A High-resolution Method to Assess Cell Multinucleation with Cytoplasm-localized Fluorescent Probes

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Contents

1. CLFP development

1.1. General information of Chemistry

1.2. Compound information (\(^1\)H, \(^{13}\)C, HRMS)

1.3. Probe characterization (photophysical parameters, cell cytoxicity, cell distribution, incubation concentration)

2. Information of NMR spectra
1, CLFP development

1.1 General information

General: All the solvents and chemicals were purchased from commercial sources: J&K® Chemical Corporation, Beijing Ouhe Reagents Corporation with a purity > 95%. Flash column chromatography was performed on Biotage Isolera one. $^1$H NMR and $^{13}$C NMR were recorded on Bruker AVANCEIII 400 spectrometer. Chemical shifts are referenced to the residual solvent peak and reported in ppm (δ scale) and all coupling constant (J) values are given in Hertz (Hz). The following multiplicity abbreviations are used: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. ESI-HRMS data were measured on Thermo Exactive Orbitrap plus spectrometer. Purity was determined using HPLC, LCMS and NMR spectroscopy. All of the synthesized compounds have the purity over than 95%.

1.2 Compound information ($^1$H, $^{13}$C, HRMS)

*Series 1, BODIPY derivatives*

We designed and synthesized 13 BODIPY derived probes, the structures of them are listed in table S1.

Table S1, The structure information of 13 BODIPY derived CLFPs.

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Synthesis protocol and compound characterization (\(^1\text{H NMR}, \, \text{\textsuperscript{13}C NMR and HRMS}\)) \(^{1,2}\)

Scheme S1, Synthesis of B1-B4. (a) POCl\(_3\), DCM; TEA, BF\(_3\)-Et\(_2\)O, DCM

B1

3-Carbonitrile-4, 4-difluoro-1, 3, 5, 7-tetramethyl-4-bora-3a, 4a-diaza-s-indacene
3, 5-dimethyl-pyrrole-2-carbaldehyde (100mg, 0.81mmol) and 2,4-dimethyl-pyrrole-3-carbonitrile (89mg, 0.74mmol) were dissolved in dry DCM (15 mL), the reaction mixture cooled to 0 °C and stirred for 10 min under argon atmosphere, then POCl₃ (124mg, 0.81mmol) was slowly added in 5 mins. The reaction mixture was stirred at 0°C for 1h, then another 4h at 25°C. Dry TEA (750mg, 7.4 mmol) was added, and after 15 min BF₃·Et₂O (0.93ml, 7.4 mmol) was added. After 2 h, the reaction mixture was evaporated in vacuum, and was extracted by EtOAc (200 mL), then washed with H₂O (3× 50 mL) and dried by Na₂SO₄. The crude product was purified by silica gel column chromatography (hexane/EtOAc 5:1) to yield 99 mg (49%) of B1 as red crystal.

^1H NMR (400MHz, CDCl₃): δ = 2.30 (s, 3H), 2.36 (s, 3H), 2.59 (s, 3H), 2.62 (s, 3H), 6.23(s, 1H), 7.13 (s, 1H). ^13C NMR (101MHz, CDCl₃): δ = 10.58, 11.49, 13.34, 15.29, 100.97, 114.89, 121.27, 122.39, 130.18, 136.81, 140.54, 146.31, 155.37, 164.63. HRMS (ESI): m/z [M + H]^+ calculated for C₁₄H₁₅N₃BF₂: 274.13216; found: 274.13153

B2

4, 4-Difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene

B2 (109 mg, 60%) was obtained from 3, 5-dimethyl-pyrrole-2-carbaldehyde (100mg, 0.81mmol) and 2, 4-dimethyl-pyrrole (70 mg, 0.74mmol) as red powder.

^1H NMR (400MHz, CDCl₃): δ = 2.24 (s, 6H), 2.53 (s, 6H), 6.04 (s, 2H), 7.04 (s, 1H). ^13C NMR (101MHz, CDCl₃): δ = 11.27 (2C), 14.66 (2C), 119.01 (2C), 120.08, 133.40 (2C), 141.20 (2C), 156.71 (2C). HRMS (ESI): m/z [M + H]^+ calculated for C₁₃H₁₆N₂BF₂: 249.13691; found: 249.13643

B3

2, 2-Trichloroethyl-2-(4, 4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-yl)acetate
B3 (63mg, 21%) was obtained from 3, 5-dimethyl-pyrrole-2-carbaldehyde (100mg, 0.81mmol) and 2,2,2-trichloroethyl-2-(pyrrol-2-yl) acetate (188mg, 0.74mmol) as red powder.

$^1$H NMR (400MHz, CDCl$_3$): $\delta = 2.25$ (s, 3H), 2.57 (s, 3H), 4.18 (s, 2H), 4.81 (s, 2H), 6.14 (s, 1H), 6.48 (d, $J = 4.0$Hz, 1H), 6.90 (d, $J = 4.0$Hz, 1H), 7.13 (s, 1H). $^{13}$C NMR (101MHz, CDCl$_3$): $\delta = 11.35$, 15.08, 33.86, 74.37, 94.74, 117.75, 121.12, 124.30, 127.28, 133.03, 136.01, 145.16, 146.98, 162.29, 168.12. HRMS (ESI): m/z [M + H]$^+$ calculated for C$_{13}$H$_{15}$O$_2$BF$_2$Cl$_3$: 409.02547; found: 409.02542

B4

2-Ethyl-4, 4-difluoro-1, 3, 5, 7-tetramethyl-4-bora-3a, 4a-diaza-s-indacene

B4 (83 mg, 41%) was obtained from 3, 5-dimethyl-pyrrole-2-carbaldehyde (100 mg, 0.81 mmol) and 3-ethyl-2,4-dimethyl-pyrrole (91mg, 0.74 mmol) as green crystal.

$^1$H NMR (400MHz, CDCl$_3$): $\delta = 1.07$ (t, $J = 8.0$Hz, 3H), 2.17 (s, 3H), 2.23 (s, 3H), 2.39 (q, $J = 8.0$Hz, 2H), 2.51 (s, 6H), 6.00 (s, 1H), 6.99 (s, 1H). $^{13}$C NMR (101MHz, CDCl$_3$): $\delta = 9.41$, 11.23, 12.68, 14.50, 14.55, 17.28, 118.23, 119.26, 132.41, 132.79, 133.05, 137.64, 139.89, 155.09, 156.48. HRMS (ESI): m/z [M + H]$^+$ calculated for C$_{13}$H$_{20}$N$_2$BF$_2$: 277.16821; found: 277.16763
Scheme S2: Synthesis of B5-B9 (a) TEA, BF₃·Et₂O, DCM

B5

8-(Dec-9-yn-1-yl)-4, 4-difluoro-1, 3, 5, 7-tetramethyl-4-bora-3a, 4a-diaza-s-indacene

[Chemical structure image]

2, 4-Dimethyl-pyrrole (200 mg, 2.1 mmol) were dissolved in dry DCM (30 mL), undec-10-ynoyl chloride (210 mg, 1.05 mmol) was added under argon atmosphere at 25°C. Then the reaction mixture was heated at 50 °C and stirred for 2h, and cooled to 25°C. The solution was evaporated in vacuum, dry toluene (30 mL) and dry DCM (5 mL) was added to reaction mixture. After the reaction mixture was stirred for 5 min at 25°C, dry TEA (505mg, 5mmol) was added at 25°C, and after 15mins BF₃•Et₂O (760mg, 5mmol) was added by dropwise. The reaction mixture was heated to 50 °C for 1 h. The reaction mixture was evaporated in vacuum, and was extracted by EtOAc (300 mL), then washed with H₂O (3×50mL) and dried by Na2SO4. The crude product was purified by silica gel column chromatography (hexane/DCM 2:1) to yield 15 mg (3.7%) of B5 as red powder.

¹H NMR (400MHz, CDCl₃): δ = 1.32-1.44 (m, 6H), 1.45-1.56 (m, 4H), 1.59-1.67 (m, 2H), 1.94 (s, 1H), 2.17-2.21 (m, 2H), 2.41(s, 6H), 2.51(s, 6H), 2.91-2.95 (m, 2H), 6.05(s, 2H). ¹³C NMR (101MHz, CDCl₃): δ = 14.44 (2C), 16.39 (2C), 18.37, 28.41, 28.47, 28.62, 29.02, 29.27, 30.32, 31.89, 68.17, 84.64, 121.56 (2C), 131.44, 140.27 (2C), 146.62(2C), 153.74 (2C). HRMS (ESI): m/z [M + H]⁺ calculated for C₂₃H₃₂N₂BF₂: 385.26211; found: 385.26135

B6

4, 4-Difluoro-8-(hex-5-yn-1-yl)- 1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene
B6 (25 mg, 7.2%) was obtained from hept-6-ynoyl chloride (151 mg, 1.05 mmol) and 2, 4-dimethyl-pyrrole (200 mg, 2.1 mmol) as red powder.

$^1$H NMR (400MHz, CDCl$_3$): $\delta = 1.71$-$1.80$ (m, 4H), 1.96 (s, 1H), 2.26-$2.29$ (m, 2H), 2.43(s, 6H), 2.52(s, 6H), 2.95-2.99(m, 2H), 6.06(s, 2H). $^{13}$C NMR (101MHz, CDCl$_3$): $\delta = 14.46$ (2C), 16.39 (2C), 18.26, 27.94, 28.96, 30.70, 69.05, 83.66, 121.67 (2C), 131.42, 140.30 (2C), 145.85(2C), 153.95 (2C). HRMS (ESI): m/z [M + H]$^+$ calculated for C$_{19}$H$_{24}$N$_2$BF$_2$: 329.19951; found: 329.19904

B7

8- (Chloropropyl)-4, 4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene

B7 (30 mg, 8.7%) was obtained from 4-chlorobutanoyl chloride (147 mg, 1.05 mmol) and 2, 4-dimethyl-pyrrole (200 mg, 2.1 mmol) as red powder.

$^1$H NMR (400MHz, CDCl$_3$): $\delta = 2.05$-$2.12$ (m, 2H), 2.44 (s, 6H), 2.52 (s, 6H), 3.11-3.16 (m, 2H), 3.70 (t, $J = 8.0$Hz, 2H), 6.06 (s, 2H). $^{13}$C NMR (101MHz, CDCl$_3$): $\delta = 14.48$ (2C), 16.60 (2C), 25.95, 34.03, 44.75, 121.86 (2C), 131.43, 140.33 (2C), 144.43(2C), 154.37 (2C). HRMS (ESI): m/z [M + H]$^+$ calculated for C$_{16}$H$_{21}$N$_2$BClF$_2$: 325.14489; found: 325.14423

B8

8-[4-(Chloromethyl)phenyl]-4, 4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene
B8 (351 mg, 17.8%) was obtained from 4-(chloromethyl)benzoyl chloride (1.0 g, 5.32 mmol) and 2,4-dimethyl-pyrrole (1.01 g, 10.6 mmol) as red powder.

$^1$H NMR (400MHz, CDCl$_3$): $\delta = 1.38$ (s, 6H), 2.55 (s, 6H), 4.66 (s, 2H), 5.98 (s, 2H), 7.29 (d, $J = 8.0$Hz, 2H), 7.52 (d, $J = 8.0$Hz, 2H). $^{13}$C NMR (101MHz, CDCl$_3$): $\delta = 14.47$ (2C), 14.59 (2C), 45.61, 121.34 (2C), 128.42 (2C), 129.26(2C), 131.32, 135.09(2C), 138.60(2C), 140.94, 143.03, 155.67 (2C). HRMS (ESI): m/z [M + H]$^+$ calculated for C$_{20}$H$_{21}$N$_2$BClF$_2$: 373.14489; found: 373.14429

B9

4, 4-Difluoro-8-methyl-1, 3, 5, 7-tetramethyl-4-bora-3a, 4a-diaza-s-indacene

B9 (50 mg, 18.4%) was obtained from acetyl chloride (82 mg, 1.05 mmol) and 2, 4-dimethyl-pyrrole (200 mg, 2.1 mmol) as red powder.

$^1$H NMR (400MHz, CDCl$_3$): $\delta = 2.40$ (s, 6H), 2.51 (s, 6H), 2.56 (s, 3H), 6.05 (s, 2H). $^{13}$C NMR (101MHz, CDCl$_3$): $\delta = 14.42$ (2C), 16.37, 17.32 (2C), 121.22 (2C), 132.06, 140.99 (2C), 141.42 (2C), 153.60 (2C). HRMS (ESI): m/z [M + H]$^+$ calculated for C$_{14}$H$_{18}$N$_2$BF$_2$: 263.15256; found: 263.15210
Scheme S3: Synthesis of B10-B13. (a) Cs$_2$CO$_3$, KI, CH$_3$CN; (b) HCl, DCM

B10

8-[4-((decylamino)methyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene

8-[4-(chloromethyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (B8) (50mg, 0.13mmol) , Cs$_2$CO$_3$ (87 mg, 0.26 mmol) and KI (45 mg, 0.26 mmol) were dissolved in dry CH$_3$CN (30mL) , decan-1-amine (210mg, 1.3mmol) was added under argon atmosphere at 25°C. Then the reaction mixture was heated at 80 °C and stirred for 2 h, and cooled to 25 °C. The reaction mixture was evaporated in vacuum, and was extracted by EtOAc (200 mL), then washed with H$_2$O (3×50mL) and dried by Na$_2$SO$_4$. The crude product was purified by silica gel column chromatography (DCM/CH$_3$OH 50:1). The HCl salt of B10 was obtained by using a solution of HCl in DCM, and yielded 50 mg (70%) as red powder.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta = 0.86$ (br, 3H), 1.22-1.27 (m, 14H), 1.35 (s, 6H), 1.89 (br, 2H), 2.55 (s, 6H), 2.76 (br, 2H), 4.29 (br, 2H), 5.97 (s, 2H), 7.38 (d, $J = 4.0$Hz, 2H), 7.80 (d, $J = 4.0$Hz, 2H), 10.14 (br, NH-HCl, 2H). $^{13}$C NMR (101MHz, CDCl$_3$): $\delta = 14.10$, 14.52 (2C), 14.62 (2C), 22.66, 26.03, 26.87, 28.98, 29.25, 29.44, 29.46, 31.86, 45.53, 49.89, 121.51 (2C), 129.24
(2C), 130.98 (2C), 131.05, 131.16 (2C), 136.60, 140.18, 142.64 (2C), 155.97 (2C). HRMS (ESI) : m/z [M + H]^+ calculated for C_{30}H_{43}N_{3}BF_{2}: 494.35126; found: 494.35129

B11

8-[4-((hexylamino)methyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene

![Chemical Structure]

B11 (+HCl) (80 mg, 90%) was obtained from 8-[4-(chloromethyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (B8) (70 mg, 0.19 mmol) and hexan-1-amine (190 mg, 1.9 mmol) as red powder.

^1^H NMR (400MHz, CDCl\_3): δ = 0.84 (t, J = 8.0Hz, 3H), 1.26-1.30 (m, 6H), 1.35 (s, 6H), 1.90(br, 2H), 2.55 (s, 6H), 2.76 (br, 2H), 4.29 (br, 2H), 5.98 (s, 2H), 7.39 (d, J = 8.0Hz, 2H), 7.81 (d, J = 8.0Hz, 2H), 10.13 (br, NH-HCl, 2H). ^13^C NMR (101MHz, CDCl\_3): δ = 13.90, 14.51 (2C), 14.62 (2C), 22.41, 25.93, 26.48, 31.05, 45.52, 49.91, 121.52 (2C), 129.25 (2C), 130.97 (2C), 131.04, 131.16 (2C), 136.60, 140.18, 142.65 (2C), 155.97 (2C). HRMS (ESI): m/z [M + H]^+ calculated for C_{26}H_{35}N_{3}BF_{2}: 438.28866; found: 438.28851

B12

8-[4-((dodecylamino)methyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene

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11
B12 (+HCl) (70 mg, 93%) was obtained from 8-[4-(chloromethyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (B8) (50 mg, 0.13 mmol) and dodecan-1-amine (248 mg, 1.3 mmol) as red powder.

$^1$H NMR (400MHz, CDCl$_3$): $\delta = 0.87$ (t, $J = 8.0$Hz, 3H), 1.20-1.27 (m, 18H), 1.35 (s, 6H), 1.89 (br, 2H), 2.55 (s, 6H), 2.75 (br, 2H), 4.29 (br, 2H), 5.97 (s, 2H), 7.38 (d, $J = 8.0$Hz, 2H), 7.80 (d, $J = 8.0$Hz, 2H), 10.10 (br, NH-HCl, 2H). $^{13}$C NMR (101MHz, CDCl$_3$): $\delta = 14.12$, 14.50 (2C), 14.62 (2C), 22.69, 25.98, 26.86, 28.98, 29.35, 29.45, 29.52, 29.60, 29.63, 31.91, 45.52, 49.87, 121.51 (2C), 129.23 (2C), 130.97 (2C), 131.05, 131.16 (2C), 136.59, 140.19, 142.64 (2C), 155.96 (2C). HRMS (ESI): m/z [M + H]$^+$ calculated for C$_{32}$H$_{47}$N$_3$BF$_2$: 522.38256; found: 522.38239

B13

8-[4-(((10-aminodecyl)amino)methyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene

B13 (+2HCl) (80 mg, 73%) was obtained from 8-[4-(chloromethyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (B8) (70 mg, 0.19 mmol) and decane-1,10-diamine (323 mg, 1.9 mmol) as red powder.
$^1$H NMR (400MHz, CDCl3): $\delta = 1.30$ (m, 12H), 1.35 (s, 6H), 1.86 (br, 4H), 2.55 (s, 6H), 2.79 (br, 2H), 3.06 (br, 2H), 4.34 (br, 2H), 5.98 (s, 2H), 7.37 (br, 2H), 7.81 (br, 2H), 8.28 (br, NH2-HCl, 3H), 9.88 (br, NH-HCl, 2H). $^{13}$C NMR (101MHz, CDCl3): $\delta = 14.56$ (2C), 14.63 (2C), 25.70, 25.93, 26.25, 27.15, 28.17 (3C), 29.72, 40.19, 45.77, 50.20, 121.52 (2C), 129.17 (2C), 131.12 (2C), 131.16 (3C), 136.51, 140.22, 142.66 (2C), 155.95 (2C). HRMS (ESI) : m/z [M + H]$^+$ calculated for C$_{30}$H$_{44}$N$_4$BF$_2$: 509.36216; found: 509.36160

**Series 2, Dansyl chloride derivatives**

Table S2, The structure information of 20 DNS derived CLFPs

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General procedure of the synthesis of 5-(dimethylamino)-naphthalene-1- sulfonamide derivatives (dansyl chloride based probes).

Scheme S4. Reaction conditions: a), D1 5 mmol, D2 5 mmol, TEA (10 mmol) solvent DCM (2 ml), for 2 hours; b), HCl, Ethanol (2.5 ml).

Dansyl chloride was added to a solution of alkyl amine and TEA in DCM at room temperature. The reaction mixture was kept to stir for 15h, and DCM was then evaporated. Final compound was obtained by column chromatography (MeOH/DCM). The product was kept as the HCl via re-crystalization in HCl of ethanol solution, all of the probes was produced as the form of HCl salt.

D1

5-(Dimethylamino)-N-propynaphthalene-1-sulfonamide
D1 (HCl-salt) (114 mg, 94%) was obtained from propylamine (22 mg, 0.37 mmol) and DNS-Cl (100mg, 0.37mmol) as white power. $^1$H NMR (400MHz, CD$_3$OD): $\delta = 0.76$ (t, $J = 7.4$ Hz, 3H), 1.33-1.43 (m, 2H), 2.83 (t, $J = 7.0$ Hz, 2H), 3.51 (s, 6H), 7.85-7.93 (m, 2H), 8.13 (d, $J = 7.8$ Hz, 1H), 8.38 (d, $J = 7.3$ Hz, 1H), 8.59 (d, $J = 8.7$ Hz, 1H), 8.95 (d, $J = 8.8$ Hz, 1H). $^{13}$C NMR (101MHz, CD$_3$OD): $\delta = 11.40$, 24.05, 45.79, 47.81 (2C), 120.52, 126.48, 127.25, 128.01, 128.55, 128.68, 130.74, 131.10, 139.17, 140.61. HRMS (ESI): m/z [M + H]$^+$ calculated for C$_{16}$H$_{21}$N$_2$O$_2$: 293.13183; found: 293.13162.

D2

5-(Dimethylamino)-N-hexynaphthalene-1-sulfonamide

D2 (HCl-salt) (124mg, 91%) was obtained from hexylamine (37mg, 0.37mmol) and DNS-Cl (100mg, 0.37mmol) as white power. $^1$H NMR (400MHz, CD$_3$OD): $\delta = 0.76$ (t, $J = 6.9$ Hz, 3H), 1.07-1.12 (m, 6H), 1.28-1.33(dd, 2H), 2.85 (t, $J = 6.9$ Hz, 2H), 3.51 (s, 6H), 7.84-7.91 (m, 2H), 8.15 (d, $J = 7.8$ Hz, 1H), 8.36 (d, $J = 7.3$ Hz, 1H), 8.64 (d, $J = 8.7$ Hz, 1H), 8.93 (d, $J = 8.8$ Hz, 1H); $^{13}$C NMR (101MHz, CD$_3$OD): $\delta = 14.25$, 23.43, 27.17, 30.58, 32.29, 43.91, 47.88 (2C), 120.64, 126.56, 127.17, 128.04, 128.57, 128.77, 130.71, 131.18, 139.12, 140.44; HRMS (ESI) : m/z [M + H]$^+$ calculated for C$_{18}$H$_{27}$N$_2$O$_2$: 335.17878; found: 335.17871.

D3

N-decyl-5-(dimethylamino) naphthalene-1-sulfonamide

D3 (HCl-salt) (137mg, 87%) was obtained from decylamine (58mg, 0.37mmol) and DNS-Cl (100mg, 0.37mmol) as white power. $^1$H NMR (400MHz, CD$_3$OD): $\delta = 0.89$ (t, $J = 6.9$ Hz, 3H), 1.12-1.36 (m, 16H), 2.87 (t, $J = 7.0$ Hz, 2H), 3.50 (s, 6H), 7.85-7.92 (m, 2H), 8.12 (d, $J = 7.8$ Hz,
1H), 8.38 (d, J = 7.3 Hz, 1H), 8.59 (d, J = 8.7 Hz, 1H), 8.94 (d, J = 8.7 Hz, 1H); 13C NMR (101MHz, CD3OD): δ = 14.42, 23.70, 27.54, 30.11, 30.37, 30.52, 30.57, 30.69, 33.01, 43.94, 47.76 (2C), 120.39, 126.56, 127.35, 127.93, 128.50, 128.57, 130.77, 131.13, 139.15, 140.96.

HRMS (ESI): m/z [M + H] calculated for C22H35N2O2S: 391.24138; found: 391.24127.

D4

(Z)-5-(dimethylamino)-N-(octadec-9-en-1-yl) naphthalene-1-sulfonamide

D4 (HCl-salt) (180 mg, 91%) was obtained from octadece-9-nyl amine (110mg, 0.37mmol) and DNS-Cl (100mg, 0.37 mmol) as white wax. 1H NMR (400MHz, CD3OD): δ = 0.86 (t, J = 6.6 Hz, 3H), 1.11-1.26 (m, 26H), 1.94-2.00 (m, 2H), 2.84 (t, J = 6.9 Hz, 2H), 3.48 (s, 6H), 5.28-5.35 (m, 2H), 7.82-7.91 (m, 2H), 8.10 (d, J = 7.5 Hz, 1H), 8.35 (dd, J1 = 7.5 Hz, J2 = 0.9 Hz, 1H), 8.57 (d, J = 8.7 Hz, 1H), 8.92 (d, J = 8.7 Hz, 1H); 13C NMR (101MHz, CD3OD): δ = 14.45, 23.71, 27.55, 28.11, 30.10, 30.19, 30.32, 30.40, 30.42, 30.58, 30.71, 30.74, 30.77, 30.81, 33.03, 43.94, 47.83 (2C), 120.57, 126.56, 127.23, 128.01, 128.57, 128.73, 130.75, 130.76, 130.86, 131.13, 139.15, 140.58; HRMS (ESI) : m/z [M + H] calculated for C30H49N2O2S: 501.35393; found: 501.35303.

D5

N, N’-didecyl-5-(dimethylamino) naphthalene-1-sulfonamide

D5 (HCl-salt) (179mg, 93%) was obtained from didecylamine (100mg, 0.34mmol) and DNS-Cl (91mg, 0.34mmol) as white wax. 1H NMR (400MHz, CD3OD): δ = 0.90 (t, J = 6.7 Hz, 6H), 1.19-1.27 (m, 28H), 1.50 (br, 4H), 3.28 (br, 2H), 3.35 (br, 2H), 3.45 (s, 6H), 7.83-7.92 (m, 2H),
8.05 (d, J = 7.7 Hz, 1H), 8.33 (d, J = 7.2 Hz, 1H), 8.55 (d, J = 8.8 Hz, 1H), 8.81 (d, J = 8.8 Hz, 1H); $^{13}$C NMR (101MHz, CD$_3$OD): $\delta$ = 14.43 (2C), 23.72 (2C), 27.61 (2C), 29.33 (2C), 30.19 (2C), 30.41 (4C), 30.56 (2C), 30.59 (2C), 33.04 (2C), 47.63 (2C), 120.21, 126.93, 127.76 (2C), 128.19, 128.63, 129.54, 131.22 (2C), 138.62. HRMS (ESI) : m/z [M + H] calculated for C$_{32}$H$_{55}$N$_2$O$_2$S: 531.39788; found: 531.39752.

D6

$N$-(2-aminoethyl)-5-(dimethylamino) naphthalene-1-sulfonamide

![D6 structure](image)

D6 (HCl-salt) (110 mg, 80%) was obtained from ethane-1,2-diamine (22mg, 0.37mmol) and DNS-Cl (100mg, 0.37mmol) as white power. $^1$H NMR (400MHz, CD$_3$OD): $\delta$ = 3.07-3.11 (m, 4H), 3.49 (s, 6H), 7.89-7.95 (m, 2H), 8.13 (d, J = 7.7 Hz, 1H), 8.41 (d, J = 7.3 Hz, 1H), 8.66 (d, J = 8.7 Hz, 1H), 8.90 (d, J = 8.7 Hz, 1H). $^{13}$C NMR (101MHz, CD$_3$OD): $\delta$ = 40.81, 41.23, 47.83(2C), 120.85, 127.36, 127.40, 127.97, 128.30, 129.02, 130.55, 131.51, 137.69, 140.83. HRMS (ESI) : m/z [M + H] calculated for C$_{14}$H$_{20}$N$_3$O$_2$S: 294.12707; found: 294.12665.

D7

5-(Dimethylamino)-N-(4-hydroxybutyl) naphthalene-1-sulfonamide

![D7 structure](image)

D7 (HCl-salt) (110 mg, 83%) was obtained from 4-Amino-1-butanol (33mg, 0.37mmol) and DNS-Cl (100mg, 0.37mmol) as white power. $^1$H NMR (400MHz, CD$_3$OD): $\delta$ = 1.33-1.47 (m, 4H), 2.89 (t, J = 6.5 Hz, 2H), 3.39 (t, J = 6.0 Hz, 2H), 3.51 (s, 6H), 7.85-7.92 (m, 2H), 8.13 (d, J = 7.8 Hz, 1H), 8.38 (d, J = 7.4 Hz, 1H), 8.61 (d, J = 8.7 Hz, 1H), 8.94 (d, J = 8.8 Hz, 1H); $^{13}$C NMR (101MHz, CD$_3$OD): $\delta$ = 27.24, 30.47, 43.80, 47.83 (2C), 62.19, 120.60, 126.59, 127.21,
128.01, 128.60, 128.66, 130.69, 131.15, 139.00, 140.52; HRMS (ESI) : m/z [M + H] calculated for C_{16}H_{23}N_{2}O_{3}S: 323.14239; found: 323.14233.

D8

N-(6-aminohexyl)-5-(dimethylamino) naphthalene-1-sulfonamide

D8 (2HCl-salt) (142mg, 74%) was obtained from 1,6-hexanediamine (429mg, 3.7mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ¹H NMR (400MHz, CD_{3}OD): δ = 1.25 (br, 4H), 1.41 (br, 2H), 1.54 (br, 2H), 2.82-2.89(m, 4H), 3.51 (s, 6H), 7.86-7.92 (m, 2H), 8.15 (d, J = 7.7 Hz, 1H), 8.36 (d, J = 7.3 Hz, 1H), 8.67 (d, J = 8.6 Hz, 1H), 8.94 (d, J = 8.7 Hz, 1H); ¹³C NMR (101MHz, CD_{3}OD): δ = 26.83, 26.96, 28.35, 30.52, 40.61, 43.72, 44.14, 47.88 (2C), 120.77, 126.75, 127.17, 128.04, 128.64, 128.79, 130.71, 131.09, 139.06, 140.36; HRMS (ESI) : m/z [M + H] calculated for C_{18}H_{28}N_{3}O_{2}S: 350.18967; found: 350.18900.

D9

N-(8-aminooctyl)-5-(dimethylamino) naphthalene-1-sulfonamide

D9 (2HCl-salt) (113mg, 68%) was obtained from 1,8-diaminooctane (535mg, 3.7mmol) and DNS-Cl (100mg, 0.37mmol) as white power. ¹H NMR (400MHz, CD_{3}OD): δ = 1.21-1.40 (br, 10H), 1.60-1.63 (m, 2H), 2.86-2.91 (m, 4H), 3.48(s, 6H), 7.85-7.92 (m, 2H), 8.10 (d, J = 7.8 Hz, 1H), 8.36 (d, J = 7.1 Hz, 1H), 8.62 (d, J = 8.8 Hz, 1H), 8.92 (d, J = 8.8 Hz, 1H); ¹³C NMR (101MHz, CD_{3}OD): δ = 27.28, 27.32, 28.46, 29.76, 29.93, 30.60, 40.73, 43.84, 47.80 (2C), 120.60, 126.93, 127.31, 127.89, 128.48, 128.63, 130.71, 131.09, 139.00, 140.87; HRMS (ESI) : m/z [M + H] calculated for C_{20}H_{32}N_{3}O_{2}S: 378.22097; found: 378.22049.
D10

\[ N-(2-(2-(2\text{-aminoethoxy})\text{ethoxy})\text{ethyl})-5-(\text{dimethylamino})naphthalene-1-sulfonamide \]

\[ \text{H}_2\text{N} \quad \text{O} \quad \text{O} \quad \text{N} \quad \text{S} \quad \text{O} \quad \text{N} \quad \text{S} \quad \cdot \text{2HCl} \]

D10 (2HCl-salt) (110mg, 66%) was obtained from 1,8-diamino-3,6-dioxaoctane (551mg, 3.7mmol) and DNS-Cl (100mg, 0.37mmol) as white power. \(^1\text{H}\) NMR (400MHz, CD\(_3\)OD): \(\delta = 3.05-3.08\) (m, 4H), 3.40-3.43 (m, 4H), 3.48 (br, 8H), 3.64 (t, \(J = 4.8\) Hz, 2H), 7.84-7.89 (m, 2H), 8.13 (d, \(J = 7.7\) Hz, 1H), 8.36 (d, \(J = 7.3\) Hz, 1H), 8.72 (d, \(J = 8.6\) Hz, 1H), 8.89 (d, \(J = 8.7\) Hz, 1H); \(^{13}\text{C}\) NMR (101MHz, CD\(_3\)OD): \(\delta = 40.63, 43.68, 47.82\) (2C), 49.85, 67.74, 70.69, 71.12, 120.70, 127.12, 127.28, 127.88, 128.44, 128.70, 130.63, 130.95, 138.90, 140.82; HRMS (ESI): m/z [M + H] calculated for C\(_{18}\)H\(_{28}\)N\(_3\)O\(_4\)S: 382.17950; found: 382.17908.

D11

\[ N-(10\text{-aminodecyl})-5-(\text{dimethylamino})naphthalene-1-sulfonamide \]

\[ \text{H}_2\text{N} \quad \text{N} \quad \text{O} \quad \text{S} \quad \cdot \text{2HCl} \]

D11 (2HCl-salt) (99.5mg, 56%) was obtained from 1,10-Diaminodecane (640mg, 3.7mmol) and DNS-Cl (100mg, 0.37mmol) as white power. \(^1\text{H}\) NMR (400MHz, CD\(_3\)OD): \(\delta = 1.16-1.37\) (br, 14H), 1.60-1.67 (br, 2H), 2.84-2.92 (br, 4H), 3.49(s, 6H), 7.85-7.92 (m, 2H), 8.13 (d, \(J = 7.8\) Hz, 1H), 8.37 (d, \(J = 7.3\) Hz, 1H), 8.64 (d, \(J = 8.7\) Hz, 1H), 8.94 (d, \(J = 8.8\) Hz, 1H); \(^{13}\text{C}\) NMR (101MHz, CD\(_3\)OD): \(\delta = 27.42, 27.47, 28.55, 30.02, 30.12, 30.31, 30.36, 30.70, 40.78, 43.91, 47.81(2C), 120.52, 126.78, 127.34, 127.91, 128.52, 128.60, 130.76, 131.08, 139.09, 140.90; HRMS (ESI): m/z [M + H] calculated for C\(_{22}\)H\(_{36}\)N\(_3\)O\(_2\)S: 406.25227; found: 406.25177.

D12

\[ N-(12\text{-aminododecyl})-5-(\text{dimethylamino})naphthalene-1-sulfonamide \]
D12 (2HCl-salt) (200.3mg, 71%) was obtained from 1,12-diaminododecane (1.1g, 5.6mmol) and DNS-Cl (150mg, 0.56mmol) as white power. $^1$H NMR (400MHz, CD$_3$OD): $\delta = 1.16$-1.37 (br, 18H), 1.62-1.67 (m, 2H), 2.85-2.92 (m, 4H), 3.49(s, 6H), 7.85-7.92 (m, 2H), 8.11 (br, 1H), 8.38 (d, $J = 7.3$ Hz, 1H), 8.64 (br, 1H), 8.94 (d, $J = 8.7$ Hz, 1H). $^{13}$C NMR (101MHz, CD$_3$OD): $\delta = 27.44, 27.51, 28.56, 30.08, 30.19, 30.46, 30.49, 30.55(2C), 30.69, 40.77, 43.92, 47.80(2C), 120.55, 126.79, 127.31, 127.91, 128.53, 128.60, 130.73, 131.11, 139.05, 140.86. HRMS (ESI) : m/z [M + H] calculated for C$_{24}$H$_{40}$N$_3$O$_2$S: 434.28357; found: 434.28326.

D13

$N, N'$-(ethane-1, 2-diyil) bis(5-(dimethylamino)naphthalene-1-sulfonamide)

D13 (2HCl-salt) (99mg, 92%) was obtained from 1,2-Diaminoethane (11mg, 0.18mmol) and DNS-Cl (100mg, 0.37mmol) as white power. $^1$H NMR (400MHz, CD$_3$OD): $\delta = 2.87$ (s, 4H), 3.50 (s, 12H), 7.88 (dd, $J_1 = 17.6$ Hz, $J_2 = 8.8$ Hz, 4H), 8.12 (d, $J = 7.7$ Hz, 2H), 8.30 (d, $J = 7.3$ Hz, 2H), 8.58 (d, $J = 8.8$ Hz, 2H), 8.84 (d, $J = 8.8$ Hz, 2H). $^{13}$C NMR (101MHz, CD$_3$OD): $\delta = 43.75$ (2C), 47.77 (4C), 120.48 (2C), 126.70 (2C), 127.42 (2C), 127.91 (2C), 128.38 (2C), 128.74 (2C), 130.65 (2C), 131.18 (2C), 138.56 (2C), 140.89 (2C). HRMS: m/z [M + H] calculated for C$_{26}$H$_{31}$N$_4$O$_4$S$_2$: 527.17812; found: 527.17810.

D14

$N, N'$-(butane-1, 4-diyil)bis(5-(dimethylamino)naphthalene-1-sulfonamide)
D14 (2HCl-salt) (146mg, 88%) was obtained from 1,4-diaminobutane (23mg, 0.26mmol) and DNS-Cl (150mg, 0.56mmol) as white power. \(^1\)H NMR (400MHz, CD\(_3\)OD): \(\delta = 1.23\) (br, 4H), 2.68 (br, 4H), 3.50(s, 12H), 7.87-7.89 (m, 4H), 8.12 (d, \(J = 7.1\) Hz, 2H), 8.32 (d, \(J = 7.1\) Hz, 2H), 8.58 (d, \(J = 8.5\) Hz, 2H), 8.89 (d, \(J = 7.9\) Hz, 2H). \(^{13}\)C NMR (101MHz, CD\(_3\)OD): \(\delta = 27.52\) (2C), 43.12(2C), 47.73(4C), 120.36(2C), 126.67(2C), 127.45(2C), 127.89(2C), 128.24(2C), 128.67(2C), 130.68(2C), 131.11(2C), 138.92(2C), 141.21(2C). HRMS (ESI) : m/z [M + H] calculated for C\(_{28}\)H\(_{35}\)N\(_4\)O\(_4\)S\(_2\): 555.2094; found: 555.20935.

D15

5-(Dimethylamino)-N-(2-(2-(8-(dimethylamino)naphthalene-2-sulfamido)ethoxy)ethyl)naphthalene-1-sulfonamide

D15 (2HCl-salt) (91.7mg, 53%) was obtained from 2, 2'-oxybis(ethylamine) (28mg, 0.26mmol) and DNS-Cl (150mg, 0.56mmol) as white power. \(^1\)H NMR (400MHz, CD\(_3\)OD): \(\delta = 2.83\) (t, \(J = 5.3\) Hz, 4H), 3.04 (t, \(J = 5.3\) Hz, 4H), 3.49 (s, 12H), 7.85-7.90 (m, 4H), 8.11 (d, \(J = 7.8\) Hz, 2H), 8.34 (d, \(J = 7.3\) Hz, 2H), 8.61 (d, \(J = 8.7\) Hz, 2H), 8.88 (d, \(J = 8.8\) Hz, 2H). \(^{13}\)C NMR (101MHz, CD\(_3\)OD): 43.61(2C), 47.67(4C), 70.26(2C), 120.31(2C), 126.98(2C), 127.53(2C), 127.75(2C), 128.00(2C), 128.74(2C), 130.70(2C), 130.93(2C), 139.03(2C), 141.58(2C); HRMS (ESI) : m/z [M + H] calculated for C\(_{28}\)H\(_{35}\)N\(_4\)O\(_5\)S\(_2\): 571.20434; found: 571.20422.

D16

\(N, N'-(\text{hexane-1, 6-diyl})\text{bis}(5-(\text{dimethylamino})\text{naphthalene-1-sulfonamide})\)
D16 (2HCl-salt) (118mg, 95%) was obtained from 1,6-Hexanediame (21mg, 0.18mmol) and DNS-Cl (100mg, 0.37mmol) as white powder. $^1$H NMR (400MHz, CD$_3$OD): $\delta = 0.95$ (br, 4H), 1.17 (br, 4H), 2.77 (t, $J = 6.9$ Hz, 4H), 3.50 (s, 12H), 7.85-7.93 (m, 4H), 8.12 (d, $J = 7.8$ Hz, 2H), 8.36 (d, $J = 7.3$ Hz, 2H), 8.59 (d, $J = 8.7$ Hz, 2H), 8.92 (d, $J = 8.8$ Hz, 2H); $^{13}$C NMR (101MHz, CD$_3$OD): $\delta = 26.80$ (2C), 30.44 (2C), 43.68 (2C), 47.78 (4C), 120.46 (2C), 126.66 (2C), 127.36 (2C), 127.95 (2C), 128.45 (2C), 128.63 (2C), 130.74 (2C), 131.12 (2C), 139.07 (2C), 140.97 (2C); HRMS (ESI) : m/z [M + H] calculated for C$_{30}$H$_{39}$N$_4$O$_4$S$_2$: 583.24072; found: 583.24054.

D17

$N, N'$-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(5-(dimethylamino)naphthalene-1-sulfonamide)

D17 (2HCl-salt) (106mg, 62%) was obtained from 1,8-diamino-3,6-dioxaoctane (38mg, 0.25mmol) and DNS-Cl (150mg, 0.50mmol) as yellow power. $^1$H NMR (400MHz, CD$_3$OD): $\delta = 3.05$ (t, $J = 5.4$ Hz, 4H), 3.24 (s, 4H), 3.35 (t, $J = 5.4$ Hz, 4H), 3.48 (s, 12H), 7.88 (dd, $J_1 = 17.5$ Hz, $J_2 = 8.8$ Hz, 4H), 8.10 (d, $J = 7.7$ Hz, 2H), 8.39 (d, $J = 7.3$ Hz, 2H), 8.58 (d, $J = 8.7$ Hz, 2H), 8.91 (d, $J = 8.7$ Hz, 2H); $^{13}$C NMR (101MHz, CD$_3$OD): $\delta = 43.77$ (2C), 47.85 (4C), 70.65 (2C), 71.00 (2C), 120.63 (2C), 126.75 (2C), 127.26 (2C), 128.04 (2C), 128.67 (4C), 130.71 (2C), 131.04 (2C), 139.09 (2C), 140.63 (2C); HRMS (ESI) : m/z [M + H] calculated for C$_{30}$H$_{39}$N$_4$O$_6$S$_2$: 615.23055; found: 615.22974.
$N, N'$-(octane-1, 8-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide)

![Compound D18](image)

D18 (2HCl-salt) (109mg, 89%) was obtained from 1,8-Diaminoctane (26mg, 0.18mmol) and DNS-Cl (100mg, 0.37mmol) as white powder. $^1$H NMR (400MHz, (CD$_3$)$_2$SO): $\delta = 0.79$ (br, 4H), 0.92 (br, 4H), 1.17-1.20 (m, 4H), 2.72-2.74 (m, 4H), 3.01 (s, 12H), 7.59 (br, 2HNH), 7.69 (dd, $J_1 = 15.8$ Hz, $J_2 = 8.0$ Hz, 4H), 7.95 (br, 2H), 8.14 (d, $J = 7.2$ Hz, 2H), 8.50 (d, $J = 7.9$ Hz, 2H), 8.68 (d, $J = 8.0$ Hz, 2H); $^{13}$C NMR (101MHz, (CD$_3$)$_2$SO): $\delta = 25.58$ (2C), 28.05 (2C), 28.82 (2C), 42.21 (2C), 45.53 (4C), 124.51 (2C), 127.51 (4C), 128.43 (4C), 128.49 (4C), 128.84 (4C), 136.47 (2C); HRMS (ESI) : m/z [M + H] calculated for C$_{32}$H$_{43}$N$_4$O$_4$S$_2$: 611.27202; found: 611.27045.

D19

$N, N'$-(decane-1,10-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide)

![Compound D19](image)

D19 (2HCl-salt) (163.5mg, 86%) was obtained from 1,10-Diaminodecane (46mg, 0.26mmol) and DNS-Cl (150mg, 0.56mmol) as white power. $^1$H NMR (400MHz, CD$_3$OD): $\delta = 1.05$-1.23 (br, 12H), 1.30-1.36 (br, 4H), 2.86 (t, $J = 6.9$ Hz, 4H), 3.49(s, 12H), 7.85-7.92 (m, 4H), 8.12 (d, $J = 7.8$ Hz, 2H), 8.37 (d, $J = 7.3$ Hz, 2H), 8.58 (d, $J = 8.7$ Hz, 2H), 8.94 (d, $J = 8.8$ Hz, 2H); $^{13}$C NMR (101MHz, CD$_3$OD): $\delta = 27.43$(2C), 29.95(2C), 30.27(2C), 30.63(2C), 43.89(2C), 47.82(4C), 120.53(2C), 126.60(2C), 127.28(2C), 127.99(2C), 128.60(4C), 130.74(2C), 136.47(2C), 142.13(2C), 150.62(2C), 153.02(2C), 154.31(2C), 160.51(2C), 161.87(2C).
131.14(2C), 139.11(2C), 140.74(2C); HRMS (ESI) : m/z [M + H] calculated for C_{34}H_{47}N_{4}O_{2}S_{2}: 639.30332; found: 639.30310.

D20

\textit{N, N'}-(dodecane-1, 12-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide)

\begin{center}
\includegraphics[width=0.5\textwidth]{D20.png}
\end{center}

D20 (2HCl-salt) (180.5mg, 92%) was obtained from 1,12-diaminododecane (53mg, 0.26mmol) and DNS-Cl (150mg, 0.56mmol) as white power. $^1$H NMR (400MHz, CD$_3$OD): $\delta = 1.11$ (br, 16H), 1.33-1.36 (m, 4H), 2.86 (t, $J = 6.9$ Hz, 4H), 3.49(s, 12H), 7.85-7.92 (m, 4H), 8.12 (d, $J = 7.8$ Hz, 2H), 8.37 (d, $J = 7.3$ Hz, 2H), 8.59 (d, $J = 8.7$ Hz, 2H), 8.94 (d, $J = 8.7$ Hz, 2H); $^{13}$C NMR (101MHz, (CD$_3$)$_2$SO): $\delta = 25.63$(2C), 28.18(2C), 28.57(2C), 28.59(2C), 28.81(2C), 42.13(2C), 45.70(4C), 118.00(2C), 123.56(2C), 124.97(2C), 126.87(2C), 127.32(4C), 127.92(2C), 128.55(2C), 128.61(2C), 136.51(2C); HRMS (ESI) : m/z [M + H] calculated for C_{36}H_{51}N_{4}O_{4}S_{2}: 667.33462; found: 667.33411.

1.3. Probe characterization (photophysical parameters, cell cytotoxicity, cell distribution, incubation concentration)

General information: The fluorescence microscopy was performed with fluorescence microscopy Olympus IX71. Cancer cell lines, HepG2, MCF-7 and A2780 were obtained from cell center of Chinese Academy of Medical Sciences & Peking Union Medical College. They were cultured in DMEM medium (Invitrogen) with 10% fetal bovine serum (Gibco) at 37°C with 5% CO$_2$.

\textbf{Screening of photophysical parameters (\lambda_{ex}, \lambda_{em}) for all CLFP probes.}
Fluorescence excitation and emission spectra were measured and calculated with HITACHI F-7000 Fluorescence Spectrophotometer. Each compound was dissolved in PBS buffer, pH 7.4 with the concentration of 10 μM.

Table S3, The photophysical parameters ($\lambda_{\text{ex}}$, $\lambda_{\text{em}}$) for all of the CLFP probe in this study.

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Cell Cytoxicity Assay

Probe B10 (0.5 μM) and D13 (25 μM) were incubated with three cell line HepG2, A2780 and MCF-7 for 1 hour, which is similar to the staining condition in this study. After that, the dyes were washed away using PBS buffer and cells were kept culturing under regular condition, and we did not observe cell toxicity for all three cell lines.

Nevertheless, we also evaluated cell cytotoxicities for all 33 fluorescent probes using MTT method. Most of the compounds have the IC\textsubscript{50} over than 50 μM for all tested cancer cell lines, but a few of them do show some cell cytotoxicities.

Table S4: Evaluation of cell cyto-toxicity of CLFP compounds

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### Screening of fluorescence intensities of the probes in living cells

HepG2 cells were seeded in cell culture plates, and when the cells reached 50% confluence, two series of fluorescent probes (BODIPY and DNS) were separately added into the each well with the final concentration of 0.5μM, 1μM (BODIPY), 10μM, 25μM (DNS). After incubation for 10 to 30 min, the fluorescent probes were washed away with PBS buffer. HepG2 cells were imaged under fluorescent microscopy. The relative fluorescence intensities of all probes were compared to choose the feasible CLFP probes (Figure S1-S4).

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Figure S1. Screening of cellular distribution of fluorescent probes B1 to B13 in HepG2 cells at 0.5 μM after 30min of incubation.
Figure S2. Screening of cellular distribution of fluorescent probes B1 to B13 in HepG2 cells at 1 μM after 30 min of incubation.

Figure S3. Screening of cellular distribution of fluorescent probes D1 to D20 in HepG2 cells at 10 μM after 30 min of incubation.
Figure S4. Screening of cellular distribution of fluorescent probes D1 to D20 in HepG2 cells at 25 μM after 30 min of incubation.

For BODIPY derivatives, at a concentration of 0.5 and 1 μM, the fluorescent intensities of many probes in HepG2 cells were sufficiently bright (Figure S1, S2) after 10 to 30 min incubation. For DNS derivatives, at a concentration of 10 μM, the fluorescent intensities of many probes in HepG2 cells were sufficiently bright (Figure S3, S4) after 10 to 30 min incubation. Generally, at the same molar concentration, probes with two DNS moieties exhibited stronger fluorescent intensity than probes with a single DNS moiety.

We finally chose probe B10 (0.5 μM) and D13 (25 μM) as the CLFP for this study, considering the sub-cellular distribution and fluorescence intensity.

Sub-cellular localization of fluorescent probes in various cell lines

According to fluorescence intensities and the physical chemistry properties, probe B10 and D13 was selected to further detect the sub-cellular localization in various cancer cell lines. Cells were cultured in 24-well plates with low density. Notably, in the imaging experiment, probes D13 was only needed to incubate with cells for 0.5 hour, but in this sub-cellular localization experiment, we decided to incubate the probe with cells for at least 3 hours to evaluate the localization of these probes. So this make sure that probe D13 will not stain cell nuclei after long period of
incubation, which is important to maintain the reliability of CLFP assay of this study. After incubation, the medium was washed away with PBS buffer, HepG2 cells were imaged under confocal microscopy.
Copy of NMR spectra of all compounds

B1

3-carbonitrile-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene
B2
4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene

12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

158.71 141.30 123.60 120.68 119.01 77.85 76.68 76.72 14.45

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
2,2,2-trichloroethyl-2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-yl)acetate
B4

2-ethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene
8-(dec-9-yn-1-yl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene
4,4-difluoro-8-(hex-5-yn-1-yl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene
8- (chloropropyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene
8-[4-(chloromethyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-1,4-bora-3a,4a-diaza-s-indacene
4,4-difluoro-8-methyl-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene
8-{4-[(decylamino)methyl]phenyl}-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene
8-[(hexylamino)methyl]phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene
B12

8-[4-((dodecylamino)methyl)phenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene
8-[(10-aminodecyl)amino)methyl]phenyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene
Copy of NMR spectra of series 2

5-(Dimethylamino)-N-propynaphthalene-1-sulfonamide (D1)
5-(Dimethylamino)-N-hexynaphthalene-1-sulfonamide (D2)
N-decyl-5-(dimethylamino) naphthalene-1-sulfonamide (D3)
(Z)-5-(dimethylamino)-N-(octadec-9-en-1-yl) naphthalene-1-sulfonamide (D4)
$N, N'$-didecyl-5-(dimethylamino) naphthalene-1-sulfonamide (D5)
$N$-(2-aminoethyl)-5-(dimethylamino) naphthalene-1-sulfonamide (D6)
5-(Dimethylamino)-N-(4-hydroxybutyl) naphthalene-1-sulfonamide (D7)
$N$-(6-aminohexyl)-5-(dimethylamino) naphthalene-1-sulfonamide (D8)
$N$-(8-aminoctyl)-5-(dimethylamino) naphthalene-1-sulfonamide (D9)
N-(2-(2-aminoethoxy)ethoxy)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (D10)
$N$-(10-aminodecyl)-5-(dimethylamino) naphthalene-1-sulfonamide (D11)
N-(12-aminododecyl)-5-(dimethylamino) naphthalene-1-sulfonamide (D12)
$N, N'$-(ethane-1, 2-diyl) bis(5-(dimethylamino)naphthalene-1-sulfonamide) (D13)
$N, N'-(\text{butane-1, 4-diy})\text{bis(5-(dimethylamino)naphthalene-1-sulfonamide})$ (D14)
5-(Dimethylamino)-N-(2-(2-(8-(dimethylamino)naphthalene-2-sulfonamido)ethoxy)ethyl)naphthalene-1-sulfonamide (D15)
$N, N'$-(hexane-1, 6-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide) (D16)
$N, N'-(\text{ethane-1,2-diylbis(oxy)})\text{bis(ethane-2,1-diyl)}\text{bis(5-(dimethylamino)}$

naphthalene-1-sulfonamide$)$ (D17)

![Chemical Structure Image]
$N, N'-(\text{octane-1, 8-diy})\text{bis}(5-(\text{dimethylamino})\text{naphthalene-1-sulfonamide})$ (D18)

![Chemical structure and NMR spectra](image-url)
$N,N'$(decane-1,10-diyl)bis(5-(dimethylamino)naphthalene-1-sulfonamide) (D19)
$N, N'-(\text{dodecane-1, 12-diyl})\text{bis}(5-(\text{dimethylamino})\text{naphthalene-1-sulfonamide})$ (D20)