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A pyrene-based fluorescent sensor for Zn^{2+} ions: a molecular 'butterfly'†

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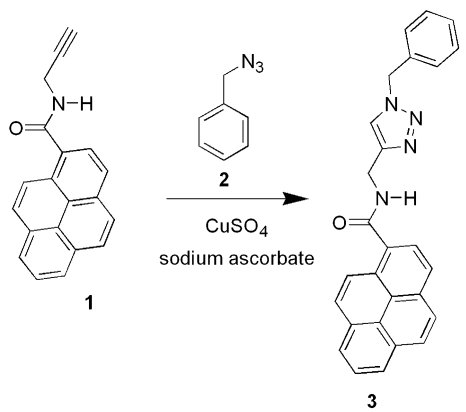
A simple pyrene-based triazole receptor has been synthesised and shown to self-assemble in the presence of $ZnCl_2$ in an exclusively 2 : 1 ratio, whereas a mixture of 2 : 1 and 1 : 1 ratios are observed for other Zn^{2+} salts. The pyrene units are *syn* in orientation; this is supported by a strong excimer signal observed at 410 nm in the presence of $ZnCl_2$ in acetonitrile. DFT calculations and 2D NMR support the proposed structure.

The design and synthesis of molecular receptors to target metal analytes *via* changes in fluorescence are topics of current interest as fluorescence signals are easily perturbed with any change to the fluorophore's local environment.[1,2] Many fluorescence mechanisms have been employed in molecular sensing and their applications in the field of supramolecular chemistry have been elegantly reviewed.[3] Numerous recent examples have incorporated the pyrene motif as a spectroscopic handle to detect ion pairs, cations,[4,5] anions[4] and neutral species.[6] This is due to a broad strong excimer signal observed typically in the 450 nm range when two pyrene units come into close proximity. We have applied this fluorescent mechanism to the problem of Zn^{2+} detection. The detection of Zn^{2+} is of particular interest both *in vitro* and *in vivo* due to the biological importance of Zn^{2+}.[7,8] Zinc complexes (in particular $ZnCl_2$) also play an important role, as a moderate strength Lewis acid, in Friedel–Crafts acylations.[9,10] Unfortunately, Zn^{2+} is spectroscopically silent making it difficult to detect Zn^{2+} ions directly.

Here we report a pyrene-derived molecule (**3**) that contains an amide functional group and a triazole moiety that self-assembles around the metal and anion. The signal is generated by the self-assembled induced excimer that forms upon the addition of Zn^{2+}. The Zn^{2+} ion coordinates to the oxygen atom of the amide functional group rather than a nitrogen atom in the triazole moiety, as seen in other Zn^{2+}-binding receptors.[11] Compound **3** was prepared by reacting pyrene carboxylic acid with $SOCl_2$ to form the acid chloride, which was subsequently reacted with an equimolar amount of propargylamine, to produce compound **1**.[12] This was purified by silica column chromatography to afford compound **1** in 60% yield. Compound **1** was then tethered to benzylazide *via* azide-alkyne Huisgen cycloaddition to afford the target molecule in 50% yield, (Scheme 1).

The binding affinity of compound **3** towards Zn^{2+} salts was investigated by NMR and fluorescence spectroscopy. ^{1}H-NMR titrations were carried out in acetonitrile-d_3 solution. There were significant downfield ^{1}H NMR chemical shifts for the NH proton, the triazole proton and, to a lesser extent, for the singlet of CH_2 on the benzyl group. Upon the addition of $ZnCl_2$, for example, the changes in chemical shift for the NH, CH (triazole) and CH_2 (Bz) protons were 0.90 ppm, 0.25 ppm and 0.1 ppm, respectively. The binding affinity (K_{21}) of **3** for Zn^{2+} was calculated to be greater than 1.0×10^5 M^{-1} (see ESI† Table S1*). Interestingly, the reaction of **3** with $ZnCl_2$ produced a **3**:Zn^{2+} complex that was exclusively in the 2 : 1 ratio, whereas non-linear curve fitting analysis can only be successfully refined for the other Zn^{2+} salts if other ligand-to-metal stoichiometries are taken into consideration. This was further confirmed by Job's Plot analysis (see ESI,† Fig. S11). The NMR shifts can be rationalized through close contacts between the triazole hydrogen and chloride atom on the Zn^{2+} centre. Surprisingly, the NH proton is *trans* to the metal centre, and does not participate in the binding event (*vide infra*). The chemical shift seen for the amide proton can be rationalised by the inductive effect. The amide oxygen atom coordinates to the Zn^{2+} centre becoming more negatively



Scheme 1 Synthesis of molecular receptor **3**.

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charged, the carbonyl carbon is affected (+ve) as is the amide nitrogen (−ve). On closer inspection of the NMR spectra, specifically the aromatic region around 7.0 ppm, compound **3** shows two sets of multiplets assigned to the benzyl protons. Upon the addition of $ZnCl_2$, only one set of equivalent signals is observed (ESI,† Fig. S10). This can be explained by the prevention of free rotation of the benzyl group and the resultant symmetry of $\mathbf{3}_2ZnCl_2$. We can rule out the possibility of the second ligand binding in the *anti* orientation, as an excimer band is seen in the fluorescence experiments (see below).

The binding behaviour of compound **3** was also studied by investigating the photophysical properties of **3** towards various Zn^{2+} salts in acetonitrile solution at 298 K. A 6.5×10^{-4} M solution of compound **3** was excited at 355 nm; the monomer showed two distinct emission bands at 382 and 402 nm with a broad shoulder at 420 nm, typical of pyrene-derived monomers, assigned to the π–π* electron transitions.[13] Upon the addition of sub-equimolar amounts of Zn^{2+} salts (F^-, Cl^-, Br^-, I^-, BF_4^-, $CH_3CO_2^-$ SO_4^{2-}, $H_2PO_4^-$, ClO_4^-, CN^-, NO_3^-) different fluorescence changes were observed. Full titrations were carried out for those Zn^{2+} salts that showed excimer formation only. For example, the excimer band at 410 nm was observed for F^-, Cl^-, (Fig. 1) Br^-, I^-, NO_3^-, whereas no excimer band was seen for the other anions. However, an increase in the fluorescence intensity of the monomer at 382 and 402 nm was seen for the remaining anions CN^-, $CH_3CO_2^-$, SO_4^{2-} and $H_2PO_4^-$. It is well known that many organic functional groups, such as amines, effectively PET quench the fluorescence of pyrene units.[1] The amide functional group can also

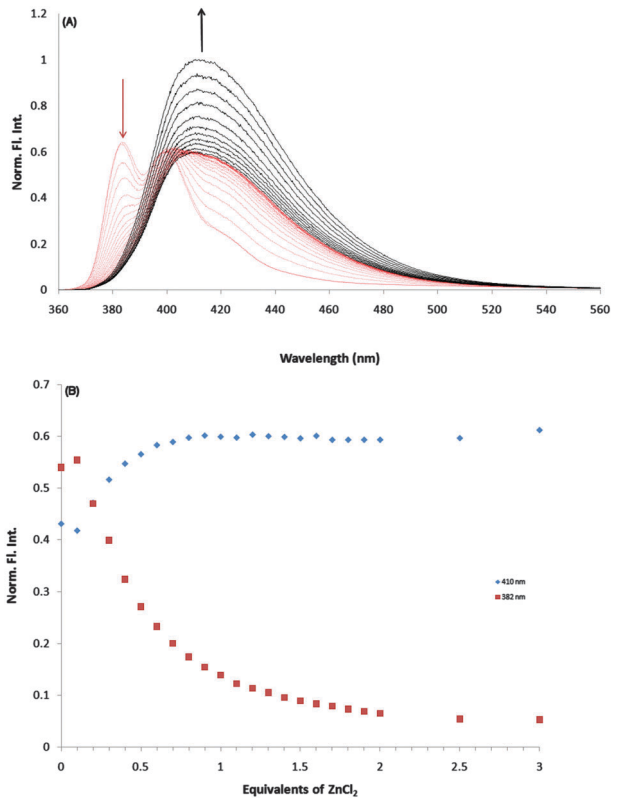

Fig. 1 Fluorescence titration of compound **3** and $ZnCl_2$ in CH_3CN (6.5×10^{-4}, M), λ_{Ex} = 355 nm. (A) Fluorescence spectra and (B) binding isotherm.

PET quench the pyrene moiety, but to a lesser degree. The intensity of the excimer signal is dependent on the proximity of the two pyrene units. In absence of the Zn^{2+} ion, the excimer peak is not observed, as was confirmed by the analogous spectroscopic studies with tetrabutylammonium salts. This suggest that the Zn^{2+} halide is templating the pyrene units. The geometry around the Zn^{2+} ion is tetrahedral and the pyrene moieties adopt a *syn* configuration. The fluorescence titrations are in good agreement with the NMR studies showing a 2 : 1 non-linear fitting predominated for the $ZnCl_2$ complex with a calculated binding constant (K_{21}) of 1.8×10^6 M^{-1} (ESI,† Table S1). In the later stage of the titration (>3 equivalents) it is clear from the fluorescence spectra (Fig. 1, black lines) that there is a loss of defined spectroscopic transitions, giving a broad and featureless band. This is entirely consistent with a second Zn^{2+} coordination event of a much weaker affinity, such as the formation of complexes with higher metal to ligand ratios.

Zn^{2+} is versatile with respect to the number of ligands it can adopt in its coordination sphere. Zn^{2+} is known to be tetrahedrally coordinated with halides in the inner coordination sphere, whereas octahedral Zn^{2+} is most common in aqueous solutions.[14] However, there are many tetrahedral biological Zn^{2+} complexes, and some of these systems are supported by extensive DFT studies to show the preference for tetrahedral Zn^{2+}.[15] Therefore it is reasonable to assume that compound **3** would self-assemble around the Zn^{2+} salt in a tetrahedral fashion for the halides. Nitrate, on the other hand, can coordinate to the metal centre *via* two oxygen atoms and form an octahedral geometry. The fluorescence experiments clearly show the formation of an excimer, meaning that the pyrene units had to be in close proximity for the π–π* transition and in the *syn* orientation. To investigate this further we turned to molecular modelling calculations.[16] Molecular mechanics calculations for $\mathbf{3}_2ZnX_2$ (where X = F^-, Cl^- or NO_3^-) showed that complexes of the form *syn*-$\mathbf{3}_2Zn(NO_3)_2$ should be the most stable geometries. This is evident for the $\mathbf{3}_2Zn(NO_3)_2$ complex where there is a difference of approximately 16 kJ mol^{-1} between the *anti* and *syn* isomers, in excellent agreement with the fluorescence and NMR studies.

DFT calculations revealed that only two structures, *anti*-$\mathbf{3}_2Zn(NO_3)_2$ and *syn*-$\mathbf{3}_2Zn(NO_3)_2$, appeared to remain viable once bonding constraints had been removed. These were also two of the three lowest energy structures identified by Monte Carlo molecular dynamics and subsequent geometry refinement by molecular mechanics. The pyrene–pyrene interactions suggested that, of these structures, the more stable *syn*-$\mathbf{3}_2Zn(NO_3)_2$ was an excimer, with the two pyrene groups separated by 6.8 Å at an angle of 36°. When the same protocol was applied to the $\mathbf{3}_2ZnF_2$ and $\mathbf{3}_2ZnCl_2$ complexes, the pyrene groups in the resulting $\mathbf{3}_2ZnF_2$ structure were a similar distance apart, however, the angle between the pyrene moieties was now 76°, explaining the lack of excimer signal in the fluorescence study (ESI,† Fig. S12 and Table S2). In the analogous $\mathbf{3}_2ZnCl_2$ complex, the interplane distance was calculated to be 4.4 Å at an angle of 4.9° (Fig. 2). This explains why the excimer signal observed in the fluorescence studies is more intense for the chloride than the other Zn^{2+} salts studied. The result can also be attributed to the ionic size of

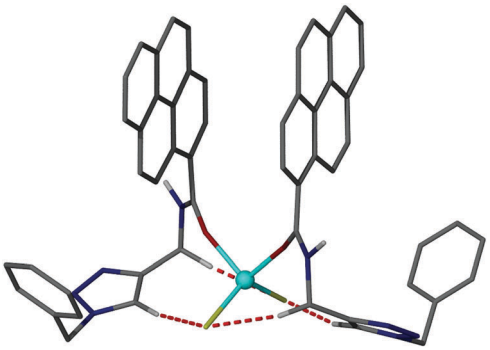

Fig. 2 DFT fully optimised structure of $\mathbf{3}_2ZnCl_2$. Calculated pyrene distance 4.4 Å and angle 4.9°.

F^- (1.33 Å), Cl^- (1.81 Å) and NO_3^- (1.96 Å). The larger size of the chloride forces the pyrene units closer together. Another factor is the electronegativity of the fluorine and oxygen atoms, which spreads the pyrene units further apart. The observation is supported by the calculated bond distances between the anion and CH (triazole), CH (methyl amide) and CH (benzyl) protons. The distance is shorter for anions that are more electronegative than chloride (ESI, Table S2). This is in agreement with the less intense excimer behaviour observed in the NO_3^- and F^- fluorescence studies.

Further evidence to support the tetrahedral arrangement of $\mathbf{3}_2ZnCl_2$ was determined by 2D NMR spectroscopy. An rOe spectrum recorded for compound **3** in CD_3CN showed five strong rOe signals (ESI,† Fig. S16). The signal that is of greatest significance is the rOe between the NH proton and the hydrogen atom attached to C(10) on the pyrene moiety. Upon the addition of $ZnCl_2$, this signal disappears and an rOe is observed between the NH and C(2) proton. This clearly demonstrates that rotation about the C(1) pyrene has occurred. This correlation is very distinctive, the calculated bond distance between the NH group and C(2)H is greater than 4 Å for compound **3**, too far to feel an rOe effect. However, the calculated bond distance between the NH and C(2)H is 2.386 Å for the complex, a reasonable distance for an rOe signal. The proposed geometry is also supported by an additional weak rOe signal between the triazole proton and the *ortho* proton of the benzyl group (ESI,† Fig. S17), absent in the free ligand. This clearly supports that $ZnCl_2$ acts as a template for two equivalents of compound **3** in the proposed geometry seen in Fig. 2.

It is clear from the studies described that $ZnCl_2$ produced the most intense excimer signal but only when present in a 2 : 1 ratio of **3** : metal. In order to see if compound **3** could act as a sensor in a practical application, we carried out a Friedel–Crafts acylation reaction by preparing 1-(2,4-dihydroxyphenyl)-ethanone (ESI,† Scheme S1), which uses $ZnCl_2$ in the synthetic procedure, to determine how much residual $ZnCl_2$ remained after the chemical work up. Initial results (ESI,† Fig. S18) show that we do observe excimer formation. However, the amount of water in the system competes with the self-assembly process, preventing qualitative detection limits; this is currently been investigated and will be reported in due course.

In summary a pyrene-based triazole receptor has been synthesised and shown to self-assemble in the presence of $ZnCl_2$; due to the size of $ZnCl_2$, this occurs in an exclusively 2 : 1 ratio. The calculated binding constants show a high affinity for the Zn^{2+} salt and 2D NMR and DFT calculations support the proposed structure.

All new compounds were characterised by ^1H NMR, ^{13}C NMR, 2D NMR (COSY, ROSEY, HMBC) ESI-MS and elemental analysis. Binding studies were determined from ^1H NMR and fluorescence titrations using HypNMR 2008 and HypSpec 2008, respectively. Financial support for this work was provided by the NSF grant OCE-0963064, additional acknowledgments in ESI.†

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