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

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, including cys-loop receptors and other ligand-activated channels. Most recently, we have obtained cryo-EM structures of the ryanodine-sensitive calcium-release channel known as the ryanodine receptor (Zalk *et al*., 2015; des Georges *et al*., 2016).

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. F. Mancia, Z. Assur, A, Herman, R Siegel and W.A. Hendrickson, Ligand Sensitivity in Dimeric Associations of the Serotonin 5HT2c Receptor. *EMBO Reports* **9**, 363-369 (2008). PMCID: PMC2271072

c. J.O. Moore and W.A. Hendrickson, An Asymmetry-to-Symmetry Switch in Signal Transmission by the Histidine Kinase Receptor for TMAO. *Structure* **20**, 729-741 (2012). PMCID: PMC3625974

d. 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

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).

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. 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

5. *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 interractions 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 model for the chaperone cycle and also the generation of mutant-stabilized ATP states that have succumbed to crystallization. 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 the 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

d. R. Qi, E.B. Sarbeng, Q. Liu, K.Q. Le, X. Xu, H. Xu, J. Yang, J.L. Wong, C. Vorvis, W.A. Hendrickson, L. Zhou and Q. Liu. Allosteric Opening of the Polypeptide-binding Site when an Hsp70 Binds ATP. *Nat. Struct. Mol. Biol.* **20**, 900-907 (2013). PMCID: PMC3772632

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

D. Research Support

ACTIVE RESEARCH SUPPORT

P01 GM056550-21 (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 GM107462-05 (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.

P41 GM116799-01 (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.

PENDING RESEARCH SUPPORT

R01 NS109366-01 (Siegelbaum) 09/01/19 - 08/31/24
NIH
Structural Studies of HCN Channels in Health and Disease

This application proposes support of efforts to study the structure, regulation and disease-causing mutations in HCN4 cAMP-activated cation channels. I will participate as a PI together with Steve Siegelbaum (contact PI) and other investigators.

R01 GM132656-01 (Hendrickson) 04/01/20 - 03/31/25
NIH
Allosteric Regulation and Chaperone Activity of Hsp70 Proteins

This application proposes support of our efforts to a develop comprehensive mechanistic understanding of chaperone activity and allosteric regulation in Hsp70 molecular chaperones. We will perform x-ray and cryo-EM structural analyses, biochemical analyses of function, and theoretical and computational studies on mechanism.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Zhen Gong

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

POSITION TITLE: Postdoctoral Research Scientist

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
China Pharmaceutical University, Nanjing, China	B.S.	08/2004	07/2008	Traditional Chinese Medicine
Arizona State University, Phoenix, AZ	Ph.D.	08/2009	12/2014	Chemistry/Biochemistry
Arizona State University, Phoenix, AZ	Postdoc	01/2015	01/2016	Chemistry/Biochemistry
Columbia University, New York City	Postdoc	02/2016	present	Biochemistry/Molecular Biophysics

A. Personal Statement

I worked in the Center of Membrane Proteins in Infectious Diseases (MPID) at Arizona State University as a Ph.D. graduate student. MPID is one of the 9 membrane protein centers funded by NIH through the PSI:Biology program and my Ph.D. supervisor Prof. Petra Fromme is the director. I was working on a very interdisciplinary and challenging Ph.D. project with an aim to determine the structure of the membrane proximal region (MPR) and transmembrane (TM) domain of HIV-1 gp41 using X-ray crystallography. Gp41 is an envelope glycoprotein of HIV-1 which plays a crucial role in HIV infection cycle.

I have accumulated substantial experience in construct design, gene cloning, membrane protein expression, purification, biophysical characterization and crystallization from my graduate research. As a member of MPID center, I worked closely with scientists from different fields including medicine, biochemistry, molecular biology, and physics. Such interdisciplinary research experience has greatly expanded my scope of knowledge and scientific expertise. In the meanwhile, I was actively involved in several parallel projects in MPID center and shared my valuable experience of membrane proteins with my colleagues.

In addition to crystallization of membrane proteins in detergent micelles, I have also travelled to Ireland to explore crystallization of membrane proteins in lipidic cubic phase (LCP) in Prof. Martin Caffrey's laboratory at Trinity College in Dublin. Furthermore, I have research experience with electron microscopy (EM). When I was a graduate student, I took a graduate-level bioimaging class containing both lecture and laboratory. The lecture covered introduction and theory of current bioimaging techniques while the laboratory is based on gradate students' own research project. I brought three newly discovered cyanobacteria to the lab, prepared them for EM imaging and observed their sub-cellular components using EM.

With exceptional working on structural determination of membrane proteins, I feel well qualified to carry out the proposed research in Prof. Hendrickson's laboratory at Columbia University. Prof. Hendrickson is not only a great scientist with tremendous contributions to the field of structural biology, but also a well-known mentor who has supervised numerous distinguished scientists from his laboratory. I will continue to build on my previous expertise in structure biology. Furthermore, I will expand my study of interest to eukaryotic system, strengthen my understanding of X-ray crystallography, and master the application of cryo-EM to solve

biological problems. The goal of my postdoctoral training is to become an independent principle investigator in structural biology.

B. Positions and Honors

2008 - 2009 Research and teaching assistant, China Pharmaceutical University, Nanjing, China
2009 - 2014 Research and teaching assistant, Arizona State University, Phoenix, AZ
2015 - 2016 Postdoctoral scholar, Arizona State University, Phoenix, AZ
2016 - Postdoctoral research scientist, Columbia University, New York City

Excellent Cadre of Students in Henan Province (2004)
Honor of the three-good student in City Zhengzhou (2004)
The Premier Prize Scholarship in China Pharmaceutical University (2005)
The Second Prize Scholarship in China Pharmaceutical University (2006)
Outstanding volunteer for the 70th anniversary of China Pharmaceutical University (2006)
Government Scholarship in Jiangsu Province (2007)
The Premier Prize Scholarship in China Pharmaceutical University (2007)
China National Scholarship (2008)
Excellent Undergraduate Thesis (2008)
Special Scholarship for new graduate students (2009)
Poster award in Membrane Protein Structures 2015 Meeting (2015)

C. Contributions to Science

Undergraduate Career:

1. *Metabolic pathways of Danggui Buxue Tang (DBT).* I participated in a study of metabolic pathways of DBT when I was an undergraduate student. DBT is a well-known Chinese herbal formula consisting of *Radix Astragali* and *Radix Angelica sinensis*. It has been used as a classical prescription since 1247 AD for invigorating qi and nourishing blood (raising vital energy and stimulating red blood cell production). Nowadays, DBT is used not only as an effective medicine, but also as a common food supplement. However, metabolism pathways and bioactive components of DBT remain elusive. We have developed a method coupling liquid chromatography with electrospray ionization time-of-flight mass spectrometry (LC/ESI-TOF/MS) for rapid and sensitive analysis of rat urinary metabolite profile of DBT (Li *et al.*, 2009). A total of 68 compounds including 13 parent compounds and 55 metabolites were detected in the drug-containing urines compared with blank urines. 43 metabolites of DBT were finally identified, among which 27 metabolites were isoflavonoids and other 16 metabolites were phthalides. Our results indicated that glucuronidation and sulfation were the major metabolic pathways of isoflavonoids, while glutathione conjugation, glucuronidation and sulfation were the main metabolic pathways of phthalides. My specific role in this project was to optimize preparation of urine samples for LC/ESI-TOF/MS analysis.

    a. Li, C. Y., Qi, L. W., Li, P., Wen, X. D., Zhu, Y. F., Liu, E. H., Gong, Z., Yang, X. L., Ren, M. T., Li, Y. J., and Ge, X. X. (2009) Identification of metabolites of Danggui Buxue Tang in rat urine by liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry, Rapid Commun Mass Spectrom 23, 1977-1988.

Graduate Career:

2. *Structural analysis of the membrane proximal region (MPR) and transmembrane (TM) domain of HIV-1 gp41.* I worked on structural analysis of the MPR and TM domain of HIV-1 gp41 using X-ray crystallography for my doctoral dissertation study. Gp41 is a transmembrane subunit of HIV-1 envelop glycoprotein. The MPR-TM domain of HIV-1 gp41 contains epitopes for broadly neutralizing antibodies (Abs) against HIV-1, which makes it an attractive target for vaccine design. Thirty years of HIV research have yielded substantial advances in development of antiretroviral drugs, but there is no cure for HIV/AIDS yet. We aim to determine atomic structure of the MPR-TM domain of HIV-1 gp41 in fusion-active state, in

which epitopes for broadly neutralizing Abs are accessible for Ab binding. This work will enable further investigation into the structure of MPR-TM, which will be important for structure-based vaccine design against HIV-1.

In order to improve membrane protein expression, I explored multiple strategies to overexpress the MPR-TM domain of HIV-1 gp41 in *E. coli* and was able to purify the MPR-TM protein in monodisperse distribution. Significantly, the purified MPR-TM protein reacts strongly with broadly neutralizing Abs 2F5 and 4E10 with nanomolar to subnanomolar affinities, thereby may represent an immunologically relevant conformation mimicking a pre-hairpin intermediate of gp41 (Gong *et al.,* 2014). However, no crystal with diffraction quality was obtained. Another construct was designed to overexpress MPR-TM as a maltose binding protein (MBP) fusion, which might provide hydrophilic contacts for crystal formation. Crystals of MBP/MPR-TM were obtained after rational construct optimization. In addition, a series of biophysical criteria were established to monitor the quality of membrane proteins for crystallization (Gong *et al.,* 2015). Further analysis of diffraction data indicated that crystals of MBP/MPR-TM were twinned. The structure was solved under the help of Dr. Lebedev and Dr. Keegan in CCP4 workshop at Argonne National Laboratory (Gong *et al.,* 2015). The final structure demonstrated that MBP oligomerized as dimers of trimers, but the electron density did not extend beyond the linker region between MBP and MPR-TM. Further biochemical analysis confirmed that the crystal contained MBP only. Based on comparison of our MBP trimer with published trimeric MBP-fusion structures, and the fact that MBP on its own crystalizes as a monomer, we proposed that MBP might form such a dimer of trimers induced by MPR-TM prior to the latter's cleavage. A crystal formation hypothesis was proposed to explain the MBP hexamerization phenomena (Gong *et al.,* 2015).

a. Gong, Z., Kessans, S. A., Song, L., Dorner, K., Lee, H. H., Meador, L. R., LaBaer, J., Hogue, B. G., Mor, T. S., and Fromme, P. (2014) Recombinant expression, purification, and biophysical characterization of the transmembrane and membrane proximal domains of HIV-1 gp41, Protein Sci 23, 1607-1618.
b. Gong, Z., Martin-Garcia, J. M., Daskalova, S. M., Craciunescu, F. M., Song, L., Dorner, K., Hansen, D. T., Yang, J. H., LaBaer, J., Hogue, B. G., Mor, T. S., and Fromme, P. (2015) Biophysical characterization of a vaccine candidate against HIV-1: The transmembrane and membrane proximal domains of HIV-1 gp41 as a maltose binding protein fusion, PLoS One 10, e0136507.

3. *Characterization of Protein Nanocrystals.* Femtosecond nanocrystallography using X-ray free electron laser (XFEL) is a novel powerful technique for structural determination. In contrast to conventional crystallography, which requires growing crystals of sufficient size, XFEL allows the structural analysis of protein nanocrystals in a range of 100 nm to 10 μm. However, nanocrystal growth cannot be monitored with common methods used in conventional protein crystallography because the resolution of bright field microscopy is not sufficient. A high-performance method to screen for nanocrystals is second order nonlinear imaging of chiral crystals (SONICC), but the high cost prevents its use in every laboratory and some protein nanocrystals may be "invisible" to SONICC. Therefore, methods to characterize nanocrystals are in strong demand to facilitate sample preparation for XEFL. We developed an approach to screen protein nanocrystals based on the reversibility of crystallization using a common crystallization robot and an imaging system, from which precipitation comprised of nanocrystals and precipitation caused by aggregated proteins can be distinguished (Dorner *et al.*, 2016). I started this project using the MPR-TM protein as a study model and participated in data analysis as well as data interpretation for publication.

a. Dorner, K., Martin-Garcia, J. M., Kupitz, C., Gong, Z., Mallet, T. C., Chen, L. Q., Wachter, R. M., and Fromme, P. (2016) Characterization of protein nanocrystals based on the reversibility of crystallization, *Cryst Growth Des 16*, 3838-3845.

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

YEAR	SCIENCE COURSE TITLE	GRADE
	CHINA PHARMACEUTICAL UNIVERSITY	
2004	College English I	95
2004	Advanced Mathematics	97
2004	Inorganic Chemistry I	99
2004	Physics I	93
2004	Fundamental of Law	90
2004	Military Theory	88
2004	Survey of Pharmacy	84
2004	Physical Education I	90
2005	Inorganic Chemistry II	74
2005	Inorganic Chemistry Laboratory	90
2005	Physics II	76
2005	Physics Laboratory	90
2005	Cultivation of Qualities and Virtues	80
2005	The Basic of Traditional Chinese Medicine	75
2005	Computer Application Fundamentals	91
2005	Survey of Mao Zedong thought	70
2005	College English III	87
2005	Liner Algebra	79
2005	Computer Programming Language	89
2005	Physical Education III	86
2005	Principles of Science Political Economics	86
2005	Traditional Chinese Drugs	81
2005	Organic Chemistry I	88
2005	Organic Chemistry Laboratory I	88
2005	Analytical Chemistry I	88
2005	Analytical Chemistry Laboratory I	92
2006	College English IV	85
2006	Physical Education IV	84
2006	Organic Chemistry II	77
2006	Organic Chemistry Laboratory II	85
2006	Analytical Chemistry II	90
2006	Analytical Chemistry Laboratory II	84
2006	Mathematical Statistics	86
2006	Biochemistry	87
2006	Biochemistry Laboratory	86
2006	Spectral Analysis	87
2006	Medicinal Botany	93
2006	Medicinal Botany Laboratory	87
2006	Philosophy	87
2006	Public Relations Psychology	93
2006	Physical Chemistry I	90
2006	Physical Chemistry Laboratory	93
2006	Pharmacognosy	84
2006	Pharmacognosy Laboratory	94
2006	Medicinal Chemistry of Natural Products	91
YEAR	SCIENCE COURSE TITLE	GRADE
2006	Medicinal Chemistry of Natural Products Laboratory	91

2006	The human Anatomy Physiology	97
2006	The human Anatomy Physiology Laboratory	93
2006	Pharmaceutical Latin	94
2006	Microbiology	89
2006	Microbiology Laboratory	80
2006	Physical Education V	90
2006	Appreciation of Film and Television Arts	91
2006	Computer network	93
2007	Physical Chemistry II	90
2007	Literature Search	84
2007	Pharmaceutics of Chinese Materia Medica	84
2007	Pharmaceutics of Chinese Materia Medica Laboratory	97
2007	Special English in Pharmacy	92
2007	Processing of Chinese Materia Medica	87
2007	Processing of Chinese Materia Medica Laboratory	89
2007	Microscopic Identification of Chinese Drugs	70
2007	Microscopic Identification of Chinese Drugs Laboratory	91
2007	Instrument Analysis	98
2007	Instrument Analysis Laboratory	90
2007	Pharmacology	78
2007	Pharmacology Laboratory	89
2007	Survey of Deng Xiaoping Theory	79
2007	Computational Pharmacy	82
2007	Computational Pharmacy Laboratory	95
2007	Pharmaceutical Analysis	84
2007	Pharmaceutical Analysis Laboratory	84
2007	Analysis of Crude Chinese Medicinal Materials	73
2007	Analysis of Crude Chinese Medicinal Materials Laboratory	91
2007	The design of Career	85
2008	Social Practice	95
2008	Political Science	95
2008	Thesis Work	94
	ARIZONA STATE UNIVERSITY	
2009	Chemical Biology	B^-
2010	Advanced Topics in Biochemistry	A
2010	Analytical Chemistry Seminar	A
2010	Introduction to Nanoscience	A
2010	Trans Electron Microscopy	A
2010	Bioimaging: Current Techniques	A^+
2011	Tech in Molecular Bio/Genetics	A^-
2011	Tech in Molecular Bio/Genetics Lab	A
2011	Current Topics in Biochemistry (Seminar)	A
2012	Current Topics in Biochemistry (Seminar)	A
2012	Current Topics in Biochemistry (Seminar)	A
2013	Current Topics in Biochemistry (Seminar)	A