Supporting Information for

Emission modulation of fluorescent turn-on mode Dibenzothienyl sulfonyl ethene photoswitches embedded in a polymer film

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1. Synthesis and analysis of SW-1 and SW-2

The 1,2-Bis(2-methyl-1-benzothiophene-1,1-dioxide-3-yl)perfluorocyclpentene (SW-1) and its phenyl derivative, 1,2-bis(2-methyl-6-phenyl-1-benzothiophene-1,1-dioxide-3-yl)perfluorocyclopentene (SW-2) in figure 1 were synthesized by adapting published procedures. The respective non-oxidized forms were synthesized by elimination reactions of perfluorocycloalkenes with organolithium compounds as described in.¹ For obtaining SW-1 with methyl and SW-2 with ethyl substituents, oxidation using H2O2 was adopted.² For obtaining the phenyl derivative SW2, the oxidized compound, 1,2-bis(2-ethyl-1-benzothiophene-1,1-dioxide-3-yl)perfluorocyclopentene was first iodinated as shown below followed by a Suzuki-Miyaura coupling reaction using the 6,6′-diiodo derivative and phenylboronic acid.

1,2-Bis(2-methyl-1-benzothiophene-1,1-dioxide-3-yl)perfluorocyclpentene SW-1









Chemical Formula: $C_{23}H_{14}F_6O_4S_2$ Molecular Weight: 532,48

Synthesis: Weigh 1,2-bis[2-methylbenzo[b]thiophen-3-yl]-3,3,4,4,5,5-hexafluoro-1cyclopentenene (0.200g, 0.43mmol) into a 25ml multi-neck flask, add 10ml acetic acid and heat to boiling point with stirring. Slowly drop in 1ml 50% H_2O_2 and reflux for 48h. Drop the reaction mixture into 25ml cold water, filter off the precipitate and wash a few times with water, then dry under vacuum. The product was purified by recrystallization from MeOH and drying under vacuum: 31% yield, white solid.

Analyses: 1H-NMR (d6-DMSO, 300MHz): δ (ppm) 8.18-7.47 (m, 8H), 2.20 (s, 3H), 2.09 (s, 3H). MALDI: m/z = 532 (M+) 1,2-bis(2-methyl-6-phenyl-1-benzothiophene-1,1-dioxide-3-yl)perfluorocyclopentene SW-2



Synthesis: Dissolve 1,2-bis(2-ethyl-6-iodo-1-benzothiophene-1,1-dioxide-3yl)perfluorocyclopentenes (0.5g, 0.616mmol) and phenylboronic acid (0.188g, 1.54mmol) in 33ml THF, add 33ml aq. saturated solution of K_2CO_3 ., then add Pd2(bda)₃ (0.1g, 0.11mmol), then 0.33ml tricyclohexylphosphine (18% in toluene solution). Stir at RT for 20 minutes. Neutralize with dilute HCl. Extract three times with chloroform, dry the organic phase over Na₂SO₄, remove solvent at the evaporator. Purification was done using column chromatography (n-Hex/EA 9:1), followed by freeze-drying with dioxane and vacuum drying for 24h at 80°C. Yield: 49% dark yellow solid

Analyses: 1H-NMR (DioxanD8, 300 MHz): δ (ppm) 8.26 (d, 1H), 8.21 (d, 1H), 7.92 (d, 1H), 7.90 (d, 1H), 7.79-7.35 (m, 12H), 2.75-2.42 (m, 4H), 1.45-1.35 (t, 3H), 1.10-1.00 (t, 3H), Molecular weight (EI): m/z 712 and MALDI: m/z = 712 (M⁺).

2. Saturation effects

The fluorescence signal from the photochromic molecules is directly related to the populations in the open and closed state, and is given by

$$F(t) = \frac{I_{420/488}}{h \nu_{420/488}} \sigma_{420/488,c} \eta_{det} \Phi_c N_{closed}(t) + \frac{I_{325}}{h \nu_{325}} \eta_{det} \left[\sigma_{325,c} \Phi_c N_{closed}(t) + \frac{I_{325}}{(51)} \right]$$

Here I refers to the laser intensity at the wavelength given by the index, v to the corresponding

frequency, h denotes Plancks constant (in fact $h\nu$ gives the number of photons at the wavelength given by the index), σ to the absorption cross section of the particular conformation (o for open and c for closed) at that wavelength, Φ to the respective fluorescence quantum yield, and η_{det} to the detection efficiency. However, the linear relationship between the detected emission signal F(t) and the excitation intensity $I_{420/488}$ that is assumed in eq. (S1) is only valid as long as saturation effects can be neglected. In order to test whether saturation effects are relevant we detected the emission intensity from a distinct spot on the sample as a function of the excitation $I_{420/488}$ intensity in the presence of a fixed UV intensity. For SW-1, I_{325} was adjusted to 3.8 W/cm² and I_{420} was varied between 5 and 1250 W/cm². For each data point the sample was illuminated with both lasers for 400 ms in order to minimize bleaching effects. During this period a series of 7 frames with an illumination time of 50 ms each was recorded. The average of these frames corresponds to the signal for the applied I_{420} , in Fig. S1a. For SW-2, I_{325} was adjusted to 6 W/cm² and I_{488} was varied between 12.5 and 1250 W/cm². For each data point the sample was illuminated with both lasers for 200 ms, and 5 frames with an illumination time of 30 ms each were recorded. The average of these frames corresponds to the signal for the applied I₄₈₈, in Fig. S1b. For both molecules we find a clear deviation from the linear relationship, see Fig. S1.



Fig. S1. *a*) Saturation experiment on SW-1. The red curve shows a fit according to eq. (S8). To verify that the fluorescence signal is not limited by a too low UV intensity, the measurement at $I_{420} = 1250 \text{ W/cm}^2$ was repeated for $I_{325}=5 \text{ W/cm}^2$ (green). The fit yields a saturation intensity of $I_{S,420} = (1733 \pm 534)$ W/cm² with fit parameters of $a = (7.9 \pm 0.9) \text{EMCCDcts cm}^2/0.05J$ and $b = (490.1 \pm 124.2)$ EMCCDcts/50ms. b) Saturation experiment on SW-2. The red curve shows a fit according to eq. (S8). To verify that the fluorescence signal is not limited by a too low UV intensity, the measurement at $I_{488}=1250 \text{ W/cm}^2$ was repeated for $I_{325}=10 \text{ W/cm}^2$ (green). The fit yields a saturation intensity of $I_{S,488} = (2764 \pm 416)$ W/cm² with fit parameters of $a = (34.7 \pm 1.6) \text{ CCDcts cm}^2/0.03J$ and $b = (98.7 \pm 215.3) \text{ CCDcts/30ms}$.

At higher excitation intensities the absorption cross section has to be modified to³

$$\sigma_{420/488,c}(I_{420/488}) = \frac{I_{S,420/488}}{I_{420/488} + I_{S,420/488}} \sigma_{420/488,c}^{(0)} ,$$

(S2)

where $\sigma_{420/488,c}^{(0)}$ denotes the cross section for low excitation intensities, and $I_{S,420/488}$ refers to the saturation intensity of the particular compound. Inserting (S2) into eq. (S1) yields for the fluorescence signal

$$F(t) = \frac{I_{420/488}}{h \nu_{420/488}} \frac{I_{5,420/488}}{I_{420/488} + I_{5,420/488}} \sigma_{420/488,c} \eta_{det} \Phi_c N_{closed}(t) + \frac{I_{325}}{h \nu_{325}} \eta_{det} \left[\sigma_{325,c} \Phi_c N_{closed}(t) + \sigma_{325,o} \Phi_o N_{open}(t) \right]$$

Using an effective excitation intensity

(S3)

$$I_{420/488,eff} = I_{420/488} \frac{I_{S,420/488}}{I_{420/488} + I_{S,420/488}}$$
(S4)

eq. (S3) can be rewritten as

$$F(t) = \frac{I_{420}}{\frac{488}{488}, eff}}{\frac{h v_{420}}{\frac{420}{488}} \sigma_{\frac{420}{488}, c}^{(0)}} \eta_{det} \Phi_c N_{closed}(t) + \frac{I_{325}}{h v_{325}} \eta_{det} \left[\sigma_{325, c} \Phi_c N_{closed}(t) + \frac{h v_{325}}{\frac{h v_{325}}{488}} \sigma_c N_{close$$

(S5)

Using the abbreviations

$$a = \frac{\sigma_{420/488,c}}{h \nu_{420/488}} \eta_{det} \Phi_c N_{closed}(t)$$
(S6)

$$b = \frac{I_{325}}{h v_{325}} \eta_{det} \left[\sigma_{325,c} \Phi_c N_{closed}(t) + \sigma_{325,o} \Phi_o N_{open}(t) \right]$$
(S7)

and

eq. (S5) has the form

$$F(I_{420/488}) = a \cdot I_{420/488, eff} + b = a \cdot I_{420/488} \frac{I_{S,420/488}}{I_{420/488} + I_{S,420/488}} + b$$

(S8)

The red line in Fig. S1 corresponds to a fit of the data points to eq. (S8). From several independent experiments on different spots on the sample the averaged saturation intensities of $I_{S,420} = (1070 \pm 835)$ W/cm² for SW-1 and $I_{S,488} = (2344 \pm 655)$ W/cm² for SW-2 were extracted. Hence, using excitation intensities in the range of 1 kW/cm² saturation effects cannot be fully neglected.

3. Kinetic model

3.1. Simple model

Under the assumption that the total number of photoswitchable molecules is constant during the experiment the change of the number of molecules in either isomeric state is given by

$$\frac{d}{dt}N_{open}(t) = -k_{oc}N_{open}(t) + k_{co}N_{closed}(t) = losed constant: me of cycleo$$

$$\frac{decay}{rise}of \ fluorescence \ signal$$

$$= -k_{oc}N_{open}(t) + k_{co}[N_{total} - N_{open}(t)] = -\frac{d}{dt}N_{closed}(t)$$
(S9)

n

where N_{open} and N_{closed} refer to the number of molecules in the open and closed state, respectively, and k_{oc} and k_{co} to the open-to-close and close-to-open conversion rates. This yields

$$N_{open} = \frac{k_{co}}{k_{co} + k_{oc}} N_{total}$$
(S10a)

$$N_{closed} = \frac{k_{oc}}{k_{oc} + k_{co}} N_{total}$$
(S10b)

and

for the steady state populations. The conversion rates can be written as

$$k_{oc} = \frac{I_{325}}{h \nu_{325}} \sigma_{325,o} \varphi_{oc}$$
(S11a)

$$k_{co} = \frac{I_{420/488,eff}}{h v_{420/488}} \sigma_{420/488,c} \varphi_{co}$$
(S11b)

where φ refers to the respective photoconversion quantum yields. For the time intervals of low fluorescence intensity, denoted as t_{low} , we have $I_{325} = 0$, which yields $k_{oc} = 0$, $N_{closed}(t_{low}) = 0$, $N_{open}(t_{low}) = N_{total}$, and $F(t_{low}) = 0$ (cf. eq. (S5)). Similarly, for time intervals of high fluorescence intensity, t_{high} , we find $N_{closed}(t_{high}) \neq 0$, $N_{open}(t_{high}) \neq 0$, and $F(t_{high}) \neq 0$, from which a contrast

$$C = \frac{F(t_{high}) - F(t_{low})}{F(t_{high})} = 1$$
(S12)

is deduced irrespective of the excitation intensities, and in disagreement with the observations.

3.2. Refined model

Under the assumption that only a fraction of the molecules in the closed conformation is photoactive we define

$$N_{total} = \frac{N_{closed}^{(hindered)} + N_{closed}^{(active)}}{N_{closed}} + N_{open}$$
(S13)

where $N_{closed} = N_{closed}^{(hindered)} + N_{closed}^{(active)}$ denotes all molecules in the closed conformation. Using $s = \left(N_{closed}^{(active)} + N_{open}\right)/N_{total}$ for the fraction of the photoactive molecules we find

$$N_{closed}(t) = N_{closed}^{(active)}(t) + (1-s)N_{total}$$
(S14)

Hence, now the number of photoactive molecules is given by $N_{closed}^{(active)}(t) + N_{open}(t) = s N_{total}$, and eq. (S9) is changed to

$$\frac{d}{dt}N_{open}(t) = -k_{oc}N_{open}(t) + k_{co}N_{closed}^{(active)}(t) = losed constant: me of cycleo the cycleo the constant is the constant in the constant is the cons$$

Using eq. (S15), the steady-state populations of the photoactive molecules at the times t_{high} are modified to

$$N_{closed}^{(active)}(t_{high}) = \frac{k_{oc}}{k_{oc} + k_{co}} \cdot s N_{total}$$
(S16a)

$$N_{open}(t_{high}) = \frac{k_{co}}{k_{oc} + k_{co}} \cdot s N_{total}$$
(S16b)

and

and, given that $I_{\rm 325}$ = 0 and therefore $k_{\rm oc}$ = 0, we find for $t_{\rm low}$

$$N_{closed}^{(active)}(t_{low}) = 0$$
(S17a)

and

$$N_{open}(t_{low}) = s N_{total}$$
 (S17b)

From eq. (S14) we find

$$N_{closed}(t_{high}) = \frac{k_{oc}}{k_{oc} + k_{co}} \cdot s N_{total} + (1 - s)N_{total}$$
(S18a)

$$N_{closed}(t_{low}) = (1-s)N_{total}$$
(S18b)

for the total number of molecules in closed conformation, N_{closed} , at t_{high} and t_{low} . The fluorescence signal follows from eq. (S5), yet using the new definition for $N_{closed}(t)$ see eq. (S14). Taking $I_{325} = 0$ into account for time intervals of low fluorescence intensity it follows from eq. (S5)

$$F(t_{low}) = \frac{I_{420/488,eff}}{h \nu_{420/488}} \eta_{det} \sigma_{420/488,c} \Phi_c (1-s) N_{total}$$
(S19a)

and for time intervals of high fluorescence intensity it follows

$$F(t_{high}) = \frac{I_{420/488,eff}}{h v_{420/488}} \eta_{det} \sigma_{420/488,c} \Phi_c \left(\frac{k_{oc}}{k_{oc} + k_{co}} s N_{total} + (1 - s) N_{total} \right) + \frac{I_{325}}{h v_{325}} \eta_{det} \sigma_{325,c} \Phi_c \left[\frac{k_{oc}}{k_{oc} + k_{co}} s N_{total} + (1 - s) N_{total} \right] + \sigma_{325,o} \Phi_o \frac{k_{co}}{k_{oc} + k_{co}} s N_{total} \right]$$
(S19b)

4. Calculation of the Contrast

Using the expressions for $F(t_{high})$ and $F(t_{low})$ (cf. eqs. (S19a, S19b), the definitions of k_{oc} and k_{co}

$$\gamma = \frac{\Phi_c}{r}$$

(cf. eqs. (S11a, S11b)), and the abbreviations ${}^{\prime}$ Φ_o for the ratio of the fluorescence quantum

$$\delta = \frac{\varphi_{co}}{\varphi_{co}}$$

yields in the closed and open conformation, and φ_{oc} for the ratio of the quantum yields for the light-induced ring-opening and the ring-closure reactions, respectively, the contrast (cf. eq. (S12)) is obtained as

$$C\left(\frac{I_{325}}{I_{420/488,eff}}\right)$$

$$= \frac{\gamma \frac{\sigma_{325,c} \quad v_{420/488}}{\sigma_{420/488,c} \quad v_{325}} \cdot \frac{I_{325}}{I_{420/488,eff}} + (1-s) \gamma \delta \frac{\sigma_{325,c}}{\sigma_{325,o}} + s (\gamma + \delta)}{\gamma \frac{\sigma_{325,c} \quad v_{420/488}}{\sigma_{420/488,c} \quad v_{325}} \cdot \frac{I_{325}}{I_{420/488,eff}} + (1-s) \gamma \delta \left[\frac{\sigma_{325,c}}{\sigma_{325,o}} + \frac{\frac{\sigma_{420/488,c}}{\sigma_{325,o}}}{\frac{v_{420/488}}{v_{325}} \cdot \frac{I_{325}}{I_{420/488,eff}}}\right] - \frac{1}{1-s}$$

$$K = \frac{I_{325}}{I_{420/488,eff}}$$

Using

(S20)

for the (effective) ratio of the number of photons applied in the UV and the VIS spectral range we can rewrite eq. (S20) as

$$C\left(\frac{I_{325}}{I_{420/488,eff}}\right) = \frac{\gamma \frac{\sigma_{325,c}}{\sigma_{420/488,c}} K + (1-s) \gamma \delta \frac{\sigma_{325,c}}{\sigma_{325,o}} + s (\gamma + \delta)}{\gamma \frac{\sigma_{325,c}}{\sigma_{420/488,c}} K + (1-s) \gamma \delta \left[\frac{\sigma_{325,c}}{\sigma_{325,o}} + \frac{1}{K} \frac{\sigma_{420/488,c}}{\sigma_{325,o}}\right] + \gamma + s \delta}$$
(S22)

5. Calculation of the ring opening rate $k_{\rm co}$

For calculating the decrease of the fluorescence intensity upon ceasing the UV illumination we have to determine the number of photoactive molecules in the open/closed conformation as a function of time for the time interval $(t - t_{high})$. This can be obtained from the solutions of eq. (S15) for the initial conditions of $N_{closed}^{(active)}(t_{high})$ and $N_{open}(t_{high})$, see eqs. (16a, 16b), which are

$$N_{closed}^{(active)}(t - t_{high}) = \frac{k_{oc}}{k_{oc} + k_{co}} \cdot s \cdot N_{total} \cdot e^{-k_{co}t}$$
(S23a)

$$N_{open}(t - t_{high}) = N_{total} - (1 - s)N_{total} - N_{closed}^{(active)}(t - t_{high})$$
(S23b)

In order to take into account also the molecules that are not photoactive in the closed conformation, eq. (S23a) is inserted into in eq. (S14) which yields

$$N_{closed}(t - t_{high}) = \frac{k_{oc}}{k_{oc} + k_{co}} \cdot s \cdot N_{total} \cdot e^{-k_{co}t} + (1 - s)N_{total}$$
(S24)

The corresponding temporal decay of the fluorescence signal is then obtained from eq. (S5) for $I_{325} = 0$ as

$$F(t - t_{high}) = \frac{I_{420/488,eff}}{h v_{420/488}} \eta_{det} \sigma_{420/488,c} \Phi_c \frac{k_{oc}}{k_{oc} + k_{co}} \cdot s \cdot N_{total} \cdot e^{-k_{co}t} + (1 - s)N_{total}$$
(S25)

Using the abbreviations

$$c = \frac{I_{420/488,eff}}{h \nu_{420/488}} \eta_{det} \sigma_{420/488,c}^{(0)} \Phi_c \frac{k_{oc}}{k_{oc} + k_{co}} \cdot s \cdot N_{total}$$
$$d = \frac{I_{420/488,eff}}{h \nu_{420/488}} \eta_{det} \sigma_{420/488,c}^{(0)} \Phi_c (1-s) N_{total}$$

and

eq. (S25) has the form

$$F(t - t_{high}) = c \ e^{-k_{co}t} + d$$
(S26)

Unfortunately, the temporal resolution of the experiment does not allow for a meaningful analysis of the data for the SW-1 molecules. For the SW-2 molecule, Fig. S2 shows a few examples for the decrease of the fluorescence intensity as a function of time together with a fit according to eq. (S26).



 $k_{co} = 1.23 \ s^{-1}$

The examples in Fig. S2 testify that the decrease of the fluorescence is not properly described by eq. (S26). Studies on single photochromic molecules revealed that the rates k_{co} feature a distribution,⁴ which is reflected in a non-monoexponential decay of the fluorescence intensity for the ensemble.⁵ Since these distributions can be very broad⁴ we refrained from introducing more fit parameters as for example using stretched exponentials for fitting, and give only a very crude estimate of $\phi_{co} \approx 10^{-3} - 10^{-5}$ for the quantum yield of the ring opening reaction of the SW-2 molecules.

6. Calculation of the ring closing rate $k_{\rm oc}$

For calculating the increase of the fluorescence intensity upon UV illumination at t = t_{low} we have to determine the number of photoactive molecules in the open/closed conformation as a function of time for the time interval ($t - t_{low}$). This can be obtained from the solution of eq. (S15) for the initial conditions $N_{closed}^{(active)}(t_{low})$ and $N_{open}(t_{low})$, see eq. (S17a, S17b), which results in

$$N_{closed}^{(active)}(t - t_{low}) = \frac{k_{oc}}{k_{oc} + k_{co}} \cdot s \cdot N_{total} \cdot \left(1 - e^{-(k_{oc} + k_{co})t}\right)$$
(S27a)

$$N_{open}(t - t_{low}) = N_{total} - (1 - s)N_{total} - N_{closed}^{(active)}(t - t_{low})$$
(S27b)

In order to take also the molecules in the closed conformation that are not photoactive into account, eq. (S27a) is inserted into in eq. (S14), which yields

$$N_{closed}(t - t_{low}) = \frac{k_{oc}}{k_{oc} + k_{co}} \cdot s \cdot N_{total} \cdot \left(1 - e^{-(k_{oc} + k_{co})t}\right) + (1 - s)N_{total}$$
(S28)

The corresponding increase of the fluorescence signal as a function of time is then obtained by inserting eq. (S28) and eq. (S27b) into eq. (S5)

$$\begin{split} F(t-t_{low}) &= \left[\frac{I_{325}}{h\nu_{325}} \Phi_c \sigma_{325,c} + \frac{I_{420/488,eff}}{h\nu_{420/488}} \cdot \Phi_c \sigma_{420/488,c}\right] (1-s) N_{total} \eta_{det} + s N_{total} \eta_{det} \\ &+ \left[\frac{I_{325}}{h\nu_{325}} \Phi_o \sigma_{325,o} - \frac{I_{325}}{h\nu_{325}} \Phi_c \sigma_{325,c} - \frac{I_{420/488,eff}}{h\nu_{420/488}} \Phi_c \sigma_{420/488,c}\right] \frac{k_{oc}}{k_{oc} + k_{co}} \cdot s \\ &\cdot N_{total} \cdot \eta_{det} \cdot e^{-(k_{co} + k_{oc})t} \\ &(S29) \end{split}$$

Introducing the abbreviations

е

$$= \left[\frac{I_{325}}{h v_{325}} \Phi_c \sigma_{325,c} + \frac{I_{420/488,eff}}{h v_{420/488}} \cdot \Phi_c \sigma_{420/488,c}^{(0)}\right] (1-s) N_{total} \eta_{det} + s$$

and

$$f = \left[\frac{I_{325}}{h v_{325}} \Phi_o \sigma_{325,o} - \frac{I_{325}}{h v_{325}} \Phi_c \sigma_{325,c} - \frac{I_{420/488,eff}}{h v_{420/488}} \Phi_c \sigma_{420/488,c} \right] \frac{k_{oc}}{k_{oc} + k_{co}} \cdot s \cdot N_{total} \cdot \eta_{det}$$

eq. (S29) has the form

$$F(t - t_{low}) = e + f \ e^{-(k_{co} + k_{oc})t}$$
(S30)

Yet, for both types of photochromic molecules the rise of the fluorescence upon UV illumination is too fast to allow for a quantitative analysis of the data.

7. Calculation of the relative spike intensities

For obtaining a quantitative expression for the relative spike intensities as observed in Fig. 5, we have to determine the number of photoactive molecules in the open/closed conformation as a function of the time interval $(t - t_0)$, where t_0 refers to the time where the visible light applied. Since the UV illumination prior to t_0 initiates all photoactive molcules in the closed state this requires to solve eq. (S15) for the initial conditions

$$N_{open}(t_0) = 0 \tag{S31a}$$

$$N_{closed}^{(active)}(t_0) = s N_{total.}$$
(S31b)

which yields

$$N_{closed}^{(active)}(t-t_{0}) = \frac{s N_{total}}{k_{co} + k_{oc}} \left(k_{oc} + k_{co} e^{-(k_{co} + k_{oc})t} \right)$$
(S32a)

$$N_{open}(t-t_0) = N_{total} - (1-s)N_{total} - N_{closed}^{(active)}(t-t_0)$$
(S32b)

In order to consider also the non-photoactive molecules in the closed conformation eq. (S32a) is inserted into eq. (S14), giving

$$N_{closed}(t - t_0) = \frac{s N_{total}}{k_{co} + k_{oc}} \left(k_{oc} + k_{co} e^{-(k_{co} + k_{oc})t} \right) + (1 - s)N_{total}$$
(S33)

for the number of molecules in the closed states as a function of time after starting the illumination in the VIS. The corresponding fluorescence signal is then obtained by inserting eq. (S33) and eq. (S32b) into eq. (S5), and reads

$$F(t-t_{0}) = \frac{I_{420/488,eff}}{h \nu_{420/488}} \eta_{det} \sigma_{420/488,c} \Phi_{c} \left[\frac{s N_{total}}{k_{co} + k_{oc}} (k_{oc} + k_{co} e^{-(k_{co} + k_{oc})t}) + (1-s)N + \frac{I_{325}}{h \nu_{325}} \eta_{det} \right]$$

$$+ \frac{I_{325}}{h \nu_{325}} \eta_{det} \left[\sigma_{325,c} \Phi_{c} \left[\frac{s N_{total}}{k_{co} + k_{oc}} (k_{oc} + k_{co} e^{-(k_{co} + k_{oc})t}) + (1-s)N_{total} \right] + \sigma_{325,o} \Phi_{c} \left[\frac{s N_{total}}{k_{co} + k_{oc}} (k_{oc} + k_{co} e^{-(k_{co} + k_{oc})t}) + (1-s)N_{total} \right] \right]$$
(S34)

For the limiting cases t = 0 and t_{∞} (for $t = \infty$) this results in

$$F(t_0) = \frac{I_{420/488,eff}}{h \nu_{420/488}} \eta_{det} \,\sigma_{420/488,c} \,\Phi_c N_{total} + \frac{I_{325}}{h \nu_{325}} \eta_{det} \sigma_{325,c} \,\Phi_c N_{total}$$
(S35)

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and

$$F(t_{\infty}) = \frac{I_{420}}{\frac{488}{488}} \eta_{det} \sigma_{420}^{(0)} \Phi_c \left[s \, N_{total} \frac{k_{oc}}{k_{co} + k_{oc}} + (1-s) N_{total} \right] + \frac{I_{325}}{h \, \nu_{325}} \eta_{det} \left(\sigma_{325,0} \right)$$
(S36)
$$\frac{F(t_0)}{F(t_0)}$$

Using the definitions of k_{oc} and k_{co} , see eqs. (S11a, S11b), we obtain for the ratio $F(t_{\infty})$

$$\frac{F(t_{0})}{F(t_{\infty})} = \left\{ \gamma + \gamma \frac{I_{325} \quad \nu_{420/488} \quad \sigma_{325,c}}{I_{420/488,eff} \quad \nu_{325} \quad \sigma_{420/488,c}} \right\} \cdot \left\{ \frac{\frac{I_{325} \quad \nu_{420/488}}{I_{420/488,eff} \quad \nu_{325}}}{\frac{\sigma_{420/488,c}}{\sigma_{325,o}} \delta + \frac{I_{325} \quad \nu_{420/488}}{I_{420/488,eff} \quad \nu_{325}}} \right[\gamma s + \frac{I_{325} \quad \nu_{420/488,eff}}{I_{420/488,eff} \quad \nu_{325}}} (S37)$$

 $\gamma = \frac{\Phi_c}{\Phi_o}$ As before $\gamma = \frac{\Phi_c}{\Phi_o}$ corresponds to the ratio of the fluorescence quantum yields in the closed

$$\delta = \frac{\varphi_{co}}{\omega}$$

and open conformation, and φ_{oc} corresponds to the ratio of the quantum yields for the light-induced ring-opening and the ring-closure reactions. Using the abbreviation K, see eq. (S21), we obtain

$$\frac{F(t_{0})}{F(t_{\infty})} = \frac{\gamma + \gamma K \frac{\sigma_{325,c}}{\sigma_{420/488,c}}}{\frac{K}{\sigma_{420/488,c}} \delta + K} \left[\gamma s + K \cdot s \cdot \left(\gamma \frac{\sigma_{325,c}}{\sigma_{420/488,c}} - \frac{\sigma_{325,o}}{\sigma_{420/488,c}} \right) \right] + \gamma (1 - s) + K \left(\gamma (1 - s) \frac{\sigma_{325,o}}{\sigma_{420/488,c}} \right) \right]$$
(S38)

References

- 1 M. Hanazawa, R. Sumiya, Y. Horikawa and M. Irie, J. Chem. Soc., Chem. Commun., 1992, 206.
- 2 K. Uno, H. Niikura, M. Morimoto, Y. Ishibashi, H. Miyasaka and M. Irie, *J. Am. Chem. Soc.*, 2011, **133**, 13558–13564.
- 3 A. B. Myers, P. Tchenio, M. Z. Zgierski and W. Moerner, J. Phys. Chem., 1994, **98**, 10377–10390.
- 4 J. Maier, M. Pärs, T. Weller, M. Thelakkat and J. Köhler, *Sci. Rep.*, 2017, **7**, 41739.
- 5 A. F. van Driel, I. S. Nikolaev, P. Vergeer, P. Lodahl, D. Vanmaekelbergh and W. L. Vos, *Phys. Rev. B*, 2007, **75**, 693.