BIOGRAPHICAL SKETCH

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NAME: **Stephen B. Long, PhD**

eRA COMMONS USER NAME: LONGSB

POSITION TITLE: **Member, Structural Biology Program, MSK**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Amherst College, Amherst, MA	BA	05/1994	Physics
Duke University, Durham, NC	PhD	05/2001	Biochemistry/Structure
Duke University, Durham, NC	Postdoc	07/2003	Biochemistry/Structure
Rockefeller University, New York, NY	Postdoc	05/2007	Structural Neurobiology

A. PERSONAL STATEMENT

The objectives of my laboratory are to understand the biophysical mechanisms of eukaryotic ion channels and of enzymes that catalyze chemical reactions within biological membranes and to relate these mechanisms to physiology and disease. The enzymes and ion channels we study have important biological functions and are implicated in cancer and in eye, heart, and immune disorders. For ion channels, we seek to understand the ways in which potassium, calcium, and chloride channels conduct ions across cellular membranes, the chemistry of ion coordination that imputes the channels with ion selectivity, and the mechanisms for how channels are gated by ligands, properties of the membrane, and protein-protein interactions. For membrane-embedded enzymes, which catalyze chemical reactions within the context of lipid membranes, the salient questions include how both water-soluble and lipophilic substrates access catalytic active sites, what conformational changes in the enzymes occur and what constraints the lipid membrane places on these. The approaches include biochemistry, electrophysiology, X-ray crystallography, cryo-electron microscopy, enzymatic assays, and other approaches to probe protein function. I possess the necessary skills, experience, expertise, knowledge, scientific environment, and leadership to successfully guide and complete our studies of ion channels and membrane enzymes.

I am committed to providing rigorous doctoral and postdoctoral training. As a testament to this, all doctoral and postdoctoral alumni from my laboratory are currently engaged in scientific research. Additionally, nearly all the technician alumni from my laboratory are pursuing careers in research or medicine. I have mentored numerous graduate students as both their primary thesis advisor (6) and by serving on doctoral thesis committees (>20). Regarding graduate coursework, I lecture each year on topics related to ion channels and transporters, biophysics, and structural biology. I am committed to fostering a scientifically rich and exciting environment for postdoctoral fellows and graduate students both within my individual laboratory as well as at an institutional level. I work to fulfill the importance of trainees obtaining their Ph.D. degrees in timely fashions and with the skills, credentials, and experiences to be successful in their scientific careers. Ph.D. graduates of my laboratory (3) have gone on to receive postdoctoral training in excellent laboratories (e.g. at UCSF, Janelia campus of the HHMI, Rockefeller U.) and are beginning to launch independent research careers. Postdoctoral fellows trained in my laboratory (2 current and 7 alumni) have been highly successful. Of those who have completed their postdoctoral training, five hold tenure-track Assistant/Associate Professor positions – three in the USA and two abroad. All three positions in the USA are held by women – Assistant Professor Dr. Xiaowei Hou at the U. of Cincinnati, Assistant Professor Dr. Melinda Diver at Memorial Sloan Kettering Cancer Center, and Assistant Professor Dr. Alexandria Miller at the University of Iowa.

I am committed to scientific integrity, rigor, and experimental reproducibility. I work to create an environment that cultivates and rewards these bedrocks of research. I am also committed to fostering diversity, equity and inclusion within my own laboratory, within our institution, in the broader scientific enterprise, and within society. Members of my laboratory self-identify as having disadvantaged backgrounds and as underrepresented minorities. I am committed to training students, postdocs and technical staff in rigorous and unbiased experimental design, analysis, interpretation, and the reporting of results. I am dedicated to fostering the careers of my trainees and to helping them obtain the necessary skills to transition into successful careers in the biomedical workforce that are consistent with their skills, interests, and values.

In relation to Institutional Service, I serve on the Oversight Committees for the Antibody and Bioresource Core Facility (as Chair) and for the Structural Biology Core Facility (as a Member). I work to foster their continued high-productivity and effectiveness. To this end, I regularly interact with the Directors of these Core Facilities (e.g. to discuss technology advancement, data interpretation, and to provide operational advice). I currently serve as a Member of the Committee for Appointments and Promotions at Sloan Kettering Institute. I also serve as the Scientific Director of the Stephen and Barbara Friedman Cryo-EM Imaging Laboratory at MSKCC.

B. Positions and Honors

Positions

2001-2002	Facility Manager, X-ray Crystallography Resource, Duke University, Durham, NC
2002-2003	Postdoctoral Fellow (with Bruce Sullenger, PhD), Duke University, Durham, NC
2003-2007	Postdoctoral Associate (with Roderick MacKinnon, MD), Rockefeller University, New York, NY
2007-2009	Adjunct Faculty, Rockefeller University, New York, NY
2007-2013	Assistant Prof., Weill Graduate School of Medical Sciences of Cornell University, New York, NY
2007-2013	Assistant Member, Program in Structural Biology, Sloan-Kettering Institute, New York, NY
2013-2017	Associate Prof., Weill Graduate School of Medical Sciences of Cornell University, New York, NY
2013-2016	Associate Member, Program in Structural Biology, Sloan-Kettering Institute, New York, NY
2016-pres	Member, Program in Structural Biology, Sloan-Kettering Institute, New York, NY
2016-pres	Professor, Gerstner Sloan Kettering Graduate School of Biomedical Sciences, New York, NY
2017-pres	Professor, Weill Graduate School of Medical Sciences of Cornell University, New York, NY
2022-pres	Scientific Director, Friedman Cryo-EM Imaging Lab, Sloan-Kettering Institute, New York, NY

Honors

1994	Magna Cum Laude, Physics, Amherst College
1996	National Science Foundation Graduate Fellowship Honorable Mention Recipient
1998-1999	American Heart Association Pre-doctoral research fellowship
2006-2014	Burroughs Wellcome Career Award in the Biomedical Sciences
2008-2010	V Foundation Scholar Award
2008-2011	Louis V. Gerstner, Jr. Young Investigators Fund Award
2014-2016	Bressler Scholars Award
2016	Boyer Award for Basic Research

C. Contributions to Science

1. Potassium channels (roles: postdoc and PI). Voltage-dependent potassium (Kv) and sodium (NaV) channels propagate action potentials by altering the permeability of the axon to potassium and sodium ions in response to changes in the voltage across the membrane. During my postdoctoral work, I determined a series of X-ray structures of a mammalian Kv channel that ultimately revealed the complete structure of the channel embedded in a membrane-like arrangement of lipid molecules (Long et al. & Mackinnon, 2005a, 2005b, 2007). The structures provided a foundational understanding for the molecular mechanisms of voltage sensing in such channels and in other proteins that contain voltage sensor domains. The work also represented two technical advances in the field of membrane protein structural biology. It represented the first crystal structure of a eukaryotic membrane protein obtained using heterologously expressed protein. Previous structures were obtained by isolation of protein from natural sources. Secondly, rather than the standard practice of completely replacing the lipid membrane with detergent during purification, we found that using mixtures of detergents and lipids preserved the proper fold of the protein and were necessary for crystallization. This technique became

standard practice for structural determination of eukaryotic membrane proteins using X-ray crystallography and cryo-EM. The publications have been cited more than 1500 times.

a) **Long, S.B.**, Campbell, E.B., and Mackinnon, R. (2005). "Crystal structure of a mammalian voltage-dependent *Shaker* family K^+ channel." *Science.* 309, 897-903. PMID: 16002581

b) **Long, S.B.**, Campbell, E.B., and Mackinnon, R. (2005). "Voltage sensor of Kv1.2: structural basis of electromechanical coupling." *Science.* 309, 903-908. PMID: 16002579

c) **Long, S.B.**, Tao, X., Campbell, E.B., and Mackinnon, R. (2007). "Atomic structure of a voltage-dependent K^+ channel in a lipid membrane-like environment." *Nature*. 450, 376-382. PMID: 18004376

In work on mammalian potassium channels as an independent investigator, my laboratory studies the structure and function of two-pore domain potassium (K2P) channels (role: PI). These channels control the resting potential of eukaryotic cells and regulate cell excitability by conducting K^+ ions across the plasma membrane. Our work on the TWIK-1 (K2P1) channel (Miller & Long, *Science* 2012) represented the first structure of a K2P channel and the first structure of any human potassium channel. It defined novel structural features of this channel family and provided a foundation to understand and further investigate the diverse modes of regulation of K2P channels, which are modulated by lipids, protein modifications, mechanical tension, and changes in pH or temperature. Two distinguishing features of the channels that we discovered are an "extracellular cap" domain that may be a site for regulation by extracellular factors and lateral "fenestrations" within the transmembrane region that expose the ion pore to the lipid bilayer. The physical connection between the membrane and the ion pore has subsequently been shown to couple channel function with properties of the membrane such as lipid composition and mechanical tension. Our work revealed a novel binding site for potential inhibitors, which have subsequently been developed and could have utility as neuromodulatory agents. In ongoing work, we are addressing the structure, gating, and regulation of other the K2P channels TWIK-2 and TRESK.

d) Miller, A.M. and **Long, S.B.** (2012). "Crystal Structure of the Human Two-Pore Domain Potassium Channel K2P1." *Science.* 335, 432-436. PMID: 22282804

2. The Calcium-release activated calcium (CRAC) channel Orai (initially supported by R01GM094273 and subsequently by R35GM13192, role: PI). The CRAC channel Orai generates sustained intracellular Ca^{2+} signals that, among other functions, are necessary for activation of immune response genes in T cells. In 2012, we determined an X-ray structure of Orai, which represents a closed conformation of the channel (Hou, Pedi, Diver & Long, *Science* 2012). The work was a milestone achievement: it was the first structure of any Ca^{2+}-selective ion channel and remained the only structure of Orai until recent work from my laboratory. The work revealed structural and mechanistic principles relevant to ion selectivity and gating by the channel. In subsequent studies, we determined structures of Orai in open and intermediate conformations (Hou et al., *eLife* 2018, Hou et al. *eLife* 2020). Together, these structural and mechanistic studies provided a sequence of molecular transitions between closed and open conformations of the ion pore. Dr. Xiaowei Hou, who is the first author on these studies, was appointed as an Assistant Professor at the University of Cincinnati in 2022. In support of her career, Dr. Hou will be carrying out efforts to study gating of the channel by the protein STIM in her independent laboratory.

a) Hou, X., Pedi, L., Diver, M.M., and **Long, S.B.** (2012). "Crystal Structure of the Calcium Release-Activated Calcium Channel Orai." *Science*. 338, 1308-1313. PMID: 23180775 PMCID: PMC3695727

b) Hou, X., Burstein, S.R., and **Long, S.B.** (2018). "Structures reveal opening of the store-operated calcium channel Orai." *eLife.* PMID: 30160233. PMCID: PMC6170153

c) Hou, X.*, Outhwaite, I.R.*, Pedi, L., & **Long, S.B.** (2020). "Cryo-EM structure of the calcium release-activated calcium channel Orai in an open conformation." *eLife*. PMID: 33252040 PMCID: PMC7723414

3. The Mitochondrial Calcium Uniporter (supported by R35GM13192, role: PI). The mitochondrial calcium uniporter is a highly regulated multi-subunit ion channel complex present in the inner mitochondrial membrane. The channel serves as the primary conduit for Ca^{2+} entry into mitochondria and it thereby regulates ATP synthesis and programmed cell death. The core constituents of the channel complex are the proteins MCU, EMRE, MICU1, and MICU2. Using reconstitution biochemistry, functional studies, and structural analyses, we have investigated the molecular mechanisms of the channel's function – namely its regulation by EMRE, its control by the calcium-dependent MICU1-MICU2 complex, and its mechanisms for exquisite ion selectivity. In 2018, we presented a cryo-EM structure of the pore-forming MCU subunit of the channel (Baradaran et al., *Nature* 2018). We subsequently reconstituted the function of an MCU-EMRE complex, identified its function was

dependent on cardiolipin, and determined its cryo-EM structure (Wang et al., *J Mol. Biol.* 2020). Subsequently, we investigated the basis of Ca^{2+}-dependent control of MCU by the MICU1-MICU2 complex. In this tour de force effort, we demonstrated that MICU1-MICU2 controls Ca^{2+} permeation through the channel by operating like a regulatable toxin (Wang et al., *eLife* 2020). In a recent major body of work (Delgado & Long, *Science Advances* 2022), we probed the ion selectivity mechanism of the uniporter using detailed electrophysiological studies of the purified channel. The studies represented the first bone fide electrical measurements of ion currents through the purified uniporter and the first electrophysiological studies of mutations within the pore. We determined that the exquisite ion selectivity of the uniporter is governed by a single 'E-locus' within the pore, comprised of glutamate residues. Our experimental measurements showed that the E-locus can contain two calcium ions simultaneously. The presence of multiple ions is necessary for selective and high-throughput Ca^{2+} conduction by channel. Comparison with our structural work thereby yielded mechanistic and structural foundations of Ca^{2+} selectivity. Graduate student alumnus Bryce Delgado (a URM, supported by 1F31GM143882) performed all the electrophysiological studies. In ongoing work, we are interrogating the ion selectivity mechanisms using structural biology of complexes with a variety of cations and studying gating by the channel's other regulatory subunits, MCUb and MCUR1.

a) Baradaran, R.*, Wang, C.*, Siliciano, A. F. & **Long, S. B.** (2018). "Cryo-EM structures of fungal and metazoan mitochondrial calcium uniporters." *Nature.* 559, 580-584. PMID: 29995857. PMCID: PMC6336196

b) Wang, C., Baradaran, R., & **Long, S.B.** (2020). "Structure and Reconstitution of an MCU-EMRE Mitochondrial Ca(2+) Uniporter Complex." *J Mol. Biol.* 432, 5632-5648. PMID: 32841658 PMCID: PMC7577567

c) Wang, C., Jacewicz, A., Delgado, B.D., Baradaran, R., & **Long, S.B.** (2020). "Structures reveal gatekeeping of the mitochondrial Ca(2+) uniporter by MICU1-MICU2." *eLife. 9*. PMID: 32667285 PMCID: PMC7434445

d) Delgado, B.D. & **Long, S.B.** (2022). "Mechanisms of ion selectivity and throughput in the mitochondrial calcium uniporter." *Science Advances.* 8(50). PMID: 36525497 PMCID: PMC9757755

4. Chloride channels (initially supported by R01GM110396 and currently by R35GM13192, role: PI). Our studies on chloride channels have focused on two families: bestrophin calcium-activated chloride channels and the proton-activated channel ASOR.

Bestrophin calcium-activated chloride channels (CaCCs) regulate the flow of chloride and other monovalent anions across cellular membranes in response to cytosolic Ca^{2+} levels. Mutations in bestrophin 1 (BEST1) cause certain eye diseases. We have described X-ray structures of BEST1-Fab complexes with permeant anions and Ca^{2+} (Dickson et al., *Nature* 2014). The work represented the first structure of any CaCC. It included demonstration of channel function using purified protein and thereby resolved doubts about whether BEST1 forms an ion pore. The studies revealed a novel architecture for an ion channel and new paradigms for mechanisms of ion selectivity and channel gating. In subsequent work, we studied the function of purified BEST1 using electrophysiology and thereby identified the principal components of the gating apparatus (Vaisey et al., *PNAS* 2016). Through this work, which was the first electrophysiological study of purified BEST1 protein, we determined that distinct regions of the pore control Ca^{2+}-dependent activation and ion selectivity. Further, we found that BEST1 undergoes inactivation, and we identified the mechanism of this process, wherein a C-terminal peptide binds to a surface receptor to allosterically control a distant gate (Vaisey & Long, *JGP* 2018). These studies laid the foundation to capture a series of cryo-EM structures that represent all major gating transitions of the channel, which revealed a novel choreography for ion channel gating (Miller*, Vaisey* & Long, *eLife* 2019).

a) Dickson, V.K., Pedi, L. and **Long, S.B.** (2014). "Structure and insights into the function of a Ca2+ -activated Cl- channel." *Nature.* 516, 213-8. PMID: 25337878 PMCID: PMC4454446

b) Vaisey, G. & **Long, S. B.** (2018) "An allosteric mechanism of inactivation in the calcium-dependent chloride channel BEST1." *J Gen Physiol*. PMID: 30237227 PMCID: PMC6219684

c) Miller, A.N.*, Vaisey, G.* & **Long, S. B.** (2019) **"**Molecular mechanisms of gating in the calcium-activated chloride channel bestrophin." *eLife*. PMID: 30628889 PMCID: PMC6342527

The proton-activated chloride channel ASOR (TMEM206/PAC) permeates anions across cellular membranes in response to acidification and thereby regulates endocytosis. Luminal (or extracellular) pH controls the gating of the channel, but the identity of the proton sensor(s) and the mechanism of pore opening were unclear prior to our efforts. In published work (Wang et al., *Science Advances* 2022), we reconstituted the function of human

ASOR from purified protein and determined a series of atomic structures that represent the major gating conformations of the channel. Combined with electrophysiological studies performed in collaboration with Dr. Thomas Jentsch, the work revealed the mechanisms of proton-sensing and ion pore gating. We found that clusters of extracellular acidic residues function as pH sensors and coalesce when protonated. The proton-induced conformational changes result in the metamorphosis of the transmembrane domain to fashion an ion conduction pathway in the activated state from a disparate collection of helices in the resting state – a new paradigm of channel gating. The work represents the most comprehensive mechanistic understanding for an ion channel reported in a single publication from my laboratory. Dr. Chongyuan Wang, the first author on the study and now an Assistant Professor, is continuing to work on the channel in his independent laboratory.

d) Wang, C.*, Polovitskaya, M. M.*, Delgado, B. D., Jentsch, T. J., & **Long, S. B.** (2022) "Gating choreography and mechanism of the human proton-activated chloride channel ASOR." *Science Advances*. 8(5), PMID: 35108041 PMCID: PMC8809534

5. Structural and mechanistic studies of integral membrane enzymes (role: PI). Other major contributions pertain to the molecular mechanisms of integral membrane enzymes. Our published work has focused on two enzymes that reside in the membrane of the endoplasmic reticulum: ICMT and HHAT.

In work on the prenylation pathway stemming from my graduate work (Long et al, *Nature* 2002), my lab studies the integral membrane enzyme ICMT (isoprenylcysteine carboxyl methyltransferase) that methylates the C-terminal carboxylate of Ras and other CAAX proteins that have a prenyl lipid attached at their C-terminus. This methylation is necessary for the proper cellular function of Ras and other CAAX proteins. Inhibitors of ICMT have potential as anticancer agents. ICMT resides in the membrane of the endoplasmic reticulum and has no homology with soluble methyltransferases. Through a tour de force effort, Dr. Melinda Diver and colleagues determined a 2.3 Å resolution X-ray structure of ICMT (Diver et al, *Nature* 2018). Combined with mutagenesis and functional assays (Diver et al, *JBC* 2014, PMC4176209), the structure identified the active site and gave insight into substrate specificity. It suggests that the two reactants reach the enzyme by distinct routes – the methyl donor, AdoMet, reaches it from the cytosol while the CAAX substrate reaches it by diffusing laterally in the membrane. In ongoing efforts, we aim to capture structures that represent steps of the reaction coordinate to inform our understanding of the mechanisms of substrate specificity, substrate access, and catalysis.

a) **Long, S. B.**, Casey, P. J., & Beese, L. S. (2002) "Reaction path of protein farnesyltransferase at atomic resolution." *Nature*. 419, 645–650. PMID: 12374986

b) Diver, M.M, Pedi, L., Koide, A., Koide, S., and **Long, S.B**. (2018) "Atomic structure of the eukaryotic intramembrane RAS methyltransferase ICMT." *Nature.* 553, 526-529. PMID: 29342140 PMCID: PMC5785467

Mechanistic studies of the enzyme Hedgehog acyltransferase (HHAT) represent the other major contribution regarding integral membrane enzymes. HHAT attaches a palmitate lipid onto the Hedgehog family of secreted morphogens. The attached lipid is required for the signaling activity of sonic Hedgehog during development and for aberrant Hedgehog signaling that is upregulated in certain beast, lung and pancreatic cancers. To investigate the molecular mechanisms of substrate specificity and catalysis, we determined cryo-EM structures of human HHAT that represent snapshots of its reaction coordinate: a 2.7-Å resolution structure of a complex with palmitoyl-CoA, and a 3.2-Å resolution structure with a palmitoylated Hedgehog peptide product (Jiang, Benz and Long, *Science* 2021). The structures revealed how HHAT overcomes the challenges of bringing together substrates that have different physiochemical properties from opposite sides of the endoplasmic reticulum membrane within a membrane-embedded active site for catalysis. These principles are relevant to related enzymes that catalyze acylation of Wnt and the appetite-stimulating hormone ghrelin. Ongoing work seeks to further understand mechanisms of substrate specificity and inhibition of the enzyme, which may be useful for combating certain cancers.

d) Jiang, Y., Benz, T. L., & **Long, S. B.** (2021). "Substrate and product complexes reveal mechanisms of Hedgehog acylation by HHAT." *Science*. 372, 1215–1219. PMID: 34112694 PMCID: PMC8734478

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