Synthesis of Fluorescent Probes for Specific Recognition and Imaging Applications

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By

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Conclusion of the Thesis

The thesis entitled "*Synthesis of Fluorescent Probes for Specific Recognition and Imaging Applications*" describes the design and synthesis of new fluorescent molecules that are capable of detecting and monitoring the various analytes having biological significance. Bioimaging applications of the probes were explored through modern microscopy techniques. The first chapter is the introductory chapter, which describes the importance of fluorescent probes in analyte monitoring and bioimaging. Some of the existing fluorescent probes in the literature for the detection of biologically important analytes and the reagents that are available for imaging are briefly described.

Chapter 2 describes a chemodosimetric reagent **L** capable of specific detection of Cys in an aqueous medium as well as in Hct116 living cells. This reagent is capable of specific detection of Cys in the presence of various amino acids, competing bio thiols and ions of biological significance with a visual colour change as well as turn ON fluorescence response. This colorimetric and fluorescence response was utilized to develop simple, cost-effective test strips for Cys detection. Moreover, **L** could monitor the Cys released from a commercial Cys supplement drug NAC in hepatocytes. In addition to this, the application of **L** in the fluorescence detection of Cys residues present in proteins was demonstrated by using whey proteins extracted from cow milk.

Chapter 3 describes a coumarin-acrylate based enzyme specific reagent **CA,** capable of specific detection and quantification an important enzyme and biomarker aminoacylase-1 (ACY-1). Moreover, this reagent is also capable of detecting Cys as well as reporting the oxidative stress induced by reactive oxygen species inside the living cells. Photophysical properties of the probe were studied in detail. Application of the probe in monitoring intracellular Cys levels in normal HEK 293T cells as well as in cancerous SW 480 cells was demonstrated by confocal imaging. Moreover, **CA** could detect and quantify the ACY-1 in an aqueous solution as well as in blood serum samples. ACY-1 is an important biomarker in patients with delayed DGF and the treatment is based on the quantity of the biomarker released. The possible application of CA in quantifying ACY-1 in stimulated blood serum samples was demonstrated and the results were compared with the existing commercial reagents.

Conclusion

Chapter 4 deals with a BODIPY based hydrogen polysulfide (H_2S_n, n>1) probe **MB-S_n** capable of specific detection of H_2S_n in an aqueous medium. Moreover, this reagent is specific to endoplasmic reticulum region of the cells and it could detect the H_2S_n localized in the sub-cellular organelle. In addition to this, an important feature of this probe is its compatibility with super-resolution microscopy, precisely structured illumination microscopy (SIM). Further, application of the probe in imaging intracellular H_2S_n in RAW 264.7 macrophages was demonstrated using super-resolution SIM and 3D-SIM microscopy.

Cell membrane is a foremost barrier responsible for the movement of ions or molecules in and outside of the cell. Cell membrane plays many important roles and it is the outer most layer accessible to drug targets. Chapter 5A describes the three near-infrared emissive silicon-rhodamine (SiR) based membrane probes **SiR-C12**, **SiR-C18** and **SiR-C12DA**. Reversible ON/OFF switching behaviour of SiR with respect to the surrounding environment was utilized to develop environment-sensitive membrane probes. Among the probes, **SiR-C18** and **SiR-C12DA** showed excellent affinity to model membranes with turn ON fluorescence response. Further application of the probes in imaging plasma membrane was demonstrated in live KB cells using fluorescence microscopy. Long chain hydrophobic carbon chains present on the probes helped the unbound probes to self-assemble in the aqueous medium, and in turn, converted to non-fluorescent form. This helped to carry out live cell imaging under 'no wash' conditions.

Apoptosis or 'programmed cell death' is an important phenomenon which is happening inside the body. Apoptosis has immense biological significance and it is one of the prime methods used to determine the efficacy of any cancer treatment. Chapter 5B deals with a BODIPY based molecular rotor BPDPA-Zn, capable of reporting apoptosis through turn ON fluorescence response. Cationic DPA-Zn ligand is linked with the probe to recognise the anionic phosphatidylserine present on the apoptotic membrane surface. Photophysical properties of the molecular rotor were studied in detail. BPDPA-Zn showed strong affinity to anionic membrane surface in model membranes with turn ON fluorescence response. Further application of the probe in imaging early stages of apoptosis was demonstrated using apoptotic HeLa cells. Efficacy of the reagent was compared with the commercially available Annexin V based apoptosis probe.

Overall an attempt has been made to develop new fluorescent probes for specific recognition and imaging. Analyte responsive probes for Cys, ACY-1, H_2S_n and imaging reagents for

apoptosis and cell membrane were developed and their optical responses with the targets were studied in detail. Along with the solution studies, application of the probes for biological imaging is also explored. Some of the probes could also be used for imaging without washing steps. Wherever is possible, the results were compared with the commercially available methods or toolkits. Optical properties of the probes were fine-tuned with the synthetic modifications and the turn-ON emission probes ranging from visible to near-infrared were achieved. Some of the probes also showed excellent compatibility with the modern microscopic techniques which demand more photostable fluorophores. So, the design strategies and the results discussed in this thesis are interesting and it will certainly be useful in designing better and efficient molecules for specific detection and imaging.