BIOGRAPHICAL SKETCH

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NAME: Evan Michael Billings

eRA COMMONS USERNAME (credential, e.g., agency login): EBILLIN

POSITION TITLE: Postdoctoral Research Scientist

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
Purdue University	B.S.	05/2018	Biochemistry, minors in biology and philosophy
Purdue University	PhD	05/2024	Membrane protein structural biology
Columbia University Irving Medical Campus	Postdoctoral		Membrane enzyme biochemistry & structural biology

A. Personal Statement

I am currently a Postdoctoral Research Scientist at Columbia University Irving Medical Campus. Since the beginning of my scientific training, I have developed a strong interest in the biochemistry and structural biology of membrane protein transporters and enzymes related to human health. As a graduate student in the lab of Dr. Nicholas Noinaj at Purdue University, I investigated, at a molecular level, membrane protein machinery complexes involved in the biogenesis of β barrel membrane proteins in Gram-negative bacteria. I studied the β barrel assembly machinery (BAM) complex, which folds and inserts β barrel membrane proteins into the outer membrane of Gram-negative bacteria. My time in the lab gave me extensive training and exposure to structural biology techniques and various biophysical methods. Using single particle cryo-electron microscopy (cryo-EM), I determined the structure of BAM from the human pathogen *Neisseria gonorrhoeae* (*Ng*BAM), which provided valuable insight into its protein folding mechanism. Additionally, I also characterized a novel antibiotic natural product, darobactin, which functions as a BAM inhibitor. I determined the mode of interaction between darobactin and its higher potency derivatives by determining inhibitor-bound cryo-EM structures of *Ng*BAM. I also complemented this work by performing DEER spectroscopy to characterize the conformational changes *Ng*BAM undergoes upon darobactin binding. As a graduate student, I was heavily involved in several grant and fellowship applications both on my own projects and others in the lab. I was fortunate to have this research funded by a predoctoral fellowship through the American Heart Association, which I independently wrote and applied for. Additionally, I wrote several smaller pilot grants, most if not all of which were successful.

Wanting to increase my experience in membrane protein biochemistry, I joined the lab of Dr. Filippo Mancia at Columbia University in 2024 to continue my scientific training as a postdoctoral researcher. I am now studying the glycosyl-transferase enzymes responsible for building the unique cell wall in *Mycobacteria tuberculosis*. Here, I am working to purify the endogenous enzymes and map their native interactome using genetic, biochemical, and structural techniques. This project is a great avenue for me to apply my previous experience working with bacterial membrane proteins and contribute to the exciting field of structural biology performed under endogenous, physiological conditions. I am also working to apply for and secure my own fellowship funding to support this work. My long term goal is to obtain a scientist position in industry or a public or private research institution and use my experience to bring together structural biology, *in vivo* techniques, and biophysics to better understand membrane proteins related to human health and disease.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2024-pres Postdoctoral Research Scientist, Columbia University Irving Medical Campus, Department of Physiology and Cellular Biophysics
2018-2024 Graduate student, Purdue University, Department of Biological Sciences

Academic and Professional Honors

2022-2024 American Heart Association Predoctoral Fellow
2023 ACA Linus Pauling Poster Prize
2021 2022 BPS Annual Meeting Travel Award
2020 College of Science International Travel Award
2019-2021 NIH T32 Molecular Biophysics Training Program Trainee
2017 College of Agriculture Undergraduate Research Scholarship

Professional Memberships

2022-2024 Member, American Heart Association
2020-pres Member, Biophysical Society
2019-pres Member, American Crystallographic Association
2018-pres Member, certified with distinction, American Society for Biochemistry and Molecular Biology

Meetings and Conferences

2023 29th Midwest Microbial Pathogenesis Conference, Chicago, Illinois
2023 73rd American Crystallographic Association Annual Meeting, Baltimore, Maryland
2022 Hitchhikers Guide to the Biomolecular Galaxy Symposium, Purdue University
2022 66th Biophysical Society Annual Meeting, San Francisco, California
2021 Hitchhikers Guide to the Biomolecular Galaxy Symposium, Purdue University
2021 American Crystallographic Association Annual Meeting, attended virtually
2021 ASBMB PDB50 Symposium, attended virtually
2021 Experimental Biology Annual Meeting, attended virtually
2021 S^2C^2 Cryo-EM Specimen Preparation and Data Collection Workshop, attended virtually
2021 65th Biophysical Society Annual Meeting, attended virtually
2020 6th MicroED Imaging Center Course, attended virtually due to COVID-19
2020 American Crystallographic Association Annual Meeting, attended virtually due to COVID-19
2020 S^2C^2 Image Processing Workshop, virtually hosted by SLAC due to COVID-19
2019 Everything BioSAXS V Workshop, APS, Argonne National Lab, Lemont, Illinois
2019 American Crystallographic Association Annual Meeting, Cincinnati, Ohio
2019 Hitchhikers Guide to the Biomolecular Galaxy Symposium, Purdue University
2018 Purdue Cryo-EM Symposium, Purdue University
2018 Hitchhikers Guide to the Biomolecular Galaxy Symposium, Purdue University
2017 Hitchhikers Guide to the Biomolecular Galaxy Symposium, Purdue University

C. Contributions to Science

1. During my time as a graduate student in the lab of Dr. Noinaj, I studied the β barrel assembly machinery (BAM) complex, a 200 kDa protein assembly in the outer membrane of Gram-negative bacteria that is responsible for the biogenesis of outer membrane proteins. In *E. coli*, this machine consists of 5 component proteins, a β barrel insertase BamA, and 4 accessory lipoproteins, BamB, C, D, and E. My research focused on the BAM complex from *Neisseria gonorrhoeae* (*Ng*BAM), a pathogen rapidly showing signs of antibiotic resistance. Interestingly, this complex only consists of 4 component proteins, as there is no Neisserial homolog of BamB. In order to understand the mechanism by which *Ng*BAM may function, I determined the high-resolution structure of the intact *Ng*BamACDE complex using cryo-EM. This revealed architectural distinctions between *Ng*BAM and the previously characterized complex from *E. coli*, particularly in the orientation of *Ng*BamC and the conformational state of *Ng*BamA. Using my work, we were able to then perform molecular dynamics simulations with our collaborator, showing how

the BAM proteins may act to regulate the dynamics of the *Ng*BamA barrel domain. This project allowed us to build a model of how *Ng*BAM folds its outer membrane proteins and allowed me an avenue to receive extensive training in membrane protein structural biology.

- **a.** Billings, E. Fan, Z., Sooreshjani, M. Gumbart, JC, and Noinaj, N. (2025). Structural Insights into outer membrane protein biogenesis in pathogenic *Neisseria*. *Structure.* In review.
- **b.** Overly Cottom, C. Billings, E. Bush, M. Drozario, D. Scherschel, W. Noinaj. N. (2025) Surface protein machineries in gram-negative bacteria. *Journal of Cell Science.* In review.
- **c.** Billings, E., Lundquist, K., Overly, C., Srinivasan, K., and Noinaj, N. (2021). Structural Determination of Membrane Proteins Using X-Ray Crystallography. *Methods in Molecular Biology*. 2302, 101-136. DOI: 10.1007/978-1-0716-1394-8_7
- **d.** Lundquist, K. Billings, E, Bi, M., Wellnitz, J., and Noinaj, N. (2021). The assembly of β-barrel membrane proteins by BAM and SAM. *Molecular Microbiology*, 115,(3). 425-435. DOI: 10.1111/mmi.14666

2. In addition to my work studying *Ng*BAM, towards the second half of my graduate training, I became involved in a collaborative project studying darobactin, a novel natural product that was shown to inhibit BAM. Our lab previously contributed to work initially characterizing darobactins antibiotic activity and inhibition mechanism in *E. coli*, and we wanted to expand this work into other bacterial pathogens. Using cryo-EM, I determined high resolution structures of *Ng*BAM in complex with darobactin and its higher potency chemical derivatives. These structures gave insight into darobactin's recognition mechanism and provided a foundation for designing *Neisseria* specific darobactin compounds. Surprisingly, our structural studies also indicated that upon binding darobactin, *Ng*BAM undergoes significant conformational changes. To better understand the conformational dynamics of the apo and darobactin bound states, I performed DEER spectroscopy on the complex and observed that darobactin enhanced the conformational rigidity of the complex. Finally, this work was complemented with *in vivo* studies of the efficacy of these darobactin compounds in *N. gonorrohoeae,* performed by our collaborators.

- **a.** Billings, E., Wolske, N., Seyfert, C., Stein, R., Mchaourab, H., Sikora, A., Mueller, R., and Noinaj, N. (2025). An improved class of antibiotics against pathogenic *Neisseria*. In prep.

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: Ryan Timothy Morgan

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

POSITION TITLE: Predoctoral candidate

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of South Florida	B.S.	08/2019	05/2022	Cell and Molecular Biology
Columbia University	M.A.	08/2022	05/2024	Cellular Molecular & Biomedical Sciences
Columbia University	Ph.D.	08/2022	05/2027	Cellular Molecular & Biomedical Sciences

A. Personal Statement

I first became involved in biomedical research when I started college in 2019, joining Dr. Richard Pollenz's lab at the University of South Florida (USF) to study the molecular determinants of bacteriophage virulence. Dr. Pollenz, a renowned researcher in molecular biology and molecular toxicology, led the joint HHMI-USF SEA-PHAGES program, where undergraduate students identify, analyze, and catalog bacteriophages. Working in Dr. Pollenz's lab, I gained hands-on experience in microbiology techniques and bioinformatics, learning how to sequence and purify bacteriophage particles as well as TEM grid preparation. This experience laid the foundation for much of my scientific career thus far as I learned how to balance a team and independent environment. In my sophomore year, I joined Dr. Yu Chen's lab at the USF Morsani College of Medicine, utilizing structural biology methods for the first time, a field I would later base my PhD training on. Dr. Chen's research focuses on understanding the structural mechanisms of bacterial antibiotic resistance, particularly in the formation of lipopolysaccharide molecules which decorate the outer membrane of Gram-negative bacteria and serve as a major impediment to the design of novel antibiotic therapies. This transition to structural biology felt like a natural progression from my initial work with bacteriophages, as it allowed me to explore the molecular structures that underpin biological functions. My experience in Dr. Chen's lab provided me with a deeper understanding of how structural insights can drive biological discoveries. My initial interest in structural biology eventually evolved into a more specific interest in membrane protein biology, as most cellular processes are influenced in some way by membrane-dependent pathways. With this in mind, I chose to complete my second rotation of my Ph.D. program at Columbia University in the lab of Dr. Filippo Mancia, who uses structural biology techniques to study the molecular mechanisms of membrane proteins involved in drug resistance, ion transport, and membrane biosynthesis. In particular, I was very interested in beginning to learn methods related to cryogenic electron microscopy (cryo-EM). During my rotation, I had excellent training in membrane protein expression and purification, including the reconstitution of these proteins into lipid-filled nanodiscs to mimic their native lipid environment, as well as bacterial and mammalian cell culture. Since joining the Mancia lab, I have taken on two projects – endogenous-based purification for structural determination and a time-resolved cryo-EM study of the membrane glycosyltransferase GtrB.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2023 – Present	*Student Recruiting Mentor,* Integrated PhD program at Columbia
2022 – Present	*Graduate Research Assistant*, Columbia University
2021 – 2022	*Research Assistant*, University of South Florida (USF) College of Medicine
2020 – 2021	*Teaching Assistant,* Department of Cell and Molecular Biology, USF
2019 – 2021	*USF-HHMI STEM Academy Scholar*, USF

Honors

2024	Physiology Distinguished Research Award
2024	Poster Presenter, Annual NYC Cryo-EM Meeting
2024	Oral Presenter, Physiology Department Retreat
2022	B.S. awarded with *Summa Cum Laude*, University of South Florida
2022	King O' Neal Scholar Award, USF
2019	USF Scholars Gold Award

C. Contributions to Science

1. **Undergraduate Research:**

Prior to my first semester at the University of South Florida (USF), I applied and was accepted into a Microbiology Research Program hosted by USF and the Howard Hughes Medical Institute called SEA-PHAGES. My role as a researcher part of Dr. Richard Pollenz's SEA-PHAGES group was to isolate, characterize, and sequence bacteriophages collected from the environment. During my time in Dr. Pollenz group, I sequenced and functionally characterized 7 bacteriophages leading to the discovery of a novel "lytic island" of genes in a diverse set of phages. This work ultimately resulted in several poster presentations, one of which was selected for the HHMI-SEA Symposium held in Washington D.C. (moved to virtual format) and two minor publications. Transitioning from the Pollenz group, I joined Dr. Yu Chen's group in 2021 at the Morsani College of Medicine. Here, I used x-ray crystallography to structurally characterize soluble proteins, in particular two enzymes relevant for antibiotic drug design. I worked closely with an Associate Researcher in the lab, Dr. Eric Lewandowski, to crystallize and structurally solve the main protease from Covid-19 variants resistant to Nirmatrelvir, an orally-administered Covid treatment. I determined the structures of 11 mutant Mpro variants and using a novel yeast-based assay functionally characterized their effects on activity. This work led to two publications high-impact publications for the structural determination, (Hu et al. ACS Cent. Sci., 2023), and functional assays, (Ou el al. PLoS Path, 2023). I simultaneously worked with a graduate student in the lab, Dr. Michael Sacco, to functionally determine the metallo-selectivity of penicillin-binding proteins (PBPs) from *C. diff.* This work led to a publication in Nature Communications (Sacco et al Nature Comm., 2022).

Publications:

1. Ou J, Lewandowski E, Hu Y, Lipinski A, **Morgan R**, Jacobs L, Zhang X, Bikowitz M, Langlais P, Tan H, Wang J, Chen Y, Choy J. A yeast-based system to study SARS-CoV-2 Mpro structure and to identify nirmatrelvir resistant mutations. PLoS Pathogens. 2023 Aug 31.

2. Hu Y, Lewandowski E, Tan H, Zhang X, **Morgan R**, Zhang X, Jacobs L, Butler S, Gongora M, Choy J, Deng X, Chen Y, Wang J. Naturally occurring mutations of SARS-CoV-2 main protease confer drug resistance to nirmatrelvir. ACS Cent. Sci. 2023 Jul 24.

3. Sacco M, Wang S, Adapa S, Zhang X, Lewandowski E, Gongora M, Keramisanou D, Atlas Z, Townsend J, Gatdula J, **Morgan R**, Hammond L, Marty M, Wang J, Eswara PJ, Gelis I, Jiang R, Sun X, Chen Y. A unique class of Zn2+-binding serine-based PBPs underlies cephalosporin resistance and sporogenesis in *Clostridioides difficile*. Nat. Commun. 2022 Jul 28.

Presentations:

1. **Morgan R.**, Pollenz R. Functional Analysis of Gene 89 in *Mycobacterium* phage Girr through Cytotoxicity and Immunity Assays. Poster presented at 13th HHMI-SEA Symposium, April 2021.

2. Graduate Research:

As a member of Dr. Filippo Mancia's lab at Columbia University, I have been working to establish two platforms to study the physiological structures and dynamics of membranes proteins – endogenous-based purification and time-resolved cryo-EM. By using the Lambda Red homologous recombination system in bacteria, I developed a protocol to insert high-affinity tags into the genomic sequence of target proteins intended for *in vivo* cross-linking and cryo-EM analysis. Using membrane-permeable cross-linkers, such as SDA and DSSO, we aim to retain endogenous protein complexes before pulling-down and enriching for mass spec analysis. I've applied this method to the *E. coli* O-antigen ligase WaaL which our lab recently structurally determined from *Cupriavidus metallidurans.* We hypothesize that proteins involved in O-antigen biosynthesis, lipid A import, and LPS export associate directly with WaaL to form a coordinated supercomplex, named the Lipolysaccharide Assembly Complex (LAC), at the bacterial periplasm that mediates rapid modification and transport of LPS to the outer membrane. In addition to working on the WaaL ligase, I have spent considerable time working to develop and perform a novel time-resolved cryo-EM method that combines caged chemical derivatives with a photoactivable Vitrobot system. I've focused my efforts on a bacterial polyprenyl-glycosyltransferase called GtrB to study the fundamental processes of glycosylation in the cell and as a model system for DPM1-related congenital disorders of glycosylation. I've made significant progress on this project, obtaining several high-resolution structures of catalytic intermediates of GtrB as well as mutant expression to study the mechanism of glycolipid biosynthesis culminating in a first-author paper recently at review in *Nature Communications*. Additionally, a fellow graduate student in the lab and I have prepared a review encompassing the biology and structure of the diverse family of polyprenyl-glycosyltransferases which was recently published in *Structure*.

Publications:

1. **Morgan R**, Motta S, Gil-Iturbe E, Bhattacharjee B, Anwar MT, di Muccio G, Romagnoli A, Mishra B, Ashraf KU, Bang I, di Marino D, Lowary TL, Quick M, Petrou VI, Stowell MHB, Nygaard R, Mancia F. Mechanistic snapshots of lipid-linked sugar transfer. Nat. Commun. In review. 2025
2. **Morgan R***, Zinkle A*, Nygaard R, Mancia F. Structural insights into phosphopolyisoprenyl-binding glycosyltransferases. Structure. 2025 Apr 3

Presentations:

1. **Morgan R.** Time-Resolved cryo-EM of Lipid-Linked Sugar Transfer. Presented at the Biophysical Society Annual Meeting in Los Angeles CA, Feb 2025.
2. **Morgan R.** Time-resolved cryo-EM of the membrane glycosyltransferase GtrB. Presented at the Annual Physiology Retreat in New Paltz, NY, May 2024.
3. **Morgan R.**, Motta S., Romagnoli A., Bhattacharjee B., Mishra B., Anwar M., Bang I., Petrou V., Quick., Stansfeld P., Lowary T., Stowell., Nygaard R., and Mancia F. Time-resolved cryo-EM of the membrane glycosyltransferase GtrB. Poster presented at the NY Area NYC Cryo-EM Meeting in New York, NY, June 2024.

* Authors contributed equally

BIOGRAPHICAL SKETCH

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NAME: Mancia, Filippo

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

POSITION TITLE: 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
Università di Pavia, Pavia, Italy	M.Sc.	03/1992	Chemistry
MRC Laboratory of Molecular Biology & University of Cambridge, Cambridge, England	Ph.D.	12/1996	Biology (adviser Dr. Philip R. Evans, FRS)
Columbia University Medical Center (CUIMC), New York, NY	Postdoctoral	12/2000	Struct Biol (adviser Dr. Wayne Hendrickson)

A. Personal Statement

I am a membrane protein biochemist and structural biologist with experience in x-ray crystallography and in single particle cryo-electron microscopy (cryo-EM), and in production and characterization of membrane proteins for both structural and functional studies. We use a structure-based integrated approach to investigate the molecular details of biological processes that occur at the cell membrane and we pioneered the development of structural genomics methods to achieve these goals. My labs main research interests are (a) to understand how the membrane bilayer and specific membrane enzymes and transporters, most often nutrients, interact to accommodate lipidic substrates and their constituents. To cite two examples, we have solved the structure of STRA6, the cell surface receptor for retinol-binding protein (RBP) bound vitamin A (in the form of retinol) allowing us to investigate the mechanism of transport of this essential nutrient across the membrane; we have integrated structural biology, functional assays, native mass spectrometry and molecular dynamics simulations to investigate how omega-3 fatty acids are transported specifically across the blood-brain barrier by MFSD2A. (b) To understand the molecular bases of drug resistance. Again, to cite two examples, we have determined the structure and probed the mechanism of aminoarabinose transferase (ArnT), the enzyme that mediates the resistance to polymyxins, last resort antibiotics. We have also determined the structure of the chloroquine resistance transporter from *Plasmodium falciparum* (PfCRT), combining cryo-EM, biochemistry, genetics and parasitology to start to unveil the molecular basis of resistance to the common antimalarials of the 4-aminoquinoline family (chloroquine and piperaquine).

In respect to my services to the scientific community, I have been a key member of the NIH Protein Structure Initiative-funded New York Consortium of Membrane Protein Structure (NYCOMPS), then the NIH-P41 funded Center on Membrane Protein Production and Analysis (COMPPÅ). I have also been the lead organizer of the two COMPPÅ Membrane Protein Production and Analysis Symposia (2018 and 2022), highlighting the recent developments in membrane protein molecular-level research, and each bringing together over 250 participants from all over the world in a 3-day format gathering. Furthermore, I am a grant reviewer for many European entities, and was also a permanent member and Chair of the Biochemistry and Biophysics of Membranes (BBM) NIH Study Section, a key grant review structure for the membrane biophysics community. Finally, I was recently elected to Chair-Elect of the Channels, Receptors & Transporters Subgroup of the Biophysical Society.

Ongoing projects that I would like to highlight include:

R35GM132120, NIH/NIGMS (PI Mancia, F.) 05/01/2019 – 04/30/2029
"*Structural basis of integral membrane enzyme function*"

The goal of this project is to determine the basic principles that govern how lipidic substrates are recognized and processed my membrane enzymes.

R01AI147628, NIH/NIAID (MPI; Mancia, F., Fidock, D. & Quick, M.) 07/01/2019 – 06/30/2029
"Leveraging PfCRT structure to discern function and predict emergence of drug-resistant Malaria"
The goal of this proposal is to combine structural, biochemical and genetic experiments to understand the molecular basis for resistance to anti-malarial drugs mediated by the membrane transporter pfCRT.

R01CA275005 (MPI; Mancia, F. & Virshup, D.) 09/18/2023 – 08/31/2028
"*Molecular Mechanisms of Wnt Transport*"
The goal of this proposal is to provide a molecular-level understanding of how transport of Wnts by their sole specific carrier Wntlss (WLS) occurs.

R01EY027405, NIH/NEI (MPI; Mancia, F. & Khelashvili, G.) 04/01/2017 – 04/30/2028
"*Structural basis of receptor-mediated cellular vitamin A uptake*"
Our objective is to investigate at the molecular level the relationship between the structure and the function of STRA6 as it may point at novel targets for therapeutic approaches in treatment of developmentally-related pathologies and diseases of the visual system.

Citations:

a. Chen, Y., Clarke, O.B., Kim, J., Stowe, S., Kim, Y.K., Assur, Z., Cavalier, M., Godoy-Ruiz, R., von Alpen, D.C. Manzini, C. Blaner, W.S., Frank, J., Quadro, L., Weber, D.J., Shapiro, L., Hendrickson, W.A. and **Mancia, F.** (2016). Structure of the STRA6 receptor for retinol uptake. *Science,* doi: 10.1126/science.aad8266. PMCID: PMC5114850.

b. Cater, R.J., Chua, G.L., Erramilli S.K., Keener, J.E., Choy, B.C., Tokarz, P., Chin, C.F., Quek, D.Q.Y., Kloss, B., Pepe, J.G., Parisi G., Kossiakoff A.A., Khelashvili, G., Silver, D. and **Mancia, F.** (2021). Structural basis of omega-3 fatty acid transport across the blood-brain barrier. *Nature*, doi: 10.1038/s41586-021-03650-9. PMCID: PMC8266758.

c. Petrou, V.I., Herrera, C.M., Schultz, K.M., Clarke, O.B., Vendome, J., Tomasek, D., Banerjee, S., Rajashankar, K.R., Belcher Dufrisne, M., Kloss, B., Kloppmann, E., Rost, B., Klug, C.S., Trent, M.S., Shapiro, L. and **Mancia, F.** (2016). Structures of aminoarabinose transferase ArnT suggest a molecular basis for resistance to polymyxins. *Science*, doi: 10.1126/science.aad1172. PMCID: PMC4963604.

d. Kim, J., Tan, Y.Z., Wicht, K.J., Erramilli, S.K., Dhingra, S.K., Okombo, J., Vendome, J., Hagenah, L.M., Giacometti, S.I., Warren, A.L., Nosol, K., Roepe, P.D., Potter, C.S., Carragher, B., Kossiakoff, A.A., Quick, M., Fidock, D.A. and **Mancia, F.** (2019). Structure and drug resistance of the *Plasmodium falciparum* transporter PfCRT. *Nature*, doi: 10.1038/s41586-019-1795-x. PMCID: PMC6911266.

B. Positions, Scientific Appointments and Honors

Positions and Employment

2022- Vice Chair, Dept of Physiology & Cellular Biophysics, CUIMC, New York
2021- Professor, Dept of Physiology & Cellular Biophysics, CUIMC, New York
2018- Co-Director, Graduate Program in Physiology, Columbia University, New York
2017 – 2021 Associate Professor, Dept of Physiology & Cellular Biophysics, CUIMC, New York
2009 – 2017 Assistant Professor, Dept of Physiology & Cellular Biophysics, CUIMC, New York
2003 – 2009 Assoc Res Scientist, Dept of Biochem & Molecular Biophysics, CUIMC, New York
2000 – 2003 Associate, Howard Hughes Medical Institute, CUIMC, New York

Honors / Other Experiences / Professional Memberships

2025 Chair-Elect, Channels, Receptors & Transporters Subgroup, Biophysical Society
2024- Foreign Member, Academy of Sciences of Lisbon, Portugal
2024- Chair, Standing Independent Evaluation Committee, Human Technopole, Milano, Italy
2023- Chair, CUIMC Scientific Research Advisory Committee (reports to the Dean)
2023 Visiting Professor, Università del Piemonte Orientale, Novara, Italy
2021 – 2023 Chair, Biochemistry and Biophysics of Membranes (BBM) Study Section, NIH
2019 Visiting Professor in Biochemistry, Università La Sapienza, Rome, Italy
2018 Structural Biology Lecturer, University of Warwick, Coventry, UK
2019 – 2022 Permanent Member, Biochemistry and Biophysics of Membranes (BBM) Study Section, NIH
2017 Visiting Professor, Università Politecnica delle Marche, Ancona, Italy

2017 Clyde and Helen Wu Assistant Professor of Physiology and Cellular Biophysics, CUIMC
2016 Visiting Professor in Biochemistry, Università La Sapienza, Rome, Italy
2016 Schaefer Research Scholar
1997 Human Science Frontier Program Organization (HSFPO) long-term fellowship
1996 European Molecular Biology Organization (EMBO) long-term fellowship
1995 Max Perutz student prize for the work as a graduate student.

C. Contributions to Science

1. Structural genomics of membrane proteins and CysZ-mediated sulfate uptake. I have developed methods for production of membrane proteins and complexes, for productions of reagents to aid in structural biology of membrane proteins, and for high-throughput screening of prokaryotic and eukaryotic membrane proteins to identify well-expressed, detergent-stable candidates for in-depth structural investigation. The development of methodology and technology in my lab and in collaboration with NYCOMPS and COMPPÅ has resulted in substantial contributions to the membrane protein field, leading to the solution of numerous problems, which could not have been otherwise approached. Our association with NYCOMPS has also led us to make use of a structural genomics approach to identify membrane proteins suitable for in-depth structural investigation. This has revealed many new and unexpected structures, yielding surprises and shedding light on the mechanistic details of several important biological processes. One such example from my lab is the transporter for sulfate CysZ, in which three structures of different orthologs have revealed an unprecedented fold comprising four TM helices of an inverted transmembrane topology dimer, arranged in a hexamer. We were able to study the function of these CysZ proteins combining multiple assays, shedding light on this novel transmembrane transport system for sulfate.

a. Assur-Sanghai, Z., Liu, Q., Clarke, O.B., Belcher-Dufrisne, M., Wiriyasermkul, P., Giese, M.H., Leal Pinto, E., Kloss, B., Tabuso, S., Love, J., Punta, M., Banerjee, S., Rajashankar, K.R., Rost, B., Logothetis, D., Quick, M., Hendrickson, W.A. and **Mancia, F.** (2018). Structure-based analysis of CysZ-mediated cellular uptake of sulfate. *Elife*, doi: 10.7554/eLife.27829. PMCID: PMC5967866.

b. Assur, Z., Hendrickson, W.A. and **Mancia, F**. (2012). Tools for Co-producing Multiple Proteins in Mammalian Cells. *Methods in Molecular Biology*, doi: 10.1007/978-1-61779-352-3_12. PMCID: PMC3773504.

c. **Mancia, F**. and Love, J. (2010). High-throughput expression and purification of membrane proteins. *J. Struct. Biol.*, doi: 10.1016/j.jsb.2010.03.021. PMCID: PMC2933282.

d. Love, J., **Mancia, F**., Shapiro, L., Punta, M., Rost, B., Girvin, M., Wang, D.N., Zhou, M., Hunt, J.F., Szyperski, T., Gouaux, E., MacKinnon, R., McDermott, A., Honig, B., Inouye, M., Montelione, G. and Hendrickson, W.A. (2010). The New York Consortium on Membrane Protein Structure (NYCOMPS): a high-throughput platform for structural genomics of integral membrane proteins. *J Struct Funct Genom*, doi: 10.1007/s10969-010-9094-7. PMCID: PMC3099345.

2. Structure and function of integral membrane lipid-modifying enzymes. Cellular membranes are critical components of all free-living organisms. However, knowledge of their biosynthesis and modification has been hindered by the hydrophobicity engendered by their lipid constituents. To shed light on this question, we are studying the structure and function of distinct families of integral membrane lipid-modifying enzymes. We have focused our attention on glycosyltransferases that use sugar-charged polyprenyl (PP) donors to assemble or modify the lipopolysaccharide (LPS) or peptidoglycan (PG) layers of Gram-negative bacteria. For example, we have employed an integrated structural biology approach to show how the last step in the assembly of LPS is catalyzed by the O-antigen ligase, which transfers the PP-linked O-antigen onto the LPS Lipid A core. We have then shown how LPS is modified by ArnT, which transfers an aminoarabinose from a PP donor to a phosphate of Lpid A. Furthermore, the enzyme RodA, takes a PP-linked glycan termed Lipid II to assemble the bacterial PG layer, which is then crosslinked via a transpeptidase reaction. We determined the structure of RodA in complex with the transpeptidase PBP2 and combined this information with biochemical, genetic, spectroscopic, and computational analyses to propose a mechanism for Lipid II polymerization. Finally, we have determined the cryo-EM structures of arabinofuranosyltransferases B (AftB), D (AftD) and EmbB, representative enzymes that transfer a PP-linked sugar (arabinofuranose) to the nascent glyco-lipid mesh that constitutes the impermeable cell wall of mycobacteria. We have combined structural information, with biochemical assays and genetics to begin to understand how these enzymes function and are regulated.

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3. Cellular uptake of Vitamin A. Vitamin A is an essential nutrient for all mammals. Many biological processes, including and foremost vision, are crucially dependent on its adequate supply for proper function. Alterations of vitamin A metabolism can result in a wide spectrum of ocular defects and lead to blindness. Retinol (vitamin A alcohol) is the predominant circulating vitamin A form in the fasting state. In times of need (i.e. in the absence of dietary vitamin A intake), in order to distribute vitamin A to the target peripheral tissues, retinol is released in the bloodstream from the liver, the main body storage site of the vitamin, bound to retinol- binding protein (RBP). Inside the cells, retinol binds specific intracellular carriers, namely cellular retinol-binding proteins, and it serves as a precursor for the active vitamin A forms: retinaldehyde, critical for vision, and retinoic acid, the ligand for specific nuclear receptors that regulate the transcription of hundreds of target genes. How retinol is released from the retinol-RBP complex and internalized by the cell has been subject of debate for decades. STRA6, the putative plasma membrane receptor for RBP, was identified in 2007. However, its mechanism of action has remained elusive, not least due to the absence of any structural information. We have determined the structure of STRA6 determined to 3.9 Å resolution by single-particle cryo-electron microscopy (improved to 3.1 Å resolution with protein reconstituted in nanodisc). The atomic model of STRA6 provides a template to guide our understanding at a molecular level on how this protein may function.

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4. Molecular mechanisms of drug resistance. Drug resistance in *Plasmodium falciparum* (Pf), the deadliest of the malaria parasites that threatens almost half the world's population, has been associated with mutations in specific genes. The protein responsible for parasite resistance to both previously and currently used first-line antimalarials, chloroquine (CQ) and piperaquine (PPQ), is the 48-kDa *P. falciparum* chloroquine resistance transporter (PfCRT). PfCRT resides on the DV membrane and mediates drug resistance via active drug efflux. Using antigen-binding fragment technology and single-particle cryo-electron microscopy (cryo-EM), we have determined the structure of a CQ-resistant isoform of PfCRT to 3.2 Å resolution. Combining structural information, with biochemistry, genetics and parasitology, we have gained insights on the molecular mechanism of PfCRT-mediated drug resistance and identified markers for the development of resistance. We have also

explored the mechanistic structure of Arabinosyltransferase B (EmbB), an enzyme that belongs to a family of membrane-bound mycobacterial glycosyltransferases that build the lipidated polysaccharides of the mycobacterial cell envelope. EmbB is the known target of an anti-tuberculosis drug, ethambutol. We determined the structure of EmbB not only showing the overall fold of the enzyme and providing insight on the reaction mechanism, but also allowing us to map all the drug resistance-causing mutations, thus providing a valuable platform from which to understand and predict their effects

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5. Transfer and transport of lipids and their constituents. Wnts are evolutionarily conserved ligands that signal at short range to regulate morphogenesis, cell fate and stem cell renewal. The first and essential steps in Wnt secretion are their O-palmitoleation by the enzyme PORCN and subsequent loading onto the dedicated transporter WLS/Evi. O-palmitoleated Wnts associated with WLS then travel from the ER to the plasma membrane, where they are transferred to receptors, such as Frizzled, on the membranes of target cells, in turn triggering the activation of signaling pathways. We determined the 3.2 Å resolution cryo-EM structure of palmitoleated human WNT8A in complex with WLS. We show, how the lipid is harbored, and that the WLS membrane domain has close structural homology to GPCRs. A large opening to the bilayer within WLS may delineate the route for how the PAM is shuttled from PORCN to WLS in an energetically favorable way. By comparing our structure to that of Wnt in complex with the binding domain of Frizzled, we noticed a large conformational change on a separate Wnt hairpin which may be the key to its one-way transfer to receiving cells.

The blood-brain barrier omega-3 fatty acid transporter Major Facilitator Superfamily Domain containing 2A (MFSD2A) is an atypical MFS protein because it transports large amphiphilic lysolipids as opposed to small, soluble substrates. We have determined the structure of MFSD2A in an inward-facing conformation complexed with a lysolipid substrate to 3Å resolution. Using an integrated approach of structural biology, cell-based functional assays, molecular dynamics simulations and native mass-spectrometry, we revealed details of how MFSD2A interacts with substrates and how Na+-dependent conformational changes allow for substrate release into the membrane. This work provides insight into the mechanism by which this atypical MFS transporter mediates uptake of omega-3 fatty acids into the brain.

Choline is an essential nutrient that humans need in vast quantities for cell membrane synthesis. The brain has a particularly high demand for choline, but how it enters the brain has remained elusive. Recently, we demonstrated both *in vivo* and *in vitro* that the MFS transporter FLVCR2 is a BBB choline transporter and is responsible for the majority of choline uptake into the brain. We determined structures of choline-bound FLVCR2 in the inward- and outward-facing states using cryo-EM to 2.49 and 2.77 Å resolution, respectively. Our work shows how the brain obtains choline and provides molecular-level insights into how FLVCR2 binds and mediates choline uptake.

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