Bend and twist intramolecular charge transfer and emission for selective metal ion sensing

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Abstract: Zn^{2+} and Cu^{2+} complexation of cyclam-triazolyl-naphthalimide fluoro-ionophores lead to increased and decreased fluorescence respectively. The differences between the two metals are accounted for by their Lewis acid and base properties. This difference means the system can be described as an optical diode with characteristics that suggest measurable differences in fluorescence rise and decay times for the mechanical suppression of bend- and/or twist-induced emissions from intramolecular charge transfer. Different fluorescence evolution profiles are observed offering a new way of distinguishing metal ions for applications in biomedical and environmental sensing.

OCIS codes: (280.4788) Optical sensing and sensors; (260.2510) Fluorescence; (230.2090) Electro-optical devices; (230.5170) Photodiodes; (170.4580) Optical diagnostics for medicine.

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1. Introduction

Specific molecular interactions are key to an increasing range of optical devices which operate at the molecular level, including molecular transistors and diodes [1–4]. Given the dimensions of such systems, it is anticipated that once molecular fabrication, practical interconnectivity and reproducability are all achieved, these devices will herald the arrival of sub-nanoscale technologies. However, this dream is far from realised both in terms of fully understanding the physics of these systems and controling their fabrication. Detailed molecular simulations can predict some properties and demonstrate features such as charge induced transfer within molecules. Unfortunately, simple physical insight akin to that taken for granted with well-established semiconductor optolectronics (which includes newer nanoscale approaches exploiting plasmons [5]) remains rudimentary. There is considerable uncertainty about the reliability of such devices and how to best utilise them. This arises partly from the scale on which these interactions occur, a scale at which quantum effects such as electron interference and anomolous charge transport must also be taken into consideration [6]. These complicate the treatment of systems employing multiple effects (as is common with a functional circuit), and mean these are not accessible to traditional *ab initio* calculations and density functional theories. Given the immense scope of this field, we concentrate on a particular application area in which single molecule properties have an immediate practical potential: detecting and discriminating metal ions using specific fluorescent chemosensor dyes [7] for biomedical and environmental applications. Although there is a tremendous amount of literature reporting the properties of such dyes and numerous descriptions of their fluorescence behaviour, the physical origins and implications are not well described. Here, we identify a simple mechanistic description in terms of optical diodes. By exploiting temporal differences in emission, discrimination between ions arising from voltage (V) induced charge transfer is demonstrated.

Tuning structural conformation to shape molecular electronic interactions is an important tool for optimizing the photophysical properties of potential chemical and biological sensors. Dual or multiple emissions can identify fluorescence arising from such conformational changes. This is most readily assessed for smaller molecules with well defined planar and orthogonal structural orientations. The dual fluorescence bands of *p*-cyano-*N*,*N*-dimethylaniline (CDMA) first repored by Lippert *et al.* [8] were sufficiently distinct for a phenomenon of charge transfer enabled by a twisted state of the molecule to be inferred, and the source of the observed second, red-shifted band to be identified. This overall mechanism was later labelled "twisted intramolecular charge transfer (TICT)" [9]. Dual emission of this type involves two processes: direct excitation of a conjugated ligand and conformation-based charge transfer between the ligand and an attached moiety. Solvent polarity influences the conformational change of such molecules between planar and orthogonal states: TICT is generally observed in polar solvents [10]. However, for dual or multiple emitting bands within larger molecules where photo-induced electron transfer (PET) pathways may also occur, spectral overlap and the asymmetry that arises from molecular bending make detailed attribution to TICT processes challenging.

Complexation with metal ions such as Zn^{2+} can lead to a large increase in fluorescence within many organic complexes, which in some cases has been attributed directly to a TICT band [11]. With smaller molecules this appears relatively simple because the only degree of mechanical freedom is twist, and clear separation of non-degenerate bands after twist might be anticipated. For larger species such as naphthalimide-based ligands with large cyclam chelating moieties (Fig. 1) [12,13] it is rare that the chelating regions of the molecule are strictly planar or planar-aligned relative to the conjugated system that defines ligand emission, particularly in polar solvents. Rather, such molecules are bent to enable interactions between different groups within the complex (e.g. chelation of binding groups to the metal). This situation is complicated by twisting of the fluorophore itself, triggered by charge heterogeneity, which can in principle break any electronic degeneracy of the entire molecule. In such cases, pre-existing TICT-like fluorescent bands that are closely overlapping with

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ligand emission bands fluoresce with a common excitation wavelength. The nature of the optically excited process remains a photo-induced electron (*e*$^-$) excitation, followed by charge transfer between neighboring sites. Given the extended distances involved with coupling to the larger conjugated system, the ligand emission is red-shifted relative to an isolated system with no attached groups. The degree of red shift is determined by the structure of the attached moieties, the amount of bending and twisting, and the extent to which degeneracy may be lifted to create additional excited states. We note that the breaking of degenerate electromagnetic field states by increasing geometric dimensions in space through twisting or bending of quantized systems is universal. It is directly related to the resonant solutions possible within a quantized cavity in 1, 2 or 3 dimensions. In these molecular cases where the electronic resonance frequencies are close, leading to overlap in spectra, Gaussian spectral fitting can be used to identify emission peaks.

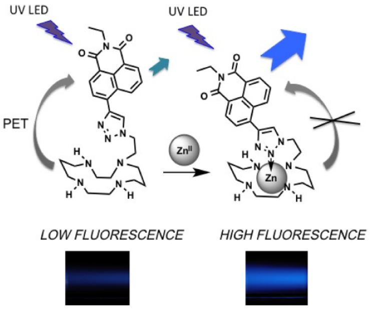

Fig. 1. Blue emission (λ_{ex} ~370 nm, λ_{em} ~458 nm) from *1* before and after Zn^{2+} incorporation in HEPES buffer (pH ~8). The structures are likely solvated. Smartphone spectrometer images @λ_{em} ~ 458 nm are shown below (from [14]).

2. Experiment and discussion

The 6-(1,4,8,11-tetraazacyclotetradecane)-2-ethyl-naphthalimide fluoro-ionophore (**1**) (Fig. 1) investigated herein was synthesized as in [12]. Figure 2(a) shows the emission from **1** in HEPES buffer (pH ~8) where strong conformation-assisted interactions are present. Experimental data were collected on a benchtop spectrofluorimeter (excitation wavelength λ_{ex} = 370 nm; source UV diodes). Complexation with Zn^{2+} (used by Aoki *et al.* [11] for example) shown in Fig. 2 (b) leads to increased fluorescence, correlating with substantial increases in both ligand and TICT-like emissions. Peak fitting (OriginPro 2015) suggests the presence of a third, weaker band. Figure 1 summarises the processes ascribed for the cyclam-naphthalimide system [12,13] where PET is considered to be the main mechanism impacted by twisting or bending. The addition of Zn^{2+} to **1** leads to a blue shift ($\Delta\lambda$ ~10 nm) as well as a 5-6 fold increase in net fluorescence intensity, making this an important approach for the detection of Zn^{2+} in diagnostic applications [13]. For example, Zn^{2+} detection was recently demonstrated using a smartphone fluorimeter [14] - CMOS images are shown in Fig. 1.

The addition of Zn^{2+}, a strong *e*$^-$ acceptor that makes the conjugated ligand a Lewis base, leads to a Lewis adduct (Fig. 1) which enhances both the ligand and TICT-related emissions. This is explained by an elimination of direct PET at the ring whilst enhancing the photo-induced interaction through the triazole nitrogen to which the Zn^{2+} is attracted. Consistent with this, Aoki *et al.* [11] observed a general decrease in the TICT emission within related large structures based on cyclen-pyridyl ligands. This was in the form of a decreasing TICT wavelength relative to the ligand wavelength only when external anions (imidates, phosphates, thiolates and dicarboxylates) were present. These anions, stronger Lewis bases than the pyridine nitrogen in [11], which plays a similar role to the triazole nitrogen of **1**, bind to Zn^{2+} and force the twisted pyridine ring to relax. The combination prevents the Zn^{2+} from having direct interactions with the nitrogen which in turns prevents charge transfer, eliminating TICT-like emission. Variations between the two emission bands arise from solvation effects of a polar solvent, in this case hydroxyl anions in slightly basic water. In the Zn^{2+}-free probe **1**, the naphthalimide represents an e^- acceptor relative to the cyclam group which provides an e^- when excited by UV light (PET); the subsequent relaxation of a transient exciplex state, which has a larger quantized volume to an isolated naphthalimide state, releases a lower energy photon. The emission is red-shifted relative to that of just the fluorophore. Although chelation with Zn^{2+} bends and twists the structure more towards the triazole group, the cyclam moiety is now the strong e^- acceptor because Zn^{2+} is a strong acceptor – the opposite of the pristine situation. As Zn^{2+} interacts in **1** directly with the triazole *N* it is plausible to assume it prevents PET; this leads to enhancement and a blue shift of the conjugated fluorescence and increased TICT-related emission which is neither blue nor red shifted. The absence of a detectable TICT band shift indicates that it is not coupled to the conjugated system but instead acts as an independent transition.

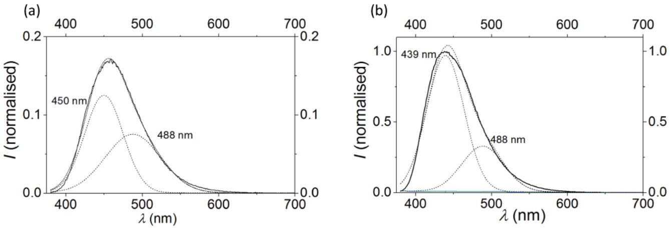

Fig. 2. Normalised emission spectra (λ_{ex} ~370 nm) of (a) **1** in HEPES buffer, and (b) with added Zn^{2+} as [**1**:Zn]$^{2+}$. Peak fitting (χ^2 = 0.0025) reveals two bands in the blue that accounts for the asymmetric spectra, corresponding to the conjugated ligand emission at shorter wavelengths and the TICT-related emission.

In this work, we consider two aspects of these processes which are important to understand and utilize them. The above arguments suggest strongly *there must be a significant temporal distinction between the mainly electronic, and therefore fast, naphthalimide ligand emission and the structurally-assisted PET suppression and TICT-like emission.* Mechanical torsions and relaxations take finite times (t > ms) longer than electronic excitations (t ~ns). The interplay can be measured by monitoring the ligand emission as a function of time – the slow evolution of net fluorescence intensity will be driven by the slow mechanical relaxation of bending and/or twisting. A temporal contribution makes it difficult to quantify key properties and mechanisms in absolute terms if time is not considered. The response of ligand **1** to Zn^{2+} and to Cu^{2+} versus time should therefore be noticeably different, given the contrasting redox potentials of these ions. Cu^{2+} has a positive reduction potential in the free state and shows much more metal-like behavior than does Zn^{2+}, which has a negative potential. Zn^{2+} accepts charge more strongly than the napthalimide whereas Cu^{2+} donates charge to the naphthalimide.

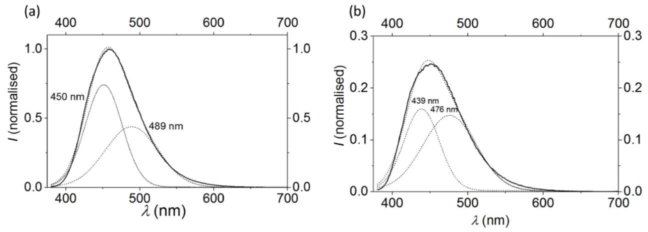

Fig. 3. Normalised emission spectra (λ_{ex} ~370 nm) of (a) **1** in HEPES buffer, and (b) with added Cu^{2+} as [**1**:Cu]$^{2+}$. Peak fitting (χ^2 = 0.084) reveals two bands corresponding to the conjugated ligand and red-shifted TICT emissions.

Figure 3 shows the emission from **1** with and without Cu^{2+}. As predicted, Cu^{2+} reduces the emission intensity significantly, consistent with an increased ability to donate charge through conventional PET quenching. A similar blue shift ($\Delta\lambda$ ~11 nm) is observed for the conjugated ligand, though it is slightly larger which may be attributed to the difference in size between Zn^{2+} and Cu^{2+} ions. However, there also appears to be a substantial blue shift of the TICT-related band, along with a reduction in emission here. These changes suggest an increase in the required excitation energy as a consequence of the charge transfer reversal at the triazole linker, which may be consistent with electrostatic repulsive packing of electron density within the distributed π system. Although the ligand-derived and TICT-like emissions appear to be electronically independent of each other, for the Zn^{2+} case where pristine PET is removed, the increase in fluorescence signal must share a common dependency on the rate of mechanical twisting in response to metal ion binding over time. The emission peak at λ_{em} = 458 nm of the free ligand and both the Cu^{2+} and Zn^{2+} complexes was monitored as a function of time at varying temperatures (T = 15 to 55 °C) using an excitation wavelength of λ_{ex} = 370 nm. The results are shown in Fig. 4. The free ligand emission was measured at 15 and 55 °C and found to be constant over six minutes (within the experiment temporal resolution); this is consistent with a predominantly electronic transition with a risetime t_{rise} << 5s. By contrast, the rate of change is strongly temperature dependent for both the Cu^{2+} and Zn^{2+} complexes, as anticipated. For the Cu^{2+} measurements, the signal-to-noise ratio deteriorates as the fluorescence signal decays below the levels of the ligand emission alone (shaded region of Fig. 4). In the free ligand case it is evident that there is no twist-related contribution to the conjugated system emission. The main impact of temperature on the ligand emission is the reduction of quantum efficiency. At the highest temperature measured, the onset of a peak emission for the chelated Zn^{2+} complex is within 30 s after a very rapid rise (t_{rise} < 15s at T = 55 °C). At cooler temperatures this risetime stretches to minutes (t_{rise} > 6 min at T = 15 °C). The lengthy timescales observed for the Zn^{2+} complex demonstrate that the fluorescence at 458 nm is increasing on a timescale that might be more consistent with phosphorescence; however, no delayed luminescence was observed with excitation turned off. Further, the lifetime of $[\mathbf{1}{:}Zn]^{2+}$ has previously been measured to be τ ~2 ns [12]. This points strongly to a local change in the number of excitation pathways to existing fluorescence bands; only slow mechanical conformational change can account for this. The fact that the decay of the existing band in the Cu^{2+} complex mirrors these results (within error) is consistent not with new phosphorescent bands but with a changing number of sites where altered energy transfer efficiencies to existing bands occurs.

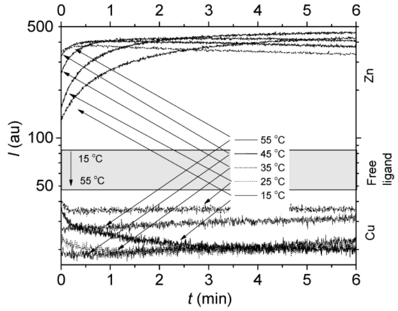

Fig. 4. Evolution of florescence intensity, I, at λ_{em} = 458 nm (λ_{ex} = 370 nm) versus time, t, for different temperatures (T = 15 to 55 °C) for Cu^{2+} and Zn^{2+} complexes of **1**. The free ligand range measurements at 15 °C and 55 °C were constant and flat over this time period, defining the border of the shaded region in which the ligand emission is affected.

In many ways, the simplest description of the system is as an electronic battery from the cyclam to the conjugated triazolyl-naphthalimide moiety via the triazole nitrogen bridge, with which the metal ion acceptor or donor interacts. The direction of the potential of the system determines the overall efficiency of emission from the states, as depicted in Fig. 5.

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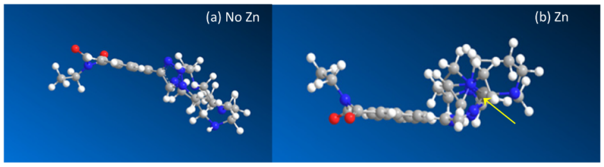

Fig. 5. ChemBioDraw structures of **1** (a) without and (b) with Zn^{2+} shown with yellow arrow. As a result of electrostatic interactions, the cyclam group is more twisted and bent out of plane with the naphthalimide group.

In terms of fluorescence, the asymmetry of this system describes an optical diode. Optical excitation provides the electromotive force to drive an e^- towards the Zn^{2+}-cyclam acceptor via the triazole *N* charge transfer bridge (rather than being directed elsewhere, where recombination may occur with no emission), leaving a triazolyl-naphthalimide hole. This behavior is characteristic of the way normal diodes function. Recovery occurs when the e^-, driven by electrostatic repulsion, can return and recombine at the triazolyl-naphthalimide hole and light is emitted. Cu^{2+} on the other hand, as a hard metal, is a donor and will suppress e^- flow across the triazole *N* bridge when it is bound; no hole is formed and the emission is quenched. These processes are dependent on conformation and the conformations themselves are driven by the overall electrostatic properties of the system (namely attraction of the metal ion to the triazole N). Hence the excited state generated by optical pumping is likely to lead to flexural changes in both bending and twisting, and these changes are the source of the relaxation of both emission in the Zn^{2+} complex and signal recovery in the Cu^{2+} complex.

This description of ligand **1** as an optically driven diode (Fig. 6) raises interesting prospects. Photons interact with a planar naphthalimide system that has an electrical field distribution of significant spatial extent. The conformation and orientation of the system determine the success of the *V*-driven hole generation and recombination. It seems logical that the polarisation of the exciting light relative to the molecular orientation will influence excitation of the ligand, which in turn affects the induced *V* across the triazole *N*. Since this bridge plays a key role in enhancing emission intensity, this offers a path to improving individual molecular responses beyond the net average typically measured in experiments reported to date. For single molecule excitation and sensing there may be profound effects.

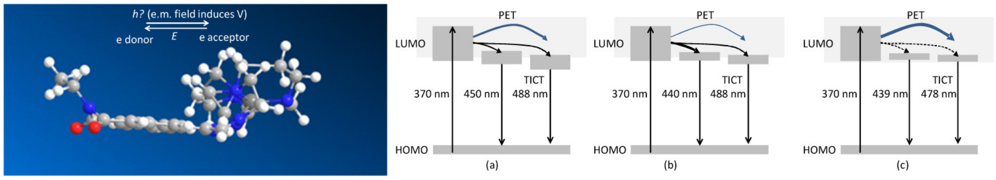

Fig. 6. A molecular optical diode: charge transport through the bridging triazole *N* determines hole generation and recombination. On the right are allowed transitions for (a) no complexation where emission is poor due to PET to an exciplex-like state; (b) Zn^{2+} complex leading to a higher energy for emission when the bias is preferentially through the triazole *N* instead of PET; (c) Cu^{2+} complex leading to higher energy of emission but with induced bias in the wrong direction, so emissions are quenched (dashed lines) as PET charge transfer is consequentially increased.

3. Conclusions

The fluorescence of a naphthalimide dye attached via a bridging triazole group to a cyclam receptor was studied. Consistent with published work on dual emitting species, a twist/bend assisted non-degenerate emission analogous to TICT is observed. Twisting or bending enables formation of a secondary emission band, while metal chelation brings further active deformation based on electrostatic repulsion of electron density fields. On this basis it was predicted and demonstrated that there should be an associated temporal dependence of fluorescence upon UV excitation, arising from charge transfer via the *N* through which the metal ion interacts. This was measured to be $t > 4$ minutes at room temperature, far too long to be accounted for by an electronic transition or charge diffusion. The observed temperature dependence is further evidence of a mechanical mechanism, suggesting formation of a highly deformable electronic system upon optical excitation. Zn^{2+} acts as a strong e^- acceptor preventing PET in the ligand by encouraging charge transfer through the triazole N with which it interacts. Electrostatic confinement of charge distributions across the system lead to quantisation of the main ligand emission band when blue shifted to higher energy. Within experimental and fitting uncertainty the non-degenerate band is unaffected. Therefore, we predicted that a photo-driven diode junction using a harder metal than Zn^{2+} (as determined by the sign of the free ion redox potential) would have the opposite effect. This was confirmed through chelation with Cu^{2+} which leads to a blue shift of both bands. Consequently, whilst this indicates that quantisation of charge still occurs, it also shows that Cu^{2+} is an e^- donor inhibiting charge transfer, encouraging PET and suppressing both emissions. Such contrasting diode behaviour between the Cu^{2+} and Zn^{2+} complexes suggests a way to distinguish between metal ions. The long-lived nature of the mechanical bending and twisting of the molecule is consistent with dynamic photo-induced charge separation in 3-D space. Eventually mechanical relaxation competes with electrostatic repulsive and attractive forces that lead to partial recovery of the system, observed at lower temperatures. From these observations, it is possible to conclude that for large conjugated molecular systems with chelating groups, the strain energies are moderate and readily accessible to near-UV light. Finally, it is plausible that the battery dynamics identified here can help clarify and optimise the selectivity of metal ion binding within living cells and in photolytically driven systems such as chlorophyll, where supressing PET processes optimises energy conversion.