BIOGRAPHICAL SKETCH

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NAME: Nilesh K. Banavali

eRA COMMONS USER NAME (credential, e.g., agency login): WADS099

POSITION TITLE: Research Scientist / 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
University of Mumbai, Mumbai, India	B.S. Pharmacy	05/1995	Pharmaceutical Sciences
University of Maryland, Baltimore	Ph.D.	05/2001	Pharmaceutical Sciences
Weill Medical College of Cornell University	Postdoc	12/2005	Biophysics
University of Chicago, Chicago	Postdoc	08/2006	Biophysics

A. Personal Statement

This proposal will characterize the translating and hibernating ribosome structures for the Lyme disease pathogen, *Borrelia burgdorferi (Bb)*, using single-particle cryo-electron-microscopy (cryo-EM) techniques. My previous accomplishments are in understanding molecular mechanisms for local structural transitions (e.g. base flipping), global structural transitions (e.g. A- vs. B-form equilibrium in nucleic acids), allostery (e.g. tyrosine kinases), and biochemical pathways (e.g. protein splicing) through implementation and application of computational biophysics methods. At Wadsworth Center, I discovered B-form structures for RNA in localized single-strand-dinucleotide contexts and delineated the precise mechanism of DNA strand slippage leading to indel mutations. With local collaborations, I have also predicted multiple macromolecular structures using experimental data, including protein splicing precursors, a tubulin helical filament, a ryanodine receptor, and a dynein motor protein; and identified multiple new flavivirus inhibitors through structure-based drug design. I have recently switched to primarily using single-particle cryo-EM in collaboration with Dr. Rajendra Agrawal, and we have three joint publications through our collaboration on translation mechanisms. This is my first independent proposal that uses cryo-EM methods, in which I will apply both my prior computational expertise and recently acquired cryo-EM experience to characterize structural and functional properties of *Bb* translation mechanisms and antibiotic interactions.

B. Positions and Honors

Professional Experience:

1995-1997	Graduate Research Assistant University of Maryland, Baltimore	Supervisor: Professor Kevin A. Reynolds Department of Pharmaceutical Sciences
1997-2001	Graduate Research Assistant University of Maryland, Baltimore	Mentor: Professor Alexander D. MacKerell, Jr. Department of Pharmaceutical Sciences
2001-2005	Postdoctoral Fellow Cornell University Medical College	Mentor: Professor Benoît Roux Department of Physiology and Biophysics

2006	Postdoctoral Fellow	Mentor: Professor Benoît Roux
	University of Chicago	Department of Biochemistry and Molecular Biology
2006-present	Assistant Professor	Department of Biomedical Sciences
	School of Public Health, State University of New York at Albany	
2006-present	Research Scientist IV	Laboratory of Computational and Structural Biology
	Division of Genetics, Wadsworth Center, New York State Department of Health	

Other Experience:

2008-2013	Hudson Valley RNA Club steering committee
2009-present	Wadsworth Center Research Experience for Undergraduates (REU) steering committee
2010-2017	National Academy of Sciences Anton supercomputing grant review panel
2020	Guest Editor (2 issues), *Journal of Computational Chemistry*

Honors:

1992	Jagtiani Award (best academic performance), KMK College of Pharmacy, University of Mumbai
1998	Rho Chi Pharmacy Honors Society Member
1999	Graduate Merit Award, School of Pharmacy, University of Maryland, Baltimore
2003	W. M. Keck Foundation Postdoctoral Fellowship

C. Contributions to Science

Experimental data-driven structure prediction

With structural biology groups at the Wadsworth Center, we interpreted structural and biochemical data through structure prediction for macromolecular contexts including tubulin helical filaments, a protein splicing precursor, a ryanodine receptor, and mitochondrial ribosomal interactions.

1. Structures of the human mitochondrial ribosome bound to EF-G1 reveal distinct features of mitochondrial translation elongation. R. K. Koripella, M. R. Sharma, K. Bhargava, P. P. Datta, P. S. Kaushal, P. Keshavan, Linda L. Spremulli, Nilesh K. Banavali, Rajendra K. Agrawal, *Nature Comm.* 2020, 11 (1), 1-11.

2. Maintenance of electrostatic stabilization in altered tubulin lateral contacts may facilitate formation of helical filaments in foraminifera. D. M. Bassen, Y. Hou, S. S. Bowser, **N. K. Banavali**, *Scientific Rep.*, 2016, 6, 31723.

3. Modeling a Ryanodine Receptor N-terminal Domain Connecting the Central Vestibule and the Corner Clamp Region' L. Zhu, X. Zhong, S. R. W. Chen, **N. K. Banavali,** Z. Liu, *J. Biol. Chem.*, 2013, 288, 903-914.

4. Insertion domain within mammalian mitochondrial translation initiation factor 2 serves the role of eubacterial initiation factor 1. A. S. Yassin, M. E. Haque, P. P. Datta, K. Elmore, **N. K. Banavali**, L. L. Spremulli, R. K. Agrawal. *Proc. Natl. Acad. Sci. USA,* 2011, 108 (10), 3918-3923.

Structure-based inhibitor design

In close collaborations at the Wadsworth Center, we have used pathogen proteins in West Nile Virus, Dengue Virus, and *Mycobacterium tuberculosis* as virtual screening targets to identify inhibitors and/or predict their bound structures.

1. Chemical activation of adenylyl cyclase Rv1625c inhibits growth of Mycobacterium tuberculosis on cholesterol and modulates intramacrophage signaling. R. M. Johnson, G. Bai, C. M. DeMott, **N. K. Banavali**, C. R. Montague, C. Moon, A. Shekhtman, B. VanderVen, K. A. McDonough, *Mol. Microbiol.* 2017, doi: 10.1111/mmi.13701.

2. Identification and Characterization of Novel Broad-Spectrum Inhibitors of the Flavivirus Methyltransferase. M. Brecher, H. Chen, Z. Li, **N. K. Banavali**, S. A. Jones, J. Zhang, L. D. Kramer, H. Li, *ACS Infectious*

Diseases, 2015, 1 (8), 340-349.

3. Novel Broad Spectrum Inhibitors Targeting the Flavivirus Methyltransferase. M. Brecher, H. Chen, B. Liu, **N. K. Banavali**, S. A. Jones, J. Zhang, Z. Li, L. D. Kramer, H. Li, *PLoS One*, 2015, 10 (6), e0130062.

4. Selective inhibition of the West Nile virus methyltransferase by nucleoside analogs. H. Chen, L. Liu, S. A. Jones, **N. K. Banavali**, J. Kass, Z. Li, J. Zhang, L. D. Kramer, A. K. Ghosh, H. Li, *Antiviral Res.*, 2013, 97, 232-239.

Nucleic acid structure and mechanisms

We discovered that B-form structures, previously thought to not occur in RNA, are present in localized single strand RNA dinucleotides. I elucidated the first three-dimensional pathways for the decades-old Streisinger strand slippage mechanism for indel errors by DNA polymerases, and showed how only partial base pair separation was required for such errors to occur (reference in section A). I identified how stabilizing non-canonical base interactions of a partially separated base with the neighboring sequence can induce such errors. We showed how variable energetic costs could influence such errors for bases near or in template overhangs (reference in section A). We were the first to determine the precise free energy difference between the A- and B-forms of DNA using an explicit solvent MD simulation-based free energy estimation method. We were the first to show that the mechanism of base flipping used by many nucleic acid modification and repair enzymes can occur through both duplex grooves, and to predict free energy landscapes for this process in explicit solvent (reference in section A). We also showed that the protein could enable such a conformational change through attractive pulling interactions rather than the previously hypothesized steric pushing interactions.

1. RNA approaches the B-form in stacked single strand dinucleotide contexts. A. Sedova, **N. K. Banavali**, *Biopolymers*, 2016, 105 (2), 65-82 **(cover article).**

2. Analyzing the relationship between single base flipping and strand slippage near DNA duplex termini. **N. K. Banavali**. *J. Phys. Chem B,* 2013, 117 (46), 14320-14328.

3. Free energy landscape of A-DNA to B-DNA conversion in aqueous solution. **N. K. Banavali**, B. Roux. *J. Am. Chem. Soc.* 127 (18), 6866-6876, 2005.

4. Protein-facilitated base flipping in DNA by cytosine-5-methyltransferase. N. Huang, **N. K. Banavali**, A. D. MacKerell Jr. *Proc. Natl. Acad. Sci. USA,* 2003, 100 (1), 68-73.

Free energy estimation methods for complex conformational changes and allostery

By developing restrained and unrestrained simulation-based methods to determine free energy profiles, we identified tyrosine kinase structural intermediates that were better target structures for inhibitor design, showed how free energies along unrestrained coordinates could be determined from restrained simulations, how secondary structure elements respond to a phosphorylation event that changes their direct interactions, and move as rigid bodies to enable structural change across long distances through a macromolecule, and how a small linker region at the N-terminal end of a Src tyrosine kinase catalytic domain could act as a hinge to relay information between its active site and a remote regulatory site.

1. Mapping the conformational transition in Src activation by cumulating the information from multiple molecular dynamics trajectories. S. Yang, **N. K. Banavali**, B. Roux *Proc. Natl. Acad. Sci. USA,* 2009, 106 (10), 3776-3781.

2. Characterizing structural transitions using localized free energy landscape analysis. **N. K. Banavali**, A. D. MacKerell Jr. *PLoS One*, 2009, 4 (5), e5525.

3. Anatomy of a structural pathway for activation of the catalytic domain of Src kinase Hck. **N. K. Banavali**, B. Roux. *Proteins*, 2007, 67 (4), 1096-1112.

4. The N-terminal end of the catalytic domain of Src kinase Hck is a conformational switch implicated in long-range allosteric regulation. **N. K. Banavali**, B. Roux. *Structure,* 2005, 13 (11), 1715-1723.

Nucleic acid force fields

I was one of three authors contributing to a major release of the CHARMM nucleic acid force field (CHARMM27) that has been in wide use since 2000. We also developed force field parameters for multiple modified nucleic acid backbones, and in doing so, corrected the previous understanding of the stereoelectronic contribution of the O3' atom to the sugar pucker preference of DNA and RNA.

1. Reevaluation of stereoelectronic contributions to the conformational properties of the phosphodiester and N3'-phosphoramidate moieties of nucleic acids. **N. K. Banavali**, A. D. MacKerell Jr. *J. Am. Chem. Soc.,* 2001, 123 (28), 6747-6755.

2. Re-examination of the intrinsic, dynamic and hydration properties of phosphoramidate DNA. **N. K. Banavali**, A. D. MacKerell Jr. *Nucleic Acids Res.*, 2001, 29 (15), 3219-3230.

3. All-atom empirical force field for nucleic acids: II. Application to molecular dynamics simulations of DNA and RNA in solution. A. D. MacKerell Jr., **N. K. Banavali**. *J. Comp. Chem.,* 2000, 21 (2), 105-120.

4. Use of oligodeoxyribonucleotides with conformationally constrained abasic sugar targets to probe the mechanism of base flipping by Hha I DNA (cytosine C5)-methyltransferase. P. Wang*, A. S. Brank*, **N. K. Banavali***, M. C. Nicklaus, V. E. Marquez, J. D. Christman, A. D. MacKerell Jr. *J. Am. Chem. Soc,* 2000*,* 122 (50), 12422-12434. * equal contribution authors.

A link to the full list of my published work in NCBI's 'My Bibliography' is given below.
https://www.ncbi.nlm.nih.gov/sites/myncbi/12Ip7txwpDFQb/bibliography/52164512/public/?sort=date&direction=descending

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

Research Support:

Wadsworth Center Seed Funding Nilesh Banavali (PI) 01/01/2015 – present
Using Next-generation sequencing to quantify sequence dependent errors in replication
The goal is to optimize a simple next-generation sequencing assay for sequence-dependent replication errors.

Completed Research Support:

State University of New York FRAP-A Award Nilesh Banavali (PI) 05/01/2015 – 04/30/2017
A Next-Generation Sequencing Assay for Sequence-Dependent Polymerase Errors
The goal is to use designed all k-mer template sequences to assess sequence-dependent polymerase errors using next-generation sequencing.

BIOGRAPHICAL SKETCH

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NAME: MANJULI R. SHARMA

eRA COMMONS USER NAME (credential, e.g., agency login): WADS258

POSITION TITLE: Research Scientist II

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
Banaras Hindu University (BHU), Varanasi, India	B.Sc.	1987	Chemistry (Hons)
Banaras Hindu University (BHU), Varanasi, India	M.Sc.	1989	Biochemistry
Banaras Hindu University (BHU), Varanasi, India	Ph.D.	1995	Clinical Biochemistry

A. Personal Statement

The proposed research aims to characterize the translating and hibernating ribosomes of the spirochete Lyme disease pathogen *Borrelia burgdorferi*. Cutting-edge cryo-EM and 3D image processing techniques will be primarily used in this project. My research interests, from the start, have been to study molecular complexes and their role in human health and diseases. After obtaining my Ph.D. in clinical biochemistry, I received postdoctoral fellowships from 1996-2001 consecutively from the American Heart Association and NIH, NRSA (National Research Service Award) to obtain training in state of art cryo-EM and image processing techniques and utilize them to study architecture of the Calcium Release Channel in Dr. Terence Wagenknecht's laboratory. I determined the first 3D cryo-EM structures of cardiac and brain ryanodine receptors (RyRs). These studies shed light on understanding the structural and functional relationships among various isoforms of RyRs and had implications in understanding the excitation-contraction coupling mechanism. Further, utilizing my expertise in cryo-EM and 3D image reconstruction techniques, I have continued to study macromolecular complexes involved in protein synthesis. I obtained the first cryo-EM structure of the mammalian mitochondrial ribosome (mito-ribosome). Since joining the Agrawal lab, I have studied several ribosome-ligand complexes, including various complexes of the 55S mito-ribosome and mycobacterial ribosomes, at near-atomic resolutions. I have expertise in biochemistry, cryo-EM data collection, image-processing and modelling aspects of the proposed research. In addition, I have been actively involved in training and supervising interns/students and new postdocs who have joined the group. Based on prior experience and training in biochemistry and 3D cryo-EM, and my proven record, I am confident of making further useful contributions in achieving goals of the proposed research.

B. Positions and Honors

Positions and Employment

1990 - 1992	Junior Research Fellow, Institute of Medical Sciences, BHU, Varanasi, India
1993 - 1995	Senior Research Fellow, Institute of Medical Sciences, BHU, Varanasi, India
1996 - 1996 (Jun)	Research Affiliate, Wadsworth Center, New York State Dept. of Health (NYSDOH), Albany, NY
1996 (Jul) - 1998 (Jun)	American Heart Research Affiliate, Wadsworth Center, NYSDOH, Albany, NY
1998 (Jul) - 1999 (Mar)	Research Affiliate, Wadsworth Center, NYSDOH, Albany, NY
1999 (Apr) - 2001 (Mar)	NRSA (NIH) Fellow at the Wadsworth Center, NYSDOH, Albany, NY
2001 (Apr) - 2006 (Jan)	Research Affiliate, Wadsworth Center, NYSDOH, Albany, NY
2006 (Feb) - present	Research Scientist II, Wadsworth Center, NYSDOH, Albany, NY

Awards and Honors

1989	First in Order of Merit (M.Sc), Awarded University Gold Medal, BHU, India.
1990	Rameshwardas Birla Smarak Kosh Pre-Doctoral Research Project Award, Bombay, India.
1990 (Oct) -1995 (Dec)	Pre-doctoral Research Fellowship by Council of Scientific and Industrial Research (CSIR), New Delhi, India.
1996 (Jul) - 1998 (Jun)	Postdoctoral Fellowship (# 960124) from American Heart Association, NY, USA
1999 (Apr) - 2001 (Mar)	National Research Service Award (NRSA # 1F32H20060), from NIH, USA
1999	Travel Award from the Biophysical Society (USA) to attend and present a paper in the XIII Intl. Biophysics Congress (Sept. 19-24), New Delhi, India
2000	Travel Award from the IUBMB to present a paper in the 18th Intl. Congress of Biochemistry & Molecular Biology (July 16-20), Birmingham, U.K.

C. Contributions to Science

1) Cryo-EM Studies of the Mitochondrial Ribosomes: My expertise and experience has been intensely directed into studies of structure and function of mammalian mitochondrial ribosome, including determination of first structure of the mammalian mitochondrial ribosome from bovine liver, which provided insights into its structural novelty as compared to all previously studied cytosolic ribosomes. The structure became the foundation for all subsequent studies in the Agrawal lab. I have since then been able to help capture and analyze different functional complexes of mammalian mitochondrial ribosome to understand the mechanism of protein translation in mitochondria.

a. **Sharma, MR**, Koc EC, Datta PP, Booth TM, Spremulli LL, Agrawal RK. (2003) Structure of the mammalian mitochondrial ribosome reveals an expanded functional role for its component proteins. ***Cell*** *115*, 97-108.
b. Mears JA*, **Sharma MR***, Gutell RR., McCook AS, Richardson PE, Caulfield TR, Agrawal RK, and Harvey SC. (2006) A structural model for the large subunit of the mammalian mitochondrial ribosome. ***J. Mol. Biol.*** *358,* 193-212. **equal first authorship.*
c. Koripella, RK, **Sharma, MR,** Risteff, P, Keshavan, P, and Agrawal, RK. (2019). Structural insights into unique features of the human mitochondrial ribosome recycling. ***Proc. Natl. Acad. Sci. USA*** *116*, 8283-8288.
d. Koripella, RK*, **Sharma, MR*,** Bhargava, K, Datta, PP, Kaushal, PS, Keshavan, P, Spremulli, LL, Banavali, NK, Agrawa,l RK. (2020). Structures of the human mitochondrial ribosome bound to EF-G1 reveal distinct features of mitochondrial translation elongation. ***Nat Commun*****.** 11(1):3830. **equal first authorship.*

2) Architecture of Mammalian Ryanodine Receptors: During my post- doctoral NIH NRSA and American Heart Research Affiliate award period, I determined the first three-dimensional cryo-EM structures of cardiac (RyR2) and brain (RyR3) isoforms of ryanodine receptors (RyR) that contributed to understand their structural relationship to excitation-coupling (E-C) mechanism. Efforts to visualize RyR2 and RyR3 had been ongoing for almost a decade, but I was able to standardize a modified method to make cryo-grids for these RyR isoforms which helped maintained their structural integrity that was crucial for their 3-D structure determinations in open and close conformational states, as well as in complex with FKBP12.6 (FK506-binding protein).

a. **Sharma MR**, Penzeck P, Grassucci R, Xin H-B, Fleischer S, and Wagenknecht T. (1998) Cryo-electron microscopy and image analysis of the cardiac ryanodine receptor. ***J. Biol. Chem*****.** *273*,1829-1834.
b. **Sharma MR**, Jeyakumar L, Fleischer S, and Wagenknecht T. (2000) Three-dimensional structure of ryanodine receptor isoform three in two conformational states as visualized by cryo-electron microscopy. ***J. Biol. Chem.*** *275*, 9485-9591.
c. Liu Z, Zhang J, **Sharma MR**, Li P, Wayne Chen SR, and Wagenknecht T. (2001) Three-dimensional reconstruction of recombinant type-3 ryanodine receptor and localization of its amino terminus. ***Proc. Natl. Acad. Sci., USA*****.** *98*, 6104-6109.
d. **Sharma, MR**, Jeyakumar LH, Fleischer S, Wagenknecht T. (2006) Three-dimensional visualization of FKBP12.6 binding to an open conformation of cardiac ryanodine receptor. ***Biophys. J.*** *90*, 164-72.

3) **Cryo-EM Studies of the Cytosolic Ribosomes:** After transitioning to Dr. Agrawal's lab, I explored the cytoplasmic ribosomes, implementing my expertise in cryo-EM and image processing techniques. My results included one of the first structural determinations of ribosome recycling factor (RRF) and Era (*E. coli ras*-like protein) in their ribosome-bound states. I also discovered that the previously annotated plastid-specific ribosomal protein 1 (PSRP1) was in fact a functional homologue of *E. coli* cold-shock protein pY rather than a component chloroplast ribosomal protein.
   a. **Sharma, MR**, Barat C, Wilson DN, Booth TM, Kawazoe M, Hori-Takemoto C, Shirouzu M, Yokoyama S, Fucini P, Agrawal RK. (2005) Interaction of Era with the 30S ribosomal subunit implications for 30S subunit assembly. ***Mol. Cell*** *18*, 319-329.
   b. Agrawal RK, **Sharma MR**, Kiel MC, Hirokawa G, Booth TM, Spahn CM, Grassucci RA, Kaji A, Frank J. (2004) Visualization of ribosome-recycling factor on the *Escherichia coli* 70S ribosome: Functional implications. ***Proc. Natl. Acad. Sci. USA.*** *101*, 8900-8905.
   c. Datta PP, **Sharma MR**, Qi L, Frank J, & Agrawal RK. (2005) Interaction between the G' domain of elongation factor-G and the C-terminal domain of ribosomal protein L7/L12 during translocation, as revealed by cryo-EM. ***Mol. Cell*** *20*, 723-731.
   d. **Sharma MR,** Dönhöfer A, Barat C, Marquez V, Datta PP, Fucini P, Wilson DN, and Agrawal RK. (2010) PSRP1 is not a ribosomal protein, but a ribosome binding factor that is recycled by RRF and EF-G. ***J. Biol. Chem.*** *285*, 4006-4014.

4) **Structure Analysis of alternate ribosome form of *Mycobacterium* species:** I have also contributed with my expertise towards analyzing the three-dimensional structure of *Mycobacterium smegmatis* 70S ribosome that provided novel insights into its mechanism of hibernation and drug resistance activity.
   a. Li, Y., Sharma, M.R., Koripella, R.K., Yang, Y., Kaushal, P.S., Lin, Q., Lee, R.E., Wade, J. T., Gray, T.A., Derbyshire, K.M., Agrawal, R.K., Ojha, A.K. (2018). Zinc depletion induces ribosome hibernation in mycobacteria. ***Proc. Natl. Acad. Sci. USA*** *115,* 8191-8196
   b. Li Y, Sharma MR, Koripella RK, Wade JT, Gray TA, Derbyshire KM, Agrawal RK, Ojha AK. (2019). Zinc depletion is a specific signal for induction of ribosome hibernation in mycobacteria. ***Proc Natl Acad Sci*** U S A. 116(7):2398-2399.
   c. Li Y, Sharma MR, Koripella RK, Sharma MR, Lee, RE, Agrawal RK, Ojha AK. (2020). Replacement of S14 protein in ribosomes of zinc-starved mycobacteria reduces spectinamide sensitivity. ***Antimicrob Agents Chemother*** (in press), doi:10.1128/AAC.01833-20.

5) **Reviews and Book Chapters**
   In addition to more than 26 peer-reviewed publications, I have contributed to reviews and book chapters that are directly related to the area of research proposed here:
   a. Agrawal RK, **Sharma MR**, Yassin A, Lahiri I, and Spremulli L (2011) Structure and function of organellar ribosomes as revealed by cryo-EM. In ***Ribosomes: Structure, Function, and Dynamics****,* Rodnina, M., Wintermeyer, W., & Green, R. eds. (SpringerWien, New York), pp. 83-96.
   b. Agrawal RK and **Sharma MR** (2012) Structural aspects of mitochondrial translational apparatus. ***Curr. Opin. Struct. Biol.*** *22(6), 22,* 797-803.
   c. **Sharma MR**, Kaushal PS, Gupta M, Banavali NK, and Agrawal RK (2013) Insights into structural basis of mammalian mitochondrial translation. In ***Translation in mitochondria and other organelles***, Duchene, A.M. ed. (Springer-Verlag, Berlin/ Heidelberg), pp. 1-28, DOI: 10.1007/978-3-642-39426-3_1.

D. Research Support

None