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 whole 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 trainings equip me with the know-how to tackle pertinent scientific questions with the 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 and, when wedded harmoniously, they offer a more comprehensive understanding of any human illness. In my 25 years as an independent investigator, I have upheld this three-pronged approach and made tremendous progress in each area and as a whole (described in **C. Contribution to Science**; also exemplified by the four key papers listed below). With more than two decades of continuous NIH funding, I have established my laboratory as a formidable force in pushing the frontier of molecular medicine for CF. Three decades of fundamental research on the structure/function relationship of CFTR have placed me at a vantage point to take full advantage of the newly developed structural biology initiative at the University of Missouri. By recruiting my former student Dr Xiaolong Goa to launch cryoEM studies of this medically important protein, I believe that achieving the goal of the current proposal will move us one step closer to a comprehensive understanding of this mysterious protein that straddles between ion channels and active transporters.

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. Tzyh-Chang Hwang, Jiunn-Tyng Yeh, Jingyao Zhang, Ying-Chun Yu, Han-I Yeh, and Samantha Destefano. (2018). Structural mechanisms of CFTR function and dysfunction. J. Gen. Physiol. 150:539-570. On the most read article list since its publication in April 2018.

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 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

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. Molecular Physiology of 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 employed non-hydrolyzable ATP analogs as a tool (*a*). This study provides the first piece of evidence explaining how a transporter-turned channel utilizes ATP hydrolysis as the free energy source 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*). Of note, a crystallographic report of a bacterial ABC exporter (Hohl et al., 2012) later documented a structure predicted in our functional study. Using two independent methods, we lately showed evidence for a non-strict coupling between ATP hydrolysis cycle and the gating cycle (*e, f*). This new concept of an energetic coupling between gating conformational changes in CFTR's transmembrane domains (TMDs) and nucleotide binding domains (NBDs) provides not only a mechanism explaining numerous puzzling data in the literature, but also a conceptual framework to account for the action of a now clinically applied medicine Ivacaftor.

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)
e. Jih, K, Sohma, Y, Li, M & Hwang, TC. (2012). Identification of a post-hydrolytic state in CFTR gating. J. Gen. Physiol. 139:359-370.
f. 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)
g. Jingyao Zhang, Ying-Chun Yu, Jiunn-Tyng Yeh, and Tzyh-Chang Hwang. (2018). Functional characterization of zebrafish CFTR reveals preferable closed channel conformation. PlosOne 13(12):e0209862.

2. 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 started to employ SCAM to study CFTR's TMDs in the past 7 years with fruitful results. In addition to the expected designation of individual transmembrane segments (TMs) to the pore construction, 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*); 4) A group of mutations in CFTR's TMDs reveals ATP hydrolysis-driven open-to-open state transition (*a, e*). 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 (see Jih and Hwang, Physiology 27:351-361 for details). It is important to note that the findings described in 1) and 2) are verified recently by the cryo-EM structures of CFTR. On the contrary, our propositions noted in 3) and 4) are contested by the cryo-EM structures, demanding more thorough studies proposed in the current application.

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.
e. Zhang, J & Hwang, TC. (2017). Electrostatic tuning of the pre- and post-hydrolytic open states in CFTR. J. Gen. Physiol. 149:355-372. (Feature article on the cover)

3. 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,d*). These latest studies again demonstrate proof-of-concept results for future development of compounds that can complement or supplant VX-770. This is important as VX-770, albeit providing significant symptomatic relief for patients carrying the mutations with gating defects, is not effectively enough to completely rectify their gating defects. In collaboration with pharmaceutical industry, my lab has just completed a project looking into a newly developed CFTR potentiator GLPG1837, which is more efficacious but less potent than VX-770. In this paper (*e*), we provided evidence that VX-770 and GLPG1837 act through a common binding site. 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. Then, using a combinational approach of computational and electrophysiological techniques, we identified the potential binding sites for GLPG1837 and VX-770 (*f*)

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. Lin, W, Sohma, Y & Hwang, TC. (2016). Synergistic potentiation of CFTR gating by two chemically distinct potentiators ivacaftor (VX-770) and NPPB. Mole. Pharm. 90:275-285.
e. Yeh, H, Sohma, Y, Conrath, K & Hwang, TC. (2017). A common mechanism for CFTR potentiators. J. Gen. Physiol. 149:1105-1118.
f. 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

4. 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 feed back 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 (*b*). 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. Although not acknowledged by the cryo-EM investigators, our finding preceded the cryo-EM picture of a closed state with dimerized NBDs (Zhang et al., 2017) by a full year of time. Furthermore, our accurate assessment of the gating defect associated with the R117H mutation was validated lately by an in vivo study on patients carrying the R117H mutation (Char et al., 2017). Our latest studies show that the outcomes of PTC mutations are variable and depend of the position of PTC. We are the first to demonstrate, at a single-channel level, the biophysical and pharmacological properties of read-through products and show that the missense mutation accompanying read-through can be affected by read-through reagents (*f, g*).

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. Lin, W, Jih, K & Hwang, TC. (2014). A single amino acid substitution converts ATP into an inhibitory ligand. J. Gen. Physiol.144:311-320.
c. 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.
d. 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.
e. Samantha Destefano, Maarten Gees, and Tzyh-Chang Hwang. (2018). Physiological and pharmacological characterization of the N1303K mutant CFTR. J. Cys. Fibro. 17:573-581.
f. 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.
g. 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.

5. 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 (Jih and Hwang 2013). A comprehensive characterization of GLPG1837 was done immediately after Galapagos developed this new CFTR potentiator (Yeh et al., 2017). 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 offering not only know-how or mechanistic insights, but also the intellectual rigor that is essential for successful drug discovery.

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

R01 DK55835 (NIDDK) Hwang (PI) 06/01/19 – 05/31/23
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.
Role: PI

HWANG19G0 (CFF) Hwang (PI) 11/01/19 – 10/31/21
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.
Role: PI

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.
Role: PI

Completed Research in the Past Three Years

Startup fund (National Yang-Ming University) Hwang (PI) 09/01/2019 – 08/31/2020
The startup fund will allow the PI to establish a state-of-the art laboratory to study molecular medicine of ion channel diseases.

Seed Funding (National Yang-Ming University) Hwang (PI) 08/01/2020 – 12/31/2020
This Institutional fund is used to support the three labs (Hwang, Chiou, and Her) involved in the current project.

R01 DK55835 Hwang (PI) 09/01/14 – 08/31/19
Molecular pathophysiology of cystic fibrosis
The goal of this study is to investigate the functional role of CFTR's transmembrane domains, to tackle the mechanism underlying gating defects in G551D, the third most common pathogenic mutation, and to address the mechanism of action for VX-770 or Ivacaftor, a drug targeting the CFTR protein.

Sponsored Research (AbbVie) Hwang (PI) 01/01/17 – 12/31/17
Patch-clamp electrophysiological investigation of CFTR modulators
The goal of this proposal is to characterize latest drug GLPG1837 that has been undergone Phase II clinical trial on CFTR gating.

HWANG15G0 (CFF) Hwang (PI) 09/01/15 – 08/31/17
Structure-based drug design for cystic fibrosis
The goal of this study is to investigate the mechanism of action for a proprietary compound that improves the gating of G551D and delF508-CFTR.

BIOGRAPHICAL SKETCH

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NAME: Gao, Xiaolong

eRA COMMONS USER NAME (credential, e.g., agency login): 0000-0001-8933-9286

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)*	Completion Date MM/YYYY	FIELD OF STUDY
Jilin Medical College, China	BS	06/2011	Biomedical Engineering
University of Missouri-Columbia	PhD	12/2015	Biological Engineering
Weill Cornell Medicine	Postdoctoral Associate	09/2021	Physiology and Biophysics

A. Personal Statement

As a well-trained electrophysiologist and structural biologist, my passion lies in unraveling the function and structure of biological protein molecules. During my PhD study, I invested myself in the investigation of structure and function of CFTR chloride channel, the culprit behind a lethal genetic disease cystic fibrosis (CF) affecting mostly Caucasians. Four years of 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. My achievements on functional and structural study of cyclic nucleotide-gated (CNG) ion channels using Cryo-EM, a technique that initiated the resolution revolution for biological studies, has led to a much better understanding of ligand-gated and voltage-modulated ion channels. These ten years of intense scientific research have resulted in several publications that provide pivotal insights into the working mechanism of these physiologically important channel proteins. Equipped with a plethora of biophysical/biochemical techniques, intense curiosity and willingness to serve, I will continue my research to further our understanding on CFTR, and use the knowledge I gained to explore better remedies for CF patients.

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 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*.
4. Xiaolong Gao, Chen Fan, Vladimir Berka, Vasanthi Jayaraman, Crina M. Nimigean. (2021). Dynamic pore opening of CNG channels revealed with cryo-EM. *Manuscript in preparation*.

B. Positions and Honors

Positions

2011 – 2015	Graduate Research Assistant, University of Missouri-Columbia
2015 – 2021	Postdoctoral Associate, Weill Cornell Medicine
2021 – Present	Research Scientist, University of Missouri-Columbia

Honors

2007	First-class Scholarship, Jilin Medical College
2015	Outstanding PhD Award, University of Missouri-Columbia
2018	Travel Award, Society of General Physiologists

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 (*a*). 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 (*b*). 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 (*c*). All above discoveries are confirmed by later solved cryo-EM structures in high resolution. While all current molecular structures of CFTR are in closed state, by adopting new mutations and various compounds, 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.

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. (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*.

c. 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*.

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 (*a*-*c*). Using the cryo-EM technique, I solved a series of conformations of SthK from closed to its fully open state, which allow me to depict a complete picture of conformational changes taking place in a gating cycle of the CNG channel. The conformational differences observed among different states unequivocally pinpointed how cAMP binding to the nucleotide-binding domain opens the channel gate. 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 manuscript is currently under preparation. In addition, functional characterization of ion channels at single channel level established by me was successfully implanted to other membrane transport proteins to study the function and structure correlation between electrophysiology and atomic force microscopy measurements (*d*).

*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.*

d. Raghavendar Reddy Sanganna Gari, Joel José Montalvo-Acosta, George R. Heath, Yining Jiang, Xiaolong Gao, Crina M. Nimigean, Christophe Chipot, and Simon Scheuring. (2021). Correlation of Membrane Protein Conformational and Functional Dynamics. *Nature Communications*, 12, 4363.