Chapter-6

Naphthalimide based chemosensor for Zn\(^{2+}\), pyrophosphate and H\(_2\)O\(_2\): Sequential logic operations at the molecular level

Abstract

Probe 3 based on 1,8-naphthalimide appended with quinoline moiety has been designed and synthesized. Probe 3 exhibits selective ratiometric fluorescence response in presence of Zn\(^{2+}\) ions in mixed aqueous media and is also used for the detection of these ions in the living cells. Further, the in situ prepared Zn\(^{2+}\) complex of probe 3 was used as a chemosensing system for the detection of pyrophosphate (PPi) and hydrogen peroxide (H\(_2\)O\(_2\)). Based on the fluorescence behaviour of 3 with Zn\(^{2+}\), PPi and H\(_2\)O\(_2\) sequential logic circuits have been designed where the chemically encoded information as input was converted into fluorescent output.
6.1. Introduction

The idea of using of electronic logic devices for information processing is presently extended to the design of molecular systems that mimic logic operations analogous to those executed by their macroscopic electronic analogues.¹ Chemical systems are able to perform logic operations at the molecule level resulting in the nanoscaling of computing units.² Molecular information processing is a common feature of natural systems. For example, molecular recognition and signal transduction in cellular systems process information at the molecular level based on YES and NOT logic functions.³ These molecular systems can be switched to two different states i.e. on and off states related to their photophysical or electronic properties modulated by using inputs such as ions, molecule or light. Molecular sensors are promising candidates for the realization of systems having logic application along with fluorescence technique to monitor the operation of molecular level devices.⁴ Logic gates are the basic building blocks for the information processing devices. Most of the logic gates are binary logic i.e. have two states of "0" and "1" and the input or output of logic gates can only exist in one of these states. A number of fluorescent chemosensors have been developed from the formulation of single logic gates mimicking Boolean operations including AND, OR, XOR, NOR, NAND, INHIBIT and XNOR logic functions.⁵ Further, the combination of various chemical logic gates resulted in simple computing devices performing basic arithmetic operations.⁶ The outputs of combinational logic circuits are determined by current input level at any given instant in time and any changes to their inputs will immediately have an effect at the output as combinational circuits have "no memory" or "feedback loops" (Chart A). However, sequential logic circuits (Chart B) are essential for realization of memory devices which are capable of storing information and operate through the feedback loop where one of the outputs of the device function as input and is memorized as “memory element”.⁷ Memory elements can store binary
information which defines the state of sequential circuit at any given time. Further, the output of the sequential circuit is determined by input and present state of the memory element. The development of such sequential circuits is important as combinational logic is not enough to construct memory devices. In combinational logic, output depends only on the inputs irrespective of the requirements of the system. However, by adding a memory element to it, we can generate system which can store encoded information and implement them at the time at which they are required. For example, Yamato et al.\(^8\) reported a fluorescent probe \(1a\) based on pyrene-linked triazole-modified homooxacalix[3]arene which functions as a sequential logic circuit (R-S latch logic circuit) with memory unit in combination with an INHIBIT logic gate upon sequence addition of chemical inputs of \(\text{Zn}^{2+}\) and \(\text{H}_2\text{PO}_4^-\) ions. van der Boom et al.\(^9\) reported a surface-immobilized \(\text{Os}^{2+}\) polypyridyl complex \(1b\) which functions as sequential logic devices with oxidizing/reducing reagents as inputs, \(\text{Os}^{2+}\) to \(\text{Os}^{3+}\) conversion as output and further uses this sequential logic approach to mimic the memory function of random access memory (RAM). However, the development of systems that can store encoded information at the molecular level is still in the halfway as compared to the well-developed field of molecular logic gates. Keeping this point in the mind, in the present work we have designed and synthesized 1,8-naphthalimide linked quinoline based probe \(3\) which shows a selective ratiometric emission shift of more than 100 nm with \(\text{Zn}^{2+}\) ions in mixed aqueous media. Further, we used \(3-\text{Zn}^{2+}\) complex as a system for the detection of pyrophosphate (PPi) and hydrogen peroxide (\(\text{H}_2\text{O}_2\)). The fluorescence variations of \(3\) with chemical inputs of \(\text{Zn}^{2+}\), pyrophosphate and hydrogen peroxide are used to mimic the functions of sequential logic circuits capable of storing information at the molecular level where the chemically encoded information as input was converted into fluorescent output. The design of such signalling devices is of great significance
as such systems are capable to execute molecular level logic operations in presence of chemical inputs and thus can be applied to the biological systems.

6.2. Results and discussion

The condensation of 1,8-naphthalimide derivative 10 with 2-quinolinecarboxaldehyde 2 furnished compound 3 in 68% yield (Scheme 6.1). The structure of compound 3 was confirmed from its spectroscopic and analytical data. The $^1$H NMR spectrum of compound 3 showed two triplets (3H and 2H) at 1.37 and 8.32 ppm corresponding to the methyl and aromatic protons, a quartet (2H) at 4.25-4.33 ppm corresponding to the methylene protons, three multiplets (5H, 3H and 2H) at 7.52-7.64, 7.71-7.80 and 8.65-8.68 ppm corresponding to the aromatic protons, three doublets (1H each) at 7.90, 8.19 and 8.41 ppm corresponding to the aromatic protons and a singlet (1H) at 8.90 ppm corresponding to imino proton. A parent ion peak at $m/z$ 456.18 (M+1)$^+$ corresponding to the product 3 was observed in the mass spectrum. These spectroscopic data corroborate the structure 3 for this compound.

The binding behaviour of compound 3 was studied toward different metal ions ($\text{Hg}^{2+}$, $\text{Cu}^{2+}$, $\text{Ni}^{2+}$, $\text{Zn}^{2+}$, $\text{Co}^{2+}$, $\text{Cd}^{2+}$, $\text{Pb}^{2+}$, $\text{Fe}^{3+}$, $\text{Fe}^{2+}$, $\text{Ag}^+$, $\text{Mg}^{2+}$, $\text{Ba}^{2+}$, $\text{Ca}^{2+}$, $\text{Li}^+$, $\text{K}^+$ and $\text{Na}^+$) as their perchlorate salts by UV-vis and fluorescence spectroscopy. The absorption spectrum of receptor 3 (5 $\mu$M) in $\text{H}_2\text{O}:\text{CH}_3\text{CN}$ (2:8, v/v) shows typical absorption band of naphthalimide moiety at 363 nm along with characteristic absorptions of quinoline moiety at 214 and 239 nm (Figure 6.1). The addition of only $\text{Zn}^{2+}$ ions to the solution of 3 results in the red shift of absorption band centred at 363 nm to 375 nm along with the formation of a clear isosbestic point at 368 nm (Figure 6.1). The addition of other transition and alkali metal ions did not alter the absorption spectrum of receptor 3 (Figure 6.2). The fluorescence spectrum of receptor 3 (2 $\mu$M) is characterized by a blue coloured emission band at 448 nm related to the quinoline.

![Scheme 6.1 Synthesis of compound 3.](image-url)
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Figure 6.1 UV-vis spectra of 3 (5 μM) with Zn$^{2+}$ ions (0-15 equiv) in H$_2$O:CH$_3$CN (2:8, v/v) buffered with HEPES, pH = 7.0.

Figure 6.2 UV-vis spectra of 3 (5 μM) with various metal ions (15 equiv each) in H$_2$O:CH$_3$CN (2:8, v/v) buffered with HEPES, pH = 7.0.

moiety when excited at 360 nm in H$_2$O:CH$_3$CN (2:8, v/v; Figure 6.3). However, emission from the naphthalimide moiety was not observed owing to the electron transfer from the imino nitrogen to the naphthalimide moiety. Upon addition of only Zn$^{2+}$ ions (0-26 μM) a new emission band corresponding to the naphthalimide moiety appeared at 550 nm (Figure 6.3) along with the orange coloured fluorescence (Inset of Figure 6.3). While, the emission band at 448 nm shows remarkable decrease in the emission intensity. This ratiometric fluorescence behaviour of 3 in the presence of Zn$^{2+}$ ions is attributed to the complexation of Zn$^{2+}$ ion with the electron-donating imino nitrogen responsible for the inhibition of the photoinduced electron transfer mechanism. In $^1$H NMR spectrum, the imino proton of 3 undergoes a downfield shift of Δδ = 1.32 ppm on the addition of Zn$^{2+}$ ions proving the interaction of receptor 3 with Zn$^{2+}$ through nitrogen atom of imino moiety (Figure 6.4). Further, by considering the ratio of the fluorescence intensity (I$_{550}$/I$_{448}$), a 6.5-fold emission

Figure 6.3 Fluorescence spectra of 3 (2 μM) in H$_2$O:CH$_3$CN (2:8, v/v) buffered with HEPES, pH = 7.0; at 25 °C; $\lambda_{ex}$ = 360 nm in the presence of Zn$^{2+}$ ions (0-26 μM). Inset showing the fluorescence change (a) before (b) after the addition of Zn$^{2+}$ ions.
enhancement at 550 nm was observed in the case of the 3-Zn$^{2+}$ complex. The fluorescence quantum yield of 3-Zn$^{2+}$ complex was calculated to be 0.74 (at 550 nm) as compared to that of free 3 (0.22 at 448 nm). Further, the nonlinear regression analysis program SPECFIT gave a good fit and demonstrated that 1:1 stoichiometry (host:guest) was the most stable species in the solution with a binding constant ($\log \beta$) of 5.19 ± 0.07. The method of continuous variation (Job’s plot) was also used to prove 1:1 stoichiometry (Figure 6.5). The binding of Zn$^{2+}$ with 3 is also proved by mass spectroscopy which showed a peak at $m/z$ 396.9 [3 + Zn$^{2+}$ + 2ClO$_4^-$ + 2H$_2$O + K$^+$ + H$^+$]$^{2+}$ corresponding to the 1:1 stoichiometry of host and guest species. The detection limit of 3 for Zn$^{2+}$ ions was found to be of $20 \times 10^{-8}$ mol L$^{-1}$ which is sufficiently low for the detection of Zn$^{2+}$ ions found in the many chemical and biological systems. This type of ratiometric fluorescence behaviour is not observed by the addition of any other metal ions (Figure 6.6). The presence of other metal ions did not alter the fluorescence behaviour of 3-Zn$^{2+}$ complex system (Figure 6.7). Thus, receptor 3 acts as efficient ratiometric fluorescence probe for Zn$^{2+}$ ions with red shift of more than of 100 nm.

**Figure 6.4** $^1$H NMR spectra of 3 in CDCl$_3$:CD$_3$CN (9:1); (A) Free 3; (B) 3 + 1.0 equiv of zinc perchlorate.

**Figure 6.5** Job’s plot for determining the stoichiometry of 3 and Zn$^{2+}$ ions.
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The potential biological application of the chemosensor 3 was evaluated for the \textit{in vitro} detection of Zn$^{2+}$ ions in prostate cancer (PC3) cell lines. The prostate cancer (PC3) cell lines incubated with receptor 3 (5 μM) showed intracellular fluorescence in the blue channel which indicated the emissive nature of 3 in the intracellular system (Figure 6.8c). However, the cells with receptor 3 (5 μM) further treated with Zn$^{2+}$ ions (10 μM) shows fluorescence in the red channel (Figure 6.8f). These results suggest that the probe 3 is cell permeable and an effective intracellular Zn$^{2+}$ ions imaging agent with the change in fluorescence emission from blue to red.

Next, we utilized 3-Zn$^{2+}$ complex system for the detection of anions and reactive oxygen species. The detection mechanism is based on the variation in the emission of ligand-Zn$^{2+}$ complex directed by the strength of coordination between the Zn$^{2+}$ centre and anionic species. The addition of pyrophosphate i.e. PPI (0-60 μM) to the solution...
of 3-Zn\(^{2+}\) complex (Figure 6.9) results in the appearance of blue coloured fluorescence emission at 448 nm (Inset of Figure 6.9) along with simultaneous decrease in the fluorescence intensity at 550 nm. The coordination of PPi with the Zn\(^{2+}\) centre results in the weakening of interaction between probe and Zn\(^{2+}\) responsible for the revival of probe fluorescence at 448 nm. The addition of other inorganic anions (HPO\(_4^{2-}\), H\(_2\)PO\(_4^-\), CN\(^-\), F\(^-\), Br\(^-\), Cl\(^-\), AcO\(^-\), CO\(_3^{2-}\) and HSO\(_4^-\)) did not introduce this type of fluorescence behaviour (Figure 6.10). Moreover, the fluorescence behaviour of 3-Zn\(^{2+}\) complex with PPi does not change with the additions of other anions (Figure 6.11). Thus, 3-Zn\(^{2+}\) complex provides a ratiometric system for the detection of pyrophosphate in the mixed aqueous media with detection limit of 40 × 10\(^{-8}\) mol L\(^{-1}\). We further employed 3-Zn\(^{2+}\) complex as a chemosensing system for the detection of reactive oxygen species. The addition of H\(_2\)O\(_2\) to the solution of free 3 did not introduce any evident changes in the emission spectrum of 3.

Figure 6.9 Fluorescence spectra of 3 (2 μM) upon addition of PPi (0-60 μM) to the 3-Zn\(^{2+}\) complex in H\(_2\)O:CH\(_3\)CN (2:8, v/v) buffered with HEPES, pH = 7.0; \(\lambda_{ex}\) = 360 nm. Inset showing the fluorescence change; (a) 3 + Zn\(^{2+}\); (b) 3 + Zn\(^{2+}\) + PPi.

Figure 6.10 Fluorescence spectra of 3 (2 μM) upon addition of various inorganic anions (60 μM each) to the 3-Zn\(^{2+}\) complex in H\(_2\)O:CH\(_3\)CN (2:8, v/v) buffered with HEPES, pH = 7.0; \(\lambda_{ex}\) = 360 nm.

Figure 6.11 Competitive fluorescence selectivity (I\(_{448}/I_{550}\)) of 3-Zn\(^{2+}\) complex towards PPi (60 μM) in the presence of other anions (60 μM each) in H\(_2\)O:CH\(_3\)CN (2:8, v/v) buffered with HEPES, pH = 7.0 (at 25 °C; \(\lambda_{ex}\) = 360 nm). 1, 3 + Zn\(^{2+}\); 2, PPi; 3, HPO\(_4^{2-}\); 4, H\(_2\)PO\(_4^-\); 5, CN\(^-\); 6, F\(^-\); 7, Br\(^-\); 8, Cl\(^-\); 9, Γ; 10, AcO\(^-\); 11, CO\(_3^{2-}\) and 12, HSO\(_4^-\).
However, the addition of H$_2$O$_2$ (0-80 µM) to the aqueous solution of 3-Zn$^{2+}$ complex initially results in the decrease in the emission intensity at 550 nm which finally blue shifted to 510 nm (Figure 6.12). The whole detection process involved the fluorescence colour change (Inset of Figure 6.12) from blue (3) to orange (3-Zn$^{2+}$) and orange to green (3-Zn$^{2+}$-H$_2$O$_2$). No significant change in the emission of 3-Zn$^{2+}$ complex was observed with other biologically relevant reactive oxygen species (ClO$^-$, 'OH, 'O'Bu and t-BuOOH; Figure 6.13). The detection limit of $10 \times 10^{-7}$ mol L$^{-1}$ of 3-Zn$^{2+}$ complex for H$_2$O$_2$ suggests this system as proficient sensing platform for detection of H$_2$O$_2$ found in many chemical systems. Therefore, the complex of 3 with Zn$^{2+}$ acts as a chemosensing system for the detection of both pyrophosphate and hydrogen peroxide. However, the different fluorescence response of 3-Zn$^{2+}$ complex...
toward pyrophosphate and H$_2$O$_2$ is probably due to the strong binding of PPi with Zn$^{2+}$ centre as compared to H$_2$O$_2$ owing to the difference in total anionic negative charge density of PPi and H$_2$O$_2$ that are involved in the complexation process.$^{11}$

Recently, the development of sequential logic devices involving the conversion of chemically encoded information into fluorescent signals has emerged as an active research area of unconventional computing.$^{12}$ Sequential logic circuits are important for the development of systems which are capable of storing encoded information owing to the presence of a memory element. Thus, depending upon the different chemical inputs (Zn$^{2+}$, PPi and H$_2$O$_2$) and fluorescent signals of compound 3 as outputs, sequential logic circuits are constructed. The three chemical inputs of Zn$^{2+}$, PPi and H$_2$O$_2$ are designated as In$Z$, In$P$, and In$H$, respectively. The threshold values of fluorescence intensities specified at out-1 (448 nm), out-2 (550 nm) and out-3 (510 nm) are 90, 200 and 140, respectively. Fluorescence intensities higher than the threshold values are assigned as “1” while the fluorescence intensities lower than the threshold value are assigned as “0” corresponding to the “On” and “Off” state of the readout signals, respectively. First, we constructed sequential logic circuit with two inputs (In$Z$ and In$P$) and two outputs (Figure 6.14) measured as the fluorescence emissions at 448 nm (Out-1) and 550 nm (Out-2). The truth table 6.1 reveals various combinations of inputs for “Out-1” and the sequential logic circuit of “Out-1” emission representing the set/reset element corresponds to the memory device. The sequential logic operations are presented by two inputs: reset (In$Z$) and set (In$P$) as a function of the memory element (Figure 6.15). The reversible and reconfigurable sequences of set/reset logic operations in a feedback loop demonstrate the memory feature with “write-read-erase-read” functions (Figure 6.15) through the output

![Figure 6.14](image-url)  
**Figure 6.14** Sequential logic circuit displaying memory unit with two inputs (In$Z$ and In$P$) and two outputs (Out-1 and Out-2).  

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>In$Z$</th>
<th>In$P$</th>
<th>Out-1 448 nm</th>
<th>Out-2 550 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
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<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1(1st)</td>
<td>1(2nd)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1(2nd)</td>
<td>1(1st)</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 6.1** Truth table for the sequential logic circuit displaying memory unit with two inputs (In$Z$ and In$P$) and two outputs (Out-1 and Out-2).
signals at 550 nm and 448 nm. The reset input ($In Z = 1$) results in the fluorescence off at 448 nm and this encoded information is “read” out the system as “erased” and saves “Out-1 = 0”. The stored information was “written” by set input ($In P = 1$) with the fluorescence on at 448 nm and off at 550 nm and the system write and save “Out-1 = 1”. Further, the write/erase cycles were performed on the receptor 3 through

**Figure 6.15** Schematic representation of the reversible logic operations for memory element possessing “write-read-erase” functions.

“On-Off” fluorescence intensity (at 550 nm and 448 nm) with good rewritable characteristics as well as visual fluorescence changes by adding PPi and Zn$^{2+}$ in the alternate sequence (**Figure 6.16**). Similarly, the second constructed sequential circuit displays memory elements with two inputs ($In Z$ and $In H$) and three outputs at 448 nm (Out-1), 550 nm (Out-2) and 510 nm (Out-3) (**Figure 6.17**). The combination of different inputs as shown in the truth table 6.2 permits the execution of a sequential logic circuit involving “Out-2” and “Out-3” (**Figure 6.17**) representing the set/reset element corresponds to the memory device. The sequential logic operations are presented by two inputs: set ($In Z$) and reset ($In H$) as a function of the memory element. As shown in logic circuit 6.17, the “0” or “1” state of “Out-2” and “Out-3” is defined by “Out-3”. Similarly, the On/Off state of “Out-1” depends upon the “Out-1” which defines the presence or absence of $In Z$. The development of such sequential
circuits is important to store encoded information and their implementation at the time at which they are required.

Figure 6.17 Sequential logic circuit displaying memory units with two inputs (In Z and In H) and three outputs (Out-1, Out-2 and Out-3).

In conclusion, we synthesized naphthalimide based fluorescent probe 3 which shows ratiometric emission shift with Zn$^{2+}$ ions in solution as well in intracellular systems. Further, the 3-Zn$^{2+}$ complex used as a platform for the sensing of pyrophosphate and hydrogen peroxide. In addition, the various fluorescence signals obtained are used to mimic the function of logical memory devices at the molecular level.

6.3. Experimental

6.3.1. General methods and instrumentations

Same as given on page 63 in chapter 2.

6.3.1.1. Procedure for fluorescence imaging

Same as given on page 114 in chapter 4.

6.3.2. Synthesis of compound 3

A mixture of compound 10 (0.1 g; 0.31 mmol) and 2-quinolinecarboxaldehyde 2 (0.04 g; 0.31 mmol) in ethanol was refluxed for 24 h. After the completion of the reaction, solvent was evaporated and the residue left was crystallized from CHCl$_3$/CH$_3$OH to give compound 3 in 68% yield (0.09 g). Mp: > 250°C. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ = 1.37 (t, J = 6 Hz, 3H, CH$_3$), 4.25-4.33 (q, 2H, CH$_2$), 7.52-7.64 (m, 5H, ArH), 7.71-7.80 (m, 3H, ArH), 7.90 (d, J = 9 Hz, 1H, ArH), 8.19 (d, J = 9 Hz, 1H, ArH), 8.32 (t, J = 9 Hz, 2H, ArH), 8.41 (d, J = 9 Hz, 1H, ArH), 8.65-8.68 (m, 2H, ArH) and 8.90 (s, 1H, HC=N) ppm. $^{13}$C NMR (CDCl$_3$, 75.45 MHz): $\delta$ = 13.76, 35.92, 115.38, 13.76, 35.92, 115.38.
121.43, 121.66, 121.73, 123.35, 125.19, 125.37, 126.86, 127.18, 127.88, 128.15, 129.14, 129.25, 130.31, 130.57, 131.11, 131.27, 131.37, 131.51, 133.33, 136.32, 147.68, 157.00, 159.06, 164.49 and 164.69 ppm. MS ES+ m/z 456.18 (M+1)+. Anal Calcd for C\textsubscript{30}H\textsubscript{21}N\textsubscript{3}O\textsubscript{2}: calcd. C, 79.10%; H, 4.65%; N, 9.22%. Found C, 79.02%; H, 4.78%; N 9.47%.

References


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10 Synthesis of compound 10 is discussed in chapter 4.