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An Aqueous Ratiometric Fluorescence Probe for Zn(II)

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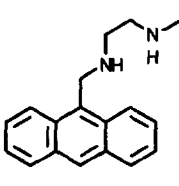
Abstract. We report herein the first example of the use of metal promoted intramolecular excimer formation using a system derived from a simple linear polyamine. The system demonstrates that considerably less preorganization is necessary than that used in cyclic systems. The water solubility results in the first aqueous operative ratiometric fluorosensor for Zn^{2+} ions.Bis-9-anthrylpolyamines (e.g. **2**) were prepared by the 4Å molecular sieve promoted condensation of the polyamine with a stoichiometrically appropriate amount of 9-anthraldehyde in $CHCl_2$. Compound **2** (25μM) was titrated with $Zn(ClO_4)_2$ in CAPS buffer (0.05 M) at pH 10.5. The most notable feature of this titration is the nearly 4-fold increase in fluorescence intensity at about 495 nm. In fact, after the addition of 1 equivalent of Zn^{+2} the normal anthracene fluorescence profile is no longer the dominant feature of the spectrum. Subtracting the spectrum with no Zn^{+2} from that with 1 equivalent reveals the dominant excimer spectrum. The mono substituted control shows only the expected chelation enhanced fluorescence based upon the complexation state of the benzylic amine nitrogen.

Fluorescent chemosensors for metal ions in solution are varied and the history of these species dates to the 19'th century.[1] The design and development of these tools remains a highly active area of research. We report here the use of a simple bis-substituted linear polyamine as a ratiometric fluorescence probe for the Zn^{2+} ion in aqueous solution.[2]

The mechanism for the effect in most fluoroionophores is dependent on a direct change in the electronics of the sensor upon the binding event leading to an increase or reduction in the fluorescence intensity at a characteristic wavelength. The more desirable means of fluorescence detection is ratiometric.[3] We are interested in designing molecules that use the non-covalent bonds of molecular recognition to perform specific tasks, one of which is fluorescence signaling. To effect these tasks, we seek large changes in molecular conformation upon the binding event. These large conformational changes could bring fluorescent species into proximity thereby enhancing energy transfer and the fluorescence of the associated species. This strategy has been effectively employed by a few others {for alkali,[4] for Pb(II)[5], and for Na^+, Ag^+, Tl^+ and Th(IV)[6]}but all are reported to work only in organic solvents. Fages has recently introduced a self-assembled system for Fe(III) in acetonitrile that operates similarly.[7]

One binding interaction that has been exploited in water is ionophore complexation of metals.[8] This chemistry is dominated by the use of macrocyclic polyethers and polyamines mainly because of the large binding affinities (relative to their open-chained counterparts) resulting from preorganization (the macrocyclic effect).[9] Nonetheless, the template effect[10] on the synthesis of some of these macrocycles suggests that the binding affinity of the open-chain species is strong enough to do useful work on the molecular scale. A few aqueous allosteric or self-assembling systems have been designed on this principle.[11] Notably, none of these are based on a linear polyether or polyamine that would correspond directly to the simplest of the macrocyclic hosts. In fact, in at least one case where this strategy might have been used with bis-9-anthryl-polyethers for the template directed synthesis of photocrowns,[12] no effect was seen on the photocyclomerization quantum yield upon the addition of alkali metal salts. In contrast to this polyether evidence, linear polyamines are well known to bind transition metal ions in aqueous solution and have been used in the design of fluorescent signaling devices for ion recognition.[13] We report herein the first example of the use of metal promoted intramolecular excimer formation using a system derived from a simple linear polyamine. The system demonstrates that considerably less preorganization is necessary than that used in the cyclic systems as evidenced by the less prominent excimer fluorescence in the unbound state.[14] The water solubility results in the first aqueous operative ratiometric fluorosensor for Zn^{2+} ions.

9-Anthrylpolyamine **1**[15] and bis-9-anthrylpolyamine **2** were prepared by the 4Å molecular sieve promoted condensation of triethylenetetramine with a stoichiometrically appropriate amount of 9-anthraldehyde in CH_2Cl_2.[16] The putative imine was not isolated but was dissolved in hot methanol and treated with an excess of $NaBH_4$. Both compounds were isolated and characterized as their HCl salts.[17]



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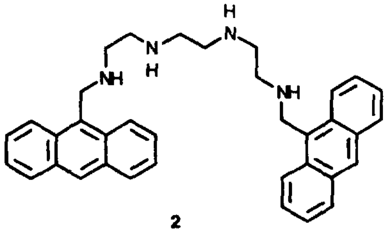

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In accordance with the protocol described by Czarnik[18], compounds **1** (10 μM) and **2** (25μM) were titrated with $Zn(ClO_4)_2$. CAPS buffer (0.05 M) at pH 10.5 was used to obtain an appropriate initial anthracene fluorescence while maintaining conditions for maximum Zn^{+2} complexation.[19] As expected, compound **1** shows a chelation-enhanced fluorescence (CHEF) of about 6-fold at λ_{max} (414 nm) based upon the complexation state of the benzylic amine nitrogen. A representative overlay demonstrating this increase in fluorescence intensity is shown for the full spectra in **Figure 1**.

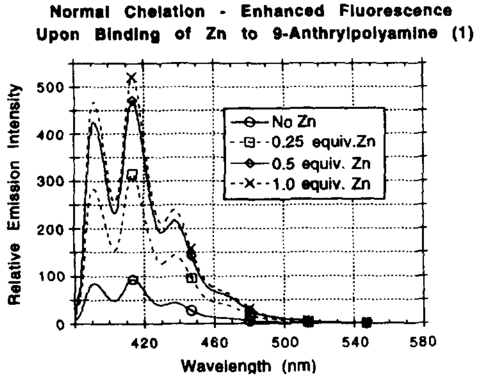

Figure 1. Fluorescence spectra for 10μM solutions of **1** with increasing concentration of $Zn(ClO_4)_2$. Excitation wavelength was set at 335 ± 3 nm.

Similar titration of **2** shows a completely different effect. The most notable feature of this titration is the nearly 4-fold increase in fluorescence intensity at about 495 nm. In fact, after the addition of 1 equivalent of Zn^{+2} the normal anthracene fluorescence profile is no longer the dominant feature of the spectrum (**Figure 2**). Subtracting the spectrum with no Zn^{+2} from that with 1 equivalent reveals the dominant excimer spectrum (**Figure 3**). We propose this as the first example of chelation-promoted intramolecular excimer formation for aqueous solution. As apparent from the difference spectrum in **Figure 3**, there is some growth at 415 nm (the normal anthracene λ_{max}) that can best be attributed to the expected CHEF effect as a result of complexation. Furthermore, examination of the fluorescence spectrum of **2** indicates that there is some excimer already apparent even without Zn^{+2} present. This is consistent with Desvergne's observation for the polyether analog alone in methlcyclohexane.[20] The small additional excimer observed here in aqueous solution with no cation may arise from the hydrophobic interaction between aromatics promoting their proximity. The differential response to Zn^{2+} at 414 nm and 495 nm allows the fluorescence ratio at these two wavelengths to be used for concentration determination.

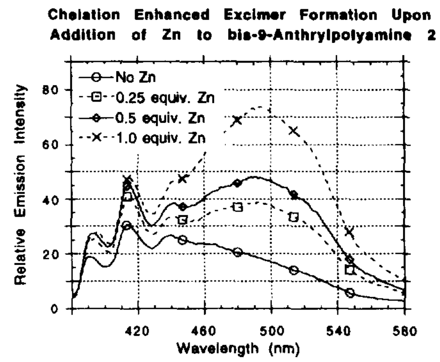

Figure 2. Fluorescence spectra for 25μM solutions of **2** with increasing concentration of $Zn(ClO_4)_2$. Excitation wavelength was set at 335 ± 3 nm.

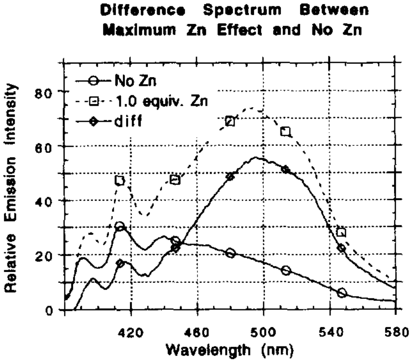

Figure 3. Difference spectrum between compound **2** (25 μM) with 1 equivalent of Zn^{+2} (maximum effect) and compound **2** (25 μM) with no Zn^{+2}.

This work provides one example of how small molecule three dimensional structure can be greatly influenced by non-covalent bonding interactions *in aqueous solution*. We are in the process of designing new receptors on the basis of this paradigm and examining their behavior in the presence of a variety of potential metallic and non-metallic guests. Based on coordination geometries of metal ions in solution, this could lead to selective signaling devices. Small allosteric or self-assembled systems for catalysis or transport is an additional likely result of this simple strategy.

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14. Compare to the Anthraceno-crown ether reported in reference 3d for a non-aqueous example.
15. One of a series of 9-anthrylpolyamines reported in the Ph.D. dissertation of Scott A. Van Arman and in Van Arman, S.A.; Czarnik, A.W. *J. Am. Chem. Soc.* **1990**, *112*, 5376.
16. Exactly 2 equivalents of 9-anthraldehyde per polyamine were used to obtain (ultimately) the bis adduct (**2**) in 69% yield. A 10-fold excess of the polyamine was used to obtain (ultimately) the mono adduct (**1**) in 76% yield.
17. This procedure is outlined in a separate manuscript submitted elsewhere for publication. All new compounds have been fully characterized by ^{1}H NMR, ^{13}C NMR, and high resolution FAB MS or MS and elemental analysis.
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