CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200902547

Squaraine Rotaxane as a Reversible Optical Chloride Sensor

Jeremiah J. Gassensmith,[a] Sarah Matthys,[a] Jung-Jae Lee,[a] Aleksandra Wojcik,[b, c] Prashant V. Kamat,[a, b] and Bradley D. Smith*[a]

Abstract: A mechanically interlocked squaraine rotaxane is comprised of a deep-red fluorescent squaraine dye inside a tetralactam macrocycle. NMR studies show that Cl^- binding to the rotaxane induces macrocycle translocation away from the central squaraine station, a process that is completely reversed when the Cl^- is removed from the solution. Steady-state fluorescence and excited-state lifetime measurements show that this reversible machine-like motion modulates several technically useful optical properties, including a three-fold increase in deep-red fluorescence emission that is observable to the naked eye. The excited states were characterized quantitatively by time-correlated single photon counting, femtosecond transient absorption spectroscopy, and nanosecond laser flash photolysis. Cl^- binding to the rotaxane increases the squaraine excited singlet state lifetime from 1.5 to 3.1 ns, and decreases the excited triplet state lifetime from >200 to 44 μs. Apparently, the surrounding macrocycle quenches the excited singlet state of the encapsulated squaraine dye and stabilizes the excited triplet state. Prototype dipsticks were prepared by adsorbing the lipophilic rotaxane onto the ends of narrow, C18-coated, reverse-phase silica gel plates. The fluorescence intensity of a dipstick increased eighteen-fold upon dipping in an aqueous solution of tetrabutylammonium chloride (300 mM) and was subsequently reversed by washing with pure water. It is possible to develop the dipsticks for colorimetric determination of Cl^- levels by the naked eye. After dipping into aqueous tetrabutylammonium chloride, a dipstick's color slowly fades at a rate that depends on the amount of Cl^- in the aqueous solution. The fading process is due primarily to hydrolytic bleaching of the squaraine chromophore within the rotaxane. That is, association of Cl^- to immobilized rotaxane induces macrocycle translocation and exposure of the electrophilic C_4O_2 core of the squaraine station, which is in turn attacked by the ambient moisture to produce a bleached product.

Keywords: anions · fluorescence · molecular recognition · rotaxanes · supramolecular chemistry

Introduction

There is a growing need for improved optical chemosensors that can detect Cl^- in various types of biological and environmental samples.[1,2] At present, most of the commercially available fluorescent probes are structurally simple quinolinium- and acridinium-based compounds that have several technical limitations, chief among them are no intrinsic Cl^- recognition ability and emission wavelengths in the UV/Vis.[3] Cl^- is a collisional quencher for these compounds, and thus sensing is indicated by a loss in fluorescence intensity. Not surprisingly, these non-selective probes are susceptible to interference by other analytes that can also induce quenching. Improved performance would be gained from next-generation Cl^- chemosensors that exhibit "turn-on" fluorescence, large changes in excited-state lifetimes, and operate at the deep-red wavelengths or beyond (>650 nm) that are optimal for biological imaging applications.[4]

Operationally, optical chemosensors can be divided into two groups; single-use sensors for batch-wise assays, and reversible sensors for continuous flow applications.[5] Dye displacement assays are good examples of the former group.[6] To convert them into reversible sensing systems, however, typically requires covalent tethering of the dye to the host

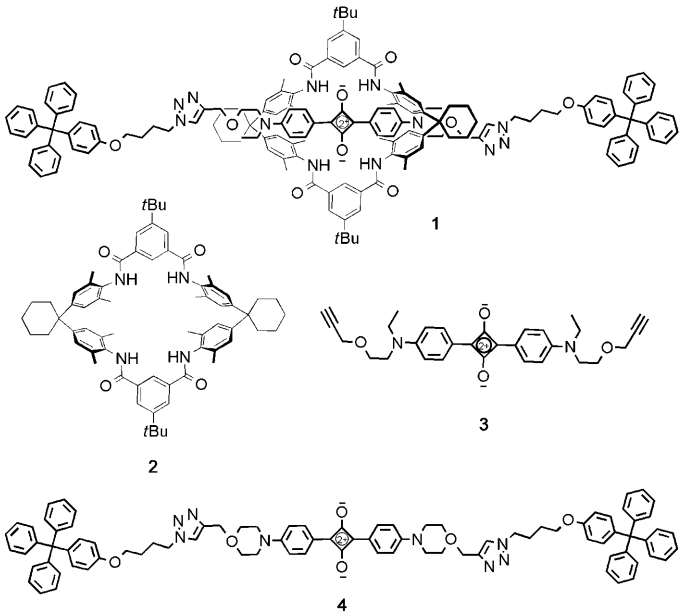
[a] Dr. J. J. Gassensmith, S. Matthys, Dr. J.-J. Lee, Prof. Dr. P. V. Kamat, Prof. Dr. B. D. Smith
Department of Chemistry and Biochemistry
University of Notre Dame, Notre Dame, IN 46556 (USA)
Fax: (+1) 574-631-6652
E-mail: smith.115@nd.edu

[b] Dr. A. Wojcik, Prof. Dr. P. V. Kamat
Radiation Laboratory, University of Notre Dame
Notre Dame, IN 46556 (USA)

[c] Dr. A. Wojcik
Faculty of Chemistry, Adam Mickiewicz University
Grunwaldzka 6, 60-780 Poznan (Poland)

scaffold which is synthetically challenging and also susceptible to problems with self-dimerization.[7] One-potential solution is to permanently encapsulate the dye inside a macrocycle to make a mechanically interlocked [2]rotaxane that exhibits binding-induced changes in co-conformation.[8] Despite the ongoing advances in rotaxane development, the number of published optical rotaxane sensors is surprisingly small and are primarily limited to fluorescent systems that respond to changes in solvent polarity or metal cation concentration.[9] Recently, several new designs of anion-binding rotaxanes have appeared;[10] however, the translation of these systems into practically useful optical sensors remains as a significant challenge in multiparameter molecular design.[11]

Herein, we report on squaraine rotaxane **1** as a prototype Cl^- sensor that is endowed with several favorable optical chemosensing features. The design of rotaxane **1** is based on recent work in our group that demonstrated macrocycle **2** can encapsulate the deep-red, fluorescent squaraine dye **3** to form a non-covalent, pseudorotaxane complex causing a three-fold decrease in fluorescence quantum yield of the dye.[12] Furthermore, addition of Cl^- to the pseudorotaxane solution induced displacement of the squaraine dye and a rescue of the fluorescence intensity. In the same study, we reported that "clicked capping" of stopper groups to the ends of the pseudorotaxane produced the [2]rotaxane **1** but its photophysical and chemosensing properties were not evaluated. We now describe a series of NMR, steady-state fluorescence, and excited-state lifetime measurements that reveal the ability of **1** to reversibly sense Cl^- in solution. Additionally, we show that immobilization of this chemosensor creates a prototype dipstick system for fluorometric and "naked eye" colorimetric detection of Cl^- in aqueous solution.



Results and Discussion

We have previously described the ^{1}H NMR spectrum of squaraine rotaxane **1** in $CDCl_3$ and compared it to spectra of the free macrocycle and thread components.[12] The rotaxane exhibits strong anisotropic shielding of diagnostic squaraine and macrocycle peaks indicating that the surrounding macrocycle resides over the central squaraine station in the thread.[13] Squaraine rotaxane **1** is a permanently interlocked molecule; the stopper groups are sufficiently large to ensure that it does not dissociate upon dilution.[14] Furthermore, mixing free thread component **4** and macrocycle **2** does not produce any evidence of pseudorotaxane formation. For example, incremental addition of a large molar excess of **2** to a solution of **4** in $CHCl_3$ does not induce any changes in the NMR chemical shifts or fluorescence quantum yield of **4**.

Shown in Figure 1 a and b are the comparative changes in ^{1}H NMR spectra for rotaxane **1** induced by the addition of one molar equivalent of tetrabutylammonium chloride (TBA^+Cl^-), and the subsequent complete reversal of these spectral changes when the Cl^- is removed from the solution by precipitation with a source of Na^+. The reversible changes in chemical shift for the thread and macrocycle protons suggest that Cl^- association promotes macrocycle translocation from the central squaraine station to one of the two triazole linkages in the thread component (Figure 2).[15] An

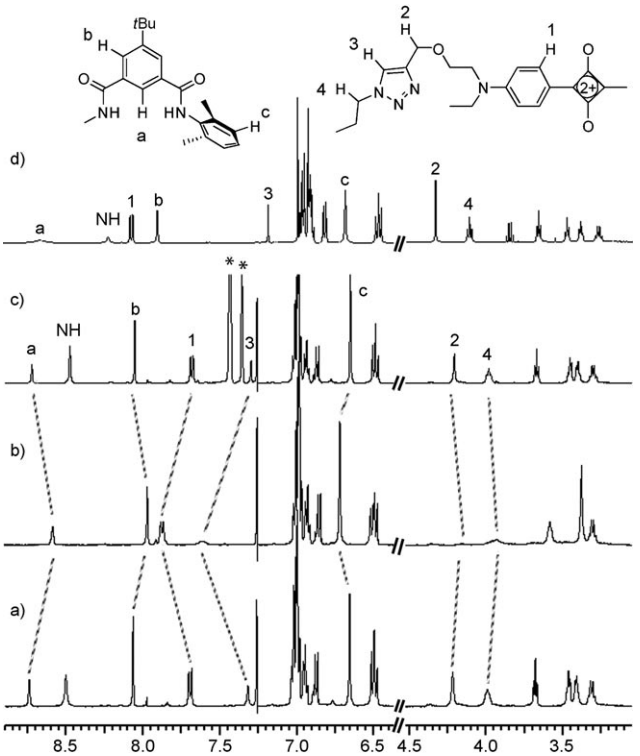
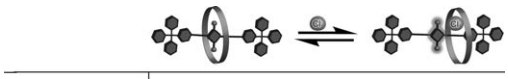


Figure 1. Partial ^{1}H NMR spectra in $CDCl_3$ and 22 °C: a) **1**, b) **1** plus one molar equivalent of TBA^+Cl^-, c) addition of one molar equivalent of $Na^+B[3,5\text{-}(CF_3)_2C_6H_3]_4^-$ to the previous mixture, which immediately produced a solid precipitate of NaCl (the $B[3,5\text{-}(CF_3)_2C_6H_3]_4^-$ peaks are denoted with asterisks), d) a 1:1:1 molar mixture of **2**:**4**:TBA^+Cl^-.



Fluorescence emission intensity	Low	High
Excited singlet state lifetime	Short	Long
Excited triplet state lifetime	Long	Short
Rate of chemical bleaching	Slow	Fast

Figure 2. Cl^- binding to rotaxane **1** induces macrocycle translocation and relative changes in the listed molecular properties.

alternative explanation, that the added Cl^- completely displaces the thread component from the rotaxane, can be dismissed for several reasons. The most obvious is direct comparison of the NMR spectrum of a solution of rotaxane **1** mixed with one molar equivalent of TBA^+Cl^- (Figure 1b) to the spectrum of a 1:1:1 mixture of macrocycle **2**, thread **4**, and TBA^+Cl^- (Figure 1d). In the latter case, the signals for the thread protons 1–4 correspond to those observed independently for the free thread molecule, and they are very different to the signals for the analogous protons 1–4 in Figure 1b.[16]

A quantitative assessment of Cl^- association was gained by fluorescence titration studies. As shown in Figure 3a, incremental addition of TBA^+Cl^- to a solution of **1** in $CHCl_3$ at 25 °C produced an increase in fluorescence emission at 655 nm. The titration curve fitted very closely to a 1:1 binding model with a Cl^- association constant of $(340 \pm 10)\,M^{-1}$. This is much lower than the Cl^- association constant of $>10^5\,M^{-1}$ reported previously for the free macrocycle **2** in CH_2Cl_2[17] and reflects the energetic cost to translocate the macrocycle away from the squaraine station, a process that disrupts squaraine/macrocycle NH hydrogen-bonding interactions. Saturation of the rotaxane with Cl^- produces a three-fold increase in fluorescence emission intensity at 655 nm, which is easily observed by the naked eye as a much brighter red color (Figure 3b). Taken together, the fluorescence and NMR titration data strongly indicate that hydrogen bonding of Cl^- to the surrounding macrocycle in **1** induces macrocycle translocation away from the mechanically encapsulated squaraine station and alleviates partial squaraine fluorescence quenching (Figure 2). The three-fold change in fluorescence is similar to that seen previously with pseudorotaxane complex **2**⊃**3**. Another result that is consistent with the behavior of **2**⊃**3** is the semi-quantitative finding that rotaxane **1** exhibits a Cl^--selective response. For example, addition of TBA^+ salts (5 mM) to separate samples of **1** (1 μM) in $CHCl_3$ produced the following normalized fluorescence enhancements: Cl^- (2.7) > OAc^- (1.6) > $H_2PO_4^-$ (1.4) > I^- (1.0).

The excited states of rotaxane **1** and free squaraine thread component **4** were characterized quantitatively by time-correlated single photon counting, femtosecond transient absorption spectroscopy, and nanosecond laser flash photolysis.

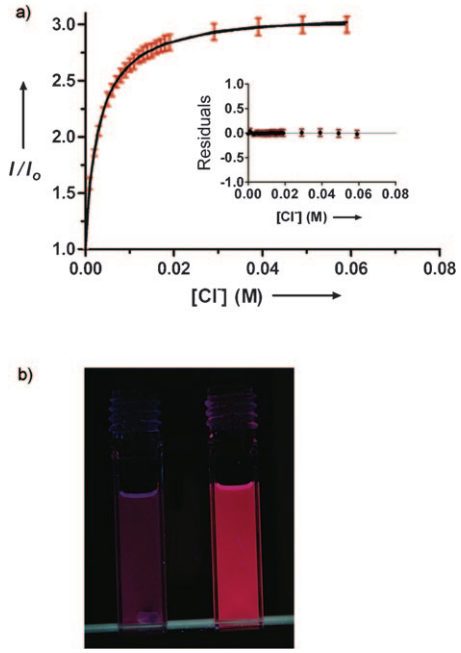


Figure 3. a) Titration of TBA^+Cl^- into a solution of **1** (1 μM) in $CHCl_3$ at 25 °C. Error bars for $n=3$. b) Fluorescence emission from cuvettes irradiated with 365 nm light and containing **1** in $CHCl_3$ before (left) and after (right) saturation with TBA^+Cl^-.

The transient absorption spectroscopy and flash photolysis revealed the characteristic squaraine absorption maxima for singlet and triplet excited states at 470–480 nm and 560–570 nm, respectively.[18] They occur at higher energies than the ground-state absorption maxima of **1** and **4** at 645 and 635 nm, respectively.[12] The data from time-correlated single photon counting (Figure 4 and Table 1) show that the excited singlet state lifetime, τ_S, for squaraine thread component **4** (3.1 ns) is more than two-times longer than rotaxane **1** (1.5 ns). The shorter lifetime of the rotaxane excited singlet state is consistent with quenching of the encapsulated squaraine station by the surrounding macrocycle. Addition of excess Cl^- increases the excited singlet state lifetime for **1** to

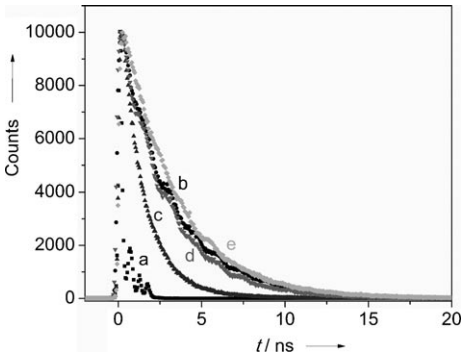


Figure 4. Fluorescence decays in $CHCl_3$ measured by time-correlated single photon counting: a) prompt, b) **4** (52 μM), c) **1** (40 μM), d) **4** (52 μM) and TBA^+Cl^- (52 mM), e) **1** (44 μM) and (44 mM) TBA^+Cl^-. $\lambda_{ex}=$ 373 nm and fluorescence monitored at corresponding emission maxima.

Table 1. Excited state data in $CHCl_3$.[a]

	1	4	1+TBA$^+$Cl$^-$	4+TBA$^+$Cl$^-$
excited singlet state				
λ_{max} (S_1-S_n) [nm][b]	472	480	480	–
τ_S [ns][c]	1.5	3.1	3.2	2.8
excited triplet state				
λ_{max} (T_1-T_n) [nm][d]	570	560	560	560
τ_T [μs][d]	>200	85	44	38
$\Delta\varepsilon_{560}$ [$M^{-1}cm^{-1}$][d,e]	56500	39800	–	–
ϕ_{ISC}[d,f]	0.025	0.059	–	–

[a] Concentrations are listed in Figures 4 and 5, TBA$^+$Cl$^-$ is 1000-fold excess. [b] Femtosecond transient absorption spectroscopy. [c] Time-correlated single photon counting, $\lambda_{ex}=373$ nm. [d] Nanosecond laser flash photolysis. [e] Molar absorption coefficient, determined by triplet-triplet energy transfer using 9,10-dibromoanthracene as sensitizer.[20,21] [f] Intersystem crossing (triplet) quantum yield, determined by relative actinometry using benzophenone as a standard.[20,21]

3.2 ns so that it nearly matches that observed with free squaraine **4** (2.8 ns) under the same conditions. This Cl^--promoted lengthening of rotaxane singlet state lifetime confirms the picture of Cl^- association with **1** inducing lateral translocation of the macrocycle so that it no longer quenches the excited singlet state of the encapsulated squaraine station.

Both the encapsulated and free squaraine fluorophores, **1** and **4**, respectively, undergo moderately efficient intersystem crossing (ISC) to excited triplet states with ISC yields (ϕ_{ISC}) of 0.025 and 0.059, respectively (Table 1).[18] The relatively lower ISC yield for **1** is due to quenching of the singlet excited state by the surrounding macrocycle as a competing pathway. Interestingly, the presence of the surrounding macrocycle in **1** substantially prolongs the excited triplet state lifetime τ_T (>200 μs for **1** versus 85 μs for **4**). This suggests that the surrounding macrocycle sterically inhibits excited triplet state deactivation pathways.[19] As shown in Figure 5, addition of excess Cl^- promotes efficient triplet state quenching for both fluorophores, however, the substantial reduction of triplet-state lifetime for **1** (from >200 to 44 μs) is much greater than the reduction observed for **4** (from 85 to 38 μs). This further supports the dynamic process in Figure 2 showing Cl^--promoted macrocycle translocation away from the encapsulated squaraine station so that it no longer stabilizes the squaraine excited triplet state.

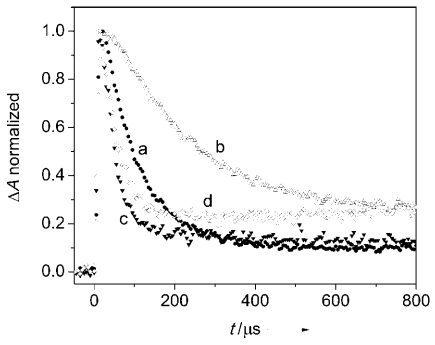


Figure 5. Decay profiles for excited triplet states in $CHCl_3$ generated by pulse-irradiation ($\lambda_{ex}=337$ nm): a) **4** (135 μM), b) **1** (82 μM), c) **4** (135 μM) and TBA$^+$Cl$^-$ (135 mM), d) **1** (82 μM) and TBA$^+$Cl$^-$ (82 mM). Monitoring wavelengths were 570 and 560 nm for **1** and **4**, respectively.

The next goal was to evaluate the practical potential of chemosensor **1** by fabricating prototype dipsticks for detecting Cl^- in aqueous samples. The dipsticks were prepared by adsorbing **1** onto the ends of narrow, C18-coated, reverse-phase silica gel TLC plates. This was achieved by dipping the ends of the dipsticks into a chloroform solution of **1** and allowing the solvent to evaporate (Figure 6a). The ability to act as a reversible fluorescence sensor was evaluated using a reflected fluorescence imaging machine that was equipped with a charge-coupled device.[22] Shown in Figure 6 are a series of fluorescence intensity images of one dipstick after each of the following experimental steps: 1) no treatment, 2) dipping into an aqueous solution of TBA$^+$Cl$^-$ (300 mM) and quickly drying, a process that increased the integrated intensity by a factor of eighteen, 3) rinsing with pure water and drying, which removed the adsorbed TBA$^+$Cl$^-$ and returned the dipstick to low fluorescence intensity, 4) a second dipping into aqueous TBA$^+$Cl$^-$ (300 mM) and drying, which reverted the dipstick to high fluorescence intensity. The sensing reversibility is readily apparent,[23] and the remarkable eighteen-fold modulation in fluorescence intensity is much higher than the three-fold change seen in homogeneous organic solution (see Figure 3). It appears that immobilization of **1** onto the dipstick greatly improves the signal dynamic range. The reasons for this unexpectedly favorable finding are the focus of future studies. Control experiments showed that the sensing effect is lost if the TBA$^+$Cl$^-$ is replaced with TBA$^+$$PF_6^-$ or TBA$^+$$ClO_4^-$ demonstrating the need for a hydrogen bonding anion. Also, there is no sens-

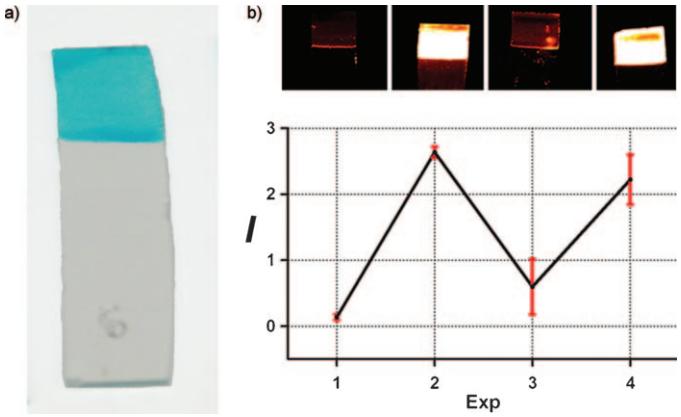


Figure 6. a) Photograph of a typical dipstick composed of rotaxane **1** adsorbed onto the end of a C18-coated, reverse-phase silica gel plate. b) Graph of integrated fluorescence intensity, *I*, (filter set, ex: (630±30) nm, em: (700±30) nm) for the coated end of the same dipstick after each of the following experimental steps: 1) no treatment, 2) dipping into aqueous solution of TBA$^+$Cl$^-$ (300 mM) and drying for 10 min, 3) rinsing with pure water and drying for 10 min, 4) second dipping into a aqueous solution of TBA$^+$Cl$^-$ (300 mM) and drying for 10 min. Located above each experiment number (Exp) is the false-colored fluorescence intensity image.

ing response if the TBA^+Cl^- is replaced with Na^+Cl^-, matching the literature expectation that only lipophilic ion-pairs can transfer from aqueous solution into the reverse-phase coating of the dipstick.[24] This means that in practical applications the sample under analysis would have to be spiked with a source of phase transfer cation, which in some cases may be a technical limitation.

As shown in Figure 7, it is also possible to "develop" the dipsticks for colorimetric detection of Cl^- by the naked eye. After dipping in aqueous TBA^+Cl^-, a dipstick's color slowly fades at a rate that depends on the amount of Cl^- in the aqueous solution. The images in Figure 7 show dipsticks that

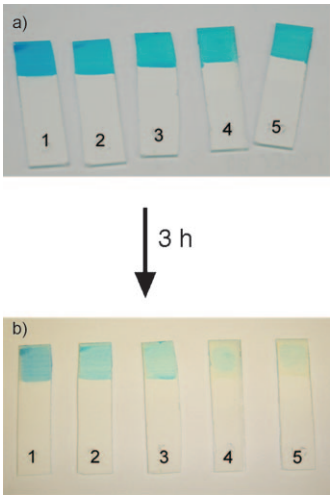


Figure 7. Naked eye detection of Cl^- concentration using dipsticks coated with immobilized **1**. The five identical dipsticks were dipped into aqueous solutions containing: 1) 0 M, 2) 0.2 M, 3) 0.5 M, 4) 0.8 M, 5) 1.0 M, TBA^+Cl^-. a) After drying for 10 min and sitting for 20 min; b) after sitting for a further 3 h.

have faded after sitting on the bench top for three hours. The differences in color intensity allows aqueous Cl^- concentrations to be distinguished semi-quantitatively over the range 0.2–1.0 M. Control experiments show that dipstick fading occurs in the dark (albeit at a slightly lower rate) and that it is greatly accelerated at higher temperatures. This suggests that the fading process is due primarily to hydrolytic bleaching of the squaraine chromophore in **1**. That is, association of Cl^- to immobilized rotaxane **1** induces macrocycle translocation and exposure of the electrophilic C_4O_2 core of the squaraine station (Figure 2), which is in turn attacked by the ambient moisture to produce a bleached product. This rationalization is supported by previous studies demonstrating that the rate for nucleophilic bleaching of squaraine rotaxanes depends heavily on the steric protection provided by the encapsulating macrocycle.[13b,25]

Conclusion

Association of Cl^- with squaraine rotaxane **1** causes a substantial change in rotaxane co-conformation. This reversible, machine-like motion induces a concurrent modulation of technically useful optical properties, including "turn-on" deep-red fluorescence that is highly compatible with biological and environmental samples, significant differences in excited-state lifetimes, and changes in the developed color of prototype Cl^--sensing dipsticks. It should be possible to improve the sensing performance by producing next generation squaraine rotaxanes with increased Cl^- affinity and selectivity,[10,11] increased amount of fluorescence quenching relief upon binding, and optimized dipsticks that can detect the presence of hydrophilic salts like Na^+Cl^-.[26,27]

Experimental Section

Compounds **1**, **2**, **3** and **4** were synthesized according to previously published methods.[12,13b] Femtosecond transient absorption experiments were conducted using a 1 kHz amplified Ti/sapphire laser system combined with an optical detection system. The fundamental output of the laser (775 nm, 1 mJ per pulse, pulse width 150 fs) was directed through a second harmonic generator to provide the 387 nm excitation wavelength used in all the experiments. Nanosecond laser flash photolysis experiments were carried out with a 355 nm laser pulse from a Nd/YAG laser operated at 1 mJ and a 337 nm laser pulse from a nitrogen laser operated at 2 mJ. All solutions were deoxygenated by bubbling N_2 for 10 min prior to the experiment. Emission lifetimes were measured using a time-correlated single photon counting system with a 373 nm diode at a 1 kHz repetition rate and a 1.1 ns pulse width.

General procedure to prepare and image dipsticks: Commercial C18-coated, reverse-phase silica plates (5×1.5 cm) that did not contain any fluorescent indicator were submerged in a $CHCl_3$ solution of **1** (1 mM) for 1–2 s and then allowed to dry in the open air. A fluorescence intensity image of the coated end of the dipstick was acquired using a wide-field fluorescent imaging station configured for epifluorescence (filter set, ex: (630±30) nm, em: (700±30) nm, 1 s acquisition time, no binning, f-stop 2.8). After background correction, Region of Interest image analysis was conducted using the ImageJ v1.40 software program available for free download at http://rsb.info.nih.gov/ij. The dipstick was submerged in an aqueous solution of TBA^+Cl^- (300 mM) for 1–2 s and allowed to dry superficially by firmly pressing against paper towels for 10 min, then imaged as described above.

Acknowledgements

B.D.S. thanks the NSF and the Notre Dame Integrated Imaging Facility for funding support. P.V.K. and A.W. acknowledge the support of Office of Basic Energy Sciences of the Department of Energy. This is contribution number NDRL 4817 from the Notre Dame Radiation Laboratory.

[1] a) F. Davis, S. D. Collyer, S. P. J. Higson, *Top. Curr. Chem.* **2005**, *255*, 97–124; b) L. D. Lavis, R. T. Raines, *ACS Chem. Biol.* **2008**, *3*, 142–155.

[2] For recent research on optical probes for Cl^-, see: a) R. B. C. Jagt, M. S. Kheibari, M. Nitz, *Dyes Pigm.* **2009**, *81*, 161–165; b) Y. M. Zhang, Q. Lin, T. B. Wei, D. D. Wang, H. Yao, Y. L. Wang, *Sensors Actuat. B* **2009**, *137*, 447–455; c) A. Graefe, S. E. Stanca, S. Nietzsche, L. Kubicova, R. Beckert, C. Biskup, G. J. Mohr, *Anal. Chem.*

2008, *80*, 6526–6531; d) M. J. Ruedas-Rama, E. A. H. Hall, *Analyst* **2008**, *133*, 1556–1566; e) U. I. Kim, J. M. Suk, V. R. Naidu, K. S. Jeong, *Chem. Eur. J.* **2008**, *14*, 11406–11414; f) P. A. Gale, *Chem. Commun.* **2008**, 4525–4540; g) B. Schazmann, N. Alhashimy, D. Diamond, *J. Am. Chem. Soc.* **2006**, *128*, 8607–8614.

[3] a) C. D. Geddes, K. Apperson, J. Karolin, D. J. S. Birch, *Anal. Biochem.* **2001**, *293*, 60–66; b) J. Biwersi, B. Tulk, A. S. Verkman, *Anal. Biochem.* **1994**, *219*, 139–143; c) B. A. McNally, A. V. Koulov, B. D. Smith, J. B. Joos, A. P. Davis, *Chem. Commun.* **2005**, 1087–1089.

[4] J. Rao, A. Dragulescu-Andrasi, H. Yao, *Curr. Opin. Biotechnol.* **2007**, *18*, 17–25.

[5] A. P. Demechenko, *Introduction to Fluorescence Sensing*, Springer, New York, **2008**.

[6] For a summary of dye displacement assays, see: B. T. Nguyen, E. V. Anslyn, *Coord. Chem. Rev.* **2006**, *250*, 3118–3127. Dye displacement systems are technically straightforward and excellent choices for batch-wise assays, but they are inherently limited as reversible sensors for many types of longitudinal studies because the ratio of host to dye can change over time. This is particularly problematic for intracellular sensing, as the cell permeation and localization properties of host and dye will often differ. Another major area of sensing technology is the development of optodes for monitoring continuous flows. Optodes are fiber optic devices that have their tips coated with sensing molecules. Differential leaching of host or dye from the fiber tip would obviously be a major performance drawback. For an excellent tutorial on sensing devices, see Chapter 9 in reference [5].

[7] a) H. Onagi, C. J. Easton, S. F. Lincoln, *Org. Lett.* **2001**, *3*, 1041–1044; b) Y. Liu, Z. X. Yang; Y. Chen, *J. Org. Chem.* **2008**, *73*, 5298–5304; Y. Chen, *J. Org. Chem.* **2008**, *73*, 5298–5304.

[8] a) M. J. Chmielewski, J. J. Davis, P. D. Beer, *Org. Biomol. Chem.* **2009**, *7*, 415–424; b) H. Tian, Q. C. Wang, *Chem. Soc. Rev.* **2006**, *35*, 361–374.

[9] a) D. A. Leigh, M. Á. F. Morales, E. M. Pérez, J. K. Y. Wong, C. G. Saiz, A. M. Z. Slawin, A. J. Carmichael, D. M. Haddleton, A. M. Brouwer, W. J. Buma, G. W. H. Wurpel, S. León, F. Zerbetto, *Angew. Chem.* **2005**, *117*, 3122–3127; *Angew. Chem. Int. Ed.* **2005**, *44*, 3062–3067; b) N. Kameta, Y. Nagawa, M. Karikomi, K. Hiratani, *Chem. Commun.* **2006**, 3714–3716; c) H. Onagi, J. Rebek, Jr., *Chem. Commun.* **2005**, 4604–4606; d) K. Hiratani, M. Kaneyama, Y. Nagawa, E. Koyama, M. Kanesato, *J. Am. Chem. Soc.* **2004**, *126*, 13568–13569; e) B. W. Laursen, S. Nygaard, J. O. Jeppesen, J. F. Stoddart, *Org. Lett.* **2004**, *6*, 4167–4171; f) G. W. H. Wurpel, A. M. Brouwer, I. H. M. van Stokkum, A. Farran, D. A. Leigh, *J. Am. Chem. Soc.* **2001**, *123*, 11327–11328; g) M. Montalti, L. Prodi, *Chem. Commun.* **1998**, 1461–1462.

[10] a) L. M. Hancock, P. D. Beer, *Chem. Eur. J.* **2009**, *15*, 42–44; b) K. M. Mullen, J. Mercurio, C. J. Serpell, P. D. Beer, *Angew. Chem.* **2009**, *121*, 4875–4878; *Angew. Chem. Int. Ed.* **2009**, *48*, 4781–4784; c) M. J. Barrell, D. A. Leigh, P. J. Lusby, A. M. Z. Slawin, *Angew. Chem.* **2008**, *120*, 8156–8159; *Angew. Chem. Int. Ed.* **2008**, *47*, 8036–8039; d) Y. L. Huang, W. C. Hung, C. C. Lai, Y. H. Liu, S. M. Peng, S. H. Chiu, *Angew. Chem.* **2007**, *119*, 6749–6753; *Angew. Chem. Int. Ed.* **2007**, *46*, 6629–6633; e) C. F. Lin, C. C. Lai, Y. H. Liu, S. M. Peng, S. H. Chiu, *Chem. Eur. J.* **2007**, *13*, 4350–4355.

[11] a) K. M. Mullen, J. J. Davis, P. D. Beer, *New J. Chem.* **2009**, *33*, 769–776; b) A. Brown, K. M. Mullen, J. Ryu, M. J. Chmielewski, S. M. Santos, V. Felix, A. L. Thompson, J. E. Warren, S. I. Pascu, P. D. Beer, *J. Am. Chem. Soc.* **2009**, *131*, 4937–4952; c) D. Curiel, P. D. Beer, *Chem. Commun.* **2005**, 1909–1911.

[12] a) J. J. Gassensmith, L. Barr, J. M. Baumes, A. Paek, A. Nguyen, B. D. Smith, *Org. Lett.* **2008**, *10*, 3343–3346; b) J. J. Gassensmith, J. M. Baumes, B. D. Smith, *Chem. Commun.* **2009**, 6329–6338.

[13] a) M. Xue, C. F. Chen, *Chem. Commun.* **2008**, 6128–6130; b) J. J. Gassensmith, E. Arunkumar, L. Barr, J. M. Baumes, K. M. DiVittorio, J. R. Johnson, B. C. Noll, B. D. Smith, *J. Am. Chem. Soc.* **2007**, *129*, 15054–15059.

[14] G. Hübner, G. Nachtsheim, Q. Y. Li, C. Seel, F. Vögtle, *Angew. Chem.* **2000**, *112*, 1315–1318; *Angew. Chem. Int. Ed.* **2000**, *39*, 1269–1272.

[15] As seen in Figure 1b, Cl^- binding to **1** induces severe broadening of triazole related protons 2, 3 and 4. At low temperature and excess Cl^-, the spectral patterns for the thread and macrocycle become unsymmetric and the chemical shifts indicate that the macrocycle resides predominantly over one of the triazoles. It is possible that the macrocycle-bonded Cl^- simultaneously interacts with the triazole CH residue as recently described by others. See, Y. J. Li, A. H. Flood, *Angew. Chem.* **2008**, *120*, 2689–2692; *Angew. Chem. Int. Ed.* **2008**, *47*, 2649–2652; H. Juwarker, J. M. Lenhardt, D. M. Pham, S. L. Craig, *Angew. Chem.* **2008**, *120*, 3800–3803; *Angew. Chem. Int. Ed.* **2008**, *47*, 3740–3743; R. M. Meudtner, S. Hecht, *Angew. Chem.* **2008**, *120*, 5004–5008; *Angew. Chem. Int. Ed.* **2008**, *47*, 4926–4930.

[16] In Figure 1b, the signals corresponding to macrocycle protons a, b, and c for rotaxane **1** mixed with one molar equivalent of TBA^+Cl^- are also different to the signals for a 1:1:1 mixture of macrocycle **2**, thread **4**, and TBA^+Cl^- (Figure 1d). This is expected because macrocycle **2** is fully occupied with Cl^- in Figure 1d, but it is not saturated with Cl^- in Figure 1b (the Cl^- affinity is weaker) and it is also remains threaded by squaraine.

[17] G. M. Hübner, J. Gläser, C. Seel, F. Vögtle, *Angew. Chem.* **1999**, *111*, 395–398; *Angew. Chem. Int. Ed.* **1999**, *38*, 383–386.

[18] P. V. Kamat, S. Das, K. G. Thomas, M. V. George, *J. Phys. Chem.* **1992**, *96*, 195–199. The excitation wavelengths in Figures 3, 4 and 5 were chosen for experimental convenience, since molar absorptivity is not a factor in these measurements.

[19] C. Bohne, M. Barra, R. Boch, E. B. Abuin, J. C. Sciano, *J. Photochem. Photobiol. A* **1992**, *65*, 249–265.

[20] I. Carmichael, G. L. Hug, *J. Phys. Chem. Ref. Data* **1986**, *15*, 1–250.

[21] S. L. Murov, I. Carmichael, and G. L. Hug, *Handbook of Photochemistry*, **1993**, Marcel Dekker, New York.

[22] In addition to fluorescence intensity imaging, it is likely that the dipsticks can be monitored using fluorescence lifetime imaging technology. For a review, see: J. A. Levitt, D. R. Matthews, S. M. Ameer-Beg, K. Suhling, *Curr. Opin. Biotechnol.* **2009**, *20*, 28–36.

[23] The question arises if rotaxane **1** has any sensing advantages over the synthetically simpler dye displacement system composed of macrocycle **2** and dye **3**. In chloroform solution, the dye displacement system exhibits essentially the same reversible changes in fluorescence as rotaxane **1**. That is, the intensity enhancement upon Cl^- addition to the solution is reversed when it is removed by precipitation. However, the inherent limitations of the dye displacement system become apparent when an admixture of macrocycle **2** and dye **3** are employed in a dipstick that mimics the experiment in Figure 6. Sensing performance of the dye displacement dipstick is poor. Dipping into an aqueous solution of TBA^+Cl^- produces a relatively small, unstable fluorescence enhancement that decreases irreversibly if the dipping time is lengthened. There are three reasons for this loss of performance, the displaced free squaraine dye **3** forms non-fluorescent self-aggregates in the immobilized dipstick phase, it leaches irreversibly into the aqueous phase, and it is rapidly bleached by the water. As discussed in ref. [6], this loss of sensing function for the dye displacement system is not surprising.

[24] A. Aydogan, D. J. Coady, V. M. Lynch, A. Akar, M. Marquez, C. W. Bielawski, J. L. Sessler, *Chem. Commun.* **2008**, 1455–1457.

[25] a) E. Arunkumar, N. Fu, B. D. Smith, *Chem. Eur. J.* **2006**, *12*, 4684–4690; b) E. Arunkumar, C. C. Forbes, B. C. Noll, B. D. Smith, *J. Am. Chem. Soc.* **2005**, *127*, 3288–3289; c) S. Y. Hsueh, C. C. Lai, Y. H. Liu, Y. Wang, S. M. Peng, S. H. Chiu, *Org. Lett.* **2007**, *9*, 4523–4526.

[26] Dipsticks that can assess NaCl levels in urine may allow regular monitoring of dietary NaCl intake. See, for example: M Büngel, R. Kluthe, P. Fürst, *Ann. Nutr. Metab.* **2001**, *45*, 169–174.

[27] It may be possible to create dipsticks that sense NaCl by immobilizing a binary mixture of **1** and a neutral cationophore. See, for example: A. Aydogan, D. J. Coady, S. K. Kim, A. Akar, C. W. Bielawski, M. Marquez, J. L. Sessler, *Angew. Chem.* **2008**, *120*, 9794–9798; *Angew. Chem. Int. Ed.* **2008**, *47*, 9648–9652.

Received: September 15, 2009
Published online: January 15, 2010

