

Abstract

Squaraine based fluorescent probes for sensing and imaging of various bio-analytes

The creation of novel bioanalytical tools for the detection and monitoring of a range of important target substances and biological events *in vivo* and *in vitro* is a great challenge in clinical diagnostics and chemical biology. Among the various techniques available, optical assay based on fluorescent probes and photoacoustic materials allows direct visualization of complex biological structures and processes with high temporal and spatial resolution. In this context, design of small molecular dyes and exploration of new mechanisms for the detection of various analytes with improved sensing capability is of interest among researchers. Recently, recognition-driven disassembly of molecular self-assembly has been used as a novel sensing mechanism. The first chapter of the thesis gives an overview of the recent developments in the construction of fluorescent probes based on small molecules and their assemblies for sensing and imaging of various analytes.

The Chapter 2 of the thesis deals with the design and synthesis of a water soluble squaraine dye (**USq**) for the real time *in vivo* and *in situ* fluorescence and photoacoustic bimodal signaling of amino thiols in living animals. **USq** exhibits excellent solubility in aqueous conditions. Since they did not form aggregates in aqueous condition, it exhibited intense absorption and fluorescence properties with a maximum absorption at 670 nm. These signals selectively disappear in the presence of thiols. In addition, the excellent cell permeability, low cytotoxicity and high stability in aqueous conditions inspired us to explore the dye for the real time imaging of biothiol variations in a living system. We utilized **USq** for establishing a known metabolic activity of modulating the amino thiol production by altering the food intake. The experiments performed with live mice in fasting condition and post-food conditions is well matched with our expectations implying the versatility of our system in understanding the metabolic activity in a living system.

In the third chapter, we have demonstrated the versatility of an organic dye nanoparticle (**Sq-NPs**) for the sensing of serum albumin protein (SAP) in a pool of other biomolecules. **Sq** dye in its native molecular form is reactive to a variety of thiol-containing molecules. However, when the dye self-assembles to form nanoparticles, only SAP could selectively interact with the dye thereby opening the access for a thiol attack. Thus, the dormant fluorescence moiety present in the **Sq** dye gets activated latently, allowing the specific sensing of SAP by “turn-on” green fluorescence. The fact that this selective covalent modification of SAP is achieved only with the self-assembled system and not with the monomeric dye does make the **Sq-NPs** a selective supramolecular fluorescent sensor. This selective response of the dye nanoparticles allowed detection and quantification of HSA in blood serum with a sensitivity limit of 3 nM. The described self-assembly approach using a small organic NIR dye having a

dormant fluorophore, which is latently activatable through a nucleophilic attack is a model example for empowering a small molecular fluorophore to a reaction-specific nanosensor by self-assembly. (*J. Am. Chem. Soc.* **2014**, 136, 13233–13239).

In the fourth chapter, we have designed an array of dye-protein hybrid sensors whose interaction can be accurately triggered with specific pH values, which is used as an ultra-sensitive probe for compartmental pH sensing. The nanosensor array is constructed using different ratios of a NIR squaraine dye nanoparticles and a protein, BSA. The dye noncovalently interacts with the protein at lower pH and covalently at higher pH triggering two distinguishable fluorescence read outs at 480 and 700 nm. The reversible dye-protein interaction allows ratiometric monitoring of minute spatial and temporal dynamic pH variations in cells with high sensitivity as demonstrated by the fluorescence imaging of pH variations in HeLa cells with a 1:6 dye-protein hybrid sensor.

List of publications

1. Self-Assembled Near-Infrared Dye Nanoparticles as a Selective Protein Sensor by Activation of a Dormant Fluorophore **P. Anees**, S. Sreejith & A. Ajayaghosh *J. Am. Chem. Soc.* **2014**, 136, 13233–13239.
2. A. Ajayaghosh & **P. Anees**. A Squaraine Based Fluorescent Probe and a Process for the Preparation thereof. WO 2015/029050 A1, **2015**.
3. Near-IR Squaraine Dye-Loaded Gated Periodic Mesoporous Organosilica for Photo-Oxidation of Phenol in Continuous-Flow Device. P. Borah, S. Sreejith, **P. Anees**, N. V. Menon, Y. Kang, A. Ajayaghosh & Y. Zhao. *Science Advances*. **2015** (accepted).
4. Heteroaromatic Donors in Donor–Acceptor–Donor Based Fluorophores Facilitate Zinc ion Sensing and Cell Imaging. S. Sreejith, K. P. Divya, P. Jayamurthy, J. Mathew, V. N. Anupama, D. S. Philips, **P. Anees** & A. Ajayaghosh. *Photochem. Photobiol. Sci.* **2012**, 11, 1715–1723.
5. A Zn²⁺-Specific Fluorescent Molecular Probe for the Selective Detection of Endogenous Cyanide in Biorelevant Samples. K. P. Divya, S. Sreejith, B. Balakrishna, P. Jayamurthy, **P. Anees** & A. Ajayaghosh. *Chem. Commun.* **2010**, 46, 6069–6071.
6. Tailor-made dye-protein hybrid nanosensor array for segmental pH sensing and bioimaging. **P. Anees**, J. Joseph, S. Sreejith, N. V. Menon, S. W. K. Yu, Y. Kang, Y. Zhao & A. Ajayaghosh. (To be communicated).
7. Real Time Monitoring of Amino-thiol Level in Blood Using a Near Infrared Dye Assisted Deep Tissue Fluorescence and Photoacoustic Bimodal Imaging. **P. Anees**, K. V. Sudheesh, P. Jayamurthy, R. C. Arun, R. V. Omkumar, & A. Ajayaghosh (To be communicated).