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: ARTEMYEV, NIKOLAI

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

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
St. Petersburg Institute of Technology, St. Petersburg	BS	04/1984	Biotechnology
Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg	PHD	10/1988	Biochemistry
Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg	Postdoctoral Fellow	12/1989	Biochemistry
University of Illinois at Chicago, Chicago, IL	Postdoctoral Fellow	06/1994	Physiology and Biophysics

A. Personal Statement

My research focuses on visual transduction in rod and cone photoreceptors and the mechanisms leading to associated visual disorders. My laboratory has accumulated extensive expertise in research on phototransduction proteins, particularly the visual G protein transducin and the effector enzyme phosphodiesterase 6 (PDE6). I have directed two NEI R01 grants on the regulation of transducin and PDE6 for over 20 years. The results of our studies on transducin and PDE6 have been documented in numerous publications. In our research of the phototransduction proteins, we have been successfully utilizing structural and biophysical approaches, including X-ray crystallography, cryo-EM, and small-angle x-ray scattering.

1. Yadav RP, Boyd K, Yu L, **Artemyev NO**. Interaction of the tetratricopeptide repeat domain of aryl hydrocarbon receptor-interacting protein-like 1 with the regulatory Pγ subunit of phosphodiesterase 6. J Biol Chem. 2019 Oct 25;294(43):15795-15807. PMID: 31488544; PMCID: PMC6816093.
2. Yadav RP, Gakhar L, Yu L, **Artemyev NO**. Unique structural features of the AIPL1-FKBP domain that support prenyl lipid binding and underlie protein malfunction in blindness. Proc Natl Acad Sci U S A. 2017 Aug 8;114(32):E6536-E6545. PubMed PMID: 28739921; PubMed Central PMCID: PMC5559027.
3. Srivastava D, Gakhar L, **Artemyev NO**. Structural underpinnings of Ric8A function as a G-protein α-subunit chaperone and guanine-nucleotide exchange factor. Nat Commun. 2019 Jul 12;10(1):3084.. PMID: 31300652; PMCID: PMC6625990.
4. Barren B, Gakhar L, Muradov H, Boyd KK, Ramaswamy S, **Artemyev NO**. Structural basis of phosphodiesterase 6 inhibition by the C-terminal region of the gamma-subunit. EMBO J. 2009 Nov 18;28(22):3613-22. PubMed PMID: 19798052; PubMed Central PMCID: PMC2782096.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

1988 - 1989	Postdoctoral Fellow, St. Petersburg Institute of Evolutionary Physiology and Biochemistry, St. Petersburg
1990 - 1994	Postdoctoral Research Associate, Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL
1994 - 1995	Research Assistant Professor, Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL
1995 - 1999	Assistant Professor, Department of Physiology and Biophysics, University of Iowa, Iowa City, IA
1999 - 2004	Associate Professor, Department of Physiology and Biophysics, University of Iowa, Iowa City, IA
2004 -	Professor, Department of Molecular Physiology and Biophysics, University of Iowa, Iowa City, IA
2010 -	Professor, Department of Ophthalmology and Visual Sciences, University of Iowa , Iowa City, IA

1992 -	Member, Association for Research in Vision and Ophthalmology
2000 - 2003	Ad hoc reviewer, Visual Sciences C Study Section, CSR, NIH
2003	Ad hoc reviewer, Biology and Diseases of the Posterior Eye Study Section, CSR, NIH
2004 - 2004	Reviewer, Special Emphasis Panel Pathophysiology of the Retina, CSR, NIH
2004 - 2007	Member, Biology and Diseases of the Posterior Eye Study Section, CSR, NIH
2009	Member, Challenge Grants Panel, CSR, NIH
2012	Ad hoc reviewer, Biology of the Visual System Study Section, CSR, NIH
2013	Ad hoc reviewer, Great Lakes Fishery Commission Research Program
2015	Ad hoc reviewer, German Research Foundation
2016	Reviewer, Special Emphasis Panel Retinal Development, Signaling and Circuitry, CSR, NIH
2016	Reviewer, Special Emphasis Panel Retinal Degeneration, Signaling and Circuitry, CSR, NIH

Honors

1994	Young Investigator Award, National Alliance for Research on Schizophrenia and Depression
1996	Carver Trust Medical Research Initiative Award, University of Iowa
2001	Established Investigator Award, American Heart Association
2004	Alberta Heritage Foundation for Medical Research (AHFMR) visiting lecturer award, University of Calgary

C. Contributions to Science

1. Determined structure-function relationships of PDE6 and its specialized chaperone AIPL1. To study the structure-function relationships of PDE6 we expressed PDE6/PDE5 chimeras, as well as cone PDE6 and rod/cone PDE6 chimeras, in the rods of transgenic *X. laevis*. Extensive mutational analyses based on these systems allowed us to identify the dimerization determinants of PDE6 catalytic subunits, identify the PDE6 residues that are involved in binding to Pγ and noncatalytic cGMP, and establish that the catalytic domains of rod PDE6 are functionally equivalent. Using biochemical and structural approaches, we elucidated the molecular details of PDE6 inhibition by Pγ and demonstrated that Pγ physically occludes the opening of the catalytic pocket, thereby preventing cGMP from binding to the active site. We have developed a novel heterologous system for expressing PDE6 in cultured cells, in which PDE6 catalytic subunits are co-expressed with AIPL1 and Pγ. The use of this system is markedly advancing our understanding of PDE6 biology and pathology. We solved the crystal structures of the FKBP and TPR domains of AIPL1 and described unique interactions of these domains with the prenyl moieties and the Pγ-subunit of PDE6. Recently, we solved the cryo-EM structure of the complex of AIPL1 with HSP90, and the structure of human cone PDE6C. These structures have established a framework for studies the HSP90/AIPL1-mediated folding of PDE6.

a. Singh S, Srivastava D, Boyd K, **Artemyev NO.** Structural and functional dynamics of human cone cGMP-phosphodiesterase important for photopic vision. Proc Natl Acad Sci U S A. 2025 Jan 7; 122(1):e2419732121. doi: 10.1073/pnas.2419732121. Epub 2024 Dec 31. PMID: 39739818
b. Yadav RP, Gakhar L, Yu L, **Artemyev NO**. Unique structural features of the AIPL1-FKBP domain that support prenyl lipid binding and underlie protein malfunction in blindness. Proc Natl Acad Sci U S A. 2017 Aug 8;114(32):E6536-E6545. PubMed PMID: 28739921; PubMed Central PMCID: PMC5559027.
c. Gopalakrishna KN, Boyd K, Yadav RP, **Artemyev NO**. Aryl Hydrocarbon Receptor-interacting Protein-like 1 Is an Obligate Chaperone of Phosphodiesterase 6 and Is Assisted by the γ-Subunit of Its Client. J Biol Chem. 2016 Jul 29;291(31):16282-91. PubMed PMID: 27268253; PubMed Central PMCID: PMC4965576.
d. Muradov H, Boyd KK, **Artemyev NO**. Rod phosphodiesterase-6 PDE6A and PDE6B subunits are enzymatically equivalent. J Biol Chem. 2010 Dec 17;285(51):39828-34. PubMed PMID: 20940301; PubMed Central PMCID: PMC3000964.
e. Barren B, Gakhar L, Muradov H, Boyd KK, Ramaswamy S, **Artemyev NO**. Structural basis of phosphodiesterase 6 inhibition by the C-terminal region of the gamma-subunit. EMBO J. 2009 Nov 18;28(22):3613-22. PubMed PMID: 19798052; PubMed Central PMCID: PMC2782096.

2. Identified mechanisms underlying rhodopsin-dependent activation of transducin and transducin-dependent activation of PDE6, as well as the regulation of transducin by RGS-proteins and Ric8A. We used chimeric rhodopsins and transducins and mutational analyses to map the interaction interface and identify residues in both proteins that are critical for activating transducin. Our studies provided evidence that the transducin-α C-terminus and α5-helix act as a dominant channel that transmits the R*-induced conformational change, leading to G protein activation. We have mapped the effector surface of transducin, provided insights into the transducin/PDE6 interface, and developed a comprehensive model of PDE6 activation by transducin. Recently, we solved the crystal structures of the resistance to inhibitors of cholinesterase 8 homolog A (Ric-8A), a guanine nucleotide exchange factor (GEF) and putative chaperone of transducin-α, and developed a model of the complex formed by Ric-8A and Gα. This study laid the groundwork for understanding Ric-8A function at the molecular level, as well as potential roles of the protein in the retina.

   a. Srivastava D, Gakhar L, **Artemyev NO**. Structural underpinnings of Ric8A function as a G-protein α-subunit chaperone and guanine-nucleotide exchange factor. Nat Commun. 2019 Jul 12;10(1):3084.. PMID: 31300652; PMCID: PMC6625990.
   b. Natochin M, Granovsky AE, **Artemyev NO**. Identification of effector residues on photoreceptor G protein, transducin. J Biol Chem. 1998 Aug 21;273(34):21808-15. PubMed PMID: 9705319.
   c. Natochin M, Moussaif M, **Artemyev NO**. Probing the mechanism of rhodopsin-catalyzed transducin activation. J Neurochem. 2001 Apr;77(1):202-10. PubMed PMID: 11279276.
   d. Natochin M, Barren B, Ahmad ST, O'Tousa JE, **Artemyev NO**. Probing rhodopsin-transducin interaction using Drosophila Rh1-bovine rhodopsin chimeras. Vision Res. 2006 Dec;46(27):4575-81. PubMed PMID: 16979689.

3. Delineated mechanisms whereby mutations in the genes encoding transducin, PDE6, and AIPL1 cause visual dysfunction. Our studies have revealed novel pathogenic mechanisms underlying mutant photoreceptor-specific proteins. Using a mouse model of the Nougaret form of dominant stationary night blindness, we identified a unique combination of functional deficiencies of the mutant transducin that alter visual signaling. Our investigation of the mouse *atrd3* model demonstrated that a missense mutation in the gene that encodes PDE6 causes retinal degeneration by aberrant splicing of the pre-mRNA. We discovered that disruption of binding of the PDE6 farnesyl moiety to mutant AIPL1 is a novel cause of Leber congenital amaurosis. Recently, we successfully developed a system for heterologous expression of PDE6 and utilized it to screen PDE6 and AIPL1 variants for pathogenicity.

   a. Gopalakrishna KN, Boyd K, Yadav RP, **Artemyev NO**. Aryl Hydrocarbon Receptor-interacting Protein-like 1 Is an Obligate Chaperone of Phosphodiesterase 6 and Is Assisted by the γ-Subunit of Its Client. J Biol Chem. 2016 Jul 29;291(31):16282-91. PubMed PMID: 27268253; PubMed Central PMCID: PMC4965576.
   b. Majumder A, Gopalakrishna KN, Cheguru P, Gakhar L, **Artemyev NO**. Interaction of aryl hydrocarbon receptor-interacting protein-like 1 with the farnesyl moiety. J Biol Chem. 2013 Jul 19;288(29):21320-8. PubMed PMID: 23737531; PubMed Central PMCID: PMC3774400.

c. Muradov H, Boyd KK, Kerov V, **Artemyev NO**. Atypical retinal degeneration 3 in mice is caused by defective PDE6B pre-mRNA splicing. Vision Res. 2012 Mar 15;57:1-8. PubMed PMID: 22326271; PubMed Central PMCID: PMC3285400.
d. Moussaif M, Rubin WW, Kerov V, Reh R, Chen D, Lem J, Chen CK, Hurley JB, Burns ME, **Artemyev NO**. Phototransduction in a transgenic mouse model of Nougaret night blindness. J Neurosci. 2006 Jun 21;26(25):6863-72. PubMed PMID: 16793893.

4. Described mechanisms underlying trafficking of transducin in rods. Initially, we focused on the mechanism underlying the light-dependent translocation of transducin. Collectively, these studies supported a "diffusion model", which at present is commonly accepted. We subsequently generated a mouse model in which light-dependent translocation of transducin is impaired to probe the role of this phenomenon. Our analysis of this mouse model led to two major discoveries. It provided strong experimental support for a previously hypothesized role of transducin translocation in protecting rod photoreceptors. Secondly, it showed that transducin translocation in rods enhances signaling to rod bipolar cells. This finding markedly expands the classical paradigm of transducin signaling.

a. Kerov V, Chen D, Moussaif M, Chen YJ, Chen CK, **Artemyev NO**. Transducin activation state controls its light-dependent translocation in rod photoreceptors. J Biol Chem. 2005 Dec 9;280(49):41069-76. PubMed PMID: 16207703.
b. Kerov V, Rubin WW, Natochin M, Melling NA, Burns ME, **Artemyev NO**. N-terminal fatty acylation of transducin profoundly influences its localization and the kinetics of photoresponse in rods. J Neurosci. 2007 Sep 19;27(38):10270-7. PubMed PMID: 17881533.
c. Gopalakrishna KN, Doddapuneni K, Boyd KK, Masuho I, Martemyanov KA, **Artemyev NO**. Interaction of transducin with uncoordinated 119 protein (UNC119): implications for the model of transducin trafficking in rod photoreceptors. J Biol Chem. 2011 Aug 19;286(33):28954-62. PubMed PMID: 21712387; PubMed Central PMCID: PMC3190703.
d. Majumder A, Pahlberg J, Boyd KK, Kerov V, Kolandaivelu S, Ramamurthy V, Sampath AP, **Artemyev NO**. Transducin translocation contributes to rod survival and enhances synaptic transmission from rods to rod bipolar cells. Proc Natl Acad Sci U S A. 2013 Jul 23;110(30):12468-73. PubMed PMID: 23836670; PubMed Central PMCID: PMC3725049.

5. Defined the evolution of cone and rod phototransduction components and the isoform differences that contribute to the distinct physiology of cones and rods. We examined pigments, transducins, and PDE6 in lamprey *P. marinus*, an ancient vertebrate species. Our studies revealed duplications of the Pγ- and transducin-encoding genes in the stem of the vertebrate lineage. These duplications gave rise to mixed rod/cone forms of these proteins, which is thought to represent an essential step in the evolution of scotopic vision in early vertebrates. We also found that the key activation and inactivation properties of cone and rod transducins *in vitro* are comparable. On the other hand, using a transgenic mouse model that expresses cone PDE6 in rods lacking rod PDE6, we demonstrated that the PDE6 isoforms play an essential role in the physiological differences between cone and rod photoreceptors.

a. Muradov H, Boyd KK, Kerov V, **Artemyev NO**. PDE6 in lamprey Petromyzon marinus: implications for the evolution of the visual effector in vertebrates. Biochemistry. 2007 Sep 4;46(35):9992-10000. PubMed PMID: 17685558.
b. Muradov H, Kerov V, Boyd KK, **Artemyev NO**. Unique transducins expressed in long and short photoreceptors of lamprey Petromyzon marinus. Vision Res. 2008 Sep;48(21):2302-8. PubMed PMID: 18687354; PubMed Central PMCID: PMC2613798.
c. Gopalakrishna KN, Boyd KK, **Artemyev NO**. Comparative analysis of cone and rod transducins using chimeric Gα subunits. Biochemistry. 2012 Feb 28;51(8):1617-24. PubMed PMID: 22324825; PubMed Central PMCID: PMC3291952.
d. Majumder A, Pahlberg J, Muradov H, Boyd KK, Sampath AP, **Artemyev NO**. Exchange of Cone for Rod Phosphodiesterase 6 Catalytic Subunits in Rod Photoreceptors Mimics in Part Features of Light Adaptation. J Neurosci. 2015 Jun 17;35(24):9225-35. PubMed PMID: 26085644; PubMed Central PMCID: PMC4469743.

Complete List of Published Work in My Bibliography:
http://bit.ly/2ks4Pni

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: Srivastava, Dhiraj

eRA COMMONS USER NAME (credential, e.g., agency login): dsrivastava1

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
D.D.U. Gorakhpur University, India	BS	05/2001	Zoology, Botany, Chemistry
Jawaharlal Nehru University, India.	MS	05/2004	Biotechnology
University of Missouri-Columbia	PHD	07/2011	Chemistry
University of California- San Diego	Postdoctoral	05/2012	Biochemistry
University of Iowa, Iowa	Postdoctoral	01/2019	Chemistry/Physiology and Biophysics

A. Personal Statement

I am an associate research scientist in the Molecular physiology and biophysics department at University of Iowa and my current research focus is on biophysical studies of regulation of synaptic output in rod and cone photoreceptors. I have a broad background in biophysics, with specific training and expertise in X-ray crystallography and small-angle X-ray scattering. During my Ph.D. and postdoctoral training, I have worked on various projects including proline metabolic enzymes, pyruvate kinase, and Ric8A. For the last few years, I am studying photoreceptor proteins and their complexes using biochemical and biophysical methods. Besides extensive documented expertise in structural biology, I gained significant experience in all the proposed biochemical and cell biological techniques.

1. **Srivastava D**, Schuermann JP, White TA, Krishnan N, Sanyal N, Hura GL, Tan A, Henzl MT, Becker DF, Tanner JJ. Crystal structure of the bifunctional proline utilization A flavoenzyme from Bradyrhizobium japonicum. Proc Natl Acad Sci U S A. 2010;107(7):2878-83. Epub 2010/02/06. doi: 10.1073/pnas.0906101107. PubMed PMID: 20133651; PMCID: PMC2840367.
2. **Srivastava D**, Singh RK, Moxley MA, Henzl MT, Becker DF, Tanner JJ. The three-dimensional structural basis of type II hyperprolinemia. J Mol Biol. 2012;420(3):176-89. Epub 2012/04/21. doi: 10.1016/j.jmb.2012.04.010. PubMed PMID: 22516612; PMCID: PMC3372638.

3. **Srivastava D**, Artemyev NO. Large-scale conformational rearrangement of the alpha5-helix of Galpha subunits in complex with the guanine nucleotide exchange factor Ric8A. J Biol Chem. 2019;294(47):17875-82. Epub 2019/10/19. doi: 10.1074/jbc.AC119.011135. PubMed PMID: 31624147; PMCID: PMC6879328.
4. **Srivastava D**, Gakhar L, Artemyev NO. Structural underpinnings of Ric8A function as a G-protein alpha-subunit chaperone and guanine-nucleotide exchange factor. Nat Commun. 2019;10(1):3084. Epub 2019/07/14. doi: 10.1038/s41467-019-11088-x. PubMed PMID: 31300652; PMCID: PMC6625990.

B. Positions, Scientific Appointments, and Honors

Positions and scientific appointments

2022-present- Associate research scientist, University of Iowa
2019-2022 - Assistant research scientist, University of Iowa.
2013-2019 - Postdoctoral research associate, University of Iowa.
2011-2012 - Postdoctoral research associate, Department of Biochemistry, University of California-San Diego.

Honors

1. Scholarships from the Department of Biotechnology, India for M.S. (Biotechnology)
2. Qualified for the Joint Council for Scientific and Industrial Research-University Grant Commission (CSIR-UGC) National Eligibility Test (28/12/2003) for Lectureship-NET in Life science.
3. Qualified for the Joint CSIR-UGC National Eligibility Test (27/06/2004) for Junior Research Fellowship in the subject Life science under CSIR Fellowship Scheme.
4. The Breckenridge/Lyon's Outstanding Graduate Research Award. (2010)
5. Travel award from American Crystallographic Association to attend ACA 2009 meeting.

C. Contributions to Science

1 – structural studies of proline catabolic enzyme- During my Ph.D. I studied the bacterial and mammalian enzymes involved in protein catabolism. Proline is catabolized to glutamate by two enzymes, proline dehydrogenase, and P5C dehydrogenase. In most of the bacteria, these enzyme activities are in the same polypeptide chain (PutA) and the product of Proline dehydrogenase is directly transferred to the P5C dehydrogenase active site by the process of substrate channeling. I solved the atomic structure of PutA from *Bradyrhizobium japonicum* using X-ray crystallography. The structure reveals a tunnel connecting the two active sites. SAXS was used to deduce the oligomeric state and kinetic analysis suggests the advantage of substrate channeling in accelerating the overall rate of kinetics besides protecting the labile intermediate.

I also solved the structure of P5C dehydrogenase from yeast, human, and mouse with substrates and products. The structure of mutant enzymes explained the molecular mechanism of type II hyperprolinemia.

1. **Srivastava, D.**, Schuermann, J. P., White, T. A., Krishnan, N., Sanyal, N., Hura, G. L., Tan, A., Henzl, M. T., Becker, D. F., and Tanner, J. J. (2010) Crystal structure of the bifunctional proline utilization A flavoenzyme from Bradyrhizobium japonicum. *Proc Natl Acad Sci U S A* **107**, 2878-2883
2. **Srivastava, D.**, Zhu, W., Johnson, W. H., Jr., Whitman, C. P., Becker, D. F., and Tanner, J. J. (2010) The structure of the proline utilization a proline dehydrogenase domain inactivated by N-

propargylglycine provides insight into conformational changes induced by substrate binding and flavin reduction. *Biochemistry* **49**, 560-569

3. **Srivastava, D.**, Singh, R. K., Moxley, M. A., Henzl, M. T., Becker, D. F., and Tanner, J. J. (2012) The three-dimensional structural basis of type II hyperprolinemia. *J Mol Biol* **420**, 176-189
4. Pemberton, T. A., **Srivastava, D.**, Sanyal, N., Henzl, M. T., Becker, D. F., and Tanner, J. J. (2014) Structural studies of yeast Delta(1)-pyrroline-5-carboxylate dehydrogenase (ALDH4A1): active site flexibility and oligomeric state. *Biochemistry* **53**, 1350-1359

2 – Allosteric modulation of PKM2 - PKM2 exists in highly active tetramer and less active dimer/monomer. Fructose, 1-6, bisphosphate (FBP) binds to PKM2 and induces its tetramerization. In the absence of FBP, PKM2 exists in dimeric/monomeric form. Various ligands and post-translation modification affect oligomerization and thus nuclear localization of PKM2. In the nucleus, PKM2 interacts with various proteins and regulate transcription. Due to copurification of FBP, PKM2 purified from E. coli always exists in tetrameric form making biophysical and structural studies on PKM2-protein complex difficult. I have made a mutant of PKM2 which cannot bind to FBP and thus always exists in dimeric form. This mutant was crystallized, and the solution structure was studied using SAXS and analytical ultracentrifugation showing that in the absence of FBP, PKM2 exists in monomer/dimer equilibrium. I also studied the allosteric regulation of PKM2 by cysteine and serine using a variety of biophysical, biochemical, and theoretical methods including ITC, SAXS, crystallography, and molecular dynamics simulation. Our results suggest that contrary to amino acids with bulky side chain, smaller amino acids like serine and cysteine does not affect the conformation of PKM2 raising the possibility that allosteric modulation of PKM2 activity by smaller amino acids might be entropy driven.

1. **Srivastava, D.**, Razzaghi, M., Henzl, M. T., and Dey, M. (2017) Structural Investigation of a Dimeric Variant of Pyruvate Kinase Muscle Isoform 2. *Biochemistry* **56**, 6517-6520
2. **Srivastava, D.**, Nandi, S., and Dey, M. (2019) Mechanistic and Structural Insights into Cysteine-Mediated Inhibition of Pyruvate Kinase Muscle Isoform 2. *Biochemistry* **58**, 3669-3682
3. Nandi, S., Razzaghi, M., **Srivastava, D.**, and Dey, M. (2020) Structural basis for allosteric regulation of pyruvate kinase M2 by phosphorylation and acetylation. *J Biol Chem* **295**, 17425-17440

3 – Structural studies of Ric8A and its complex with Gα - Ric-8A is a chaperone and guanine nucleotide exchange factor for Gα. I studied the Ric8A-Gα complex using crystallography, SAXS, and other biophysical and computational technique. I solved the structure of apo as well as peptide bound Ric8A using X-ray crystallography which showed that the Ric-8A structure has N terminus armadillo repeat followed by flexible and disordered C terminus. The structure, binding studies, XL-MS and SAXS suggest the critical role of the disordered C terminus in its stability and interaction with Gα. XL-MS and Molecular modeling with Rosetta suggest that the acidic proximal C terminus of Ric8A interacts with the basic patch at the C terminus end of armadillo repeat while the distal C-terminus interacts with Gα. Using XL-MS and SAXS, we showed that Ric-8A induces large scale conformational changes in Gα.

1. **Srivastava, D.**, and Artemyev, N. O. (2019) Large-scale conformational rearrangement of the alpha5-helix of Galpha subunits in complex with the guanine nucleotide exchange factor Ric8A. *J Biol Chem* **294**, 17875-17882
2. **Srivastava, D.**, Gakhar, L., and Artemyev, N. O. (2019) Structural underpinnings of Ric8A function as a G-protein alpha-subunit chaperone and guanine-nucleotide exchange factor. *Nat Commun* **10**, 3084

3. **Srivastava, D.**, and Artemyev, N. O. (2020) Ric-8A, a GEF, and a Chaperone for G Protein alpha-Subunits: Evidence for the Two-Faced Interface. *Bioessays* **42**, e1900208
4. **Srivastava, D.**, Yadav, R. P., Inamdar, S. M., Huang, Z., Sokolov, M., Boyd, K., and Artemyev, N. O. (2020) Transducin Partners Outside the Phototransduction Pathway. *Front Cell Neurosci* **14**, 589494

4 – Structural basis of NRL-CRX transcription factor cooperation – CRX, a homeodomain transcription factor, is expressed in postmitotic photoreceptor precursors, where it induces expression of photoreceptor-specific genes signifying a commitment to photoreceptor fate. NRL, a basic motif–leucine zipper (bZIP) domain transcription factor of the Maf family, is expressed at a later stage of photoreceptor development in cells that are destined to become rods. In coordination with CRX and other TFs, NRL induces the expression of rod-specific genes while simultaneously suppressing cone-specific gene expression. I solved the structure of CRX in complex with its cognate binding site at the Ret4 promoter. This structure reveals that CRX binds to the Ret4 promoter and causes significant bending of DNA which was verified by SEC-SAXS-MALS. The future goal of this project is to understand the mechanism by which CRX modulates NRL binding to DNA.

1. **Srivastava D**, Gowribidanur-Chinnaswamy P, Gaur P, Spies M, Swaroop A, Artemyev NO. Molecular basis of CRX/DNA recognition and stoichiometry at the Ret4 response element. Structure. 2024;32(10):1751-9.e4. doi: 10.1016/j.str.2024.07.004

5 – **Structure, folding and allosteric regulation of phosphodiesterase 5 and 6** – PDE5 and PDE6 are enzymes responsible for the hydrolysis of cGMP. PDE5 is widely expressed in several tissues where it regulates various physiological processes like vasodilation, neurotransmission, and calcium homeostasis. PDE6 expression is limited to the photoreceptor where it plays an important role in the visual signal transduction. Both the proteins have similar domain organization, GafA, GafB and catalytic domain. While PDE5 is a homodimer, PDE6 has four subunits, PDE6A and A' in rods, PDE6C in cones and 2 inhibitory pγ subunits. GafA domain harbor an allosteric binding site for the substrate cGMP. cGMP binding to GafA domain activate the catalytic activity in the catalytic domain of PDE5 while it serve mostly structural role in PDE6. PDE6 activity is regulated by the transducin α subunit ($G\alpha_t$). I am studying the structure, regulation and folding of catalytic activity in PDE5 and PDE6. We have solved the structure of HSP90-AIPL1 complex and PDE6C using cryoEM. Currently, I am trying to understand the allosteric regulation of PDE5 and regulation of PDE6 by $G\alpha_t$.

1. Singh S*, **Srivastava D***, Boyd K, Artemyev NO. Reconstitution of the phosphodiesterase 6 maturation process important for photoreceptor cell function. Journal of Biological Chemistry. 2024;300(1).

2. Singh S*, **Srivastava D***, Boyd K, Artemyev NO. Structural and functional dynamics of human cone cGMP-phosphodiesterase important for photopic vision. Proceedings of the National Academy of Sciences. 2025;122(1):e2419732121.

3. **Srivastava D***, Yadav RP*, Singh S, Boyd K, Artemyev NO. Unique interface and dynamics of the complex of HSP90 with a specialized cochaperone AIPL1. Structure. 2023;31(3):309-17. e5.

Complete list of published work:

https://scholar.google.com/citations?user=aLctLJIAAAAJ&hl=en

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: Singh, Sneha

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

POSITION TITLE: Postdoctoral Research Scholar

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
Guru Gobind Singh Indraprastha University, India	B.Tech	06/2013	Biotechnology
CSIR- National Chemical Laboratory, India	PhD	10/2021	Biological Sciences

A. Personal Statement

I am currently working as a Postdoctoral researcher in the Department of Molecular Physiology and Biophysics at the University of Iowa. During my Doctoral research I have worked on varied projects involving structural and biochemical studies of plant enzymes like Sesquisabinene synthase 1 and human proteins like DOCK and NEDD9. As a part of my postdoctoral training, currently, I am exploring the field of proteins crucial for visual transduction and the structure-function relationship of these proteins as defects in these proteins lead to visual disorders. I have a comprehensive expertise in biochemistry and structural biology including X-ray crystallography and Cryo-EM techniques.

1. Singh S, Thulasiram H. V., Sengupta D, & Kulkarni K. (2021). Dynamic coupling analysis on plant sesquiterpene synthases provides leads for the identification of product specificity determinants. *Biochemical and biophysical research communications*, *536*, 107–114. https://doi.org/10.1016/j.bbrc.2020.12.041. PMID: 33387748.

2. Srivastava, D., Yadav, R. P., **Singh, S**., Boyd, K., & Artemyev, N. O. (2023). Unique interface and dynamics of the complex of HSP90 with a specialized cochaperone AIPL1. *Structure (London, England : 1993)*, S0969-2126(22)00523-8. Advance online publication. https://doi.org/10.1016/j.str.2022.12.014. PMID: 36657440

3. **Singh S**, Srivastava D, Boyd K, Artemyev NO. (2024). Reconstitution of the phosphodiesterase 6 maturation process important for photoreceptor cell function. *J Biol Chem*. ;300(1):105576. https://doi.org/10.1016/j.jbc.2023.105576. Epub 2023 Dec 16. PMID: 38110033; PMCID: PMC10819763.

4. **Singh S**, Srivastava D, Boyd K, Artemyev NO. (2025). Structural and functional dynamics of human cone cGMP-phosphodiesterase important for photopic vision. *Proc Natl Acad Sci U S A*.;122(1):e2419732121. https://doi.org/10.1073/pnas.2419732121. Epub 2024 Dec 31. Erratum in: *Proc Natl Acad Sci U S A*. (2025) Apr 8;122(14):e2505043122. doi: 10.1073/pnas.2505043122. PMID: 39739818; PMCID: PMC11725853.

B. Positions, Scientific Appointments, and Honors

Positions

2022-Present: Postdoctoral Research associate, Department of Molecular Physiology and Biophysics at the University of Iowa

Honors

1. Senior Research Fellowship from the Council of Scientific and Industrial Research (2016)
2. Junior Research Fellowship from the Council of Scientific and Industrial Research (2016)
3. Qualified the Gratitude Aptitude Test in Engineering fellowship in Biotechnology (2014)

C. Contributions to Science

1. Structural studies of Sesquisabinene Synthase 1 (Sasqs1):

I began my Doctoral work with the mechanistic and structural studies of the enzyme Sasqs1 which is responsible for the formation of sesquisabinene, a terpene, in plants. This terpene is a major component of fragrant essential oil of sandalwood and thus is commercially very important. I studied the structural and functional aspects of this enzyme which shed light on the structure-mechanism relation of Sasqs1. Further, computational, and biochemical analysis of Sasqs1 have provided insights to the residues which might play role in the catalysis as it is an interesting fact that all the sesquiterpene synthases catalyze the same substrate to form different products. I used a novel approach of statistical coupling analysis to identify the product specificity determinants of Sasqs1. This approach combining sequence, structural and dynamical information of plant sesquiterpene synthases (SSQs) can be used to predict product modulating residues (PMRs) of the plant SSQs.

A. **Singh, S.**, Thulasiram, H. V., Sengupta, D., & Kulkarni, K. (2021). Dynamic coupling analysis on plant sesquiterpene synthases provides leads for the identification of product specificity determinants. *Biochemical and biophysical research communications*, *536*, 107–114. https://doi.org/10.1016/j.bbrc.2020.12.041.PMID: 33387748.

2. Structural basis of interaction of DOCK3-NEDD9:

My other doctoral project involved studies of family of proteins known as Dedicator of Cytokinesis (DOCK). DOCK include family of proteins that are atypical Guanine nucleotide Exchange Factors (GEFs) and regulate the activation of GTPases. This regulation by GEFs is involved in cell migration. Mutations in these GEFs were found to play role in the onset of metastasis and thus cancer. The objectives of the study included structural and interaction studies of two such GEFS, DOCK2 and DOCK3, with their interacting partners using cryo-EM. The major challenges of the aim were the huge molecular size of the complex (~600kDa) and post-translational modification that might play role in the interaction and downstream signaling. Several heterologous expression trials were done to obtain good quality protein for vitrification. However, further optimizations are required for the freezing of the sample as initial screening of the sample provided micrographs with very few particles which will not be sufficient to obtain good classes after data processing.

3. Molecular mechanism of PDE6 maturation:

Photoreceptor phosphodiesterase 6 (PDE6) is a key player in the visual excitation pathway and is crucial in both rod and cone photoreceptor cells. The maturation and activity of PDE6 is critically dependent on a chaperone complex of aryl hydrocarbon receptor-interacting protein-like 1 (AIPL1) and HSP90. Despite the crucial roles of these proteins in the visual transduction pathway and availability of biochemical data, there is a lack of understanding of the molecular mechanism of PDE6 maturation and the underlying mutations of PDE6 and AIPL1 that lead to onset of retinal problems. The recent work has provided insights to the interaction of the members of this chaperone complex, AIPL1 and HSP90. My current objective includes study of mechanism of PDE6 maturation and interaction of this client protein with the chaperone complex that will aid in understanding of the interaction interface of both and the mechanism of mutations that are responsible for retinal degeneration and visual defects. I have optimised the expression and purification of PDE6 with AIPL1 and performed initial screening for the cryo-EM studies of the samples. I am presently optimising the stability and vitrification conditions of the protein.

A. Srivastava, D., Yadav, R. P., **Singh, S**., Boyd, K., & Artemyev, N. O. (2023). Unique interface and dynamics of the complex of HSP90 with a specialized cochaperone AIPL1. *Structure (London, England : 1993)*, S0969-2126(22)00523-8. Advance online publication. https://doi.org/10.1016/j.str.2022.12.014. PMID: 36657440

B. **Singh S**, Srivastava D, Boyd K, Artemyev NO. (2024). Reconstitution of the phosphodiesterase 6 maturation process important for photoreceptor cell function. *J Biol Chem*. ;300(1):105576. https://doi.org/10.1016/j.jbc.2023.105576. Epub 2023 Dec 16. PMID: 38110033; PMCID: PMC10819763.

C. **Singh S**, Srivastava D, Boyd K, Artemyev NO. (2025). Structural and functional dynamics of human cone cGMP-phosphodiesterase important for photopic vision. *Proc Natl Acad Sci U S A*.;122(1):e2419732121. https://doi.org/10.1073/pnas.2419732121. Epub 2024 Dec 31. Erratum in: *Proc Natl Acad Sci U S A*. (2025) Apr 8;122(14):e2505043122. doi: 10.1073/pnas.2505043122. PMID: 39739818; PMCID: PMC11725853.

Complete list of published work:
My Bibliography - NCBI