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# Detection of Zinc Ions by Differential Circularly Polarized Fluorescence Excitation

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Accurate determination of metal ion concentration is important for environmental protection[1] and health-related[2] research. For example, determination of zinc concentration and imaging of zinc distribution in brain tissues is of interest concerning brain diseases such as epilepsy, Alzheimer's, and Parkinson's diseases.[3] Although several agents are available that give fluorescence enhancement on zinc ion binding,[4−6] improved contrast is of general interest. Additionally, in vivo studies may suffer from strong fluorescence background from proteins and amino acids and fluorescence changes from aggregation or other nonspecific interactions. High contrast is desirable in eliminating both false positives and false negatives.

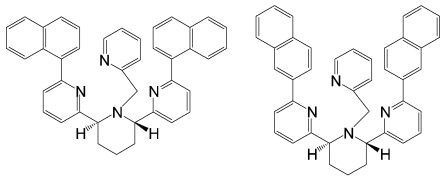
Our interest in chiroptical materials leads us to examine a new approach to metal ion sensing using fluorescence-detected circular dichroic detection, which integrates fluorescence and exciton coupled circular dichroism methods to give better contrast than those that can be achieved in either of the two parent methods.

Fluorescence-detected circular dichroism (FDCD) has been shown to be more sensitive than absorption CD.[7,8] Typically, the two channels of raw FDCD data, which correspond to the difference in emission ($F_L - F_R$) and the total emission ($F_L + F_R$) resulting from differential absorption of left- and right-circularly polarized light, are converted to CD by established methods,[7−9] which gives a normal CD spectrum if fluorescence polarization is negligible.[10] FDCD is also advantageous in displaying greater selectivity relative to CD and fluorescence in that not all absorbing chiral compounds fluoresce[8,11] and not all fluorescent compounds are chiral. The improved discrimination can be utilized to minimize background signals and interferences. Traditionally, this technique has been used to study proteins,[12] nucleic acids,[13] and absolute configuration of chiral organic compounds[9] and enantiomeric excess.[7]

We suggest here that an adaptation of the FDCD technique can provide a unique and powerful new strategy for sensor applications by using the $\Delta F$ ($F_L - F_R$)[9] component of FDCD data directly, without conversion to CD. We are not aware of a prior published report where only $\Delta F$ was used for analysis. To distinguish this new approach from traditional FDCD and to avoid confusion, we call this method differential circularly polarized fluorescence excitation (CPE), although no new instrument is required and all of the advantages of FDCD still pertain. The theoretical basis of this approach is shown in eq 1 and can be derived from established equations.[7,9] In the first part of eq 1, A is the absorbance, $\theta$ is CD ellipticity, and $k$ is a derived instrumental constant. If $\Delta A = A_L - A_R \leq 0.1$ and $\Delta A/A \leq 0.1$, total emission ($F_L + F_R$) should be proportional to fluorescence induced by nonpolarized light, i.e., $F_L + F_R = k_2F = k_2\Phi I^0(1 - 10^{-A})$, where $k_2$ is another instrumental constant. Then the equation can be simplified as shown, where $K$ is a constant that incorporates all other constants and $\Phi_F$ is fluorescence quantum yield. Materials with higher ellipticity $\theta$ and higher fluorescence quantum yield $\Phi_F$ will lead to an even larger $\Delta F$. Substances lacking either fluorescence or CD properties will not be observed.

$$\Delta F = \frac{\theta \cdot (F_L + F_R) \cdot \ln 10}{33 \cdot 2 \cdot (10^A - 1) \cdot k} = K \cdot \frac{\theta \cdot \Phi_F \cdot I^0}{10^A} \qquad (1)$$

To examine this prospect experimentally, we compared fluorescence, CD, UV, and $\Delta F$ spectra of ligands **1** and **2** and their enantiomers. Ligand **1**[6] has been shown to be a selective sensor for Zn. The corresponding spectra of compound **2** [(*S*,*S*) form], titrated with Zn(II), are shown in Figure 1. Fluorescence, CD, and $\Delta F$ signals increase proportionally with total zinc concentration added until 1:1 saturation. UV decreases with the increase in Zn concentration in the range from 220 to 320 nm. The contrast, namely extent of change, observed by CD or UV is not very high. The contrast in the fluorescence method is better. But the CPE ($\Delta F$) method gives almost no signal for the free ligand in the range from 220 to 320 nm, but strong response to Zn(II).



(*S*,*S*)-**1** (*S*,*S*)- **2**

The basis for the remarkable contrast observed in the $\Delta F$ titration derives from combined CD and fluorescence effects. The CD spectra for titration of ligands **1** and **2** indicate a 4−7-fold enhancement upon complexation with Zn(II), presumably due to changes in the orientation of chromophores in the complexes.[14,15] Zinc complexation also greatly enhanced the fluorescence emission.[6] In the ΔF spectra, the zinc complex's already larger CD signals were magnified by the increase in fluorescence emission. This effect is most obvious in compound **2**, whose fluorescence is stronger than that of **1**. The maximum observed enhancements after addition of 1 equiv of zinc (fluorescence quantum yield, fluorescence maxima value, CD ellipticity at 307 nm, and $\Delta F$ value) for **1** are 55, 90, 4.5, and greater than 500, respectively; while those for **2** are 19, 16, 6.4, and greater than 200, respectively. Traditional FDCD (Figure 1c) and $\Delta F/F$ do not offer such advantages because they cancel out the contribution from fluorescence.

The issue of competitive background fluorescence was addressed by conducting titrations with hen egg white lysozyme, which

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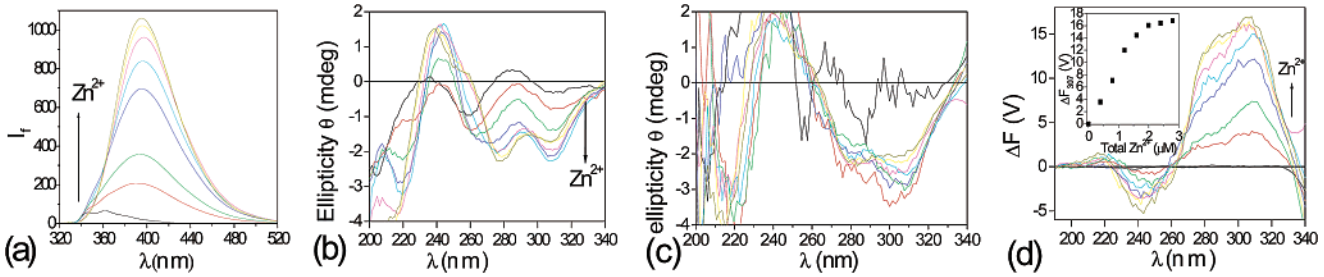


***Figure 1.*** Spectral response of 2 $\mu$M (*S*,*S*)-**2** to 0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8 $\mu$M $Zn^{2+}$ in acetonitrile. (a) Fluorescence (Ex: 300 nm). (b) CD. (c) FDCD. (d) $\Delta F$ (700 V, filter: 360 nm, polarizer at 81°. Spectra taken with JASCO FDCD J-405 device attached to J-810 spectrometer). Inset: titration curve of 2 $\mu$M (*S*,*S*)-**2** with $Zn(ClO_4)_2$.

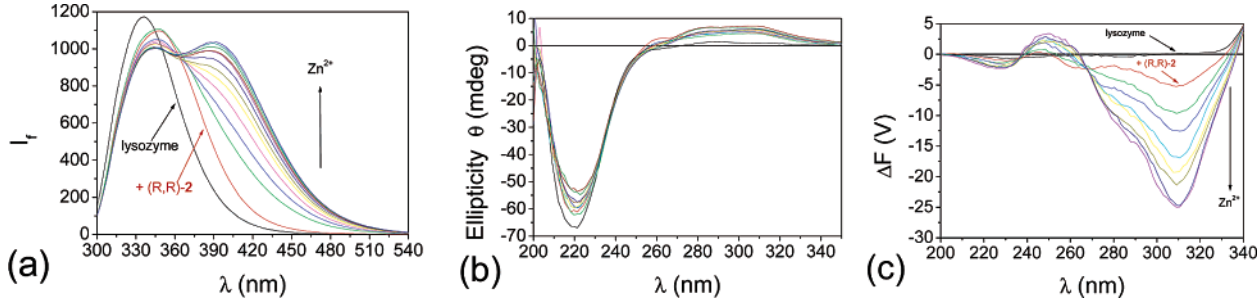


***Figure 2.*** Spectral responses of 3.2 $\mu$M (*R*,*R*)-**2** to 0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 3.2, 4.0, 4.8 $\mu$M $Zn^{2+}$ in the presence of 1.0 mg/mL HEW lysozyme in 60% acetonitrile/water. (a) Fluorescence (Ex: 280 nm). (b) CD. (c) $\Delta F$ (600 V, 81°, filter: 360 nm).

contains several tryptophan residues, a common source of background fluorescence in cells.[16] $\Delta F$ titrations of (*R*,*R*)-**2** with zinc in the presence of 1.0 mg/mL lysozyme (Figure 2) showed excellent contrast and linearity, while fluorescence, UV (see in Supporting Information), and CD measurements were significantly obscured by the background signals from the protein. These spectra illustrate the exquisite selectivity of $\Delta F$, as the protein gives a relatively weak $\Delta F$ signal since the n-$\pi^*$ absorbance that accounts for the CD spectrum does not induce an excited state of the tryptophan that leads to fluorescence. Fluorescence polarization values of all the systems studied were measured and found to be relatively small.[9]

Paramagnetic $Cu^{2+}$ can quench the fluorescence of these ligands, while enhancing their CD. As a consequence, $\Delta F$ of $Cu^{2+}$ complex of (*R*,*R*)-**1** is not stronger than the free ligand. Thus, selective response may depend on intrinsic electronic properties of the metal ion.[5]

In conclusion, the CPE approach has the potential to improve contrast and diminish interference from background fluorescence in zinc sensing. For example, the protocol offers an alternative to addressing problems with overlapping fluorescence signals besides redesign of the probe with alternative fluorophores. Although not as sensitive as isotropic chelation-enhanced fluorescence, the unique characteristics of $\Delta F$ augment the toolbox of optical methods available for solution probes of metal ion binding and recognition and detection of other organic material. These results lay the groundwork for development of imaging tools to be used in conjunction with isotropic fluorescence and circular dichroism microscopy,[17] potentially offering better contrast and unique advantages such as turbid solution analysis.

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**Supporting Information Available:** Synthesis and characterization of compound **2**, fluorescence polarization and all other UV, fluorescence, CD, $\Delta F$ spectra of compounds **1** and **2**, and measurement conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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