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Photoactivatable turn-on fluorescence based on the photo-cleavage of the C–Br bond in 1,2-bis(5-(bromoethyl)benzoxazol-2-yl)ethane

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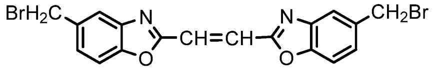
This paper describes photoactivatable turn-on fluorescence based on the photo-cleavage of the C–Br bond in 1,2-bis(5-(bromoethyl)benzoxazol-2-yl)ethane (**1**) in both solution and in a polymeric thin film. It was found that **1** exhibited very weak fluorescence in both solution and in a PMMA thin film, upon irradiation with 365 nm light, a strong fluorescence was detected. The strong fluorescence attributed to the photo-product resulted from the photo-induced cleavage of the C–Br bond in **1**. Moreover, it was also found that the photo-cleavage of the C–Br bond in **1** can be performed using a two-photon induced process, and upon irradiation on a **1**-PMMA thin film with a 780 nm femtosecond laser, finely resolved fluorescence images are obtained.

Introduction

Photoactivatable organic fluorophores play an important role in biology[1–4] and material science.[5–7] Several strategies have been reported in the design of photoactivatable organic fluorophores including photorelease of fluorophores from the photolabile linkers,[8] photo-uncaging of "caged" fluorophores,[9–13] and photoswitching of fluorophores based on the photoisomerization reaction.[14,15] In these systems, the latent fluorophores are already in place, which may compromise the photoactivation efficiency due to the filtering effect.[16] Recent advance in the super-resolution microscopic techniques such as photoactivated localization microscopy (PALM)[17] and stochastic optical reconstruction microscopy (STORM)[18] also demands new strategies for designing photoactivatable fluorophores with turn-on efficiency. Some elegant examples of photoactivatable fluorophores that exhibit high turn-on fluorescence and are suitable for super-resolution imaging have been reported.[19,20]

In this paper, we report a system in which the fluorophores are generated *in situ* through photo-triggered bond-breaking reaction. Photo-triggered, especially, two-photon-triggered bond-breaking appears as a promising candidate since it can provide a powerful tool with spatial and temporal control, resulting in many practical applications such as photolithography,[21–24] optical storage[25–29] and photorelease.[30–32] Herein, 1,2-bis(5-(bromomethyl)benzoxazol-2-yl)ethane **1** (Scheme 1), used as a model compound, is prepared, it is found that **1** undergoes single- or two-photon-induced cleavage of C–Br bond resulting in fluorophores as photolysis products in both solution and a polymeric thin film. A strong turn-on fluorescence is beneficial for applications, such as fluorescence imaging; with a 780 nm femtosecond laser irradiation on a **1**-PMMA thin film, finely resolved fluorescence images are obtained.



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Scheme 1 Chemical structure of **1**.

Experimental

General

^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, with TMS as an internal reference. MS spectra were recorded on a TOC-MS spectrometer. UV absorption spectra and fluorescence spectra were measured on an absorption spectrophotometer (Hitachi U-3010) and a fluorescence spectrophotometer (F-2500), respectively. Fabrication in a **1**-PMMA thin film using a two-photon process was performed on a Ti–sapphire laser system from Tsunami, Spectra-Physics. The output from the femtosecond laser (tuned to 780 nm, 100 fs pulse width, 80 MHz repetition rate) was used as two-photon writing source.

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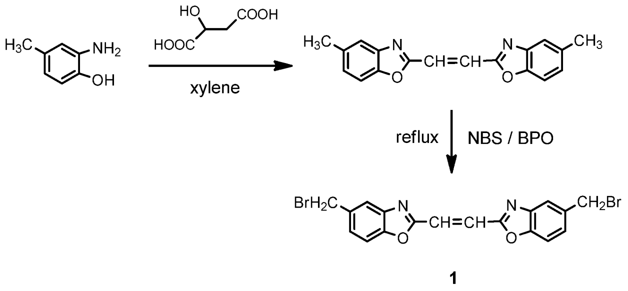
In the fabrication process, a 3D piezostage (P-563.3CD, Physik Instrument) was used as the scanning device. The laser beam was tightly focused using a high numerical aperture (NA) objective lens (100×, NA = 1.4, Olympus), and the scanning speed is 10 μm s^{-1}. Fluorescence imaging was performed on a fluorescence microscope (Ti-U, CFI Plan Fluor 40X, NA = 0.75, Nikon) equipped with a Color Cooled Digital Camera (DS-Ri1, Nikon). A metal mercury lamp (C-LHGFI, Nikon) was used as an excitation source with an excitation filter (340–380 nm) and absorbing filters (435–485 nm). All the chemicals for the synthesis were purchased from commercial suppliers, and solvents were purified according to standard procedures. The reaction was monitored using TLC silica gel plates (60F-254). Column chromatography was performed on silica gel (70–230 mesh). PMMA thin films were prepared as follows: the target compound **1** (1.0 mg) was dissolved in 1.0 ml of a PMMA–cyclohexanone solution (10%, w/w). A film was obtained by spin coating on quartz glass with a gradient of 700 rpm (10 s), followed by 1200 rpm (30 s) (20 °C), and dried in air at room temperature. The concentration of the thin film was about 2.24×10^{-6} mol g^{-1}.

Material

The synthetic route for target compound **1** is presented in Scheme 2 and detailed procedure was as follows: (1) to the solution of 2-amino-4-methylphenol (10 mmol) in xylene (100 ml) was added 2-hydroxysuccinic acid (10 mmol) and H_3BO_3 (0.1 mmol). The solution was refluxed till no water was distilled; the solution was then cooled down to room temperature. The solvent was removed by rotary evaporation, and the residue was recrystallized from *N*,*N*-dimethylformamide (DMF) to afford 1,2-bis-(5-methylbenzoxazol-2-yl)ethane in 56% yield. (2) To the solution of 1,2-bis(5-methylbenzooxazol-2-yl)ethane (10 mmol) in DMF (50 ml) was added *N*-bromosuccinimide (22 mmol) and benzoyl peroxide (0.1 mmol). The mixture was refluxed for 6 h till the starting material had disappeared (detected by a TLC plate). The reaction mixture was cooled, and the yellow product was filtered off, and washed with water and ethanol, respectively. The crude was purified by recrystallization with EtOH to afford the target compound 1,2-bis(5-(bromoethyl)benzoxazol-2-yl)ethane (**1**) in 80% yield. M.p. = 283–286 °C. ^{1}H NMR (DMSO-d_6): δ (ppm) 7.82 (s, 2H), 7.76 (d, 2H, *J* = 8.0 Hz), 7.54 (m, 4H), 4.40 (s, 4H). ^{13}C NMR (DMSO-d_6): δ (ppm) 156.3, 150.2, 142.2, 134.6, 131.8, 125.7, 116.4, 108.7, 34.5. TOC-MS (*m*/*z*): [M^+] calcd. For $C_{18}H_{12}Br_2N_2O_2$: 445.9266. Found: 445.9261.



Scheme 2 Synthesis of the target compound **1**.

The photolysis products **2** and **3** were prepared by photolysis of the solution of **1** with a 500 W high-pressure mercury lamp (wavelength ≤ 400 nm, exposure: 39. 5 mW cm^{-2}) in a glass beaker of 500 ml. The solution of **1** (30 mg) in EtOH or CH_3CN (300 ml) was stirred under light irradiation till no starting material was detected using a TLC plate (irradiation time 6 h). After evaporation of the solvent, the product was purified using a flash column (elute: dichloromethane/methanol = 20 : 1, v/v) to afford **2** and **3**, respectively.

2. Yield: 40%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.52 (s, 2H), 7.76 (d, 2H, *J* = 8.0 Hz), 7.54 (m, 4H), 4.63 (s, 4H), 4.46–4.41 (q, 4H), 1.46–1.42 (t, 6H). ^{13}C NMR ($CDCl_3$): δ (ppm) 156.5, 150.8, 143.7, 134.3, 131.8, 126.7, 117.2, 109.3, 74.7, 68.9, 17.5. TOC-MS (*m*/*z*): [M^+] calcd. For $C_{22}H_{22}N_2O_4$: 378.1580. Found: 378.1588.

3. Yield: 34%. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 10.05 (s, 2H), 8.13 (s, 2H), 7.68 (m, 4H), 7.65 (d, *J* = 8.0 Hz, 2H). IR: ν (cm^{-1}): 3074.26, 2926.47, 1687.96 (strong), 1608.23, 1523.28, 1384.18, 1159.07. TOC-MS (*m*/*z*): [M^+] calcd. For $C_{18}H_{10}N_2O_4$: 318.0641. Found: 318.0658.

Results and discussion

The optical properties of **1** were measured in EtOH solution (10 μM) at ambient temperature (25 °C). The absorption spectral (Fig. 1) showed that the maximum absorption of **1** appeared at λ_{max} = 356 nm ($\varepsilon_{max} = 2.54 \times 10^4$ M^{-1} cm^{-1}). Upon irradiation with 365 nm light as the excitation wavelength, a weak fluorescence which appeared at λ_{em} = 422 nm was detected (Fig. 1), and a very small fluorescence quantum yield of **1** (ϕ_f = 0.04, in EtOH) was obtained using quinine sulfate (ϕ_f = 0.557, in H_2SO_4) as the reference.

The magnitude of the turn-on fluorescence was measured by the photo-induced reaction of **1** in EtOH solution (10 μM) with 365 nm light irradiation. Upon irradiation with 365 nm light, the fluorescence intensity at 422 nm was increased, as shown in Fig. 2, the intensity of fluorescence increased with the increase of irradiation time, and the largest fluorescence intensity was obtained when the solution of **1** was irradiated for 180 s. Comparing the intensity of fluorescence before and after irradiation

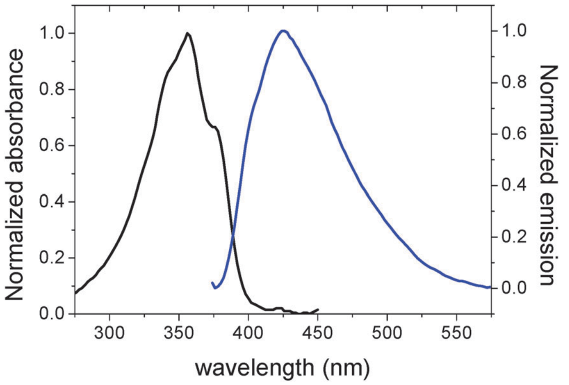


Fig. 1 Absorption and fluorescence of **1** (10 μM) in EtOH, λ_{ex} = 365 nm.

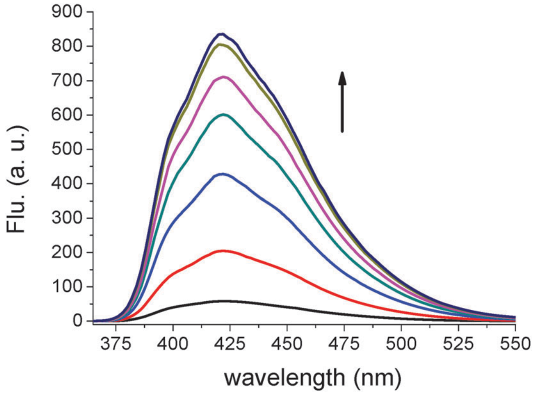
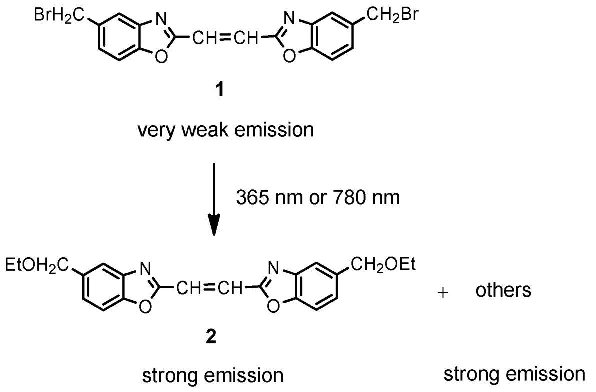


Fig. 2 Fluorescence changes of **1** (10 μM, in EtOH) irradiating with 365 nm light (periods: 0, 30, 60, 90, 120, 150, 180 s), λ_{ex} = 365 nm.

it was found that a 15.6-fold fluorescence turn-on effect was obtained.

To investigate the mechanism of the photo-reaction of **1** and to elucidate the fluorescence properties of the product, the photolysis of **1** in EtOH is performed. Upon irradiation of solution of **1** in EtOH under a high pressure Hg lamp with wavelength filter ($\lambda \leq 400$ nm), more than three new spots were detected using the TLC plate, and all of them showed strong blue fluorescence. The main product **2** (Scheme 3) was obtained by chromatography and its structure was identified using H NMR spectroscopy and MS spectroscopy (see Experimental section). The confirmed structure of **2** demonstrated that **1** underwent the cleavage of C–Br bond upon irradiation in solution. It was found that **2** exhibited a strong emission appeared at λ_{em} = 422 nm (Fig. 3), and a large fluorescence quantum yield (ϕ_f = 0.63, in EtOH) was obtained using quinine sulfate (ϕ_f = 0.557, in H_2SO_4) as the reference. Comparing Fig. 3 with Fig. 1 it was found that both the spectral profile and the position of peaks are similar, this is because the skeletal structure of the molecule is not changed when Br in **1** is replaced by OEt. An enhancement fluorescence of **2** is probably because of the leave of Br in **1**. It is known that the fluorescence is usually quenched by internal heavy-atoms such as Br and I due to intersystem crossing.



Scheme 3 Photolysis of **1** in EtOH with 365 nm light and 780 nm light, respectively.

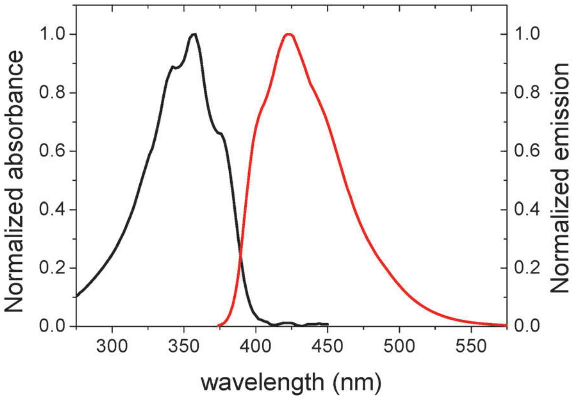
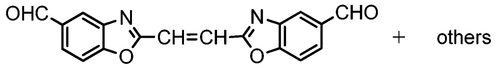


Fig. 3 Absorption and fluorescence of **2** (10 μM) in EtOH, λ_{ex} = 365 nm.



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major

Scheme 4 Photolytic product of **1** in CH_3CN or toluene with 365 nm light or 780 nm light, respectively.

A different photolytic product (main product) was obtained when the photo-induced reaction of **1** in an aprotic solvent such as CH_3CN or toluene. 2,2′-(Ethane-1,2-diyl)bis(benzooazole-5-carbaldehyde) (**3**) (Scheme 4) as main product was obtained when the photochemical reaction of **1** was conducted in CH_3CN or toluene solution with 365 nm irradiation (its characterization data are presented in Experimental section). It was found that **3** also showed a strong fluorescence in CH_3CN (Fig. 4) with the maximum emission at 424 nm (a similar result was obtained in toluene), which is similar to the emission of **2** in EtOH. The photolytic compound **3** was probably formed *via* a two-step reaction mechanism: photolysis of **1** produced free radicals, followed by oxidation with oxygen in the air. The obtained result suggested that the photo-induced cleavage of C–Br bond in **1** could also be performed in aprotic solvents, and also resulted in a strong fluorescent compound as the main product.

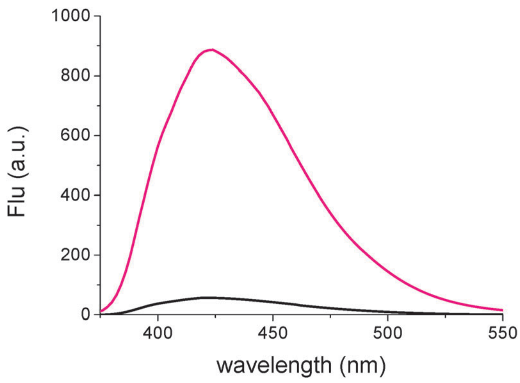

Fig. 4 Fluorescence change of **1** (10 μM, in CH_3CN) before (black) and after (red) irradiation (20 min) with 365 nm light (λ_{ex} = 365 nm).

It is worth noting that the turn on fluorescence was also detected when the solution of **1** was irradiated with 780 nm femtosecond laser, which implied that the cleavage of C–Br bond of **1** could also be performed by a two-photon induced process. Control experiments confirmed that **2** or **3** as main product together with other photolysis products were detected using a TLC plate when the solution of **1** in EtOH or CH_3CN was irradiated with a 780 nm femtosecond laser. Further investigation found that **1** exhibited nonlinear optical properties (NLO), and a small two-photon absorption cross-section (σ = 20 GM) in EtOH was obtained using the 800 nm femtosecond Z-scan technique.[33,34]

The photoactivatable turn-on fluorescence of **1** in the polymeric matrix was performed by doping **1** in a PMMA thin film. **1**-PMMA thin film showed very weak fluorescence, which appeared at 418 nm, as compared to in solution, the fluorescence of **1** in the PMMA thin film occurred 4 nm blue shift. Upon irradiation with 365 nm light, the intensity at 418 nm was increased (Fig. 5), and the largest fluorescence intensity was obtained after the **1**-PMMA thin film was irradiated for 12 min under 365 nm light (3.7 mW cm^{-2}). As shown in Fig. 5, photon-induced turn on fluorescence spectral change of **1** in the PMMA thin film showed a similar profile to that of **1** in solution, which implied that the C–Br bond of **1** in the PMMA thin film was also cleaved by photo-induction.

Two-photon induced turn on fluorescence and consequent fluorescence microscopic imaging were performed on the **1**-PMMA thin film, which was obtained by spin coating a solution of PMMA (in cyclohexanone) contained **1** on a quartz glass substrate. A two-photon fabrication pattern was completed by a femtosecond laser (writing wavelength: 780 nm, 100 fs pulse width, 80 MHz repetition rate, scanning speed: 10 μm s^{-1}) with a different power. Upon excitation, a finely resolved fluorescent pattern in which written portions appeared bright while the unwritten portions that appeared dark was obtained (Fig. 6) under fluorescence microscopy (Ti-U, CFI Plan Fluor 40X, N.A. 0.75, Nikon) with an color cooled digital camera. A metal mercury lamp was used as the excitation source with a excitation filter (340–380 nm). As presented in Fig. 6, the laser power used for writing plays a role in both the size of the pattern lines and fluorescence imaging. The width of lines was enhanced with the increase of laser writing power due to the following fact: two-photo absorption probability is proportional to the square of light intensity, therefore, the fabricated line-width is increased with the increase of laser writing power.[35,36] Rough calculations showed that the width of the line in the **1**-PMMA thin film widened double when the laser writing power was increased from 1.66 mW to 5.13 mW.

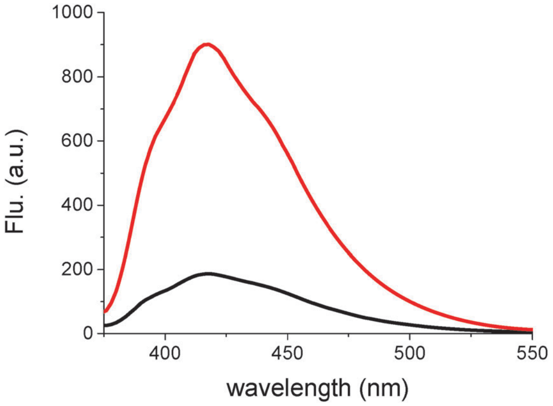

Fig. 5 Fluorescence change of the **1**-PMMA thin film before (black) and after (red) irradiation with 365 nm light (λ_{ex} = 365 nm).

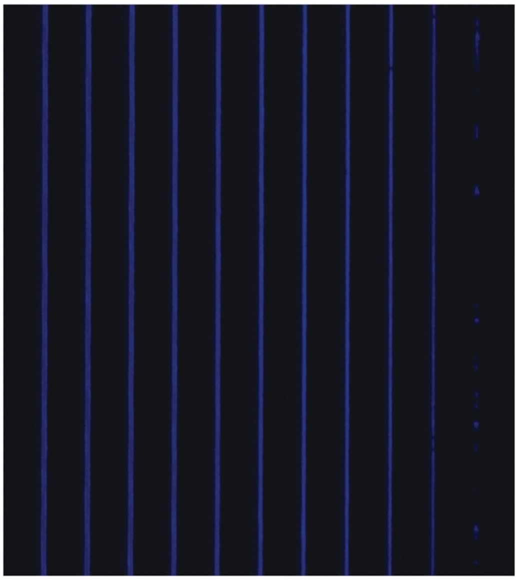

Fig. 6 Fluorescence imaging on the **1**-PMMA with 780 nm femtosecond laser irradiation at different laser powers (from right to left: 1.18, 1.40, 1.66, 1.98, 2.39, 2.96, 3.60, 4.38, 5.13, 5.52, 5.53 mW).

Further investigation showed that the laser writing power also affects the photo-induced fluorescence of the **1**-PMMA thin film. As presented in Fig. 7, the fluorescence intensity increased with the increase of laser power till it reached *ca.* 2.0 mW. Fig. 7 also showed that the fluorescence intensity was slightly decreased when the laser power is between 3.6–5.5 mW. The decrease of fluorescence probably resulted from the damage (photo-degradation) of material (fluorescent molecules) due to

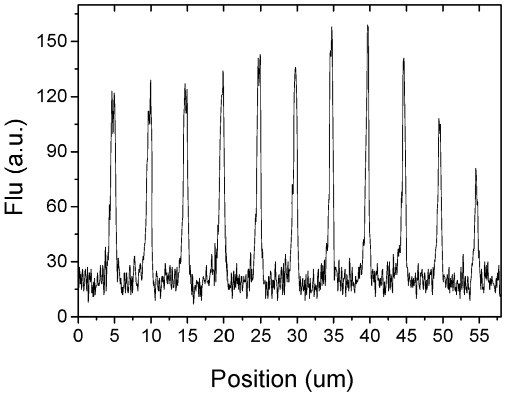

Fig. 7 Two-photon induced fluorescence intensity *vs.* the laser powers at 780 nm (from right to left: 1.18, 1.40, 1.66, 1.98, 2.39, 2.96, 3.60, 4.38, 5.15, 5.52, 5.53 mW).

high laser power. To confirm it, larger laser power was used for fabrication experiment, and it was found that the fluorescence of lines in the **1**-PMMA thin film was significantly decreased when the laser writing power is more than 6.5 mW, which suggests that too high laser power will degrade the material and results in the decrease of fluorescence. Besides, preliminary experiment showed that the threshold writing power for the fabrication is *ca.* 1.40 mW, below the power, it was difficult to obtain a completed line (see Fig. 6, the first line on the right). This is probably because the photo-cleavage of C–Br bond in the **1**-PMMA thin film could not be performed when the laser writing power was too small.

Conclusions

In summary, a new system for photoactivatable turn-on fluorescence has been developed. It has demonstrated that 1,2-bis(5-(bromoethyl)-benzoxazol-2-yl)ethane undergoes single- and two-photon-induced the cleavage of C–Br bond in both solution and polymeric matrix, resulting in significant enhancement of fluorescence. Photo-activatable turn-on fluorescence is beneficial for fluorescent images, with 780 nm femtosecond laser irradiation; finely resolved fluorescence images on polymeric thin films containing 1,2-bis-(5-(bromoethyl)benzoxazol-2-yl)ethane are obtained.

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Notes and references

1 M. Fernandez-Suarez and A. Y. Ting, *Nat. Rev. Mol. Cell Biol.*, 2008, **9**, 929.
2 E. D. Prieto and H. A. Garda, *Biochemistry*, 2011, **50**, 466.
3 H.-M. Lee, D. R. Larson and D. S. Lawrence, *ACS Chem. Biol.*, 2009, **4**, 409.
4 R. H. Newman, M. D. Fosbrink and J. Zhang, *Chem. Rev.*, 2011, **111**, 3614.
5 R. Sakai, A. Nagai, Y. Tago, S. Sato, Y. Nishimura, T. Arai, T. Satoh and T. Kakuchi, *Macromolecules*, 2012, **45**, 4122.
6 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.
7 P. Yang and W. Yang, *Chem. Rev.*, 2013, **113**, 5547.
8 D. Maurel, S. Banala, T. Laroche and K. Johnsson, *ACS Chem. Biol.*, 2010, **5**, 507.
9 G. A. Krafft, W. R. Sutton and R. T. Cummings, *J. Am. Chem. Soc.*, 1988, **110**, 301.
10 Y. Zhao, Q. Zheng, K. Dakin, K. Xu, M. L. Martinez and W. H. Li, *J. Am. Chem. Soc.*, 2004, **126**, 4653.
11 K. R. Gee, E. S. Weinberg and D. J. Kozlowski, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2181.
12 G. Zheng, Y. M. Guo and W. H. Li, *J. Am. Chem. Soc.*, 2007, **129**, 10616.
13 R. H. Pawle, V. Eastman and S. W. Thomas III, *J. Mater. Chem.*, 2011, **21**, 14041.
14 J. Folling, V. Belov, R. Kunetsky, R. Medda, A. Schonle, A. Egner, C. Eggeling, M. Bossi and S. W. Hell, *Angew. Chem., Int. Ed.*, 2007, **46**, 6266.
15 N. Xie and Y. Chen, *J. Mater. Chem.*, 2007, **17**, 861.
16 A. E. Keating and M. A. Garcia-Garibay, in *Organic and Inorganic Photochemistry*, ed. V. Ramamurthy and K. Schanze, Marcel Dekker, New York, 1998, vol. 2, pp. 195–248 and references therein.
17 E. Betzig, G. H. Patterson, R. Sougrat, O. W. Lindwasser, S. Olenych, J. S. Bonifacino, M. W. Davidson, J. Lippincott-Schwartz and H. F. Hess, *Science*, 2006, **313**, 1642.
18 M. J. Rust, M. Bates and X. Zhuang, *Nat. Methods*, 2006, **3**, 793.
19 H. L. Lee, S. J. Lord, S. Iwanaga, K. Zhan, H. Xie, J. C. Williams, H. Wang, G. R. Bowman, E. D. Goley, L. Shapiro, R. J. Twieg, J. Rao and W. E. Moerner, *J. Am. Chem. Soc.*, 2010, **132**, 15099.
20 Z. Yu, L. Y. Ho and Q. Lin, *J. Am. Chem. Soc.*, 2011, **133**, 11912.
21 L. Ionov and S. Diez, *J. Am. Chem. Soc.*, 2009, **131**, 13315.
22 C. Zhou, P. R. Rajasekaran, J. Wolff, X. Li and P. Kohli, *Langmuir*, 2010, **26**, 17726.
23 P. Prompinit, A. S. Achalkumar, X. Han, R. J. Bushby, C. Wälti and S. D. Evans, *J. Phys. Chem. C*, 2009, **113**, 21642.
24 E. Deniz, M. Tomasulo, R. A. DeFazio, B. D. Watson and F. M. Raymo, *Phys. Chem. Chem. Phys.*, 2010, **12**, 11630.
25 A. S. Dvornikov, E. P. Walker and P. M. Rentzepis, *J. Phys. Chem. A*, 2009, **113**, 13633.
26 K. D. Belfield and K. J. Schafer, *Chem. Mater.*, 2002, **14**, 3656.
27 C. C. Corredor, Z.-L. Huang, K. D. Belfield, A. R. Morales and M. V. Bondar, *Chem. Mater.*, 2007, **19**, 5165.
28 C. O. Yanez, C. D. Andrade, S. Yao, G. Luchita, M. V. Bondar and K. D. Belfield, *ACS Appl. Mater. Interfaces*, 2009, **1**, 2219.
29 T. Kopplmayr, M. Cardinale, G. Jakopic, G. Trimmel, W. Kern and T. Griesser, *J. Mater. Chem.*, 2011, **21**, 2965.
30 J. Cui, R. A. Gropeanu, D. R. Stevens, J. Rettig and A. del Campo, *J. Am. Chem. Soc.*, 2012, **134**, 7733.
31 J. B. Edson, L. P. Spencer and J. M. Boncella, *Org. Lett.*, 2011, **13**, 6156.
32 X. Zhang and Y. Chen, *Phys. Chem. Chem. Phys.*, 2010, **12**, 1177.
33 J. Szeremeta, R. Kolkowski, M. Nyk and M. Samoc, *J. Phys. Chem. C*, 2013, **117**, 26197.
34 D. Sajan, N. Vijayan, K. Safakath, R. Philip and I. Hubert Joe, *J. Phys. Chem. A*, 2011, **115**, 8216.
35 H. B. Sun, T. Tanaka and S. Kawata, *Appl. Phys. Lett.*, 2002, **80**, 3673.
36 S. H. Wu, J. Serbin and M. Gu, *J. Photochem. Photobiol., A*, 2006, **181**, 1.