BIOGRAPHICAL SKETCH

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NAME: Sobolevsky, Alexander

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

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
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

My lab studies structure and function of ion channels, including ionotropic glutamate receptors (iGluRs) and transient receptor potential (TRP) channels, using a combination of biochemical and biophysical methods and cryo-electron microscopy (cryo-EM) in particular. 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 channel function, including patch-clamp, double-electrode voltage-clamp and single-channel recordings as well as 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, open and desensitized 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. We also solved the first structure of a plant glutamate receptor-like channel (GLR). My lab solved the first crystal structure of a TRP channel (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. We then solved structures of TRPV3 in temperature-dependent closed, intermediate, and open states, which for the first time uncovered the structural basis of TRP channel activation by temperature. We also solved the first structure of a TRP channel from alga and the first structures of two (human and squirrel) out of three (plus rat) orthologs of TRPV1 for which structures are available. We solved structures of TRPM7 in different conformations and proposed the mechanisms of ligand-induced and spontaneous opening as well as inhibition of this channel. For many TRP channels, we solved structures in complex with different agonists or antagonists and proposed the mechanisms of activation and inhibition, respectively. 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)
01/12/2017-12/31/2027
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

R37 NS083660
Sobolevsky (PI)
09/30/2013-07/31/2029
Structure and Function of Ionotropic Glutamate Receptors

R01 NS107253
Sobolevsky (PI)
08/01/2018-03/31/2028
Single-particle cryo-EM characterization of AMPA receptor functional states

HFSP Research Grant Award, Human Frontier Science Program
Sobolevsky (co-PI)
10/01/2024-09/31/2027
Understanding the molecular basis of animal cold thermosensation

Citations:

1. Gangwar S. P., Yelshanskaya M. V., Nadezhdin K. D., Yen L. Y., Newton T. P., Aktolun M., Kurnikova M. G., and *Sobolevsky A. I.* (2024) Kainate receptor channel opening and gating mechanism. ***Nature*** 630: 762-768. PMCID: PMC11186766.
2. 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.
3. 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.
4. 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.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2024 -	Professor, Columbia University, New York, NY
2017 - 2024	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

2024	Javits Neuroscience Investigator Award, NINDS/NIH
2024	HFSP grant award from the Human Frontier Science Program
2024	Keynote speaker at 9th German Pharm-Tox Summit, München, Germany
2023	Keynote speaker at Three-Dimensional Electron Microscopy GRS, Newry, ME
2023	Keynote speaker at XXIX Symposium on Bioinformatics and Computer-Aided Drug Discovery, Moscow, Russia

2017	Amgen Young Investigator Award, Amgen
2017	Keynote speaker at Cryo-Electron Microscopy Conference RICCEM2017, MSU, Moscow
2015	Irma T. Hirschl Career Scientist Award, Irma T. Hirschl Trust
2015	Future of Biophysics Symposium Speaker, Biophysical Society 59th Annual Meeting, Baltimore
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 basis of TRPV6 allosteric regulation and calcium-induced calmodulin-mediated inactivation. We also solved the first structures of TRPV3 and determined structural basis 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

NAME	POSITION TITLE
Yelshanskaya, Maria V.	Research Assistant
eRA COMMONS USER NAME (credential, e.g., agency login) MVYELSH	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Moscow Institute of Physics and Technology	B.S.	06/97	Bioengineering
Moscow Institute of Physics and Technology	Ph.D.	11/01	Biophysics
State University of New York at Stony Brook	Postdoctoral	08/04	Physiology and Neuroscience

A. Personal Statement

My work has focused on understating the complex regulation of the ionotropic glutamate receptors (iGluRs) ion channel gating. I started to address this problem while being undergraduate student in Moscow Institute of Physic and Technology in the B.I. Khorov lab. During my PhD study at the Moscow Institute of Physics and Technology, I carried out electrophysiological studies of NMDA subtype iGluRs in hippocampal neurons acutely isolated from the rat brain and studied interaction of NMDA receptor ion channels with blockers and modulators such as arachidonic acid. After I finished my PhD study, 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 to work on the structure and function of ionotropic glutamate receptors using a combination of biophysical and biochemical approaches including X-ray crystallography. Dr. Sobolevsky's experience in crystallizing the first full length structure of iGluR as well as my enthusiasm and extensive expertise in iGluR structure and function are the best assets to successful completion of this project. Specifically, I plan to determine structures of iGluR in different conformational states and with different ligands and to study their function using mutagenesis and electrophysiology. My future research interests lie in investigating the molecular mechanisms and regulation of ion channel conduction using a combination of biochemical and biophysical approaches. My versatile training provides me with strong biophysical, biochemical, molecular and structural biology background indispensable to successfully achieve my research goals.

Technical skills

Electrophysiology
Patch-clamp recordings in whole cell and outside-out configurations (neurons, HEK cell lines), fast solution-exchange techniques (piezo and magnetic devices), multi-channel perfusion systems.

Molecular biology
PCR, DNA restriction analysis, subcloning of DNA fragments, DNA sequencing, site-directed mutagenisis, chimera construction, RNA synthesis, RNA and DNA purification, agarose gel electrophoresis, spectrophotometry.

Cell culture and Protein expression: Worked with a wide range of mammalian cell lines, both adherent and in suspension. Generated multiple stable cell lines by expressing proteins using adenovirus and transient expression systems in mammalian cells. Expressed proteins using baculovirus in insect cells. Acute isolation of neurons from brain (rats), defolliculation and RNA injection of *Xenopus laevis* oocytes.

Biochemistry and crystallography
Membrane protein purification and crystallization, affinity and size-exclusion chromatography, SDS PAGE and western blotting, crystallography robotics (Mosquito, Rock Imagers), experience working at the APS and NSLS beamlines.

Data analysis and computation
Kinetic modelling (receptor activation, desensitisation and inhibition; ion channel block); mathematical calculations and statistics (Igor Pro, Origin, Excel); image processing and graphics (Photoshop, Canvas, Nikon software); DNA construct design and analysis (DNA strider, Vector NTI, Prism).

B. Positions and Honors

Positions and Employment

1997-1998 – *Research Technician.*
Dept. Neurophysiology, Bogomoletz Institute of Physiology, Kiev, Ukraine.

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.

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.

2004-2010 – Maternity leave.

2010-present – Research Associate, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY. Structure and function of iGluRs. Supervisor – Alexander I. Sobolevsky.

Honors

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).
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).
2000 - Young Scientist Award, 2-d Pathology Congress, Moscow, Russia.
2000-2001 - International Soros Science Education Program Grant (# A2001-358), Moscow, Russia
2001 - Young Scientist Award from the Russian Foundation for Basic Research.
2001 - International Brain Research Organization Travel Grant for participation in the 31st Annual Meeting of the Society for Neuroscience (San Diego, California, November 2001).
2001-2002 - NSF-NATO Fellowship (#10108434 1 22060).

Teaching

1996-1997 – Teaching chemistry, "Phystekh-college", Dolgoprudny, Russia.
1993-2001 – Teaching physics and mathematics, Correspondence courses, MIPT, Dolgoprudny, Russia.

2011-2020 – Supervising rotation students, undergraduates, graduate students and postdocs on their projects.

C. Selected Peer-reviewed Publications

1. 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.
2. Sobolevsky A. I. and **Yelshansky M. V.** (2000) The trapping block of NMDA-receptor channels in acutely isolated rat hippocampal neurones. *J. Physiol.* **526:** 493-506.
3. **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. (in Russian)* **64:** 18-21.
4. Vergun O., Sobolevsky A. I., **Yelshansky M. V.,** Keelan J., Khodorov B. I. and Duchen M. R. (2001) Exploration of the role of reactive oxygen species in glutamate neurotoxicity in rat hippocampal neurones in culture. *J. Physiol.* **531:** 147-163.
5. **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. *Biolog. Membranes (in Russian)* **19:** 93-108.
6. Sobolevsky A. I., **Yelshansky M. V.** and Wollmuth L. P. (2003) Different Gating Mechanisms in Glutamate Receptor and K^+ Channels. *J. Neurosci.* **23:** 7559-7568.
7. Sobolevsky A. I., **Yelshansky M. V.** and Wollmuth L. P. (2004) The Outer Pore of the Glutamate Receptor Channel has Two-fold Rotational Symmetry. *Neuron* **41:** 367-378 (Cover; Preview by Y. Stern-Bach. AMPA Receptor Activation: Not a Square Dance. *Neuron* **41:** 309-311).
8. **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.
9. Sobolevsky A. I., **Yelshansky M. V.** and Wollmuth L. P. (2005) State-dependent changes in the electrostatic potential in the pore of a GluR channel. *Biophys. J*. Jan 88(1):235-42.
10. Sobolevsky A. I., Prodromou M. L., **Yelshansky M. V.** and Wollmuth L. P. (2007) Subunit-specific Contribution of Pore-Forming Domains to NMDA Receptor Channel Structure and Gating. *J. Gen. Physiol.* 129: 509-525.
11. **Yelshanskaya M. V**., Li M. and *Sobolevsky A. I.* (2014) Structure of an agonist-bound ionotropic glutamate receptor. ***Science*** 345: 1070-1074.
12. **Yelshanskaya M. V**., Saotome K., Singh A. K. and *Sobolevsky A. I*. (2016) Probing Intersubunit Interfaces in AMPA-subtype Ionotropic Glutamate Receptors. ***Scientific Reports*** 6: 19082.
13. Saotome K., Singh A. K., **Yelshanskaya M. V**. and *Sobolevsky A. I*. (2016) Crystal structure of the epithelial calcium channel TRPV6. ***Nature*** 534: 506–511.
14. Twomey E. C., **Yelshanskaya M. V**., Grassucci R. A., Frank J. and *Sobolevsky A. I.* (2016) Elucidation of AMPA receptor-stargazin complexes by cryo-electron microscopy. ***Science*** 353: 83-86.
15. **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 (**Preview** by Regan M. C. and Furukawa H. Deeper Insights into the allosteric modulation of ionotropic glutamate receptors. ***Neuron*** 91: 1187-1189).
16. **Yelshanskaya M. V.**, Mesbahi-Vasey S., Kurnikova M. and *Sobolevsky A. I*. (2017) Role of the Ion Channel Extracellular Collar in AMPA Receptor Gating. ***Scientific Reports*** 7: 1050.
17. 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.
18. Twomey E. C., **Yelshanskaya M. V.**, Grassucci R. A., Frank J. and *Sobolevsky A. I.* (2017) Channel opening and gating mechanism in AMPA-subtype glutamate receptors. ***Nature*** 549: 60-65.
19. McGoldrick L. L., Singh A. K., Saotome K., **Yelshanskaya M. V**., Twomey E. C., Grassucci R. A. and *Sobolevsky A. I.* (2018) Opening of the Human Epithelial Calcium Channel TRPV6. ***Nature*** 553: 233-237.
20. 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 (**Cover**).
21. Twomey E. C., **Yelshanskaya M. V**. and *Sobolevsky A. I.* (2019) Transmembrane AMPA receptor regulatory protein complexes. Submitted to ***Journal of General Physiology*** 151 (12): 1347–1356.
22. **Yelshanskaya MV**, Nadezhdin KD, Kurnikova MG, Sobolevsky AI. (2020) Structure and function of the calcium-selective TRP channel TRPV6. ***Journal of Physiology***: (00) 1-25.

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 USERNAME (credential, e.g., agency login): SPGANGWA

POSITION TITLE: Associate 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, USA	Postdoc	01/2019	Biophysics/Structural Biology
Columbia University, USA	Postdoc	06/2022	Biophysics/Structural Biology
Columbia University, USA	Associate Research Scientist	07/2023-current	Biophysics/Structural Biology

A. Personal Statement

I am an Associate Research Scientist working under the mentorship of Prof. Alexander Sobolevsky in the Department of Biochemistry and Molecular Biophysics at Columbia University Irving Medical Center, New York. My primary research focuses on understanding the structural and functional complexities of iGluR subtypes, specifically Kainate and AMPA receptors, and their roles in brain function and neuropathologies. To achieve this, I utilize a multidisciplinary approach combining biochemical and biophysical techniques, with a particular emphasis on cryo-electron microscopy (cryo-EM). I specialize in determining the structures of integral membrane proteins and synapse organizers using techniques such as X-ray crystallography and cryo-EM, as well as biophysical methods like electrophysiology, protein engineering, and kinetic modeling. Leveraging this expertise, I investigate the mechanisms of iGluR activation, their modulation by synaptic components, and their inhibition by potential drug candidate molecules. Over the course of my career, I have made significant contributions in the iGluR field, including elucidating the molecular mechanism of AMPA receptor regulation by type II auxiliary protein γ5, providing the first structural evidence of modulation of Kainate receptor by the antiepileptic drug Perampanel, demonstrating the first structural evidence of endogenous polyamine binding to the AMPA receptor-cornichon-2-γ5 complex, solving the first open state structure of Kainate receptor and describing their gating mechanism, and their modulation by ion channel blockers. Currently, I am advancing my research by delving deeper into the modulation and signaling mechanisms of Kainate receptors in the brain. This research seeks to uncover their roles in essential brain functions, neural activity, synaptic plasticity, and neurodevelopmental disorders like autism spectrum disorder. My goal is to better understand how Kainate receptor dynamics contribute to nervous system development and pathology, paving the way for novel therapeutic development and interventions. In summary, I possess the expertise, leadership, training, and motivation required to successfully carry out the proposed research and make meaningful contributions to the field.

Ongoing and recently completed projects that I would like to highlight include:
R21 NS139087
Gangwar (PI)
07/01/2024-06/30/2026
Molecular Mechanism of Kainate Receptors

Citations:

1. **Gangwar SP.**, Yelshanskaya MV, Nadezhdin KD, Yen LY, Newton TP, Aktolun M, Kurnikova MG, and Sobolevsky AI. Kainate receptor channel opening and gating mechanism. *Nature* (2024). PMID: 38778115.
2. **Gangwar SP.,** Yen LY., Yelshanskaya MV., Korman, A., Jones, DR., and Sobolevsky AI. (2023) Modulation of GluA2–γ5 synaptic complex desensitization, polyamine block and antiepileptic perampanel inhibition by auxiliary subunit cornichon-2. *Nat Struct Mol Biol.* PMID: 37653241.
3. **Gangwar SP.,** Yen LY., Yelshanskaya MV., Sobolevsky AI. (2023) Positive and negative allosteric modulation of GluK2 kainate receptors by BPAM344 and antiepileptic perampanel. *Cell Rep.* Feb 21;42(2):112124. PMID: 36857176.
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,* PMID: 28641112.

Positions, Scientific Appointments, and Honors

Position and Scientific Appointments

2022 - 2023 Associate Research Scientist, Columbia University, New York, NY
2019 - 2022 Postdoctoral Researcher, Columbia University, New York, NY
2014 - 2019 Postdoctoral Researcher, University of Texas Medical Branch, Galveston, TX

Honors

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.

B. 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 significant importance, providing insights into the fact that full-length Erg is a highly non-globular protein, which is subjected to DNA binding auto inhibition 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. PMID: 30279318.
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. PMID: 24699733.
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. PMID: 22761914.

2. Neuroligins (Nrxn) and Neurexins (Nlgn) facilitate synapse formation and stabilization by forming a trans-synaptic bridge across the synaptic cleft. The cell-surface molecules MDGA1/2 modulate the trans-synaptic adhesion between Nrxn and Nlgn. In this study, we have uncovered the molecular mechanism of Nrxn-Nlgn regulation by MDGA1. We observed that MDGA1 binds to the same site on Nlgn where Nrxn binds, suggesting that MDGA1 regulate Nrxn-Nlgn bridge formation by sterically blocking Nrxn access to Nlgn. In a follow-up study, we investigated the interaction between MDGA, Hevin, and SPARC, as well as their role in modulating the Nrxn-Nlgn trans-synaptic bridge. Our findings revealed that Hevin, which promotes the Nrxn-Nlgn interaction, competes with MDGA1 for binding sites on Nlgn, ultimately destabilizing the Nrxn-Nlgn trans-synaptic bridge (Fan et al., Structure 2021). Finally, the results suggest that the structure-function relationships of Hevin and SPARC support both *synergistic, antagonistic*, and *independent* roles in shaping synaptic signaling.

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. PMID: 33535026.
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. PMID: 28641112.
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. PMID: 27457813.

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. PMID: 34647037.
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. PMID: 34161757.
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. PMID: 33027636. **(Cover-page)**

4. Gating in iGluRs controls the opening and closing of the ion channel pore in response to glutamate binding, ultimately regulating the flow of ion across the neuronal membrane. This process determines the precision molecular operation of iGluRs underlying propagation of excitatory signals throughout the brain. Using time-resolve cryo-electron microscopy, I solved the first structure of KAR in the activated state and provided the first molecular description of their activation gating mechanism[1]. In this study, a combination of two positive allosteric modulators, BPAM344 and Concanavalin A, synergistically inhibited receptor desensitization and prolonged the lifetime of the activated state. I showed that receptor activation is initiated by glutamate-induced closure of individual ligand binding domain clamshells, leading to movement of pore-forming helices and ion channel opening (**Fig.1A)**. This work uncovers the molecular basis of KAR gating and paves the road to development of therapeutic strategies to regulate KAR activity in disease conditions.

AMPA (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 a 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 its ligand-binding domain (LBD) interaction with the γ5 and GSG1L head domains. The closed-state structures of GluA2-γ5 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 the 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. **Gangwar SP.**, Yelshanskaya MV, Nadezhdin KD, Yen LY, Newton TP, Aktolun M, Kurnikova MG, and Sobolevsky AI. Kainate receptor channel opening and gating mechanism. *Nature* (2024). PMID: 38778115.
b. **Gangwar SP.,** Yen LY., Yelshanskaya MV., Sobolevsky AI. (2023) Positive and negative allosteric modulation of GluK2 kainate receptors by BPAM344 and antiepileptic perampanel. *Cell Rep.* Feb 21;42(2):112124. PMID: 36857176.
c. **Gangwar SP.,** Yen LY., Yelshanskaya MV., Korman, A., Jones, DR., and Sobolevsky AI. (2023) Modulation of GluA2–γ5 synaptic complex desensitization, polyamine block and antiepileptic perampanel inhibition by auxiliary subunit cornichon-2. *Nat Struct Mol Biol.* PMID: 37653241.

5. Symposia/Conference paper presentations

a. Mechanism of Kainate receptor regulation by positive and negative allosteric modulator at iGluR-2023, (2023), Chicago.
b. Cryo-EM studies of AMPA receptor-auxiliary subunits complexes at Minisymposium entitled ‘The Intricate Regulation of AMPA Receptors by Transmembrane Accessory Factors’ Society for Neuroscience (2022), San Diego.
c. Molecular Mechanism of AMPA Receptor Modulation by Auxiliary subunits at Biochemistry and Biophysics (BMB) seminar series in Columbia University (2022), New York.
d. Structural and functional dissection of the human Ergp55 oncoprotein at 42nd National Seminar on Crystallography and international workshop on the application of X-ray diffraction for drug discovery, (2013), New Delhi

Complete List of Published Work in My Bibliography

https://www.ncbi.nlm.nih.gov/myncbi/shanti.gangwar.1/bibliography/public/

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)	START DATE MM/YYYY	END DATE MM/YYYY	FIELD OF STUDY
Georgia Institute of Technology	BS	06/2008	08/2010	Biology
Georgia Institute of Technology	MS	09/2010	12/2012	Biology
Columbia University	MA	09/2020	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 mechanistic details of pharmacological inhibition and/or activation, disease mutation function, 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 near-native, heteromeric state, probing the modulatory effects of auxiliary membrane protein binding on channel gating and inhibition. 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 requirements for my development as an independent researcher.

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. There was one extraordinary event that was particularly disruptive: during my sophomore year my father suffered from a collapsed lung and pneumonia, which hospitalized him for two weeks and bedridden for another one week. During those three weeks my mother, sister and I took shifts to manage 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 (GPA). Thankfully, it was my senior research experience that shifted my academic performance: 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. I earned a final GPA of 3.7 and currently hold a GPA of 3.8 at Columbia University. I do not consider my past academic performance an impediment to my current research or future career goals and believe I will continue to improve and excel in my professional and academic endeavors.

1. Gangwar, S. P., **Yen, L.** Y., Yelshanskaya, M. V., & Sobolevsky, A. I. (2023). Positive and negative allosteric modulation of GluK2 kainate receptors by BPAM344 and antiepileptic perampanel. *Cell reports*, *42*(2), 112124.
2. 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.
3. 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.
4. 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.

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

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

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. α-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)-γ5, 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-γ5 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 LY**, 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

YEAR	COURSE TITLE	GRADE
2008	Genetics Laboratory	C
2008	Inorganic Chemistry I	D
2008	Inorganic Chemistry Lab I	C
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	
2010	Biochemistry I	B
2010	Cancer Biol/Tech	A
2011	Macromolecular Structure	A
2011	Enzymology and Metabolism	B
	COLUMBIA UNIVERSITY – PHD	
2020	Biochemistry, Molecular, and Cell Biology I	A-
2020	Molecular Biophysics	B
2021	Mechanisms in Human Disease	A-
2022	Biochemistry, Molecular, and Cell Biology I	A-
2022	Statistics for Basic Science	

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: Newton, Thomas Payne

eRA COMMONS USERNAME (credential, e.g., agency login): TPNEWTON

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Rutgers University	BA	08/2018	05/2022	Molecular Biology & Biochemistry
Columbia University	MA	08/2022	10/2024	Cellular, Molecular, and Biomedical Studies
Columbia University	MPh	08/2022	05/2025 (expected)	Cellular, Molecular, and Biomedical Studies
Columbia University	PhD	08/2022	05/2027 (expected)	Cellular, Molecular, and Biomedical Studies

A. Personal Statement

Structural biology is an ever-advancing field utilized for the determination of 3D reconstructions of biological molecules. Starting with observations of photosynthetic proteins in their native chloroplast environment using cryo-electron tomography (cryoET), I knew that I had a passion for resolving the unknowns of the inner workings of cells using structural techniques. I now work in the lab of Dr. Alexander Sobolevsky at Columbia University Irving Medical Center in New York, NY. Combining the methods of electrophysiology and single-particle cryo-electron microscopy (cryoEM), we study the structure, function, and pharmacology of ionotropic glutamate receptors (iGluRs), including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARS) and kainate receptors (KARs). Our work has, and will continue to, contribute to the understanding of how iGluRs facilitate neurotransmission, aid in high cognitive functions, such as learning and memory, and how they can be targeted and modulated by small molecules. My contributions have led to revelations in KAR biology, specifically in the gating mechanism of GluK2 KARs and their trapping of polyamines. Currently, my work focuses on understanding modulatory alterations of AMPAR function by peripheral auxiliary subunits and how association with these small protein cofactors play a role in the trafficking, localization, and proper functioning of tetrameric receptors. Training and mentorship under Dr. Sobolevsky will provide the necessary environment for the execution of highly relevant biological research and in my growth as research scientist.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments-

2023 – current Graduate Research Assistant, Columbia University, CMBS (Cellular, Molecular, and Biomedical Studies) Umbrella Program, New York, NY
2024 – 2025 Teaching Assistant, Columbia University, Department of Biochemistry & Molecular Biophysics, New York, NY
2023 – 2023 Microscopy Society of America (MSA)
2023 – 2023 Microscopy Society of America Student Council

Honors-

2018 – 2022	The Harriet and Robert Druskin Endowed Scholarship, Rutgers University
2018 – 2022	Rutgers Scarlet Scholarship, Rutgers University
2018 – 2022	Dean's List, Rutgers University
2020 – 2021	The School of Arts and Sciences Scholarship, Rutgers University

C. Contributions to Science

1. Investigation of native biological environments is the future for structural biology. The use of cryo-electron tomography (cryoET) allows for the visualization of vitreous-frozen biological samples *in situ*. As an undergraduate research assistant at the Institute for Quantitative Biomedicine, we applied this technique to understand various biological questions, ranging from amyloid fibril formation to anti-fungal therapeutics. Our lab worked to optimize structure determination pipelines for subtomogram averaging (STA) using marina diatom species, *Phaeodactylum tricornutum*, as a model species. This work was presented at two symposia at Rutgers University, and the methods are actively being utilized for structure determination using cryoET.
    a. **Newton, T.P.**, Jiang, J., Dai, W. "Structural and Functional Analyses of Photosynthetic Thylakoid Membrane Proteins in *Phaeodactylum tricornutum*." *Rutgers University Molecular Biology & Biochemistry Honors Thesis Poster Symposium*, March 2022.

2. Cryo-electron microscopy (cryoEM) is a state-of-the-art technique for the determination of high-resolution structures for macromolecular machinery. As a graduate rotation student at the Simons Electron Microscopy Center (SEMC), we worked to establish methods for effective structure determination of highly heterogenous samples using cryoEM. This work was performed using genetically sterilized virus-like-particles (VLPs) of Qβ virions, yielding geometrical VLP ensembles for downstream biotechnological and biomedical applications.
    a. **Newton, T.**, Zhao, L., Finn, M.G., Kopylov, M. "Diversity in Qβ Virus-like Particle Cage Assembly via Coat Protein Monomers and AYGG-linked Dimers." *Microscopy and Microanalysis*, 2023. **29**(1): p. 906-910. https://doi.org/10.1093/micmic/ozad067.450
    b. **Newton, T.**, Zhao, L., Finn, M.G., Kopylov, M. "CryoEM of Qβ Virus-like Particle Cage Assemblies Reveal Extreme Heterogeneity." *Microscopy and Microanalysis Platform Talk*, 2023.

3. Ionotropic glutamate receptors (iGluRs) are glutamate-gated ion channels that facilitate that vast majority of excitatory neurotransmission in the central nervous system. One subclass of iGluRs, the kainate receptor (KAR), is of particular significance for their roles in neuronal circuitry and synaptic plasticity. Using time-resolved cryoEM and electrophysiology, we solved the first structure of the GluK2 KAR in the activated state with the help of positive allosteric modulators, BPAM344 and Concanavalin A, elucidating the mechanism of gating for this family of receptors. Furthermore, we resolved structures of GluK2 in the presence of ion channel blockers, revealing means by which KARs undergo trapping via exogenous blockers.
    a. Gangwar, S.P., Yelshanskaya, M.V., Nadezhdin, K.D., Yen, L.Y., **Newton, T.P.**, Aktolun, M., Kurnikova, M.G., Sobolevsky, A.I. "Kainate receptor channel opening and gating mechanism." *Nature*, 2024. **630**(8017): p. 762-768. PMCID: PMC11186766.
    b. Gangwar, S.P., Yelshanskaya, M.V., Aktolun, M., Yen, L.Y., **Newton, T.P.**, Strømgaard, K., Kurnikova, M.G., Sobolevsky, A.I. "Trapping of spermine, Kukoamine A, and polyamine toxin blockers in GluK2 kainate receptor channels." *Nature Communications*, 2024. **15**(10257): p. 1-14. PMCID: PMC11599716.

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	ADVANCED PLACEMENT (COLLEGE CREDIT EARNED)	
2017	Psychology	CREDIT
2018	Biology	CREDIT
2018	Calculus AB	CREDIT
2018	Physics I: Algebra Based	CREDIT
	RUTGERS UNIVERSITY	
2018	Honors General Chemistry I	A
2018	Honors Calculus II	A
2019	Honors General Chemistry II	A

YEAR	COURSE TITLE	GRADE
2019	Intro to Chemistry Experimentation	A
2019	Multivariable Calculus	A
2019	Intro to Linear Algebra	A
2019	Organic Chemistry I	A
2019	Genetics	A
2019	Elementary Differential Equations	A
2019	Biochemistry	A
2020	Organic Chemistry Lab	A
2020	Molecular Biology & Biochemistry	B+
2020	General Physics II	A
2020	General Physics Lab II	A
2020	Organic Chemistry II	A
2020	Dynamical Models for Biology	A
2020	Mathematical Theory of Probability	A
2020	Honors Intro to Molecular Biology Lab	B+
2020	Molecular Biology & Biochemistry Seminar	A
2021	Physical Chemistry II	A
2021	Proteomics and Functional Genomics	A
2021	Nucleotide Sequence Analysis	A
2021	Physical Chemistry I	A
2021	Biophysical Chemistry	A
2022	Molecular Biology & Biochemistry Seminar	A
2022	Structural Biophysics	A
	COLUMBIA UNIVERSITY	
2022	Biochemistry/Molecular/Cell Biology I	A-
2022	Molecular Genetics	A-
2023	Cryo-Electron Microscopy	A
2023	Biochemistry/Molecular/Cell Biology II	B+
2023	Statistics for Basic Sciences	A
2023	Synaptic Transmission & Plasticity	A
2023	Responsible Research Conduct & Ethics	P