BIOGRAPHICAL SKETCH

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NAME: Stephen Barstow Long

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POSITION TITLE: Member, Memorial Sloan Kettering Cancer Center, New York, NY
Professor, Weill-Cornell Graduate School of Medical Sciences of Cornell University, NY, NY

EDUCATION/TRAINING

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

A. Personal Statement

The objectives of my laboratory are to understand the biophysical mechanisms of eukaryotic ion channels and enzymes that catalyze chemical reactions within biological membranes. The membrane proteins we study have crucial biological functions and are implicated in eye, heart and immune disorders, and in cancer. We focus on eukaryotic membrane proteins that do not have prokaryotic counterparts because unique aspects of biology surround them and because their 3D structures and molecular mechanisms represent uncharted territory. 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 each channel with its 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, and how the chemistry of the reaction proceeds. The tools used include biochemistry, electrophysiology, X-ray crystallography, cryo-electron microscopy, enzymatic assays, and other biophysical and chemical approaches to study protein function. I possess all of the necessary skills, experience, expertise, knowledge, scientific environment, and leadership to successfully guide and complete our studies of ion channels and membrane enzymes.

I have mentored several graduate students as both their primary thesis advisor and by serving on doctoral thesis committees. With regard to 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 PhD students both within my individual laboratory as well as at an institutional level. PhD graduates of my laboratory have accepted postdoctoral positions in excellent laboratories (e.g. at UCSF, Janelia campus of the HHMI, Rockefeller U.). Postdoctoral fellows that I trained are beginning to launch independent research careers. I am committed to scientific integrity, scientific rigor, and experimental reproducibility, and I work to create an environment that fosters and rewards these bedrocks of research. I am also committed to training students and postdocs in rigorous and unbiased experimental design, analysis, interpretation and the reporting of results. I am dedicated to fostering the careers of my trainees and helping them to obtain the necessary skills to transition into successful careers in the biomedical workforce that are consistent with their skills, interests and values. I work to fulfill the need of trainees to obtain their Ph.D. degrees in a timely fashion and with the skills, credentials and experiences to be successful in their scientific careers.

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
2017-pres	Professor, Weill Graduate School of Medical Sciences of Cornell University, New York, NY

Honors

1994	Magna Cum Laude, 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, MSKCC
2016	Boyer Award for Basic Research, MSKCC

C. Contributions to Science

Concepts derived from three-dimensional structures and functional studies of four eukaryotic ion channels and one eukaryotic membrane enzyme are my major scientific achievements thus far.

Postdoctoral:

1. Early work on Kv channels. Voltage-dependent potassium (Kv) and sodium (NaV) channels propagate action potentials by altering the permeability of the axon to potassium (K^+) and sodium (Na^+) ions in response to changes in the voltage across the membrane. In the channels, voltage sensor domains (VSDs) respond to changes in membrane voltage by changing conformation. VSDs are also present in voltage-dependent calcium (CaV) and voltage-dependent proton (Hv) channels, and they have been identified in certain enzymes in which the membrane voltage modulates enzymatic activity. Mechanical work, done by the electrical field on the VSD, drives a conformational change. In a series of three publications, we presented the X-ray structure of a mammalian Kv channel that reveal the complete structure of the channel embedded in a membrane-like arrangement of lipid molecules. The structures allowed us to reach three major conclusions about Kv channels. First, rather than associating with the pore as a tightly bundled arrangement of helices, which was the prevailing view at the time, VSDs are separate domains, largely independent from the pore. Secondly, the VSDs are poised to perform mechanical work on the pore through linker α-helices that couple them to the pore. In this way, VSDs convert work done by the electric field on positive charged arginine residues into mechanical work done on the pore. Third, we explain how arginine amino acids that sense the voltage are stabilized within the span of the membrane and we suggest a mechanism for VSD movements and pore gating that is compatible with a large body of functional data. Until recently, these structures were the only structures of eukaryotic voltage-dependent cation channels determined. The structures provide a foundational understanding for the molecular mechanisms of voltage sensing in eukaryotic Kv, NaV, CaV, and Hv channels and in other proteins that contain VSDs. The publications have been cited more than 1000 times.

The work represented two technical advances in the field of membrane protein structural biology. It represents 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. Second, 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.

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

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

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.

Faculty:

2. Two-pore domain potassium channels. Two-pore domain potassium (K^+) channels (K2P channels) control the resting potential of eukaryotic cells and regulate cell excitability by conducting K^+ ions across the plasma membrane. Our work on the K2P1 channel (Miller & Long, *Science* 2012) represents the first structure of a two-pore-domain potassium channel and the first structure of any human potassium channel. It defined novel structural features of this channel family that probably serve as regulatory elements and are targetable by inhibitors. It also provides a foundation to understand and further investigate the diverse modes of regulation of K2P channels, which are modulated by lipids, protein modification, mechanical tension, and changes in pH or temperature. The studies, which include reconstitution of K2P channel activity in liposomes, revealed two major features that were not observed in other crystal structures of K^+ channels. K2P channels are dimers of concatenated "pore domains". While K2P channels recapitulate the fundamental properties of tetrameric K^+ channels including exquisite selectivity for K^+, we discovered that K2P channels contain an "extracellular cap" domain that could not be accommodated with a tetrameric architecture. The domain is located above the selectivity filter and restricts the ion pathway such that K^+ ions flow through side portals. The extracellular cap may be a site for regulation by extracellular factors whose identities are unknown and the extracellular cap protects K2P channels from protein toxins that inhibit other K^+ channels. A second new feature is that the channel contains lateral openings within its transmembrane region that expose the ion pore to the lipid bilayer. The openings are filled with electron density attributable to lipids. The physical connection between the membrane and the ion pore may be a means of coupling properties of the membrane such as lipid composition or mechanical tension with K2P channel function and it reveals a novel site for the development of K2P-specific inhibitors that could have utility as antidepressants, anesthetics or other neuromodulatory agents. I served as the principal investigator in these studies.

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

3. Calcium channels (supported by R01GM094273 and subsequently by R35GM13192). The calcium (Ca^{2+}) channel Orai generates sustained intracellular Ca^{2+} signals that, among other functions, are necessary for activation of immune response genes in T cells. We determined an X-ray structure of Orai in a closed conformation (Hou et al., *Science* 2012). The work was a milestone achievement: it represents the first structure of any Ca^{2+}-selective ion channel and was the only structure of Orai until recent additional work from my laboratory. Several surprising discoveries were made with the structure. First, the channel is assembled as a hexamer of subunits, rather than the expected tetrameric architecture. Some people questioned the oligomeric state at first, but it has now been shown by several research groups (including Richard Lewis and Donald Gill) that the Orai channel functions as a hexamer. Second, the structure revealed the architecture of the pore, which is distinct from other channels: the pore is long, narrow, and contains anions that stabilize the closed state and plug the pore. In a recent manuscript that has been deposited into the bioRxiv preprint server, we describe two additional conformations of the channel that we observed using X-ray crystallography. This work reveals the open conformation of the channel and an intermediate state between the closed and open conformations. With the new structures, we determine a sequence of molecular transitions in Orai that switch the pore between closed and open conformations. These structures, and supporting functional data presented in the manuscripts, establish the foundation for our current efforts to study ion selectivity and gating of the channel by the protein STIM. In other work on calcium channels, we recently determined cryo-EM structures of the mitochondrial calcium uniporter MCU. MCU is a highly selective and highly regulated calcium channel that serves as the major route of calcium entry into mitochondria. The structures revealed principals of ion selectivity and ion permeation. I served as the principal investigator in these studies.

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. PMCID: PMC3695727

b) Hou, X., Burstein, S.R., and **Long, S.B.** (2018). “Structures reveal opening of the store-operated calcium channel Orai.” *eLife* 2018;7:e36758, PMID: 30160233. PMCID: PMC6170153

c) 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, (2018) PMID: 29995857. PMCID: PMC6336196

d) 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*. doi:10.7554/eLife.59991 PMID: 32667285 PMCID: PMC7434445

e) 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*, *9*. http://doi.org/10.7554/eLife.62772 PMID: 33252040 PMCID: PMC7723414

4. Calcium-activated chloride channels (supported by R01GM110396 and subsequently by R35GM13192). Bestrophin calcium-activated chloride channels (CaCCs) regulate the flow of chloride and other monovalent anions across cellular membranes in response to intracellular calcium (Ca^{2+}) levels. Mutations in bestrophin 1 (BEST1) cause certain eye diseases. In work supported by R01GM110396, we presented X-ray structures of chicken BEST1-Fab complexes, at 2.85 Å resolution, with permeant anions and Ca^{2+} (Dickson et al., *Nature* 2014). The work represented the first structure of a bestrophin channel and the first structure of any CaCC. Our reconstitution of channel function using purified BEST1 resolved doubts about whether the protein, which had previously been studied only in cellular contexts where endogenous Cl^- channels are present, actually forms an ion pore. The work revealed a novel architecture for an ion channel and new paradigms for mechanisms of ion selectivity and channel gating. In work supported by R01GM110396, we studied BEST1 using electrophysiology and 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. We found that, unlike most ion channels, ion selectivity among permeant anions and anion-versus-cation selectivity are governed by different regions of the pore. In further electrophysiological work (Vaisey & Long, *JGP* 2018), we determined that BEST1 undergoes inactivation and we identified the mechanism of this process wherein a C-terminal peptide binds to a surface receptor to control a distant gate through allosteric means. These studies laid the foundation for cryo-EM studies of BEST1 that define the gating transitions of the channel and reveal a novel molecular choreography for gating among ion channels (Miller*, Vaisey* & Long, *eLife* 2019). I served as the principal investigator in these studies.

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**(7530):213-8, Epub 2014 Oct 22. PMID: 25337878 PMCID: PMC4454446

b) Vaisey, G., Miller, A.M. and **Long, S.B.** (2016). “Distinct regions that control ion selectivity and calcium-dependent activation in the bestrophin ion channel.” *PNAS* 113(47):E7399-E7408. Epub 2016 Nov 7. PMID: 27821745 PMCID: PMC5127342

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

d) Miller*, A.N, Vaisey*, G. & **Long, S. B.** (2019) **“**Molecular mechanisms of gating in the calcium-activated chloride channel bestrophin.” *eLife* 2019;8:e43231 PMID: 30628889 PMCID: PMC6342527

5. Integral membrane enzymes. The integral membrane enzyme isoprenylcysteine carboxyl methyltransferase (ICMT) methylates the C-terminal carboxylate of Ras and other CAAX proteins that have an isoprenoid lipid attached at their C-terminus. Methylation is necessary for the proper cellular function of Ras and other CAAX proteins and inhibitors of ICMT have potential as anticancer agents. ICMT is located in the membrane of the endoplasmic reticulum and has no homology with soluble methyltransferases. Through work spanning many years, we recently determined an X-ray structure of ICMT, at 2.3 Å resolution (Diver et al, *Nature* 2018). The structure was resolved in a lipid-membrane like environment and in complex with a monobody inhibitor that we developed. Combined with extensive mutagenesis and functional assays (Diver et al, *JBC* 2014), the structure identified the active site and gave insight into substrate specificity. It reveals 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 translate our structural and

mechanistic findings into ways to develop more effective ICMT inhibitors. I served as the principal investigator in these studies.

a) Diver, M.M. and **Long, S.B.** (2014) "Mutational Analysis of the Integral Membrane Methyltransferase ICMT Reveals Potential Substrate Binding Sites." *J Biol. Chem*. **289**(38):26007-20. PMID: 25059662 PMCID: PMC4176209

b) Diver, M.M, Pedi, L., Koide, A., Koide, K., and **Long, S.B**. (2018) "Atomic structure of the eukaryotic intramembrane RAS methyltransferase ICMT." *Nature.* Jan 25; 553(7689):526-529. PMID: 29342140 PMCID: PMC5785467

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/stephen.long.1/bibliography/44141595/public/?sort=date&direction=ascending

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

Ongoing Research Support:

R35 GM131921 Long (PI) 4/1/2019 - 3/31/2024
Mechanisms of Ion Channels in Calcium Signaling
The objectives of this research program are to understand the molecular mechanisms of ion permeation, ion selectivity, and gating in eukaryotic ion channels that generate or respond to intracellular calcium signals. Techniques are combined that address structure (e.g. X-ray crystallography and cryo-electron microscopy) with electrophysiology and other approaches that address channel function.

Pending Research Support:

None.

Completed Research Support:

R01 GM094273 Long (PI) 9/1/2010 - 7/31/2019
3D Structure and Function of CRAC Channels
The goals of this project are to determine the structures and mechanisms of calcium release-activated calcium (CRAC) channels. We use structural biology combined with approaches that address function to investigate the channels.

R01 GM110396 Long (PI) 2/1/2015 - 1/31/2019
Structure and function of retinal calcium-activated chloride channels
The goals of this project are to determine the structures and molecular mechanisms of bestrophin (BEST) calcium-activated chloride channels. Techniques are combined that address structure (e.g. X-ray crystallography and cryo-electron microscopy) with electrophysiology and other approaches that address channel function.