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Metal Ion Induced FRET OFF–ON in Tren/Dansyl-Appended Rhodamine

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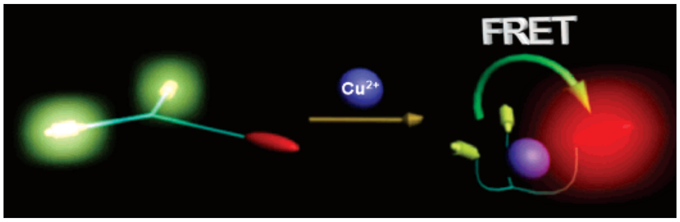
ABSTRACT



A series of new fluorescent probes bearing tren-spaced rhodamine B and dansyl groups have been synthesized. Compound 1 exhibits selective changes in the absorption and the emission spectra toward Cu^{2+} ion over miscellaneous metal cations. Among 1–3, 1 shows the best FRET efficiency through dansyl emission to rhodamine absorption for the Cu^{2+} ion.

Selective detection of target metal ions has been of great interest because of their importance in biological and environmental processes.[1–3] Many detection schemes rely on fluorescence changes upon sensing specific metal ions.[4–6] We report here a novel fluorescent probe for copper(II) cation, based on fluorescence resonance energy transfer (FRET). The FRET is defined as an excited-state energy interaction between two fluorophores in which excited donor energy is nonradiatively transferred to an acceptor unit. The metal ion induced FRET ON→OFF has been observed in previous studies.[5] To the best of our knowledge, however, the FRET OFF→ON module, inherently more sensitive owing to its zero background, is reported here for the first time.

We have synthesized a series of rhodamine/dansyl fluorophores (**1**−**3**) incorporated into a tren spacer (Scheme 1) and measured their optical properties upon the metal cations.

The absorption and the emission spectra of individual constituents of **1** are shown in Figure 1a. Rhodamine has two constitutional isomers with distinctively different absorption properties. The spirolactam form (**4**) absorbs the UV light and shows a band at ~250 nm, and thus it is colorless and nonfluorescent. Upon adding Cu^{2+} ion, **4** changes its color to pink, indicating that the absorption shifts to the visible region. The tetraaza group has been reported to show high affinity to Cu^{2+} ion, and the complexation of Cu^{2+} leads to opening of the spirolactam ring.[7] Notably, the resulting absorption spectrum of the **4**•Cu^{2+} complex overlaps with the emission spectrum of dansyl chloride (**6**), fulfilling a favorable condition for the FRET.

The absorption spectrum of **1**, shown in Figure 1b, closely resembles that of the combination of **4** and **6**, suggesting

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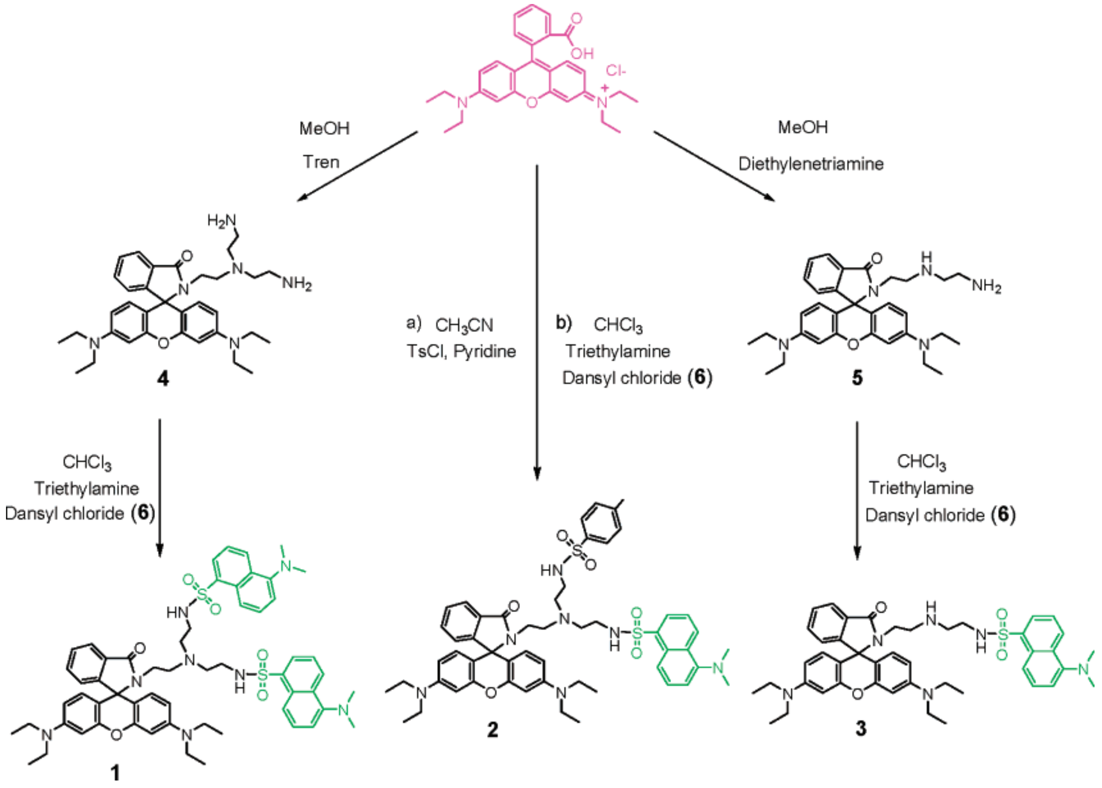
Scheme 1. Rhodamine Derivatives **1**−**5**



that the rhodamine moiety remains a ring-closed spirolactam form. Upon irradiation at 420 nm, strong emission at $\sim$507 nm is observed, attributed to the fluorescence emitted from the dansyl energy donor unit. When Cu^{2+} ion is added, the fluorescence spectrum of **1** shifts to $\sim$580 nm, the region of the energy acceptor. The binding of Cu^{2+} ion induces opening of the spirolactam ring in **1**, shifting the absorption spectrum of rhodamine. Subsequently, an increased overlap[8] between emission of the energy donor and absorption of the energy acceptor greatly enhances the intramolecular FRET, producing the fluorescence from the energy acceptor unit in **1**. We measured such FRET enhancement factors by considering the ratio of the fluorescence intensity of the energy acceptor at 574 nm (F_{rho}) to that of the energy donor at 507 nm (F_{dns}) and observed 13-fold fluorescence increase in the case of the Cu^{2+} ion complexation (Figure 4).

For more detailed studies of FRET occurring in **1**+Cu^{2+}, we modified the energy donor systematically. Table 1 summarizes FRET efficiencies of **1**−**3** and also lists association constants toward Cu^{2+} ions, respectively. Reducing the number of the dansyl energy donor groups by one-half (**2**+Cu^{2+}, **3**+Cu^{2+}) lowers the FRET efficiency and thus decreases the fluorescence intensity from the rhodamine energy acceptor as shown in Figure 1b. FRET efficiency of **2**+Cu^{2+} which is higher than that of **3**+Cu^{2+} is due to the additional presence of a sulfonamide group which presumably assists in trapping the Cu^{2+} ion. Furthermore, extremely weak fluorescence from the energy acceptor in the absence of the energy donor (**4**+Cu^{2+}) confirms that the FRET indeed occurs from the dansyl to the rhodamine group.

Density functional theory (DFT) calculations provide further structural evidence of the switching-ON of the FRET upon addition of Cu^{2+} ion. We calculated the optimized geometry of **1** and **1**•Cu^{2+} complexes and the corresponding electronic structures using the Vienna Ab initio Simulation Package (VASP).[10,11] The PBE-type generalized gradient approximation is used for the exchange correlation,[12] and the plane-wave basis set is employed with the energy cutoff of 400 eV.[11]

Figure 2 shows that **1** has an extended form due to the tren spacer, while the **1**•Cu^{2+} complex is relatively compact. The coordination of Cu^{2+} with nearby oxygen and nitrogen atoms in **1**, as indicated by the dashed lines in Figure 2b, pulls in the sulfonamide groups and brings the dansyl groups close to the rhodamine group. The optimized location of Cu^{2+} ion is found between the oxygen atom of the sulfonyl group and the nitrogen atom of the acyclic lactam group, confirming

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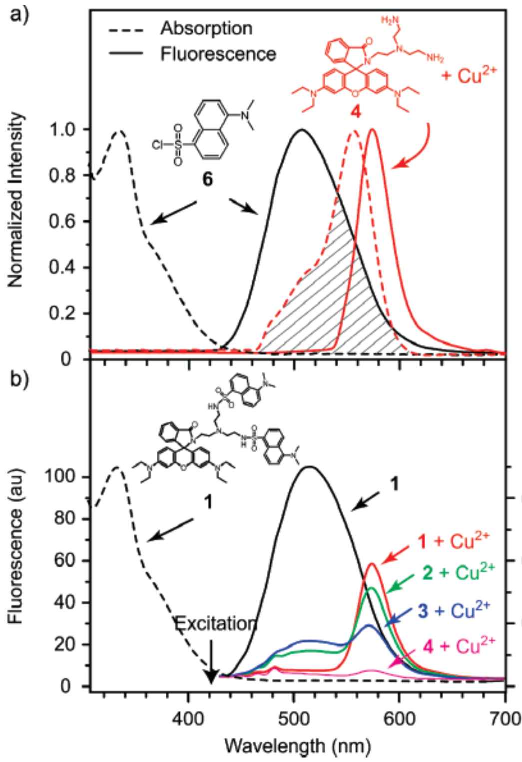

Figure 1. (a) Normalized absorption and emission spectra of dansyl chloride (**6**) and rhodamine (**4**)•Cu^{2+} complex. Shaded area indicates the spectral overlap between the emission of the former and the absorption of the latter. (b) Fluorescence changes of **1**−**4** (15.0 μM) upon addition of 7.5 mM of $Cu(ClO_4)_2$ in H_2O/CH_3CN (1/9, v/v) buffered at pH 7.0 with Tris buffer-HCl with an excitation at 420 nm marked by a downward arrow.

that the complexation indeed requires the participation of sulfonamide and opening of the spirolactam ring. These structural changes are found to affect the HOMO/LUMO energy levels of dansyl and rhodamine chromophores.

When the lactam ring is closed, the HOMO−LUMO energy gap of the rhodamine energy acceptor is greater than that of the dansyl energy donor, suppressing the energy transfer from dansyl to rhodamine. As the Cu^{2+} is bound and the spirolactam ring opens, the energy gap of rhodamine is markedly reduced just to match that of the dansyl group, enabling the intramolecular FRET.

Experimental observations and theoretical calculations consistently explain how the addition of Cu^{2+} turns on the FRET from the energy donor to the energy acceptor. Figure 3 summarizes this process along with the visual fluorescence changes. In the absence of Cu^{2+} ion, the energy mismatch between the energy donor and the acceptor blocks the energy transfer, hence fluorescence is observed only from the energy donor. The binding of Cu^{2+} to the sulfonamide and the spirolactam moieties drives the structural changes, most importantly the spirolactam ring opening, and accordingly changes absorption and fluorescence spectra. Then, the enhanced spectral overlap between the donor and the acceptor turns FRET on to give the rhodamine fluorescence ON. On the other hand, to confirm 1:1 stoichiometry between **1** and Cu^{2+} ion, Job's plot analysis was also executed (Figure S1, Supporting Information).

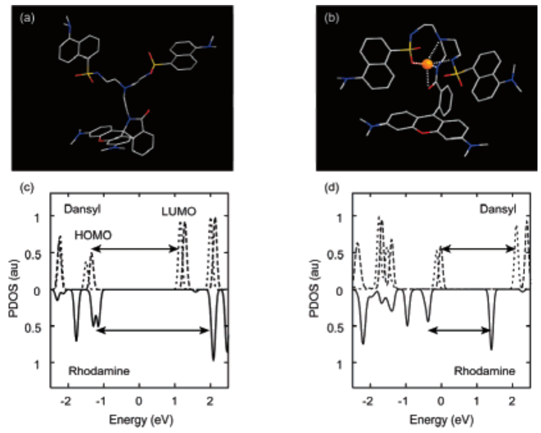

Figure 2. Optimized geometries of (a) **1** and (b) **1**•Cu^{2+} complex and the calculated partial density of states (PDOS) of (c) **1** and (d) **1**•Cu^{2+} complex obtained by the density functional calculations. Dashed lines and dotted lines in (c) and (d) indicate the energy states of two dansyl groups. Solid lines represent the states of the rhodamine group. The energy in the horizontal axis refers to the Fermi level, and thus the first state appearing below and above 0 eV is defined as HOMO and LUMO, respectively. Double-sided arrows in (c) and (d) indicate the HOMO−LUMO energy gap of the dansyl and the rhodamine group.

To gain insight into the metal ion selectivity related to the FRET, we performed similar fluorescence measurements for a series of metal ions. We then found that the Cu^{2+} ion exclusively exhibits such a high FRET enhancement (defined above as F_{rho}/F_{dns}) (Figures 4 and S2−S7, Supporting Information). The contrast in visual color changes, shown in Figures S8 and S9, confirms the Cu^{2+} ion selectivity as well.

In conclusion, we herein for the first time report a rhodamine/dansyl ionofluorophore showing metal ion induced FRET OFF→ON behavior. The complexation of Cu^{2+} ion opens the spirolactam ring of the rhodamine to give a specific color change as well as a fluorescence enhancement at ∼580 nm. Theoretical studies support that the Cu^{2+}

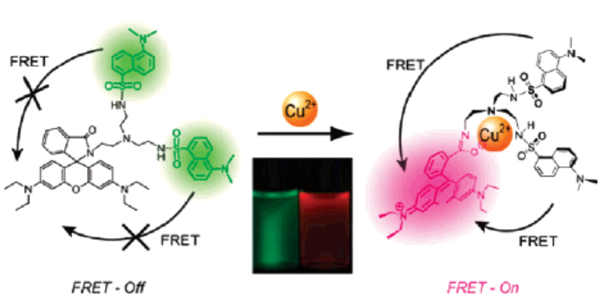

Figure 3. Cu^{2+}-induced FRET OFF→ON along with visual color changes upon irradiation at 420 nm.

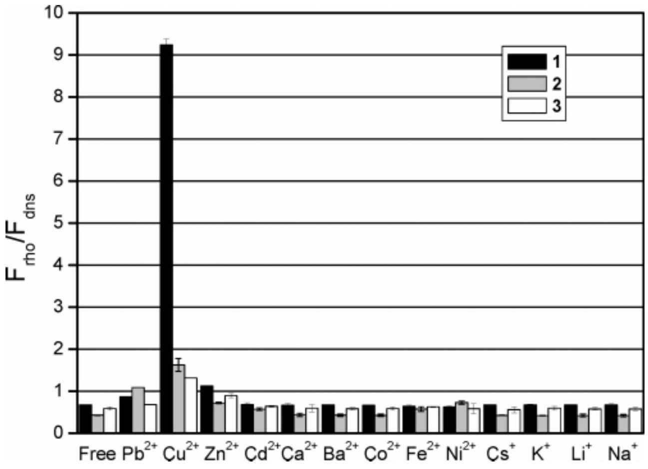

Figure 4. In the presence of a various metal ions (7.5 mM), **1** (black), **2** (gray), and **3** (white) (15.0 μM of each) show emission intensity ratios (F_{rho}/F_{dns}) in H_2O/CH_3CN (1/9, v/v) buffered at pH 7.0 with Tris buffer-HCl with an excitation at 420 nm. F_{rho} and F_{dns} denote the fluorescence intensity of rhodamine at 574 nm and that of the dansyl unit at 507 nm, respectively.

Table 1. Summarized Photochemical Properties of **1**, **2**, and **3** (15.0 μM) for Cu^{2+} Ion (7.5 mM), Such as FRET Efficiency (E_{fret}),[8] Association Constants (K_a)[9]

compound	E_{fret} (%)	K_a (M^{-1})
1•Cu(II)	0.93	7.0×10^3
2•Cu(II)	0.69	7.0×10^3
3•Cu(II)	0.56	5.1×10^3

binding to sulfonamide and acyclic amide drives the structural changes, leading to the well-matched HOMO−LUMO gap between the donor and the acceptor to provide an enhanced FRET efficiency. The FRET technology on the basis of tren/dansyl-appended rhodamine has a great potential as a novel detection method for Cu^{2+} ion over other metal cations.

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Supporting Information Available: Synthetic details, NMR copies, and additional spectra data. This material is available free of charge via the Internet at http://pubs.acs.org.

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