BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Jason T Kaelber

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

POSITION TITLE: Assistant Research 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
Cornell University	B.A.	05/2011	Chemistry; Biology
Baylor College of Medicine	Ph.D.	09/2017	Molecular Virology & Microbiology

A. Personal Statement

I lead the Rutgers New Jersey Cryo-Electron Microscopy and Tomography Core Facility and serve as a field expert in cryoelectron microscopy technologies for our state. I am a structural biologist and virologist with experience in every aspect of the cryoelectron microscopy workflow. My broad background in virology and structural biology spans from classical virology and cell biology techniques to purification to cryoelectron microscopy, structure determination, and qualitative and quantitative interpretation of electron density maps. At the Rutgers New Jersey CryoEM/ET Core Facility, my recent structure of AAV strain hu.37 is among the highest-resolution cryoEM structures ever published from a 200 kV instrument (paper *a*), demonstrating that my team and I are operating at an internationally-competitive level. In addition to work I initiated or drove, I have a demonstrated track record of catalyzing investigator-initiated projects in microbiology, cryoelectron microscopy, and image processing.

At Rutgers I wear two hats: director of the core facility, and as a researcher/educator. These roles interact in a virtuous cycle of workflow optimization, methods development, and training. Through my independent projects (e.g. paper *a*) I continually push the limits of technology at our site, leading to improved capabilities for the community. My active research helps me better relate to and communicate with collaborators with a biochemistry (e.g. paper *b*) or cell biology emphasis. I also serve on the ORED Advisory Committee for Core Facilities.

Building on my prior experience as a teaching assistant of software workshops in cryoEM and as co-organizer of the Center for HIV RNA cryoEM workshop, I co-taught the Rutgers course “Cryo-Electron Tomography” in 2018 and lectured on single-particle reconstruction in 2019 and 2020 at Rutgers and Cornell Universities. I have also trained numerous scientists hands-on in vitrification, operation of cryoelectron microscopes, and image processing. I’ve also invented novel methods to deal with specimen heterogeneity (paper *c*), which may be beneficial in resolving discretely heterogeneous complexes such as Project III ribosome.

a. Kaelber JT, Yost SA, Webber KA, Firlar E, Liu Y, Mercer AC. Structure of the AAVhu.37 capsid by cryoelectron microscopy. *Acta Cryst* 2020 February 5; F76(2). PMID: 32039886

b. Yin Z, Kaelber JT, Ebright R. Structural basis of Q-dependent antitermination. *Proc Natl Acad Sci U S A*. 2019 Sep 10;116(37):18384-18390. PMID: 31455742. PMCID: PMC6744881
c. Kaelber JT, Jiang W, Weaver SC, Auguste AJ, Chiu W. Arrangement of the Polymerase Complexes inside a Nine-Segmented dsRNA Virus. *Structure*, 2020 Feb 7. PMID: 32049031. PMC Journal - In Process
d. Sun SY, Kaelber JT, Chen M, Dong X, Nematbakhsh Y, Shi J, Dougherty M, Lim CT, Schmid MF, Chiu W, He CY. Flagellum couples cell shape to motility in *Trypanosoma brucei*. *PNAS* 2018 Jun 11; PMID: 29891682. PMCID: PMC6042131.

B. Positions and Honors

2009	Stagaire, Unité de populations virales & pathogenèse, Institut Pasteur
2009	Intern, Microbiology Division, New England Primate Research Center, Harvard Medical School
2007-2011	Laboratory Asst, Dept of Microbiol & Immunol, Baker Institute for Animal Health, Cornell Univ
2011-2017	Predoctoral fellow, National Center for Macromolecular Imaging, Baylor College of Medicine
2017-	Assistant Research Professor, Institute for Quantitative Biomedicine, Rutgers Univ
2017-	Director, Rutgers New Jersey Cryo-Electron Microscopy and Tomography Core Facility
2019-	Member, Cancer Institute of New Jersey

C. Contributions to Science

<u>Reconstructing ancient events in viral evolution</u>

The rapid evolution of viruses and lack of universally conserved genes obscure insights into their ultimate provenance from sequence comparison of extant viruses. While the most common approach to paleovirology is searching for endogenized viruses in host genomes, these events are rare and only practicable for events within the last few hundred million years. To query the history of a non-endogenized virus, I used the signatures of selective pressure it left in host genomes. Integrating reverse genetics, bioinformatics, and cell biology, I reconstructed ancient host-cell binding events to reconstruct the history of canine parvovirus. I showed its ancestor infected ancient canids until they evolved a defense, but the virus was maintained in other Carnivora until a 20th-century spillover (paper *e*). Because this technique cannot reconstruct the most ancient events, I moved to **structure-based inference of deep evolutionary events**. After establishing the Fako virus system and considering the architecture of the last universal ancestor of *Spinareovirinae* (paper *f*), I solved the atomic structure of Fako virus and am using this to shed light on the evolutionary mechanisms of architectural variation in this family (conference presentation *g*). To enhance my skills in this area I also collaborate with George Fox (co-discoverer of Archaea) to solve structures of archaean ribosomal insertions and use these to better understand ribosomal evolution (conference proceedings *h*). My long-term goal is to determine how many origins of viruses there were, where they came from, and to elucidate principles involved in transitions in virion architecture.

e. Kaelber JT, Demogines A, Harbison CE, Allison AB, Goodman LB, Sawyer SL, Parrish CR. Evolutionary reconstructions of the transferrin receptor of Caniforms supports canine parvovirus being a re-emerged and not a novel pathogen in dogs. *PLos Pathogens* 2012 May;**8**(5):e1002666. PMID: 22570610 PMCID: PMC3342950
f. Auguste JA,* Kaelber JT,* Fokam E,* Guzman H, Carrington C, Erasmus JH, Kamgang B, Popov VL, Jakana J, Liu X, Wood TG, Widen SG, Vasilakis N, Tesh RB, Chiu W, Weaver SC. A newly-isolated reovirus has the simplest genomic and structural organization of any reovirus. *J Virol*. 2015 Jan;89(1):676-687 PMID: 25355879 PMCID: PMC4301156 (* denotes co-first authors)

g. Kaelber JT, Jiang W, Weaver SC, Auguste AJ, Chiu W. CryoEM structures of the capsid, non-icosahedral replicases, and dsRNA in the simplest reovirus. Presented at: Gordon Research Conference Physical Virology; 2017 Jan 30; Lucca, Italy.
h. Tirumalai MR, Kaelber JT, Park D, Chiu W, Fox GE. Complexity in Ribosomal Evolution — A Case Study of an Evolutionarily Divergent Recent Insertion in the 5S RNA. In: *XVIIIth International Conference on the Origin of Life*. 2017 Jul 16-21; San Diego, California. Houston: Lunar and Planetary Institute; 2017. #4225.

Facilitating investigator-initiated discovery through technical excellence

For projects driven by other investigators (such as this one), in fields ranging from heart disease (paper *l*) to vaccine development (paper *j*), I have a track record of enabling discoveries by providing cutting-edge technical expertise and collaboration and by creating bespoke analyses for these projects. In each of the selected examples below I provided a key technique on a collaborative basis, such as applying cryoEM image-processing tools to recalcitrant histological data or performing rapid cryoEM reconstruction. Note also that there are no other authors in common between any of these studies, showing that I can **facilitate discovery for diverse investigators**.

i. Thomas WC, Brooks FP 3rd, Burnim AA, Bacik JP, Stubbe J, Kaelber JT, Chen JZ, Ando N. Convergent allostery in ribonucleotide reductase. Nat Commun. 2019 Jun 14;10(1):2653. doi: 10.1038/s41467-019-10568-4. PMID: 31201319. PMCID: PMC6572854.
j. Erasmus JH, Auguste AJ, Kaelber JT, Luo H, Rossi SL, Fenton K, Leal G, Kim DY, Chiu W, Wang T, Frolov I, Nasar F, Weaver SC. A chikungunya fever vaccine utilizing an insect-specific virus platform. *Nat Med*. 2017 Feb;23(2):192-199. PMID: 27991917 PMCID: PMC5296253
k. Reineke LC, Tsai W, Jain A, Kaelber JT, Jung SY, Lloyd RE. Casein Kinase 2 is linked to stress granule dynamics through phosphorylation of the stress granule nucleating protein G3BP1. *Mol Cell Biol*. 2017 Feb;37(4). PMID: 27920254 PMCID: PMC5288577
l. Suh JH, Lai L, Nam D, Kim J, Jo J, Taffet GE, Kim E, Kaelber JT, Lee HK, Entman ML, Cooke JP, Reineke EL. Steroid Receptor Coactivator-2 (SRC-2) coordinates cardiomyocyte paracrine signaling to promote pressure overload-induced angiogenesis. *J Biol Chem*. 2017 Nov 10. PMID: 29127200 PMCID: PMC5766961

Elucidating the basis of cellular phenotypes through cryoelectron tomography

Cryoelectron tomography can provide an untargeted **ultrastructural census of whole cells**. This makes it ideal to pursue known phenotypes with unknown mechanisms. For example, it was known that ovarian cancer causes a change in platelets but the nature of that change was unknown. I showed that based only on tomograms of platelets I can predict whether the person whence they were derived has an ovarian malignancy (paper *m*). I contributed data analysis to my colleagues who demonstrated that the malignancy-related changes to platelets include alterations to the mitochondria and to the marginal band, a platelet-specific cytoskeletal feature. To understand why lateral attachment of the *Trypanosoma* flagellum is required for directional motion, I imaged mutants by cryoET, wrote new code to analyze intermicrotubule relationships and cytoskeletal bending, proposed a model for force transduction in this organism, and collaborated closely with several colleagues to find that beating of the laterally-attached *Trypanosoma* flagellum contorts the cell body in a way necessary for directional motion (paper *n*). Currently, using our recently-acquired cryogenic focused ion beam, my lab is applying cryoelectron tomography of bacterial cells to understand their metabolic regulation as well as collaborating with the Ebright lab to understand heterogeneous transcription structures.

m. Wang R, Stone RL, Kaelber JT, Rochat RH, Nick AM, Vijayan KV, Afshar-Kharghan V, Schmid MF, Dong JF, Sood AK, Chiu W. Electron cryotomography reveals ultrastructure alterations in platelets from patients with ovarian cancer. *PNAS* 2015 Nov 17;112(46):14266-71 PMID: 26578771 PMCID: PMC4655568

n. Sun SY, Kaelber JT, Chen M, Dong X, Nematbakhsh Y, Shi J, Dougherty M, Lim CT, Schmid MF, Chiu W, He CY. Flagellum couples cell shape to motility in *Trypanosoma brucei*. *PNAS* 2018 Jun 11; PMID: 29891682 PMCID: PMC6042131

List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/jason.kaelber.1/bibliography/public/

D. Additional Information: Research Support

Sponsored research agreement, RegenXBio 05/2019-04/2021
"Characterization of AAV structures by cryo-electron microscopy"
Using structural biology tools I am revealing the mechanisms underpinning host cell tropism and cell penetration.
Role: PI

Pilot grant, Brain Health Institute 01/2020-01/2021
"Architecture of ribosome heterogeneity in developing neocortex under the control ofRNA binding proteins"
I aim to determine the atomic structure of specialized ribosomes purified from neocortical tissue to elucidate mechanisms of translational control by development-specific cofactors.
Role: co-PI

New faculty start-up funds, Rutgers University, Institute for Quantitative Biomedicine 09/2017-09/2020
"The origin(s) of viruses: how many were there?"
I aim to combine high-throughput determination of virion structure with structural bioinformatics to reconstruct the deep phylogeny of viral hallmark proteins, concentrating first on unifying the dsRNA families and understanding transitions between the ssDNA families.
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: Ebright, Richard H.

eRA COMMONS USER NAME (credential, e.g., agency login): EBRIGHT1

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
Harvard University, Cambridge MA	A.B.	05/1981	Biology (*summa cum laude*)
Harvard University, Cambridge MA (with Jon Beckwith)	Ph.D.	01/1987	Microbiology and Molecular Genetics

A. Personal Statement

Ebright's research focusses on the structure, mechanism, and regulation of bacterial transcription complexes, and on the development of inhibitors of bacterial transcription as antibacterial therapeutic agents. His research employs tools of structural biology, biophysical chemistry, and drug discovery. His research contributions include defining the structural organization of transcription initiation complexes, defining the "scrunching" mechanism of initial transcription, defining the "recruitment" mechanism of transcription activation, defining novel antibacterial targets in the bacterial transcription machinery, and identifying novel antibacterial agents that exhibit activity against drug-resistant bacterial pathogens. He directs a laboratory of approximately fifteen postdoctoral associates, graduate students, and technicians and serves as project leader on two NIH grants.

Ebright will lead the proposed project on *Staphylococcus aureus* RNA polymearse.

Four relevant publications:

Lin, W., Mandal, S., Degen, D., Liu, Y.., Ebright, Y., Li, S., Feng, Y., Zhang, Y., Mandal, S., Jiang, Y., Liu, S., Gigliotti, M., Talaue, M., Connell, N., Das, K., Arnold, E., and Ebright, R. (2017) Structural basis of *Mycobacterium tuberculosis* transcription and transcription inhibition. *Mol. Cell* **166**, 169-179. PMCID: PMC5438085.

Lin, W., Das, K., Degen, D., Mazumder, A., Duchi, D., Wang, D., Ebright, Y., Ebright, R.Y., Sineva, E., Gigliotti, M., Mandal, S., Jiang, Y., Liu, Y., Yin, R., Zhang, Z., Eng, E., Thomas, D., Donadio, S., Zhang, C., Kapanidis, A., and Ebright, R. (2018) Structural basis of transcription inhibition by fidaxomicin (lipiarmycin A3). *Mol. Cell* **70**, 60-71. PMCID: PMC6205224.

Lin, W., Mandal, S., Degen, D., Cho, M., Feng, Y., Das, K., and Ebright, R.H. (2019) Structural basis of ECF-σ-factor-dependent transcription initiation. *Nature Commun.* **10**, 710. PMCID: PMC6372665.

Yin, Z., Kaelber, J., and Ebright, R.H. (2019) Structural basis of Q-dependent antitermination. *Proc. Natl. Acad. Sci. USA* **116**, 18384-18390. PMCID: PMC6744881.

B. Positions and Honors

Positions and Employment

1984-1987 Junior Fellow, Society of Fellows, Harvard University, Cambridge, MA
1987- Laboratory Director, Waksman Institute of Microbiology, Piscataway NJ
1987-1992 Assistant Professor, Department of Chemistry, Rutgers University, New Brunswick, NJ
1992-1995 Associate Professor, Department of Chemistry, Rutgers University, New Brunswick, NJ
1995-2013 Professor, Department of Chemistry, Rutgers University, New Brunswick, NJ
1997-2013 Investigator, Howard Hughes Medical Institute
2013- Board of Governors Professor, Department of Chemistry, Rutgers University, New Brunswick, NJ

Honors

1980	Phi Beta Kappa
1989	Searle Scholar Award
1990	Johnson and Johnson Discovery Research Fellowship
1995	American Society for Biochemistry and Molecular Biology Schering-Plough Award
1996	Fellow, American Academy of Microbiology
1998	Rutgers University Board of Trustees Research Excellence Award
2004	Fellow, American Association for the Advancement of Science
2011	Fellow, Infectious Diseases Society of America
2012	Theobold Smith Society Waksman Award
2013	National Institutes of Health MERIT Award
2016	Member, American Academy of Arts and Sciences

C. Contributions to Science

1. Sequence-Specific Protein-DNA Interaction

Ebright helped define the basis of sequence-specific protein-DNA interaction and developed artificial sequence-specific DNA cleaving agents.

Using genetic approaches and photocrosslinking approaches, Ebright established that sequence-specific DNA binding proteins recognize DNA sequences through direct contacts between amino acids and DNA bases. By conjugating a DNA cleaving agent to a sequence-specific DNA binding protein in a manner that permitted activity in specific complexes but not in nonspecific complexes, Ebright constructed a high-specificity DNA cleaving agent able to cleave megabase DNA substrates at single sites.

Ebright, R., Cossart, P., Gicquel-Sanzey, B., and Beckwith, J. (1984) Mutations that alter the DNA sequence specificity of the catabolite gene activator protein of *E. coli*. *Nature* **311**, 232-235.

Ebright, R. (1986) Evidence for a contact between glutamine-18 of lac repressor and base pair 7 of *lac* operator. *Proc. Natl. Acad. Sci. USA* **83**, 303-307.

Blatter, E., Ebright, Y., and Ebright, R. (1992) Identification of an amino acid-base contact in the GCN4-DNA complex by bromouracil-mediated photocrosslinking. *Nature* **359**, 650-652.

Pendergrast, P.S., Ebright, Y., and Ebright, R. (1994) High-specificity DNA cleavage agent: design and application to kilobase and megabase DNA substrates. *Science* **265**, 959-961.

2. Transcription: Transcriptional Activation

Ebright provided the first mechanistic and structural description of transcription activation.

Ebright analyzed transcription activation by *Escherichia coli* catabolite activator protein (CAP) at the *lac* promoter. He showed that transcription activation requires a small patch of the activator ("activating region") and a small patch of a flexibly tethered module of RNA polymerase ("activation target"), showed that transcription activation involves direct interaction between activating region and activation target, determined a crystal structure of the complex between activating region and activation target, and, most recently, determined an EM structure of the intact transcription-activation complex. His results establish that transcription activation by CAP at *lac* proceeds by a "recruitment" mechanism, in which interactions between CAP and RNA polymerase facilitate binding of RNA polymerase to DNA.

Zhou, Y., Busby, S., and Ebright, R. (1993) Identification of the functional subunit of a dimeric transcription activator protein by use of "oriented heterodimers." *Cell* **73**, 375-379.

Chen, Y., Ebright, Y., and Ebright, R. (1994) Identification of the target of a transcription activator protein by protein-protein photocrosslinking. *Science* **265**, 90-92.

Blatter, E., Ross, W., Tang, H., Gourse, R., and Ebright, R. (1994) Domain organization of RNA polymerase α subunit: C-terminal 85 amino acids constitute a domain capable of dimerization and DNA binding. *Cell* **78**, 889-896.

Benoff, B., Yang, H., Lawson, C., Parkinson, G., Liu, J., Blatter, E., Ebright, Y., Berman, H., and Ebright, R. (2002) Structural basis of transcription activation: structure of the CAP-αCTD-DNA complex. *Science* **297**, 1562-1566.

3. Transcription: Structures of Transcription Initiation Complexes

Ebright defined the structural organization of the nucleoprotein complexes that perform transcription initiation.

Using distance restraints from systematic photocrosslinking and systematic fluorescence resonance energy transfer (FRET), Ebright constructed the first structural models of bacterial, archaeal, and eukaryotic transcription-initiation complexes. Using x-ray crystallography, Ebright determined the first atomic structure of a promoter-dependent, initiation-factor-dependent, functional transcription initiation complex. More recently, using x-ray crystallography, Ebright determined atomic structures of transcription initiation complexes engaged in *de novo* initiation and initial transcription, providing comprehensive structural descriptions of the protein-DNA interactions involved in promoter recognition, promoter unwinding, *de novo* initiation, and initial transcription. Most recently, using x-ray crystallography, Ebright determined the first atomic structures of a gene-specific transcription activation complex and of Mycobacterial transcription initiation complexes.

Naryshkin, N., Revyakin, A., Kim, Y., Mekler, V., and Ebright, R. (2000) Structural organization of the RNA polymerase-promoter open complex. *Cell* **101**, 601-611.

Mekler, V., Kortkhonjia, E., Mukhopadhyay, J., Knight, J., Revyakin, A., Kapanidis, A., Niu, W., Ebright, Y., Levy, R., and Ebright, R. (2002) Structural organization of RNA polymerase holoenzyme and the RNA polymerase-promoter open complex. *Cell* **108**, 599-614.

Zhang, Y., Feng, Y., Chatterjee, S., Tuske, S., Ho, M., Arnold, E., and Ebright, R. (2012) Structural basis of transcription initiation. *Science* **338**, 1076-1080. PMCID: PMC359305.

Feng, Y., Zhang, Y., and Ebright, R. (2016) Structural basis of transcription activation. *Science* **352**, 1330-1333. PMCID: PMC4905602.

4. Transcription: Mechanisms of Transcription Initiation and Transcription Elongation

Ebright elucidated the mechanisms of initial transcription and promoter escape in transcription initiation and defined sequence determinants and mechanisms for transcriptional pausing in transcription elongation.

Using ensemble and single-molecule FRET, Ebright showed that the transcription initiation factor σ is not obligatorily released in promoter escape but, instead, can remain bound to RNA polymerase, translocate with RNA polymerase, and recognize regulatory DNA sequence elements during transcription elongation. Using single-molecule FRET and single-molecule nanomanipulation, Ebright showed that initial transcription involves a "scrunching" mechanism, in which RNA polymerase remains stationary on promoter DNA and reels in downstream DNA, and that promoter escape involves the accumulation of stress through scrunching, followed by the use of accumulated stress to break RNA polymerase-promoter interactions. More recently, using high-throughput sequencing approaches, Ebright and collaborators showed that transcription start-site selection also involves scrunching, defined, genome-wide, the DNA sequence determinants for pausing during transcription elongation, and demonstrated roles of a newly identified DNA sequence element recognized by RNA polymerase--the "core recognition element"--in transcription initiation, transcription elongation, and transcriptional pausing.

Revyakin, A., Liu, C., Ebright, R. & Strick, T. (2006) Abortive initiation and productive initiation by RNA polymerase involve DNA scrunching. *Science* **314**, 1139-1143. PMCID: PMC2754787.

Kapanidis, A., Margeat, E., Ho, S.O., Kortkhonjia, E., Weiss, S. & Ebright, R. (2006) Initial transcription by RNA polymerase proceeds through a DNA-scrunching mechanism. *Science* **314**, 1144-1147. PMCID: PMC2754788.

Chakraborty, A., Wang, D., Ebright, Y., Korlann, Y., Kortkhonjia, E., Kim, T., Chowdhury, S., Wigneshweraraj, S., Irschik, H., Jansen, R., Nixon, B.T., Knight, J., Weiss, S., and Ebright, R. (2012) Opening and closing of the bacterial RNA polymerase clamp. *Science* **337**, 591-595. PMCID: PMC3626110.

Vvedenskaya, I., Vahedian-Movahed, H., Bird, J., Knoblauch, J., Goldman, S., Zhang, Y., Ebright, R., and Nickels, B. (2014) Interactions between RNA polymerase and the "core recognition element" counteract pausing. *Science* **344**, 1285-1289. PMCID: PMC4277259.

5. Transcription: Transcription Inhibitors, Antibacterial Drug Discovery Targeting Transcription

Ebright is elucidating binding sites and mechanisms of antibacterial agents that function by inhibiting bacterial transcription and is developing small-molecule inhibitors of bacterial transcription as antituberculosis drugs and broad-spectrum antibacterial drugs.

Ebright defined the binding sites and mechanisms of the antibiotics microcin J25, streptolydigin, myxopyronin, corallopyronin, ripostatin, GE23077, salinamide, pseudouridimycin, and lipiarmycin, and of synthetic antibacterial agents of the phloroglucinol and aroyl-aryl-phenylalaninamide classes.

Ebright validated myxopyronins, phloroglucinols, and pseudouridimycins as advanced leads for broad-spectrum antibacterial therapy (with potent activities against priority pathogens in culture and in animals) and validated aroyl-aryl-phenylalaninamides as leads for antituberculosis therapy (with potent activities against *Mycobacterium tuberculosis* in culture). Guided by crystal structures of bacterial RNA polymerase in complex with these leads, Ebright and colleagues are designing, synthesizing, and evaluating analogs of these leads, seeking novel compounds with improved antibacterial activities and improved pharmacological properties.

Prompted by crystal structures indicating that rifamycins--a class of RNA polymerase inhibitors currently used as antibacterial drugs--and GE23077 interact with adjacent binding sites on RNA polymerase and can bind simultaneously to RNA polymerase, Ebright and colleagues linked a rifamycin to GE23077 and showed that the resulting "bipartite inhibitor" had exceptional potency and exceptional ability to overcome target-dependent resistance. Guided by crystal structures, Ebright and colleagues are designing, synthesizing, and evaluating additional novel bipartite inhibitors comprising rifamycin-site ligands linked to GE23077-site ligands.

Mukhopadhyay, J., Das, K., Ismail, S., Koppstein, D., Jang, M., Hudson, B., Sarafianos, S., Tuske, S., Patel, J., Jansen, R., Irschik, H., Arnold, E., and Ebright, R. (2008) The RNA polymerase "switch region" is a target of inhibitors *Cell* **135**, 295-307. PMCID: PMC2580802.

Zhang, Y., Degen, D., Ho, M., Sineva, E., Ebright, K., Ebright, Y., Mekler, V., Vahedian-Movahed, H., Feng, Y., Yin, R., Tuske, S., Irschik, H., Jansen, R., Maffioli, S., Donadio, S., Arnold, E., and Ebright, R. (2014) GE23077 binds to the RNA polymerase "i" and "i+1" sites and prevents the binding of initiating nucleotides. *eLife*, **3**, e02450. PMCID: PMC3994528.

Maffioli, S., Zhang, Y., Degen, D., Carzaniga, T., Del Gatto, G., Serina, S., Monciardini, P., Mazzetti, C., Guglierame, P., Candiani, G., Chiriac, A.I., Facchetti, G., Kaltofen, P., Sahl, H.-G., Dehò, G., Donadio, S., and Ebright, R. (2017) Antibacterial nucleoside-analog inhibitor of bacterial RNA polymerase. *Cell* **169**, 1240-1248. PMCID: PMC5542026.

Lin, W., Das, K., Degen, D., Mazumder, A., Duchi, D., Wang, D., Ebright, Y., Ebright, R.Y., Sineva, E., Gigliotti, M., Mandal, S., Jiang, Y., Liu, Y., Yin, R., Zhang, Z., Eng, E., Thomas, D., Donadio, S., Zhang, C., Kapanidis, A., and Ebright, R. (2018) Structural basis of transcription inhibition by fidaxomicin (lipiarmycin A3). *Mol. Cell* **70**, 60-71. PMCID: PMC6205224.

6. Complete List of Published Work in MyBibliography

https://www.ncbi.nlm.nih.gov/myncbi/richard.ebright.1/bibliography/public/

D. Research Support

1. Ongoing Research Support

Bacterial Transcription Complexes
NIH-NIGMS, R37-GM041376 (Ebright)
02/01/13-01/31/23
The major goal of this project is analysis of the structural and mechanistic basis of bacterial transcription.

Center to Develop Therapeutic Countermeasures to High-Threat Bacterial Agents
NIH-NIAID, U19-AI142731 (Perlin)
05/01/19-04/30/24
The major goal of this project is operation of a Center of Excellence for Translational Research focussed on development of compounds effective against drug-resistant bacterial pathogens. The major goal of the Ebright component of the project is synthesis and efficacy testing of novel arylmyxopyronins and arylalkylcarboxamido phloroglucinols effective against drug-resistant bacterial pathogens.

New Antibacterials: Library Screening
Janssen Pharmaceuticals (Ebright)
10/29/18-10/28/20
The major goal of this project is library screening for new broad-spectrum antibacterial agents and new antituberculosis agents.

2. Completed Research Support

Center to Develop Therapeutic Countermeasures to High-Threat Bacterial Agents
NIH-NIAID, U19-AI109713 (Perlin)
04/25/14-04/30/19
The major goal of this project was establishment of a Center of Excellence for Translational Research focussed on development of compounds effective against drug-resistant bacterial pathogens. The major goal of the Ebright component of the project was synthesis and efficacy testing of novel phloroglucinols and aroyl-aryl-phenylalaninamides effective against drug-resistant bacterial pathogens.

Therapeutics for Drug-Resistant Bacteria: Pseudouridimycins
NIH-NIAID, R01-AI090837 (Ebright)
01/15/13-12/31/18
The major goal of this project is structure-based design, synthesis, and efficacy testing of novel pseudouridimycin analogs effective against drug-resistant bacterial pathogens.

New Antibiotics: Microbial Extract Screening and Mutational De-Replication
Rutgers TechAdvance Fund (Ebright)
04/25/18-04/24/19
The major goal of this project is validation of a novel antibiotics-discovery platform involving microbial extract screening and mutational de-replication.

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: Fan, Huizhou

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

POSITION TITLE: Professor of Pharmacology

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
Hunan Medical University	BM	07/1983	Medicine
Hunan Medical University	MS	07/1986	Clinical microbiology
University of Manitoba	PhD	05/1994	Medical microbiology
Manitoba Institute of Cell Biology	Postdoc	12/1997	Cancer biology
University of California San Francisco	Postdoc	04/2001	Cell biology

A. Personal Statement

My experience in leading a productive research group funded by NIH mostly continuously since 2005 demonstrates my ability to conduct the proposed research on *Chlamydia* biology. The proposed research is built on the work revealing that the peptide deformylase (PDF) is an essential enzyme in chlamydiae; we subsequently demonstrated that PDF is a promising therapeutic target (papers listed in Contribution 2 under Section 3). While studying transcription regulation of the PDF, we identified a novel *Chlamydia*-specific transcription factor that we call GrgA. We initially recognized GrgA as a transcription activator for σ^{66}-dependent genes (paper 1). We recently documented that GrgA can also activate σ^{28}-dependent transcription (paper 2). Coincidentally, a separate line of research suggested that GrgA may serve as a target for selective antichlamydial benzal acylhydrazones (BAH) (papers 3). This hypothesis is further supported by paper 4 and unpublished preliminary data (Table 4 & 5 in the scientific proposal). Collectively, these prior studies provide a strong justification for our proposed studies that will examine the mechanism underlying GrgA.

The intellectual environment at Rutgers University is outstanding, with a large number of eminent microbiologists, transcription biologists and structural biologists. We interact with a number of these investigators, including Dr. Richard Ebright (one of the most distinguished investigator of bacterial transcription), Drs. Masayori Inouye, Bryce Nickels, Smita Patel and Nancy Woychik.

The following publications are most closely related to the work proposed in this application:

1. Bao X, Nickels B, **Fan H.** 2012. *Chlamydia trachomatis* protein GrgA activates transcription by contacting the nonconserved region of σ^{66}. **Proc. Nat. Acad. Sci. USA**. 109:16870-16875. PMCID: PMC3479454. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3479454/

2. Desai M, Wurihan W, Di R, Fondell JD, Nickels B, Bao X, **Fan H.** 2018. A role for GrgA in regulation of σ^{28}-dependent transcription in the obligate intracellular bacterial pathogen *Chlamydia trachomatis*. **J. Bacteriol.** 200:e00298-18. DOI: 10.1128/JB.00298-18 (featured JB Spotlight). PMCID: PMC6153665. https://www.biorxiv.org/content/biorxiv/early/2018/05/15/322701.full.pdf,.

3. Bao X, Gylfe Å, Sturdevant GL, Gong Z, Xu S, Caldwell HD, Elofsson M, **Fan H.** Benzylidenen acylhydrazides inhibit obligate intracellular pathogens chlamydiae in a type III secretion system- and iron chelation-independent manner. **J. Bacteriol.** 2014. 196: 2989-3001. PMCID: PMC4135636. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4135636/pdf/zjb2989.pdf

4. Zhang H., Vellappan S., Tang MM, Bao X., **Fan H.** GrgA as a potential target of selective antichlamydials. *PLoS ONE.* 2019.. **PLoS ONE** 14:e0212874. PMCID: PMC6396966. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6396966/

B. Positions and Honors

Employment and appointments:

1990 – 1994 Graduate research assistant, University of Manitoba, Winnipeg, Canada
1994 – 1997 Postdoctoral fellow, Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada
1997 – 2000 Postgraduate researcher, University of California, San Francisco, CA
2001 – 2001 Assistant research cell biologist, University of California, San Francisco, CA
2001 – 2009 Assistant professor, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ
2001 – Member, Molecular Biosciences Graduate Program, Piscataway, NJ
2002 – Member, Cancer Institute of New Jersey, Piscataway, NJ
2009 – 2013 Associate professor, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ
2010 – Director, Physiology and Integrative Biology Graduate Program at Rutgers, Piscataway, NJ
2013 – 2018 Associate professor, Rutgers-Robert Wood Johnson Medical School, Piscataway, NJ
2018 – Professor, Rutgers-Robert Wood Johnson Medical School, Piscataway, NJ

Professional Memberships:

2003 – American Association for Biochemistry and Molecular Biology
2005 – Chlamydial Basic Research Society
2008 – American Society for Microbiology

Major Academic Services:

2003 – Reviewer for 50+ journals (*Scientific Reports, mBio, J Biol Chem, J Infect Dis, J Immunol,* etc)
2004 Reviewer for Susan G. Komen Breast Cancer Foundation
2005 Reviewer for **NIH** Macromolecular Structure and Function B Study Section
2006 Israel Science Foundation Grant Reviewer
2011 – National Natural Science Foundation of China Grant Reviewer
2013, '14, '18 Reviewer for **NIH** study section ZRG1 IDM-S 81 A
2015 Hungarian National Research Grant Reviewer
2016 VELUX STIFTUNG Foundation Grant Reviewer
2017 Reviewer for **NIH** study section ZRG1 IDM B [81] S
2019 Reviewer for **NIH** study section ZRG1 IDM B [80] S
2018 Reviewer for **NIH** Infectious Diseases and Microbiology Fellowship Study Section [F31]

Awards and Honors:

2011 – 2014 Central South University Guest Professorship
2017 Hunan Province (China) Highly Accomplished Educator Overseas Award
2017 – 2021 Xiangnan University Guest Professorship

C. Contributions to Science

1. **Chlamydial metabolism and molecular biology.** My PhD dissertation research examined how chlamydiae obtain thymine nucleotides and folic acid, which are needed for the synthesis of thymidylate and other metabolites. I had **two technical breakthroughs** during this time. The first was related to the difficulty in producing large volumes of chlamydiae culture for biochemical analyses. I successfully developed a method for growing chlamydiae in 5-10 liters of cells each day. The second breakthrough permitted efficient cloning of chlamydial genes by using genetic complementation. These technical innovations were key to my discovery that 1) *C. trachomatis* synthesizes its own thymine nucleotides (paper a); 2) *Chlamydia spp* obtain folates through different mechanisms (paper b); 3) synthesis of thymidylate (dTMP) is carried out by a novel thymidylate synthase (paper c), and 4) *C. trachomatis* encodes the DNA repair enzyme RecA (paper d).

   a) **Fan H,** G McClarty, RC Brunham. 1991. Biochemical evidence for the existence of thymidylate synthase in the obligate intracellular parasite *Chlamydia trachomatis*. ***J. Bacteriol.*** 173, 6670-6677. PMCID: PMC209014. http://www.ncbi.nlm.nih.gov/pubmed/1938873

b) **Fan H,** RC Brunham, G McClarty. 1992. Acquisition and synthesis of folates by obligate intracellular bacteria of the genus *Chlamydia*. ***J. Clin. Invest.*** 90, 1803-1811. PMCID: PMC443239. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC443239/pdf/jcinvest00053-0177.pdf

c) **Fan H.** 1994. Thymidylate Synthesis and folate metabolism in the obligate intracellular parasite Chlamydiae: metabolic studies and molecular cloning. University of Manitoba, Winnipeg, Canada. http://mspace.lib.umanitoba.ca/bitstream/1993/22224/1/Fan_Thymidylate_synthesis.pdf

d) Zhang D-J, **H Fan,** G McClarty, RC Brunham. 1995. Identification of the *Chlamydia trachomatis* RecA-encoding gene. ***Infect. Immun.*** 63, 676-680. PMCID: PMC1730748. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC173048/pdf/630676.pdf

2. **Chlamydial transcription regulation, novel antichlamydial target identification and antichlamydial development.** For several reasons, there is a need to identify new antichlamydial targets and develop novel antichlamydials, particularly highly selective antichlamydials without adverse effects on the microbiome. I identified the chlamydial peptide deformylase (PDF) as a promising therapeutic target (papers e-g). In studying regulation of PDF gene expression, my lab identified the ***Chlamydia*-specific transcription factor** GrgA (paper 1 under Personal Statement). GrgA is most likely a target for benzal acylhydrazones (BAHs), a group of antichlamydials first developed by my group and my collaborators (papers 3-4 under PS and paper h below). Three features displayed by these compounds make them attractive candidates for prophylactic and therapeutic treatment of chlamydiae: 1) while effectively inhibiting chlamydial growth, they do not have any detectable toxicity for host cells; 2) they do not affect the growth of beneficial lactobacilli in the female genital tract; and 3) chlamydiae do not readily develop resistance to these inhibitors (papers 3-4 under PS).

e) Balakrishnan A, B Patel, S Siebel, N Pachikara, D Chen, G Zhong, B Cravatt, **H Fan.** 2006. Matrix metalloprotease inhibitors GM6001 and TAPI-0 inhibit *Chlamydia trachomatis* by targeting polypeptide deformylase of the bacterium. ***J. Biol. Chem.*** 281:16691-16699. PMCID: N/A. http://www.ncbi.nlm.nih.gov/pubmed/16565079

f) Bao X, Pachikara ND, Oey C, Lewis C, Balakrishnan A, Tan M, Chase T, Nickels BE, Fan H. Noncoding nucleotides and amino acids near the active site regulated deformylase expression and inhibitor susceptibility in *Chlamydia trachomatis*. **Microbiology**. 2011; 157: 2569-81. PMCID: PMC3352175. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3352175/pdf/2569.pdf

g) Balakrishnan A, L Wang, X Li, P Ohman-Strickland, P Malatesta, **H Fan.** 2009. Inhibition of chlamydial infection in the genital tract of female mice by topical application of a peptide deformylase inhibitor. ***Microbiol. Res.*** 164:338-346. PMCID: PMC2735082. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2735082/

h) Zhang H, Kunadia A, Lin Y, Fondell JD, Seidel D, Fan H. 2017. Identification of a strong and specific antichlamydial N-acylhydrazone. **PLOS ONE** 12:e0185783. PMCID: PMC5626472. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5626472/

3. **Chlamydial-host interaction.** Many aspects of the human-chlamydial interaction and vaginal microbiome-chlamydial interaction remain undefined. Autophagy, a lysosome-dependent, self-degrading function provides an innate defense mechanism against most pathogens, but has been exploited by some pathogens to support their growth. Although autophagy can serve as an innate defense mechanism against chlamydiae, this parasite is also able to effectively block autophagy. My lab was the very first to demonstrate the existence of a delicate interplay between *Chlamydia* and its host cell, and such an interaction might have contributed to host-species adaptation (papers i and j). We, for the first time, also documented inactivation of the infectivity of *C. trachomatis* by physiological concentrations of lactic acid found in the vagina of healthy reproductive women (paper k).

i) Pachikara N, H Zhang, Z Pan, S Jin, **H Fan**. 2009. Productive infection of *Chlamydia trachomatis* lymphogranuloma venereum 434 in cells with augmented or inactivated autophagy. ***FEMS Microbiology Letters.*** 292: 240-249. PMCID: PMC2671565. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2671565/

j) Yasir M, Pachikara ND, Bao X, Pan Z, **Fan H.** 2011. Regulation of chlamydial infection by autophagy and vacuolar ATPase-bearing organelles. ***Infect. Immun.*** 79:4019-4028. PMCID: PMC3187247. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3187247/

k) Gong Z, Luna Y, Yu P, Fan H. 2014. Lactobacilli inactivate *Chlamydia trachomatis* through lactic acid

but not H_2O_2. **PLoS One** 9:e107758. PMCID: PMC4162611. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4162611/

4. **Development of new *Chlamydia* research tools.** As described above (contribution 1), as a PhD student, I developed a genetic complementation system for cloning chlamydial genes in *E. coli.* In the past years, my lab has contributed two significant technologies to the chlamydia research field. One is for genetic manipulation of chlamydiae. There are limited selection markers for chlamydial transformation studies, thus making studies requiring multiple selection markers difficult. Contrary to a widely-held view, we demonstrated that a chloramphenicol acetyltransferase can serve as a selectable marker (paper l). This marker is now used by many *Chlamydia* research group.

   We contributed the other technology to researchers interested in the chlamydial type III secretion system (cT3SS). cT3SS, made of more than 30 proteins, is presumably essential for establishment and maintenance of infection. Due to the obligate intracellular parasitic nature of *Chlamydia,* very limited approaches are available to study cT3SS. We have successfully generated *E. coli* expressing all genes of the chlamydial T3SS (paper m). This serves as a basis for optimal expression and assembly of a recombinant chlamydial T3SS, which is useful for characterization of the T3SS and for studying its role in chlamydial pathogenicity.

   l) Xu S, Battaglia L, Bao X, **Fan H.** 2013. Chloramphenicol acetyltransferase as a selection marker for chlamydial transformation. **BMC Res Notes** 6:377. PMCID: PMC3849861. http://www.biomedcentral.com/content/pdf/1756-0500-6-377.pdf.

   m) Bao X, Beatty WL, **Fan H.** 2012. Exploration of chlamydial type III secretion system reconstitution in *Escherichia coli*. **PLoS One** 7:e50833. PMCID: PMC3519817. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3519817/

5. **Protein ectodomain shedding.** I studied protein ectodomain shedding in mammalian cells during my second postdoctoral fellowship under the guidance of Dr. Rik Derynck at UCSF. Ectodomain shedding is a protease-mediated cleavage of membrane proteins at the cell surface, and is required for normal development and pathogenesis in many diseases. Although shedding had been previously described, the mechanism that regulated this process was unknown. I found that growth factors, tumor promoters and DNA damage could activate the shedding mechanism through Erk and p38 MAP kinase-mediated signaling pathways (paper n). Independent work in my lab has characterized the phosphorylation of the major sheddase TNF-α converting enzyme (TACE) in response to shedding inducers and MAPKs (paper o). These two papers represented significant contributions to ectodomain shedding biology.

   In 2004, my group described mutations, in TACE, which effectively inactivates the catalytic activity of the enzyme, in two shedding-defective CHO cell lines (paper p). These key studies showed that the previous understanding of this mechanism was incorrect. As a result, these cell lines are now accepted as the model system for characterizing shedding (eg, paper q).

   n) **Fan H,** R Derynck. 1999. Ectodomain shedding of TGF-α and other transmembrane proteins is induced by receptor tyrosine kinase activation and MAP kinase signaling cascades. ***EMBO J.*** 18, 6962-6972. PMCID: PMC1171759. (**Featured Science Magazine STKE "paper of the week"**).

   o) **Fan H***, CW Turck, R Derynck. 2003. Characterization of growth factor-induced serine phosphorylation of tumor necrosis factor-α converting enzyme (TACE) and of an alternatively translated polypeptide. ***J. Biol. Chem.*** 278, 18617-18627. PMID: 12621058. *Dr. Fan is the sole corresponding author.

   p) Li X, **H Fan.** 2004. Loss of ectodomain shedding due to mutations in the catalytic domain and cysteine-rich/disintegrin domain of the tumor necrosis factor-α converting enzyme (TACE). ***J. Biol. Chem.*** 279. 27365-27375. PMID: 15075334.

   q) Mo, X, Nguyen, NX, Mu, FT, Yang, W, Luo, SZ, **Fan, H,** Andrews, RK, Berndt, MC, Li, R. 2010. Trans-membrane and trans-subunit regulation of ectodomain shedding of platelet glycoprotein Ibα. ***J. Biol. Chem.*** 285:32096-32104. PMCID: PMC2952221.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/1Fg3dgum54CkO/bibliography/44354762/public/?sort=date&direction=ascending

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

Ongoing research support

1R21AI140167 (Fan) 3/1/2019 – 2/28/2021
NIH/NIAID
GrgA: A Multifunctional Transcription Factor that Controls Chlamydial Gene Expression
The goals are to 1) demonstrate the interaction of GrgA with *Chlamydia trachomatis* RNAP core enzyme, and 2) determine roles of GrgA in chlamydial development and growth.
Role: PI

PC98-20 (Fan) 3/1/2020 – 2/28/2021
New Jersey Health Foundation
Development of Novel Selective Antichlamydial Leads
The goal is to develop new lead chemical compounds that selectively inhibit *Chlamydia*
Role: PI

Completed research support for the past five years

PC 20-18 (Fan) 2/1/2018 – 1/31/2020
New Jersey Health Foundation
A potentially Druggable Transcription Regulator in the Human Pathogen Chlamydia
The major goal of this project was to develop a chemical probe for identifying the target of antichlamydials benzal acylhydrazones (BAH) and to determine the role of GrgA in transcription regulation in *Chlamydia*.
Role: PI

1R21AI122034 (Fan) 4/1/2016 – 3/31/2019
NIH/NIAID
GrgA: Key Regulator of Chlamydial Physiology and potential Antichlamydial Target
Role: PI

PC45-16 (Fan) 2/1/2016 – 1/31/2018
New Jersey Health Foundation
GrgA: A Key Physiological Regulator of the Human Pathogen *Chlamydia*
Role: PI

Busch Biomedical Research Grant (Fan & Nickels) 8/1/2015 – 7/31/2016
Charles and Johanna Busch Memorial Fund
Systematic analysis of transcription in the human pathogen *Chlamydia trachomatis*
Role: PI (CoPI: Bryce Nickels)

PC7-13 (Fan) 2/1/2013 – 1/31/2015
New Jersey Health Foundation
Development of a Gene-targeting System for the Human Pathogen *Chlamydia*
Role: PI

1R33AI071954 (Fan) 9/1/2009 – 8/31/2014
NIH/NIAID
Peptide Deformylase Inhibitor LBM415 for Sexually Transmitted Infections
Role: PI

1R21AI071954 (Fan) 9/1/2007 – 8/31/2009
NIH/NIAID
Peptide Deformylase Inhibitor LBM415 for Sexually Transmitted Infections
Role: PI

1R03AG029859 (Fan) 6/1/2007 – 5/31/2009
NIH/NIA
Role of Klotho ectodomain release in suppression of aging
Role: PI

1R21AI064441 (Fan) 3/1/2005 – 2/28/2007
NIH/NIAID
Inhibition of *Chlamydia* with hydroxamates
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: 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 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/R00 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.

In 2005, I entered the Neuroscience Ph.D. Program at Icahn School of Medicine at Mount Sinai and joined the lab of Dr. Diomedes Logothetis to study ion channel physiology and biophysics. I contributed to studies on the regulation of NMDA receptor channels and inwardly rectifying potassium (Kir) channels by phosphoinositides and other effectors using *Xenopus laevis* oocyte electrophysiology. My dissertation project involved the study of delta 2 glutamate receptor (GluD2), an atypical glutamate receptor, whose role in cerebellar physiology is increasingly appreciated. I showed that activation of Gq-coupled receptors (i.e. mGluR1) and manipulations of membrane phosphoinositide levels evoke changes in the cell surface localization of the GluD2 receptor.

After being exposed to structure-function studies of ion channels extensively in the Logothetis lab, I pursued my postdoctoral training in structural biology of membrane proteins in the lab of Dr. Filippo Mancia at Columbia University. In 2013, I started studying the bacterial enzyme ArnT, which catalyzes the 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 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). Currently, my research revolves around further characterization of ArnT structure and function by cryo-EM with an emphasis on the elucidation of substrate binding events needed for catalysis.

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 cryo-EM and other techniques, with a focus on: i) bacterial membrane enzymes involved in antibiotic resistance, and ii) eukaryotic receptors relevant to mammalian synaptic physiology and pathology, expanding on earlier work performed during my graduate studies into the physiology and regulation of delta glutamate receptors.

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. 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.

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 (PI: Diomedes Logothetis)
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)
07/2017-06/2019	Associate Research Scientist, Department of Physiology and Cellular Biophysics, Columbia University, New York, NY (PI: Filippo Mancia)
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

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. PMID: 17507574.

(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 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.

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
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.

Completed 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 other techniques. A significant training component in Cryo-EM is included.

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: 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 structures of bacterial proteins and protein-inhibitor complexes, 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, PLoS Biology
2017 Ad-hoc Reviewer, eLife,
2017 Reviewer, NIH Topics in Bacterial Pathogenesis Study Section
2017 Reviewer, Individual Research Grants, Israeli Science Foundation
2018 Ad-hoc Reviewer, eLife, PNAS, PLOS Biology, PLOS ONE
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)
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 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.

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., and Neiditch, M.B. (2019). Structural basis of DSF recognition by its receptor RpfR and its regulatory interaction with the DSF synthase RpfF. **PLoS Biol** 17, e3000123. PubMed 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. (2013b). 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 MJ, Neiditch MB. Rgg protein structure-function and inhibition by cyclic peptide compounds. **Proc Natl Acad Sci U S A**. 2015;112(16):5177-82. PubMed PMID: 25847993.

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

D. Research Support

Current Research Support

R01 GM057720 Dubnau and Neiditch (PIs) 7/1/2019-6/30/2024
NIH/NIGMS
Understanding the mechanism of genetic transformation
Natural transformation is an important mode of horizontal gene transfer in bacteria, contributing to the spread of antibiotic resistance and virulence genes as well as to immune evasion. The major goal of this proposal is to elucidate molecular mechanisms that enable the uptake of environmental DNA.
Role: PI

R01 AI125452 Neiditch and Federle (PIs) 07/19/16 – 06/30/20
NIH/NIAID
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

U19 AI142731 Perlin (PI) 4/1/2019-3/31/2024
NIH/NIAID
Center to develop innovative therapeutics to multidrug resistant high-threat bacterial agents
The goal is to establish a Center of Excellence in Translational Research (CETR) for early drug development targeting multidrug-resistant bacteria of clinical significance that helps reinvigorate the drug development pipeline by identifying novel compounds and developing them as selected optimized Leads and Preclinical Development Candidates (PDC) suitable for downstream preclinical IND enabling studies.
Role: I

R01 GM109259 Waters (PI) 7/1/2015-6/30/2020
NIH/NIGMS
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

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

R41 AI134561 Eakins (PI) 2/7/2018-1/31/2019
NIH/NIAID
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 KasA with enhanced in vivo efficacy as compared to the early lead DG167, in preparation for a Phase II application for pre-clinical studies.
Role: I

U19 AI109713 Perlin (PI) 3/1/2014-2/28/2019
NIH/NIAID
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

R01 GM110444 Cooper (PI) 1/1/2015-12/31/2019
NIH/NIGMS
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

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: Arek Kulczyk

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

POSITION TITLE: Assistant 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)*	Years	FIELD OF STUDY
Jagiellonian University, Poland	M.Sc.	10/92-06/97	Molecular Biology
University of Cambridge – MRC LMB, UK	Ph.D.	10/99-07/03	Biophysics
University of Cambridge – MRC LMB, UK	Postdoctoral	08/03-12/04	Biophysics
Harvard University	Postdoctoral	01/05-02/13	Biophysics

A. Personal Statement

My group (http://kulczyk-lab.cryoemcorp.com) integrates structural approaches, in particular single-particle cryo-EM, single-molecule methods and novel correlative light and electron microscopy (CLEM) techniques to study large macromolecular assemblies with medical relevance. By using the above methods, we expect to reveal mechanistic information that may subsequently facilitate development of novel therapeutics. Currently, our main focus is on cryo-EM structure determination and single-molecule studies of the dynamics of human mitochondrial replisome, and on cryo-EM structure determination of laminin and ribosome-toxin complexes. Prior to joining Rutgers on 09/01/2017, I held a junior faculty appointment at Harvard Medical School, where I determined a cryo-EM structure of the 650-kDa bacteriophage T7 replisome. The structure defines an architecture of the entire replisome, and provides the first such structure of a functioning replication complex assembled on DNA resembling a replication fork. During my postdoctoral studies, also at Harvard, I developed several single-molecule methods combining the flow stretching of DNA and the total-internal reflection fluorescence microscopy to study DNA replication. My graduate work in the MRC Laboratory of Molecular Biology at the University of Cambridge in the UK focused on NMR structure determination and biochemical characterization of DNA ligase III and PAPR, the two medically important proteins involved in human base excision repair.

1. Kulczyk, A.W., Akabayov, B., Lee, S-J., Bostina, M., Berkowitz, S.A., Richardson, C.C. (2012). An interaction between DNA polymerase and helicase is essential for the high processivity of the replisome. *Journal of Biological Chemistry*, 287(46), 39050-39060. PMCID: PMC3493946
2. Kulczyk, A.W., Richardson, C.C. (2012). Molecular interactions in bacteriophage T7 priming complex. *Proceedings of the National Academy of Sciences of the USA*, 109, 9408-9413. PMCID: PMC3386087
3. Geertsema, H.J., Kulczyk, A.W., Richardson, C.C., van Oijen A. (2014). Single-molecule studies of polymerase dynamics and stoichiometry at the bacteriophage T7 replication machinery. *Proceedings of the National Academy of Sciences of the USA*, 111, 4073-4078. PMCID: PMC3964090
4. Kulczyk, A.W., Moeller, A., Meyer P., Sliz, P., Richardson, C.C. (2017). Cryo-EM structure of the replisome reveals multiple interactions coordinating DNA synthesis. *Proceedings of the National Academy of Sciences of the USA*, 114, E1848-E1856. PMC5347612

B. Positions and Employment

1997-1998 Research Assistant**,** Jagiellonian University, Poland
1998 Research Assistant**,** University of London, UK
1999 Research Assistant**,** Northeastern University, MA

2008 Lecturer, Brandeis University, MA
2013-2017 Instructor, Harvard University, MA
2017-Present Assistant Professor, Rutgers University, NJ

Professional Memberships

2009-Present American Chemical Society
2009-Present American Society for Biochemistry and Molecular Biology
2009-Present American Society for Microbiology
2009-Present Biochemical Society
2009-Present Biophysical Society
2013-2014 Protein Society
2018-Present Full Member of Cancer Institute of New Jersey
2019-Present RNA Society

Honors

1995 ERASMUS Scholarship awarded by the European Community - University of Wolverhampton, UK
1998 Jagiellonian University Scholarship - University of London, UK
1999 Association for International Cancer Research Scholarship - University of Cambridge, UK
2005-Present Reviewer for: Nature, Science, Molecular Cell, Nucleic Acids Research, Scientific Reports, Journal of Biological Chemistry, Journal of Molecular Biology, Journal of Structural Biology
2013 Invited Speaker at the Keystone Symposium on DNA Replication and Recombination, Banff, Canada
2014 Invited Speaker at the International Biophysics Congress, Brisbane, Australia
2015 Invited Speaker at the Physical Society meeting, Krakow, Poland
2017 Invited Speaker at the European Physical Society meeting, Podlesice, Poland

C. Contributions to Science

1. My laboratory integrates structural approaches, in particular single-particle cryo-EM, single-molecule methods, and novel CLEM techniques that allow simultaneous visualization of an enzymatic activity and structure determination to study mitochondrial DNA repair and replication. By using the above methods we expect to reveal mechanistic information that may subsequently facilitate development of novel therapeutics. Prior to joining Rutgers, I determined a cryo-EM structure of the 650-kDa bacteriophage T7 replisome. The cryo-EM structure defines an architecture of the entire replisome, and provides the first such structure of a functioning replication complex assembled on DNA resembling a replication fork.

a. Kulczyk, A.W., Akabayov, B., Lee, S-J., Bostina, M., Berkowitz, S.A., Richardson, C.C. (2012). An interaction between DNA polymerase and helicase is essential for the high processivity of the replisome. *Journal of Biological Chemistry*, 287(46), 39050-39060. PMCID: PMC3493946
b. Kulczyk, A.W., Richardson, C.C. (2012). Molecular interactions in bacteriophage T7 priming complex. *Proceedings of the National Academy of Sciences of the USA*, 109, 9408-9413. PMCID: PMC3386087
c. Kulczyk, A.W., Richardson, C.C. (2016). The Replication System of Bacteriophage T7. A book chapter in *The Enzymes,* 39, 89-136. PMID: 27241928
d. Kulczyk, A.W., Moeller, A., Meyer P., Sliz, P., Richardson, C.C. (2017). Cryo-EM structure of the replisome reveals multiple interactions coordinating DNA synthesis. *Proceedings of the National Academy of Sciences of the USA*, 114, E1848-E1856. PMC5347612

2. The transient nature of the protein-protein interactions that occur during synthesis of DNA make it challenging to study the dynamics of these interactions by ensemble-averaging techniques. Together with my collaborators, I developed several single-molecule methods combining the flow stretching of DNA and the total-internal reflection fluorescence (TIRF) microscopy to study bacteriophage T7 replication. The DNA was labeled with quantum dots (QD), and TIRF was used to observe many DNA substrates at a given time. A multi-color imaging scheme was applied to continuously track QD position while stroboscopically observing fluorescently labeled proteins. Using these methods we determined the mechanism of polymerase exchange on leading- and lagging-strand by correlating stoichiometry of individual fluorescently labeled T7 DNA polymerases at the replication fork with DNA synthesis.

a. Loparo, J., Kulczyk, A.W., Richardson, C.C., van Oijen A. (2011). Observing polymerase exchange by simultaneous measurements of replisome structure and function at the single-molecule level. *Proceedings of the National Academy of Sciences of the USA*, 108, 3584-3589. PMCID: PMC3048139
b. Geertsema, H.J., Kulczyk, A.W., Richardson, C.C., van Oijen A. (2014). Single-molecule studies of polymerase dynamics and stoichiometry at the bacteriophage T7 replication machinery. *Proceedings of the National Academy of Sciences of the USA*, 111, 4073-4078. PMCID: PMC3964090
c. Duderstadt, K.E., Geertsema, H.J., Stratmann, S.A., Punter, C.M., Kulczyk, A.W., Richardson, C.C., van Oijen, A.M. (2016). Simultaneous real-time imaging of leading- and lagging-strand synthesis reveals the coordination dynamics of single replisomes. *Molecular Cell,* 64, 1035-1047. PMID: 27889453

3. We developed single-molecule methods for the quantitative measurement of rates and processivities of DNA synthesis catalyzed by individual multi-protein replisomes. Linearized λ DNA is modified to form a structure resembling a replication fork at one end, and by attaching a 2.8 μm magnetic bead to the opposite end of DNA. A constant laminar flow of 3 pN is applied such that the resultant drag on the bead stretches the molecule of λ DNA. At 3 pN the elasticity of DNA is determined by entropic contributions with ssDNA being shorter than dsDNA due to coiling of the ssDNA. Consequently measuring the decrease in length of the DNA molecule can monitor the conversion of dsDNA to ssDNA as a result of leading-strand DNA synthesis. These measurements are accomplished by imaging the bead that is attached to the end of λ DNA, and by tracking the change of its position as a function of time. I applied the above methods to investigate the conformational dynamics of the T7 DNA polymerase-processivity factor interaction, and assessed the 3'-5'-pyrophosphorolysis of a DNA primer by the T7 DNA polymerase in the presence of pyrophosphate.

a. Kulczyk, A.W., Tanner N., Loparo J., Richardson C., van Oijen A. (2010). Direct observation of enzymes replicating DNA using a single-molecule DNA stretching assay. *Journal of Visual Experiments*, 37, id: 1689, doi: 10.3791/1689. PMCID: PMC2913797
b. Akabayov, B., Akabayov, S.R., Lee, S.J., Tabor, S., Kulczyk, A.W., Richardson, C.C. (2010). Conformational dynamics of bacteriophage T7 DNA polymerase and its processivity factor, *Escherichia coli* thioredoxin. *Proceedings of the National Academy of Sciences of the USA*, 107, 15033-15038. PMCID: PMC2930546
c. Akabayov, B., Kulczyk, A.W., Akabayov, S., McLaughlin, L., Theile, C., Beauchamp, B., Richardson, C.C. (2011). Pyrovanadolysis: a pyrophosphorolysis-like reaction mediated by pyrovanadate, Mn2+, and DNA polymerase of bacteriophage T7. *Journal of Biological Chemistry*, 286(33), 29146-29157. PMCID: PMC3190772

4. I also applied single-molecule methods described above to investigate the coupling between the nucleotide hydrolysis and DNA unwinding by T7 DNA helicase, and assessed the subunit composition of the hexameric ring of T7 DNA helicase.

a. Satapathy, A.K., Kulczyk, A.W., Ghosh, S., van Oijen, A.M., Richardson, C.C. (2011). A critical residue for coupling dTTP hydrolysis with DNA unwinding by the helicase of bacteriophage T7. *Journal of Biological Chemistry*, 286(39), 34468-34478. PMCID: PMC3190778
b. Zhang, H., Lee, S-J. Kulczyk, A.W., Zhu, B., Richardson, C.C. (2012). Heterohexamer of 56- and 63-kD helicase-primase of phage T7 in DNA replication. *Journal of Biological Chemistry*, 287(41), 34273-34287. PMCID: PMC3464535

5. I applied NMR, X-ray crystallography and EM for structural analysis of various protein and protein-nucleic acid complexes. I determined NMR structure of the DNA-binding domain from human DNA ligase III, the first structure of the zinc-finger from this group, and investigated its interaction with DNA using chemical shift mapping. In other projects, we applied X-ray crystallography to determine structures of PorZ and vimentin. PorZ is an important component of the type-IX secretion system (T9SS) for secretion of virulence factors from *Porphyromonas gingivalis*. I used negative-stain EM combined with immunogold labeling to visualize the cellular localization of PorZ. I also applied negative-stain EM to image the nucleotide kinase from bacteriophage T7, the first reported kinase that does not require a metal ion for its activity.

a. Kulczyk, A.W., Yang, J., Neuhaus, D. (2004). Solution structure and DNA binding of the zinc finger domain from DNA ligase IIIa. *Journal of Molecular Biology*, 341, 723-738. PMID: 15288782

b. Qimron, U., Kulczyk, A.W., Hamdan, S.M., Tabor, S., Richardson, C.C. (2008). Inadequate inhibition of host RNA polymerase restricts T7 bacteriophage growth on hosts overexpressing udk. *Molecular Microbiology*, 67, 448-457. PMID: 18067538
c. Lasica, A et al. (2016). Structural and functional probing of PorZ, an essential bacterial-surface component of the type-IX secretion system of human oral-microbiomic *P. gingivalis*. *Scientific Reports*, 6, 37708. PMCID: PMC5121618
d. Pang, A., Obiero J., Kulczyk, A.W., Sviripa, V., Tsodikov, O. (2018). A crystal structure of coli 1B of vimentin in the filamentous from provides a model of a high-order assembly of a vimentin filament. *FEBS Journal*, doi: 10.1111/febs. 14585. PMCIS: PMC29905014

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/pubmed/?term=Kulczyk+AW

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

Ongoing Research Support

Busch Biomedical Grant Kulczyk (PI) 09/01/19-08/31/21
Title: Cryo-EM structure determination of the mitochondrial replisome.
The main objectives of the project are: (i) to obtain samples of the mitochondrial replisome suitable for high-resolution data acquisition, and (ii) to determine a structure of the replisome by single-particle cryo-EM.