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. The research in my laboratory 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 (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. We also solved the structures of a near-full length CaS receptor 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. In this application, we aim to understand the molecular basis of G-protein activation by the $GABA_B$ and CaS receptor by solving structures of each receptor bound to its cognate G proteins. We will collaborate with Dr. Jonathan A. Javitch, an expert on intracellular signaling, to examine physiological relevance of our structural findings. Given our combined expertise, we are poised to make significant contributions to understanding the signaling mechanisms of the $GABA_B$ and CaS receptor.

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

R01 GM112973
Fan (PI)
08/01/15-06/30/20
Structural studies of human extracellular calcium-sensing 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. (*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.*** Structural mechanism of ligand activation in human calcium-sensing receptor. *eLife*. 5, e13662 (2016). PMID: 27434672. (*Corresponding author.) Funding: R01GM112973 (NIGMS).
3. 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).
4. 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. (*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
2015-present	Associate Professor of Pharmacology and Pathology and Cell Biology Department of Molecular Pharmacology & Therapeutics, Columbia University, New York, NY
2015-present	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. (*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. (*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. (*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 (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-electron microscopy (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. (*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 GPCR that maintains extracellular Ca^{2+} homeostasis through the regulation of parathyroid hormone secretion.
   We solved crystal structures of the extracellular domain of CaS receptor 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 recently determined the structures of a near-full length CaS receptor in the presence and absence of allosteric modulators. We found that activation of CaS receptor 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.

    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.) 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. (*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.
    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.
    c. **Fan, Q. R.** and Hendrickson, W. A. Comparative structural analysis of the binding domain of the follicle stimulating hormone receptor. *Proteins* 72, 393-401 (2008). 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 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.
    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.
    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. Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

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 structures of near full-length $GABA_B$ receptor and calcium-sensing (CaS) receptor by cryo-EM techniques. 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.

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

b. 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.

c. 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. 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. (*Corresponding authors.) Funding: R35GM141871 (NIGMS).

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

N/A