BIOGRAPHICAL SKETCH

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NAME: Hwang, Tzyh-Chang

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

POSITION TITLE: Professor of Pharmacology

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
National Yang-Ming University, Taiwan	MD	06/1982	Medicine
Johns Hopkins University, School of Medicine	PhD	05/1990	Physiology
Rockefeller University	Post-doc	06/1993	Electrophysiology

A. Personal Statement

I have devoted my entire academic career to the study of CFTR chloride channels, whose defective function is responsible for cystic fibrosis (CF), the most common life-shortening hereditary disease in Caucasian populations. On one hand, my PhD and post-doctoral training equips me with the know-how to tackle pertinent scientific questions with state-of-the-art, exquisitely sensitive patch-clamp electrophysiological techniques. On the other hand, it is my MD background that fosters the vision that the fundamental understanding of a disease process at the molecular level opens the door to new possibilities in treatment. I, among very few investigators in the field, take full advantage of the enduring lesson learned in the history of medicine: physiology, pharmacology, and pathology, the trilogy of basic medical sciences, complement each other down to the molecular level; when wedded harmoniously, they offer a more comprehensive understanding of any human illness. In my 29 years as an independent investigator, I have upheld this three-pronged approach and made tremendous progress in each area (described in **C. Contribution to Science**; also exemplified by the four key papers listed below). In the past two years, with the help of my former student Dr. Xiaolong Gao who had mastered the cryo-EM technology in his postdoctoral training, I have embarked on an ambitious endeavor of solving the atomic structure of CFTR and CFTR/drug complexes. Dr. Han-I Yeh, a postdoctoral fellow who has been well trained by Dr. Gao and Dr. Min Su, the Director of the EM Core at the University of Missouri, in the past two years, is now fully equipped with the technical prowess of cryo-EM technology. Moreover, the University of Missouri has made a critical investment of ~$30 M worth in building a state-of-the-art National Cryo-EM Center (see details in **Facilities Available**) on the campus. Thus, my coworkers and I are now ready to steer an already fecund research program to a new frontier. Indeed, at the moment of this writing, we have solved three high-resolution structures of CFTR or CFTR/drug complexes (details in the Proposal). Our first two cryo-EM structures of CFTR are now published in Nature Communications. I therefore believe that this new direction of research, in the long run, will move us one step closer to a comprehensive understanding of CF physiology, pharmacology, and pathology to an atomic detail.

1. Jih, K. Sohma, Y. & Hwang, TC**.** (2012). Non-integral stoichiometry in CFTR gating revealed by a pore-lining mutation. J. Gen. Physiol. 140:347-359. (Feature article on the cover, recipient of the Paul Cranefield Student Award, Society of General Physiologists) Discovering a mutant that allows us to “visualize” ATP hydrolysis-driven gating transition. This result was reproduced in several other mutations (Zhang and Hwang, JGP 2017).

2. Jih, K. & Hwang, TC. (2013). Vx-770 potentiates CFTR function by promoting decoupling between the gating cycle and ATP hydrolysis cycle. Proc. Natl. Acad. Sci. USA. 110:4404-4409. Unraveling the mechanism by which the first CFTR targeted drug (ivacaftor) works as a gating potentiator.
3. Gao, X & Hwang, TC. (2015). Localizing a gate in CFTR. Proc. Natl. Acad. Sci. USA. 112:2461-2466. Using channel permeant thiol-reagent to identify the location of CFTR's gate.
4. Gao, X et al., (2024). Allosteric inhibition of CFTR gating by CFTRinh-172 binding in the pore. Nat. Commun. doi.org/10.1038/s41467-024-50641-1. First cryo-EM structure of CFTR solved in PI's lab.

B. Positions and Honors

Positions

1993 - 1994 Assistant Professor, Lab. Cardiac/Membrane Physiology, Rockefeller University
1994 - 1999 Assistant Professor, Department of Physiology, University of Missouri-Columbia
1994 - Research Investigator, Dalton Cardiovascular Research Center
1999 - 2004 Associate Professor, Department of Physiology, University of Missouri-Columbia
2004 - 2019 Professor, Department of Medical Pharmacology and Physiology, University of Missouri
2019 - Adjunct Professor, Department of Medical Pharmacology and Physiology, University of Missouri
2019 - Professor, Department of Pharmacology, National Yang Ming Chiao Tung University, Taiwan

Professional Experiences

1999 Cardiovascular A Study Section, NIH, *ad hoc* reviewer.
2000 General Medicine B Study Section, NIH, *ad hoc* member.
2003 - 2006 Molecular, Cellular and Developmental Neurosciences 3 Study Section, NIH, regular member.
2004 - Editorial Board, Journal of General Physiology
2006 Biophysics of Neural Systems Study Section, *ad hoc* member.
2006 - 2012 Editorial Board, Biophysical Journal.
2006 - 2010 Consultant, Cystic Fibrosis Foundation Therapeutics
2007 *Ad hoc* member, Board of Scientific Council, NIH/NINDS.
2008 *Ad hoc* member, Special Emphasis Panel/Scientific Review Group ZRG1 RUS-C
2013 *Ad hoc* member, Lung Cellular, Molecular, and Immunobiology Study Section, NIH
2014 - 2016 *Ad hoc* member, ZRG1 F10A (Physiology and Pathobiology of Cardiovascular and Respiratory Systems) Study Section, NIH
2015 *Ad hoc* member of Research Development Program Review Committee, Cystic Fibrosis Foundation
2015 *Ad hoc* member of the site visit team for the review of the NCI Laboratory of Cell Biology, NIH
2017 *Ad hoc* member, Lung Cellular, Molecular, and Immunobiology Study Section, NIH
2020 *Ad hoc* member, Lung Cellular, Molecular, and Immunobiology Study Section, NIH
2024 - Reviewing Editor, Journal of Physiology

Honors and Awards

2000 Paul Cranefield Award, Society of General Physiologists.
2007 Honorary Visiting Professorship, Osaka Medical College, Japan
2009 Outstanding Alumni Award, National Yang Ming University, Taiwan
2010 Kwan-Hwa Honorary Professorship, Xian Jaotong University, China

C. Contribution to Science (Only original research articles are included.)

1. Roles of ATP binding and hydrolysis in CFTR gating: The first major breakthrough I made as a postdoctoral fellow was the finding that ATP hydrolysis is coupled to the closing of the CFTR channel when I used non-hydrolyzable ATP analogs as a tool (*a*). This study provided the explanation of how a transporter-turned channel utilizes ATP hydrolysis as the free energy to control gating conformational changes. Through the development of high-affinity, *hydrolyzable* ATP analogs (*b*), my lab demonstrated that the two ATP binding sites play different roles in controlling CFTR gating (*c*), a conclusion backed up by not only studies on pathogenic mutations described below, but also by ligand exchange experiments that showed a tight binding of ATP in CFTR's catalysis-incompetent site (or sit 1), but a fast turnover in site 2 (*d*). In this latter paper, we proposed that normal gating of CFTR does not require a constant turnover of ATP at site 1 and that ATP binding and subsequent hydrolysis at catalysis-competent site 2 are sufficient to complete a gating cycle (*d*).

a. Hwang, TC, Nagel, G. Nairn, AC & Gadsby, DC. (1994). Regulation of the gating of CFTR Cl channels by phosphorylation and ATP hydrolysis. Proc. Natl. Acad. Sci. USA. 91:4698-4702.
b. Zhou, Z, Wang, X, Li, M, Sohma, Y, Zou, X & Hwang, TC. (2005). High affinity ATP/ADP analogs as new tools for studying CFTR gating.

c. Zhou, Z, Wang, X, Liu, H, Zou, X, Li, M & Hwang, TC. (2006). The two ATP binding sites of Cystic Fibrosis Transmembrane conductance Regulator (CFTR) play distinct roles in gating kinetics and energetics. J. Gen. Physiol. 128:413-422.
d. Tsai, M, Li, M & Hwang, TC**.** (2010). Stable ATP binding mediated by a partial NBD dimer of the CFTR chloride channel. J. Gen. Physiol. 135:399-414. (Feature article on the cover)

2. Coupling mechanism of CFTR gating
Using two independent methods, we showed evidence for a non-strict coupling between ATP hydrolysis cycle and the gating cycle (*a, b*). This concept of an energetic coupling between gating conformational changes in CFTR's transmembrane domains (TMDs) and the nucleotide binding domains (NBDs) provides not only a mechanism explaining how the G551D mutation converts ATP into an inhibitory ligand (*c*), but also a conceptual framework to account for the action of a now clinically applied medicine Ivacaftor (see 4 below). While ATP binding and hydrolysis at site 2 play a moment-to-moment role in opening/closing of CFTR, the importance of site 1 has been neglected. Cryo-EM studies suggest that NBDs association and dissociation are driven by site 2 whereas movements of site 1 are passive in each gating cycle. A new paper selected as Editor's Choice (Journal of Physiology) demonstrated tight binding of ATP in site 1 serves an indispensable role in maintaining the functional stability of CFTR in the cell membrane (*d*). In this latest report, we showed that allowing ATP to dissociate from site 1—and hence a complete separation of the NBD dimer—has undesirable consequences: the channel becomes sluggishly responsive or totally unresponsive to ATP.

a. Jih, K, Sohma, Y, Li, M & Hwang, TC. (2012). Identification of a post-hydrolytic state in CFTR gating. J. Gen. Physiol. 139:359-370.
b. Jih, K, Sohma, Y & Hwang, TC. (2012). Non-integral stoichiometry in CFTR gating revealed by a pore-lining mutation. J. Gen. Physiol. 140:347-359. (Feature article on the cover, recipient of the Paul Cranefield Student Award, Society of General Physiologists)
c. Lin, W, Jih, K & Hwang, TC. (2014). A single amino acid substitution converts ATP into an inhibitory ligand. J. Gen. Physiol.144:311-320.
d. Han-I Yeh, Ying-Chun Yu, Pei-Lun Kuo, Chun-Kuang Tsai, Hsin-Duan Huang, and Tzyh-Chang Hwang. (2021). Functional stability of CFTR depends on tight binding of ATP at its degenerate ATP-binding site. J. Physiol. 599:4625-4642 (Editor's Choice).

3. CFTR's pore and gate in its TMDs: While the studies discussed above focus on the role of CFTR's two NBDs in gating modulation, my laboratory employed scanning cysteine accessibility methods (SCAM) to study CFTR's TMDs with fruitful results. In addition to the expected designation of individual transmembrane segments (TMs) to the pore construction, which were mostly confirmed by recent cryo-EM structure of CFTR, we made several mechanistically insightful findings: 1) TM1 and TM6 are involved in both gating and ion permeation (*a, b*); 2) Contrary to the assumed two-fold pseudo-symmetry conserved in ABC proteins' TMDs, CFTR's two TMDs play asymmetrical role in pore construction (*c*); 3) CFTR's gate and selectivity filter may reside in the same region that only encompasses 1 – 2 helical turns (*d*); These findings, out of our attentiveness to microscopic details during our SCAM studies, afford direct "visualization" of hydrolysis-driven gating events and hence plays a key role in formulating our new gating model that champions an energetic coupling between CFTR's gate and the gating machinery NBDs (described in 2 above).

a. Bai, Y, Li, M & Hwang, TC. (2010). Dual roles of the sixth transmembrane segment of the CFTR chloride channel in gating and permeation. J. Gen. Physiol. 136:293-309.
b. Gao, X, Bai, Y & Hwang, TC. (2013). Cysteine scanning of CFTR's first transmembrane segment reveals its plausible roles in gating and permeation. Biophys. J. 104:786-797. (Feature article on the cover)
c. Gao, X & Hwang, TC**.** (2016). Spatial positioning of pore-lining residues affirms an asymmetrical contribution of CFTR's transmembrane segments to its anion permeation pathway. J. Gen. Physiol. 147:407-422.
d. Gao, X & Hwang, TC. (2015). Localizing a gate in CFTR. Proc. Natl. Acad. Sci. USA. 112:2461-2466.

4. Molecular Pharmacology of CFTR: The very first project launched when the applicant started his independent research lab was to demonstrate that the gating defect manifested in delF508-CFTR can be rectified by a pharmacological reagent that targets the CFTR protein (*a*). This paper thus provided the proof-of-concept evidence for later efforts in drug discovery that a decade later leads to successful development of CFTR potentiator Ivacaftor (or VX-770) by Vertex Pharmaceutical Inc. As soon as the FDA approved VX-770, my laboratory reported the mechanism underlying the gating effects of this drug (*b*). Subsequently we showed how small molecules can work synergistically with VX-770 through an independent (energetically additive) or

dependent mechanism (*c*). Our latest manuscript shows that CFTRinh-172 inhibits CFTR gating through an allosteric mechanism involving conformational changes in the TMDs of CFTR.

a. Hwang, TC, Wang, F, Yang, I & Reenstra, WW. (1997). Genistein potentiates wild-type and △F508 CFTR channel. Am. J. Physiol. 273:C988-C998.
b. Jih, K & Hwang, TC. (2013). Vx-770 potentiates CFTR function by promoting decoupling between the gating cycle and ATP hydrolysis cycle. Proc. Natl. Acad. Sci. USA. 110:4404-4409.
c. Yeh, H, Yeh, J & Hwang TC. (2015). Modulation of CFTR gating by permeant ions. J. Gen. Physiol. 145:47-60. (Feature article on the cover)
d. Gao, X et al., (2024). Allosteric inhibition of CFTR gating by CFTRinh-172 binding in the pore. Nat. Commun. doi.org/10.1038/s41467-024-50641-1.

5. Defective mechanisms for pathogenic mutations in CFTR: Disease-associated mutations offer a unique opportunity for us to not only understand how mutations cause channel dysfunction, the results could also feedback to addressing the essential role of the mutated loci in modulating CFTR function. Our studies did just that. By studying mutations located in the ABC protein signature sequences (G551D in site 2 and G1349D in site 1), we demonstrate two very different gating behaviors supporting different functional roles for the two ATP-binding sites in CFTR gating (*a*). Lately, by looking into more details of the G551D-CFTR gating, we showed that site 2 in this mutant becomes paradoxically an “inhibitory” ATP-binding site as described above. Because of the critical location of this glycine residue between the bound ATP and the signature sequence of NBD, this observation, also seen with the G551E but not G551K or G551S, turns out an expected result based on our idea of an energetic coupling between NBD dimerization and gating. Our studies of the gating defects associated with the most common pathogenic mutation delF508 also unveil molecular mechanisms that have never been reported or suspected before (*c*): destabilized NBD dimer state by the mutation. Just very recently, by studying the R117H mutation that is associated with mild-form CF, we were able to demonstrate the existence of an elusive state—a closed state with NBD already dimerized (*d*). Again, this state is exactly what is predicted by the energetic coupling mechanism proposed by my coworkers and me. It is thus particularly rewarding to see the cryo-EM picture of a closed state with dimerized NBDs (Zhang et al., 2017). In light of a lack of effective medicines for CF patients carrying premature termination codon (PTC) mutations, we have started to work on the functional outcomes of PTC mutations in CFTR (*d*). The first paper out of this new research direction reports the structural basis for a PTC mutation at the C-terminus of CFTR.

a. Bompadre, SG, Sohma, Y, Li, M & Hwang, TC. (2007). G551D and G1349D, two CF-associated mutations, exhibit distinct gating defects. J. Gen. Physiol. 129(4):285-98.
b. Jih, K, Li, M, Hwang, TC & Bompadre, SG. (2011). The most common cystic fibrosis associated mutation destabilizes the dimeric state of the nucleotide-binding domains of CFTR. J. Physiol. 589:2719-2731.
c. Yu, Y, Sohma, Y & Hwang, TC. (2016). On the mechanism of gating defects caused by the R117H mutation in cystic fibrosis transmembrane conductance regulator. J. Physiol. 594:3227-3244.
d. Jiunn-Tyng Yeh, Yingchun Yu, and Tzyh-Chang Hwang. (2019). Defective CFTR function caused by the Q1412X mutation, a severe form Class VI pathogenic mutation in cystic fibrosis. J. Physiol. 597:543-560.

6. Participants in new drug development: Ever since Vertex Pharmaceuticals developed the first CFTR targeted drug ivacaftor, the applicant had launched a project tackling the mechanism for action for ivacaftor. A comprehensive characterization of GLPG1837 was done immediately after Galapagos developed this CFTR potentiator that is more efficacious than ivacaftor (*a*). In this paper, we provided evidence that VX-770 and GLPG1837 act through a common binding site (latter confirmed by cryo-EM studies). Employing classical allosteric modulation principles, we were able to propose a physicochemical mechanism for the different efficacy and potency exhibited by these two CF drugs. Lately, using a combinational approach of computational and electrophysiological techniques, we identified the potential binding sites for GLPG1837 and VX-770 (*b*). We also started testing these pharmacological reagents on disease-associated mutations to assess their potential roles in CF treatment (*c, d*). Because of the high quality and reliability of our work, my lab has served as a gateway for the authentication, at a single-channel level, of new CFTR potentiators developed by biotech companies such as AbbVie, Pfizer. Proteostasis, and Flatley Discovery Lab. The applicant is particularly proud of this role as it testifies the rigor and integrity of his research. The current proposal follows my long-standing philosophy that academic researchers play a critical role in translating research from bench to bedside by not only offering know-how or mechanistic insights, but also instilling the intellectual rigor that is essential for successful drug discovery.

a. Yeh, H, Sohma, Y, Conrath, K & Hwang, TC. (2017). A common mechanism for CFTR potentiators. J. Gen. Physiol. 149:1105-1118.
b. Yeh, Qiu, L, Sohma, Y, Conrath, K, Zou, X & Hwang, TC. (2019). Identification of the molecular target sites for CFTR potentiators GLPG1837 and VX-770. J. Gen. Physiol. 151:912-928.
c. Samantha Destefano, Maarten Gees, and Tzyh-Chang Hwang. (2018). Physiological and pharmacological characterization of the N1303K mutant CFTR. J. Cys. Fibro. 17:573-581.
d. Jiunn-Tyng Yeh, and Tzyh-Chang Hwang. (2020). Positional effects of premature termination codon on the biochemical and biophysical properties of CFTR. J. Physiol. 598:517-541. (Editor's choice; JT Yeh was awarded the Early Investigator Award by the Physiological Society, UK.)

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40866447/?sort=date&direction=ascending

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

NHRI-EX112-11236SI (NHRI, Taiwan) Hwang (PI) 01/01/2023 – 12/31/2025
Structure-based drug design for the CFTR protein
The goal of this proposal is to design novel compounds that enhance CFTR gating based on known structures of CFTR potentiators and their binding sites in the CFTR protein.

Research Grant (Hwang24G0) Hwang (PI) 11/01/24 – 10/30/2026
Cystic Fibrosis Foundation
Structure/function mechanism of CFTR correctors.
The goal of this proposal is to address how CFTR correctors work at a molecular level by solving the structures of CFTR/corrector complexes with cryo-EM.

Completed Research in the Past Three Years

Research Grant (Hwang22G0) Hwang (PI) 11/01/22 – 10/30/2024
Cystic Fibrosis Foundation
Structural basis of CFTR function and dysfunction.
Aim 1: Structural determination of closed, intermediate and open states of CFTR in high resolution with single particle cryo-EM. Aim 2: Identification of structures for defective variants of CFTR.

109-2320-B-010-049-MY2 (MOST, Taiwan) Hwang (PI) 08/01/2020 – 07/31/2022
On the mechanism of CFTR activation
The goal of the proposal is to study the role of the R domain in CFTR activation through PKA-dependent phosphorylation.

HWANG19G0 (CFF) Hwang (PI) 11/01/2019 – 10/31/2021
Therotyping Class I mutation in cystic fibrosis
The goal of this study is to investigate the biochemical and biophysical effects of premature termination codon mutations on CFTR function.

R01 DK55835 (NIDDK) Hwang (PI) 06/01/19 – 05/31/2024
Molecular pathophysiology of cystic fibrosis
The goal of this study is to investigate the gating mechanism of CFTR by fully exploiting the molecular insights out of recently-solved cryo-EM structure human and zebrafish CFTR, and to combine computational approaches to identify the binding site(s) for CFTR potentiators including VX-770 and GLPG1837.

Sponsored Research (AbbVie) Hwang (PI) 10/01/23 – 06/30/2024
Cryo-EM studies of AbbVie potentiators
The goal of this proposal is to solve the structures of CFTR complexes with two novel CFTR potentiators (X283649, X316761).

BIOGRAPHICAL SKETCH

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NAME: Gao, Xiaolong

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

POSITION TITLE: 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
Jilin Medical College, Jilin, China	BS	09/2006	06/2011	Biomedical Engineering
University of Missouri-Columbia, Missouri, USA	PhD	08/2011	12/2015	Biological Engineering
Aix-Marseille University, Provence-Alpes-Côte d'Azur, France	Postdoctoral	02/2016	10/2016	Physiology and Biophysics
Weill Cornell Medicine, New York, USA	Postdoctoral	10/2016	08/2021	Physiology and Biophysics

A. Personal Statement

My over ten years' scientific training and track record put me in a right spot to tackle topics of structure-function relationship of membrane ion channels. During my PhD study, I invested myself in the functional and structural studies of cystic fibrosis transmembrane conductance regulator (CFTR), the culprit behind the genetic disease cystic fibrosis (CF), with patch clamp technique that is the most powerful tool for functional study of ion channels. Four years' training not only allowed me to become an expert of the exquisite patch clamp technique on top of other biophysical and biochemical assays, but also infused critical thinking, scientific rigor and research integrity into my mind. My skill set is greatly broadened upon acquisition of techniques in the structural biology field during my postdoc training. Five-year training in protein purification, lipid bilayer recording, reconstitution of ion channels into lipid nanodiscs and cryo-EM techniques made me a versatile researcher who can tackle scientific questions on both functional and structural frontiers. Equipped with a full pipeline of knowledge in cryo-EM technology from sample preparation to cryo-EM data analysis, I first determined a series of different conformations of a cyclic nucleotide-gated (CNG) ion channel (SthK) in high resolution during my postdoc training, which greatly advanced our understanding of the working mechanism of CNG channels. After I returned to CF field as a research scientist, in three years' time, I have determined multiple high-resolution structures of CFTR and CFTR/drug complexes, including NBD-dimerized E1371Q-CFTR (3.4 Å), CFTR bound with inhibitor 172 (3.6 Å), CFTR bound with two drugs under clinical trial (3.2 Å and 2.6 Å, respectively) and several disease-associated G551D-CFTR bound with various ATP analogs and channel modulators (resolutions ranging from 4 Å to 7 Å). My ten years of intense scientific research have already resulted in several publications that provide exquisite insights into how physiologically important channel proteins work. On top of these achievements, I am continuously delving deeper into CFTR studies to uncover the full picture of CFTR's physiology and pathophysiology, for the benefit of the whole CF community.

Citations:

1. Xiaolong Gao, Yonghong Bai, Tzyh-Chang Hwang. (2013). Cysteine Scanning of CFTR's First Transmembrane Segment Reveals Its Plausible Roles in Gating and Permeation. *Biophysical Journal*, volume 104, issue 4, p786-797. *Selected as featured article with appearance on the journal cover.*

2. Xiaolong Gao and Tzyh-Chang Hwang. (2015). Localizing a gate in CFTR. *Proceedings of the National Academy of Sciences of the United States of America*, volume 112, No. 8, 2461-2466. *CFTR's gate is localized for the first time*.
3. Xiaolong Gao*, Philipp A. M. Schmidpeter*, Vladimir Berka, Ryan J. Durham, Chen Fan, Vasanthi Jayaraman, Crina M. Nimigean. (2022). Gating intermediates reveal inhibitory role of the voltage sensor in a cyclic nucleotide-modulated ion channel. *Nature Communications*. Volume 13, page 6919. * *denotes co-first authors*.
4. Xiaolong Gao†, Han-I Yeh, Zhengrong Yang, Chen Fan, Fan Jiang, Rebecca J. Howard, Erik Lindahl, John C. Kappes, Tzyh-Chang Hwang†. (2024). Allosteric inhibition of CFTR gating by CFTRinh-172 binding in the pore. *Nature Communications*. Volume 15, page 6668. † *denotes correspondence authors*.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

09/2021 – present	Research Scientist, University of Missouri-Columbia, Missouri, USA
10/2016 – 08/2021	Postdoctoral Associate, Weill Cornell Medicine, New York, USA
02/2016 – 09/2016	Postdoctoral Associate, Aix-Marseille University, Provence-Alpes-Côte d'Azur, France
2011 – 2015	Graduate Research Assistant, University of Missouri-Columbia, Missouri, USA

Honors

2018	Travel Award, Society of General Physiologists, USA
2015	Outstanding Ph.D. Award, University of Missouri-Columbia, USA
2007	First-class Scholarship, Jilin Medical College, China

C. Contributions to Science

1. Molecular Understanding of CFTR's Function and Structure: Throughout my PhD study, my research focused on the function and structure of CFTR chloride channel, whose dysfunction causes genetic disease cystic fibrosis (CF). To begin with, by introducing cysteines into the first transmembrane segment (TM1) of CFTR, I found several of the TM1 residues can be accessed by bulky thiol-reactive reagents, indicating their contribution to the permeation pathway construction. Especially, state-dependent accessibility of identified pore-lining residues demonstrated TM1 moves out of the pore in the closed state, defining a dynamic motion of TM1 in CFTR's gating. On top of this, cross-linking experiments on cysteines engineered into TMs 1, 6 and 12 identified multiple cross-linkable pairs by Cd^{2+} among these three TMs, depicting a pore of CFTR in which both TM1 and TM6 contribute to the narrow region in the pore while the spatial positioning of TM12 residues are more intracellular than they were previously reported. More interestingly, by applying channel permeant probe $Au[CN]_2^-$, I, for the first time, discovered the location of CFTR's gate which governs ion flow through the pore. Such a position coincides with the narrow region in the pore which potentially serves as the selectivity filter for the channel. All above discoveries are confirmed by later solved cryo-EM structures in high resolution. In the past three years, as mentioned above in **Personal Statement**, I have determined multiple CFTR structures in high resolution with cryo-EM technology. Very recently, a manuscript where I serve as both the first author and a corresponding author detailing the inhibition mechanism of inhibitor 172 on CFTR was published by *Nature Communications*. With my exceptional skills in structural and functional study of ion channels, I am developing more advanced strategies to capture new conformations of CFTR in hope of deciphering its complete physiological roles and directing future design of better CF drugs by determining more high-resolution structures of CFTR and CFTR/drug complexes.

a. Xiaolong Gao, Yonghong Bai, Tzyh-Chang Hwang. (2013). Cysteine Scanning of CFTR's First Transmembrane Segment Reveals Its Plausible Roles in Gating and Permeation. *Biophysical Journal*, volume 104, issue 4, p786-797. *Selected as featured article with appearance on the journal cover.*
b. Xiaolong Gao and Tzyh-Chang Hwang. (2015). Localizing a gate in CFTR. *Proceedings of the National Academy of Sciences of the United States of America*, volume 112, No. 8, 2461-2466. *CFTR's gate is localized for the first time*.

c. Xiaolong Gao and Tzyh-Chang Hwang. (2016). Spatial positioning of CFTR's pore-lining residues affirms an asymmetrical contribution of transmembrane segments to the anion permeation pathway. *The Journal of General Physiology*, volume 147, No. 5, 407-422. *First author was nominated for Cranefield Student Award*.
d. Xiaolong Gao†, Han-I Yeh, Zhengrong Yang, Chen Fan, Fan Jiang, Rebecca J. Howard, Erik Lindahl, John C. Kappes, Tzyh-Chang Hwang†. (2024). Allosteric inhibition of CFTR gating by CFTRinh-172 binding in the pore. *Nature Communications*. Volume 15, page 6668. † *denotes correspondence author*.

2. Gating Mechanism of Cyclic Nucleotide-Gated (CNG) Potassium Channel: CNG channels play important roles in visual and olfactory perception in sensory neurons as well as pace-making activity in heart and brain. By characterizing SthK, a prokaryotic potassium channel originated from *Spirochetae thermophila*, I established a terrific study model to investigate the function and structure of cyclic nucleotide-activated and voltage-modulated CNG channels. Functional measurements in lipid bilayers revealed SthK prefers cAMP over cGMP as its agonist, and the channel activity is elevated with more depolarized membrane potential. Using the cryo-EM technique, I solved a series of high-resolution conformations of SthK from closed to its fully open state, which reveal the complete conformational changes taking place in a gating cycle of the CNG channel. Specifically, the conformational differences observed among different states unequivocally pinpointed how cAMP binding to the cyclic nucleotide-binding domains opens the channel gate in its transmembrane domains. In addition, based on the pore opening pattern, I proposed a voltage-modulation mechanism for CNG channels that also extends its application to other voltage-activated/modulated potassium channels.

a. Arin Marchesi, Xiaolong Gao, Ricardo Adaixo, Jan Rheinberger, Henning Stahlberg, Crina M. Nimigean, Simon Scheuring. (2018). An iris diaphragm mechanism to gate a cyclic nucleotide-gated ion channel, *Nature communications*, Sep 28; 9 (1): 3978.
b. Jan Rheinberger, Xiaolong Gao, Philipp A.M. Schmidpeter, and Crina M. Nimigean. (2018). Ligand discrimination and gating in cyclic nucleotide-gated ion channels from apo and partial agonist-bound cryo-EM structures. *elife*, 2018; 7: e39775.
c. Philipp A.M. Schmidpeter*, Xiaolong Gao*, Vikrant Uphadyay, Jan Rheinberger, and Crina M. Nimigean. (2018). Ligand binding and activation properties of the purified bacterial cyclic nucleotide-gated channel SthK. *The Journal of General Physiology*, volume 150, No. 6, 821-834. **Co-first authors. Received compliments in Research News and selected into special collection: Molecular Biophysics of Membranes 2018 of JPG.*
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