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A Scalable Synthesis of Adjuvanting Antigen Depots Based on Metal-Organic Frameworks

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KEYWORDS: ZIF-8 encapsulation, MOF degradation analysis, biomimetic mineralization, immune response enhancement

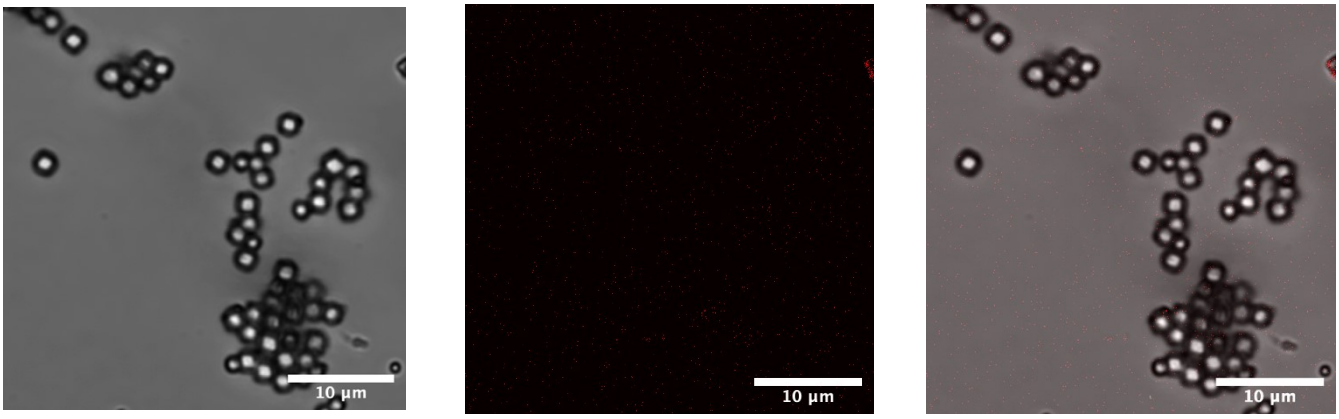

Figure S1. CLSM Images of µ-ZIF (negative control). Brightfield (left), fluorescent filter (middle), and merged channel (right) are shown. The lack of fluorescence in the fluorescent channel demonstrates that fluorescence is not from the ZIF-8.

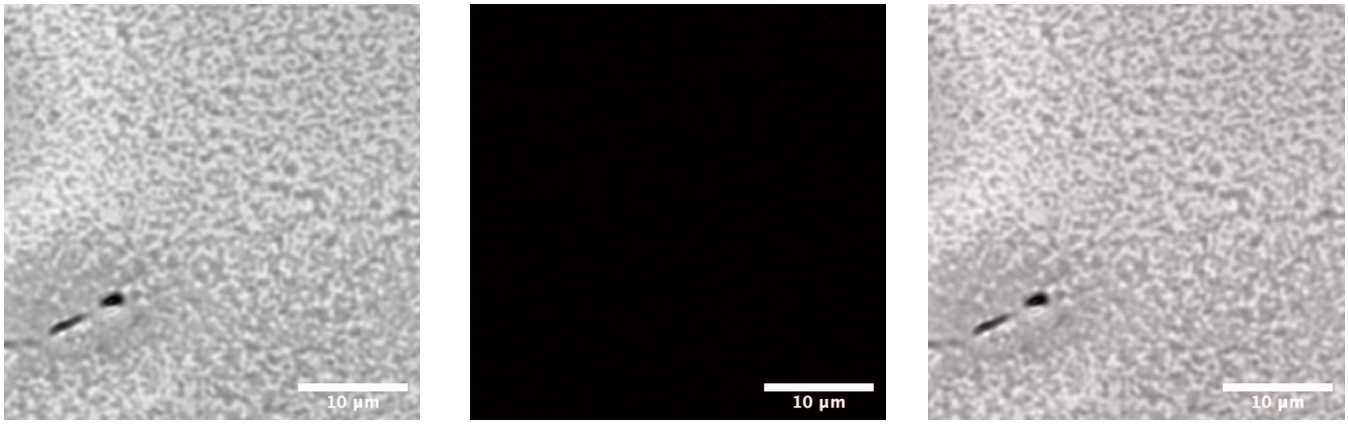

Figure S2. CLSM images of n-ZIF (negative control). Brightfield (left), fluorescent filter (middle), and merged channel (right) are shown. The lack of fluorescence in the fluorescent channel shows that fluorescence is not from the ZIF-8.

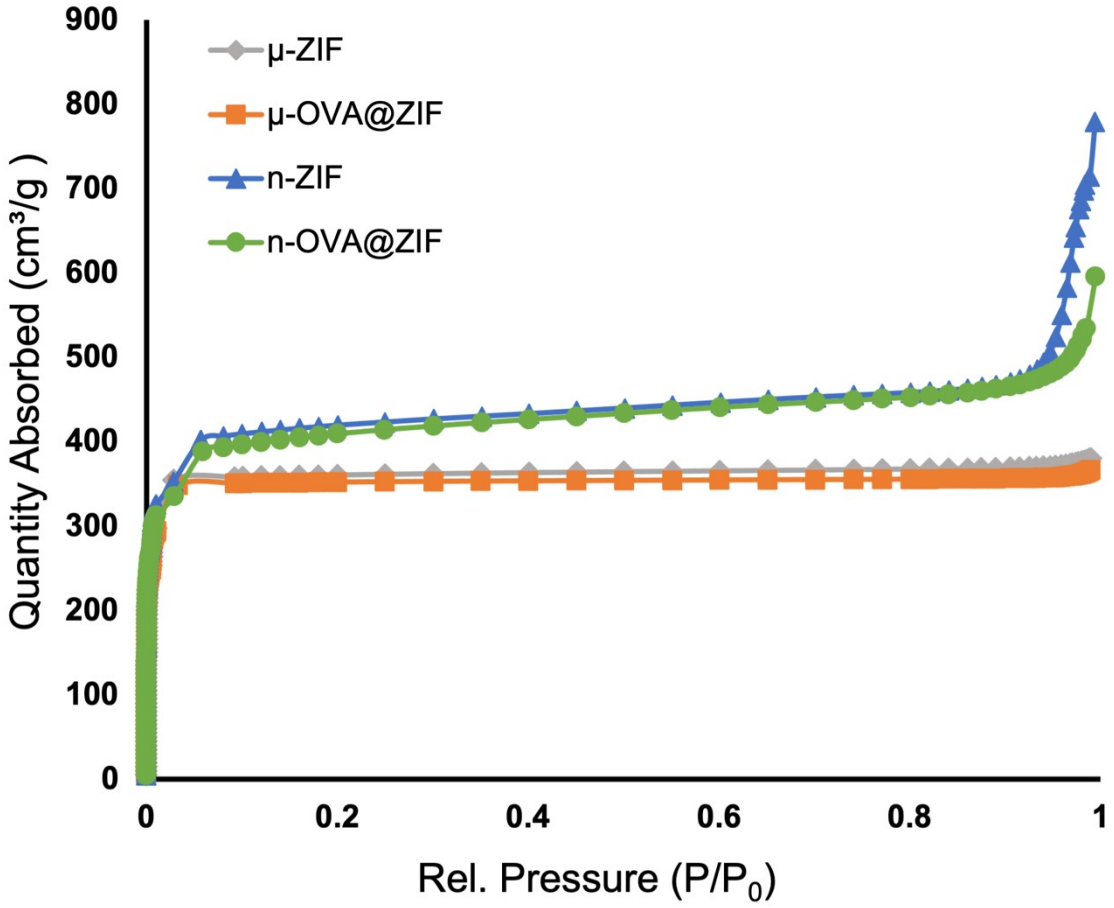

Figure S3. N_2 isotherms of ZIF and OVA@ZIF composites. Additionally, the Brunauer, Emmett, and Teller (**BET**) surface area was determined for all formulations (μ-ZIF:1405.5495 m²/g, μ-OVA@ZIF: 1376.5082 m²/g, n-ZIF: 1645.9917 m²/g, and n-OVA@ZIF: 1601.5486 m²/g).

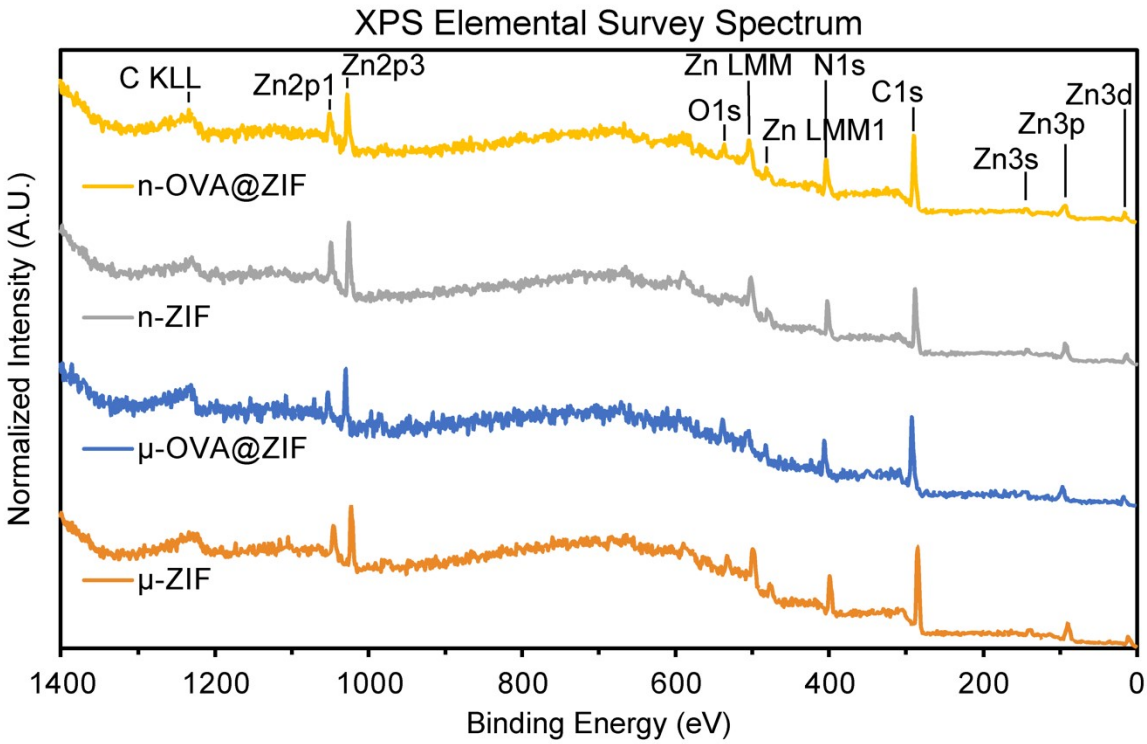

Figure S4. XPS elemental survey spectra for n-OVA@ZIF, n-ZIF, μ-OVA@ZIF, and μ-ZIF.

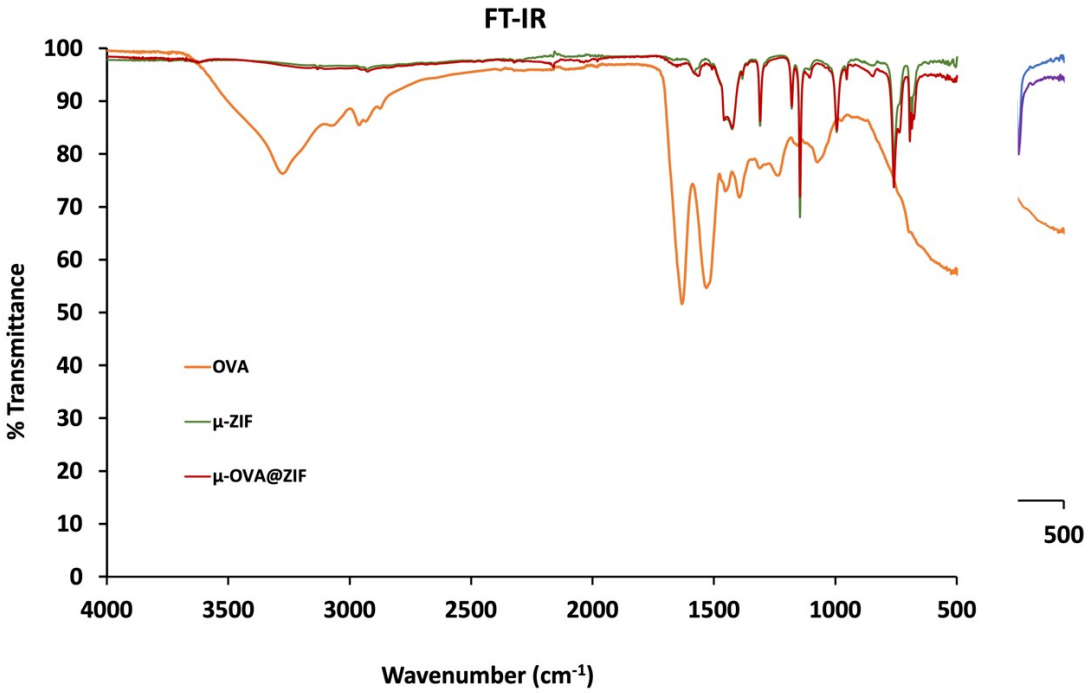

Figure S5. FT-IR spectra of n-ZIF and n-OVA@ZIF (top) and μ-ZIF and μ-OVA@ZIF (bottom).

Figure S6. Slight inflammation—raised red skin—at the injection site of n-OVA[Cy7]@ZIF post-injection.

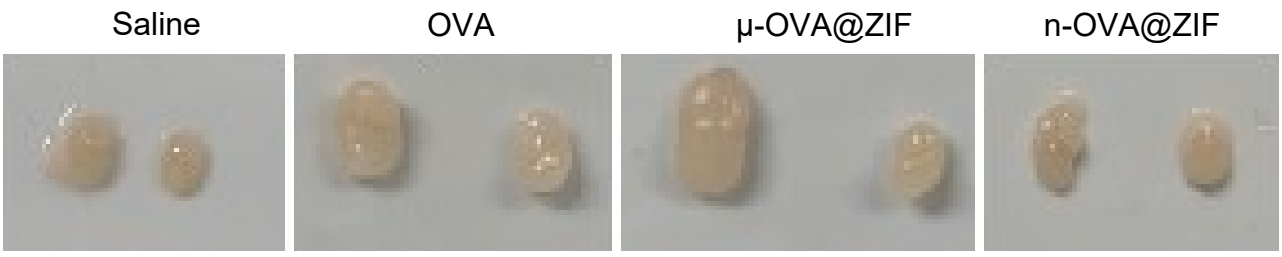

Figure S7. Extracted ILN (left) and CILN (right) on day 14 post single-dose vaccination.

Figure S8. Post-mortem surgery to extract μ-OVA@ZIF crystals 24 h post-vaccination.

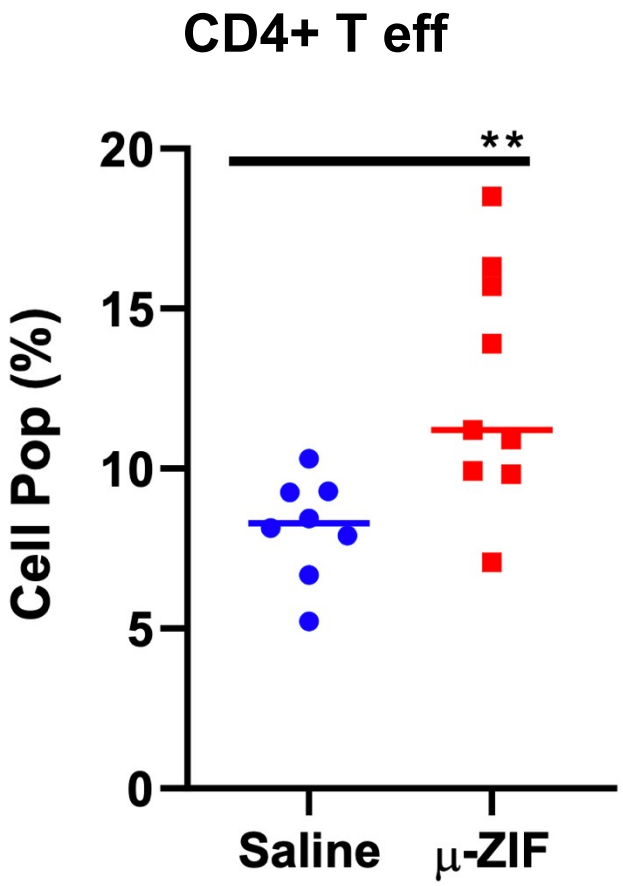

Figure S9. Percent cell populations (%) of CD4+ T eff cells (T helper cells) (CD3+/CD4+/CD44+CD62L-) in the ILN of female BALB/c mice 72 h post vaccination. Statistical significance was determined by an unpaired student's T-test (P>0.05 is ns, P≤ 0.05 is *, P ≤ 0.01 is **, P ≤ 0.001 is ***, and P ≤ 0.0001 is ****).

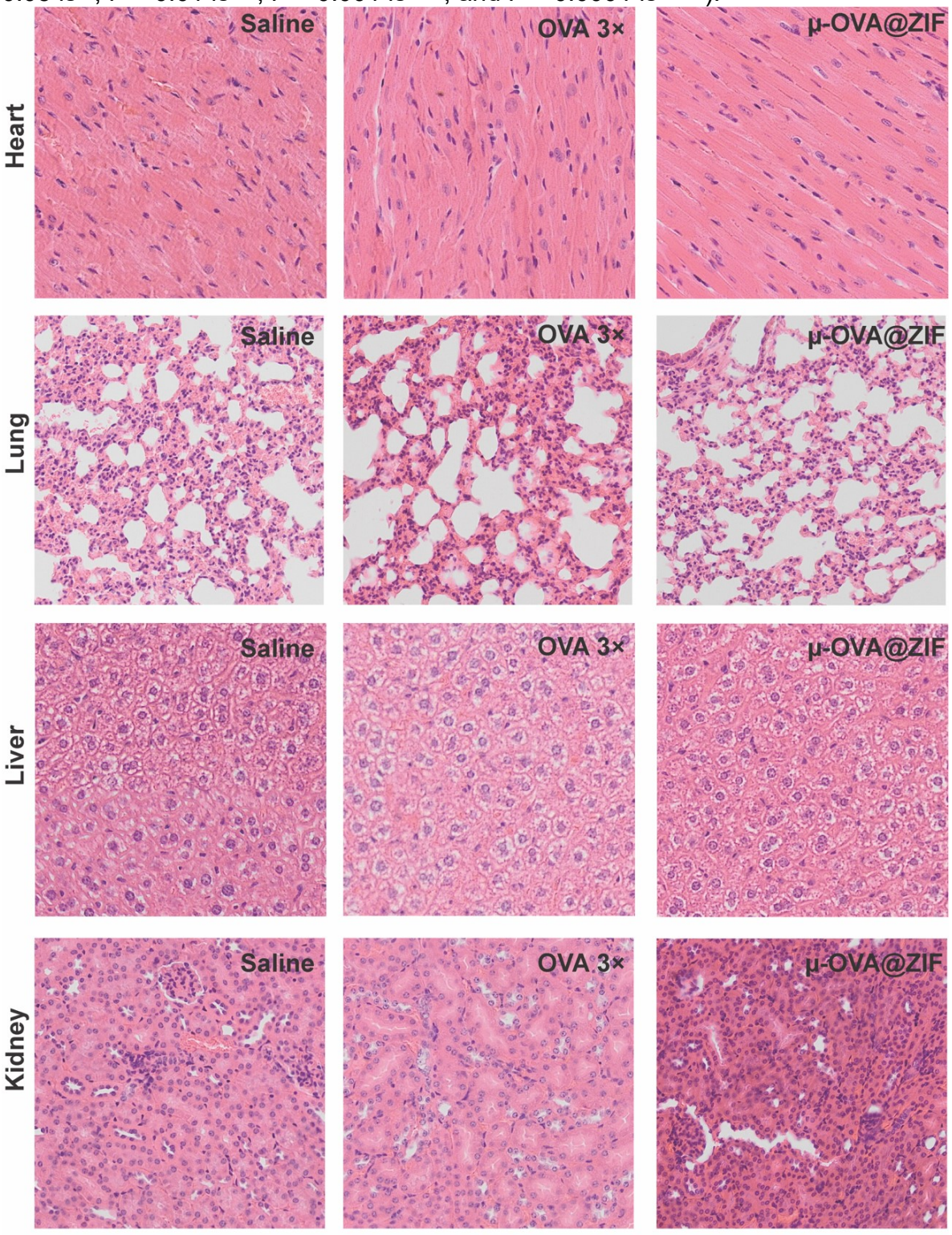

Figure S10. Day 21 H&E tissue staining of major organs.

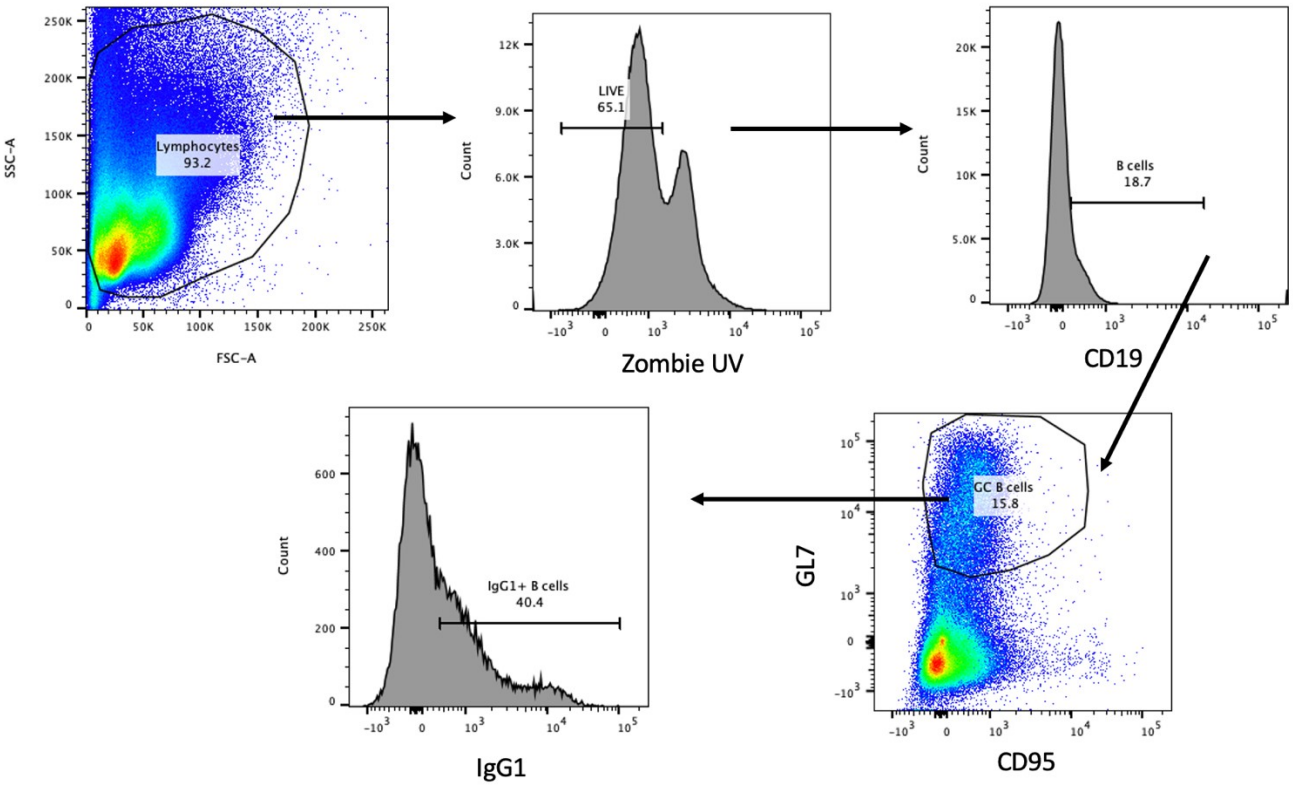

Figure S11. Gating strategy for GC B cells and IgG1+ GC B cells.

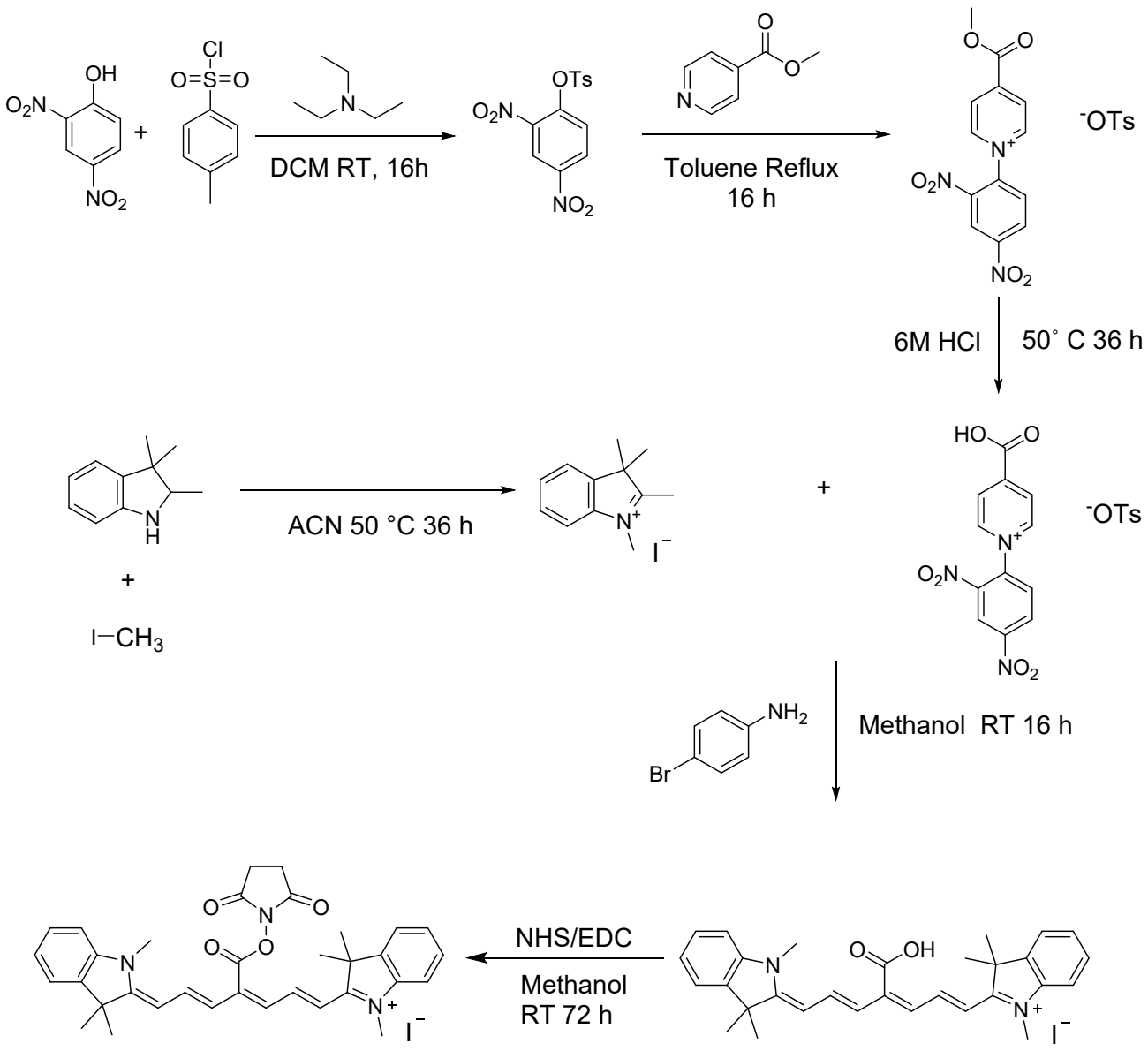

Figure S12. Synthetic Scheme for Cy7-NHS synthesis.

Cy7-NHS Synthesis

2,4-Dinitrophenyl p-Toluenesulfonate.
2,4-Dinitrophenol (2.50 g, 13.6 mmol) was dissolved in dichloromethane (50 mL), p-toluenesulfonyl chloride (2.84 g, 14.9 mmol), and triethylamine (3.45 g, 34.0 mmol) were added successively, and the mixture was stirred for 16 h at room temperature. 50 mL water was added, and the mixture was extracted with dichloromethane (3×30 mL). The organic phase was washed with $NaHCO_3$ (3×30 mL), brine (3×30 mL), and the organic fraction was evaporated under reduced pressure. The crude product was purified by trituration with hot methanol (30 mL) to give the pure product (white solid). Yield: 2.57 g (56%).
^{1}H NMR (500 MHz, d6– DMSO): δ (ppm) 8.83 (d, J = 2.8 Hz, 1H), 8.69 – 8.47 (m, 1H), 7.91 – 7.71 (m, 2H), 7.68 – 7.35 (m, 3H), 2.46 (s, 3H).

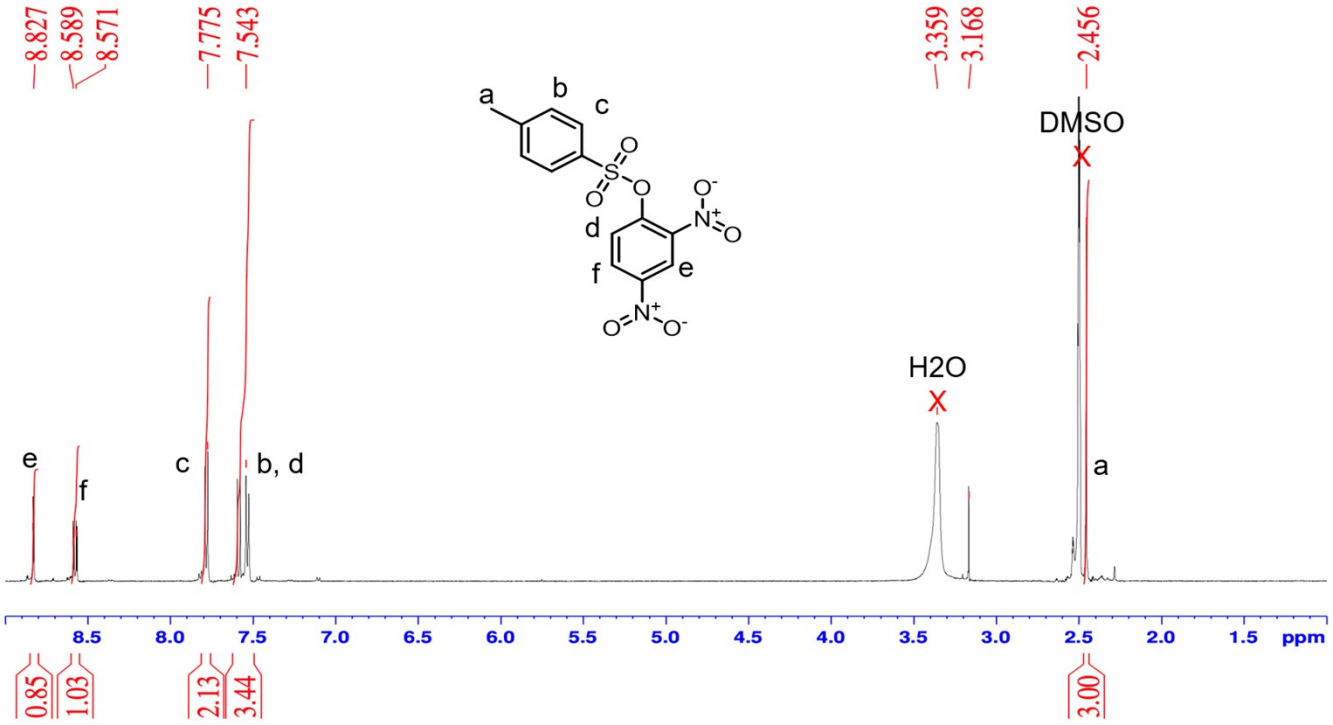

Figure S13. ^{1}H NMR of 2,4-Dinitrophenyl p-Toluenesulfonate

1-(2,4-Dinitrophenyl)-4-(methoxycarbonyl) pyridin-1-ium p-Toluenesulfonate

A mixture of 2,4-dinitrophenyl p-toluenesulfonate (2.57 g, 2.57 mmol) and methyl isonicotinate (0.940 g, 6.90 mmol) were dissolved in toluene (7 mL/mmol). The reaction was refluxed for 16 h and left to cool to room temperature. The precipitate was filtered, washed with toluene (2 × 5 mL) and Et_2O (2×5 mL), and dried. Yield: 0.78 g (24%).

^{1}H NMR (500 MHz, DMSO) δ 9.59 (dd, *J* = 6.7, 1.6 Hz, 2H), 9.12 (t, *J* = 1.8 Hz, 1H), 8.98 (dd, *J* = 8.7, 2.5 Hz, 1H), 8.88 – 8.75 (m, 2H), 8.40 (d, *J* = 8.7 Hz, 1H), 7.45 (dd, *J* = 8.2, 1.9 Hz, 2H), 7.10 (d, *J* = 7.8 Hz, 2H), 4.05 (s, 3H), 2.29 (s, 3H).

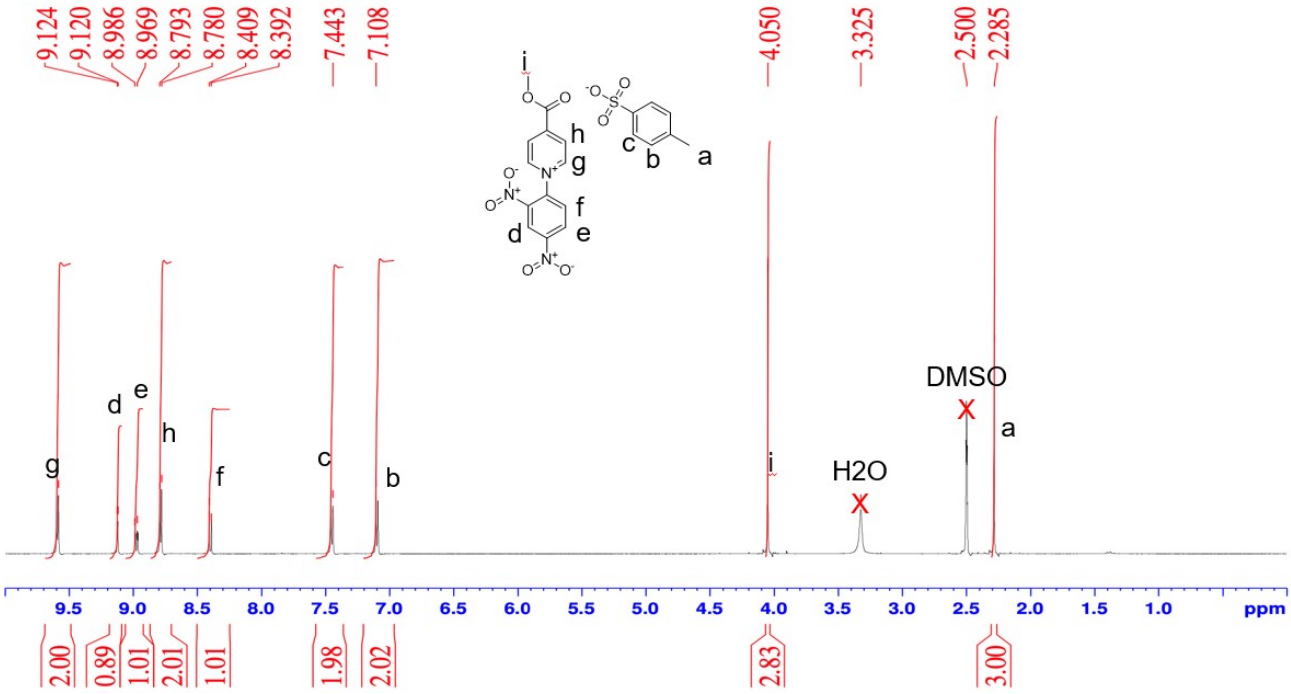

Figure S14. ^{1}H NMR of 1-(2,4-Dinitrophenyl)-4-(methoxycarbonyl) pyridin-1-ium p-Toluenesulfonate

4-Carboxy -1-(2,4-dinitrophenyl) pyridin-1-ium p-Toluenesulfonate

The carboxylic acid was prepared by hydrolysis of 1-(2,4-Dinitrophenyl)-4-(methoxycarbonyl) pyridin-1-ium p-Toluenesulfonate in aq HCl (6M, 50 mL) at 50 °C for 36 h. The solvent was evaporated at reduced pressure, and the crude product was purified by recrystallization from methanol (50 mL) to give the pure product as a white solid. Yield: 0.68 g (91%).

^{1}H NMR (500 MHz, DMSO) δ 9.59 (dd, *J* = 6.7, 1.6 Hz, 2H), 9.12 (t, *J* = 1.8 Hz, 1H), 8.98 (dd, *J* = 8.7, 2.5 Hz, 1H), 8.85 – 8.70 (m, 2H), 8.40 (d, *J* = 8.7 Hz, 1H), 7.45 (dd, *J* = 8.2, 1.9 Hz, 2H), 7.10 (d, *J* = 7.8 Hz, 2H), 2.29 (s, 3H).

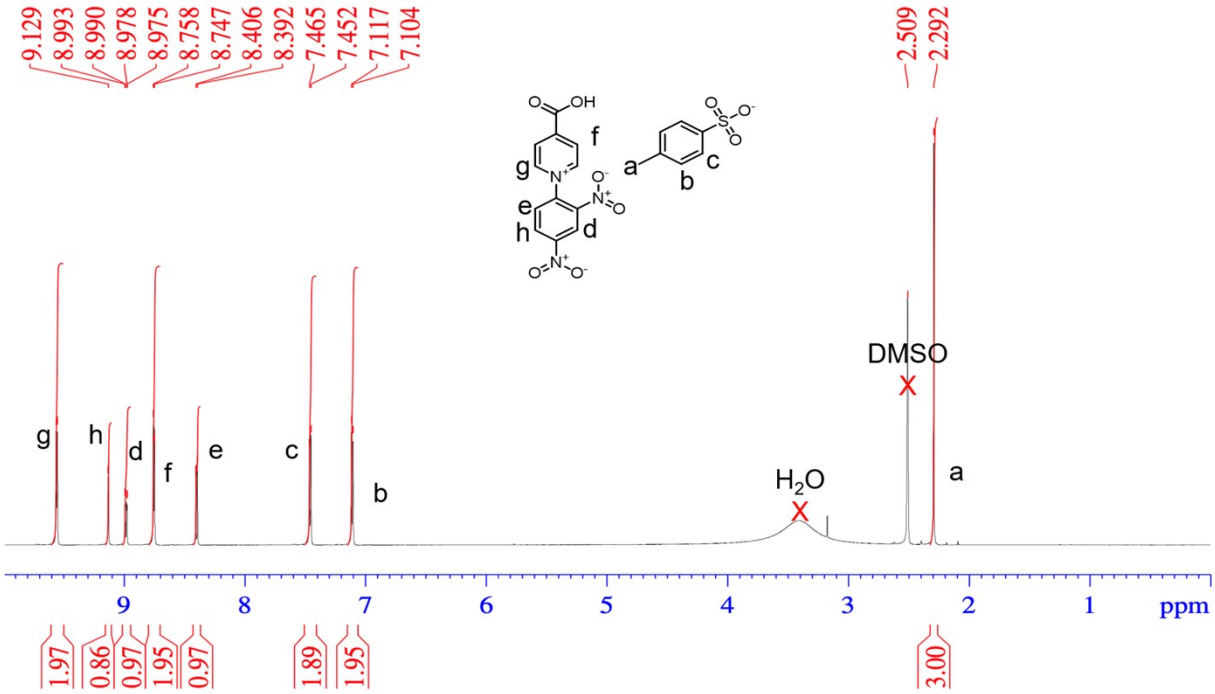

Figure S15. ^{1}H NMR of 4-Carboxy 1-(2,4-Dinitrophenyl)-4-(methoxycarbonyl)pyridin-1-ium p-Toluenesulfonate

1,2,3,3-tetramethyl-3H-indol-1-ium iodide

2,3,3-trimethylindoline (2.50 g, 15.7 mmol) and iodomethane (6.68 g, 47.1 mmol) were added to 25 ml of acetonitrile and refluxed for 5 h and cooled to RT. The white precipitate obtained was filtered and washed with acetone. The solid was dried under reduced pressure to afford 1,2,3,3-tetramethyl-3H-indol-1-ium iodide as a white solid. Yield: 2.79 g (59%). ^{1}H NMR (500 MHz, d6-DMSO) δ 7.94 – 7.86 (m, 1H), 7.86 – 7.79 (m, 1H), 7.66 – 7.57 (m, 2H), 3.97 (d, J = 1.0 Hz, 3H), 2.76 (d, J = 1.0 Hz, 3H), 1.52 (s, 6H). ^{13}C NMR (500 MHz, d6–DMSO): δ (ppm) 196.48, 124.58,142.08,129.79, 129.28, 123.76, 115.60, 54.40, 35.18, 22.18, 14.62.

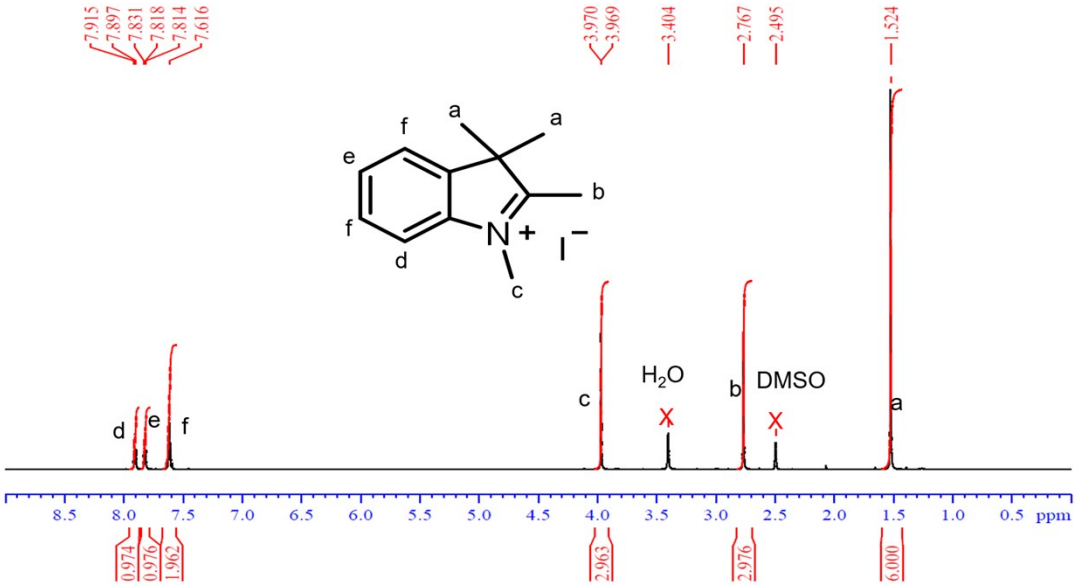

Figure S16. ^{1}H NMR of 1,2,3,3-tetramethyl-3H-indol-1-ium iodide.

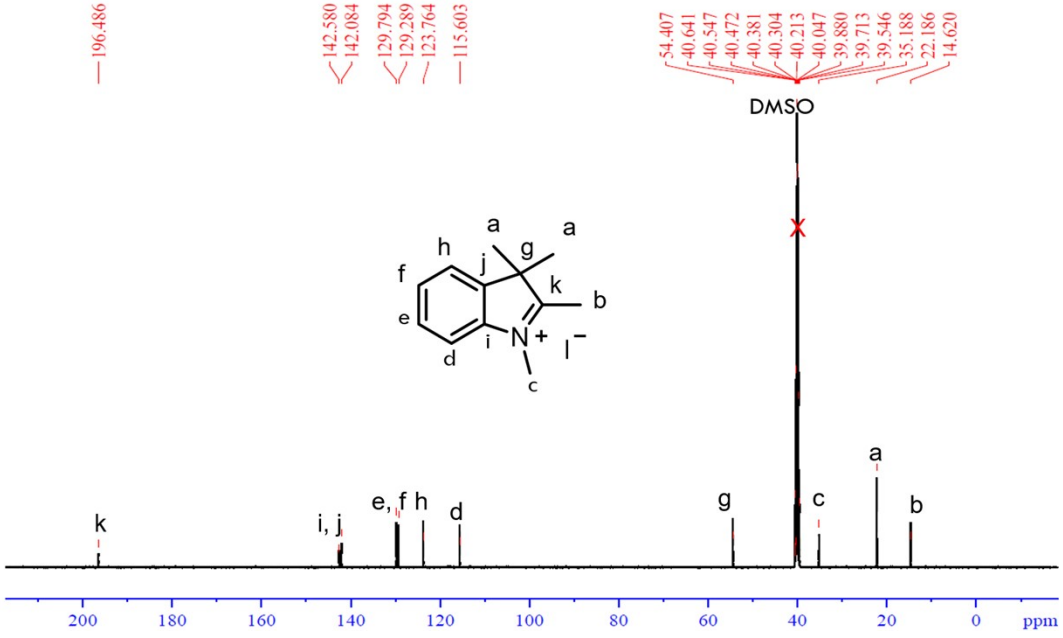

Figure S17. ^{13}C NMR of 1,2,3,3-tetramethyl-3H-indol-1-ium iodide.

2-((1E,3Z,5E)-4-carboxy-7-((Z)-1,3,3-trimethylindolin-2-ylidene) hepta-1,3,5-trien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide (Cy7)

4-Carboxy -1-(2,4-dinitrophenyl) pyridine-1-im p-Toluenesulfonate (161 mg, 0.540 mmol) and 4-bromoaniline (250 mg, 0.54 mmol) were dissolved in methanol (7 mL/mmol), and the mixture was stirred at RT for 30 min. Next, 1,2,3,3-tetramethyl-3H-indol-1-ium iodide (316 mg, 1.05 mmol) and sodium acetate (532 mg, 3.24 mmol) were added, and the reaction mixture was stirred for an additional 16 h at room temperature. Afterward, Et_2O (21 mL/mmol) was added, and the mixture was placed in the freezer (−20 °C). The resulting precipitate was filtered and purified by column chromatography (silica gel, dichloromethane/methanol, 10:1, then switched to 5:1). Green solid Yield: 548 mg (69%).

^1H NMR (600 MHz, d4–CD3OD): δ (ppm) 8.05 (dd, J1 = 13.1 Hz, J2 = 13.1 Hz, 2H), 7.39 (d, J = 7.5 Hz, 2H), 7.38–7.31 (m, 2H), 7.17– S12 7.15 (m, 4H), 6.26 (dd, J1 = 12.6 Hz, J2 = 12.6 Hz, 2H), 6.17 (d, J = 13.5 Hz, 2H), 3.50 (s, 6H), 1.59 (s, 12H).
^{13}C NMR (500 MHz, d4–CD3OD): δ (ppm) 173.83, 150.62, 152.9, 144.52, 142.45, 129.701, 125.92, 124.55, 123.35, 111.05, 104.59, 50.24, 31.41, 28.39.

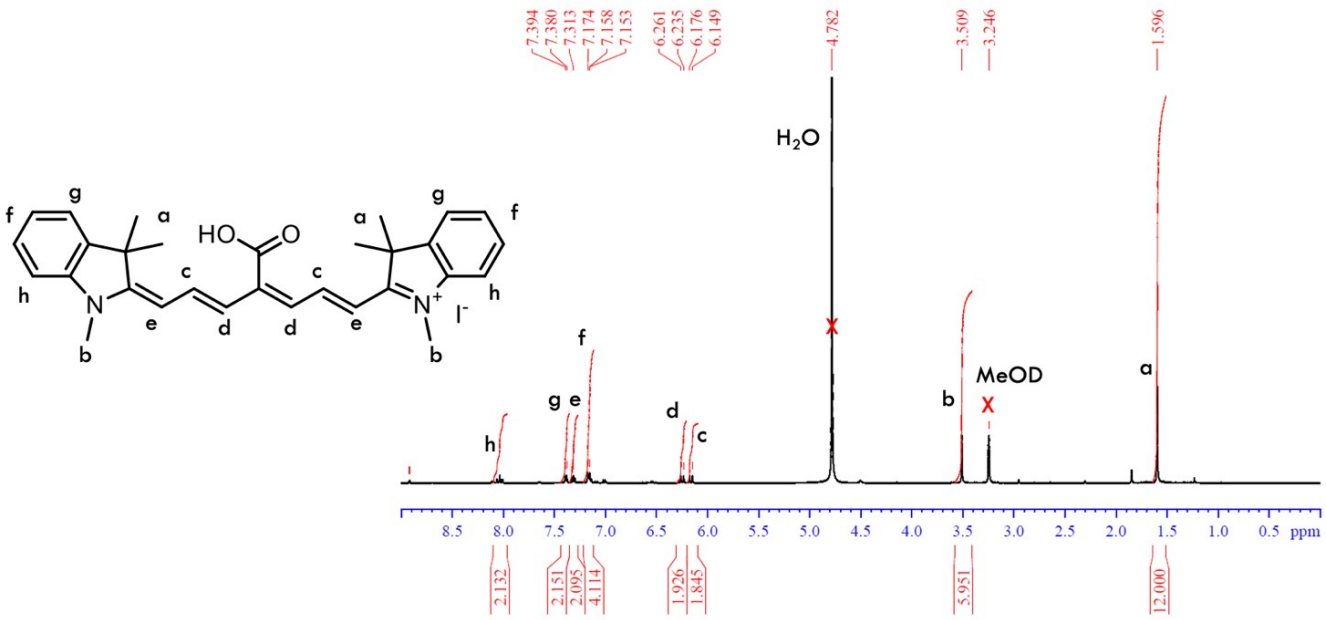

Figure S18.^1H NMR of 2-((1E,3Z,5E)-4-carboxy-7-((Z)-1,3,3-trimethylindolin-2-ylidene) hepta-1,3,5-trien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide (Cy7).

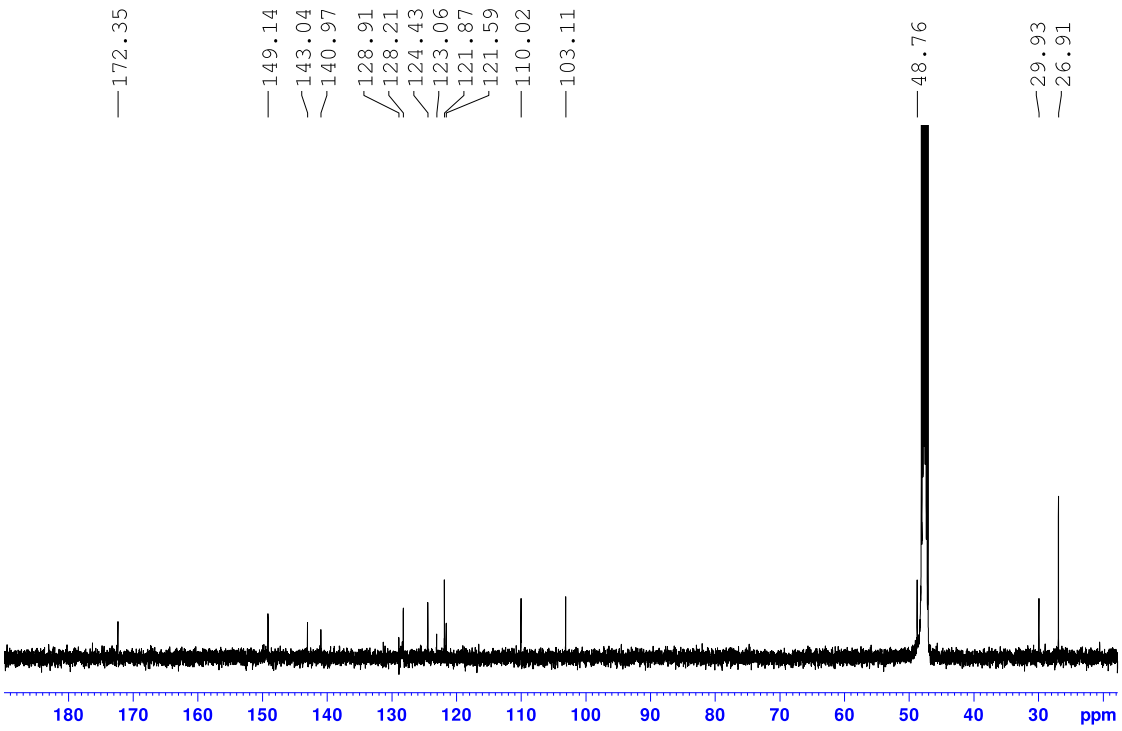

Figure S19.[13]C NMR of 2-((1E,3Z,5E)-4-carboxy-7-((Z)-1,3,3-trimethylindolin-2-ylidene) hepta-1,3,5-trien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide (Cy7).

2-((1E,3Z,5E)-4-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)-7-((Z)-1,3,3-trimethylindolin-2-ylidene)hepta-1,3,5-trien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide (Cy7-NHS).

2-((1E,3Z,5E)-4-carboxy-7-((Z)-1,3,3-trimethylindolin-2-ylidene)hepta-1,3,5-trien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide (250 mg, 0.43 mmol), N-Hydroxysuccinimide (74 mg, 0.64 mmol)and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (100 mg, 0.5 mmol) were added to 10 ml of methanol and reacted for 3 days at RT. The resulting solution was purified by column chromatography. (Silica gel, dichloromethane/methanol, 10:1 then switched to 5: 1). The fraction obtained was dried under reduced pressure to afford 2-((1E,3Z,5E)-4-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)-7-((Z)-1,3,3-trimethylindolin-2-ylidene)hepta-1,3,5-trien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide as a green solid. Yield: 182 mg (63%).

^{1}H NMR (500 MHz d4–CD3OD) δ (ppm) 1.67 (12H, s), 3.58 (6H, s), 4.61 (4H, s), 5.50 (1H, s), 6.24 (2H, d, J=13.57 Hz), 6.32 (2H, d, J=13.19 Hz), 7.08 (1H, d, J=9.40 Hz), 7.23 (4H, m, J=6.64 Hz), 7.38 (2H, dd, J=8.16 Hz), 7.46 (1H, d, J=7.33 Hz), 8.10 (1H, t, J=13.40 Hz)

^{13}C NMR (500 MHz, d4–CD3OD): δ (ppm) 173.36, 172.26, 149.15, 143.04, 140.97, 131.27, 128.91, 124.43, 123.07, 121.57, 110.01, 103.10, 48.78, 35.22, 29.92, 26.91.

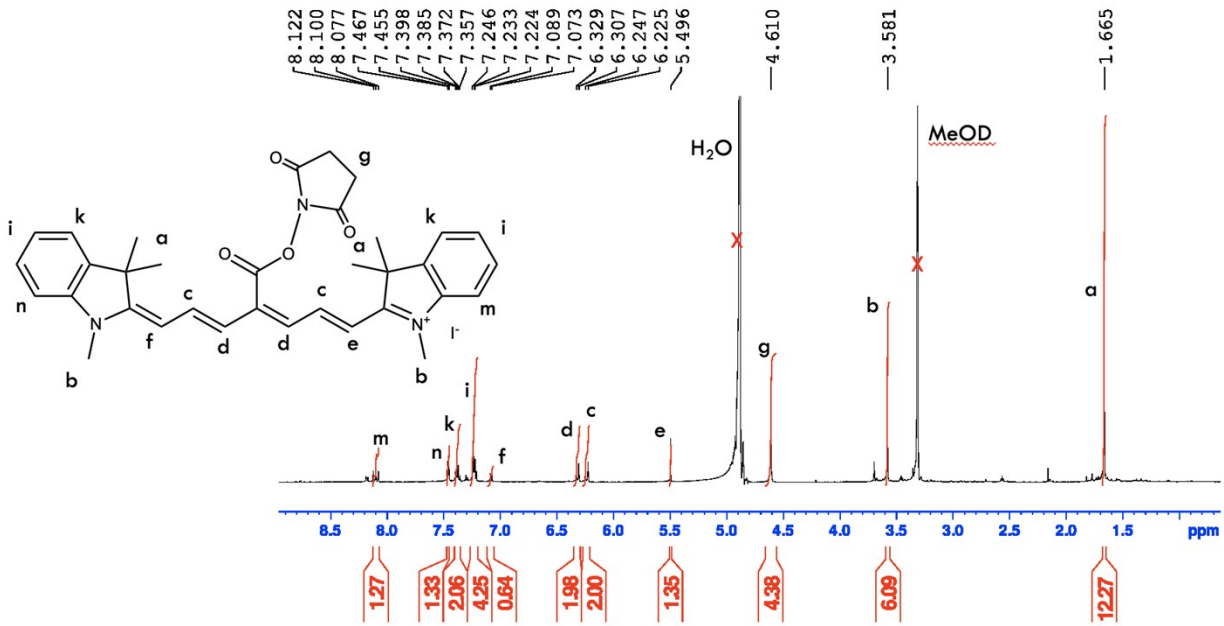

Figure S20. ^{1}H NMR of 2-((1E,3Z,5E)-4-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)-7-((Z)-1,3,3-trimethylindolin-2-ylidene)hepta-1,3,5-trien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide (Cy7-NHS).

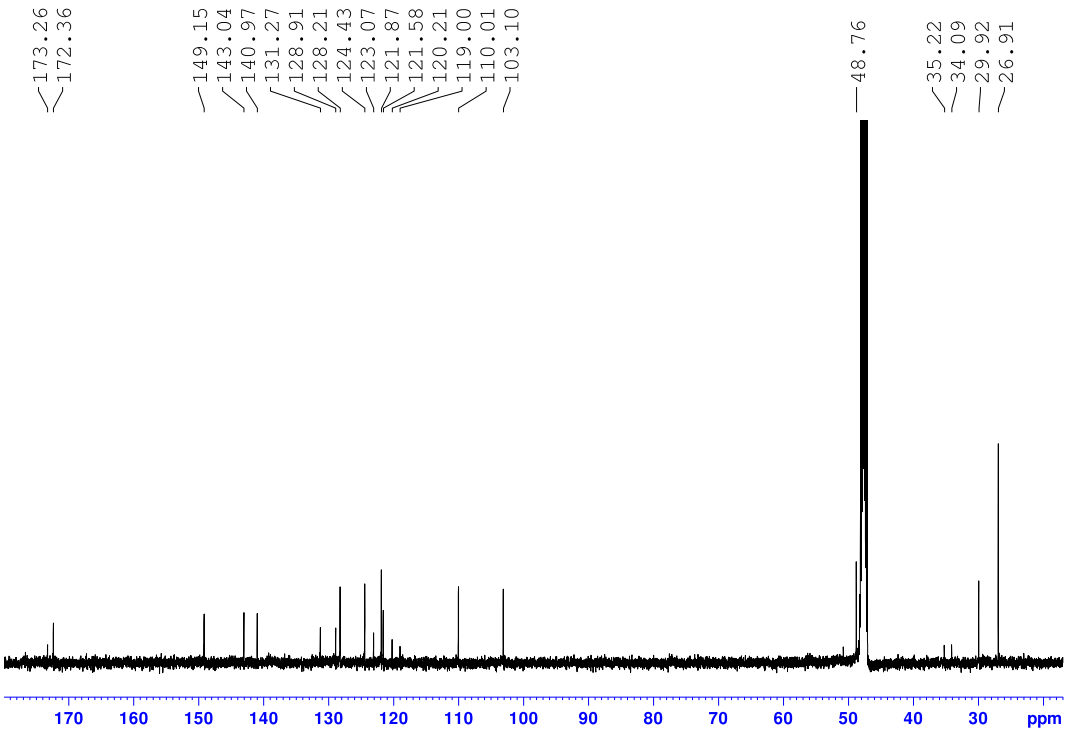

Figure S21. ^{13}C NMR of 2-((1E,3Z,5E)-4-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)-7-((Z)-1,3,3-trimethylindolin-2-ylidene)hepta-1,3,5-trien-1-yl)-1,3,3-trimethyl-3H-indol-1-ium iodide (Cy7-NHS

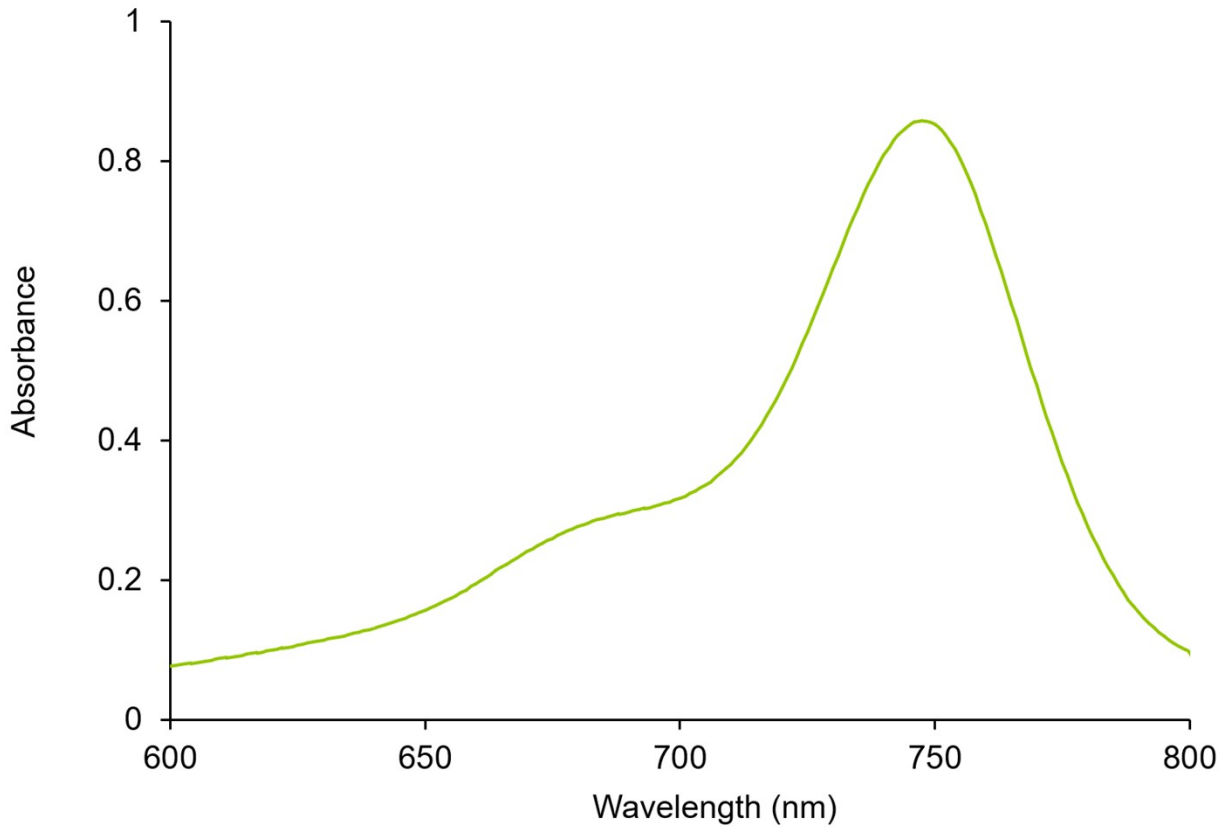

Figure S22. Absorbance spectrum of Cy7-NHS.

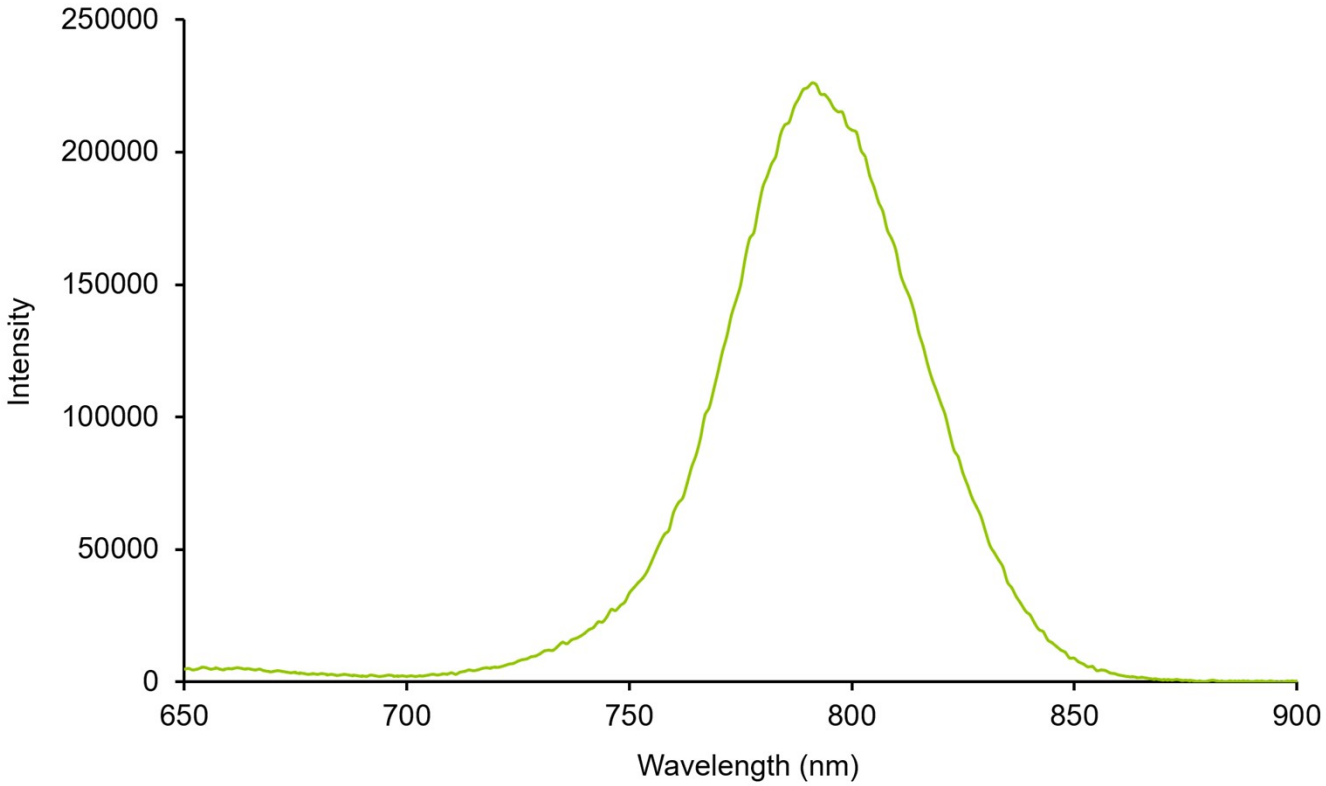

Figure 23. Emission spectrum of Cy7-NHS.