Fluorescence anisotropy in deciphering biophysical properties of membranes and proteins

Membranes and proteins are integral components of a cell. They perform a wide range of function within the cells. To get insights into the fundamental principles underlying their function, scientists are taking help of various fluorescence-based spectrometry techniques. Fluorescence, because of its high sensitivity and high specificity, allows the researchers to explore the macromolecular dynamics at single-molecule level in real time. Scientists mostly use techniques such as fluorescence quenching, fluorescence polarization, fluorescence-lifetime measurement, fluorescence microscopy, fluorescence correlation spectroscopy and time-resolved fluorescence, to study the biophysical properties of an analyte. Meanwhile, fluorescence anisotropy (FA) has created a unique niche for itself because of its wide range of applications in biochemical research.

FA measurements have been used to study protein folding kinetics. This can help scientists get new clues on how the proteins, like tau and amyloid-β within the neurons, misfold and give rise to diseases like Alzheimer, Parkinson and Huntington.

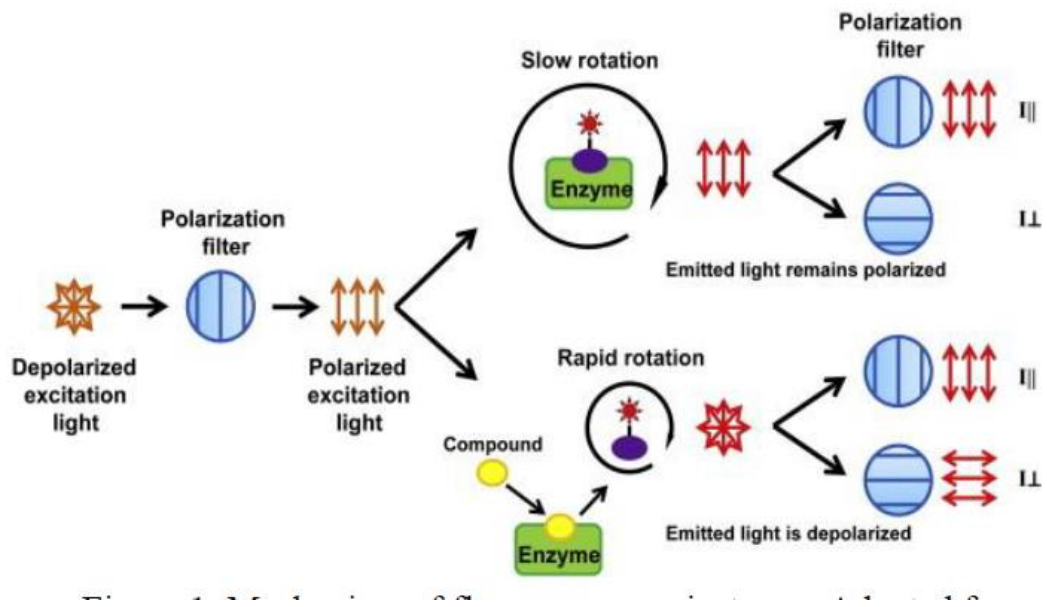

Figure 1. Mechanism of fluorescence anisotropy. Adapted from Zeng et al. 2018

FA can be used in the study of DNA-protein binding. With the help of this, scientists can look at how the interaction of certain proteins with genes, control its expression pattern and lead to several diseases like cancer and autoimmune disorders.

FA measurements of membrane probes like DPH and TMA-DPH have been used to elucidate different membrane properties like membrane potential, membrane fluidity, phase-transition temperature, under varying degrees of cholesterol-phospholipid composition. Researchers can use these insights to explain the mechanism behind diseases like obesity and atherosclerosis.

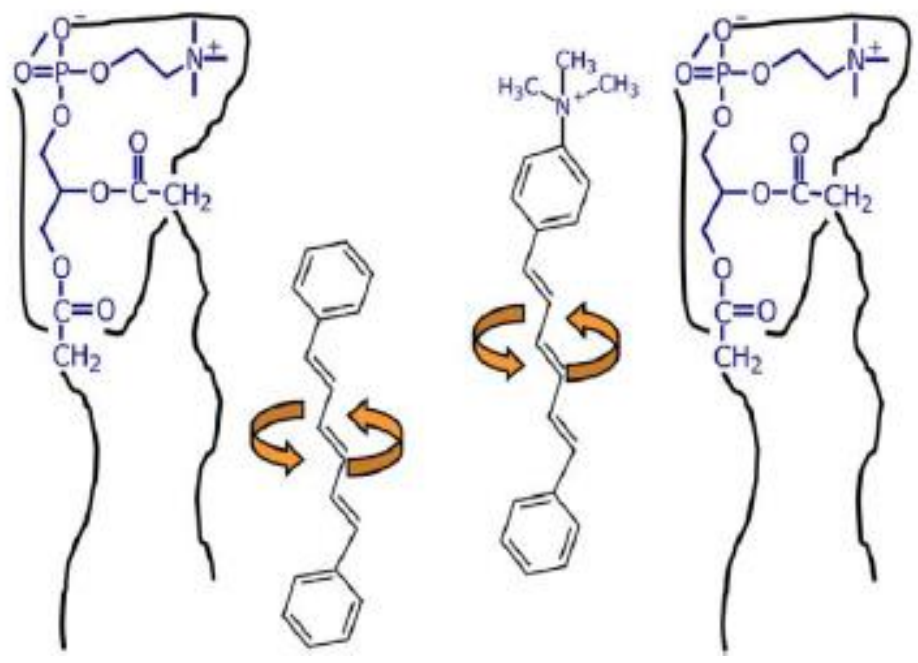

Figure 2. Membrane probes DPH and TMA-DPH inside in phospholipid layer Adapted from Shrivastava et al. 2016

FA can also be used in the high-throughput screening of cholesterol-lowering agents and anesthetic drugs and elucidation of their mechanism of action based on their partition coefficient and effect on membrane fluidity. I amassed this enormous amount of knowledge during my internship at CSIR-CCMB, Hyderabad. I worked as an intern in the membrane biophysics lab of Dr. Amitabha Chattopadhyay, SERB distinguished scientist. His group introduced me to the world of biophysics from the perspective of phospholipids and synthetic liposomes.

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I prepared model membranes using liposomes and carried out its fluorescence anisotropy measurement. I investigated the fluidizing effect of a novel cholesterol-lowering drug 1-octacosanol on model membranes of three different phases i.e. liquid-crystalline, gel and liquid-ordered phases. The reason of exploring this drug molecule was that it has fewer side effects and that too of lower magnitude as compared to statins. Moreover, it increases the level of HDL cholesterol and is extracted from plant wax.

I always wanted to propagate this cutting-edge research technology of fluorescence anisotropy but I felt that through all these times, I didn't get the right audience to present it to. But I have a strong belief that things come to you at the right time and at the right place. And recently, I along with my partner Susmita Pati from CET Bhubaneswar, won the 'ACS best poster award' for presenting the poster on topic 'Exploring Biophysical Properties of Membranes and Proteins using Fluorescence Anisotropy' at the 1st National Students' Conference on Spectroscopy-2020, jointly organized by CRSI and SciRox-Science Club, GNDU, Amritsar.

This achievement is really special because it embarks the beginning of my journey in the scientific community. And what makes it more special is that I got it after joining IIT Hyderabad!!!

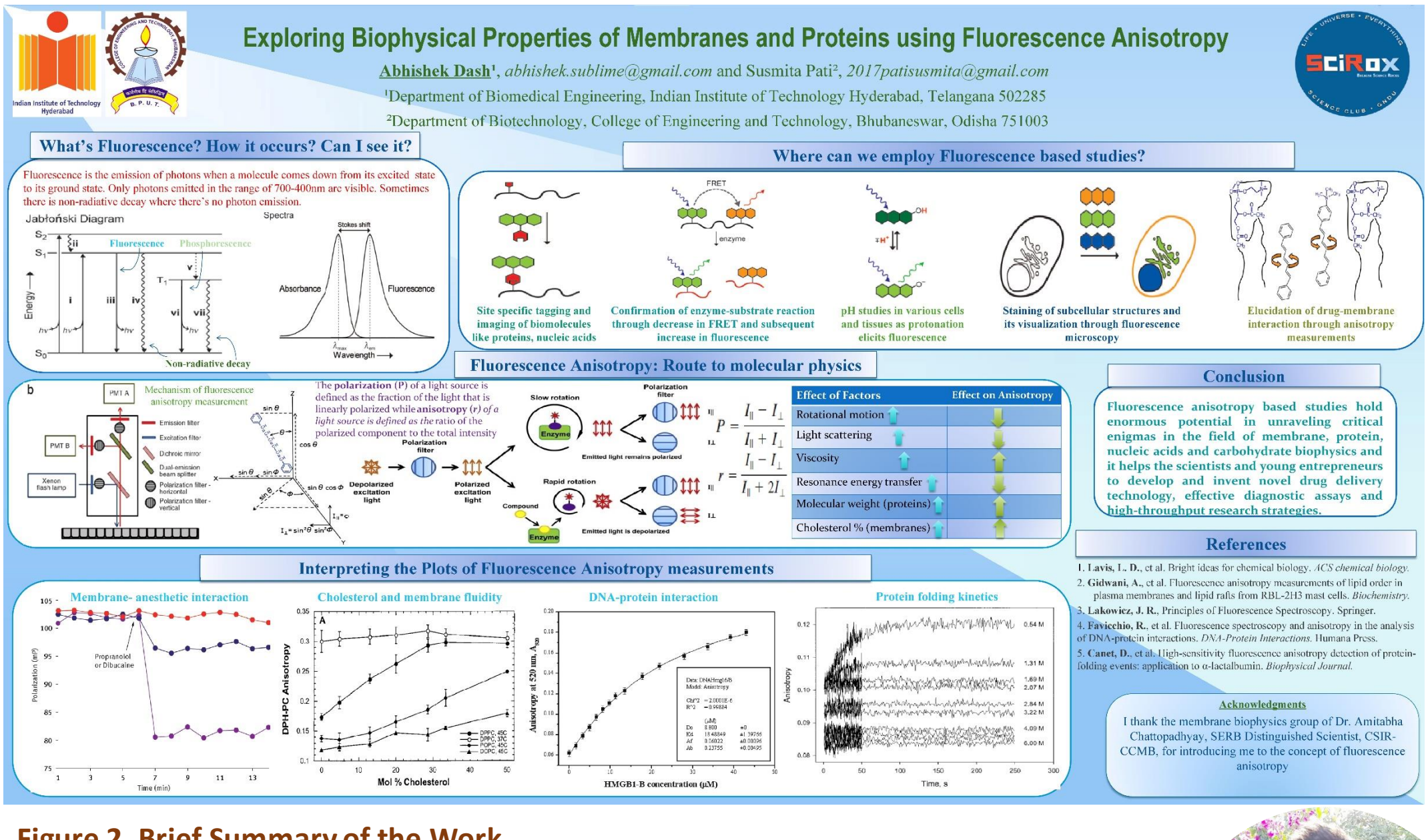

Figure 2. Brief Summary of the Work

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