

A Selective and Sensitive Fluoroionophore for Hg^{II}, Ag^I, and Cu^{II} with Virtually Decoupled Fluorophore and Receptor Units

Knut Rurack,^{*,†} Matthias Kollmannsberger,[‡]
Ute Resch-Genger,[†] and Jörg Daub^{*,‡}

Federal Institute for Materials
Research and Testing (BAM)
Richard-Willstaetter Str. 11
D-12489 Berlin, Germany
Institute of Organic Chemistry
University of Regensburg
D-93040 Regensburg, Germany

Received July 26, 1999

Revised Manuscript Received October 18, 1999

The development of sensitive and selective fluorescent sensor molecules is a fundamental goal in fluorometric metal ion analysis.¹ Besides the search for new fluoroionophores for alkali and alkaline-earth metal ions,² much attention has been focused lately on the design of probes for heavy and transition metal (HTM) ions.³ Here, not only selectivity, i.e., discrimination between chemically closely related cations, but sensitivity as well represents a major problem, as many of these cations are known as fluorescence quenchers via enhanced spin-orbit coupling (e.g., Hg^{II}),⁴ energy or electron transfer (e.g., paramagnetic Cu^{II}).⁵ Fluorescence quenching is not only disadvantageous for a high signal output upon complexation but also hampers temporal separation of spectrally similar complexes with time-resolved fluorometry.⁶ However, most common fluorescent probes undergo nonspecific quenching with analytes such as Hg^{II} or Cu^{II}.⁷ Only very few examples for probes showing a fluorescence enhancement with HTM ions have been yet reported.⁸ Recently, we found an extraordinary large fluorescence enhancement (> 2000) upon complexation of alkali and alkaline-earth metal ions to donor-substituted boron dipyrromethene (BDP) dyes.⁹ BDP dyes combine high photostability¹⁰ with high absorption coefficients ($\epsilon > 50\,000\text{ M}^{-1}\text{ cm}^{-1}$) and high fluorescence quantum yields ($\phi_f > 0.5$)¹¹ and can be excited at rather long wavelengths ($\sim 500\text{ nm}$). Combining this virtually decoupled probe design allowing for

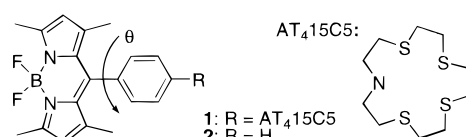


Figure 1. Chemical structures of **1** and its model compound **2**.

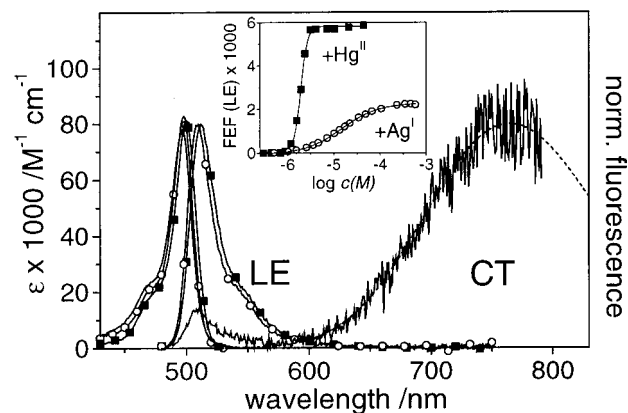


Figure 2. Absorption and emission spectra ($\lambda_{\text{exc}} 480\text{ nm}$) of **1** ($1.13 \times 10^{-6}\text{ M}$) in the absence (no symbols, dotted line: fit of the CT emission²⁴) and presence (at full complexation) of Hg^{II} (■, 10-fold excess) and Ag^I (○, 400-fold excess) in acetonitrile. Insert: Fluorometric titration of **1** ($1.13 \times 10^{-6}\text{ M}$) with Hg^{II} (■) and Ag^I (○) perchlorates in acetonitrile ($\lambda_{\text{exc}} 480\text{ nm}$, FEF (LE) determined from the integrated fluorescence intensity of the LE band).

efficient fluorescence switching with the selectivity of a recently developed thia aza crown receptor^{12,13} yields dye **1** (Figure 1) presented here which shows a strong fluorescence enhancement selectively with Hg^{II}, Ag^I, and Cu^{II}.

In **1** the π -systems of the BDP acceptor and anilino donor unit are highly twisted¹⁴ and thus largely decoupled. The absorption spectra in solvents of any polarity show a narrow band at $\sim 500\text{ nm}$ ($\epsilon = 78\,000\text{ M}^{-1}\text{ cm}^{-1}$) characteristic of the BDP chromophore. No significant charge transfer (CT) band can be observed. In contrast, the emission spectra are strongly solvent-dependent. In solvents more polar than alkanes the fluorescence is strongly quenched, and dual emission is observed (Table 1; Figure 2). Excitation at the BDP absorption band leads to a locally excited (LE) state which can undergo an ultrafast excited-state reaction to a highly polar emissive CT state.^{9,15} Whereas the BDP localized, narrow LE emission band of mirror image shape at $\sim 510\text{ nm}$ is insensitive to solvent polarity, the red-shifted, broad, and structureless CT band shifts to lower energies with increasing solvent polarity due to stabilization of the CT state. Time-resolved fluorescence measurements reveal the precursor–successor equilibrium of the two excited states. The photophysical behavior of analogous systems is discussed in more detail in ref 9.

In acetonitrile used for the complexation experiments, both the ultrafast charge-transfer reaction and strongly forbidden character of the CT emission follow from the low fluorescence quantum yields of both the LE and CT emission, respectively. Taking the

[†] Federal Institute for Materials Research and Testing.

[‡] University of Regensburg.

(1) Czarnik, A. W. *Chem. Biol.* **1995**, *2*, 423–428.

(2) 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.

(3) (a) Fabbrizzi, L.; Poggi, A. *Chem. Soc. Rev.* **1995**, 197–202. (b) Krämer, R. *Angew. Chem., Int. Ed.* **1998**, *37*, 772–773.

(4) McClure, D. S. *J. Chem. Phys.* **1952**, *20*, 682–686.

(5) Varnes, A. W.; Dodson, R. B.; Wehry, E. L. *J. Am. Chem. Soc.* **1972**, *94*, 946–950.

(6) Rurack, K.; Resch-Genger, U.; Rettig, W. *J. Photochem. Photobiol., A Chem.* **1998**, *118*, 143–149.

(7) (a) Akkaya, E. U.; Huston, M. E.; Czarnik, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 3590–3593. (b) Alfimov, M. V.; Fedorov, Y. V.; Fedorova, O. A.; Gromov, S. S.; Hester, R. E.; Lednev, I. K.; Moore, J. N.; Oleshko, V. P.; Vedernikov, A. I. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1441–1447. (c) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Perotti, A.; Taglietti, A.; Sacchi, D. *Chem. Eur. J.* **1996**, *2*, 75–82. (d) Yoon, J.; Ohler, N. E.; Vance, D. H.; Aumiller, W. D.; Czarnik, A. W. In *Chemosensors of Ion and Molecule Recognition*; Desvergne, J.-P., Czarnik, A. W., Eds.; Kluwer Academic: Dordrecht, 1997; pp 189–194.

(8) (a) Unterreitmaier, E.; Schuster, M. *Anal. Chim. Acta* **1995**, *309*, 339–344. (b) Ghosh, P.; Bharadwaj, P. K.; Mandal, S.; Ghosh, S. *J. Am. Chem. Soc.* **1996**, *118*, 1553–1554. (c) Ramachandram, B.; Samanta, A. *J. Phys. Chem. A* **1998**, *102*, 10579–10587. (d) Hennrich, G.; Sonnenschein, H.; Resch-Genger, U. *J. Am. Chem. Soc.* **1999**, *121*, 5073–5074.

(9) Kollmannsberger, M.; Rurack, K.; Resch-Genger, U.; Daub, J. *J. Phys. Chem. A* **1998**, *102*, 10211–10220.

(10) BDP dyes are used as laser dyes (cf. Pavlopoulos, T. G.; Shah, M.; Boyer, J. *Appl. Opt.* **1988**, *27*, 4998–4999).

(11) (a) Vos de Wal, E.; Pardoën, J.; van Koevring, J. A.; Lugtenburg, J. *Recl. Trav. Chim. Pays-Bas* **1977**, *96*, 306–309. (b) Karolin, J.; Johansson, L. B.-A.; Strandberg, L.; Ny, T. *J. Am. Chem. Soc.* **1994**, *116*, 7801–7806.

(12) Rurack, K.; Bricks, J. L.; Slominskii, J. L.; Resch-Genger, U. In *Near-Infrared Dyes for High Technology Applications*; Dähne, S., Resch-Genger, U., Wolfbeis, O. S., Eds.; Kluwer Academic: Dordrecht, 1998; pp 191–200.

(13) Ishikawa, J.; Sakamoto, H.; Wada, H. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1273–1279.

(14) $\theta = 78^\circ$ for a derivative of **2** (Kollmannsberger, M.; Gareis, T.; Heinel, S.; Breu, J.; Daub, J. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1333–1335.)

(15) Rettig, W. *Top. Curr. Chem.* **1994**, *169*, 253–299.

Table 1. Spectroscopic Data of **1** in Various Solvents and of **1**Cu^{II}, **1**Ag^I, and **1**Cu^{II} in Acetonitrile at Room Temperature

	solvent	λ (abs)	λ (em, LE)	λ (em, CT)	ϕ_f (LE)	ϕ_f (CT)	τ_f (1)	τ_f (2)	τ_f (2, LE), a ^{rel}	logK _S
		nm	nm	nm			ps	ps	ps	
1	<i>n</i> -hexane	501	510		0.32		1850			
	diethyl ether	499	508	589	0.23	0.022	552	3030		
	acetonitrile	497	509	749	0.0001	0.0014	<3	495		
1 Cu ^{II}	acetonitrile	500	511		0.59		3060			>5.75 ^a
1 Ag ^I	acetonitrile	498	508		0.22		2790		1290, 0.08	4.79 ^b
1 Cu ^{II}	acetonitrile	501	513		0.25		3190		810 ^c	5.39 ^{b,d}

^a Too high to be determined with acceptable accuracy with the method employed. ^b log K_S corresponds to an overall log K_S of the two conformers, cf. ref 9. ^c Relative amplitudes vary with Cu^{II} concentration. ^d Titration spectra recorded 5 min after each step of cation addition.

effective rate constant for radiative CT* deactivation $k_f^{\text{eff}}(\text{CT})$ as a measure,¹⁶ the low value of $0.03 \times 10^8 \text{ s}^{-1}$ calculated from the data in Table 1 supports these findings. For instance, for the related pre-twisted compound 3-methyl-4-(methylamino)-benzotrile showing dual fluorescence, a much larger $k_f^{\text{eff}}(\text{CT})$ of $1.12 \times 10^8 \text{ s}^{-1}$ was reported.¹⁷

As shown in Figure 2, reaction of **1** with the thiophilic ions Hg^{II} and Ag^I leads to complex formation. Among all the other HTM ions (Cu^{II}, Ni^{II}, Co^{II}, Zn^{II}, Cd^{II}, Pb^{II}) investigated, a similar effect is only observed for Cu^{II}. The changes in absorption are minor, but even in the case of the fluorescence quenchers Hg^{II} and Cu^{II} cation binding is accompanied by a large increase in LE fluorescence. In acetonitrile, fluorescence enhancement factors (FEF) of 5900 for Hg^{II} (more or less reaching the fluorescence quantum yield of the reference compound **2**),¹⁸ 2500 for Cu^{II}, and 2200 for Ag^I can be realized. The orthogonal setup prevents any ground-state interaction of both molecular fragments in the complexes, and the shape and position of the absorption and emission bands resemble closely those of **2** lacking a donor.¹⁸ In agreement with the observed chelation-enhanced fluorescence, the fluorescence decay times of the complexes increase drastically. Monoexponential decay kinetics are found for **1**Cu^{II}, whereas for **1**Ag^I and **1**Cu^{II} the decays are biexponential. The relative amplitudes of the two decay components of **1**Ag^I remain constant over the entire concentration range and are independent of excitation and observation wavelength. This suggests the formation of two different complex conformers⁹ which are closely related to the *endo-exo* conformational equilibrium of crown ethers in solution¹⁹ and involves a multistep Eigen-Winkler scheme for the kinetics of cation macrocycle binding.²⁰ Different geometries (e.g., with respect to the intramolecular twist angle, degree of pyramidalization at the anilino nitrogen atom, and orbital overlap) and differences in cation–nitrogen atom interaction for both complex conformers influence the CT process and lead to different CT inhibition rates, thus resulting in a biexponential decay of the LE fluorescence.

Fits of the fluorometric titration curves yield 1:1 stoichiometries for the Hg^{II} and Ag^I complex in acetonitrile. In the case of Cu^{II},

(16) $k_f^{\text{eff}}(\text{CT}) = \phi_f(\text{CT})/\tau_f(\text{CT})$

(17) Rotkiewicz, K.; Rettig, W. *J. Lumin.* **1992**, *54*, 221–229.

(18) Data of **2** in acetonitrile 497/505 nm, $\phi_f = 0.60$, $\tau_f = 3.17 \text{ ns}$ (ref 9).

(19) Buschmann, H.-J. In *Stereochemical and Stereophysical Behaviour of Macrocycles*; Bernal, I., Ed.; Elsevier: Amsterdam, 1987; pp 103–185.

(20) Gokel, G. W.; Echegoyen, L.; Kim, M. S.; Eyring, E. M.; Petrucci, S. *Biophys. Chem.* **1987**, *26*, 225–233.

the picture is more complex since actual binding of Cu^{II} to the crown is fast but seems to include slower complex rearrangement kinetics.²¹

The high complex stability constants of **1**Cu^{II} and **1**Ag^I (Table 1) and the strong FEF distinguish **1** as a very sensitive fluorescent probe in acetonitrile. Moreover, with a probe concentration of $1.13 \times 10^{-6} \text{ M}$, Hg^{II}, Ag^I, and Cu^{II} could be detected up to the ppb concentration range, and no quenching hampers the cation detection for metal ion concentrations <50-fold excess for Hg^{II} and concentrations <10-fold excess for Cu^{II}, respectively.²²

The high selectivity for these ions is manifested by the at least 2 orders of magnitude smaller FEF for Ni^{II} (FEF = 5.3), Co^{II} (2.5), Zn^{II} (1.5), Cd^{II} (3.0), and Pb^{II} (35).²³ Studies of the complexes in aqueous media (water/acetonitrile: 3:1) also show strong fluorescence enhancement in the case of Hg^{II} ($\phi_f = 0.58$, FEF = 223) and Ag^I ($\phi_f = 0.24$, FEF = 92), but only a minor effect is observed in the presence of Cu^{II} (FEF = 2). Both **1**Cu^{II} and **1**Ag^I show monoexponential fluorescence decay kinetics with clearly distinguishable lifetimes (**1**Cu^{II}: $\tau_f = 3550 \text{ ps}$, **1**Ag^I: $\tau_f = 2620 \text{ ps}$) which allow a simultaneous determination of Hg^{II} and Ag^I.

These results demonstrate that highly sensitive and selective detection of Ag^I and the widely known quenchers Hg^{II} and Cu^{II} can be achieved with a simple orthogonally decoupled fluorescent probe in an analytically advantageous wavelength region.

Acknowledgment. We thank Dr. J. L. Bricks (National Academy of Sciences, Kiev, UKR) for synthesizing the crowned aldehyde used as precursor for **1**. We gratefully acknowledge the financial support by the Deutsche Forschungsgemeinschaft.

Supporting Information Available: Synthesis, experimental details, and spectroscopic data of **1** in 1,4-dioxane and tetrahydrofuran (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>. JA992630A

(21) Westerby, B. C.; Juntunen, K. L.; Leggett, G. H.; Pett, V. B.; Koenigbauer, M. J.; Purgett, M. D.; Taschner, M. J.; Ochrymowycz, L. A.; Rorabacher, D. B. *Inorg. Chem.* **1991**, *30*, 2109–2120.

(22) The dynamic working range for Cu^{II} sensing is limited because at higher excess, displacement of the BF₂ group takes place and Cu^{II} binds to the dipyrromethene unit. Reference measurements with **2** and Cu^{II} confirmed these findings.

(23) FEF (LE band) for a 10-fold excess of metal ion in the solution.

(24) For a description of the fitting procedure, see ref 9.