MCW
GRADUATE SCHOOL

Doctoral Dissertation Defense Announcement

"Investigating the Molecular Determinants of Superagonism at the Mu-Opioid Receptor"

Nicholas J. Malcolm

Candidate for Doctor of Philosophy in Cell and Developmental Biology

School of Graduate Studies, Medical College of Wisconsin

Committee in Charge:

John D. McCorvy, PhD (Mentor)

Cheryl Stucky, PhD

Michael T. Lerch, PhD

Jonathan Marchant, PhD

Tao Che, PhD

Date: Monday, June 17, 2024

Time: 9:00 AM – 10:00 AM (CST)

Defense Location: Alumni Center

Zoom: https://mcw-edu.zoom.us/j/92014516955?pwd=THhCN0QzRjB0emo0U2dtcEJmNWU1UT09

Meeting ID: 920 1451 6955 **Passcode**: jmcQr4MX

Graduate Studies:

MCW Medical School Discovery Curriculum M1 & M2

Principles of Quantum Mechanics I & II

Statistical Mechanics

Theoretical Physics - Dynamics

Statistical Models & Methods I

Nuclear Magnetic Resonance

Introduction to Probability Models

Neurobiology of Pain

Ethics & Integrity in Science

Research Ethics Discussion Series

Doctoral Dissertation

Dissertation

"Investigating the Molecular Determinants of Superagonism at the Mu-Opioid Receptor"

The ongoing opioid epidemic is responsible for thousands of deaths annually. Synthetic opioids are responsible for many of those deaths, and the emergence of novel opioids hampers our ability to control the epidemic. These drugs, sometimes termed "designer opioids", display a wide range of pharmacological profiles, with many able to achieve potent agonism at the μ-opioid receptor (MOR) above the level reached by endogenous ligands. Although the potency and efficacy of opioid drugs are directly related to their ability to cause harmful side effects like respiratory depression, the specific MOR pharmacological profiles that leads to overdose and death remain poorly predictable. In fact, strategies toward generating safer opioid drugs are severely limited without deep structural knowledge on the underpinnings of MOR activation processes. To develop the next generation of safer opioid therapeutics, it is paramount to understand the structural and biophysical determinants of MOR activation and signaling. Synthetic opioids are valuable tools toward assessing the structural basis of varying degrees of agonism displayed by MOR ligands. This is because the stability of conformations induced by a ligand is directly proportional to their intrinsic efficacy. Computational studies have suggested that the degree of ligand-binding induced conformational heterogeneity in the MOR intracellular coupling domain is inversely correlated with that ligand's efficacy, with partial agonists displaying increased motional dynamics at key microswitch motifs compared to full agonists. Learning how signal transmission is mediated by binding pocket interactions, and how signals propagate to the intracellular side of the receptor will help us understand why these interactions correspond to specific degrees of agonism. Here, we present data showing that members of the nitazene family of MOR ligands contain compounds capable of achieving supraphysiologic levels of signaling efficacy for both the G protein and β-arrestin signaling pathways. These superagonists can potently induce both analgesia and respiratory depression in animal models. The high selectivity shown by these nitazenes for MOR over the δ- and κ-opioid receptors indicates they can serve as useful tool compounds to interrogate MOR signaling. We utilize this to our advantage by harnessing computational tools to aid in the targeted design of novel nitazene compounds displaying partial G protein agonism at MOR. We show that these compounds can induce analgesia but are unable to induce apnea at relevant doses. We use molecular dynamics and Markovian modeling to show that nitazene partial agonists adopt an alternative binding orientation within the MOR orthosteric binding pocket relative to nitazene superagonists and conventional opioids. These findings lead us to propose a model of nitazene mediated MOR activation where ligand positioning between TMs 2 and 7 promote partial agonism, positioning between TMs 2 and 3 promotes full agonism, and the positioning of Q2.60 relative to ECL1 functions as a switch to trigger superagonism.

Nicholas J. Malcolm
Curriculum Vitae
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Education

Medical College of Wisconsin — Milwaukee, WI
Doctor of Medicine - Candidate — June 2018 - Expected May 2026
- USMLE Step 1: 249 — June 2020
- USMLE Step 2 CK: TBD

Doctor of Philosophy - Candidate — June 2018 –Expected June 2024
- Cell and Developmental Biology

University of Wisconsin - Parkside — Kenosha, WI
Bachelor of Science, *Magna Cum Laude* — Jan. 2015 – Dec. 2017
Major in Molecular Biology and Bioinformatics – Honors
Major in Mathematics
- GPA: 3.79

Honors & Awards

Membership, Sigma Xi Scientific Research Honor Society; Member at large — Sept. 2023

Outstanding Graduate Award, University of Wisconsin - Parkside; Kenosha, WI — Dec. 2017

Fellowship Award, The Thomson Research Fellowship Award; Kenosha, WI — 2016 - 2017

Membership, Phi Eta Sigma National Honor Society; Kenosha, WI — Feb. 2016

Research & Laboratory Experience

Medical College of Wisconsin; Milwaukee, WI — July 2020 – June 2024
Graduate Student (MSTP), Program in Cell and Developmental Biology
Advisor: Dr. John D. McCorvy, PhD
Project*: Investigating the Molecular Determinants of Superagonism at the μ-Opioid Receptor*

Medical College of Wisconsin; Milwaukee, WI — June 2017 – Aug 2017
SPUR Student,
Advisor: Jeffrey Medin, PhD
Project*: Mitochondrial activity in mouse models of lysosomal storage disorders*

University of Wisconsin - Parkside; Kenosha, WI — Jan 2016 – Dec 2017
Undergraduate Student,
Advisor: Daphne Pham, PhD
Project*: Inhibitors of ribonucleotide reductase oligomerization as a novel mosquito control mechanism*

Publications

Peer-Reviewed Journal Articles

1. **Malcolm, Nicholas J.**, Barbara Palkovic, Daniel J. Sprague, Maggie M. Calkins, Janelle K. Lanham, Adam L. Halberstadt, Astrid G. Stucke, and John D. McCorvy. "*Mu-Opioid Receptor Selective Superagonists Produce Prolonged Respiratory Depression.*" iScience 26, no. 7 (July 21, 2023): 107121. https://doi.org/10.1016/j.isci.2023.107121.

2. Rohr, Claudia M., Daniel J. Sprague, Sang-Kyu Park, **Nicholas J. Malcolm**, and Jonathan S. Marchant. "*Natural Variation in the Binding Pocket of a Parasitic Flatworm TRPM Channel Resolves the Basis for Praziquantel Sensitivity.*" Proceedings of the National Academy of Sciences 120, no. 1 (January 3, 2023): e2217732120. https://doi.org/10.1073/pnas.2217732120.

3. Lewis, V., Bonniwell, E. M., Lanham, J. K., Ghaffari, A., Sheshbaradaran, H., Cao, A. B., Calkins, M. M., Bautista-Carro, M. A., Arsenault, E., Telfer, A., Taghavi-Abkuh, F.-F., **Malcolm, N. J.**, El Sayegh, F., Abizaid, A., Schmid, Y., Morton, K., Halberstadt, A. L., Aguilar-Valles, A., & McCorvy, J. D. (2023). "*A non-hallucinogenic LSD analog with therapeutic potential for mood disorders*." Cell Reports, 42(3), 112203. https://doi.org/10.1016/j.celrep.2023.112203

Manuscripts in Preparation

1. Daniel J. Sprague [*], **Nicholas J. Malcolm** [*], Barbara Palkovic, Maggie M. Calkins, Natalie G. Cavalco, Josie Lammers, Allison A. Clark, Robert F. Keyes, Anastasia Boutris, Carly A. George, Philip D. Mosier, Brian C. Smith, Adam L. Halberstadt, Astrid G. Stucke, Tao Che, and John D. McCorvy. "*Target-based Discovery and Mechanism of Action of Non-Respiratory-Depressive Nitazene Opioids.*"
   *Contributed Equally

Presentations

Poster Presentations

1. **Nick Malcolm**, Barbara Palkovic, Daniel J. Sprague, Natalie Cavalco, Maggie M. Calkins, Janelle K. Lanham, Adam L. Halberstadt, Astrid G. Stucke, John D. McCorvy (2023, June 10-16) "*Markovian Modeling of the Conformational Dynamics Involved in μ Opioid Receptor Superagonism"* [Poster presentation] Gordon Research Conference "Complexity in Proteins", Holderness, NH, USA

Leadership Experience

Medical College of Wisconsin — Milwaukee, WI
MD-PhD Student Interviewer — Jan 2021 – Present
MSTP Student Council G2 Representative — 2021-2022
Principles of Drug Action Student Liaison — Feb 2019 – May 2019

Volunteer Experience

Greater Milwaukee Free Clinic — Milwaukee, WI
- Medical Student Volunteer — 2018 – 2019

Saturday Clinic for the Uninsured — Milwaukee, WI
- Medical Student Volunteer — 2018 – 2019

Princeton Fire Department – Mercer County Engine Co. 3 — Princeton, NJ
- Firefighter — 2012 – 2015

Westfield Rescue Squad Westfield, NJ

- Emergency Medical Technician 2013 – 2014

Princeton First Aid and Rescue Squad Princeton, NJ

- Emergency Medical Technician 2012 – 2015

Teaching/Mentoring Experience

University of Wisconsin - Parkside Kenosha, WI

- Introductory Biology Supplemental Instructor Jan 2016 – May 2016
- Biology Tutor Jan 2016 – May 2016

Professional Societies

American Chemical Society (ACS) 2015 - present

American Mathematical Society (AMS) 2023 - present