BIOGRAPHICAL SKETCH

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NAME: Fan, Qing Rong

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

POSITION TITLE: Associate Professor of Pharmacology and Pathology

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

This application focuses on the structural studies of human $GABA_B$ receptor, a G protein-coupled receptor (GPCR) central to inhibitory neurotransmission in the brain. Our goal is to define the molecular structures of essential components of the receptor signaling complex, and elucidate the mechanisms of receptor activation and modulation. Specifically, we propose to develop structural models for full-length $GABA_B$ receptor, its auxiliary subunits and their complexes with G-protein. We will test structure-generated hypotheses through functional analysis of $GABA_B$-mediated effector channel activities. Finally, we will examine the allosteric coupling between $GABA_B$ receptor and G-protein. I have extensive experience studying the structural mechanisms of cell surface receptors. As a graduate student in late Professor Don Wiley's laboratory, I determined the 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 structure of human follicle stimulating hormone (FSH) bound to the extracellular domain of its receptor ($FSHR_{HB}$), a GPCR important for the regulation of reproduction in mammals. As an independent investigator, my research has focused on the structural and functional analysis of class C GPCRs, specifically human $GABA_B$ receptor and human calcium-sensing receptor. My laboratory determined the crystal structures of the $GABA_B$ receptor extracellular domain in multiple functional states, including apo, antagonist- and agonist-bound forms. We also solved the structure of an intracellular coiled-coil heterodimer of $GABA_B$ receptor. Recently, we determined the extracellular domain structures of human calcium-sensing receptor in both the resting and active conformations. This proposal builds upon our previous work on $GABA_B$ receptor and class C GPCR in general. Therefore, I am well positioned to continue the research on human $GABA_B$ receptor.

1. **Fan, Q. R.** and Hendrickson, W. A. (2005). Structure of human follicle-stimulating hormone in complex with its receptor. *Nature* 433:269-277. PMID: 15662415.
2. 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.*** (2012). Structure and functional interaction of the extracellular domain of human $GABA_B$ receptor GBR2. *Nature Neuroscience* 15, 970-978. PMID: 22660477. (*Corresponding author.)

3. Geng, Y., Bush, M., Mosyak, L., Wang, F., and **Fan, Q. R.*** (2013). Structural mechanism of ligand activation in human $GABA_B$ receptor. *Nature* 504, 254-259. PMID: 24305054. (*Corresponding author.)
4. 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. PMID: 27434672. (*Corresponding author.)

B. Positions and Honors

Positions

2007-2015	Assistant Professor of Pharmacology and Pathology, Department of Pharmacology, Columbia University, New York, NY
2015-present	Associate Professor of Pharmacology and Pathology, Department of Pharmacology, Columbia University, New York, NY

Honors

1994	Radcliffe Valedictorian, class of 1994 (Harvard-Radcliffe Colleges)
1994	B.A. in chemistry, Summa Cum Laude (Harvard-Radcliffe Colleges)
1994	David Wittes Master's Scholarship (United Federation of Teachers)
1995	National Science Foundation (NSF) Predoctoral Fellowship
2001	Jane Coffin Childs Memorial Fund for Medical Research Postdoctoral Fellowship (Agouron Institute Fellow in Structural Biology)
2008	Columbia University Fellowship for Minority and Women Junior Faculty
2009	Pew Scholar in the Biomedical Sciences
2009	Irma T. Hirschl Career Scientist
2011	McKnight Scholar in Neuroscience
2013	Schaefer Research Scholar

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 GBR1 and GBR2 subunits. GBR1 is responsible for ligand binding, while GBR2 is involved in G-protein coupling.
   We solved the first crystal structure of GBR2 extracellular domain, and demonstrated that GBR2 ectodomain directly interacts with GBR1 ectodomain to increase agonist affinity by selectively stabilizing the agonist-bound conformation of GBR1.
   Subsequently, we succeeded in assembling a complex between the extracellular domains of the ligand-binding subunit (GBR1) and the modulatory subunit (GBR2), and determined the crystal structures of the heterodimer in three states, in the apo form, bound to six different antagonists and bound to two different agonists. These are the first crystal structures of human $GABA_B$ receptor extracellular domain. Structural comparison indicates a unique activation mechanism for the inhibitory $GABA_B$ receptor that involves the formation of a novel heterodimer interface between its subunits. Our structures also reveal the molecular basis of ligand recognition by the $GABA_B$ receptor.
   In addition, we solved the first crystal structure of an intracellular coiled-coil heterodimer of $GABA_B$ receptor. Our structure reveals the heterodimeric interaction that is responsible for promoting the surface transport of the intact receptor.

   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.*** (2012). Structure and functional interaction of the extracellular domain of human $GABA_B$ receptor GBR2. *Nature Neuroscience* 15, 970-978. PMID: 22660477. (*Corresponding author.)

b. Geng, Y., Bush, M., Mosyak, L., Wang, F., and **Fan, Q. R.*** (2013). Structural mechanism of ligand activation in human $GABA_B$ receptor. *Nature* 504, 254-259. PMID: 24305054. (*Corresponding author.)
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. (*Corresponding author.)
d. Frangaj, A., **Fan, Q. R.***. Structural biology of $GABA_B$ receptor. *Neuropharmacology* 136, 68-79 (2018). PMIC: 29031577. PMID: 29031577. (*Corresponding author.)

2. I have created an independent research program to study the structure and function of human calcium-sensing receptor (CaSR), a G-protein coupled receptor that maintains extracellular Ca^{2+} homeostasis through the regulation of parathyroid hormone secretion. CaSR activates multiple signaling pathways and responds to a variety of ligands. The general consensus is that the principal agonist of CaSR is extracellular Ca^{2+}. Aromatic and aliphatic L-amino acids such as L-Phe and L-Trp increase the sensitivity of CaSR toward Ca^{2+} and are considered as positive allosteric modulators of the receptor.
We solved the first crystal structures of the entire extracellular domain of CaSR in the resting and active conformations. We provide direct evidence that L-amino acids are 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. Our structures reveal multiple binding sites for Ca^{2+} and PO_4^{3-} ions. Both ions are crucial for structural integrity of the receptor. While Ca^{2+} ions stabilize the active state, PO_4^{3-} ions reinforce the inactive conformation. The activation mechanism of CaSR involves specific association of membrane-proximal domains.

   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. (*Corresponding author.)

3. As a postdoctoral fellow in Professor Wayne Hendrickson's laboratory, I studied the structure of human follicle stimulating hormone receptor (FSHR). Follicle stimulating hormone (FSH) is essential for the regulation of reproduction in mammals. FSH induces the maturation of ovarian follicles in females and supports spermatogenesis in males; it is used clinically to treat infertile patients. FSH belongs to the family of glycoprotein hormones, which act through specific G-protein coupled receptors (GPCRs) in the target cell membrane. The large extracellular domains of glycoprotein hormone receptors mediate ligand binding, whereas the transmembrane domains are responsible for signal transduction across the membrane. I have determined the crystal structure of human FSH bound to the extracellular hormone-binding domain of its receptor ($FSHR_{HB}$). The FSH-$FSHR_{HB}$ complex structure provides a molecular understanding of their interactions, which may be utilized to design FSH mimics as alternative agonists and contraceptive antagonists. This was the first structure of a glycoprotein hormone-receptor complex.

   a. **Fan, Q. R.** and Hendrickson, W. A. (2005). Structure of human follicle-stimulating hormone in complex with its receptor. *Nature* 433:269-277. PMID: 15662415.
   b. **Fan, Q. R.** and Hendrickson, W. A. (2007). 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. PMID: 17045735.
   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.

4. 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 inhibitory receptor KIR2D, and identified its relationship to haematopoietic

receptors. This was the first structure of a natural killer cell receptor. I also determined the structure of the class I MHC molecule HLA-Cw4 bound to a consensus peptide, and the structure of the KIR2D–HLA-Cw4 complex. The specificity determinants as indicated by the complex structure provide an explanation of independent mutational data. The KIR2D–HLA-Cw4 complex structure also suggests a common binding mode for 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. (1996). 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. PMID: 8692965.
b. **Fan, Q. R.**, Mosyak, L., Winter, C. C., Wagtmann, N., Long, E. O. and Wiley, D. C. (1997). Structure of the inhibitory receptor for human natural killer cells resembles haematopoietic receptors. *Nature* 389:96-100. PMID: 9288975.
c. **Fan, Q. R.** and Wiley, D. C. (1999). Structure of human leukocyte antigen (HLA)-Cw4, a ligand for the KIR2D natural killer cell inhibitory receptor. *J. Exp. Med.* 190:113-123. PMID: 10429675.
d. **Fan, Q. R.**, Long, E. O. and Wiley, D. C. (2001). 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

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

Ongoing Research Support

1R01GM112973-01A1 Fan (PI) 08/01/2015 - 06/30/2019
NIH/NIGMS
Structural studies of human extracellular calcium-sensing receptor
The goal of this project is to investigate the molecular mechanisms of ligand recognition and activation of human extracellular calcium-sensing receptor.
Role: PI

2 R01 AA018734 Slesinger (PI) 04/15/2016 - 03/31/2021
NIH/NIAAA
Structural analysis of alcohol-dependent activation of GIRKs
The major goal of this competitive renewal project is to investigate the molecular and structural mechanisms underlying ethanol-dependent activation of neuronal potassium channels.
Role: Co-investigator

Completed Research Support

AHA SDG 0835183N Fan (PI) 07/01/2008 - 06/30/2012
American Heart Association
American Heart Association Scientist Development Grant
Structural and functional analysis of GABA(B) receptors
The aim of this study is to understand the signal transduction mechanism of GABA(B) receptor in relation to hypertension and stroke.
Role: PI

Pew Scholars Award Fan (PI) 07/01/2009 - 06/30/2013
Pew Scholars Program in the Biomedical Sciences
Structural studies of the human $GABA_B$ receptors
The Pew Scholarship is a junior faculty award that aims to support the overall research program of the principal investigator.

Role: PI

Irma T. Hirschl Career Scientist Award Fan (PI) 01/01/2009 - 12/31/2013
The Irma T. Hirschl/Monique Weill-Caulier Trust
Structural studies of the human $GABA_B$ receptors
This grant supports medical research at six lead institutions in New York City, and is given to the principal investigator through Columbia University as a junior faculty research award.
Role: PI

Chemical Probe Synthesis (CPS) Facility Ignition Grant Fan (PI) 09/01/2013 - 12/31/2013
Columbia University Technology Ventures
Novel $GABA_B$ receptor ligands
The goal of this project is to discover novel ligands of human $GABA_B$ receptor based on the structures of its extracellular domain.
Role: PI

McKnight Scholar Award Fan (PI) 07/01/2011 - 06/30/2014
The McKnight Endowment Fund for Neuroscience
Molecular mechanism of metabotropic GABA receptor function
The goal of this project is to identify the activation mechanism of human $GABA_B$ receptor in relation to neurological disorders.
Role: PI

Schaefer Research Scholar Award Fan (PI) 07/01/2013 - 06/30/2014
Dr. Ludwig Schaefer Fund
Structural studies of human extracellular calcium-sensing receptor
The goal of this project is to carry out structural and functional analysis of human extracellular calcium-sensing receptor.
Role: PI

1R01GM088454-05 Fan (PI) 07/01/2009 - 07/31/2016
NIH/NIGMS
Structural studies of metabotropic GABA receptors
The goal of this project is to develop structural models for the various components of metabotropic $GABA_B$ receptor and analyze their function in the receptor activation process.
Role: PI

AHA 15GRNT22740035 Fan (PI) 07/01/2015 - 06/30/218
American Heart Association
American Heart Association Grant-in-aid
Molecular Mechanisms of human extracellular calcium-sensing receptor function
The aim of this study is to understand the signal transduction mechanism of calcium-sensing receptor in relation to hypertension and vascular calcification.
Role: PI

BIOGRAPHICAL SKETCH

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NAME: Aurel Frangaj

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

POSITION TITLE: Technician

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
Fordham College, Rose Hill	B.S.	06/2012	Biology

A. Personal Statement

My goal is to determine the three-dimensional structure of full-length human $GABA_B$ receptor in multiple functional states, and in complex with its downstream signaling molecules. Human $GABA_B$ receptor is a G protein coupled receptor that mediates inhibitory neurotransmission in the brain. It functions as a heterodimer. Our laboratory has recombinantly expressed full-length $GABA_B$ receptor in mammalian cells. I have learned to solve structures by cryo-electron microscopy, and I'm proficient at all techniques including sample preparation, data collection and data processing. I'm determined to unravel the mechanism of action of this important receptor.

B. Positions and Honors

2014 Assistant Animal Caretaker
Department of Biology, Fordham University, New York, NY
2017-present Technician
Department of Pharmacology, Columbia University, New York, NY

C. Contributions to Science

1. I performed molecular biology, protein expression and purification experiments that was part of a study to understand the role of p60 and NamA autolysins in primary host cell invasion, the inflammatory response, and the differentiation of functional memory CD8(+) T-cells.

a. Chandrabos, C., M'Homa Soudja, S., Weinrick, B., Gros, M., Frangaj, A., Rahmoun, M., Jacobs, W.R. Jr., Lauvau, G. The p60 and NamA autolysins from Listeria monocytogenes contribute to host colonization and induction of protective memory. Cell Microbiol. 17, 147-63 (2015). PMIC: 4457399. PMID: 25225110.

2. I am currently pursuing the structure of full-length $GABA_B$ receptor from human by cryo-EM method. I have written a review on the structural biology of $GABA_B$ receptor, with specific emphasis on the molecular

mechanism of receptor activation and modulation. I am also a co-author on a manuscript under review that describes the structural and functional interaction between $GABA_B$ receptor and its auxiliary signaling proteins.

a. Frangaj, A., Fan, Q. R. Structural biology of GABAB receptor. Neuropharmacology 136, 68-79 (2018). PMIC: 29031577. PMID: 29031577.

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

None.

BIOGRAPHICAL SKETCH

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NAME: Fu, ZIAO

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

POSITION TITLE: GRADUATE STUDENT

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
JILIN UNIVERSITY, CHANGCHUN, CHINA	B.S.	2012	CHEMISTRY

A. Personal Statement

I have been working in the cryo-EM field for five years in Joachim Frank's lab as a graduate student. Mainly I am working on time-resolved cryo-EM method development and application. I showed a few successful cases to demonstrate the time-resolved cryo-EM method can capture short-lived intermediates which have half-life time from 10 ms to 1 s. Apart from this main project I am working on, I am also involved in a few other projects to help the researchers to succeed in solving structures using cryo-EM technique. One exciting example is a collaboration with Wayne Hendrickson's lab on membrane protein extraction method development. Avoiding detergent in protein extraction from cell membrane, we can preserve native lipids and we observed for the native lipid bilayer directly extracted from native cell membrane. The method would help researchers working with membrane protein to gain more insight in terms of protein lipid interaction and lipid functional and structure roles. With the experience of cryo-EM and membrane protein, I am planning to collaborate with Dr. Fan's lab to determine GABA(B) receptor structures in various functional states.

B. Positions and Honors

Research Assistant 2014-2018 Columbia University.

C. Contributions to Science

1. The structural basis for release factor activation during translation termination revealed by time resolved cryo-EM. 2018 **Ziao Fu*, Gabriele Indrisiunaite*, Sandip Kaledhonkar*, Binita Shah, Ming Sun, Bo Chen, Robert A. Grassucci, Måns Ehrenberg, Joachim Frank** (in preparation)

   We determined high-resolution structures of short-lived intermediates in the translation termination process using time-resolved Cryo-EM technique.

2. Real-time structural dynamics of late steps in bacterial translation initiation visualized using time-resolved cryogenic electron microscopy. 2018 **Sandip Kaledhonkar* , Ziao Fu* , Kelvin Caban* , Wen Li , Bo Chen , Ming Sun , Ruben Gonzalez Jr, Joachim Frank** （Nature in review）

We determined high-resolution structures of short-lived intermediates in the translation initiation process using time-resolved Cryo-EM technique.

3. Structure and Activity of Lipid Bilayer within a Membrane Protein Transporter 2018 **Weihua Qiu*, Ziao Fu*, Guoyan G. Xu, Robert A. Grassucci, Yan Zhang, Joachim Frank, Wayne A. Hendrickson, Youzhong Guo** (PNAS in review)
   We solved native lipid bilayer structure by Cryo-EM technique at high resolution about 3 A.

4. A Fast and Effective Microfluidic Spraying-Plunging Method for High-Resolution Single-Particle Cryo-EM 2017 **Xiangsong Feng*, Ziao Fu*, Sandip Kaledhonkar, Yuan Jia, Binita Shah, Amy Jin, Zheng Liu, Ming Sun, Bo Chen, Robert A Grassucci, Yukun Ren, Hongyuan Jiang, Joachim Frank, Qiao Lin** Structure 25 (4), 663-670. e3
   We describe a spraying-plunging method for preparing cryoelectron microscopy (cryo-EM) grids with vitreous ice of controllable, highly consistent thickness using a microfluidic device. The new polydimethylsiloxane (PDMS)-based sprayer was tested with apoferritin. We demonstrate that the structure can be solved to high resolution with this method of sample preparation. Besides replacing the conventional pipetting-blotting-plunging method, one of many potential applications of the new sprayer is in time-resolved cryo-EM, as part of a PDMS-based microfluidic reaction channel to study short-lived intermediates on the timescale of 10-1,000 ms.

5. Key intermediates in ribosome recycling visualized by time-resolved cryoelectron microscopy 2016 **Ziao Fu*, Sandip Kaledhonkar*, Anneli Borg*, Ming Sun, Bo Chen, Robert A Grassucci, Måns Ehrenberg, Joachim Frank** Structure 24 (12), 2092-2101

   We determined the structures of short-lived intermediates in the translation recycling process using time-resolved cryo-EM technique. Upon encountering a stop codon on mRNA, polypeptide synthesis on the ribosome is terminated by release factors, and the ribosome complex, still bound with mRNA and P-site-bound tRNA (post-termination complex, PostTC), is split into ribosomal subunits, ready for a new round of translational initiation. Separation of post-termination ribosomes into subunits, or “ribosome recycling,” is promoted by the joint action of ribosome-recycling factor (RRF) and elongation factor G (EF-G) in a guanosine triphosphate (GTP) hydrolysis-dependent manner. Here we used a mixing-spraying-based method of time-resolved cryo-electron microscopy (cryo-EM) to visualize the short-lived intermediates of the recycling process. The two complexes that contain (1) both RRF and EF-G bound to the PostTC or (2) deacylated tRNA bound to the 30S subunit are of particular interest. Our observations of the native form of these complexes demonstrate the strong potential of time-resolved cryo-EM for visualizing previously unobservable transient structures.

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

None.