Chapter 1

Introduction
Chapter 1A

1A. Synthetic Receptors for Cations

1A.1. Introduction

Detection of metal ions, which are biologically important and have detrimental effect on the environments, is of great interest to many scientists around the world.\textsuperscript{1,2} Recent advances in the knowledge on the neuromodulatory roles that various alkali, alkaline earth, d-block and certain f-block metal ions play, along with their involvement as neurotransmitters, as cofactors in neuroproteins, and as neurotoxins have contributed significantly to the exponential growth of interest in "Metal ion Recognition" as the research area and its subsequent development.\textsuperscript{3} Traditional analytical methods to provide direct and quantitative information about metal ions concentrations in water samples employ a number of analytical techniques that include flame photometry, atomic absorption spectroscopy, ion sensitive electrodes, cold vapour atomic fluorescence spectrometry, neutron activation analysis and gas chromatography. But, many of the above mentioned techniques are expensive and need multistep sample preparation and/or sophisticated instrumentation. These procedures often require samples of large size and do not allow continuous monitoring. Further, these sophisticated instrumental techniques are neither well-suited for quick in-field detection of these metal ions nor for \textit{in–vivo} studies.

To address these issues, recognition and detection of one of the desired analytes using purpose made chromogenic/or fluorogenic host molecules, which show selectivity towards a specific analyte, have gained significant interest in the last decade in field of host-guest chemistry. These colorimetric and fluorogenic receptor molecules allow the detection of the targeted analyte through binding induced changes in the optical response in the form of electronic and fluorescence spectral changes. Sometimes, biologically important metal ions can be toxic to life when present at certain concentrations in the environment, water supplies, the food chain, and industrial
chemicals and products. Consequently, an intensive effort has been devoted to develop various sensory molecular receptors capable of recognizing, sensing, and selectively transporting these positively charged substrates, so that the concentrations of these metal ions in aqueous or non-aqueous media can be monitored quantitatively, metal ions of commercial value can be recovered from waste solutions, and certain toxic transition and post transition metal ions in the environment can be removed.  
4 The methods, to detect metal cation, based on fluorescent sensors hold distinct advantages over colorimetric sensors in terms of sensitivity, selectivity, response time, and local observation by fluorescence imaging spectroscopy.  
5 The design and construction of sensors by assembling an ion-specific metal ion-receptor fragment, coupled to a signalling subunit through a conduit for binding induced signal transduction. The role of the signalling subunit is to generate spectral output as response for the occurrence of receptor-substrate interaction. Thus, the efficiency of any such sensor is based on factors like, selectivity or specificity, sensitivity, reversibility in the binding process and ultrafast response time. More importantly, such receptor molecules designed for biological applications should be non-toxic to the living organisms for probable in-vitro application.

Designing of an efficient sensor is basically a delicate balance of several factors. Aquation or solvation energy for the cation used for the study is crucial for its recognition in highly polar solvent like water. Thus, for efficient recognition of the desired cation in water, change in free energy (-ΔG) for the receptor-analyte binding should be much higher than the -ΔGSolvation for the respective ion. For higher sensitivity a strong analyte-receptor binding is desirable; however too strong binding would affect the reversibility phenomena adversely. But, one has to keep it in mind that the designed receptor should be able to bind selectively or show preference for the desired ion in presence of any other competing ions present in the medium.
The first synthesis of crown ethers by Charles Pedersen at DuPont in 1960s and the realization that depending upon the cavity size different crown ether derivatives show preference for binding towards different alkali metal ions initiated the active research in the area of cation recognition. Better insight in the cation recognition chemistry has also helped to understand the role of various of metal ions and their corresponding complexes in many biological processes, such as transmission of nerve impulses, muscle contraction, regulation of cell activity, treatment for manic depression, and possible implication in Alzheimer’s disease. Apart from these, many metal ions are known to have detrimental influences in human and other living organisms when present in certain amounts higher than the permissible level. Thus, specific role of different metal ions in various physiological processes is a research area of an immense interest and constitutes one major part of the contemporary research in chemistry and biology.

1A.2. Design of Synthetic Receptors

After initial report on crown ether by Pederson, reports on coronands and cryptands by Cram and J. M. Lehn in late 1960s had helped enormously in initiating an exponential growth in the research on cation recognition. Since then, cation recognition chemistry has matured and enormous data available in the literature allowed the effective correlation between the structure of the receptors and specificity in binding to a particular cationic species. This was further aided by the concurrent growth in user friendly software for molecular modelling and computational facility led chemists to the more successful design of the tailored-made receptors for targeted cation(s) for practical applications, e.g, for *in-vivo* or *in-vitro* detection of a metal ion in live organisms or in environmental samples. Apart from these, recent advances in the basic understanding of various weak interactions like ion-dipole, dipole-dipole, C-H–π, π-stack, and hydrogen bonding interactions have contributed significantly in developing
appropriate receptor for organic cations like quarternary ammonium, pyridinium and imidazolium ions. However, the idea of coupling receptor fragment having high complimentarity to the specific metal ion or organic cations, to colorimetric or fluorogenic centers has actually emerged in 1985, which has formally led to the development of the research on colorimetric and fluorogenic sensors. \(^7\)

### 1A.3. Photophysical Mechanisms Involved on Photoresponsive Cation Receptors

Signalling subunit constitutes one of the major components of any efficient sensors. Change in output signal, either in the form of fluorescence or electronic spectral changes(s), of the reporter functionality in response to the receptor-analyte interaction is basically the manifestation of the relative change in the difference in the energies of the frontier orbitals (Highest occupied molecular orbital (HOMO) and lowest occupied molecular orbital (LUMO)), as well as the differences in the ground and excited state properties of the photoactive unit in the free and the bound form. Such spectral response processes generally involve mechanistic pathways like, photo-induced electron transfer (PET), photo-induced energy transfer, excimer or exciplex formation, \(\pi-\pi\) interaction, intra or intermolecular charge transfer (CT), and excited state proton transfer, etc. \(^8\)-\(^10\) In few cases, changes associated with the redox potential of the reporter group is also used for designing appropriate sensor molecule. Few of these processes, which have a direct bearing in describing the new sensor molecules in this thesis, are to be discussed in the following section.

#### 1A.3.1. Photoinduced Electron Transfer (PET)

The working principle of the PET based sensors is shown in the following figure 1A.1. The photoinduced electron transfer (PET) is an attractive photophysical mechanism to recognize cations due to its simplicity. The designing principle for the PET based sensors involve a fluorophore connected with a receptor unit via a spacer.
Since PET based sensors rely on electron transfer, electroactivity is the main factor and thus molecular components of known redox potential\textsuperscript{11} would be helpful to choose the appropriate receptors, so that electron transfer between receptor and the fluorophore is energetically feasible due to low oxidation potential. The energetically feasible electron transfer quenches the fluorescence of the lumophore and thus fluorescence is absent at this stage. When cation binds with the receptor unit, it is expected that oxidation potential of the receptor unit will increase so that electron transfer is no longer energetically feasible. Therefore, the arrest of electron transfer after cation binding allows all the excited state electrons come back to the ground state and make the lumophore highly fluorescent.

**Figure 1A.1.** Photophysical mechanism of PET and fluorescence enhancement.

In the orbital picture of PET mechanism, when a lone pair is located in a receptor’s orbital, the HOMO of a receptor’s orbital lies between the HOMO and LUMO of the fluorophore. Efficient electron transition from HOMO of the lone pair to hole created in the HOMO of the fluorophore, during photo excitation, will not allow the transition of the photoexcited electron in the LUMO to the HOMO of the fluorophore to make the molecule fluorescent. Such an electron transfer process induces the non-radiative deactivation of excited state leading to the quenching of the fluorescence. The loss of fluorescence of the fluorophore when a lone pair orbital sits in the between the HOMO and the LUMO of the fluorophore can be recovered if the lone pair is engaged by protonation or metal ion coordination.\textsuperscript{12,13}
Thus, protonation or metal ion coordination brings the lone pair orbital lower than the HOMO of the fluorophore and inhibits the electron transfer quenching. The inhibition of PET thus allows the photoexcited electron present in the LUMO to come back to the HOMO of the lumophore by radiative fluorescence. Fluorescence PET sensor with an aza crown ether receptor, which is known to bind efficiently to Na\(^+\) ions, is an ideal demonstration of such PET based signal transaction mechanism and is used to measure the blood electrolyte levels quickly and reliably.

Siegerman et. al reported\(^{14}\) a PET sensor molecule which has an N–phenyl mono aza crown receptor (1) to bind Na\(^+\) ion at physiological conditions. Due to lower oxidation potential (0.8 V vs saturated calomel electrode) of the aniline motif, this unit participate effectively photoinduced electron transfer quenching upon photoexcitation, hence lack of luminescence in the switched OFF state. The very presence of the Na\(^+\) ion rises the oxidation potential of the aniline motif\(^{15}\) and this rise in oxidation potential explains the energetically impossible photoinduced electron transfer in the Na\(^+\) bound sensor.

![Diagram of molecule 1](image)

Most of the PET fluorescence sensors based on this mechanism involves in fluorescence enhancement when alkali and alkaline earth metal ions bind to the amine receptor. But, other than PET mechanism, some other photophysical signal transaction can take place with the transition metal ions like Ni(II) and Cu(II) and does not induce chelation enhance fluorescence and causes fluorescence to be quenched by electron transfer and energy transfer to the bound metal ions or vice versa.\(^{16}\)

**1A.3.2. Energy Transfer Quenching (ET)**

A metal ion can quench the fluorescence of the excited state fluorophore by an energy transfer mechanism, when metal ion posses empty or half–filled d orbital. R.
Bergonzi et al. reported that Cu(II) ion, with elongated-octahedron d^9 configuration, is capable of quenching the fluorescence of the naphthalene moiety in 2 by energy transfer (ET) mechanism. The d^9 metal ion quenches the fluorescence through double electron exchange as shown in the figure 3 without net distribution of an electron (Dexter type electron transfer).\(^{17}\)

![Energy transfer mechanism (double-electron exchange) between excited fluorophore and d\(^9\) metal ion (elongated-octahedral arrangement).](image)

In order to discriminate the possibility of ET and eT and support the ET quenching in [Cu\(^{II}\)(2)]\(^{2+}\), fluorescence experiment was carried out at 77 K in the glass forming solvent butyronitrile and was found to be non-fluorescent. Thus, low temperature fluorescence experiment supports a simple circulation of electrons, which does not induce any net charge creation or redistribution and thus it does not cause reorientation of the solvating molecules. As a consequence of this, ET is equally efficient in both liquid and frozen glass solution.
1A.3.3. Electron Transfer Quenching (eT)

The other mechanistic pathway that is involved in some recognition processes is Electron Transfer Quenching, which basically is an extension of the ideology of the PeT process. Here PeT is operational with a particular oxidation state of a metal ion; while the other accessible oxidation state of that metal ion does not participate in the PeT process and results in a different fluorescence pattern as output response. Such an example is shown in the figure 1A.6. Ni$^{2+}$ ion with a d$^8$ configurations do not participate in the PeT; while Ni$^{3+}$ does and thus the recognition phenomena works for the covalently attached dansyl moiety.

![Electron transfer mechanism](image)

**Figure 1A.3:** Electron transfer mechanism.

1A.3.4. Energy Transfer (ET) Vs Electron Transfer (eT) Quenching Mechanisms

Only few empirical guidelines are available in the literature to differentiate between the ET and eT mechanism, which could be operational for a particular system.19
1. The ET mechanism operates in a system (2, 4) where the saturated methylene spacer (−CH₂−) is the amine nitrogen-based receptor fragment to the fluorogenic receptor unit.

2. Distance between the metal centre (M²⁺) and the fluorescent moiety (C) should be less. C is the closest carbon atom of the fluorophore which is bound to the spacer. M−C = 4.5 Å for 5, 4.4 Å for 6.

3. Van der Walls contact (occasional collision) between the fluorophore and the metal ion is a pre-requisite condition for Dexter type ET mechanism.

4. Saturated spacers are needed.

But, in the case of eT mechanism,

1. The eT process takes place in systems (3, 5, 6, 7) in which the spacer is linked to a carbon atom (5, 6) of the ring or to a nitrogen atom, which are not involved in the coordination.

2. M−C distance is considerably large in the eT systems. 6.0 for 4, 6.1 for 5, 6.9 for 6.

3. eT system requires quite a rigid bridges like sulphonamide group, ester group.

4. Largely delocalised π orbitals, which connect the fluorophore and the receptor, are needed for electron transfer.

1A.3.5. Energy Transfer Mechanisms

Interaction between excited and ground states of two molecular chromophores has been the subject of considerable interest as occurrence of such a process is common in nature.¹⁹b This interaction is better described as electronic energy transfer (EET) and generally occurs at distances ranging from 1Å to 100Å over a wide time range from femtoseconds to milliseconds. Electronic energy transfer process either involves Radiative Energy Transfer or Non–radiative Energy Transfer from an excited donor molecule (D*) to an acceptor molecule (A).

\[ D^* + A \rightarrow D + A^* \]

1A.3.5.1. Radiative Energy Transfer

Radiative energy transfer is two–step processes which involve the absorption of a photon by a molecule A (Accepter) emitted from a molecule D* (Donor), in its excited
state and this transfer happens when the average distance between D and A is larger than the excitation wavelength.

\[ \text{D}^* \rightarrow \text{D} + \text{hv} \]

\[ \text{hv} + \text{A} \rightarrow \text{A}^* \]

The radiative transfer does not require any molecular interaction between the interacting donor and the acceptor, but it depends on the spectral overlap and on the concentration. Such an energy transfer is often called trivial energy transfer.

### 1A.3.5.2. Non-radiative Energy transfer

Non-radiative energy transfer is an electrodynamic phenomenon and transfers the excitation energy by long range dipole-dipole interactions between the donor and acceptor moieties. The rate of energy transfer depends upon the extent of spectral overlap of the emission spectrum of the donor and the absorption spectrum of the acceptor, the quantum yield of the donor, the relative orientation of the donor and acceptor dipoles and the distance between the donor and acceptor molecules.

\[ \text{D}^* + \text{A} \rightarrow \text{D} + \text{A}^* \]

Spectral overlap between the donor and the acceptor allows the several vibronic transitions of the donor to be coupled with the corresponding transition of the acceptors that have the same energy levels. Thus, such energy transfer processes are also called as resonance energy transfer process (RET).

**Figure 1A.4:** Forster Resonance Energy Transfer mechanism.
1A.4. Spherical Inorganic Cation Recognition

Participation of various metal ions in innumerable critical biological processes and in environmental pollution is well documented and the need for the development of a reliable as well as an efficient sensor molecule for such metal ions is well emphasized in the area of cation recognition research. Among alkaline earth metal ions, calcium ion (Ca\(^{2+}\)) is an important secondary messenger and thus large number of biological processes are regulated by temporal and spatial fluctuation of Ca\(^{2+}\) concentrations.\(^{20}\) In clinical diagnosis, measurement of concentration of Ca\(^{2+}\) ions in blood or serum is very important due to the involvement of Ca\(^{2+}\) ions in hypoparathyroidism, tumor metastasis and renal failure related problems.\(^{21}\) Magnesium ion (Mg\(^{2+}\)) involves in the regulation of enzymes and molecules of different structure and function. This ion is also involved in many processes like cell proliferation and cell death. Most ATP in cells is bound to Mg\(^{2+}\) since MgATP\(_2^−\) is the active species in enzyme binding and the energy-producing form in active transport and muscular contraction. Therefore, change in concentration of Mg\(^{2+}\) can have significant consequences in cell metabolism and cell functions.\(^{22}\)

The heavy transition metal ions such as Hg\(^{2+}\), Cu\(^{2+}\) which are highly toxic, poses severe risk for human health and environmental related problems. Thus, detection of these toxic metal ions are very significant important to resolve the environmental problem. Mercury contamination is widespread and the environment is getting contaminated by volcanic emissions. Oxidation of mercury vapor in atmosphere to water-soluble Hg\(^{2+}\) ions and its consequent metabolism by aquatic microbes produces methyl mercury, which bioaccumulates through the food chain. This is expected to have severe effect on human health and the environment. This methyl mercury is neurotoxic and can cause brain damage and minamata disease.\(^{23}\)

Cu\(^{2+}\) ion at lower concentrations is an essential trace nutrient to all higher plant and animal lives. In animals, including humans, it is found widely in tissues, liver,
muscles, and bones.\textsuperscript{24} It functions as a co-factor in various enzymes and in copper-based pigments. Copper is the third in abundance among the essential heavy transition metal ions in human body. However, its toxic effect, when present in concentration higher than the permissible level is known to cause different neurodegenerative diseases.\textsuperscript{25}

\textbf{1A.4.1. Synthetic Receptors for Hg\textsuperscript{2+} Ion}

As mentioned earlier, analytical techniques only provide direct and quantitative information about the Hg\textsuperscript{2+} ion present in any samples. However, these techniques are not suitable for quick detection of Hg\textsuperscript{2+} ion in real-time analysis. Further, these techniques are not suitable for \textit{in-vivo} detection of Hg\textsuperscript{2+} ion in biological sample. These limitations have actually contributed to the recent development of small molecule chemosensors for different metal ions. Recent advancement in Hg\textsuperscript{2+} ion sensor developed a number of considerations that influences the design of sensor molecules to probe Hg\textsuperscript{2+} ions.

1. Hg\textsuperscript{2+} ion being soft acid, receptors should be designed with soft donors like thiols and thioethers. Other donor atom includes, nitrogen and phosphorous.

2. Due to lack of ligand field stabilization, Hg\textsuperscript{2+} ion can have different coordination geometries. Among many coordination geometries linear and tetrahedral geometries are common. Thus, while designing suitable receptor for Hg\textsuperscript{2+} ion, these probable coordination geometries should be considered.

3. For live cell imaging application, water soluble sensor molecules are required that function at physiological pH.

\textbf{1A.4.1.1. Colorimetric sensor for Hg\textsuperscript{2+} ion}

Colorimetric sensors allow simple in-field visual detection and semi-quantitative analysis of Hg\textsuperscript{2+} ion. This simplicity in the detection process is the primary reason for the present emphasis in the search of efficient colorimetric sensors for Hg\textsuperscript{2+} ion.
Kim et al. reported a new series of benzoylthiourea derivatives 8, 9, 10 as a selective chemodosimeters for the Hg$^{2+}$ ion based on the mercury-promoted desulfurization, which lead to an irreversible chemical event with entirely different colorimetric response in DMSO/H$_2$O (4:1, v/v) medium. The azo-unit present in 8, 9, 10 acts as the reporter functionality.

Huang et al. reported an azo 8–hydroxyquinoline benzoate compound (11) as a colorimetric sensor for Hg$^{2+}$ ion in acetonitrile media. The Hg$^{2+}$ ion on binding to N$_{\text{quinoline}}$ N together with O$_{\text{carbonyl}}$ of 11, the characteristic strong absorption band of 11 at 445 nm ($\varepsilon = 3.1 \times 10^4$ mol$^{-1}$ L cm$^{-1}$) disappeared, while a new spectral band developed at 518 nm. The metal ion coordination transforms the D–π–D structure to D–π–A structure, which favoured the ICT process and thereby the red shift in the absorption spectra. The stoichiometry and binding constant of 11 with Hg$^{2+}$ was calculated to be 1:1 and 4.4 x 10$^5$ mol$^{-1}$ L, respectively.
Sensor 12 was found to be selective for Hg$^{2+}$ when screened for twenty different metal ions. The CT band for 12 at 460 nm in dioxane was shifted to lower energy (∼ 50 nm) on binding to Hg$^{2+}$ with associated colour change from yellow to red. $^1$H NMR investigations indicate a strong interaction between Hg(II) and the aza–oxo chain, though no binding constant was reported for such interaction.

Sensor 13 was found to be active in EtOH/H$_2$O (1:10) medium at neutral pH. This hemicyanine based selective dye for Hg$^{2+}$ ion consists of an aniline donor and benzothiazolium acceptor. Addition of Hg$^{2+}$ to 13 causes an appreciable (∼100 nm) blue-shift in the $\lambda_{\text{max}}$ from ∼550 to ∼450 nm, with an isosbestic point at ∼480 nm with the change in solution from pink to green. The response of 13 to Hg$^{2+}$ was pH dependent and optimal response was observed at pH 7. The stoichiometry (by Job’s plot method) and binding constant were found to be 1:1 and $1.0 \times 10^7$ M$^{-1}$, respectively.

Yuliang Li et al. reported a visible near-infrared sensor 14, having cyanine as a chromophoric unit in methanol. The absorption band maxima for 14 at 695 nm was found to decrease on binding to Hg$^{2+}$ ion and a new absorption band with maxima at 817 nm appeared along with an isosbestic point 740 nm. The 1:1 stoichiometry and the association constant $K_f = 4.335 \times 10^4$ M$^{-1}$ were determined from Benesi–Hilderbrand plot.
The N-azo coupled macrocycle 15 with a NO$_2$S$_2$ donor was selective towards Hg$^{2+}$ ion. Compound 15 exhibits an intense absorption band at 480 nm and results in a large cation-induced blue shift of 133 nm on binding to Hg$^{2+}$ ion with a colour change from red to pale-yellow. Crystals of the complex of 15 with Hg(ClO$_4$)$_2$ salt were pale-yellow in colour with Hg$^{2+}$ ion adopting a square planar environment. Its coordination sites were occupied by two S and one N atom arranged in an endo-fashion. Weakly bound ClO$_4^-$ at fourth coordination site favours the Hg-N coordination and the colour change to pale-yellow. But crystal obtained with iodide ion found to be colourless, as Hg$^{2+}$ ion remained coordinated to one S and three I$^-$ in an exo-fashion. Here, strong coordination of I$^-$ ion prevents the Hg-N bond formation and the recognition process.

1A.4.1.2. Fluorescent sensors for Hg$^{2+}$ ion

Fluorescent probes have their apparent advantage over others due to their higher sensitivity and most importantly for probable application in imaging processes. Among these, receptors that work in the visible to NIR region are more suitable for use with biological samples, as light within this spectral range are not known to affect the live cells. Fluoresceins, rhodamines, 4,4-difluoro-4-bora-3a,4a-diaza-3-indacenes (BODIPY dyes), squaraines, and cyanines belong to this class of fluorophore and are thus ideally suitable for biological applications and in-vitro imaging processes. Among these long emitting fluorophores, rhodamine derivatives received special attention, since it was first synthesized by Noelting and Dziewonsky in 1905$^{32}$, due to their excellent photophysical properties.

Rhodamine derivatives in their spirolactam form are non-fluorescent and colorless; whereas strongly emissive and have visually detectable pink colour when present in their respective ring-opened xanthene form. Czarnik et al. reported a rhodamine B-based chemodosimeter, 16 for detection of Cu$^{2+}$ in 0.01 M HEPES buffer-CH$_3$CN (4:1, v/v; pH 7). The reported molecule 16 was designed to provide the binding
site analogous to a $\alpha$-amino ester, whose hydrolysis is promoted by Cu$^{2+}$. Also
hydrazide groups are well known to bind Cu$^{2+}$ which results in an enhanced
transacylation reactivity. The reported sensitivity of 16 towards Cu$^{2+}$ is reported to be
10 nM.$^{33}$

Xu et al. converted the chemosensor 16 into rhodamine thiocarbazide (17) by treating
16 with Lawesson’s reagent for achieving better selectivity towards Hg$^{2+}$ ion. Thus, the
introduction of sulphur–based functional group successfully recognises the Hg$^{2+}$ ion
due to soft acid–soft base interaction. The binding constant of 17 with Hg$^{2+}$ for a 2:1
complex formation was found to be $4.5 \times 10^{10}$ M$^{-2}$. The lower detection limit is reported
to be $1.0 \times 10^{-7}$ M.$^{34}$
The advantage of the soft nature of the sulphur atom was also exploited by Ma et al.\textsuperscript{35} They reported a thiolactone derivative (18) for specific detection of Hg\textsuperscript{2+} in aqueous media.

Kim et al. reported a series of rhodamine (acceptor) and dansyl (donor) based sensor molecules (20–22) linked by tren-based spacer. Specific choice of the two fluorophore groups allowed to achieve FRET on binding to Cu\textsuperscript{2+} ion.\textsuperscript{36} In the absence of Cu\textsuperscript{2+} ion the spirolactum form prevailed and thus on excitation of 22 at 420 nm ($\lambda_{\text{exc}}^{\text{Dansyl}}$), only dansyl based emission at 507 nm was observed. On binding to Cu\textsuperscript{2+}, xanthenes form prevailed and a substantial spectral overlap between dansyl emission and spirolactum absorption bands was achieved to initiate a switch-ON FRET emission maximum at 580 nm.

Huang et al. reported a rhodamine based multisignaling sensor 23, which displays changes in UV/vis absorption, fluorescence and electrochemical measurement. 23 showed a weak absorption and weak emission band at 500 nm and 580 nm respectively in ethanol/HEPES buffer (1:1, v/v, pH 7.2) solution due to the existence of colourless spirolactam form. Upon addition of Hg\textsuperscript{2+} to a 20 \(\mu\)M solution of 23 showed a new peak appearing at 560 nm in the absorption spectra and high fluorescence enhancement at 580 nm, clearly suggesting the formation of the ring–opened xanthene form of 23. The association constant for 1:1 stoichiometry model was found to be 3.7 x 10\textsuperscript{3} mol\textsuperscript{-1}. Differential pulse voltammetric (DPV) of 23 in the presence and absence of Hg\textsuperscript{2+} ion showed an evolution of a new oxidation peak at 0.15 V from 0.4 V versus
The Fc/Fc\(^+\) redox couple in ethanol solution containing 0.1 M \(n\)-tetrabutylammonium hexafluorophosphate as a supporting electrolyte. Confocal images of Caov–3 cells treated with 5 \(\mu\)M of 23 and 10 \(\mu\)M of Hg\(^{2+}\) showed strong intracellular fluorescence suggesting that the subcellular distribution of Hg\(^{2+}\) within the biological samples could be detected by 23.\(^{37}\)

Shin. et al reported a chemodosimetric detection of intra-cellular Hg\(^{2+}\) ion in living organism.\(^{38}\) This reagent (24) undergoes Hg\(^{2+}\) ion induced cyclization transformation from thiosemicarbazide to 1, 3, 4-oxadiazole in aqueous media and this resulted an instant colour change from colorless to pink (\(\varepsilon = 4.67\)) and a strong fluorescence (\(\Phi = 0.52\)) at 557 nm. Experiments were carried out to detect the Hg\(^{2+}\) in the living organisms like 3 months old zebrafish in E3 media. Fluorescence images were recorded when organs treated with 5 nM HgCl\(_2\) and 10\(\mu\)M 24. Fluorescence images of dissected tissues and organs of zebra fish suggested that Hg\(^{2+}\) ions could be detected in the brain, heart, liver and gall bladder due to the ability of 24 to reach all the organs.

A thioether derivative, 25 was found to be selective for Hg\(^{2+}\) ions in the presence of other competing metal ions under biological conditions. The chemosensor 25 has a strong absorption at 485 nm with a very weak emission band at 514 nm (\(\Phi = 0.02\)). On binding to Hg\(^{2+}\) ion, a 44-fold fluorescence enhancement (\(\Phi = 0.72\)) in HEPES buffer media at pH 7 was observed. In order to improve the membrane permeability, 25 was
converted to acetoxy methyl ester (25-AE) derivative and this was further used to track the Hg^{2+} ion in living cell (HEK 293T cells). Confocal images of live HEK 293T cells loaded with 1\(\mu\)M of 25-AE up to 60 minutes showed a low background intracellular fluorescence. But, cells, which were incubated with 25-AE and then treated with 4 ppm of Hg^{2+} showed enhanced intracellular fluorescence.\(^{39}\)

![Chemical Structure 25](image1)

Xuhong Qian et al. reported ratiometric fluorescent probe, 26 to detect Hg^{2+} ion present in MCF–7 (breast cancer) cells.\(^{40}\) In the presence of Hg^{2+}, emission maximum of this sensor molecule, when excited at 488 nm, changes from 514 nm, which is characteristic to BODIPY, to rhodamine emission at 589 nm. The shift in fluorescence emission wavelength allowed detection of the Hg^{2+} ion ratiometrically by FRET process with associated change in the observed fluorescence from green to orange-red. The Forster resonance energy transfer between BODIPY and rhodamine was calculated to be 99% with the FRET distance of 58.9 Å. In confocal fluorescence imaging experiment, MCF-7 cells incubated with 26 (5 \(\mu\)M) showed a clear green intracellular fluorescence. But, addition of HgCl\(_2\) (5 \(\mu\)M) in the cells stained with 26 showed a remarkable red fluorescence.

![Confocal Images](image2)
1A.5. Recognition of Organic Cations such as Imidazoilium/pyridinium and Ammonium Cations: Pseudorotaxane Based Interlocked Molecules

In the molecular evolution of biological systems, it is believed that highly selective molecular complexation between organic compounds must have played a central role.\textsuperscript{41} In this view, due to the important role of substituted ammonium and imidazolium/pyridinium ions in chemistry and in biology, development of synthetic receptors for the recognition of these organic cations such as ammonium cations and imidazolium cations are of great interest. Thus, studies of such recognition processes with tailored-made receptors and ammonium or imidazolium ion-based guests are expected to help in developing a better insight in unravelling the complicated multitopic recognition process in biology, which are operational through weak interaction forces like H-bonding, dipole-dipole/induced dipole interactions, and $\pi$-$\pi$ stack interactions.

Some physiologically active amines like histamine, dopamine and acetylcholine exist as ammonium ions under physiological pH. The interaction of these small ammonium ions with proteins influences many biological signal transduction processes. Binding of histamine to the human H1 receptor, a G-coupled protein receptor, results in lower blood pressure and failure of dopamine responsive neurons causes Parkinson’s disease.\textsuperscript{42} The understanding of the multiple interactions involved in the recognition of histamine and dopamine class of ammonium ions in physiological condition is the key to the development of tools to study these processes.

Protonated and non–protonated forms of imidazole have a key role in the biological function of various enzymes and nucleic acids, as it acts as an active structural unit at the active sites of various enzymes and nucleic acids.\textsuperscript{43} In its non–protonated form, imidazole easily co–ordinates to transition metal ions like Zn$^{2+}$ and also can participate in forming strong H–bonded adducts.\textsuperscript{44} However, the protonated form of imidazole, which exists during enzymatic reactions, may interact
with the substrate by direct electrostatic or $\pi-\pi$ interactions.\textsuperscript{44} Thus, analogous to these biological systems, functioning of the supramolecular host–guest system, derived from ammonium or imidazolium/pryridinium cations, occur by multiple non–covalent interactions. So the binding mechanism of the host–guest complex can be understood, and finally thus be correlated to understand the difficult biological mechanisms, by the combined efforts of several non–covalent interactions that are operational. Therefore, the investigation of ammonium, imidazolium/pryridinium ion recognition is of considerable fundamental and practical interest.

1A.5.1 Molecular Complementarity, Molecular Interactions and Thermodynamic factors in Molecular Recognition

1A.5.1.1. Molecular Complementarity

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{image.png}
\caption{Molecular complementarity and positive allosteric effect in molecular recognition.}
\end{figure}

Molecular recognition process could be of two types, namely, static and dynamic. Static molecular interaction is a simple 1:1 complex formation between host and guest molecule. The necessary condition to static molecular recognition is highly specific receptor site to sterically fit into guest molecule like recognition between key and keyhole. But, in dynamic molecular recognition the binding of the first guest to the first binding site of a host affects favourably or adversely the association constant of second guest with a second binding site.\textsuperscript{45} If the first binding event favours the association of second binding, the system is termed as positive allosteric effect. If the first binding event does not favour and thus decreases the association of second
binding event, the system is called negative allosteric system. To design a receptor with high specificity based on lock and key model, Emil Fischer proposed that complimentarity between host and guest is the most important factor.46 A combination of excellent steric fit with a good match of the charge distributions of guest surface and the hosts cavity maximizes the selectivity in the recognition events.

1A.5.1.2. Molecular Interactions

In addition to molecular complementarity, various molecular interactions between host and guest help to achieve selective and effective molecular recognition. Various molecular interactions like, electrostatic, hydrogen bonding, ion–dipole, dipole–dipole, hydrophobic and van der Walls interactions work together to influence the molecular association cooperatively. Thus, designing of an effective molecular recognition needs a thorough understanding of various prevailing interactions. Molecular interactions range from coordination bonds with a strength ranging from 150 KJ mol\(^{-1}\) to 500 KJ mol\(^{-1}\) to weak vander Walls interactions of < 5 KJ mol\(^{-1}\).

1A.5.1.2.1. Ionic and dipolar interactions

Ionic and dipolar interaction can be divided into three categories based on the columbic interactions: (1) ion–ion interaction, (2) ion–dipole interaction, and (3) dipole–dipole interaction. Electrostatic ion–ion interaction occurs between oppositely charged molecules and thus the magnitude of this interaction is relatively high compared to the other interaction. Due to its non-directionality nature, this interaction can occur from any direction and thus it can stabilise wide range of molecular pairings. But, ion–dipole and dipole–dipole interactions are orientation dependent and directional in nature.
Directional nature of these interactions only allows the mutually complementary species to form ion pairs. Among these three interactions, dipole–dipole interaction is the weakest one (5 - 50 KJ mol\(^{-1}\)) but, it is highly directional in nature.\(^{47}\)

1A.5.1.2.2. \(\pi\)-\(\pi\) interactions

\(\pi\)-\(\pi\) interactions play an important role in various molecular recognition processes. Three different \(\pi\) interactions, namely, edge-face \(\pi\) interaction (CH-\(\pi\)), offset \(\pi\)-\(\pi\) interaction and face-to-face \(\pi\)-\(\pi\) interaction are generally observed.\(^{48}\) Although benzene has no net dipole, it has an uneven distribution of electron density, with greater electron density on the face of the ring and reduced electron density on the edge. The edge-face geometry (CH-\(\pi\)) is found in the \(\pi\)-\(\pi\) interaction appears to be most stable in benzene. The offset \(\pi\)-\(\pi\) interaction is more common when electron density on the face of one or both rings is reduced.

Thus, these edge-face \(\pi\) interaction and offset \(\pi\)-\(\pi\) interaction arise from the attraction between the negatively charged \(\pi\) electron cloud on the face of benzene ring and the
positively charged sigma-framework of the edge of a neighbouring molecule (Figure 1A.7). A third geometry is the face-to-face $\pi - \pi$ interaction, where parallel ring systems are separated by ca. 3.5 Å. This kind of interaction is commonly observed in donor-acceptors pairs and compounds have opposite quadrupole moments. The benzene and perfluorobenzene interaction is an example of this type aromatic interaction and calculated to provide -15.5 KJ mol$^{-1}$ in stability.

1A.5.1.2.3. Hydrogen bonding interactions

Hydrogen bonding plays an important role in molecular recognition processes although it is weaker than that of electrostatic interaction. Hydrogen bonding interaction is a kind of dipole-dipole interaction between proton donor and proton acceptor and its strength ranges from 4 - 120 KJ mol$^{-1}$. The strength of the hydrogen bond is determined by its geometry and type of donor and acceptor involved in the hydrogen bonded adduct formation. The interaction of polarised hydrogen atom with an electron rich atom results in direction specific hydrogen bonding between functional groups. Hydrogen bonding can be classified into three categories. In strong hydrogen bond, the hydrogen atom is close to the centre point of the donor and acceptor atoms. For example, in HF$_2^-$ ion, linear hydrogen bonding formation between two fluorine atoms [F···H···F]$^-$ makes stronger interaction similar to covalent bond interaction. Moderate hydrogen bonding interaction does not have linear geometry but bent in nature. Here interaction takes place between neutral donor and neutral acceptor via its lone pairs of electron. Slightly bent hydrogen bonding is observed in self-association of carboxylic acids due to moderate interaction between donor and acceptor atoms. Weak hydrogen bonds are even less linear and in some cases found to form perpendicular interactions like C-H···$\pi$ interactions.
1A.5.1.3. Thermodynamic Factors

Molecular recognition results a complex formation between two molecular entities, which is entropically ($\Delta S$ value) not favoured. However, this is counterbalanced through enthalpy release ($\Delta H$ value) on host-guest adduct formation. This is further favoured if more than one binding site in host molecule can be incorporated; as more than one interaction sites favour the higher enthalpy release ($\Delta H$ value); while the $\Delta S$ remains same. This effect is called as the chelate effect. Donald Cram developed an idea of pre-organization to reduce the entropic costs stemming from conformationally flexible hosts during guest binding. Thus, he developed a host which has the binding sites in a conformationally fixed way, perfectly complimentary to the guest's need, will bind more strongly than a flexible host which needs to be rigidified in the binding process. Thus, comparison of binding constants of alkali metal ions with conformationally flexible 18-crown-6 and the spherand (displays the six oxygen donor atoms in a preorganized manner) reveals that the spherand has binding constant higher by 10 orders of magnitude than that for 18-crown-6. Binding systems/events which are enthalpically disfavoured and entropically favoured can also exist, which is exact opposite of the above discussed process. If a free host contains few solvent molecules as a guest, which on binding to guest ions/molecules releases more number of solvent molecules from the host-solvent complex and thus contributes to the enhanced entropy.

1A.5.2 Ammonium ion binding

Pedersen was the first to report the use of different crown ether derivatives for the recognition of alkali metal cations as well as ammonium cations. Since then innumerable reports on recognition of different quarternary ammonium ions have appeared. Crown ether-ammonium ion interaction occurs by H-bonding between $OCrown$
and other free electron pair in hetero atoms of the crown ethers and N*-H bonds of the ammonium ion.\textsuperscript{53}

\textbf{Figure 1A.8:} Possible modes of interaction between O\textsubscript{Crown} and H\textsubscript{NR2H+2} and H\textsubscript{NR3H+}.

Michael et al. reported the binding ability and the change in interaction energy of C\textsubscript{6}H\textsubscript{11}NH\textsubscript{3}+ with mono-, bi-, tri- and tetra-dentate ethers.\textsuperscript{54} He investigated that bi-dentate bonding of C\textsubscript{6}H\textsubscript{11}NH\textsubscript{3}+ with diethers and reveal that use of CH\textsubscript{3}O(CH\textsubscript{2})\textsubscript{2}OCH\textsubscript{3} (Figure 1A.8 (a)) increases the interaction energy by 7 Kcal mol\textsuperscript{-1} compared to that of monoether (C\textsubscript{2}H\textsubscript{5})\textsubscript{2}O. Molecular modelling study shows that optimal H-N-H bond angle for a bi-dentate geometry is about 140°, which is less than the optimum value 180°. But triether, CH\textsubscript{3}(OCH\textsubscript{2}CH\textsubscript{2})\textsubscript{2}OCH\textsubscript{3} allows the formation of an additional third hydrogen bond and increases the NH\textsuperscript{+}…O angle about \textasciitilde160°. This additional gain from stability from third hydrogen bond results in the decrease in geometrical constrain by 10 Kcal mol\textsuperscript{-1}. Interestingly, for tetraether, CH\textsubscript{3}(OCH\textsubscript{2}CH\textsubscript{2})\textsubscript{3}OCH\textsubscript{3} a further increase in the interaction energy is observed, although the three H atoms on –NH\textsubscript{3}+ are already hydrogen bonded in the triether complex. Therefore, the increased interaction energy with the tetraether must be due to further geometry optimization, or possibly due to a –CH\textsuperscript{δ+}….O hydrogen bond.

\textbf{Figure 1A.9:} Projection of ammonium ion binding into the crown ether ring.

In the case of cyclic polyethers the increasing stabilities of the complexes with increasing size of the cyclic crown ethers was observed due to the preorganised
geometry and additional $-\text{CH}^{5\ast}\cdots\text{O}-$ or dispersive interaction. Molecular models show that the $-\text{NH}^+\cdots\text{O}$ angles becomes more favourable for the larger ethers. The figure (1A.9) depicts Newman projection formula of methyl ammonium ion and 18-crown-6 which has the three N-H$^+\cdots\text{O}$ and three N$^+\cdots\text{O}$ binding sites. In these structures, the C-N bond is perpendicular to the best plane of the oxygen atoms of the crown, and the tripod arrangement of the hydrogen bonds accounts for the rigid structure of the complex.

Among the three cyclic crown ether derivatives 12-crown-4, 15-crown-5 and 18-Crown-6, 18-Crown-6 shows the highest affinity for primary ammonium ions. Ionic diameter of an ammonium ion is 286 pm and this is very close to the cavity size of 18-crown-6 (260 - 320 pm). The ionic diameter of an ammonium ion is 286 pm, which is very similar to potassium ions with 266 pm; however, ammonium ions prefers a tetrahedral binding and potassium ions needs an octahedral co-ordination for strong binding.$^{55}$

Interestingly, secondary ammonium ions prefer larger crown ethers and slips through the crown ether ring forming “pseudorotaxane” (27) like structures. The binding strength of ammonium ion with crown ethers decreases in the order primary $>$ secondary $>$ tertiary-ammonium ion and this correlates well with the number of H-bonds that can be formed with the crown ether-based guest.

Balzani et al. took the advantage of this reversible interwoven complex, pseudorotaxane, formation for designing a unique molecular level plug/socket system using a crown ether derived from the binaphthyl (binap) unit and a secondary ammonium ion-based guest molecule (28), functionalized with an anthracenyl unit.$^{56}$ As
anticipated, the association of binap-crown ether with an anthracenyl ammonium ion through hydrogen bonding leads to an electronic energy transfer from binap moiety to anthracene unit. The association constant for 1:1 binding stoichiometry was found to be $10^5$ M$^{-1}$, with energy transfer rate constant between binap and anthracene was found to be $> 4 \times 10^9$ s$^{-1}$.

Recently, Schalley et al. demonstrated the concept of integrative self-sorting in ammonium ion based hetero[3]pseudorotaxane system. They designed a four component self sorting system (29 - 32) on the basis of literature reports where the association constant for 29:30 and 31:32 are found to be more stable than 31:30 and 29:32. Because of two stoppers (anthracene and phenyl) attached to 30 exist as a barrier for 31 to slip into the axle 30. Using this thermodynamic property of these systems, they designed an integrative self-sorting system by combining two axles 30 and 32 to form a divalent counterpart 33. In non-polar solvents, an equimolar mixture of 33 and 29/31, site A is occupied by 29 and site B is occupied by 31 due to their complementary nature and corresponding high binding constant. Thus, irrespective of the mixing order of the three components, integrative self-sorting of crown ethers by their corresponding binding sites has been achieved and proved by NMR and MS experiments.
Stoddart et al. in 1998, reported series of secondary dialkyl ammonium ions and their binding properties and kinetic stabilities towards the dibenzo crown ether 29\(^{58}\). The self-assembly of 29 with 34\(^+\) is primarily stabilised by [N\(^+\)-H⋯O] and [C-H⋯O] hydrogen bonds and π-π interaction. In order to understand the threading ability of ammonium ions into the dibenzo crown ether 29, they modified the 34\(^+\) cation with varying stopper sizes. Their idea was, different size of stoppers would reach a state where formation of an inter-woven complex would be failed at ambient temperature. However, kinetically stable rotaxane-like entity could be achieved under the influence of appropriate thermal energy.

The proton NMR spectra at room temperature proved that a 1:1 mixture of 29 and 35\(^+\), in CDCl\(_3/\)CD\(_3\)CN (3:1) medium, form H-bonded inclusion complex after 9.5 days. But the decomplexation of this mixture at ambient temperature confirms that, the 29.35\(^+\) is
a *pseudorotaxane* and not a rotaxane. On the other hand, the bulky stopper t-butyl group $36^+$ failed to form any interwoven like complexes even at higher temperatures, as slippage of $29$ was not possible. At 20ºC, very rapid slippage of $29$ over $37^+$ was observed and the system reached the equilibrium in ~ 90 min after dissolving the 1:1 mixture of $29$ and $37^+$ in CDCl$_3$/CD$_3$CN (3:1) medium. In contrast, $38^+$ did not show any inclusion complex formation with $29$ at 20ºC. But, at 40ºC the system reached the equilibrium after 36 days and almost of 98% of $38^+$ was converted into its complex with $29$. The extrusion of $29.38^+$ complexes in DMSO solution suggests that $29.38^+$ complex is kinetically inert in non polar solvents, but kinetically labile in high polar solvents like DMSO. A 1:1 mixture of $29$ and $39^+$ failed to form any complex due to the bulky nature of stopper.

**1A.5.3. Imidazolium/pyridinium cation binding**

Imidazolium derivatives have been investigated as a suitable guests for interwoven complex formation with macrocyclic ligands such as crown ethers, calixarenes, and cucurbituril because of the acidic nature of all the protons in the 1,3–disubstituted imidazolium salts; e.g., the pK$_a$ of the C$_2$ proton is 16 - 24, depending on the nature of the substituents on the two imidazolium nitrogens. $^{59}$ These acidic protons act as hydrogen bond donor and participate in the formation of supramolecular complexes and with appropriate design it is possible to use for the formation of a supramolecular pseudorotaxane complexes. Bis-functional nature of imidazolium ions can interact with crown ether type host structures both by hydrogen bonding to the ether oxygen atoms and/or by charge–transfer interactions to the electron rich phenyl rings of the crown ether.
In 1979 Lehn et al. have shown that a chiral 1, 2, 10, 11, 19, 20-hexacarboxylate-27-crown-9 derivative (40), forms a relatively stable complex with an imidazolium ion, even in aqueous solutions with the binding constant of 350 M$^{-1}$.\textsuperscript{60} They opined that the imidazolium ion got bound to the macrocyclic receptor through two N-H…O bonds with the involving O\textsubscript{Crown} atoms.

Stolwijk et. al. found that for the efficient binding of imidazolium ion, a ring size of at least 30 ring atoms of a crown ether is needed.\textsuperscript{61} Further, results of their studies could reveal that the incorporation of aromatic ring in the crown ether decreases electron density in the adjacent oxygen atoms and thus resulted in weaker stability of crown ether-imidazolium ion complexes. A 1:1 complex formation between benzo-30-crown-10 with imidazolium perchlorate was proved by single crystal X-ray analysis. In the encapsulated cavity, short non-bonding distances between N-H and C-H groups of the cation and ether O\textsubscript{Crown} of benzo-30-crown-10 are observed which can be described as weak nonlinear hydrogen bonds.

![Figure 1A.10: Crystal structure of benzo-30-crown-10-imidazolium perchlorate, with atom numbering. Hydrogen bonds are indicated by dashed lines.](image)

Later, in 2000, pursiainen et al. reported the complexation of the imidazolium cation with crown ethers with smaller size as well as with varying numbers of aromatic units in acetonitrile.\textsuperscript{62} H-bonding was the primary influence that was responsible for 1:1 complex formation in acetonitrile solution. Minor contribution from $\pi$-$\pi$ stacking or charge transfer interactions was also observed in the benzo crown ether derivatives.
Among these different crown ethers and imidazolium cation derivatives, the highest binding constant was observed for $41.46$ with the affinity value of $40 \text{ M}^{-1}$ and the lowest value observed for $45.46$ ($8 \text{ M}^{-1}$). The binding affinity constants for complexes $43.46$ ($27 \text{ mol}^{-1}$) and $42.46$ ($25 \text{ mol}^{-1}$) are lower than the corresponding values for the complex $41.46$. In complexes $43.46$ and $42.46$, the presence of aromatic rings in the crown ether lowers the electron density of the adjacent oxygen atoms, and thus adversely influence the strength of any H-bonding and this explains the lower stability constants. However, complex $43.46$ has higher binding constant than the complex $42.46$ due to the additional π-stacking and/or charge transfer interactions. The stability constants of the 1-substituted methyl- ($47$) and phenyl-imidazolium ($48$) cations with $43$ ($18 \text{ mol}^{-1}$ and $16 \text{ mol}^{-1}$, respectively) are nearly 50% lower than the stability constants of the unsubstituted imidazolium complex.

The host–guest complexation of a macrocyclic ligand with a pyridinium ion has been studied on several occasions previously. Piepers and Kellogg observed, in 1980, that pyridine-substituted crown ethers form complexes with n-alkylpyridinium salts. The 1:1 complex between 18-crown-6 and pyridinium chlorochromate has been prepared in order to study its oxidation properties towards alcohols. New catenanes and rotaxanes have been assembled on the basis of donor ± acceptor interactions between bipyridinium ions and benzo and naphthalene crownethers.
Figure 1A.9. Ball-and-stick representation of the solid-state structure of the complex between DB18C6 (45) and pyridinium tetrafluoroborate (49).

The formation of complexes between 18C6 (41) and pyridinium ions (Py) shows that hydrogen bonding contributes to the stability of complexes. Comparison of the observed intensities, in mass spectra, of complex peaks suggests that pyridinium ion forms more stable complexes with crown ethers than do substituted pyridinium salts (Py > PyNH₂ > PyCH₃) and that complexes of monobenzo-substituted crown ethers are somewhat more stable than those of disubstituted crown ethers. The small pyridinium ring can penetrate deeper into the cavity of the macrocycle than substituted pyridinium ions, and this allows increased interaction between the phenyl ring(s) of the crown ethers and the electron-deficient pyridinium cation. The amino group is smaller than the methyl substituent and can form hydrogen bonds, which explains the greater $K_a$ values for the 1-aminopyridinium complexes than the 1-methylpyridinium complexes. Markku et al. reported the FAB mass spectrum for 18C6 (41) and pyridinium tetrafluoroborate, which showed a peak at m/z 344. This corresponds to the 1:1 [18C6 – Py] complex after loss of its BF₄⁻ counterion and found to be most stable with stability constant of 113 dm³mol⁻¹. However, in the crystal structures of the DB18C6–pyridinium complexes (Figure 1A.9), hydrogen bonding seems to play a secondary role and the π–π interaction is dominant in these complexes, because the benzene rings decrease the negative charge of the oxygen atoms and hence their ability to undergo hydrogen bonding. This is in full agreement with the observed stability constants, which decrease in the order unsubstituted crown ether > benzo-substituted crown ether > dibenzo-substituted crown ether.
1A. References


1B. Molecular Logic Gates

1B.1. Introduction

Till date, the miniaturization of electronic devices continues to deliver high capacity memories and faster processors. However, development of very smaller electronic devices with more number of transistors on the silicon chips becomes more difficult and expensive as the nanometer scale is approached. The development of reliable techniques to produce conventional silicon–based semiconductor devices with dimensions lower than 100 nm is a formidable technological challenge due to fundamental and technological barrier. More importantly, the bulk properties of semiconductors vanish at the nanometer level. Thus, the operating principles of present devices cannot survive the shrinking process. Other limitations are imposed by the material used, the device type used, the circuit concept, and the system configuration. Material limits are related to the properties of semiconductor materials used for fabrication of the device: switching energy, transit time, thermal conductance, and a dopant fluctuation limit. Device, circuit, and system limitations are associated with heat dissipation, transition time, circuit latency, cross–talk, signal contamination, and the chip size.\(^1\) These limitations in developing nano scale semiconductor device will slow down and finally stop the miniaturization process. Thus, to overcome these limitations alternative materials that can mimic the function of semiconductors devices must be identified.

Although the properties of semiconductors can be tuned by utilizing size effects or adopting doping strategies, the vast array of chemical reactions that are available in the data base allow the synthetic chemist to access a diverse range of molecular structures. The properties of molecules can be modified or tuned rationally in a systematic process with appropriate structural variations, which offers a much greater degree of variability and control of their properties. Moreover, assemblies of molecules
(supermolecules)² containing multiple modules, each with its own property and
function, are particularly promising for information handling. Because, for example, one
module could be used to respond to a specific chemical input and another would then
be triggered to give an output of some kind. Thus, recent advances in supramolecular
chemistry have opened up the possibility of constructing simple photonic and
chemically driven systems that can function as molecular level devices. In this regard,
organic molecules are the promising candidates for the realization of future digital
processors. The most important feature of the organic molecules is their synthetic
tunability in the nano scale dimension.

1B.1.1. From Boolean logic to Molecular Logic

The mathematical Boolean logic is being used in uncountable electronic
devices and is one of the basic functions of all digital electronics for information
processing, although initially it was established as a pure theoretical science.³ One of
the predominant features of Information Technology is the processing of input signals
by means of combinatorial operations, using the so-called Boolean logic gates. Data
processing in conventional computers based on the silicon circuitry requires the
Boolean binary encoding of information contained in electrical signal;⁴ for each signal a
threshold value and a logic convention are defined. Positive logic convention relates to
0 for a signal value below the threshold and to 1 for a value above the threshold.
Negative logic convention leads to the reverse situation. It should be noted that binary
logic is a general concept and applies, therefore, to any type of signal, including
chemical and optical ones. This makes it possible to use molecular systems for
mimicking logic functions. More importantly, information processing at the molecular
level is a common feature of numerous biological and chemical systems.⁵ In many
biological systems, molecules and their corresponding supramolecular assemblies
carry information and act as information processing devices. All of the regulatory
processes in living cells, cellular signalling, and of course all of the neurobiological activities process information at the molecular level. Molecular recognition in biological systems, activation of enzymes by small molecules and signal transduction are also processes based on YES–NOT logic. Thus, many biochemical bifurcate pathway undergoes Boolean logic rules at the molecular level in a sense that every single molecule can follow only one reaction path; therefore, its fate can be described in terms of Boolean algebra. All these led to the realization that molecular components could be used to describe logic operations with specific ionic or chemical inputs or with a specific sequence of inputs.

1B.1.2. Molecular Switches to replace the existing silicon chips

Any chemical system that can exist in at least two forms with distinctly different spectral, electrochemical, or magnetic properties can be regarded as a molecular switch. The discrete transition between these two forms should only be possible upon stimulation with chemical, optical and electrochemical inputs and certainly not spontaneously. Such chemical systems that can exist in two discrete forms can be considered to mimic the logic functions existing in conventional electronic devices. If one state of the switch is assigned to logical 1 (ON) and the other to logical 0 (OFF), the switch should be regarded as one input logic gate: YES or NOT. Thus, these simple switches can be considered as one input logic gates. Any chemical system which can switch over between its two states (0 and 1) can easily be considered as molecular logic gates if two states are distinguishable as follows.

1. Fluorescence quantum yield of one state should change from very low (\(\Phi<1\)) to high (\(\Phi \approx 1\)) when changes are monitored by fluorescence technique.
2. In case of absorbance changes, significant spectral shift of the absorption band which should be larger than the band half-width.

3. Significant changes in the redox potential values.

The first practical implementation of Feynman’s ideas came after 30 years\(^9\) when the first molecular machines were reported in 1992 by Balzani and co-workers\(^{10}\) and then by A. P. de Silva in 1993.\(^{10b}\) Both exploited ion-binding (ionic inputs) induced spectral changes in tailor-made molecular receptors functionalized with fluorophores for optical output in these molecular systems. Latter, various articles described input information in the form of specific ionic or neutral analytes, along with the respective concentration; while signal output were generally in the form of optical (light absorption or fluorescence) and electrical (Redox potential) in nature.

1B.2. Different Types of Logic Gate in Chemical System

1B.2.1. Single Input logic gate

Single input logic system refers to one input and one out values of a logic gate. This class of logic gate is divided into four categories, PASS 1, PASS 0, YES and NOT as shown in the truth table below. These logic gates are considered to be trivial in semiconductor electronic devices.

<table>
<thead>
<tr>
<th>Input</th>
<th>Output (Fluorescence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H⁺</td>
<td>PASS 0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 1B.1.** Simplest electric circuits representing the YES (a) and NOT (b) logic gates. Moving the switch (logical 1) turns on the light in the first system (YES gate), but turns it off in the second one (NOT gate).
The PASS 1 gate simply yields 1 output that is independent of the input value. Here, a fluorescent dye, \textbf{L1} immobilised on Tentagel–S–NH$_2$ polymer bead by peptide coupling is simply a PASS 1 gate. But, the fluorescent dyes \textbf{L2}, \textbf{L3}, and \textbf{L4} are non-fluorescent due to the photoinduced electron transfer (PET) which deactivates its fluorescence. The YES logic gate function of \textbf{L2}, \textbf{L3} and \textbf{L4} can be achieved when they are activated with H$^+$, which binds to the unshared pair of electron of the amine receptor to stop the PET, to exceed a chosen threshold fluorescence value.$^{11}$ NOT logic gate is represented by \textbf{L5}.$^{12}$ A NOT, one of the principal Boolean logic, operator has one input and one output and thus, a two-terminal gate. It executes NOT operations transducing a chemical input (proton) into an optical output (fluorescence). In a mixture of CH$_3$OH and H$_2$O (1:4, v/v) the fluorescence quantum yield of \textbf{L5} is 0.13 in the presence of only 0.1 M hydrochloric acid. The quantum yield drops to $10^{-3}$ when the concentration of H$^+$ increased to $10^3$ M. The fluorescence of the protonated form is quenched by the PET from the central pyrazoline unit to the pendant benzoic acid. Thus, switching the emission intensity from a high to a low value can be achieved by simply changing the H$^+$ concentration from a low to a high value. The inverse relationship between the chemical input and the optical output translates into the truth table of a NOT operation.
1B.2.2. Two Input Logic Gate

Sixteen Verities of the double–input and single–output variety are available,¹¹ which are being commonly used in electronics. Some examples of the two input logic gates are OR, AND, XOR, INH, NOR, NAND, XNOR.

<table>
<thead>
<tr>
<th>Input</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>In1</td>
<td>In2</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Figure 1B.2** Electric circuits representing the OR (a) and AND (b) logic gates. System (a) is switched on by moving any of the switches, but in (b) to turn on the light both switches must be moved.

The sensor molecule, L₆ which was found to bind Ca²⁺, Mg²⁺ was reported as a molecular OR logic device.¹³ OR logic gate is one of the basic logic gates, which produces high output value only when any one of the inputs is in the ON state. Fluorescence switching "on" of L₆ arises in the presence of Ca²⁺, Mg²⁺ and H⁺ due to PET suppression and increased oxidation potential of L₆ and their corresponding binding constant (logβ) are 5 M⁻¹, 3M⁻¹, 6M⁻¹. This non selectivity constitutes the basis for OR logic gate.
Cation-induced rearrangement of L7 upon binding to any one of the receptors (polyether chain and bipyridine unit) is matched to OR logic gate. Cations like, Na⁺ and Hg²⁺ bind with polyether chain and bipyridine unit, respectively and bring the two anthracene fragments closer due to guest-enforced rearrangements. Thus, the rearrangement (rotation of bipyridine unit) of L7, irrespective of the cation supports the OR logic function of L7.¹⁴

D. Shabat and co–workers recently reported¹⁵ the application of OR logic gate in antitumor therapy. Compound L8 contains two target sites (phenylacetamide and 4–hydroxy–4–methylhexan–2–one, shown in red colour) can selectively be hydrolysed by penicillin G amidase and 38C2 catalytic antibodies, respectively, to cause the liberation of 4–nitrophenol. Here, in the presence of any one of the antibodies, amido group gets cleaved and thus produces free amino group. This free amino group in turn catalyses the cleavage of second amido bond of L8 and finally releases 4–nitrophenol which can be used for diagnostic purpose. Thus, liberation (on) of 4–nitrophenol in
compound L8 in the presence of either penicillin G amidase or 38C2 catalytic antibody is correlated with OR logic functions. Similarly, compound L9 releases the antitumor drug, doxorubicin (shown in green colour), by enzymatic reaction.

Molecular switches able to process chemical inputs into optical outputs with AND functions were first reported by A. P. De Silva in 1993. Since then, reports on various small molecular switches have been reported by other researchers which can function as AND logic gate. In contrast to OR logic gate, AND logic gate converts two simultaneous inputs into output signal.

In the first reported model, A. P. De Silva and co-workers designed a ditopic receptor L10 with PET photophysical mechanism to quench the fluorescence of anthracene unit from its two receptors, namely benzo-5-crown-5-ether receptor and tertiary amine, connected by a covalent bond. Either binding of sodium ion to the crown ether receptor or the binding of proton in the tertiary amine receptor does not induce significant fluorescence of L10 and L11. But, simultaneous presence of two inputs, Na+ and H+, induces very high fluorescence enhancement due to the prohibition of two PET mechanisms. AND Boolean logic is also observed in the compound L12, which is a calcium selective chelator.

Bignozzi and Radmond et al. reported the NOR logic function of ruthenium polypyridyl complex which is self-assembled at the surface of nanoporous nanocrystalline TiO2 (anatase) thin film. Chelation of L13 onto the TiO2 substrate via its pendant carboxylate groups allows both electrical and chemical switching. Under
negative applied potentials and in the absence of Cu$_{2}^{+}$ (0,0), excitation at 467 nm results in a strong metal to ligand charge transfer (MLCT) based luminescence at 668 nm. Large negative potential (than TiO$_{2}$) reduces the charge injection yield and a strong MLCT emission may be switched on.$^{20}$ The emission output is switched off by either in positive applied potential and in the absence of Cu$_{2}^{+}$ ions (1,0) or in the presence of Cu$_{2}^{+}$ ions and the application of negative potential (0,1). Thus, switching on function of L13 in the absence of any inputs demonstrates the NOR logic function.

An XOR gate is one in which the presence of only one high input value registers a high output value, while concomitant presence of both inputs should leave the system in the OFF state. Balzani, Stoddart and co–workers first demonstrated the XOR logic at the molecular level, utilizing the threading/dethreading processes of a pseudorotaxane.$^{21}$ A. P de Silva et al. reported XOR logic function on integrated “ICT chromophore–receptor” systems that show ion–induced shifts in their electronic absorption spectra. As shown in the compound L14, in addition to the carboxylate functionality (a Ca$_{2}^{+}$ binding site), this compound was further derivatized with a second receptor namely a heterocyclic nitrogen group, which can be protonated to modify the ICT nature of the parent molecule. Binding of the Ca$_{2}^{+}$ to the carboxylate receptor and the proton to heterocyclic nitrogen group destabilize the ICT by 41 KJ mol$^{-1}$ and stabilizes the ICT by 54 KJ mol$^{-1}$, respectively. The corresponding blue and red shift in the absorption spectra of L14, in the presence of Ca$_{2}^{+}$ and H$^{+}$ respectively, was used to express the XOR logic operation.$^{22}$
Gunnlaugsson et al. reported \textbf{L15},\textsuperscript{23} a tetrazamacrocyclic-Tb(III) complex, and demonstrated the first INHIBIT function of compound \textbf{L15} at molecular level. INHIBIT logic gate is combination of AND and NOT logic gate. Upon protonation of quinoline nitrogen in O\textsubscript{2} free condition and excitation at the protonated quinoline moiety, fluorescence of the Tb\textsuperscript{3+} centre was observed due to electronic energy transfer from quinolinium ion to the terbium fluorophore. But, the molecular O\textsubscript{2} present in the medium quenches the fluorescence of terbium by triplet quenching mechanism. Thus, compound \textbf{L15} behaves as INHIBIT logic gate with proton and molecular oxygen inputs and fluorescence output.

\textbf{1B.2.3. Combinatorial Logic gate}

Combination Logic Circuits are made up from basic logic NAND, NOR and NOT gates that are "combined" or connected together to produce more complicated switching circuits. These logic gates are the building blocks of combinational logic circuits. Combinational logic circuits can be very simple or very complicated and any combinational circuit can be implemented with only NAND and NOR gates as these are classed as "universal" gates. Usually, combinational logic circuits are made by connecting simple logic gates called AND, NOT, and OR gates. The NAND and NOR operators and the enabled OR (EOR), exclusive NOR (XNOR), exclusive OR (XOR), two- and three-input inhibit (INH) operators are simple examples of combinational logic circuits. The most important are binary half-adder and half-subtractor.\textsuperscript{24a} These circuits enable arithmetic operations on bits of information in binary fashion, which are one of the pillars on which the entire information technology has been built. In practical computer electronic circuits, arithmetic logic unit (ALU), does mathematical calculations is constructed using combinational logic. Other circuits used in computers, like half adders, full adders, half subtractors, full subtractors, multiplexers, demultiplexers, encoders and decoders are also made by using combinational logic.\textsuperscript{24}
1B.2.3.1. Half-subtractor

Molecular implementation of the binary half-subtractor is much more difficult than any of the logic devices discussed so far. Half-subtractors are composed of an INHIBIT and an XOR gate. They produce a BORROW (B) and a DIFFERENCE (D) output, resulting in the following operations (I₁−I₂): 0 - 0 = 0, 1 - 0 = 1, and 1 – 1 = 0. In the case of 0 – 1, a 1 is borrowed from a higher stage, which makes the difference become 2 – 1 = 1. S. J. Langford and co-workers reported the first molecular half-subtractor in 2003 using 5,10,15,20-tetraphenylporphyrin (L₁₆) in dimethyl formamide (DMF) solution. Amphophilic nature of L₁₆ allows the two pyrrolene nitrogen atoms to accept protons and weakly acidic (pKₐ > 16) two inner peripheral nitrogen atoms allow easy deprotonation by potassium tert-butoxide (t-BuOK). The absorption spectra of either the porphyrin dianion or the dication at 400–420 nm show little absorption when compared to the free L₁₆. The change in the absorption maxima of the Soret band at this wavelength region can be used to derive a truth table for XOR logic function based on the chemical inputs H⁺ and t-BuOK and transmittance as the output variable. Monitoring the fluorescence at 637 nm upon the addition of acid, base, and an equimolar mixture of acid and base simultaneously yields a truth table that leads to an INHIBITION function. Thus, Integration of XOR and INH within one molecule with the same inputs and different output channels results in the molecular scale binary half-subtractor.

Akkaya et al. reported unimolecular half-subtractor operation on a boradiazaindacene-type ICT (internal charge transfer) fluorophore, L₁₇. The luminescence of the L₁₇ central unit is controlled by two processes: PET from the phenol moiety and ICT involving the dimethylaniline moiety. The results associated with fluorescence changes at 565 nm and 660 nm upon addition of acid and base were used to correlate the half-subtractor operation. Spectral response on specific ionic
inputs in the form of acid and base could be used to correlate the INHIBIT and XOR logic function, respectively. Here, Akkaya used negative logic convention at 660 nm to obtain the XOR logic function.\textsuperscript{26}

Shanzer and co-workers introduced another half-subtractor (L18), where fluorescence response was used as output or readout signal. The siderophore-like compound, L18 contains two fluorophores, pyrene and fluorescein. With acid (HCl) and base (sodium acetate) as inputs, the Fe\textsuperscript{3+} complex of L18 can perform XOR and INHIBIT logic operations in a reconfigurable manner. The fluorescein emission at 525 nm (INHIBIT) and the simultaneous emissions of pyrene at 390 nm and fluorescein at 525 nm (XOR) serve as outputs. In absence of both inputs, metal-induced fluorescence quenching results. Addition of sufficient amount of base (pH > 8) leads to the generation of dianionic form of fluorescein and its green fluorescence is induced by electronic energy transfer from the selectively excited pyrene. On the other hand, addition of acid favours the formation of free L18 with fluorescein in its protonated form, which is non fluorescent. This nullifies the possibility of energy transfer and the blue fluorescence of the pyrene unit (390 nm) is observed. If both inputs are present simultaneously, a buffer is formed and no significant changes of the output signals are observed. When
HCl is replaced with the weaker acid and metal-ion chelator N,N,N’,N’-ethylenediaminetetraacetic acid, the fluorescence readout signals of the Fe$^{3+}$ complex of L18 can be interpreted as an AND logic gate. This, along with the XOR gate described above could be used to describe the half adder function. However, scope of such a half adder is hypothetical, as two different sets of inputs would have to be applied in parallel.

1B.2.3.2. Molecular Half–Adder

A half–adder is a device that adds two binary digits (bits) and implements AND and exclusive OR (XOR) logic gates in a parallel way. The two gates share the same inputs (I₁ and I₂) and produce outputs that code for the CARRY (C) and SUM (S) digits. These digits stand for the binary realization of 0 + 0 = 0, 1 + 0 = 1, and 0 + 1 = 1. In the case of 1 + 1 the SUM is 0, but the CARRY is set to 1, yielding 1 + 1 = 2.

Shanzer et al. reported a fluorescein molecular switch as a molecular half-adder$^{27}$ that can interchange between its four different ionization forms (cation, neutral, anion and dianion, each of which has distinct spectral properties) in a controlled manner through a selective change of the solution pH. In the absorption spectra, the dianionic form (pH > 8) has the strongest absorption peak at 490 nm, with a shoulder around 475 nm, whereas the monoanion (pH ~5.5) has a weaker absorption, with peaks at 472 and 453 nm. The neutral species (pH ~ 3.3) has by far the lowest absorption, with a maximum at 434 nm, and the cationic form (pH < 2) has a maximum at 437 nm.
Performance of algebraic $a+b$ operation, half-adder, by fluorescein was obtained by two base inputs to create mono and dianionic form of fluorescein and monitoring the fluorescence output at 447 and 501 nm. Each base (0.013 M) input predominately generates monoanion form by mere deprotonation of carboxylic group with a high output at 447 nm. Addition of extra amount of base (0.013 M) to the monoanionic form generates the dianionic species, where phenol group is also deprotonated, which has absorption output maximum at 501 nm. Consequently, by monitoring at 447 nm, a XOR gate is obtained, whereas observing at 501 nm results in an AND logic gate.
1B. References


Aim and outline of the thesis

1. Chapter 1A briefly provides the detailed literature survey on the development of cation recognition. Discussion and examples of some of the chemosensor for biologically important metal ions are provided. Chapter 1B basically describes the work done so far on molecular logic gate with brief literature survey and various examples of well cited molecular logic gates in literatures.

2. Chapter 2 deals with design and synthesis of tailored made fluorescent receptors for specific recognition of Hg$^{2+}$ ion either in aqueous solution or in physiological condition. Attempt has been made to demonstrate the possibility of utilizing fluorescence resonance energy transfer (FRET) for designing receptor for more efficient recognition with lesser possibility of spectral interference. Further, possibility of using these fluorescent probes for detection of the Hg$^{2+}$ present in lower microbes like *Pseudomonas putida*, has been discussed.

3. Chapter 3 briefs the utilization of newly synthesized coumarin and anthraquinone-based receptor molecules for preferential recognition of Ca$^{2+}$ and Mg$^{2+}$ and it is discussed how the binding induced output signals could be influenced by two different photophysical mechanisms (Photoinduced electron Transfer and Intramolecular Charge Transfer) on sensing phenomena.

4. In chapter 4, the concept of pseudorotaxane formation between host-guest components has been used to recognize N-alkylated cationic species like imidazolium ions, secondary ammonium ions and pyridinium ions by host molecules derived from crown ethers. With appropriate design and choice of the fluorescent signalling unit as probe component, it is demonstrated that how conformational changes and extent of folding-unfolding motion of the crown ether derivative in [2]pseudorotaxane can be
studied by FRET mechanism. Observed results and interpretations have been further corroborated using molecular modelling studies.

5. Finally in chapter 5, it has been shown how the simple newly synthesized receptors or some commercially available fluorogenic receptors with specific binding sites for cations (in the form of H\(^+\) or Cu\(^{2+}\)) and anions (in the form of OH\(^-\) or F\(^-\)) have been used to construct truth table for demonstrating complicated molecular half-subtractor operation and their potential application in molecular computers has been discussed.