Synthesis and Application of water soluble BODIPY Fluorophores for live cellular imaging

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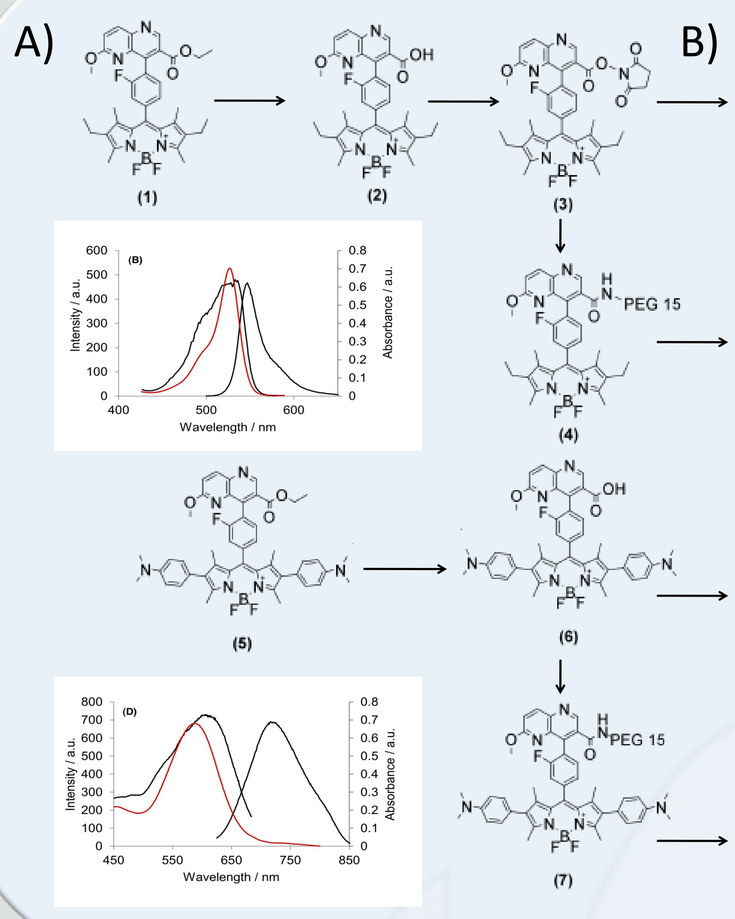
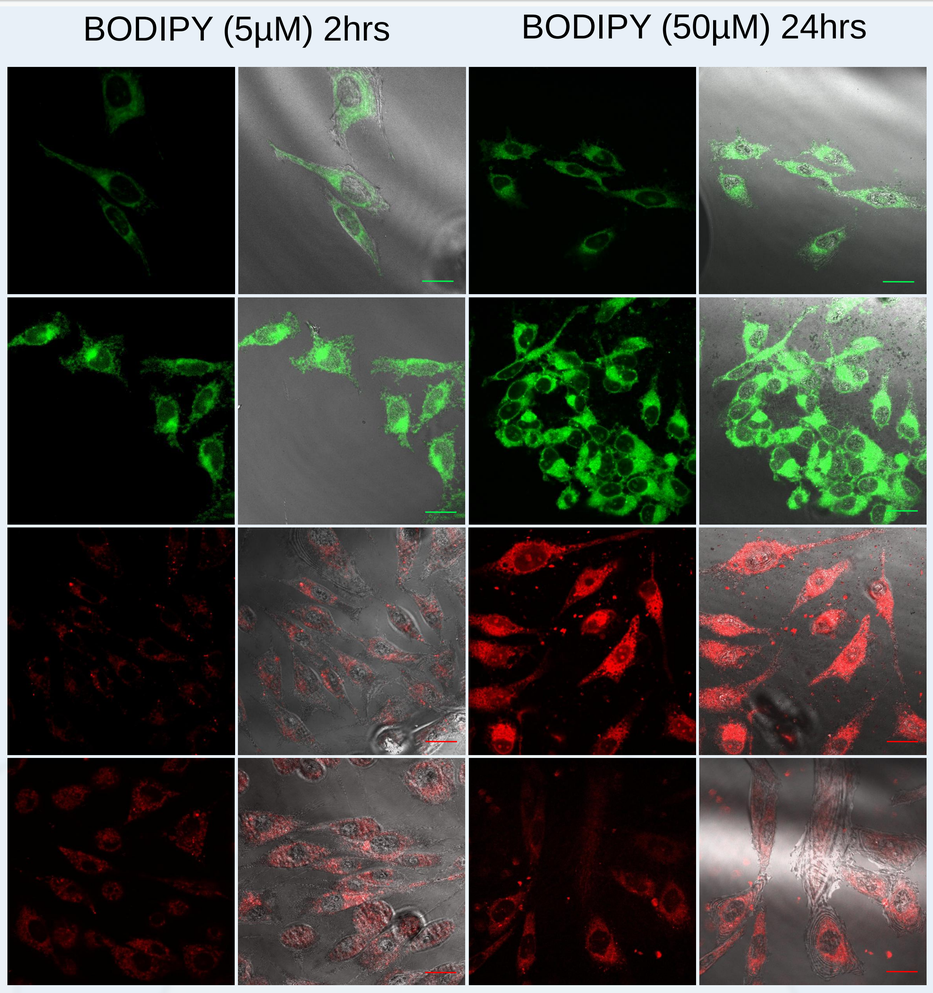
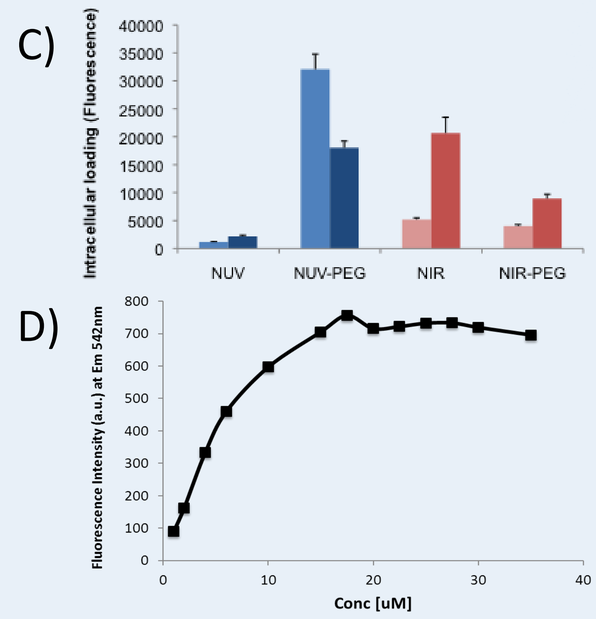
BODIPY (4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene) fluorophores have been used extensively in bioimaging to label many different proteins, lipids and nucleotides. They are powerful chormophores, due to high quantum yields and have bio-imaging applications such as detection systems for Fe^{3+}, Cd^{2+}, Cu^{2+} and esterase activity within cells.(1-4) Here we describe a novel family of BODIPY derivatives with a Mega-Stokes shift and near infrared (NIR) fluorescence emission which reverses the high concentration dependent self-quenching seen with the visible counterpart. Live CHO and HeLa cell imaging, cellular uptake, cellular localisation and a potential application for pH sensing within cells is described for these new compounds.

The structure, absorbance/emission spectra and live cell imaging and uptake of BODIPY complexes





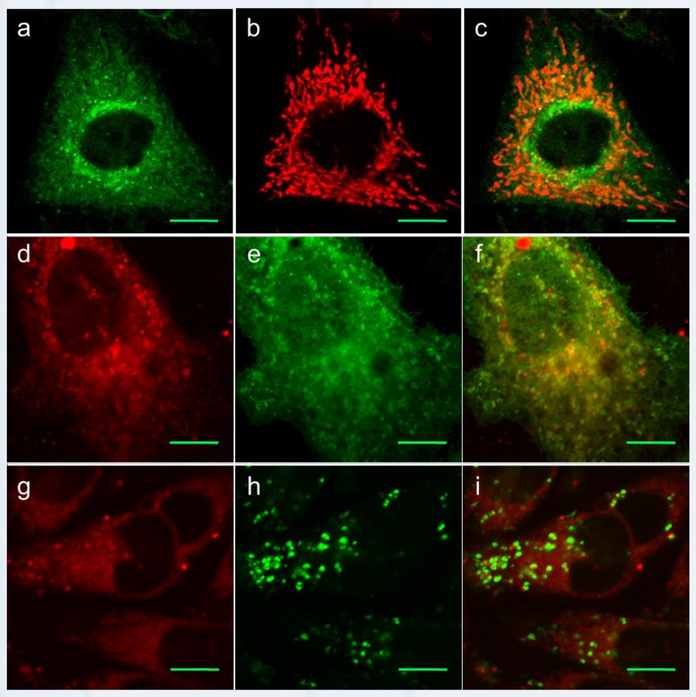


Schematics BODIPY structures and preceding synthetic steps with corresponding Abs/Ex/Em spectra for PEG linked dyes (A). Live HeLa cell uptake after incubation with BODIPY overnight at 5 and 50 µM in culture media imaged using a Zeiss LSM510 Meta confocal microscope, bar 20 µm (B) and quantified using ImageJ for 2 hours (light coloured bars) and 24 hours (dark coloured bars) at 50 µM (C). Quenching of NUV-PEG dye at concentrations above 17.5 µM (D).

Near ultraviolet (NUV) and infrared (NIR) BODIPY dyes were taken up by cells and uptake was increased with the aid of polyethylene glycol (PEG) linker for NUV but not for NIR dyes. Quenching of NUV-PEG dye at concentrations above 17.5 µM (D) explain the apparent decrease from 2 to 24 hours exposure (C).

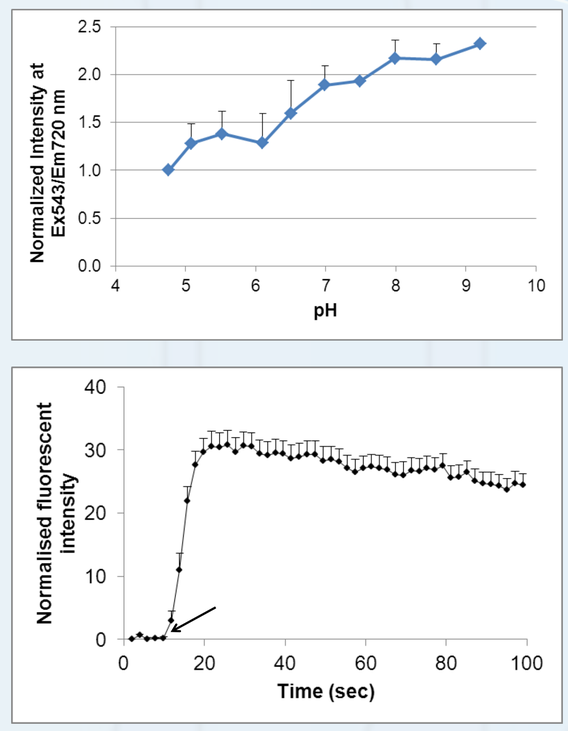
BODIPY Localisation

Localisation of NUV-PEG and NIR parent BODIPY dyes. HeLa cells were incubated for 16 hours with NUV-PEG (5µM) (a) or NIR dye (50 µM) (d & g) in culture media followed by mitochondrial stain, MitoTracker® (25 µM) (b, merged with a in c) or mitochondrial and endoplasmic reticulum stain, DiOC6 (5 µM) (e, merged with NIR in f) or lysosomal stain, LysoTracker® (75nM) (h, merged with NIR in i) for 2 hours at 37°C with 5% CO_2. Live cell images were obtained using a Zeiss LSM510 Meta confocal microscope and 63x oil immersion lens, line bars 10 µm.



NIR dye can be used to detect intracellular pH

Near infrared BODIPY emission is pH sensitive within the physiological pH range. Normalized fluorescent intensity of near infrared BODIPY (100 µM) in 10 mM phosphate buffer increases with increasing pH at excitation 543, emission 720nm (A). Cells preloaded with NIR BODIPY (50 µM) for 24 hours were imaged live followed by treatment of ammonium chloride (20 mM) at image 5 (arrow). This neutralises acidic organelles within the cells and BODIPY emission was expressed as normalized fluorescent intensity (B).



The BODIPY derivatives were excluded from the nucleus and localise within lipophillic regions of intracellular organelles most likely the endoplasmic reticulum.

NIR BODIPY emission is pH sensitive and neutralisation of acidic organelles resulted in a dramatic rise in dye emission within the cells indicating further localisation in the lysosomes. Therefore, NIR BODIPY can detect a shift in intracellular pH.

Taken together these BODIPY dyes, especially the near infrared derivatives may act as a new class of high quantum yield, photostable dyes that can be used for bioimaging and pH sensing within cells.

References: 1. X. Qu, Q. Liu, X. Ji, H. Chen, Z. Zhou and Z. Shen. Chem. Commun. 2012, **48**, 4600-4602. 2. X. Peng, J. Du, J. Fan, J. Wang, Y. Wu, J. Zhao, S. Sun, T. Xu, J. Am. Chem. Soc. 2007, **129**, 1500 – 1501. 3. H. Y. Lee, H. Son, J. M. Lim, J. Oh, D. Kang, W. S. Han and J. H. Jung. Analyst, 2010, **135**, 2022–2027. 4. T. Komatsu, Y. Urano, Y. Fujikawa, T. Kobayashi, H. Kojima, T. Terai, K. Hanaokaa and T. Nagano. Chem. Commun. 2009, 7015-7017.

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