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Activation of BODIPY fluorescence by the photoinduced dealkylation of a pyridinium quencher

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The photoinduced cleavage of a 2-nitrobenzyl group from a pyridinium quencher covalently attached to the *meso* position of a BODIPY fluorophore activates the emission of the latter. This photochemical transformation prevents the transfer of one electron from the BODIPY platform to its heterocyclic appendage upon excitation and, as a result, permits the radiative deactivation of the excited fluorophore. This versatile mechanism for fluorescence switching can translate into the realization of an entire family of photoactivatable fluorophores based on the outstanding photophysical properties of BODIPY chromophores.

The borondipyrromethene (BODIPY) platform offers outstanding photophysical properties in conjunction with long-term stability and synthetic versatility.[1–5] In fact, established synthetic protocols permit the manipulation of the substituents on the two pyrrole rings as well as on the boron and carbon atoms joining the two heterocycles. In turn, such structural modifications can be exploited to regulate the spectroscopic signature of the BODIPY chromophore and produce molecules able to emit light across the visible region with quantum efficiencies approaching unity. In addition, the synthetic accessibility of these derivatives permits the integration of the BODIPY skeleton into a diversity of multicomponent molecular and supramolecular constructs. Indeed, BODIPY chromophores have become valuable building blocks for the assembly of electroluminescent materials, fluorescent probes, laser dyes, light harvesters and sensitizing agents.

In spite of the remarkable combination of attractive properties associated with the BODIPY platform, mechanisms to photoactivate its fluorescence remain limited to one representative example.[6] Molecules able to switch from a nonemissive to an emissive state under illumination at an activating wavelength, however, offer the opportunity to switch fluorescence on exclusively within a defined region of space at a given interval of time. Such spatiotemporal control of fluorescence can translate into the possibility of monitoring dynamic events in real time[7–14] as well as recording images with resolution at the nanometer level.[15–22] Indeed, these attractive prospects are stimulating the identification of photochemical strategies to activate the emission of organic dyes.[23–28] In fact, several examples of photoactivatable coumarins,[29–33] diarylethenes,[34,35] dihydrofurans,[36] fluoresceins,[37–44] rhodamines[43,45–48] and spiropyrans[49] have been developed already, relying on either photocleavable protecting groups or photochromic transformations. Structural designs to extend these operating principles for fluorescence switching also to BODIPY chromophores can be a valuable addition to this emerging field of study and might lead to the realization of versatile photoactivatable probes with improved photophysical properties.

Literature data demonstrate that the introduction of a 1-alkylpyridinium-4-yl substituent on the *meso* position of the BODIPY skeleton results in fluorescence suppression.[50,51] In these molecular constructs, the excitation of the BODIPY chromophore is followed by the transfer of one electron to the pyridinium appendage. This process deactivates nonradiatively the excited BODIPY platform and quenches its fluorescence effectively. By contrast, the presence of a pyrid-4-yl ring in the very same position has negligible influence on the emissive behavior of the BODIPY chromophore, which retains its fluorescence with high quantum efficiency. These observations suggest that the photoinduced dealkylation of a pyridinium quencher can be exploited to activate the fluorescence of a BODIPY chromophore under optical control. Indeed, this article reports the implementation of these operating principles for BODIPY photoactivation with a representative example.

The absorption spectrum (a in Fig. 1) of compound **1** shows a band for the BODIPY chromophore at 526 nm in acetonitrile at 25 °C. Upon illumination at an excitation wavelength positioned within this band, the characteristic BODIPY fluorescence appears in the emission spectrum (b in Fig. 1) at 545 nm with

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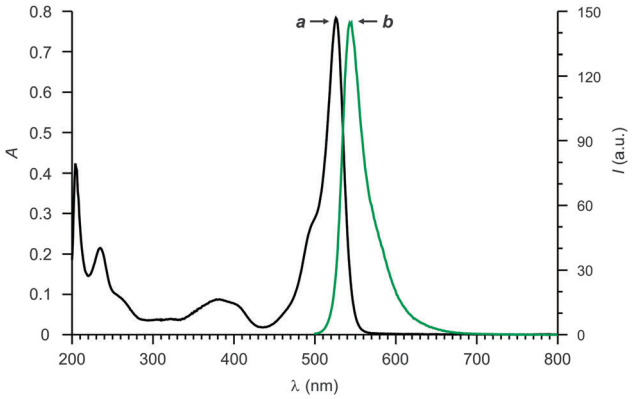

Fig. 1 Absorption (a) and emission (b) spectra of **1** (10 μM, MeCN, 25 °C, λ_{Ex} = 470 nm).

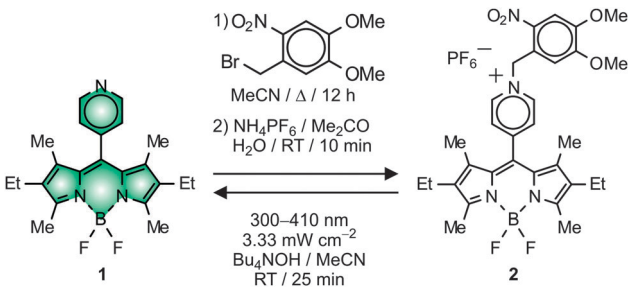

Fig. 2 Synthesis of the hexafluorophosphate salt of **2** from **1** and photoinduced generation of the latter from the former.

a quantum yield of 0.50, in agreement with literature data.[50a] Treatment of this compound with (1-bromomethyl)-4,5-dimethoxy-2-nitrobenzene (Fig. 2) results in the N-alkylation of its pyrid-4-yl appendage. After counterion exchange, the hexafluorophosphate salt of the corresponding pyridinium cation **2** can be isolated in a yield of 17%.

The absorption spectrum (a in Fig. 3) of the hexafluorophosphate salt of **2** reveals a band for the BODIPY chromophore at 534 nm. In contrast to the behavior of **1**, however, illumination at an excitation wavelength positioned within this band does not produce any significant fluorescence (b in Fig. 3). The drastic difference between the emission spectra of the two compounds demonstrates that the cationic pyridinium-4-yl appendage of **2** quenches the excited state of the adjacent BODIPY component, in agreement with literature observations on similar compounds.[50,51] Nonetheless, the characteristic emission band (c–g in Fig. 3) of **1** develops upon ultraviolet illumination of **2**.† The photoinduced enhancement in fluorescence is indicative of the cleavage of the 2-nitrobenzyl group from the nonemissive species **2** (Fig. 2) with the concomitant formation of its emissive counterpart **1**. The temporal evolution of the integrated emission intensity (a in Fig. 4) under irradiation, in the presence of a proton scavenger, indicates the quantum yield for this photochemical transformation to be 0.001.

† Exposure of a solution of **2** to ambient light does not result in any detectable change in fluorescence even after 48 hours.

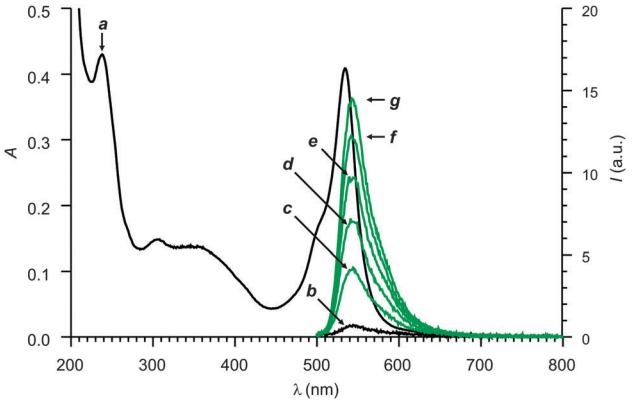

Fig. 3 Absorption (a) and emission (b) spectra of an equimolar solution of the hexafluorophosphate salt of **2** and Bu_4NOH (10 μM, MeCN, 25 °C, λ_{Ex} = 470 nm). Emission spectra of the same solution after irradiation (300–410 nm, 3.33 mW cm^{-2}) for 5 (c), 10 (d), 15 (e), 20 (f) and 25 min (g).

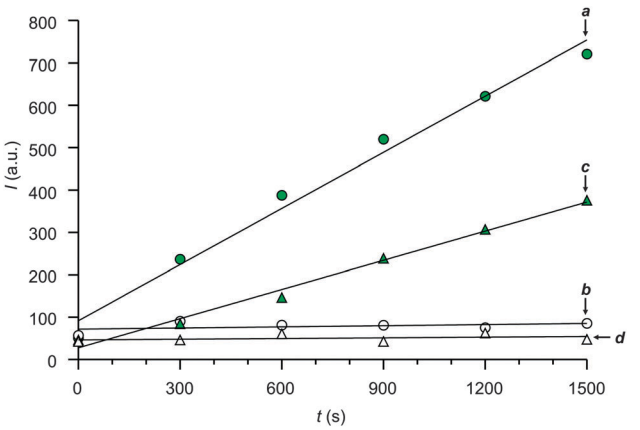

Fig. 4 Evolution of the integrated emission intensity detected for solutions of the hexafluorophosphate salt of **2** (10 μM, MeCN, 25 °C, λ_{Ex} = 470 nm) with (a and b) or without (c and d) Bu_4NOH (1 eq.) under irradiation (a and c, 300–410 nm, 3.33 mW cm^{-2}) or in the dark (b and d).

This value is consistent with the quantum efficiencies reported in the literature for the photoinduced cleavage of the very same 2-nitrobenzyl group from a diversity of compounds.[28] By contrast, the emission intensity (b in Fig. 4) of an identical solution maintained in the dark over the very same period of time does not change, demonstrating that irradiation is essential to activate fluorescence.

The photochemical transformation of the 2-nitrobenzyl group of **2** into the corresponding 2-nitrosobenzaldehyde is accompanied by the release of a proton.[32f] In turn, the protonation of the pyrid-4-yl appendage of **1** is known to quench the emission of the adjacent BODIPY fluorophore.[50b] Furthermore, the literature value (2.8×10^5 M^{-1}) of the association constant for the protonation of **1** in acetonitrile indicates that *ca.* 50% of this compound is protonated at the concentration of the photolysis experiment, in the absence of a proton scavenger. Indeed, the evolutions of the integrated emission intensity during the photolysis of **2** in the presence (a in Fig. 4) and absence

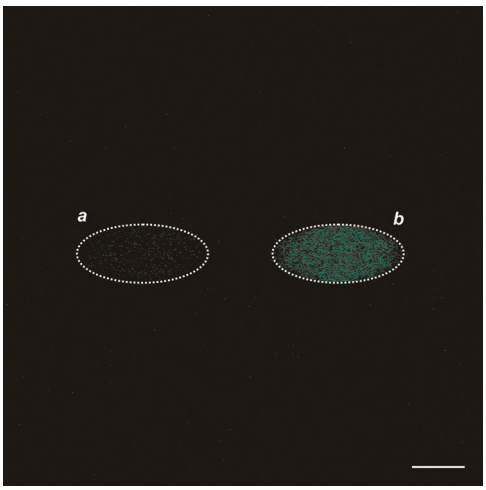

Fig. 5 Confocal laser-scanning fluorescence image (λ_{Em} = 500–700 nm, scale bar = 100 μm) of a solution of the hexafluorophosphate salt of **2** (10 μM, MeCN, 25 °C) recorded upon exclusive illumination of two ellipsoidal areas within the imaging field at 476 (a) and 405 nm (b) respectively.

(c in Fig. 4) of one equivalent of tetrabutylammonium hydroxide are significantly different. Specifically, this particular base scavenges the photogenerated protons and prevents the protonation of the emissive species, ensuring a twofold increase in fluorescence.‡ Furthermore, identical solutions with (b in Fig. 4) and without (d in Fig. 4) base, maintained in the dark, do not reveal any change in the integrated emission intensity over the same period of time of the photolysis experiments. These observations confirm, once again, that ultraviolet irradiation is essential to activate fluorescence and that the presence of base alone has no influence of the emission behavior.

The enhancement in emission intensity associated with the photoinduced transformation of **2** into **1** is also evident from a fluorescence image (Fig. 5) of the corresponding acetonitrile solution, recorded under simultaneous illumination with a pair of independent lasers. Specifically, the irradiation of an ellipsoidal area (a in Fig. 5) within the imaging field at 476 nm results in negligible fluorescence. By contrast, the illumination of an identical and adjacent area (b in Fig. 5) at 405 nm produces significant fluorescence. Indeed, the laser operating at 476 nm can only excite the resulting nonemissive BODIPY of **2**, while that at 405 nm can first cleave the 2-nitrobenzyl group of **2** and then excite the emissive BODIPY of **1**. Consistently, the ratio between the integrated emission intensities of the two areas indicates an eightfold enhancement in fluorescence with excitation at 405 nm, under these experimental conditions.

These results demonstrate that the attachment of a photocleavable quencher, in the form of a pyridinium cation, to the *meso* position of a BODIPY platform translates into the assembly of a photoactivatable fluorophore. Indeed, the photoinduced conversion of the pyridinium-4-yl quencher into a pyrid-4-yl appendage prevents the electron-transfer process responsible for quenching the BODIPY emission and, hence, activates fluorescence. As a result, these viable operating principles for the photochemical manipulation of excitation dynamics, coupled to the synthetic accessibility of such structural design, can lead to the development of an entire family of photoactivatable fluorophores based on the outstanding photophysical properties of BODIPY chromophores.

‡ The fluorescence quantum yield of **1** does not change in the presence of one equivalent of tetrabutylammonium hydroxide.

Experimental procedures

Materials and methods

Chemicals were purchased from commercial sources and used as received with the exception of MeCN, which was distilled over CaH_2. Compound **1** was prepared according to a literature procedure.[50c] Absorption spectra were recorded with a Varian Cary 100 Bio spectrometer, using quartz cells with a path length of 1.0 cm. Emission spectra were recorded with a Varian Cary Eclipse spectrometer in aerated solutions. Samples were irradiated at 300–410 nm (3.33 mW cm^{-2}) within the chamber of a Luzchem Research LZC-4V photoreactor. The fluorescence quantum yield of **1** was measured with a fluorescein standard, following a literature protocol.[52] The quantum yield for the photochemical conversion of **2** into **1** was determined with a potassium ferrioxalate actinometer, according to an established procedure.[53] Electrospray ionization mass spectra were recorded with a Bruker micrOTO-Q II spectrometer. Nuclear magnetic resonance spectra were recorded with a Bruker Avance 400 spectrometer. Fluorescence images were recorded with a Leica SP5 confocal laser-scanning microscope. The imaged solution was positioned between two glass slides separated by a Teflon ring with a thickness of 0.44 mm.

Hexafluorophosphate salt of 2

A mixture of **1** (56 mg, 0.2 mmol) and (1-bromomethyl)-4,5-dimethoxy-2-nitrobenzene (59 mg, 0.3 mmol) in MeCN (10 mL) was heated under reflux for 12 hours. After cooling down to ambient temperature, the solvent was removed under reduced pressure. The residue was suspended in EtOAc (50 mL), washed with H_2O (2 × 20 mL) and brine (20 mL) and dried over Na_2SO_4. The solvent was distilled off under reduced pressure and the residue was purified by column chromatography [Al_2O_3 (activated, neutral), CH_2Cl_2–MeOH (100 : 0 → 95 : 5, v/v)]. The resulting solid was dissolved in CH_2Cl_2 (2 mL). The addition of hexane (10 mL) caused the formation of a precipitate, which was filtered off and dissolved in Me_2CO (5 mL). After the addition of a saturated aqueous solution of NH_4PF_6 (5 mL), the mixture was concentrated under reduced pressure to half of its original volume and the resulting precipitate was filtered off to give the hexafluorophosphate salt of **2** (20 mg, 17%) as a red powder. ESIMS: m/z = 577.2830 $[M]^+$ (m/z calcd for $C_{31}H_{36}BF_2N_4O_4$ = 577.2798); ^{1}H NMR ($CDCl_3$): δ = 0.96 (6H, t, 8 Hz), 1.33 (6H, s), 2.30 (4H, q, 8 Hz), 2.55 (6H, s), 4.04 (3H, s), 4.18 (3H, s), 6.15 (2H, s), 7.72 (1H, s), 7.84 (1H, s), 7.97 (2H, d, 8 Hz), 8.73 (2H, d, 8 Hz); ^{13}C NMR ($CDCl_3$); δ = 11.8, 12.0, 13.4, 13.8, 16.5, 22.4, 29.0, 31.2, 56.3, 56.4, 62.5, 109.3, 116.5, 119.7, 128.8, 132.5, 134.3, 138.2, 141.1, 145.4, 150.7, 153.8, 154.1, 156.0.

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Notes and references

1 (*a*) A. Loudet and K. Burgess, *Chem. Rev.*, 2007, **107**, 4891–4932; (*b*) A. Kamkaew, S. H. Lim, H. B. Lee, L. V. Kiew, L. Y. Chung and K. Burgess, *Chem. Soc. Rev.*, 2013, **42**, 77–88.

2 (*a*) R. Ziessel, G. Ulrich and A. Harriman, *New J. Chem.*, 2007, **31**, 496–501; (*b*) G. Ulrich, R. Ziessel and A. Harriman, *Angew. Chem., Int. Ed.*, 2008, **47**, 1184–1201.

3 M. Benstead, G. H. Mehl and R. W. Boyle, *Tetrahedron*, 2011, **67**, 3573–3601.

4 A. B. Nepomnyashchii and A. J. Bard, *Acc. Chem. Res.*, 2012, **45**, 1844–1853.

5 N. Boens, V. Leen and W. Dehaen, *Chem. Soc. Rev.*, 2012, **41**, 1130–1172.

6 T. Kobayashi, T. Komatsu, M. Kamiya, C. Campos, M. González-Gaitán, T. Terai, K. Hanaoka, T. Nagano and Y. Urano, *J. Am. Chem. Soc.*, 2012, **134**, 11153–11160.

7 (*a*) G. A. Krafft, R. T. Cummings, J. P. Dizio, R. H. Furukawa, L. J. Brvenik, W. R. Sutton and B. R. War, in *Nucleocytoplasmic Transport*, ed. R. Peters and M. Trendelenburg, Springer-Verlag, Berlin, 1986, pp. 35–52; (*b*) B. R. Ware, L. J. Brvenik, R. T. Cummings, R. H. Furukawa and G. A. Krafft, in *Applications of Fluorescence in the Biomedical Sciences*, ed. D. L. Taylor, A. S. Waggoner, R. F. Murphy, F. Lanni and R. B. Birge, Liss, New York, 1986, pp. 141–157.

8 S. R. Adams and R. Y. Tsien, *Annu. Rev. Physiol.*, 1993, **55**, 755–784.

9 J. P. Y. Kao and S. R. Adams, in *Optical Microscopy: Emerging Methods and Applications*, ed. B. Herman and J. J. Lemasters, Academic Press, San Diego, 1993, pp. 27–85.

10 J. C. Politz, *Trends Cell Biol.*, 1999, **9**, 284–287.

11 R. W. Dirks, C. Molenaar and H. J. Tanke, *Histochem. Cell Biol.*, 2001, **115**, 3–11.

12 *Dynamic Studies in Biology: Phototriggers, Photoswitches and Caged Biomolecules*, ed. M. Goeldner and R. Givens, Wiley-VCH, Weinheim, 2005.

13 G. C. Ellis-Davies, *Nat. Methods*, 2007, **4**, 619–628.

14 Y. Xu, T. J. Melia and D. T. Toomre, *Curr. Opin. Chem. Biol.*, 2011, **15**, 822–830.

15 M. Fernández-Suárez and A. Y. Ting, *Nat. Rev. Mol. Cell Biol.*, 2008, **9**, 929–943.

16 B. Huang, M. Bates and X. Zhuang, *Annu. Rev. Biochem.*, 2009, **78**, 993–1016.

17 S. W. Hell, *Nat. Methods*, 2009, **6**, 24–32.

18 D. Toomre and J. Bewersdorf, *Annu. Rev. Cell Dev. Biol.*, 2010, **26**, 285–314.

19 S. van de Linde, M. Heilemann and M. Sauer, *Annu. Rev. Phys. Chem.*, 2012, **63**, 519–540.

20 T. Ha and P. Tinnefeld, *Annu. Rev. Phys. Chem.*, 2012, **63**, 595–617.

21 W. E. Moerner, *J. Microsc.*, 2012, **246**, 213–220.

22 F. M. Raymo, *J. Phys. Chem. Lett.*, 2012, **3**, 2379–2385.

23 T. J. Mitchison, K. E. Sawin, J. A. Theriot, K. Gee and A. Mallavarapu, *Methods Enzymol.*, 1998, **291**, 63–78.

24 L. M. Wysocki and L. D. Davis, *Curr. Opin. Chem. Biol.*, 2011, **15**, 752–759.

25 D. Puliti, D. Warther, C. Orange, A. Specht and M. Goeldner, *Bioorg. Med. Chem.*, 2011, **19**, 1023–1029.

26 F. M. Raymo, *ISRN Phys. Chem.*, 2012, 619251.

27 W.-H. Li and G. Zheng, *Photochem. Photobiol. Sci.*, 2012, **11**, 460–471.

28 P. Klán, T. Šolomek, C. G. Bochet, A. Blanc, R. Givens, M. Rubina, V. Popik, A. Kostikov and J. Wirz, *Chem. Rev.*, 2013, **113**, 119–191.

29 (*a*) Y. Zhao, Q. Zheng, K. Dakin, K. Xu, M. L. Martinez and W.-H. Li, *J. Am. Chem. Soc.*, 2004, **126**, 4653–4663; (*b*) G. Zheng, L. Cochella, J. Liu, O. Hobert and W.-H. Li, *ACS Chem. Biol.*, 2011, **6**, 1332–1338.

30 (*a*) N. Gagey, P. Neveu and L. Jullien, *Angew. Chem., Int. Ed.*, 2007, **46**, 2467–2469; (*b*) N. Gagey, M. Emond, P. Neveu, C. Benbrahim, B. Goetz, I. Aujard, J.-B. Baudin and L. Jullien, *Org. Lett.*, 2008, **10**, 2341–2344.

31 C. Orange, A. Specht, D. Puliti, E. Sakr, T. Furuta, B. Winsor and M. Goeldner, *Chem. Commun.*, 2008, 1217–1219.

32 (*a*) E. Deniz, S. Sortino and F. M. Raymo, *J. Phys. Chem. Lett.*, 2010, **1**, 3506–3509; (*b*) E. Deniz, M. Tomasulo, J. Cusido, S. Sortino and F. M. Raymo, *Langmuir*, 2011, **27**, 11773–11783; (*c*) E. Deniz, M. Tomasulo, J. Cusido, I. Yildiz, M. Petriella, M. L. Bossi, S. Sortino and F. M. Raymo, *J. Phys. Chem. C*, 2012, **116**, 6058–6068; (*d*) J. Cusido, M. Battal, E. Deniz, I. Yildiz, S. Sortino and F. M. Raymo, *Chem.–Eur. J.*, 2012, **18**, 10399–10407; (*e*) E. Deniz, N. Kandoth, A. Fraix, V. Cardile, A. C. E. Graziano, D. Lo Furno, R. Gref, F. M. Raymo and S. Sortino, *Chem.–Eur. J.*, 2012, **18**, 15782–15787; (*f*) S. Swaminathan, M. Petriella, E. Deniz, J. Cusido, J. D. Baker, M. L. Bossi and F. M. Raymo, *J. Phys. Chem. A*, 2012, **116**, 9928–9933.

33 X.-Y. Duan, B.-C. Zhai and Q.-H. Song, *Photochem. Photobiol. Sci.*, 2012, **11**, 593–598.

34 (*a*) Y.-C. Jeong, S. I. Yang, K.-H. Ahn and E. Kim, *Chem. Commun.*, 2005, 2503–2505; (*b*) Y.-C. Jeong, S. I. Yang, E. Kim and K.-H. Ahn, *Tetrahedron*, 2006, **62**, 5855–5861.

35 K. Uno, H. Niikura, M. Morimoto, Y. Ishibashi, H. Miyasaka and M. Irie, *J. Am. Chem. Soc.*, 2011, **133**, 13558–13564.

36 (*a*) S. J. Lord, N. R. Conley, H. D. Lee, R. Samuel, N. Liu, R. J. Twieg and W. E. Moerner, *J. Am. Chem. Soc.*, 2008, **130**, 9204–9205; (*b*) S. J. Lord, N. R. Conley, H. D. Lee, S. Y. Nishimura, A. K. Pomerantz, K. A. Willets, Z. Lu, H. Wang, N. Liu, R. Samuel, R. Weber, A. Semyonov, M. He, R. J. Twieg and W. E. Moerner, *ChemPhysChem*, 2009, **10**, 55–65; (*c*) S. J. Lord, H. D. Lee, R. Samuel, R. Weber, N. Liu, N. R. Conley, M. A. Thompson, R. J. Twieg and W. E. Moerner, *J. Phys. Chem. B*, 2010, **114**, 14157–14167.

37 G. A. Krafft, W. R. Sutton and R. T. Cummings, *J. Am. Chem. Soc.*, 1988, **110**, 301–303.

38 T. J. Mitchison, *J. Cell Biol.*, 1989, **109**, 637–652.
39 J. P. Vincent and P. H. O'Farrell, *Cell*, 1992, **68**, 923–931.
40 J.-P. Pellois, M. E. Hahn and T. W. Muir, *J. Am. Chem. Soc.*, 2004, **126**, 7170–7171.
41 T. Kobayashi, Y. Urano, M. Kamiya, T. Ueno, H. Kojima and T. Nagano, *J. Am. Chem. Soc.*, 2007, **129**, 6696–6697.
42 G. Zheng, Y.-M. Guo and W.-H. Li, *J. Am. Chem. Soc.*, 2007, **129**, 10616–10617.
43 D. Maurel, S. Banala, T. Laroche and K. Johnsson, *ACS Chem. Biol.*, 2010, **5**, 507–516.
44 L. Yuan, W. Lin, Z. Cao, L. Long and J. Song, *Chem.–Eur. J.*, 2011, **17**, 689–696.
45 J. Ottl, D. Gabriel and G. Marriott, *Bioconjugate Chem.*, 1998, **9**, 143–151.
46 K. R. Gee, E. S. Weinberg and D. Kozlowski, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2181–2183.
47 (*a*) J. Fölling, V. N. Belov, R. Kunetsky, R. Medda, A. Schönle, A. Egner, C. Eggeling, M. Bossi and S. W. Hell, *Angew. Chem., Int. Ed.*, 2007, **46**, 6266–6270; (*b*) J. Fölling, V. N. Belov, D. Riedel, A. Schönle, A. Egner, C. Eggeling, M. Bossi and S. W. Hell, *ChemPhysChem*, 2008, **9**, 321–326; (*c*) I. Testa, A. Schönle, C. von Middendorf, C. Geisler, R. Medda, C. A. Wurm, A. C. Stiel, S. Jakobs, M. L. Bossi, C. Eggeling, S. W. Hell and A. Egner, *Opt. Express*, 2008, **16**, 21093–21104; (*d*) V. N. Belov, M. L. Bossi, J. Fölling, V. P. Boyarskiy and S. W. Hell, *Chem.–Eur. J.*, 2009, **15**, 10762–10776; (*e*) V. N. Belov, C. A. Wurm, V. P. Boyarskiy, S. Jakobs and S. W. Hell, *Angew. Chem., Int. Ed.*, 2010, **49**, 3520–3523; (*f*) K. Kolmakov, C. Wurm, M. V. Sednev, M. L. Bossi, V. N. Belov and S. W. Hell, *Photochem. Photobiol. Sci.*, 2012, **11**, 522–532; (*g*) H. Montenegro, M. D. Paolo, D. Capdevila, P. F. Aramendía and M. L. Bossi, *Photochem. Photobiol. Sci.*, 2012, **11**, 1081–1086.
48 L. M. Wysocki, J. B. Grimm, A. N. Tkachuk, T. A. Brown, E. Betzig and L. D. Lavis, *Angew. Chem., Int. Ed.*, 2011, **50**, 11206–11209.
49 (*a*) D. Hu, Z. Tian, W. Wu, W. Wan and A. D. Q. Li, *J. Am. Chem. Soc.*, 2008, **130**, 15279–15281; (*b*) Z. Tian, A. D. Q. Li and D. Hu, *Chem. Commun.*, 2011, **47**, 1258–1260.
50 (*a*) G. Ulrich and R. Ziessel, *J. Org. Chem.*, 2004, **69**, 2070–2083; (*b*) A. Harriman, L. J. Mallon, G. Ulrich and R. Ziessel, *ChemPhysChem*, 2007, **8**, 1207–1214; (*c*) M. A. H. Alamiry, A. Harriman, L. J. Mallon, G. Ulrich and R. Ziessel, *Eur. J. Org. Chem.*, 2008, 2774–2782.
51 D. Wang, R. Miyamoto, Y. Shiraishi and T. Hirai, *Langmuir*, 2009, **25**, 13176–13182.
52 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New York, 2006.
53 J. C. Scaiano, *Handbook of Organic Photochemistry*, CRC Press, Florida, 1989.