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

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 G protein-coupled receptor (GPCR) important for the regulation of reproduction in mammals. As an independent investigator, my research has focused on the structure and functionl of a family of dimeric GPCRs, specifically human $GABA_B$ receptor and human calcium-sensing (CaS) receptor. 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 CaS receptor in both the resting and active conformations. Based on these structures, we found that amino acids function as orthosteric agonists of the CaS receptor. Recently, my lab has been acquiring expertise in single particle Cryo-EM reconstruction for our projects. With the support from our collaborators, Dr. Joachim Frank and Dr. Oliver Clarke, we are in an ideal environment to expand into the cryo-EM field. In summary, given my expertise and my collaborative network, we are poised to make significant contributions to understanding the molecular mechanisms of dimeric GPCR activation and regulation.

1. 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.) Funding: R01GM088454 (NIGMS).
2. 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.*** (2016). Structural mechanism of ligand activation in human calcium-sensing receptor. *eLife*. 5, e13662. PMID: 27434672. (*Corresponding author.) Funding: R01GM112973 (NIGMS).

3. 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.*** (2019). Structural basis for auxiliary subunit KCTD16 regulation of the $GABA_B$ receptor. *Proc. Natl. Acad. Sci. USA.* 116, 8370-8379. PMID: 30971491. (*Corresponding author.) Funding: R01GM088454 (NIGMS) and R01GM12580 (NIGMS).
4. 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. (*Corresponding authors.) Funding: R01GM088454 (NIGMS) and R01GM12580 (NIGMS).

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 $GABA_{B1}$ 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 succeeded in assembling a complex between the extracellular domains of the ligand-binding subunit ($GABA_{B1}$) and the modulatory subunit ($GABA_{B2}$), 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. 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 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. Recently, we also 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.*** (2012). Structure and functional interaction of the extracellular domain of human $GABA_B$ receptor GBR2. *Nature Neuroscience* 15, 970-978. PMID: 22660477. (*Corresponding author.) Funding: R01GM088454 (NIGMS).
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.) Funding: R01GM088454 (NIGMS).
c. Burmakina, S., Geng, Y., Chen, Y., and **Fan, Q. R.*** (2014). Heterodimeric coiled-coil interactions of the human $GABA_B$ receptor. *Proc. Natl. Acad. Sci. USA*. 111, 6958-6963. PMID: 24778228. (*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.*** (2019). Structural basis for auxiliary subunit KCTD16 regulation of the GABAB receptor. Proc. Natl. Acad. Sci. USA. 116, 8370-8379. PMID: 30971491. (*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 determined a near full-length structure of human $GABA_B$ receptor to atomic resolution, captured in the inactive state by cryo-EM. The clear visualization of side chain densities allows us to describe critical heterodimer interactions in the transmembrane region that control receptor activation. Specifically, our structure reveals a novel heterodimer interface between the transmembrane helices 3 and 5 of both $GABA_B$ subunits. This interface represents the signature heterodimer arrangement of $GABA_B$ TM domains in the inactive conformation. Furthermore, we identify a unique ′intersubunit latch′ motif within this TM interface that maintains the inactive state of the receptor. We show that disruption of the ‘intersubunit latch’ through single point mutations renders the receptor constitutively active. To our surprise, we discover multiple endogenous ligands pre-associated with $GABA_B$ receptor, including two large phospholipids embedded within the transmembrane domains. These lipids serve as architectural braces to uphold the receptor integrity. In addition, the lipids may function as negative allosteric modulators since mitigation of receptor-lipid interaction results in higher receptor activity.

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. (*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 (CaS) receptor, a G-protein coupled receptor that maintains extracellular Ca^{2+} homeostasis through the regulation of parathyroid hormone secretion. CaS receptor activates multiple signaling pathways and responds to a variety of ligands. The general consensus is that the principal agonist of CaS receptor is extracellular Ca^{2+}. Aromatic and aliphatic L-amino acids such as L-Phe and L-Trp increase the sensitivity of CaS receptor toward Ca^{2+} and are considered as positive allosteric modulators of the receptor.
We solved the crystal structures of the entire extracellular domain of CaS receptor 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.*** (2016). Structural mechanism of ligand activation in human calcium-sensing receptor. *eLife*. 5, e13662. PMID: 27434672. (*Corresponding author.) Funding: R01GM112973 (NIGMS).

4. 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.

   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.

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 inhibitory receptor KIR2D, and identified its relationship to haematopoietic receptors. 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) (no-cost extension) 08/01/2015 - 06/30/2020
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

1R01GM12580-01A1 Fan, Slesinger and Quick (PI) 09/01/2018 - 07/31/2022
NIH/NIGMS
Mechanism of activation and modulation in human GABA(B) receptor
The goal of this project is to investigate the molecular mechanisms of activation and modulation of human GABA(B) 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

Berrie Obesity Research Initiative Grant Fan (PI) 04/01/2018 - 07/31/2020
Russell Berrie Foundation
Structural analysis of sweet taste receptors
The goal of this project is to determine the structure and activation mechanism of human sweet taste receptors.
Role: PI

Completed Research Support

AHA 15GRNT22740035 Fan (PI) 07/01/2015 - 06/30/2018
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: Frangaj, Aurel

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

POSITION TITLE: Technician B

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 University, Rose Hill	B.S.	05/2016	Biological Sciences

A. Personal Statement

My goal is to determine the three-dimensional structures of full-length human $GABA_B$ and calcium-sensing (CaS) 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, and it functions as a heterodimer. Human CaS receptor controls extracellular calcium absorption, and it functions as an obligate homodimer. Our laboratory has recombinantly expressed full-length $GABA_B$ and CaS receptors in mammalian cells. I have learned to solve structures by cryo-electron microscopy, and I'm proficient at various techniques including sample preparation, data collection and data processing. I'm determined to unravel the mechanism of action of these important receptors.

B. Positions and Honors

2017-present Technician
Department of Pharmacology, Columbia University, New York, NY
2020-present Student
Biotechnology M.A. program,
Department of Biological Sciences, Columbia University, New York, NY

C. Contributions to Science

1. I performed molecular biology, protein expression and purification experiments that were 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 have helped to resolve the structure of near full-length $GABA_B$ receptor from human by cryo-EM techniques. It is in the same family as CaS receptor and experience with this protein has proven beneficial when pursuing CaS receptor structural information. 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 have also contributed to work surrounding the structural and functional interaction between $GABA_B$ receptor and its auxiliary signaling proteins.

Frangaj, A., Fan, Q. R. Structural biology of $GABA_B$ receptor. Neuropharmacology 136, 68-79 (2018). PMID: 29031577. PMCID: PMC5897222.

Zuo, H., Glaaser, I., Zhao, Y. L., Kurinov, I., Mosyak, L., Wang, H. N., Liu, J., Park, J., Frangaj, A., Sturchler, E., Zhou, M., McDonald, P., et al. Structural basis for auxiliary subunit KCTD16 regulation of the GABA(B) receptor. Proceedings of the National Academy of Sciences of the United States of America 116:8370-8379 (2019). PMID: 30971491. PMCID: PMC6486783.

Park, J., Fu, Z., Frangaj, A. et al. Structure of human $GABA_B$ receptor in an inactive state. Nature 584, 304–309 (2020). PMID: 32581365.

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