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 and X-ray crystallography and single-particle cryo-electron microscopy (cryoEM) for structural studies. My dissertation project involved the study of an atypical glutamate receptor (GluD2) and its regulation by Gq-coupled receptors (i.e. mGluR1) and membrane phosphoinositide levels (**1**). During my postdoc I was able to determine the structure of the bacterial enzyme ArnT in two conformations using X-ray crystallography (**2**). ArnT is responsible for modifying Lipid A to enable resistance to polymyxin antibiotics. These structures were subsequently utilized for early-phase drug discovery (**3**). A K99/R00 award from NIGMS has 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. The first product of the Petrou lab, a collaborative project focusing on quorum-sensing proteins of Gram-positive bacteria, reporting the solution of a small transcription factor solved by cryoEM in complex with its peptide activator, has now been published (**4**).

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. In addition to the rigorous curriculum that all graduate students at NJMS follow, students supported in my lab will be instructed in state-of-the-art methodologies for experimental design, data interpretation, and data reporting relevant to the field of structural biology. Moreover, I am committed in supporting the career development of mentees under my supervision, through both formal measures (i.e. IDP, support for workshop participation, etc.) and informal discussions, to help them identify and transition into biomedical careers that utilize their skills and expertise to further our collective knowledge and advance treatments for human diseases.

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 [published online ahead of print, 2020 Sep 9]. PNAS. doi:10.1073/pnas.2008427117 [*co-corresponding authors]

B. Positions and Honors

Positions and Employment

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

Other Experience and Professional Memberships

2005-	Member, New York Academy of Sciences
2006-	Member, Biophysical Society
2007	Teaching Assistant, Icahn School of Medicine at Mount Sinai, Cellular and Molecular Neurobiology (G-351)
2017-	Member, American Association for the Advancement of Science (AAAS)
2017	Ad-hoc Reviewer, Nature Communications, PLOS Pathogens, Biochimica et Biophysica Acta (BBA) - General Subjects
2018	Ad-hoc Reviewer, Biochimica et Biophysica Acta (BBA) - General Subjects, Journal of Structural Biology, ACS Chemical Biology
2019-	Ad-hoc Reviewer, Journal of Molecular Biology
2020	Ad-hoc Reviewer, Science

Academic and Professional Honors

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

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.

Complete List of Published Work: [My Bibliography]

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

Current Research Support

R00 GM123228 (Petrou, V.I.) 09/13/2019 - 08/31/2022
NIH/NIGMS $492,018 (DC)
Role: PI
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.

Pending Research Support

R03 TR003666 (Petrou, V.I.) 02/01/2021 - 01/31/2022
NIH/NCATS $100,000 (DC)
Role: PI
Title: Structural studies of the GRID1-encoded orphan glutamate receptor GluD1
Description: The goal of the proposal is to determine the structure of the orphan glutamate receptor GluD1 by cryoEM and investigate the structural correlates that enable delta receptors to behave differently compared to other members of the ionotropic glutamate receptor family. Understanding how delta glutamate receptors operate at a molecular level may facilitate our understanding of associated neurological diseases.

Completed Research Support

K99 GM123228 (Petrou, V.I.) 07/01/2017 - 06/30/2019
NIH/NIGMS $166,666 (DC)
Role: PI
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.

2017 Interdisciplinary Research Initiatives Seed (IRIS) Fund Program
(Uhlemann, A.C./Mancia F.) 07/01/2017 - 06/30/2018
Columbia University $100,000 (DC)
Role: Co-I
Title: Combating resistance to last resort antibiotics through combined genomic and structure-guided approaches
Description: The aims of the project involve genomic characterization of polymyxin resistant clinical isolates, validation of potential ArnT inhibitors and co-crystallization of ArnT/drug candidates, with the goal of synthesizing this information to refine structure guided drug design approaches based on the ArnT structures.