Electronic Supplementary Information

For

Pentafluorophenyl dipyrin as probe for transition metal ion detection and its bioremediation in Bacillus subtilis and Bacillus cereus

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Experimental
Materials and methods

All the chemicals used for the synthesis were reagent grade unless otherwise specified. Pyrrole purchased from Spectrochem (India) was distilled over CaH₂ before use. 2,3-Dichloro-5,6-dicyano-p-benzoquinone (DDQ) obtained from Sigma Aldrich and were used as such. Trifluoroacetic acid (TFA) was purchased from Merck (India) and was distilled over P₂O₅. Dimethylsulphoxide (DMSO), cyclohexane, diethyl ether, 1,2-dichloroethane, 1,4-dioxane, dimethylformamide (DMF), dimethyl sulphoxide (DMSO) were also purchased from Spectrochem (India) and were used as such. 2,3,4,5,6-Pentafluorobenzaldehyde and tetrabutylammonium hexafluorophosphate (NBu₄PF₆) was purchased from Alfa Aesar. Chloroform, dichloromethane, hexane, acetone, ethyl acetate and methanol were purchased from Avra Synthesis Pvt. Ltd. and were purified by distilling over K₂CO₃. Toluene and acetonitrile were purchased from Fischer Scientific. Tetrahydrofuran (THF) was obtained from SRL Chemicals Ltd. India. Nutrient broth was purchased from HiMedia, India.

¹H NMR spectra were recorded with a Bruker 400 MHz FT-NMR spectrometer in CDCl₃ using tetramethylsilane as the internal reference. IR data was collected on a JASCO FT/IR-4700 with KBr pellets. UV-Visible spectra were recorded on a Shimadzu double beam spectrometer 2600 instrument using quartz cuvettes at room temperature. Fluorescence spectra were recorded on a Perkin-Elmer LS-55 Luminescence spectrophotometer with a slit width of 10 at 480 nm excitation wavelength and emission from 500 to 800 nm. The fluorescence quantum
yield ($\Phi_t$) computed for ligand in presence of Zn(II) and Hg(II) ions in PBS 1X buffer used the below equation,

$$\Phi_t = \Phi_f R F S A R \eta^2_S / F R A S \eta^2_R,$$

where $F_S$ and $F_R$ are the integrated fluorescence intensities of the sample and reference, $A_S$ and $A_R$ are the absorbance of the sample and reference at excitation wavelength, $\eta_S$ and $\eta_R$ are the refractive indices of the solvents used for the sample and reference. Fluorescein in 0.1 M NaOH solution was used as the reference ($\Phi_f = 0.90$, $\lambda_{ex} = 470$ nm). All $\Phi$ values are corrected for changes in refractive index. Both the dipyrrin and reference solutions were prepared with the same absorbance at the excitation wavelength (between 0.1 and 0.05 in a 1 cm quartz cell).

Single crystal structure X-ray data collection of the title compound was performed on a Bruker AXS Kappa Apex II CCD diffractometer with graphite monochromated Mo K$_\alpha$ radiation ($\lambda = 0.71073$ Å) at 298 K. Also, the crystal structure is quantitatively analyzed with the help of Crystal Explorer 3.1. Electrochemical measurements were performed on a CH-instruments Inc, USA (Model: CH1660E) equipped with potentiostat / galvanostat with Fourier Transform AC voltammeter. The electrochemical system utilized a three-electrode configuration consisting of a glassy carbon (working), platinum wire (auxillary) and Standard Calomel reference (SCE) electrodes. The concentrations of the samples were maintained as 0.1 M containing tetrabutylammonium hexafluorophosphate (NBu$_4$PF$_6$) as supporting electrolyte (0.1 M) in methanol at 25 $^\circ$C under N$_2$ atmosphere at a scan rate of 100 mV/s. The fluorescence imaging was performed using LEICA DM5000B fluorescent microscope (Germany) at 100x magnification (oil immersion) and the images were processed by using ImageJ (NIH, USA). Mass spectra of BODIPY complexes were performed using a Waters Xevo G2 Quadrapole-Time-of-Flight (Q-TOF) mass spectrometer (ESI-MS).

**Synthesis of meso-(2,3,4,5,6-pentafluorophenyl)dipyrrromethane and meso-(2,3,4,5,6-pentafluorophenyl)dipyrrin**

5-(Pentafluorophenyl)dipyrrromethane$^1$ and 5-(pentafluorophenyl)dipyrrin$^2$ were synthesized by literature procedures. A solution of pyrrole (25 equivalents) and 2,4,5,6-pentafluorobenzaldehyde under nitrogen was stirred for 10 min after the addition of TFA (0.1 equivalents). The reaction was quenched with 10 mL of NaOH (0.1M), washed with water and
was extracted in ethyl acetate. The solvent was evaporated and the product was recrystallized in EtOH / water mixture (Yield: 60%).

*Meso*(2,3,4,5,6-pentafluorophenyl)dipyrromethane was dissolved in 15 mL of THF and was degassed under nitrogen for 2-3 min. To this resulting solution, was added DDQ (2.4 equiv.) and was stirred for 4 hrs at room temperature. The reaction mixture was washed with H₂O and extracted in dichloromethane after removing THF. The organic layer was collected, dried over anhydrous Na₂SO₄, filtered and evaporated the solvent. The crude sample was further purified by silica gel column chromatography using 50/50 chloroform/hexane. The first eluting yellow fraction on removal of solvent and vacuum drying gave the dark brownish yellow powder as the product 1. Yield = 85 %. ¹H NMR (400 MHz, CDCl₃, δ in ppm): 6.46 (2H, d, py-H), 6.41 (2H, d, CH β to pyrrole NH), 7.65 (2H, s, CH α to pyrrole NH), 7.25 (1H, s, br, py-NH). UV/vis (CHCl₃): λ_max, nm (log ε) = 437 (4.19). IR (KBr, cm⁻¹): 3226, 2921, 1650, 1585, 1522, 1496, 1437, 1382, 1348, 1255, 1147, 1115, 1093, 1045, 992, 911, 878, 822, 768, 746, 597, 570.

**Preparation of stock solutions**

The dipyrrin ligand (1) as well as the metal salt stock solutions was prepared in methanol.³,⁴ The dipyrrin (1 mg, 3 mmol) was dissolved in methanol (1 mL) and 20 μL of it was made up to 3 mL with PBS 1X buffer (pH = 7.4) to make a final concentration of 21 μM. Metal chlorides, MCl₂ (M = Mn, Co, Ni, Cd, Ca, Mg, Ba, Sn), MCl₃ (M = Fe, Al, Cr) and MCl (M = K, Na) were weighed and dissolved in methanol to give solutions of 16 mM. 4 μL of these metal ion solutions when added to 3 mL of dipyrrin solution in PBS 1X gives a concentration of 21 μM. Thus, to 3 mL of dipyrrin solution (21 μM) in PBS 1X buffer, 4 μL of metal ions (16 μM) were added to get a mixture of 1:1 dipyrrin / cation. The ability of dipyrrin to detect different metal cations was studied by colorimetric (naked-eye) analysis, UV-visible and fluorescence spectroscopic studies. All the experiments were performed at room temperature.

**UV-Visible and fluorescence titrations**

As described earlier, to the ligand (1) in methanol diluted with PBS 1X buffer, metal chloride solutions (0-2 equivalents) in methanol were added. After mixing for a few seconds, UV-Visible and fluorescence spectra were taken.
In order to validate the selectivity of the sensor, experiments were performed in the presence of 1 equiv. of other competing metal ions, such as, Ba\(^{2+}\), Ca\(^{2+}\), Cd\(^{2+}\), Co\(^{2+}\), Cr\(^{3+}\), Fe\(^{3+}\), K\(^{+}\), Mg\(^{2+}\), Mn\(^{2+}\), Na\(^{+}\), Sn\(^{2+}\) and Al\(^{3+}\). For instance, to check the interference of other ions for the detection of Zn(II), 20 \(\mu\)L of the receptor 1 (3 mM) solution was diluted with 3 mL of PBS 1X buffer to make the final concentration of 21 \(\mu\)M. 4 \(\mu\)L of each competing metal ion solution (16 mM of Ba\(^{2+}\), Ca\(^{2+}\), Cd\(^{2+}\), Co\(^{2+}\), Cr\(^{3+}\), Fe\(^{3+}\), K\(^{+}\), Mg\(^{2+}\), Hg\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), Mn\(^{2+}\), Na\(^{+}\), Sn\(^{2+}\) and Al\(^{3+}\) ions) were taken and added to 3 mL of the ligand solution to make 1 equivalent. Then, 4 \(\mu\)L of Zn(II) solution (16 mM) was added into the mixed solution of each metal ions and ligand to make 1 equivalent (21 \(\mu\)M). After mixing for few seconds, fluorescence spectra were taken at room temperature. Selectivity for Hg(II) was also investigated by the same token. Likewise, selectivity for Zn(II), Hg(II), Ni(II) or Cu(II) ions in a complex background of each potentially competing species were examined using UV-Visible absorption studies.

**Job’s plot measurements**

About 10 mM solutions of dipyrrin and metal ions were prepared in methanol. 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 \(\mu\)L of 1 solution were taken and transferred to vials containing 2.976 mL of PBS 1X buffer. To this, 24, 22, 20, 18, 16, 14, 12, 10, 8, 6, 4, 2 and 0 \(\mu\)L of the metal solution were added to each diluted 1 solution such that each vial had a total volume of 3 mL. After shaking the vials for few minutes, UV-Visible or fluorescence spectra were taken at room temperature.

**Biosorption studies**

*Bacillus subtilis* and *Bacillus cereus* were used for the present biosorption studies. *B. subtilis* strain BKDS1 deposited in GeneBank as KT004506.1 was used for the studies and *B. cereus* (BC) was a wild isolate, the details of which are reported elsewhere\(^5a\). The cells were precultured in nutrient broth for 48 hrs at 37 \(^\circ\)C and 110 rpm to achieve active growth. Then, they were allowed to grow in sublethal concentrations of different metals (450 \(\mu\)g/mL Ni and Cu, 25 \(\mu\)g/mL Hg and 33 \(\mu\)g/mL Zn) in nutrient broth\(^5b,c\). The culture conditions were maintained at 37 \(^\circ\)C with constant shaking at 110 rpm for 24 h. Uninoculated flasks containing broth and different heavy metals served as control.
**UV-Visible/ fluorescence spectroscopy**

Their biosorption levels were estimated using UV-Visible spectroscopic method for Ni and Cu. Zn and Hg biosorption were analyzed using fluorescence spectroscopy. After incubation, 1 mL of culture was centrifuged at 6000 rpm for 5 min to separate out biomass at different time intervals for 70 hrs. The metal ions remaining in supernatant was tested by recording the UV-visible or fluorescence spectra of the supernatant in presence of ligand. To 100 µL of the supernatant, 3 mL of PBS 1X buffer, 20 µL of dipyrrin ligand, 1 (0.2 mM) was added and spectra were recorded.

**Fluorescence microscopy**

The amount of metal ions (Zn(II)/Hg(II)) absorbed by the organism was determined by fluorescence microscopic analysis of the cell mass. The bacterial pellets separated from the broth were washed twice with PBS 1X buffer by centrifugation at 6000 rpm for 5 min. About 20 µL of dipyrrin ligand 1 (0.2 mM) buffered with PBS 1X was added to the pellet and incubated at 37 °C for 30 min for the uptake of the ligand. Subsequent washing with PBS 1X buffer (centrifugation at 6000 rpm for 5 min twice) removed excess of ligand. The bacterial cells were resuspended in 1mL of PBS 1X buffer and were transferred to glass slides, fixed with glyceraldehyde to observe under fluorescence microscope at 40x magnification. The microscopic images revealed the intracellular presence of Zn(II) and Hg(II).

**Single crystal X-ray structure analysis**

Single crystal of 1 was mounted on a glass capillary with suitable size and the data collections were performed on a Bruker AXS Kappa Apex II CCD diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71073 Å) at 298 K. The reflections with $I > 2\sigma(I)$ were employed for structure solution and refinement. The SIR926 (WINGX32) program was used for solving the structure by direct methods. Successive Fourier synthesis was employed to complete the structures after full-matrix least squares refinement on $|F|^2$ using the SHELXL977 software.

**References**


2. E. H. L. Yu, K. Muthukumaran, I. V. Sazanovich, C. Kirmaier, J. S. Lindsey, J. R. Diers,


**Figure S1.** $^1$H NMR spectrum of dipyrrin (1)

**Figure S2.** IR spectrum of dipyrrin (1)

**Figure S3.** Absorption and emission spectra of dipyrrin, 1 in solvents of different polarity.

**Figure S4.** (a) Absorption spectra of dipyrrin (21 μM) after addition of increasing amounts of Hg(II) ions (0 to 2 equiv.) in PBS 1X buffer - CH$_3$OH (99.33 : 0.67, v/v) at room temperature. (b) Absorbance at 520 nm versus the number of equivalents of Hg(II): Optical density as a function of concentration of Hg(II). (c) Absorbance of Hg(II) complex with dipyrrin in the presence of various metal ions and its (d) absorption spectra.

**Figure S5.** (a) Absorption spectra of dipyrrin (21 μM) after addition of increasing amounts of Cu(II) ions (0 to 2 equiv.) in PBS 1X buffer - CH$_3$OH (99.33 : 0.67, v/v) at room temperature. (b) Absorbance at 486 nm versus the number of equivalents of Cu(II): Optical density as a function of concentration of Cu(II). (c) Absorbance of Cu(II) complex with dipyrrin in the presence of various metal ions and (d) its absorption spectra.

**Figure S6.** (a) Absorption spectra of dipyrrin (21 μM) after addition of increasing amounts of Ni(II) ions (0 to 2 equiv.) in PBS 1X buffer - CH$_3$OH (99.33:0.67, v/v) at room temperature. (b) Absorbance at 486 nm versus the number of equivalents of Ni(II): Optical density as a function of concentration of Ni(II). (c) Absorbance of Ni(II) complex with dipyrrin in the presence of various metal ions and its (d) absorption spectra.
Figure S7. ESI-mass spectrum (positive-ion mode) of I (1.0 x 10^{-5} M) upon addition of 1 equiv. of Ni^{2+} (0.5 x 10^{-5} M) in MeOH.

Figure S8. ESI-mass spectrum (positive-ion mode) of I (1.0 x 10^{-5} M) upon addition of 1 equiv. of Cu^{2+} (0.5 x 10^{-5} M) in MeOH.

Figure S9. ESI-mass spectrum (positive-ion mode) of I (1.0 x 10^{-5} M) upon addition of 1 equiv. of Zn^{2+} (0.5 x 10^{-5} M) in MeOH.

Figure S10. The working curve plot for the detection of (a) Ni(II), (b) Cu(II) solution in PBS 1X buffer - CH_{3}OH (99.33:0.67, v/v) at room temperature based on the optical density value against various concentrations: 0, 2.1, 4.2, 6.3, 8.4 and 10.5 µM. The working curve plot for the detection of (c) Zn(II), (d) Hg(II) solution in PBS 1X buffer - CH_{3}OH (99.33:0.67, v/v) at room temperature based on the fluorescence intensity value against various concentrations: 0, 2.1, 4.2, 6.3, 8.4 and 10.5 µM.

Figure S11. Cyclic voltammogram of (a) dipyrin (1) (b) copper complex (c) nickel complex (d) zinc complex (e) mercury complex.

Figure S12. (a, b) Emission spectra of supernatant of BC and BS maintained with Hg(II) and (c, d) with Zn(II) in presence of dipyrin ligand.

Table S1. Absorption and emission data of dipyrin ligand in solvents of different polarity at \lambda_{ex} (nm) = 480 nm.
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