BIOGRAPHICAL SKETCH

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NAME: Filippo Mancia

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

POSITION TITLE: Associate 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
Politecnico di Milano, Milano, Italy		09/1988	Chemical Engineering
Università di Pavia, Pavia, Italy	B.A.	03/1992	Chemistry
MRC Laboratory of Molecular Biology & University of Cambridge, Cambridge, England	Ph.D.	12/1996	Structural Biology

A. Personal Statement

I am an Associate Professor and Co-Director of Graduate Education in the Department of Physiology & Cellular Biophysics at Columbia University. I am a structural biologist with considerable experience in x-ray crystallography, and more recently in single particle cryo-electron microscopy (cryo-EM), and in production and characterization of membrane proteins for structural studies. I have developed methods for functional overexpression of eukaryotic membrane proteins and membrane protein complexes. Related to this, I have also been a key member of the NIH Protein Structure Initiative-funded New York Consortium of Membrane Protein Structure (NYCOMPS) – now the NIH-P41 funded Center on Membrane Protein Production and Analysis (COMPPÅ) – where I have played a pivotal role in the design, development, implementation and optimization of the high-throughput cloning and protein production and characterization platform for membrane proteins successfully functioning there. My main research interests, which often overlap are (1) to understand how the membrane bilayer and specific membrane enzymes and transporters interact to accommodate lipidic substrates and (2) to use structural biology techniques to understand the molecular bases of drug resistance. We have been able to determine atomic-level structures and characterize the molecular mechanisms of exemplar membrane enzyme families which process lipidic substrates. Retinoids (Vitamin A derivatives) are also lipids, and we have determined the structure of STRA6, the receptor for retinol (bound to retinol-binding protein, its sole specific carrier in the circulation), by single-particle cryo-EM allowing us to begin to understand how this essential nutrient is transferred in or out of the cell. Recently, we have 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).

1. 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*, **351,** 608-612. PMCID: PMC4963604.

3. Ardiccioni, C., Clarke, O.B., Tomasek, D., Issa, H.A., von Alpen, D.C., Pond, H.L., Banerjee, S., Rajashankar, K.R., Liu, Q., Guan, Z., Li, C., Kloss, B., Bruni, R., Kloppmann, E., Rost, B., Manzini, M.C., Shapiro, L. and **Mancia, F.** (2016). Structure of the polyisoprenyl-phosphate glycosyltransferase GtrB and insights into the mechanism of catalysis. *Nat. Commun.,* 7:10175. doi: 10.1038/ncomms10175. PMCID: PMC4728340.

3. 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,* **353,** pii: aad8266. doi: 10.1126/science.aad8266. PMCID: PMC5114850.

4. 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. [Epub ahead of print]. PMCID: In progress.

B. Positions and Honors

Positions and Employment

1997-2000 Post-doc, Dept of Biochemistry & Molecular Biophysics, Columbia University, New York
2000-2003 Associate, Howard Hughes Medical Institute, Columbia University, New York
2003-2009 Assoc Res Scientist, Dept of Biochem & Molecular Biophysics, Columbia University, New York
2009-2017 Assistant Professor, Dept of Physiology & Cellular Biophysics, Columbia University, New York
2017- Associate Professor, Dept of Physiology & Cellular Biophysics, Columbia University, New York
2018- Co-Director, Graduate Program in Physiology, Columbia University, New York

Honors / Other Experiences / Professional Memberships

1992-1995 Recipient for three years of an annual renewable fellowship from the Italian Consiglio Nazionale delle Ricerche.
1992-1995 Recipient of a three year fellowship to cover University and College fees from Sigma-Tau Industrie Farmaceutiche riunite s.p.a., Pomezia, Italy.
1995 Recipient, within the MRC Laboratory of Molecular Biology, of the 1995 Max Perutz student prize for the work as a graduate student.
1996 Recipient of a European Molecular Biology Organization (EMBO) long-term fellowship for the duration of 1 year.
1997 Recipient of a Human Science Frontier Program Organization (HSFPO) long-term fellowship for the duration of 2 years.
2016 Schaefer Research Scholar
2016 Burroughs Wellcome Fund Collaborative Research Travel Award
2016 Visiting Professor in Biochemistry, Department of Biochemistry, University of Rome La Sapienza, Rome, Italy
2017 Clyde and Helen Wu Assistant Professor of Physiology and Cellular Biophysics, Columbia University
2017 Visiting Professor, Department of Life Sciences, University Politecnica delle Marche, Ancona, Italy
2018 Structural Biology Lecturer, University of Warwick, Coventry, UK
2018 – Permanent Member, Biochemistry and Biophysics of Membranes (BBM) Study Section, NIH
2019 Visiting Professor in Biochemistry, Department of Biochemistry, University of Rome La Sapienza, Rome, Italy

C. Contributions to Science

1. Structure and function of G-protein coupled receptors. I have studied the structure and function of G-protein coupled receptors (GPCRs), and developed methods for their production, and for the production of stabilizing, conformation-sensitive ligands such as monoclonal antibodies. Work on the serotonin receptor subtype 2c (5HT2c) allowed us to show that this receptor forms dimeric assemblies, to map the dimer interface, and to show ligand-state conformational changes at this interface.

A. **Mancia, F.,** Assur, Z., Herman, A.G., Siegel, R. and Hendrickson, W.A. (2008). Asymmetry and Ligand Sensitivity in Dimeric Associations of the Serotonin 5HT2c Receptor. *EMBO Rep*. **9,** 363-9. PMCID: PMC2271072.

B. **Mancia, F.** and Hendrickson, W.A. (2007). Expression of recombinant G-protein coupled receptors for structural biology. *Mol Biosyst*. **3,** 723-34.

C. **Mancia F,** Brenner-Morton S, Siegel R, Assur Z, Sun, Y., Schieren, I., Mendelsohn, M., Axel, R. and Hendrickson, W.A. (2007). Production and characterization of monoclonal antibodies sensitive to conformation in the 5HT2c serotonin receptor. *Proc Natl Acad Sci U S A*.**104,** 4303-4308. PMCID: PMC1838597.

2. 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. This work has been instrumental to the success of the New York Consortium of Membrane Protein Structure (NYCOMPS), which applied a structural genomics approach, combined with high-throughput technologies to identify membrane protein with increased likelihood of yielding structural information. I co-directed the protein production center of NYCOMPS, and I am now on the Executive and Operations Committees of its successor, the Center on Membrane Protein Production and Analysis (COMPPÅ). 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 transport system for sulfate across the membrane.

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*. pii: e27829. doi: 10.7554/eLife.27829. PMCID: in progress.

B. Assur, Z., Hendrickson, W.A. and **Mancia, F**. (2012). Tools for Co-producing Multiple Proteins in Mammalian Cells. *Methods in Molecular Biology*, **801,** 173-187. PMCID: PMC3773504.

C. **Mancia, F**. and Love, J. (2010). High-throughput expression and purification of membrane proteins. *J. Struct. Biol.*, **172,** 85-93. 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*, **11**, 191-9. PMCID: PMC3099345.

3. 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. Lipids are synthesized and modified primarily by integral membrane enzymes embedded, at least in part, in the bilayer, but the atomic-level details of lipid/enzyme interactions and the determinants of their specificity remain poorly understood. To shed light on this question, we are studying the structure and function of three distinct families of integral membrane lipid-modifying enzymes. (1) GtrB, a polyisoprenyl phosphate glycosyltransferase attaches glucose to a lipid carrier for membrane translocation and a glycosyl donor for subsequent reactions. This reaction represents the first step in all protein glycosylation and glycosylation of the cell wall. (2) ArnT uses sugar-charged donors produced by GtrB-like enzymes, and transfers the saccharide to lipid A on the cell surface of bacteria, altering antibiotic resistance properties. (3) We have determined the structures of *Af*2299 and of *Renibacterium salmoninarum* phosphatidylinositol-phosphate (PIP) synthase – an enzyme required for inositol-lipid synthesis – with a bound CDP-diacylglycerol substrate. Both enzymes are members of the CDP-alcohol phosphotransferase family (CDP-APs), which catalyze the defining step in glycerophospholipid biosynthesis across all kingdoms of life. We are exploring substrate recognition by these enzymes with a combination of experimental approaches including x-ray crystallography, cryo-EM, and structure-guided mutagenesis coupled to functional readouts in bacteria, yeast, and zebrafish.

A. 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*, **351,** 608-612. PMCID: PMC4963604.

B. Ardiccioni, C., Clarke, O.B., Tomasek, D., Issa, H.A., von Alpen, D.C., Pond, H.L., Banerjee, S., Rajashankar, K.R., Liu, Q., Guan, Z., Li, C., Kloss, B., Bruni, R., Kloppmann, E., Rost, B., Manzini, M.C., Shapiro, L. and **Mancia, F.** (2015). Structure of the polyisoprenyl-phosphate glycosyltransferase GtrB and insights into the mechanism of catalysis. *Nat. Commun.,* 7:10175. doi: 10.1038/ncomms10175. PMCID: PMC4728340.

C. Clarke, O.B., Tomasek, D., Jorge, C.D., Belcher Dufrisne, M., Kim, M., Banerjee, S., Rajashankar, K.R., Shapiro, S., Hendrickson, W.A., Santos, H. and **Mancia, F.** (2015) Structural basis for phosphatidylinositol-phosphate biosynthesis. *Nat. Commun.,* 6:8505. doi: 10.1038/ncomms9505. PMCID: PMC4634129.

D. Sciara, G., Clarke, O.B., Tomasek, D., Kloss, B., Tabuso, S., Byfield, R., Cohn, R., Banerjee, S., Rajashankar, K.R., Slavkovic, V., Graziano, J.H., Shapiro, L. and **Mancia, F.** (2014) Structural basis for catalysis in a CDP-alcohol phosphotransferase. *Nat. Commun.,* 5:4068. doi: 10.1038/ncomms5068. PMCID: PMC4098843.

4. Structure of the STRA6 receptor. 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, and to further investigate its physiological role.

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,* 353, pii: aad8266. doi: 10.1126/science.aad8266. PMCID: PMC5114850.

B. Varney, K.M., Wilder, P.T., Godoy-Ruiz, R., **Mancia, F.** and Weber, D.J. (2019). $^{1}H^{N,}$ ^{13}C, and ^{15}N resonance assignments of human calmodulin bound to a peptide derived from the STRA6 vitamin A transporter (CaMBP2). *Biomol NMR Assign*., **13,** 275-278. PMCID: In progress.

5. Structure and drug resistance of the *Plasmodium falciparum* transporter PfCRT. 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. Our progress in understanding the molecular basis of PfCRT-mediated drug resistance, has been seriously hampered by the lack of an atomic model of this transporter. 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, identified markers for the development of resistance, and set the bases for future prospects in structure-guided drug design.

4. 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. [Epub ahead of print]. PMCID: In progress.

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/46202800/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

R01EY027405, NIH/NEI (Mancia, F.) 04/01/2017 – 03/31/2021
Role: PI
"*Structural basis of receptor-mediated cellular vitamin A uptake*"
Understanding 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.

R35GM132120, NIH/NIGMS (Mancia, F.) 05/01/2019 – 04/30/2024
Role: PI
"*Structural basis of integral membrane enzyme function*"
The goal of this structural biology focused proposal is to determine the basic principles that govern how lipidic substrates are recognized and processed my membrane enzymes, and how substrates, proteins and lipid bilayer interact for catalysis to occur.

R01AI137338, NIH/NIAID (Niederweis, M.) 01/01/2019 – 12/31/2023
Role: Subcontract PI
"*Heme and hemoglobin utilization by Mycobacterium turberculosis*"
The aim of this proposal is to reveal the molecular mechanism how *M. tuberculosis* obtains iron from heme and hemoglobin, the most prevalent iron sources in the human body.

R01AI147628, NIH/NIAID (MPI; Mancia, F., Fidock, D. & Quick, M.) 07/01/2019 – 08/31/2024
Role: Contact PI
"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.

R01NS109366, NIH/NINDS (Siegelbaum, S.) 07/01/2019 – 08/31/2024
Role: Co-PI
"*Structural studies of HCN channels in health and disease*"
The goal of this proposal is to understand how different forms of HCN function in physiological and pathological states, using mainly structural biology techniques.

Completed Research Support

R01 GM111980, NIH/NIGMS (Mancia, F.) 09/01/2015 – 08/31/2019
Role: PI
"Lipid Biosynthesis And Modification By Integral-Membrane Enzymes"
The goal of this proposal is to investigate the molecular determinants of specificity for membrane enzymes and their cognate lipidic substrates.

R01 GM109882, NIH/NIGMS (Muniswamy, M.) 08/15/2014 – 05/31/2018
"Spectral revelations of mitochondrial $Ca2^{+}$ flux interactome"
The goal of the subcontract is to produce recombinant proteins for functional analysis of the mitochondrial $Ca2^{+}$ flux interactome and make progress towards its structure determination.
Role: Subcontract PI

R21 AI119672, NIH/NIAID (Mancia, F.) 07/01/2015 – 12/31/2017
"Structural basis of phosphoinositide biosynthesis in Mycobacterium tuberculosis"
The goal of this proposal is to validate phosphatidylinositol-phosphate synthase as a potential target for the development of structure-based novel drugs to combat tuberculosis.

R01 GM098617, NIH/NIGMS (Mancia, F.) 05/01/2012 – 02/28/2017
Role: PI
"CysZ proteins: A family of sulfate transporters with remarkable architecture"
We have determined the structure of the sulfate transporter CysZ, revealing a protein of remarkable architecture. The objective of this proposal is to understand how sulfate ions are transported within the cell.

BIOGRAPHICAL SKETCH

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NAME: Cater, Rosemary

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

POSITION TITLE: Postdoctoral Fellow

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)	END DATE MM/YYYY	FIELD OF STUDY
University of Sydney, Sydney, NSW	BSc (Adv)	11/2011	Biochemistry and Pharmacology
University of Sydney, Sydney, NSW	Honours	11/2012	Pharmacology
University of Sydney, Sydney, NSW	PHD	12/2016	Pharmacology

A. Personal Statement

I am a current Postdoctoral Fellow in Filippo Mancia's lab at Columbia University, New York and am a current Simons Society Junior Fellow. I am a structural biologist with strong experience in single particle cryo-electron microscopy (cryo-EM), production and characterization of membrane proteins for structural studies, two-electrode-voltage-clamp electrophysiology, and x-ray crystallography. My passions lie in understanding the structures of membrane transport proteins and the mechanisms they use to move membrane-impermeable substrates across cell membranes. My current research focuses on using cryo-EM to understand fundamental questions related to the transport of lipidic molecules across membranes. More specifically, I am interested in the mechanisms behind the transportation of dietary omega-3 fatty acids into the brain. Prior to my post-doc, I completed my PhD at the University of Sydney under the supervision of Renae Ryan and Robert Vandenberg where I employed a wide range of molecular, electrophysiological, and x-ray crystallographic techniques to determine how glutamate transporters serve a secondary function as chloride channels.

1. Ichia Chen*, Shashank Pant*, Qianyi Wu*, **Rosemary J. Cater**, Meghna Sobti, Robert Vandenberg, Alastair G. Stewart, Emad Tajkhorshid**, Josep Font** & Renae Ryan**. Glutamate transporters contain a conserved chloride channel with two hydrophobic gates. Nature 2020. *Under review*.

2. Brendon Choy*, **Rosemary J. Cater***, Filippo Mancia**, Edward E. Pryor, Jr.**. Detergents and Membrane Mimetics in Membrane Protein Structural Biology: a 10-Year Meta-Analysis. BBA-Biomembranes 2020. *In Preparation.*

3. *Krycer JR, *Fazakerley DJ, **Cater RJ**, C Thomas K, Naghiloo S, Burchfield JG, Humphrey SJ, Vandenberg RJ, Ryan RM, James DE. The amino acid transporter, SLC1A3, is plasma membrane-localised in adipocytes and its activity is insensitive to insulin. FEBS Lett. 2017 Jan;591(2):322-330. PubMed PMID: 28032905. * Denotes equal contribution.

4. **Cater RJ,** Vandenberg RJ, Ryan RM. Tuning the ion selectivity of glutamate transporter-associated uncoupled conductances. J Gen Physiol. 2016 Jul;148(1):13-24. PubMed PMID: 27296367; PubMed Central PMCID: PMC4924932.

5. **Cater RJ**, Ryan RM, Vandenberg RJ. The Split Personality of Glutamate Transporters: A Chloride Channel and a Transporter. Neurochem Res. 2016 Mar;41(3):593-9. PubMed PMID: 26303507.

6. **Cater RJ,** Vandenberg RJ, Ryan RM. The domain interface of the human glutamate transporter EAAT1 mediates chloride permeation. Biophys J. 2014 Aug 5;107(3):621-629. PubMed PMID: 25099801; PubMed Central PMCID: PMC4129490.

B. Positions and Honors

Positions and Employment

2013 - 2017	Casual academic (Laboratory demonstrator, marker, and tutor), Discipline of Pharmacology and School of Molecular Bioscience, University of Sydney, Sydney
2016 - 2016	Casual Academic (tutor), Sydney University Medical Program, Sydney
2017 -	Postdoctoral Fellow, Department of Physiology and Cellular Biophysics, Columbia University Irving Medical Center, New York, NY

Other Experience and Professional Memberships

2014 - 2016	Postgraduate Student Representative, Discipline of Pharmacology Research Committee
2016 -	Presenter: 'Science at University and a Career in Research.', St Columba's High School, Springwood
2016 -	Postgraduate Seminar Series and Careers Fair Organizing Committee, Discipline of Pharmacology, University of Sydney
2018 -	Symposium Volunteer, COMPPÅ
2018 -	Paper Reviewer, American Chemical Society
2020 -	Paper Reviewer, Journal of Molecular Biology
2020 -	Presenter and representative post-doc at the structural biology day for prospective graduate students, Columbia University

Honors

2012	The Australian Society for Biophysics Satellite Meeting: Membrane Transporters and their Role in Human Disease Student Poster Prize, The Australian Society for Biophysics
2012	Honors Scholarship ($6,000), University of Sydney
2012	The University of Sydney Medal for outstanding honors, University of Sydney
2013	Alumni Scholarship ($6,000), University of Sydney
2013 - 2016	John A Lamberton Scholarship ($6,000 per annum), University of Sydney
2013 - 2016	Australian Postgraduate Award Scholarship, Australian Government
2014	International Union for Pure and Applied Biophysics Congress Travel Award for Young Scientists, Australian Society for Biophysics
2014	Student Poster Prize, International Union for Pure and Applied Biophysics Congress
2015	Gibson/Cox Prize for Best Student Poster Presentation, Gage Ion Channels and Transporters Conference
2015	Student Travel Award, Gordon Research Conference – Mechanisms of Membrane Transport
2016	Best Student Paper, Australian Physiological Society
2017	Bercovici Prize for Best Student Paper, Bosch Institute, University of Sydney
2018 - 2021	Simons Society Junior Fellow, Simons Society of Fellows
2018	COMPPÅ Symposium Fisher Award, COMPPÅ
2020	Robin Anders Young Investigator Award, Lorne Proteins

C. Contribution to Science

1. **Molecular determinants of the human glutamate transporter EAAT1 mediates chloride permeation.** My PhD research investigated how glutamate transporters can function as both glutamate transporters and chloride channels. Glutamate is the predominant excitatory neurotransmitter within the brain and glutamate transporters make up 3% of total brain protein. The glutamate transporter chloride channels are critical for glutamatergic signaling in the retina and the regulation of astrocytic volume, and their dysfunction is associated with episodic ataxia. Several crystal structures of glutamate transporters at different stages of the transport cycle have been solved, but in none can the chloride channel be seen indicating that the channel is only transiently open throughout the transport cycle. In my first PhD project, I investigated several polar residues that I predicted were important in chloride permeation. This involved mutating them to non-polar residues and testing the effect of these mutations on chloride conductance properties. This led to the identification of several residues that are involved in chloride permeation through glutamate transporters. I

published this work in the Biophysical Journal where it was featured as a new and notable article. This review article detailed recent advances in our understanding of how glutamate transporters act as both transporters and channels.

In my second project, I investigated an arginine that I and others predicted was responsible for selecting anions over cations to enter the chloride channel. I mutated this arginine to a histidine (an amino acid with a pKa of ~6), which allowed me to remove or introduce a positively charged residue at this position by simply manipulating the pH of my experimental set-up. I used this technique to demonstrate that the positive charge of the native arginine residue is crucial for anion selectivity, and that the absence of this positive charge causes the channel to instead become purely cation selective. I published this work in the Journal of General Physiology where it was selected to feature on the issue cover and have since won two awards for best student paper in association with this work.

For my third PhD project, I sought to obtain a crystal structure of the glutamate transporter chloride channel in an open conformation. I introduced pairs of cysteine residue that I predicted would be in close proximity when the chloride channel was open and used disulfide crosslinking in a bid to trap the channel in this open state. I was able to express, purify, and obtain crystals for a range of different double cysteine mutant transporters that functionally demonstrated properties of being trapped in a chloride conducting conformation. I obtained structures of two of these crosslinked transporters however in these structures, no open channel conformation was observed. I also obtained poorly ordered crystals and low-resolution structures of a number of other crosslinked transporters with open channel functional properties, however at this stage of the project I needed to leave my PhD lab to commence my postdoctoral studies abroad. Thanks to other lab members working on this project, this work has been ongoing since and we are currently preparing a manuscript for publication which I expect will be published this year.

a. **Cater RJ,** Ryan RM, Vandenberg RJ. The Split Personality of Glutamate Transporters: A Chloride Channel and a Transporter. Neurochem Res. 2016 Mar;41(3):593-9. PubMed PMID: 26303507.
b. **Cater RJ,** Vandenberg RJ, Ryan RM. The domain interface of the human glutamate transporter EAAT1 mediates chloride permeation. Biophys J. 2014 Aug 5;107(3):621-629. PubMed PMID: 25099801; PubMed Central PMCID: PMC4129490.
c. **Cater RJ,** Vandenberg RJ, Ryan RM. Tuning the ion selectivity of glutamate transporter-associated uncoupled conductances. J Gen Physiol. 2016 Jul;148(1):13-24. PubMed PMID: 27296367; PubMed Central PMCID: PMC4924932.

2. **Localization and insulin gensitivity of glutamate transporters in adipocytes.** Throughout my PhD, I also had the honor of collaborating with Professor David James, a leader in the field of metabolic disease research, and his team. Together, we demonstrated that human glutamate transporters are localized in the cell membranes of adipocytes, where they are the major regulator of acidic amino acid uptake. We further demonstrated that its localization and activity are unaffected by insulin or mutation of the insulin-regulated phosphosite, which indicates that this glutamate transporter maintains a constant import of acidic amino acids independently of nutritional status in adipocytes. I performed and analyzed the data from all oocyte-based experiments in this project and wrote the sections of the report relevant to my results.

   a. *Krycer JR, *Fazakerley DJ, **Cater RJ**, C Thomas K, Naghiloo S, Burchfield JG, Humphrey SJ, Vandenberg RJ, Ryan RM, James DE. The amino acid transporter, SLC1A3, is plasma membrane-localised in adipocytes and its activity is insensitive to insulin. FEBS Lett. 2017 Jan;591(2):322-330. PubMed PMID: 28032905. * Denotes equal contribution.

3. **Structure and mechanism of w-3 fatty acid transport into the brain.** Docosahexaenoic acid (DHA) is an ω-3 fatty acid found at incredibly high concentrations within the brain and is essential for normal brain functioning and development. Despite this, the brain cannot synthesize DHA de novo and we rely heavily on systemic and dietary sources of DHA to be transported across the blood-brain barrier (BBB) as a means of supply. It was recently demonstrated that a member of the Major Facilitator Superfamily (MFS) of transporters - MFSD2A - is highly expressed by endothelial cells of the BBB and is the primary route via which the brain acquires DHA. As well as serving a vital physiological function, LPC-DHA transport via MFSD2A is biophysically and evolutionarily curious given that the majority of transporters from the MFS mediate transport of small, water-soluble molecules. MFSD2A has a molecular weight of 58 kDa and is only able to transport DHA that is chemically complexed with lysophosphatidylcholine (LPC-DHA). Here I have

used single particle cryogenic electron microscopy (cryo-EM) to obtain a 3.45 Å resolution structure of apo-MFSD2A reconstituted in nanodisc and complexed with a synthetic FABs (50 kDa) to introduce a soluble feature for cryo-EM purposes. This structure provides our first insight as to how MFSD2A transports DHA across the BBB and will provide insight into how MFS transporters can transport lipid molecules across cell membranes. Furthermore, this research has the potential to lay the foundation for the rational design of neurotherapeutics that "hijack" MFSD2A for delivery across the blood brain barrier, which is a major bottleneck in neurotherapeutic development. We are currently preparing this work for publication and I expect it will be published later this year.

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

Ongoing Research Support

578646, Simons Society
Cater, Rosemary (PI)
07/01/18-06/30/21
Structural investigation of ω-3 fatty acid transport into the brain
Role: PI

APPLICANT BIOGRAPHICAL SKETCH

Use only for individual predoctoral and postdoctoral fellowships, dissertation research grants (R36),and Research Supplements to Promote Diversity in Health-Related Research (Admin Suppl). DO NOT EXCEED FIVE PAGES.

NAME OF APPLICANT: Jonathan Young Kim

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

POSITION TITLE: Post-Doctorate

EDUCATION/TRAINING *(Most applicants will begin with baccalaureate or other initial professional education, such as nursing. Include postdoctoral training and residency training if applicable. High school students should list their current institution and associated information. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	START DATE MM/YYYY	END DATE *(or expected end date)* MM/YYYY	FIELD OF STUDY
Syracuse University	B.S.	08/2005	05/2009	Biochemistry
Columbia University	M.A.	08/2010	05/2012	Biotechnology
Columbia University	M.A. & M.Phill	09/2012	02/2015	Physiology and Cellular Biophysics
Columbia University	Ph.D.	09/2012	10/2019	Physiology and Cellular Biophysics

NOTE: The Biographical Sketch may not exceed five pages. Follow the formats and instructions below.

A. Personal Statement

Currently, I am working as a Post-Doctoral researcher in Dr. Filippo Mancia's Laboratory at Columbia University Irving Medical Center. After obtaining a Ph.D. degree under Dr. Mancia's supervision, I gained considerable experience and specialized in expressing and subsequently purifying proteins for structural analysis using both x-ray crystallography and single-particle cryogenic electron microscopy (cryo-EM).

During my time as a Ph.D. student, I was allowed to work closely with Dr. Yunting Chen, a senior and experienced post-doc in the lab, who awakened my passion for understanding the compositional anatomy of integral membrane proteins. Together, Dr. Chen and I were able to determine the structure of STRA6 (stimulated by retinoic acid 6), a plasma membrane receptor for retinol (vitamin A alcohol) bound RBP (retinol-binding protein), that mediates cellular uptake of retinol by cryo-EM. Genetic mutations that deform STRA6 have been implicated in numerous diseases including Matthew-Wood syndrome, anophthalmia, congenital heart defects, diaphragmatic hernia, alveolar capillary dysplasia, lung hypoplasia, and mental retardation. By determining the structure of STRA6, to an initial resolution of 3.9 Å (now improved to 3.0 Å), we have opened pathways to elucidate the mechanistic action of STRA6-mediated retinol translocation and potentially relevant pharmaceutical target.

The second half of my graduate school career was driven by my goal to understand the structure of membrane proteins within *Plasmodium* (the parasite responsible for Malaria), and their role in evolving drug resistance, which characterizes the disease and our constant, decades- if not centuries-long fight to halt it. My project aimed to determine the structure of PfCRT (*P*lasmodium *f*alciparum chloroquine resistance transporter), a 49-kilodalton integral membrane protein localized to the digestive vacuole membrane of the parasite, which serves as a central determinant by which *Plasmodium falciparum* acquires drug resistance. To overcome the crucial hurdle of obtaining a sufficient amount of pure and homogeneous protein as well as of size, I was able to express the protein in mammalian cells and reconstitute the detergent-extracted and purified sample in lipid-filled nanodiscs, and implemented antigen-binding fragment technology, which was instrumental in determining the structure of PfCRT, a sub-50-kilodalton membrane protein – one of the smallest membrane proteins solved

by cryo-EM at the current state of size limitation. Subsequently, we achieved a 3.2 Å resolution, which revealed a central, charged cavity of the protein as a putative drug-binding site where mutations that cause drug resistance are located. Our work enhances a substantial advance in understanding the molecular mechanisms underlying *Plasmodium falciparum* resistance to antimalarial drugs in clinical use.

Columbia University has allowed me to dedicate myself to science and medicine; as a result, I have been fortunate to develop a passion for progressing the effort to cure disease. My long-term goal is to integrate structural biology, biochemistry, and physiology into elucidating the molecular mechanisms of drug resistance in malaria to pave the way for potential therapeutic avenues. I believe that my education and training coupled with my enthusiasm and curiosity makes me an ideal candidate for the Blavatnik Awards for Young Scientists. I am continuously motivated by the obscurity of macromolecules associated with human disease and the potential for advancements in this field to positively impact millions of people globally.

B. Positions and Honors

ACTIVITY/ OCCUPATION	START DATE	ENDING DATE	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Research Intern	05/2008	08/2008	Biochemistry	Han-Yang University	Yong-Hoon Chung
Lab Technician	04/2011	08/2012	Structural Biology	Columbia University	Filippo Mancia/Larry Shapiro
Graduate Student & Research Assistant	09/2012	08/2019	Structural Biology	Columbia University	Filippo Mancia
Post-Doctorate	09/2019	present	Structural Biology	Columbia University	Filippo Mancia

Academic and Professional Honors

Dean's Scholarship, Syracuse University, New York, *2005-2009*

College of Arts & Sciences 6 out of 8 semesters Dean's Lists, Syracuse University, New York, *2005-2009*

Golden Key International Honour Society, *2006*

Phi Eta Sigma National Honor Society, *2006*

The National Scholars Honor Society, *2006*

The National Society of Collegiate Scholars, *2006*

Member of the Renee Crown University Honors Program, Syracuse University, New York, *2006-2009*

B.S. awarded Cum Laude, Syracuse University, New York, *2009*

Biology Undergraduate Research Achievement Certificate, Syracuse University, New York, *2009*

Thermo Fischer Scientific Award for COMPPÅ Meeting, 2018

Columbia University Graduate Student Organization Travel Scholarship, 2018

Physiology Retreat Poster Award, 2019

C. Contributions to Science

(**i**) As an undergraduate student at Syracuse University, I worked in Dr. Mark Braiman's laboratory where I first developed a nascent appreciation for structural biology. The goal of my research was to develop and optimize a purification method to selectively precipitate proteorhodopsin (pR), an archaeal transmembrane protein that utilizes retinal (vitamin A aldehyde) as a chromophore for light-driven proton pump. To accomplish this, I systematically explored the various concentrations of phosphate and citrate in the presence of an octyl-glucoside detergent until discovering an optimal ratio. Subsequently, I obtained purity of approximately 50% which was evaluated by UV/Visible spectroscopy. This was the highest pR purity that had been achieved without the use of column purification methods.

Honor Thesis Project:
"Purification of Proteorhodopsin (pR) by Using Citrate and Phosphate to Induce Selective Precipitation."

Abstract:
Syed, F.F., **Kim, J.Y.**, Ha, K.Y. and Braiman, M.S. (2010). Citrate-Binding Site in Proteorhodopsin Involves Two Lysines in the First Cytoplasmic Loop. *Biophysical Journal.* 98(3),174a.

(**ii**) I was a part of a research team whose goal was to understand how STRA6 (stimulated by retinoic acid 6) receptor mediates cellular retinol uptake. My role was to screen STRA6 orthologs from different species for expression and stability in non-ionic detergents as GFP-fusions in transiently transfected HEK293 by western blot and florescence-coupled size exclusion chromatography (FSEC). Based on the small-scale screen results, we chose an ortholog from a particular organism for large-scale production. I worked with Dr. Yunting Chen, a leading author of the project, to express and purify the protein of interest using the baculovirus system for structural determination. Moreover, I expressed and purified the ligands of STRA6 for a HEK293 cell-based retinol uptake assay and the formation of complexes.

Publication:
Chen, Y.*, Clarke, O.B.*, **Kim, J.**, Kim, Y.K., Assur, Z., Frank, J., Blaner, W.S., Quadro, L., Shapiro, L., Hendrickson, W.A. and Mancia, F. (2016). Structure of the STRA6 receptor for retinol uptake. *Science.* 353(6302).

* Co-first authors

(**iii**) I have led a project in which we integrated structural biology, biochemistry, and genetic approaches to develop a comprehensive understanding of the function of PfCRT, a primary antimalarial drug resistance determinant that mediates drug efflux, at a molecular level. After producing functional PfCRT in baculovirus-transduced mammalian cells, I used cryo-EM to solve the structure of the PfCRT reconstituted in lipid-filled nanodisc and bound to Fab fragment to 3.2 Å resolution. This work sheds unprecedented light on the mechanism of drug resistance in the malaria parasite. I am pursuing an antimalarial drug bound state structure of PfCRT as well as other different conformations in combined with functional studies to further enhance greater insights on the mechanistic basis of PfCRT-mediated drug resistance.

Publication:
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*. 576(315-320).

* Co-first authors

Invited Presentation:
New York Structural Biology Discussion Group (NYSBDG) Winter Meeting, NY. "Role of *Plasmodium falciparum* chloroquine resistance transporter in anti-malarial drug resistance." January 14th, 2019

Biophysical Society Annual Meeting - Ion Channel and Receptor Pharmacology: Insights from Correlating CryoEM Structures with Functional Data Symposium, San Diego, CA, February 15th, 2020

Mini-Symposium on Advances in Malaria Research. June 29th, 2020

Cambridge Healthtech Institute's 8th Annual Antibodies Against Membrane Protein Targets, Boston, MA, September 17th, 2020