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.

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.

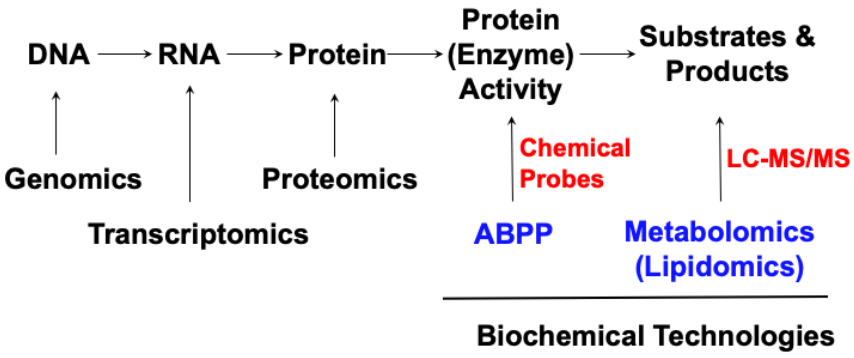

Figure 1: Overview of candidate global profiling methods*. Standard genomics, transcriptomics and proteomics measure DNA, RNA and Protein abundance respectively. In contrast, the emerging biochemical technologies, ABPP and metabolomics measure key functional aspects of enzymes, i.e. their activity and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

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.

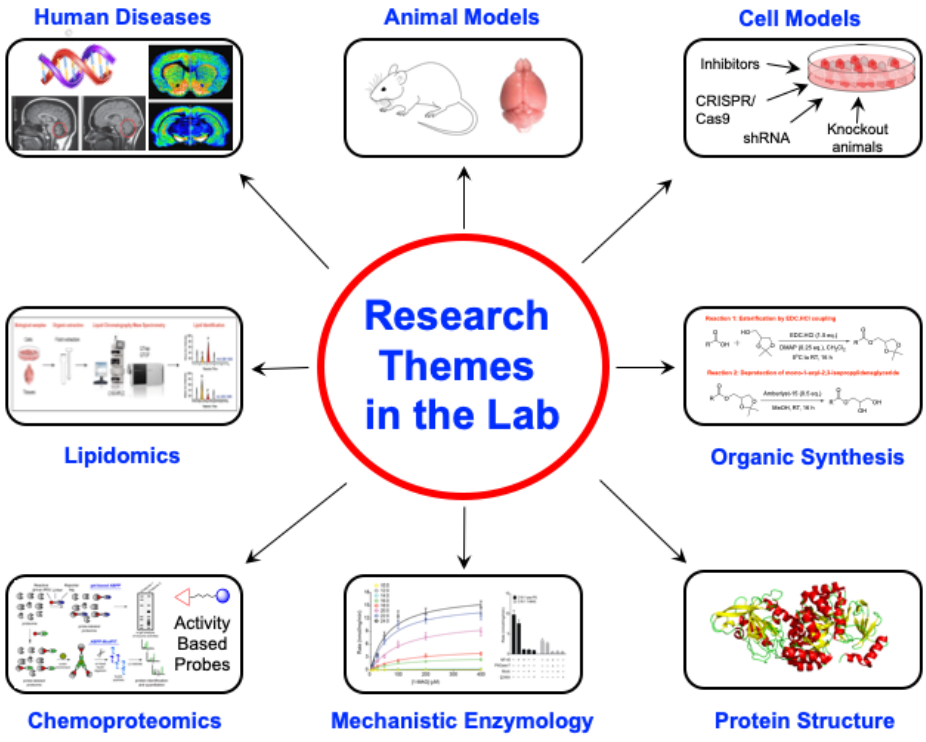

Figure 2: *Overview of the different research themes in lab at the interface of biology and chemistry.*

ONGOING PROJECTS IN LAB.

1. **Discovering lysophosphatidylserine (lyso-PS) signalling networks in mammalian physiology.**

Lysophospholipids are important hormone-like signalling molecules that regulate many facets of mammalian physiology, and deregulation in their metabolism is linked to human diseases. Sphingosine 1-phosphate and lysophosphatidic acid are the best characterized members for this lipid family, and drugs targeting their metabolic pathways are currently used for treating diverse human diseases. Over the past decade, lysophosphatidylserine (lyso-PS) has emerged as yet another signaling lysophospholipid that regulates many important physiological functions and has direct causative associations to several human neurological and autoimmune diseases. My lab has been at the forefront of research on this signaling lipid, and made seminal contributions in understanding how this lipid functions and is regulated in humans. Specifically, we have identified, biochemically and anatomically characterized the lipases that are responsible for the biosynthesis (ABHD16A) and degradation (ABHD12) of lyso-PS in humans (Joshi et al., 2018; Kelkar et al., 2019; Singh et al., 2020) (***Figure 3***). Further, we are the first lab globally to successfully synthesize lyso-PS lipids with varying lipid tails, and have shown that besides the recognition of the head group, the length of lipid tail determines which immunological function is modulated by lyso-PS in humans (Khandelwal et al., 2021). We have also identified Toll-like Receptor 2 as the major receptor for very-long chain lyso-PSs in the human brain and immune cells (Khandelwal et al., 2021), and shown it to be responsible for numerous inflammatory conditions associated with neurological and autoimmune diseases caused by dysregulated lyso-PS metabolism. Following up, we have also shown that increased lyso-PS in the mammalian brain results in (neuro)-inflammation, which is the underlying cause for the initiation and progression of a human neurodegenerative disease called PHARC (Singh & Kamat, 2021). Lastly, given our expertise, we have been invited to write a couple of reviews on lyso-PSs, including an exhaustive review in ***Chemical Reviews*** (Journal Impact Factor ~ 52) (Chakraborty & Kamat, 2024; Shanbhag et al., 2020).

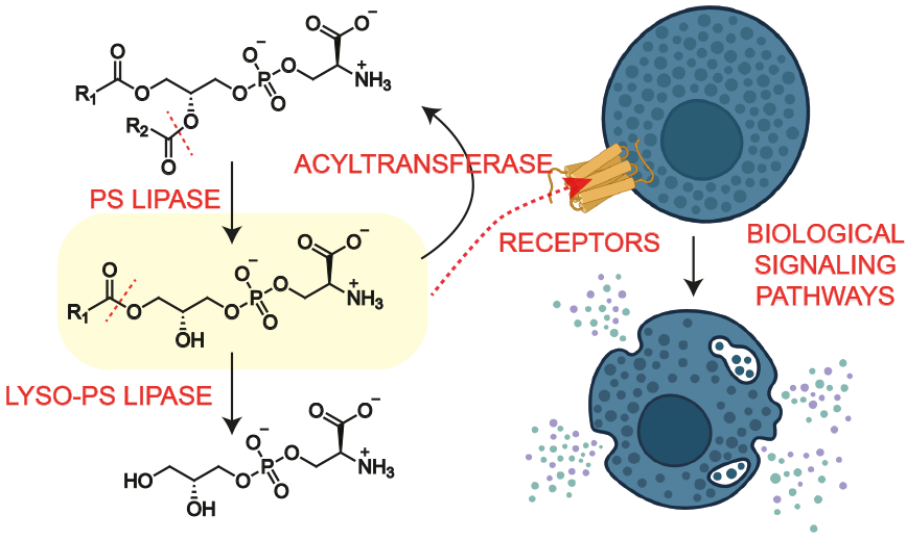

Figure 3. A schematic representation of the metabolic pathways for the biosynthesis and degradation of lyso-PS lipids identified by our lab in mammalian physiology and disease.

2. **Mapping lipid pathways during immunological processes**

Phagocytosis is an important innate immunological response involved in clearing infectious pathogens (e.g. bacteria, viruses). This process involves immune cells (mostly monocytes) engulfing invading pathogens in lipid vesicles and transporting them to the lysosome for degradation. Despite extensive involvement of lipids, the lipidomic compositions of phagosomes during this immune response have remained elusive till recently. Our lab has performed the first exhaustive lipidomics measurements on phagosomes at different times during phagocytosis and have shown that cholesterol and ceramides play a critical role during this immune response (Pathak et al.,

2018). Following up, we have further shown that sphingolipid metabolism is central to this process, and perturbations to this metabolism hamper phagocytosis (Mehendale et al., 2021). Given our expertise in this field, we have been invited to write a couple of reviews on this topic in reputed journals, where we describe in detail the lipidomes during the different stages of phagocytosis (***Figure 4***) (Saharan & Kamat, 2023; Saharan et al., 2022).

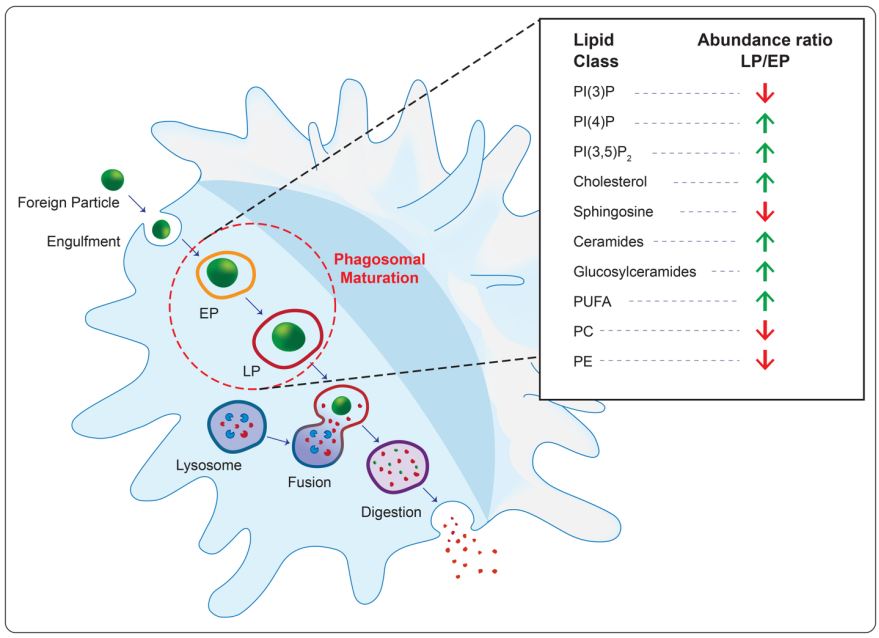

Figure 4. *A summary of the lipid pathways mapped by our lab during phagosomal maturation.*

Ferroptosis is a type of programmed cell death dependent on iron and characterized by the intracellular accumulation of oxidatively damaged lipids (e.g. lipid peroxides). Immune and cancer cells are resistant to this form of cell death, and have dedicated enzymes capable of metabolizing such oxidatively damaged lipids produced from the elevated reactive oxygen species generated during this process. Oxidized phosphatidylserine lipids in particular have potent immunogenic and pro-apoptotic properties, and yet very little remained known about them for the past few decades. Using advanced LCMS-based lipidomics technologies coupled with animal and cell models, we elucidated the chemical structures of these oxidized phosphatidylserine lipids, and have shown that the lipase ABHD12 is responsible for their metabolism in different cancer cells, the immune and nervous system (Kelkar et al., 2019) (***Figure 5***). Since our studies, drugs inhibiting ABHD12 are currently under investigation as anti-cancer therapy.

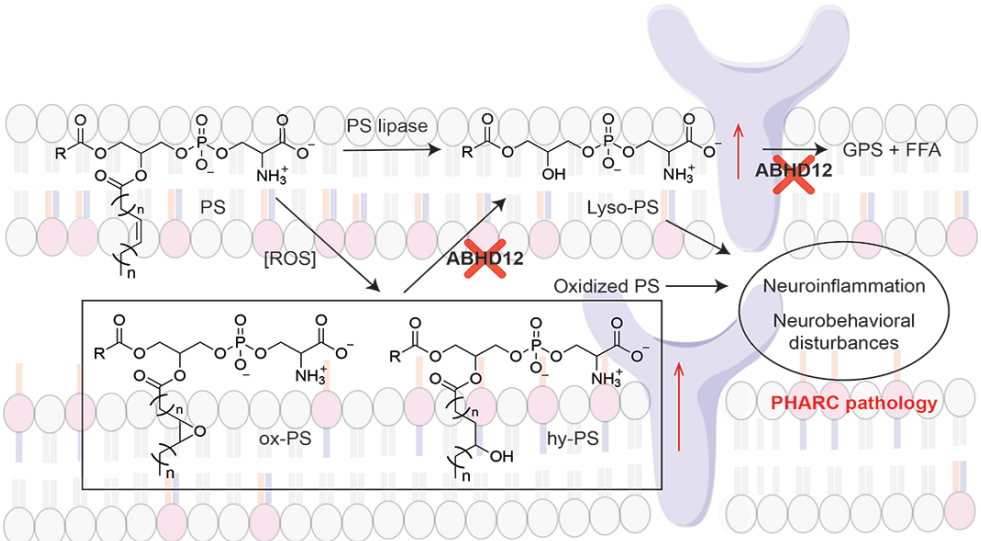

Figure 5. *Schematic representation of the oxidised PS and lyso-PS lipase activities of ABHD12 identified by our lab, and the contribution of these signalling lipids to the pathology of PHARC.*

3. **Discovery of a novel lysine deacetylase in mammals**

The sirtuins and histone deacetylases are evolutionarily conserved members of the lysine deacetylase (KDAC) family, that have been extensively characterized, and defects in their activities have been linked to diverse metabolic human diseases. Our lab has recently identified an orphan enzyme ABHD14B as another member of KDAC family, and expanded the repertoire of biochemical activities for this catalytic reaction (Rajendran et al., 2020). We have shown that the KDAC ABHD14B in present only in highly evolved organisms (in mammals particularly), where it regulates systemic glucose and lipid metabolism via its biochemical activity in the liver (***Figure 6***) (Rajendran et al., 2022; Vaidya et al., 2023). This discovery has tremendous translational potential, as our studies show that inhibitors to ABHD14B can potentially treat metabolic syndrome and obesity.

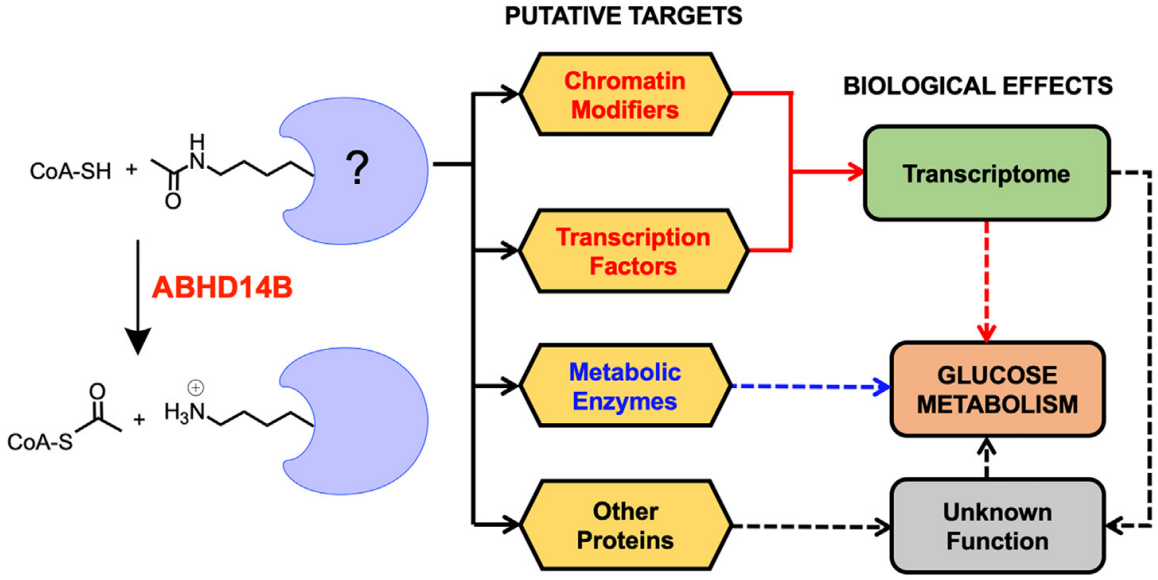

Figure 6. *The lysine deacetylase activity of the orphan metabolic SH enzyme ABHD14B identified by our lab, and its role in regulating glucose metabolism in mammals.*

4. **Development of LC-MS based chemoproteomics and lipidomics/metabolomics technologies**

Chemoproteomics is an emerging functional proteomic strategy which uses tailored chemical probes (typically substrate or ligand mimics) that react with proteins (or enzymes) based on their biochemical activities or on principles of protein-ligand affinity, and allows for their detection, enrichment and quantitative identification from native and complex biological systems like cells and tissues (part of this technology is reviewed by us: (Shanbhag et al., 2023)) (***Figure 7***). On the other hand, lipidomics/metabolomics are large scale tandem qualitative and/or quantitative analysis of biological pathways and networks of cellular lipids and (polar) metabolites respectively using mass spectrometry (LCMS) as a readout (***Figure 8***).

The chemoproteomics and lipidomics/metabolomics platforms developed in our lab afford the following unique advantages: (1) Protein-substrate relationships can be studied in physiological settings without the need of purifying proteins. (2) All endogenous lipids/metabolites, most of which are expensive to purchase or difficult to synthesize, can be quantitatively profiled as a function of the protein's *in vivo* activity. (3) No *a priori* knowledge is needed for the function of the protein(s) of interest, as these technologies reports only on the functional proteins, circumventing effects from post-translational modifications. (4) Both technologies can be used in a high-throughput manner to discover inhibitors for biological pathways and have tremendous translational potential.

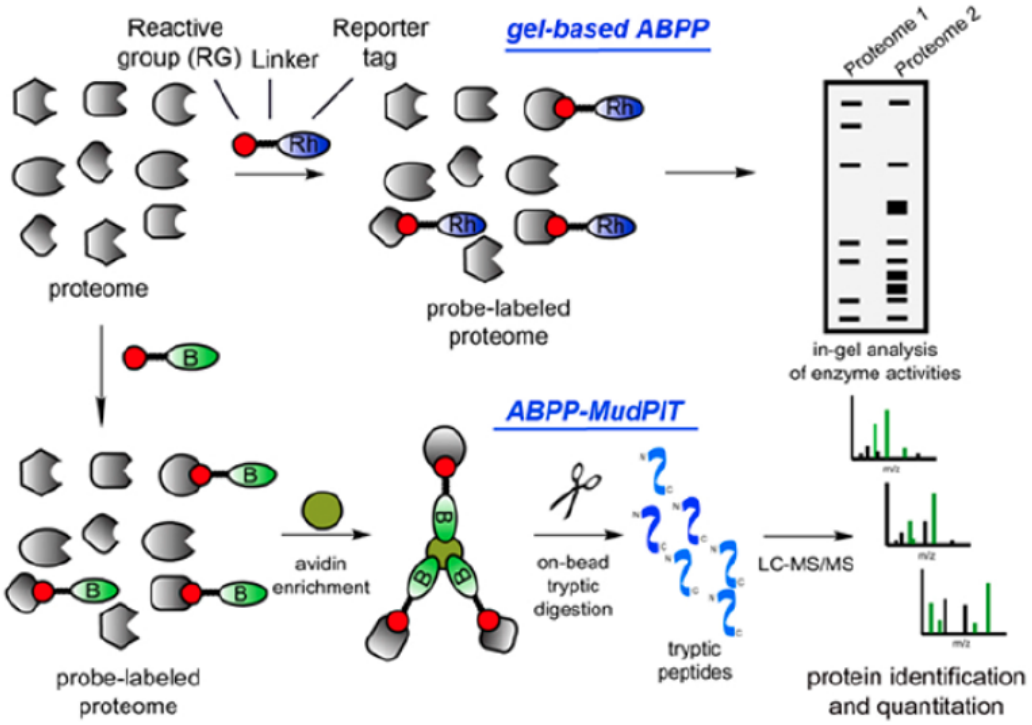

Figure 7. *Activity-Based Protein Profiling (ABPP). Gel-based ABPP and LC-MS/MS based ABPP platforms for the detection, and quantification of enzyme activities from same functional class from complex biological proteomes. (Rh = Rhodamine, B = Biotin, MudPIT = Multidimensional Protein Identification Technology).*

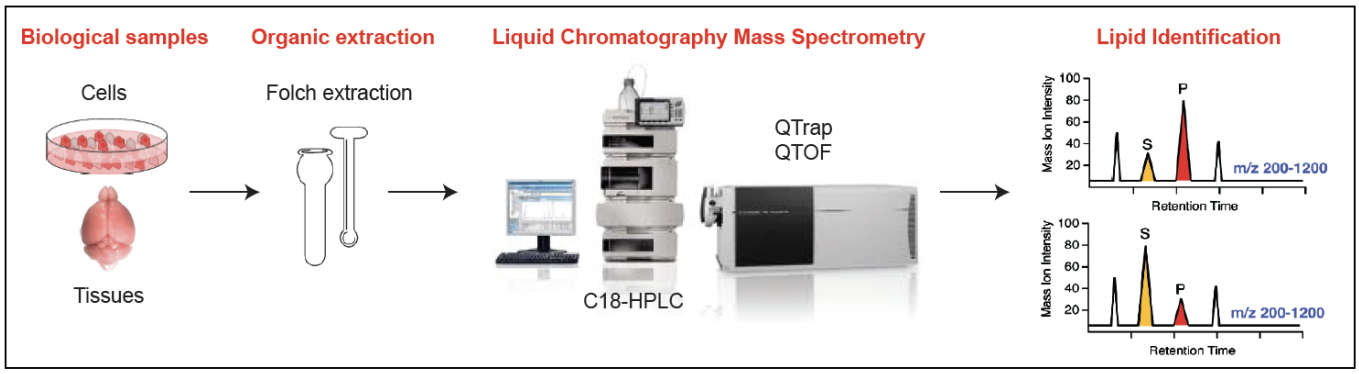

Figure 8: *Schematic of a pipeline to quantitatively measure lipids (and other metabolites) from complex biological samples (e.g. cells, tissues) developed by our lab.*

We have successfully used our chemoproteomics platforms to identify new druggable targets in antibiotic resistant bacteria (Kulkarni et al., 2019; Kumari et al., 2023), and for mapping activities for serine hydrolase enzymes during development in fly models (K. Kumar et al., 2021).

Besides our core research problems, with collaborators, we have applied our lipidomics/metabolomics platforms towards understanding several biological processes such as: (i) regulation of systemic lipid and glucose secretion from the liver in mammals including humans (Chattopadhyay et al., 2020; M. Kumar et al., 2019; Malik et al., 2019; Rai et al., 2017; Sen et al., 2023; Talwadekar et al., 2024), (ii) regulation of systemic lipid homeostasis in non-mammalian model systems (Abhyankar et al., 2018; Chaplot et al., 2019; Mondal et al., 2022) and (iii) pathogenicity of hitherto uncharacterized metabolic (lipid) pathways and secondary metabolites from bacteria including Mycobacterium tuberculosis (S. Kumar et al., 2022; Mehdiratta et al., 2023; Mehdiratta et al., 2022).

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