Visible light excitable pyrene-naphthalene conjugate for ON fluorescence sensing of histidine in living cells†

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Experimental

Materials and methods

N1-(naphthalen-1-yl) ethane-1, 2-diamine and pyrene-1-carbaldehyde were purchased from Sigma Aldrich (India). The amino acids kit was purchased from Himedia (India). Other chemicals were of analytical reagent grade and used without further purification except when specified. Milli-Q Milipore 18.2 MΩ cm−1 water was used throughout all experiments.

A Shimadzu-1800 UV-Vis spectrophotometer was used to measure UV-Vis spectra. Mass spectra were recorded on a QTOF MicroYA263 mass spectrometer in ES positive mode. Steady state emission and excitation spectra were recorded with a Perkin Elmer LS55 fluorescence spectrometer. 1H NMR spectra were recorded in DMSO-d6 on a Bruker Advance 600 MHz instrument using TMS as internal standard. Systronics digital pH meter (model 335) was used to measure the solution pH. Either 50 mM HCl or KOH was used for pH adjustment. Geometries of NEDAP and NEDAP-His adduct are optimized by DFT (B3LYP/6-31G basis set) using Gaussian ’03 software package.1

Imaging system
The imaging system is composed of an inverted fluorescence microscope (Leica DM 1000 LED), digital compact camera (Leica DFC 420C), and an image processor (Leica Application Suite v3.3.0). The microscope is equipped with a 50 W mercury arclamp and UV filter.

**Preparation of cells**

*Candida albicans* cells (IMTECH No. 3018) from an exponentially growing culture in a yeast extract glucose broth medium (pH 6.0; incubation temperature, 37 °C) were centrifuged at 3000 rpm for 10 min, washed twice in normal saline, and then treated with 50 μM histidine for 30 minutes in normal saline. After incubation, the cells were washed again in normal saline, incubated with **NEDAP** (1 μM) for 15 minutes, and observed under a highpower fluorescence microscope. Cells loaded with **NEDAP** but not with histidine were used as the control.

A Gram positive bacterial strain was isolated from a tea garden soil, later identified as *Bacillus sp*. On the basis of 16S rDNA sequence homology and physio-biochemical characteristics. The bacteria isolate was grown in nutrient broth (pH 7.00 at 37 °C for 24 hours. After 24 hrs incubation one ml culture broth containing the bacterial cells was centrifuged at 3000 rpm for five minutes, the supernatant was discarded and the cell pellet was washed twice in normal saline and then incubated in a solution of histidine (1 mg/ml) for one hour at 37 °C. After incubation they were again washed in normal saline and observed under fluorescence microscope using appropriate UV filter in presence and absence of the **NEDAP**.

**Calculation of Quantum Yield**

Fluorescence quantum yields (Φ) were estimated by integrating the area under the fluorescence curves using the equation²,
Where, \( A \) was the area under the fluorescence spectral curve, \( OD \) was optical density of the compound at the excitation wavelength and \( \eta \) was the refractive indices of the solvent. \( Tris(2,2\text{-bi pyridyl}) \) ruthenium(II) was used as a reference with a known \( \phi_{\text{ref}} \) value of 0.042 in water.\(^4\)

**Synthesis of NEDAP \([(E)-N^1-(naphthalen-1-yl)-N^2-(pyren-1-ylmethylene) ethane-1,2-diamine] (Scheme 1)**

To a solution of \( N^1-(naphthalen-1-yl) \)ethane-1,2-diamine hydrochloride (466.2mg, 2.1 mmol in 10 mL dry methanol), pyrene 1-carboxaldehyde (500 mg, 2.10 mmol) was added in presence of excess \( \text{NaHCO}_3 \). The reaction mixture was refluxed for 4 h. After removing the solvent using rotary evaporator, the crude product was subjected to column chromatography (hexanes: \( \text{EtOAC} = 85: 15, \text{v/v} \)). Yield, 80%.\(^1\)HNMR (600 MHz, DMSO-\(d_6\)) (Fig. S6, S7) in ESI):\( \delta \) (ppm): 9.3 (1H, s); 8.9 (1H, d, \( J = 9.6 \) Hz); 8.5 (1H, d, \( J = 7.8 \) Hz); 8.3 (2H, m) 8.2 (4H, m); 8.1 (3H, m); 7.8 (1H, s); 7.5 (1H, s); 7.3 (1H, d, \( J = 10.2 \) Hz); 7.3 (1H, s); 6.4 (1H, d, \( J = 7.8 \) Hz) 6.3 (1H, m); 4.0 (2H, m,); 3.5 (2H, m,) QTOF – MS ES\(^+\): [M +H\(^+\)]\(^+\) (Fig. S8), calcd., 399.1; found, 399.1 (100%);

**Calculation of detection limit**

Fluorescence titration of NEDAP with histidine was carried out by adding aliquots of micromolar concentration of histidine. From the concentration at which there was a sharp change in the fluorescence intensity multiplied with the concentration of NEDAP gave the detection limit.\(^5\)

**Equations used for calculating detection limit (DL)**

\[
DL = C_L \times C_T
\]
$C_L = \text{Conc. of NEDAP}$; $C_T = \text{Conc. of histidine at which fluorescence enhanced.}$

Thus;

$$DL = 1 \, \mu M \times 0.01 \, \mu M = 0.01 \, \mu M = 1 \times 10^{-8} \, M$$

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**Fig. S1** Plot of fluorescence intensity vs. externally added [His] (1-10 $\mu$M). Inset: linear portion of the plot (up to 1.0 $\mu$M histidine).
Fig. S2 Job’s plot for determination of stoichiometry of the [NEDAP – His] adduct ($\lambda_{\text{Ex}} = 410$ nm, $\lambda_{\text{Em}} = 458$ nm).

Fig. S3 Mass spectrum of [NEDAP-His] adduct
**Fig. S4** Determination of the binding constant of NEDAP with histidine using \((F_\infty - F_0)/(F_x - F_0) = 1 + (1/K) \times (1/[C]^n)\), where \(F_\infty\), \(F_0\) and \(F_x\) are fluorescence intensities of NEDAP in presence of histidine at saturation, free NEDAP and at any intermediate histidine concentration, respectively. Here \([C] = [\text{Histidine}]\).

**Fig. S5** Determination of binding constant of NEDAP with histidine using equation (ii).
Fig. S6 $^1$H NMR spectrum of NEDAP (aliphatic region) in DMSO-d$_6$. 
Fig. S7 $^1$H NMR spectrum (aromatic region) of NEDAP in DMSO-$d_6$. 
Fig. S8 Mass spectrum of NEDAP

References

1. Gaussian 03, Rev.C.02 (Gaussian Inc., Wallingford CT), 2004.


