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Supplemental information

A light-switching pyrene probe to detect phase-separated biomolecules

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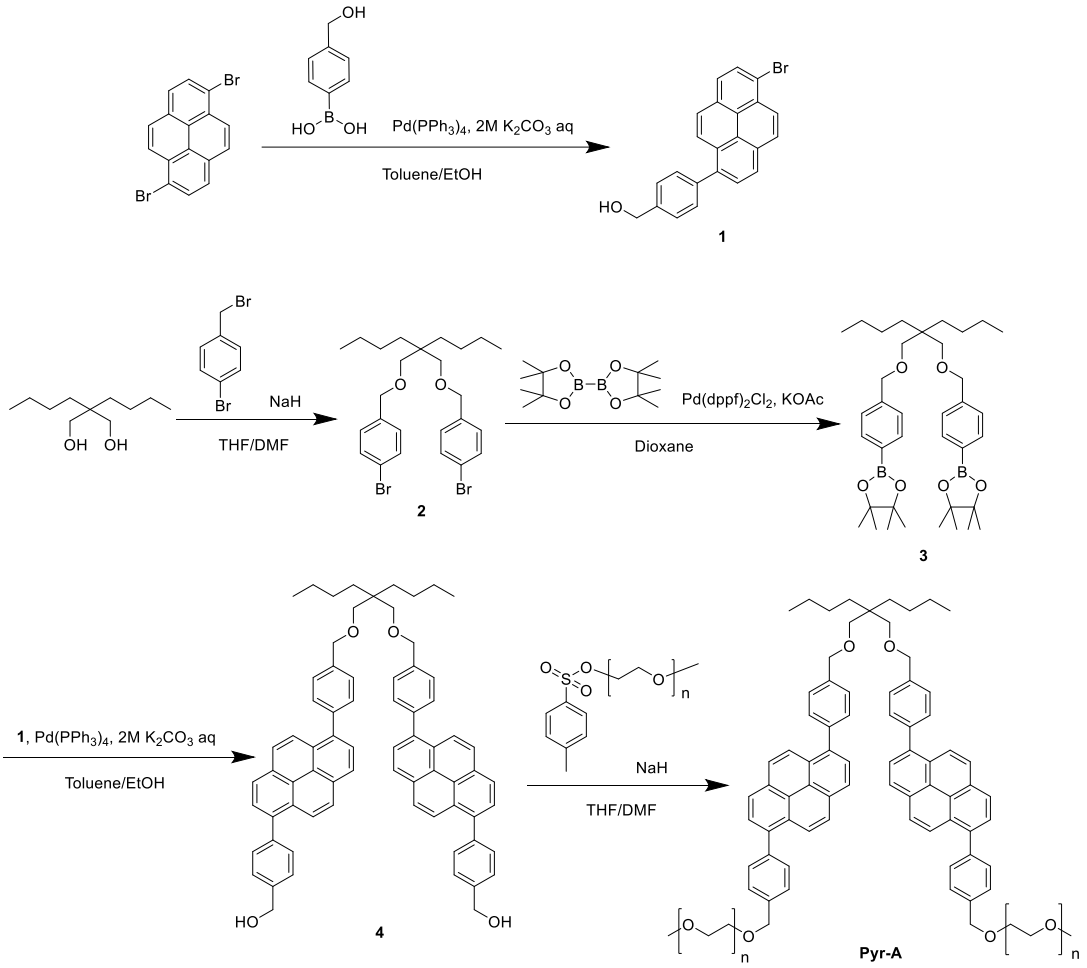
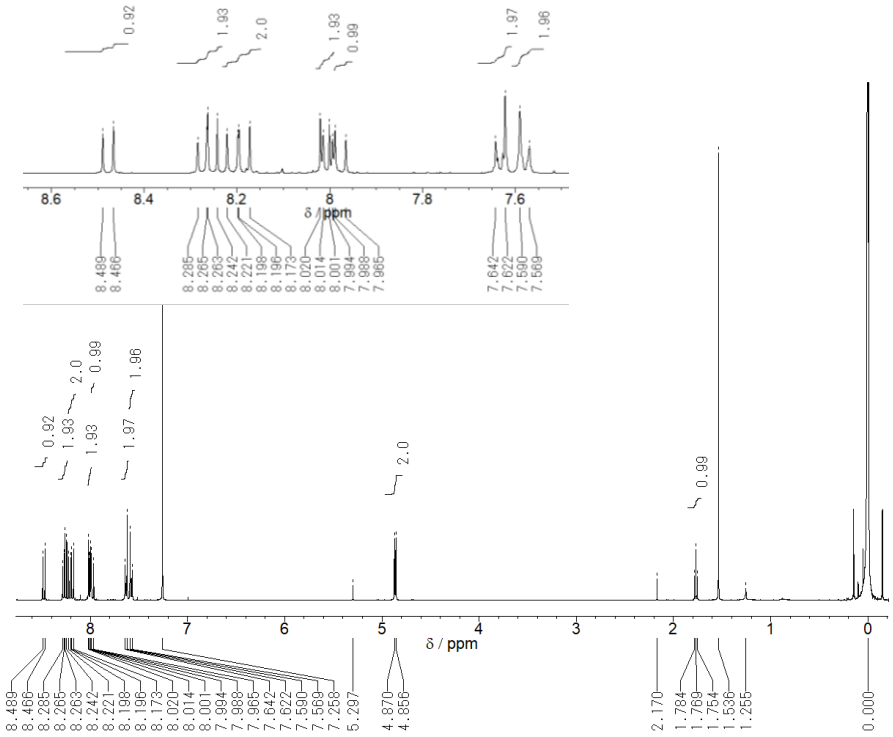
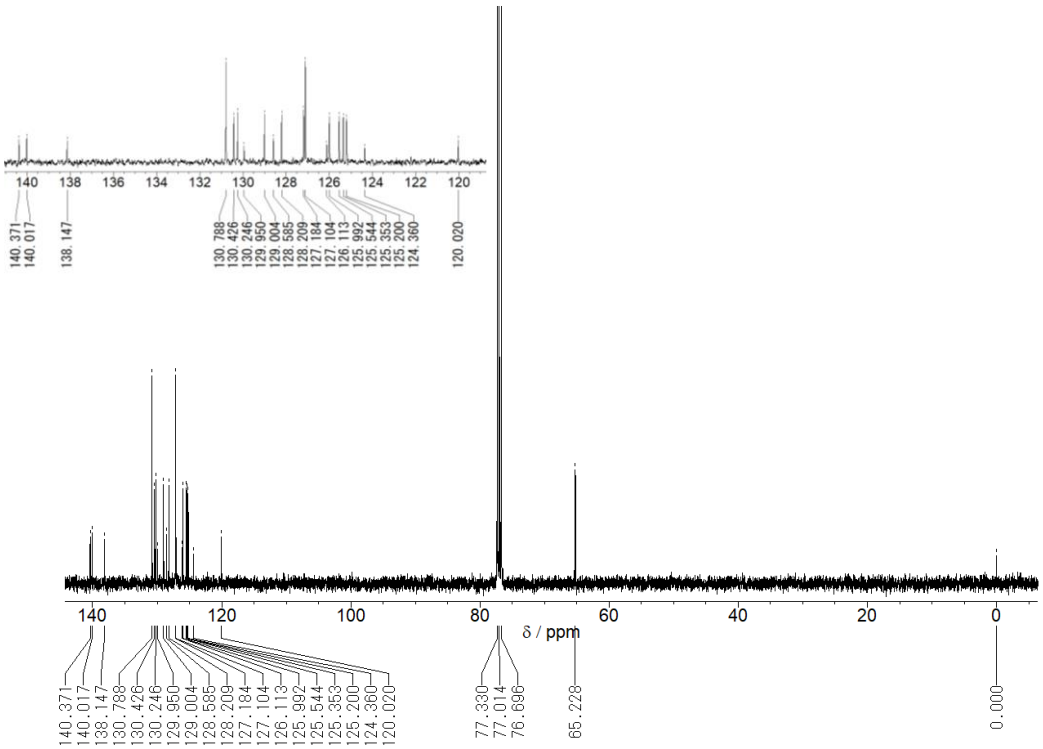
SUPPLEMENTAL INFORMATION



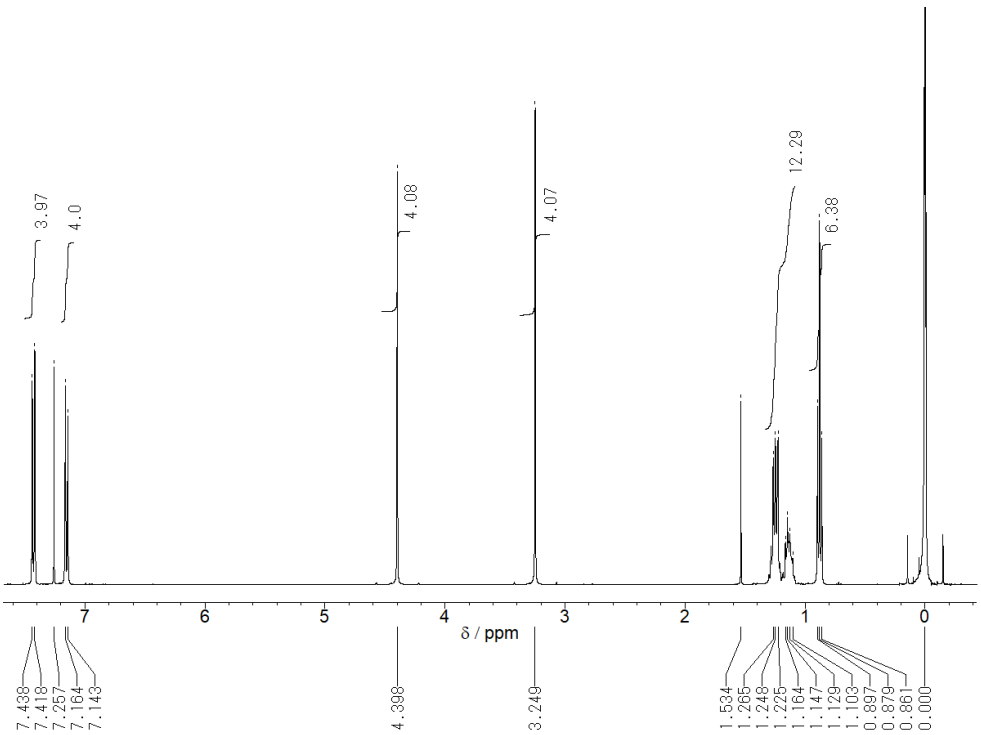
Scheme S1. Synthetic route of Pyr-A, related to **Figure 2** and **STAR Methods.**



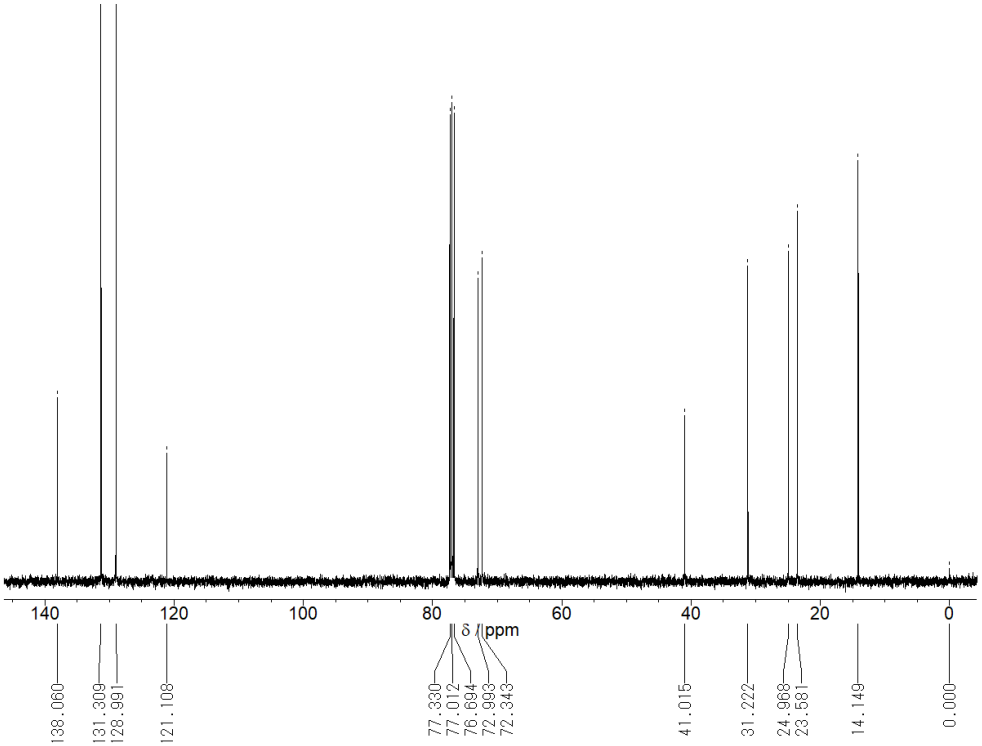
Data S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS standard) of 1, related to **Scheme S1.** ^{1}H NMR (400 MHz, $CDCl_3$, TMS standard): δ (ppm) 1.77 (t, *J* = 5.9 Hz, 1 H, O*H*), 4.86 (t, *J* = 5.9 Hz, 2 H, CH_2), 7.58 (d, *J* = 8.3 Hz, 2 H, Ar*H*), 7.63 (d, *J* = 8.3 Hz, 2 H, Ar*H*), 7.98 (d, *J* = 9.3 Hz, 1 H, Ar*H*), 8.00 (d, *J* = 8.3 Hz, 1 H, Ar*H*), 8.01 (d, *J* = 7.9 Hz, 1 H, Ar*H*), 8.18 (d, *J* = 9.3 Hz, 1 H, Ar*H*), 8.21 (d, *J* = 9.2 Hz, 1 H, Ar*H*), 8.25 (d, *J* = 8.3 Hz, 1 H, Ar*H*), 8.27 (d, *J* = 7.9 Hz, 1 H, Ar*H*), 8.48 (d, *J* = 9.2 Hz, 1 H, Ar*H*).



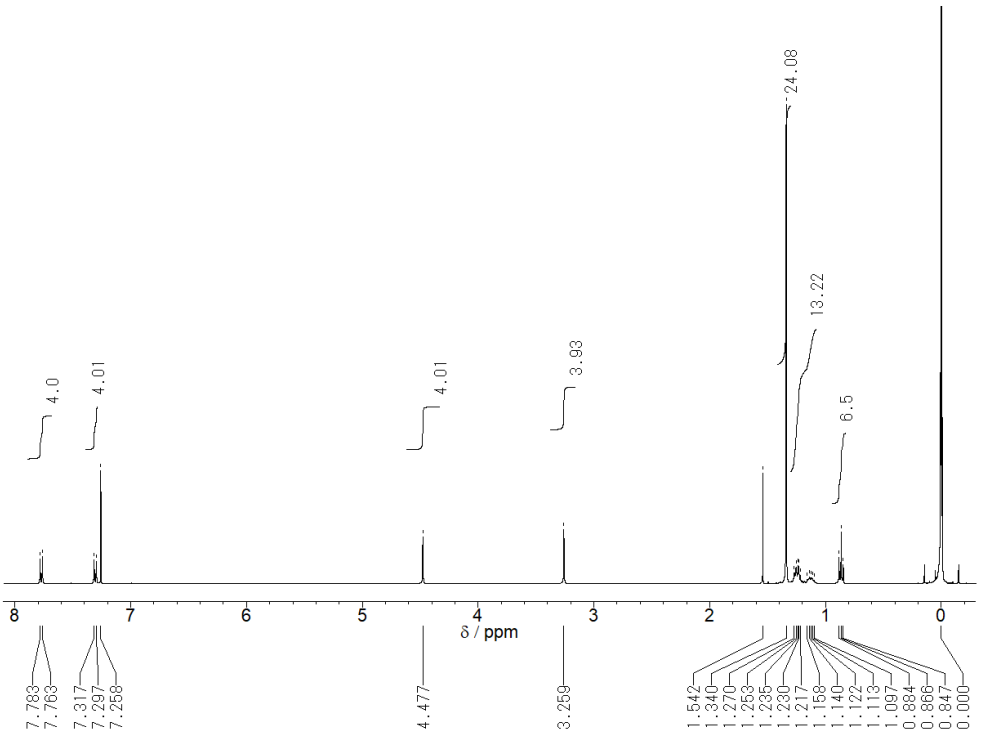
Data S2. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, TMS standard) of 1, related to **Scheme S1**. ^{13}C NMR (100 MHz, $CDCl_3$, TMS standard): δ (ppm) 65.23, 120.02, 124.36, 125.20, 125.35, 125.54, 125.99, 126.11, 127.10, 127.18, 128.21, 128.59, 129.00, 129.95, 130.25, 130.43, 130.79. 138.15, 140.02, 140.37. HRMS(EI) Calcd for $C_{23}H_{15}BrO$ [M^+]: m/z 386.0306, Found: m/z 386.0312. Elemental analysis; Calcd for $C_{23}H_{15}BrO$: C 71.33, H 3.90, Found: C 71.28, H 4.10.



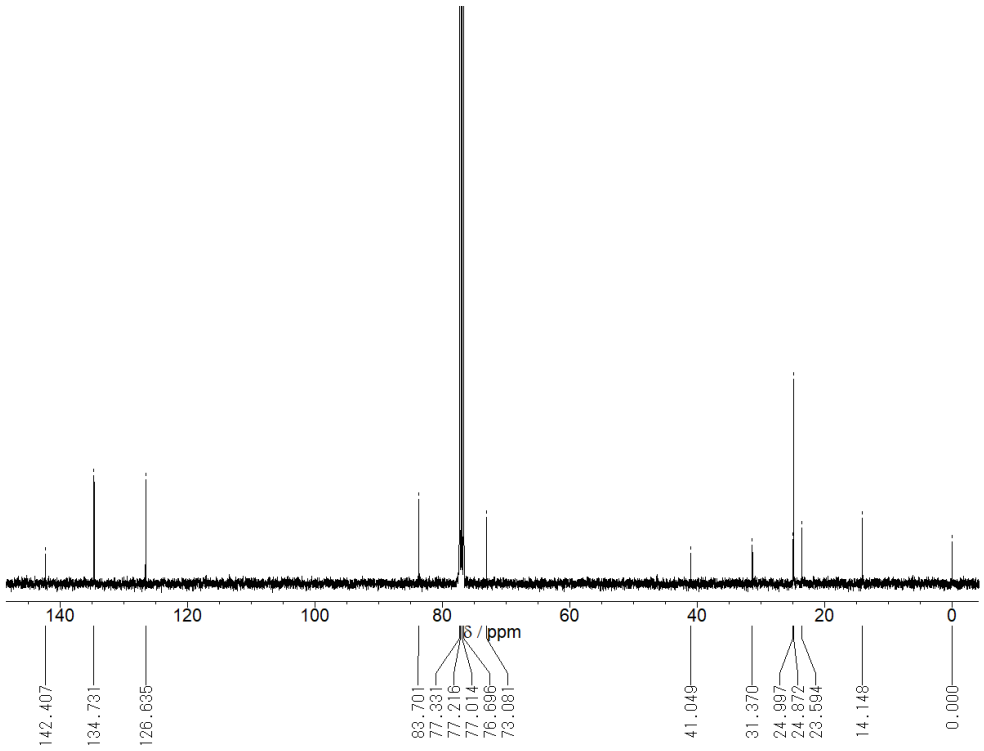
Data S3. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS standard) of 2, related to **Scheme S1**. ^{1}H NMR (400 MHz, $CDCl_3$, TMS standard): δ (ppm) 0.88 (t, *J* = 7.2 Hz, 6 H, C*H*$_3$), 1.08-1.32 (m, 12 H, C*H*$_2$), 3.25 (s, 4 H, C*H*$_2$), 4.40 (s, 4 H, C*H*$_2$), 7.15 (d, *J* = 8.3 Hz, 4 H, Ar*H*), 7.43 (d, *J* = 8.3 Hz, 4 H, Ar*H*).



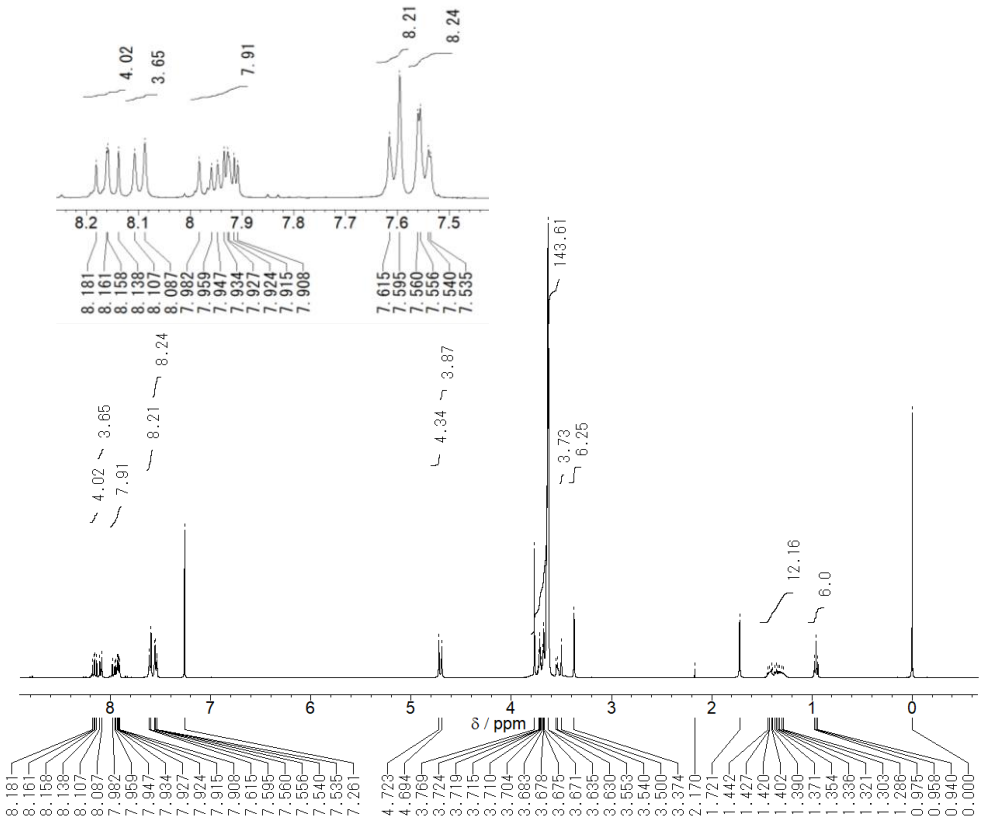
Data S4. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, TMS standard) of 2, related to **Scheme S1.** ^{13}C NMR (100 MHz, $CDCl_3$, TMS standard): δ (ppm) 14.15, 23.58, 24.97, 31.22, 41.02, 72.34, 72.99, 121.11, 128.99, 131.31, 138.06. HRMS(EI) Calcd for $C_{25}H_{34}Br_2O_2$ [M^+]: m/z 524.0926, Found: m/z 524.0938. Elemental analysis; Calcd for $C_{25}H_{34}Br_2O_2$: C 57.05, H 6.51, Found: C 56.84, H 6.39.



Data S5. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS standard) of 3, related to **Scheme S1**. ^{1}H NMR (400 MHz, $CDCl_3$, TMS standard): δ (ppm) 0.87 (t, *J* = 7.3 Hz, 6 H, C*H*$_3$), 1.08-1.30 (m, 12 H, C*H*$_2$), 1.34 (s, 24 H, C*H*$_3$), 3.26 (s, 4 H, C*H*$_2$), 4.48 (s, 4 H, C*H*$_2$), 7.31 (d, *J* = 8.1 Hz, 4 H, Ar*H*), 7.77 (d, *J* = 8.1 Hz, 4 H, Ar*H*).



Data S6. ^{13}C NMR spectrum (100 MHz, $CDCl_3$, TMS standard) of 3, related to **Scheme S1**. ^{13}C NMR (100 MHz, $CDCl_3$, TMS standard): δ (ppm) 14.15, 23.59, 24.87, 25.00, 31.37, 41.05, 73.08, 77.22, 83.70, 126.64, 134.73, 142.41. HRMS(FAB) Calcd for $C_{37}H_{59}O_6B_2$ [$(M+H)^+$]: m/z 621.4498, Found: m/z 621.4500.



Data S7. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, TMS standard) of Pyr-A, related to **Scheme S1**. ^{1}H NMR (400 MHz, $CDCl_3$, TMS standard): δ (ppm) 0.96 (t, *J* = 7.0 Hz, 6 H, C*H*$_3$), 1.24-1.45 (m, 12 H, C*H*$_2$), 3.37 (s, 6 H, OC*H*$_3$), 3.50 (s, 4 H, OC*H*$_2$CH), 3.52-3.80 (m, 144 H, OC*H*$_2$C*H*$_2$O), 4.69 (s, 4 H, OC*H*$_2$Ar), 4.72 (s, 4 H, OC*H*$_2$Ar), 7.53-7.58 (8 H, Ar*H*), 7.61 (d, *J* = 8.0 Hz, 8 H, Ar*H*), 7.90-7.99 (8 H, Ar*H*), 8.10 (d, *J* = 8.0 Hz, 4 H, Ar*H*), 8.13-8.19 (4 H, Ar*H*).

Table S1. Spectroscopic parameters of Pyr-A in various solvents, related to **Figure 2.**

solvent[a]	λ_{max}^{abs} (nm)	ε (M^{-1} cm^{-1})[b]	Φ_f	τ_M (ns)	τ_E (ns)	$I_{470/400}$	$E_T(30)$[c]
benzene	359	7.6×10^4	0.46	3.9	4.2	0.29	34.3
THF	358	8.1×10^4	0.53	3.5	4.7	0.29	37.4
$CHCl_3$	359	8.0×10^4	0.48	3.7	5.1	0.28	39.1
acetone	356	7.8×10^4	0.45	3.8	11	0.69	42.2
DMSO	360	8.0×10^4	0.47	4.5	9.5	0.39	45.1
ACN	356	8.0×10^4	0.39	4.5	12	1.17	45.6
1-OcOH	358	7.9×10^4	0.52	3.9	10	0.41	48.3
1-PrOH	357	8.1×10^4	0.47	4.1	10	0.67	50.7
EtOH	356	8.0×10^4	0.44	4.0	11	0.90	51.9
MeOH	356	8.0×10^4	0.40	4.2	12	1.54	55.4
water	356	4.1×10^4	0.08	2.4	2.5	25.8	63.8

a THF: tetrahydrofuran; $CHCl_3$: chloroform; DMSO: dimethylsulfoxide; ACN: acetonitrile; 1-OcOH : 1-octanol; 1-PrOH : 1-propanol; EtOH: ethanol; MeOH: methanol

b Measured at absorption peak wavelength

c Reichardt 1994

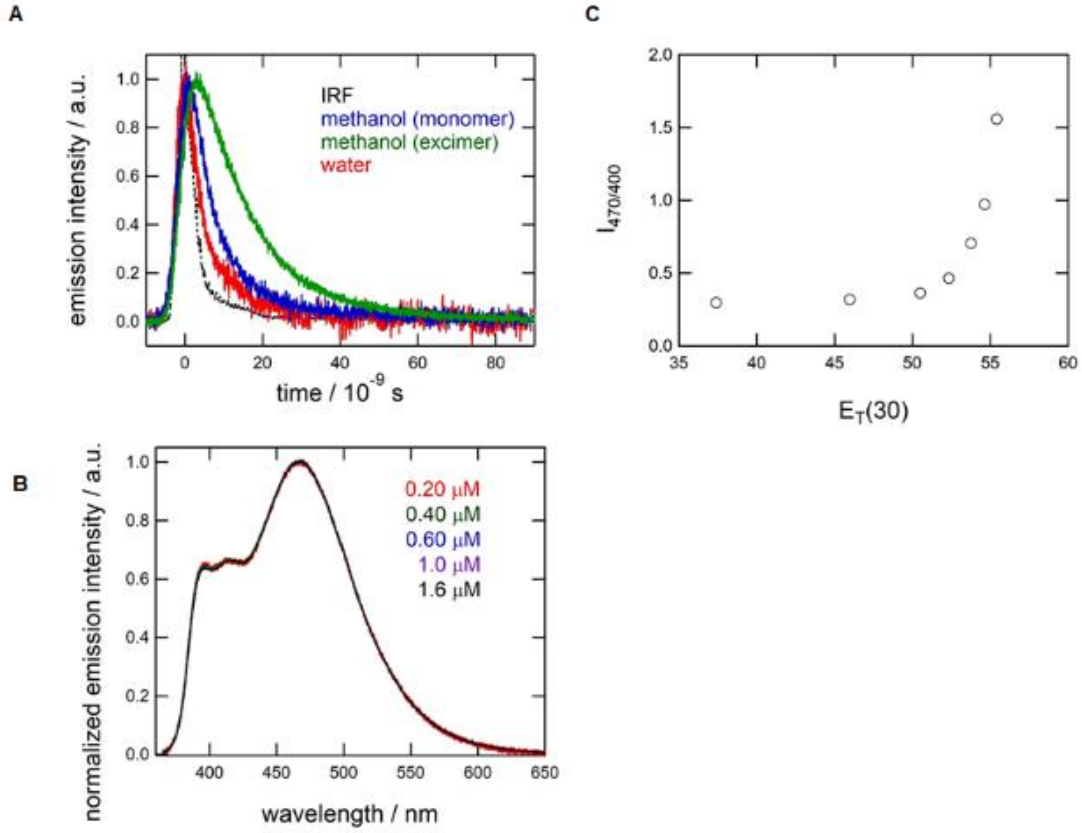


Figure S1. ***In Vitro*** **Spectroscopic Properties of Pyr-A**, related to **Figure 2.** (A) Time-resolved fluorescence curves of Pyr-A in methanol (blue, 400 nm; green, 470 nm) and water (red, 470 nm). A dotted curve is the instrumental response function (IRF). (B) Emission spectra of Pyr-A in methanol at 0.20, 0.40, 0.60, 1.0 and 1.6 μM concentrations. (C) Fluorescence intensity ratio of excimer emission (at 470 nm) to monomer emission (at 400 nm), $I_{470/400}$, plotted against $E_T(30)$ of binary mixed solvents of methanol and tetrahydrofuran.

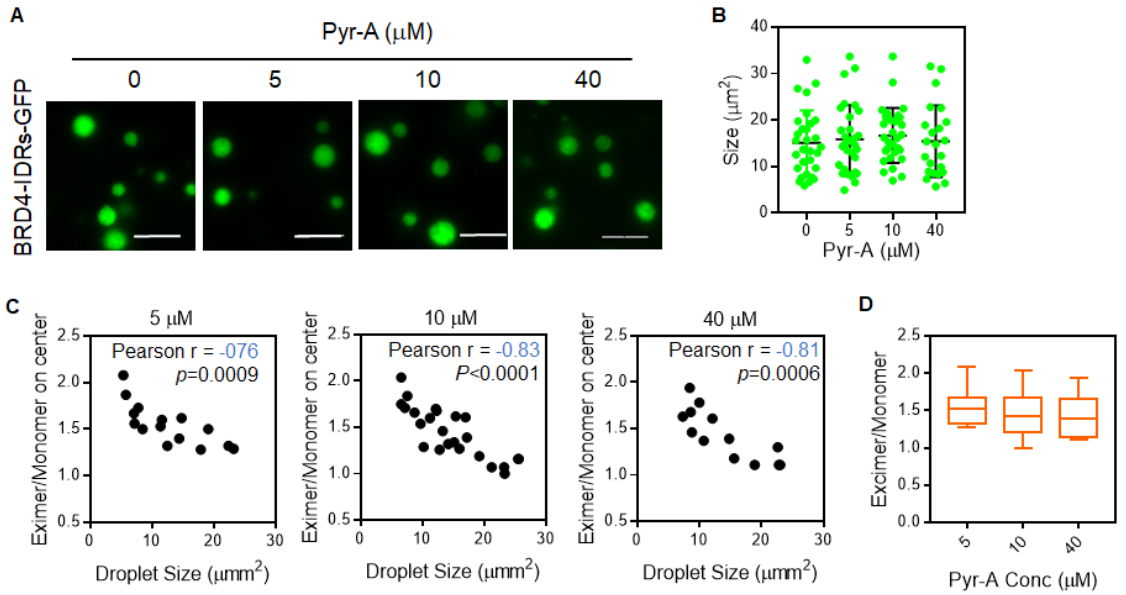


Figure S2. The Size and E/M ratio Profiles of BRD4-IDR-mEGFP Droplets in The Presence of Different Concentration of Pyr-A, related to **Figure 3**. (A) Representative image of BRD4-IDRs-GFP in the presence of different concentration of Pyr-A from the droplets formation assays. Bar=10μm. (B) Quantitative analysis was performed (Mean +/- SD; n=23-30). There is no statistical difference. (C) E/M ratio on the center of droplets was analyzed. Correlation analysis was performed using GraphPad Prizm. (D) E/M values were quantified. Box plot element: medians with interquartile range and whiskers (min to max). There is no statistical difference between conditions.

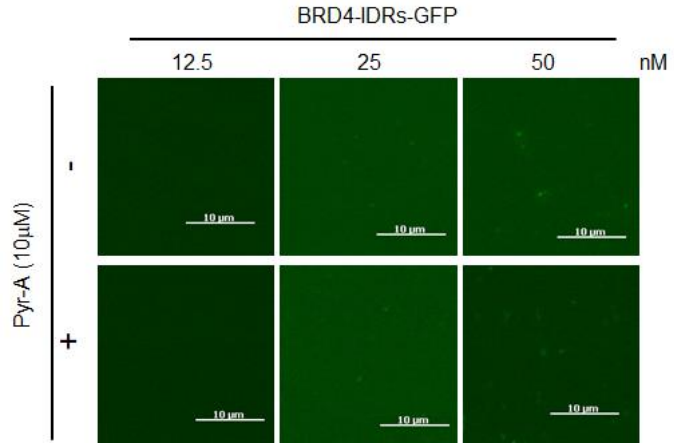


Figure S3. The Effects of Pyr-A on The Csat of BRD4-IDR-mEGFP, related to **Figure 3.** Droplets formation assay of BRD4-IDRs-GFP was performed in the absence of Pyr-A (Upper) or presence of Pyr-A (Lower). Csat is between 12.5 and 25 nM in the both conditions.

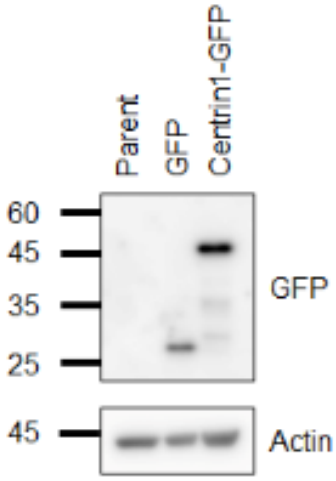


Figure S4, The Expression of Exogenous Centrin1-GFP in HeLa Cells, related to **Figure 4.** Western blotting analysis of centrin1-GFP protein levels. Lysate was obtained from parental cells (MOCK), GFP expressed cells or centrin1-GFP expressed cells. Cell lysates were subjected to SDS-PAGE and immunoblotting using anti-GFP antibody.