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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: Yadav, Gaya Prasad

eRA COMMONS USERNAME (credential, e.g., agency login): gyadav (11368210)

POSITION TITLE: Associate Research Scientist

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
Univ. of Lucknow, Lucknow, UP, India	BS	08/2000	Botany & Chemistry
Univ. of Lucknow, Lucknow, UP, India	MS	09/2003	Chemistry
CSJM Univ. Kanpur, UP, India	B.Ed.	05/2004	Biological and physical sciences
Jawaharlal Nehru Univ. New Delhi, India	PhD	05/2011	Biochemistry & Structural Biology
UT Southwestern Medical Center, Dallas, TX, USA	Postdoctoral	01/2016	Biochemistry & Structural Biology
Univ. of Florida, Gainesville, FL, USA	Postdoctoral	01/2019	Biochemistry & Structural Biology

A. Personal Statement

My research interests are largely direct towards understanding Molecular and Cellular Physiology and Molecular Biophysics. I am particularly interested in learning how the molecular mechanism involved in the regulated secretion and how the granin proteins are involved in different steps of regulated secretion. As a graduate student in the laboratory of Dr. R. Ravishankar at central drug research Institute, Lucknow, India, was trained in the fundamental methodologies of Molecular Biology and Structural Biology that aided me in investigating the molecular mechanisms underlying cellular physiology and molecular biophysics. I found that phosphoserine phosphatase, an essential protein for the mycobacterium survival, is involved in the chromatin remodeling of the THP-1 cell.

During my postdoctoral training, I have investigated the molecular role of a granin protein (CHGB) involved in the regulated secretion, forms anion channels in the secretory granules and facilitate the maturation of immature secretory granules to dense core secretory granules which was prerequisite for the release of the hormones as insulin. My research has led to important discoveries that have advanced our understanding of molecular roles of chromogranin B in the regulated secretion and in neuroendocrine caners.

Our study of ion channels in the regulated secretory pathway is a new direction which is centered around a completely new ion channel function we discovered in a granin family protein (***Life Science Alliance; 2018***). This study involved the use of different levels of imaging systems as light microscopy, confocal microscopy, super-resolution imaging and electron microscopy (TEM). I have used the confocal fluorescence microscope to image the individual secretory granule for ratiometric analysis. Individual secretory granules were imaged, and emission intensity was measure using imaging software and the intensity was converted to pH using standard curve prepared in similar way. I have used different image processing software to conduct these experiments and conclude the results of the results obtained from the images.

Before staring my graduate research, I have completed my Bachelor of Education, which is basically teachers training course. This degree requires the teaching of at least two months at high school grades. After my master's degree, I have worked as part time teacher in a degree college and used to teach

Physical Chemistry for one year. Currently, in the laboratory I am training multiple undergraduate students in the lab techniques and helping them in performing different lab experiments. I have trained graduate students for the use of electron microscope and set-up pipeline for the automatic data collection. Having these teaching and training experiences for different levels of students and working experience on different kinds of microscopes, I am confident that I will be able to pull forward the new responsibilities required for the imaging scientist.

(**1**). **Gaya P. Yadav**, Hui Zheng, Qing Yang, Lauren G Douma, Linda B Bloom and Qiu-Xing Jiang; Secretory granule protein chromogranin B (CHGB) forms an anion channel in membrane. Life Science Alliance, Sept 2018, 1(5) e201800139; DOI:10.26508/lsa.201800139.
(**2**). **Yadav GP, Current Dilemma on Granin Proteins:** Proteins involved in various cellular functions without known mechanisms. Cell Cellular Life Science J (2017), 2(2):000115.
(**3**). **Yadav GP**, Shree S, Maurya R, Rai N, Singh DK, Srivastava KK, et al. (2014) Characterization of *M. tuberculosis* SerB2, an Essential HAD-Family Phosphatase, Reveals Novel Properties. PLoS ONE 9(12): e115409.
(**4**). Singh P, **Yadav GP** (equal first author) , Gupta S, Tripathi AK, Ramachandran R, et al. A Novel Dimer-Tetramer Transition Captured by the Crystal Structure of the HIV-1 Nef (2011) A Novel Dimer-Tetramer Transition Captured by the Crystal Structure of the HIV-1 Nef. PLOS ONE 6(11): e26629. https://doi.org/10.1371/journal.pone.0026629.
(**5**). **Gaya P. Yadav**, Haiyuan Wang, Joke Ouwendijk, Mani Annamalai, Stephen Cross, Qiaochu Wang, D. Walker Hagan, Clayton Mathews, Edward A. Phelps, Paul Verkade, Michael X. Zhu, and Qiu-Xing Jiang; Membrane insertion of chromogranin B for granule maturation in regulated secretion (**under review**).

B. Positions and Employment

2019-2020 Biological Scientist III, Department of Microbiology and Cell Science, Univ. of Florida, Gainesville, FL, USA.
2020- present Associate Research Scientist, Hauptman-Woodward Medical Research Institute, Buffalo, NY, USA

Professional Memberships

2014 -- Present Member, Biophysical Society
2013 -- 2014 Member, American Heart Association

Honors and Awards

2005-2007 CSIR, Junior research fellowship, UP, India
2007-2010 CSIR, senior research fellowship, UP, India

C. Contributions to Science

1. Granin family proteins are by default granule proteins that chaperone other secretory molecules through regulated secretion. They have both intracellular and extracellular functions. Chromogranins (CHGs) are believed to function in all three steps of the regulated secretory pathway. It was proposed that CHGs interact with cargos and serve as a low-affinity, high-capacity Ca^{2+} reserve. Their extracellular functions are executed by CHG-derived peptides that are associated with various human diseases. However, the molecular mechanisms for all CHGs' intracellular functions remain to be elucidated. We have investigated the functional properties of CHGB proteins in different membrane systems and demonstrated that CHGB inserts into membrane and by itself suffices to form an unconventional chloride channel. We have investigated that CHGB has dual functional states: as soluble protein, it processed into small bioactive peptide while when reconstituted in the membrane it functions as chloride channel.

(**a**). **Yadav GP,** Current Dilemma on Granin Proteins**:** Proteins involved in various cellular functions without known mechanisms. Cell Cellular Life Science J (2017), 2(2):000115.
(**b**). **Gaya P. Yadav**, Hui Zheng, Qing Yang, Lauren G Douma, Linda B Bloom and Qiu-Xing Jiang; Secretory granule protein chromogranin B (CHGB) forms an anion channel in membrane. Life Science Alliance, Sept 2018, 1(5) e201800139; DOI:10.26508/lsa.201800139.

(**c**) **Gaya P. Yadav**, Haiyuan Wang, Joke Ouwendijk, Mani Annamalai, Stephen Cross, Qiaochu Wang, D. Walker Hagan, Clayton Mathews, Edward A. Phelps, Paul Verkade, Michael X. Zhu, and Qiu-Xing Jiang; Membrane insertion of chromogranin B for granule maturation in regulated secretion (under review).

2. Tuberculosis (TB), one of the oldest known human diseases, is still is one of the major causes of mortality, since two million people die each year from this malady. TB has many manifestations, affecting bone, the central nervous system, and many other organ systems, but it is primarily a pulmonary disease that is initiated by the deposition of *Mycobacterium tuberculosis*, contained in aerosol droplets, onto lung alveolar surfaces. Though there many medicines are available for tuberculosis but still Its therapy takes prolong time to get cured. Due to that researcher are still working to find the better inhibitor of the mycobacterium tuberculosis. Phosphoserine phosphatase is an essential enzyme for the survival of the mycobacterium. So, we targeted this protein and characterize biochemically and biophysically. we find that exogenously added MtSerB2 induces microtubule rearrangements in THP-1 cells, a cell- line that can differentiate into macrophage-like cells. The experiments with mutant MtSerB2 demonstrates that the phosphatase activity is co-related to the elicited microtubule rearrangements.
(**a**). **Yadav GP**, Shree S, Maurya R, Rai N, Singh DK, Srivastava KK, et al. (2014) Characterization of *M. tuberculosis* SerB2, an Essential HAD-Family Phosphatase, Reveals Novel Properties. PLoS ONE 9(12): e115409.
(**b**). Shree, S. Singh, A.K., Saxena, R et. al. Cell. Mol. Life Sci. (2016) 73:3401.

3. Through collaborative studies, we discovered, how changes to the oligomeric state of Nef can help it to distinguish between protein partners. HIV-1 Nef is an important accessory protein that is attributed with multiple distinct functions such as immune evasion, virion infectivity and support for viral replication and survival. These functions are regulated through the interactions of Nef with more than 30 different partner proteins in the membrane anchored and cytoplasmic states respectively, it is puzzling as to how this small ~27 kDa protein can control multiple important functions. Vicinal to membranes, it exists as smaller oligomers such as a dimer or a trimer, as seen in the core domain (Nef_{core}) complexes with the SH3 domain of Fyn Kinase (Fyn_{SH3}). On the other hand, small angle X-ray scattering, dynamic light scattering, and analytical centrifugation studies show that the cytoplasmic form exists as a tetramer or higher oligomers that result when multiple tetramers associate together. Despite earlier efforts, structural elucidation of a full-length Nef as also of the elusive '*closed*' oligomeric form has not been reported so far. This has led to a gap in understanding, in molecular terms, as to how Nef modulates its interactions with partner proteins. Using X-ray Crystallography, we showed, how the interaction of the Asp shifts to the second Arg residue of the motif leading to the novel dimer-tetramer transition.

(**a**). Singh P, **Yadav GP** (equal first author) , Gupta S, Tripathi AK, Ramachandran R, et al. A Novel Dimer-Tetramer Transition Captured by the Crystal Structure of the HIV-1 Nef (2011) A Novel Dimer-Tetramer Transition Captured by the Crystal Structure of the HIV-1 Nef. PLOS ONE 6(11): e26629. https://doi.org/10.1371/journal.pone.0026629.

D. <u>Technical expertise</u>:

- Good skills in various software packages for CryoEM data analysis.
- Hands-on experience with nanoscale Confocal microscopes (Zeiss LSM 880 Airyscan confocal microscope, Zeiss LSM 780 confocal microscope/multiphoton, Andor spinning disk confocal with FRAP and TIRF).
- Hands-on experience with nanoscale Wide Field microscopes (Deltavision deconvolution microscopes, Zeiss Axio Observer epifluorescence microscope).
- Hands-on experience with nanoscale Electron Microscopes (Tecnai F20, Titan Krios, Tecnai F12, JEOL 2200FS and CM120BT).
- Sample preparation for CryoEM using vitrobot.
- Negative stained and Cryo-imaging for Single particle reconstruction in Electron Microscope.
- Single particle reconstruction to get 3D model for proteins (using EMAN, IMAGIC, BOXER and RELION).

- Preparation of Nanodiscs for membrane protein cryoEM.
- Handling mammalian (HEK293, PC-12, INS-1, CHO and hPho1), insect (Sf9 and Hi5) and bacterial cell cultures.
- Gene cloning, site directed mutagenesis, over-expression and Purification of recombinant proteins, western blotting.
- Affinity chromatography, ion exchange methods and gel permeation chromatography.
- Purification of soluble as well as membrane proteins.
- Protein purification using FPLC and HPLC system.
- Circular Dichroism, fluorescence and UV-VIS spectrophotometry, Isothermal calorimetry, protein-ligand interaction analysis and enzymatic assays.
- Crystallization, crystal harvesting, diffraction data collection and processing.
- Small molecule structure determination using X-ray crystallography.

Complete List of Published Work in My Bibliography:

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

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

Ongoing Research Support

None

Completed Research Support

None