BIOGRAPHICAL SKETCH

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NAME: Hasan, Syed Saif

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

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
Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, India	BSc	06/2004	Biochemistry
Department of Biotechnology, University of Pune, Pune, India	MSc	06/2006	Biotechnology
Department of Biological Sciences, Purdue University, West Lafayette (IN) USA	PhD	05/2013	Structural Biology
Department of Biological Sciences, Purdue University, West Lafayette (IN) USA	Post-doc	01/2019	Structural Biology

A. Personal Statement

I am an Assistant Professor in the Department of Biochemistry and Molecular Biology, University of Maryland Baltimore MD. I am applying for Titan Krios time at NCCAT. My graduate and post-doctoral training are in the field of structural biology. I have published 23 research articles, 11 review articles, 9 book chapters, 10 protein crystal structures, and 10 single particle cryo-electron microscopy (cryoEM) structures of enveloped RNA-containing viruses. The proposed project on SARS-CoV-2 spike protein will utilize my expertise in structure determination by single particle cryoEM to advance my research interests in structural virology.

Graduate research- I received my doctoral degree in the laboratory of Prof. William A. Cramer (Purdue University). As a graduate student, I elucidated the structural basis of lipid functions in the cytochrome b6f complex. This 16-subunit, membrane-embedded enzyme activates charge transfer, membrane remodeling, and protein trafficking during photosynthesis. For this research, I employed membrane protein purification in detergents and detergent-lipid mixtures, enzyme assays and X-ray crystallography. My research yielded 14 original research articles including 9 first-author articles in *PNAS, Structure, JBC, JMB, Biophysical Journal,* and *Biochemistry*, 6 review articles (3 as first-author), 8 book chapters (one as first-author), and seven membrane protein crystal structures.

Post-doctoral research- I trained in the laboratory of Prof. (late) Michael G. Rossmann (Purdue University), where I employed single particle cryo-electron microscopy (cryoEM) to investigate the structural basis of antibody binding and host hijacking by epidemic causing enveloped Zika virus (ZIKV) and Eastern equine encephalitis virus (EEEV). During this time, I gained hands-on experience in mammalian and insect cell culture for protein and virus production. My research yielded two first-author original research articles in *Nature Communications* and *Cell Reports*, two co-authored articles in *PNAS*, one in *Journal of Virological Methods*, a first-author review article in *Nature Structural and Molecular Biology*, and nine single particle cryoEM structures of Zika virus and EEEV, and one single particle cryoEM structure of Venezuelan equine encephalitis virus.

Current research- My laboratory is interested in elucidating the structural and biophysical basis of SARS-CoV-2 spike biogenesis and maturation. We are focused on investigating the trafficking of the spike in ER-Golgi, which provides glycosylation and glycan-modifying enzymes during secretory biogenesis of the spike. We employ host trafficking factors such as coatomer proteins to regulate spike trafficking, thus ensuring compartment-specific glycosylation of the spike. *Our recent work applying these approaches to investigate coatomer interactions of the SARS-CoV-2 spike has yielded an original research manuscript in Communications Biology.* This publication includes three high-resolution (1.2-1.8Å) crystal structures of a

coatomer domain and its mutants. Our group has published two review articles in *Pathogens* and *Virus Research*, and a book chapter on virus structural biology has been accepted for publication in *Medical Microbiology*.

Mentoring- I have mentored three post-doctoral researchers (two current). Dr. Debajit Dey and Dr. Suruchi Singh are co-first authors on our recent article in *Communications Biology*. Dr. Asma Rehman, a former post-doc, is currently a scientist in nearby Novavax in Gaithersburg MD. I have mentored three undergraduate researchers (one current). Of the previous two undergraduates, Niki Gooya is now in the Neuroscience Graduate Program at Johns Hopkins Medical School, and, Matthew Martin, who is a co-author on our article in *Communications Biology*, is now in the Molecular Biophysics and Structural Biology Graduate Program, run jointly by University of Pittsburg and Carnegie Mellon University.

In summary, I have the training, leadership, published preliminary data, and motivation to perform the proposed research.

Ongoing and recently completed projects that I would like to highlight include:
American Thoracic Society/Glaxo Smith Kline
Hasan (PI)
Molecular Lipidomics of a Therapeutic Target in Coronavirus Assembly
02/15/21/-02/14/23

University of Maryland MPower COVID19 Response Fund Award
Hasan (co-PI), Orban (co-PI), MacKerell (co-PI)
Molecular Investigations of SARS-CoV-2 Spike Protein
08/07/20-08/06/21

Citations:

1. **Hasan SS**, Yamashita E, Baniulis D, Cramer WA. Quinone-dependent proton transfer pathways in the photosynthetic cytochrome b6f complex. *Proc Natl Acad Sci U S A*. 2013;110(11):4297-302. PMID: 23440205; PMCID: PMC3600468 *(techniques used: membrane protein purification in detergent, lipid reconstitution, and X-ray crystallography)*

2. **Hasan SS**, Miller A, Sapparapu G, Fernandez E, Klose T, Long F, Fokine A, Porta JC, Jiang W, Diamond MS, Crowe JE Jr, Kuhn RJ, Rossmann MG. A human antibody against Zika virus crosslinks the E protein to prevent infection. *Nat Commun*. 2017;8:14722. PMID: 28300075; PMCID: PMC5356071 *(techniques used: mammalian cell culture, virus purification, and single particle cryoEM)*

3. **Hasan SS**, Sun C, Kim AS, Watanabe Y, Chen CL, Klose T, Buda G, Crispin M, Diamond MS, Klimstra WB, Rossmann MG. Cryo-EM Structures of Eastern Equine Encephalitis Virus reveal mechanisms of virus disassembly and antibody neutralization. *Cell Rep*. 2018;25(11):3136-3147.e5. PMID: 30540945; PMCID: PMC6302666 *(techniques used: mammalian and insect cell culture, virus purification, single particle cryoEM, mass spectrometry to identify viral glycoprotein glycans)*

4. Dey D*, Singh S*, Khan S, Martin M, Schnicker NJ, Gakhar L, Pierce BG, **Hasan SS**. An extended motif in the SARS-CoV-2 spike modulates binding and release of host coatomer in retrograde trafficking. *Commun Biol*. 2022;5,115. DOI: 10.1038/s42003-022-03063-y (*equal contribution) PMID: 35136165; PMCID: PMC8825798 *(techniques used: COPI WD40 expression and purification from E. coli and Expi293 cells, BLI assay of spike tail peptide and WD40 domains, X-ray crystallography, and molecular modeling) (independent publication from Hasan lab)*

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2019-present	Assistant professor, Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore MD
2013-19	Post-doctoral research associate, Department of Biological Sciences, Purdue University, West Lafayette IN
2008-13	Graduate research assistant, Department of Biological Sciences, Purdue University, West Lafayette IN
2007-08	Graduate teaching assistant, Department of Biological Sciences, Purdue University, West Lafayette IN
2006-07	Junior research fellow, National Centre for Cell Science, Pune, India

Other Scientific Appointment

2022 Early Career Reviewer, NIH Study Section: Biochemistry and Biophysics of Membranes (BBM); meeting dates June 2nd and 3rd 2022

Honors

2019 Biophysical Society travel award to present research at the 63rd Annual Biophysical Society Meeting, Baltimore MD (USA)

2018 Best talk by a post-doctoral researcher at The Hitchhiker's Guide to the Biomolecular Galaxy: A Purdue Mini-Symposium on Integrating Structure, Function, and Interactions of the Biomolecular Universe at Purdue University, West Lafayette IN (USA)

2018 Best short talk by a post-doctoral researcher at the Third Annual Life Sciences Postdoc Symposium at Purdue University, West Lafayette IN (USA)

2012 Student Research Achievement Award at the 56th Annual Biophysical Society Meeting, San Diego CA (USA)

2011 Best talk by a graduate student (co-winner) at the 37th Midwest-Southeast Photosynthesis Meeting, Marshall IN (USA)

2005 Nationally competed scholarship for higher studies awarded by the Bharat Petroleum Corporation Limited (India)

2005 University Medal for highest marks in Bachelor of Science (Honors) Biochemistry awarded by the Aligarh Muslim University, Aligarh (India)

2005 University Medal for highest marks in the Faculty of Life Sciences awarded by the Aligarh Muslim University, Aligarh (India)

2004 'JNCASR – Summer Research Fellowship' awarded by the Jawaharlal Nehru Centre for Advanced Scientific Research (Bangalore), Rajiv Gandhi Foundation (New Delhi) and Department of Science and Technology (Government of India)

2003 'JNCASR – Summer Research Fellowship' awarded by the Jawaharlal Nehru Centre for Advanced Scientific Research (Bangalore), Rajiv Gandhi Foundation (New Delhi) and Department of Science and Technology (Government of India)

C. Contributions to Science

1. *Ongoing research: Coatomer dependent trafficking of coronavirus spike protein*- The spike is synthesized in the ER and is then trafficking to Golgi where it completes post-translational modifications. This trafficking is regulated by cellular factors such as the coatomer complex, which recycles the spike from Golgi to ERGIC for viral progeny assembly. This recycling protects the spike from glycan maturation until its export to the plasma membrane. Using a combination of X-ray crystallography and BLI binding assays, we have shown that the trafficking sequence in the SARS-CoV-2 spike tail demonstrates molecular mimicry of the host coatomer-binding sequences. Furthermore, regulation of the spike-coatomer interaction is mapped to residues outside the canonical coatomer-binding sequence, thus establishing a new paradigm for spike trafficking and recognition of coatomer proteins.
    a. Dey D*, Singh S*, Khan S, Martin M, Schnicker NJ, Gakhar L, Pierce BG, **Hasan SS**. An extended motif in the SARS-CoV-2 spike modulates binding and release of host coatomer in retrograde trafficking. *Commun Biol*. 2022;5(115):1-. DOI: 10.1038/s42003-022-03063-y (*equal contribution) PMID: 35136165; PMCID: PMC8825798
    b. Dey D*, Singh S*, Khan S, Martin M, Schnicker NJ, Gakhar L, Pierce BG, **Hasan, SS**. An extended coatomer binding motif in the SARS-CoV-2 spike protein. *PDB 50th Anniversary Symposium in Asia: 50 Years of the Protein Data Bank and the Frontier of Structural Biology in Asia* (Virtual poster presentation), 2021 (*equal contribution)

2. *Post-doctoral research: Structural basis of immune evasion and host hijacking by alphaviruses*- Alphaviruses are enveloped icosahedral viruses responsible for encephalitis and arthritis in humans. In a first-author article in *Cell Reports*, I published the first single particle cryoEM structure of the alphavirus EEEV. This revealed cryptic viral glycosylation epitopes for evasion from the host's immune system. This cryoEM structure suggested an electrostatic basis for pH-triggered membrane fusion with the viral envelope protein and capsid disassembly. This investigation mapped the main-chain atoms of a capsid protein segment that is implicated in host ribosome hijacking by molecular mimicry of host ribosome-

binding sequences. This study was complemented by structural investigations of virus-like particles of Chikungunya virus, an arthritogenic alphavirus. Overall, our research elucidated the structural basis of alphavirus envelope and capsid protein trafficking in infected host cells.

a. Yap ML, Klose T, Urakami A, **Hasan SS**, Akahata W, Rossmann MG. Structural studies of Chikungunya virus maturation. *Proc Natl Acad Sci U S A*. 2017;114(52):13703-13707. PMID: 29203665; PMCID: PMC5748190
b. **Hasan SS**, Sun C, Kim AS, Watanabe Y, Chen CL, Klose T, Buda G, Crispin M, Diamond MS, Klimstra WB, Rossmann MG. Cryo-EM Structures of Eastern Equine Encephalitis Virus reveal mechanisms of virus disassembly and antibody neutralization. Cell Rep. 2018;25(11):3136-3147.e5. PMID: 30540945; PMCID: PMC6302666
c. Chen CL, **Hasan SS**, Klose T, Sun Y, Buda G, Sun C, Klimstra WB, Rossmann MG. Cryo-EM structure of eastern equine encephalitis virus in complex with heparan sulfate analogues. Proc Natl Acad Sci U S A. 2020;117(16):8890-8899. PMID: 32245806; PMCID: PMC7183182

3. *Post-doctoral research: Structural basis of ZIKV neutralization by a therapeutic antibody*- ZIKV is an enveloped flavivirus that is responsible for paralytic Guillain-Barre syndrome in adults and microcephaly in fetuses of infected pregnant women. Using single particle cryoEM, I demonstrated the structural basis of ZIKV neutralization by a therapeutic antibody, ZIKV117. The Fabs of ZIKV117 were found to cross-link the viral envelope leading to inhibition of membrane fusion. This investigation demonstrated that the epitope of ZIKV117 is unique to ZIKV. This provided an explanation for the observed specificity of ZIKV117 to ZIKV. The quaternary organization of ZIKV117 epitope on ZIKV envelope protein provides a paradigm for the design of antibodies with specificity to selected flaviviruses, which has the potential to reduce antibody-dependent enhancement of infection, which is a major concern in flavivirus vaccine development.

a. **Hasan SS**, Miller A, Sapparapu G, Fernandez E, Klose T, Long F, Fokine A, Porta JC, Jiang W, Diamond MS, Crowe JE Jr, Kuhn RJ, Rossmann MG. A human antibody against Zika virus crosslinks the E protein to prevent infection. Nat Commun. 2017;8:14722. PMID: 28300075; PMCID: PMC5356071
b. **Hasan SS**, Sevvana M, Kuhn RJ, Rossmann MG. Structural biology of Zika virus and other flaviviruses. Nat Struct Mol Biol. 2018;25(1):13-20. PMID: 29323278 (review article)
c. Dey D, Poudyal S, Rehman A, **Hasan SS***. Structural and biochemical insights into flavivirus proteins. Virus Res. 2021;296:198343. PMID: 33607183 (review article; *corresponding author)

4. *Graduate research on ligand-interactions of cytochrome b6f complex:* The membrane embedded cytochrome b6f complex functions as a quinol/quinone oxidoreductase to generate an electrochemical potential for ATP synthesis. Using X-ray crystallography in a mixed detergent-lipid environment, I demonstrated the structural basis of quinol-dependent proton transfer across the cytochrome complex. This research elucidated conservation of proton exit pathway between photosynthetic cytochrome b6f and respiratory cytochrome bc1 complexes. This investigation laid the foundation for crystallographic, biophysical, and *in silico* analyses of proton release and domain motion in the cytochrome as modulated by membrane lipids. This research advanced the understanding of how cytochrome function, i.e., proton-electron transfer, is controlled by association with lipids and showed that these critical lipid sites are conserved between cytochrome b6f and bc1 complexes. Furthermore, these results lay a foundation for mechanistic investigations of photosynthetic membrane remodeling, which is initiated by cytochrome b6f activation.

a. **Hasan SS**, Yamashita E, Ryan CM, Whitelegge JP, Cramer WA. Conservation of lipid functions in cytochrome bc complexes. J Mol Biol. 2011;414(1):145-62. PMID: 21978667; PMCID: PMC3215850
b. **Hasan SS**, Yamashita E, Baniulis D, Cramer WA. Quinone-dependent proton transfer pathways in the photosynthetic cytochrome b6f complex. Proc Natl Acad Sci U S A. 2013;110(11):4297-302. PMID: 23440205; PMCID: PMC3600468
c. **Hasan SS***, Cramer WA. Internal lipid architecture of the hetero-oligomeric cytochrome b6f complex. Structure. 2014;22(7):1008-15. PMID: 24931468; PMCID: PMC4105968 (*corresponding author)

d. **Hasan SS**, Proctor EA, Yamashita E, Dokholyan NV, Cramer WA. Traffic within the cytochrome b6f lipoprotein complex: Gating of the quinone portal. Biophys J. 2014;107(7):1620-8. PMID: 25296314; PMCID: PMC4190601

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1hUCiihpyaych3/bibliography/public/

BIOGRAPHICAL SKETCH

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NAME: Singh, Suruchi

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

POSITION TITLE: Postdoctoral 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)*	Completion Date MM/YYYY	FIELD OF STUDY
Banasthali University, Rajasthan, India	BSc.	06/2009	Biotechnology
Banasthali University, Rajasthan, India	MSc.	06/2011	Biotechnology
CSIR- Institute of Microbial Technology (JNU), Chandigarh, India	Ph.D.	06/2018	Protein Structure Biology
Fred Hutchinson Cancer Research Center, WA, USA	Postdoctoral	01/2021	Protein Structure Biology

A. Personal Statement

I am a post-doctoral research associate in the Department of Biochemistry and Molecular Biology, University of Maryland, Baltimore, in the lab of Dr. S. Saif Hasan. My long-term research interests lie in understanding the three-dimensional structure of protein assemblies in essential biological functions. A major focus of my research is on structural characterization of protective antibodies and stabilized vaccine immunogens, which are critical to combat human diseases.

In the Hasan lab, my current research seeks to elucidate the fundamental principles of biogenesis and glycosylation of the spike protein in SARS-CoV-2. This is highly relevant for the design of improved genetic vaccines against coronaviruses that utilize the host cellular machinery for spike biogenesis and glycosylation, which is a key determinant of spike immunogenicity. Hence, I am utilizing a structure-function approach to gain mechanistic atomic-level insights into spike-host interactions that modulate spike synthesis, glycosylation, and glycan maturation. My investigations involve hands-on production of the full-length spike protein in mammalian cells, its purification from cell membranes in detergents, optimization of vitrification conditions on Quantifoil and lacey carbon grids, and structural characterization by single particle cryo-electron microscopy (cryoEM). This structural research is complemented by collaborative cellular and mass spectrometric investigations of spike glycosylation and glycan maturation. My latest samples of the spike protein displaying immature state glycans has reached a resolution of 8.2Å on our in-house Talos-Arctica/Falcon 3EC DED setup.

Before joining the University of Maryland, I was a postdoctoral research fellow in Dr. Marie Pancera's lab at Fred Hutchinson Cancer Research Center, Seattle, USA. My research in the Pancera lab focused on the structural characterization of antigens from Human Papilloma Virus (HPV) and their interactions with neutralizing antibodies using a combination of X-ray crystallography, negative staining electron microscopy, and high-resolution single particle cryoEM. HPV affects nearly 300 million people worldwide and is a leading cause of a variety of cancers. During this research, I received hands-on training in cryoEM at the University of Washington, Seattle and at the screening and data collection facility at PNCC (Pacific Northwest Center for CryoEM). I determined a cryoEM structure of a neutralizing antibody in complex with antigen protein L1 from HPV at 3.2Å. Overall, in my two and a half years in the Pancera lab, I determined six crystal structures, one cryoEM structure, and published six co-author research articles. One first author manuscript on HPV-antibody complex cryoEM structure is in preparation for publication.

Prior to moving to USA, I received my Ph.D. from the CSIR-Institute of Microbial Technology (IMTECH), India. During my PhD, I applied X-ray crystallography, biochemistry, and biophysics skills to elucidate the structure and function of an essential transcriptional regulator in *Mycobacterium tuberculosis (Mtb),* which is the causative agent of TB that affects 10 million people annually. During my graduate studies at IMTECH, I solved two crystallographic structures and published one first-author and two co-author manuscripts.

Hence, I have the training and expertise to complete the cryoEM-based investigation proposed in the present application to NCCAT.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2021-Present Postdoctoral Research Associate, University of Maryland, School of Medicine, Baltimore, MD
2018 – 2020 Postdoctoral Fellow, Fred Hutchinson Cancer Research Center, Seattle, WA
2012 – 2018 PhD scholar, CSIR-IMTECH, Chandigarh, India

Honors

2014 Awarded Senior Research Fellowship by CSIR-UGC
2012 Qualified Graduate Aptitude Test in Biotechnology with 6th All India Ranking
2011 Awarded Junior Research Fellowship by CSIR-UGC
2009 Scholarship for Masters in Biotechnology by Department of Biotechnology (DBT), India

C. Contributions to Science (reverse chronological order)

1. **Postdoctoral research (Hasan lab): Structural basis of SARS-CoV-2 spike protein biogenesis and immunogenicity-** I have demonstrated that the tail of the SARS-CoV-2 spike mimics host signals for recycling in the ER-Golgi secretory pathway. My work has shown that modulation of these tail signals dramatically alters interactions with the coatomer complex, which is responsible for spike recycling, retention, and release in the secretory ER-Golgi pathway. This is highly relevant for the processing and release of the spike protein encoded in mRNA vaccines to the plasma membrane and thus, its presentation to the immune system. This secretory pathway houses the glycosylation machinery, and hence, modified interactions of the tail signals have direct consequences on spike glycan maturation, which is a key modulator of immunogenicity. Using this information, I have now generated spike constructs that display distinct levels of glycan maturation (unpublished). Preliminary cryoEM reconstructions of the spike displaying immature-state glycans have been improved to 8.2Å. Overall, these constructs will be investigated by high-resolution single particle cryoEM at NCCAT.

   Dey D*, **Singh S***, Khan S, Martin M, Schnicker NJ, Gakhar L, Pierce BG, Hasan SS. An extended motif in the SARS-CoV-2 spike modulates binding and release of host coatomer in retrograde trafficking. Commun Biol. (2022) 5(1):115. doi: 10.1038/s42003-022-03063-y. PMID: 35136165; PMCID: PMC8825798. (*equal first authorship)

2. **Postdoctoral research (Pancera lab): Structural basis of antibody recognition of L1 protein in Human Papilloma Virus (HPV)-** Vaccines against HPV elicit a strong immune response compared to natural infection. Yet, limited information is available on the molecular features of neutralizing antibodies and different B cell responses, thus limiting the fundamental understanding of vaccine-induced immunity. My research was focused on elucidating the structural details of this immune response following HPV vaccination. A collaborative investigation on the Merck 9-valent HPV vaccine (GARDASILÒ9) identified human monoclonal antibodies (hmAbs) from HPV16-specific memory B-cells and plasmablasts. These potent hmAbs served as targets for structural characterization. I purified the HPV L1 protein antigen, Fab fragments of these hmAbs, generated their complexes, optimized vitrification conditions on grids, and determined the single particle cryoEM structures of these L1-Fab complexes. This generated novel insights into the structural basis of recognition and binding of L1 by hmAbs.

Singh S, Carter J, Cohen K, McElrath J, Galloway D, Pancera M. Structural characterization of immune responses to HPV infection and HPV vaccination. Microsc Microanal. (2019) 25(S2):1224-1225. doi:10.1017/S1431927619006858

3. **Graduate research (Karthikeyan lab): Structural and biochemical characterization of an essential transcriptional regulator from *Mycobacterium tuberculosis*-** Rv1828 is an essential gene for survival of the pathogenic bacterium, *M. tuberculosis*. Yet, little is known about its function and the underlying structural basis of its activity. My graduate research annotated this gene as a family of MerR transcription factors that regulate gene expression in response to drugs. Using X-ray crystallography, I determined a unique dimeric structure of this gene product and demonstrated for the first time that it was bound to a fatty acid ligand. These studies suggest that Rv1828 is involved in a fatty acid biosynthesis pathway essential for the survival of *M. tuberculosis*.

   Singh S, Sevalkar RR, Sarkar D, Karthikeyan S. Characteristics of the essential pathogenicity factor Rv1828, a MerR family transcription regulator from Mycobacterium tuberculosis. FEBS J. 2018 Dec;285(23):4424-4444. doi: 10.1111/febs.14676. Epub 2018 Oct 25. PMID: 30306715.

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