Chapter 1

Introduction and review of literature

Abstract

This chapter deals with a brief introduction of the supramolecular chemistry and review of literature about fluorescent receptors based on (thia)calix[4]arene scaffold, resonance energy transfer architectures and chemical reaction-based approach. The chemically modified thiacalix[4]arene scaffold with different types of fluorophores and binding sites can act as excellent host for different types of guest species. In addition, the resonance energy transfer and irreversible chemical reactions based fluorescent probes have advantages owing to their highly sensitive nature and real time monitoring with fast response time in biological systems. The objectives of present work are defined by keeping in mind the significance of these reports.
1.1. Introduction and review of literature

Supramolecular chemistry\(^1\) referred as the chemistry that goes beyond the covalent bond, beyond the individual molecule, is the study of non-covalent interactions between molecules and the resulting multi-molecular complexes. It is concerned with the organization and properties of molecules held together by weak interactions and spatially organized films can also be conjured out of small building blocks by the careful exploitation of hydrogen bonds, metal–ligand binding, \(\pi-\pi\) interactions and hydrophobic effects. Natural molecules, such as proteins, oligonucleotides, lipids and their multimolecular complexes have been the major source of inspiration for supramolecular chemists. Supramolecular chemistry also finds role in the wide varieties of areas including material science,\(^2\) catalysis,\(^3\) medicine,\(^4\) green chemistry,\(^5\) data storage\(^6\) and molecular recognition.\(^7\) Thus, there has been significant progress in these areas of supramolecular chemistry. Out of the different areas of supramolecular chemistry, molecular recognition is the key component which aims at the design, synthesis and specific binding of molecular receptors to complementary guest molecules. The term molecular recognition refers to the specific interaction between two or more molecules through non-covalent bonding such as hydrogen bonding.\(^1,8\) A variety of synthetic organic receptors like crown ethers,\(^9\) cryptands,\(^10\) spherands,\(^11\) porphyrins,\(^12\) calixarenes,\(^13\) thiacalixarenes\(^14\) and cyclodextrins\(^15\) have been used as molecular recognition reagents with their vast applications as host-guest systems,\(^16\) supramolecular assemblies,\(^17\) ion selective electrodes,\(^18\) bioinspired complexes\(^19\) drug delivery systems,\(^20\) photochemical and electron transfer devices.\(^21\) For a molecular receptor to be an effective host, it should be easily synthesized, undergo chemical modification and have potential molecular recognition ability for drawing out the best outcome of the receptor molecule for a specified application. However, the efficiency of a recognition process largely relies on the available analytical methods for monitoring the detection process. Several methods, including atomic absorption spectroscopy, flame photometry, inductively coupled plasma atomic emission spectrometry and ion sensitive electrodes are often used to detect different types of analytes.\(^22\) However, these methods require expensive instruments, large amount of sample and do not allow continuous monitoring. In contrast, the method based on fluorescence spectroscopy in conjunction with suitable
fluorogenic chemosensors offers distinct advantages in terms of sensitivity, selectivity, response time and provides platform to analyze and measure the amount of guest species as well as sense biologically important species in vitro and in vivo to clarify their functions in living systems. Fluorescent chemosensor is a molecular system for which the photophysical characteristics of the fluorophore, such as fluorescence intensity, emission wavelength and fluorescence lifetime, will change via different mechanisms upon interaction with a chemical species and such a change provides a signal that indicates guest binding. The selective recognition of biologically and environmentally important species by synthetic fluorescent receptors is therefore a topic of great interest from both supramolecular chemistry and analytical application point of view.

Thus, keeping in view the significance of fluorescent chemosensors as an effective recognition tool, in the present investigation we have designed and synthesized fluorescent receptors and evaluated their recognition behaviour toward different types of analytes. Our approach for the development of fluorescent receptors involves: (1) the use of thiacalix[4]arene as a molecular scaffold which can be decorated with different number/nature of fluorophores and ligating sites; (2) the combination of two different fluorogenic moieties such as rhodamine and naphthalimide to develop energy transfer based probes; (3) incorporation of different types of recognition or reaction sites in the fluorescent sensors for the desired recognition/sensing behaviour. The results have been divided into five different chapters depending upon the type of fluorescent chemosensors. However, before the presentation of our results a brief review of literature about (thia)calix[4]arenes based fluorogenic receptors, energy transfer based probes and reaction based fluorescent sensors is given below.

1.2. (Thia)calix[4]arenes based fluorogenic receptors

Calix[n]arenes obtained from p-tert-butylphenol and formaldehyde under basic conditions are one of the most actively studied molecular scaffolds in supramolecular chemistry (Scheme 1.1). These calix[n]arenes have well defined conformational properties and cavities of molecular dimensions. The chemically modified calix[n]arene derivatives can be tuned to serve as efficient host toward different guest species either by controlling their conformations or by
changing the nature and number of ligating sites. On the other hand, thiacalix[4]arene, in which all four methylene bridges of conventional calix[4]arene are substituted by sulfide bonds (Scheme 1.1), makes thiacalix[4]arene very interesting molecule, with many features that are not present in the chemistry of “classical” calix[4]arenes.\textsuperscript{26} Thiacalix[4]arene scaffold like that of conventional calix[4]arene has a lower narrow rim of phenolic groups and upper wider rim of para-substituents (Figure 1.1A). The orientation of phenolic units with respect to each other provides four different conformations of thiacalix[4]arene\textsuperscript{27} i.e. ‘Cone’ (uuuu); ‘Partial cone’ (uuud); ‘1,2-Alternate’ (uudd) and ‘1,3-Alternate’ (udud) (Figure 1.1B). However, the intramolecular circular hydrogen bonding between the adjacent phenolic groups in (thia)calix[4]arene favours the cone conformation in the solution.\textsuperscript{28} The chemical modifications at the upper as well as lower rim make (thia)calix[4]arene a unique scaffold for preparing a variety of derivatives selective for different types of guest.

Scheme 1.1 General method of synthesis of (thia)calixarenes.

Figure 1.1A Structure of thiacalix[4]arene.

Figure 1.1B Thiacalix[4]arene in different conformations.
species. Therefore, (thia)calix[4]arene scaffold can be appended with various types of fluorophores and binding sites. A number of fluorogenic receptors based on the (thia)calix[4]arenes have been reported in the past, a brief account of representative examples of fluorogenic receptors during the period 1998-2012 based on (thia)calix[4]arenes is reviewed below.

Ji et al.\textsuperscript{29} reported calixarene-anthracene derivatives 1-3 for the selective detection of Cs\textsuperscript{+} ions. In the absence of metal ions, fluorescence of these derivatives 1-3 is weak due to the photoinduced electron transfer (PET) from the oxygen atoms of the benzo crown moiety to the excited singlet state of the anthracene fluorophore. The maximum fluorescence enhancement in the emission of 1 and 2 upon the addition of Cs\textsuperscript{+} ions is 8.2 and 11.7-folds, respectively. This enhancement can be explained by the fact that complexation of Cs\textsuperscript{+} ions with the oxygen atoms of the crown ring inhibits PET, i.e. there is a chelation-enhanced fluorescence (CHEF) effect. Compound 3 with the same fluorophore as 1 but based on a slightly different calixarene platform shows not only an increased selectivity toward Cs\textsuperscript{+} ions but also a remarkable enhancement in the fluorescence emission intensity by Cs\textsuperscript{+} ions compared with the other systems. Even addition of only a small amount of Cs\textsuperscript{+} (1.0 × 10\textsuperscript{-7} M) to 3 leads to a 20-fold fluorescence enhancement, while there is no detectable response to other alkali metal ions except Rb\textsuperscript{+} (2-fold enhancement) at the same concentration. The fluorescence quantum yield of 3 is 54 times greater than that of the free ligand when fully complexed by Cs\textsuperscript{+} ions.

Fluorescence quenching by PET processes involving an electron transfer mechanism was also reported in the case of compounds 4\textsuperscript{30} and 5.\textsuperscript{31} Compound 4 is
the first example of a Hg\(^{2+}\) selective fluorescent sensor based on calix[4]arene. The addition of Hg\(^{2+}\) ions to the solution of 4 results in the fluorescence quenching due to electron transfer from excited dansyl moiety to Hg\(^{2+}\) ions. Similar type of fluorescence quenching with Hg\(^{2+}\) ions was also observed in the case of receptor 5. Calixarene-based fluorescent sensor 6\(^{32}\) containing two cation recognition sites, a crown ether ring and two facing pyrene amide moieties, shows a drastic change in the fluorescence and absorption spectra upon complexation with Pb\(^{2+}\), Cu\(^{2+}\) and K\(^{+}\) ions with different binding modes. N---Cu\(^{2+}\)--N chelation makes the distance between the two pyrenes shorter, resulting in a static excimer evidenced by a red shift in the excitation spectrum of its complex. On the other hand, C=O---Pb\(^{2+}\)--O=C coordination forces the two pyrene units to move apart, allowing no static excimer formation at all. The addition of K\(^{+}\) ions result in the increase in excimer emission, presumably because polyether ring of calix[4]crown-5 is more suitable for complexation of K\(^{+}\) ions.

Kim et al.\(^{33}\) synthesized calix[4]arene based fluorescent chemosensors 7 and 8. 1,2,3-triazole units in compound 7 based on calix[4]arene act as both spacers and cation-binding sites in combination with pyrene units. Receptor 7 exhibits selectivity toward Cd\(^{2+}\) and Zn\(^{2+}\) ions. Binding of Cd\(^{2+}\) and Zn\(^{2+}\) ions occur in a ratiometric manner through an enhanced monomer and declining excimer emission spectrum. Calix[4]arene derivative 8 locked in the 1,3-\textit{alternate} conformation bearing two pyrene and rhodamine fluorophores shows high selectivity towards Hg\(^{2+}\) ions. The sensing mechanism is based on fluorescence resonance energy transfer (FRET) from pyrene excimer emission to ring-opened rhodamine absorption upon complexation with the Hg\(^{2+}\) ions. Addition of Hg\(^{2+}\) ions to the solution of 8 gives significantly enhanced fluorescence at 576 nm \textit{via} FRET on excitation at 343 nm. Further, the
pyrene excimer emission formed by the intramolecular $\pi-\pi$ interactions is more effective in obtaining strong FRET bands than those by intermolecular $\pi-\pi$ interactions.

Calix[4]arene based fluorescent chemosensors 9 and 10 with excimer emission properties have been synthesized by Paul and co-workers where two pyrene moieties act as fluorophores.¹⁴ Both 9 and 10 exhibit strong excimer emission at around 515 nm due to the face to face $\pi-\pi$ stacking of the pyrene moieties which is used to monitor the interaction of metal ions. The presence of Hg$^{2+}$, Pb$^{2+}$, Cu$^{2+}$ and Ni$^{2+}$ in acetonitrile–chloroform resulted in the quenching of the excimer emission due to break down of the $\pi-\pi$ stacking of the pyrene moieties owing to the separation of pyrenes from each other on complex formation. Dramatic change in selectivity is observed in THF–H$_2$O as the metal ion hydration and the effective size of the first hydration shell of metal ion with six water molecules played crucial role in determining the selectivity. The more selectivity of Cu$^{2+}$ in the aqueous media is attributed to the size of the hydrated shell of the Cu$^{2+}$ ion which enters the ionophore cavity to form complex. Further, both compounds exhibit strong interaction with F$^-$ ions with sharp colour change. In addition to this, Paul et al. also reported calix[4]arene based fluorescent chemosensors 11 and 12 containing amide as binding site and quinoline as a fluorogenic unit which are characterized by single crystal X-ray diffraction study.³⁵ The cation binding behaviour of these fluoroionophores has

been monitored by fluorescence and UV-vis spectral changes. These compounds exhibit strong complexation with Hg$^{2+}$, Pb$^{2+}$, Fe$^{3+}$ and Cu$^{2+}$ ions which is supported by the fluorescence and UV-vis spectroscopy. The complexation of metal ions resulted in a new emission band at lower energy ascribed to the excimer emission, which was supported by the DFT calculations. Calixarene-based fluoroionophore 13 bearing crown ether and an azacrown ether as two binding sites was reported by Kim
and Yoon et al. An interesting “molecular taekwondo” process between Ag\(^+\) and K\(^+\) ions was easily monitored via fluorescence changes. The fluorescence of 13 in ethanol upon addition of Ag\(^+\) exhibited chelation-enhanced fluorescence (CHEF) effect due to the inhibition of the PET mechanism ascribed to the binding of Ag\(^+\) in the azacrown site. However, when K\(^+\) ion was added to a solution containing ligand and Ag\(^+\), fluorescence quenching was observed as the complexation of K\(^+\) into the crown ether ring induced the decomplexation of Ag\(^+\) from the azacrown site.

A highly selective fluorescent chemosensor 14 for sensing of Pb\(^{2+}\) ions based on calix[4]arene pre-organized in the partial cone conformation with dansylcarboxamide groups has been reported by Talanova and co-workers. In acidic CH\(_3\)CN:H\(_2\)O (1:1 v/v) solution, the partial cone fluoroionophore allowed detection of Pb\(^{2+}\) ions at the levels as low as 2.5 ppb, which is within the acceptable limit of Environmental Protection Agency and the World Health Organization for the allowable limit of Pb\(^{2+}\) ions in drinking water. Calix[4]arene derivative 15 with modified bipyridine as binding sites has been synthesized by Kim et al. that can detect Zn\(^{2+}\) ions selectively with respect to ratiometric fluorescent changes and red shift. The fluorescence titration of 15 (10 µM) with Zn\(^{2+}\) ions in CH\(_3\)CN showed the decrease in the fluorescence intensity at 363 nm along with a new red shifted emission band centered at 408 nm. The appearance of red shifted emission band is attributed to the stabilization of intramolecular charge transfer (ICT) excited state of 15 and the formation of 15-Zn\(^{2+}\) complex. Further, no considerable change in optical properties of 15 was observed by changing the perchlorate counter ions with sulfate, acetate or chloride. Fluorescent chemosensor 16 based on calix[4]arene bearing four iminoquinoline subunits on the upper rim was reported by Huang and co-workers. The fluorescence spectra of 16 in acetonitrile showed weak fluorescence at 412 nm. However, the addition of Cu\(^{2+}\) resulted in remarkably enhanced fluorescence at 412 nm (1200-fold). Under the same conditions, Zn\(^{2+}\) only induced emission enhancement.
of 24-fold. No significant fluorescence changes were observed with other transition as well as alkaline-earth metal ions. The nonlinear fitting of the fluorescence spectra indicated a 1:1 stoichiometry for the 16-Cu\(^{2+}\) complex with association constant of \(3.67 \times 10^7\) M\(^{-1}\), which indicates a high affinity of 16 for Cu\(^{2+}\) ions.

A triazole-modified calix[4]crown based chemosensor 17 with two different types of cationic binding sites was synthesized by Chung and co-workers.\(^{40}\) The fluorescence spectrum of 17 (10 µM) in CH\(_3\)CN:CHCl\(_3\) exhibited a characteristic monomer emission of anthracene moiety at 415 nm when excited at 367 nm. The fluorescence of 17 was quenched by the addition of Hg\(^{2+}\), Cu\(^{2+}\), Cr\(^{3+}\) and Pb\(^{2+}\) ions. However, it showed enhancement after addition of K\(^+\), Ba\(^{2+}\), and Zn\(^{2+}\) ions ascribed to their binding in the crown ring. It was found that only single triazole group is required for the coordination of Hg\(^{2+}\), Cu\(^{2+}\) and Cr\(^{3+}\) ions whereas, the involvement of the two triazole groups is required for complexation of Pb\(^{2+}\) ions. The observed quenching is explained by either a reverse PET or a heavy atom effect. Further, the authors observed interesting off-on switching process between K\(^+\) and Pb\(^{2+}\) based on negative allosteric regulations.

Li et al. synthesized a fluorescent probe 18 bearing boron-dipyrromethene (BODIPY) and rhodamine moieties on calix[4]arene platform for the detection of Hg\(^{2+}\) and Ba\(^{2+}\) ions.\(^{41}\) Due to the significant PET effect from the oxygen atoms of the crown-6 ring to the excited BODIPY fluorophore, 18 exhibits weak fluorescence emission at 505 nm when excited at 490 nm. However, the addition of Ba\(^{2+}\) (20 equiv) results in a 15-fold enhancement in fluorescence intensity at 505 nm as Ba\(^{2+}\) ions complexed by the oxygen atoms of the crown ring and hence, remove the PET effect. On the other hand, addition of Hg\(^{2+}\) led to the spirolactam form of rhodamine to ring-
opened state with a strong emission at 585 nm upon excitation at 490 nm attributed to the FRET from BODIPY to the ring-opened form of rhodamine. Upon addition of both Hg\(^{2\text{+}}\) and Ba\(^{2\text{+}}\) to the solution of 18 the emission at 505 nm undergoes fluorescence quenching and the emission at 585 nm exhibits significant fluorescence enhancement owing to the FRET. The stoichiometry for the binding between 18 and Ba\(^{2\text{+}}\) or Hg\(^{2\text{+}}\) was determined to be 1:1 using the Job’s plot method with association constants 1.68 × 10\(^{5}\) and 1.15 × 10\(^{5}\), respectively. Further, by using Ba\(^{2\text{+}}\) and Hg\(^{2\text{+}}\) as chemical inputs an INHIBIT logic gate was developed.

Vicens and co-workers reported a chemosensor 19 based on calix[4]arene of cone conformation appended with pyrene and rhodamine moieties exhibits Hg\(^{2\text{+}}\)-induced fluorescence resonance energy transfer.\(^{42}\) The gradual addition of Hg\(^{2\text{+}}\) ions to 19 results in the emission enhancement of pyrenyl monomer (at 400 nm), pyrenyl excimer (at 475 nm) and rhodamine moiety at 575 nm when excited at 343 nm. The rhodamine emission arises due to the energy transfer from the pyrenyl excimer to rhodamine moiety. Because of the presence of tren structure, the tertiary \(N\) atom quenches the pyrenyl monomer and excimer emissions in the absence of Hg\(^{2\text{+}}\) due to the PET process. However, when Hg\(^{2\text{+}}\) is present, PET is suppressed and the fluorescence is increased due to chelation-enhanced fluorescence (CHEF) and favors the energy transfer. On the other hand, the addition of Al\(^{3\text{+}}\) induced a significant increase of pyrenyl excimer emission at 475 nm and no energy transfer was observed in this case.

Kim et al.\(^{43}\) reported a chemosensor based on calix[4]crown 20 bearing additional diazo-phenyl moieties which selectively forms a complex with Pb\(^{2\text{+}}\) ions over other metal ions. Receptor 20 is weakly emissive in nature because of the FRET.
Upon addition of Pb$^{2+}$ ions, the fluorescence of 20 was observed to revive due to the less overlapped bands between the donor (emission) and the acceptor (absorption) responsible for the hypsochromical shift of diazo units by the metal ion complexation, resulting in a diminished FRET effect. In addition, the Pb$^{2+}$ ion selectivity was also observed by the selective colour change of 20 from pale green to colourless. A calix[4]arene based fluorescent chemosensor 21 bearing one naphthocrown-6 and two coumarin amide units at the lower rim in partial cone conformation exhibiting fluorescence energy transfer was synthesized by Kim and co-workers.$^{44}$ With an excitation at 245 nm, 21 shows very weak emission around 340 nm corresponding to the naphthalene along with coumarin emission band at 422 nm ascribed to the energy transfer from naphthalene to the coumarin moiety. Upon addition of F$^-$ ions, red-shifted coumarin emissions appear at 536 nm due to the PCT mechanism while the naphthalene emission at 340 nm remarkably enhanced. This is attributed to the minimized spectral overlap between the donor emission and the acceptor absorption band and hence no energy transfer. Further, the coumarin emission in 21 is significantly enhanced by the addition of Cs$^+$ ions owing to the chelation-enhanced fluorescence (CHEF) upon complexation of Cs$^+$ ion by the crown-6 ring which trammels the PET from oxygen atoms to the naphthalene moiety and thus, more effective spectral overlap and ultimately energy transfer. Receptors 22 and 23 containing 1,2-alternate tetrahomodioxacalix[4]arene functionalized with pyrene-amides exhibited both monomer and excimer bands in the fluorescence spectra.$^{45}$ However, the addition of Pb$^{2+}$ resulted in the fluorescence quenching effect on the monomer and excimer emissions.

A series of fluorescent chemosensors 24-27 based on triazole linked calix[4]arene have been reported by Rao and co-workers. The receptor 24 exhibits very weak
fluorescence emission owing to photoelectron transfer at 450 nm when excited at 380 nm in 1:4 water/methanol mixture. The addition of only Zn$^{2+}$ ions results in the enhancement of fluorescence intensity at 450 nm. The plot of fluorescence intensity as a function of added [Zn$^{2+}$]/[L] mole ratio shows a stoichiometry of 1:1 between 24 and Zn$^{2+}$ ions. Further, the biological applicability of 24 to sense Zn$^{2+}$ is carried out in blood serum as well as related albumin proteins, viz., human serum albumin (HSA) and bovine serum albumin (BSA). Derivative 25 based on N,N-dimethylamine ethylimino appended triazole-linked calix[4]arene conjugate showed selectivity toward Cd$^{2+}$ and the structure of 25-Cd$^{2+}$ complex was characterized by computational calculations. Time-dependent density functional theory calculations were performed to demonstrate the electronic properties of 25-Cd$^{2+}$ complex. In addition, the highly fluorescing 25-Cd$^{2+}$ complex has been used to recognize cysteine with a minimum detection limit of 58 ppb. The triazole linked thiophenyl conjugate of calix[4]arene, 26 showed fluorescence enhancement at ~450 nm with Zn$^{2+}$ ions. The in situ prepared 26-Zn$^{2+}$ complex has been used for the detection of pyrophosphate. Chemosensor 27 showed weak fluorescence emission at ~535 nm and the addition of metal ions such as Mn$^{2+}$, Fe$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$ resulted in the quenching of fluorescence emission at 535 nm. Moreover, the in situ prepared complexes of these metal ions were used as chemo-sensing systems for the recognition of Glu, Asp, His and Cys.

Kumagai et al. synthesized thiacalix[4]arene derivatives 28 and 29 containing the dansyl fluorophore exhibited fluorescent enhancement upon complexation with Cd$^{2+}$ ions and some other transition-metal ions. Both derivatives are soluble in water and thus can be used to sense metal ions directly in aqueous solution by fluorescence changes. Compound 28 with only two dansyl moieties is sterically less congested than 29 and is thus a stronger host and a better sensor. Both also show the ability to sense some neutral molecules such as borneol, nerol, menthol, cyclohexanol, cyclooctanol.
and 1-adamantanecarboxylic acid, the greatest sensitivity of both being to nerol because of its close fit to their cavities.

Varma et al.\textsuperscript{51} reported a 1,3-\textit{alternate} thiacalix[4]arene derivative \textbf{30} possessing four 8-quinolinoloxo groups through flexible propyl chains which exhibits a high affinity toward Hg\textsuperscript{2+} ions. The highly selective fluorescence quenching behaviour of the receptor toward Hg\textsuperscript{2+} ions, its sensing ability in partially aqueous systems and its reversible binding with Hg\textsuperscript{2+} ions, reinstating its fluorescence in the presence of stronger ligands suggest that it can be used as an on-off fluorescent sensor toward Hg\textsuperscript{2+} ions. The receptor exhibited only marginal affinity toward Cr\textsuperscript{3+} and Ag\textsuperscript{+} ions and no affinity to other transition metal ions tested including Cu\textsuperscript{2+}, thus reducing the possibility of interference by these metal ions. Fluorescent sensor \textbf{31}, comprised of two rhodamine B moieties as fluorophores linked to thiacalix[4]arene scaffold showed fluorescence enhancement with Fe\textsuperscript{3+} and Cr\textsuperscript{3+}.\textsuperscript{52} The fluorescence enhancement is ascribed to the spirocyclic opening reaction of rhodamine moiety in the presence of Fe\textsuperscript{3+} and Cr\textsuperscript{3+}. The stoichiometric ratios and association constants of the complexes between \textbf{31} and these ions showed that Fe\textsuperscript{3+} or Cr\textsuperscript{3+} form stable 1:1 complexes with receptor \textbf{31}.

Kumar et al.\textsuperscript{53} reported fluorescent chemosensors \textbf{32-34} based on thiacalix[4]arene of 1,3-\textit{alternate} conformations. Compound \textbf{32} with two dansyl moieties and a crown-5 ring shows significant quenching in fluorescence emission upon addition of Hg\textsuperscript{2+} ions. This remarkable quenching induced by Hg\textsuperscript{2+} ion is ascribed to reverse PET from the dansyl moieties to the nitrogen atom of which the electron density was diminished upon metal complexation. Further, compound \textbf{32} behaves as an “on-off” reversible switch for two chemical inputs Hg\textsuperscript{2+} and K\textsuperscript{+} ions and mimics a molecular level keypad lock in the presence of F\textsuperscript{−} ions. Thiacalix[4]crown derivative \textbf{33} of 1,3-\textit{alternate} conformation possessing anthracene moieties exhibits fluorescence enhancement with Fe\textsuperscript{3+} ions. Further, negative
allosteric behaviour between Fe$^{3+}$/K$^+$ ions is observed with high selectivity and fluorescence amplification. Chemosensor 34 bearing two pyrene groups demonstrates ratiometric sensing with Ag$^+$ and fluorescence quenching with Fe$^{3+}$ ions in mixed aqueous media. The ‘in situ’ prepared Ag$^+$ and Fe$^{3+}$ complexes showed high selectivity toward cysteine. The molecular switching between three chemical inputs (Ag$^+$, Fe$^{3+}$ and cysteine) results in various molecular logic gates which have been integrated sequentially to generate a sequential information processing device.

Yamato et al.$^{54}$ reported fluorescent chemosensors 35 and 36 based on thiacalix[4]arene of 1,3-alternate conformation with pyrenyl-appended triazole moieties. The fluorescence spectra changes suggested that chemosensors 35 and 36 are highly selective for Ag$^+$ ions over other metal ions by enhancing the monomer emission of pyrene in neutral solution. However, other heavy metal ions such as Cu$^{2+}$ and Hg$^{2+}$ quench both the monomer and excimer emission of pyrene. The $^1$H NMR results indicate that Ag$^+$ ions can be selectively recognized by the triazole moieties on the receptors 35 and 36 together with the ionophoricity cavity formed by the two inverted benzene rings and sulfur atoms of the thiacalix[4]arene.

1.3. Resonance energy transfer based fluorescent probes

A fluorescence measurement which involves the emission enhancement without any spectral shift tend to be affected by factors such as probe concentration, environmental conditions (pH, polarity and temperature) and emission efficiency which can interfere with the signal output.$^{55}$ In practice, ratiometric fluorescence signals which consider the ratio of emissions at two different wavelengths are able to eliminate the influence of such factors.$^{56}$ For the development of ratiometric fluorescent probes resonance energy transfer (RET) is an efficient approach where the excitation of a donor (D) fluorophore results in emission of the acceptor (A) at a longer wavelength. Resonance energy transfer is a spectroscopic process by which energy is transferred non-radiatively between molecules over biologically relevant distances (10–100 Å). The non-radiative energy transfer from a donor to the acceptor molecule occurs either via Förster mechanism or Dexter mechanism.$^{57}$ Förster or fluorescence resonance energy transfer (FRET), is generally known as through-space energy transfer based on resonance between two oscillating dipoles of donor and acceptor.$^{58}$ On the other hand, Dexter energy transfer is known as through bond
energy transfer (TBET) mechanism which involves electron exchange between the donor and acceptor molecular orbitals.\(^{59}\)

Besides numerous applications of resonance energy transfer in biological systems, it is further an active field in molecular recognition. RET induced by guest binding is a proficient approach to design fluorescence probes which can emit at two different wavelengths with a single excitation source. Thus, it is worth to discuss some examples of fluorescent chemosensors exhibiting resonance energy transfer. For convenience, the discussion has been categorized into fluorescence resonance energy transfer (FRET) based fluorescent probes and through bond energy transfer (TBET) based fluorescent cassettes.

### 1.3.1. Fluorescence resonance energy transfer (FRET) based fluorescent probes

Förster or fluorescence resonance energy transfer is a well-known and useful spectroscopic phenomenon that has been extensively employed for a variety of biological analyses.\(^{60}\) For instance, enzyme actions in biological systems have been examined using synthetic fluorescent dyes to understand their functions. Toward this, Farber and Pack \textit{et al.} reported a synthetic fluorescent phospholipid reporter 37 which acts as a substrate for phospholipase A\(_2\) (PLA\(_2\)) that catalyzes the cleavage of the \(sn\)-2 fatty acyl bond of glycerophospholipids.\(^{61}\) Reporter 37 with donor and acceptor moieties upon excitation of donor at 505 nm led to an emission at 568 nm attributed to the FRET from donor to acceptor. However, the cleavage of probe introduced by the action of PLA\(_2\) separated the donor–acceptor pair and excitation of donor results in the emission at 515 nm. Further, the zebrafish loaded with 37 exhibits strong green fluorescence in the gall bladder as it is associated with PLA\(_2\) activity. Similarly, Schultz \textit{et al.}\(^ {62}\) developed a phospholipid based reporter 38 with phosphatidylethanolamine (PE) backbone in which the ester in the \(sn\)-1 position was altered to nonhydrolyzable ether and thus exhibits high specificity for PLA\(_2\). The
incorporation of FRET pair, 7-nitrobenzo-2-oxa-1,3-diazole amine (NBD) as donor and 2-hydroxy derivative of 9-diethylamino-5H-benzo[α]phenoxazin-5-one (Nile red) as acceptor in 38 provides a ratiometric monitoring of PLA$_2$ activity in HeLa or MDCK II cells and in living embryos during development phases. The activity of PLA$_2$ resulted in an immediate increase in green fluorescence (NBD) that was accompanied by a substantial decrease in red fluorescence (Nile red) indicating the deactivation of the FRET process as two fluorophores are spatially separated.

Nagano et al.\textsuperscript{63} reported a protein labeling probe 39 based on hydroxycoumarin as an energy donor and fluorescein as an energy acceptor having β-galactopyranosyl-protected phenol moiety. Upon excitation at 400 nm, 39 exhibits emission at around 515 nm corresponding to the acceptor fluorescein moiety and energy transfer efficiency was calculated to be >93%. The addition of β-galactosidase trigger an enzymatic (quinone methide formation) reaction that cleaves phenol protective group to give a free phenol and ultimately remove the acceptor moiety. Thus, fluorescence related to the donor moiety at 460 nm was observed which indicates the protein labeling as well as its detection. In addition to this, Nagano and co-workers developed a FRET based ratiometric fluorescent probe 40 with a coumarin donor, fluorescein acceptor and two phenyl linkers having the phosphodiester moiety for monitoring the phosphodiesterase activity.\textsuperscript{64} Free 40 in aqueous buffer upon excitation at 370 nm exhibits fluorescence emission of the fluorescein acceptor at 515 nm. This indicates that energy transfer takes place from the coumarin donor to the fluorescein acceptor. The addition of phosphodiesterase to aqueous solution of 40 resulted in an increase in the donor fluorescence at 450 nm and a decrease in the acceptor fluorescence. Thus, 40 showed a large shift in its emission spectrum after the hydrolysis of the phosphodiester group by the enzyme and further may be readily applicable to other hydrolytic enzymes.

Akkaya et al.\textsuperscript{65} reported a boradiazaindacene based chemosensors 41a and 41b appended with dithiaazacrown-substituted benzaldehyde having increasing
interchromophoric distances. The presence of dithiazacrown makes the receptor 41a and 41b selective for Hg$^{2+}$ ions and thus, responsible for the emission ratio changes. Compound 41b exhibits an emission at 540 nm which upon addition of 25 µM of Hg$^{2+}$ ions undergoes small decrease in the intensity at 540 nm and appearance of a strong emission band at 600 nm. The coordination of Hg$^{2+}$ ions causes a red shift in the absorbance results in the enhanced spectral overlap between the energy donor and the acceptor and hence enhanced emission peak is observed.

Das et al. developed a ratiometric FRET based fluorescent chemosensor 42 having rhodamine 6G and dansyl moieties (Scheme 1.2). The free receptor showed the characteristic absorption and emission bands of the dansyl moiety at 310 nm and 500 nm in CH$_3$CN:H$_2$O solution. However, upon addition of Hg$^{2+}$ or Cu$^{2+}$ to the solution of 42, a new absorption band appeared at 530 nm with clear colour change. In the case of fluorescence spectrum, the addition of Hg$^{2+}$ or Cu$^{2+}$ results in the ring opening of the rhodamine moiety and thus there is FRET from the dansyl moiety to the rhodamine 6G fluorophore which generates respective emission bands at 555 and 545 nm when excited at 340 nm. A 1:1 binding mode of the host-guest was observed in both cases by the Job’s plot method. In addition, Gram-negative bacterial cells after loading with 42 and further stained with Hg$^{2+}$ exhibit fluorescence in the red channel when wavelength of 405 nm was used as excitation source. Shang and co-workers synthesized an irreversible Hg$^{2+}$ selective ratiometric fluorescence probe 43 based on fluorescein fluorophore linked to a rhodamine B hydrazide by a thiourea spacer (Scheme 1.2). Free receptor exhibits fluorescence emission at 520 nm when excited at 490 nm. Addition of Hg$^{2+}$ to 43 results in the intramolecular FRET from fluorescein to the rhodamine B moiety. This energy transfer leads to the formation of a new emission band at 591nm which corresponds to the emission of the ring-opened rhodamine B moiety. Further, the linear range and the detection limit of this
ratiometric fluorescence probe for Hg$^{2+}$ were 0.0–10.0 × 10$^{-6}$ and 5 × 10$^{-8}$ M, respectively.

A conjugated polymer sensor 44 for Hg$^{2+}$ ions based on poly[μ-(phenylene ethynylene)-alt-(thienylene ethynylene)] bearing covalently linked rhodamine B moieties has been reported by Li et al. (Scheme 1.3). The emission spectrum of 44 showed an emission band at 487 nm when excited at 441 nm. An efficient FRET process takes place from polymer backbones to the rhodamine group upon addition of Hg$^{2+}$ ions resulting in a decrease in the emission band at 487 nm with the appearance of a new emission band at 575 nm. Liu et al. developed a FRET based system 45 (Scheme 1.3) for detection and intracellular sensing of Hg$^{2+}$ ions, comprises of blue fluorescent conjugated oligoelectrolytes substituted polyhedral oligomeric silsesquioxane (POSSFF) and blue fluorescent gold nano clusters (R-AuNC). Owing to their opposite charges and efficient spectral overlap, fluorescence energy transfer from POSSFF to R-AuNC occurs upon electrostatic complex formation in aqueous solution leading to dual-emission at 435 and 565 nm (λ$_{ex}$ = 390 nm) corresponding to both POSSFF and R-AuNC, respectively. However, the emission at longer wavelength turns blue in the presence of only Hg$^{2+}$ ions because of the strong metallophilic interaction between Hg$^{2+}$ and Au$^{+}$ that quenches the red fluorescence from R-AuNC. Further, the linear fluorescence response of the hybrid complex toward Hg$^{2+}$ ions allows the quantification of mercury ions with a detection limit of ~0.1 nM in aqueous solution.

Wong et al. reported bichromophoric sensory assemblies 46a and 46b by using the combination of a rhodamine derivative and luminescent cyclometalated iridium (III) complex and evaluated their metal ion sensing behaviour as well as energy-
transfer properties.\textsuperscript{70} Free 46a exhibited a very weak fluorescence emission at 555 nm upon excitation at 365 nm as no energy transfer takes place due to the spirolactam form of rhodamine moiety. The titration with Hg\textsuperscript{2+} or acid in methanol results in the appearance of an emission band of iridium (III)-based MLCT at 675 nm owing to the energy transfer from the ring-opened to the iridium (III) luminophore. On the other hand, no significant change in the emission intensity of the iridium (III) luminophore in 46b upon the addition of acid was observed. This inefficient energy transfer in 46b is ascribed to the non-conjugated ethyl group linker between rhodamine and the iridium (III) luminophore.

A flexible 8-hydroxyquinoline benzoate (8-HQ-B) linked BODIPY-porphyrin dyad 47 was reported by Jiang \textit{et al.} which facilitates the energy transfer process and also provides versatile binding affinity for Hg\textsuperscript{2+} and Fe\textsuperscript{2+} ions.\textsuperscript{71} The Hg\textsuperscript{2+} binding promotes the energy transfer process and Fe\textsuperscript{2+}-binding just inhibits this process. The dyad 47 gives the BODIPY emission at 516 nm along with the FRET induced porphyrin emission at 650 nm when irradiated at BODIPY absorption (470 nm). The addition of Hg\textsuperscript{2+} led to the simultaneous decrease in the BODIPY emission and a slight increase in the porphyrin emission. The addition of Fe\textsuperscript{2+} resulted in the enhancement of BODIPY emission and simultaneous quenching of the porphyrin emission. The opposite influence of Hg\textsuperscript{2+}/Fe\textsuperscript{2+}-binding with the 8-hydroxyquinoline benzoate moiety in dyad 47 is further supported by the DFT calculations.

Chang \textit{et al.} reported fluorescent chemosensor 48\textsuperscript{72} based on the combination of coumarin and fluorescein moieties which is used for the ratiometric sensing of Hg\textsuperscript{2+} ions in aqueous media. The fluorescence spectrum of 48 showed strong emission ($\lambda_{\text{ex}} = 340$ nm) at ~525 nm which is characteristic of the fluorescein moiety owing to the FRET from coumarin donor to fluorescein and a relatively weak emission for the coumarin moiety around ~440 nm. The addition of Hg\textsuperscript{2+} ions results in the quenching
of the emission at 525 nm and a slightly increased fluorescence for the coumarin moiety ascribed to the Hg$^{2+}$ induced suppression of FRET between coumarin and fluorescein moiety. The detection limits for sensing Hg$^{2+}$ ions by 48 was estimated to be 9.25 μM.

Ramaiah et al.$^{73}$ synthesized receptors 49a and 49b based on dansyl and naphthalimide units linked through the polymethylene group. Upon excitation at 339 nm, both 49a and 49b exhibit dual emission centered at 375 and 525 nm, respectively, attributed to the locally excited state of the naphthalimide moiety and energy transfer-mediated emission (FRET) from the dansyl moiety. The addition of only Cu$^{2+}$ ions trammels the FRET mediated emission at 525 nm with concurrent enhancement in the emission intensity of the naphthalimide chromophore at 375 nm. 49a and 49b form stable 2:1 stoichiometric host-guest complex involving sulfonamide functionality. The dyad 49b with longer spacer length (octamethylene unit) showed efficient FRET-mediated emission with a fluorescence intensity ratio $I_{525}/I_{375}$ of 1.2 when compared to the ratio of 0.5 observed with the dyad 49a having a shorter spacer group (hexamethylene unit). Lehn et al.$^{74}$ synthesized fluorogenic system 50 comprising of naphthalene and acridine moieties. The free receptor adopts a U shape and energy transfer takes place form the donor naphthalene to the acceptor acridine moiety resulting in an emission band at 440 nm when excited at 280 nm. However, after the addition of Cu (I) ions the conformation of 50 becomes W-shape and emission corresponding to the both the naphthalene and acridine was observed.

Kim and co-workers developed a rhodamine appended dansyl ionofluorophore 51 showing Cu$^{2+}$ induced fluorescence resonance energy transfer off-on behaviour.$^{75}$ The excitation of 420 nm results in a strong emission band at 507 nm in H$_2$O:CH$_3$CN (1:9; v/v) attributed to the dansyl energy donor unit. Upon addition of Cu$^{2+}$ ions, the fluorescence spectrum shifted to 580 nm, the region of energy acceptor. The binding of Cu$^{2+}$ results in the increased overlap between emission of the energy donor and absorption of the energy acceptor which greatly enhances the intramolecular FRET,
producing the fluorescence from the energy acceptor in receptor 51. Das et al. reported naphthalimide–rhodamine derivative 52 which binds specifically to Hg$^{2+}$ or Cr$^{3+}$ in presence of large excess of other competing ions and was used as a ratiometric sensor for detection of Cr$^{3+}$ and Hg$^{2+}$ based on the FRET process involving the donor naphthalimide and the acceptor Cr$^{3+}$/Hg$^{2+}$-bound xanthene fragment.\(^7\)

By changing the mode of connection and the binding site, Duan et al. developed FRET based systems 53a–53d, comprising of a coumarin donor and a rhodamine acceptor for the selective and quantitative detection of metal ions.\(^7\) Chemosensors 53a and 53b connected by 1,2-diethylamine unit showed excellent selectivity towards Cu$^{2+}$ in CH$_3$CN and thus, fluorescence enhancement related to the acceptor part when excited at donor absorption wavelength. As the spectral overlap between the acceptor and donor of 53a was larger, the FRET efficiency of 53a (90%) is higher than that of 53b (33%). Hydrazide linked probes 53c and 53d functioned as ratiometric receptors for Cu$^{2+}$ ions in CH$_3$CN:H$_2$O (9:1, v/v) owing to the FRET process. Lin et al. constructed FRET based ratiometric fluorescent chemodosimeters 54a and 54b by using the combination of coumarin and rhodamine fluorophores connected by a piperazine linker which restricts the static fluorescence quenching and provides a suitable distance between the donor coumarin and acceptor rhodamine to ensure high energy transfer efficiency.\(^7\) Without Cu$^{2+}$ ions, the excitation of the donor results in the emission of the coumarin as the rhodamine acceptor is in the closed form corresponding to the FRET off state. The addition of Cu$^{2+}$ promoted the hydrolysis of rhodamine B hydrazide to rhodamine B responsible for the energy transfer and emission from the acceptor rhodamine moiety.

Hamachi et al. reported FRET based ratiometric Zn$^{2+}$-complexes 55a and 55b, comprising of a xanthene moiety as a FRET acceptor together with a coumarin FRET
The addition of Zn\(^{2+}\) ions to the free ligands result in the dying out of the possibilities of FRET owing to the decreased spectral overlap between the coumarin emission and xanthene absorption ascribed to the formation of a 2:1 Zn\(^{2+}\)-ligand complexes. Further, the titration of Zn\(^{2+}\)-ligand complexes, 55a and 55b in aqueous media with a range of nucleotides resulted in turn-on of the FRET process from the coumarin to the xanthene moiety which indicates that 55a and 55b bind strongly to polyphosphate derivatives including nucleoside triphosphates, nucleoside diphosphates and pyrophosphate.

Yi et al. reported a naphthalimide–rhodamine derivative 56 as a fluorescence turn-on chemosensor for Sn\(^{4+}\) ions. Upon excitation at 420 nm in ethanol–HEPES buffer, 56 shows fluorescence related to the naphthalimide at 523 nm. The addition of Sn\(^{4+}\) to a solution of 56 caused marked enhancement of emission band at 580 nm and the intensity of the fluorescent peak at 523 nm gradually decreased. This is ascribed to the FRET process from naphthalimide to the rhodamine. Further, the ratio of emission intensity (I\(_{580}/I_{523}\)) varied from 0.72 to 2.46 with an association constant of 4.73 ± 0.05 × 10\(^3\) M\(^{-1}\).

Lin et al. developed a ratiometric probe 57 based on the combination of the BODIPY dye and rhodamine fluorophore for the sensitive detection of Au\(^{3+}\) ions. Upon excitation at 470 nm, free probe in phosphate buffer/C\(_2\)H\(_5\)OH showed emission at 514 nm attributed to the BODIPY unit. The addition of Au\(^{3+}\) leads to the decrease in the emission intensity of the BODIPY and simultaneously, a significant enhancement in the rhodamine emission peak at 594 nm. The Au\(^{3+}\) mediated transformation of a thioamide-phenyl-substituted alkyne to 5-ketothiazole facilitate the ring-opening reaction of the rhodamine which favors the FRET from BODIPY to the rhodamine fluorophore. A turn-on fluorescent probe 58 for Fe\(^{3+}\) based on the rhodamine conjugated with quinoline donor group was reported by Meng and Zhu et
The addition of Fe$^{3+}$ ions to the solution of 58 led to the appearance of a new emission band at 584 nm and the emission at 482 nm also changed along with the fluorescence colour changing from pale green to light orange. This could be attributed to the FRET from conjugated quinoline to rhodamine acceptor. Davidson and Zhu et al. selected 5-(4-methoxystyryl)-5'-methyl-2,2'-bipyridine as the FRET donor and diamino-substituted naphthalenediimide (NDI) as the FRET acceptor to synthesize Zn$^{2+}$ fluorescent chemosensors, 59a and 59b. The fluorescence spectra of 59a and 59b when excited at 400 nm, exhibit a weak emission of NDI at 630 nm. Upon gradual addition of Zn$^{2+}$, the emission band at 630 nm shows remarkable fluorescence enhancement. In mechanism, the Zn$^{2+}$ coordination at the bipy moiety results a bathochromic shift of donor emission to enable a significant spectral overlap and hence fluorescence resonance energy transfer.

Anzenbacher, Jr. et al. reported two approaches to signal amplification based on 2,3-di(1H-2-pyrrolyl)quinoxaline (DPQ) appended with a chromophore capable of resonance energy transfer to the parent sensor. The visual inspection of the solutions of sensors 60a and 60b illuminated by black light (365 nm) shows enhanced emission for 60a and 60b compared to the parent compound (DPQ). The presence of pyrene antenna moieties (donor) results in significant increase in the emission from the DPQ core ($\lambda_{\text{max}}$ 495 nm) in case of 60a while in sensor 60b emission is shifted to $\lambda_{\text{max}}$ 550 nm. In sensor 60b, the signal amplification is achieved through effective excited state delocalization as confirmed by the shift of emission wavelength from $\lambda_{\text{max}}$ 495 nm to $\lambda_{\text{max}}$ 550 nm. Further, the additions of fluoride, pyrophosphate and phosphate to 1 µM solutions of sensors 60a and 60b caused significant quenching of the fluorescence emission intensity. Li et al. synthesized FRET off-on based chemosensor 61 comprising of 1,8-naphthalimide donor and a rhodamine acceptor linked by a flexible multi-chelating site. Without any metal ion, energy transfer is suppressed and only the yellow emission of the 1,8-naphthalimide donor is observed at 536 nm. The addition of Cr$^{3+}$ ions results in the formation of 1:1 host-guest complex and induces ring
opening reaction of the rhodamine moiety which leads to emission at 594 nm of rhodamine moiety due to energy transfer.

Bojinov et al. reported a bichromophoric system 62 based on rhodamine 6G and 4-(N-methylpipеразинил)-1,8-naphthalimide moieties which behaves as a wavelength-shifting FRET chromophore. The electron transfer from the N-methylpipеразинил moiety to the excited state of the fluorophore quenches the fluorescence emission of the 1,8-naphthalimide unit, leading to the off state of the system. However, the protonation of the pipеразинь N-amine restricts the electron transfer which switches on the emission of the donor. Thus, this system was used as a pH indicator. At pH 8.0, the system is weakly fluorescent due to the PET effect as well as ring closed form of rhodamine 6G. However, the spirolactam ring opening of rhodamine at pH 2.5 and removal of the PET process results in a new emission band at 562 nm upon excitation at 390 nm in water–DMF (4:1, v/v) solution, attributed to the energy transfer from the donor 1,8-naphthalimide to the ring-opened form of rhodamine.

Resonance energy transfer based sensors, 63a and 63b, which contain two naphthalimide donors and a squaraine acceptor, have been developed by Xiao and co-workers. Upon excitation at the donor absorption, an emission band corresponding to the squaraine acceptor was observed in both cases ascribed to the FRET mechanism. The nucleophilic addition of analytes (F⁻ and CN⁻ ions) to the electron deficient squaraine center hampers the spectral overlap between the donor and acceptor groups. Thus, restricts the FRET phenomenon responsible for the enhancement of the donor emission and provided the basis of ratiometric detection.

Xiao and Qian et al. coupled a BODIPY donor to the 5’ position of a tetramethylrhodamine (TMR) acceptor by using a rigid biphenyl group as the spacer to construct FRET sensors, 64a and 64b having high energy transfer efficiencies. Free probes exhibit emission band of BODIPY donor at 510 nm in ethanol/water. However, upon addition of only Hg²⁺ ions, the emission intensity at 510 nm decreased

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and the emission band of rhodamine moiety at 584 nm undergo fluorescence enhancement. Probe 64a followed the irreversible Hg$^{2+}$-promoted oxadiazole-forming reaction to induce FRET. In contrast to this, probe 64b coordinate with Hg$^{2+}$ ions in 2:1 (H:G) mode to make FRET process feasible. Further, the response of 64a towards Hg$^{2+}$ ions is very fast as compared to the 64b with a detection limit of ppb scale. Further, both the ratiometric probes are used for the imaging of Hg$^{2+}$ ions in living cells and 64a exhibited higher sensitivity and a faster response.

Hulme et al.\textsuperscript{89} presented a novel approach for detecting Cu(I) based on the 1,3-dipolar cycloaddition reaction between an alkyne 65a and an azide 65b (Scheme 1.4). They used Cu(I) ion complexed with the carboxylate anion of glutathione complex, GS$^-$-Cu(I), to catalyze formation of triazole 65 which results in the europium luminescence enhancement through the FRET from the dansyl moiety to the Eu complex.

Wang et al.\textsuperscript{90} developed a sensitive, homogeneous and real-time protocol by combining cationic conjugated polymer, poly(9,9-bis(6'-'N,N,N-trimethylammonium-hexyl) fluorine phenylene) (PFP-NMe$_3^+$) and peroxyfluor-1 with boronate protecting groups 66 to detect H$_2$O$_2$ by taking advantage of the amplified fluorescence-quenching ability of cationic conjugated polymers (Scheme 1.5). The absence of electrostatic interactions between the cationic PFP-NMe$_3^+$ and the neutral 66 in the free protocol keeps 66 well separated from the PFP-NMe$_3^+$ and as a results it shows emission band at 425 nm when excited at 380 nm. The addition of H$_2$O$_2$ results in the
hydrolysis of boronic ester groups to produce anionic fluorescein moieties and thus, strong electrostatic interactions between the PFP-NMe$_3^{3+}$ and fluorescein. Therefore, efficient fluorescence quenching of the PFP-NMe$_3^{3+}$ occurs which is responsible for the fluorescence resonance energy transfer (FRET) from the PFP-NMe$_3^{3+}$ to the fluorescein.

Ono et al.$^{91}$ developed an oligodeoxyribonucleotide (ODN)-based sensing system $^{67}$ that showed selectivity toward Hg$^{2+}$ ions in aqueous solution (Scheme 1.6). The sensor $^{67}$ consists of an ODN functionalized with a fluorophore (fluorescein derivative) and a quencher moiety (dabcyl derivative) at the 3’ and 5’-termini, respectively and further divided into thymine-rich mercury-binding sequence and a linker sequence. In the presence of Hg$^{2+}$ ions, a hairpin structure emerge out due to the mercury-mediated base pairs (T–Hg–T) formation between thymine residues from two Hg-binding sequences in the ODN. Thus, termini of the ODN are brought close to each other which lead to fluorescence resonance energy transfer process between the fluorophore and quencher moieties which result in significant quenching of the fluorescent emission relative to the random coil. The emission intensity of $^{67}$ decreased as the concentration of Hg$^{2+}$ ions increased and exhibited a linear correlation between the emission intensity and concentration of Hg$^{2+}$ ions in the concentration range $40 \text{ nm} < [\text{Hg}^{2+}] < 100 \text{ nm}$.

A BODIPY-rhodamine FRET off–on system $^{68}$ as a ratiometric and intracellular Hg$^{2+}$ sensor was reported by Qian and co-workers.$^{92}$ Excitation at donor absorption (488 nm) in ethanol/water mixture results in the fluorescence emission of BODIPY
moiety at 514 nm. The emission at 514 nm decreases regularly upon addition of Hg\(^{2+}\) ions among various metal ions tested and a new emission band at 589 nm corresponding to the rhodamine moiety appeared. This confirms that the addition of Hg\(^{2+}\) ions promoted the formation of the ring-opened compound hence, energy transfer with fluorescence colour change from green to orange. The energy transfer efficiency between BODIPY and rhodamine in the presence of Hg\(^{2+}\) ions was found to be 99%. Kaewtong et al. reported a reversible ditopic receptor 69 based on rhodamine-naphthalene capable of undergoing excimer-fluorescent resonance energy transfer (Em-FRET).\(^{93}\) Addition of Cu\(^{2+}\) ions to the solution of 69 induced a ring-opened conformation of spirolactam and thus, the fluorescent spectrum of 69 shifted from excimer emission at 490 nm to 584 nm, the region of the energy acceptor (Em-FRET ON). Further, the selectivity of 69 for anions in the presence of Cu\(^{2+}\) was carried out. The addition of CH\(_3\)COO\(^-\) to the solution of 69-Cu\(^{2+}\) resulted in the colour change from pink to colourless and the fluorescence and UV-vis spectra immediately turned off (Em-FRET Off), which indicates a closed-ring form of rhodamine. The addition of other anionic species (Cl\(^-\), Br\(^-\), I\(^-\), NO\(_3\)\(^-\), ClO\(_4\)\(^-\)) did not introduce any significant change in the fluorescence behaviour of 69-Cu\(^{2+}\) complex.

A 4-(N,N-dimethylamino)benzamide–fluorescein based fluorescence probe 70 for CN\(^-\) ions was reported by Guo and co-workers.\(^{94}\) The presence of activated hydrazine functionality facilitated the nucleophilic attack by CN\(^-\), followed by fast acidic phenol proton transfer to the developing nitrogen anion which ultimately triggers the ring-opening process in the fluorescein unit to generate a long-wavelength fluorescein monoanion fluorophore that can act as the energy acceptor. Without CN\(^-\) ions, the fluorescein moiety exists in non-fluorescent spirolactone form and only the blue emission of donor 4-(N,N-dimethylamino)benzamide at 450 nm was observed in DMF/H\(_2\)O solution upon excitation at 350 nm. The addition of CN\(^-\) induces spirolactone opening of fluorescein moiety which results in emission of fluorescein owing to FRET at 550 nm.
Zeng et al. utilized a solid film-based approach for the ratiometric detection of Hg$^{2+}$ ions in aqueous media (Scheme 1.7). The solid film consists of a fluorescent dye, nitrobenzoxadiazolyl derivative (NBD) as a donor which was covalently confined in the film while an acceptor probe, spirolactam rhodamine derivative was grafted onto the film surface as the receptor for Hg$^{2+}$ ions. The film exhibits a fluorescence emission at 528 nm which clearly indicated that the rhodamine moiety is in ring closed form. However, FRET in the presence of mercury ions occurred from the donor layer to the acceptor which is on the film surfaces owing to the fact that the addition of Hg$^{2+}$ opened the spirolactam form of rhodamine moiety and an emission related to the acceptor was observed at 588 nm.

**Scheme 1.7** Solid film-based approach for the ratiometric detection of Hg$^{2+}$ ions.

1.3.2. Through bond energy transfer (TBET) based fluorescent cassettes

In contrast to the fluorescence resonance energy transfer (FRET), through bond energy transfer (TBET) is usually a much faster mechanism where donor transmits resonance energy to an acceptor predominantly *via* twisted π-electron system. Energy transfer *via* through space required spectral overlap between the emission of donor and adsorption of acceptor which essentially restricts the construction of FRET based systems as well as Stoké’s shift in these systems. In this concern, through-bond energy transfer is much beneficial as there is no known requirement of spectral overlap.

In the past, the phenomenon of through bond energy transfer was used for the development of oligomeric conjugated materials and models for biological systems. For example, Lindsey and co-workers developed a TBET cassette comprising of BODIPY dye as an optical input and a free base porphyrin as an optical output connected by a linear array of three zinc porphyrins as a model for photosynthesis. Burgess and co-workers developed a number of through-bond energy transfer cassettes for the labeling of biological systems where donor connected *via* a conjugated linker to acceptor components with functional groups for...
the attachment of the cassettes to the biological molecules. The rigid conjugated linker prevents the donor and acceptor parts from becoming flat or planar and thus these cassettes did not behave as a single conjugated dye. These energy transfer cassettes exhibit desirable spectral benefits such as large emission and Stokes shifts as this type of systems absorb at a wavelength characteristic of a donor and then emits via acceptor part at longer wavelength. Such spectral benefits are difficult to obtain by using a single fluorophore system. Thus, more attention has been paid to the development of multi-fluorophore systems with donor-acceptor pairs for the use of fluorescent dyes in biotechnology, photosynthetic systems and material science. However, the use of such systems for the fluorogenic determination of metal ions is not much explored till now.

Kumar and co-workers synthesized rhodamine-pentaquinone cassettes, 79 and 80 which undergo through bond energy transfer in the presence of Hg\(^{2+}\) ions with nearly 100% energy transfer efficiency and large pseudo-Stokes shifts of more than 200 nm. The attachment of pentaquinone moiety with rhodamine through a conjugated spacer like benzene provides a system for the phenomenon of through bond energy transfer. The absorption spectrum of 79 and 80 in the presence of Hg\(^{2+}\) show bands characteristic of both the donor and acceptor components which indicate that the donor and acceptor moieties in both compounds are interacting with Hg\(^{2+}\) ions independent of each other and thus, these compounds behave like cassettes and not as...
planar totally conjugated dye. Whereas, the fluorescence spectrum of 79 and 80 upon addition of Hg$^{2+}$ ions showed emission corresponding to the rhodamine acceptor owing to the energy transfer from pentaquinone to rhodamine moiety. Further, 79 and 80 operate well in the pH range of 4.0−7.0 for the detection of mercury ions. In addition, the potential biological application of 79 and 80 was carried out for the in vitro detection of mercury ions.

Shen et al. reported TBET cassettes 81a and 81b comprising of 5-(quinolin-2-yl)thiophen-2-yl as an energy donor linked to a BODIPY or NIR tetrastyrylsubstituted BODIPY acceptor. These cassettes exhibit fluorescence emission with energy transfer efficiency up to 99% and large pseudo Stokes shift of about 400 nm. Upon excitation at 334 nm strong emissions typical for the BODIPY acceptor at 732 nm for 81b and 524 nm for 81a, were observed as the emission from the donor moiety in both cassettes is almost quenched completely because of the TBET process. The addition of Fe$^{3+}$ ions result in the quenching of fluorescence from the acceptor moiety in both cases ascribed to the inhibition of the energy transfer from 2-(thiophen-2-yl)quinoline to the BODIPY after binding with Fe$^{3+}$. Recently, Das et al. reported a coumarin donor–rhodamine acceptor based chemosensor 82 which exhibits PET coupled TBET behaviour with Hg$^{2+}$ ions. Free receptor in MeOH−HEPES buffer exhibits a weak emission band at 405 nm when excited at 320 nm. Upon addition of Hg$^{2+}$ ions, a new emission band appears at 582 nm related to the energy acceptor part. The binding of Hg$^{2+}$ ions results in the inhibition of the PET process which enabled
the coumarin donor to take part in the energy transfer process responsible for the fluorescence emission of ring-opened rhodamine. A 1:1 binding stoichiometry of host-guest species was confirmed from Job’s plot and mass analysis. The association constant for the Hg$^{2+}$-82 was found to be $(7.0 \pm 0.5) \times 10^4$ M$^{-1}$. Further, probe 82 was applied for the intracellular imaging of Hg$^{2+}$ ions.

1.4. Reaction based fluorescent probes

Fluorescent chemosensors have been developed on the basis of non-covalent interactions between the host and guest molecules. However, the use of reaction-based fluorescent probes, also known as chemodosimeters has also been research area of significant interest.$^{104}$ The approach involves the use of irreversible chemical reactions induced by a target analytes to generate observable optical changes. Czarnik et al. reported a pioneering work for the sensing of Cu$^{2+}$ ions utilizing a rhodamine B hydrazide 83 through the ring opening reaction of rhodamine moiety (Scheme 1.8)$^{105}$ This reactive probe can selectively detect Cu$^{2+}$ ions and further undergoes Cu$^{2+}$ promoted hydrolysis to produce rhodamine B as a fluorescent product. This initial work resulted in a great attention to the application of reaction based fluorescent sensors for the analyte detection. Based on the Hg$^{2+}$-promoted oxadiazole cyclization of the thiosemicarbazide, Tae and co-workers reported a rhodamine based reactive probe 84 for the selective detection of Hg$^{2+}$ ions (Scheme 1.9)$^{106}$ The addition of Hg$^{2+}$ ions (1.0 equiv) to a solution of 84 in water-methanol (4/1, v/v) resulted in a 26-fold fluorescence enhancement. The interaction of Hg$^{2+}$ with 84 is accountable for the appearance of pink colour, indicating the formation of ring-opened form of rhodamine moiety. The detection limit of 84 was found to be < 2.0 ppb for Hg$^{2+}$ which is within
the Environmental Protection Agency (EPA) drinking water limits for inorganic Hg$^{2+}$ ions. A ratiometric fluorescent chemodosimeter 85 for the selective detection of Hg$^{2+}$ based on the mercury-promoted intramolecular cyclic guanylation of thiourea was reported by Tian and co-workers.\textsuperscript{107} The addition of Hg$^{2+}$ ions transformed the thiourea unit of the 85 into the weaker electron-donating imidazoline moiety responsible for a significant fluorometric and colorimetric change. Chemodosimeter 85 exhibited good selectivity and sensitivity toward Hg$^{2+}$ ions. The fluorescence emission changed from light green (530 nm) to blue (475 nm) with an isoemissive point at 510 nm. While, the absorption spectra changed from 435 to 350 nm with an isosbestic point at 391 nm.

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\begin{align*}
\text{85} & \quad \text{86} & \quad \text{87}
\end{align*}
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Kim \textit{et al.} reported chemodosimeters 86\textsuperscript{108} and 87\textsuperscript{109} based on the rhodamine 6G and Nile Blue fluorophores, respectively for Hg$^{2+}$ ions. The detection mechanism is based on the Hg$^{2+}$-induced cyclic guanylation of thiourea. Probe 86 exhibited high selectivity and sensitivity for Hg$^{2+}$ ions even in the presence of both blood plasma and albumin samples.

Mercury-induced thiol elimination reaction is another strategy for the development of reaction based fluorescent probes for Hg$^{2+}$ ions. Mercury ions are known to convert mercaptan protected aldehyde groups into free aldehydes. By employing this reaction mechanism a number of Hg$^{2+}$ selective sensors, for example 88\textsuperscript{110} and 89\textsuperscript{111} with fluorescence turn-on response or spectral shift have been reported in the literature. Probe 90 utilizes Hg$^{2+}$-induced thiol elimination followed by intermolecular cyclization reaction with phenylene diamine to introduce fluorescence changes.\textsuperscript{112} Similarly, in the case of chemodosimeter 91, the addition of Hg$^{2+}$ resulted
in the thiol elimination reaction and leading to the recovery of the intramolecular charge transfer (ICT) process which ultimately switches on the fluorescence.\(^{113}\)

Because of the affinity of Hg\(^{2+}\) toward alkynes, Hg\(^{2+}\) can catalyze the hydration of alkynes to their corresponding ketone. This mechanism has been used to generate excellent Hg\(^{2+}\) ions chemodosimeters. Koide \textit{et al.} reported a non-emissive fluorescein derivative 92 containing an alkyne moiety that detects Hg\(^{2+}\) based on oxymercuration-elimination reaction.\(^{114}\) Addition of Hg\(^{2+}\) resulted in the formation of a new compound which exhibited 219-fold enhancement in fluorescence intensity. The same group further developed Hg\(^{2+}\) sensitive probe 93, taking the advantage of vinyl ether oxymercuration mechanism for detection of Hg\(^{2+}\). The addition of Hg\(^{2+}\) to 93 promoted the hydrolysis of the non-fluorescent vinyl ether to highly fluorescent product.\(^{115}\) In the case of probe 94, the Hg\(^{2+}\)-promoted hydrolysis of vinyl ether resulted in the excited-state intramolecular proton transfer (ESIPT) process responsible for ratiometric fluorescence behaviour from blue to cyan by virtue of the ESIPT process.\(^{116}\) Mercuration of a fluorescence dye can be used for designing Hg\(^{2+}\)-selective chemodosimeter. For example, the 2-hydroxy derivative of Nile Red 95 exhibits a significant fluorescent change with Hg\(^{2+}\) ions in aqueous environments by selective dimercuration at the 1,6-positions.\(^{117}\)

Chang \textit{et al.} reported turn-on fluorescent probes for hydrogen peroxide (H\(_2\)O\(_2\)) based on the chemical conversion of arylboronates to the phenols by the reactive oxygen species which proceeds \textit{via} addition followed by rearrangement. For example, a fluorescein based diboronate 96 reacts with H\(_2\)O\(_2\) to generate the fluorescein.\(^{118}\) This oxidative cleavage is highly selective to H\(_2\)O\(_2\) over the other reactive oxygen species. They further elaborated this work by introducing the lipophilic phosphonium cation to develop an advanced probe 97 that targeted mitochondrial H\(_2\)O\(_2\) in living HeLa cells.\(^{119}\)

Nagano \textit{et al.} reported a number of diaminofluorescein derivatives 98-100 as fluorescent indicators for NO.\(^{120}\) Initially, the NO-sensitive probe is non-emissive in nature owing to the photoinduced electron transfer (PET) effect from \(\sigma\)-diamine
moeity in the excited state. However, after reaction of NO with o-diamine, cyclization occurs to produce triazole moiety which suppress the PET effect and the fluorescence of the probe is restored. The fluorescence quantum efficiencies in the case of 98-100 are increased more than 100 times after the transformation of 98-100 into the highly green-fluorescent triazole form and offer a simple protocol for the direct detection of NO with detection limit 5 nM. Further, derivative 98 with acetate groups can be used for real-time imaging of NO with fine temporal and spatial resolution in the living system. Based on the similar NO-induced diamine cyclization strategy, Xiao and Jin et al. reported a lysosome-targeted two-photon fluorescent probe 101 based on the combination of NO-capturing o-phenylenediamine, lysosome targeting (aminoethyl)morpholine along with two-photon fluorophore naphthalimide. Probe 101 exhibited fluorescence turn-on selectivity for NO over other reactive oxygen species with a nanomolar-scale limit of detection. The lower cytotoxic nature of probe 101 favoured the lysosomal localization as well as capturing of endogenous NO in lysosomes of macrophage cells.

Chang and co-workers reported rhodamine-based probes 102a and 102b with azide functionalities for selective fluorescence turn-on detection of H₂S (Scheme 1.10) in the solution as well as in intracellular systems via H₂S-mediated reduction of the aryl azide to the corresponding fluorescent aryl amine. The probes exhibit high selectivity for H₂S over other biologically reactive sulphur, oxygen and nitrogen species. The biological applications of these probes to visualize changes in H₂S levels in the living cells provide a potentially powerful approach for probing H₂S chemistry in biological systems.

Wang et al. synthesized a dansyl azide based probe 103 for H₂S as the reduction of sulfonyl azide by H₂S into sulfonamide triggered a change in the fluorescence
properties of the dansyl moiety.\textsuperscript{123} Probe 103 because of the azide group is non-emissive in nature. However, upon addition of hydrogen sulfide, the solution of 103 showed a strong fluorescence enhancement ascribed to the formation of dansyl amide. The addition of 25 µM of hydrogen sulfide led to a 40-fold fluorescence enhancement in 20 mM sodium phosphate buffer (pH 7.5) with the detection limit of 1 µM level. Han \textit{et al.} reported a fluorescence probe 104 based on the heptamethine cyanine platform for cellular H\textsubscript{2}S where intramolecular charge transfer (ICT) mechanism results in absorption and emission shifts upon reaction with H\textsubscript{2}S.\textsuperscript{124} The H\textsubscript{2}S mediated reduction of azide to amine modifies an electron-withdrawing azido group into an electron-donating amino group which switches a system for the ratiometric detection of H\textsubscript{2}S. Cho \textit{et al.} reported a two-photon (TP) probe 105 derived from 7-(benzo[d]thiazol-2-yl)-9,9-(2-methoxyethoxy)ethyl-9\textit{H}-fluorene (BMF) as the reporter and azide moiety as the H\textsubscript{2}S reaction site.\textsuperscript{125} Probe showed a 21-fold emission enhancement with H\textsubscript{2}S and exhibit good selectivity for H\textsubscript{2}S over other the biologically relevant reactive sulfur (RSS), oxygen (ROS) and nitrogen species (RNS). Therefore,
the probe can detect the intracellular sulfide without interference from other biologically relevant analytes under physiological pH conditions.

Pluth et al. reported a fluorescent probe 106 based on naphthalimide fluorophore that was selective for H$_2$S over cysteine, glutathione, and other reactive sulphur, nitrogen and oxygen species. The detection strategy involves the masking of a fluorogenic amine as an azido or nitro group, followed by the mild reduction with H$_2$S which regenerates the parent amine responsible for the fluorescence turn-on changes. Lin and co-workers synthesized a new fluorescent chemosensor 107 for H$_2$S based on a phenanthroimidazole scaffold. The fluorescence of the phenanthroimidazole dye was quenched by the presence of azido group. However, the H$_2$S mediated reduction of azide to amine resulted in a significant emission enhancement. Further, the probe is suitable for monitoring intracellular H$_2$S levels. Han et al. reported the selective detection of hydrogen sulfide based on the 7-o-2′-(azidomethyl)benzoyl-4-methylcoumarin fluorophore 108 where H$_2$S mediated reductive removal of the 2′-(azidomethyl)benzoyl generate a fluorescent species 7-hydroxy-4-methylcoumarin which showed emission at 450 nm. Further, the hydrogen sulfide detection in living cells was carried out by using probe 108 suggesting the selectivity of 108 towards H$_2$S in living cells over a broad variety of chemical and biological species.

1.5. Observations drawn from literature

From the above review of literature, it is clear that:-

- Thiacalix[4]arene scaffold is a unique host with enormous possibilities of functionalization at the upper as well as at lower rim.
- The chemically modified thiacalix[4]arene scaffold with different types of fluorophores and binding sites can act as excellent host for different kinds of guest species.
- Resonance energy transfer induced by guest binding is a proficient approach to design ratiometric fluorescence probes as they can emit at two different wavelengths with a single excitation source. Thus, independent of factors, such as probe concentration and environmental conditions, permitting the signaling rationing, increase the dynamic range and improving the sensitivity of the method.
The detection method based on the irreversible chemical reaction between the probe molecule and the analyte is a proficient methodology for the development of fluorescent chemosensor for an analyte owing to its highly selective nature.

In addition, fluorogenic moieties such as naphthalimide, rhodamine and fluorescein can be appended with different types of recognition sites for the detection of various types of analytes.

Thus, keeping in view of these considerations, in the present investigation we have designed and synthesized fluorescent chemosensors based on the thiacalix[4]arene scaffold, resonance energy transfer mechanisms and chemosensors having different types of recognition or reaction sites for various types of analytes. The results of our findings have been divided in five chapters.

**Chapter 2: Thiacalix[4]arene based fluorogenic receptors**

**Chapter 3: Molecular switches based on thiacalix[4]arene**

**Chapter 4: Resonance energy transfer based fluorescent probes**

**Chapter 5: Reaction-based fluorescent probes**

**Chapter 6: Naphthalimide based chemosensor for Zn$^{2+}$, pyrophosphate and H$_2$O$_2$: Sequential logic operations at the molecular level**

**References**


Chapter 1

Introduction & review of literature


Chapter 1: Introduction & review of literature


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