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First report of Zn^{2+} sensing exclusively at mesoscopic interfaces†

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We present a simple but unique, new probe 1-pyrenyl-methyl-bis(2-picolyl) amine (Pybpa) that selectively senses interfacially bound Zn^{2+} ions while being completely silent towards Zn^{2+} in bulk water.

Fluorescent sensors are practical tools for visualizing metal ions at the molecular level. Numerous sensors for metal ions, *e.g.* Zn^{2+} have been devised,[1] but most of them use organic solvents as the detection medium. Zn^{2+} sensing is particularly important, as these ions are essential ingredients of biological systems. Indeed, specific, cell permeable sensors for the detection of Zn^{2+} ions in water have been the focus of much recent attention.[2] However, all the above mentioned studies focused on sensing Zn^{2+} in the solution phase. The experimental approaches for probing of Zn^{2+} ion distributions at micro-heterogeneous interfaces such as micellar or vesicular surfaces have not been developed. This is a challenging but important task since such interfaces have a profound relevance in chemistry and biology. It requires the design of a probe that does not report the presence of Zn^{2+} in water, while being able to respond exclusively to interfacial Zn^{2+}.

Fig. 1 shows the fluorescence emission spectrum of **Pybpa** at pH ~ 7. The probe fluorescence in water is quenched partly due to a photoinduced electron transfer (PET) process[1] operating from the pyrenyl nitrogen ($PyCH_2$–N) to the excited pyrenyl (Py*) chromophore. The spectrum has two components, slightly structured emission in the 380–420 nm region and a broad, emission band centered at ~ 500 nm. Significantly, the broad, red-shifted emission band is not seen when solubilized in organic solvents (Inset, Fig. 1). The 380–420 nm emission band can be attributed to 'monomeric' **Pybpa** while the broad ~ 500 nm band may be due to 'pyrene excimer' emission.[3] The appearance of 'pyrene excimer' emission suggests that **Pybpa** molecules are undergoing self-association in water. Note that at this concentration range (1–2 μM) unsubstituted pyrene does not show excimer emission.

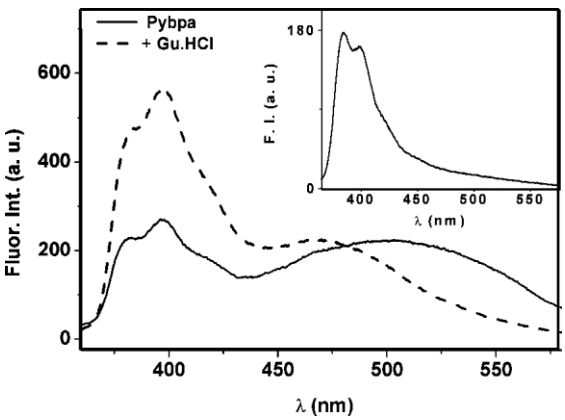

Fig. 1 The emission spectrum of **Pybpa** (2 μM) in water (50 mM HEPES, pH 7) and in the presence of 4.8 M guanidinium hydrochloride. Inset: **Pybpa** (2 μM) emission spectrum in methanol.

Unambiguous evidence of the aggregation of **Pybpa** in water comes from experiments using a chaotropic salt, guanidinium hydrochloride (Gu·HCl). Gu·HCl, a widely used protein denaturant is known to disrupt hydrophobic associations.[4] Gu·HCl in water has a substantial effect on the **Pybpa** emission (Fig. 1). At 4.8 M Gu·HCl the **Pybpa** 'monomer' emission showed a threefold increase over that in water. Similarly, the monomer to excimer emission intensity ratio increased ~ 3 times along with a noticeable change in the shape of the excimer fluorescence. There was a clear blue shift in the excimer λ_{max} and this band became considerably less broadened. Thus, Gu·HCl appears to disrupt the aggregates formed by **Pybpa** in water, in keeping with its ability to suppress hydrophobic interactions. On the other hand a salt like LiCl known to promote aggregation of apolar solutes in water, simply induced further quenching of the **Pybpa** emission.†

The association of **Pybpa** in water can either be an excited state phenomenon, or in the ground state itself the pyrene units of **Pybpa** molecules may stack leading to aggregation. This distinction was made by examining the fluorescence excitation spectrum of **Pybpa**.† The excitation spectrum of the 'excimer' band (~ 450 nm) was found to be extended towards the red as compared to the excitation spectrum for the **Pybpa** monomer emission (~ 400 nm). This broadening and the extension of the excitation spectrum suggests that the pyrenyl chromophores are associated in the ground state. For the excimer formation to be an excited state process, the excitation spectra corresponding to the monomer and excimer emission have to be the same. Taken together the above studies clearly indicate that **Pybpa** aggregates only in water.

Due to the presence of bis(2-picolyl amine) unit in **Pybpa**, we then examined this probe for metal ion sensing. Interestingly addition of different metal ions, such as Zn^{2+}, Cu^{2+}, Ca^{2+}, Fe^{2+} etc. had practically no effect on the emission properties of **Pybpa** in water.† This implies that since the **Pybpa** molecules are in aggregated state in water, the ion binding, bis(2-picolyl) amine unit may not be accessible to the added metal ions. This observation is in contrast to a closely related probe (9-anthryl) methyl-bis(2-picolyl amine)[5] that senses Zn^{2+} in water effectively.[5,6] The difference between the two molecules must originate from the fact that while the pyrenyl probe aggregates in water, the anthryl probe does not. It is notable that in the presence of 4.8 M Gu·HCl, the **Pybpa** fluorescence starts to respond to the added Zn^{2+} ion (not shown).

Pybpa aggregation in water and its lack of sensitivity for Zn^{2+} in water prompted us to exploit this probe towards Zn^{2+} sensing in a context-dependent manner. We wanted to see if **Pybpa** could sense Zn^{2+} effectively in microenvironments[7,8] that could convert its aggregates in water to monomers. Micellar solutions have polar interfaces in water with hydrophobic core.[9,10] Hence we sought to examine the influence of different surfactant micelles on the aggregation of **Pybpa**. The fluorescence spectrum of **Pybpa** in micelles showed no excimer and the monomer emission was substantially enhanced. This was true irrespective of the charge or the type of surfactant used for the preparation of micelles. This suggests that micelles 'break'

† Electronic supplementary information (ESI) available: additional Figs. 1–3. See http://www.rsc.org/suppdata/cc/b3/b301364b/

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open the **Pybpa** aggregates. This observation led us to examine the effect of Zn^{2+} ions on the probe solubilized in micelles. The change in **Pybpa** fluorescence on Zn^{2+} addition to micelle bound probe at pH ~7 is shown in Fig. 2. **Pybpa** not only responds to Zn^{2+} ions, but the sensing by **Pybpa** is clearly dependent on the surface charge of the micellar aggregate. The sensing of Zn^{2+} on cationic cetyltrimethyl ammonium bromide (CTABr) micelles was the poorest while it was most efficient on neutral polyoxyethylene (20) sorbital monolaurate (TWEEN 20) micelles. The Zn^{2+} binding to **Pybpa**on the TWEEN 20 micelles was in fact stoichiometric (1:1). Sensing of Zn^{2+} on anionic sodium dodecyl sulfate (SDS) micelles is in between that in CTABr and TWEEN 20 micelles. This impressive surface charge discrimination can be rationalized as follows. In micelles, for Zn^{2+} sensing to be accomplished by the 'monomeric' **Pybpa**, the bis-picolyl unit should be facing water with hydrophobic pyrene units buried in the micellar interior. Now, Zn^{2+} ions are not easily accessible to the CTABr surface due to its unfavorable electrostatic disposition and this is reflected in the inefficiency of Zn^{2+} sensing at the CTABr/water interface. At the surface of the anionic SDS micelles, only a fraction of the Zn^{2+} ions would be available for binding to **Pybpa** as the sulfate headgroups of the surfactant would also compete for Zn^{2+} binding. In contrast at the micellar interface of neutral TWEEN 20 micelles, the **Pybpa** molecules can bind to Zn^{2+} ions without encumbrance from headgroup charge leading to a dramatic enhancement in fluorescence emission.

Micelles are highly dynamic aggregates[9,10] and it was of interest to see if the approach could work in kinetically more ordered aggregates, *e.g*. lipid bilayer vesicles.[9,11] For these experiments, **Pybpa** doped vesicles of natural phospholipids, soyabean PC and dipalmitoyl phosphatidylcholine (DPPC) were prepared. The fluorescence spectra of the probe in various lipid suspensions indicate that indeed the probe does get incorporated in the vesicles, as evident from increased emission intensity and a lack of 'excimer' emission (not shown). This was similar to the observation in micelles. Addition of Zn^{2+} to **Pybpa** incorporated in PC vesicles led to a large fluorescence enhancement (not shown). A comparison of the sensing efficiency in DPPC and soyabean PC is interesting. The increase is much greater with the probe doped in soya PC vesicles and the saturation appears to occur at ~1:1 stoichiometry (probe: Zn^{2+}). The major difference between DPPC and soya PC vesicles at 25 °C is in the relative order of their organizations. At 25 °C, while the DPPC vesicles would be in solid-like gel phase, the soya PC vesicles would be in the more fluid, liquid-crystalline phase. The recognition of Zn^{2+} by the probe is more facile at the soya PC interface probably because of its greater fluidity. Thus, the ability of **Pybpa** extends impressively to the sensing of Zn^{2+} ions at biologically relevant lipid/water interfaces.‡

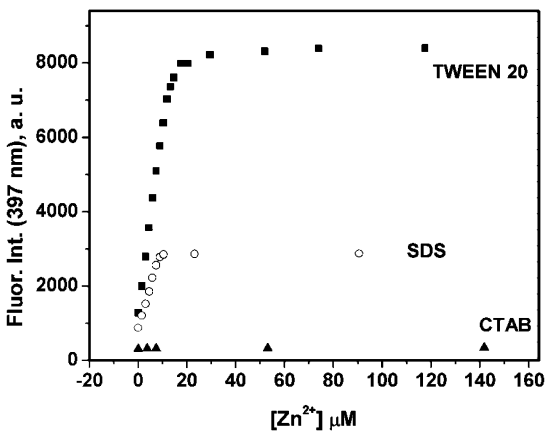

Fig. 2 Zn $^{2+}$ sensing by **Pybpa** (10 μM) solubilised in CTABr, SDS and TWEEN 20 micelles at pH 7 (50 mM HEPES). Shown here is the change in fluorescence at 397 nm on $ZnCl_2$ addition. [surfactant] = 10 mM.

In summary, we have synthesized a very simple PET sensor, **Pybpa** that remains aggregated in water even at very low concentrations. In this situation, **Pybpa** is unable to recognize Zn^{2+} ions. As these aggregates are disrupted by self-assembled entities like micelles and vesicles, **Pybpa** becomes highly responsive to Zn^{2+}. In effect mesoscopic surfaces of micelles and vesicles 'chaperone' Zn^{2+} sensing by **Pybpa**. The recognition process is modulated by the surface charge and order of the aggregates that template the recognition process. Thus **Pybpa** can provide useful information about the presence and activity of Zn^{2+} on microheterogeneous aqueous interfaces. To our knowledge this is the first instance where a fluorescent probe failed to recognize Zn^{2+} in water but the Zn^{2+} sensing got 'switched on' as soon as the probe came in contact with hydrophobic surfaces. Thus, the fluorescent sensor has been used in a context dependent manner. Work is currently underway to further extend this concept of multifunctional hydrophobic probe design.

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Notes and references

‡ An important concern in metal ion sensing is the interference by other ions. The ions, Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} that are commonly present in significant concentrations in biological systems, did not affect **Pybpa** emission and its Zn^{2+} sensing. This is consistent with the reported properties of the **bpa** (bis-2-picolyl) unit.[2]

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