# BIOGRAPHICAL SKETCH

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NAME: Sobolevsky, Alexander

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

POSITION TITLE: Associate 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 |
|---|---|---|---|
| Moscow Inst. of Physics and Technology, Moscow | MS | 06/1996 | Bioengineering |
| Moscow Inst. of Physics and Technology, Moscow | PHD | 11/1999 | Biophysics |
| Stony Brook University, NY | Post-Doc | 08/2004 | Neurobiology |
| Columbia University, NY/Vollum Institute, OHSU, OR | Post-Doc | 08/2010 | Structural Biology |

## A. Personal Statement

The goal of the BAG proposal is to apply single-particle cryo-EM to study structure and function of ionotropic glutamate receptors (iGluRs). Our experiments will help better understanding molecular bases of excitatory neurotransmission in the central nervous system and will result in a new structure-functional model of iGluR that can serve as a dynamic template for theoretical prediction, in silico fitting and chemical synthesis of new compounds that can be tested in different models of neurological diseases and eventually become safe and effective medications. I have an expertise in solving structures of integral membrane proteins by both X-ray crystallography and cryo-EM and an extensive experience in using methods of characterizing ion channels function, including patch-clamp, double-electrode voltage-clamp recordings and Fura-2-based ratiometric fluorescent measurements of intracellular calcium. I also have an expertise in analyzing different types of ion channel inhibition using a combination of electrophysiology, protein engineering and kinetic modeling. With such expertise and experiences, I studied the mechanisms of ionotropic glutamate receptor (iGluR) inhibition by ion channel blockers, including the only FDA-approved NMDA receptor channel blocker Memantine, currently used for treatment of Alzheimer's disease. I solved the first full length crystal structure of ionotropic glutamate receptor. My lab solved numerous structures of full-length iGluRs, including the first agonist-bound, desensitized and different conductance open state structures and proposed the first complete structural model of iGluR gating. Using X-ray crystallography, my lab determined the structural mechanism of iGluR inhibition by noncompetitive inhibitors, including Perampanel that is currently used for treatment of epilepsy. My lab also solved the first TRP channel crystal structure (the structure of TRPV6). Using cryo-EM, my lab determined structures of human TRPV6 in different conformations and proposed the mechanism of TRPV6 activation. Similarly, my lab solved the first TRPV3 structure and structures of TRPV3 in different conformations and proposed the mechanism of ligand-induced TRPV3 activation. Recently, we solved structures of TRPV3 in temperature-dependent closed, intermediate and open states, which for the first time uncovered the structural bases of TRP channel activation by temperature. As a result of my previous experiences, I am aware of the importance of frequent communication among project members and of constructing a realistic research plan, timeline, and budget. My current research plans build logically on my prior work.

Ongoing and recently completed projects that I would like to highlight include:

R01 CA206573
Sobolevsky (PI)
01/12/2017-12/31/2021
Structure and Function of Transient Receptor Potential channels

R01 AR078814
Sobolevsky (PI)
02/01/2022-1/31/2027

Structural and functional principles of activation and regulation of the transient receptor potential channel TRPV3

R01 NS083660
Sobolevsky (PI) / Kurnikova (co-PI)
09/30/2013-06/30/2023
Structure and Function of AMPA subtype ionotropic glutamate receptors

R01 NS107253
Sobolevsky (PI)
08/01/2018-05/31/2023
Single-particle cryo-EM characterization of AMPA receptor functional states

NSF 1818086
Sobolevsky/Kurnikova/Stern-Bach (MPI)
08/01/2018-07/31/2022
Collaborative Research: Towards development of the structural determinants of the Glutamate receptor gating regulation by auxiliary membrane anchored proteins

Citations:


1. Yelshanskaya MV, Patel D. S., Kottke C. M., Kurnikova M. G. and *Sobolevsky A. I.* (2022) Opening of glutamate receptor channel to subconductance levels. ***Nature*** 605: 172-178. PubMed Central PMCID: PMC9068512.
2. McGoldrick LL, Singh AK, Saotome K, Yelshanskaya MV, Twomey EC, Grassucci RA, Sobolevsky AI. (2018) Opening of the human epithelial calcium channel TRPV6. ***Nature*** 553: 233-237. PubMed Central PMCID: PMC5854407.
3. Twomey EC, Yelshanskaya MV, Grassucci RA, Frank J, Sobolevsky AI. (2017) Channel opening and gating mechanism in AMPA-subtype glutamate receptors. ***Nature*** 549: 60-65. PubMed Central PMCID: PMC5743206.
4. Saotome K, Singh AK, Yelshanskaya MV, Sobolevsky AI. (2016) Crystal structure of the epithelial calcium channel TRPV6. ***Nature*** 534: 506-11. PubMed Central PMCID: PMC4919205.


## B. Positions, Scientific Appointments and Honors

### Positions and Scientific Appointments

| | |
|---|---|
| 2017 - | Associate Professor, Columbia University, New York, NY |
| 2010 - 2017 | Assistant Professor, Columbia University, New York, NY |
| 2005 - 2010 | Post-doctoral Research Fellow, Vollum Institute, Oregon Health and Science University, Portland, OR |
| 2004 - 2005 | Post-doctoral Research Fellow, Columbia University, New York, NY |
| 2000 - 2004 | Post-doctoral Research Fellow, Stony Brook University, Stony Brook, NY |
| 1996 - 1999 | Pre-doctoral Research Fellow, Moscow Institute of Physics and Technology, Moscow |
| 1993 - 1996 | Pre-diploma Research Fellow, Moscow Institute of Physics and Technology, Moscow |

### Honors

| | |
|---|---|
| 2017 | Amgen Young Investigator Award, Amgen |
| 2015 | Irma T. Hirschl Career Scientist Award, Irma T. Hirschl Trust |
| 2013 | Pew Scholar Award, Pew Charitable Trusts |
| 2012 | Schaefer Research Scholar Award, Dr. Ludwig Schaefer Fund |
| 2011 | Klingenstein Fellowship Award in the Neurosciences, Esther A. & Joseph Klingenstein Fund |
| 2002 | Postdoctoral Travel Award for participation in the 32nd Annual Meeting of the Society for Neuroscience, Burroughs Wellcome Fund |
| 2000 | Travel Grant for participation in the 31st Annual Meeting of the Society for Neuroscience, International Brain Research Organization |

1999 International Soros Science Education Program Grant, Soros Foundation
1998 International Soros Science Education Program Grant, Soros Foundation
1998 Travel Grant for participation in the 29th Annual Meeting of the Society for Neuroscience, International Brain Research Organization

## C. Contribution to Science

1. N-methyl-D-aspartate (NMDA) receptors are a subtype of ionotropic glutamate receptors that is critical to neuronal development and synaptic plasticity, associated with memory formation and learning and implicated in acute and chronic neuronal death, associated with brain trauma and neurological disorders. Ion channel blockers of NMDA receptors therefore have an enormous drug potential. We have been among the first research groups to study the mechanism of ion channel block of NMDA receptors by various derivatives of aminoadamantane, one of which, Memantine (NAMENDA), have become the first and so far the only drug acting at NMDA receptors that has been approved by FDA for treatment of moderate to severe Alzheimer's disease. We developed a set of new kinetic criteria to analyze the mechanism of blocker interaction with ion channel gating machinery. Using this set, we were the first to discover that $Mg^{2+}$ interacts with NMDA receptors via the trapping block mechanism. The discovery of the trapping block of NMDA receptor channels by $Mg^{2+}$ led to reevaluation of the role of $Mg^{2+}$ and NMDA receptors in neurotransmission across excitatory synapses in the brain.

    a. Sobolevsky AI, Yelshansky MV. The trapping block of NMDA receptor channels in acutely isolated rat hippocampal neurones. **J Physiol**. 2000 Aug 1;526 Pt 3:493-506. PubMed Central PMCID: PMC2270033.
    b. Sobolevsky AI, Koshelev SG, Khodorov BI. Probing of NMDA channels with fast blockers. **J Neurosci**. 1999 Dec 15;19(24):10611-26. PubMed Central PMCID: PMC6784965.
    c. Sobolevsky AI, Koshelev SG, Khodorov BI. Interaction of memantine and amantadine with agonist-unbound NMDA-receptor channels in acutely isolated rat hippocampal neurons. **J Physiol**. 1998 Oct 1;512 (Pt 1):47-60. PubMed Central PMCID: PMC2231181.
    d. Sobolevsky A, Koshelev S. Two blocking sites of amino-adamantane derivatives in open N-methyl-D-aspartate channels. **Biophys J**. 1998 Mar;74(3):1305-19. PubMed Central PMCID: PMC1299478.

2. Before the structures of the full length iGluR become available, one could only guess what are the structural organization of the iGluR channel and the mechanisms of pore opening and closure. To gain insights into the structure of the NMDA receptor ion channel pore and the structural rearrangements during gating, we used the substituted cysteine accessibility method (SCAM). The NMDA receptor is an obligate heterotetramer composed of two or more different subunits. We individually mutated residues in the transmembrane portion of the two major subtypes of NMDA receptor subunits, NR1 and NR2. We identified the boundaries and the pore-facing surfaces of the transmembrane domains, their relative contribution to the ion channel pore and gating and the amino acid residues in the pore involved into receptor activation and desensitization as well as binding of the channel blockers. We were among the first to discover the asymmetrical contribution of the NR1 and NR2 subunits to channel pore structure and gating and the central role of the M3 segment in NMDA receptor gating.

    a. Sobolevsky AI, Prodromou ML, Yelshansky MV, Wollmuth LP. Subunit-specific contribution of pore-forming domains to NMDA receptor channel structure and gating. **J Gen Physiol**. 2007 Jun;129(6):509-25. PubMed Central PMCID: PMC2151626.
    b. Wollmuth LP, Sobolevsky AI. Structure and gating of the glutamate receptor ion channel. **Trends Neurosci**. 2004 Jun;27(6):321-8. PubMed PMID: 15165736.
    c. Sobolevsky AI, Rooney L, Wollmuth LP. Staggering of subunits in NMDAR channels. **Biophys J**. 2002 Dec;83(6):3304-14. PubMed Central PMCID: PMC1302406.
    d. Sobolevsky AI, Beck C, Wollmuth LP. Molecular rearrangements of the extracellular vestibule in NMDAR channels during gating. **Neuron**. 2002 Jan 3;33(1):75-85. PubMed PMID: 11779481.

3. We used SCAM and patch-clamp recordings to study structure and function of homotetrameric AMPA subtype iGluRs. We identified pore-forming elements and residues involved in AMPA receptor gating. We

discovered mutations outside the ligand binding domain (LBD) – in the linkers connecting the LBD to the ion channel – that resulted in either enhancement or nearly complete oblation of AMPA receptor desensitization. We found that AMPA receptors are unique compared to other tetrameric ion channels and that despite the subunit assembly is homomeric, contribution of individual subunits to the ion channels pore is different leading to the overall two- rather than four-fold rotation symmetry of the ion channel in the active state.

a. Sobolevsky AI, Yelshansky MV, Wollmuth LP. State-dependent changes in the electrostatic potential in the pore of a GluR channel. **Biophys J**. 2005 Jan;88(1):235-42. PubMed Central PMCID: PMC1305001.
b. Yelshansky MV, Sobolevsky AI, Jatzke C, Wollmuth LP. Block of AMPA receptor desensitization by a point mutation outside the ligand-binding domain. **J Neurosci**. 2004 May 19;24(20):4728-36. PubMed Central PMCID: PMC6729461.
c. Sobolevsky AI, Yelshansky MV, Wollmuth LP. The outer pore of the glutamate receptor channel has 2-fold rotational symmetry. **Neuron**. 2004 Feb 5;41(3):367-78. PubMed PMID: 14766176.
d. Sobolevsky AI, Yelshansky MV, Wollmuth LP. Different gating mechanisms in glutamate receptor and K+ channels. **J Neurosci**. 2003 Aug 20;23(20):7559-68. PubMed Central PMCID: PMC6740752.

4. The transient receptor potential (TRP) channels are a superfamily of cation permeable ion channels that are widely known for their role as transducers of sensory modalities, including temperature, taste, olfaction, vision, hearing and touch. TRP channels are also crucial for a diverse range of physiological processes, such as neurite outgrowth, hormone secretion and control of vascular tone. Accordingly, mutations or malfunction of TRP channels are associated with numerous human diseases, including cardiovascular, renal, nociceptive and metabolic disorders. We solved the first crystal structure of TRP channel, $Ca^{2+}$-selective channel TRPV6 that plays vital roles in calcium homeostasis as a $Ca^{2+}$ uptake channel in epithelial tissues and is implicated in development and progression of numerous forms of cancer. We also determined the structural bases of TRPV6 allosteric regulation and calcium-induced calmodulin-mediated inactivation. We also solved the first structures of TRPV3 and determined structural bases of TRPV3 activation by both ligands and temperature. Our results provide structural foundations to understand the role of TRP channels in physiology and disease, and provide information necessary for drug design.

a. Nadezhdin KD, Neuberger A, Trofimov YA, Krylov N, Sinica V, Kupko N, Vlachova V, Zakharian E, Efremov RG and *Sobolevsky AI.* Structural mechanism of heat-induced opening of a temperature-sensitive TRP channel. **Nat Struct Mol Biol**. 2021 Jul 8;28(7):564-572. PubMed Central PMCID: PMC8283911.
b. Singh AK, McGoldrick LL, Sobolevsky AI. Structure and gating mechanism of the transient receptor potential channel TRPV3. **Nat Struct Mol Biol**. 2018 Sep;25(9):805-813. PubMed Central PMCID: PMC6128766.
c. McGoldrick LL, Singh AK, Saotome K, Yelshanskaya MV, Twomey EC, Grassucci RA, Sobolevsky AI. Opening of the human epithelial calcium channel TRPV6. **Nature**. 2018 Jan 11;553(7687):233-237. PubMed Central PMCID: PMC5854407.
d. Saotome K, Singh AK, Yelshanskaya MV, Sobolevsky AI. Crystal structure of the epithelial calcium channel TRPV6. **Nature**. 2016 Jun 23;534(7608):506-11. PubMed Central PMCID: PMC4919205.

5. High resolution structural information about ionotropic glutamate receptors opens new horizons to understanding their gating mechanism and regulation at the molecular level as well as makes iGluRs a novel pharmacological platform for characterizing new compounds with diverse activities for use as therapies in neurological diseases. My lab has solved the first crystal structure of the full length AMPA receptor in complex with agonist, crystallographically discovered novel binding sites of antiepileptic drugs, obtained the first cryo-EM structures of AMPA receptor complexes with the auxiliary subunits stargazin, gamma5 and GSG1L, and solved the first structures of AMPA receptor in the open and desensitized states, and in complex with ion channel blockers.

a. Yelshanskaya MV, Patel D. S., Kottke C. M., Kurnikova M. G. and *Sobolevsky A. I.* (2022) Opening of glutamate receptor channel to subconductance levels. **Nature** 605: 172-178. PubMed Central PMCID: PMC9068512.

b. Twomey EC, Yelshanskaya MV, Grassucci RA, Frank J, Sobolevsky AI. Channel opening and gating mechanism in AMPA-subtype glutamate receptors. **Nature**. 2017 Sep 7;549(7670):60-65. PubMed Central PMCID: PMC5743206.
c. Twomey EC, Yelshanskaya MV, Grassucci RA, Frank J, Sobolevsky AI. Elucidation of AMPA receptor-stargazin complexes by cryo-electron microscopy. **Science**. 2016 Jul 1;353(6294):83-6. PubMed Central PMCID: PMC5125255.
d. Yelshanskaya MV, Li M, Sobolevsky AI. Structure of an agonist-bound ionotropic glutamate receptor. **Science**. 2014 Aug 29;345(6200):1070-4. PubMed Central PMCID: PMC4383034.

**Complete List of Published Work in PubMed:**
https://www.ncbi.nlm.nih.gov/myncbi/alexander.sobolevsky.1/bibliography/public/

# BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Yelshanskaya, Maria

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

POSITION TITLE: Research Assistant

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 |
|---|---|---|---|
| Moscow Inst. of Physics and Technology, Moscow | BS | 06/1997 | Bioengineering |
| Moscow Inst. of Physics and Technology, Moscow | PHD | 11/2001 | Biophysics |
| Stony Brook University, NY | Post-Doc | 08/2004 | Physiology and Neuroscience |

## A. Personal Statement

The goal of the BAG proposal is to apply single-particle cryo-EM to study structure and function of ionotropic glutamate receptors (iGluRs). Our experiments will help better understanding molecular bases of excitatory neurotransmission in the central nervous system and will result in a new structure-functional model of iGluR that can serve as a dynamic template for theoretical prediction, in silico fitting and chemical synthesis of new compounds that can be tested in different models of neurological diseases and eventually become safe and effective medications. My previous work has been focused on understating the complex regulation of ionotropic glutamate receptors (iGluRs) and ion channel gating. I started to address this problem while being an undergraduate student at the Moscow Institute of Physics and Technology (MIPT) in the lab of Prof. B.I. Khodorov. During my PhD study at MIPT, I carried out electrophysiological recordings from NMDA subtype iGluRs in hippocampal neurons acutely isolated from rat brain and studied interaction of NMDA receptor ion channels with blockers and modulators, such as arachidonic acid. After receiving my PhD from MIPT, I joined the laboratory of Dr. Wollmuth at the Stony Brook University for my postdoctoral training, where I studied recombinant iGluRs using mutagenesis and outside-out patch-clamp recordings. In 2010, after maternity leave, I joined the laboratory of Dr. Sobolevsky and continued to work on structure and function of iGluRs, but also representatives of the transient receptor potential (TRP) channels. My research interests lie in investigating the molecular mechanisms of ion channel gating, ion conductance and regulation by small molecules and auxiliary proteins using a combination of biochemical and biophysical approaches, including X-ray crystallography and single-particle cryo-electron microscopy. My versatile training provides me with strong biophysical, biochemical, molecular and structural biology background, indispensable to successfully achieve my research goals.

Citations:


1. *Yelshanskaya M. V.*, Patel D. S., Kottke C. M., Kurnikova M. G. and Sobolevsky A. I. (2022) Opening of glutamate receptor channel to subconductance levels. ***Nature*** 605: 172-178. PubMed Central PMCID: PMC9068512.
2. *Yelshanskaya M. V.*, Nadezhdin K. D., Kurnikova M. G. and Sobolevsky A. I. (2021) Structure and Function of the calcium-selective TRP channel TRPV6. ***Journal of Physiology*** 599: 2673-2697. PubMed Central PMCID: PMC7689878.
3. *Yelshanskaya M. V.*, Singh A. K., Sampson J. M., Narangoda C., Kurnikova M. and Sobolevsky A. I. (2016) Structural Bases of Noncompetitive Inhibition of AMPA-Subtype Ionotropic Glutamate Receptors by Antiepileptic Drugs. ***Neuron*** 91: 1305-1315. PubMed Central PMCID: PMC5033713.
4. *Yelshanskaya M. V.*, Li M. and Sobolevsky A. I. (2014) Structure of an agonist-bound ionotropic glutamate receptor. ***Science*** 345: 1070-1074. PubMed Central PMCID: PMC4383034.

## B. Positions, Scientific Appointments and Honors

### Positions and Scientific Appointments

| | |
|---|---|
| 2010-present | Research Associate, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY. Structure and function of iGluRs. Supervisor – Alexander I. Sobolevsky. |
| 2004-2010 | Maternity leave. |
| 2001-2004 | Post-doctoral Research Fellow. Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, NY, USA. Studies of structure, function and molecular biology of glutamate receptors. Supervisor – Lonnie P. Wollmuth. |
| 1998-2001 | Pre-doctoral Research Fellow. Institute of General Pathology & Pathophysiology and Moscow Institute of Physics and Technology, Moscow, Russia. The title of the doctoral thesis: "Study of the effects of arachidonic acid on biophysical and chemoreceptive properties of NMDA receptor channels". Supervisor – Prof. Boris I. Khodorov. |
| 1997-1998 | Research Technician. Dept. Neurophysiology, Bogomoletz Institute of Physiology, Kiev, Ukraine. |

### Honors

| | |
|---|---|
| 2001-2002 | NSF-NATO Fellowship (#10108434 1 22060). |
| 2001 | International Brain Research Organization Travel Grant for participation in the 31st Annual Meeting of the Society for Neuroscience (San Diego, California, November 2001). |
| 2001 | Young Scientist Award from the Russian Foundation for Basic Research. |
| 2000-2001 | International Soros Science Education Program Grant (# A2001-358), Moscow, Russia. |
| 2000 | Young Scientist Award, 2-d Pathology Congress, Moscow, Russia. |
| 2000 | Women in Neuroscience/Eli Lilly Student Travel Award for participation in the 30th Annual Meeting of the Society for Neuroscience (New Orleans, Lousiana, November 2000). |
| 1999 | Travel grant from Russian Foundation for Basic Research for participation in the 29th Annual Meeting of the Society for Neuroscience (Miami, Florida, October 1999). |

### Teaching

| | |
|---|---|
| 2011-2020 | Supervising postdocs, undergraduate and graduate students in Dr. Sobolevsky lab. |
| 1993-2001 | Teaching physics and mathematics, Correspondence courses, MIPT, Dolgoprudny, Russia. |
| 1996-1997 | Teaching chemistry, "Phystekh-college", Dolgoprudny, Russia. |

## C. Contribution to Science

1. N-methyl-D-aspartate (NMDA) receptors are a subtype of ionotropic glutamate receptors that is critical to neuronal development and synaptic plasticity, associated with memory formation and learning and implicated in acute and chronic neuronal death, associated with brain trauma and neurological disorders. Small-molecule regulators of NMDA receptors therefore have an enormous drug potential. As a member of Prof. Khodorov group, I have been studying the mechanisms of small-molecule regulation and ion channel block of NMDA receptors using whole-cell patch-clamp recordings from acutely isolated rat hippocampal neurons. We developed a set of new kinetic criteria to analyze the mechanism of blocker interaction with ion channel gating machinery. Using this set, we were the first to discover that $Mg^{2+}$ interacts with NMDA receptors via the trapping block mechanism. The discovery of the trapping block of NMDA receptor channels by $Mg^{2+}$ led to reevaluation of the role of $Mg^{2+}$ and NMDA receptors in neurotransmission across excitatory synapses in the brain.

    a. Sobolevsky A. I., *Yelshansky M. V*. (2000) The trapping block of NMDA receptor channels in acutely isolated rat hippocampal neurones. ***J Physiol.*** 526: 493-506. PubMed Central PMCID: PMC2270033.

    b. *Yelshansky M. V.,* Sobolevsky A. I. and Khodorov B. I. (2002) Study of the Effect of Arachidonic Acid on NMDA Channels in Acutely Isolated Rat Hippocampal Neurons. ***Biological Membranes*** 19: 93-108.

c. *Elshanskaia M. V.,* Sobolevskii A. I., Val'dman E. A. and Khodorov B. I. (2000) Interaction of a new adamantane derivative (A-7), a potential antiparkinsonian drug, with NMDA receptor channels. ***Exp. Clinical Pharmacol.*** 64: 18-21. PubMed Central PMID: 11544796.

d. Sobolevsky A. I., *Yelshansky M. V.*, and Khodorov B. I. (2000) Eosine-induced blockade of N-Methyl-D-Aspartate Channels in Acutely Isolated Rat Hippocampal Neurones. ***Molecular Pharmacology*** 57: 334-341. PubMed Central PMID: 10648643.

2. AMPA-subtype iGluRs mediate fast signaling between neurons and contribute to high cognitive processes. Since AMPA receptors are also implicated in numerous neurological disorders, including Alzheimer's disease, amyotrophic lateral sclerosis, epilepsy and ischemia, the ability to regulate them represents an important clinical goal. Better understanding structure and function of AMPA receptors facilitates the design of drugs. As a member of Dr. Wollmuth lab, I have been studying structure and function of AMPA receptors using the recombinant expression systems, site-directed mutagenesis and outside-out and whole-cell patch-clamp recordings. We identified an LBD-TMD linker region critically involved in AMPA receptor desensitization, determined the overall two-fold rotational symmetry of the channel and studied the key features of AMPA receptor gating machinery and the shape and electrostatic potential of the channel pore.

   a. *Yelshansky M. V.*, Sobolevsky A. I., Jatzke C. and Wollmuth L. P. (2004) Block of AMPA Receptor Desensitization by a Point Mutation outside the Ligand-Binding Domain. ***J. Neurosci.*** 24: 4728-4736. PubMed Central PMCID: PMC6729461.

   b. Sobolevsky AI, *Yelshansky MV*, Wollmuth LP. The outer pore of the glutamate receptor channel has 2-fold rotational symmetry. **Neuron**. 2004 Feb 5;41(3):367-78. PubMed PMID: 14766176.

   c. Sobolevsky AI, *Yelshansky MV*, Wollmuth LP. Different gating mechanisms in glutamate receptor and K+ channels. **J Neurosci**. 2003 Aug 20;23(20):7559-68. PubMed Central PMCID: PMC6740752.

   d. Sobolevsky AI, *Yelshansky MV*, Wollmuth LP. State-dependent changes in the electrostatic potential in the pore of a GluR channel. **Biophys J**. 2005 Jan;88(1):235-42. PubMed Central PMCID: PMC1305001.

3. The transient receptor potential (TRP) channels are a superfamily of cation permeable ion channels that are widely known for their role as transducers of sensory modalities, including temperature, taste, olfaction, vision, hearing and touch. TRP channels are crucial for a diverse range of physiological processes, such as neurite outgrowth, hormone secretion and control of vascular tone. Accordingly, mutations or malfunction of TRP channels are associated with numerous human diseases, including cardiovascular, renal, nociceptive and metabolic disorders. As a member of Dr. Sobolevsky team, I have been studying representatives of the vanilloid family of TRP channels. We solved the first crystal structure of TRP channel, $Ca^{2+}$-selective channel TRPV6 that plays vital roles in calcium homeostasis as a $Ca^{2+}$ uptake channel in epithelial tissues and is implicated in development and progression of numerous forms of cancer. Our results provide structural foundations to understand the role of TRP channels in physiology and disease, and provide information necessary for drug design.

   a. Saotome K, Singh AK, *Yelshanskaya MV*, Sobolevsky AI. Crystal structure of the epithelial calcium channel TRPV6. **Nature**. 2016 Jun 23;534(7608):506-11. PubMed Central PMCID: PMC4919205.

   b. McGoldrick LL, Singh AK, Saotome K, *Yelshanskaya MV*, Twomey EC, Grassucci RA, Sobolevsky AI. Opening of the human epithelial calcium channel TRPV6. **Nature**. 2018 Jan 11;553(7687):233-237. PubMed Central PMCID: PMC5854407.

   c. *Yelshanskaya M. V.*, Nadezhdin K. D., Kurnikova M. G. and Sobolevsky A. I. (2021) Structure and Function of the calcium-selective TRP channel TRPV6. ***Journal of Physiology*** 599: 2673-2697. PubMed PMID: PMC7689878.

   d. *Yelshanskaya M. V.* and Sobolevsky A. I. (2022) Ligand-binding sites in vanilloid-subtype TRP channels. ***Frontiers in Pharmacology*** 13: 900623. PubMed Central PMCID: PMC9149226.

4. In synapses, AMPA receptors exist as complexes with numerous regulatory proteins, many of which are transmembrane auxiliary subunits. These subunits not only tightly regulate functional properties of AMPA receptors but also trafficking to the plasma membrane and synaptic localization. Some of the complexes are directly linked to the pathophysiology of neurological and psychiatric disorders and therefore represent an

important drug target. As a member of Dr. Sobolevsky lab, I have been studying assembly, structure and regulation of AMPA receptor-auxiliary subunit complexes. We solved the first structures of the AMPA receptor synaptic complexes with auxiliary subunits type I TARP γ2 or stargazin (STZ), type II TARP γ5 and germline-specific gene 1-like (GSG1L).

a. Twomey EC, *Yelshanskaya MV*, Grassucci RA, Frank J, Sobolevsky AI. Elucidation of AMPA receptor-stargazin complexes by cryo-electron microscopy. **Science**. 2016 Jul 1;353(6294):83-6. PubMed Central PMCID: PMC5125255.
b. Klykov O., Gangwar S. P., *Yelshanskaya M. V*., Yen L. and Sobolevsky A. I. (2021) Structure and desensitization of AMPA receptor complexes with type II TARP γ5 and GSG1L. ***Molecular Cell*** 81: 4771-4783. PubMed Central PMCID: PMC8642297.
c. Twomey E. C., *Yelshanskaya M. V*., Vassilevski A. A. and Sobolevsky A. I. (2018) Mechanisms of Channel Block in Calcium-Permeable AMPA Receptors. ***Neuron*** 99: 956-968. PubMed Central PMCID: PMC6181147.
d. Twomey E. C., *Yelshanskaya M. V*., Grassucci R. A., Frank J. and Sobolevsky A. I. (2017) Structural Bases of Desensitization in AMPA Receptor-Auxiliary Subunit Complexes. ***Neuron*** 94: 569-580. PubMed Central PMCID: PMC5492975.

5. Communication between neurons is mediated by AMPA receptors that open or close their channels for ion conduction in a process termed gating. The two major gating functions are activation, which in response to agonist binding leads to ion channel opening, and desensitization, which results in ion channel closure in the prolonged presence of agonist bound to the receptor. As a member of Dr. Sobolevsky team, I have been studying activation and desensitization gating of AMPA-subtype iGluRs. We succeeded to solve the first structures of the agonist-bound, open and desensitized states of the receptor as well as provided the first structural evidence of iGluR subconductance states. We showed that AMPA receptor desensitization occurs through rupture of the ligand-binding domain dimer interface but can proceed differently depending on the composition of the receptor.

a. *Yelshanskaya MV*, Patel D. S., Kottke C. M., Kurnikova M. G. and Sobolevsky A. I. (2022) Opening of glutamate receptor channel to subconductance levels. **Nature** 605: 172-178. PubMed Central PMCID: PMC9068512.
b. Twomey EC, *Yelshanskaya MV*, Grassucci RA, Frank J, Sobolevsky AI. Channel opening and gating mechanism in AMPA-subtype glutamate receptors. **Nature**. 2017 Sep 7;549(7670):60-65. PubMed Central PMCID: PMC5743206.
c. *Yelshanskaya MV*, Li M, Sobolevsky AI. Structure of an agonist-bound ionotropic glutamate receptor. **Science**. 2014 Aug 29;345(6200):1070-4. PubMed Central PMCID: PMC4383034.
d. *Yelshanskaya M. V*., Singh A. K., Sampson J. M., Narangoda C., Kurnikova M. and Sobolevsky A. I. (2016) Structural Bases of Noncompetitive Inhibition of AMPA-Subtype Ionotropic Glutamate Receptors by Antiepileptic Drugs. ***Neuron*** 91: 1305-1315. PubMed Central PMCID: PMC5033713.

**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: Gangwar, Shanti Pal

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

POSITION TITLE: Post-Doctoral Research Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| Kumaun University, India | M.Sc. | 07/2007 | Biotechnology |
| Jawaharlal Nehru University, India | Ph.D. | 02/2014 | Biophysics/Structural Biology |
| University of Texas Medical Branch (UTMB), USA | Postdoc | 01/2019 | Biophysics/Structural Biology/Neuroscience |
| Columbia University, USA | Postdoc | Current | Biophysics/Structural Biology/Neuroscience |

## A. Personal Statement

My long-term goal is to understand the structural and functional perspective of ion channels involved in neuronal communication and several neurological disorders including Alzheimer's disease, amyotrophic lateral sclerosis, epilepsy, and ischemia. My academic and research training has provided me skills in biophysics, X-ray crystallography, molecular biology, biochemistry, and Cryo-EM. As a graduate student, I learned X-ray crystallography studying transcription factors from human and *Mycobacterium tuberculosis*. As a postdoc under the guidance of **Dr. Gabrielle Rudenko at UTMB,** I was introduced to the scope and importance of neurodevelopmental/neuropsychiatric disorders and I started studying on synaptic proteins. Subsequently, my research findings on the structural and mechanistic details of synapse adhesion molecules (Neuroligin-MDGA1) have suggested strategies to design structure-guided peptides/small molecules modulating protein-protein interactions as therapeutics for neurodevelopmental disorders. Now I am advancing my research further by studying the gating mechanism of neuronal ionotropic glutamate receptors under the supervision of **Dr. Alexander Sobolevsky at Columbia University**. The research outlines a set of career developmental activities such as grant writing, public speaking, management in the lab, mentoring students, and altogether enhancing my abilities in becoming a successful independent investigator. Given the competitive nature of the proposed research area, it will be an excellent opportunity for me to work on the structure-function relationship of ion channels.

Citations:


1. Klykov, OV*., **Gangwar, SP*.,** Yelshanskaya, MV*., Sobolevsky, AI. (2021) Structure and desensitization of AMPA receptor complexes with type II TARP g5 and GSG1L. *Molecular Cell,* (23):4771-4783.e7 (*Equal contribution)
2. Green, MN*., **Gangwar, SP*.,** Michard, E., Simon, AA., Portes, MT., Barbosa-Caro, J., Wudick, MM., Lizzio, MA., Klykov, O., Yelshanskaya, MV., Feijo, JA., and Sobolevsky, AI. (2021) Structure of the Arabidopsis thaliana Glutamate Receptor-Like Channel GLR3.4. *Molecular Cell,* (15):3216-3226.e8 (*Equal contribution)
3. **Gangwar, SP*.,** Green, MN*., Michard, E*., Simon, AA., Feijo, JA., and Sobolevsky, AI. (2019) Structure of the *Arabidopsis* Glutamate Receptor-like Channel GLR3.2 Ligand-Binding Domain. *Structure,* 29(2):161-169.e4 (Cover-page)

4. **Gangwar, SP.,** Zhong, X., Seshadrinathan, S., Chen, H., Machius, M., and Rudenko, G. (2017). Molecular Mechanism of MDGA1: Regulation of Neuroligin 2: Neurexin Trans-synaptic Bridges**.** *Neuron,* 21;94(6):1132-1141.e4.

## B. Positions, Scientific Appointments, and Honors

### Position and Scientific Appointments

2019-Current Postdoctoral Researcher, Columbia University, New York, NY
2014-2019 Postdoctoral Researcher, University of Texas Medical Branch, Galveston, TX

### Honors and Awards

2018 **Best Poster** Prize in a poster presentation at 23rd Annual Sealy Center for Structural Biology Symposium, 28th April 2018, University of Texas Medical Branch, Texas USA.

2010 **Best Poster** Prize in a poster presentation at a 4th International Symposium on "Recent trends in Macromolecular Structure and Function", Jan 21-23, 2010, University of Madras, Chennai, India.

2006 **Junior Research Fellowship** and **Senior Research Fellowship** from Council of Scientific and Industrial Research **(CSIR)** and University Grants Commission (UGC) India and Qualified Graduate Aptitude Test in Engineering **(GATE)** 2006 by Department of Higher Education, MHRD, Government of India.

### Mentorship

Laura Y. Yen (Ph.D. candidate, 2021-current)
Jeffery Khau (Undergraduate, 2020-current)
Marriah N. Green (Ph.D. candidate, 2019-current)
Hubert Lee (Ph.D. candidate, 2019)
Srinivasan Sundararaj (Undergraduate, 2010)
Farhana Yasmin (Summer intern, 2008)

### AD HOC Reviewer for Scientific Journals

Biochemistry, eLife, Schizophrenia, PLOS One

## C. Contributions to Science

**1.** My graduate research contributions focused on the transcription factors from human and *Mycobacterium tuberculosis*. The outcomes of the research were of significate importance that provided insights that full-length Erg is a highly nonglobular protein, which is subjected to DNA binding autoinhibition mechanism. The DNA binding domain (ETS domain) of human Erg is a winged helix-turn-helix and binds to DNA using its particular helix. Modulation of this DNA-Protein interaction by small molecules/peptides may open up new therapeutic avenues in the field of prostate cancer. The *Mycobacterium tuberculosis* transcriptional regulator EspR contains an N-terminal helix–turn–helix DNA binding domain and a C-terminal dimerization domain. Structural study and comparison of EspR in different crystal forms indicated that the N-terminal helix–turn–helix domain of EspR acquires a rigid structure in different crystal forms. However, significant structural differences were observed in the C-terminal domain of EspR. The interaction, stabilization energy and buried surface area analysis of EspR in the different crystal forms have provided information about the physiological dimer interface of EspR.

a. Sharma, R*., **Gangwar, SP*.,** Saxena, A.K. (2019) Comparative structure analysis of the ETSi domain of ERG3 and its complex with the E74 promoter DNA sequence. *Acta Crystallogr. Section F Biol. Crystallogr,*75(Pt 5):397-398 (*Equal contribution)
b. **Gangwar, SP.,** Meena, SR., and Saxena, AK. (2014). Comparison of four different crystal forms of Mycobacterium tuberculosis ESX-1 secreted protein regulator, EspR. *Acta Crystallogr. Section F Biol. Crystallogr,* 70(Pt 4):433-7
c. **Gangwar, SP.,** Dey, S., and Saxena, AK. (2012). Structural modeling and DNA binding auto-inhibition analysis of Ergp55, a critical transcription factor in prostate cancer. *PLoS ONE*, 7(6): e39850

**2.** During my postdoc at UTMB, Texas, I have studied the structural perspective of the synapse-related, organizers/adhesion, proteins critical in brain development using X-ray crystallography, and other biophysical methods. The outcomes of this research focus on how a synapse organizer, MDGA1, interacts with Neuroligin and regulates the interaction with Neurexin and Neuroligin. On the basis of this structural information, we designed small peptides modulating the Neuroligin and MDGA1 interaction and tested their efficacy by related biophysical methods. The next goal of this project is to increase the binding affinity of these peptides by optimizing the peptide sequence making them protease-resistant and then test *in vivo* / in animal models to explore the therapeutic potential to recalibrate excitation-inhibition imbalances at the synapse.

a. Fan, S., **Gangwar, SP.,** Machius, M., and Rudenko, G. (2020) Interplay between hevin, SPARC, and MDGAs: modulators of neurexin-neuroligin trans-synaptic bridges. *Structure,* 29(7):664-678.e6
b. **Gangwar, SP.,** Zhong, X., Seshadrinathan, S., Chen, H., Machius, M., and Rudenko, G. (2017). Molecular Mechanism of MDGA1: Regulation of Neuroligin 2: Neurexin Trans-synaptic Bridges**.** *Neuron,* 21;94(6):1132-1141.e4.
c. Kim, MJ., Biag, J., Fass, DM., Lewis, MC., Zhang, Q., Fleishman, M., **Gangwar, SP.**, Machius, M., Fromer, M., Purcell, SM., Premont, RT., McCarroll, SA., Rudenko, G., Scolnick, EM., Haggarty, SJ. Functional analysis of rare variants found in schizophrenia implicates a critical role for GIT1-PAK3 signaling in neuroplasticity. *Molecular Psychiatry*, 2017, 22(3):417-429.

**3.** Glutamate receptor-like channels (GLR) are found in various plant lineages, including moss, rice, tomato, and Arabidopsis. Physiological studies uncovered many functions of GLR in plants, including regulating nitrogen and carbon metabolism, water balance, ion distribution, and response to environmental stress. However, the molecular bases of these GLR functions have remained an enigma. It was unclear, for example, whether GLRs from a channel pore for ion permeation or auxiliary subunits modulate them. Similarly, it was not easy to establish the structural and functional relationship between GLRs and their mammalian counterparts iGluRs. In our research, we have solved the long-awaited puzzle of the structural organization of plant GLRs. We discovered that plant GLRs are tetrameric assemblies of four identical or similar subunits reminiscent of animal iGluRs and have a 3-layer architecture, which includes amino-terminal domains (ATDs), ligand-binding domains (LBDs), and ion channel-forming transmembrane domains (TMDs). Furthermore, the binding of glutathione to the ATD and Glutamate to the LBD suggested that, unlike iGluRs, GLRs involve both types of extracellular domains in the modulation of the ion channel activity. We also discovered that the presence of the auxiliary subunit cornichon (CNIH) is critical for GLR function.

a. **Gangwar, SP*.,** Green, MN*., Yelshanskaya, MV., and Sobolevsky, AI. (2021) Purification and cryo-EM structure determination of Arabidopsis thaliana GLR3.4. *STAR Protocols,* (4):100855.
b. Green, MN*., **Gangwar, SP*.,** Michard, E., Simon, AA., Portes, MT., Barbosa-Caro, J., Wudick, MM., Lizzio, MA., Klykov, O., Yelshanskaya, MV., Feijo, JA., and Sobolevsky, AI. (2021) Structure of the Arabidopsis thaliana Glutamate Receptor-Like Channel GLR3.4. *Molecular Cell,* (15):3216-3226.e8 (*Equal contribution)
c. **Gangwar, SP*.,** Green, MN*., Michard, E*., Simon, AA., Feijo, JA., and Sobolevsky, AI. (2019) Structure of the *Arabidopsis* Glutamate Receptor-like Channel GLR3.2 Ligand-Binding Domain. *Structure,* 29(2):161-169.e4 (Cover-page)

**4.** AMPA receptors (AMPARs) mediate the majority of excitatory neurotransmission. Auxiliary subunits regulate their surface expression, trafficking, gating, and pharmacology. Of the two types of TARP auxiliary subunits, type I TARPs assume activating roles. In contrast, type II TARPs serve a generally suppressive function. We have recently solved the cryo-EM structures of GluA2 AMPAR in complex with type II TARP γ5, which reduces steady-state currents, increases single-channel conductance, and slows recovery from desensitization. GluA2-γ5 complex shows maximum stoichiometry of two TARPs per AMPAR tetramer, different from type I TARPs but reminiscent of the auxiliary subunit GSG1L. Regulation of AMPAR function depends on the interaction of its ligand-binding domain (LBD) with the γ5 and GSG1L head domains. The closed-state structures of GluA2-g5 and GluA2-GSG1L complexes appear similar, but their desensitized-state structures are different. While desensitization of both GluA2-GSG1L and GluA2-γ5 complexes is accompanied by rupture of LBD dimer interface, GluA2-γ5 but not GluA2-GSG1L LBD dimers remain 2-fold symmetric. Different structural architectures and desensitization mechanisms of complexes with auxiliary subunits endow AMPARs with broad functional capabilities.

a. Klykov, OV*., **Gangwar, SP*.,** Yelshanskaya, MV*., Sobolevsky, AI. (2021) Structure and desensitization of AMPA receptor complexes with type II TARP g5 and GSG1L. *Molecular Cell,* (23):4771-4783.e7 (*Equal contribution)

**3. Symposia/Conference paper presentations**

a. **Gangwar SP.,** Zhong X., Seshadrinathan S., Chen H., Machius M., and Rudenko G. Structural Insights into The Regulation of Neuroligin2: Neurexin Trans-Synaptic Bridge by MDGA1. 27th Annual Keck Center Research Conference: Innovations in Interdisciplinary Neuroscience, October 27, 2017, Houston, Texas.
b. **Gangwar, SP.,** Structural and functional dissection of the human Ergp55 oncoprotein.42nd National seminar on crystallography and international workshop on the application of X-ray diffraction for drug discovery, November 21-23, 2013, New Delhi- India.
c. **Gangwar, SP.,** Meena, SR., and Saxena, AK. Structure and functional analysis of key proteins involved in Mtb ESX-1 protein export pathway: potential drug targets. National symposium of microbes in Health and Agriculture, March 12-13, 2012, JNU, New Delhi, India. (Best poster presentation award).
d. **Gangwar, SP.,** Meena, SR., and Saxena, AK. Structure analysis of ERG oncoprotein: A potential target to develop a prostate cancer drug. International Symposium on Novel Strategies for Targeted Prevention and Treatment of Cancer, JNU, India. 2008

**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: Yen, Laura Yaunhee

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

POSITION TITLE: PhD Candidate

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 |
|---|---|---|---|
| Georgia Institute of Technology | BS | 08/2010 | Biology |
| Georgia Institute of Technology | MS | 12/2012 | Biology |
| Columbia University | MA | 05/2022 | Physiology and Cellular Biophysics |

**A. Personal Statement**

My first exposure to biomedical research was during my senior year of undergraduate at the Georgia Institute of Technology, studying an enzyme involved in the production of inflammatory mediators by a method called two-dimensional crystallography. Since then, I've obtained my MS degree in 2012, furthered my scientific training with 3 years of work at the National Institutes of Health (Bethesda, MD) followed by another 4 years at the Simons Electron Microscopy Center (New York, NY), both in research support roles with a focus on using electron cryo-microscopy (cryo-EM) to elucidate the three-dimensional structure of target proteins. My research experience prior to entering the PhD program at Columbia University has resulted in 14 co-authored publications.

My long-term career goals are to use interdisciplinary research tools to address the biochemical and molecular details of human health related disease processes. In particular, I want to leverage my extensive background in cryo-EM to understand the atomic level details of pharmacological inhibition and/or activation, disease mutations, and protein-protein interactions that are involved in and dictate disease onset and progression. The research proposed in this application focuses on elucidating functional states of ionotropic glutamate receptors (iGluRs), ligand-gated ion channels that mediate excitatory neurotransmission in the central nervous system. Using interdisciplinary approaches like cryo-EM and electrophysiology, I want to investigate iGluR biology in their more-native, heteromeric state, probing the modulatory effects of auxiliary membrane protein binding on channel gating. Findings from this research will provide novel insights into the structural basis for excitatory neurotransmission across a wide range of neurological disorders, which would be critical in the design of more specific and efficacious therapeutics. I am uniquely qualified to carry out this F31 fellowship: as a non-traditional student, going back to school to pursue my PhD after working for almost 8 years, and also being the first in my family to pursue a doctoral degree, I have the skills, expertise, motivation and potential needed to carry out the proposed work. The specialized and focused training environment and the mentorship of Dr. Alexander Sobolevsky will provide the necessities for my development as a successful research scientist. Overall, I believe that my research setting alongside my proposed training plan will provide the foundation for my long-term goal as a research scientist.

During my undergraduate education, there were some circumstances that impacted my academic performance. My parents are owners of a small dry-cleaning business and as a family operated business, it has required me to work part-time for most of my young adult life, including when I was in university. Obviously, this took time away from my studies. There was one extraordinary event that was particularly disruptive: during my sophomore year my father suffered from a collapsed lung, which was further complicated by pneumonia in both lungs, that kept him hospitalized for two weeks and bedridden for another. During those three weeks my mother, sister and I took shifts to take care of the store as well as visit and care for my father. Through most of my university education I struggled to form a solid academic routine and this reflects clearly in my low grade-point average. It was actually my senior research experience that shifted my learning patterns: working in a research environment helped concretize scientific theories/principles for me, helping me to engage more in my education. By the end of my senior year, my grades improved dramatically, and I achieved the academic accolades of Dean's List (GPA

> 3.0) and Faculty Honors (GPA = 4.0) for the last two semesters of my undergraduate. Since then, my academic record has improved significantly and I do not consider it an impediment to my current research or future career goals.


1. Vallese F, Kim K, Yen LY, Johnston JD, Noble AJ, Calì T, Clarke OB. Architecture of the human erythrocyte ankyrin-1 complex. Nat Struct Mol Biol. 2022 Jul;29(7):706-718. PubMed PMID: 35835865.
2. Park J, Zuo H, Frangaj A, Fu Z, Yen LY, Zhang Z, Mosyak L, Slavkovich VN, Liu J, Ray KM, Cao B, Vallese F, Geng Y, Chen S, Grassucci R, Dandey VP, Tan YZ, Eng E, Lee Y, Kloss B, Liu Z, Hendrickson WA, Potter CS, Carragher B, Graziano J, Conigrave AD, Frank J, Clarke OB, Fan QR. Symmetric activation and modulation of the human calcium-sensing receptor. Proc Natl Acad Sci U S A. 2021 Dec 21;118(51) PubMed Central PMCID: PMC8713963.
3. Klykov O, Gangwar SP, Yelshanskaya MV, Yen L, Sobolevsky AI. Structure and desensitization of AMPA receptor complexes with type II TARP γ5 and GSG1L. Mol Cell. 2021 Dec 2;81(23):4771-4783.e7. PubMed Central PMCID: PMC8642297.
4. Kim LY, Johnson MC, Schmidt-Krey I. Cryo-EM in the study of membrane transport proteins. Compr Physiol. 2012 Jan;2(1):283-93. PubMed PMID: 23728976.


**B. Positions, Scientific Appointments and Honors**

**Positions and Scientific Appointments**

| | |
|---|---|
| 2020 - | Graduate Research Assistant, Columbia University, Physiology and Cellular Biophysics, New York, NY |
| 2016 - 2020 | Staff Scientist, New York Structural Biology Center, Simons Electron Microscopy Center, New York, NY |
| 2013 - 2016 | Biologist, National Institutes of Health, National Heart, Lung and Blood Institute, Bethesda, MD |
| 2012 - 2013 | Graduate Research Assistant, Case Western Reserve University, Cleveland, OH |
| 2010 - 2012 | Graduate Research Assistant, Georgia Institute of Technology, Atlanta, GA |

**Honors**

| | |
|---|---|
| 2005 - 2007 | Hope Scholarship, Georgia State University |
| 2015 | Distinguished Achievement Award, Kelly Government Services |
| 2010 | Dean's List, Georgia Institutes of Technology |
| 2010 | Faculty Honors, Georgia Institute of Technology |

**C. Contribution to Science**

1. Two-dimensional crystallography is a method used for high-resolution protein structure determination using the electron microscope. Using two-dimensional crystallography, we studied leukotriene C4 synthase, a membrane protein that converts leukotriene A4 and glutathione to create leukotriene C4. Leukotrienes have been implicated as mediators of inflammation and inflammatory conditions like anaphylaxis and bronchial asthma. Our lab produced a number of book chapters and reviews detailing the specifics of two-dimensional crystallization of membrane proteins.

   a. Johnson MC, Dreaden TM, Kim LY, Rudolph F, Barry BA, Schmidt-Krey I. Two-dimensional crystallization of membrane proteins by reconstitution through dialysis. Methods Mol Biol. 2013;955:31-58. PubMed PMID: 23132054.
   b. Dreaden TM, Metcalfe M, Kim LY, Johnson MC, Barry BA, Schmidt-Krey I. Screening for two-dimensional crystals by transmission electron microscopy of negatively stained samples. Methods Mol Biol. 2013;955:73-101. PubMed PMID: 23132056.
   c. Kim LY, Johnson MC, Schmidt-Krey I. Cryo-EM in the study of membrane transport proteins. Compr Physiol. 2012 Jan;2(1):283-93. PubMed PMID: 23728976.

2. Single particle electron cryo-microscopy (cryo-EM) is a powerful technique for the high-resolution structure determination of challenging targets, especially those intractable to x-ray crystallization. At the Simons Electron Microscopy Center, we worked on methods development projects to optimize cryo-EM data

collection and sample characterization, with the overall goal of improving utilization of expensive and precious EM time. This included protocols on optimizing a cryo-EM workflow using test specimen aldolase, using beam-image shift for increasing data throughput, using energy filters and aperture scattering to routinely determine ice thickness, and routine sample characterization using tomography.

a. Cheng A, Eng ET, Alink L, Rice WJ, Jordan KD, Kim LY, Potter CS, Carragher B. High resolution single particle cryo-electron microscopy using beam-image shift. J Struct Biol. 2018 Nov;204(2):270-275. PubMed Central PMCID: PMC6163078.
b. Rice WJ, Cheng A, Noble AJ, Eng ET, Kim LY, Carragher B, Potter CS. Routine determination of ice thickness for cryo-EM grids. J Struct Biol. 2018 Oct;204(1):38-44. PubMed Central PMCID: PMC6119488.
c. Kim LY, Rice WJ, Eng ET, Kopylov M, Cheng A, Raczkowski AM, Jordan KD, Bobe D, Potter CS, Carragher B. Benchmarking cryo-EM Single Particle Analysis Workflow. Front Mol Biosci. 2018;5:50. PubMed Central PMCID: PMC6009202.
d. Noble AJ, Dandey VP, Wei H, Brasch J, Chase J, Acharya P, Tan YZ, Zhang Z, Kim LY, Scapin G, Rapp M, Eng ET, Rice WJ, Cheng A, Negro CJ, Shapiro L, Kwong PD, Jeruzalmi D, des Georges A, Potter CS, Carragher B. Routine single particle CryoEM sample and grid characterization by tomography. Elife. 2018 May 29;7 PubMed Central PMCID: PMC5999397.

3. $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) represent the fastest sub-type of ionotropic glutamate receptors (iGluRs) that mediate the majority of excitatory neurotransmission in the central nervous system. The majority of neuronal AMPARs function as central elements of synaptic complexes being surrounded by auxiliary subunits, which regulate receptor trafficking, scaffolding, stability, turnover, and various physiological responses. Using cryo-EM, we solved the structure of GluA2 AMPAR in complex with type II transmembrane AMPAR regulating protein (TARP)-$\gamma$5, uncovering a stoichiometric binding ratio of two TARPs per AMPAR tetramer (2:1), which is reminiscent of another auxiliary subunit, germ cell-specific gene 1-like (GSG1L). While the closed states of GluA2-$\gamma$5 and GluA2-GSG1L were similar, we found that desensitized states of the two complexes were distinct, stressing the unique functional roles of different auxiliary subunits and their modulation on AMPAR gating.

a. Klykov O, Gangwar SP, Yelshanskaya MV, Yen L, Sobolevsky AI. Structure and desensitization of AMPA receptor complexes with type II TARP γ5 and GSG1L. Mol Cell. 2021 Dec 2;81(23):4771-4783.e7. PubMed Central PMCID: PMC8642297.

**D. Scholastic Performance**

| YEAR | COURSE TITLE | GRADE |
|---|---|---|
| | GEORGIA STATE UNIVERSITY | |
| 2006 | General Chemistry I | B+ |
| 2006 | Chem I Concept Development | A |
| 2006 | Critical Thinking | A |
| 2007 | Principles of Biology I | B |
| 2007 | Chem II Concept Development | B |
| 2007 | General Chemistry II | A- |
| 2007 | Calculus of One Variable I | C |
| 2007 | Calculus of One Variable II | C |
| 2007 | Principles of Biology I | B |
| 2007 | Microbiology & Public Health | C |
| 2007 | Principles of Physics I | B |
| | GEORGIA INSTITUTE OF TECHNOLOGY - BS | |
| 2008 | Genetics | C |
| 2008 | Genetics Laboratory | C |
| 2008 | Inorganic Chemistry I | D |
| 2008 | Inorganic Chemistry Lab I | C |

| YEAR | COURSE TITLE | GRADE |
|---|---|---|
| 2008 | Organic Chemistry I | C |
| 2008 | Linear Algebra for Calc | C |
| 2008 | Intro Physics II | D |
| 2008 | Cell Biology | D |
| 2008 | Organic Chemistry II | B |
| 2009 | Synthesis Lab I | B |
| 2009 | Intro to Computing | C |
| 2009 | Math Models in Biol | B |
| 2009 | Anatomy & Physiology | C |
| 2009 | Animal Physiology | B |
| 2010 | Immunology & Immunochem | B |
| 2010 | Eukaryotic Mol Genetics | C |
| | GEORGIA INSTITUTE OF TECHNOLOGY – MS | B |
| 2010 | Biochemistry I | A |
| 2010 | Cancer Biol/Tech | A |
| 2011 | Macromolecular Structure | B |
| 2011 | Enzymology and Metabolism | |
| | COLUMBIA UNIVERSITY – PHD | A- |
| 2020 | Biochemistry, Molecular, and Cell Biology I | B |
| 2020 | Molecular Biophysics | A- |
| 2021 | Mechanisms in Human Disease | B |
| 2022 | Biochemistry, Molecular, and Cell Biology I | A- |
| 2022 | Statistics for Basic Science | |