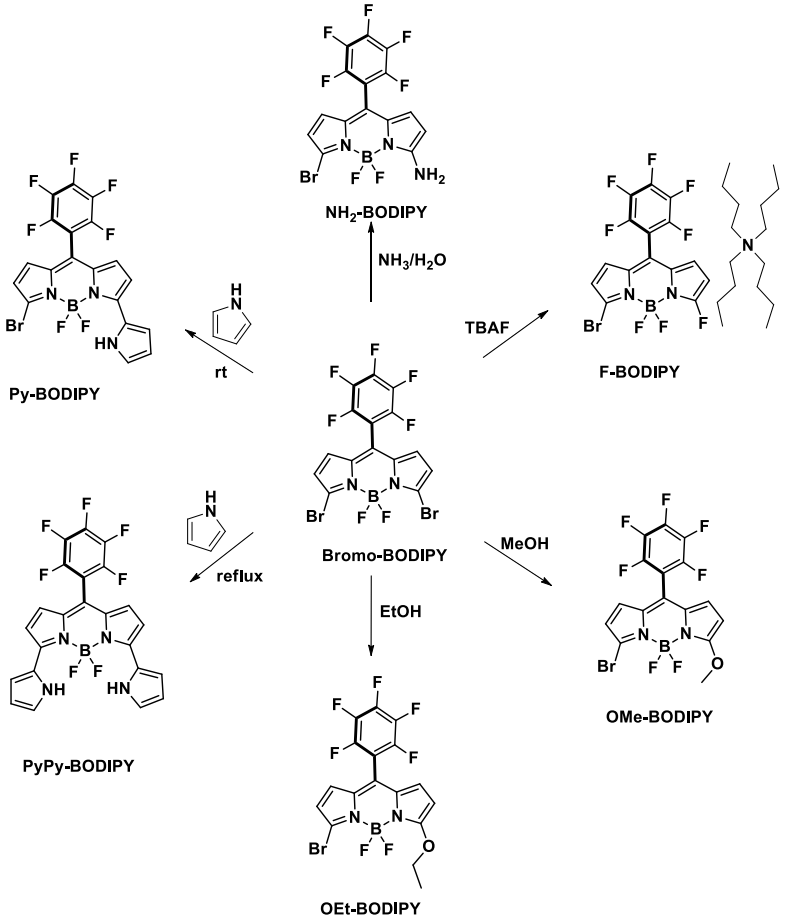
Supplementary Information

Materials and Methods

General synthetic procedure

Pentafluorophenyl dibromo-BODIPY (100 mg) was dissolved in 10 ml of CH_2Cl_2. Excess equivalents of respective nucleophile was added to the reaction mixture at room temperature, stirred this mixture for over-night in open atmospheric condition. The reaction mixture was concentrated by evaporation of solvent under reduced pressure. The crude mixture was subjected to silica column chromatography. The desired compound was purified using ethyl acetate and hexane as elutants [1]. In the case of pyrrole substituted pentafluorophenyl-bromo BODIPY, reaction was performed under neat condition. For mono substituted pyrrolic product, reaction was performed at room temperature with pyrrole as solvent and for di-substituted pyrrolic product reaction was performed under reflux condition [2].

Scheme 1. Synthesis of substituted pentafluorophenyl- bromo BODIPYs.



Cell culture and transduction

HeLa cells (cell line derived from cervical tumour) were cultured in DMEM (Dulbecco's Modified Eagle Medium) medium supplemented with 10% fetal bovine serum (FBS), 1X penicillin and streptomycin at 37 ºC in an atmosphere containing 5% CO_2. The experiment was performed by seeding the cells at a density of 1×10^5 cells/ml in a 6-well tissue culture plate. The Golgi bodies were labelled by transfecting the cells with CellLight® Golgi-RFP over night followed by incubating NH_2-BODIPY (100 nM) for 10 minutes. The CellLight® Golgi-RFP, BacMam 2.0, is a fusion construct of human Golgi resident enzyme (N-acetylgalactosaminyl transferase) and TagRFP, providing accurate and specific targeting to cellular Golgi-RFP. This fusion construct is packaged in the baculovirus, which does not replicate in human cells.

Confocal microscopy

For live and fixed cell imaging, cells were treated with NH_2-BODIPYs at a concentration of 100 nM, 1 μM for ER-Tracker™ Red and Mito-Tracker™ Red (Invitrogen) grown on a coverslip for 10 minutes, washed with PBS (phosphate-buffered saline) two times and mounted in confocal microscope slide. Cells were imaged using Airyscan LSM 800 or LSM 780 Zeiss confocal laser scanning microscope with the 40X or 63X objective lens (excited with 488 nm laser for NH_2-BODIPY and 561 nm for ER-Tracker™ Red and Mito-Tracker™ Red).

Super resolution imaging

The essence of STED microscopy is the use of two overlapping beams which act as excitation and depletion laser consecutively. In Leica TCS SP8 STED 3X, the STED laser possess zero-fields in the center and maximum in the periphery which exhibits a doughnut-shaped phase pattern. The doughnut-shaped depletion laser wavefield in STED effectively narrows the point-spread function of the excitation laser to increase resolution beyond the diffraction limit, which in the best cases can approach 20 nanometers in the lateral dimension. The quality of the doughnut phase pattern of the depletion beam which overlaps with the excitation beam determines the ultimate resolution of the setup. Moreover, we have used Leica SP8 3X STED microscope equipped with high sensitive 3X HyD detectors with HC PL APO CS2 63x oil/1.4 (WD = 0.14 mm) objective lens. All lasers were set at zero before imaging and laser power was slowly adjusted below the saturation limit. To image cells labelled with NH_2-BODIPYs, 484 nm

excitation and 592 nm depletion lasers were used. For ER-Tracker™ Red, 558 nm excitation and 660 nm depletion lasers were used.

For super resolution imaging, HeLa cells are grown in a glass bottom petriplate at a density of 1×10^5 cells/ml in DMEM medium supplemented with 10% fetal bovine serum, 1X penicillin and streptomycin at 37 ºC in an atmosphere containing 5% CO_2. For live imaging, cells were treated with NH_2-BODIPYs at a concentration of 150 nM, 1 μM for ER-Tracker™ Red (Invitrogen) for 20 minutes, washed with PBS (phosphate-buffered saline) two times. Cells were imaged using Leica SP8 3X STED microscope with the 63X objective lens. To image cells labelled with NH_2-BODIPYs, 484 nm excitation and 592 nm depletion lasers were used. For ER-Tracker™ Red, 558 nm excitation and 660 nm depletion lasers were used.

Cellular toxicity assay

For assaying cytotoxic effect of NH_2-BODIPYs, HeLa were treated with MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) which involves the reduction of MTT tetrazolium to MTT formazan pigment by the metabolic activity of living cells. HeLa cells were seeded at a density of 1×10^5 cells/ml in a 96-well plate. After 24 h of cell attachment, HeLa cells were treated with DMSO and the NH_2-BODIPYs at a concentrations of (50, 100, 150, 200, 250 nM) for 12 hours. Three replicate wells were used for each control and tested concentrations. After incubation for 12 h, the medium was removed and cells were washed with PBS twice. MTT tetrazolium solution (150 μL of 0.5 mg/ml in PBS) was added to each well, and the cells further incubated at 37 ^{0}C for 4 h in a 5% CO_2 humidified atmosphere. Excess MTT tetrazolium solution was then carefully removed and the coloured formazan was dissolved in 200 μL dimethyl sulfoxide (DMSO). The plate was shaken for 10 minutes and the absorbance was measured at 570 and 630 nm using a microplate reader.

Statistical analysis

For comparisons of cell viability between control and treated cells at various concentrations of the NH_2-BODIPYs (50 nM, 100 nM, 150 nM, 200 nM, 250 nM) in living HeLa cells, the number of live cells were counted by MTT assay and were analyzed using one-way ANOVA with post-hoc Tukey's test. The statistical analysis was specifically used for multiple comparisons of the samples. No significant difference was observed between control and the treated cells. The P

value for this test was calculated to be 0.0211. The value for each test was analyzed in the supplementary table S1.

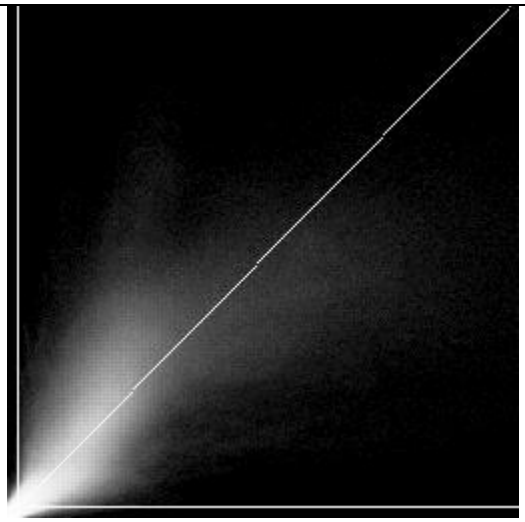
Table S1

Tukey's Multiple Comparison Test	Mean ± SEM	q value	Significant? P < 0.05?
Control vs 50 nM	101.5 ± 4.53	0.3597	No
Control vs 100 nM	100.6 ± 3.22	0.1359	No
Control vs 150 nM	104.3 ± 3.64	1.807	No
Control vs 200 nM	98.05 ± 2.45	0.4796	No
Control vs 250 nM	94.92 ± 3.09	3.549	No

Colocalization analysis

To quantify we performed colocalization analysis through Image J software with Colocalization threshold and Coloc^2 plugins. On colocalization experiment we used synthetic 100 nM and commercial dyes 1μM with 20 min incubation time, we got week intensity fluorescence images. Unlike Manders tM1 and tM2 values Pearson's R value is independent of signal intensity, so we got similar values in in both calculations as 0.89. In Coloc^2 calculation due to high autothreshold pixel signal intensity, we end up with very less values in Manders tM1 and tM2 values as 0.000 and 0.008 respectively. However in Colocalization Threshold calculation we got considerable results which are mentioned in manuscript and shown in Table S2.[3]

Table S2

Colocalization threshold Calculation	
	
Images	LC 2.czi - C=1 & LC 2.czi - C=2
Rtotal	0.891
Rcoloc	0.7805
tM1	0.9383
tM2	0.9526

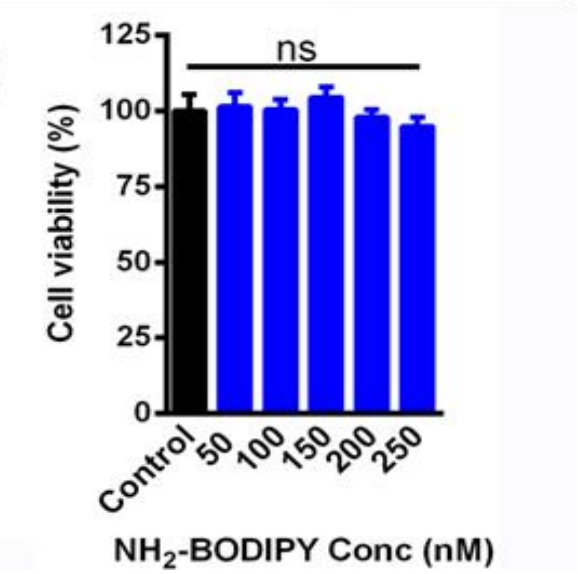


Figure S1. A cytotoxicity study via an MTT assay shows the cell-viabilities of control (in 10% DMSO). The error bars represent standard error of the mean (+SEM). Statistical analysis based

on one-way ANOVA followed by post-hoc Tukey's multiple comparison test (ns represent 'not significant').

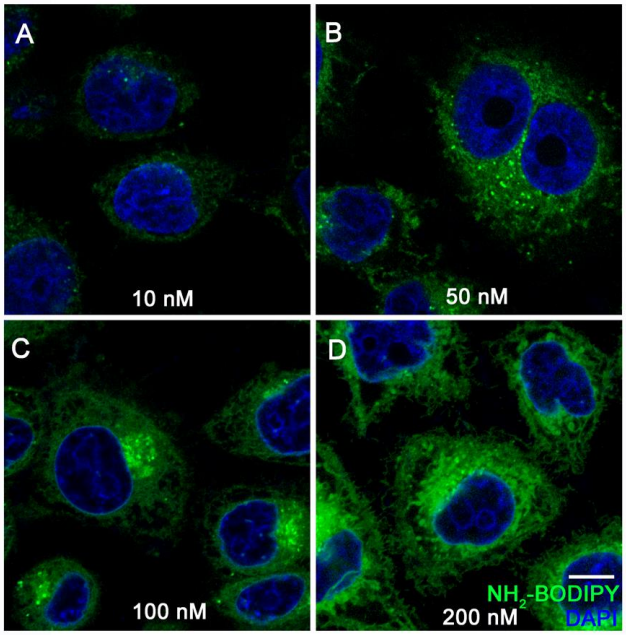


Figure S2. Confocal images of HeLa cells labelled with NH_2-BODIPY at a concentration of 10 nM (A), 50 nM (B), 100 nM (C) and 200 nM (D) (green) and DAPI (blue, to label the cell nuclei). The cells were incubated with NH2-BODIPY for 10 minutes in living HeLa cells. Scale bar =10 μm.

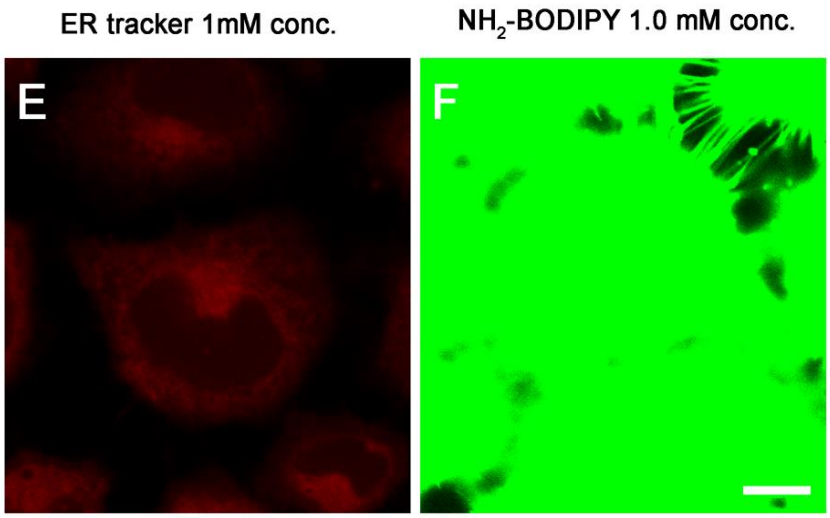


Figure S3. Confocal images of HeLa cells colabelled with ER-tracker Red at a concentration of 1.0 mM (E) or 1.0 mM NH_2-BODIPY (F) Cells were imaged under the same laser power. The signal becomes highly saturated with 1.0 mM NH_2-BODIPY.

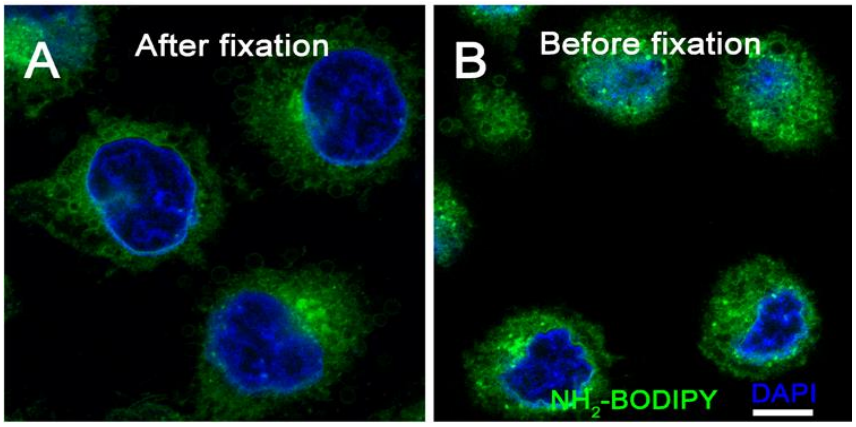


Figure S4. Confocal images of HeLa cells labelled with NH_2-BODIPY after fixation (A), before fixation (B), (green) and DAPI (blue). The cells were incubated with NH_2-BODIPY for 10 minutes in HeLa cells. Note that this NH_2-BODIPY retains the fluorescence significantly, and hence can be used on fixed cells. Scale bar =10 μm.

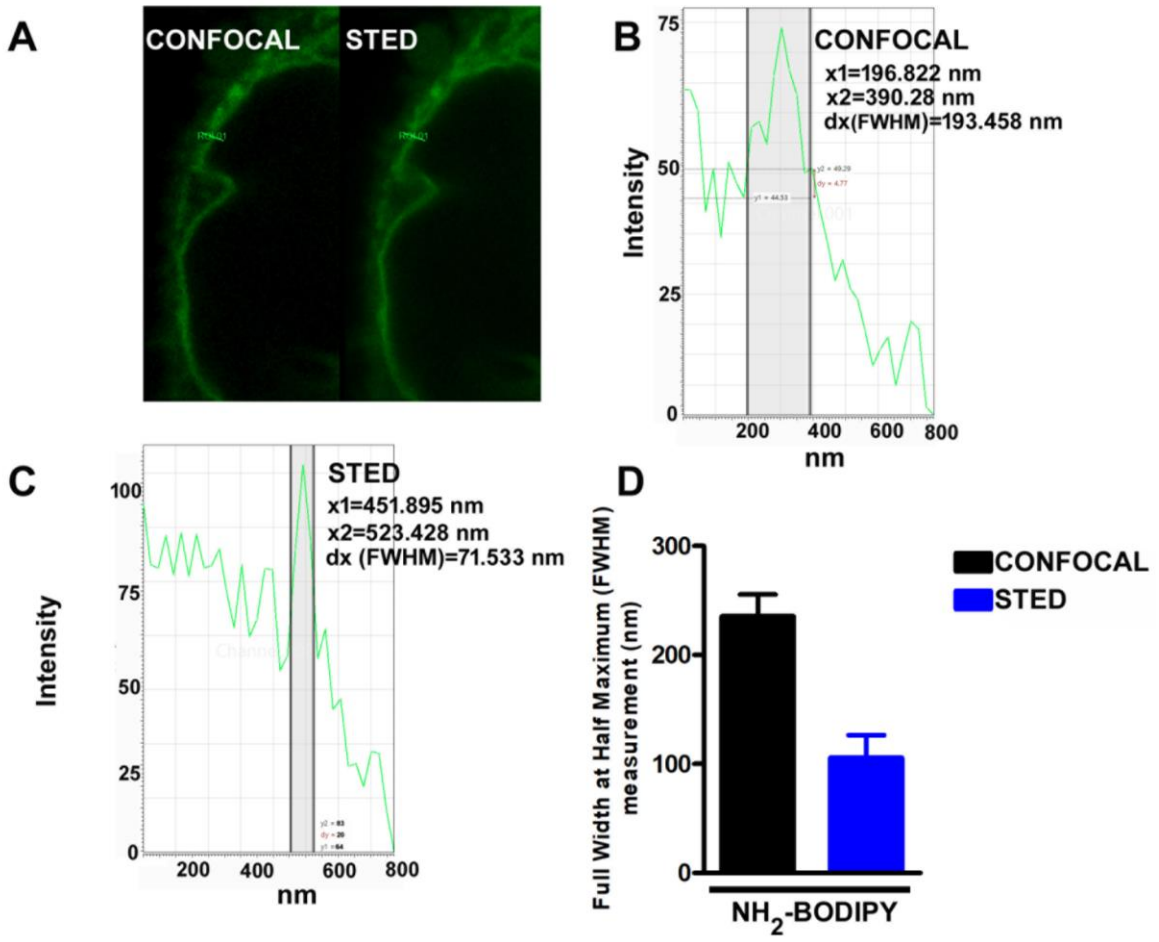


Figure S5. (A) Confocal and STED microscopic images of HeLa cells labelled with NH2-BODIPY (green). (B-C) Intensity profile of confocal and STED images with indicated curve points (confocal: x1=196.822 nm, x2=390.28 nm), (STED: x1= 451.895 nm, x2=523.428 nm). The FWHM values of confocal and STED were calculated as 193.458 nm and 71.534 nm respectively. (D) The bar graph showing comparisons of FWHM value between confocal (235.6 ± 19.98 nm) and STED 105.7 ± 20.86 nm) images. Error bars represent standard error of mean.

FWHM Calculation

To quantify the improvement in image resolution, we took Gaussian profile of signal intensity of confocal and STED images. The confocal and STED images were analyzed by selecting region of interest (ROI) in Leica LAS X software to get respective data graph. Full width at half

maximum (FWHM) was determined by drawing a vertical line on the data graph from the peak maximum to the base line. After determining the peak, two additional lines were drawn on the data graph in either side of the peak to find out respective curve points and FWHM values were determined by calculating the distance between the curve points. At least five images of confocal and STED were analysed to obtain FWHM values. Smaller the value of FWHM indicates improved resolution.

References

1. Sekhar, A.R., M.A. Kaloo, and J. Sankar, *Dual-mode chemodosimetric response of dibromo-BODIPY with anions. Org Biomol Chem*, **2015**. *13*(40): p. 10155-61.
2. Jiang, T., et al., *Straightforward Synthesis of Oligopyrroles through a Regioselective SNAr Reaction of Pyrroles and Halogenated Boron Dipyrrins. Org. Lett*., **2014**, *16(*7): p. 1952-1955.

3 https://imagej.net/Coloc_2