Electronic Supplementary Information (ESI)

for

Pyrrolic Molecular Rotors Acting as Viscosity Sensors with High Fluorescent Contrast

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Synthesis

The reagents 1*H*-pyrrole-2-carbaldehyde, bromoethane, 1-bromobutane and 1-phenyl-1*H*-pyrrole-2-carbaldehyde are purchased from Sigma Aldrich and used as received. The intermediate 2-(3,5,5-trimethylcyclohex-2-en-1-ylidene)malononitrile was synthesized according to the literature.[S1] MP and DAP compounds were synthesized following the synthetic protocols described previously.[S1,S2]

A. Synthesis of Intermediates

1-Ethyl-1H-pyrrole-2-carbaldehyde: 1*H*-pyrrole-2-carbaldehyde and potassium carbonate (K_2CO_3) were dissolved in dimethylformamide (DMF) (20 ml) and the solution was stirred at 40 ^oC for 1 h. After adding bromoethane, the solution was stirred and heated at 70 ^oC. After 20 h, the solution was filtered to remove residual K_2CO_3 and DMF is eliminated by evaporation. The resulting solution was separated using dichloromethane and water. A white product was obtained using column chromatography (dichloromethane). Yield = 40%. ^{1}H-NMR ($CDCl_3$, δ): 9.52 (s, 1H, -CHO), 6.96-6.91 (m, 2H, Py-H), 6.21 (m, H, Py-H), 4.34 (m, 2H, -CH_2-), 1.38 (t, 3H, -CH_3).

1-Butyl-1H-pyrrole-2-carbaldehyde: 1*H*-pyrrole-2-carbaldehyde was dissolved in DMF (20 ml) followed by addition of K_2CO_3. After stirring at 40 ^oC for 1 h, 1-bromobutane was added and the solution was stirred and heated at 70 ^oC for 20 h. Residual K_2CO_3 was removed by filtration and DMF was eliminated by evaporation. The solution was separated using dichloromethane and water. A white product was obtained using column chromatography (dichloromethane). Yield = 45%. ^{1}H-NMR ($CDCl_3$, δ): 9.48 (s, 1H, -CHO), 6.90-6.87 (m, 2H, Py-H), 6.16 (m, H, Py-H), 4.27 (m, 2H, -CH_2-), 1.70 (m, 2H, -CH_2-), 1.28 (m, 2H, -CH_2-), 0.88 (m, 3H, -CH_3).

B. Synthesis of Pyrrolic Molecular Rotors

The intermediate 2-(3-(2-(1-ethyl-1H-pyrrol-2-yl)vinyl)-5,5-dimethylcyclohex-2-en-1-

ylidene)malononitrile and corresponding pyrrolic aldehydes were dissolved in ethanol and then piperidine was added. The solution was stirred and heated at 60 ^{o}C for 20 h. The resulting solution was cooled at room temperature and the precipitate was filtered. The precipitate was purified by recrystallization method. Residue solvent was evaporated in a vacuum oven at 60 ^{o}C.

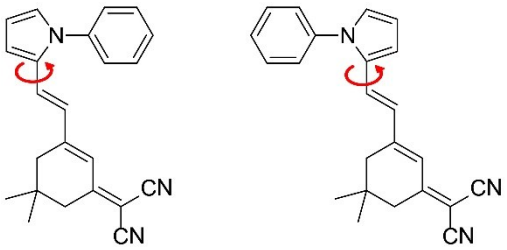
2-(5,5-dimethyl-3-(2-(1-phenyl-1H-pyrrol-2-yl)vinyl)cyclohex-2-enylidene)malononitrile (PP): 29 % yield. ^{1}H-NMR ($CDCl_3$, δ): 7.54-7.50 (m, 2H, Ar-H), 7.47 (m, H, Ar-H), 7.33-7.30 (m, 2H, Ar-H), 6.98 (m, 1H, Py-H), 6.83 (d, 1H, Py-H), 6.69 (t, 1H, Py-H), 6.75-6.79 (d, 1H, *J* = 15.6 Hz, -CH=CH-), 6.69-6.73 (d, 1H, *J* = 16 Hz, -CH=CH-), 6.39 (m, 1H, -C=CH-), 2.52 (s, 2H, -CH_2-), 2.20 (s, 2H, -CH_2-), 1.00 (s, 6H, -CH_3). ^{13}C-NMR ($CDCl_3$, δ): 168.94, 154.52, 139.04, 131.52, 129.66, 128.22, 126.98, 126.44, 122.14, 114.19, 113.38, 112.02, 111.34, 76.82, 43.31, 39.18, 32.30, 28.36. MS Calcd. 339.17; Found 339.03. λ_{abs} (ethanol): 464 nm.

2-(3-(2-(1-ethyl-1H-pyrrol-2-yl)vinyl)-5,5-dimethylcyclohex-2-enylidene)malononitrilee (EP): 25 % yield. ^{1}H-NMR ($CDCl_3$, δ): 6.83 (d, 1H, Py-H), 6.73 (d, 1H, Py-H), 6.69 (m, 1H, Py-H), 6.90-6.94 (d, 1H, *J* = 16 Hz, -CH=CH-), 6.69-6.73 (d, 1H, *J* = 15.6 Hz, -CH=CH-), 6.23 (m, 1H, -C=CH-), 4.04 (m, 2H, -CH_2-), 2.58 (s, 2H, -CH_2-), 2.41 (s, 2H, -CH_2-), 1.43 (t, 3H, -CH_3), 1.08 (s, 6H, -CH_3). ^{13}C-NMR ($CDCl_3$, δ): 168.90, 154.64, 130.21, 125.50, 125.33, 124.80, 121.86, 114.32, 113.53, 111.74, 110.42, 76.43, 43.33, 42.19, 39.45, 32.38, 30.09, 28.45, 17.24. MS Calcd. 291.17; Found 290.98. λ_{abs} (ethanol): 478 nm.

2-(3-(2-(1-butyl-1H-pyrrol-2-yl)vinyl)-5,5-dimethylcyclohex-2-enylidene)malononitrile (BP): 20 % yield. ^{1}H-NMR ($CDCl_3$, δ): 6.80 (d, 1H, Py-H), 6.75 (d, 1H, Py-H), 6.68 (m, 1H, Py-H), 6.89-6.93 (m, 1H, *J* = 15.6 Hz, -CH=CH-), 6.69-6.73 (d, 1H, *J* = 16.4 Hz, -CH=CH-), 6.28 (m, 1H, -C=CH-), 3.98 (t, 2H, -CH_2-), 2.58 (s, 2H, -CH_2-), 2.40 (s, 2H, -CH_2-), 1.71 (m, 2H, -CH2-), 1.32 (m, 2H, -CH2-), 1.08 (s, 6H, -CH_3), 0.96 (t, 3H, -CH_3). ^{13}C-NMR ($CDCl_3$, δ): 168.87, 154.59, 130.43, 126.38, 125.27, 124.90, 121.83, 114.32, 113.51, 111.56, 110.23, 76.43, 47.17, 43.32, 39.45, 33.95, 32.38, 28.46, 20.35, 14.10. MS Calcd. 319.20;

Found 319.07. λ_{abs} (ethanol): 478 nm.

Rotational Isomers



Scheme S1. Proposed two rotational isomers of pyrrolic molecular rotors: *e.g.,* R- and L-isomers of PP.

Fluorescence Measurement in Viscous Mediums

A. Absorption and Fluorescence Measurements

The absorption and fluorescence of all molecular rotors were meaused at room temperature. The solution concetrations were 1.0×10^{-5} M. The ethanol is used as solvent in the absorption measurements, for fluorescence measurements in viscous mediums, the mixed solvent consisted of ethanol, ethylene glycol and glycerol with various different ratios are used. UV-Vis absorption and fluorescence spectra were mesured using a Jasco V-670 and Ocean Optics Mya 2000 pro spectrometer at an excitation wavelength of 405 nm from Ergonomic LED Light Sources LLS-405, respectively.

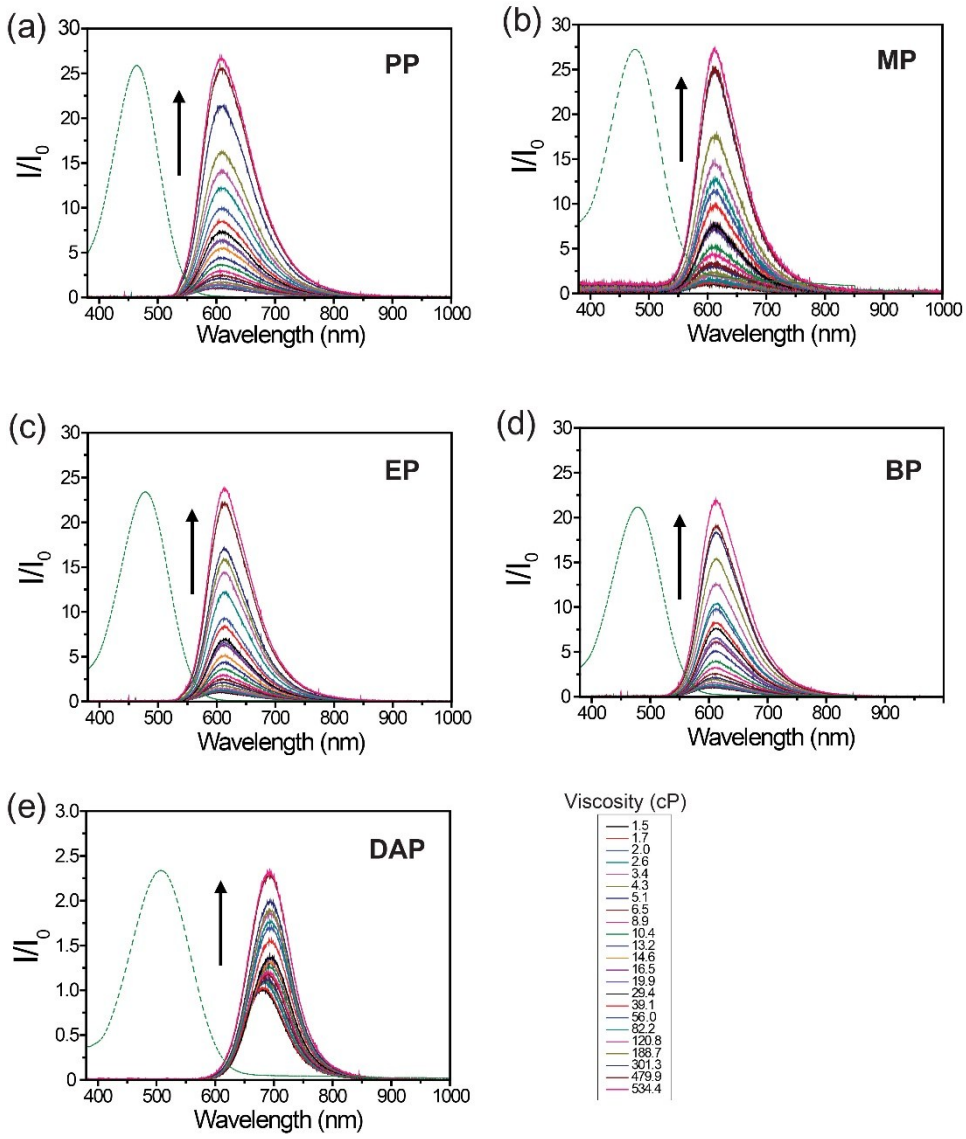


Fig. S1. The absorption and fluorescence spectra of (a) PP, (b) MP, (c) EP, (d) BP and (e) DAP under various viscosity mediums.

B. Fluorescence Measurements under Various Sovents

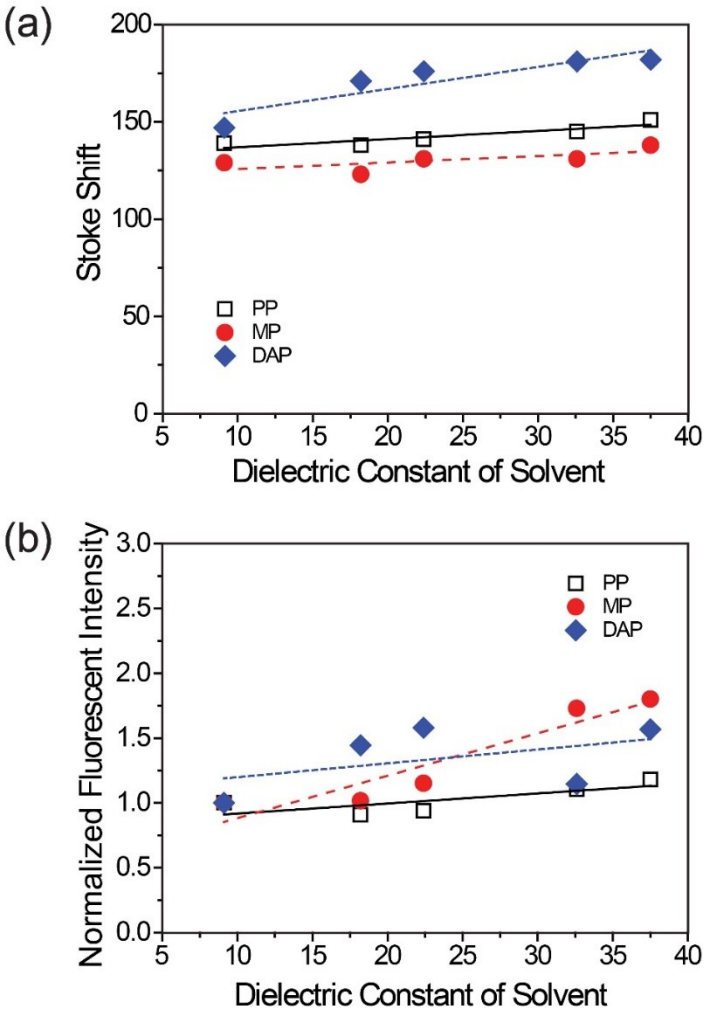


Fig. S2. (a) Stoke shift and (b) normalized fluorescent intensity of PP, MP and DAP under various solvents: methylene chloride, buthanol, ethanol, methanol and acetonitrile, for which dielectric constant is 8.93, 18.2, 24.5, 32.7 and 37.5, respectively, obtained from Ref. [S3].

C. Time-Correlated Single Photon Counting (TCSPC) Measurements

Time-correlated single photon counting (TCSPC) was performed to investigate the kinetics of fluorescence intensity change in pyrrolic viscosity sensors with different *N*-substituted group according to media viscosity. The second harmonic (SHG =405 nm) of a tunable Ti:sapphire laser (Mira900, Coherent) with ~150 fs pulse width and 76 MHz repetition rate was used as an excitation source. The fluorescence emission was spectrally resolved using some collection optics and a monochromater (SP-2150i, Acton). The TCSPC module (PicoHarp 300, PicoQuant) with MCP-PMT (R3809U-59, Hamamatsu) was used for ultrafast detection. The total instrument response function (IRF) was less than 140 ps, and the temporal time resolution was less than 10 ps. The deconvolution of the fluorescence decay curve, which separates the IRF and actual fluorescence decay signal, was performed using fitting software (FluoFit, PicoQuant) to deduce the time constant associated with each exponential decay curve.

Table S1. Fluorescence lifetimes of PP in solution.[a)]

Mateials/Viscosity	τ_1/ns, (f_1)	τ_2/ns, (f_2)	$\chi^{2b)}$	τ_{avr}/ns [c)]
PP/Viscosity : 10	0.07 (1.00)	-	1.182	0.07
PP/Viscosity : 15	0.10 (1.00)	-	1.070	0.10
PP/Viscosity : 19	0.11 (1.00)	-	1.085	0.11
PP/Viscosity : 55	0.15 (0.56)	0.24 (0.44)	1.040	0.19
PP/Viscosity : 88	0.15 (0.36)	0.28 (0.64)	1.055	0.23
PP/Viscosity : 534	0.25 (0.25)	0.70 (0.75)	1.076	0.59

[a)] Monitored wavelength was 609 nm. The fluorescence decay curves were fitted by a single and bi-exponential function to calculate the material lifetimes. [b)] Parameter χ^2 is the reduced chi-squared value. [c)] The intensity-weighted average lifetime (τ_{avr}) is defined as $f_1\tau_1 + f_2\tau_2$, where f_1 and f_2 are fractional intensities and τ_1 and τ_2 are lifetimes.

Table S2. Fluorescence lifetimes of MP in solution.[a)]

Mateials/Viscosity	τ_1/ns, (f_1)	τ_2/ns, (f_2)	$\chi^{2b)}$	τ_{avr}/ns [c)]
MP/Viscosity : 10	0.08 (1.00)	-	1.118	0.07
MP/Viscosity : 15	0.10 (1.00)	-	1.031	0.10
MP/Viscosity : 19	0.11 (1.00)	-	1.066	0.11
MP/Viscosity : 55	0.12 (0.41)	0.23 (0.59)	1.047	0.18
MP/Viscosity : 88	0.12 (0.36)	0.26 (0.64)	0.991	0.21
MP/Viscosity : 534	0.25 (0.26)	0.73 (0.74)	1.054	0.61

[a)] Monitored wavelength was 604 nm. The fluorescence decay curves were fitted by a single and bi-exponential function to calculate the material lifetimes. [b)] Parameter χ^2 is the reduced chi-squared value. [c)] The intensity-weighted average lifetime (τ_{avr}) is defined as $f_1\tau_1 + f_2\tau_2$, where f_1 and f_2 are fractional intensities and τ_1 and τ_2 are lifetimes.

Table S3. Fluorescence lifetimes of DAP in solution.[a)]

Mateials/Viscosity	τ_1/ns, (f_1)	τ_2/ns, (f_2)	$\chi^{2b)}$	τ/ns
DAP/Viscosity : 2	0.63 (1.00)	-	0.978	0.63
DAP /Viscosity : 10	0.73 (1.00)	-	1.033	0.73
DAP /Viscosity : 15	0.77 (1.00)	-	0.976	0.77
DAP /Viscosity : 19	0.82 (1.00)	-	1.066	0.82
DAP /Viscosity : 55	1.03 (1.00)	-	1.233	1.03
DAP /Viscosity : 88	1.13 (1.00)	-	1.280	1.13
DAP /Viscosity : 534	1.50 (1.00)	-	1.271	1.50

[a)] Monitored wavelength was 682 nm. The fluorescence decay curves were fitted by a single exponential function to calculate the material lifetimes. [b)] Parameter χ^2 is the reduced chi-squared value.

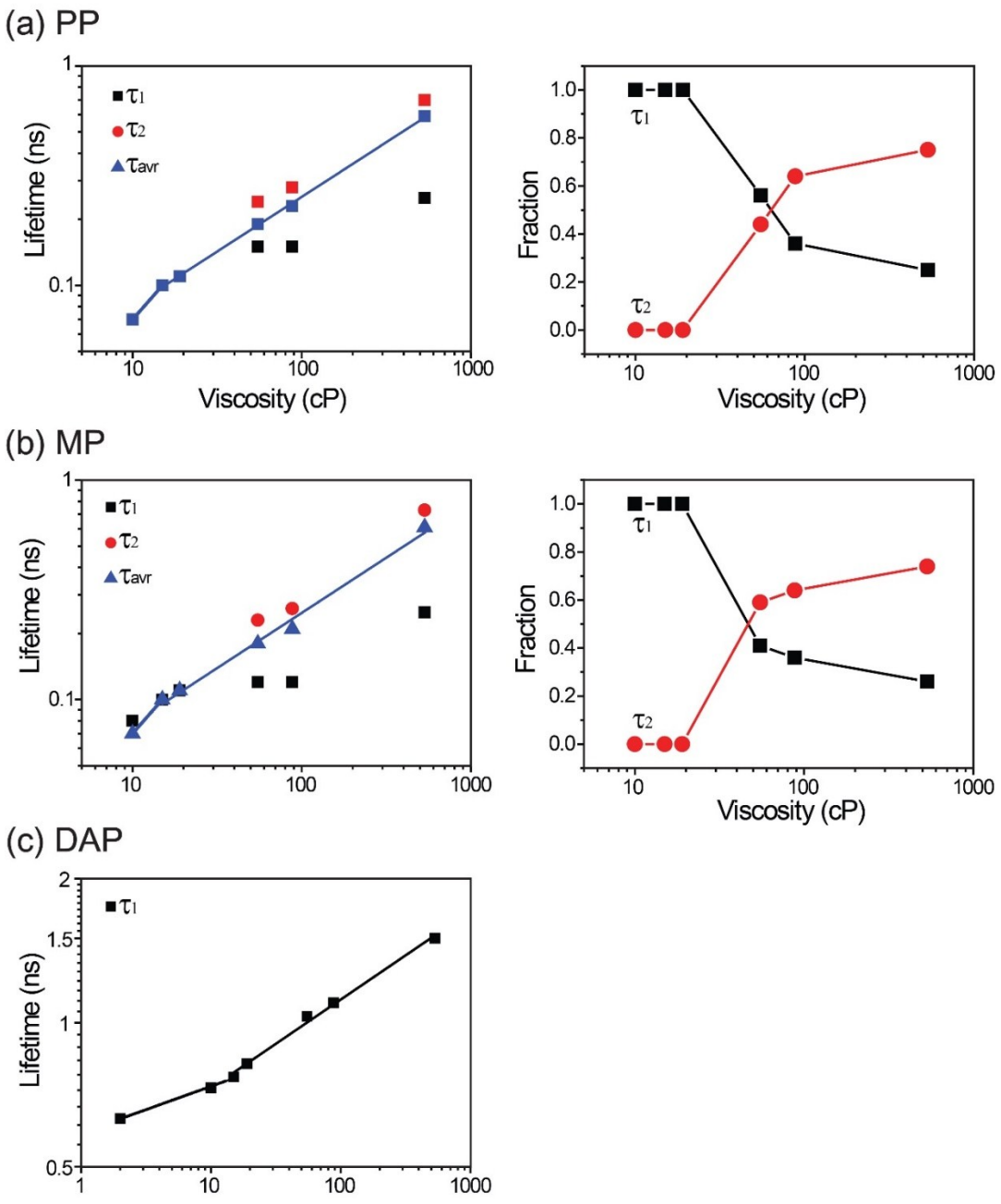


Fig. S3. A log-log plot of short, long and average lifetimes and fraction of lifetimes under various viscosity mediums: bi-exponential (a) PP, (b) MP and (c) single-exponential DAP.

Table S4. Gradient values obtained from Figure S3 and viscosity sensitivity obtained from Figure 2 of molecular rotors.

Molecular rotors	Gradient at low viscosity	Gradient at high viscosity	Viscosity sensitivity at low viscosity	Viscosity sensitivity at high viscosity
PP	0.88	0.50	0.77	0.40
MP	0.88	0.50	0.77	0.40
DAP	0.1	0.18	0.11	0.16

Fluorescence Imaging of Live Cells

A. Preparation and Staining of Cells

HeLa cells were cultured in DMEM media (Gibco, Grand Island, NY) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in a humidified 5% CO_2 incubator at 37 ^oC. For microscopic imaging, HeLa cells were seeded at a density of 10^4 cells onto a 35-mm cover glass bottom dishes and allowed to grow to confluence for 24 h. The fluorescence images were obtained after incubation with 5 μM DMSO solution of PP and MP in culture media for 1 h and then with 5 μM DMSO solution of MitoTracker Deep Red or LysoTracker Deep Red (Invitrogen) for 10 min.

B. Fluorescence Imaging of Live Cells

Spectral fluorescence imaging was performed with a Nuance FX multispectral imaging system (Cambridge Research & Instrumentation, Inc., USA) set to the excitation at 455 ± 35 nm. Co-localized fluorescence imaging was conducted by a DeltaVison imaging system with filter sets (excitation/emission) of DAPI/DAPI (nucleus), FITC/TRITC (PP and MP), and Cy5/Cy5 (MitoTracker and LysoTracker), using a 60× oil immersion objective.

C. Fluorescence Lifetime Imaging Microscopy (FLIM) of Live Cells

FLIM images were taken using an inverted-type scanning confocal microscope (MicroTime-200, Picoquant, Germany) with a 60× water immersion objective. The measurements were performed at the Korea Basic Science Institute (KBSI), Daegu Center, South Korea. A single-mode pulsed diode laser (470 nm with a pulse width of ~100 ps, 40 MHz repetition rate, and an average power of < 1 μW) was used as an excitation source. A dichroic mirror (490 DCXR, AHF), a longpass filter (HQ500lp, AHF), a 50 μm pinhole, and a single photon avalanche diode (PDM series, MPD) were used to collect emission through 600 ± 20 and 700 ± 20 nm band-pass filters from the samples. Time-correlated single-photon counting (TCSPC) technique was used to count emission photons.

Exponential fittings for the obtained fluorescence decays were performed by the iterative least-squares deconvolution fitting method using the Symphotime software (Picoquant, Version 5.3).

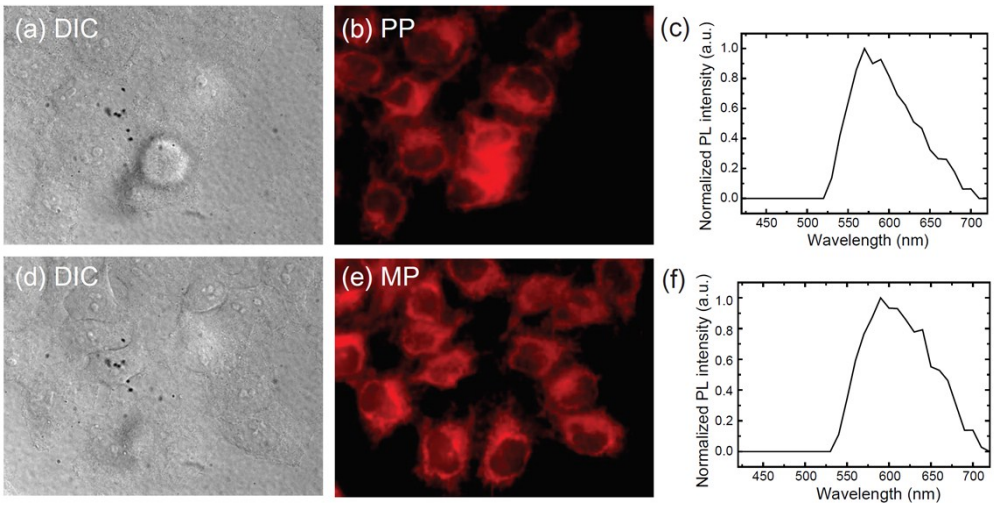


Fig. S4. Fluorescence image of HeLa cells stained with PP (a-c) and MP (d-f); (a, d) bright-field images, (b, e) fluorescence images, and (c, f) fluorescence spectra. The intracellular fluorescence spectra revealed maximum peaks at ca. 580 and 590 nm for PP and MP, respectively, well accordant to the corresponding *in vitro* emission spectra.

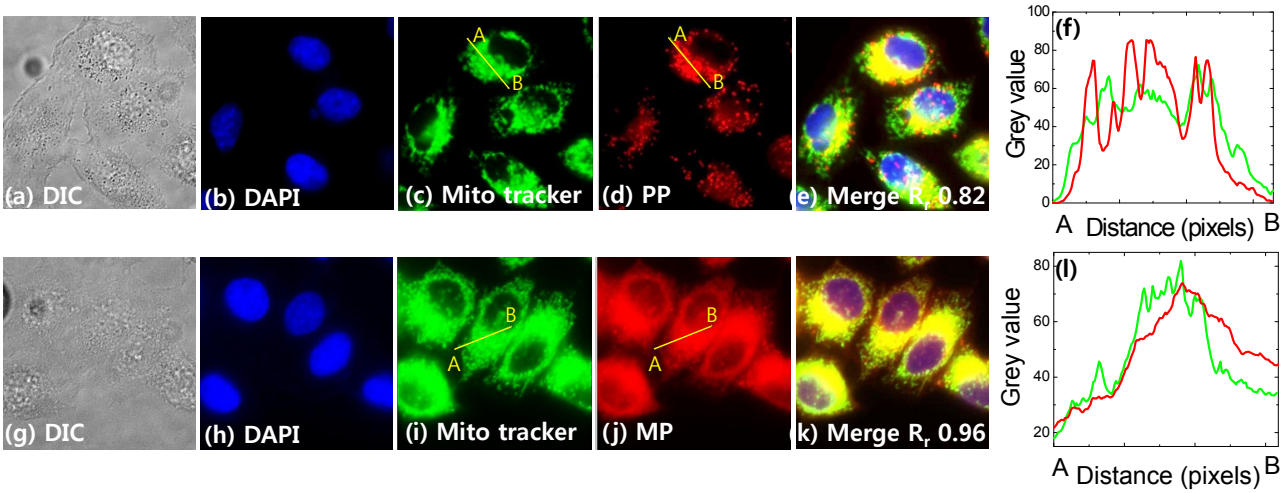


Fig. S5. Fluorescence co-localization images of HeLa cells stained with PP, MP, DAPI and Mito trackers; (a) bright-field image, (b) image from DAPI, (c) image from Mito tracker, (d) image from PP, (e) merged image (b+c+d), (f) line profiles of fluorescence intensity indicated by line AB of Mito tracker (c, green line) and PP (d, red line), (g) bright-field image, (h) image from DAPI, (i) image from Mito tracker, (j) image from MP, (k) merged image (g+h+i), (l) line profiles of fluorescence intensity indicated by line AB of Mito tracker (i, green line) and PP (l, red line).

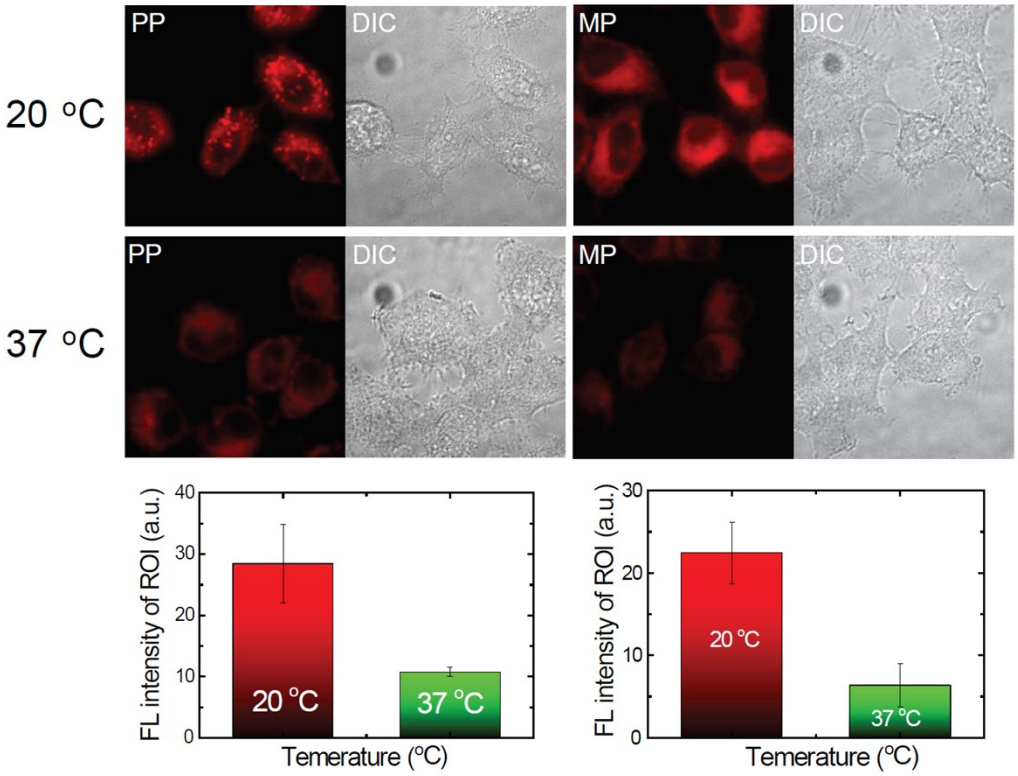


Fig. S6. Intracellular fluorescence responses of HeLa cells stained with PP and MP at different temperatures (20 and 30 °C).

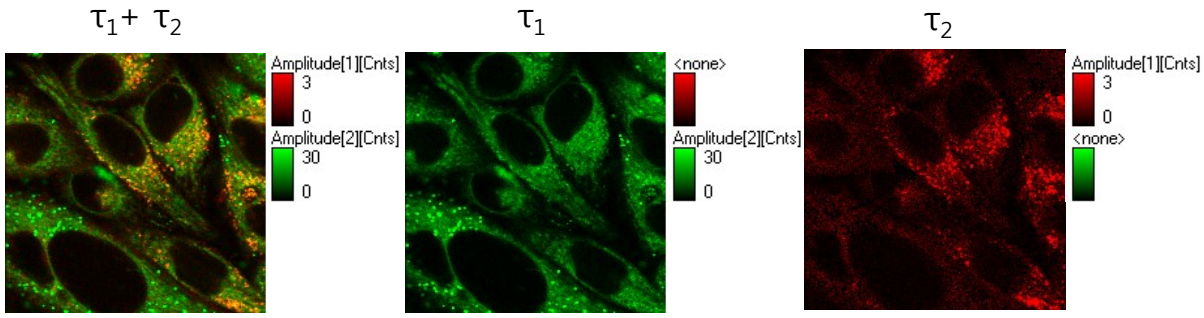


Fig. S7. FLIM images (Fig. 5a) constructed in the time windows of the fast and slow components, τ_1 and τ_2, respectively; $\tau_1 + \tau_2$, overlay image.

References

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