BIOGRAPHICAL SKETCH

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NAME: Vasileios I. Petrou

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

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
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	-	Structural Biology

A. Personal Statement

My research training from undergraduate trainee to postdoctoral researcher has enabled me to develop a unique skillset, encompassing molecular biology, biochemistry, electrophysiology and x-ray crystallography. In the past few years, I have been expanding this skillset to incorporate single-particle cryo-electron microscopy (cryo-EM) for structural studies. A recent K99 award from NIGMS has enabled me to focus on cryo-EM for the study of small transmembrane enzymes in their close-to-native lipidic environment, using lipidic nanodiscs as a membrane substitute.

Shortly after joining the lab of Dr. Filippo Mancia at Columbia University, I started studying the bacterial enzyme ArnT, which catalyzes transfer of an aminoarabinose sugar from a carrier lipidic donor to Lipid A, the major lipid of the outer membrane of Gram-negative bacteria. Modification of Lipid A by ArnT leads to the development of resistance to polymyxins, a class of last resort antibiotics. I was previously able to determine the structure of ArnT in two conformations using X-ray crystallography (*Science*, first author), which was subsequently utilized for early-phase drug discovery (patent application). My current research revolves around further characterization of ArnT structure and function with an emphasis on the elucidation of substrate binding events that enable catalysis in the ArnT family of enzymes by cryo-EM.

In the summer of 2019, I will be starting my lab in the Department of Microbiology, Biochemistry and Molecular Genetics at Rutgers-New Jersey Medical School. The research focus of my lab will be on the structural biology of membrane proteins, particularly using single particle cryo-EM and other techniques. The lab will aim to characterize the structure and function of bacterial membrane enzymes involved in antibiotic resistance, and eukaryotic receptors and enzymes relevant to human physiology and pathology.

The proposed study will be the first collaborative project between my lab and the labs of Dr. Matthew Neiditch and Dr. David Dubnau. The target of this project is a small soluble complex of bacterial proteins with high biological significance. We are proposing to use single-particle cryo-EM to study the complete complex, since other structural biology techniques have not yet been successful in solving the structure of the complex. In this project, I will be contributing expertise in utilizing cryo-EM for the study of small enzyme proteins based on my current work on the structure determination of ArnT using cryo-EM.

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. 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.

3. **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.

4. **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.

B. Positions and Honors

Positions and Employment

01/2005-07/2005	Research Assistant, Department of Surgery, Section of Neuropathology, Yale School of Medicine, New Haven, CT (PI: Laura Manuelidis)
08/2008-07/2012	Visiting Ph.D. student, Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond, VA (PI: Diomedes Logothetis)
08/2012-04/2013	Postdoctoral Fellow, Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond, VA (PI: Diomedes Logothetis)
05/2013-06/2017	Postdoctoral Research Scientist, Department of Physiology and Cellular Biophysics, Columbia University, New York, NY (PI: Filippo Mancia)
07/2017-	Associate Research Scientist, Department of Physiology and Cellular Biophysics, Columbia University, New York, NY (PI: Filippo Mancia)

Other Experiences 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

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**) 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. PMID: 17507574.

(**ii**) 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 electrophysiological 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. A manuscript for peer-reviewed publication of this work is under preparation.

1. **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.

2. **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.

3. **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**) 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.,** 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.

6. **Petrou V.I.**, Clarke O.B., Schultz K.M., Tomasek D., Kloss B., Banerjee S., Rajashankar K.R., Klug C.S., Shapiro L., Mancia F. (2015). Crystal structure of the bacterial aminoarabinose transferase ArnT. Poster presentation, 59th Biophysical Society Annual Meeting. Biophys. J. **108**(2) Supplement 1: p. 253a, 1280-Pos. Baltimore, MD, February 2015.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/1Tiost6ux7k5H/bibliography/45398425/public/?sort=date&direction=descending

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

Ongoing Research Support

K99 GM123228 (Petrou, V.I.) 07/01/2017 - 06/30/2019
NIH/NIGMS
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 Cryo-EM, X-ray crystallography and Electron (EPR). A significant training component in Cryo-EM and EPR techniques is included.

Completed Research Support

2017 Interdisciplinary Research Initiatives Seed (IRIS)
Fund Program (Uhlemann, A.C./Mancia F.) 07/01/2017 - 06/30/2018
Columbia University
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.

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: Matthew B. Neiditch

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

POSITION TITLE: ASSOCIATE PROFESSOR

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
Rutgers University, NJ	B.S.	05/1996	Biology
Baylor College of Medicine, TX	Ph.D.	09/2002	Microbiology and Immunology
Princeton University, NJ	Postdoc.	08/2007	Structural Biology, Biochemistry, and Microbiology

A. Personal Statement

My lab uses diverse methods including biochemical, genetic, computational, and biophysical (mainly X-ray crystallographic) techniques to study fundamentally important and broadly conserved cellular processes in bacteria. The basic science research in my lab encompasses structure-function studies of the following areas: genetic competence, oligopeptide-mediated bacterial cell-cell communication, c-di-GMP second messenger signal transduction, phosphorelay signal transduction, phosphatases, and transcriptional anti-activators, among others. Our translational work focuses on developing inhibitors of *Mycobacterium tuberculosis* mycolic acid biosynthesis and respiration, as well as broad-spectrum inhibitors of bacterial biofilm growth and cell-cell communication. We have extensive experience determining X-ray crystal structures of proteins bound to small molecules, peptides, proteins, and, more recently, DNA. We employ X-ray crystallography as a first-pass technique because of the spectacular mechanistic insight that can be revealed from crystal structures of proteins and, in particular, protein complexes. Based on the information gleaned from our structural studies, we generate testable models of protein function. These models are then rigorously evaluated using *in vitro* biochemical and *in vivo* genetic approaches. I am well-qualified to carry out the proposed studies not only because my lab has determined numerous crystal structures of *Bacillus subtilis* proteins and protein-peptide complexes that regulate genetic competence and sporulation, but also based on my graduate training in microbiology and immunology, my postdoctoral training in the structural biology of bacterial quorum-sensing signal transduction (performed at Princeton University in Fred Hughson's lab and in close collaboration with Bonnie Bassler's lab), and, most importantly, my lab's existing track record studying mechanistic aspects of bacterial structure and function at the atomic level.

B. Positions and Honors

Positions and Employment

1997-2002 Graduate Student, Baylor College of Medicine Department of Microbiology and Immunology - Howard Hughes Medical Institute
David, B. Roth, M.D., Ph.D., (advisor)
Research: Molecular mechanism of V(D)J recombination

2002-2007 Postdoctoral Fellow, Princeton University Department of Molecular Biology
Frederick M. Hughson, Ph.D. (advisor)
Research: Structural biology of bacterial quorum-sensing signal transduction

2007-2013* Assistant Professor of Microbiology and Molecular Genetics, UMDNJ - New Jersey Medical School
2013-2013* Associate Professor of Microbiology and Molecular Genetics, UMDNJ - New Jersey Medical School
2013- Associate Professor of Microbiology, Biochemistry, and Molecular Genetics, Rutgers University - New Jersey Medical School (Rutgers Biomedical and Health Sciences)

*UMDNJ merged with Rutgers University on July 01, 2013 to form Rutgers Biomedical and Health Sciences

Other Experience and Professional Memberships

1998-2000 Member, Baylor College of Medicine Microbiology and Immunology Curriculum Committee
2004 Volunteer, Princeton University/HHMI Molecular Biology Outreach Program
2004 Ad-hoc Reviewer Molecular Microbiology
2004 Representative, Princeton University Department of Molecular Biology Advisory Committee
2004- Member, American Society for Microbiology
2005 Lecturer, Princeton University, Cellular Biochemistry (MOL504)
2006 Lecturer, Princeton University, Cellular Biochemistry (MOL504)
2006 Ad-hoc Reviewer, Molecular Microbiology
2006 Volunteer, Princeton University/HHMI Molecular Biology Outreach Program
2007 Ad-hoc Reviewer, Molecular Microbiology
2008 Ad-hoc Reviewer, Molecular Microbiology
2009 Ad-hoc Reviewer, Molecular Microbiology, Biochemistry, Structure, PNAS
2009 Reviewer, NIH Study Section Biological Chemistry and Macromolecular Biophysics (BCMB) Competitive Revisions A- Special Emphasis Panel
2010 Reviewer, UMDNJ Foundation Grants
2010 Reviewer, NIH Study Section Biological Chemistry and Macromolecular Biophysics (BCMB) ZRG1 BCMC-B 02, Members Conflicts in Biological Chemistry and Macromolecular Biophysics Panel
2010 Ad-hoc Reviewer, Science, Acta Crystallographica Section F
2011 Ad-hoc Reviewer, CUNY Collaborative Incentive Research Grants (CIRG)
2011 Ad-hoc Reviewer, National Science Foundation, Division of Molecular and Cellular Biosciences
2011- Class Advisor, Infection, Inflammation, and Immunity Ph.D. Graduate Program
2011- Member, NJMS Laboratory Safety Committee
2011 Ad-hoc Reviewer, Journal of Bacteriology, PLOS One
2012 Ad-hoc Reviewer, Journal of Bacteriology, Biochemistry, Protein Science
2013 Ad-hoc Reviewer, PLOS Biology, Nature, PNAS
2013 Ad-hoc Reviewer, NIH/NIGMS Center of Biomedical Research Excellence (COBRE) Awards
2014 Reviewer, French National Research Agency (ANR)
2014 Rutgers Strategic Planning Working Group - Drug Discovery, Development, and Pharmacoepidemiology
2014 Ad-hoc Reviewer, Structure, PLOS Biology
2015 Reviewer, French National Research Agency (ANR), Major Societal Challenges Proposals
2015 Reviewer, French National Research Agency (ANR), All-Knowledge Challenge Proposals
2015 Ad-hoc Reviewer, Cell, PLOS Pathogens, Molecular Microbiology
2016 Ad-hoc Reviewer, Cell, PLOS Pathogens
2017 Ad-hoc Reviewer, eLife
2018 Ad-hoc Reviewer, eLife, PNAS, PLOS Biology, PLOS ONE, Journal of Medicinal Chemistry
2018 Editorial Board Member, PLOS ONE, Review Editor of the Editorial Board Molecular Bacterial Pathogenesis, Frontiers in Cellular and Infection Microbiology
2019 Ad-hoc Reviewer, PLOS Biology

Honors

1992-1996 Johnson & Johnson National Merit Scholar
2005-2007 Ruth L. Kirschstein National Research Service Award (F32)
2008, 2013 Honors Thesis Examiner, Swarthmore College Department of Chemistry and Biochemistry
2019 New Jersey Health Foundation Excellence in Research Award

C. Contribution to Science

1. V(D)J Recombination. My early publications focused on mechanistic aspects of antigen receptor generation. The immune system of jawed vertebrates can recognize billions of different antigens, but rather than having an intact gene for each antigen receptor, antigen receptors are created by a combinatorial DNA rearrangement mechanism termed V(D)J recombination. The V(D)J recombinase, a complex formed by the recombination activating gene (RAG) proteins, introduces double strand breaks at the borders of chromosomal V(D)J recombination signal sequences and antigen receptor coding gene segments. DNA repair enzymes ligate the ends containing the coding gene segments together creating intact antigen receptor genes. Similarly, the ends of the excised chromosomal DNA, which contain the recombination signal sequences, are joined together intramolecularly creating a circular DNA element. The joint formed from the ligation of two DNA ends encoding recombination signal sequences is called a signal joint, and it had been widely accepted as dogma that signal joints convert reactive broken DNA ends into safe, inert products. The most important contribution from my dissertation was my discovery that signal joints are, in fact, not at all inert; they are cleaved efficiently *in vivo* and *in vitro* by a novel RAG-mediated nick-nick mechanism and form an excellent substrate for RAG-mediated transposition, the integration of linear DNA into target DNA. These findings, and the data from follow-up studies that resulted from the discovery of signal joint cleavage, suggest that rather than safeguarding our genome, signal joints may actually pose a serious threat to genomic stability in lymphocytes.

a. Neiditch, M.B., Lee, G.S., Landree, M.A., and Roth, D.B. (2001). RAG transposase can capture and commit to target DNA before or after donor cleavage. **Mol Cell Biol** *21*, 4302-4310. PMID:11390658.
b. Neiditch, M.B., Lee, G.S., Huye, L.E., Brandt, V.L., and Roth, D.B. (2002). The V(D)J recombinase efficiently cleaves and transposes signal joints. **Mol Cell** *9*, 871-878. PMID:11983177.
c. Lee, G.S., Neiditch, M.B., Sinden, R.R., and Roth, D.B. (2002). Targeted transposition by the V(D)J recombinase. **Mol Cell Biol** *22*, 2068-2077. PMID:11884595.
d. Lee, G.S., Neiditch, M.B., Salus, S.S., and Roth, D.B. (2004). RAG proteins shepherd double-strand breaks to a specific pathway, suppressing error-prone repair, but RAG nicking initiates homologous recombination. **Cell** *117*, 171-184. PMID:15084256.

2. Structure-function studies of autoinducer-2 (AI-2)- and diffusible signal factor (DSF)-mediated cell-cell communication in Gram-negative bacteria. The focus of my postdoctoral work was X-ray crystallographic and biochemical studies of bacterial quorum-sensing signal transduction. Quorum sensing is a cell-cell communication process mediated by secreted molecules called autoinducers. Bacteria respond to autoinducers by synchronizing the gene expression of the community, allowing groups of bacteria to act in unison. A quorum-sensing signal called autoinducer-2 (AI-2) is unusual both in its chemical structure (a furanosyl borate diester) and in that it is produced and detected by many different species of Gram-negative and Gram-positive bacteria, allowing inter-species cell-cell communication. AI-2 signaling has been most extensively studied in *Vibrio harveyi*, a bioluminescent marine bacterium that controls light production, as well as other behaviors, through quorum sensing. The *V. harveyi* AI-2 receptor is composed of two polypeptides, LuxP and LuxQ. LuxP is a periplasmic protein that binds AI-2 by clamping it between two domains, whereas LuxQ is an integral membrane protein of the two-component sensor kinase family whose cytoplasmic activities (kinase and phosphatase) are regulated by its interactions with LuxP in the periplasm. The most important work I performed as a postdoc was the determination of X-ray crystal structures of LuxP-LuxQ receptor complexes with and without bound AI-2. By comparing these structures, which represent the first structures of a bacterial periplasmic protein in complex with a membrane protein, and carrying out complementary functional analysis, I discovered how ligand binding by a periplasmic protein is coupled to two-component signal transduction across the bacterial membrane. This work provides the first evidence in favor of a ligand-induced asymmetry model for receptor regulation that is proving to have general significance for bacterial signal transduction.

More recently, my lab has focused on the mechanistic basis of DSF signaling. DSFs are fatty acids, containing a signature *cis*-2 double bond critical for their activity. We have carried out the first structure–function studies of DSF binding to a receptor, RpfR. We determined X-ray crystal structures of the RpfR DSF-binding domain in complex with an active DSF, *cis*-2-dodecenoic acid, and in complex with its inactive saturated isomer, dodecanoic acid (C12:0). These X-ray crystal structures, together with extensive complementary *in vivo* and *in vitro* functional studies, revealed the molecular basis of DSF recognition and the importance of the *cis*-2 double bond to DSF function. In addition, we have discovered a previously overlooked domain at the RpfR N-terminus, and we showed that this domain binds to and negatively regulates the DSF synthase RpfF. We solsved X-ray

crystal structures of this domain alone and in complex with RpfF, which—together with in vivo and in vitro functional studies—showed how RpfR binds to RpfF, negatively regulating the synthesis of its own AI.

a. Neiditch, M.B., Federle, M.J., Miller, S.T., Bassler, B.L., and Hughson, F.M. (2005). Regulation of LuxPQ receptor activity by the quorum-sensing signal autoinducer-2. **Mol Cell** *18*, 507-518. PMID:15916958.
b. Neiditch, M.B., Federle, M.J., Pompeani, A.J., Kelly, R.C., Swem, D.L., Jeffrey, P.D., Bassler, B.L., and Hughson, F.M. (2006). Ligand-induced asymmetry in histidine sensor kinase complex regulates quorum sensing. **Cell** *126*, 1095-1108. PMID:16990134.
c. Neiditch, M.B., and Hughson, F.M. (2007). The regulation of histidine sensor kinase complexes by quorum sensing signal molecules. **Methods Enzymol** *423*, 250-263. PMID:17609135.
d. Waldron E.J., Snyder D., Fernandez N.L., Sileo E., Inoyama D., Freundlich J.S., Waters C.M., Cooper V.S., Neiditch M.B. (2019). Structural basis of DSF recognition by its receptor RpfR and its regulatory interaction with the DSF synthase RpfF. **PLoS Biology** 17: e3000123. PMID: 30716063.

3. Structure-function studies of cell-cell communication in Gram-positive bacteria. I am actively studying the mechanistic basis of Gram-positive bacterial cell-cell communication, which is mediated by secreted peptide pheromones. The pheromones are synthesized in the cytoplasm as pro-peptides. During secretion, the pro-peptides are proteolytically processed to their mature forms. The pheromone peptides bind to and regulate receptors on the bacterial surface or in the cytoplasm. My lab uses biochemical, genetic, biophysical (mainly X-ray crystallographic), and computational approaches to study all aspects of pheromone receptor function and regulation. Furthermore, we are targeting these receptors for therapeutic intervention. For example, we have recently determined how cyclosporin A and its non-immunosuppressive derivative valspodar inhibit pheromone receptor function in *Streptococcus* species.

a. Parashar, V., Mirouze, N., Dubnau, D.A., and Neiditch, M.B. (2011). Structural basis of response regulator dephosphorylation by Rap phosphatases. **PLoS Biol** *9*, e1000589. PMID:21346797.
b. Baker, M.D., and Neiditch, M.B. (2011). Structural basis of response regulator inhibition by a bacterial anti-activator protein. **PLoS Biol** *9*, e1001226. PMID:22215984.
c. Parashar, V., Jeffrey, P.D., and Neiditch, M.B. (2013). Conformational change-induced repeat domain expansion regulates Rap phosphatase quorum-sensing signal receptors. **PLoS Biol** *11*, e1001512. PMID:23526881.
d. Parashar V., Aggarwal C., Federle M.J., Neiditch M.B. (2015). Rgg protein structure-function and inhibition by cyclic peptide compounds. **Proc Natl Acad Sci U S A** 112(16):5177-82. PMID: 25847993.

4. Antibacterial drug discovery targeting *Mycobacterium tuberculosis*. I am actively participating in collaborative efforts to develop antibacterial compounds (antibiotics) targeting *Mycobacterium tuberculosis* proteins important for cell wall biosynthesis. Cellular targets of these antibacterial compounds include Pks13 and KasA. My lab has been responsible for X-ray crystallographic and biochemical components of structure-activity relationship studies revealing the mechanistic basis underlying the activity of hit and lead compounds.

a. Wilson, R., Kumar, P., Parashar, V., Vilcheze, C., Veyron-Churlet, R., Freundlich, J.S., Barnes, S.W., Walker, J.R., Szymonifka, M.J., Marchiano, E., Shenai, S., Colangeli, R., Jacobs, W.R., Jr., Neiditch, M.B., Kremer, L. and Alland, D. (2013). Antituberculosis thiophenes define a requirement for Pks13 in mycolic acid biosynthesis. **Nature Chemical Biology**. 9: 499-506. PMID: 23770708.

b. Kumar P., Capodagli G.C., Awasthi D., Shrestha R., Maharaja K., Sukheja P., Li S.G., Inoyama D., Zimmerman M., Ho Liang H.P., Sarathy J., Mina M., Rasic G., Russo R., Perryman A.L., Richmann T., Gupta A., Singleton E., Verma S., Husain S., Soteropoulos P., Wang Z., Morris R., Porter G., Agnihotri G., Salgame P., Ekins S., Rhee K.Y., Connell N., Dartois V., Neiditch M.B.*, Freundlich J.S., Alland D. (2018). Synergistic Lethality of a Binary Inhibitor of Mycobacterium tuberculosis KasA. **MBio** 9: 02101-17. PMID: 30563908. *Co-corresponding author

Complete List of Published Work:
http://www.ncbi.nlm.nih.gov/pubmed/?term=Neiditch+MB

D. Research Support

Current Research Support

R01 AI125452 Neiditch and Federle (PIs) 07/19/16 – 06/30/20
Molecular Mechanisms of Rgg Receptor Regulation by Pheromones and Inhibitors
The major goals are structure-function studies are to elucidate the mechanisms of Rgg signal transduction responses to peptide pheromones. Design and identification of specific inhibitors of Rgg proteins will be conducted.
Role: PI

R01 GM109259 Waters (PI) 7/1/2015-6/30/2020
From structure to systems: Understanding cyclic di-GMP control of transcription
The overall goals of this proposal are to explore how the second messenger cyclic di-GMP controls gene transcription by understanding molecular mechanism and global gene regulatory networks.
Role: I

R01 GM110444 Cooper (PI) 10/1/2014-9/30/2019
Molecular mechanisms of adaptive diversity in *Burkholderia* biofilms
The major goals of the project are to identify the genetic and physiological basis of adaptations that enable *Burkholderia* to form biofilms and adapt to colonize distinct niches.
Role: I

U19 AI109713 Perlin (PI) 3/1/2014-2/28/2019
Center to develop therapeutic countermeasures to high-threat bacterial agents
The major goal of the project is to perform process and pathway based discovery of novel anti-TB drugs.
Role: I

R41 AI134561 Ekins and Freundlich (PIs) 2/7/2018-1/31/2019
Structure-Guided Optimization of an In Vivo Active Small Molecule Antitubercular Targeting KasA
The major goal of the project is to deliver an optimized lead candidate inhibitor of Mtb KasA with enhanced in vivo efficacy as compared to the early lead DG167, in preparation for a Phase II application that would target critical pre-clinical studies.
Role: I

Completed Research Support

R03 AI101669 Neiditch (PI) 6/1/2013-5/31/2016
X-ray Crystallographic Analysis of Diguanylate Cyclase Enzyme-Inhibitor Complexes
The major goal of this exploratory project is to determine X-ray crystal structures of diguanylate cyclase enzyme-inhibitor complexes to begin to reveal the mechanism of inhibitor function.
Role: PI

R01 AI081736 Neiditch (PI) 08/01/2009 – 07/31/2015
Structural Biology of Multifunctional Bacterial Phosphatases
The major goals of this project are to use biochemical, genetic, and X-ray crystallographic techniques to determine how the large family of multifunctional bacterial Rap proteins inhibits the activity of response regulators.
Role: PI

F32 AI060174 Neiditch (PI) 3/1/2005-2/28/2007
Structural Analysis of Bacterial Quorum Sensing Proteins
The major goals of this project are to elucidate the regulation of autoinducer-2 signal transduction by describing the interaction of LuxP and LuxQ in mechanistic detail.
Role:PI

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: David A. Dubnau

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

POSITION TITLE: Professor

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
Lafayette College, Easton, PA	AB	9/56	Biology/chemistry
Columbia University, New York, NY	MA	6/58	Biology
Columbia University, New York, N	PhD	6/61	Biology

A. Personal Statement

My laboratory investigates the biology of the model organism *Bacillus subtilis* in the belief that this powerful model reveals fundamental aspects of biology with wide application to other fields, including the study of pathogens. We focus on two areas. The first is the regulation of developmental processes, including the K-state (which includes genetic competence for transformation), sporulation and biofilm formation. A second major goal is to gain an understanding of how transforming DNA is internalized. For all of our studies we use a combination of microbiological, microscopic, biochemical, genetic and biophysical approaches, based on the conviction that adopting a variety of experimental avenues of attack greatly expands the power of our research. Our work has been consistently productive and NIH has continuously funded us for nearly 50 years. Given our extensive experience and track record with the study of bacterial transformation and with the genetic manipulation of *Bacillus subtilis*, I believe that our lab is well positioned to carry out the investigations proposed in this application.

I believe strongly in a team approach to research. My group meets weekly so that students and postdocs may present their work and valuable ideas emerge from these discussions. I also believe strongly in collaborations when progress requires an expertise that is lacking in my group. These practices have made our work both productive and enjoyable. Over the years I have helped train many doctoral and postdoctoral students, several of whom have gone on to successful careers in academia, in the private sector and as editors of premiere journals. Several of these individuals have taken possession of projects that were initiated in our lab and this practice continues.

B. Positions and Honors

9/61 - 2/64 Postdoctoral Fellow (National Cancer Institute), National Institute for Medical Research, London, England (with Dr. M.R. Pollock)

3/64-12/65 Postdoctoral Fellow (National Cancer Institute), Dept. of Biochemistry, Albert Einstein College of Medicine, Bronx, New York (with Dr. J. Marmur)

7/66-8/71 Research Assistant, Professor, Department of Microbiology, New York University School of Medicine, New York, New York

9/71-8/79 Research Associate Professor, Department of Microbiology, New York University School of Medicine, New York, New York

9/79-1/04 Research Professor, Department of Microbiology, New York University School of Medicine, New York, New York

1/66-6/70 Associate, Department of Microbiology, The Public Health Research Institute, New York, New York

7/70-6/79 Associate Member, Department of Microbiology, The Public Health Research Institute, New York, New York

7/79-Present Member, Department of Microbiology, The Public Health Research Institute, Newark, New Jersey

7/84-6/88 Member of the Microbial Physiology & Genetics Study Section, NIH

7/86-6/88 Chairman, of the Microbial Physiology & Genetics Study Section, NIH
5/90-1993 Member, Genbank Advisory Committee, NIH

1996-present Fellow of AAAS

2003-2016 Professor, Department of Microbiology and Molecular Genetics, New Jersey Medical School, University of Medicine and Dentistry of New Jersey

2016-present Professor, Department of Microbiology, Biochemistry and Molecular Genetics, New Jersey Medical School, Rutgers University

2008 Mentor of the Year Award, voted by UMDNJ Graduate students.

C. Contributions to Science

a. As a postdoctoral fellow I generated the first nearly contiguous genetic map of the *Bacillus subtilis* chromosome. This study helped establish *B. subtilis* as the most widely used Gram-positive bacterial model system.

Dubnau D, Goldthwaite C, Smith I, Marmur J. Genetic mapping in Bacillus subtilis. J Mol Biol. 1967 **27**:163–185.

Dubnau D, Smith I, Marmur J. Gene conservation in Bacillus species. II. The location of genes concerned with the synthesis of ribosomal components and soluble RNA. Proc Natl Acad Sci U S A. 1965. **54**:724–730.

b. My lab isolated and characterized the first systematically derived set of mutants deficient in transformation. These carried either regulatory mutations (see "d") or mutations in the genes that encode DNA uptake proteins. The DNA uptake proteins identified in these studies have now been found in all the bacterial transformation systems (except for the pilus-related proteins that are absent from *Helicobacter*) and are still being actively investigated in many labs.

Hahn J, Albano M, Dubnau D. Isolation and characterization of Tn917lac-generated competence mutants of *Bacillus subtilis.* Bacteriol 1987. **169**:876–885

Albano, M, Hahn, J, Dubnau, D. Expression of competence genes in *Bacillus subtilis.*. J. Bacteriol. 1987. **169**: 3110-7.

c. **We identified the enzyme that methylates bacterial 23S rRNA to confer resistance to macrolide antibiotics and discovered a unique mechanism of regulation of synthesis of this enzyme by translational attenuation. These studies have expanded our understanding of resistance to an important class of antibiotics.**

Shivakumar AG, Dubnau, D. Characterization of a plasmid-specified ribosome methylase associated with macrolide resistance. Nuc. Acids Res. 1981. **9**: 2549-2562.

Hahn J, Grandi G, Gryczan TJ, Dubnau D. Translational attenuation of *ermC*: a deletion analysis. Mol Gen Genet. 1982. **186**:204-16.

Dubnau D. Induction of *ermC* requires translation of the leader peptide. EMBO J. 1985. **4:** 533–537.

Narayanan CS, Dubnau D. An in vitro study of the translational attenuation model of *ermC* regulation. J Biol Chem. 1987. **262**:1756-65.

d. **Our laboratory has been a leader in elucidating the complex network of protein-protein and protein-DNA interactions that regulates the expression of competence genes. This network is embedded in an even more complex set of interactions that governs spore and biofilm formation, and we have continued to investigate this expanded regulatory system.**

Turgay K, Hahn J, Burghoorn J, Dubnau D Competence in Bacillus subtilis is controlled by regulated proteolysis of a transcription factor. EMBO J 1998. **17**: 6730-6738. PMC1171018

Albano M, Smits WK, Ho LT, Kraigher B, Mandic-Mulec I, Kuipers OP, Dubnau D.
The Rok protein of Bacillus subtilis represses genes for cell surface and extracellular functions.
Journal of bacteriology. 2005. **187**:2010-9. PMC1064057

Prepiak P, Dubnau D A peptide signal for adapter protein-mediated degradation by the AAA+ protease ClpCP . Mol Cell 2007. **26**: 639-647. PMC2041856

Miras M, Dubnau D. A DegU-P and DegQ-Dependent Regulatory Pathway for the K-state in *Bacillus subtilis.* Frontiers in Microbiol 2016 .**22**:1868. PMC5118428

e. We continue to investigate the mechanisms involved in the uptake of DNA during transformation, using the *B. subtilis* system. Our initial studies used radioactive and heavy isotope labeled DNA to elucidate the fate of transforming DNA in wild type and transformation mutant strains. We have more recently characterized transformation proteins and made progress in understanding their interactions and cellular locations. This subject is the focus of the current application and we believe that we are poised to make significant gains in our understanding of the mechanism of DNA uptake to the periplasm and transport across the cell membrane**.**

Provvedi, R, Dubnau, D. ComEA is a DNA receptor for transformation of competent *Bacillus subtilis*. Mol. Microbiol. 1999. **31**: 271-80.

Draskovic I, Dubnau D. Biogenesis of a putative channel protein, ComEC, required for DNA uptake: membrane topology, oligomerization and formation of disulphide bonds. Mol. Microbiol. 2005 **55**:881-896. PMC3835657

Hahn J, Maier B, Haijema BJ, Sheetz M, Dubnau D. Transformation Proteins and DNA Uptake Localize to the Cell Poles in *Bacillus subtilis*. Cell 2005. **122**: 59-71. PMC4442496

Maier, B, Chen, I, Dubnau, D, Sheetz, MP. DNA transport in *Bacillus subtilis* requires proton motive force to generate large molecular forces. Nature structural and molecular biology. 2004. **11**:643-9. PMC3832999.

Briley K, Jr., Dorsey-Oresto A, Prepiak P, Dias MJ, Mann JM, Dubnau D. The secretion ATPase ComGA is required for the binding and transport of transforming DNA. Mol Microbiol 2011. **81**: 818-830. PMC3781931

f. We were among the first to recognize that the expression of competence development is governed stochastically and that cells are selected on the basis of transcriptional noise. Our investigations of the bistable expression of competence genes stimulated much related work in other labs and are often cited as a model for other developmental systems.

Maamar, H., and Dubnau, D. Bistability in the *Bacillus subtilis* K-state (competence) system requires a positive feedback loop, Mol. Microbiol. 2005. **56**, 615-624. PMC3831615

Maamar H, Raj A and Dubnau D Noise in gene expression determines cell fate in Bacillus subtilis. Science 2007. **317**: 526-529. PMC3828679

Mirouze N, Prepiak P and Dubnau D. Fluctuations in spo0A transcription control rare developmental transitions in *Bacillus subtilis*. PLoS genetics. 2011. 7(:e1002048. PMC3084206

Mirouze N, Desai Y, Raj A, Dubnau D Spo0A~P imposes a temporal gate for the bimodal expression of competence in Bacillus subtilis. PLoS Genet. 2012. 8: e1002586. PMC3297582

g. Recently we have discovered a complex of three proteins (RicAFT) needed for biofilm formation, sporulation and genetic competence in *B. subtilis*. This complex carries two Fe-S clusters as well as a single molecule of FAD. These proteins are widely conserved among the firmicutes and although their precise roles are still poorly understood, they are clearly important for developmental processes.

Carabetta VJ, Tanner AW, Greco TM, Defrancesco M, Cristea IM, Dubnau D A complex of YlbF, YmcA and YaaT regulates sporulation, competence and biofilm formation by accelerating the phosphorylation of Spo0A. Mol Microbiol 2013. **88**: 283-300. PMC3781937

Dubnau, EJ, Carabetta VJ, Tanner AW, Miras M, Diethmaier C, Dubnau D A protein complex supports the production of Spo0A-P and plays additional roles for biofilms and the K-state in *Bacillus subtilis*. Mol. Microbiol. 2016. **101**:606-624. PMC4978174

Tanner, AW, Carabetta, VJ, Martinie, RJ, Mashruwala, AA, Boyd, JM, Krebs, C, Dubnau, D. The RicAFT ((YmcA-YlbF-YaaT) complex carries two $[4Fe\text{-}4S]^{+2}$ clusters and may respond to redox changes. Mol Microbiol 2017.**104**: 837-8500. PMC5444954

h. We have initiated an ongoing study of protein acetylation and its roles in bacterial physiology and development. We began by characterizing the acetylome of *B. subtilis*. We have discovered a potential role for acetylation in the control of DNA compaction in the nucleoid. My ex-postdoc Valerie Carabetta has established her own lab will seek independent funding for this project, which we will not work on in our lab.

Carabetta VJ, Greco, TM, Tanner AW, Cristea IM, Dubnau D (2016) Temporal Regulation of the Bacillus subtilis Acetylome and Evidence for a Role of MreB Acetylation in Cell Wall Growth. mSystems 1(3). pii: e00005-16. Epub 2016. PMC4927096

i. We have investigated the role of the bacterial flagellum as a sensing device for the regulation of gene expression and have identified viscous drag on the flagellum as a

signal that regulates the phosphorylation of DegU. The control of gene expression by mechanosensing signaling involving flagella and cilia is widespread biology but the signal transduction pathways are not well understood in bacteria. This work continues as part of Aim 2

Diethmaier, C, Chawla, R, Canzoneri, A, Kearns, DB, Lele, PP, Dubnau D Viscous drag on the flagellum activates Bacillus subtilis entry into the K-state. Mol Microbiol 2017.**106**: 367-380. PMC5653444

j. We have shown that competent cells of *B. subtilis* are arrested in growth and DNA replication, reminiscent of persistent cells during antibiotic exposure. In fact competent cells are tolerant of antibiotics. We have partially elucidated the mechanism of this arrest.

Haijema, B. J., Hahn, J., Haynes, J., and Dubnau, D. . A ComGA-dependent checkpoint limits growth during the escape from competence, Mol. Microbiol. 2001. **40**: 52-64.

Briley, K Jr, Prepiak, P, Dias, MJ, Hahn, J, Dubnau D. . Maf acts downstream of ComGA to arrest cell division in competent cells of *B. subtilis*. Mol. Microbiol. 2011. **81**:23-39. PMC3781949

Hahn J, Tanner AW, Carabetta VJ, Cristea IM, Dubnau D. ComGA-RelA interaction and persistence in the *Bacillus subtilis* K-state. Mol. Microbiol. 2015.**81**:23-39. 97: 454-471. PMC4722805

Complete list of publications in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/41438987/?sort=date&direction=descending

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

RO1 GM057720 D. Dubnau (PI) Last Funding Period: 07/01/2014 - 06/30/2018
Now on no-cost extension.
Title Regulation of genetic competence in *Bacillus subtilis*
The goal of this study is to understand the regulation of stationary phase developmental pathways in *Bacillus subtilis*, particularly genetic competence. Role: PI

RO1 GM043756 D. Dubnau (PI) Last Funding Period: 05/01/2013 - 02/28/2017
Title Genetic competence apparatus of *Bacillus subtilis*
The goal of this study is to understand the mechanisms of transformation in *Bacillus subtilis*. Role: PI