BIOGRAPHICAL SKETCH

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

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

POSITION TITLE: Assistant Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Lomonosov Moscow State University, Moscow, Russia	MS (Diploma)	06/2012	Organic Chemistry
BAM Federal Institute for Materials Research and Testing, Berlin, Germany	-	09/2015	Bioanalytical Chemistry
Utrecht University, Utrecht, The Netherlands	Ph.D.	10/2019	Pharmaceutical Sciences
Columbia University	Postdoctoral	01/2022	Molecular Biochemistry and Biophysics
New York Structural Biology Center (NYSBC)	Staff	04/2023	Structural Biology

A. Personal Statement

During my academic career, I have acquired a broad background in the applications of cutting-edge biophysical and bioanalytical chemistry approaches for protein imaging coupled with structure and interactome determination. In the field of biomolecular mass spectrometry (MS), I took part in establishing proteome-wide *in-situ* structural proteomics approaches covering the whole pipeline from sample preparation to data analysis and interpretation. As a result, the structural proteomics technique of crosslinking MS has become widely accepted and applicable to whole-cell and complex tissue-like biological samples as opposed to purified protein samples of limited complexity. In the field of cryoelectron microscopy and tomography (cryo-EM and -ET), I focused on the democratization and automation of high throughput and high-resolution *in situ* cryo-ET techniques. At the New York Structural Biology Center (NYSBC), we successfully optimized approaches for high-pressure freezing vitrification of thick and tissue-like biological samples followed by cryo-Fluorescence Light Microscopy (cryo-FLM) screening, Focus Ion Beam (FIB) sample thinning, and high-resolution ET data acquisition and analysis. While both fields are still rapidly developing, their current state allows for protein characterization at unprecedented detail with minimal interference in their physiological function and without disrupting their close-to-native interactome. The combination of these technically challenging state-of-art technologies opens new frontiers in the structural characterization of macromolecular machinery involved in physiological processes and aims to establish a solid basis for efficient structure-based drug discovery.

From the biological perspective, I am interested in the neuronal membrane protein complexes that define chemical synapse function. Throughout my early career, I have sought to apply the newly developed techniques to the relevant membrane protein targets through several collaborative projects. During my graduate studies, I successfully implemented in-house designed approaches to pinpoint the details of interactions within neuronal signaling complexes and gained insights into the mechanism of neuronal dense-core vesicle transport.
Currently, I focus on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (**AMPARs**) and their complexes. AMPARs are a subtype of synaptic ionotropic glutamate receptors that mediate our perception as humans and are involved in numerous neurological disorders and brain diseases. For my postdoctoral training, I joined the Department of Molecular Biochemistry and Biophysics at Columbia University to focus on the gating and biophysical properties of AMPARs. There, I gained relevant expertise in the structural and functional

characterization of purified AMPAR complexes with auxiliary subunits which mediate most of the fast excitatory synaptic transmission. I have also used AMPAR complexes as a model system for establishing the *in-situ* high-resolution cryo-ET pipeline.

For the next step of my career, I aim to further delve into the molecular mechanisms of how our brain functions by focusing on fundamental studies of synaptic transmission mechanisms. The main targets of my group are AMPARs and AMPAR complexes which are crucial for the functioning of the chemical synapses. I have the expertise, training, and motivation to conduct high-quality academic research and to address relevant scientific questions through a variety of multidisciplinary approaches in combination with state-of-art non-invasive *in-situ* methods. My laboratory is also highly interested in translational studies. To perform translational studies, we have teamed up with several scientists across the US. I would specifically highlight the project focused on traumatic brain injury (TBI), where we work together with Dr. Fabio Vigil, a junior faculty in Louisiana State University. I oversee cryo-imaging part of the project, and we have already prepared 14 mouse brain samples. Currently, we are waiting for the microscope time through our instrument access proposal at Stanford-SLAC NIH-dedicated center for cryo-ET.

Selected publications:

1. **Klykov O.,** Kopylov M., Carragher B., Heck A.J.R., Noble A.J., Scheltema R.A.S. Label-free visual proteomics: Coupling MS- and EM-based approaches in structural biology. *Mol. Cell*, 82 (2), 2022, 285-303
2. Kelley K.†, Raczkowski A.M.*, **Klykov O.*,** Jaroenlak P.*, Bobe D.*, Kopylov M., Eng E.T., Bhabha G., Potter C.S., Carragher B., Noble A.J. Waffle Method: A general and flexible approach for FIB-milling small and anisotropically oriented samples. *Nat. Commun.,* 13, 2022, 1857
3. **Klykov O.*,** Gangwar S.P.*, Yelshanskaya M.V.*, Yen L., Sobolevsky A.I. Structure and desensitization of AMPA receptor complexes with type II TARP gamma-5 and GSG1L. *Mol. Cell*, 81 (23), 2021, 4771-4783

B. Other Experience and Professional Memberships

2015 – present	Member, NVMS, Dutch Society for Mass Spectrometry
2016	Oral presentation, 6th Structural Proteomics Symposium, Dortmund, Germany
2018	Organizing committee, 1st Integrative Structural Biology Autumn School, Utrecht University, Utrecht, The Netherlands
2019	Oral presentation, 4th Dutch-Belgian Mass Spectrometry Society meeting, Kerkrade, The Netherlands
2019	Oral presentation, American Society for Mass Spectrometry (Sanibel), St. Petersburg, FL, USA
2020 – present	AHA partner, American Heart Association
2021 – present	Member, Biophysical Society
2022	Invited speaker, seminar series, Dept. of Physiology and Biophysics, Case Western Reserve University
2023	Invited speaker, seminar series, Dept. of Cellular and Integrative Physiology, UT Health San Antonio
2023	Invited speaker, seminar series, Dept. of Microbiology, Immunology & Molecular Genetics, UT Health San Antonio
2023 – present	Seminar Committee, Dept. of Biochemistry and Structural Biology, UT Health San Antonio
2023 – present	Search Committee, Postdoctoral Leadership Program, Dept. of Biochemistry and Structural Biology, UT Health San Antonio
2024	Invited speaker, Southwest Regional Meeting of the American Chemical Society

C. Contributions to Science

Publications 15; Citations 839; H-index 14 (according to Google Scholar, as of October 2024)

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1L7HiZMtMuu5z/bibliography/public/

BIOGRAPHICAL SKETCH

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NAME:	Haven Tillmon
eRA COMMONS USERNAME (credential, e.g., agency login):	TILLMONH
POSITION TITLE:	Graduate Student 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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
St. Mary's University	B.S.	09/2017	05/2021	Biochemistry
UTHSCSA	PhD	209/2021	12/2026 (Expected)	Neuroscience

A. Personal Statement

I first became interested in scientific research when I was diagnosed with Type 1 Diabetes at twelve years old, which required me to constantly monitor my blood sugar and take insulin injections with each and every carbohydrate I ate. This sudden diagnosis pushed me to think about the causes and consequences of living with a chronic disease, which is what brough my attention to a career in scientific research. I pursued my desire to work in research as an undergraduate at St. Mary's University, where I received my Bachelor of Science in Biochemistry, while also conducting research in Dr. Karl Rodriguez's lab in the Barshop Institute at UTHSCSA, where I studied the role of heat shock protein 25 in longevity and aging as well as developed my ability to perform various molecular techniques. This resulted in a third-author publication. Although I entered my undergraduate career Pre-Med, I quickly realized my place was in research, and after a family member was diagnosed with early-onset Alzheimer's Disease, I knew that I wanted to study the brain. Upon graduating, I applied to several neuroscience PhD programs and chose to attend UTHSCSA's Integrated Biomedical Science program in the neuroscience discipline. Upon entering the program, I joined Dr. Gek Ming-Sia's lab, where I studied the role of complement in chronic stress conditions. My work in Dr. Sia's allowed me to develop knowledge in mice models as well as additional molecular techniques, including immunohistochemistry and microglial engulfment assays. This work also resulted in my first, first author publication. I then joined Dr. Oleg Klykov's lab, where I am currently studying the proteomic alterations that occur in progressive supranuclear palsy (PSP). This lab has given me the opportunity to develop technical training, while also allowing me career development and public speaking and presentation opportunities. Overall, I believe that my current research and research environment in addition to training and public speaking opportunities will provide an excellent foundation to reach my goal of research scientist.

B. Positions, Scientific Appointments and Honors

1. **Positions and Employment**
   - 2021 – Present Graduate Student Research Assistant, UT Health Science Center at San Antonio
   - 2018 – 2021 Undergraduate Research Assistant, UTHSCSA
   - 2018 Volunteer Summer Student, The Barshop Institute, UTHSCSA

2. **Scientific and Professional Memberships**
   - 2022-2024 Society for Neuroscience (SFN)
3. **Honors**
   - 2022 – 2023 Early-Stage Neuroscience T32 Training Grant

2017 – 2021 Trustee Scholarship, St. Mary's University

C. Contributions to Science

1.Undergraduate Research: I spent three years working in the laboratory of Dr. Karl Rodriguez at the UTHSCSA. Dr. Rodriguez's laboratory studied the role of heat shock protein 25 (hsp-25), also known as hsp-27 in humans, and its role in extending lifespan in the model *C. elegans,* as well as investigated the role of specific transcription factors involved in the pathway. This model demonstrated increased longevity as well as resistance to heat stress. RNAi experiments demonstrated that the transcription factors in the Skn-1 pathway, Nrf2 and Hsf1, are involved mechanistically in the action of hsp-25. My contributions to this work were included in a publication recently accepted in the Journal of Gerontology. The work was particularly interesting because it aims to understand the role of heat shock proteins in aging and longevity, which can aid in humans aging healthier as well as living longer.

1. Alexander CC, Munkáscy E, Tillmon H, Fraker T, Scheirer J, Holstein D, Lozano D, Khan M, Gidalevitz T, Lechleiter JD, Fisher AL, Zare H, Rodriguez KA. HspB1 Overexpression Improves Life Span and Stress Resistance in an Invertebrate Model. J Gerontol A Biol Sci Med Sci. 2022 Feb 3;77(2):268-275. doi: 10.1093/gerona/glab296. PMID: 34610126; PMCID: PMC8824566.

2. Graduate Research: My predoctoral research initially focused on the role of the complement pathway in chronic stress conditions. I believe the data resulting from this research is highly relevant to human health as it will provide insights into the symptoms we see as a result of chronic stress, including depression and memory alterations, as well as identify the regions of the brain affected that cause these symptoms. This research resulted in a first author publication in Nature Communications. My current research in Dr. Klykov's lab centers on understanding the proteomics of PSP at the protein level using techniques including mass spectrometry and cryogenic electron microscopy (cryo-EM). This research will allow us to highlight the mechanistic features of PSP and thus pinpoint potential strategies for the efficient therapeutic interventions.

1. Tillmon, H., Soteros, B.M., Shen, L. et al. Complement and microglia activation mediate stress-induced synapse loss in layer 2/3 of the medial prefrontal cortex in male mice. Nat Commun 15, 9803 (2024). https://doi.org/10.1038/s41467-024-54007-5.

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	ST. MARY'S UNIVERSITY	
2015	Second Year Latin I	A
2015	College Algebra	A
2016	Second Year Latin II	A
2016	Precalculus	A
2016	Rhetoric and Composition	A-
2017	British Literature	A
2017	General Biology for Majors I	A
2017	General Chemistry I	B+
2017	Fundament. Of Oral Comm	A-
2017	Calculus I	B+
2017	Personal and Academic Develop	P
2018	General Biology for Majors II	A
2018	General Chemistry II	A-
2018	Calculus II	A
2018	Foundations of Reflection: Self	A-
2018	Cell & Molecular Methods	A
2018	Cell Biology	A-
2018	Organic Chemistry I	C

2018	Foundations Of Reflection: Nature	A
2018	Foundations of Practice: Lit.	A
2019	Organic Chemistry II	C+
2019	University Physics I	C+
2019	Foundations of Reflection: God	A
2019	Foundations Practice: Ethics	A
2019	Genetic Principles	A-
2019	Biochemistry I	A-
2019	Foundations of Pract.: Civ Eng	A
2019	Foundations of Practice: Fine Arts and Creative Process: Theatre Emphasis	A
2020	Intro to Bioinformatics	A
2020	Biochemistry II	A-
2020	University Physics II	A-
2020	Major Old Testament Themes: Man and Masculinity in the Hebrew Bible	A
2020	Analytical Chemistry	A-
2020	Physical Chemistry I	C
2020	Enzyme Chemistry	A
2020	Capstone Seminar: Prospects for Community & Civilization	A-
2021	Neurophysiology	A-
2021	Advanced Biochemistry	A-
2021	General Psychology	A-

UTHSCSA

2021	Funds of Biomedical Sciences	A
2021	Lab Rotations	S
2021	Responsible Conduct of Research	S
2022	Experimental Design and Data Analysis	B
2022	Seminar (Neuroscience)	S
2022	Research (Neuroscience)	H
2022	Student Journal Club & Research Presentation (Neuroscience)	A
2022	Fundamentals of Neuroscience 1	A
2022	Principles of Pharmacology and Physiology 1	B
2022	Excitable Membranes	B
2022	Practical Optical Microscopy	S
2022	Rigor and Reproducibility	A
2022	Seminar (Neuroscience)	S
2022	Research (Neuroscience)	H
2022	Student Journal Club & Research Presentation (Neuroscience)	A
2022	Fundamentals of Neuroscience 2	A
2022	Neuroanatomy	B
2022	Basics of Research Design	A
2022	Research Practicum	A
2023	Seminar (Neuroscience)	S
2023	Research (Neuroscience)	H
2023	Qualifying Exam (Neuroscience)	S
2023	Student Journal Club & Research Presentation (Neuroscience)	A
2023	Clinical Practicum (Neuroscience)	H
2023	Seminar (Neuroscience)	S
2023	Research (Neuroscience)	S
2023	Student Journal Club & Research Presentation (Neuroscience)	A

2024	Seminar (Neuroscience)	S
2024	Research (Neuroscience)	H
2024	Student Journal Club & Research Presentation (Neuroscience)	A

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NAME: Gao, Fei

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

POSITION TITLE: 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)	END DATE MM/YYYY	FIELD OF STUDY
Shandong Jianzhu University	BENG	06/2010	Bioengineering
Shandong University	PHD	06/2016	Neuroscience
Qingdao University	Postdoctoral Fellow	08/2018	Neuroscience
LSU Health New Orleans	Postdoctoral Fellow	03/2019	Neuroscience
UT Health San Antonio	Postdoctoral Fellow	08/2023	Neuroscience

A. Personal Statement

I am a research scientist with over a decade of experience investigating the mechanisms underlying neurodegenerative diseases, with a particular focus on hippocampal neurogenesis and protein dysregulation under pathological conditions. My work seeks to understand how abnormal neurogenesis and dysregulated protein expression contribute to cognitive impairments in conditions such as epilepsy, traumatic brain injury (TBI), and Alzheimer's disease. Specifically, I have explored how hippocampal neurogenesis contributes to the imbalance of excitatory and inhibitory projections and cognitive deficits, targeting neurogenesis as a potential therapeutic avenue to mitigate symptoms such as impaired learning, memory, and seizure activity. My research has also identified key players, such as TDP-43 and MAGL, in TBI-induced Alzheimer's-like pathology. I demonstrated that preventing the abnormal accumulation of TDP-43 could attenuate cognitive impairment and delay Alzheimer's progression after repeated mild TBI. These findings highlight the potential of therapeutic interventions to improve outcomes for individuals suffering from neurodegenerative disorders. Motivated by the profound impact of dementia on patients and their families, I have dedicated my career to identifying novel therapeutic targets. I bring a robust background in neuroscience, with expertise in electrophysiology, behavioral testing, viral vector techniques, and molecular biology. These skills have enabled me to bridge the gap between molecular mechanisms and functional outcomes in animal models. My current research integrates my expertise in neuroscience with emerging structural biology tools to investigate synaptic function. AMPA receptors, as key mediators of excitatory neurotransmission, are fundamental to learning and memory, yet their structural changes under pathological conditions remain poorly understood. By leveraging advances in cryo-electron microscopy, I aim to elucidate these changes at the molecular level, complementing my prior work and paving the way for the development of multimodal therapeutic approaches ranging from gene therapy to small-molecule interventions. Participation in this grant will enable me to advance our understanding of the molecular and structural mechanisms of synaptic function. This work will provide a foundation for developing innovative treatments aimed at addressing the cognitive impairments that affect millions of patients worldwide."

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2023 - Research Scientist, UT Health San Antonio
2019 - 2023 postdoctoral Fellow, UT Health San Antonio
2018 - 2019 Postdoctoral Fellow, LSU Health New Orleans
2016 - 2018 Postdoctoral Fellow, Qingdao University
2016 - 2018 Lecturer, Qingdao University
2013 - 2014 Teaching Assistant, Shandong University

Honors

2024 Research Symposium Poster Award Winners, UT Health San Antonio

C. Contribution to Science

1. TDP-43 and Traumatic Brain Injury in Alzheimer's Pathology My research has established the pivotal role of TDP-43 in promoting Alzheimer's disease (AD) neuropathology and cognitive decline following traumatic brain injury (TBI). We demonstrated that a single mild closed head injury (CHI) accelerates AD neuropathology in APP transgenic mice, while repeated CHI induces cognitive impairments in wild-type animals. Crucially, these pathological and cognitive changes were alleviated by silencing TDP-43, and reverted when TDP-43 knockdown was rescued. Overexpression of TDP-43 in the hippocampus mimicked the detrimental effects of CHI, underscoring its role in synaptic and cognitive deterioration. Our work further uncovered that TBI-induced neuroinflammation activates NF-κB, driving excessive TDP-43 expression, which in turn promotes tau phosphorylation and Aβ formation. These findings position TDP-43 as a critical mediator of AD-related pathologies following TBI and a potential therapeutic target to mitigate cognitive impairments.

    a. Gao F, Hu M, Zhang J, Hashem J, Chen C. TDP-43 drives synaptic and cognitive deterioration following traumatic brain injury. Acta Neuropathol. 2022 Aug;144(2):187-210. PubMed Central PMCID: PMC9945325.

2. Role of 2-Arachidonoylglycerol in Cognitive Function and Neuroprotection My research has significantly advanced our understanding of the role of 2-Arachidonoylglycerol (2-AG), an endocannabinoid, in maintaining cognitive function and promoting neuroprotection under pathological conditions. Through a series of studies, we have explored how modulation of 2-AG signaling impacts glial immunity, synaptic functionality, and recovery from neurodegenerative insults: • Enhancing Glial Immunity and Synaptic Functionality: Using single-cell transcriptomic analysis, we demonstrated that inhibiting 2-AG degradation enhances glial immune responses, which are critical for neuroprotection in disease contexts. Additionally, we found that astrocyte-specific augmentation of 2-AG signaling regulates miRNA-30b, preserving synaptic functionality. • Recovery from Traumatic Brain Injury (TBI): Our studies revealed that enhancing 2-AG signaling in astrocytes promotes synaptic repair and cognitive recovery post-TBI. These findings underline the therapeutic potential of endocannabinoid modulation in mitigating TBI-induced damage. • Cognitive Improvement in Alzheimer's Disease Models: In a tau mouse model of Alzheimer's disease, inhibition of 2-AG metabolism alleviated neuropathological changes and improved cognitive function. These results highlight 2-AG's role in counteracting the synaptic and cognitive impairments characteristic of neurodegenerative diseases. Collectively, our work emphasizes the critical role of 2-AG in preserving cognitive function and points to its therapeutic potential in addressing synaptic dysfunction and neurodegeneration.

    a. Zhu D, Zhang J, Gao F, Hu M, Hashem J, Chen C. Augmentation of 2-arachidonoylglycerol signaling in astrocytes maintains synaptic functionality by regulation of miRNA-30b. Exp Neurol. 2023 Mar;361:114292. PubMed Central PMCID: PMC9892245.
    b. Zhu D, Zhang J, Hashem J, Gao F, Chen C. Inhibition of 2-arachidonoylglycerol degradation enhances glial immunity by single-cell transcriptomic analysis. J Neuroinflammation. 2023 Jan 30;20(1):17. PubMed Central PMCID: PMC9885699.
    c. Gao F, Hu M, Zhang J, Hashem J, Chen C. TDP-43 drives synaptic and cognitive deterioration following traumatic brain injury. Acta Neuropathol. 2022 Aug;144(2):187-210. PubMed Central PMCID: PMC9945325.
    d. Hu M, Zhu D, Zhang J, Gao F, Hashem J, Kingsley P, Marnett LJ, Mackie K, Chen C. Enhancing endocannabinoid signalling in astrocytes promotes recovery from traumatic brain injury. Brain. 2022 Mar 29;145(1):179-193. PubMed Central PMCID: PMC8967103.

3. Hippocampal Neurogenesis and Cognitive Function My research has focused on the role of hippocampal neurogenesis in cognitive function, particularly in pathological conditions like epilepsy and chemotherapy, and has provided valuable insights into the underlying mechanisms of cognitive impairments: • Cyclophosphamide and Hippocampal Neurogenesis: We found that cyclophosphamide, a chemotherapy

agent, disrupts dendritic development in adult-born hippocampal granule cells, potentially contributing to long-term cognitive dysfunction • Status Epilepticus and Granule Cell Activity Our research examined the impact of status epilepticus (SE) on neurogenesis and synaptic function. We found that SE leads to an increase in mature and ectopic granule cells in the molecular layer, which may contribute to impaired cognitive function and spontaneous seizure activity. In contrast, granule cells born in the normal location did not show significant differences from control animals. These cells exhibited a higher density of mushroom spines, but their synaptic current amplitudes were smaller. Despite these morphological and functional changes, granule cells did not show sustained hyperactivity. This suggests that transient neurogenesis in the normal position following SE does not lead to long-term cognitive impairments or increased activity, highlighting the potential impact of ectopic granule cells on epileptic pathology.

a. Wu L, Guo D, Liu Q, Gao F, Wang X, Song X, Wang F, Zhan RZ. Abnormal Development of Dendrites in Adult-Born Rat Hippocampal Granule Cells Induced by Cyclophosphamide. Front Cell Neurosci. 2017;11:171. PubMed Central PMCID: PMC5478697.

b. Gao F, Song X, Zhu D, Wang X, Hao A, Nadler JV, Zhan RZ. Dendritic morphology, synaptic transmission, and activity of mature granule cells born following pilocarpine-induced status epilepticus in the rat. Front Cell Neurosci. 2015;9:384. PubMed Central PMCID: PMC4596052.

c. Liang Z, Gao F, Wang F, Wang X, Song X, Liu K, Zhan RZ. Status epilepticus increases mature granule cells in the molecular layer of the dentate gyrus in rats. Neural Regen Res. 2013 Mar 5;8(7):609-15. PubMed Central PMCID: PMC4145990.