Chapter V

Naphthalimide based fluorescent probe: Detection of Hg$^{2+}$ via PET-inhibition and Cu$^{2+}$ via paramagnetic quenching
5.1 Introduction

Chemosensors for the detection of heavy and transition metal (HTM) ions are especially important because some of these metal ions play crucial roles in living systems, and most of them exert a toxic impact in the environment.\textsuperscript{1-4} Mercury, as one of the most toxic metal ions, gets accumulated in human body and other higher organisms through the food chain.\textsuperscript{5} Though copper plays a crucial role in several physiological responses,\textsuperscript{6,7} excessive intake of copper leads to childhood cirrhosis (ICC).\textsuperscript{8,9} Excess concentrations of Cu\textsuperscript{2+} is also an environmental pollutant.\textsuperscript{10} Therefore, it is important to develop new multianalyte fluorescent probes for simultaneous detection of toxic metal ions including Hg\textsuperscript{2+} and Cu\textsuperscript{2+} ions present in aqueous samples.

Research on the development of such fluorescent probes has been attaining importance in recent years. These probes are also useful for designing molecular logic gates,\textsuperscript{11} and molecular keypad lock devices.\textsuperscript{12-14} The most important feature of molecular keypad lock over simple molecular logic gate is that its output signals are dependent not only the proper combination of the chemical inputs but also the correct sequence of inputs. Such a device can be used for numerous applications in which access is restricted to the exact password to open a device like the keypad lock. Although great effort has been put into the simultaneous detection of Hg\textsuperscript{2+} and Cu\textsuperscript{2+} ions,\textsuperscript{13} fluorescent sensor that can differentiate these two metal ions at molecular level remains a great challenge to modern researchers.\textsuperscript{14} Hence, chemical modification and screening of the currently available ligands are being explored to attain multianalyte fluorescent probes with different signal transduction modes for information protection.

The initiation or inhibition of photo-induced electron transfer (PET) processes as a mode of signal transduction has attracted considerable attention in the design of molecular level information processors.\textsuperscript{15} The derivatives of 1,8-naphthalimide fluorophore are the most appropriate examples for PET process, because the modulation of the fluorescence properties with different chemical inputs usually yields ‘turn-on’ or ‘turn-off’ type signal.\textsuperscript{16} Further, prevalent in the design of new probes for practical purposes, is the need for reversibility in order to provide continuous monitoring of metal ions. On the basis of the above considerations, herein, we have developed a new naphthalimide based single molecular probe \textbf{IV} that acts as a reversible molecular switch with different chemical inputs (Hg\textsuperscript{2+} and Cu\textsuperscript{2+} ions) and works as a molecular keypad lock.
5.2 Experimental section

5.2.1 Materials and methods

All the materials for synthesis were purchased from Sigma Aldrich, USA, and used as received. Column chromatography was performed on silica gel (200-400 mesh). Analytical grade solvents and double distilled water were used in all experiments. Pre-coated plates supplied by Merck, UK (silica gel 60 GF254, 0.25 mm) were used for TLC analysis. The solutions of different metal ions (Na\(^+\), K\(^+\), Zn\(^{2+}\), Co\(^{2+}\), Ca\(^{2+}\), Ni\(^{2+}\), Ba\(^{2+}\), Mn\(^{2+}\), Cd\(^{2+}\), Pb\(^{2+}\), Cu\(^{2+}\), Hg\(^{2+}\), Fe\(^{2+}\), Fe\(^{3+}\), Cr\(^{3+}\), and Al\(^{3+}\)) were prepared using their chloride salts, and for Ag\(^+\) solution, silver nitrate was used. \(^1\)H and \(^13\)C NMR spectra were recorded on a BRUKER 400 MHz spectrometer using TMS as internal standard. ESI-MS analysis was performed by a Thermo Finnigan LCQ Advantage MAX 6000 mass spectrometer. Absorption spectra were recorded on a SPECORD 200 PLUS UV-Visible spectrophotometer. Fluorescence measurements were performed on a Cary Eclipse spectrofluorometer (Excitation wavelength: 405 nm, Excitation and emission slit width: 5 nm). All measurements were carried out at room temperature.

5.2.2 Synthesis of 4-(4-piperazinyl)-N-propyl-1,8-naphthalimide (A)

The title compound (A) was synthesized according to the procedure reported in the section 2.2.3.

5.2.3 Synthesis of 2-bromo-N-(2-(benzothiazol-2-yl)phenyl)acetamide (b)

The title compound (b) was synthesized according to the procedure reported in the section 3.2.2.

5.2.4 Synthesis of naphthalimide derivative (IV)

The naphthalimide derivative IV, N-(2-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(1,3-dioxo-2-propyl-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)piperazin-1-yl)acetamide, was synthesized using the following procedure. To a solution of 4-(4-piperazinyl)-N-propyl-1,8-naphthalimide (A, 0.45 g, 1.4 mmol) and 2-bromo-N-(2-(benzothiazol-2-yl)phenyl)acetamide (b, 0.52 g, 1.5 mmol) in DMF (10 mL), K\(_2\)CO\(_3\) (0.19 g, 1.4 mmol) was added and stirred at 70°C for 3 h until the starting materials were consumed. The reaction mixture was diluted with cold water. The precipitate formed was separated by filtration and purified by column chromatography using hexane-ethyl acetate (60:40, v/v) mixture as eluent to afford V as yellow solid in 0.65 g yield (79%). Mp: 220-222°C.
**NMR and Mass data of N-(2-(benzo[d]thiazol-2-yl)phenyl)-2-(4-(1,3-dioxo-2-propyl-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)piperazin-1-yl)acetamide (IV)**

$^1$H NMR (400 MHz, CDCl$_3$): δ (ppm) 12.27 (s, 1H), 8.86 (d, $J = 8.4$ Hz, 1H), 8.57 (d, $J = 7.2$ Hz, 1H), 8.47 (d, $J = 8.0$ Hz, 1H), 8.37 (d, $J = 8.4$ Hz, 1H), 8.14 (d, $J = 7.4$ Hz, 1H), 7.96 (d, $J = 7.5$ Hz, 1H), 7.82 (d, $J = 7.8$ Hz, 1H), 7.68 (t, $J = 7.8$ Hz, 1H), 7.54-7.45 (m, 3H), 7.21 (t, $J = 7.6$ Hz, 1H), 6.95 (d, $J = 8.1$ Hz, 1H), 4.13 (t, $J = 7.5$ Hz, 2H), 3.46 (s, 2H), 3.23 (bs, 4H), 2.94 (bs, 4H), 1.79-1.73 (m, 2H), 1.01 (t, $J = 7.3$ Hz, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$): δ (ppm) 169.55, 168.20, 164.46, 164.07, 155.58, 153.41, 136.88, 134.28, 132.29, 131.79, 131.14, 130.52, 130.04, 129.80, 126.48, 126.23, 125.83, 125.82, 123.74, 123.32, 123.11, 121.79, 121.50, 120.75, 117.14, 115.14, 63.61, 53.59, 52.50, 41.81, 21.45, 11.56.

ESI-MS calculated for [C$_{34}$H$_{31}$N$_5$O$_3$S+H]$^+$: 590.17; found: 591.

![Scheme 5.1 Synthesis of naphthalimide derivative IV](image)

**5.2.5 Quantum chemical calculations**

The ground state geometry of IV was optimized using density functional theory (DFT) with B3LYP functional and 6-31G** basis set. Frequency calculations were performed on the same level of theory to ensure no imaginary frequencies were present to confirm that the optimized geometry was the local minima on potential energy surface. On the basis of ground state optimized geometry, the vertical excitation energies and associated oscillator strengths were calculated by performing time dependent DFT (TDDFT) method at CAMB3LYP/6-31G** level basis set. The CAM-B3LYP functional was useful to get accurate results for the excited state properties when dealing with the system with a
significant charge transfer characteristic. All calculations were performed using a Gaussian 09 program package.

5.3 Results and discussion

The 1,8-naphthalimide based fluorescent probe IV was synthesized as outlined in Scheme 5.1 and characterized using $^1$H, $^{13}$C-NMR and ESI-MS techniques (Figure 5.1-5.3). The $^1$H NMR spectrum of IV in CDCl$_3$ displayed a multiplet at ~1.78 ppm, triplets at 4.13 and 1.01 ppm arising from the propyl group attached to the naphthalimide moiety. The two broad peaks at 3.23 and 2.94 ppm were assigned to the two types of methylene protons on the piperazine moiety. The presence of a singlet at 3.46 ppm corresponding to the linker moiety (N-CH$_2$-C=O) confirmed the attachment of 2-bromo-N-(2-(benzothiazol-2-yl)phenyl)acetamide to the 4-(4-piperazinyl)-N-propyl-1,8-naphthalimide. The signals seen in the $^{13}$C NMR spectrum were in good agreement with the proposed structure. ESI-MS analysis of the probe IV also supported the proposed structure.

![Figure 5.1 $^1$H NMR spectrum of naphthalimide derivative IV in CDCl$_3$](image-url)
Figure 5.2 $^{13}$C NMR spectrum of naphthalimide derivative IV in CDCl$_3$

Figure 5.3 ESI-MS spectrum of naphthalimide derivative IV
The metal ion sensing ability of IV was tested using MeCN/H$_2$O (70% v/v) mixture containing different metal ions (Na$^+$, Ag$^+$, Zn$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Ba$^{2+}$, Pb$^{2+}$, Cu$^{2+}$, Mn$^{2+}$, Hg$^{2+}$, Cd$^{2+}$, Fe$^{3+}$, Cr$^{3+}$ and Al$^{3+}$), and UV-Visible and fluorescence techniques (Figure 5.4). A neat solution of probe IV (10 µM) showed fluorescence emission in the characteristic region of naphthalimide moiety with moderate efficiency ($\lambda_{\text{max}} = 534$ nm, $\Phi = 0.12$) due to the intramolecular charge transfer (ICT) process. The ICT process could also be inferred from the shift in absorption spectra and significant quenching of fluorescence intensity by solvent polarity (Figure 5.5). Further, in all solvents, probe IV displayed a single emission corresponding to naphthalimide moiety, indicating that the effect of APBT moiety on the emission is not significant (Figure 5.5) as the APBT is known to display ESIPT process. Moreover, the fluorescence intensity of probe IV in neat MeCN was gradually decreased by the addition of incremental quantities of water, indicating the absence of aggregation induced emission (AIE) effect through the restricted ICT process in IV (Figure 5.6).

**Figure 5.4** Change in the absorption (a) and fluorescence (b) spectra of IV (10 µM) in MeCN/H$_2$O (70% v/v) mixture upon addition of 10 equiv. of various metal ions

**Figure 5.5** The absorption (a) and fluorescence (b) spectra of IV (10 µM) in different solvents
Amongst all tested metal ions, only Hg\(^{2+}\) and Cu\(^{2+}\) induced distinct changes in the fluorescence profile of IV as shown in Figure 5.4b. Clearly, addition of 10 equiv. of Hg\(^{2+}\) ions enhanced the fluorescence intensity of IV by 2.3 fold due to the formation of IV-Hg\(^{2+}\) complex, whereas the Cu\(^{2+}\) binding led to the complete quenching of fluorescence emission at 534 nm. These results revealed that IV could differentiate Hg\(^{2+}\) and Cu\(^{2+}\) ions on the basis of distinct emission responses. Under similar conditions, the UV-Visible spectra of IV displayed a broad band centered at ~404 nm that could be ascribed to the naphthalimide moiety, and it was impassive to the addition of indicated metal ions except for Hg\(^{2+}\) and Cu\(^{2+}\) ions. The addition of these divalent metal ions shifted the absorption maxima of IV towards ~397 and 388 nm, respectively (Figure 5.4a). The ICT sensors usually exhibit large shift in the absorption as well as fluorescence spectra in comparison with photo-induced electron transfer (PET) sensors.\(^{19}\) Thus, the observed blue shift in absorption maxima and enhancement in fluorescence intensity of IV upon addition of Hg\(^{2+}\) might be attributed to the Hg\(^{2+}\)-induced reduction in the PET from lone pair electrons of ‘N’-atoms to the naphthalimide moiety. Significant fluorescence quenching of IV induced by Cu\(^{2+}\) ion is well known to its paramagnetic property due to the reverse PET process.

To further investigate the recognition mechanism, frontier molecular orbital energy levels were calculated using DFT and TDDFT calculations at the CAMB3LYP/6-31G** level. The energetically optimized structure of IV is provided in Figure 5.7. The results from TDDFT calculations on IV revealed that electronic transitions from HOMO to LUMO, HOMO-2 to LUMO and HOMO-1 to LUMO+1 have considerable contribution to state S1. The HOMO-2 was mainly assigned to the naphthalimide moiety and piperazine group, whereas the electron density of the LUMO orbital was predominantly located over the

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*Figure 5.6* The fluorescence spectra of IV (10 μM) in MeCN and with different amounts of water (0-90%)
naphthalimide moiety. The calculated energy values of molecular orbitals signified that the HOMO-1 was located above the HOMO-2 of the probe. This orbital arrangement enables electron transfer from piperazine, amide and benzothiazole ‘N’-atoms (HOMO-1) to naphthalimide moiety (HOMO-2) and forbids the electron comeback from LUMO and thereby results in weak emission of naphthalimide (Figure 5.8). From the results of DFT calculations, it was inferred that the enhancement in fluorescence of IV-Hg$^{2+}$ complex (Figure 5.2) could be a result of the inhibition of PET process.

![Figure 5.7](image)

**Figure 5.7** Optimized geometry of IV as predicted by quantum-chemical calculations

![Figure 5.8](image)

**Figure 5.8** Frontier molecular orbitals of IV obtained from TDDFT calculations. For the transition of HOMO-1 to LUMO, it is clearly seen that there is no charge transfer as both orbitals are mainly delocalized on the benzothiazole moiety. Therefore, probe IV emits fluorescence emission in the characteristic region of naphthalimide moiety.
H-NMR experiments were performed to explore the binding environments of Hg\textsuperscript{2+} and Cu\textsuperscript{2+} ions (Figure 5.9 and 5.10). On addition of 1 equiv. of Hg\textsuperscript{2+} or Cu\textsuperscript{2+} ions to IV, the resonance position of amide proton (H\textsubscript{8}) at 12.27 ppm was shifted downfield to 12.49 and 12.43 ppm, respectively, indicating the increased electron-withdrawing ability of amide group induced by ‘N’-metal coordination to shift the -OH resonance downfield. The proton resonances of piperazine (H\textsubscript{5}, H\textsubscript{6}), naphthalimide (H\textsubscript{1}, H\textsubscript{2}, H\textsubscript{3} and H\textsubscript{4}) and linker (-N-CH\textsubscript{2}-C=O, H\textsubscript{7}) moieties were also shifted downfield significantly (Figure 5.9). In addition, the resonances position of APBT (H\textsubscript{9}, H\textsubscript{10}, H\textsubscript{11} and H\textsubscript{12}) protons showed distinct differences (Figure 5.10). These observations confirmed the involvement of the ‘N’-atom of amide functionality and the ‘N’-atoms of piperazine and benzothiazole moieties in IV-Hg\textsuperscript{2+} and IV-Cu\textsuperscript{2+} complex formation.

**Table 5.1** Calculated photophysical parameters of IV at CAMB3LYP/6-31G** level

<table>
<thead>
<tr>
<th>Excitation energy (eV)</th>
<th>Nature of S1 transition</th>
<th>Wavelength (nm)</th>
<th>Oscillator Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6440 eV</td>
<td>HOMO → LUMO (0.66569)</td>
<td>340.24</td>
<td>0.3947</td>
</tr>
<tr>
<td>3.9828 eV</td>
<td>HOMO-1 → LUMO+1 (0.64207)</td>
<td>311.30</td>
<td>0.4052</td>
</tr>
</tbody>
</table>

Figure 5.9 \textsuperscript{1}H-NMR spectral change of IV (a) and in the presence of Hg\textsuperscript{2+} (b) and Cu\textsuperscript{2+} (c) ions.
Figure 5.10 $^1$H-NMR spectral change of IV(a) and in the presence of Hg$^{2+}$ (b) and Cu$^{2+}$ (c) ions

The absorbance and fluorescence profiles of IV (10 µM) in MeCN/H$_2$O (70% v/v) mixture at various concentrations of Hg$^{2+}$ and Cu$^{2+}$ ions are provided in Figure 5.11 and 5.12. Successive addition of serial concentrations of Hg$^{2+}$ (0–10 equiv.) shifted the absorption band of IV at ~404 nm, and the maximum absorption was observed with 5 equiv. of Hg$^{2+}$ ions (Figure 5.11a). A similar titration with Hg$^{2+}$ ions gradually increased the fluorescence emission intensity of IV at ~534 nm (Figure 5.12a). On the other hand, addition of serial concentrations of Cu$^{2+}$ (0–10 equiv.) to IV (10 µM) resulted in a steady blue shift in the absorption maximum at ~404 nm, and upon addition of 10 equiv. of Cu$^{2+}$, the absorption maximum appeared at 388 nm (Figure 5.11b). Under similar conditions, a systematic decrease in the fluorescence intensity of IV was observed as shown in Figure 5.12b.

Both the quenching of fluorescence intensity of IV by Cu$^{2+}$ and enhancement in fluorescence by Hg$^{2+}$ yielded linear relationships in response to the increase in the concentration of Hg$^{2+}$ and Cu$^{2+}$ ions (0-10 µM). The detection limits were evaluated to be 2.1×10^{-7} M (R$^2$ = 0.9928) and 2.5×10^{-7} M (R$^2$ = 0.9966) for Hg$^{2+}$ and Cu$^{2+}$ ions, respectively (Figure 5.13). The binding constant values of 4.0×10^4 M$^{-1}$ (R$^2$ = 0.9860) and 2.6×10^4 M$^{-1}$ (R$^2$ = 0.9823) were obtained for Hg$^{2+}$ and Cu$^{2+}$ ions, respectively. The ESI-MS spectrum analysis confirmed the formation of IV-Hg$^{2+}$ (m/z = 849) and IV-Cu$^{2+}$ (m/z = 651) complex in a 1:1 stoichiometry (Figure 5.14 and 5.15).
Figure 5.11 Change in the absorption spectra of IV (10 μM) in MeCN/H₂O (70% v/v) mixture and upon serial addition (0-10 equiv.) of Hg²⁺ (a) and Cu²⁺ (b) ions

Figure 5.12 Change in the fluorescence spectra of IV (10 μM) in MeCN/H₂O (70% v/v) mixture upon serial addition (0-10 equiv.) of Hg²⁺ (a) and Cu²⁺ (b) ions

Figure 5.13 Linear response of IV (10 μM) in the presence of Hg²⁺ (a) and Cu²⁺ (b) ions in MeCN/H₂O (70% v/v) mixture. The detection limit was calculated using 3σ/S, where σ is the standard deviation of the blank solution, and S is the slope of the calibration curve
Figure 5.14 ESI-MS spectrum of IV-Hg$^{2+}$

Figure 5.15 ESI-MS spectrum of IV-Cu$^{2+}$
The comparable binding constant values of IV-Hg\(^{2+}\) and IV-Cu\(^{2+}\) complexes suggested that these metal ions could compete against each other when they coexist in a system. To explore this possibility the competitive complexation of IV between Hg\(^{2+}\) and Cu\(^{2+}\) ions were also investigated. Upon treatment with Cu\(^{2+}\) (0-10 equiv.) the fluorescence profile of IV-Hg\(^{2+}\) was transformed into almost that of IV-Cu\(^{2+}\) system as shown in Figure 5.16a, while the fluorescence of IV-Cu\(^{2+}\) was significantly enhanced upon adding Hg\(^{2+}\) (0-50 equiv.) ions (Figure 5.16b). Thus, the comparable coordinating ability of Hg\(^{2+}\) and Cu\(^{2+}\) for binding with IV results in the transformation of IV-Hg\(^{2+}\) to IV-Cu\(^{2+}\) and *vice versa*. Moreover, a specific signal pattern (Figure 5.17) was also observed upon changing the sequence of addition of Hg\(^{2+}\) (5 equiv.) and Cu\(^{2+}\) (5 equiv.) ions.

**Figure 5.16** Change in the fluorescence spectra of IV (10 \(\mu\)M) in MeCN/H\(_2\)O (70% v/v) mixture upon titration with different concentration of Cu\(^{2+}\) (0-10 equiv.) in the presence of 5 equiv. Hg\(^{2+}\) (a) and Hg\(^{2+}\) (0-50 equiv.) in the presence of 5 equiv. Cu\(^{2+}\) (b) ions

**Figure 5.17** Change in the fluorescence spectra of IV (10 \(\mu\)M) in MeCN/H\(_2\)O (70% v/v) mixture upon addition of different concentration of Cu\(^{2+}\) and Hg\(^{2+}\): First (5 equiv.) Hg\(^{2+}\) + second (5 equiv.) Cu\(^{2+}\) (a) and First (5 equiv.) Cu\(^{2+}\) + Second (5 equiv.) Hg\(^{2+}\) (b)
On the basis of sequence dependent fluorescence behaviors of IV, the sequential logic gate was constructed using the chemical inputs of Hg$^{2+}$ (5 equiv.) and Cu$^{2+}$ (5 equiv.) designated as In 1 and In 2, respectively (Figure 5.18). The output value at 534 nm was assigned as ‘0’ (when $I/I_0 < 1.20$) and ‘1’ (when $I/I_0 > 1.20$). In the first input (addition of Hg$^{2+}$ ions) the fluorescence intensity ($I/I_0$) was enhanced above 1.20 (‘ON’ state). In the second input addition of Cu$^{2+}$ ions to IV-Hg$^{2+}$ the $I/I_0$ value remained above 1.20 (output = 1, ‘ON’ state). Interestingly, when the order of inputs was reversed, the output signals changed (output = 0, ‘OFF’ state), suggesting the suitability of IV for designing molecular logic gate.

Figure 5.18 Output ($I/I_0$) resulting from different input sequences: (a) first (5 equiv.) Hg$^{2+}$ and second (5 equiv.) Cu$^{2+}$; (b) first (5 equiv.) Cu$^{2+}$ and second (5 equiv.) Hg$^{2+}$

![Figure 5.18](image1.png)

Figure 5.19 Fluorescence spectra of IV-Hg$^{2+}$ (a) and IV-Cu$^{2+}$ (b) complexes at different concentration of EDTA in MeCN/H$_2$O (70% v/v) mixture

The reversibility in molecular logic gates is important to realize a system reset. This could be achieved by the addition of a strong chelating reagent, EDTA. As seen in Figure
5.19a, the enhanced fluorescence intensity of IV-Hg\(^{2+}\) was reduced into that of free IV by the treatment with EDTA. Again, an excess addition of Hg\(^{2+}\) recovered the fluorescence of the IV-Hg\(^{2+}\). Similarly, addition of EDTA to the solution of IV-Cu\(^{2+}\) complex resulted in a significant enhancement in the fluorescence intensity at ~517 nm as shown in Figure 5.19b. The reversibility of IV-Hg\(^{2+}\) and IV-Cu\(^{2+}\) complexes could make the molecular logic gate reusable. Thus, it is apparent that this system can be utilized to develop the sequence dependent logic circuit.

![Diagram of molecular keypad lock with input sequence C, A, N resulting in "ON" state and F, C resulting in "OFF" state]

**Figure 5.20** Fluorescent keypad to access secret code at 534 nm with different input sequences

The results can be exploited for the design of molecular keypad lock as seen in Figure 5.20. To generate an input sequence as a password entry for a keypad lock, inputs Hg\(^{2+}\) and Cu\(^{2+}\) ions could be designated as ‘C’ and ‘A’, respectively. When the input ‘C’ was added first followed by ‘A’, the emission I/I\(_{0}\) at 534 nm would be above 1.20 and create the secret code ‘CAN’ (N defines the ‘ON’ state). Reversal of the input sequence *(i.e. the first input is ‘A’ and the second input is ‘C’)* result in the ‘OFF’ state and generate a wrong password ‘ACF’ (F defines the ‘OFF’ state) fails to open the keypad lock. This demonstrates that exact codes can be generated by the correct sequence of input signals so that only one code would work.
5.4 Conclusion

In conclusion, we have developed a new naphthalimide based fluorescent probe IV that forms a 1:1 complex with Hg$^{2+}$ and Cu$^{2+}$ with binding constant values $4.0 \times 10^4$ M$^{-1}$ and $2.6 \times 10^4$ M$^{-1}$, respectively. Reversible and controllable OFF-ON and ON-OFF fluorescent switch could be constructed by varying the sequence of chemical inputs, viz., Hg$^{2+}$ and Cu$^{2+}$ ions. The comparable binding constant values and the reversibility of IV-Hg$^{2+}$ and IV-Cu$^{2+}$ complexes were exploited to construct molecular keypad lock. As the molecular keypad lock has the potential for protecting information at the molecular level, the molecular switch proposed from this study would be useful to develop more sophisticated security systems.
5.5 References

Summary

Heavy and transition metal (HTM) ions play critical roles in many biological and environmental processes but their abnormal homeostasis in cells leads to several adverse health effects. Owing to their significant impact on the biosphere and human health, it is highly desirable to develop convenient and efficient analytical methods to provide precise and quantitative determination of HTM ions. Fluorescence chemosensors have been a central focus of recent efforts in the context of sensing environmentally and biologically relevant metal ions because of their advantageous features including operational simplicity, rapid response time, high sensitivity and selectivity, and for their potential in bioimaging application. Fluorescent chemosensor is a molecular system that interacts with target metal ions and gives detectable output signal through changes in the fluorescence properties (such as fluorescence intensity, wavelength, and lifetime).

The spectrometric techniques exploited for designing fluorescent probes include Photo-induced electron transfer (PET), Excited state intramolecular proton transfer (ESIPT), Fluorescence resonance energy transfer (FRET), Intramolecular charge transfer (ICT)/Photo-induced charge transfer (PCT), Excimer formation, C=N Isomerization, Aggregation induced emission (AIE)/Aggregation induced emission enhancement (AIEE) and Chelation-induced enhancement in fluorescence (CHEF).

The focal theme of this thesis is to develop new and efficient fluorescent probes for the detection of environmentally and biologically relevant metal ions. In the present investigation, we have explored the photo-induced electron transfer approach to develop chemosensor for Fe$^{3+}$, Al$^{3+}$ and Cr$^{3+}$ ions using naphthalimide as the fluorophore moiety. We have also investigated the utility of another naphthalimide based chemosensor for selective detection of Cu$^{2+}$ and Hg$^{2+}$ ions and for the molecular keypad lock application. Exploiting the ESIPT and C=N isomerization techniques, we have obtained a chemosensor for the detection of Zn$^{2+}$ ions. In addition, the combination of twisted intramolecular charge transfer and aggregation induced emission processes have been explored to get new chemosensors for Hg$^{2+}$ and Ag$^+$ ions. The thesis is composed of five chapters and the data obtained from this investigation are presented in Chapters II-V.

A general introduction on the development of fluorescent chemosensors for the selective detection of Fe$^{3+}$, Al$^{3+}$, Cr$^{3+}$, Cu$^{2+}$, Hg$^{2+}$, Zn$^{2+}$ and Ag$^+$ ions is provided in Chapter I. The strategy used for the development of fluorescent chemosensors, method used for the determination of detection limit, selectivity and possible application of the
probes are presented in this chapter. The literature survey provided in Chapter I is limited to the scope of the chemosensors presented in this thesis.

The development of ‘turn-on’ sensors for Fe$^{3+}$, Cr$^{3+}$ and Al$^{3+}$ ions has been found to be comparatively more difficult than for other metal ions due to the paramagnetic quenching nature of Fe$^{3+}$ and Cr$^{3+}$ as well as the weak coordinating ability of Al$^{3+}$ ions. A new method for the selective detection of Fe$^{3+}$, Al$^{3+}$ and Cr$^{3+}$ ions without interference from other competitive metal ions, is presented in Chapter II. Our naphthalimide based non-fluorescent probe I emits fluorescence upon complex formation with these trivalent cations through inhibition of PET process. Formation of a 1:1 chelation with Fe$^{3+}$, Al$^{3+}$ or Cr$^{3+}$ results in the strong fluorescence emission from the probe. This chelation enhanced fluorescence confirms the inhibition of PET operating in I, and the direct involvement of piperazine ‘N’-atoms in complex formation. The selective sensing ability of I towards Fe$^{3+}$, Al$^{3+}$ and Cr$^{3+}$ ions can also be observed by the naked eye by illuminating the sample under UV-light. Moreover, the probe I is stable at biologically relevant pH range, membrane permeable, non-lethal at the experimental conditions and useful for imaging intracellular Fe$^{3+}$, Al$^{3+}$ or Cr$^{3+}$ levels in live HaCaT cells exposed to these trivalent cations.

Zinc(II) is the second most abundant transition metal in humans after iron. Since Zn$^{2+}$ is spectroscopically and magnetically silent due to its d$^{10}$ electronic configuration, development of fluorescent probes for the selective determination of Zn$^{2+}$ ions in trace levels, particularly with ‘turn-on’ response is a great challenge. In Chapter III, selective detection of Zn$^{2+}$ ions using a new ESIPT based 2-(2’-aminophenyl)benzothiazole (APBT) derivative II is described. Probe II has ‘N-N-N-O’ combination of coordinating atoms for chelation with Zn$^{2+}$ ions. Binding of Zn$^{2+}$ with probe II inhibits ESIPT and –CH=N– isomerization processes permits the selective detection of Zn$^{2+}$ ions. The observed limit of detection of Zn$^{2+}$ ions in aqueous medium was 4.5×10$^{-9}$ M. Probe II displayed good thermal stability and would be useful for designing new APBT derivatives for tunable fluorescent sensors.

Mercury (Hg$^{2+}$) and silver (Ag$^{+}$) are two of the potentially toxic heavy metal ions that can get into human body through food chain in the instance of persistent contamination of soil or effluent water. Selective detection of Hg$^{2+}$ over Ag$^{+}$ is achieved by exploiting the thiophilic nature of these metal ions. However, development of a novel ‘turn-on’ fluorogenic probe for the differential detection Hg$^{2+}$ and Ag$^{+}$ ions through new mechanisms remains still a challenge. In Chapter IV, the synthesis of an AIE active dual analyte probe III derived from 1,8-naphthalimide is presented. Probe III allows the
quantification of Hg\textsuperscript{2+} and Ag\textsuperscript{+} ions in a single medium through metal ion induced AIE activity stemming from two different aggregation modes of the metal complexes formed. The amorphous and crystalline nature of \textbf{III-Hg}\textsuperscript{2+} and \textbf{III-Ag}\textsuperscript{+} aggregates are demonstrated. Also, the difference in the ability of Hg\textsuperscript{2+} and Ag\textsuperscript{+} ions to induce aggregation of \textbf{III} in aqueous media of different polarity permits the selective detection of Hg\textsuperscript{2+} and Ag\textsuperscript{+} ions. The other common metal ions do not induce AIE of \textbf{III}, and therefore do not interfere with the selective detection of Hg\textsuperscript{2+} and Ag\textsuperscript{+} ions.

Copper plays a crucial role in several physiological responses, but excessive intake of copper leads to childhood cirrhosis. Mercury is one of the most toxic metal ions, and gets accumulated in human body and other higher organisms through the food chain. We have described in \textbf{Chapter V}, a new naphthalimide based single molecular probe \textbf{IV} that acts as a reversible molecular switch with different chemical inputs (Hg\textsuperscript{2+} and Cu\textsuperscript{2+} ions) and works as a sensor and a molecular keypad lock. The fluorescent probe \textbf{IV} forms a 1:1 complex with Hg\textsuperscript{2+} and Cu\textsuperscript{2+} ions with binding constant values 4.0×10\textsuperscript{4} M\textsuperscript{-1} and 2.6×10\textsuperscript{4} M\textsuperscript{-1}, respectively. The comparable binding constant values and the reversibility of \textbf{IV-Hg}\textsuperscript{2+} and \textbf{IV-Cu}\textsuperscript{2+} complexes were exploited to construct molecular keypad lock. Reversible and controllable OFF-ON and ON-OFF fluorescent switch could be constructed by varying the sequence of chemical inputs, viz., Hg\textsuperscript{2+} and Cu\textsuperscript{2+} ions. As the molecular keypad lock has the potential for protecting information at the molecular level, the molecular switch proposed from this study would be useful to develop more sophisticated security systems.