Supporting Information for:

Photoactivatable Red Chemiluminescent AIEgen Probe for In Vitro/Vivo Imaging Assay of Hydrazine

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**MTT assay.** 4T1 cells (1×10^6 cells/well) were seeded into replicate 96-well microtiter plates in a total volume of 200 µL·well⁻¹. Plates were maintained at 37 °C in a 5% CO₂ and 95% air incubator for 24 h. The fresh culture medium containing different concentrations of ACLD (0, 2 µM, 5 µM, 10 µM, 20 µM, 40 µM, 60 µM, 80 µM, 100 µM) was added to each well after the original medium was removed. The 4T1 cells were incubated for 24 h. Then, 100 µL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added to each well. After a 4 h incubation, the remaining MTT solution was removed, and 150 µL of DMSO was added to each well to dissolve the formazan crystals. The absorbance of the wells was measured at 570 nm on an RT 6000 microplate reader.

**Synthesis of the unactivated Chemiluminescent AIEgen probe (ACL).**
Scheme S1. The synthetic routes of ACL and HCLD.

Compound 1 was synthesized according to the reported literature.\textsuperscript{1} \textsuperscript{1}H NMR (400 MHz, Chloroform-d) δ 7.66 (d, J = 8.3 Hz, 2H), 7.59 (t, J = 6.5 Hz, 1H), 7.47 (dd, J = 12.4, 7.9 Hz, 6H), 7.39 (d, J = 8.2 Hz, 2H), 7.12 (d, J = 7.8 Hz, 5H), 7.05 (d, J = 7.7 Hz, 2H), 6.96 (dd, J = 11.4, 8.7 Hz, 4H), 6.70 – 6.60 (m, 4H), 3.75 (s, 6H). \textsuperscript{13}C NMR (101 MHz, Chloroform-d) δ 174.51, 158.29, 158.21, 145.24, 145.00, 144.05, 140.94, 138.41, 136.46, 136.17, 136.15, 134.48, 132.64, 132.61, 132.13, 131.44, 131.16, 130.52, 128.88, 127.83, 127.01, 126.39, 126.30, 114.17, 114.07, 113.17, 113.04, 80.87, 55.14, 55.12. MALDI-TOF MS (DHB, linear mode, m/z): 620.06 [M\textsuperscript{+}] 620.25 calc. for
Synthesis of compound 2. Compound 1 (1.24 g, 2 mmol) was dissolved in 20 mL ultradry dichloromethane, boron tribromide (964 μL, 10 mmol) in 10 mL dichloromethane was added dropwise to the above solution at 0 °C. The solution was stirred for 4 h until the reaction was complete. Water (10 mL) was added dropwise and the solution was exacted with dichloromethane for three times and the organic phase was dried with anhydrous magnesium sulfate for 20 min. The solvent was evaporated under reduced pressure and the residue was subjected to silica gel with dichloromethane as the eluent. Compound 2 was obtained as a dark red solid with a yield of 0.81 (959 mg, 1.62 mmol). 1H NMR (400 MHz, Chloroform-d) δ 7.66 (m, 3H), 7.58 – 7.39 (m, 8H), 7.13 (m, 7H), 6.95 (dd, J = 11.0, 8.5 Hz, 4H), 6.61 (dd, J = 8.2, 6.1 Hz, 4H), 5.22 (s, 2H). 13C NMR (101 MHz, Chloroform-d) δ 174.82, 174.78, 154.40, 154.30, 145.27, 144.94, 143.99, 140.87, 138.48, 136.50, 136.22, 136.21, 136.11, 134.45, 132.83, 132.81, 132.74, 132.12, 131.44, 131.20, 130.54, 128.91, 127.86, 127.04, 126.43, 126.35, 114.78, 114.65, 114.18, 114.08, 80.66. MALDI-TOF MS (DHB, linear mode, m/z): 592.01 [M+] 592.22 calc. for C₄₂H₂₈N₂O₂. (Figure S1-S3)

Synthesis of compound 3. Compound 2 (592 g, 1 mmol) was dissolved in 10 mL dimethylformamide, 1,4-dibromobutane (1.2 mL, 10 mmol) and potassium carbonate (415 mg, 3 mmol) was added to the solution, then the solution was stirred overnight at 80 °C. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica gel with DCM/petroleum ester (1/1) as
the eluent to afford a red solid compound 3 resulting in a yield of 0.71 (611 mg, 0.71 mmol). $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.71 (d, $J = 8.2$ Hz, 2H), 7.67 – 7.60 (m, 1H), 7.57-7.53 (m, 6H), 7.46 (d, $J = 8.1$ Hz, 2H), 7.23-7.15 (m, 7H), 7.07 (dd, $J = 13.6$, 7.9 Hz, 4H), 6.72 (t, $J = 9.5$ Hz, 4H), 3.97 (q, $J = 6.0$ Hz, 4H), 3.51 (t, $J = 7.0$ Hz, 4H), 2.09 (p, $J = 6.8$ Hz, 4H), 1.99 – 1.92 (m, 4H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 174.37, 157.76, 157.66, 145.11, 144.17, 141.09, 138.55, 136.50, 136.31, 136.28, 136.24, 132.77, 132.27, 131.55, 131.31, 130.63, 129.00, 128.01, 127.06, 126.53, 126.47, 114.32, 113.90, 113.75, 81.05, 66.82, 66.78, 33.75, 29.61, 28.02. MALDI-TOF MS (DHB, linear mode, m/z): 861.16 [M$^+$] 860.16 calc. for C$_{50}$H$_{42}$Br$_2$N$_2$O$_2$. (Figure S7-S9)

**Synthesis of compound 4.** Compound 3 (612 g, 0.72 mmol) was dissolved in acetonitrile and sodium azide (514 mg, 7.9 mmol) was added to the solution with care, the solution was stirred at 80 °C for 5 h, and the reaction was monitored by mass analysis. Then the solution was filtered through a flash silica gel chromatography to remove the excessive sodium azide. The solution was evaporated under reduced pressure and the crude product was purified by column chromatography on silica gel with DCM/petroleum ester (1/1) as the eluent to afford a red solid compound 4 resulting in a yield of 0.65 (368 mg, 0.47 mmol). $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.91 – 7.41 (m, 11H), 7.21 – 7.07 (m, 7H), 7.17-7.11 (m, 4H), 6.68 (t, $J = 7.8$ Hz, 4H), 3.95 (t, $J = 6.3$ Hz, 4H), 3.38 (t, $J = 7.2$ Hz, 4H), 1.84 (dt, $J = 13.0$, 6.5 Hz, 8H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 174.55, 157.62, 157.53, 145.22, 145.02, 144.08,
Synthesis of compound 5. 3-butyn-1-ol (750 μL, 10 mmol) and triethylamine (4.17 mL, 30 mmol) was added into dry dichloromethane (50 mL), then chloroacetyl chloride (800 μL, 10 mmol) in 20 mL anhydrous dichloromethane was added to the solution dropwise at 0 °C, the solution was stirred for 2 h and subjected to a flash chromatography on silica gel to remove the salt. The filtered solution was evaporated under reduced pressure to remove the solvent and excessive triethylamine. 1H NMR analysis showed that compound 5 had a good purity without further purification. Compound 5 was obtained as a colorless oil with a yield of 0.82 (1.2 g, 8.2 mmol). 1H NMR (400 MHz, Chloroform-d) δ 4.33 – 4.26 (m, 2H), 4.12 – 4.07 (m, 2H), 2.60-2.55 (m, 2H), 2.02 (t, J = 2.7 Hz, 1H). (Figure S13)

Synthesis of compound 7. Compound 5 (760 mg, 5.2 mmol) and triphenylphosphine (1.52 g, 5.8 mmol) was dissolved in toluene (60 mL), the solution was stirred at 110 °C overnight, the solution was cooled to 0 °C and the precipitate was filtered and washed with petroleum ester for 3 times to obtain compound 6 without further purification. Compound 6 (1.7 g, 4.2 mmol) was dissolved in water (20 mL), and sodium hydroxide (168 mg, 4.2 mmol) in water (20 mL) was added dropwise to the above solution, white precipitate was produced during the addition, the solution was stirred for 1 h and the
precipitate was filtered and washed with water for 3 times. The precipitate was freeze-dried in vacuum to obtain compound 7 without further purification.

**Synthesis of compound 9.** Compound 8 (664 mg, 2.0 mmol) and compound 7 (818 mg, 2.2 mmol) was dissolved in anhydrous chloromethane (20 mL) and the solution was stirred for 2 h at room temperature. The reaction was monitored by thin layer chromatography and the solvent was evaporated under reduced pressure upon the completion of the reaction. The crude product was purified by column chromatography on silica gel with DCM/petroleum ester (2/1) as the eluent. Compound 9 was obtained as a white solid with a yield of 0.75 (639 mg, 1.5 mmol). \(^1\)H NMR (400 MHz, Chloroform-d) δ 8.00 (d, J = 16.1 Hz, 1H), 7.39 (d, J = 7.9 Hz, 1H), 6.87 (d, J = 7.9 Hz, 1H), 6.64 (d, J = 16.1 Hz, 1H), 6.59-6.54 (m, 1H), 4.34 (t, J = 6.8 Hz, 2H), 3.32 (s, 3H), 3.27 (s, 1H), 2.68 – 2.59 (m, 2H), 2.12 (s, 1H), 2.04 (s, 1H), 1.98 – 1.71 (m, 14H). \(^{13}\)C NMR (101 MHz, Chloroform-d) δ 166.97, 150.78, 139.72, 139.41, 136.68, 132.80, 126.71, 123.56, 121.91, 121.56, 119.37, 80.14, 70.00, 62.29, 57.30, 39.03, 37.02, 32.88, 29.70, 19.09. MALDI-TOF MS (DHB, linear mode, m/z): 426.07 [M\(^+\)] 426.16 calc. for C\(_{25}\)H\(_{27}\)ClO\(_4\). (Figure S14-S16)

**Synthesis of HCL.** Compound 9 (468 mg, 1.1 mmol) was dissolved in anhydrous dichloromethane (25 mL), triethylamine (445 \(\mu\)L, 3.2 mmol) was added and the solution was cooled to 0 °C. Acetyl chloride (92 \(\mu\)L, 1.3 mmol) in anhydrous dichloromethane (10 mL) was added dropwise to the solution at 0 °C and the solution was stirred for 2 h. The mixture was filtered through a flash column chromatograph on
short silica gel to remove the salt and the solution was evaporated under reduced pressure. The crude product was purified by column chromatograph on silica gel with DCM/petroleum ester (1/1) as the eluent. HCL was obtained as a white solid with a yield of 0.85 (438 mg, 0.94 mmol). $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.71 (d, $J = 16.2$ Hz, 1H), 7.54 (d, $J = 8.1$ Hz, 1H), 7.20 (d, $J = 8.1$ Hz, 1H), 6.49 (d, $J = 16.0$ Hz, 1H), 4.31 (t, $J = 6.7$ Hz, 2H), 3.30 (s, 3H), 3.27 (d, $J = 2.9$ Hz, 1H), 2.42 (s, 3H), 2.10 (d, $J = 3.4$ Hz, 1H), 2.05 (t, $J = 2.7$ Hz, 1H), 1.97 – 1.62 (m, 14H). (Figure S17-S18)

**Synthesis of ACL.** HCL (239 mg, 0.51 mmol), compound 4 (197 mg, 0.25 mmol), CuBr (22 mg, 0.15 mmol) and PMDETA (N, N', N'', N'''-pentamethyl diethylenetriamine, 53 mg, 0.31 mmol) were added to 25 mL flask, the flask was vacuumed and recharged with inert gas for 3 times, then anhydrous and oxygen-free dichloromethane (10 mL) was added to the flask. The solution was stirred overnight at room temperature. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatograph on silica gel with ethyl acetate/DCM (1/1) as the eluent. ACL was obtained as a dark red solid with a yield of 0.42 (181 mg, 0.11 mmol). $^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.74 – 7.62 (m, 4H), 7.61 – 7.37 (m, 13H), 7.21 (d, $J = 8.0$ Hz, 2H), 7.15 – 7.10 (m, 5H), 7.06 (d, $J = 7.0$ Hz, 2H), 6.96 (m, 4H), 6.68 – 6.43 (m, 6H), 4.55 – 4.39 (m, 8H), 3.92 (t, $J = 6.0$ Hz, 4H), 3.32 (s, 6H), 3.29 (s, 2H), 3.17 (q, $J = 6.3$ Hz, 4H), 2.43-2.09 (m, 5H), 2.09 (m, 8H), 1.98 – 1.73 (m, 28H). MALDI-TOF MS (DHB, linear mode, m/z): 1722.86 [M$^+$] 1722.68 calc. for C$_{104}$H$_{100}$Cl$_2$N$_8$O$_{12}$. (Figure S19-S20)
**Synthesis of HCLD.** HCL (203 mg, 0.43 mmol) and a few milligrams of methylene blue were dissolved in dichloromethane and the solution was stirred at 0 °C under irradiation with white light. The reaction was monitored by TLC (thin-layer chromatography). The solvent was evaporated upon the completion of the reaction and the crude product was purified by column chromatograph on silica gel with ethyl acetate/DCM (1/1) as the eluent. *HCLD* was obtained as a white solid with a yield of 0.85 (183 mg, 0.37 mmol).

$^1$H NMR (400 MHz, Chloroform-d) δ 8.04 (d, $J$ = 8.5 Hz, 1H), 7.75 – 7.64 (m, 2H), 6.55 (d, $J$ = 16.0 Hz, 1H), 4.33 (t, $J$ = 6.7 Hz, 2H), 3.21 (s, 3H), 3.01 (s, 1H), 2.61 (m, 2H), 2.43 (s, 3H), 2.05 – 1.97 (m, 2H), 1.89 – 1.48 (m, 10H), 1.34 (m, 2H).

MALDI-TOF MS (DHB, linear mode, m/z): 500.67 [M$^+$] 500.16 calc. for C$_{27}$H$_{29}$ClO$_7$.

(Figure S21-S22)
Figure S1. $^1$H spectrum of compound 1 in CDCl$_3$.

Figure S2. $^{13}$C spectrum of compound 1 in CDCl$_3$. 
Figure S3. Mass spectrum of compound 1 in CH₃CN.

Figure S4. ¹H spectrum of compound 2 in CDCl₃.
Figure S5. $^{13}$C spectrum of compound 2 in CDCl$_3$.

Figure S6. Mass spectrum of compound 2 in CH$_3$CN.
Figure S7. $^1$H spectrum of compound 3 in CDCl$_3$.

Figure S8. $^{13}$C spectrum of compound 3 in CDCl$_3$.  

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**Figure S9.** Mass spectrum of compound 3 in CH$_3$CN.

**Figure S10.** $^1$H spectrum of compound 4 in CDCl$_3$. 
Figure S11. $^{13}$C spectrum of compound 4 in CDCl$_3$.

Figure S12. Mass spectrum of compound 4 in CH$_3$CN.
Figure S13. $^1$H spectrum of compound 5 in CDCl$_3$.

Figure S14. $^1$H spectrum of 9 in CDCl$_3$. 
**Figure S15.** $^{13}$C spectrum of 9 in CDCl$_3$.

**Figure S16.** Mass spectrum of 9 in CH$_3$CN.
**Figure S17.** $^1$H spectrum of HCL in CDCl$_3$.

**Figure S18.** Mass spectrum of HCL in CH$_3$CN.
**Figure S19.** $^1$H spectrum of ACL in CDCl$_3$.

**Figure S20.** Mass spectrum of ACL in CH$_3$CN.
Figure S21. $^1$H spectrum of HCLD in CDCl$_3$.

Figure S22. Mass spectrum of HCLD in CH$_3$CN.
**Figure S23.** Mass spectrum of ACLD in CH$_3$CN.

**Figure S24.** (A-B) Stability investigation of the Schaap’s dioxetane. After storing the HCLD at 4 °C for different time (0, 2, 4, 6, 8, 10, 12, and 14 days), chemiluminescent image (A) and the corresponding chemiluminescence intensity (B) of the 5-μM HCLD-contained PBS solution (pH 7.4) upon addition of hydrazine (the final concentration is 100 μM). Exposure time: 5 s.
**Figure S25.** Absorbance spectra (A) and the absorbance intensity at 378 nm (B) of the mixture solution of ABDA (50 μM) and ACL (10 μM) after being irradiated by using a white light source (50 mW cm$^{-2}$) at an interval of 20 s.

**Figure S26.** The possible activation strategy of C=C bond depended on singlet oxygen ($^{1}\text{O}_2$).
**Figure S27.** Absorbance spectrum of TPEDC (40 μM) in water containing 0.1% DMSO (red line) and the normalized fluorescence spectrum of HCL (blue line).

**Figure S28.** Chemiluminescence kinetic profiles (at 620 nm) of ACLD (100 μM) upon addition of N₂H₄ (10 mM)
Figure S29. Cell viability of 4T1 cells incubated with different concentrations of ACLD.

References