MCW
GRADUATE SCHOOL

Doctoral Dissertation Defense Announcement

"Altering Chemokine Receptor Signaling and Selectivity with Nanobodies"

Roman R. Schlimgen

Candidate for Doctor of Philosophy

Biochemistry

School of Graduate Studies

Medical College of Wisconsin

Committee in Charge:

Brian Volkman, PhD (Mentor)

Michael Dwinell, PhD

Blake Hill, PhD

John McCorvy, PhD

Francis Peterson, PhD

Date: Friday, July 19, 2024

Time: 11:00 AM (CST)

Defense Location: Alumni Center

Zoom: https://mcw-edu.zoom.us/j/91240747345?pwd=q0izhlD9hOzEZRwKnP92xvshnwLu4p.1

Meeting ID: 912 4074 7345 Passcode: 92216096

Graduate Studies:

Foundations in Biomedical Sciences I-IV

Techniques in Molecular and Cell Biology

Professional Development I-II

Reading and Research

Statistics for Basic Sciences

Protein Chemistry Applications

Protein Chemistry Principles

Understanding Cell Signaling - Therapeutic Drugs

Contemporary Methods in X-ray Crystallography

Ethics and Integrity in Science

Writing a Scientific Paper

Writing an Individual Fellowship

Biophysical Techniques in Biochemistry

Research Ethics Discussion Series

Metabolism

Biomolecular NMR Structure and Molecular Recognition

Biochemistry Seminar

Dissertation

"Altering Chemokine Receptor Signaling and Selectivity with Nanobodies"

Over 800 human G protein-coupled receptors (GPCRs) permit cells throughout the body to respond to a myriad of extracellular cues. The fine-tuned control of these receptors has driven them to become the most abundant class of therapeutic target. When successful drugs are developed that are selective for their target, GPCR drugs can significantly aid in disease treatment. However, current therapeutic strategies are not always successful. Only 17% of all GPCRs have FDA-approved drugs, despite the critical role many other receptors play in regulating disease. Chemokine receptors, for instance, are highly sought-after drug targets due to their involvement in inflammation and cancer, but traditional small-molecule therapeutic strategies have yet to yield successful treatments for either drug model.

In this dissertation, I explored the use of truncated llama-derived antibodies, or nanobodies, as a new therapeutic strategy to target GPCRs. These small (12-15 kDa), easily produced proteins use a small paratope of three complimentary-determining regions (CDRs) to bind GPCR epitopes that are difficult to target. Focusing specifically on the chemokine receptor field, we collaborated with the lab of Dr. Martine Smit to immunize two llamas against the Atypical Chemokine Receptor 3 (ACKR3), resulting in the generation of a nanobody termed VUN701.

Using bioluminescence resonance energy transfer (BRET) assays for GPCR activation, we established VUN701 as a selective ACKR3 inhibitor with extracellular therapeutic potential. Solving the solution structure of VUN701 revealed an unusual motif that enables VUN701's inhibition. While uncommon in most nanobody structures, we find this distinctive motif is a frequent feature of GPCR targeting nanobodies. New tools in molecular modeling then allowed us to map the inhibitory ACKR3-VUN701 interface and define a molecular mechanism by which ACKR3 and other GPCRs can be inhibited.

The results of our modeling were validated by solving the cryoEM structure of ACKR3 bound to VUN701. Using the active and inactive ACKR3 structures, along with Nuclear Magnetic Resonance (NMR) spectroscopy, we defined a molecular mechanism by which ACKR3 and other chemokine receptors can be pharmacologically tuned. By modifying the CDR3 motif of VUN701, we developed ACKR3-specific inverse agonists, partial agonists, and agonist nanobodies. These results have now been extended to alternative GPCRs.

This dissertation demonstrates the utility of nanobodies as new therapeutics and establishes the small proteins as tunable tools for understanding GPCR function. These findings pave the way for the development of a highly specific, biologic toolkit for the chemokine receptor and GPCR fields.

Roman Richard Schlimgen

Curriculum Vitae

June 19, 2024

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EDUCATION

Medical College of Wisconsin — Milwaukee, WI
Ph.D., Department of Biochemistry — Anticipated July 2024

University of Wisconsin – La Crosse — La Crosse, WI
B.S., Department of Chemistry and Biochemistry and Department of Biology — September 2015-May 2019

RESEARCH EXPERIENCE

Medical College of Wisconsin — Milwaukee, WI
Graduate Student; Advisor: Brian F. Volkman, Ph.D. — 2019 – Present

- Identified and altered the mechanism by which VHH-based immunoglobin single domain monomers can alter the pharmacology of the Atypical Chemokine Receptor 3 (ACKR3).
- Utilized Nuclear Magnetic Resonance (NMR) to characterize the structural flexibility of a viral chemokine termed vCXCL1.
- Established a high-throughput technique to create and validate computational models of Chemokine-Chemokine Receptor complexes.

University of Wisconsin – La Crosse — La Crosse, WI
Undergraduate Student; Advisors: Daniel Grilley, Ph.D. and Dr. Todd Weaver, Ph.D. — 2018 – 2019

- Systematically disrupted the nonpolar core of Hemolysin A to understand the role of the β-helix structure in enabling protein structure, function, and secretion.
- Altered the binding pocket of an *E. coli* alkaline phosphatase to enable competitive inhibition by free amino acids.

AWARDS AND ACCOMPLISHMENTS

- Outstanding Commitment to Service Through Volunteerism Award, Spring 2015
- Marie D. Sanders Scholarship, 2015
- Horace and Gladys Moran Fund Scholarship, 2015
- Wisconsin Academic Excellence Scholarship, 2015-2019
- University of Wisconsin – La Crosse Soaring Eagle Scholarship, 2015
- Mayo Clinic Foundation Scholarship, 2018 – 2021
- University of Wisconsin – La Crosse Dean's List, 6 semesters, 2015-2019
- ASBMB Degree Certification, 2019
- B.S. awarded with Honors, University of Wisconsin – La Crosse, 2019
- ASBMB Undergraduate Poster Session Honorable Mention, April 2019
- ASBMB Travel Award Recipient, April 2022
- Gordon Research Conference on Chemotactic Cytokines - Poster Award, June 2024

GRANTS AND FELLOWSHIPS

Completed:

Source: University of Wisconsin - La Crosse Undergraduate Research Committee
Title: Segmented Disruption of the Nonpolar β-Helix of HpmA and its Effect on Protein Structure, Function, and Secretion
Role: PI
Dates: 09/2018-02/2019

PEER-REVIEWED PUBLICATIONS

PUBLICATIONS:

Schlimgen RR, Peterson FC, Heukers R, Smit MJ, McCorvy JD, Volkman BF. Structural Basis for Selectivity and Antagonism in Extracellular GPCR-Nanobodies. Nat. Comm. 2024.

Zhou AL, Jensen DR, Peterson FC, Thomas MA, **Schlimgen RR**, Dwinell MB, Smith BC, Volkman BF. Fragment-based drug discovery of small molecule ligands for the human chemokine CCL28. SLAS. 2023.

Berg C, Wedemeyer MJ, Melynis M, **Schlimgen R**, Hansen LH, Våbenø J, Peterson FC, Volkman BF, Rosenkilde MM, Lüttichau HR. The non-ELR CXC chemokine encoded by human cytomegalovirus UL146 genotype 5 contains a C-terminal b-hairpin and induces neutrophil migration as a selective CXCR2 agonist. PLOS Pathogens. 2022.

MANUSCRIPTS SUBMITTED:

Zhang X*, **Schlimgen RR*,** Volkman BF, Zhang C. Structural basis for the ligand recognition and signaling of XCR1. *(In Revision)*

POSTERS AND ABSTRACTS

1. "Controlling the Selectivity and Pharmacology of Chemokine Receptors Using Engineered Nanobodies." Abstract for poster presentation. Gordon Research Seminar – Chemotactic Cytokines. University of Southern Maine, Portland, ME. June 1-7, 2024. *Poster Award*
2. "Selectively Modulating Chemokine Receptor Activity with Nanobodies." Abstract for poster presentation. Gordon Research Seminar – Molecular Pharmacology. Les Diablerets Conference Center, Les Diablerets, Switzerland. July 11 – 17, 2022.
3. "Structural Basis of Nanobody Induced ACKR3 Inhibition." Abstract for poster presentation. Chicago Area NMR Discussion Group (CANMDG) Annual Meeting. Abstract for poster presentation. Dominican University, River Forest, Illinois. November 5, 2022.
4. "Structural Basis of Nanobody Induced ACKR3 Inhibition." Abstract for poster presentation. Gordon Research Seminar – Cytotactic Chemokines. Les Diablerets Conference Center, Les Diablerets, Switzerland. July 11 – 17, 2022.
5. "Structural Basis of Nanobody Induced ACKR3 Inhibition." Abstract for poster presentation. Experimental Biology. Pennsylvania Convention Center, Philadelphia, PA. April 2 – 5, 2022. *Travel Award Recipient.*
6. "Systematic Disruption of the Nonpolar β-Helix Core of Hemolysin A and its Site-Specific Effect on Protein Structure, Function, and Secretion." Abstract for poster presentation. Experimental Biology. Orange County Convention Center Orlando, FL. April 6 – 9, 2019. *Undergraduate Poster Session Honorable Mention.*

INVITED TALKS

1. "Controlling the Selectivity and Pharmacology of Chemokine Receptors Using Engineered Nanobodies." Abstract for poster presentation. Gordon Research Seminar – Chemotactic Cytokines. University of Southern Maine, Portland, ME. June 1-7, 2024.
2. "Structural Basis of Nanobody Induced ACKR3 Inhibition." Gordon Research Seminar – Cytotactic Chemokines. Les Diablerets Conference Center, Les Diablerets, Switzerland. July 11 – 17, 2022.
3. "Structural Basis of Nanobody Induced ACKR3 Inhibition." Experimental Biology. Pennsylvania Convention Center, Philadelphia, PA. April 2 – 5, 2022.

WORKSHOPS AND OUTSIDE TRAINING

Laboratory of Dr. Aashish Manglik San Francisco, CA
Visiting student, University of California at San Francisco October 2023
Worked with Assistant Prof Researcher Christian Billesbølle to learn about the collection and processing of cryo-electron microscopy data of a membrane protein-nanobody complex.

Introduction to Molecular Modeling in Drug Discovery Virtual Workshop
Workshop attendee, Schrödinger October 2020
Learned how to utilize Schrödinger Maestro computational software to screen virtual compound libraries on numerous drug targets.

TEACHING EXPERIENCE

Association for Biomolecular and Computational Simulation (ABACUS) Milwaukee, WI
President, Medical College of Wisconsin 2023 – 2024
- ABACUS is a student-created and student-led club designed to teach students, professors, and other researchers how to utilize a wide-range of computational techniques at the Medical College of Wisconsin (MCW); (bi-)monthly meetings regularly attract 20-30 students and faculty members.

- Topics include: The command line interface, Alphafold, PyMOL, R Studio, Python, ImageJ, molecular dynamics (MD) simulations, and more.

Fundamentals of Biomedical Sciences I – Biochemistry Milwaukee, WI

Student Facilitator August 2022
- Assisted first-year Ph.D. students with in-class activities and take-home assignments.

One-on-One Lab Mentoring:

Student Name:	**Position:**	**Timeframe:**
Angela Zhou	Undergraduate Student	2020-2022
Julia Wendland	Undergraduate Student	2021-2021
Neha Ajjampore	Undergraduate Student	2022-2022
Shawn Jenjak	Graduate Student	2022-Present
Yushin Kim	Undergraduate Student	2023-Present
Alexa De La Sancha	Research Technician	2023-Present
Joey DeMeyer	Research Technician	2023-Present

PROFESSIONAL ORGANIZATIONS

American Society of Biochemistry and Molecular Biology (ASBMB) 2021 - Present

COMPETENCIES AND SKILLS

Laboratory: Soluble/membrane protein expression/purification (E. coli, insect cell/baculovirus), protein chromatography (HPLC, gel filtration chromatography, affinity chromatography, AKTA), flow cytometry, cloning, site-directed mutagenesis, mammalian cell culture, receptor functional assays (BRET, NanoLuc), uniform labeling in *E. coli* for NMR; 2D NMR data collection/analysis (Bruker; TopSpin; NMRpipe; XEasy); 3D NMR data collection/analysis, cryo-electron microscopy data collection/processing, thermostability assays.

Computational: Unix/Linux, AlphaFold, molecular dynamics (Gromacs), structure visualization and analysis software (VMD, Maestro, Chimera, PyMOL), RFDiffusion, ProteinMPNN, Adobe Illustrator, GraphPad Prism.