BIOGRAPHICAL SKETCH

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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

My role in this project is as a principal investigator. 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. 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. I have determined the structural-functional relationships of several of the RND efflux proteins and published several important crystal and cryo-EM structures of the RND-type efflux pumps, including E. coli AcrB, *E. coli* CusA, *N. gonorrhoeae* MtrD and *C. jejuni* CmeB. 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. Recently, I focus on studying membrane protein structure and function using cryo-electron microscopy (cryo-EM). I have solved several membrane protein structures by cryo-EM, including *A. baumannii* AdeB, *M. smegmatis* MmpL3, *N. gonorrhoeae* MtrD and *Plasmodium falciparum* PfFNT.

My rapid progress in the field has positioned me to carry out the research proposed in this application. I have recently developed a bottom-up iterative method that enables identifying and determining cryo-EM structures of various protein complexes of different sizes and dimensions from a heterogeneous, impure protein sample. 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
2017-2019 Scientist, Department of Pharmacology, Case Western Reserve University, Cleveland, OH
2019- 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. A methodology to simultaneously solve cryo-EM structures of membrane proteins:
Single-particle cryo-electron microscopy (cryo-EM) has become a powerful technique in the field of structural biology. However, the inability to reliably produce pure, homogeneous membrane protein samples hampers the progress of their structural determination. We develop a bottom-up iterative method, Build and Retrieve (BaR), that enables the identification and determination of cryo-EM structures of a variety of inner and outer membrane proteins, including membrane protein complexes of different sizes and dimensions, from a heterogeneous, impure protein sample. We also use the BaR methodology to elucidate structural information from *Escherichia coli* K12 crude membrane and raw lysate. The findings demonstrate that it is possible to solve high-resolution structures of a number of relatively small (<100 kDa) and less abundant (<10%) unidentified membrane proteins within a single, heterogeneous sample. Our study will facilitate the development of a new era of systems structural biology, capable of elucidating the cell membrane proteome at atomic resolution.

a. **Su CC**, Lyu M, Morgan CE, Bolla JR, Robinson CV, Yu EW. A 'Build and Retrieve' methodology to simultaneously solve cryo-EM structures of membrane proteins. **Nat Methods**. 2021 Jan;18(1):69-75. PMID: 33408407; PMCID: PMC7808410

2. Structural basis of transport and inhibition of the Plasmodium falciparum transporter PfFNT:
Malaria is an extremely devastating disease as it infects more than 200 million people with nearly half a million deaths worldwide each year (WHO, 2018). It appears that more than 90% of pregnant women and children with Plasmodium falciparum infection reside in sub-Saharan Africa (WHO, 2019). Plasmodium falciparum formate-nitrite transporter (PfFNT), a 34-kDa transmembrane protein, has been identified as a novel drug target as it exports lactate from inside the parasite to the surrounding parasitophorous vacuole within the erythrocyte cytosol. We have determined structures of apo-PfFNT and PfFNT bound with MMV007839 using single-particle cryo-electron microscopy (cryo-EM) to resolutions of 2.56 Å and 2.78 Å. Coupled with genetic analysis and transport assay, these structures suggest that PfFNT contains transient binding sites for lactate and lactic acid. This channel sequentially shuttles lactate ions and converts these ions into lactic acids via a stepwise displacement mechanism. Our work provides molecular insights into the mechanism of lactate extrusion that involves substrate binding and displacement, as well as proton transfer via the PfFNT membrane protein to facilitate substrate transport across the membrane.

a. Lyu M, **Su CC**, Kazura JW, Yu EW. Structural basis of transport and inhibition of the Plasmodium falciparum transporter PfFNT. **EMBO Rep**. 2021 Jan 20:e51628. PMID: 33471955.

3. Structure and mechanism of *Mycobacterium* MmpL3 lipid transporter:

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*). The cell envelope of *Mycobacterium tuberculosis* is notable for the abundance of mycolic acids (MAs), essential to mycobacterial viability, and of other species-specific lipids. Mycobacterial membrane protein Large 3 (MmpL3) is essential and required for transport of trehalose monomycolates (TMMs), precursors of MA-containing trehalose dimycolates (TDM) and mycolyl arabinogalactan peptidoglycan. We have determined a crystal structure of *Mycobacterium smegmatis* MmpL3 at a resolution of 2.59 Å. A previously unknown MmpL3 ligand, phosphatidylethanolamine (PE), was discovered inside this transporter. We also show, via native mass spectrometry, that MmpL3 specifically binds both TMM and PE, but not TDM, in the micromolar range. These observations provide insight into the function of MmpL3 and suggest a possible role for this protein in shuttling a variety of lipids to strengthen the mycobacterial cell wall.

a. **Su CC**, Klenotic PA, Bolla JR, Purdy GE, Robinson CV, Yu EW. MmpL3 is a lipid transporter that binds trehalose monomycolate and phosphatidylethanolamine. **Proc Natl Acad Sci**. 2019 Jun 4;116(23):11241-11246. PMID: 31113875; PMCID: PMC6561238.

4. 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. The research help to improve our knowledge of silver and copper resistance in pathogenic bacteria, and will provide a platform for thinking about novel metal-based antimicrobial therapeutic strategies that will lead to new treatments.

a. Delmar JA, Su CC, Yu EW. Bacterial multidrug efflux transporters. **Annu Rev Biophys.** 2014;43:93-117. PMID: 24702006; PMCID: PMC4769028.
b. **Su CC,** Long F, Lei HT, Bolla JR, Do SV, Rajashankar KR, Yu EW. Charged amino acids (R83, E567, D617, E625, R669, and K678) of CusA are required for metal ion transport in the Cus efflux system. J Mol Biol. 2012 Sep 21;422(3):429-41. PMID: 22683351; PMCID: PMC3423576.
c. **Su CC,** Long F, Zimmermann MT, Rajashankar KR, Jernigan RL, Yu EW. Crystal structure of the CusBA heavy-metal efflux complex of Escherichia coli. **Nature**. 2011 Feb 24;470(7335):558-62. PMID: 21350490; PMCID: PMC3078058**.** (Reviewed by F1000)
d. Long F, **Su CC**, Zimmermann MT, Boyken SE, Rajashankar KR, Jernigan RL, Yu EW. Crystal structures of the CusA efflux pump suggest methionine-mediated metal transport. **Nature**. 2010 Sep 23;467(7314):484-8. PMID: 20865003; PMCID: PMC2946090. (Reviewed by F1000)

5. 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 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 JR, **Su CC**, Delmar JA, Radhakrishnan A, Kumar N, Chou TH, Long F, Rajashankar KR, Yu EW. Crystal structure of the Alcanivorax borkumensis YdaH transporter reveals an unusual topology. **Nat Commun**. 2015 Apr 20;6:6874. PMID: 25892120; PMCID: PMC4410182
b. **Su CC**, Bolla JR, Kumar N, Radhakrishnan A, Long F, Delmar JA, Chou TH, Rajashankar KR, Shafer WM, Yu EW. Structure and function of Neisseria gonorrhoeae MtrF illuminates a class of antimetabolite efflux pumps. **Cell Rep**. 2015 Apr 7;11(1):61-70. PMID: 25818299; PMCID: PMC4410016. (Made the cover of the issue)

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/chih-chia.su.1/bibliography/public/

D. Research Support

Ongoing Research Support

None