भारतीय विज्ञान शिक्षा एवं अनुसंधान संस्थान पुणे
INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH PUNE
डॉ. होमी भाभा मार्ग, पुणे 411008, महाराष्ट्र, भारत | Dr. Homi Bhabha Road, Pune 411008, Maharashtra, India
T +91 20 2590 8001 W www.iiserpune.ac.in
IISER PUNE

25th August 2024

THIS IS TO CERTIFY THAT THE RESEARCH WORK UNDER REFERENCE IS RECENT AND HAS NOT BEEN GIVEN ANY AWARD IN THE PAST.

Our lab has made original and significant contributions in the field of functional annotation of uncharacterized enzymes and biological pathways, particularly lipid signaling and metabolic pathways associated with various human disorders. In particular, my lab has made seminal contributions to the understanding the metabolism and bioactivities of a class signaling lipids, called lysophosphatidylserines (lyso-PSs). We have now identified the major biosynthetic and degradative enzymes (lipases) that make and break lyso-PS respectively, and studied how genetic mutations to these enzymes results in dysregulated lyso-PS signaling, which is associated with several human neurological and autoimmune diseases that are currently incurable.

Paper # 1: Chakraborty, A., **Kamat, S. S.*** (2024) Lysophosphatidylserine: a signaling lipid with implications in human diseases, ***Chemical Reviews***, 124 (9), 5470 – 5504. (IF = 51.4)
Article featured on the May 2024 issue cover of Chemical Reviews.

Chemical Reviews invites very limited exhaustive articles on topics that are considered very contemporary and of interest to the chemistry and/or chemical biology community. Additionally, the lead author invited to contribute an article, is considered to be a leader of the field and/or should have made substantial contribution to the field in which the review is being written. Given our lab's contributions towards mapping the biological pathways associated with lysophosphatidylserine (lyso-PS) metabolism and signaling over the past decade, we were invited to write an up to date, comprehensive and authoritative review on lyso-PS lipids. In this review, we discuss our lab's contributions towards annotating functions to lipases associated with lyso-PS metabolism, and various pharmacological studies that have led to the identification of signaling pathways modulated by lyso-PS in different immunological contexts. Since this review was listed as a "*Hot Paper*", we were invited to submit an artwork for the journal cover, which ended up on the *May 2024 edition of Chemical Reviews*.

Authors' contribution: Siddhesh S. Kamat is the corresponding author, has conceptualized and written this exhaustive review with his PhD student Arnab Chakraborty. This work was funded by a SwanaJayanti Fellowship and a SERB-CRG grant to Siddhesh S. Kamat. Arnab Chakraborty is a Ph.D. student with Siddhesh S. Kamat, and this review will be a part of his Ph.D. dissertation.

Paper # 2: Khandelwal, N., Shaikh, M., Mhetre, A., Singh, S., Sajeevan, T., Joshi, A., Balaji, K. N., Chakrapani, H., **Kamat, S. S.*** (2021) Fatty acid chain length drives lysophosphatidylserine dependent immunological outputs, ***Cell Chemical Biology*** 28, 1169 – 1179. (IF: 10.1)
Article featured on August 2021 issue cover of Cell Chemical Biology.

Following up on our lab's discovery of ABHD12's lipase activity, and its association to the human neurological disorder PHARC via modulation of immunomodulatory lysophoshatidylserine (lyso-PS) and pro-apoptotic oxidized phosphatidylserine lipids (Kelkar et al., *Nature Chemical Biology, 2019*), we have

reported the first successful synthesis of the lyso-PS lipids, and using these, have shown that as a function of the lipid tail length, these hormone like signalling lipids act through TLRs to elicit pro-inflammatory responses to cause neuroinflammation or via GPCRs to cause activation of macrophages and histamine release from mast cells (**Khandelwal et al., *Cell Chemical Biology, 2021***). This study has paved the wave for the development of bioorthogonal lyso-PS lipids, that can now be used to map the protein ligands for this biomedically important signalling lipid class, and for development of TLR2 antagonists as therapeutic targets in treating neuroinflammation and PHARC. Since this review was also listed as a "*Hot Paper*", we were invited to submit an artwork for the journal cover, which ended up on the *August 2021 edition of Cell Chemical Biology*.

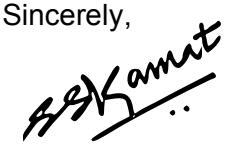
Authors' contribution: Siddhesh S. Kamat is the lead corresponding author and has conceptualized and supervised these studies, that were funded by a DBT/Wellcome Trust India Alliance grant to Siddhesh S. Kamat. The co-first authors are Postdocs and Ph.D. students of Siddhesh S. Kamat and the work done by the Ph.D students has been included in their respective dissertations. We collaborated with Prof. Harinath Chakrapani for the synthesis aspects of this project, and Prof. K. N. Balaji provided the TLR2 null mice used in this paper.

Paper # 3: Kelkar, D. S., Ravikumar, G., Mehendale, N., Singh, S., Joshi, A., Sharma, A., Mhetre, A., Rajendran, A., Chakrapani, H., **Kamat, S. S.*** (2019) A chemical genetic screen identifies ABHD12 as an oxidized phosphatidylserine lipase, ***Nature Chemical Biology***, 15, 169 – 178. (IF: 12.9)

In this paper, my lab has mapped different lipid pathways that are involved in pathological outcomes due to oxidative stress. Specifically,we devised a novel synthetic reactive oxygen species (ROS)-generating small-molecule that makes oxidized phosphatidyl-serine (PS), a lipid species implicated in programmed cell death. Utilizing MS-based chemical genetic and pharmacological methods, we screened a broad spectrum of lipase inhibitors to identify compounds that altered the levels of oxidized PS in multiple mammalian cell lines. Consequently, we successfully identified diverse classes of serine hydrolase inhibitors that lead to a dramatic elevation of cellular oxidized PS. Building on this discovery, we went on to identify the target of one of the identified inhibitors as the lipase ABHD12, previously known to be involved in the PHARC syndrome. Subsequent validation of these findings in animal models and patient derived samples established how elevated levels of ROS contribute to pathological outcomes in human diseases via dysregulated lipid signalling pathways, paving the way for the development of novel therapeutic targets to treat PHARC. Our studies thus annotated ABHD12 as a major regulator of both the lysophosphatidylserine lipids and oxidized PS lipids in different disease outcomes.

Authors' contribution: Siddhesh S. Kamat is the lead corresponding author and has conceptualized and supervised these studies, that were funded by a DBT/Wellcome Trust India Alliance grant to Siddhesh S. Kamat. The co-first authors are a Postdoc and Ph.D. students of Siddhesh S. Kamat and the work done by the Ph.D students has been included in their respective dissertations. We collaborated with Prof. Harinath Chakrapani for the synthesis aspects of this project.

Sincerely,



Siddhesh Kamat, Ph.D.
IISER Pune.