BIOGRAPHICAL SKETCH

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NAME: Mueller, David Michael

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

POSITION TITLE: Professor of Biochemistry and Molecular 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
The University of Michigan, Ann Arbor	B.S.	05/1976	Chemistry
Wayne State University, Detroit	Ph.D.	12/1981	Biochemistry
The University of Chicago, Chicago	Postdoctoral	12/1986	Molecular Biology

A. Personal Statement

The strength of my laboratory is being able to integrate a number of disciplines in asking and answering key questions. My educational background is broad. As a chemistry major at the University of Michigan, I did research with Dr. Jim Fee in the Biophysical Institute. In graduate school at Wayne State University, I worked with C.P. Lee who had just come from a faculty position at the Johnson Research Foundation at the University of Pennsylvania. Dr. Lee was a leader in Bioenergetics and worked with the likes of Britton Chance. With Dr. Lee, I used biochemical and biophysical methods in the study of mitochondrial bioenergetics with a considerable use of fluorescence spectroscopy. My post-doctoral studies with Dr. Murray Rabinowitz at the University of Chicago were exclusively on gene expression studies with heavy utilization of yeast genetics and molecular biology. In 2000, I spent a sabbatical at the MRC in Cambridge working with Drs. John Walker and Andrew Leslie on crystallography. As a PI, my laboratory has investigated genetic, cellular, biochemical, biophysical, X-ray crystallographic and more recently, cryo-EM studies on the mitochondrial ATP synthase. We have a general interest in membrane proteins and studied the role of codon usage in the expression and folding of CFTR. We have recently renewed our interest in the study of Batten disease, as we were the first lab to localized the Batten protein to the lysosome, which is the vacuole in yeast. We have expertise in a broad area of research and continually expand on our expertise and as needed, set up collaborations. Careful inspection of the list of publications will reveal studies that are entirely genetic, biochemical, cellular, crystallographic, or cryo-EM. My entry into crystallography has been noticeable as we have been successful in the solving the structure of the yeast F_1- ATPase, a major accomplishment as many established laboratories have tried to crystallize the F_1 from yeast of other species, without success. John Walker was awarded the Nobel Prize in Chemistry for solving the first complete structure of the bovine F_1 and we continue close discussions with him and Dr. Andrew Leslie. My lab has completed the structure of 5 mutant structures of the yeast F_1 and an apo form of the enzyme. We have also solved the crystal structure of yeast c-ring at pH 8.3, 6.1, 5.5, and at 5.5 modified with the inhibitor, DCCD from 2.0-2.5Å resolution. We have solved the structure of oligomycin bound to the yeast c-ring at 1.9Å. Most recently, by cryo-EM in collaboration with Maofu Liao at Harvard Medical School, we have solved at near atomic resolution the structure of the entire monomeric mitochondrial ATP synthase, with and without, bound oligomycin. It is hard to over-emphasize this accomplishment as this has been the goal of many labs across the globes for decades. Our expertise on the ATP synthase, in yeast genetics, molecular biology, crystallography, and cryo-EM are unique and critical for the success in this project.

B. Positions and Honors
Positions and Employment

August 2000 to Sept. 2001, Visiting Professor, Medical Research Council, Dunn Human Nutrition, Unit, Cambridge, U.K.

July 1999 to present, Professor, Department of Biochemistry and Molec. Biology, The Chicago Medical School, North Chicago, IL

1990 to July 1999, Associate Professor, Department of Biochemistry and Molec. Biology, The Chicago Medical School, North Chicago, IL.

1986 to 1990, Assistant Professor, Department of Biochemistry and Molec. Biology, The Chicago Medical School, North Chicago, IL.

1984-1986, Research Associate, Department of Medicine, The University of Chicago, Chicago, IL

1981-1984, Postdoctoral Fellow, Department of Medicine, The University of Chicago, Chicago, IL.

1977-1980, Research Assistant, Department of Biochemistry, Wayne State University, Detroit, MI.

1977, Teaching Assistant, Department of Chemistry, Wayne State University, Detroit, MI.

Other Experience and Professional Memberships

1989-	Member of Genetic Society
1990-present	Member of the American Society For Biochemistry and Molecular Biology
1990	Board of Trustees Research Award, The Chicago Medical School
1998-2002	Editorial Board, *The Journal of Biological Chemistry*
1999	Scientific Review Board for the American Heart Association
2000	NIH, Fogarty Senior International Fellowship
2002, 2005	Ad Hoc Reviewer for Wellcome Trust Foundation, UK.
2005-present	Reviewer for Argonne National Laboratory, APS, General User Program**.**
2005	Ad Hoc Reviewer for NHBLI Program Project Study Section
2005	Ad Hoc Reviewer for BBM Study Section
2006	Ad Hoc Reviewer for GCMB Study Section
2007	Ad Hoc Reviewer for BBM Study Section
2007	Ad Hoc Reviewer for GCMB Study Section
2009	Ad Hoc Reviewer for BBM Study Section
2009	Ad Hoc Reviewer for BCMB Integrated Review Group
2010, 2011	Ad Hoc Reviewer for NHBLI Program Project Study Section
Current	Recent *Ad hoc* reviewer for *Science, Nature, Nature Cell Biology*, *Biochemistry, PNAS, EMBO J., Eukary. Cell, BBA, J., Bioenerg. Biomemb., JBC*, *Structure, Human Genetics, Biochemical J., Molec. Biol. and Evolution, Protein Science, PLOS Pathogens, PLOS Biology, IUBMB Life, Frontiers Genetics*
2013-Current	Academic Editor, Microbial Cell
2014	Ad Hoc Reviewer for Netherlands Organisation for Scientific Research (NWO)

Honors

1981-1984	NIH Training Grant
1990	Board of Trustees Research Award, The Chicago Medical School
2010	Morris L. Parker Award research award, The Chicago Medical School
2009, 2013	Guest Editor, PNAS

Recent Invited Talks.
2010 FASEB summer meeting on “Transport ATPases”, Snowmass Co.
2010 European Bioenergetic Conference, Warsaw, Poland, (turned down).
2011 Gordon Research Conference on “Bioenergetics”, Andover, N.H.
2012 FASEB summer meeting on “Transport ATPases”, Snowmass Co.
2013 Gordon Research Conference on “Bioenergetics”, Andover, N.H.
2014 FASEB summer meeting on “Transport ATPases”, Barga, Italy. (Meeting was canceled)

C. Contribution to Science

1. Structure of the monomeric mitochondrial ATP synthase incorporated in nanodiscs by cryo-EM. Obtaining the structure of the entire monomeric form of the mitochondrial ATP synthase has been the culmination of many years of study. The structure was solved by cryo-EM, but while that was a critical tool, it was not sufficient which is why we were able to succeed. The key to the structure determination was creating a fusion between the rotor and peripheral stalk which locked the enzyme in a single rotomer conformation. In addition, it is possible, that incorporation into nanodiscs helped in the sample preparation, but that has not been critically evaluated. Nonetheless, the structure, for the first time, revealed the twisting of the rotor that certainly occurs during proton translocation and the process of ATP synthesis. While this twisting was only about 9^{o}, it illustrates that we can obtain twisted intermediates. We also observed a slight rotation of the c-ring which was likely caused by the torque of the twisting. We observed that the side chain carboxylate of cGlu59 could be in the "open conformation" in the membrane. This is discussed more in the next paragraph, but much has been discussed on the open and closed conformation in the course of proton translocation. This result contradicted the prior conclusions which were based on x-ray crystal structure of the purified c-ring crystallized in detergent. We also obtained the structure with bound oligomycin and it showed that the mode of binding was the same as we determined for the isolated c-ring (see below). However, the structure provided a clue as to how extragenic mutations in a-subunit can cause resistance to oligomycin. This was a key discovery as it provides a better understanding of drug resistance as well as the plasticity of, in this case, the c_{10}-ring of the yeast ATP synthase. **Associated pdbs: 6CP3, 6CP5, 6CP6, 6CP7.**

A. Srivastava, A. P., Luo, M., Zhou, W., Symersky, J., Bai, D., Chambers, M. G., Faraldo-Gomez, J. D., Liao, M., and Mueller, D. M. (2018) *High-resolution cryo-EM analysis of the yeast ATP synthase in a lipid membrane*. Science. 360(6389). pii: eaas9699. doi: 10.1126/science.aas9699. Epub 2018 Apr 12. PMID:29650704

2. Structure of the c-ring and structural basis of inhibitor binding. These studies addressed both the structure/function relationship of the mitochondrial ATP synthase and the molecular basis of drug binding We have made critical discoveries during the course of these studies. We have solved structure of the mitochondrial c_{10}-ring at 1.9Å resolution. This high resolution is the best structure of a eukaryotic c-subunit today. The structure gave new insight into the proton translocation mechanism as it showed the carboxylate of the critical Glu in the "open" conformation – ready to accept protons. We also showed the open conformation could occur in low and high pH. We also determined the structure with the inhibitor, DCCD, bound. This study gave evidence against studies and hypotheses, which suggested that the α-helix twists during the transition from the protontated and unprotonated form. The structure also identified some key features, which might determine the ion specificity. Besides DCCD, we also determined the structure of oligomycin bound to the c-ring. Oligomycin has been known to inhibit the mitochondrial but not the bacterial enzyme since about 1950. This study explained the selectivity of oligomycin and pointed to the understanding of other inhibitors to the ATP synthase and even the vacuolar ATPase. We also have data that shows that the antibiotic effective against *Mycobacterium tuberculosis* also inhibits that yeast ATP synthase. This is a critical finding as a similar inhibition of the human ATP synthase may be the cause of the high rate of deaths due to its usage. Indeed, we are currently studying this. **Associated pdbs: 5BPS, 5BQ6, 5BQA, 5BQJ, 4F4 S, 3U2F, 3UTUY, 3U32, 3UD0, 3ZIA.**

A. Symersky, J., Pagadala, V., Osowski, D., Krah, A., Meier, T., Faraldo-Gómez, J., and Mueller, D.M., (2012) Structure of the c_{10} Ring of the Yeast Mitochondrial ATP Synthase in the Open Conformation. *Nat. Struct. Molec. Biol.* 19: 485-491. PMCID: PMC3343227
B. Symersky, J., Osowski, D., Walters, D.E., and Mueller, D.M. (2012) Oligomycin frames a common drug-binding site in the ATP synthase. *PNAS USA*, 109: 13961-13965. PMCID: PMC3435195
C. Pagadala, V., Vistain, L., Symersky, J., and Mueller, D.M. (2011) Characterization of the mitochondrial ATP synthase from yeast *Saccharomyces cerevisiae*, *J. Bioener. Biomemb.* 43, 333-347. PMID: 21748405

3. Structure of the yeast F_1 ATPase. The recent studies were paved by my entry into protein crystallography in 2000 when I did a sabbatical in the lab of John Walker. I brought a yeast strain with a modified β-subunit and a purification scheme for the yeast F_1 ATPase, which gave high yield and high purity. In a matter of months I obtained diffracting crystals (3.0Å), a data set from 4 crystals, and the tools needed to get a get a 2.8Å data set from a single crystal upon my return to Chicago. The structure of the yeast enzyme provided

new insight into the mechanism of the ATP synthase, provided the Pi binding site, and was only bettered by the structure of the bovine F_1 ATPase. We then went on to determine the crystal structure of the yeast F_1 ATPase in the absence of nucleotides. This study provided the role of the nucleotides in determining the conformation of the active site. **Associated pdbs: 2HLD, 3FKS.**

A. Kabaleeswaran V, Puri N, Walker JE, Leslie AG, Mueller DM. (2006) Novel features of the rotary catalytic mechanism in the structure of the yeast F_1 ATPase. *EMBO J.*, 25, 5433-5442. PMCID: PMC1636620
B. Kabaleeswaran, V, Shen, H., Symersky, J., Walker, JE, Leslie, AGW, Mueller, DM, (2009) Asymmetric structure of the yeast F_1 ATPase in the absence of bound nucleotides, *J. Biol Chem.* 284: 10546-10551. PMCID: PMC2667741

4. Structural basis for mutations that uncouple the ATP synthase. My laboratory has also studied the relationship between loss of mitochondrial DNA and the uncoupling of the ATP synthase. These studies included structural studies, which investigated the structure function relationship in the ATP synthase involved in the coupling of rotation of the central stalk with the synthesis of ATP. The initial studies evolved from the identification of an epistatic effect of null mutations in the genes encoding the ATP synthase. The genetic study suggested that the ATP synthase assembled in subcomplexes, which were uncoupled due to the loss of essential subunits. This established the effect of mutations that uncouple the ATP synthase with the loss of mitochondrial DNA. We next provided biochemical support for this hypothesis and other labs have since provided independent evidence in support of these hypotheses. Next we studied a group of mutations in yeast that were related to loss of mitochondrial DNA (*mgi* mutations) and established that these mutations uncoupled the ATP synthase. Next we crystallized the F_1 ATPase from 4 of these mutants and established the structural basis for the uncoupling. We have determined that a **human disease** mutation in the α-subunit has properties similar to mutations that uncouple. Most critically, the patient likely died due to the loss of mitochondrial DNA. **Associated pdbs: 3OE7, 34OEE, 3OEH, 3OFN**.

A. Lai-Zhang, J., Xiao, Y., Mueller DM (1999) Epistatic interactions of deletion mutants in the genes encoding the F_1-ATPase in yeast *Saccharomyces cerevisiae*. *EMBO J.*: 18: 58-64. PMCID: PMC1171102
B. Xiao, Y., Metzl, M. and Mueller, D.M. (2000) Partial uncoupling of the mitochondrial membrane by a heterozygous null mutation in the gene encoding the γ- or δ-subunit of the yeast mitochondrial ATPase *J. Biol. Chem*. 275: 6963-6968. PMID:10702258
C. Wang, Y., Singh, U., and Mueller, D.M. (2007) Mitochondrial genome integrity mutations uncouple the yeast *Saccharomyces cerevisiae* ATP Synthase *J. Biol. Chem*. 282: 8228-8236. PMID:17244612
D. Arsenieva, D., Symersky, J., Wang, Y., Pagadala, V., and Mueller, D.M. (2010) Crystal Structure of mutant forms of the yeast F_1ATPase reveal two modes of uncoupling. *J. Biol. Chem*. 285: 36561-36569. PMCID: PMC2978584
E. Little, M.A, Steel, B.C., Bai, F., Sowa, Y., Bilyard, T., Mueller, D.M., Berry, R.M.Jones, N.S (2011) Steps and bumps: precision extraction of discrete states of molecular machines, *Biophysical Journal*, 101(2):477-485. PMCID: PMC3136774
F. Lieber, D.S., Calvo,S.E., Slate, N.G., Liu, S.L., Hershman, S.G., Gold, N.B., Borowsky, M.L., Thorburn, D.R, Berry, G.T., Schmahmann, J.D., Mueller, D.M., Sims, K.B., Mootha, V.K., Targeted exome sequencing of suspected mitochondrial disorders. (2013) Neurology 80:1762-1770. PMID: 23596069.

5. Structure/function of the ATP synthase. My early work investigated the structure function relationship of the yeast ATP synthase as analyzed by genetic and biochemical studies. We were one of the first labs to use site-directed mutagenesis to study the ATP synthase. These initial studies allowed us to evaluate residues in the ATP synthase that were identified via chemical modification to be critical for function. We also used a novel approach to study the amino acid requirements in the P-loop of the yeast ATP synthase. We determined the structural variations allowed in each of the 9 residues. This study was broadly important for all enzymes that bound nucleotides and had a P-loop.

A. Mueller, D.M. (1988) Arginine-328 of the β-subunit of the mitochondrial ATPase in yeast is essential for protein stability, *J. Biol. Chem*. 263, 5634-5639. PMID:2895771
B. Mueller, D.M. (1989) A mutation altering the kinetic responses of the yeast mitochondrial F_1-ATPase. *J. Biol. Chem*., 264, 16552-16556. PMID:2528546
C. Shen,H.-G. Yao, B-y. and Mueller, D.M., (1994) Primary structural requirements of the glycine-rich ATP binding motif of the yeast *S. cerevisiae* mitochondrial F_1-ATPase, *J. Biol. Chem.*

269, 9424-9428. PMID:8144526

D. Shen H, Sosa-Peinado A, Mueller DM. Intragenic suppressors of P-loop mutations in the β-subunit of the mitochondrial ATPase in the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem*. 1996; 271(20):11844-51. PMID:8662632

6. Batten disease and cystic fibrosis. My laboratory has also studied the Batten disease using yeast as a model organism and more recently, studied role of codon usage on the expression and folding of CFTR. The identification of the human gene responsible for the juvenile form of Batten disease, Cln3, did not help in understanding the cause of the disease because the function of the gene product was not, and still is not, known. The gene product has weak homology to nucleoside transporters and we believe it is a transporter. We set out to study the yeast homologue to determine the role in yeast which then would give insight to the role in human. However, knocking out the gene in yeast did not give a phenotype, and although phenotypes have since been reported, they are not robust. We moved to determine the cellular location of the gene product and determined that it was located in the lysosome. This result has since been confirmed in human cells. We have also studied CFTR and the role of codon usage in the expression and folding of CFTR. The hypothesis tested was if translation rate affected folding of CFTR. We determined profound effects on the expression of CFTR, but this was due to nonsense mediated decay of the mRNA, and not directly due to translation rate or folding. However, we were able to rescue the mutant form of CFTR, CFTR ΔF508, which is the major mutation responsible for cystic fibrosis. We hypothesize that codon usage alters the association of chaperonins with CFTR ΔF508 allowing it to escape ERAD.

A. Shah K, Cheng Y, Hahn B, Bridges R, Bradbury NA, Mueller DM. *J. Molec. Biol*. 2015; 427(6 Pt B):1464-79. PMID:25676312.

B. Croopnick JB, Choi HC, Mueller DM. The subcellular location of the yeast *Saccharomyces cerevisiae* homologue of the protein defective in the juvenile form of Batten disease. *Biochem. Biophys. Res. Comm*. 1998; 250(2):335-41. PMID: 9753630

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/41151196/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

NIH/NIGMS R01GM066223 (Mueller, PI) 2007 – 2019
Structure and function of the yeast ATPase. The major goal of this project is to determine structure/function relationship of the mitochondrial ATP synthase. A combination of genetic, biochemical, biophysical, X-ray crystallographic, and cryo-EM studies are used to probe the molecular mechanism of the ATP synthase.
Role: PI

Completed Research Support

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: **Liao, Maofu**

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

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
Tsinghua University, Beijing, China	B.S.	07/1999	Biology
Albert Einstein College of Medicine, Bronx, NY	Ph.D.	05/2006	Biochemistry and Virology
Albert Einstein College of Medicine, Bronx, NY	Postdoctoral	12/2007	Biochemistry
University of California, San Francisco	Postdoctoral	06/2014	Electron microscopy

A. Personal Statement

I have gained expertise and knowledge in a number of areas including biochemistry, virology and high-resolution electron microscopy. As a postdoctoral fellow, I acquired extensive training of using single particle cryo-electron microscopy (cryo-EM) to study the structure and function of a variety of protein complexes. My research interests focus on combining biochemical approaches and cryo-EM techniques to gain mechanistic insights into the function and regulation of protein machineries. Previously I obtained cryo-EM structures at near-atomic resolution for the TRPV1 membrane ion channel in distinct functional states, thus revealing the mechanism of channel activation. This work paved the way for determining high-resolution cryo-EM structures of membrane proteins without the need for crystallization. Since starting my own research group in July 2014, we have focused on elucidating the molecular mechanisms of dynamic membrane protein and protein-DNA/RNA complexes including RAG1-RAG2 synaptic complex, CRISPR, ERAD Hrd1-Hrd3 complex and ABC transporters. With all the experiences in related areas, I am well qualified for carrying out the proposed studies.

1. Mi W, Li Y, Yoon SH, Ernst RK, Walz T, Liao M. Structural basis of MsbA-mediated lipopolysaccharide transport. Nature. 2017 Sep 14;549:233-237.
2. Schoebel S*, Mi W*, Stein A, Ovchinnikov S, Pavlovicz R, DiMaio F, Baker D, Chambers MG, Su H, Li D, Rapoport TA, Liao M. Cryo-EM structure of the protein-conducting ERAD channel Hrd1 in complex with Hrd3. Nature. 2017 Aug 17;548:352-355.
3. Xiao Y*, Luo M*, Hayes RP, Kim J, Ng S, Ding F, Liao M[#], Ke A[#]. Structure Basis for Directional R-loop Formation and Substrate Handover Mechanisms in Type I CRISPR-Cas System. Cell. 2017 Jun 29;170(1):48-60.
4. Ru H, Chambers MG, Fu TM, Tong AB, Liao M[#], Wu H[#]. Molecular mechanism of V(D)J recombination captured by structures of RAG1-RAG2 synaptic complexes. Cell. 2015 Nov 19;163(5):1138-52. PMCID: PMC4690471
5. Liao M*, Cao E*, Julius D, Cheng Y. Structure of the TRPV1 ion channel determined by electron cryo-microscopy. Nature. 2013 Dec 5;504(7478):107-12. PMCID: PMC4078027
6. Cao E*, Liao M*, Cheng Y, Julius D. TRPV1 structures in distinct conformations reveal activation mechanisms. Nature. 2013 Dec 5;504(7478):113-8. PMCID: PMC4023639

(* and [#] denote equal contribution)

B. Positions and Honors

Positions and Employment

2014 - Assistant Professor, Harvard Medical School, Department of Cell Biology, Boston, MA

Other Experience and Professional Memberships

Honors

1999 Outstanding Graduate Award, Tsinghua University, Beijing, China
2006 Julius Marmur Research Award, Albert Einstein College of Medicine, Bronx, NY

C. Contributions to Science

1. **Molecular mechanism underlying the ABC transporter-driven lipopolysaccharide transport in Gram-negative bacteria.** Lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria is critical for membrane biosynthesis and bacterial survival, and the LPS transport pathway is of great potential to be targeted by novel antibiotics. Nascent LPS synthesized in the cytoplasmic leaflet of the inner membrane is flipped to the periplasmic leaflet by MsbA, an ATP-binding cassette transporter. Despite substantial efforts, the structural mechanisms underlying MsbA-driven LPS flipping remain elusive. We have used single particle cryo-EM to determine the structures of lipid nanodisc-embedded MsbA in three functional states. The 4.2 Å-resolution structure of the transmembrane domains of nucleotide-free MsbA reveals the long-sought-after structural basis of MsbA-LPS interactions. Two subnanometer-resolution structures of MsbA with ADP-vanadate and ADP reveal an unprecedented closed and an inward-facing conformation, respectively. Our results delineate the conformational transitions of MsbA to flip LPS, present a great opportunity for structure-guided antibiotic development targeting bacterial membrane biosynthesis, and pave the way to uncover the structural basis of many other lipid flippases.
    a. Mi W, Li Y, Yoon SH, Ernst RK, Walz T, Liao M. Structural basis of MsbA-mediated lipopolysaccharide transport. Nature. 2017 Sep 14;549:233-237.

2. **Cryo-EM structure of the ERAD Hrd1-Hrd3 protein channel.** Misfolded endoplasmic reticulum proteins are retro-translocated through the membrane into the cytosol and degraded by the proteasome, a pathway termed endoplasmic reticulum-associated protein degradation (ERAD). ERAD pathways require the ubiquitin ligase Hrd1. In collaboration with Dr. Tom Rapoport's laboratory, we determined the cryo-EM structure of S. cerevisiae Hrd1 in complex with its endoplasmic reticulum luminal binding partner, Hrd3. Hrd1 forms a dimer within the membrane with one or two Hrd3 molecules associated at its luminal side. Each Hrd1 molecule has eight trans-membrane segments, five of which form an aqueous cavity extending from the cytosol almost to the endoplasmic reticulum lumen, while a segment of the neighboring Hrd1 molecule forms a lateral seal. Our results suggest that Hrd1 forms a retro-translocation channel for the movement of misfolded polypeptides through the endoplasmic reticulum membrane.
    a. Schoebel S*, Mi W*, Stein A, Ovchinnikov S, Pavlovicz R, DiMaio F, Baker D, Chambers MG, Su H, Li D, Rapoport TA, Liao M. Cryo-EM structure of the protein-conducting ERAD channel Hrd1 in complex with Hrd3. Nature. 2017 Jul 6.

3. **Structure basis for R-loop formation and substrate recognition in type I CRISPR-Cas System.** Type I CRISPR systems feature a sequential dsDNA target searching and degradation process, by crRNA-displaying Cascade and nuclease-helicase fusion enzyme Cas3, respectively. In collaboration with Dr. Ailong Ke's laboratory at Cornell University, we have generated a set of cryo-EM snapshots of the type I-E Cascade bound with different DNA substrates. These structures provide the much-needed temporal and spatial resolution to resolve key mechanistic steps leading to Cas3 recruitment. In the early steps, PAM recognition causes severe DNA bending, leading to spontaneous DNA unwinding to form a seed-bubble. The full R-loop formation triggers conformational changes in Cascade, licensing Cas3 to bind. The same process also generates a bulge in the non-target DNA strand, enabling its handover to Cas3 for cleavage.

The combination of both negative and positive checkpoints ensures stringent yet efficient target degradation in type I CRISPR-Cas systems.

a. Xiao Y*, Luo M*, Hayes RP, Kim J, Ng S, Ding F, Liao M#, Ke A#. Structure Basis for Directional R-loop Formation and Substrate Handover Mechanisms in Type I CRISPR-Cas System. Cell. 2017 Jun 29;170(1):48-60.

4. **Cryo-EM structures of RAG1-RAG2 synaptic complexes in V(D)J recombination.** Diverse repertoires of antigen-receptor genes that result from combinatorial splicing of V, D and J gene segments are hallmarks of vertebrate immunity. The $(RAG1\text{-}RAG2)_2$ recombinase precisely recognizes and cleaves two different recombination signal sequences (12-RSS and 23-RSS), and forms synaptic complexes only with one 12-RSS and one 23-RSS, a dogma known as the 12/23 rule that governs the recombination fidelity. In collaboration with Dr. Hao Wu's laboratory at Boston Children's Hospital, we have used single-particle cryo-EM to determine the structures of synaptic RAG1-RAG2 complexes at up to 3.4 Å resolution. These structures elaborate the molecular mechanisms for DNA recognition, catalysis and the unique synapsis underlying the 12/23 rule, provide new insights into the RAG-associated human diseases, and represent a most complete set of complexes in the catalytic pathways of any DDE family recombinases, transposases or integrases.

   a. Ru H, Chambers MG, Fu TM, Tong AB, Liao M#, Wu H#. Molecular mechanism of V(D)J recombination captured by structures of RAG1-RAG2 synaptic complexes. Cell. 2015 Nov 19;163(5):1138-52. PubMed PMID: 26548953. PMCID: PMC4690471

5. **Cryo-EM structures and activation mechanism of the TRPV1 ion channel.** The vast majority of membrane protein structures have been determined by X-ray or electron crystallography, necessitating formation of three-dimensional (3D) or two-dimensional (2D) crystals. In either case, obtaining well-diffracting crystals is absolutely necessary and remains a major roadblock to success. Single particle cryo-EM circumvents the requirement of well-ordered crystals for structure determination and therefore represents a transformative approach for studying membrane proteins. Taking advantage of very recent technological breakthroughs, such as direct electron detection camera, dose fractionation imaging, correction of motion-induced image blurring, and the maximum-likelihood algorithm to sort out heterogeneous protein conformations, I determined the near-atomic resolution cryo-EM structures for the TRPV1 ion channel. The structures of TRPV1 in three distinct conformations revealed the mechanisms of ligand-induced channel activation. These studies showcase the power of single-particle cryo-EM for achieving high resolution structures of integral membrane proteins in multiple function states.

   a. Liao M*, Cao E*, Julius D, Cheng Y. Structure of the TRPV1 ion channel determined by electron cryo-microscopy. Nature. 2013 Dec 5;504(7478):107-12. PubMed PMID: 24305160. PMCID: PMC4078027
   b. Cao E*, Liao M*, Cheng Y, Julius D. TRPV1 structures in distinct conformations reveal activation mechanisms. Nature. 2013 Dec 5;504(7478):113-8. PMCID: PMC4023639

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D. Additional Information: Research Support and/or Scholastic Performance

RESEARCH SUPPORT

Ongoing Research Support

R01GM066223 Mueller (PI) 07/2016 - 06/2019
"Structure and function of the ATP synthase"
Study the structure and function of the ATP synthase in its functional and inhibited states.
Role: Co-investigator

R01AI125535 Wu (PI) 07/2016 - 06/2021
“Recombination activating gene (RAG) in V(D)J recombination and immunodeficiency”
Reveal the functions and regulatory mechanisms of the RAG complex.
Role: Collaborator

R35GM118174 Ke(PI) 07/2016 - 06/2018
“Super-resolution EM snapshots of Type I CRISPR interference”
Cryo-EM training to solve high resolution structures of the CRISPR Cascade complex.
Role: Collaborator

EMD Serono, Inc. Award A29868 Liao (PI) 10/2016 - 10/2019
“Study pharmaceutical important protein targets using single particle cryo-electron microscopy”
Structurally characterize the protein complexes of pharmaceutical importance.
Role: PI

Smith Family Foundation Liao (PI) 02/2017 - 01/2020
“Cryo-EM studies of multidrug resistance conferred by human ABC transporters”
Study the structural basis of human ABC transporter-mediated multidrug export.
Role: PI

The Broad Institute Liao (PI) 04/2017 - 01/2019
“Structural Biology of $Ca_V3.3$ using Cryo-EM”
Use single particle cryo-EM to study the structure and function of $Ca_V3.3$.
Role: PI

R01GM122797 Liao (PI) 08/2017 - 04/2022
“Molecular mechanisms of lipopolysaccharide transport driven by ABC transporters”
Gain structural and functional insights into the mechanisms of the lipopolysaccharide transport mediated by the MsbA and Lpt proteins.
Role: PI

The Broad Institute Liao (PI) 10/2017 - 09/2019
“Structural Biology of $Na_V1.2$ using Cryo-EM”
Use single particle cryo-EM to study the structure and function of $Ca_V3.3$.
Role: PI

Completed Research Support

Biogen Idec, Award A20335 Liao (PI) 07/01/16 - 06/30/17
“Structural studies on tenase complexes”
Use cryo-EM to determine the structures of both extrinsic and intrinsic Xase complexes assembled on the surface of phospholipid nanodiscs in both the presence and absence of the substrate, FX.
Role: PI