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Research paper

# Turn on ESIPT based chemosensor for histidine: Application in urine analysis and live cell imaging

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ABSTRACT

A vitamin $B_6$ cofactor containing excited-state intramolecular proton transfer (ESIPT) based fluorescent sensor $[Cu(LH_2)Cl_2]\cdot 2H_2O$ [$LH_2$ is pyridoxal-semicarbazide Schiff base] is used as a selective "naked-eye" fluorescent sensor of L-histidine at pH 7.4 in aqueous media via the ligand displacement approach. Uv-Vis spectrophotometry and cyclic voltammetry can also be used to detect L-histidine by the Cu(II)-complex. The DFT calculation also supports the sensing phenomena. The fluorescence imaging studies indicated that the Cu(II)-complex can be applied to visualize the intracellular histidine in living cells. Our chemosensor can also be used for quantitative determination of histidine in urine. It acts as a helpful tool for physiological and pathological estimation of histidine. It also shows high efficiency in 'dip-stick' method.

## 1. Introduction

Pyridoxal, a vitamer of vitamin $B_6$, is an important biological cofactor [1–3], and it helps in various enzymatic processes, such as catalytic transamination [4], racemization [5], decarboxylation [6] desulphydration of amino acids [7] and dehydration of serine and threonine [8]. Pyridoxal and its derivatives have also been investigated for their roles in prevention and treatment of cancer [9,10]. Study of Schiff bases of pyridoxal and their metal complexes are attractive because Mn(II) chelates of N,N′-bis-(pyridoxal)-alkylenediamine-N,N′-diacetic acids, N,N′-bis-(pyridoxal)-1,2-cycloalkylene-N,N′-diacetic acids and N,N′-bis-(pyridoxal)-1,2 arylenediamine- *N*-acetic acids, as well as their salts and esters have been reported as superior contrast agents for NMR imaging [11], whereas Cu(II) complex of pyridoxal amino guanidine Schiff base helps in the treatment of diabetic complications[12]. Pyridoxal being a fluorogenic moiety [13], the modulation of fluorescence properties of pyridoxal Schiff bases and their metal complexes are profitable area of development of fluorogenic sensors, particularly for biologically important ions [14–17]. Pyridoxalaminoguanidine complexes of Cu(II) and Co(III) are reported to be highly photosensitive and can be potentially used for the development of electronic system for the detection and monitoring of UV radiation and concentrations of atmospheric oxygen [18]. The same group also reported Zn(II) complexes with the same Schiff base ligand which has strong photoluminescence property and has application in optical sensors and white light-emitting optical sources [19]. These advantageous features motivated us to search for new pyridoxal based sensors and in this context we have developed a pyridoxal based chemosensor for the detection of histidine in aqueous medium. Despite their attractive coordinating and photophysical properties, pyridoxal derivatives have received scant attention as a sensor. Histidine, being an essential amino acid, plays a crucial role in biochemistry due to its aromatic imidazole moiety. It is essential for human growth [20–22] and serves as a neurotransmitter [23–25] and controls the transmission of metal ions in biological bases [26]. In recent times, it has been found that deficiency of histidine results in impaired nutritional state of patients with chronic kidney disease [27] whereas abnormal level of histidine-rich protein is an indicator of a variety of diseases such as asthma, liver cirrhosis and rheumatoid arthritis [28–32]. Hence, accurate and rapid detection and quantification of histidine in biological fluids has become a challenging area for both clinical and biochemical analysis. Numerous methods such as chromatography [33–35], capillary electrophoresis [36–39], voltammetry [40–43], resonance light scattering techniques [44], mass spectrometry [45] and spectrophotometry [46,47] have been developed for the detection of histidine. However, the drawbacks of these approaches are that they are destructive, time consuming, expensive and require homogenization which makes them unsuitable for detection of histidine in living bodies. In past few years many efforts were made by researchers for the development of fluorescent probes which can detect histidine [48–51]. Zhang *et al.* reported a fluorescent probe for the detection of histidine [52]. Recently, many research groups paid their attention towards Cu(II)-containing fluorescent probes of histidine

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because of the discovery of presence of Cu(II)-His species in human body which is helpful in the treatment of Menkes disease [53]. A Cu(II)-containing chemosensor has been developed by Chan and coworkers which has high selectivity and sensitivity towards histidine and has applicability towards intracellular fluorescence imaging [54]. $Al^{3+}$ complex of a pyridoxal derivatized polyether ligand was found to detect histidine in urine with a detection limit of 0.3 μM in HEPES buffer [55]. However, displacement of Cu(II) by His to turn on excited-state intramolecular proton transfer (ESIPT) is unknown yet. ESIPT mechanism is a very rapid process which ranges from fraction of picoseconds to tens of picoseconds, as proton transfer is much easier and speedy than that of electron transfer in excited state [56,57]. ESIPT compounds have drawn much attention in recent years due to their potential applications in optical devices that may take advantage of the salient properties such as the ultra-fast reaction rate and extremely large fluorescence Stokes shift [58] which helps to avoid spectral overlap between absorption and emission spectra.

Vidovic *et al.* reported copper complexes of pyridoxal-semicarbazide Schiff base [59]. Herein, we report a pyridoxal-semicarbazone based Cu (II) complex, $[Cu(LH_2)Cl_2]\cdot 2H_2O$, which is a solvatomorph of the earlier reported Cu(II) complex of the same lignad, as ESIPT based turn-on fluorescent probe for selective detection of L-histidine not only in aqueous medium but also in living cells. Though various metal complexes of the pyridoxal-semicarbazone Schiff base are known [60–62] the possibility of vast range of applications using these complexes are yet to be adequately explored. The Cu(II) present in the chemosensor quenches the fluorescence of the ligand and constructs a non fluorescent off state due to its intrinsic paramagnetic property and due to the deprotonation of the particular −OH proton which participates in ESIPT. Upon addition of copper binding analyte His, the strong and effective chelation of Cu(II) by His leads to displacement of the fluorescent ligand from the Cu(II) coordination sphere leading to switch on of the fluorescence. Using this principle, one can also detect histidine in human urine with our reported Cu(II)-complex.

## 2. Experimental

### 2.1. Materials and methods

Pyridoxal hydrochloride and $CuCl_2\cdot 2H_2O$ were obtained from Aldrich and semicarbazide hydrochloride was purchased from BDH Ltd. All other chemicals and solvents were of reagent grade and used as such while solvents for spectroscopic and cyclic voltammetry studies were of HPLC grade obtained from Merck or Aldrich. Elemental analyses were performed on a Perkin-Elmer 2400C, H, and N analyzer. Infrared spectra were recorded as KBr pellets on a JASCO FT-IR-460 spectrophotometer. UV-Vis spectra were recorded using a JASCO V-530 spectrophotometer. Cyclic and differential pulse voltammetry experiments were carried out using a CH1106A potentiostat. A three-electrode configuration, with glassy-carbon working electrode and Pt-auxiliary electrode, Ag, AgCl/saturated KCl reference electrode and TEAP as supporting electrolyte, was used. Under our experimental conditions the ferrocene/ferrocenium couple was observed at $E^0$ ($\triangle E_p$) = 0.48 V (100 mV). $^1$H NMR spectra were recorded on a Bruker AVANCE DPX 300 MHz or 400 MHz spectrometer using, $Si(CH_3)_4$ as internal standard. ESI-MS spectra of the samples were recorded on JEOL JMS 600 instrument. Fluorescence titration experiments were carried out using PTI made QuantaMaster40 spectrofluoremeter.

### 2.2. Preparation of ligand $LH_2\cdot 2H_2O$

Though the ligand was reported earlier [59], we synthesized it in a modified procedure to grow single crystals suitable for X-ray diffraction studies. To a vigorously stirred methanolic solution (10 ml) of the pyridoxal hydrochloride (0.203 g, 1 mmol) a solution of semicarbazide hydrochloride (0.111 g, 1 mmol) dissolved in methanol (10 ml) was added drop by drop. The mixture was neutralised by adding small amount of anhydrous $Na_2CO_3$ solution (0.158 g, 1.5 mmol dissolved in 5 ml $H_2O$) and stirred at room temperature for two hours and then filtered [61,63]. The filtrate was allowed to evaporate slowly at room temperature. After 2 days white, rod shaped, shiny crystals of ligand $LH_2\cdot 2H_2O$, suitable for X-ray diffraction studies, were obtained. Since the quality of the single crystals were found to be not of a satisfying quality, therefore, only preliminary structural parameters are being reported here. The details of X-ray diffraction studies with ORTEP diagram were given in Electronic Supporting Information (Fig. S1, Table S1, ESI).

Yield: 225 mg (88%). Anal.calc. for $C_9H_{16}N_4O_5$ (M.Wt.:260.25): C, 41.54; H, 6.20; N, 21.53. Found: C, 41.93; H, 6.37; N, 21.81%. $^1$HNMR (DMSO-$d_6$): δ(ppm): 10.61(1H,s), 8.38(1H,s), 7.92(2H,s), 6.44(1H,s), 5.25(1H,s), 4.55(2H,s), 2.44(3H,s) (Fig. S2). Electronic spectrum in $H_2O$ (pH 7.4, 0.01 M HEPES buffer solution, $\lambda_{max}$/nm ($\varepsilon_{max}$/$M^{-1}cm^{-1}$): 290 (57292), 372 (42330) (Fig. S3). MS: ESI-MS- *m/z*: 225.12 [$LH_2$ + $H^+$] (Fig. S4). Selected IR bands ($cm^{-1}$): 3436($\nu^{asym}_{NH2}$), 3358($\nu^{sym}_{NH2}$), 3173($\nu_{NH}$), 1709($\nu_{C=O}$), 1596($\nu_{C=N}$) and 1476 ($\nu_{C\text{-}O}$) (Fig. S5).

### 2.3. Preparation of $[Cu(LH_2)Cl_2]\cdot 2H_2O$

To a vigorously stirred methanolic solution (10 ml) of the pyridoxal hydrochloride (0.203 g, 1 mmol) a solution of $CuCl_2\cdot 2H_2O$ (170 mg, 1 mmol) in methanol (10 ml) was added drop wise. Next, semicarbazide hydrochloride (0.111 g, 1 mmol) dissolved in methanol (10 ml) was added drop by drop to the suspension. The solution turned deep green. The mixture was stirred at room temperature for half an hour, refluxed for another 2 h and then filtered. The filtrate was allowed to evaporate slowly at room temperature. After 2 days green, square shaped, shiny crystals of $[Cu(LH_2)Cl_2]\cdot 2H_2O$, suitable for X-ray diffraction studies, were obtained (Fig. 1). The details of X-ray diffraction studies are given in Electronic Supporting Information (Table S2 and S3 in ESI). A comparison of the crystallographic refinement details along with bond lengths and bond angles of our Cu(II) complex with the previously reported complex are presented in Tables S4 and S5 in ESI.

Yield: 333 mg (84%). Anal.calc. for $C_9H_{16}Cl_2N_4O_5Cu$ (M.Wt.:394.70): C, 27.36; H, 4.05; N, 14.19. Found: C, 27.32; H, 4.07; N, 14.21%. MS: ESI-postive ion mode- *m/z*: 358.13 [$Cu(LH_2)Cl_2$] (Fig.

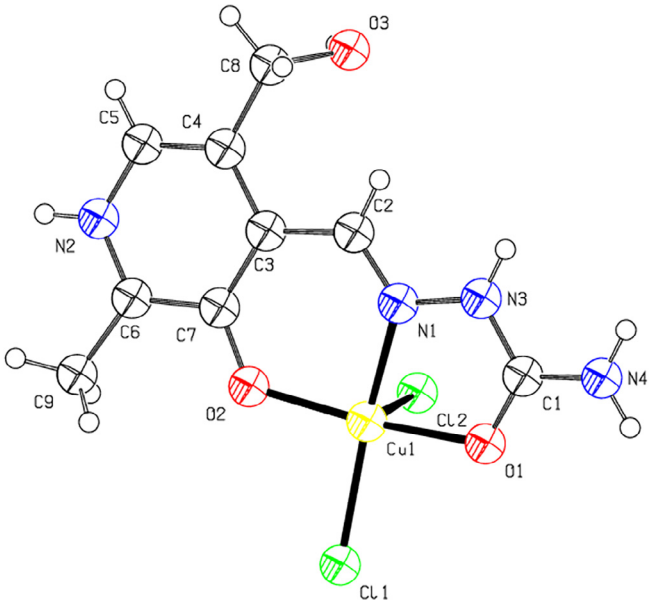



**Fig. 1.** Molecular Structure of $[Cu(LH_2)Cl_2]\cdot 2H_2O$. Only non hydrogen atoms are labeled and their thermal ellipsoids are drawn at 50% probability. The water molecules of crystallization are omitted for clarity.

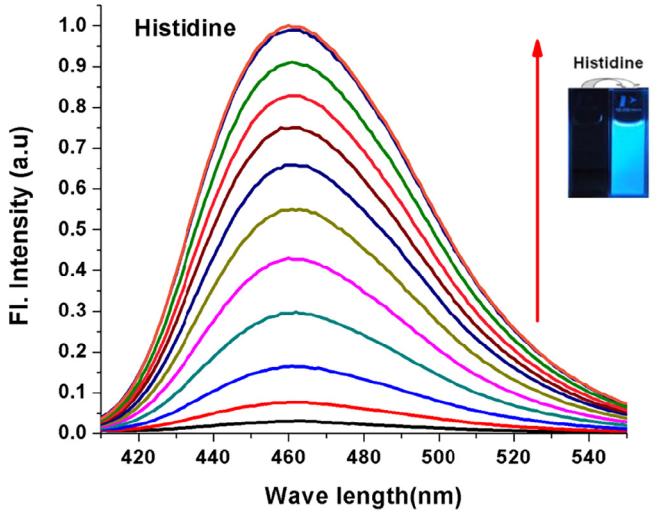



**Fig. 2.** Normalized fluorescence titration spectra of $[Cu(LH_2)Cl_2]\cdot 2H_2O$ ($c = 1.0 \times 10^{-5}$ M) in the presence of histidine ($c = 1.0 \times 10^{-4}$ M) in $H_2O$ (pH 7.4, 0.01 M HEPES buffer solution) with 'naked-eye' color change of $[Cu(LH_2)Cl_2]\cdot 2H_2O$ in presence of histidine under hand held UV lamp (inset).

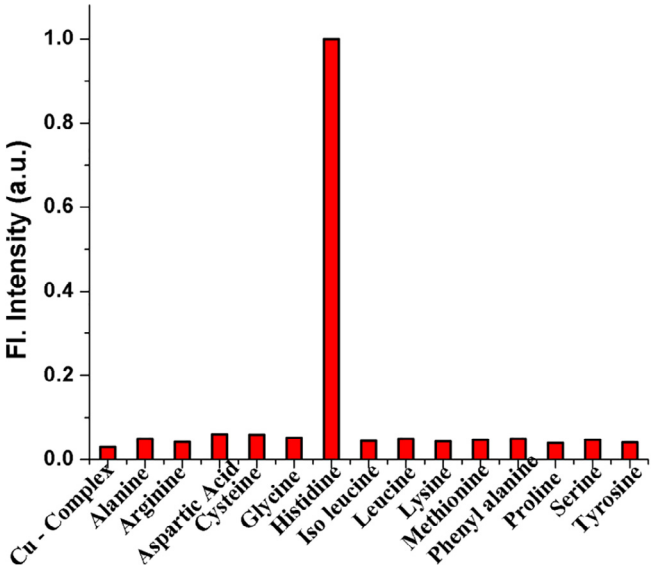



**Fig. 3.** Normalized fluorescence response of $[Cu(LH_2)Cl_2]\cdot 2H_2O$ in presence of various amino acids at 460 nm.

S6). Electronic spectrum in $H_2O$ (pH 7.4, 0.01 M HEPES buffer solution, $\lambda_{max}$/nm ($\varepsilon_{max}/M^{-1}cm^{-1}$): 296 (52906), 310 (55411), 324 (51835), 382(55391), 703 (227) (Fig. S7). Selected IR bands ($cm^{-1}$): 3529($\nu_{OH}$), 3433($\nu^{asym}_{NH2}$), 3353($\nu^{sym}_{NH2}$), 3184($\nu_{NH}$), 1659($\nu_{C=O}$), 1569($\nu_{C=N}$) and 1459 ($\nu_{C\text{-}O}$) (Fig. S8).

## 3. Results and discussions

### 3.1. Spectrophotometric titration

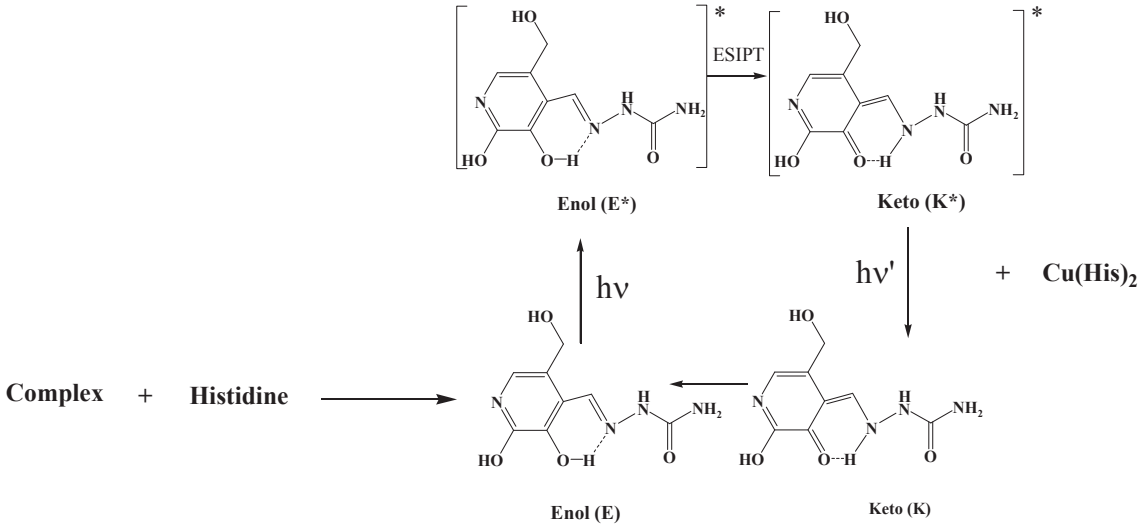
Considering the fact that amino acids can strongly bind with metal ions especially Cu(II) we measured the fluorescence response of $[Cu(LH_2)Cl_2]\cdot 2H_2O$ in presence of various amino acids. The fluorescence titrations were carried out with a series of amino acids including His, Ala, Arg, Asp, Cys, Gly, Ile, Leu, Lys, Met, Phe, Pro, Ser and Tyr at biological pH, at room temperature, in aqueous medium. Only with the addition of His, a significant, 32.8 fold, fluorescence intensity enhancement at 460 nm (Fig 2 and Fig. S9 in ESI) is observed with a 'naked eye' color change from colourless to blue (under UV light) in aqueous HEPES buffer (pH 7.4). No such change is observed on addition of other amino acids to the Cu(II)-complex (Fig. 3). The association constant (K) of binding with histidine was determined as $1.25 \times 10^4\ M^{-1}$ by Benesi-Hilderbrand equation (Fig. S10). This indicates that as $[Cu(LH_2)Cl_2]\cdot 2H_2O$ has a relatively high binding affinity for histidine. The fluorescence titration experiments also demonstrate that the title Cu(II) complex exhibits a high selectivity for histidine binding over other amino acids and it can be used as a selective off-on chemosensor for histidine. Previous reports in literature suggest that cooperative chelation takes place between Cu(II) ion and carboxyl and imidazole moieties of histidine [54,64]. We therefore can predict that histidine captures the Cu(II) ion from $[Cu(LH_2)Cl_2]\cdot 2H_2O$ in aqueous solution and recovers the fluorescence of $LH_2\cdot 2H_2O$ which was quenched by complexation with Cu(II).

The origin of the fluorescence of the free ligand may be as follows. Excitation at 410 nm resulted rapid conversion (within picoseconds) by ESIPT from excited state of enol (E*) to the excited state of keto (K*) tautomer. The excited keto tautomer (K*) emits at 460 nm to come back to its ground state (K) (Scheme 1). Then the less stable keto form (K) lapses to more stable enol form (E) via reverse proton transfer. The ratio of keto and enol emission depends on solvent polarity [65]. As we carried out the experiment in a polar protic solvent water, the keto amine form predominates here whereas the normal (phenol-imine) emission band is not prominent.

Time-Correlated Single Photon Counting (TCSPC) experiment of the isolated product, in aqueous buffer (HEPES, pH 7.4), shows (Fig. 4) a bi-exponential decay of the excited state. On deconvolution, two components of significantly differing lifetimes and amplitudes were obtained. The fast component of ~300 ps having amplitude of ~98% has been attributed to the keto form since this is the only emissive species observed from steady-state emission measurements. The other component which is comparatively much slower (~4.29 ns) is expected to be the signature of the enol form, attaining stability via the existing hydrogen bond within the framework. The corresponding amplitude being too low (~2%) accounts for the absence of the emission band of the enol form in steady-state measurements.

The UV-vis spectrum of complex $[Cu(LH_2)Cl_2]\cdot 2H_2O$ in aqueous medium (pH 7.4, 0.01 M HEPES buffer solution) consists of one sharp band at 382 nm and a broad absorption with peaks at 324, 310 nm and 296 (sh) nm (Fig. 5). A weak, broad d-d band is observed at 703 nm (Fig. S7). Spectrophotometric titrations of 0.01 mM solution of the Cu (II) complex with 0.1 mM solution of histidine in aqueous medium (pH 7.4, 0.01 M HEPES buffer solution) reveals growth of a new peak at around 286 nm along with disappearance of the peaks at 324, 310 nm and 296 nm; the intensity of the band at 382 nm decreases and it undergoes a hypsochromic shift to 370 nm. An isosbestic point is observed at 298 nm. This clearly shows that on sequential addition of histidine to the Cu(II) complex, histidine displaces the ligand($LH_2\cdot 2H_2O$) to bind with Cu(II).

To confirm that the mechanism of the sensing reaction is displacement of the pyridoxal ligand from the coordination sphere of Cu(II) by histidine, we added an excess amount of histidine (complex: histidine = 1:3 mol ratio) solution to the aqueous solution of the $[Cu(LH_2)Cl_2]\cdot 2H_2O$ complex and the mixture was stirred for 5 h. The dark green colour of the solution faded to light green. The reaction mixture was evaporated to dryness and a white coloured substance was separated through column chromatography. The ESI-MS (225.12 [$LH_2 + 2H^+$ (100%)], 371.53 [$Cu(His)_2$ complex]) of the reaction mixture of Cu(II)-complex with L-histidine (Fig. S11), $^1H$ NMR (Fig. S12), $^{13}C$ NMR (Fig. S13), UV-vis and fluorescence spectral study of the white colored solid obtained by reacting excess histidine with the copper complex also confirmed the formation of the free ligand (Figs. S14 and S15, details isolation procedure is described in ESI). A Job's plot analysis was performed to further investigate the binding of the




**Scheme 1.** Sensing mechanism with 4-level ESIPT diagram of the free ligand ($LH_2{\cdot}2H_2O$).

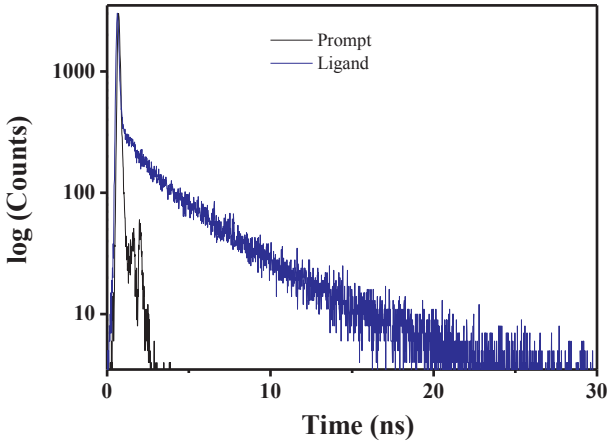



**Fig. 4.** Time-Correlated Single Photon Counting (TCSPC) plot of the isolated product in water.

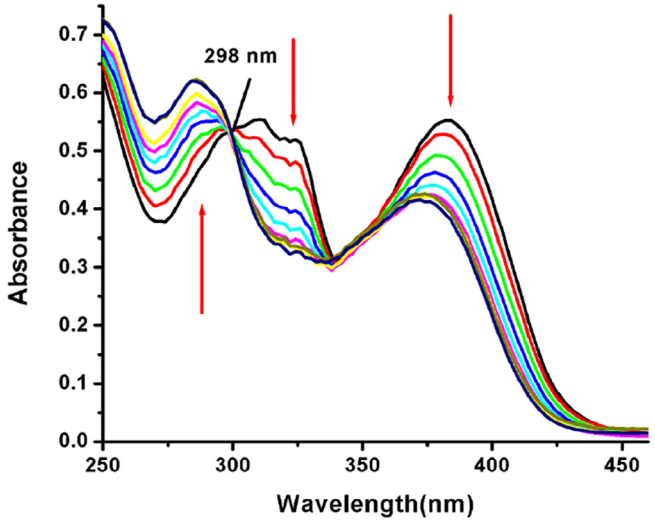



**Fig. 5.** UV-Visible titration spectra of $[Cu(LH_2)Cl_2]{\cdot}2H_2O$ ($c = 1.0 \times 10^{-5}$ M) in the presence of histidine ($c = 1.0 \times 10^{-4}$ M) in $H_2O$ (pH 7.4, 0.01 M HEPES buffer solution).

title Cu(II) complex with histidine (Fig. S16). The 1:2 stoichiometric ratio between Cu(II)-His also confirmed our prediction.

To examine the selectivity of the sensing of His, a competition experiment was carried out upon addition of histidine into solutions that already contained the chemosensor $[Cu(LH_2)Cl_2]{\cdot}2H_2O$ with 5 times excess of other amino acids. As shown in Fig. S17 it is clear that while no significant fluorescence intensity changes were observed with excess amount of other amino acids, under identical conditions presence of histidine shows fluorescence enhancement.

As histidine is one of the most essential bioactive amino acid components of different proteins we next tried to further evaluate the interferences of co-existing substances (biological species) on the fluorescence response of our proposed turn-on sensor towards histidine. No significant fluorescence change was observed when excess amount of these biological species including ascorbic acid, $Ca^{2+}$ , $Fe^{3+}$, glucose, $K^+$, $Na^+$ and urea were added. But, addition of histidine, under identical condition, showed fluorescence enhancement (Fig. S18) which indicated the potential utility of our fluorescent probe $[Cu(LH_2)Cl_2]{\cdot}2H_2O$ for the determination of histidine in biological fluids. The detection limit for histidine was determined as 1.89 μM with $[Cu(LH_2)Cl_2]{\cdot}2H_2O$ based on K* Sb1/S, where Sb1 is the standard deviation and S is the slope of the calibration curve (Fig. S19).

### 3.2. Electrochemical studies

We studied the cyclic voltammogram (CV) of $[Cu(LH_2)Cl_2]{\cdot}2H_2O$ upon treatment with histidine (Table S6 in ESI) [66]. Initially, a reduction at around −0.14 V is observed for the Cu(II) complex in DMF. On successive addition of histidine in DMF containing TEAP as supporting electrolyte, the peak is shifted to −0.04 V. On the anodic side, an initial quasi-reversible couple at 0.45 V ($\Delta E_p$ = 130 mV) is replaced by an irreversible oxidative peak at 0.48 V upon treatment with various equivalents of histidine (Fig. 6a). Very similar cyclic voltammograms were observed when $CuCl_2{\cdot}2H_2O$ solution was treated with various equivalents of histidine (Fig. S20 in ESI). The similarity of cyclic voltammograms of $[Cu(LH_2)Cl_2]{\cdot}2H_2O$ after treatment with excess histidine with those of $CuCl_2{\cdot}2H_2O$ with histidine also confirmed that histidine displaces the Cu(II) center of $[Cu(LH_2)Cl_2]{\cdot}2H_2O$ to release free ligand ($LH_2$) and restore its fluorescence.

To study the selectivity of histidine over other amino acids we carried out the CV of $[Cu(LH_2)Cl_2]{\cdot}2H_2O$ upon treatment with excess phenyl alanine. No significant change from the initial CV of $[Cu(LH_2)Cl_2]{\cdot}2H_2O$ confirmed that phenyl alalnine is unable to release free ligand (Fig. 6b).

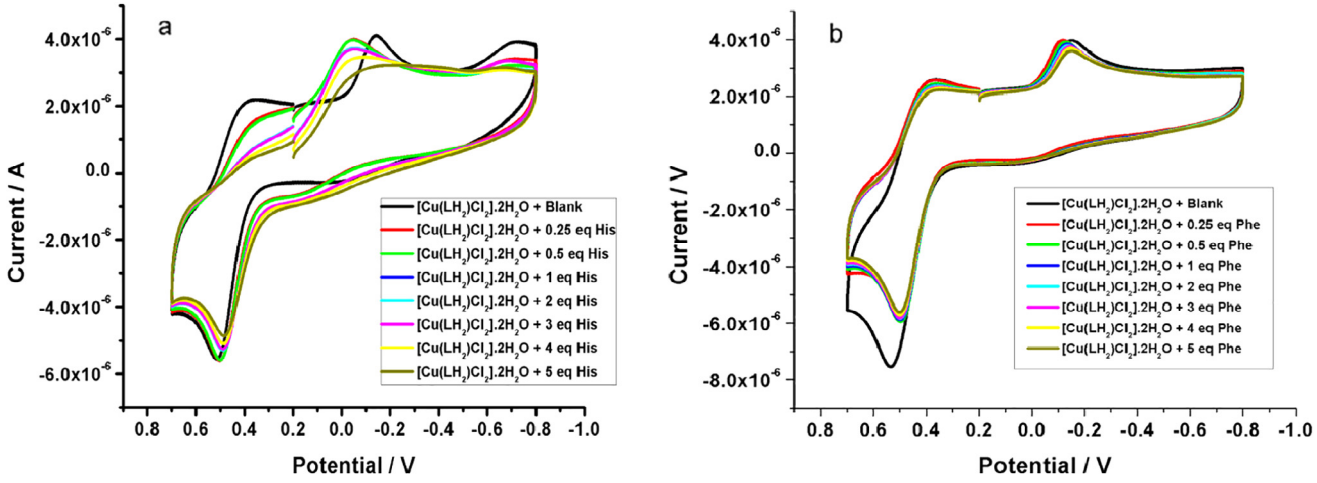



**Fig. 6.** CV titration profiles of (a) $[Cu(LH_2)Cl_2]\cdot 2H_2O$ (c = $1.0 \times 10^{-3}$ M) with various amounts of Histidine and (b) $[Cu(LH_2)Cl_2]\cdot 2H_2O$ (c = $1.0 \times 10^{-3}$ M) with various amounts of Phenyl alanine.

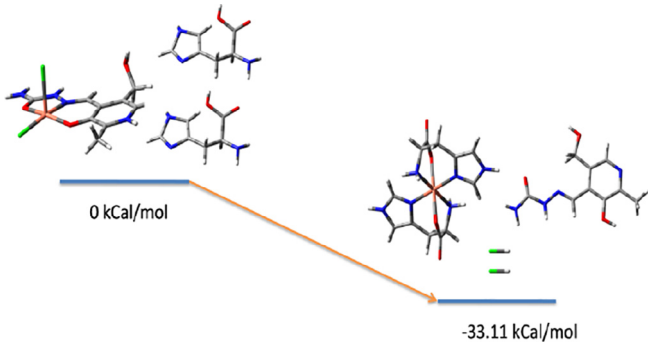



**Fig. 7.** Energy profile diagram of the sensing mechanism.

**Table 1**
Analytical results for different histidine concentrations in human urine samples.

| Sample No. | Added(μM) | Found (mean μM) | Recovery (%) |
|---|---|---|---|
| 1 (Female) | 10 | 9.92 | 99.20 ± 3 |
| | 20 | 19.57 | 97.85 ± 2 |
| | 30 | 28.43 | 94.76 ± 5 |
| 2 (Male) | 10 | 9.89 | 98.90 ± 4 |
| | 20 | 19.33 | 96.65 ± 5 |
| | 30 | 28.95 | 96.50 ± 2 |

**Table 2**
Recovery values for different histidine concentrations in human urine samples by various complexes.

| Sl No. | Complex | Recovery (%) | Ref. |
|---|---|---|---|
| 1 | $CuL^1$ | 94.7–98.8 | 67 |
| 2 | D5-Ag NCs/$Cu^{2+}$ | 90.2–102.3 | 68 |
| 3 | HCy-CDTe QDs | 94.4–106 | 69 |
| 4 | Biomembrane electrode | 98.6–113.2 | 72 |

$L^1$ – derived from 2-(2-aminophenyl)benzimidazole and 2,3-dihydroxybezaldehyde.

### 3.3. Theoretical calculation

DFT calculation was performed using Gaussian 03 package (revision B.04). When we calculate the overall energy of the following reaction, the right hand side is more stable by 33.11 kcal/mol.

$[Cu(LH_2)Cl_2]$ + 2 Histidine = $Cu(histidine)_2$ + 2HCl + Free ligand ($LH_2$).

On addition of histidine to the Cu(II)-complex the ligand gets displaced from the Cu(II)-complex and the fluorescence is restored (Fig. 7 and Figs. S21–S23; details description given in ESI). This is due to the extra stability which leads to the formation of Cu-histidine complex and free ligand.

### 3.4. Detection of histidine in urine samples

To explore the practical applicability of the fluorescence probe $[Cu(LH_2)Cl_2]\cdot 2H_2O$ in biological fluids, we collected human urine samples from healthy adults and diluted 150 times with water before using [67–70]. On addition of the urine sample to $[Cu(LH_2)Cl_2]\cdot 2H_2O$ fluorescence enhancement was observed (Fig. S24). On comparison of the intensity with that of the fluorescence titration upon addition of histidine, the average histidine concentrations in two urine samples were determined to be 450 μM (female) and 900 μM (male) respectively (normal level of histidine in human urine: 130–2100 μM) [71,72].

Next different amounts of histidine were spiked into the urine and the fluorescence of the mixtures was measured to detect the histidine concentration. The recovery values ranging from 94.8 to 99.2% are calculated for 10 μM, 20 μM and 30 μM of histidine respectively (Table 1). The error bar in each analysis is represented in Fig S25.

The % of recovery values for various complexes have been reported in literature and summarized in Table 2.The results suggested that $[Cu(LH_2)Cl_2]\cdot 2H_2O$ is a promising chemosensor for the detection of histidine in biological systems.

### 3.5. Cell imaging study

To examine whether $[Cu(LH_2)Cl_2]\cdot 2H_2O$ can sense histidine in living cells, *Saccharomyces cerevisiae* (Baker's yeast), one of the most intensively studied eukaryotic model organisms in molecular and cell biology, was used (detailed description is given in ESI). The yeast cells were incubated with $[Cu(LH_2)Cl_2]\cdot 2H_2O$ alone for overnight, which exhibited no background fluorescence initially but after 2 h a weak fluorescence was observed due to hydrolysis. Then the cells were incubated with 50 μM histidine solution and after 30 mins with $[Cu(LH_2)Cl_2]\cdot 2H_2O$, when the incubated yeast cell displayed a enhanced blue fluorescence in DAPI range (Fig. 8). From Differential interference contrast (DIC) microscopic image it is also clear that the structure of the cell remain unaffected during the whole sensing process. These experiments indicate $[Cu(LH_2)Cl_2]\cdot 2H_2O$ can be used to detect histidine in living cells. The MTT assay for $[Cu(LH_2)Cl_2]\cdot 2H_2O$ was conducted, and the results showed that the sensor can be used with a concentration of 50 μM safely (Fig. S26).

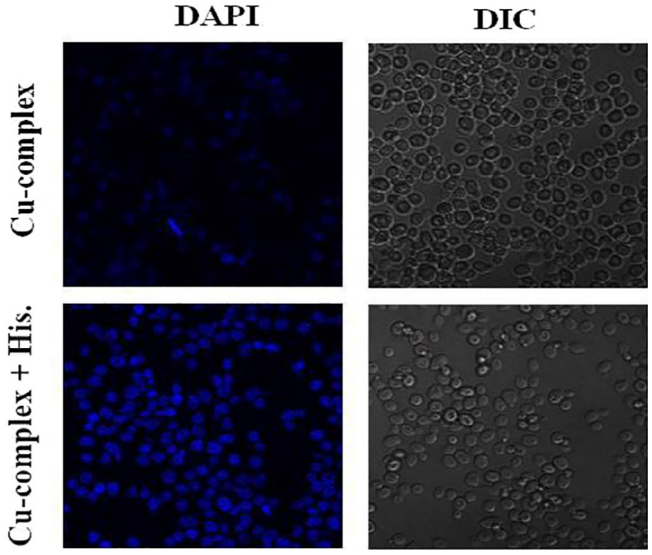



**Fig. 8.** Fluorescence image of yeast cell treated with only $[Cu(LH_2)Cl_2]\cdot 2H_2O$ and $[Cu(LH_2)Cl_2]\cdot 2H_2O$ with addition of histidine.

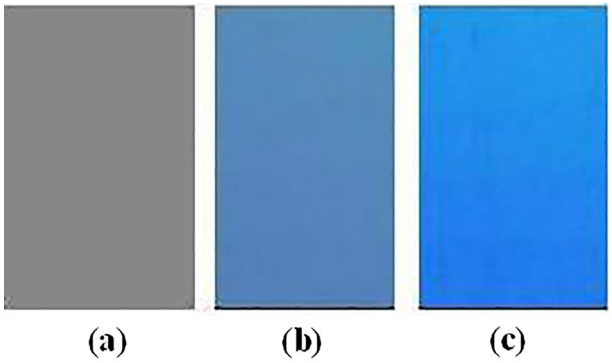



**Fig. 9.** Color changes visualized on TLC plate strips of (a) $[Cu(LH_2)Cl_2]\cdot 2H_2O$ (c = $1.0 \times 10^{-3}$ M) and during addition of Histidine at (b) $2.0 \times 10^{-5}$ M and (c) $2.0 \times 10^{-4}$ M in water.

### 3.6. Application in development of low cost devices

Prompted by the high sensitivity and selectivity of our complex towards histidine we prepared test strips to demonstrate its practical application. TLC plates coated with $[Cu(LH_2)Cl_2]\cdot 2H_2O$ were immersed in aqueous solution of histidine in different concentrations and then dried in air to determine the suitability of a "dip stick" method for the detection of histidine. These test strips confirmed clear color changes under irradiation with a UV lamp, and the detectable histidine could be as low as 20 μM (Fig. 9). As instant qualitative information is obtained, without any instrumental requirement, development of such dipsticks is very useful.

## 4. Conclusions

Using a ONO donor tridentate ligand we have synthesized a square-pyramidal Cu(II) complex $[Cu(LH_2)Cl_2]\cdot 2H_2O$. The Cu(II) complex can be used as a selective, multichannel sensor for histidine in biological samples using Uv-Vis and fluorescence spectroscopy, cyclic voltammetry or a dip stick method. It is shown that in presence of histidine, the tridentate ligand as well as the chlorides are displaced from the coordination sphere yielding $[Cu(his)_2]$complex. The free ligand shows ESIPT driven fluorescence, signaling the presence of histidine. The estimation of histidine in human urine samples and imaging of histidine in yeast cells demonstrate the application of the complex in detection of histidine in biological samples.

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## Appendix A. Supplementary data

X-ray crystallographic data in CIF format, mass spectra, $^1$H NMR spectra, $^{13}$C NMR spectra, IR spectra, FMOs of complex **1** and other characterization data. Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ica.2018.06.017.

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