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 am well qualified to oversee this research project, which is in an area of longstanding and continuing interest to me. The project relates directly to our work on glycoprotein hormones and their receptors, and by mechanistic implications to our efforts on transmembrane signal transmission, allosteric control, and molecular evolution. My research interests and motivation, as well as my experience and accomplishments, are well aligned with aims of the proposed research. 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 membrane receptors and cellular signaling, on molecular chaperones and protein folding, on viral proteins and HIV infection, and on technology for structural analysis at an atomic level of resolution.

Four of our publications are especially relevant to the proposal:

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. 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

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

My work is supported by the following grants:

NIH R01 AI165904-01 (Sodroski, Contact PI) 07/10/23 - 06/30/27
Probing functional HIV-1 envelope glycoprotein conformations with novel potent CD4-mimetic compounds

This grant supports a multi-investigator project (Joseph G. Sodroski, Dans-Farber Cancer Institute, Contact P.I.) aimed at understanding the intervention of HIV 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*, Facing the phase problem. *IUCrJ* 10, 521-543 (2023). PMCID: PMC10478523

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), follicle-stimulating hormone (Fan & Hendrickson, 2005) and invertebrate homologs (Gong et al., 2024) as well as 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; Qin *et al.*, 2024).

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; Fritschi *et al*., 2023).

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: 10068826

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

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: Fan, Qing Rong

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

POSITION TITLE: Professor of Molecular Pharmacology and Therapeutics and Pathology and Cell Biology

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
Harvard-Radcliffe Colleges, Cambridge	B.A.	06/1994	Chemistry
Harvard University, Cambridge	M.A.	06/1996	Chemistry
Harvard University, Cambridge	Ph.D.	06/1999	Chemistry
Harvard University, Cambridge	Postdoctoral	06/2000	Structural Biology
Columbia University, New York	Postdoctoral	12/2006	Structural Biology

A. Personal Statement

I am interested in understanding the signaling mechanisms of cell surface receptors and how dysregulation of these receptors impact disease processes. I have been pursuing this goal through structural biology. As a graduate student in late Professor Don Wiley's laboratory, I determined the crystal structure of a human natural killer cell receptor and that of its complex with a class I major histocompatibility complex (MHC) molecule. As a postdoctoral fellow in Professor Wayne Hendrickson's laboratory, I solved the crystal structure of human follicle stimulating hormone (FSH) bound to the extracellular domain of its receptor ($FSHR_{HB}$), a leucine-rich repeat-containing G-protein-coupled receptor (LGR) important for the regulation of reproduction in mammals. As an independent investigator, my research has focused on the structure and function of a family of dimeric G-protein-coupled receptors (GPCRs), specifically human $GABA_B$ receptor and human calcium-sensing receptor (CaSR). My research goal is to understand how these dimeric GPCR assemblies transmit extracellular signals across the membrane. My laboratory determined the crystal structures of the $GABA_B$ receptor extracellular domain in multiple functional states, including apo, antagonist- and agonist-bound forms. Recently, we captured the inactive structure of a near full-length $GABA_B$ receptor by cryo-electron microscopy (cryo-EM). This structure revealed an important heterodimeric interaction motif that controls receptor activation. We also discovered multiple endogenous ligands of $GABA_B$ receptor that include two phospholipids embedded in the transmembrane domains. We have determined the extracellular domain structures of human CaSR in both the resting and active conformations. Based on these structures, we found that Ca^{2+} and amino acids function as co-agonists of the CaSR. We also solved the structures of a near-full length CaSR in multiple functional states. We found that a critical development during receptor activation arises from a helix-breaking event that facilitates the formation of a novel transmembrane homodimer interface. Recently, we determined structures of CaSR complexed with G proteins from three different subfamilies: G_q, G_i and G_s. These structures revealed mechanism of G-protein activation and selectivity as well as the molecular basis of promiscuous G-protein coupling by the CaSR. In this application, I have teamed up again with my postdoctoral advisor, Professor Wayne Hendrickson to continue our journey to unravel the mystery of LGR signaling. We aim to understand how an evolutionarily preserved hormone activates dimeric LGRs in C. elegans and human.

Ongoing and recently completed projects that I would like to highlight include:
R35 GM141871
Fan (PI)
04/01/21-03/31/26
Molecular mechanism of dimeric G protein-coupled receptor signaling

R01 GM12580
Fan, Slesinger and Quick (PI)
09/01/18-07/31/22
Mechanism of activation and modulation in human GABA(B) receptor

Citations:

1. Geng, Y., Bush, M., Mosyak, L., Wang, F., and **Fan, Q. R.*** Structural mechanism of ligand activation in human $GABA_B$ receptor. *Nature* 504, 254-259 (2013). PMID: 24305054. PMC3865065. (*Corresponding author) Funding: R01GM088454 (NIGMS).
2. Park, J., Fu Z., Frangaj, A., Liu, J., Mosyak, L., Shen, T., Slavkovich, V.N., Ray, K.M., Taura, J., Cao, B., Geng, Y., Zuo, H., Kou, Y., Grassucci, R., Chen, S., Liu, Z., Lin, X., Williams, J.P., Rice, W.J., Eng, E.T., Huang, R.K., Soni, R.K., Kloss, B., Yu, Z., Javitch, J.A., Hendrickson, W.A., Slesinger, P.A., Quick, M., Graziano, J., Yu, H., Fiehn, O., Clarke, O.B.*, Frank, J.*, **Fan, Q.R.*** Structure of human $GABA_B$ receptor in an inactive state. *Nature* 584, 304-309 (2020). PMID: 32581365. PMC7725281. (*Corresponding authors) Funding: R01GM088454 (NIGMS) and R01GM12580 (NIGMS).
3. Park, J., Zuo, H., Frangaj, A., Fu, Z., Yen, L.Y., Zhang, Z., Mosyak, L., Slavkovich, V.N., Liu, J., Ray, K.M., Cao, B., Vallese, F., Geng, Y., Chen, S., Grassucci, R., Venkata, P.D., Tan, Y.Z., Eng, E., Lee, Y., Kloss, B., Liu, Z., Hendrickson, W.A.*, Potter, C.S., Carragher, B., Graziano, J., Conigrave, A.D.*, Frank, J.*, Clarke, O.B.*, and **Fan, Q.R.*** Symmetric activation and modulation of the calcium-sensing receptor. *Proc. Natl. Acad. Sci. USA* 118, e2115849118 (2021). PMID: 34916296. PMC8713963 (*Corresponding authors) Funding: R35GM141871 (NIGMS).
4. Zuo, H., Park, J., Frangaj, A., Ye, J., Lu, G., Manning, J.J., Asher, W.B., Lu, Z., Hu, G., Wang, L., Mendez, J., Eng, E., Zhang, Z., Lin, X., Grassucci, R., Hendrickson, W.A., Clarke, O.B., Javitch, J.A.*, Conigrave, A.D.*, and **Fan, Q.R.*** Promiscuous G-protein activation by the calcium-sensing receptor. *Nature* 629, 481-488 (2024). PMID: 38632411. (*Corresponding authors) Funding: R35GM141871 (NIGMS).

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2021	NIH Molecular and Integrative Signal Transduction Study Section, ad hoc reviewer
2020	NIH Peer Review Committee on Pilot Project (R03), ad hoc reviewer Understudied G Protein-Coupled Receptors, Ion Channels, and Protein Kinases
2020-present	Member, Faculty Opinions Cell Signaling & Trafficking Structures Section
2019-2020	Member, F1000Prime Cell Signaling & Trafficking Structures Section
2018-2020	Consultant, PSY Therapeutics, INC, Consultant
2022-present	Professor of Pharmacology and Pathology and Cell Biology Department of Molecular Pharmacology & Therapeutics, Columbia University, New York, NY
2022-present	Professor of Pharmacology and Pathology and Cell Biology Department of Pathology & Cell Biology, Columbia University, New York, NY
2015-2022	Associate Professor of Pharmacology and Pathology and Cell Biology Department of Molecular Pharmacology & Therapeutics, Columbia University, New York, NY
2015-2022	Associate Professor of Pharmacology and Pathology and Cell Biology Department of Pathology & Cell Biology, Columbia University, New York, NY
2007-2015	Assistant Professor of Pharmacology and Pathology and Cell Biology Department of Pharmacology, Columbia University, New York, NY

2007-2015 Assistant Professor of Pharmacology and Pathology and Cell Biology
Department of Pathology & Cell Biology, Columbia University, New York, NY

Honors

2021-2026	NIH Maximizing Investigators' Research Award (MIRA)
2016	HHMI Faculty Scholars Competition Semifinalist
2013-2014	Schaefer Research Scholar
2011-2014	McKnight Scholar in Neuroscience
2009-2014	Pew Scholar Award in Biomedical Sciences
2009-2013	Irma T. Hirschl Career Scientist Award
2008-2009	Columbia University Fellowship for Minority and Women Junior Faculty
2001-2004	The Jane Coffin Childs Memorial Fund for Medical Research Postdoctoral Fellowship (Agouron Institute Fellow in Structural Biology)
1995-1998	National Science Foundation Predoctoral Fellowship
1994	Radcliffe Valedictorian, Harvard-Radcliffe Colleges

C. Contributions to Science

1. I have developed an independent research program to investigate the structure and function of human $GABA_B$ receptor, a class C GPCR important for inhibitory neurotransmission in the brain. $GABA_B$ receptor functions as an obligatory heterodimer of the $GABA_{B1}$ and $GABA_{B2}$ subunits. $GABA_{B1}$ is responsible for ligand binding, while $GABA_{B2}$ is involved in G-protein coupling.
   The first part of my work focuses on the molecular structures of various components of $GABA_B$ receptor. First, we solved the crystal structure of $GABA_{B2}$ extracellular domain, and demonstrated that $GABA_{B2}$ ectodomain directly interacts with $GABA_{B1}$ ectodomain to increase agonist affinity by selectively stabilizing the agonist-bound conformation of $GABA_{B1}$. Subsequently, we assembled a complex between the extracellular domains of the ligand-binding subunit ($GABA_{B1}$) and the modulatory subunit ($GABA_{B2}$). We determined crystal structures of the heterodimer in three states, in the apo form, bound to six different antagonists and bound to two different agonists. Structural comparison indicates a unique activation mechanism for the $GABA_B$ receptor that involves the formation of a novel heterodimer interface between its subunits. Our structures also revealed the molecular basis of ligand recognition by the $GABA_B$ receptor. In addition, we solved the crystal structure of an intracellular coiled-coil heterodimer of $GABA_B$ receptor. Our structure revealed the heterodimeric interaction that is responsible for concealing an endoplasmic reticulum retention signal in $GABA_{B1}$ and promoting the surface transport of the intact receptor. Recently, we solved the complex structure of the oligomerization domain of a intracellular KCTD protein bound to a $GABA_{B2}$-derived peptide. We found that KCTD forms a pentameric assembly and binds to $GABA_{B2}$ at a 5:1 molar ratio. The structure revealed the $GABA_{B2}$-KCTD interface and the residues that control the effect of KCTD on $GABA_B$ receptor activation and desensitization.

   a. Geng, Y., Xiong, D., Mosyak, L., Malito, D. L., Kniazeff, J., Chen, Y., Burmakina, S., Quick, M., Bush, M., Javitch, J. A., Pin, J.-P., and **Fan, Q. R.*** Structure and functional interaction of the extracellular domain of human $GABA_B$ receptor GBR2. *Nature Neuroscience* 15, 970-978 (2012). PMID: 22660477. PMC3374333. (*Corresponding author) Funding: R01GM088454 (NIGMS).
   b. Geng, Y., Bush, M., Mosyak, L., Wang, F., and **Fan, Q. R.*** Structural mechanism of ligand activation in human $GABA_B$ receptor. *Nature* 504, 254-259 (2013). PMID: 24305054. PMC3865065. (*Corresponding author) Funding: R01GM088454 (NIGMS).
   c. Burmakina, S., Geng, Y., Chen, Y., and **Fan, Q. R.*** Heterodimeric coiled-coil interactions of the human $GABA_B$ receptor. *Proc. Natl. Acad. Sci. USA*. 111, 6958-6963 (2014). PMID: 24778228. PMC4024898. (*Corresponding author) Funding: R01GM088454 (NIGMS).
   d. Zuo, H., Glaaser, I., Zhao, Y., Kourinov, I., Mosyak, L., Wang, H., Liu, J., Park, J., Frangaj, A., Sturchler, E., Zhou, M., McDonald, P., Geng, Y., Slesinger, P.A. and **Fan, Q.R.*** Structural basis

for auxiliary subunit KCTD16 regulation of the GABAB receptor. Proc. Natl. Acad. Sci. USA. 116, 8370-8379. PMID: 30971491. PMC6486783. (2019). (*Corresponding author) Funding: R01GM088454 (NIGMS) and R01GM12580 (NIGMS).

2. The second part of my work on $GABA_B$ receptor focuses on its transmembrane signaling mechanism. We recently captured the inactive-state structure of a near full-length human $GABA_B$ receptor by cryo-EM. Our structure revealed critical heterodimer interactions in the transmembrane region that control receptor activation. Specifically, the structure features a novel heterodimer interface between the transmembrane 3 (TM3) and 5 (TM5) helices of both $GABA_B$ subunits, which embodies the signature dimer arrangement of $GABA_B$ TM domains in the inactive conformation. Furthermore, we identified a unique ′intersubunit latch′ motif within this TM interface that maintains the inactive state of the receptor. We showed that disruption of the 'intersubunit latch' through single point mutations renders the receptor constitutively active. To our surprise, we discovered multiple endogenous ligands pre-associated with $GABA_B$ receptor, including two large phospholipids embedded within the transmembrane domains. These lipids are necessary structural components that maintain the receptor integrity.

    a. Park, J., Fu Z., Frangaj, A., Liu, J., Mosyak, L., Shen, T., Slavkovich, V.N., Ray, K.M., Taura, J., Cao, B., Geng, Y., Zuo, H., Kou, Y., Grassucci, R., Chen, S., Liu, Z., Lin, X., Williams, J.P., Rice, W.J., Eng, E.T., Huang, R.K., Soni, R.K., Kloss, B., Yu, Z., Javitch, J.A., Hendrickson, W.A., Slesinger, P.A., Quick, M., Graziano, J., Yu, H., Fiehn, O., Clarke, O.B.*, Frank, J.*, **Fan, Q.R.*** Structure of human $GABA_B$ receptor in an inactive state. *Nature* 584, 304-309 (2020). PMID: 32581365. PMC7725281. (*Corresponding authors) Funding: R01GM088454 (NIGMS) and R01GM12580 (NIGMS).

3. I have created an independent research program to study the structure and function of human calcium-sensing receptor (CaSR), a GPCR that maintains extracellular Ca^{2+} homeostasis through the regulation of parathyroid hormone secretion.
   We solved crystal structures of the extracellular domain of CaSR in the resting and active conformations. These structures provided direct evidence that L-amino acids and Ca^{2+} are co-agonists of the receptor. In the active structure, L-Trp occupies the orthosteric agonist-binding site at the interdomain cleft and is primarily responsible for inducing extracellular domain closure to initiate receptor activation. We also identified multiple binding sites for Ca^{2+} and PO_4^{3-}. Both ions are crucial for structural integrity of the receptor. While Ca^{2+} stabilizes the active state, PO_4^{3-} reinforces the inactive conformation. Binding of Ca^{2+} induces specific association of membrane-proximal domains to achieve full receptor activation.
   We also determined the cryo-EM structures of a near-full length CaSR in the presence and absence of allosteric modulators. We found that activation of CaSR requires a break in the transmembrane 6 (TM6) helix of each subunit, which facilitates the formation of a TM6-mediated homodimer interface. Allosteric modulators increase or decrease receptor activity by either reinforcing the disruption of TM6 helix or stabilizing its integrity.
   We recently solved the cryo-EM structures of CaSR complexed with four different G proteins from three subfamilies, G_q, G_i, and G_s. The restraint imposed by the receptor dimer and an intracellular loop (ICL2) of CaSR enables G-protein activation by facilitating conformational transition of Gα. We identified a single Gα residue that determines G_q and G_s vs G_i selectivity. Finally, the length and flexibility of ICL2 allows CaSR to bind all three Gα subtypes, thereby conferring capacity for promiscuous G-protein coupling.

    a. Geng, Y., Mosyak, L., Kurinov, I., Zuo, H., Sturchler, E. Cheng, T.C., Subramanyam, P., Brown, A.P., Brennan, S.C., Mun, H., Bush, M., Chen, Y., Nguyen, T.X., Cao, B., Chang, D.D., Quick, M., Conigrave, A.D., Colecraft, H.M., McDonald, P. and **Fan, Q.R.*** Structural mechanism of ligand activation in human calcium-sensing receptor. *eLife*. 5, e13662 (2016). PMID: 27434672. PMC4977154. (*Corresponding author) Funding: R01GM112973 (NIGMS).
    b. Park, J., Zuo, H., Frangaj, A., Fu, Z., Yen, L.Y., Zhang, Z., Mosyak, L., Slavkovich, V.N., Liu, J., Ray, K.M., Cao, B., Vallese, F., Geng, Y., Chen, S., Grassucci, R., Venkata, P.D., Tan, Y.Z., Eng,

E., Lee, Y., Kloss, B., Liu, Z., Hendrickson, W.A.*, Potter, C.S., Carragher, B., Graziano, J., Conigrave, A.D.*, Frank, J.*, Clarke, O.B.*, and **Fan, Q.R.*** Symmetric activation and modulation of the calcium-sensing receptor. *Proc. Natl. Acad. Sci. USA* 118, e2115849118 (2021). PMID: 34916296. PMC8713963. (*Corresponding authors) Funding: R35GM141871 (NIGMS).

c. Zuo, H., Park, J., Frangaj, A., Ye, J., Lu, G., Manning, J.J., Asher, W.B., Lu, Z., Hu, G., Wang, L., Mendez, J., Eng, E., Zhang, Z., Lin, X., Grassucci, R., Hendrickson, W.A., Clarke, O.B., Javitch, J.A.*, Conigrave, A.D.*, and **Fan, Q.R.*** Promiscuous G-protein activation by the calcium-sensing receptor. *Nature* 629, 481-488 (2024). PMID: 38632411. (*Corresponding authors) Funding: R35GM141871 (NIGMS).

4. As a postdoctoral fellow in Professor Wayne Hendrickson's laboratory, I studied the structure of human follicle stimulating hormone (FSH) receptor. FSH is essential for the regulation of reproduction in mammals, as it induces the maturation of ovarian follicles in females and supports spermatogenesis in males. FSH belongs to the family of glycoprotein hormones, which act through specific GPCRs in the target cell membrane. I determined the crystal structure of human FSH bound to the extracellular hormone-binding domain of its receptor and described their binding mode. Our structure also provides an explanation for the specificity of recognition between glycoprotein hormones and their receptors.

    a. **Fan, Q. R.** and Hendrickson, W. A. Structure of human follicle-stimulating hormone in complex with its receptor. *Nature* 433:269-277 (2005). PMID: 15662415. PMC5514322
    b. **Fan, Q. R.** and Hendrickson, W. A. Assembly and structural characterization of an authentic complex between human follicle stimulating hormone and a hormone-binding ectodomain of its receptor. *Mol. Cell. Endocrinol.* 260-262:73-82 (2007). PMID: 17045735. PMC2012943
    c. **Fan, Q. R.** and Hendrickson, W. A. (2008). Comparative structural analysis of the binding domain of the follicle stimulating hormone receptor. *Proteins* 72, 393-401. PMID: 18214954. PMC3078555.

5. As a graduate student in late Professor Don Wiley's laboratory, I studied the structure and function of the human natural killer (NK) cell receptor KIR2D and its class I major histocompatibility complex (MHC) ligand HLA-Cw4. Natural killer cells are a class of lymphocytes that lyse transformed and virally infected cells deficient in class I MHC expression. Inhibitory receptors on NK cell surface down-regulate the cytotoxicity of NK cells upon recognition of specific MHC molecules on target cells. I determined the extracellular domain structure of the inhibitory receptor KIR2D, the class I MHC molecule HLA-Cw4, and their complex. These structures revealed the binding mode and specificity determinants of inhibitory NK receptors and their MHC ligands.

    a. **Fan, Q. R.**, Garboczi, D. N., Winter, C. C., Wagtmann, N., Long, E. O. and Wiley, D. C. Direct binding of a soluble natural killer cell inhibitory receptor to a soluble human leukocyte antigen-Cw4 class I major histocompatibility complex molecule. *Proc. Natl. Acad. Sci. USA* 93:7178-7183 (1996). PMID: 8692965. PMC38956
    b. **Fan, Q. R.**, Mosyak, L., Winter, C. C., Wagtmann, N., Long, E. O. and Wiley, D. C. Structure of the inhibitory receptor for human natural killer cells resembles haematopoietic receptors. *Nature* 389:96-100 (1997). PMID: 9288975.
    c. **Fan, Q. R.** and Wiley, D. C. Structure of human leukocyte antigen (HLA)-Cw4, a ligand for the KIR2D natural killer cell inhibitory receptor. *J. Exp. Med.* 190:113-123 (1999). PMID: 10429675. PMC2195553
    d. **Fan, Q. R.**, Long, E. O. and Wiley, D. C. Crystal structure of the human natural killer cell inhibitory receptor KIR2DL1 bound to its class I MHC ligand. *Nature Immunology* 2: 452-460. PMID: 11323700. This work was featured in a News and Views commentary in *Nature Immunology* 2, 379-380 (2001).

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/qing.fan.1/bibliography/40773268/public/?sort=date&direction=ascending

BIOGRAPHICAL SKETCH

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

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

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. 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	BS	08/2004	07/2008	Traditional Chinese Medicine
Arizona State University, Phenix, AZ	PHD	08/2009	12/2014	Chemistry/Biochemistry
Arizona State University, Phenix, AZ	Postdoc	01/2015	01/2016	Chemistry/Biochemistry
Columbia University, New York City	Postdoc	02/2016	01/2021	Biochemistry/Molecular Biophysics
Columbia University, New York City	Associate Research Scientist	02/2021	Present	Biochemistry/Molecular Biophysics

A. Personal Statement

My academic training and research experience have provided me with an excellent background in multiple disciplines including molecular biology, biochemistry, biophysics, and structural biology. As an undergraduate, I conducted study of metabolic pathways of Danggui Buxue Tang with rapid and sensitive analysis of its rat urinary metabolite profile using a method coupling liquid chromatography with electrospray ionization time-of-flight mass spectrometry (LC/ESI-TOF/MS) with Dr. Changyin Li in China Pharmaceutical University. I was a co-author of the identification of metabolites of Danggui Buxue Tang in rat urine. During my undergraduate careers, I received several important academic awards, including China National Scholarship, Government Scholarship of Jiangsu Province, and Excellent Undergraduate Thesis.

I was admitted to the graduate school in Department of Chemistry and Biochemistry at Arizona State University, from where I was fascinated with structural biology and health-related research. I joined Professor Petra Fromme's laboratory and worked in the Center of Membrane Proteins in Infectious Diseases (MPID) as a graduate student. MPID is one of the 9 membrane protein centers funded by NIH through the PSI:Biology program and my Ph.D. supervisor Professor 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 purified the MPR-TM domain of HIV-1 gp41 alone and with maltose binding protein fusion partner (MBP-MPR-TM). Both proteins are in monodisperse conformation and are able to interact with broadly neutralizing antibodies 2F5 and 4E10, which makes them attractive targets for vaccine design and suitable candidates for structural determination. I have published two first-author research papers on this project. Beyond that, I participated in method development to characterize protein nanocrystals for X-ray free electron laser beamtime and I am a co-author of this paper. I have accumulated substantial experience in construct design, gene cloning, membrane protein expression, purification, biophysical characterization, and crystallization from my graduate research.

For my postdoctoral training, I was eager to expand my expertise in data processing, phase determination, membrane protein expression in eukaryotic system (both insect and mammalian cells) and especially structural

determination using cryo-electron microscope (cryo-EM). My mentor Professor Wayne Hendrickson is a world-renowned biophysicist and has an extensive successful record of training postdoctoral fellows. My research interest focuses on structural determination of a full-length glycoprotein hormone receptor in complex with its ligand using X-ray crystallography and single-particle cryo-EM. I have solved the first crystal structure of an ancient glycoprotein hormone homologue called thyrostimulin in *C. elegans* using single-wavelength anomalous diffraction and AlphaFold guided molecular replacement. This work has been published in a major journal and I am the first author. Another method paper related to this work is under revision with me as a co-author. A few years later, I solved the structure of the receptor to thyrostimulin in *C. elegans* using cryo-EM. The manuscript has been prepared for publication and I am the first author. In addition to the glycoprotein hormone receptor project, I took part in the development of CD4-mimetic compounds (CD4mcs) with potent and broad HIV-1 Inhibition. I have determined structures of seven noval indoline CD4mcs in complex with HIV-1 gp120 from 1.9 to 2.5 Å using X-ray crystallography, from which the structural basis of indoline CD4mcs to gain potency against HIV-1 has been uncovered. This work has recently been published in a major journal and I am a co-first author. My long-term research goals involve becoming an independent principal investigator and carry out health-related research using structural biology as a powerful tool.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 – Present	Associate Research Scientist, Columbia University
2016 – 2021	Postdoctoral Research Scientist, Columbia University
2015 – 2016	Postdoctoral Scholar, Arizona State University
2009 – 2014	Graduate Research and Teaching Assistant, Arizona State University
2009 – 2008	Undergraduate Research and Teaching Assistant, China Pharmaceutical University

Honors

2023	Travel Grant Award for American Crystallographic Association Meeting
2019 to 2020	NIH Endocrinology Training Grant
2018	COMPPÅ Symposium Fisher Awards
2015	Poster award in Membrane Protein Structures 2015 Meeting
2009	Special Scholarship for new graduate students, Arizona State University
2008	Excellent Undergraduate Thesis, China Pharmaceutical University
2007	China National Scholarship
2006	Government Scholarship of Jiangsu Province
2006	Outstanding volunteer for the 70th anniversary of China Pharmaceutical University
2004	Excellent Cadre of Students of Henan Province
2003	Honor of the three-good student of Zhengzhou City
2002	Advanced individual of Henan Province

C. Contributions to Science

1. **Undergraduate Career:** I participated in a study of metabolic pathways of Danggui Buxue Tang (DBT) when I was an undergraduate student. More specifically, I worked with a team of postdoctoral fellows and graduate students to develop 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. My role in this project was to optimize preparation of urine samples for the 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.

2. **Graduate Career:** My graduate research contributions focused on expression, purification, and biophysical characterization of the membrane proximal region and transmembrane (MPR-TM) domain of HIV-1 gp41, the transmembrane subunit of HIV-1 envelope glycoproteins, for structural analysis using X-ray crystallography. I have purified the MPR-TM domain of HIV-1 gp41 alone and with maltose binding

protein fusion partner (MBP-MPR-TM). Both proteins are in monodisperse conformation and are able to interact with broadly neutralizing antibodies 2F5 and 4E10, which makes them attractive targets for vaccine design and suitable candidates for structural studies.

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.

Another contribution of my graduate research is characterization of protein nanocrystals. Femtosecond nano-crystallography using X-ray free electron laser (XFEL) is a novel powerful technique for structural determination. 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. 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. 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.

3. **Postdoctoral and Associate Research Scientist Career:** As a postdoctoral and associate research scientist, my research interest focuses on structural analysis of glycoprotein hormone (GPH) in complex with its full-length receptor using X-ray crystallography and single-particle cryo-EM. GPHs are central to the complex endocrine system that regulates normal growth, sexual development, and reproductive function. These hormones are heterodimers of α and β subunit. Ancestral glycoprotein hormone homologues are identified as α2β5. The human α2β5 heterodimer activates TSH receptors but not the FSH or LH/CG receptor counterparts; and it was named thyrostimulin because of this specificity. I have solved the first crystal structure of thyrostimulin in *C. elegans* using single-wavelength anomalous diffraction and AlphaFold guided molecular replacement. A few years later, I solved the structure of the receptor to thyrostimulin in *C. elegans* using cryo-EM.

a. **Z. Gong**, W. Wang, K. El Omari, A. A. Lebedev, O. B. Clarke, W. A. Hendrickson, Crystal structure of LGR ligand alpha2/beta5 from Caenorhabditis elegans with implications for the evolution of glycoprotein hormones. *Proc Natl Acad Sci U S A* 120 (1), e2218630120 (2023).

b. W. Wang, **Z. Gong**, W. A. Hendrickson, AlphaFold-guided molecular replacement for solving challenging crystal structures, Second revision under consideration in *Acta Crystallographica Section D*.

c. **Z. Gong**, S. Chen, Z. Fu, B. Kloss, C. Wang, O. B. Clarke, Q. Fan, W. A. Hendrickson, Structure of an LGR dimer – an evolutionary predecessor of glycoprotein hormone receptors. Under considertion in *Nature Structural & Molecular Biology*.

Another contribution of my postdoctoral and associate research scientist career is the structural analysis of CD4-mimetic compounds in complex with HIV-1 envelope glycoprotein gp120. I have determined structures of seven noval indoline CD4mcs in complex with HIV-1 gp120 from 1.9 to 2.5 Å using X-ray crystallography. These CD4mcs exhibit increases in potency and breadth against HIV-1 variants, as well as greater ability to sensitize HIV-1 infected cells to antibody-dependent cellular cytotoxicity. Our crystal structures indicate that the indoline CD4mcs gain potency compared to the indane CD4mcs through more

favorable π-π overlap from the indoline pose and by making favorable contacts with the vestibule of the CD4-binding pocket on gp120.

a. C. J. 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. F. Abrams, A. Finzi, W. A. Hendrickson, J. G. Sodroski, A. B. Smith, Indoline CD4-mimetic Compounds Mediate Potent and Broad HIV-1 Inhibition and Sensitization to Antibody-dependent Cellular Cytotoxicity. *Proc Natl Acad Sci U S A* 120 (13), e2222073120 (2023). (*, co-first author)

b. C. Chaplain, C. J. Fritschi, S. Anang, **Z. Gong**, J. Richard, C. Bourassa, S. Y. Liang, M. Mohammadi, J. Park, A. Finzi, N. Madani, J. G. Sodroski, C. F. Abrams, W. A. Hendrickson, A. B. Smith, Structural and Functional Characterization of Indane-Core CD4-Mimetic Compounds Substituted with Heterocyclic Amines. *ACS Med Chem Lett* 14, 51-58 (2023).

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	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

BIOGRAPHICAL SKETCH

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

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

POSITION TITLE: Ph.D. candidate

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Zhejiang University, Hangzhou, Zhejiang	BS	07/2020	Biological Sciences
Columbia University, New York, NY	MS	07/2023	Biological Science
Columbia University, New York, NY	MPHIL	07/2024	Biological Sciences
Columbia University, New York, NY	PHD	current	Biological Sciences

A. Personal Statement

I am a fourth-year Ph.D. student at Columbia University, specializing in the structural and functional characterization of glycoprotein hormone receptors. My academic journey began with rigorous training in molecular and cellular biology during my undergraduate studies, complemented by practical experience in molecular dynamics simulations during an internship at Dr. John Hunt's laboratory. Throughout graduate school, I also expanded my expertise to include sequencing and image analysis through course projects.

1. Zuo H, Park J, Frangaj A, Ye J, Lu G, Manning JJ, Asher WB, Lu Z, Hu GB, Wang L, Mendez J, Eng E, Zhang Z, Lin X, Grassucci R, Hendrickson WA, Clarke OB, Javitch JA, Conigrave AD, Fan QR. Promiscuous G-protein activation by the calcium-sensing receptor. Nature. 2024 May;629(8011):481-488. PubMed PMID: 38632411.
2. Banayan NE, Loughlin BJ, Singh S, Forouhar F, Lu G, Wong KH, Neky M, Hunt HS, Bateman LB Jr, Tamez A, Handelman SK, Price WN, Hunt JF. Systematic enhancement of protein crystallization efficiency by bulk lysine-to-arginine (KR) substitution. Protein Sci. 2024 Mar;33(3):e4898. PubMed Central PMCID: PMC10868448.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 - Ph.D. candidate, Columbia University, New York, NY

Honors

2023 Completion of the Teaching Development Program Foundational Track, Columbia University

C. Contribution to Science

1. During my undergraduate research, I investigated kinases involved in phosphorylating TET2, a crucial enzyme in DNA demethylation. Additionally, I explored the role of N-glycosylation in modulating the signaling pathways of the orexigenic neuropeptide QRFP receptor GPR103. This work highlighted N-glycosylation as a significant regulatory mechanism influencing the strength and timing of GPR103 signal transduction.

2. During my internship, I employed computational methods to evaluate the impact of lysine to arginine mutations on enhancing protein crystallization. I utilized molecular dynamics (MD) simulations to predict that lysine to arginine mutations in the protein PDI-a would enhance its crystallization propensity. This computational insight guided subsequent experimental efforts within our team, which confirmed a

significant increase in crystallization success rates for engineered PDI-a mutants compared to the wild-type protein.

   a. Banayan NE, Loughlin BJ, Singh S, Forouhar F, Lu G, Wong KH, Neky M, Hunt HS, Bateman LB Jr, Tamez A, Handelman SK, Price WN, Hunt JF. Systematic enhancement of protein crystallization efficiency by bulk lysine-to-arginine (KR) substitution. Protein Sci. 2024 Mar;33(3):e4898. PubMed Central PMCID: PMC10868448.

3. During graduate school, I engineered constructs and produced corresponding baculoviruses for a project investigating the structural basis for the Calcium-sensing receptor's capability of coupling to multiple classes of G proteins. This research identified a single Gα residue conferring G protein Gq and Gs vs Gi selectivity, and uncovered that the length and flexibility of intracellular loop 2 (ICL2) of the Calcium-sensing receptor enable promiscuous G protein coupling.

   a. Zuo H, Park J, Frangaj A, Ye J, Lu G, Manning JJ, Asher WB, Lu Z, Hu GB, Wang L, Mendez J, Eng E, Zhang Z, Lin X, Grassucci R, Hendrickson WA, Clarke OB, Javitch JA, Conigrave AD, Fan QR. Promiscuous G-protein activation by the calcium-sensing receptor. Nature. 2024 May;629(8011):481-488. PubMed PMID: 38632411.

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