## Supporting information

# Exploiting Fast Exciton Diffusion in Dye-Doped Polymer Nanoparticles to Engineer Efficient Photoswitching

Kateryna Trofymchuk, Luca Prodi, Andreas Reisch, Yves Mély, Kai Altenhöner, Jochen Mattay, and Andrey S. Klymchenko

### Experimental Methods

Poly(D,L-lactide-co-glycolide) (lactide 50 mole%, glycolide 50 mole%, Mn 24 000; PDI 1.7) and rhodamine B octadecyl ester (R18) perchlorate (≥98.0 %) were purchased from Sigma-Aldrich and used as received. Salts of R18 with hydrophobic counterions: R18/TPB, R18/F1-TPB and R18/F5-TPB were synthesized as described elsewhere.[1] The synthesis of photochromic dye PCD1 was described earlier.[2] Sodium phosphate monobasic (>99.0 %, Sigma-Aldrich) and sodium phosphate dibasic dihydrate (>99.0 %, Sigma-Aldrich) were used to prepare 20 mM phosphate buffer solutions at pH 7.4. MilliQ-water (Millipore) was used in all experiments.

**Preparation of fluorescent NPs.** PLGA (lactide:glycolide, 1:1, mole:mole, Mn 24 000) was dissolved at 2 mg/mL$^{-1}$ in acetonitrile containing different amounts of dye (from 0 to 3 wt% relative to the PLGA). These solutions were added quickly and under stirring (shaking) using a micropipette to a 10-fold volume excess of 20 mM phosphate buffer at pH 7.4. The particle solution was then quickly diluted five-fold with the same buffer.

**Characterization of NPs.** Dynamic light scattering measurements were performed on a Zetasizer Nano series DTS 1060 (Malvern Instruments S.A.). It uses a laser source at 633 nm, which excludes any light excitation of our dye-doped NPs. Absorption and emission spectra were recorded on a Cary 400 Scan UV-visible spectrophotometer (Varian) and a FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon) equipped with a thermostated cell compartment, respectively. For standard recording of fluorescence spectra, the excitation wavelength was set to 520 nm. The fluorescence spectra were corrected for detector response and lamp fluctuations. For fluorescence switching analysis, peak fluorescence intensity at the band maximum was used after correction from the background noise of the blank. To realize photoswitching in solution a small volume (200 μL) of the sample in 1 mL quartz cuvette was irradiated by a laser at 645 nm with a light flux ~ 200 mW/cm$^2$ or a lamp of a fluorometer at 405 nm with a light flux ~1 mW/cm$^2$. The time of illumination was varied dependent on the sample. For solution of the photoswitch in dioxane it was 2 min at 405 nm and 20 min at 645 nm, while for PLGA NPs it was 10 min at 405 nm and 40 min at 645 nm.

**Calculation of FRET efficiency.** In a model where one donor is surrounded with multiple acceptors the theoretical FRET efficiency $E_n$ can be calculated using the following equation (1):[3]

$$E_n = \frac{nE_0}{1+(n-1)E_0} \qquad (1)$$

where *n* is the number of acceptors and $E_0$ is the FRET efficiency in a classical one donor – one acceptor system:

$$E_0 = \frac{1}{1+\left(\frac{r}{R_0}\right)^6} \qquad (2)$$

where *r* is a donor-acceptor distance and $R_0$ is the Förster radius.

**Fluorescence microscopy.** For single particle fluorescence measurements, the NPs were immobilized on glass surfaces covered by polyethyleneimine (PEI), as described earlier.[1] The solutions of NPs were diluted to a particle concentration of about 6 pM with buffer. 400 µL of these solutions per $cm^2$ were then brought in contact with the PEI covered glass for 15 min, followed by extensive rinsing with milliQ-water. The surfaces were left in milliQ-water during the microscopy measurements.
Single particle measurements were performed in the TIRF (Total Internal Reflection Fluorescence) mode on a home-made wide-field setup based on an Olympus IX-71 microscope with an oil immersion objective (NA = 1.49, 100x). A DPPS (Cobolt) continuous wave (CW) laser emitting at 532 nm was used for excitation of dye-doped NPs. The laser intensity for image recording was set to 0.2 $W/cm^2$ by using a polarizer and a half-wave plate (532 nm). The fluorescence signal was recorded with an EMCCD (ImagEM Hamamatsu) with an integration time of 50 ms. To switch the particles to the on state, the observation area was illuminated by 645 nm laser with 97 $W/cm^2$ power for 60 s. Then to switch them off 5 s illumination at 405nm with laser power 8.7 $W/cm^2$ was used. The image recording was realized after each illumination step. After background subtraction, the individual particle intensity was determined as a mean intensity in a circle with a fixed radius that included the signal from one NP. The average intensity was evaluated as an arithmetic mean of all NPs in one image. The average intensity variation was calculated as a ratio between average NPs intensity after each illumination and average fluorescence intensity at the off state in the first illumination cycle.

**Table 1.** Hydrodynamic diameter and polydispersity (PDI) of PLGA NPs encapsulating R18 with different counterions and PCD1 measured by DLS.[a]

| NPs content | Fluorophore only | | With 0.3% PCD1 | |
|---|---|---|---|---|
| | Diameter (nm) | PDI | Diameter (nm) | PDI |
| 1% ClO4 | 86 | 0.06 | 129 | 0.03 |
| 1% TPB | 46 | 0.06 | 46 | 0.11 |
| 1% F1-TPB | 44 | 0.13 | 44 | 0.1 |
| 1% F5-TPB | 46 | 0.11 | 43 | 0.04 |
| 0.1% F5-TPB | 47 | 0.13 | 42 | 0.07 |
| 3% F5-TPB | 43 | 0.06 | 45 | 0.09 |

[a] Statistics by volume was used in the analysis.

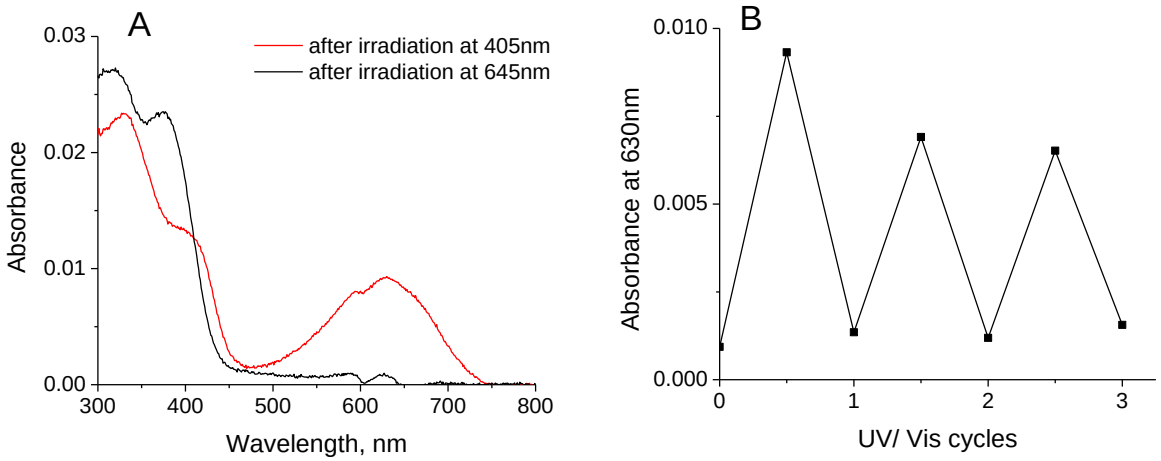


**Fig. S1.** (A) Absorption spectra of 1 wt% of photochromic dye PCD1 in PLGA matrix. (B) Absorbance at 630 nm of 1 wt% of PCD1 inside PLGA matrix under alternate illumination at 405 nm (higher values) and 645 nm (lower values).

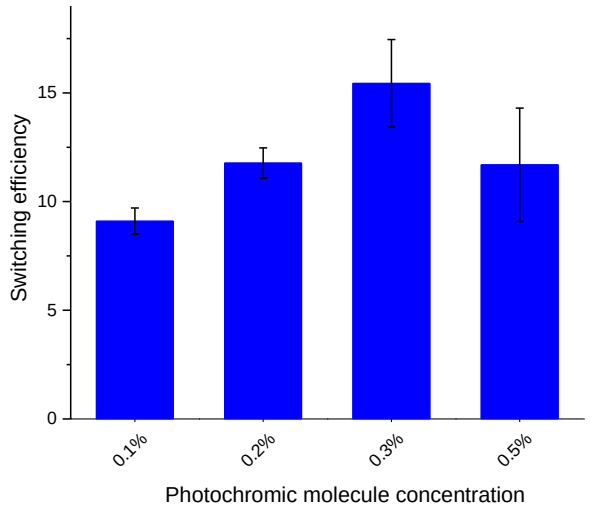


**Fig. S2.** Average switching efficiency ($I_{on}/I_{off}$) from four complete on/off cycles of NPs loaded with 1 wt% of R18/F5-TPB and different amounts (wt%) of photochromic molecule PCD1.

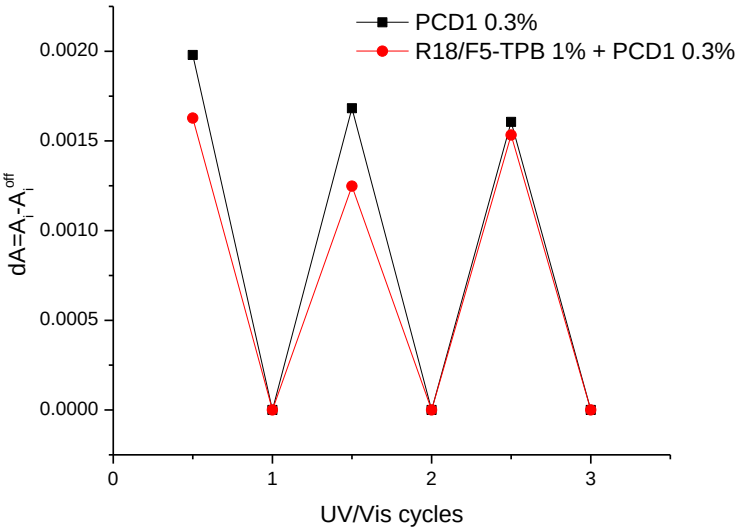


**Fig. S3.** Variation of absorbance at 630 nm under alternate irradiation presented as $dA=A_i- A_i^{off}$ of NPs loaded with PCD1 0.3% with and without 1 wt% of R18/F5-TPB.

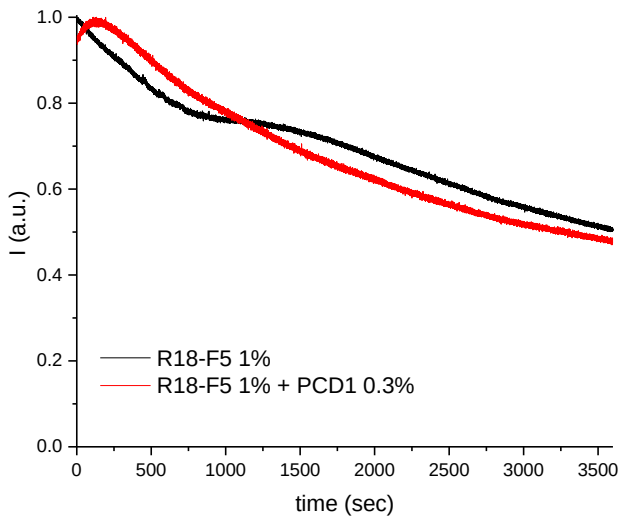


**Fig. S4.** Photostability of NPs loaded with 1 wt% of R18/F5-TPB in the presence or absence of PCD1 0.3%. Experiment was performed in low volume cuvette (50µl) under the illumination of the lamp of spectrofluorometer at excitation wavelength 560 nm. The illumination power was ~ 1mW/cm$^2$.

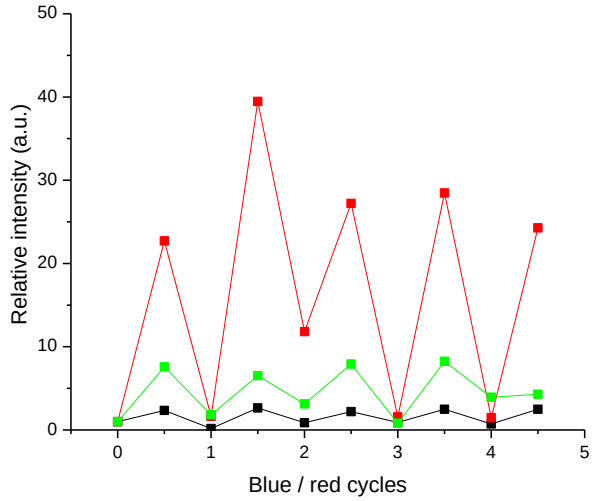


**Fig. S5.** Photoswitching of individual NPs under the microscope. Relative intensity of different NPs in the on- and off-state obtained from microscopy images after 405 and 645 nm illumination. The optimal conditions for photoswitching were 60 s illumination at 645 nm with laser power 97 W/cm$^2$, and 5 s illumination at 405nm with laser power 8.7 W/cm$^2$. To record fluorescence images the power of the laser at 532 was set to 0.2 W/cm$^2$.

## References

(1) Reisch, A.; Didier, P.; Richert, L.; Oncul, S.; Arntz, Y.; Mely, Y.; Klymchenko, A. S. Collective fluorescence switching of counterion-assembled dyes in polymer nanoparticles. *Nat. Commun.* **2014**, *5*, 4089.
(2) Altenhoener, K.; Lamm, J.-H.; Mattay, J. Novel Dithienylethenes with Extended pi-Systems: Synthesis by Aldol Condensation and Photochromic Properties. *Eur. J. Org. Chem.* **2010**, 6033.
(3) Fabian, A. I.; Rente, T.; Szoellosi, J.; Matyus, L.; Jenei, A. Strength in Numbers: Effects of Acceptor Abundance on FRET Efficiency. *ChemPhysChem* **2010**, *11*, 3713.