BIOGRAPHICAL SKETCH

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NAME: Aaron P. Owji

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

POSITION TITLE: Predoctoral Researcher, Graduate 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)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Central Florida, Orlando, FL	BS	08/2012	Biotechnology & Molecular Biology
University of Central Florida, Orlando, FL	MS	06/2015	Biotechnology (w/ Thesis)
Columbia University, Graduate School of the Arts and Sciences, New York, NY	MA	08/2016	Pharmacology & Molecular Signaling
Columbia University, Graduate School of the Arts and Sciences, New York, NY	MPhil	08/2017	Pharmacology & Molecular Signaling
Columbia University, Graduate School of the Arts and Sciences, New York, NY	PhD (In Progress)	09/2021	Pharmacology & Molecular Signaling

A. Personal Statement

My long-term research interest lies in elucidating the structures of integral membrane proteins related to human health and disease. Specifically, I seek to utilize single-particle cryogenic electron microscopy (cryoEM) and X- ray crystallography to determine the structure and functional relationship of biomedically-relevant protein targets. My academic coursework and training have provided me with an excellent understanding of molecular biology, physiology, biochemistry, and, more recently, a variety of structural techniques. As an undergraduate at the University of Central Florida, I sought my initial training in biomedical research in the lab of Dr. Steven N. Ebert, where I developed a strong interest in cardiac physiology. Upon graduating with my Bachelor's degree, I began my Master's coursework and continued working with Dr. Ebert for my Master's thesis. My research aim was to determine how a specific population of progenitor cells, which express the biosynthetic enzyme for adrenaline, contribute to heart development and adult cardiac function. I presented work from my Master's thesis at two major conferences with the American Heart Association and I was also selected for 1st Place Master's Presentation at the University of Central Florida 9th Annual Graduate Research Symposium. This initial work in cardiac physiology, which entailed in-depth mouse echocardiography, led to a fascination with ion channel function and my ultimate pursuit of a PhD in the Pharmacology and Molecular Signaling program at Columbia University. By the time I joined Columbia, I had a keen interest in electrophysiology and this interest grew as I learned more about the structural mechanisms underlying ion channel function. For my doctoral dissertation, I am generating a structural model to explain calcium-dependent activity of mammalian bestrophins. Other projects I am developing include elucidation of the molecular mechanisms underlying activity of the Tweety homolog family of volume regulated anion channels, as well as the structural basis of organic anion transport by an OATP transporter. The common theme of these projects is that they are membrane proteins of biomedical significance that require thorough biochemical optimization for successful structural analysis. Our access to NCCAT microscope resources will further the development of these membrane protein projects and will directly contribute to my development as a scientist in training.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2011-2012	Undergraduate Research Volunteer, 1 year, University of Central Florida
2012-2014	Graduate Teaching Assistant in Microbiology, University of Central Florida
2014-2015	Graduate Research Assistant, University of Central Florida
2013-2015	Event Planning Committee Member, UCF Biomedical Sciences Graduate Student Association
2015-Present	Graduate Research Assistant, Columbia University
2015-2016	Graduate Student Organization Social Committee, Columbia University
2017-Present	Mentor for High School Students in the Minds Matter Science Matters Research Internship

Academic and Professional Awards

2008-2012	Selected as Florida Bright Futures Medallion Scholar, which paid 75% of tuition at all Florida public universities for four years
2009-2012	Dean's List, University of Central Florida, 6 semesters
2011-2012	Active Member of Delta Epsilon Iota UCF Chapter, Academic Honor Society
2014	Selected for Kalyani Parthasarathy Award for 1st Place M.S. Presentation at the UCF 9th annual Graduate Research Symposium, which included a cash prize
2017-2018	Selected for the Training Program in Molecular Biophysics, Training Grant T32 5T32GM008281-30, NIGM
2018	Selected as Fisher Award Recipient based on research progress (Columbia Internal Award - covered registration costs at the COMPPÅ Symposium on Membrane Protein Production and Analysis)

Memberships in Professional Societies

2013-2015	American Heart Association, Student/Trainee Member
2015-2016	NYAS, Student Member
2020-2021	American Crystallographic Association, Student Member
2021-2022	Biophysical Society, Student Member

Professional Meetings, Posters, and Presentations

2014	Selected for Poster Presentation, "Genetically-programmed suicide of adrenergic cells in mice produces left ventricular dysfunction as revealed by high-resolution echocardiography." Abstract #17028. At the American Heart Association Scientific Sessions in Chicago, IL.
2015	Selected for Poster Presentation, "Selective destruction of adrenergic cells in mice leads to severe left-ventricular dysfunction at rest with apparent stress-induced recovery." Abstract #197. At the American Heart Association Basic Cardiovascular Sciences (BCVS) Scientific Sessions in New Orleans, LA.
2016	Attended New York Structural Biology Discussion Group Summer Meeting
2017	Attended New York Structural Biology Discussion Group Winter Meeting
2017	Attended New York Structural Biology Discussion Group Summer Meeting
2017	Attended Center on Membrane Protein Production and Analysis (COMPPÅ) Annual Meeting
2018	Attended New York Structural Biology Discussion Group Winter Meeting
2018	Attended New York Structural Biology Discussion Group Summer Meeting
2018	Attended Center on Membrane Protein Production and Analysis (COMPPÅ) Symposium on Membrane Protein Production and Analysis, Fisher Award Recipient
2019	Registered to Attend New York Structural Biology Discussion Group Winter Meeting
2020	Poster Presentation (Canceled due to COVID-19) at Understanding Biology Through Structure 2020.
2021	Selected for Poster Presentation, "Structural and Functional Characterization of the Bestrophin-2 Anion Channel." At the Biophysical Society 2021 Annual Meeting. (Virtual).

C. Contributions to Science

C.1 Undergraduate Research at the University of Central Florida. I spent one year volunteering in the lab of Dr. Steven Ebert at the University of Central Florida. During this time, I learned basic lab techniques used to study heart development. This was my first exposure to hands-on biomedical research and it led to my pursuit of a Master's of Science with a thesis.

C.2 Master of Science Thesis at The University of Central Florida. I worked in Dr. Ebert's lab for one year prior to beginning my thesis work in the Biotechnology MS program. I found the field of cardiovascular development exciting and led a study to identify the role of a specific cardiomyocyte progenitor cells in heart development. My work focused on the role of progenitor cells that express phenylethanolamine-N-methyltransferase (Pnmt), the biosynthetic enzyme for adrenaline, and their contribution to working myocardium in the adult. I received an award for 1st place Master's Presentation for my oral presentation of this work at the UCF 9th Annual Graduate Research Symposium in 2014. My completion of this program required formation of a thesis committee, an oral thesis defense, and a written thesis submission. I was also a Graduate Teaching Assistant for these three years and received a full tuition waver and a yearly stipend.

1. **Owji, AP**, Genetically-programmed suicide of adrenergic cells in the mouse leads to severe left ventricular dysfunction, impaired weight gain, and symptoms of neurological dysfunction. (**2015**). *Electronic Theses and Dissertations*. 1492. https://stars.library.ucf.edu/etd/1492
2. **Owji AP**, Varudkar N, Ebert SN. Therapeutic potential of Pnmt+ primer cells for neuro/myocardial regeneration. American Journal of Stem Cells. **2013**;2(3):137-54. Epub 2014/01/08. PMID:24396707
3. **Owji AP**, Baker CN, Jacob JL, Tumuluri L, Ebert SN. Genetically-programmed suicide of adrenergic cells in the mouse leads to severe left ventricular dysfunction, impaired weight gain, and neurological dysfunction. (Manuscript in preparation)
4. Baker CN, Katsandris R, **Owji AP**, Goldblatt G, Van C, and Ebert SN. Echocardiographic and Histological Analysis of Left Ventricular Function in Stress-Challenged Aged Mice: Effects of Gender and Menopause. (Manuscript in preparation)

C3. Graduate Research at Columbia University

My ongoing predoctoral research is focused on understanding the molecular mechanisms of calcium-dependent activation and inactivation in mammalian bestrophin channels. Specifically, I use cryoEM to study how this channel responds to activating and inactivating levels of calcium, as well as the mechanism of potentiation by ATP. Bestrophins are a family of Ca^{2+}-activated Cl^- channels expressed in a variety of human tissues. The Best2 isoform is localized to the basolateral plasma membrane of nonpigmented ciliary epithelial cells of the nonpigmented epithelium of the ciliary body and is required for the maintenance of intraocular pressure. I have recently used cryoEM to solve the first structure of a mammalian bestrophin channel, which is also the first Best2 structure. These structures, coupled with functional experiments, reveal regions of the channel responsible for gating and selectivity and have distinct differences from the Best1 channel. Ongoing areas of investigation on this project include structural analysis of human bestrophins and mechanisms of general chloride channel inhibitors.

1. **Owji AP**, Zhao Q, Ji C, Kittredge A, Hopiavuori A, Fu Z, Ward N, Clarke OB, Shen Y, Zhang Y, Hendrickson WA, Yang T. Structural and functional characterization of the bestrophin-2 anion channel. Nat Struct Mol Biol. 2020 Apr;27(4):382-391. doi: 10.1038/s41594-020-0402-z. Epub 2020 Apr 6. PMID: 32251414; PMCID: PMC7150642.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Tingting Yang

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

POSITION TITLE: Assistant Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Fudan University, Shanghai, China	B.S.	07/2001	Biological sciences
Fudan University, Shanghai, China	M.S.	07/2004	Microbiology
Johns Hopkins University, Baltimore, MD mm	M.S.E.	05/2008	Applied Math and Statistics
Johns Hopkins University, Baltimore, MD	Ph.D.	05/2010	Ion channel function (Colecraft lab)
Columbia University, New York, NY	Postdoc	05/2010-08/2012*	Ion channel function (Colecraft lab)
Columbia University, New York, NY	Postdoc	09/2012-12/2015	Ion channel structure (Hendrickson lab)

**09/10-08/11: left science for family reasons. Colecraft lab moved from Hopkins to Columbia in 2007.*

A. Personal Statement

Employing multidisciplinary approaches including cryoEM, crystallography, electrophysiological recording, CRISPR/Cas9, gene therapy and stem cell reprogramming/differentiation, my lab studies the structure, function and regulation of disease-related ion channels.

1. Owji AP, Zhao Q, Ji C, Kittredge A, Hopiavuori A, Fu Z, Ward N, Clarke OB, Shen Y, Zhang Y, Hendrickson WA, **Yang T**. Structural and functional characterization of the bestrophin-2 anion channel. *Nat Struct Mol Biol*, 2020; 27(4): 382-391
2. Zhang Y, Kittredge A, Ward N, Ji C, Chen S, **Yang T**. ATP activates bestrophin ion channels through direct interaction. *Nat Commun*, 2018; 9(1): 3126
3. Ji C, Kittredge A, Hopiavuori A, Ward N, Chen S, Fukuda Y, Zhang Y, **Yang T**. Dual Ca^{2+}-dependent gates in human Bestrophin1 underlie novel disease-causing mechanisms of gain-of-function mutations. *Commun Biol*, 2019; 2:240
4. Li Y, Zhang Y, Xu Y, Kittredge A, Ward N, Chen S, Tsang SH, **Yang T**. Patient-specific mutations impair BESTROPHIN1's essential role in mediating Ca^{2+}-dependent Cl^- currents in human RPE. *eLife*, 2017; 6. pii: e29914
5. Ji C, Li Y, Kittredge A, Hopiavuori A, Ward N, Yao P, Fukuda Y, Zhang Y, Tsang SH, **Yang T**. Investigation and restoration of BEST1 activity in patient-derived RPEs with dominant mutations. *Sci Rep*, 2019; 9(1):19026

B. Positions and Honors

Positions and Employment

Assistant Professor, Ophthalmology, Columbia University	2019-present
Assistant Professor, Pharmacology and Physiology, University of Rochester	2016-2019
Associate Research Scientist, Biochemistry and Molecular Biophysics, Columbia University	2015
Postdoc Research Scientist, Biochemistry and Molecular Biophysics, Columbia University	2012-2015
Postdoc Research Scientist, Physiology and Cellular Biophysics, Columbia University	2010, 2011-2012

Honors

Schaefer Research Scholar Award	2021
Irma T. Hirsch/Monique Weill-Caulier Trusts Research Award	2021
Target-of-Opportunity Faculty Recruitment Award, CUIMC	2019
NIH Pathway to Independence Award (K99/R00)	2015
Symposium Award, Society of General Physiologists	2015
Travel Award, Biophysical Society	2012
Phi Beta Kappa National Academic Honor Society	2010
Student Research Achievement Award, Biophysical Society	2009
Student Travel Grant, Biophysical Society	2009
Physiology Retreat Poster Award, 1st Prize, Columbia University	2009

C. Contributions to Science

1. Bestrophins belong to a family of calcium-activated chloride channels that have four members (Best1-4) in mammals and play important roles in humans. We solved the first Best1 and Best2 structures, discovered critical channel properties, and identified ATP as an evolutionarily conserved interacting activator of bestrophins. These findings provide valuable information of the biophysics of bestrophin ion channels.

a. Owji AP, Zhao Q, Ji C, Kittredge A, Hopiavuori A, Fu Z, Ward N, Clarke OB, Shen Y, Zhang Y, Hendrickson WA, **Yang T**. Structural and functional characterization of the bestrophin-2 anion channel. *Nat Struct Mol Biol*, 2020; 27(4): 382-391
b. Ji C, Kittredge A, Hopiavuori A, Ward N, Chen S, Fukuda Y, Zhang Y, **Yang T**. Dual Ca^{2+}-dependent gates in human Bestrophin1 underlie novel disease-causing mechanisms of gain-of-function mutations. *Commun Biol*, 2019; 2:240
c. Zhang Y, Kittredge A, Ward N, Ji C, Chen S, **Yang T**. ATP activates bestrophin ion channels through direct interaction. *Nat Commun*, 2018; 9(1): 3126
d. **Yang T**, Liu Q, Kloss B, Bruni R, Kalathur RC, Guo Y, Kloppmann E, Rost B, Colecraft HM, Hendrickson WA. Structure and selectivity in bestrophin ion channels. *Science*, 2014; 346(6207): 355-9

2. Genetic mutations of the human *BEST1* gene are associated with retinal degenerative diseases. We demonstrated the physiological role of Best1 in mediating Ca^{2+}-dependent Cl^- current in RPE cells, elucidated disease-causing mechanisms of *BEST1* patient-derived mutations, and established gene therapy for bestrophinopathies.

a. Ji C, Li Y, Kittredge A, Hopiavuori A, Ward N, Yao P, Fukuda Y, Zhang Y, Tsang SH, **Yang T**. Investigation and restoration of BEST1 activity in patient-derived RPEs with dominant mutations. *Sci Rep*, 2019; 9(1):19026
b. Kittredge A, Ji C, Zhang Y, **Yang T**. Differentiation, maintenance and analysis of human retinal pigment epithelium cells: a disease-in-a-dish model for BEST1 mutations. *J Vis Exp*, 2018; (138). doi:10.3791/57791
c. Li Y, Zhang Y, Xu Y, Kittredge A, Ward N, Chen S, Tsang SH, **Yang T**. Patient-specific mutations impair BESTROPHIN1's essential role in mediating Ca^{2+}-dependent Cl^- currents in human RPE. *eLife*, 2017; 6. pii: e29914
d. **Yang T**, Justus S, and Li Y, Tsang SH. BEST1: the best target for gene and cell therapies. *Molecular Therapy*, 2015; 23(12): 1805-9

3. TMEM16A and TMEM16B are two Ca^{2+}-activated Cl^- channels in the TMEM16 family of membrane proteins. They both have important (patho)/physiological roles and are potential pharmaceutical targets. We discovered the regulatory mechanisms of calmodulin in modulating the activities of TMEM16A/TMEM16B channels.

a. **Yang T**, Colecraft HM. Calmodulin regulation of TMEM16A and 16B Ca^{2+}-activated chloride channels. *Channels,* 2016; 10(1): 38-44
b. **Yang T***, Hendrickson WA*, Colecraft HM*. Preassociated apocalmodulin mediates Ca^{2+}-dependent sensitization of activation and inactivation of TMEM16A/16B Ca^{2+}-gated Cl^- channels. *PNAS*, 2014; 111(51): 18213-8 (*corresponding authors)

4. High-voltage-activated Ca^{2+} channel blockers have broad biotechnological and therapeutic applications. We established a general method for developing novel genetically encoded Ca^{2+} channel blockers, termed 'channel inactivation induced by membrane-tethering of an associated protein' (ChIMP). Importantly, this method can be extended to other ion channels.

a. **Yang T**, He LL, Chen M, Fang K, Colecraft HM. Bio-inspired voltage-dependent calcium channel blockers. *Nat Commun,* 2013; 4: 2540
b. **Yang T**, Suhail Y, Dalton S, Kernan T, Colecraft HM. Genetically encoded molecules for inducibly inactivating Ca_V channels. *Nat Chem Biol,* 2007; 3(12): 795-804

5. RGK proteins belong to the Ras superfamily of monomeric G-proteins, and are the most potent known intracellular inhibitors of high-voltage-activated Ca^{2+}channels. We extensively dissected the mechanisms of RGK mediated inhibition.

a. **Yang T**, Colecraft HM. Regulation of voltage-dependent calcium channels by RGK proteins. *Biochim Biophys Acta,* 2013; 1828(7): 1644-54
b. **Yang T***, Puckerin A, Colecraft HM*. Distinct RGK GTPases differentially use α_1- and auxiliary β- binding-dependent mechanisms to inhibit $Ca_V1.2$/$Ca_V2.2$ channels. *PLoS One,* 2012; 7(5): e37079 (*corresponding authors)
c. **Yang T**, Xu X, Kernan T, Wu V, Colecraft HM. Rem, a member of the RGK GTPases, inhibits recombinant $Ca_V1.2$ channels using multiple mechanisms that require distinct conformations of the GTPase. *J physiol,* 2010; 588(Pt 10): 1665-1681 (Cover Article)

Complete List of Published Work
https://www.ncbi.nlm.nih.gov/sites/myncbi/143HCMDekGtAQ/bibliography/48622784/public/?sort=date&direction=descending

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

Ongoing Research Support

R01 GM127652 (Yang, PI) 05/01/2018 – 03/30/2023
NIH/NIGMS
Mechanistic Characterization of Calcium-Activated Chloride Channels in Retinal Pigment Epithelium
This project aims to define the physiological roles of three candidate calcium-activated chloride channels (BEST1, TMEM16A and TMEM16B) in RPE.

R24 EY028758 (Yang, I) 06/01/2020 – 05/31/2025
NIH/NEI
Therapeutic gene editing and multimodal imaging in juvenile macular degeneration
This project aims for the clinical treatment of juvenile macular degeneration.

Irma T. Hirschl Research Award (Yang, PI) 01/01/2021 – 12/31/2025
Irma T. Hirschl Trusts
Structural and functional investigations of BEST1 patient-derived mutations
This project aims to investigate patient-derived mutations that results in distinct clinical phenotypes in different patients with the same *BEST1* genotype and newly identified gain-of-function mutations.

BIOGRAPHICAL SKETCH

NAME:	Wayne A. Hendrickson
eRA COMMONS USER NAME:	hendricksonw
POSITION TITLE:	University Professor

EDUCATION/TRAINING:

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Wisconsin at River Falls	B. A.	05/1963	Physics / Biology
Johns Hopkins University, Baltimore, MD	Ph. D.	01/1968	Biophysics
Johns Hopkins University, Baltimore, MD	Postdoc	09/1969	Biophysics
Naval Research Laboratory, Washington, DC	Postdoc	09/1971	Structure of Matter

A. Personal Statement

I feel well qualified to have substantial impact in the proposed program of research; my research interests and motivation as well as my experience and accomplishments are well aligned with aims of the proposal. Our laboratory works to advance diffraction and cryo-EM methods for analyzing biological structure, and we use such technology together with biochemical and cellular analyses to study biological molecules in atomic detail. Our current emphasis is on molecular chaperones and protein folding, on membrane receptors and cellular signaling, on viral proteins and HIV infection, and on technology for membrane protein production and analysis.

B. Positions and Honors

1971 - 1984 Research Biophysicist, Naval Research Laboratory, Washington, DC
1986 - 2012 Investigator, Howard Hughes Medical Institute (HHMI)
2009 - 2015 Chief Life Scientist, Photon Sciences Directorate, Brookhaven National Laboratory
1984 - Professor of Biochemistry and Molecular Biophysics,
College of Physicians and Surgeons, Columbia University, New York, NY
2001 - University Professor, Columbia University
2008 - Violin Family Professor of Physiology & Cellular Biophysics, Columbia University
2010 - Scientific Director, New York Structural Biology Center (NYSBC)

Washington Academy of Sciences Award in Biological Sciences (1976)
Arthur S. Flemming Award for Outstanding Young Federal Employees (1979)
A.L. Patterson Award of the American Crystallographic Association (1981)
Distinguished Alumnus Award, University of Wisconsin at River Falls (1984)
Fellow of the American Association for the Advancement of Science (1984)
Johns Hopkins Society of Scholars (1986)
Fritz Lipmann Award of the American Society for Biochemistry and Molecular Biology (1991)
Fellow of the American Academy of Arts and Sciences (1992)
Stevens Triennial Prize, Columbia University, College of Physicians and Surgeons (1992)
Member of the National Academy of Sciences (1993)
Doctor of Philosophy *honoris causa*, Uppsala University (1995)
Aminoff Prize, Royal Swedish Academy of Sciences (1997)
Christian B. Anfinsen Award, Protein Society (1997)
Alexander Hollaender Award, National Academy of Sciences (1998)
Doctor of Science *honoris causa*, Mount Sinai School of Medicine (2000)
Compton Award, Advanced Photon Source of Argonne National Laboratory (2001)
Academy Medal, New York Academy of Medicine (2003)
Gairdner International Award (2003)
Paul Janssen Prize (with M.G. Rossmann), Rutgers University (2004)

B. Honors, continued

Harvey Prize, Technion - Israel Institute of Technology (2004)
Mayor's Award for Excellence in Science & Technology, New York City (2005)
Kaj Linderstrøm-Lang Prize, Carlsberg Laboratory (2008)
Einstein Professorship of the Chinese Academy of Sciences (2012)
Doctorate *honoris causa* in Biochemistry, Sapienza University of Rome (2016)
iHuman Structure of Life Award, ShanghaiTech University (2018)

C. Contribution to Science

1. *Diffraction methods and synchrotron radiation.* Our laboratory has been engaged in the development of methods for diffraction analysis of biological structure for a long time. Early contributions include widely used phasing coefficients (Hendrickson & Lattman, 1970), the introduction of stereochemically restrained refinement of crystal structures (Hendrickson & Konnert, 1980; Konnert & Hendrickson, 1980), and the structural analysis of crambin based solely on anomalous scattering from sulfur atoms (Hendrickson & Teeter, 1981). The crambin structural analysis established what is now known as the single-wavelength anomalous diffraction (SAD) method and paved the way for his development of the multi-wavelength anomalous diffraction (MAD) method (Hendrickson, 1985; Hendrickson et al., 1988). Broad utility of the MAD method followed when we recognized that selenium could serve as a rich source for the required diffraction signals (Hendrickson et al., 1989) and that selenomethionine (SeMet) could be substituted readily for the natural amino acid methionine (Hendrickson *et al*., 1990; Yang *et al*., 1990). We tested MAD phasing in applications at synchrotrons around the world, and we developed National Synchrotron Light Source (NSLS) beamlines X4A and X4C at Brookhaven National Laboratory to optimize the MAD experiment (Staudenmann *et al*., 1989). Subsequentlly, we advanced methods for SAD phasing analysis of native macromolecules, using low x-ray energy to enhance anomalous signals and multiple crystals to reduce noise (Liu *et al*., 2012; Liu *et al*., 2013). MAD beamlines were emulated around the world; and MAD and SAD methods now dominate in biological crystallography, producing many hundreds of new structures each year (Hendrickson, 2014). We are now developing new synchrotron beamlines at NSLS-II for optimized anomalous diffraction analyses.

a. W.A. Hendrickson* and M.M. Teeter, Structure of the Hydrophobic Protein Crambin Determined Directly from the Anomalous Scattering of Sulfur. *Nature* **290**, 107-113 (1981). PMCID: PMC 5536114

b. W.A. Hendrickson*, J.R. Horton and D.M. LeMaster, Selenomethionyl Proteins Produced for Analysis by Multiwavelength Anomalous Diffraction (MAD): A Vehicle for Direct Determination of Three-Dimensional Structure. *EMBO J.* **9**, 1665-1672 (1990). PMCID: PMC551863

c. Q. Liu, T. Dahmane, Z. Zhang, Z. Assur, J. Brasch, L. Shapiro, F. Mancia and W.A. Hendrickson*. Structures from Anomalous Diffraction Data of Native Biological Macromolecules. *Science* **336**, 1033-1037 (2012). PMCID: PMC3769101

d. W.A. Hendrickson*, Anomalous Diffraction in Crystallographic Phase Evaluation. *Quarterly Reviews of Biophysics* **47**, 49-93 (2014). PMCID: PMC4128195

2. *Efficient production and analysis of membrane proteins.* Working with several colleagues, I lead the Consortium on Membrane Protein Production and Analysis (COMPPÅ), a Biomedical Technology Research Resource that is a successor to our New York Consortium on Membrane Protein Structure (NYCOMPS). With NYCOMPS, we created an efficient pipeline for the expression and production of membrane proteins nominated by the community at large and for our own effort to improve characterization of the universe of membrane proteins. Candidates identified by the protein production group at NYSBC were distributed to associated laboratories for scaled-up protein production and structure analysis. The NYCOMPS pipeline became highly productive (Punta *et al*., 2009; Love *et al*., 2010) and it led to several published structural analyses, most of which were accompanied by substantial functional characterization.

My own laboratory participates actively in the COMPPÅ development of technology for protein production and structure determinations (Liu *et al*., 2012), and we are highly engaged in resulting structure determinations for membrane proteins. Our first NYCOMPS structure was that of bacterial TehA, which proved to be homologous to the SLAC1 anion channel that control stomatal closure in plant leaves in response to darkness and to environmental factors such as drought and high CO_2 levels. We determined TehA structures with extraordinary detail (down to 1.15Å resolution), and we characterized the channel activity of both TehA and

Arabidopsis SLAC1 (Chen et al., 2010). More recently, we determined the structure of a bacterial homolog of human bestrophin 1 (Yang *et al*., 2014), known for its association with early-onset macular degeneration; and we have analyzed tryptophan-rich sensory proteins (TSPOs), for which we tested structure-inspired hypotheses to establish a previously unappreciated role of TSPO proteins in degrading porphyrins for the control of reactive oxygen species (Guo *et al.*, 2015). I have also contributed to several other NYCOMPS/COMPPÅ structure analyses; these include a homolog of the anti-apoptotic calcium-leak channel, BI-1 (Chang *et al.,* 2014), the retinol uptake receptor STRA6 (Chen *et al.*, 2016), and trimeric intracellular cation (TRIC) channels (Su *et al*., 2017; Wang et al., 2019).

a. Y.-H. Chen, L. Hu, M. Punta, R. Bruni, B. Hillerich, B. Kloss, B. Rost, J. Love, S.A. Siegelbaum and W.A. Hendrickson*, Homologue Structure of the SLAC1 Anion Channel for Closing Stomata in Leaves. *Nature* **467**, 1074-1080 (2010). PMCID: PMC3548404

b. T. Yang, Q. Liu, B. Kloss, R. Bruni, R. Kalathur, Y. Guo, E. Kloppmann, B. Rost, H.M. Colecraft and W.A. Hendrickson*, Structure and Selectivity in Bestrophin Ion Channels. *Science* **346**, 355-359 (2014). PMCID: PMC4341822

c. Y. Guo, R. Kalathur, Q. Liu, B. Kloss, R. Bruni, C. Ginter, E. Kloppmann, B. Rost and W.A. Hendrickson*, Structure and Activity of Tryptophan-rich TSPO Proteins. *Science* **347**, 551-555 (2015). PMCID: PMC4341906

d. X. Wang, M. Su, F. Gao, W. Xie, Y. Zeng, D. Li, X. Liu, H. Zhao, L. Qin, F. Li, Q. Liu, O.B. Clarke, S.M. Lam, G. Shui, W.A. Hendrickson* and Y. Chen*. Structural basis for activity of TRIC counter-ion channels in calcium release. *Proc. Natl. Acad. Sci. USA* **116**, 4238-4243 (2019). PMCID: PMC6410872

3. *Molecular chaperones and protein folding.* The 70kD family of heat shock protein (Hsp70) chaperones is ubiquitous, having involvement in diverse activities in all organisms. Others had characterized the ATPase domain of Hsp70s and we determined the first structure of an Hsp70 substrate-binding domain, that of DnaK as associated with a high-affinity peptide (Zhu *et al*., 1996). The nature of allosteric interaction between the ATPase and substrate-binding units in the chaperone cycle remained elusive, however. Our structure of yeast Sse1 (Liu & Hendrickson, 2007), an Hsp110 family member and clear relative of Hsp70s based on its structure, provided a clear picture for these interactions. It showed remarkable change in conformation relative to that in domains from Hsp70s. Biochemical tests of a battery of interface mutations in Sse1 and its DnaK homologs informed us about general modes of conformational change and ATPase action. The Sse1-inspired model for allosteric interactions was confirmed in a full-length Hsp70 structure (Qi et al., 2013), for which we collaborated. *In vitro* biochemical tests of several of the DnaK mutants inspired a new theory for the chaperone cycle (Hendrickson, 2000) and this theory has inspired the generation of mutant-stabilized ATP states that have succumbed to crystallization (Wang & Hendrickson, 2021; Wang *et al*., 2021). In addition to our work on Hsp70 molecules, we have also made progress on other molecular chaperones including trigger factor (Martinez-Hackert & Hendrickson, 2009) and Boca/MESD (Collins & Hendrickson, 2011). In addition, we have analyzed the role played by coiled-coil interactions in aggregations associated with protein folding disorders (Fiumara *et al*., 2010).

a. X. Zhu, X. Zhao, W.F. Burkholder, A. Gragerov, C.M. Ogata, M.E. Gottesman and W.A. Hendrickson*, Structural Analysis of Substrate Binding by the Molecular Chaperone DnaK. *Science* **272**, 1606-1614 (1996). PMCID: PMC5629921

b. Q. Liu and W.A. Hendrickson*, Insights into Hsp70 Chaperone Activity from a Crystal Structure of the Yeast Hsp110 Sse1. *Cell* **131**, 106-120 (2007). PMCID: PMC2041797

c. F. Fiumara, L. Fioriti, E.R. Kandel* and W.A. Hendrickson*, Essential Role of Coiled-Coils for Aggregation and Activity of Q/N-rich Prions and PolyQ Proteins. *Cell* **143**, 1121-1135 (2010). PMCID: PMC3472970

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4. *Membrane receptors and cellular signaling.* An important emphasis of our research concerns the initial phases of cellular signal transduction, focusing primarily on the biochemical and biophysical aspects of signal transduction across membranes by major signaling systems (Hendrickson, 2005). In most cases, the signal-initiating stimulus from the environment is chemical; it may be a small compound, a macromolecular hormone or growth factor, or even another cell. Receptors embedded in the cellular membrane mediate transmission of signaling into the cell. Our interest lies in the mechanisms by which biochemical signals are transduced across

the membrane. We concentrate on the integral membrane receptor proteins, but relevant extra-membranous components are also studied.

Much of our earlier work related to receptor tyrosine kinases, including the T-cell co-receptor CD4 (Ryu *et al*., 1990; Wu *et al*., 1997), the insulin-receptor tyrosine kinase (Hubbard *et al*., 1994), lymphocyte kinase (Yamaguchi & Hendrickson, 1996), and fibroblast growth factor receptors (Stauber *et al*., 2000). We also work on G-protein coupled receptor systems, including glycoprotein hormone receptors for chorionic gonadotropin (Wu *et al*., 1994) and follicle-stimulating hormone (Fan & Hendrickson, 2005) and canonical receptors for serotonin (Mancia *et al*., 2008). Histidine kinase receptors are another major focus. These efforts have produced many results on sensory domains (PhoQ: Cheung *et al*., 2008; DcuS and DctB: Cheung & Hendrickson, 2008; NarX: Cheung & Hendrickson, 2009; HK1: Zhang & Hendrickson, 2010; TorT/TorS: Moore & Hendrickson, 2012; HK3, Zhang *et al*., 2014) and some on cytoplasmic domains, including the first entire cytoplasmic portion (Marina & Hendrickson, 2005). Finally, we are studying ion-channel receptors, including cys-loop receptors and other ligand-activated channels. Recent focus is on cryo-EM studies on calcium-release channel known as the ryanodine receptor (Zalk *et al*., 2015; des Georges *et al*., 2016) and on bestrophins (Owji et al., 2020).

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5. *Viral proteins and HIV infection.* The foundation of our work on interactions of the HIV envelope proteins with cellular receptors lies in structures of complexes between HIV gp120 and both its the cellular receptor CD4 and a neutralizing antibody bound to the co-receptor binding site. These were determined both for a laboratory adapted R4 strain, HxBc2 (Kwong et al., 1998), and for a primary R5 isolate, Yu2 (Kwong et al. 2000); in each case CD4 was represented by the D1D2 binding fragment and the antibody component was the human 17b Fab fragment. We had previously determined structures for soluble CD4 (Ryu *et al*., 1990; Wu *et al*., 1997). We subsequently carried out studies on the thermodynamics of gp120-ligand interactions (Myszka et al., 2000; Kwong et al., 2002), and we have determined a number of additional structures including complexes with CD4 mimetics (Huang et al., 2005). Recent work focuses on the development of antagonists of the gp120-CD4 interaction. Toward this end, we devised a chemical design for derivatives of F43C CD4 (D1D2) in which cysteine adducts bind into the Phe43 interfacial cavity (Xie et al., 2007), and we have determined four structures of such complexes. More recently, we have determined structure of small-molecule entry inhibitors and are using structure-based design methods to develop these compounds (Melillo *et al*., 2016).

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b. P.D. Kwong, R. Wyatt, J. Robinson, R.W. Sweet, J. Sodroski and W.A. Hendrickson*, Structure of an HIV gp120 Envelope Glycoprotein in Complex with the CD4 Receptor and a Neutralizing Human Antibody. *Nature* **393**, 648-659 (1998). PMCID: PMC5629912

c. H. Xie, D. Ng, S.N. Savinov, B. Dey, P.D. Kwong, R. Wyatt, A.B. Smith III and W.A. Hendrickson*, Structure-Activity Relationships in the Binding of Chemically Derivatized CD4 to gp120 from Human Immunodeficiency Virus. *J. Med. Chem.* **50**, 4898-4908 (2007). PMCID: PMC2532594

d. B. Melillo, S. Liang, J. Park, A. Shön, J.R. Courter, J.M. LaLonde, D.J. Wendler, A.M. Princiotto, M.S. Seaman, E. Friere, J. Sodroski, N. Madani, W.A. Hendrickson and A.B. Smith, III*. Small-molecule CD4-mimics: Structure-based Optimization of HIV-1 Entry Inhibitors. *ACS Med. Chem. Lett.* **7**, 330-334 (2016). PMCID: PMC4789667

Complete List of Published Work
in NCBI MyBibliography: http://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/47371322/

D. Research Support

ACTIVE RESEARCH SUPPORT

R01 GM107462-07 (Hendrickson, PI) 09/21/18 - 08/31/22
NIH
Atomic Level Analysis of Biomolecular Structure

This grant supports our efforts to improve methods for structural analysis of biological macromolecules. Proposed activities include efforts at analyses by cryo-EM as well as by x-ray diffraction, particularly exploiting phase information from anomalous diffraction. We drive methods with challenging applications.

P01 AI150471-24 (Chaiken, PI) 09/01/18 - 08/31/23
NIH
Structure-Based Antagonism of HIV-1 Envelope Function in Cell Entry

This grant (Irwin M. Chaiken, Drexel University, P.I.) supports a program project aimed at designing effective drugs to treat AIDS by blocking HIV-1 entry into cells. Our component concerns the structural analysis of HIV-1 entry inhibition by crystallography, biochemical analysis and computation.

Role: PI of Project 5 of this Program Project

R01 NS109366-02 (Siegelbaum, contact PI) 08/15/19 - 06/30/24
NIH
Structural Studies of HCN Channels in Health and Disease

This grant supports studies on the structure, regulation and disease-causing mutations in HCN4 cAMP-activated cation channels. I participate as a PI together with Steve Siegelbaum (contact PI) and other investigators.

Role: PI

P41 GM116799-05 (Hendrickson, PI) 05/01/16 - 04/30/21
NIH
Center on Membrane Protein Production and Analysis (COMPPÅ)

This grant to NYSBC supports a Biomedical Technology Research Resource that enables structural and functional studies on membrane proteins through technological research and development and through service to and collaboration with the research community. It provides no direct support to my laboratory.