Chapter One

Introduction.
1. Introduction- An overview

Metals, anions and amino acids are essential for life and play important roles in biological and environmental processes. All life forms have a necessity for metals as they play prominent roles in fundamental processes, including osmotic regulation, catalysis, metabolism, biomineralization, and signalling. Metals like Na\(^+\), K\(^+\) and Mg\(^{2+}\) control metabolic reactions via signalling by means of their flux through cell membranes and other boundary layers. Calcium is mainly involved in structural integrity of large molecules as well as bones and teeth. Transition metals that are generally recognized as playing critical roles in biology include iron, zinc, copper, manganese, cobalt, nickel, molybdenum, tungsten, chromium, vanadium. These are essential for maintenance of human metabolic processes such as oxygen transport, regulating cell activity. Copper is a cofactor for many enzymes, participates in critical biological processes, such as respiration, antioxidant defense, and iron metabolism. However, free copper ions are toxic since they catalyze the generation of highly reactive hydroxyl radicals which cause cellular damage. Disorders of copper metabolism process cause neurodegenerative diseases, Alzheimer’s disease, Wilson’s disease and Menkes syndrome. Iron is the most abundant transition metal in the body and performs many important biological processes such as respiration, reproduction and mammalian cellular growth. Increase in brain iron concentration which accompanies aging, along with disruption in homeostasis can create a severe imbalance in the amount of labile iron. Ferric iron in particular is known to react with hydrogen peroxide to produce hydroxyl radical. This reactive oxygen species has been suspected to be a key player in the pathogenesis of neurodegenerative diseases. Zinc ion is the second most abundant metal ion and it works with the enzymes that make genetic material, manufacture heme, digest food, metabolize carbohydrate, protein and fat, liberate vitamin A from storage in the liver and dispose of...
damaging free radicals. Its overload induces apoptosis, epilepsy, ischemia and Alzheimer’s disease while its deficiency in the body leads to several malfunctions like growth retardation, diarrhoea, impotence and delayed sexual maturation\textsuperscript{11}. It is obvious that homeostasis of these ions is required in human body.

On the other hand heavy metal like Hg\textsuperscript{2+}, if ingested into the body through food, could cause a variety of symptoms in vivo, including digestive, cardiac, kidney, and neurological diseases\textsuperscript{12}. Ag\textsuperscript{+} ions are well-known because of their antimicrobial activities and various applications in electronic, photographic, and imaging industries\textsuperscript{13}. Excessive exposure of cadmium cause serious diseases such as renal dysfunctions and calcium metabolic disorders, and pulmonary, prostatic, and renal cancer\textsuperscript{14}. Similar to essential metals, anions like fluoride, acetate and phosphate attract great attention since they play crucial role as co-factors and enzyme substrates in bio systems. Apart from these anion channels are known to be involved in cell migration, cell proliferation in chemical and biological processes. Notably, fluorides are routinely used for the prevention of dental cavities and the treatment for osteoporosis\textsuperscript{15}. In several countries fluoride as the main additive in drinking water prevents the bone and dental problems. However, high levels of fluoride in drinking water can give rise to a number of adverse effects such as fluorosis\textsuperscript{16}. Cyanide is also highly toxic and its absorption in the lungs, gastrointestinal track, and skin may cause severe damage to the human body. It has affinity with active site of cytochrome a\textsubscript{3} that may cause cell death by inhibiting the cellular respiration\textsuperscript{17}.

Amino acids are the building block of proteins and serves as key factor in enzyme reactions\textsuperscript{18}. Biological thiol-containing amino acids play crucial roles in the cellular antioxidant defense system. Specifically, the level of cysteine and homocysteine have been linked to many diseases, such as Alzheimer’s, AIDS and cancers\textsuperscript{19}. Both essential metal ions, anions and
aminoacids are essential to life, while the higher concentration of these is extremely toxic. Therefore a great deal of attention has been devoted to the detection of guest metal/anions and neutral species.

A variety of analytical methods are available for the detection of these analytes\(^\text{20}\). Although these methods offer good limits of detection and wide linear ranges they require high cost analytical instruments. The necessary collection, transportation, and pre-treatment of a sample are also time consuming process. Among the different methods used in chemical sensing applications, fluorescence based techniques are well suited to investigate the fundamental processes in life sciences due to broad versatility, low cost and simple instrumentation\(^\text{21}\). Further fluorescent sensors have been demonstrated to be powerful tools for the non-invasive visualization of intracellular detection in single cells.

1.2 Optical chemosensors

Molecular recognition and sensing of biologically important species is surging in recent years and different protocols have been widely applied to the design of probes for anions, cations and neutral molecules\(^\text{22}\). Chemosensors are systems that rely on the use of coordinative forces for analyte binding. A chemosensor is defined as “a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal” \(^\text{23}\). Several methods have been developed to analyse the guest species.

An optical chemical sensor involves the interaction between guest species and a recognition unit that is signalled by an easily detectable change\(^\text{24}\). Generally chemosensor depends on a binding event or a chemical reaction between guest and recognition unit to change the absorption/fluorescence characteristics of an appended receptor\(^\text{25}\). Other chemosensor changes redox potential\(^\text{26}\) or conformational change\(^\text{27}\) for signalling presence of analyte. Many
chemosensor have been synthesised based on this concept for detection of analytes.

1.3 Fluorescence – a phenomenon

When a molecule is electronically excited, one of its electrons is promoted to a higher energy level: the molecule is considered to be in an electronically excited state. The excited state ultimately will relax back to the ground state via three ways i) Photochemical reaction ii) Non-radiative decay, iii) Radiative decay. The Jablonski diagram sketches a more detailed description of the above processes that can occur in the excited state. $S_0$ is the ground state, $S_1$ and $S_2$ are the excited states where $S$ denotes for singlet state; while the first triplet excited state is $T_1$. Each electronic state is characterized by different vibrational energy levels. According to the Franck-Condon principle, electronic transition between states are vertical. The fluorophore is excited to a vibrationally excited level of an excited electronic state—for simple organic dyes, usually $S_1$ or $S_2$ and it rapidly transfers this excess vibrational energy to the surrounding solvent molecules through vibrational relaxation. The system will revert from $S_2$ to $S_1$ via internal conversion quickly, i.e. a non-radiative transition between two electronic states of the same spin multiplicity, followed by vibrational relaxation and this process is very fast. From $S_1$, the excited molecule can relax to the ground state through an internal conversion/vibrational relaxation pathway or through the emission of a photon, defined as fluorescence, i.e. a radiative transition between states of the same multiplicity. Fluorescence time scale is generally in the range $10^{-9}$-$10^{-6}$ s; for this reason, fluorescence takes place only from the lowest vibrational level of $S_1$ (known also as Kasha’s rule). Photons emitted by fluorescence are typically at higher wavelengths than the photons absorbed, because of the energy lost during internal conversion/vibrational relaxation. An additional pathway is represented by intersystem crossing, i.e., a non-radiative transition between two iso energetic vibrational levels of electronic states with different
multiplicity. As in the case of internal conversion, intersystem crossing is quickly followed by vibrational relaxation, leaving the excited molecule in the lowest vibrational level of T₁. Emission from T₁ is called phosphorescence and is generally shifted to longer wavelengths with respect to fluorescence; the time scale for this transition is in the range 10⁻²-10² S, because it is spin forbidden as well as intersystem crossing. In spite of its long lifetime it is difficult to observe this transition in solution at room temperature (Fig. 1.1).

Fig. 1.1 Jablonski Diagram

1.4 Fluorescent chemosensors

The design of fluorescent chemosensors for biologically/environmentally relevant chemical species (cation/anion and neutral species) has important impacts in many applications, and for this reason it has been the subject of active research in sensor field. As far as fluorescent chemosensors is concerned, molecules that change their fluorescence property i.e. intensity or emissive colour in response to analyte bindings offer an extremely sensitive optical method for the real-time monitoring of molecular interactions. Such chemosensors are finding increased use as diverse as...
biology, medical diagnostics, and environmental monitoring. Designing of fluorescent sensors for biological applications has even become a wider field of research with materials ranging from the molecular level to the micro and nano scale\textsuperscript{31}. Basic requirements for the fluorescent sensors are stability, solubility, high response time, brightness, selectivity and sensitivity towards the target analyte. For biological applications, other issues of crucial importance such as the biocompatibility of the sensor water solubility and quantitative determination of the target analyte in real time have to be taken into account. Further the fluorescence measurement involving either excitation or emission wavelength can be influenced/interfered by many factors, such as the localization of the probe, changes of environment around the molecule (pH, polarity, temperature, and so forth), emission collection efficiency, effective cell thickness in the optical beam and changes in the excitation intensity. To avoid these factors, ratiometric measurement is utilized. In other words, simultaneous recording of the fluorescence intensities at two wavelengths and calculation of their ratio is suitable for cellular imaging but are difficult to design\textsuperscript{32}. While many fluorescent sensors are based on macro molecules, small molecule-based fluorescent chemosensors are superior in that they are stable, easier to be tuned in changing emission wavelength and also provide faster rates of labelling. Thus, such fluorescent chemosensors are indispensable tools in biology.

The two main strategies used in the design of fluorescent and colorimetric chemosensors for guest detection are molecular recognition and signal transduction. The chemosensor contains three parts viz., i) recognition unit, ii) signalling moiety, iii) spacer that is attached in between the recognition unit and signalling unit at times. When the analyte binds to the recognition unit the signalling unit changes either the colour or its fluorescence behaviour. The displacement approach is based on the release of the signalling unit into the solution with a concomitant change in their optical properties from molecular
assemblies of binding site and signalling unit, upon interaction of analyte with the recognition site. In the chemodosimeter approach a specific analyte-induced chemical reaction occur which results in a fluorescent signal (Fig. 1. 2).

**Fig. 1. 2 Binding site-signalling unit approach**

**1. 5 Fluorescence signalling mechanisms**

In the design of new fluorescent chemosensors, exploration of sensing mechanisms between recognition and signal reporting units is of continuing interest. Based on different photophysical process, conventional sensing mechanisms such as Photo-induced electron transfer (PET), Intramolecular charge transfer (ICT), Fluorescence resonance energy transfer (FRET), Twisted intramolecular charge transfer (TICT) and Monomer-excimer/exciplex formation have been invoked. In addition, Aggregation-induced emission (AIE), Excited-state intramolecular proton transfer and C=N isomerization mechanism can be ascribed to fluorescence changes via conformational restriction.
1.5.1 Photo induced electron Transfer

The most common class of fluorescent sensors for guest species are based on Photoinduced electron transfer mechanism (PET). PET sensor can be classified into two types i) turn-on, ii) turn-off. In the absence of analyte, electron in the highest occupied molecular orbital (HOMO) of fluorophore is promoted to the lowest unoccupied molecular orbital (LUMO), which facilitates PET from the HOMO of free receptor to HOMO of fluorophore causing fluorescence quenching. Upon binding of the analyte the redox potential of the donor is raised thereby the relevant HOMO becomes lower in energy than that of fluorophore; this decreases the driving force of PET process effectively thus preventing the quenching effect to turn-on the fluorescence of the chromophore (Fig. 1.3).

![Figure 1.3: Oxidative PET](image)

In some cases the receptor takes part indirectly in the photophysical process. If the LUMO level of the analyte bound receptor is between the energy levels HOMO and LUMO of the fluorophore the binding of the analyte by the receptor provides a non-radiative pathway to dissipate the excitation energy, resulting in quenching of the fluorescence. The difference between two mechanisms is that the PET process takes place either before or after the analyte binding. In turn-on sensor the PET process is participated by the HOMO and LUMO of the fluorophore, and HOMO of the receptor before
analyte binding. In turn-off sensor, the PET process involves the HOMO and LUMO of the fluorophore, and the LUMO of the analyte after complex formation (Fig. 1.4).

**Fig. 1.4** Reductive PET

Qian *et al.* and co-workers developed naphthalimide based fluorescent probe towards Ag\(^{+}\) in aqueous solution\(^{34}\). The probe showed a weak fluorescence in aqueous solution due to the efficient Photoinduced electron transfer process from the electron rich receptor to the excited fluorophore (Scheme 1.1). Upon addition of Ag\(^{+}\) significant enhancement of fluorescence at 553 nm was observed.

**Scheme 1.1** Proposed binding mode of naphthalimide probe with Ag\(^{+}\)

Duan *et al.* have developed a Cu\(^{2+}\) specific “turn-on” fluorescence sensor using the incorporation of coumarin fluorophores with benzylidihydrazone moiety\(^{35}\). Due to its excellent sensitivity, high selectivity, good water solubility and favourable spectroscopic properties (Scheme 1.2). It acts as an efficient sensing probe for the detection of Cu\(^{2+}\) in living cells.
Chellappa et al. and co-workers have designed, synthesised a new pyrene based turn-on fluorescent chemosensor 1 for Hg$^{2+}$ in aqueous CH$_3$CN. Photoinduced electron transfer from imine nitrogen to the pyrene fluorophore and C=N isomerisation quenches the fluorescence of probe. After chelation with Hg$^{2+}$ ion PET and C=N isomerisation are inhibited and enhances the fluorescence.

**Scheme 1. 2** Proposed binding mode of Cu$^{2+}$.

1.5.2 Photo induced internal charge transfer

When a fluorophore contains an electron-donating (often an amino group) conjugated to an electron-withdrawing group, it undergoes intramolecular charge transfer (ICT) from the donor to acceptor upon excitation by light. Normally ICT mechanism displays large stoke shift and ratiometric response. When a receptor interacts with a cation, the electron donating character of the receptor is reduced; blue shift of the emission
spectrum is expected. In the same direction, if a cation plays the role of an electron receptor the interaction between the receptor and the cation would further strengthen the push-pull effect. So red shift in emission would be observed. In this process the fluorophore undergoes a transition from Local excited state to charge transfer state (Fig. 1.5). The ICT results normal shift the emission.

![Fig. 1.5 Principle of ICT process](image)

Qian and co-workers have developed a selective ratiometric fluorescence sensor 2 for Cu$^{2+}$ ion based on naphthalimide$^{38}$. In the presence of Cu$^{2+}$ ion fluorescence emission centred at 525 nm decreased with concomitant increase in fluorescence emission at 475 nm based on the internal charge transfer mechanism.
Pitchumani et al. and his co-workers have reported 4-methoxy-N-((thiophen-2-yl) methyl)benzenamine as a highly selective chemosensor for Ag$^+$ ion, a strong fluorescence enhancement is observed which is attributed to an increase in intramolecular charge transfer$^{39}$ (Scheme 1.3).

![Scheme 1.3 Charge transfer mechanism of silver ion detection](image)

Chellappa et al. and his coworkers reported 6-imidazo-2-yl-5, 6-dihydro-benzo-(4, 5)imidazo(1, 2-c)-quinazoline 3 as selective sensor for Al$^{3+}$ in aqueous DMSO solution$^{40}$. Addition of Al$^{3+}$ induces ratiometric behaviour based on charge transfer mechanism.

![3](image)

### 1.5.3 Twisted intra molecular Charge Transfer (TICT)

A twisted intramolecular charge transfer TICT model proposed by Grabowski has been widely accepted$^{41}$. Usually the electron donor and acceptor groups are linked in the ground state and undergoes significant charge transfer in the excited state. In addition, TICT is a strong intramolecular CT occurring in the excited state that involves solvent relaxation around the molecule to yield a continuing rotation of electron donor and acceptor until it is twisted about 90°C$^{42}$. The local excited state and TICT are in equilibrium and both LE and TICT bands are observed with the latter being observed normally.
in longer wavelength region (Fig. 1.6). Meanwhile the rotation and charge separation of the TICT state mainly depends on polarity, temperature and steric hindrance of the molecule. In non-polar solvents only one fluorescence band appears because of the locally excited state. Changing the solvent nature from non-polar to polar solvents facilitates the charge transfer state band.

**Fig. 1.6** Energy diagram of TICT formation

Tharmaraj *et al.* reported an efficient fluorescent chemosensor for Hg$^{2+}$ based on 5-(dimethylamino)-N-(2-mercaptophenyl) naphthalene-1-sulfonamide$^{43}$. Addition of Hg$^{2+}$ exhibits selective fluorescent quenching behaviour through twisted electron transfer mechanism, which is confirmed by TD-DFT calculations (Scheme 1.4).

**Scheme 1.4** TICT sensing mechanism of Hg$^{2+}$ sensor.
Liu et al. developed novel chemosensor comprising biarylpypyridine and crown ether moieties\textsuperscript{44} for detection of Mg\textsuperscript{2+}. In the absence of acid, no selective changes were observed upon addition of all metal ions. Addition of acid quenches the fluorescence behaviour, and the subsequent addition of metal ions causes a fluorescence enhancement only for Mg\textsuperscript{2+} ion (Scheme 1. 5). The ON-OFF-ON fluorescence response indicates that the conversion to the TICT state plays prominent role in sensing.

\textbf{Scheme 1. 5} Schematic representation of the proton-triggered ON-OFF and the OFF-ON fluorescence response induced by Mg\textsuperscript{2+}.

Li et al. reported a new near-IR “turn-on” fluorescent chemosensor 4 for Hg\textsuperscript{2+} ion based on naphthalimide bearing dipcolyl amine group. The presence of Hg\textsuperscript{2+} enhances the fluorescence at 660 nm based on TICT mechanism. The receptor can be used to image intracellular Hg\textsuperscript{2+} ions in living Hela cells\textsuperscript{45}.
1.5.4 Forster resonance energy transfer

Forster resonance energy transfer (FRET) is a physical phenomenon which is the distance-dependent interaction between the electronic excited states of two chromophores in which excited energy is transferred from a donor to an acceptor through non-radiative dipole–dipole coupling\textsuperscript{46}. When both compounds are fluorescent in nature, the term “fluorescence resonance energy transfer” is often used instead\textsuperscript{47}. The efficiency of FRET is dependent on the intermolecular separation, such that it can only occur over very short distances typically within 10 nm. Beyond the spatial proximity requirement, the emission spectrum of the donor must overlap effectively with the absorption spectrum of the acceptor (Fig. 1.7). By exciting the donor and then monitoring the relative ratio of donor and acceptor emissions, either sequentially or simultaneously, the presence and efficiency of the FRET interaction can be monitored.
Qian and Xiao co-workers developed ratiometric probes 5 that can selectively detect Hg$^{2+}$ ions based on FRET mechanism\textsuperscript{48}. Addition Hg$^{2+}$ induced change in the intensity ratio of the two well-separated and comparably strong emission bands of BODIPY and Rhodamine. Confocal laser scanning microscopy experiments shows that probe has practical application in living cells.

Li \textit{et al.} reported a fluorophore dyad containing rhodamine and a naphthalimide moiety as a Cr$^{3+}$-selective fluorescent probe on the basis of fluorescent resonance energy transfer mechanism\textsuperscript{49} (Scheme 1. 6). Confocal
fluorescence microscopy confirmed that probe can be used for monitoring intracellular Cr\(^{3+}\) levels in living cells.

**Scheme 1.** Proposed mechanism of Cr\(^{3+}\) selective sensor.

Kim *et al.* designed and reported Rhodamine-dansyl group incorporated with tren spacer 6 as a selective sensor for Cu\(^{2+}\) ion\(^{50}\). Addition of Cu\(^{2+}\) ion induces opening of the spirolactam ring and leads to a shift in the absorption spectrum of Rhodamine. Subsequently, increased overlap between the emission of the dansyl and the absorption of the rhodamine greatly enhances the intramolecular FRET, producing an emission from the energy acceptor unit in molecule.
1.5.5 Monomer-Excimer Formation

The formation of excited state collision complexes (excited dimers) can take place between two identical fluorophores (excimer) or two distinct fluorophores (exciplex). Both excimer and exciplex involve the association of an excited state species with a ground state entity if one comes into close approach to another during the lifetime of the excited state. The net stabilization that results from this type of interaction lengthens the lifetime of the excited state dimer. Several fluorophores like anthracene and pyrene can form excimer (excited dimer) when an excited molecule can come in close approach to another one during the lifetime of the excited state. Dual fluorescence is then observed with a monomer band and, at longer wavelengths, a structure less broad band due to excimer formation. The ratio of the fluorescence intensities corresponds to monomer and excimer emission on molecular mobility and ‘micro viscosity’. When a fluorescent probe contains two fluorophores whose mutual distance is affected by analyte (usually metal cation) complexation, recognition of this analyte can be monitored by the monomer/excimer fluorescence–intensity ratio. Cation binding may favour or hinder excimer formation. In any case, such a ratiometric method allowing self-calibration is of great interest for practical applications.

Das and co-workers developed ratiometric fluorescence probe 7 for Al\(^{3+}\) by coupling of 4, 5 diaminopyridine with 1-pyrenecarbaxaldehyde in DMSO: H\(_2\)O (4:1 v/v) solution. Addition of Al\(^{3+}\) exhibits excimer emission at 445 nm along with decrease of its monomer emission at 368 nm. The detection value for Al\(^{3+}\) is 0.2 μM.
A novel naphthalene based fluorescent probe for Ni\(^{2+}\) has been investigated and demonstrated by excitation spectra, life time and \(^1\)H-NMR titration\(^{54}\). Addition of Ni\(^{2+}\) quenches its monomer emission with subsequent enhancement of the excimer emission at 430 nm. Lifetime of Ni\(^{2+}\) is higher than probe and it detected Ni\(^{2+}\) in \(1 \times 10^{-6}\) M range (Scheme 1. 7).

\[ \text{Scheme 1. 7 Schematic representation of the plausible mechanism of the Ni}^{2+} \text{induced intramolecular excimer formation} \]

Das \textit{et al.} displayed an efficient fluorescent probe derived from pyrene carboxaldehyde and diethylenetriamine\(^{55}\). The probe can selectively detect Cd\(^{2+}\) ion and detect in \(1.8 \times 10^{-8}\) M range (Scheme 1. 8). The presence of Cd\(^{2+}\) converts the fluorescence from excimer emission to monomer emission.
Scheme 1. 8 Proposed mechanism for selective sensing of Cd$^{2+}$

1.5.6 Aggregation-induced emission (AIE)

Aggregation induced emission (AIE) has become more popular and has also been applied to many areas of science by Tang et al. during the period 2001$^{56}$. Molecules with AIE characteristics have been found to serve as chemosensors, bioprobes, stimuli-responsive nanomaterials and active layers in the construction of efficient organic light-emitting diodes. It is also known that fluorescent emission of organic fluorophores is often quenched in aggregated form; this effect is denoted aggregation-caused quenching (ACQ)$^{57}$. Because of the ACQ effect, the application of many organic fluorophores in organic light-emitting diodes (OLEDs) and as sensing materials (chemosensors, biosensors) has been greatly limited$^{58}$. To overcome the ACQ effect, branched chains, bulky cyclic species and dendritic wedges have been covalently attached to the fluorophores to suppress the formation of aggregates$^{59}$. However, some organic molecules that are almost non-fluorescent in solution have been shown to become strongly fluorescent upon aggregation, which is an abnormal behaviour. AIE phenomenon have been successfully utilized to design sensitive and selective bio/chemosensors. The aggregation of AIE molecules can be tuned by guest molecules through electrostatic interaction, coordination interaction, hydrophobic interaction, steric hindrance, a particular mercapto reaction, or the influence of polarity and viscosity. As a result, a variety of new AIE-active fluorescent bio/chemosensors have been developed to detect ionic
species, biomolecules, and gases and explosives, as well as assay the activities of nuclease activities.

Vandana bhalla et al. has reported star shaped highly fluorescent triphenylene derivative, which forms nano aggregates in the presence of Cu$^{2+}$ in a mixed aqueous medium. Addition of CN$^-$ induces dissociation of Cu$^{2+}$ ensemble via displacement approach, thereby restores the fluorescence of triphenylene derivative (Scheme 1.9).

**Scheme 1.9** Pictorial picture showing the ‘turn-on’ sensing of CN$^-$ ions with the nano aggregates of a copper ensemble of triphenylene derivative.

Lin et al. developed novel pyrene-anthracene based Schiff base derivative 8 and 9 as a turn-on fluorescence sensor for Cu$^{2+}$ and Fe$^{3+}$ ion through aggregation induced emission in CH$_3$CN and THF solution. Turn-on response is observed for Cu$^{2+}$ and Fe$^{3+}$ ions due to chelation enhanced fluorescence through pyrene-pyrene excimer and anthracene-anthracene excimer formation.
Zhang et al. reported selective and somewhat sensitive chemosensor for cyanide ion by the AIE feature of silole compounds and high nucleophilic reactivity of cyanide ion in aqueous solution (Scheme 1.10).

Scheme 1.10 Fluorescence Turn-on detection of cyanide by making Use of the AIE Feature of Silole Compounds

1.5.7 C=N isomerization

Generally, compounds containing unbridged C=N bonds are non-fluorescent, where C=N isomerization is the predominant decay process of excited states. However, compounds either containing a cyclic C=N bond or complexed with a guest species to restrict the rotation about the C=N bond are strongly-fluorescent. This leads to the expectation that luminescence turn-on sensors towards specific analytes can be sophisticatedly designed by virtue of the suppression of C=N isomerization. According to this thought, currently, some sensitive organic fluorescence sensors have been developed for the detection of metal ions based on the C=N isomerization. They show significant fluorescence enhancement upon N donor of the C=N bond binding to a metal ion so that the isomerization is significantly inhibited. Instead of the suppression of C=N isomerization, it is also reasonable to deduce that the fluorescence intensity can be enhanced by removal of the C=N bond in the sensor molecule through a chemical reaction promoted by special analytes.
Wu et al. reported a simple Schiff base as selective detection of Zn$^{2+}$ ion\textsuperscript{64}. Upon addition of Zn$^{2+}$, formation of rigid structure between imine and Zn$^{2+}$ ion inhibits the C=N isomerisation and excited state intramolecular proton transfer leading to fluorescence enhancement (Scheme 1.11).

\begin{center}
\includegraphics[width=0.8\textwidth]{Scheme11.png}
\end{center}

Scheme 1.11 Proposed binding mechanism receptor and Zn$^{2+}$

Kim et al. developed coumarin based turn-on sensor for Zn$^{2+}$ ion\textsuperscript{65}. The probe is non fluorescent because of pseudo planarity and nitrogen lone pair orbital contribution to the excitation. Upon Zn$^{2+}$ coordination, probe exhibited a fluorescence enhancement due to the blocking of nitrogen lone pair orbital by metal coordination (Scheme 1.12). TD-DFT calculation also supports the above mechanism.

\begin{center}
\includegraphics[width=0.8\textwidth]{Scheme12.png}
\end{center}

Scheme 1.12 Schematic representation of C=N isomerisation of receptor with Zn$^{2+}$.

Liu et al. synthesised a fluorescent probe benzene-1, 2-dicarbaldehyde bis-benzoyl hydrazide\textsuperscript{66}. In the absence of Zn$^{2+}$, the probe is non-fluorescent. Upon binding with Zn$^{2+}$, C=N isomerisation was eliminated, meanwhile, the
ICT effect in the ligand was reduced and a rigid framework was formed, which causes CHEF effect (Scheme 1.13)

![Scheme 1.13 Proposed mechanism for the fluorescence enhancement of L upon the addition of Zn$^{2+}$](image)

**Scheme 1.13** Proposed mechanism for the fluorescence enhancement of L upon the addition of Zn$^{2+}$

1. 5. 8 **Excited-state intramolecular proton transfer (ESIPT)**

ESIPT is a very important reaction in chemical and biological systems$^{67}$. It has received considerable interest in the areas of photochemistry, photophysics and photobiology. The basic photophysical properties of the ESIPT chromophores are illustrated in Fig. 1.8. Excited state intramolecular proton-transfer mechanism is a phototautomerization in the electronically excited state. The ESIPT chromophores exist in the $cis$ enol form in the ground state which may be stabilised by intramolecular hydrogen bond. Upon photoexcitation the singlet excited state of the enol form is populated. After excitation, an ultrafast ESIPT process occurs. Since, the ESIPT is much faster than the fluorescence process, the fluorescence observed for the ESIPT chromophore is very often due to the keto tautomer, although exceptions do exist$^{68}$. Another deactivation channel is isomerisation to the $trans$-keto form.
Chellappa *et al.* and co-workers designed and reported 1, 2, 4 triazole motif as a fluorescence chemosensor for Zn\(^{2+}\) ion\(^69\). The emission bands at 416 nm and 529 nm corresponds to enol form and keto form which is stabilised by hydrogen bonding (Scheme 1.14). After addition of Zn\(^{2+}\), the band at 416 nm decreased along with increase of band at 529 nm selectively. The probe was successfully applied for intracellular imaging of Zn\(^{2+}\).

**Scheme 1.14** Enol and keto tautomers of triazole receptor

Pang *et al.* reported a bis(benzoazole) derivative with dipcolylamine exhibits a large fluorescence turn-on effect upon Zn\(^{2+}\) binding\(^70\). Addition of Zn\(^{2+}\) enables the probe via excited state intramolecular proton transfer and giving a new band at 710 nm with large stockes shift (scheme 1. 15).
Scheme 1. 15 Schematic representation of the ESIPT process

Kim et al. developed a new thiazole based ratiometric chemosensor 10 for detection of zinc ion\textsuperscript{71}. Coordination of zinc to the phenolic oxygen and nitrogen atoms of thiazole and pyridine disrupts ESIPT mechanism, which in turn leads to a blue shift in the emission spectrum.

1. 6 Small molecule based Fluorophores

Fluorescent probes capable of selectively and sensitively sensing guest species are receiving considerable interest because of their potential applications in environmental detection, molecular catalysis, and biological fluorescence imaging, together with the advantages of spatial and temporal resolution of fluorescent assays. Even though considerable efforts have been devoted to develop fluorescent probes for various species over the last few decades, there is a strong need for fluorophore that can meet particular applications. Currently organic dyes have been extensively used for conjugation with biomolecules, owing to their excellent photophysical properties. Fluorescent small molecule is a highly sensitive analytical tool and an obvious choice for visualizing cellular function to respond specifically to a cellular event or analyte. Numerous small molecule fluorescent sensors have been designed for a variety of purposes, including labelling of cellular
organelles and membranes, indication of pH, assessment of cell viability, and detection of metal cations and small molecules\textsuperscript{72}. However, many probes have some undesirable characteristics related to their compatibility with living cells, pH-dependent fluorescence, water solubility, and membrane permeability. In other designs, the sensor’s recognition site explicitly reacts with an intracellular small molecule, as with the pH and metal indicators, to elicit a change in its fluorescence emission\textsuperscript{73}. Hence nurturing of low-cost small molecule as a sensor for guest species, by a more straightforward method that would utilize few synthetic steps and would be easy to scale up.

Pyrene, anthracene, coumarin, bodipy, Rhodamine and fluorescein derivatives were evidenced as excellent small fluorophore and widely used in the developments of fluorescence sensors because of their excellent photoluminescence properties and chemical stabilities\textsuperscript{74}. Anthracene as a good fluorescent probe (~400 nm) is used in various fields such as pH sensor, cell surface labelling and medicinal diagnosis. It has also been intensively studied as an attractive building block and starting materials for organic light emitting diode (OLED), due to their unusual photoluminescence and electroluminescence properties and excellent electrochemical properties. Furthermore, pyrene and anthracene fluorescent probes self-assembled to form dimeric structures upon the addition of certain metal cations to give P–P\textsuperscript{*} and A–A\textsuperscript{*} excimer fluorescence and also provided the AIE characteristics by tuning the solvent conditions\textsuperscript{75}. Fluorescent coumarin (~500 nm) are popular as dyes, sensors for metal cations, and dopants for OLEDs, due to their excellent spectroscopic properties such as high quantum yields, good extinction coefficients, and large Stokes shifts. These features promoted their use as promising fluorescent tags for intracellular use\textsuperscript{76}. Rhodamine dyes (~550 nm) are fluorophores that belong to the family of xanthenes along with fluorescein and eosin dyes. The general structures of xanthene chromophore and rhodamine dyes are same. Due to their excellent photostability and
photophysical properties, rhodamines are used as laser dyes, fluorescence standards, and imaging in living cells. Rhodamine derivatives are non-fluorescent and colorless; whereas influence of analyte it triggers ring-opening of the corresponding spirolactam, lead to strong fluorescence emission and a pink colour. Inspired by this strategy, spirolactam (nonfluorescent) to ring opened amide (fluorescent) process was utilized for the detection of guest species. 4, 4-Difluoro-4-bora-3a, 4a-diaza-s-indacene (BODIPY) (~600 nm) is a versatile fluorophore because of its high quantum yield, tuneable fluorescence characteristics, high photostability, and narrow emission bandwidth, relative inertness under physiologically relevant conditions, and resistance to photo bleaching. Numerous labeling agents derived from the BODIPY scaffold have been reported and even commercialized. Their spectroscopic and photophysical properties can be finely tuned by substitution on the dipyrrromethene core. Near-infrared light is much more favourable for biological imaging due to its minimum photo damage to biological samples, good tissue penetration, and weak auto fluorescence interference from the complicated living systems.
Govindaraju et al. have reported coumarin-conjugated thiocarbanohydrazone 11 as new highly selective colorimetric chemosensor for Co$^{2+}$ ion$^{80}$. The ligand 11 senses Co$^{2+}$ in solution by changing its colour from light yellow to deep pink. The sensor has been applied in the development of practically viable colorimetric kits and also used as a staining agent for Co$^{2+}$ in microorganisms. Zeng et al. have developed a new fluorescent sensor 12 based on the coumarin fluorophore appended with benzohydrazide$^{81}$. It shows high sensitivity and excellent selectivity toward Cu$^{2+}$ in aqueous solution. In the presence of Cu$^{2+}$ it quenches the fluorescence at 523 nm and it’s confirmed by DFT calculations. The probe can detect Cu$^{2+}$ in living cells.

Yoon et al. reported rhodamine 6G thiolactone derivative 13 as a selective fluorescent and colorimetric sensor for Hg$^{2+}$ in neutral aqueous solution$^{82}$. Fluorescent and colorimetric changes were observed only for Hg$^{2+}$, which can be attributed to a spirothiolactone ring-opening process. Since sensor can detect Hg$^{2+}$ in the nanomolar range, this sensor has great potential for biological imaging and environmental purposes. Shyamaprosad Goswami et al. developed rhodamine derivative 14 bearing an 8-amino-quinoline moiety for selective sensing of Pd$^{2+}$ in the presence of other competing metal ions in aqueous media$^{83}$. Pd$^{2+}$ induced spirolactam ring opening of rhodamine is confirmed for the first time and it is confirmed by the X-ray crystal structure of the bound Pd$^{2+}$ complex.
Molina *et al.* reported new probe 15 based on an anthryl derivative bearing an azadiene side chain selectively senses Cu$^{2+}$ in CH$_3$CN$^{84}$. In the presence of Cu$^{2+}$, the yellow-to-orange color change and a remarkable enhancement of the fluorescence was noticed. Wu *et al.* developed a fluoroionophore sensor 16 derived from tryptophan-pyrene moiety that shows high sensitivity and specific selectivity for lead ion Pb$^{2+}$ in aqueous solution$^{85}$. The detection limit is up to 0.15 mM.

Rao *et al.* developed a new glucose based receptor 17 linked to anthracene through an imine moiety as a selective sensing of Hg$^{2+}$ ion$^{86}$. Presence of Hg$^{2+}$ exhibits a 13-fold fluorescence enhancement among the biological and ecological important ions in aqueous solution of CH$_3$OH. The reversibility of the Hg$^{2+}$ was checked with Na$_2$EDTA. This probe 17 is correspondingly sensitive towards Hg$^{2+}$ in the presence of albumin proteins and
in blood serum and milk. Kim et al. reported new 1-(anthracen-9-yl)-N-(pyridin-2-ylmethyl)-N-(quinolin-2-ylmethyl)methanamine 18 by incorporating a dipicolylamine derivative as a binding unit and an anthracene group as a fluorescence signaling unit for zinc ion detection\textsuperscript{87}. The anthracene-based receptor was highly selective for Zn\textsuperscript{2+} with a fluorescence enhancement and a remarkable red shift in aqueous solution.

Lin et al. developed a new coumarin-bodipy based novel ratiometric chemosensor 19 for the detection of F\textsuperscript{-} in DMSO solution\textsuperscript{88}. The sensor exhibits a large red-shift in absorption and dramatic fluorescence enhancement for the addition of F\textsuperscript{-} anion. DFT and TD-DFT calculations were conducted to rationalise the optical response of the sensor. Thilagar group synthesised 20, a novel borane-thiophene-bodipy triad via a facile synthetic route\textsuperscript{89}. Triad 1 exhibits tricolour emission when excited at the borane and also displays high energy absorption band. Additions of fluoride gives rise a new band at 624 nm because of ICT between borate anion and boradiazaindacanene. The receptor shows highly selective, colorimetric and ratiometric fluorescence sensor for fluoride with visual colour change. The experimental results are supported by computational studies.
Trivedi et al. reported 21 indole-3-carboxaldehyde functionalized with fluorescein hydrazine, which selectively detects Cu$^{2+}$ in vivo and in vitro by the “turn-on” mechanism followed by fluorescence “turn-off” with NO gas generated by the lipopolysaccharide action$^{90}$. The in vivo experiment performed in the cellular system indicates that probe loaded RAW264.7 cells showed bright fluorescence in the presence of Cu$^{2+}$, while other metals did not influence the receptor fluorescence. In addition, the fluorescence of probe Cu$^{2+}$ was efficiently quenched by NO generated in macrophages through LPS stimulation.

Yang et al. developed a novel fluorescent sensor, 7-hydroxy-4-methylcoumarin-8-carbaldehyde-hydrazone 22 for selective recognition of Zn$^{2+}$ ion$^{91}$. This sensor displays an extreme selectivity, sensitivity and color change for Zn$^{2+}$ over other earth- and transition metal ions, which was mainly due to the spirolactam ring-opening power of Zn$^{2+}$. The detection limit was low as 6.54 ppb. Enhancement of fluorescence enhancement was mainly due to
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the photoinduced electron transfer process and intramolecular charge transfer process.

![Chemical Structure](image)

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The fluorescent chemosensors must possess few key properties:

(i) To produce a viable signal with high quantum yield upon selective interaction with the desired analyte.

(ii) To exhibit an emission wavelength ($\lambda_{em}$) approaching 500 nm to avoid auto fluorescence issues from species native to the cell.

(iii) Cell permeability and non-cytotoxic property.

(iv) High sensitivity – to get maximal signal change in the presence of the analyte.

(v) High selectivity – to be able to distinguish only one analyte in a complex mixture.

1.7 Fluorescence based Live cell imaging

Fluorescent probes for imaging cellular molecules have become a hot research topic in chemical biology during last two decades. Compared to other technologies, such as radioisotope labeling, MRI, ESR, and electrochemical detection, fluorescence imaging has many advantages for this purpose, as it enables highly sensitive, non-invasive, and safe detection using readily available instruments. Another advantage of fluorescence imaging is that the fluorescence signal of a molecule can be drastically modulated. Molecular imaging technologies enable visualization of (bio) molecules and ions in cells, tissues and organisms with the aim of gaining information about
the biological effects of the analytes. Fluorescent chemosensors have been
developed to be a useful tool to sense biologically important species such as
metal ions and anions in vitro and in vivo because of the simplicity and high
sensitivity of fluorescence assays. Fluorescent probes, such as synthetic organic
dyes, poly aromatic hetero cycles, fluorescent proteins and quantum dots, have
become popular tools for molecular imaging owing to their high sensitivity,
simple manipulation and the lack of a need for sophisticated instrumentation. In
particular, fluorescent probes that respond to analytes with high selectivity and
sensitivity have been widely employed to monitor biomolecules and
biologically relevant species as well as to probe biological events in a spatio-
temporal manner. The fluorescent probe for imaging must have water
solubility, low cytotoxicity, and have good cellular uptake property, crossing
the outer lipid membrane rapidly. Beyond these general requirements, an
excellent luminescent chemosensor for imaging should exhibit luminescence
“turn-on” or (and) a clear shift in emission wavelength upon reacting with the
analyte. Finally, luminescent chemosensors should show high selectivity,
excellent sensitivity, and rapid response in detecting the analyte in biological
samples. Moreover, fluorescent imaging studies using organisms that are
genetically close to humans have become highly attractive.

Today, a significant number of interdisciplinary groups are working
towards developing new fluorescent sensor materials to be used as common
tools for faster, safer and more reliable medical diagnostics and research. In
line with these groups, this thesis also pertains to studies that aim to nurture
fluorescent sensors for imaging.

1.8 References

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