BIOGRAPHICAL SKETCH

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NAME: Laura Santambrogio

eRA COMMONS USER NAME: paolo6

POSITION TITLE: Associate Director of the Englander Institute of Precision Medicine at

Weill-Cornell Medicine, Professor of Physiology and Biophysics

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
Perugia University School of Medicine	MD	07/1987	Medicine
Padua University School of Medicine	PhD	07/1993	Immunology

A. Personal Statement

I am currently the Associate Director of Precision Immunology for the Englander Institute of Precision Medicine and Professor of Physiology, Biophysics and Radiation Oncology at Weill-Cornell Medical. Current efforts in my laboratory are focused on the different mechanisms of antigen processing and presentation, peptide binding to MHC class II molecules and the overall role of dendritic cells in the innate and adaptive immune response. We are tackling these questions using and integrated approach that combine cell biology, biochemistry and biophysics. Important questions addressed by my laboratory relate to the different cellular pathways utilized for antigen processing and presentation which include autophagy, endosomal processing and surface MHC II loading. Additionally, my laboratory is also involved in the analysis of peptides or metabolites inducing tolerogenic immune responses. I serve as an *ad hoc* reviewer on over 20 peer- review journals. I serve as reviewer on several NIH and non-NIH study sections. I have trained over 40 PhD, and post-doctoral scientists, many of which now hold faculty positions.

Santambrogio L, Marrack P. The broad spectrum of pathogenic autoreactivity. **Nat Rev Immunol**. 2022 Nov 23:1-2. doi: 10.1038/s41577-022-00812-2.

C. C. Clement, P. P. Nanaware, T. Yamazaki, M.P. Negroni, K. Ramesh, K. Morozova, S. Thangaswamy, A. Graves, H.J. Kim, T.W. Li, M. Vigano', R.K. Soni, M. Gadina, H. Y. Tse L. Galluzzi, L.A. Roche, L. K. Denzin, L.J. Stern, **L. Santambrogio**. Pleiotropic consequences of metabolic stress on the major histocompatibility complex class II molecule antigen processing and presentation machinery. **Science Immunology** 2022 Aug 12;7(74):eabl3795. doi: 10.1126/sciimmunol.abl3795.

C. C. Clement, P. P. Nanaware, T. Yamazaki, M.P. Negroni, K. Ramesh, K. Morozova, S. Thangaswamy, A. Graves, H.J. Kim, T.W. Li, M. Vigano', R.K. Soni, M. Gadina, H. Y. Tse L. Galluzzi, L.A. Roche, L. K. Denzin, L.J. Stern, **L. Santambrogio**. Pleiotropic consequences of metabolic stress on the major histocompatibility complex class II molecule antigen processing and presentation machinery. **Immunity** 2021 Apr 13;54(4):721-736.e10. doi: 10.1016/j.immuni.2021.02.019. Epub 2021 Mar 15.PMID: 33725478

Wan X, Zinselmeyer BH, Zakharov PN, Vomund AN, Taniguchi R**, Santambrogio L,** Anderson MS, Lichti CF, Unanue ER. Pancreatic islets communicate with lymphoid tissues via exocytosis of insulin peptides. **Nature.** 2019 Aug;560(7716):107-111. doi: 10.1038/s41586-018-0341-6. Epub 2018 Jul 18. PMID: 30022165

B. Positions, Scientific Appointments, and Honors

1991-1994	Visiting PhD Student, Department of Pathology, NYU Medical Center, New York, NY
1996-2003	Post-Doctoral fellow and Instructor of Pathology, Harvard Medical School, Boston, MA
2003-2007	Assistant Professor of Pathology, Albert Einstein College of Medicine, New York, NY
2008-2012	Associate Professor of Pathology, Immunology and Microbiology, Albert Einstein College of Medicine, New York, NY
2012-2019	Professor of Pathology, Immunology and Microbiology, Albert Einstein College of Medicine, Medicine, New York, NY
2019-present	Associate Director of the Englander Institute of Precision Medicine, Professor of Physiology Biophysics and Radiation Oncology at Weill-Cornell Medical

Professional Memberships

1993- present Member, American Association of Immunologists
2006- present Member of the Society of Experimental Pathologist
(PLUTO) 2011- present Member of the "The Henry Kunkel Society"

***Ad hoc* reviewer** Science, Immunity, Nature Immunology, Nature Biotechnology, Cell Molecular Biology, Cell Reports, PNAS., Journal of Clinical Investigation, FASEB, Journal of Immunology, Aging Cell, PLOS One, FASEB, EMBO, European Journal of Immunology, Cell Biology International, Journal of Neuroimmunology, International Immunology, Molecular Immunology, Immunology, Analytical Biochemistry, Nature Immunology, Autophagy, Frontiers of Immunology, Journal of the American Aging Association,

Editorial Board Member: Scientific Reports
Associate Editor: Frontiers of Immunology

Grant Reviewer

2005-present	Italian Ministry of Science; *Ad hoc* grant reviewer
2005-present	United States-Israel Bi-national Science Foundation; *Ad hoc*
2007-present	Arthritis Foundation; *Ad hoc* grant reviewer
2008-present	Singapore Network Immunology; *Ad hoc* grant reviewer
2009-present	The Netherlands Organization for Scientific Research; *Ad hoc*
2010-2016	NIH Member of the BMBI study section
2008-present	NIH *Ad hoc* reviewer on CMI-B, BST-Z 95, ZRG1CB-J, IDM-C, IMM-E.
2019-2024	NIH Member of CMI-A
2014-present	Swiss National Science Foundation; *Ad hoc* grant reviewer
2014-present	Austrian Science Fund; *Ad hoc* grant reviewer

Honors

2003 Irene Diamond Professorship of Immunology
2023 Amaranth Foundation prize for most impactful research in immunosenescence

C. Contributions to Science

1) Our laboratory was the first one to identify endosomal microautophagy (eMI) as an important pathway for delivering cytosolic antigens to late endosomes and exosomes in dendritic cells. The pathway relies on hsc- 70 and the ESCORT system to form multivesicular bodies and transport cytosolic proteins into the organelles. Additional chaperones involved in this pathway, as well as the NMR structure of hsc-70 with its endosomal receptor, are currently under investigation in my laboratory.

R. Sahu, S. Kaushik, C. C. Clement, E. S. Cannizzo, B. Scharf, A. Follenzi, I. Potolicchio, E. Nieves, AM Cuervo, **L. Santambrogio** (2011) Microautophagy of cytosolic proteins by late endosomes. ***Developmental Cell*** Jan 18;20(1):131-9. doi: 10.1016/j.devcel.2010.12.003. (**Cover Article and News and Views in Nature Review of Cell Biology**). PMID: 21238931

L. Santambrogio, I. Potolicchio, S. Fessler, S-H Wong, G. Raposo and J.L. Strominger. (2005) Role of caspase-cleaved and intact AP-1 complex during endosomal remodeling in maturing dendritic cells. ***Nature Immunol****.* 6: 1020-1028. PMID: 16170319. doi: 10.1038/ni1250.

Morozova K, Zolla V, Sidhar S, Clement CC, Scharf B, Verzani Z, LaRocca J, Hajjar K, Cuervo AM, Santambrogio L. Role of Annexin A2 in Atg16 Vesicle Biogenesis and Homotypic Fusion. **Nature Comms**, 2015 Jan 19;6:5856. doi: 10.1038/ncomms6856

M. Bourdenx, A. Martín-Segura, A. Scrivo, J. A. Rodriguez-Navarro, S. Kaushik, I Tasset, A. Diaz, N. J. Storm, Q. Xin, Y. R. Juste, E. Stevenson, E Luengo, C.C. Clement, S.J. Choi, N. J. Krogan, E. V. Mosharov, L. Santambrogio, F. Grueninger, L. Collin, D. L. Swaney, D. Sulzer, E. Gavathiotis, Ana Maria Cuervo Chaperone-mediated autophagy prevents collapse of the neuronal metastable proteome **Cell** in press

2) Our laboratory was the first one to perform a proteomic analysis of the human lymph and characterize the self- antigen repertoire available for MHC II presentation. Additionally, we also analyze the role of lymphatic Endothelial Cells in antigen Processing and Presentation

Clement C, Becerra A, Yin L, Zolla V, Merlin S, Follenzi A, Shafer S, Stern L, **Santambrogio L**. The dendritic cell MHC II peptidome derives from a variety of processing pathways and includes peptides with a broad spectrum of HLA-DM sensitivity. ***J Biol Chem***. **2016** Jan 6. PMID: 26740625. doi:10.1074/jbc.M115.655738

The Antigen Processing and Presentation Machinery in Lymphatic Endothelial Cells. **Laura Santambrogio,** Stella J. Berendam, Victor H. Engelhard. ***Frontiers of Immunology***,in press

Tumor-associated factors are enriched in lymphatic exudate compared to plasma in metastatic melanoma patients. Broggi MAS, Maillat L, Clement CC, Bordry N, Corthésy P, Auger A, Matter M, Hamelin R, Potin L, Demurtas D, Romano E, Harari A, Speiser DE, **Santambrogio L**, Swartz MA. ***J Exp Med***. 2019 Apr 11. pii: jem.20181618. doi: 10.1084/jem.20181618. PMID: 30975896

3) Our laboratory established for the first time the connection between impaired endosomal proteostasis and MHC II presentation. Our work identified the importance of glycation, lipoxidation and carbonylation, as occurring during conditions of oxidative and metabolic stress and aging, in endosomal antigen processing and MHC II- restricted presentation. These data indicate how qualitative changes in the cellular proteome contribute to immunosenescence and the decline in MHC-II restricted immune responses.

Tanase M, Urbanska A, Zolla V, Clement C, Morozova K, Roda B, Reschiglian P, **Santambrogio L**. Role of Carbonyl Modifications on Aging-Associated Protein Aggregation. ***Scientific Reports*** 2016 Jan 18;6:19311 PMID:26776680 doi: 10.1038/srep19311.

E. S. Cannizzo, C. C. Clement, K. Morozova, R. Valdor, S. Kaushik , L. Almeida, C. Follo, A.M. Cuervo, F. J. Macian, **L. Santambrogio**. (2012) Age-related Oxidative Stress Compromises Endosomal Proteostasis. ***Cell Reports*** 2012 Jul 26;2(1):136-49. doi: 10.1016/j.celrep.2012.06.005.

Tanase M, Zolla V, Clement CC, Borghi F, Urbanska A, Rodriguez-Navarro JA, Roda B, Zattoni A, Reschiglian P, Cuervo AM, **Santambrogio L**. Hydrodynamic-size based separation and characterization of protein aggregates from total cell lysates. ***Nature Protocols*** *2015 Jan;10(1):134-48.* doi: 10.1038/nprot.2015.009.

Complete List of Published Work in My Bibliography
https://www.ncbi.nlm.nih.gov/pubmed/?term=santambrogio+laura

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, which often overlap 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 have been a grant reviewer for many European entities, and also been a permanent member (2019-2023) and Chair of the Biochemistry and Biophysics of Membranes (BBM) NIH Study Section.

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 – 08/31/2024
"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

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. 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 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. These include (1) ArnT uses sugar-charged polyprenyl donors, and transfers the saccharide to lipid A on the cell surface of bacteria, altering antibiotic resistance properties. We have determined crystal structures of ArnT with and without a partial substrate, shedding light on the mechanism of this reaction. (2) We have determined the crystal structures of phosphatidylinositol-phosphate (PIP) synthase – an enzyme required for inositol-lipid synthesis – with a bound CDP-diacylglycerol substrate. This is a member of the CDP-alcohol phosphotransferase family (CDP-APs), which catalyze the defining step in glycerophospholipid biosynthesis across all kingdoms of life. (3) We have focused on enzyme that are involved in assembling the glycolipids of the mycobacterial cell envelope, crucial for growth and virulence and a major contributor to resistance against common antibiotics. We have determined the cryo-EM structures of arabinofuranosyltransferases D (AftD) and EmbB, representative enzymes that transfer a lipid carrier-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. (4) We have employed an integrated structural biology approach to show how the last step in the assembly of intact lipopolysaccharide, an essential constituent of the outer membrane of Gram-negative bacteria, is catalyzed by the O-antigen ligase.

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*, doi: 10.1126/science.aad1172. PMCID: PMC4963604.

b. 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.,* doi: 10.1038/ncomms9505. PMCID: PMC4634129.

c. Tan, Y.Z., Zhang, L., Rodrigues, J., Zheng, R.B., Giacometti, S.I., Rosário, A.L., Kloss, B., Dandey, V.P., Wei, H., Brunton, R., Raczkowski, A.M., Athayde, D., Catalão, M.J., Pimentel, M., Clarke, O.B., Lowary, T.L., Archer, M., Niederweis. M., Potter, C.S., Carragher, B. and **Mancia, F.** (2020). Cryo-EM structures and regulation of arabinofuranosyltransferase AftD from mycobacteria. *Mol Cell.,* doi: 10.1016/j.molcel.2020.04.014. PMCID: PMC7263364.

d. Ashraf, K.U., Nygaard, R., Vickery, O.N., Erramilli, S.K., Herrera, C.M., McConville, T.H., Petrou, V.I., Giacometti, S.I., Belcher Dufrisne, M., Nosol, K., Zinkle, A.P., Graham, C.L.B., Loukeris, M., Kloss, B., Skorupinska-Tudek, K., Swiezewska, E., Roper, D.I., Clarke, O.B., Uhlemann, A.C., Kossiakoff, A.A., Trent, M.S., Stansfeld, P.J. and **Mancia, F.** (2022). Structural basis of lipopolysaccharide maturation by the O-antigen ligase. *Nature*, doi: 10.1038/s41586-022-04555-x. PMCID: PMC9884178.

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.

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. 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*., doi: 10.1007/s12104-019-09890-1. PMCID: PMC7154012.

c. Costabile, B.K., Kim, Y.K., Chen, Y., Clarke, O.B., Quadro, L. and **Mancia, F.** (2020). Sample preparation for structural and functional analyses of the STRA6 receptor for retinol-binding protein. *Methods Enzymol.,* doi: 10.1016/bs.mie.2020.03.005. PMCID: PMC9394758.

d. Young, B.D., Varney, K.M., Wilder, P.T., Costabile, B.K., Pozharski, E., Cook, M.E., Godoy-Ruiz, R., Clarke, O.B., **Mancia, F.** and Weber, D.J. (2021). Physiologically relevant free Ca2+ ion concentrations regulate STRA6-Calmodulin complex formation via the BP2 region of STRA6. *J Mol Bio*., doi: 10.1016/j.jmb.2021.167272. PMCID: PMC8568335.

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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b. Tan, Y.Z., Rodrigues, J., Keener, J.E., Zheng, R.B., Brunton, R., Kloss, B., Giacometti, S.I., Rosário, A.L., Zhang, L., Niederweis, M., Clarke, O.B., Lowary, T.L., Marty, M.T., Archer, M., Potter, C.S., Carragher, B. and **Mancia, F.** (2020). Cryo-EM structure of arabinosyltransferase EmbB from *Mycobacterium smegmatis*. *Nat Commun*., doi: 10.1038/s41467-020-17202-8. PMCID: PMC7341804.

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 3.2Å 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.

a. Nygaard, R., Yu, J., Kim, J., Ross, D., Parisi, G., Clarke, O.B., Virshup, D.M. and **Mancia, F.** (2021). Structural basis of WLS/Evi-mediated Wnt transport and secretion. *Cell*, doi:10.1016/j.cell.2020.11.038. PMCID: PMC7797000.

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., Wong, B.H., Clarke, O.B., Marty, M.T., 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. Cater, R.J., Mukherjee, D., Gil-Iturbe, E., Erramilli, S.K., Chen, T., Koo, K., Santander, N., Reckers, A., Kloss, B., Gawda, T., Choy, B.C., Zhang, Z., Katewa, A., Larpthaveesarp, A., Huang, E.J., Mooney, S.W.J., Clarke, O.B., Yee, S.W., Giacomini, K.M., Kossiakof, A.A., Quick, M., Arnold, T. and **Mancia, F.** (2024). Structural and molecular basis of choline uptake into the brain by FLVCR2. *Nature*, doi: 10.1038/s41586-024-07326-y. *In press*.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/46202800/?sort=date&direction=descending

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: Gelardi, Edoardo

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

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)	END DATE MM/YYYY	FIELD OF STUDY
University of Piemonte Orientale, Novara	PHMD	03/2017	Pharmaceutical Chemistry and Technology
University of Pavia, Pavia	OTH	07/2017	Professional Pharmacy Qualification
University of Piemonte Orientale, Novara	PHD	04/2021	Chemistry & Biology
University of Piemonte Orientale, Novara	Fellow	10/2017	Predoctoral Fellow
Columbia University Irving Medical Center, New York, NY	Other training	08/2020	Visiting Ph.D. Student
European Institute of Oncology, Milan	Postdoctoral Fellow	03/2023	
Columbia University Irving Medical Center, New York, NY	Postdoctoral Fellow	present	

A. Personal Statement

After completing my research internship for my master's degree in Pharmaceutical Chemistry & Technology, I actively worked for one year in the Garavaglia group at University of Piemonte Orientale (UPO) in determining the X-ray crystallography structure of Aldehyde dehydrogenase isoform 3 (ALDH1A3). This enzyme is essential for the irreversible oxidation, in an NAD+-dependent manner, of retinaldehyde to retinoic acid and is considered a promising target for developing small active molecules for cancer treatment, especially against glioblastoma. The structure was obtained in the presence of its natural cofactor and product. As a result, I received extensive training in basic molecular biology and biochemistry techniques, including DNA cloning, bacterial cell growth and maintenance, and recombinant protein purification. As a result of my dedication to research I was awarded a 6-month fellowship from UPO, targeted to students seeking to continue their research after completing their master's thesis and before applying for Ph.D. programs. During this time, I collaborated with Prof. Robert Sobol from Brown University, USA, who shared a strong interest in developing a specific inhibitor for ALDH1A3. I worked on validating a novel testing method for rapidly screening potential inhibitors against this target. The promising preliminary results and my enthusiasm to continue this project led me to apply for a PhD program. Subsequently, I was awarded a 3-year PhD Fellowship

(XXXIII cycle) from the "Ministero dell'Istruzione e del Merito," the Italian government, to join the group of Prof. Menico Rizzi at UPO. My Ph.D. project focused on targeting ALDH1A3 using small active inhibitors rationally designed by our collaborators based on our high-resolution structure. We developed a small library of active compounds with high potency and selectivity against ALDH1A3 over other ALDH1A isoforms. My work included optimizing the expression, purification, in vitro assays, and crystallization conditions of ALDH1A3. As a result of these efforts, I published several articles in this specific field. Additionally, we developed a small family of fluorescent probes that selectively bind to ALDH1A3 over other isoforms, both in vitro and in vivo. This novel approach resulted in another publication and in an international patent, for which I am part of.
Throughout my Ph.D., I acquired extensive knowledge on general and specific techniques in biochemistry and structural biology, particularly in acquired extensive knowledge on general and specific techniques in biochemistry and structural biology, particularly in determining the structure of soluble proteins complexed with small molecules using X-ray crystallography and small-angle X-ray scattering. I strongly believe in the significance of structure-based rational design of compounds as novel therapies, enabling unprecedented treatment strategies against cancer and infectious diseases. After completing my Ph.D., I pursued a two-year postdoctoral position in the Lab of Marina Mapelli at the European Institute of Oncology (Italy), where I received multiple Fellowships. The project focused on the biochemical dissection of Nuclear Mitotic Apparatus Proteins and determining their structure in complex with the Wnt-dependent Destruction Complex (DC). To achieve this, I further honed my skills in eukaryotic cell culture (both mammalian and insect cells), genome editing, more advanced purification approaches, single-particle cryo-EM, and cross-linking Mass Spectrometry (XL-MS). During this period, I also established a collaboration with Prof. Laura Santambrogio from Weill Cornell University to investigate the structure of Aromatic L-amino acid decarboxylase 1 (AADC1) in complex with a novel immunomodulator discovered by the Santambrogio Lab. The manuscripts describing our results from these two projects are currently under revision. After dedicating five years to cancer research, I felt the need to expand my scientific interests, explore other research areas, while maintaining the focus on structural biology, and embrace the most recent techniques, such as cryo-ET, mixed with approaches outside my comfort zone. As a result, in May 2023, I officially became a postdoctoral researcher within the Mancia Lab The project focuses on the biochemical and structural dissection of Malaria-related proteins involved in the parasite's mechanism of resistance to commonly used therapies.

1. AI is a viable alternative to high throughput screening: a 318-target study. Sci Rep. 2024 Apr 2;14(1):7526. PubMed Central PMCID: PMC10987645.
2. Gelardi ELM, Caprioglio D, Colombo G, Del Grosso E, Mazzoletti D, Mattoteia D, Salamone S, Ferraris DM, Aronica E, Nato G, Buffo A, Rizzi M, Magrassi L, Minassi A, Garavaglia S. Curcuminbased-fluorescent probes targeting ALDH1A3 as a promising tool for glioblastoma precision surgery and early diagnosis. Commun Biol. 2022 Sep 1;5(1):895. PubMed Central PMCID: PMC9437101.
3. Gelardi ELM, Colombo G, Picarazzi F, Ferraris DM, Mangione A, Petrarolo G, Aronica E, Rizzi M, Mori M, La Motta C, Garavaglia S. A Selective Competitive Inhibitor of Aldehyde Dehydrogenase 1A3 Hinders Cancer Cell Growth, Invasiveness and Stemness In Vitro. Cancers (Basel). 2021 Jan 19;13(2) PubMed Central PMCID: PMC7835878.
4. Quattrini L, Gelardi ELM, Coviello V, Sartini S, Ferraris DM, Mori M, Nakano I, Garavaglia S, La Motta C. Imidazo[1,2-a]pyridine Derivatives as Aldehyde Dehydrogenase Inhibitors: Novel Chemotypes to Target Glioblastoma Stem Cells. J Med Chem. 2020 May 14;63(9):4603-4616. PubMed PMID: 32223240.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2023 - Postdoctoral Research Scientist, Columbia University Irving Medical Center, Department of Physiology and Cellular Biophysics (PI Prof. Filippo Mancia), New York, NY

2021 - 2023 Postdoctoral Researcher Scientist (PI Dr. Marina Mapelli), European Institute of Oncology, Milan

2020 - 2020 Visiting Ph.D. Student , Columbia University Irving Medical Center , New York, NY

2017 - 2021 Ph.D. student and Graduate Research Assistant (PI Prof. Menico Rizzi and Prof. Silvia Garavaglia), University of Piemonte Orientale, Department of Pharmaceutical Science, Novara

Honors

2023 - 2025 3-years Postdoctoral Fellowship (DECLINED), Associazione Italiana Ricerca Cancro

2023 - 2024 1-year Postdoctoral Fellowship , American Italian Cancer Foundation

2022 - 2023 1-year Postdoctoral Fellowship (DECLINED), Fondazione Umberto Veronesi (FUV)

2022 - 2023 1-year Postdoctoral Fellowship , Associazione Italiana Ricerca Cancro

2017 - 2021 3-years Ph.D. Fellowship (XXXIII cycle), Ministero dell'Istruzione e del Merito, Italian Government

2022 Travel and registration award for the EMBO WNT 2022 meeting, EMBO

2022 Travel and registration award for the EMBO Practical and integrative Structural Biology 2022 practical course, EMBO

2021 Honorary Fellow in "Biochemistry", University of Piemonte Orientale, Novara, Italy

2020 COVID-19 service award, New York Presbyterian Hospital

2017 6-months Predoctoral Fellowship, University of Piemonte Orientale

C. Contribution to Science

1. As an undergraduate and PhD student at UPO in Novara, Italy, I worked under Professors Silvia Garavaglia and Menico Rizzi. Here, I gained my initial interest in structural biology and biochemistry.
   My primary research focused on perfecting a purification method for three isoforms of Aldehyde Dehydrogenase 1A (1A1, 1A2, and 1A3) found in bacterial cells. These enzymes play a crucial role in Vitamin A metabolism and are potential targets for treating various solid cancers. We analyzed the Xray structures of ALDH1A3 bound with its natural cofactor NAD+ and the reaction product, retinoic acid. Collaborating with other academic institutions and a company called Atomwise, we combined expertise in chemical synthesis, molecular dynamics, artificial intelligence, and in vitro and in vivo assays to develop selective inhibitors against ALDH1A3. We determined the structures of four ALDH1A3-inhibitor complexes, understanding the inhibition mechanism and affinity through various assays like spectrophotometry and surface plasmon resonance. Additionally, we designed a series of curcumin-based fluorescent probes, now internationally patented under PCT/IB2022/053216.

   a. AI is a viable alternative to high throughput screening: a 318-target study. Sci Rep. 2024 Apr 2;14(1):7526. PubMed Central PMCID: PMC10987645.
   b. Gelardi ELM, Caprioglio D, Colombo G, Del Grosso E, Mazzoletti D, Mattoteia D, Salamone S, Ferraris DM, Aronica E, Nato G, Buffo A, Rizzi M, Magrassi L, Minassi A, Garavaglia S. Curcuminbased-fluorescent probes targeting ALDH1A3 as a promising tool for glioblastoma

precision surgery and early diagnosis. Commun Biol. 2022 Sep 1;5(1):895. PubMed Central PMCID: PMC9437101.

c. Gelardi ELM, Colombo G, Picarazzi F, Ferraris DM, Mangione A, Petrarolo G, Aronica E, Rizzi M,
Mori M, La Motta C, Garavaglia S. A Selective Competitive Inhibitor of Aldehyde Dehydrogenase 1A3 Hinders Cancer Cell Growth, Invasiveness and Stemness In Vitro. Cancers (Basel). 2021 Jan 19;13(2) PubMed Central PMCID: PMC7835878.

d. Quattrini L, Gelardi ELM, Coviello V, Sartini S, Ferraris DM, Mori M, Nakano I, Garavaglia S, La Motta C. Imidazo[1,2-a]pyridine Derivatives as Aldehyde Dehydrogenase Inhibitors: Novel Chemotypes to Target Glioblastoma Stem Cells. J Med Chem. 2020 May 14;63(9):4603-4616. PubMed PMID: 32223240.

2. During my PhD, I dedicated six months to enhancing my expertise in the expression, purification, biophysical characterization, and structural analysis of small membrane proteins. Specifically, I concentrated on improving my skills in these areas within the context of the Vitamin A transporter while working in Prof. Filippo Mancia's group at Columbia University Irving Medical Center. Vitamin A is vital for mammals, supporting functions like vision and embryonic development. When dietary vitamin A is lacking, the body releases retinol, its main form, into the bloodstream using a specific carrier called retinol-binding protein (RBP). This retinol is then transported to various tissues where it's taken up by cells. Inside cells, retinol is converted into active forms like retinaldehyde for vision and retinoic acid for gene regulation. Understanding how retinol is released from RBP and taken up by cells has been a major question. Our research focused on a protein called STRA6, involved in this process. We used cryo-electron microscopy to determine the 3D structure of a mammalian version of STRA6 bound to RBP. By optimizing expression and purification conditions, we achieved a highresolution structure, shedding light on how STRA6 facilitates retinol transport across cell membranes.

3. During my first postdoc at the European Institute of Oncology with Dr. Marina Mapelli, I focused on studying novel complexes relevant to colorectal cancer treatment. Specifically, I investigated potential interactions between Nuclear Apparatus Mitotic Protein (NuMA) and components of the Wnt-related Destruction Complex, including Adenomatous Polyposis Coli, AXIN1, β-catenin, GSK3β, and CK1α. Using the biGBac system, I optimized the expression and purification of these proteins, either individually or in combination, to explore their binding interfaces. Additionally, I conducted biophysical experiments to determine the binding affinity of sub-complexes using techniques like surface plasmon resonance and isothermal titration calorimetry. Collaborating with Dr. Andrea Graziadei from the Human Technopole Foundation, we employed cross-linking mass spectrometry to obtain complementary structural insights, given the transient nature and high disorder content of these complexes.

4. For my second postdoc, I returned to Prof. Filippo Mancia's group at Columbia University Irving Medical Center. Here, my focus is on studying various malaria-related proteins, including Pf chloroquine resistance transporter (PfCRT), Pf amino acid transporter (PfAAT1), and Pf ATP-binding cassette transporter i3 (PfABCi3). The goal is to understand drug resistance mechanisms and design new active compounds using a structure-based approach. Malaria, caused by the Plasmodium falciparum parasite, poses a significant public health threat worldwide, affecting over 200 million people annually. Resistance to antimalarials like chloroquine (CQ) and piperaquine (PPQ) is linked to specific mutations in PfCRT. We determined the structure of a CQ-resistant PfCRT isoform using cryo-EM combined with antigen-binding fragment technology. Currently, I'm working on obtaining the PfCRT-CQ and PfCRT-PPQ complexes using a similar approach, including expressing the proteins of interest using a BacToMam pipeline and incorporating UV-activated photocrosslinked modified versions of CQ and PPQ. Additionally, I'm expressing and

purifying PfAAT1 and PfABCi3, which are crucial targets for malaria resistance mechanisms. Collaborating with Prof. David Fidock, a malaria expert, we aim to understand the physiological functions of these proteins and the consequences of treatments by conducting cryo-CLEM and cryo-ET experiments on the digestive vacuole, where these proteins are expressed, to gain a broader understanding of how the parasite survives.

5. Between my first and second postdoc positions, I initiated a collaboration with Prof. Laura Santambrogio from Weill Cornell University. Our project focuses on characterizing a novel compound called 3-hydroxy-L-kynurenamine (3HKA), which is a biogenic amine with immunomodulatory properties and potential anticancer effects, derived from tryptophan metabolism. In this collaboration, I expressed and purified two key components: Aromatic L-amino acid decarboxylase 1 (AADC1), which is essential for 3HKA biosynthesis and also involved in PLP DOPA decarboxylation, and the sigma-1 receptor (σ1R), considered the primary target for 3HKA. For AADC1, I optimized a commercially available kit to study the kinetics of 3HKA biosynthesis and used X-ray crystallography to determine the structure of the AADC1-3HKA-PLP complex. For σ1R, I obtained a small library of potential inhibitors for 3HKA interaction and used Microscale Thermophoresis (MST) to investigate the binding between our target and these compounds. Additionally, I'm using cryo-electron microscopy (cryo-EM) to study the APO- and drug-bound states of σ1R