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 viral proteins and HIV infection, on molecular chaperones and protein folding, on membrane receptors and cellular signaling.

This application seeks an allocation of electron microscope time for analyses related to our work on Hsp70 molecular chaperones. We have ample experience in Hsp70 crystallography, but we have not yet published any cryo-EM structures on Hsp70 proteins. We do, however, have several publications on other cryo-EM structural studies. Four of our publications are especially relevant to the proposal:

a. W. Wang, Qun Liu, Qinglian Liu and W.A. Hendrickson*. Conformational Equilibria in Allosteric Control of Hsp70 Chaperones. *Molecular Cell* **81**, 3919–3933 (2021). PMCID: PMC8500941
b. A. des Georges, O.B. Clarke, R. Zalk, Q. Yuan, K.J. Condon, R.A. Grassucci, W.A. Hendrickson*, A.R. Marks* and J. Frank*. Structural Basis for Gating and Activation of RyR1. *Cell* **167**, 145-157 (2016). PMCID: PMC5142848
c. A.P. Owji, Q. Zhao, C. Ji, A. Kittredge, A. Hopiavuori, Z. Fu, N. Ward, O.B. Clarke, Y. Shen*, Y. Zhang*, W. A. Hendrickson* and T. Yang*. Structural and Functional Characterization of Bestrophin2 Anion Channels. *Nat. Struct. Mol. Biol.* **27**, 382-391 (2020). PMCID: PMC7150642
d. Y. Deng, H. Kashtoh, Q. Wang, G. Zhen, Q. Li, L. Tang, H. Gao, C. Zhang, L. Qin, M. Su, F. Li, X. Huang, Y. Wang, Q. Xie, O.B. Clarke*, W.A. Hendrickson* and Y. Chen*. Structure and Activity of SLAC1 Channels for Stomatal Signaling in Leaves. *PNAS* 118, e2015151118 (2021). PMCID: PMC8106318

My research is supported by the following grants:

NIH P01 AI150471-25 (Chaiken, PI) 09/01/18 - 08/31/23
Structure-Based Antagonism of HIV-1 Envelope Function in Cell Entry
This grant (Irwin M. Chaiken, Drexel University, P.I.; Hendrickson, PI of Project 5) 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.

NIH R01 NS109366-03 (Siegelbaum, contact PI) 08/15/19 - 06/30/24
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.

B. Positions, Scientific Appointments, and Honors

Positions

2008 - Violin Family Professor of Physiology & Cellular Biophysics, Columbia University
2001 - University Professor, Columbia University
1984 - Professor of Biochemistry and Molecular Biophysics,
College of Physicians and Surgeons, Columbia University, New York, NY
2010 - 2023 Scientific Director, New York Structural Biology Center (NYSBC)
2009 - 2015 Chief Life Scientist, Photon Sciences Directorate, Brookhaven National Laboratory
1986 - 2012 Investigator, Howard Hughes Medical Institute (HHMI)
1971 - 1984 Research Biophysicist, Naval Research Laboratory, Washington, DC

Honors

Ewald Prize, International Union of Crystallography (2023)
iHuman Structure of Life Award, ShanghaiTech University (2018)
Doctorate *honoris causa* in Biochemistry, Sapienza University of Rome (2016)
Einstein Professorship of the Chinese Academy of Sciences (2012)
Kaj Linderstrøm-Lang Prize, Carlsberg Laboratory (2008)
Mayor's Award for Excellence in Science & Technology, New York City (2005)
Harvey Prize, Technion - Israel Institute of Technology (2004)
Paul Janssen Prize (with M.G. Rossmann), Rutgers University (2004)
Gairdner International Award (2003)
Academy Medal, New York Academy of Medicine (2003)
Compton Award, Advanced Photon Source of Argonne National Laboratory (2001)
Doctor of Science *honoris causa*, Mount Sinai School of Medicine (2000)
Alexander Hollaender Award, National Academy of Sciences (1998)
Christian B. Anfinsen Award, Protein Society (1997)
Aminoff Prize, Royal Swedish Academy of Sciences (1997)
Doctor of Philosophy *honoris causa*, Uppsala University (1995)
Member of the National Academy of Sciences (1993)
Stevens Triennial Prize, Columbia University, College of Physicians and Surgeons (1992)
Fellow of the American Academy of Arts and Sciences (1992)
Fritz Lipmann Award of the American Society for Biochemistry and Molecular Biology (1991)
Johns Hopkins Society of Scholars (1986)
Fellow of the American Association for the Advancement of Science (1984)
Distinguished Alumnus Award, University of Wisconsin at River Falls (1984)
A.L. Patterson Award of the American Crystallographic Association (1981)
Arthur S. Flemming Award for Outstanding Young Federal Employees (1979)
Washington Academy of Sciences Award in Biological Sciences (1976)

C. Contributions 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). Subsequently, 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. *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. W.A. Hendrickson*. Theory of Allosteric Equilibria in Hsp70 Molecular Chaperones. *QRB Discovery,* **1**, e7 1-12 (2020). PMCID: PMC7968864

d. W. Wang, Qun Liu, Qinglian Liu and W.A. Hendrickson*. Conformational Equilibria in Allosteric Control of Hsp70 Chaperones. *Molecular Cell* **81**, 3919–3933 (2021). PMCID: PMC8500941

3. *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 with recent focus is on cryo-EM studies, notably the ryanodine receptor calcium-release channel (Zalk *et al*., 2015; des Georges *et al*., 2016), bestrophins (Owji *et al.,* 2020) and the plant stomatal channel SLAC1 (Deng *et al*., 2021).

a. Q.R. Fan and W.A. Hendrickson*, Structure of Human Follicle Stimulating Hormone in Complex with its Receptor. *Nature* **433**, 269-277 (2005). PMCID: PMC5514322

b. A. des Georges, O.B. Clarke, R. Zalk, Q. Yuan, K.J. Condon, R.A. Grassucci, W.A. Hendrickson*, A.R. Marks* and J. Frank*. Structural Basis for Gating and Activation of RyR1. *Cell* **167**, 145-157 (2016). PMCID: PMC5142848

c. A.P. Owji, Q. Zhao, C. Ji, A. Kittredge, A. Hopiavuori, Z. Fu, N. Ward, O.B. Clarke, Y. Shen*, Y. Zhang*, W. A. Hendrickson* and T. Yang*. Structural and Functional Characterization of Bestrophin2 Anion Channels. *Nat. Struct. Mol. Biol.* **27**, 382-391 (2020). PMCID: PMC7150642

d. Z. Gong, W. Wang, K. El Omari, A.A. Lebedev, O.B. Clarke and W.A. Hendrickson. Crystal structure of LGR ligand α2/β5 from *C. elegans* with implications for the evolution of glycoprotein hormones. *Proc. Natl. Acad. Sci. USA* **120**, e2218630120 (2023). PMCID: PMC9910494

4. *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; Fritschi *et al*., 2021).

a. S.-E. Ryu, P.D. Kwong, A. Truneh, T.G. Porter, J. Arthos, M. Rosenberg, X. Dai, Ng.-h. Xuong, R. Axel, R.W. Sweet and W.A. Hendrickson*, Crystal Structure of an HIV-binding Recombinant Fragment of Human CD4. *Nature* **348**, 419-426 (1990). PMCID: PMC5638305

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. C. Fritschi, S. Anang, Z. Gong, M. Mohammadi, J. Richard, C. Bourassa, K.T. Severino, H. Richter, D. Yang, H.-C. Chen, T.-J. Chiu, M. Seaman, N. Madani, C. Abrams, A. Finzi, W.A. Hendrickson, J. Sodroski, and A.B. Smith, III. Indoline CD4-mimetic Compounds Mediate Potent and Broad HIV-1 Inhibition and Sensitization to Antibody-dependent Cellular Cytotoxicity. *Proc. Natl. Acad. Sci. USA* **120**, e2222073120 (2023). PMCID: PMC10068826

5. *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 TehA and *Arabidopsis* SLAC1 (Chen *et al*., 2010). We also determined structures of a bacterial homolog of human bestrophin 1 (Yang *et al*., 2014), associated with early-onset macular degeneration, and tryptophan-rich sensory proteins (TSPOs), establishing 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), and detergent-free extraction of AcrB captured a segment of lipid bilayer (Qiu *et al*., 2018).

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. 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

c. W. Qiu, Z. Fu, G. Xu, R.A. Grassucci, Y. Zhang, J. Frank, W.A. Hendrickson* and Y. Guo*. Structure and Activity of Lipid Bilayer within Multidrug Exporter AcrB. *Proc. Natl. Acad. Sci. USA* **115**, 12985-12990 (2018) PMCID: PMC4341822

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

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

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Wang, Wei

eRA COMMONS USER NAME: WANG2283

POSITION TITLE: Postdoctoral Research Scientist, Columbia University Irving Medical Center

EDUCATION/TRAINING:

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Columbia University Irving Medical Center, USA	Postdoctoral	10/2018-	Biochemistry, Structural Biology
Columbia University in the City of New York, USA	Ph.D.	09/2018	Biochemistry, Structural Biology
Tsinghua University, China	BS	07/2009	Biological Sciences

A. Personal Statement

My primary scientific interest lies in unraveling the complex processes underlying enzymatic reactions and the dynamic structural adaptations of proteins facilitating these processes. My passion for utilizing structural biology methods to decipher the functions of biological macromolecules took root early in my scientific career and has been a constant driving force ever since. My commitment was recognized when I received the prestigious NIH career transition K99/R00 award, a testament to my contribution to the development of structural biology methods aimed at understanding a broad spectrum of enzymatic reactions. Notably, my research focuses on the 70 kilo-dalton heat shock protein (Hsp70) system, a critical cellular machinery. This application seeks to deploy single-particle cryo-EM techniques to investigate the intricate interplay between Hsp70 and its co-chaperone Hsp40 during ATP hydrolysis, a key step in the allosteric reaction cycle of Hsp70. Insights gleaned from this study may offer new strategies to modulate their involvement in disease states.

My research is supported by the following grants:

NIH K99 GM147598-01 (WANG, WEI, contact PI) 08/15/22 - 07/31/27
Deciphering atomic-level enzymatic activity by time-resolved crystallography and computational enzymology
This grant aims to investigate enzymatic reactions through a combination of time-resolved crystallography and computational enzymology. Alongside this methodological advancement, I aim to devise a suite of innovative tools benchmarked against the 70-kDa heat shock protein (Hsp70) to further enhance the analytical capability.

Publications:

1. Z. Gong, W. Wang, K.E. Omari, A.A. Lebedev, O.B. Clarke and W.A.Hendrickson. *Crystal structure of LGR ligand α2/β5 from C. elegans with implications for the evolution of glycoprotein hormones.* Proc Natl Acad Sci USA. 120(1):e2218630120 (2023). PMCID: PMC9910494.
2. C. Wang, Z. Yang, B.J. Loughlin, H. Xu, G. Veit, S. Vorobiev, O.B. Clarke, F. Jiang, Y. Li, S. Singh, Z. Rich, E.R. Menten, R.A. Grassucci, W. Wang, A. Mezzell, Z. Fu, K.H. Wong, J. Wang, D.R. Wetmore, R.B. Sutton, C.G. Brouillette, I.L. Urbatsch, J.C. Kappes, G.L. Lukacs, J. Frank and J.F. Hunt. *Mechanism of dual pharmacological correction and potentiation of human CFTR*. bioRxiv 2022.10.10.510913
3. W. Wang, Qinglian Liu, Qun Liu and W.A. Hendrickson. *Conformational equilibria in allosteric control of Hsp70 chaperones*. Mol Cell. 81, 3919–3933 (2021). PMCID: PMC8500941.
4. W. Wang and W.A. Hendrickson. *Intermediates in allosteric equilibria of DnaK-ATP interactions with substrate peptides.* Acta Crystallogr D Struct Biol. 77, 606-617 (2021). PMCID: PMC8098474.
5. W. Wang, L. Wei, A. Yang, T. He, K.Y. Yuen, C. Chen and Z. Rao. *Expression, crystallization and preliminary crystallographic study of human coronavirus HKU1 nonstructural protein 9.* Acta Crystallogr Sect F Struct Biol Cryst Commun. 65, 526-528 (2009). PMCID: PMC2675602.

B. Positions and Scientific Presentations

Positions and Employment

2018 –	Postdoctoral Research Scientist, Columbia University Medical Center
2014 – 2015	Teaching Assistant, Biochemistry W3300 and Biology C2005/F2401, Columbia University in the City of New York
2012 – 2018	Graduate Research Assistant, Columbia University in the City of New York
2010 – 2012	Technician, Columbia University in the City of New York

Presentations

2023	ACA Annual Meeting, Baltimore, Maryland
2019	Summer meeting of New York Structural Biology Discussion Group

Honors

2009	Outstanding Undergraduate Research Award, Tsinghua University

C. Contributions to Science

1. **Early Career:** Crystallographic study of human coronavirus HKU1 nonstructural protein 9 (B.S. with Dr. Zihe Rao)

   Historical background: Since the outbreak of SARS coronavirus in 2003, several other human coronaviruses have been discovered. Nonstructural protein 9 was believed to be essential for viral RNA synthesis and replication. This work was among the earliest efforts trying to determine the nsp9 structure from human coronaviruses.

   Central findings: Being proposed as an RNA stabilizing protein, HKU1 nsp9 can be stabilized and crystallized without RNA substrate in the presence. The nature of two

molecules in the asymmetric unit seems resonant with the possibility of working as a dimer, as did with SARS-CoV nsp9.

My specific roles: I carried out cloning, expression, and crystallization trials leading to final successful crystals, which diffracted well to 2.7 Å. This work was the central part of my bachelor's degree thesis and helped grow my interest in structural biology, which I have pursued ever since.

Reference:

1. **Wang W**, Wei L, Yang A, He T, Yuen KY, Chen C, Rao Z. Expression, crystallization and preliminary crystallographic study of human coronavirus HKU1 nonstructural protein 9. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2009 May 1;65(Pt 5):526-8. PMCID: PMC2675602.

2. **Graduate Career:** Discovery of the intermediate states of Hsp70. (with Dr. Wayne Hendrickson)

Historical background: Hsp70s play a preeminent role in guarding the proper folding of proteins in all life forms, thus indispensable for cell functions. Hsp70 binds and hydrolyzes ATP at its nucleotide-binding domain (NBD), while it binds and releases the hydrophobic regions of peptide substrates at its substrate-binding domain (SBD). Hsp70s are allosteric molecular machines: ATP binding dramatically decreases substrate affinity for peptide substrates, and peptide substrates binding stimulates ATP hydrolysis. I postulated and determined the structure of two intermediate states, which reside downstream of the uncoupled apo state, but upstream of the restraining state. Before my work, only one state was known, no knowing it was the restraining state which restricts ATP hydrolysis; thus, the allostery details of Hsp70s were poorly understood.

Central findings: Besides the known stable state, I proved that intermediate states exist, determined the structures of two intermediate states, and explained the potential physiology importance associated with those two states.

My Specific roles: I was the lead author of the Hsp70 papers listed below. I was responsible for experiment design and conducting, structure determination, refinement and interpretation, and manuscript preparation.

Reference:

1. **Wang W**, Hendrickson WA. Intermediates in allosteric equilibria of DnaK-ATP interactions with substrate peptides. Acta Crystallogr D Struct Biol. 2021 May 1;77(Pt 5):606-617. PMCID: PMC8098474.

3. **Postdoctoral Career:** Discovery of the allosteric controlling mechanism of Hsp70. (with Dr. Wayne Hendrickson)

Central findings: I determined that the previously published stable state was the Hsp70 restraining state (R), which restricts ATP hydrolysis and binds peptide poorly. I proved the existence of a stimulating state (S), which hydrolyzes ATP rapidly and has high intrinsic polypeptide substrate affinity but rapid binding kinetics. For the first time, we described in this work the equilibria between the R and S states. I determined the S state structure, explained the biochemistry observation associated with the S state, and improved the R state structure by unambiguous polypeptide substrate-binding domain definition. I explained why the S state can have a high ATP hydrolysis rate, high polypeptide substrate

affinity, and high on-off rate simultaneously, by examining its structural details. Those findings from the S state provided foundations for the work described in this proposal.

My Specific roles: I was the lead author of the Hsp70 papers listed below. I am responsible for experiment design and conducting, structure determination, refinement and interpretation, and manuscript preparation. I presented the results at the meeting of the New York Structural Biology Discussion Group in Aug 2019.

Reference:

1. **Wang W**, Liu Q, Liu Q, Hendrickson WA. Conformational equilibria in allosteric control of Hsp70 chaperones. Mol Cell. 2021 Aug 26:S1097-2765(21)00623-7. PMCID: PMC8500941.

Development of new crystallographic structure solving method. (with Dr. Wayne Hendrickson)

Central findings: I developed a new method of solving protein crystal structures, by incorporation of *AlphaFold* and *Rosetta* force field. My colleague had a long-time intractable moderate-resolution dataset from a follicle-stimulating hormone complex that cannot be solved by current molecular replacement methods. Experimental phasing methods proved to be equally challenging. I predicted the model from *AlphaFold*, and morphed the model by *Rosetta* force field to allow straightforward molecular replacement solution of that structure. This is a new development of the method in submission.

My Specific roles: I was the lead author of the paper listed below. I am responsible for experiment design and conducting, scripts deployment, structure determination, refinement and interpretation, and manuscript preparation.

Reference:

1. **Wang W**, Hendrickson WA. Combining *AlphaFold* and *phenix.mr_rosetta* for solving challenging structures. Acta Crystallogr D Struct Biol. 2023 *Submitted*
2. Z. Gong, **W. Wang**, K.E. Omari, A.A. Lebedev, O.B. Clarke and W.A.Hendrickson. *Crystal structure of LGR ligand α2/β5 from C. elegans with implications for the evolution of glycoprotein hormones.* Proc Natl Acad Sci USA. 120(1):e2218630120 (2023). PMCID: PMC9910494.

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BIOGRAPHICAL SKETCH

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NAME: Wang, Chi

eRA COMMONS USER NAME (agency login): NA

POSITION TITLE: Associate Research Scientist

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Jilin University, China	B.S.	07/2000	Chemistry
Jilin University, China	M.S.	07/2003	Chemistry
Columbia University, USA	Ph.D.	02/2011	Biological Sciences

A. Personal Statement

My research has been using biochemical and biophysical methods to understand the mechanisms of complex biomolecular processes related to human diseases. Under the mentorship of Prof. John Hunt at Columbia University, my graduate research focused on investigating the molecular pathogenesis of cystic fibrosis (CF) – a fatal human genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Specifically, my study examined the thermodynamic defects caused by the most common CF-causing mutation, F508del, which involves a single phenylalanine deletion at residue number 508 in the CFTR gene. I discovered that the F508del mutation facilitates the aggregation-prone carrying domain, nucleotide domain 1 (NBD1), resulting in almost 100% of F508del-CFTR being aggregated and degraded in the ER membrane.

After obtaining my Ph.D. degree, I developed a novel and effective high-throughput drug screening method to identify chemical compounds that correct the thermodynamic defects caused by the F508del mutation. I successfully identified several nucleotide analogs with moderate corrector activity using NBD1 as the protein target. Furthermore, my postdoctoral research has focused exclusively on CFTR and the development of more advanced screening methods based on the original design. These methods should enable the identification of new types of corrector compounds with higher potency, providing more insight into the molecular design and gating mechanism of CFTR. These compounds may also serve as lead compounds for drug development.

To enhance the depth and impact of my thermodynamic and ligand-discovery studies, I have also worked on determining high-resolution structures of CFTR domains and interdomain interfaces using X-ray crystallography. Additionally, I have used cryo-electron microscopy (cryo-EM) to investigate the structure of full-length CFTR varients.

Another area of my research interest is related to DnaK, an *E. Coli* version of Heat Shock Protein 70 (Hsp70), which plays central role as a molecular chaperone in all living organisms. My investigation focuses on the structural details of DnaK functioning as a folding catalyst on misfolded proteins by utilizing X-ray crystallography and cryo-EM.

B. Positions and Honors

Positions and Employment

2011- 2016 Post-doctoral research associate, Columbia University, New York, NY
2016- present Associate Research Scientist, Columbia University, New York, NY
2020-2022 Assistant Director, Cryo-Electron Microscopy Center, Columbia University, New York, NY

Honors

1997-2000, member of National Training Base for Fundamental Scientific Research &Teaching, Jilin University, China

C. Contribution to Science

Investigations into the molecular pathology caused by the predominant disease-causing F508del mutation in the CFTR protein have been conducted. I contributed to elucidating the thermodynamic defects in the nucleotide-binding domain 1 (NBD1) of human CFTR resulting from the F508del mutation. This mutation destabilizes the protein, leading to aggressive aggregation of NBD1 in vitro. This conformation likely triggers efficient degradation of the protein in vivo, laying a new foundation for the development of high-throughput compound screens aimed at identifying small molecules to treat CF caused by the F508del mutation. Additionally, I have developed a robust and accurate fluorescence-based high-throughput assay, through which I have identified several drugable nucleotide derivatives that warrant further investigation.

To further our understanding of the molecular pathogenesis of CF, I conducted structural determinations of full-length human CFTR protein with disease-causing mutations. Using the state-of-the-art cryo-EM technique, I successfully solved the structure of the full-length human F508del-CFTR protein. This achievement represents the first direct observation, at the molecular level, of how F508del causes the molecular pathogenesis of CFTR, thirty years after the mutation was identified. Furthermore, by investigating the dual function of pharmacological correction and potentiation drug binding to CFTR, we discovered the drug binding site at the first transmembrane domain of CFTR and, more importantly, elucidated the mechanism by which the drug functions as a CFTR potentiator as well as a corrector.

Related publications:

1. S. Vorobiev*, **C. Wang***, Z. Yang*, O.B. Clarke, F. Jiang, A.A. Aleksandrov, Z. Fu, S. Wu, R.A. Grassucci, A. Mezzell, K. Wong, R.M. Vernon, S. Goeta, E. Hildebrandt, J.F. Fay, S. Patel, E. Werth, L.M. Brown, E. Joseloff, J.D. Forman-Kay, J.R. Riordan, D.R. Wetmore, C.G. Brouillette, I.L. Urbatsch†, J.C. Kappes†, J. Frank†, and J.F. Hunt† Cryo-EM structures of the human cystic fibrosis transmembrane conductance regulator harboring the predominant disease-causing F508del mutation in open- and closed-channel conformations, in preparation [*Co-first authors]
2. **C Wang***, Z Yang*, B.J. Loughlin*, H. Xu, G. Veit, S. Vorobiev, O.B. Clarke, F. Jiang, Y. Li, S. Singh, Z.Rich, E.R. Menten, R.A. Grassucci, W. Wang, A. Mezzell, Z. Fu, K. Wong, J. Wang, Diana R. Wetmore, R. Bryan Sutton, Christie G. Brouillette, Ina L. Urbatsch, John C. Kappes, G.L. Lukacs, J. Frank, J.F. Hunt Mechanism of dual pharmacological correction and potentiation of human CFTR bioRxiv 2022.10.10.510913 doi: https://doi.org/10.1101/2022.10.10.510913 [*Co-first authors]
3. M.S. Bahia, N. Khazanov, Q. Zhou, Z. Yang, **C. Wang**, J. S. Hong, A. Rab, E.J. Sorscher, C.G. Brouillette, J.F. Hunt, H. Senderowitz. Stability prediction for mutations in the cytosolic domains of cystic fibrosis transmembrane conductance regulator *Journal of Chemical Information and Modeling* 2021 61 (4), 1762-1777 DOI: 10.1021/acs.jcim.0c01207
4. **C. Wang**, A.A. Aleksandrov, Z. Yang, E. Proctor, P. Kota, J. An, F. Forouhar, G. Boël, N.V. Dokholyan, J.R. Riordan, C.G. Brouillette, J.F. Hunt Thermodynamic correction of the F508del mutation in human CFTR by binding of thymidine analogs to a remote site in the mutant domain *J. Biol. Chem.* 2018, 293(46):17685-17704
5. Z. Yang, **C. Wang**, Q. Zhou, J. An, E. Hildebrandt, L.A. Aleksandrov, J.C. Kappes, L.J. Delucas, J.R. Riordan, I.L. Urbatsch, J.F. Hunt, C.G. Brouillette. Membrane protein stability can be compromised by detergent interactions with the extramembranous soluble domains *Protein Sci.* 2014 23(6):769-89. doi: 10.1002/pro.2460. PMID: 24652590

6. J.F. Hunt, **C. Wang**, & R.C. Ford. Cystic Fibrosis Transmembrane Conductance Regulator (ABCC7) Structure *Cold Spring Harb Perspect Med.* 2013 1;3(2):a009514. doi: 10.1101/cshperspect.a009514. Review. PMID: 23378596

7. **C. Wang**, I. Protasevich, Z. Yang, D. Seehausen, T. Skalak, X. Zhao, S. Atwell, J. S. Emtage, D.R. Wetmore, C.G. Brouillette, and J.F. Hunt*. Integrated biophysical studies implicate partial unfolding of NBD1 of CFTR in the molecular pathogenesis of F508del cystic fibrosis. *Protein Sci.* 2010 19(10):1932-47. doi: 10.1002/pro.480. PMID: 20687163

8. I. Protasevich, Z. Yang, **C. Wang**, X. Zhao, S. Atwell, J. S. Emtage, D.R. Wetmore, J.F. Hunt, and C.G. Brouillette*. Thermal unfolding studies show the disease causing F508del mutation in CFTR thermodynamically destabilizes nucleotide-binding domain 1. *Protein Sci.* 2010 19(10):1917-31. doi: 10.1002/pro.479. PMID: 20687133

9. H.A. Lewis, **C. Wang**, X. Zhao, Y Hamuro, K. Conners, M.C. Kearins, F. Lu, J.M. Sauder, K. Molnar, S.J. Coales, P.C. Maloney, W.B. Guggino, D.R. Wetmore, P.C. Weber, and J.F. Hunt.* Structure and dynamics of NBD1 from CFTR characterized using crystallography and hydrogen/deuterium exchange mass spectrometry *J Mol Bio.* 2005 14;280(2):1346-53. PMID: 19944699

10. H.A. Lewis, X. Zhao, **C. Wang**, J.M. Sauder, I. Rooney, B.W. Noland, D. Lorimer, M.C. Kearins, K. Conners, B. Condon, P.C. Maloney, W.B. Guggino, J.F. Hunt, S. Emtage. Impact of the deltaF508 mutation in first nucleotide-binding domain of human cystic fibrosis transmembrane conductance regulator on domain folding and structure *J Biol Chem.* 2005, 280(2):1346-53 PMID: 15528182

D. Research Support

No independent support at this time.