Table of Contents

Application Summary

Competition Details

Competition Title:	P*3 Full Application 2023 Cycle
Category:	
Submission Deadline:	06/30/2023 11:59 PM

Application Information

Application Title:	CF-ReBI3LD: Research into the Biogeography of Inflammation-Infection-Injury and Lung Disrepair
Application ID:	936
Submission Date:	06/28/2023 3:00 PM

Personal Details

PI First Name:	Nael
PI Last Name:	McCarty
PI Email:	namccar@emory.edu
Primary Institution:	Emory University School of Medicine
PI Position Title:	Marcus Professor and CF-AIR Center Director
PI Degree:	PhD
Project Title:	CF-ReBI3LD: Research into the Biogeography of Inflammation-Infection-Injury and Lung Disrepair
MPI 1 Name:	Mike Koval
MPI 1 Primary Institution:	Emory University School of Medicine
MPI 1 Position Title:	Professor of Medicine and Cell Biology
MPI 1 Degree:	PhD
Human Subjects:	No
Vertebrate Animals:	Yes

CF-ReBI^{3}LD: Research into the Biogeography of Inflammation-Infection-Injury and Lung Disrepair

SPECIFIC AIMS Cystic fibrosis (CF) is the second most-common life-shortening genetic disease among American children. Airway damage in the CF lung is focal, not evenly distributed across the respiratory tree. The ***overarching question*** we seek to answer is this: What are the properties of local areas of the CF airway that sensitize them to focal damage and disrepair? This project (CF-ReBI^{3}LD) seeks to support multi-omic Research into the Biogeography of Inflammation-Infection-Injury and Lung Disrepair.

Goals of the Enabled NIH (U01, P01, or large R01) or CFF Collaborative Research Grant Application

Beginning with the end in mind, we describe here the goals of the planned extramural application.

- **Goal 1: Develop experimental platforms to enable research at the cellular, tissue, and whole animal levels.** This will include establishing three different sets of Platforms, as follows. **Track A**: *In Vitro* Models; **Track B**: *Ex Vivo* Models; **Track C**: Longitudinal *In Vivo* Models. These are described below, in Table 1.
- **Goal 2: Determine the topological relationships between mucus accumulation, infection, inflammation, and the recruitment of innate immune cells.**
- **Goal 3: Determine how sites of focal inflammation and infection alter the transcriptional profiles of airway epithelial and mesenchymal cells and cause airway damage and loss of barrier function.**
- **Goal 4: Determine how mutant CFTR predisposes the airway to damage and disrepair.**

Aims of this P*3 Program Planning Grant Application

The major goal of the planning grant is to establish experimental platforms that will enable the future application of high-content approaches to characterize the changes in four cellular compartments at the sites of focal infection and damage: (a) infecting bacteria, (b) airway epithelial cells, (c) the mesenchyme, and (d) transmigrating neutrophils and monocytes/macrophages. This will include multiple applications of mass spectrometry-based metabolomics (both fluid-phase and mass spec imaging), bacterial transcriptomics (RNAseq), spatial transcriptomics of both bacteria and host cells, and scRNAseq of airway epithelium in both *in vivo* and *in vitro* platforms (to determine potential changes in cellular composition of the tissue). These approaches will be used iteratively in downstream experiments as we test the importance of individual genes/proteins in knockdown experiments after those genes/proteins are identified in initial experiments.

(1) Develop and organize the investigative team, listed on the following page, whose members bring diverse expertise and research methodologies to solve the complex problems underlying airway damage.

(2) Continue the development, in the McCarty lab, of Transwell-based models of the airway epithelium, with human airway monolayers (both CF and non-CF) based upon Koval's well differentiated primary human airway cell models [1], then use these models for optical imaging, mass spec imaging, spatial transcriptomics, etc., as described below in Table 1. Test a lung-on-a-chip system (EmulateBio, *see* LoS) and generate versions of the novel 96-well plate microphysiological system developed by Takayama [2, 5, 6] and Tirouvanziam [7, 8] that will enable mass spec imaging and spatial transcriptomics on a mesoscale platform.

(3) In these *in vitro* systems, identify conditions to establish chronic bacterial infection of *S. aureus* and/or *P. aeruginosa* and investigate the structural changes in the infected airway models by light-sheet imaging via the ICI Core, and other approaches. Bacteria will be added apically to establish infection, then neutrophils and monocytes will be added basolaterally. This will enable imaging of transmigrating neutrophils and monocytes in real time, and analysis of accruing damage in the epithelium and mesenchyme.

(4) Using the *Ex Vivo* Porcine Lung model (Diggle lab [3, 4]) and whole murine lung imaging (Azimi lab), we will assess how changes in spatial patterning of bacteria in the airway epithelium leads to changes in the biogeography of the airways, airway injury, and disrepair. These two models enable the establishment of infection over several days. Long term, we will leverage a new collaboration with UPitt Medical Center investigators to extend this method to human lung explants.

(5) Establish chronic murine infection models in new CF mouse models, available from CF-AIR, to identify molecular mechanisms and signaling pathways that lead to structural damage in response to polymicrobial infection; this will enable the future application of MRI for measures of fibrosis in murine infection models in longitudinal studies during the development stages of tissue damage, using Yang's approach [9-12].

OUTCOMES This project will identify genes/proteins/signaling pathways that can be manipulated to circumvent structural damage and/or enhance repair thereof, leading to the development of new therapies or potentially existing therapeutics that can be repurposed to treat CF. Furthermore, lessons learned from this CF-focused project also will be applicable to other pulmonary diseases such as Non-CF bronchiectasis (NCFB) and chronic obstructive pulmonary disease (COPD). Hence, a secondary consequence of this funding may be extension to NCFB, COPD, and other pulmonary disorders to be funded under additional grant proposals.

BACKGROUND AND SIGNIFICANCE TO CHILD HEALTH CF is a genetic disease impacting children of all demographics. There are ~40,000 children and adults living with CF in the USA, a recent increase due to a rapidly growing (and surviving!) adult population. The disease begins *in utero*, and its care becomes the lifelong focus for the patient and their families. This project targets the fundamental basis of life-shortening lung disease in our patients. While CF is a multiorgan disease, lung failure is the main cause of morbidity and mortality. Importantly, while new highly effective modulator therapies (HEMTs) that directly target the primary defect in CF – loss of CFTR function – have greatly improved life expectancy in this population, many people with CF (PwCF) still suffer and die at an early age. These new therapies are not a cure, but are only disease-modifying – slowing many, but not all, aspects of disease. Indeed, the use of HEMTs does not lead to resolution of inflammation and immune dysregulation or clearance of invasive bacteria from the airway; roughly 80% of our children with CF are infected with *S. aureus*, usually along with other pathogens such as *P. aeruginosa*. ***These great successes at the single-protein level have now bought us time, slowing disease so that we can undertake groundbreaking studies to tackle the most important problem: understanding what connects loss of CFTR function to structural lung damage.***

Most research in the CF lung considers the airway as a whole, likely averaging out all of the signals as if the whole lung were impacted equally and homogeneously; this undoubtedly leads to loss of information that is specific to the sites of infection and subsequent damage. New approaches must be applied to understand the focal nature of damage in the CF airway so that new therapies may be designed to target the changes in gene expression, protein function, and inflammatory processes that contribute to bronchiectasis. The biogeography of infection in the CF lung has been studied in two dimensions [13-16], across the epithelium, considering the topological relationship between two sites of infection by bacteria of the same or different species. We will extend biogeography into three dimensions by considering the relationship between those infection sites and the damage that occurs through the depth of the tissue, including the mesenchyme, extracellular matrix, and endothelium. It is a well-accepted fact that it is not the bacteria that kills the CF patient, but the unrelenting host inflammation [17], which is intensified by the presence of chronic pathogens. The robust transmigration [18, 19] of neutrophils and monocytes and their release of proteases in CF lungs generates damage, induces epithelial-mesenchymal transition and fibrosis, and induces signaling pathways that extend inflammation [20, 21]. Airway-resident macrophages normally activate repair pathways, but these are defective in CF lungs [22, 23].

This project seeks to build upon recent successes that have led to deeper understanding of the earliest inciting events underlying lung damage in our youngest patients. Work from the IMPEDE-CF program (led by Drs. Tirouvanziam, Guglani, and Chandler, with funding from CF-AIR, the NIH/NHLBI, the CFF, and philanthropy) has given us the ability to recognize the earliest changes in the airway that underlie structural lung disease, evident by CT imaging, and have established that inflammation in CF airways precedes infection. We propose to build upon this new understanding at the tissue level to identify molecular targets that may serve as the basis of new therapeutic development that will circumvent structural damage, in the lungs of our youngest patients, and perhaps even reverse the damage in the lungs of our older patients. This will require a multidisciplinary approach, from the following muti-investigator team based at Emory (EU, including in the Dept. of Pediatrics, DoP), Georgia Tech (GT), Georgia State (GSU), and the U. Alabama at Birmingham (UAB).

(PI/PD) Nael McCarty, PhD, EU-DoP; airway barrier function, mouse models, *in vitro* airway models
Mike Koval, PhD, EU; epithelial barrier function, regulation by tight junctions, *in vitro* airway models
Steve Diggle, PhD, GT; airway microbiology, *ex vivo* models of infection using lung tissue explants
Sheyda Azimi, PhD, GSU; microbiologist in murine airway infection models, novel microscopy methods
Shuichi Takayama, PhD, GT; biomedical engineer, development of lab-on-a-chip model systems
Rabindra Tirouvanziam, PhD, EU-DoP; airway neutrophils, early CF, extracellular vesicles, inflammation
Ben Kopp, MD, EU-DoP; pediatric CF pulmonologist, airway macrophage biology, inflammation, repair
Vibha Lama, MD, EU, regenerative and fibrotic pathways in lung disease, mesenchymal biology
Viranuj Sueblinvong, MD, EU; adult CF pulmonologist, expert in lung injury, repair, and fibrosis
Arlene Stecenko, MD, EU-DoP; pediatric and adult CF pulmonologist, expert in lung injury and fibrosis
Lokesh Guglani, MD, EU-DoP; pediatric CF pulmonologist, early CF lung disease
Jenny Yang, PhD, GSU; use of MR imaging to study injury and fibrosis in multiple tissues
Camilla Margaroli, PhD; UAB, spatially resolved transcriptomics in the lung
Joshua Chandler, PhD, EU-DoP; metabolomic analysis of airway secretions and blood
Neha Garg, PhD, GT; metabolomic analysis of airway bacteria
Ahmet Coskun, PhD, GT; mass spec imaging, spatial omics in general
Marvin Whiteley, PhD, GT; airway microbiologist, biogeography of polymicrobial infections
Joanna Goldberg, PhD, EU-DoP; airway microbiologist, mouse models of infection

Table 1. Experimental Tracks and Assays (red font identifies objectives for this planning grant)

Track A: *In Vitro* Models

Model Platform or Objective	Assays
A1: Epithelial models in traditional Transwells with 3 μm pores, +/- HEMTs; apical *S.a.* ± *P.a.*, basolateral PMNs and monocytes	
• CRC Airway epithelial cells only	TEER, I-TJ, I-B, I-TMP&M, EMT, CytoS, P&M-TMR, TP&M-FC, TP&M-Tx, MS-M, SRT, MSI-M, MSI-P
• CRC Airway epithelial cells plus mesenchymal cells	Above plus: C-Dep, Sma
• CRC Airway epithelial cells plus mesenchymal plus endothelial cells	Above plus: C-Dep, Sma
A2: Epithelial models in Lung-chip models (Emulate), +/- HEMTs; apical *S.a.* ± *P.a.*, basolateral PMNs and monocytes, Lightsheet optical imaging	
• CRC Airway epithelial cells only	TEER, I-TJ, I-B, I-TMP&M, EMT, CytoS, P&M-TMR, TP&M-FC, TP&M-Tx, MS-M
• CRC Airway epithelial cells with mesenchymal +/- endothelial cells	Above plus: C-Dep, Sma
A3: Generation and testing of 96-well Transwell models, based on Takayama's systems [2] for MSI-M, MSI-P, SRT	
• CRC Airway epithelial cells	MSI-M, MSI-P, SRT
A4: Epithelial models on 96-well systems, +/- HEMTs; apical *S.a.* ± *P.a.*, basolateral PMNs and monocytes	
• CRC Airway epithelial cells only	TEER, I-TJ, I-B, I-TMP&M, EMT, CytoS, P&M-TMR, TP&M-FC, TP&M-Tx, MS-M, SRT, MSI-M, MSI-P
• CRC Airway epithelial cells with mesenchymal +/- endothelial cells	Above plus: C-Dep, Sma

Track B: *Ex Vivo* Models

Model Platform or Objective	Assays
B1: Murine lung tissue explanted from infected mice; hCFTR models +/- HEMTs, SCNN1B-Tg model	
• Acute infection, *S.a.* ± *P.a.* • Chronic infection, *S.a.* ± *P.a.*	I-TJ, I-B, I-TMP&M, EMT, CytoS, MS-M, SRT, MSI-M, MSI-P, C-Dep, Sma, Histology
B2: Murine lung tissue explanted from healthy mice, on 8 μm Transwells, then infected with *S.a.* ± *P.a.*; hCFTR models +/- HEMTs, SCNN1B-Tg model	
• *P.a.* ± *S.a.* added apically 1-7 days • PMNs and monocytes added basolaterally	I-TJ, I-B, I-TMP&M, EMT, CytoS, MS-M, SRT, MSI-M, MSI-P, C-Dep, Sma, Histology
B3: Generation of novel Lung-Chip models, to fit explanted tissues	
• Tested with mouse airway	MSI-P, MSI-M, SRT
B4: Murine lung tissue explanted from healthy mice, on 8 μm Transwells, infected with *S.a.* ± *P.a.*; hCFTR models +/- HEMTs, SCNN1B-Tg model	
• *S.a.* ± *P.a.* added apically 1-7 days • PMNs and monocytes added basolaterally	I-TJ, I-B, I-TMP&M, EMT, CytoS, MS-M, SRT, MSI-M, MSI-P, C-Dep, Sma, Histology
B5: Ferret lung tissue explanted from healthy ferets, on 8 μm Transwells, infected with *S.a.* ± *P.a.*; +/- HEMTs	
• Bacteria added apically 1-7 days • PMNs and monocytes added basolaterally	I-TJ, I-B, I-TMP&M, EMT, CytoS, MS-M, SRT, MSI-M, MSI-P, C-Dep, Sma, Histology
B6: Murine lung tissue explanted from healthy mice, on 8 μm Transwells, infected with *S.a.* ± *P.a.*; hCFTR models +/- HEMTs, SCNN1B-Tg model	
• Bacteria added apically 1-7 days • PMNs and monocytes added basolaterally	I-TJ, I-B, I-TMP&M, EMT, CytoS, MS-M, SRT, MSI-M, MSI-P, C-Dep, Sma, Histology
B7: Generate new breed of mice by crossing hCFTR model with SCNN1B-Tg model, to add CFTR mutation to muco-obstructive phenotype; then use with platforms in B1, B2, B4, B6.	

KEY

Assays:

TEER: Transepithelial electrical resistance

I-TJ: Imaging of tight junctions

I-B: Imaging of bacteria

I-TMP&M: Imaging of transmigrating PMNs and monocytes

EMT: Evidence of epithelial-mesenchymal transition

CytoS: Secretion of cytokines and inflammatory mediators in apical or basolateral medium, or bronchoalveolar lavage fluid and blood

P&M-TMR: P&M transmigration rate

TP&M-FC: Flow cytometry analysis of transmigrated PMNs and monocytes

TP&M-Tx: Transcriptomic analysis of transmigrated PMNs and monocytes

MS-M: Untargeted MS metabolomics of apical and basolateral medium, or bronchoalveolar lavage fluid and blood

SRT: Spatially resolved transcriptomics

MSI-M: Mass spec imaging – metabolomics

MSI-P: Mass spec imaging – proteomics

C-Dep: Collagen deposition

Sma: Abundance of smooth-muscle actin

C-MRI: ProCA32.collagen^{+} MRI

Other Abbreviations:

HEMTs: Highly effective CFTR modulator therapies

S.a.: *Staphylococcus aureus*

P.a.: *Pseudomonas aeruginosa*

CRC: Conditional reprogramming culture

PMNs: Neutrophils

hCFTR: Mice expressing humanized CFTR

SCNN1B-Tg: Mice overexpressing ENaC β-subunit; muco-obstructive model

Track C: Longitudinal *In Vivo* Models

Model Platform	Assays
C1: Mice with humanized CFTR, infected intratracheally with *S.a.* ± *P.a.*; +/- HEMTs	
• F_{508}del-CFTR, G551D-CFTR	C-MRI, CytoS
C2: Ferrets with mutant CFTR, infected intratracheally with *S.a.* ± *P.a.*; +/- HEMTs	
• G551D-CFTR	C-MRI, CytoS
C3: Humans, with endogenous infections with a variety of airway bacteria; +/- HEMTs	
• Various CF-associated CFTR genotypes	C-MRI, CytoS

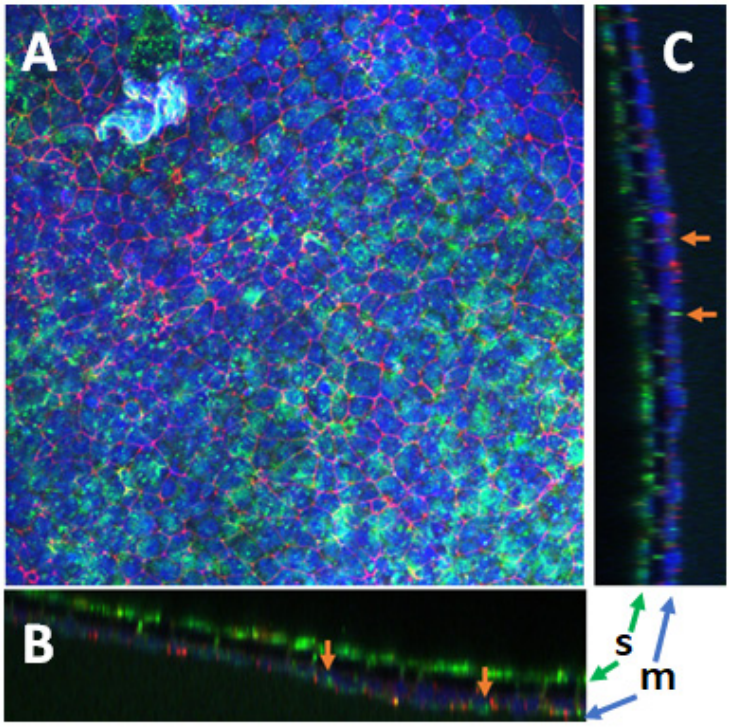

Fig 2. Confocal images of neutrophils transmigrating through a monolayer of airway epithelial cells, on a Transwell with 3 µm pores. **(A)** Overlay image. Red: ZO-1 staining, showing the location of tight junctions. Green: CD35 staining, showing the location of neutrophils. Blue: DAPI staining for nuclei. **(B)** XZ, and **(C)** YZ views of overlay image showing neutrophils transmigrating through the monolayer as highlighted by the orange arrows. **s**: serosal side of Transwell, to which neutrophils are added. **m**: mucosal side, including epithelium.

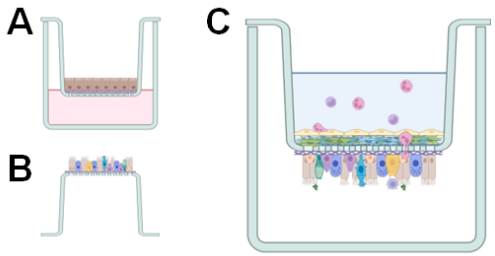

Fig 1. Transwell models. (A) Traditional Transwell model (0.3 µm pores) with standard epithelial cells cultured on collagen coating, at Air/Liquid Interface. **(B)** Inverted Transwell model with large pores (3 µm) to enable transmigration of neutrophils and monocytes, shown using CRC [1] epithelial cells that construct a complex monolayer of all relevant cell types. **(C)** Inverted model of CRC epithelial cells at Air/Liquid Interface, generated as in (B) with addition of mesenchymal and endothelial cells, reconstructing full tissue. Bacteria added to the mucosal surface, then neutrophils and monocytes are added to the serosal surface. Serosal side up, mucosal side down.

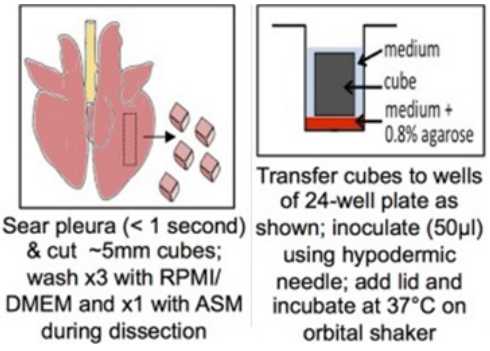

Fig 3. *Ex Vivo* Lung Explants. Model developed by the Diggle lab, first in the porcine lung. Can be inoculated with bacteria enabling chronic infection, > 7 days. ASM: artificial sputum medium. [4]

Box 1. Existing Tools That Will Be Leveraged to Develop Novel Models of Lung Injury and Disrepair

In vitro models based upon Transwells with various pore sizes and various arrangements of the mesenchyme/lamina propria and epithelial/endothelial interfaces to enable transmigration of neutrophils and monocytes (Fig. 1, 2), including a 96-well miniaturized version developed by Takayama [2].

CRC epithelial cells, derived from airway brushings of our patients and non-CF subjects, reprogrammed to express all cell types making up the complex airway monolayer, which are stable over many passages (Fig. 1B,C). [1] Cells expressing mutant CFTR, or wildtype CFTR as *controls* for *in vitro* platforms. Cells are already on-hand.

Ex vivo lung models, developed by Diggle, which provide the entire airway in culture, lasting > 7 days (Fig. 3). [3]

SCNN1B-Tg mice expressing β subunit of the ENaC sodium channel, inducing muco-obstructive phenotype, available via the CF Animal Models Core of CF-AIR.

Mice expressing humanized CFTR (hCFTR), including disease-associated mutants that respond to HEMTs *in vivo*.

Non-CF animals on the same genetic background, as *controls* for *in vivo* platforms.

Ferret models expressing G551D-CFTR mutant that is responsive to HEMTs *in vivo*, located at GaState and UGA as part of other funded grants.

Human lung explants (CF or non-CF) from colleagues at the University of Pittsburgh CF program.

Patient-derived bacterial isolates from the CF-Biospecimen Repository (part of the CF Discovery Core of CF-AIR).

DESCRIPTION OF PLANNING GRANT ACTIVITIES

As noted above, the primary goals of this planning grant are to both build the team to refine the plan for the large extramural grant application, and to develop key preliminary data that will be required to show momentum and feasibility for the extramural application.

Detailed Timeline and Milestones of Planning Grant

(a) Continue gathering team, developing goals of programmatic extramural grant. Establish the **administrative structure for the programmatic grant** to include at least the following faculty: McCarty, Koval, Stecenko, Tirouvanziam, Kopp, and Lama. Dr. Kymry Jones (Associate Director, CF@LANTA) will serve as Program Manager. We have begun these discussions by ZOOM/hybrid meetings thus far (and this already has had good consequences including establishing new collaborations between team member labs), but we would propose to additionally host a quarterly in-person gathering over lunch. At these sessions, each of the teams focusing on experimental platforms (Table 1) and experimental goals (Aims page) would be asked to present their progress. This will lead to refinement of the plan for the extramural grant application.

(b) Continue development of the *in vitro* model systems based upon well differentiated human airway epithelial cell cultures in Transwells, to be led by McCarty and Koval. This builds upon work already underway that has been supported by a CF Fdn. grant ending this year. (Table 1, A1)

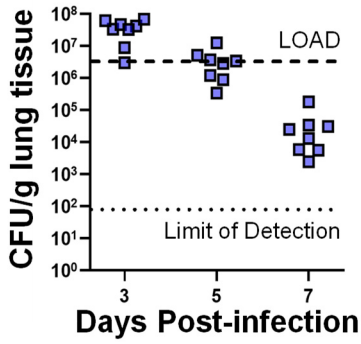

Fig 4. Murine chronic infection model development. Scatterplot of lung homogenate CFUs from individual mice (n=8 per group) infected with biofilm-adapted *P. a.* (Goldberg lab)

(c) Rent commercially available Emulate lab-on-a-chip system, using chips designed for lung biology studies. (See quote attached to Budget Justification). This will be led by the Koval lab at Emory, so that we can test the extension of the airway cell models to traditional chips for optical evaluation in real time during infection, neutrophils/monocytes transmigration, and development of tissue damage, and untargeted MS metabolomics (Chandler). (Table 1, A2)

(d) Develop new systems for application of high-content experimental studies such as MS imaging and spatially resolved transcriptomics. This will be led by Takayama for system construction, and Coskun [24-28] and Margaroli [29, 30] for MSI-M and SRT, respectively, with help from 10x Genomics (see LoS). (Table 1, A3)

(e) Develop *ex vivo* model systems using tissue from mice that have received intratracheal infection over at least five days, which we already have achieved (Fig. 4). This will be led by the McCarty lab in collaboration with Diggle. (Table 1, B1).

(f) Develop chronic infection model in F_{508}del-hCFTR mice or SCNN1B-Tg mice, to at least 2 weeks duration (already IACUC-approved). This will be led by McCarty, working with Dr. Guiying Cui (Research Assistant Professor, Dept. of Pediatrics, affiliated with the McCarty lab), who has led the development of multiple studies in CF mouse models. At the same time, we will continue to work with the Azimi lab to extend their novel approaches for imaging bacterial infection in murine lung tissues, to determine if these can be applied to the *ex vivo* platforms described above.

(g) Throughout this process, we will be in frequent communication with Drs. Marrah Lachowicz-Scroggins and Roy Sutliff, Program Directors, NHLBI, and Dr. Patrick Thibodeau, VP for Basic Science Research, CFF.

(g) Finalize structure of extramural proposal by May 1, 2024, including budgets. Through this time, we will work with Dr. Julie Hawk as grants consultant.

(h) Achieving these initial experimental goals will involve labs at Emory (McCarty, Koval, Chandler, Kopp, Tirouvanziam), Georgia Tech (Takayama, Diggle, Coskun), GaStateU (Azimi), and UAB (Margaroli). Expenses will include for lab personnel (including Cui and Cegla), initial costs for lab-on-a-chip system (a rental from Emulate, see quote attached with Budget), components for generation of 96-well plate Transwell systems, airway epithelial cell culture, neutrophil/monocyte isolation from human blood, initial MSI-M and SRT experiments, to show feasibility, and mouse models from the CF-AIR Animal Models Core. All independent faculty are cost-sharing their FTE and thus are not included in the budget.

Timeline for submission of Extramural Grant: NHLBI Program Officer advises us that relevant mechanisms for a project of this scope include: P01, U19, U54, RC2, large R01 (over $500k direct costs per year), and RM1, among others. The CF Foundation also provides multiple appropriate mechanisms including the Path to a Cure Collaborative Research Grant, allowing direct costs ≤ $1M per year for three years. We may also attempt an ARPA-H submission, based on the Broad Agency Announcement 3/15/23.

Deadlines: CF Fdn: July 2024; NIH P01, etc.: Standard dates apply. Would target October 2024.

REFERENCES CITED

1. Morgan, R., C. Manfredi, K.F. Easley, L.D. Watkins, W.R. Hunt, S.L. Goudy, E.J. Sorscher, M. Koval, and S.A. Molina, *A medium composition containing normal resting glucose that supports differentiation of primary human airway cells.* Sci Rep, 2022. **12**(1): p. 1540.
2. Viola, H., K. Washington, C. Selva, J. Grunwell, R. Tirouvanziam, and S. Takayama, *A High-Throughput Distal Lung Air-Blood Barrier Model Enabled By Density-Driven Underside Epithelium Seeding.* Adv Healthc Mater, 2021. **10**(15): p. e2100879.
3. Harrison, F., A. Muruli, S. Higgins, and S.P. Diggle, *Development of an ex vivo porcine lung model for studying growth, virulence, and signaling of Pseudomonas aeruginosa.* Infect Immun, 2014. **82**(8): p. 3312-23.
4. Harrison, F. and S.P. Diggle, *An ex vivo lung model to study bronchioles infected with Pseudomonas aeruginosa biofilms.* Microbiology (Reading), 2016. **162**(10): p. 1755-1760.
5. Kamm, R.D., R. Bashir, N. Arora, R.D. Dar, M.U. Gillette, L.G. Griffith, M.L. Kemp, K. Kinlaw, M. Levin, A.C. Martin, T.C. McDevitt, R.M. Nerem, M.J. Powers, T.A. Saif, J. Sharpe, S. Takayama, S. Takeuchi, R. Weiss, K. Ye, H.G. Yevick, and M.H. Zaman, *Perspective: The promise of multi-cellular engineered living systems.* APL Bioeng, 2018. **2**(4): p. 040901.
6. Mertz, D.R., T. Ahmed, and S. Takayama, *Engineering cell heterogeneity into organs-on-a-chip.* Lab Chip, 2018. **18**(16): p. 2378-2395.
7. Forrest, O.A., S.A. Ingersoll, M.K. Preininger, J. Laval, D.H. Limoli, M.R. Brown, F.E. Lee, B. Bedi, R.T. Sadikot, J.B. Goldberg, V. Tangpricha, A. Gaggar, and R. Tirouvanziam, *Frontline Science: Pathological conditioning of human neutrophils recruited to the airway milieu in cystic fibrosis.* J Leukoc Biol, 2018. **104**(4): p. 665-675.
8. Dobosh, B., V.D. Giacalone, C. Margaroli, and R. Tirouvanziam, *Mass production of human airway-like neutrophils via transmigration in an organotypic model of human airways.* STAR Protoc, 2021. **2**(4): p. 100892.
9. Xue, S., J. Qiao, J. Jiang, K. Hubbard, N. White, L. Wei, S. Li, Z.R. Liu, and J.J. Yang, *Design of ProCAs (protein-based Gd(3+) MRI contrast agents) with high dose efficiency and capability for molecular imaging of cancer biomarkers.* Med Res Rev, 2014. **34**(5): p. 1070-99.
10. Xue, S., H. Yang, J. Qiao, F. Pu, J. Jiang, K. Hubbard, K. Hekmatyar, J. Langley, M. Salarian, R.C. Long, R.G. Bryant, X.P. Hu, H.E. Grossniklaus, Z.R. Liu, and J.J. Yang, *Protein MRI contrast agent with unprecedented metal selectivity and sensitivity for liver cancer imaging.* Proc Natl Acad Sci U S A, 2015. **112**(21): p. 6607-12.
11. Salarian, M., R.C. Turaga, S. Xue, M. Nezafati, K. Hekmatyar, J. Qiao, Y. Zhang, S. Tan, O.Y. Ibhagui, Y. Hai, J. Li, R. Mukkavilli, M. Sharma, P. Mittal, X. Min, S. Keilholz, L. Yu, G. Qin, A.B. Farris, Z.R. Liu, and J.J. Yang, *Early detection and staging of chronic liver diseases with a protein MRI contrast agent.* Nat Commun, 2019. **10**(1): p. 4777.
12. Salarian, M., O.Y. Ibhagui, and J.J. Yang, *Molecular imaging of extracellular matrix proteins with targeted probes using magnetic resonance imaging.* Wiley Interdiscip Rev Nanomed Nanobiotechnol, 2020. **12**(4): p. e1622.
13. Azimi, S., G.R. Lewin, and M. Whiteley, *The biogeography of infection revisited.* Nat Rev Microbiol, 2022. **20**(10): p. 579-592.
14. Armbruster, C.R., C.W. Marshall, A.I. Garber, J.A. Melvin, A.C. Zemke, J. Moore, P.F. Zamora, K. Li, I.L. Fritz, C.D. Manko, M.L. Weaver, J.R. Gaston, A. Morris, B. Methe, W.H. DePas, S.E. Lee, V.S. Cooper, and J.M. Bomberger, *Adaptation and genomic erosion in fragmented Pseudomonas aeruginosa populations in the sinuses of people with cystic fibrosis.* Cell Rep, 2021. **37**(3): p. 109829.
15. Barraza, J.P. and M. Whiteley, *A Pseudomonas aeruginosa Antimicrobial Affects the Biogeography but Not Fitness of Staphylococcus aureus during Coculture.* mBio, 2021. **12**(2): p. e00047-21.
16. Whiteson, K.L., B. Bailey, M. Bergkessel, D. Conrad, L. Delhaes, B. Felts, J.K. Harris, R. Hunter, Y.W. Lim, H. Maughan, R. Quinn, P. Salamon, J. Sullivan, B.D. Wagner, and P.B. Rainey, *The upper respiratory tract as a microbial source for pulmonary infections in cystic fibrosis. Parallels from island biogeography.* Am J Respir Crit Care Med, 2014. **189**(11): p. 1309-15.
17. Law, S.M. and R.D. Gray, *Neutrophil extracellular traps and the dysfunctional innate immune response of cystic fibrosis lung disease: a review.* J Inflamm (Lond), 2017. **14**: p. 29.
18. Margaroli, C., D. Moncada-Giraldo, D.A. Gulick, B. Dobosh, V.D. Giacalone, O.A. Forrest, F. Sun, C. Gu, A. Gaggar, H. Kissick, R. Wu, G. Gibson, and R. Tirouvanziam, *Transcriptional firing represses bactericidal activity in cystic fibrosis airway neutrophils.* Cell Rep Med, 2021. **2**(4): p. 100239.

19. Ford, B.D., D. Moncada Giraldo, C. Margaroli, V.D. Giacalone, M.R. Brown, L. Peng, and R. Tirouvanziam, *Functional and Transcriptional Adaptations of Blood Monocytes Recruited to the Cystic Fibrosis Airway Microenvironment In Vitro.* Int J Mol Sci, 2021. **22**(5): p. 2530.
20. Twaddell, S.H., K.J. Baines, C. Grainge, and P.G. Gibson, *The Emerging Role of Neutrophil Extracellular Traps in Respiratory Disease.* Chest, 2019. **156**(4): p. 774-782.
21. Hoenderdos, K., K.M. Lodge, R.A. Hirst, C. Chen, S.G. Palazzo, A. Emerenciana, C. Summers, A. Angyal, L. Porter, J.K. Juss, C. O'Callaghan, E.R. Chilvers, and A.M. Condliffe, *Hypoxia upregulates neutrophil degranulation and potential for tissue injury.* Thorax, 2016. **71**(11): p. 1030-1038.
22. Assani, K., C.L. Shrestha, F. Robledo-Avila, M.V. Rajaram, S. Partida-Sanchez, L.S. Schlesinger, and B.T. Kopp, *Human Cystic Fibrosis Macrophages Have Defective Calcium-Dependent Protein Kinase C Activation of the NADPH Oxidase, an Effect Augmented by Burkholderia cenocepacia.* J Immunol, 2017. **198**(5): p. 1985-1994.
23. Jaganathan, D., E.M. Bruscia, and B.T. Kopp, *Emerging Concepts in Defective Macrophage Phagocytosis in Cystic Fibrosis.* Int J Mol Sci, 2022. **23**(14): p. 7750.
24. Cai, S., T. Hu, M. Venkatesan, M. Allam, F. Schneider, S.S. Ramalingam, S.Y. Sun, and A.F. Coskun, *Multiplexed protein profiling reveals spatial subcellular signaling networks.* iScience, 2022. **25**(9): p. 104980.
25. Ganesh, S., T. Hu, E. Woods, M. Allam, S. Cai, W. Henderson, and A.F. Coskun, *Spatially resolved 3D metabolomic profiling in tissues.* Sci Adv, 2021. **7**(5): p. eabd0957.
26. Kumar, A., S. Cai, M. Allam, S. Henderson, M. Ozbeyler, L. Saiontz, and A.F. Coskun, *Single-Cell and Spatial Analysis of Emergent Organoid Platforms.* Methods Mol Biol, 2023. **2660**: p. 311-344.
27. Venkatesan, M., N. Zhang, B. Marteau, Y. Yajima, N.O. De Zarate Garcia, Z. Fang, T. Hu, S. Cai, A. Ford, H. Olszewski, A. Borst, and A.F. Coskun, *Spatial subcellular organelle networks in single cells.* Sci Rep, 2023. **13**(1): p. 5374.
28. Zhou Fang, A.J. Ford, T. Hu, N. Zhang, A. Mantalaris, and A.F. Coskun, *Subcellular spatially resolved gene neighborhood networks in single cells.* Cell Reports Methods, 2023. **3**: p. 100476.
29. Margaroli, C., P. Benson, M.G. Gastanadui, C. Song, L. Viera, D. Xing, J.M. Wells, R. Patel, A. Gaggar, and G.A. Payne, *Spatial transcriptomic profiling of coronary endothelial cells in SARS-CoV-2 myocarditis.* Front Med (Lausanne), 2023. **10**: p. 1118024.
30. Margaroli, C., T. Fram, N.S. Sharma, S.B. Patel, J. Tipper, S.W. Robison, D.W. Russell, S.D. Fortmann, M.M. Banday, Y. Soto-Vazquez, T. Abdalla, S. Saitornuang, M.C. Madison, S.M. Leal, Jr., K.S. Harrod, N.B. Erdmann, and A. Gaggar, *Interferon-dependent signaling is critical for viral clearance in airway neutrophils.* JCI Insight, 2023. **8**(10): p. e167042.

BUDGET JUSTIFICATION – CF-ReBI^{3}LD

A. Key Personnel for the Subsequent Extramural Application:

Nael McCarty, PhD, Emory; airway barrier function, mouse models, *in vitro* airway models
Mike Koval, PhD, Emory; epithelial barrier function, regulation by tight junctions, *in vitro* airway models
Steve Diggle, PhD, GaTech; airway microbiology, *ex vivo* models of infection using lung tissue explants
Sheyda Azimi, PhD, GaState; microbiologist in murine airway infection models, novel microscopy methods
Shuichi Takayama, PhD, GaTech; biomedical engineer, development of lab-on-a-chip model systems
Rabindra Tirouvanziam, PhD, Emory; airway neutrophils, early CF, extracellular vesicles, inflammation
Ben Kopp, MD, Emory; pediatric CF pulmonologist, airway macrophage biology, inflammation, repair
Vibha Lama, MD, Emory; regenerative and fibrotic pathways in lung disease, mesenchymal biology
Viranuj Sueblinvong, MD, Emory; adult CF pulmonologist, expert in lung injury, repair, and fibrosis
Arlene Stecenko, MD, Emory; pediatric and adult CF pulmonologist, expert in lung injury and fibrosis
Lokesh Guglani, MD, Emory; pediatric CF pulmonologist, early CF lung disease
Jenny Yang, PhD, GaState; use of MR imaging to study injury and fibrosis in multiple tissues
Camilla Margaroli, PhD, UAB; spatially resolved transcriptomics in the lung
Joshua Chandler, PhD, Emory; metabolomic analysis of airway secretions and blood
Neha Garg, PhD, GaTech; metabolomic analysis of airway bacteria
Ahmet Coskun, PhD, GaTech; mass spec imaging, spatial omics in general
Marvin Whiteley, PhD, GaTech; airway microbiologist, biogeography of polymicrobial infections
Joanna Goldberg, PhD, Emory; airway microbiologist, mouse models of infection

B. Key Personnel for this P*3 Planning Grant:

1) **Nael A. McCarty, Ph.D., PI/PD (1.2 calendar months, cost-shared).** Dr. McCarty is the Marcus Professor of Cystic Fibrosis (full Professor), Department of Pediatrics, Emory University School of Medicine, and is Associate Chief for Research in the Division of Pulmonology, Asthma, Cystic Fibrosis, and Sleep. Dr. McCarty also is the Director of ***CF@LANTA***: the Emory+Children's CF Center of Excellence, which is the comprehensive Cystic Fibrosis program that includes the institutionally supported Center for Cystic Fibrosis and Airways Disease Research (***CF-AIR***). Dr. McCarty has a history of research in studying CFTR, its role in airway epithelial cells, and the consequences of its mutation at a variety of physiological levels; he also has a history of both clinical research and studies of receptor-mediated signaling. Relevant to the present proposal, Dr. McCarty will be responsible for overseeing the administration of the proposed research program, including leading the multidisciplinary team of investigators at multiple institutions in the planning of the extramural proposal. Dr. McCarty initiated the present project, and he has led its development thus far. It grows out of multiple streams of research within the CF-AIR team, including: (a) the collaboration between Drs. McCarty and Koval on the impact of mutant CFTR on epithelial barrier function; (b) the collaboration between Drs. Tirouvanziam, Chandler, and Guglani in identifying the earliest markers of airway disease in CF; (c) the collaboration between Drs. McCarty, Koval, and Sueblinvong on identifying markers of fibrosis and injury in the CF airway; and (d) the collaboration between Drs. Whiteley, Goldberg, and McCarty, to develop a chronic infection model. Experiments within the McCarty lab, *per se*, will include the work of Dr. Guiying Cui and doctoral student Mrs. Analia Vazquez Cegla. Dr. McCarty will oversee the work of all team members. He will ensure robust communication between the 18 faculty members involved, as they plan the extramural application and develop preliminary data to show feasibility; all are members of CF-AIR, or are in process of becoming members.

2) **Mike Koval, Ph.D., (0.6 calendar months, cost-shared).** Dr. Koval is full Professor in the Department of Medicine at the Emory University School of Medicine, and is Associate Chief for Research in the Division of Pulmonary, Allergy, and Critical Care Medicine. A cell biologist by training, Dr. Koval is an international authority on the role of tight junction proteins in regulating barrier function in multiple epithelia, including the lung. Dr. Koval has collaborated with Dr. McCarty over several years on their CF and CFRD projects, as noted in our publication record. This has included work both in epithelial cells and in the development of the CFRD mouse model. Dr. Koval will serve as Project Director in Dr. McCarty's absence.

3) **Guiying Cui, M.D./Ph.D., Assistant Professor (3.3 calendar months).** Dr. Cui is Assistant Professor of Pediatrics in the Emory University School of Medicine, and is an outstanding molecular physiologist, a top experimentalist. She has been associated with the McCarty lab for ~19 years and has expertise that extends from molecular biology to electrophysiology. Dr. Cui has spent the past three years developing Transwell assays to enable study of the interactions between transmigrating neutrophils/monocytes and the airway epithelial cells. Much of the data that has enabled this Planning grant came from her hands. She will play a crucial role in the continued development of Transwell-based systems, and will also test the extension of these systems to the lung-on-a-chip models that make use of the Emulate platform.

4) **Rabindra Tirouvaziam, Ph.D., Associate Professor (0.6 calendar months, cost-shared).** Dr. Tirouvanziam is well known in the CF research community as the "Prince of the CF Neutrophil." He was the first faculty member recruited to the Center for CF Research (before we became CF-AIR), and was targeted for recruitment due to his desire to contribute to an understanding of the systems biology of the CF airway. In this regard, he brings outstanding expertise in studying the linkage between innate immunity, infection, and disrepair in the airway tissue. It was he who established the first *in vitro* models enabling the study of neutrophil transmigration across airway epithelial cells, and he has been instrumental in the McCarty lab's work noted above where these models have been expanded upon.

5) **Joshua Chandler, Ph.D., Assistant Professor (0.6 calendar months, cost-shared).** Dr. Chandler is a junior faculty member hired for his expertise in both targeted and untargeted metabolomics, particularly as relates to redox signaling, NADPH biology, and Cystic Fibrosis. Dr. Chandler has special expertise in generating high-content data from (primarily) metabolomics approaches, as well as in bioinformatics approaches required for interpretation of the high-content data from such omics approaches. He characterizes the oxidant landscape in airway diseases in great detail and investigates the roles of different oxidants in disease pathogenesis. He also has developed some novel approaches for analyzing both redox markers (e.g., glutathione) and glucose in either BAL fluid or exhaled breath condensate, highly relevant to our team's other projects on CF-related diabetes. He will be particularly important for bulk metabolomics assays of fluids from the mucosal and serosal sides of the lung-on-a-chip platforms, upon either infection or transmigration.

6) **Ben Kopp, M.D., Associate Professor (0.6 calendar months, cost-shared).** Dr. Kopp is the most recent recruit to the CF-AIR team in the PACS Division of Pediatrics. He is expert in the role of the monocyte/macrophage in the CF lung, particularly in the repair mechanisms that tissue-resident macrophages provide in the non-CF lung that are missing in the CF lung. Dr. Kopp is thus an expert in the immunology of the airway, and will provide guidance with respect to extending the airway models to include monocytes/macrophages.

7) **Shu Takayama, Ph.D., Professor (0.6 calendar months, cost-shared).** Dr. Takayama is Professor of Biomedical Engineering, based on the Georgia Tech campus, and is a Georgia Research Alliance Eminent Scholar and Price Gilbert, Jr. Chair in Regenerative Engineering and Medicine. Dr. Takayama has a background in engineered microphysiological systems including the first lung-on-a-chip system. Working with Dr. Tirouvanziam, the Takayama lab has recently developed a method to produce Transwell 96-well-based air-blood barriers with an underside epithelia in a high-throughput manner using flotation-based cell seeding. He also has experience in high throughput lung fibroblast cell culture systems as well as in engineering biomaterials that mimic neutrophil extracellular traps to study their contributions to lung disease. In this proposal, he will further develop the air-blood barrier array for CF studies by incorporating the appropriate primary lung cells and CF relevant bacteria while also testing the latest CF drugs.

8) **Ahmet Coskun, Ph.D., Assistant Professor (0.6 calendar months, cost-shared).** Dr. Coskun is an Assistant Professor of Biomedical Engineering on the Georgia Tech campus, and is the Bernie Marcus Early-Career Professor in Therapeutic Cell Characterization and Manufacturing. He directs an interdisciplinary team at the Single Cell Biotechnology Laboratory (SCBL) at Georgia Tech, which develops purpose-driven multiplex bioimaging technologies that can visualize two/three-dimensional (2D/3D) spatial heterogeneity of biological systems at the single cell and subcellular level. Dr. Coskun will mainly be responsible for spatial omics assays and machine learning data analysis of the tissue specimens from the *in vitro* and *ex vivo* models, using single cell molecular analysis.

9) **Camilla Margaroli Bell, Ph.D., Assistant Professor (0.6 calendar months, cost-shared).** Dr. Bell is Assistant Professor of Pathology in the School of Medicine at the University of Alabama at Birmingham. She is a recent graduate of Emory's Immunology and Molecular Pathogenesis doctoral program, where she received her training in the lab of Dr. Tirouvanziam. She then received postdoctoral training at UAB with Dr. Amit Gaggar, focusing on the immunology of the CF airway. She has the necessary skills to conduct transcriptional analyses, including the acquisition of the necessary expertise to conduct spatial transcriptomics experiments using the Nanostring GeoMx platform, and that equipment is housed in her new lab at UAB. She will bring this expertise to our study of the immune pathological response to CF in the lung.

10) **Steve Diggle, Ph.D., Professor (0.6 calendar months, cost-shared).** Dr. Diggle is Professor of Biological Sciences at Georgia Tech; McCarty and CF-AIR were involved in his recruitment to this department in 2015. Dr. Diggle is a microbiologist, with special focus on understanding bacterial-bacterial communications, especially as relates to cystic fibrosis infections. Dr. Diggle has shown that the phenotypic and genotypic diversity in *Pseudomonas aeruginosa* is driven by recombination and that this has implications for antibiotic resistance and susceptibility testing. Dr. Diggle established the *Ex Vivo* Lung Explant model, first working with porcine lung tissue, to enable the study of long-term (7-10 days) interactions between airway microbes and the lung epithelium. Dr. Diggle will be crucial for developing these models for the proposed work. Dr. Diggle also currently serves as the Director of the Center for Microbial Dynamics and Infection at Georgia Tech, which partners strongly with CF-AIR in all of our team grant applications.

11) **Sheyda Azimi, Ph.D., Assistant Professor (0.6 calendar months, cost-shared).** Dr. Azimi is a newly minted independent faculty member in the Department of Biology at Georgia State University. She received her Ph.D. with CF-AIR member Dr. Sam Brown in England, and then began her postdoctoral training with Dr. Steve Diggle before moving with the Diggle lab to Atlanta in 2015. In her most recent work, including in her own lab, Dr. Azimi has developed a framework to study the effects of microbial population heterogeneity on pathogenesis, microbiogeography, and host-pathogen interactions, including new methods to explore the relationship between pathogens infecting the airway and subsequent tissue damage. Dr. Azimi will work with Drs. McCarty and Cui to analyze tissue from CF mouse models of infection, thus playing a critical role in the development of the *In Vivo* Platforms.

Note: Biosketches for all faculty engaged in the P*3 Planning Grant are provided.
Note: No faculty salary is requested within the P*3 budget.

C. Other Personnel

1) **Analia Vazquez Cegla, Graduate Student (2.4 calendar months).** Mrs. Cegla joined the McCarty lab in 2020, and is studying the impact of CF-related diabetes on airway barrier function. She will be supervised by Dr. McCarty. Mrs. Cegla is currently a third-year student in the Molecular and Systems Pharmacology doctoral program. Her prior training was in the field of bioengineering, and she was strongly recruited to Emory based on her past research experiences at Smith College and then two years in the biotech industry. For this project, Mrs. Cegla will contribute cell culture expertise and will perform most of the optical imaging of preparations from both the *In Vitro* and *Ex Vivo* platforms.

D. Equipment:
Three-month rental of the Emulate lab-on-chip system, including consumables: $25,405
This will be a shared resource, which we may purchase via the subsequent extramural grant. The rental fee can be applied to the purchase cost. This would then be added to CF-AIR's Common Equipment.

E. Supplies, core services, etc.:
This cost category includes costs for Transwells, cell culture, antibodies, reagents for mass-spec imaging, and Core lab fees for spatial transcriptomics using the 10x Genomics platforms (see Letter of Support, from 10x Genomics)

F. Travel:

N/A.

G. Other Expenses:

This cost category includes charges for CF mice from the CF Animal Models Core, part of CF-AIR, and for lunches to support the planning meetings of the 18 faculty members engaged in the larger project.

Quote Number 00002216

Quotation From:

Company Address 27 Dry Dock Avenue
Boston, Massachusetts 02210
United States

Prepared By Eunan Hendron

Email eunan.hendron@emulatebio.com

Created Date 6/26/2023

Expiration Date 7/31/2023

Quotation To:

Emory University

Ship To:

, Georgia, 30322
United States

Michael Koval

mhkoval@emory.edu

(404) 712-2976

Product	Product Code	Sales Price	Quantity	Total Price
Hardware Evaluation - Return Fee	Hardware Evaluation - Return Fee	USD 6,000.00	1.00	USD 6,000.00
Human Emulation System Monthly Rental - (Qty1) Zoë Culture Module 2 & (Qty1) Orb Hub Module monthly rental	RENT - 1 MTH - 1O1Z	USD 6,335.00	3.00	USD 19,005.00

Payment Terms Net 30

Incoterms FCA Free Carrier

Shipping and Handling USD 400.00

Grand Total USD 25,405.00

Send Purchase Orders To:
support@emulatebio.com

*** Shipping / handling charges and taxes stated on the quotation are estimates and may differ from actual amounts due.**

****If you are exempt from the payment of sales tax or other assessments, you may provide documentary proof of such exemption at the time of your order.**

Terms and Conditions

This quotation concerns the purchase and sale of products of Emulate, Inc ("Emulate"). Emulate's Standard Terms and Conditions of Sale, which are located at www.emulatebio.com/legal ("Standard Terms"), apply to the products listed on this quotation and are incorporated herein by reference. By signing this quotation, issuing a purchase order for the products listed herein and/or accepting such products from Emulate, you conrm that you agree to be bound by the Standard Terms to the exclusion of all other terms, whether written or oral, relating to the purchase and sale of Emulate products listed in this quotation.

You acknowledge and agree that you have received and reviewed the Standard Terms prior to signing and delivering this quotation and/or issuing a purchase order for the products listed herein.

No addition to, or modication of, any provision of the Standard Terms shall be effective unless made by a written instrument, signed by you and Emulate, that expressly states a mutual intention to modify the Standard Terms. No purchase order or similar document issued by you will modify the Standard Terms. Emulate's failure to object to such terms will not be construed as acceptance of such terms or a waiver of any term of the Standard Terms by Emulate.

emulate

Additional Terms and Conditions of Quote

1. This quotation shall apply only to direct order purchases. In order to receive quoted prices, the quotation number must be referenced at time of order.
2. The effective date and expiration date of this quotation appear on the rst page unless otherwise noted herein. We may terminate this quotation at any time upon written notice to you.
3. This quotation contains our confidential pricing information, which if disclosed to third parties could cause competitive harm to us. Subject to overriding obligations to third party funding agencies or governmental entities, the customer agrees to keep all pricing information contained herein confidential.
4. If installation and/or training services are included in this quotation, Emulate shall provide such installation and/or training services at your address as it appears on the rst page of this quotation unless otherwise noted herein.

Platform Rental Agreement

This Hardware and Consumables Rental Agreement ("**Agreement**") is entered into as of the date of the last signature below by and between Emulate, Inc. ("**Emulate**") and the undersigned organization or institution ("**User**"). Capitalized terms used but not otherwise defined in this Agreement will have the meaning given to them in Emulate's Standard Terms and Conditions of Sale, available at https://www.emulatebio.com/legal, which Terms and Conditions of Sale are incorporated herein by reference ("**Standard Terms**") and apply to the purchase, supply and use of Emulate Products, including those described in this Agreement. For purposes of the application of the Standard Terms to this Agreement, references in the Standard Terms to these "Standard Terms" shall be deemed to include this Agreement as well.

1. **HARDWARE.** Emulate will supply the items of Hardware identified in the table below ("**Hardware**") to User for use at the User facility identified below ("**User Facility**"). User authorizes Emulate to file precautionary Uniform Commercial Code ("**UCC**") financing statements and other similar filings and recordings with respect to the Hardware. User agrees not to file any corrective or termination statements or partial releases with respect to any UCCs or other similar filings or recordings filed by Emulate in connection with the Hardware except (a) if Emulate fails to file a corrective or termination statement or release on request from User following User purchase of the Hardware in accordance with this Agreement or (b) with Emulate's consent.

No.	Hardware	Quantity	Retail Price
1.	Zoe CM2	1	$90000
2.	Orb HM1	1	$19500
3.	Equipment return and decontamination fee	1	$6000
4.	Basic Research Kit 24pk	1	16200

User Facility: Emory University School of Medicine – Koval Lab

2. **PAYMENTS.** During the Term (as defined below) and where applicable until return to, and acceptance thereof by, Emulate of the Hardware provided hereunder, in consideration for, among other things, the provision of the Hardware and Consumables hereunder, User will, make non-cancelable monthly payments in advance in the following amount **$6335.** User's failure to strictly comply with this Section 2 will be considered a material breach of this Agreement. Unless title to the Hardware transfers to User under Section 5(a) below or the User has exercised the purchase option set forth in Section 5(b) below, User shall promptly return the Hardware as provided for herein.

   The equipment return and decontamination fee ("Return Fee") set forth in Section 1 is not included in the monthly payment amount set forth in this Section 2 and shall be invoiced upon execution of this Agreement. The Return Fee shall be credited against the purchase price of the equipment upon completion by the User of a rental term of at least 20 months or the purchase of the equipment by the User.

3. **TRAINING AND SUPPORT.** Emulate will provide that training and support set forth in Section 11 of the Standard Terms.

4. **OPERATION.** In connection with this Agreement, User shall (a) comply with all applicable laws, statutes, rules, regulations, and ordinances and (b) maintain in effect all the licenses, permissions, authorizations, consents, and permits that it needs to carry out its obligations under this Agreement. User shall not remove the Hardware from the User Facility without prior written approval of Emulate. User shall allow Emulate to enter User's premises at all reasonable times to locate and inspect the state and condition of the Hardware. User shall, at its expense, keep and maintain the Hardware in a good state of repair, normal wear and tear excepted, and shall use the Hardware only for its intended purpose and follow Emulate's instructions regarding the use and maintenance of the Hardware.

5. **TRANSFER OF TITLE, PURCHASE OR RETURN OF HARDWARE.**

(a) Where the Term of this Agreement is no less than two (2) years, so long as no User default exists hereunder and the Agreement has not been earlier terminated, at Agreement expiration or the earlier payment of the full aggregate minimum purchase commitment as set forth in Section 2 above for the entire Term, title to the Hardware shall automatically transfer to User on an AS IS, WHERE IS BASIS without recourse to or warranty from Emulate, express or implied, following which User shall then have sole ownership of such Hardware.

(b) So long as no User default exists hereunder, at any time during the Term, User may upon prior written notice to Emulate purchase any item of Hardware for cash equal to its then applicable Purchase Price (plus all applicable taxes), on an AS IS, WHERE IS BASIS without recourse to or warranty from Emulate, express or implied. The "Purchase Price" shall be the greater of (i) the Retail Price specified above in Section 1 less all amounts paid by the User under Section 2 above or (ii) zero dollars ($0.00).

(c) If upon early termination of the Term by Emulate, Emulate determines in good faith that any item of Hardware is damaged beyond ordinary wear and tear or has been lost, stolen or encumbered in any manner, Emulate will be entitled to require User to purchase such Hardware at the Purchase Price as determined in Section 5(b) above.

(d) In the event of termination of this Agreement where ownership of the Hardware does not transfer to User under Section 5(a) above and User does not exercise the purchase option set forth in Section 5(b) above, User shall, at its risk and expense, promptly (i) de-install, inspect, and properly pack the Hardware; and (ii) return the Hardware, freight prepaid, to Emulate's facility set out on the signature page of this Agreement (or such other delivery address designated by Emulate in writing) by delivering the Hardware on board such carrier as Emulate may specify. User will include in its return notice a reasonably detailed explanation regarding its decision to return the Hardware and a statement as to whether any viruses, other pathogens, or biohazardous materials were used with the Hardware, and if they were so used, shall at User's sole cost and expense, appropriately decontaminate such Hardware. Further, User shall cause the Hardware returned to (1) be free and clear of all liens (other than liens of Emulate) and rights of third parties; (2) be in the same condition as when delivered to User, ordinary wear and tear excepted; (3) have all User's insignia or markings removed or painted over and the areas where such markings were removed or painted over refurbished as necessary to blend with adjacent areas; and (4) be in compliance with applicable law, rules, regulations and ordinances. Further, on a net 30 day basis, User shall pay a reasonable biological decontamination fee to Emulate for all returned Hardware.

6. **TITLE.** Title to the Hardware will remain with Emulate (a) until the expiration of this Agreement if Term of this Agreement is no less than two (2) years or (b) where termination is prior to two (2) years unless and until User purchases the Hardware pursuant to Section 5(b) above. Prior to transfer of title to User under Section 5(a) above or User purchase of Hardware under Section 5(b) above, User shall not pledge or encumber the Hardware in any way except for liens of Emulate. User shall bear all risk of loss, damage, destruction, theft, and condemnation to or of the Hardware from any cause whatsoever ("**Loss**") until the Hardware has been returned to Emulate as provided for in this Agreement. User shall notify Emulate in writing promptly (but in no event more than five (5) days) of any such Loss.

7. **TERMINATION.** This Agreement will expire [____Three (3)_____] [months] following installation of the Hardware at the User Facility (the "**Term**"), unless extended by the parties in writing. Either party may terminate this Agreement upon written notice if the other party (a) fails to pay any amount due under this Agreement and such failure is not cured within ten (10) days of written notice thereof, (b) materially breaches this Agreement unless the breaching party cures the breach prior within thirty (30) days following in initial written notification of the breach, or (c) becomes insolvent, files a petition for bankruptcy, or commences or has commenced against it proceedings relating to bankruptcy, receivership, reorganization, or assignment for the benefit of creditors. If User is in default of any of the terms and conditions of this Agreement, Emulate, and its agents, at User's risk, cost, and expense may at any time enter User's premises where the Hardware is stored or used and recover the Hardware. Further, Emulate may, but shall not be required to, sell Hardware at private or public sale, in bulk or in parcels, with or without notice, and without having the Hardware present at the place of sale; or Emulate may, but shall not be required to, lease, otherwise dispose of or keep idle all or part of the Hardware; and Emulate may use User's premises during reasonable hours and in a reasonable manner for any or all of

the foregoing without liability for rent, costs, damages or otherwise, except to the extent caused by Emulate's gross negligence or willful misconduct. User waives notice of sale or other disposition (and the time and place thereof), and the manner and place of any advertising. All proceeds of sale, lease or other disposition, if any, shall be retained by Emulate, and Emulate shall have no obligation to remit any such proceeds to the User or for User's benefit. User shall promptly pay any difference between the net proceeds of such sale and amounts still due to Emulate hereunder. Upon expiration or termination of this Agreement, neither party will have any further obligations under this Agreement, except that: (x) User will pay Emulate any monies due and owing to Emulate under this Agreement, (y) if applicable, User will return the Hardware to Emulate in accordance with Section 5, and (c) the Standard Terms and the terms and conditions of Sections 5 through and including 11 will survive any such expiration or termination.

8. **CONFIDENTIAL INFORMATION**. Emulate and User may exchange Confidential Information in connection with this Agreement. For purposes of this Section 8, "**Confidential Information**" means any and all nonpublic scientific technical, financial, or business information, in whatever form (oral, written, electronic, or other form), that is made available by one party ("**Disclosing Party**") to the other party ("**Receiving Party**") whether or not such information is marked, designated, or otherwise identified as "confidential," or proprietary. Confidential Information also includes all notes and other materials prepared by the Receiving Party that incorporate or are based on the Disclosing Party's Confidential Information.

(a) The Receiving Party agrees to (i) hold in confidence all of the Disclosing Party's Confidential Information, and not disclose the Disclosing Party's Confidential Information except as expressly provided in this Section 8 without the prior written consent of the Disclosing Party; (ii) use the Disclosing Party's Confidential Information solely to carry out the Receiving Party's rights or obligations under this Agreement and (iii) treat the Disclosing Party's Confidential Information with the same degree of care the Receiving Party uses to protect the Receiving Party's own confidential information but in no event with less than a reasonable degree of care.

(b) The Receiving Party may provide the Disclosing Party's Confidential Information solely to its directors, officers, managers, members, partners, employees, consultants, subcontractors, agents, and financial, technical and legal advisors ("**Representatives**") on a need-to-know basis and solely as necessary to carry out the Receiving Party's rights or obligations under this Agreement; provided, that the Receiving Party will (i) remain liable for the compliance of its Representatives with the terms of this Agreement and (ii) promptly notify the Disclosing Party in writing of any misuse or misappropriation of any Confidential Information by the Receiving Party's Representatives of which the Receiving Party has actual knowledge.

(c) The Receiving Party's non-disclosure and non-use obligations under this Agreement will not apply to any portion of the Disclosing Party's Confidential Information that the Receiving Party can demonstrate by competent proof:

(i) is generally known to the public at the time of disclosure or becomes generally known through no wrongful act on the part of the Receiving Party;

(ii) is in the Receiving Party's possession at the time of disclosure other than as a result of the Receiving Party's breach of any legal obligation;

(iii) becomes known to the Receiving Party on a non-confidential basis through disclosure by sources other than the Disclosing Party who, to the Receiving Party's knowledge after reasonable inquiry, have the legal right to disclose such Confidential Information; or

(iv) is independently developed by the Receiving Party without reference to or reliance upon the Disclosing Party's Confidential Information by persons who had no access to such Confidential Information.

(d) If the Receiving Party is required by a governmental authority or by order of a court of competent jurisdiction to disclose any Confidential Information, the Receiving Party will, to the extent permitted by law, give the Disclosing Party prompt written notice of such requirement or order and the Receiving Party will take all reasonable and lawful actions to avoid or minimize the degree of such disclosure. The Receiving Party will,

at the Disclosing Party's expense, cooperate reasonably with the Disclosing Party in any efforts to seek a protective order.

9. **INDEMNIFICATION; INSURANCE.**

(a) Except to the extent covered under Emulate's indemnification obligations in Section 13 of the Standard Terms, User shall indemnify, defend, and hold harmless Emulate and its officers, directors, employees, agents, affiliates, successors, and permitted assigns against any and all Actions incurred by any of the foregoing indemnitees relating to, arising out of or resulting from Products, or User's use thereof, or the negligence, willful misconduct, or breach of this Agreement by User or any of User's officers, directors, employees, agents, affiliates, successors, or permitted assigns.

(b) If and until title to the Hardware transfers to User, User agrees, at its own expense, to keep all Hardware insured for such amounts and against such hazards as Emulate may reasonably require, including, but not limited to, insurance for damage to or loss of such Hardware and liability coverage for personal injuries, death or property damage, with Emulate named as additional insured and with a loss payable clause in favor of Emulate, as its interest may appear, irrespective of any breach of warranty or other act or omission of User. All such policies shall be with companies, and on terms, reasonably, satisfactory to Emulate. User agrees to deliver to Emulate evidence of insurance satisfactory to Emulate. No insurance shall be subject to any co-insurance clause. User hereby appoints Emulate as User's attorney-in-fact to make proof of loss and claim for insurance, and to make adjustments with insurers and to receive payment of and execute or endorse all documents, checks or drafts in connection with payments made as a result of such insurance policies. Any reasonable expense of Emulate in adjusting or collecting insurance shall be borne by User. User will not make adjustments with insurers except (i) with respect to claims for damage to any unit of Hardware where the repair costs do not exceed ten percent (10%) of such unit's fair market value, or (ii) with Emulate's written consent. Said policies shall provide that the insurance may not be altered or cancelled by the insurer until after thirty (30) days' written notice to Emulate. Emulate may, at its option, apply proceeds of insurance, in whole or in part, to (1) repair or replace Hardware or any portion thereof, or (2) satisfy any obligation of User to Emulate hereunder.

10. **NOTICES**. To be effective, all notices must be in writing and delivered personally, emailed or mailed by overnight U.S. mail, postage prepaid, by certified U.S. mail, return receipt requested, postage prepaid, or sent by Federal Express or another (inter)nationally recognized courier service (billed to sender), to the parties at the address set forth below their signatures below. Any notice will be deemed to have been given or made on the earlier of the date on which it is actually received or (a) on the date on which it is delivered if personally delivered or sent if sent by email (with written confirmation of receipt or read receipt confirmation, whichever occurs first), (b) five (5) days after the date it is mailed if mailed by certified U.S. mail and (c) three (3) days after the date it is mailed if mailed by overnight U.S. mail, Federal Express or another (inter)nationally recognized courier service.

11. **MISCELLANEOUS**. Each party, for all purposes related to this Agreement, will be deemed an independent contractor, and nothing in this Agreement will be deemed to create a relationship of employment or agency or to constitute the parties as partners or joint ventures, nor will anything in this Agreement give either party the authority to bind or commit the other party in any respect. This Agreement, along with the Standard Terms, constitutes the entire understanding and agreement of the parties with respect to the subject matter hereof and supersedes all prior and contemporaneous written or oral agreements and understandings with respect to the subject matter hereof. If any provision of this Agreement conflicts with the Standard Terms, this Agreement will govern. If any provision of this Agreement is held invalid or unenforceable, such provision will be enforced to the maximum extent permitted by applicable law so as to give effect to the intent of the parties, and the remaining terms will continue in full force and effect and the provision that is invalid or unenforceable shall be revised by the court to the least amount to achieve as nearly as possible the same effect as was originally intended by the parties. No amendment to this Agreement or waiver of any right, condition or obligation herein will be effective unless made in a writing signed by both parties. The failure of either party to exercise any right granted herein or to require performance of any term or the waiver by either party of any breach will not prevent a subsequent exercise or enforcement of, or be deemed a waiver of any subsequent breach of, the same or any other term. The remedies set forth in this Agreement and the Standard Terms are cumulative, and any or all thereof may be exercised in lieu of or in addition to each other or any remedies at law, in equity, or under

statute. User shall pay Emulate's reasonable attorney's fees incurred in connection with the enforcement, assertion, defense or preservation of Emulate's rights and remedies hereunder, or if prohibited by law, such lesser sum as may be permitted. User may not assign, delegate or otherwise transfer any of its rights or obligations under this Agreement without Emulate's prior written consent. Any attempted assignment, delegation, or other transfer in violation of this Section will be null and void. Emulate may, without the consent of User, assign this Agreement or any interests therein. User agrees that if User receives written notice of an assignment from Emulate, User will pay all amounts payable to such assignee or as instructed by Emulate. User further agrees to confirm in writing receipt of the notice of assignment as may be reasonably requested by assignee. User hereby waives and agrees not to assert against any such assignee any defense, set-off, recoupment claim or counterclaim which User has or may at any time have against Emulate for any reason whatsoever. This Agreement set forth a net lease. User's obligation to pay amounts due hereunder shall be absolute and unconditional. User shall not be entitled to any abatement or reductions of, or set-offs against, said amounts, including, without limitation, those arising or allegedly arising out of claims (present or future, alleged or actual, and including claims arising out of strict tort or negligence of Emulate) of User against Emulate under this Agreement or otherwise. Nor shall this Agreement terminate or the obligations of User be affected by reason of any defect in or damage to, or loss of possession, use or destruction of, any Hardware from whatsoever cause. It is the intention of the parties that amounts due hereunder shall continue to be payable in all events in the manner and at the times set forth herein unless the obligation to do so shall have been terminated pursuant to the express terms hereof. This Agreement, and all claims relating to or arising out of this Agreement (including the Standard Terms), or the breach thereof, will be governed by and construed in accordance with the laws of the Commonwealth of Massachusetts without regard to any choice of law principles that would require the application of the laws of another jurisdiction. If the parties are unable to settle a dispute relating to this Agreement, the dispute will be adjudicated exclusively in the state or federal courts located in the Commonwealth of Massachusetts (Suffolk County) (and the appropriate appellate courts therefrom), and each party irrevocably submits to the exclusive jurisdiction of such courts in any such suit, action or proceeding. This Agreement may be executed (including by use of industry standard signature software, such as DocuSign®) in counterparts, each of which will be deemed an original, but all of which together will constitute one and the same instrument. An executed copy of this Agreement that is delivered by facsimile or other electronic means will be sufficient to show execution and delivery thereof.

EMULATE, INC.

Signature: _____
Name:
Title:
Date:

Address for Notices:
27 Drydock Avenue, 5th Floor
Boston, MA 02210
Attn: Legal Dept.
Email: legal@emulatebio.com

[INSERT USER NAME]

Signature: _____
Name:
Title:
Date:

Address for Notices:
[_____]
[_____]
Attn: [_____]
Email: [_____]

10x GENOMICS

6230 Stoneridge Mall Road
Pleasanton, CA 94588-3260
925 401 7300

June 23, 2023

Nael McCarty, PhD
Marcus Professor of Cystic Fibrosis
Department of Pediatrics
Emory University
Center for CF and Airways Disease Research
Emory University and Children's Healthcare of Atlanta
Atlanta, GA 30322

Dear Dr. McCarty,

We are pleased to hear about the exciting project you are launching with colleagues at Emory University, Georgia Tech, Georgia State University, and the University of Alabama at Birmingham and agree that our state-of-the-art technologies can be best brought to bear on the research problems you are seeking to solve.

10x Genomics is proud to be support our customers, independent investigators and Core labs at Emory and Georgia Tech to enable transformative research programs, such as the one you have proposed. Relevant to your specific needs, the 10x platforms operating at the Core labs include the Chromium and Visium systems that enable discovery analysis at the single-cell level and in localized spatial transcriptomics systems, as well as, plans to acquire the new Xenium platform. These platforms are complementary in nature, especially for projects which include co-registration of spatially resolved transcriptomics and markers of protein localization enabled by outside technologies, including mass-spec imaging and fluorescence microscopy. Our technologies have been very well received by the scientific community, as recognized by inclusion in over 5,000 customer publications.

The 10x Genomics toolkit has the capabilities to support the gene expression studies you plan in this exciting proposal. We look forward to seeing your results.

Sincerely

DocuSigned by:
Danae Van Oene
2A9FF2A84C8E4C4...

Danae Van Oene
Vice President of the Americas

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: Sheyda Azimi

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

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)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Tehran, Tehran/Iran	BSc	04/2004	Molecular Cell Biology/Microbiology
University of Nottingham, Nottingham, UK	MSc	12/2010	Molecular Medical Microbiology
University of Nottingham, Nottingham, UK	PhD	03/2015	Microbiology

A. Personal Statement

My academic training and research experience have provided me with an excellent background in multiple biological disciplines, including molecular biology, microbiology, and genetics. I gained my BSc degree from the University of Tehran, Iran. I completed four years of experience in industry as a molecular cell biologist before enrolling in MSc course at the University of Nottingham, UK. In 2010, I enrolled in Microbiology Ph.D. program to study the ''Interactions between *Neisseria meningitidis* and its human host''. I authored two publications on findings from my graduate research, demonstrating the impact of host proinflammatory cytokines on the virulence of *N. meningitidis* and introducing a novel tissue-specific receptor for *N. meningitidis* cells on endothelial cells forming the blood-brain barrier. As a postdoctoral fellow I studied the Social Evolution of *Pseudomonas aeruginosa* during chronic infections. Since then, my research has been focused on understanding the role of intra-strain population diversity of *P. aeruginosa* in chronic infections. My research on microbial population heterogeneity and its role on shaping microbial functional phenotypes has been very impactful. As a Cystic Fibrosis Foundation postdoctoral fellow (2018-2021), I have developed a framework to study the effects of microbial population heterogeneity on pathogenesis, microbiogeography, and host-pathogen interactions.
My long-term goal is to develop a framework that allows us to monitor and understand the changes in microbial interactions that shape host-pathogen interactions and microbiogeography that shape the physiology of the pathogens during chronic infections.

Citations:

1. **Azimi S,** Thomas J, Cleland SE, Curtis JE, Goldberg JB, Diggle SP. O-Specific Antigen-Dependent Surface Hydrophobicity Mediates Aggregate Assembly Type in Pseudomonas aeruginosa. mBio. 2021:e0086021.
2. **Azimi S,** Wheldon LM, Oldfield NJ, Ala'Aldeen DAA, Wooldridge KG. A role for fibroblast growth factor receptor 1 in the pathogenesis of Neisseria meningitidis. Microb Pathog. 2020;149:104534.

3. **Azimi S,** Roberts AEL, Peng S, Weitz JS, McNally A, Brown SP, Diggle SP. Allelic polymorphism shapes community function in evolving Pseudomonas aeruginosa populations. ISME J. 2020.

4. Mahdavi J, Royer PJ, Sjolinder HS, **Azimi S,** Self T, Stoof J, Wheldon LM, Brannstrom K, Wilson R, Moreton J, Moir JW, Sihlbom C, Boren T, Jonsson AB, Soultanas P, Ala'Aldeen DA. Pro-inflammatory cytokines can act as intracellular modulators of commensal bacterial virulence. Open Biol. 2013;3(10):130048.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022- Present	Assistant Professor, Department of Biology, Georgia State University, Atlanta, GA
2018-2021	Cystic Fibrosis Foundation Postdoctoral Fellow, Georgia Institute of Technology, Atlanta, GA
2017-2019	CF Scholar, Emory Children's Hospital- Georgia Institute of Technology, Atlanta, GA
2016-2017	Postdoctoral Fellow, Department of Respiratory Medicine, University of Nottingham, UK
2015-2016	Postdoctoral Fellow, School of Life Sciences, Diggle lab, University of Nottingham**,** UK

Honors

2016	Collaborative Early Career Fellowship scheme on AMR, University of Warwick
2014	Travel Award from University of Nottingham School of Life Sciences
2014	Travel Grant from Microbiology Society
2009	University of Nottingham Iranian Masters Scholarship

C. Contributions to Science

My research contributions are in two main categories:

1. Role of host-derived factors in host-microbe interactions

As a Ph.D. trainee, my research was focused on investigating the host factors used by bacterial pathogens to develop the disease. My research showed that changes in host proinflammatory responses regulate the expression of virulence factors of the obligate human pathogen *Neisseria meningitidis.* I further identified a novel receptor for *N. meningitidis* explicitly expressed by endothelial cells forming the blood-brain barrier. Recruitment of his receptors by *N. meningitidis* demonstrates the bacterial tissue tropism leading to the development of bacterial meningitis.

a. Mahdavi, J, Royer, P. J., Sjolinder, H. S., **Azimi, S.,** Self, T., Stoof, J., Wheldon, L. M. Brannstrom, K., Wilson, R., Moreton, J., Moir, J. W., Sihlbom, C., Boren, T., Jonsson, A. B. Soultanas, P., Ala'Aldeen, D. A. (2013). Pro-inflammatory cytokines can act as intracellular modulators of commensal bacterial virulence. Open Biol, Volume 3 Issue 10

b. **Sheyda Azimi,** Lee M Wheldon, Neil J Oldfield, Dlawer AA Ala'Aldeen, Karl G Wooldridge. (2020). A role for Fibroblast Growth Factor Receptor 1 in the pathogenesis of *Neisseria meningitidis.* Microbial Pathogenesis.

2. Role of microbial intra-species population diversity in chronic infections

To determine the impact of microbial intra-specific genetic heterogeneity on pathogenesis, I focused on chronic infection of airways in individuals with cystic fibrosis (CF) with *Pseudomonas aeruginosa* (*Pa*) as a model system. My research underlines that the accumulation of *Pa* genetic variants changes the population dynamics and alters the functional phenotype of the whole population. Furthermore, this study highlights that this change in *Pa* population dynamics leads to an increase in antibiotic tolerance of the whole population.

I further investigated how intra-species bacterial population heterogeneity changes the microbiogeography of infection. I showed that changes in bacterial cell surface properties alter the spatial organization of bacterial aggregates in CF airways. This finding underscores how changes in the bacterial population can shape the ecosystem of chronic infections.

a. **Sheyda Azimi,** Aled E. L. Roberts, Shengyun Peng, Joshua S. Weitz, Alan McNally, Samuel P. Brown, Stephen P. Diggle. (2020). Allelic polymorphism shapes collective phenotypes in evolving *Pseudomonas aeruginosa* populations. The ISME Journal.volume 14.

b. Jelly Vanderwoude, Derek Fleming, **Sheyda Azimi,** Urvish Trivedi, Kendra P. Rumbaugh, Stephen P. Diggle. (2020). The evolution of virulence in *Pseudomonas aeruginosa* during chronic wound infection. Proc. R. Soc. B.28720202272.

c. **Sheyda Azimi**, Jacob Thomas, Sara E. Cleland, Jennifer E. Curtis, Joanna B. Goldberg, and Stephen P. Diggle. (2021) O-Specific Antigen-Dependent Surface Hydrophobicity Mediates Aggregate Assembly Type in *Pseudomonas aeruginosa.* mBio. 12:e00860-21.

Complete List of Published Work:

My Bibliography

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: Joshua D. Chandler

eRA COMMONS USER NAME (credential, e.g., agency login): JCHAN31

POSITION TITLE: Assistant Professor of Pediatrics

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Drury University, Springfield, MO	BA	05/2009	Biology, Chemistry
University of Colorado, Aurora, CO	PhD	05/2014	Toxicology
Emory University, Atlanta, GA	Postdoc	07/2017	Toxicology, Metabolomics

A. Personal Statement

I am an Assistant Professor in the Emory University School of Medicine, Department of Pediatrics, Division of Pulmonary, Allergy & Immunology, Cystic Fibrosis and Sleep Medicine.

My research program, launched in August of 2017, utilizes state-of-the-art technologies to study redox biochemistry and metabolism. We are internationally recognized for rigor and creativity in research using LC-MS-based metabolomics, redox chemistry, and mammalian cell and animal biology. We have developed exquisite methods to simultaneously profile hundreds of metabolites with high accuracy and sensitivity in a single experiment. We complement this with data-dependent MS/MS acquisition in unbiased experiments to identify novel compounds, which can be validated and added to future screens. We are well recognized by our colleagues for applying advanced biostatistical and bioinformatic techniques to our research data. In addition to LC-MS and other advanced methods, our lab is well-versed in gold standard approaches in quantitative biochemistry including high-throughput enzyme activity assays, redox-accurate determination of oxidant and antioxidant species, quantitative ELISA, and western blotting.

Discerning the causes and consequences of inflammatory pathology in the earliest stages of cystic fibrosis (CF) airways disease has been a major emphasis of research in my laboratory. We identified a pathway of neutrophilic inflammation occurring in CF toddlers with accumulation of myeloperoxidase, chlorine bleach production by this enzyme, oxidation of the airway fluid metabolome, and bronchiectasis (PMC7034951). More recent findings suggest these metabolic pathways, and others, are active in asthma and relate to failures of medication to prevent exacerbations (PMC9825634). At the same time, we are aware that clinical specimen availability is predicated on willingness of patients to participate in research and are advancing methods to monitor metabolites non-invasively via exhaled breath (PMC6166650). I have significant experience with cell culture and animal models to test mechanisms of toxicology and pathology as well (e.g., PMC3689984, PMC4566044, PMC6536378). My intensive interdisciplinary training has allowed me to oversee a rigorous and expansive research program of trainees conducting experiments in model systems and with clinical specimens.

I am delighted to participate in the current proposal, led by Dr. McCarty, that asks the question, "What are the properties of local areas of the CF airway that sensitize them to focal damage and disrepair?" I will contribute to this project via my knowledge of metabolomics, inflammation, and redox biology. Specifically, my lab will provide high content, rigorous metabolomics analysis of airway secretions and blood to define phenotypes that can be linked to focal points of inflammation in patient samples and basic/translational models. I will work closely with Dr. McCarty and the rest of the team to ensure the success of the Aims of this proposal.

Relevant ongoing research:

NIH/NHLBI: R01 HL150658 12/1/2022 – 11/30/2026
Neutrophil hyperexocytosis and hypochlorous acid exposure in early cystic fibrosis
Role: PI
Using state of the art translational models, test impact of neutrophil pathobiology on bleach generation via myeloperoxidase in cystic fibrosis airways and downstream impact on lung epithelial cells.

NIH/NHLBI: R01 HL159058 7/1/2022 – 6/30/2026
Extracellular vesicle-driven neutrophilic inflammation in cystic fibrosis lungs
Role: Co-I (PI: Tirouvanziam)
This project explores a new hypothesis related to progressive lung inflammation in patients with cystic fibrosis.

NIH/NIAID: R01 AI166988 7/7/2022 – 6/30/2027
Inflammation and Fibrosis in Pulmonary TB: the INFIN-TB Study
Role: Co-I (PI: Auld)
By characterizing the role of neutrophilic and profibrotic activity as risk factors for post-TB lung damage, this study will inform future treatment strategies to prevent or reduce this devastating complication of TB.

CF Foundation: GUGLAN19A0 1/1/2022 – 12/31/2023
Trikafta effects in 6-12 year olds with CF: clinical and home-based monitoring
Role: Co-I (PI: Guglani)
This study will establish a cohort of matched clinical and home-based sputum sampling and advance our goal of enabling home-based monitoring of airway inflammation and treatment responses in young children with CF.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2017 –	Assistant Professor of Pediatrics, Division of Pulmonary, Allergy & Immunology, Cystic Fibrosis and Sleep, Emory University School of Medicine, Atlanta, GA, USA
2014 – 2017	Postdoctoral Fellow (PI: Dean P. Jones), Division of Pulmonary, Allergy and Critical Care Medicine, Emory University School of Medicine, Atlanta, GA, USA
2010 – 2014	PhD Student (PI: Brian J. Day), Medicine Office of Research, National Jewish Health Department of Medicine, Denver, CO, USA
2009 – 2010	Teaching Assistant, Department of Pharmaceutical Sciences, Skaggs School of Pharmacy, University of Colorado, Denver, CO, USA
2008 – 2009	Teaching Assistant, Department of Chemistry, Drury University, Springfield, MO, USA
2008	Undergraduate Research Intern, Department of Pharmaceutical Science, University of Kansas, Lawrence, KS, USA

Honors

2021	Emory School of Medicine Educator Appreciation Day Recognition
2015 – 2018	Society of Toxicology: Biomarkers (2015); Oxidative Stress, Metals (2016, 2018); Inflammation in Disease (2017)
2017	Invited Presenter, Society of Toxicology 56th Annual Meeting
2017	Invited Presenter, 10th International Human Peroxidase Meeting
2016 – 2017	Ruth L. Kirschstein NRSA Individual Postdoctoral Fellowship (NHLBI F32 HL132493)
2016	Junior Investigator Basic Science Runner-Up, North American Cystic Fibrosis Conference
2016	Emory CF-AIR Research Development Plan Postdoctoral Fellow
2016	Cystic Fibrosis Foundation Individual Postdoctoral Fellowship (CHANDL16F0)
2015 – 2016	Ruth L. Kirschstein Institutional NRSA Postdoctoral Fellowship (NIEHS T32 ES012870, PI: Miller)
2015	Young Investigator Award, Society for Redox Biology and Medicine
2014	Occupational and Public Health Specialty Section Best Student Abstract, Society of Toxicology

2013 Society of Toxicology Domestic ToxScholar

Other Professional Activities

2022- Females in Mass Spectrometry (FeMS) Pod Mentor
2019- American Society for Mass Spectrometry
2012- Society for Redox Biology and Medicine (2012 - present)
2012-2021 Society of Toxicology
Peer review *AJP-Lung, Crit Rev Toxicol, Eur Respir J, Free Radic Biol Med, Int J Environ Res Public Health, J Cyst Fibros, Metabolites, Microbiol Spectr, Mol Cell Biochem, Nat Commun, Toxicol Rep, Toxicol Sci, Toxicology*

C. Contributions to Science

1. Lung hypothiocyanous acid metabolism impacts inflammation and host defense. During graduate training, I discovered a novel mechanism of hypothiocyanous acid (HOSCN) detoxification in mammalian bronchoepithelial cells. HOSCN is an important product of neutrophils generated when myeloperoxidase is released into mucosae in the presence of hydrogen peroxide. Thiocyanate is oxidized to HOSCN upon the reduction of more potent and reactive oxidants, either enzymatically (via chordate peroxidases) or by direct reactions. Previous research had shown HOSCN is well tolerated by mammalian cells, unlike related oxidants. I discovered that mammalian thioredoxin reductase (TrxR) metabolizes HOSCN by transferring electrons from NADPH in a catalytic selenocysteine residue-dependent manner. In contrast, HOSCN inhibited bacterial TrxR, which lacks selenocysteine and is evolutionarily distinct from that of eukaryotes. Also, TrxR in mammals did not reduce hypochlorous acid (HOCl), the other major oxidant product of neutrophils at inflamed tissue. Human bronchoepithelial cells were killed by HOCl, but not HOSCN, while clinical isolates of *Pseudomonas aeruginosa* were vulnerable to both oxidants. My lab continues to investigate the impact of HOSCN on lung cells during to better understand how cells cope with and adapt to inflammation.

a. **Chandler JD** and Day BJ. Thiocyanate: a potentially useful therapeutic agent with host defense and antioxidant properties. *Biochem Pharmacol* 2012, 84:1381-1387. PMC3983948
b. **Chandler JD**, Nichols DP, Nick AJ, Hondal RJ and Day BJ. Selective metabolism of hypothiocyanous acid by mammalian thioredoxin reductase promotes lung innate immunity and antioxidant defense. *J Biol Chem* 2013, 288:18421-18428. PMC3689984
c. Day BJ, Bratcher PE, **Chandler JD**, Kilgore MB, Min E, LiPuma JJ, Hondal RJ, Nichols DP. The thiocyanate analog selenocyanate is a more potent antimicrobial pro-drug that also is selectively detoxified by the host. *Free Radic Biol Med* 2020 146:324-332. PMC6951815

2. Thiocyanate promotes lung immunity and resolution of inflammation. Because HOSCN is well tolerated in lung cells (see above), I tested the impact of thiocyanate (precursor to HOSCN) on mice with lung infection or with sterile inflammation. I tested intratracheal exposure to mucoid *P. aeruginosa* isolated from a CF patient. 24 hours later, mice were nebulized with thiocyanate or vehicle saline every 12 hours. Mice transgenic for club cell beta-epithelial sodium channel (βENaC) overexpression were used, in addition to wild type littermates, to phenocopy CF, as they have spontaneous inflammation and muco-obstruction. Thiocyanate accelerated bacterial clearance, decreased neutrophil inflammation, and promoted more rapid recovery. Uninfected βENaC mice also exhibited less inflammation (littermate controls had no inflammation). Thiocyanate decrease airway HOCl, demonstrated by lower glutathione sulfonamide, and lung TrxR activity increased, indicating higher capacity to metabolite HOSCN. Later collaboration with Dr. Balazs Rada lab showed that HOSCN is also an important anti-influenza agent in mice.

a. **Chandler JD**, Min E, Huang J, Nichols DP and Day BJ. Nebulized thiocyanate improves lung infection outcomes in mice. *Brit J Pharmacol* 2013, 169:1166-1177. PMC3696337
b. **Chandler JD**, Min E, Huang J, McElroy CS, Dickerhof N, Mocatta TJ, Fletcher AA, Evans CM, Liang L, Patel M, Kettle AJ, Nichols DP, Day BJ. Anti-inflammatory and anti-microbial effects of thiocyanate in a cystic fibrosis mouse model. *Am J Respir Cell Mol Biol* 2015, 53:193-205. PMC4566044
c. **Chandler JD** and Day BJ. Biochemical mechanisms and therapeutic potential of pseudohalide thiocyanate in human health. *Free Radic Res* 2015, 49:695-710. PMC4959427
d. Sarr D, Gingerich AD, Asthiwi NM, Almutairi F, Sautto GA, Ecker J, Nagy T, Kilgore MB, **Chandler JD**, Ross TM, Tripp RA, Rada B. Dual oxidase 1 promotes antiviral innate immunity. *Proc Natl Acad Sci U S A* 2021, 118(26). PMC8256044

3. Metabolomic perturbations of pediatric and adult lung diseases. My laboratory has been designed to facilitate high-dimensional, rigorous metabolomic analyses in lung diseases. This includes CF and many other lung diseases of children and adults, such as asthma and COVID-19. Metabolites are the currency of cell biology, and perturbations in metabolite steady-state provide important clues to pathological perturbations which may lead to discoveries of new biomarkers and therapeutic strategies. The studies below reflect my track record of both high-dimensional untargeted and quantitative metabolomic analyses in airway samples (including bronchoalveolar lavage and exhaled breath) and blood from patients with lung diseases. We have found important associations in airway fluid metabolites and lipids from CF toddlers to myeloperoxidase activity and lung damage noted by chest computed tomography, prior to the onset of major symptoms. Research on children with asthma revealed an extensive metabolomic signature of resistance to corticosteroid therapy. Additionally, our research has shown the power of high-resolution mass spectrometry to enable breath-based patient sampling.

a. **Chandler JD**, Margaroli C, Horati H, Kilgore MB, Veltman M, Liu HK, Taurone AJ, Peng L, Guglani L, Uppal K, Go YM, Tiddens HAWM, Scholte BJ, Tirouvanziam R, Jones DP, Janssens HM. Methionine oxidation by myeloperoxidase associates with early cystic fibrosis lung disease. *Eur Respir J* 2018 52(4). PMC7034951
b. **Chandler JD**, Horati H, Walker DI, Pagliano E, Tirouvanziam R, Veltman M, Scholte BJ, Janssens HM, Go YM, Jones DP. Determination of thiocyanate in exhaled breath condensate. *Free Radic Biol Med* 2018 126:334-340. PMC6166650
c. Horati H, Janssens HM, Margaroli C, Veltman M, Stolarczyk M, Kilgore MB, Chou J, Peng L, Tiddens HAWM, **Chandler JD**, Tirouvanziam R, Scholte BJ. Airway profile of bioactive lipids predicts early progression of lung disease in cystic fibrosis. *J Cyst Fibros* 2020 S1569-1993:30034-5. PMC7415592
d. Cottrill KA, Stephenson ST, Mohammad AF, Kim SO, McCarty NA, Kamaleswaran R, Fitzpatrick AM, **Chandler JD**. Exacerbation-prone pediatric asthma is associated with arginine, lysine, and methionine pathway alterations. *J Allergy Clin Immunol* 2023 151(1):118-127.e10. PMC9825634

4. Impact of chronic, low-dose cadmium exposure on pulmonary diseases. As a postdoctoral fellow, I studied the impact of oral cadmium (Cd) exposure and its importance to pro-inflammatory pulmonary disease. I analyzed lung transcriptomic and metabolomic responses to chronic low-dose Cd exposure using a mouse model that achieves comparable lung tissue Cd burden to that of adult humans, as determined by tissue explant analysis. Cd administration led to increased airway hyperresponsiveness and autonomic receptor and agonist abundances, indicating Cd might impact pulmonary function through altered autonomic signaling. Furthermore, Cd predisposed mice to worse myeloid cell-driven inflammation following sub-lethal H1N1 influenza A virus infection compared to vehicle-exposed controls. Cd exposure alone insufficient to cause lung inflammation but potentiated the inflammatory response to H1N1, including along metabolomic axes which we previously established for H1N1 exposure alone. I identified a novel myeloid cell-specific pathway that mediates inflammatory response to influenza, suggesting Cd specifically alters metabolic patterns and behavior of this cell type to affect inflammation more broadly.

a. Go YM, Sutliff RL, **Chandler JD**, Khalidur R, Kang BY, Anania FA, Orr M, Hao L, Fowler B and Jones DP. Low-dose cadmium causes metabolic and genetic dysregulation associated with fatty liver disease in mice. *Toxicol Sci* 2015 147:524-534. PMC4598795
b. **Chandler JD,** Wongtrakool C, Banton SA, Li S, Orr ML, Barr DB, Neujahr DC, Sutliff RL, Go YM, Jones DP. Low-dose oral cadmium increases airway reactivity and lung neuronal gene expression in mice. *Physiol Reports* 2016 4:1-12. PMC4945833
c. **Chandler JD**, Hu X, Ko EJ, Park S, Lee YT, Orr ML, Fernandes J, Uppal K, Kang SM, Jones DP, Go YM. Metabolic pathways of lung inflammation revealed by high-resolution metabolomics (HRM) of H1N1 influenza virus infection in mice. *AJP-Regu* 2016 311:906-916. PMC5243214
d. **Chandler JD**, Hu X, Ko EJ, Park SJ, Fernandes J, Lee YT, Orr ML, Hao L, Smith MR, Neujahr D, Uppal K, Kang SM, Jones DP, Go YM. Low-dose cadmium potentiates lung inflammatory response to 2009 pandemic H1N1 influenza virus in mice. *Environment International* 2019 127:720-729. PMC6536378

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1hSSghNPFFPku/bibliography/public/

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: Coskun, Ahmet F.

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

POSITION TITLE: Assistant Professor of Biomedical Engineering; Bernie Marcus Early-Career Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Koc University, Turkey	B.S.	06/2008	Physics
Koc University, Turkey	B.S.	06/2008	Electrical Engineering
University of California, Los Angeles	M.S.	06/2009	Electrical Engineering
University of California, Los Angeles	Ph.D.	06/2013	Electrical Engineering
California Institute of Technology	Postdoctoral	12/2016	Single Cell Genomics
Stanford University School of Medicine	Postdoctoral	05/2018	Systems Immunology
Stanford University School of Medicine	Instructor	07/2019	Systems Immunology

A. Personal Statement

Dr. Coskun is currently an Assistant Professor of Biomedical Engineering at the Georgia Institute of Technology and Emory University. He directs an interdisciplinary team at the Single Cell Biotechnology Laboratory (SCBL) at Georgia Tech. SCBL develops purpose-driven multiplex bioimaging technologies that can visualize two/three-dimensional (2D/3D) spatial heterogeneity of biological systems at the single cell and subcellular level. Dr. Coskun trained with Garry Nolan as a postdoctoral fellow and then as a faculty member at Stanford University. Previously, Dr. Coskun performed his postdoctoral work at Caltech in Single Cell Genomics with Long Cai and his doctoral work at UCLA in Bioimaging instrumentation with Aydogan Ozcan. Primarily, using quantitative microscopy tools at all scales, from single molecules to multi-cellular systems, Dr. Coskun explores how the “spatial” nature of cell-to-cell interactions and subcellular variations in lung cancers.

Dr. Coskun has contributed to a few key techniques in the single-cell analysis field. At Caltech, Dr. Coskun was instrumental in the development of a **single cell spatial genomics** profiling method, known as **seqFISH** (Lubeck & Coskun, Nat Methods 2014 and Coskun, Nat Methods 2016). Multiplexing fluorescent in situ hybridization (FISH) allowed spatially resolved gene expression measurements in complex cells and tissues. At Stanford, Dr. Coskun developed **three-dimensional (3D) multiplexed, subcellular metabolic, and proteomics** molecular profiling methods based on ion beam imaging (Coskun & Nolan, Nat Comms, 2021). In his lab at Georgia Tech, Dr. Coskun’s team demonstrated a **3D spatially resolved metabolic profiling framework** that provides correlative networks of 20 distinct cell types and up to 168 metabolites in human tissues (Ganesh, Science Advances, 2021).

In this **proposal**, Coskun Lab will be working with Dr. Nael A. McCarty on the Cystic Fibrosis project. Coskun Lab is mainly responsible for spatial omics assays and machine learning data analysis of the tissue specimens from the preclinical, in vitro, or clinical models. We are fully supportive of this exciting project to help develop novel Cystic Fibrosis (CF) therapies using single cell molecular analysis.

Ongoing Research Support

NIH R21 NIA (Coskun, PI) 05/15/2023 - 02/28/2025
Tissue systems biology of immune dysregulation in aging by single cell spatial metabolomics
The purpose of this project is to use multiple lymphoid tissues (tonsil and lymph node) to study the metabolic regulation and senescence phenotypes of lymphoid cells during aging using a newly developed three-dimensional spatially resolved metabolic profiling technique.

NIH R01 NCI (Coskun, co-I) 07/01/2022 – 06/30/2027
Hydrogel-based Organoids of African-American Lymphomas to Study B Cell Receptor Pathway Inhibitors
The purpose of this project is to decipher BCR signaling in AA lymphomas.

BWF 1015739.02 (Coskun, PI) 07/01/2018 – 06/30/2025
Burroughs Wellcome Fund Career Award at the Scientific Interface
Computational single-molecule imaging and barcoding: Exploring cellular identity at the single cell transcript level.
This project aims to decode cellular identity from single-cell transcript profiles.

NIH 5K25AI140783-01 (Coskun, PI) 07/01/2018 – 06/30/2024
Spatial Epigenomic Profiling of Immune Cell Signatures at Subcellular Resolution in Health and Disease
The purpose of this project is to visualize the epigenetic states of B cell subsets in normal and leukemic samples.

Welcome LEAP AWD-002202 (Coskun, co-PI) 04/01/2021 – 03/31/2024
Decoding specificity between antibodies and vaccines using integrated human Lymphoid, Gut, and Lung organoids.
The proposed work is a significant leap in (a) generating a human lymphoid-on-chip system capable of driving bona fide human GC reactions and antibodies, and (b) creating interconnected lung-lymphoid and gut-lymphoid organ model systems to better understand the potency of immune responses.

American Lung Association (Coskun, PI) 07/01/2021 – 06/30/2023
Deciphering Single Cell Immunometabolism of T-Cell Differentiation in Lung Tumor Organoid on a Chip
This project will evaluate the T cell differentiation in lung tumor organoids using an integrated spatially resolved single-cell transcriptional and metabolic analysis.

NIH Lung SPORE pilot grant (Coskun, PI) 06/01/2021 – 05/30/2023
Spatial Subcellular Signaling Networks for Non-Small Cell Lung Cancer Therapies
This project aims to create a subcellular signaling network model for deciphering therapies in NSCLCs

Oak Ridge National Lab CNMS grant (Coskun, PI) 01/01/2022 – 12/31/2023
Correlative 3D metabolic and structural in situ imaging of human tissues.
This project implements a user proposal for a year for TOF-SIMS cancer imaging of tumors.

Marcus Foundation (Coskun, PI) 01/01/2020 – 12/31/2024
Bernie Marcus Early-Career Professorship in Therapeutic Cell Characterization and Manufacturing
This project focuses on understanding the heterogeneity of stem cells for regenerative medicine.

Regenerative Medicine Center Grant (Coskun, PI) 09/01/2022 – 8/31/2024
Elucidating predictive potency of IFN-ɣ primed mesenchymal stromal cells in Graft vs Host Disease using Spatial Single Cell Transcriptomics
This project focuses on understanding the potency of stem cells for treating GVHD in leukemias.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2021-present Member, Cell and Molecular Biology, Winship Cancer Institute, Emory University
2019-present Assistant Professor, BioEngineering Interdisciplinary Program, Georgia Institute of Technology
2019-present Assistant Professor of Biomedical Engineering, Georgia Institute of Technology and Emory
2017-2019 Postdoctoral Fellow, Systems Immunology, Stanford University and
Instructor of Radiology, Molecular Imaging Program at Stanford (with Prof. Garry P. Nolan)
2013-2016 Postdoctoral Fellow, Single Cell Systems Biology, Caltech (with Research Prof. Long Cai)
2008-2013 Graduate Research Assistant, Bio-Photonics, UCLA (with HHMI Prof. Aydogan Ozcan)
2007 Undergraduate Research Assistant, Materials Science, UIUC (with Prof. John Rogers)

Honors

2023 BMES-CMBE Rising Star Junior Faculty Award
2022 American Lung Association Innovation Award (2022-2024)
2022 American Cancer Society, Institutional Research Grant Award (2022-2023)
2022 Career Enhancement Program (CEP) Pilot Grant, Winship Lung Cancer SPORE (2021-2023).
2022 Multi-cellular engineered living systems (M-CELS), Seed Grant Awards.
2022 Student Recognition of Excellence in Teaching: Spring Semester 2022 CIOS Honor Roll
2021 Student Recognition of Excellence in Teaching: Class of 1934 CIOS Award
2021 Georgia Tech Institute for Electronics and Nanotechnology (IEN) Core Facilities Seed Grant.
2020 Bernie Marcus Early-Career Professorship in Therapeutic Cell Characterization & Manufacturing
2020 Healthy Longevity Award, Interstellar initiative, The New York Academy of Sciences
2020 Cell Manufacturing Technologies (CMaT) Center, Georgia Tech, Seed Grand (2020-2021)
2018 Maternal and Child Health Research Institute K-grant supplement (2018-2019)
2018 National Institutes of Health K25 Career Development Award (2018-2023)
2018 Kenan Sahin Award, Turkish American Scientists & Scholars Association.
2016 Burroughs Wellcome Fund CASI Award (2018-2023)
2015 Leukemia & Lymphoma Research Fellowship (2015-2018)

Service

2023 Panelist, P01 panel reviewer for NCI calls, NIH
2022 Reviewer, Chan Zuckerberg Initiative, Advancing Imaging Through Collaborative Projects
2022 Reviewer, RE: NWO, The Netherlands: Invitation to review a research proposal (National Roadmap for Large-Scale Research Facilities) 184.036.013, Netherlands
2022 Reviewer, Interdisciplinary approaches in oncogenic processes and therapeutic perspectives: Contributions of mathematics and informatics to oncology, Inserm, France
2022 External Reviewer, University of California-Irvine, California
2021 Panelist, IMAT panel reviewer for Biospecimen calls, R21/R33, NIH
2021 Panelist, NIH SBIR/STTR panel, R41/R42/R43/R44, NIH, December/June/March
2021 Panelist, NIH Shared Equipment panel, S10, ZRG1 CB-B (30) I study section, NIH
2021 Reviewer, National Research Foundation Prime Minister's Office, Singapore
2021 Reviewer, Georgia Tech IEN Seed grant, Atlanta
2020 Panelist, Breast Cancer Panel, Department of Defense
2019-now Reviewer, BMES Annual Meeting, 2020/2021/2022
2020-now Reviewer, Nature Biotechnology, Nature Methods, Science Advances, Nature Communications, Cell Systems, Communications Biology; Cell Reports Methods; Genome Biology

Committees

2021-2022 Graduate Recruitment and admissions committee
2021-2022 Diversity and inclusion committee
2020-2021 Undergraduate Committee
2019-2020 BME curriculum sub-committee
2019-2020 Graduate Committee

C. Contributions to Science

1. Spatial multi-omics and multiplexed data visualization. In the Single Cell Biotechnology Laboratory at the Georgia Institute of Technology, my team now applies spatial omics technologies to cells and tissues to decipher subcellular and cellular neighborhoods and signaling networks in healthy and diseased biological systems.

a. Allam, M., Hu, T., Lee, J., Aldrich, J., Badve, S., Polar-Gokmen, Y., Bhave, M., Ramalingam, S., Schneider, F., and **Coskun, A.F.** (2022) Spatially variant immune infiltration scoring in human cancer tissues. *npj Precision Oncology*, 6, 60, DOI: 10.1038/s41698-022-00305-4.
b. Cai, S., Hu, T., Venkatesan, M., Allam, M., Schneider, F., Ramalingam, S. S.,. Sun, S.Y., and **Coskun, A.F.** (2022) "Multiplexed protein profiling reveals spatial subcellular signaling networks," *iScience*, 25, 9, 104980, DOI: 10.1016/j.isci.2022.104980.
c. Ganesh, S., Hu, T., Woods, E., Allam, M., Cai, S., Henderson, W., **Coskun, A.F.** (2021) Spatially resolved 3D metabolomic profiling in tissues. *Science Advances*, 7, 5, DOI: 10.1126/sciadv.eabd0957
d. Allam M, Hu T, Cai S, Laxminarayanan K, Hughley R B, and **Coskun, A.F.** (2021) Spatially visualized single-cell pathology of highly multiplexed protein profiles in health and disease. *Communications Biology*, 10.1038/s42003-021-02166-2.

2. Single cell transcriptional profiling. Single-cell RNA sequencing has transformed systems biology research. This approach has the potential to reveal the heterogeneity of populations, which would have been lost due to averaging in conventional ensemble techniques such as genomics. However, single-cell sequencing methods lose the spatial context of the samples during the measurements. On the other hand, single-cell imaging approaches have been limited to a few parameters at a time. Therefore, there is still unmet demand for spatial genomics technologies in single cells based on imaging. To provide a solution to this important need, I developed (with a graduate student) a sequential FISH method that could profile gene expression in single cells by multiplexing a large number of mRNAs directly in tissue specimens. Using computational solutions, I have then provided an alternative single-cell transcriptional profiling method to quantify dense transcripts in situ using image correlations. We call this method a correlation FISH. It provides a general strategy to decode high-density molecules with multiplexing.

a. Fang, Z., Ford, A., Hu, T., Zhang, N., Mantalaris, A., **Coskun, A.F.** (2023) Subcellular spatially resolved gene neighborhood networks in single cells. *Cell Reports Methods, 100476*
b. **Coskun, A.F.** and Cai, L. (2016). Dense Transcript Profiling in Single Cells by Image Correlation Decoding. *Nature Methods*, 13, 657–660
c. **Coskun, A.F.**, Eser, U., and Islam, S. (2016) Cellular Identity at the Single-Cell Level, *Molecular BioSystems,*12, 2965-2979
d. Lubeck, E.*, Coskun, A.F.*, Zhiyentayev, T., Ahmad, M., and Cai, L. (2014). Single-cell in situ RNA profiling by sequential hybridization. *Nature Methods*, 11, 360-361 (*Co-first)

3. Spatially resolved omics for precision oncology. In the Single Cell Biotechnology Laboratory at the Georgia Institute of Technology, my team now applies spatial omics technologies to regenerative medicine and cancers to quantify cell-to-cell interactions in visually encoded precision medicine. The team integrates deep learning to classify patients into subgroups based on the spatial multi-omics maps of hundreds of genes.

a. S. Shah, C. Carlson, K. Lai, Z. Zhong, G. Marsico, K. Lee, N. Félix-Velez, E. Abeles, M. Allam, T. Hu, L. Walter, K. Martin, K. Gandhi, S. Butler, R. Puri, A. McCleary-Wheeler, W. Tam, O. Elemento, K. Takata, C. Steidl, D. Scott, L. Fontan, H. Ueno, B. Cosgrove, G. Inghirami, A. García, **A. F. Coskun**, J. Koff, A. Melnick, A. Singh, (2023) Combinatorial Targeting of Aberrant Signaling Pathways in Lymphomas Rescues Lymphoid Tumor Microenvironment-mediated attenuation of MALT1 inhibitors, *Nature Materials*, 22, 511–523.
b. Mosquera, M.J., Kim, S., Bareja, R., Fang, Z., Cai, S., Pan, H., Asad, M., Martin, M.L., Sigouros, M., Rowdo, F. M., Ackermann, S., Capuano, J., Bernheim, J., Cheung, C., Doane, A., Brady, N., Singh, R., Rickman, D. S., Prabhu, V., Allen, J.E., Puca, L., **Coskun, A.F.**, Rubin, M., Beltran, H., Mosquera, J. M., Elemento, O., Singh, A. (2022) Extracellular Matrix in Synthetic Hydrogel-based Prostate Cancer Organoids Regulate Therapeutic Response to EZH2 and DRD2 inhibitors, *Advanced Materials* 2100096 DOI: 10.1002/adma.202100096

c. Guo, Y., Lee, H., Fang, Z., Velalopoulou, A., Kim, J., Thomas, M. B. Liu, B. J., Abramowitz, R. G., Kim, Y., **Coskun, A. F.**, Krummel, D. P., Sengupta, S., MacDonald, T. J., and Costas Arvanitis. Single-cell analysis reveals effective siRNA delivery in brain tumors with microbubble-enhanced ultrasound and cationic nanoparticles. *Science Advances Vol. 7, no. 18, eabf7390DOI: 10.1126/sciadv.abf7390 (2021)*
d. Allam, M., Cai, S. and **Coskun, A.F.** (2020). Multiplex Bioimaging of Single Cell Spatial Profiles for Precision Cancer Diagnostics and Therapeutics. *NPJ Precision Oncology*, vol 4, 11 (2020) DOI: 10.1038/s41698-020-0114-1

4. Subcellular Protein and Metabolite Profiling. Immune regulation has been studied by flow and mass cytometers. These regulators revealed mechanisms of signaling and surface proteins for large cellular populations, but with limited spatial details. However, intracellular circuits interact with each other within subcellular volumes to make cellular decisions. Thus, we have developed subcellular assays based on ion beam imaging at a sub-100-nm resolution to visualize and quantitate chromatin, metabolic regulators, organelles, and protein factors.

a. Venkatesan, M., Zhang, N., Marteau, B., Yajima, Y., Ortiz De Zarate Garcia, N., Fang, Z., Hu, T., Cai, S., Ford, A. Olszewski, H., Borst, A., and **Coskun, A. F.** (2023) Spatial subcellular organelle networks in single cells. *Scientific Reports* 13, 5374.
b. **Coskun, A. F.**, Han, G., Ganesh, S. Chen, S.Y., Rovira-Clave, X., Harmsen, S., Jiang, S., Schürch, C. M., Bai, Y., Hitzman, C., Nolan, G. P. (2021) Nanoscopic subcellular imaging enabled by ion beam tomography, *Nature Communications*. 12, 789
c. Harmsen, S. *, **Coskun, A.F.** *, Nolan, G.P., Gambhir, S. S. (2019). Isotopically encoded nanotags for highly multiplexed ion beam imaging. *Advanced Mat Technologies*, DOI: 10.1002/admt.202000098 (2020) (*Co-first and Corresponding)
d. Rovia-Clave, X., Jiang, S., Bai, Y., Barlow, G. L., Bhate, S., **Coskun, A.F.**, Han, G., Zhu, B., Ho, C. M., Hitzman, C., Chen, S. Y., Bava, F. A. and Nolan, G.P. (2021) Subcellular localization of biomolecules and drug distribution by high-definition ion beam imaging, *Nature Communications*. 12, 4628.

5. Bioengineering educational tools. To translate data into discoveries, my team has launched the Bioengineering Media Lab at Georgia Tech, with the mission to implement visual representations of spatial omics data. To achieve this, his team interfaced emerging digital technologies with spatial omics data, to visually encode precision medicine using interactive and 3D representations and stimulate novel data interpretation and discovery.

a. Venkatesan, M., and **Coskun, A.F.** (2019). Digital posters for interactive cellular media and bioengineering education. *Communications Biology*.2:455. doi: 10.1038/s42003-019-0702-1
b. Venkatesan, M., Mohan, H., Ryan, J., Schurch, C. M., Nolan, G.P., Frakes, D.H., and **Coskun, A.F.** (2021). Virtual and Augmented Reality for Biomedical Applications. *Cell Reports Medicine*. 2, 7 100348

Complete List of Published Work in MyBibliography:

https://pubmed.ncbi.nlm.nih.gov/?term=coskun+af&sort=date

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: Cui, Guiying

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

POSITION TITLE: Assistant Professor of Pediatrics, Emory University School of Medicine

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
Yanbian Medical College	M.D.	07/1992	Medicine
China Medical University	MB	07/1997	Pharmacology
China Medical University	Ph. D	07/2001	Physiology

A. Personal Statement

I have a broad background in physiology and pharmacology, with specific training and expertise in key research areas for this application. My current research mainly focuses on two research areas: (1) Cystic Fibrosis lung physiology in hyperglycemia condition utilizing both cell models and mice models. Focusing on bronchial epithelial cells to test different ion channels and tight junction proteins modulated by hyperglycemia by combining multiple techniques to do this study, including Ussing chamber recording, immunostaining, western blotting, transcriptomic analysis, and more. Using CF related diabetes mice model to test lung function, airway inflammation, and airway infection by combining multiple techniques, including cell count of bronchoalveolar lavage fluid, lung histology, RNAseq analysis of lung tissue and airway cells, and more. My data thus far suggest that CF bronchial epithelial is severely “leaky” compared to wildtype bronchial epithelial which resulted in changes in multiple ion channels and tight junction proteins. (2) To probe modulation of CFTR channel by lipids, including cholesterol. I have found that CFTR channel behavior was significantly affected by changes in the plasma membrane cholesterol level modified by methyl-β-cyclodextrin, cholesterol oxidase, and cholesterol esterase. I have further found that function of the sole potentiator for CF patients Kalydeco also modulated by the plasma membrane cholesterol level change.

B. Positions and Honors

Positions and Employment

2001-2005 Postdoctoral fellow, School of Biology, Georgia Institute of Technology, Atlanta, GA
2006-2007 Postdoctoral fellow, Department of Pharmacology, Emory University, Atlanta, GA
2008-2015 Instructor, Department of Pediatrics, Emory University, Atlanta, GA
2015- Assistant professor, Department of Pediatrics, Emory University, Atlanta, GA

Professional Memberships

2001-2005 Biophysical Society
2006-2007 Society for Neuroscience
2007- Biophysical Society

C. Contributions to Science

1) Regulation of CFTR function and pharmacology by plasma membrane cholesterol and lipids. My main project is to understand the mechanism that the plasma membrane lipid modulates CFTR channel function and alter the potentiation efficiency by CFTR modulator. I have found that the plasma membrane cholesterol changes significantly affect CFTR channel behavior and VX-770 potentiation efficiency. I have established the techniques to move this project forward.

a) Stauffer BB, **Cui G,** Infield DT, and McCarty NA. (2017). Bacterial Sphingomyelinase is a state-dependent inhibitors of CFTR. *Sci Rep,* **7**:2931. PMID: 28592822

b) Cui, G., K.A. Cottrill, and N.A. McCarty (2021) Electrophysiological approaches for the study of ion channel function. *Methods Mol Biol* **2302**:49-67. PMID: 33877622

c) Cui, G., K.A. Cottrill, K.M. Strickland, B.R. Imhoff, S.A. Mashburn, M. Koval, and N.A. McCarty (2021) Alteration of membrane Cholesterol content plays a key role in regulation of CFTR channel activity. *Front. Physiol.* **12**:652513. PMID: 34163370.

2) Molecular pharmacology of CFTR I have studied CFTR structure/function and pharmacology since I was a postdoc in the McCarty lab. The majority of our papers have reflected the use of various compounds as probes of CFTR structure and function, including my most highly-cited work. These are highly mechanistic studies. In particular, we used this mechanistic understanding of CFTR to better understand the mechanism by which CFTR is potentiated by compounds such as VX-770.

a) Cui G, Song B, Turki HW, and McCarty NA (2012) Differential contribution of TM6 and TM12 to the pore of CFTR identified by three sulphonylurea-based blockers. *Pflügers Archiv.* **463**:405-418. PMID: 22160394

b) Cui G, and McCarty NA (2015) Murine and human CFTR exhibit different sensitivities to CFTR potentiators. *Am. J. Physiol: Lung*. **309**:L687-699. PMID: 26209275.

c) Cui G, Khazanov N, Stauffer BB, Infield DT, Imhoff BR, Senderowitz H, and McCarty NA (2016) Potentiators exert distinct effects on human, murine, and *Xenopus* CFTR *Am.J.Physiol: Lung.* **311**: L192-207. PMID: 27288484.

d) Cui G, Stauffer BB, Imhoff BR, and McCarty NA (2019) VX-770 potentiation of numerous human CFTR disease mutants influenced by CFTR phosphorylation level**.** *Scientific Report*, 2019, 9 (1): 13460. PMID: 31530897

3) The pore of the CFTR channel, and pore-gating: The major focus of the McCarty lab, during the time that I have been a member of it, has been understanding how CFTR functions as a Cl^- channel. We have used mutagenesis and high-resolution electrophysiology to identify elements of this important protein that contribute to channel activity. These studies have helped to identify the regions underlying ion selectivity, pore gating and stability, and the pharmacology of this protein in relation to both inhibition and potentiation.

a) Cui G, Freeman C, Knotts T, Prince CZ, Kuang C, and McCarty NA (2013) Two salt bridges differentially contribute to the maintenance of CFTR channel function. *J. Biol. Chem.* **288**:20758-67. (PMC:3711338)

b) Cui G, Rahman KS, Infield DT, Kuang C, Prince CZ, and McCarty NA (2014) Three charged amino acids in extracellular loop 1 are involved in maintaining outer pore architecture of CFTR. *J. Gen. Physiol.* **144**:159-179. PMID: 22160394 (PMC:4113900)

c) Infield DT, **Cui G**, Kuang C, and McCarty NA (2016) The positioning of the N-terminal end of extracellular loop 1 significantly affects pore gating of the Cystic Fibrosis Transmembrane Conductance Regulator. *Am. J. Physiol: Lung* **310**:L403-14. PMID: 2668425

d) Strickland, K.M., G. Stock, **Cui G**, H. Hwang, D.T. Infield, I. Schmidt-Krey, N.A. McCarty, and J.C. Gumbart (2019) ATP-Dependent signaling in simulations of a revised model of cystic fibrosis transmembrane conductance regulator (CFTR). *J Phys Chem B*. 123:3177-3188. PMID: 30921517

e) Moffett AS, **Cui G**, Thomas PJ, Hunt WD, McCarty NA, Westafer R, and Eckford AW. 2022 A factor graph EM algorithm for inference of kinetic microstates from patch clamp measurements. *Biophysical Reports* 2, 100083

f) Hunt WD, McCarty NA, Marin EM, Westafer RS, Yamin PR, **Cui G**, Eckford AW, and Dension DR. 2023. A transistor model for the cystic fibrosis transmembrane conductance regulator. *Biophysical Reports 3*, 100108

4) Evolution of channel activity in CFTR: CFTR is the only member of the ABC Transporter Superfamily known to function as a channel. This fact makes CFTR a great target for the study of molecular evolution. We have combined bioinformatics approaches, computational modeling, and functional interrogation to understand

how CFTR, alone, evolved the ability to interrupt the alternating access function of a transporter by stabilizing a state with the pore open at both ends. In collaboration with the lab of Dr. King Jordan at Georgia Tech, my lab was the first to explain the origin and evolution of the regulatory domain of CFTR – a domain that is not found in any other member of the ABC Transporter Superfamily member. The regulatory domain arose between 550 and 650 million years ago. We also determined the mechanism by which this important domain of CFTR evolved since it arose, and described how that links to function.

a) Zhang Z-R, **Cui G**, Liu XH, Song BL, Dawson DC, McCarty NA. Determination of the Functional unit of the Cystic Fibrosis Transmembrane Conductance Regulatior Chloride channel. *Journal of Biological Chemistry,* 2005, 280: 458-468. PMID: 15504728

b) Jordan IK, Kota K, **Cui G.**, Thompson CH, and McCarty NA (2008) Evolutionary and functional divergence of CFTR from ABC transporters. *Proc.Natl.Acad.Sci. U.S.A.***105**:18865-18870. (PMC:2585040)

c) Rahman KS, **Cui G**, Harvey SC, and McCarty NA (2013) Modeling the conformational change underlying channel opening in CFTR. *PLoS One*. 8: e74574. (PMC:3785483)

d) Cui G, Hong J, and Chung-Davidson Yu-Wen et al. (2019) Lamprey express an ancient CFTR ortholog with unique structural and biophysical properties. *Dev Cell* 51 (4): 421-430. PMID:31679858.

5) Other ion channels: structure, pharmacology, and regulation: Collaborative work done in the labs of H.C. Hartzell and A. Lee at Emory has contributed to our understanding of the structure and mechanisms of calcium channels and calcium-activated chloride channels.

a) Yu K, Xiao Q, **Cui G**, Lee A, Hartzell HC (2008) The best disease-linked Cl- channel hBest1 regulates CaV1 (L-type) Ca^{2+} channels via src-homology-binding domains. *J Neurosci*. 28:5660-70 (PMC2587081)

b) Lee A, Jimenez A, **Cui G**, Haeseleer F (2007) Phosphorylation of the Ca^{2+}-binding protein CaBP4 by protein kinase C zeta in photoreceptors. *J Neurosci*. 27:12743-54 (PMC2703458)

c) **Cui G**, Meyer AC, Calin-Jageman I, Neef J, Haeseleer F, Moser T, Lee A (2007). Ca^{2+}-binding proteins tune Ca^{2+}-feedback to Cav1.3 channels in mouse auditory hair cells. *J Physiol*. 585:791-803 (PMC2375505)

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/50579628/?reload=settingSaved

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: Stephen Diggle

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

POSITION TITLE: Professor, School of Biological Sciences Georgia Institute of Technology

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
Salford University (UK)	BSc	06/1997	Biological Sciences
Nottingham University (UK)	PhD	09/2001	Molecular Microbiology
Royal Society; Nottingham University (UK)	Fellowship	09/2013	Social Evolution

A. Personal Statement

I have over 25 years research experience in the field of bacterial cell-to-cell communication (quorum sensing, QS). My group was the first to demonstrate that QS is a social trait, that it is costly for cells to perform, but is exploitable by social cheats *in vitro, in vivo* and in biofilms. We also showed that QS is likely to be maintained in natural populations at high relatedness because of kin selection. We experimentally showed, that the fitness benefits provided by QS are most beneficial at high cell densities, something that had long been assumed in the literature but never tested. Our work encompasses both molecular and evolutionary approaches giving me unique insights into how bacteria cause disease and resist treatment. I am committed to supporting Societies and have a track record of service to the Microbiology Society in the UK. I was an elected Member of the UK Microbiology Society Council and a member of their conference committee (2012-2017). After my term ended, I became a Senior editor for their flagship journal 'Microbiology'. I am committed to promoting microbiology to students, early career scientists and the general public. In the UK, I have been a guest on live BBC Radio Science shows to discuss quorum sensing. I have also been interviewed by New Scientist about how we can use bacterial cheats to treat infection (cheatobiotics). Finally, our work with a 1000-year old ancientbiotic recipe, led to TV, radio and newspaper interviews (ie: Washington Post, CNN) and the work was featured for a full-length episode of Radio Lab (Staph Retreat). My work is often interdisciplinary in nature and highlights how scientists and researchers from different fields can work together to create new ideas and conceptually change research fields.

B. Positions, Scientific Appointments and Honors

Positions

2022 –	Full Professor, School of Biology, Georgia Institute of Technology, Atlanta GA
2017 – 2022	Associate Professor, School of Biology, Georgia Institute of Technology, Atlanta GA
2013 – 2017	Associate Professor in Sociomicrobiology, University of Nottingham, UK
2006 – 2014	Royal Society University Research Fellow, University of Nottingham, UK
2004 – 2006	Post Doctoral Research Fellow, University of Nottingham, UK
2001 – 2004	Post Doctoral Research Associate, University of Nottingham, UK

Scientific Appointments

2021 – 2023	Member of the ASM Distinguished Lecturer Program
2017 –	Editor for Microbiology (Senior Editor from 2021)

2016 – 2017	Member of the Microbiology Society Policy Committee
2016	Member of the advisory board for the ASM 7th International Conference on Cell-to-Cell Signaling in Bacteria
2014 – 2017	Associate Editor for Royal Society 'Open Science'
Journal2013 – 2017	Microbiology Society elected Council Member
2013	Member of the advisory board for the ASM 6th International Conference on Cell-to-Cell Signaling in Bacteria (San Antonio, Texas)
2012 – 2014	Associate Editor for BMC Microbiology
2011 – 2017	Editor for Microbiology Open
2011	Organized a 2-day session on social evolution in microbes at the 2011 Microbiology Society General meeting in Harrogate
2008 – 2012	Member of the Editorial Board for FEMS Microbiology Letters

Honors

2023	Elected to the American Academy of Microbiology
2020	Cullen-Peck Scholar Award (Georgia Tech)
2006 – 2014	Royal Society University Research Fellowship awarded by the Royal Society of London.
2010	Microbiology Society Fleming Award; outstanding achievement by early stage microbiologist
1997	Final year course prize for highest achievement (University of Salford, UK)

C. Contributions to Science

Chronic infection and biofilms

We have shown that phenotypic and genotypic diversity in *Pseudomonas aeruginosa* cystic fibrosis infections is driven by recombination and that this has implications for antibiotic resistance and susceptibility testing (a). We have worked on R-pyocins (bacteriocins) produced by *P. aeruginosa* to show that susceptibility can vary in heterogenous populations of *P. aeruginosa* sourced from cystic fibrosis (CF) lungs (b), but also that most CF 'variants' remain susceptible to R2-pyocin killing (c). We have demonstrated that genotypic diversity in evolving *Pseudomonas aeruginosa* populations can impact on community functions including antibiotic resistance (d).

(a) Darch, S. E., McNally, A., Harrison, F., Corander, J., Barr, H. L., Paszkiewicz, K., Holden, S., Fogarty, A., Crusz, S. A. & **Diggle, S. P.** (2015) Recombination is a key driver of genomic and phenotypic diversity in a *Pseudomonas aeruginosa* population during cystic fibrosis infection. *Scientific Reports*. 5: 7649.

(b) Mei, M., Thomas, J. & Diggle, S. P. (2021) Heterogenous susceptibility to R-pyocins in populations of *Pseudomonas aeruginosa* sourced from cystic fibrosis lungs. mBio. e00458-21.

(c) Mei, M., Pheng, P., Kurzeja-Edwards, D. & Diggle, S. P. (2023) High prevalence of lipopolysaccharide mutants and R2-pyocin susceptible variants in *Pseudomonas aeruginosa* populations sourced from cystic fibrosis lung infections. BioRxiv.

(d) Azimi, S., Roberts, A. E. L., Peng, S., Weitz, J. S., McNally, A., Brown, S. P. & **Diggle, S. P.** (2020) Allelic polymorphism shapes community function in evolving *Pseudomonas aeruginosa* populations. *ISME J*. 14: 1929-1942.

Quorum sensing and Sociomicrobiology

I made significant contributions to our understanding of the mechanistic basis of bacterial quorum sensing. Specially, I spent many years understanding the role of the AHL-dependent and PQS-dependent QS systems in regulating virulence in the opportunistic pathogen *Pseudomonas aeruginosa.* My research then switched into asking more adaptive questions relating to QS. As a key member of an interdisciplinary team, we

comprehensively reviewed how social evolution theory applies to microbes and the implications of this for virulence. We were the first to demonstrate that density is an important component of QS, something which is assumed in the literature but had not previously been empirically demonstrated (a). Experimentally we were also the first to show that QS is a cooperative social behavior, which can be exploited by cheats *in vitro* (b), *in vivo* (c) and in biofilms (d) and that a potential solution to this problem is cooperation between relatives (kin selection) (b).

(a) Darch, S. E., West, S. A., Winzer, K. & **Diggle, S. P.** (2012) Density-dependent fitness benefits in quorum sensing bacterial populations. *Proceedings of the National Academy of Sciences USA.* 109: 8259-8263.

(b) **Diggle, S. P.,** Griffin, A. S., Campbell, G. S. & West S. A. (2007) Cooperation & conflict in quorum sensing bacterial populations. *Nature.* 450: 411-414.

(c) Rumbaugh, K. P., **Diggle, S. P.,** Watters, C., Ross-Gillespie, A., Griffin, A. S., & West, S. A. (2009) Quorum sensing and the social evolution of bacterial virulence. *Current Biology*. 19: 341-345.

(d) Popat, R., Crusz, S. A., Messina, M., Williams, P., West, S. A. & **Diggle, S. P.** (2012) Quorum sensing and cheating in bacterial biofilms. *Proceedings of the Royal Society B*. 279: 4765-4771.

Complete List of Published Work in MyBibliography:

https://pubmed.ncbi.nlm.nih.gov/?term=diggle+sp&sort=date

BIOGRAPHICAL SKETCH

NAME: **Benjamin T. Kopp**

eRA COMMONS USER NAME: KOPPB1

POSITION TITLE: Associate Professor of Pediatrics

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Miami University, Oxford, OH	B.A.	05/2002	Zoology
The Ohio State University, Columbus, OH	M.D.	06/2006	Medicine
Nationwide Children's Hospital	Residency	06/2009	Pediatrics
Nationwide Children's Hospital	Fellowship	06/2012	Pediatric Pulmonology
The Ohio State University, Columbus, OH	MPH	06/2017	Public Health

A. Personal Statement

My laboratory is dedicated to the immunologic control of microbial infections in cystic fibrosis (CF), in addition to a burgeoning interest in sickle cell lung disease. We specifically focus on host-pathogen-environment interactions in children, with the goal to develop targeted therapeutics for dysfunctional host-immunity. We have extensive experience in handling human immune cells from patients with CF, particularly alveolar and monocyte-derived macrophages. Over the past fifteen years, we have identified key deficiencies in human CF macrophage function including defective assembly of the NADPH oxidase[1] and reduced macrophage autophagy. Autophagy and NADPH oxidase signaling are among many pathways of dysregulated immunity in CF that interact together in our current research investigating mechanisms of signaling between host phagocytes, pathogens, and environmental stressors that lead to bacterial persistence in CF. Environmental stressors of interest include secondhand tobacco exposure, which critically influences early disease in CF.[2] Our in vitro work resulted in exciting new insights into gene-expression networks that regulate inflammatory signaling induced by tobacco exposure. Additionally, we have contributed critical findings on the role of CFTR in macrophage function[3] and deficits in macrophage function that persist post-CFTR modulator therapy[4]. I am the former Co-Director for the Cure CF Columbus (C3) Research & Development Program (RDP) Immune Core and have been a part of the CF Foundation Research and Training review panel since 2018. In 2022, I was recruited to Emory University to Co-Direct the Center for CF and Airways Disease Research (**CF-AIR**) and Direct the Pulmonary Sickle Cell Disease program.

Information pertinent to this proposal: In the current P*3 proposal CF-MORBI[3]D, I will be part of a multidisciplinary team of scientists investigating aberrant responses to CF lung injury to help develop new therapeutic approaches. I will provide expertise in monocyte and macrophage biology and their relation to lung repair to the proposed in vitro and ex vivo model studies and actively participate in planning grant activities. Although I am new to Emory in 2022, I have active collaborations with Drs. McCarty, Tirouvanziam, Chandler, and Takayama, and have had extensive scientific discussions with the other PIs for ongoing work and am excited to be a part of this highly collaborative team.

Ongoing relevant support includes:

R01 HL148171-01A1 NIH NHLBI
Kopp (PI), **Wozniak** (Co-I), **Partida-Sanchez** (Co-I)
04/01/2020 – 03/31/2025
The role of CFTR during macrophage-mediated killing of bacteria

R01 HL158747-01A1 NIH NHLBI
Kopp (Contact MPI), Partida-Sanchez, Amer, Hall-Stoodley
04/01/2022 – 03/31/2026
Rescue of CF phagocyte function with CFTR modulator therapy

R21 AI174000-01 NIH NIAID
Kopp (Contact PI), Creary (MPI), Hood (MPI), Mejias (MPI), Ramilo (MPI)
09/01/2022 – 08/31/2024
SMILE: Sickle Cell Disease Microbiologic and Immunologic Links to Health Equity

Citations:

1. Assani K, Shrestha CL, Robledo-Avila F, Rajaram MV, Partida-Sanchez S, Schlesinger LS, **Kopp BT.** "Human Cystic Fibrosis Macrophages Have Defective Calcium-Dependent Protein Kinase C Activation of the NADPH Oxidase, an Effect Augmented by *Burkholderia cenocepacia*". *J Immunol*. 2017 Jan 16. PMID: 28093527.
2. **Kopp BT**, Thompson R, Kim J, Konstan R, Diaz A, Smith B, Shrestha C, Rogers LK, Hayes D, Tumin D, Woodley F, Ramilo O, Sanders DB, Groner J, Mejias A. Secondhand smoke alters arachidonic acid metabolism in infants and children with cystic fibrosis. *Thorax.* Jan 19, 2019. PMID: 30661024.
3. Zhang, S, Shrestha CL, Wisniewski B, Pham H, Hou X, Li W, Dong Y**, Kopp BT**. "Consequences of CRISPR-Cas9-mediated CFTR knockout in human macrophages." Frontiers in Immunology. 2020 July. PMID: 32973772.
4. Zhang S, Shrestha C, Robledo F, Jaganathan D, Wisniewski B, Brown N, Pham H, Carey K, Amer A, Hall-Stoodley L, McCoy K, Bai S, Partida-Sanchez S, **Kopp BT**. Cystic fibrosis macrophage function and clinical outcomes after elexacaftor/tezacaftor/ivacaftor. European Respiratory Journal, 2022.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2023 – Present	Co-Director, Pediatric Residency Investigative Scholars at Emory (PRISE)
2023 – Present	Co-Director, CF Scholars Program, Emory University
2023 – Present	Hercules Exposome Research Center Member, Emory University
2022 – Present	Associate Professor of Pediatrics, Emory University
2022 – Present	Co-Director, Center for CF and Airways Disease Research (CF-AIR), Emory University
2022 – Present	Director of Pulmonary Sickle Cell Program, Emory University
2021 – 2022	Associate Professor of Pediatrics, The Ohio State University, Columbus, OH
2021 – 2022	Director of Pulmonary Research, Nationwide Children's Hospital (NCH), Columbus, OH
2022 – 2023	Program Chair, Pediatrics Assembly of the American Thoracic Society
2020 – 2022	Faculty, Center for Microbiome Science, The Ohio State University (OSU/NCH, Columbus, OH
2020 – Present	Fellow, American Thoracic Society
2019 – 2021	Editorial Board, Scientific Reports (Nature)
2019 – 2022	Cystic Fibrosis Canada Scientific Review Panel
2018 – Present	Standing Grant Reviewer, CF Foundation Research and Research Training Committee
2017 – 2022	Co-Director, Cure CF Columbus (C^3) Immune Core, NCH/OSU, Columbus, OH
2017 – Present	Appointed Member, American Academy of Pediatrics Tobacco Consortium
2017 – 2022	Faculty, Infectious Disease Institute, OSU/NCH, Columbus, OH
2017 – 2019	Member, Vertex Cystic Fibrosis Advisory Board
2012 – 2022	Principal Investigator, Center for Microbial Pathogenesis, Abigail Wexner Research Institute at NCH Assistant Professor of Pediatrics, OSU, Columbus, OH Faculty, Division of Pulmonary Medicine, NCH, Columbus, OH Courtesy appointment, Dept Microbial Infection & Immunity, OSU, Columbus, OH
2009 – 2012	Post-doctoral Fellow, Amal Amer Laboratory
2009 – 2012	Fellow, Pediatric Pulmonology, Nationwide Children's Hospital, Columbus, OH
2006 – 2009	Resident, Pediatrics, NCH, Columbus, OH

Honors

2019	Best Scientific Abstract Award, American Thoracic Society Assembly on Pediatrics
2017	Outstanding Junior Faculty Investigator, Certificate of nomination
2016	John and Ruth Weimer Mount Award, OSU
2016	Best Mentor, Center for Microbial Interface Biology, OSU

2015 Columbus' Finest
2015 Junior Faculty Innovation Award, Certificate of nomination
2015 Outstanding Mentor Award for Basic Research, Research Institute at NCH
2014 – 2017 NIH Loan Repayment renewal
2012 OSU Medical Center Research Travel Award
2012 ATS Travel Award
2011 Distinguished Clinical Fellow of the Year
2010 NIH Loan Repayment Award

Medical Licensures and certifications
2022 Georgia Medical License (active)
2012 Sub-board Pediatric Pulmonology (active)
2009 American Board of Pediatrics (active)
2009 Ohio Medical license (inactive)

Patents
Kopp, B., et al. Compositions and methods for inhibiting the growth of multi-drug resistant microbes. **U.S. Patent application** 10028933. 2018.
Kopp, B., et al. Compositions and methods for improving CFTR function in CF-affected cells. U.S. Provisional Patent application 62/626,766. 2018.

C. Contributions to Science

C1. **Discovered fundamental defects in macrophage function dependent on CFTR.** Why bacterial infections in the CF airway are established so early in life remains poorly understood. We recently discovered that CF macrophages have a defective assembly of the NADPH oxidase, which leads to reduced reactive oxygen species (ROS) production, and therefore reduced bacterial killing. The defective assembly is characterized by reduced calcium-dependent protein-kinase-C-mediated phosphorylation of cytosolic NADPH components.[a] As ROS production is one of the earliest responses to infection, we have determined a crucial initiating step for the early establishment of intracellular survival in CF macrophages. This critical finding is a focus of ongoing studies in the CF host immune response to bacterial pathogens. As part of a large project to determine how loss of CFTR influences macrophage function, we developed a CRISPR-Cas9 CFTR human macrophage model in which we verified that defective NADPH oxidase assembly is directly dependent on CFTR.[b] We also helped identify similar deficits in CF neutrophil ROS production.[c] Finally, we identified mechanisms by which CFTR modulation partially restores macrophage function and immunophenotypic markers of clinical responses. I served as the senior author on 3 studies and co-author on the other.

a. Assani K, Shrestha CL, Robledo-Avila F, Rajaram MV, Partida-Sanchez S, Schlesinger LS, **Kopp BT.** "Human Cystic Fibrosis Macrophages Have Defective Calcium-Dependent Protein Kinase C Activation of the NADPH Oxidase, an Effect Augmented by *Burkholderia cenocepacia*". ***J Immunol.*** 2017 Jan 16. PMID: 28093527.
b. Zhang, S, Shrestha CL, Wisniewski B, Pham H, Hou X, Li W, Dong Y, **Kopp BT**. "Consequences of CRISPR-Cas9-mediated CFTR knockout in human macrophages." ***Frontiers in Immunology***. 2020 July. PMID: 32973772
c. Robledo-Avila FH, Ruiz-Rosado JD, Brockman KL, **Kopp BT**, Amer AO, McCoy K, Bakaletz LO, Partida-Sanchez S. Dysregulated Calcium Homeostasis in Cystic Fibrosis Neutrophils Leads to Deficient Antimicrobial Responses. ***J Immunol.*** 2018 Oct 1;201(7):2016-2027. PMID: 30120123
d. Zhang S, Shrestha C, Robledo F, Jaganathan D, Wisniewski B, Brown N, Pham H, Carey K, Amer A, Hall-Stoodley L, McCoy K, Bai S, Partida-Sanchez S, **Kopp BT**. Cystic fibrosis macrophage function and clinical outcomes after elexacaftor/tezacaftor/ivacaftor. ***European Respiratory Journal***, 2022.

C2. **Determined critical pathways of CF macrophage-mediated bacterial killing that respond to therapeutics.**
We have determined that human CF macrophages have reduced autophagy, which leads to impaired macrophage-mediated bacterial killing. Reduced macrophage autophagy is dependent on transglutaminase 2 (TG2)-mediated sequestration of autophagy initiating molecules, which is reversed by administration of the TG2 inhibitor cysteamine.[a] Autophagy and CFTR can be independently stimulated through the novel agent,

AR-13, which results in clearance of multi-drug resistant bacteria from primary immune cells (U.S. Patent application 10028933).[b] Further, macrophage responses to CFTR modulation reveals additional targets for therapeutic manipulation of host defenses.[c] Using RNA-Seq we have also determined novel therapeutic pathways induced by CFTR modulators that can be targeted for future immunomodulation in CF.[d] I was the senior author on these studies.

a. Shrestha CL, Assani KD, Rinehardt H, Albastroiu F, Zhang S, Shell R, Amer AO, Schlesinger LS, **Kopp BT**. Cysteamine-mediated clearance of antibiotic-resistant pathogens in human cystic fibrosis macrophages. ***PLoS One****.* 2017 Oct 5;12(10). PMID: 28982193.
b. Assani K, Shrestha CL, Rinehardt H, Zhang S, Robledo-Avila F, Wellmerling J, Partida-Sanchez S, Cormet-Boyaka E, Reynolds SD, Schlesinger LS, **Kopp BT**. AR-13 reduces antibiotic-resistant bacterial burden in cystic fibrosis phagocytes and improves CFTR function. ***J Cyst Fibros***. 2018 Oct 23. PMID: 30366849.
c. Zhang S, Shrestha CL, **Kopp BT.** Cystic fibrosis transmembrane conductance regulator (CFTR) modulators have differential effects on cystic fibrosis macrophage function. ***Sci Rep***. 2018 Nov 20;8(1):17066. PMID: 30459435.
d. **Kopp BT**, Fitch J, Jaramillo L, Shrestha CL, Robledo-Avila F, Zhang S, Palacios S, Woodley F, Hayes D Jr, Partida-Sanchez S, Ramilo O, White P, Mejias A. Whole-blood transcriptomic responses to lumacaftor/ivacaftor therapy in cystic fibrosis. ***J Cyst Fibros***. 2019 Aug 29; PMID: 31474496.

C3. **Characterized factors determining *Burkholderia cenocepacia* survival in human CF macrophages**. To model bacterial persistence in CF and the efficacy of potential therapeutics, I have studied the persistence of *B. cenocepacia* in human CF macrophages. *B. cenocepacia* is highly virulent in patients with CF, underscoring its importance in modeling. We first determined that *B. cenocepacia* uses an O antigen to stimulate caspase-1 dependent IL-1β production and resulting host damage.[a] We then determined that human macrophages respond to *B. cenocepacia* with an exaggerated inflammatory cytokine response including IL-1β.[b] Recently, we discovered that bacterial intracellular survival occurs via reduced autophagy and therefore results in excess cytokine production. Reduced autophagy can be reversed with autophagy stimulating agents such as IFN-γ[c] and is controlled by a microRNA cluster that also negatively regulates CFTR and is predictive of clinical outcomes.[d] I was the lead, co-lead or senior author on these publications.

a. Kotrange S, **Kopp B**, Akhter A, Abdelaziz D, Abu Khweek A, Caution K, Abdulrahman B, Wewers MD, McCoy K, Marsh C, Loutet SA, Ortega X, Valvano MA, Amer AO. *Burkholderia cenocepacia* polysaccharide chain contributes to caspase-1-dependent IL-1beta production in macrophages*.* ***J Leukoc Biol.*** 2011 Mar;89(3):481-8. PMID: 21178113; PMCID: PMC3040464.
b. **Kopp BT**, Abdulrahman BA, Khweek AA, Kumar SB, Akhter A, Montione R, Tazi MF, Caution K, McCoy K, Amer AO." Exaggerated inflammatory responses mediated by *Burkholderia cenocepacia* in human macrophages derived from Cystic fibrosis patients." ***Biochem Biophys Res Commun***. 2012 Jul 27;424(2):221-7. PMID: 22728038; PMCID: PMC3408781.
c. Assani K, Tazi MF, Amer AO, **Kopp BT**. "IFN-γ stimulates autophagy-mediated clearance of *Burkholderia cenocepacia* in human cystic fibrosis macrophages." ***PLoS One***. 2014 May 5;9(5):e96681. PMID: 24798083
d. Krause K*, **Kopp BT***, Tazi MF, Caution K, Hamilton K, Badr A, Shrestha C, Tumin D, Hayes D Jr, Robledo-Avila F, Hall-Stoodley L, Klamer BG, Zhang X, Partida-Sanchez S, Parinandi NL, Kirkby SE, Dakhlallah D, McCoy KS, Cormet-Boyaka E, Amer AO. The expression of Mirc1/Mir17-92 cluster in sputum samples correlates with pulmonary exacerbations in cystic fibrosis patients. ***J Cyst Fibros***. 2018 Jul;17(4):454-461. PMID: 29241629

C4. **Identified the impact of secondhand smoke exposure on early disease progression and survival in children with CF.** Concurrent with our studies on host immune function, I am interested in how environmental modifiers can modulate immunity in CF. I was the first investigator to identify second-hand smoke as a risk factor for poor growth and bacterial acquisition in infants with CF.[a] Currently we are prospectively examining the correlation between nicotine levels in infants and inflammatory gene expression and immune cell function in CF. We have discovered that secondhand smoke exposure alters arachidonic acid metabolism, which is associated with heightened inflammation and increased bacterial burden.[b] Further, we have identified alterations in the intestinal microbiome that are correlated with smoke exposure in young children.[c] Finally, we identified critical metabolic pathways altered by tobacco exposure in the same cohort.[d] I was the lead or senior author on these studies and coordinated all study efforts.

1. **Kopp BT**, Sarzynski L, Khalfoun S, Hayes D Jr, Thompson R, Nicholson L, Long F, Castile R, Groner J. Detrimental effects of secondhand smoke exposure on infants with cystic fibrosis. ***Pediatr Pulmonol*.** 2014 Mar 9. PMID: 24610820.
2. **Kopp BT**, Thompson R, Kim J, Konstan R, Diaz A, Smith B, Shrestha C, Rogers LK, Hayes D, Tumin D, Woodley F, Ramilo O, Sanders DB, Groner J, Mejias A. Secondhand smoke alters arachidonic acid metabolism in infants and children with cystic fibrosis. ***Thorax***. Jan 19, 2019. PMID: 30661024
3. Loman BR, Shrestha CL, Thompson R, Groner JA, Mejias A, Ruoff KL, O'Toole GA, Bailey MT, **Kopp BT**. Age and environmental exposures influence the fecal bacteriome of young children with cystic fibrosis. ***Pediatr Pulmonol***. 2020 Apr 10. PMID: 32275127
4. Wisniewski B, Shrestha CL, Zhang S, Thompson R, Gross M, Groner J, Uppal K, Mejias M, **Kopp BT**. Metabolomics profiling of tobacco exposure in children with cystic fibrosis. ***J Cyst Fibros***. 2020 May. PMID: 32487493.

C5. **Discovered novel alterations in gene regulatory networks and microbial interactions during acute chest syndrome in children with sickle cell disease (SCD).** We are currently using a multi-omics approach to better understand acute and chronic lung disease in children with SCD. We are the first to publish alterations in the upper airway microbiome of children with SCD at baseline, during acute chest syndrome, and during vaso-occlusive episodes.[a] We are also the first to describe whole-blood RNA-seq transcriptional profiles in this same cohort.[b] Overall, these findings will continue to help drive the research and clinical recommendations for lung disease in children with SCD,[c] including the provision of multi-disciplinary care models which improve patient outcomes.[d]

a. Creary S, Loman BR, Kotha K, Shrestha CL, Minta A, Zhang S, Pinto S, Thompson R, Mejias A, Bailey MT, and **Kopp BT**. Upper airway microbiome changes in children with sickle cell during acute chest syndrome. ***Am J Hematology***. 2020 July 9. PMID: 32644239.
b. Creary S, Shrestha CL, Kotha K, Minta A, Fitch J, Jaramillo L, Zhang S, Pinto S, Thompson R, Ramilo O, White P, Mejias A, **Kopp BT**. Baseline and disease-induced transcriptional profiles in children with sickle cell disease. ***Sci Reports (Nature***). 2020 Jun. PMID: 32487996
c. Ruhl AP, Sadreameli SC, Allen JL, Bennett DP, Campbell AD, Coates TD, Diallo DA, Field JJ, Fiorino EK, Gladwin MT, Glassberg JA, Gordeuk VR, Graham LM, Greenough A, Howard J, Kato GJ, Knight-Madden J, **Kopp BT**, Koumbourlis AC, Lanzkron SM, Liem RI, Machado RF, Mehari A, Morris CR, Ogunlesi FO, Rosen CL, Smith-Whitley K, Tauber D, Terry N, Thein SL, Vichinsky E, Weir NA, Cohen RT, Klings ES. Identifying Clinical and Research Priorities in Sickle Cell Lung Disease. An Official American Thoracic Society Workshop Report. ***Ann Am Thorac Soc***. 2019 Sep. PMID: 31469310
d. Zeno RN, Stanek J, Pugh C, Gillespie ML, **Kopp BT**,* Creary SE*. Outcomes before and after providing interdisciplinary hematology and pulmonary care for children with sickle cell disease. ***Blood Adv***. 2022 Dec 28PMID: 36576975

Complete List of Published Work in MyBibliography (75 total):

http://www.ncbi.nlm.nih.gov/sites/myncbi/benjamin.kopp.1/bibliography/40532994/public/?sort=date&direction=ascending

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: Michael Koval, PhD

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

POSITION TITLE: Professor of Medicine and Cell Biology

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
University of California, Berkeley, CA	AB	06/82	Applied Mathematics and Biophysics
Johns Hopkins University, Baltimore, MD	PhD	01/90	Cell Biology
Washington University School of Medicine	Postdoctoral	06/96	Cell Biology

A. Personal Statement:

I have a long-standing interest and expertise in studying cell membranes, with a particular emphasis in understanding the molecular basis for formation and remodeling of intercellular junctions. A major research direction in my lab is to understand how tight junctions regulate the pulmonary air/liquid barrier. We use molecular and cell biological approaches to define roles for several tight junction proteins (claudins, occludin, ZO-1, ZO-2) in normal lung barrier function and in pathologic conditions such as acute respiratory distress syndrome (ARDS). We were the first lab to characterize claudin expression by lung epithelial cells and to identify specific claudin extracellular loop motifs which regulate the ability of different lung claudins to heterotypically interact which enables them fine tune paracellular permeability. To study pulmonary tight junctions at a molecular level, we have developed several tools including adenovector and lentiviral constructs, which enable us to transduce primary cells to express fluorescently tagged proteins and mutant claudins and targeted shRNAs. A long-term goal of our research is to identify control points which augment barrier function as a means to improve the outcome of patients with ARDS and other forms of lung injury.

We have collaborated with the McCarty lab for over a decade studying how pulmonary junctions are impaired in Cystic Fibrosis (CF) and CF-related diabetes (CFRD). This includes working with the McCarty lab to develop a CFRD mouse model which demonstrates that CF and diabetes synergistically impair clearance of bacterial infections. We have developed primary human airway cell models that faithfully recapitulate their function *in vivo* and found that tight junctions and gap junctions are impaired in the CF airway. Developing a novel medium formulation was a key step required to support these experiments:

- Morgan, R., C. Manfredi, K. F. Easley, L. Watkins, W. R. Hunt, S. Goudy, E. J. Sorscher, **M. Koval** and S. A. Molina. 2022. A medium composition containing normal resting glucose that supports differentiation of primary human airway cells, Sci. Reports, 12:1540. PMCID: PMCID: PMC8795386.

We are using CF and non-CF cells cultured with this method to create several novel in vitro platforms. These cells will be use as source material for lung on a chip models to be developed in this project.

The McCarty and Koval laboratories have 6 co-authored research articles related to the impact of CF on airway cell function, including analysis of cell signaling and -omics approaches to identify changes in gene expression and lipid metabolism:

- Molina, S.A., H. K. Moriarty, D. T. Infield, B. R. Imhoff, A. H. Kim, J. M. Hansen, W. R. Hunt, **M. Koval**, and **N. A. McCarty**. 2017. Insulin signaling via the PI3K/Akt pathway regulates airway glucose uptake and

barrier function in a CFTR-dependent manner. Amer. J. Physiol. Lung Cell. Mol. Biol., 312:L688-L702. PMCID: PMC5451595.

- Cui, G., K. A. Cottrill, K. M. Strickland, S. A. Mashburn, **M. Koval** and **N. A. McCarty**. 2021. Alteration of membrane cholesterol content plays a key role in regulation of CFTR channel activity. Front Physiol, 12(705). doi: 10.3389/fphys.2021.652513. PMCID: PMC8215275.
- Cottrill, K.A., R. J. Peterson, C. F. Lewallen, **M. Koval**, R. J. Bridges, and **N. A. McCarty**. 2021. Sphingomyelinase decreases transepithelial anion secretion in airway epithelial cells in part by inhibiting CFTR-mediated apical conductance. Physiol Rep. 9:e14928. PMCID: PMC8358481.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments:

2023	Co-Organizer, "Tight Junctions: from Structure and Development to Therapeutics" Conference, Leysin, Switzerland
2022-present	At Large Executive Committee Member, Biochemistry, Cell and Developmental Biology Graduate Group, Emory University
2021-present	Member, Atlanta Society of Mentors (Certificate in 2021)
2018-present	Editorial Board, American Journal of Physiology, Lung Cellular and Molecular Biology
2017	American Physiological Society, Respiration Section, EB Joint Programming Committee
2016	Co-Organizer, 2016 FASEB Conference "The Lung Epithelium in Health and Disease"
2015-present	Professor of Medicine and Cell Biology, Emory Univ. School of Medicine, Atlanta, GA
2015-2020	American Cancer Society, Development, Differentiation and Cancer (DDC) and Cell Structure and Metastasis (CSM) Review Panels
2015-2019	Core Director, Experimental Models, CF@LANTA CFF-RDP
2014-2021	Program Director, Biochemistry, Cell and Developmental Biology Graduate Group, Emory University
2013	Chair and Co-Organizer, International Gap Junction Conference, Charleston, SC.
2012-present	Editorial Board, PLOS ONE
2012-present	Editorial Board, Tissue Barriers
2010-2014	Director of Graduate Studies, Biochemistry, Cell and Developmental Biology Graduate Group, Emory University
2009-present	Associate Director for Research, Emory Univ., Div. of Pulmonary, Allergy and Critical Care
2009-2014	Editorial Board, Journal of Biological Chemistry
2009-2017	American Physiological Society, Respiration Section, Awards Committee
2008-2015	Associate Professor of Cell Biology, Emory Univ. School of Medicine, Atlanta, GA
2007-2015	Associate Professor of Medicine, Emory Univ. School of Medicine, Atlanta, GA
2006-2008	NHLBI Exploratory/Developmental Grants in Systems Biology Panel
2005-2007	Assistant Professor of Medicine, Emory Univ. School of Medicine, Atlanta, GA
2004-2008	American Heart Association, Mid-Atlantic and Southeastern Affiliate Peer Review Panels.
2002-present	NIH, ad hoc member: including CDF3, CSD, LIRR, RIBT and ICI study sections; Cellular Membranes SEP, CBB High Throughput Structure Determination SEP, Cell Biology, Developmental Biology, and Bioengineering F31 Panel, NIH Research Enhancement Award (R15) Panel, NIAAA Review Subcommittee Member Conflict Review Panel, Multiple Respiratory Sciences SEPs, NIGMS R13 Conference Grant Application Panel
1997-2005	Assistant Professor of Physiology, Univ. Pennsylvania School of Medicine, Philadelphia, PA
1996-1997	Instructor, Internal Medicine, Washington U. School of Medicine, St. Louis, MO
1993-1996	Postdoctoral Fellow, Internal Medicine, Washington U. School of Medicine, St Louis, MO
1990-1993	Postdoctoral Fellow, Cell Biol. and Physiol., Washington U. School of Medicine, St. Louis, MO

Honors:

2024	Keynote Speaker, International Gap Junction Conference, Washington, DC.
2021	Invited Speaker, Annual Meeting of the Alcohol and Immunology Research Interest Group
2021	Carroll Cross Distinguished Lectureship, University of California, Davis, Division of Pulmonary, Critical Care, and Sleep Medicine
2019	Plenary Lecture, DFG Research Training Group Summer School program, Berlin, Germany
2014	Guest of Honor, 2nd International Conference on Cell Signaling & Network (CeSiN), Council of Scientific & Industrial Research (CSIR), Kolkata, India.
2006	Keynote Speaker, "Gap Junction Group Research Day", University of Western Ontario

2001 American Physiological Society, Frontiers in Physiology Award (Mentor).
1997-2000 Hulda Irene Duggan Arthritis Investigator of the Arthritis Foundation.
1991-1994 Amgen Fellow of the Life Sciences Research Foundation.

C. Contributions to Science

PubMed bibliography (139 PubMed indexed publications (67 first/last author)):
https://www.ncbi.nlm.nih.gov/myncbi/michael.koval.1/bibliography/public/

1. Claudins in lung barrier function. Acute respiratory distress syndrome (ARDS) is exacerbated by a catastrophic failure of the alveolar barrier. Although it was known that tight junctions were critical for lung barrier function, their molecular composition had not been determined. My lab was the first to identify the claudin-family tight junction proteins expressed by alveolar epithelial cells, including claudin-3, claudin-4 and claudin-18. My lab has also developed strategies to directly manipulate claudin expression by primary alveolar epithelial cells in the physiologic range. This enabled the differential function of claudin-3 and claudin-4 in alveolar epithelial cells to be measured, suggesting that heterocellular type II-type I cell junctions have higher permeability than type I-type I cell junctions and demonstrating that increased claudin-4 has a barrier protective effect. We also identified claudin-5 as an alveolar epithelial tight junction protein upregulated in response to chronic alcohol exposure and found that claudin-5 has a deleterious effect on the alveolar barrier by interfering with the productive integration of claudin-18 into alveolar epithelial tight junctions. Candidate pharmacologic agents targeting claudin-5 are currently being evaluated for efficacy in preventing ARDS associated with alcoholic lung syndrome. We also are defining the molecular mechanisms that control lung vascular barrier function, focusing on a pannexin1-dependent purinergic signaling cascade that regulates the assembly of claudin-11 into endothelial tight junctions.

- Schlingmann, B., C. E. Overgaard, S. A. Molina, K. S. Lynn, L. A. Mitchell, S. Dorsainvil White, A. L. Mattheyses, D. M. Guidot, C. T. Capaldo, **M. Koval**. 2016. Regulation of claudin / zonula occludens-1 complexes by hetero-claudin interactions. Nature Comm. 7:12276 doi: 10.1038/ncomms12276. PMCID: PMC4962485.
- Smith, P., L. A. Jeffers, **M. Koval**. 2019. Effects of different routes of endotoxin injury on barrier function in alcoholic lung syndrome. Alcohol, 80:81-89. PMCID: PMC6613986.
- Lynn, K.S., K. F. Easley, F. J. Martinez, R. C. Reed, B. Schlingmann, and **M. Koval**. 2021. Asymmetric distribution of dynamin-2 and β-catenin relative to tight junction spikes in alveolar epithelial cells. Tissue Barriers, doi: 10.1080/21688370.2021.1929786. PMCID: PMC8489912.
- Maier-Begandt, D., H. S. Comstra, S. A. Molina, N. Krüger, C. A. Ruddiman, Y. L. Chen, X. Chen, L. A. Biwer, S. R. Johnstone, A. W. Lohman, M. E. Good, L. J. DeLalio, K. Hong, H. M. Bacon, Z. Yan, S. K. Sonkusare., **M. Koval**, B. E. Isakson. 2021. A venous-specific purinergic signaling cascade initiated by Pannexin 1 regulates TNFalpha-induced increases in endothelial permeability, Sci Signal 14:eaba2940. PMCID: PMC8011850.

2. Epithelial responses to nanoscale particles and topographies. My lab has collaborated with Vladimir Muzykantov at Penn to develop antibody-nanoparticle conjugates which recognize Cell Adhesion Molecules (CAMs) such as PECAM-1 or ICAM-1. Anti-CAM nanoparticles can be used to target antioxidants to vascular endothelium as anti-inflammatory agents. In developing this platform, we discovered a novel internalization pathway, CAM-mediated endocytosis, which is regulated by src kinase, Rho kinase and Na,H Exchanger proteins to internalize multivalent nanoparticles targeted to CAMs. CAM-mediated endocytosis has unique properties which enable nanoparticle localization and turnover to be pharmacologically modulated to fine tune their therapeutic efficacy. We also have collaborated with Tejal Desai at UCSF to develop novel nanostructured surfaces that are biologically active. Specifically, we have identified classes of nanostructured surfaces that interact with epithelial cell integrins to stimulate transepithelial delivery of macromolecules through the paracellular and transcellular pathways. We are currently using this approach to design novel transdermal drug delivery devices and to identify novel signaling pathways linking integrin stimulation to the regulation of tight junctions and transcytosis.

- Muro, S., M. Mateescu, C. Gajewski, M. Robinson, V. R. Muzykantov and **M. Koval**. 2006. Control of intracellular trafficking of ICAM-1-targeted nanocarriers by endothelial Na+/H+ exchanger proteins. Amer. J. Physiol. Lung Cell. Mol. Biol. 290:L809-L817.

- Walsh, L., J. Ryu, S. Bock, **M. Koval**, T. Mauro, R. Ross and T. Desai. 2015. Nanotopography facilitates in vivo transdermal delivery of high molecular weight therapeutics through an integrin-dependent mechanism. Nano Letters, 15:2434-2441. PMCID: PMC4478088
- Stewart, T., W. T. Koval, S. A. Molina, S. M. Bock, J. W. Lillard, Jr., R. F. Ross, T. A. Desai and **M. Koval**. 2017. Calibrated flux measurements reveal a nanostructure-stimulated transcytotic pathway. Exp. Cell Res., 355:153-161. PMCID: PMC5501187.
- Huang, X., X. Shi, M. E. Hansen, I. Setiady, C. Nemeth, A. Ceili, B. Huang, T. Mauro, **M. Koval**, and T. A. Desai. 2020. Nanotopography enhances dynamic remodeling of tight junction proteins through cytosolic liquid complexes. ACS Nano.14:13192-13202 doi: 10.1021/acsnano.0c04866. PMCID: PMC7606830

3. Metabolic and hormonal control of airway epithelial cells. Diabetes is a major co-morbidity associated with Cystic Fibrosis, particularly as patients reach early adulthood. CF-related diabetes (CFRD) accelerates the progression of lung disease so that mortality in CF with diabetes is twice that of CF without diabetes. However mechanistic links between CFRD and pulmonary disease are not well established. We developed a mouse model of CFRD and demonstrated that the effects of CF and diabetes synergistically impair the ability to clear *Pseudomonas Aeruginosa* infection more so than either CF or diabetes alone. We also have developed primary human airway cell models that faithfully recapitulate their function *in vivo*. Using this system, we found that normal airway cells express functional insulin receptors and that they respond to insulin by increasing barrier function and glucose uptake. By contrast, airway cells expressing the disease-causing mutant F508del CFTR become leakier in response to insulin and do not increase glucose uptake. These data suggest that insulin has a deleterious effect on the CF airway epithelium. We are currently using several model systems to delineate mechanisms by which CFTR mislocalization and the lack of chloride transporter activity exacerbate the deleterious effects of metabolic imbalance and inflammation associated with CFRD on lung airway epithelial barrier function and repair.

- Hunt, W. R., S. M. Zughaier, D. E. Guentert, M. A. Shenep, **M. Koval**, N. A. McCarty, and J. M. Hansen. 2014. Hyperglycemia impedes lung bacterial clearance in a murine model of cystic fibrosis-related diabetes. Amer. J. Physiol. Lung Cell. Mol. Biol., 306:L43-L49. PMCID: PMC3920212.
- Overgaard, C.E., B. Schlingmann, S. Dorsainvil White, C. Ward., X. Fan, S. Swarnakar, L. A. Brown, D. M. Guidot and **M. Koval**. 2015. The relative balance of GM-CSF and TGFβ1 regulates lung epithelial barrier function. Amer. J. Physiol. Lung Cell. Mol. Biol., 308:L1212-1223. PMCID: PMC4499033.
- Molina, S.A., B. Stauffer, H. K. Moriarty, A. Kim, N. A. McCarty, and **M. Koval**. 2015. Junctional abnormalities in human airway epithelial cells expressing F508del CFTR. Amer. J. Physiol. Lung Cell. Mol. Biol., 309:L475-87. PMCID: PMC4556929.
- Molina, S.A., H. K. Moriarty, D. T. Infield, B. R. Imhoff, A. H. Kim, J. M. Hansen, W. R. Hunt, **M. Koval**, and N. A. McCarty. 2017. Insulin signaling via the PI3K/Akt pathway regulates airway glucose uptake and barrier function in a CFTR-dependent manner. Amer. J. Physiol. Lung Cell. Mol. Biol., 312:L688-L702. PMCID: PMC5451595

4. Regulation of gap junction channel assembly and function. Gap junction channels allow the passage of ions and small aqueous molecules from the cytoplasm of one cell to another as a form of intercellular communication. Cells express multiple different gap junction protein (connexin) genes which have the capacity to specifically interact to form heteromeric channels with unique permeability. Although most multimeric protein complexes form in the endoplasmic reticulum (ER), a gap junction proteins related to connexin43 (Cx43), exist as monomers in the ER and subsequently oligomerize in the Golgi complex. However, beta connexins, such as Cx32, use a more classical pathway and oligomerize in the ER. We used structural differences between Cx43 and Cx32 to provide the first identification of a component of the connexin quality control pathway, an ER-localized, 29-kDa thioredoxin-family protein (ERp29), which stabilizes monomeric Cx43. ERp29 provides a mechanistic basis for control of connexin hetero-oligomerization which, in turn, regulates intercellular communication. We also have also found that diseases that affect ERp29 interfere with gap junction assembly and impair intercellular communication. In addition, we have identified roles for connexin multiplicity in lung epithelium, determining roles for distinct connexins in regulating the formation of intercellular communication networks that regulate calcium signaling, surfactant secretion and how gap junction dysregulation contributes to fibrosing lung disease.

- Maza, J., J. Das Sarma and **M. Koval**. 2005. Defining a minimal motif required to prevent connexin oligomerization in the endoplasmic reticulum, J. Biol. Chem., 280:21115-21.

- Das, S., J. Das Sarma, J. D. Ritzenthaler, T. Smith, J. Maza, B. E. Kaplan, L. A. Cunningham, L. Suaud, R. C. Rubenstein, **M. Koval**. 2009. Regulation of connexin43 oligomerization and trafficking by ERp29, Mol. Biol. Cell, 20:2593-2604. PMCID: PMC2682600
- **Koval, M**., M. Billaud, A. C. Straub, A. Zarbock, B. R. Duling, B. E. Isakson, 2011. Spontaneous lung dysfunction and fibrosis in mice lacking connexin40 and endothelial cell connexin43, Amer. J. Path., 178:2536-2546. PMCID: PMC3124229.
- Smith, T.D., A. Mohankumar, P. J. Minogue, E. C. Beyer, V. M. Berthoud, **M. Koval**. 2012. Cytoplasmic amino acids within the membrane interface region influence connexin oligomerization, J. Membr. Biol., 245:221-230. PMCID: PMC3501836.

5. Plasma membrane lipid recycling. It was well established that several transmembrane proteins, such as transferrin receptors, are recycled between the plasma membrane and intracellular compartments. Since intracellular vesicles are involved in all known steps of the recycling process, we hypothesized that there should also be considerable lipid transport in conjunction with protein recycling. Using a fluorescent sphingomyelin analogue (C6-NBD-SM), we provided the first direct demonstration that plasma membrane lipids also recycled between the plasma membrane and intracellular compartments. C6-NBD-SM labeling of normal and Niemann-Pick, type A disease fibroblasts enabled lipid sorting between the recycling and lysosomal delivery pathways to be measured and also provided the basis for diagnostic assays developed by the Pagano lab to screen for sphingolipidoses.

- **Koval, M.**, and R. E. Pagano. 1989. Lipid recycling between the plasma membrane and intracellular compartments: Transport and metabolism of fluorescent sphingomyelin analogues in cultured fibroblasts. J. Cell Biol. 108:2169-2181. PMCID: PMC2115574
- **Koval, M.**, and R. E. Pagano. 1990. Sorting of an internalized plasma membrane lipid between recycling and degradative pathways in normal and Niemann-Pick, type A fibroblasts. J. Cell Biol. 111:429-442. PMCID: PMC2116198
- **Koval, M.,** and R. E. Pagano. 1991. Intracellular transport and metabolism of sphingomyelin. Biochim. Biophys. Acta 1082:113-125. (peer reviewed)
- **Koval, M**., A. H. Futerman and R. E. Pagano. 1995. Sphingomyelin synthesis in endosomes? Trends. Cell Biol., 5:148-149. (peer reviewed)

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: **Camilla Margaroli Bell**

eRA COMMONS USER NAME: **CMARGA2**

POSITION TITLE: **Assistant Professor**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Fribourg, Switzerland	BSc	06/2013	Biochemistry/Functional Medical Sciences
University of Lausanne, Switzerland	MSc	01/2015	Medical Biology: Immunology and Oncology
Emory University, Atlanta, USA	PhD	06/2019	Immunology and Molecular Pathogenesis
University of Alabama at Birmingham, Alabama, USA	Postdoctoral Training	09/2022	Immunology

A. Personal Statement

Career goals: My objectives are to develop new technical skills and deepen my basic knowledge to advance towards the career goal of serving as a long-standing academic investigator in one of the leading academic CF research centers in the USA. My research focus is on understanding the behavior of inflammatory cells in both chronic and acute lung disease, and to exploit new findings to develop novel therapies.

Experience: My past and current academic training has set a solid background toward achieving my career goal. During my graduate years, I gained expertise in conducting human research and establishing international collaborations (Sophia Children's hospital in Rotterdam, The Netherlands, and the AREST-CF group in Perth, Australia), a skillset that I am currently applying to our studies in COVID-19 patients. Moreover, part of my graduate work in Dr. Tirouvanziam's group focused on the discovery and mechanistic understanding of de novo transcription occurring during neutrophil adaptation to the cystic fibrosis airway milieu, which has allowed me to acquire the necessary skills to conduct transcriptional analyses, including the acquisition of the necessary expertises to conduct spatial transcriptomics experiments using the Nanostring GeoMx platform. My post-doctoral training in Dr. Gaggar's group at the University of Alabama at Birmingham, was designed to further my training in mentoring and leadership, as well as provide me with a stronger background on preclinical models, exosome research, and clinical perspective on airway diseases. My new position as an Assistant Professor in the Department of Pathology at the University of Alabama at Birmingham will allow me to apply the skillset acquired during my training to develop my research program in pathological immune response in CF. Overall, the skillset acquired so far will be leveraged to complete the research proposed in this application.

Ongoing research support include:

Cystic Fibrosis Foundation - MARGAR21F5; Margaroli Bell (PI) 08/01/21– 07/31/25
Title: Role of Epidermal Growth Factor Receptor in CF airway neutrophils

Vertex Pharmaceuticals – Research Innovation Award; Margaroli Bell (PI) 04/01/23– 03/31/26
Title: Impact of pathogenic immunity in CF-related diabetes

UAB CFRC – Research Pilot; Margaroli Bell (PI) 07/01/23– 06/30/25
Title: Characterization of adaptive immune responses to NTM infection in CF

a. Genschmer KR, Russell DW, Lal C, Szul T, Bratcher PE, Noerager BD, Abdul Roda M, Xu X, Rezonzew G, Viera L, Dobosh BS, **Margaroli C**, Abdalla TH, King RW, McNicholas CM, Wells JM, Dransfield MT, Tirouvanziam R, Gaggar A, Blalock JE. “Activated PMN exosomes: pathogenic entities causing matrix destruction and disease in the lung”, Cell, 2019, 176(1-2):113-126, PMID: 30633902
b. **Margaroli C**, Benson P, Sharma NS, Madison MC, Robison SW, Arora N, Ton K, Liang Y, Zhang L, Patel RP, Gaggar A. Spatial mapping of SARS-CoV-2 and H1N1 Lung Injury Identifies Differential Transcriptional Signatures. Cell Rep Med. 2021 Mar 23;:100242. doi: 10.1016/j.xcrm.2021.100242. PMID: 33778787; PMCID: PMC7985929.
c. **Margaroli C**, Madison MC, Viera L, Russell DW, Gaggar A, Genschmer K, Blalock JE. An in vivo model for extracellular vesicles-induced emphysema. JCI insight. 2022 Jan 25;7(4):e153560. doi: 10.1172/jci.insight.153560.PMID: 35077395

B. Positions, Scientific Appointments and Honors

2022-current	Assistant Professor, Department of Pathology, University of Alabama at Birmingham
2019-2022	Post-doctoral trainee, University of Alabama at Birmingham, Dr. A. Gaggar
2017-2018	Instructor for Undergraduate Biology laboratory, Emory University (USA), Dr. M.F. Cole
2016-2019	PhD, Emory University (USA), Dr. R. Tirouvanziam
2015	Lab Technician, Cardiocentro Ticino Foundation – Swiss Institute for Regenerative Medicine (Switzerland), Dr. T. Tallone
2014-2015	Masters Student, University of Lausanne (Switzerland), Dr. H. Acha-Orbea
2013	Lab Technician, Oncology Institute of Southern Switzerland (Switzerland), Prof. F. Bertoni
2013	Lab Technician, University of Queensland (Australia), Dr. R. Thomas

Professional Memberships

2018-current	Member of the Association for Women in Science (AWIS)
2017-current	Member of the American Association of Immunologists (AAI)
2017-2019	Student representative for the Immunology graduate program at Emory University
2017-2018	Vice-president of the Immunology Graduate Group at Emory University

Honors

2019	Best basic science publication for the Department of Pediatrics (Emory University) and Children’s Healthcare of Atlanta. (Publication: Margaroli C et al, “Elastase exocytosis by airway neutrophils associates with early lung damage in cystic fibrosis children”, Am J Respir Crit Care Med, 2019, 199(7):873-881,PMID:30281324)
2017	Contestant in the Young Investigator award at the 31st Annual North American CF Conference, Indianapolis, IN; 10/2017
2016	Eugene Gangarosa Laboratory Research Mentoring Fellowship. Teaching and mentoring in the laboratory of high school students in the Emory Pre-College Program Infectious Disease Institute.
2010	Best presentation award at Youth and Science Event, Switzerland. High school thesis “Nanotechnology from natural porous tissues: Metal micro coating of spongy bone tissue to achieve catalytic systems with high active surface.”
2009	Best presentation award at EPFL, Switzerland. High school thesis “Nanotechnology from natural porous tissues: Metal micro coating of spongy bone tissue to achieve catalytic systems with high active surface.”

C. Contributions to Science

1. **Role of apoptosis in the immune response to infection.** My early work consisted in investigating the immunological response to Listeria monocytogenes infection, which induces apoptosis of hepatocytes and splenic cells, and becomes fatal for pregnant women and immunocompromised patients. This project led us to discover that pro-apoptotic BH3-only proteins (Bim, Bid and Noxa) affect the immune response, with emphasis on neutrophil and monocyte/macrophage behavior. Specifically, during the early phase of infection, pro-apoptotic BH3-only proteins regulate the TNFα response, reactive oxygen

species and neutrophil fate, shaping the outcome of the disease. This work contributed to the understanding of basic neutrophil biology and effector function during acute infection.

a. **Margaroli** C, Oberle S, Lavanchy C, Scherer S, Rosa M, Strasser A, Pellegrini M, Zehn D, Acha- Orbea H, Ehirchiou D, “Role of pro-apoptotic BH3-only in Listeria Monocytogenes Infection”, Eur. J. Immunology, 2016; 46(6):1427-37; PMID: 27064265

2. **Basic mechanisms of neutrophil biology and exosome roles in airway diseases**. During my graduate studies, I have investigated the basic mechanisms involved in the acquisition of the pathogenic fate of neutrophils in CF airway disease. Remarkably, we showed that neutrophil adaptation to the CF airway microenvironment is dependent upon de novo transcription, challenging the paradigm that neutrophils are short-lived, pre-programmed and with little opportunity for plasticity. In collaboration with the Blalock and Gaggar laboratories at University of Alabama in Birmingham, I also participated as a member of the Tirouvanziam group in studies describing neutrophil-derived extracellular vesicles in patients with COPD and in a mouse model of the disease. Together, this body of work represent a new biological mechanism of neutrophil behavior in inflammation and opens opportunities for neutrophil-directed immunotherapies.
    a. **Margaroli C**, Tirouvanziam R, “Neutrophil plasticity enables the development of pathological microenvironments: implications for cystic fibrosis airway disease”, Mol Cell Pediatr, 2016; 3(1):38; PMID: 27868161
    b. **Margaroli C**, Moncada Giraldo D, Arafat-Gulick D, Dobosh BS, Giacalone V, Forrest O, Sun F, Gu C, Gaggar A, Kissick H, Wu R, Gibson G, Tirouvanziam R. Broad transcriptional firing represses bactericidal activity in human airway neutrophils. Cell Rep Med. doi: doi.org/10.1016/j.xcrm.2021.100239. PMID: 33948572.
    c. Genschmer KR, Russell DW, Lal C, Szul T, Bratcher PE, Noerager BD, Abdul Roda M, Xu X, Rezonzew G, Viera L, Dobosh BS, **Margaroli C**, Abdalla TH, King RW, McNicholas CM, Wells JM, Dransfield MT, Tirouvanziam R, Gaggar A, Blalock JE. “Activated PMN exosomes: pathogenic entities causing matrix destruction and disease in the lung”, Cell, 2019, 176(1-2):113-126, PMID: 30633902
    d. **Margaroli C**, Madison MC, Russell D, Viera L, Gaggar A, Genschmer K, Blalock JE. An in vivo model for extracellular vesicles-induced emphysema. JCI insight. 2022 Jan 25;7(4):e153560. doi: 10.1172/jci.insight.153560. PMID: 35077395

3. **Clinical studies of neutrophilic inflammation in CF and other pediatric airway diseases** During my graduate studies in the Tirouvanziam laboratory at the CF@LANTA Center in Emory University and Children’s Healthcare of Atlanta (USA), I worked with collaborators in the AREST-CF pediatric program at Telethon Kids Institute (Perth, Australia), in the I-BALL CF monitoring program at Sophia Children Hospital and Erasmus Medical Center (Rotterdam, The Netherlands), in the Mall laboratory at Charité Hospital (Berlin, Germany) to study the advent of neutrophil and macrophage dysfunction in the airways of CF infants, and in infants with various non-CF aerodigestive disorders. I also collaborated on the first comprehensive study of airway neutrophil phenotype during acute respiratory failure in infants. Together, this body of work provided evidence for the development of early airway inflammation in chronic and acute respiratory diseases, and contributes to a better understanding of airway neutrophilic responses in infants. Moreover, it opens opportunities for the development of better disease biomarkers for early disease, which are necessary to assess efficacy of novel therapies.
    a. **Margaroli C**, Garratt LW, Horati H, Dittrich AS, Rosenow T, Montgomery ST, Frey DL, Brown MR, Schultz C, Guglani L, Kicic A, Peng L, Scholte BJ, Mall MA, Janssens HM, Stick SM, Tirouvanziam R; AREST-CF, and IMPEDE-CF, “Elastase exocytosis by airway neutrophils associates with early lung damage in cystic fibrosis children”, Am J Respir Crit Care Med, 2019, 199(7):873-881,PMID:30281324
    b. **Margaroli C**, Horati H, Garratt LW, Giacalone V, Dittrich AS, Rosenow T, Lim HS, Frey DL, Veltman M, Silva GL, Brown MR, Schultz C, Tiddens HAWM, Ranganathan S, Chandler JD, Qiu P, Peng L, Scholte BJ, Mall MA, Kicic A, Guglani L, Stick SM, Tirouvanziam R, Janssens HM; AREST-CF, and IMPEDE-CF, “Airway Macrophage PD-1 expression signals neutrophil takeover, infection, and structural lung damage in children with cystic fibrosis”, Cell Med, 2020 (in review)

c. Chandler JD, **Margaroli C**, Horati H, Kilgore MB, Veltman M, Liu HK, Taurone AJ, Peng L, Guglani L, Uppal K, Go YM, Tiddens HAWM, Scholte BJ, Tirouvanziam R, Jones DP, Janssens HM, "Myeloperoxidase oxidation of methionine associates with early cystic fibrosis lung disease", Eur Respir J, 2018;52(4); PMID: 30190273
d. Grunwell JR, Giacalone VD, Stephenson S, **Margaroli C**, Dobosh BS, Brown MR, Fitzpatrick AM, Tirouvanziam R (2018), "Neutrophil dysfunction in the airways of children with acute respiratory failure due to lower respiratory tract infections", Sci Rep, 2019, 9(1):2874, PMID: 30814584

4. **Systemic and local immune responses to SARS-CoV-2 infection** Since the beginning of the pandemic, while in Dr. Gaggar's group, I shifted my research effort to study the systemic and local immune responses upon SARS-CoV-2 infection. In the first study, we identified unique transcriptional pathways in the lungs of COVID-19 patients that were absent in another virally-induced form of acute lung injury. While the second study, characterized myeloid systemic and airway immune responses and identified key markers that when measured upon admittance to the intensive care unit were associated with worsen patient outcomes, including death.
    a. **Margaroli C**, Benson P, Sharma NS, Madison MC, Robison SW, Arora N, Ton K, Liang Y, Zhang L, Patel RP, Gaggar A. Spatial mapping of SARS-CoV-2 and H1N1 Lung Injury Identifies Differential Transcriptional Signatures. Cell Rep Med. 2021 Mar 23;:100242. doi: 10.1016/j.xcrm.2021.100242. PMID: 33778787; PMCID: PMC7985929.
    b. **Margaroli C**, Fram T, Sharma NS, Patel S, Tipper J, Robison SW, Russell D, Fortmann SD, Banday MM, Abdalla T, Saitornuang S, Madison MC, Leal SM, Harrod K, Erdmann NB, Gaggar A. A novel neutrophil population demonstrates viral clearance via type I interferon. JCI insight, in press
    c. Burkett A, McElwee S, **Margaroli C**, Bajpai P, Elkholy A, Manne U, Wille K, Benson PV. Positive Retrospective SARS-CoV-2 testing in a Case of Acute Respiratory Distress Syndrome of Unknown Etiology. Case Rep Pulmonol. 2021 Aug 28;2021:5484239. doi: 10.1155/2021/5484239. eCollection 2021. PMID: 34513107.
    d. Benson PV, Litovsky SH, Steyn AJC, **Margaroli C**, Iriabho E, Anderson PG. Use of telepathology to facilitate COVID-19 research and education through an online COVID-19 autopsy biorepository. J Pathol Inform. 2021 Dec 1;12(1):48. doi: 10.4103/jpi.jpi_15_21. eCollection 2021. PMID : 34934523.

5. **Development of novel immunotherapies** During my graduate training, I have contributed within the Selvaraj group at Emory to the validation of new therapeutic approaches for breast cancer treatment, where EGFR and HER-2 are commonly mutated and constitutively active. We showed that treatment post-surgery with engineered vesicles from tumor tissue, induced immunological memory and rejection of tumor relapses in a mouse model of HER-2+ breast cancer. Currently, Dr. Selvaraj is moving this therapy to a phase I clinical trial for post-surgery treatment of HER-2+ breast cancer. Moreover, I worked within the Tirouvanziam group, in collaboration with the Roy group at Georgia Tech in Atlanta, and the Gaggar group at University of Alabama in Birmingham, on developing a new delivery system to target lung neutrophils in an in vivo mouse model.
    a. Giacalone VD, Dobosh BS, Gaggar A, Tirouvanziam R, **Margaroli C**. "Immunomodulation in Cystic Fibrosis: why and how", Int J Mol Sci, 2020, 8;21(9):3331, PMID: 32397175
    b. Mejías JC, Forrest OA, **Margaroli C**, Frey Rubio DA, Gaggar A, Tirouvanziam R, Roy K, "Nanoparticle-in-microgel protease sensitive drug delivery system for the targeting of neutrophils in the inflamed lung", JCI insight, 2019, 4(23):e131468, PMID: 31661469

Complete list of published work
https://www.ncbi.nlm.nih.gov/myncbi/camilla.margaroli.1/bibliography/public/

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: McCarty, Nael A.

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

POSITION TITLE: Marcus Professor of Cystic Fibrosis, Dept. of Pediatrics

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
University of Kansas, Lawrence, KS	BS	06/1983	Systematics & Ecology
University of NC at Wilmington, Wilmington, NC	MS	06/1986	Marine Physiology
Univ. of Texas Health Science Center, Houston, TX	PhD	06/1990	Cell Physiology
California Institute of Technology, Pasadena, CA	Postdoctoral	06/1994	Molecular Physiology

A. Personal Statement

My training included work at several levels of organization, from ecology as an undergraduate student to single molecule physiology as a postdoc. Since becoming an independent scientist, I have continued to study the structure and function of individual macromolecules (ion channels, transporters, receptors), primarily focusing on proteins involved in Cystic Fibrosis, while also engaging in more integrative work, including translational research related to CF.

The main focus of the McCarty lab over the past 29 years has been the function and regulation of CFTR, the protein defective in CF, which forms a chloride/bicarbonate channel with roles in both plasma membranes and intracellular membranes. We combine molecular and pharmacological tools with quantitative biophysical assays at high resolution. We determined the oligomeric structure of the functional CFTR channel. We identified the first peptide toxin inhibitors of any Cl^- channel of known identity: GaTx1, active at CFTR, and GaTx2, active at ClC-2 channels. We used small organic inhibitors as probes of channel structure and identified the region of strongest anion selectivity in CFTR's pore, providing insights borne out by subsequent Cryo-EM studies. We used chemical modification to study permeation and conformational change during gating. We used MD simulations of a novel CFTR homology model to understand how the major domains move during gating, and then explored those conformational changes to understand the response to ATP binding. We have made multiple important contributions to understanding the evolution of CFTR. We showed that ABCC4 is the closest relative to CFTR, sharing a common ancestor that is distinct from the remaining ABCC subfamily members. We explored the wide spectrum of CFTR disease-associated mutations in order to relate site-specific evolutionary parameters with the propensity and severity of these mutations. We determined the mechanisms underlying the invention of the R-domain and its further development during evolutionary time. We cloned and characterized the sea lamprey ortholog of CFTR, which is now the evolutionarily oldest CFTR ortholog known. We have described the regulation of CFTR function by lipid-mediated signaling, now a major focus of our group. We developed novel approaches to purification of CFTR protein and now are the first to study CFTR in nanodiscs enabling the study of purified CFTR protein within a lipid bilayer environment using biochemical approaches that do not rely upon electrophysiology.

Over the past ten years, the McCarty lab has undergone a major shift in research directions. While still strongly focused on CF, we have extended our work into the roles of CFTR in regulation of barrier function in the airway epithelium, including new understandings of how the expression of mutant CFTR impacts airway glucose

handling, and the defects that underlie the development of pulmonary complications in CF-related diabetes (CFRD), the most common comorbidity in CF. This includes working with the Koval lab at Emory to develop a CFRD mouse model which demonstrates that CF and diabetes synergistically impair clearance of bacterial infections. We have developed primary human airway cell models that faithfully recapitulate their function *in vivo* and found that tight junctions and gap junctions are impaired in the CF airway. We also provided the first demonstration that airway epithelial cells express functional insulin receptors and GLUT4 transporters that provide the function of insulin-stimulated glucose uptake. Non-CF airway cells respond to insulin by increasing barrier function and glucose uptake. By contrast, CF airway cells become leakier in response to insulin and do not increase glucose uptake, suggesting that insulin has a deleterious effect on the CF airway epithelium. We also showed that insulin-mediated signaling is altered in cells expressing mutant CFTR. I am an expert in understanding the role of CFTR in epithelial cell function and dysfunction using models from the molecular scale through animal models and human subjects. This line of research now has positioned us to lead a team effort to understand how the expression of mutant CFTR in airway epithelial cells, innate immune cells, and tissue fibroblasts predispose the airway epithelium to infection, damage, and disrepair. This addresses the fact that the loss of lung function is the primary source of morbidity and mortality in CF patients.

Ongoing research projects include:

a) Pathophysiology of CF-related diabetes – Innate immunity in the airway
b) Mechanisms and consequences underlying the lung pathophysiology of CF-related diabetes
c) Role of membrane lipids in CFTR channel function and pharmacology
d) Evolution within the ABC transporter superfamily
e) Bacteriophage therapy to eliminate cystic fibrosis infections

Through my work as Director of CF-AIR (the Children's Center for CF and Airways Disease Research), I have built a team of investigators to study the role of CFTR in epithelial cell function and dysfunction using models from the molecular scale through animal models and human subjects, and the multifactorial consequences of loss of CFTR function in various organ systems. This has included recruitment of investigators to Emory, Georgia Tech, and Georgia State University, as well as drawing already-local investigators to CF research when their research expertise is relevant. The present P*3 Planning Grant represents the effort by McCarty and Koval to gather a large group of investigators with multidisciplinary approaches to target these important questions that will lead to new therapeutic options for our patients.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2016-2019	Director, Graduate Division of Biological & Biomedical Science, Laney Graduate School, Emory University, Atlanta, GA
2016	Member, Cystic Fibrosis Foundation Mucocilliary Clearance Consortium Review Committee
2015-pres.	Professor of Pediatrics and Senior CF Scientist, Emory University Medical School (tenured), Atlanta, GA
2013	Abstract Reviewer for Program Committee, North American CF Conference
2013	Chair of organizing committee for "The Enigmatic Chloride Ion: Transport, Regulation, and Roles in Physiology", International Meeting of the Society of General Physiologists, Woods Hole, MA
2013-2017	Ad hoc member, MBPP Study Section, NIH (2013, 2015-2017)
2012-pres.	Marcus Professor of Cystic Fibrosis, Emory University Medical School, Atlanta, GA
2010-pres.	Director, Children's Healthcare of Atlanta Center for Cystic Fibrosis and Airways Disease Research, and Emory+Children's CF Center of Excellence, Atlanta, GA
2010-pres.	Associate editorial Board Member, *Frontiers in Membrane Physiology and Biophysics*
2007-2015	Associate Professor of Pediatrics and Senior CF Scientist, Emory University Medical School (tenured), Atlanta, GA
2007	Member of Faculty Senate, Georgia Institute of Technology, Atlanta, GA
2006-2007	Adjunct Prof. of Chemistry & Biochemistry, Georgia Institute of Technology, Atlanta, GA
2006-pres.	Member, Cystic Fibrosis Foundation Research & Research Training Review Committee
2004-2017	Member, Special Emphasis Panels: (a) MDCN-A IRG (2004, 2005, 2006); (b) ZDK1 IRG (2005);(c) ZRG1-BST-L (2006, 2007); (d) ZRG1 BST-Q (2007); ZRG1 CB-J (03)M (2009); (e) ZDK1 GRB-7(J1)P, NIH P30 program for CF Core Centers (2011); (f) NIH Office of the Director

for BEST awards (July 2014); (g) ZGM1 PPBC-0 (GL), NIH/NIGMS U54 grants (2015); (h) ZRG1 CVRS-Q (11) B for SBIR/STTR, NIH (2017)

2004-2006 External Grant Reviewer, NSF
2004-2007 Adjunct Prof. of Applied Physiology, Georgia Institute of Technology, Atlanta, GA
2003-pres. Associate Director for Research, Children's Healthcare of Atlanta + Emory University CF Center
2002-2007 Chairman, Institute Biosafety Committee, Georgia Institute of Technology, Atlanta, GA
2001-2021 Adjunct Professor of Physiology, Emory University Medical School, Atlanta, GA
2001-2007 Associate Professor of Biology, Georgia Institute of Technology (tenured 2004), Atlanta, GA
2001-2003 Cellular Cardiovascular Physiology & Pharmacology Study Group, American Heart Association
2001-2002 Editorial board member for *Am. J. Physiol., Renal Physiology* section
1996-2002 Editorial board member for *Am. J. Physiol., Cell Physiology* section
1995-2001 Adjunct Assistant Professor of Pediatrics, Emory University Medical School, Atlanta, GA
1994-2001 Assistant Professor of Physiology, Emory University Medical School, Atlanta, GA
1993-pres. External grant reviewer for the Canadian Cystic Fibrosis Foundation
1990-1994 Research fellow and senior research fellow in biology, Caltech, Pasadena, CA
1986-1990 Graduate research assistantship, U. TX Health Science Center, Houston, TX
1983-1986 Graduate teaching and research assistantships, UNC, Wilmington, NC

Honors, Professional Memberships, and Other Experience

2006 Chairman, Membrane Biophysics Subgroup, Biophysical Society (2006-2007)
2006 Georgia Tech - Hesburgh Teaching Fellow
2005 Georgia Tech - BP Junior Faculty Teaching Award
2001 Established Investigator of the American Heart Association (2001-2004)
2000 NIH Certified Clinical Investigator
1990 Drown Foundation Postdoctoral Fellowship in Molecular Neurobiology, Caltech
1990 NIH Individual National Research Service Award (NRSA)
1989 Proctor and Gamble Professional Opportunity Award in Renal Physiology
1984 Sigma Xi Grant-in-Aid of Research

C. Contributions to Science

1. **Cystic Fibrosis-Related Diabetes**: CFRD is the most common co-morbidity among CF patients, but the basic mechanisms are yet unknown. We have recently shown that CF epithelial cells exhibit a defect in tight junction proteins, and this is worsened in the context of hyperglycemia.
   a. Hunt, W.R., S.M. Zughaier, D. Guentert, M.A. Schenep, M. Koval., **N.A. McCarty**, and J.M. Hansen (2014) Hyperglycemia impedes lung bacterial clearance in a murine model of cystic fibrosis-related diabetes. *Am. J. Physiol. Lung* 306: L43-9. PMCID: PMC3920212
   b. Molina, S.A., B. Stauffer, H.K. Moriarty, A. Kim, **N.A. McCarty**, and M. Koval (2015) Junctional abnormalities in human airway epithelial cells expressing delF508 CFTR. *Am. J. Physiol. Lung* 309: L475-87. PMCID: PMC4556929
   c. Hunt, W.R., B.R. Helfman, **N.A. McCarty**, and J.M. Hansen (2016) Advanced glycation end products are elevated in cystic fibrosis related diabetes and correlate with worse lung function. *J. Cyst Fibros* 15:681-8.
   d. Molina, S.A., H.K. Moriarty, D.T. Infield, B.R. Imhoff, R.J. Vance, A.H. Kim, J.M. Hansen, W.R. Hunt, M. Koval, and **N.A. McCarty** (2017) Insulin signaling via the PI3K/Akt pathway regulates airway glucose uptake and barrier function in a CFTR-dependent manner. *Am. J. Physiol. Lung* 312: L688-L702. PMCID: PMC5451595

2. **Translational research in the CF or asthmatic lung**: Our group has over the past 14 years established new arms of research that address the complex ecosystem of the CF lung and how that ecosystem changes during early establishment of CF lung disease, during acute pulmonary exacerbations, and during the development of CF-related diabetes.
   a. Zang, X., J.J. Pérez, C.M. Jones, M.E. Monge, **N.A. McCarty**, A.A. Stecenko, and F.M. Fernández (2017) Comparison of ambient and atmospheric ion sources for cystic fibrosis exhaled breath condensate ion mobility-mass spectrometry metabolomics. *J. Am. Soc. Mass Spec*. 28:1489-1496.
   b. Agarwal, R., C.T. Johnson, B.R. Imhoff, D.M. Donlan, **N.A. McCarty**, and A.J. García (2018) Inhaled

bacteriophage-polymeric particles ameliorate acute cystic fibrosis lung infections. *Nature BME 2:841-*849.

c. Zang, X., M.E. Monge, D.A. Gaul, **N.A. McCarty**, A.A. Stecenko, and F.M. Fernández (2020) Early detection of cystic fibrosis acute pulmonary exacerbations by exhaled breath condensate metabolomics. *J. Proteome Res.* 3:144-152. PMID: 31621328.

d. Cottrill, K.A., S.T. Stephenson, A.F. Mohammad, S.O. Kim, **N.A. McCarty**, R. Kamaleswaran, A.M. Fitzpatrick, J.D. Chandler. 2022. Exacerbation-prone pediatric asthma is associated with arginine, lysine, and methionine pathway alterations. *J Allergy Clin Immunol*. 9(22):01127-7. PMID: 36096204.

3. **Molecular pharmacology and regulation of CFTR by lipids:** The study of CFTR pharmacology is a primary endeavor. Many of our papers reflect use of various compounds as probes of CFTR structure and function, including my most highly cited work. These are highly mechanistic studies. Specifically, use of this CFTR knowledge heightens understanding of the mechanism by which CFTR is potentiated by compounds such as VX-770/Ivacaftor. Our interest in the molecular evolution of CFTR channel activity also leads us to study more than just human CFTR, which has enabled the comparative molecular pharmacology approach that is key to our work. This also includes the arm of our program that seeks to understand how CFTR impacts cellular and extracellular lipids, and how lipids and lipid-mediated signaling impact CFTR function. This is a new and major area of focus for the McCarty lab.

   a. Cottrill, K.A., C.M. Farinha, and **N.A. McCarty** (2020) The bidirectional relationship between CFTR and lipids. *Commun. Biol.* 3:179. PMCID: PMC7170930

   b. Cui, G., N. Khazanov, B.B. Stauffer, D.T. Infield, B.R. Imhoff, H. Senderowitz, and **N.A. McCarty** (2016) Potentiators exert distinct effects on human, murine, and *Xenopus* CFTR *Am. J. Physiol. Lung* 311: L192- 207. PMCID: PMC5142458

   c. Cottrill, K.A., R.J. Peterson, C.F. Lewallen, M. Koval, R.J. Bridges, and **N.A. McCarty** (2021) Sphingomyelinase decreases transepithelial anion secretion in airway epithelial cells in part by inhibiting CFTR conductance. *Physiol. Rep.* 9:e14928. PMID: 34382377.

   d. Cui, G., K.A. Cottrill, K.M. Strickland, B.R. Imhoff, S.A. Mashburn, M. Koval, and **N.A. McCarty** (2021) Alteration of membrane Cholesterol content plays a key role in regulation of CFTR channel activity. *Front. Physiol.* 12:652513. PMID: 34163370.

4. **Evolution of channel activity in CFTR:** CFTR is the only member of the ABC Transporter Superfamily known to function as a channel. This makes CFTR a great target for study of molecular evolution. We have combined bioinformatics approaches, computational modeling, and functional interrogation to understand how CFTR, alone, evolved the ability to interrupt the alternating access function of a transporter by stabilizing a state with the pore open at both ends. In collaboration with the lab of Dr. King Jordan at Georgia Tech, my lab was the first to explain the origin and evolution of the regulatory domain of CFTR – a domain that is not found in any other member of the ABC Transporter Superfamily member. We also determined the mechanism by which this important domain of CFTR evolved since it arose and described how that links to function. We also now have worked with Dr. Amit Gaggar at UAB to clone and characterize the sea lamprey ortholog of CFTR, now the oldest known ortholog. This enables the proposed studies.

   a. Jordan, I.K., K. Kota, G. Cui, C.H. Thompson, and **N.A. McCarty** (2008) Evolutionary and functional divergence of CFTR from ABC transporters. *Proc. Natl. Acad. Sci., U.S.A..*105:18865-18870. PMCID: PMC2585040

   b. Cui G, Hong JS, Chung-Davidson YW, Infield DT, Xu X, Li J, Simhaev L, Khazanov N, Stauffer B, Imhoff B, Cottrill KA, Blalock JE, Li W, Senderowitz H, Sorscher EJ, **McCarty NA**, and A. Gaggar (2019). An ancient CFTR ortholog informs molecular evolution in ABC Transporters. *Dev Cell.* 51:421-430. PMCID: PMC7665244

   c. Infield, D.T., K.M. Strickland, A. Gaggar, and **N.A. McCarty**. The molecular evolution of function in the CFTR chloride channel. Invited Review. *J. Gen. Physiol*. 153:e202012625. PMID: 34647973

   d. **McCarty, N.A.** (2023) Tweaking the catalytic efficiency of the CFTR ion channel (Commentary). *J Gen Physiol.* 155:e202313343. PMID: 37014352

5. **The pore of the CFTR channel, and pore-gating:** The major focus of the McCarty lab has been understanding how CFTR functions as a Cl^- channel. We have used mutagenesis and high-resolution electrophysiology to identify elements of this important protein that contribute to channel activity. These

studies have helped to identify the regions underlying ion selectivity, pore gating and stability, and the pharmacology of this protein in relation to both inhibition and potentiation.

a. Simhaev, L., **N.A. McCarty**, R.C. Ford, and H. Senderowitz (2017) Molecular dynamics flexible fitting (MDFF) simulations identify new models of closed state CFTR. *J. Chem. Informatics Modeling* 57:1932-1946.
b. Strickland, K.M., G. Stock, G. Cui, H. Hwang D.T. Infield, I. Schmidt-Krey, **N.A. McCarty**, and J.C. Gumbart (2019) ATP-Dependent signaling in simulations of a revised model of cystic fibrosis transmembrane conductance regulator (CFTR). *J Phys Chem B*. 123:3177-3188.
c. Moffett AS, Cui G, Thomas PJ, Hunt WD, **McCarty NA**, Westafer R, and Eckford AW (2022) A factor graph EM algorithm for inference of kinetic microstates from patch clamp measurements. *Biophysical Reports* 2, 100083. //doi.org/10.1016/j.bpr.2022.100083. PMID: 36425670
d. Hunt WD, **McCarty NA**, Marin EM, Westafer RS, Yamin PR, Cui G, Eckford AW, and Denison DR (2023) A transistor model for the cystic fibrosis transmembrane conductance regulator. *Biophysical Reports 3*, 100108. //doi.org/10.1016/j.bpr.2023.100108.

Complete List of Published Work in MyBibliography: (81 indexed articles in PubMed)
//www.ncbi.nlm.nih.gov/sites/myncbi/1IcyGvbuU6Jkl/bibliography/44167237/public/?sort=date&direction=ascending

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: Shuichi Takayama

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

POSITION TITLE: Professor of Biomedical Engineering, Georgia Tech and Emory

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
University of Tokyo, Japan	B. S.	03/1992	Agricultural Chemistry
University of Tokyo, Japan	M. S.	03/1994	Agricultural Chemistry
The Scripps Research Institute, La Jolla, CA	Ph. D.	06/1998	Chemistry & Chemical Biology
Harvard University, Cambridge, MA	Postdoc	06/2000	Chemistry & Chemical Biology

A. Personal Statement

Dr. Takayama is Professor, Georgia Research Alliance Eminent Scholar, and Price Gilbert, Jr. Chair in Regenerative Engineering and Medicine in the Wallace H. Coulter Department of Biomedical Engineering at the Georgia Institute of Technology and Emory University School of Medicine since Summer 2017. He received his B.S. and M.S. from the University of Tokyo, Ph.D. from the Scripps Research Institute (1998), and worked as a Leukemia and Lymphoma Society Postdoctoral Fellow at Harvard University. He started as assistant professor at the University of Michigan, Department of Biomedical Engineering in 2000 and rose through the ranks before moving to Georgia. He has worked on many multi-investigator collaborative projects including an MPI R01 on “Microfluidic Tissue Engineering of Small Airway Injuries” as well as a multi-PI FDA project that uses a Transwell-96 based lung air-blood barrier platform for toxicology studies. Dr. Takayama has published over 250 journal articles including many on microphysiological system platforms.

Dr. Takayama has a background in engineered microphysiological systems including the first lung-on-a-chip system.[1] Most relevant for this proposal, we have recently developed a method to produce Transwell 96-based air-blood barriers with an underside epithelia in a high-throughput manner using flotation-based cell seeding.[2] He also has experience in high throughput lung fibroblast cell culture systems[3] as well as in engineering biomaterials that mimic neutrophil extracellular traps to study their contributions to lung disease.[4] In this proposal, he will further develop the air-blood barrier array for cystic fibrosis (CF) studies by incorporating the appropriate primary lung cells and CF relevant bacteria while also testing the latest CF drugs.

8404520 Takayama (contact), Ng 09/01/2022-8/31/2025
EPA
High-Throughput Lung Damage and Inflammation Assessment of Polyaromatic Hydrocarbon Mixtures
The goal is to use a high-throughput lung microphysiological system to study PAH immunotoxicity
Role: Co-PI (contact)

75F40122C00146 Takayama (contact), Ioachimescu, Tirouvanziam, Zhang, Ng 10/01/2022-9/30/2025
FDA
In Vitro Assessment of Chemical Mixture-induced Airway Inflammation in Healthy and Diseased Lungs
Role: Co-PI (contact)

Takayama
Georgia Tech EPICenter 11/01/2021-10/30/2024

Secondary organic aerosol high-throughput toxicology to enhance epidemiological models used for energy and environmental policy making
The goal of this project is to obtain secondary organic aerosol toxicity data for improving epidemiological models of pollution.
Role: Co-PI

COMPLETED
HL136141 Takayama (contact PI), Grotberg, Nemzek 06/03/2017-11/30/2022
NIH R01
Microfluidic Tissue Engineering of Small Airway Injuries
The goal is to understand how sub-lethal fluid mechanical stresses may exacerbate lung injury.
Role: Co-PI (contact)

Select citations:

1. Huh D, Fujioka H, Tung Y-C, Futai N, Paine R, Grotberg JB, Takayama S. (2007) Acoustically Detectable Cellular-Level Lung Injury Induced by Fluid Mechanical Stresses in Microfluidic Airway Systems. *Proc. Natl. Acad. Sci. U.S.A. 104*, 18886–18891. PMC2141877.
2. Viola H, Washington K, Selva C, Grunwell J, Tirouvanziam R, Takayama S. (2021) A high-throughput distal lung air-blood barrier model enabled by density-driven underside epithelium seeding. *Adv Healthcare Mater*. 10, 2100879. PMC8349865.
3. Robinson S, Chang S, Parigoris E, Hecker L, Takayama S. (2021) Aqueous two-phase printing and fibrinolysis of fibroblast-laden fibrin micro-scaffolds. *Biofabrication. 13,* 035013. PMC8934703
4. Yang T, Yu J, Ahmed T, Nguyen K, Nie F, Zan R, Li Z, Han P, Shen H, Zhang X, Takayama X,* Song Y.* (2023) Synthetic Neutrophil Extracellular Traps Dissect Bactericidal Contribution of NETs under Regulation of α-1-Antitrypsin. *Sci Adv*, adf2445. PMC10146876

B. Positions, Scientific Appointments, and Honors

2017- Georgia Research Alliance Price Gilbert Chair Professor, Department of Biomedical Engineering, Georgia Tech/Emory University
2010-2017 Professor, Dept Biomed Eng and Macromolecular Sci & Eng, University of Michigan, Ann Arbor
2014-2017 Adjunct Professor, Ulsan National Institute of Science and Technology (UNIST), Korea
2010-2013 WCU Professor, Ulsan National Institute of Science and Technology (UNIST), Korea
2006-2010 Associate Professor, Dept Biomed Eng and Macromolecular Sci & Eng, University of Michigan
2000-2006 Assistant Professor, Dept Biomed Eng and Macromolecular Sci & Eng, University of Michigan

Other Experience and Professional Memberships

2019- Director, GT Nakatani Research and International Experience for Students (RIES) Program
2015-2021 Editorial Board, *Scientific Reports*
2014- Associate Editor, *Integrative Biology*
2013- Editorial Adv Board, *Innovation and Emerging Technologies*
2013- Editorial Adv Board, *Biomedical Engineering Letters*
2013-2017 Director, NIH Microfluidics in Biomedical Sciences Training Program
2010-2016 National Institutes of Health (NIH) ISD standing member
2008- Editorial Adv Board, *Microfluidics & Nanofluidics*
2002-2017 Regional Editor, then 2013 Editorial Adv Board *J. Mech. Med. Biol.*

Honors

2019 BioEngineering Program Best Advisor Award (Georgia Tech)
2015 AIMBE Fellow
2014 Rackham Graduate Student Mentor Award (U Michigan)
2013 3Rs Highly Commended Award (http://www.nc3rs.org.uk/) for Spheroid Culture System
2013 Pioneers of Miniaturization Prize (Royal Society of Chemistry & Corning Inc)
2013 Collegiate Inventor's Award Finalist (ATPS), Natl Inventors Hall of Fame & USPTO
2012 Wall Street Journal Technology Innovation Awards Runner Up (3D Biomatrix Spheroid Culture)
2009 College of Engineering, George J Huebner Research Excellence Award
2006 Biomedical Engineering Department Award for Outstanding Accomplishment
2004 Collegiate Inventor's Award (Wei Gu's Advisor), National Inventors Hall of Fame & USPTO

2003 NSF Career Award, NSF
2002 Ralph E. Powe Junior Faculty Enhancement Award, ORAU
2000 Green Chemistry Challenge Award (From EPA)
1998 Life Sciences Research Institute Fellowship (declined)
1998 Damon Runyon - Walter Winchell Cancer Research Fund Fellowship (declined)
1998 Leukemia and Lymphoma Society (formerly Leukemia Society of America) Fellow
1997 Lesly Shelton Moreaux Excellence in Chemistry Graduate Studies Award (Scripps Research Institute)

C. Contributions to Science

C1. Air-liquid interface (ALI) culture platforms: We have developed a variety of platforms to perform air-liquid interface (ALI) cultures. We developed a method to incorporate porous membranes reliably into microfluidic channels (*Anal Chem* 2007) and used the technique to perform the first in-channel ALI culture and differentiation of primary human small airway cells for lung-on-a-chip applications (*PNAS* 2007). Our lab also developed a Transwell 96-based co-culture model of the air-blood barrier with the epithelia on the underside of the porous filter inserts (*Adv Healthcare Mater* 2021). This was enabled by engineering the cell culture media density and performing flotation-based epithelial cell seeding. This platform has been used to study how viral infection (beta-coronavirus and influenza A) of the air-side epithelia induces blood-side endothelial cell response in a virus dose (MOI)-dependent manner.

a) Chueh, B.H.; Huh, D.; Kyrtsos, C. R.; Houssin, T.; Futai, N.; Takayama, S. "Leakage-Free Bonding of Porous Membranes into Layered Microfluidic Array Systems" *Anal. Chem.* **2007**, *79*, 3504-3508. PMID: 17388566. PMC2517097.
b) Huh D, Fujioka H, Tung Y-C, Futai N, Paine R, Grotberg JB, Takayama S. (2007) Acoustically Detectable Cellular-Level Lung Injury Induced by Fluid Mechanical Stresses in Microfluidic Airway Systems. *Proc. Natl. Acad. Sci. U.S.A. 104*, 18886–18891. PMC2141877
c) Douville NJ, Tung Y-C, Li R, Wang JD, El-Sayed MEH, Takayama S. (2010) Fabrication of Two-Layered Channel System with Embedded Electrodes to Measure Resistance Across Epithelial and Endothelial Barriers. *Anal Chem. 82*, 2505-2511. PMC2839931
d) Viola H, Washington K, Selva C, Grunwell J, Tirouvanziam R, Takayama S. (2021) A high-throughput distal lung air-blood barrier model enabled by density-driven underside epithelium seeding. *Adv Healthcare Mater*. 10, 2100879. PMC8349865

C2. Microscale Lung Fibroblast Culture and Fibrosis Models: Extracellular matrix (ECM) molecules can be both contributor towards and result of fibrosis. Using aqueous two-phase system bioprinting technology pioneered by our lab, we have developed one of the world's smallest and highest throughput ECM bioprinting and cellular response assays. One example is the collagen contraction assay (down to 1 μL and few hundred fibroblasts) (*Biomaterials* 2013, *Front Biotechnol Bioeng* 2020). The microscale collagen contraction assays are not only high throughput and cost-saving, it has the advantage that growth factor, cytokine, and drug penetration throughout the gel is orders of magnitude faster than the more conventional larger collagen contraction assays. We have also incorporated time-lapse imaging and artificial intelligence (AI)-assisted image processing to enable more in-depth quantification of the cell behaviors (*Front Biotechnol Bioeng* 2020). Bioprinting of other ECM materials such as fibrin has also been demonstrated (*Biofabrication* 2021). Fibrin degradation was also observed to be delayed with pro-fibrotic, senescence-promoting stimulations such as TGF-β1 or H_2O_2, and with higher passage numbers.

a) Moraes C, Simon AB, Putnam AJ, Takayama S. (2013) High-throughput aqueous two-phase printing of contractile collagen microgels. *Biomaterials 34*, 9623-9631. PMC3819461.
b) Yamanishi C, Parigoris E, Takayama S. (2020) Kinetic Analysis of Label-Free Microscale Collagen Gel Contraction Using Machine Learning-Aided Image Analysis. *Front Biotechnol Bioeng*. *8*, 582602. PMC7537788
c) Robinson S, Chang J, Parigoris E, Hecker L, Takayama S. (2021) Aqueous two-phase printing and fibrinolysis of fibroblast-laden fibrin micro-scaffolds. *Biofabrication. 13,* 035013. PMC8282251.
d) Robinson S, Parigoris E, Chang JC, Hecker L, Takayama S. (2022) Contracting Scars from Fibrin Drops. *Integr Biol. 14,* 1-12. PMC8934703.

C3. Lung-on-a-chip: Our lab developed one of the first lung on a chip published in *PNAS* 2007. We have particularly demonstrated the role of fluid mechanical stress associated with airway closure and reopening in epithelial injury. We collaborate with experts in pulmonary biology, pulmonary fluid mechanics, and critical care

to guide device designs and link our *in vitro* studies to physiology and disease *in vivo*. In an easy to understand clinical correlation, our lung-on-a-chip recreate fluid mechanical stresses associated with stethoscope sounds to reveal how surfactant dysfunction can cause lung injury. We have also developed a related fluid mechanical stress-mediated injury model for the alveoli (*Lab Chip* 2011; bioRxiv 2023).

a) Huh D, Fujioka H, Tung Y-C, Futai N, Paine R, Grotberg JB, Takayama S. (2007) Acoustically Detectable Cellular-Level Lung Injury Induced by Fluid Mechanical Stresses in Microfluidic Airway Systems. *Proc. Natl. Acad. Sci. U.S.A. 104*, 18886–18891. PMC2141877
b) Douville NJ, Zamankhan P, Tung Y-C, Li R, Vaughan BL, White J, Grotberg JB, Takayama S. (2011) Combination Fluid and Solid Mechanical Stresses Contribute to Cell Death and Detachment in a Microfluidic Alveolar Model. *Lab Chip 11*, 609-619.
c) Tavana H, Zamankhan P, Christensen PJ, Takayama S, Grotberg JB. (2011) Epithelium Damage and Protection During Reopening of Occluded Airways in a Physiologic Microfluidic Pulmonary Airway Model. *Biomed Microdev. 13*, 731–742.
d) Viola H, Vasani V, Washington K, Lee J-H, Selva C, Li A, Llorente CJ, Murayama N, Grotberg J, Romano F, Takayama S. (2023) Liquid plug propagation in computer-controlled microfluidic airway-on-a-chip with semi-circular microchannels. *bioRxiv* https://doi.org/10.1101/2023.05.24.542177

C4. Microscale Bacteria Cultures: Our lab has developed microscale culture methods to study chemical communication and cooperation, as well as predation between different bacteria species in a community. Technologically, we pioneered the use of microscale bacteria culture using aqueous two phase system (ATPS droplets) (*Analyst* 2010). We have used the systems to analyze chemical communication via quorum sensing molecules (*JACS* 2013), how proximity to an antibiotic-resistant strain can help a susceptible strain (*Biomacromolecules* 2012), how periodic relocation can enhance cooperation between micro-colonies (*JACS* 2013), and how a predatory bacteria can “eat” bad bacteria to protect epithelial cells (*PLoSONE* 2013). We have also micropatterned biofilms (*Biomacromolecules* 2012).

a) Yaguchi T, Lee S, Choi WS, Kim D, Kim T, Mitchell RJ, Takayama S. (2010) Micropatterning Bacterial Suspensions Using Aqueous Two Phase Systems. *Analyst 135*, 2848-2852.
b) Yaguchi T, Dwidara M, Byun CK, Leung B, Lee S, Cho Y-K, Mitchell RJ, Takayama S. (2012) Aqueous two-phase system-derived biofilms for bacterial interaction studies. *Biomacromolecules 13*, 2655-61.
c) Byun CK, Hwang H, Choi WS, Yaguchi T, Park J, Kim D, Mitchell RJ, Kim T, Cho Y-K, Takayama S. (2013) Productive Chemical Interaction Between a Bacterial Micro-colony Couple is Enhanced by Periodic Relocation. *J. Am. Chem. Soc. 135*, 2242–2247.
d) Dwidar M, Leung B, Yaguchi T, Takayama S, Mitchell RJ. (2013) Patterning bacterial communities on epithelial cells. *PLoSONE 8*, e67165.

C5. Neutrophil Extracellular Trap Inspired Biomaterials: Our lab has pioneered the preparation and use of reconstituted chromatin nano-meshes in a manner that mimics the function of neutrophil extracellular traps (NETs). A key advantage of our bioengineered materials being that unlike cell-produced NETs that are complex, variable, and undefined in its composition and structure, we can produced defined compositions to reveal what components of NETs is responsible for its different functions. Using this capability, we showed that the histone content is critical for bacteria capture and killing activity of NETs (*Adv Mater* 2019). We have also demonstrated that NETs containing non-methylated DNA (as may be produced from mitochondrial DNA) rather than methylated DNA (as would be present in nuclear DNA) elicit a stronger immune response from dendritic cells (*Adv Healthcare Mater* 2019). In our latest work, we study the impact of how multiple proteins from NET and lung tissue environment interact to combat *Pseudomonas aeruginosa* infection (*Sci Adv* 2023).

a) Song Y, Kadiyala U, Weerappuli P, Valdez JJ, Yalavarthi S, Louttit C, Knight JS, Moon JJ, Weiss DS, VanEpps JS, Takayama S. (2019) Antimicrobial Microwebs of DNA-Histone Inspired from Neutrophil Extracellular Traps. *Adv Mater. 31*, 1807436. PMC6467213
b) Weerappuli PD, Louttit C, Kojima T, Brennan L, Yalavarthi S, Xu Y, Ochyl LJ, Maeda M, Kim HS, Knight JS, Takayama S,* Moon JJ.* (2019) Extracellular trap-mimicking DNA-histone mesostructures synergistically activate dendritic cells. *Adv Healthcare Mater 8*, 1900926. PMC6872909
c) Yang T, Song Y, Ahmed T, Nguyen K, Yu J, Cao X, Zhang X, Shen H, Fay ME, Williams EK, Lam WA, van Epps JS, Takayama S,* Song Y.* (2021) Dosage-dependent antimicrobial activity of DNA-histone complexes against *Staphylococcus aureus*. *Adv Mater Interface 8*, 2100717. PMC8447838

d) Yang T, Yu J, Ahmed T, Nguyen K, Nie F, Zan R, Li Z, Han P, Shen H, Zhang X, Takayama S,* Song Y.* (2023) Synthetic Neutrophil Extracellular Traps Dissect Bactericidal Contribution of NETs under Regulation of α-1-Antitrypsin. *Sci Adv*, adf2445. PMC10146876

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: Tirouvanziam, Rabindra

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

POSITION TITLE: Associate Professor of Pediatrics

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
University Paris XII, Paris, France	MSc	06/1994	Lung physiology
Agro Paris-Tech, Paris, France	Engineer	06/1994	Bioengineering
Institute of Embryology of CNRS and College de France, Nogent-sur-Marne, France	PhD	12/1998	Developmental biology
Stanford University, Stanford, CA, USA	Postdoctoral	06/2006	Genetics, Immunology

A. PERSONAL STATEMENT

It is my privilege to serve as co-I with Dr. McCarty and colleagues on this PRA P*3 planning grant focused on mechanisms of bronchiectasis and abormal tissue repair underlying chronic cystic fibrosis (CF) lung disease.

Career and productivity: I earned PhD and engineering degrees in Paris, and pursued postdoctoral training at Stanford before joining Emory as faculty, where I head a translational research lab of 15+ members. I count 25+ years of experience in human immunology, notably in CF lung disease, spanning studies of pathogenesis in models, prospective studies in pediatric and adult patients, biomarker, and interventional trials. I served as PI on a multicenter R01 focused on CF and MPI on DARPA programs focused on malaria and flu. I currently am PI on 1 NHLBI R01 focused on cell reprogramming in CF, 4 CF Foundation awards, and 2 pilot awards focused on epigenetic drug development, and plague; as co-PI on 1 Australian NHMRC program grant focused on CF; and as co-I on 1 NHLBI R01 focused on neutrophil myeloperoxidase and 1 NIAID R01 focused on lung repair in tuberculosis. As of June 2023, I count 86 papers, 160 abstracts, and 5 patents.

Information pertinent to this proposal: My group investigates immunometabolic dysregulation in human disease using omics methods to yield deep phenotyping of pathological states in patients and in vitro models to deconstruct mechanistic processes leading to them. Our prior studies identified adaptations in myeloid cells (neutrophils, monocytes) recruited from blood into stressed peripheral tissues (mucosae, tumor) *in vivo*. We developed a modular transmigration model to mass-produce tissue-recruited myeloid cells *in vitro*, and used it for mechanistic studies of diseases like CF, chronic obstructive pulmonary disease, acute lung injury, asthma, lung cancer, COVID-19, and plague. Prior work from my lab, some in collaboration with colleagues included on this planning grant (McCarty, Chandler, Takayama, Margaroli, Goldberg) have established prime roles for a progressive downregulation of resident macrophages and dominance of recruited, adaptively reprogrammed live neutrophils in the development of focal areas of disease leading to CF lung bronchiectasis. This work has led to the development of a new paradigm for early CF lung pathogenesis (inflammation prior to colonization by proinflammatory pathogens), which has changed the view on how to approach and treat this disease. For example, my group is working with the pharma company Insmed on repurposing their non-CF bronchiectasis, neutrophil-targeted, drug brensocatib to CF. To further probe this core process, my group will contribute conceptual insights, technical and analytical expertise to this planning grant and to the ensuing program project grant. Based on my scientific credentials and prior and current track record of innovative work in CF lung pathogenesis, I am well positioned to contribute to this collaborative effort, and ensure its successful execution.

References below illustrate my commitment to innovative translational research relevant to human pathology:

1. Genschmer KR, Russell DW, Lai C, Szul T, Bratcher PE, Noerager BD, Roda MA, Xu X, Rezonzew G, Viera L, Dobosh BS, Margaroli C, Abdalla TH, King RW, McNicholas CM, Wells JM, Dransfield MT, **Tirouvanziam R**, Gaggar A, Blalock JE. Activated PMN exosomes: pathogenic entities inducing matrix destruction and disease in the lung. Cell (2019); 176(1-2):113-126. PMID: 30633902.
2. Margaroli C, Moncada-Giraldo D, Gulick DA, Dobosh B, Giacalone VD, Forrest OA, Sun F, Gu C, Gaggar A, Kissick H, Wu R, Gibson G, **Tirouvanziam R**. Transcriptional firing represses bactericidal activity in cystic fibrosis airway neutrophils. Cell Rep Med. (2021); 2(4):100239. doi: 10.1016/j.xcrm.2021.100239. PMID: 33948572.
3. Margaroli C, Horati H, Garratt LW, Giacalone VD, Schofield C, Dittrich AS, Rosenow T, Dobosh BS, Lim HS, Frey DL, Veltman M, Silva GL, Brown MR, Schultz C, Tiddens HAWM, Ranganathan S, Chandler JD, Qiu P, Peng L, Scholte BJ, Mall MA, Kicic A, Guglani L, Stick SM, Janssens HM, **Tirouvanziam R**. Macrophage PD-1 associates with neutrophilia and reduced bacterial killing in early cystic fibrosis airway disease. J Cyst Fibros. (2022);21(6):967-976. PMID: 35732550
4. Dobosh B, Zandi K, Moncada Giraldo D, Goh S, Musall K, Aldeco M, LeCher J, Giacalone V, Yang J, Eddins D, Bhasin M, Ghosn E, Sukhatme V, Schinazi RF, **Tirouvanziam R**. Baricitinib attenuates the proinflammatory phase of COVID-19 driven by lung-infiltrating monocytes. Cell Rep. (2022); doi: 10.1016/j.celrep.2022.110945. PMID: 35688145.
5. Forrest OA*, Dobosh B*, Ingersoll SA, Rao S, Rojas A, Laval J, Alvarez JA, Brown M, Tangpricha V, **Tirouvanziam R**. Neutrophil-derived extracellular vesicles promote feed-forward inflammasome signaling in cystic fibrosis airways. *J Leukoc Biol.* 2022; doi: 10.1002/JLB.3AB0321-149R. PMID: 35172381. * denotes co-first authors.

Ongoing projects I would like to highlight include:

1R01HL158059 Tirouvanziam (PI) 07/01/2022-06/30/2026
NIH/NHLBI (R01)
Extracellular vesicle-driven neutrophilic inflammation in cystic fibrosis lungs
Role: PI

1R01AI166988 Auld, Bisson (MPIs) 07/01/2022-06/30/2027
NIH/NIAID (R01)
Inflammation and fibrosis in pulmonary tuberculosis; the INFIN-TB study
Role: Co-I

21170A01 Coppinger (PI) 11/01/2021-10/31/2023
Cystic Fibrosis Foundation (Basic Science Grant)
Investigating the role of bronchial EVs in activating neutrophils in early CF disease
Role: Co-I

APP1183640 Stick (PI) 01/01/2020-12/31/2025
National Health and Medical Research Council of Australia (Program Project Grant)
Preventing early cystic fibrosis lung disease by solving the host inflammation-infection conundrum
Role: Co-PI

No number Tirouvanziam (PI) 09/01/2021-08/31/2023
Emory MP3 Initiative (Pilot Host-Pathogen Interaction Grant)
Cystic fibrosis trait carrier advantage: protecting against ancient and modern plague.
Role: PI

B. POSITIONS, SCIENTIFIC APPOINTMENTS, AND HONORS

Positions and scientific appointments

2023-Present Associate Professor with tenure, Emory University, Atlanta, GA
2023-Present Adjunct Associate Professor, Biomedical Engineering Program at Georgia Tech, Atlanta, GA
2022-Present Member, Cystic Fibrosis Foundation Program Planning Committee
2022-Present Review Editor*, Frontiers in Respiratory Drug Delivery*
2022-Present Scientific Consultant, Microbion Corp., Bozeman, MT
2021 *Ad hoc* reviewer, NIH SBIR/STTR Program

2021	Guest Editor*, Frontiers in Immunology*
2020-Present	*Ad hoc* reviewer, NSF SBIR/STTR Program
2019-Present	Member, Executive Committee, Immunology and Molecular Pathogenesis Graduate Program, Emory University, Atlanta, GA
2018-Present	Associate Professor, Emory University, Atlanta, GA
2018-Present	Member, American Thoracic Society
2017-Present	Permanent reviewer, Cystic Fibrosis Foundation Clinical Research Grant Program
2017-Present	Member, Society for Leukocyte Biology
2015-Present	*Ad hoc* reviewer, Italian CF Foundation
2015-Present	Scientific Consultant, Calithera Biosciences, Incorporated, South San Francisco, CA
2015-2016	Scientific Consultant, ProterixBio, Incorporated, Billerica, MA
2013-Present	Editor, *Frontiers in Pediatric Pulmonology*
2013-Present	Member, American Association of Immunologists
2011-Present	Member, American Society for the Advancement of Science
2011-2020	Scientific Advisory Board Member, Celtaxsys, Incorporated, Atlanta, GA
2011-2018	Assistant Professor, Emory University, Atlanta, GA
2006-2011	Instructor (PI status), Stanford University, Palo Alto, CA

Honors

2023	Chair, 36th North American CF Conference / Omics symposium
2022	Chair, Respi-DART Conference / Respiratory viruses in children and inflammation
2021	Chair, 35th North American CF Conference / Airway inflammation and repair workshop
2019	Chair, Immunology 2019 / Block symposium workshop
2019	Chair, 32nd North American CF Conference / Treating CF sequelae and monitoring
2019	Best basic science publication, Emory Department of Pediatrics, Atlanta, GA
2015	Chair, European CF Conference / Inflammation and Remodeling workshop
2014	Chair, 27th North American CF Conference / Endocrine Update workshop
2003	Chair, European CF Conference / Human-mouse models of CF workshop

C. CONTRIBUTIONS TO SCIENCE

1. Xenograft model for human lung development and gene transfer studies. I started my research career with the development of a xenograft model of human lung ontogeny. This model offered opportunities to study gene transfer to the human fetal lung [Ref. a], the ontogeny of airway mucosal immunity [Ref. b], and the state of human CF airways prior to infection [Refs. c, d], which prefigured my investigations in patients (below).

a. Péault B, **Tirouvanziam R**, Sombardier MN, Chen S, Perricaudet M, Gaillard D. Gene transfer to human fetal pulmonary tissue developed in immunodeficient SCID mice. *Hum Gene Ther*. 1994 Sep;5(9):1131-7. PMID: 7530495.

b. **Tirouvanziam R**, Khazaal I, N'Sondé V, Peyrat MA, Lim A, de Bentzmann S, Fournié JJ, Bonneville M, Péault B. Ex vivo development of functional human lymph node and bronchus-associated lymphoid tissue. *Blood*. 2002; 99:2483-9. PMID: 11895783.

c. **Tirouvanziam R**, de Bentzmann S, Hubeau C, Jacquot J, Péault B, Puchelle E. Inflammation and infection in naive human CF airway grafts. *Am J Respir Cell Mol Biol*. 2000; 23(2):121-7. PMID: 10919974.

d. **Tirouvanziam R**, Khazaal I, Péault B. Primary inflammation in human CF small airways. *Am J Physiol Lung Cell Mol Physiol.* 2002; 283: L445-51. PMID: 12114207.

2. Patient-based studies of CF lung inflammation. Based on results in the xenograft model (above), I set out as a research fellow at Stanford to study CF lung inflammation in patients. Using a new approach toward lung sample analysis using high-content cytometry, I showed that most CF airway neutrophils remain alive and release toxic effectors actively, driving lung destruction [Ref a]. This finding changed the paradigm of CF lung inflammation and opened multiple research avenues for my group at Emory, improving basic understanding of CF lung neutrophils [Refs b, c] notably via a novel transmigration model geared at mechanistic studies [Ref d].

a. Makam M, Diaz D, Laval J, Gernez Y, Conrad CK, Dunn CE, Davies ZA, Moss RB, Herzenberg LA, Herzenberg LA, **Tirouvanziam R**. Activation of critical, host-induced, metabolic and stress pathways mark neutrophil entry into CF lungs. *Proc Natl Acad Sci USA.* 2009; 106:5779-83. PMCID: PMC2667067.
b. Laval J, Touhami J, Herzenberg LA, Conrad C, Taylor N, Battini JL, Sitbon M, **Tirouvanziam R**. Metabolic adaptation of neutrophils in CF airways involves distinct shifts in nutrient transporter expression. *J Immunol*. 2013; 190:6043-50. PMID: 23690474.
c. Ingersoll SA, Laval J, Forrest OA, Preininger M, Brown MR, Arafat D, Gibson G, Tangpricha V **Tirouvanziam R**. Mature cystic fibrosis airway neutrophils suppress T cell function: evidence for a role of arginase 1 but not programmed death-ligand 1. *J Immunol*. 2015; 194:5520-8. PMCID: PMC4433848.
d. Forrest OA, Ingersoll SA, Preininger MK, Laval J, Limoli DH, Brown MR, Lee FE, Bedi B, Sadikot RT, Goldberg JB, Tangpricha V, Gaggar A, **Tirouvanziam R**. Pathological conditioning of human neutrophils recruited to the airway milieu in CF. *J Leukoc Biol*. 2018; 104:665-75. PMCID: PMC6956843.

3. Drug trials and biomarker studies. Using the approach for rapid *ex vivo* profiling of patient samples mentioned above, I discovered a basic defect in redox regulation of CF blood neutrophils, culminating in a phase 2b trial for a redox intervention [Ref. a]. These studies use optimized procedures for airway sample collection and analysis that my group leverages for all our patient-based studies, which we expanded to fluid-based proteomic, metabolomic and EV biomarkers, enabling deep phenotyping in children [Refs. b, c, d].

a. Conrad C, Lymp J, Thompson V, Dunn C, Davies Z, Chatfield B, Nichols D, Clancy J, Vender R, Egan ME, Quittell L, Michelson P, Antony V, Spahr J, Rubenstein RC, Moss RB, Herzenberg LA, Goss CH, **Tirouvanziam R**. Long-term treatment with oral NAC: affects lung function but not sputum inflammation in CF. A phase II randomized placebo-controlled trial. *J Cyst Fibros*. 2015; 14:219-27. PMID: 25228446.
b. Chandler JD, Margaroli C, Horati H, Kilgore MB, Veltman M, Liu HK, Taurone AJ, Peng L, Guglani L, Uppal K, Go YM, Tiddens HAWM, Scholte BJ, ***Tirouvanziam R**, Jones DP, Janssens HM. Myeloperoxidase oxidation of methionine associates with early cystic fibrosis lung disease. *Eur Respir J*. 2018; 52(4):1801118. PMCID: PMC7034951 [*co-senior, corresponding author].
c. Margaroli C, Garratt LW, Horati H, Dittrich AS, Rosenow T, Montgomery ST, Frey DL, Brown MR, Schultz C, Guglani L, Kicic A, Peng L, Scholte BJ, Mall MA, Janssens HM, Stick SM, **Tirouvanziam R**. Elastase exocytosis by airway neutrophils associates with lung damage in CF children. *Am J Respir Crit Care Med*. 2019; 199:873-881. PMCID: PMC6444666.
d. Forrest OA, Dobosh B, Ingersoll SA, Rao S, Rojas A, Laval J, Alvarez JA, Brown M, Tangpricha V, **Tirouvanziam R**. Neutrophil-derived extracellular vesicles promote feed-forward inflammasome signaling in cystic fibrosis airways. *J Leukoc Biol.* 2022; doi: 10.1002/JLB.3AB0321-149R. PMID: 35172381.

4. Clinical studies in conditions other than CF. Using the approach and know-how developed in CF studies, my group has contributed to several studies in allergy [Ref. a], transfusion reactions [Ref. b], ARDS [Ref. c], COVID-19 [Ref. d] and various other conditions. Our work spans prospective cohort studies and clinical trials.

a. Gernez Y, **Tirouvanziam R**, Nguyen KD, Herzenberg LA, Krensky AM, Nadeau KC. Altered phosphorylated STAT profile of CD4+CD161+ T cells in asthma: modulation by allergic status and oral corticosteroids. *J Allergy Clin Immunol*. 2007;120(6):1441-8. PMCID: PMC2679255.
b. Fontaine MJ, Shi H, Schubert R, Wong W, Andrews J, Jeng M, **Tirouvanziam R**. Leukocyte and plasma activation profiles in chronically transfused patients with a history of allergic reactions. *Transfusion.* 2017; 57(11):2639-2648. PMID: 28880378.
c. Grunwell JR, Giacalone VD, Stephenson S, Margaroli C, Dobosh BS, Brown MR, Fitzpatrick AM, **Tirouvanziam R**. Neutrophil dysfunction in the airways of children with acute respiratory failure due to lower respiratory tract viral and bacterial coinfections. *Sci Rep*. 2019;9(1):2874. PMCID: PMC6393569.
d. Eddins DJ, Yang J, Kosters A, Giacalone V, Pechuan X, Chandler JD, Eum J, Babcock BR, Dobosh BS, Hernández MR, Abdulkhader F, Collins GL, Orlova DY, Ramonell RP, Sanz I, Moussion C, Lee FE, **Tirouvanziam R**, Ghosn E. Transcriptional reprogramming of infiltrating neutrophils drives lung disease in severe COVID-19 despite low viral load. *Blood Adv*. 2022; doi: 10.1182/bloodadvances.2022008834. PMID: 36399523.

5. Myeloid cell development and function. In parallel with translational studies [Refs. a, b], I advanced basic knowledge on effector and regulatory subsets of myeloid cells in Drosophila [Ref. c], and mice [Refs d]. A better understanding of myeloid poise in blood and in the tissues is of relevance to this proposal.

a. Mejías JC, Forrest OA, Margaroli C, Frey Rubio DA, Viera L, Li J, Xu X, Gaggar A, **Tirouvanziam R***, Roy K*. Neutrophil-targeted, protease-activated pulmonary drug delivery blocks airway and systemic inflammation. *JCI Insight*. 2019;4(23): e131468. PMCID: PMC6962027 [* co-senior authors].
b. Genschmer KR, Russell DW, Lal C, Szul T, Bratcher PE, Noerager BD, Abdul Roda M, Xu X, Rezonzew G, Viera L, Dobosh BS, Margaroli C, Abdalla TH, King RW, McNicholas CM, Wells JM, Dransfield MT, **Tirouvanziam R**, Gaggar A, Blalock JE. Activated PMN exosomes: pathogenic entities inducing matrix destruction and disease in the lung. *Cell.* 2019; 176(1-2):113-126. PMCID: PMC6368091.
c. **Tirouvanziam R**, Davidson CJ, Lipsick JS, Herzenberg LA. Fluorescence-activated cell sorting (FACS) of Drosophila hemocytes reveals important functional similarities to mammalian leukocytes. *Proc Natl Acad Sci USA*. 2004; 101(9):2912-7. PMCID: PMC365719.
d. Napier RJ, Norris BA, Swimm A, Giver CR, Harris WA, Laval J, Napier BA, Patel G, Crump R, Peng Z, Bornmann W, Pulendran B, Buller RM, Weiss DS, **Tirouvanziam R**, Waller EK, Kalman D. Low doses of imatinib induce myelopoiesis and enhance host anti-microbial immunity. *PLoS Pathog*. 2015; 11(3): e1004770. PMCID: PMC4379053.

Complete list of published work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/1R_g8tk0GuCkD/bibliography/41448653/public