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Fluorescent Dyes

Through-Bond Energy Transfer Cassettes with Minimal Spectral Overlap between the Donor Emission and Acceptor Absorption: Coumarin–Rhodamine Dyads with Large Pseudo-Stokes Shifts and Emission Shifts**

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Small-molecule organic dyes have been widely used in fluorescent probes,[1] labels,[2] logic gates,[3] light-emitting materials,[4] and light-harvesting systems.[5] However, the undesirable photophysical properties of various fluorophores still constrain the full potential of their applications. For instance, many bright organic dyes including rhodamine, fluorescein, boron dipyrromethane (BODIPY), and cyanine derivatives have the serious disadvantage of very small Stokes shifts (typically less than 25 nm), which can lead to serious self-quenching and fluorescence detection errors because of excitation backscattering effects.[6] Therefore, there is a need to develop dyes with improved properties.

Since it is still difficult to judiciously design single organic dyes with desirable photophysical properties, considerable attention has recently been paid to the exploration of multifluorophores with energy-donor–acceptor architectures.[1m,6b,7–9] In this regard, some energy-donor–acceptor systems based on fluorescence resonance energy transfer (FRET) have been constructed.[6b,8] FRET dyads are usually linked by a nonconjugated spacer, and the energy transfer occurs through space. Although the pseudo-Stokes shifts (the wavelength discrepancy between the donor absorption and the acceptor emission in an energy transfer system with almost 100% energy transfer efficiency[7]) of FRET-based energy cassettes are larger than the Stokes shifts of either the donor or acceptor dyes, FRET-based cassettes are still limited by the requirement that the donor emission must have strong overlap with the acceptor absorption.[10] This requirement essentially restricts the pseudo-Stokes shifts as well as the emission shifts (the emission wavelength shift between the donor and acceptor) of FRET-based systems. Like the pseudo-Stokes shift, the emission shift is also an important parameter in energy-transfer dyads. A large emission shift in energy transfer systems should result in two well-separated emission peaks, which is favorable for the precise measurement of the peak intensities and ratios.[6b,11] Thus, energy-transfer dyads with large pseudo-Stokes shifts and emission shifts are desirable.

By contrast, through-bond energy transfer (TBET) is theoretically not subjected to the constraint of intense spectral overlap between the donor emission and the acceptor absorption.[9] Thus, TBET cassettes may have large pseudo-Stokes shifts and emission shifts. Unlike through-space energy-transfer cassettes, in TBET cassettes, the donor and the acceptor units are joined by a conjugated spacer. Burgess and co-workers have developed elegant TBET systems based on the conjugated fluorescein–rhodamine system.[9a,b] However, the fluorescein (donor) emission overlaps significantly with the rhodamine (acceptor) absorption and the advantage of TBET, that is, no requirement of strong spectral overlap between the donor emission and the acceptor absorption, was not really capitalized upon in these conjugated fluorescein–rhodamine energy transfer cassettes. Not surprisingly, the pseudo-Stokes shifts (<120 nm) and emission shifts (20–90 nm) in these fluorescein–rhodamine TBET systems are rather restricted.[9a,b]

Although it is believed that TBET systems do not require a strong spectral overlap between the donor emission and the acceptor absorption, to the best of our knowledge, this challenge has not been met in small-molecule dual-fluorescent dye systems. Thus, we were interested in creating novel TBET platforms that only have minimal spectral overlap between the donor emission and the acceptor absorption. The merits of such a new class of TBET systems should include large pseudo-Stokes shifts and emission shifts. These advantageous spectral properties are desirable for the applications of fluorescent dyes in chemistry, biology, medicine, and materials science. Herein, as a proof-of-concept, we present the coumarin–rhodamine TBET cassettes **1a–d** as a small-molecule dual-fluorescent dye energy-transfer platform with minimal spectral overlap between the donor emission and the acceptor absorption (Scheme 1). In addition, this TBET platform was applied to develop a new TBET-based pH probe. As expected, the probe exhibited the key features of our TBET platform, namely a large pseudo-Stokes shift and a significant ratiometric value that arise from a significant emission shift.

The choice of dyes with a minimal spectral overlap as the donors and acceptors is straightforward. To exemplify the general concept of our TBET design, coumarin and rhodamine dyes were selected as the energy donors and acceptors, respectively (Scheme 2), as the coumarin emission has

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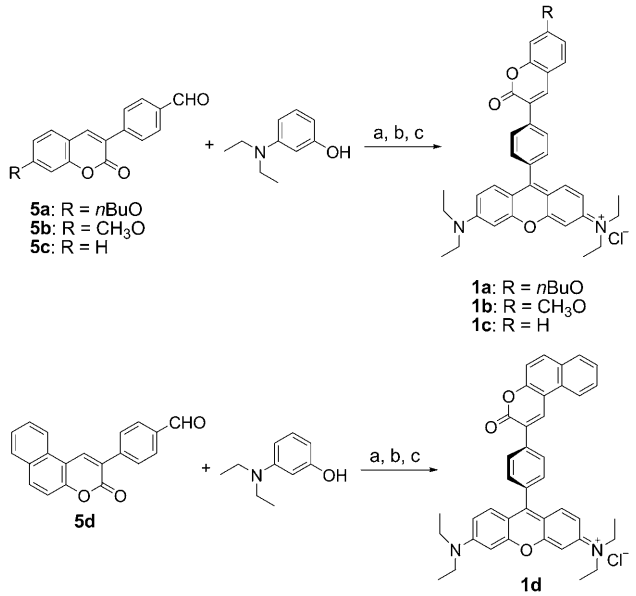
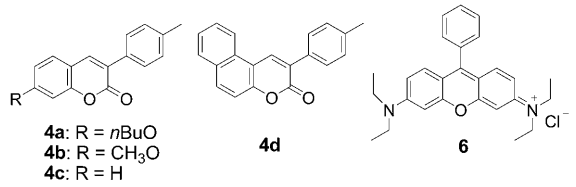


Scheme 1. Synthesis of the coumarin–rhodamine TBET cassettes **1a–d**. a) CH_3CH_2COOH, 4-toluenesulfonic acid. b) Chloranil, CH_2Cl_2, MeOH. c) $NaBH_4$, MeOH, then chloranil, CH_2Cl_2, MeOH, standard concentrated HCl.



Scheme 2. Structures of the energy donors **4a–d** and the acceptor **6**.

negligible overlap with the rhodamine absorption (Figure 1). However, the main challenge in the development of the new TBET system is to connect the coumarin donor with the rhodamine acceptor with a suitable linker that may prevent the donor and the acceptor moieties from becoming planar and facilitate the through-bond energy transfer process. Furthermore, from a practical point of view, such a construct should also be synthetically accessible. These considerations led us to select a phenyl moiety as a rigid and conjugated linker.

The new class of TBET cassettes **1a–d** was synthesized by condensation of coumarin aldehydes **5a–d** with 3-(diethylamino)phenol, followed by oxidation with chloranil (Scheme 1). However, the purification of the products **1a–d** by column chromatography on silica gel proved to be very challenging. Alternatively, we employed a reduction–oxidation process to successfully purify these products (see the Supporting Information). The donors **4a–d** were prepared by following standard procedures (see the Supporting Information). The new coumarin–rhodamine compounds were fully characterized by ^1H and ^{13}C NMR spectroscopy, and HRMS.

The absorption spectra of the TBET cassettes **1a–d** (5 μM) displayed the characteristic absorption signal of rhodamine

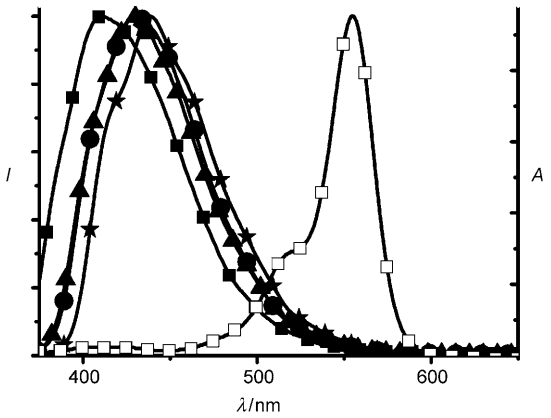


Figure 1. Normalized emission spectra of donor coumarin dyes **4a** (●), **4b** (▲), **4c** (■), and **4d** (★) and the normalized absorption spectra of the acceptor rhodamine **6** (□) in pH 7.0 phosphate buffer/MeOH (3:2).

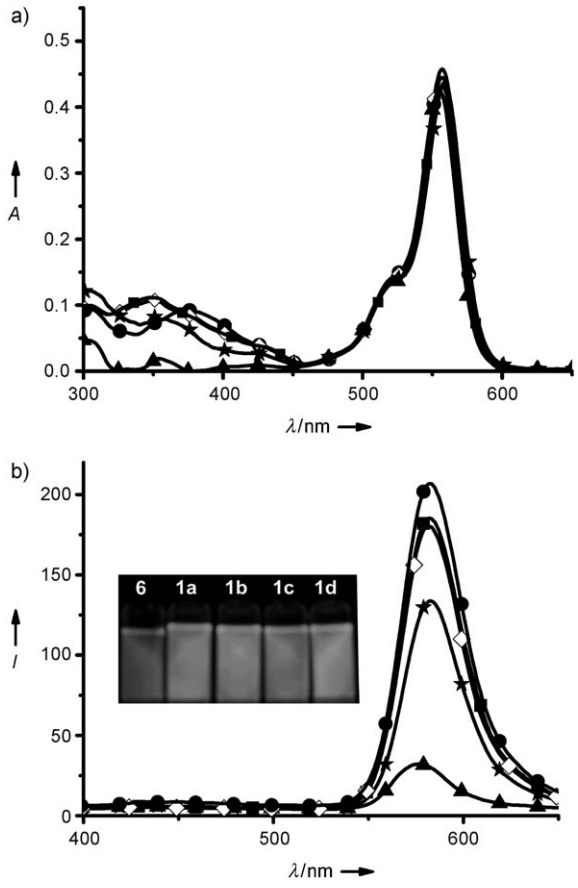


Figure 2. a) Absorption spectra of equimolar **1a** (◇), **1b** (■), **1c** (★), **1d** (●) and **6** (▲) in pH 7.0 phosphate buffer/MeOH (3:2). b) Fluorescence spectra of equimolar **1a** (◇), **1b** (■), **1c** (★), **1d** (●), and **6** (▲) excited at 372 nm in phosphate buffer/MeOH (3:2) (see Figure S2–6 in the Supporting Information for the fluorescence spectra at other excitation wavelengths in phosphate buffer or other solvent systems.). The inset shows the visual fluorescence color of compounds **6**, **1a**, **1b**, **1c**, **1d** excited at 365 nm using a hand-held UV lamp. The concentration of all the compounds is 5 μM. Figure S14 in the Supporting Information is a color version of Figure 2b.

Table 1: Photophysical data of **1a–d** and acceptor **6**.[a]

Compound	Absorption				Emission				
	λ_{abs} [nm][b]	log ε_{max}	λ_{abs} [nm][c]	Log ε_{max}	λ_{em} [nm][d]	FEF[e]	Φ_f[f]	$\Delta\lambda$[g]	$\Delta\lambda_{em}$[h]
1a	353	4.29	560	4.92	582	5.8	0.27	229	153
1b	351	4.30	560	4.92	582	6.0	0.26	231	150
1c	354	4.22	561	4.98	582	4.3	0.25	228	172
1d	372	4.28	561	4.92	582	6.7	0.27	210	143
6	–	–	559	4.96	576	1.0	0.25	17	–

[a] Measurements recorded in 25 mM phosphate buffer/MeOH (3:2). [b] The maximal absorption of the coumarin component; [c] The maximal absorption of the rhodamine component. [d] The maximal emission of the cassettes. [e] Fluorescence enhancement relative to the acceptor **6**. [f] Fluorescence quantum yields were determined using rhodamine 6G ($\Phi_f = 0.95$ in water) as a standard.[10,15] [g] Pseudo-Stokes shifts of the cassettes **1a–d** and the Stokes shift of the acceptor **6**. [h] Emission wavelength shifts between the donor emission and acceptor emission in cassettes **1a–d**.

derivatives around 560 nm and the typical absorption signal of coumarin derivatives around 350 nm (in the cases of **1a–c)** or 370 nm (in the case of **1d**; Figure 2a and Table 1). This result reveals that there are very weak electronic interactions between the donor and the acceptor in the ground state, and the compounds **1a–d** behave as cassettes[12] instead of single dye molecules.

Upon excitation of the cassettes **1a–d** (5 μM; phosphate buffer/MeOH 3:2) in the coumarin absorption band, only the characteristic emission band of the acceptor rhodamine (around 582 nm) was observed (Figure 2b and Figure S1 in the Supporting Information). The characteristic emission of coumarin (410–439 nm) was not observed, thus indicating that the energy-transfer efficiencies were nearly perfect (>99%).[13] For comparison, we also examined the fluorescence spectra of an equimolar mixture of the coumarin donor and the rhodamine acceptor. For instance, in an equimolar mixture of coumarin **4a** and rhodamine **6**, no visible quenching of the fluorescence of **4a** and no marked enhancement of the fluorescence of **6** was observed upon excitation of the coumarin absorption band (Figure S7 in the Supporting Information), thus suggesting that there is essentially no intermolecular energy transfer between coumarin **4a** and rhodamine **6** in the mixture. The same conclusion can be drawn for an equimolar mixture of rhodamine **6** and coumarin **4b**, **4c**, or **4d**, respectively (Figure S8–10 in the Supporting Information). Thus, the superiority of the TBET cassettes for energy transfer is evident.

For an energy-transfer cassette to be useful in practical applications, the fluorescence intensity of the acceptor component in the cassettes must be greater than that of the energy acceptor (without the donor) when it is excited at the donor absorption wavelength. As is evident from Figure 2b, the fluorescence enhancement factors (FEFs) are 5.8-, 6.0-, 4.3-, and 6.7-fold for the cassettes **1a–d** compared to the acceptor **6**, respectively. Notably, these enhancement factors are much higher than those of other typical FRET-based rhodamine systems (<4.0-fold).[14] Moreover, the cassette fluorescence is significantly brighter than that of the acceptor **6** (Figure 2b, inset), thus confirming the enhanced fluorescence of the cassette.

The photophysical data of the cassettes **1a–d** and acceptor **6** are given in Table 1 and Tables S1 and S2 in the Supporting Information. Indeed, the TBET cassettes have very large pseudo-Stokes shifts of up to 230 nm, which are much larger than those of fluorescein–rhodamine TBET cassettes (<120 nm)[9a,b] and those of the typical FRET-based rhodamine systems (<100 nm).[6b,14c,d] Furthermore, the emission shifts between the coumarin donor and rhodamine acceptor in our TBET cassettes are large (up to 172 nm; Table 1 and Figure S1 in the Supporting Information), which are also much larger than those of the fluorescein–rhodamine TBET cassettes (20–90 nm)[9a,b] and those of the typical FRET-based rhodamine systems (<75 nm).[6b,8a–c]

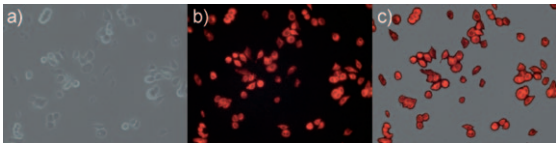
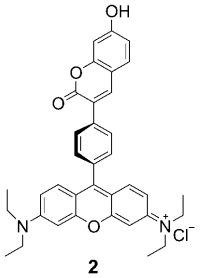


Figure 3. a) Brightfield image of nasopharyngeal carcinoma cells treated with **1b** (15 μM); b) fluorescence image of nasopharyngeal carcinoma cells treated with **1b** (15 μM) excited around 355 nm; c) the overlay image of (a) and (b).

To examine the potential use of these new coumarin–rhodamine cassettes for fluorescence imaging in living cells, nasopharyngeal carcinoma cells were incubated with the representative cassette **1b** or **1d** for 30 min at 37 °C. As shown in Figure 3 and Figure S12 in the Supporting Information, the novel cassettes were cell-permeable and can be employed for cell imaging when the coumarin absorption band is excited. This characteristic suggests that the coumarin–rhodamine TBET cassettes are potentially useful for biological applications in living systems.



2

As a preliminary illustration of the utility of our system, we created the water-soluble fluorescent probe **2** as a new candidate for a TBET-based pH probe. The 7-hydroxycoumarin moiety not only improves the water solubility of the probe, but also functions as the H^+ sensing unit.[16] Figure 4 shows the ratiometric fluorescence response of compound **2** to variations of the pH value. The increase of pH from 3.0 to 8.6 induced a significant decrease (13-fold overall) in the rhodamine emission signal around 587 nm and a large increase (19-fold overall) in the coumarin emission signal around 465 nm. Thus, the ratios of emission intensities at 587 and 465 nm (I_{587}/I_{465}) exhibit a dramatic change from 46.5 at pH 3.0 to 0.2 at pH 8.6. It should be noted that such a large change of emission intensity ratios at two wavelengths is desirable for ratiometric fluorescent probes, as the sensitivity as well as the dynamic range of ratiometric probes are

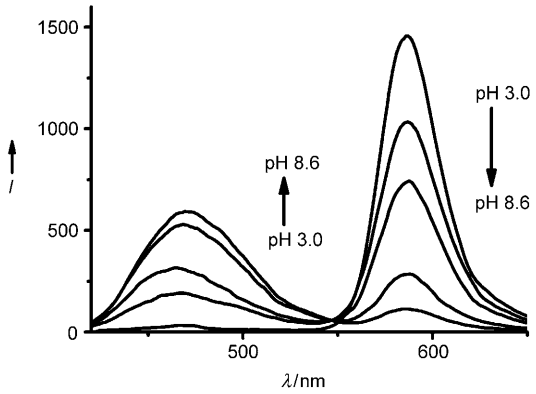


Figure 4. The pH-dependence of the fluorescence intensity of fluorescent probe **2** (5 μM) in phosphate buffer excited at 400 nm.

controlled by the emission ratio.[7b] Furthermore, the emission changes evolved with a well-defined isoemissive point at 555 nm. The difference in emission wavelength between the coumarin donor and rhodamine acceptor is very large (122 nm). This difference not only contributes to the accurate measurement of the intensities of the donor and acceptor emission peaks,[6b,11] but also results in a huge ratiometric value.[7b] Thus, the new ratiometric fluorescent pH probe **2** displays the advantageous properties of our new TBET platform.

In conclusion, we have described coumarin–rhodamine TBET cassettes as a novel paradigm of small-molecule dual-fluorescent dye energy-transfer systems with minimal spectral overlap between the donor emission and the acceptor absorption. The key features of the novel class of the TBET platform include large pseudo-Stokes shifts (up to 230 nm) and emission shifts (up to 170 nm). These advantageous spectral properties should allow use of the TBET cassettes in many areas. In addition, we have demonstrated that the new TBET cassettes are cell-membrane-permeable and potentially useful for biological applications. For a preliminary application, we created a ratiometric fluorescent TBET pH probe. This probe showed a large pseudo-Stokes shift as well as a dramatic change of the ratios of emission intensities at 587 and 465 nm (I_{587}/I_{465}) from 46.5 at pH 3.0 to 0.2 at pH 8.6 because of a large emission shift. We expect that our general design concept of the new class of the TBET platform with minimal spectral overlap between the donor emission and the acceptor absorption should be applicable to other small-molecule dual-fluorescent dye energy transfer systems based on a wide variety of dyes. This work, as well as the application of our TBET platform to the development of ratiometric fluorescent probes for various analytes of interest is in progress.

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[1] For some reviews and examples, see: a) T. Terai, T. Nagano, *Curr. Opin. Chem. Biol.* **2008**, *12*, 515–521; b) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515–1566; c) E. M. Nolan, S. J. Lippard, *Chem. Rev.* **2008**, *108*, 3443–3480; d) W. Lin, L. Yuan, Z. Cao, Y. Feng, L. Long, *Chem. Eur. J.* **2009**, *15*, 5096–5103; e) S. Mizukami, R. Takikawa, F. Sugihara, M. Shirakawa, K. Kikuchi, *Angew. Chem.* **2009**, *121*, 3695–3697; *Angew. Chem. Int. Ed.* **2009**, *48*, 3641–3643; f) D. W. Domaille, E. L Que, C. J Chang, *Nat. Chem. Biol.* **2008**, *4*, 168–175; g) L. Marbella, B. Serli-Mitasev, P. Basu, *Angew. Chem.* **2009**, *121*, 4056–4058; *Angew. Chem. Int. Ed.* **2009**, *48*, 3996–3998; h) A. L. Garner, C. M. St Croix, B. R. Pitt, G. D. Leikauf, S. Ando, K. Koide, *Nat. Chem.* **2009**, *1*, 316–321; i) T. Schwarze, H. Müller, C. Dosche, T. Klamroth, Wu. Mickler, A. Kelling, H.-G. Löhmannsröben, P. Saalfrank, H.-J. Holdt, *Angew. Chem.* **2007**, *119*, 1701–1704; *Angew. Chem. Int. Ed.* **2007**, *46*, 1671–1674; j) M. Taki, M. Desaki, A. Ojida, S. Iyoshi, T. Hirayama, I. Hamachi, Y. Yamamoto, *J. Am. Chem. Soc.* **2008**, *130*, 12564–12565; k) S. Malashikhin, N. S. Finney, *J. Am. Chem. Soc.* **2008**, *130*, 12846–12847; l) A. Palma, M. Tasior, D. O. Frimannsson, T. T. Vu, R. Méallet-Renault, D. F. O'Shea, *Org. Lett.* **2009**, *11*, 3638–3641; m) R. Guliyev, A. Coskun, E. U. Akkaya, *J. Am. Chem. Soc.* **2009**, *131*, 9007–9013.

[2] For some reviews and examples, see: a) M. S. T. Gonçalves, *Chem. Rev.* **2009**, *109*, 190–212; b) U. Resch-Genger, M. Grabolle, S. Cavaliere-Jaricot, R. Nitschke, T. Nann, *Nat. Methods* **2008**, *5*, 763–775; c) A. Waggoner, *Curr. Opin. Chem. Biol.* **2006**, *10*, 62–66; d) M. J. Hangauer, C. R. Bertozzi, *Angew. Chem.* **2008**, *120*, 2428–2431; *Angew. Chem. Int. Ed.* **2008**, *47*, 2394–2397.

[3] For some reviews and examples, see: a) S. D. Straight, P. A. Liddell, Y. Terazono, T. A. Moore, A. L. Moore, D. Gust, *Adv. Funct. Mater.* **2007**, *17*, 777–785; b) A. Coskun, E. Deniz, E. U. Akkaya, *Org. Lett.* **2005**, *7*, 5187–5189; c) U. Pischel, *Angew. Chem.* **2007**, *119*, 4100–4115; *Angew. Chem. Int. Ed.* **2007**, *46*, 4026–4040; d) K. Szaciłowski, *Chem. Rev.* **2008**, *108*, 3481–3548; e) S. Kou, H. N. Lee, D. van Noort, K. M. K. Swamy, S. H. Kim, J. H. Soh, K.-M. Lee, S.-W. Nam, J. Yoon, S. Park, *Angew. Chem.* **2008**, *120*, 886–890; *Angew. Chem. Int. Ed.* **2008**, *47*, 872–876; f) K. Rurack, C. Trieflinger, A. Kovalchuck, J. Daub, *Chem. Eur. J.* **2007**, *13*, 8998–9003; g) L. Mu, W. Shi, G. She, J. C. Chang, S.-T. Lee, *Angew. Chem.* **2009**, *121*, 3521–3524; *Angew. Chem. Int. Ed.* **2009**, *48*, 3469–3472.

[4] For some reviews and examples, see: a) B. Li, J. Li, Y. Fu, Z. Bo, *J. Am. Chem. Soc.* **2004**, *126*, 3430–3431; b) B. Domercq, C. Grasso, J. Maldonado, M. Halik, S. Barlow, S. R. Marder, B. Kippelen, *J. Phys. Chem. B* **2004**, *108*, 8647–8651; c) Z. H. Li, M. S. Wong, *Org. lett.* **2006**, *8*, 1499–1502; d) C. -T, Chen, *Chem. Mater.* **2004**, *16*, 4389–4400.

[5] For some examples, see: a) T. Ishi-i, K. Murakami, Y. Imai, S. Mataka, *Org. Lett.* **2005**, *7*, 3175–3178; b) K. Becker, J. M. Lupton, *J. Am. Chem. Soc.* **2006**, *128*, 6468–6479; c) D. S. Tyson, F. N. Castellano, *Inorg. Chem.* **1999**, *38*, 4382–4383; d) S. Zrig, P. Remy, B. Andrioletti, E. Rose, I. Asselberghs, K. Clays, *J. Org. Chem.* **2008**, *73*, 1563- 1566; e) X. Zhang, Z.-K. Chen, K. P. Loh, *J. Am. Chem. Soc.* **2009**, *131*, 7210–7211; f) A. Ajayaghosh, V. K. Praveen, C. Vijayakumar, S. J. George, *Angew. Chem.* **2007**, *119*, 6376–6381; *Angew. Chem. Int. Ed.* **2007**, *46*, 6260–6265.

[6] a) L. Tolosa, K. Nowaczyk, J. Lakowicz, *An Introduction to Laser Spectroscopy, 2nd ed.*, Kluwer, New York, **2002**; b) X. Zhang, Y. Xiao, X. Qian, *Angew. Chem.* **2008**, *120*, 8145–8149; *Angew. Chem. Int. Ed.* **2008**, *47*, 8025–8029.

[7] a) A. Burghart, L. H. Thoresen, J. Chen, K. Burgess, F. Bergström, L. B.-Å. Johansson, *Chem. Commun.* **2000**, 2203–

2204; b) A. Coskun, E. U. Akkaya, *J. Am. Chem. Soc.* **2006**, *128*, 14474 – 14475.
[8] a) M. Suresh, S. Mishra, S. K. Mishra, E. Suresh, A. K. Mandal, A. Shrivastav, A. Das, *Org. Lett.* **2009**, *11*, 2740 – 2743; b) M. H. Lee, H. J. Kim, S. Yoon, N. Park, J. S. Kim, *Org. Lett.* **2008**, *10*, 213 – 216; c) J. Yang, H. Chen, I. R. Vlahov, J.-X. Cheng, P. S. Low, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 13872 – 13877; d) A. E. Albers, V. S. Okreglak, C. J. Chang, *J. Am. Chem. Soc.* **2006**, *128*, 9640 – 9641; e) T. Komatsu, K. Kikuchi, H. Takakusa, K. Hanaoka, T. Ueno, M. Kamiya, Y. Urano, T. Nagano, *J. Am. Chem. Soc.* **2006**, *128*, 15946 – 15947.
[9] a) G.-S. Jiao, L. H. Thoresen, K. Burgess, *J. Am. Chem. Soc.* **2003**, *125*, 14668 – 14669; b) R. Bandichhor, A. D. Petrescu, A. Vespa, A. B. Kier, F. Schroeder, K. Burgess, *J. Am. Chem. Soc.* **2006**, *128*, 10688 – 10689; c) J. Han, J. Jose, E. Mei, K. Burgess, *Angew. Chem.* **2007**, *119*, 1714 – 1717; *Angew. Chem. Int. Ed.* **2007**, *46*, 1684 – 1687.
[10] J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, *2nd ed.*, Kluwer/Plenum, New York, **1999**.
[11] E. Kimura, T. Koike, *Chem. Soc. Rev.* **1998**, *27*, 179 – 184.
[12] The term "cassette" refers to a multifluorophore system in which the individual chromophores behave independently in the ground state but can interact in the excited state because of a spacer between chromophores that prevents full conjugation. We thank an anonymous reviewer for providing the definition of cassette.
[13] According to Ref. [9b], the energy transfer efficiency of TBET cassettes was calculated from 100 % × [(fluorescence of donor) − (fluorescence of donor in cassette)/(fluorescence of donor)].
[14] a) J. Su, H. Tian, K. Chen, *Dyes Pigm.* **1996**, *31*, 69 – 77; b) H. Tian, Y. Tang, K. Chen, *Dyes Pigm.* **1994**, *26* 159 – 165; c) L. G. Lee, S. L. Spurgeon, B. U. S. Rosenblum, Patent 7,169,939, **2007**; d) L. G. Lee, S. L. Spurgeon, C. R. Heiner, S. C. Benson, B. B. Rosenblum, S. M. Menchen, R. J. Graham, A. Constantinescu, K. G. Upadhya, J. M. Cassel, *Nucleic Acids Res.* **1997**, *25*, 2816 – 2822.
[15] D. Magde, G. E. Rojas, P. Seybold, *Photochem. Photobiol.* **1999**, *70*, 737 – 744.
[16] M. Adamczyk, M. Cornwell, J. Huff, S. Rege, T. V. S. Rao, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1985 – 1988.

