Fluorescence resonance energy transfer across a mechanical bond of a rotaxane†

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Fluorescence transfer across a donor-acceptor tagged rotaxane was studied and a small conformational change of the rotaxane observed using fluorescent spectroscopy and ROESY NMR.

Fluorescence resonance energy transfer (FRET) is widely used to monitor asssociation and dynamic processes of biological molecules,[1] and has been used to monitor the self-assembly of molecular capsules.[2] There are only a few systems featuring FRET across mechanically interlocked components,[3–7] and we proposed a new rotaxane with FRET labels. Rotaxanes have been extensively studied for the development of mechanically bonded molecular machines[8] (Fig. 1a),[8d,e] such as shuttles,[9] switches[10] and even muscles.[11] We report here the synthesis and characterization of the system, and observations relevant to their use as moving parts.

The relevant structural feature of the rotaxane **1** is a macrocycle bearing a fluorophore and the axle bearing another fluorophore at one end. Coumarin 2 was the donor on the axle and coumarin 343 was the acceptor on the macrocycle. The motion of the interlocked molecule was based on the behavior of rotaxanes developed by Leigh and co-workers[12] (Fig. 1b). The most straightforward synthesis, involving macrocyclization[13] of the fully labeled components around **2** was thwarted by low solubility. Instead, an extended alkyl chain was attached to the macrocycle precursor **3** on an Alloc group using olefin metathesis (see electronic supplementary information). The macrocycliation of precursor **3** with **4** to gave the [2]rotaxane **5** (Scheme 1). The standard deprotection using Pd(0) removed the modified Alloc group, and gave the amino-[2]rotaxane **6**. The installation of coumarin 343 on the macrocycle through the amide condensation using EDCI concluded the synthesis of the FRET labeled [2]rotaxane **1**.

The absorption and emission spectra of the [2]rotaxane **1** were recorded as illustrated in Fig. 2. Labeled **1** possesses the characteristic absorption of both donor and acceptor coumarins, and the fluorescence of the acceptor (λ_{max} = 470 nm in $CHCl_3$) when excited at 345 nm shows that efficient FRET occured. This emission property of **1** was not affected under high dilution, as

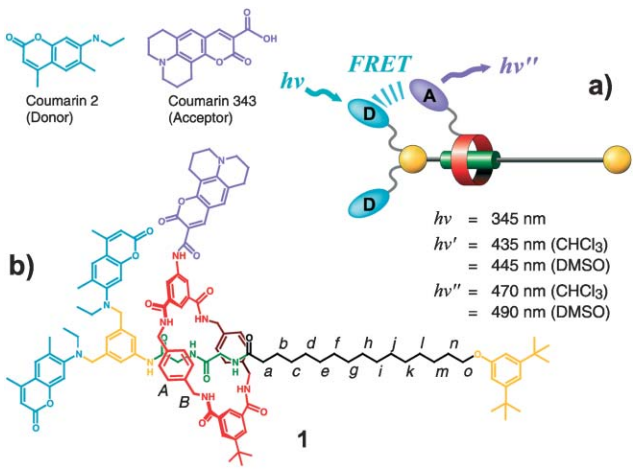

Fig. 1 (a) Schematic representation of the rotaxane showing that a macrocycle (red) was mechanically interlocked by a dumbbell composed of an axle (black) with a hydrogen bonding site (green), terminated by the bulky end group (yellow). Interlocked components were tagged by the donor D (blue) and acceptor A (purple). (b) Chemical structure of the [2]rotaxane **1**.

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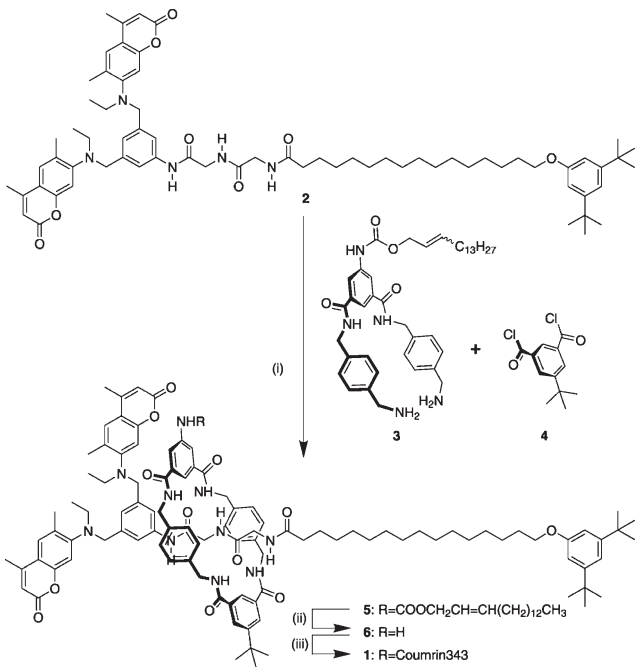
† Electronic supplementary information (ESI) available: Synthetic and characterisation details for **1** and additional fluorescence experiments. See http://dx.doi.org/10.1039/b506177f



Scheme 1 Synthesis of the [2]rotaxane **1**: (i) Et_3N, anhydrous $CHCl_3$, RT, **3** and **4** were added to a solution of **2** by syringe pump over 4 h then stirred for 10h; (ii) $Pd(PPh_3)$, $PhSiH_3$, CH_2Cl_2, RT, 12 h, 8.4% (through procedures i and ii); (iii) EDCI, $CHCl_3$, RT, 10h, 69%.

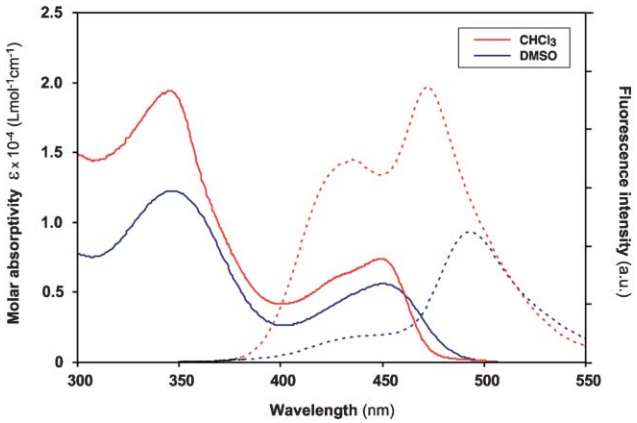


Fig. 2 Absorption (—) and fluorescence (.....) spectra of the [2]rotaxane **1** in $CHCl_3$ (red) and DMSO (blue), (50 μM, λ_{exc} = 345 nm, 298 K).

expected by the mechanical bonding of the donor and acceptor (see ESI†). Absorption and fluorescence properties of **1** were also investigated under polar and non-polar environments. Although it is clear that the overall intensity of both absorption and emission are lower in polar solvent (DMSO), the fluorescence intensity in DMSO is relatively lower than that in $CHCl_3$. This can be attributed to the release of the macrocycle from the Gly-Gly station through the disruption of hydrogen bonding by DMSO. The Förster distances of the dyes are typically around 20–80 Å;[1a] a distance that mostly exceeds the length of the axle. Accordingly, the system maintains FRET as long as the rotaxane is intact.

The motion of the macrocycle was also observed using NMR analysis. The conformation of the rotaxane **1** was elucidated by ^{1}H and ROESY NMR spectra as shown in Fig. 3. In a non-polar environment,[14] the macrocycle was predominantly located around one of the glycine residues and the proton H_a. In the polar environment, the macrocycle shifts towards the center of the axle as is apparent from the upfield shifts (up to 0.8 ppm) of protons that also show NOE interactions with the macrocycle.

In summary, the present research demonstrated that FRET labeling can be used to detect small motions in mechanically interlocked systems.

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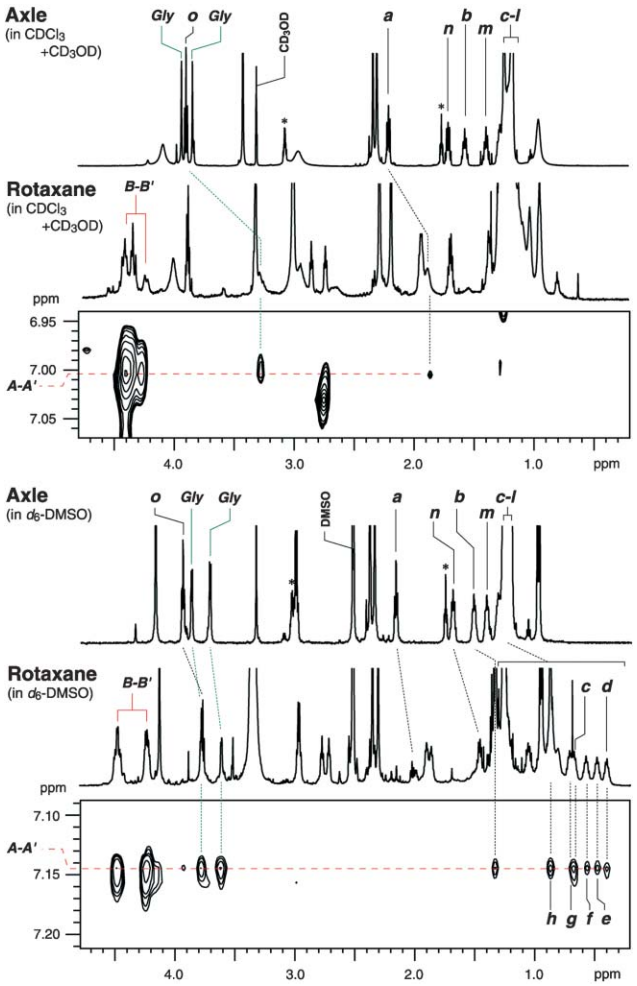

Fig. 3 A section of the 600 MHz ^{1}H spectra of the axle **2** and ^{1}H and ROESY NMR spectra of the [2]rotaxane **1** recorded in $CDCl_3$ + CD_3OD (600 : 50 μL) and d_6-DMSO, showing the NOEs between the axle (*x*-axis) and the $H_{A-A'}$ protons of the macrocycle (*y*-axis).

Notes and references

1 For reviews on FRET theory and applications, see: (*a*) P. Wu and L. Brand, *Anal. Biochem.*, 1994, **218**, 1; (*b*) J. Szöllősi, S. Damjanovich and L. Mátyus, *Cytometry*, 1998, **34**, 159.

2 R. K. Castellano, S. L. Craig, C. Nuckolls and J. Rebek, Jr., *J. Am. Chem. Soc.*, 2000, **122**, 7876.

3 M. Tamura and A. Ueno, *Chem. Lett.*, 1998, 369.

4 For related examples where a macrocycle itself quenches fluorescence of a donor, see: (*a*) G. Giro, M. Cocchi, V. Fattori, G. Gadret, G. Ruani, M. Cavallini, F. Biscarini, R. Zamboni, T. Loontjens, J. Thies, D. A. Leigh, A. F. Morales and R. F. Mahrt, *Synth. Met.*, 2001, **122**, 63; (*b*) E. M. Perez, D. T. F. Dryden, D. A. Leigh, G. Teobaldi and F. Zerbetto, *J. Am. Chem. Soc.*, 2004, **126**, 12210.

5 For related systems but fluorophores are covalently attached, see: (*a*) D. H. Qu, G. C. Wang, J. Ren and H. Tian, *Org. Lett.*, 2004, **6**, 2085; (*b*) K. Hiratani, M. Kaneyama, Y. Nagawa, E. Koyama and M. Kanesato, *J. Am. Chem. Soc.*, 2004, **126**, 13568.

6 For examples of non-interlocked systems, see: (*a*) E. Ishow, A. Credi, V. Balzani, F. Spadola and L. Mandolini, *Chem. – Eur. J.*, 1999, **5**, 984; (*b*) L. Jullien, J. Canceill, B. Valeur, E. Bardez, J.-P. Lefèvre, J.-M. Lehn, V. Marchi-Artzner and R. Pensu, *J. Am. Chem. Soc.*, 1996, **118**, 5432.

7 For examples of electron/energy transfer, see: (*a*) M. Andersson, M. Linke, J.-C. Chambron, J. Davidsson, V. Heitz, L. Hammarström and J.-P. Sauvage, *J. Am. Chem. Soc.*, 2002, **124**, 4347; (*b*) K. Li, P. J. Bracher, D. M. Guldi, M. Á. Herranz, L. Echegoyen and D. I. Schuster, *J. Am. Chem. Soc.*, 2004, **126**, 9156.

8 For reviews on molecular machines, see: (*a*) P. F. Barbara, *Acc. Chem. Res.*, 2001, **34**, 409, special issue on Molecular Machines, ed. J. F. Stoddart; (*b*) V. Balzani, M. Venturi and A. Credi, *Molecular Devices and Machines: A Journey into the Nanoworld*, Wiley-VCH, Weinheim, 2003; (*c*) C. J. Easton, S. F. Lincoln, L. Barr and H. Onagi, *Chem.-Eur. J.*, 2004, **10**, 3120. Specific to rotaxanes and catenanes, see: (*d*) V. Balzani, M. Gómez-López and J. F. Stoddart, *Acc. Chem. Res.*, 1998, **31**, 405; (*e*) J.-P. Sauvage, *Acc. Chem. Res.*, 1998, **31**, 611.

9 (*a*) P. L. Anelli, N. Spencer and J. F. Stoddart, *J. Am. Chem. Soc.*, 1991, **113**, 5131; (*b*) H. Murakami, A. Kawabuchi, K. Kotoo, M. Kunitake and N. Nakashima, *J. Am. Chem. Soc.*, 1997, **119**, 7605; (*c*) A. S. Lane, D. A. Leigh and A. Murphy, *J. Am. Chem. Soc.*, 1997, **119**, 11092; (*d*) N. Armaroli, V. Balzani, J.-P. Collin, P. Gaviña, J.-P. Sauvage and B. Ventura, *J. Am. Chem. Soc.*, 1999, **121**, 4397.

10 (*a*) M. Cavallini, F. Biscarini, S. Leon, F. Zerbetto, G. Bottari and D. A. Leigh, *Science*, 2003, **299**, 531; (*b*) A. H. Flood, J. F. Stoddart, D. W. Steuerman and J. R. Heath, *Science*, 2004, **306**, 2055.

11 M. C. Jiménez, C. Dietrich-Buchecker and J.-P. Sauvage, *Angew. Chem., Int. Ed.*, 2000, **39**, 3284.
12 For example, see: T. Da Ros, D. M. Guldi, A. F. Morales, D. A. Leigh, M. Prato and R. Turco, *Org. Lett.*, 2003, **5**, 689.
13 A. G. Johnston, D. A. Leigh, R. J. Pritchard and M. D. Deegan, *Angew. Chem., Int. Ed.*, 1995, **34**, 1209.
14 In order to determine the conformation in a non-polar environment, a mixture of $CDCl_3$ with a small amount of CD_3OD was used. This is because the spectrum of **1** in pure $CDCl_3$ was broad (258 K to 308 K), and was not suitable for accurate analysis. The broadening is caused by slow rotation of the macrocycle on the axle.