BIOGRAPHICAL SKETCH

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NAME: Jaigeeth Deveryshetty

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POSITION TITLE: Senior Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE	Completion Date MM/YYYY	FIELD OF STUDY
Osmania University	B.Sc	03/2000	Biochemistry, Microbiology Chemistry
Bangalore University	M.Sc	04/2003	Biotechnology
Indian Institute of Technology - Bombay	Ph.D.	08/2010	Biotechnology
Institute of Glycomics, Griffith University, Australia	Postdoc	12/2012	X-ray Crystallography and Glycobiology
Carl Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, USA	Postdoc	10/2016	Biochemistry and X-ray Crystallography
School of Medicine, St Louis University, USA	Postdoc	10/2019	Biochemistry and cell biology

A. Personal Statement

My long-term goal is to understand the fundamental mechanistic action of multiprotein complexes. Presently my work is focused on understanding the protein-protein interactions of recombination mediator proteins like Rad52 in presynaptic stage of Homologous recombination. Through my research work I would address the following two questions. 1) How does Rad52 gain access to ssDNA bound by RPA? and 2) How is ssDNA hand over by Rad52 to Rad51? Specifically, I would like to address with the structural details of the protein complexes involved in presynaptic state using CryoEM. The end goal is to use to develop structure-based chemotherapeutics to treat cancers. My research work ultizes pre-steady state kinetics, biophysical techniques, and unnatural amino acid incorporation to tease out the mechanistic details of functional activity and specificity of presynaptic complexes.

B. Positions, Scientific Appointments, and Honors

Positions

Oct 2019 -	Senior Scientist, School of Medicine, St Louis University, USA
Oct 2016-Dec 2018	Postdoc, School of Medicine, St Louis University, USA
May 2019-Oct 2020	Postdoc, School of Medicine, St Louis University, USA
Mar 2013 -Oct 2016	Postdoc, Carl Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, USA
Apr 2010 Dec 2012	Post Doc - Institute for Glycomics, Griffith University, Australia
Jan 2004 Aug 2010	Ph.D (Biotechnology), Indian Institute of Technology-Bombay, India

Academic and professional honors

Feb 2022	Session Co-Chair, Biophysical Society Annual Meeting, "*Platform: DNA Replication, Recombination, and Repair*"
Feb 2022	Poster Judge, Biophysical Society Annual Meeting, Macromolecular Machines Subgroup.
Oct 2015	Reviewer research paper for Microbiology Journal
Nov 2018	Reviewed research paper for Applied Environmental Microbiology
2005-2007	Awarded Junior Research Fellowship (JRF), University Grants Commission (UGC), India
2008-2010	Awarded Senior Research Fellowship (JRF) CSIR-UGC, India

C. Contributions to Science

C1. Structural details of pre-synaptic step in HR

Homologous recombination (HR) dependent repair of double stand DNA breaks (DSB) maintains genomic integrity. HR is broadly divided into three broad stages: presynapsis, synapsis and postsynapsis. In presynapsis, end resection machinery generates long stretches of ssDNA from DSBs that become coated by Replication Protein A (RPA). RPA protects ssDNA from nucleases and prevents formation of secondary structural features of ssDNA. Rad51 is the recombinase that searches for homologous genomic regions and catalyzes synapsis. Mediator proteins such as Rad52 promote HR by remodeling RPA and stabilize the RAD51 filament. Structural details of how the proteins interact and orchestrate this complex process is unknown. Using cryoEM we have uncovered a wealth of knowledge that upends conventional models of Rad52 interaction with HR proteins. Our structure of full-length yeast Rad52 reveals a homodecameric ring rather than the canonically ascribed heptamer. Preliminary cryoEM analysis of Rad52-RPA and Rad52-Rad51 complexes shows a surprising intrinsic allostery restricting protein-protein interactions to a subunit of Rad52. To understand this process high resolution structural data is required.

C2. Dynamics of RPA interaction with ssDNA

Replication Protein A (RPA) is an essential single-strand DNA binding protein that protects ssDNA from nucleases and removes secondary structures of ssDNA. RPA binding to ssDNA forms a platform for more than three dozen proteins and coordinates DNA metabolic processes including replication, repair, and recombination. RPA is composed of a series of DNA binding domains (DBDs A-E) and protein-interaction domains (PIDs). Previous work has revealed that DBDs-A & B are very dynamic whereas DBDs-C, D and E stably interact with DNA. My work using HDX-MS analysis presents a different model wherein RPA functions as two halves: a 'dynamic half' and a 'less-dynamic half' (Ahmad *et. al.* NAR. 2021). The dynamic half consisting of DBDs-A and B and less-dynamic half consisting of DBDs-C, D and E. This model lays the foundation for understanding how interacting proteins modulate these dynamic halves gain access to ssDNA.

1. Ahmad F., Patterson A., Deveryshetty J. Mattice J., Pokhrel N., Bothner B., and **Antony E.** Hydrogen–deuterium exchange reveals a dynamic DNA-binding map of replication protein A. *Nucleic Acids Research*. 2021. 49(3):1455-1469. PMID: 33444457

C3. Novel function of tumor suppressor protein PALB2

Tumor suppressor protein Partner and localizer of BRCA2 (PALB2) is has emerged as one of key protein of Homologous recombination. Using fluorescence based biochemical assay, I discovered a novel strand exchange function of PALB2 that contributes to the homologous recombination dependent DNA repair. I have determined the key DNA binding region in PALB2 and demonstrated this region is very good drug target. Following on this novel finding, I have screened 14000 compounds by high-throughput drug screening method targeting DNA binding site and identified 8 active compounds which are at different stages of validation. Simultaneously, I have crystallized and solved structure of PALB2 peptide bound to MRG

(histone). This structure allowed us to map mutations in cancer patient revealing essential amino acid residues in this interaction. This gave us insights into how cancer mutations can disrupt functions.

1. Novel RNA and DNA strand exchange activity of the PALB2 DNA binding domain and its critical role for DNA repair in cells. **Deveryshetty J**, Peterlini T, Ryzhikov M, Brahiti N, Dellaire G, Masson JY, Korolev S. ***Elife***. 2019 Apr 29;8. pii: e44063. doi: 10.7554/eLife.44063.

2. Redington J, Deveryshetty J, Kanikkannan L, Miller I, Korolev S. Structural Insight into the Mechanism of PALB2 Interaction with MRG15. Genes (Basel). 2021 Dec 17;12(12):2002. doi: 10.3390/genes12122002. PMID: 34946951; PMCID: PMC8701324.

C4. Structure of BioW

In 2013, I joined lab of Prof. Satish Nair at University of Illinois Urbana Champaign (UIUC), USA as Post-Doctoral Research Associate. Structural and mechanistic studies of novel enzymes in natural products (potential drugs) biosynthesis. Idea here is to understand the chemistry involved in the synthesis of novel natural products (potential drugs and antibiotics) and engineer these enzymes and organisms for large scale synthesis. I successfully crystallized and solved the novel structure of the biotin biosynthetic pathway enzyme, BioW in apo state. Apart from revealing the mechanistic details, BioW structure revealed a novel protein fold never reported before. X-ray diffraction data for this project was collected at synchrotron located in Argonne national lab, Chicago.

The pimeloyl-CoA synthetase BioW defines a new fold for adenylate-forming enzymes. Estrada P, Manandhar M, Dong SH, **Deveryshetty J**, Agarwal V, Cronan JE, Nair SK. ***Nat Chem Biol***. 2017 Jun;13(6):668-674. doi: 10.1038/nchembio.2359.

C. Rotavirus spike protein VP8* interaction with host factors

Rotavirus is the cause of 200,000 deaths due to severe childhood gastroenteritis in developing countries but also causes gastroenteritis in older population and in immune compromised individuals. Trypsin activated VP8* binds to sialic acids present in the glycans that are located on the cell surface and acts as a receptor for virus particles. Strains (animal strains like NCDV, CRW-8, RRV) whose binding is affected by treatment of sialidase are called sialidase sensitive and strains (human strains like Wa, RV-3) with unaffected binding are sialidase insensitive.

To understand the interaction of VP8* and VP5* of human rotavirus strains with host cells our group collaborated with virologists. GM1 is a complex glycan with an internal sialic acid commonly found on the cell surface of human cells. My work established the role of GM1 (bound by VP8*) and α2β1 integrin (bound by VP5*) in retroviral infection human cells. Sialidase treatment of host cells was found to enhance cellular GM1 surface exposure and the infectivity of several rotaviruses. Cholera Toxin B (which binds to GM1) and/or a-GM1 competition reduced the infectivity of these viruses to human cells, indicating human strains bind to GM1. Saturation Transfer Difference NMR spectra revealed that recombinant VP8* of RV-3 rotavirus recognized a-GM1, whereas RRV VP8* showed only weak STD NMR effects for a-GM1, suggesting a low affinity for the ligand. These data provide evidence that several human rotaviruses utilize GM1 glycans during infection.

Further, I studied structural features of protein interaction with carbohydrate ligands using X-ray crystallography. My research resulted in crystal structures that identified key interactions of Rotavirus spike protein with host carbohydrate and human galectin-1, a tumor suppressor protein, with galactose. Knowledge from the structure details of substrate binding was exploited in designing potent anti-viral and anti-cancer drugs.

Relative roles of GM1 ganglioside, N-acylneuraminic acids, and α2β1 integrin in mediating rotavirus infection. Fleming FE, Böhm R, Dang VT, Holloway G, Haselhorst T, Madge PD, **Deveryshetty J**, Yu X, Blanchard H, von Itzstein M, Coulson BS. ***J Virol***. 2014 Apr;88(8):4558-71. doi: 10.1128/JVI.03431-13

C. **Enzymology of Phenanthrene biodegradation**

Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds containing carbon and hydrogen atoms arranged in aromatic rings making PAHs stable. PAHs are carcinogenic pollutants that damage DNA leading to cancers. Bacterial metabolism of PAHs is the cleanest means to degrade these stable pollutants by enzymes called oxygenases. Phenanthrene (three-ringed) is one of the smallest PAHs, therefore is used as a model substrate to study the metabolism of high molecular weight PAHs. In bacteria, phenanthrene is metabolized to a two-ring key intermediate, 1-hydroxy-2-naphthoic acid (1-H2NA), which is further channelized to the central carbon pathway either via a 'naphthalene' or 'phthalic acid' routes.

In the 'naphthalene' route, 1-H2NA is metabolized via 1,2-dihydroxynaphthalene by 1-H2NA monooxygenase. Salicylate hydroxylase is thought to be the enzyme that catalyzes 1-H2NA to 1,2-dihydroxynaphthalene. Like salicylate hydroxylase (one ring substrate), 1-H2NA monooxygenase (2 ring substrate) replaces a carboxyl group with hydroxyl group to form a diol substrate that is suspectable to ring cleavage by dioxygenases. However, 1-H2NA monooxygenase was never purified and characterized. In the 'phthalic acid' route, 1-H2NA is cleaved by a novel 1-H2NA dioxygenase (1-HNDO) by novel mechanism to yield 2-carboxybenzalpyruvic acid, which is further metabolized via phthalic acid to tricarboxylic acid cycle intermediates. 1-HNDO cleaves carbon carbon bond between a carboxyl and hydroxyl groups not well characterized.

For the above reasons studying phenanthrene degradation by both pathways was vital for the general understanding of PAHs bioremediation and specific understanding of the biochemistry involved in 1H2NA metabolism. My work resulted in isolation and characterization of two Bacterial strains that utilized Phenanthrene as sole source of carbon and energy. I demonstrated these bacterial strains could utilize a mixture of Hydrocarbons simultaneously by activating all three PAH metabolic pathways. A key trait, useful in bioengineering microbes to accept wide range of substrates in bioremediation of contaminated sites. Most importantly I characterized two novel enzymes (proteins) that metabolize the same key intermediate (1-hydroxy 2-naphthoic acid) of Phenanthrene metabolic pathway into two different products that are channeled into their respective metabolic pathways.

1. Biodegradation of phenanthrene by Alcaligenes sp. strain PPH: partial purification and characterization of 1-hydroxy-2-naphthoic acid hydroxylase. **Deveryshetty J**, Phale PS. FEMS Microbiol Lett. 2010 Oct;311(1):93-101. doi: 10.1111/j.1574-6968.2010.02079.x.
2. Biodegradation of phenanthrene by Pseudomonas sp. strain PPD: purification and characterization of 1-hydroxy-2-naphthoic acid dioxygenase. **Deveryshetty J**, Phale PS. *Microbiology*. 2009 Sep;155(Pt 9):3083-91. doi: 10.1099/mic.0.030460-0. Epub 2009 Jul 2.
3. Metabolism of 2-, 3- and 4-hydroxybenzoates by soil isolates Alcaligenes sp. strain PPH and Pseudomonas sp. strain PPD. **Deveryshetty J**, Suvekbala V, Varadamshetty G, Phale PS. *FEMS Microbiol Lett.* 2007 Mar;268(1):59-66.
4. Metabolic diversity in bacterial degradation of aromatic compounds. Phale PS, Basu A, Majhi PD, **Deveryshetty J**, Vamsee-Krishna C, Shrivastava R. *OMICS*. 2007 Fall;11(3):252-79. Review.