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

As a well-trained electrophysiologist and structural biologist, my passion lies in unraveling the function-structure relationship of membrane channel proteins. During my PhD study, I invested myself in the functional and structural studies of CFTR chloride channel, the culprit behind cystic fibrosis (CF) which is a lethal hereditary disease prevalent mostly among Caucasians in North America. Four years of training not only allowed me to become an expert with 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 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 determined a series of different conformations of a cyclic nucleotide-gated (CNG) ion channel in high resolution, which greatly advanced our understanding of the working mechanism of CNG channels. My more than ten years of intense scientific research have already resulted in several publications that provide insights into how these physiologically important channel proteins work. After my postdoc training, I am thrilled that I got an opportunity to bring my structural biology expertise back to the CF field. Within less than two years so far, I have determined several high-resolution molecular structures of CFTR and CFTR/drug complex. I will continue to delve deeper into the working mechanism of CFTR molecule and use the structural and functional insights gained from my study to guide the development of better treatment strategies for an ultimate cure for all CF patients.

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 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*, 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, issue 1, page 6919.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 – Present	Research Scientist, University of Missouri-Columbia, Missouri, USA
2016 – 2021	Postdoctoral Associate, Weill Cornell Medicine, New York, USA
02/2016 – 10/2016	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 PhD 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 determined by me and other labs. 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, especially the open state(s), of CFTR. Meanwhile, I have solved CFTR structures bound with different investigational compounds that shed lights on how CFTR protein interacts with these medicinal drugs. These protein/drug complex act as valuable guides for development of novel drug treatments for all CF patients.

Ongoing projects that I would like to highlight:

HWANG22G0 (CFF)
Hwang (PI), Role: Co-Principal Investigator (Co-PI)
11/01/2022 – 10/31/2024
Structural Basis of CFTR Function and Dysfunction
Major goals: To determine various conformations in CFTR's gating cycle by means of the most advanced single particle cryo-EM technology introduced lately by the Co-PI to the laboratory (Aim 1), and to delve into the pathophysiological mechanism of CF-causing mutations through structural analysis of these mutant CFTR proteins (Aim 2).

NCCAT-BAG-XG211223 (NCCAT)

Hwang (PI), Role: Spokesperson and Primary User
03/10/2022 – 03/09/2024
Structural Basis of CFTR Dysfunction and Pharmacology
Aim 1: To obtain the open channel and physiologically relevant intermediate channel structures of CFTR.
Aim 2: To identify the binding sites on CFTR for clinically approved drugs and compounds with known pharmacological effects on CFTR gating.

Citations:

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*.

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 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. 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.

*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. 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, issue 1, page 6919. **Co-first authors*.

e. 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.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Hwang, Tzyh-Chang (TC)

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

POSITION TITLE: Adjunct Professor of Medical Pharmacology & Physiology

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
National Yang-Ming University, Taiwan	MD	09/1975	06/1982	Medicine
Johns Hopkins University, School of Medicine	PhD	09/1986	05/1990	Physiology
Rockefeller University	Post-doc	07/1990	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 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; when wedded harmoniously, they offer a more comprehensive understanding of any human illness. In my 28 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. In the past two years, I was very fortunate to recruit my former graduate student Dr. Xiaolong Gao, who had mastered the cryo-EM technology in his postdoctoral training at Weill Cornell Medicine, to my lab. His joining my lab, together with the fact that 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**) in the campus, serves as a driving force that catapults my research program to a new frontier. Of note, the projects proposed in the current application are almost all based on our past and present mechanistic studies of CFTR and CF pathogenesis. The molecular insights amassed from almost three decades of rigorous investigations place the applicant's lab at a vantage point to tackle unaddressed questions of both basic and clinical significance. Answering these questions will move us one step closer to a comprehensive understanding of CF physiology, pharmacology and pathology to an atomic detail.

Ongoing projects:

R01 DK55835 (NIDDK) Hwang (PI) 05/01/2019 – 04/30/2024 (NCE)
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

HWANG22G0 (CFF) Hwang (PI) 11/01/2022 – 10/31/2024
Structural Basis of CFTR Function and Dysfunction
Major goals: To determine various conformations in CFTR's gating cycle by means of the most advanced single particle cryo-EM technology introduced lately by the Co-PI to the laboratory (**Aim 1**), and to delve into the pathophysiological mechanism of CF-causing mutations through structural analysis of these mutant CFTR proteins (**Aim 2**).
Role: PI

NCCAT-BAG-XG211223 (NCCAT) Hwang (PI) 03/10/2022 – 03/09/2024
Structural Basis of CFTR Dysfunction and Pharmacology
Aim 1: To obtain the open channel and physiologically relevant intermediate channel structures of CFTR.
Aim 2: To identify the binding sites on CFTR for clinically approved drugs and compounds with known pharmacological effects on CFTR gating.
Role: PI

Sponsored Research (AbbVie Inc.) Hwang (PI) 01/01/2023 – 12/31/2023
Structure/function studies of CFTR potentiator
The goal of this proposal is to characterize two novel CFTR potentiators (X283649, X316761) on CFTR gating.

NHRI-EX112-11236SI (NHRI, Taiwan) Hwang (PI) 01/01/2023 – 12/31/2025
Structure-based drug design for CFTR
Major goals: The major goal of the project is to use cryo-EM structures of CFTR/drug complex to design CFTR potentiators with higher potency and efficacy (**Aim 1**), and to understand the molecular mechanism for the gating effects of VX-445 (**Aim 2**).
Role: PI

VGHUST112-G7-5-1 (Taipei Veteran General Hospital, Taiwan). Hwang (PI) 01/01/2023 – 12/31/2023
Molecular characterization of physiological and pharmacological properties of CFTR PTC mutations
Major goals: Biochemical and biophysical characterization of S308X-CFTR and other CFTR PTC mutations (**Aim 1**), and pharmacological rescue of S308X-CFTR and other CFTR PTC mutations (**Aim 2**).
Role: PI

Citations:

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 physical location of CFTR's gate, which coincides with the obstructed segment in CFTR's pore as revealed in the atomic structure of CFTR.
4. 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.

A more complete list of published work can be found in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40866447/?sort=date&direction=ascending

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2022 – present Member, the User Review Committee (URC) of National Center for Cryo-EM Access and Training (NCCAT)
2022 *Ad hoc* member, Lung Cellular, Molecular, and Immunobiology Study Section, NIH

2020	*Ad hoc* member, Lung Cellular, Molecular, and Immunobiology Study Section, NIH
2019 - present	Professor, Department of Pharmacology, National Yang Ming Chiao Tung University, Taiwan
2019 - present	Adjunct Professor, Department of Medical Pharmacology and Physiology, University of Missouri
2019 - present	Professor Emeritus, Department of Medical Pharmacology and Physiology, University of Missouri
2019	*Ad hoc* member, ZDK1 GRB-M (J1) P Review Panel, Cystic Fibrosis Clinical and Translation Centers, NIDDK
2017	*Ad hoc* member, ZDK1 GRB-M (J1) P Review Panel, Cystic Fibrosis Clinical and Translation Centers, NIDDK
2017	*Ad hoc* member, Lung Cellular, Molecular, and Immunobiology Study Section, NIH
2015	*Ad hoc* member of the site visit team for the review of the NCI Laboratory of Cell Biology, NIH
2015	*Ad hoc* member of Research Development Program Review Committee, Cystic Fibrosis Foundation
2014 - 2016	*Ad hoc* member, ZRG1 F10A (Physiology and Pathobiology of Cardiovascular and Respiratory Systems) Study Section, NIH
2013	*Ad hoc* member, Lung Cellular, Molecular, and Immunobiology Study Section, NIH
2008	*Ad hoc* member, Special Emphasis Panel/Scientific Review Group ZRG1 RUS-C
2007	*Ad hoc* member, Board of Scientific Council, NIH/NINDS.
2006 - 2010	Consultant, Cystic Fibrosis Foundation Therapeutics
2006 - 2012	Editorial Board, Biophysical Journal
2006	Biophysics of Neural Systems Study Section, *ad hoc* member
2004 - 2019	Professor, Department of Medical Pharmacology and Physiology, University of Missouri
2004 - present	Editorial Board, Journal of General Physiology
2003 - 2006	Molecular, Cellular and Developmental Neurosciences 3 Study Section, NIH, regular member
2000	General Medicine B Study Section, NIH, *ad hoc* member
1999	Cardiovascular A Study Section, NIH, *ad hoc* reviewer
1999 - 2004	Associate Professor, Department of Physiology, University of Missouri-Columbia
1994 - present	Research Investigator, Dalton Cardiovascular Research Center
1994 - 1999	Assistant Professor, Department of Physiology, University of Missouri-Columbia
1993 - 1994	Assistant Professor, Lab. Cardiac/Membrane Physiology, Rockefeller University

Honors

2010	Kwan-Hwa Honorary Professorship, Xian Jaotong University, China 2015
2009	Outstanding Alumni Award, National Yang Ming University, Taiwan
2007	Honorary Visiting Professorship, Osaka Medical College, Japan
2000	Paul Cranefield Award, Society of General Physiologists

C. Contributions to Science

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 provided evidence for a non-strict coupling between the 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) offers 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 not been studied extensively. Cryo-EM data however suggest that NBDs association and dissociation are driven by site 2 whereas movements of site 1 are passive in each gating cycle. A recent 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 functional 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. Yeh, H, Yu, Y, Kuo, P, Tsai, C, Huang, H, and Hwang, TC. (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 ended in demonstrating 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.

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.

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. A recent paper (*d*) out of this new research direction reports the positional effects of PTC on CFTR function. The paper was chosen as Editor’s choice, and the first author was awarded the Early Investigator Award by the Physiological Society, UK.

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. Yeh, JT, and TC 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.)