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**  
Doctor of Medicine - Candidate  
June 2018 - Expected May 2026  
- USMLE Step 1: 249  
- USMLE Step 2 CK: TBD

**University of Wisconsin - Parkside**  
Bachelor of Science, *Magna Cum Laude*  
Major in Molecular Biology and Bioinformatics – Honors  
Major in Mathematics  
- GPA: 3.79

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**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

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**Research & Laboratory Experience**

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

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

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

Peer-Reviewed Journal Articles


Manuscripts in Preparation

Presentations

Poster Presentations

Leadership Experience

Medical College of Wisconsin
- MD-PhD Student Interviewer Milwaukee, WI 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
- Medical Student Volunteer Milwaukee, WI 2018 – 2019

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

Princeton Fire Department – Mercer County Engine Co. 3
- Firefighter Princeton, NJ 2012 – 2015
Westfield Rescue Squad  
- Emergency Medical Technician  
Westfield, NJ  
2013 – 2014

Princeton First Aid and Rescue Squad  
- Emergency Medical Technician  
Princeton, NJ  
2012 – 2015

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Teaching/Mentoring Experience

University of Wisconsin - Parkside  
- Introductory Biology Supplemental Instructor  
  Kenosha, WI  
  Jan 2016 – May 2016
- Biology Tutor  
  Jan 2016 – May 2016

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Professional Societies

American Chemical Society (ACS)  
2015 - present

American Mathematical Society (AMS)  
2023 - present