Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

The small molecule probe PT-Yellow labels the renal proximal tubules in zebrafish

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Synthetic Material and Method:

(Synthetic procedure as of reference 1)

All reactions were performed in oven-dried glassware under a positive pressure of nitrogen. Unless otherwise noted, starting materials and solvents were purchased from Aldrich and Acros organics and used without further purification. Analytical TLC was carried out on Merck 60 F_{254} silica gel plate (0.25 mm layer thickness) and visualization was done with UV light. Column chromatography was performed on Merck 60 silica gel (230 - 400 mesh). NMR spectra were recorded on a Bruker Avance 300 MHz NMR spectrometer. Chemical shifts are reported as δ in units of parts per million (ppm) and coupling constants are reported as a *J* value in Hertz (Hz). Mass of BDNCA3-D2 compound was determined by LC-MS of Agilent Technologies with an electrospray ionization source. Spectroscopic measurements were performed on a fluorometer and UV/VIS instrument, Synergy 4 of bioteck company and Gemini XS fluorescence plate reader. Relative quantum yield was calculated by comparing the areas under the corrected emission spectrum. The following equation was used to calculate quantum yield.

$$\Phi_x = \Phi_{st}(I_x/I_{st})(A_{st}/A_x)(\eta_x^2/\eta_{st}^2)$$

where Φ_{st} is the reported quantum yield of the standard, I is the integrated emission spectrum, *A* is the absorbance at the excitation wavelength, and η is the refractive index of the solvents used. The subscript "x" denotes unknown and "st" denotes standard. Rhodamine B was used as standard.

Data for BDNCA3-D2:

Yield: 55 %

Quantum Yield (Φ): 0.024 (Solvent: Ethanol, Reference Dye: Rhodamine B, Excitation wavelength (λ_{ex}): 510nm)

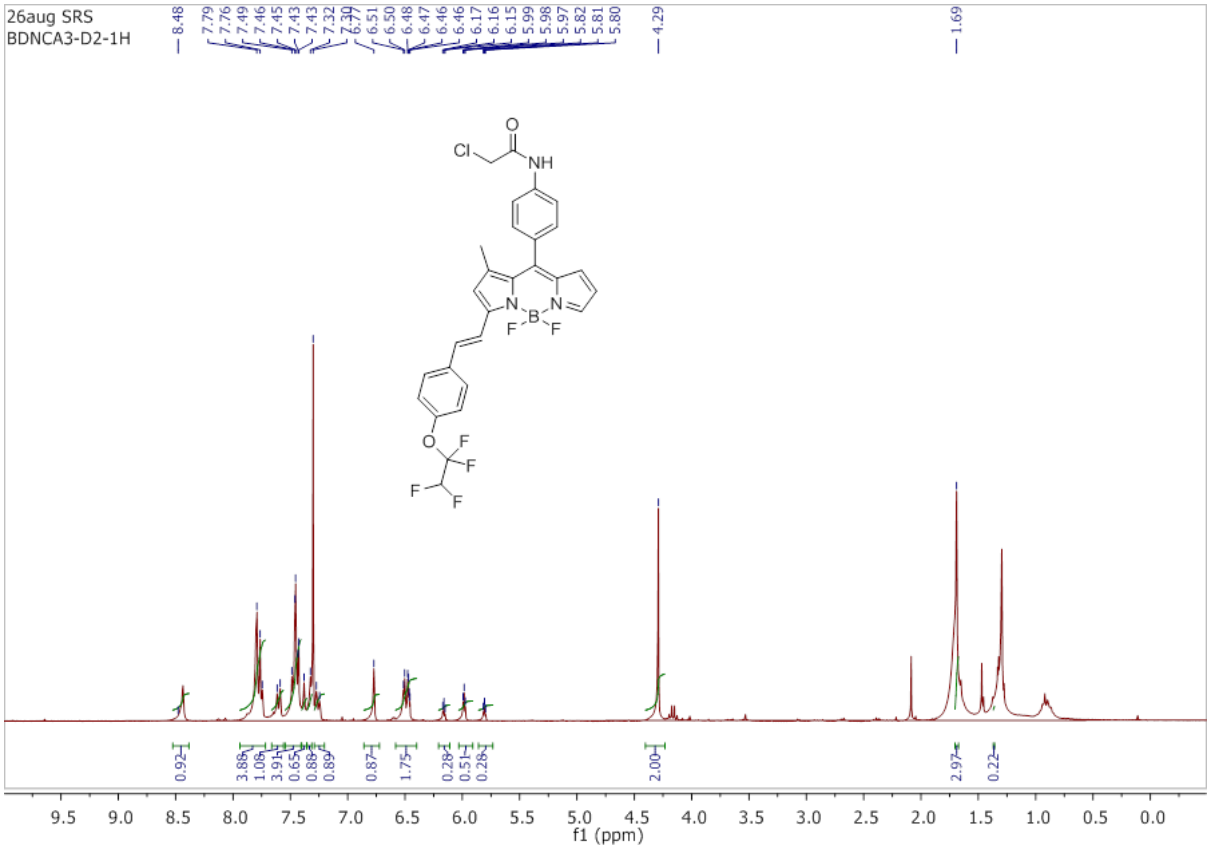
Extinction coefficient (ε): 114969 $M^{-1}cm^{-1}$ (Solvent: Ethanol, Wavelength (λ): 548 nm)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.69 (s, 3H), 4.29 (s, 2H), 5.98 (tt, J_1 = 54 Hz, J_2 = 3 Hz, 1H), 6.46-6.51 (m, 2H), 6.77 (s, 1H), 7.25 (d, J = 9 Hz, 1H), 7.38 (s, 1H), 7.49-7.38 (m, 4H), 7.6 (d, J = 6 Hz, 1H), 7.79-7.74 (m, 4H), 8.48 (s, 1H).

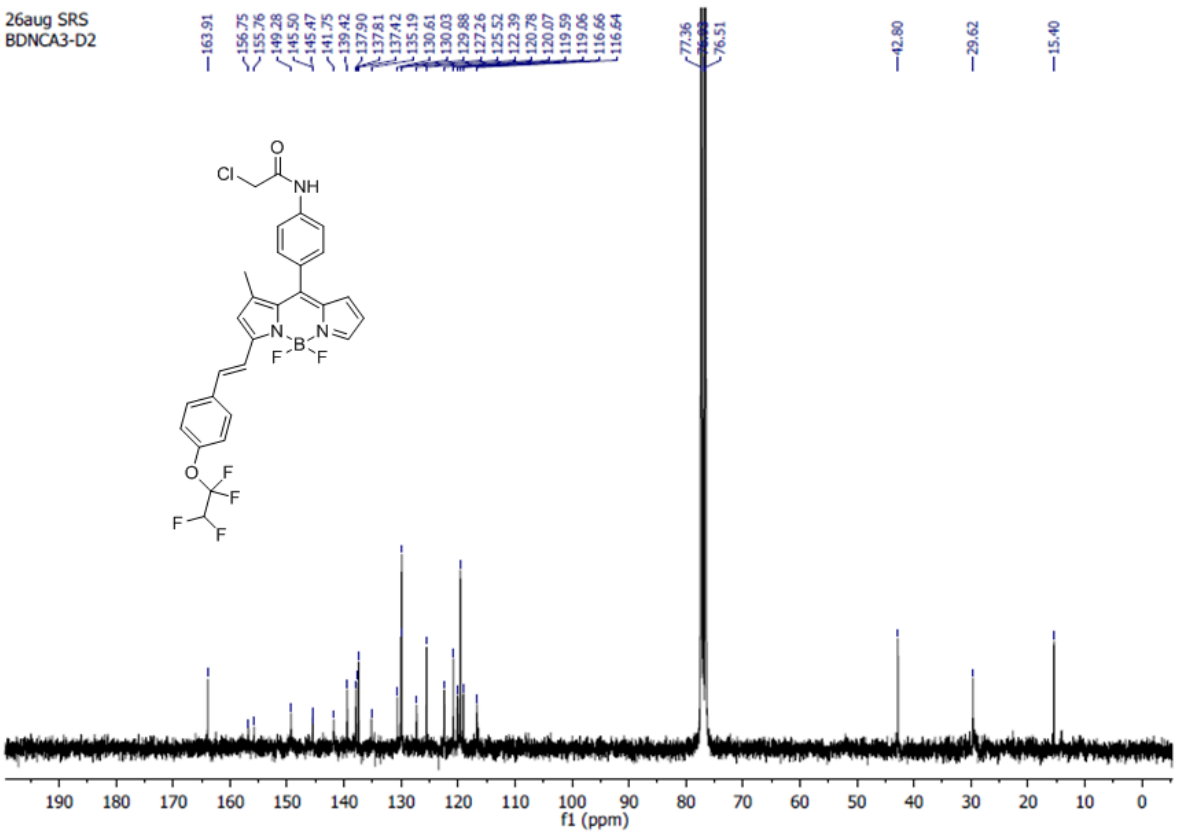
^{13}C NMR ($CDCl_3$, 75 MHz): δ 15.4, 29.6, 42.8, 116.6, 116.7, 119.1, 119.6, 120.1, 120.8, 122.4, 125.5, 127.3, 129.9, 130.0, 130.6, 135.2, 137.4, 137.8, 137.9, 139.4, 141.7, 145.47, 145.5, 149.3, 155.8, 156.7, 163.9

HRMS: m/z ($C_{28}H_{21}BClF_6N_3O_2Na$) calculated: 614.1217, found: 614.1227 (M+Na).

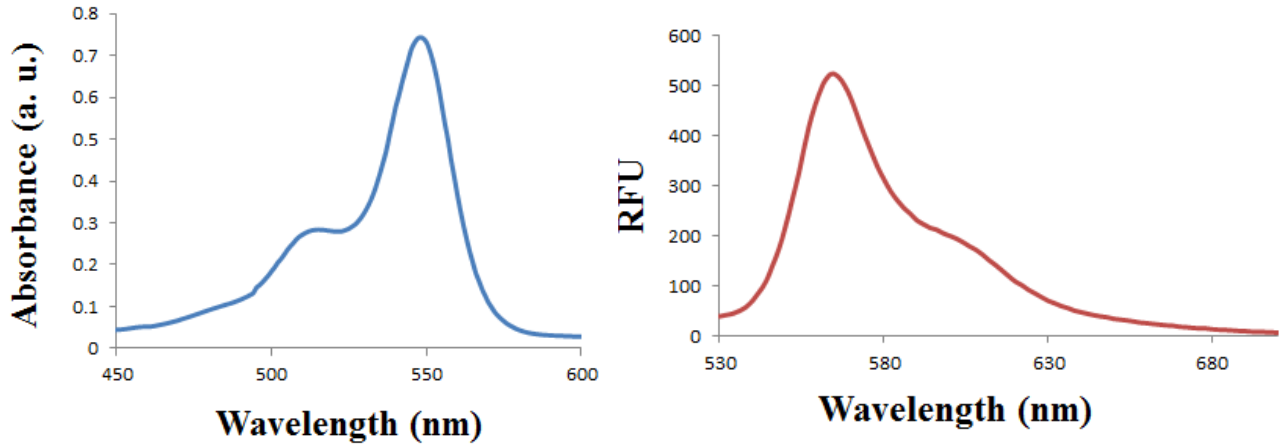
Spectral Data for BDNCA3-D2:



^{1}H NMR (300 MHz, $CDCl_3$) spectrum of BDNCA3-D2



^{13}C NMR (75 MHz, CDCl3) spectrum of BDNCA3-D2



Absorption and emission spectra of BDNCA3-D2, spectra were measured at the concentration of 6.3 μM in Ethanol, Excitation wavelength (λ_{ex}) = 510nm.

PT-Yellow
A stock solution of 100 µM in dimethyl sulphoxide (DMSO) was prepared and stored at -20°C.

Zebrafish maintenance and stocks
Zebrafish (Tuebingen wild type) were maintained at 28°C under standard conditions. Embryos were raised in E3 medium[2]. For imaging purposes, embryos were incubated in 1% phenylthiourea (PTU) in E3 from 24 hpf on to suppress pigment formation. Generation of *Tg(PT::EGFP)*, *Tg(nphs2::EGFP)*, and *hi1843* has been described elsewhere[3-6].

Zebrafish treatment
Zebrafish larvae at 3 dpf were incubated for 20 min in 1 ml of 100 nM PT-Yellow in E3 in 12 well plates covered with aluminum foil. Larvae were subsequently washed twice with E3 and left to develop until imaging at 4 dpf. Adult zebrafish were anesthetized with 0.2 mg/ml tricaine (Sigma). 50 µl of 100 µM PT-Yellow (in water) were injected intraperitonally (IP). Gentamicin (Gibco) was IP-injected the following day at a concentration of 2 mg/ml. Fish were starved for 3 days post-gentamicin injection and sacrificed at 6 days post-injury. Whole kidneys were excised, fixed overnight in 4% paraformaldehyde (PFA), and further processed for histology as described below. Hnf1bb morpholino (5'-CTTGGACACCATGTCAGTAAA-3'[7]) injection was performed as described[8]. For cardiac arrest, 3 dpf larvae were put in E3 medium containing 20 mM 2,3-butanedione monoxime (BDM, Sigma) and 100 nM PT-Yellow for 24 hrs.

Imaging
Live embryos and histological sections were imaged using a fluorescent compound microscope (Nikon Eclipse 80i) equipped with a digital camera (Hamamatsu C4742-80-12AG). 510/560 nm and 600/660 nm emission filters were used for imaging. Embryos were anesthetized with 0.2 mg/ml tricaine and mounted in 1% methylcellulose solution in a concave slide for immobilization.

Dose response analysis
5 dpf larvae were incubated for 5, 10, 20 and 60 min. Larvae were washed and imaged immediately after the specific incubation periods (t=0). The exposure values for each larva were noted and the same individuals were imaged again after 24 hours (t=24), using the same exposure settings as for t=0.

Histology
4 dpf larvae and adult kidneys were fixed overnight in 4% PFA at 4°C. After washing with PBST, fixed larvae were transferred into an embedding mold and filled with embedding medium (1% low-melting agarose, 0.9% agar, 5% sucrose). After the embedding medium had solidified, the blocks were transferred to a 30% sucrose solution in 2 ml tubes and incubated at 4°C until they sank to the bottom. The blocks were then removed from the sucrose solution and stored at -20°C. Sections of 10 µm (for larval zebrafish) and 12 µm (for adult kidneys) were obtained using a Leica cryostat (CM-3050-S), collected on Superfrost slides, dried at room temperature overnight and stored at -20°C. For imaging, sections were thawed at room

temperature for 20 min, washed in PBS and mounted in Prolong Gold (Life technologies).

Survival analysis

3 dpf larvae were incubated for 20 min in PT-Yellow (100 nM in E3), or, for controls, in DMSO (1 µl/ml in E3). Larvae were raised in 3.5-liter tanks of Tecniplast's ZebTec Zebrafish housing system. Surviving fish were counted 27 days post-treatment (at 30 dpf).

References:

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