OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

NAME: Philipp A.M. Schmidpeter

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

POSITION TITLE: Research Associate

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Bayreuth (Germany)	B.Sc.	10/2005	08/2008	Biochemistry
University of Bayreuth (Germany)	M.Sc.	10/2008	07/2010	Biochemistry and Molecular Biology
University of Bayreuth (Germany)	Ph.D.	09/2010	10/2014	Molecular Biosciences
Weill Cornell Medicine (NY, USA)	Postdoc	03/2015	02/2021	Physiology/ Biophysics

A. Personal Statement

Already early on in my education I became fascinated with the structural and functional diversity of proteins and with my work on different classes of proteins this fascination only has grown stronger. Understanding kinetics of protein function and regulation in combination with high-resolution structural data is an extremely powerful approach to decipher the workings of these molecules in molecular detail, it allows for detailed structure-function correlations of proteins, and it is necessary for drug development.

Initially, my education and research were focused on protein folding and stability, as well as the characterization of enzymatic activities. I gained significant expertise in optical spectroscopy (absorbance, fluorescence, and circular dichroism) and calorimetry (DSC and ITC). Combined with detailed studies of reaction kinetics from manual mixing as well as stopped-flow single- and sequential-mixing experiments, I developed a pronounced mechanistic thinking based on thermodynamic concepts.

During my tenure as Postdoctoral researcher, I extended my knowledge to membrane proteins, in particular ion channels. Building on my existing, experimental skills, I quickly learned to express and purify different ion channels from bacteria, yeast and mammalian cells. Furthermore, I became familiar with single-channel recordings to study ion channel function. Being already proficient in stopped-flow spectroscopy to study protein folding and function, I quickly learned to apply a stopped-flow based flux assay, developed in the lab of Dr. Nimigean, to my studies of ion channel activity, and successfully used this assay for my own research and for collaborations with other groups. Within one of my major projects, I also reconstituted ion channels into lipid nanodiscs for structural studies using single particle cryoEM and acquired profound knowledge in solving high-resolution structures.

I believe, that, with my broad experimental and analytical experience, I am ready to take on the next project, which will require a combination of all the skills I developed so far and also provide the opportunity to learn new techniques. My current research combines two topics of my postdoctoral research and is an example of how I continuously develop ideas and think about my results in a bigger, more connected picture. I propose to study how ligand binding, native-state prolyl isomerization, and the membrane environment work together to regulate a pacemaker channel homolog. Usually, these mechanisms are studied separately, however, my preliminary data indicate that, in this case, they are coupled. I established assays that allow for studying this coupling. Quantitative analysis of coupled equilibria is not trivial and will further strengthen my thermodynamic profile.
My research will be performed in the laboratory of Dr. Crina Nimigean at Weill Cornell Medicine, within the Tri-Institutional environment that combines researchers from Weill Cornell Medicine, Memorial Sloan Kettering Cancer Center, and The Rockefeller University to form a strong and supportive community. I have access to all the equipment needed for protein biochemistry and functional studies within the lab space. For all structural studies, I have access to the in-house cryoEM facility at Weill Cornell Medicine, as well as to resources at the New York Structural Biology Center (NYSBC).

B. Positions and Honors

Positions and Employment

2021 -	Research Associate, Weill Cornell Medicine, NY, USA
2015 - 2021	Postdoctoral Associate, Weill Cornell Medicine, NY, USA
2010 - 2014	Ph.D. student at the University of Bayreuth, Germany ("*summa cum laude*")

Professional Memberships

2019 -	Member, Society of General Physiologists
2018 -	Member, American Heart Association
2017 -	Member, Biophysical Society
2016 -	Member, New York Academy of Sciences
2012 -	Member, The Protein Society
2009 -	Member, German Society of Biochemistry and Molecular Biology (GBM)

Honors

2021	Protein Society Anniversary Award for the Annual Symposium
2019	Travel award of the Society of General Physiologists
2018 - 2020	Postdoctoral Fellowship, American Heart Association (18POST33960309)
2015 - 2016	German Research Foundation (DFG) Research Fellowship (SCHM 3198/1-1)
2013	BOGS scholarship of the Leopoldina (German Academy of Sciences) for the International Symposium on Cyclophilins and other Foldases

C. Contributions to Science

Prolyl isomerization and prolyl isomerases

Peptidyl-prolyl *cis*/*trans* isomerization (prolyl isomerization) is known as rate limiting step during protein folding reactions. It also can be used as a molecular switch to regulate and fine-tune protein function. Prolyl isomerization is an intrinsically slow process that can be efficiently catalyzed by a class of enzymes called prolyl isomerases (PPIases). During my undergraduate and my graduate studies, I contributed to the understanding of how these enzymes work by analyzing their sequence specificity[1] as well as their adaptation to a broad spectrum of environmental conditions[2]. At the same time, I worked towards understanding how a proline switch in the adapter protein Crk-II works. By studying the folding and ligand-binding characteristics of a two-domain protein construct, I was able to reveal that the proline switch in Crk-II is not conserved between species and which residues are necessary for this switch to be functional[3]. Building on these results, I designed kinetic experiments of interrupted unfolding or refolding, and combined these experiments with ligand binding studies to establish the energetics of this proline switch[4]. For this study I developed a kinetic mechanism that shows how the *cis*/*trans* equilibrium at a specific proline is shifted towards the intrinsically less favorable *cis* conformation during protein folding, and how ligand binding breaks up important interactions leading to a relaxation and a shift in the *cis*/*trans* equilibrium back towards mostly *trans* proline. Finally, I used a mutational approach to show how switching between *cis* and *trans* proline is structurally propagated throughout one domain, thereby restructuring the domain interface of the two-domain protein construct to regulate the ligand binding activity[5].

1. **Schmidpeter PA**, Jahreis G, Geitner AJ, Schmid FX. Prolyl isomerases show low sequence specificity toward the residue following the proline. Biochemistry 2011;50(21):4796-803.
2. Godin-Roulling A, **Schmidpeter PA**, Schmid FX, Feller G. Functional adaptations of the bacterial chaperone trigger factor to extreme environmental temperatures. Environ Microbiol 2015;17(7):2407-20.
3. **Schmidpeter PA**, Schmid FX. Molecular determinants of a regulatory prolyl isomerization in the signal adapter protein c-CrkII. ACS Chem Biol 2014;9(5):1145-52.
4. **Schmidpeter PA**, Schmid FX. Prolyl isomerization as a molecular memory in the allosteric regulation of the signal adapter protein c-CrkII. J Biol Chem 2015;290(5):3021-32.
5. **Schmidpeter PA**, Ries LK, Theer T, Schmid FX. Long-Range Energetic Changes Triggered by a Proline Switch in the Signal Adapter Protein c-CrkII. J Mol Biol 2015;427(24):3908-20.

A model system to study CNG and HCN channels

Cyclic nucleotide-gated channels (CNG) are important for electrical signaling related to vision and olfaction. The closely related hyperpolarization-activated and cyclic nucleotide-modulated channels (HCN) are key players for pacemaking activity in the heart and brain as well as pain sensation. These proteins are extremely hard to express and purify for biophysical and structural studies under defined conditions and knowledge about their workings mostly is obtained from proteins heterologously expressed in cells followed by electrophysiological measurements. In order to understand the function and regulation of these channels in molecular detail, I introduced a bacterial homologue, SthK, as a model system to study CNG and HCN channels[6]. The amount of purified SthK obtained from expression in *E. coli* allowed for a detailed functional analysis. Single-channel recordings and data from a stopped-flow flux assay both showed that SthK is differentially regulated by cAMP and cGMP. Ligand binding in equilibrium, however, showed similar dissociation constants for the two ligands. Structural studies of SthK

reconstituted into lipid nanodiscs revealed the apo state as well as the cAMP- and the cGMP-bound, closed state of SthK[7]. Together, these studies established structural and functional homology between SthK and the eukaryotic CNG and HCN channels laying the groundwork for more detailed questions regarding the regulation of these proteins in molecular detail.

6. **Schmidpeter PA**, Gao X, Uphadyay V, Rheinberger J, Nimigean CM. Ligand binding and activation properties of the purified bacterial cyclic nucleotide-gated channel SthK. J Gen Physiol 2018;150(6):821-834.
7. Rheinberger J, Gao X, **Schmidpeter PA**, Nimigean CM. Ligand discrimination and gating in cyclic nucleotide-gated ion channels from apo and partial agonist-bound cryo-EM structures. Elife 2018;7.

A proline switch to regulate ion channel activation

Analysis of the activation kinetics of SthK revealed bi-phasic activation upon exposure of the channel to cAMP, similar to the slow increase in current elicited by HCN2 upon rapid perfusion of voltage-activated channels with cAMP. I was able to show that this bi-phasic activation of SthK is due to *cis*/*trans* heterogeneity at a proline residue in the siphon, the transition region of the C-linker into the CNBD[8]. Mutation of this proline residue to alanine or valine abolishes the bi-phasic activation and the channel only shows fast activation. The same effect is observed in the presence of prolyl isomerases. This effect is dependent on the isomerase concentration and can be reversed by specific isomerase inhibitors. Furthermore, I was able to show that the single-channel open-closed gating equilibrium is not affected by these enzymes. In conclusion, *cis*/*trans* heterogeneity at a single proline residue in SthK leads to bi-phasic activation kinetics. With *trans* proline, SthK activates fast and displays high apparent affinity for cAMP, with *cis* proline, SthK activates slowly and shows reduced affinity for cAMP. During activation of the channel, the *cis*/*trans* equilibrium is thus shifted towards the *trans* proline species, which happens on a physiological timescale only in the presence of prolyl isomerases. High-resolution structural data obtained from cryoEM data of SthK P300A reconstituted into lipid nanodiscs support this mechanism. This work constitutes the first characterization of how a proline switch can be used to fine-tune ion channel activity and it opens up a novel field of research.

8. **Schmidpeter PA**, Rheinberger J, Nimigean CM. Prolyl isomerization controls activation kinetics of a cyclic nucleotide-gated ion channel. Nat Commun 2020;11(1):6401.

Attended scientific conferences

Year	Conference title	Contribution
2021	65th Annual Meeting of the Biophysical Society (virtual)	Poster presentation
2020	64th Annual Meeting of the Biophysical Society (San Diego, CA, USA)	Platform presentation
2019	SGP 73rd Annual Symposium and SOBLA Annual Meeting (Valparaiso, Chile)	Poster presentation
2019	Protein Society 33rd Annual Symposium (Seattle, WA, USA)	Young Investigator talk
2019	1st NYC cryoEM meeting (New York, NY, USA)	Invited talk
2019	63rd Annual Meeting of the Biophysical Society (Baltimore, MD, USA)	Poster presentation

2018	Protein Society 32nd Annual Symposium (Boston, MA, USA)	Poster presentation
2018	62nd Annual Meeting of the Biophysical Society (San Francisco, CA, USA)	Poster presentation
2017	61st Annual Meeting of the Biophysical Society (New Orleans, LA, USA)	Poster presentation
2016	Membrane Proteins Friends Lab Meeting (Porquerolles, France)	Talk
2016	GRC – Ligand Recognition and Molecular Gating (Il Ciocco, Italy)	Poster presentation
2015	Proteins: A central research topic in Industry and Academia (Bayreuth, Germany)	Talk
2013	"Faltertage" (Regensburg, Germany)	Poster presentation
2013	International Symposium on Cyclophilins and other Foldases (Halle, Germany)	Poster presentation
2012	"Faltertage" (Regensburg, Germany)	Poster presentation
2012	4th Symposium of the Graduate School for Molecular Biosciences of the University of Bayreuth (Selb, Germany)	Talk
2011	Rabensteiner Kolleg (Pottenstein, Germany)	Talk
2011	"Faltertage" (Regensburg, Germany)	Poster presentation

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

Completed Research Support

18POST33960309 Schmidpeter (PI) 07/2018 - 06/2020
Postdoctoral Fellowship, American Heart Association
Title: Molecular determinants of the modulation of HCN pacemaker channels by cAMP
Role: PI

SCHM 3198/1-1 Schmidpeter (PI) 03/2015 - 02/2016
Research Fellowship, German Research Foundation (DFG)
Title: Insights into the molecular mechanism of gating of eukaryotic cyclic nucleotide-modulated ion channels using structural and functional tools
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: NIMIGEAN, CRINA M

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

POSITION TITLE: Professor

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)	END DATE MM/YYYY	FIELD OF STUDY
University of Bucharest, Bucharest	MS	06/1995	Physics
University of Bucharest, Bucharest	BS	06/1995	Physics
University of Miami School of Medicine	PHD	12/1999	Physiology and Biophysics
HHMI/Brandeis University	Postdoctoral Fellow	04/2003	Biochemistry
Brandeis University, Waltham, MA	NIH training grant	04/2005	Biochemistry

A. Personal Statement

Research in my laboratory is geared toward understanding how ion channel protein structure and mechanism interrelate at the molecular level to allow channels to elaborate various biological properties. We use a combination of molecular, biochemical, structural, and electrophysiological approaches to evaluate in a complete fashion fundamental channel properties. The main focus of the lab is to elucidate the mechanisms of gating, selectivity, ligand modulation, and lipid/membrane modulation in ion channels. We approach these questions using a range of biological and biophysical techniques including molecular biology, electrophysiology, NMR spectroscopy, X-ray crystallography, stopped-flow fluorescence assays and single-particle cryo-electron microscopy (cryo-EM). We have long-time collaborations with Simon Scheuring (Weill Cornell) for high speed AFM, and Toby Allen (RMIT, Australia) for MD simulations. About four years ago we have acquired expertise with cryo-EM in the laboratory as shown by our recent articles in Nature, eLife, Nature communications, and Nature methods. Currently 5 of the postdocs in the lab have cryo-EM expertise ranging from expert to enthusiastic novice. There is massive amount of information that cryo-EM can bring towards understanding ion channel mechanisms by revealing multiple conformations sampled during gating. And when combined with high-resolution single-channel recordings, flux assays, and AFM imaging/force spectroscopy, the wealth of information is unparalleled. Philipp Schmidpeter has been a very successful postdoctoral fellow in my lab, who is now transitioning into a faculty position. He is performing his research mostly independently, but he is also learning new techniques to improve his skill set. Specifically, he will be learning patch-clamp electrophysiology on cells, which I will teach him personally, and hands-on grid preparation and data collection for cryo-EM to determine ion channel structures, for which there is ample expertise from fellow postdocs as well as Devrim Acehan, the WCM cryo-EM facility manager. I have been and will continue to support and mentor Philipp to become a successful PI by sharing my experiences with him, being accessible for advice, and guiding him throughout this process.

1. Schmidpeter PAM, Rheinberger J, Nimigean CM. Prolyl isomerization controls activation kinetics of a cyclic nucleotide-gated ion channel. Nat Commun. 2020 Dec 16;11(1):6401. PubMed Central PMCID: PMC7744796.
2. Fan C, Sukomon N, Flood E, Rheinberger J, Allen TW, Nimigean CM. Ball-and-chain inactivation in a calcium-gated potassium channel. Nature. 2020 Apr;580(7802):288-293. PubMed Central PMCID: PMC7153497.
3. Rheinberger J, Gao X, Schmidpeter PA, Nimigean CM. Ligand discrimination and gating in cyclic nucleotide-gated ion channels from apo and partial agonist-bound cryo-EM structures. Elife. 2018 Jul 20;7 PubMed Central PMCID: PMC6093708.

4. Schmidpeter PAM, Gao X, Uphadyay V, Rheinberger J, Nimigean CM. Ligand binding and activation properties of the purified bacterial cyclic nucleotide-gated channel SthK. J Gen Physiol. 2018 Jun 4;150(6):821-834. PubMed Central PMCID: PMC5987880.

B. Positions and Honors

Positions and Employment

2005 - 2008	Assistant Professor, UNIVERSITY OF CALIFORNIA AT DAVIS
2008 - 2011	Assistant Professor, WEILL MEDICAL COLL OF CORNELL UNIV
2011 - 2020	Associate Professor, WEILL MEDICAL COLL OF CORNELL UNIV
2020 -	Professor, Weill Cornell Medical College, New York, NY

Other Experience and Professional Memberships

2006 -	Scientific reviewer, Science, PNAS, Biophysical Journal, Journal of General Physiology, Structure, Neuron, PLOS, EMBO Reports/Journal, eLife, Nature communications, Scientific Reports, Nature, JACS, Nature Structural and Molecular Biology, Nature Neuroscience
2006 - 2007	Program committee member, Biophysical Society
2007 - 2007	Grant reviewer, Wellcome Trust
2007 - 2007	Peer review board member, American Heart Association, Western States Affiliate
2007 - 2014	Grant reviewer, NSF
2008 - 2012	Secretary-treasurer, Vice-chair, Chair, Permeation and Transport subgroup Biophysical Society
2009 - 2009	Grant reviewer, Italian Ministry of Health - NIH
2011 -	Editorial board member, Journal of General Physiology
2011 - 2013	Councilor, Society of General Physiologists
2012 - 2012	Grant Reviewer, Romanian National Council for Scientific Research and Israeli Science Foundation
2012 - 2012	Member of Membrane Transport and Biophysics review panel, National Science Foundation
2012 - 2014	member of the Proteins and Crystallography 2 Peer Review Group, American Heart Association
2014 - 2014	Grant reviewer, INSERM/CNRS Avenir
2014 - 2016	Vice chair, then chair of the Ligand Recognition and Molecular Gating GRC, Gordon Research Conferences
2014 - 2017	Member of Special Emphasis Panel Review Groups ZRG1 F104B-D, NIH
2014 - 2018	Member of Special Emphasis Panel Review Groups ZRG1 MDCN Biophysics and BCMB, NIH
2015 - 2015	Grant reviewer, Research Foundation - Flanders (Fonds Wetenschappelijk Onderzoek - Vlaanderen, FWO)
2016 - 2016	Scientific reviewer, German Research Foundation (Deutsche Forschungsgemeinschaft, DFG)
2016 - 2016	ad-hoc member of BPNS study section, NIH
2018 - 2019	Chair-elect of the Membrane biophysics subgroup, Biophysical Society
2018 - 2020	President-elect, Society of General Physiologists
2019 - 2019	Reviewing member, NIH Special emphasis Panel ZRG1 MDCN-Q(04) M
2019 - 2019	Review panel member, NIH Program project
2019 - 2020	Chair of the Channels, Receptors & Transporters subgroup, Biophysical Society
2019 - 2021	Panel member, NIH Fellowship study section ZRG1 F03B-R (20) L
2020 -	Associate Editor, Journal of General Physiology
2020 - 2020	Scientific reviewer , German Research Foundation (Deutsche Forschungsgemeinschaft, DFG)
2020 - 2022	President, Society of General Physiologists

Honors

1995	National Scholarship Award, University of Bucharest, Romania
1995	Fellowship award, TEMPUS, University of Coimbra, Portugal
1998	Predoctoral fellowship, American Heart Association - Florida affiliate

1999	Academic excellence merit award, University of Miami Graduate School
2000	Postdoctoral fellowship, Howard Hughes Medical Institute
2006	Scientist Development Award, American Heart Association - National
2010	Most Promising Young Woman in Biophysics Margaret Dayhoff Award, Biophysical Society
2011	Career Scientist Award, Irma T. Hirschl Trust
2012	Biophysicist in profile, Biophysical Society Newsletter

C. Contribution to Science

1. We characterized pH gating of the potassium channel KcsA using mutagenesis, single-channel recording, X-ray crystallography, NMR, and modeling. We identified a histidine and a glutamate at the intracellular mouth of the pore, which are necessary to render KcsA pH dependent. The KcsA pH sensor residues are located within a lager network of ionizable residues at the bundle crossing of KcsA and we dissected the individual contributions of each of the amino acid residues at this location to pH sensing. Furthermore, using NMR, we showed that KcsA displays several conformations for both open and closed channels.

    a. Xu Y, Zhang D, Rogawski R, Nimigean CM, McDermott AE. Identifying coupled clusters of allostery participants through chemical shift perturbations. Proc Natl Acad Sci U S A. 2019 Feb 5;116(6):2078-2085. PubMed Central PMCID: PMC6369819.

    b. Kim DM, Dikiy I, Upadhyay V, Posson DJ, Eliezer D, Nimigean CM. Conformational heterogeneity in closed and open states of the KcsA potassium channel in lipid bicelles. J Gen Physiol. 2016 Aug;148(2):119-32. PubMed Central PMCID: PMC4969796.

    c. Posson DJ, Thompson AN, McCoy JG, Nimigean CM. Molecular interactions involved in proton-dependent gating in KcsA potassium channels. J Gen Physiol. 2013 Dec;142(6):613-24. PubMed Central PMCID: PMC3840921.

    d. Thompson AN, Posson DJ, Parsa PV, Nimigean CM. Molecular mechanism of pH sensing in KcsA potassium channels. Proc Natl Acad Sci U S A. 2008 May 13;105(19):6900-5. PubMed Central PMCID: PMC2383984.

2. We contributed to the understanding of the mechanism of selectivity for potassium (K+) and against sodium (Na+) ions in K+ channels by combining single-channel recording, X-ray crystallography, and molecular dynamics (MD) simulations. Rejection of Na+ from K+ channel pores is crucial in maintaining a resting potential and generating an action potential in electrically active cells. Understanding this process is of fundamental importance, evidenced by the 60-year long search for this elusive mechanism. Our findings challenged commonly held ideas about permeation and selectivity in potassium channels as we proposed that Na+ and Li+ have favorable binding in the selectivity filter, albeit at different sites than K+, and that the initial selectivity from the inside is due to a large entry barrier for the smaller monovalent cations. We also investigated the mechanism by which different K+ channels with the same signature sequence for K+ (GYG) achieve different selectivities by taking advantage of a non-inactivating KcsA variant that displays less K+ selectivity. We investigated the perturbed selectivity using Na+ block studies at the single-channel level with lipid bilayer electrophysiology and X-ray crystallography of the channel with different ions in the pore. We found that filters that do not collapse/inactivate are also less selective for K+ against Na+.

    a. Thompson AN, Kim I, Panosian TD, Iverson TM, Allen TW, Nimigean CM. Mechanism of potassium-channel selectivity revealed by Na(+) and Li(+) binding sites within the KcsA pore. Nat Struct Mol Biol. 2009 Dec;16(12):1317-24. PubMed Central PMCID: PMC2825899.

    b. Cheng WW, McCoy JG, Thompson AN, Nichols CG, Nimigean CM. Mechanism for selectivity-inactivation coupling in KcsA potassium channels. Proc Natl Acad Sci U S A. 2011 Mar 29;108(13):5272-7. PubMed Central PMCID: PMC3069191.

    c. Nimigean CM, Allen TW. Origins of ion selectivity in potassium channels from the perspective of channel block. J Gen Physiol. 2011 May;137(5):405-13. PubMed Central PMCID: PMC3082928.

    d. McCoy JG, Nimigean CM. Structural correlates of selectivity and inactivation in potassium channels. Biochim Biophys Acta. 2012 Feb;1818(2):272-85. PubMed Central PMCID: PMC3253935.

3. We identified and characterized two prokaryotic channels as good models for eukaryotic cyclic nucleotide-modulated (CNG/HCN) channels. These channels are central to visual and olfactory signal transduction, as well as the pacemaker activity in heart and brain. By relating the function of these channels to their structure, it will ultimately be possible to develop/identify pharmaceutical agents that could tune channel activity. In 2004, we identified MloK1 from M. loti. Using MloK1, we were able to measure, for the first time for this channel family, direct binding of ligand (cAMP and cGMP) to the channel together with activity assays. In collaboration, we determined a series of MloK1 structures at higher and higher resolution, using single particle and cryo electron crystallography. Also in collaboration, we were able to directly visualize MloK1 conformational changes upon ligand binding with high-speed AFM (HS-AFM). One problem with MloK1 was that it lacked an important domain present in eukaryotic channels, and it was not possible to record ionic currents from it. We then identified a better model for CNG channels: the SthK from S. thermophila. SthK is highly homologous with the eukaryotic channels, we optimized its expression for high protein yields suitable for structural work and found that the channel is functional in both ensemble assays and single-channel recordings. Using cryo-EM, we solved high-resolution SthK structures in different ligand-bound conformations (and the first apo conformation for this channel family), which were assigned to functional states with single-channel recordings. We used HS-AFM to image SthK dynamics upon ligand exchange. The high-resolution structures together with the dynamics from AFM constrain a comprehensive model for CNG channel gating. Most recently, we identified yet another way to modulate SthK activation, by prolyl isomerization, which could allow cellular enzymes to fine tune the channel activity. This could play an important role in the regulation of pacemaker channels in vivo, and allow for heart rhythm tuning.

    a. Schmidpeter PAM, Rheinberger J, Nimigean CM. Prolyl isomerization controls activation kinetics of a cyclic nucleotide-gated ion channel. Nat Commun. 2020 Dec 16;11(1):6401. PubMed Central PMCID: PMC7744796.
    b. Rheinberger J, Gao X, Schmidpeter PA, Nimigean CM. Ligand discrimination and gating in cyclic nucleotide-gated ion channels from apo and partial agonist-bound cryo-EM structures. Elife. 2018 Jul 20;7 PubMed Central PMCID: PMC6093708.
    c. Schmidpeter PAM, Gao X, Uphadyay V, Rheinberger J, Nimigean CM. Ligand binding and activation properties of the purified bacterial cyclic nucleotide-gated channel SthK. J Gen Physiol. 2018 Jun 4;150(6):821-834. PubMed Central PMCID: PMC5987880.
    d. Kowal J, Biyani N, Chami M, Scherer S, Rzepiela AJ, Baumgartner P, Upadhyay V, Nimigean CM, Stahlberg H. High-Resolution Cryoelectron Microscopy Structure of the Cyclic Nucleotide-Modulated Potassium Channel MloK1 in a Lipid Bilayer. Structure. 2018 Jan 2;26(1):20-27.e3. PubMed PMID: 29249605.

4. We investigated the mechanism of gating in K+ channels with the prokaryotic MthK, a Ca2+-activated K+ channel, and KcsA, as models. Ca2+-activated K+ channels have important physiological roles, and understanding their gating is a fundamental knowledge quest as well as a gateway towards pharmacological modulation of this channel in disease states. Using structural, and biophysical techniques, we characterized the channel and identified the location of the gates that open and close the pore. We proposed that the activation gate is at the selectivity filter rather than at the "bundle-crossing", the location where voltage-gated ion channels and KcsA are believed to gate, in agreement with existing functional/structural data on eukaryotic Ca2+-activated K+ channels. This finding opened the door to the question of whether other channels believed to gate at the bundle crossing, such as KcsA, may also gate at the selectivity filter. Using MD simulations and single-channel recordings of KcsA mutants, we found that KcsA also gates at the selectivity filter. We proposed a universal mechanism of activation in K channels where the central gate for ions is located at the selectivity filter and the movement of the inner helices couples ligand binding to the filter gate. Recently, using single-particle cryo-EM we identified the key conformations in the gating cycle of MthK. In the absence of Ca2+ we obtained one structure in closed state, while in Ca2+-bound conditions, we obtained several structures. The closed conformations displays a tightly shut bundle-crossing, re-opening the question of the location of the calcium gate. The calcium-bound conformations have open pores and dynamic gating rings (also seen with simulations).

    a. Fan C, Sukomon N, Flood E, Rheinberger J, Allen TW, Nimigean CM. Ball-and-chain inactivation in a calcium-gated potassium channel. Nature. 2020 Apr;580(7802):288-293. PubMed Central PMCID: PMC7153497.

b. Heer FT, Posson DJ, Wojtas-Niziurski W, Nimigean CM, Bernèche S. Mechanism of activation at the selectivity filter of the KcsA K^+ channel. Elife. 2017 Oct 10;6 PubMed Central PMCID: PMC5669632.

c. Posson DJ, Rusinova R, Andersen OS, Nimigean CM. Calcium ions open a selectivity filter gate during activation of the MthK potassium channel. Nat Commun. 2015 Sep 23;6:8342. PubMed Central PMCID: PMC4580985.

d. Posson DJ, McCoy JG, Nimigean CM. The voltage-dependent gate in MthK potassium channels is located at the selectivity filter. Nat Struct Mol Biol. 2013 Feb;20(2):159-66. PubMed Central PMCID: PMC3565016.

5. Inactivation is a universal process by which ion channels terminate ion flux through their pores while opening stimulus is still present. In neurons, channel inactivation is crucial for action potential generation and firing frequency regulation. N-type and C-type inactivation are two major inactivation described for K+ channels. N-type inactivation was proposed to involve a cytoplasmic domain plugging the open pore via a “ball-and-chain” mechanism. C-type inactivation was proposed to involve a selectivity filter constriction to stop permeation. Although "ball-and-chain" inactivation was coined as early as 1973, structural evidence was first provided by us in 2020 when we determined structures of a Ca-gated and inactivating channel (MthK) in a lipid environment using single-particle cryo-EM. The open channel conformations revealed that the N-terminus of one subunit of the tetramer sticks into the pore and plugs it. Deletion of N-terminus leads to non-inactivating channels indicating that this N-terminal peptide is responsible for ball-and-chain inactivation. Since only open states are accessed by inactivation peptides, this work underscored the importance of obtaining structures of the functional state necessary to capture the process of inactivation. MthK also proved a good model for illustrating that some K channels, despite having the same selectivity filter structure and sequence, do not undergo C-type inactivation because they have a higher binding affinity for ions, due to molecular interactions occurring behind the filter.

   a. Boiteux C, Posson DJ, Allen TW, Nimigean CM. Selectivity filter ion binding affinity determines inactivation in a potassium channel. Proc Natl Acad Sci U S A. 2020 Nov 24;117(47):29968-29978. PubMed Central PMCID: PMC7703589.

   b. Dandey VP, Budell WC, Wei H, Bobe D, Maruthi K, Kopylov M, Eng ET, Kahn PA, Hinshaw JE, Kundu N, Nimigean CM, Fan C, Sukomon N, Darst SA, Saecker RM, Chen J, Malone B, Potter CS, Carragher B. Time-resolved cryo-EM using Spotiton. Nat Methods. 2020 Sep;17(9):897-900. PubMed Central PMCID: PMC7799389.

   c. Fan C, Sukomon N, Flood E, Rheinberger J, Allen TW, Nimigean CM. Ball-and-chain inactivation in a calcium-gated potassium channel. Nature. 2020 Apr;580(7802):288-293. PubMed Central PMCID: PMC7153497.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/crina.nimigean.1/bibliography/public/

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

Ongoing Research Support

1R01GM124451-01, NIH
NIMIGEAN, CRINA M (PI)
09/01/17-08/31/21 (renewal application for this grant was reviewed on 2/5/2021 and is in the 3rd percentile)
Structural dynamics in cyclic nucleotide-modulated channels
Role: PI

R01 GM088352, National Institute of General Medical Sciences (NIGMS)
NIMIGEAN, CRINA M (PI)
09/01/10-12/01/23
Molecular mechanisms of potassium channel permeation and gating
Role: PI