Fluorescent PET (Photoinduced Electron Transfer) Sensors with Targeting/Anchoring Modules as Molecular Versions of Submarine Periscopes for Mapping Membrane-bounded Protons

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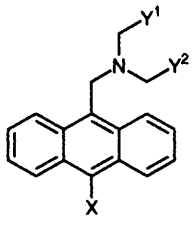
The two families of fluorescent PET (photoinduced electron transfer) sensors (**1–9**) show that the effective proton density near the surface of several micelle membranes changes over 2–3 orders of magnitude as the microlocation of the sensor (with respect to the membrane) is altered *via* hydrophobic tuning.

Ion gradients near membranes play a vital role in biological energy conversion[1] and signal processing[1–3] and chemical catalysis.[4–6] We now demonstrate that these ion densities can be mapped (for protons near detergent micelles in the first instance) by employing two families of fluorescent PET sensors with the 'fluorophore–spacer–receptor' format[7,8] with two additional targeting/anchoring moieties of varying hydrophobicity (Fig. 1). These sensors can be anchored at the membrane/water interface, with the receptor module protruding into the water to an extent which is determined by the hydrophobicity of the targeting head and tail groups.[9] This allows the production of a fluorescence signal from each member of the sensor families after sampling the proton density near its receptor unit at microlocations of gradually varying polarities on a scale between bulk water and the less polar micelle interior† in terms of the Hansch hydrophobicity constant for the targeting group.[10] The proton receptor module of each sensor behaves like a periscope on a submarine. Fluorescent PET sensors are particularly suited to such applications since they measure thermodynamically valid ground-state ion-binding constants even during excited-state experiments of short time duration with high sensitivity of signal detection.[8,11] While fluorescent and absorptiometric sensors have long been used to study membrane-bounded protons,[4,13,14] the fine tuning of sensor location to shed light on the spatial distribution of protons near the micelle surface is virtually unknown. Quina[15a] and Kraayenhof[15b] reported preliminary experiments aimed towards this goal, but these differ considerably in approach from our work. Since the fluorescent PET sensor principle is quite general,[8,16] it should also be possible to visualize other ion gradients near membranes by the present strategy. Anchoring, targeting and steering/orienting of molecular units in ion gradients can also be useful for the improvement of ion input–photon output logic devices.[17]

Aminomethyl anthracenes are established as fluorescent PET sensors for protons.[8,16,18] Protonation of the amino group blocks an intramolecular PET channel, resulting in the 'switching on' of fluorescence. The members of the two families **1–4** and **5**, **2**, **6–9**‡ are based on this core structure with a set of additional units of graded hydrophobicity at the head (amine) and tail (anthracene 10-position), respectively. The intrinsic hydrophobicity of the anthracene fluorophore would also contribute to the retention of these sensors in the micelle phase. The fluorescence of these sensors was examined as a function of pH (a space-averaged value as measured with a macroscopic glass electrode) in three representative micelle media of different charge types. Owing to their molecular nature, the sensors will respond to the local pH at their receptor sites and this information will be reflected in their pK_a values. Of all the electronic absorption and fluorescence emission spectral properties, the fluorescence intensity (or quantum yield ϕ_F) is unique in showing drastic changes and these are well fitted by the equation:[18]

$$\log[(\phi_{F,max} - \phi_F)/(\phi_F - \phi_{F,min})] = pH - pK_a \quad (1)$$

Note that the polarities of micellar microenvironments of aromatic probes[13] are not low enough to 'switch on' the fluorescence of the present sensors in the absence of protonation to any significant extent except where noted otherwise. We have previously shown that extremely low polarities are needed to 'switch on' the fluorescence of dialkylaminomethyl anthracenes.[19] The pK_as of the sensors in Table 1 can be interpreted once we note and allow for the fact that the targeting/anchoring substituents at the head and tail will also influence their intrinsic acid/base properties. The intrinsic acidity/basicity of the sensors ($pK_{a,intrins}$ values) were obtained as their pK_a values (or those of more soluble model compounds as noted in Table 1) in water with 20% (v/v) methanol to prevent aggregation. The microenvironmental effect on the protons neighbouring the receptor module of a given sensor is then given by ΔpK_a, the difference between the pK_a value for that sensor in a chosen micelle medium and its $pK_{a,intrins}$ value.[11] As the receptor module of each sensor will be anchored at different locations relative to the micelle surface, ΔpK_a should be a smooth function of the hydrophob-



1 X = n-C_8H_{17}; $Y^1 = Y^2 = CH_3$
2 X = n-C_8H_{17}; Y^1–$Y^2 = CH_2OCH_2$
3 X = n-C_8H_{17}; $Y^1 = Y^2 = CH_2OH$
4 X = n-C_8H_{17}; $Y^1 = Y^2 = CO_2^-$
5 X = n-$C_{18}H_{37}$; Y^1–$Y^2 = CH_2OCH_2$
6 X = Cl; Y^1–$Y^2 = CH_2OCH_2$
7 X = H; Y^1–$Y^2 = CH_2OCH_2$
8 X = SO_3^-; Y^1–$Y^2 = CH_2OCH_2$
9 X = $CH_2NH^+(CH_2CH_2)_2O$; Y^1–$Y^2 = CH_2OCH_2$
10 X = H; $Y^1 = Y^2 = CH_3$
11 X = H; $Y^1 = Y^2 = CH_2OH$
12 X = H; $Y^1 = Y^2 = CO_2^-$

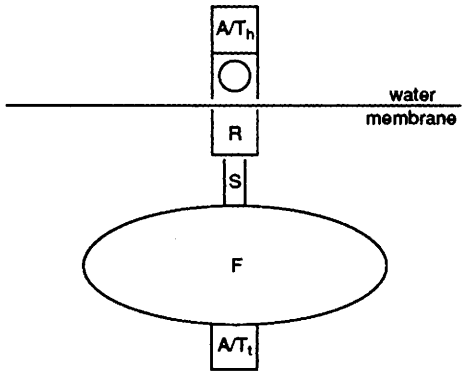


Fig. 1 Schematic representation of a periscopic ion sensor near a membrane/water interface. F, Fluorophore; S, spacer; R, receptor; A/T_h, head group for anchoring/targeting and A/T_t, tail group for anchoring/targeting.

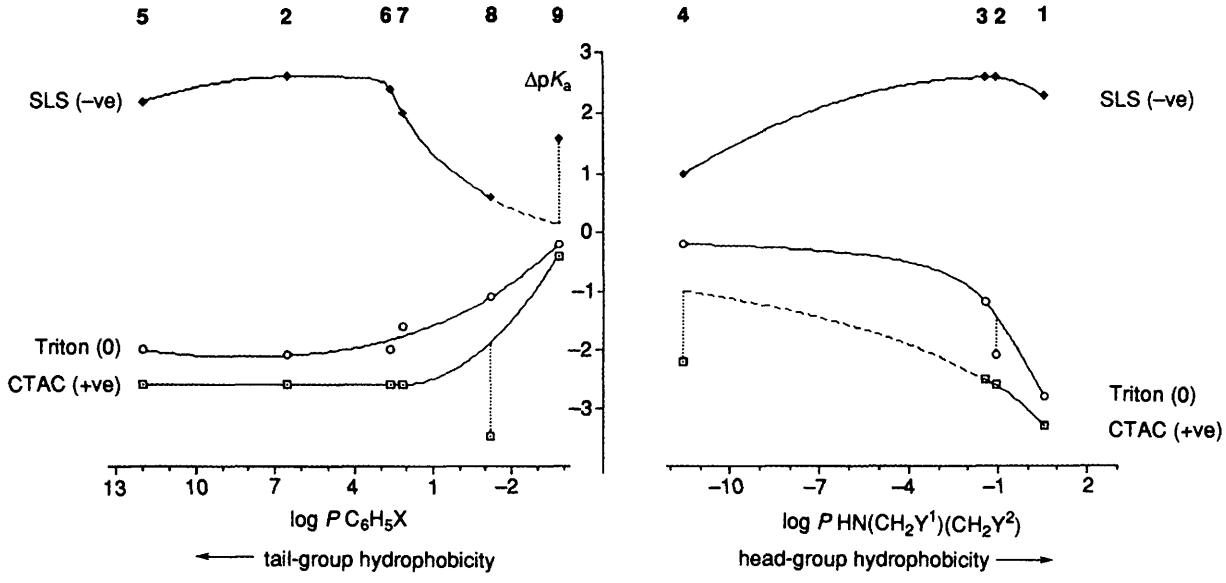


Fig. 2 ΔpK_a–log P profiles for **1–9** in various micellar media

Table 1 Microenvironmental effects on the fluorescence and acidity properties of the periscopic sensors **1–9**[a]

				ΔpK_a[c]			$\varphi_{F,max}$[e]		
Sensor	$\log P_{HN(CH_2Y^1)(CH_2Y^2)}$[b]	$\log P_{C_6H_5X}$[b]	$pK_{a,intrins}$[c,d]	CTAC	Triton X-100	SLS	CTAC	Triton X-100	SLS
1	+0.53		9.2	−3.3	−2.8	+2.3	0.47	0.46	0.45
2	−1.08	+6.57	6.3	−2.6	−2.1	+2.6	0.43	0.41	0.41
3	−1.43		7.1	−2.5	−1.2	+2.6	0.42	0.36	0.34
4	−11.57[f]		8.9[g]	−2.2	−0.2	+1.0	0.41	0.36	0.40
5		+11.97[f]	6.3	−2.6	−2.0	+2.2	0.34	0.41	0.37
6		+2.57[f]	6.0	−2.6	−2.0	+2.4	0.36	0.21	0.34
7		+2.10	6.3	−2.6	−1.6	+2.0	0.59	0.59	0.58
8		−1.2[h]	6.6	−3.5	−1.1	+0.6	0.68	0.75	0.88
9		−3.80[f]	5.1[i]	−0.4	−0.2	+1.6	0.60	0.52	0.45

[a] 10^{-6} mol dm^{-3} sensors and model compounds were used in aerated solutions of H_2O–CH_3OH (4:1, *v/v*) (non-micellar medium), 5.0×10^{-3} mol dm^{-3} hexadecyl trimethylammonium chloride (CTAC, cationic micellar medium), 5.2×10^{-4} mol dm^{-3} polyoxyethylene (E 9-10) octyl phenol (Triton X-100, neutral micellar medium) and 2.0×10^{-1} mol dm^{-3} sodium dodecyl sulfate (SLS, anionic micellar medium) in water. The c.m.c.s. of CTAC, Triton X-100 and SLS are 1.4×10^{-3}, 2.6×10^{-4} and 8.0×10^{-3} mol dm^{-3}, respectively.[13] In the case of **4** and **12**, 2×10^{-4} mol dm^{-3} EDTA was present during fluorescence titrations in order to avoid effects due to trace metal ions. [b] Logarithm of the partition coefficient between octan-1-ol and water.[10] [c] I_F–pH data obtained from fluorescence titrations gave good linear least-squares fits to eqn. (1) with gradient = 0.88–1.15 and average r = 0.99 (average n = 10). The pK_a values are reproducible within ±0.1 pH unit. The only unusually large gradients are encountered with **9** and suggest non- unity activity coefficients. These can arise from the interaction between the dicationic form of **9** and cationic micelles. The bis ether **9** can engage in hydrogen bonding with the terminal hydroxy groups of Triton X-100. It is notable that **9** possesses two suitable interaction sites for each of these cases. This non-ideal behaviour may increase the uncertainty of the corresponding pK_a. Similar non-ideal behaviour has been noted during the binding of α,ω alkane diammonium cations with bis crown ethers.[22] [d] **1–5** could not be employed here owing to their high hydrophobicity and poor solubility. Instead, the corresponding 9-anthracenyl methyl amines (**10**, **7**, **11**, **12** and **7**, respectively) were used as reasonable models to obtain pK_a values. [e] Obtained by comparison of quantum corrected emission spectra with that of 9,10 diphenyl anthracene in CH_3OH.[23] For these sensors, the spectra position and shape is essentially constant and the determination of ϕ_F values is therefore simplified. [f] Calculated value.[10] [g] pK_{a3} value of this tribasic system. [h] Estimated from values in diethyl ether/water, isobutyl alcohol/water and relationships between these solvents and octan-1-ol.[24] [i] The fluorescence of this dibasic probe is 'switched on' only when both nitrogen atoms are protonated, so fluorescence titrations measure pK_{a1} only.

icity (log P) of the targeting unit. Such Gibbs energy relationships with hydrophobic Gibbs energy rather than distance as a coordinate are graphic maps of membrane-bounded proton densities. The absolute distance from the amine receptor to the dynamic and convoluted micelle surface is much less accessible.

Owing to their relatively low polarity, membraneous microenvironments are less likely to solvate protonated amine receptors. Thus protons are effectively less available at such locations of the sensors, resulting in more negative ΔpK_a values. Fig. 2 confirms that the effective proton densities near neutral Triton X-100 micelles decrease as the interface is approached by the amine receptor units of the sensors which are driven by the increasing hydrophobicity of the targeting groups. Additionally any surface charge on the membrane, depending on its sign (negative or positive) and magnitude, will electrostatically concentrate or deplete protons causing more positive or more negative ΔpK_a values, respectively. The ΔpK_a–log P function for Triton X-100 is nicely bracketed by those for the SLS and CTAC media. Fig. 2 also shows that the nett effect of these dielectric and charge influences decrease in magnitude to near zero as the location of the amine receptor is gradually shifted towards bulk water and away from the micellar interface, confirming the periscope action of the sensors. It is notable that a range of tail-group hydrophobicities lead to virtually constant ΔpK_a values in a

given micelle medium. At high enough hydrophobicities, the distal tail group has little influence on the microlocation of the receptor module in the ion and polarity gradient at the micelle/water interface.

The several outliers on the ΔpK_a–log P curves deserve comment. They are **9**–SLS, **8**–CTAC, **4**–CTAC and **2**–Triton sensor–micelle pairs and all display an apparent enhanced hydrophobicity due to binding interactions with the micelle head groups. The deviation of the first three pairs can be attributed to ion pairing[20] and the fourth can be ascribed to hydrogen bonding of the morpholino oxygen atom of **2** with the hydroxy terminii of Triton head groups.

$\Phi_{F,max}$ for each sensor is expected to be approximately constant in each micellar medium and this is generally found to be the case. Some of the sensor–micelle pairs where some quenching is seen correspond to those where deviations in ΔpK_a were found, *e.g.* **9**–SLS and **8**–CTAC. The case of **6**–Triton is unique as the 10-chloroanthracenyl 9-methylammonium moiety, the most electron-deficient system employed in this study, suffers a donor–acceptor interaction from the phenoxy moieties of the micelle head groups. $\phi_{F,min}$ values are uniformly low in all cases, as expected for efficient fluorescent PET sensors in microenvironments of sufficiently high polarity. Two exceptions to this generalization are **2** and **5** in Triton X-100 micelles and can be attributed to the hydrogen-bonding interaction mentioned above in connection with the deviation of ΔpK_a values. This indirectly increases the already high oxidation potential of the morpholino nitrogen atom,[21] causing a retardation of the PET process which originally suppressed the fluorescence.

In conclusion, ΔpK_a–log P profiles of periscopic pH sensors provide a window on the distribution of protons near micelle surfaces. Extension of this strategy to other ions and membranes of biological significance should follow.

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Footnotes

† See ref. 5 for an overview of theoretical models of micelles with varying degrees of water penetration.

‡ The syntheses are straightforward and will be detailed in the full paper.

References

1 F. M. Harold, *The Vital Force—A Study of Bioenergetics*, Freeman, New York, 1986.

2 S. McLaughlin, *Annu. Rev. Biophys. Biophys. Chem.*, 1989, **18**, 113.

3 Z. W. Hall, *An Introduction to Molecular Neurobiology*, Sinauer, Sunderland, MA, 1992.

4 E. J. Fendler and J. H. Fendler, *Catalysis in Micellar and Macromolecular Systems*, Academic Press, New York, 1975.

5 J. H. Fendler, *Membrane Mimetic Chemistry*, Wiley, New York, 1982.

6 C. A. Bunnett, F. Nome, F. H. Quina and L. S. Romsted, *Acc. Chem. Res.*, 1991, **24**, 357.

7 A. J. Bryan, A. P. de Silva, S. A. de Silva, R. A. D. D. Rupasinghe and K. R. A. S. Sandanayake, *Biosensors*, 1989, **4**, 169.

8 R. A. Bissell, A. P. de Silva, H. Q. N. Gunaratne, P. L. M. Lynch, G. E. M. Maguire and K. R. A. S. Sandanayake, *Chem. Soc. Rev.*, 1992, **21**, 187.

9 M. J. Minch and S. S. Shah, *J. Org. Chem.*, 1979, **44**, 3252 examine micellar effects on solvatochromic dyes with various hydrocarbon chains. J. P. Otruba and D. G. Whitten, *J. Am. Chem. Soc.*, 1983, **105**, 6503; K. S. Schanze, D. M. Shin, J. P. Otruba, P. E. Brown and D. G. Whitten, *Isr. J. Chem.*, 1987/88, **28**, 37; T. Miyashita, T. Murakata and M. Matsuda, *J. Phys. Chem.*, 1989, **93**, 1426. These explicitly consider hydrophobicity control of probe microlocation in micelles with respect to charge-transfer complexation and surface diffusion but not concerning ion sensing.

10 C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979. See also: A. J. Leo, *Chem. Rev.*, 1993, **93**, 1281.

11 M. S. Fernandez and P. Fromherz, *J. Phys. Chem.*, 1977, **81**, 1755 exemplifies the use of conventional fluorescent pH sensors (ref. 12) under restricted wavelength conditions to achieve this end.

12 G. Kirkbright, in *Indicators*, ed. E. Bishop, Pergamon Press, Oxford, 1972, ch. 9.

13 K. Kalyanasundaram, *Photochemistry in Microheterogeneous Systems*, Academic Press, Orlando, 1987.

14 J. K. Thomas, *The Chemistry of Excitation at Interfaces*, ACS Monograph 181, American Chemical Society, Washington DC, 1984; F. Grieser and C. J. Drummond, *J. Phys. Chem.*, 1988, **92**, 5580.

15 (*a*) F. H. Quina, R. M. S. G. Naal, E. O. Alonso, J. B. S. Bonilha and A. C. Tedesco, *Abstr. IUPAC Symp. Photochem.*, 1992, **14**, 66 and (*b*) R. Kraayenhof, G. J. Sterk, H. S. Walraven, F. A. de Wolf, K. Krab and H. W. W. F. Sang, in *Fluorescent Biomolecules: Methodologies and Applications*, ed. D. M. Jameson and G. D. Rinehart, Plenum Press, New York, 1989, p. 365 employ a photochemically or photophysically active unit held at varying distances into the aqueous phase by means of hydrophilic spacers of various lengths attached to a hydrophobic unit anchored in the membrane.

16 R. A. Bissell, A. P. de Silva, H. Q. N. Gunaratne, P. L. M. Lynch, G. E. M. Maguire, C. P. McCoy and K. R. A. S. Sandanayake, *Top. Curr. Chem.*, 1993, **168**, 223.

17 A. P. de Silva, H. Q. N. Gunaratne and C. P. McCoy, *Nature (London)*, 1993, **364**, 42.

18 R. A. Bissell, A. P. de Silva, S. A. de Silva, H. Q. N. Gunaratne, J. L. Habib-Jiwan, S. L. A. Peiris, R. A. D. D. Rupasinghe, T. K. S. D. Samarasinghe, K. R. A. S. Sandanayake and J.-P. Soumillion, *J. Chem. Soc., Perkin Trans. 2*, 1992, 1559.

19 R. A. Bissell, A. P. de Silva, W. T. M. L. Fernando, S. T. Patuwathavithana and T. K. S. D. Samarasinghe, *Tetrahedron Lett.*, 1991, **32**, 425.

20 M. J. Minch, J. P. Sevenair and C. Henling, *J. Org. Chem.*, 1979, **44**, 3247; F. Diederich, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 362; C. J. Drummond, F. Grieser and T. W. Healy, *J. Chem. Soc., Faraday Trans. 1*, 1989, **85**, 536.

21 For photoelectrochemical evidence, see: R. Grigg and W. D. J. A. Norbert, *J. Chem. Soc., Chem. Commun.*, 1992, 1298. See also: C. K. Mann and K. K. Barnes, *Electrochemical Reactions in Nonaqueous Systems*, Dekker, New York, 1970.

22 A. P. de Silva and K. R. A. S. Sandanayake, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 1173.

23 A. Maciejewski and R. P. Steer, *J. Photochem.*, 1986, **35**, 59; I. H. Leaver and D. E. Rivett, *Mol. Photochem.*, 1974, **6**, 113.

24 R. Collander, *Acta Chem. Scand.*, 1949, **3**, 717; 1950, **4**, 1085; 1951, **5**, 774.