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Tb(III) functionalized vesicles for phosphate sensing: membrane fluidity controls the sensitivity†

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We report a modular design of vesicular chemosensors by co-embedding a Tb(III) complex and a receptor–sensitizer conjugate in phospholipid vesicles. The binding of phosphate anions to the vesicle surface in aqueous media is detected by a decrease in Tb(III) phosphorescence. The sensory response can be modulated by a variation in the membrane fluidity.

Analyte detection at low concentrations is a key issue in the fields of analytical, biological and medicinal chemistry. Fluorescent chemosensors, with their high sensitivity and fast response times, are frequently employed for this purpose.[1] Functionalized artificial membranes/bilayers with embedded fluorescent probes[2] represent a special class of chemosensors. They mimic the functions of biomembranes, known to play pivotal roles in the intracellular signalling pathways.[3] The molecular recognition events at the cellular interface are governed by the self-assembly of membrane lipids and proteins through the formation of domains or clusters.[4] We recently reported the detection of various biological analytes in aqueous buffer (pH = 7.4) by utilizing the interface of functionalized luminescent vesicles.[5] The vesicles were prepared by co-embedding amphiphilic metal complex receptors and amphiphilic fluorescent dyes in synthetic DSPC (1,2-distearoyl-*sn-glycero*-3-phosphocholine) lipid membranes. The analyte binding at the interface alters the composition of the self-assembled mixed dye/receptor patches. This results in a partial expulsion of the dyes from these patches, thereby triggering an emission response.

In spite of their widespread use, a common drawback of fluorescent probes with short excited state lifetimes is the interference from the background fluorescence, especially for bioanalyte detection and *in vivo* measurements.[6] Therefore, time delayed measurement using long life-time luminescent probes has evolved as a technique. Phosphorescent complexes of heavy metals such as Pt(II), Ru(II), Re(I) and Ir(III) are effective,[7] but particularly attractive are trivalent lanthanide [Ln(III)] complexes. They possess a combination of several desirable properties for sensor design: long excited state life-times (μs to ms range), sharp line-like emissions and large Stokes shifts (>200 nm).[8] Hence, lanthanide based probes have found uses in sensors for metal ions and other analytes,[9] in immunoassays and bio-imaging[10] and for monitoring of enzyme activity.[11]

We envisaged that the modular nature of the design of our vesicular chemosensors would allow us to replace the fluorescent dyes with lanthanide based long excited state life-time probes; a modification that would certainly broaden their applicability. In the literature, to the best of our knowledge, the only example of such Ln(III) based vesicular chemosensors is of Kimizuka *et al.*[12] They reported the sensing of ATP and ADP by phosphorescent vesicles made from an amphiphilic, positively charged Tb(III) complex. The binding event was accompanied by an increase in Tb(III) emission, a consequence of the removal of H_2O molecules from its inner co-ordination sphere. Herein, we report a conceptually different approach by co-embedding a Tb(III) complex, functioning as a phosphorescent reporter, along with a phosphate receptor in the phospholipid vesicles (~100 nm) for the detection of different phosphate analytes. Furthermore, we demonstrate the tuning of the sensitivity of these chemosensors by varying the fluidity of the lipid bilayer.

The intra 4f transitions in Ln(III) ions are Laporte forbidden resulting in very weak emissions by their direct excitations. Therefore, the Ln(III) ions are usually excited through organic chromophores, which act as antennas to absorb light much more strongly and transfer the excited energy to the lanthanides (*antenna effect* or *sensitization*).[13] This energy transfer is strongly dependent on the mutual proximity of the sensitizer and Ln(III).[14] We reasoned that if we could design a functionalized vesicle surface where the binding of an analyte leads to a variation in the distance of the membrane embedded Ln(III)–sensitizer, the concomitant change in the Ln(III) emission would provide a sensitive method for analyte detection. Accordingly, we synthesized an amphiphilic Tb(III) complex (**Tb-1**, Fig. 1) of diethylene triamine pentaacetic acid (DTPA), reported previously by Duhme-Klair *et al.*[15] The octadentate chelating ligand DTPA occupies most of the co-ordination sites of Tb(III) (typically co-ordination numbers of 7–9)[8a] thereby minimizing the quenching

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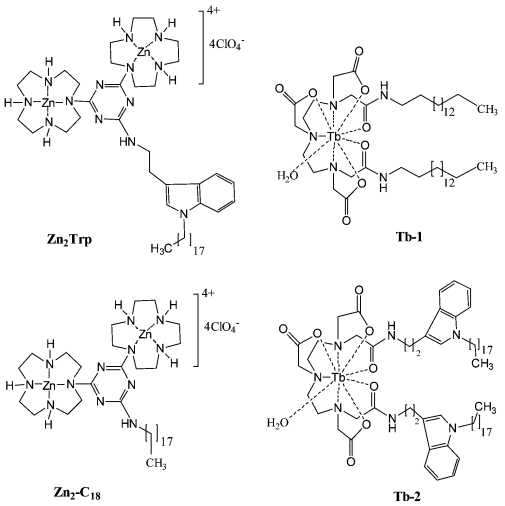


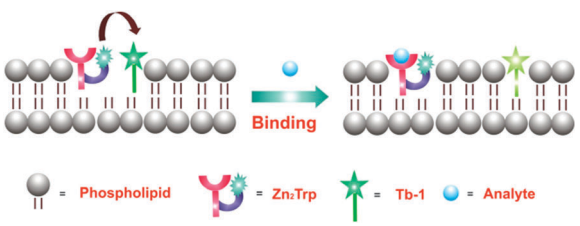
Fig. 1 Amphiphilic Tb(III) and Zn(II)–cyclen complexes.

of the Tb(III) excited state by the OH vibrations[8a] of H_2O molecules at the interface. We also synthesized an amphiphilic receptor–sensitizer conjugate in which tryptamine is covalently attached to a binuclear zinc cyclen headgroup (**Zn_2Trp**, Fig. 1, see ESI† for the synthesis). The indole chromophore in tryptophan is an efficient sensitizer of Tb(III) and has been previously employed by others[16] and us.[17] Bis-zinc cyclen complexes are chosen as they are well-known strong phosphate ion binders even in aqueous media.[18,19]

Complex **Tb-1** embedded DSPC vesicles (in 25 mM HEPES buffer, pH = 7.4, see ESI† for the preparation of vesicles) exhibited the characteristic emission bands (λ_{ex} = 285 nm) of Tb(III) at 490, 544, 584 and 621 nm,[8a] but with very low intensities. The intensities of these bands, however, increased significantly when **Zn_2Trp** was co-embedded in the vesicles (Fig. S5, ESI†). The Tb(III) intensity (for a fixed concentration of **Tb-1**) was found to depend on the ratio of these two components and ratios close to 1 : 1 gave the maximum initial intensity. When phosphate analytes such as UTP, GTP, ATP, PP_i, pSer, ADP and AMP were added to the **Zn_2Trp/Tb-1** (1 : 1) co-embedded vesicles, a gradual decrease in the Tb(III) intensity was observed. Presumably, co-embedded **Zn_2Trp** and **Tb-1** initially form self-assembled mixed patches, bringing Tb(III) ions in close proximity to the sensitizer. The analyte addition, thereafter, disrupts the patches causing an increase in their mutual separation and a concomitant decrease in the Tb(III) emission (Scheme 1).[20] The response towards analytes depends critically on the ratio of **Zn_2Trp** and **Tb-1**; with ratios close to 1 : 1 giving the best response. This highlights the need for a particular composition in the patches as observed previously for our fluorescent vesicles.[5] Vesicles with the following composition were chosen for the studies: lipid (1.6×10^{-4} M)/**Zn_2Trp** (2×10^{-5} M)/**Tb-1**(2×10^{-5} M).[21]

All lipid bilayers undergo a phase transition at a particular temperature (T_m) from the gel phase to a more fluidic liquid crystalline phase.[22] In the present context, the membrane fluidity influences two important factors related to the sensory response of the vesicles: the assembly–disassembly of the vesicular components[23] and the extent of vibrational relaxation (a major non-radiative deactivation pathway of Ln(III) excited states)[8a] of the embedded Tb(III). Our recent studies with another amphiphilic complex, **Tb-2** (Fig. 1), embedded in the vesicles of different phospholipids, clearly demonstrated this fact.[17] For example, at ambient temperatures, DOPC (1,2-dioleoyl-*sn-glycero*-3-phosphocholine, T_m = −20 °C, liquid crystalline)[22b] vesicles exhibited significantly lower emission intensity for **Tb-2** than DSPC (T_m = 54 °C, gel phase)[22a] vesicles, an outcome of the higher vibrational relaxation of Tb(III) in DOPC. Hence, an optimum value of fluidity is required to provide a fine balance between the analyte induced disassembly of the mixed patches and the vibrational deactivation of the Tb(III) luminescence.

DPPC (1,2-dipalmitoyl-*sn-glycero*-3-phosphocholine) and DMPC (1,2-dimyristoyl-*sn-glycero*-3-phosphocholine) possess lower T_m values[22c] than DSPC due to their shorter C16 and C14 chains. DMPC (T_m = 23 °C) vesicles, already at a fluidic phase at 25 °C, showed a significantly lower intensity than DSPC suggesting a much higher vibrational deactivation of the Tb(III) excited state. Analyte addition to these vesicles generated very poor response.[24] On the other hand, DPPC (T_m = 41 °C) vesicles exhibited comparable intensities but a better response than those of DSPC.[25] Therefore, they were chosen for the binding studies at 25 °C. The decrease in Tb(III) intensity was plotted against the concentration of the added analytes and fitted using the Hill equation. The binding constants were dependent on the charge as well as the steric demands of the phosphate anions.[26] The nucleotide triphosphates UTP (Fig. 2), ATP and GTP along with PP_i showed the highest binding affinities (Table 1). Nucleotide mono and di-phosphates, ADP and AMP, showed binding affinities that are roughly one and two orders lower than that of ATP, respectively. A phosphorylated protein, α-casein, also could be sensed using DSPC/DSPE-PEG 350 (1 : 1) vesicles (see ESI†). The addition of other monovalent and divalent anions such as Cl^-, Br^-, OAc^-, CO_3^{2-} and SO_4^{2-} resulted in only minor changes in the Tb(III) emission (see Fig. S9 and S10, ESI,† for Cl^- and CO_3^{2-}), thus demonstrating the specificity of these vesicles against phosphates.



Scheme 1 Principle of phosphorescence response from Tb(III) embedded vesicles upon analyte addition.

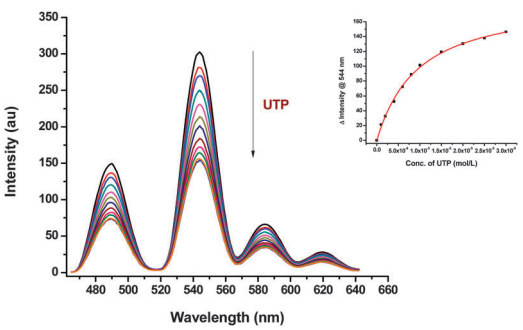


Fig. 2 Emission titration of DPPC vesicles *vs.* UTP, ([DPPC] = 1.6×10^{-4} M), ([**Zn_2Trp**] = [**Tb-1**] = 2×10^{-5} M); deviation of intensities < ±10% from 3 indep. titrations; inset: Hill plot.

Table 1 Apparent binding constants of the **Zn_2Trp** receptor embedded in vesicles

Lipid (temperature)	Analyte	Binding constant (lgK)[a]
1. DPPC (25 °C)	UTP	5.3
	ATP	5.2
	GTP	5.0
	PP_i	5.1
	ADP	4.2
	pSer	4.0
	AMP	3.4
2. DMPC (15 °C)	UTP	5.2
	ATP	5.1
3. DSPC (37 °C)	UTP	5.0

[a] Error in determining the binding constant values is ±0.2.

The reversibility of the coordinative bonds between phosphates and zinc–cyclen allowed the separation of the vesicles from the bound analytes using size exclusion chromatography (see ESI† for details) and the vesicles were reused for emission titrations with very similar binding constants (Fig. S21, ESI†).

The different sensory responses of three lipid vesicles (DSPC, DPPC and DMPC) at ambient temperatures suggested that it would be possible to modulate their sensitivity by temperature. Indeed, when the temperature was decreased to 15 °C, DMPC vesicles exhibited a significant increase in the embedded Tb(III) intensity (Fig. S8, ESI†) and more importantly, a much higher sensitivity towards analytes. In a similar way, DSPC vesicles at 37 °C exhibited better response compared to that at 25 °C. The binding affinities obtained for UTP and ATP at 15 °C in DMPC vesicles or for UTP at 37 °C in DSPC vesicles were comparable to those obtained with DPPC at 25 °C (Table 1).

Tb-1, a neutral complex, is not expected to have appreciable binding interactions with the analytes. Such interactions might lead to an increase in Tb(III) emission through elimination of H_2O molecule(s) from its co-ordination sphere[8a,b] and complicate the signalling event. To verify this, DPPC vesicles (at 25 °C) were co-embedded with only **Tb-2**, possessing an identical neutral headgroup to **Tb-1**, but with the tagged sensitizer. Upon phosphate addition to them, only minor variations in the Tb(III) intensity could be observed (Fig. S11, ESI†), suggesting a rather weak interaction between the neutral Tb(III) and the analytes. Even the vesicles co-embedded with **Zn_2–C_{18}** (Fig. 1)[5] and **Tb-2** did not show any significant change upon analyte addition (Fig. S12, ESI†). It is well-known that the Ln(III) luminescence is almost independent of the chemical environment due to the shielding of 4f orbitals by 5s and 5p closed shells.[8] Thus the expulsion of **Tb-2**, from the pre-formed mixed patches following analyte binding to **Zn_2–C_{18}**, does not lead to any significant variation in Tb(III) intensity as the Ln(III)–sensitizer distance remains unaltered.

In conclusion, we have reported a new approach for the detection of phosphates in aqueous media (pH = 7.4) by Tb(III) embedded lipid vesicles. Analyte binding induces a higher mutual separation of the embedded Tb(III)–sensitizer pair, resulting in a ''switch-off'' sensor. The sensitivity is modulated by the membrane fluidity and can be adapted to any desired temperature range. The modular nature of the strategy allows the design of chemosensors for other analytes by choosing the appropriate amphiphilic receptors.

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