

Modulation of a Photoswitchable Dual-Color Quantum Dot containing a Photochromic FRET Acceptor and an Internal Standard

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Supporting information

1. Synthesis and Characterization of PCahx

A. Synthesis of PCahx

6-amino-N-(3-(3,3,4,4,5,5-hexafluoro-2-(2-methylbenzo[b]thiophen-3-yl) cyclopent-1-enyl)-2-methylbenzo[b]thiophen-6-yl) hexanamide.

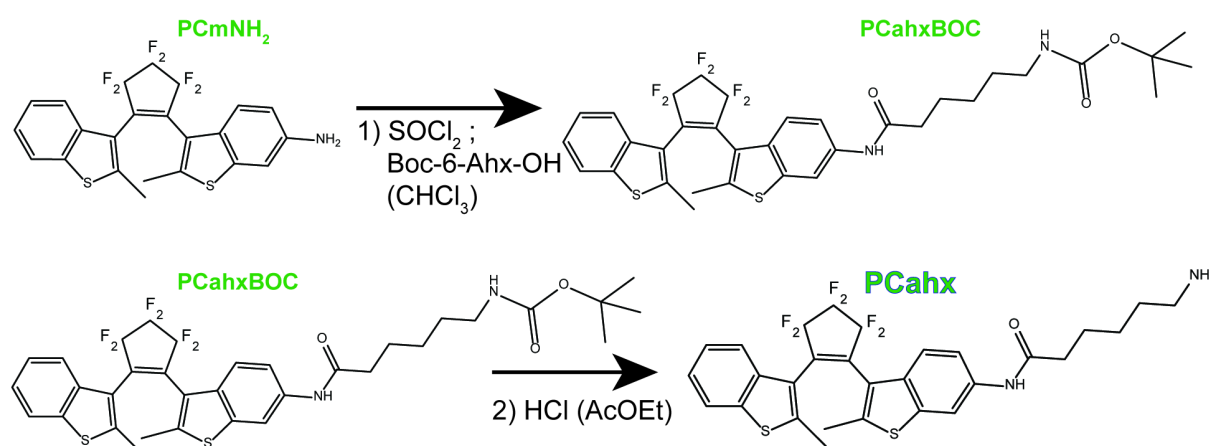


Figure S1. Synthetic scheme for PCahx

1) Commercially available 6-(tert-butoxycarbonylamino) hexanoic acid (46 mg, 200 μ moles, Sigma-Aldrich CAS: 6404-29-1), was added to a flask that had been previously dried and flushed with argon. Thionyl chloride (20 μ l, 275 μ moles, CAS:7719-09-7) was added, the flask was flushed with argon and allowed to react for 30 min at 50 $^{\circ}$ C. The flask was dried in a rotovap system to obtain freshly formed tert-butyl 6-chloro-6-oxohexylcarbamate (CAS: 903891-59-8).

A solution containing PCmNH₂ (50 mg, 100 μ moles) prepared in the lab¹ was dissolved in dry CHCl₃. The solution was added to the dry flask containing the tert-butyl 6-chloro-6-oxohexylcarbamate and allowed to react at 30 $^{\circ}$ C for 2 h. The reaction was neutralized with NaOH solution and the crude was extracted with CH₂Cl₂, a greenish-brownish oil was obtained. Purification was performed on a silica gel column with cyclohexane:ethyl acetate

(90:10) mobile phase. The intermediate product tert-butyl 6-(3-(3,3,4,4,5,5-hexafluoro-2-(2-methylbenzo[b]thiophen-3-yl)cyclopent-1-enyl)-2-methylbenzo[b]thiophen-6-ylamino)-6-oxohexylcarbamate was obtained (38 mg, 54.4 μ moles) as a pink oil.

^1H NMR (500 MHz, CDCl_3) δ 1.25 (dd, 2.04H, $\text{CH}_2\text{-CH}_2\text{-CH}_2$), 1.41 (s, 11H, $\text{C-(CH}_3)_3$), 1.68 (m, 2.81H, CH_2), 1.75 (dd, 2.18H, CH_2), 2.13 (s, 2.80H, CH_3 ap), 2.16 (s, 2.89H, CH_3 ap), 2.35 (t, 2.02H, NH-CO-CH_2), 2.43 (2s, 3.3H, CH_3 p), 3.10 (bp, 2.36H, $\text{CH}_2\text{-NH-CO}$), 6.85-7.70 (m, 6H, ArH), 8.18 (s, 0.35H, H-7 p), 8.22 (s, 0.65H, H-7 ap).

Parallel (p) to antiparallel (ap) isomers 40:60

2) The BOC protective group was released from the aliphatic amine by addition of 20% fuming HCl in ethyl acetate for 10 min at room temperature. The reaction was quenched with NaOH and extracted with CH_2Cl_2 . The product was purified by creating a silica gel filter, impurities were removed with washes of cyclohexane: ethyl acetate (from 100% cyclohexane to 100% ethyl acetate) and then the product was obtained by eluting with DMF. Solvent was evaporated in a rotovap system and the final product, 6-amino-N-(3-(3,3,4,4,5,5-hexafluoro-2-(2-methylbenzo[b]thiophen-3-yl) cyclopent-1-enyl)-2-methylbenzo[b]thiophen-6-yl) hexanamide (20 mg, 33 μ moles) was obtained as a pink/orange oil.

^1H NMR (400 MHz, CDCl_3) δ 1.25 (m, 1.94H, $\text{CH}_2\text{-CH}_2\text{-CH}_2$), 1.51 (m, 2.30H, CH_2), 1.72 (m, 2.07H, CH_2), 2.13 (s, 1.20H, CH_3 ap), 2.16 (s, 1.18H, CH_3 ap), 2.34 (t, 2.04H, NH-CO-CH_2), 2.43 (2s, 2.66H, CH_3 p), 2.71 (m, 2.00H, $\text{CH}_2\text{-NH}_2$), 6.85-7.70 (m, 6H, ArH), 8.18 (s, 0.35H, H-7 p), 8.22 (s, 0.65H, H-7 ap).

Parallel (p) to antiparallel (ap) conformers 40:60

B. Characterization of PCahx

The analysis is based on the quantitative formalism for the photostationary stage presented in our previous publications.^{1,2} Structural similarities to a previously studied diheteroarylethene, [4-((3-(3,3,4,4,5,5-hexafluoro-2-(2-methylbenzo[b]thiophene-3-yl)cyclopent-1-en-1-yl]-2-methylbenzo[b]thiophene-6-yl)amino)-4-oxobutanoic acid],¹ suggested that the photophysical properties would be comparable. The values calculated for the PCahx are consistently within the expected range as detailed below. A sample of PCahx was dissolved in CDCl_3 and ^1H NMR (400 MHz) was carried out on the sample in its open and photostationary forms.

Table S1. Integrated values for ^1H NMR signals of interest in PCahx

State	Peaks (chemical shift, ppm)		
	1.99	2.15	2.43
open	3922	4459	3724
photostationary	4964	3401	2955

The spectral changes display the same chemical shifts as in our previous publication. The methyl peaks presented the clearest area for quantification, preferable to the complex aromatic region. Irradiation at 340 nm led to decreases of 24% and 21%, of the CH₃ ap (2.13 and 2.16 ppm) and CH₃ p (2.43 ppm) hydrogens respectively, as well as an increase of the 1.99 ppm peak. The increase in the 1.99 peak is hard to quantify due to a partial overlap with another pre-existing peak. We conclude that in the photostationary state, $\alpha_{PS} = 0.22$ of the photochromic acceptor is in the closed form. We assumed that the same functional photoconversion applied to the PC- in the polymer within the dual-color psQDs, i.e. in the hypothetical absence of intervention by the QD.

Solutions of known concentrations of PCahx were prepared in CHCl₃ and EtOH. Assuming the validity of Lambert-Beer conditions, the extinction coefficients were calculated by applying the established 22% photoconversion in the photostationary state. The spectra are presented in Figure S2 and the results in Table S2.

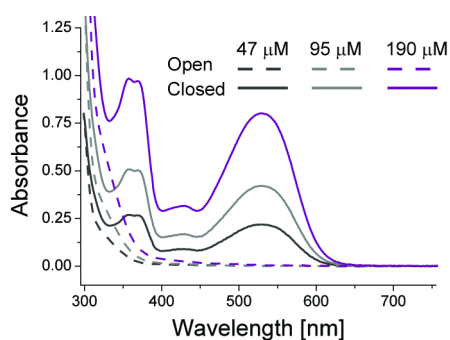


Figure S2. Absorbance spectra of PCahx solutions of varying concentrations in chloroform. The open form (---) shown as dashed lines and the closed (photostationary) state (—) as solid lines. Not all the concentrations used to calculate the extinction coefficients are presented in the figure.

Table S2. Extinction coefficients ($M^{-1} cm^{-1}$) at different wavelengths of interest in the open and closed states of PCahx

State	Wavelength [nm]				
	340	365	400	540	550
Open (oPC)	2000	665	175	25	20
Closed (cPC)	9100	19000	5730	17530	16500

An isosbestic point occurs at 301 nm in EtOH. Using equation S1 and the values in Table S3 we calculated α_{PS} for the photostationary state in EtOH. The value was 0.22, corroborating the determination by NMR spectroscopy.

$$\alpha_{\text{PS}} = -\left(\frac{\varepsilon_{-}^{540}}{\varepsilon_{+}^{540} - \varepsilon_{-}^{540}}\right) + \left(\frac{\varepsilon^{isos}}{\varepsilon_{+}^{540} - \varepsilon_{-}^{540}}\right) \left(\frac{A_{\text{PS}}^5}{A^{isos}}\right) \quad [\text{S1}]$$

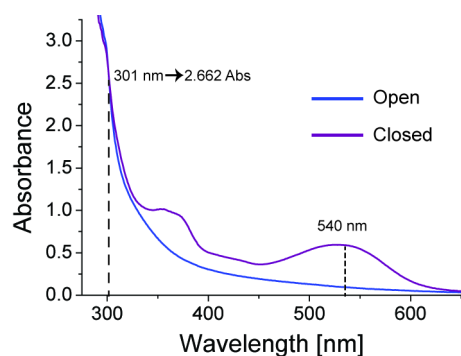


Figure S3. Absorbance of PCahx solution in ethanol in the open and open/closed (photostationary) states.

Table S3. Extinction coefficients and absorbance values of PCahx in EtOH.

Solvent	ε_{-}^{540} ($\text{M}^{-1}\text{cm}^{-1}$)	ε_{+}^{540} ($\text{M}^{-1}\text{cm}^{-1}$)	ε^{isos} ($\text{M}^{-1}\text{cm}^{-1}$)	A_{PS}^{535}	A^{isos}	α_{PS}
EtOH	25 ^a	17530 ^a	17250	0.592	2.662	0.22

a. Values previously calculated using CHCl_3 as solvent.

2. TEM Images

Samples were of $\sim 0.1 \mu\text{M}$ concentration. They were placed on a carbon grid and imaged with a Philips 120 kV BioTwin microscope equipped with a 1024×1024 pixel GATAN CCD camera (Gatan, Inc.).

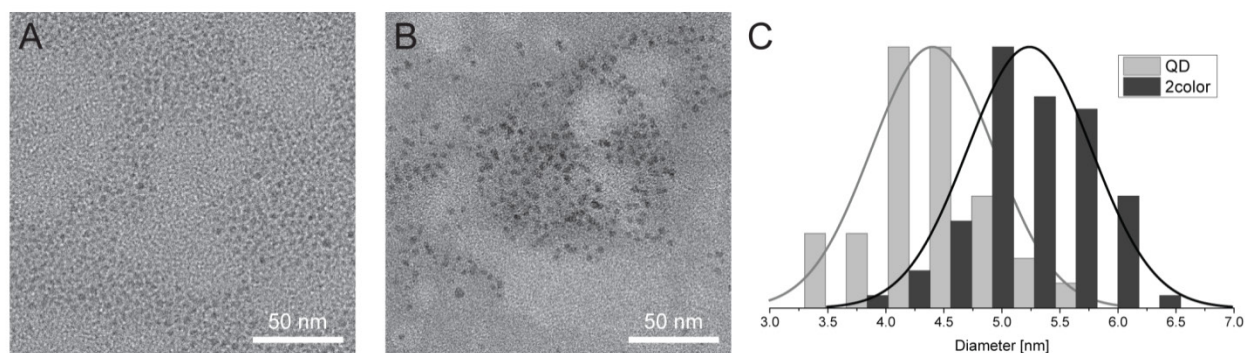


Figure S4. TEM images (A) CANdot[®] Series A CSS 540 nm, CAN GmbH, Hamburg in hexane as provided by manufacturer. (B) Dual-color psQDs in SBB 50 mM. (C) Size distributions of samples ($N > 70\text{QDs}$)

The addition of the polymer coating to the QDs leads to an increase in the mean diameter from $4.4 \pm 0.5 \text{ nm}$ to $5.3 \pm 0.5 \text{ nm}$. Outliers, $>2\sigma$, were assigned to aggregates and eliminated from calculations.

3. Photophysical Data and Evaluations

In the experimental set-up for the determination of the kinetic constants of photoconversion, photoswitching of the PC probe was achieved by irradiation of the dual-color psQD with UV light (340 ± 10 nm, 1.1 mW cm^{-2}) and, in the case of the photoreversal reaction (Figure S5), by visible/green light (545 ± 10 nm, 6.2 mW cm^{-2}). Measurements were realized in $100 \mu\text{l}$ microcuvettes with a 10 mm optical path on a Cary 100 UV-Vis Spectrophotometer and a Cary Eclipse Fluorimeter (Varian). Samples were allowed to reach room temperature and were irradiated for a short time (normally 30 s) with green light to assure that the PC moieties were in the open state. Short pulses ($\sim 1\text{-}15$ sec) of UV irradiation were utilized, with spectral determinations after each irradiation, until the photostationary stage was achieved.

The PC conversion and QD quenching data (Figure 3, UV irradiation; Figure S5, visible light irradiation) were fitted by creating a composite difference function based on evaluation of the differential equations for the closed forms of class 1 and 2 PC (Equation 1) and applying the FindMinimum subroutine of *Mathematica* (Wolfram Research) with a minimal least squares criterium for convergence. The fitted parameters are those given in the first row of Table 1 with $Q_{oc,uv}$ linked to $Q_{co,uv}$ as indicated in the text. The FindMinimum subroutine does not provide error estimations. A rough estimation of parameter sensitivity was based on systematic relative perturbation of each fitted quantity, as indicated in Table 1.

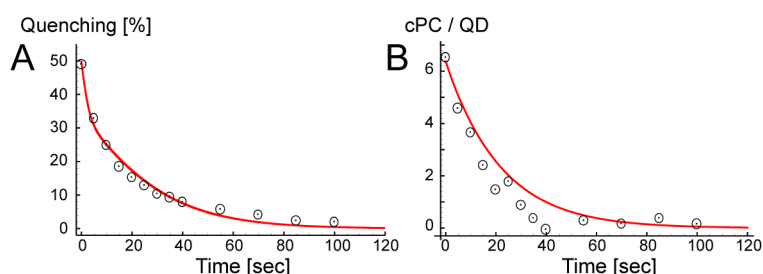


Figure S5. Photoreversal of psQD by irradiation with visible light. Dual-color psQDs in the photostationary state were irradiated with successive 5-10 s pulses of visible light (5-10 s, 545 ± 10 nm, irradiance 6.2 mW cm^{-2}). Absorbance and fluorescence spectra were recorded after each pulse. (A) Fluorescence quenching of QD as a function of irradiation time. [\odot , Data]; [$—$, Fit] (B) Mean value of cPC molecules per QD as a function of irradiation time. [\odot , Data]; [$—$, Fit].

References

1. Díaz, S. A.; Menendez, G. O.; Etchehon, M. H.; Giordano, L.; Jovin, T. M.; Jares-Erijman, E. A. *ACS Nano* **2011**, *5*, 2795-2805.
2. Giordano, L.; Jovin, T. M.; Irie, M.; Jares-Erijman, E. A. *J. Am. Chem. Soc.* **2002**, *124*, 7481-9.