BIOGRAPHICAL SKETCH

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NAME: Petrou, Vasileios I.

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

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date (MM/YYYY)	FIELD OF STUDY
Democritus University of Thrace, Alexandroupolis, Greece	Ptychion (B.S. equivalent)	07/2005	Molecular Biology and Genetics
Icahn School of Medicine at Mount Sinai, New York, USA	Ph.D.	09/2012	Neuroscience
Virginia Commonwealth University, Virginia, USA	Postdoctoral	04/2013	Physiology and Biophysics
Columbia University, New York, USA	Postdoctoral	06/2019	Structural Biology

A. Personal Statement

My research training has enabled me to develop a unique skillset, encompassing molecular biology, biochemistry, electrophysiology, X-ray crystallography and single particle cryo-electron microscopy (cryo-EM) for structural studies. During my postdoc, I trained in structural biology, and I was able to determine the structure of the bacterial enzyme ArnT in two conformations using X-ray crystallography (**1**). These structures were subsequently utilized for early-phase drug discovery (**2**). A K99/R00 award from NIGMS supported my specialization in cryo-EM for the study of small transmembrane enzymes in their close-to-native lipidic environment using lipidic nanodiscs as a membrane substitute.

In July 2019, I opened my laboratory in the Department of Microbiology, Biochemistry and Molecular Genetics at Rutgers New Jersey Medical School. The Petrou Lab is aiming to characterize the structure and function of membrane proteins using single particle cryo-EM and other techniques, with a focus on: i) bacterial membrane enzymes involved in antibiotic resistance, and ii) eukaryotic receptors and effectors relevant to mammalian physiology and pathology. It is my absolute pleasure and privilege to support the training of the next generation of molecular microbiologists that will use X-ray crystallography and cryo-EM to decipher biological processes relevant to antibiotic resistance and microbial pathogenesis; and molecular physiologists aiming to decipher the structural basis of synaptic physiology and intracellular signaling cascades involved in cancer. I am fully committed to building an inclusive scientific environment that will enable mentees to grow into accomplished scientists.

The first product of the Petrou lab, a collaborative project focusing on quorum-sensing proteins of Gram-positive bacteria, was published in 2020 (**3**). In this publication, together with X-ray crystallography data generated by the Neiditch Lab, we reported the structure of a small transcription factor (66kDa) in complex with its peptide activator determined by cryo-EM. The most recent publication I co-authored (**4**) is a product of collaboration with my postdoctoral lab, but I highlight it here because it showcases approaches we routinely utilize in my own lab.

The aminoarabinose pathway and polymyxin resistance continue to be a major focus of my lab, both in terms of understanding ArnT structure and function, and also targeting other enzymes of the aminoarabinose pathway. This program is supported by an NIGMS MIRA award since July 2023. The present application to NCCAT focuses again on ArnT and utilizing cryo-EM to begin understanding the metal-dependence of the enzyme and whether a metal is necessary to return to the apo conformation. We will be exploring this by solving EDTA-treated and metal-bound structures of the ArnT from C. metallidurans (which we know binds to zinc and other metals) in 1E3D1 nanodiscs.

1. **Petrou, V.I.**, Herrera, C.M., Schultz, K.M., Clarke, O.B., Vendome, J., Tomasek, D., Banerjee, S., Rajashankar, K.R., Belcher Dufrisne, M., Kloss, B., Kloppmann, E., Rost, B., Klug, C.S., Trent, M.S., Shapiro, L., Mancia, F. (2016). Structures of aminoarabinose transferase ArnT suggest a molecular basis for Lipid A glycosylation. *Science*, **351**(6273): 608-612. PMCID: PMC4963604.

2. Mancia F., **Petrou V.**, Clarke O.B., Vendome J.P. (inventors); The Trustees of Columbia University in the City of New York (assignee). Rational drug design targeting resistant Gram-negative bacterial infections to polymyxin-class antibiotics. United States patent US 10,925,857 B2. 2021 Feb 23.

3. Capodagli G.C., Tylor K.M., Kaelber J.T., **Petrou V.I.***, Federle M.J.*, Neiditch M.B.* (2020) Structure-function studies of Rgg binding to pheromones and target promoters reveal a model of transcription factor interplay PNAS, **117**(39): 24494-24502. doi: 10.1073/pnas.2008427117. PMCID: PMC7533842. [*co-corresponding authors]

4. Ashraf K.U., Nygaard R., Vickery O.N., Erramilli S.K., Herrera C.M., McConville T.H., **Petrou V.I.**, Giacometti S.I., Dufrisne M.B., Nosol. K., Zinkle A.P., Graham C.L.B., Loukeris M., Kloss B., Skorupinska-Tudek K., Swiezewska E., Roper D.I., Clarke O.B., Uhlemann A.C., Kossiakoff A.A., Trent M.S., Stansfeld P.J., Mancia F. (2022) Structural basis of lipopolysaccharide maturation by the O-antigen ligase. Nature, **604**(7905): 371–6. PMCID: PMC9884178.

Ongoing and recently completed projects:

R35 GM150831 (NIGMS)
Petrou, V.I. (PI)
07/10/2023 - 04/30/2028
Title: Structure and mechanism of membrane enzymes responsible for bacterial lipid modification and polymyxin resistance
Description: Polymyxins are cationic peptides that associate with the bacterial outer membrane of Gram-negative bacteria through electrostatic interactions and are considered a last line of defense against multi-drug resistant infections. Resistance to polymyxins is acquired through enzymatic modifications of Lipid A. Addition of an aminoarabinose sugar to Lipid A phosphates is one such modification, which is produced by the aminoarabinose pathway. The core goals of this research program are: (1) Structure determination and substrate-binding characterization for the three bona fide membrane enzymes that operate in the aminoarabinose biosynthetic pathway (the polyprenol phosphate glycosyltransferase ArnC, the deformylase ArnD and the lipid-to-lipid glycosyltransferase ArnT), and (2) Investigating the mechanistic basis of enzymatic function, metal cofactor coordination, and catalysis, in each of the three membrane enzymes under study.

R03 AG081787 (NIA)
Petrou, V.I. (PI)
04/01/2023 - 03/31/2025
Title: Structural characterization of APP family proteins
Description: The amyloid precursor protein (APP) is a type I transmembrane glycoprotein that plays a central role in the pathogenesis of Alzheimer's disease. As part of this project, we will establish an expression and purification pipeline for medium throughput screening of APP, APLP1 and APLP2 orthologs, investigate an alternative tagging procedure and compare extraction and reconstitution methods for a subset of orthologs. Finally, we will utilize single particle cryo-electron microscopy (cryo-EM) to elucidate atomic-level details of the molecular architecture of APP family proteins.

R01 CA285713 (NCI)
Poulikakos, P.I. (PI), Petrou, V.I. (Co-I)
12/22/2023 – 11/30/2028
Title: Mechanisms determining tumor-selective potency of RAS pathway inhibitors
Description: The RAS/RAF/MEK/ERK (RAS/MAPK) signaling pathway is an established driver of many cancers, commonly hyperactivated by genetic alterations in RAS or BRAF. The proposed research will develop experimental tools and concepts that delineate mechanisms determining differential therapeutic potency of RAFi and MEKi in Ras mutant tumors over normal cellular contexts. The Petrou lab will be responsible for performing heterologous protein expression, purification, and biochemical characterization of target proteins belonging to the RAS/MAPK signaling complex, and will be performing biophysical studies using microscale thermophoresis (MST) to characterize the response of the RAS/MAPK signaling complex to

pharmacologic inhibition.

K99/R00 GM123228 (NIGMS)
Petrou, V.I. (PI)
07/1/2017 - 06/30/2023 (Completed)
Title: Structural basis of aminoarabinose biosynthesis linked to polymyxin resistance
Description: The K99 phase of the project focused on investigating substrate binding in the ArnT enzyme by utilizing cryo-EM, X-ray crystallography and other techniques. A significant training component in cryo-EM was included. The R00 phase focused on complete structural characterization of substrate binding in the enzyme ArnT, and structure determination of other transmembrane enzymes participating in the aminoarabinose biosynthetic pathway, utilizing cryo-electron microscopy.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

07/2019 - present	Assistant Professor and Chancellor Scholar, Department of Microbiology, Biochemistry and Molecular Genetics, Rutgers University-New Jersey Medical School, Newark, NJ
07/2017 - 06/2019	Associate Research Scientist, Department of Physiology and Cellular Biophysics, Columbia University, New York, NY
05/2013 - 06/2017	Postdoctoral Research Scientist, Department of Physiology and Cellular Biophysics, Columbia University, New York, NY
08/2012 - 04/2013	Postdoctoral Fellow, Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond, VA
08/2008 - 07/2012	Visiting Ph.D. student, Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond, VA
08/2005 - 07/2012	Ph.D. student, Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, NY

Other Experience and Professional Memberships

2022-	Review Editor, Frontiers in Physiology, Membrane Physiology and Membrane Biophysics specialty section
2022	Ad-hoc Reviewer, Nature Chemical Biology, Nature Communications
2022	Member, Society for General Physiologists
2021	Ad-hoc Early Career Reviewer (NIH-CSR, BBM 2022/01)
2021	Ad-hoc Reviewer, Structure
2020	Ad-hoc Reviewer, Science
2019	Ad-hoc Reviewer, Journal of Molecular Biology
2018	Ad-hoc Reviewer, BBA - General Subjects, Journal of Structural Biology, ACS Chemical Biology
2017	Ad-hoc Reviewer, Nature Communications, PLOS Pathogens, BBA - General Subjects
2017-	Member, American Association for the Advancement of Science (AAAS)
2006-	Member, Biophysical Society
2005-	Member, New York Academy of Sciences

Academic and Professional Honors

2018	Regeneron Prize for Creative Innovation (Finalist)
2017-2023	NIH NIGMS K99/R00 Pathway to Independence Award
2005	B.S. awarded with honors, Democritus University of Thrace, Alexandroupolis, Greece
2001	Academic merit award, State Scholarship Foundation of Greece (I.K.Y.)

C. Contributions to Science

(i) Early career. During my graduate career, I was involved in the study of ion channel regulation by phosphoinositides, a class of minority polar lipids, and other membrane lipids (i.e. cholesterol). Phosphatidylinositol-4,5-bisphosphate (PIP_2), one of the more abundant plasma membrane phosphoinositides, has emerged as a master regulator of the activity of most ion channel classes, and a point where many regulatory signals converge to adjust the activity of ion channels. In the Logothetis lab, I contributed experimentally to studies examining the regulation of NMDA receptor channels by the phosphoinositide PIP_2 through interactions with the membrane-associated protein alpha-actinin (*J. Neurosci.*, co-author), and the intersection of regulation of inwardly rectifying potassium (Kir) channels by PIP_2 and cholesterol (*J. Biol. Chem.*, co-author). I also contributed to two state-of-the-field review articles, meant to present up-to-date information of phosphoinositide regulation of ion channels. The first examined the link between deregulation of phosphoinositide control of ion channels and potential for disease (Pflugers Arch., second author). The second, in Annual Review of Physiology, provided an up-to-date overview of phosphoinositide regulation of ion channels and how that can be extended in mechanistic terms to explain regulation of membrane proteins (in more general terms) by phosphoinositides (Annual Rev. Physiol., second author).

1. Logothetis D.E., **Petrou V.I.**, Zhang M., Mahajan R., Meng X.-Y., Adney S.K., Cui M., Baki L. (2015). Phosphoinositide control of membrane protein function: a frontier led by studies on ion channels. *Annu. Rev. Physiol.* **77**: 81–104. PMCID: PMC4485992.

2. Rosenhouse-Dantsker, A., Noskov, S., Han, H., Adney, S.K., Tang, Q.-Y., Rodríguez-Menchaca, A.A., Kowalsky, G.B., **Petrou, V.I.**, Osborn, C.V., Logothetis, D.E., Levitan, I. (2012). Distant cytosolic residues mediate a two-way molecular switch that controls the modulation of inwardly rectifying potassium (Kir) channels by cholesterol and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2). *J. Biol. Chem.* **287**(48): 40266-40278. PMCID: PMC3504743.

3. Logothetis D.E., **Petrou V.I.**, Adney S.K., Mahajan R. (2010) Channelopathies linked to plasma membrane phosphoinositides. *Pflugers Arch.* **460**(2): 321-341. PMCID: PMC4040125.

4. Michailidis I.E., Helton T.D., **Petrou V.I.**, Mirshahi T., Ehlers M.D., Logothetis D.E. (2007) Phosphatidyl inositol-4,5-bisphosphate regulates NMDA receptor activity through alpha-actinin. *J. Neurosci.* **27**(20): 5523-5532. PMCID: PMC6672336.

(ii) Regulation of delta 2 glutamate receptor. My dissertation project involved the study of an atypical ionotropic glutamate receptor, the δ2 glutamate receptor (GluD2), considered an orphan receptor by some since it remains controversial whether it can be gated. GluD2 is highly expressed in the parallel fiber-Purkinje cell (PF-PC) synapse and its role in cerebellar physiology is increasingly appreciated. I used a single point mutant of GluD2 (lurcher mutation) that renders GluD2 constitutively active to examine the regulation of the receptor by phosphoinositides using electro-physiological techniques. I also adapted a chemiluminescence-based assay for use in 96-well trays that allowed me to quantify the surface population of the GluD2 receptor in single *Xenopus laevis* oocytes. I showed that manipulations of membrane phosphoinositide levels evoke changes in the cell surface localization of both wild-type and mutant receptors. Moreover, I showed that changes in PIP_2 and PIP_3 levels result in antagonistic actions towards the size of GluD2 membrane population, thus, uncovering a dual-regulation scheme controlling the surface localization of GluD2 through the cellular levels of PIP_2 and PIP_3.

1. **Petrou V.I.** (2012) Phosphoinositides regulate the surface localization of the delta 2 ionotropic glutamate receptor (Doctoral dissertation). Icahn School of Medicine at Mount Sinai. Available from ProQuest Dissertations & Theses Global (1285517826).

2. **Petrou V.I.**, Logothetis D.E. (2012) Phosphoinositide signaling regulates the surface localization of the δ2 ionotropic glutamate receptor. Poster presentation, 56th Biophysical Society Annual Meeting. *Biophys. J.* **102**(3) Supplement 1: p. 115a, 580-Pos. San Diego, CA, February 2012.

3. **Petrou V.I.**, Logothetis D.E. (2011) The lurcher mutant of δ2 ionotropic glutamate receptor is regulated by phosphoinositides. Poster presentation, 55th Biophysical Society Annual Meeting. *Biophys. J.* **100**(3) Supplement 1: p. 268a, 1460-Pos. Baltimore, MD, March 2011.

(iii) Structure and function of the aminoarabinose transferase ArnT. My postdoctoral project shifted my research focus more towards membrane enzymes, though retaining a theme of protein-lipid interactions, as it involves study of an integral lipid-to-lipid glycosyltransferase, an enzyme that accommodates two lipidic

substrates. ArnT (4-amino-4-deoxy-L-arabinose transferase) is located in the inner membrane of Gram-negative bacteria and catalyzes the transfer of a modified arabinose moiety from an undecaprenyl phosphate donor to lipid A, the major lipidic component of bacterial lipopolysaccharide (LPS). The modification of lipid A by aminoarabinose causes a charge modification of the bacterial outer membrane and enables bacteria to develop resistance to polymyxin-class antibiotics and natural antimicrobial peptides. I determined the structure of ArnT from *Cupriavidus metallidurans*, a Gram-negative bacterium, in the apo conformation and in complex with the lipid carrier undecaprenyl phosphate, at 2.8 and 3.2Å resolution, respectively. I identified cavities that seem suitable to accommodate its lipidic substrates and observed a significant coil-to-helix structural transition upon binding of undecaprenyl phosphate that seems to stabilize the carrier lipid near the active site. Using mutagenesis experiments and a polymyxin growth assay, I was able to identify critical residues for the function of the protein that were grouped based on their potential to participate in substrate-binding or catalysis and proposed a model for catalysis by ArnT family enzymes. I am currently utilizing single-particle cryo-EM to provide a complete characterization of substrate binding in ArnT by incorporating the protein into lipid-filled nanodiscs.

1. **Petrou, V. I.,** Ashraf, K.U. Mancia, F. (2022) Structural basis of Lipid A modification by the aminoarabinose transferase ArnT linked to polymyxin resistance. Oral presentation, Biophysical Society Thematic Meeting, Physical and Quantitative Approaches to Overcome Antibiotic Resistance, Stockholm, Sweden, August 2022.

2. Dufrisne, M. B., **Petrou, V. I.**, Clarke, O. B. & Mancia, F. (2017) Structural basis for catalysis at the membrane-water interface. *Biochim Biophys Acta BBA - Mol Cell Biol Lipids* **1862**: 1368-1385. PMCID: PMC5449265.

3. Mancia, F., **Petrou, V.**, Clarke, O.B., Vendome, J.P. (inventors); The Trustees of Columbia University in the City of New York (applicant). Rational drug design targeting resistant Gram-negative bacterial infections to polymyxin-class antibiotics. United States patent US 10,925,857 B2. 2021 Feb 23.

4. **Petrou, V.I.**, Herrera, C.M., Schultz, K.M., Clarke, O.B., Vendome, J., Tomasek, D., Banerjee, S., Rajashankar, K.R., Belcher Dufrisne, M., Kloss, B., Kloppmann, E., Rost, B., Klug, C.S., Trent, M.S., Shapiro, L., Mancia, F. (2016). Structures of aminoarabinose transferase ArnT suggest a molecular basis for Lipid A glycosylation. *Science*, **351**(6273): 608-612. PMCID: PMC4963604.

(iv) Structure of the peptide-bound state of transcription factor Rgg3. The first published work from my laboratory reports the structure of a complex between the transcription factor Rgg3 and its cognate ligand, the peptide pheromone SHP3. Rgg proteins are quorum-sensing receptors in *Streptococcus* species that regulate virulence, antibiotic resistance and competence. Rgg3 is capable of crystallization either alone or in complex with its DNA box, but it proved impervious to crystallization when its SHP3 peptide ligand is added to the mix. In order to solve the structure of the complex we utilized single particle cryo-EM, and we managed to obtain a reconstruction of the Rgg3-SHP3 complex at 3Å resolution. The structure of the complex unveiled a significant conformational transition taking place upon binding of the SHP3 ligand that may explain the inability of the complex to form crystal contacts in the bound state. Moreover, the Rgg3-SHP3 complex structure explained the mode of action of cyclosporin A (CsA), a previously identified inhibitor of Rgg function, by showing that SHP3 binds to the same groove that CsA has been shown to bind on Rgg proteins, thus proving that CsA acts as a competitive inhibitor of SHP-triggered Rgg function. This cryo-EM structure constitutes a technical achievement as the total size of the Rgg3-SHP3 complex is 66kDa, close to the size limit of the current cryo-EM instrumentation.

1. Capodagli G.C., Tylor K.M., Kaelber J.T., **Petrou V.I.***, Federle M.J.*, Neiditch M.B.* (2020) Structure-function studies of Rgg binding to pheromones and target promoters reveal a model of transcription factor interplay PNAS, **117**(39): 24494-24502. doi: 10.1073/pnas.2008427117. PMCID: PMC7533842. [*co-corresponding authors]

Complete List of Published Work:
https://www.ncbi.nlm.nih.gov/myncbi/vasileios.petrou.1/bibliography/public/