BIOGRAPHICAL SKETCH

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NAME: **Wacker, Daniel**

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

POSITION TITLE: **Assistant Professor of Pharmacological Sciences, Neuroscience**

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
University of Munich, Munich, Germany	BSc	2003-2007	Chemistry
University of Munich, Munich, Germany	MSc	2007-2009	Biochemistry
Rockefeller University, New York, NY	Technician	2008-2009	Cell Biology
The Scripps Research Institute, La Jolla, CA	PhD	2009-2013	Biophysics
University of North Carolina, Chapel Hill, NC	Postdoc	2014-2018	Pharmacology

A. Personal Statement

The long-term goal of my laboratory at the Icahn School of Medicine is a comprehensive mechanistic understanding of important drug targets involved in a variety of pathologies using structural and pharmacological methods. We are further leveraging these insights in structure-based drug discovery towards studying and treating a variety of illnesses, including neuropsychiatric disorders, hemolytic anemias, Alzheimer's Disease, and cancer. We are particularly interested in the structure and function of membrane receptors, transporters, and channels, and aim to design novel, target selective compounds that help delineate the role of these proteins in human health and disease.

Much of my work has focused on crystallographic and pharmacological studies of GPCRs, the largest class of drug targets in the human genome. These studies are aimed at elucidating how GPCRs are activated and inhibited by a variety of therapeutic and illicit drugs. Among others, I solved crystal structures of serotonin, dopamine, and opioid receptors. In addition, I have begun investigating mechanisms of transport and drug modulation at solute carrier (SLC) transporters, a neglected and understudied family of membrane protein drug targets. The main objective herein not only lies in characterizing distinct transporter states and elucidating substrate binding sites and potentially modulatory surfaces, but also identification and characterization of previously unknown SLC substrates. Moreover, we aim to directly use our molecular and pharmacological data in structure-based small molecule discovery in an effort to interrogate SLC (patho)physiology and explore therapeutic avenues. Towards our goals we use cutting-edge technologies including membrane protein crystallization and cryo-electron microscopy, quantitative pharmacology, and structure-based computational drug design. Over the past 4 years we have solved 23 cryoEM structures of receptors and transporters, and developed novel modulators for several SLCs using structure based drug discovery.

Ongoing and recently completed project funding that I would like to highlight include:

R35 GM133504 (PI: Wacker) 08/01/19 – 04/30/24
NIH/NIGMS
Structural Studies and Drug Discovery Illuminate Serotonin Pharmacology
Using cryo-EM and x-ray crystallography, we investigate molecular mechanisms of serotonin transporters and receptors, and leverage our insights in computational structure-based ligand discovery.

McKnight Scholar Award (PI: Wacker) 07/01/20 – 06/30/23
The McKnight Endowment Fund for Neuroscience
Accelerating Drug Discovery for Cognitive Disorders through Structural Studies of a Serotonin Receptor
We study the structure and function of the 5-HT7 receptor, a key mediator of the pro-cognitive effects of several antipsychotic, and will discover novel tool compounds to delineate the receptors role in cognition

Irma T. Hirschl/Monique Weill-Caulier Scholar Award (PI: Wacker) 01/01/23 – 12/31/27
Irma T. Hirschl/Monique Weill-Caulier Trust
Structural Characterization and Probe Discovery of an Understudied Bicarbonate Transporter
We seek to generate novel molecular insights and tool compounds to elucidate the role of the NBCn2 transporter in Alzheimer's disease, epilepsy and other neurological disorders.

Relevant publications (*corresponding author, #first author) I would like to highlight include:

Capper MJ, Yang S, Stone AC, Vatansever S, Zilberg G, Mathiharan YK, Habib R, Hutchinson K, Schlessinger A, Mezei M, Osman R, Zhang B, *__Wacker D__. *Substrate Binding and Inhibition of the Anion Exchanger 1 Transporter* | **BioRxiv**, DOI:10.1101/2022.02.11.480130, (2022) (accepted at Nat Struct Mol Biol)

#Sakloth F, #Sanchez-Reyes OB, Pryce KD, Ruiz A, Serafini RA, Bertherat F, Gomes I, Devi L, *__Wacker D__, *__Zachariou V__. *A regional and projection-specific role of RGSz1 in the ventrolateral periaqueductal grey in the modulation of morphine reward* | **Mol Pharmacol.** Online ahead of print. DOI: 10.1124/molpharm.122.000528 (2022)

*#Wang S, ***#__Wacker D__**, #Levit A, Che T, Betz RM, McCorvy JD, Venkatakrishnan AJ, Huang XP, Dror RO, *Shoichet BK, *Roth BL. *D4 dopamine receptor high-resolution structures enable the discovery of selective agonists* | **Science** 358, 381-386, (2017), PMCID: PMC5856174

***#__Wacker D__**, #Wang S, #McCorvy JD, Betz RM, Venkatakrishnan AJ, Levit A, Lansu K, Schools Z, Che T, Nichols DE, Shoichet BK, *Dror RO, *Roth BL. *Crystal structure of an LSD-bound human serotonin receptor* | **Cell** 168, 377-389, (2017), PMCID: PMC5289311

I confirm that I have not published under a different name

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

07/2018 – Present	Associate Director, Mount Sinai Center for Therapeutics Discovery
02/2018 – Present	Assistant Professor, Department of Pharmacological Sciences, Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York (NY), USA
01/2014 – 01/2018	Postdoctoral Associate: Structural and functional characterization of G protein-coupled receptor signaling. University of North Carolina, Chapel Hill (NC), USA, Prof. Bryan Roth
07/2009 – 12/2013	Ph.D. and Postdoctoral Research: Structural basis of hallucinogen signaling through serotonin and opioid receptors. The Scripps Research Institute, La Jolla (CA), USA, Prof. Raymond Stevens
03/2008 – 07/2009	Master's Thesis: Structural and functional analysis of yeast Nup133 of the nuclear pore complex. Rockefeller University, New York (NY), USA, Prof. Günter Blobel
08/2006 – 12/2007	Undergraduate Research: Electron-microscopic single particle reconstruction of ribosome complexes. Gene Center Munich, Germany, Prof. Roland Beckmann

Honors

2023	Irma T. Hirschl/Monique Weill-Caulier Trust Research Award
2020	McKnight Endowment Fund for Neuroscience Scholarship
2020	Edwardt Mallinckrodt, Jr Foundation Grant
2019	Sloan Research Fellowship in Neuroscience

2016 Keystone Symposia Future of Science Fund scholarship for Keystone Symposia
2010-12 Boehringer Ingelheim Fonds PhD Fellowship
2008 DAAD Foreign Exchange Research Fellowship

C. Contributions to Science

*#first author, *corresponding author*

1. I have made major contributions towards a structural understanding of G Protein Coupled Receptor (GPCR) function. I contributed to the structural elucidation of numerous therapeutically important GPCRs, including the first crystal structures of the kappa opioid receptor, the glucagon receptor, the D2 and D4 dopamine receptors, and the 5-HT serotonin receptor 5-HT_{2B}, 5-HT_{2C}, and 5-HT_{1B}.
   a. ***#Wacker D**, #Wang S, #McCorvy JD, Betz RM, Venkatakrishnan AJ, Levit A, Lansu K, Schools Z, Che T, Nichols DE, Shoichet BK, *Dror RO, *Roth BL. Crystal structure of an LSD-bound human serotonin receptor | **Cell** 168, 377-389, (2017), PMCID: PMC5289311 - COVER
   b. **Wacker D**, Wang C, Katritch V, Han GW, Huang XP, Vardy E, McCorvy JD, Jiang Y, Chu M, Siu FY, Liu W, Xu HE, Cherezov V, *Roth BL, *Stevens RC. Structural Features for Functional Selectivity at Serotonin Receptors | **Science** 340, 615-619, (2013), PMCID: PMC3644390
   c. *Wang S, Che T, Levit A, Shoichet BK, ***Wacker D**, *Roth BL. Structure of the D2 dopamine receptor bound to the atypical antipsychotic drug risperidone | **Nature** 555, 269-273, (2018), PMCID: PMC5843546
   d. *#Wang S, ***#Wacker D**, #Levit A, Che T, Betz RM, McCorvy JD, Venkatakrishnan AJ, Huang XP, Dror RO, *Shoichet BK, *Roth BL. D4 dopamine receptor high-resolution structures enable the discovery of selective agonists | **Science** 358, 381-386, (2017), PMCID: PMC5856174
2. Through combining structural studies with quantitative pharmacological studies, I have contributed to resolving several of the underlying molecular principles that govern how ligands modulate GPCR activity to engage differential signaling pathways. This phenomenon termed functional selectivity or biased signaling can be exploited to design drugs that specifically activate therapeutic over pathological pathways, thereby drastically reducing on-target side effects.
   a. **Wacker D**, Wang C, Katritch V, Han GW, Huang XP, Vardy E, McCorvy JD, Jiang Y, Chu M, Siu FY, Liu W, Xu HE, Cherezov V, *Roth BL, *Stevens RC. Structural Features for Functional Selectivity at Serotonin Receptors | **Science** 340, 615-619, (2013), PMCID: PMC3644390
   b. ***#Wacker D**, #Wang S, #McCorvy JD, Betz RM, Venkatakrishnan AJ, Levit A, Lansu K, Schools Z, Che T, Nichols DE, Shoichet BK, *Dror RO, *Roth BL. Crystal structure of an LSD-bound human serotonin receptor | **Cell** 168, 377-389, (2017), PMCID: PMC5289311
   c. Che T, Majumdar S, Zaidi SA, Kormos C, McCorvy JD, Wang S, Mosier PD, Uprety R, Vardy E, Krumm BE, Han GW, Lee MY, Pardon E, Steyaert J, Huang XP, Strachan RT, Tribo AR, Pasternak GW, Carroll IF, Stevens RC, Cherezov V, Katritch V, ***Wacker D**, *Roth BL. Structure of a nanobody-stabilized active state of the kappa opioid receptor | **Cell** 172, 55-67, (2018), PMCID: PMC5802374
   d. **Wacker D**, Stevens RC, *Roth BL. How ligands illuminate GPCR molecular pharmacology | **Cell** 170, 414-427, (2017), PMCID: PMC5560499
3. I have also made major contributions to structure-based drug discovery efforts providing GPCR structures for virtual ligand screening campaigns, and identifying and characterizing novel chemical matter for challenging target receptors such as the D4 dopamine receptor. I also determined the first crystal structure of a GPCR ligand developed by structure-based methods, which substantially contributed to the validation and optimization of virtual ligand screening efforts towards novel GPCR compounds.
   a. *#McCorvy JD**, #Wacker D**, #Wang S, Agegnehu B, Liu J, Lansu K, Tribo AR, Olsen RHJ, Che T, Jin J, *Roth BL. Structural determinants of 5-HT2B receptor activation and biased agonism | **Nat Struct Mol Biol** 25, 787-796 (2018), PMCID: PMC6237183
   b. *#Wang S, ***#Wacker D**, #Levit A, Che T, Betz RM, McCorvy JD, Venkatakrishnan AJ, Huang XP, Dror RO, *Shoichet BK, *Roth BL. D4 dopamine receptor high-resolution structures enable the discovery of selective agonists | **Science** 358, 381-386, (2017), PMCID: PMC5856174

c. **Wacker, D.**, Stevens, R.C., *Roth, B.L. How ligands illuminate GPCR molecular pharmacology. **Cell** 170, 414-427, (2017), PMCID: PMC5560499

d. #**Wacker D**, #Fenalti G, #Brown MA, Katritch V, Abagyan R, Cherezov V, *Stevens RC. Conserved binding mode of human β2 adrenergic receptor inverse agonists and antagonist revealed by X-ray crystallography | **J Am Chem Soc** 132, 11443-11445, (2010), PMCID: PMC2923663

4. In addition to small molecule compounds, antibodies are becoming increasingly useful probes to study GPCR pharmacology. I used both conventional IgG derived antigen-binding fragments (Fabs) and camelid single-chain antibodies, termed nanobodies, to structurally elucidate GPCR activation mechanisms based on distinct antibody-stabilized GPCR conformations.

   a. Che T, Majumdar S, Zaidi SA, Kormos C, McCorvy JD, Wang S, Mosier PD, Uprety R, Vardy E, Krumm BE, Han GW, Lee MY, Pardon E, Steyaert J, Huang XP, Strachan RT, Tribo AR, Pasternak GW, Carroll IF, Stevens RC, Cherezov V, Katritch V, ***Wacker D**, *Roth BL. Structure of a nanobody-stabilized active state of the kappa opioid receptor | **Cell** 172, 55-67, (2018), PMCID: PMC5802374

   b. Ishchenko A, **Wacker D**, Kapoor M, Zhang A, Han GW, Basu S, Patel N, Messerschmidt M, Weierstall U, Liu W, Katritch V, Roth BL, Stevens RC, *Cherezov V. Structural insights into the extracellular recognition of the human serotonin 2B receptor by an antibody | **Proc Natl Acad Sci USA** 114, 8223-8228, (2017), PMCID: PMC5547598

   c. *English JG, Olsen RHJ, Lansu K, Patel M, White K, Cockrell AS, Singh D, Strachan RT, **Wacker D**, *Roth BL. VEGAS as a Platform for Facile Directed Evolution in Mammalian Cells | **Cell** 178, 748-761, (2019), PMCID: PMC6660416

5. I have recently begun investigating the structure and function of several classes of solute carrier (SLC) transporters, such as SLC4 family bicarbonate transporters. In my lab we have already determined several cryoEM structures of different SLC4 transporters bound to substrates or chemically and pharmacologically different drugs, and begun elucidating mechanisms of bicarbonate transport and distinct mechanisms of inhibition.

   a. Capper MJ, Yang S, Stone AC, Vatansever S, Zilberg G, Mathiharan YK, Habib R, Hutchinson K, Schlessinger A, Mezei M, Osman R, Zhang B, ***Wacker D**. Substrate Binding and Inhibition of the Anion Exchanger 1 Transporter. **BioRxiv**. DOI:10.1101/2022.02.11.480130 (accepted at Nat Struct Mol Biol)

List of published work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/wacker.daniel.1/bibliography/54351448/public/?sort=date&direction=ascending

BIOGRAPHICAL SKETCH

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NAME: **Yang, Shifan**

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

POSITION TITLE: **Senior 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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Huazhong Agricultural University	BA	09/2004	06/2008	Bioengineering
Huazhong Agricultural University	PhD	09/2008	06/2014	Structural Biology
ShanghaiTech University	Postdoc	07/2014	10/2017	Structural Biology
Indiana University	Postdoc	01/2018	04/2020	Structural Biology
Icahn School of Medicine at Mount Sinai	Postdoc	04/2020	05/2022	Structural Pharmacology
Icahn School of Medicine at Mount Sinai	Senior Scientist	05/2022	Current	Structural Pharmacology

A. Personal Statement

I am currently a senior scientist in the labs of Dr. Zhang and Dr. Wacker in the Department of Genetics & Genomic Sciences and the Department of Pharmacological Sciences at the Icahn School of Medicine at Mount Sinai. I am mostly interested in the structure and function of membrane proteins impacting a wide variet of diseases including homological disorders, as well as neurodegenerative diseases.

I have always been fascinated by mechansims of protein function including enzymatic reactions, signaling of G protein-coupled receptors (GCPRs), membrane transport, and the basics of how proteins interact with other proteins, drugs, or nucleic acids. During my undergraduate and graduate work in Wuhan, China I worked mostly on archaeal proteins, before I begun investigating GPCR function in the lab of Dr. Xu in Shanghai. There I was intrigued by the impact of structural biology on drug discovery and human disease due to the importance of membrane proteins in human pathophysiology.

Due to this interest I joined the Icahn School of Medicine at Mount Sinai (ISMMS) to work in the laboratory of Dr. Daniel Wacker and additional mentorship by Dr. Bin Zhang. In a collaboration between these labs I am focused on elucidating the structure-function relationship of solute carrier (SLC) transporters and GPCRs using cryo-electron microscopy (cryo-EM) and x-ray crystallography in combination with biochemical uptake and signaling assays. In collaboration with computational structural biologists, I am further working on the development of novel small molecule modulators for these drug targets to explore their roles in a wide variety of disorders.

In compliance with NIH requirements, I confirm that I have not published under a different name.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2022-Current	Postdoc, Icahn School of Medicine at Mount Sinai, Department of Pharmacological Sciences, Zhang and Wacker Labs
2020-2022	Postdoc, Icahn School of Medicine at Mount Sinai, Department of Pharmacological Sciences, Zhang and Wacker Labs
2018-2020	Postdoc, Indiana University Bloomington, Department of Molecular and Cellular Biochemistry,
2014-2017	Postdoc, iHuman institute, ShanghaiTech University, Xu lab
2008-2014	Ph.D., Huazhong Agricultural University, Institute of High Energy Physics, He lab
2004-2008	Bachelor of Bioengineering, Huazhong Agricultural University

C. Contributions to Science

1. **Structural and pharmacological study of SLC4 family of bicarbonate (HCO3-) transporters:** In the Wacker and Zhang labs I am elucidating the structure and function of several SLC4 bicarbonate membrane transporters, and develop novel probes targeting different members of this family. I have already determined several cryoEM structures of different SLC4 transporters including that of SLC4A1 (Anion Exchanger 1) bound to substrates and chemically and mechanistically distinct inhibitors.

    i. #Capper MJ, #**Yang S**, #Stone AC, Vatansever S, Zilberg G, Mathiharan YK, Habib R, Hutchinson K, Schlessinger A, Mezei M, Osman R, Zhang B, *Wacker D. Substrate Binding and Inhibition of the Anion Exchanger 1 Transporter. **BioRxiv**. doi: https://doi.org/10.1101/2022.02.11.480130 (Accepted in Nat Struct Mol Biol)

2. **Structural insights into Frizzled receptor mechanisms:** In the Xu lab I studied the structure and function of Frizzled receptors, an important class of GPCRs involved in development. I determined the high-resolution crystal structure of the human Frizzled 4 receptor and uncovered unique features such as a tightly packed helix VI, as well as a narrow and highly hydrophilic pocket that cannot bind traditional GPCR ligands.

    ii. **Yang S**, Wu Y, Xu TH, de Waal PW, He Y, Pu M, Chen Y, DeBruine ZJ, Zhang B, Zaidi SA, Popov P, Guo Y, Han GW, Lu Y, Suino-Powell K, Dong S, Harikumar KG, Miller LJ, Katritch V, Xu HE, Shui W, Stevens RC, Melcher K, Zhao S, Xu F. Crystal structure of the Frizzled 4 receptor in a ligand-free state | **Nature** 560, 666-670, (2018)

3. **Structural insights into the DNA replication machinery in a hyperthermophilic archaea:** In the He lab I conducted structural and biochemical studies on DNA replication in several hyperthermophilic organisms. Among this work are several studies in which I elucidated how different DNA polymerases are regulated through interaction with origin recognition complexes and replication initiators. I also determined a high resolution structure of a Tet repressor bound to DNA and uncovered a unique binding mode in which the recognition helix only inserted slightly into the DNA major groove.

    i. **Yang S**, Gao Z, Li T, Yang M, Zhang T, Dong Y, He ZG. Structural basis for interaction between Mycobacterium smegmatis Ms6564, a TetR family master regulator, and its target DNA | **J Biol Chem** 288, 23678-23695, (2013)

    i. **Yang S**, Gong H, Zhang L, Liu Y, He ZG. Characterization of physical and functional interactions between eukaryote-like Orc1/Cdc6 proteins and Y-family DNA polymerase in the hyperthermophilic archaeon Sulfolobus solfataricus | **Biochem Biophys Res Commun** 396, 755-762, (2010)

i. Zhang L, Zhang L, Liu Y, **Yang S**, Gao C, Gong H, Feng Y, He ZG. Archaeal eukaryote-like Orc1/Cdc6 initiators physically interact with DNA polymerase B1 and regulate its functions | **Proc Natl Acad Sci USA** 106, 7792-7797, (2009)

4. **Biochemical studies of protein kinases and their interaction partners in a hyperthermophilic archaea:** In the He lab I also investigated kinase function and interaction networks in archaea. I made major contributions to our understanding of how the Ser/Thr kinase ST1565 interacts with the forkhead-associated (FHA) domain containing protein ST0829.

ii. Wang B, **Yang S**, Zhang L, He ZG. Archaeal eukaryote-like serine/threonine protein kinase interacts with and phosphorylates a forkhead-associated-domain-containing protein | **J Bacteriol** 192, 1956-1964, (2010)

BIOGRAPHICAL SKETCH

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NAME: Gregory Zilberg

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

POSITION TITLE: Graduate Student Research Assistant

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Wisconsin - Madison	BS	08/2013	12/2017	Biochemistry, Neurobiology
Icahn School of Medicine at Mount Sinai	PhD	08/2018	XX/2024	Neuroscience

A. Personal Statement

My long term research interests primarily focus on the biochemical determinants of the function of macromolecular complexes that enable pre- and post-synaptic signal transduction and scaffolding, and discovery of drugs relating to their modulation for therapeutic ends. In pursuing that, my undergraduate academic coursework focused on establishing a foundational knowledge of the molecular biology involved in synaptic development and plasticity, and the structure-function relationships of proteins and enzymes. I additionally worked as an undergraduate in Dr. Kate O'Connor-Giles's lab at UW-Madison studying the mechanisms of multiple proteins involved in synaptic development in Drosophila. The four years in this lab resulted in authorship on a publication and a poster presentation at UW-Madison's annual Undergraduate Research Symposia. In my graduate education, in the lab of Dr. Daniel Wacker, I have shifted from phenotypic studies in Drosophila to protein purification and structural determination of GPCR-G protein complexes. For my thesis project, I am focusing on studying the structural and functional basis for the activation of understudied aminergic receptor subtypes that may yield novel clinically applicable compounds.

I confirm that I have not published under a different name

B. Positions, Scientific Appointments and Honors

Positions and Employment

2013 - 2017 Undergraduate Research Assistant, University of Wisconsin - Madison
2018 - Graduate Student Research Assistant, Icahn School of Medicine at Mount Sinai

Other Experience and Professional Memberships

2015 – 2016 Content Editor, Journal of Undergraduate Science and Technology
2016 – 2017 Senior Content Editor, Journal of Undergraduate Science and Technology

Honors

2013 Undergraduate Research Scholars Program, UW College of Letters and Sciences
2014 Engineering Great People Scholarship, UW Foundation

C. Contributions to Science

1. **Undergraduate Research:** I was involved in a project in the lab of Dr. Kate O'Connor-Giles at UW – Madison, focusing on the effects of the Drosophila synaptic adaptor protein, Nervous Wreck (Nwk), on pre- and post-synaptic morphological characteristics. This resulted in a poster presentation at UW – Madison's annual Undergraduate Research Symposium. I also assisted in the analysis of electron microscopy tomograms of wild-type and mutant Fife-deficient Drosophila presynaptic densities, leading to an authorship on a publication in the Journal of Cell Biology.

    a. (2015, May). "The Role of Nervous Wreck in Dendritic Patterning and Growth in Drosophila." Poster presented at UW-Madison Undergraduate Research Symposium, Madison, WI.

    b. Bruckner, J.J.; Zhan, H.; Gratz, S.J.; Rao, M.; Ukken, F.; **Zilberg, G.**; O'Connor-Giles, K.M. (2017) "Fife organizes synaptic vesicles and calcium channels for high-probability neurotransmitter release." *J. Cell Biol.* 216(1):231-246.

2. **Graduate Research**: My ongoing predoctoral research focuses on the structural determination and pharmacological characterization of understudied aminergic receptors and solute carrier (SLC) transporters that may yield insights into the basic atomic level mechanisms of membrane protein function. I am also interested in elucidating mechanisms of stimulant drug action in neurons, as well as an unappreciated mechanism of action for antidepressants. I have collected structural and functional data demonstrating novel signaling properties and novel agonists for aminergic receptors that I hope to publish in the coming years alongside relevant structures of the receptors in complex with their G protein heterotrimer transducer counterparts. I have additionally worked on the structural elucidation and drug discovery of understudied bicarbonate transporters.

    a. Zilberg, G.; Wacker, D (2020). "A Novel Cryo-EM Structure Enables Development of Selective Cannabinoid Receptor Drugs." *Biochemistry.* 5;59(17):1643-1644.
    b. Capper MJ, Yang S, Stone AC, Vatansever S, Zilberg G, Mathiharan YK, Habib R, Hutchinson K, Schlessinger A, Mezei M, Osman R, Zhang B, *Wacker D. Substrate Binding and Inhibition of the Anion Exchanger 1 Transporter. BioRxiv. doi: https://doi.org/10.1101/2022.02.11.480130 (accepted in Nat Struct Mol Biol)

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NAME: **Warren, Audrey Louise**

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

POSITION TITLE: **PhD Candidate**, Graduate School of Biomedical Sciences at the Icahn School of Medicine at Mount Sinai

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Columbia University	BA	09/2012	05/2016	Biological Sciences
Icahn School of Medicine at Mount Sinai	PhD	08/2019	In Progress	Biomedical Sciences *Training Area- Pharmacology and Therapeutics Discovery*

A. Personal Statement

I am currently a fourth-year PhD student in the Department of Pharmacological Sciences at the Icahn School of Medicine at Mount Sinai. I am interested in the structure and function of membrane proteins impacting neuropharmacology. After completing my PhD, I would like to pursue this field of study as a postdoctoral fellow and eventually establish my own laboratory.

I have always been fascinated by neuroscience, and this interest has driven my selection of research experiences. My first experience was under the supervision of Dr. Kristen Brennand studying the cellular phenotypes associated with schizophrenia using induced pluripotent stem cells. Later as a technician with Dr. Vincent Racaniello and Dr. Amy Rosenfeld, I worked to understand the molecular determinants of tissue tropism allowing viruses like Zika, Enterovirus D-68, and poliovirus to replicate in the brain. Following my work in virology, I spent two years in the laboratory of Dr. Matthias Quick. His lab specializes in the study of neurotransmitter transporters. Under his guidance, I learned the principles of pharmacology. My work cloning and purifying recombinant proteins for functional studies also cultivated an interest in structural biology. Learning to examine the atomic detail of a protein seemed to me the next logical step of inquiry as structural biology in coordination with functional work has the potential to explain how and why drugs work.

This interest drove me to pursue a PhD at the Icahn School of Medicine at Mount Sinai (ISMMS) in the laboratory of Dr. Daniel Wacker. His lab focuses on the structure-function relationship of G-protein coupled receptors (GPCR) and transporters. Under the guidance of Dr. Wacker, I have learned new techniques to answer fundamental questions about the action of drugs and small molecules on proteins. This experience has allowed me to learn about X-ray crystallography and cryo-electron microscopy (cryo-EM)—the dominant techniques in the field of structural biology. I am learning how to apply these tools to answer questions about existing drugs and develop new compounds.

In compliance with NIH requirements, I confirm that I have not published under a different name.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2019-Present	Graduate Research Assistant, Icahn School of Medicine at Mount Sinai Department of Pharmacological Sciences
2017-2019	Research Technician, Columbia University Department of Psychiatry
2016-2017	Research Technician, Columbia University Department of Microbiology
2014-2016	Undergraduate Research Assistant, Icahn School of Medicine at Mount Sinai Department of Neuroscience

Honors

2022	Selected Speaker, Department of Pharmacology Training Symposium, Icahn School of Medicine at Mount Sinai
2022	Selected Speaker, Friedman Brain Institute Retreat, Icahn School of Medicine at Mount Sinai
2021	Poster Presentation Department of Pharmacology Training Symposium, Icahn School of Medicine at Mount Sinai
2020	Irving L. Schwartz Poster Prize, Icahn School of Medicine at Mount Sinai
2013, 2015, 2016	Dean's List (3 semesters), Columbia University

Grants

2022-2025	F31 awardee (1F31MH132317-01), Icahn School of Medicine at Mount Sinai
2022	T32 awardee (5T32DA053558-02), Icahn School of Medicine at Mount Sinai
2020-2022	T32 awardee (5T32GM062754-21), Icahn School of Medicine at Mount Sinai

C. Contributions to Science

1. **Research Technician, Columbia University Department of Microbiology**
    a. As a technician working with Dr. Rosenfeld and Dr. Racaniello, I assisted in multiple studies of Zika virus. The disease received worldwide attention in 2016 due to the outbreaks in South America that were associated with a rise of microcephaly cases. However, Zika virus was identified many decades prior with a number of previous outbreaks. Using organotypic slice cultures from mouse brains, our work demonstrated that Zika infects multiple sites in the developing brain. All lineages of Zika were found to be neurotropic in early and midgestation brain slice cultures, indicating that neurotropism was not a result of mutations present in the virus from recent Brazilian and Colombian outbreaks. I assisted with the tissue culture work. A similar phylogenetic inquiry into Enterovirus-D68, a pathogen associated with the development of acute flaccid myelitis, revealed that all isolates of the virus are capable of replicating in murine brain slice cultures. For these inquiries, I performed the confocal microscopy and assisted in all of the tissue culture experiments. My contributions were recognized with co-authorship on the resulting manuscripts.

        i. Rosenfeld AB, Doobin DJ, **Warren AL**, Racaniello VR, Vallee RB. Replication of early and recent Zika virus isolates throughout mouse brain development. **Proc Natl Acad Sci USA**. 2017 Nov 14;114(46):12273-12278.

        ii. Rosenfeld AB, **Warren AL**, Racaniello VR. Neurotropism of Enterovirus D68 Isolates Is Independent of Sialic Acid and Is Not a Recently Acquired Phenotype. **mBio**. 2019 Oct 22;10(5):e02370-19.

2. **Research Technician, Columbia University Department of Psychiatry**
    a. I was instrumental in a project in the laboratory of Dr. Matthias Quick evaluating transport mechanisms in secondary active transporters. The work, performed in collaboration with Dr. Scott Blanchard and Dr. Jonathan Javitch, established a fluorescence-based assay for assessing the activity of a single transporter using a hydrophobic amino acid sensor. The project also demonstrated the impact of different substrates on the rates of the first and second half cycles of transport by the protein MhsT. As MhsT is a homolog of neurotransmitter sodium

symporters, these observations may have relevance for related proteins. I cloned, expressed, and purified all of the MhsT used for study. I was responsible for optimizing MhsT reconstitution into proteliposomes and preparing bacterial membrane vesicles for uptake studies. For my contribution, I was a co-author on the resulting publication.

i. Fitzgerald GA, Terry DS, **Warren AL**, Quick M, Javitch JA, Blanchard SC. Quantifying secondary transport at single-molecule resolution. **Nature**. 2019 Nov;575(7783):528-534.

b. I also played an important role in another transporter project that resulted in a top-tier publication. The *Plasmodium falciparum* chloroquine resistance transporter (pFCRT) is a transporter located in the membrane of the digestive vacuole of the malaria parasite. Resistance to chloroquine and piperaquine, the first-line treatment for malaria, is associated with the development of mutations in pFCRT. The project solved the structure of pFCRT and demonstrated the functional differences between different mutations in the protein conferring drug resistance. Using skills learned from previous transporter studies, I contributed setting up the reconstitution, binding, and transport assays to study the molecular mechanism of this key drug target.

i. Kim J, Tan YZ, Wicht KJ, Erramilli SK, Dhingra SK, Okombo J, Vendome J, Hagenah LM, Giacometti SI, **Warren AL**, Nosol K, Roepe PD, Potter CS, Carragher B, Kossiakoff AA, Quick M, Fidock DA, Mancia F. Structure and drug resistance of the Plasmodium falciparum transporter PfCRT. **Nature**. 2019 Dec;576(7786):315-320.

3. Graduate Research, ISMMS Department of Pharmacological Sciences

a. As a graduate student with Dr. Wacker, I have contributed to multiple projects on the structure and activity of receptors and transporters that modulate serotonin signaling. In collaboration with another trainee in the lab, I am studying the receptor 5-HT1E, which is the one of the few serotonin receptors with an undefined physiological role. To help delineate the role of 5-HT1E, we aim to identify receptor-selective compounds. We have identified a number of tricyclic agonists of the receptor, which is novel as tricyclics are typically antagonists at GPCRs, and we have solved structures of 5-HT1E bound to these drugs. I have also performed extensive structural and functional studies on the 5-HT1A receptor, evaluating its role as a major target of psychedelic drugs. To date, I have solved nine structures of 5-HT1A in complex with different therapeutic and research compounds and bound to different G-protein subtypes.