BioF:GREAT Glycoscience Research Education and Training

External User: Project Proposal

Project Title: Unraveling the role of protein O-mannosylation in the regulation of LAR Receptor Protein Tyrosine Phosphatases
BioF:GREAT Code: VP092225F
PI/Co-PI Name(s): Vladislav Panin
BioF:GREAT Project Liaison Name: Lance Wells
Submission Date: October 21, 2025
Funding Sources and Grant No's.: No other funding sources are available for the proposed project.

Section I: Project Summary (1 page)

1. Project Background, Project Overview and Goals

a. Motivation for the proposed work (small paragraph 3-4 sentences):

Protein O-mannosylation (POM) is a crucial type of protein posttranslational modification that plays conserved vital roles in muscle and brain development across animal species, from fruit flies to humans. Defects in the genes involved in the POM pathway, including Protein O-Mannosyltransferases (POMTs), cause severe muscular dystrophies and brain malformations in humans, the disorders collectively known as dystroglycanopathies, while resulting in analogous phenotypes in Drosophila, which highlights the importance of the POM pathway. While research in this area has been long focused on a single POM substrate, Dystroglycan (Dg)[1], our recent studies using the Drosophila model has discovered that POMTs also modify a Receptor Protein Tyrosine Phosphatase 69D, which represents a novel type of substrates that play essential roles in the nervous system[2]. These results open new avenues for understanding mechanisms of POM function in RPTP regulation and could lead to new therapeutic approaches for POMT-associated disorders, which highlights the main motivation for our proposed work.

b. Desired aims: (2-3 sentences)

Our overarching goal is to test our central hypothesis that POM regulates LAR-type RPTPs. These RPTPs are highly conserved between Drosophila and mammals, with provides rationale for our approach focused on the following aims.

Specific Aim 1: *To characterize POM modifications of Drosophila LAR and a human LAR homologue expressed in Drosophila.*

Specific Aim 2: *To elucidate the function of POM modification in the regulation of LAR in the nervous system.*

2. Relevant to Glycoanalytics, Glycoenzymes and Bioinformatics, the proposed research activities are expected to advance:

a. Tools and methods: (1-2 sentences)

The project will advance tools and methods in several areas of glycoscience, including (i) MS-based glycoproteomics of POM-modified substrates, (ii) characterization of substrate specificity of POMTs in vivo using endogenous and transgenic substrates, (iii) contributing to bioinformatic database of POM substrates and POM modification sites.

b. Knowledge: (1-2 sentences)

Our project will expand our understanding of POMT function in the nervous system and elucidate mechanisms of POM-mediated regulation of RPTPs.

c. Technologies: (1-2 sentences)

We will develop novel Drosophila genotypes for further in vivo research on molecular and cellular mechanisms of POM. The technology and principles of these in vivo models can be extended to studies of other conserved disease-associated glycosylation pathways.

d. Training of next-generation glycoscience researchers: (1-2 sentences)

The project will provide an excellent platform for multidisciplinary training of undergraduate and graduate students in the Panin's lab in the areas of protein glycosylation and congenital disorders of glycosylation. Collaborative work in the Wells's lab will offer the opportunity to train junior researchers in advance MS-based glycoproteomics.

3. Broader Impacts:

a. Outcomes to be shared with BioF:GREAT community: (1-2 sentences)

The results of the project are expected to be of interest for broad scientific community, including researchers working in clinical and biomedical areas of CDGs, as well as scientists working on fundamental principles of neurodevelopment, along with experts interested in development of improved analytical glycoproteomics and MS analyses of glycoconjugates.

b. Material(s), data or model(s) to be made available to BioF:GREAT databases: (1-2 sentences)

Mutant and transgenic Drosophila strains and in vivo models of POMT defects-associate conditions, along with detailed information on POM modification sites will be published in peer-reviewed papers and deposited to public databases and genetic collections. All materials and data generated by the project will be freely available to the BioF:GREAT community.

c. Planned collaborations within and outside of BioF:GREAT (1-2 sentences).

We will collaborate with Lance Wells's group at CCRC. We plan to expand our strategy of POM analysis to vertebrate RPTP proteins in collaboration with Chiara Manzini's lab (Rutgers University, NJ) that accumulated expertise in mammalian and zebrafish models of dystroglycanopathies.

Section II: Research Plan (1-2 pages)

1. Approaches and Proposed Activities

a. Scientific approaches explained:

We will employ an interdisciplinary strategy combining neurobiological analyses with genetic and biochemical approaches. We will build upon our previous success with this strategy, preliminary data, as well as the advantages of unique Drosophila strains that we generated for this project [2, 6, 7].

Our project will concentrate on the following aims:

Specific Aim 1: *To characterize POM modifications of Drosophila LAR and a human LAR homologue.*

The LAR proteins will be transgenically expressed in vivo in genetic strains with POMT loss-of-function mutations, as well as in genotypes with upregulation of POMTs' activity (gain-of-function conditions). We

will isolate the expressed LAR proteins using affinity purification with anti-LAR antibodies. POM will be analyzed on LAR proteins using MS-based glycoproteomics in the laboratory of Lance Wells (CCRC), a collaborator on this project. We plan to purify and send for MS analysis samples of Drosophila, zebrafish, and human LAR proteins, each expressed in 3 genetic backgrounds, wildtype, POMT mutants, and Drosophila with co-upregulation of POMTs. Each sample will be generated and analyzed at least in triplicates (3 biological repeats). Altogether, approximately 12 samples will be sent to Well's laboratory at CCRC for MS analyses.

Specific Aim 2: *To unveil the function of POM in the regulation of LAR in the nervous system.*
We will analyze the genetic interactions between LAR and POMT mutant alleles in the development of axonal connections of sensory and circadian rhythm pacemaker neurons. The effect of POM on LAR function will be examined at a single-cell level using combinations of mutant and transgenic expression genotypes.

2. Research Team

The research team at Texas A&M University includes the PI (Vlad Panin, PhD), a Research Scientist (Boris Novikov, PhD), a graduate student (Caden Summers, a PhD student in Biochemistry Program), and 3 undergraduate student researchers who participate in the team's activities by helping with simple research tasks and lab chores.

Travel to BioF:GREAT facility:

The PI plans to travel to BioF:GREAT facility and visit Well's lab for project discussions, planning experiments, and other collaborative activities of the BioF:GREAT project.

3. Sample Information

i. Sample details, quantity (as appropriate):

Aim 1 of the project will require MS instrumentation and glycoproteomics expertise of Wells's research team, our collaborators at CCRC.

ii. Expected analysis, data, and data format:

MS glycoproteomic data will be generated and their analyses performed in Aim 1. High-resolution immunofluorescent confocal images will be generated and analyzed using image analysis tools in Aim 2. All these data will be freely available to the broad scientific community via publications and public databases/ depositories of research information.

c. Project Deliverables and Timeline:

Project Deliverable/Milestone	Expected Timeline (months)
Aim 1: (1a) Generation and testing Drosophila genotypes for LAR expression	3 months
(1b) In vivo expression and protein purification - pilot experiments	6 months
(1c) Large-scale collection of material and protein purification	9 months
(1d) MS-based glycoproteomics of purified LAR proteins (Wells lab)	12 months
Aim 2: (2a) Generation and testing Drosophila genotypes for functional analyses	4 months
(2b) Genetic interaction studies and analyses of functional phenotypes	11 months
(2c) Image and statistical analyses, re-assessing significance and results	12 months

ESTIMATED budget expenditure of BioF:GREAT Users In-Residence funds:

Budget Expenditures	Proposed Budget (USD)
Food medium, vials, and other materials for Drosophila rearing and genetics	$5,000.00
Antibodies and affinity reagents for protein purification (approx. 12 protein samples will be generated for MS analysis)	$5,000.00
Tissue dissection, immunohistochemistry reagents, materials for microscopy, reagents and materials for western blot assays, general molecular biology	$5,000.00
Total expected budget	**$15,000.00**

ESTIMATED budget expenditure of BioF:GREAT PI Resources:

Budget Expenditures	Proposed Budget (USD)
Payment of personnel (TBD), 80 hours	$2,400
Consumables for Aim 2:	$600
Mass spec time (**12** injections @ $250 per injection), Wells Lab	$3,000
Total expected budget	**$6,000**

Certification and agreement of proposed work by User:

User Name (print): _ Vladislav Panin

User Signature: _____

Date: ___October 21, 2025_____

Section III: References:

Section IV: PI/Co-PI Biosketch(es).

1. Yoshida-Moriguchi, T and Campbell, KP, Glycobiology, 2015. **25**: p. 702-13.
2. Monagas-Valentin, P, et al., J Biol Chem, 2023. **299**: p. 102890-102890.
3. Vester-Christensen, MB, et al., Proc Natl Acad Sci U S A, 2013. **110**: p. 21018-23.
4. Raducu, M, et al., J Child Neurol, 2014. **29**: p. 289-94.
5. Lommel, M, et al., Proc Natl Acad Sci U S A, 2013. **110**: p. 21024-9.
6. Baker, R, et al., J Neurosci, 2018. **38**: p. 1850-1865.
7. Nakamura, N, et al., Glycobiology, 2010. **20**: p. 381-94.