

## Customer case study

### Enzyme function annotation by chemical proteomics and metabolomics

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#### Project goal

Annotate endogenous functions to uncharacterized enzymes associated with human diseases and map the biochemical pathways that they physiologically regulate using an integrated chemical proteomics and metabolomics approach.

#### Biggest challenges right now

- The advent of genome sequencing technologies has resulted in an explosion of known protein sequences without any experimental validation for them. Hence, there is a large disconnect in tying protein functions, especially enzyme functions, to the biochemical pathways that they modulate.
- While genomics, transcriptomics and proteomics have emerged as versatile techniques for studying DNA, RNA and proteins, respectively, there is a dearth of techniques for studying enzymes and the corresponding metabolites they regulate on a system-level scale. Hence, developing such technologies will greatly facilitate our understanding of the physiological relationship between enzymes and metabolic pathways.
- Algorithms for searching and analyzing complex LC-MS/MS metabolomics data as well as universally standardized sample preparation protocols are still limited.
- A genuine absence of mapping lipase activities and deregulated lipid signaling pathways in emerging human neurological and immunological disorders.

#### The solution

- **Chemical proteomics or activity-based protein profiling (ABPP)** is a functional proteomic strategy that uses a chemical probe that reacts with mechanically or functionally related enzymes that allows for their detection, enrichment and quantification from various biological systems. By coupling ABPP to an in-house, post-tryptic, reductive demethylation labelling strategy, we have been able to simultaneously profile activities of > 100 enzymes from a particular enzyme superfamily in a single experiment. In my lab, and with ongoing collaborations, we are now using this approach to study 5 enzyme superfamilies and assessing > 1,000 unique enzyme activities.
- **Metabolomics** is a large-scale tandem, quantitative analysis of biological pathways and networks of cellular metabolites from complex biological systems such as cells and tissue. The metabolomics workflows we have developed, allow the detection and quantification of > 1,000 unique metabolites spanning many different classes of biological molecules (lipids, glycolytic metabolites, amino acids, nucleotides, etc.) in a single experiment. Using metabolomics, we have also identified very low-abundant transient metabolites, such as oxidized phosphatidylserine lipids, which exist at femtomolar concentrations in cells and are potent pro-apoptotic signaling lipids.
- **High-resolution mass spectrometry** is the readout for both ABPP and metabolomics, and using these techniques in tandem with pharmacological and genetic tools has proven to be a powerful way to assess enzyme activities and the pathways that they regulate in physiological settings. For LC-MS/MS based ABPP experiments, we used the TripleTOF® LC-MS/MS 6600 System with the Eksigent 425 NanoLC™ HPLC System, while for metabolomics applications, we used the SCIEX X500R QTOF System with the Exion LC UHPLC. IDA and MRM-HR workflows were leveraged for discovery and quantitative applications, respectively.

#### Outcomes of research

- We have annotated functions to several uncharacterized enzymes and mapped new biochemical pathways that are involved in lipid metabolism and signaling in humans. These new findings have resulted in an in-depth mechanistic understanding of various human pathophysiological conditions such as neurodegenerative diseases, immunological disorders, metabolic obesity and diabetes.
- For example, we have recently shown that the lipase ABHD12 is an oxidized phosphatidylserine lipase (Nature Chemical Biology, 2019), and how this lipase prefers very-long chain lysophosphatidylserine as substrates (JBC, 2018) to regulate neuroinflammation and other immunological phenotypes, such as histamine release, during infections (Cell Chemical Biology, 2021). Recently, we have also annotated the orphan enzyme ABHD14B as a novel lysine deacetylase (Biochemistry, 2020) thus expanding the biological repertoire for this enzyme class, which is central to the regulation of metabolism, with its deregulation being associated with obesity and diabetes.

*“Assigning functions to proteins, particularly enzymes associated with human diseases, represents a grand challenge in modern research in the post-genomic era.”*

#### Type of organization

The Indian Institute of Science Education and Research (IISER) Pune is a research-intensive teaching institute interested in investigating and solving questions in all disciplines of science that are beyond conventional thinking.

#### Goal

Train the next-generation of scientists and researchers through innovative pedagogical experiences, and provide solutions to important socioeconomic, health and energy problems in India via multidisciplinary research.

#### SCIEX products

- Triple TOF® 6600 LC-MS/MS System
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*“SCIEX instruments at IISER Pune have high scan speed, sensitivity, mass accuracy as well as the quantitative power that suits our proteomics and metabolomics platforms perfectly. SCIEX service and technical support has been top-notch, both which have ensured high uptime and peak instrument performance to generate high quality data.”*