Small molecule based optical monitors: synthesis, characterization, and applications in bioimaging

A Thesis

submitted

by

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for the award of the degree of

Doctor of Philosophy

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To my loving father

&

To the memory of my mother

Declaration by the Research Scholar

This is to certify that the thesis entitled "**Small molecule based optical monitors: synthesis, characterization, and applications in bioimaging**", submitted by me to the Indian Institute of Technology Mandi for the award of the degree of Doctor of Philosophy is a bonafide record of research work carried out by me under the supervision of Dr. Subrata Ghosh. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

In keeping with the general practice of reporting scientific observation, due acknowledgements have been made wherever the work described is based on the findings of other investigators.

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All the corrections suggested by the referees have been made and provided as addendum at the end of this thesis.

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Thesis Certificate

This is to certify that the thesis entitled "**Small molecule based optical monitors: synthesis, characterization and applications in bioimaging**", submitted by Mr. Sougata Sinha to the Indian Institute of Technology, Mandi for the award of the degree of Doctor of Philosophy is a bonafide record of research work carried out by him under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

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Research Guide

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Table of Contents

Chapter 6 Small molecule based Zn^{2+}- specific molecular bioimaging materials: from animal to plant cells

Abstract

Given the potential applications of sensing materials in our daily life and also the market size of such materials, starting from electronics to biomedical applications, efforts are being made throughout the world toward their development with improved properties.

On the other hand, development of cell permeable materials for staining/imaging bio-relevant analytes in living system has attracted great attention of scientific community as this non-destructive technique helps in understanding various biological events. Often chemical sensing agents with low toxicity are employed extensively for bioimaging.

The present thesis entitled 'Small molecule based optical monitors: synthesis, characterization and applications in bioimaging' contains six chapters. Chapter 1 provides brief introduction about fluorescent chemical sensors, their sensing mechanisms, and about the importance of metal ion sensing. Chapter 2 describes an approach for fabrication of fluorescent imine and the applications of such imine as a ratiometric sensor (**CRZ 1**) for paramagnetic metal ions Fe^{3+}/Cu^{2+}. Probe **CRZ 1** contains one carboxylated unit, which act as a 'locking unit' restricting the possibility of E/Z-isomerization and ESIPT making **CRZ 1** fluorescent. **CRZ 1** was found to be an efficient fluorescent chemical sensor for the selective sub-micromolar ratiometric detection of Fe^{3+}/Cu^{2+} over a wide range of metal ions. Chapter 3 mainly focuses on the utility of an unsymmetric benzophenone-based imine. A benzophenone-based half condensed Schiff base **BZP** has been applied for selective detection of Al^{3+}. Due to combined effect of photoinduced electron transfer (PET), excited state intramolecular proton transfer (ESIPT) and E/Z-isomerization, the half condensed probe was found to be almost non-fluorescent. Remarkable fluorescence enhancement was noticed upon the addition of Al^{3+} into a methanolic solution of

BZP. This probe (**BZP)** can detect micromolar level of Al^{3+} and can distinguish Al^{3+}, Ga^{3+}, In^{3+} from the absorption and emission profile. The selectivity was tested over 24 different metal and non-metal ions (Li^{+}, Na^{+}, K^{+}, NH_4^{+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Sn^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}, Sr^{2+}, Ag^{+} and Pb^{2+}).

As demonstrated in the beginning, in many occasions fluorescent chemical sensors are widely applied as bioimaging agents for tracking/imaging metal ions in living systems. Chapter 4 offers a quick look on the importance of metal ions in living systems followed by a brief history on the development of bioimaging materials for metal ion tracking/imaging. Utilization of a benzo[h]chromene derivative (synthesized following a multicomponent one pot procedure), 2-amino-4-phenyl-4H-benzo[h]chromene-3-carbonitrile **1**, as a chemical sensor for Pb^{2+} via 'turn on' fluorescence signaling was described in Chapter 5. The selectivity was tested over a range of seventeen different metal and non-metal ions. Addition of Pb^{2+} to a methanolic solution of **1** changed the weak blue emission of **1** to a glowing green emission along with a prominent red shift (26 nm) in the emission band. Sensor **1** can easily penetrate the cell membrane without causing any harm to the living cells and can image Pb^{2+} inside living system. Chapter 6 describes our systematic approach towards the development of cell permeable molecular probes with higher wavelength excitation and emission profile for imaging of zinc in living systems. Probes such as **Tris 2**, **L5** and **TRZ** were found to be efficient fluorescent chemical sensors for zinc in biofriendly medium. All these three probes were non-fluorescent initially. The metal-induced turn-on fluorescence was used as a potential tool to image zinc in living cells, both *in vitro* and *in vivo*.