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

BIOGRAPHICAL SKETCH

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NAME: Slesinger, Paul A.

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

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)*	Completion Date MM/YYYY	FIELD OF STUDY
Reed College, Portland, OR	BA	05/1984	Biology
University of California, San Francisco, CA	PHD	06/1991	Neuroscience
University of California, San Francisco, CA	Postdoctoral Fellow	12/1997	Neuroscience

A. Personal Statement

My long-term objective is to discover new drugs for treating mental health, addiction to drugs and alcoholism. Currently, there are a small number of FDA approved drugs available for treating these diseases; more are greatly needed. Many of the neurotransmitters in the drug-reward pathway exert their effects by activating G protein-coupled receptors, which, in turn, communicate with specific G proteins and affect down-stream signaling pathways. One of the down-stream targets is the G protein-gated inwardly rectifying potassium (GIRK) channel. GIRK (also referred to as Kir3) channels provide a key source of neuromodulatory inhibition in the brain, by controlling the membrane excitability of neurons. My laboratory has been addressing fundamental questions concerning the function of GIRK channels in the brain, taking a broad approach of combining structural biology, biochemistry, electrophysiology and behavior. We have contributed significant work on the mechanism underlying G protein-regulation and gating of GIRK channels, provided evidence for the assembly of GIRK channels in macromolecular signaling complexes with receptors and G proteins, and identified novel proteins that regulate GIRK channels (e.g., SNX27). Recently, we have been focusing on identifying small molecule modulators of GIRK channels, including cholesterol, alcohol-like compounds and PIP_2. Our work on the structural mechanism of cholesterol modulation of GIRK2 channels using cryoEM is now published in *Cell Reports*.

My specific scientific contributions are outlined in *Section C*. Here, I highlight review papers that include some of our major findings on GPCRs and GIRK channels. The recent TIPS review outlines our strategy for using structural information to identify novel therapeutic compounds that target GIRKs.

1. Zhao Y, Gameiro-Ros I, Glaaser IW, Slesinger PA (2021) Advances in Targeting GIRK Channels in Disease. Trends Pharmacol Sci. 2021 Jan 16:S0165-6147(20)30283-2. doi: 10.1016/j.tips.2020.12.002. PMID: 33468322; PMCID: in progress

2. Rifkin RA, Moss SJ, Slesinger PA. G Protein-Gated Potassium Channels: A Link to Drug Addiction. Trends Pharmacol Sci. 2017 Apr;38(4):378-392. PubMed PMID: 28188005; PubMed Central PMCID: PMC5368012

3. Lüscher C, Slesinger PA. Emerging roles for G protein-gated inwardly rectifying potassium (GIRK) channels in health and disease. Nat Rev Neurosci. 2010 May;11(5):301-15. PubMed PMID: 20389305; PubMed Central PMCID: PMC3052907.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2018 - current Lillian and Henry M. Stratton Professor of Neuroscience, ISMMS
2018 - current Director, Center for Neurotechnology and Behavior at Mount Sinai, ISMMS
2012 - current Professor, Icahn School of Medicine at Mount Sinai, Dept. Neuroscience, New York, NY
2012 - 2015 Adjunct Professor, The Salk Institute for Biological studies, La Jolla, CA
2006 - 2014 Adjunct Associate Professor, University of California, San Diego, Dept. Neuroscience
2005 - 2012 Associate Professor, The Salk Institute for Biological studies, La Jolla, CA
1998 - 2006 Adjunct Assistant Professor, University of California, San Diego, Dept. Neuroscience,
1997 - 2005 Assistant Professor, The Salk Institute for Biological studies, La Jolla, CA

Other Experience and Professional Memberships

2019 - Co-chair of Mount Sinai's MSTP Admissions Committee
2019 - Editorial Board member at Nature Scientific Reports
2017 - 2023 Standing member on NMB NIH study section
2012 - Member, Admissions Committee for Mount Sinai's MD/PHD program
2010 - Member, Research Society on Alcoholism
2008 - 2011 Editorial Board, CNS and Neurological Disorders – Drug Targets
2008 - Review Editor, Frontiers in Molecular Neuroscience
2004 - 2012 Member, Admissions Committee for UCSD Neurosciences Graduate and MSTP Programs
1999 - Ad hoc reviewer for, NAL, NMB, NTRC, NIDA CEBRA grants, NINDS EUREKA grants, NIDA P50 Addiction Center grants
1999 - Reviewer for Nature Neurosci, Cell, Nature Chem. Biol., Neuron, PNAS, Science Signaling, Nature Comm., Elife, Cell Reports, American J. Physiology, FEBS Letters, J. Clinical Invest., J. Comp. Neurol., J. Gen. Phys., J. Neurosci., J. Physiology, Mol. Cell. Neurosci., Mol. Pharm.
1988 - Member, Biophysical Society
1985 - Member, Society for Neuroscience

Honors

2018 - present Director, Center for Neurotechnology and Behavior at Mount Sinai
2018 - present Lillian and Henry M. Stratton Professor of Neuroscience Chair
2006 Independent Investigator Award, NARSAD
2003 Technological Innovations in Neuroscience, McKnight Foundation
2001 Young Investigator Grant, Human Frontiers Science Program
1999 Scholars Award in Neuroscience, McKnight Foundation
1998 Research Fellow, Alfred P. Sloane Foundation
1991 Postdoctoral Fellowship, Muscular Dystrophy Association

Organized Conference/Symposia/Books

2019 Keynote speaker at MolTag Doctoral Program, Pharmacoinformatics Research Group Department of Pharmaceutical Chemistry, University of Vienna, Austria
2018 Monitoring Molecules in Neuroscience, Oxford, England, Symposium Organizer, Chair and Speaker
2015 International Review of Neurobiology; Editor for volume on GIRK channels
2013 RSA Meeting Symposium, Orlando, FL; Symposium Organizer, Chair and Speaker
2012 Membrane Biophysics Subgroup Symposium, 56th Annual Biophysical Society Meeting, San Diego, CA; Symposium Organizer, Chair and Speaker

C. Contribution to Science

1. **Regulation of GIRK channels by G proteins, KCTDs, and small molecules**. We have contributed major studies that determine the structural mechanisms underlying Gβγ activation of GIRK channels as well as structural studies on the interaction of KCTD regulatory proteins and $GABA_B$ receptors. These studies also

establish that both Gβγ and Gα interact directly with GIRK channels, creating a macromolecular signaling complex. We have also recently provided insights into the molecular mechanism by which cholesterol and ethanol directly activate GIRK channels in the absence of G proteins.

a. Mathiharan YK*, Glaaser IW*, Zhao Y*, Robertson MJ, Skiniotis G, Slesinger PA (2021) Structural insights into GIRK2 channel modulation by cholesterol and PIP_2. Cell Rep. 2021 Aug 24;36(8):109619. doi: 10.1016/j.celrep.2021.109619. PMID: 34493831

b. Glaaser IW, Slesinger PA. Dual activation of neuronal G protein-gated inwardly rectifying potassium (GIRK) channels by cholesterol and alcohol. Sci Rep. 2017 Jul 4;7(1):4592. PubMed PMID: 28676630; PubMed Central PMCID: PMC5496853.

c. Lacin E, Aryal P, Glaaser IW, Bodhinathan K, Tsai E, Marsh N, Tucker SJ, Sansom MSP, Slesinger PA. Dynamic role of the tether helix in PIP_2 -dependent gating of a G protein-gated potassium channel. J Gen Physiol. 2017 Jul 18;PubMed PMID: 28720589; PubMed Central PMCID: PMC5560777.

d. Aryal P, Dvir H, Choe S, Slesinger PA. A discrete alcohol pocket involved in GIRK channel activation. Nat Neurosci. 2009 Aug;12(8):988-95. PubMed PMID: 19561601; PMCID: PMC2717173.

2. **Structural insights into $GABA_BR$-GIRK function**. We have contributed several important studies on the structures of GIRK channels, $GABA_B$ receptors, and KCTD proteins. These studies revealed the structural mechanisms underlying cholesterol/ethanol modulation of GIRK channels, the activation mechanisms of $GABA_B$ receptors, and the interaction of KCTD regulatory proteins and $GABA_B$ receptors.

a. Park J, Fu ZJ, Frangaj, Liu J, Mosyak L, Shen T, Slavkovich VN, Ray KM, Cao B, Geng Y, Zuo H, Kou Y, Grassucci R, Chen S, Liu Z, Rice W, Eng E, Huang RK, Soni RK, Kloss B, Yu Z, Potter C, Carragher BO, Slesinger PA, Hendrickson WA, Quick M, Graziano J, Yu H, Fiehn O, Henderson R, Clarke OB, Frank J, Fan QR (2020) Cryo-EM structure of human GABAB G protein-coupled receptor. Nature. 2020 Jun 24. doi: 10.1038/s41586-020-2452-0. PMID: 32581365 PMCID: in progress

b. Zuo H, Glaaser IW, Zhao Y, Kurinov I, Mosyak L, Wang H, Liu J, Park J, Frangaj A, Sturchler E, Zhou M, McDonald P, Geng Y, Slesinger PA, and Fan QR (2019) Structural basis for auxiliary subunit KCTD16 regulation of the GABAB receptor. Proc Natl Acad Sci USA 116(17):8370-8379. doi: 10.1073/pnas.1903024116. PMID: 30971491 PMCID: PMC6486783

3. **Plasticity in $GABA_B$ - GIRK signaling with drugs of abuse**. My laboratory has provided some of the first evidence for drug-dependent plasticity in the slow inhibitory signaling pathway in the VTA. Most recently, we described a novel pathway in VTA GABA neurons for psychostimulant-dependent depression of $GABA_B$-GIRK currents involving de-phosphorylation of the receptor and the protein phosphatase PP2a. Subsequently, we described a different subcellular mechanism of psychostimulant-dependent depression of $GABA_B$-GIRK currents in VTA DA that involves the GIRK3 subunit and an endosomal trafficking protein SNX27. Recently, we found that mice show enhanced sensitivity to cocaine when $GABA_B$ receptor activated GIRK currents are reduced in size by conditionally knocking out SNX27 in VTA DA neurons. Together, these studies highlight GIRK channels as an emerging drug-target for treating addiction and alcoholism.

a. Li X, Terunuma M, Deeb TG, Wiseman X, Pangalos MN, Nairn AC, Moss SJ and Slesinger PA. (2020) Direct interaction of PP2A phosphatase with GABAB receptors alters functional signaling. J Neurosci. 2020 Feb 26. pii: 2654-19. doi: 10.1523/JNEUROSCI.2654-19.2020. PMID: 32111696 PMCID: PMC7117905

b. Rifkin RA, Huyghe D, Li X, Parakala M, Aisenberg E, Moss SJ and Slesinger PA (2018) GIRK currents in VTA dopamine neurons control the sensitivity of mice to cocaine-induced locomotor sensitization. Proc Natl Acad Sci U S A. 2018 Oct 2;115(40):E9479-E9488. doi: 10.1073/pnas.1807788115. Epub 2018 Sep 18. PMID: 30228121.

c. Padgett CL, Lalive AL, Tan KR, Terunuma M, Munoz MB, Pangalos MN, Martínez-Hernández J, Watanabe M, Moss SJ, Luján R, Lüscher C, Slesinger PA. Methamphetamine-evoked depression of GABA(B) receptor signaling in GABA neurons of the VTA. Neuron. 2012 Mar 8;73(5):978-89. PubMed PMID: 22405207; PubMed Central PMCID: PMC3560416.

d. Munoz MB, Padgett CL, Rifkin R, Terunuma M, Wickman K, Contet C, Moss SJ, Slesinger PA. A Role for the GIRK3 Subunit in Methamphetamine-Induced Attenuation of GABAB Receptor-Activated GIRK Currents in VTA Dopamine Neurons. J Neurosci. 2016 Mar 16;36(11):3106-14. PubMed PMID: 26985023; PubMed Central PMCID: PMC4792929.

4. ***GIRK channels and alcohol abuse***. Ethanol's actions on neural circuits in the brain involve the direct modulation of ion channel activity. Atomic-resolution structures of alcohol pockets provide clues on the mechanism. One of our major contributions has been describing the first 3D structure of an ion channel bound to an alcohol. Using this structure, we elucidated structural and chemical components of alcohol-mediated activation of GIRK channels. We recently used an alcohol-tagging procedure to reveal the chemical requirements for activating GIRK channels; the hydroxyl in ethanol is required for stabilizing the alcohol in the pocket through hydrogen bonding, and the hydrophobic elements of alcohol promote channel gating. We recently provided evidence for a definitive role of the GIRK3 subunit in binge-drinking, and most recently identified a GIRK1/2 activator that targets the alcohol pocket in GIRKs.

   a. Zhao Y, Ung PMU, Zahoranszky-Kohalmi G, Zakharov A, Martinez N, Simeonov A, Glaaser IW, Bantukallu G, Schlessinger A, Marugan JJ, Slesinger PA (2020) Discovery of a G protein-independent activator (GiGA1) of GIRK channels. Cell Rep. 2020 Jun 16;31(11):107770. doi: 10.1016/j.celrep.2020.107770. PMID: 32553165. PMCID: PMC7401321

   b. Herman MA, Sidhu H, Stouffer DG, Kreifeldt M, Le D, Cates-Gatto C, Munoz MB, Roberts AJ, Parsons LH, Roberto M, Wickman K, Slesinger PA, Contet C. GIRK3 gates activation of the mesolimbic dopaminergic pathway by ethanol. Proc Natl Acad Sci U S A. 2015 Jun 2;112(22):7091-6. PMID: 25964320; PMCID: PMC4460485.

   c. Bodhinathan K, Slesinger PA. Molecular mechanism underlying ethanol activation of G-protein-gated inwardly rectifying potassium channels. Proc Natl Acad Sci U S A. 2013 Nov 5;110(45):18309-14. PMID: 24145411; PMCID: PMC3831446.

   d. Aryal P, Dvir H, Choe S, Slesinger PA. A discrete alcohol pocket involved in GIRK channel activation. Nat Neurosci. 2009 Aug;12(8):988-95. PMID: 19561601: PMC2717173.

Current NCBI listing:
http://www.ncbi.nlm.nih.gov/sites/myncbi/paul a.slesinger.1/bibliography/43983971/public/?sort=date&direction=descending

BIOGRAPHICAL SKETCH

NAME: **Mathiharan, Yamuna Kalyani**

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

POSITION TITLE: Postdoctoral Research Fellow

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	START DATE MM/YYYY	COMPLETION DATE MM/YYYY	FIELD OF STUDY
Government College of Technology, Coimbatore, India	B.Tech	08/2002	05/2006	Industrial Biotechnology
Alagappa College of Technology, Chennai, India	M.Tech	08/2006	05/2008	Biotechnology
Indian Institute of Science, Bengaluru, India	PhD	08/2008	06/2015	Crystallography
University of Michigan, MI & Stanford University, CA	Postdoctoral Fellow	02/2016	02/2020	Cryo-Electron Microscopy
Icahn School of Medicine, Mount Sinai, NY	Postdoctoral Fellow	05/2021	Present	Cryo-Electron Microscopy

A. Personal Statement

My research training is in structural biology. I've expertise to solve protein and protein-RNA structures by using cryo-electron microscopy and macromolecular crystallography. Overall goals of my research have been to use these techniques to determine protein structures, resolve conformational heterogeneity and understand their functional relevance. I have studied various proteins involved in important regulatory machinery like reverse transcription and of membrane proteins. Long-term goal of my research is to understand the role of membrane proteins in neurotransmission and their significance in human diseases. One of the key directions of my future research will be to study the role of GIRK channels in VTA DA and their implication in addiction. My work on GIRK2 will form basis for these studies, I have elucidated the role of lipids in GIRK2 gating and a "novel" apo conformation with cytoplasmic domain detached from membrane.

I have discussed in detail about my contributions to science in section C. Here are few publications which highlights my expertise in cryoEM, macromolecular crystallography and GIRK channels.

a) ***Mathiharan YK****, Glaaser IW*, Zhao Y*, Robertson MJ, Skiniotis G, Slesinger PA, "Structural basis of GIRK2 channel modulation by cholesterol and PIP_2", **Cell Reports**, 36, 109619, August 2021 **(*equally contributed)**.

b) Glassman CR, ***Mathiharan YK****,* Jude KM*, Su L, Panova O, Lupardus PJ, Spangler JB, Ely LK, Thomas C, Skiniotis G, Garcia KC, "Structural basis for IL-12 and IL-23 shared receptor usage reveals a gateway for shaping actions on T versus NK cells", **Cell**, 184, 983-999, February 2021 **(*equally contributed)**.

c) Larsen KP*, ***Mathiharan YK****, Kappel K, Coey AT, Chen DH, Barrero D, Madigan L, Puglisi JD, Skiniotis G and Puglisi EV, "Architecture of an HIV-1 reverse transcription initiation complex", **Nature**, 557 (7703): 118-122, May 2018 **(*equally contributed)**.

d) ***Mathiharan YK***, Savithri HS and Murthy MRN, "Insights into stabilizing interactions in the distorted domain swapped dimer of *Salmonella typhimurium* survival protein", **Acta Crystallographica Section D**, 71:1812-1823, September 2015.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2021 – Present	**Postdoctoral Fellow, Mount Sinai**
2017 – 2020	**Postdoctoral Fellow, Stanford**
2016 – 2017	**Postdoctoral Fellow, University of Michigan**
2015	**Project officer, IIT Madras**

Honors

2013	**Department of Biotechnology (DBT), Government of India travel/stay grant** to visit European Synchrotron Radiation Facility (ESRF),
2012	**International Union of Crystallography (IUCr) travel grant,** attend AsCA 12/CRYSTAL 28
2011	**International travel grant by Indian Council of Medical Research (ICMR)** to attend PDB40 symposium
2008 – 2013	**GATE Life Science PhD Fellowship**
2006 – 2008	**GATE Life Science M.Tech Fellowship**

C. Contributions to Science

1. **Structural studies to understand lipid-dependent gating in GIRK2:** As a postdoctoral fellow in Skiniotis lab, I helped to develop a project where the objective was to capture "open state" conformation of G protein-coupled inwardly rectifying potassium channels (GIRK2) upon alcohol/G protein binding, done in collaboration with Dr. Paul Slesinger, Icahn School of Medicine, Mount Sinai. I collected cryoEM data, processed and analyzed GIRK2 structures with different lipid modulators like PIP_2 and cholesterol, also prepared and optimized GIRK2 nanodisc reconstituted sample with lipid composition similar to brain plasma membrane. These studies revealed the mechanism of PIP_2 and cholesterol gating in GIRK2.

   a) ***Mathiharan YK****, Glaaser IW*, Zhao Y*, Robertson MJ, Skiniotis G, Slesinger PA, "Structural basis of GIRK2 channel modulation by cholesterol and PIP_2", **Cell Reports**, 36, 109619, August 2021 **(*equally contributed)**.

2. **Cryo-electron microscopy (EM) to resolve conformational heterogeneity in GIRK2 and HIV-1 reverse transcriptase initiation complex:** In the presence of PIP_2, I have shown that GIRK2 homotetramer exists in multiple conformations; the predominant state is the one with four PIP_2 bound in the TMD-CTD interface while other states with detached CTD like apo suggest that GIRK2 has low affinity for PIP_2. I have solved structures of HIV-1 reverse transcriptase initiation complex (RTIC), a protein-RNA complex done in collaboration with Dr. Elisabetta Viani Puglisi and Dr. Joseph D Puglisi, Stanford. I have solved RTIC structures with different conformations of the viral and tRNA. The RNA plasticity observed in the RTIC

might be the reason for slow initiation in the HIV-1. I have used different cryoEM data processing tools like 3DVA and 3D multi-body refinement to understand the heterogeneity in the sample.

a) ***Mathiharan YK****, Glaaser IW*, Zhao Y*, Robertson MJ, Skiniotis G, Slesinger PA, "Structural basis of GIRK2 channel modulation by cholesterol and PIP_2", **Cell Reports**, 36, 109619, August 2021 **(*equally contributed)**.
b) Larsen KP*, ***Mathiharan YK****, Kappel K, Coey AT, Chen DH, Barrero D, Madigan L, Puglisi JD, Skiniotis G and Puglisi EV, "Architecture of an HIV-1 reverse transcription initiation complex", **Nature**, 557 (7703): 118-122, May 2018 **(*equally contributed)**.

3. **Structural studies to understand substrate binding and inhibition in SLCs:** I have helped solve cryoEM structures of other membrane proteins like solute carrier (SLC) transporters. CryoEM structures of SLC4A1 with different inhibitors and substrate have helped to understand its mechanism of bicarbonate transport and inhibition.

   a) Capper MJ, Yang S, Stone AC, Vatansever S, Zilberg G, ***Mathiharan YK***, Habib R, Hutchinson K, Schlessinger A, Mezei M, Osman R, Zhang B, Wacker D, "Substrate Binding and Inhibition of the Anion Exchanger 1 Transporter", **BioRxiv**, February, 2022 (**doi:** https://doi.org/10.1101/2022.02.11.480130).

4. **Structural and functional studies to understand the role of domain swapping in protein oligomerization:** My PhD thesis dwelt on the importance of hinges involved in domain swapping, their implication in protein oligomerization and function - stress protein from *Salmonella typhimurium* and *Sesbania mosaic* virus coat protein was studied. By X-ray crystallography, biochemical, biophysical and computational tools, I was able to demonstrate that the hinge sequence indeed has a profound impact on protein structures and the stress protein's function. By these studies, I was able to highlight the importance of intricate network of short- and long-range protein interactions and how it influences the function.

   a) ***Mathiharan YK*** and Murthy MRN, "Molecular dynamics studies on the domain swapped *Salmonella typhimurium* survival protein SurE: insights on the possible reasons for catalytic cooperativity", **Journal of Biomolecular Structure and Dynamics**, 1-9, August 2017.
   b) ***Mathiharan YK***, Savithri HS and Murthy MRN, "Insights into stabilizing interactions in the distorted domain swapped dimer of *Salmonella typhimurium* survival protein", **Acta Crystallographica Section D**, 71:1812-1823, September 2015.
   c) ***Mathiharan YK***, Pappachan A, Savithri HS and Murthy MRN, "Dramatic structural changes resulting from the loss of a crucial hydrogen bond in the hinge region involved in C-terminal helix swapping in SurE: a survival protein from *Salmonella typhimurium*", **PLoS ONE**, 8(2): e55978, February 2013.

List of published work in My Bibliography:

https://www.ncbi.nlm.nih.gov/myncbi/yamuna%20kalyani.mathiharan.1/bibliography/public/