Chapter 1

Introduction
1A. Receptor design for the recognition of biologically important analytes through reversible binding

In the last few decades research in field of molecular recognition and sensing has received considerable attention of the researchers from all over the world. In order to develop an artificial receptor that is selective towards a specific analyte, multiple interactions between host and guest in a complementary fashion have to be considered. Several strategies can be followed in the design of molecular receptors with optimal selectivity toward to a particular analyte and be able to convert their recognition process into a read out signal, either in the form of spectroscopic (Optical/Fluorescence/NMR) or electrochemical signal.

1A.1. Synthetic molecular probes for Cations

1A.1.1. Introduction

“Cation Recognition” as a research area has been actively pursued by many scientists including chemists, biologists, clinical biochemists and environmentalists during last 45 years. Sodium, potassium, magnesium and calcium ions are involved in many crucial biological processes such as transmission of impulses, muscle contraction, regulation of cell activity, etc. Moreover, various metal ions perform important functions in metalloenzymes. In medicine it is important to control the serum levels of Li⁺ in patients under treatment for manic depression, and K⁺ in the case of high blood pressure. In chemical oceanography, it has been demonstrated that some nutrients required for the survival of microorganisms in sea water. Such nutrients contain zinc (Zn²⁺), iron (Fe²⁺/³⁺), copper (Cu²⁺), cobalt (Co²⁺), chromium (Cr³⁺) and manganese (Mn²⁺) as enzyme cofactors. Biological and environmental importance of different lanthanide ions are well-known due to their involvement in biological/therapeutic/imaging processes and catalytic reactions. Among different lanthanide ions, smaller trivalent lanthanide metal ions are reported to be the best T-channel antagonists. Ln³⁺ ions are also being
used as biochemical probes to study calcium transport in mitochondria and other organelles. Metal ions like Hg$^{2+}$, Pb$^{2+}$ and Cd$^{2+}$ are important due to their biological significance as well as their toxic influence on various living organism. Therefore early detection and sensing of these metal ions are desirable. Among the numerous analytical methods available for the detection of cations, techniques like flame photometry, atomic absorption spectrometry, ion sensitive electrodes, electron microprobe analysis, neutron activation analysis etc are common. But, most of these above mentioned techniques are expensive and often require samples of large size do not allow continuous monitoring and involve pre-treatment for sample preparation. Furthermore, these sophisticated instrumental techniques are neither well-suited for quick in-field detection nor for in vivo studies. In contrast the method based on chemical sensors for the detection of cations offer distinct advantages in terms of binary response (yes/no type) with high selectivity and sensitivity, faster response time, and local observation (e.g. by fluorescence imaging spectroscopy). Moreover remote sensing is possible by using optical fibres with a molecular sensor immobilized at the tip. Chromogenic sensor that allows visual detection are of special interest as it allows semiquantitative naked eye detection of targeted analytes without resorting to any spectroscopic instrumentation. Whereas fluorescent sensors are crucial because of its simplicity, high sensitivity, reliability and the possibility of using such reagent for bioimaging application in living cells with temporal and spatial resolution. It is possible to further tune the luminescence responses of a fluorophore using a range of photoinduced processes; e.g., Charge Transfer (CT), Electron Transfer (ET), Energy Transfer (eT) and Förster Resonance Energy Transfer (FRET). Presence of heavy metal or paramagnetic ions also significantly influence the photophysical behaviour of the luminescent chromophores and the destabilization of the excited states. Apart from
these, change(s) in conformation/structural flexibility on receptor-analyte binding can also influence the change(s) in luminescence response(s).

In this context, receptors that can act both as a colorimetric and fluorescence-based sensor are even more desirable as they offer the possibility of bimodal recognition and thus offer the advantages associated with both detection processes.

Fig. 1A.1. Pictorial representation of recognition of analyte by a chemosensor.

The design of a chemosensor involves integration three important components, namely receptor unit for specific binding to the desired analyte, a conduit for efficient signal transduction and signalling unit for reporting the binding induced changes as a read-out signal through changes in optical, magnetic and redox behaviour(s) (Fig 1.A.1). As mentioned earlier, choice of appropriate reporter functionality allows us to probe the binding induced process through changes in optical properties that are conducive for an “in-field” application.8

In order to develop an efficient artificial receptor that is specific towards a particular cationic analyte, multiple interactions between receptor and guest analyte in a complementary fashion have to be considered. The topology of the receptor is of importance in determining the overall receptor analyte interaction as well as efficient signal transduction to the receptor functionality.9 In order to achieve higher sensitivity a strong analyte-receptor binding is desirable; however too strong binding would affect the reversibility phenomena adversely—a concern for designing a reversible chemosensor. Also, an efficient sensor ought to have a very high specificity with distinct preference for certain analyte in presence of large excess of all other
competing analytes. For ionic analytes, electrostatic interaction between the analyte and the receptor plays an important role; while for neutral analytes, interactions like ion-dipole, dipole-dipole, or ion-induced dipole interactions are important. These interaction forces being weak in nature as compared to electrostatic interactions, designing of an efficient receptor for a neutral analyte is a challenging issue for chemists as well as for the material scientists. On the contrary, for anionic analytes with higher solvation or more precisely the hydration energy also affect the recognition process in polar solvents and more importantly in aqueous medium. Thus, recognition of these ionic analytes with higher charge density and thus, the higher hydration energy is also a challenging issue for the chemists for developing an efficient reagent for environmental sample analysis, clinical/diagnostic application, biological sample analysis and for inter./intra-cellular imaging applications.

1A.1.2. Design of synthetic molecular receptor

First synthesis of crown ethers was reported by Charles Pedersen at DuPont in 1960s and the realization that different crown ethers derivatives show binding preference for different alkali metal ions depending upon the cavity size of crown ether, which commenced the dynamic research in the area of cation recognition. After this initial report on crown ether by Pedersen, coronands and cryptand based receptors were reported by Cram and J. M. Lehn in late 1960s. These together had helped enormously in initiating an exponential growth in the area of cation recognition and all three were conferred with Nobel Prize for their contribution in host-guest chemistry and molecular recognition in 1987. Since then cation recognition chemistry has matured and considerable attention continues to be devoted to the design and synthesis of cation receptors possessing high affinity and selectivity towards a particular analyte with desired utility for practical in-field applications in environmental studies or biological sample analysis or developing imaging studies for in-vivo applications.
1A.1.3. Photoinduced signalling mechanism associated with photoresponsive cation receptors

Signaling subunit is one of the key components of any chemosensor and converts the recognition process into a read out signal either in the form of a spectroscopic (Optical/Fluorescence/NMR/EPR) or electrochemical signal. As mentioned earlier, sensor molecules that work on changes in Uv-vis or luminescence spectral changes as the read-out signal are generally preferred. Such output signals are basically the manifestation of the relative changes of 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 bound state. These spectral responses generally involve mechanistic pathways like, photoinduced electron transfer (PET), photoinduced/resonance energy transfer, excimer or exciplex formation, $\pi-\pi$ interaction, intra or intermolecular charge transfer (CT), and excited state proton transfer, etc.\textsuperscript{10-12} However, changes in molecular flexibility or rigidity and conformation due the receptor-analyte interaction may also lead to associated changes in luminescence property. In few cases, changes associated with the redox potential of the reporter group are also used for designing appropriate sensor molecule. Few of these processes, which have a direct bearing in describing the new chemosensors in this thesis, are to be discussed in the following section.

1A.1.3.1. Photoinduced Electron Transfer (PET)

Photoinduced electron transfer (PET) is an attractive photophysical signalling mechanism that has been extensively used for cation recognition studies due to its simplicity. The working principle of the PET based sensor is shown in Figure 1A.2. This illustrates how a cation binding to a receptor could actually interrupt the PET-based luminescence quenching process. Cation binding to the receptor unit makes the PET
process from the cation-bound receptor to the HOMO of the excited luminophore an energetically inaccessible process. This generally results a substantial enhancement in luminescence. Turn-On luminescence response is the most common manifestation of such interruption of the PET process on binding to certain cationic analyte.\textsuperscript{13}

Various representative examples of PET based cation sensors are given in Fig. 1A.3. 1 is the first and simplest coronand PET sensor reported.\textsuperscript{14} Fluorescence quantum yield of 1 is reported to be enhanced from 0.003 to 0.14 upon binding of K\textsuperscript{+} in methanol. Siegerman et.al reported a PET sensor molecule which has an N–phenyl mono aza crown receptor (2) to bind Na\textsuperscript{+} ion at physiological conditions.\textsuperscript{15} Due to lower oxidation potential (0.8 V vs saturated calomel electrode) of the aniline motif, this unit participate
effectively in PET-based quenching process upon photoexcitation and this results a luminescence switched OFF state. The very presence of the Na⁺ ion raises the oxidation potential of the aniline motif and this makes the PET process photoinduced electron transfer an energetically impossible process for the Na⁺ bound sensor.¹⁶ The cavity size of reagent 3 fits well the ionic radius of K⁺,¹⁷ whereas the cavity size for reagent 4 is better suited for binding with Na⁺ and thus, its detection.¹⁸ Receptor 5 containing polyazamacrocycle was more appropriate for the recognition of soft metal ions like Zn²⁺.¹⁹ For reagent 6,²⁰ the recognition moiety is similar to Tsien’s PCT-based sensor in which the methoxy groups in ortho position with respect to the nitrogen atoms of the crown participate in the complexation process (according to design of lariat-ethers). This shows preference for Na⁺ binding with associated turn ON fluorescence response.

1A.1.3.2. Energy transfer Quenching (ET)

![Diagram of Energy Transfer Quenching](image)

Fig. 1A.4. (A) Electron transfer mechanism: double electron exchange between excited fluorophore and d⁹ metal ion.

Most PET fluorescent sensors are designed utilizing the scheme shown in Fig. 1A.2, however, other PET-based processes can also be operational with transition metal ions.²¹ In fact 3d metals exhibit redox activity and electron transfer can occur from the fluorophore to the bound metal ion, or vice versa. In some cases electron exchange is
possible which results in quenching of the fluorophore by nonradiative energy transfer according to the Dexter mechanism in which a metal ion can quench the fluorescence of the excited state of the fluorophore by an energy transfer mechanism.

Bergonzi et al. reported that Cu(II) ion, with elongated-octahedron d⁹ configuration, is capable of quenching the fluorescence of the naphthalene moiety in 7 by energy transfer (ET) mechanism. The d⁹ metal ion quenches the fluorescence through double electron exchange as shown in the Fig. 1A.4 without affecting the net distribution of electron.

1A.1.3.3.  **Resonance Energy transfer**

Förster (Fluorescence) resonance energy transfer (FRET), is a photo physical phenomenon that describes the energy transfer between two chromophores. The donor chromophore, initially in its electronically excited state, transfers energy to an acceptor through nonradiative long range dipole–dipole coupling (Fig. 1A.5A).

![Resonance Energy Transfer Diagram](image)

**Fig. 1A.5.** (A) Förster resonance energy transfer mechanism. (B) Spectral overlap between donor based emission and acceptor based absorption bands.

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 and the relative orientation of the donor and acceptor dipoles and the distance between the donor and acceptor units (Fig. 1A.5A). 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 (Fig. 1A.5B). The critical or Förster distance $R_0$ (at which distance 50% energy transfer is possible) is calculated using the following expression,

$$R_0 = 0.211 [(J(\lambda)) Q (\eta^{-4}) (\kappa^2)]^{1/6}$$

where $\eta$ is the refractive index of the medium, $\kappa^2$ is the dipole orientation factor, $Q$ is the fluorescence quantum yield of the donor in the absence of acceptor and $J(\lambda)$ is the spectral overlap integral between the emission spectrum of the donor and the absorption spectrum of the acceptor.

FRET is particularly wildly used to determine distances in biomolecules and supramolecular associations and assemblies. In the area of modern analytical research FRET as an operating principal has been extensively used by several researchers for metal ion recognition studies. Report from Kim et al. includes a naphthalene-pyrene based FRET system 8 for the recognition of Cu$^{2+}$ (Fig. 1A.6).$^{24}$ Folded conformation for 8 allows an efficient energy transfer from naphalene (donor) to pyrene (acceptor), which is interrupted on coordination of Cu$^{2+}$ to the the crown ether fragment. Quenching of the naphthalene luminescence on binding to paramagnetic Cu$^{2+}$ ion (d$^9$) could have also contributed to this interrupted FRET process. Das and his co-workers have developed a FRET based off-on fluorogenic sensor for Hg$^{2+}$ (9) (Fig. 1A.6).$^{25}$

![Fig. 1A.6. Structures of the different FRET based chemosensors 8-10.](image-url)
In this receptor (9) rhodamine moiety in its spirolactam form doesn't not has any absorption and emission spectral band in the visible region, however, on binding to a Hg²⁺ a strong absorption band at ~ 525 nm and emission band at ~ 555 nm are observed. This acyclic Hg²⁺-bound rhodamine moiety acts as an acceptor to constitute a FRET pair with the donor dansyl moiety. This initiates an efficient FRET process and the switched on fluorescence response at ~ 555 nm on excitation at the dansyl unit at ~ 420 nm. In another example a fluorophore dyad 10 containing rhodamine and naphthalimide moiety is reported for the ratiometric fluorescent detection of Cr³⁺.²⁶ In presence of Cr³⁺, reagent 10 on excitation at 405 nm shows a bleaching of emission intensity at 544 nm with concomitant growth of a new fluorescent band with maxima at 592 nm (isoemissive point at 558 nm). An associated change in solution luminescence from yellow to red is reported. This observation is consistent with increased FRET between the donor naphthalimide and the acceptor Cr³⁺-bound acyclic form of the rhodamine (acceptor) moiety.

1A.1.3.4. **Intramolecular Charge Transfer**

Excitation of a chromophore induces the transition of an electron from one orbital to another. If the initial and final orbital are separated in space, the electronic transition is accompanied by an almost instantaneous change in the dipole moment of the chromophore. When the latter possesses and electron donating group (e.g. –NH₂, –NMe₂, –OCH₃) conjugated to an electron withdrawing group (e.g. >C=O, –CN) the increase in dipole moment is quite large. Consequently excited state reached upon excitation (called the *Frank-Condon* State or *locally excited* state, LE) is not in equilibrium with the surrounding solvent molecule if the later is polar. In a medium that is sufficiently fluid, the solvent molecules rotate during the lifetime of the excited state until the solvation shell is in thermodynamic equilibrium with the chromophore. A relaxed intramolecular charge transfer (ICT) state is then reached. Such a solvent
relaxation explains the increase in the red shift of electronic spectrum (absorption and emission spectrum) as the polarity of the solvent increases. Moreover, when a cation is linked to an ICT-active chromophore so that the bound cation is capable of interacting with either the donor group or the acceptor group, energy for the ICT process is modulated and consequently the associated physical properties of the chromophore. Such phenomenon are being utilized for achieving optical readout signal in recognition processes of cations.27

Interaction of an electron donor that is a part of a chromophore, with a cation reduces its electron donating character and thereby the extent of conjugation.

![Fig. 1A.7. Spectral displacements of ICT based sensors resulting from interaction of abound cation with an electron-donating or withdrawing group.](image)

This induces an anticipated blue shift in the absorption spectrum with a decrease in the extinction co-efficient (Fig. 1A.7). Conversely, interaction of the cation with an acceptor group enhances its electron withdrawing character and this is expected to induce a red shift with increase in the molar absorption coefficient for ICT transition band (Fig. 1A.7). The emission spectra are in principle is expected to shift in the same direction as those of the absorption spectra. All these photophysical effects are obviously dependent on the charge and size of cation and selectivity of these effects is expected.

Many synthetic cation receptors have been designed according to following principle. The change in photophysical properties on cation binding depends upon the charge in dipole interaction and motivates the recognition process. Various representative examples of ICT based cation sensors are given figure Fig. 1A.8. Among these 11,28
12 and 13 show shifts in absorption and emission spectra upon cation binding which are hypsochromic in nature.\textsuperscript{29,30} On the other hand 14,\textsuperscript{31} 15 \textsuperscript{32} and 16 \textsuperscript{33} are another class of ICT based chemosensors where the receptor-metal ion binding favoured the ICT process and induces red shifts in changes in absorbance and emission bands.

![Structures of the chemosensors 11-16.](image1)

**1A.1.3.5. Twisted Intramolecular Charge Transfer (TICT)**

The relaxation towards an ICT state may be accompanied by internal rotation within the chromophore. The prime example of this type of chromophore is 4-$N,N$-dinethyl amino benzonitrile (DMABN). It exhibits dual fluorescence in polar solvents.

![Representation of the TICT model for 17.](image2)

This intriguing phenomenon laying in this fact is that in the ground state the molecule is almost planar According to the Franck-Condon principle, the locally excited state (LE) is still planer, but solvent relaxation takes place with a concomitant rotation of the di-methylamino group until it is twisted at right angles and the conjugation is lost. In the resulting TICT state, stabilized by polar solvent molecules and the intramolecular
charge separation between the di-methylamino group (donor) and cyano phenyl group (acceptor) is most favourable in that twisted conformation.\textsuperscript{34}

1A.1.4. Synthetic Receptors for the detection of lanthanides

Interest in the photo physical properties of lanthanide ions complexes has been grown considerably since Lehn proposed that such complexes could be seen as light conversion molecular devices (LCMDs),\textsuperscript{35} coining the term antenna effect to denote the absorption, energy transfer, emission sequence involving distinct absorbing (the ligand) and emitting (the lanthanide ion) components, thus overcoming the very small absorption coefficients of the lanthanide ions. In this regard design and development of efficient receptor reagents for lanthanides has become an important research goal, being pursued by several groups working with many different classes of ligands. Rare earth (RE) elements, because of their diverse physical and chemical effects, have been widely used in the pharmacological and electronic industries and in agriculture. More and more RE elements are entering the environment and eventually accumulating \textit{in vivo}. RE elements could induce chromosome damage to blood lymphocytes,\textsuperscript{36} liver damage,\textsuperscript{37} and metabolism disturbance.\textsuperscript{38} Currently available techniques for screening RE elements such as ICP-MS, ICP-AES, and ICP OES are labor intensive, expensive and involve multi-step sample preparation.\textsuperscript{39} Thus, the development of highly selective and sensitive sensors for RE ions is of great current interest. However, at present there are only a few reports that describe detection of RE ions based on optical readout signal. Among such reagents, dyes based on the fluorescence as the output signal are more common as all these utilizes the rich physicochemical properties of RE ions,\textsuperscript{40} while examples of the colorimetric reagent that allows the optical detection of a specific RE ions among other such ions are uncommon in the contemporary literature.
1A.1.4.1. Colorimetric Sensors for Lanthanide Ions

Colorimetric sensors are very useful in the analysis of biomolecules and metal ions due to their simplicity, speed and the use of uncomplicated apparatus.\textsuperscript{41} Gold nanoparticle (AuNP) based sensors are a specific class of colorimetric sensor that has generated much interest due to their unique and tunable optical and photophysical properties.\textsuperscript{42} In this context Hutchison and coworkers reported a selective and sensitive molecular sensor 18 for trivalent lanthanide (Ln\textsuperscript{3+}) ions based upon a malonamide functionalized gold nanoparticle, was developed for colorimetric detection in aqueous medium.\textsuperscript{43} A new synthetic approach permits nanoparticle synthesis, stabilization, and incorporation of a selective lanthanide binding site in a single, direct step. The design incorporates a specifically tailored dual function precursor ligand that bears a sodium thiosulfate (Bunte salt) group that links to the gold nanoparticle core and a tetramethylmalonamide (TMMA) group that serves as a selective Ln\textsuperscript{3+} binding site (Fig 1.A.10).

Fig. 1.A.10. Synthesis of Malonamide-functionalized AuNPs and NP Cross-Linking through Ln\textsuperscript{3+}. 
Fig. 1.A.11. (A) Absorption spectra, aggregation of 18 AuNPs and corresponding colour change upon introduction of Eu\(^{3+}\) ions to solutions. (B) TEM images of the 17 AuNPs before and after addition of Eu(NO\(_3\))\(_3\) is added to the solution of 18. (C) Color response of 18 AuNPs after incubation with uranyl nitrate and excess HAuCl\(_4\). In order to assess the difference between simple ion binding and cross-linking.

Optical responses of the receptor towards lanthanide ions is reported to be immediate (Fig 1.A.11) with sensitivity as low as \(\sim 50\) nM for Eu\(^{3+}\) and Sm\(^{3+}\). This study demonstrates a general strategy for direct, convenient nanoparticle synthesis that enables the incorporation of analyte binding groups directly to the nanoparticle surface. Appropriately functionalized surface bound ligands can cross-link nanoparticles in the presence of an analyte, which leads to an aggregation and thus a bathochromic shift in absorbance. The resulting color change from red to blue indicates particle cross-linking.\(^{44}\) This demonstrates the possibility of developing nanoparticle-based colorimetric sensors for widespread use. This unique one-step synthesis offers uniform surface ligand composition, reduces the volume of waste generated during synthesis of nanoparticle and its purification as well as produces functionalized gold nanoparticles that are stable in non-modified aqueous environments at ambient temperature.
Similarly, silver nanoparticles (AgNPs) having uniform size (~ 4.5 nm) distribution are suitable as a colorimetric indicator, as the color due to the surface plasmon resonance effect is strongly influenced by particle size and aggregation. The use of AgNPs for colorimetric detection has increased in recent years. Through appropriate functionalization of the NPs, one can modulate their optical properties and increase their selectivity towards a certain analyte and thus, consequently their application potential. A report by Li et al. reveals a novel AgNPs material (\(\{4\text{-DPD} \subset \beta\text{-CD}\}_2\text{-AgNP}\), 19), generated using an inclusion complex \(\{4\text{-DPD} \subset \beta\text{-CD}\}_2\text{-AgNP}\) as a receptor for Yb\(^{3+}\). Binding to Yb\(^{3+}\) induced an aggregation process. The AgNLs (\(\{4\text{-DPD} \subset \beta\text{-CD}\}_2\text{-AgNP}\)) are reported to be remarkably stable in water and yields a stable, clear yellow solution. Fig 1.A.13 displays the UV-vis spectra and color changes of the solution of 19 before and after addition of various RE ions (0.2 mM). Only Yb\(^{3+}\) ion is found to induce a distinct color change from yellow to red, which corresponds to a dramatic increase in the absorbance intensity at ~ 610 nm. To quantify the spectral changes at 401 nm and 610 nm, the absorbance ratio at two wavelengths (R = \(A_{610}/A_{401}\)) in the presence of 0.2 mM RE ions was determined (Fig 1.A.13 inset). An enhancement of the R value only in presence of Yb\(^{3+}\) is reported and thus confirms the selectivity of \(\{4\text{-DPD} \subset \beta\text{-CD}\}_2\text{-AgNP}\) towards Yb\(^{3+}\). This specific response for Yb\(^{3+}\) is attributed to the lanthanide contraction, a gradual decrease in ionic radius from La to Lu, as the smaller ionic radius of Yb\(^{3+}\) is responsible for its higher charge density of Yb\(^{3+}\) and this probably accounts for a stronger binding to the pyridine donor. Molecular modeling studies confirms that the steric hindrance induced by the bulky \(\beta\text{-CD}\) moiety prevents approach of the larger ions.
(i.e., La$^{3+}$, Ce$^{3+}$, Pr$^{3+}$, Nd$^{3+}$, Sm$^{3+}$ and Eu$^{3+}$) and thus, the binding process. Binding of 19 to Yb$^{3+}$ induces aggregation and thus, the optical properties, which eventually accounts for the optical recognition.

**Fig. 1.A.13.** (A) The UV-vis spectra and (B) photographic images of β-CD–4-DPD-Ag NPs solution in the presence of 0.2 mM different RE ions. Inset: the absorbance ratio R of β CD–4-DPD-Ag NPs solution in the presence of 0.2 mM different RE ions. (c) Supramolecular aggregates and TEM images of β-CD–4-DPD-Ag NPs in the presence 2 x 10$^{-4}$ M Yb$^{3+}$.

Photochromism of spirobenzopyran derivatives has been extensively utilized for designing photo-switchable molecular devices.$^{50}$ On irradiation with ultraviolet light (or in dark conditions), the colorless, neutral spiropyran (SP) forms isomerize to the colored, zwitterionic merocyanine forms (MC) (Fig. A.12).$^{51}$ This zwitterionic merocyanine forms have stronger affinity for lanthanide ions than the precursor neutral host for greater electrostatic interaction.$^{52}$ This phenomenon is utilized by Gao and co-workers for designing a novel receptor 20 having two spirobenzopyran groups (Fig. 1.A.14).$^{53}$ Remarkable shifts of the UV–vis spectra (68–84 nm) and the emission spectra (42 nm for Eu$^{3+}$) for the solution of receptor 21 are reported on formation of lanthanide complexes. It has been argued that the calix[4]-arene cavity contributed to the stability of the complex Calix-2MC.Ln$^{3+}$. Higher affinity of the reagent 21 towards lanthanide ions is due to the combination of several favorable effects, like, higher electrostatic interaction, hard acid–hard base interaction and size-fit effect.
Fig. 1.A.14. Sketches of equilibrium reaction of compound 20 in absence and presence of lanthanide ions.

Fig. 1.A.15. Color change of compound 20 (50 mM) in acetonitrile induced by addition of 2.0 equiv of metal nitrate (from left to right: compound 20 without metal ion; addition of Na⁺; Ca²⁺; Zn²⁺; La³⁺; Pr³⁺; Eu³⁺; Gd³⁺; Er³⁺).

Fig. 1.A.16. (A) Synthetic route of 22. (B) Changes in the absorption spectra of compound 22 (0.1 mM) in CH₃CN upon addition Yb³⁺ ion, from 0 (black) to 1 (purple) equivalent.

Recently Molina et al. have reported a simple chemosensor 22 that could operate through absorption and emission channels in acetonitrile medium. Receptor 22 exhibits significant changes in its UV-Vis spectrum in the presence of Lu³⁺ and Yb³⁺. Detailed UV-Vis analysis results reveal that stepwise addition of such lanthanide cations induce the appearance of new and weak low-energy band at $\lambda = 503$ nm and $\lambda$
= 508 nm, respectively, for Lu$^{3+}$ and Yb$^{3+}$ with associated changes in color for the “naked-eye” detection. However, among these two ions, only Yb$^{3+}$ is reported to be useful for inducing changes in the emission spectra of 22 and thus, could be used as a specific fluorescence-based receptor for Yb$^{3+}$.

Focus of the research work, presented in this thesis, is to develop sensitive and specific colorimetric sensor for lanthanide. Thus, discussion in this section is restricted to the recent developments pertaining to the colorimetric recognition of lanthanides.

1A.1.5. **Synthetic Receptors for the recognition of Hg$^{2+}$ and Cr$^{3+}$ ions**

As mentioned earlier, analytical techniques provide direct and quantitative information about the metal ion concentration in different samples. However, these sophisticated techniques are generally not appropriate for quick in-field detection of these metal ions. These limitations have actually attributed to the recent surge in research activity in the design and development of different molecular probes capable of producing a binary response (YES for presence and NO for absence) for the desired analyte along with a semiquantitative estimation, which are appropriate for a quick in-field application.

Among various metal ions, Hg$^{2+}$ is one of the most toxic metal ions. Though, this metal ion is being used widely in industrial and agriculture. Despite efforts to curb the mercury emission in the environment, the global mercury contamination from natural processes such as oceanic and volcanic emission, coal-burning, gold mining, and solid incineration have posed a big threat to the human society in the last few decades.$^{55}$ Mercury being added in the surrounding atmosphere from various natural or industrial sources and eventually add to aqua body.$^{56,57}$ Metabolic pathways of lower aquatic microbes are known to convert inorganic mercury to the methyl mercury, a potent neurotoxin known to have severe adverse influences on human physiology. Methyl mercury is known to bioaccumulate in higher living organisms through the food chain. It easily passes through biological membranes such as skin, respiratory, and
gastrointestinal tissues,\textsuperscript{58} which eventually damages the central nervous and endocrine system.\textsuperscript{59} Bioaccumulation of Hg\textsuperscript{2+} from atmospheric deposition is also known to happen in certain mosses and tree leaves and this adversely affects photosynthesis and transpiration in plants.\textsuperscript{60}

Unlike Hg\textsuperscript{2+}, Cr\textsuperscript{3+} is less harmful to human life, though chromium present in other higher oxidation states (+4 and +6) has a grave consequence on human health. Cr\textsuperscript{3+} is an effective nutrient and gives immunity power to human body to prevent various diseases like diabetes, cardiovascular disease etc.\textsuperscript{61} Further, its deficiency is known to influence the metabolism of glucose and lipids adversely and causes maturity-onset diabetes, cardiovascular diseases and nervous system disorders.\textsuperscript{62} However, an exposure to higher concentration of Cr\textsuperscript{3+} is known to inflict a negative effect on the normal enzymatic activities. A recent study reveals that the soluble Cr\textsuperscript{3+} at pH 6-8 can be found transiently in significant concentrations and has an adverse influence on microorganisms, like Shewanella sp. MR-4.\textsuperscript{63} Cr\textsuperscript{3+} ion, present in the cytoplasm, is known to bind non-specifically to DNA and other cellular components and these processes become important when concentration of Cr\textsuperscript{3+} exceeds certain threshold value. These are known to inhibit DNA transcription and possibly replication.\textsuperscript{64} The concern over their deleterious effects on human health has led the chemistry and, more broadly, sensing community to develop new detection methods that are cost effective, rapid, facile, for efficient and selective detection of these two ions present in trace quantity and applicable to the environmental and biological sample analysis. Hg\textsuperscript{2+} through its effective spin orbit coupling and Cr\textsuperscript{3+} through its paramagnetic influence, are commonly known to quench the receptor fluorescence effectively. In this regard, receptor molecules that are capable of providing optical feedback on binding to these metal ions in the form of visually detectable change in colour and \textit{turn-ON} fluorescence response rather than a \textit{turn-OFF} response are expected to find \textit{in-field} application for
easy and facile detection. However, designing a suitable receptor for these ions (Hg$^{2+}$, Cr$^{3+}$) in aqueous environment poses a challenge to chemists due to their high and deleterious solvation enthalpy. Some of the recent advancements in area of reversible optical sensors of Hg$^{2+}$ and Cr$^{3+}$ are narrated in the following section.

![Fig. 1A.17.](image)

Das et al. has reported a new quinoline-rhodamine-based conjugate (23)$^{,65}$ that shows remarkable preference toward Hg$^{2+}$ and Cr$^{3+}$. Upon binding to either of these two ions, a visually detectable change in the color and emission were observed because of conversion of the lactam form of the rhodamine derivative to the acyclic xanthene form. This offers the possibility of using this reagent for use either as a colorimetric or as a fluorescence based reagent for the detection of these ions, especially in the case of Hg$^{2+}$, which binds at a micromolar level under physiological conditions. Conversion from the cyclic lactam form to the acyclic xanthene on binding to the metal ions is reported to be reversible, and thus this reagent could actually be used as a reversible sensor for the detection of Hg$^{2+}$ or Cr$^{3+}$. More importantly, reagent 23 is found to be cell membrane permeable and thus, could be used as an cell imaging reagent for detection of the uptake of these ions in breast cancer cell MCF7.

In an another article Das and coworkers have described the incorporation of a rhodamine B derivative, in its spirolactam form (24)$^{,66}$, onto the alginate backbone and use of this modified polymer for recognition of Hg$^{2+}$ and Cr$^{3+}$ in homogeneous aqueous media of pH 7.1 (1 mM HEPES buffer).$^{,66}$
Fig. 1A.18. Structure of chemosensor 24 and the naked eye colour variation of the Hg$^{2+}$/Cr$^{3+}$ bound gel bead.

Presence of the rhodamine moiety allows the colorimetric as well as fluorogenic detection of the targeted cations. The gel forming ability of modified alginate in the presence of Ca$^{2+}$ is utilized for developing beads to act as a self-indicating sponge that can effectively bind and scavenge Hg$^{2+}$ or Cr$^{3+}$ in aq. buffer (1 mM HEPES) of pH 7.1.

Fig. 1A.19. (A) Structure of chemosensor 25. (B) Color changes of 25 (50 μM) after addition of 10 equiv of different metal ions. (C) Test papers having 25 before and after immersion in 9, 20, and 100 ppm Hg$^{2+}$ in distilled water, respectively.

A new chemosensor 25 having a ferrocene unit and rhodamine-6G fragment linked via carbohydrazone binding unit, is reported by Wu et al.\textsuperscript{67} This receptor exhibits an excellent selectivity towards Hg$^{2+}$, which could be probed by both electrochemical and optical detection methods. The obvious and characteristic color change of the solution from colorless to pink upon the addition of Hg$^{2+}$ demonstrates that 25 can be used for visual detection of Hg$^{2+}$ in water; while, results of the fluorescence studies confirm a lower detection limit of 1 parts per billion (ppb). Investigations in natural water samples including seawater and freshwater indicate that 25 offers a direct and immediate Hg$^{2+}$
detection in complex media, pointing out its potential utility in environment monitoring and assessment. Even this reagent is reported to be suitable for developing test paper kit for semi-quantitative detecting of Hg$^{2+}$ in natural water (Fig. 1A.19).

**Fig. 1A.20.** (A) Structure of chemosensor 26 (MSIR). (B) MSIR-coated glass substrate (a) without Hg$^{2+}$, (b) $1.0 \times 10^{-5}$, (c) $1.0 \times 10^{-4}$; and (d) $1.0 \times 10^{-3}$ M.

A mesoporous silica immobilized rhodamine derivative **MSIR (26)** is also reported in the literature for selective recognition of Hg$^{2+}$ ion over other metal ions. Report also reveals a decrease in BET surface area and pore volume of 1119.72 m$^2$/g and 0.49 cm$^3$/g, respectively, for mesoporous silica to 377.21 m$^2$/g and 0.26 cm$^3$/g for the modified silica and these decreases are due to the attachment of rhodamine molecules to the mesoporous silica.

**Fig. 1A.21.** (A) Structure of chemosensor 27, 28. (B) Color changes of 28 after addition of different metal ions. (C) Mesoporous nanocrystalline TiO$_2$ sensitized with 28 before (left) and after immersion in 9 ppm HgCl$_2$ (center) and 300 ppm HgCl$_2$ in distilled water (right).

The adsorption ability of 26 for Hg$^{2+}$ is 70% and glass plate-coated MSIR is shown to exhibit excellent visual and fluorescence changes with Hg$^{2+}$. In an another report Coronado et al. have demonstrated that the interactions between Hg$^{2+}$ ions and the NCS groups of the ruthenium dyes 27 and 28 are responsible for the color change of
the corresponding dye. One would expect to see a blue shift on binding of Hg$^{2+}$ to the NCS and thus, the observed color change. The limit of quantification of Hg$^{2+}$ using UV-vis spectroscopy in homogeneous aqueous solutions is estimated to be \(~20\) ppb for 27 and \(~150\) ppb for 28. Supporting of these dyes on mesoporous TiO$_2$ film could also be used for the dip sensing of Hg$^{2+}$ in aqueous solution. Reversibility of binding of Hg$^{2+}$ to this dye on TiO$_2$ surface is also reported on treatment with an aqueous solution of KI.

Fig. 1A.22. Structures of the chemosensors 29. (B) Demonstration of polymer 1d coated “dip-sticks” for three different concentrations of Hg$^{2+}$.

Recently, a new colorimetric mercury sensor (29), derived from a terpyridyl derivatives, is reported. It is able to selectively detect Hg$^{2+}$ ions over a number of environmentally relevant ions. Development of paper strips coated with 29d and suitability of these paper sticks for detecting Hg$^{2+}$ following the “di stick” method, similar to that commonly used for pH measurements, is also reported. Color of the litmus strips coated with 29d is reported to turn pink on dipping into an aqueous solution of Hg$^{2+}$ and this methodology is found to be valid for [Hg$^{2+}$] of 2ppb, the EPA standard for safe drinking water. Thus, this method for Hg$^{2+}$ is ideally suited for simple in-field detection of Hg$^{2+}$ without the need for special equipment.

However, reports on selective recognition and detection of Cr$^{3+}$ are not so common in the literature. Two rhodamine-based receptor molecules (30 and 31) are reported for the selective recognition and binding to Cr$^{3+}$ in aqueous solution at physiological pH (Fig. 1A.23).71,72 Binding to the Cr$^{3+}$ induces spirolactam ring opening reaction of the rhodamine moiety, which leads to an appreciable changes in electronic as well as in
fluorescence response having $\lambda_{Abs}^{Max}$ of 500 nm and $\lambda_{Ems}^{Max}$ of 560 nm ($\lambda_{Ext} = 500$ nm). Thus, an associated change in solution colour and fluorescence are also observed.

![Fig. 1A.23. Structures of the chemosensors 30, 31 and 32.](image)

Compound 31, by virtue of having a ferrocene (Fc) fragment comprised with acts as a reagent for bimodal detection of Cr$^{3+}$ in ethanol-water (1:1, v/v; pH 7.4) solutions. An associated changes in Fc/Fc$^+$-based redox potential allows probing the Cr$^{3+}$-binding process. Little interference of the Hg$^{2+}$ is also reported. This reagent in ethanol-PBS buffer (1:99, v/v; pH 7.4) could be used for imaging application for seeing the subcellular distribution of local concentration of Cr$^{3+}$ in HeLa cells.

In this context Samanta et al. describes an OFF-ON fluorescence chemosensor (32) for specific detection of Cr$^{3+}$, which exploits the selective binding ability of SCN ligand to Cr$^{3+}$. Other environmentally and biologically relevant metal ions, such as alkali/alkaline earth metal ions, divalent first row transition metal ions and Group-12 metal ions fails to interfere in the detection process. The chelation enhanced fluorescence response in THF is attributed to the disruption of PET communications between the receptor and the fluorophore unit.

Focus of the research work, presented in this thesis, is to develop an aza based dual responsive (both chromogenic and fluoregenic) molecular probe for the recognition of Hg$^{2+}$ and Cr$^{3+}$ and explore the possibility of using this host reagent for the development of an easy-to-use paper test strip for the semi-quantitative colorimetric detection of Hg$^{2+}$ and Cr$^{3+}$ present in neutral aqueous media. Thus, discussions in this section are
mostly restricted to those receptor molecules that are able to recognise these two ions simultaneously. Recent development of easy-to-use test strip for in-field application in neutral aqueous medium is also discussed. However there are past reports based on different chemosensors for Hg$^{2+}$ and Cr$^{3+}$, which are extensively covered in the review article by Juyoung Yoon et al. and Jong Seung Kim et al.\textsuperscript{74} Those are not included in the present account.

1A.2. Synthetic Receptors for Anions

1A.2.1. Introduction

Anions play crucial roles in various physiological functions as well as in numerous industrial processes. They carry genetic information (DNA is a polyanion) and the majority of enzyme substrates and co-factors are anionic. Anions also play crucial roles in the areas of medicine and catalysis. Consequently, in the environment, anionic species are essential for sustaining growth, while presence of many anionic analytes beyond a permissible level has a detrimental influence to the human health and the environment. It is therefore not surprising that in the last decade the design and development of synthetic receptor that act as colorimetric and luminescent sensors for anions have received considerable attention of the researchers. Consequently, new efficient receptors have emerged that allow monitoring the function, concentration and location of the negatively charged.\textsuperscript{75} The first report on a synthetic receptor for inorganic anions appeared in 1968, which described the size selective binding of Cl$^{-}$ anions by diprotonated 1,1,1-diazabicyclo-[9.9.9] nonacosane.\textsuperscript{76} The field started to develop in 1976 when Graf and Lehn reported protonated cryptate, which could encapsulates F$^{-}$, Br$^{-}$ and Cl$^{-}$ ions.\textsuperscript{77} Since then several other anion receptors have been reported and the anion receptor as a research area has become enriched.\textsuperscript{78} The design of appropriate receptor(s) for anion(s) is particularly more challenging, when it is compared with that for the cations, due to several reasons e.g.
Anions are relatively large and therefore require receptors of considerably greater size than cations. For example, one of the smallest anions, \(F^-\), is comparable in ionic radius to \(K^+\) (1.33 Å versus 1.38 Å).

Even simple inorganic anions occur in a range of shapes and geometries, e.g. spherical (halides), linear (SCN\(^-\), \(N_3^-\)), planar (NO\(_3^-\), PtCl\(_4^-\)), octahedral (PF\(_6^-\), Fe(CN)\(_6^{3-}\)) as well as more complicated examples as in the case of biologically important oligophosphate anions.

In comparison to cations of similar size, anions generally have high free energies of solvation and hence anion-receptor interaction must compete effectively with the solvation energy; e.g. \(\Delta G_{\text{hydration}}(F^-) = -465 \text{ KJ mol}^{-1}\), \(\Delta G_{\text{hydration}}(K^+) = -295 \text{ KJ mol}^{-1}\).

Many anions exist only in a relatively narrow pH window, which may not be favourable for a certain receptor to exist in appropriate form for binding to that anion.

Over the past few decades various methodologies have been adopted for achieving desired anion recognition and designing of effective anion receptors, namely, strong electrostatic attractions,\(^{79}\) metal-anion complexation,\(^{80}\) Lewis acid–base interactions,\(^{81}\) and various weak—but more directional—nonbonding interactions. These non-bonded interaction include anion-\(\pi\) interaction,\(^{82}\) \(\pi-\pi\) stack interactions, dipole-dipole or dipole-induced dipole interactions and hydrophobic effects\(^{83}\) and hydrogen bonding interactions.\(^{84}\) Among these, H-bonding as the motif,\(^{85}\) has been extensively used by several researchers due to the relatively high binding energy, directionality, and possibility of achieving multiple binding and thus, the higher stability for the adduct formed. Moreover, the precise arrangement of H-bonding points, supported by the rational design, potentially allows the geometry of a binding pocket to be adjusted to the size and topology of a desired anion, which contribute significantly in achieving its strong and selective binding for achieving the desired specificity.

1A.2.2. Design of receptor molecules for the recognition of Fluoride

In this field the most investigated individual of the vast family of anions is undoubtedly fluoride due to its duplicitous nature. \(F^-\) is essential for the prevention of dental carries and treatment of osteoporosis.\(^{86}\) \(F^-\) is a common ingredient in anesthetic, hypnotic, psychiatric drugs, military nerve gases and contaminant in drinking water.\(^{87}\) However a
high intake of $F^-$ can cause fluorosis, nephrotoxic changes in both human and animals and lead to urolithiasis.\textsuperscript{88} Recently, it is believed that higher [$F^-$] may cause osteosarcoma.\textsuperscript{89} Studies seem to reveal some correlations between lower IQ in humans and the presence of high [$F^-$] in water supplies.\textsuperscript{90} Inhibition of neurotransmitter biosynthesis in fetuses is caused by high [$F^-$].\textsuperscript{91} Hence, the need for a methodology having fast measurable output or for a real time monitoring with efficient and specific recognition of $F^-$ over all other competitive anions has become an unfortunate reality. These together with a very high solvation energy in polar solvents like water have posed a serious challenge to the sensing community for designing an effective sensor molecules for efficient recognition of the $F^-$ in aqueous medium at a concentration level that is allowed by the world regulatory agency for the safe drinking water by using synthetically simple receptors and involving minimal instrumental assistance.

One way to achieve this is to develop appropriate receptors that are capable of translating the binding induced process into a measurable optical output signal. Anion receptors can be mainly divided into two categories: 1) neutral anion receptors consist of binding block, like urea, thiourea, imidazole, indole, amine, amide, pyrrole, phenol, and sulphonamide moieties, having hydrogen bond donor functionalities or lewis acid in the recognition sites. 2) Positively charged sensors can generally be ammonium, guanidium, quinolinium, and protonated quinoxaline salts and metal complexes that provide electrostatic interactions with the guest anions. These have been utilized for specific recognition of certain anionic analytes in organic aprotic solvents. More recently, the sensors made by immobilization of amides and metal complexes on nanoparticle and anion–$\pi$ interaction have shown improved sensitivity towards certain anions.
1A.2.2.1 Different types of synthetic receptors for $F^-$

Among, two types of receptors shall be discussed in some details to match the emphasis of this thesis. First one being the strong H-bond donor moieties that bind the anionic analytes through H-bonded interactions; while, the second type of receptors are coordination complexes, where the coordination of the anion to the metal centre modulate the energy gap of the frontier orbitals (HOMO and LUMO) and thereby the spectroscopic behaviour of the indicator (Scheme 1).

In recognition through hydrogen-bonding interactions, acidity of the hydrogen atom of the H-bond donor fragment and the charge density or basicity of the anionic analyte play a crucial role. In case of the lower acidity of the H-atom (R-H) involved in H-bonding and lower basicity of the anionic analyte ($A^-$), the binding process is generally governed by the simple H-bonded adduct formation [Eq. (1)]; while, for a situation where both acidity of the H-atom and the basicity of the anionic analyte are significant, binding processes are generally governed by the deprotonation process [Eq. (2)].

\[
\begin{align*}
\text{RH} + A^- & \leftrightarrow \text{RH} \cdots A^- & \text{(1)} \\
\text{RH} + A^- & \leftrightarrow R^+ + AH & \text{(2)}
\end{align*}
\]

For moderate basicity of $A^-$ and acidity of the H-atom of the H-bond donor fragment (R-H), H-bonded adduct formation precedes the deprotonation process and the deprotonation process may further be favored due to formation of hydrogen-bonded anion dimer $A_2H^-$ [Eq. (3)] or higher oligomers.

\[
\begin{align*}
\text{RH} \cdots A^- & \leftrightarrow R^+ + AHA^- & \text{(3)}
\end{align*}
\]

The above mentioned stepwise changes are generally crucial for the selective optical detection of the anionic species like $F^-$ by H-bond donor receptors.

In this context Das and co workers reported two novel colorimetric receptors ($33$ and $34$) for selective $F^-$ recognition having anthraquinone as chromogenic signaling subunit with urea ($33$) and thiourea ($34$) as H-bond donor sites (Fig. 1A.24). These receptors
have shown no affinity for other halide ions (Cl⁻, Br⁻, and I⁻ ions). Well-defined color change (Fig. 1A.24) in the visible region of the spectrum makes these two reagents appropriate for visual detection of F⁻ in DMSO/CH₃CN solution. More interestingly, authors have also performed a detailed theoretical calculation for rationalizing the preferential binding of F⁻ to these two receptors.

However, a more recent DFT study predicted a trend for the interaction of anions of different shapes with urea/thiourea receptor molecules, which do not always follow the basicity scale of anions.⁹³b The order of selectivity predicted at B3LYP/6-311+G** level with urea/thiourea are: F⁻ > CH₃COO⁻ > H₂PO₄⁻ > Cl⁻ ~ NO₃⁻ > Br⁻ > ClO₄⁻. These results are further substantiated by calculations performed at Hartree-Fock and MP2 levels using the 6-311+G** basis set. These results revealed that the interactions are electrostatic in nature, but cannot be related solely to the intrinsic basicity of anions. Optimal geometric arrangement of –N(H) donors and acceptors (A⁻) is also important to achieve the maximum stability.

A substituted naphthalene diimide receptor (35, Φ = 0.34)⁹⁴ bearing a bis-sulfonamide group is described by Langford et al. The compound shows a unique selectivity towards F⁻ over other competing anions in CHCl₃, while a two-stage deprotonation process leads to a colorimetric response. In DMSO solution, affinity of the receptor towards F⁻ is reported to be even higher (Kₐ ~ 10⁶ M⁻¹) with more pronounced changes in absorption characteristics.
Lin et al. have reported pyreno[2,1-b]pyrrole and its dimeric derivative 36,95 which show excellent preference and sensitivity for detection of F¯ in presence of all other competing anions. Eventual deprotonation and formation of the stable HF₂⁻ provide remarkable changes in electronic and fluorescence spectra for visual detection as well as for real-time and on-site application.

A simple tris(indolyl)methene receptors 37-39 having conjugated bisindole skeletons are reported by Shao et al. These receptors show high selectively towards F¯ based on two stages of proton transfer with associated stepwise color changes.96 Acidity of the H-atoms involved in H-bonding could be tuned through substitution of the appropriate electron withdrawing or donating groups into indole unit, which has a direct influence on the occurrence of the deprotonation of receptor. In another article Kim and his co-workers have reported a macrocyclic anion receptor 40 that has an array of positively charged imidazolium units and has shown significant preference for F¯ ions.97

Fabbrizzi et al. reported a novel urea-based receptor 41,98 in which a urea subunit has been substituted with two electron-withdrawing naphthalenimide moieties (Fig. 1A.27).
In DMSO medium, this receptor fails to form any hydrogen bonded adduct even in the presence of varying excess of \( F^- \), however, it undergoes stepwise deprotonation of two N-H fragments. Presumably, the high stability of the \( HF_2^- \) species to the deprotonation of the N-H fragment and these stepwise processes could be distinguished through two visually detectable colour changes. Double deprotonation is also reported in the presence of \( OH^- \). Less basic anions (\( CH_3COO^- \), \( H_2PO_4^- \)) could induce only single deprotonation of only one of two N-H functionalities.

![Fig. 1A.27. Structures of the chemosensors 41. Color changes observed on addition of \([Bu_4N]F\) to a DMSO solution of receptor 41 (LH₂). Left to right: no addition (dominant species: LH₂); plus 5 equiv of \([Bu_4N]F\) (dominant species: LH⁻); plus 40 equiv of \([Bu_4N]F\) (dominant species: L₂⁻).](image-url)

Report on two Ru(II)-polypyridyl)-based receptors (42 and 43) with pendant phenol or catechol functionality as an efficient receptor for \( F^- \) is also available in the literature (Fig. 1A.28A). Experiments have revealed that hydrogen bond formation occurs with a slight excess (1.2 mole equivalence) of \( F^- \). However, with higher \([F^-]\), deprotonation of the O-H functionality occurs. A new absorption band at longer wavelength appears for both H-bonded adduct formation and deprotonation process, effect being more prominent for deprotonation process. Detailed TD-DFT calculations in acetonitrile reveal that this new absorption band at 560 nm upon complexation with \( F^- \) arises primarily due the inter-ligand CT process. However, at higher \([F^-]\), Brønsted acid-base interactions prevail, a situation similar for urea/thiourea-based receptors.
Min et al. reported a new phosphonium derivative of naphthalene (44, Fig. 1A.28B).\textsuperscript{100} Synthesis of this reagent is reported to be achieved by the reaction of 1,8-dibromomethyl-naphthalene with triphenylphosphine. A distinct color change from colourless to yellow is reported only when this reagent solution is treated with F\textsuperscript{−} (Fig. 1A.28B). The high specificity of 44 towards F\textsuperscript{−} is attributed to the acidity of the methylene protons as well as the appropriate fitting of the small F\textsuperscript{−}. \textsuperscript{1}H NMR experiments also supports the deprotonation process in presence of excess F\textsuperscript{−} due to the appearance of a characteristic triplet signal at \( \sim 16 \) ppm for the HF\textsubscript{2}\textsuperscript{−} species. The shift in absorption maxima from 477 (in presence of 20 equiv. of F\textsuperscript{−}) to 407 nm in presence of excess F\textsuperscript{−} further confirms the deprotonation behavior.

More recently Bu and his co-workers have described two polydentate conjugate molecules 45 and 46 (Fig. 1A.29) having a rigid quinoxaline plane with six indole NH
moieties as the recognition sites.\textsuperscript{101} They display an excellent selectivity toward the detection of F\textsuperscript{−} in DMSO, with distinct changes in color from yellow to golden-red and visually detectable fluorescence changes. In particular, receptor 46 displays a 42 nm red-shift for emission band on binding to F\textsuperscript{−}. An enhancement in the ICT process from the donor indole anion (on deprotonation) to the phenyl rings affords this red shift in a ratiometric manner.

However, the above mentioned synthetic probes has mapped out methods to achieve molecular recognition and sensing of anionic guests mainly tetrabutyl ammonium (TBA) fluoride only in organic solvent media. However, for practical application and using such F\textsuperscript{−} specific receptor for analysis of environmental samples, it is imperative to develop appropriate chemosensors that works under competitive conditions in aqueous environments, which is a difficult issue owing to the high hydration energy of F\textsuperscript{−} (-457 kJ Mol\textsuperscript{−1}) compared to other anions (-\Delta H\textsuperscript{0} = 100-110 kcal mol\textsuperscript{−1}). Relatively smaller ionic radius (1.47Å) is also not conducive for the convergent positioning of multiple binding sites. Further F\textsuperscript{−} being a hard base, is less easily distinguishable from water, which is hard as well. Thus, even though many chemosensors have been developed over the past few decades, there are still difficulties in the detection of F\textsuperscript{−} in highly polar or aqueous solution. In solvents with high dielectric constants such as DMSO and water, H-bond formation between F\textsuperscript{−} and solvent molecules compete successfully with the stabilization that can be achieved through H-bonded adduct formation between F\textsuperscript{−} and the H-bond donor fragment of the chemosensor and in some cases, selective recognition of fluoride over oxygen-containing anions (e.g., AcO\textsuperscript{−}, H\textsubscript{2}PO\textsubscript{4}\textsuperscript{−} and CH\textsubscript{3}CO\textsubscript{2}\textsuperscript{−}) is restricted.\textsuperscript{102} Further, for any bioanalytical application as well as for analysis of environmental samples, it is essential to develop a chemosensor that can selectively recognize and detect inorganic fluoride (e.g. NaF) in aqueous medium. NaF
is known to influence various cell signaling processes and thus, its detection has significance in molecular and cell biology also.\textsuperscript{103}

In this context with an objective of recognition of fluoride and cyanide ions in aqueous medium Gabbai and his co-workers have investigated the anion binding properties of two isomeric ammonium boranes, namely \([p-(\text{Mes}_2\text{B})\text{C}_6\text{H}_4(\text{NMe}_3)]^+ (47a)\) and \([o-(\text{Mes}_2\text{B})\text{C}_6\text{H}_4(\text{NMe}_3)]^+ (47b)\textsuperscript{104}\) (Fig. 1A.30). Both reagents react with \(\text{F}^-\) and \(\text{CN}^-\) in organic solvents to afford the corresponding fluoroborate or cyanoborate ammonium zwitterions \(47a\text{F}, 47a\text{CN}, 47b\text{F},\) and \(47b\text{CN}\).

![Fig. 1A.30. (A) Structures of the chemosensors 47a, 47b.](image)

This reaction is utilized for designing a chemodosimeter for these two ions in aqueous solution. In aq. HEPES buffer-DMSO medium (v/v: 3:2; pH ~ 7), \(47a\) is found to form a complexe with \(\text{CN}^-\) \((K_a = 3.9 \times 10^8 \text{M}^{-1})\), while \(47b\) only forms complex with \(\text{F}^-\) \((K_a = 910 \text{ M}^{-1})\). The unusual cyanide binding properties of \(47a\) can be assigned to favorable columbic effects which increase the lewis acidity of the boron atom and strengthen the receptor-cyanide interaction. In \(47b\) the trimethyl ammonium functionality is positioned ortho to the boron center as in \(47b\), the Lewis acidity of the ammonium borane is increased, making fluoride binding possible.

Receptors having a Lewis acidic boron based receptors bind \(\text{F}^-\) through a strong covalent interaction and cause fluorescence quenching due to intramolecular charge transfer between the boron-p\(\pi\) orbital and electrons from \(\text{F}^-\).\textsuperscript{105} High affinity of \(\text{F}^-\) for silicon induces a cleavage of the silyl ethers (e.g., TBDMS, TBDPS ethers).\textsuperscript{106} This phenomenon is used successfully by yang et. al. in designing a highly sensitive and selective fluorogenic probe for \(\text{F}^-\) (Fig. 1A.31), 4-methylumbelliferyl tert-
butyldimethylsilyl ether (48).\textsuperscript{107} 48 is reported to have a weak fluorescence. Upon interaction with F\textsuperscript{−} in acetone-water solution (7:3, v/v), cleavage at the Si-O bond of 48 takes place and highly fluorescent 4-methylumbelliferone (4-MU) is found to be generated with associated fluorescence enhancement for solution. This fluorescence increase is reported to vary linearly with [F\textsuperscript{−}] in the range 50-8000 nmol l\textsuperscript{−1} with a detection limit of 19 nmol l\textsuperscript{−1}. This method has been successfully applied to the F\textsuperscript{−} estimation in toothpaste and tap water samples.

Fig. 1A.31. (A) Feasible Mechanism for the cleavage of Si–O bond in 48 by fluoride ion.

Hong and co-workers have developed a novel chromogenic and fluorescent chemodosimeter 49 (Fig. 1A.32), which shows drastic changes in absorption and emission intensities upon addition of NaF in CH\textsubscript{3}CN:H\textsubscript{2}O medium (50:50, v/v).\textsuperscript{108} Reagent 49 is found to be extreme selectivity for F\textsuperscript{−} over other anions in aqueous medium. Si-O bond cleavage through interaction of F\textsuperscript{−} results in the formation of a highly fluorescent resorufin, which accounts for the observed enhanced fluorescence.

Fig. 1A.32. (A) Feasible Mechanism for the Spectroscopic Changes of 49 in the Presence of F\textsuperscript{−}. (B) Change in emission colour only 49 (1) and 49 + F\textsuperscript{−} (2) excited by UV lamp (λ\textsuperscript{ext}=365 nm).

However, for any biological and cell imaging application, chemosensors for F\textsuperscript{−} should meet the following criteria: (1) needs to be specific for F\textsuperscript{−} in pure aqueous medium or
aq. buffer medium having pH of ~7, (2) needs to be cell membrane permeable, (3) non-toxic to the living organisms or cells, (4) display fluorescence ON response upon interaction with F⁻ ions in cellular systems. In an effort to address these issues for biological applications, Hong and co-workers have developed a 7-hydroxy coumarin (Fig. 1A.33) based chemodosimeter (50). The cleavage of Si-O bond by F⁻ leads to a “turn-ON” luminescence response and this could be used as an imaging reagent for NaF detection in A549 human epithelial lung cancer cells under physiological condition.

Fig. 1A.33. (A) Structure of the chemosensor 50. (B) Bright-field image of A549 cells incubated with 50 (20 mM) for 30 min subsequently incubated for 3 h at 37°C (a). Fluorescence image of A549 incubated with TBPCA (20 mM) for 30 min and subsequently incubated without NaF (b) and with NaF (50mM) (c) for 3 h at 37°C.

Apart from these chemodosimetric approach base on the F⁻ induced Si-O bond cleavage, Bai and co-workers have reported a remarkable fluoride selective Ru(II)-polypyridyl based colorimetric and fluorogenic chemosensor 51. In the presence of F⁻, proton transfer from the quinonehydrazone tautomer (Fig. 1A.34) to F⁻, which induces the formation of azophenol tautomer and a dramatic change in color from orange to blue-violet. They have also presented a rational strategy for the development of easy to prepare fluoride test paper capable of detecting F⁻ in natural aqueous environments with a lowest detection limit of 10 ppm (10 mg L⁻¹).

Fig. 1A.34. (A) Proposed mode of binding of receptor 51 to fluoride. (B) The color changes of the test papers for detecting fluoride ion in neutral aqueous solution with different F⁻ concentrations.
1A.2.3. Recognition of Phosphate ion having Biological Significance

One of the major focuses of the research work presented in this thesis is development of suitable and effective sensor for biologically important phosphate ion, more specifically Pyrophosphate (PPI). So, our discussion in this section will be limited to the recent developments in the area of molecular receptors for phosphate anions that have biological significance. Phosphate ion and its derivatives are important because they are widespread in living cells and ubiquitously play significant roles in a myriad of biological processes. Among the various phosphate anions the selective detection of the anionic pyrophosphate (PPI) is a major research focus. PPI is a biologically important target because it is the product of ATP hydrolysis under cellular conditions, and is involved in DNA replication catalyzed by DNA polymerase. In addition detection of PPI has significance in cancer research. Patients with calcium pyrophosphate dehydrate (CPPD) crystals and chondrocalcinosis have also been shown to have a high synovial fluid PPI level. In this regard, the detection and discrimination of PPI has been the main focus of several studies over the last 10 years with particular attention being paid to the development of chemosensors for PPI in aqueous medium. In general, sensing anions in aqueous solution requires a strong affinity for anions in water as well as the ability to convert anion recognition into a measurable output in terms of changes in fluorescent or colorimetric signal. Consequently, it is almost imperative to develop colorimetric or fluorescence-based receptor that is selective for PPI over other phosphate-based anions like PO₄³⁻, H₂PO₄⁻, AMP, ADP and ATP.

1A.2.3.1. Synthetic Receptors for PPI

Considerations that generally influences the design of sensor molecules for PPI:

- PPI contains di phosphate units with four negative charges. So, charge density and possibility of the multi-point binding are important criterion for designing suitable receptors for PPI.
Hydration energy for PPI is high due to its tetra-negative charge. So receptor-PPI binding enthalpy should be more than the enthalpy for the hydration of PPI.

For live cell imaging application, one needs to design a receptor that works in aqueous environment and at physiological pH (~7.0). Further, efficient sensor for PPI needs to be non-toxic to living cells.

Designing of a receptor that is specific towards PPI in presence of other competitive anions, particularly AMP, ADP, Pi, ATP.

### 1A.2.3.1.1. Recognition of PPI Based on Hydrogen-Bonding Interaction

![Fig. 1A.35](image1)

(A) Structure of chemosensor 52 and the proposed binding mode with PPI.

In this context in 1994, Czarnik et al. have reported their pioneering work in which an anthracene derivative bearing polyamine groups (52) was used as a PPI sensor in a pure aqueous solution. This chemosensor 52 binds pyrophosphate with fluorescence enhancement, and 1:1 complexation occurred with $K_d = 2.9 \mu M$ at pH 7 (0.05 M aq. HEPES buffer). A 2200-fold increase in pyrophosphate/phosphate discrimination could be achieved using this reagent. Presumably, the molecular rigidity achieved in binding to PPI is responsible for the observed fluorescence enhancement. A real-time assay of PPI hydrolysis, catalyzed by inorganic pyrophosphatase is also reported.

![Fig. 1A.36](image2)

(A) Structure of chemosensor 53 and the proposed binding mode with PPI.

In another article Teramae et al. have described a pyrene-functionalized guanidinium receptor as a fluorescent chemosensor (53) for PPI in methanol. This particular system uses a complexation induced self-assembly approach for the detection of PPI.
An intramolecular excimer band on binding to PPi is accounted for a new emission band as output signal.

Fig. 1A.37. (A) Molecular structure of chemosensors 54-56.

A series of fluorescent chemosensors 54, 55, 56 having calixpyrrole receptors were reported by Sessler and co-workers. These systems rely on the use of dansyl (54), Lissamine rhodamine B (55), and fluorescein (56) moieties as the fluorescent reporter functionality. Calixpyrrole moiety shows high affinity toward PPi and F-. In this case, PPi can make multiple hydrogen bonds with both calixpyrrole, while for 56 additional H-bond formation with the thiourea moiety is also reported.

Fig. 1A.38. (A) A schematic drawing of synergy in a p-doped conductive polymer with integrated hydrogen-bonding receptors. Right: Structures of monomers 57 and 58, and sensor materials poly-57 and poly-58.

Dipyrrolyl quinoxalines have been actively examined by the Anzenbacher group as chromogenic as well as fluorescent sensors for PPi. These results have been applied to multiwall assays using polyurethane-embedded sensors bearing dipyrrolyl quinoxaline moieties, which allowed the colorimetric screening of aqueous phosphate-
related anions based on chromogenic conductive polymers. This method utilizes synergy between low-level p-doping in a polythiophene polymer and hydrogen bonding to increase anion-sensor affinity. These chromogenic conductive polymers exhibit reversible anion-specific changes both in color and in conductivity after increasing the concentration of anions such as PPI.\textsuperscript{114a}

![Fig. 1A.39. Structures of fluorescent imidazolium based receptors 59-62.](image)

In addition to the use of the well-established H-bond formation moieties for the anion recognition studies (such as amide, pyrrole, and urea functionalities), receptors containing imidazolium moieties have also been explored in designing chemosensors for anion.\textsuperscript{115} These hosts can produce strong and unique (C-H)⁺…X⁻ hydrogen bonding between the imidazolium moieties and various anions.\textsuperscript{115} Yoon and Kim et al. recently reported four different fluorescent imidazolium derivatives (59-62) as fluorescent chemosensors for PPI (Fig. 1A.39).\textsuperscript{116} Among the various anions, such as HSO₄⁻, CH₃CO₂⁻, I⁻, Br⁻, Cl⁻, F⁻, H₂PO₄⁻, and PPI, compounds 59-62 show the highest binding affinity with PPI in acetonitrile. The fluorescence quenching effect upon the addition of PPI was explained on the basis of a PET-based mechanism. The association constants of compounds 59-62 with PPI are reported as 5.43 x 10⁵, 1.01 x 10⁸, 3.58 x 10⁶, and 6.76 x 10⁶ M⁻¹, respectively. Among the series of hosts examined, dimer host 59 showed the largest binding constant with PPI, which suggests that a preorganized rigid binding pocket might play an important role in the binding with PPI.
Different PET-based fluorescent sensors, flanked with two binding sites, are reported for anions like dicarboxylates and pyrophosphate.\textsuperscript{111,112,117} In this context Gunnaugsson and co-workers have reported new chemosensors \textsuperscript{63, 64}, having a "receptor-spacer-fluorophore-spacer-receptor" conjugate.\textsuperscript{118} These chemosensors have two thiourea moieties that can form H-bonded adducts with anions possessing two H-bond acceptor sites, such as dicarboxylates and pyrophosphate. The anion recognition studies in DMSO takes place involving two thiourea receptor sites with concomitant PET quenching of the anthracene moiety. This is the first examples of charge neutral fluorescent PET sensors that show ideal PET behavior for bis-anions.

Although there are some limited examples of chemosensors for PPI involving hydrogen bonding interactions, such receptors usually fail to recognize or work in polar solvents like water due the deleterious and high solvation energy for PPI. Thus far, the utilization of the gain in stabilization in metal ion-PPI complex formation has been found to be the most successful strategy, as the associated enthalpy changes could compete successfully with the enthalpy of hydration of the PPI moiety in water and this strategy could be expanded for exploring the bio-analytical potential to exhibit sufficient compatibility under complicated biological conditions.
1A.2.3.1.2. Receptors for PPi Based on Metal Complexes.

Fig. 1A.41. (A) X-Ray crystal structure of the catalytic core of alkaline phosphatase (ALP). (B) The two zinc ions simultaneously bind to the substrate phosphate anion with coordination interaction.

To overcome the above mentioned demerits, sensor community have adopted metal ion-anion coordination as the most successful strategy for anion recognition and sensing in aqueous medium. In many cases, multiple metal ions are positioned on an organic scaffold at appropriate distances to allow an anion guest to bridge the metal centers, providing a means of introducing selectivity for a specific guest.

As mentioned earlier, in general, enthalpy changes for metal-phosphate coordination, are more favourable than the enthalpy of hydration for these anions in aqueous medium. Further, water being a poor Lewis base does not have any significant attraction towards the Lewis acidic cationic metal centre. Such metal-analyte coordination plays an important role in many enzymatic process and govern the activity of the respective metalloenzymes. For example, alkaline phosphatase (ALP), an important enzyme for influencing the hydrolysis of the phosphoester linkages has two zinc(II) centres in the active site and are located in close proximity suitable for bidentate binding with a phosphate anion (Fig. 1A.41) prior to the hydrolysis reaction.

The design of receptors for phosphate anions inspired by the binding sites of metalloenzymes, in which phosphates act as substrates or inhibitors by reversibly coordinating to one or more Zn(II) ions in the enzymatic pocket. By mimicking such recognition strategy in Nature, researchers have developed various transition metal -
based coordination complexes for the recognition of PPI in aqueous as well as complicated biological conditions.

The dipicolylamino (DPA) ligand is one of the most commonly used ligands for such studies as the tridentate ligand with three nitrogen donors affords good selectivity for Zn$^{2+}$ over biologically relevant metals such as Na$^+$, K$^+$, Mg$^{2+}$ and Ca$^{2+}$, and leaves available coordination sites for binding to anionic analyte. The ease of synthesis of suitable DPA derivatives and the synthetic ease in appending multiple DPA units to a scaffolds has led to widespread use of Zn(II)-DPA based complexes in anion recognition and sensing. In an effort to develop DPA-Zn$^{2+}$ based receptors for the recognition of PPI under neutral aqueous conditions, Hong et.al have developed a new chromogenic sensor 64 having an azophenol-Dpa (bis(2-pyridylmethyl)amine) system, which shows a high sensitivity and selectivity for PPI over other anions in aqueous medium over a wide pH range. More importantly, the addition of P$_2$O$_7^{4-}$ (PPI) to the solution of 64 causes bathochromic shifts from 417 nm ($\lambda_{\text{max}}$) to 465 nm in aq.HEPES buffer (pH 7.4) medium with associated changes in color from yellow to red. Association constant ($K_a$) is reported as $(6.6 \pm 1.2) \times 10^8$ M$^{-1}$ for PPI-64 binding by a standard algorithm for competitive binding in the presence of excess HPO$_4^{2-}$.

Fig. 1A.42. (A) Structure of chemosensor 65. (B) X-ray structure of the di-zinc complex. (C) Color changes of sensor 65 in 10 mM aqueous HEPES buffer solution (pH 7.4) in presence of following their different anion, left to right: no anion, PPI, citrate, HPO$_4^{2-}$, H$_2$PO$_4^{-}$, acetate, F$^-$. 
The binding mode for PPI to two Zn(II)-centers in 65 is illustrated in Fig. 1A.42B. The X-ray structure of the complex reveals that the two O\(^-\), belong to two different PO\(_4\)-unit in PPI bind to the dinuclear Zn(II)-complex as a bridging ligand and result two hexa-coordinated Zn\(^{2+}\) ions in 65. These results demonstrate that the azophenol-based chemosensor having two Zn\(^{2+}\)-DPA units can be a promising candidate for sensing PPI in aqueous systems.

![Proposed binding model for the complexation of sensor 66 with PPI](image1)

**Fig. 1A.43.** (A) Proposed binding model for the complexation of sensor 66 with PPI. (B) Fluorescence changes of 66 (6 \(\mu\)M) upon the addition of various anions (8 \(\mu\)M) in HEPES buffer (10 mM, pH 7.4).

Hong et al. has used this recognition motif in developing a fluorescence-based chemosensor for the detection of PPI. A napthalene derivative, 66 is reported to exhibit specificity towards PPI, while the binding process is monitored by probing fluorescence change on binding to PPI (Fig. 1A.43).\(^{122}\) A shift of 20 nm in \(\lambda_{\text{Max}}\) (436nm to 456 nm) with 9.5-fold fluorescence enhancement upon the addition of 1 equiv of PPI at pH 7.4 is reported (\(K_a = 2.9 \times 10^8 \text{ M}^{-1}\)). This reagent is capable of detecting nanomolar concentration of PPI in aqueous medium at physiological pH. Even the presence of large (250-fold excess) excess of ATP fails to interfere in the detection process. This is the first example of a complex that can discriminate PPI from ATP in aqueous solution. Stronger interaction between the PPI having higher charge density and cationic Zn(II)-centers in 66 could account for the observed specificity for PPI over ATP.

Hong et al. has introduced the concept of the synergistic effect of metal coordination and hydrogen bonding in order to improve the binding affinity and selectivity toward
PPi by designing a new dinuclear Zn(II)-complex (67), which shows remarkable specificity towards PPi and acts as a selective colorimetric sensor for PPi in water.123

![Chemical Structure](image)

**Fig. 1A.44.** (A) The structure of compound 67 and (B) the crystal structure of 67–PPi.

Four amide hydrogen bond donors have a rigid pre-organized orientation favorable for the interaction with the PPi, coordinated to the two Zn$^{2+}$ ions and this is presumed to contribute to the substantial improvement in binding affinity ($K_a = 5.39 \times 10^{10} \text{ M}^{-1}$) and the lowest detection limit (20 pM). This reagent shows strongest binding to PPi in water. Fig. 1A.44 shows the X-ray crystal structure of complex 67–PPi, in which additional H-bonds by the amide groups are clearly shown. This approach clearly reveals the merit of careful designing in developing an efficient and improved receptor for the target anion.

Yoon and co-workers have reported two more DPA-based receptors (68a and 68b)124 using Zn(II) and Cu(II) complexes of Zinpyr-1, respectively,125 as fluorescent and colorimetric sensors for PPi. In aqueous solution (HEPES buffer, pH 7.4), the fluorescence intensity of 68a and 68b is significantly (150%) enhanced upon binding to PPi. Respective association constants for binding of PPi to 68a ($K_a = 9.8 \times 10^4 \text{ M}^{-1}$) and 68b ($K_a = 1.7 \times 10^5 \text{ M}^{-1}$) are determined from the fluorescence titration. On the other hand, no significant spectral changes were observed upon addition of H$_2$PO$_4^-$ and anions such as HSO$_4^-$, CH$_3$COO$^-$ and halides. Yoon et al. subsequently reported the synthesis of acridine-based fluorescent chemosensor 69,126 the X-ray structure of which is shown in Fig. 1A.45B. This receptor is found to exhibit different fluorescence responses upon binding to PPi and inorganic phosphate. Upon the addition of PPi (100
equiv.) to a solution of 69 (3 mM) in 10 mM HEPES buffer (pH 7.4), a large chelation enhanced fluorescence quenching (CHEQ) effect with a bathochromic shift (~20 nm) was observed. On the other hand, a large chelation enhanced fluorescence (CHEF) effect with a bathochromic shift (~20 nm) was observed upon the addition of \( \text{H}_2\text{PO}_4^- \).

![Fig. 1A.45.](image)

No spectral change was observed upon the addition of 100 equiv. of \( \text{AcO}^- \), \( \text{HSO}_4^- \) and halides. The association constants for binding of \( \text{PPi} \) and \( \text{H}_2\text{PO}_4^- \) (Pi) to the receptor 69 are also reported (\( K_a^{PPi} = 4.85 \times 10^7 \text{ M}^{-1} \) and \( K_a^{Pi} = 9.36 \times 10^4 \text{ M}^{-1} \)), which clearly reveals the preference of the receptor for \( \text{PPi} \). The CHEQ effect observed upon with \( \text{PPi} \) was attributed to PET process from the benzylic amine of the DPA group to the acridine moiety, while the CHEF effect was attributed to the formation of hydrogen bonds between the hydrogen atoms of \( \text{H}_2\text{PO}_4^- \) and nitrogen on the acridine moiety.

![Fig. 1A.46.](image)

The energy-minimized structure of 70·Zn²⁺·PPi (Spartan '02 program, Wavefunction Inc.) and fluorescent changes of 70·Zn²⁺ (0.02 mM) upon the addition of PPi and ATP (0.4 equiv) in HEPES buffer (10 mM, pH 7.4).
An alternative approach to PPI recognition by a Bis[Zn²⁺–DPA] complex through excimer formation has also reported,

where specific binding to PPI induces self-assembly and thereby the modified fluorescence response on excimer formation of two interacting pyrene moieties. Results of the Job’s plot suggest a 2:1 (70:PPi) complex formation and further the energy minimized structure obtained by using Spartan’02 program supports the presence of π–π stacked pyrene dimer. This also corroborates the new fluorescence response due to the excimer formation.

![Proposed binding mechanism of the chemosensor 71 with PPI.](image)

Yoon et al. recently extended this concept to naphthaldimide system (71) for developing an efficient fluorescent chemosensor for PPI, which can function in a 100% aqueous solution (Fig. 1A.47).

This sensor shows a unique excimer peak at 490 nm only in the presence of PPI. Four Zn(II)-sites as well as a π-π stacking interaction induced the unique 2 + 2 type excimer in the presence of PPI, as shown in Fig. 1A.47. This 2 + 2 type excimer formation is supported by the ESI-Ms data and unique excimer fluorescence peak. Furthermore, the detection of PPI is selective over ATP or Pi. The association constant for PPI was reported to be 4.1 x 10⁵ M⁻¹.
The tetraphenylethylene-based bis[Zn(II)–DPA] receptor 72 exhibits an 11-fold fluorescence enhancement upon addition of 1 equiv. of PPI in H$_2$O-DMSO (10:1, v/v) at 25°C, although addition of 1 equiv. of either AMP or ATP also results in smaller but relatively less significant fluorescence enhancements (~3-fold and 5-fold, respectively), which restricted the use of this reagent as specific chemosensor for PPI (no association constants are reported). The sensing mechanism in this case may either be attributed to the restricted molecular flexibility of the phenyl rings as a result of PPI binding and/or the induced charge transfer process.

In recent attempts to develop a chemosensor with higher sensitivity for PPI than that observed for naphthalene derivative 66, the fluorescent chemosensor 73 was synthesized with two coumarin units appended to the two Zn(II)–DPA moieties. Compound 73 exhibits a large decrease in fluorescence intensity upon binding to PPI (9.8-fold) in 10 mM aq. HEPES buffer (pH 7.4) at 25°C. However, similar experiments with ATP also reveal a 4.8-fold decrease in fluorescence emission, while other phosphate ions, like H$_2$PO$_4^-$, AMP and ADP do not induce detectable fluorescence changes. Notably, the detection limit reported for PPI is 49 nM, which is an important improvement over that observed for 66 (83 nM). Kim and co-workers have developed fluorescent chemosensor 74 based on a 1,8-naphthalimide-bis[Zn(II)–DPA] complex as a PPI-selective Turn-Off fluorescent probe. In CH$_3$CN-aq. HEPES buffer [20 mM, pH 7.4, 5:95, v/v], 74 exhibits partial fluorescence quenching upon binding to both PPI
Fig. 1A.49. Structure of chemosensor 74 and Fluorescence image of C2C12 cells treated with 1(1.0 μM). (b) Fluorescence images of C2C12 cells treated with 74 (1.0 μM) and Zn²⁺ (5.0 μM). (c) Fluorescence images of C2C12 cells treated with 74 (1.0 μM), Zn²⁺ (5.0 μM) and PPi (0.5 mM).(d) Fluorescence images of C2C12 cells treated with 74 (1.0 μM), Zn²⁺ (5.0 μM) and PPi (1.0 mM). (e), (f), (g), (h) are their corresponding bright field images.

(~52%) and ATP (~31%). However, the binding of PPi causes a large blue shift (23 nm) in the fluorescence maximum of 74, which is not the case for ATP. In contrast to the bis[Zn(II)–DPA] complexes discussed above, where binding to PPi generally involves both Zn(II) ions, molecular modeling suggests that only one of the Zn²⁺ centers in 74 binds directly to PPi. However, Receptor 74 is also used successfully for monitoring the intracellular Zn(II) ions through intercellular fluorescence enhancement and PPi through intracellular fluorescence quenching in confocal fluorescence imaging studies with C2C12 cells.

Fig. 1A.50. (A) PPi sensing via ESIPT by fluorescent sensor 75. (B) Fluorescence spectra of 75 (12 μM) in 10 mM HEPES buffer (pH 7.4) at 25 °C in the presence of various anions (600 μM) and their fluorescent images (irradiated at 365 nm).
Pang and co-workers have recently reported a binuclear Zn(II)-complex of a 2-(2-hydroxyphenyl)-1,3-benzoxazole derivative, **75**, as a highly selective PPI sensor using excited state intramolecular proton transfer (ESIPT) as the sensing mechanism (Fig. 1A.50). In 10mM aq. HEPES buffer medium (pH 7.4) at 25°C, **75** exhibits a unique fluorescence response to PPI with a large red shift in the emission band (~100 nm) with a new maximum at 518 nm, and an almost complete quenching at 420 nm region (Fig. 1A.50) consistent with ESIPT occurring. In contrast, other anions including ATP, H$_2$PO$_4^-$, citrate and AcO$^-$ do not induce an ESIPT bathochromic shift; resulting only in a slight increase of fluorescence of **75** at 420 nm. The association constant between **75** and PPI is reported as 9.2 x 10$^7$ M$^{-1}$ in aq. HEPES buffer medium. The strong binding affinity and selectivity of **75** towards PPI should allow its use in biomedical and analytical applications and preliminary studies have indicated that **75** can be employed to monitor the amount of PPI released during the DNA polymerase chain reaction.

A number of metal ions, specially Cu$^{2+}$ besides Zn$^{2+}$, have been used to form complexes with DPA ligands and these complexes have been used as the receptor for recognition and detection of phosphate ions. Yoon and co-workers have demonstrated the difference of using Cu$^{2+}$ and Zn$^{2+}$ complexes of a coumarin derivative in mono-DPA derivatives **76-79**. They found that while the Cu$^{2+}$ complexes, **76** and **78**, exhibited large fluorescence enhancement upon addition of PPI, the Zn$^{2+}$ analogues, **77** and **79**, did not show any significant fluorescence changes with a variety of anions, including PPI and ATP. Both **76** and **78** bind selectively to PPI with association constants of around 10$^4$ M$^{-1}$, while the binding process is associated with ~ 20-fold increase in fluorescence intensity in aq. HEPES buffer (pH 7.4). The fluorescence changes associated with ATP, ADP, AMP and H$_2$PO$_4^-$ are of much less significance.
Recently, several structurally similar receptors based on mononuclear Zn\(^{2+}\) and Cu\(^{2+}\) complexes of a 2-hydroxy-6-cyanonaphthalene derivative, compounds 80-82, have been reported by Ahn and co-workers\(^{135}\). The Zn(II)-complex (80) exhibits strong fluorescence enhancement (17-fold) in aq. HEPES buffer (10 mM, pH 7.4) in the presence of 250 equiv PPi, significant but much lower fluorescence enhancement also observed upon addition of ATP. Other anions such as ADP, H\(_2\)PO\(_4\)\(^{-}\), HSO\(_4\)\(^{-}\), AcO\(^{-}\), Cl\(^{-}\) and F\(^{-}\) show little or negligible fluorescence enhancement. The association constant of 80 with PPi (K\(_a\) = 1x10\(^5\) M\(^{-1}\)) is evaluated from the fluorescence titration data. The Cu(II) complex 81 also shows a 24 fold enhancement in fluorescence on binding to PPi. Also this Cu(II)-based receptor offers better selectivity for PPi over ATP in comparison to the Zn(II) analogue 80, however the binding mode in this case is not 1:1 and no association constant is reported. Nevertheless, experimental data tend to suggest that the binding of PPi to Cu(II) in 81 is less tight than that of the Zn(II) analogue in 80. Interestingly, both 80 and 81 exhibit a time-dependent fluorescence enhancement when bound to PPi. This unusual behavior for 80 is attributed to the slow cleavage of the metal ion-phenolate bond upon binding to PPi. Compound 82, without such phenol/phenoxide group, do not exhibit such time-dependent response.

In 2007, Tian and co-workers presented a novel Cu(II) containing colorometric as well as \textit{turn ON} fluorescent sensor 83 having dicyanomethylene-4H-chromene as the reporter functionality and DPA frameworks as the receptor fragment\(^{136}\). This shows
high selectivity and sensitivity towards PPi over other competitive anions. The associated color change from pale yellow to light pink can be easily distinguished by visual inspection. The fluorescence titration profile with varying [Cu$^{2+}$] confirms that 83 forms a 1:1 complex with PPi with association constant ($K_a$) of 4.6x10^5 M$^{-1}$.

![Figure 1A.52](image)

**Fig. 1A.52.** Structure of Chemosensors 83 and Photographic images observed from DCCP-Cu$^{2+}$ with the addition of PPI anions ([DCCP-Cu$^{2+}$] = 10 μM, [PPI] = 10 μM): (a) color change; (b) fluorescent emission change irradiated at 365 nm by a portable fluorescent lamp.

Strong electrostatic interaction between PPI and Cu(II)-center in 83 is expected to reduce the extent of interaction between the Cu(II)-center and the neutral DPA fragment. This presumably enhances the electron-donating character of the DPA moiety, which eventually results in an increased efficiency of ICT. The most remarkable observation for this recognition process is the changes in fluorescence for monitoring the PPI binding is the NIR region, an important issue for good transmission and low auto fluorescence in biological samples.

![Figure 1A.53](image)

**Fig. 1A.53.** Proposed sensing mechanism of the chemosensor 84 with anion.

So far the discussion is restricted to the DPA based metal ion receptor for the recognition of PPI. There are other receptors without DPA framework, which are used for the recognition of PPI in physiological environment.
Kikuchi et al. have reported the Cd$^{2+}$-cyclen-coumarin system as a fluorescent chemosensor (84) for PPI in an aqueous solution.\textsuperscript{137} As shown in Fig. 1A.53, Cd$^{2+}$ in complex 84 is coordinated to four nitrogen atoms of cyclen and an aromatic amino group. The aromatic amino group of coumarin is displaced when different anions (A$^-$) are added to a buffer solution (aq. HEPES buffer, pH 7.4) containing complex 84 due to the preferential binding of A$^-$ to the Cd(II)-center in 84. This causes a change in the CT properties and thus, the emission responses as the output signal. Among the various anions examined, this system has shown comparable preference for citrate ($K_d = 9.0 \times 10^{-5}$ M) and PPI ($K_d = 7.5 \times 10^{-5}$ M).

![Fig. 1A.54. Structure of poly electrolyte PPE–CO$_2^-$ (85). ALP assay using PPE–CO$_2^-$/Cu$^{2+}$ system ([PPE–CO$_2^-$] = 3 $\mu$M, [Cu$^{2+}$] = 6 $\mu$M, [PPI] = 12 $\mu$M in 0.01 M HEPES buffer, pH 7.5, 37°C). Emission was monitored at 525 nm ($\lambda_{ex} = 390$ nm).](image)

Schanze et al. reported a new fluorescence turn-on sensor 85 composed of a conjugated polyelectrolyte (PPE–CO$_2^-$) and Cu$^{2+}$.\textsuperscript{138} Systematic addition of PPI into the solution of 85 (5 $\mu$M/10 $\mu$M) results in a continuous recovery of the polymer’s fluorescence intensity, and at 10 $\mu$M of added PPI (1 mole equivalent with respect to [Cu$^{2+}$]) a 17-fold enhancement of fluorescence intensity is observed. Titrations over the low concentration range (0 - 1.0 $\mu$M) indicate that the analytical detection limit for PPI is 80 nM. No such detectable change in the fluorescence intensity of 85 is observed upon addition of 50 $\mu$M of other competitive anions. This result clearly indicates that this system can detect PPI at nanomolar concentration with high selectivity over other anions.
inorganic anions, including inorganic phosphate (Pi). On the basis of this finding a real-
time turn-off assay for alkaline phosphatase from bovine intestinal mucosa (catalyzes
the hydrolysis of PPI to Pi) was developed and tested under physiological conditions.

Recently Ghosh et al reported a fluorescent chemosensor based on a quinoline
derivative, 86 (OFF state).\textsuperscript{139} This receptor selectively senses Zn\textsuperscript{2+} by effective chelate
enhanced fluorescence (ON state), which further shows selectivity toward PPI over
other competing anions like Pi, AMP, and ATP via fluorescence quenching (OFF state)
in a 100\% aq. HEPES buffer (pH 7.4). Thus, this quinoline derivative, 86, acts as an
“OFF-ON-OFF” molecular switch by Zn\textsuperscript{2+} and PPI inputs, respectively. The binding
mode of 86 and Zn\textsuperscript{2+} is also confirmed by single-crystal X-ray analysis of 86-Zn\textsuperscript{2+}. A
plausible mode for the selective binding of PPI to 86-Zn\textsuperscript{2+} has been demonstrated by
quantum mechanical density functional theory calculations and high-resolution mass
spectrometry analysis.

Although many covalently linked metal-based chromophore have been developed for
PPI sensing and recognition purposes, the inherent synthetic complexity of such
molecules has been one of the factors that is limiting the rapid development of new
receptors. An alternative and fast-growing approach is the design of metal- based
receptors suitable for use in indicator displacement assays (IDAs). The IDA method
has been a subject of extensive review in recent literature.\textsuperscript{140} In this method, a
fluorescent or colorimetric indicator is coordinated to a receptor to form a chemo-
sensing ensemble. Upon addition of an anion with similar or higher affinity for the receptor, the indicator is displaced from the ensemble and its subsequent photophysical property changes can be used to study the receptor-anion binding phenomena. The advantages of this approach include simpler synthesis and rapid indicator screening which allow faster and more efficient turn-over in receptor design. In addition, selectivity can be tuned through changing the indicator.¹⁴¹ The structures of some of the common colorimetric and fluorescent indicators are reported in the literature for developing displacement assays are shown in Fig. 1A.57.

Fig. 1A.56. Generalized Representation of the Signal Transduction Mechanism for IDAs; I) Indicator, A) Analyte, R) Receptor. Emission or absorption intensity or wavelength maxima may change between bound and unbound states of the indicator.

Fig. 1A.57. Colorimetric and fluorescent indicators used in IDAs for anion sensing.
A subsequent study by B. D. Smith et al. found that a chemo-sensing ensemble comprising a 1:1 mixture of 87 and the fluorescent indicator coumarin methylsulfonate (Fig. 1A.57) shows significantly higher indicator displacement for PPI than HPO$_4^{2-}$.\textsuperscript{142} Competitive binding analysis of fluorescence titration data affords association constants for 87 with PPI and HPO$_4^{2-}$ of $6.7 \times 10^6$ and $1.1 \times 10^5$ M$^{-1}$, respectively in aq. TES buffer (pH 7.4, 145 mM NaCl).

![Structure of chemosensor 87–90.](image)

More recently, R. C. Smith and co-workers have developed a PPI-selective colorimetric IDA using receptor 87, in which the tuning of selectivity for PPI over HPO$_4^{2-}$ is achieved via the screening of many commercially available indicators.\textsuperscript{143} The dissociation constants ($K_d$) were determined for eleven indicators (ARS, BPR, DT, EBBB, MB9, ERB, GC, MX, PAR, PV and ZC, Fig. 1A.57) by UV-vis spectroscopy in aq. HEPES buffer medium (pH 7.4). Dissociation constants are found to span over 2 orders of magnitude from $2.8 \times 10^{-4}$ M (ARS) to $2.7 \times 10^{-6}$ M (BPR). Based on the framework of 87, Smith and co-workers have reported polyglycerol-bound receptors 88-PG and 89-PG, in which the $m$-xylyl-bis(DPA)-phenoxide or $m$-xylylene-bis(DPA) scaffolds were tethered to the periphery of a water-soluble hyperbranched polyglycerol (PG).\textsuperscript{144} In aq. HEPES buffer medium (pH 7.4), these polymeric bis[Zn(II)-DPA] complexes are capable of detecting HPO$_4^{2-}$ and PPI via IDAs using six commercially available indicators (ARS, MB9, MX, PAR, PV and ZC, Fig. 1A.57). Again, selectivity for PPI
over HPO$_4^{2-}$ can be achieved with 88-PG and 89-PG by simply choosing an indicator with appropriate $K_d$ values, such as MX or PV. The same research group have later reported another new receptor 90 using a m-terphenyl scaffold for PPI-selective detection by IDAs. The increase of the spacer distance between the two Zn(II)–DPA binding sites is reported expected to enhance selectivity for PPI over HPO$_4^{2-}$ as compared to receptor 87. Different colorimetric (MX, PAR, PV and ZC) and a fluorescent (ESC) indicators are actually used to study the binding affinity of 90 for PPI following the IDA methodologies. The dissociation constants of 50 were calculated from competitive binding analysis of absorption data for PPI ($K_d = 2.4 \times 10^{-6}$ M) and HPO$_4^{2-}$ ($K_d = 3.1 \times 10^{-5}$ M), which represents a 13-fold selectivity for PPI.

Fig. 1A.59. Structure of chemosensor 91–93.

B. D. Smith and co-workers have developed an indicator displacement system for fluorescent detection of PPI using receptors 91-93. The association constants between receptors 91-93 and the indicator coumarin methylsulfonate (Fig. 1A.53) are reported to be evaluated from fluorescence titrations with value for the respective receptor being $1.0 \times 10^5$, $6.0 \times 10^5$ and $2.3 \times 10^6$ M$^{-1}$, respectively in aq. TES buffer medium (pH 7.4, 145 mM NaCl). Using competitive binding analysis of fluorescence data, receptor 93 is found to bind PPI with $K_a$ of $1.5 \times 10^7$ M$^{-1}$, a 20-fold selectivity over HPO$_4^{2-}$ ($K_a = 7.3 \times 10^5$ M$^{-1}$), which also represents a 2-fold stronger binding than that of 87 ($K_a = 6.7 \times 10^6$ M$^{-1}$ for PPI) under the same experimental conditions. Notably, all other anions (AcO$^-$, CO$_3^{2-}$, NO$_3^-$, HPO$_4^{2-}$ and SO$_4^{2-}$) tested in this study fail to displace the indicator that is coordinated to the Zn(II)-centers in 91-93.
Macrocyclic peptides bearing two Zn(II)-DPA substituted side-arms have been employed as scaffolds for pyrophosphate sensors by Jolliffe and co-workers. Receptors 94–98 possess common structural features, having the same relatively flat macrocyclic scaffold that is constructed from an oxazole modified cyclic peptide. Two Zn(II)-DPA units as the receptor fragment are appended for probable binding to PPI.

The large scaffold is expected to increase the spacing between the two zinc centers, to better complement the size of PPI, thus providing higher selectivity for PPI over HPO$_4^{2-}$. A high association constant (log $K_{ass} = 8.0$) is indeed obtained for receptor 94 with PPI in aq. HEPES buffer medium (pH 7.2, 145 mM NaCl) using a fluorescent IDA with the indicator coumarin methylsulfonate. This chemo-sensing ensemble shows complete selectivity for PPI over monophosphate derivatives, including HPO$_4^{2-}$ which shows no indicator displacement, and significant selectivity (~2 orders of magnitude) is also observed for PPI over polyphosphate nucleotides such as ATP and ADP.

The Fabbrizzi group used an azacrown-Cu$^{2+}$ complex (99) and fluorescent dye as the so-called “chemosensing ensemble (CE)” approach (Fig. 1A.61). In this metal-containing CE system, efficient quenching is provided by Cu$^{2+}$ ions, which is suppressed when the indicator is released into the solution with full revival of the
fluorescence. From the competition assay, the log $K_a$ values obtained for PPi and Pi using coumarin 343 (100) at pH 7 were 7.2 and 4.4, respectively.

Fig. 1A.61. Structure of chemosensor 99 and indicator coumarin 343 (100).
1A.3. References


29. Haugland, R. P.; *Handbook of Fluorescent Probes and Research Chemicals*, Molecular Probes, Inc, Eugene, OR, USA.


1B. Receptor design for the recognition of analytes through irreversible binding: Chemodosimeter approach

Chemodosimeter approach for the design of a specific receptor involves the use of a chemical reaction (usually irreversible) induced by the presence of the desired ionic or neutral analyte with a specific organic functionality that is associated to a change in color or emission variation. For irreversible process, the use of the term chemosensor cannot strictly be used and is generally referred as chemodosimeter or chemoreactant. Two such examples are shown schematically in Fig. 1B.1; one in which the analyte reacts with the chemodosimeter and remains covalently bonded to the product, while for the second example the analyte catalyzes a chemical reaction. In both examples, as the final compound is chemically different from the original one, one would expect to see an associated change(s) in the spectroscopic characteristic of their solution. These spectroscopic changes could be probed for the specific detection and quantitative determination of the analyte. The underlying idea of these irreversible systems is to take advantage of the specificity of the reaction of a target analyte with a chosen organic functionality for achieving the desired specificity in recognition process alongside with its quantitative estimation.

Fig. 1B.1. Representation of a Chemodosimeter with two different signalling approaches.

1B.1. Synthetic receptors for Biothiols

1B.1.1. Introduction

Certain thiols play key roles in several biological processes. Among these, three mercapto biomolecules, namely cysteine (Cys), homocysteine (Hcy) and glutathione
(GSH) have similar structures (Fig. 1B.2) and are most important in this regard. Generally, alternations in the level of these cellular thiols are linked to a number of diseases, such as leucocyte loss, psoriasis, liver damage, cancer, and AIDS.\(^1\)

![Fig. 1B.2. Structure of Cysteine (Cys), Homocysteine (Hcy) and Glutathione (GSH).](image)

Specifically, Cys deficiency is involved in many syndromes, for instances, slow growth in children, hair depigmentation, edema, lethargy, liver damage, loss of muscle and fat, skin lesions, and weakness.\(^2\) At elevated level of Hcy in blood plasma enhance the risk factor for Alzheimer’s and cardiovascular disease(CVD)\(^3,4\) as well as folate and cobalamin (vitamin B12) deficiency.\(^5\) Total homocysteine (tHcy) concentration in plasma is also related to birth defects,\(^6\) cognitive impairment in the elderly,\(^7\) etc. GSH, is the most abundant intracellular nonprotein thiol\(^8\) and serves many cellular functions, which include maintenance of intracellular redox activities, xenobiotic metabolism, intracellular signal transduction, and gene regulation.\(^9\) More specifically, GSH can keep the cysteine thiol group in proteins in the reduced state and protect the cells from oxidative stress by trapping free radicals that damage DNA and RNA.\(^10\) Accordingly, the detection of these mercapto biomolecules in biological samples are important. Among the various conventional detection techniques, such as high performance liquid chromatography (HPLC), capillary electrophoresis, electro chemical methods, mass spectrometry etc. However, all these methodologies involve intricate sample preparation and expensive instrumentation techniques. These offer us an opportunity to develop simple and suitable optical methods for detection of these important thiols present in biofluids or biological samples for obvious ease in detection process for diagnostic applications. In this regard, reagents that could be used as a colorimetric as
well as fluorescence-based sensor for a specific biothiols have an obvious significance. As mentioned in the earlier chapter, colorimetric sensors are preferred for semi-quantitative binary (yes or no) detection, while receptors that work on luminescence based response offer the possibility of achieving lower detection limit (i.e. desired sensitivity), imaging application and thus, the detection of the intercellular distribution of the targeted thiol molecule.11,12

Two significant characteristic properties of thiols, such as thiols’ strong nucleophilicity and their high binding affinity towards metal ions are generally used for designing of the appropriate optical probes. Accordingly, most of the optical probes reported so far in the literature are based on the chemodosimetric approach and involve specific reactions between probe molecule and the respective thiol, which include reactions like Michael addition, cyclization with aldehyde, cleavage of sulfonamide and sulfonate ester bond by thiols, cleavage of selenium–nitrogen bond by thiols, cleavage of disulfide by thiols, metal complexes-oxidation–reduction, metal complexes-displace coordination, nano-particles and others.

1B.1.2. Colorimetric and fluorescent-based methods for detection of thiols

1B.1.2.1. Based on Michael addition

![Chemical structures](image)

Fig. 1B.3. Structure of 1 and naphthopyranose derivative (2-6).
Design of a chemodosimetric reagent for detection of thiols by using a methodology based on Michael reaction was first reported by Sippel in 1981; N-(4′-(7-diethylamino-4-methylcoumarin-3-yl)phenyl)maleimide (1) is the first examples of such a thiol probe (Fig. 1B.3).\(^{13,14}\)

Langmuir et al. extended this concept to naphthopyranone based fluorescent probes (2–6) (Fig. 1B.3).\(^{15}\) Due to the extended conjugation, naphthopyranones displayed better photophysical properties than those of coumarins. In all cases, the quantum yield of the naphthopyranone derivatives was significantly reduced due to a low lying \(n\rightarrow\pi^*\) transition associated with the maleimide ring that was present in the probe molecules. Addition reaction of the thiol molecule to the unsaturated double bond led to the formation of a saturated compound and thus, nullifying any \(n\rightarrow\pi^*\) transition, which caused the revival of the fluorescence. Authors described that probes 3 and 4 were selective for glutathione in viable Chinese hamster V79 cells.

![Fig. 1B.4. Structure of PET type thiol probe (7-9).](image)

On the other hand, de Silva et al. introduced fluorophore via a –CH\(_2\)– (Fig. 1B.4)\(^{16}\) for designing chemodosimetric probes 7–9 for thiols in order to realize a *luminescence on* responses due to an interrupted PET-based process. PET process that was initially operational involving the donor fluorophore and the acceptor alkene moiety and responsible for luminescence quenching, was interrupted on Michael addition of thiols to an electron deficient alkene group in 7–9. This resulted in the substantial increase in fluorescence quantum yield of the fluorophore. 2-Mercaptoethanol and Cys induced large fluorescence enhancements in aqueous methanol solution (1:1, pH 7.2).
Fig. 1B.5. Structure of chemodosimeter 10 and the associated change in emission colour on treatment with thiols.

Nagano et al. reported o-maleimide derivative of BODIPY (10) as a thiol-specific fluorescence probe, in which the fluorescence was significantly quenched owing to an efficient PET process (Fig. 1B.5). This appreciable quenching of the luminescence of the BODIPY derivative was not observed for analogous meta- (m) and para- (p) derivatives, which was rationalized based on the longer distance of separation between electron donor and acceptor moieties in m and p derivatives. The strong fluorescence of BODIPY was restored on reaction with thiol, such as N-acetylcysteine (NAC). This probe was even useful for detecting extremely low concentrations of protein in the gel after SDS PAGE.

Fig. 1B.6. (A) Structure of chemodosimeter 11 and and its fluorescent turn-on response towards thiols. (B) Confocal microscopic analysis of HepG2 cells treated with 11, the cells were incubated with media containing NEM at various concentrations for 1 h at 37 °C; $\lambda_{\text{Ext}} = 488$ nm.

Kim et al. reported a coumarin-based probe molecule (11) that could effectively and selectively recognize thiols (Cys, Hcy, GSH) with associated switch on fluorescence response following a Michael type reaction. This reagent showed a preference for Cys over other biologically important molecules, including Hcy and GSH. DFT calculations further suggested an interrupted ICT based process could be accounted for this observed fluorescence amplification of 11 upon its reaction with thiols. The preference for Cys over Hcy and GSH in the cellular metabolite was also confirmed.
from the results of the LC-MS spectroscopic studies. This reagent could also be used as an imaging reagent for detection of the intracellular distribution of thiols in HepG2 cell line using laser confocal microscopic studies.

Recently Yoon and co-workers developed a fluorescein-based fluorescent probe 12 for the detection of thiol-containing molecules with high specificity and sensitivity. The fluorescence enhancement and UV-Vis spectral changes were attributed to the 1,4-addition of thiols to \( \alpha,\beta \)-unsaturated ketone functionality of the probe reagent that led to the formation of the corresponding thio-ether derivative.

![Fig. 1B.7](image1.png)

**Fig. 1B.7.** A possible mechanism of the response of 12 towards to thiols in aqueous medium.

The significance of this probe reagent was demonstrated through its use for in-vivo detection of intracellular thiols in Murine P-19 cells (Fig. 1A.8). This was the first report on use of chemodosimetric reagent for monitoring of thiols in zebrafish (Fig. 1B.8).

![Fig. 1B.8](image2.png)

**Fig. 1B.8.** Phase contrast and fluorescence images for the in-vivo detection of intracellular thiols in (A) Murine P-19 and (B) 3-days old zebrafish.

## 1B.1.2.2. Based on Cyclization reaction with aldehyde

The selective reaction of aldehydes with N-terminal cysteines to form thiazolidines has been used to label and immobilize peptides and proteins. The well-known cyclization reaction of \( \beta-/\gamma \)-aminoalkylthiols (containing both SH and NH\(_2\) groups) with organic aldehydes that lead to the formation of the corresponding thiazolidine and thiazinane
derivatives has been utilized for designing appropriate chemodosimetric reagent for \( \beta / \gamma \)-aminoalkylthiols. More recently, this methodology has been used to design specific fluorescent probes for the detection of total concentration of Cys/Hcy in biofluids as well as an imaging reagent for intercellular studies, since these reagents could react with aldehyde functionality to form a 6- or 5-membered ring with 1,3- or 1,2-aminothiols, respectively, while other biothiols like GSH fail to do so.

Strongin and co-workers revealed that xanthene dye 13 could be used for efficient detection of Cys and Hcy (Fig. 1B.9) in the range of their physiological levels in aqueous medium of appropriate pH.\(^{21}\) A visually detectable change from bright yellow to brownish orange (a shift from 480 nm to 505 nm) were observed on reaction of Cys or Hcy with 13 (H\(_2\)O, pH 9.5). Electronic spectral studies ensured that [Cys] as low as 10\(^{-5}\)–10\(^{-6}\) M could be detected using this reagent. This could also be probed by monitoring the fluorescence quenching at 510 nm. Control studies also revealed that presence of excess GSH failed to interfere in the quantitative detection of Cys or Hcy in commercial deproteinized sample of human blood plasma. A time delay of 5 min was used for all spectroscopic measurements for ensuring the completion of the reaction between Cys/Hcy and 13.

![Fig. 1B.9. Structures of probe 13-15. Reaction mechanism and naked eye colour variation of 13 with thiols (Cys:B, and Hcy:C) and other amino acids.](image-url)
Strongin et al. reported selective colorimetric detections of Cys/Hys using a commercially available xanthene dye 14 and an unsaturated aldehyde 15 (Fig. 1B.9).\textsuperscript{22} More efficient fluorescence quenching for 13 and 14 was observed on preferential reaction with Cys than that for Hcy, while a significantly high selectivity towards Cys was observed for 15 due to the formation of the more stable 5-membered rings ((Fig. 1B.9). An associated change in solution color change from yellow ($\lambda_{\text{max}} = 400$ nm) to colorless was observed in 10 min after addition of Cys to a solution of 15 (carbonate buffer, pH 9.5). For Hcy, no such change in solution color of 15 was observed under identical condition. This offered the possibility of using 15 could in combination with 13 for detecting Cys at 400 nm and total concentration of Cys/Hcy at 500 nm, as reagent 15 reacted with Cys, leaving Hcy free to react with the reagent 13, which was added subsequently. This resulted a fluorescence on response only for Hcy and this helped in evaluating the [Hcy] from [Hcy + Cys] – [Cys]. This method could even be used for relatively more complex native plasma solutions over a range of healthy (6 $\mu$M) to dangerous levels (1.3 x 10$^{-4}$ M) levels without any interference from the excess Cys.

Fig. 1B.10. Structure and Reaction mechanism of 16 with Cys

Tanaka and Barbas III et al. reported a fluorescence growth method for Cys detection employing fluorogenic aldehyde 16 at neutral pH (Fig. 1B.10).\textsuperscript{23} In aq. phosphate buffer (pH 7), the reaction of 16 with Cys could be probed by monitoring the substantial increase in fluorescent at 380 nm, while similar process for glutathione registered only a small increase. The [Cys] in the range of 100 $\mu$M to 5 mM was well correlated with the fluorescence enhancements that were recorded after a time delay of 30 min.
Huang and co-workers reported another example of aldehyde based sensor using an Ir(III)-complex 17 (Fig. 1B.11). This complex displayed unique luminescent changes for Hcy over other amino acids (including Cys) and GSH. Upon addition of increasing concentration of Hcy, solution color of 17 in DMSO-aq. HEPES buffer (9:1 v/v, pH 7.2) medium changed from orange to yellow led to the gradual decrease in absorption band at 510 nm. On the other hand upon addition Hcy, a blue-shift of approximately 90 nm of emission maxima with an associated change in emission color from deep red (λ_{Ems, Max} = 615 nm) to green (λ_{Ems, Max} = 525 nm) with substantial enhancement in the emission quantum yield. Both surface charge analysis and the electrochemical measurement indicated that a photoinduced electron-transfer-process could be only operational for the 17-Cys adduct and might be responsible for the observed specificity of luminescence enhancement toward Hcy over Cys.

Huang et al. extended their research work to develop a platinum(II)-complex (18) as a highly selective phosphorescent chemodosimeter for Cys and Hcy (Fig. 1B.12). Upon addition of Hcy or Cys a change in luminescence color from green (λ_{Ems, Max}=510 nm) to orange (λ_{Ems, Max}=555 nm) for its acetonitrile–water solution (4:1, v/v; pH 7.2) was
observed. This red-shift was attributed to thiazinane formation, which was confirmed by 
$^1$H NMR investigation. This red shift was also substantiated by DFT calculations.

**Fig. 1B.13.** Reaction mechanism of 19 with Cys and Hcy.

Lin et al. reported a ratiometric fluorescent probe 19 for Cys and Hcy (Fig. 1B.13).\(^2\) In 
19, the ICT process was operational between electron rich phenanthroimidazole moiety as donor and the aldehyde group as an acceptor. Upon addition of Cys or Hcy in aq. 
HEPES buffer-DMF (v/v, 1:3; pH 7.4), an appreciable hypsochromic shift of 125 nm in solution emission was observed; a gradual bleach in the ICT-based emission band 
intensity at 519 nm with concomitant growth of a new locally excited (LE) band intensity at 394 nm was detected. A good linear fit for the plot of the 
Intensity$_{394\text{nm}}$/Intensity$_{519\text{nm}}$ as a function of the [Cys], while the lower detection limit of 0.8 mM, which was within the physiological levels of Cys (2.4 – 3.6 x 10^-4 M).

**1B.1.2.3. Based on cleavage of sulfonamide and sulfonate ester**

**Fig. 1B.14.** (a) Structures of receptor molecules 20 and 21 and their reactions with thiols, and 
discrimination of selenols and thiols using probe 21.

Maeda et al. utilized nucleophilic aromatic substitution for designing of thiol probes (20 
and 21) as shown in Fig. 1B.14.\(^2\) Selective and large fluorescent enhancements were 
observed upon the addition of GSH or Cys in aq. HEPES buffer (10 mm, pH 7.4) 
containing 0.5% EtOH. The fluorescent intensities were stabilized after 10 mins and the
rate constants $K_{\text{obsd}}$ for reaction of 20 or 21 with GSH at pH 7.4 and 37°C were reported as $1.7 \times 10^2$ and $1.4 \times 10^2$ M$^{-1}$ s$^{-1}$, respectively. 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB or Ellman's reagent) has been used widely for the quantification of thiols, especially for assays of various enzymes including acetyl- and butyryl-cholinesterase (AChE and BChE, respectively) in which substrates release thiols through enzymatic reactions. Authors successfully demonstrated that probes 20 and 21 could be used as reliable fluorogenic chemodosimetric probe for measuring ChE inhibitory activities. Reagent 21 could also discriminate selenols from thiols by simply changing the media pH.$^{28}$ This selectivity could be achieved as the pKa(SeH) value of SeCys is 5.2, which is much lower than the pKa(SH) value of 8.3 compared to its thiol counterpart Cys. This was further favored by the higher nucleophilicity of selenols than thiols (Fig. 1B.14). A linear calibration curve for SeCys was obtained over the range 1–1000 pmol/well with lower detection limit of 0.8 pmol when a microtiter plate assay was performed. They also reported that probe 20 can be a useful tool for the identification and measurement of unknown as well as known selenoproteins, which was proved by determination of the SeCys content in selenoproteins (SePs) glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) by fluorometric assay with probe 21.

![Fig. 1B.15. The reaction mechanism of fluorescent probe 22 and Fluorescence images of 3T3 cells: (A) bright field image of cells incubated with probe 22 (25 μM) for 10 min at 37 °C, and stained with a nucleus-staining dye, Hoechst 33342; (B) fluorescence image collected with a Hoechst dye filter set; (C) fluorescence image collected with a Cy3 dye filter set.](image)

Hilderbrand et al. reported fluorescent turn-on probe (22) for the selective sensing and bioimaging of thiols (Fig. 1B.15).$^{29}$ In aq. HEPES buffer solution (10 mM, pH 7.4), thiols
cleaved the 2,4-dinitrobenzenesulfonyl group to release the red-emissive donor–acceptor fluorophore (Fig. 1B.15). Thiol-mediated cleavage of the electron-withdrawing sulfonyl group yielded an aniline donor, which enhanced the push-pull character of the dye. This resulted in improved quantum yield and large bathochromic shifts (158 nm) in the absorption and emission spectrum. Upon the addition of Cys, the solution instantly turned from yellow to pink, and fluorescence was turned on with high on/off ratios (up to 120-fold). Use of the probe 22 for the bioimaging of intracellular thiols in live cells was also demonstrated by using albino Swiss mouse embryo fibroblast (3T3) cell line.

Zhao et al. recently demonstrated how Off-On fluorescent thiol probes 23 and 24 with 2,4-dinitrobenzenesulfonyl protected ethylnylpyrene fluorophore can be designed based on DFT/TDDFT calculations (Fig. 1B.16). Theoretical calculations predicted dark state (S1) for probes 23 and 24, which was attributed to an efficient ICT-based process involving ethynylated pyrene fluorophore as donor and 2,4-dinitrobenzenesulfonyl unit as electron acceptor. Cleavage of the 2,4-dinitrobenzenesulfonyl unit by thiol could release the free fluorophore having an emissive lowest-lying excited state S1. These theoretical predictions agreed well with the photophysical properties of the molecular probes in MeOH–H2O (4/1, v/v). Upon addition of Cys, the fluorescence intensity of 24 was enhanced by 53 folds with $\lambda_{\text{Ems Max}} = 560$ nm. This reagent could be even be used...
for monitoring of thiols in living pheochromocytoma cells (PC12 cells) and onion epidermal cells, while green fluorescence of the cleaved-probe 24 was evident.

**1B.1.2.4. Based on cleavage of Se–N by thiols**

Selective cleavage of selenium–nitrogen bond by thiols was adopted for the design of thiol probes by Tang et al.\textsuperscript{31} Rhodamine-based fluorescent probe (25) having a Se–N bond displayed fluorescent enhancement on reaction with GSH in PBS (pH 7.4, 20 mM) media, which was induced by nucleophilic substitution of sulfhydryl (Fig. 1B.17).\textsuperscript{31}

![Image](https://via.placeholder.com/150)

**Fig. 1B.17.** (A) Reaction associated with the chemodosimetric response of 25 towards thiol; (B) Confocal fluorescence images of both living HL-7702 cells incubated with probe 25 (0.50 \( \mu \text{M} \)) (a) 15 min and (b) 30 min; HepG2 cells incubated with 25 (0.50 \( \mu \text{M} \)) for (d) 15 min, (e) 30 min; (c) and (f) represent the bright-field images of (b) and (e).

The limit of detection of this probe for GSH was reported as 1.4 nM. Probe 25 also could detect protein thiols, including thioredoxin, glutathione reductase, and metallothionein with an efficiency of about 2-3-fold higher than that of GSH. The new probe was successfully used for imaging of thiols in both HL-7702 cells and HepG2 cells with high sensitivity and selectivity.

![Image](https://via.placeholder.com/150)

**Fig. 1B.18.** Reaction mechanism of the probe 26 with thiol with associated change in solution color of probe 26 (15 \( \mu \text{M} \)) in absence and presence of thiols (60 \( \mu \text{M} \)).

Same group extended Se–N bond cleavage by thiols to probe 26 (Fig. 1B.18).\textsuperscript{32} For GSH, chemodosimetric reagent 26 showed a high signal-to-noise ratio (170-fold) in
PBS media (pH 7.40, 15 mM) with detection limit of 144 pM with response time ~ 5 min. Imaging of intracellular thiols was demonstrated using HL-7702 and HepG2 cells.

1B.1.2.5. Based on cleavage of disulfide by thiols

5,5′-dithiobis(2-nitrobenzoic acid) (DTNB or Ellman’s reagent) has been the most popular reagent for the quantitation of sulfhydryl contents as well as covalent modification of thiol groups on proteins. Pei et al. demonstrated that 5-(2-aminoethyl)dithio-2-nitrobenzoate (ADNB, 27) reacted with free thiols following similar kinetic pathway as those of Ellman’s reagent. However, a dramatically improved stability under alkaline conditions (Fig. 1B.19) was achieved.33 These results showed that 27 could be an excellent alternative for Ellman’s reagent for the quantitation of thiol contents and developing of enzymatic assays under basic pH conditions.

Fig. 1B.19. Reaction mechanism of the probe 27 with thiolates.

Probe 28 was designed having fluorescein as donor and rhodamine as acceptor components in the same molecule (Fig. 1B.20).34 Rhodamine and fluorescein are fluorescent at low pH (<6.0) and at high pH (>6.0), respectively, and thus, these two reagents in combination give fluorescence response over a wide pH range.

Fig. 1B.20. Structure and schematic representation of recognition process of the reagent 28.

FRET based response at pH 5.0 indicated that there was an opening of the cyclic lactam form of the rhodamine ring happened and that allowed the ET from the fluorescein moiety to the ring-opened rhodamine as acceptor. Lack of FRET at pH 7.3 indicated that the rhodamine moiety remained in non-luminescence spirolactam form.
The decrease in fluorescein-based fluorescence with change in pH from 8.2 to pH 7.3 indicated that the fluorescein was approximately 50% in the neutral and cyclized form at pH 7.3. In presence of thiol, reduction of the dithiol yielded an increase in fluorescein-based fluorescence due to removal of quencher rhodamine fragment. In Escherichia coli, decreased thiol levels were detected in cells that were deficient in glutathione synthesis. In zebrafish embryos, the DSSA reagent permitted detection of unusually high thiol levels in the zebrafish chorion.

Recently Lin and co-workers reported a new ratiometric FRET-based molecular probe, 29 having a tetrakis(4-hydroxyphenyl) porphyrin and coumarin moieties in the scaffold and acted as a fluorescent probe for thiols. This reagent was highly selective and sensitive towards thiols (Fig. 1B.21). More importantly, this novel ratiometric probe exhibited a remarkable change in emission color from red to blue with a 60-fold enhancement in the emission ratio (I459/I658) in presence of thiols. This key feature allowed 29 to be used for thiol detection by simple visual detection. Furthermore, studies revealed that 29 was cell membrane permeable and was suitable for ratiometric fluorescence imaging of intracellular thiols in living HeLa cells.

![Fig. 1B.21. Structure and schematic representation of ratiometric response of molecular probe 29 in presence of thiols.](image)

Chmielewski et al. synthesized a new rhodamine derivative (30) having a disulfide unit, which displayed large fluorescent enhancement upon reduction by cellular thiols such as GSH in vitro (Tris-HCl buffer, 80 mM, pH 8.0 at 37°C) and in cyto (Fig. 1B.22). The
reduction of disulfide bonds by intracellular GSH took place as nucleophilic sulfhydryl groups caused the cleavage of the neighboring carbamate bonds, thereby unmasking the rhodamine 110. In addition, probe 30 was also demonstrated to respond to changing levels of intracellular GSH in live HeLa cell.

**Fig. 1B.22.** Structure and mechanism of unmasking for the rhodamine moiety in the molecular probe 30 on interaction with thiols.

1B.1.2.6. **Metal complex related: oxidation–reduction involved thiols**

A simple, sensitive and selective chemiluminescence (CL) method was developed for the determination of Cys by Ma et al. This method is based on the generation of CL Ce(III)*, produced by the reaction of Cys and Ce(IV).\(^{37}\) In the presence of quinine, Ce(III)* could transfer the energy to quinine, producing excited quinine, since the emission spectrum of Ce(III) and the absorption spectrum of quinine had an overlap in the wavelength range of 300-400 nm (Fig. 1B.23). Quinine has an \(\lambda_{\text{EmsMax}}\) of about 450 nm and has a high luminescence quantum yield. The luminescence response of quinine was linear over the [Cys] range of 3.5 nM to 3.5 mM with a detection limit of 2.5 nM. Due to high sensitivity, this method could determine the total concentration of Cys in human serum through simply diluting the sample for a thousand fold. The obtained result was in agreement with that given by amino acid autoanalyzer.

**Fig. 1B.23.** Mechanism of CL of Cys and GSH in the presence of cerium(IV) and quinine.
The same group reported that this method could also be applied to the selective determination of GSH in the blood sample of rabbit in the presence of Cys, since GSH exhibited a higher CL response than Cys in the Ce(IV)–quinine system, and the concentration of GSH in whole blood is much higher than that of Cys.³⁸

Rezaei and co-worker developed a similar chemiluminescence system with Ru(phen)$_3^{2+}$ instead of quinine.³⁹ The proposed method was successfully applied for the flow injection determination of Cys in the real samples with minimum sampling rate of 90 sample/h.

**1B.1.2.7. Metal complex related: displacement of coordination by thiols**

A few displacement approaches utilizing high binding affinities of thiols with metal ions have been reported. Lam et al. reported the synthesis and characterization of a neutral trinuclear heterobimetallic cyano-bridged Ru(II)/Pt(II) complex, cis- Ru(phen)$_2$-[CN–Pt(DMSO)Cl$_2$]$_2$ (phen = 1,10-phenanthroline) (31), as a chemodosimetric ensemble for thiols, such as Cys, Hcy, methionine (Met) and GSH (Fig. 1B.24).⁴⁰ $^{3}$MLCT emission of cis-[Ru(phen)$_2$(CN)$_2$] was quenched upon coordination of the cyano moieties by the electron-accepting Pt(DMSO)Cl$_2$ moieties. Higher affinity of the Pt(II) centers towards Cys, Hcy, methionine (Met), and GSH in aqueous DMF media at pH 7 caused the cleavage of the cyano bridge and the restoration of the characteristic orange–red $^{3}$MLCT luminescence of the Ru(II)-diimine chromophore.

**Fig. 1B.24.** Mechanism of displacement approach of 31 with thiols.
Kim et al., also reported a displacement approach in which the ensemble (32) \([\text{Cd}_2(\text{TPXD}))^{4+}\) served as the receptor for Cys (Fig. 1B.25), while pyrocatechol violet was used as the indicator in sensing Cys in aq. buffer (HEPES, 10 mM; pH 7.0).\(^{41}\) The decrease of the UV-visible absorbance at 665 nm or visually detectable color change from blue to yellow was observed upon addition of an aqueous solution of Cys. The association constant for the binding of Cys to the receptor was reported as \(1.62 \times 10^7 \text{M}^{-1}\). Hcy lowered the absorbance intensity at 665 nm but in a much less extent (about 30% of the decrease caused by Cys).

**Fig. 1B.25.** The schematic representation of the rationale used for designing a Cys selective chemosensor 32.

Recently, CdTe quantum dots (QDs)-\(\text{Hg}^{2+}\) system for the detection of thiols was reported by Wang et al. (Fig. 1B.26).\(^{42}\) Fluorescence of QDs was found to be quenched efficiently by \(\text{Hg}^{2+}\).

**Fig. 1B.26.** Mechanism of fluorescent changes of CdTe quantumdots(QDs)-\(\text{Hg}^{2+}\) system with thiols.

In the presence of biothiols, such as GSH, Hcy and Cys, the fluorescence of CdTe QDs was recovered since \(\text{Hg}^{2+}\) preferred to react with them to form the \(\text{Hg}^{2+}-\text{S}\) bond because of the strong affinity with the thiols. The restoration ability followed the order GSH > Hcy > Cys due to the decreased steric hindrance effect. A good linear
relationship was obtained from 0.6 to 20.0 \( \mu \text{M} \) for GSH and from 2.0 to 20.0 \( \mu \text{M} \) for Cys, respectively. The detection limits of GSH and Cys were 0.1 and 0.6 mM, respectively. Furthermore, this method was successfully applied to detect biothiols in the Hela cell.
1B.2. References


Aim and outline of the thesis

1. Design and development of newly synthesized molecular probes for the selective recognition of biologically and environmentally important analytes through reversible binding phenomena is briefly described in Chapter 1A. Chapter 1B basically describes a brief literature survey on examples of chemodosimetric approaches, which involve the use of different chemical reactions (usually irreversible) for the recognition and quantitative detection of biothiols such as Glutathione, Cysteine and Homocysteine in physiological conditions.

2. Chapter 2 provides the Design and synthesis of tailored made unique molecular receptor for specific recognition of Nd$^{3+}$ among all others lanthanides and Hg$^{2+}$, Cr$^{3+}$ among all other common transition , alkali and alkaline earth metal ions in non-aqueous conditions. The binding mode and stoichiometry of this molecular receptor with these recognized metal ions was established by detailed spectroscopic analysis. The possibility of using this host reagent for the development of an easy-to-use paper test strip for the semi-quantitative colorimetric detection of Hg$^{2+}$ and Cr$^{3+}$ present in neutral aqueous media has been discussed.

3. Chapter 3 consist of receptors design with diverse signaling units and active acidic methylene hydrogens was found to act as an efficient binding motif for F$^-$. The binding process through C–H⋯F$^-$ hydrogen bond formation and stoichiometric assembly was probed by monitoring the changes in their spectral properties. The relative affinities of various anions and the preferential binding of F$^-$ to these reagents are also rationalized using computational studies. More importantly the methodology for selective and quantitative extraction, as well as visual detection of an ultra trace quantity of inorganic F$^-$ present in water by using this artificial receptor has been discussed.
4. In chapter 4, a completely different approach of metal-phosphate coordination has been conceived in order to overcome this deleterious hydration energy and to design a specific fluorogenic sensor for biologically imperative phosphate ions such as Pyrophosphate (PPI), Adenosine monophosphate (AMP) and Adenosine diphosphate (ADP) in physiological environment is discussed using appropriate Zn(II) and Cd(II) based probes. In order to expand the bio-analytical potential of these chemosensors, both "turn-on" and "turn-off" real-time assay, for the evaluation of the enzymatic activity of alkaline phosphatase (ALP) has been discussed.

5. Finally in the last chapter 5, design and development of new molecular probes for dual channel detection of biothiols such as Glutathione, Cysteine and Homocysteine in physiological conditions following different chemodosimetric approach has been discussed. Possibility of using these reagents for quantitative detection of Cysteine present in blood plasma by a modified column switching HPLC technique as well as the in-vivo imaging reagents for the detection of intracellular thiols in living HeLa cells was also discussed, which may have special significance in medicinal biology and diagnostic applications.