

**BIOGRAPHICAL SKETCH**

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NAME: Bhardwaj, Anshul

eRA COMMONS USER NAME (credential, e.g., agency login): axb050

POSITION TITLE: Research 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
Barkatullah University, Bhopal, India	B.S.	07/1999	Biochemistry, Microbiology
Barkatullah University, Bhopal, India	M.S.	07/2001	Biochemistry, Microbiology
Freie University, Berlin, Germany	Ph.D.	01/2006	Biochemistry
SUNY Upstate Medical University, Syracuse, NY	Postdoctoral	06/2009	Structural Biology

**A. Personal Statement**

My research background and expertise are in the use of structural biology tools and biochemical-biophysical techniques to understand protein structure and function. As a graduate student at the Freie University, Berlin and a post-doctoral fellow at SUNY upstate, I have studied molecular and regulatory mechanisms underlying nucleocytoplasmic transport, viral assembly and viral genome packaging-ejection mechanisms using a hybrid approach of combining x-ray crystallography, SAXS, electron microscopy and various structural, molecular interaction-biochemical techniques. I have determined several novel protein structures, importantly phage Sf6 tail needle knob (pdb 3RWN), importin beta bound to snurportin1 IBB-domain (pdb 3LWW), phage HK620 tail needle (pdb 5BU5, 5BVZ), HS1 knob (pdb 4K6B), gp26\_2M (pdb 4LIN), DUSP26 (pdb 4HRF), importin alpha-scrubblase 4 NLS complex (pdb 3Q5U), the small terminase subunit of phage P22 (pdb 3P9A), crystal structures of AMP-PNP and sangivamycin bound GPCR kinase 5( pdb 4TND, 4TNB), the crystal structures of asymmetry causing mutants of HIV gp41 subunit (5KA5, 5KA6) and recently GRK5-Calmodulin complex (pdb 6PJX). The series of successful structural biology-oriented research work allowed me to build a strong foundation in x-ray crystallography and biophysical interaction techniques. This allows me to play an active collaborating role in several research projects of high clinical relevance leveraging on my structural biology expertise.

From 2010 to 2019, I was a Scientific Manager for the X-ray crystallography and molecular interactions shared resource facility, at Thomas Jefferson University. This facility is one of the 6 NCI-supported research facilities at the Sidney Kimmel Cancer Center (SKCC), within Thomas Jefferson University. As a facility manager, I have overseen and trained internal and external users in x-ray crystallographic data collection and structure determination, and in-solution biophysical techniques such as small angle x-ray scattering (SAXS), Circular Dichroism (CD), Analytical Ultracentrifugation (AUC), Surface Plasmon Resonance (SPR), Nano-ITC, and VP-ITC. In summary, I am well qualified to carry out structural biology work specifically the CryoEM studies described in this application and I have the required expertise, leadership and motivation to do so.

## B. Positions and Honors

### Positions and Employment

2002-2005	Research Scientist, Max-Delbrueck-Centre for Molecular Medicine, Berlin, Germany
2006- 2009	Post-doctoral fellow, SUNY Upstate Medical University, Syracuse, NY
2010- 2019	Facility Manager, SKCC X-ray Crystallography and Molecular Interactions shared resource, Thomas Jefferson University, Philadelphia, PA
2011- 2014	Research Instructor, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA
2015 -	Research Assistant Professor, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA

### Other Experience and Professional Memberships

2000 - 2001	Research trainee, National Institute for Immunology, New Delhi, India
2001 - 2002	Research Assistant, International Centre for Genetic Engineering and Biotechnology, New Delhi, India
2007-	Member, American Association for the Advancement of Science

### Honors

1993	Governors scout award, Governor of the state Mr. Qureshi
1994	Presidents scout award, President of the India Dr. Sharma
1996 - 1999	University scholarship from Barkatullah University (earned annually)
1999	Scholarship award for securing top position in B.S.
1999	Scholarship award for securing top position in M.S first semester.
2000 - 2001	University research scholarship award to conduct research training at National Institute of Immunology, New Delhi, India

## C. Contributions to Science

1. My early research work on DNA recombination proteins and bacteriophage infectious tail machinery during graduate studies and postdoctoral training heavily utilized biochemical and biophysical techniques. This work involved molecular biology tools together with expression and purification of over dozen isolated proteins and detailed structural characterization to get a better understanding of their physiological roles. Also, importantly shed light on cross-play between various interacting partners supporting mechanisms of action. The fundamental work has a huge relevance to basic biology and to the field.

- Bhardwaj A, Welfle K, Misselwitz R, Ayora S, Alonso JC, Welfle H. (2006) Conformation and stability of the *Streptococcus pyogenes* pSM19035-encoded site-specific beta recombinase, and identification of a folding intermediate. *Biol Chem.* 2006 May;387(5):525-33. PMID: 16740123
- Bhardwaj A, Olia AS, Walker-Kopp N, Cingolani G. (2007) Domain organization and polarity of tail needle GP26 in the portal vertex structure of bacteriophage P22. *J Mol Biol.* 2007 Aug 10;371(2):374-87. PMID: 17574574
- Olia AS, Bhardwaj A, Joss L, Casjens S, Cingolani G. (2007) Role of gene 10 protein in the hierarchical assembly of the bacteriophage P22 portal vertex structure. *Biochemistry.* 2007 Jul 31;46(30):8776-84. PMID: 17620013
- Bhardwaj A, Walker-Kopp N, Casjens SR, Cingolani G. (2009) An evolutionarily conserved family of virion tail needles related to bacteriophage P22 gp26: correlation between structural stability and length of the alpha-helical trimeric coiled coil. *J Mol Biol.* 2009 Aug 7;391(1):227-45. PMID: PMC2713385

2. A significant portion of my postdoctoral training in Dr. Cingolani laboratory was dedicated to studying viral genome ejection and packaging mechanisms using phage P22 as a model system employing a hybrid approach of combining structural biology, cell biology, and biochemical-physical techniques. In collaboration with Dr. Sherwood Casjens, we studied various viral tail apparatus components that are involved in genome ejection and host cell penetration. One of the major achievements of this work was determining the first atomic resolution crystal structures of gp26 protein homologs that act as a plug to trap newly packaged genomes in the Podoviridae

virions. Furthermore, I also contributed to structural and biophysical studies of the research work focusing on studying viral genome packaging mechanisms. I resolved the first crystal structure of phage P22 small terminase unit that is part of multi-component viral genome packaging motor. My efforts led to various co-authored publications describing results of the research work.

- a. Bhardwaj A, Walker-Kopp N, Wilkens S, Cingolani G. (2008) Foldon-guided self-assembly of ultra-stable protein fibers. *Protein Sci.* 2008 Sep;17(9):1475-85. PMID: PMC2525528
- b. Bhardwaj A, Molineux IJ, Casjens SR, Cingolani G. (2011) Atomic structure of bacteriophage Sf6 tail needle knob. *J Biol Chem.* 2011 Sep 2;286(35):30867-77. PMID: PMC3162447, RCSB PDB code: 3RWN
- c. Roy A, Bhardwaj A, Cingolani G. (2011) Crystallization of the nonameric small terminase subunit of bacteriophage P22. *Acta Crystallogr Sect F Struct Biol Cryst Commun.* 2011 Jan 1;67(Pt 1):104-10. PMID: PMC3079985
- d. Roy A, Bhardwaj A, Datta P, Lander GC, Cingolani G. (2012) Small terminase couples viral DNA binding to genome-packaging ATPase activity. *Structure.* 2012 Aug 8;20(8):1403-13. PMID: PMC3563279, RCSB PDB code: 3P9A
- e. Leavitt JC, Gogokhia L, Gilcrease EB, Bhardwaj A, Cingolani G, Casjens SR. (2013) The tip of the tail needle affects the rate of DNA delivery by bacteriophage P22. *PLoS One.* 2013 Aug 12;8(8):e70936. PMID: PMC3741392, RCSB PDB code: 4K6B
- f. Bhardwaj A, Casjens SR, Cingolani G. (2014) Exploring the atomic structure and conformational flexibility of a 320Å long engineered viral fiber using X-ray crystallography. *Acta Crystallogr D Biol Crystallogr.* 2014 Feb;70(Pt2):342-353. PMID: PMC3940195, PDB code: 4LIN
- g. Bhardwaj A, Olia AS, Cingolani G. (2014) Architecture of viral genome delivery molecular machines. *Curr Opin Struct Biol.* 2014 Apr;25:1-8. PMID: PMC4040186
- h. Bhardwaj A, Sankhala RS, Olia, AS, Brooke D, Casjens SR, Taylor DJ, Prevelige PE Jr, Cingolani G. (2016) Structural plasticity of the protein plug that traps newly packaged genomes in Podoviridae virions. *J Biol Chem.* 2016 Jan 1;291(1):215-26. PMID: PMC4697157, RCSB PDB codes: 5BVZ, 5BU5, 5BU8, 4ZKP, 4ZKU, 4ZXQ

3. Structural biology of nuclear import machinery and protein trafficking pathways. I contributed my structural biology expertise to study underlying mechanisms of macromolecular nucleocytoplasmic transport. We did a comprehensive analysis of importin beta binding affinity for FG-rich nucleoporins lining the Nuclear Pore Complex. This work involved extensive use of x-ray crystallography and biophysical tools such as Surface Plasmon Resonance and Isothermal titration Calorimeter to accurately measure thermodynamic and kinetic binding parameters of the interaction. More recently, I have also contributed my expertise in studies focusing on defining guiding principles and mechanisms of membrane protein nuclear transport.

- a. Bhardwaj A, Cingolani G. (2010) Conformational selection in the recognition of the snurportin importin beta binding domain by importin beta. *Biochemistry.* 2010 Jun 22;49(24):5042-7. PMID: 20476751, RCSB PDB code: 3LWW
- b. Lott K, Bhardwaj A, Mitrousis G, Pante N, Cingolani G. (2010) The importin beta binding domain modulates the avidity of importin beta for the nuclear pore complex. *J Biol Chem.* 2010 Apr 30;285(18):13769-80. PMID: PMC2859540
- c. Lott K, Bhardwaj A, Sims PJ, Cingolani G. (2011) A minimal nuclear localization signal (NLS) in human phospholipid scramblase 4 that binds only the minor NLS-binding site of importin alpha1. *J Biol Chem.* 2011 Aug 12;286(32):28160-9. PMID: PMC3151061, RCSB PDB code: 3Q5U
- d. Lokareddy RK, Hapsari RA, van Rhee M, Pumroy RA, Bhardwaj A, Steen A, Veenhof LM, Cingolani G. (2015) Distinctive properties of the nuclear localization signals of an inner nuclear membrane proteins Heh1 and Heh2. *Structure.* 2015 Jul 7;23(7):1305-16. PMID: PMC4768490, RCSB PDB codes: 4PVZ, 4XZR

4. Therapeutic protein design and engineering. It's well known that protein fold dictates function. As a trained structural biologist at Jefferson, I have been actively involved in efforts towards the development of engineered proteins that can be utilized therapeutically. One of such collaborative effort is directed toward the development of engineered antibody-based blocker of localized fibrosis with collagen telopeptide. This research work is led by Dr. Andrzej Fertala and of highly collaborative nature that utilizes specialties of many basic sciences researchers and clinicians at Jefferson. I secured independent funding as well to pursue goals of this project.

- a. Fertala J, Steplewski A, Kostas J, Beredjikian P, Williams G, Arnold W, Abboud J, Bhardwaj A, Hou C, Fertala A. (2013) Engineering and characterization of the chimeric antibody that targets the C-terminal telopeptide of the  $\alpha 2$  chain of human collagen I: A next step in the quest to reduce localized fibrosis. *Connect Tissue Res.* 2013;54(3):187-96. PMID: PMC3896972

5. In addition to the recent contributions described above I have been actively involved in G protein-coupled receptor kinase 5 (GRK5) structure-function studies led by Prof. Jeffrey L. Benovic at Jefferson. I have determined an atomic resolution crystal structure of GRK5.AMP-PNP and GRK5.sangivamycin complexes, more recently I have also resolved 2.0 angstrom structure for GRK5-Calmodulin complex (Mol. Cell. 2021). This work further attests that I have necessary structural biology expertise and training to contribute meaningfully to the goals of the proposed application. I look forward to contributing and expanding these studies to next level.

- a. Komolov KE, Bhardwaj A\*, Benovic JL\*. (2015) Atomic structure of GRK5 reveals distinct structural features novel for G protein-coupled receptor kinases. *J Biol Chem.* Aug 21;290(34):20629-47. PMID: PMC4543624, RCSB PDB codes: 4TNB, 4TND

\*co-corresponding authors

- b. Komolov KE\*, Sulon SM\*, **Bhardwaj A**, van Keulen SC, Duc NM, Laurinavichyute DK, Lou HJ, Turk BE, Chung KY, Dror RO, Benovic JL. Structure of a GRK5-Calmodulin Complex Reveals Molecular Mechanism of GRK Activation and Substrate Targeting. *Molecular Cell* 2021 Jan 21;81(2):323-339.e11.

\*equal contributions

RCSB PDB code: 6PJX Bhardwaj, A, Komolov KE, Benovic JL

#### 6. Publications from other collaborative efforts –

- a. Thangavel C, Boopathi E, Liu Y, Haber A, Ertel A, **Bhardwaj A**, Addya S, Williams N, Ciment SJ, Cotzia P, Dean JL, Snook A, McNair C, Price M, Hernandez JR, Zhao SG, Birbe R, McCarthy JB, Turley EA, Pienta KJ, Feng FY, Dicker AP, Knudsen KE, Den RB. RB loss promotes prostate cancer metastasis. (2017) *Cancer Research.* 77(4):982-995.
- b. Khasnis MD, Halkidis K, **Bhardwaj A**, Root MJ. (2016) Receptor activation of HIV-1 Env leads to asymmetric exposure of the gp41 trimer. (2016) *PLoS Pathogens.* 12(12):e1006098  
RCSB PDB codes: 5KA5, 5KA6
- c. Thangavel C, Boopathi E, Liu Y, McNair C, Haber A, Perepelyuk M, **Bhardwaj A**, Addya S, Ertel A, Shoyele S, Birbe R, Salvino JM, Dicker AP, Knudsen KE, Den RB. Therapeutic Challenge with a CDK 4/6 Inhibitor Induces an RB-Dependent SMAC-Mediated Apoptotic Response in Non-Small Cell Lung Cancer. (2018) *Clinical Cancer Research.* 24(6):1402-1414.

#### Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/50041260/?sort=date&direction=descending>

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