Supporting Information

Protein-Based Photothermal Theranostics for Imaging-Guided Cancer Therapy

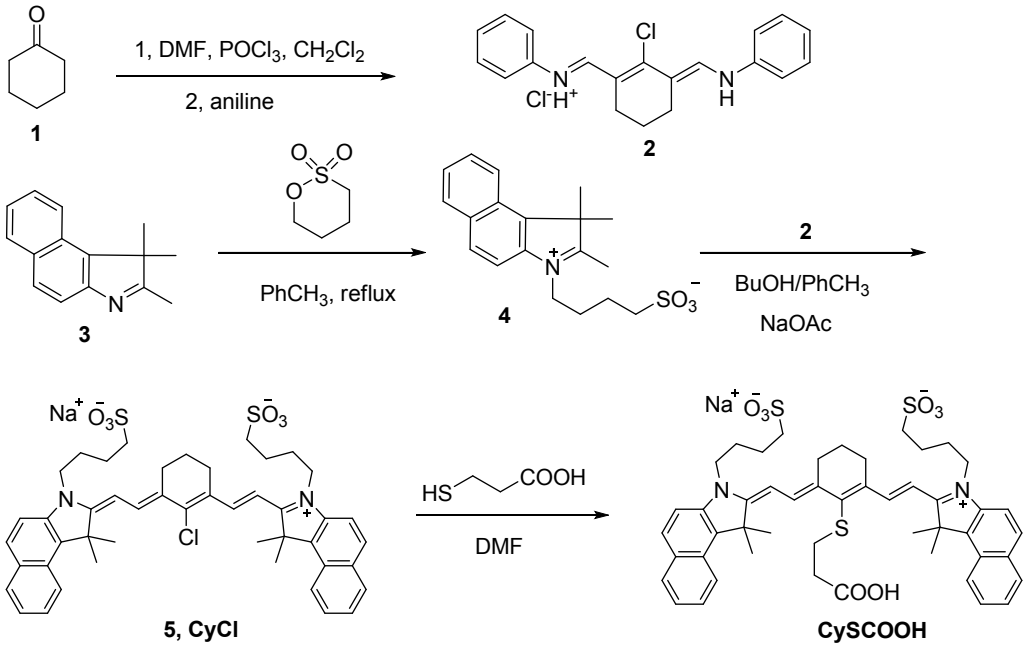

Fig. S1. Synthesis of CySCOOH.

Preparation of CySCOOH

N-[5-Anilino-3-chloro-2,4-(propane-1,3-diyl)-2,4-pentadiene-1-ylidene]anilinium Chloride (**2**). At 0 °C, phosphorus oxychloride (11 mL, 0.12 mol) was added dropwise from a pressure-equalizing addition funnel to anhydrous DMF (13 mL, 0.17 mol). After 30 min, cyclohexanone (**1**) (5.5 mL, 0.053 mol) was added and the mixture was refluxed for 1 h. Next, with constant cooling at 20 °C, an aniline/EtOH [1:1 (v/v), 18 mL] mixture was added dropwise. Reaction was continued for an additional 30 min after aniline addition, and then the deep purple mixture was poured into ice cold H_2O/ concentrated HCl (10:1, 110 mL). Crystals

were allowed to form for 2 h in an ice bath, then filtered, washed with cold H_2O and Et_2O, and then dried *in vacuo* to afford the product **2** (15.41 g, 90% yield) as a white soild. m.p. 220 °C, FAB-MS calcd for C20H20N2Cl 323.1 (M+), found 323.3; ^{1}H NMR (DMSO-*d*6, 200 MHz) δ 8.5 (s, 2H), 7.6-7.2 (m, 10H), 2.74 (t, 4H, J = 5.6 Hz), 1.85(m, 2H).

2,3,3-Trimethyl-1-(3-sulfobutyl)-3*H*-benzindolium, Inner Salt (**4**). Toluene (50 mL), 2,3,3-trimethyl-3*H*-ben**z**indolenine (**3**) (10 mL, 62.3 mmol), and 1,4-butanesultone (8.2 mL, 93.5 mmol) were heated under reflux for 18 h. The reaction mixture was allowed to cool to room temperature. The resulting pink crystals were filtered and washed with acetone (3 × 10 mL). The filtered product was crystalized from a solution of MeOH and Et_2O. The crystals were collected and dried *in vacuo* to afford the product **4** (14.88, 69.2% yield).

Bis-1,1'-(4-sulfobutyl)indoletricarbocyanine sodium salt (**5**). A solution of **4** (2.07 g, 6 mmol), **2** (0.969 g, 3 mmol), and anhydrous sodium acetate (600 mg, 7 mmol) in absolute EtOH (60 mL) under a N_2 atmosphere were heated under reflux for 3.5 h. The EtOH was removed under reduced pressure, and the residue was purified by preparative RP-HPLC to afford the product **5** (1.32, 53% yield).

To afford the desired product CySCOOH, a solution of **5** (255 mg, 0.30 mmol) in 4 mL of anhydrous DMF was added 3-mercaptopropionicacid (5 μL, 0.35 mmol) and TEA (30.5 μL, 0.35 mmol). The green solution was allowed to stir in the dark. After 15 h, the reaction was completed as monitored by HPLC. Crude CySCOOH was isolated by precipitation from the reaction by addition of 20 mL of acetone and was

washed with acetone (3 × 10 mL). Finally, CySCOOH was purified by recrystallization using DMF-acetone and afforded the desired product (143 mg, 45% yield) as a green powder. ^{1}H NMR (400 MHz, DMSO-*d*6): δ 8.88 (d, 2H, *J* = 14.0 Hz), 8.32 (d, 2H, *J* = 8.4 Hz), 8.08 (s, 4H), 7.81 (d, 2H, *J* = 8.9 Hz), 7.66 (t, 2H, *J* = 7.4 Hz), 7.52 (t, 2H, *J* = 7.4 Hz), 6.41 (d, 2H, *J* = 13.7 Hz), 4.35 (br s, 4H), 3.05 (t, 2H, *J* = 6.6 Hz), 2.74 (br s, 4H), 2.58-2.54 (m, 6H), 1.99 (s, 12H), 1.89-1.78 (m, 10H).ESI-MS [M]+ *m*/*z* calcd for C49H57N2O8S3 897.3, found 897.1.

Table S1. Characteristics of HSA@CySCOOH.

Sample	D/P ratio *	CE (%)
HSA@CySCOOH-2	0.9	45%
HSA@CySCOOH-4	1.7	43%
HSA@CySCOOH-12	4.1	34%

*D/P ratio denotes the amount of dye per HSA. CE denotes conjugation efficiency. Data represent mean ± SD, n = 3.

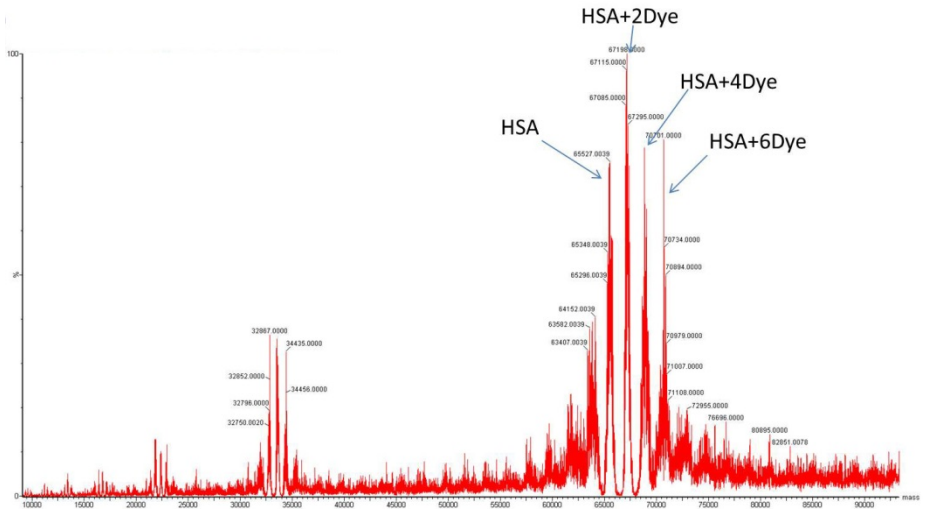

Fig. S2 LC/MS spectrum of HSA@CySCOOH.

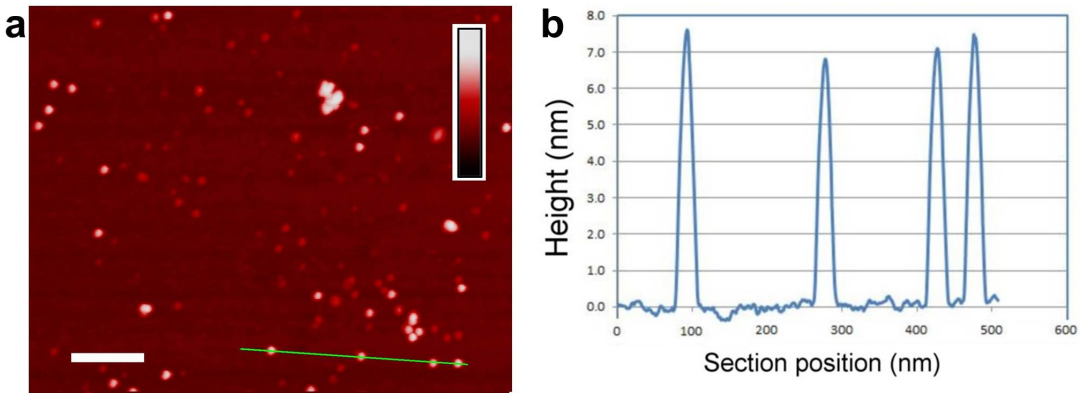

Fig. S3 AFM topography image (a) of HSA on mica substrate, with the height profile (b) along the line in the image.

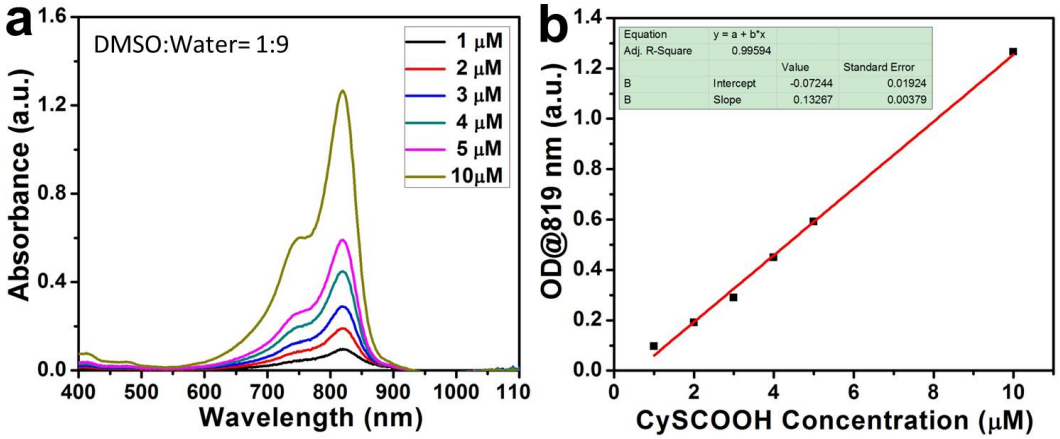

Fig. S4 (a) UV-Vis-NIR spectra of CySCOOH with different concentrations in DMSO:Water = 1:9 solution. (b) The optical density at 819 nm as a function of

CySCOOH concentration with a good linear relationship, described by the following equation: Y = 0.13267 X - 0.07244 (R^2 = 0.99594).

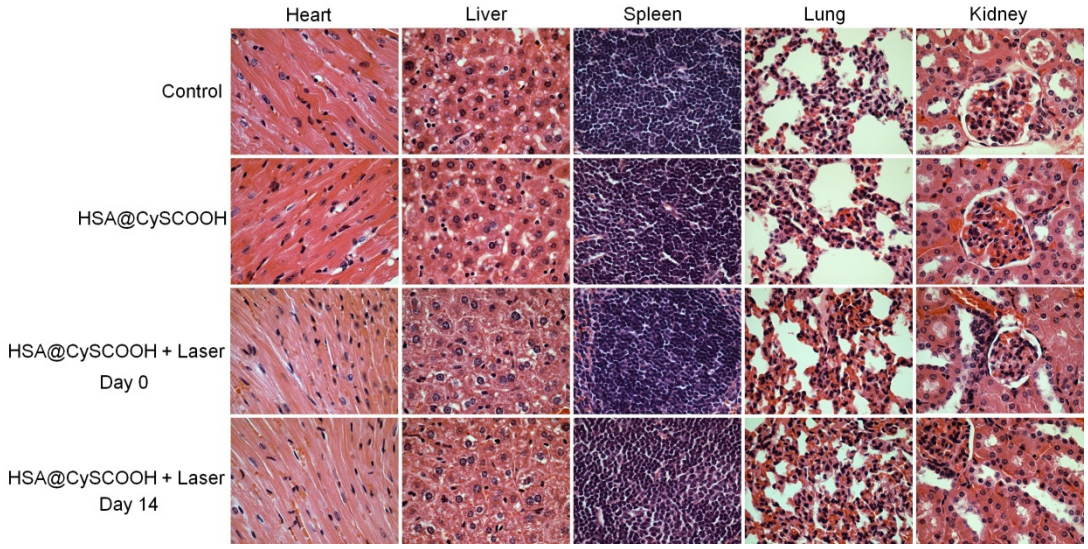

Fig. S5 H&E stained images of major organs collected from different groups of mice.

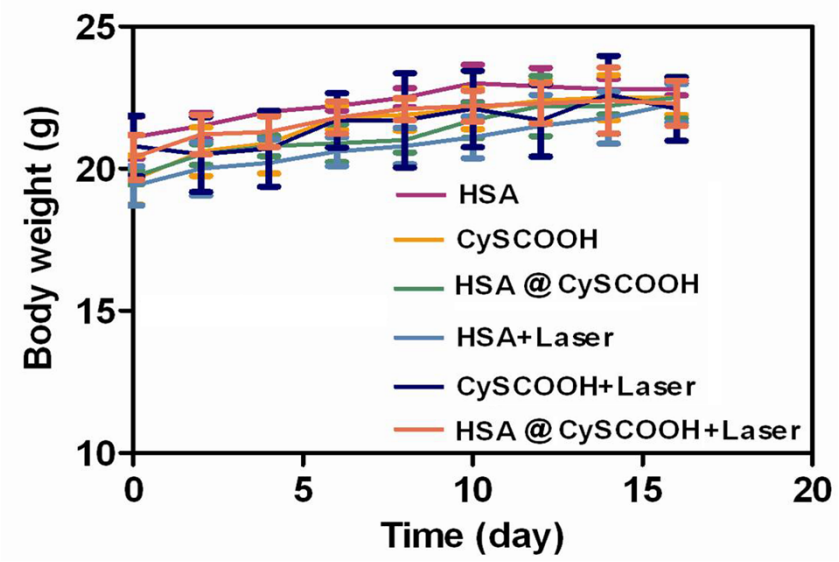

Fig. S6 Mice body weight changes of 4T1 tumor-bearing mice after various treatments indicated. The body weight of mice did not show significant change over 2 weeks.

References:

[1] Flanagan JH, Khan SH, Menchen S, Soper SA, Hammer RP. Functionalized tricarbocyanine dyes as near-infrared fluorescent probes for biomolecules. Bioconjugate Chem 1997;8:751-6

[2] Hilderbrand SA, Kelly KA, Weissleder R, Tung CH. Monofunctional near-infrared fluorochromes for imaging applications. Bioconjugate Chem 2005;16:1275-81