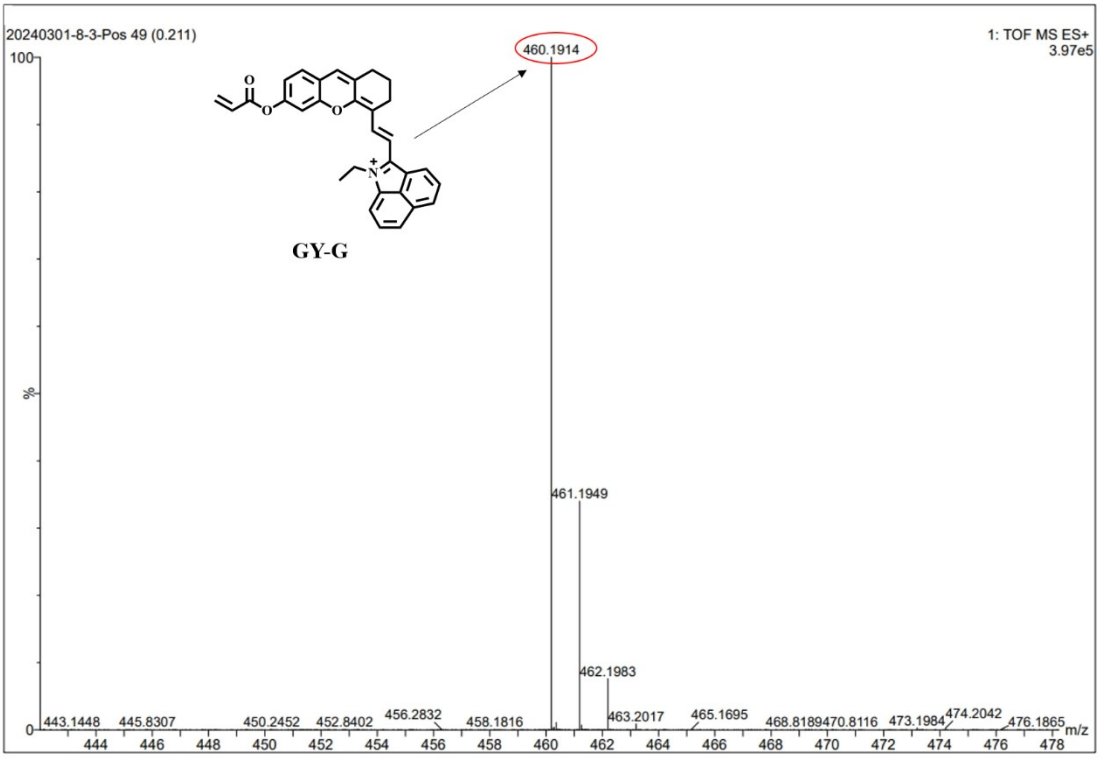


Fig. S3. HRMS spectral of compound **GY-G.**

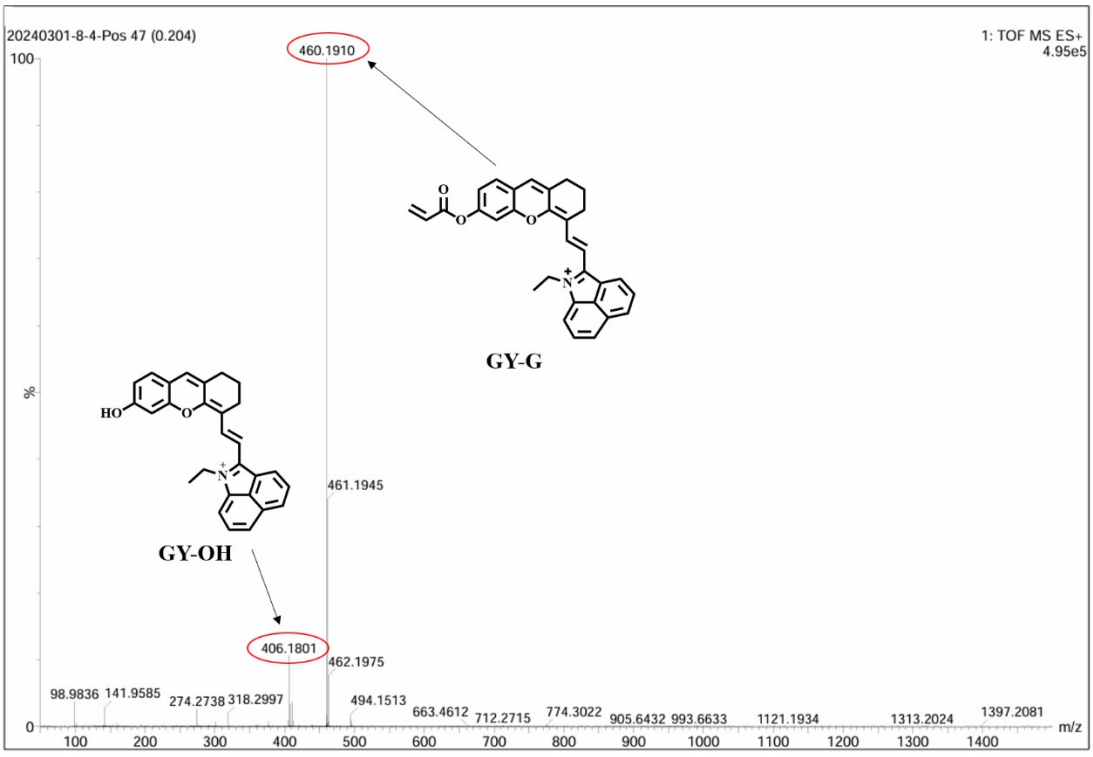


Fig. S4. HRMS spectral of compound **GY-G** respond to Cys.

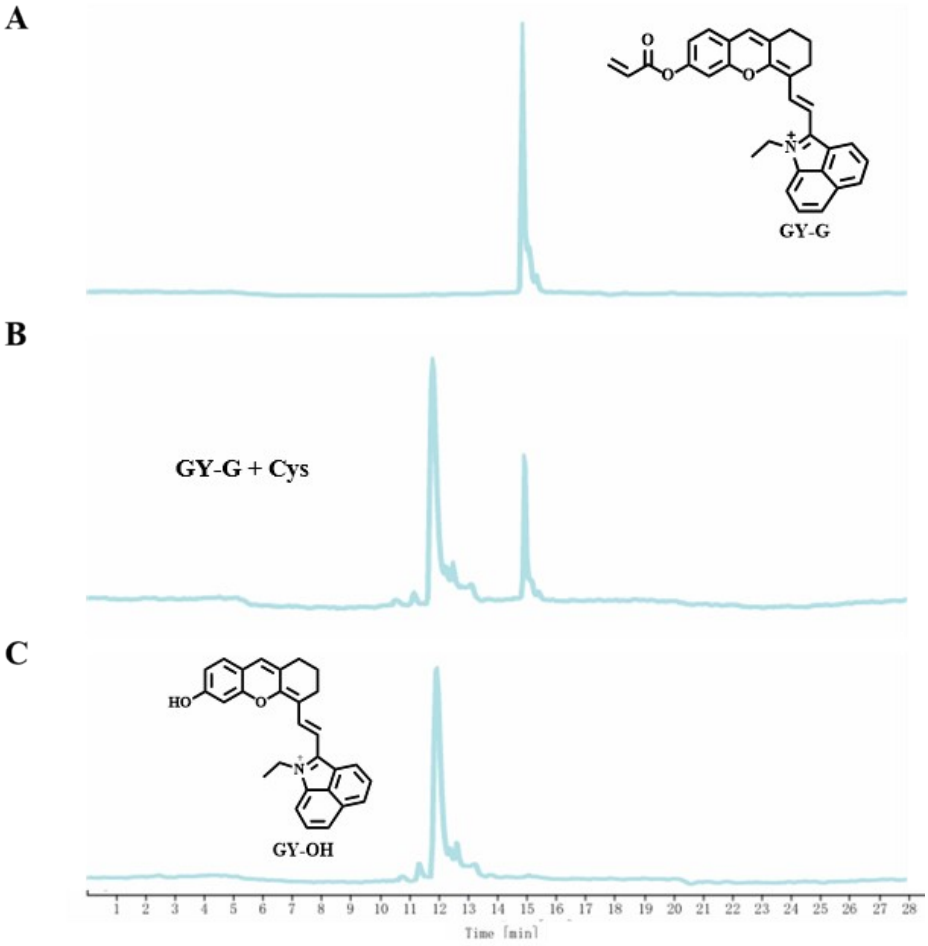


Fig. S5. The HPLC of (A) **GY-G**; (B) **GY-G** in the presence of Cys; (C) **GY-OH**.

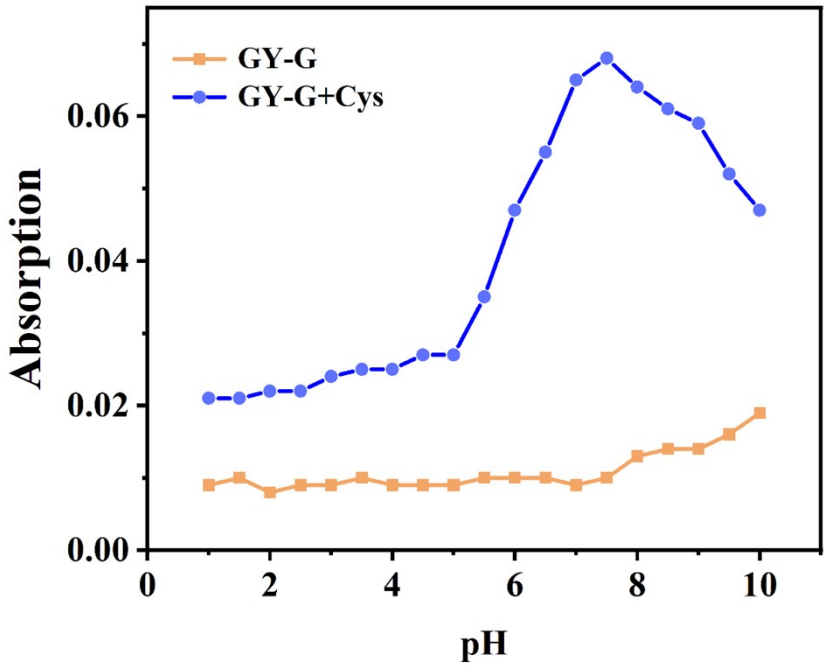


Fig. S6. The absorption of **GY-G** in the absence and presence of Cys under different pH values.

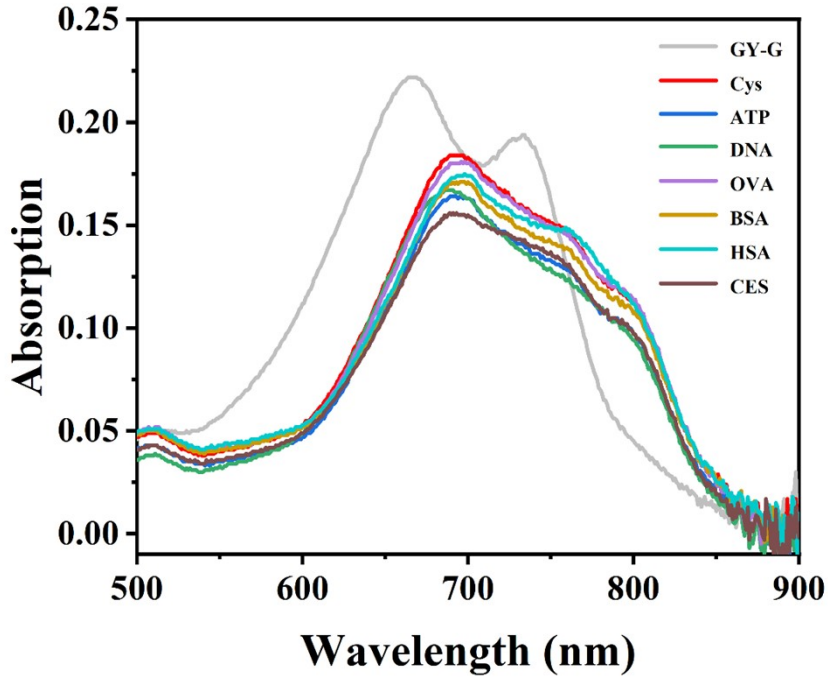


Fig. S7. Absorption spectra of probe **GY-G** in response to Cys in the presence of some biomolecules. (Cys: 30 μM; Carrier DNA: 50 μM; CES: 40 μM; and others species :100 μM)

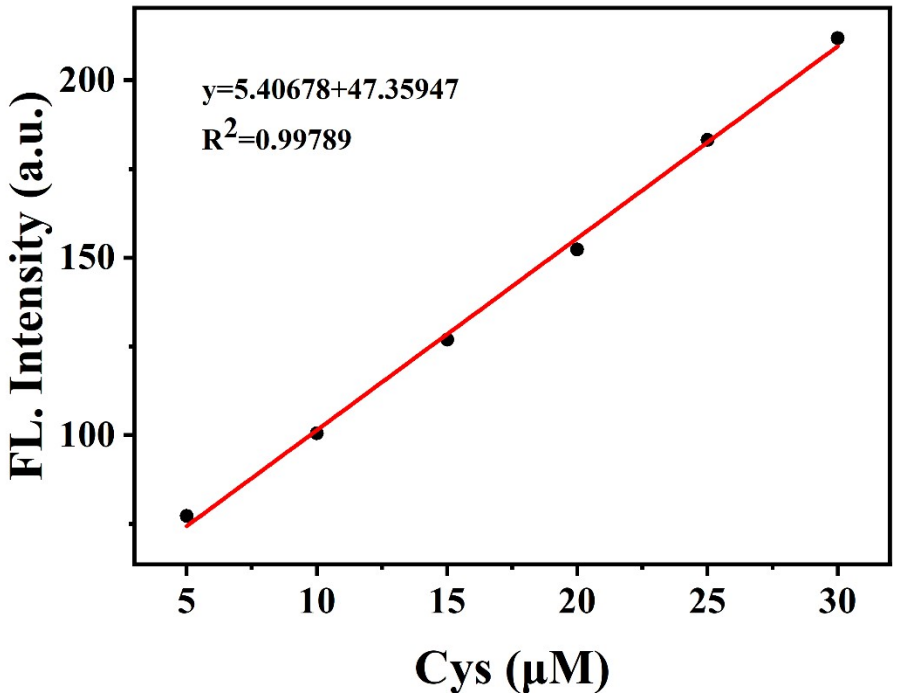


Fig. S8. Linear relationship of fluorescence intensity corresponding to varying Cys concentrations.

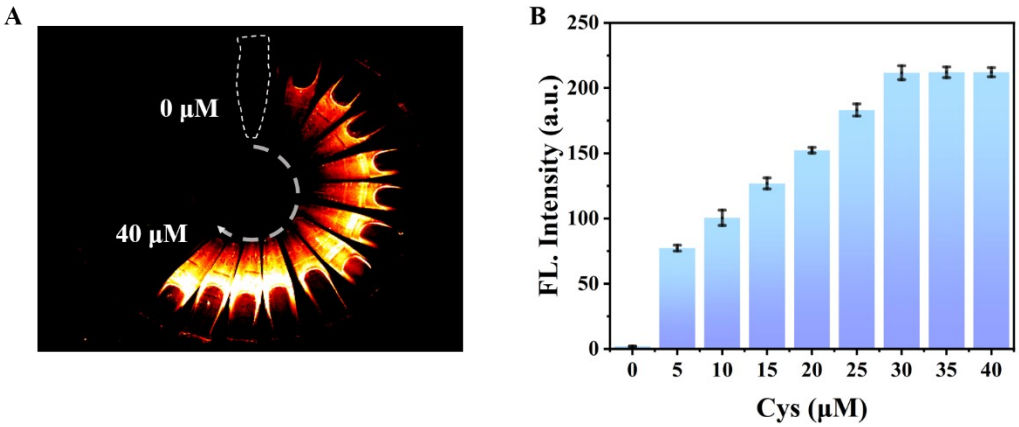


Fig. S9. (A) NIR-II FL imaging of **GY-G** after addition of different concentrations of Cys and the average fluorescence intensity of GY-G NIR-II FL imaging after adding different concentrations of Cys (0-40 μM) in a PBS solution (containing 20% DMSO). (B) The average fluorescence intensity of **GY-G** NIR-II FL imaging after adding different concentrations of Cys (0-40 μM)

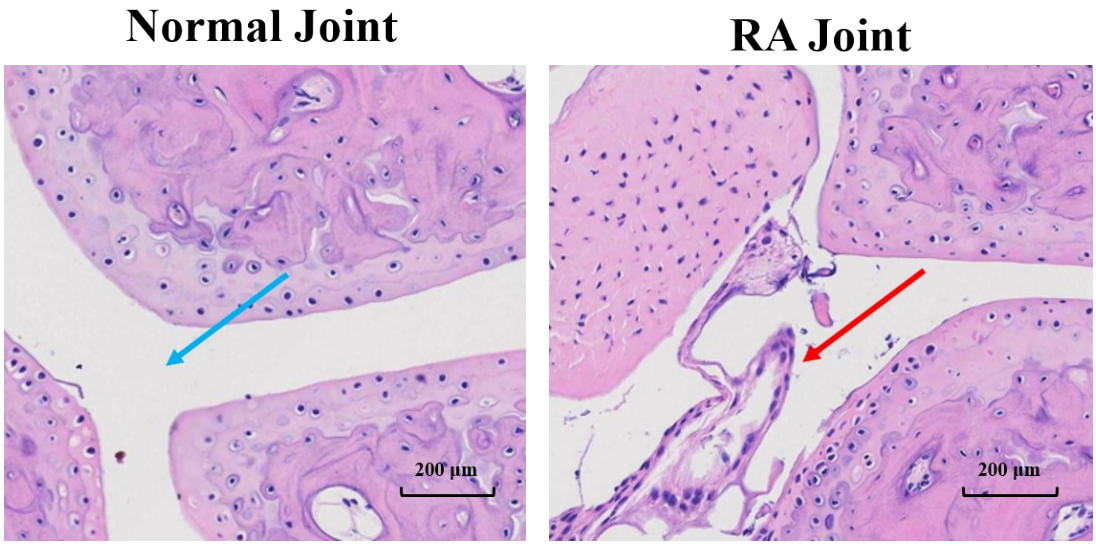


Fig. S10. H&E staining of arthritis model mice**.**

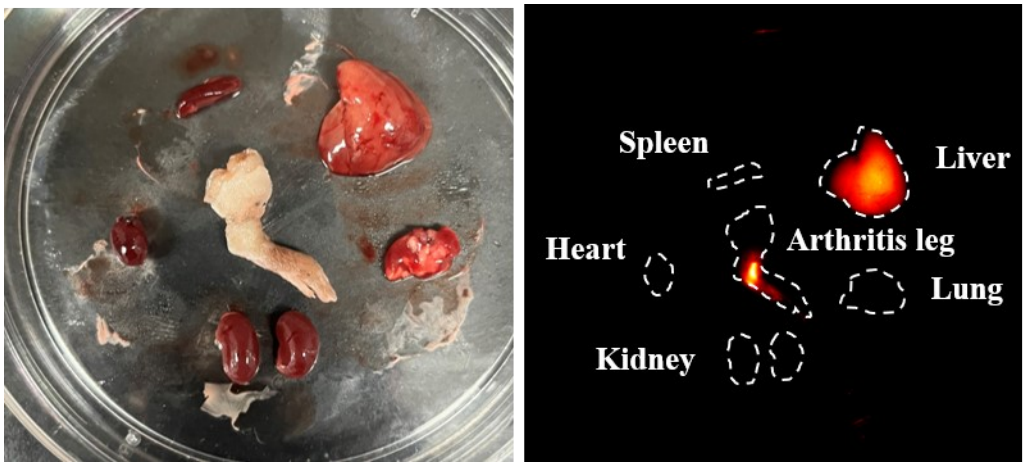


Fig. S11. NIR-II fluorescence imaging of major organs of RA model mice 24 h after injection of **GY-G**.

1. Wang, W.-X.; Chao, J.-J.; Wang, Z.-Q.; Liu, T.; Mao, G.-J.; Yang, B.; Li, C.-Y., Dual Key-Activated Nir-I/II Fluorescence Probe for Monitoring Photodynamic and Photothermal Synergistic Therapy Efficacy. *Advanced Healthcare Materials* **2023,** *12* (27), 2301230.