Biographic Sketch

Name: Kumar, Shivesh
eRA COMMONS USER NAME (credential, e.g., agency login): SHIVESHK
Email: shivesh.kumar@wustl.edu
Position Title: Research Assistant Professor
Education/Training:

INSTITUTE AND LOCATION	DEGREE (if applicable)	COMPLETION DATE (MM/YYYY)	FIELD OF STUDY
Jawaharlal Nehru University, New Delhi, India	Ph.D.	01/2010	Biochemistry and Structural Biology
Purdue University, IN	Postdoctoral associate	02/2010-08/2013	Biochemistry and Structural Biology
Duke University	Postdoctoral associate	08/2013-08/2016	Biochemistry and Structural Biology
Duke University	Senior Research Associate	09/2016-09/2022	Biochemistry and Structural Biology

Personal Statement

I started my scientific career as a structural biologist and utilized X-ray crystallography as a technique to study the role of calcium binding protein in amoebic pathogenesis. *Entamoeba histolytica*, the causative agent of amoebic colitis, has a significant impact on human health, particularly in developing countries. The parasite's ability to cause disease is heavily influenced by its interaction with calcium ions (Ca^{2+}), which play a crucial role in its pathogenesis, particularly in the process of cell invasion and cytotoxicity. The genome of *E. histolytica* encodes 27 Ca^{2+}-binding proteins, which suggests a highly complex Ca^{2+} signaling network. These proteins may regulate the parasite's responses to calcium fluctuations within its environment, including processes like motility, host cell attachment, and the secretion of lytic enzymes. During my Ph.D. I performed structural, biochemical, biophysical and functional characterization of *Eh*CaBP1 and reported the first crystal structure of calcium binding proteins from *E. histolytica* (Kumar et al, Proteins 2007). *EhCaBP1* is closely related to calmodulin but adopts a unique trimeric organization through its N-terminal EF-hand motif is particularly intriguing. This suggests that while *EhCaBP1* shares evolutionary lineage with calmodulin, its quaternary structure and calcium-binding mechanism may differ, possibly allowing it to fulfill distinct functional roles in the parasite's calcium signaling network. Further, I deciphered the ligand-binding mode of *EhCaBP1*, especially revealing phenylalanine binding in its crystal structure, represents a significant step forward in understanding the molecular mechanisms underlying *E. histolytica* pathogenesis (Jain R., Kumar S., et al., Plos ONE 2009). The identification of phenylalanine as a key ligand is a novel finding that could provide new insights into the functional regulation of calcium signaling in the parasite, as well as offer potential avenues for drug development targeting these interactions. It's an exciting piece of structural biology that contributes to a much deeper understanding of parasite biology and offers innovative possibilities for therapeutic strategies. To study the dynamic nature of this protein and further validate the trimerization of N-terminal domain at physiological conditions, I determined the crystal structure of N-terminal domain (Biophys J., 2010). The final structure consists of EF-1 and EF-2 motifs separated by a long straight helix as seen in the full-length protein. The spectroscopic and stability studies, like far and near-ultraviolet circular dichroism spectra, intrinsic and extrinsic fluorescence spectra, acrylamide quenching, thermal denaturation, and dynamic light scattering, provided clear evidence for a conversion from trimeric state to monomeric state. This piece of work provided a deep

understanding of the structural dynamics and functional properties of *EhCaBP1*, revealing how it transitions between trimeric and monomeric states in response to pH changes, and how this transition influences its functionality, especially its ability to activate endogenous kinases. These findings are pivotal in understanding the molecular mechanisms of *E. histolytica* pathogenesis and open-up potential therapeutic avenues for targeting the parasite's calcium-binding proteins and signaling pathways.

I also determined crystal structures of *Eh*CaBP1 in complex with Ba^{2+}, Pb^{2+} and Sr^{2+} and characterized interaction of heavy metals with this protein, which provided a foundation to utilize this protein for bioremediation of metal toxicity (Kumar et al, BMC Biophys., 2012). Since *EhCaBP1* interacts with various heavy metals like Ba^{2+}, Pb^{2+}, and Sr^{2+}, understanding how these metals affect its function could provide insights into potential therapeutic approaches that exploit this interaction. The structural and functional insights gained from studying *EhCaBP1* and its interaction with calcium and heavy metals open several exciting avenues for therapeutic development. This work likely holds broad implications for both structural biology and parasitology, providing a clearer picture of how *E. histolytica* regulates its complex calcium signaling network. It could also inspire further studies on other CaBPs in *E. histolytica* to decipher how the parasite coordinates such an intricate set of pathways to maintain its virulence.

During my postdoctoral training at Purdue University, I was involved in structural and functional characterization of KdpD-KdpE two component signal transduction system involved in the regulation of the KdpFABC transporter, which helps maintain potassium ion (K^+) balance in bacteria. The structure of the KdpE-DNA complex provided fundamental insights into how KdpE, a response regulator in the KdpD-KdpE two-component system, interacts with its target DNA to regulate gene expression (Narayanan[#] and Kumar[#] et al, Nat Commun. 2014). By determining the atomic details of the KdpE-DNA interface, I've contributed to our understanding of potassium homeostasis**,** bacterial stress responses**,** and transcriptional regulation. This research has broader implications for bacterial pathogenesis and can inform the development of novel antibacterial strategies targeting two-component signaling systems. KdpE is a response regulator from the OmpR/PhoB family, involved in regulating the kdpFABC operon in bacteria, which is responsible for potassium uptake under conditions of low potassium availability. This regulation is critical for maintaining potassium homeostasis and ensuring bacterial survival under stress conditions, such as during osmotic or nutrient stress. The determination of the full-length crystal structure of the KdpE-DNA complex is a landmark achievement, as it provides a comprehensive view of how KdpE functions as a transcriptional regulator. I also performed biophysical and structural characterization KdpD C-terminal histidine kinase domain (Protein Science 2020).

During my postdoctoral training at Duke University, I have utilized cryo-EM to determined structure of NPR1 (Kumar et al, Nature 2022). NPR1 in the context of plant immunity, particularly in its role as a master regulator of the defense transcriptome induced by the plant immune signal salicylic acid (SA). Indeed, NPR1 is central to plant immune responses. However, despite its clear importance in immunity, the mechanisms by which NPR1 regulates immune responses have remained somewhat elusive, particularly due to a lack of detailed structural information. Cryo-EM of NPR1 offers the potential to resolve structural details and uncover key mechanisms of NPR1 activation and transcriptional regulation. SPINDLY (SPY) in *Arabidopsis thaliana* is a novel nucleocytoplasmic protein O-fucosyltransferase (POFUT), which regulates diverse developmental processes. Sequence analysis indicates that SPY is distinct from ER-localized POFUTs and contains N-terminal tetratricopeptide repeats (TPRs) and a C-terminal catalytic domain resembling the O-linked-N-acetylglucosamine (GlcNAc) transferases (OGTs). I have used cryo-electron microscopy (cryo-EM) to reveal the structure of SPY and its complex with GDP-fucose, which adds significant insights into the structural and functional basis of its enzymatic selectivity, which had remained unclear (Nat Commun. 2023).

Currently, I'm focusing to understand the molecular mechanism of KEAP1 activation as a redox sensor. Kelch-like ECH-associated protein 1 (KEAP1) is an adaptor subunit of CUL3 based E3 ubiquitin ligase and KEAP1 mutation is associated with cancer progression, treatment resistance, metabolic reprogramming, ferroptosis, cell cycle and poor patient survival in lung cancers (Gong M, *et al*. Cell Commun Signal. 2020; Wohlhieter CA, *et al*., Cell Rep. 2020; Sitthideatphaiboon P, *et al*., Clin Cancer Res. 2021; Hellyer JA, *et al*., J Thorac Oncol. 2021). Although, KEAP1 KELCH domain has been well studied as a therapeutic target, KEAP1 target recognition and assembly of active Cullin-Ring ligase (CRL) for degradation has not been achieved. Oxidative stress, a condition implicated in various diseases such as Alzheimer's disease, atherosclerosis, and cancer, triggers a response in cells to maintain stability in their environment. This response is facilitated by cellular defense mechanisms. A key player in this defense is the transcription factor Nrf2, which orchestrates protective responses against oxidative insults. Under normal conditions without stress, Nrf2 levels remain low due to continuous ubiquitination mediated by Keap1. The Nrf2 pathway serves as a critical cellular defense mechanism, regulating the expression of genes that help cells manage oxidative damage, maintain redox balance, and protect against the development of oxidative stress-related diseases like cancer, neurodegenerative disorders, and cardiovascular diseases. The major goal is to decipher the molecular mechanism of KEAP1/NRF2 axis.

Selected Plenary Presentations at Scientific Meetings

BIOSPARKS, 14th -15th March 2008, School of Life Sciences, Jawaharlal Nehru University, New Delhi.
First Triangle Area Cryo-EM Symposium, Dec. 16-17, 2019, Research Triangle Park Headquarters, 12 Davis Drive, RTP, NC 27709.

Positions and Scientific Appointments
2022-Present: Research Assistant Professor
2016-2022: Senior Research Associate
2010-2016: Postdoctoral Associate
2004-2010: Ph.D. candidate

Honors and Awards
Best POSTER PRESENTATION AWARD in "International Symposium on Recent Trends in Molecular Structure and Function (ISRTMSF08), Madras University, Chennai, India.
Best ORAL PRESENTATION AWARD, Biosparks08, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India.
Council for Scientific and Industrial Research- University Grant Commission, Junior Research Fellowship- June 2004 - June 2006.
Council for Scientific and Industrial Research- University Grant Commission, Senior Research Fellowship- July 2006- July 2009.
The Graduate Aptitude Test in Engineering (GATE), 2004, Indian Institute of Technology, New Delhi, India with 94.54% score.

Contributions to Science
Structure and dynamics of the Arabidopsis O-fucosyltransferase SPINDLY. Kumar, S., Wang, Y., Zhou, Y., Dillard, L., Li, F. W., Sciandra, C. A., Sui, N., Zentella, R., Zahn, E., Shabanowitz, J., Hunt, D. F., Borgnia, M. J., Bartesaghi, A., Sun, T. P. & Zhou, P., Dec 2023, In: Nature communications. 14, 1, 1538.

Structural basis of NPR1 in activating plant immunity. Kumar, S., Zavaliev, R., Wu, Q., Zhou, Y., Cheng, J., Dillard, L., Powers, J., Withers, J., Zhao, J., Guan, Z., Borgnia, M. J., Bartesaghi, A., Dong, X. & Zhou, P., May 19 2022, In: Nature. 605, 7910, p. 561-566 6 p.

Oncogenic KRAS is dependent upon an EFR3A-PI4KA signaling axis for potent tumorigenic activity. Adhikari, H., Kattan, W. E., Kumar, S., Zhou, P., Hancock, J. F. & Counter, C. M., Dec 1 2021, In: Nature communications. 12, 1, 5248.

Structure and function of the juxtamembrane GAF domain of potassium biosensor KdpD. Kumar, S., Gillilan, R. E. & Yernool, D. A., Sep 1 2020, In: Protein Science. 29, 9, p. 2009-2021 13 p.

An asymmetric heterodomain interface stabilizes a response regulator-DNA complex. Narayanan, A., Kumar, S., Evrard, A. N., Paul, L. N. & Yernool, D. A., Feb 14 2014, In: Nature communications. 5, 3282.

Accurate Identification of Periplasmic Urea-binding Proteins by Structure- and Genome Context-assisted Functional Analysis. Allert, M. J., Kumar, S., Wang, Y., Beese, L. S. & Hellinga, H. W., Nov 15 2024, In: Journal of Molecular Biology. 436, 22, 168780.

Chromophore carbonyl twisting in fluorescent biosensors encodes direct readout of protein conformations with multicolor switching. Allert, M. J., Kumar, S., Wang, Y., Beese, L. S. & Hellinga, H. W., Dec 2023, In: Communications Chemistry. 6, 1, 168.

Crystal structure and trimer-monomer transition of N-terminal domain of EhCaBP1 from entamoeba histolytica. Kumar, S., Ahmad, E., Mansuri, M. S., Kumar, S., Jain, R., Khan, R. H. & Gourinath, S., Jun 16 2010, In: Biophysical Journal. 98, 12, p. 2933-2942 10 p.

N- and C-terminal domains of the calcium binding protein ehcabp1 of the parasite entamoeba histolytica display distinct functions. Jain, R., Kumar, S., Gourinath, S., Bhattacharya, S. & Bhattacharya, A., 2009, In: PloS one. 4, 4, e5269.

Crystal structure of native O-acetyl-serine sulfhydrylase from Entamoeba histolytica and its complex with cysteine: Structural evidence for cysteine binding and lack of interactions with serine acetyl transferase. Chinthalapudi, K., Kumar, M., Kumar, S., Jain, S., Alam, N. & Gourinath, S., Sep 2008, In: Proteins: Structure, Function and Genetics. 72, 4, p. 1222-1232 11 p.

Crystal structure of calcium binding protein-1 from Entamoeba histolytica: A novel arrangement of EF hand motifs. Kumar, S., Padhan, N., Alam, N. & Gourinath, S., Sep 2007, In: Proteins: Structure, Function and Genetics. 68, 4, p. 990-998 9 p.