Quinazoline Copper (II) ensemble as turn–on fluorescence sensor for Cysteine and Chemodosimeter for NO.
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Abstract

A fluorescent quinazoline based chemosensor 3-(((2-hydroxynaphthalen-1-yl) methylene) amino)-2-thioxo-2, 3-dihydroquinazolin 4 (1H)-one (QHYN) had been designed and synthesized. It exhibits high sensitivity and selectivity towards Cu$^{2+}$ over other metal ions in DMSO:H$_2$O (1:9, v/v) at pH 7.4 (HEPES buffer) by fluorescence quenching. Addition of nitric oxide to a solution of this QHYN·Cu$^{2+}$ restores the fluorescence. This is attributed to the reduction of the Cu$^{2+}$ center by nitric oxide to diamagnetic Cu$^+$. The fluorescence response of QHYN·Cu$^{2+}$ to NO is direct and specific, which is a significant improvement over commercially available small molecule-based chemodosimeter probes that are capable of detecting NO. The QHYN·Cu$^{2+}$ also acts as an efficient “off–on” fluorescent sensor for Cysteine with high sensitivity.
4.1 Introduction

Among the various transition metal ions, Cu$^{2+}$ is an important metal nutrient for metabolism of life and has been widely used in various biological processes\(^1\). It is the third most abundant soft transition metal ion in human body\(^2\). Cu$^{2+}$ involves in bio functions such as electron transport, iron transport, O$_2$ metabolism, and oxidation-reduction reactions. It is a catalytic cofactor for a variety of metalloenzymes including superoxide dismutase, cytochrome oxidase and tyrosinase\(^3\). Besides, Cu$^{2+}$ participates in hemoglobin synthesis (in Fe utilisation and Hb regeneration), connective tissue development, normal functions of central nervous systems and oxidative phosphorylation\(^4\). The brain needs much higher level of copper compared to other parts of the body\(^5\).

Despite these vital roles, exposure to a high level of copper even for a short period of time can cause gastrointestinal disturbance, and long-term exposure can cause liver or kidney damage\(^6\). In addition excess level of Cu$^{2+}$ ion can also be detrimental because it can promote the formation of reactive oxygen species. These species directly oxidises protein side chain that can cause many amyloid diseases such as Alzheimer, menkes, Parkinson, prion and dialysis-related amyloidosis\(^7\). On the other hand deficiency of copper in human body effects uncontrolled redox activity. This can cause aberrant oxidative and nitrosative stress which sometimes lead to cancer\(^8\). The world health organisation (WHO) has set the upper limit of copper in drinking water to be 2 mg/L (31 µM)\(^9\). The homeostasis of Cu$^{2+}$ necessitates developing sensors for detecting and monitoring copper ions in bio systems. Currently, variety of methods are available for detection of Cu$^{2+}$ ions. The conventional methods for detection of copper ions in water samples such as photometric methods, atomic absorption spectroscopy (AAS), inductively coupled plasma emission or mass spectrometry (ICP-ES, ICP-MS), total reflection X-Ray fluorimetry (TXRF)
and anodic stripping voltammetry (ASV) provide good detection limits but these methods are non-preferable as these techniques not only require high cost analytical instrumentation but also has operational difficulties\(^\text{10}\). These techniques when adopted for the biological sample analysis usually results in destruction of the cells. As an alternative, fluorescent sensors have been demonstrated to be powerful tools for the non-destructive imaging of intracellular distribution in single cells\(^\text{11}\). Yet mapping metals in cellular sub-compartments within the cell is a necessary step in understanding metal homeostasis. Fluorescence is well-suited for imaging small optically transparent organisms and increasingly for intra-vital imaging as well.

### 4.1.1 Fluorescence based Cu\(^{2+}\) sensor

Kim \textit{et al.} have displayed that 2-picoly diamine appended coumarin derivative 1 as efficient tridentate complexation for Cu\(^{2+}\) in preference to a variety of other common heavy and toxic metal ions\(^\text{12}\). Addition of Cu\(^{2+}\) quenches the fluorescence intensity of this probe. The quenching mechanisms were elucidated by the time-resolved fluorescence and \textit{ab-initio} calculations. It is also employed for the fluorescence changes of intracellular Cu\(^{2+}\) in cultured cells. Yang \textit{et al.} reported quinoiline based novel fluorescence sensor 2 for Cu\(^{2+}\) that works in aqueous medium\(^\text{13}\). It exhibits excellent selectivity towards Cu\(^{2+}\) ion by quenching the fluorescence of 2. This quenching phenomenon via attributed to photoinduced electron transfer. Coordination of Cu\(^{2+}\) and 2 stands as an interesting 1D chain coordination polymer framework.
Guan et al. designed a novel Rhodamine-quinoline compound 3 as a sensor for Cu\textsuperscript{2+} ions\textsuperscript{14}. It shows excellent colorimetric response towards Cu\textsuperscript{2+} ion in aqueous medium. The presence of Cu\textsuperscript{2+} induces fluorescence enhancement via spirolactum ring opening. Mukherjee et al. displayed hydroxy-naphthyl-hydrazone 4 as sensor for Cu\textsuperscript{2+} ion. In the presence of Cu\textsuperscript{2+}, fluorescence was quenched due to the reverse photoinduced electron transfer mechanism\textsuperscript{15}. It can detect Cu\textsuperscript{2+} in nanomolar range. In addition, the receptor detect Cu\textsuperscript{2+} ions in human cervical HeLa cancer cell.

![Chemical structures](image)

Chellappa et al. developed imidazoquinoline 5 as a selective fluorescent and colorimetric sensor for Cu\textsuperscript{2+} ion in aqueous CH\textsubscript{3}CN medium\textsuperscript{16}. It shows a distinct colour change from yellow to green. 5 shows turn-on response towards Cu\textsuperscript{2+} via ICT mechanism. The probe was found to be applicable for imaging intracellular Cu\textsuperscript{2+} in HeLa cells. It can detects Cu\textsuperscript{2+} in 2.6×10\textsuperscript{-9} mol L\textsuperscript{-1}.
4. 1. 2 Fluorescence sensor for nitric oxide (NO) examples

Nitric oxide, a small uncharged free radical, containing one unpaired electron, plays an important role in human physiology. It is an intra and extra cellular messenger molecule\(^\text{17}\). NO is identical with endothelium-derived relaxing factor (EDRF), which is biosynthesised in body. NO can be produced from the oxidation of ammonia, conversion of L-arginine to L-citrulline\(^\text{18}\) and incomplete combustion of gasoline from vehicles. NO at a moderate concentration plays a critical role in physiological process such as vasodilation, synaptic activity and neurotransmission\(^\text{19}\). Dysregulation of NO production has been associated with pathological conditions such as cancer, ischemia, septic shock, inflammation and neurodegeneration\(^\text{20}\). In addition NO, by virtue of relatively stable nature can react with various reactive oxygen species that cause damage to DNA, lipids and proteins\(^\text{21}\). Recently, lysosomal functions are found to be regulated by NO. NO exhibits unique effects in the autophagy process, which degrades unnecessary/dysfunctional cellular components through the lysosomal machinery to suppress energy and nutrient sources for cell growth\(^\text{22}\). It would be beneficial therefore to develop fluorescence sensors\(^\text{23}\) that allow the bioimaging which in turn aids elucidation of biological functions of NO.

Prasad et al. developed a probe 6 that bears the fluorophore anthracene moiety fastened to acyl hydrazone\(^\text{24}\). The presence of Cu\(^{2+}\) triggers aggregation of the molecules thereby leading to significant enhancement of the emission.
Cu$^{2+}$ bound probe when exposed to NO selectively quenches the fluorescence via de-aggregation system mechanism. The receptor senses Cu$^{2+}$/NO in micro and nanomolar range respectively. Lippard et al. introduced 7 dansyl aminoquinoline based Co$^{2+}$ complexes as a sensor for NO$^{25}$. Fluorescence of the ligand was significantly quenched in CH$_3$CN solvent when Co$^{2+}$ was added to it. Addition of NO to the probe-Co$^{2+}$ system enhanced fluorescence of the solution. $^1$H-NMR and IR data, also reveals the formations of Co-nitrosyl adduct with dissociation of one ligand from the cobalt center. Qian et al. developed a new naphthalimide based sensor 8 for NO$^{26}$. Cu$^{2+}$ bound naphthalimide probe 8 renders the direct NO detection in 30 nM range. It displays a large 200 nm blue-shifted signal due to the disturbance of internal charge transfer.

Nagano et al. reported 9 tricarbocyanine as a NIR fluorophore, O-phenylenediamine as a binding unit for NO in sodium phosphate buffer pH 7.4$^{27}$. O-phenylenediamine should quench the fluorescence of tricarbocyanine because electron transfer occur from o-phenylenediamine to the excited fluorophore. Addition of NO forms triazole should recover its NIR fluorescence because the triazole ring should not have sufficient electron-
donating ability for such photoinduced electron transfer. This probe was further applied in to the isolated rat kidney. Xiao et al. displayed ratiometric fluorescent probe 10 has excellent selectivity and quick response to NO\textsuperscript{28}. In the presence of NO, fluorescent colour was changed immediately from green to red. Receptor 10 was successfully employed for imaging intracellular NO.

4.1.3 Fluorescence sensor for amino acid examples

Amino acids are building blocks for proteins and especially thiol containing amino acids play crucial role in biological system\textsuperscript{29}. Intracellular thiols play a pivotal role in important functions such as bio-catalysis, metal binding, detoxification of xenobiotics and metabolic process\textsuperscript{30}. Cysteine was found to as a regulator in various diseases. In vivo the concentration of Cysteine is correlated to the physiological functions to diagnose the diseases. Cysteine and Homocysteine levels have been linked to many diseases such as Alzheimer’s, AIDS and cancer\textsuperscript{31}. The deficiency of Cys can cause slowed growth, liver damage, hair depigmentation, edema, lethargy, muscle, fat loss, skin lesions and weakness\textsuperscript{32}. So rapid sensitive analysis of intracellular thiols is highly demanded\textsuperscript{33}.
Shao et al. designed a new bodipy- based bifunctional chemosensor 11 for Cu$^{2+}$ and thiol containing aminoacids$^{34}$. Upon binding with Cu$^{2+}$ the probe becomes non-fluorescent. This chemosensing ensemble afforded real time detection of Cys with high sensitivity and selectivity via ligand to metal charge transfer mechanism (LMCT) in CH$_3$CN:H$_2$O buffer solution pH-7.4. Detection limit of Cys is 0.17 μM and Cu$^{2+}$ is 0.1 μM. Zhang et al. prepared 8-hydroxyquinoline based probe 12. Its copper complex serves as a colorimetric probe for Cysteine in water through a displacement reaction$^{35}$. Addition of Cysteine shows an obvious colour change from yellow to red.

Su et al. displays fluorescent conjugate polymer for the detection of Cys and Cu$^{2+}$ ion$^{36}$. Cu$^{2+}$ quenches the fluorescence intensity of polymer through electrostatic interaction and electron transfer mechanism between polymer and Cu$^{2+}$. In the presence of thiols like Cysteine, Cu$^{2+}$ reacts with sulphur and displaces the Cu$^{2+}$ center. So the fluorescence of polymer was restored. Detection limit for Cysteine is 4.5×10$^{-8}$ mol/L. Polymer:Cu$^{2+}$ system was further used for fluorescence imaging of thiols in HepG$_2$ cells (Scheme 4. 1).
Huang et al. developed 13, an azo dye as a colorimetric sensor for Cysteine\textsuperscript{37}. Azo compound containing aldehyde groups react with Cysteine to give a very stable derivative of thiazinanes under neutral pH conditions. Addition of Cysteine gave conspicuous colour change from pink to yellow. Kim et al. displays a coumarin fluorophore 14 as a fluorescence sensor for thiol-containing amino acids\textsuperscript{38}. Receptor bearing double bond conjugated quinolone unit undergone a Michael type addition with thiol containing amino acids. It can detect Cys in $10^{-7}$ nm range. This system was successfully applied to the fluorescence imaging of intracellular Cys in HepG\textsubscript{2} cells.
Here in we report the synthesis and photophysical characterization of Cu$^{2+}$ complexes with quinazoline bearing 2-hydroxy-1-naphthaldehyde, that shows speedy and selective response to NO and Cysteine over other species.

4.2 Results and Discussion

The receptor QHYN was synthesized by reported procedure$^{39}$ (Scheme 4.2). The structure of 2-hydroxy naphthaldehyde derived quinazoline (QHYN) was confirmed by the analytical techniques.

\[ \text{2-aminobenzohydrazide} + \text{CS}_2 \xrightarrow{\text{Reflux 15 hr}} \text{QHYN} \]

\[ \text{A1} + \text{2-Hydroxy-1-naphthaldehyde} \xrightarrow{\text{Reflux 4 hr}} \text{QHYN} \]

Scheme 4.2 Synthesis of probe QHYN

Initially the binding behaviour of QHYN towards different biologically relevant metal cations such as Na$^+$, K$^+$, Ba$^{2+}$, Ca$^{2+}$, Al$^{3+}$, Co$^{2+}$, Fe$^{3+}$, Ni$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Ag$^+$ and Cr$^{3+}$ using their chloride salts was carried out by UV-Vis absorption method as illustrated in (Fig. 4.1a). The QHYN shows three absorption bands at 246, 278, 324 nm in DMSO:Η₂Ο (1:9, v/v) at pH 7.4 (HEPES buffer). During the addition of Cu$^{2+}$ ions to the solution of QHYN the absorbance of QHYN at 246 nm increased gradually and the peak at 328 nm decreased. Moreover a new absorption band at 397 nm emerged gradually along with the addition of increased amounts of Cu$^{2+}$ ions,
accompanied by a clear colour change from yellow to green (Fig. 4.1b). Interestingly there is no significant colour change nor any spectral change of QHYN upon addition of other metal ions. Hence the receptor QHYN is a selective and sensitive sensor for Cu^{2+} ions.

![UV-Vis spectra of probe QHYN upon addition of Cu^{2+} ion and other transition metal ions such as Na^+, K^+, Ba^{2+}, Ca^{2+}, Al^{3+}, Co^{2+}, Fe^{3+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Ag^+ and Cr^{3+} (0-1 equivalent).](image-a)

![UV-Vis spectra of probe QHYN upon addition of Cu^{2+} ion (0-1 equivalent) in DMSO:H_2O (1:9, v/v) at pH 7.4 (HEPES buffer).](image-b)

Fig. 4.1 (a) UV-Vis spectra of probe QHYN upon addition of Cu^{2+} ion and other transition metal ions such as Na^+, K^+, Ba^{2+}, Ca^{2+}, Al^{3+}, Co^{2+}, Fe^{3+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Ag^+ and Cr^{3+} (0-1 equivalent). (b) UV-Vis spectra of probe QHYN upon addition of Cu^{2+} ion (0-1 equivalent) in DMSO:H_2O (1:9, v/v) at pH 7.4 (HEPES buffer).

The fluorescence spectra of QHYN in DMSO:H_2O (1:9, v/v) at pH 7.4 (HEPES buffer) exhibited a strong green emission band at 434 and 494 nm with the quantum yield value being 0.48, when excited at 325 nm. The fluorescence of QHYN is almost completely quenched with the addition of Cu^{2+} ions. No obvious changes in emission spectra were observed upon addition of Na^+, K^+, Ba^{2+}, Ca^{2+}, Al^{3+}, Co^{2+}, Fe^{3+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Ag^+ and Cr^{3+} (Fig. 4.2a). The green fluorescent QHYN when titrated with the addition of Cu^{2+} undergoes a gradual decrease in the emission bands at 434 and 494 nm. The fluorescence of QHYN was completely quenched after 1 equivalent addition of copper ions. (Fig. 4.2b). These results summarize the formation of well-defined complex between receptor QHYN and Cu^{2+} ions.
Fig. 4.2 (a) Fluorescence emission spectra of QHYN upon addition of Cu\(^{2+}\) ion and other transition metal ions such as Na\(^+\), K\(^+\), Ba\(^{2+}\), Ca\(^{2+}\), Al\(^{3+}\), Co\(^{2+}\), Fe\(^{3+}\), Ni\(^{2+}\), Mn\(^{2+}\), Zn\(^{2+}\), Cd\(^{2+}\), Hg\(^{2+}\), Ag\(^+\) and Cr\(^{3+}\) (0-1 equivalent). (b) Fluorescence emission spectra of QHYN (10 \(\mu M\)) upon addition of Cu\(^{2+}\) ion (0-1 equiv.) in DMSO:H\(_2\)O (1:9, \(V/V\)) at pH 7.4 (HEPES buffer) (\(\lambda_{ex} = 325\) nm, \(\lambda_{em} = 434, 494\) nm, (slit: 5 nm/5 nm).

The selectivity of QHYN towards Cu\(^{2+}\) was examined by the fluorescence titration experiments of QHYN with biologically relevant miscellaneous metal ions. The other metal ions such as Na\(^+\), K\(^+\), Ba\(^{2+}\), Ca\(^{2+}\), Al\(^{3+}\), Co\(^{2+}\), Fe\(^{3+}\), Ni\(^{2+}\), Mn\(^{2+}\), Zn\(^{2+}\), Cd\(^{2+}\), Hg\(^{2+}\), Ag\(^+\) and Cr\(^{3+}\) exhibit almost no fluorescence quenching under the same condition (Fig. 4.3). Hence it is obvious that the receptor QHYN is highly selective fluorescent sensor for Cu\(^{2+}\) ions. To investigate the influence of various metal ions the experiment was carried out by adding 1 equivalent of Cu\(^{2+}\) to the solution of QHYN in the presence of one equivalence of other metal ions. The result shows there is no significant change in the fluorescence quenching compared with that observed by the addition of Cu\(^{2+}\) alone to the probe QHYN solution (Fig. 4.4). So it exhibits excellent selectivity for Cu\(^{2+}\) over other cations. Further to confirm the
paramagnetic nature of Cu$^{2+}$ and geometry of complex, EPR spectra was recorded at liquid nitrogen temperature (Fig. 4. 5).

![Fig. 4. 3](image)

**Fig. 4. 3** Bar chart illustrating fluorescence response of probe QHYN and one equiv. of various metal ions. **Fig. 4. 4** Bar chart illustrating selective fluorescence response of probe QHYN ($5.0 \times 10^{-6}$ M) for Cu$^{2+}$ ion in the presence of other metal ions ($5.0 \times 10^{-6}$ M) DMSO:H$_2$O (1:9, v/v) at pH 7.4 (HEPES buffer) ($\lambda_{ex} = 325$ nm, $\lambda_{em} = 434, 494$ nm, slit: 5nm/5nm).

The QHYN-Cu$^{2+}$ exhibited the characteristic four line pattern of Cu$^{2+}$ with the $g_{\parallel} = 2.212$, $g_{\perp} = 1.998$ and $A_{\parallel} = 197.2$. $g_{\parallel} > g_{\perp}$ indicates Cu$^{2+}$ complex has unpaired electron in the $d_{x^2-y^2}$ orbital. The $g_{\parallel}/A_{\parallel}$ value of 112 cm is consistent with the square planar geometry$^{40}$. The electrochemical behaviour of copper complex was studied in CH$_3$CN solvent. Cyclic voltammetric studies revealed reduction potential of 340 mV for Cu$^{2+}$/Cu$^{+}$ couple with respect to Ag/Ag$^+$ reference electrode (Fig. 4. 6).
Fig. 4.5  EPR spectra of QHYN with the addition of Cu$^{2+}$.  Fig. 4.6  Cyclic voltammograms of QHYN and Cu$^{2+}$ in CH$_3$CN with 0.5 M (Bu$_4$N)(PF$_6$) as supporting electrolyte, Pt- working electrode, Ag/AgCl as Reference electrode and a scan rate of 50 mV/s.

The stoichiometry of the complex between QHYN and Cu$^{2+}$ was found to be 1:1 by using jobs plot method (Fig. 4.7). It was further confirmed by ESI- MS wherein the molecular ion peak at m/z = 429 corresponds to (QHYN-2H$^+$+Cu$^{2+}$+H)$^+$ (Fig. 4.8). The fluorimetric titration profile shows a steady quenching of green fluorescence with increase in concentration of Cu$^{2+}$ (Fig. 4.9). The association constant$^{41}$ and the detection limit$^{42}$ were found to be 5.91×10$^5$ M$^{-1}$ and 20.0 nM respectively.

Fig. 4.7 The Job’s plot: mole fraction of Cu$^{2+}$ in solution of Cu$^{2+}$ and probe Vs fluorescence intensity at $\lambda_{em} = 434$ nm.
4.2.1 Nitric oxide detection (NO)

The emission spectra of QHYN showed typical fluorescence emissions at 434 and 494 nm. When 100 µM of Cu$^{2+}$ was added to the buffer solution of receptor QHYN (25 µM), the fluorescence was drastically quenched. This could be attributed to the paramagnetic nature of Cu$^{2+}$. On the other hand, addition of NO (50 µM) to the Cu$^{2+}$ bound QHYN ensemble solution was found to restore the emission intensity. On the basis of fluorescence titration the fluorescence intensity was found to be steadily increasing with increasing
concentration of NO (Fig. 4. 10). Upon interaction of Nitric oxide with the \textbf{QHYN$\text{Cu}^{2+}$} ensemble, \textit{Cu}$^{2+}$ is reduced to \textit{Cu}$^{+}$ with concomitant conversion of NO to NO$^{+}$ ion. The complete reduction of \textit{Cu}$^{2+}$ to \textit{Cu}$^{+}$ was evident from the EPR silent nature\textsuperscript{43} of the \textbf{QHYN$\text{Cu}^{2+}$} ensemble after treatment with NO (Fig. 4. 11). This resembles the earlier report of Lippard \textit{et al.}\textsuperscript{44} 

In the cyclic voltammetric studies, both cathodic and anodic peaks, present initially in the \textbf{QHYN$\text{Cu}^{2+}$} ensemble, disappeared with nitric oxide addition (Fig. 4. 12). This further substantiates that \textit{Cu}$^{2+}$ is completely reduced to \textit{Cu}$^{+}$. Probably the affinity of \textit{Cu}$^{+}$ towards the ligand \textbf{QHYN} ion is lesser than those of \textit{Cu}$^{2+}$ and the complex dissociates. The observation of a new peak at 1373 cm$^{-1}$ (Fig. 4. 13) in the IR spectra\textsuperscript{45} of ensemble after the addition of NO solution clearly shows that the NO was incorporated in the free probe \textbf{QHYN}. Hence it is obvious that this sensor has the potential of being a fluorescent chemodosimeter for NO. To check the selectivity and chemodosimetric response of the probe, 100 equivalent of reactive oxygen and nitrogen species such as \textit{ClO}$^{-}$, \textit{NO$_2$}$^{-}$, \textit{NO$_3$}$^{-}$, H$_2$O$_2$, ONOO$^{-}$, OH$^{-}$, t-BuOOH and O$_2$ was added and fluorescence monitored. No detectable fluorescence responses appeared, whereas the fluorescence intensity increased only when treated with NO solution. (Fig. 4. 14).
Fig. 4. 10 The photoluminescence spectra of \( \text{QHYN} \cdot \text{Cu}^{2+} \) (125 µM) DMSO:H\(_2\)O (1:9, v/v) at pH 7.4 (HEPES buffer) in the presence of increasing concentration of nitric oxide (0-100 µM). (\( \lambda_{\text{ex}} = 325 \, \text{nm}, \lambda_{\text{em}} = 434, 494 \, \text{nm}, \text{slit: 5 nm/5 nm} \)).

Fig. 4. 11 X-band EPR spectra of \( \text{QHYN} \cdot \text{Cu}^{2+} \) before and after addition of NO in CH\(_3\)CN solvent.

Fig. 4. 12 Cyclic voltammograms of \( \text{Cu}^{2+} \cdot \text{QHYN} \) ensemble and NO in CH\(_3\)CN with 0.5 M (Bu\(_4\)N)(PF\(_6\)) as supporting electrolyte, Pt- working electrode, Ag/AgCl as reference electrode and a scan rate of 50 mV/s. Fig. 4. 13 IR spectrum of \( \text{QHYN} \) s addition of NO.
Fig. 4.14 Fluorescence emission spectra of QHYN-Cu$^{2+}$ ensemble with NO and other reactive oxygen and nitrogen species in DMSO:H$_2$O (1:9, v/v) at pH 7.4 (HEPES buffer), ($\lambda_{ex} = 325$ nm, $\lambda_{em} = 434, 494$ nm, slit: 5 nm/5 nm).

4.2.2 Detection of Cysteine (Cys)

From the above studies it could be concluded that the displacement of Cu$^{2+}$ from the ensemble QHYN-Cu$^{2+}$ would restore the emission property of receptor. To investigate the ability of ensemble QHYN-Cu$^{2+}$ as sensor for amino acids, the fluorescence changes of solution were analysed with various amino acids. Emission was changed only with the addition of Cysteine, whereas other amino acids produce negligible changes in the ensembles fluorescence spectra. To further explore the selectivity for Cysteine, fluorescence experiments were carried out with solution of ensemble (125 µM) in the presence of other amino acids such as Ala, Thr, Ser, Gly, Glu, Arg, Val, Trp, Lys, Pro, Ile, Leu, Asp, Met, Tyr, Phe, His, and GSH (500 µM) (Fig. 4.15a). The fluorescence results show no obvious changes in the emission spectra even under higher concentration of these aminob acids (Fig. 4.15b). However, incremental addition of Cysteine (Cys) (50 µM) to the ensemble restored the fluorescence (Fig. 4.16). The change in the position and shapes of the cathodic and anodic peaks of the ensemble QHYN-Cu$^{2+}$ with the addition of Cysteine displays the change in the environment around copper center (Fig. 4.17).
Yu et al. reports the same observation in the presence of amino acid in copper ensemble\textsuperscript{46}. It suggests that the Cysteine coordinates to the copper. Obviously these results show that the QHYN-Cu\textsuperscript{2+} is working as a selective and sensitive detector of Cysteine. To find the binding constant, first Cu\textsuperscript{2+} (100 \textmu M) was added to QHYN (25 \textmu M) to effect, complete fluorescence quenching. Then to the QHYN-Cu\textsuperscript{2+} ensembles Cysteine was gradually added to perform fluorescence titration (Fig. 4. 18). From the titration association constant was calculated as 1.54×10\textsuperscript{4} M\textsuperscript{-2} and the detection limit was found to be 5.41×10\textsuperscript{-7} M. The binding stoichiometry was confirmed by ESI-MS wherein the molecular ion peak at m/z = 327 corresponds to (2Cys-2H\textsuperscript{+}+Cu\textsuperscript{2+})+Na (Fig. 4. 19).

**Fig. 4. 15a** Fluorescence intensity diagram of QHYN-Cu\textsuperscript{2+} (125 \textmu M) upon addition of various amino acids (500 \textmu M). **Fig. 4. 15b** The fluorescence spectra of QHYN (25 \textmu M) and Cu\textsuperscript{2+} (100 \textmu M) in the presence of various amino acids (500 \textmu M) and Cysteine (0- 50 \textmu M) in DMSO:H\textsubscript{2}O (1:9, v/v) at pH 7.4 (HEPES buffer). (\lambda_{ex} = 325 nm, \lambda_{em} = 434, 494 nm, slit: 5 nm/5 nm).
**Fig. 4.16** Fluorescence emission spectra of QHYN + Cu$^{2+}$ upon incremental addition of Cysteine in DMSO:H$_2$O (1:9, v/v) at pH 7.4 (HEPES buffer). ($\lambda_{ex}$ = 325 nm, $\lambda_{em}$ = 434 nm, slit: 5 nm/5 nm).

**Fig. 4.17** Cyclic voltammograms of QHYN ensemble in CH$_3$CN and Cysteine with 0.5 M (Bu$_4$N)(PF$_6$) as supporting electrolyte, Pt- working electrode, Ag/AgCl as reference electrode and a scan rate of 50 mV/s.

**Fig. 4.18** The plot of fluorescence intensity of Cysteine (0-50 µM) with QHYN Cu$^{2+}$ in DMSO:H$_2$O (1:9, v/v) at pH 7.4 (HEPES buffer).
Fig. 4.19 ESI-MS spectrum of Cysteine with Cu^{2+}

4.3 DFT Calculations

In order to understand the fluorescence quenching process of QHYN with Cu^{2+}, DFT/TDDFT calculations were carried out with Gaussian 03 program\(^4^7\). The geometries of QHYN, QHYN.Cu^{2+} were optimized by B3LYP/6-31G and B3LYP/LANL2DZ methods respectively. In probe QHYN the HOMO resides on the naphthyl group and considerable amount of electron density is present in quinazoline ring, LUMO resides on the whole molecule except sulphur atom (Fig. 4. 20).

After binding of Cu^{2+} with probe QHYN most of the electron density of HOMO resides on sulphur atom, whereas LUMO is essentially centred on Cu^{2+}. LUMO+1 spread on the entire molecule except metal and sulphur. Hence HOMO-LUMO+1 transition corresponds to the transfer of electron from sulphur atom to \(\pi\) system. In other words the transition is \(n-\pi^*\) transition. The matching of the calculated energy gap of 316 nm between HOMO and LUMO+1 with that of excitation wavelength 325 nm suggests that \(n-\pi^*\) transition occurs when excited with \(\lambda_{ex} = 325\) nm. It is well known that the \(n-\pi^*\)
transition is slow as it has longer radiative lifetime\textsuperscript{48}. This slow process cannot compete with the fast non-radiative de-excitation pathways; hence the QHYN.Cu\textsuperscript{2+} is weakly fluorescent.

Fig. 4. Frontier molecular orbitals of QHYN and QHYN + Cu\textsuperscript{2+}
4. 4 Conclusion

In summary, the receptor QHYN that acts as a selective fluorescent and colorimetric sensor for Cu$^{2+}$ in DMSO:H$_2$O (1:9, v/v) at pH 7.4 (HEPES buffer) had been synthesized. Upon addition of Cu$^{2+}$, the fluorescence intensity undergoes proportional quenching thereby allowing for a sensitive and selective Cu$^{2+}$ sensing based on the fluorescence “turn-off”. More importantly, the non-fluorescent chemosensing ensemble “QHYN-Cu$^{2+}$” had been shown to be viable for the real time detection of Cysteine with high sensitivity and selectivity. Similarly, addition of nitric oxide to solution of the ensemble also resulted in a substantial increase of fluorescence intensity. The revival of the fluorescence intensity particularly in this case was attributed to the reduction of Cu$^{2+}$ to diamagnetic Cu$^{+}$. The non-fluorescent chemosensing ensemble “QHYN-Cu$^{2+}$” thus acts as sensor for Cysteine and displays chemodosimetric response for Nitric oxide.

4. 5. 1 Synthesis of Quinazoline (A1)

Ethanolic solution containing 2-aminobenzohydrazide (0.483g, 3.2 mmol), sodium hydroxide (0.13 g, 3.2 mmol) and carbon disulphide (0.25 g, 3.2 mmol) was refluxed for 15 hours. The excess solvent was distilled off under reduced pressure. The residue was washed with water and treated with 1:1 HCl and subsequently washed several times with water. Recrystallized from ethanol to get a white solid. Yield: 65%. Melting point: 205-207$^\circ$C.

4. 5. 2 Synthesis of QHYN

Ethanolic solution containing quinazoline A1 (0.618 g, 3.2 mmol), 2-hydroxy-1-naphthaldehyde (0.550 g, 3.2 mmol) was refluxed in the presence of catalytic amount of glacial acetic acid for 4 hours. The excess solvent was distilled off under reduced pressure. The residue was washed with water.
Recrystallization of crude with ethanol yields orange solid. Yield: 80%.

Melting point: 243-244°C. ESI-MS: observed: 346 (M-H)\(^-\) calculated: 347.

\(^1\)H-NMR (300 MHz, DMSO-d\(_6\)): ppm \(\delta\) 7.48 (d, 1H, \(J = 8.4\)Hz), 7.09 (d, 1H, \(J = 8.1\)Hz), 6.90 (d, 1H, \(J = 8.1\)Hz), 6.83 (d, 1H, \(J = 7.8\)Hz), 6.72 (t, 1H, \(J = 7.8\)Hz), 6.48-6.56 (m, 2H), 6.40 (t, 1H, \(J = 7.5\)Hz), 6.23 (d, 1H, \(J = 9.3\)Hz), 6.00 (d, 1H, \(J = 9.3\)Hz), 5.85 (t, 1H, \(J = 7.5\)Hz), 9.91 (s, 1H), 12.0 (s, 1H).

\(^{13}\)C-NMR (75MHz, DMSO-d\(_6\)): 189.0, 160.0, 156.9, 143.4, 134.5, 128.9, 125.4, 124.9, 123.7, 123.2, 120.3, 118.2, 114.9, 112.2, 111.9 (Fig. 4.21-23).

4. 6 References


Fig. 4. 21 $^1$H NMR spectrum of QHYN in DMSO-d$_6$.

Fig. 4. 22 $^{13}$C NMR spectrum of QHYN in DMSO-d$_6$. 
Fig. 4. 23 ESI – MS Spectrum of Probe QHYN.