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Supplementary Material

Exploring BODIPY-Based Sensor for Imaging of Intracellular Microviscosity in Human Breast Cancer Cells

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1. Normalized absorbance and fluorescence spectra

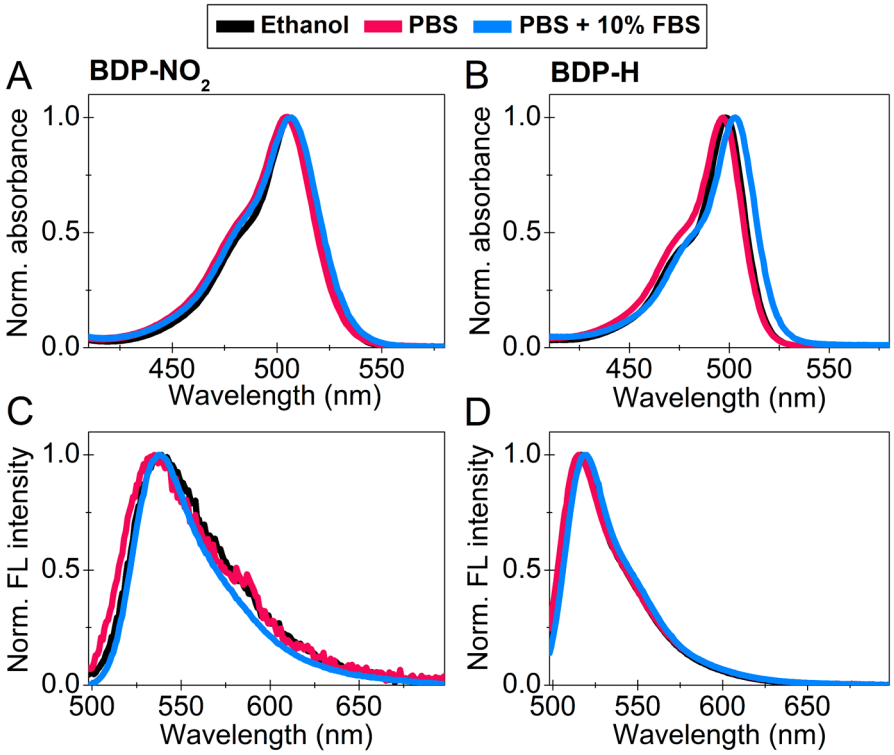


Figure S1. Normalized (Norm.) spectra of BDP-NO_2 and BDP-H diluted in ethanol (black), 7.4 pH phosphate-buffered saline (PBS) (red) or PBS with 10% fetal bovine serum (FBS) (blue). **A**, **B** – norm. absorption spectra. **C**, **D** – norm. fluorescence (FL) spectra, λ_{ex} = 488 nm.

2. Fitting parameters of fluorescence decays from the molecular rotors in aqueous media

Table S1. Fitting parameters of BDP-NO$_2$ and BDP-H fluorescence decays in ethanol and aqueous media (Figure 2E, F). Amplitudes are scaled by the integrated instrument response function. Concentration of both dyes was 9 μM.

Sample	Exponent component	α [1]	τ (ps)	f [2] (%)	τ_{Av} [3] (ps)	χ^2
BDP-NO$_2$ in ethanol	1 (τ_1)	0.2300	64	100	64	1.13
BDP-NO$_2$ in PBS	1 (τ_1) 2 (τ_2)	0.2541 0.0003	57 1958	96.69 3.31	131	1.07
BDP-NO$_2$ in PBS with 10% FBS	1 (τ_1) 2 (τ_2) 3 (τ_3)	0.0186 0.0094 0.0087	610 2822 7392	11.11 25.95 62.94	5453	1.08
BDP-H in ethanol	1 (τ_1)	0.0709	261	100	261	1.15
BDP-H in PBS	1 (τ_1)	0.0701	264	100	264	1.01
BDP-H in PBS with 10% FBS	1 (τ_1) 2 (τ_2) 3 (τ_3)	0.0280 0.0104 0.0071	364 2379 7000	12.08 29.24 58.68	4850	1.12

[1] Amplitude or pre-exponential factor.
[2] Fraction of fluorescence intensity (scales with the amplitude and the lifetime).
[3] Intensity-weighted mean lifetime.

3. Gradual decrease in BDP-NO$_2$ absorbance in aqueous media

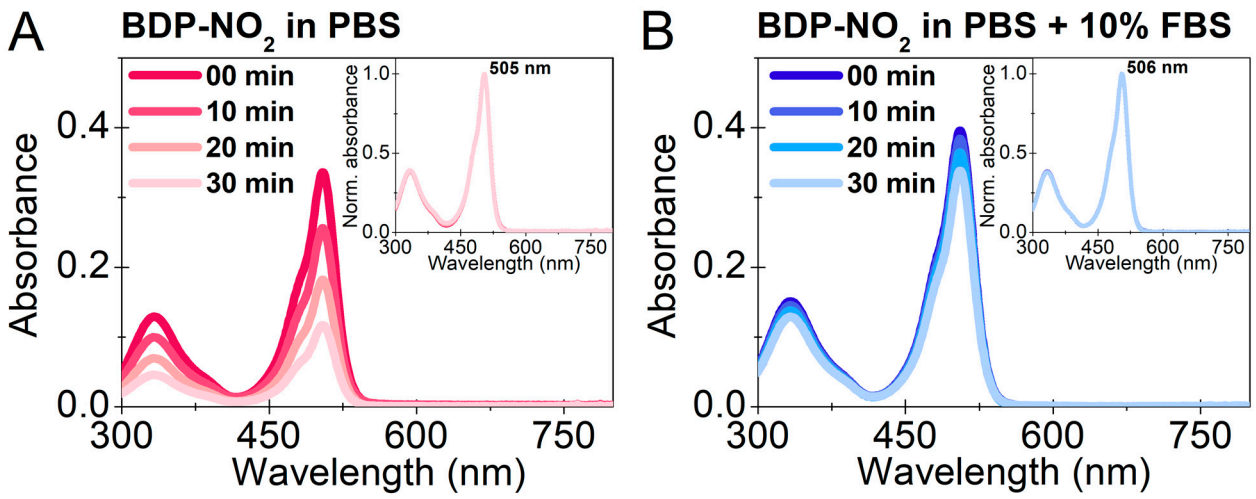


Figure S2. Absorption spectra of BDP-NO$_2$ diluted in PBS (**A**) and PBS + 10% FBS (**B**). Insets show normalized (norm.) spectra. BDP-NO$_2$ absorbance dwindles with time (65.23% decrease in 30 min) indicating that the optical properties of the molecule are not stable in aqueous media. Addition of FBS slightly improves stability of BDP-NO$_2$, however the decrease in absorbance with time was still observed, although at a much slower rate (14.58% decrease in 30 min).

4. Photostability of BDP-H in PBS

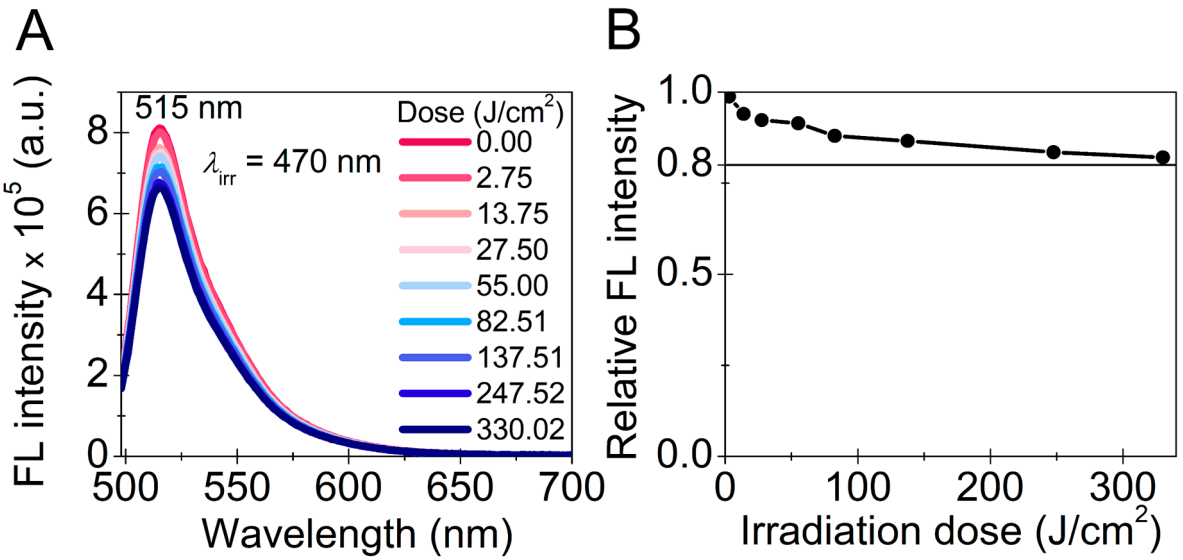


Figure S3. Photostability of BDP-H in PBS. **A** – fluorescence (FL) spectra of 9 μM BDP-H in PBS after irradiation with 470 nm light (laser power – 36 mW). FL spectra were obtained under the excitation at 488 nm. **B** – BDP-H photobleaching curve. Values of relative FL intensity were obtained by dividing the maximum FL spectrum value (at 515 nm) of non-irradiated sample from the maximum FL values after each irradiation.

5. Photophysical characterization of BDP-H interaction with BSA

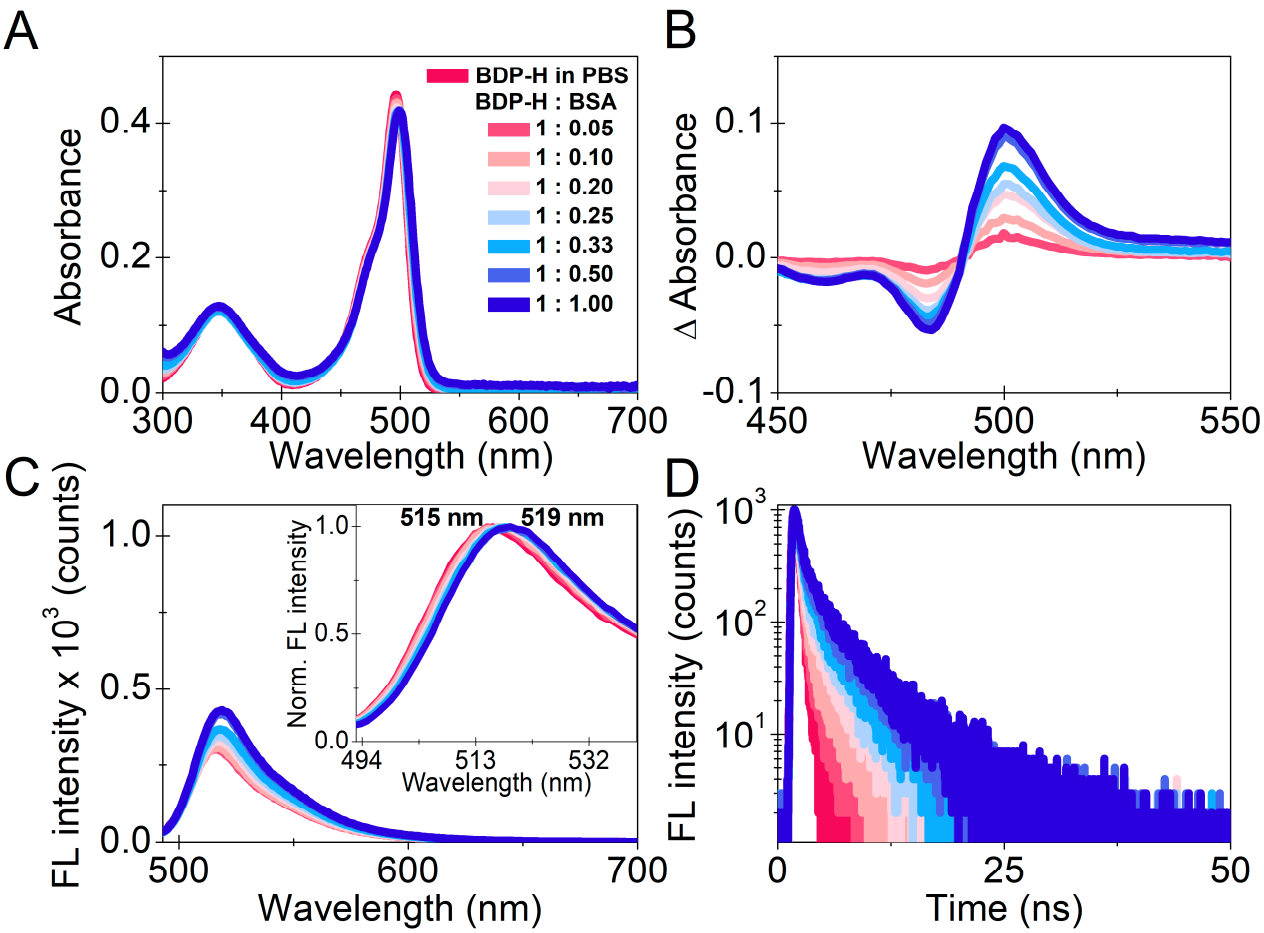


Figure S4. Photophysical characterization of 9 μM BDP-H in PBS with increasing concentrations of bovine serum albumin (BSA) (up to 9 μM) measured in PBS. The color legend of BDP-H and BSA ratio is shown in **A** panel. The absorption of BDP-H in PBS was subtracted from each BDP-H + BSA spectra (**A**), which resulted in **B**. The changes of fluorescence emission are represented in **C**, with the inset showing normalized fluorescence spectra. **D** – comparison of time-resolved fluorescence decays, λ_{ex} = 473 nm.

6. BDP-H staining in live and fixed human breast cancer cells

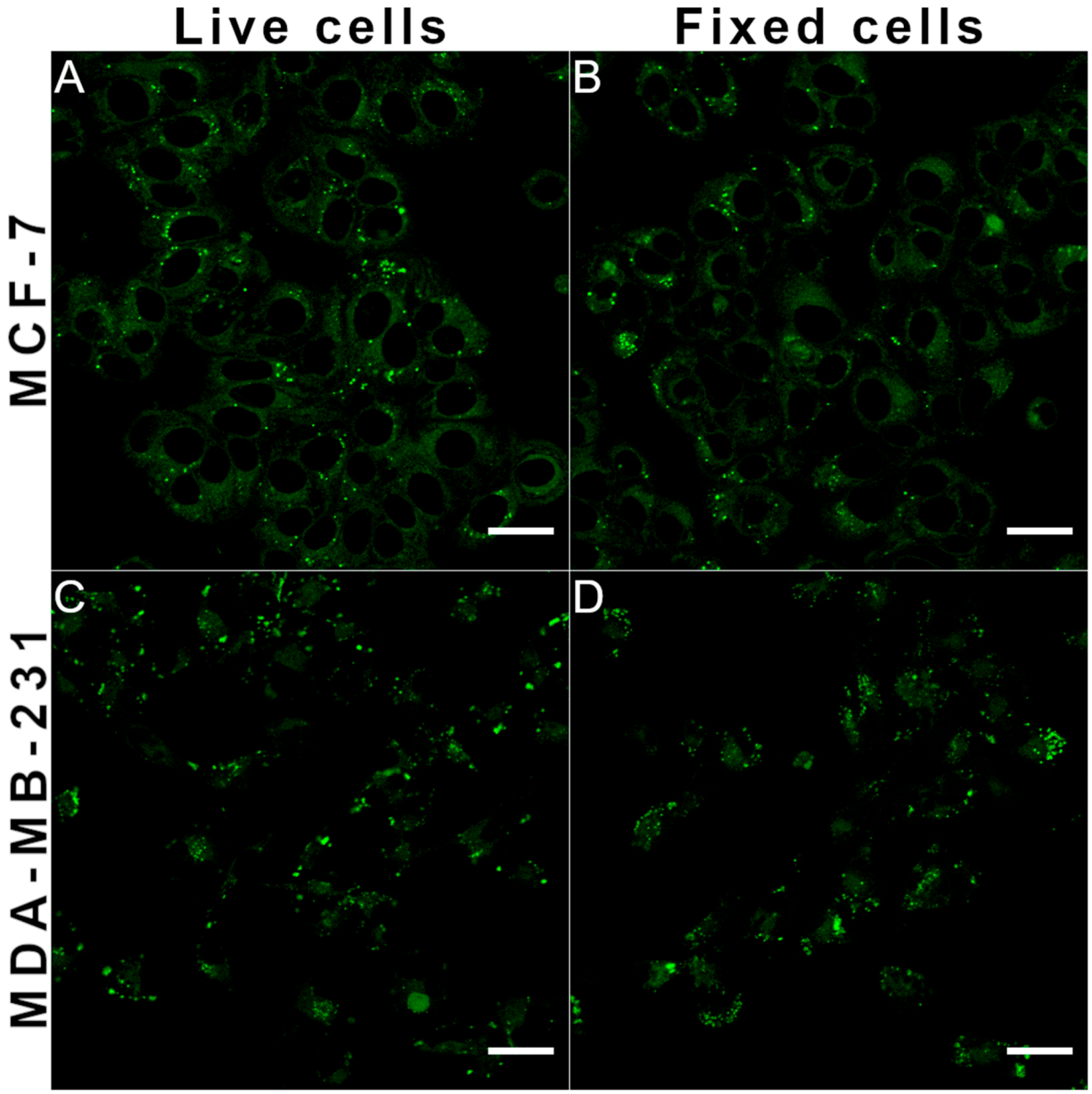


Figure S5. Comparison of BDP-H staining in live and fixed MCF-7 and MDA-MB-231 human breast cancer cells. Live cells (**A**, **C**) were incubated with 9 μM BDP-H (diluted in PBS) for 60 min. Cells in **B** and **D** were fixed using 4% paraformaldehyde (PFA) and afterwards stained with 9 μM BDP-H (diluted in PBS) for 60 min. Cells were visualized using laser-scanning confocal microscope; λ_{ex} = 480±5 nm. Scale bars: 30 μm.

7. BDP-H colocalization analysis

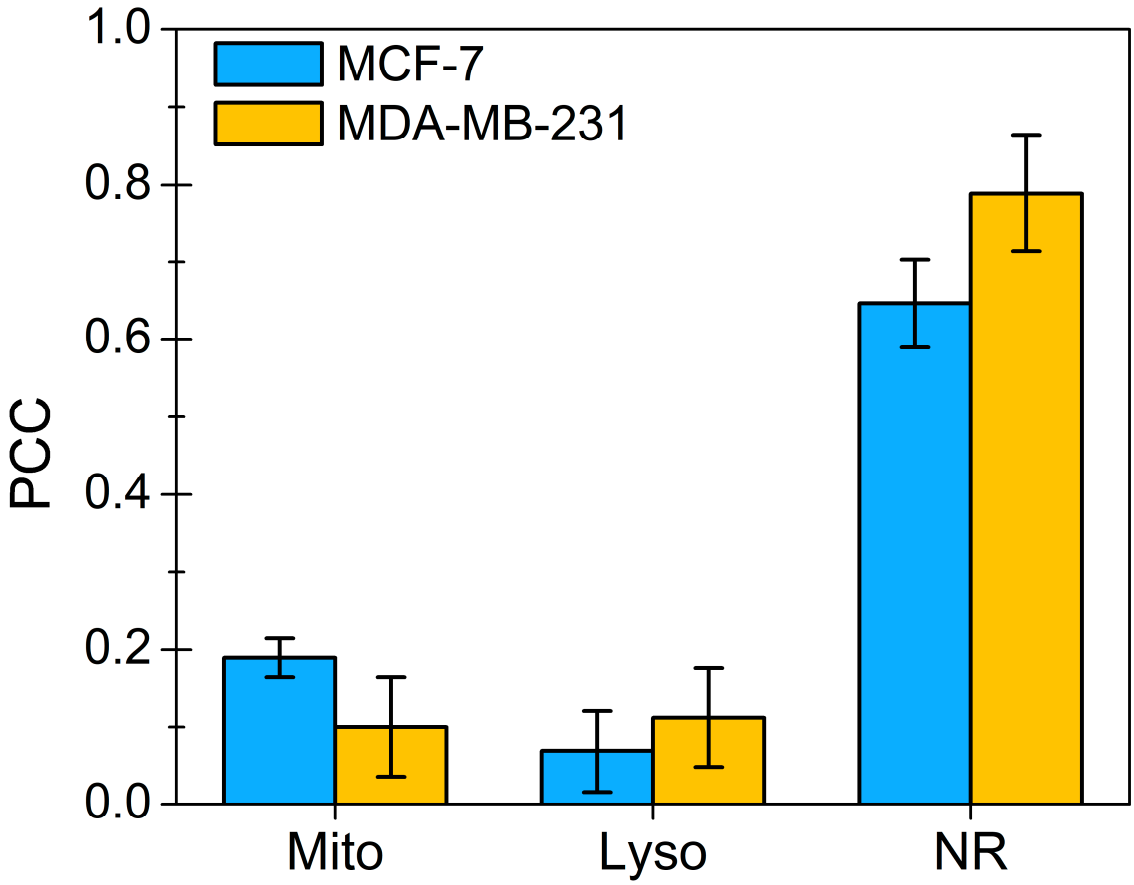


Figure S6. Pearson correlation coefficients (PCC) obtained from colocalization analysis of BDP-H and the following commercial probes: MitoTracker Red FM (Mito), LysoTracker Deep Red (Lyso), Nile Red (NR) in MCF-7 and MDA-MB-231 cells. The average PCC ± SD were calculated from ten ROI, each containing a single cell with the nuclear region excluded. Fiji's Coloc 2 plugin was used to obtain the PCC values.

8. Fluorescence and FLIM images of unstained human breast cancer cells

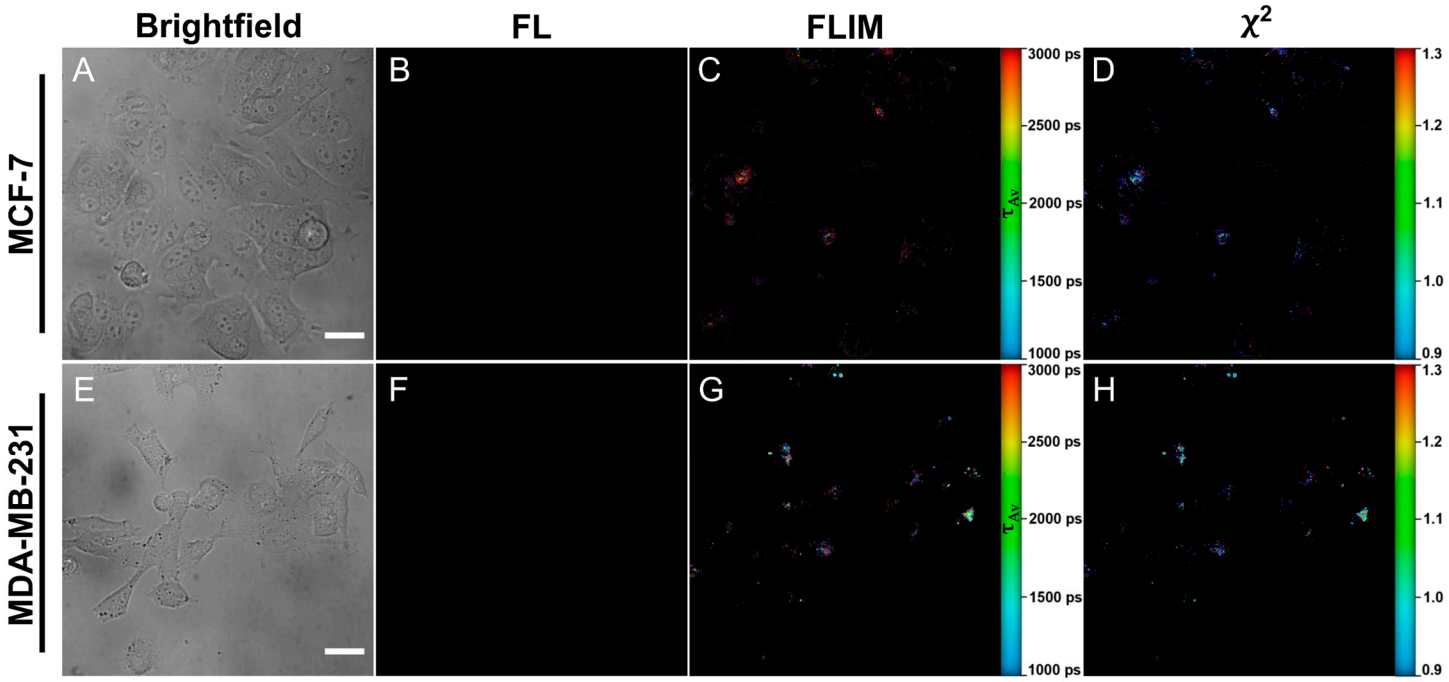


Figure S7. Confocal and fluorescence lifetime imaging microscopy (FLIM) images of unstained human breast cancer cells. **A**, **E** – brightfield images; scale bars: 30 μm. Autofluorescence of cells is not visible in fluorescence (FL) images (**B**, **F**) since imaging parameters were chosen specifically to nullify it. However, weak autofluorescence signal is evident in FLIM images (**C**, **G**); the fluorescence was excited at 480±5 nm and detected through a 578/105 nm filter. **D**, **H** – χ^2 images obtained from the analysis of the FLIM images. Fitting results of the aforementioned FLIM images are shown in Figure S8 and Table S2.

9. Fluorescence decays and residuals obtained from FLIM images of unstained cells

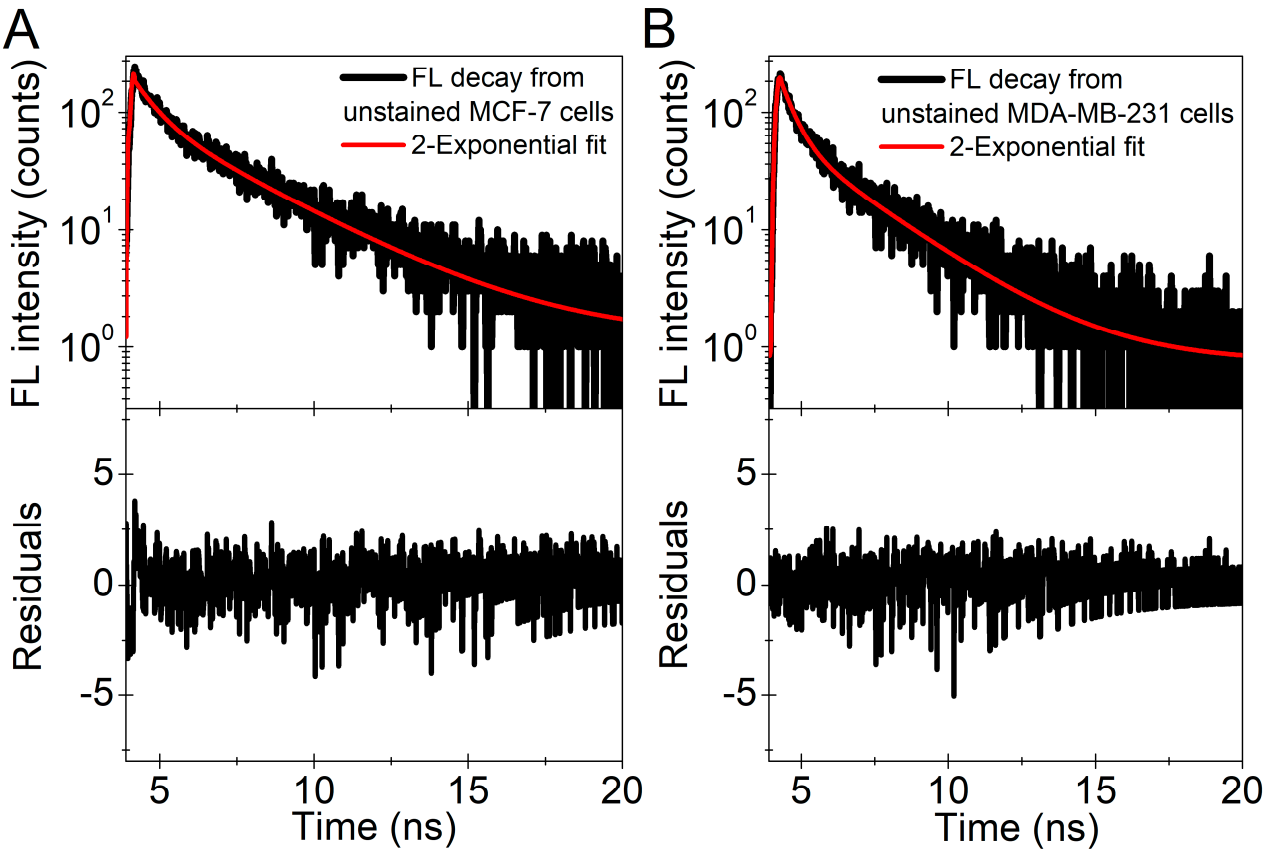


Figure S8. Fluorescence decays (black) with biexponential reconvolution fits (red line) obtained from FLIM images of unstained MCF-7 and MDA-MB-231 cells (Figure S7C, G). Residuals (difference between the fitted decay curve and raw data) are shown below the decay curves.

10. Fitting parameters of fluorescence decays obtained from FLIM images of unstained cells

Table S2. Fluorescence lifetimes, amplitudes and other fitting parameters of fluorescence decays obtained from FLIM images of unstained MCF-7 and MDA-MB-231 cells displayed in Figure S7.

Cell line (FLIM image)	Exponent component	τ (ps)	Amplitude (kCnts)	τ_{Av} [1] (ps)	χ^2
MCF-7 (Figure S6C)	1 (τ_1)	570	0.12 (57.14%)	2420	1.10
	2 (τ_2)	2920	0.09 (42.86%)		
MDA-MB-231 (Figure S6G)	1 (τ_1)	522	0.18 (78.26%)	1990	1.00
	2 (τ_2)	2900	0.05(21.74%)		

[1] Intensity-weighted mean lifetime.

11. BDP-H calibration curve

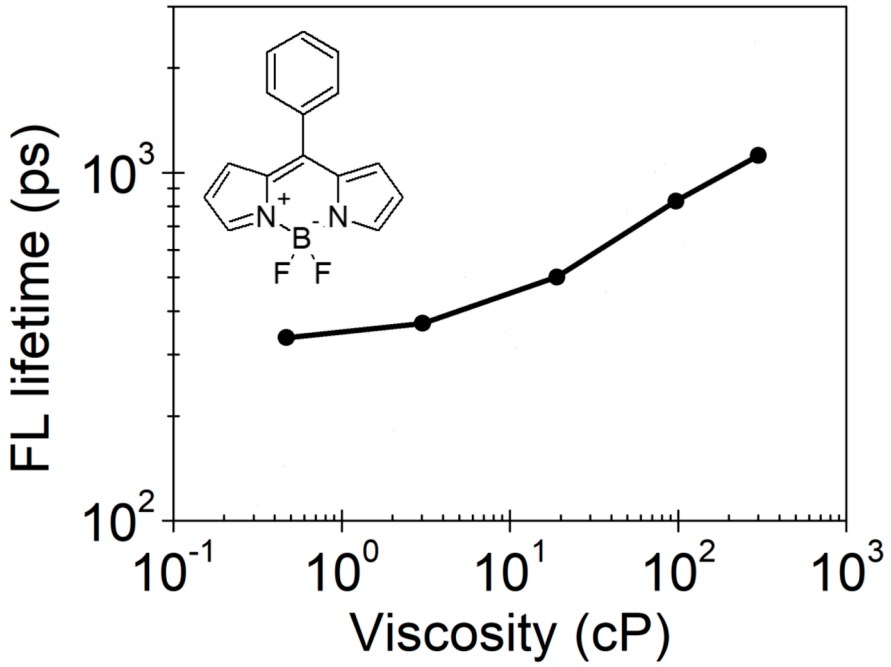


Figure S9. BDP-H calibration curve obtained at 40 °C in toluene-castor oil mixtures [1].

12. Average values of fitting parameters obtained from FLIM images of cancer cells stained with BDP-H

Table S3. Average values of fitting parameters of fluorescence decays obtained from the analysis of 40 FLIM images (20 FLIM images for each cell line).

Cell line	Exponent component	$\tau_{1,2_Av}$ [1] (ps)	Average amplitude (kCnts)	τ_{Av} [2] (ps)	χ^2_{Av}	Microviscosity [3] (cP)
MCF-7	1 (τ_{1_Av})	875±18	1.78±2.17	1158±53	1.06±0.05	119.69±8.51
	2 (τ_{2_Av})	3675±23	0.04±0.05			
MDA-MB-231	1 (τ_{1_Av})	992±64	1.08±2.06	1436±194	1.03±0.06	195.33±48.30
	2 (τ_{2_Av})	2862±396	0.16±0.30			

[1] Average lifetime of τ_1 or τ_2 components.

[2] Average intensity-weighted mean lifetime.

[3] Microviscosity calculated from τ_{1_Av} using the BDP-H calibration graph in Figure S9.

13. Fluorescence and FLIM images of human breast cancer cells stained with BDP-H

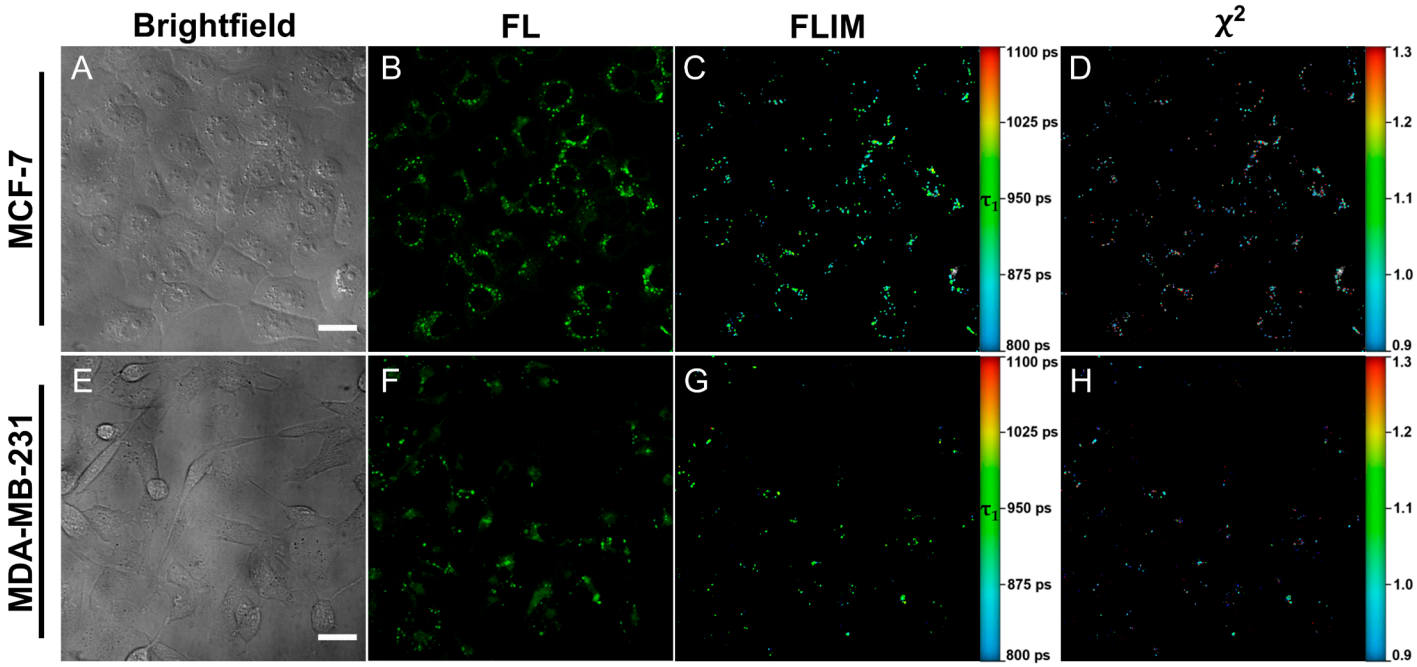


Figure S10. Confocal and FLIM images of human breast cancer cells stained with BDP-H molecular rotor. **A**, **E** – brightfield images; scale bars: 30 μm. **B**, **F** – fluorescence (FL) images. **C**, **G** – FLIM images. The fluorescence was excited at 480±5 nm and detected through a 578/105 nm filter. **D**, **H** – χ^2 images obtained from the analysis of the FLIM images. Fitting results of the aforementioned FLIM images are shown in Figure S11 and Table S4.

14. Fluorescence decays and residuals obtained from FLIM images of human breast cancer cells stained with BDP-H

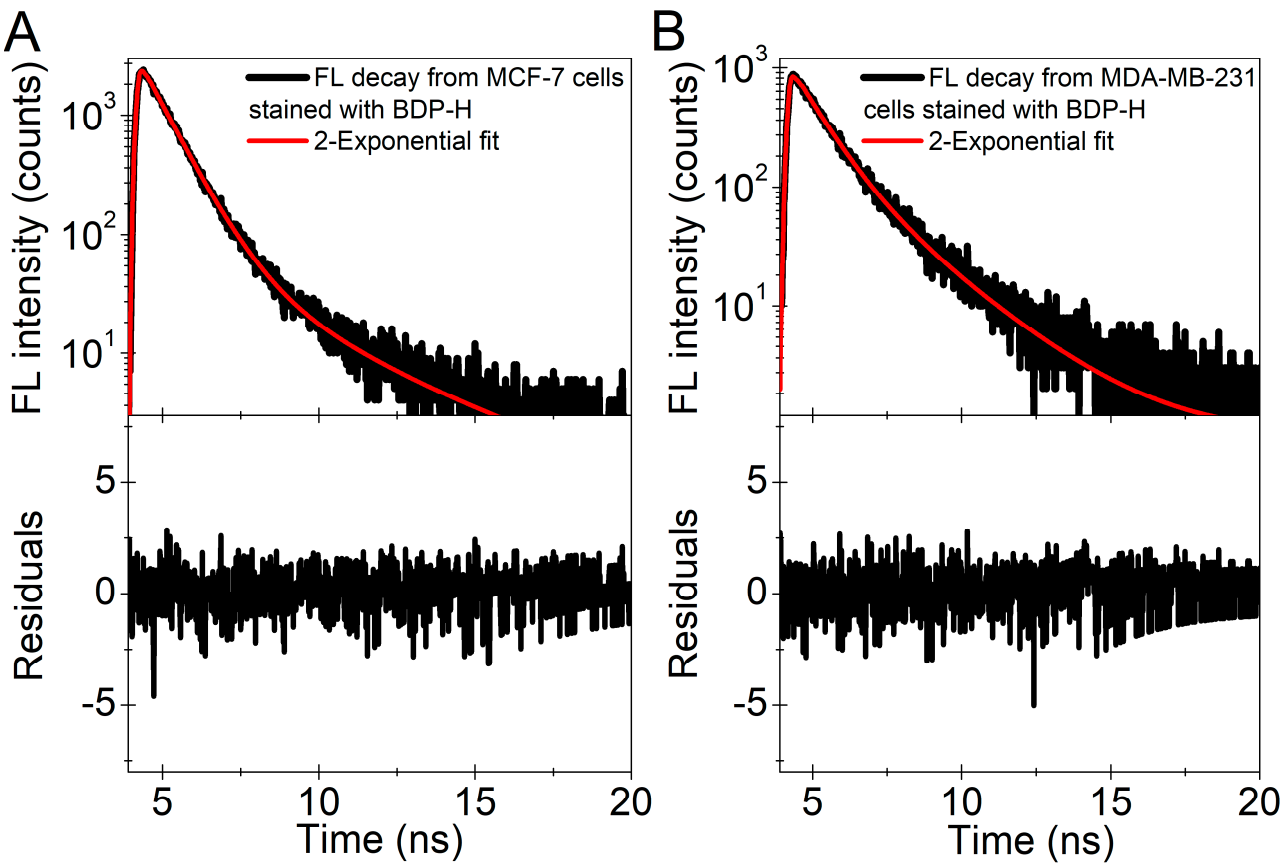


Figure S11. Fluorescence decays (black) with biexponential reconvolution fits (red line) obtained from FLIM images of MCF-7 and MDA-MB-231 cells stained with BDP-H (Figure 6B, D or Figure S10C, G). Residuals (difference between the fitted decay curve and raw data) are shown below the decay curves.

15. Fitting parameters of fluorescence decays obtained from FLIM images of human breast cancer cells stained with BDP-H

Table S4. Fluorescence lifetimes, amplitudes and other fitting parameters of fluorescence decays obtained from FLIM images displayed in Figure 6B, D or Figure S10C, G.

Cell line (FLIM image)	Exponent component	τ (ps)	Amplitude (kCnts)	τ_{Av} [1] (ps)	χ^2
MCF-7 (Figure 6B)	1 (τ_1)	836	3.04 (97.43%)	1080	0.98
	2 (τ_2)	3300	0.08 (2.56%)		
MDA-MB-231 (Figure 6D)	1 (τ_1)	927	0.93 (83.78%)	1410	1.08
	2 (τ_2)	2400	1.18 (16.22%)		

[1] Intensity-weighted mean lifetime.

Reference

1. Toliautas, S.; Dodonova, J.; Žvirblis, A.; Čiplys, I.; Polita, A.; Devižis, A.; Tumkevičius, S.; Šulskus, J.; Vyšniauskas, A. Enhancing the Viscosity-Sensitive Range of a BODIPY Molecular Rotor by Two Orders of Magnitude. *Chemistry – A European Journal* **2019**, *25*, 10342–10349, doi:10.1002/chem.201901315.