BIOGRAPHICAL SKETCH

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NAME: Vasileios I. Petrou

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 (cryoEM) for structural studies. During my graduate training I studied an atypical glutamate receptor (GluD2) and its regulation by Gq-coupled receptors and membrane phosphoinositide levels (**1**). Later, 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 (**2**). These structures were subsequently utilized for early-phase drug discovery (**3**). A K99/R00 award from NIGMS supported my specialization in cryoEM 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 lab is aiming to characterize the structure and function of membrane proteins using single particle cryoEM and other techniques, with a focus on: i) bacterial membrane enzymes involved in antibiotic resistance, and ii) eukaryotic receptors relevant to mammalian physiology and pathology. In addition, 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 cryoEM to decipher biological processes relevant to antibiotic resistance and microbial pathogenesis. I am fully committed in 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, which reported the structure of a small transcription factor in complex with its peptide activator determined by cryoEM, has now been published (**4**). In addition, the aminoarabinose pathway and polymyxin resistance will continue to be a major focus of the lab, both in terms of understanding ArnT structure and function, which continues as a collaborative effort between the Petrou and Mancia Labs, and also targeting other enzymes of the aminoarabinose pathway. The present application to NCCAT focuses on ArnT and utilizing cryoEM to map substrate binding and catalytic mechanisms in the ArnT enzyme family.

Ongoing and recently completed projects that I would like to highlight:

R00 GM123228
Petrou, V.I. (PI)
09/13/2019 - 08/31/2022
Title: Structural Basis of Aminoarabinose Biosynthesis Linked to Polymyxin Resistance
Description: The R00 phase will focus 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.

K99 GM123228
Petrou, V.I. (PI)
07/01/2017 - 06/30/2019
Title: Structural Basis of Aminoarabinose Biosynthesis Linked to Polymyxin Resistance
Description: The goal of this proposal is to investigate substrate binding in the ArnT enzyme by utilizing cryoEM, X-ray crystallography and other techniques. A significant training component in cryoEM is included.

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.**, 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.

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. Patent application PCT/US2016/61906. 2016 Nov 14.

4. 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]

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

2021	Ad-hoc Reviewer, Structure

2020 Ad-hoc Reviewer, Science
2019- Ad-hoc Reviewer, Journal of Molecular Biology
2018 Ad-hoc Reviewer, Biochimica et Biophysica Acta (BBA) - General Subjects, Journal of Structural Biology, ACS Chemical Biology
2017 Ad-hoc Reviewer, Nature Communications, PLOS Pathogens, Biochimica et Biophysica Acta (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- 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.

4. **Petrou V.I.**, Logothetis D.E. (2009) A mutant δ2 ionotropic glutamate receptor exhibits dual regulation by phosphoinositides. Poster presentation, 53rd Biophysical Society Annual Meeting. *Biophys. J.* **96**(3) Supplement 1: p. 489a, 2521-Pos. Boston, MA, March 2009.

(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 cryoEM to provide a complete characterization of substrate binding in ArnT by incorporating the protein into lipid-filled nanodiscs.

1. **Petrou, V. I.,** Mancia, F. (2018) Structural and biochemical studies of the aminoarabinose transferase ArnT linked to polymyxin resistance. Poster presentation, 62nd Biophysical Society Annual Meeting. L3799-Pos. San Francisco, CA, February 2018.

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. Patent application PCT/US2016/61906. 2016 Nov 14.

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.

5. **Petrou, V.I.**, Herrera, C.M., Schultz, K.M., Clarke, O.B., Vendome, J., Tomasek, D., Banerjee, S., Rajashankar, K.R., Kloss, B., Kloppmann, E., Rost, B., Klug, C.S., Trent, M.S., Shapiro, L., Mancia, F. (2016). ArnT: Structure and mechanism of the aminoarabinose transferase responsible for resistance to

polymyxin-class antibiotics. Oral presentation, 60th Biophysical Society Annual Meeting. *Biophys. J.* **110**(3) Supplement 1: p. 38a, 205-Plat. Los Angeles, CA, February 2016.

(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 exactly 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 cryoEM structure constitutes a technical achievement as the total size of the Rgg3-SHP3 complex is 66kDa, close to the size limit of the current cryoEM 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/sites/myncbi/1Tiost6ux7k5H/bibliography/45398425/public/