

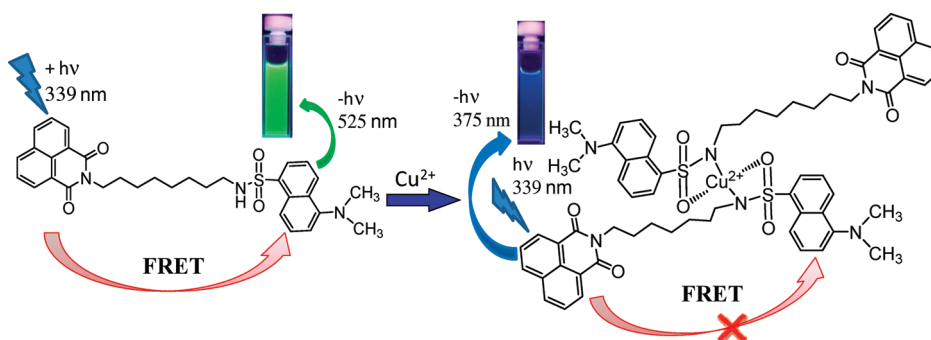
Fluorescence Ratiometric Selective Recognition of Cu^{2+} Ions by Dansyl–Naphthalimide Dyads

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Chimeric dyads **1a** and **1b** based on dansyl and naphthalimide units linked through the polymethylene group were synthesized, and their photophysical and interactions with various metal ions were investigated under different conditions. These dyads showed dual emission centered at around 375 and 525 nm, respectively, due to the locally excited state of the naphthalimide chromophore and energy-transfer-mediated emission (FRET) from the dansyl moiety. When titrated with various metal ions, these systems exhibited unusual selectivity for Cu^{2+} ions as compared to Na^+ , Li^+ , K^+ , Zn^{2+} , Pb^{2+} , Hg^{2+} , Co^{2+} , Fe^{2+} , Cd^{2+} , Mg^{2+} , and Ba^{2+} ions and signaled the binding event through inhibition of FRET mediated emission at 525 nm with concurrent enhancement in the emission intensity of the naphthalimide chromophore at 375 nm. The uniqueness of these dyads is that they form stable 2:1 stoichiometric complexes involving sulfonamide functionality and act as visual fluorescence ratiometric probes for the selective recognition of Cu^{2+} ions.

Introduction

Development of highly sensitive and specific probes for various metal ions has been a subject of intense interest due to their potential applications in clinical biochemistry and the environment.^{1,2} Of all metal ions, design of probes for Cu^{2+}

ions has received considerable attention due to their importance in several biological processes.³ At higher concentrations, Cu^{2+} ions can be highly toxic to the organisms, since they can displace other metal ions that act as cofactors in enzyme-catalyzed reactions.⁴ Also, the unregulated Cu^{2+} ions can cause oxidative stress, and their concentration in neuronal cytoplasm may contribute to the etiology of Alzheimer's or Parkinson's disease.⁵ In this context, the design of functional

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(1) (a) 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. (b) *Fluorescent Chemosensors for Ion and Molecule Recognition*; Desvergne, J. P., Czarnik, A. W., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1997.

(2) (a) Martinez-Manez, R.; Sancenon, F. *Chem. Rev.* **2003**, *103*, 4419. (b) Descalzo, A. B.; Martinez-Manez, R.; Radeaglia, R.; Rurack, K.; Soto, J. J. *Am. Chem. Soc.* **2003**, *125*, 3418. (c) Prodi, L.; Bolletta, F.; Montalti, M.; Zaccaroni, N. *Coord. Chem. Rev.* **2000**, *205*, 59. (d) Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3. (e) Nguyen, B. T.; Anslyn, E. V. *Coord. Chem. Rev.* **2006**, *250*, 3118. (f) Avirah, R. R.; Jyothish, K.; Ramaiah, D. *Org. Lett.* **2007**, *9*, 121. (g) Avirah, R. R.; Jyothish, K.; Ramaiah, D. *J. Org. Chem.* **2008**, *73*, 274.

(3) (a) Kramer, R. *Angew. Chem., Int. Ed.* **1998**, *37*, 772. (b) Linder, M. C.; Hazegh-Azam, M. *Am. J. Clin. Nutr.* **1996**, *63*, 811S.

(4) Koval, I. A.; Gamez, P.; Belle, C.; Selmecci, K.; Reedijk, J. *Chem. Soc. Rev.* **2006**, *35*, 814.

(5) (a) Barnham, K. J.; Masters, C. L.; Bush, A. I. *Nat. Rev. Drug Discovery* **2004**, *3*, 205. (b) Brown, D. R.; Kozlowski, H. *Dalton Trans.* **2004**, 1907. (c) Millhauser, G. L. *Acc. Chem. Res.* **2004**, *37*, 79. (d) Gaggelli, E.; Kozlowski, H.; Valensin, D.; Valensin, G. *Chem. Rev.* **2006**, *106*, 1995. (e) Deraeve, C.; Boldron, C.; Maraval, A.; Mazarguil, H.; Gornitzka, H.; Vendier, L.; Pitie', M.; Meunier, B. *Chem.—Eur. J.* **2008**, *14*, 682. (f) Lee, J. C.; Gray, H. B.; Winkler, J. R. *J. Am. Chem. Soc.* **2008**, *130*, 6898.

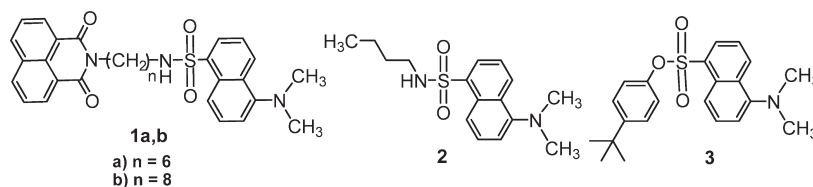


FIGURE 1. Structures of the dyads **1a,b** and the model compounds **2** and **3** under investigation.

molecules that selectively bind to Cu^{2+} ions and signal the event through sensitive and easily detectable outputs is highly important.⁶ Of the various techniques, the optoelectronic detection has several advantages, and the fluorescence-based techniques, in particular, offer high sensitivity.^{1,7}

Recently, the development of fluorescence ratiometric probes for metal ions has attracted much attention since they allow the measurement of emission intensities at two different wavelengths.⁸ This method provides a built-in correction for environmental effects (i.e., artifacts as a result of probe concentration variations) as well as increases the dynamic range of emission measurements.⁹ Since the sensitivity and dynamic range of a ratiometric probe are controlled by the ratio of emission intensities, the design of probes that selectively interact with metal ions and show high ratiometric signals has been challenging. In particular, ratiometric probes for Cu^{2+} ions is a challenge due to its inherent paramagnetic nature, and hence, the complexation generally results in quenching of the fluorescence intensity of the probe.¹⁰ However, there were also a few examples reported

wherein enhancement in the fluorescence intensity has been observed upon complexation with Cu^{2+} ions.¹¹ The low sensitivity and the high order of interference by chemically closely related metal ions has thus necessitated the design of highly selective probes for Cu^{2+} ions.^{10g,12} In this context, we synthesized two chimeric dyads **1a** and **1b** based on dansyl and naphthalimide chromophores and the model compounds **2** and **3** for comparison (Figure 1) and investigated their photophysical properties on interactions with various monovalent and divalent metal ions. We designed these dyads because of the fact that their individual units have been investigated as chemosensors¹³ and fluorescent labels¹⁴ and can, in principle, undergo intramolecular fluorescence resonance energy transfer (FRET) and photoinduced electron transfer (PET) reactions.¹⁵ Our results demonstrate that these dyads can interact selectively with Cu^{2+} ions as compared to other metal ions and signal the binding event through inhibition of FRET-mediated emission, thereby indicating their potential use as sensitive fluorescence ratiometric probes for the selective recognition of Cu^{2+} ions.

Results and Discussion

Synthesis and Photophysical Properties of the Dyads. Synthesis of the chimeric dyads **1a** and **1b** has been achieved in good yields (60–65%) by the reaction of the corresponding *N*- ω -alkylnaphthalimide with dansylchloride (Scheme 1), whereas the model compounds **2** (90%) and **3** (50%) were synthesized as per the reported procedure^{16,17} by the reaction of dansyl

(6) (a) Kukrer, B.; Akkaya, E. U. *Tetrahedron Lett.* **1999**, *40*, 9125. (b) Fabbrizzi, L.; Poggi, A. *Chem. Soc. Rev.* **1995**, *24*, 197. (c) Kim, H. J.; Hong, J.; Hong, A.; Ham, S.; Lee, J. H.; Kim, J. S. *Org. Lett.* **2008**, *10*, 1963. (d) Martinez, R.; Zapata, F.; Caballero, A.; Espinosa, A.; Tarraga, A.; Molina, P. *Org. Lett.* **2006**, *8*, 3235. (e) Qi, X.; Jun, E. J.; Xu, L.; Kim, S.-J.; Hong, J. S. J.; Yoon, Y. J.; Yoon, J. J. *Org. Chem.* **2006**, *71*, 2881. (f) Li, Y.; Cao, L.; Tian, H. *J. Org. Chem.* **2006**, *71*, 8279. (g) Choi, J. K.; Kim, S. H.; Yoon, J.; Lee, K.-H.; Bartsch, R. A.; Kim, J. S. *J. Org. Chem.* **2006**, *71*, 8011. (h) Silveira, V. C. D.; Luz, J. S.; Oliveira, C. C.; Graziani, I.; Ciriolo, M. R.; Ferreira, A. M. D. C. *J. Inorg. Biochem.* **2008**, *102*, 1090.

(7) (a) Neelakandan, P. P.; Ramaiah, D. *Angew. Chem., Int. Ed.* **2008**, *47*, 8407. (b) Xu, Z.; Xiao, Y.; Qian, X.; Cui, J.; Cui, D. *Org. Lett.* **2005**, *7*, 889. (c) Neelakandan, P. P.; Hariharan, K.; Ramaiah, D. *J. Am. Chem. Soc.* **2006**, *128*, 11334. (d) Jisha, V. S.; Arun, K. T.; Hariharan, M.; Ramaiah, D. *J. Am. Chem. Soc.* **2006**, *128*, 6024. (e) Ros-Lis, J. V.; Martinez-Manez, R.; Rurack, K.; Sancenon, F.; Soto, J.; Spies, M. *Inorg. Chem.* **2004**, *43*, 5183. (f) Ros-Lis, J. V.; Marcos, M. D.; Martinez-Manez, R.; Rurack, K.; Soto, J. *Angew. Chem., Int. Ed.* **2005**, *44*, 4405. (g) Constable, E. C.; Martinez-Manez, R.; Cargill Thompson, A. M. W.; Walker, J. V. *J. Chem. Soc., Dalton Trans.* **1994**, 1585. (h) Arun, K. T.; Ramaiah, D. *J. Phys. Chem. A* **2005**, *109*, 5571. (i) Kuruvilla, E.; Nandajan, P. C.; Schuster, G. B.; Ramaiah, D. *Org. Lett.* **2008**, *10*, 4295.

(8) (a) Zhang, X.; Xiao, Y.; Qian, X. *Angew. Chem., Int. Ed.* **2008**, *47*, 8025. (b) Mello, J. V.; Finney, N. S. *Angew. Chem., Int. Ed.* **2001**, *40*, 1536. (c) Takakusa, H.; Kikuchi, K.; Urano, Y.; Kojima, H.; Nagano, T. *Chem.—Eur. J.* **2003**, *9*, 1479. (d) Coskun, A.; Akkaya, E. U. *J. Am. Chem. Soc.* **2005**, *127*, 10464. (e) Lin, W.; Yuan, L.; Long, L.; Guo, C.; Feng, J. *Adv. Funct. Mater.* **2008**, *18*, 2366. (f) Xu, Z.; Qian, X.; Cui, J. *Org. Lett.* **2005**, *7*, 3029. (9) (a) Lewis, F. D.; Zhang, Y.; Letsinger, R. L. *J. Am. Chem. Soc.* **1997**, *119*, 5451. (b) Lou, J.; Hatton, T. A.; Laibinis, P. E. *Anal. Chem.* **1997**, *69*, 1262. (c) Reis e Sousa, A. T.; Castanheira, E. M. S.; Fedorov, A.; Martinho, J. M. G. *J. Phys. Chem. A* **1998**, *102*, 6406. (d) Nohta, H.; Satozono, H.; Koiso, K.; Yoshida, H.; Ishida, J.; Yamaguchi, M. *Anal. Chem.* **2000**, *72*, 4199. (e) Okamoto, A.; Ichiba, T.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 8364.

(10) (a) Varnes, A. V.; Dodson, R. B.; Whery, E. L. *J. Am. Chem. Soc.* **1972**, *94*, 946. (b) Kemlo, J. A.; Shepherd, T. M. *Chem. Phys. Lett.* **1977**, *47*, 158. (c) Rurack, K.; Resch, U.; Senoner, M.; Daehne, S. *J. Fluoresc.* **1993**, *3*, 141. (d) Torrado, A.; Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1998**, *120*, 609. (e) Yu, M.; Shi, M.; Chen, Z.; Li, F.; Li, X.; Gao, Y.; Xu, J.; Yang, H.; Zhou, Z.; Yi, T.; Huang, C. *Chem.—Eur. J.* **2008**, *14*, 6892. (f) Li, G.-K.; Xu, Z.-X.; Chen, C.-F.; Huang, Z.-T. *Chem. Commun.* **2008**, 1774. (g) Wen, Z.-C.; Yang, R.; He, H.; Jiang, Y.-B. *Chem. Commun.* **2006**, 106. (h) Xiang, Y.; Tong, A.; Jin, P.; Ju, Y. *Org. Lett.* **2006**, *8*, 2863.

(11) (a) Ghosh, P.; Bharadwaj, P. K.; Mandal, S.; Sanjib, G. *J. Am. Chem. Soc.* **1996**, *118*, 1553. (b) Yang, J.-S.; Lin, C.-S.; Hwang, C.-Y. *Org. Lett.* **2001**, *3*, 889. (c) Xie, J.; Menand, M.; Maisonneuve, S.; Metivier, R. *J. Org. Chem.* **2007**, *72*, 5980. (d) Park, S. M.; Kim, M. H.; Choe, J.-I.; No, K. T.; Chang, S.-K. *J. Org. Chem.* **2007**, *72*, 3550.

(12) (a) Kaur, S.; Kumar, S. *Chem. Commun.* **2002**, 2840. (b) Royzen, M.; Dai, Z.; Canary, J. W. *J. Am. Chem. Soc.* **2005**, *127*, 1612. (c) Kim, S. H.; Kim, J. S.; Park, S. M.; Chang, S.-K. *Org. Lett.* **2006**, *8*, 371. (d) Lin, W.; Yuan, L.; Tan, W.; Feng, J.; Long, L. *Chem.—Eur. J.* **2009**, *15*, 1030. (e) Li, Y.; Zheng, H.; Li, Y.; Wang, S.; Wu, Z.; Liu, P.; Gao, Z.; Liu, H.; Zhu, D. *J. Org. Chem.* **2007**, *72*, 2878. (f) Zheng, Y.; Gattás-Asfura, K. M.; Konka, V.; Leblanc, R. M. *Chem. Commun.* **2002**, 2350. (g) Grandini, P.; Mancin, F.; Tecilla, P.; Scrimin, P.; Tonellato, U. *Angew. Chem., Int. Ed.* **1999**, *38*, 3061.

(13) (a) Montalti, M.; Prodi, L.; Zaccaroni, N.; Falini, G. *J. Am. Chem. Soc.* **2002**, *124*, 13540. (b) Vicinelli, V.; Ceroni, P.; Maestri, M.; Balzani, V.; Gorka, M.; Vögtle, F. *J. Am. Chem. Soc.* **2002**, *124*, 6461. (c) Jiang, P.; Chen, L.; Lin, J.; Liu, Q.; Ding, J.; Gao, X.; Guo, Z. *Chem. Commun.* **2002**, 1424. (d) Corradini, R.; Dossena, A.; Galaverna, G.; Marchelli, R.; Panagia, A.; Sartor, G. *J. Org. Chem.* **1997**, *62*, 6283.

(14) (a) Daffy, L. M.; de Silva, A. P.; Gunaratne, H. Q. N.; Huber, C.; Lynch, P. L. M.; Werner, T.; Wolfbeis, O. S. *Chem.—Eur. J.* **1998**, *4*, 1810. (b) Gunnlaugsson, T.; Clive Lee, T.; Parkesh, R. *Org. Biomol. Chem.* **2003**, *1*, 3265. (c) Zhong, D.; Pal, S. K.; Zewail, A. H. *ChemPhysChem* **2001**, *2*, 219.

(15) (a) Abad, S.; Kluciar, M.; Miranda, M. A.; Pischel, U. *J. Org. Chem.* **2005**, *70*, 10565. (b) Lee, M. H.; Kim, H. J.; Yoon, S.; Park, N.; Kim, J. S. *Org. Lett.* **2008**, *10*, 213. (c) Battistuzzi, G. G.; Grandi, G.; Menabue, L.; Pellacani, G. C.; Sola, M. *J. Chem. Soc., Dalton Trans.* **1985**, 2363.

(16) (a) Ceroni, P.; Laghi, I.; Maestri, M.; Balzani, V.; Gestermann, S.; Gorkab, M.; Vogtle, F. *New J. Chem.* **2002**, 26, 66.

(17) Pandey, S.; Azam, A.; Pandey, S.; Chawla, H. M. *Org. Biomol. Chem.* **2009**, *7*, 269.

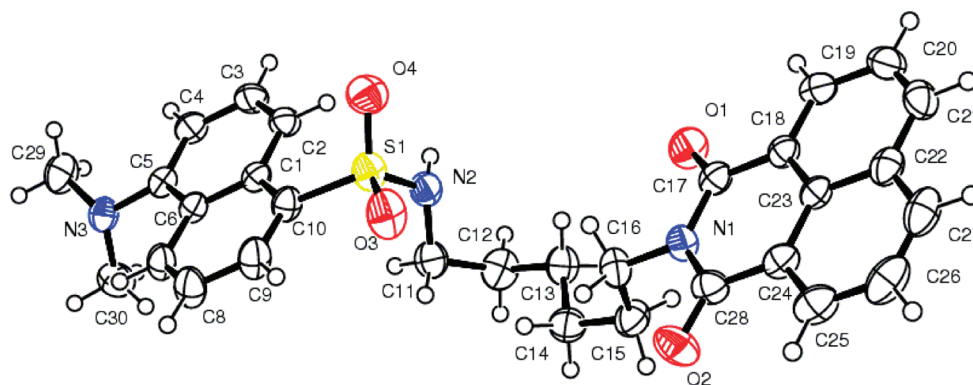
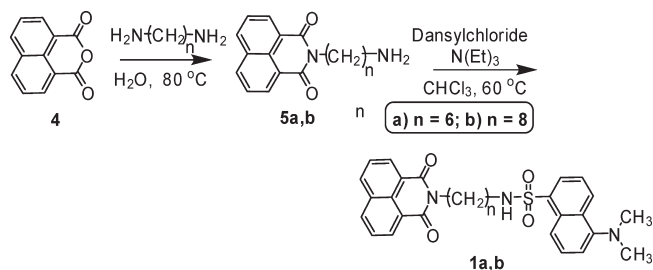


FIGURE 2. Projection view of the dyad **1a** with 50% probability ellipsoids.

SCHEME 1. Synthesis of the Dyads **1a** and **1b**



chloride with butylamine and *p*-*tert*-butylphenol, respectively. These products were purified through recrystallization and were characterized on the basis of analytical and spectral data (Figures S1–S4, Supporting Information). In addition, the structure of the dyad **1a** has been confirmed through X-ray crystallographic analysis (Figure 2 and Tables S1–S7, Supporting Information). This structure shows a distance of 11.6 Å between the naphthalimide and dansyl chromophores, which is well below the critical distance, required for an effective energy transfer between the donor and acceptor dyads.

Figure 3 shows the absorption spectra of the dyads **1a** and **1b** in acetonitrile. These dyads showed absorption maximum at 332 nm with a shoulder at 339 nm. Similar observations have been made in other solvents like methanol, ethanol, and toluene (Figure S5, Supporting Information). In all these solvents, the absorption spectrum of these dyads is found to be the sum of the individual units; indicating thereby that no significant interactions exist between the naphthalimide and dansyl chromophores in the ground state. Inset of Figure 3 shows the emission spectra of the dyads **1a** and **1b**. The fluorescence spectra of these dyads exhibited two emission maxima at 375 and 525 nm, when excited at 339 nm, where most of the photons are absorbed by naphthalimide chromophore. Based on the excitation spectral analysis and literature reports,^{15a} the bands at 375 and 525 nm, respectively, could be assigned to the locally excited state of the naphthalimide chromophore and fluorescence resonance energy transfer (FRET) mediated emission from the dansyl moiety. When compared to the dansyl-based model compound **2**, the fluorescence intensity of the dyads **1a** and **1b** is significantly quenched. This is because of the fact that the emission observed at 525 nm in the case of these dyads is due to the existence of FRET from the excited state of the naphthalimide chromophore to the dansyl unit and the

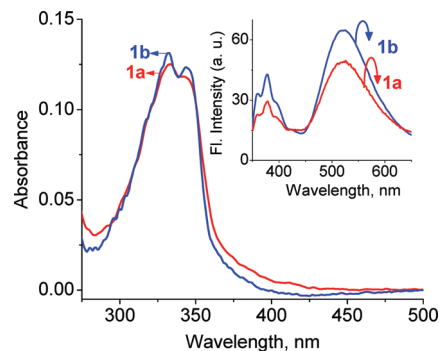


FIGURE 3. Absorption and fluorescence emission (inset) spectra of the dyads **1a** and **1b** (3 μM) in acetonitrile. Path length of the cell, 1 cm, and the excitation wavelength, 339 nm.

photoinduced electron transfer (PET) reaction from the dansyl chromophore to the naphthalimide moiety. The dyad **1b** with longer spacer length (octamethylene unit) showed efficient FRET-mediated emission with a fluorescence intensity ratio I_{525}/I_{375} of 1.2 when compared to the ratio of ca. 0.5 observed with the dyad **1a** having a shorter spacer group (hexamethylene unit). This can be attributed to increased energy transfer from the naphthalimide chromophore to the dansyl moiety with the increase in spacer length as observed in the case of the donor–acceptor systems.¹⁸

Metal Ion Binding Properties of the Dyads. As the naphthalimide- and dansyl-based dyads **1a** and **1b** interestingly exhibited intramolecular dual emission, it was our objective to evaluate their potential use as ratiometric sensors and identify the ideal conditions for the detection of metal ions in the aqueous medium. In this context, we have investigated the interactions of these dyads with various metal ions under different conditions, including the micellar medium. Of all the conditions examined, it has been observed that a solvent system consisting of a mixture (4:1) of water and acetonitrile containing neutral micelles triton X-100 (TX-100; 2 mM) has been found to be very effective with respect to the stability of dyads as well as the selectivity and sensitivity of the metal ion binding event. For example, Figure 4 shows the changes in the absorption spectrum of the dyad **1b** with the addition of

(18) (a) Joseph, J.; Eldho, N. V.; Ramaiah, D. *J. Phys. Chem. B.* **2003**, *107*, 4444. (b) Joseph, J.; Eldho, N. V.; Ramaiah, D. *Chem.—Eur. J.* **2003**, *9*, 5926. (c) Kuruvilla, E.; Joseph, J.; Ramaiah, D. *J. Phys. Chem. B.* **2005**, *109*, 21997.

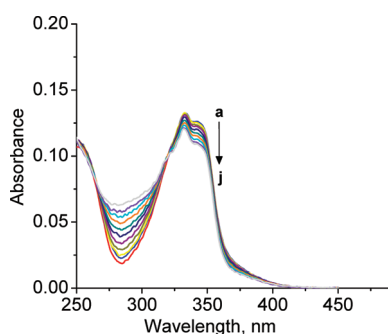


FIGURE 4. Changes in the absorption spectrum of the dyad **1b** ($3 \mu\text{M}$) in 20% acetonitrile containing micelles TX-100 (2 mM) with increase in addition of Cu^{2+} ions. $[\text{Cu}^{2+}]$ (a) 0 and (j) $20 \mu\text{M}$. Path length of the cell, 1 cm, and excitation wavelength, 339 nm.

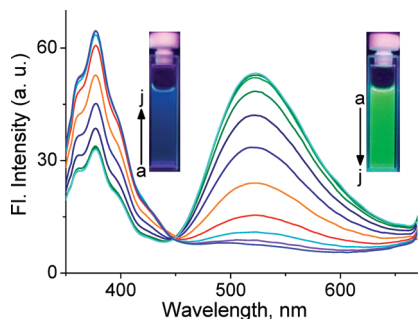


FIGURE 5. Changes in the fluorescence spectrum of the dyad **1b** ($3 \mu\text{M}$) in 20% acetonitrile containing neutral micelles TX-100 (2 mM) with increase in addition of Cu^{2+} ions. $[\text{Cu}^{2+}]$ (a) 0 and (j) $20 \mu\text{M}$. Path length of the cell, 1 cm, and excitation wavelength, 339 nm.

copper perchlorate in micellar medium. With the increase in concentration of Cu^{2+} ions, we observed a decrease in the absorption band at 340 nm with the concomitant increase in the absorbance at 284 nm with isosbestic points at 325 and 360 nm. Interestingly, in the fluorescence spectrum of the dyad **1b**, we observed a regular decrease in the intensity of FRET-mediated emission from the dansyl moiety at 525 nm with the increase in concentration of Cu^{2+} ions. Correspondingly, we observed a concomitant increase in the emission intensity of the naphthalimide chromophore at 375 nm with an isoemissive point at 450 nm. Further additions of $20 \mu\text{M}$ of Cu^{2+} ions resulted in the complete quenching of the FRET-mediated emission with ca. 12-fold increase in the fluorescence intensity ratio of I_{375}/I_{525} . The significant “turn on” intensity with a blue shift of ca. 150 nm led to the visual fluorescence ratiometric detection of Cu^{2+} ions by **1b** (insets of Figure 5). Similar observations have been made with the dyad **1a** having a shorter spacer group. In this case, we observed ca. 6-fold increase in the fluorescence intensity ratio of I_{375}/I_{525} with the addition of $20 \mu\text{M}$ of Cu^{2+} ions. Of the two systems investigated, the dyad **1b** was found to be very sensitive for the selective recognition of Cu^{2+} ions as compared to **1a** because of the efficient FRET-mediated emission observed in the former case. The selective quenching of FRET-mediated emission at 525 nm alone upon addition of Cu^{2+} ions indicate that these ions form complex

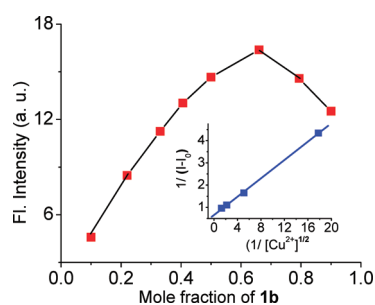


FIGURE 6. Job's plot for the complexation of the dyad **1b** with Cu^{2+} ions in 20% acetonitrile containing neutral micelles TX-100 (2 mM) with increase in addition of Cu^{2+} ions. The variation of the emission at 525 nm was plotted as a function of the mole fraction of **1b**. Inset shows the Benesi–Hildebrand analysis of the emission changes of the dyad **1b** under similar conditions.

with the dyads involving the sulfonamide group of the dansyl moiety.¹⁹ As a consequence, the dansyl moiety becomes incapable of quenching the excited state of the naphthalimide chromophore resulting in the revival of blue naphthalimide emission, thereby facilitating the visual detection of Cu^{2+} ions.

To understand the stability of the complex formed, the observed fluorescence changes of the dyad **1b** in the presence of Cu^{2+} ions were analyzed through Job's²⁰ and Benesi–Hildebrand plots²¹ (Figure 6). These analyses gave a 2:1 stoichiometry for the complex formed between the dyad **1b** and Cu^{2+} ions with an association constant of (K_{assoc}) of $5.2 \pm 0.1 \times 10^{10} \text{ M}^{-2}$. The association constant was further calculated using the curve-fitting method,^{8e} and the binding constant obtained by this method is in agreement with the value obtained from the Benesi–Hildebrand method. The MALDI-TOF mass spectral analysis of this complex showed a molecular mass of 1141.96 (Figure S6, Supporting Information), which is in agreement with the calculated molecular mass corresponding to a 2:1 stoichiometric complex between the dyad **1b** and Cu^{2+} ions. Similar observations have been made with the dyad **1a** (Figures S7–S8, Supporting Information), albeit with lesser sensitivity as compared to the dyad **1b**. In this case, we observed ca. 6-fold increase in the fluorescence ratio of I_{375}/I_{525} in the presence of Cu^{2+} ions with an association constant of $K_{\text{assoc}} = 4 \pm 0.1 \times 10^{10} \text{ M}^{-2}$.

Nature of Metal Ion Complexation. To understand the nature of the complex formed as well as the functional groups involved in the coordination, we have analyzed the ^1H NMR and Fourier transform infrared spectra (FTIR) of the dyads **1a** and **1b** in the presence and absence of Cu^{2+} ions and compared with the model compounds **2** and **3**. For example, with the addition of Cu^{2+} ions, we observed a downfield shift of $\Delta\delta = 0.02$ for the N–H proton of the dyad **1b**, while the peaks corresponding to the aromatic protons showed significant broadening as well as considerable downfield shifts in the range of $\Delta\delta = 0.03 - 0.07$ (Figure S9,

(19) (a) Lim, M. H.; Lippard, S. J. *J. Am. Chem. Soc.* **2005**, *127*, 12170. (b) Lim, M. H.; Lippard, S. J. *Inorg. Chem.* **2006**, *45*, 8980.

(20) (a) Vosburgh, W. C.; Cooper, G. R. *J. Am. Chem. Soc.* **1941**, *63*, 437. (b) Zeng, L.; Miller, E. W.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2006**, *128*, 10.

(21) (a) Benesi, H. A.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949**, *71*, 2703. (b) Yanniss, L. L. *J. Phys. Chem. B* **1997**, *101*, 4863. (c) Hariharan, M.; Neelakandan, P. P.; Ramaiah, D. *J. Phys. Chem. B* **2007**, *111*, 11940. (d) Hariharan, M.; Karunakaran, S. C.; Ramaiah, D. *Org. Lett.* **2007**, *9*, 417.

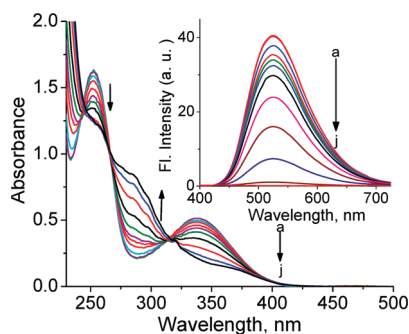


FIGURE 7. Changes in absorption and fluorescence (inset) spectrum of the model compound **2** ($100 \mu\text{M}$) in acetonitrile with increase in addition of Cu^{2+} ions. $[\text{Cu}^{2+}]$ (a) 0 and (j) $500 \mu\text{M}$. Path length of the cell, 1 cm, and the excitation wavelength, 339 nm.

Supporting Information). In contrast, negligible changes were observed in the chemical shift values of *N*-methyl protons of the dansyl unit and methylene protons of the spacer group. In the FTIR spectrum of the dyad **1b**, the characteristic sulfonamide and carbonyl groups stretching frequencies were observed at 1323 , 1654 , and 1695 cm^{-1} , while the NH stretching frequency was observed at 3284 cm^{-1} (Figure S10, Supporting Information). Upon interaction with Cu^{2+} ions, we observed significantly decreased stretching frequencies of both NH and sulfonyl groups, confirming thereby the involvement of these functional groups in the complexation with Cu^{2+} ions.

To confirm the role of sulfonamide group of the dyads **1a** and **1b** on their selective complexation with Cu^{2+} ions, we have investigated the interactions with the model compounds **2** and **3**. Of these two model derivatives, the compound **2** possess both dialkylamino and sulfonamide groups as in the case of **1a** and **1b**. In contrast, the derivative **3**, in addition to the dialkylamino group, been substituted with a sulfonate group instead of the sulfonamide functionality. With an increase in the addition of Cu^{2+} ions to a solution of the model compound **2** ($100 \mu\text{M}$), we observed a regular decrease in absorbance at 260 and 339 nm, with the concomitant increase in absorbance at 286 nm with isosbestic points at 265 and 318 nm (Figure 7). At $500 \mu\text{M}$ of Cu^{2+} ions, we observed ca. 50% hypochromicity in the absorption spectrum of the model compound **2**. In the emission spectrum of the compound **2**, we observed regular and significant fluorescence quenching at 525 nm with the addition of Cu^{2+} ions (inset of Figure 7). In contrast, the model compound **3** showed negligible changes in the absorption and fluorescence spectra with an increase in concentration of Cu^{2+} ions under identical conditions (Figure 8). These results further demonstrate that the complexation between Cu^{2+} ions and the dyads **1a** and **1b** and the model compound **2** occur due to the involvement of the sulfonamide group of the dansyl chromophore and rule out the possibility of participation of the dialkylamino group in the binding event.

Selectivity of the Metal Ion Complexation. To demonstrate the selectivity of the dyads **1a** and **1b** for Cu^{2+} ions, we have investigated their interactions with other important monovalent and divalent metal ions such as Na^+ , Li^+ , K^+ , Zn^{2+} , Pb^{2+} , Hg^{2+} , Co^{2+} , Fe^{2+} , Cd^{2+} , Mg^{2+} , and Ba^{2+} ions under identical conditions (Figure 9). As can be seen from Figure 9, the addition of these metal ions caused

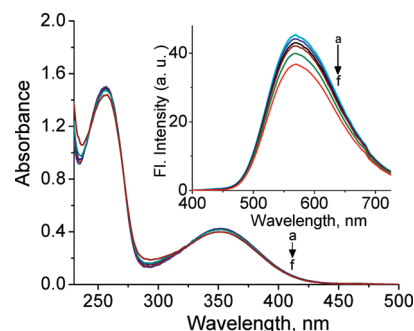


FIGURE 8. Changes in the absorption and fluorescence (inset) spectrum of **3** ($100 \mu\text{M}$) in acetonitrile with increase in addition of Cu^{2+} ions. $[\text{Cu}^{2+}]$ (a) 0 and (f) $500 \mu\text{M}$. Path length of the cell, 1 cm, and the excitation wavelength, 339 nm.

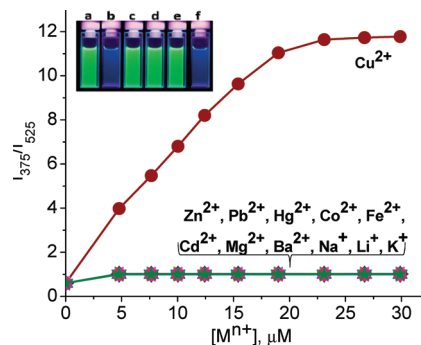


FIGURE 9. Relative changes in the fluorescence intensity of the dyad **1b** ($3 \mu\text{M}$) in the presence of various metal ions. Inset shows visual observation of fluorescence changes: (a) **1b** alone; (b–d) **1b** in the presence of Cu^{2+} , Li^+ , Hg^{2+} ; (e) equivalent mixture of various metal ions without Cu^{2+} ions; (f) equivalent mixture of various metal ions with Cu^{2+} ions.

negligible changes in the fluorescence intensity of the dyad **1b** (Figure S11, Supporting Information). Similar observations have been made with the dyad **1a** in the presence of various metal ions (Figure S12, Supporting Information). The selectivity of the dyad **1b** toward Cu^{2+} ions can be observed visually since the green fluorescence intensity of the dyad **1b** remained unchanged upon addition of these metal ions, while with Cu^{2+} ions, we observed the enhancement in blue fluorescence intensity at 375 nm (inset Figure 9). Interestingly, the presence of equimolar concentrations of all other metal ions used for the present studies showed negligible influence on the sensitivity of the detection of Cu^{2+} ions by the dyads **1a** and **1b**. Moreover, the complexation of the dyads **1a** and **1b** with Cu^{2+} ions was found to be reversible as confirmed by the addition of EDTA under identical conditions.

The novel dyads **1a** and **1b** having naphthalimide chromophore as the donor and dansyl group as the acceptor moiety showed negligible interactions in the ground state but exhibited efficient FRET reaction in the excited state. The evidence for such a reaction was obtained through excitation spectral analysis, observation of the spacer length dependent energy transfer, as well as on the basis of literature reports.^{15a} The presence of a favorable distance of 11.6 \AA between the donor and acceptor units in the case of the dyad **1a** as characterized through X-ray crystal analysis further

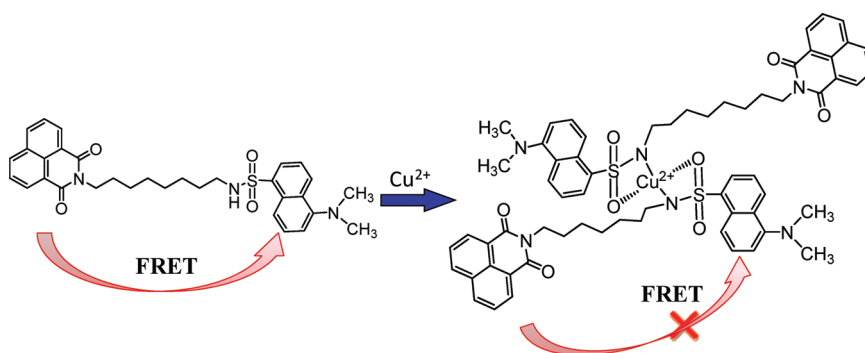


FIGURE 10. Schematic representation of the complexation between the dyad **1b** and Cu^{2+} ions.

indicates that such a reaction is quite possible in these dyads. In accordance with the above hypothesis, the emission spectrum of the dyads consisted of two emission maxima, one at 375 nm and the other at 525 nm, where the latter band is due to the FRET-mediated emission from the dansyl moiety. The observation of significant FRET-mediated emission in the case of the dyad **1b** as compared to **1a** could be attributed to the presence of a spacer with an appropriate length for an effective overlap between the donor and acceptor groups.

Investigation of the interactions with various metal ions indicates that the dyads **1a** and **1b** undergo selective interactions with Cu^{2+} ions as compared to other monovalent and divalent metal ions. Interestingly, such selective interactions with Cu^{2+} ions results in the formation of 2:1 stoichiometric complexes with significant association constants and the formation as confirmed through MALDI-TOF mass spectral analysis (Figure 10). The driving force for the selective complexation with Cu^{2+} ions is due to the presence of sulfonyl and NH groups in these dyads. The involvement of these groups in the complexation was evidenced through absorption, fluorescence, and ^1H NMR and FTIR spectral analysis and further confirmed by studies with the appropriately substituted model compounds **2** and **3**. Uniquely, the complexation of the dyads with Cu^{2+} ions involving sulfonamide group alters the interaction between the naphthalimide and dansyl chromophores leading to the disruption of FRET, thereby enabling the visual fluorescence ratiometric detection of Cu^{2+} ions.

Conclusion

In conclusion, we have developed novel donor and acceptor dyad systems **1a** and **1b** having naphthalimide and dansyl units, which exhibit spacer length dependent intramolecular energy transfer mediated emission from the dansyl moiety in addition to the emission from the naphthalimide chromophore. These dyads showed selective interactions with Cu^{2+} ions as compared to other metal ions and signal the event through inhibition of energy transfer mediated emission intensity. The uniqueness of these dyads is that they form stable complexes with Cu^{2+} ions and act as visual fluorescence ratiometric probes for the specific detection of Cu^{2+} ions.

Experimental Section

General Procedure for the Synthesis of the Dyads 1a and 1b. Dansyl chloride (5.0 mmol) was added to a stirred solution of the

corresponding *N*-(ω -aminoalkyl)-1,8-naphthalimide (4.2 mmol) and triethylamine (5.4 mmol) in dry chloroform (15 mL). The mixture was refluxed for 10 h. After the reaction mixture was cooled to room temperature (25 °C), the precipitate obtained was filtered off. The clear filtrate solution was evaporated to dryness under vacuum to give a pale yellow residue. The product mixture was chromatographed over silica gel. Elution with a mixture (1:9) of ethyl acetate and hexane gave the dyads **1a** (60%) and **1b** (65%), which were further purified through recrystallization from acetonitrile.

Dyad 1a (60%): mp 121–122 °C; IR (KBr) ν_{max} 3248, 2927, 1693, 1651 cm^{-1} ; ^1H NMR (500 MHz, CD_3CN) δ (ppm) 1.1–1.12 (m, 4H), 1.12–1.13 (m, 4H), 1.254 (d, $J = 4$ Hz, 2H), 2.80 (s, 6H), 3.93 (t, $J = 7.5$ Hz, 2H), 5.7 (t, $J = 5.5$ Hz, 1H), 7.20 (d, $J = 7.5$ Hz, 1H), 7.53–7.59 (m, 2H), 7.78–7.81 (m, 2H), 8.14–8.15 (dd, 1H), 8.25 (d, $J = 8.5$ Hz, 1H), 8.31–8.33 (dd, 2H), 8.47–8.51 (m, 3H); ^{13}C NMR (125.77 MHz, CD_3CN) δ (ppm) 25.3, 25.7, 27.1, 28.5, 29.0, 39.4, 42.40, 44.3, 114.8, 118.7, 122.5, 123.0, 126.7, 127.6, 127.7, 128.7, 129.1, 129.3, 129.6, 130.3, 131.4, 133.7, 135.4, 151.7, 163.6; HRMS (FAB) calcd for $\text{C}_{30}\text{H}_{31}\text{N}_3\text{O}_4\text{S}$ 531.21, found 531.23. Anal. Calcd for $\text{C}_{30}\text{H}_{31}\text{N}_3\text{O}_4\text{S}$: C, 68.03; H, 5.90; N, 7.93. Found: C, 67.90; H, 6.03; N, 7.89.

Dyad 1b (65%): mp 108–109 °C; IR (KBr) ν_{max} 3284, 2933, 1697, 1654 cm^{-1} ; ^1H NMR (500 MHz, CD_3CN) δ (ppm) 1.05–1.08 (m, 8H), 1.18–1.21 (m, 4H), 1.53–1.59 (m, 2H), 2.80 (s, 6H), 4.01 (t, $J = 7.5$ Hz, 2H), 5.75 (t, $J = 6$ Hz, 1H), 7.21 (d, $J = 7.5$ Hz, 1H), 7.52–7.59 (m, 2H), 7.76–7.80 (m, 2H), 8.14–8.16 (dd, 1H), 8.25 (d, $J = 8.5$ Hz, 1H), 8.29–8.31 (m, 2H), 8.47–8.49 (d, $J = 8.5$ Hz, 3H); ^{13}C NMR (125.77 MHz, CD_3CN) δ (ppm) 27.2, 27.9, 28.9, 29.7, 30.0, 30.1, 41.2, 44.0, 46.0, 116.4, 120.3, 124.1, 124.6, 128.3, 129.3, 130.4, 130.7, 130.9, 131.2, 131.9, 132.9, 135.3, 137.0, 153.3, 165.2; HRMS (FAB) calcd for $\text{C}_{32}\text{H}_{35}\text{N}_3\text{O}_4\text{S}$ 559.23, found 559.11. Anal. Calcd for $\text{C}_{32}\text{H}_{35}\text{N}_3\text{O}_4\text{S}$: C, 68.92; H, 6.33; N, 7.53. Found: C, 68.66; H, 6.51; N, 7.56.

Calculation of Association Constants. The association between the dyads and Cu^{2+} ions was analyzed using the fluorescence data. The association constants were calculated employing Benesi–Hildebrand method^{6c,21} using eqs 1 and 2

$$\frac{1}{(I-I_0)} = \frac{1}{(I-I_{\text{fc}})} + \frac{1}{K(I-I_{\text{fc}})[\text{Cu}^{2+}]} \quad (1)$$

$$\frac{1}{(I-I_0)} = \frac{1}{(I-I_{\text{fc}})} + \frac{1}{K(I-I_{\text{fc}})[\text{Cu}^{2+}]^{1/2}} \quad (2)$$

where K is the association constant, I is the fluorescence intensity of the free dyad, I_0 is the observed fluorescence intensity of the dyad– Cu^{2+} complex, and I_{fc} is the fluorescence intensity at the saturation. The plot of $1/(I-I_0)$ vs $1/[\text{Cu}^{2+}]^{1/2}$ gave a linear fitting, indicating a 2:1 stoichiometry between the dyad and Cu^{2+} ions.

Alternatively, the association constants were also calculated using the fluorescence titration data using a reported

method for a 1:2 metal–ligand binding mode^{7b,8e,22} and according to eq 3

$$\frac{\alpha^2}{(1-\alpha)} = \frac{1}{2KC_F[M]} \quad (3)$$

where C_F denotes the total concentration of ligand in the system and α is defined as the ratio between the free dyad concentration $[C]$ and the total concentration of ligand C_F . α was evaluated using eq 4

$$\alpha = \frac{[F - F_0]}{[F_1 - F_0]} \quad (4)$$

where F_1 and F_0 are the limiting emission values for $\alpha = 1$ (in the absence of metal) and $\alpha = 0$ (probe 1 is completely complexed with the metal ion), respectively.

(22) Yang, R.; Li, K.; Wang, K.; Zhao, F.; Li, N.; Liu, F. *Anal. Chem.* **2003**, *75*, 612.

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Supporting Information Available: General experimental methods and starting material used for the synthesis. Figures S1–S4 showing the ^1H and ^{13}C NMR spectra of the dyads **1a,b** and the model compounds **2** and **3**, Figures S5–S12 showing the changes in absorption and fluorescence spectra of the dyads **1a** and **1b** in the presence of various metal ions, and Tables S1–S7 showing the data of X-ray crystallographic structure analysis of the dyad **1a**. This material is available free of charge via the Internet at <http://pubs.acs.org/>.