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ARTICLE TYPE

A *'turn-on'* coordination based detection of Pd^{2+}- Application in bioimaging

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A molecular probe derived by conjugating the metal ion binding dithia-dioxa-aza crown ether with BODIPY, a fluorescent signalling handle, detects Pd^{2+} with a detection limit (1.18 ppb) complying satisfactorily with the permissible concentration by WHO in drug chemicals. On the basis of DFT/TD-DFT studies, the *'turn-on'* behaviour on binding to Pd^{2+} could be correlated to the restricted PET.

Palladium (Pd) is one of the widely used metal ions in the metal supported catalysts, employed in many industrial organic processes, such as oxidative conversion of alcohol to an aldehyde,[1] synthesis of many drugs e.g. roficoxib and cofprozil,[2] C-H functionalization,[3] cyclisation reactions of Nazarov type,[4] Suzuki-Miyaura, Buchwald-Hartwig and Sonogashira reactions,[2c,5] etc. However, immense success of its implementation is plagued by the limitation of its contamination in the products obtained in the chemical processes employing Pd based catalysts/reagents.[6] Thus the necessary, often extensive purification of such end products is an obvious operation, wherein palladium is released in the environment including soils, dust, water resources and biological plants which in fact cause serious threats to the environment and human health.[7] The presence of palladium, even in extreme low doses, can trigger serious health problems, as it can lead to the binding of palladium with biomolecules such as thiol containing amino acids, proteins, DNA and RNA,[8] and also cause allergic reactions such as eye and skin irritation.[8c] In order to restrict such hazards, the environment enacting agencies have strictly limited its concentration upto 5-10 ppm in the end products of the reactions.[9] Consequently, chemists, biologists, clinical biochemists and environmentalists in the recent years[10] have shown a great interest in the development of synthetic receptors capable of recognising Pd. Apart from the conventional analytical methods like atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), plasma emission spectroscopy (PES), solid phase micro extraction-high performance liquid chromatography and X-ray fluorescence,[11] methods based upon its use as catalyst,[12] and involving coordination mechanism[13] have been reported in the literature. However, the use of conventional analytical methods and catalysis based methods is limited by the requirement of sophisticated and time-consuming sample preparations, high cost, standardizations and the release of high level of palladium in the environment. The methods of detection based on coordination of Pd^{2+} with a sensor, invariably interpreted from the modulation of optical (absorption/fluorescence) spectra, have attained importance owing to simple and highly sensitive analysis protocols. The detection process gains additional significance when upon coordination of the paramagnetic Pd^{2+}, an otherwise fluorescence quencher,[14] the sensing event results in the fluorescence enhancement. There are very few reports[15] of such a *"turn on"* chemosensor for the detection of Pd^{2+}. Herein, we report on the synthesis of a BODIPY dye **1** (Fig. 1), where in the fluorophore BODIPY core is linked to a dithia-dioxa-aza crown ether (TOAC) which acts as a soft metal chelating site, to achieve the *"off-on"* type fluorescence enhanced sensing protocol based upon coordination method. The hallmark of its superiority over the reported methods is ease in synthesis as well as very low detection limit (1.18 ppb, *vide infra*) obtained in the detection of Pd. Further, using density functional theory (DFT) and time-dependent DFT (TD-DFT) calculations, we have unequivocally correlated the observed changes in spectrophotometric properties with the geometries of the frontier molecular orbitals involved in the sensing process. As far as our knowledge is concerned, this is one of the rare examples of the BODIPY derivative employed for the detection of Pd^{2+}. Recently, Kim et.al[15f] have reported the use

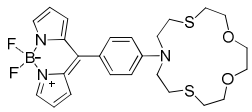

Fig. 1 BODIPY dye **1**.

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† Electronic Supplementary Information (ESI) available: [Synthetic procedure, Characterization/Spectral data of **1**, additional absorption, emission and TD-DFT data and complete ref: 16]. See DOI: 10.1039/c000000x/

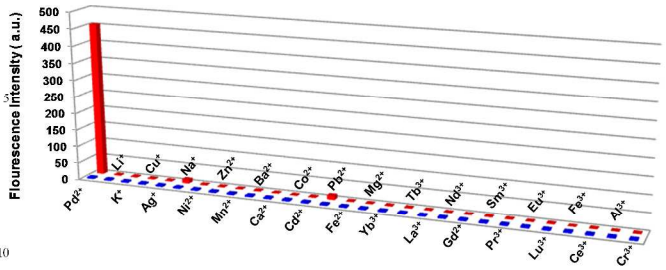

Fig. 2 Changes in the emission spectrum of **1** (5 x 10^{-6} M, in CH_3CN)upon addition of different cations (2.28 x 10^{-5} M, in water) in CH_3CN at 520 nm.

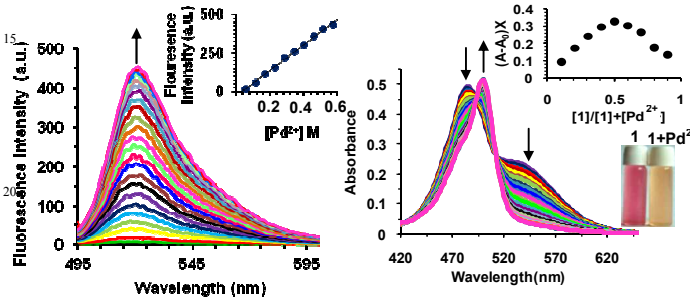

Fig. 3(a) Emission spectral changes of **1** (5 x 10^{-6} M, in CH_3CN) upon addition of Pd^{2+} (2.87 x 10^{-7} M to 5.71 x 10^{-6} M, in H_2O:DMSO, 9:1 *v/v*). Inset: Plot of the fluorescence intensity vs. concentration of Pd^{2+}; **(b)** The changes in the colour and the absorption spectra of **1** (1 × 10^{-5} M, in CH_3CN) upon addition of Pd^{2+} (5.71 x 10^{-7} to 1.54 × 10^{-5} M, in H_2O:DMSO, 9:1 *v/v*). Inset: Job's plot (x is mole fraction of **1**. A_o is absorbance at x = 1; A is absorbance at respective values of x).

of a BODIPY derivative for the detection of palladium operating via *in situ* generation of palladium nanoparticles. Thechemosensor **1** (Fig. 1) was synthesised by following standard synthetic transformations (see †ESI), in analogy with the reported methods and the dye **1** as well as the intermediates were satisfactorily characterized using spectroscopic data (Figs. S1-S4, see †ESI). Before evaluating the metal ion binding properties of **1**, theacid-basetitration experiment wasperformed which revealed the stability of **1** over a wide pH range (1-11) (Fig. S5, see †ESI), thus demonstrating an advantage for rapid monitoring of analytes in environmental and biological settings without resorting to buffered medium.

The fluorescence spectrum of chemosensor**1** (5 x 10^{-6} M, in CH_3CN) is characterised by a very weak intensity emission band at 520 nm (ϕ_f = 0.0018) when excited at 488 nm. The non-fluorescent nature of **1** has been ascribed to the photo-induced electron transfer (PET) from the TOAC unit to the BODIPY core. Our preliminary investigations revealed that the emission intensity of **1** was significantly enhanced in the presence of Pd^{2+} (as chloride salt) over a number of other cations: Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Ni^{2+}, Mn^{2+}, Co^{2+} Cu^{2+}, Zn^{2+}, Pb^{2+}, Fe^{3+}, Cr^{3+}, Al^{3+},Yb^{3+}, La^{3+}, Sm^{3+}, Lu^{3+}, Ce^{3+}, Pr^{3+}, Tb^{3+}, Nb^{3+}, Nd^{3+} (added as their perchlorate or nitrate salts), under identical experimental conditions (Fig. 2). On successive addition of an aqueous solution

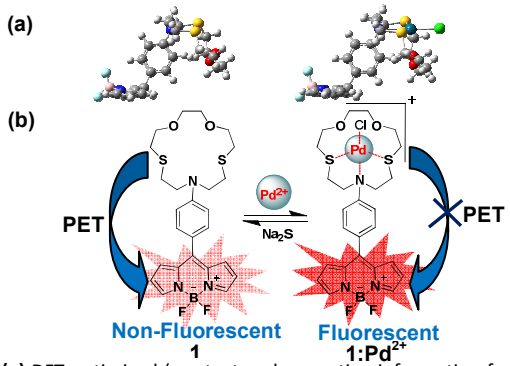

Fig. 4 (a) DFT-optimised (*see* text andsupporting information for details) structures of **1** and its complex with Pd^{2+}; **(b)** proposed complexation mode of **1**with Pd^{2+} explaining the observed fluorescence *"off-on"* mechanism.

of Pd^{2+} (2.87 x 10^{-7} M to 5.71 x 10^{-6} M, in H_2O:DMSO: 9:1 *v/v*)to a solution of **1** (5 x 10^{-6} M, in CH_3CN), the intensity of the emission band of **1** increases regularly and gets stabilised when addition of 5 x 10^{-6} M solution (corresponding to 1 equiv. Pd^{2+} ions) is achieved (Fig. 3a).

The electronic absorption band of **1** (1 x 10^{-5} M in CH_3CN) exhibits an intense absorption band at 484 nm (ε = 49400 M^{-1} cm^{-1}) and a shoulder at 538 nm (ε = 23400 M^{-1} cm^{-1}), attributed to internal charge-transfer (ICT) transitions, responsible for the pink color of **1**. Upon gradual addition of a solution of Pd^{2+} ions (5.71 x 10^{-7} - 1.54 ×10^{-5} M, in H_2O:DMSO 9:1 *v/v*), a bathochromic shift of the intense absorption band to 498 nm ($\Delta E \approx 88.57$ eV) as well as the disappearance of the shoulder, resulted along with a naked-eye color change from pink to light orange (Fig. 3b). This absorbance change reached the saturation point at the addition of 1.03 x 10^{-5} M solution of Pd^{2+}ions (corresponding to 1 equiv.), indicating the formation of a 1:1 stoichiometric complex. It is noteworthy that further addition of Pd^{2+} ion solution caused no significant change in the spectral patterns.

To get the deeper insight into the sensing protocol of **1** with Pd^{2+},DFT calculations were performed (Table S1 and Fig. S6&Table S2 and Fig.S7, see †ESI).[16]The best optimised structure of the**1**:Pd^{2+} complex (Fig. 4a) (Table S3 and S4, see †ESI), predicts that Pd^{2+} is coordinated to two sulphur atoms and one nitrogen atom of TOAC group along with one chlorine atom in a distorted square planar geometry (d_{Pd-S}=2.74 Å; d_{Pd-N} =2.68 Å; d_{Pd-Cl}=2.54 Å). Thus, the fluorescence enhancement could be interpreted as: when Pd^{2+} coordinates with the sulphur and nitrogen atoms of the TOAC unit, the PET process responsible for non-fluorescent behaviour of **1**, gets inhibited (Fig. 4b) thereby enhancing the emission intensity (ϕ_f = 0.1025). The proposed binding mode was well supported by the HRMS spectrum of the **1**:Pd^{2+} complex, which shows a peak at *m/z*658.05 possibly due to the [**1**+Pd^{2+}+Cl^-]$^+$ion (Fig. S8, see †ESI).

Additional evidence in support of the proposed binding of Pd^{2+}, could be accrued from the observed changes in the chemical shifts of the relevant protons of **1**, recorded upon incremental addition of Pd^{2+}, dissolved in d_6-DMSO(Fig. S9). Thus, the protons of the CH_2 groups attached to sulphur and nitrogen atoms of TOAC moiety observed significant downfield shifts (H^a :$\Delta\delta$ = 0.56 ppm; H^b: $\Delta\delta$ = 0.83 ppm; H^c: $\Delta\delta$ = 0.83 ppm; H^d: $\Delta\delta$ = 0.57 ppm; $H^{e\text{-}f}$: $\Delta\delta$ = 0.28 ppm; H^k: $\Delta\delta$ = 0.68 ppm)upon

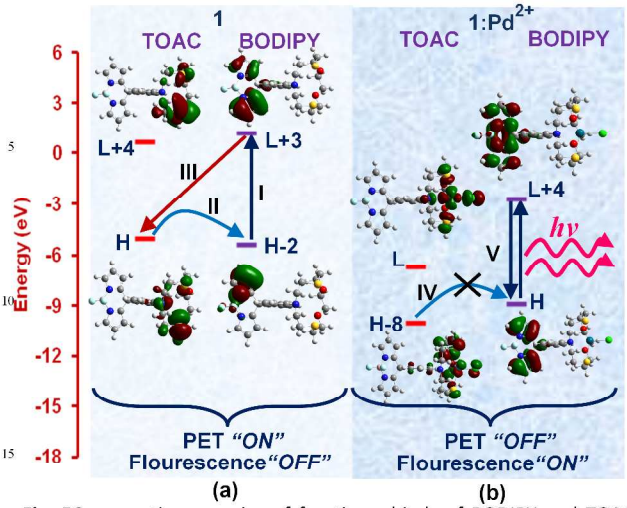

Fig. 5 Comparative energies of frontier orbitals of BODIPY and TOAC in the **(a)** free and **(b)**complexed states. H = HOMO, L = LUMO.

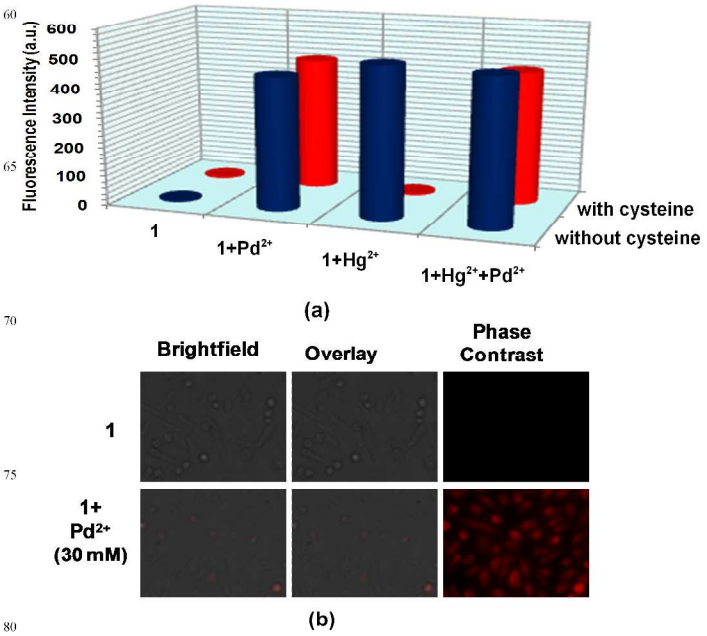

Fig. 6 (a) Emission spectral pattern of **1**, **1**+Pd^{2+}, **1**+Hg^{2+} and **1**+Pd^{2+}+Hg^{2+} in the absence (blue) and presence (red) of cysteine (5.71 x 10^{-7} M, at 520 nm). For absorption based changes see figureS14. (b) Confocal images of MCF-7 cells, supplemented with Pd^{2+} (30 μM) and **1** (1 x 10^{-5} M).

binding to Pd^{2+}(Table S5, see †ESI). The 1:1 stoichiometry was also confirmed by job's plot (a continuous variation method) where maximum absorption change was observed when the mole fraction of **1** *vs.* Pd^{2+} was 0.5 (Fig. 3b inset). Also fitting of the absorption titration data, using Hyp Spec, a non-linear least squares fitting programme,[17] established a 1:1 stoichiometry of the most stable species (**1**:Pd^{2+}) with the binding constant value, *log* $\beta_{1,1}$ = 7.23. Thecalculated detection limit of 1.18 ppb (see †ESI)is significantly lower than most of the probes reported in the literature as well as is well below the permissible limit of 5-10 ppm by WHO for Pd^{2+} in drug chemicals[10](Table S6, see †ESI). The TD-DFT calculations performed on the free receptor **1** and in the complexed state further supported the feasibility of the thermodynamic fluorescence "*off-on*" process *via* the proposed PET mechanism (Fig. 5). Upon the promotion of the electron from the HOMO-2 to LUMO+3 in the BODIPY unit (step I, Fig. 5), the HOMO of the TOAC, at slightly higher energy (-5.42 eV), could transfer the electron to the HOMO-2 (-5.65 eV) of BODIPY (step II, Fig. 5), followed by the electrontransfer from the LUMO+3 (0.66 eV) of **1** to the HOMO of TOAC (step III,Fig. 5). In the complexed form, however, the HOMO-8 and LUMO (similar to the HOMO and LUMO+4, respectively, of free TOAC) of the complexed TOAC get shifted to lower energy (-10.93 eV and -6.72, respectively) such that the electron transfer from the HOMO-8 of TOAC to the HOMO of BODIPY(-8.93 eV) is not favourable (step IV, Fig. 5) and thus the PET gets inhibited turning the fluorescence "*on*" (step V, Fig. 5). Further, the observed small magnitude (ΔE≈ 88.57 eV) of the shift in the main absorption band as a consequence of binding of Pd^{2+} by **1** is rationalized by the fact that since the BODIPY unit is oriented in almost perpendicular position to the TOAC unit (Fig. 4a), the metal binding is less likely to affect the ICT.[18]

While a number of common interfering cations did not interfere in the above detection process (Fig. S10, see †ESI), however, as anticipated,[19] the successive addition of aqueous solution of Hg^{2+} ions under similar experimental conditions, exhibited the similar emission and absorption spectral perturbations (Figs. S11, S12, see †ESI), but with lower binding constant value, log $\beta_{1,1}$ = 4.55, as compared to Pd^{2+} (log $\beta_{1,1}$=. 7.23), and is thus expected to interfere in the detection of Pd^{2+} in the situations where both of these metal ions coexist. However, to counter this problem, we performed the competition experiments in the presence of cysteine. Interestingly, as anticipated, in the presence of cysteine which selectively complexes with Hg^{2+}, **1** binds selectively to Pd^{2+}.The *in situ* formed cysteine:Hg^{2+} did not interfere in the principle detection process. The results of the emission and the absorption based competitive experiments performed are presented in the figure 6(a) and figure S13 (see †ESI). It is clear from the figure 6(a) that the presence of cysteine has no effect on the spectral behaviour of free **1**. However, the changes in the spectral behaviour of **1**:Hg^{2+} complex in thepresence ofcysteine is suggestive of a strong interaction of Hg^{2+} with cysteine as compared to **1**. A similar event of checking theinterference of the presence of Pt^{2+} during the reaction based detection of Pd^{2+} has been reported[20] through exercising pH control. Additionally, the behaviour of **1** towards Pd^{2+} and Hg^{2+} was implemented to generate a fundamental OR logic gate (Fig. S14, see †ESI).

Since reversibility is a prerequisite in developing chemosensors for practical applications, we also studied the reversibility of the sensing protocol as proposed in figure 4b. The emission intensity was quenched after the addition of the aqueous solution of Na_2S to the solution of **1**:Pd^{2+} complex with the subsequent restoration of the original spectrum indicating that the S^{2-} sequesters Pd^{2+} from the **1**:Pd^{2+} complex[13c] (Fig. S15, see †ESI).

Further we also evaluated the potential application of **1** for *in vitro* detection of Pd^{2+} in the human breast MCF7 cancer cells. Figure 6(b) shows the confocal microscope images of MCF7 cells

treated with 30 μM Pd^{2+} ions (For various concentrations see figure S16). The fluorescence was particularly visible in the perinuclear region of the cells as suggested by the overlay of fluorescence and bright fieldimages indicating the subcellular distribution and excellent membrane permeability of **1**.

Conclusions

In conclusion, we have developed a fluorescent and colorimetric chemosensor for Pd^{2+} by combining the ion binding unit of dithia-dioxa-aza crown ether with the efficient signalling handle of BODIPY, depicting the detection limit much less than the permissible limit by WHO.

Acknowledgements

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A *'turn-on'* coordination based detection of Pd^{2+}- Application in Bioimaging

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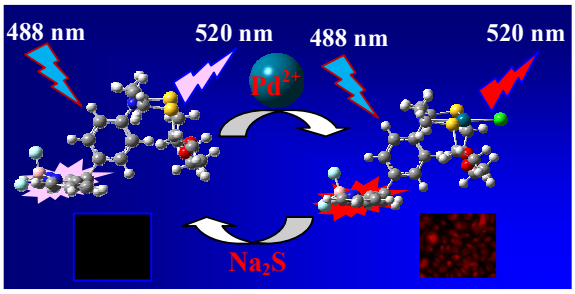
Table of Contents entry



A BODIPY based molecular probe recognises Pd^{2+} via *off-on* type fluorescence enhancement which could be correlated to the restricted PET on the basis of DFT/TD-DFT calculations.