BIOGRAPHICAL SKETCH

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NAME: Vijay Parashar

eRA COMMONS USER NAME (credential, e.g., agency login): parasharvj

POSITION TITLE: Assistant Professor

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 *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Panjab University, Chandigarh, India	BS	06/1999	Biology
Thapar University, Patiala, India	MS	06/2001	Biotechnology
Panjab University, Chandigarh, India	PhD	06/2006	Molecular Biology
Wayne State University, Detroit, MI	Post-Doctoral Fellow	12/2007	Biochemistry
Rutgers University, Newark, NJ	Post-Doctoral Fellow	12/2010	Molecular Biophysics and Biochemistry

A. PERSONAL STATEMENT

The discovery of the first antibiotic penicillin in the 1940s led to the belief that bacterial infections are completely curable. However, the enormous selective pressure exerted by these bactericidal agents soon lead to the emergence of antimicrobial resistance (AMR) in bacteria. AMR has been declared by the World Health Organization (WHO) as one of the top 10 global public health threats facing humanity today. AMR costs >$55 billion dollars and more than 35,000 lives in the USA each year. Therefore, new approaches to tackle bacterial infections and counter the development of AMR are urgently needed. The incredible ability of bacteria to adapt to a diverse array of biological (like phages) and environmental (like pH, temperature and antibiotics) stresses lies in their complex signal transduction pathways such as quorum sensing and RNA-based cyclic nucleotide second messengers (cNSMs) signaling which leads to expression of antibiotic resistance and virulence genes or the recently identified programmed cell death to ensure survival of other bacteria. A promising solution to antibiotic resistance and emergence of new virulent strains is to target these signaling pathways using “anti infectives” agents so that bacteria are unable to “turn on” undesired virulent traits or using novel “anti-microbials” agents that can trigger programmed cell death pathways in the pathogenic bacteria, *all without imposing any major selective pressure in bacterial populations*. Unfortunately, such agents are not easy to develop because most of these pathways are not completely understood at mechanistic levels.

My 20 years of academic journey has prepared me well to face the biological questions posed in this renewal application. During my doctoral studies, I discovered and characterized novel restriction endonucleases from the restriction-modification phage-defense systems. During my postdoctoral training at Wayne State University, I worked on biochemical characterization of human cytidine deaminase (AID), which is a distant homolog of bacterial adenosine deaminase family members proposed here to deaminate adenosines to Inosines. During my postdoctoral training at Rutgers University, I studied the mechanistic details of quorum sensing and cNSM biology in Gram-positive bacteria by structural biology, biochemistry and genetics. During these postdoctoral trainings, I also learnt advanced skills in phage biology (*e.g.*, phage-based genetics and phage-display) which will be useful in this proposal.

My passion for developing a detailed understanding of bacterial cNSM biology, and for discovering new agents that target these mechanisms fueled me to start my independent lab that uses a combination of biochemistry, bacterial genetics, and structural biology. With the help of ESI-MIRA award from NIGMS, we started exploration of metabolism and functions of cyclic-di-AMP (c-di-AMP), an indispensable cNSM in bacteria that regulates important physiological processes, including potassium homeostasis, virulence, and development of resistance to β-lactam antibiotics. We have identified the mechanisms of binding of c-di-AMP cNSM to the KdpD histidine kinase (HK) using X-ray crystallography, and have established KdpD-USP domains as new

structural class of USP proteins. We recently discovered a novel, c-di-AMP -dependent, function of KdpD HKs in bacterial nucleoid assembly and biofilm formation (unpublished). We also identified and characterized potent small-molecule inhibitors of c-di-AMP phosphodiesterases of the widespread GdpP class for the first time (unpublished, and a provisional patent application is in preparation). Further, we recently finished regulatory exploration of the c-di-AMP cyclase, DisA, which we found to be regulated by its reaction intermediate (unpublished). I have also been actively collaborating on the development of novel tools for single-molecule imaging of various RNA types & forms including cNSMs.

Recently, the field of bacterial signal transduction has exponentially expanded with the discovery of a variety of new cNSMs with different lengths and compositions that have important roles in bacterial conflict systems of abortive infections. However, due to lack of information on the identity, an understanding of receptor regulatory mechanisms is still in infancy. I am vested to functionally characterize the mechanisms of action of key players involved in novel process of programmed cell death in bacteria as outlined in the submitted proposal.

As a scientist and faculty member, I diligently contribute to the growth and nurturing of STEM appreciation in our community beyond the scope of my assigned workload of teaching and service at the University. In addition to serving as a peer reviewer and editor for PubMed indexed journals including being a guest editor for special issue of "*Microbial cooperation and conflict'* Collection in Scientific Reports, Nature publishing group, I am also a panelist and reviewer for the Graduate research fellowship program (GRFP) by NSF for last three years. I have been actively involved in various national and international scientific communities like American society for biochemistry and molecular biology (ASBMB) and American society for microbiology (ASM). I regularly present my work at bacterial signal transduction conferences like Gordon STIM and BLAST conferences to disseminate my findings to the research community. I am a regular member of the Science Advisory Board, where I provide input on various product concepts and designs. Above all of these, I get the most enthralling experience when I engage with young inquisitive minds either when I served as a judge for the odyssey of the mind challenge or when I participate in the annual STEAM Days for the project BrainLight where students from local high schools spend one day learning about various scientific principles driving research. I love to engage with young students to inculcate a curiosity about research. I am also implementing Course-Based Undergraduate Research Experiences (CURES) based platform called Biochemistry Authentic Scientific Inquiry Lab (BASIL) in which the students in my teaching labs are provided real research exposure instead of just educational kits. I have recently engaged with the Delaware Technology Community College to provide research internships for students from low income and under presented communities in my laboratory. I am actively participating in the culturally aware mentoring program and mentor training programs at my institute to grow as a mentor and create a nurturing environment in my lab. In future, I would like to continue these endeavors and become more engaged with service at community level as well actively participate in diversity caucuses at University of Delaware to contribute towards the next generation of scientists and visionaries.

B. POSITIONS AND HONORS

Positions and Employment

2017 -	Assistant Professor, University of Delaware, Newark, DE
2014 - 2017	Assistant Professor, Rutgers University, Newark, NJ
2013 - 2014	Research Associate, Rutgers University, Newark, NJ
2010 - 2013	Research Associate, University of Medicine and Dentistry of New Jersey, Newark, NJ
2001 - 2006	Research Scholar, Panjab University, Chandigarh
2000 - 2000	Internship, Indian Institute of Technology, Roorkee

Other Experience and Professional Memberships

2022 -	Member, American Society of Microbiology
2022 -	Member, American Society for Biochemistry and Molecular Biology
2020 -	Guest Editor for 'Microbial cooperation and conflict' Collection in Scientific Reports, Nature
2018 -	Steering committee member: Microbiology Graduate Program at UD
2015 -	Editorial board member: *Scientific Reports,* Nature publishing group
2014 -	Reviewer for multiple peer reviewed manuscripts.

Honors

2023	Nominated for 2023 Excellence in Research Award for College of Health Sciences at University of Delaware
2022	Nominated for 2022 Excellence in Research Award for College of Health Sciences at University of Delaware
2022	Featured article "New approach to reduce antibiotic dependency against diseases" in UDaily
2021	Nominated for the 2020 Excellence in Teaching Award at University of Delaware
2017	Received an excellence in research award from New Jersey Health Foundation
2016	Selected to receive Free FDA approved compound library (worth $10,000) from Rutgers Translational Science through internal competition
2013	Nominated for Blavatnik award (NYAS) by Rutgers University
2006	Technology Transfer, Licensed New England Biolabs (Ipswich, MA) for production and commercialization of a thermostable type II restriction endonuclease, TspMI, (5'C/CCGGG3'). NEB is currently selling TspMI under catalog number R0709.
2004	Selected internationally amongst 20 participants to attend Summer School in Nanobiotechnology, Osaka University, Japan. Awarded funds for travel and accommodation
2004	Received travel award for 5th NEB meeting on restriction /modification systems, Bristol, UK.

C. CONTRIBUTIONS TO SCEINCE:

1. Isolation and characterization of novel restriction endonucleases:

One of earliest understood bacterial defense systems against phage infections are the restriction modification (RM) systems. In RM systems bacteria express specific restriction endonucleases that target viral DNA while bacterial DNA is protected by methylation. During my graduate work, I isolated thermophilic bacteria from hot water springs of Himalayas, purified, and characterized several novel restriction endonucleases from these isolates. I transferred the production technology for one of them to New England Biolabs (NEB). This enzyme, TspMI, is being sold commercially by NEB as one of their time-saver enzymes. I was also involved in teaching molecular cloning to graduate students and published laboratory courses on REBASE-assisted restriction mapping.

I. Sharma, P., D'Souza, DR., Bhandari, D., **Parashar, V**., Capalash, N. (2003) Demonstration of the Principles of Restriction Endonuclease Cleavage Reactions Using Thermostable Bfl I from *Anoxybacillus flavithermus*. *Biochem Mol Biol Educ*. 31:392-96. DOI: 10.1002/bmb.2003.494031060283

II. **Parashar V**, Capalash N, Xu SY, Sako Y, Sharma P. (2006) TspMI, a thermostable isoschizomer of XmaI (5'C/CCGGG3'): characterization and single molecule imaging with DNA. *Appl Microbiol Biotechnol.* 72(5):917-23. DOI: 10.1007/s00253-006-0386-6

III. **Parashar V**, Capalash N, Sharma P. (2007) Demonstration of REBASE-assisted restriction mapping to determine the recognition site of unknown restriction endonucleases. *Biochem Mol Biol Educ*. 35(5):337-41. DOI: 10.1002/bmb.82

2. Structural biology of quorum-sensing signal transduction receptors in Gram-positive bacteria:

The highly homologous members of Rap family of proteins are important cytosolic quorum-sensing receptors involved in regulating biologically important social behaviors, including sporulation, genetic competence, antibiotic expression, and transposition of genetic elements in *Bacillus* species. At high cell density, secreted quorum-sensing signals, called Phr oligopeptides, are imported into the cells, where they bind to Rap proteins and inhibit their function. Despite 40 years of biochemical and genetic studies, the molecular mechanism of Rap protein function and its regulation by Phr quorum-sensing oligopeptides remained entirely unknown. I employed a combination of complementary experimental approaches, including X-ray crystallography, biochemistry, bacterial genetics, and computational modeling, to show at the atomic level how Rap proteins function, and how Phr oligopeptides regulate these functions. These studies provided the first example of conformational change-induced repeat domain expansion regulating protein function. Further, we elucidated the three-dimensional structure of Rgg protein (a critical quorum sensing regulator in *Streptococci*) alone, and in complex with a cyclic inhibitor peptide to obtain mechanistic details of this regulation.

I. **Parashar V**, Mirouze N, Dubnau DA, Neiditch MB. (2011) Structural basis of response regulator dephosphorylation by Rap phosphatases. *PLoS Biol.* 8;9(2):e1000589. DOI:10.1371/journal.pbio.1000589

II. Mirouze N, **Parashar V**, Baker MD, Dubnau DA, Neiditch MB. (2011) An atypical Phr peptide regulates the developmental switch protein RapH. *J Bacteriol*. 193(22):6197-206. DOI:

10.1128/JB.05860-11

III. **Parashar V**, Jeffrey PD, Neiditch MB (2013) Conformational change-induced repeat domain expansion regulates Rap phosphatase quorum-sensing signal receptors. *PLoS Biol*. 11(3): e1001512. DOI:10.1371/journal.pbio.1001512

3. Development of novel small molecule inhibitors against pathogenic bacteria and use of complementary genetic approaches to understand regulation of cellular turgor:

I have been developing and using multiple bacterial genetic systems to understand physiological relevance of the interactions that we identified using biochemical and structural approaches. Using these techniques, I have discovered novel cell-cell communication signals in Gram-positive bacteria and performed functional characterization of orphan signal transduction proteins. Furthermore, we discovered novel thiophene compounds that inhibit cell wall synthesis, and that kill *Mycobacterium tuberculosis*. Most recently, we identified the mechanism of BCCT transporter system in *Vibrio* species by modelling its interaction with the substrates.

I. Wilson R, Kumar P, **Parashar V**, Vilchèze C, Veyron-Churlet R, Freundlich JS, Barnes SW, Walker JR, Marchiano, Shenai S, Colangeli R, Jacobs WR, Neiditch MB, Kremer L, Alland D. (2013) Highly efficient antituberculosis activity of a new class of thiophene compounds which target the Mycobacterium tuberculosis Polyketide Synthase Pks13 and inhibit mycolic acid production. *Nat Chem Biol.* Aug;9(8):499-506. DOI: 10.1038/nchembio.1277

II. **Parashar V**, Aggarwal C, Federle MJ, Neiditch MB. (2015) Rgg protein structure-function and inhibition by cyclic peptide compounds. *Proc Natl Acad Sci USA*. Apr; 9(8): 499-506. DOI: 10.1073/pnas.1500357112

III. Gregory GJ, Dutta A, **Parashar V**, Boyd EF. Investigations of dimethylglycine (DMG), glycine betaine (GB) and ectoine uptake by a BCCT family transporter with diverse substrate specificity in Vibrio species. *J Bacteriol*. 2020 Aug 17;. DOI: 10.1128/JB.00314-20.

4. Transcriptional regulation and RNA-protein interactions in different biological systems:

While we are deeply vested in bacterial proteins and their function, we are also extending our expertise into mammalian systems. We have performed biochemical analysis to identify role of transcription factor NKX2.2 as a novel co-transcriptional regulator of the master regulator protein, EWSFI1, in regulating the oncogenesis of the solid tumor called Ewing's sarcoma. This work opened new avenues to understand the genome-wide implications of this NKX2.2-EWSFLI1 interaction. Further, we helped in performing Chromatin immunoprecipitation and target validation to identify genome-wide promoters that are regulated by this interaction (manuscript submitted). Currently, we are utilizing our expertise in RNA biochemistry to characterize RNA -editing enzymes as a part of this proposal. Furthermore, we are exploring a vast repertoire of regulatory RNAs in different biological systems. We worked with Batish laboratory at UD to characterize the role of microRNAs in Chronic lymphocytic leukemia and published an extensive literature review for the role of non-coding RNAs in Ewing's Sarcoma progression. One of our long-standing collaborative research directions with Batish laboratory is to develop tools for imaging different RNA species. Towards this, we published an advanced review on the methods of imaging different steps of RNA processing. Most recently, we developed a novel method for imaging circular RNA, a newly appreciated class of regulatory RNAs. Since bacterial cNSMs are close mimics of RNA, we are currently working to optimize RNA imaging tools to develop *in vivo* imaging methods to visualize their intracellular concentrations.

I. Markey FB, **Parashar V**, Batish M (2021) Methods for spatial and temporal imaging of the different steps involved in RNA processing at single-molecule resolution. Wiley Interdisciplinary Reviews on RNA 12(1), PMID: 32543077

II. Barrett C, Budhiraja A, **Parashar V**, Batish M. The landscape of regulatory non-coding RNAs in Ewing's sarcoma. Biomedicines. 2021 9(8), 933. DOI: 10.3390/biomedicines9080933

III. Markey FB, Romero B, **Parashar V,** Batish M. Identification of a new transcriptional co-regulator of STEAP1 in Ewing's Sarcoma. *Cells* 2021, *10*, 1300. DOI: 10.3390/cells10061300

IV. Koppula A, Abdelgawad A, Guarnerio J, Batish M, **Parashar V***. CircFISH: A Novel Method for the Simultaneous Imaging of Linear and Circular RNAs. ***Cancers*** (Basel). 2022 Jan 15;14(2):428. doi: 10.3390/cancers14020428.

5. Structural and functional characterization of cNSMs in prokaryotes:

The cNSM signaling in bacteria is a major research direction in my lab. I started in this field with the identification and biochemical characterization of novel anti-microbial small-molecule inhibitors of bacterial diguanylate cyclases, which are enzymes that synthesize c-di-GMP, a centrally important bacterial cNSM. I also biochemically characterized a novel class of benzimidazole compounds that inhibit bacterial biofilm formation. Currently, we are pursuing two broad areas understanding: 1) how bacterial cNSM receptors sense these signals to conduct responses, and 2) how cNSMs metabolism is regulated. We recently elucidated the cNSM-mediated regulation of potassium homeostasis and virulence in Firmicutes. The KdpD histidine kinases (HKs) of the KdpDE two-component system contain a universal stress protein (USP) domain which binds to c-di-AMP cNSM for regulating transcriptional output but the structural basis of c-di-AMP specificity within the KdpD-USP domain was not well understood. We determined a high-resolution crystal structure of the *S. aureus* KdpD-USP domain (USP_{Sa}) complexed with c-di-AMP. Binding affinity analyses of USP_{Sa} mutants targeting the observed USP_{Sa}:c-di-AMP structural interface enabled the identification of USP_{Sa} residues that are required for c-di-AMP specificity that allowed us to predict c-di-AMP binding in other KdpD HKs. Furthermore, we found that the USP_{Sa} domain contains structural features distinct from the canonical standalone USPs that bind ATP as a preferred ligand. Notably, our study established USP_{Sa}-like domains in KdpD HKs as a novel subfamily of the USPs. The biological scope and impact of bacterial cNSM signaling has significantly expanded in the last five years. Multiple different classes of bacterial cNSMs are now known to be synthesized by bacteria upon infection by phages. While these cNSMs have significant impact on bacterial physiology, critical gaps exist in the knowledge of how cOAs and PSMs function. This is due either to a lack of information on receptor identities or to a lack of structures of the known cNSM receptors. Towards this, we have recently identified a transcription factor (Csa3) receptor of a cOA (cA4) which outlines crosstalk between Type III and Type I CRISPR systems. We have elucidated the X-ray crystal structure of a cA4-bound Csa3 homolog. Structure-function studies on Csa3-cA4 interactions has identified the basis of cA4 binding and allosteric regulation of Csa3 function.

I. Sambanthamoorthy K, Gokhale AA, Lao W, **Parashar V**, Neiditch MB, Semmelhack MF, Lee I, Waters CM. (2011) Identification of a novel benzimidazole that inhibits bacterial biofilm formation in a broad-spectrum manner. *Antimicrob Agents Chemother.* 55(9):4369-78. DOI: 10.1128%2FAAC.00583-11

II. Sambanthamoorthy K, Sloup RE, **Parashar V**, Smith JM, Kim EE, Semmelhack MF, Neiditch MB, Waters CM. (2012) Identification of small molecules that antagonize diguanylate cyclase enzymes to inhibit biofilm formation. *Antimicrob Agents Chemother.* 56(10):5202-11. DOI: 10.1128/AAC.01396-12

III. Dutta A, Batish M, **Parashar V***. Structural basis of KdpD histidine kinase binding to the second messenger c-di-AMP. *J Biol Chem.* 2021 296, 100771. DOI: 10.1016/j.jbc.2021.100771

IV. Xia P, Dutta A, Gupta K, Batish M, **Parashar V*.** Structural basis of cyclic oligoadenylate binding to the transcription factor Csa3 outlines crosstalk between Type-III & Type-I CRISPR systems. ***J Biol Chem***. 2022 Jan 14:101591. doi: 10.1016/j.jbc.2022.101591.

Complete List of Published Work

https://www.ncbi.nlm.nih.gov/myncbi/121D5LPkT_RAM/bibliography/public/

BIOGRAPHICAL SKETCH

NAME:Shataer, Shadikejiang

eRA COMMONS USER NAME: SSHATAER

POSITION TITLE: Graduate Student (Research/Teaching Assistant)

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Start Date	Completion Date	FIELD OF STUDY
Shanghai Jiao Tong University	BA	09/2011	07/2015	Biotechnology (Bioinformatics)
University of Glasgow	Master of Science	08/2016	07/2017	Biotechnology
Birkbeck, University of London	Master of Research	09/2018	06/2019	Structural Biology
University of Delaware	PhD	08/2019	NA	Structural Biology

A. Personal Statement

My initial fascination with structural biology was sparked during an undergraduate summer research project. I distinctly recall encountering a crystal structure of a human GPCR, and I was instantaneously captivated by the intricate insights the structure unveiled. Progressing to my master's studies, I delved deeper into X-ray crystallography, particularly focusing on membrane proteins, while engaged at the Cogdell laboratory at the University of Glasgow. Although the power of X-ray crystallography enthralled me, the challenge of obtaining well-diffracting crystals prompted me to approach the field with cautious optimism.

The advent of the 'Resolution Revolution' within Cryo-EM propelled me to explore this method further, under the guidance of Prof. Elena Orlova at Birkbeck College, University of London. Collaborating with an adept team, I achieved the atomic resolution structure of a phage portal protein within a year using Cryo-EM. This endeavor acquainted me with the theoretical underpinnings of Cryo-EM and image processing, as I employed RELION to analyze my structure, deepening my comprehension of this field.

During my doctoral studies at the University of Delaware, under the mentorship of Dr. Parashar, I gained practical expertise in integrated structural biology, along with additional biophysical techniques such as small-angle X-ray scattering (SAXS), microscale thermophoresis (MST), and analytical ultracentrifugation (AUC). Presently, my efforts are divided between two distinct projects necessitating the fusion of these aforementioned techniques. The first project centers on the comprehensive characterization of newly discovered enzyme inhibitors within the cyclic-di-AMP signaling pathway, while the second project focuses on the structural elucidation of enzymes integral to the same pathway. Overall, I am convinced that the current landscape of my research,

coupled with my proposed training plan, will furnish a robust groundwork for my enduring aspiration to establish myself as an academic researcher.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments:

2019 – Present PhD Student, College of Health Sciences, University of Delaware

Honors:

2023 **Poster Presenter,** Health Science Research Day, University of Delaware

2022 **Poster Presenter,** Health Science Research Day, University of Delaware

2014 Chinese National Merit Scholarship Program

2013 Chinese National Merit Scholarship Program

C. Contributions to Science

1. **Masters Research Project:** While working in the Orlova lab at Birkbeck College, Universty of London, I was able to process a set of Cryo-EM data for a phage portal protein. Under the mentorship of Prof Orlova and Dr. Javed, I was able to quickly obtain an atomic structure[1].

Javed, A.; Villanueva, H.; **Shataer, S**.; Vasciaveo, S.; Savva, R.; Orlova, E. V. Cryo-EM Structures of Two Bacteriophage Portal Proteins Provide Insights for Antimicrobial Phage Engineering. *Viruses* **2021**, *13* (12), 2532. https://doi.org/10.3390/v13122532.

2. **PhD Research**: My current doctoral research is centered around exploring the metabolic regulation of the cyclic-di-AMP signaling pathway, as well as its pivotal role within bacterial genome repair processes. My work has yielded intriguing findings, indicating that the enzyme responsible for synthesizing cyclic-di-AMP in *Mycobacterium tuberculosis* displays a distinctive structural pattern. This discovery holds promise in advancing the development of improved vaccines against this pathogen. I firmly believe that the outcomes of my research will bear substantial significance for human health. By shedding new light on the intricate mechanisms of this resilient and intricate pathogen, my findings have the potential to provide valuable insights into addressing complex health challenges.