EXCELLENCE IN RESEARCH WORK STATEMENT

This is to certify that the research work described below is original and from my lab at Department of Biology, IISER Pune, and all the illustrations and figures have either been prepared by me or adapted from our published work.

Siddhesh S. Kamat

OUR RESEARCH PROBLEM AND LONG-TERM GOAL

The advent of genome sequencing technology has resulted in an explosion of available protein sequences. Concomitant with the rising number of sequences, the propagation of annotation errors or lack of annotation, have become more prominent throughout databases that rely heavily on high-throughput computational predictions of protein function without much experimental support. Understanding the genetic basis for rare hereditary human disorders has also greatly benefited from tremendous advances in DNA sequencing technologies. Human genome mapping projects and sequencing have, to date, facilitated determination of the genetic basis for over 4,000 inherited diseases, with additional pathogenic mutations still being discovered. However, it is also becoming apparent that as a greater number of disease-causing mutations are being mapped, many of the affected genes encode unannotated proteins. Thus, assigning biochemical and cellular functions to such proteins is critical to achieve a deeper mechanistic understanding of these pathological disorders and for identifying potential therapeutic interventions for them. While there are several established high throughput platforms to study DNA (genomics), RNA (transciptomics) and Proteins (proteomics), there is a large disconnect thereafter in terms of biochemical technologies available that relate protein activities (in particular enzyme activities) to their endogenous substrates and products (***Figure 1***). Our long term goal is thus, to build on emerging biochemical platforms (see next paragraph) to enable us to identify and understand as-of-yet uncharacterized lipid signalling pathways *in vivo*, annotate their metabolic enzymes and cognate receptors that regulate their biology and provide new physiological insights and treatment paradigms for orphan and/or emerging human neurological and immunological diseases.

OUR STRATEGY FOR ENZYME FUNCTION ANNOTATION

Towards achieving our goals, we have tailored two methodologies: (1) a chemoproteomics technique called activity-based protein profiling (ABPP), and (2) mass spectrometry lipidomics and metabolomics (***Figure 1***). Briefly, ABPP is a functional proteomic strategy that uses a chemical probe (often referred to as a “reactive warhead”) that reacts with enzymes that are mechanistically or functionally related and allows for their detection, enrichment and quantitative identification from native and complex biological systems like cells and tissues. On the other hand, lipidomics and metabolomics are large scale tandem quantitative analysis of biological pathways and networks of cellular lipids and polar metabolites respectively using mass spectrometry as a readout.

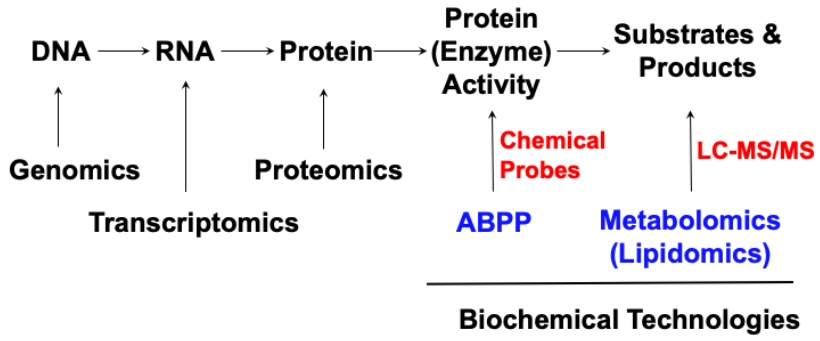

Figure 1: Overview of candidate global profiling methods. *Standard genomics, transcriptomics and proteomics measure DNA, RNA and Protein abundance respectively. In contrast, the emerging biochemical technologies, ABPP and metabolomics measure key functional aspects of enzymes, i.e. their activity and endogenous substrate/products respectively.*

Our lab uses both these technologies in tandem to reach our research goals, and together they afford the following advantages: (1) Enzyme – substrate (and/or product) relationships can be studied *in vivo* in cellular or tissue context without the need of purifying the enzyme(s) of interest. (2) All endogenous substrates and/or products, most of which are otherwise expensive to purchase or difficult to synthesize, can be quantitatively profiled in tandem as a function of the enzyme's *in vivo* activity. (3) No *a priori* knowledge is needed for the function of the enzyme(s) of interest, and these technologies reports only on the active enzyme, circumventing effects from post-translational modifications. (4) Both technologies can be used in competitive mode to discover inhibitors and/or activators for enzyme pathways, and have tremendous translational perspective.

RESEARCH THEMES IN THE LAB

While my primary appointment is in the Biology department at IISER Pune, I am also affiliated with the Chemistry department, which permits us to recruit talented students from both departments. This cross-fertilization of academic ideas between students of both backgrounds and training, had greatly benefitted the lab, and allowed for really exciting science at the interface of Biology and Chemistry (***Figure 2***). Given the diverse academic backgrounds of students in the lab, the themes in lab broadly range from development and implementation of biochemical technologies (lipidomics and chemoproteomics) to classical biochemistry (mechanistic enzymology and structural biology) to organic chemistry to physiological experiments (cell biology and animal studies) all towards understanding deregulated mechanisms in human diseases in order to develop newer diagnostics and treatments for them.

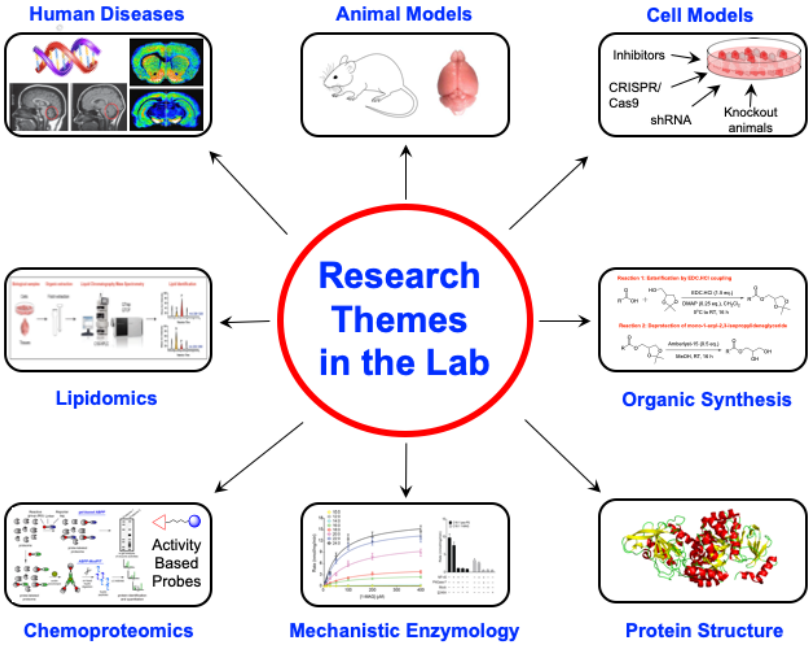

Figure 2: Overview of the different research themes in lab.

ONGOING PROJECTS

1. **UNDERSTANDING THE PATHOPHYSIOLOGY OF THE NEUROLOGICAL DISORDER PHARC.**
   Polyneuropathy, **h**earing loss, **a**taxia, **r**etinitis pigmentosa, and **c**ataract (PHARC) is an autosomal recessive early onset neurological disorder in humans caused by deleterious mutations to the *abhd12* gene that encodes a metabolic serine hydrolase (lipase) enzyme ABHD12. We have recently shown that the enzyme functions as a major lysophosphatidylserine (lyso-PS) lipase in the mammalian central nervous and immune system (***Figure 3***), where it the controls the levels of immunomodulatory lyso-PS lipids (Joshi et al., 2018; Kamat et al., 2015; Khandelwal et al., 2021; Singh, Joshi, & Kamat, 2020). While a lot is known of the immune phenotype regulated by lyso-PS lipids, their receptors and/or protein ligands remain unknown, and finding these is a major focus of this project. We have also found an upstream phosphatidylserine lipase ABHD16A that biosynthesizes lyso-PS lipids (***Figure 3***) (Kamat et al., 2015; Singh et al., 2020) and we are testing using animal models, whether the inhibition of ABHD16A can indeed of any therapeutic value in treating this neurodegenerative disease.

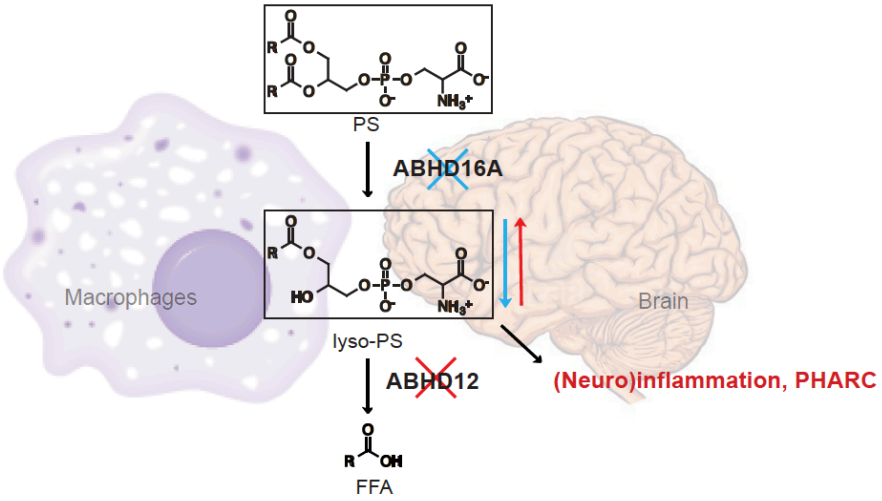

Figure 3. A schematic representation of the ABHD12/ABHD16A-lyso-PS pathway in mammals

2. **MAPPING LIPID PATHWAYS IN MAMMALIAN CELLS AND TISSUES UNDER OXIDATIVE STRESS**.
Lipids are the primary targets of oxidative damage for cells that have elevated ROS levels be use of oxidative stress. Several oxidatively damaged lipids serve as key immunological modulators, yet their chemical structures, their metabolic pathways and receptors remains unknown. We have recently elucidated the chemical structures of the pro-apoptotic oxidized phosphatidylserine (ox-PS) lipids, and shown that ABHD12 is a major lipase that controls their concentrations *in vivo* under chronic oxidative stress (***Figure 4***) (Kelkar et al., 2019). Moving ahead, we are now mapping the receptors and protein ligands for ox-PS lipids, and looking broadly at other phospholipids and sterols using the same strategy to understand their chemical structures and in vivo metabolic and signalling pathways under oxidative stress.

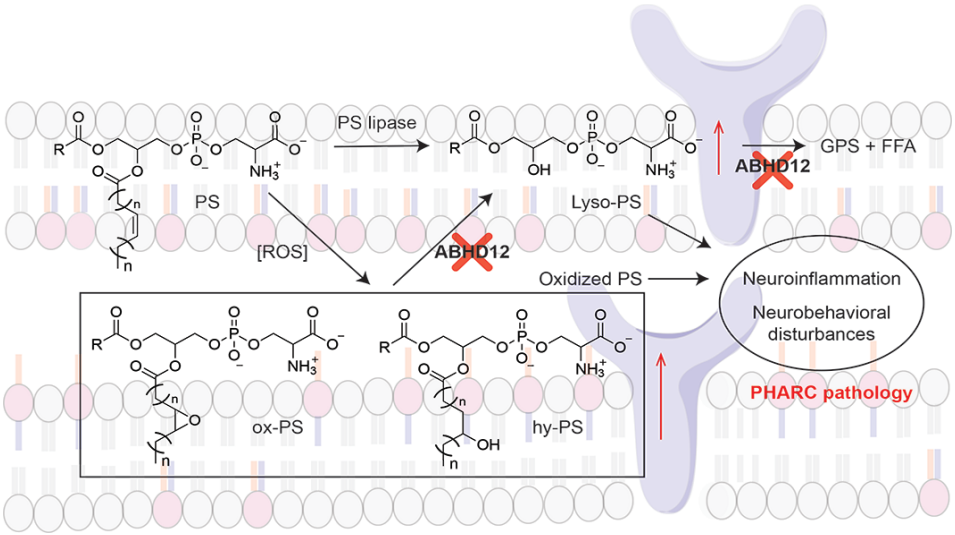

Figure 4. Schematic representation of the oxidised PS and lyso-PS lipase activities of ABHD12, and the contribution of these signalling lipids to the pathology of PHARC.

3. **FUNCTIONAL ANNOTATION OF LIPASES AND ACYLTRANFERASES FROM THE METABOLIC SERINE HYDROLASE (SH) ENZYME FAMILY**.
The metabolic SH family consists of ~220 members, of which 120 members are putative lipases and/or acyltransferases. ABHD12 as mentioned earlier regulates lyso-PS levels in the nervous and immune system, but lyso-PS lipids are present in all tissues. Hence there must exist lyso-PS lipases other than ABHD12 in other tissues. We have preliminary evidence that other lipases from the metabolic SH family are able to perform this activity, and we are validating their roles *in vivo*. Additionally, we have recently found that the orphan SH enzyme ABHD14B is indeed a novel lysine deacetylase (***Figure 5***) (Rajendran, Vaidya, Mendoza, Bridwell-Rabb, & Kamat, 2020), and are now investigating the biological pathways under the control of ABHD14B.

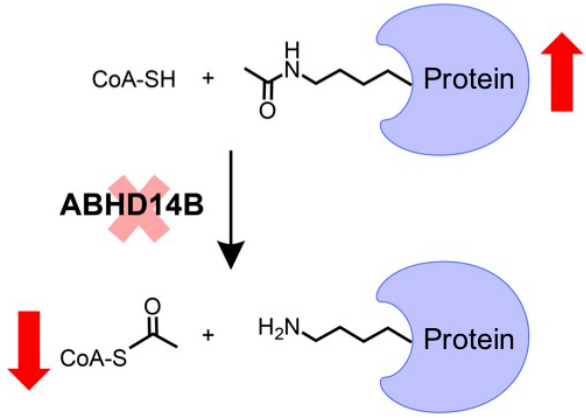

Figure 5. The lysine deacetylase activity of the orphan metabolic SH enzyme ABHD14B.

4. **MAPPING LIPID PATHWAYS DURING PHAGOCYTOSIS**.
Phagocytosis is an evolutionary conserved process, involving interplay between various cellular organelles, and their membranes. We recently performed a rigorous lipidomics study of phagosomes of various ages, and showed for the first time, that the ceramide lipids are critical for phagosomal maturation (***Figure 6***) (Pathak, Mehendale, Singh, Mallik, & Kamat, 2018). We also demonstrated that this process is orchestrated by the enzymes ceramide synthase, and its inhibition prevents phagosomal maturation. We have also mapped changes in phospholipid pools during phagocytosis, and are also interested in annotating function to putative phospholipases involved in process.

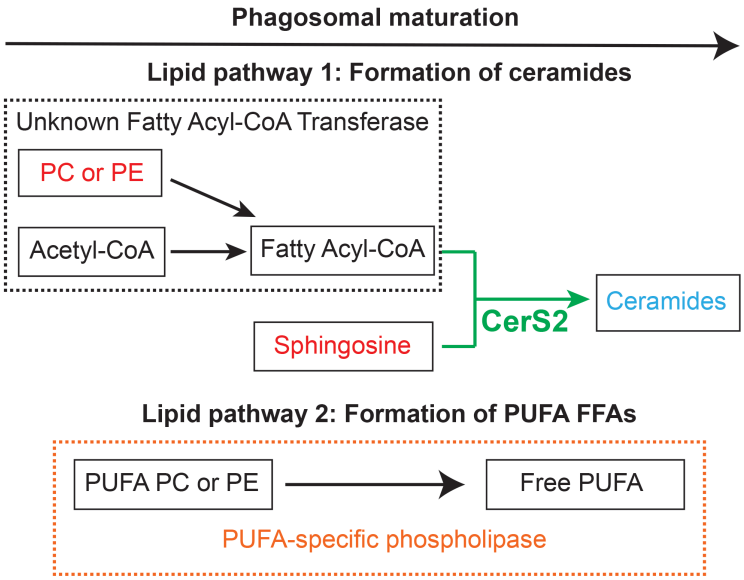

Figure 6. A summary of the lipid pathways mapped by our lab during phagosomal maturation.

5. **DEVELOPMENT OF LC-MS BASED CHEMICAL PROTEOMICS AND LIPIDOMICS PLATFORMS.**
Since liquid chromatography coupled to mass spectrometry (LC-MS) based lipidomics & proteomics are central to all the ongoing projects in the lab, we have set up a Biological Mass Spectrometry lab at the Department of Biology at IISER Pune that specializes in: (i) quantitative lipidomics platform for profiling lipids, and (ii) a discovery & quantitative chemical proteomics platform for profiling protein activities.

Our lipid (or metabolite) extractions and LC-MS platform now allows for the detection and quantitative assessment of >2000 unique lipid species, including phospholipids and their lyso-versions, neutral lipids, sterols and their esters, sphingolipids, and oxidatively truncated and/or damaged lipids of aforementioned classes (***Figure 7***), while our chemical proteomics platforms, termed activity based protein profiling (ABPP) (***Figure 8***) allow for discovery data dependent and independent chemoproteomics, and assessing endogenous enzyme activities in complex biological settings.

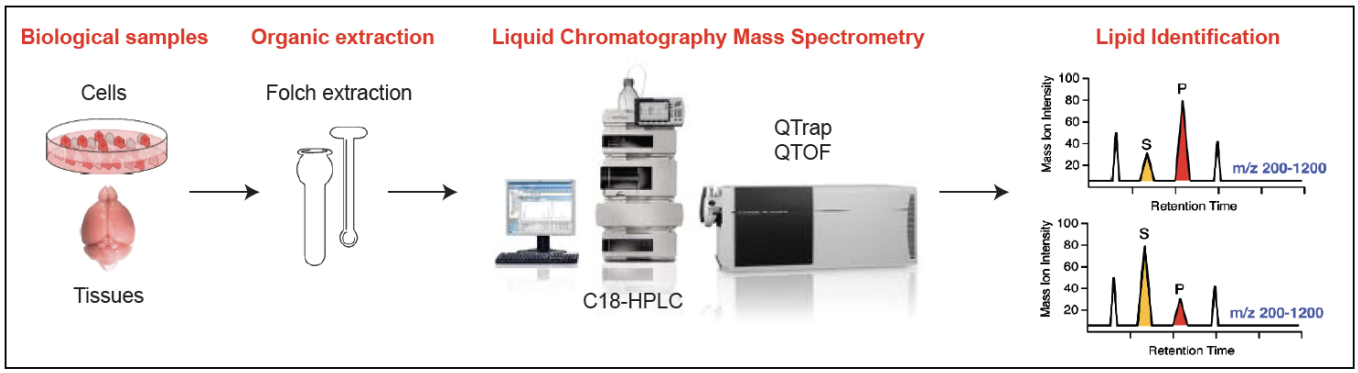

Figure 7: Schematic of a pipeline to quantitatively measure lipids (and other metabolites) from complex biological samples (e.g. cells, tissues).

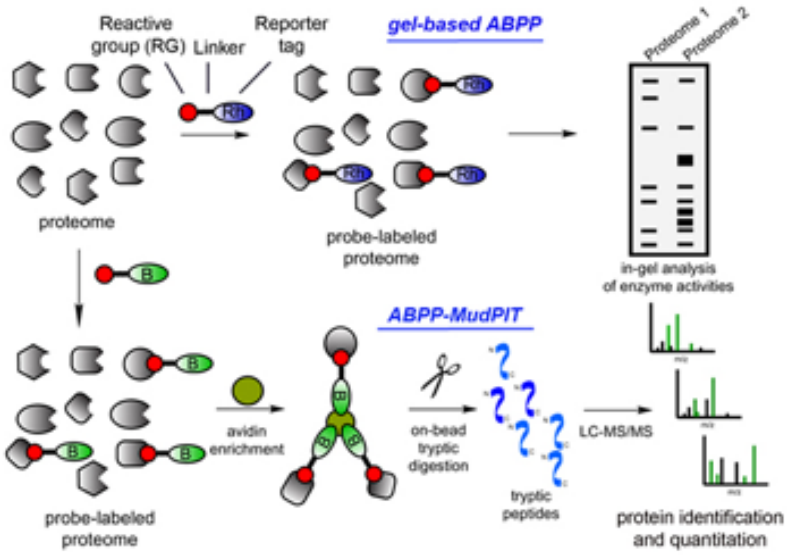

Figure 8. Activity-Based Protein Profiling (ABPP). *(**A**) Gel-based ABPP and LC-MS/MS based ABPP platforms for the detection, and quantification of enzyme activities from same functional class from complex biological proteomes. (Rh = Rhodamine, B = Biotin, MudPIT = Multidimensional Protein Identification Technology).*

Since we established our LC-MS platforms, we have actively crowd sourced them, and have established several fruitful collaborations, and solved complex problems in biology, that would not have been possible by individual labs otherwise. A few examples of such productive collaborations include:

Roop Mallik (TIFR Mumbai/IIT Mumbai): understanding the role of lipids in the functioning of molecular motors (M. Kumar et al., 2019; Rai et al., 2017).

Girish Ratnaparkhi (Biology, IISER Pune): mapping enzymatic and deregulated lipid networks in genetically altered fly models (Abhyankar, Kaduskar, Kamat, Deobagkar, & Ratnaparkhi, 2018; Chaplot et al., 2019; K. Kumar, Mhetre, Ratnaparkhi, & Kamat, 2021).

Harinath Chakrapani (Chemistry, IISER Pune): using chemoproteomics to map druggable vulnerabilities in antibiotic resistant bacteria (Kulkarni et al., 2019).

Ullas Kolthur-Seetharam (TIFR Mumbai): understanding the role of sirtuins in metabolism (Chattopadhyay et al., 2020).

Rajan Sankarnarayanan, Vinay Nandicoori (CCMB Hyderabad) & **Rajesh Gokhale** (NII): mechanisms of MTB lipid biosynthesis (Kinatukara et al., 2020).

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