

International Edition: DOI: 10.1002/anie.201607940 German Edition: DOI: 10.1002/ange.201607940

Carboxylated Photoswitchable Diarylethenes for Biolabeling and Super-Resolution RESOLFT Microscopy

Benoît Roubinet⁺, Mariano L. Bossi⁺, Philipp Alt⁺, Marcel Leutenegger, Heydar Shojaei, Sebastian Schnorrenberg, Shamil Nizamov, Masahiro Irie,* Vladimir N. Belov,* and Stefan W. Hell*

Abstract: Reversibly photoswitchable 1,2-bis(2-ethyl-6phenyl-1-benzothiophene-1,1-dioxide-3-yl)perfluorocyclopentenes (EBT) having fluorescent "closed" forms were decorated with four or eight carboxylic groups and attached to antibodies. Low aggregation, efficient photoswitching in aqueous buffers, specific staining of cellular structures, and good photophysical properties were demonstrated. Alternating light pulses of UV and blue light induce numerous reversible photochemical transformations between two stables states with distinct structures. Using relatively low light intensities, EBTs were applied in biology-related super-resolution microscopy based on the reversible saturable (switchable) optical linear fluorescence transitions (RESOLFT) and demonstrated optical resolution of 75 nm.

he optical super-resolution techniques, as well as the fluorescent proteins and synthetic dyes required for them, revived fluorescence microscopy as one of the most powerful methods in the life sciences. The diffraction limit no longer restricts the spatial resolution in optical microscopy; it was overcome by using photoinduced switching of the fluorescent markers between dark and emitting states.^[1] For example, in biology-related optical microscopy based on the RESOLFT concept,^[1a] an optical resolution of 30-40 nm has been

[*	 J Dr. B. Roubinet,^[+] Dr. M. L. Bossi,^[+] DiplBiophys. P. Alt,^[+] Dr. M. Leutenegger, Dr. H. Shojaei, M. Sc. S. Schnorrenberg, Dr. S. Nizamov, Dr. V. N. Belov, Prof. Dr. S. W. Hell Abteilung Nanobiophotonik Max-Planck-Institut für biophysikalische Chemie Am Fassberg 11, 37077 Göttingen (Germany) E-mail: vbelov@gwdg.de shell@gwdg.de Homepage: http://www.mpibpc.gwdg.de/abteilungen/200/
	Prof. Dr. M. Irie Research Center for Smart Molecules Department of Chemistry Rikkyo University Nishi-Ikebukuro 3-34-1, Toshimaku, Tokyo (Japan) E-mail: iriem@rikkyo.ac.jp
[+] These authors contributed equally to this work.
Ē	 Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under http://dx.doi.org/10. 1002/anie.201607940.
0	© 2016 The Authors. Published by Wiley-VCH Verlag GmbH & Co.

Co. KGaA. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial, and no modifications or adaptations are made.

achieved with reversibly photoswitchable fluorescent proteins (Dreiklang, rsEGFP) undergoing thousands of E/Z isomerizations.^[2] Owing to the thermal stabilities of the nonfluorescent and fluorescent states and adequate photoisomerization quantum yields, the switching between these states can be performed reversibly, with relatively low light intensities and comparatively low photobleaching. While fluorescent proteins demonstrate good performance in RESOLFT microscopy, synthetic dyes are far inferior in this respect. Man-made photochromic fluorescent dyes^[3] incorporated into silica gel nano-beads undergo tens of switching cycles and improve optical resolution.^[4] Recently, thiol-assisted photoswitching of a Cy3-Alexa647 conjugate has been implemented in the presence of potassium iodide (KI) under RESOLFT conditions and allowed, for example, to discern tubulin filaments approximately 130 nm apart.^[5] Still, RESOLFT microscopy of noteworthy subdiffraction resolution on biological samples has not yet been realized for synthetic organic fluorophores undergoing reversible photochemical transformation and switching between two stable states with distinct and stable structures.

The required properties of RESOLFT markers-sufficient photostability, high fluorescent modulation (>90%), high brightness (product of the fluorescence quantum yield $(\Phi_{\rm fl})$ and extinction coefficient (ε)), thermal stability of the fluorescent and non-fluorescent forms, satisfactory switching kinetics, large separation between the absorption bands of the two stable states-have been achieved for synthetic photochromic dyes in organic solvents.^[3,4] but their performance in purely aqueous solutions remained poor (even in the presence of solubilizing residues and polar ionic groups).^[6] In most cases, these disappointing results were obtained for bichromophoric compounds with separate photochromic and fluorescent units, in which the fluorescence quenching was effected by the resonant energy transfer (RET) from the fluorescent unit (RET donor) to the colored form of the photochromic unit (RET acceptor).^[7] Alternatively, photoswitching of the emission signal without destructive readout caused by the excitation light was realized by using the electron-transfer process (from the excited state of fluorophore to the "closed form" of the photochromic unit).^[8] In any case, it is difficult to compensate the inherent hydrophobicity of the relatively large and complex molecular switch by adding polar or charged groups.

Great expectations in the field were associated with the new photochromic sulfones based on 1,2-bis(2-alkyl-6-aryl-1benzothiophen-3-yl)perfluorocyclopentenes (see Scheme 1).^[9] By irradiation with UV light, the "open" form

Angew. Chem. Int. Ed. 2016, 55, 1-6

© 2016 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

These are not the final page numbers!

Wiley Online Library



Scheme 1. Photoswitchable EBTs 1 and 2 decorated with four or eight carboxylic acid groups; see Table 1 for spectral properties of the "open" (OF, non-fluorescent) and the "closed" (CF, fluorescent) forms.^[12] For compounds with R = H and $R = C_6H_5$ see Refs. [9a, b, c] and [9d, e], respectively.

transforms to the fluorescent yellow "closed" form.^[10] Illumination with visible (blue) light restores the "open" isomer. Remarkably, the fluorescent "closed" forms emit green light with quantum efficiencies of up to 0.92,^[11] and the photochromic (switching) and fluorescent (signal) functions are combined in one chemical entity. Using these sulfone derivatives as leads, we planned to prepare photochemically switchable fluorophores applicable in purely aqueous buffers that possess a reactive group and perform well (as bioconjugates) in commercially related RESOLFT microscopes using moderate light intensities of 1–10 kW cm⁻² and switching times in the µs-ms range.

After screening many substituents attached to positions C2(2') and C6(6') in 1,2-bis(1-benzothiophene-1,1-dioxide-3yl)perfluorocyclopentene, we have chosen a symmetrical core structure with 2(2')-ethyl and 6(6')-phenyl groups (Scheme 1, dyes **1** and **2**).^[11] These groups, and the absence of strong electron-donor residues at C6,6' provide a relatively low (but not too low) quantum efficiency of the ring-opening reaction (Table 1). It was sufficient for a high number of excitation– emission cycles of the closed isomer in a read-out process, before its back-isomerization to the open form takes place (erase or refresh step). In contrast, strong electron donors at C6,6' slow down the ring-opening reaction drastically^[11a,c] and

Table 1: Photophysical properties of free dyes 1 and 2 in PBS (pH 7.4).

Parameter	State	1	2
$\lambda_{\max abs} [nm]/$ $\varepsilon [M^{-1} cm^{-1}]$	$OF^{[a]}$	340/12400	337/16500
$\lambda_{\max abs} [nm]/$ $\varepsilon [M^{-1} cm^{-1}]$	CF	450/35000	448/45000
$\lambda_{max em}$ [nm]	CF	534, 558	522, 550
$arPsi_{fl}^{[b]}$	CF	0.48	0.57
τ [ns]	CF	1.72 ± 0.05	2.04 ± 0.05
$\Phi_{OF o CF}^{[c]}$		0.19 ± 0.04	0.23 ± 0.04
$oldsymbol{\Psi}_{CF o OF}$, ,		$1.2 \times 10 \pm 3 \times 10$	$2.0 \times 10 \pm 3 \times 10$

[a] lowest energy absorption peak. [b] fluorescence quantum yield; fluorescein in 0.1 M NaOH was used as standard. [c] At 365 nm. [d] At 470 nm (see Supporting Information for details).

© 2016 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

might promote photodegradation of the closed form. Ethyl groups at C2(2') (not methyl groups) are essential for increasing the fluorescence quantum yields in highly polar solvents.^[11b] Photophysical properties of newly synthesized dyes **1** and **2** in aqueous buffers are summarized in Table 1 (the absorption and emission spectra are presented in Figure S6). The differences in properties measured in aqueous solutions and in methanol (Table S2) are not large. For instance, the emission efficiencies and fluorescence lifetimes are reduced by only 20–30% in PBS at pH 7.4, with slightly larger variations observed for compound **1**.

A difficult task was to find and attach to the dye core the most appropriate hydrophilic residues, to prevent aggregation of the free dyes and their bioconjugates in aqueous solutions. Numerous approaches to hydrophilic, water soluble diarylethenes with sulfonic,^[13] carboxylic acid,^[11,14] hydroxy groups, or inositol residues,^[14] as well as poly(ethyleneglycol) chains,^[14a,15] have been already reported. These polar groups are necessary because the cores of the best synthetic switchers are inherently hydrophobic, and these compounds are poorly soluble in water,^[14a] form nanoaggregates,^[13b] or need cyclodextrins as additives for solubilization.^[13a] Moreover, they often lack a reactive group for bioconjugation. We have found that the presence of several carboxylic acid groups conferred the required properties to dyes 1 and 2, despite the common notion that the carboxylic acid is inferior to the sulfonic or phosphoric (phosphonic) residue in terms of solubilization. Indeed, the presence of four or eight carboxylates provided a fairly good solubility at pH > 5 and inhibited aggregation (in the micromolar range) even after photocyclization (the closed isomer aggregates more readily).^[15a] Unlike most of the other solubilizing groups, one of the carboxylates can be conveniently transformed into any reactive group. Using mono Nhydroxysuccinimidyl esters and standard labeling methods, we have successfully conjugated these two new hydrophilic diarylethenes with secondary antibodies. Remarkably, all the bioconjugates retain the photochromic and fluorescent properties of free dyes 1 and 2. They were used in conventional (confocal) microscopy and in super-resolution RESOLFT microscopy.

Diarylethenes 1 and 2 were obtained via three different routes: using a Suzuki-Miyaura cross coupling of C6(6')diiodide derivative^[11a] (Scheme S2/S3) with either 3,5-diformylphenyl boronic (route A; 23% yield over 2 steps), or 5boronoisophthalic acid (route B; 46% yield), or a pinacol ester of 3,5-di(tert-butoxycarbonyl)phenylboronic acid (route C; 13% over 2 steps).^[16] An additional Jones oxidation step in route A or an acidic treatment (with CF₃COOH in CH_2Cl_2) in route C was applied. To improve the hydrophilic properties and suppress the aggregation even further, we prepared diarylethene 2 with eight carboxylic groups. This was achieved in 41% yield by a similar strategy using a Suzuki-Miyaura cross-coupling followed by cleavage of tert-butyl esters under acidic conditions.[16] The amino-reactive mono NHS esters of these dyes were prepared by the gradual and controlled addition of the measured quantities of N,N'-dicyclohexylcarbodiimide (DCC) or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) to solutions of 1 or 2 and N-hydroxysuccinimide in DMF with catalytic amounts of

Angew. Chem. Int. Ed. 2016, 55, 1-6

These are not the final page numbers!

4-(*N*,*N*-dimethyl)aminopyridine (DMAP).^[16] The stable mono-NHS ester of dye **1** was isolated by preparative RP-HPLC, while mono-NHS ester of dye **2** could be only generated in solution without isolation. Then, the secondary antibodies were labeled with these mono-NHS esters and purified by gel-filtration.^[16] MALDI-TOF mass spectrometry and UV/Vis spectroscopy were used to evaluate the degrees of labeling (DOL; see Table S1 in the Supporting Information). We varied the DOL values to achieve the best immunofluorescence staining and acquire the best-possible images under confocal and RESOLFT conditions.

The conjugates obtained with secondary antibodies were highly fluorescent and photochromic; we used them for immunostaining of tubulin filaments and nucleopore complexes in Vero cells. Confocal measurements (Figure S1) showed their selective binding with primary antibodies and tubular network in the whole fixed cells. Cells immunolabeled with dyes 1 and 2 were used for pump-probe fluorescence measurements in a modified confocal microscope using a wide-field illumination (Figure S2). For that, the stained cells were mounted onto an object slide with a cavity filled with PBS (pH 7.4). Switching between the open and closed forms was performed by laser light of 375 nm and 491 nm wavelength, respectively. The measurement was initialized by a 10 ms pulse of 491 nm light (17 kW cm^{-2}) to switch all the fluorophores in the observation area to the "dark" state. Then a pulse sequence was applied consisting of a 20 µs pulse of 375 nm light (0.8 kW cm^{-2}), a 500 µs delay, a 10 ms pulse of 491 nm light (17 kW cm^{-2}) and another 2 ms delay. This pulse sequence was repeated 200 times (in Figure 1 and Figure S3,



Figure 1. Fluorescence emission of cells immunolabeled with dye 1 (top) and 2 (bottom) showing the on/off switching cycles upon irradiation with the light pulse sequence mentioned in the text (gray). The red line indicates the maximum fluorescence signal detected within a switching cycle, while the black line shows the overall fluorescence gained per cycle. Both plots were normalized to their maximum value. The insets show one switching cycle as the mean value of 100 cycles (blue). All measurements were taken with 20 μ s bins. Displayed intensities were corrected according to the dead-time of the sensors.

100 and 200 cycles are shown, respectively). These measurements showed that half of the initial fluorescence is bleached after performing about 11 switching cycles for dye **1** and 21 switching cycles for dye **2**, respectively. A bi-exponential fit of the obtained decay curve of the ring-opening ("off"-switching) reaction gave the time constants of $\tau_1 = 241 \mu s$ and $\tau_2 = 1.31$ ms for dye **1** (79% of the amplitude belongs to τ_1 and 21% to τ_2). Under these irradiation conditions, the corresponding decay times for dye **2** are $\tau_1 = 126 \mu s$ (77%) and $\tau_2 = 674 \mu s$ (23%). The residual fluorescence per cycle was evaluated by comparison of the maximal fluorescence in a cycle and the average value of the residual fluorescence recorded during the last 100 μs illumination with 491 nm light (as a mean value of 100 cycles). The residual fluorescence was found to be 4.3% for dye **1** and 1.7% for dye **2**, respectively.

To demonstrate the applicability of dyes 1 and 2 for RESOLFT microscopy, fixed immunolabeled Vero cells were imaged using a modified 1C RESOLFT QUAD Scanning microscope (Abberior Instruments, Göttingen). The imaging was performed by applying the following pulse sequence: switching the diarylethenes to their fluorescent "closed" isomers with 355 nm light (130 $W \text{ cm}^{-2}$) for 50 µs, 200 µs delay, application of the doughnut-shaped 488 nm beam (36 kW cm^{-2}) for 1.2 ms to switch the diarylethenes in the periphery of the focal spot to their non-fluorescent "open" isomers. Finally, the remaining area with the fluorescent isomers was probed for 80 µs with a Gaussian shaped 488 nm light beam (9.7 kW cm^{-2}) . The same pulse sequence was applied to record the confocal images except that the offswitching was omitted. Using this imaging scheme, it was possible to acquire RESOLFT images with an optical resolution of 74 nm FWHM^[17] and confocal images with a resolution of 175 nm FWHM.^[17] The confocal and RESOLFT images are given in Figure 2, Figure S4 (tubulin filaments), and Figure S5 (nuclear pore complexes). The relatively high confocal resolution (Figure 2 and Figure S4) results from the 355 nm activation spot that is significantly smaller than the excitation spot at 488 nm wavelength. This two-step process of activation and excitation vields an effective confocal point spread function (PSF) which can be described by the product of the activation, excitation, and detection PSFs.^[18]

Both diarylethenes show a high switching contrast in the pump-probe measurements. Although the switching-off rate of the free dye **2** is only about twice the rate of dye **1** (Table 1), it turned out that during RESOLFT measurements, dye **2** (attached to a protein) switches off much faster than dye **1** (1.5 ms for dye **2** versus 7 ms for dye **1**); this is probably the reason why dye **1** bleached faster and provided poorer resolution (85 nm). The resolution achieved in the RESOLFT measurements was limited mainly by photobleaching and not by the residual fluorescence after off-switching.

To date, reversibly photoswitchable fluorescent proteins still offer a significantly lower switching fatigue (about $100 \times$ more cycles),^[19] yet we have shown the possibility to prepare reversibly photoswitchable organic dyes that perform in purely aqueous solutions and are suitable for biology-related RESOLFT microscopy. Importantly, the simple EBT scaffold presented a viable core structure and was decorated with four

Angew. Chem. Int. Ed. 2016, 55, 1-6

 \odot 2016 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

These are not the final page numbers!



Figure 2. RESOLFT images of the whole fixed Vero cells immunostained with primary antibodies against α -Tubulin and with the diarylethene 2 attached to the secondary antibodies. All images show raw data. The RESOLFT images were recorded before confocal images. Scale bar: 1 μ m. The line profiles A–D (averaged over ten adjacent lines) display the regions indicated in the RESOLFT image. The data (dots) was fitted with a Lorentzian function (solid line) for the RESOLFT (red) and the confocal (blue) image. The FWHM was determined on the fits A and B as indicated by the small black arrows.

or eight carboxylic acid groups. Compounds 1 and 2 underwent reversible photochemical transformations and switched between two stable states with distinct structures in aqueous PBS buffer and without stabilizing reagents and additives. The results of the present study allow for the first time manmade photoswitchable fluorophores to be compared with photoswitchable fluorescent proteins. Though the performance of synthetic dyes in RESOLFT microscopy is still inferior to that of fluorescent proteins (in respect of bleaching, number of switching cycles, and phototoxic effects of UV light), the simplicity of EBT structures is encouraging. Indeed, low aggregation, efficient photoswitching in aqueous buffers, and chemical reactivity were gained straightforwardly, by introducing numerous freely rotating COOH groups to a highly symmetrical core structure. All these results can be very helpful for future work. It will focus on the design and characterization of reversibly photoswitchable and fatigue-resistant dyes that isomerize into a stable fluorescent form by irradiation with light of 405 nm and longer wavelengths. Moreover, their ability for labeling living cells (using SNAP-Tag or HaloTag® technologies) will be evaluated.

© 2016 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Acknowledgements

We thank Jürgen Bienert (MPIBPC), Dr. Holm Frauendorf, Dr. Michael John and co-workers (Institut für organische und biomolekulare Chemie, Georg-August-Universität, Göttingen, Germany) for recording spectra. We are indebted to Jens Schimpfhauser and Jan Seikowski (MPIBPC) for the synthesis of 1,2-bis(2-ethyl-6-iodo-1-benzothiophen-1,1-dioxide-3-yl)perfluorocyclopentene and 3,5-di(tert-butycarboxyphenyl)boronic acid, pinacol ester. We are grateful to Dr. Ellen Rothermel (MPIBPC) for the preparation and immunolabeling of cells. We thank Prof. Stefan Jakobs (MPIBPC) for lending us the Abberior RESOLFT microscope. We are indebted to the Bundesministerium für Bildung und Forschung (BMBF, Germany) for funding in the program KMUinnovativ: Photonik/ Optische Technologien (FKZ 13N12995; to SWH).

Keywords: diarylethenes \cdot fluorescence \cdot optical microscopy \cdot photochromic dyes \cdot water solubility

- For the idea of RESOLFT, see: a) S. W. Hell, S. Jakobs, L. Kastrup, *Appl. Phys. A* 2003, 77, 859-860; for examples, see: b) M. Hofmann, C. Eggeling, S. Jakobs, S. W. Hell, *Proc. Natl. Acad. Sci. USA* 2005, *102*, 17565-17569; c) E. Betzig, G. H. Patterson, R. Sougrat, O. Wolf Lindwasser, S. Olenych, J. S. Bonifacino, M. W. Davidson, J. Lippincott-Schwartz, H. F. Hess, *Science* 2006, *313*, 1642-1645; for reviews, see: d) S. W. Hell, *Nat. Methods* 2009, *6*, 24-32; e) S. W. Hell, *Science* 2007, *316*, 1153-1158.
- [2] a) T. Brakemann, A. C. Stiel, G. Weber, M. Andresen, I. Testa, T. Grotjohann, M. Leutenegger, U. Plessmann, H. Urlaub, C. Eggeling, M. C. Wahl, S. W. Hell, S. Jakobs, *Nat. Biotechnol.* 2011, 29, 942–947; b) T. Grotjohann, I. Testa, M. Leutenegger, H. Bock, N. T. Urban, F. Lavoie-Cardinal, K. I. Willig, C. Eggeling, S. Jakobs, S. W. Hell, *Nature* 2011, 478, 204–208; c) J. G. Danzl, S. C. Sidenstein, C. Gregor, N. T. Urban, P. Ilgen, S. Jakobs, S. W. Hell, *Nat. Photonics* 2016, 10, 122–128.
- [3] M. Bossi, V. N. Belov, S. Polyakova, S. W. Hell, Angew. Chem. Int. Ed. 2006, 45, 7462–7465; Angew. Chem. 2006, 118, 7623– 7627.
- J. Fölling, S. Polyakova, V. Belov, A. van Blaaderen, M. L. Bossi, S. W. Hell, Small 2008, 4, 134–142.
- [5] J. Kwon, J. Hwang, J. Park, G. R. Han, K. Y. Han, S. K. Kim, *Sci. Rep.* 2015, 5, 17804.
- [6] a) N. Soh, K. Yoshida, H. Nakajima, K. Nakano, T. Imato, T. Fukaminato, M. Irie, *Chem. Commun.* 2007, 5206–5208;
 b) S. M. Polyakova, V. N. Belov, M. L. Bossi, S. W. Hell, *Eur. J. Org. Chem.* 2011, 3301–3312.
- [7] a) J. M. Endtner, F. Effenberger, A. Hartschuh, H. Port, J. Am. Chem. Soc. 2000, 122, 3037–3046; b) L. Giordano, T. M. Jovin, M. Irie, E. A. Jares-Erijman, J. Am. Chem. Soc. 2002, 124, 7481– 7489; c) T. Fukaminato, T. Sasaki, T. Kawai, N. Tamai, M. Irie, J. Am. Chem. Soc. 2004, 126, 14843–14849; d) M. Frigoli, G. H. Mehl, Eur. J. Org. Chem. 2004, 636–642.
- [8] T. Fukaminato, T. Doi, N. Tamaoki, K. Okuno, Y. Ishibashi, H. Miyasaka, M. Irie, J. Am. Chem. Soc. 2011, 133, 4984–4990.
- [9] a) Y. C. Jeong, S. I. Yang, K. H. Ahn, E. Kim, *Chem. Commun.* 2005, 2503–2505; b) Y.-C. Jeong, D. G. Park, I. S. Lee, S. I. Yang, K.-H. Ahn, *J. Mater. Chem.* 2009, *19*, 97–103; c) Y.-C. Jeong, S. I. Yang, E. Kim, K.-H. Ahn, *Tetrahedron* 2006, *62*, 5855–5861;

Angew. Chem. Int. Ed. 2016, 55, 1-6

These are not the final page numbers!

d) T. Wu, B. Johnsen, Z. Qin, M. Morimoto, D. Baillie, M. Irie, N. R. Branda, *Nanoscale* **2015**, 7, 11263–11266; e) M. Bälter, S. Li, M. Morimoto, S. Tang, J. Hernando, G. Guirado, M. Irie, F. M. Raymo, J. Andréasson, *Chem. Sci.* **2016**, 7, 5867–5871; for a recent application in PALM/STORM microscopy, see: f) O. Nevskyi, D. Sysoiev, A. Oppermann, T. Huhn, D. Wöll, *Angew. Chem. Int. Ed.* **2016**, DOI: 10.1002/anie.201606791; *Angew. Chem.* **2016**, DOI: 10.1002/ange.201606791.

- [10] The "closed" form is yellow and emits green light.
- [11] a) K. Uno, H. Niikura, M. Morimoto, Y. Ishibashi, H. Miyasaka, M. Irie, J. Am. Chem. Soc. 2011, 133, 13558-13564; b) Y. Takagi, T. Kunishi, T. Katayama, Y. Ishibashi, H. Miyasaka, M. Morimoto, M. Irie, Photochem. Photobiol. Sci. 2012, 11, 1661-1665; c) F. Gillanders, L. Giordano, S. A. Díaz, T. M. Jovin, E. A. Jares-Erijman, Photochem. Photobiol. Sci. 2014, 13, 603-612.
- [12] The "open" form is in equilibrium between two isomers: parallel (p) and antiparallel (ap), and the fluorescent "closed" form is formed only from an anti-parallel conformer (one of the Woodward–Hoffmann rules). For reviews on DAEs, see: a) M. Irie, *Chem. Rev.* 2000, 100, 1685–1716; b) M. Irie, T. Fukaminato, K. Matsuda, S. Kobatake, *Chem. Rev.* 2014, 114, 12174–12277.
- [13] a) M. Takeshita, M. Irie, *Chem. Commun.* 1997, 2265–2266;
 b) M. Takeshita, N. Kato, S. Kawauchi, T. Imase, J. Watanabe, M. Irie, *J. Org. Chem.* 1998, *63*, 9306–9313.

- [14] a) Y. Shoji, A. Yagi, M. Horiuchi, M. Morimoto, M. Irie, *Isr. J. Chem.* 2013, *53*, 303–311; b) "Photochromic molecule": M. Irie, C. C. Yamada, JP2012172139, 2012; c) "Water-soluble photochromic molecule": H. Tokiwa, M. Irie, K. Ikeda, T. Otsubo (Rikkyo Educational Corporation and Josho Gakuen Educational Foundation), EP 2902402, 2015.
- [15] a) T. Hirose, M. Irie, K. Matsuda, Adv. Mater. 2008, 20, 2137– 2141; b) T. Hirose, M. Irie, K. Matsuda, Chem. Asian J. 2009, 4, 58–66; c) T. Hirose, K. Matsuda, M. Irie, J. Org. Chem. 2006, 71, 7499–7508.
- [16] For synthetic procedures, details and analytical data, see the Supporting Information. Dyes **1** and **2** are base-sensitive; under alkaline conditions they react with water giving products with a $-CF_2CF_2C(O)$ -fragment instead of initial perfluorocyclopentene ring. The same transformation occurred in wet DMSO.
- [17] Full width at half minimum (FWHM)of the Lorentzian fit.
- [18] The point spread function (PSF) is the spatial intensity distributon of a point-like source imaged by an optical system.
- [19] T. Grotjohann, I. Testa, M. Reuss, T. Brakemann, C. Eggeling, S. W. Hell, S. Jakobs, *eLife* **2012**, 1, e00248, DOI: 10.7554/ eLife.00248.

Received: August 15, 2016 Published online: ■■ ■■, ■■■■



Communications



Communications

VIP

Optical Microscopy

B. Roubinet, M. L. Bossi, P. Alt,
M. Leutenegger, H. Shojaei,
S. Schnorrenberg, S. Nizamov, M. Irie,*
V. N. Belov,* S. W. Hell* ____ IIII-IIII

Carboxylated Photoswitchable Diarylethenes for Biolabeling and Super-Resolution RESOLFT Microscopy



Glow the distance: Diarylethenes with a fluorescent "closed" form and carboxylic groups reversibly switch between "dark" and green-emitting states. In aqueous buffers their antibody conjugates specifically stain cellular structures. RESOLFT microscopy based on reversible saturable (switchable) optically linear fluorescence transitions (induced by UV and blue light) demonstrated optical resolution of 75 nm.

6 www.angewandte.org © 2016 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim Angew. These are not the final page numbers!