# A Simple Bifunctional Fluoroionophore Signaling Different Metal Ions Either Independently or Cooperatively

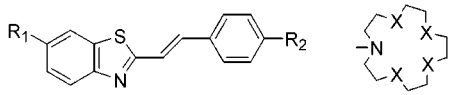
Knut Rurack,*[,†] Anton Koval'chuck,[‡] Julia L. Bricks,[‡] and Juri L. Slominskii[‡]

*Federal Institute for Materials Research and Testing (BAM)*
*Richard-Willstaetter Strasse 11*
*D-12489 Berlin, Germany*
*Institute of Organic Chemistry*
*National Academy of Sciences of the Ukraine*
*5 Murmanskaya St., Kiev-94, Ukraine*

*Received January 18, 2001*
*Revised Manuscript Received May 2, 2001*

The independent simultaneous or cooperative recognition of different chemical species is one of the key features in bio- or analytical chemistry. In combination with photophysical or electrochemical transduction processes, a cornucopia of advanced sensing architectures[1] or molecular-scale logic devices[2] has been designed. Here, modular systems that combine one or several receptor and reporter units are of particular interest, since such a constitution allows tuning sensitivity and selectivity by choice of the functional subunits.[3] When employing a fluorophore as the transduction unit, all of the advantages of the highly sensitive and versatile method of fluorometry can be utilized for the application of interest. In the field of metal ion signaling, recently, a variety of design concepts were realized to enhance sensitivity,[4] selectivity[5] or the dynamic working range[6] as well as to independently recognize but cooperatively report two different analytes.[2a,c]

Besides designing efficient fluorescent sensors and switches with increased selectivity for various environmentally or biochemically relevant metal ions,[6,7] we are interested in combining receptor-based (chemical) selectivity with analyte-mediated signaling (spectroscopic) selectivity in bifunctional probes. The aim is to design a molecule with two different receptors that possesses four distinct, spectroscopically distinguishable states, that is a molecule that indicates whether only one, the other, both, or none of the binding sites are occupied by substrates. Moreover, these states should also allow to independently quantify the respective guests. An application of such advanced sensor molecules would be particularly interesting in many fields of clinical or biochemistry, for instance, in monitoring bidirectional ion fluxes (e.g., $Ca^{2+}$ and $H^+$)[8] or the presence of competing, beneficial, or harmful metal ions (e.g., $Ca^{2+}/Zn^{2+}$ vs $Cd^{2+}$).[9]




**1**: $R_1$ = A15C5, $R_2$ = $AT_4$15C5
**2**: $R_1$ = A15C5, $R_2$ = $N(CH_3)_2$
**3**: $R_1$ = $N(CH_3)_2$, $R_2$ = $AT_4$15C5

A15C5: X = O
$AT_4$15C5: X = S

Besides improvements in spatially resolved quantitation, a reporter molecule with a built-in "alarm function" that generates a "cooperative signal" when binding to both analytes would be highly desirable. Here, we present—to the best of our knowledge—the first simple bifunctional sensor molecule (**1**) that shows such a multifold signal expression in the presence or absence of one or two different types of metal ions.

This concept of performing and transducing two recognition events for very similar analytes in the previously outlined way with a simple dye architecture relies on the following features. To be able to generate site-specific independent signals when only a single stimulus is present, an unsymmetrical molecular structure was conceived with both receptors being an integral part of the different subunits. Since in the present case, metal ions were our analytical targets, we integrated electron-rich monoaza crown ethers in a styryl base-type[10] structure with a donor−acceptor−donor ($D_1$−A−$D_2$) substitution pattern. Here, both donors are formally $\pi$-conjugated and the intramolecular charge transfer (CT) reactions ($D_1$→A and A←$D_2$) commonly observed for such compounds, that is, for **1**,[11] the monofunctionalized reference compounds **2** and **3**, or closely related molecules,[12] are rather independent yet competitive excited-state processes: whereas the CT from the *p*-phenyl donor $D_2$ (= $R_2$) most probably is connected with an excited-state species of twisted nature, resulting in moderate emission,[10] the $D_1$−A charge-transfer reaction within the substituted heterocyclic moiety of such molecules involves a $^1$CT state of predominantly planar conformation, leading to a strongly emissive species.[6a,10,12] Accordingly, both CT reactions in **1**−**3** differ in fluorescence yield and should thus allow to generate specific signals by either engaging $D_1$, $D_2$, or both receptor units of **1** in complexation. Especially these latter features are important prerequisites for achieving the desired cooperative effects. The requirement of coinciding site and analyte specificity was met by using a tetraoxa and a tetrathia monoaza-15-crown-5 as binding units with intrinsically different affinities,[6b,7c] $Ca^{2+}$ and $Ag^+$ serving as the representative cations in the present studies.

A comparison of the data of **1**−**3** in Table 1 demonstrates the suitability of the underlying design rationale. Binding of $Ca^{2+}$

† Federal Institute for Materials Research and Testing.
‡ Academy of Sciences of the Ukraine.

(1) James, T. D.; Linnane, P.; Shinkai, S. *Chem. Commun.* **1996**, 281−288. Wang, F.; Schwabacher, A. W. *J. Org. Chem.* **1999**, *64*, 8922−8928. Cooper, J. B.; Drew, M. G. B.; Beer, P. D. *J. Chem. Soc., Dalton Trans.* **2000**, 2721−2728.

(2) (a) de Silva, A. P.; McClenaghan, N. D. *J. Am. Chem. Soc.* **2000**, *122*, 3965−3966. (b) Gunnlaugsson, T.; MacDónail, D. A.; Parker, D. *Chem. Commun.* **2000**, 93−94. (c) McSkimming, G.; Tucker, J. H. R.; Bouas-Laurent, H.; Desvergne, J.-P. *Angew. Chem., Int. Ed.* **2000**, *39*, 2167−2169.

(3) Beer, P. D. *Chem. Soc. Rev.* **1989**, *18*, 409−450. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566.

(4) Glass, T. E. *J. Am. Chem. Soc.* **2000**, *122*, 4522−4523.

(5) Marquis, D.; Desvergne, J.-P.; Bouas-Laurent, H. *J. Org. Chem.* **1995**, *60*, 7984−7996. Xia, W.-S.; Schmehl, R. H.; Li, C.-J. *J. Am. Chem. Soc.* **1999**, *121*, 5599−5600. Ushakov, E. N.; Gromov, S. P.; Fedorova, O. A.; Pershina, Y. V.; Alfimov, M. V.; Barigelletti, F.; Flamigni, L.; Balzani, V. *J. Phys. Chem. A* **1999**, *103*, 11188−11193.

(6) (a) Bricks, J. L.; Slominskii, J. L.; Kudinova, M. A.; Tolmachev, A. I.; Rurack, K.; Resch-Genger, U.; Rettig, W. *J. Photochem. Photobiol., A* **2000**, *132*, 193−208. (b) Rurack, K.; Bricks, J. L.; Schulz, B.; Maus, M.; Reck, G.; Resch-Genger, U. *J. Phys. Chem. A* **2000**, *104*, 6171−6188.

(7) (a) Rurack, K.; Kollmannsberger, M.; Resch-Genger, U.; Daub, J. *J. Am. Chem. Soc.* **2000**, *122*, 968−969. (b) Kollmannsberger, M.; Rurack, K.; Resch-Genger, U.; Rettig, W.; Daub, J. *Chem. Phys. Lett.* **2000**, *329*, 363−369. (c) Rurack, K.; Resch-Genger, U.; Bricks, J. L.; Spieles, M. *Chem. Commun.* **2000**, 2103−2104.

(8) Wiegmann, T. B.; Welling, L. W.; Beatty, D. M.; Howard, D. E.; Vamos, S.; Morris, S. J. *Am. J. Physiol.* **1993**, *265*, C1184−C1190. Salvador, J. M.; Inesi, G.; Rigaud, J.-L.; Mata, A. M. *J. Biol. Chem.* **1998**, *273*, 18230−18234.

(9) Vo-Dinh, T.; Viallet, P.; Ramirez, L.; Pal, A.; Vigo, J. *Anal. Chim. Acta* **1994**, *295*, 67−72. Hirshfeld, K. M.; Toptygin, D.; Grandhige, G.; Packard, B. Z.; Brand, L. *Biophys. Chem.* **1998**, *71*, 63−72.

(10) (a) Rurack, K.; Rettig, W.; Resch-Genger, U. *Chem. Commun.* **2000**, 407−408. (b) Rettig, W.; Rurack, K.; Sczepan, M. In *New Trends in Fluorescence Spectroscopy*; Valeur, B., Brochon, J.-C., Eds.; Springer: Berlin, 2001; pp 125−155.

(11) The synthesis of **1**−**3** and a series of model compounds, including a newly developed procedure to introduce aza crowns to the benzothiazole moiety, as well as detailed mechanistical studies will be reported separately: Bricks, J. L.; Koval'chuck, A.; Resch-Genger, U.; Rettig, W.; Rurack, K.; Slominskii, J. L., manuscript in preparation.

(12) (a) Fery-Forgues, S.; Le Bris, M.-T.; Guetté, J.-P.; Valeur, B. *J. Phys. Chem.* **1988**, *92*, 6233−6237. (b) Fery-Forgues, S.; Le Bris, M.-T.; Mialocq, J.-C.; Pouget, J.; Rettig, W.; Valeur, B. *J. Phys. Chem.* **1992**, *96*, 701−710.

10.1021/ja015560s CCC: $20.00 
Published on Web 06/02/2001

**Table 1.** Spectroscopic Data of **1**−**3** in the Absence and the Presence of $Ca(ClO_4)_2$ and $AgClO_4$ in Acetonitrile at 295 K[a]

| | λ (abs) nm | λ (em) nm | $\phi_f$ | $\tau_f$ ns | log *Ks*[b] |
|---|---|---|---|---|---|
| **1** | 413 | 499 | 0.05 | 0.37 | |
| $Ca^{2+}$−**1** | 403 | 510 | 0.02 | 0.10 | 4.78 |
| **1**−$Ag^+$ | 403 | 519 | 0.27 | 1.07, 2.69[c] | 4.83 |
| $Ca^{2+}$−**1**−$Ag^+$ | 384 | 513 | 0.04 | *c* | *c* |
| **2** | 410 | 502 | 0.02 | 0.14 | |
| $Ca^{2+}$−**2** | 401 | 520 | 0.01 | 0.09 | 4.87 |
| **2**/$Ag^+$ | 411 | 504 | 0.02 | 0.15 | *d* |
| **3** | 409 | 503 | 0.05 | 0.35 | |
| **3**−$Ag^+$ | 400 | 525 | 0.27 | 1.28, 2.90[c] | 4.75 |
| **3**/$Ca^{2+}$ | 408 | 504 | 0.05 | 0.36 | *d* |

[a] $c$(dye) = 1 × $10^{-6}$ M, $c(M^{n+})$ = 1 × $10^{-4}$ M. For experimental spectroscopic details, see ref 6b. [b] From fluorescence titration. [c] For details, see Supporting Information. [d] No measurable complex formation up to a 400-fold excess of metal ion.

(at A15C5) or $Ag^+$ (at $AT_4$15C5) reduces the respective donor strength due to coordinative engagement of the nitrogen atom's lone electron pair and leads to slightly blue-shifted absorption spectra. Upon photoexcitation of $Ca^{2+}$−**1**[13] and $Ca^{2+}$−**2**, $D_1$ is converted into $A_1$, impeding population of the highly emissive planar $^1$CT state with respect to the single bond-twisted less emissive species and reduced fluorescence is found. $Ag^+$ coordination, however, yields a $D_1$−A−$A_2$ structure promoting the $D_1$−A charge transfer and leads to drastically enhanced emission.[14] Thus, analyte-specific signals are obtained. The expected ion selectivities are further evident from the fluorescence lifetime and complex stability data. Whereas $Ca^{2+}$−**1** and $Ca^{2+}$−**2** decay with very similar time constants of ca. 100 ps, **1**−$Ag^+$ and **3**−$Ag^+$ display two characteristically long fluorescence lifetimes of ca. 1.2 (major component) and ca. 2.8 ns (minor component).[15] Moreover, fits of the titration data revealed complex stability constants typical for 1:1 complexes of neutral fluoroionophores.[16]

Upon binding to both ions, **1** clearly displays cooperativity. Formation of $Ca^{2+}$−**1**−$Ag^+$ largely deprives the chromophoric system of the nitrogen atom lone electron pairs and leads to a pronounced hypsochromic shift in absorption. In accordance with excited-state processes in other complexed D−A(−D) probes,[6a,10,12a] absorption of a photon still triggers an excited-state CT reaction in doubly bound **1**, yielding a moderate overall emission which is centered at almost the same wavelength (Table 1).[15b] Figure 1 stresses the suitability of this modular design concept for selective but cooperative ion signaling: when titrating either $Ca^{2+}$−**1** with $Ag^+$ or **1**−$Ag^+$ with $Ca^{2+}$, the starting points of each titration are very similar to the reference complexes $Ca^{2+}$−**2** and **3**−$Ag^+$ and, after shifting into the expected direction, the end-point spectra of both titrations are virtually superimposed. Owing to the positive charge of the single-cation complex, the log *Ks* obtained for the titrations with the second ion are smaller but nonetheless the data in Figure 1 support the largely independent selectivities of both receptors.

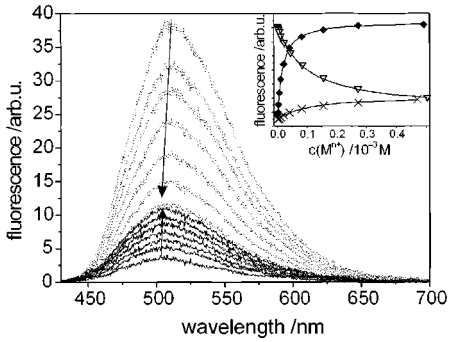



**Figure 1.** Selected (uncorrected) fluorescence spectra taken during a titration of **1**−$Ag^+$ with $Ca(ClO_4)_2$ (···) and $Ca^{2+}$−**1** with $AgClO_4$ (−) in MeCN ($\lambda_{exc}$ = 377 nm). Inset: Fit of the fluorescence intensity at 510 nm for **1**/$Ag^+$ (◆, log *Ks* = 4.83), **1**−$Ag^+$/$Ca^{2+}$ (▽, 4.03), and $Ca^{2+}$−**1**/$Ag^+$ (×, 3.97) according to a 1:1 complexation model.

On the basis of this guest- and site-specific reconfiguration of the photophysically active subunits, **1** is the prototype of a sensor molecule that combines intrinsic receptor-based binding preferences with an intramolecular, analyte-sensitive, dual-mode signaling mechanism. With such a bifunctional fluoroionophore, it is possible to cooperatively recognize and signal the presence of two metal ions by a specific shift in absorption (thus setting off an "alarm"), while allowing the independent quantification of both analytes by time-resolved fluorescence and global analysis. In terms of digital action,[17] **1** can be employed as a bimodal logic gate: a strongly decreased absorbance at 440 nm is only found in the presence of both ions (transducing an inverted "AND", that is, a "NAND" event, with digital action = 1 for $Ca^{2+}$/$Ag^+$ = 0/0, 0/1, and 1/0)[18] and a strong fluorescence signal, when excited at 440 nm, is only found for $Ag^+$. The latter case with a digital signal = 1 only for $Ca^{2+}$/$Ag^+$ = 0/1 represents a noncommutative "INHIBIT" logic function.[2b,19]

In summary, we introduced a general framework for combining two competitive yet complementary signaling mechanisms in a simple modularly configurable dye architecture that provides a promising approach for the development of molecular sensors and switches, exhibiting cooperativity.

**Acknowledgment.** Financial support by the Deutsche Forschungsgemeinschaft and BAM-MOE is gratefully acknowledged.

**Supporting Information Available:** Detailed description of the excited-state mechanisms (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA015560S

(13) **X**−$M^{n+}$, $M^{n+}$−**X**, and $M^{n+}$−**X**−$M^{n+}$ stand for binding to (a) receptor(s) at the $D_1$, $D_2$, or at both positions.

(14) Although spectrally very similar, the differences in fluorescence quantum yield of free **1** and **3** as compared to unbound **2** can be rationalized in terms of the different donor strengths of the three substituted anilino groups, the donating character increasing on the order of $AT_4$15C5 < A15C5 ≤ $N(CH_3)_2$.[6b,7c]

(15) (a) Such a biexponential decay behavior of cation complexes of crown-containing fluoroionophores has been observed before.[7a,b] (b) A description is given in conjunction with the detailed excited-state mechanisms in the Supporting Information.

(16) Rurack, K.; Sczepan, M.; Spieles, M.; Resch-Genger, U.; Rettig, W. *Chem. Phys. Lett.* **2000**, *320*, 87−94.

(17) Burger, P. *Digital Design, A Practical Course*; J. Wiley & Sons: New York, 1988.

(18) Since binding to both macrocyclic nitrogen atoms leads not only to a pronounced blue-shift but also to a decrease in extinction coefficient, the "NAND" function with measuring the absorption in the red part of the spectrum is the more efficient transduction mode.

(19) In agreement with the ion selectivites reported in refs 6b,7c, **1** can also be operated with $Hg^{2+}$ instead of $Ag^+$. In this case, **1**−$Hg^{2+}$ shows quenched fluorescence conceivable with the heavy-atom effect $Hg^{2+}$ induces in the tightly bound excited complex of $D_1$−A−$A_2$ constitution. Since $Hg^{2+}$ is a divalent ion, $Ca^{2+}$−**1**−$Hg^{2+}$ shows an even more strongly blue-shifted absorption spectrum (348 nm) than that of $Ca^{2+}$−**1**−$Ag^+$ while maintaining the quenched fluorescence of **1**−$Hg^{2+}$. Thus, with $Hg^{2+}$ and $Ca^{2+}$, **1** can be operated as a bimodal "NAND" (absorption at 440 nm) or "NOR" (moderate fluorescence only in the unbound state) logic gate.