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Bay functionalized perylenediimide as a deaggregation based intracellular fluorescent probe for perchlorate†

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The aggregates of perylenediimide based chemosensor (PDI 1) undergo de-aggregation induced fluorescence quenching selectively with ClO_4^- ions both in the solution and in the solid phase and can detect ClO_4^- ions in drinking water and fireworks. PDI 1 is permeable to C6 glioma cells, and ClO_4^- can be detected using confocal microscopy.

For the last few years, research has focused on developing chromo-fluorescent sensors as a safeguard for global ecosystems, and for monitoring of toxic anions and carcinogenic metal ions that impose serious human and environmental health hazards.[1,2] Perylenediimides (PDI) are intensively coloured dyes with high molar extinction coefficients, fluorescence quantum yields, unique light harvesting and redox properties and exceptional thermal, chemical and photochemical stability.[3] Although literature reports have considered PDIs as promising materials for various opto-electronic devices,[4,5] we have witnessed slow growth in considering PDIs as chromo-fluorescent probes for sensing applications. Probably their poor solubility in aqueous media is one of the most detrimental factors for developing molecular recognition receptors. So far, only a few reports on PDI based fluorescent probes for cations[6–10] and anions[6,11,12] have been reported and none of these were for perchlorate ions (ClO_4^-).

Perchlorates find extensive use as oxidants in solid rocket fuel, fireworks and pyrotechnic devices, flares, electrolyte in lithium batteries, gas generator, pharmaceutical for hyper-thyroid and chemistry reagents. In nature, perchlorates may be formed by photochemical transformation reactions involving chlorine precursors and volcanic eruptions.[13] Perchlorates, due to high water solubility, may be transported from soil to the surface or ground water and then become potential health concern because perchlorates can impair proper functioning of the thyroid gland.[14]

Current strategies for ClO_4^- detection require long processing times, expensive instruments, and in many cases the techniques have poor selectivity and high detection limits.[15,16] Recently, we have shown that *N*-aryl benzimidazolium moieties linked through an appropriate spacer can be used for selective detection of ClO_4^- ions.[17]

Herein we report a PDI based chemosensor, PDI **1**, which undergoes fluorescence quenching with only ClO_4^- ions in HEPES buffer (10% DMSO) (v/v) (pH 7.4) with a minimum limit of detection of 60 nM. PDI **1** was also evaluated for the detection of ClO_4^- in drinking water and fireworks. In solid phase, TLC strips doped with PDI **1** can detect 0.8 ng cm^{-2} of ClO_4^- under UV-illumination. The cell uptake studies of PDI **1** with live rat C6 glioma cells show permeability of PDI **1** into the cytoplasm followed by quenching of fluorescence with ClO_4^- ions in a concentration-dependent manner.

PDI **1** was synthesized by nucleophilic substitution of PDI **3** with hydroxyphenylbenzimidazole followed by heating of PDI **2** and 1-bromobutane mixture in DMF:CH_3CN at 100 °C (Scheme 1). (See ESI† for synthesis details and structural characterization)

Before examining the solution properties of PDI **1** towards different anions, the aggregation behaviour of PDI **1** in DMSO and a binary mixture of DMSO:H_2O (v/v) was investigated. The concentration-dependent UV-Vis and emission experiments (1×10^{-3} M to 1×10^{-6} M) show a minor propensity of PDI **1** for aggregation in DMSO with an A_{0-0}/A_{0-1} ratio greater than 1.4 (Fig. 1a). The solvent-dependent UV-Vis and emission experiments with different DMSO–H_2O compositions (starting from pure DMSO and increasing the volume fraction of H_2O) at a fixed concentration of 10 μM show that at low volume fraction of water [DMSO : H_2O 8 : 2 (v/v)], the absorption spectrum exhibits well-resolved vibronic band for A_{0-0} at 526 nm, whereas the absorption band for A_{0-1} at 500 nm was poorly resolved. The value of the ratio A_{0-0}/A_{0-1} drastically decreased from 1.4 to 0.7 as

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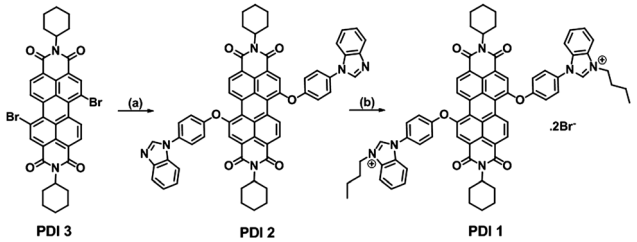
† Electronic supplementary information (ESI) available: Experimental details, additional photophysical, spectroscopic and microscopic characterization of PDI **1** and PDI **1**⊂ClO_4^- complex. See DOI: 10.1039/c4cc06765g



Scheme 1 Synthesis of PDI **1**; *reagents and conditions*: (a) NMP, K_2CO_3, (**7**), 80 °C, 8 h; (b) 1-bromobutane, DMF:CH_3CN, 100 °C, 48 h.

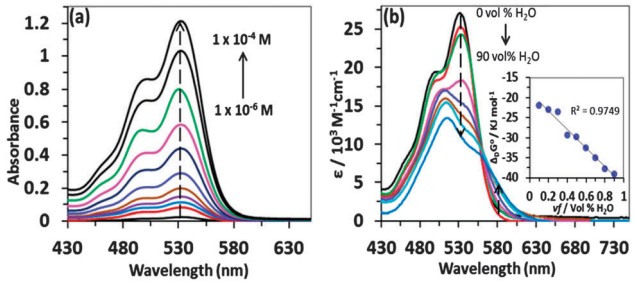


Fig. 1 (a) Concentration-dependent UV-Vis absorption spectra of PDI **1** (10^{-6} to 10^{-4} M) in pure DMSO; (b) solvent-dependent UV-Vis absorption changes of PDI **1** after incremental addition of 10 vol% of H_2O in DMSO at a concentration of 10 μM. Inset: correlation plot of ΔG° values against vf (volume fraction) of H_2O.

the percentage of H_2O increased to 90% (Fig. 1b) (see Fig. S1, ESI†). Fig. 1b also shows the appearance of new bathochromically shifted absorption band at 580 nm with isosbestic point at 553 nm, thus showing a transition from the molecularly dissolved state to the H-type excitonically coupled aggregates.[18] The correlation plot of ΔG° *versus* H_2O content in ratios from 0 to 0.9 shows a linear relationship (Fig. 1b inset). The fluorescence intensity of PDI **1** remained unaffected between pH 8.0 and 4.5 (see Fig. S2, ESI†) and allowed for the selection of HEPES buffer–10% DMSO (v/v) (pH 7.4) solutions for examining its anion sensing ability, where PDI **1** exists in an aggregated state.

The aggregation of PDI **1** in DMSO–H_2O (1 : 9) is further confirmed by SEM and DLS experiments. The SEM images of thin films of PDI **1** reveal the formation of aggregates with an average diameter of 0.8–1.5 μm and are consistent with the 1–1.2 μm size of aggregates observed in DLS experiments (Fig. 2a and c). In wide angle X-ray diffraction (WXRD) of PDI **1**, the presence of a broad peak around $2\theta \approx 18.25^\circ$ (d = 4.77 Å) points to loose packing of chains at the 1,7-positions, and peaks around $2\theta = 25.64^\circ$ (d = 3.47 Å) and $2\theta = 26.62^\circ$ (d = 3.34 Å) indicate the π–π stacking between adjacent perylene rings.

The UV-Vis spectrum of PDI **1** (10 μM), recorded in HEPES buffer–10% DMSO (v/v), shows λ_{max} at 509 nm, whereas the emission spectrum of **1** (10 μM), on excitation at 500 nm, displays emission band at λ_{max} 577 nm. The UV-Vis spectrum of PDI **1** shows insignificant change in the absorption spectrum upon addition of anions *viz.* F^-, Cl^-, I^-, CN^-, OH^-, AcO^-, ClO_4^-, CO_3^{2-}, $H_2PO_4^-$, NO_3^{2-}, SO_4^{2-} (see Fig. S3, ESI†). The emission spectrum of PDI **1** shows >90% quenching of the

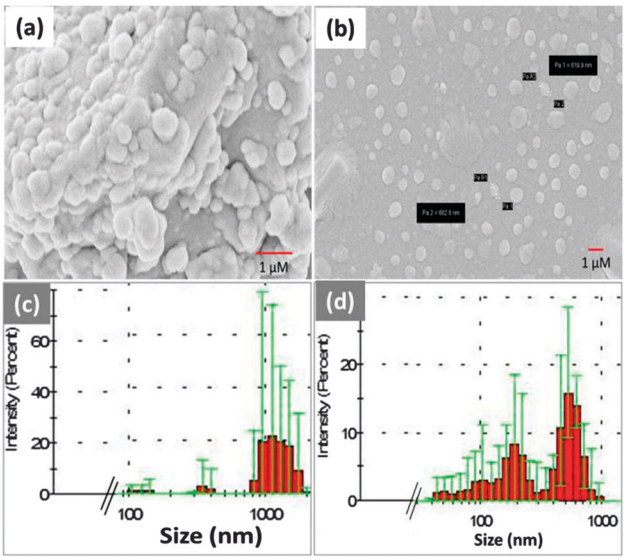


Fig. 2 SEM micrograph (a and b) and DLS bar graph (c and d) of 5 μM (90% aqueous DMSO solution) of PDI **1** alone and after addition of perchlorate ions (40 μM).

emission intensity upon addition of ClO_4^- (Fig. 3a), whereas the addition of all other anions (100 μM) causes insignificant change in its emission spectrum.

The fluorescence intensity of PDI **1** at 577 nm gradually decreases as the concentration of ClO_4^- is increased from 0–70 μM and then it achieves a plateau. The Stern–Volmer plot follows the linear relation up to the concentration range of 0–30 μM (Fig. 3b, inset). The Stern–Volmer constant (K_{sv}) is 1.65×10^5 M^{-1} and the quenching rate constant (K_q) of PDI **1** for ClO_4^- is 3.8×10^{13} M^{-1} s^{-1}. The minimum limit of detection[19] for ClO_4^- is 60 nM. The Job's plot shows inflection at the mole fraction of 0.5 with a symmetrical peak shape and suggests the formation of a PDI **1** : ClO_4^- (1 : 1) complex. The association constant (K) of PDI **1** with ClO_4^- as determined by the Benesi–Hildebrand plot is 0.56×10^5 M^{-1}. The HRMS spectrum of 1 : 1 solution of PDI **1** and $NaClO_4$ shows a parent ion peak at m/z 1183.4296 (theor. 1183.44) and confirms the formation of a 1 : 1 complex (see Fig. S4, ESI†). We also observed that the fluorescence quenching efficiency of ClO_4^-

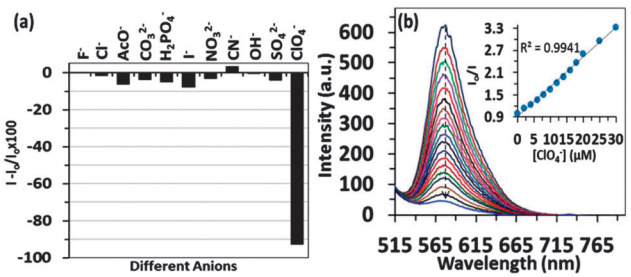


Fig. 3 (a) Fluorescence intensity changes of PDI **1** (10 μM) in the presence of various anions recorded in HEPES buffer (0.01 M, pH 7.4)–10% DMSO (v/v); (b) fluorescence spectra of PDI **1** (10 μM) upon addition of the ClO_4^- ions; excitation at 500 nm. Inset: Stern–Volmer plot at 577 nm corresponds to fluorescence titration.

was not significantly affected in the presence of other probable interfering anions (see Fig. S5, ESI†).

The DLS and SEM experiments show the de-aggregation of PDI **1** aggregates upon addition of ClO_4^- ions. The size of the aggregates is reduced from 1–1.2 μm size to mixture of 200 nm and 600–700 nm size aggregates as observed by both the techniques (Fig. 2). The SEM coupled energy dispersive X-ray spectroscopy (EDX) confirms the presence of N and Cl on the surface of the spherical structure PDI **1** + ClO_4^- complex (see Fig. S6, ESI†). The DLS experiments of the PDI **1** + ClO_4^- (1 : 4) complex also show the decrease in size of the aggregates from 1–1.2 μm to 200 and 600–700 nm (Fig. 2b and d).

Significantly, the fluorescence titration of PDI **1** with ClO_4^- in HEPES buffer–DMSO (1 : 1; v/v) (pH 7.4), *i.e.* in the non-aggregate state of PDI **1**, shows poor sensitivity towards ClO_4^- (see Fig. S7, ESI†). Therefore, the results of fluorescence studies are consistent with SEM and DLS experiments, showing that ClO_4^- mediated de-aggregation of PDI **1** is responsible for its fluorescence quenching.

The optimization of the 1 : 1 geometry of PDI **1** and ClO_4^- shows that the two C_2–H moieties of benzimidazolium groups and C–H group of phenylene ring form C–H···O interactions with the ClO_4^- anion with an H-bond distance between 2.04 Å and 2.32 Å and encapsulate the perchlorate ion perfectly in the cavity (Fig. 4). Upon addition of 3 equiv. of ClO_4^- ions to the solution of PDI **1**, the up-field shift of benzimidazolium C_2–H and N–CH_2 signals, respectively, by 0.2 and 0.07 ppm in its ^{1}H NMR spectrum (see Fig. S8, ESI†) also supports the binding of the ClO_4^- anion in the cavity of PDI **1**.

For the analytical applications of PDI **1**, we have also carried out analysis of tap water spiked with various concentrations of ClO_4^-. The concentrations thus determined by fluorescence spectroscopy agree with actual concentrations with a relative error of 2.7% or better and a RSD below 3.5% (Table S1, ESI†). The PDI **1** was also tested for detection of ClO_4^- in a sample solution prepared from flash powder of firework. The response towards ClO_4^- occurred immediately with the addition of 20 μL of sample solution to the solution of PDI **1** (10 μM, HEPES buffered–10% DMSO), and the concentration of ClO_4^- was found to be 3 μM from the standard calibration curve, leading to the total concentration of 3.7×10^{-4} M for firework sample solution (see Fig. S9, ESI†).

Contact mode analysis of ClO_4^- ions *via* TLC strips coated with PDI **1** was also studied under illumination of 365 nm UV light. We observed that the addition of 6 μL of 10^{-7} M ClO_4^- causes naked eye observable quenching of the fluorescence intensity of PDI **1**. However, complete quenching of fluorescence intensity was observed with 6 μL of 10^{-6} M ClO_4^-. PDI **1** shows no quenching of fluorescence with only water (as control). Addition of higher concentrations caused reddish colour area developed on the TLC strip itself (Fig. 5) and indicates the presence of excess ClO_4^-. Therefore, the minimum 0.8 ng cm^{-2} of ClO_4^- is detectable.

To test the biological application of PDI **1** to detect ClO_4^- in the live cells, glial cells of the rat brain (C6 glioma cells) have been used for cell imaging studies (Fig. 6). Brightfield image shows that the C6 glioma cells were healthy and viable throughout the experiment [Fig. 6(a)]. C6 glioma cells, themselves (without PDI **1**) and after incubation with ClO_4^- (40 μM) did not exhibit any fluorescence [Fig. 6(b) and (c)].

However, C6 glioma cells on incubation with PDI **1** (10 μM) for 30 min at 37 °C show bright green fluorescence in the

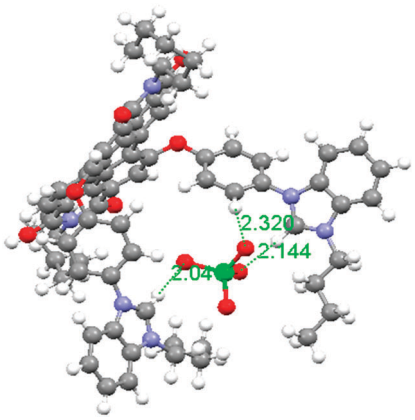


Fig. 4 The energy minimized structure (DFT 6-31G*) of PDI **1** + ClO_4^- complex.

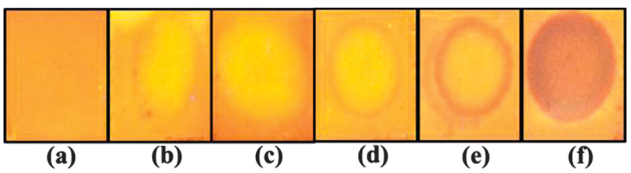


Fig. 5 Photographs of fluorescence quenching (under 365 nm UV light) of solution coated TLC strips of PDI **1** for the detection of ClO_4^-; (a) TLC strip with a drop of water; TLC strips on addition of 6 μL of different concentration of ClO_4^- (b) 10^{-7} M (c) 10^{-6} M (d) 10^{-5} M (e) 10^{-4} M (f) 10^{-3} M. [The size of each TLC strip is 1 cm^2.]

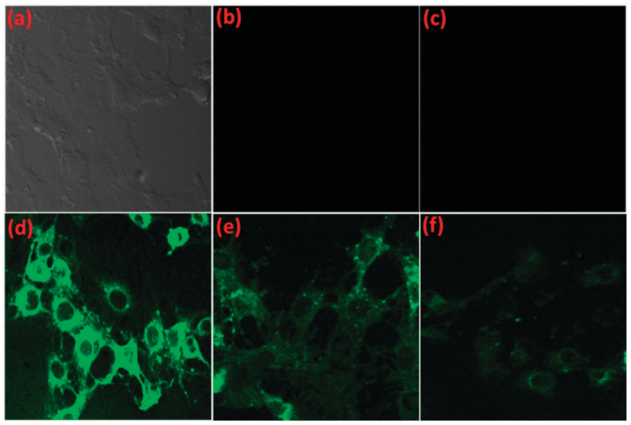


Fig. 6 Images of C6 glioma cells: (a) brightfield image of C6 glioma cells, (b) fluorescence image of C6 glioma cells without PDI **1**, (c) fluorescence image of C6 glioma cells incubated with ClO_4^- (40 μM) for 30 min, (d) fluorescence image of C6 glioma cells incubated with PDI **1** (10 μM) for 30 min (e) fluorescence image of C6 glioma cells incubated with PDI **1** (10 μM) for 30 min and then incubated with ClO_4^- (40 μM) for another 30 min and (f) fluorescence image of C6 glioma cells incubated with PDI **1** (10 μM) for 30 min and then incubation with ClO_4^- (80 μM) for another 30 min.

cytoplasmic region upon using 488 nm excitation laser [Fig. 6(d)]. This indicated that PDI **1** is permeable to C6 cells and can be used for bioimaging of ClO_4^- in live cells. When C6 glioma cells pretreated with PDI **1** were incubated with ClO_4^- (40 μM) for another 30 min, bright green fluorescence decreased [Fig. 6(e)]. The bright green fluorescence was further quenched when C6 Glioma cells pretreated with PDI **1** were incubated with a higher concentration of ClO_4^- ions (80 μM) [Fig. 6(f)]. MTT assay using PDI **1** shows no significant difference in the proliferation of the C6 glioma cells in the absence or presence (98% cell viability) of PDI **1** (see Fig. S10, ESI†).

In conclusion, PDI **1** could be useful for the detection of perchlorate ions in aqueous buffer, live rat C6 glioma cells, drinking water and firework flash powder in solution state. In addition, TLC strips doped with PDI **1** could detect ClO_4^- ions 0.8 ng cm^{-2}.

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