Supporting information for:

Optical Imaging of Bacterial Infection in Living Mice Using a Fluorescent Near-Infrared Molecular Probe

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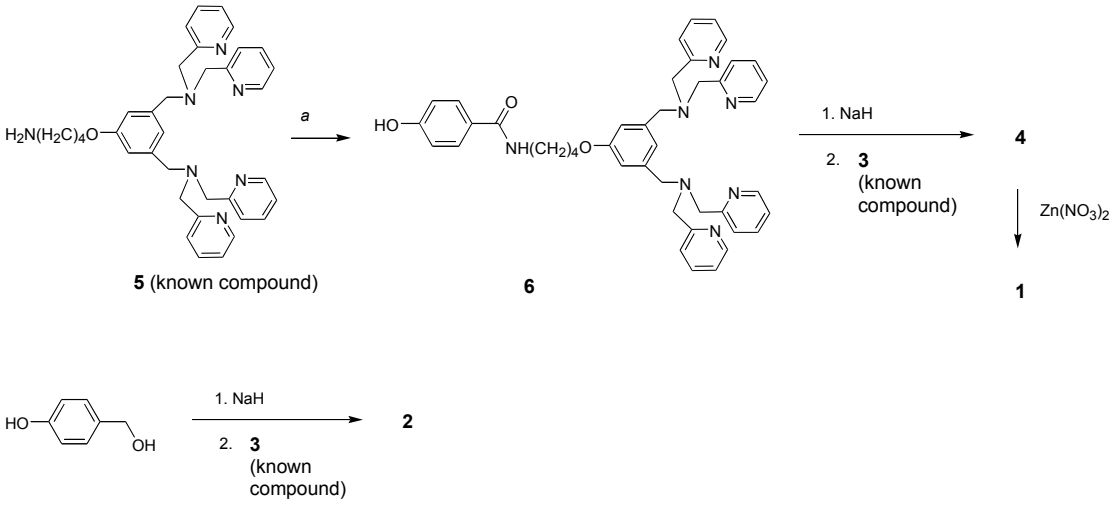
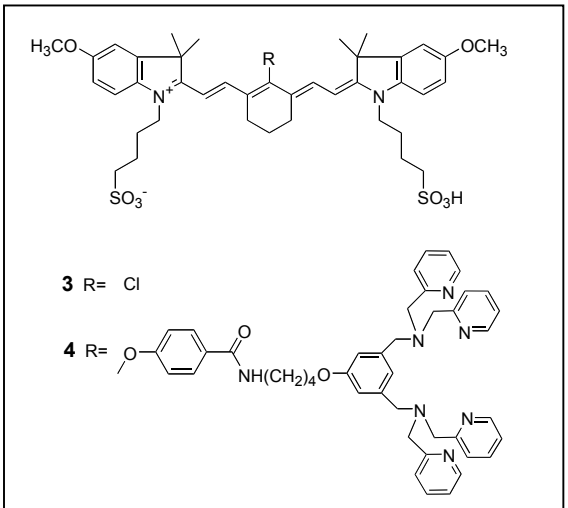
1) Synthesis of 1 and 2



Scheme S1. *a*. 4-hydroxybenzoic acid, EDC/HOBt, DMF.



Scheme S2. Structures of synthetic intermediates

Compound **6**:

To a solution of 4-hydroxybenzoic acid (0.15g) in DMF (10mL) was added EDC (0.74g) and HOBt (15mg), and after 10 min, amine **5**[1] (0.5g) was added. The solution was stirred at ambient temperature for 24 h. The solvent was removed by evaporation, and the crude compound was purified by silica gel column (chloroform/5%methanol) to afford **6** (0.39g). ^{1}H NMR ($CDCl_3$, ppm): 8.48 (4H, d, J=4.5Hz), 7.56-7.66 (10H, m), 7.16 (4H, m), 7.06 (1H, s), 6.81 (1H, t, J=5.9), 6.73 (4H, m), 3.97 (2H, t, J=5.4Hz), 3.77 (8H, s), 3.59 (4H, s), 3.49 (2H, m), 1.85 (4H, m). ^{13}C

NMR ($CDCl_3$, ppm): 167.84, 160.68, 159.54, 158.96, 148.80, 140.42, 136.93, 128.91, 125.74, 123.03, 122.30, 121.79, 115.82, 113.92, 67.77, 59.92, 58.73, 39.95, 27.19, 26.19. Mass (FAB^+): m/z 708 (M+1).

Dye conjugate **4**:
Under nitrogen atmosphere, a slurry of NaH (60% in mineral oil, washed with hexane, 9 mg, 1.2 eq.) and DMF (5mL) was added to a stirring solution of **6** (155 mg, 1.1 eq.) in anhydrous DMF at 0°C. After 30 min the reaction was allowed to warm up to ambient temperature. This solution was added to a solution of **3**[2] (150 mg, 1eq.) in anhydrous DMF. The reaction was stirred for 2 h, then quenched with water. The solvent was evaporated by vacuum at 40 °C. The dye conjugate **4** was purified by column chromatography (silica gel, chloroform/20-30% methanol) to yield 200 mg of a solid. ^{1}H NMR (CD_3OD/10% $CDCl_3$, ppm): 8.45 (4H, d, J=5Hz), 7.96 (2H, d, J=9.1Hz), 7.85-7.74 (6H, m), 7.62 (4H, d, J=8Hz), 7.26 (4H, m), 7.18 (4H, m), 7.00 (1H, s), 6.90 (4H, m), 6.82 (2H, s), 6.11 (2H, d, J=14Hz), 4.08 (4H, br), 3.98 (2H, t, J=5.4Hz), 3.84 (8H, s), 3.77 (6H, s), 3.68 (4H, s), 3.45 (2H, m), 2.89 (4H, t, J=3.6Hz), 2.74 (4H, br), 1.93 (14H, m), 1.32 (12H, s). ^{13}C NMR (CD_3OD/10% $CDCl_3$, ppm): 172.46, 168.90, 163.43, 163.30, 160.57, 159.51, 149.35, 143.98, 141.71, 140.51, 138.83, 136.90, 131.01, 129.69, 124.64, 123.94, 122.93, 122.13, 115.73, 115.35, 114.60, 112.70, 109.94, 100.80, 68.59, 60.45, 59.64, 56.45, 51.74, 50.32, 45.05, 40.69, 28.37, 27.80, 27.24, 25.24, 23.49, 22.35. Mass (FAB^+): m/z 1459 $(M+1)^+$; LC-ESMS: single peak at 7.60 min (see Figure S3) with m/z 730.6 $(M+2)^{2+}$.

Probe **1**:
To a solution of dye conjugate **4** (15 mg) in 10 mL methanol was added zinc nitrate (6 mg, 2.0 equiv.). The solution was stirred at room temperature for 30 min. The solution was evaporated to give the molecular probe **1** without further purification. The ^{1}H NMR spectrum for probe **1** in water is strongly broadened due to aggregation (see Section 3 below). Photophysical properties of probe **1** in water: Absorption λ_{max} = 794 nm, emission λ_{max} = 810 nm, ε = 1.1 x 10^5 $M^{-1}cm^{-1}$, quantum yield = 0.14. The quantum yield was measured in water at 25 °C using IR-125 as a reference dye with a quantum yield of 0.13 in DMSO.[3]

Control fluorophore **2**:
The procedure is similar to the preparation of **4**. Condensation of dye **3** (20 mg, 1equiv.), NaH (1 mg, 1.2 eq) and 4-hydroxybenzyl alcohol (4 mg, 1.1 equiv.) generated fluorophore **2** (18 mg). The ^{1}H NMR spectrum for compound **2** in water is strongly broadened due to aggregation (see Section 3 below). LC-ESMS: single peak at 6.75 min (see Figure S4) with m/z 875.6 $(M+1)^+$, 437.6 $(M+1)^{2+}$, 438.6 $(M+2)^{2+}$. Photophysical properties in water: Absorption λ_{max} = 787 nm (ε = 1.5 x 10^5 $M^{-1}cm^{-1}$), emission λ_{max} = 810 nm.

References: (1) Lakshmi, C.; Hanshaw, R. G.; Smith, B. D. *Tetrahedron*. **2004**, *60*, 11307-11315.
(2) Narayanan, N.; Patonay, G. *J. Org. Chem.*, **1995**, *60*, 2391-95. (3) Benson, R. C.; Kues, H. *J. Chem. Eng. Data* **1977**, *22*, 379-383.

2) NMR and LC-MS spectra

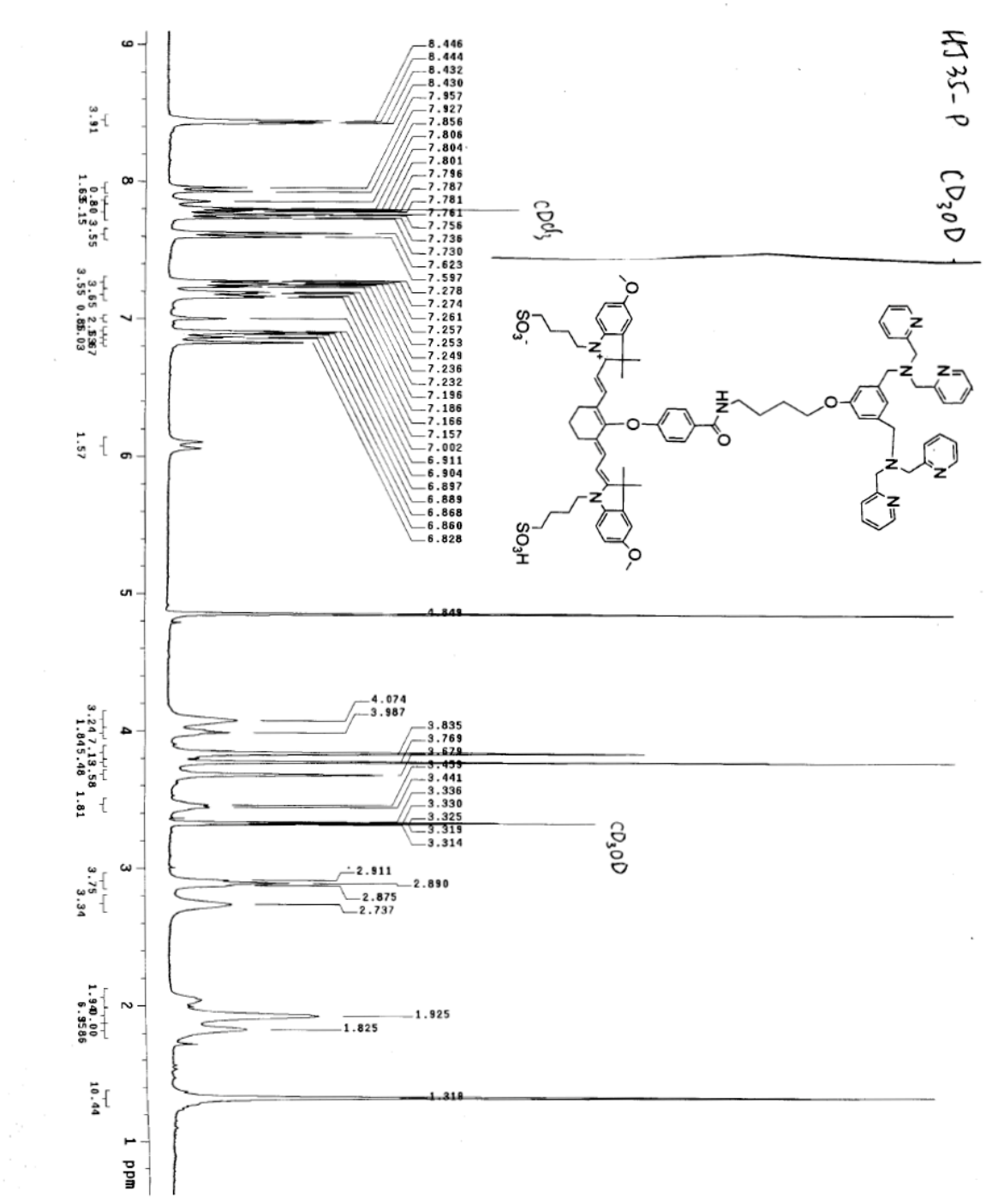


Figure S1. ^{1}H NMR of **4** in CD_3OD/10% $CDCl_3$.

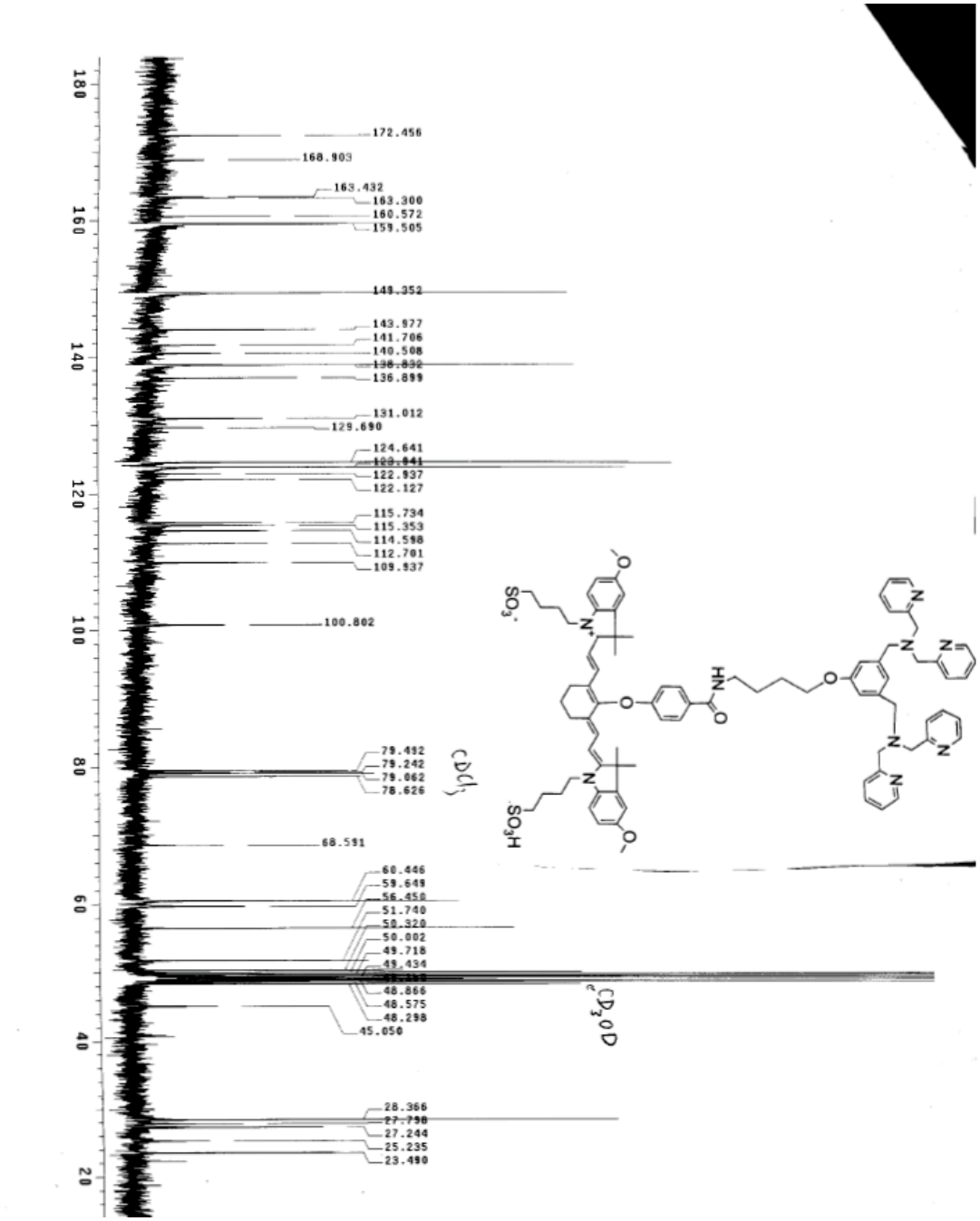


Figure S2. ^{13}C NMR of **4** in CD_3OD/10% $CDCl_3$.

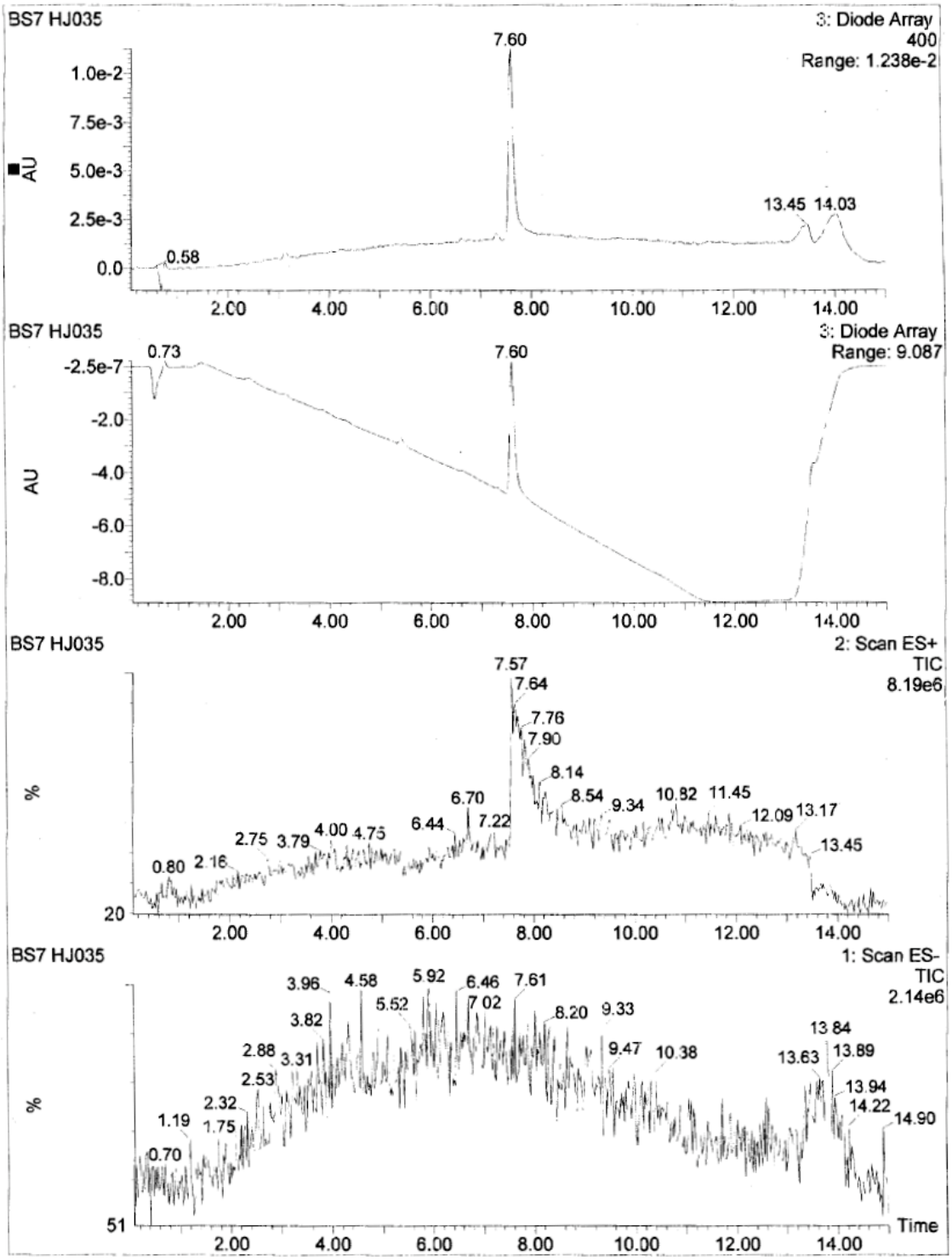


Figure S3a. LC-MS of **4**.

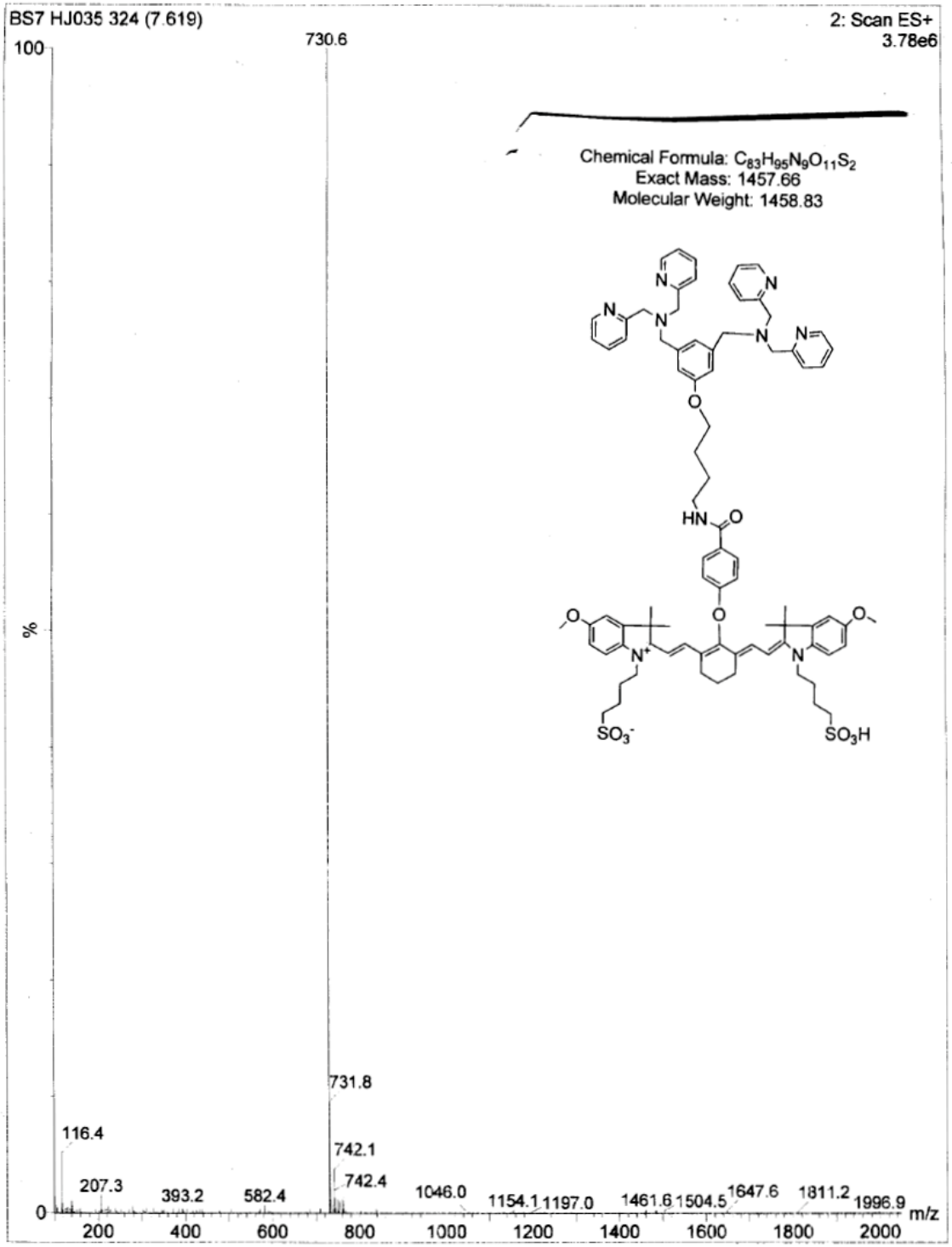


Figure S3b. LC-MS of **4**.

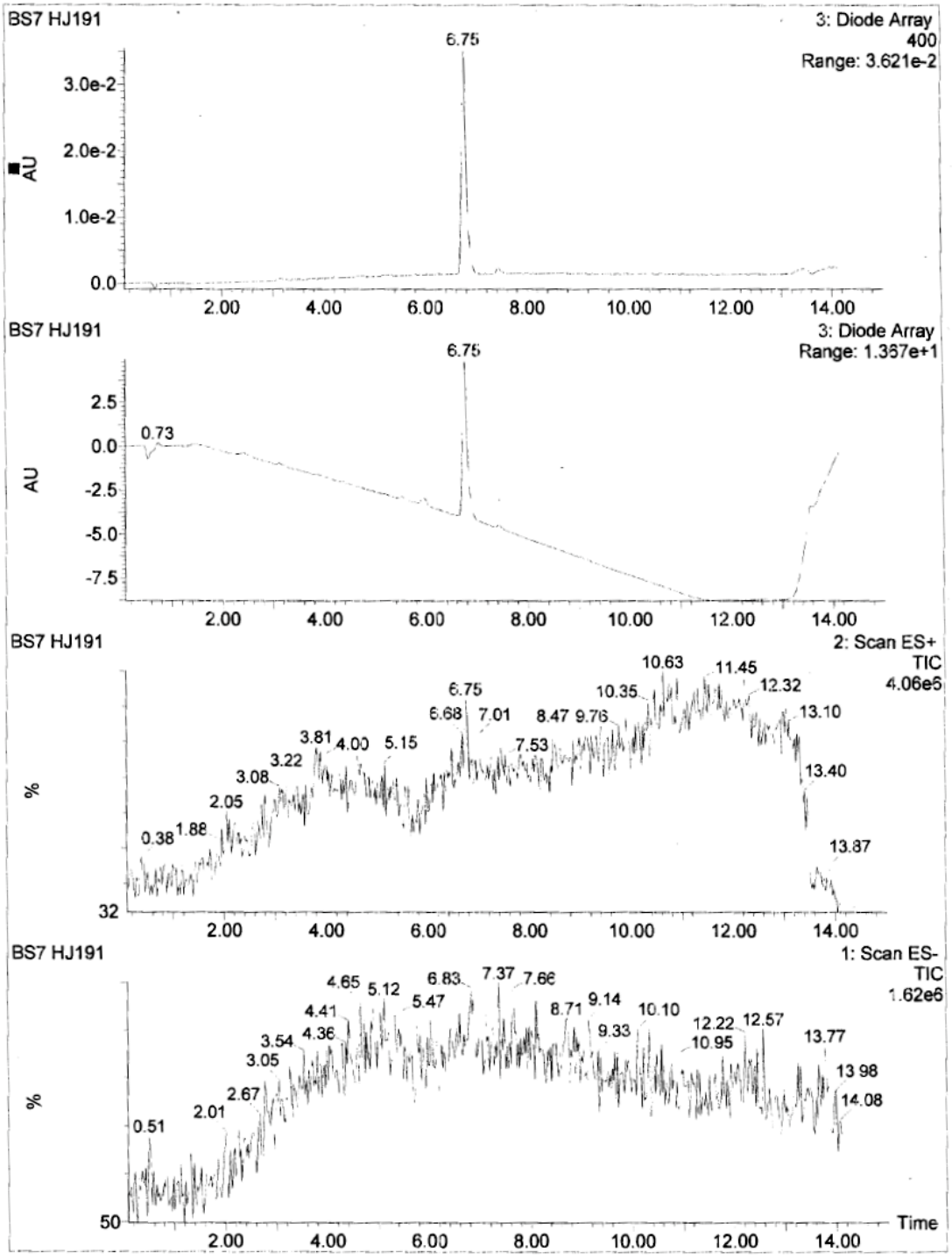


Figure S4a. LC-MS of **2**.

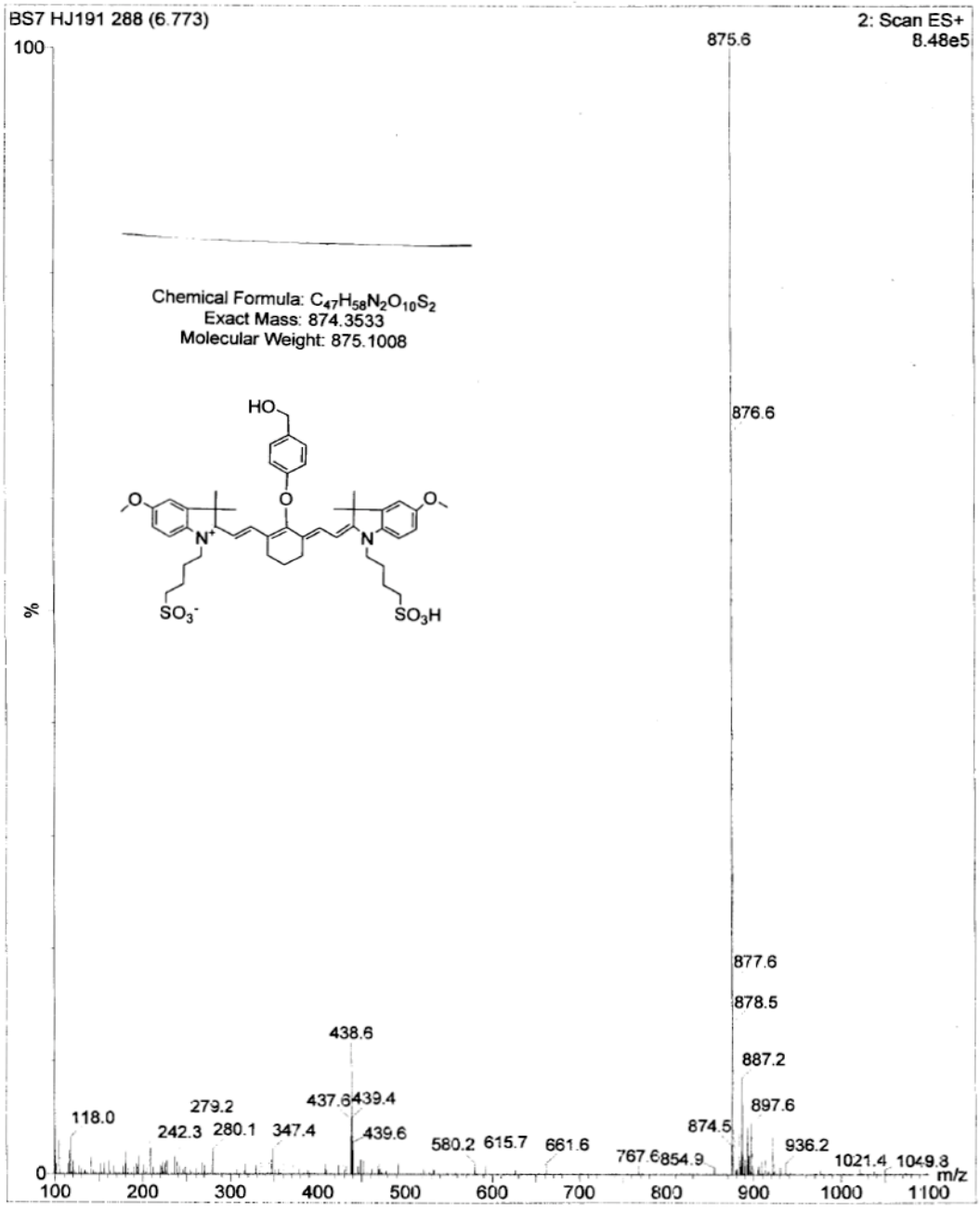


Figure S4b. LC-MS of **2**.

3) Spectral properties of 1 and 2

Like most cyanine dyes (Mishra et al. *Chem. Rev.* **2000**, *100*, 1973), probe **1** and control fluorophore **2** aggregate in pure water, which induces absorption broadening and modest self-quenching of the fluorescence (Figure S5). This means that the spectral properties of these dyes in pure water may not have much relevance to the spectral properties inside a living animal. For example, fluorescence emission from control fluorophore **2** is higher than probe **1** in pure water (Figure S6); however, addition of anionic liposomes induces a large, 15-fold increase in fluorescence intensity for probe **1** but no intensity change for control fluorophore **2** (Figure S7). We have previously reported on this effect in some detail (Lakshmi et al. *Tetrahedron*, **2004**, *60*, 11307-11315. Koulov et al. *Israel. J. Chem.* **2005**, *45*, 373-379). The emission from most fluorescent Zn-DPA probes increases when they bind to anionic membranes, apparently because of probe de-aggregation, chromophore rigidfication, and/or protection from the quenching effects of water. The data in Figure S7 is consistent with the observation in Figure 1 of the paper that probe **1** strongly stains anionic bacterial cell membranes. In contrast, control dye **2** has no measurable affinity for anionic membranes and does not associate with bacterial cells.

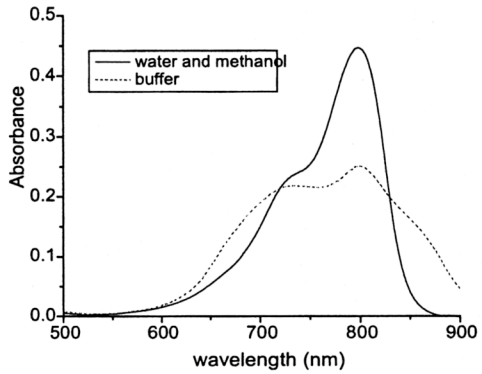


Figure S5. Absorption spectra of probe **1** (5 μM) in aqueous buffer (pH 7) and in 1:1 water/methanol. The broadened absorption in buffer is due to probe aggregation which induces exciton coupling and a blue-shifted "H-aggregate" band at 710 nm.

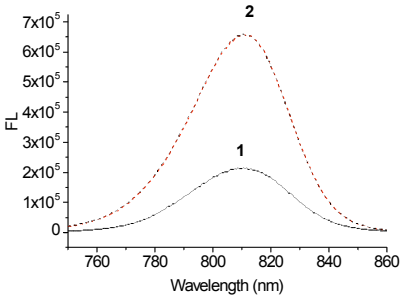


Figure S6. Fluorescence emission of **1** and **2** (1 μM) in water. λ_{ex} = 725 nm.

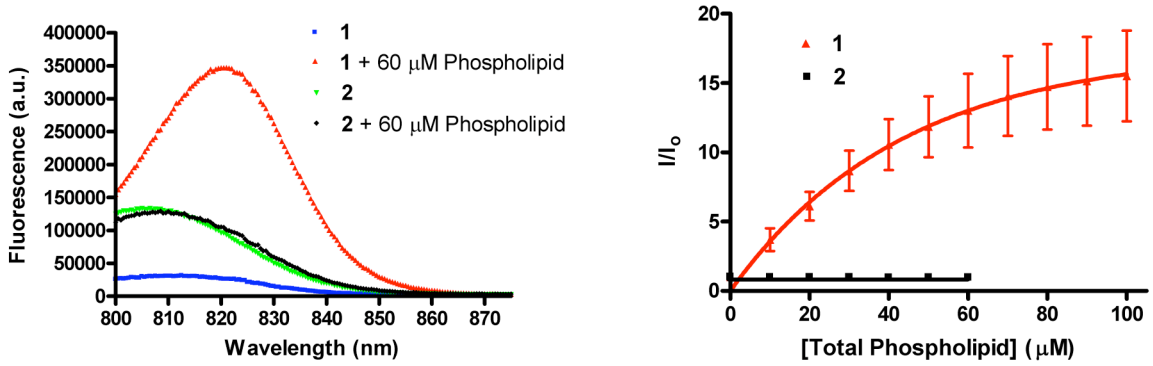


Figure S7. (*Left*) Fluorescence spectra for probe **1** and control fluorophore **2** in the presence and absence of anionic liposomes composed of POPC:POPG. (*Right*) Change in fluorescence emission for probe **1** and control fluorophore **2** upon addition of anionic liposomes composed of POPC:POPG. λ_{ex} = 750 nm.

4) Imaging procedures and MIC measurements.

4.1 *In vitro* imaging of bacteria

S. aureus NRS11 cells were grown to mid-log phase (OD = 0.5) in Luria Bertani (LB) Miller broth (5 g/L yeast extract, 10 g/L Tryptone, 10g/L NaCl), centrifuged (3500 R.P.M., 3.5 min.) and the pellet resuspended in 1 mL of buffer (5mM TES (N-Tris-(hydroxymethyl)-methyl-2-aminoethanesulfonic acid), 145 mM NaCl, pH = 7.4). Probe **1** or control fluorophore **2** were subsequently added (10 μM final conc.). Bacterial suspensions containing **1** and **2** were allowed to incubate for 10 min., and subsequently washed twice with buffer (as above) to reduce background fluorescence. Images were acquired on a Nikon Eclipse TE2000-U epifluorescence microscope using a 100X objective, 10X ocular and 1.5X optivar to obtain images at 1500X. Near infrared fluorescence images were captured using a Photometrics Cascade 512B CCD and a Cy7 filter set (Exciter HQ710/75x, Dichroic Q750LP, Emitter HQ810/90m).

4.2 *In vivo* imaging of mice injected with pre-labeled bacteria

S. aureus NRS11 or *E. coli* UTI89 cells were grown to OD_{600} = 0.5 in LB media and 1 ml aliquots were treated with either probe **1** or control fluorophore **2** (10 μM final). After incubation for 5 min, the cells were pelleted by centrifugation (17,000 RPM for 30 s) and the supernatant removed. The cells were resuspended in 50 μL of LB, and the resulting suspensions injected into the thigh muscles of nude mice (male, 5-6 week old, Strain NCRNU, Taconic, Hudson, NY). One mouse was given an injection of *S. aureus* and another mouse was injected with *E. coli*. The left thigh of each mouse was injected with cells pre-labeled with probe **1**, while the right side was injected with bacteria that had been pre-incubated with control fluorophore **2.** The mice were imaged using a Kodak 4000MM Imaging Station with the recommended Cy7 filter set (Exciter x720, Emitter e790WA). Images were background subtracted (rolling ball method, 500 pixels) and scaled with *Fire Look-Up Table* using the ImageJ software (v1.36b). The image of a mouse after injection of pre-labeled *E. coli* is given in the left side of Figure S8.

4.3 *In vivo* targeting of a bacterial infection with probe 1

The experimental details are described in the text of the paper. Shown in the right side of Figure S8 is targeting of probe **1** to an *E. coli* infection in a living mouse.

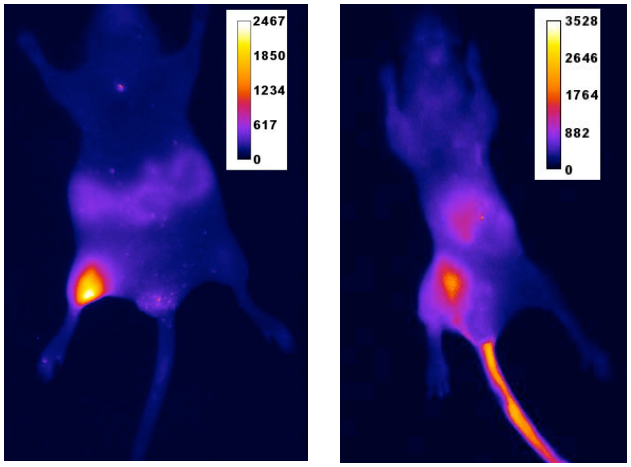


Figure S8. *Left*: Optical image of a mouse with intramuscular injections of *E. coli* cells that have been pre-labeled with **1** or **2** (left and right leg, respectively). *Right*: Optical image of a mouse given an intramuscular injection of unlabeled *E. coli* (left leg) followed by an intravenous injection of **1**. Image taken 18 h post injection with **1**. Scale represents relative fluorescence intensity.

4.4 Measurements of MIC

The minimum inhibitory concentration (MIC) is the lowest serial 2-fold dilution that prevents bacterial growth as outlined by the National Committee for Clinical Laboratory Standards (NCCLS). *S. aureus* NRS11 cells were tested using a bacterial load of 5×10^5 C. F. U./mL. Cells were grown at 37 ◦C in 2 mL of Luria Bertani (LB) Miller media (10 g/L peptone, 5 g/L yeast extract, 10 g/L, NaCl) that were 2-fold serially diluted with compound. The MIC is the lowest concentration that inhibited growth after 24 h as judged by visual turbidity. However, there was no inhibition of *S. aureus* growth by probe **1** or control **2** at the maximum tested concentrations of 100 μM and 400 μM, respectively.

4.5 Tissue distribution of probe 1.

Shown in Figure S9 are fluorescence images of the organs from six athymic nude mice that underwent the imaging procedure described in Figure 2 of the paper. Mice 4-6 had thigh injections of *S. aureus* followed 6 hours later by intravenous injection of probe **1**; whereas mice 1-3 are controls that were not infected and did not receive probe **1**. Mice 4-6 were sacrificed 21 h after probe **1** injection and dissected immediately. The major organs and tissue were imaged using the Kodak 4000MM Imaging Station. The selective accumulation of probe **1** in the infected thigh is immediately obvious. More minor accumulations of probe **1** are observed in the liver and kidney. The moderately high fluorescent signal from the intestines in all six mice is due to autofluorescence from digested food.

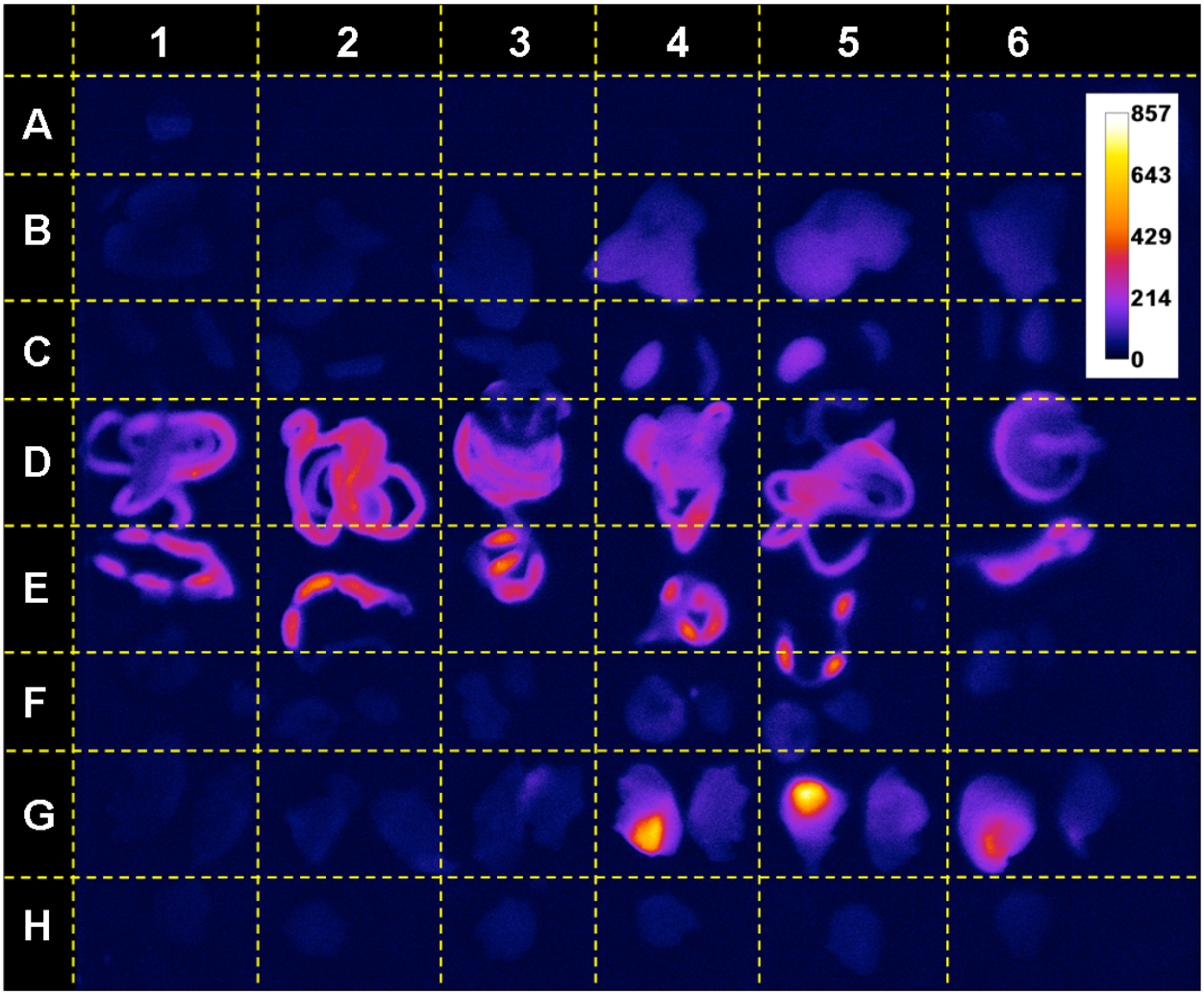


Figure S9. Tissue layout: Row A: Drop of Blood, Row B: Liver, Row C: One Kidney (left) and the Spleen (right),* Row D: Small Intestine, Row E: Large Intestine, Row F: Lungs (left) and Heart (Right), Row G: Left Rear Thigh Muscle (site of infection) and Right Rear Thigh Muscle, and Row H: Brain. *Location order of kidney and spleen is reversed for mouse 6.