BIOGRAPHICAL SKETCH

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NAME: Uday Suryan Ganapathy

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POSITION TITLE: Research Assistant Member

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, College of Arts and Sciences, Ithaca, NY	B.A.	05/2008	Biological Sciences (*magna cum laude*)
Weill Cornell Graduate School of Medical Sciences, Cornell University, New York, NY	Ph.D.	05/2015	Immunology and Microbial Pathogenesis
Stony Brook University, Stony Brook, NY	Postdoctoral	07/2017	Chemical Biology, Microbiology
Rutgers University, New Jersey Medical School, Newark, NJ	Postdoctoral	03/2019	Drug Discovery, Microbiology
Hackensack Meridian Health, Center for Discovery and Innovation, Nutley, NJ	Postdoctoral	01/2022	Drug Discovery, Microbiology

A. Personal Statement

I am a microbiologist with 15 years of experience in mycobacteriology, including expertise in bacterial physiology, biochemistry, chemical biology, and most recently, antimycobacterial drug discovery. I am currently a Research Assistant Member at Hackensack Meridian Health's Center for Discovery and Innovation (CDI; Nutley, NJ) where I conduct research in close collaboration with Dr. Thomas Dick. My PhD and early postdoctoral work focused on Tuberculosis (TB), first applying bacterial genetics and later chemical biology to the study of *Mycobacterium tuberculosis*. After joining Dr. Dick's group in 2017, my research focus shifted to non-tuberculous mycobacteria (NTM). I have since led several productive NTM drug discovery projects. This includes an ongoing, productive collaboration with GlaxoSmithKline in which I have tested TB active lead compounds for activity against NTM. This project has led to the identification of several novel drug classes and lead compounds for NTM drug discovery and is supported by **NIH/NIAID R01 AI132374** (*Combatting natural resistance and persistence in non-TB mycobacterial disease*, Thomas Dick PI). Since 2019, I have been leading a project to redesign rifamycins for *M. abscessus* lung disease. This project is an active collaboration with Drs. Thomas Dick, Véronique Dartois, Courtney C. Aldrich, and Richard H. Ebright and is supported by **NIH/NIAID R01 AI177342** (*Optimization of rifamycins to overcome intrinsic resistance of nontuberculous mycobacteria to improve treatment of NTM lung disease*, Thomas Dick PI). As this project's team head, I have overseen the identification and characterization of several C25-modified rifabutin analogs with activity against *M. abscessus* and am coordinating our development of a pre-clinical candidate for *M. abscessus* lung disease. Unexpectedly, I have also found that several C25-modified rifabutin analogs have improved activity against two multidrug-resistant TB (MDR-TB) strains. This promising finding is the basis for the current NIH R21 grant proposal to evaluate the potential of C25-modified rifabutin analogs as a novel strategy to treat MDR-TB. This new project will also be a collaboration with Drs. Dick, Dartois, Aldrich, and Ebright. Given my expertise in rifamycin drug discovery, prior experience in the TB research field, and continued mentorship from Dr. Thomas Dick, I am ideally positioned to lead this project as a PI and expand the applications of C25-modified rifabutin analogs to include combatting TB drug-resistance.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022-Present Research Assistant Member, Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ
2019-2022 Senior Research Associate, Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ
2017-2019 Postdoctoral Research Fellow, New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, NJ
2015-2017 Postdoctoral Associate, Dept. of Pharmacological Sciences, Stony Brook University, Stony Brook, NY

Honors

2016 F1000Research Poster Award, Emerging Paradigms in Drug Discovery and Chemical Biology
2012-2014 Stony-Wold Herbert Fund Fellowship Award for Ph.D. work
2008 *Magna cum laude* for B.A. honors thesis
2007 Cornell Hughes Scholar

C. Contributions to Science

1. Candidate TB Drug Target Characterization and Tool Development for TB Drug Discovery.

M. tuberculosis likely utilizes host fatty acids as a carbon source during infection. Gluconeogenesis is essential for the conversion of fatty acids into biomass. As part of my PhD work in the laboratory of Dr. Sabine Ehrt (Weill Cornell Medicine), I studied the role of gluconeogenesis in *M. tuberculosis* virulence. My project focused on the genetic characterization of GLPX, the only annotated fructose bisphosphatase (FBPase) in the *M. tuberculosis* genome. As an FBPase, GLPX controls the rate-limiting step of gluconeogenesis, making it a potential *M. tuberculosis* virulence factor and a candidate TB drug target. Unexpectedly, my work showed that an *M. tuberculosis* mutant lacking GLPX could grow on gluconeogenic carbon sources and had detectable FBPase activity. Further study showed that the *M. tuberculosis* genome encodes an alternative FBPase (GPM2, Rv3214) that can maintain gluconeogenesis in the absence of GLPX. Consequently, deletion of both GLPX and GPM2 was required for disruption of gluconeogenesis and attenuation of *M. tuberculosis* in a mouse model of infection. My work affirmed the role of gluconeogenesis in *M. tuberculosis* virulence and revealed previously unidentified metabolic redundancy at the FBPase-catalyzed reaction step of the pathway. It also had serious implications for the development of GLPX inhibitors for TB chemotherapy.

To enable the study of mycobacterial periplasmic proteins for TB drug discovery, my postdoctoral work in the laboratory of Dr. Jessica C. Seeliger (Stony Brook University) sought to adapt peroxidase-mediated biotinylation to label periplasmic proteins. My work showed that peroxidase-mediated biotinylation can be performed in mycobacteria and *Escherichia coli*. To eliminate detection artifacts from natively biotinylated mycobacterial proteins, I validated two alternative labeling substrates, tyramide azide and tyramide alkyne, which enabled biotin-independent detection of labeled proteins. This novel application of peroxidase-mediated protein labeling will advance efforts to characterize the role of the periplasm in TB pathogenesis and identify new TB drug targets.

i. **Ganapathy U**, Marrero J, Calhoun S, Eoh H, de Carvalho LPS, Rhee K, Ehrt S. Two enzymes with redundant fructose bisphosphatase activity sustain gluconeogenesis and virulence in Mycobacterium tuberculosis. **Nat Commun**. **2015** Aug 10;6:7912. PubMed Central ID: PMC4535450.
ii. **Ganapathy US**, Bai L, Wei L, Eckartt KA, Lett CM, Previti ML, Carrico IS, Seeliger JC. Compartment-Specific Labeling of Bacterial Periplasmic Proteins by Peroxidase-Mediated Biotinylation. **ACS Infect Dis**. **2018** Jun 8;4(6):918-925. PubMed Central ID: PMC6767932.

2. New Drugs and Drug Targets for *M. abscessus* Lung Disease

Unlike TB, *M. abscessus* lung disease is a highly drug-resistant bacterial infection with no reliable treatment options. *De novo M. abscessus* drug discovery is urgently needed but is hampered by the bacterium's extreme drug resistance profile, leaving the current drug pipeline underpopulated. One proposed strategy to accelerate *de novo M. abscessus* drug discovery is to prioritize screening of advanced TB-active compounds for anti-*M.*

abscessus activity. As part of my postdoctoral work in the laboratory of Dr. Thomas Dick (Rutgers University and Hackensack Meridian Health), I collaborated with GlaxoSmithKline (GSK) to test several advanced TB-active compounds for activity against *M. abscessus*. First, I screened a series of TB-active benzoxaboroles – a class of boron-heterocyclic molecules that target the editing domain of leucyl-tRNA synthetase (LeuRS). This work led to the identification of EC/11770 as a preclinical candidate for *M. abscessus* lung disease. In addition, this work further benefitted *M. abscessus* drug discovery by identifying a new drug class (benzoxaboroles) and drug target (LeuRS). My findings also prompted the study of epetraborole, a non-halogenated benzoxaborole with anti-Gram-negative activity, as a candidate for *M. abscessus* drug development. Indeed, my work showed that epetraborole has *in vitro* activity against the *M. abscessus* complex that was comparable to EC/11770 and was also active in a murine *M. abscessus* infection model.

Fluoroquinolones – the only clinically used DNA gyrase inhibitors – are effective against TB but are in limited clinical use *M. abscessus* lung infections due to intrinsic drug resistance. In this context, I investigated the anti-*M. abscessus* activity of EC/11716, a lead *Mycobacterium tuberculosis* gyrase inhibitor (MGI) developed by GSK. My work showed that EC/11716 has *in vitro* activity against all subspecies of the *M. abscessus* complex and was also active against *M. abscessus* in mice, providing *in vivo* proof-of-concept. Thus, my work identified EC/11716 as an alternative gyrase inhibitor-based drug candidate for *M. abscessus* lung disease that could overcome the present limitations of fluoroquinolones. Taken together, my efforts have helped populate the *M. abscessus* drug pipeline with several new lead compounds while also identifying two new drug classes (benzoxaboroles and MGIs) and a novel drug target (LeuRS) for *M. abscessus* drug discovery.

i. **Ganapathy US**, Del Rio RG, Cacho-Izquierdo M, Ortega F, Lelièvre J, Barros-Aguirre D, Lindman M, Dartois V, Gengenbacher M, Dick T. A Leucyl-tRNA Synthetase Inhibitor with Broad-Spectrum Anti-Mycobacterial Activity. **Antimicrob Agents Chemother**. **2021** Apr 19;65(5):e02420-20 PubMed Central ID: PMC8092876.
ii. **Ganapathy US**, Gengenbacher M, Dick T. Epetraborole Is Active against Mycobacterium abscessus. **Antimicrob Agents Chemother**. **2021** Sep 17;65(10):e0115621. PubMed Central ID: PMC8448144.
iii. **Ganapathy US**, Del Río RG, Cacho-Izquierdo M, Ortega F, Lelièvre J, Barros-Aguirre D, Aragaw WW, Zimmerman MD, Lindman M, Dartois V, Gengenbacher M, Dick T. A Mycobacterium tuberculosis NBTI DNA Gyrase Inhibitor Is Active against Mycobacterium abscessus. **Antimicrob Agents Chemother**. **2021** Nov 17;65(12):e0151421. PubMed Central ID: PMC8597734.
iv. **Ganapathy US**, Dick T. Why Matter Matters: Fast-Tracking Mycobacterium abscessus Drug Discovery. **Molecules**. **2022** Oct 17;27(20) PubMed Central ID: PMC9608607.

3. Redesigning Rifamycins for *M. abscessus* Non-Tuberculous Mycobacteria Lung Disease

The clinical utility of rifamycins against non-tuberculous mycobacterial (NTM) lung disease is limited by intrinsic drug resistance. As part of my postdoctoral work in the laboratory of Dr. Thomas Dick (Rutgers University and Hackensack Meridian Health), I investigated the mechanism of intrinsic rifamycin resistance in *M. abscessus*, the most common rapidly growing NTM pathogen. My work revealed a dual mechanism of intrinsic rifamycin resistance in *M. abscessus* subsp. *abscessus*. First, bacterial monooxygenases can oxidize rifamycins that contain a naphthohydroquinone core (e.g., rifampicin), but this enzymatic oxidation is prevented in rifamycins with a naphthoquinone core (e.g., rifabutin), explaining why rifabutin is 5–10 times more potent than rifampicin against *M. abscessus* subsp. *abscessus*. Second, the ADP-ribosyltransferase Arr_{Mab} can conjugate an ADP-ribose to the C23 hydroxyl group of rifamycins, including rifampicin and rifabutin. In collaboration with Dr. Courtney C. Aldrich (University of Minnesota), we have designed C25-modified rifabutin analogs that block the activity of Arr_{Mab} and are more than 200 times more potent against *M. abscessus* than rifampicin. Recently, I determined the potency of a representative C25-modified rifabutin analog (RFB-5m) against a panel of Arr-positive and Arr-negative species representing a range of rapidly and slowly growing NTM. RFB-5m had strongly enhanced potency against all subspecies of the *M. abscessus* complex, other clinically relevant rapidly growing NTM (*M. chelonae* and *M. fortuitum*), and the slowly growing NTM *M. simiae*, all of which encode an Arr homolog. Therefore, our Arr-tolerant rifamycins in development for *M. abscessus* lung disease can be applied to other Arr-positive NTM. This finding expands the potential therapeutic utility of our novel rifamycins to include several currently difficult-to-cure NTM lung disease pathogens beyond *M. abscessus*.

i. **Ganapathy US**, Lan T, Krastel P, Lindman M, Zimmerman MD, Ho H, Sarathy JP, Evans JC, Dartois V, Aldrich CC, Dick T. Blocking Bacterial Naphthohydroquinone Oxidation and ADP-Ribosylation Improves Activity of Rifamycins against Mycobacterium abscessus. **Antimicrob Agents Chemother. 2021** Aug 17;65(9):e0097821. PubMed Central ID: PMC8370238.
ii. Lan T*, **Ganapathy US***, Sharma S, Ahn YM, Zimmerman M, Molodtsov V, Hegde P, Gengenbacher M, Ebright RH, Dartois V, Freundlich JS, Dick T, Aldrich CC. Redesign of Rifamycin Antibiotics to Overcome ADP-Ribosylation-Mediated Resistance. **Angew Chem Int Ed Engl. 2022** Nov 7;61(45):e202211498. PubMed Central ID: PMC9633546.
iii. **Ganapathy US***, Lan T*, Dartois V, Aldrich CC, Dick T. Blocking ADP-ribosylation expands the anti-mycobacterial spectrum of rifamycins. **Microbiol Spectr. 2023** Sep 8;11(5):e0190023. PubMed Central ID: PMC10580999.
iv. Aldrich, C. C.; Lan, T.; Dick, T.; **Ganapathy, U**.; Dartois, V. "Rifamycins for nontuberculous mycobacteria" **PCT Application** No.PCT/US2023/034573, filed October 5, **2023**.

*Authors contributed equally to this work

A complete list of my published work can be found in MyBibliography at:
https://www.ncbi.nlm.nih.gov/myncbi/uday.ganapathy.1/bibliography/public/