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Supporting Information

Water-soluble Pyrrolopyrrole Cyanine (PPCy) near-infrared fluorescent pH indicators for strong acidity†

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I: Experimental section

General

Solvents were purified and dried according to standard procedures. All commercially available reagents were used without further purification unless otherwise noted. Column chromatography was performed on Roth silica gel 60 (40–63 µm). 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 ($CHCl_3$: d = 7.26 ppm; C_2DHCl_4: d = 5.91 ppm). For ^{1}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. MALDI-TOF mass spectra were recorded on a Bruker Microflex. Elemental analysis was performed with a CHN analyser Vario EL from Elementar. For the titration experiments the pH values were determined with a pH meter "Lab 850" from Schott Instruments. 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.[1] 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 **7'** and **8''**: 690 nm, 19 mW, model ACM19/1203, Power Technology, Little Rock, AR; for **8'** and **9'**: 804 nm, 30 mW, model ACM30/1476, Power Technology) were used for excitation. BF_2-PPCy **10e** (Φ_F = 0.59 in $CHCl_3$; for **7'** and **8''**) and BPh_2–PPCy **11e** (Φ_F = 0.53 in $CHCl_3$; for **8'** and **9'**) from reference [2] were used as standards to determine the quantum yields. The syntheses of aminophenylsubstituted DPP **1** and chlorodiphenylborane (BPh_2Cl) were published previously.[2-3] The synthesis of the heteroaryl acetonitrile **2'** was performed as published by Zhou *et al*.[4]

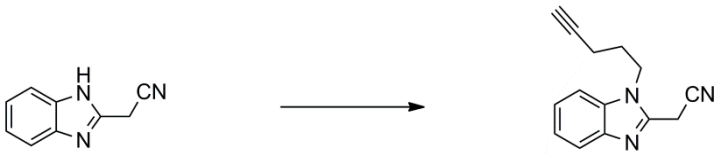
Chlorodi-*n*-hexylborane ($BHex_2Cl$)

Under nitrogen 3.73 ml (30 mmol) 1-hexene and 30 ml (30 mmol) of a 1 M solution of BCl_3 in heptane were cooled to -78 °C. 4.79 ml (30 mmol) triethylsilane were added and stirred under cooling for 15 min. The mixture was allowed to warm to room temperature and stirred for another 15 min. After cooling again to -78 °C another 3.72 ml (30 mmol) 1-hexene and 4.79 ml (30 mmol) triethylsilane were added and stirred under cooling for 15 min. The mixture was allowed to warm to room temperature and stirred for 26 hours. The heptane, triethylsilylchloride and byproducts were removed at 60 °C in vacuum. $BHex_2Cl$ was obtained as a colorless liquid.
Yield: 4.01 g (19 mmol, 63%)
^{1}H-NMR (400 MHz, $CDCl_3$): δ / ppm = 1.55-1.40 (m, 4 H; BCH_2) , 1.39–1.19 (m, 16 H; $BCH_2(CH_2)_4$), 0.88 (t, ^{3}J = 7 Hz, 6 H; CH_3);
^{11}B-NMR (400 MHz, $CDCl_3$): δ / ppm = 79.47 (s; *B*Hex_2Cl).

5-Iodo-pent-1-yne[5]

10.4 ml (97.50 mmol) 5-chloro-pent-1-yne and 71.0 g (473.68 mmol) NaI were dissolved in 150 ml acetone and heated to reflux for 21 h. After cooling to room temperature the reaction mixture was filtrated and 100 ml water were added to the filtrate. The aqueous phase was extracted with diethylether (4x100 ml). The combined organic phases were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was obtained as a clear, yellow liquid.
yield: 15.51 g (79.94 mmol, 82%)
^{1}H-NMR (400 MHz, $CDCl_3$): δ / ppm = 3.30 (t, ^{3}J = 6.8 Hz, 2 H; ICH_2), 2.33 (dt, ^{3}J = 6.8 Hz, ^{4}J = 2.7 Hz, 2 H; $I(CH_2)_2CH_2$), 2.00 (qui, ^{3}J = 6.8 Hz, 2 H; ICH_2CH_2), 1.99 (t, ^{4}J = 2.7 Hz, 1 H; CH_2CCH).

2-(1-(Pent-4-yne-1-yl)benzimidazol-2-yl)acetonitrile 2''



0.50 g (3.18 mmol) 2-(1H-Benzo[d]imidazol-2-yl)acetonitrile and 1.23 g (6.36 mmol) 5-Iodo-pent-1-yne were heated to 125 °C in 40 minutes. After addition of 0.5 ml abs. ethanol the reaction mixture was heated to reflux. After 1 h 0.4 ml abs. ethanol were added and the mixture was heated to reflux for another 2 h. The product was purified by column chromatography (chloroform) to obtain colorless needles.

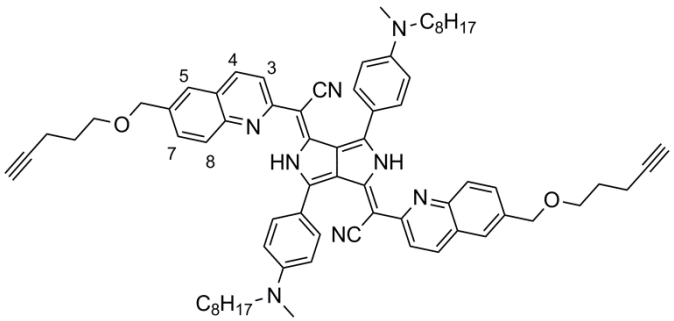
yield: 0.15 g (0.69 mmol, 22%)

^{1}H-NMR (400 MHz, $CDCl_3$): δ / ppm = 7.77 (m, 1 H; benzimidazole), 7.43 (m, 1 H; benzimidazole), 7.32 (m, 2 H; benzimidazole), 4.39 (t, ^{3}J = 6.8 Hz, 2 H; NCH_2), 4.17 (s, 2 H; CH_2CN), 2.26 (dt, ^{3}J = 6.8, ^{4}J = 2.7 Hz, 2 H; $N(CH_2)_2CH_2$), 2.17 (t, ^{4}J = 2.7 Hz, 1 H; CH_2CCH), 2.11 (qui, ^{3}J = 6.8 Hz, 2 H; NCH_2CH_2).

MALDI-MS: calc. for $C_{14}H_{14}N_3[M+H]^+$ 224.1; found 224.2;

elemental analysis: calc. [%] for $C_{14}H_{13}N_3$ (M = 223.27 g/mol): C 75.31, H 5.87, N 18.82; found.: C 75.10, H 6.06, N 18.61.

H-PPCy 3'



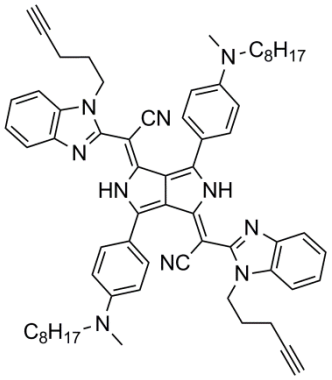
400 mg (0.701 mmol) DPP **1** (1 eq) and 463 mg (1.752 mmol) heteroarylacetonitrile **2'** (2.5 eq) were heated to reflux in 40 ml of anhydrous toluene under nitrogen. 859 mg (5.61 mmol, 512 µl) phosphoryl chloride (8 eq) were then added. The reaction was monitored by UV/Vis/NIR spectroscopic analysis (neutralization with aqueous $NaHCO_3$ solution) and thin-layer chromatography. As soon as either **1** was used up or the concentration of the short-wavelength absorbing byproducts increased, 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 $NaHCO_3$ 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 dark violet solid.

yield: 410 mg (0.386 mmol, 55%)

^{1}H-NMR (400 MHz, $C_2D_2Cl_4$): δ /ppm = 14.65 (s, 2 H; NH), 7.98 (d, ^{3}J = 8.9 Hz, 2H; quinoline), 7.79 (d, ^{3}J = 8.9 Hz, 2 H; quinoline), 7.75-7.60 (m, 10 H; 6 H quinoline, 4 H AA'), 6.86 (m, 4 H; XX'), 4.60 (s, 4 H; $HCC(CH_2)_3OCH_2$), 3.57 (t, ^{3}J = 6.2 Hz, 4 H; $HCC(CH_2)_2CH_2O$), 3.39 (t, ^{3}J = 6.8 Hz, 4 H; NCH_2), 3.06 (s, 6 H; NCH_3), 2.30 (dt, ^{3}J = 7.1 Hz, ^{4}J = 2.5 Hz, 4 H; $HCCCH_2$), 1.93 (t, ^{4}J = 2.5 Hz, 2 H; $HCC(CH_2)_3O$), 1.81 (qui, ^{3}J = 6.6 Hz, 4 H; $HCCCH_2CH_2CH_2O$), 1.61 (qui, ^{3}J = 6.8 Hz, 4 H; NCH_2CH_2), 1.38-1.15 (m, 20 H; $N(CH_2)_2(CH_2)_5$), 0.82 (t, ^{3}J = 6.8 Hz, 6 H; $N(CH_2)_7CH_3$).

MALDI-MS: calc. for $C_{70}H_{79}N_8O_2$ $[M+H]^+$ 1063.6; found 1063.7.

H-PPCy 3''

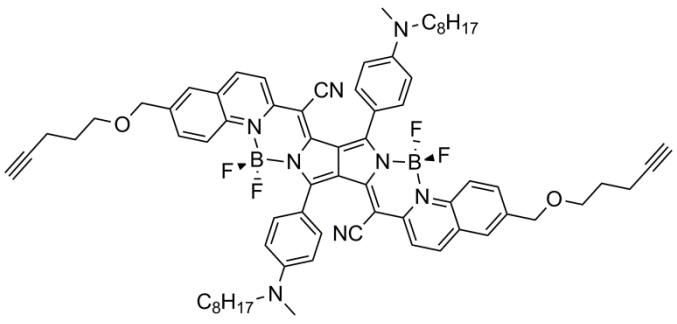


409 mg (0.72 mmol) DPP **1** (1 eq) and 400 mg (1.79 mmol) heteroarylacetonitrile **2''** (2.5 eq) were heated to reflux in 35 ml of anhydrous toluene under nitrogen. 890 mg (5.81 mmol, 530 µl) phosphoryl chloride (8 eq) were then added. The reaction was monitored by UV/Vis/NIR spectroscopic analysis (neutralization with aqueous $NaHCO_3$ solution) and thin-layer chromatography. As soon as either **1** was used up or the concentration of the short-wavelength absorbing byproducts increased, 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 $NaHCO_3$ 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 violet solid.
yield: 379 mg (0.39 mmol, 54%)
^{1}H-NMR (400 MHz, $C_2D_2Cl_4$): δ / ppm = 13.60 (s, 2 H; NH), 7.62 (m, 6 H; 4 H AA', 2 H benzimidazole), 7.35 (m, 2 H; benzimidazole), 7.21 (m, 4 H; benzimidazole), 6.80 (m, 4 H; XX'), 4.61 (t, ^{3}J = 6.8 Hz, 4 H; NC*H*$_2$(CH$_2$)$_2$CCH), 3.40 (t, ^{3}J = 6.8 Hz, 4 H; NCH$_2$), 3.05 (s, 6 H; NCH$_3$), 2.30 (dt, ^{3}J = 6.8 Hz, ^{4}J = 2.4 Hz, 4 H; HCCC*H*$_2$), 2.04 (t, ^{4}J = 2.4 Hz, 2 H; *H*CC(CH$_2$)$_3$N), 1.81 (qui, ^{3}J = 6.6 Hz, 4 H; HCCCH$_2$C*H*$_2$CH$_2$N), 1.66 (qui, ^{3}J = 6.7 Hz, 4 H; NCH$_2$C*H*$_2$), 1.42-1.26 (m, 20 H; N(CH$_2$)$_2$(C*H*$_2$)$_5$), 0.89 (t, ^{3}J = 6.8 Hz, 6 H; N(CH$_2$)$_7$C*H*$_3$);
MALDI-MS: calc. for $C_{64}H_{73}N_{10}$ $[M+H]^+$ 981.6; found 981.7.

BR_2-PPCys 4-6

General procedure:
Under nitrogen 1 eq of H-PPCy **3** and 20 eq (for reaction with $BF_3{\cdot}Et_2O$) or 3 eq (for reaction with BPh_2Cl or $BHex_2Cl$) *N*,*N*-diisopropylethylamine (DIPEA) were heated to reflux in CH_2Cl_2. 40 eq $BF_3{\cdot}Et_2O$ or 4.6 eq BPh_2Cl or 9.2 eq $BHex_2Cl$, respectively, were added and the mixture was heated to reflux until the reaction was complete (UV/Vis/NIR). The mixture was washed with saturated, aqueous $NaHCO_3$ solution and dried over $MgSO_4$. After removal of the solvent, the crude product was purified by column chromatography (CH_2Cl_2 or mixtures with EtOAc).

BF_2-PPCy 4'



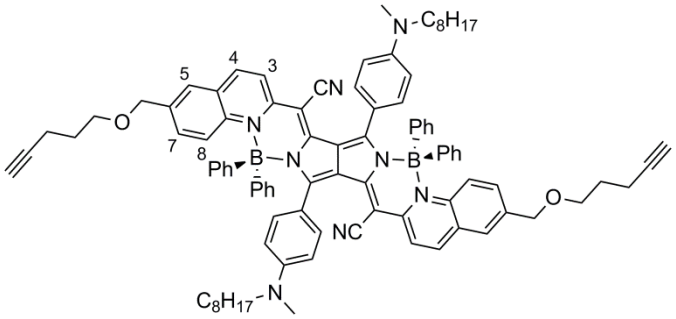
Reaction batch:

PPCy **3'**	150 mg	141 µmol	
$BF_3{\cdot}Et_2O$	801 mg	5.64 mmol	709 µl
DIPEA	364 mg	282 mmol	466 µl
CH_2Cl_2			25 ml

Column chromatography (gradient from CH_2Cl_2 to CH_2Cl_2:EtOAc, 100:1) yielded **4'** as a dark blue solid in 52% yield (85 mg, 73 µmol).

^{1}H-NMR (400 MHz, $C_2D_2Cl_4$): δ / ppm = 8.45 (b, 2 H; quinoline), 8.00 (d, ^{3}J = 9.1 Hz, 2 H; quinoline), 7.73 (m, 4 H, AA'), 7.67-7.53 (m, 6 H; quinoline), 6.76 (m, 4 H, XX'), 4.56 (s, 4 H; HCC$(CH_2)_3$OCH_2), 3.54 (t, ^{3}J = 5.8 Hz, 4 H; HCC$(CH_2)_2$CH_2O), 3.36 (t, ^{3}J = 6.8 Hz, 4 H; NCH_2), 3.03 (s, 6 H; NCH_3), 2.30 (dt, ^{3}J = 7.1 Hz, ^{4}J = 2.5 Hz, 4 H; HCCCH_2), 1.91 (t, ^{4}J = 2.5 Hz, 2 H; *H*CC$(CH_2)_3$O), 1.81 (qui, ^{3}J = 6.4 Hz, 4 H; HCCCH_2CH_2CH_2O), 1.61 (b, 4 H; NCH_2CH_2), 1.38-1.13 (m, 20 H; N$(CH_2)_2(CH_2)_5$), 0.83 (t, ^{3}J = 6.5 Hz, 6 H; N$(CH_2)_7CH_3$).
MALDI-MS: calc. for $C_{70}H_{77}B_2F_4N_8O_2$ $[M+H]^+$ 1159.6; found 1158.7;
elemental analysis: calc. [%] for $C_{70}H_{76}B_2F_4N_8O_2$ (M = 1159.02 g/mol): C 72.54, H 6.61, N 9.67; found: C 72.44, H 6.99, N 9.71.

BPh_2-PPCy 5'



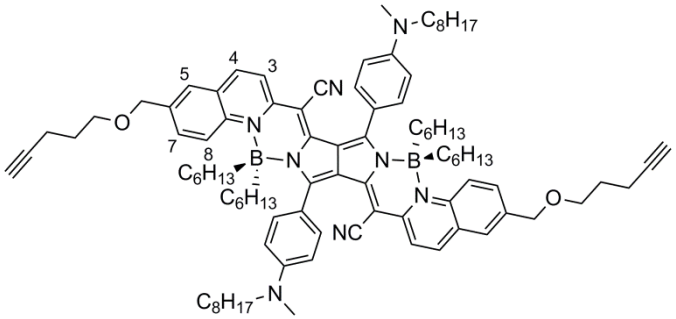
Reaction batch:

PPCy **3'**	370 mg	348 µmol	
BPh_2Cl	321 mg	1.60 mmol	287 µl
DIPEA	135 mg	1.04 mmol	173 µl
CH_2Cl_2			60 ml

Column chromatography (CH_2Cl_2) yielded **5'** as a turquoise solid in 32% yield (155 mg, 111 µmol).

^{1}H-NMR (400 MHz, $C_2D_2Cl_4$): δ / ppm = 8.06 (d, ^{3}J = 9.4 Hz, 2 H; H-8), 7.72 (d, ^{3}J = 9.2 Hz, 2 H; H-4), 7.53 (d, ^{3}J = 9.2 Hz, 2 H; H-3), 7.28 (s, 2 H; H-5), 7.24 (m, 8 H; BPhH), 7.01 (m, 12 H; BPhH), 6.95 (d, ^{3}J = 9.4 Hz, 2 H; H-7), 6.26 (m, 4 H; XX'), 5.96 (m, 4 H; AA'), 4.27 (s, 4 H; HCC$(CH_2)_3$OCH_2), 3.40 (t, ^{3}J = 6.1 Hz, 4 H; HCC$(CH_2)_2$CH_2O), 3.33 (t, ^{3}J = 7.1 Hz, 4 H; NCH_2), 2.94 (s, 6 H; NCH_3), 2.16 (dt, ^{3}J = 7.1 Hz, ^{4}J = 2.5 Hz, 4 H; HCCCH_2), 1.80 (t, ^{4}J = 2.5 Hz, 2 H; *H*CC$(CH_2)_3$O), 1.66 (m, 8 H; 4 H NCH_2CH_2, 4 H HCCCH_2CH_2CH_2O), 1.40-1.22 (m, 20 H; N$(CH_2)_2(CH_2)_5$), 0.85 (t, ^{3}J = 6.6 Hz, 6 H; N$(CH_2)_7CH_3$).
MALDI-MS: calc. for $C_{94}H_{97}B_2N_8O_2$ $[M+H]^+$ 1392.8; found 1391.3;
elemental analysis: calc. [%] for $C_{94}H_{96}B_2N_8O_2$ (M = 1391.44 g/mol): C 81.14, H 6.95, N 8.05; found: C 80.87, H 7.35, N 8.03.

$BHex_2$-PPCy 6‘



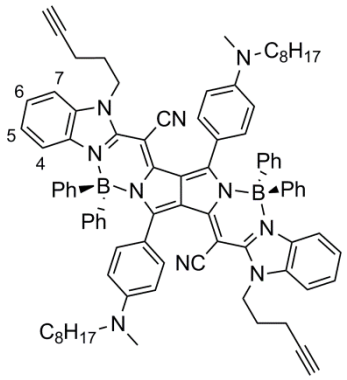
Reaction batch:

PPCy **3‘**	100 mg	94 µmol	
$BHex_2Cl$	187 mg	865 µmol	
DIPEA	37 mg	282 µmol	47 µl
CH_2Cl_2			20 ml

Column chromatography (CH_2Cl_2) yielded **6‘** as a dark blue solid in 43% yield (57 mg, 40 µmol).

^{1}H-NMR (400 MHz, $C_2D_2Cl_4$): δ / ppm = 8.37 (d, ^{3}J = 9.2 Hz, 2 H; H-8), 7.77 (d, ^{3}J = 9.2 Hz, 2 H; H-4), 7.48 (m, 4 H; H-3, H-5), 7.35 (d, ^{3}J = 9.2 Hz, 2 H; H-7), 7.29 (m, 4 H; AA'), 6.67 (m, 4 H; XX'), 4.50 (s, 4 H; $HCC(CH_2)_3OCH_2$), 3.54 (t, ^{3}J = 6.1 Hz, 4 H; $HCC(CH_2)_2CH_2O$), 3.32 (t, ^{3}J = 7.1 Hz, 4 H; NCH_2), 2.95 (s, 6 H; NCH_3), 2.26 (dt, ^{3}J = 7.1 Hz, 4J = 2.5 Hz, 4 H; $HCCCH_2$), 1.90 (t, ^{4}J = 2.5 Hz, 2 H; $HCC(CH_2)_3O$), 1.77 (qui, ^{3}J = 6.6 Hz, 4 H; $HCCCH_2CH_2CH_2O$), 1.57 (m, 4 H; NCH_2CH_2), 1.33-1.15 (m, 20 H; $N(CH_2)_2(CH_2)_5$), 1.11-0.87 (m, 32 H; $BCH_2(CH_2)_4$), 0.81 (t, ^{3}J = 6.8 Hz, 6 H; $N(CH_2)_7CH_3$), 0.69 (t, ^{3}J = 7.2 Hz, 12 H; $B(CH_2)_5CH_3$), 0.64-0.46 (m, 8 H; BCH_2).
MALDI-MS: calc. for $C_{94}H_{129}B_2N_8O_2$ $[M+H]^+$ 1425.1; found 1424.2;
elemental analysis: calc. [%] for $C_{94}H_{128}B_2N_8O_2$ (M = 1423.70 g/mol): C 79.30, H 9.06, N 7.87; found: C 79.01, H 9.63, N 7.91.

BPh_2-PPCy 5‘‘



Reaction batch:

PPCy **3‘‘**	126 mg	130 µmol	
BPh_2Cl	118 mg	0.59 mmol	106 µl
DIPEA	52 mg	0.40 mmol	67 µl
CH_2Cl_2			15 ml

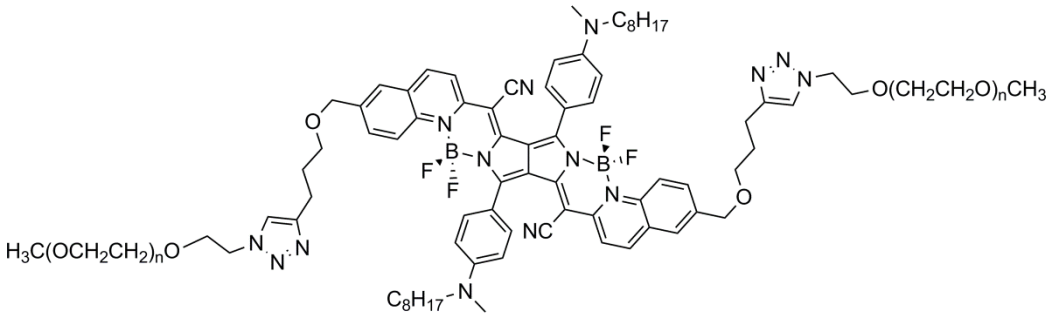
Digesting the crude product in MeOH (*i. e.* suspending in MeOH, treating in an ultrasonic bath, heating and filtrating several times) yielded **5''** as a blue solid in 80% yield (127 mg, 97 µmol).

^{1}H-NMR (400 MHz, $C_2D_2Cl_4$, 100 °C): δ / ppm = 7.25 (m, 8 H; BPhH), 7.15 (d, ^{3}J = 7.5 Hz, 2 H; H-4), 7.10-6.99 (m, 14; 12 H BPhH, 2 H H-5), 6.82 (t, ^{3}J = 8 Hz, 2 H; H-6), 6.62 (m, 4 H; AA'), 6.43 (d, ^{3}J = 8 Hz, 2 H; H-7) 6.19 (m, 4 H; XX'), 4.37 (t, ^{3}J = 6.8 Hz, 4 H; HCC$(CH_2)_2$CH_2N), 3.20 (t, ^{3}J = 7.1 Hz, 4 H; NCH_2), 2.84 (s, 6 H; NCH_3), 2.14 (b, 4 H; HCCCH_2), 2.00-1.86 (m, 6 H; 4 H HCCCH_2CH_2CH$_2$N, 2 H *H*CCCH_2), 1.55 (b, 4 H; NCH_2CH_2), 1.40-1.24 (m, 20 H; N$(CH_2)_2(CH_2)_5$), 0.89 (t, ^{3}J = 6.9 Hz, 6 H; N$(CH_2)_7CH_3$).
MALDI-MS: calc. for $C_{88}H_{91}B_2N_{10}$ $[M+H]^+$ 1309.8; found 1309.8.

PEG-BR_2-PPCys 7-9

General procedure:
Under nitrogen atmosphere 1 eq of BR_2-PPCy (**4**-**6**), 3 eq poly(ethylene glycol) methyl ether azide (PEG-azide, M_n ≈ 1000 g/mol, Sigma-Aldrich), 0.6 eq $CuSO_4 \cdot 5\ H_2O$ and 1.5 eq Na-L-(+)-ascorbat in degassed DMF were stirred at room temperature overnight. The solvent was removed in vacuo. The crude product was purified by column chromatography (mixtures of CH_2Cl_2 and MeOH). Excess PEG-azide could not be removed completely due to almost identical elution behavior.

PEG-BF_2-PPCy 7'



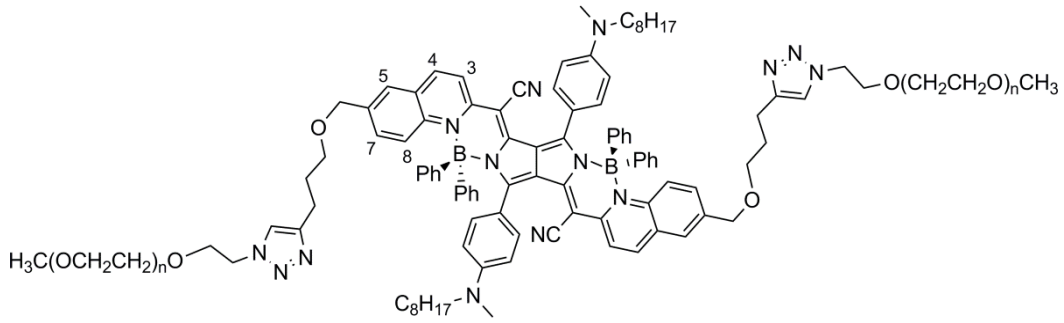
Reaction batch:

PPCy **4'**	40 mg	38 µmol
PEG-azide	113 mg	113 µmol
$CuSO_4 \cdot 5\ H_2O$	3.6 mg	23 µmol
Na-L-(+)-ascorbat	11.2 mg	56 µmol
degassed DMF		4 ml

Column chromatography (CH_2Cl_2:MeOH, 15:1) yielded **7'** as a blue, waxy solid (60 mg).

^{1}H-NMR (400 MHz, $C_2D_2Cl_4$): δ / ppm = 8.44 (b, 2 H; quinoline), 8.02 (d, ^{3}J = 9.3 Hz, 2 H; quinoline), 7.72 (m, 4 H, AA'), 7.65-7.55 (m, 6 H; quinoline), 7.39 (s, 2 H; triazole-H), 6.75 (m, 4 H, XX'), 4.56 (s, 4 H; CH_2O$(CH_2)_3$-triazole), 4.40 (t, ^{3}J = 5.2 Hz, 4 H; triazole-NCH_2CH$_2$OPEG), 3.76 (t, ^{3}J = 5.2 Hz, 4 H; triazole-NCH_2CH_2OPEG), 3.64-3.47 (m; PEG-chain), 3.45 (m, 4 H; OC$H_2(CH_2)_2$-triazole), 3.34 (t, ^{3}J = 5.4 Hz, 4 H; NCH_2CH_2), 3.27 (s, 6 H; PEG-OCH_3), 3.02 (s, 6 H; NCH_3), 2.74 (t, ^{3}J = 7.5 Hz, 4 H; O$(CH_2)_2$CH_2-triazole), 1.94 (qui, ^{3}J = 7.0 Hz, 4 H; $OCH_2CH_2CH_2$-triazole), 1.61 (b, 4 H; NCH_2CH_2), 1.34-1.18 (m, 20 H; N$(CH_2)_2(CH_2)_5$), 0.82 (t, ^{3}J = 6.7 Hz, 6 H; N$(CH_2)_7CH_3$).

PEG-BPh_2-PPCy 8‘



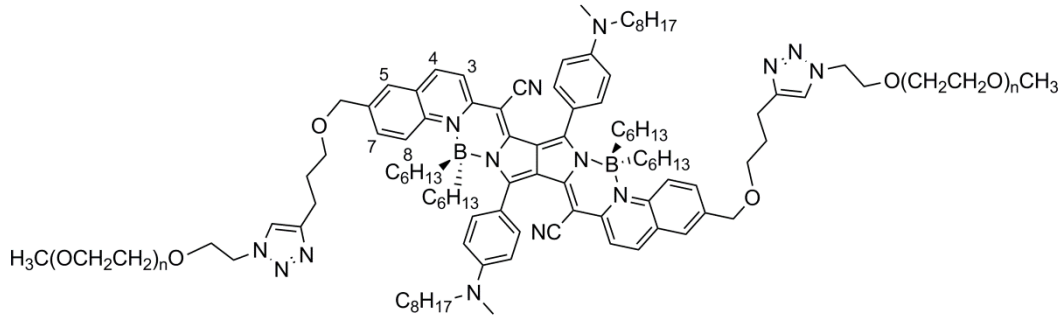
Reaction batch:

PPCy **5‘**	40 mg	29 µmol
PEG-azide	86 mg	86 µmol
$CuSO_4 \cdot 5\ H_2O$	2.8 mg	17.3 µmol
Na-L-(+)-ascorbat	8.5 mg	43 µmol
degassed DMF		4 ml

Column chromatography (CH_2Cl_2:MeOH, 15:1) yielded **8‘** as a turquoise, waxy solid (40 mg).

^{1}H-NMR (400 MHz, $C_2D_2Cl_4$, 100 °C): δ / ppm = 8.12 (d, ^{3}J = 9.5 Hz, 2 H; H-8), 7.73 (d, ^{3}J = 9.1 Hz, 2 H; H-4), 7.58 (d, ^{3}J = 9.1 Hz, 2 H; H-3), 7.37-7.26 (m, 12 H; 8 H BPhH, 2 H H-5, 2 H triazole-H), 7.03 (m, 12 H; BPhH), 6.95 (d, ^{3}J = 9.5 Hz, 2 H; H-7), 6.32 (m, 4 H; AA'), 6.08 (m, 4 H; XX'), 4.36 (t, ^{3}J = 5.4 Hz, 4 H; triazole-NC*H*$_2$CH$_2$OPEG), 4.31 (s, 4 H; C*H*$_2$O(CH$_2$)$_3$-triazole), 3.77 (t, ^{3}J = 5.4 Hz, 4 H; triazole-NCH$_2$C*H*$_2$OPEG), 3.70-3.46 (m; PEG-chain), 3.43 (t, ^{3}J = 6.5 Hz, 4 H; OC*H*$_2$(CH$_2$)$_2$-triazole), 3.34 (t, ^{3}J = 7.4 Hz, 4 H; NC*H*$_2$CH$_2$), 3.32 (s, 6 H; PEG-OCH$_3$), 2.97 (s, 6 H; NCH$_3$), 2.68 (t, ^{3}J = 7.5 Hz, 4 H; O(CH$_2$)$_2$C*H*$_2$-triazole), 1.89 (qui, ^{3}J = 7.0 Hz, 4 H; OCH$_2$C*H*$_2$CH$_2$-triazole), 1.69 (qui, ^{3}J = 6.5 Hz, 4 H; NCH$_2$C*H*$_2$), 1.57-1.31 (m, 20 H; N(CH$_2$)$_2$(C*H*$_2$)$_5$), 0.90 (t, ^{3}J = 6.7 Hz, 6 H; N(CH$_2$)$_7$C*H*$_3$).

PEG-$BHex_2$-PPCy 9‘



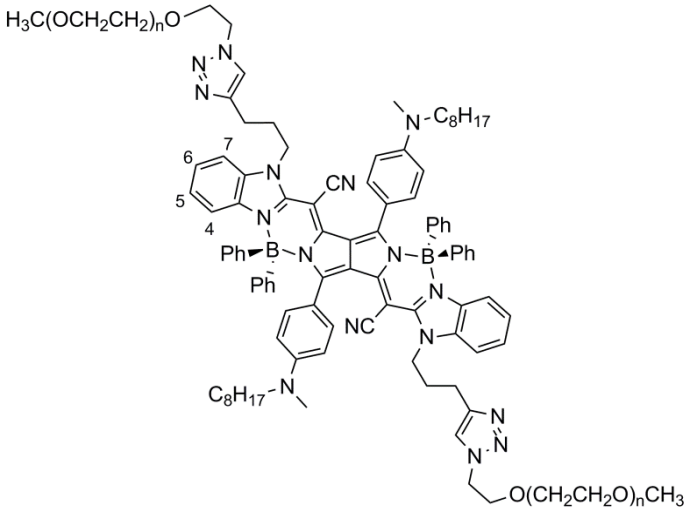
Reaction batch:

PPCy **6‘**	40 mg	28 µmol
PEG-azide	84 mg	84 µmol
$CuSO_4 \cdot 5\ H_2O$	2.7 mg	17 µmol
Na-L-(+)-ascorbat	8.3 mg	42 µmol
degassed DMF		4 ml

Column chromatography (CH_2Cl_2:MeOH, 15:1) yielded **9‘** as a turquoise, waxy solid (35 mg).

^{1}H-NMR (400 MHz, $C_2D_2Cl_4$): δ / ppm = 8.41 (d, ^{3}J = 8.6 Hz, 2 H; H-8), 7.83 (d, ^{3}J = 8.8 Hz, 2 H; H-4), 7.57-7.49 (m, 4 H; H-3, H-5), 7.43 (s, 2 H; triazole-H), 7.40 (d, ^{3}J = 8.6 Hz, 2 H; H-7), 7.33 (m, 4 H; AA'), 6.71 (m, 4 H; XX'), 4.54 (s, 4 H; C*H*$_2$O(CH$_2$)$_3$-triazole), 4.45 (t, ^{3}J = 5.1 Hz, 4 H; triazole-NC*H*$_2$CH$_2$OPEG), 3.81 (t, ^{3}J = 5.1 Hz, 4 H; triazole-NCH$_2$C*H*$_2$OPEG), 3.66-3.52 (m; PEG-chain), 3.50 (m, 4 H; OC*H*$_2$(CH$_2$)$_2$-triazole), 3.36 (m, 4 H; NC*H*$_2$CH$_2$), 3.32 (s, 6 H; PEG-OCH$_3$), 2.99 (s, 6 H; NCH$_3$), 2.78 (t, ^{3}J = 7.6 Hz, 4 H; O(CH$_2$)$_2$C*H*$_2$-triazole), 1.98 (qui, ^{3}J = 7.0 Hz, 4 H; OCH$_2$C*H*$_2$CH$_2$-triazole), 1.61 (b, 4 H; NCH$_2$C*H*$_2$), 1.34-1.20 (m, 20 H; N(CH$_2$)$_2$(C*H*$_2$)$_5$), 1.12-0.93 (m, 32 H; BCH$_2$(C*H*$_2$)$_4$), 0.85 (t, ^{3}J = 6.8 Hz, 6 H; N(CH$_2$)$_7$C*H*$_3$), 0.73 (t, ^{3}J = 7.2 Hz, 12 H; B(CH$_2$)$_5$C*H*$_3$), 0.66-0.50 (m, 8 H; BCH$_2$).

PEG-BPh$_2$-PPCy 8‘‘



Reaction batch:

PPCy **5‘‘**	60 mg	46 µmol	
PEG-azide	137 mg	137 µmol	
$CuSO_4 \cdot 5\ H_2O$	5 mg	31 µmol	
Na-L-(+)-ascorbat	17 mg	86 µmol	
degassed DMF			7 ml

Column chromatography (CH_2Cl_2:MeOH, 15:1) yielded **8’‘** as a blue, waxy solid (38 mg).

^{1}H-NMR (400 MHz, $C_2D_2Cl_4$): δ / ppm = 7.28 (s, 2 H; triazole-H), 7.22 (m, 8 H; BPhH), 7.08-6.96 (m, 16 H; 12 H BPhH, 2 H H-4, 2 H H-5), 6.78 (t, ^{3}J = 8 Hz, 2 H; H-6), 6.60 (m, 4 H; AA'), 6.32 (d, ^{3}J = 8 Hz, 2 H; H-7) 6.13 (m, 4 H; XX'), 4.36 (t, ^{3}J = 5.4 Hz, 4 H; triazole-NC*H*$_2$CH$_2$OPEG), 4.30 (t, ^{3}J = 6.8 Hz, 4 H; NC*H*$_2$(CH$_2$)$_2$-triazole), 3.73 (t, ^{3}J = 5.4 Hz, 4 H; triazole-NCH$_2$C*H*$_2$OPEG), 3.60-3.44 (m; PEG-chain), 3.28 (s, 6 H; PEG-OCH$_3$), 3.14 (t, ^{3}J = 6.8 Hz, 4 H; NC*H*$_2$CH$_2$) 2.77 (s, 6 H; NCH$_3$), 2.61 (t, ^{3}J = 7.1 Hz, 4 H; N(CH$_2$)$_2$C*H*$_2$-triazole), 2.05 (b, 4 H; NCH$_2$C*H*$_2$CH$_2$-triazole), 1.46 (b, 4 H; NCH$_2$C*H*$_2$), 1.32-1.19 (m, 20 H; N(CH$_2$)$_2$(C*H*$_2$)$_5$), 0.83 (t, ^{3}J = 6.9 Hz, 6 H; N(CH$_2$)$_7$C*H*$_3$).

Cell imaging

$5 \cdot 10^5$ CHO cells (wild-type Chinese Hamster Ovary) were seeded in a 35 mm µ-dish (Ibidi, Planegg, Germany) and incubated at 37 °C and 5 % CO_2 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). **7'** or **8'** was dissolved in Milli-Q water (~1 mM). Cells were incubated with 1 µM of **7'** or **8'** in culture medium for 30 minutes at 37 °C. Cells were washed three times with 1 ml PBS (Gibco) and fixed with formalin (4 % PFA in PBS, 1 ml). The cells were washed three times with 1 ml PBS and then treated with 1 ml Triton X-100 (c = 0.1 %) for 5 minutes at room temperature. The cells were washed again three times with 1 ml PBS and 1 ml of distilled H_2O was added. Observations were performed using a scanning confocal microscope (Axiovert 200, Zeiss, Jena, Germany) equipped with a piezo stage in z-scan mode. **7'** or **8'** were 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 710 nm long-pass filter (HQ710LP, Chroma) or a 770 nm long-pass filter (HQ770LP, Chroma) for **7'** or **8'**, respectively. Fluorescence was detected with an avalanche photodiode (SPCM-AQR-14, Perkin Elmer, Dumberry, Canada). Z-scans of the same volumina were recorded at different pH values. The pH was adjusted to the desired value by adding 1 N HCl and it was waited for 15 minutes prior to the recording of images.

II: Synthesis scheme of failed route to sulfonated, aminophenyl substituted PPCys

The synthesis route was analog to the one described by us for the ionic (sulfonated), water-soluble alkoxyphenyl substituted PPCys.[6] The synthesis failed in step e), radical addition of mercapto acetic acid was not possible for aminophenyl substituted PPCys.

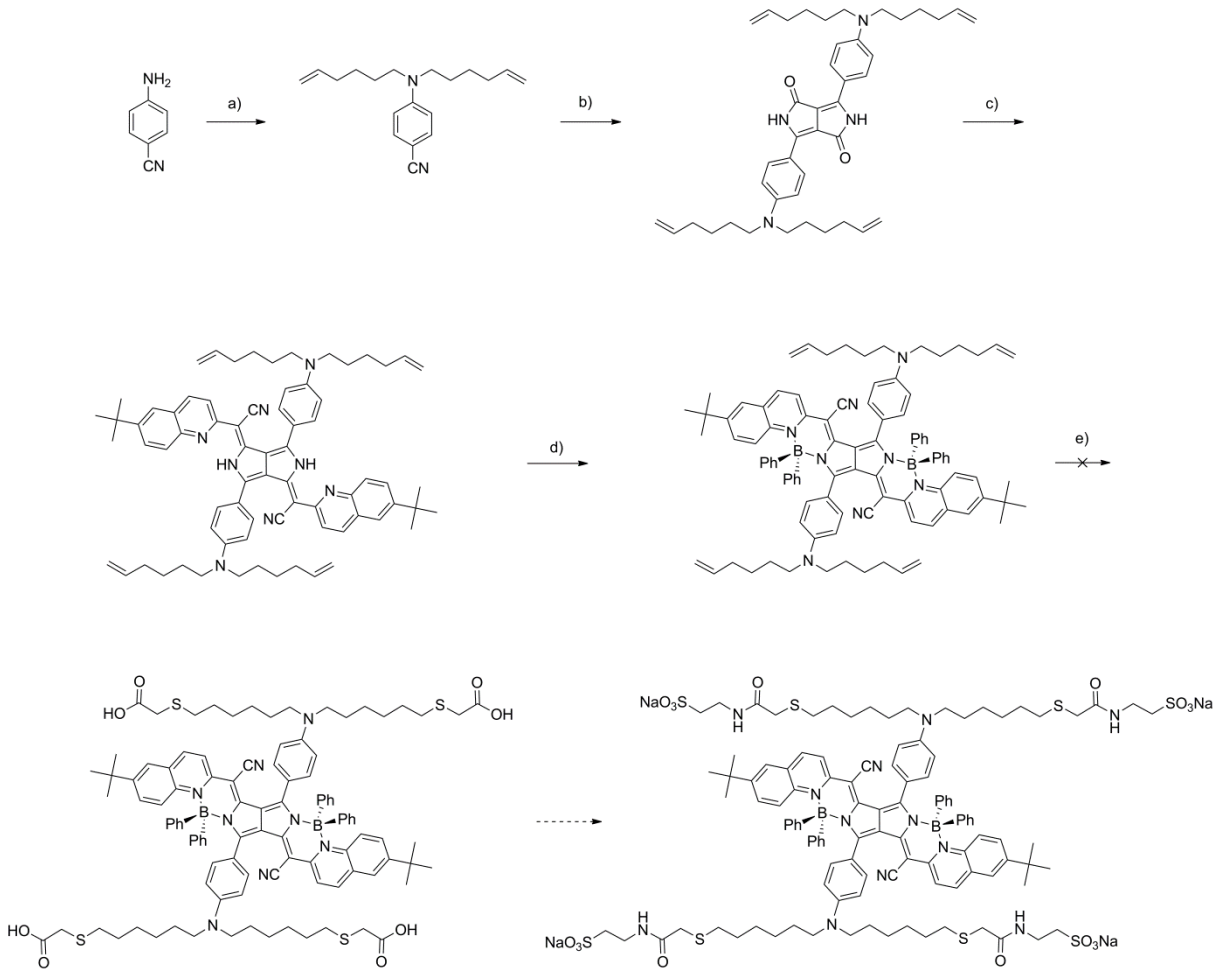


Fig. S 1 Failed route to ionic (sulfonated), water-soluble aminophenyl substituted PPCys (pH indicators); a) abs. THF/NaH/6-bromo-1-hexene, b) *tert*-amylalcohol/*tert*-amylalcoholat/di-*tert*-butyl succinate, c) abs. toluene/heteroaromatic acetonitrile/$POCl_3$, d) CH_2Cl_2/DIPEA/BPh_2Cl, e) abs. 1,4-dioxane/mercapto acetic acid/AIBN.

III: Fluorescence titration spectra of **7'**, **9'** and **8''** in water:methanol (1:1)

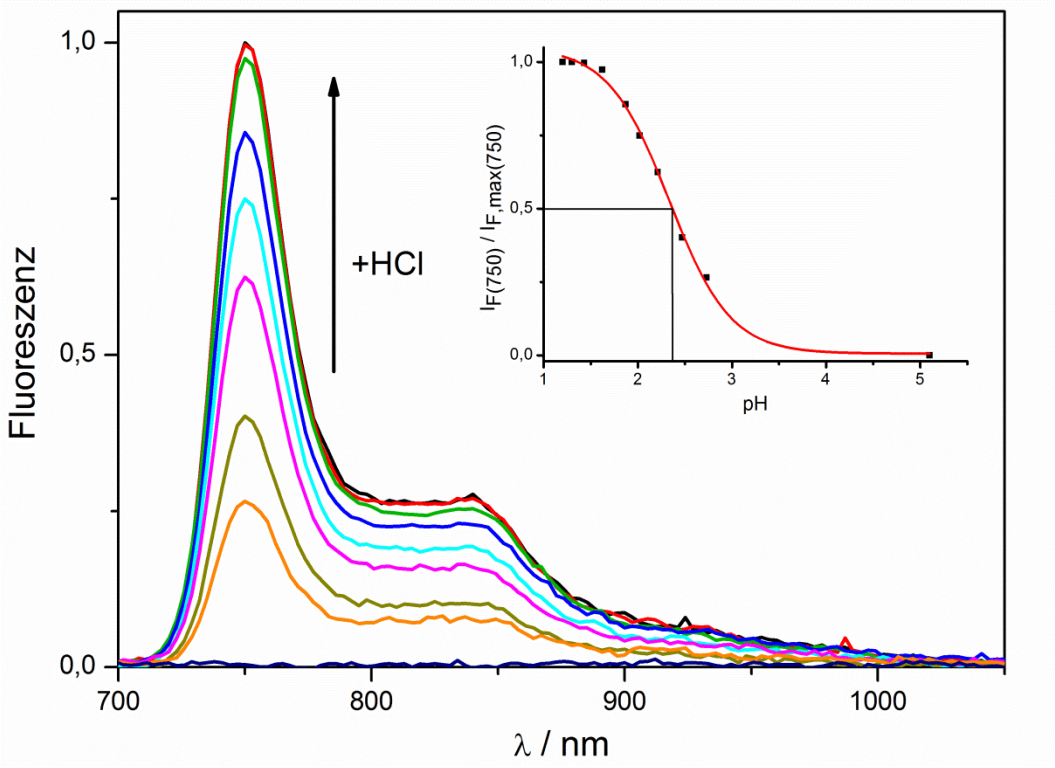


Fig. S2 Fluorescence titration spectra of PPCy **7'** in water:methanol (1:1) by successive addition of HCl.

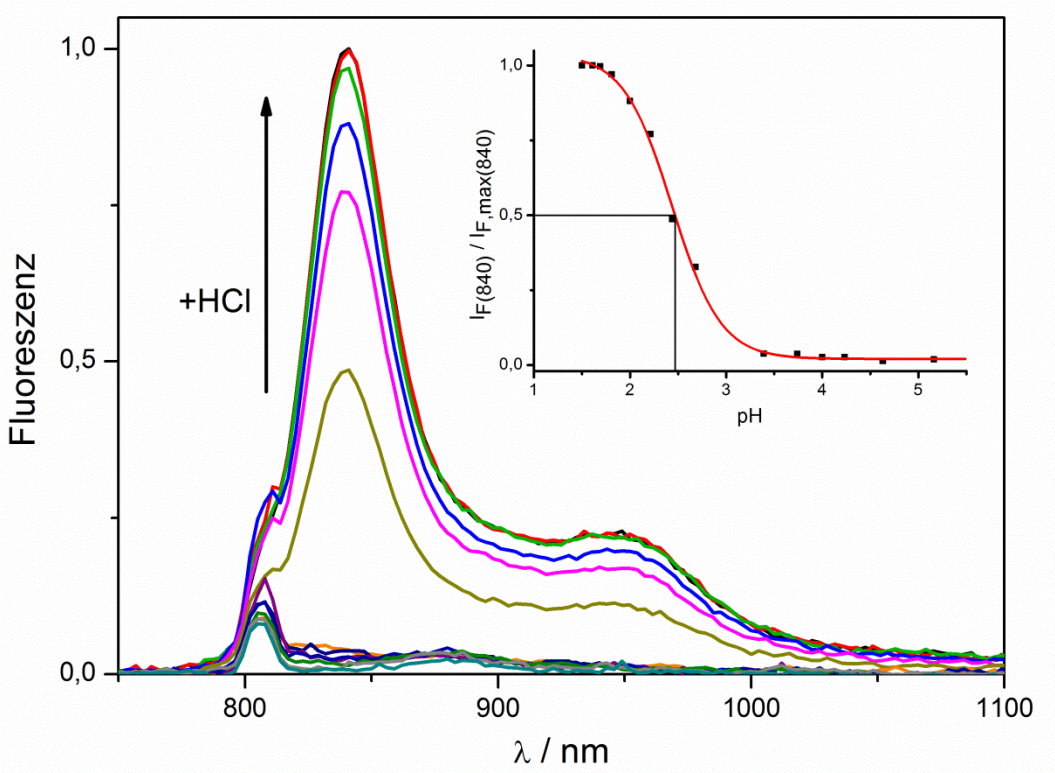


Fig. S3 Fluorescence titration spectra of PPCy **9'** in water:methanol (1:1) by successive addition of HCl (peak at 804 nm: stray light of λ_{exc} = 804 nm).

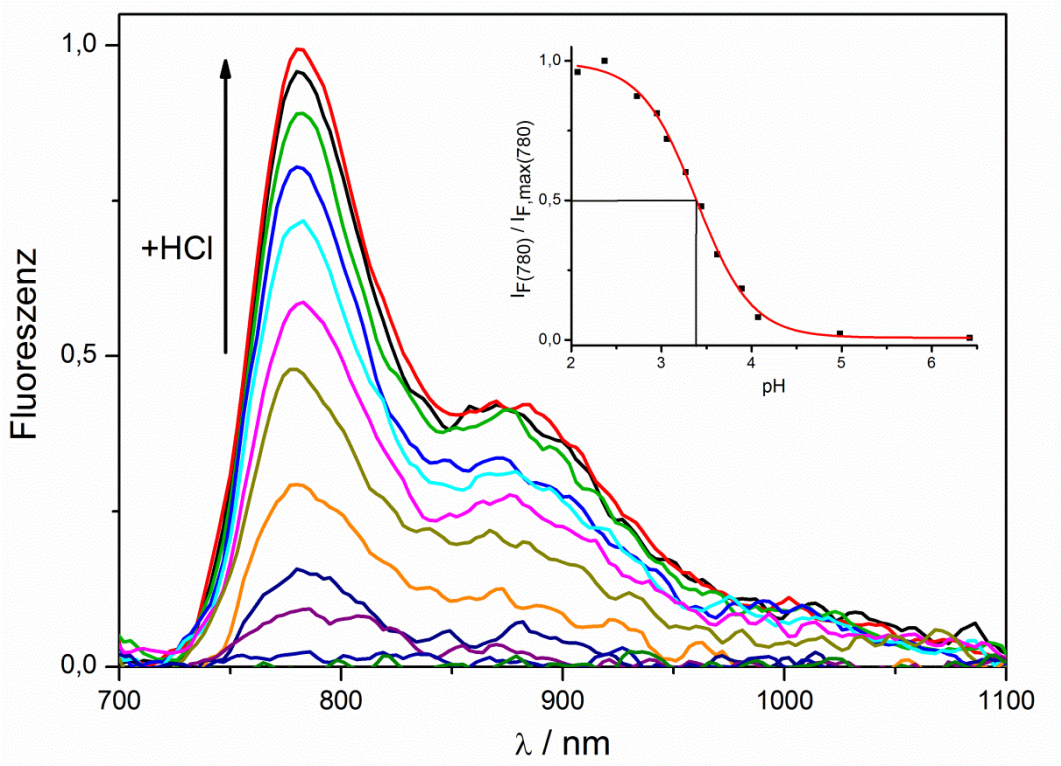


Fig. S4 Fluorescence titration spectra of PPCy **8''** in water:methanol (1:1) by successive addition of HCl.

IV: Absorption spectra of final compounds **7'**, **8'**, **9'** and **8''** in CH_2Cl_2 and H_2O

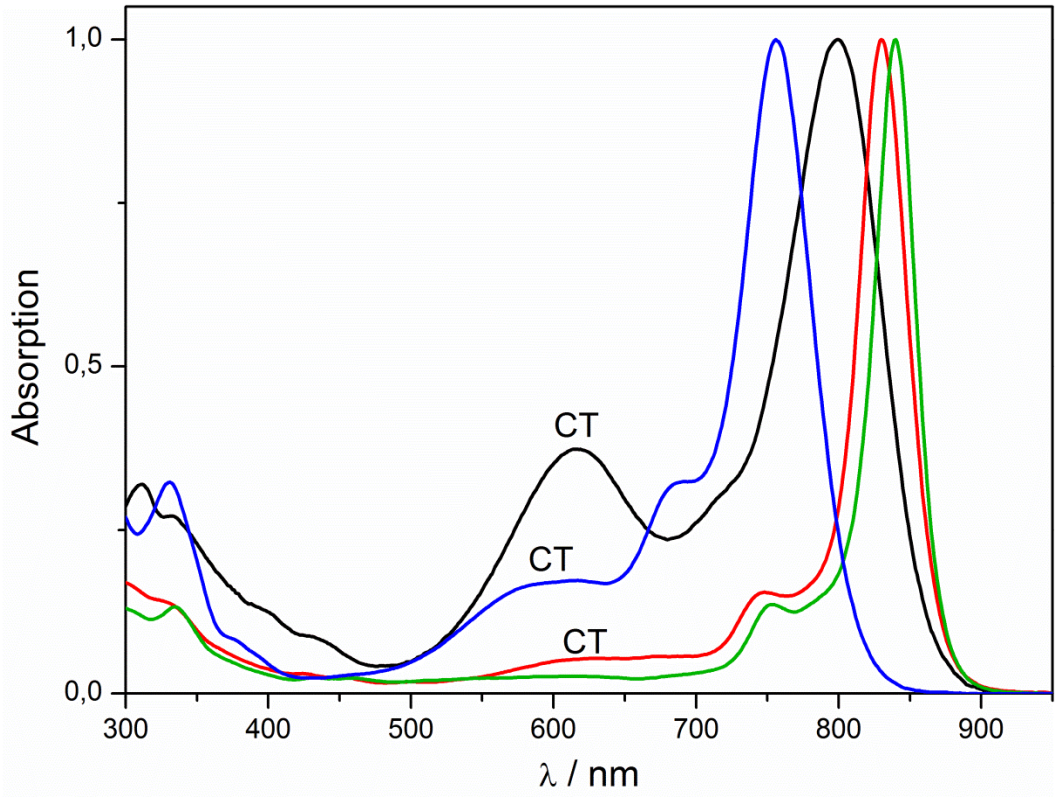


Fig. S5 Absorption spectra of PPCys **7'** (black), **8'** (red), **9'** (green) and **8''** (blue) in CH_2Cl_2.

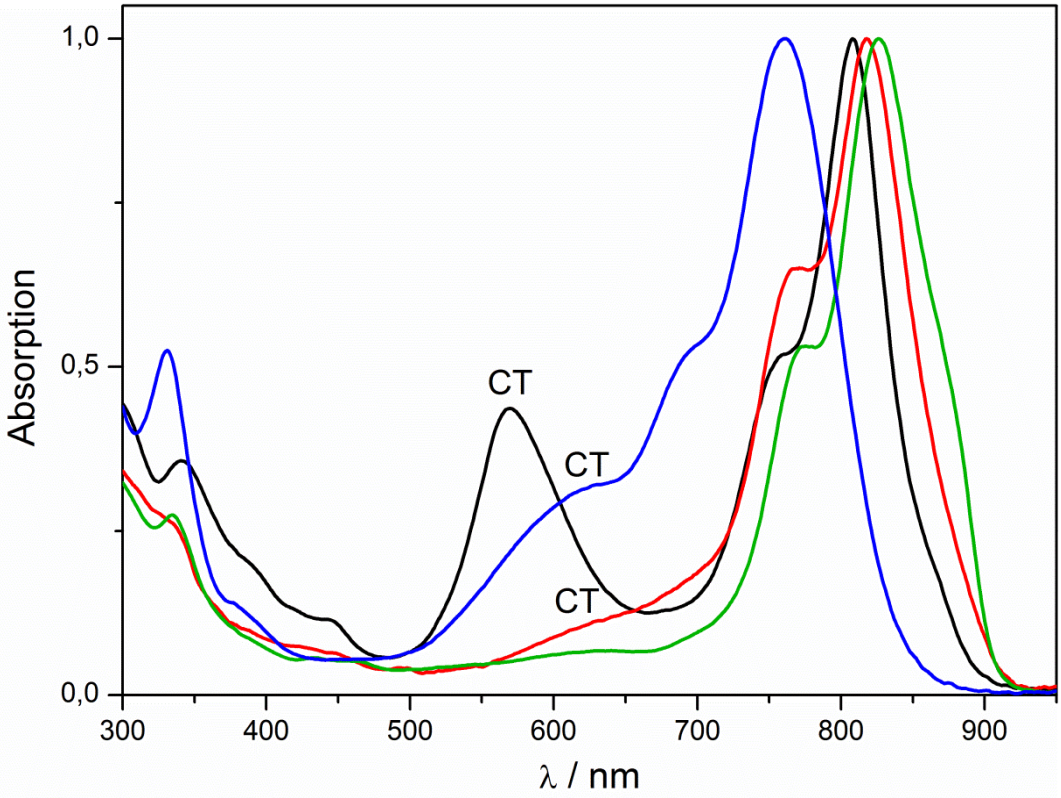


Fig. S6 Absorption spectra of PPCys **7'** (black), **8'** (red), **9'** (green) and **8''** (blue) in H_2O.

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