BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Kenny, Sebastian

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

POSITION TITLE: Graduate Research Assistant

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)	START DATE MM/YYYY	COMPLETION DATE MM/YYYY	FIELD OF STUDY
Purdue University, West Lafayette, IN	BS	08/2015	05/2018	Biochemistry (ACS)
Purdue University, West Lafayette, IN	PHD	05/2018	CURRENT	Chemistry (Biochemistry)

A. Personal Statement

I am a Ph.D. candidate aspiring to obtain an MD/Ph.D. degree with a career goal as a clinical researcher, specifically in pediatric medicine. I grew up in Surabaya, Indonesia and migrated to the United States to get the necessary experience for me to be a leading scientist who can utilize the most recent research technology. I have experience in both organic compound synthesis as well as molecular biology, including site-directed mutagenesis, cloning, protein expression, and protein purification. During my undergraduate studies, I helped develop photoacoustic water-soluble contrast agents for cardiovascular blockage detection. Currently, I am training in the field of structural enzymology, employing the techniques of X-ray crystallography, cryo-electron microscopy, and other biophysical methods to better understand the mechanism of enzymes.

In the Das Lab, we study the mechanism of ubiquitinating and deubiquitinating proteins and seek to identify sites that can be targeted by small molecule therapeutics. I am currently working towards determining the structure of a five-protein complex that forms during Human Papillomavirus (HPV) infection. If this complex can be modulated by small molecules, it may be possible to prevent tumor development and heart failure that is a potential consequence of HPV infection. In addition to my research experience, I have also been a part of an NIGMS-funded Molecular Biophysics Training Program. This program has given me additional experience in networking with academics, extra coursework to strengthen my biophysical chemistry skills, and greater opportunities to communicate my research work to others at Purdue and national conferences. Finally, I believe that my personality sets me apart from other candidates for this fellowship. I am an extremely organized, highly motivated, and experienced leader. I am currently an American Heart Association Predoctoral Fellowship. As a fellow, I have boosted my research capability, resulting in the first cryo-EM structures from the Das Lab. The fellowship has also given me opportunities to form a network with other professionals in the field of structural enzymology and biophysics.

B. Positions and Honors

Positions and Scientific Appointments

2019 -	Graduate Research Assistant, Department of Chemistry, Purdue University, West Lafayette, IN
2018 - 2019	Teaching Assistant - General Chemistry and Biochemistry, Department of Chemistry, Purdue University, West Lafayette, IN
2017 - 2018	Organic Chemistry Supplemental Instruction Leader, Student Success at Purdue University, West Lafayette, IN
2016 - 2019	Resident Assistant, University Residences at Purdue University, Purdue Village, West Lafayette, IN

Honors

2021 - 2022	PCCR SIRG Research Assistantship, Purdue Center for Cancer Research
2020 - 2021	PI4D/PCCR Training Grant Fellowship, Purdue University
2019 - 2020	Graduate School Training Grant Fellowship, Purdue University
2018 - 2019	Ross Fellowship, Purdue University
2017 - 2018	Mark Fesenmeyer Scholarship, Department of Chemistry, Purdue University
2021	Student Research Achievement Award, Biophysical Society
2021	Diversity Award, The Protein Society
2021	Graduate Poster Competition Award, The Protein Society
2019	Phi Lambda Upsilon Travel Award, Phi Lambda Upsilon
2018	Mortar Board Fellowship, Purdue University
2017	Class of 1937 Scholarship, Student Activities and Organization, Purdue University
2017	John Leighty Award, Department of Chemistry, Purdue University
2017	College of Science Scholarship, College of Science, Purdue University
2016	College of Science Outstanding Student Award, College of Science, Purdue University
2016	Undergraduate Wolinsky – Summer Research Award, Department of Chemistry, Purdue University
2016	Ruth Skillman Award, Department of Chemistry, Purdue University

C. Contribution to Science

1. **Undergraduate Research**: I started research early in my undergraduate career under Dr. Mingji Dai, an uprising name in total synthesis methodology. Under Dr. Dai, I helped develop a carbon-carbon cross-linking synthesis method using cyclopropanols. Cyclopropanols are easily synthesized using the Kulinkovich reaction, allowing novel synthesis of previously redundant synthesis pathways. I joined Dr. Jianguo Mei's research group to learn more organic chemistry, specifically polymer and oligomer synthesis. My project was to develop a photoacoustic contrast agent using donor-acceptor-donor monomers. Such contrast agents give a higher resolution in blood clot imaging compared to the currently available contrast agents. Given the fact that the human body is mostly composed of water, my main contribution was making this oligomer water-soluble by introducing ionic side chains. I employed small molecule organic synthesis (functional group protection & deprotection, alkylation, and oligomerization) as well as spectroscopic methods to validate each intermediate, including nuclear magnetic resonance (NMR) spectroscopy, infrared spectroscopy (IR), and mass spectrometry (MS). This experience trained me to employ chemical methods in approaching biological questions.

    a. **Kenny S**, Chaudhry S, Mei J. *Synthesis of a Water-Soluble Organic Conjugated Donor-Acceptor Oligomer*. Purdue University Undergraduate Research Symposium; 2018 April; West Lafayette, IN.

2. **Graduate Research:** My ongoing research in the Das Lab focuses on understanding mechanistic pathways of protein-protein interaction, specifically in ubiquitinating and deubiquitinating proteins. Bacterial effector often plays a role in pathogen survival, either by deubiquitinating proteins that are essential for pathogen survival, hence avoiding proteasomal degradation or by ubiquitinating proteins that pose as a threat for the survival of the pathogen. We do this through structure determination of the protein complexes involving the pathogenic effectors, as well as quantification and characterization of their activity utilizing various biochemical and biophysical techniques including but not limited to BLI, ITC, Protein NMR, X-ray crystallography. More recently we have started looking into cryo-Electron Microscopy for structure solution of some of the larger molecular assemblies of certain pathogenic effectors. Structural Enzymology and biochemical assays of mutated enzymes and their activity allow me to develop hypotheses on how proteins utilize their side chain's characteristics in its functioning mechanism. In a broader sense, understanding the enzyme mechanism allows us to study the variability of the active site – which leads us to better development for drugs that target the activity of the enzyme. This development would allow better provision of medical treatments related to similar-acting enzymes. I am currently in the process of writing two manuscripts, one on a product release mechanism that we studied with BLI which I will be the first co-author on, and one on the mechanism of a bacterial effector MavC, in which I contributed my binding studies of MavC and its substrates and product.

a. Sheedlo MJ*, **Kenny S***, Podkorytov IS, Brown K, Ma J, Iyer S, Hewitt CS, Arbough T, Mikhailovskii O, Flaherty DP, Wilson MA, Skrynnikov NR, Das C. Insights into Ubiquitin Product Release in Hydrolysis Catalyzed by the Bacterial Deubiquitinase SdeA. Biochemistry. 2021 Mar 2;60(8):584-596. PubMed PMID: 33583181.

b. Puvar K, Iyer S, Fu J, **Kenny S**, Negrón Terón KI, Luo ZQ, Brzovic PS, Klevit RE, Das C. Legionella effector MavC targets the Ube2N~Ub conjugate for noncanonical ubiquitination. Nat Commun. 2020 May 12;11(1):2365. PubMed Central PMCID: PMC7217864.

c. Hausman JM, **Kenny S**, Iyer S, Babar A, Qiu J, Fu J, Luo ZQ, Das C. The Two Deubiquitinating Enzymes from *Chlamydia trachomatis* Have Distinct Ubiquitin Recognition Properties. Biochemistry. 2020 Apr 28;59(16):1604-1617. PubMed Central PMCID: PMC7700883.

*indicates co-first authorship

D. Scholastic Performance

Scholastic Performance

YEAR	COURSE TITLE	GRADE
	PURDUE UNIVERSITY	
2015	Introduction to Chemistry I	A+
2015	Freshman Chemistry Orientation	A
2015	Chemistry Freshman Honor Research	A
2015	First-Year Composition	A+
2015	Plane Analytic Geometry And Calculus I	A+
2015	Introductory Sociology	A
2016	Introduction to Chemistry II	A+
2016	Plane Analytic Geometry And Calculus II	A+
2016	Chinese Level VI	A
2016	Modern Mechanics	A+
2016	Elementary Psychology	A+
2016	C Programming	A+
2016	Multivariate Calculus	A+
2016	Intro to Statistics	A+
2016	Fundamentals Of Biology I	A+
2016	Organic Chemistry I	A+
2016	Organic Chemistry Laboratory Honors I	A+
2016	Sophomore Chemistry Seminar	A
2016	Science Writing and Presentation	A
2016	Residential Leadership Seminar	A
2016	Electric And Magnetic Interactions	A+
2017	Fundamentals of Biology II	A+
2017	Introductory Inorganic Chemistry	A
2017	Organic Chemistry II	A+
2017	Organic Chemistry Laboratory Honors II	A+
2017	Analytical Chemistry I Honors	A+
2017	Junior-Senior Chemistry Seminar	A-
2017	Introduction To Cognitive Psychology	A
2017	Biology III: Cell Structure And Function	A
2017	Laboratory In Biology III: Cell Structure And Function	A+
2017	Great Issues Genomics And Society	A
2017	Physical Chemistry I	A+
2017	Physical Chemistry Laboratory	A

2017	Introductory Biochemistry	A+
2018	Genetics	A
2018	Genetics Laboratory	A-
2018	Neural Systems	A
2018	Inorganic Chemistry	A
2018	Physical Chemistry II	A+
2018	Physical Chemistry Laboratory II	A
2018	Molecular Biotechnology	A
	PURDUE UNIVERSITY	
2018	Biochemistry: Dynamic Aspects	A+
2018	Safety in Laboratory	S
2018	Membranes: Structure And Function	A+
2018	Biochemistry: Structural Aspects	A
2018	Bioinorganic Chemistry	A+
2018	Advanced Organic Chemistry	A
2018	Biochemistry Seminar	S
2018	Teaching in Chemistry	S
2019	X-ray Crystallography	AU
2019	Synthetic Organic Chemistry	AU
2019	Biochemistry Seminar	S
2019	Life of a Faculty Entrepreneur	A
2019	Methods and Measurements in Biophysical Chemistry	A
2019	Structural Biology Research Seminar	A
2020	CryoEM 3D Reconstruction	AU
2020	Biophysics Grant Writing	A
2020	Biophysical Methods	A
2020	Biophysical Chemistry	AU

The grading system at Purdue University gives the same amount of grade point for A+ and A. Some classes give A+ while some do not.
Zero-Credit Courses are graded as follows: S-Satisfactory, U-Unsatisfactory, SI-Incomplete, IU-Unremoved Incomplete, WU-Withdrew Unsatisfactory.
Classes that are audited are given a grade of "AU". Classes that are currently taken are given a grade of "IN PROGRESS".

Research Support

Ongoing Research Support

American Heart Association Predoctoral Fellowship 01/01/2022 - 12/31/2023
American Heart Association, 905924
p53 is a protein that is important for cell survival and one that is heavily regulated. This regulation is altered in patients with Human papillomavirus (HPV) infection. HPV produces a protein that aids in degradation of p53. This degradation results in many diseases, including many types of cancer. This project aims to understand how p53 is degraded by this protein produced by HPV. Using cryo-EM and biophysical methods, this project aims to identify important regions of the complex that are needed for its activity. Identifying these regions will allow us to design drugs to prevent p53 degradation by HPV.
Role: Principal Investigator

Completed Research Support

PCCR SIRG Graduate Research Assistantship 07/01/2021 - 12/31/2021
Purdue University Center for Cancer Research, P30CA023168
The Purdue University Center for Cancer Research has allocated SIRG Graduate Research Assistantships from the Office of the Executive Vice President for Research and Partnerships (EVPRP) to support center faculty members with outstanding graduate students undertaking cancer-related research.
Role: Principal Investigator

PI4D/PCCR Training Fellowship (PI: John J.G. Tesmer) 07/01/2019 - 06/30/2021
(linked to 1T32GM132024)

Purdue University Molecular Biophysics Training Program

The goal of this program are: (i) to provide enhanced training in the application of molecular biophysics in a rigorous and reproducible way to modern problems in human health and disease, (ii) to foster effective and inclusive teamwork, and (iii) to provide career development opportunities tailored to the goals of individual trainees.

Role: Graduate Student Trainee

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Iyer, Shalini

eRA COMMONS USERNAME (credential, e.g., agency login): IYER59
POSITION TITLE: Post-doctoral Research Associate

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Delhi, New Delhi, India	BSc	07/1993	05/1996	Zoology (Specialization in Biochemistry and Genetics)
University of Delhi, New Delhi, India	MSc	07/1996	05/1998	Zoology (Specialization in Biochemistry, Cell & Molecular Biology, and Immunology)
NIIT, Delhi, India	Certification course	06/1998	07/1999	Computing: UNIX
University of Bath Bath, United Kingdom	PhD	01/2000	03/2003	Biochemistry "Structural Studies on Angiogenic Proteins"
University of Bath Bath, United Kingdom	Post-doc (Funded by Wellcome Trust)	02/2003	12/2013	Probing structure-function relationships of proteins involved in angiogenesis
University of Bath Bath, United Kingdom	Post-doc (Funded by Motor Neuron Disease Association)	12/2013	05/2017	Structure-function studies on C9ORF72, a protein associated with familial FTD and/or ALS
University of Bath Bath, United Kingdom	Post-doc (Funded by AngioDesign Ltd)	06/2017	05/2018	Structure-guided design of novel, next generation inhibitors for Neprilysin
Purdue University, West Lafayette, IN	Post-doc (Funded by NIH)	06/2018	present	Mechanism of atypical ubiquitination and deubiquitination by bacterial effectors

A. PERSONAL STATEMENT

I was born and raised in Delhi, India, a country where gender (especially a few decades ago) and caste (sadly to some extent even now) plays a huge role in education. I grew up in a Brahmin family (strike 1) where although girls (strike 2) received education, they were expected to marry early and start a family. But my story didn't end there. I shall always be thankful to my parents who decided not to follow the norm and believed that I was the best person to choose what I wanted to do with my life. I chose the sciences, and I was the first female to have obtained a master's degree in my family.

Twenty-four years old, I moved from India all the way to Bath, UK when I decided that my interests lay in doing scientific research. I started my PhD in Biochemistry at the University of Bath under the tutelage of Prof. Ravi Acharya, and the moment I saw the three-dimensional structure an antibody, I fell in love with X-ray crystallography. Fast-forward to the present day, I have over 19 years of experience in the field of biochemistry, molecular biology, and structure biology. I have solved 18 crystal structures of proteins and protein-protein complexes. I am proficient in structure-function studies using various biophysical/biochemical techniques (e.g., ITC, assay development) and inhibitor/ligand/protein-protein analyses. In addition to my experience in structure biology, I am also well versed with molecular biology and cell culture techniques. I established the use of yeast expression system in my current place of work, using *Pichia pastoris* as the eukaryotic host to produce five different proteins including growth factors and molecules involved in hypertension. I received training at the Oxford Protein Production Facility (Oxford, UK), which enabled me to establish a purpose-built cell culture facility (both insect and mammalian cells) in my laboratory to meet the ongoing needs of expressing mammalian proteins with post-translational modifications. I also worked on structure-guided design of novel next generation therapeutics with AngioDesign Ltd, a UK-based drug discovery and development company. I have mentored undergraduate and graduate students alike.

Rosalind Franklin is known to have said, "Science and life cannot and should not be separated". I am sure you are probably wondering why I am still a post-doc. Why have I not made the next obvious career move? Well, life happened. It did not stop me from doing what I loved but I faced an odd kind of disparity. Not because of gender for I was given recognition for my academic achievements. I made all the mistakes one can make in their post-doc career, sidelining me from my desired vocation. I relied too much on my advisor believing that they had my best interests at heart. I didn't realize it then, but I was being treated as a technician. When realization set in, albeit late, I decided to make a clean break and travelled across the proverbial pond to come to the US to pursue my dream of succeeding in academia.

For the past 4 years, I have contributed to the research projects in the Das lab at Purdue University (Department of Chemistry). The group mainly focuses on protein ubiquitination and its relationship with bacterial pathogens, especially *Legionella pneumophila*. We use X-ray crystallography, in combination with biochemical and biophysical techniques, to probe the mechanisms of action of these bacterial effectors with aim to understand how they impact host cellular processes. I was instrumental in elucidating the structural basis and mechanism of a novel ubiquitinating enzyme, MavC, from *Legionella* that catalyzes ubiquitination of a host protein using non-canonical means. I am currently involved in new effectors from the same pathogen. It seems these also potentially play a role in manipulation of the host ubiquitination machinery. The current project gives me the opportunity to extend my skillset to include cryoEM, genetic code expansion, mass spectrometry and proteomics on one hand and also microbiology techniques which I have been fortunate to learn in the lab of Dr Zhao-Qing Luo, a pioneer in the field of *Legionella* biology.

B. POSITIONS, SCIENTFIC APPOINTMENTS, AND HONORS

Positions

Jan 2000 – Present	Member Biochemistry
Jan 2001 – 2018	Member Southwestern Structural Biology Consortium (UK)
October 2018 – Present	Member American Society for Biochemistry and Molecular Biology

Awards and Honors

1. Best Poster Prize – (Bath, UK – Jun 2014) South-Western Structural Biology Consortium
2. Overseas Research Student Award (2001-2002, 2000-2001)
3. Ede & Ravenscroft Prize – For Best Postgraduate Student (2001) University of Bath
4. N Prakash Memorial Gold Medal – Highest score in Biochemistry (1998) University of Delhi

C. CORE COMPETECIES

Biochemistry: Protein Purification (Affinity, Ion-exchange, Hydrophobic-Interaction, Size-Exclusion Chromatography), Protein Quantification, Enzyme Kinetics, Protein Detection (SDS PAGE, Western Blotting, Far western analysis), Protein Refolding (Area of Expertise), Protein-Protein Interactions, Heparin Oligosaccharide Fragment Purification, ELISAs, Pull-Down Assays, Fluorescence Polarization

Biophysics: Circular Dichroism Spectroscopy, Dynamic Light Scattering, Biolayer Interferometry, and Isothermal Calorimetry

Bioinformatics: Sequence and Structural Analysis, Various Biological Database Utilities, Sequence / Structural Data Mining, Homology Modelling, Protein-Protein Docking and Analysis

Cell Biology: Immunohistochemistry, Immunoprecipitation, Cell/tissue Culture, Fluorescence Microscopy

Molecular Biology: Polymerase Chain Reaction, Cloning, Transformation (Heat Shock and Electroporation) in bacterial and yeast Strains, Insect / Mammalian Cell Transfections, Transduction, PCR-Based Site-Directed Mutagenesis (Insertion, Deletion, Point-Mutations), Recombinant protein expression in bacteria and Pichia (Yeast), Insect cells (sf9 and sf21), and Mammalian cells (HEK293T and SH-SY5Y), Establishment of Insect/Mammalian Cell Culture Facility Laboratory (in the Acharya Lab, Bath UK)

Structural Biology: X-ray Crystallography: Crystallization of Proteins and Protein-Protein/Protein-Ligand Complexes, X-Ray Data Collection, Structure Solution (Molecular Replacement, Anomalous Dispersion, Isomorphous Replacement), Refinement (CNS, Phenix Suite, CCP4 Suite), Validation and Analysis, Small-Angle X-Ray Scattering (SAXS)

Computers: Unix, Linux, Mac, and Windows-Based Operating Systems

D. CONTRIBUTIONS TO SCIENCE

1. Mechanism of atypical ubiquitination and deubiquitination by bacterial effectors

Legionella pneumophila is a facultative intracellular pathogen capable of replicating within macrophages and amoebae in a unique membrane-bound compartment known as the *Legionella*-containing vacuole (LCV). Essential for its intracellular growth is the Dot/Icm type IVB translocation system, which mediates translocation of over 300 different protein substrates in *L. pneumophila.* Among these effectors, the SidE family remodels host endoplasmic reticulum and Golgi dynamics via an atypical ubiquitination pathway. Regulation of this pathway results in the recovery of native host substrates and accumulation of phosphoribosylated ubiquitin (PR-Ub). The aim of this project is to determine the structure of these effectors (alone and in complex with their host targets), use biochemical and biophysical data to unravel mechanism of action of these enzymes, including regulation of catalytic activity and substrate selection.

– I successfully elucidated three-dimensional structures of both substrate- and product-bound complexes of MavC, a transglutaminase from *Legionella pneumophila*, that uses a non-canonical mode to crosslink ubiquitin on to the host ubiquitin-conjugating enzyme E2, Ube2N. (**PDB codes: 6P5B, 6P5H, 6ULH, 6UMP, 6UMS**)

2. Structure-guided design of novel, next generation inhibitors for Neprilysin

Neprilysin is a zinc-dependent enzyme expressed most abundantly in kidney and lung tissues. Neprilysin inhibition represents a potential alternative strategy to produce antihypertensive and anti-proliferative

effects. The aim of this project is to determine crystal structures of this enzyme in complex with known inhibitors of Neprilysin to facilitate design of effective next generation inhibitors with superior efficacy.

– I successfully elucidated three-dimensional structures of Neprilysin in complex with three inhibitors.

3. Structure-function studies on C9ORF72, a protein associated with familial FTD and/or ALS

ALS and FTD are complex neurodegeneration disorders, both of which exhibit a hexa-nucleotide repeat expansion. The aim of the project is to gain insights into the specific physiological role played by C9ORF72 by means of structure-function studies.

– Successfully cloned, expressed, and purified native C9orf72 and 5 different C9orf72 mutants from insect as well as mammalian cells.
– Successfully cloned, expressed, and purified 4 different Rab-GTPases to characterise potential interaction between C9orf72 and selective Rab-GTPases.
– Successfully proved for the first time that C9orf72 is a guanine-exchange factor for Rab-GTPases.
– Identified (for the first time) that C9orf72 can interact with select Rho-GTPases as well.
– Published a comprehensive bioinformatics-based genomic study on C9orf72.
– Currently carrying out co-localisation studies in neuronal cells.

4. Probing structure-function relationships of proteins involved in angiogenesis

The main aims of the projects were to study the structural characteristics of angiogenic proteins. I worked on
several different kinds of angiogenic molecules ranging from growth factors to enzymes (RNases and MMPs) and their inhibitors, from receptors and antibodies to peptides and heparin fragments. This work resulted in 8 structures that have been deposited with the Protein Data Bank.

– *Collagen breakdown by collagenases (e.g., MMP-1) is an essential part of extracellular matrix turnover during angiogenesis and can be regulated by their inhibitors (TIMPs).* I successfully elucidated three dimensional structures of MMP-1 and its complex with TIMP-1. The study helped provide a crucial platform for further engineering of MMP-selective TIMPs. (**PDB codes: 2CLT and 2J0T**).

– *Angiogenin (Ang) is a potent inducer of neovascularisation*. Point mutations in human Ang have been linked to cancer progression and two neurodegenerative diseases: amyotrophic lateral sclerosis and Parkinson's disease. (**PDB codes: 3ZBV and 3ZBW**).

– *Angiogenesis, formation of vascular sprouts from pre-existing microvasculature is the result of an extensive interplay between factors that display spatially and temporally coordinated activities*. I structurally characterised native proteins as well as complexes of VEGF-A, VEGF-B and PlGF with their receptor/antibody to get an insight into how signal-transduction pathways get initiated via the VEGF receptors. (**PDB codes: 2C7W, 2VWE, 2XAC, and 2BEX**).

E. PUBLICATIONS

1. The unity of opposites: Strategic interplay between bacterial effectors to regulate cellular homeostasis. (2022) *J. Biol. Chem.* **297**, 101340-101359. DOI: 10.1016/j.jbc.2021.101340.

2. Insights into ubiquitin product release in hydrolysis catalyzed by the bacterial deubiquitinase, SdeA. (2021). *Biochemistry*. DOI: 10.1021/acs.biochem.0c00760*.

3. *Legionella* effector MavC targets the Ube2N~Ub conjugate for noncanonical ubiquitination. (2020). *Nat. Commun.* DOI: 10.1038/s41467-020-16211-x.

4. The two deubiquitinating enzymes from *Chlamydia trachomatis* have distinct ubiquitin recognition properties. (2020) *Biochemistry*. DOI: 10.1021/acs.biochem.9b01107*.

5. Purification and functional characterization of the DUB domain of SdeA. (2019). *Methods Enzymol*. DOI: 10.1016/bs.mie.2018.12.024.

6. A comparative bioinformatic analysis of C9orf72. (2018). *PeerJ*. 6:e4391 DOI: 10.7717/peerj.4391.

7. Prediction of structural consequences for disease causing variants in C21orf2 protein using computational approaches. (2018). *J Biomol Struct Dyn*. DOI: 10.1080/07391102.2018.1429313 (Epub ahead of print].

8. Crystal structure of X-prolyl aminopeptidase from Caenorhabditis elegans: A cytosolic enzyme with a di-nuclear active site. (2015). *FEBS Open Bio*. **5**, 292-302. DOI: 10.1016/j.fob.2015.03.013.

9. Crystal structures of murine angiogenin-2 and -3-probing 'structure--function' relationships amongst angiogenin homologues. (2013). *FEBS J*. **280**, 302-318. DOI: 10.1111/febs.12071.

10. Tying the knot: the cysteine signature and molecular-recognition processes of the vascular endothelial growth factor family of angiogenic cytokines. (2011). *FEBS J*. 278, 302-318. DOI: 10.1111/j.1742-4658.2011.08350.x.

11. Structural insights into the binding of vascular endothelial growth factor-B by VEGFR-1(D2): recognition and specificity. (2010). *J. Biol. Chem.* **285**, 23779-23789. DOI: 10.1074/jbc.M110.130658.

12. Structures of native human thymidine phosphorylase and in complex with 5-iodouracil. (2009). *Biochem Biophys Res Commun.* **386**, 666-670. DOI: 10.1016/j.bbrc.2009.06.104*.

13. Crystal structure of vascular endothelial growth factor-B in complex with a neutralizing antibody Fab fragment. (2008). *J. Mol. Biol.* **384**, 1203-1217. DOI: 10.1016/j.jmb.2008.09.076*.

14. Characterization of human angiogenin variants implicated in amyotrophic lateral sclerosis. (2007). *Biochemistry* **46**, 11810-11818. DOI: 0.1021/bi701333h*.

15. Crystal structure of the catalytic domain of matrix metalloproteinase-1 in complex with the inhibitory domain of tissue inhibitor of metalloproteinase-1. (2007). *J. Biol. Chem.* **282**, 364-371. DOI: 10.1074/jbc.M607625200.

16. Crystal structure of an active form of human MMP-1. (2006). *J. Mol. Biol.* **362**, 78-88. DOI: 10.1016/j.jmb.2006.06.079.

17. Molecular recognition of human eosinophil-derived neurotoxin (RNase 2) by placental ribonuclease inhibitor. (2005). *J. Mol. Biol.* **347**, 637-655. DOI: 10.1016/j.jmb.2005.01.035.

18. Identification of placenta growth factor determinants for binding and activation of Flt-1 receptor. (2004). *J. Biol. Chem.* **279**, 43929-43939. DOI: 10.1074/jbc.M401418200.

19. Role of placenta growth factor in cardiovascular health. (2002). *Trends Cardiovasc. Med.* **12**, 128-134. DOI: 10.1016/S1050-1738(01)00164-5*.

20. The crystal structure of human placenta growth factor-1 (PlGF-1), an angiogenic protein, at 2.0 A resolution. (2001). *J. Biol. Chem.* **276**, 43929-43939. DOI: 10.1074/jbc.M008055200.

*- publications marked with an asterisk are not my first author papers.

F. SEMINARS AND POSTER PRESENTATIONS

1. **Title**: Same but different: Modulation of host signaling by two syntenic genes of *Legionella pneumophila*. - ***9/7/2020; The Joseph F. Foster Memorial Biochemistry***; Seminar Series, Purdue University
2. Structure-function studies on Aminopeptidase P-1: a pita-bread fold enzyme, from *Caenorhabditis elegans* (2013) – University of Bath, UK
3. Structural insights into the binding of VEGF-B by VEGFR-1: Recognition and Specificity (2011) – University of Reading, UK
4. Clinical Implications of Basic Research Crystal structures of MMP-1 and its complex with TIMP-1 (2008) - University of Exeter, UK
5. Vascular Endothelial Growth Factor-B: Identification of amino acids important for receptor binding (2006) - University of Bath, UK
6. Molecular Recognition of Human Eosinophil Derived Neurotoxin by Placental Ribonuclease Inhibitor (2005) University of Bristol, UK
7. Structural Studies on Placenta Growth Factor: An Angiogenic Protein (2002) – University of Exeter
8. Placenta Growth Factor: Structure and Function – (2001) University of Bath, UK (Presentation for Ede & Ravenscroft Prize)

G. MENTORING

Mentoring/training students has always been one of my top priorities. My role as a mentor not only involves training students/young researchers in experimental techniques but also focuses on conveying ideas and influencing them to be able to think independently. I have had the privilege of mentoring 19 undergraduate and 11 graduate students in all (UK and here). More than 90% of the students I mentored proceeded to do graduate and/or post-doctoral studies.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: DAS, CHITTARANJAN

eRA COMMONS USER NAME: CHITTARANJAND

POSITION TITLE: PROFESSOR

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Delhi University, New Delhi, India	M.Phil.	04/1994	Physical Chemistry
Indian Institute of Science, Bangalore	Ph.D.	01/2001	Biophysical Chemistry
Harvard Medical School, Boston	Postdoctoral	04/2004	Biochemistry
Brandeis University, Waltham	Postdoctoral	06/2007	Protein Crystallography

A. Personal Statement

Research in my laboratory is aimed at developing mechanistic understanding of ubiquitin signaling. Our focus has primarily been on structural and biochemical properties of enzymes involved in ubiquitin pathways, particularly deubiquitinases (DUBs), enzymes that remove ubiquitin from modified proteins. Using protein crystallography combined with biochemical and biophysical analysis, we are trying to uncover mechanism of action of DUBs. My group has crystallized some important human and prokaryotic DUBs in complex with mechanism-based inhibitors. Examples of DUBs and their ubiquitin complexes crystallized in my laboratory include UCHL1, the proteasome-bound DUB UCHL5, and the zinc-metallo deubiquitinase AMSH. X-ray structures of these DUB complexes have revealed significant insights into their catalytic mechanism.

DUBs have emerged as new targets for drug discovery aimed at therapeutic development for cancer and immune system related disorders. The UCH family of DUBs are particularly interesting because of their involvement in a number of cancers and neurodegeneration. However, drug discovery efforts targeting these DUBs have been sparse, although lately there has been an uptick in the number of programs in industry and academia targeting some of these enzymes. In an effort to contribute to this relatively unexplored but significant area, my group along with Dr. Flaherty and Dr. Wendt (both MCMP) have initiated a platform for UCH-targeted drug discovery. Recently, my lab has produced co-crystal structures of optimized compounds from a covalent fragment screen performed by the Flaherty group. We are about to initiate similar fragment screen and optimization strategy for UCHL3 and UCHL5, both important targets for anticancer therapeutics.

In an ongoing project related to this proposal, we are using cryo-EM 3D reconstruction techniques to visualize the structure of the p53-degron complexes underlying cancers caused by high-risk humanpapilloma virus (HPV) in order to understand the mechanism of ubiquitin transfer catalyzed by the HPV E6 protein in complex with an E3 ubiquitin ligase of the host, E6AP. Along with this structural work, we seek to develop robust functional assays for a better understanding of the mechanism. The current proposal (PCCR Pilot Award) is aimed to colleting critical functional data that would complement the ongoing structural work towards the goals of a future multidisciplinary R01 proposal.

1. Boudreaux, D.A., Maiti, T.K., Davies, C.W. and **Das C** (2010). Ubiquitin vinyl methyl ester binding orients the misaligned active site of the ubiquitin hydrolase UCHL1 into productive conformation. **Proc. National Acad. of Sci. USA**, 107:9117-9122.

2. Sheedlo M, Qiu J, Tan Y, Paul L, Luo Z and **Das C** (2015). Structural basis of substrate recognition by a bacterial deubiquitinase important for dynamics of phagosome ubiquitination. **Proc. National Acad. of Sci. USA** 112*:* 15090-95.
3. Qiu J, Sheedlo M, Yu K, Tan Y, Nakayasu E, **Das C**, Liu X and Luo Z (2016). Ubiquitination independent of E1 and E2 enzymes by bacterial effectors. **Nature** 533:120-124.
4. Puvar K, Iyer S, Fu J, Kenny S, Negron-Teron K, Luo ZQ, Brzovic P, Klevit R, **Das C** (2020). *Legionella* effector MavC targets the Ube2N~Ub conjugate for noncanonical ubiquitination. ***Nature Communications*** May 12;11(1):2365. doi: 10.1038/s41467-020-16211-x.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2001-2004 Postdoctoral Fellow, Center for Neurologic Diseases, Harvard Medical School, Boston, MA
2004-2007 Postdoctoral Fellow, Brandeis University, Waltham, MA
2005-2007 Visiting Scientist, Center for Neurologic Diseases, Harvard Medical School, Boston, MA
2007-2014 Assistant Professor of Chemistry, Purdue University, West Lafayette, IN
2014-2021 Associate Professor of Chemistry, Purdue University, West Lafayette, IN
2021- Professor of Chemistry, Purdue University, West Lafayette, IN

Other Experience and Professional Memberships

2005- Member, American Chemical Society
2010- Member, American Society for Biochemistry and Molecular Biology 2012- Member, Protein Society
2015 Reviewer, Human Frontier Science Program
2015 Reviewer, The Netherlands Cancer Institute

Honors and Awards

1992 University Grants Commission Award (India) for Post-Graduate Research
1998 Senior Research Fellowship, Indian Institute of Science
2011 Seed for Success Award, Purdue University

C. Contribution to Science

1. During my graduate studies, I investigated the role of unnatural amino acids in directing peptide folding to generate conformationally constrained structures in designed peptides. Specifically, I used ^{D}Pro-Gly as a β-turn nucleator to stabilize β-hairpins and β-sheet structures in designed peptides and characterized them by NMR spectroscopy and x-ray crystallography. My work led to successful design principles that can be used to stabilize de novo designed secondary structures in relatively small peptides. Subsequently as a postdoctoral fellow in Harvard Medical School, I was able to apply the knowledge of conformational constrain to generate designed peptide inhibitors of gamma-secretase, an enzyme that catalyzes formation of β-amyloid peptides. I was able to convert these inhibitors into molecular probes that revealed some interesting features of substrate binding by this complex intramembrane protease.
   a) **Das, C.**, Raghothama, S. and Balaram, P (1998). A designed three stranded β-sheet peptide as a multiple β-hairpin model. **J. Am. Chem. Soc**.,120:5812-5813.
   b) Karle, I.L., **Das, C.** and Balaram, P (2000). De novo protein design: crystallographic characterization of a synthetic peptide containing independent helical and hairpin domains. **Proc. National Acad. of Sci. USA**, 97:3034-3037.
   c) **Das, C.**, Berezovska, O., Diehl, T.S., Genet, C., Buldyrev, I., Tsai. J.Y., Hyman, B.T. and Wolfe, M.S (2003). Designed helical peptides inhibit an intramembrane protease. **J. Am. Chem. Soc.,** 2003;125: 1794-11795.
   d) Kornilova, A.Y., Bihel, F.**, Das, C**. and Wolfe, M.S (2005). The initial substrate-binding site of gamma- secretase is located on presenilin near the active site. **Proc. National Acad. of Sci. USA,**102:3230-3235.
2. As a postdoctoral fellow at Brandeis University, I was able to crystallize and solve the structure of the Parkinson's disease associated deubiquitinase UCHL1, an abundant neuronal enzyme with

neuroprotective properties. Surprisingly, the structure revealed a misaligned catalytic center inconsistent with its DUB activity. Later as an independent faculty at Purdue University, I was able to solve the structure of the enzyme in complex with a ubiquitin-based suicide inhibitor which revealed a remarkable mechanism of substrate-induced activation of the enzyme. These structures have provided a sound basis of catalytic activity of this important enzyme paving the way for an understanding of how mutations in this enzyme can lead to neurodegeneration.

a) **Das, C.**, Hoang, Q.Q., Kreinbring, C.A., Luchansky, S.J., Meray, R.K., Ray, S.S., Lansbury, P.T., Ringe, D. and Petsko, G.A (2006). Structural basis of conformational plasticity of the Parkinson's disease-associated ubiquitin hydrolase UCHL1. **Proc. National Acad. of Sci. USA**, 103:4675-4680.

b) Andersson, F.I., McMorran, L., Werrell, E.F., Crone, W.J., **Das, C**., Hsu, S.D. and Jackson, S.E (2011). The effect of Parkinson's disease associated mutations on the stability, structure and dynamics of the deubiquitinating dnzyme UCH-L1. **J. Mol. Biol.**, 407: 261-72.

c) Davies, C.W., Chaney, J., Korbel, G., Ringe, D., Ploegh, H., Petsko, G.A. and **Das C** (2012). The co-crystal Structure of Ubiquitin Carboxy-terminal hydrolase L1 (UCHL1) with a tripeptide fluoromethyl ketone (Z-VAE(OMe)-FMK) **Bioorg. and Med. Chem. Letters**, 22: 3900-3904.

3. My group has crystallized and solved the structure of several important DUBs including UCHL1 bound to ubiquitin vinyl methyl ester (Ub-VME), the proteasome DUB UCHL5 bound to Ub-VME, the metallo DUB AMSH bound to ubiquitin and diubiquitin substrate and more recently the legionella DUB SdeA bound to Ub-VME. In addition to the crystallographic studies, we have uncovered quite a few interesting properties of these human DUBs that are critical regulator of key biological processes including proteasomal degradation (UCHL5, also known as UCH37) and ESCRT-mediated lysosomal degradation (AMSH). Our studies have unraveled some salient features of these enzymes, how they function as DUBs and how their catalytic activities are regulated through protein-protein interaction.

   a) Davies CW (G), Paul LN, Kim M (P), M and **Das C** (2011). Structural and Thermodynamic Comparison of the Catalytic Domain of AMSH and AMSH-LP: Nearly Identical Fold but Different Stability. **J. Mol. Biol**. 413:416-29.

   b) Davies, C.W., Paul, L.N. and **Das C** (2013). Mechanism of Recruitment and Activation of the Endosome- Associated Deubiquitinase AMSH. **Biochemistry**, 52:7818-29.

   c) Morrow M, Kim M, Ronau JA, Chaney J, Rhiannon W, Sheedlo M, Paul LN, Lill M, Artavanis-Tsakonas K, and **Das C** (2013). Stabilization of an unusual salt bridge in ubiquitin by the extra C-terminal domain of the proteasome-associated deubiquitinase UCH37 as a mechanism of its exo specificity. **Biochemistry** 52:3564-3578.

   d) Hausman J, Kenny S, Iyer S, Babar A, Qiu J, Fu J, Luo ZQ and **Das C** (2020). The two deubiquitinating enzymes from *Chlamydia trachomatis* have distinct ubiquitin recognition properties. **Biochemistry** 59: 1604-1617.

4. Our research on DUBs has led us to a novel group of bacterial effectors from intracellular pathogens such as *Legionella* and *Salmonella*. Recently, my group reported the first structural study of a prokaryotic DUB, present in an effector in *Legionella pneumophila*, revealing some interesting properties of CE clan bacterial DUBs including a distinct preference for Lys63-linked polyubiquitin chains. We discovered that preference for this specific type of polyubiquitin chain is used to counteract host cellular defense, thus revealing some remarkable aspects of host-pathogen interaction based on ubiquitin signaling. This work has laid the foundation of a very productive collaboration with Zhao-Qing Luo at the Department of Biological Sciences here at Purdue. Our collaboration has unraveled an enzymatic system in bacterial effectors capable of catalyzing ubiquitination independent of E1 and E2 enzymes.

   a) Sheedlo M, Qiu J, Tan Y, Paul L, Luo Z and **Das C** (2015). Structural basis of substrate recognition by a bacterial deubiquitinase important for dynamics of phagosome ubiquitination. **Proc. National Acad. of Sci. USA** 112*:* 15090-95.

   b) Qiu J, Sheedlo M, Yu K, Tan Y, Nakayasu E, **Das C**, Liu X and Luo Z (2016). Ubiquitination independent of E1 and E2 enzymes by bacterial effectors. **Nature** 533:120-124.

   c) Gan N, Zhen X, Liu Y, Xu X, He C, Qui J, Liu Y, Fujimoto GM, Nakayasu ES, Zhao B, Zhao L, Puvar K, **Das C**, Ouyang S, Luo ZQ (2019). Regulation of phosphoribosyl ubiquitination by a calmodulin-dependent glutamylase. **Nature** 572:387-391.

   d) Gan N, Guan H, Huang Y, Tinh Y, Nakayasu E, Puvar K, **Das C**, Wang D, Ouyang S, Luo ZQ (**2020**).

Legionella pneumophila regulates the activity of UBE2N by deamidase-mediated deubiquitination. ***EMBO J*** Feb 17;39(4):e102806. doi: 10.15252/embj.2019102806.

List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/1RYB_x1G1vQ5l/bibliography/public/

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

1R01GM12629601 Multi PI: Das and Luo 1/16/2018-12/30/2022
"Mechanism of atypical ubiquitination and deubiquitination by bacterial effectors"
Major goals of this proposal are to develop structural and mechanistic understanding of atypical ubiquitin conjugation and deconjugation catalyzed by bacterial enzymes.

R01AI134685 Das Co-I, Flaherty Co-I, Dunman, PI 9/01/18 – 8/31/2023
NIH/NIAID
"Antibacterial inhibitors of RnpA"
The goal of the project is to use a targeted ligand and structure-based design approach to develop novel inhibitors of *Staphylococcus aureus* RnpA.

Completed Research Support (last 3 years)

2R01GM103401 (PI: Garth Simpson, Role: Co-PI) 4/1/2015-3/31/2020
Nonlinear Optical Imaging for Guiding Protein Structure Determination
The goal of this study is to use second order nonlinear imaging of chiral crystals (SONICC) to detect incipient crystal formation and assess the quality of protein crystals.

Purdue PI4D Life Science Pillar Team Science Program (PI: Luo, Role: Co-PI) 6/1/2016-5/31/2017
Structural and functional study of E1/E2-indepenent ubiquitin ligases
The goal of this study is to understand biochemical mechanism of a novel type of ubiquitination.