Project ID: NCCAT- GUP1-CS190401
Project name: Structural studies of MmpL transporter
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Institution: Case Western Reserve University
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Abstract:
The MmpL family of transporters transloates gylcolipids and siderophores across the cell membrane mycobacteria for cell wall remodeling. Although these transporters are highly significant in terms of virulence and physiology of these bacteria, little is known about their structure and function. This project focuses on the MmpL protein, which is responsible for shuttling long-chain mycolic acid molecules to the cell wall. It is expected that the cryo-EM structure will serve as the first structural model for this important family of transporters. It will also shed light to the fundamental mechanisms that govern substrate translocation across the membrane via these transporters.

Scientific Impact:
Tuberculosis (TB) is one of the deadliest infectious diseases and was responsible for the death of 1.7 million people in 2016. The disease is caused by the bacterium Mycobacterium tuberculosis (Mtb) has developed resistance to commonly used anti-TB agents. It is obvious that the emergence of these drug-resistant TB strains has evolved into a major threat and challenges our global prospects for TB control. Thus, knowledge of the molecular mechanisms underlying drug resistance in M. tuberculosis is essential for the development of new strategies to combat this disease. This project focuses on the MmpL protein, which are responsible for cell wall remodeling and drug resistance. It is expected that the cryo-EM structure will serve as the first structural model for this important family of transporters. It will also shed light to the fundamental mechanisms that govern substrate translocation across the membrane via these transporters and thereby improve rational development of anti-tuberculosis drugs.

Scientific Feasibility:
Cryoelectron microscopy (cryo-EM) in association with a single particle analysis method (SPA) is a powerful tool to determine the structures of membrane protein and their macromolecular complexes.

Technical Feasibility:
We have expressed and purified one of MmpL family transporter. We have also embedded this pump in nanodiscs for structural studies using cryo-electron microscopy (cryo-EM) single particle imaging. We have obtained a small set of cryo-EM data (Fig. 1A- D), indicating the feasibility of the proposed structural work. We will continue our effort to resolve the atomic resolution structures of this transporters.

Resources Requested:
Due to the relatively small size of MmpL transporters (~100 kDa) and multiple classes, we expect 3-4 Krios days will be required for high-resolution structure.
Geographic/Demographics:
Case Western Reserve University at Cleveland is located proximity to New York.

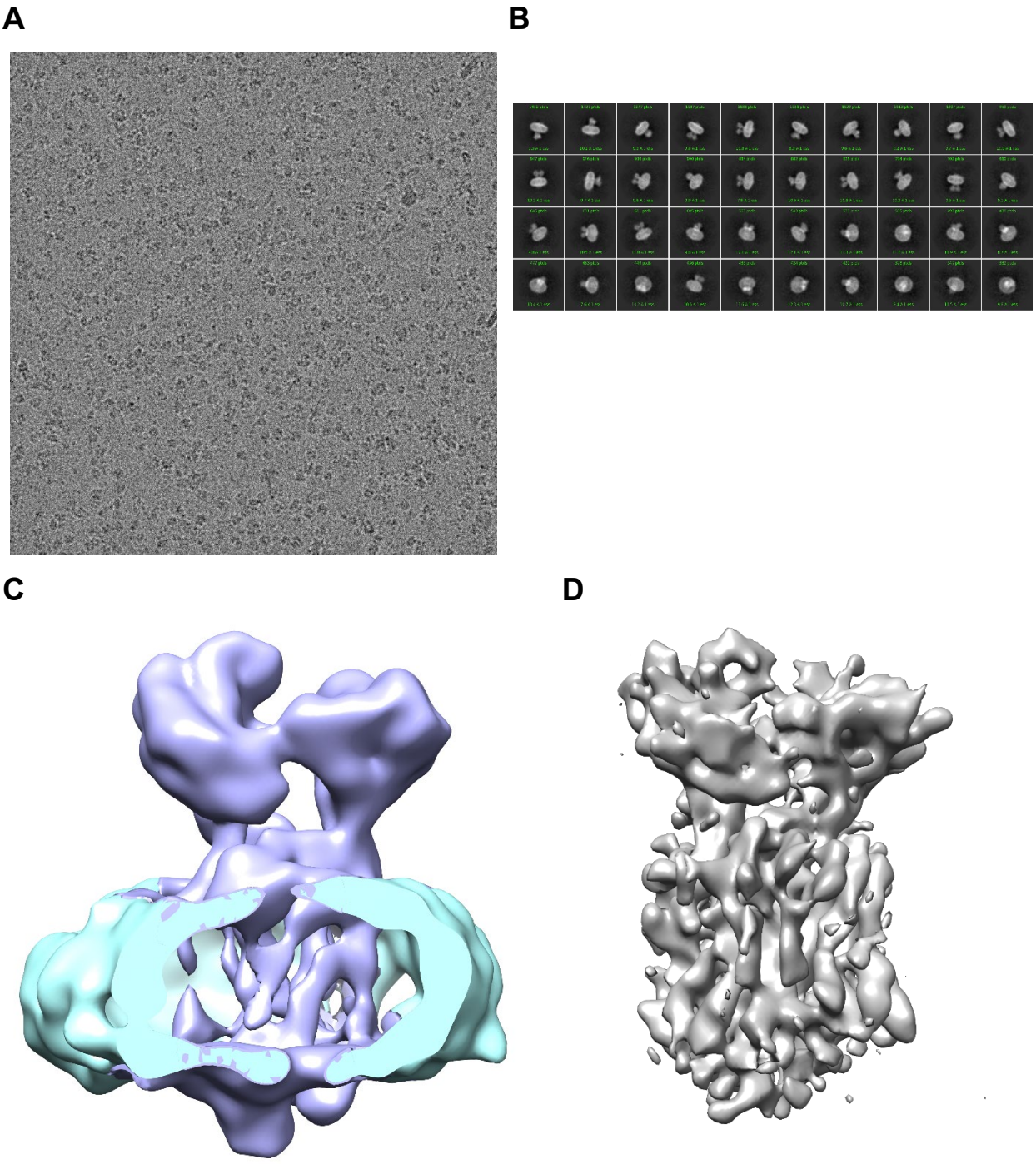

Fig. 1. (A) Representative micrograph of *Mycobacterium* MmpL transporter in nanodiscs collected on a Titan Krios. **(B)** Representative 2D class averages of this pump **(C)** 8.0 Å cryoEM reconstruction of monomeric MmpL transporter (purple) in lipid bilayer (cyan). **(D)** 4.5 Å local refinement reconstruction of MmpL protein based on 32K particles.

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: Chih-Chia Su

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

POSITION TITLE: Instructor

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
National Dong Hwa University, Taiwan	B.S	2001	Life Science
National Dong Hwa University, Taiwan		2003	Chemistry
Iowa State University, Ames, IA	Ph.D.	2009	Biochemistry and Biophysics
Iowa State University, Ames, IA	Postdoctoral	2012	Biochemistry and Biophysics

A. Personal Statement

Since I started my graduate career, I have concentrated on elucidating the structure, assembly and mechanism of the resistance-nodulation-cell division (RND)-superfamily efflux pumps as well as their regulation. In gram-negative bacteria RND family efflux system is composed of an inner membrane pump, an outer membrane channel and a periplasmic adaptor protein. They are assembled as a tripartite efflux complex to form ducts inside cell facilitating drug exit across both membranes. These efflux pumps are key components for Gram-negative pathogens to ensure their survival in toxic environments.

I have determined the structural-functional relationships of several of the RND efflux proteins as well as their transcriptional regulators in Gram-negative bacteria. I have published several important crystal structures of the RND-type efflux pumps, including *E. coli* AcrB, *E. coli* CusA and *N. gonorrhoeae* MtrD. I have also involved in resolving several crystal structures of outer membrane channels, including *E. coli* CusC, *N. gonorrhoeae* MtrE and *C. jejuni* CmeC, which work with their corresponding efflux pumps to export toxic chemicals from cells. In addition, I have studied the assembly of these efflux protein complexes by determining detailed co-crystal structure of the CusBA adaptor–transporter complex. This is the only adaptor-transporter efflux complex structure that has been determined using X-ray crystallography.

My rapid progress in the field has positioned me uniquely to fully unravel the structure and function of the *Mycobacterium tuberculosis* MmpL family lipid transporters, which belong to the RND-superfamily transporters. MmpL proteins play a predominant role in mediating lipids trafficking and cell wall remodeling in *Mycobacterium.* Recently, I have cloned, expressed and purified several of these MmpL transporters. I have also reconstituted these pumps in nanodiscs for single particle imaging. These data clearly demonstrate the feasibility of the proposed work.

B. Positions and Honors

Positions and Employment

2012-2015 Assistant Scientist, Department of Physics & Astronomy, Iowa State University, Ames, IA
2015-2017 Associate Scientist, Department of Physics & Astronomy, Iowa State University, Ames, IA
2017-2018 Scientist, , Department of Pharmacology, Case Western Reserve University, Cleveland, OH
2018- Instructor, Department of Pharmacology, Case Western Reserve University, Cleveland, OH

Honors

2003 Academic Excellence, National Dong Hwa University, Taiwan
2012 National Institute of General Medicine Science (NIGMS) Scholarship for Keystone Symposia of Structural Biology of Cellular Processes: From Atoms to Cells
2015 Best Paper Award, Protein Science.

C. Contribution to Science

1. The CusCBA heavy metal efflux system of *Escherichia coli*:

Bacteria such as *Escherichia coli* have developed various mechanisms to overcome toxic environments that are otherwise unfavorable for their survival. One important strategy that bacteria use to subvert toxic compounds, including heavy metal ions, is the expression of membrane transporters that recognize and actively export these toxic compounds out of bacterial cells, thereby allowing the bugs to survive in extremely toxic conditions. The *E. coli* CusCBA efflux system that recognizes and extrudes silver and copper ions out of the bacterial cell. CusA is an inner membrane transporter, which belongs to the resistance-nodulation-division (RND) protein superfamily. CusC forms an outer membrane channel in *E. coli*. These two membrane proteins interact with each other, in conjunction with a membrane fusion protein CusB, to mediate the extrusion of heavy metal ions across both membranes of *E. coli*. Recently, we have determined the crystal structures of the CusA heavy-metal efflux pump. The structure suggested that CusA utilizes methionine residues to bind and export Ag(I) and Cu(I). We have also resolved the first detailed crystal structure of the CusBA adaptor-transporter efflux complex. The structure of the CusBA efflux complex depicted that the transporter CusA, which is presented as a trimer, interacts with six CusB protomers forming a continuous channel. In addition, we have determined two mutant structures of the CusC efflux channel, reviewing conformational changes accompanying folding and transmembrane channel formation of this outer membrane protein.

a. Delmar, J. A., **C.-C. Su** and E. W. Yu. 2014. Bacterial multidrug efflux transporters. Annu. Rev. Biophys. 43:93-117. (PMC4769028)
b. **Su, C.-C**., F. Long, H.-T. Lei, J. B. R. Bolla, S. V. Do, K. R. Rajashankar, and E. W. Yu**.** 2012. Charged amino acids (R83, E567, D617, R669, and K678) of CusA are required for metal ion transport in the Cus efflux system. J. Mol. Biol. 422: 429-441. (PMC3423576)
c. **Su, C.-C.**, F. Long, M. T. Zimmermann, K. R. Rajashankar, R. L. Jernigan, and E. W. Yu. 2011. Crystal structure of the CusBA heavy-metal efflux complex of *Escherichia coli*. Nature 470:558-562. (Reviewed by F1000) (PMC3078058)
d. Long, F., **C.-C. Su**, M. T. Zimmermann, S. E. Boyken, K. R. Rajashankar, R. L. Jernigan, and E. W. Yu. 2010. Crystal structures of the CusA heavy-metal efflux pump suggest a methionine-mediated transport mechanism. Nature 467:484-488. (Reviewed by F1000) (PMC2946090)

2. The MtrCDE tripartite multidrug efflux system of *Neisseria gonorrhoeae*:

Neisseria gonorrhoeae is a Gram-negative obligate human pathogen. It is the causative agent of the sexually-transmitted infection (STI) gonorrhea and rare cases of disseminated disease. This STI is the second most commonly reported infection in the United States and more than 78 million cases occur annually worldwide. Over the decades the gonococcus has developed resistance to a range of clinically relevant antibiotics, including beta-lactams, tetracyclines, macrolides and quinolones. Multidrug efflux is considered to be one of the major causes of failure of drug-based treatments of bacterial infections, which appear to be proliferating in prevalence. Therefore, we have targeted the *N. gonorrhoeae* MtrCDE (multiple transferable resistance) tripartite multidrug efflux system, which has been demonstrated to contribute to the resistance of both antibiotics and host-derived antimicrobials, forbidding this system to piece together and work properly. The MtrCDE tripartite efflux pump is composed of the MtrD inner membrane transporter, MtrC periplasmic membrane fusion protein and MtrE outer

membrane channel. These three efflux proteins contact one another at the periplasmic space and assemble to form a powerful tripartite system. This system allows direct efflux of substrates across both membrane of the Gram-negative cellular envelope, mediating resistance to a broad spectrum of antimicrobial agents and gonadal steroidal hormones. Our working hypothesis is that MtrC, MtrD and MtrE must specifically interact with each other at the protein-protein interfaces (PPIs) in order to assemble and function. Recently, we have determined the crystal structures of the MtrD and MtrC membrane proteins. Based on our structural information, we will rationally design peptides that tightly attach to these efflux proteins at the PPIs, prohibiting them to unite and function. We will also apply phage display methodology to identify novel peptides that strongly contact MtrC, MtrD or MtrE, inhibiting the assembly of the MtrCDE tripartite efflux complex, which we posit will produce unique efflux pump inhibitors that render gonococci susceptible to antibiotics and host antimicrobials during infection.

a. Delmar, J. A., J. R. Bolla, **C.-C. Su** and E. W. Yu. 2015. Crystallization of membrane proteins by vapor diffusion. Meth. Enzymol. 557:363-392. (PMC4755347)
b. Bolla, J. R., **C.-C. Su**, S. V. Do, A. Radhakrishnan, N. Kumar, T.-H. Chou, J. A. Delmar, H.-T. Lei, K. R. Rajashankar, W. M. Shafer and E. W. Yu. 2014. Crystal structures of the *Neisseria gonorrhoeae* MtrD inner membrane multidrug efflux system. PLoS One 9 (6):e97903. (PMC4046932)
c. Lei, H.-T., T.-H. Chou, **C.-C. Su**, J. R. Bolla, N. Kumar, A. Radhakrishnan, F. Long, J. A. Delmar, S. V. Do, K. R. Rajashankar, W. M. Shafer and E. W. Yu. 2014. Crystal structure of the open state of the *Neisseria gonorrhoeae* MtrE outer membrane channel. PLoS One 9 (6):e97475. (PMC4046963)

3. Structure and mechanism of the AbgT-family transporters:

The AbgT family of transporters was thought to contribute to bacterial folate biosynthesis by importing the catabolite p-aminobenzoyl-glutamate for producing this essential vitamin. Approximately 13,000 putative transporters of the family have been identified. Surprisingly, among proteins in this diverse family, only *E. coli* AbgT and *N. gonorrhoeae* MtrF have been partially characterized. To elucidate the structure and function of the AbgT family of transporters, we recently determined the X-ray structures of the full-length Alcanivorax borkumensis YdaH and Neisseria gonorrhoeae MtrF membrane proteins. Our novel findings strongly suggest that both YdaH and MtrF behave as antibiotic efflux pumps, which are able to remove sulfonamides from the cell and effect bacterial resistance to this class of antimetabolites. Two research papers on the structural studies of the YdaH and MtrF transporters have been published.

a. Bolla, J. R., **C.-C. Su**, J. A. Delmar, A. Radhakrishnan, N. Kumar, T.-H. Chou, F. Long, K. R. Rajashankar and E. W. Yu**.** 2015. Crystal structure of the *Alcanivorax borkumensis* YdaH transporter reveals an unusual topology. Nature Commun. 6:6874. (PMC4410182)
b. **Su, C.-C**., J. R. Bolla, N. Kumar, A. Radhakrishnan, F. Long, J. A. Delmar, T.-H. Chou, K. R. Rajashankar, W. M. Shafer and E. W. Yu. 2015. Structure and function of *Neisseria gonorrhoeae* MtrF illuminates a class of antimetabolite efflux pumps. Cell Reports 11:61-70. (Made the cover of the issue) (PMC4410016)

4. Drug Efflux and Transcriptional Regulation in *Campylobacter jejuni*:

Campylobacter jejuni is the leading bacterial cause of food-borne diarrhea in the USA as well as other developed countries. It is also in the list of the *National Institute of Allergy and Infectious Diseases (*NIAID) Category B Priority Pathogens. *Campylobacter* infection may also trigger an autoimmune response, which is associated with the development of Guillain-Barre syndrome, an acute flaccid paralysis caused by degeneration of the peripheral nervous system. For antibiotic treatment of human campylobacteriosis, fluoroquinolones and macrolides are frequently prescribed. Unfortunately, *Campylobacter* has developed resistance to these antimicrobials, especially fluoroquinolones. According to the genomic sequence of NCTC 11168, *C. jejuni* harbors 13 putative antibiotic efflux transporters that mediate resistance to antimicrobial agents. Among them, the *Campylobacter* multidrug efflux system CmeABC, which is a RND-type efflux pump, is the primary antibiotic efflux system and is the best functionally characterized transporter in *C. jejuni*. CmeABC consists of three components including an outer membrane protein (CmeC), an inner membrane drug transporter (CmeB), and a periplasmic membrane fusion protein (CmeA). This efflux system is regulated by the CmeR transcriptional regulator. To understand how this multidrug efflux system works, we have defined several crystal structures of these efflux proteins, including the CmeC outer membrane channel and CmeR transcriptional regulator (with our collaborator Dr. Qijing Zhang).

a. **Su C.-C**, Yin L, Kumar N, Radhakrishnan A, Dai L, Chou TH, Delmar JA, Rajashankar KR, Zhang Q. Shin YK. Yu EW. Structures and transport dynamics of the *Campylobacter jejuni* multidrug efflux pump CmeB. Nat Commun**.**2017 ;8:171 (PMC5537355)

b. **Su, C.-C**., A. Radhakrishnan, N. Kumar, F. Long, J. R. Bolla, H.-T. Lei, J. A. Delmar, S. V. Do, T.-H. Chou, K. R. Rajashankar, Q. Zhang and E. W. Yu. 2014. Crystal structures of the *Campylobacter jejuni* CmeC outer membrane channel. Prot. Sci. 23:954-961. (Made the cover of this issue) (Won the 2015 Protein Science Best Paper Award) (PMC4088979)
c. Lei H. T., Z. Shen, P. Surana, M. D. Routh, **C.-C. Su**, Q. Zhang, and E. W. Yu**.** 2011. Crystal structures of CmeR-bile acid complexes from *Campylobacter jejuni*. Prot. Sci. 20:712-23. (PMC3081549)
d. Gu, R., **C.-C. Su**, F. Shi, M. Li, G. McDermott, Q. Zhang, and E. W. Yu**.** 2007. Crystal structure of the transcriptional regulator CmeR from *Campylobacter jejuni*. J. Mol. Biol. 372:583-593. (PMC2104645)

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/collections/57442314/

D. Research Support

Ongoing Research Support

None