MCW
GRADUATE SCHOOL

Doctoral Dissertation Defense Announcement

"The Nutrient Sensing *O*-GlcNAc Modification in Development and Neuroendocrine Function"

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Committee in Charge:
Stephanie Olivier-Van Stichelen, PhD (Mentor)
Nancy Dahms, PhD
Daisy Sahoo, PhD
Christopher J. Kristich, PhD
Meredith Cruz, MD, MPH, MBA
Gerald W. Hart, PhD

Date: Tuesday, March 19, 2024
Time: 11:00 AM (CST)
Defense Location: Bolger Auditorium
Zoom: https://mcw-edu.zoom.us/j/97227093238?pwd=bUVuSStJY2svVHJ1alpYSnRVc2dKdz09

Meeting ID: 972 2709 3238 Passcode: qMpXrG4x

Graduate Studies:

Biochemistry of the Cell

Techniques in Molecular & Cell Biology

Molecular and Cellular Biology

Mechanism of Cellular Signaling

Classical and Molecular Genetics

Ethics & Integrity in Science

Introduction to Biomedical Research

Research Ethics Discussion Series

Protein Chemistry Principles

Protein Chemistry Applications

Writing an Individual Fellowship

Biostatistics Health Sciences

Reading and Research

Biochemistry Seminar

Doctoral Dissertation

Dissertation

"The Nutrient Sensing *O*-GlcNAc Modification in Development and Neuroendocrine Function"

The *O*-GlcNAcylation is a ubiquitous, nutrient-sensing, and reversible post-translational modification of nucleocytoplasmic and mitochondrial proteins. It is a unique glucose rheostat for cell signaling since the availability of the nucleotide-sugar donor, UDP-GlcNAc, reflects extracellular glucose.

In Chapter 1, we have curated thousands of *O*-GlcNAcylated proteins from the published literature and created the *O*-GlcNAc database. The meta-analysis of the human *O*-GlcNAcome confirmed that numerous physiological processes are *O*-GlcNAc-regulated, such as development, cell cycle, transcriptional/translational regulation, protein localization, and degradation. Moreover, *O*-GlcNAc deregulation has been linked to pathologies like diabetes, cardiovascular diseases, neurodegeneration, and cancers. Therefore, *O*-GlcNAcylation is a molecular bridge linking dietary glucose levels and proper signaling regulation, and the *O*-GlcNAc database now serves as a tool for continued research and advancement of the field.

In Chapter 2, we explored the vital role of *O*-GlcNAcylation in cell signaling during development, especially in the brain. Thus, we investigated the role of *O*-GlcNAcylation in regulating the homeobox protein OTX2, which contributes to various brain disorders, such as combined pituitary hormone deficiency, retinopathy, and medulloblastoma. Our research demonstrated that OTX2 is degraded by the proteasome and, when aggregated, by the lysosome. We demonstrated that *O*-GlcNAcylation enhances the solubility of OTX2, thereby limiting the formation of these aggregates. Additionally, we unveiled an interaction between OTX2 and the chaperone protein CCT5 at the *O*-GlcNAc sites, suggesting a potential collaborative role in preventing OTX2 aggregation. Finally, our study demonstrated that while OTX2 physiologically promotes cell proliferation, an *O*-GlcNAc-depleted OTX2 is detrimental to cancer cells. These findings suggest that the aggregation and autophagic degradation of OTX2 may serve as a protective mechanism against aberrant OTX2 expression, as observed in medulloblastoma. However, in the context of cancer, marked by hyper-*O*-GlcNAcylation, this mechanism could lead to OTX2 stabilization, potentially contributing to cancer progression.

Finally, in Chapter 3, we studied *O*-GlcNAcylation's roles in development *in vivo*. We previously delved into the consequences of hyper-*O*-GlcNAcylation in the brain with a mouse model. Among phenotypes such as early onset obesity and growth defects, the anterior pituitary gland was generally hypotrophic and mice presented signs of Growth Hormone (GH) deficiency. To follow up on this observation, we interrogated the importance of *O*-GlcNAcylation in the anterior pituitary gland by specifically knocking out the enzyme that removes the *O*-GlcNAc modification, *O*-GlcNAcase (*Oga*) in this organ ($Oga^{\Delta Pit}$). Male knockout (KO) mice presented an initial higher-than-expected Mendelian distribution, but about 15% of the $Oga^{\Delta Pit}$ failed to be metabolically self-sufficient and died shortly after weaning, with incomplete penetrance phenotypes including impaired eye development, hydrocephalus, and growth defects. Additionally, GH deficiency was observed in living adult $Oga^{\Delta Pit}$ mice compared to wild-type

characterized by a decrease of the downstream effector of GH, Insulin-like growth factor 1 (IGF-1) levels. These results suggest that a controlled *O*-GlcNAcylation level is essential for the anterior pituitary gland development and endocrine function. More importantly, growth hormone-secreting cells (somatotrophs) seemed highly affected by *O*-GlcNAc cycling perturbation. Ongoing characterization of somatotroph-specific *Oga* KO, and equivalent *Ogt* KO mice, will further define the importance of the nutrient-dependent *O*-GlcNAc cycling in the pituitary gland's development and its various hormonal secretion.

To conclude, our work highlights the growing significance of the *O*-GlcNAc modification, revealing the modification of thousands of proteins and continuing to expand. Additionally, we have elucidated the critical function of *O*-GlcNAcylation in modulating the stability of the homeobox protein OTX2. This discovery is further emphasized by *O*-GlcNAc's profound effects on the maturation of the pituitary gland, a process in which OTX2 plays a crucial role. In light of these findings, our work calls for further exploration into the regulatory mechanisms mediated by *O*-GlcNAc modification and its implications for various biological processes.

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EDUCATION

MEDICAL COLLEGE OF WISCONSIN (MCW)- PhD candidate, Biochemistry Dept.- Expected March 2024. Interdisciplinary PhD Program in Biomedical Sciences Fall 2018 cohort, under guidance of faculty mentor Dr. Stephanie Olivier-Van Stichelen (solivier@mcw.edu). Research focus: nutrient sensing glycosylation (*O*-GlcNAcylation) and its effects on pre- and post-natal development, hormone secretion and disease.

UNIVERSITY OF WISCONSIN-STEVENS POINT (UWSP), WI- December 2017.
Bachelor of Science in Biology and Biochemistry, *Magna Cum Laude* (GPA: 3.85/4.00).

PROFESSIONAL EXPERIENCE

MCW DEPARTMENT OF BIOCHEMISTRY- Graduate Research Assistant- Spring 2019-present.
-Under guidance of faculty mentor Dr. Stephanie Olivier-Van Stichelen.

MCW DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY- Research Technician- Spring 2018.
-Collaborate on project studying underlying mechanisms of intrinsic antibiotic resistance by bacterium *E. faecalis* under guidance of faculty mentor Dr. Christopher Kristich.

UWSP CHEMISTRY DEPARTMENT- Undergraduate Research Student-
Summer terms 2014 and 2015 (full-time), Fall 2014-2017 (part-time).
-Collaborate on water-gas-shift catalysis project under guidance of faculty mentor Dr. Jason D'Acchioli.

UWSP TUTORING AND LEARNING CENTER- Biology and Chemistry Tutor- Fall 2014, Spring and Fall 2016.
-Assist students with understanding of fundamental chemistry and microbiology course material through collaboration and reflection.
-Model and promote successful study skills and test-taking strategies, within one-on-one and group settings.
-Training for this position included a workshop in academic success strategies, which enhanced my understanding in a variety of learning styles.

PUBLICATIONS

-Wulff-Fuentes E, Boakye J, Kroenke K, Berendt RR, Martinez-Morant C, Pereckas M, Hanover JA, Olivier-Van Stichelen S. *O*-GlcNAcylation regulates OTX2's proteostasis. iScience. 2023 Oct 12;26(11):108184. doi: 10.1016/j.isci.2023.108184. PMID: 38026167; PMCID: PMC10661118, in press.

-Wulff-Fuentes E*, Berendt RR*, Massman L, Danner L, Malard F, Vora J, Kahsay R, and Olivier-Van Stichelen S, The Human *O*-GlcNAcome Database and Meta-Analysis. Scientific Data 2021 8(1):25. January 21 2021, PMID: 33479245, PMCID: PMC7820439, in press. *Authors contributed equally.

> **Contribution as co-first author:** alongside two other members of the lab, did the initial literature curation of 1703 *O*-GlcNAc articles published until May 5th 2020 (of which 948 articles were excluded out of the review and summarization that followed), extracting the initial *O*-GlcNAcome list and manuscript writing.

-Malard F, **Wulff-Fuentes E**, Berendt RR, Didier G, Olivier-Van Stichelen S, Automatization and self-maintenance of the *O*-GlcNAcome catalog: a smart scientific database. Database (Oxford). Volume 2021, baab039. doi: 10.1093/database/baab039. July 19 2021, PMID: 34279596; PMCID: PMC8288053.

Contribution as middle author: alongside two other members of the lab, confirmed the results from the Neural Network algorithm to validate presence or absence of identification of *O*-GlcNAcylated proteins and sites in 732 articles categorized as non-human models in our previous curation.

ORAL PRESENTATIONS

"OTX2's Dual Mode of Degradation is Regulated by *O*-GlcNAcylation", April 3, 2022**.** Experimental Biology 2022 in Philadelphia, PA, USA. American Society for Biochemistry and Molecular Biology (ASBMB) Spotlight Session on "New links between glycoproteins, tissue development and disease**.**"

"Pituitary Gland Hyper-*O*-GlcNAcylation drives Growth Hormone Deficiency", November 9, 2020**.** Society for Glycobiology (SFG) 2020 Virtual Annual Meeting, short poster talk.

"Linking Maternal Sugar Consumption to Progenies' Developmental Defects: A Focus on OTX2's *O*-GlcNAcylation", June 23, 2020. American Society for Biochemistry and Molecular Biology (ASBMB) Virtual Spotlight in Glycobiology Session, short talk.

POSTER PRESENTATIONS

Eugenia Wulff-Fuentes, Jeffrey Boakye, Kaeley Kroenke, Rex Berendt, Carla Martinez-Morant, Michaela Pereckas, John A Hanover and Stephanie Olivier-Van Stichelen. "*O*-GlcNAcylation regulates OTX2's proteostasis"

-September 28, 2023 **–** 2023 Translational Glycomics Symposium at Versiti.
-November 6, 2023 - Society for Glycobiology 2023 Annual Meeting in Hawaii, USA.
-December 5, 2023 **–** MCW 33rd Annual Graduate School Research Poster Presentation.

Eugenia Wulff, Jeffrey Boakye, Michael Pereckas, Rex Berendt, John A Hanover and Stephanie Olivier-Van Stichelen**.** "OTX2's Dual Mode of Degradation is Regulated by *O*-GlcNAcylation"

-November 8, 2021 - Society for Glycobiology 2021 Annual Meeting Poster Session. *Award recipient.
-April 4, 2022 **–** ASBMB/Experimental Biology 2022 in Philadelphia, PA, USA.
-July 9, 2022 **–** *O*-GlcNAc Regulation of Cellular & Pathophysiology Conference in Athens, GA, USA.

Eugenia Wulff, Rex Berendt, John A Hanover and Stephanie Olivier-Van Stichelen**.** "Pituitary Gland Hyper-*O*-GlcNAcylation drives Growth Hormone Deficiency"

-May 15, 2020 - 2020 NIH & FDA Glycoscience Research Day (Virtual), abstract only. *Award recipient.
-November 9, 2020 - Society for Glycobiology (SFG) 2020 Annual Virtual Meeting. *Award recipient.

Eugenia Wulff, Jeffrey Boakye, Rex Berendt, John A Hanover and Stephanie Olivier-Van Stichelen. "Linking maternal sugar consumption to progenies' developmental defects: a focus on OTX2's *O*-GlcNAcylation"

-August 9, 2019 - MCW 5th Annual Graduate Student Welcome Poster Session.
-November 7, 2019 - MCW 29th Annual Graduate School Research Poster Presentation.

HONORS AND AWARDS

-2023 MCW Graduate Student Travel Award	November 7, 2023
-2023 SFG Graduate Student Travel Award	October 2, 2023
-2022 MCW Graduate Student Travel Award	July 2022
-2022 ASBMB Graduate Student Diversity, Equity, and Inclusion Travel Award	April 2022
-2021 Society for Glycobiology (SFG) Annual Meeting Student Poster Award*	November 8, 2021

-2021 SFG Graduate Student Travel Award	August 26, 2021
-2020 SFG Annual Meeting Graduate Student Poster Award*	November 12, 2020
-MCW 2020 Women in Science Student Award	October 19, 2020
-NIH & FDA Glycosciences Research Day Outstanding Abstract Award*	May 15, 2020
-2022 ASBMB Graduate Student Travel Award (Cancelled due to COVID-19)	January 2020
-UWSP Bachelor of Science in Biology and Biochemistry, Magna Cum Laude (GPA: 3.85/4.00).	December 16, 2017
-UWSP 2017 Chancellor's Leadership Award	December 15, 2017
-UWSP Academy of Letters and Science 2016 Distinguished Achievement Award	April 9, 2016
-UWSP Outstanding Sophomore Biology Student Award	May 14, 2015
-UWSP College of Letters & Science Dean's Distinguished Achievement	Fall 2013-Fall 2017

MENTORING EXPERIENCE

Medical College of Wisconsin – undergraduate, high school, and rotation students

Kaeley Kroenke	01/2022-08/2022, high school senior, Students Understanding Principles of Research Education through Medicine, Engineering, and Science (SUPREMES)
Uchechi Nworah	06/2021-07/2021, Summer Program for Undergraduate Research
Janelle Lanham	11/2020-12-2020, rotation student, Interdisciplinary Program in Biomedical Sciences
Carla Martinez	06/2018-07/2018, Summer Program for Undergraduate Research

COMMUNITY OUTREACH ACTIVITIES

-Summer Program for Undergraduate Research Ambassador for Underrepresented Groups	Summer 2022, 2023
-15th Annual Milwaukee Public Schools STEM Fair volunteer.	May 5, 2022
-Women's Mount Mary University, STEM outreach seminar series.	November 30, 2021
-Tonawanda Elementary School Science Fair student project judge.	March 12, 2020
-Wauwatosa STEM Mentorship Program, volunteer mentor of Wilson Elementary School students, biweekly commitment.	January 13-March 9, 2020
-12th Annual Milwaukee Public Schools STEM Fair, volunteer and student project Spanish-speaking judge.	April 4, 2019

MEMBERSHIPS IN PROFESSIONAL SOCIETIES

Student member

American Heart Association	Spring 2022-present
Society for Glycobiology (SFG)	Fall 2020-present
American Society for Biochemistry and Molecular Biology (ASBMB)	Fall 2019-present

PERSONAL

Fully bilingual in Spanish and English. Passionate about reading, puzzles, art, and nature.