CURRICULUM VITAE

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Education:

- **Oct, 2016 – Apr, 2019-** Post Doctoral visiting fellow under supervision of Dr. Robert J Crouch, NICHD, NIH, Bethesda, MD, USA. Project- **RNase H2 mutant mouse model- perinatal lethality to viability**.
- **Aug, 2014 - Dec, 2014: Postdoc** under supervision of Dr. Utpal Bhadra, CCMB, Hyderabad, AP, India. Work title: **Role of gas41 gene in suppression of virus infection in Drosophila**.
- **Jul, 2008 – Jul, 2014: PhD** under supervision of **Prof. Rakesh Bhatnagar,** Laboratory of Genetic engineering and molecular biology, JNU, New Delhi, India. Thesis title: **"Structural and functional characterization of PemK-PemI module of *Bacillus anthracis".***
- **Aug, 2006 – June, 2008: M.Sc. Biotechnology**, Utkal University, Bhubaneswar, India.

Achievements and awards:

- Secured first rank in all India JNU, SBT Ph.D. entrance test (2008).
- Qualified Council of Scientific and Industrial Research-Junior Research Fellowship (CSIR-JRF), a national level test and eligibility for lectureship held in Dec 2009**.**
- Qualified Graduate Aptitude Test in Engineering (GATE) -2008 in Life Sciences with GATE Score- 450, Percentile Score- 97.07.
- Qualified All India Combined Entrance Test (A.I.C.E.T.) 2006 conducted by J.N.U., New Delhi, on behalf of Department of Biotechnology (DBT) Govt. of India for admission to M.Sc. Biotechnology Programme.
- Availed the scholarship from Department of Biotechnology, Govt. of India for Post-graduation (2006-2008)

Publications:

1. **Shashikala Verma** and Rakesh Bhatnagar (2014); MoxT toxin of *Bacillus anthracis* exhibits sequence specific ribonuclease activity. *Biochemical and Biophysical Research Communications 2014 Jul 25;450(2):998-1004.*

2. **Shashikala Verma**, Sudhir Kumar, Ved Prakash Gupta, Samudrala Gourinath, Sonika Bhatnagar and Rakesh Bhatnagar (2014); Structural basis of *Bacillus anthracis* MoxXT disruption and modulation of MoxT ribonuclease activity by rationally designed peptides. *Journal of Biomolecular Structure and Dynamics 2015;33(3):606-24*.
3. Nikita Chopra, Shivangi Agarwal, **Shashikala Verma**, Sonika Bhatnagar and Rakesh Bhatnagar (2011); Modeling of the structure and interactions of the B. anthracis antitoxin, MoxX: deletion mutant studies highlight its modular structure and repressor function. *J Comput Aided Mol Des 2011 Mar;25(3):275-91*.

Technical skills:

- **Molecular Biology:** Plasmid DNA isolation, Cloning and other general molecular biology applications, PCR, site-directed mutagenesis (SDM), Prokaryotic expression and purification of recombinant proteins by Affinity chromatography and Gel filtration chromatography, Production of polyclonal antiserum, Western Blotting, Northern blotting, *In vitro* transcription/translation, Primer Extension, Sequencing gel electrophoresis, Beta-gal assay, Site directed mutagenesis, Reverse Transcriptase PCR, 5' RACE (Rapid Amplification of cDNA ends), Electrophoretic Mobility Shift Assay (EMSA), Polysome analysis, FACS.
- **Bacteria:** Bacterial culturing, growth curve, β-galactosidase assay.
- **Biophysical studies:** Fluorescence and Circular dichroism (CD) spectroscopy, Confocal microscopy
- **Mouse**- mouse breeding and genotyping, whole mount skeleton staining
- **Next generation sequencing**- RNA-seq, Methyl-seq, Ribo-methyl seq, PAR-CLIP
- **Tissue culture**- Transfection and induction in cell line
- **Drosophila:** Drosophila culture and crosses, Virus infection in Drosophila.

Meeting and conferences:

- **Shashikala Verma,** Sudhir Kumar, Sonika Bhatnagar, Samudrala Gourinath and Rakesh Bhatnagar. Structural and functional characterization of PemK-PemI module of *Bacillus anthracis*. Poster presented in 81st Annual meeting of the Society of Biological Chemists (India) and Symposium on Chemistry and Biology: Two weapons against diseases held in Science City, Kolkata on November 8-11, 2012.

- ❖ Seminar-cum-workshop on "Freshwater algae and their utilization" held in Post Graduate Department of Biotechnology, Utkal University, Bhubaneswar on 18th and 19th March 2007.
- ❖ Attended BIOEPOCH 2012, 1st to 4th Annual Symposium, SBT, JNU, New Delhi.
- ❖ Presented postdoctoral work in NICHD Monday AM seminar, NIH, Bethesda, USA.
- ❖ Attended RNA Biology symposium, Apr 11-16, 2019, NCI, NIH, Bethesda, USA.

Summary of the research work for thesis:

PhD Thesis Title: "Structural and Functional Characterization of PemK-PemI Module of Bacillus anthracis".

Thesis- *Bacillus anthracis*, a Gram-positive bacterium, causes anthrax and has been classified by NIH as a category A agent on its bioterrorism threat list. In *B. anthracis*, a PemIK toxin antitoxin (TA) module renamed as **MoxXT** system has been identified as a type II TA system. PemIK module encodes PemI, a labile protein and PemK, a ribonuclease. PemI inhibits PemK toxic activity by forming an inactive complex. Over expression of PemK inhibits protein synthesis and arrests cell growth. PemI binds to the promoter of its own operon and acts as a repressor. PemIK module plays an important role in stress conditions as PemK found to be up-regulated in different stress conditions. One of the applications of the study of TA systems is an antimicrobial strategy where disruption of TA interaction and activation of the toxin would lead to bacterial cell death. In *B. anthracis* few peptides have been reported as inhibitor of PemIK interaction with concomitant decrease in PemK ribonuclease activity. Our study demonstrated the structure of PemK, mechanism of PemK ribonuclease activity, effect of PemK on binding of PemI to DNA and effects of designed peptides on PemIK interaction as well as on PemK ribonuclease activity. Structure of PemK was determined using molecular replacement at 1.8Å resolution. PemK was crystallized as dimer which is consistent with the gel elution profile which suggests that PemK exists as dimer. Overall structure of the PemK is similar to the structure of YdcE from *B. subtilis* (PDB id: 1NE8). The structure of PemK showed that it is a globular protein pointing towards its stability. Elucidation of ribonuclease activity of PemK showed that PemK is a sequence specific ribonuclease which recognizes UACAU sequence in ss RNA and cleaves between U and A. Moreover, cleavage of RNA requires 2′ OH group of first residue, U of UACAU RNA sequence. PemK cleaves phosphodiester linkage in RNA and produces 2′, 3′-cyclic phosphate on one side and 5′-OH group on the other. An interesting finding was also observed, i.e. its ability to cleave RNA in DNA-RNA hybrid. PemK increases the DNA binding affinity of PemI. E9, I10, V11 and R13 residues of PemI were found to be crucial for binding to DNA. We have also studied the effect of several peptides in disrupting the PemIK interaction as well as augmentation of PemK ribonuclease activity by binding to PemK *in vitro*. Docking studies on the peptides were carried out in order to explain the observed structure activity relationships. The peptides with distinct structure and activity profile are proposed to bind to two distinct site of PemK. The docking of the active peptides with PemK showed that they possess an aromatic group that occupies a conserved hydrophobic pocket. Additionally, the peptides inducing high ribonuclease activity were anchored

by a negatively charged group near a cluster of positively charged residues present near the pocket. Our study provides a structural basis and rationale for the observed properties of the peptides and may aid the development of small molecule to disrupt the TA interaction.

Summary of the research work for postdoctoral work:

Title: "Role of gas41 gene in suppression of virus infection in Drosophila".

Innate immunity is the most ancient line of defence against pathogens. Invertebrates and plants rely solely on innate mechanisms to combat infections. Recent studies have identified RNA interference (RNAi) as an ancient, cell-intrinsic immune mechanism that controls RNA viruses in plants and insects. In Drosophila, Gas41 is required for proper functioning of RNA interference (RNAi) machinery. Thus, gas41 gene might be a key factor in control of RNA virus infection. To find the role of gas41, the effect of virus infection on the life span of drosophila was checked and it was found that in case of gas41 mutant, the life span or survival decreases, which suggests that gas41 gene might play a role in control of RNA virus infection.

Title: RNase H2 mutant mouse model- perinatal lethality to viability

Aicardi-Goutieres syndrome (AGS) is an inherited encephalopathy that affects newborn infants and usually results in severe mental and physical handicaps. Mutations in several nucleic acid transacting genes are associated with AGS. More than 50% of AGS patients have mutations in any of the three genes encoding RNase H2. In numerous situations, RNA associates with DNA forming RNA-DNA hybrids, R-loops and single ribonucleotides in dsDNA which are resolved by RNase H2. Our lab works on a AGS mutation which affects catalytic activity, the *RNASEH2A*-G37S, to generate a mouse model for AGS. Our lab has published data showing that *Rnaseh2a*$^{G37S/G37S}$ mice are perinatal lethal and exhibit an innate immune response activated by the DNA-sensing pathway. Later, we found a single viable male mouse that was *Rnaseh2a*$^{G37S/G37S}$ and subsequently, we have obtained more than 70 live homozygous mice, all of which are small with variable phenotypic characters including white spot on the ventral side, kinky tail, small eyes and malocclusion. There is sex difference of 5:1 male: female ratio in viability and females usually are more severely affected. Females are mostly sterile or just gave one or two pups per litter. Thus, from lethality to viability of G37S homozygous mice suggests that there is a suppressor of G37S mutation which permits viability and our goal is to find that modifier gene. Our long-term goal is to understand how *RNASEH2A*-G37S mutation lead to AGS development and lethality. The objective defined to determine the mutation or genetic aberration permitting G37S homozygous mice viability at very low frequencies. We hypothesized that impaired ribosomal biogenesis is related to the lethality or viability of G37S homozygous mice. *RNASEH2A*-G37S mutation can have wide range effects on ribosomal biogenesis. In *S. cerevisiae*, 40% of R-loops are produced during rDNA transcription and removal of R-loops are dependent on hybrid activity of RNase H2. This suggests that inefficient removal of R-loops in rRNA in G37S homozygous mice leads to impaired ribosomal biogenesis. Phenotypes similar to G37S homozygous has been reported in mice with mutation in small ribosomal protein 7 (RPS7) gene. RPS7 mutation impairs ribosomal RNA processing resulting in mice with smaller body size, white ventral spotting, kinky tail, skeletal and CNS developmental defects Similarity in phenotypes of G37S homozygous mice and RPS7 mice suggests a possible role of defect in ribosomal biogenesis in G37S homozygous mice. Elucidation

of genetic variation and ribosomal biogenesis in G37S homozygous mice will provide insight into cause of lethality/viability. The rationale of this work is to understand role of RNase H2 mutation in the manifestation of AGS and will provide a good mouse model for the AGS study.