Synopsis

The thesis entitled **'Engaging Small Organic Molecules and Self-Assemblies for 'Label-Free' Recognition of Biologically Relevant Analytes'** mainly deals with design and synthesis of various types of small molecular probes or molecular assemblies for fluorometric recognition of a large number of biologically relevant analytes in water.

The first chapter begins with the general overview of various types of biosensors and their working principles. Then we discussed about various small organic molecules or molecular assemblies based systems in optical biosensing. The strategies for designing these sensors have also been discussed in connection with frequently encountered photophysical interactions. Finally, the design principles of some biosensors were highlighted on the basis of the structural aspects of the concerned analytes.

The second chapter describes the design and synthesis of various optical probes for selective detections of different warfare materials in water.

Chapter 2A: **Cyanide Mediated Facile Michael Addition Reaction: A Simple Protocol to Distinguish 'Tabun Mimic' DCNP in Solution as well as in Vapor Phase**

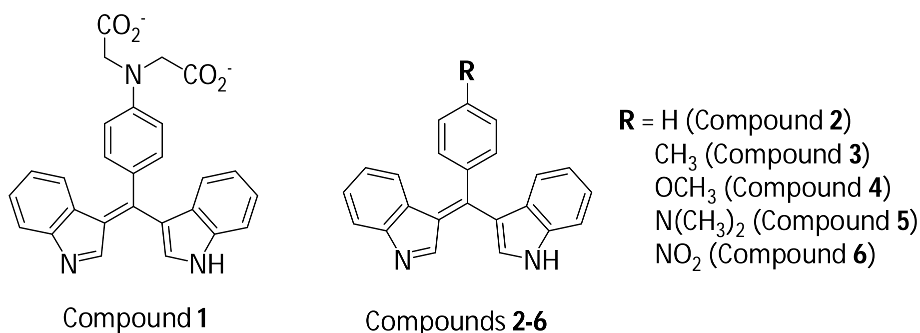

Figure 1. Structures of the molecular probes used in chapter 2A.

This chapter discussed about involvement of a series of water soluble bis-indolyl compounds for selective detection of nerve gas mimicking agent, DCNP (Diethyl Cyanophosphonate) at pH 8.0 in water. The mechanism of interaction was proposed as the bis-indole assisted hydrolysis of phosphoester nerve gas agents through hydrogen bonding interaction. Then the Michael addition of the liberated CN^- ion to the electron deficient C=C bond of the bis-indolyl moiety. This led to a remarkable color change from red to colorless at ambient condition. Further the protocol was exploited in vapor phase detection of DCNP (Tabun mimic) below its toxic level.

Chapter 2B: **Selective and Efficient Detection of Nitro-Aromatic Explosives in Multiple Media including Water, Micelles, Organogel, and Solid Support**

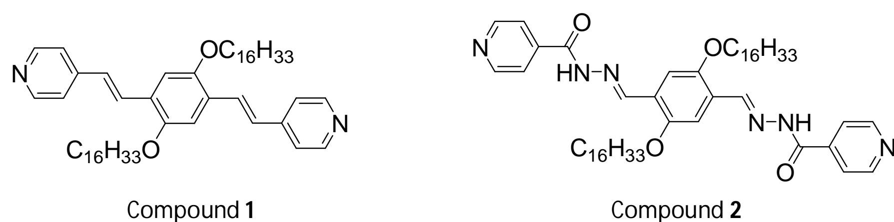

Figure 2. Structures of the molecular probes used in chapter 2B.

This chapter demonstrated development of phenylenevinylene based optical probes for the selective detection of both DNP (2,4-Dinitrophenol) and TNP (2,4,6-Trinitrophenol) in pure water as well as in micellar medium. The mechanistic investigation suggested that the initial proton transfer caused electrostatic association between the picrate anions and the protonated pyridine ends, which could further promote electron transfer from the electron-rich fluorophore moiety to the electron-deficient nitroaromatics (NACs). Thus the selectivity depends on the pK_a of the probe molecules as well as the NACs under consideration. Further portable test strips were made successfully for rapid on-sight detection purpose.

The third chapter describes identification of different enzymes in water as well as in biological fluids via disaggregation of template mediated molecular assemblies.

Chapter 3A: **Employment of ATP-Sensitive Lanthanide Ensemble for the Estimation of Creatine Kinase Level in Blood Serum and Live Cell Imaging**

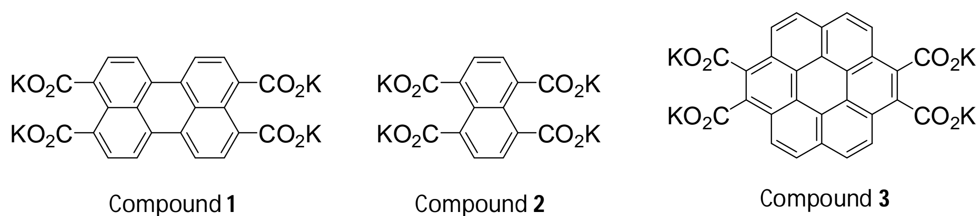

Figure 3. Structures of the molecular probes used in chapter 3A.

This chapter presents the use of in-situ generated luminescent lanthanide complexes in sensing of adenosine triphosphate (ATP) in water. The indicator tetracarboxylate salt showed rapid quenching in green molecular emission upon coordination with highly paramagnetic lanthanide ion (Gd^{3+}). The addition of ATP could displace the lanthanide ion from the vicinity of the compound and retrieved its original emission. Further this selective 'Turn-On' response of the probe towards ATP was utilized to discriminate between the live and dead

cells depending upon their ATP/ADP content. Moreover, excellent selectivity of the probe for ATP over ADP was utilized for the detection of Creatine Kinase in human blood serum samples.

Chapter 3B: **Estimation of Albumin Content in Different Biological Fluids and Monitoring Trypsin Mediated Digestion of HSA**

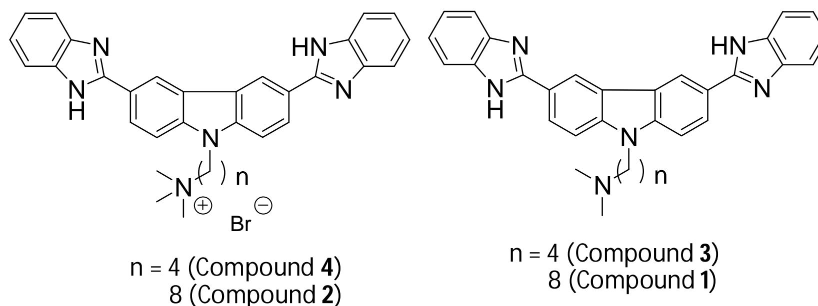

Figure 4. Structures of the molecular probes used in chapter 3B.

This chapter describes design of several amphipathic carbazole dyes, which could form thermo sensitive, pH dependent fluorescent nano-aggregates in water. This facile nanoclustering behavior of the compounds was then utilized for detection of human serum albumin (HSA). The site-specific nature of the interaction was appeared to be the key reason for the unique selectivity of the probes towards HSA over other structurally resembled albumin proteins. Further the estimation of HSA was achieved in different biological fluids like human urine, blood serum and saliva *etc*. Finally the protease mediated digestion of protein template was applied as an alternative strategy for detection of trypsin.

The fourth chapter describes fluorometric detection of different physiologically relevant monosaccharides by exploiting multi-point hydrogen bonding network.

Chapter 4A: **Analyte Induced Alteration in the Aggregation Property of Fluorescent Organic Nano-particles: A Simple Recognition Strategy for *D*-(-)-Ribose in Water**

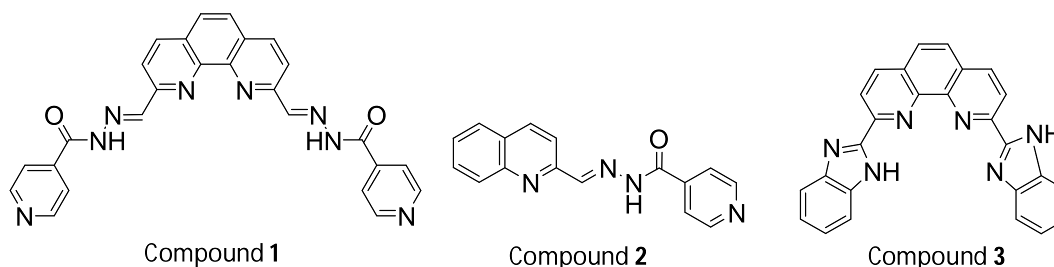

Figure 5. Structures of the molecular probes used in chapter 4A.

This chapter demonstrated selective detection of D(-)-ribose and ribose containing biomolecules (Adenosine, riboflavin etc.) in water via hydrogen bonding interactions from aceylhydrazone and pyridine units. The interaction with ribose was found to be responsible for dissociation of the preformed molecular assemblies, resulting drastic quenching in luminescent signal. Finally determination of ribose was also performed in presence of complex biological matrix like human urine.

Chapter 4B: **Restriction in Keto-Enol Tatumerization through Multipoint Hydrogen Bonding Network with *D*-(+)-Glucosamine**

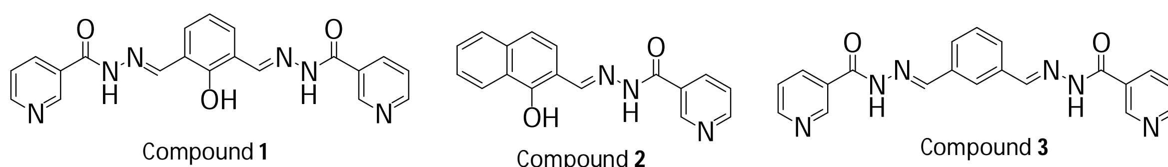

Figure 6. Structures of the molecular probes used in chapter 4B.

This chapter presents design and synthesis of different chromogenic probes by connecting nicotinic hydrazide with different α-hydroxy aromatic aldehydes via carbonyl-nucleophile addition protocol. Addition of D-glucosamine was found to modulate the keto-enol tatumerization of the molecules via formation of multi-point hydrogen bonding network. The rapid change in color from bright yellow to colorless with prominent emission quenching led detection of up to 5.3 mg/L of glucosamine in water. Then monitoring of glucosamine level was also achieved in human blood serum as well as pharmaceutical formulations. Moreover, the probes were found to be competent in detection of glucosamine even in intracellular condition.

The fifth chapter describes development of new fluorometric probes for identifying different inflammatory-response markers in water.

Chapter 5A: **Heparin Triggered Multicolor Emission Switching in Water: A Convenient Protocol for Heparinase I Assay**

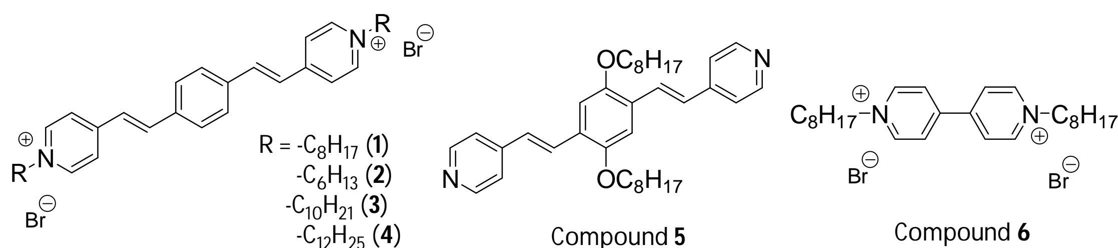

Figure 7. Structures of the molecular probes used in chapter 5A.

In this chapter, conjugated bis-pyridinium salts of phenylenevinylene (PPV) have been used for selective detection of heparin in water. The unique dose-dependent emission switching ability of the probes ensured quantitiative estimation of heparin without involving any sofisticated facilities. The higher negative charge density and preferential conformation of sugar dimers faciliated interaction between compounds and heparin. Finally, the heparin induced reversible aggregate formation was utilized to develop an alternative easy-to-operate heparinase I assay protocol.

Chapter 5B: **Application of Neurotransmitter Induced Emission Switching for Histamine Detection in Biological Fluids and Macrophage Cells**

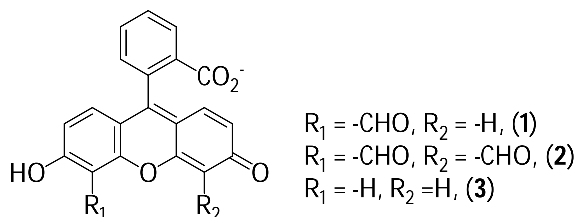

Figure 8. Structures of the molecular probes used in chapter 5B.

This chapter presents the involvement of well-known fluorescein aldehyde dyes in recognition of histamine below nanomolar concentration in water. Here, the dual mode of detection of histamine was achieved at pH 7.0 without involving any toxic metal ions. The selective response towards histamine was confirmed through secondary hydrogen bonding interaction followed by imine formation. Then the probes were also employed for detection of histamine in different biological fluid matrices (human urine and blood serum). Finally, the rise in endogenous histamine level in macrophage RAW 264.7 was identified under stimuli-responsive condition (treatement with thapsigargin).

The sixth chapter describes fluorometric detection of purine alkaloids via their preferential stacking over the electron rich planer dye molecules.

Chapter 6A: **Hydrogen Bonding Triggered Crumbling of Pyrene Excimer: A Simple Method for Detection of Uric Acid in Human Serum and Infested Grain Extracts**

This chapter deals with design and synthesis of different amphipathic pyrene molecules for nanomolar detection of uric acid in water. Hydrogen bonding interaction of uric acid with amide functional groups found to push two adjacent pyrene units away from each other, resulting into quenching of blue excimer emission. The optimized liposomal formulation derived from the doping of probe molecules into soya lecithin vesicles was further utilized for estimating uric acid content in blood serum samples. Finally, the present protocol was used for identifying the rise in uric acid content in insect-infested grain samples.

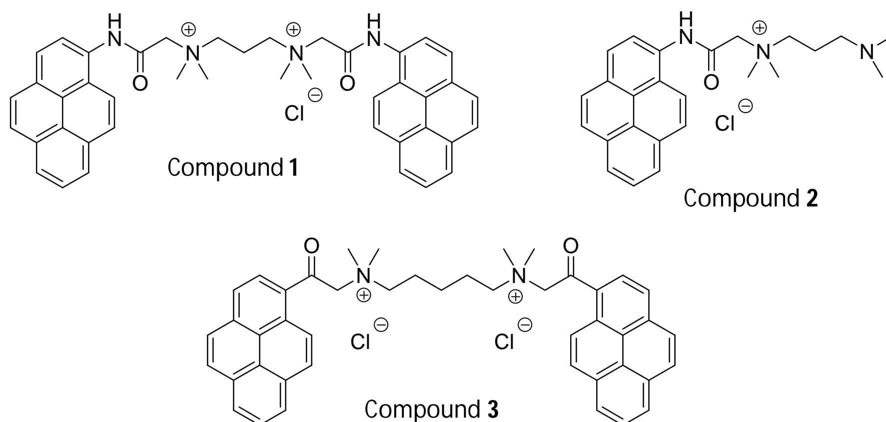

Figure 9. Structures of the molecular probes used in chapter 6A.

Chapter 6B: **Caffeine Modulated Dissociation of Carbazole Based FONs in Water: Excitation Triggered Alteration in Sensing Property**

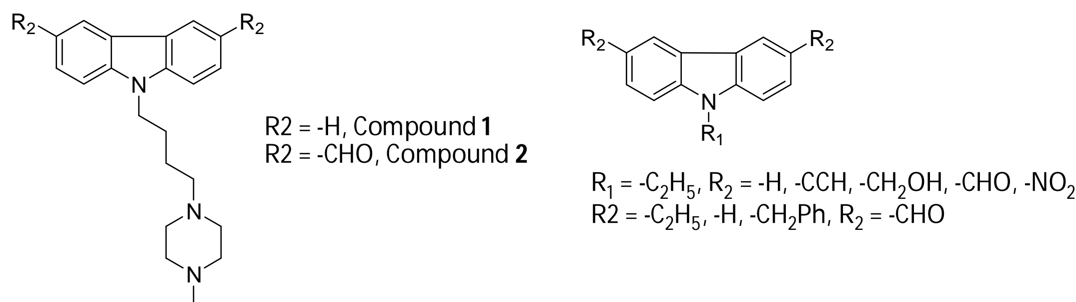

Figure 10. Structures of the molecular probes used in chapter 6B.

This chapter deals with self aggregation properties of a series of carbazole based ampiphilic dyes, which could be modulated by the micro polarity of the local environment and electronic influence from the functional groups attached to the core organic scaffold. In comparison to other structurally related purine analogs, caffeine exerted most prominent emission response due to its planar electron deficient structure. An optimal balance between selectivity and sensitivity was achieved by varying the excitation wavelengths or electron density over the carbazole core. In application, the sensor was successfully exploited to address diverse real-life problems ranging from applications in 'dope-analysis in human urine' to carbonated drinks.