BIOGRAPHICAL SKETCH

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NAME: Allison Mattern

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

POSITION TITLE: Graduate student

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
DeSales University	B.S.	05/2023	Biochemistry and Molecular Biology
University of Delaware	Ph.D.		Biological Sciences

A. Personal Statement

I am a third-year Biological Sciences PhD candidate at the University of Delaware advised by Molly C. Sutherland. My research career began as an undergraduate at DeSales University where I completed independent research projects in two labs. With advisor Dr. Lara Goudsouzian, I analyzed bacterial composition in different soil environments using next-generation sequencing and presented this work at two local conferences. With Dr. Joshua Slee, I investigated the effect of Bromelain and Epigallocatechin gallate (EGCG) on inflammation and biomaterial rejection using cell-based models. I received a Summer Undergraduate Research Fellowship (SURF) in this lab, presented at one national and one local conference, and published one first-author publication. As a graduate student at UD, I was funded for my first two years as a NIH T32 Chemistry-Biology Research Interface Fellow, during which I completed three laboratory rotations as well as a research sabbatical with the University of Delaware School of Marine Science and Policy. In the Sutherland lab, we are interested in understanding mechanisms of heme handling and transport at a detailed, molecular level using prokaryotic cytochrome *c* biogenesis pathways as a model system. My dissertation research focuses on elucidating mechanisms of heme reduction in the bacterial System I pathway, with a focus on understanding the function of the cytochrome *c* synthase complex, CcmF/H. I have completed initial biochemical analysis of key CcmF variants to elucidate its function, however, further biochemical analysis as well as structural determination are crucial next steps for this project. The expertise of those at NCCAT will be invaluable towards this goal and I am also supported by a highly collaborative and interdisciplinary network at University of Delaware as a Chemistry-Biology Interface fellow.

Ongoing projects I'd like to highlight:

T32-GM133395
Grimes (PI)
Role: CBI Fellow
09/01/2024-08/31/2025

B. Positions, Scientific Appointments, and Honors

Positions

2023-Present Graduate Student, Laboratory of Molly C. Sutherland, Department of Biological Sciences, University of Delaware, Newark, DE

Fellowships

2024-2025 NIH/NIGMS Chemistry Biology Interface Program Fellowship T32-GM133395
2025 CBI Professional Development Award, University of Delaware
2023-2024 NIH/NIGMS Chemistry Biology Interface Program Fellowship T32-GM133395
2024 CBI Professional Development Award, University of Delaware
2022 Summer Undergraduate Research Fellowship (SURF), DeSales University

Professional Memberships

2025 Member, American Society for Biochemistry and Molecular Biology

C. Contributions to Science

1. Investigation of a heme reduction mechanism via CcmF/H in the System I cytochrome *c* biogenesis pathway. Cytochromes *c* are a structurally and functionally diverse set of proteins, performing critical functions in bacteria such as respiration, detoxification and noncanonical functions (e.g., iron uptake, extracellular nanowires). Despite their diversity, a common feature across all cytochromes *c* is the requirement for covalent binding of a heme molecule to a conserved CXXCH motif that is accomplished by one of three systems: System I (prokaryotes), System II (prokaryotes) or System III (eukaryotes). System I involves 8 integral membrane proteins (CcmABCDEFGH) that collaboratively transport and attach heme to cytochrome *c*. A requirement for heme attachment to the CXXCH motif by the holocytochrome *c* synthase CcmF/H is that the heme must be in the reduced state. The literature suggests that CcmF may not only attach heme to cytochrome *c* but also facilitate its reduction. CcmF co-purifies in a 1:1 ratio with a stable *b*-type heme that is liganded by two conserved transmembrane localized histidines. CcmF binds a different heme molecule in its active site that must be reduced prior to heme attachment. We hypothesize that CcmF/H is a heme reductase that functions by transferring electrons via the stable *b*-heme and two conserved aromatic residues. I have completed initial biochemical and functional analysis of CcmF variants with alanine mutations at each of the key residues with techniques including *in vivo* cytochrome *c* biogenesis assays, membrane protein purifications and heme stains, UV-vis spectra, and pyridine hemochrome assays. I presented a poster of this project at the American Society of Biochemistry and Molecular Biology (ASBMB) annual meeting in Chicago and anticipate at least one first-author publication from this work.

    a) **Mattern AE**, Kranz RG, Sutherland MC. Heme reduction in CcmFH, a key enzyme within the bacterial System I cytochrome *c* biogenesis pathway (*Poster*). American Society for Biochemistry and Molecular Biology Annual Meeting. Chicago, IL

2. Determining the effect of Epigallocatechin-gallate on inflammation and biomaterial rejection. Chronic inflammation plays a central role in the development of various health conditions, including heart disease and diabetes mellitus. For centuries, naturally occurring compounds, especially those found in plants, have been explored for their potential health benefits. One such compound is Epigallocatechin gallate (EGCG), a molecule found in green tea, which has been reported to offer a range of anti-inflammatory and antioxidant effects. However, our cell culture-based assays determined that EGCG is strongly proinflammatory and cytotoxic at concentrations utilized in other published reports, in both vascular endothelial cells and monocyte-derived macrophages. Additional tests using wound healing assays, *in vitro* THP-1 cell adhesion assays and *ex vivo* Chandler Loop Apparatus with whole equine blood indicated that EGCG possesses proinflammatory characteristics and may promote the rejection of biomaterials. I presented a poster of this project at the American Society of Cell Biology (ASCB) annual meeting in Washington D.C. and published a first-author publication as an undergraduate researcher.

a) **Mattern AE**, Azar C, Slee JB. (2022) Is green tea all it is brewed up to be? Our results suggest it may not be. Journal of the Pennsylvania Academy of Science, 10.5325/jpennacadscie.96.1.0046

b) **Mattern AE**, Azar C, Slee JB. Is green tea all it is brewed up to be? Our results suggest it may not be (*Poster*). American Society for Cell Biology (ASCB) Annual Meeting. Washington DC

BIOGRAPHICAL SKETCH

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NAME: Molly C. Sutherland

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

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 Maryland – College Park, MD	B.S.	5/2006	General Biology/Music
Washington University in St. Louis	Ph.D.	5/2013	Microbiology
Washington University in St. Louis	Postdoctoral	12/2019	Biochemistry

A. Personal Statement

I am an Assistant Professor at the University of Delaware, and I am the advisor of Allison Mattern who is submitting a GUP3 Grid Preparation and Screening proposal to the National Center for CryoEM Access and training. This proposal will serve to train Allison in Cryo-EM techniques and to bring Cryo-EM studies to my lab. My research group studies the mechanisms of heme delivery, trafficking, modification and attachment by the prokaryotic cytochrome *c* biogenesis pathways. I am uniquely qualified to lead this research due to my technical skills in microbial genetics, heme proteins and membrane protein biochemistry. I have a long-standing interest in membrane spanning protein complexes. My research career began as an undergraduate at the University of Maryland-College Park where I studied ecology and animal behavior. As an undergraduate I then received an NSF REU where I performed genetic characterization of bacterial conjugation machinery, sparking my interest in membrane complexes that transport a molecule, in this case DNA, out of bacterial cells. My graduate training in microbial genetics and pathogenesis at Washington University in St. Louis focused on the Type IVB Dot/Icm Secretion System of *Legionella pneumophila*, the causative agent of Legionnaires' disease, which transports over 300 protein effectors. I performed genetic screens and in-depth structure-function studies on one T4SS component, resulting in a new model for the molecular mechanism of substrate secretion. This work sparked my interest in biochemical characterization of membrane proteins, which I pursued during my postdoctoral training on the prokaryotic cytochrome *c* biogenesis pathways at Washington University in St. Louis. My NRSA supported postdoctoral work led to the development of novel approaches to study heme trafficking in these pathways, which consist of integral membrane proteins that transport heme across the bacterial membrane for covalent attachment to cytochromes *c*. Cytochromes *c* play essential and diverse roles within electron transport chains and while hundreds of cytochromes *c* exist, all prokaryotic cytochromes *c* are matured by two pathways. Thus, understanding their mechanisms represents a fundamental biological question. In my independent lab I have worked to develop these pathways as tractable model systems to study heme delivery, binding and trafficking, which are essential, but not well understood cellular processes.

Ongoing projects I would like to highlight:

R35 GM142498
Sutherland (PI)
07/01/2021-05/31/2026
Heme trafficking in prokaryotic cytochrome c biogenesis

Citations

a) Kranz RG, **Sutherland MC.** Mechanisms and Control of Heme Transport and Incorporation into Cytochrome *c*. 2025. *Annu Rev Microbiol*. 2025 Jun 3. doi: 10.1146/annurev-micro-050624-031631. Epub ahead of print. PMID: 40460019

b) Kreiman AN, Garner SE, Carroll SC, **Sutherland MC.** Biochemical mapping reveals a conserved heme transport mechanism via CcmCD in System I bacterial cytochrome *c* biogenesis. 2025. *mBio*. Apr 1:e03515-24. PMID: 40167305. doi: https://doi.org/10.1128/mbio.03515-24.

c) Yeasmin T, Carroll SC, Hawtof DH, **Sutherland MC**. *Helicobacter pylori* and *Campylobacter jejuni* bacterial holocytochrome *c* synthase structure-function analysis reveals conservation of heme binding. 2024. *Communications Biology*. 7(1):984. PMID: 39138305; PMCID: PMC11322641. doi: 10.1038/s42003-024-06688-3.

d) Grunow AL, Carroll SC, Kreiman AN, **Sutherland MC**. Structure-function analysis of the heme-binding WWD domain in the bacterial holocytochrome *c* synthase, CcmFH. 2023. *mBio*. 2023 mBio 14:e01509-23. PMID: 37929956; PMCID: PMC10746174. doi: 10.1128/mbio.01509-23.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2020-present	Assistant Professor, Department of Biological Sciences, University of Delaware, Newark DE
2019	Research Scientist, Laboratory of Robert G. Kranz, Washington University, St. Louis, MO
2013-2019	Postdoctoral Researcher, Lab of Robert G. Kranz, Washington University, St. Louis, MO
2006-2103	Graduate Student, Laboratory of Joseph P. Vogel, Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO

Honors

2015-2017	Ruth L. Kirschstein National Research Award (F32 NRSA) from NIGMS of the nIH
2013	W. M. Keck Postdoctoral Fellowship in Molecular Medicine
2010	Sondra Schlesinger Graduate Student Fellowship, Dept. of Molecular Microbiology, Washington University
2009	Federation of European Microbiological Societies (FEMS), Young Scientists Meeting Grant
2009	Stephen I. Morse Fellowship, Dept. Of Molecular Microbiology, Washington University School of Medicine
2009	Milton and Sondra Schlesinger and Paul Olivio Travel Grant, Dept. of Molecular Microbiology, Washington University School of Medicine
2005	National Science Foundation Research Experience for Undergraduates (REU), University of Texas at Austin

C. Contributions to Science

1. *Determination of heme trafficking pathway in the System I pathway of cytochrome c biogenesis in prokaryotes via heme redox potentials*. The System I holocytochrome *c* biogenesis pathway consists of eight membrane proteins (CcmABCDEFGH) that are proposed to traffick heme from the cytoplasm to the holocytochrome *c* synthase for attachment to apocytochrome *c* via two thioether bonds at a conserved CXXCH motif. During my postdoc, advances in genetics and biochemical approaches allowed for purification of key System I intermediates with endogenous heme at levels high enough to determine heme redox potentials. These measurements provide information on the environment of heme as it moves through the pathway and allows for speculation of potential heme oxidants and reductants. It is important to note that these pathway intermediates are composed of integral membrane proteins, thus were of high difficulty to obtain. This study resulted in a model in which heme is oxidized during transport from the cytoplasm by CcmABCD, allowing for

attachment via a single covalent bond to CcmE. CcmE then trafficks heme to the synthetase where it is re-reduced for attachment to apocytochrome *c*. These heme redox potentials provided the first evidence that heme is actively trafficked and modified in this pathway. In my independent lab, these results were expanded and we biochemically mapped the pathway of heme transport from the cytoplasm to the periplasmic CcmC WWD. Cysteine/heme crosslinking was used to determine specific residues within CcmCD that directly interact with heme, overlay of these residues on a recently published cryo-EM structure of CcmCD revealed the pathway of heme trafficking. These results determined that CcmCD is a dedicated heme transporter, answering a long-standing question in the cytochrome *c* biogenesis field. Bioinformatic and structural predication assays suggest that the heme channel is conserved across bacteria that encode System I, but the cytoplasmic heme acceptance domain does not appear to be conserved, suggesting that heme delivery may occur via multiple distinct avenues.

a) Kreiman AN, Garner SE*, Carroll SC, **Sutherland MC.** Biochemical mapping reveals a conserved heme transport mechanism via CcmCD in System I bacterial cytochrome *c* biogenesis. 2025. *mBio*. Apr 1:e03515-24. PMID: 40167305. doi: https://doi.org/10.1128/mbio.03515-24.

b) **Sutherland MC**, Rankin JR, Kranz RG. Heme trafficking and modification during System I cytochrome *c* biogenesis: insights from heme redox potentials of Ccm proteins. Biochemistry. 55(22):3150-3156. PMID: 27198710. PMCID: PMC5554621

c) San Francisco B, **Sutherland MC**, Kranz RG. The CcmFH complex is the system I holocytochrome *c* synthetase: engineering cytochrome *c* maturation independent of CcmABCDE. Mol Microbiol. 2014. 91(5):996-1008. PMID: 24397552. PMCID: PMC3959880

2. *Identification of discrete heme binding domains in prokaryotic cytochrome c biogenesis.* Trafficking of heme from the site of synthesis (inside) to the site of attachment to apocytochrome *c* (outside) is an essential function of the System I and System II cytochrome *c* biogenesis pathways. However, due to the tight regulation of intracellular heme and the transient nature of heme trafficking, direct evidence for heme binding has remained elusive. Mechanisms of heme binding and transport are critical to basic cellular function in eukaryotes and prokaryotes; however they are not well understood. I have worked to develop the bacterial cytochrome *c* biogenesis assays as model systems to study general mechanisms of heme binding and transport. Here, a novel cysteine/heme crosslinking approach was developed that exploits the natural propensity of cysteine and heme to form a covalent bond (crosslink) when in close proximity. Using this technique, heme binding domains were defined in CcmC, CcmE, CcmF and CcsA to identify specific residues that directly interact with heme. Subsequent work in CcmC determined that heme must be stereospecifically positioned for attachment. In collaboration with Sergey Ovchinnikov and David Baker (University of Washington, Seattle) these experimental constraints and genomic co-evolution data were used to model heme in the CcmC and CcsBA heme binding domains. Subsequently, cryo-EM structures with heme in the WWD domain validated the heme interacting residues, demonstrating the utility of the cysteine/heme crosslinking approach. Comparison of the WWD domain across these proteins suggest that heme is bound via conserved mechanism. We have hypothesized that the WWD domain is the active site of these three proteins, thus conservation of heme interaction suggests the WWD could be an attractive therapeutic target.

a) Yeasmin T, Carrol SC, Hawof DH*, **Sutherland MC**. *Helicobacter pylori* and *Campylobacter jejuni* bacterial holocytochrome *c* synthase structure-function analysis reveals conservation of heme binding. 2024. *Communications Biology*. 7(1):984. PMID: 39138305; PMCID: PMC11322641

b) Grunow AL, Carroll SC, Kreiman AN, **Sutherland MC**. Structure-function analysis of the heme-binding WWD domain in the bacterial holocytochrome *c* synthase, CcmFH. 2023. *mBio*. 2023 mBio 14:e01509-23. PMID: 37929956; PMCID: PMC10746174.

c) **Sutherland MC**, Jarodsky JM, Ovchinnikov S, Baker D, Kranz RG. 2018. Structurally Mapping Endogenous Heme in the CcmCDE Membrane Complex for Cytochrome *c* Biogenesis. J Mol Biol. *430:1065-1080.* PMID:29518410. PMCID: PMC5889519

d) Mendez DM, Lowder EP*, Tillman DE*, **Sutherland MC**, Collier AL, Rau MJ, Fitzpatrick JAJ, Kranz RG. 2022. Cryo-EM of CcsBA reveals the basis for cytochrome *c* biogenesis and heme transport. Nature Chemical Biology: 18, 101-108. PMID: 34931065. PMCID: PMC8712405.

3. *Identification of differential mechanisms of secretion for the Legionella pneumophila type IVB secretion system substrate classes.* The *Legionella pneumophila* type IVB secretion system (T4SS) is critical for the virulence of this intracellular pathogen. The membrane spanning T4SS is composed of 27 proteins that secrete over 300 effector substrates that are required for virulence and the formation of the bacterium's intracellular replicative vacuole. The type IV coupling protein (T4CP) subcomplex was identified and consists of DotL, DotM, DotN and IcmS/W. DotL is proposed to function as the T4SS substrate receptor due to its homology to known T4CPs, which function as receptors in other systems. However, the mechanism of secretion by this system is poorly understood. Prior to this work, it was known that at least two classes of substrates existed, those dependent on the chaperone-like proteins IcmS/W (S/W-dependent) and those that were not (S/W-independent). The prevailing model in the field was that substrates were bound by S/W in the cytoplasm, purportedly to be maintained in a partially unfolded state for secretion. In this work, a genetic approach was taken to identify the function of DotL. One mutant, DotL$^{-S/W}$, did not interact with S/W and showed that DotL and IcmS/W directly and stably interact. This result was unexpected and led to the identification of a minimal S/W binding domain on DotL. Interestingly, DotL$^{-S/W}$ was able to secrete S/W-independent substrates, but not S/W-dependent substrates, demonstrating that DotL requires a direct interaction with IcmS/W for secretion of one class of substrates. This differential mechanism of secretion for the T4SS substrate classes resulted in a paradigm shift for understanding substrate secretion in the T4BSS.

a) **Sutherland MC**, Nguyen TL*, Tseng V*, Vogel JP. The Legionella IcmSW complex directly interacts with DotL to mediate translocation of adaptor-dependent substrates. PLoS Pathog. 2012 Sep;8(9):e1002910. PMID: 23028312. PMCID: PMC3441705

b) Vincent CD, Friedman JR, Jeong KC, **Sutherland MC**, Vogel JP. Identification of the DotL coupling protein subcomplex of the Legionella Dot/Icm type IV secretion system. Mol Microbiol. 2012 Jul;85(2):378-91. PMID: 22694730. PMCID: PMC3391322

c) **Sutherland MC**, Binder KA*, Cualing PY*, Vogel JP. Reassessing the role of DotF in the *Legionella pneumophila* type IV secretion. PLoS One. 2013 Jun 7;8(6):e65529. PMID: 23762385. PMCID: PMC3676331

*Denotes undergraduate researcher
A complete list of Published Work can be found in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/167Xji3ysr5A5/bibliography/public/