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## Supporting Information

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### **Water-soluble Pyrrolopyrrole Cyanine (PPCy) NIR fluorophores**

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**S1:** Experimental section

25 **S2:** Supplemented spectroscopic data

**S3:** Time-dependent change in absorption spectra of **6'** and **6''** in water

**S4:** Photostability of **6''**

**S5:** In-cell emission of **6''**

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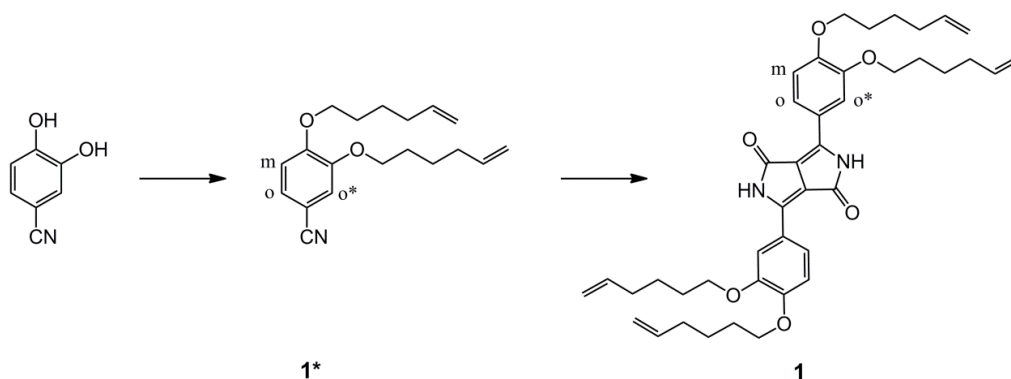
**S1:** Experimental section

## General

Solvents were purified and dried according to standard procedures. All commercially available reagents were used without further purification unless otherwise noted. Normal phase column chromatography was performed on Roth silica gel 60 (40–63  $\mu\text{m}$ ). Reversed phase column chromatography was performed on Macherey-Nagel C-18 silica gel Polygoprep 60-50. All solvents used for UV/Vis/NIR and fluorescence measurements were of spectroscopic grade. NMR spectra were recorded with a Bruker Avance III-400 (400 MHz). The residual solvent peak was used as internal reference ( $\text{CHCl}_3$ :  $d = 7.26$  ppm;  $\text{C}_2\text{D}_2\text{HCl}_4$ :  $d = 5.91$  ppm). For  $^1\text{H}$  NMR, multiplicity ( $s =$  singlet,  $d =$  doublet,  $dd =$  doublet of doublets,  $t =$  triplet,  $q =$  quartet,  $quint =$  quintet,  $m =$  multiplet,  $b =$  broad signal) and coupling constants are reported whenever possible. ESI-ion trap mass spectra were recorded with a Bruker Esquire 3000 plus. High-resolution (HR) ESI mass spectra were recorded on a Bruker MicroTOF. Elemental analysis was performed with a CHN analyser Vario EL from Elementar. Absorption and emission spectra were recorded at ambient temperature by using 1 cm quartz cuvettes (3 mL). UV/Vis/NIR absorptions were recorded with a Varian spectrometer, model Cary 50; the spectra were processed with Spekwin32.<sup>19</sup> Fluorescence spectra were recorded with a self-assembled NIR fluorescence spectrometer with a nitrogen-cooled Ge diode (EO-817L, Northcoast, USA) as detector. Diode lasers (for **6'**: 690 nm, 19 mW, model ACM19/1203, Power Technology, Little Rock, AR; for **6''**: 804 nm, 30 mW, model ACM30/1476, Power Technology) were used for excitation.  $\text{BF}_2\text{-PPCy}$  **10e** ( $\Phi_F = 0.59$  in  $\text{CHCl}_3$ ; for **6'**) and  $\text{BPh}_2\text{-PPCy}$  **11e** ( $\Phi_F = 0.53$  in  $\text{CHCl}_3$ ; for **6''**) from reference <sup>12a</sup> were used as standards to determine the quantum yields. For a correct presentation of the fluorescence spectra they were multiplied by  $\lambda^2$  in the wavelength scale, then converted to the wavenumber scale and normalized. The syntheses of heteroarylacetonitriles **2'** and **2''** as well as of chlorodiphenylborane ( $\text{BPh}_2\text{Cl}$ ) were published previously.<sup>12a</sup> 2-aminoethanesulfonic acid sodium salt was synthesized as described in literature and the elemental analysis was according to calculated values.<sup>20</sup> The  $^1\text{H}$ -NMR data showed that **6''** crystallizes with three and **6'** with ten molecules of water per formula unit (see elemental analyses). The strong high-field shift of the o-H and o\*-H protons of the bisalkoxyphenyl substitutes upon complexation ( $\Delta\delta \approx 1.6$  ppm for **4''**;  $\Delta\delta \approx 1.3$  ppm for **4'**) is to be interpreted as a consequence of the spatial closeness of these protons to the field-enhancing part of the anisotropy cone of one of the  $\text{BPh}_2$  groups. The inequivalence (different proton chemical shifts!) of seemingly equivalent protons (protons m-H, m'-H; o-H, o'-H; o\*-H, o\*'-H; H-8, H-8') found upon complexation we assign to a deviation of the molecules' symmetry from  $C_i$ .

### 3,6-bis(3,4-bis(hex-5-en-1-yloxy)phenyl)pyrrolo[3,4-c]pyrrole-1,4(2H,5H)-dione (DPP) **1**:

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### 3,4-bis(hex-5-en-1-yloxy)benzonitrile **1\***:

35 5.18 g (38.3 mmol) 3,4-dihydroxybenzonitrile, 25.0 g (153.5 mmol) 6-bromo-1-hexene, 42.4 g (306.7 mmol)  $\text{K}_2\text{CO}_3$  and 11.5 g (76.7 mmol) NaI in 200 ml of acetone were heated under reflux overnight.  $\text{K}_2\text{CO}_3$  was filtered off and washed with acetone. The filtrate was evaporated under reduced pressure. The crude product was dissolved in dichloromethane and washed twice with water and brine. The solvent was dried over  $\text{MgSO}_4$  and evaporated under reduced pressure. The crude product was kept at 100  $^\circ\text{C}$  under vacuum until constant weight and was then cooled down. After some time the product solidified. **1\*** was obtained as a white solid in 84 % yield (9.6 g, 40 32.1 mmol).

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 7.23 (dd,  $^3\text{J} = 8.4$  Hz,  $^4\text{J} = 1.9$  Hz, 1 H; o-H), 7.07 (d,  $^4\text{J} = 1.9$  Hz, 1 H; o\*-H), 6.86 (d,  $^3\text{J} = 8.4$  Hz, 1 H; m-H), 5.82 (m, 2 H;  $\text{O}(\text{CH}_2)_4\text{CHCH}_2$ ), 5.03 (m,  $^3\text{J} = 17.2$  Hz, 2 H;  $\text{H}_{\text{trans}}$ ), 4.97 (m,  $^3\text{J} = 10.3$  Hz, 2 H;  $\text{H}_{\text{cis}}$ ), 4.04 (t,  $^3\text{J} = 6.5$  Hz,

2 H; OCH<sub>2</sub>), 3.99 (t, <sup>3</sup>J = 6.5 Hz, 2 H; OCH<sub>2</sub>), 2.13 (m, 4 H; O(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.85 (m, 4 H; OCH<sub>2</sub>CH<sub>2</sub>), 1.58 (m, 4 H; O(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>). Elemental analysis (%): C<sub>42</sub>H<sub>52</sub>N<sub>2</sub>O<sub>6</sub> (299.41): calcd. C 76.22, H 8.42, N 4.68; found C 76.07, H 8.49, N 4.76.

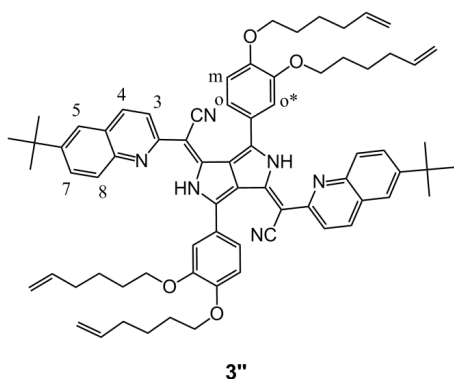
### 5 3,6-bis(3,4-bis(hex-5-en-1-yloxy)phenyl)pyrrolo[3,4-c]pyrrole-1,4(2H,5H)-dione (DPP) 1:

Under nitrogen atmosphere 2.41 g (104.7 mmol) Na were dissolved in 23.6 ml *tert*-amylalcohol at reflux with a spatula tip of water-free FeCl<sub>3</sub>. After complete dissolving of Na the solution was cooled to ca. 90 °C and 9.5 g (31.7 mmol) **1**\* were added and the mixture was heated to reflux again. 2.64 ml (15.8 mmol) diethyl succinate were added dropwise via a syringe pump over a period of 5 h and the mixture was then refluxed overnight. The reaction mixture was cooled to room temperature and methanol was added. 4 ml formic acid were added slowly. The precipitated solid was filtered off and washed with methanol. The solid was digested in refluxing methanol for 30 min and filtered. This procedure was repeated until the filtrate went colorless. The solid was then digested in refluxing acetone for 30 min and filtered. This procedure was repeated until the filtrate went colorless. **1** was obtained as a red solid in 59 % yield (6.4 g, 9.4 mmol).

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<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ (ppm) = 11.18 (s, 2 H; NH), 8.18 (d, <sup>4</sup>J = 1.5 Hz, 2 H; o\*-H), 8.11 (dd, <sup>3</sup>J = 8.6 Hz, <sup>4</sup>J = 1.5 Hz, 2 H; o-H), 7.13 (d, <sup>3</sup>J = 8.6 Hz, 2 H; m-H), 5.83 (m, 4 H; O(CH<sub>2</sub>)<sub>4</sub>CHCH<sub>2</sub>), 5.03 (d, <sup>3</sup>J = 17.2 Hz, 4 H; H<sub>trans</sub>), 4.97 (d, <sup>3</sup>J = 10.3 Hz, 4 H; H<sub>cis</sub>), 4.08 (m, 8 H; OCH<sub>2</sub>), 2.10 (m, 8 H; O(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.75 (m, 8 H; OCH<sub>2</sub>CH<sub>2</sub>), 1.54 (m, 8 H; O(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>). ESI-MS: calcd. for C<sub>42</sub>H<sub>53</sub>N<sub>2</sub>O<sub>6</sub> [M + H]<sup>+</sup> 681.4 ; found 681.3. Elemental analysis (%): C<sub>42</sub>H<sub>52</sub>N<sub>2</sub>O<sub>6</sub> (680.87): calcd. C 74.09, H 7.70, N 4.11; found C 73.29, H 7.62, N 4.33.

### H-PPCy 3'':

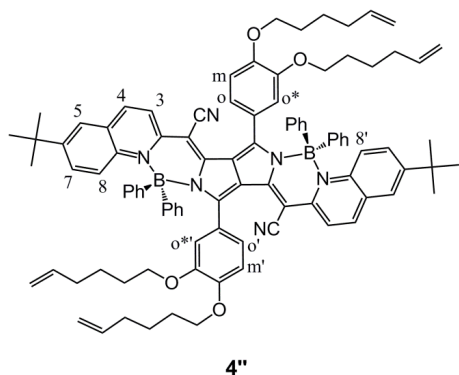


25 2.0 g (2.94 mmol) **1** and 1.65 g (7.34 mmol) heteroarylacetonitrile **2''** were heated to reflux in anhydrous toluene under nitrogen. 2.15 ml (23.52 mmol) phosphoryl chloride was then added. The reaction was monitored by UV/Vis/NIR spectroscopic analysis and thin layer chromatography. As soon as **1** was used up (around 1 h) the reaction was stopped. The solvent and excess phosphoryl chloride were removed under vacuum, the crude product was dissolved in dichloromethane and washed with saturated, aqueous NaHCO<sub>3</sub> solution. The solvent was again removed under vacuum and the crude product was treated with methanol in an ultrasonic bath. The solid was collected **30** by filtration and washed with methanol until the filtrate became colorless. **3''** was obtained as a green solid in 69 % yield (2.23 g, 2.0 mmol). For analytics, a small amount was purified by column chromatography (dichloromethane).

<sup>1</sup>H NMR (400 MHz, C<sub>2</sub>Cl<sub>4</sub>D<sub>2</sub>): δ (ppm) = 14.69 (s, 2 H; NH), 7.96 (d, <sup>3</sup>J = 8.9 Hz, 2 H; H-4), 7.70 (m, 4 H; H-7, H-8), 7.60 (s, 2 H; H-5), 7.59 (d, <sup>3</sup>J = 8.9 Hz, 2 H; H-3) 7.34 (dd, <sup>3</sup>J = 8.3 Hz, <sup>4</sup>J = 1.5 Hz, 2 H; o-H), 7.24 (d, <sup>4</sup>J = 1.5 Hz, 2 H; o\*-H), 7.06 (d, <sup>3</sup>J = 8.3 Hz, 2 H; m-H), 5.79 (m, 4 H; O(CH<sub>2</sub>)<sub>4</sub>CHCH<sub>2</sub>), 5.02 (qd, <sup>3</sup>J = 17.2 Hz, <sup>2</sup>J ≈ <sup>4</sup>J = 1.7 Hz, 2 H; H<sub>trans</sub>), 4.95 (qd, <sup>3</sup>J = 17.2 Hz, <sup>2</sup>J ≈ <sup>4</sup>J = 1.7 Hz, 2 H; H<sub>trans</sub>), 4.945 (md, <sup>3</sup>J = 10.3 Hz, 2 H; H<sub>cis</sub>), 4.885 (md, <sup>3</sup>J = 10.3 Hz, 2 H; H<sub>cis</sub>), 4.07 (t, <sup>3</sup>J = 6.2 Hz, 8 H; OCH<sub>2</sub>), 2.11 (m, 8 H; O(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 1.84 (m, 8 H; OCH<sub>2</sub>CH<sub>2</sub>), 1.57 (m, 8 H; O(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>), 1.35 (s, 18 H; *tert*-BuH). ESI-MS: calcd. for C<sub>72</sub>H<sub>81</sub>N<sub>6</sub>O<sub>4</sub> [M + H]<sup>+</sup> 1093.6 ; found 1093.8. Elemental analysis (%): C<sub>72</sub>H<sub>80</sub>N<sub>6</sub>O<sub>4</sub> (1093.44): calcd. C 79.09, H 7.37, N 7.69; found C 78.89, H 7.49, N 7.70.

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### BPh<sub>2</sub>-PPCy 4'':



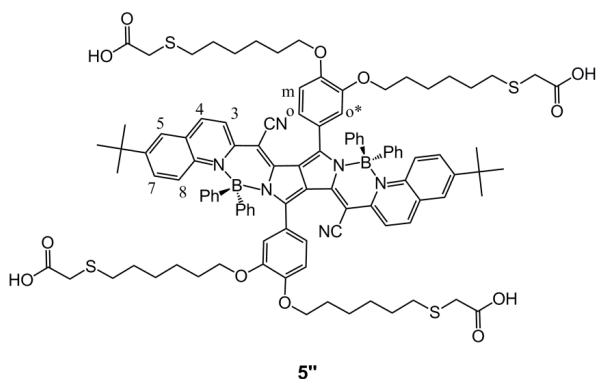
**4''**

280 mg (256.1  $\mu\text{mol}$ ) **3''** and 0.127 ml (768.2  $\mu\text{mol}$ ) *N,N*-diisopropylethylamine were heated to reflux in  $\text{CH}_2\text{Cl}_2$ . 0.212 ml (1.18 mmol) chlorodiphenylborane were added, and the mixture was heated at reflux until the reaction was complete (UV/Vis/NIR). The mixture was washed with saturated, aqueous  $\text{NaHCO}_3$  solution and dried over  $\text{MgSO}_4$ . After removal of the solvent, the crude product was treated with methanol in an ultrasonic bath. The solid was collected by filtration and washed with methanol. The crude product was purified by column chromatography (dichloromethane) to afford **4''** as a brown solid in 60 % yield (220 mg, 154.8  $\mu\text{mol}$ ).

$^1\text{H}$  NMR (400 MHz,  $\text{C}_2\text{Cl}_4\text{D}_2$ ):  $\delta$  (ppm) = 8.07 (d,  $^3J = 9.7$  Hz, 1 H; H-8), 8.06 (d,  $^3J = 9.7$  Hz, 1 H; H-8'), 7.77 (d,  $^3J = 9.2$  Hz, 2 H; H-4), 7.51 (d,  $^3J = 9.2$  Hz, 2 H; H-3), 7.36-7.23 (m, 10 H; BPhH, H-5), 7.12 (dd,  $^3J = 9.7$  Hz,  $^4J = 1.7$  Hz, 2 H; H-7), 7.09-6.97 (m, 12 H; 10 BPhH), 6.43 (d,  $^3J = 8.2$  Hz, 1 H; m-H), 6.42 (d,  $^3J = 8.2$  Hz, 1 H; m'-H), 5.83 (m, 4 H;  $\text{O}(\text{CH}_2)_4\text{CHCH}_2$ ), 5.752 (d,  $^4J = 1.8$  Hz, 1 H; o\*-H), 5.746 (d,  $^4J = 1.8$  Hz, 1 H; o\*' -H), 5.63 (dd,  $^3J = 8.2$  Hz,  $^4J = 1.8$  Hz, 1 H; o-H), 5.61 (dd,  $^3J = 8.2$  Hz,  $^4J = 1.8$  Hz, 1 H; o'-H), 5.05 (qd,  $^3J = 17.2$  Hz,  $^2J \approx ^4J = 1.7$  Hz, 2 H;  $\text{H}'_{\text{trans}}$ ), 4.985 (qd,  $^3J = 17.2$  Hz,  $^2J \approx ^4J = 1.7$  Hz, 2 H;  $\text{H}'_{\text{trans}}$ ), 4.97 (md,  $^3J = 10.3$  Hz, 2 H;  $\text{H}_{\text{cis}}$ ), 4.91 (md,  $^3J = 10.3$  Hz, 2 H;  $\text{H}'_{\text{cis}}$ ), 3.98 (t,  $^3J = 6.3$  Hz, 4 H;  $\text{OCH}_2$ ), 3.50 (m, 2 H; OCH), 3.38 (m, 2 H;  $\text{OCH}'$ ), 2.15 (q,  $^3J = 7.0$  Hz, 4 H;  $\text{O}(\text{CH}_2)_3\text{CH}_2$ ), 2.07 (q,  $^3J = 7.0$  Hz, 4 H;  $\text{O}(\text{CH}_2)_3\text{CH}'_2$ ), 1.82 (m, 4 H;  $\text{OCH}_2\text{CH}_2$ ), 1.62 (m, 12 H;  $\text{OCH}_2\text{CH}'_2$ ,  $\text{O}(\text{CH}_2)_2\text{CH}_2$ ), 1.11 (s, 18 H; *tert*-BuH). ESI-MS: calcd. for  $\text{C}_{96}\text{H}_{99}\text{B}_2\text{N}_6\text{O}_4$  [ $\text{M} + \text{H}$ ] $^+$  1422.8 ; found 1422.8. Elemental analysis (%):  $\text{C}_{96}\text{H}_{98}\text{B}_2\text{N}_6\text{O}_4$  (1421.47): calcd. C 81.12, H 6.95, N 5.91; found C 80.82, H 6.99, N 6.12.

#### BPh<sub>2</sub>-PPCy **5''**:

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**5''**

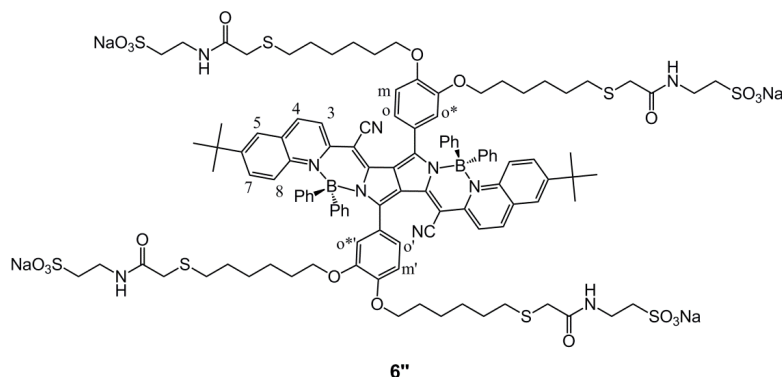
200 mg (140  $\mu\text{mol}$ ) **4''** were dissolved in 10 ml absolute 1,4-dioxane under nitrogen. 0.294 ml (4.22 mmol) mercapto acetic acid and 23.2 mg (140  $\mu\text{mol}$ ) azobisisobutyronitrile (AIBN) were added and the reaction mixture was heated to 90  $^\circ\text{C}$  for 4 h. After each of the first three hours additional 23.2 mg (140  $\mu\text{mol}$ ) AIBN were added (560  $\mu\text{mol}$  AIBN whole). 20 ml water were added and the precipitate **5''** was filtered off. The precipitate was suspended in water, treated in an ultrasonic bath and centrifuged (2 x). After drying **5''** was afforded as a brown solid in 80 % yield (200 mg, 112  $\mu\text{mol}$ ).

$^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  (ppm) = 12.53 (s, 4 H; COOH), 8.27 (d,  $^3J = 8.1$  Hz, 2 H; H-8), 8.06 (d,  $^3J = 7.8$  Hz, 2 H; H-4), 7.65 (b, 2 H; H-5), 7.56 (d,  $^3J = 7.8$  Hz, 2 H; H-3), 7.42 (b, 2 H; H-7), 7.35-7.00 (m, 20 H; BPhH), 6.55 (b, 2 H; m-H), 5.75 (b, 1 H; o\*-H), 5.70 (b, 1 H; o\*' -H), 5.68 (b, 1 H; o-H), 5.59 (b, 1 H; o'-H), 4.01 (m, 4 H;  $\text{OCH}_2$ ), 3.43 (m, 4 H; OCH,  $\text{OCH}'$ ), 3.24 (s, 4 H;

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$SCH_2COOH$ ), 3.19 (s, 2 H;  $SCHCOOH$ ), 3.17 (s, 2 H;  $SCH'COOH$ ) 2.65 (t,  $^3J = 7.2$  Hz, 4 H;  $SCH_2$ ), 2.55 (m, 4 H;  $SCH$ ,  $SCH'$ ), 1.83-1.31 (m, 32 H;  $OCH_2(CH_2)_4CH_2S$ ), 1.15 (s, 18 H; *tert*-BuH). ESI-MS: calcd. for  $C_{104}H_{115}B_2N_6O_{12}S_4$  [ $M + H$ ] $^+$  1790.8; found 1790.6.

### 5 BPh<sub>2</sub>-PPCy 6<sup>''</sup>:



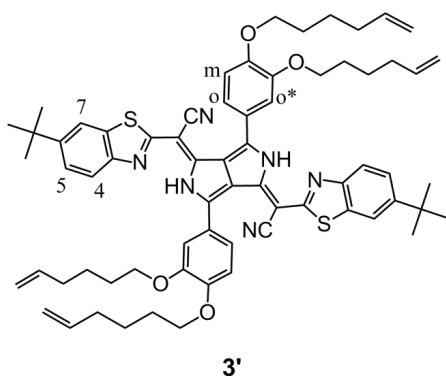
6<sup>''</sup>

*Preparation of 4-fold NHS ester:* 200 mg (111.7  $\mu$ mol) 5<sup>''</sup>, 230 mg (1.12 mmol) dicyclohexylcarbodiimide (DCC) and 130 mg (1.12 mmol) *N*-hydroxysuccinimide (NHS) were dissolved in 10 ml dichloromethane. The reaction mixture was stirred at room temperature 10 overnight. The solvent was removed in vacuum. The crude product was suspended in methanol, treated in an ultrasonic bath and filtered off (2 x). The so prepared NHS ester (200 mg) was dried.

*Preparation of 6<sup>''</sup>:* 73 mg (496  $\mu$ mol) 2-aminoethanesulfonic acid sodium salt was suspended in 27 ml DMF and treated in an ultrasonic bath for several minutes. 180 mg (82.6  $\mu$ mol) of the NHS ester was added. The reaction mixture was stirred at room temperature until only product peaks in the ESI mass spectra were found ( $m/z = 1129.9$ , 745.6; around 10 min). 50 ml  $CH_2Cl_2$  were added and the 15 precipitate was filtered off. Reversed phase C-18 column chromatography ( $H_2O:MeOH$  1:1 to  $H_2O:MeOH$  2:3) afforded 6<sup>''</sup> as a brown solid in 18 % yield (34 mg, 14.7  $\mu$ mol).

$^1H$  NMR (400 MHz,  $MeOD-d_4$ ):  $\delta$  (ppm) = 8.24 (d,  $^3J = 9.6$  Hz, 2 H; H-8), 8.12 (d,  $^3J = 9.2$  Hz, 2 H; H-4), 7.59 (d,  $^3J = 9.2$  Hz, 2 H; H-3), 7.56 (d,  $^4J = 2.2$  Hz, 2 H; H-5), 7.46-7.36 (m, 8 H; BPhH), 7.27 (dd,  $^3J = 9.6$  Hz,  $^4J = 2.2$  Hz, 2 H; H-7), 7.18-7.06 (m, 12 H; BPhH), 6.56 (d,  $^3J = 8.4$  Hz, 2 H; m-H), 5.89 (m, 2 H; o\*-H, o\*-H), 5.75 (dd, 1 H; o-H), 5.72 (dd, 1 H; o'-H), 4.10 (m, 4 H;  $OCH_2$ ), 3.66 (t,  $^3J = 6.7$  Hz, 4 H;  $CONHCH_2$ ), 3.63 (t,  $^3J = 6.7$  Hz, 4 H;  $CONHCH'_2$ ), 3.56 (m, 4 H;  $OCH$ ,  $OCH'$ ), 3.24 (s, 4 H;  $SCH_2CONH$ ), 3.14 (s, 2 H;  $SCHCONH$ ), 3.13 (s, 2 H;  $SCH'CONH$ ), 3.00 (t,  $^3J = 6.7$  Hz, 4 H;  $CH_2SO_3Na$ ), 2.97 (t,  $^3J = 6.7$  Hz, 4 H;  $CH'_2SO_3Na$ ), 2.68 (t,  $^3J = 7.2$  Hz, 4 H;  $SCH_2$ ), 2.54 (t,  $^3J = 6.9$  Hz, 2 H;  $SCH$ ), 2.52 (t,  $^3J = 6.9$  Hz, 2 H;  $SCH'$ ), 1.89 (quint,  $^3J = 6.4$  Hz, 4 H;  $OCH_2CH_2$ ), 1.72 (quint,  $^3J = 6.4$  Hz, 4 H;  $OCH_2CH'_2$ ), 1.69 (quint,  $^3J = 6.4$  Hz, 4 H;  $SCH_2CH_2$ ), 1.60 (m, 12 H;  $SCH_2CH'_2$ ,  $O(CH_2)_2CH_2$ ), 1.44 (m, 8 H;  $S(CH_2)_2CH_2$ ), 1.21 (s, 18 H; *tert*-BuH). HR ESI-MS: calcd. for  $C_{112}H_{130}B_2N_{10}NaO_{20}S_8$  [ $M - 3 Na$ ] $^3-$  745.5782; found 745.5801; calcd. for  $C_{112}H_{130}B_2N_{10}O_{20}S_8$  [ $M - 4 Na$ ] $^4-$  553.4362; found 553.4333; Elemental analysis (%):  $C_{112}H_{130}B_2N_{10}Na_4O_{20}S_8 \cdot 3 H_2O$  (2360.43): calcd. C 56.99, H 5.81, N 5.93; found C 55.95, H 5.75, N 6.03.

### 30 H-PPCy 3<sup>'</sup>:

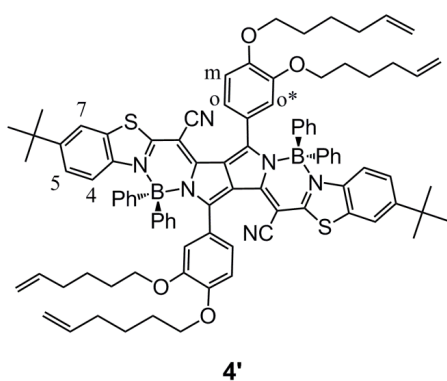


3<sup>'</sup>

1.55 g (2.3 mmol) **1** and 1.28 g (5.7 mmol) heteroarylacetonitrile **2'** were heated to reflux in anhydrous toluene under nitrogen. 1.66 ml (18.2 mmol) phosphoryl chloride was then added. The reaction was monitored by UV/Vis/NIR spectroscopic analysis and thin layer chromatography. As soon as **1** was used up (around 3.5 h) the reaction was stopped. The solvent and excess phosphoryl chloride were removed under vacuum, the crude product was dissolved in dichloromethane and washed with saturated, aqueous NaHCO<sub>3</sub> solution. The solvent was again removed under vacuum and the crude product was treated with methanol in an ultrasonic bath. The solid was collected by filtration and washed with methanol until the filtrate became colorless. **3'** was obtained as a green solid in 69 % yield (1.73 g, 1.6 mmol). For analytics, a small amount was purified by column chromatography (dichloromethane).

<sup>1</sup>H NMR (400 MHz, C<sub>2</sub>Cl<sub>4</sub>D<sub>2</sub>): δ (ppm) = 13.07 (s, 2 H; NH), 7.651 (d, <sup>3</sup>J = 8.5 Hz, 2 H; H-4), 7.648 (b, 2 H; H-7), 7.45 (dd, <sup>3</sup>J = 8.5 Hz, <sup>4</sup>J = 1.7 Hz, 2 H; H-5), 7.25 (dd, <sup>3</sup>J = 8.3 Hz, <sup>4</sup>J = 1.5 Hz, 2 H; o-H), 7.14 (d, <sup>4</sup>J = 1.5 Hz, 2 H; o\*-H), 6.98 (d, <sup>3</sup>J = 8.3 Hz, 2 H; m-H), 5.81 (m, 4 H; O(CH<sub>2</sub>)<sub>4</sub>CHCH<sub>2</sub>), 5.02 (qd, <sup>3</sup>J = 17.2 Hz, <sup>2</sup>J ≈ <sup>4</sup>J = 1.7 Hz, 2 H; H<sub>trans</sub>), 4.99 (qd, <sup>3</sup>J = 17.2 Hz, <sup>2</sup>J ≈ <sup>4</sup>J = 1.7 Hz, 2 H; H<sub>trans</sub>'), 4.91 (md, <sup>3</sup>J = 10.3 Hz, 2 H; H<sub>cis</sub>), 4.95 (md, <sup>3</sup>J = 10.3 Hz, 2 H; H<sub>cis</sub>'), 4.05 (t, <sup>3</sup>J = 6.2 Hz, 4 H; OCH<sub>2</sub>), 4.00 (t, <sup>3</sup>J = 6.2 Hz, 4 H; OCH<sub>2</sub>'), 2.12 (m, 8 H; O(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>), 1.84 (m, 8 H; OCH<sub>2</sub>CH<sub>2</sub>'), 1.58 (m, 8 H; O(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>'), 1.33 (s, 18 H; *tert*-BuH). ESI-MS: calcd. for C<sub>68</sub>H<sub>77</sub>N<sub>6</sub>O<sub>4</sub>S<sub>2</sub> [M + H]<sup>+</sup> 1105.5 ; found 1104.8. Elemental analysis (%): C<sub>68</sub>H<sub>76</sub>N<sub>6</sub>O<sub>4</sub>S<sub>2</sub> (1105.50): calcd. C 73.88, H 6.93, N 7.60; found C 73.49, H 6.83, N 7.75.

### BPh<sub>2</sub>-PPCy **4'**:



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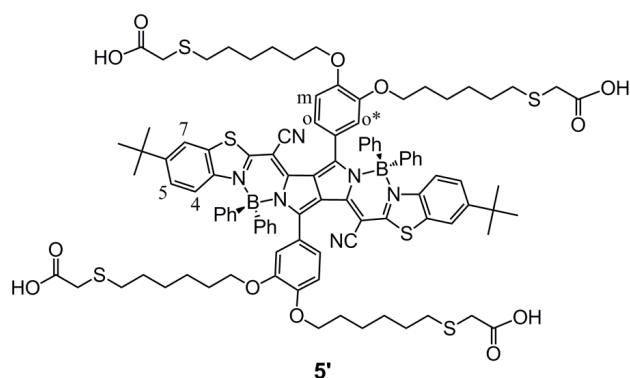
1.58 g (1.44 mmol) **3'** and 1.11 ml (4.3 mmol) *N,N*-diisopropylethylamine were heated to reflux in CH<sub>2</sub>Cl<sub>2</sub>. 0.84 ml (6.7 mmol) chlorodiphenylborane were added, and the mixture was heated at reflux until the reaction was complete (UV/Vis/NIR). The mixture was washed with saturated, aqueous NaHCO<sub>3</sub> solution and dried over MgSO<sub>4</sub>. After removal of the solvent, the crude product was treated with methanol in an ultrasonic bath. The solid was collected by filtration and washed with methanol. The crude product was purified by column chromatography (dichloromethane) to afford **4'** as a green solid in 63 % yield (1.29 g, 0.902 mmol).

<sup>1</sup>H NMR (400 MHz, C<sub>2</sub>Cl<sub>4</sub>D<sub>2</sub>): δ (ppm) = 7.43 (d, <sup>4</sup>J = 1.8 Hz, 2 H; H-7), 7.27 (m, 4 H, BPhH), 7.20-7.03 (m, 16 H, BPhH), 7.01 (dd, <sup>3</sup>J = 9.2 Hz, <sup>4</sup>J = 1.8 Hz, 2 H; H-5), 6.86 (d, <sup>3</sup>J = 9.2 Hz, 2 H; H-4), 6.32 (d, <sup>3</sup>J = 8.3 Hz, 1 H; m-H), 6.31 (d, <sup>3</sup>J = 8.3 Hz, 1 H; m'-H), 5.987 (d, <sup>4</sup>J = 1.7 Hz, 1 H; o\*-H), 5.980 (d, <sup>4</sup>J = 1.7 Hz, 1 H; o\*' -H), 5.936 (dd, <sup>3</sup>J = 8.3 Hz, <sup>4</sup>J = 1.7 Hz, 1 H; o-H), 5.933 (dd, <sup>3</sup>J = 8.3 Hz, <sup>4</sup>J = 1.7 Hz, 1 H; o'-H), 5.82 (m, 4 H; O(CH<sub>2</sub>)<sub>4</sub>CHCH<sub>2</sub>), 5.03 (md, <sup>3</sup>J = 17.2 Hz, 2 H; H<sub>trans</sub>), 5.00 (md, <sup>3</sup>J = 17.2 Hz, 2 H; H<sub>trans</sub>'), 4.95 (md, <sup>3</sup>J = 10.3 Hz, 2 H; H<sub>cis</sub>), 4.93 (md, <sup>3</sup>J = 10.3 Hz, 2 H; H<sub>cis</sub>'), 3.90 (m, 4 H; OCH<sub>2</sub>), 3.46 (m, 2 H; OCH), 3.13 (m, 2 H; OCH'), 2.11 (q, <sup>3</sup>J = 7.0 Hz, 4 H; O(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>), 2.06 (q, <sup>3</sup>J = 7.0 Hz, 4 H; O(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>'), 1.76 (m, 4 H; OCH<sub>2</sub>CH<sub>2</sub>), 1.66-1.40 (m, 12 H; OCH<sub>2</sub>CH<sub>2</sub>'), O(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>), 1.13 (s, 18 H; *tert*-BuH). ESI-MS: calcd. for C<sub>92</sub>H<sub>95</sub>B<sub>2</sub>N<sub>6</sub>O<sub>4</sub>S<sub>2</sub> [M + H]<sup>+</sup> 1433.7 ; found 1432.9. Elemental analysis (%): C<sub>92</sub>H<sub>94</sub>B<sub>2</sub>N<sub>6</sub>O<sub>4</sub>S<sub>2</sub> (1433.52): calcd. C 77.08, H 6.61, N 5.86; found C 76.61, H 6.59, N 5.89.

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### BPh<sub>2</sub>-PPCy 5':

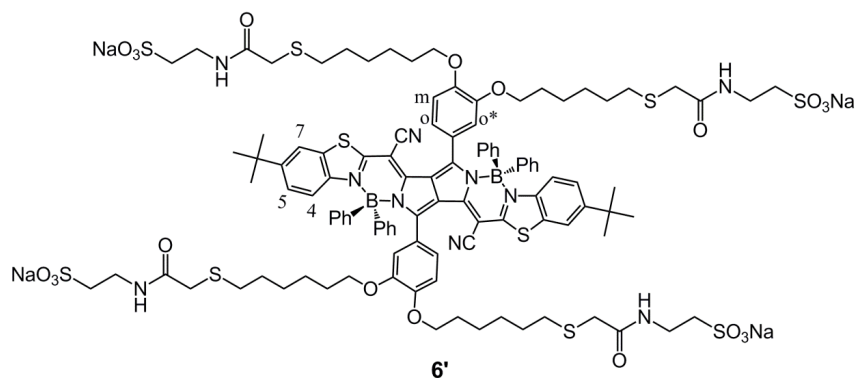


636 mg (440  $\mu$ mol) **4'** were dissolved in 30 ml absolute 1,4-dioxane under nitrogen. 0.92 ml (13.3 mmol) mercapto acetic acid and 72.9 mg (444  $\mu$ mol) azobisisobutyronitrile (AIBN) were added and the reaction mixture was heated under reflux for 4 h. After each of the first three hours additional 72.9 mg (444  $\mu$ mol) AIBN were added (1.78 mmol AIBN whole). 45 ml water were added and the precipitate was filtered off. The precipitate was suspended in water, treated in an ultrasonic bath and centrifuged (2 x). After drying **5'** was afforded as a green solid in 93 % yield (735 mg, 408  $\mu$ mol).

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  (ppm) = 12.49 (s, 4 H; COOH), 7.96 (s, 2 H; H-7), 7.41-7.03 (m, 22 H; BPhH, H-5), 6.85 (d, <sup>3</sup>J = 9.2 Hz, 2 H; H-4), 6.44 (d, <sup>3</sup>J = 8.3 Hz, 1 H; m-H), 6.43 (d, <sup>3</sup>J = 8.3 Hz, 1 H; m'-H), 6.02 (b, 2 H; o\*-H), 5.985 (d, <sup>3</sup>J = 8.3 Hz, 1 H; o-H), 5.965 (d, <sup>3</sup>J = 8.3 Hz, 1 H; o'-H), 3.93 (t, <sup>3</sup>J = 5.8 Hz, 4 H, OCH<sub>2</sub>), 3.40 (b, 4 H; OCH, OCH'), 3.24 (s, 4 H; SCH<sub>2</sub>COOH), 3.213 (s, 2 H; SCHCOOH) 3.207 (s, 2 H; SCH'COOH), 2.62 (m, 8 H; SCH<sub>2</sub>), 1.73 (m, 4 H; OCH<sub>2</sub>CH<sub>2</sub>), 1.65-1.53 (m, 12 H; OCH<sub>2</sub>CH'<sub>2</sub>, SCH<sub>2</sub>CH<sub>2</sub>), 1.46 (m, 8 H; O(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>), 1.37 (m, 8 H; S(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>), 1.13 (s, 18 H; *tert*-BuH). ESI-MS: calcd. for C<sub>100</sub>H<sub>111</sub>B<sub>2</sub>N<sub>6</sub>O<sub>12</sub>S<sub>6</sub> [M + H]<sup>+</sup> 15 1801.7; found 1800.8.

### BPh<sub>2</sub>-PPCy 6':

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*Preparation of 4-fold NHS ester:* 730 mg (405  $\mu$ mol) **5'**, 835 mg (4.05 mmol) dicyclohexylcarbodiimide (DCC) and 466 mg (4.05 mmol) *N*-hydroxysuccinimide (NHS) were dissolved in 350 ml dichloromethane. The reaction mixture was stirred at room temperature for 3 25 days. The solvent was removed in vacuum. The crude product was suspended in methanol, treated in an ultrasonic bath and filtered off (2 x). The so prepared NHS ester (805 mg) was dried.

*Preparation of 6':* 283 mg (1.93 mmol) 2-aminoethanesulfonic acid sodium salt was suspended in 40 ml DMF and treated in an ultrasonic bath for several minutes. 703 mg (321  $\mu$ mol) of the NHS ester was added. The reaction mixture was stirred at room temperature until only product peaks in the ESI mass spectra were found (*m/z* = 749.6, 556.4; around 50 min). 40 ml CH<sub>2</sub>Cl<sub>2</sub> were added 30 and the precipitate was filtered off. The precipitate was dissolved in a little amount of water and purified twice by reversed phase C-18 column chromatography (H<sub>2</sub>O:MeOH 1:2 to H<sub>2</sub>O:MeOH 1:3) to afford **6'** as a green solid in 21 % yield (156 mg, 67  $\mu$ mol).

<sup>1</sup>H NMR (400 MHz, MeOD-*d*<sub>4</sub>): δ (ppm) = 7.76 (d, <sup>4</sup>J = 1.6 Hz, 2 H; H-7), 7.64-7.08 (m, 20 H; BPh-H), 7.14 (dd, <sup>3</sup>J = 9.2 Hz, <sup>4</sup>J = 1.6 Hz, 2 H; H-5), 6.96 (d, <sup>3</sup>J = 9.2 Hz, 2 H; H-4), 6.44 (d, <sup>3</sup>J = 8.2 Hz, 1 H; m-H), 6.43 (d, <sup>3</sup>J = 8.2 Hz, 1 H; m'-H), 6.195 (b, 1 H; o\*-H), 6.175 (b, 1 H; o\*-H), 6.08 (dd, <sup>3</sup>J = 8.2 Hz, <sup>4</sup>J = 1.5 Hz, 2 H; o-H), 4.00 (t, <sup>3</sup>J = 6.4 Hz, 4 H; OCH<sub>2</sub>), 3.66 (t, <sup>3</sup>J = 6.8 Hz, 4 H; CONHCH<sub>2</sub>), 3.63 (t, <sup>3</sup>J = 6.8 Hz, 4 H; CONHCH'<sub>2</sub>), 3.55 (m, 2 H; OCH), 3.29 (m, 2 H; OCH'), 3.23 (s, 4 H; SCH<sub>2</sub>CONH), 3.19 (s, 2 H; SCHCONH), 3.17 (s, 2 H; SCH'CONH), 2.99 (t, <sup>3</sup>J = 6.8 Hz, 4 H; CH<sub>2</sub>SO<sub>3</sub>Na), 2.97 (t, <sup>3</sup>J = 6.8 Hz, 4 H; CH'<sub>2</sub>SO<sub>3</sub>Na), 2.66 (t, <sup>3</sup>J = 7.2 Hz, 4 H; SCH<sub>2</sub>), 2.61 (t, <sup>3</sup>J = 6.9 Hz, 2 H; SCH), 2.59 (t, <sup>3</sup>J = 6.9 Hz, 2 H; SCH'), 1.82 (m, 4 H; OCH<sub>2</sub>CH<sub>2</sub>), 1.74-1.60 (m, 12 H; OCH<sub>2</sub>CH'<sub>2</sub>, SCH<sub>2</sub>CH<sub>2</sub>), 1.55 (m, 8 H; O(CH<sub>2</sub>)<sub>2</sub>CH<sub>2</sub>), 1.45 (m, 8 H; S(CH<sub>2</sub>)<sub>2</sub>CH'<sub>2</sub>), 1.23 (s, 18 H; *tert*-BuH). HR ESI-MS: calcd. for C<sub>108</sub>H<sub>126</sub>B<sub>2</sub>N<sub>10</sub>O<sub>20</sub>S<sub>10</sub> [M - 4 Na]<sup>+</sup> 556.4144; found 556.4180; Elemental analysis (%): C<sub>108</sub>H<sub>126</sub>B<sub>2</sub>N<sub>10</sub>Na<sub>4</sub>O<sub>20</sub>S<sub>10</sub> · 10 H<sub>2</sub>O (2498.59): calcd. C 51.92, H 5.89, N 5.61; found C 51.80, H 5.76, N 6.07.

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## Cell imaging

5·10<sup>5</sup> CHO cells (wild-type Chinese Hamster Ovary) were seeded in a 35 mm μ-dish (Ibidi, Planegg/Martinsried, Germany) and incubated at 37 °C and 5 % CO<sub>2</sub> overnight. They were grown in Eagle's minimum essential medium (Gibco-Invitrogen, Carlsbad, CA) supplemented with 8 % heat inactivated fetal bovine serum (Gibco), 0.584 g/l L-glutamine (Gibco), 3.5 g/l D-glucose (Sigma, St. Louis, MO), 2.95 g/l tryptose-phosphate (Sigma), 100 U/ml penicillin (Gibco), 100 mg/l streptomycin (Gibco) and BME vitamins (Sigma). **6''** was dissolved in Milli-Q water (1 mM). Cells were incubated with 1 μM of **6''** in culture medium for 30 minutes at 37 °C. Cells were washed three times with 1 ml PBS (Gibco) and 1 ml of colorless culture medium (without phenol red; prevent possible disturbance of fluorescence measurements) was added. Observations were performed using a scanning confocal microscope (Axiovert 200, Zeiss, Jena, Germany) equipped with a piezo stage in z-scan mode. **6''** was excited using a pulsed laser at 690 nm (LDH-P-C-690, Picoquant, Berlin, Germany; repetition rate: 10 MHz). Excitation and fluorescence signals were separated using a dichroic mirror (z690RDC, Chroma Technology Corp., Bellows Falls, VT) and a 770 nm long-pass filter (HQ770LP, Chroma). Fluorescence was detected with an avalanche photodiode (SPCM-AQR-14, Perkin Elmer, Dumberry, Canada). Fluorescence lifetime imaging data were simultaneously collected by employing a TCSPC module (HydraHarp 400, Picoquant) with an integrated histogrammer in the TTTR mode. The emission spectra of **6''** inside living CHO cells were collected using a dispersion prism (PS 857, Thorlabs Inc., Newton, NJ) and a CCD camera (DU970N-BV, Andor Technology, Belfast, UK).

For the incubation experiments at 4 °C about 5·10<sup>5</sup> CHO cells were cultured overnight. The next day the cells were kept at 4 °C for 10 minutes to inhibit any cell-driven internalization processes (*e.g.* endocytosis). The cells were then incubated with a cooled culture medium containing 100 μM of **6''** and kept at 4 °C for 30 minutes. The cells were washed three times with PBS (1 ml, 4 °C) and fixed with formalin (4 % PFA in PBS, 1 ml, 4 °C). The cells were washed again three times with 1 ml of PBS. To avoid drying of the sample PBS was added. Observations were performed using the same microscopy setup as described above. For the control experiments, incubation with **6''** was carried out at 37 °C for 30 minutes without prior incubation at 4 °C. Washing and cell fixation was performed at room temperature.

## S2: Supplemented spectroscopic data

**Supp. Table 1** Supplemented spectroscopic data of BPh<sub>2</sub>-PPCys **6'** and **6''** in different solvents.  $\lambda_{max}^A$ : wavelength of the absorption maximum,  $\tilde{\nu}_{max}^A$ : wavenumber of the absorption maximum,  $\Delta\lambda_{1/2}^A$ : half width of the 00 absorption band in nm,  $\Delta\tilde{\nu}_{1/2}^A$ : half width of the 00 absorption band in cm<sup>-1</sup>.  $\lambda_{max}^F$ : wavelength of the fluorescence maximum,  $\tilde{\nu}_{max}^F$ : wavenumber of the fluorescence maximum,  $\Delta\lambda_{1/2}^F$ : half width of the 00 emission band in nm,  $\Delta\tilde{\nu}_{1/2}^F$ : half width of the 00 emission band in cm<sup>-1</sup>. *f*: oscillator strength,  $\epsilon_{max}$ : molar decadic absorption coefficient of the absorption maximum,  $\Phi_F$ : fluorescence quantum yield,  $\epsilon_{max} \cdot \Phi_F$ : optical brightness.

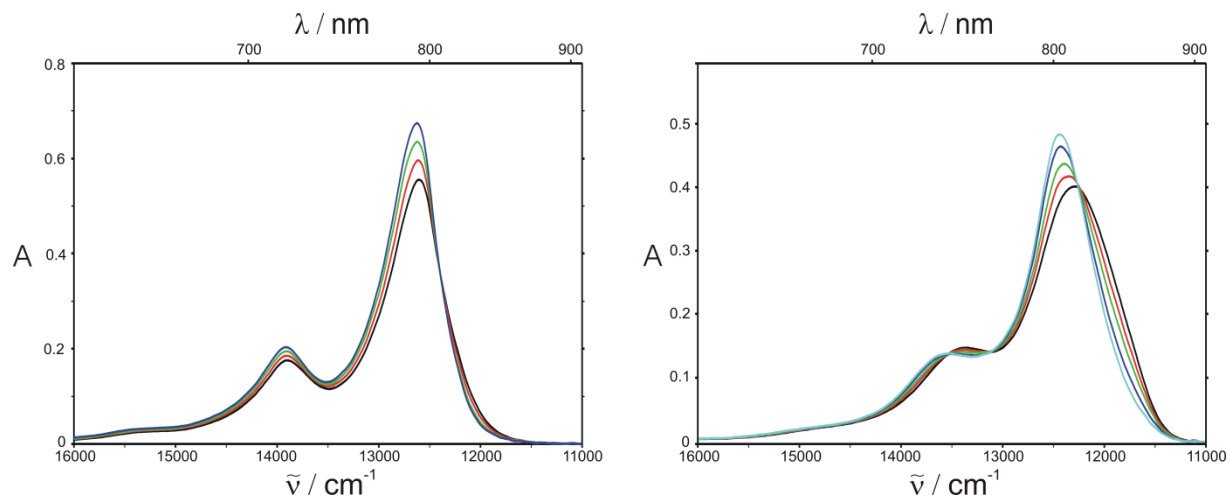
		$\lambda_{max}^A$	$\tilde{\nu}_{max}^A$	$\Delta\lambda_{1/2}^A$	$\Delta\tilde{\nu}_{1/2}^A$	$\lambda_{max}^F$	$\tilde{\nu}_{max}^F$	$\Delta\lambda_{1/2}^F$	$\Delta\tilde{\nu}_{1/2}^F$	<i>f</i>	$\epsilon_{max}^a$	$\Phi_F$	$\epsilon_{max} \cdot \Phi_F$
		[nm]	[cm <sup>-1</sup> ]	[nm]	[cm <sup>-1</sup> ]	[nm]	[cm <sup>-1</sup> ]	[nm]	[cm <sup>-1</sup> ]		[M <sup>-1</sup> cm <sup>-1</sup> ]		[M <sup>-1</sup> cm <sup>-1</sup> ]
<b>6'</b>	DMSO	794	12594	35	548	805	12422	45	698	0.66	171000	0.27	46000
	MeOH	782	12788	36	575	800	12500	44	706	0.68	166000	0.20	33000
	H <sub>2</sub> O	793	12610	43	650	800	12500	60	959	0.61	125000	0.01	1000
<b>6''</b>	DMSO	825	12121	33	485	840	11905	45	645	0.66	195000	0.34	66000
	MeOH	810	12346	35	539	825	12121	44	658	0.68	183000	0.25	46000
	H <sub>2</sub> O	805 <sup>b</sup>	12422	56	859	825	12121	59	894	0.62	110000	0.01	1000

<sup>a</sup>values corrected for ten and three molecules of water per formula unit for **6'** and **6''**, respectively (*cf.* general remarks in experimental section, ESI); <sup>b</sup>can range from 805 to 820 nm (conformation/solvation effect; see text).

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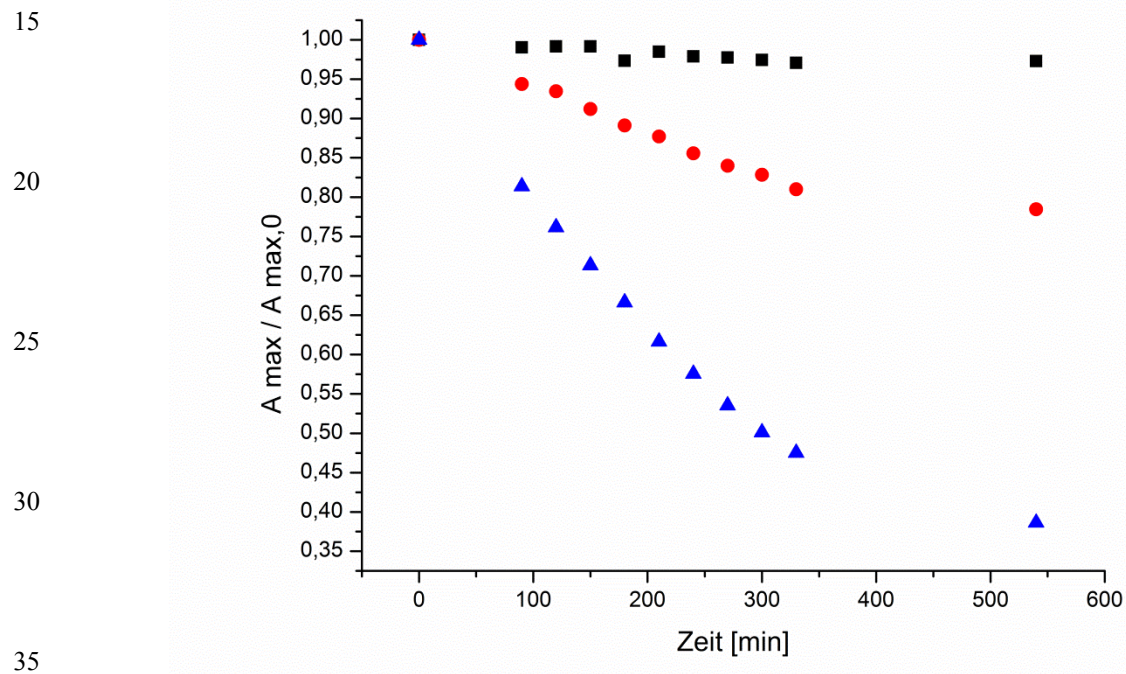


### S3: Time-dependent change in absorption spectra of 6' and 6'' in water



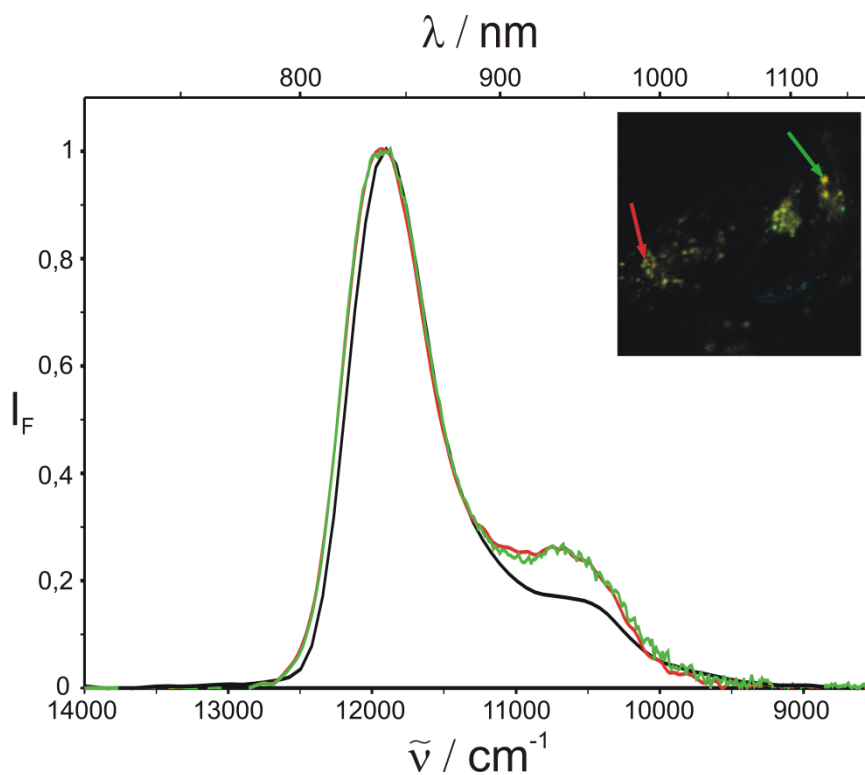
5 **Supp. Figure 1** Change in absorption spectra of benzothiazole substituted BPh<sub>2</sub>-PPCy 6' (left) and chinoline substituted BPh<sub>2</sub>-PPCy 6'' (right) over a time span of several hours (order: black, red, green, dark blue, light blue) after dissolving the compounds in water.

### 10 S4: Photostability of 6''



**Supp. Figure 2** Absorbance change of 6'' in water (circles), compound 10e from reference <sup>12a</sup> in chloroform (squares) and ICG in water (triangles) after exposition to daylight.

## S5: In-cell emission of 6''



5 **Supp. Figure 3** Normalized fluorescence spectra of 6'' in DMSO solution (black) and inside of endocytotic vesicles (red and green) marked in the FLIM inset. (The emission spectra and the fluorescence lifetime image are exemplary. For evaluation purposes a series of emission spectra and FLIM data were collected.)

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## References

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- 15 19 K. R. Bley and B. Jandeleit, WO 2013/044064 A1, 2013.