Title

Macrocyclic β-Hairpin Peptides Derived from Amyloid β, Medin, and the IQGAP1 WW Domain And Conversion of In-Person Chemistry Courses to Online Versions in Response to the COVID-19 Pandemic

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Macrocyclic β-Hairpin Peptides Derived from Amyloid β, Medin, and the IQGAP1 WW Domain
And
Conversion of In-Person Chemistry Courses to Online Versions in Response to the COVID-19 Pandemic

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Chemistry

by

William James Howitz

Dissertation Committee:
Professor James S. Nowick, Chair
Professor David L. Van Vranken
Professor Sergey Nizkorodov

2020

DEDICATION

To the educators, mentors, and many friends and family who have supported me on this journey.

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ACKNOWLEDGEMENTS

When I came to UCI in the fall of 2015 to begin my graduate school career, I did not realize how much of a roller coaster experience it would turn out to be. I experienced the lows of switching laboratories and working on projects that did not seem to have an end in sight, but I also experienced the highs of publishing my work and realizing that I wanted to pursue a career in teaching and chemistry education. The support of my advisers, mentors, colleagues, friends, and family was invaluable. They invested in my growth as a scientist and educator and encouraged me to persevere through every obstacle.

I want begin by thanking my two research advisers, Dr. Aaron Esser-Kahn and Dr. James Nowick, for their advice and their support of my professional development goals. After my first six months in the Esser-Kahn laboratory, I was not making substantial research progress. I was more interested in teaching and serving as a safety officer. I appreciate that Aaron met with me to discuss my progress and make it clear that a Ph.D. is a research degree and that I needed to find a balance between my research and other activities. It was a critique that was hard to take, but was important for me to hear early on so I could be successful as a graduate student. When Aaron chose to move his laboratory to the University of Chicago, I made the tough decision to stay at UCI. I am incredibly grateful to James for being willing to take me on as his student. Soon after I switched to the Nowick laboratory, I knew I wanted to pursue a career in teaching and chemistry education. I appreciate that James always provided his support, allowing me to participate in TAP-STEM, the Pedagogical Fellows program, serve as an instructor-of-record six times, and more. These experiences made me a competitive applicant for teaching faculty positions during a pandemic when the job market was seriously impacted.

My colleagues in the Esser-Kahn and Nowick laboratories, especially Dr. Janine Tom, Dr. Brittany Moser, Dr. Kate McKnelly, Tuan Samdin, and Gretchen Guaglianone all provided inspiration, advice, and encouragement for which I am grateful. Janine and Brittany were the first graduate student mentors I had in the Esser-Kahn laboratory and they helped me to grow into an independent researcher during my first two years of graduate school. Kate worked closely with me when I first switched to the Nowick laboratory, helping me to integrate into my new research community. Tuan and Gretchen grounded me. Our times together reminded me constantly that there is more to life than the science and the research.

The seven undergraduate student researchers I had the pleasure of mentoring in the Nowick laboratory and in the classroom are some of the brightest and most incredible people with which I have ever had the opportunity to work. Without the assistance and support of Ngoctran Tran, Ariana Motavalli, Cindy Saliba, Will Cabanela, Katelyn Haduong, Shareen Ashby, and Denise Bui I would not have been able to pursue professional development opportunities in teaching and pedagogy. I am thrilled that each of them have graduated and are going on to the next chapters in their own careers. I will miss working with them on a regular basis.

My mentors in teaching and chemistry education, Dr. Renée Link, Dr. Kim Edwards, and Dr. Susan King, were instrumental to my development as an instructor and as a mentor to students. If it were not for my first teaching experience in the chemistry department being with Renée, I am not sure if I would have remained at UCI after Aaron moved his laboratory to Chicago. She was inspiring and introduced me to many of the teaching and mentorship opportunities available at and beyond UCI. I am especially thankful that she took me on as a mentee through the TAP-STEM program because that experience served as the catalyst for later collaborations on chemistry

education research projects. I am appreciative of Kim and Susan because were the first to tell me that they saw me as a colleague and not just a graduate student. They empowered me to speak more freely by emphasizing that the ideas I had for teaching and chemistry education had merit.

Outside of the chemistry department, I want to thank Dr. Daniel Mann, Dr. Adrienne Williams, and Bri McWhorter, who exposed me to ideas and perspectives that made me a better educator and public speaker. Danny leads the Pedagogical Fellows Program through the Division of Teaching Excellence and Innovation. Through that program I saw how my peers in other disciplines approached teaching challenges and I found ways to adapt their strategies to improve the quality of my own instruction. Adrienne was the first to introduce me to teaching as research. I gained an understanding of the methods used to conduct education research and was able to apply much of what I learned to the chemistry education projects I worked on with Renée, Kim, and Susan. Bri is a speech and communications coach who is the founder and CEO of Activate to Captivate. I had the good fortune of being mentored by Bri through programs like TAP-STEM, the Mentoring Excellence Program, Improv for Teaching, and Activate to Captivate, all of which helped me to hone my presentation and teaching skills.

My friends and colleagues at UCI played integral roles in my graduate school journey. Dr. Sam Beasley and Mike Fazio provided transparent and honest advice to me when I came to UCI over the visitation weekend. Were it not for them, I would not have chosen to come to UCI for graduate school. I also want to thank Taylor Thane, Hayley Glicker, Taylor Frey, and Sarah Wang who I have had the pleasure of working with in teaching and in course/workshop design and implementation. They have been fantastic collaborators, colleagues, and friends. Finally, I want to give a special thank you to my partner in crime, Dr. Kate McKnelly. Together we made tremendous strides in research, teaching, mentorship, and chemistry education. I am so grateful for all of her support, especially when I switched into the Nowick laboratory part way through graduate school. I look forward to our continued friendship and collaborations in Atlanta.

My friends and family back home always had an encouraging word for me. I am especially grateful to my parents, Anna Schroder and Michael Howitz, who gave so much of their time and effort to mold me into the person I am today. I also want to thank my close friends from back home in Minnesota: Hannah Frey, Tessa Anderson, Miranda Chimzar, and Brittney Widmer. They were always interested in how I was doing personally and professionally and consistently made the time to talk with me despite the time difference. After chatting with them I always felt recharged and ready to tackle the next challenges to come my way.

Finally, I want to thank my fiancé, Dang Nguyen. He is the most incredible man. Without his support I would not have made it to the end of this journey. I look forward to now providing the support for him that he has provided to me over the last five years as he pursues his own career advancement opportunities.

VITA

Education

2020 Ph.D., Department of Chemistry, University of California, Irvine – Irvine, CA
2015 B.S., Hamline University – St. Paul, MN
- Majors: Chemistry, Biology
- Minor: Economics

Teaching and Mentorship Experience

Instructor of Record, University of California, Irvine (2018 – 2020)

2020 CHEM 51C Organic Chemistry Lecture
- Instructed ~150 students online using elements from specifications grading
- Engaged students with problem-solving sessions at the end of each lecture using breakout rooms moderated by peer tutors
- Developed a final project for students to have them identify a pharmaceutically relevant compound, summarize the history behind it, and propose a synthesis of the compound

2020 CHEM 51LC Organic Chemistry Laboratory Lecture
- Instructed ~125 students online using a specifications grading system
- Engaged students during class using Zoom chat, Google forms, and small group work in breakout rooms using Google slides
- Administered online assessments of laboratory techniques using Chemix
- Helped develop and refine a final project where students drafted a scientific journal article or poster based on a provided background handout and sample data

2019 CHEM 51C Organic Chemistry Lecture
- Instructed ~160 students through question-and-response and storytelling lecture styles
- Developed group problem-solving activities for discussion sections to compliment concepts covered in lecture with emphasis placed on explanations of underlying chemical principles
- Exams were restructured to include short answer sections to assess student comprehension of fundamental chemical principles

2019 CHEM 51B Organic Chemistry Lecture
- Instructed ~205 students through a question-and-response lecture style
- Incorporated in-class activities to expose students to relevant study strategies (e.g. assessing their content knowledge in the absence of notes/resources)
- Included examples of data from primary literature on reactions pertinent to the course for students to evaluate and identify trends in that they could correlate to fundamental chemical principles

2018 CHEM 51C Organic Chemistry Lecture
- Instructed ~140 students through question-and-response and storytelling lecture styles
- Developed in-class problem-solving activities to formatively assess conceptual understanding of reaction mechanisms and synthesis of target molecules
- Connected course material to historical events in chemical synthesis

2018 CHEM 51LD Organic Chemistry Laboratory Lecture

- Instructed ~60 students
- Emphasis put on strengthening critical thinking and written communication skills through group problem-solving activities
- Developed additional pre-lecture/laboratory assignments to encourage review and analysis of laboratory protocols and concepts

Teaching Assistant, University of California, Irvine (2016 – 2020)

2020 Departmental Head Teaching Assistant (TA)

- Responsible for supporting the conversion of the ~1400-student CHEM 1LC general chemistry laboratory and two ~300-student sections of CHEM 51C organic chemistry lecture to remote learning formats
- Assisted with scripting, directing, videotaping, and captioning experiments for students to watch
- Built video quizzes emphasizing fundamentals of laboratory techniques to supplement experiment videos
- Designed and implemented a new final exam for the laboratory course consisting of a multiple-choice test and two essay questions addressing proper laboratory techniques and procedures
- Segmented 28 existing organic chemistry lecture videos by topic to make 86 videos (all less than 20 min) and added captions for accessibility
- Developed video quizzes to accompany lecture videos to incentivize student accountability for course material
- Provided Zoom and YuJa trainings to ~25 graduate TAs and five faculty

2019-2020 CHEM 1L/2L General Chemistry Laboratory

- Designated head TA for the year (Fall to Spring); role changed to Departmental Head TA in Spring 2020
- Provided support for eight courses (four Fall quarter; four Winter quarter)
- Instructed laboratory TAs on weekly experiments including logistics, safety, troubleshooting, and general student instruction
- Worked with the instructor to design and implement active learning group activities to improve student comprehension of concepts including clock reactions, phase diagrams, extraction, and TLC

2018-2019 CHEM 51L Organic Chemistry Laboratory

- Designated head TA for the year (Fall to Spring), covering four laboratory courses total (two Fall quarter; one Winter quarter; one Spring quarter)
- Instructed laboratory TAs on weekly experiments including logistics, safety, troubleshooting, and general student instruction
- Developed grading calibration activities to ensure more consistent grading amongst TAs
- Prepared laboratory practicals each quarter (5-15 versions depending on the quarter)
- Implemented pre-laboratory video quizzes to improve student preparation for experiments
- Worked with the instructor to design and develop a specifications-based grading approach to the organic chemistry laboratory courses

2016-2018 CHEM 51L Organic Chemistry Laboratory

- Instructed two laboratory sections per quarter (~20 students per section) for three quarters

2017 CHEM 51C Organic Chemistry Lecture
- Instructed ~200 students across ten discussion sections per week
- Proctored and graded all exams

2016 PHRMSCI 173 Pharmacotherapy Lecture
- Led one discussion section per week (~50 students)
- Assisted course instructor with exam proctoring and grading

Research Mentor, University of California, Irvine (2017 – 2020)

2019-2020 Denise Bui, Undergraduate Student Researcher
- Degree: B.S. Biochemistry
- Date of Graduation: June 2020
- Analytical Chemist at ERC Inc.

2018-2020 Shareen Ashby, Undergraduate Student Researcher
- Degree: B.S. Chemistry
- Date of Graduation: June 2020
- Applying to Master's program in chemistry

2018-2020 Katelyn Haduong, Undergraduate Student Researcher
- Degree: B.S. Pharmaceutical Sciences
- Date of Graduation: June 2020
- Applying for jobs in pharmaceutical sciences

2018-2019 Will Cabanela, Undergraduate Student Researcher
- Degree: B.S. Neurobiology
- Date of Graduation: June 2019
- UROP Fellow (2018-2019)
- Advisor at the Learning and Academic Resource Center (LARC) at UCI

2018-2019 Cindy Saliba, Undergraduate Student Researcher
- Degree: B.S. Chemistry
- Date of Graduation: June 2019
- UROP Fellow (2018-2019)
- Applying to Master's programs in sustainable engineering

2017-2019 Ariana Motavalli, Undergraduate Student Researcher
- Degree: B.S. Chemistry, B.S. Biology
- Date of Graduation: June 2019
- UROP Fellow (2016-2017, 2017-2018)
- Dental Student at the University of California, Los Angeles (UCLA) School of Dentistry

2017-2018 Ngoctran Tran, Undergraduate Student Researcher
- Degree: B.S. Pharmaceutical Sciences
- Date of Graduation: June 2018
- UROP Fellow (2016-2017, 2017-2018)
- Pharmacy Student at the University of Southern California (USC) School of Pharmacy

Teaching Assistant, Hamline University (2012 – 2015)

2014-2015 CHEM 3450/3550 Organic Chemistry Laboratory
- Taught two laboratory sections (~20 students per section)

2012-2015 CHEM 1130/1140 General Chemistry Laboratory
- Taught a total of eight laboratory sections (~20 students per section)
- Served as the head TA for 2014-2015 academic year

Pedagogical Development & Service

2020 Division of Teaching Excellence and Innovation (DTEI) Course Design Certification Program, University of California, Irvine
- Co-facilitated this eight-hour program that served 95 graduate students, post-docs, and faculty
- Covered content including backwards course design, student learning objectives, sequencing, scaffolding, campus resources, types of grading systems, types of assessments, instructional methods, and flexibility in assessments and course policies
- Content was delivered with consideration for both in-person and online courses

2020 DTEI Summer Fellow Coordinator, University of California, Irvine
- Mentored 48 DTEI Fellows across the physical science and engineering schools as they worked to support the design and implementation of online courses for Fall 2020
- Helped develop one-hour workshops on inclusive pedagogy and flexibility in assessment and course policies
- Offered group and individual consultations for troubleshooting challenges with the transition of in-person course elements to comparable online versions

2020 Transforming Your Research into Practice (TYRIT), University of California, Irvine
- Served as a co-facilitator for this multi-institutional 7-week course design program that served 25 graduate students at UCI (and ~210 graduate student in total across all eight institutions)
- Developed workshop content covering student learning objectives, types of grading systems, design of assessment rubrics, exam blueprints, and curriculum mapping
- Assisted with the design of surveys to assess program success

2018-2020 Chemistry TA Mentor Program (CTAMP), University of California, Irvine
- Founding member
- Mentored six (2018-2019) or eight (2019-2020), first-year graduate students to be effective TAs
- Ran a two-day orientation program before fall covering lesson planning, evaluations, grading, electronic tools, and TA-student communication
- Met with mentees twice a quarter to prepare for teaching assignments, set goals, develop good teaching practices, discuss mid-quarter feedback, and reflect on progress in teaching
- Conducted one teaching observation of each mentee in winter quarter (2018-2019) or fall quarter (2019-2020)

2017-2020 Graduate Safety Team, University of California, Irvine
- Founding member and head of the Education & Resources Committee
- Set up seminars featuring industry professionals, professors, and graduate student researchers on laboratory safety topics
- Drafted quarterly departmental newsletters

2015-2020 Chemistry Department Outreach Assistant, University of California, Irvine
- Explained chemistry concepts to K-12 students
- Emphasis on short, visual demonstrations to generate student interest in science

2019 CHEM 199 Science Olympiad: Training for Event Writers, University of California, Irvine
- Drafted this hybrid online course for first-time undergraduate and graduate students interested in participating in Science Olympiad as event writers and proctors
- Assisted in the instruction of ~55 students (~50 undergraduate and ~5 graduate)
- Topics covered included writing questions using knowledge of Bloom's taxonomy and readability, consideration of the pitfalls of grading open answer questions and scaffolding in information to assist student comprehension, and building and testing the event prior to implementation.

2018-2019 DTEI Pedagogical Fellow, University of California, Irvine
- Responsible for implementing the 12-hour TA Professional Development Program (TAPDP) through construction of workshops covering topics such as lesson planning/instruction, active learning and dynamic lecturing, diversity and inclusion, campus resources, online teaching tools, and student health and academic challenges
- Completed University Studies 390A, B, and C (100 hours of training)

2016-2019 Science Olympiad Orange Country Regional Event Writer and Proctor
- Developed and led the Crime Busters B event three years in a row

2017-2018 Teaching Apprentice Program in STEM (TAP-STEM) Trainee, University of California, Irvine
- Program name changed to Summer Teaching Apprenticeship Program (STAP)
- Mentored by Professor Renee Link on course design and evidence-based teaching practices
- Guest lectured at least three times per quarter
- Received scientific communication and public speaking coaching from Bri McWhorter, Founder and CEO of Activate to Captivate
- Program culminated with teaching CHEM 51LD as an instructor-of-record

2018 University Studies 395, Teaching as Research, University of California, Irvine
- Instruction given on research into teaching practices stressing knowledge of survey design, IRB approval, and course observations (COPUS)

2018 University Studies 390X, Developing Teaching Excellence, University of California, Irvine
- Instruction given on Bloom's taxonomy, identification of student learning outcomes, the learning process, evidence-based practices, and lesson/course development

2016-2017 Laboratory Safety Officer, Esser-Kahn Laboratory, University of California, Irvine
- Wrote and updated SOPs, maintained chemical inventory, and conducted laboratory safety orientations with new laboratory members

Research

2018-2020 Chemical Education Research, Department of Chemistry, University of California, Irvine
- Advisor: Professor Renee Link
- Design and implementation of a specifications grading system in organic chemistry laboratory courses and the development of a grading calibration exercise to reduce grading variation between TAs in large laboratory courses

2017-2020 Graduate Research, Department of Chemistry, University of California, Irvine
- Advisor: Professor James Nowick
- Synthesis and characterization of macrocyclic peptide models to study the self-assembly of biologically relevant proteins including medin, responsible for vascular aging, and amyloid beta, responsible for Alzheimer's disease

2015-2017 Graduate Research, Department of Chemistry, University of California, Irvine
- Advisor: Professor Aaron Esser-Kahn
- Validation of a library of NLRP3 inflammasome-activating polymers as novel vaccine adjuvants elucidating their *in vitro* mode of action using bone marrow-derived dendritic cells and monocytes

2014-2015 Departmental Honors Research, Department of Chemistry, Hamline University
- Advisor: Professor Rita Majerle
- Thesis: The roles of electrophiles and aromaticity on the stability of isocyanides prepared from the deprotonation/metalation of oxazolic species

Honors, Awards, and Certificates

2020	Activate to Captivate Public Speaking Certificate
2020	Most Promising Future Faculty in Organic Chemistry Award
2020	Graduate Award for Extraordinary Departmental Service
2020	DTEI Summer Fellow Coordinator Fellowship
2020	Improv for Teaching Certificate
2019	Teaching Excellence Certificate
2019	DTEI Pedagogical Fellowship
2018	Outstanding Contributions to the Chemistry Department Teaching Program Award
2018	Mentoring Excellence Certificate
2018	Course Design Program Certificate
2018	CIRTL Associate
2016	Vertex Fellowship
2015	Hamline University Honors
2015	American Institute of Chemists Award
2015	Lund Speaking Competition, First Place
2011-2015	Hamline University Trustee Scholarship
2014	Phi Beta Kappa, Junior Induction
2014	3M/Ronald A. Mitsch Chemistry Scholarship
2014	Hervey Biology Scholarship
2014	ACS Undergraduate Award in Inorganic Chemistry
2014	ACS Undergraduate Award in Analytical Chemistry
2014	Carolyn V. Beggs Endowed Scholarship in Chemistry
2013	Batchelder Memorial Scholarship in Chemistry
2013	Carolyn V. Beggs Endowed Scholarship in Chemistry

Publications

Howitz, W. J.*; McKnelly, K. J.*; Thane, T. A.; Link, R. D. Scaling up a specifications grading system in an organic chemistry laboratory course with over 1,000 students. (Manuscript in Preparation)
(* indicates co-first authors)

Howitz, W. J.; McKnelly, K. J.; Haduong, K.; Guaglianone, G.; Ashby, S.; Laayouni, M.; Nowick, J. S. Macrocyclic peptides derived from familial Alzheimer's disease mutants show charge-dependent oligomeric assembly and toxicity. (Manuscript in Preparation)

McKnelly, K. J.; **Howitz, W. J.**; Kreutzer, A. G.; Haduong, K. P.; Yoo, S.; Hart, C.; Nowick, J. S. Effects of familial Alzheimer's disease mutations on the assembly of a constrained β-hairpin peptide derived from Aβ. (Manuscript in Preparation)

Kimani, F.; Ajit, J.; Galluppi, A.; Manna, S.; **Howitz, W. J.**; Tang, S.; Esser-Kahn, A. P. Receptor-ligand kinetics influence the mechanism of action of covalently linked TLR ligands. (Manuscript Submitted)

Howitz, W. J.*; McKnelly, K. J.*; Link, R. D. Developing and implementing a specifications grading system in an organic chemistry laboratory course. *J. Chem. Educ*. **2020**.
(* indicates co-first authors)

Howitz, W. J.; Wierzbicki, M.; Cabanela, W.; Saliba, C.; Motavalli, A.; Tran, N; Nowick, J. S. Interpenetrating cubes in the X-ray crystallographic structure of a peptide derived from medin$_{19\text{-}36}$. *J. Am. Chem. Soc*. **2020**, *142*, 15870-15875.

Howitz, W. J.; Guaglianone, G.; King, S. M. Converting a third quarter organic chemistry course to an online format in two weeks: Design and implementation. *J. Chem. Educ*. **2020**, *97*, 2581-2589.

Howitz, W. J.*; Thane, T. A.*; Frey, T. L.*; Wang, X. S.*; Gonzales, J. C.; Tretbar, C. A.; Seith, D. D.; Saluga, S.; Lam, S.; Nguyen, M. M.; Tieu, P; Link, R. D.; Edwards, K. D. Online in no time: Design and implementation of a remote learning first quarter general chemistry laboratory and second quarter organic chemistry laboratory. *J. Chem. Educ*. **2020**, *97*, 2624-2634.
(* indicates co-first authors)

McKnelly, K. J.; **Howitz, W. J.**; Lam, S.; Link, R. D. Extraction on paper activity: An active learning technique to facilitate student understanding of liquid-liquid extraction. *J. Chem. Educ*. **2020**, *97*, 1960-1965.

Samdin, T. D.; Wierzbicki, M.; Kreutzer, A. G.; **Howitz, W. J.**; Valenzuela, M. R.; Smith, A.; Sahrai, V.; Truex, N. L.; Klun, M.; Nowick, J. S. Effects of *N*-terminal residues on the assembly of constrained β-hairpin peptides derived from Aβ. *J. Am. Chem. Soc*. **2020**, *142*, 11593-11601.

Manna, S.*; **Howitz, W. J.***; Oldenhuis, N. J.; Eldredge, A. C.; Shen, J.; Nihesh, F. N.; Lodoen, M. B.; Guan, Z.; Esser-Kahn, A. P. Immunomodulation of the NLRP3 inflammasome through structure-based activator design and functional regulation via lysosomal rupture. *ACS Cent. Sci.* **2018**, *4*, 982-995.
(* indicates co-first authors)

Presentation

2020 Biennial Conference on Chemical Education (BCCE) – Corvallis, OR (Oral)
- "Development of a Specifications Grading Approach to the Organic Chemistry Laboratory"
- "Improving Consistency and Equity in Grading through a Collaborative Calibration Exercise"
- Because of the global COVID-19 pandemic, the 2020 Biennial Conference on Chemical Education was terminated on April 2, 2020, by the Executive Committee of the Division of Chemical Education, American Chemical Society; and, therefore, these presentations could not be given as intended.

2020 Chemistry and Biology of Peptides Gordon Research Conference – Ventura, CA (Poster)
- "Interpenetrating Cubes in the X-Ray Crystallographic Structure of a Peptide Derived from Medin$_{19\text{-}36}$"

2020 Society for the Advancement of Biology Education Research (SABER) West – Irvine, CA (Poster)
- "Development of a Specifications Grading Approach to the Organic Chemistry Laboratory"

2018 University of California Chemical Symposium – Lake Arrowhead, CA (Oral)
- "Minuscule Variations in Chemical Structure Lead to Significant Differences in NLRP3 Inflammasome Activation Solely due to Variations in Lysosomal Rupture"

2017 15th Annual UCI Immunology Fair – Irvine, CA (Poster)
- "Minuscule Variations in Chemical Structure Lead to Significant Differences in NLRP3 Inflammasome Activation Solely due to Variations in Lysosomal Rupture"

2016 American Chemical Society National Meeting – San Francisco, CA (Poster)
- Quantification of the Minimum Number of TLR2 Agonists Necessary to Elicit a Detectable Immune Response"

2015 National Conference on Undergraduate Research – Spokane, WA (Oral)
- The Role of Aromaticity on the Stability of Isocyanides Prepared from the Deprotonation/Metalation of Oxazolic Species"

2015 American Chemical Society National Meeting – Denver, CO (Poster)
- Effect of Aromaticity on the Stability of Isonitriles Prepared from the Deprotonation/Metalation of Oxazolic Species"

2014 Associated Colleges of the Twin Cities Chemistry Summer Research Meeting – St. Paul, MN (Poster)
- The Effect of Aromaticity on the Stability of Isocyanides Prepared from the Deprotonation of Oxazolic Species"

ABSTRACT OF THE DISSERTATION

Macrocyclic β-Hairpin Peptides Derived from Amyloid β, Medin, and the IQGAP1 WW Domain
And
Conversion of In-Person Chemistry Courses to Online Versions in Response to the COVID-19 Pandemic

by

William James Howitz

Doctor of Philosophy in Chemistry

University of California, Irvine, 2020

Professor James S. Nowick, Chair

The dissertation is composed of two sections. The first section is composed of chapters one, two, and three and describes the study of macrocyclic β-hairpin peptides derived from β-sheet peptides. The second section is composed of chapters four and five and discusses the curricular innovations made to support the remote instruction of an organic chemistry lecture course, a general chemistry laboratory course, and an organic chemistry laboratory course at the onset of the COVID-19 pandemic.

Chapter 1 describes the use of acetylation as a means to modulate and study the effect of charge on the oligomeric assembly and toxicity of familial Alzheimer's disease (FAD) mutants of Aβ. FAD is an inherited form of Alzheimer's disease that has an earlier onset due to point mutations within the sequence of Aβ that alter the rate of aggregation and toxicity of the peptide. The most common site of these mutations is position 22 in which the native glutamic acid may be replaced with a glycine (E22G), glutamine (E22Q), or lysine (E22K). Previous work in our lab using a macrocyclic chemical model system of the Aβ peptide that incorporates residues 16-22

and 30-36 established that there was a correlation between the net charges of these mutant peptides and their oligomeric assemblies and toxicities as measured by SDS-PAGE, LDH-release assays, and dye leakage assays. In this chapter I further probe the effect of charge on the oligomeric assembly and toxicity of the FAD mutant peptides using our lab's chemical model system of Aβ. To control for the hydrophobicity and size of the residues that vary between the mutants, I used acetylation as a tool to manipulate the net charge of the peptides. This work demonstrates that the toxicities of the peptides strongly correlate with their net charges based on LDH-release assays and dye leakage assays. The oligomeric assemblies of the peptides assessed by SDS-PAGE suggest that charge is a factor that impacts their assembly, but that the position of acetylation also influences the assembly.

Chapter 2 discusses the synthesis and X-ray crystallographic structure of a macrocyclic peptide derived from an amyloidogenic peptide called medin. Amyloidogenic peptides and proteins are rich sources of supramolecular assemblies. Sequences derived from well-known amyloids, including Aβ, human islet amyloid polypeptide, and tau have been found to assemble as fibrils, nanosheets, ribbons, and nanotubes. The supramolecular assembly of medin, a 50-amino acid peptide that forms fibrillary deposits in aging human vasculature, has not been heavily investigated. In this chapter, I present an X-ray crystallographic structure of a cyclic β-sheet peptide derived from the 19−36 region of medin that assembles to form interpenetrating cubes. The edge of each cube is composed of a single peptide, and each vertex is occupied by a divalent metal ion. This structure may be considered a metal−organic framework (MOF) containing a large peptide ligand. This work demonstrates that peptides containing Glu or Asp that are preorganized to adopt β-hairpin structures can serve as ligands and assemble with metal ions to form MOFs.

Chapter 3 presents the development of an IQGAP1 WW domain-derived peptide with the ability to bind p110α. IQGAP1 is a scaffold protein that mediates the PI(3)K-Akt pathway that is upregulated in many cancers. The WW domain within the scaffold directly binds to p110α, one of the subunits of PI(3)K. Disrupting the interaction between p110α and the WW domain using a competitive inhibitor has been proposed as a promising approach to selectively negatively affecting cancer cells dependent on the PI(3)K-Akt pathway. In this chapter, I share the design and synthesis of a β-hairpin mimic peptide derived from the WW domain of IQGAP1, and I demonstrate that the peptide can compete for binding to p110α against the native IQGAP1 WW domain. This study is ongoing and future work will focus on elucidating the secondary structure of the peptide inhibitor by NMR and X-ray crystallography and determining if the secondary structure the peptide adopts is essential for its binding capability.

Chapters 4 and 5 describe the conversion of in-person chemistry courses to online versions in response to the COVID-19 pandemic. As the SARS-CoV-2 pandemic spread throughout the world, universities were faced with extraordinary challenges. Shelter-in-place orders were given, in-person classes were cancelled, and at the University of California Irvine, instructors had less than two weeks to convert spring quarter classes from a face-to-face to an online format. A team-based approach was essential to making this transition. In chapter 4 I share the insights gained during the design and implementation of the final quarter of a large-enrollment online organic chemistry course, as well as student perspectives on the efficacy of key components of the course. In chapter 5, I describe how the curricular, administrative, and logistical challenges of high enrollment general and organic chemistry laboratories were addressed in the transition to remote teaching. I discuss the reasoning behind the approach, how the existing web-based course content was leveraged, the additions and alterations to the curriculum, the replacement of experimental

work with videos, the results of both student and TA surveys, and the lessons learned for iterations of these courses in the near future.

Chapter 1: Macrocyclic Peptides Derived from Familial Alzheimer's Disease Mutants Show Charge-Dependent Oligomeric Assembly and Toxicity

1.1 Preface

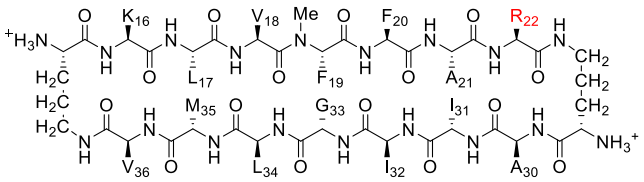
When I joined the Nowick laboratory at the beginning of my third year of graduate school, I had the opportunity to be mentored by Dr. Kate McKnelly. At the time, she was also a third year graduate student, but she had an established project investigating how familial mutants of Aβ altered supramolecular assembly and toxicity. She was able to finish collecting all of the data needed for her project during her third year and began working on a new project that was an extension of her work which has since developed into the project I will discuss in this chapter. Dr. McKnelly was unable to complete this project because she developed an allergy to uronium coupling agents at the beginning of her fourth year. At that time I took over the project and worked to complete it with the assistance of her two undergraduate students, Katelyn Haduong and Shareen Ashby, whom I continued to train and mentor. Of the twelve peptides in this study, the three non-acetylated peptides were previously synthesized, purified, and characterized by Dr. McKnelly. Katelyn and Shareen assisted me with the synthesis, purification, and characterization of the nine acetylated peptides. I also ran the SDS-PAGE gels and performed the LDH-release assays. The dye leakage assays were conducted in collaboration with fellow lab member, Gretchen Guaglianone.

1.2 Introduction

Charge plays an essential role in molecular recognition. Phosphorylation of receptor-tyrosine kinases triggers the transduction of signals that allow the release of growth factors and hormones like insulin by converting neutral hydroxy groups to anionic phosphate groups.[1] Removal of these phosphate groups by phosphatases halts downstream signaling.[2] C-terminal amidation increases the binding affinity of corticotropin-releasing hormone, thyrotropin-releasing hormone, and other peptide hormones to their G-protein coupled receptors and thus enhances cell signaling by eliminating the negative charge of the C-terminal carboxylate group.[3] Acetylation of the positively charged ammonium groups of the lysine residues of histone proteins by acetyltransferases increases gene expression by reducing the electrostatic interactions between those proteins and DNA.[4] Removal of the acetyl groups by histone deacetylases has the opposite effect.[4]

In the current study, we set out to use acetylation to study the impact of charge on the assembly and toxicity of peptides derived from familial Alzheimer's disease (FAD) mutants of Aβ. We had previously found that a β-hairpin peptide derived from $A\beta_{16–36}$, peptide **1a**, assembles in both the crystal state and in SDS-PAGE to form a hexamer (Figure 1).[5] We recently found that familial Alzheimer's disease mutations at position 22 of this peptide alters the oligomeric assembly and cytotoxicity of this peptide.[6] In this study, we compared a peptide derived from wild-type Aβ (peptide **1a**) to peptides containing the E22Q and E22K mutations (peptides **2a** and **3a**) and found increasing toxicities across this series of peptides, all of which formed hexamers by SDS-PAGE (Figure 1). Each of these macrocyclic peptides is composed of two heptapeptide strands ($A\beta_{16–22}$ and $A\beta_{30-36}$) joined by δ-linked ornithine turn units ($^{\delta}$Orn).[7,8] *N*-Methylation of the peptide backbone attenuates uncontrolled aggregation.[9] The peptides differ

only in the residue at position 22, and the resulting increasing charge of the peptides at physiological pH (+2, peptide **1a**; +3, peptide **2a**; +4 peptide **3a**) correlates with increasing toxicity toward SH-SY5Y cells in an LDH-release assay and increasing membrane disruption of negatively charged LUVs in a dye leakage assay.



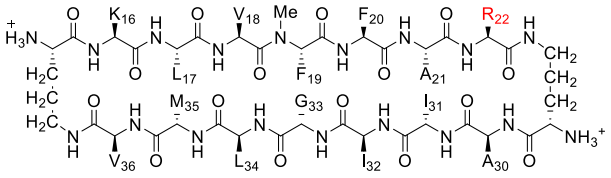
peptide **1a** (R_{22} = E); **1b** (R_{22} = Q); **1c** (R_{22} = K)

Familial mutations at position 22 have also been studied extensively by other researchers. The E22G, E22Q, and E22K mutants of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ aggregate into oligomers at faster rates than wild-type Aβ and have enhanced cellular toxicity.[10-25] Some studies have attributed the enhanced aggregation rates and toxicity of the E22G and E22Q mutants in part to the greater hydrophobicity of the glycine and glutamine residues relative to the charged glutamic acid residue.[11,21,23,25] A separate study has attributed the increased aggregation rates of E22G, E22Q, and E22K primarily to differences in the size of the amino acid residues.[17] The authors of that study suggest that as the steric bulk of the side chain decreases, the rate of aggregation increases. Still other studies suggest that it is the charge of the amino acid residues that is responsible for the differences in the aggregation rates and toxicities of the peptides.[10,22] Replacement of the charged glutamic acid residue with a neutral glycine or glutamine may reduce the electrostatic repulsion between adjacent peptides in the oligomeric state, stabilizing the oligomer.[10] Although replacement of glutamic acid with lysine would be expected to still have some electrostatic repulsion, the researchers suggest that the repulsion is not as substantial likely due to the greater conformational freedom of the lysine side chain relative to the glutamic acid side chain.

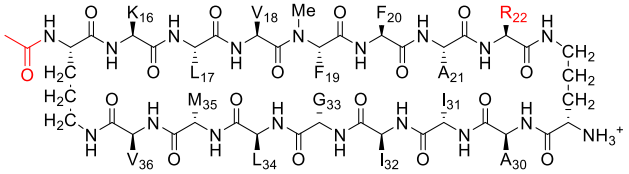
No single factor — hydrophobicity, size, or charge — can fully explain the observed differences in the aggregation rates and toxicities of the E22 mutants. A method other than amino acid mutation is necessary to understand the contribution of each factor independently because amino acid mutation may affect all three factors at once.

In the current study we set out to further probe the role of charge in the oligomeric assembly and toxicity of the E22 mutants of Aβ using the chemical model system we developed. To control for the hydrophobicity and size of the amino acid residues and focus on the impact of net charge, we acetylated the α-amino group of either or both of the $^{\delta}$Orn residues in peptides **1a**, **2a**, and **3a** (Chart 1).[26] Peptides **1b**, **2b**, and **3b** are acetylated on the top strand, peptides **1c**, **2c**, and **3c** are acetylated on the bottom strand, and peptides **1d**, **2d**, and **3d** are acetylated on both strands. This approach allows for a controlled decrease of the net charge of each peptide without altering the amino acid sequence of each peptide. We hypothesized that acetylating the more positively charged peptides would cause them to behave like the less positively charged peptides.

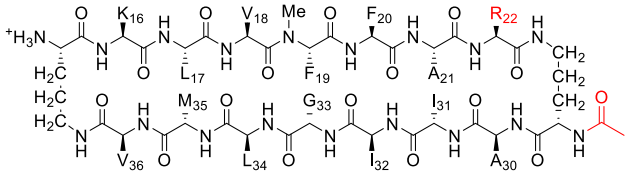
Chart 1.1. Summary of peptides, location of acetylation, and their net charges.



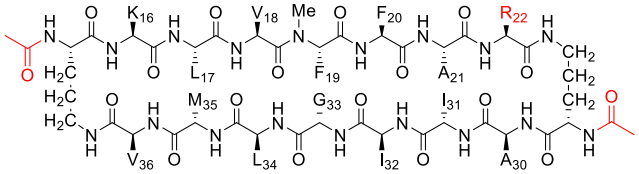
peptides **1a, 2a, 3a**



peptides **1b, 2b, 3b**



peptides **1c, 2c, 3c**



peptides **1d, 2d, 3d**

peptide	R_{22}	acetylated position	net charge
1a	E	none	+2
1b	E	top strand	+1
1c	E	bottom strand	+1
1d	E	top and bottom strands	0
2a	Q	none	+3
2b	Q	top strand	+2
2c	Q	bottom strand	+2
2d	Q	top and bottom strands	+1
3a	K	none	+4
3b	K	top strand	+3
3c	K	bottom strand	+3
3d	K	top and bottom strands	+2

1.3 Results and Discussion

We evaluated the effects of charge on the assembly and toxicity of peptides **1–3** by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), LDH-release assays with SH-SY5Y cells, and dye leakage assays using liposomes.

1.3.1 SDS-PAGE

We assessed how the net charge of the twelve peptides affect the propensity of those peptides to assemble into oligomers using SDS-PAGE. In SDS-PAGE, non-acetylated peptides **1a**, **2a**, and **3a** all migrate near the 10 kDa marker band at molecular weights consistent with hexamers (~10.6 kDa) although the shapes of the bands differ (Figure 1.1). Peptide **1a** forms a band in which the higher molecular weight region is rounded and intense and the lower molecular weight region is more diffuse, suggesting a hexamer in equilibrium with lower-order oligomers. The comet-like appearance of this band suggests that lower-order oligomers in rapid equilibrium with the hexamer migrate more quickly as the band migrates down the gel. As the lower-order oligomers migrate more quickly, they become more dilute, peeling away from the more concentrated region of the band. The greater dilution shifts the equilibrium further toward lower order oligomers, leading to yet more rapid migration and the formation of an elongated diffuse region. In contrast to peptide **1a**, peptides **2a** and **3a** form tighter bands, suggesting the formation of more stable oligomers. The more compact shape of these bands suggests that the equilibrium more strongly favors the hexamer over any other lower-order oligomers.

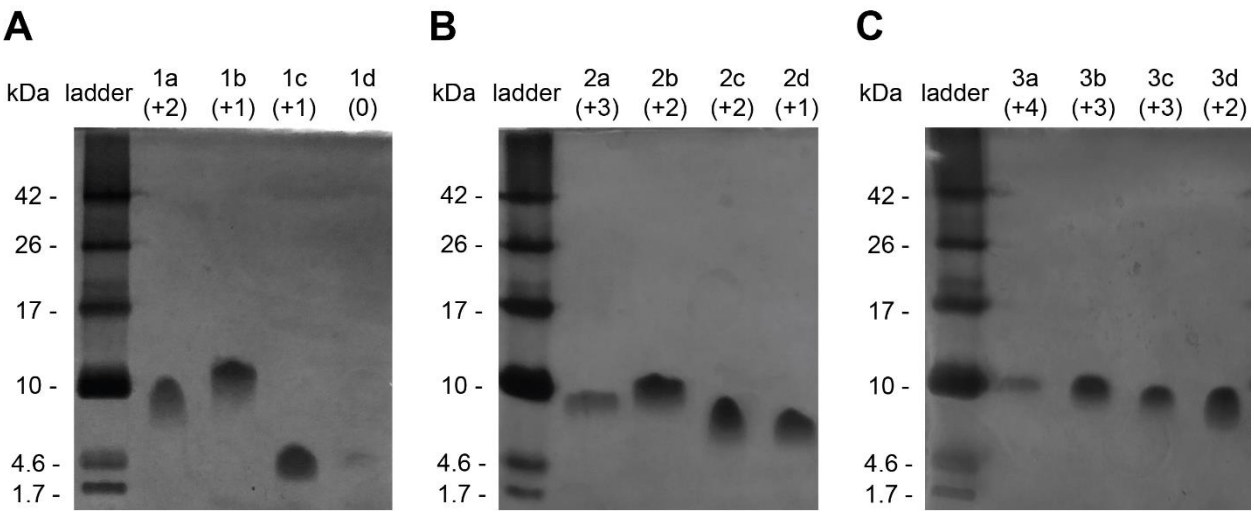


Figure 1.1. Oligomeric assembly of peptides determined by SDS-PAGE. All peptides were run at a concentration of 0.2 mg/mL and silver stained. A) Peptides **1a** (+2), **1b** (+1), **1c** (+1), and **1d** (0). B) Peptides **2a** (+3), **2b** (+2), **2c** (+2), and **2d** (+1). C) Peptides **3a** (+4), **3b** (+3), **3c** (+3), and **3d** (+2).

The diacetylated peptides **1d**, **2d**, and **3d** migrate lower on the gels than their non-acetylated counterparts. Peptide **1d** forms a tight band that migrates near the 4.6 kDa marker band at a molecular weight consistent with a trimer (~5.6 kDa). Peptides **2d** and **3d** form elongated bands that migrate slightly below the 10 kDa marker band. The elongated bands formed by peptides **2d** and **3d** have a more intense higher molecular weight region and a more diffuse lower molecular weight region, similar to the band formed by peptide **1a**. The different net charges of peptide **1a** (+2) and peptide **1d** (neutral) could be responsible for the differences in the oligomeric assemblies of the peptides on the gel. Similarly, differences in net charge could be responsible for the differences in the types of bands formed by peptides **2a** and **3a** compared to peptides **2d** and **3d**. The change in the shape of the bands from tighter to more elongated correlates with a reduction in the net charge of the peptides (+4, peptide **3a** versus +2 peptide **3d**; +3, peptide **2a** versus +1, peptide **2d**).

The monoacetylated peptides **1b** and **1c** migrate differently in SDS-PAGE, even though both peptides have the same net charge (+1). Peptide **1b** migrates as an elongated band above the

10 kDa marker band and slightly above peptide **1a**, while peptide **1c** migrates as an elongated band at the 4.6 kD marker band. Peptide **2b** also migrates as an elongated band above the 10 kDa marker band and slightly above peptide **2a**, while peptide **2c** migrates as a comet-shaped band below the 10 kDa marker band. Peptides **3b** and **3c** show similar, but less pronounced differences in migration. The differences in migration of each of these pairs of peptides is surprising, because both members of each pair have comparable net charge. The differences in their behavior suggest that the position of acetylation may also have an effect upon the oligomeric assembly of the peptides.

1.3.2 Cytotoxicity

We treated SH-SY5Y cells with the twelve peptides to assess how the net charge of the peptides may correlate to their cytotoxicity. Of the non-acetylated mutant peptides, peptide **1a** proved the least cytotoxic, exhibiting toxicity at 50 μM (Figure 1.2). Peptide **2a** is more toxic, exhibiting toxicity at 25 μM. Peptide **3a** is the most toxic of the non-acetylated peptides, exhibiting toxicity at 12.5 μM. The increasing cytotoxicity correlates with the increasing net charge of these peptides: +2, peptide **1a**; +3, peptide **2a**; +4 peptide **3a**.

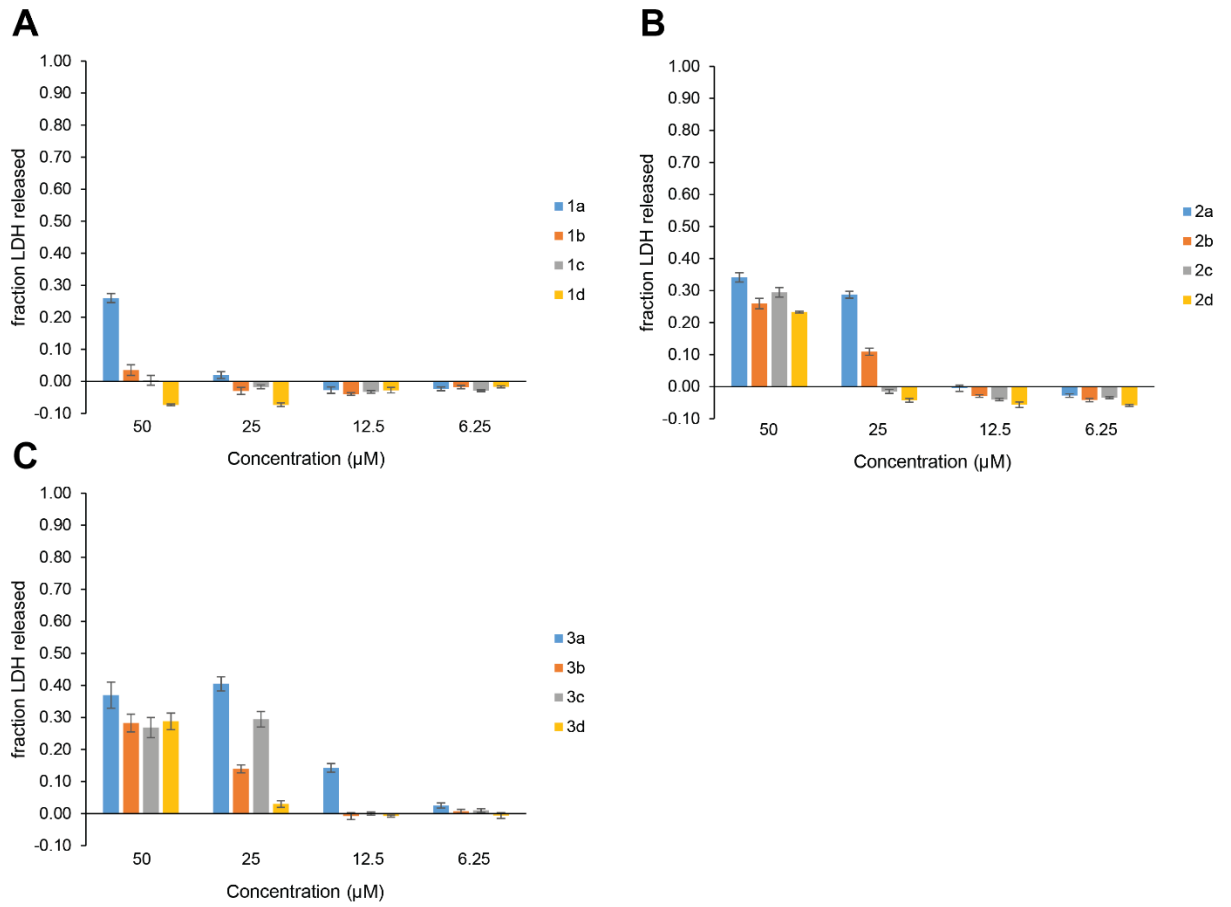


Figure 1.2. Cytotoxicity of peptides determined by LDH-release assay. Concentrations of peptides ranging from 6.25 to 50 μM were tested with water (vehicle) as the negative control and lysis buffer as the positive control. A) Peptides **1a** (+2), **1b** (+1), **1c** (+1), and **1d** (0). B) Peptides **2a** (+3), **2b** (+2), **2c** (+2), and **2d** (+1). C) Peptides **3a** (+4), **3b** (+3), **3c** (+3), and **3d** (+2).

The acetylated variants of peptides **1a**, **2a**, and **3a** are less toxic than their non-acetylated counterparts. Peptides **1b**, **1c**, and **1d** do not exhibit toxicity at any of the concentrations tested. Peptide 2b exhibits toxicity at 25 μM and peptides **2c**, and **2d** exhibit toxicity at 50 μM. Peptides **3b** and **3c** exhibit toxicity at 25 μM, and peptide **3d** exhibits toxicity at 50 μM.[27] The cytotoxicities thus correlate with the net charges of the peptides. The peptides with a net charge of +3 (peptides **2a**, **3b**, and **3c**) exhibit toxicity at 25 μM. The peptides with a net charge of +2 (peptides **1a**, **2c**, and **3d**) exhibit toxicity at 50 μM except peptide 2b which exhibits toxicity at 25 μM. And, the peptides with net charges of +1 or 0 do not exhibit toxicity at any of the

concentrations tested (peptides **1b**, **1c**, **1d**) except for peptide **2d** which exhibits toxicity at 50 μM.

1.3.3 Membrane Destabilization

The cytotoxicity of Aβ is associated with the binding to and disruption of cell membranes, and previous studies have demonstrated that Aβ binds and disrupts negatively charged lipid bilayers better than neutrally charged ones.[28,29-38] To investigate whether the cytotoxicity of the twelve peptides in our study also correlates with membrane disruption, we assessed the extent to which the peptides destabilized the membranes of negatively charged large unilamellar vesicles (LUVs) composed of 1:1 phosphatidylcholine:phosphatidylserine (PC:PS) in a dye leakage assay. In a dye leakage assay, LUVs which encapsulate a fluorescent dye are exposed to increasing concentrations of peptide. Destabilization of the membranes of the LUVs by the peptides releases the encapsulated dye and the reduced self-quenching and increased fluorescence is detected spectrophotometrically.[39] Lysis buffer is used as a positive control and is normalized to 100% dye leakage; water is used as a negative control and is normalized to 0% dye leakage. A nonlinear regression curve is fit to the normalized data points for each peptide (Figure 3). The effective concentrations at which the non-acetylated peptides cause 50% dye leakage (EC_{50}) are 1625 nM for peptide **1a**, 520 nM for peptide **2a**, and 211 nM for peptide **3a** (Figure 1.3). The membrane destabilization exhibited by these peptides follows the same trend as their cytotoxicities. The increasing membrane destabilization by these peptides also correlates with the increasing net charge of the peptides (+2, peptide **1a**; +3, peptide **2a**; +4 peptide **3a**).

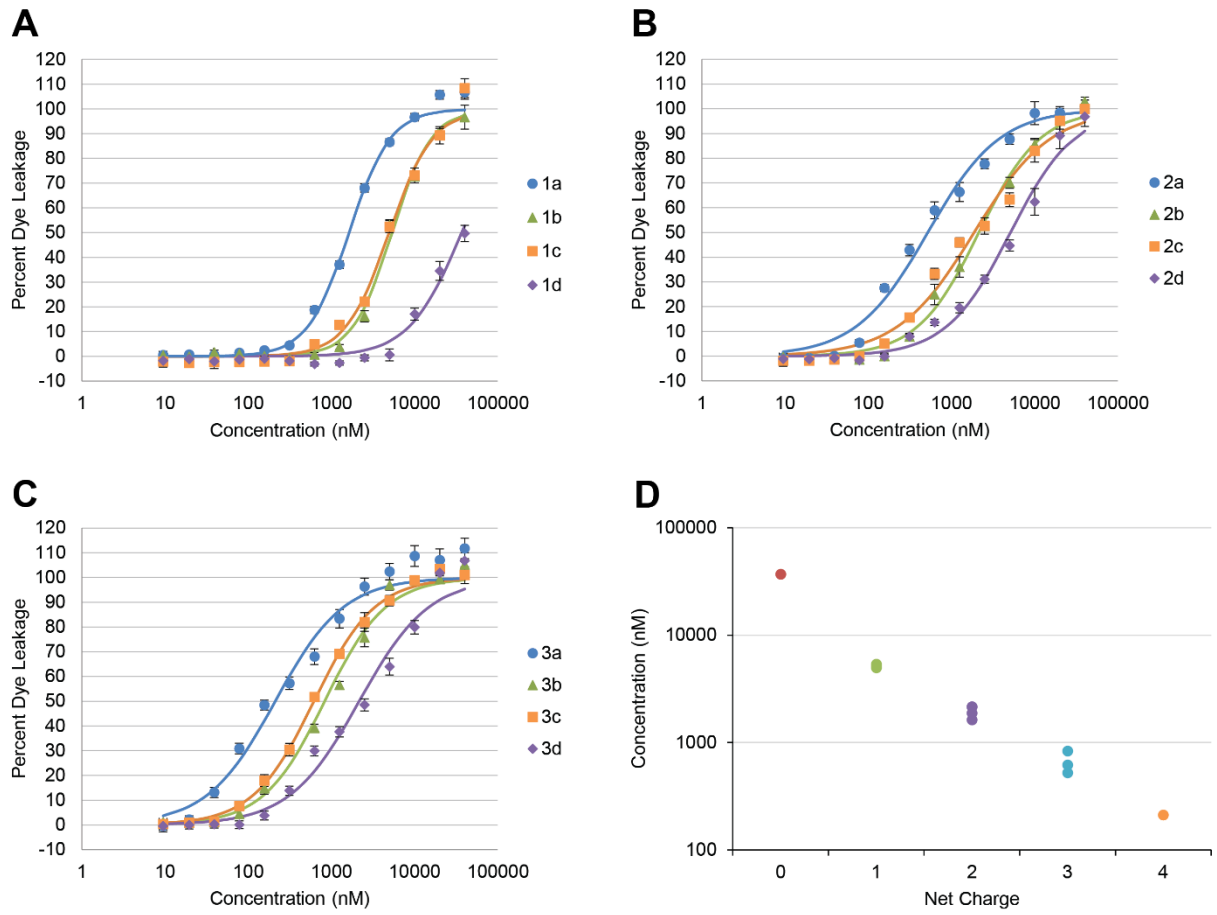


Figure 1.3. Propensity of peptides to cause membrane destabilization of negatively charged LUVs determined using a dye leakage assay. A) Peptides **1a** (+2), **1b** (+1), **1c** (+1), and **1d** (0). B) Peptides **2a** (+3), **2b** (+2), **2c** (+2), and **2d** (+1). C) Peptides **3a** (+4), **3b** (+3), **3c** (+3), and **3d** (+2). D) EC_{50} values of peptides grouped by net charge.

The acetylated variants of peptides **1a**, **2a**, and **3a** cause membrane destabilization at higher concentrations than their non-acetylated counterparts. The EC_{50} values for peptides **1b**, **1c**, and **1d** are 5347 nM, 4950 nM, and 36,869 nM, respectively. The EC_{50} values for peptides for peptides **2b**, **2c**, and **2d** are 2139 nM, 1873 nM, and 5118 nM, respectively. The EC_{50} values for peptides **3b**, **3c**, and **3d** are 829 nM, 618 nM, and 2147 nM, respectively.

The concentrations at which these peptides cause membrane destabilization correlate strongly with their net charges. The peptides with a net charge of +3 (peptides **2a**, **3b**, and **3c**) have EC_{50} values below that of the peptide with a net charge of +4 (peptide **3a**) and above the

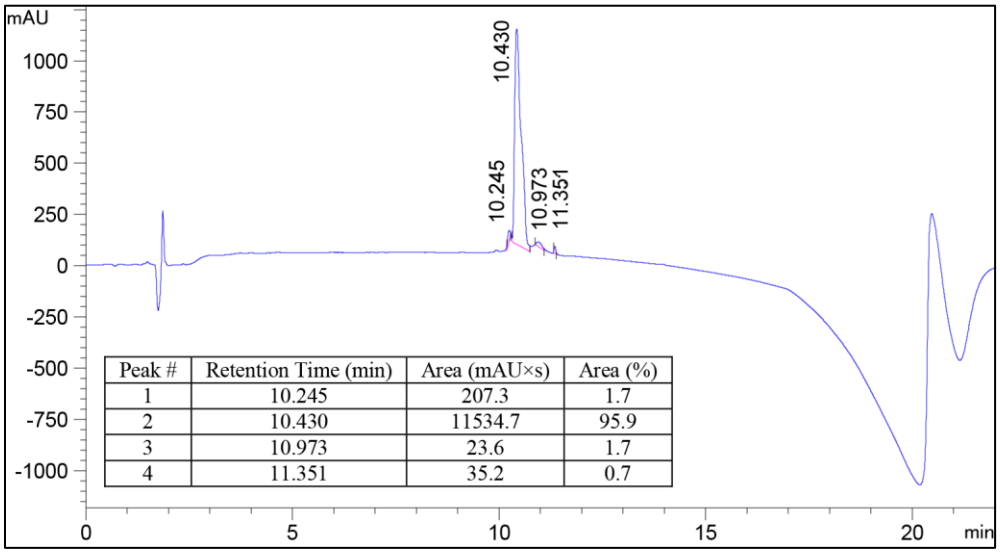
peptides with a net charge of +2 (peptides **1a**, **2b**, **2c**, and **3d**). The peptides with a net charge of +2 have EC_{50} values below those of the peptides with a net charge of +3 and above those peptides with a net charge of +1 (peptides **1b**, **1c**, and **2d**). The peptides with a net charge of +1 have EC_{50} values below those of the peptides with a net charge of +2 and above the peptide with a net charge of 0 (peptide **1d**).

1.4 Summary and Conclusion

Acetylation permits the modulation of charge within a series of peptides derived from Aβ mutants and permits the investigation of the role of charge in supramolecular assembly, cytotoxicity, and membrane disruption. By SDS-PAGE all of the peptides ran on the gel with molecular weights consistent with a hexamer except peptides **1c** and **1d** which were consistent with a trimer. Differences in the oligomeric assemblies between the non-acetylated and diacetylated peptides correlate with the differences in the net charges of the peptides. However, the position of acetylation also appears to affect the oligomeric assemblies because each pair of monoacetylated peptides (**1b** and **1c**, **2b** and **2c**, **3b** and **3c**), despite having the same net charge, migrated differently. In cytotoxicity studies, the peptides exhibited increasing cytotoxicity with increasing net charge. Dye leakage experiments revealed that a greater propensity to cause membrane destabilization correlates strongly with net charge. In both of these cases an increasing propensity to cause membrane destabilization correlated with an increasing net charge of the peptides: peptide **3a** (+4) > peptides **2a**, **3b**, **3c** (+3) > peptides **1a**, **2b**, **2c**, **3d** (+2) > peptides **1b**, **1c**, **2d** (+1) > peptide **1d** (0).

1.5 Appendix

1.5.1 Supplementary Figures



Peak #	Retention Time (min)	Area (mAU×s)	Area (%)
1	10.245	207.3	1.7
2	10.430	11534.7	95.9
3	10.973	23.6	1.7
4	11.351	35.2	0.7

Figure 1.4. Analytical HPLC trace for peptide **1b**.

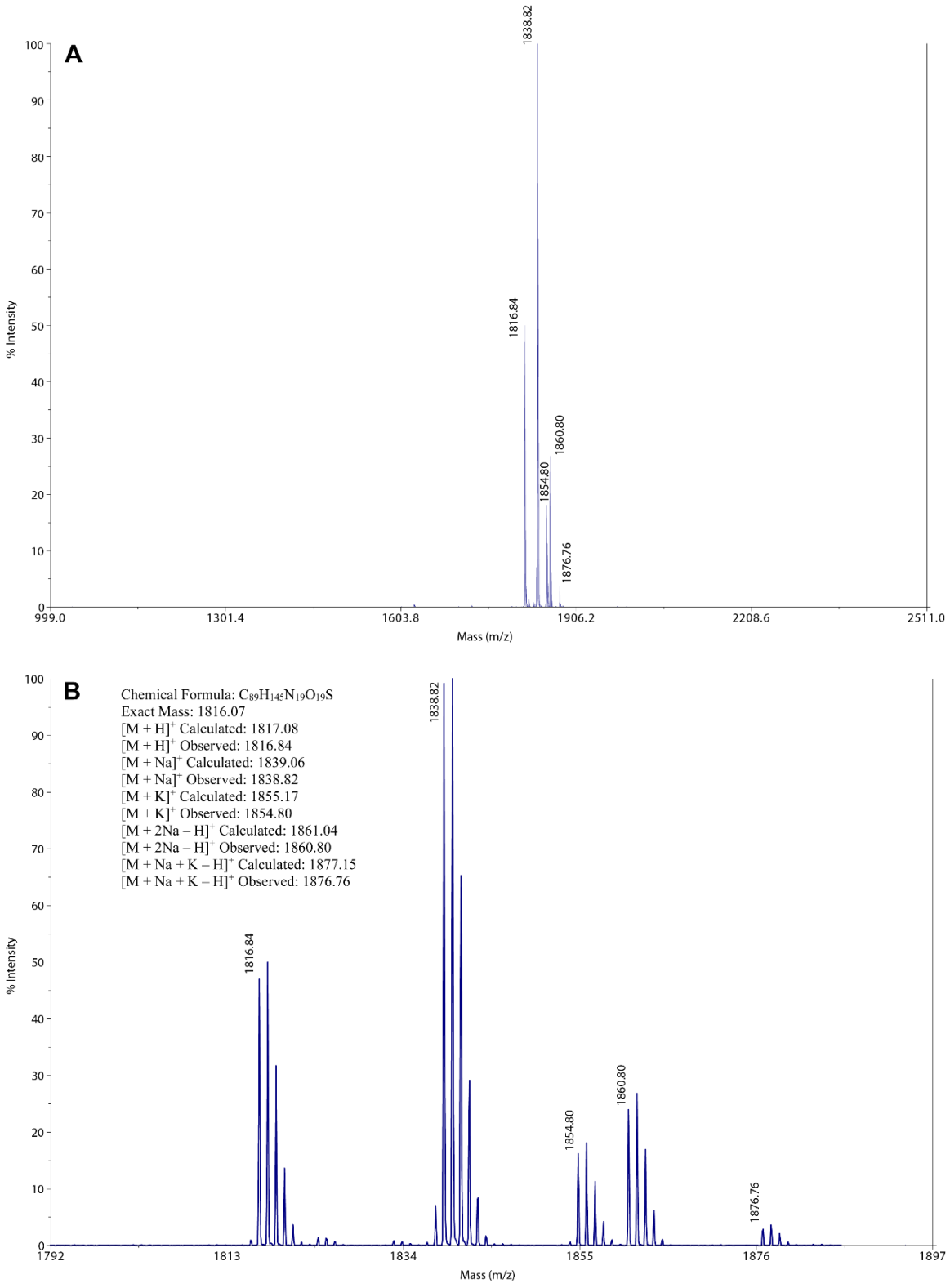


Figure 1.5. MALDI-TOF mass spectrum of peptide **1b**. A) Full spectrum. B) Zoomed in spectrum.

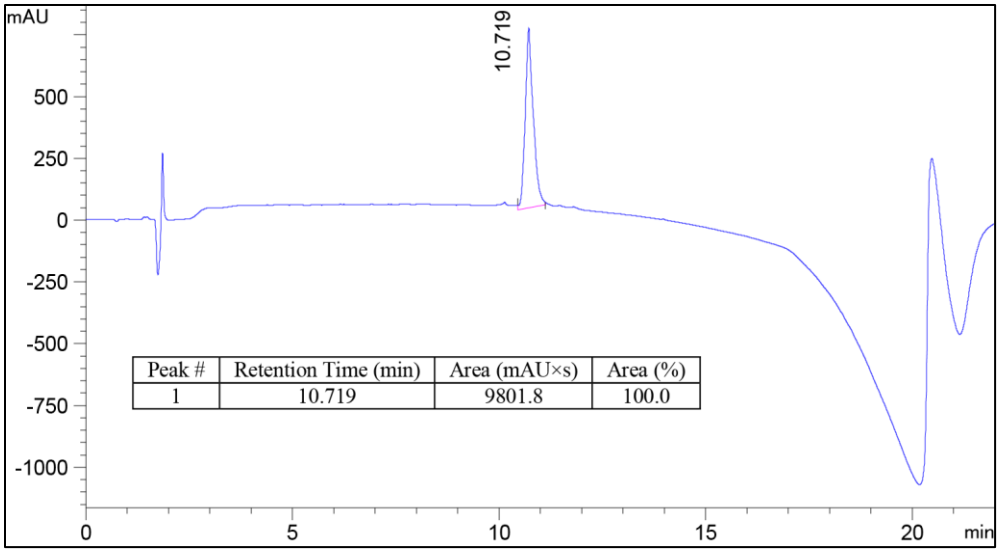


Figure 1.6. Analytical HPLC trace for peptide **1c**.

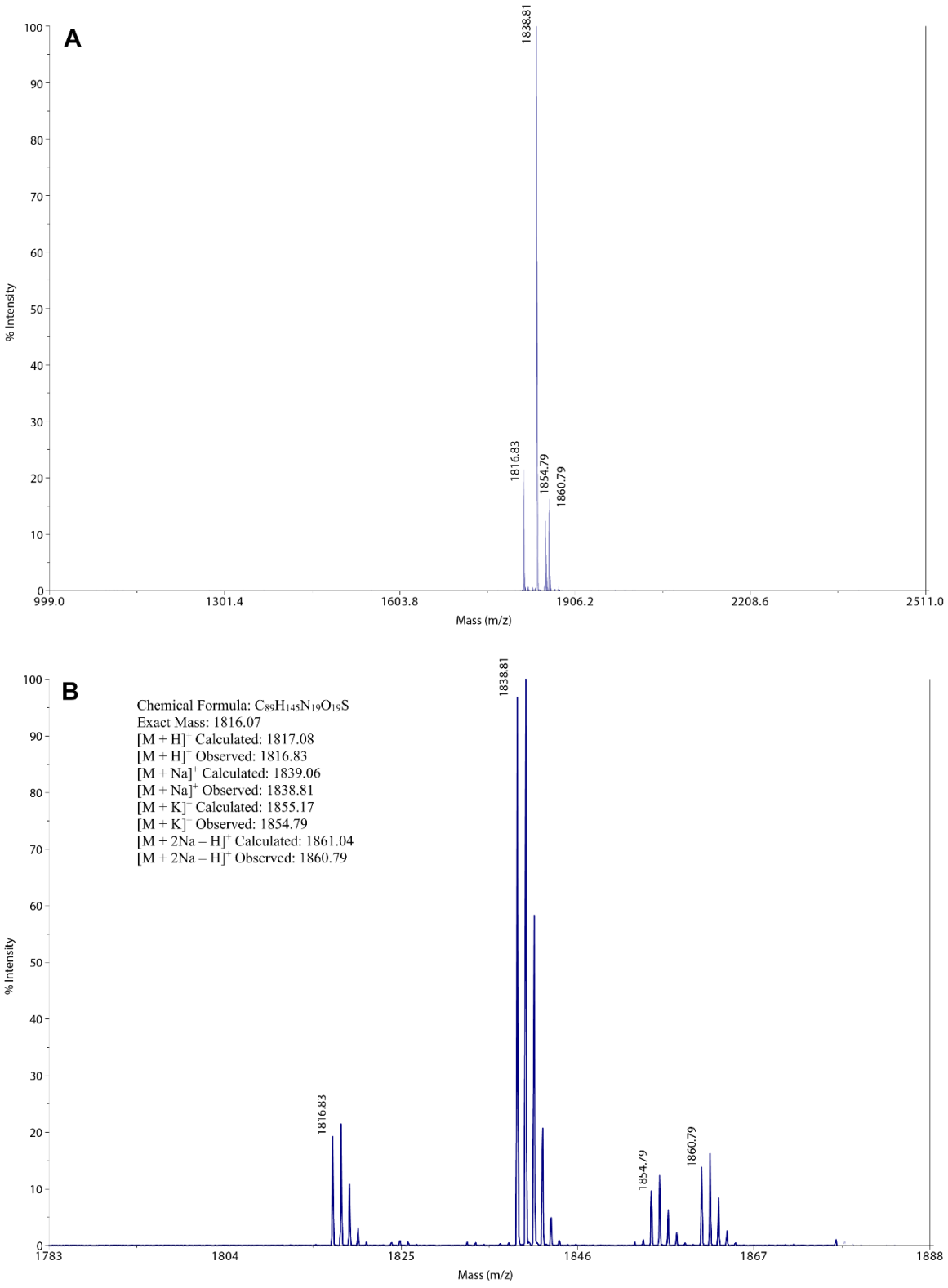
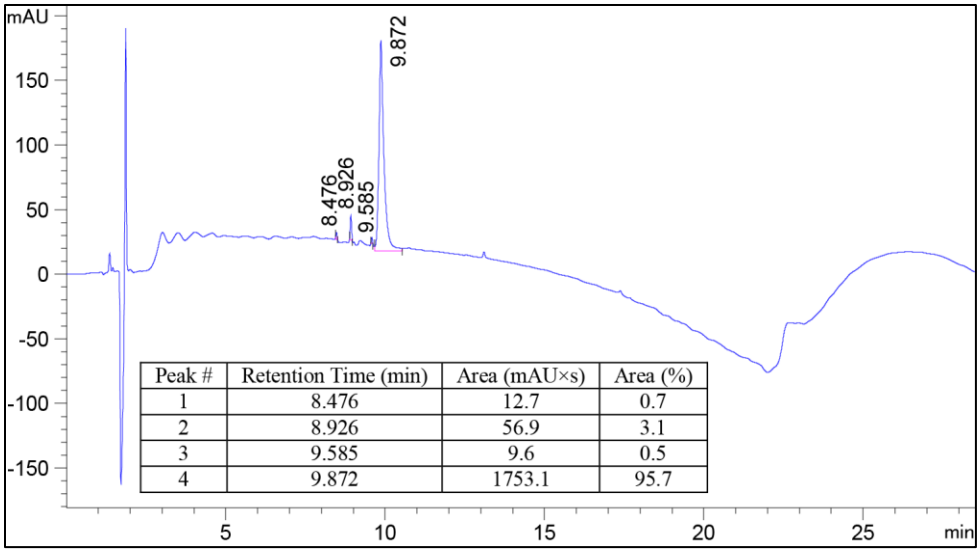


Figure 1.7. MALDI-TOF mass spectrum of peptide **1c**. A) Full spectrum. B) Zoomed in spectrum.



Peak #	Retention Time (min)	Area (mAU×s)	Area (%)
1	8.476	12.7	0.7
2	8.926	56.9	3.1
3	9.585	9.6	0.5
4	9.872	1753.1	95.7

Figure 1.8. Analytical HPLC trace for peptide **1d**.

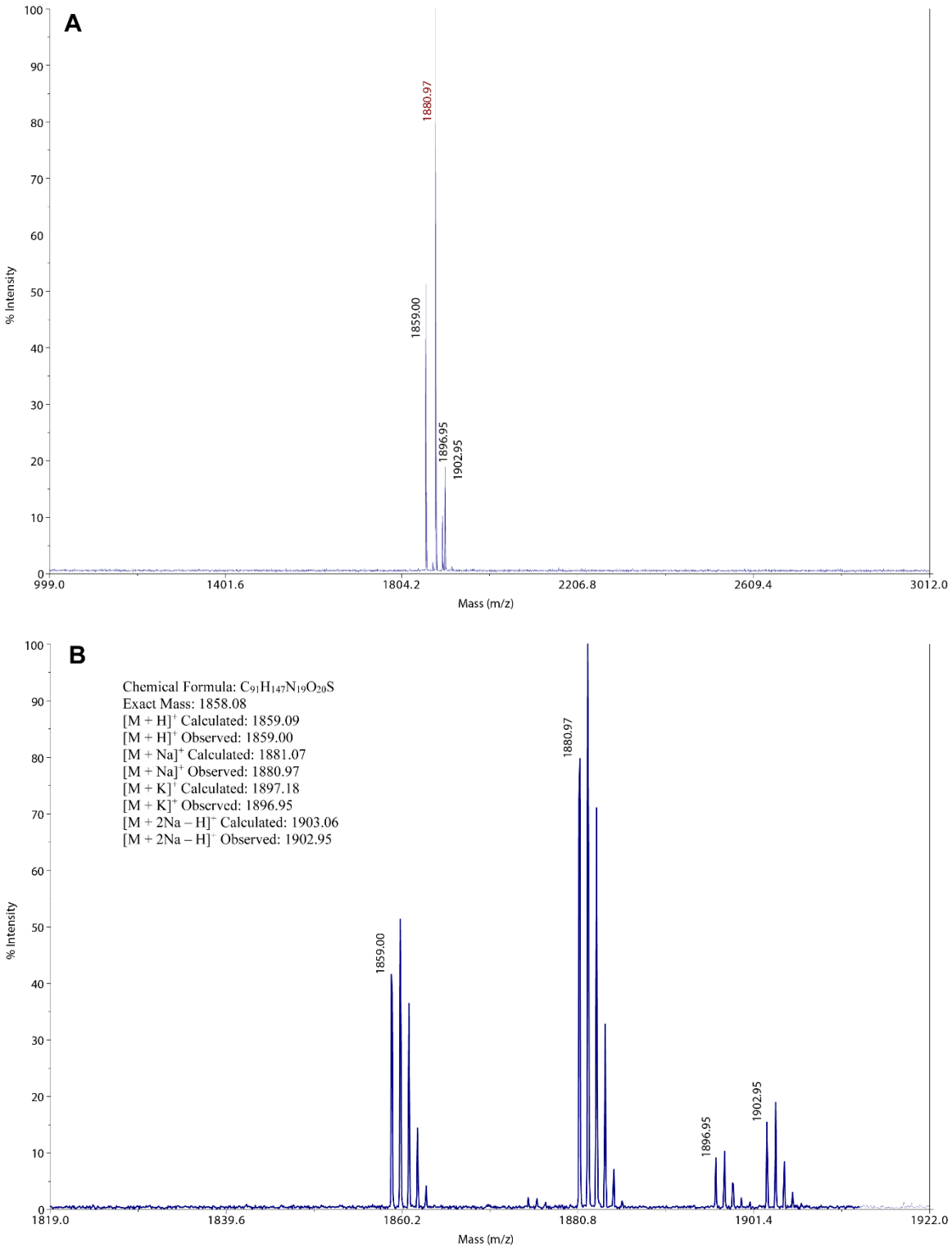


Figure 1.9. MALDI-TOF mass spectrum of peptide **1d**. A) Full spectrum. B) Zoomed in spectrum.

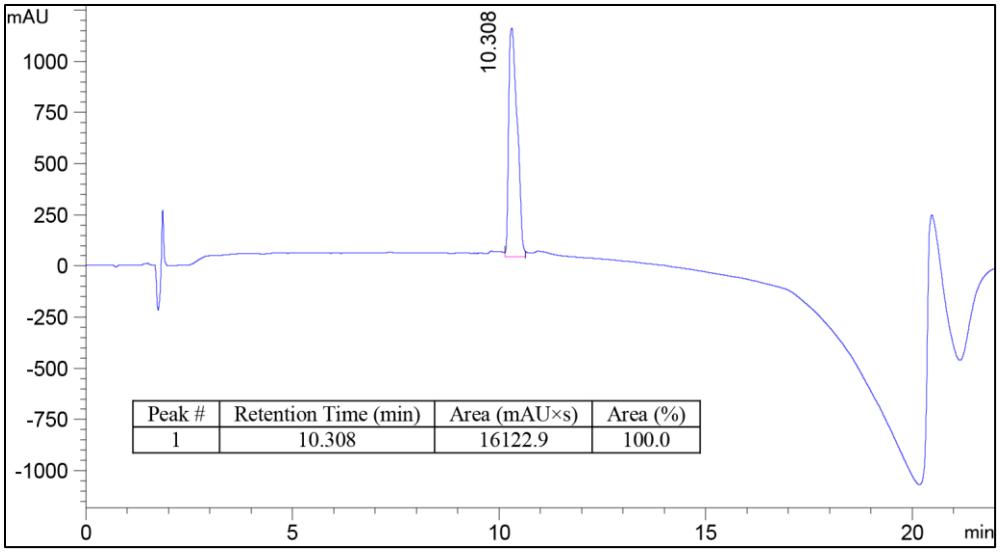


Figure 1.10. Analytical HPLC trace for peptide **2b**.

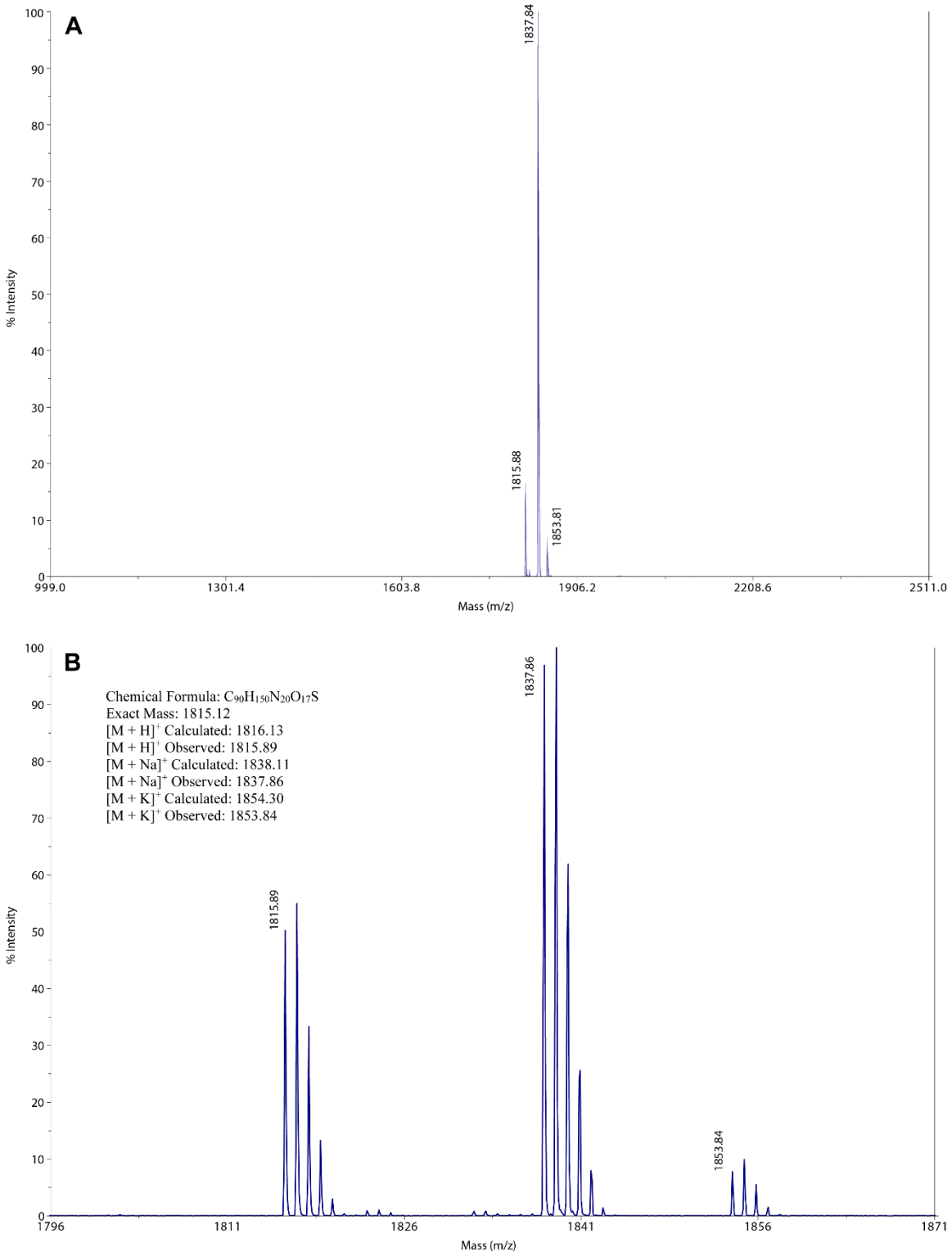


Figure 1.11. MALDI-TOF mass spectrum of peptide **2b**. A) Full spectrum. B) Zoomed in spectrum.

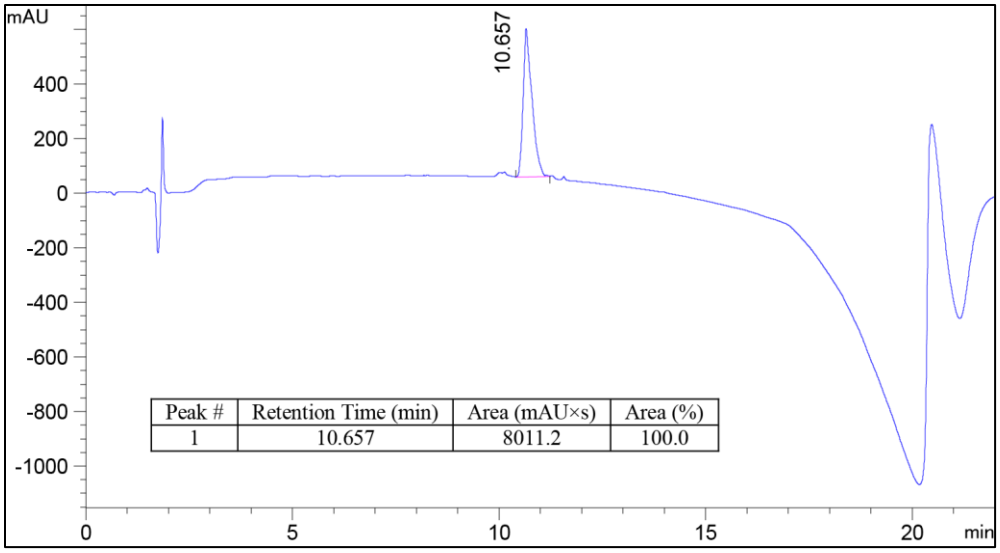


Figure 1.12. Analytical HPLC trace for peptide **2c**.

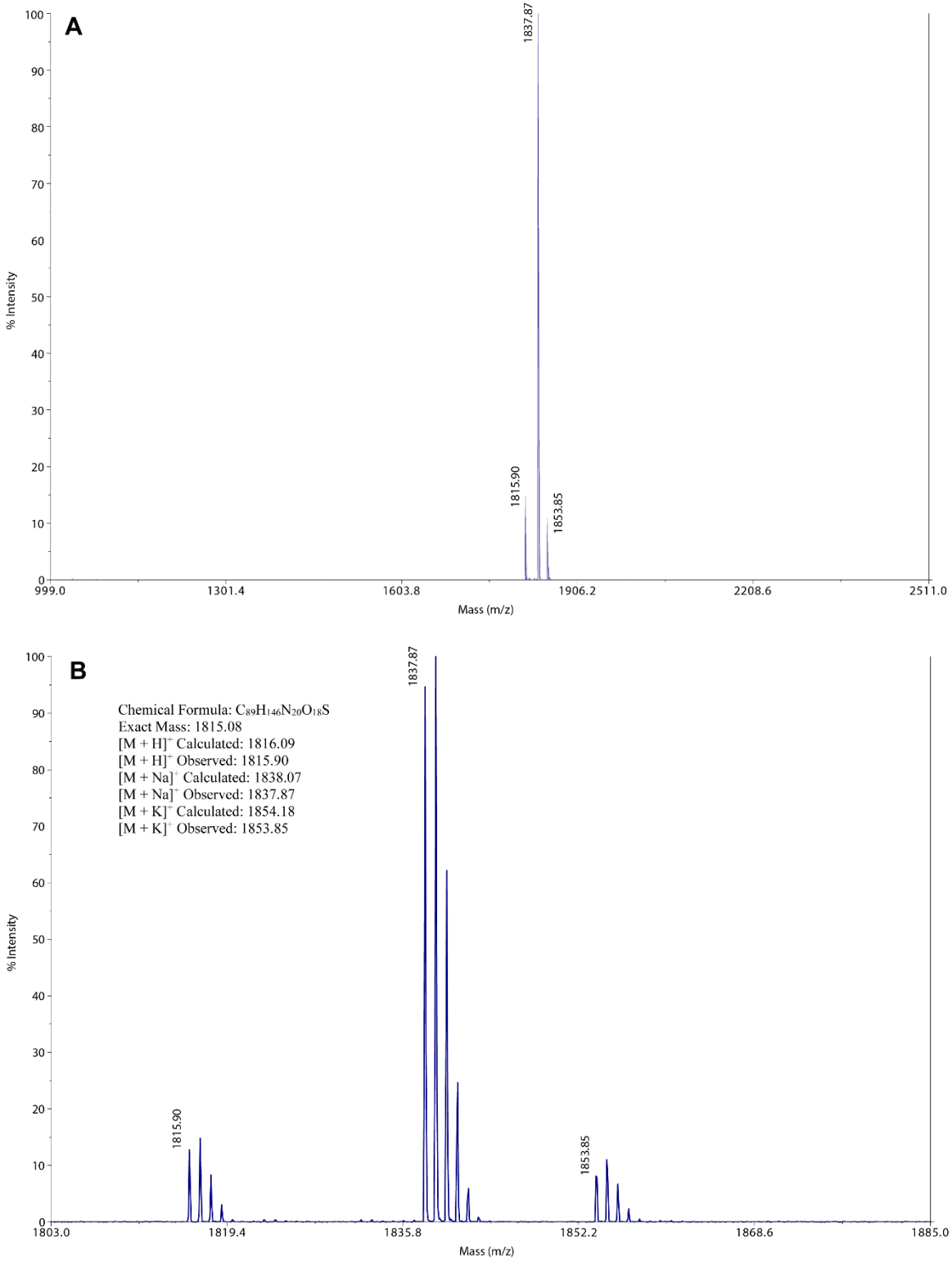
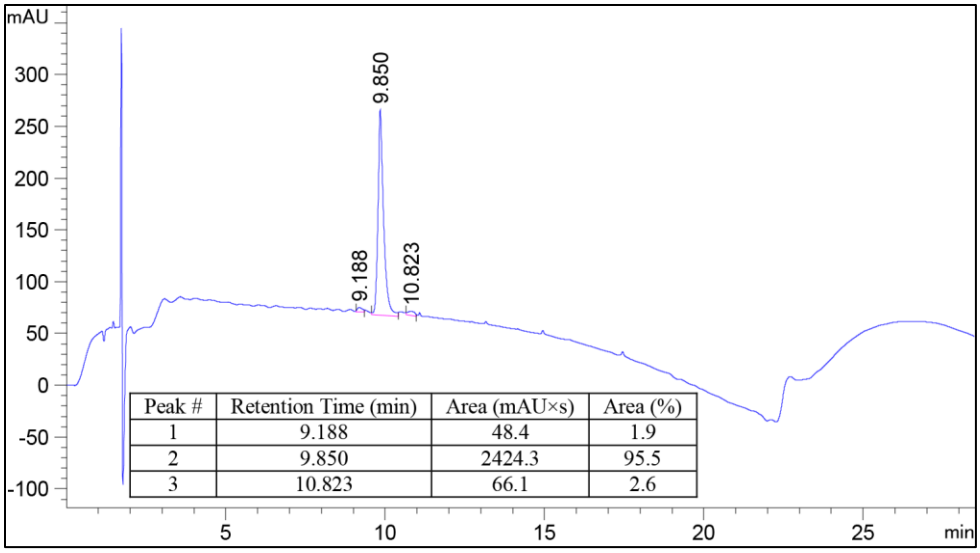


Figure 1.13. MALDI-TOF mass spectrum of peptide **2c**. A) Full spectrum. B) Zoomed in spectrum.



Peak #	Retention Time (min)	Area (mAU×s)	Area (%)
1	9.188	48.4	1.9
2	9.850	2424.3	95.5
3	10.823	66.1	2.6

Figure 1.14. Analytical HPLC trace for peptide **2d**.

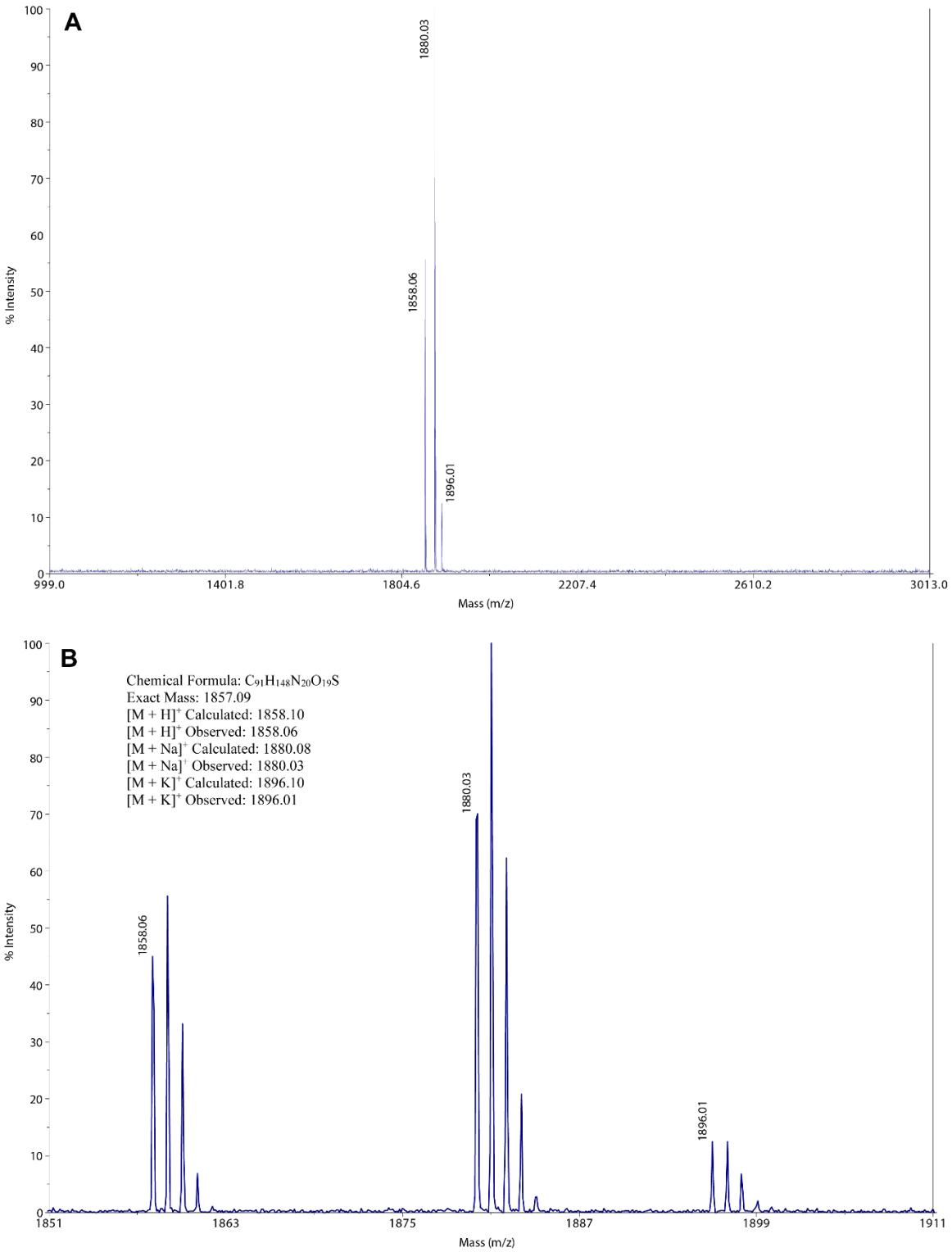


Figure 1.15. MALDI-TOF mass spectrum of peptide **2d**. A) Full spectrum. B) Zoomed in spectrum.

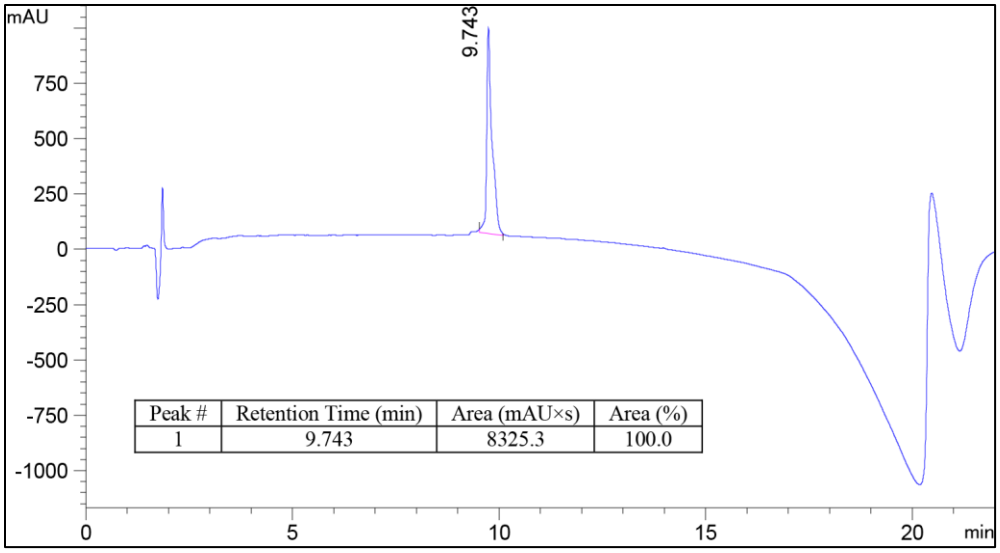


Figure 1.16. Analytical HPLC trace for peptide **3b**.

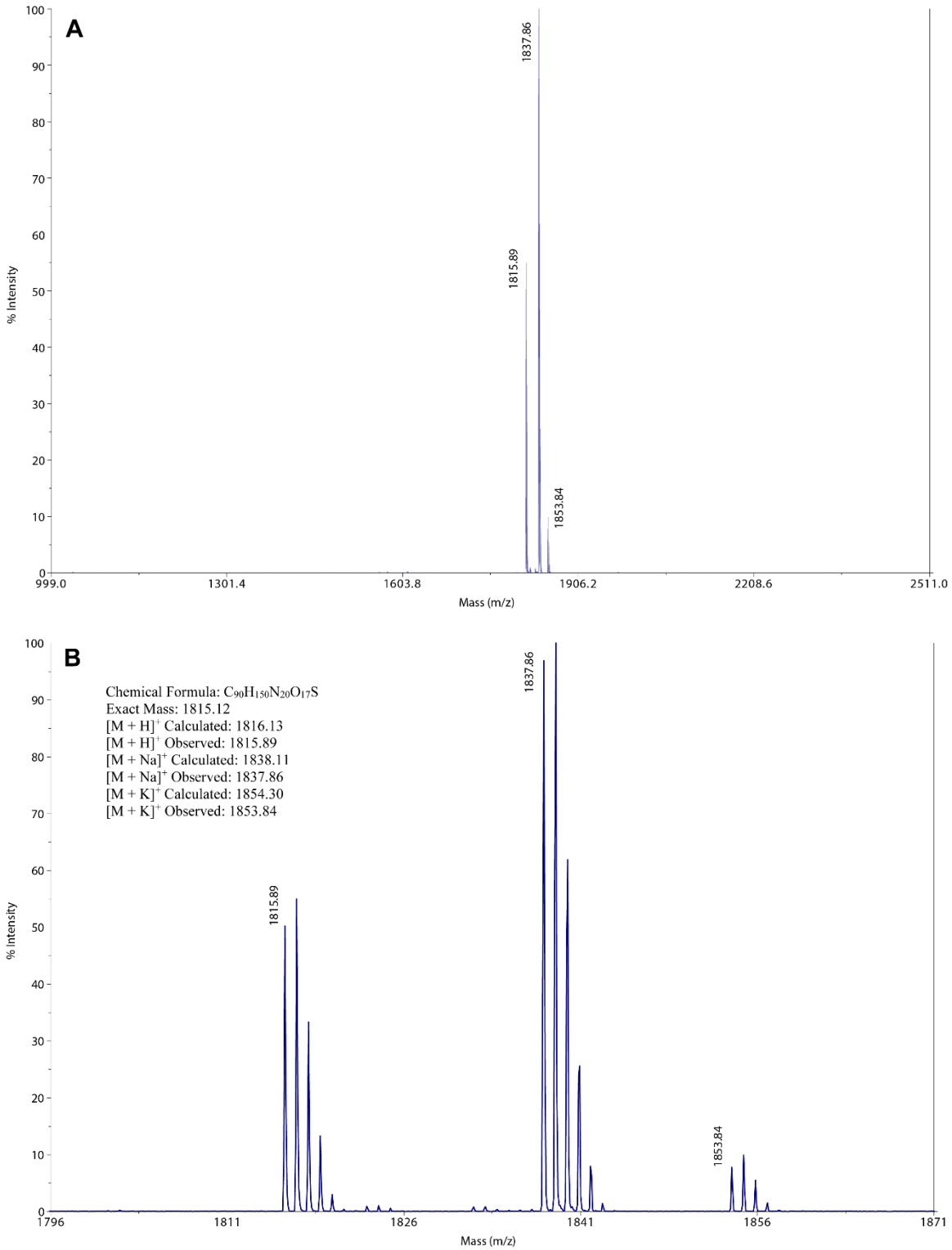
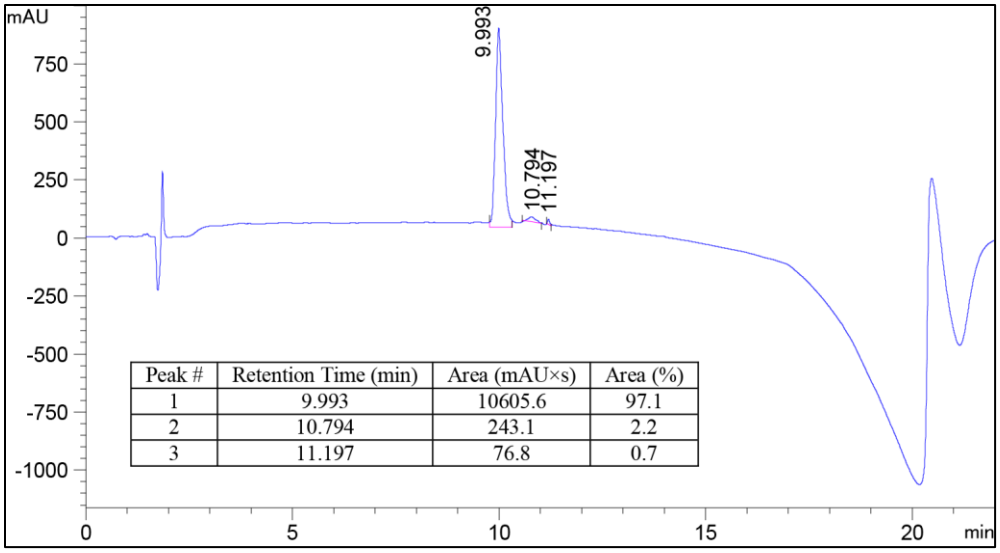


Figure 1.17. MALDI-TOF mass spectrum of peptide **3b**. A) Full spectrum. B) Zoomed in spectrum.



Peak #	Retention Time (min)	Area (mAU×s)	Area (%)
1	9.993	10605.6	97.1
2	10.794	243.1	2.2
3	11.197	76.8	0.7

Figure 1.18. Analytical HPLC trace for peptide **3c**.

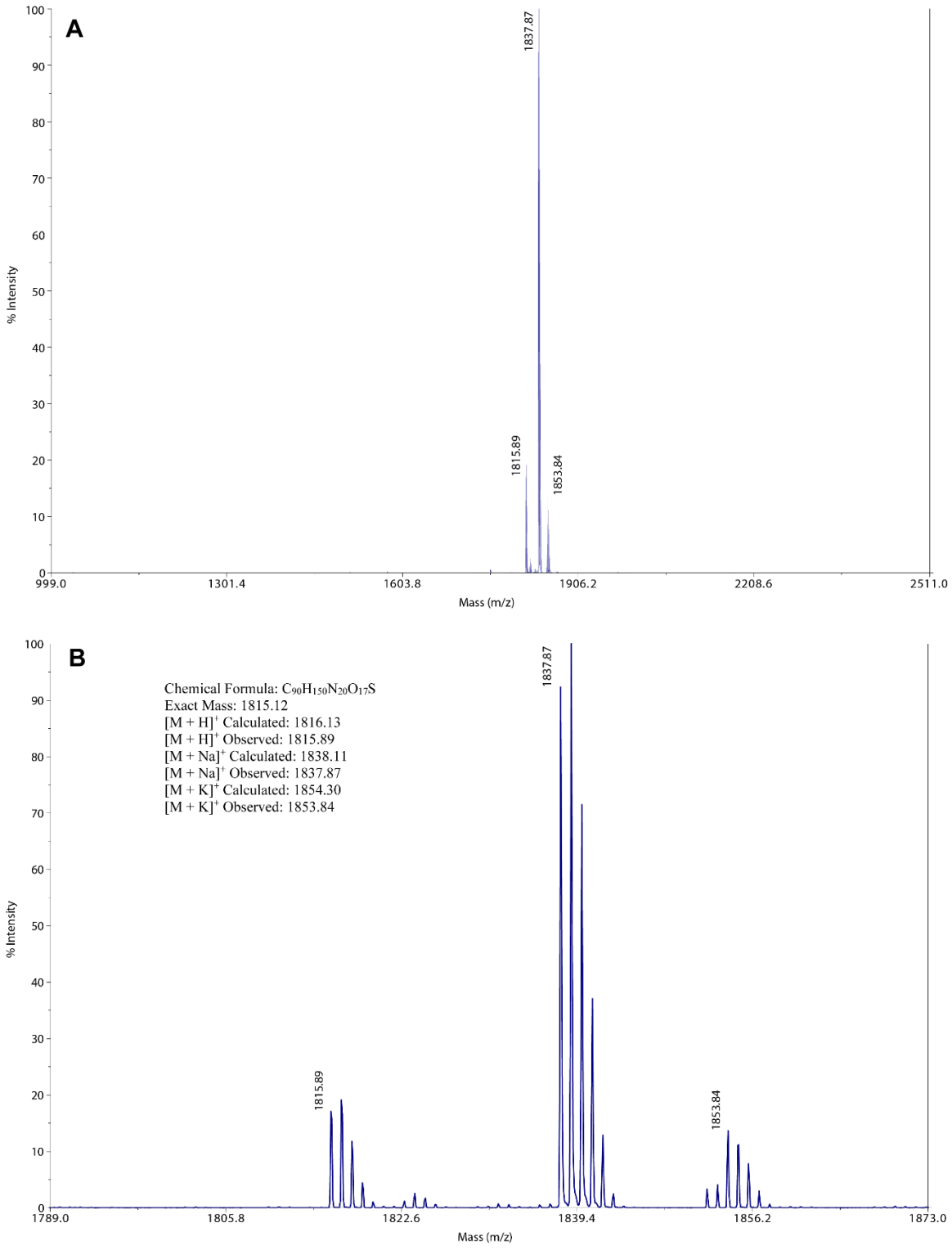


Figure 1.19. MALDI-TOF mass spectrum of peptide **3c**. A) Full spectrum. B) Zoomed in spectrum.

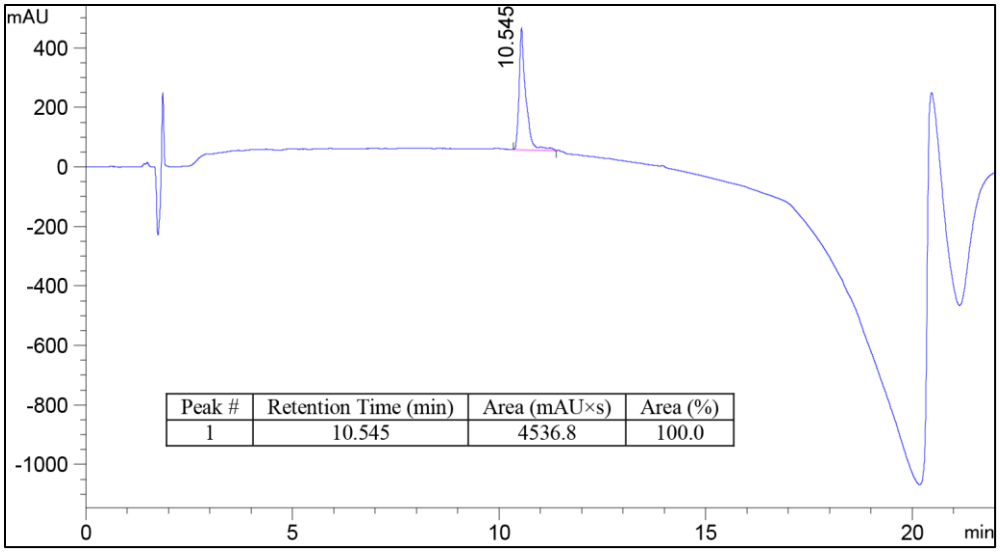


Figure 1.20. Analytical HPLC trace for peptide **3d**.

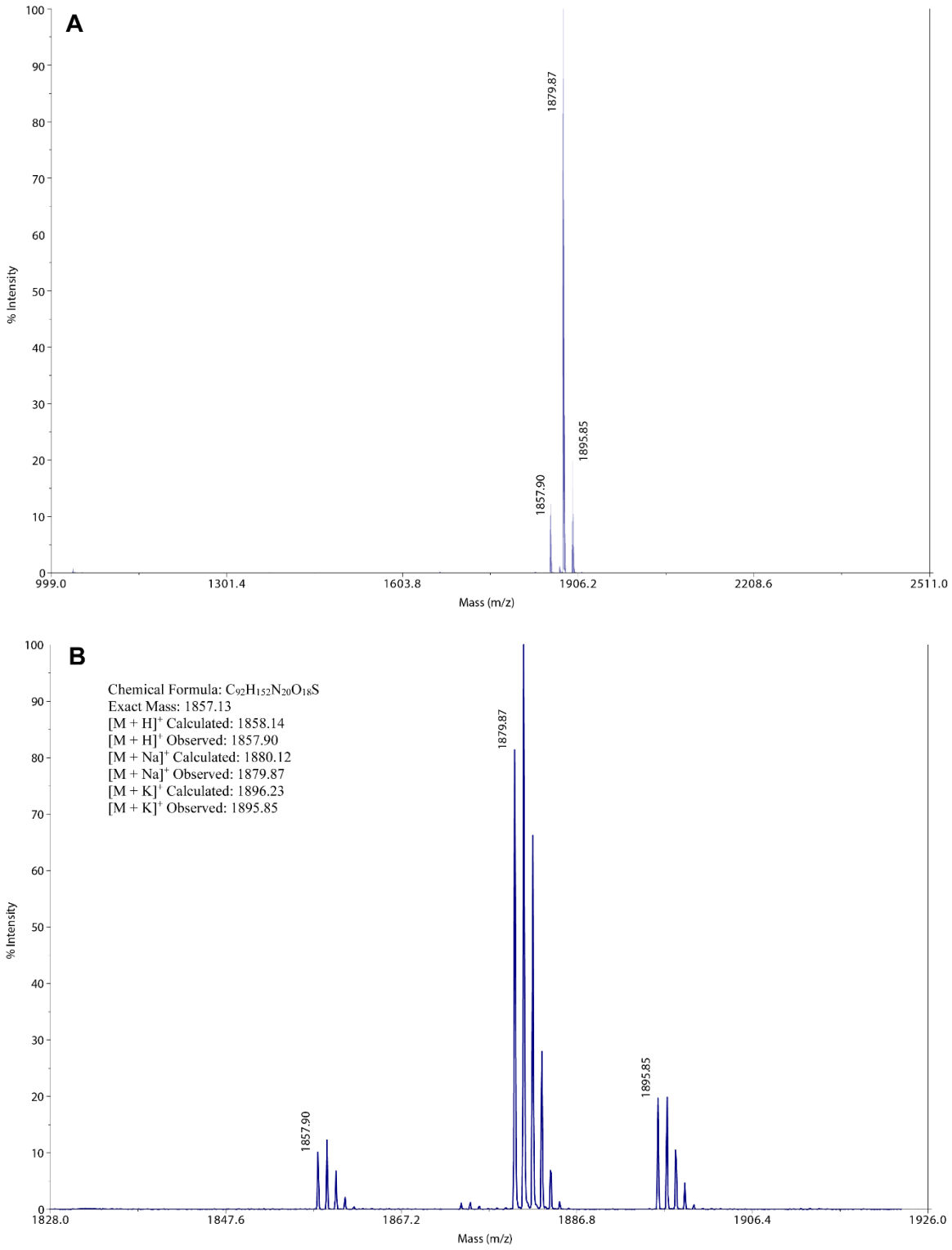
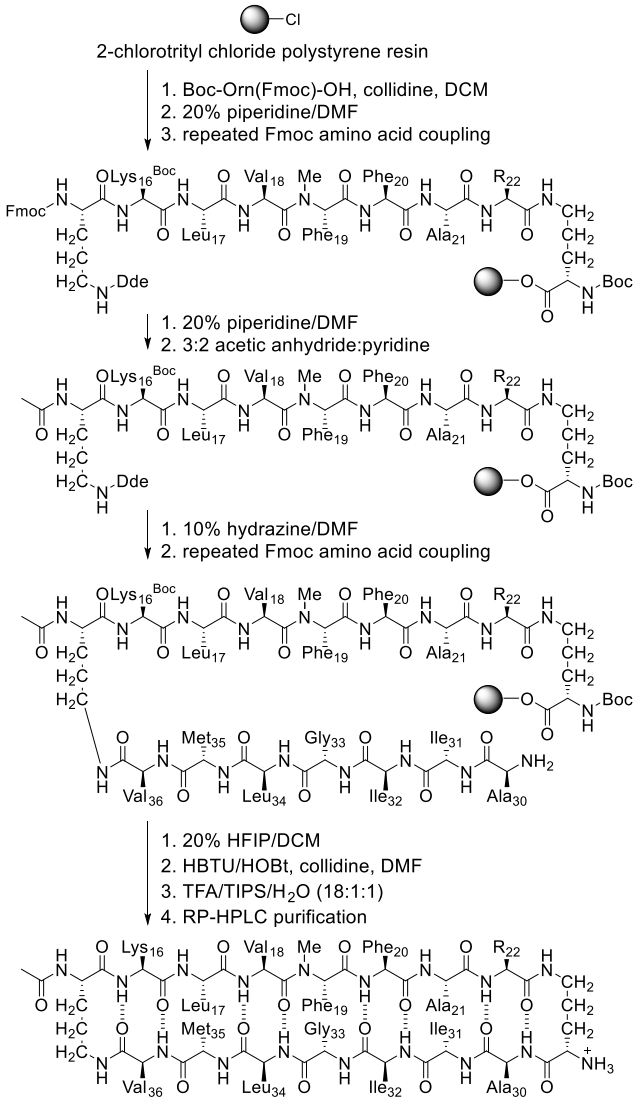
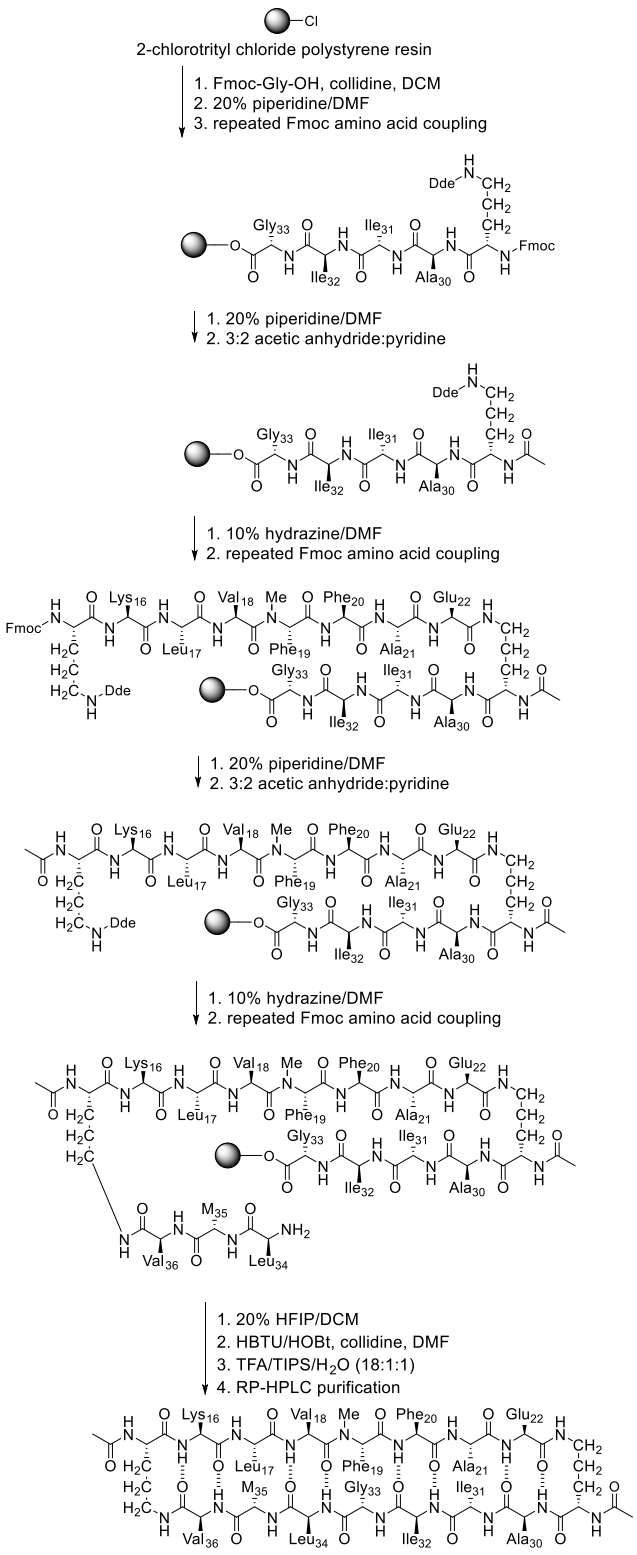


Figure 1.21. MALDI-TOF mass spectrum of peptide **3d**. A) Full spectrum. B) Zoomed in spectrum.

1.5.2 Supplementary Schemes



Scheme 1.1. Synthesis of monoacetylated peptides **1b**, **1c**, **2b**, **2c**, **3b**, and **3c**.



Scheme 1.2. Synthesis of diacetylated peptides **1d**, **2d**, and **3d**.

1.5.3 General Information[40]

All Fmoc-protected amino acids including the unnatural amino acids, Boc-ornithine(Fmoc)-OH and Fmoc-ornithine(Dde)-OH, were purchased from Chem-Impex or Anaspec. 2-Chlorotrityl chloride resin was purchased from Chem-Impex. Trifluoroacetic acid (TFA), and HPLC grade acetonitrile (MeCN) were purchased from Fischer Scientific. Water was purified to 18 MΩ with a ThermoFisher GenPure Pro water purification system. All other solvents and chemicals were purchased from Alfa Aesar and Sigma Aldrich. All amino acids, resins, solvents, and chemicals were used as received, with the exception that dichloromethane (DCM) and *N*,*N*-dimethylformamide (DMF) were dried by passage through dry alumina under argon. Analytical HPLC chromatograms were obtained using an Agilent 1260 Infinity II HPLC equipped with Phenomenex bioZen C18 column (150 mm × 4.6 mm, 2.6 μm particle size). A 1 mL/min flow rate was used with peak detection at 214 nm, all monitored using the provided HPLC OpenLAB software. Preparative-scale purification of peptides was done using an Agilent Zorbax SB-C18 PrepHT column (21.2 mm x 250 mm, 7 μm particle size) on a Rainin Dynamax HPLC with a flow rate of 7.0 mL/min, monitored at 214 nm with the accompanying DA Rainin HPLC software. MALDI mass spectrometry was performed using an Applied Biosystems SCIEX TOF/TOF under reflector positive ion mode using 2,5-dihydroxybenzoic acid matrix. Spectra were analyzed using the accompanying TOF/TOF Series Explorer software.

1.5.4 Abbreviations

DCM	dichloromethane
DIPEA	diisopropylethylamine
DMF	*N*,*N*-dimethylformamide
HATU	*N*,*N*,*N'*,*N'*-tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate

HBTU	*N*,*N*,*N'*,*N'*-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate
HCTU	*N*,*N*,*N'*,*N'*-tetramethyl-O-(6-chlorobenzotriazol-1-yl)uraniumhexafluorophosphate
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
HPLC	high-performance liquid chromatography
HOBt	hydroxybenzotriazole
MALDI	matrix-assisted laser desorption ionization
MeOH	methanol
MeCN	acetonitrile
TFA	trifluoroacetic acid
TIPS	triisopropylsilane

1.5.5 Synthesis of Macrocyclic Peptides[41]

The synthesis, purification, and characterization of peptides **1a**, **1b**, and **1c** have been reported previously.[6] The synthesis of peptides **1b**, **1c**, **1d**, **2b**, **2c**, **2d**, **3b**, **3c**, and **3d** involved the following sequence of operations: (1) resin loading, (2) solid-phase amino acid couplings, (3) acetylation of either or both of the α-amino groups of the δ-linked ornithines, (4) hydrazine deprotection of the Dde protecting group of the δ-linked ornithine, (5) cleavage of the linear peptides from the resin, (6) solution-phase cyclization of the linear peptides, (7) global deprotection of acid-labile protecting groups, and (8) purification with preparative reverse-phase HPLC. The purified peptides were characterized by analytical HPLC and MALDI mass spectrometry.

1.5.6 Resin Loading

2-Chlorotrityl chloride resin (0.300 g, 1.6 mmol/g, 100-200 mesh) was added to a 10-mL Bio-Rad Poly-Prep chromatography column (8 mm x 40 mm). The resin was suspended in dry DCM (8 mL) and allowed to swell undisturbed for 30 min. The solution was drained from the resin using nitrogen. For the monoacetylated peptides **1b**, **1c**, **2b**, **2c**, **3b**, and **3c** a solution of Boc-ornithine(Fmoc)-OH (150.0 mg, 0.33 mmol) in 20% (v/v) 2,4,6-collidine in dry DCM (8 mL) was added immediately. For the diacetylated peptides **1d**, **2d**, and **3d** a solution of Fmoc-glycine-OH (75.0 mg, 0.23 mmol) in 20% (v/v) 2,4,6-collidine in dry DCM (8 mL) was added immediately. The suspension was gently agitated for 24 h. The solution was then drained using nitrogen and washed with dry DCM (3x). After washing, a mixture of DCM/MeOH/DIPEA (8.5:1:0.5, 8 mL) was added immediately. The suspension was gently agitated for 30 min to cap any unreacted sites on the resin. The resin was washed with DMF (3x) and dried by passing nitrogen through the chromatography column. Each batch of resin loading was determined to be between 0.60 and 0.65 mmol/g based on UV analysis (290 nm) of the Fmoc cleavage product.

1.5.7 Solid-Phase Peptide Synthesis

The loaded resin was added to a Chemglass solid phase peptide synthesis vessel and subjected to cycles of amino acid couplings using Fmoc-protected amino acid building blocks. The linear peptides were synthesized from the C-terminus to the N-terminus. Each coupling consisted of: (1) Fmoc deprotection with 20% (v/v) piperidine in DMF for 5 min (2x); (2) resin washing with DMF (3x); (3) activation of the Fmoc protected amino acid (4 equiv) with 20% (v/v) 2,4,6-collidine in DMF (8 mL) in the presence of HCTU (4 equiv); (4) coupling of the activated Fmoc-protected amino acid; (5) resin washing with DMF (3x). All amino acid couplings took 20 min except for the valine that followed the *N*-methyl-phenylalanine. The

valine that followed the *N*-methyl-phenylalanine (4 equiv) was coupled twice for 1 h each with HATU (4 equiv) and HOAt (4 equiv) in 20% (v/v) 2,4,6-collidine in DMF (8 mL), with no Fmoc deprotection in between the two coupling reactions. This modification ensured complete amino acid coupling onto a more sterically hindered, secondary amine. After the last amino acid was coupled, and its Fmoc protecting group deprotected, the resin was transferred from the synthesis vessel to a new Bio-Rad Poly-Prep chromatography column. The resin was washed with DCM (3x), and dried by passing nitrogen through the column.

1.5.8 Acetylation of the α-Amino Group of δ-Linked Ornithine

After Fmoc-Orn(Dde)-OH was incorporated into the peptide sequence, the Fmoc group was deprotected with 20% (v/v) piperidine in DMF for 5 min (2x) and then the resin washed with DMF (3x). A 3:2 mixture of acetic anhydride:pyridine (8 mL) was added to the resin in the coupling vessel for 30 min to acetylate the exposed α-amino group of the ornithine. The resin was subsequently washed with DMF (3x).

1.5.9 Hydrazine Deprotection of Dde

Following the deprotection of the Fmoc group of Fmoc-Orn(Dde)-OH and the acetylation of the exposed α-amino group, the Dde protecting group was deprotected using 10% hydrazine in DMF (8 mL) for 10 min. The resin was washed after deprotection with DMF (5x).

1.5.10 Protected Cleavage of the Linear Peptide

The protected linear peptide was cleaved from the resin by subjecting the resin to a cleavage solution of 20% (v/v) HFIP in DCM (8 mL). The resin and the suspension immediately turned red. The suspension was gently agitated for 1 h. The suspension was filtered, and the filtrate was collected in a 250-mL round-bottom flask. The resin was washed with additional

cleavage solution (8 mL) and agitated for another 30 min. The suspension was filtered and the filtrate collected into the same round-bottom flask as the previous filtrate. The suspension was then washed with DCM (3 x 2 mL) until the resin was no longer red with all washes collected into the round-bottom flask. The combined filtrates were concentrated under reduced pressure and then the protected linear peptide was cyclized without prior purification.

1.5.11 Cyclization

The protected linear peptide was dissolved in dry DMF (125 mL) in the same 250-mL round-bottom flask as the previous step. HOBt (0.150 g, 1.11 mmol) and HBTU (0.300 g, 0.79 mmol) were dissolved in 8 mL of dry DMF in a test tube to which 300 μL of 2,4,6-collidine was added and the solution mixed until homogenous. The solution was then added to the flask and the mixture was stirred under nitrogen at room temperature for 96 h. The reaction mixture was concentrated under reduced pressure and then the crude product was immediately subjected to global deprotection.

1.5.12 Global Deprotection of Acid-Labile Protecting Groups and Ether Precipitation

The protected cyclic peptide was dissolved in a mixture of TFA/TIPS/H_2O (9.0:0.5:0.5, 10 mL) in the same 250-mL round-bottom flask as the previous step. The reaction mixture was then stirred under nitrogen at room temperature for 1 h. During the 1 h deprotection, two 50-mL conical tubes containing 40 mL of dry ether each were chilled on dry ice. Following the 1 h time, the peptide solution was split between the two conical tubes of ether. The tubes were then centrifuged at 500 *x g* for 15 min. The ether supernatant was poured off and the pelleted peptides dried under nitrogen for 15-20 min.

1.5.13 Purification of Macrocyclic Peptides

The crude peptide pellets were dissolved in 10% MeCN/H_2O (4 mL), and the solution was initially purified using a Biotage® Isolera One. Fractions containing the desired peptide were identified by mass spectrometry, combined, and reduced under pressure to a volume less than 5 mL. That solution was then purified by reverse-phase HPLC following a gradient whereby the percentage of MeCN in the mobile phase was increased at a rate of 0.2%/min. Elution of the monoacetylated peptides occurred within the range of 27-34% MeCN/H_2O and elution of the diacetylated peptides occurred within the range of 31-38% MeCN/H_2O. The pure fractions were lyophilized to afford 10.27 mg of peptide **1b** (2.81% based on resin loading), 7.34 mg of peptide **1c** (2.01% based on resin loading), 3.65 mg of peptide **1d** (1.04% based on resin loading), 32.08 mg of peptide **2b** (8.78% based on resin loading), 1.88 mg of peptide **2c** (0.51% based on resin loading), 4.85 mg of peptide **2d** (1.38% based on resin loading), 3.15 mg of peptide **3b** (0.82% based on resin loading), 2.09 mg of peptide **3c** (0.54% based on resin loading), and 2.50 mg of peptide **3d** (0.67% based on resin loading). All peptides were obtained as the trifluoroacetate salts and were white powders.

1.5.14 SDS-PAGE[42]

Solutions of the peptides were prepared gravimetrically by dissolving the lyophilized peptide in the appropriate amount of 18 MΩ deionized water to achieve a 10 mg/mL stock. Aliquots of the 10 mg/mL stock solutions were diluted with 18 MΩ deionized water to create 1 mg/mL solutions. These 1 mg/mL solutions were further diluted with 6X SDS-PAGE sample loading buffer (G Biosciences) to create 0.2 mg/mL working solutions. A 5 μL aliquot of each working solution was run on a 16% polyacrylamide gel with a 4% stacking polyacrylamide gel. The gels were run at a constant 80 volts for approximately 3 h. Reagents and gels for Tricine

SDS-PAGE were prepared according to recipes and procedures detailed in Schägger, H. *Nat. Protoc*. **2006**, *1*, 16–22.[43]

Staining with silver nitrate was used to visualize the peptides in the SDS-PAGE gel. Reagents for silver staining were prepared according to procedures detailed in Simpson, R. J. *Cold Spring Harb. Protoc.* **2007**.[44] Briefly, the gel was removed from the casting glass and rocked in fixing solution (50% (v/v) methanol and 5% (v/v) acetic acid in 18 MΩ deionized water) for 20 min. Next, the fixing solution was discarded and the gel was rocked in 50% (v/v) aqueous methanol for 10 min. The 50% methanol was subsequently discarded and the gel was rocked in 18 MΩ deionized water for 10 min. After the water was discarded, the gel was rocked in 0.02% (w/v) sodium thiosulfate in 18 MΩ deionized water for 1 min. The sodium thiosulfate was discarded and the gel was rinsed with 18 MΩ deionized water for 1 min (2x). After the last rinse, the gel was submerged in chilled 0.1% (w/v) silver nitrate in 18 MΩ deionized water and rocked at 4 °C for 20 min. The silver nitrate solution was then discarded and the gel was rinsed with 18 MΩ deionized water for 1 min (2X). To develop the gel, the gel was incubated in developing solution (2% (w/v) sodium carbonate, 0.04% (w/v) formaldehyde) until the desired intensity of staining was reached (~2–5 min). When the desired intensity of staining was reached, the development was stopped by discarding the developing solution and submerging the gel in 5% aqueous acetic acid.

1.5.15 Cell Culture and LDH-Release Assay[45,46]

SH-SY5Y neuroblastoma cell cultures (ATCC® CRL-2266™) were maintained in 1:1 mixture of Dubelcco's modified Eagle medium and Ham's F12 (DMEM:F12) medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin at pH 7.4 in a humidified 5% CO2 atmosphere at 37 ºC using a Fischer

Scientific Forma Series 3 Water Jacketed CO_2 Incubator. All experiments were performed using ca. 60–80% confluent cells on passages ranging from 3–12.

SH-SY5Y cells were seeded at 30,000 cells per well in the inner 60 wells of 96-well plates to a total volume of 100 µL using 1:1 DMEM/F12 media supplemented with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin at pH 7.4. The outer wells of the plate were filled with 100 µL of media without any cells. The plates were incubated for 24 h after plating. Prior to treatment, the media was removed by pipet and replaced with 90 µL of serum-free, phenol-red free 1:1 DMEM/F12 media with no added penicillin or streptomycin. Solutions of the peptides were prepared gravimetrically by dissolving the lyophilized peptide in the appropriate amount of 18 MΩ deionized water to achieve a 10 mg/mL stock. From the 10 mg/mL stock solutions, 10X solutions were made by dilution with 18 MΩ deionized water. To each well of the 96-well plate was added 10 µL of the 10X solutions, bringing the total volume of each well to 100 µL. Each treatment was run in quintuplicate. An additional ten wells were used as controls. Five wells received 10 µL of 18 MΩ deionized water (vehicle, negative control) and the other five wells were left untreated, to be subsequently treated with lysis buffer (positive control). Cells were incubated for 72 hours.

After the 72 hours, the LDH-release assay (Pierce LDH Cytotoxicity Assay Kit, Thermo Fisher) was performed according to the manufacturer's instructions. First, 10 µL of 10X lysis buffer was added to the five untreated wells and the cells incubated for an additional 45 min. Then a 50 µL aliquot from each well was transfered to a new 96-well plate and 50 µL of LDH substrate solution was added to each well. The treated plates were stored in the dark for approximately 30 min. The absorbance of each well was measured at 490 and 680 nm (A_{490} and

A_{680}). Data were analyzed by calculating the differential absorbance for each well (A_{490} - A_{680}) and comparing those values to those of the lysis buffer controls and the untreated controls:

$$\%\ LDH\ release\ =\ \frac{(A_{490}-A_{680})_{peptide}-(A_{490}-A_{680})_{vehicle}}{(A_{490}-A_{680})_{lysis}-(A_{490}-A_{680})_{vehicle}}\times\ 100$$

1.5.16 Dye Leakage Assay[47,48]

Chicken egg-derived L-α-phosphatidylcholine (PC, product number: 840051C) and porcine brain-derived L-α-phosphatidylserine (PS, product number: 840032) were purchased from Avanti Polar Lipids as 10 mg/mL solutions in chloroform. Liposomes were prepared using 2.6 micromoles of lipids as a 1:1 molar ratio of PC and PS. A solution of 2.6 micromoles lipid in chloroform was placed into a 12 x 75 mm disposable culture tube. Chloroform was removed under a stream of dry N_2 gas to yield a lipid film. The culture tube was put under vacuum (< 1 mmHg) for ca. 12 h to ensure complete removal of chloroform from the lipid film.

For dye leakage assays, LUVs were prepared in leakage buffer, comprising 10 mM Tris (pH 7.4), 150 mM NaCl and 1 mM EDTA supplemented with 70 mM calcein by extrusion through 100 nm filters. The LUVs were separated from free calcein by passage through a 10 × 1 cm column of Sephadex G-50 and collection of the yellow fractions that did not fluoresce under long-wave UV light. After removal of free calcein from LUVs encapsulating calcein, the concentration of lipids was determined using a modified phosphorus assay, as follows.[49] To a 12 x 75 mm disposable culture tube was added 50 µL of the LUV suspension. Then 30 µL of a 10% (w/v) solution of $Mg(NO_3)_2$ in ethanol was added to the culture tube. This mixture was ashed over a hot flame resulting in the formation of a grey precipitate. To dissolve the precipitate, 300 µL of 0.5 M HCl was added to the tube. This solution was heated for 15 minutes in a boiling water bath. After cooling to room temperature, 700 µL of a mixture of 1% (w/v) ascorbic acid

and 0.378% (w/v) ammonium molybdate tetrahydrate dissolved in 0.45 M H_2SO_4 was added to the boiled solution. This mixture was heated for 1 h at 37 ºC. Over this time the solution developed a faint blue color. This solution was then transferred to a 1 cm quartz cuvette, and the absorbance of the solution measured at 820 nm. The concentration of phosphate was determined using a molar extinction coefficient of 120 $M^{-1}cm^{-1}$. The concentration of total lipid is assumed to be equal to the concentration of phosphate measured in this assay, as one mole of phospholipid contains one mole of phosphate.

The stock LUV suspension was diluted in leakage buffer to a final concentration of 11 µM lipid. Solutions of peptides were prepared gravimetrically by dissolving the lyophilized peptide in the appropriate amount of 18 MΩ deionized water to achieve a 10 mg/mL stock. From the 10 mg/mL stock solutions, 10X solutions were made by dilution with 18 MΩ deionized water. From these 10X stock solutions, 20 uL were added to the wells of a 96-well plate in triplicate. An additional six wells were used as controls. Three wells received 20 µL of 18 MΩ deionized water (vehicle, negative control) and the other three wells received 20 µL of 10X lysis buffer (positive control). To every well was added 180 uL of the 11 µM lipid LUV suspension. The fluorescence was immediately recorded on a Thermo Scientific Varioskan Lux fluorescent plate reader. The excitation wavelength was set to 490 nm. Emission was recorded at 520 nm. Data was averaged across the three replicate wells. Data is plotted using the below equation:

$$\%\, leakage \;=\; 100 \;\times\; \frac{(F_{peptide} \;-\; F_{water})}{(F_{lysis} \;-\; F_{water})}$$

Where $F_{peptide}$ is the average fluorescence of the given peptide treatment, F_{water} is the average fluorescence of the water treatments, and F_{lysis} is the average fluorescence of the lysis buffer treatment.

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Oligomers Formed by a Toxic β-Hairpin Derived from α-Synuclein: Trimers and Higher-Order Oligomers. *J. Am. Chem. Soc*. **2016**, *138*, 4458-4467.

46. The cytotoxicites of the peptides were determined using an LDH-release assay following a protocol similar to those published previously in our laboratory. The procedures were either adapted from or taken verbatim from Spencer, R. K.; Kreutzer, A. G.; Salveson, P. J.; Li, H.; Nowick, J. S. X-ray Crystallographic Structures of Oligomers of Peptides Derived from β2-Microglobulin. *J. Am. Chem. Soc*. **2015**, *137*, 6304-6311.

47. Liposomes were prepared following a protocol similar to those published previously in our laboratory. The procedures were either adapted from or taken verbatim from Salveson, P. J.; Spencer, R. K.; Nowick, J. S. X-Ray Crystallographic Structure of Oligomers Formed by a Toxic β-Hairpin Derived from α-Synuclein: Trimers and Higher-Order Oligomers. *J. Am. Chem. Soc*. **2016**, *138*, 4458-4467.

48. The propensities of the peptides to destabilize the membranes of negatively charged LUVs were determined using a dye leakage assay following a protocol similar to those published previously in our laboratory. The procedures were either adapted from or taken verbatim from Salveson, P. J.; Haerianardakani, S.; Thuy-Boun, A.; Kreutzer, A. G.; Nowick, J. S. Controlling the Oligomerization State of Aβ-Derived Peptides with Light. *J. Am. Chem. Soc.* **2018**, *140*, 5842-5852.

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Chapter 2: Interpenetrating Cubes in the X-Ray Crystallographic Structure of a Peptide Derived from Medin$_{19–36}$

2.1 Preface

When I first joined the Nowick lab in my third year of graduate school, I knew I wanted to explore the supramolecular assemblies of amyloidogenic peptides that had not yet been researched in the lab. Prion, responsible for transmissible spongiform encephalopathies, was my initial focus. Unfortunately, the amino acids that comprise the prion protein are so hydrophobic that the macrocyclic peptides derived from the prion protein that I synthesized were difficult to dissolve in water. Even once the peptides were in solution I was unable to visualize them by SDS-PAGE following silver staining. At that point I determined it was necessary to pivot to study a different amyloidogenic peptide. I turned my attention to medin, a peptide that causes vascular degeneration. The monomeric structure of medin was published a few months prior to when I joined the Nowick lab and so there was not much known about how the peptide assembled to cause its biological effects. Despite the limited information available, I took the initiative to explore new territory. For this project I synthesized, purified, characterized, and crystallized the macrocylic peptide derivative of medin. Michał Wierzbicki collected the X-ray diffraction data and solved the crystal structure.

2.2 Introduction

Amyloidogenic peptides and proteins are rich sources of supramolecular assemblies. Sequences derived from Aβ (Alzheimer's disease), human islet amyloid polypeptide (type II diabetes), and tau (Alzheimer's disease and frontotemporal dementia) have been found to assemble as fibrils, nanosheets, ribbons, and nanotubes (Table 2.1).[1–19] Other types of assemblies have also been observed, including square channels from sequences derived from transthyretin (senile systemic amyloidosis) and cylindrins from sequences derived from αB-crystallin (cataracts).[20–21] Although these well characterized amyloidogenic peptides and proteins have yielded many novel supramolecular assemblies, the more recently discovered amyloidogenic peptide medin has not been as heavily studied and provides and exciting frontier for the discovery of interesting supramolecular assemblies.

Table 2.1. Supramolecular assemblies of selected sequences derived from amyloidogenic peptides and proteins.

peptide	sequence	assembly	citation
Aβ(10–35)	YEVHHQKLVFFAEDVGSNKGAIIGLM	fibril[a,b]	1, 2, 3
Aβ(16–22)	Ac-KLVFFAE-NH2	fibril, helical ribbon, nanotube[a,b,c,d]	4, 5, 6
	KLVF(*N*-Me)FAE, KLVFFAE	nanotube[e]	7
Aβ(16–22), Italian mutant	KLVFFAK	nanosheet[b,c]	8
Aβ(16–20)	AAKLVFF	nanotube[b]	9, 10
	βAβAKLVFF	helical ribbon[d]	11
Aβ(19–20)	FF	nanotube[b]	12
	Ac-FF-NH_2, NH_2-FF-NH_2, Boc-FF-NH2	nanotube[b,c]	13
	Fmoc-FF	flat ribbon, fibril[b,c,d]	13, 14
hIAPP(20–29)	SNNFGAILSS	flat ribbon, helical ribbon[c,d]	15
	SNNFG(*N*-Bu)AI(*N*-Bu)LS(*N*-Bu)S	helical ribbon[b]	16
hIAPP(21–29)	NNFGAILSS	helical ribbon[b,c]	17
hIAPP(23–27)	FGAIL	flat ribbon[b,c]	18
tau(306–311)	Ac-VQIVYK-NH_2	straight and twisted filament[b]	19
transthyretin (106–112, 115–121)	TIAA(*N*-Me)LLS, SFSTTAV	square channel[e]	20
αB-crystallin(90–100)	KVKVLGDVIEV	cylindrin[b,e]	21

a. Solid-state NMR (ssNMR) b. Electron microscopy (EM) c. Atomic force microscopy (AFM) d. Cryo-EM e. X-ray crystallography

Medin, also referred to as aortic medial amyloid, is a 50-amino acid peptide that forms fibrillary deposits in aging human vasculature (Figure 2.1). These deposits have been implicated in the pathogenesis of thoracic aortic aneurysm and dissection. Westermark et al. found evidence of fibrillary medin in the aortic media of 97% of subjects over the age of 50.[22] Although the amino acid sequence of medin was determined in 1999, the folding pattern of the monomer was not elucidated until 2017.[23] Using ^{13}C NMR spectroscopy, Madine et al. identified that the monomer of medin contains three β-strand regions consisting of residues 7–13, 21–25, and 30–36.[24] The

sequences 14–24 and 26–29 were suggested to form unstructured loops. Computational modeling using QUARK and ROSETTA consistently predicted the 21–35 region to fold as a β-hairpin.[24]

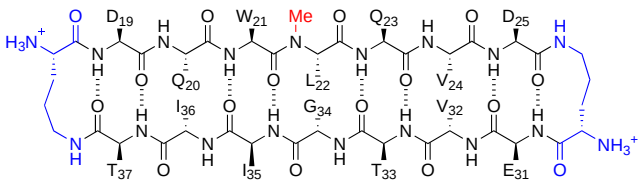
Figure 2.1. Amino acid sequence of medin. Amyloidogenic regions predicted by TANGO are highlighted in red. Underlined residues are β-strand regions identified by ^{13}C NMR spectroscopy.

Medin is predicted by TANGO to have three amyloidogenic regions (Figure 1).[25] Westermark et al. studied the medin(1–25), medin(32–41), and medin(42–49) peptides. The researchers only observed the formation of fibrils from the latter two peptides by electron microscopy, and concluded that the amyloid forming motif of medin lies in its C-terminus. Gazit et al. also observed the formation of fibrils by the medin(42–49) peptide by electron microscopy.[26] Middleton et al. subsequently used solid-state NMR spectroscopy and X-ray fiber diffraction to establish that the fibrils from medin(42–49) consist of parallel, in-register β-sheets that assemble in a face-to-back manner.[27] To our knowledge, no additional structural information regarding the assembly of medin(32–41) has been elucidated.

2.3 Results

To gain further insights into the supramolecular assembly of medin, we set out to study the two β-strand regions in the medin monomer predicted to fold as a β-hairpin. To synthesize the β-hairpin mimic peptide 1, we connected two heptapeptide strands using δ-linked ornithine ($^{\delta}$Orn) turn units (blue) to form a macrocycle.[28,29] The two heptapeptide strands are derived from medin(19–25) DQWLQVD (top strand) and medin(31–37) EVTGIIT (bottom strand). *N*-Methylation (red) of the backbone was employed to attenuate uncontrolled aggregation.[30] This

strategy to make β-hairpin mimics has been successfully used by our lab in the past to identify other amyloid assemblies using X-ray crystallography.[31]



peptide **1**

We used X-ray crystallography to study the structure and assembly of peptide 1. We began our crystallization efforts by screening peptide 1 in 576 conditions in a 96-well plate format using crystallization kits from Hampton Research. Cube-shaped crystals (Figure S4) grew in abundance in drops containing sodium acetate, calcium chloride, and 2-methyl-2,4-pentanediol. Further optimization of the crystallization conditions afforded monocrystals suitable for X-ray diffraction (optimized conditions: 0.1 M NaOAc, 0.02 M $CaCl_2$, 30% MPD). The X-ray diffraction experiments were performed at UCSD X-ray facility. Despite the relatively high resolution of the acquired dataset (ca. 1.32 Å), we were unable to solve the structure by single wavelength anomalous diffraction (SAD). In an attempt to increase the magnitude of anomalous scattering, we crystallized peptide 1 in the optimized conditions substituting barium chloride for calcium chloride. The modified conditions afforded similar cube-shaped crystals that gave rise to enough measurable anomalous signal to facilitate SAD phasing and subsequent solution of the original dataset by isomorphous replacement. The structure was thus solved in the cubic I23 space group and refined to a final R_{work} of 0.1772 (PDB 7JRH).

The X-ray crystallographic structure of peptide 1 reveals a three-dimensional network of large interpenetrating cubes, ca. 4.3 nm in size (Figure 2). Each cube is composed of molecules of peptide 1 located at the edges and coordinated to calcium ions at the vertices. In the structure,

peptide 1 folds to form a hydrogen-bonded β-sheet. The elongated β-strand conformation of the top strand places the two aspartic acid residues (D_{19} and D_{25}) at the opposite ends of the β-sheet, ca. 2.0 nm apart. The side chain carboxylate group of D_{19} binds one calcium ion, and the side chain of D_{25} binds another calcium ion. Each of the ions in turn binds either D_{19} or D_{25} residues of two other peptide molecules. This mode of coordination, in conjunction with the symmetry present in the crystal lattice, results in the formation of an assembly containing eight calcium ions and twelve molecules of peptide 1. Even though the arrangement of edges and vertices resembles a cube (Figure 2.2A and B), the point symmetry is actually tetrahedral, with four of the vertices arising from the coordination of D_{19} and the other four vertices arising from the coordination of D_{25}.

The cubes stack face-to-face in the crystal lattice in all three directions, forming an infinite 3-dimensional array (Figure 2.2C and D). Consistent with the body-centered lattice type, the complete crystal lattice contains two symmetry-equivalent mutually-interpenetrating arrays, related through a [0.5, 0.5, 0.5] Bravais translation vector (Figure 2.2E and F).[32] Such a polycatenated structure belongs to the **bcu-y** class in knot theory — a 3D 'chainmail'of linked cubes.[33]

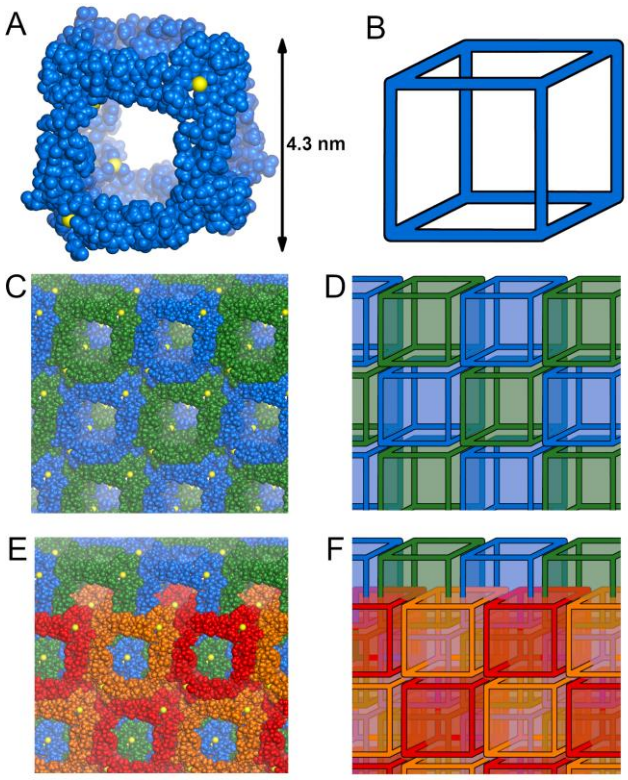


Figure 2.2. Three-dimensional assembly observed in the X-ray crystallographic structure of peptide **1**. Individual cube (A), infinite array of cubes (C), and two interpenetrating arrays (E), along with their respective schematic representations (B, D, and F). The cubes are all symmetry-equivalent, and the coloring scheme has been chosen arbitrarily for clarity.

The packing of the cubes, as shown in Figure 2.2C and D, is stabilized by hydrogen bonding and hydrophobic interactions between molecules of peptide **1** located at the edges of the cubes. The four molecules of peptide **1** that constitute the edges of every four adjacent cubes form a tetramer (Figure 2.3A). The tetramer can be interpreted as a dimer of dimers, in which each dimer is stabilized by six intermolecular hydrogen bonds involving side chains and main chains of T_{37}, Q_{23}, and I_{35} (Figure 2.3B). The dimers stack to form the tetramer. Packing of the hydrophobic side chains of I_{35}, V_{32}, and L_{22} — a total of twelve residues, three per monomer — help stabilize the tetramer. Eight hydrogen bonds between Q_{20} and V_{32} further stabilize the assembly.

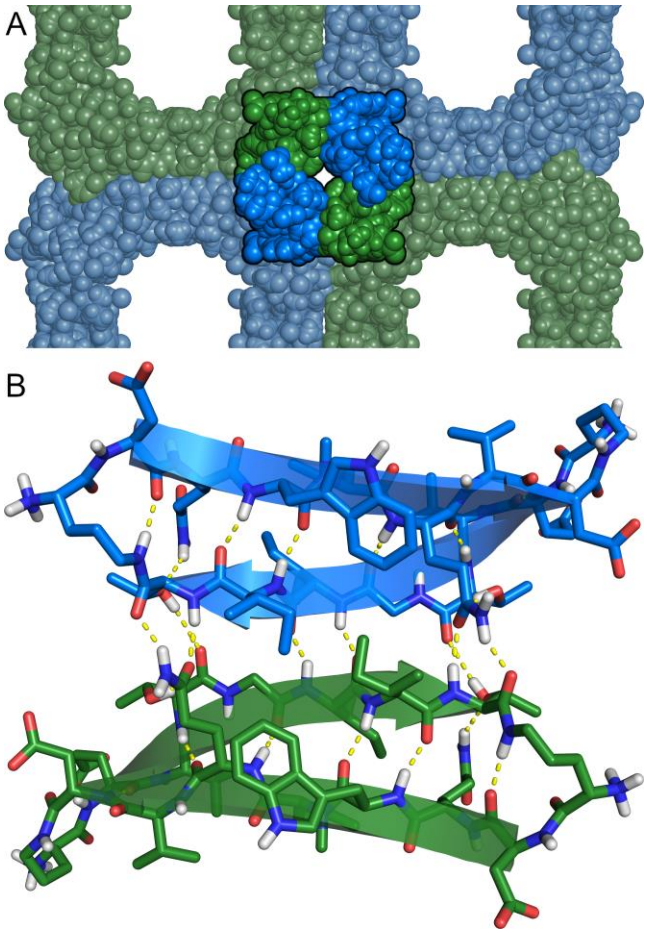


Figure 2.3. Crystal packing forms a tetramer. (A) The location of a tetramer in within the cubic assembly of peptide **1**. (B) Hydrogen-bonded dimer within the tetramer.

The monomeric β-hairpins that constitute the building blocks of the crystal lattice have an unexpected folding pattern. Peptide **1** was designed to contain two hydrogen-bonded β-strands — DQWLQVD (top strand) and EVTGIIT (bottom strand) — linked by two $^{\delta}$Orn turn units. In the X-ray crystallographic structure, the top and bottom strands do indeed form a hydrogen-bonded β-sheet, but not precisely as designed (Figure 2.4). Residues T_{33} and G_{34} in the bottom strand form a β-bulge,[34] and six of the seven residues of the top strand (Q_{20} through D_{25}) participate in β-sheet formation. The β-bulge causes a shift in the registration of the strands, which brings D_{19} into an extended loop with $^{\delta}$Orn and causes T_{37} to pair with Q_{20} instead of D_{19} (Figure 2.4B).

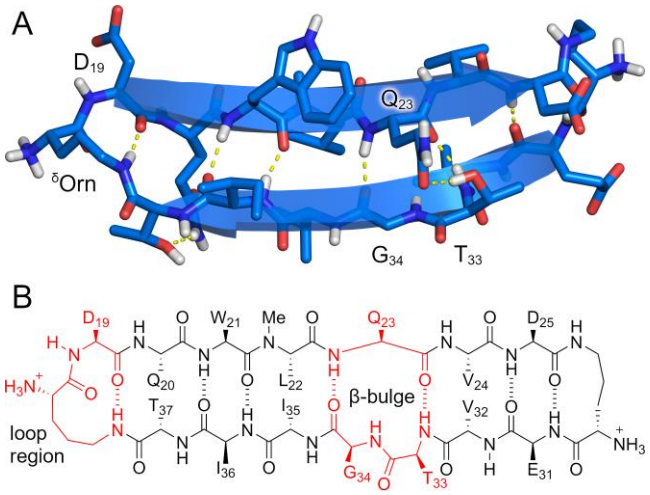


Figure 2.4. β-Bulge in the X-ray crystallographic structure of peptide **1**. (A) X-ray crystallographic structure of the monomeric β-hairpin subunit of the crystal lattice. (B) Hydrogen bonding pattern observed in the X-ray structure.

2.4 Discussion

Coordination of Ca^{2+} by carboxylates in the peptide 1 structure is reminiscent of calcium binding in structures ranging from EF-hand proteins to metal-organic frameworks (MOFs). The D_{25}-calcium binding site of peptide 1 contains three aspartate carboxylates and three water molecules in an octahedral arrangement (Figure 2.5A), while the D_{19}-calcium binding contains three aspartate carboxylates and one water molecule (Figure 2.5B). EF-hands are a class of calcium-binding proteins featuring a helix-loop-helix motif, containing multiple carboxylates and other ligands that bind Ca^{2+}. Calmodulin is a prominent example of this class of calcium-binding proteins, employing three aspartates to bind Ca^{2+}, in a fashion similar to the binding mode within the peptide 1 cubes (binding of Figure 2.5C).[35] Only a handful of calcium-based metal-organic frameworks have been reported, including MOFs suitable for removal of heavy metals from water[36] and slow release of pesticides,[37] far fewer than the number of MOFs containing transition metals.[38] Calcium-based materials, such as alginate gels, are of interest for applications in the

foodstuffs and medicines, where only non-toxic metals are permissible.[39,40] We anticipate that calcium-based MOFs may also have potential applications in these areas.

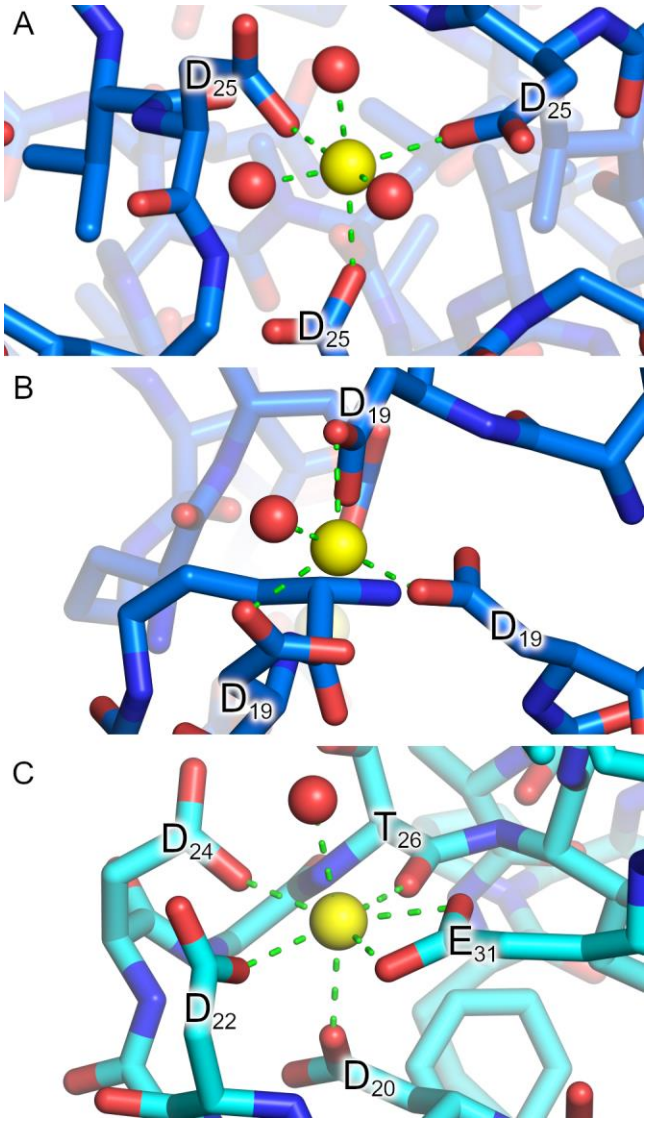


Figure 2.5. Calcium binding by carboxylate residues. (A and B) Calcium binding geometry observed in the X-ray crystallographic structure of peptide **1**. (C) Calcium binding site in the calmodulin protein (PDB 1CLL). Calcium ions are shown as yellow spheres and water molecules as red spheres.

The non-covalent interactions in the crystal structure of peptide 1 are diverse: metal coordination, hydrogen bonding, and hydrophobic packing. Although it is not possible to assess the relative contributions of each of these interactions in stabilizing the assembly, metal

coordination is critical. We have not observed growth of any stable crystals in the absence of divalent metal ions (Ca^{2+}, Ba^{2+}, or Co^{2+}). The interpenetrating cubes formed by peptide 1 and Ca^{2+} can thus be considered a calcium-based MOF with peptide ligands, and not merely an artificial calcium receptor.

The interpenetrating cubes cannot form separately, but rather must form simultaneously from tetramers, dimers, single molecules, or other assemblies of peptide 1 during the crystallization process. For this reason, the lattice may also be interpreted as a result of the assembly of tetramers, with the tetramers acting as ligands bearing eight carboxylate groups. Regardless of the process by which the lattice assembles, it can ultimately be viewed as an assembly of cubes with Ca^{2+} at the vertices, because of the crucial role metal cations play in the assembly.

Intrinsic chirality and biocompatibility have allowed peptides to act as ligands in a number of MOFs possessing remarkable properties. These structures include MOFs exhibiting selective binding of chiral molecules,[41] reversible conformational changes upon binding,[41,42] and high mechanical stability.[44] The relative flexibility of peptide chains, which imparts these interesting properties, is also a major limiting factor. In longer peptide chains, it is almost impossible to predict and control secondary structure. A notable example is a pentapeptide-cadmium complex reported by Yaghi *et al.* that exhibits no porosity.[45] In the case of the framework structure formed by peptide 1, the greatly reduced conformational flexibility of the cyclic β-sheet peptide is sufficient to allow the formation of a well-defined assembly. The preorganized spatial arrangement of the two aspartate side chains allows the peptide to behave like a rigid ligand. Thus, a design principle emerges for the construction of other peptide-based MOFs, whereby a preference for a particular secondary structure allows preorganization of metal-binding ligands (e.g., Glu, Asp, or His).

It is important to note that larger peptides with well-defined secondary structures have previously been used with metals to construct various nanostructures. Notable examples include α-helix bundles[46,47,48,49] and collagen triple helices.[50] Many of these structures rely upon the incorporation of non-natural ligands — most often various pyridine derivatives — into the peptide sequences. The three-dimensional framework formed by peptide 1 is distinctive, because the metal binding involves side chains of unmodified aspartic acid residues. We envision that it will be possible to construct other large calcium-containing MOFs from peptides with well-defined secondary structures that display Glu or Asp side chains. We further envision that this design principle might be extended to other metals and amino acid side chains such as His or Cys.

2.5 Conclusion

The assembly formed by peptide 1 in the presence of divalent metal ions constitutes a rare example of a metal-organic framework in which a large polypeptide acts as a ligand. The MOF formed by peptide 1 is among the largest peptide-based MOFs. While the structure exhibits interpenetration, and thus does not contain solvent-accessible pores, it demonstrates important rules for the construction of peptide-based MOFs. The peptide's cyclic β-sheet design exemplifies one such emergent principle, in which a preference for a particular secondary structure and supramolecular assembly allows the preorganization of metal-binding sites. We envision that different peptides with well-defined secondary structures, bearing multiple metal-binding residues may — in conjunction with metal ions — result in MOFs. Amyloidogenic peptides are particularly suited for this purpose, since they are predisposed toward supramolecular assembly. Amyloidogenic peptides other than medin might also serve as ligands for MOF structures if they contain, or are altered to contain, suitable metal-binding residues.

2.6 Appendix

2.6.1 Supplementary Figures

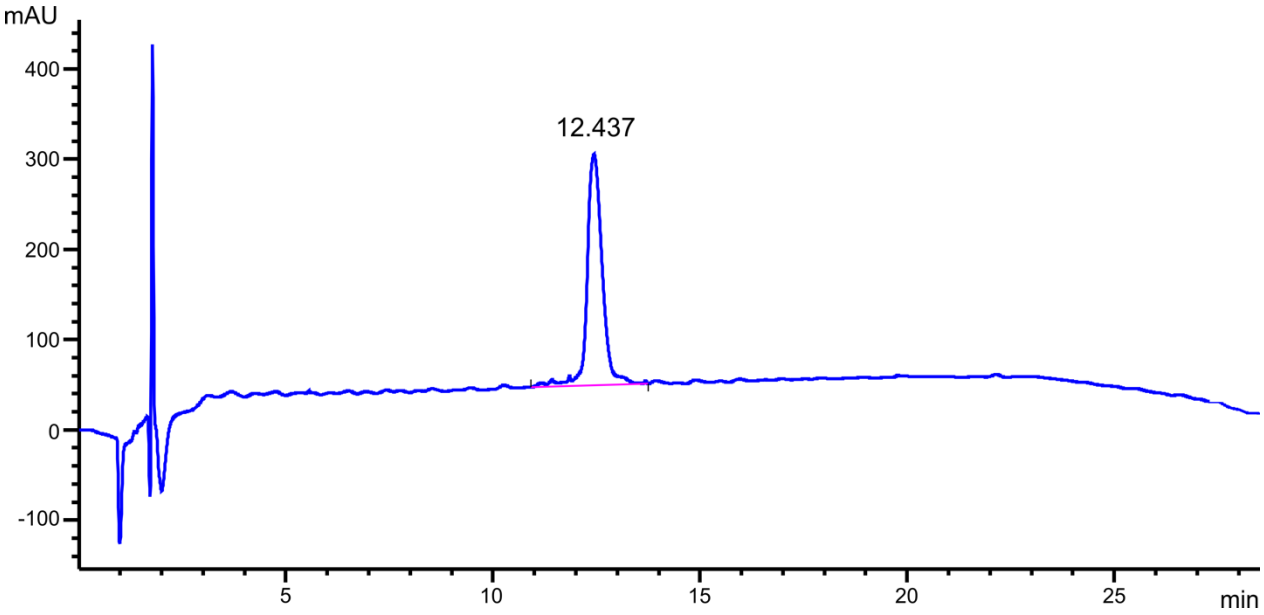


Figure 2.6. Analytical HPLC trace for peptide **1**.

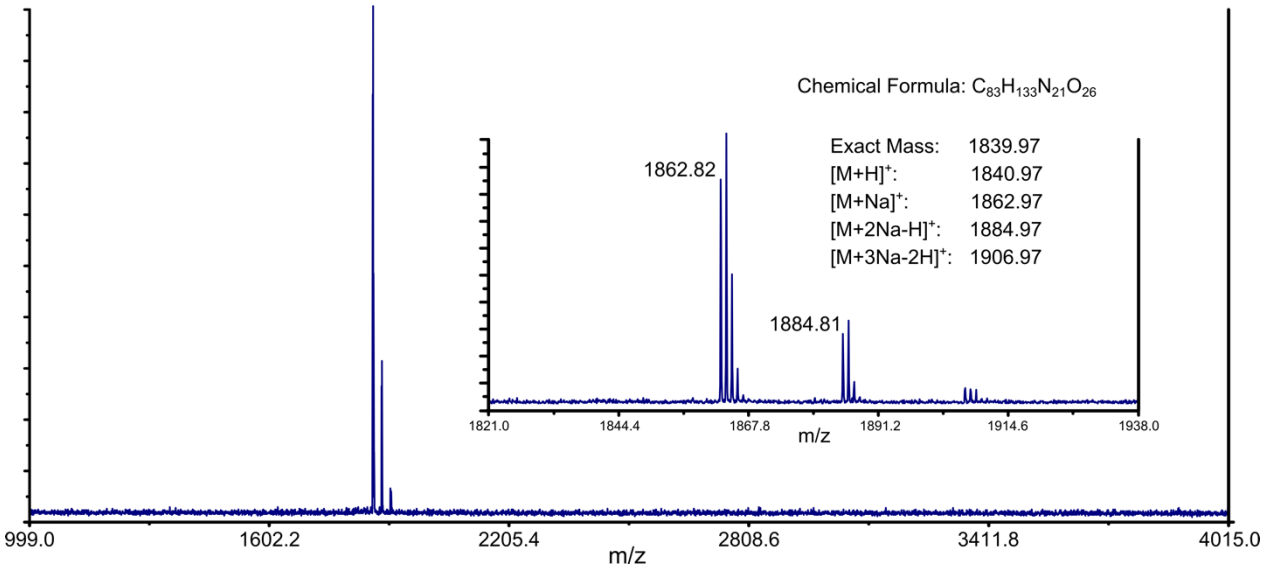


Figure 2.7. MALDI-TOF mass spectrum of peptide **1**.

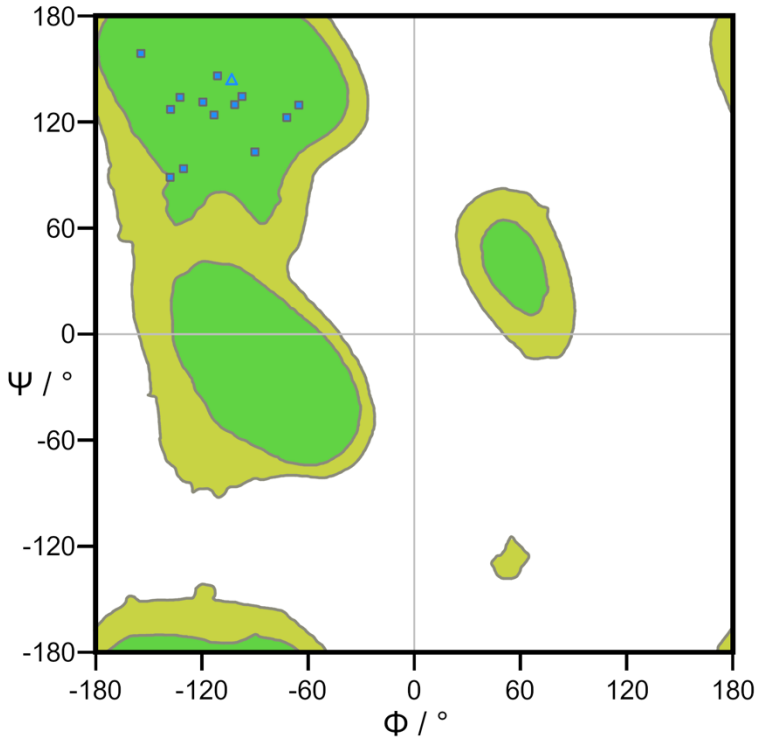


Figure 2.8. Ramachandran plot illustrating the φ and ϕ angles of residues D_{19}–D_{25} and E_{31}–T_{37} of peptide **1**. Preferred areas (0.2% probability) are shown in green and acceptable areas (2% probability) are shown in yellow-green. The glycine residue is shown as a triangle.

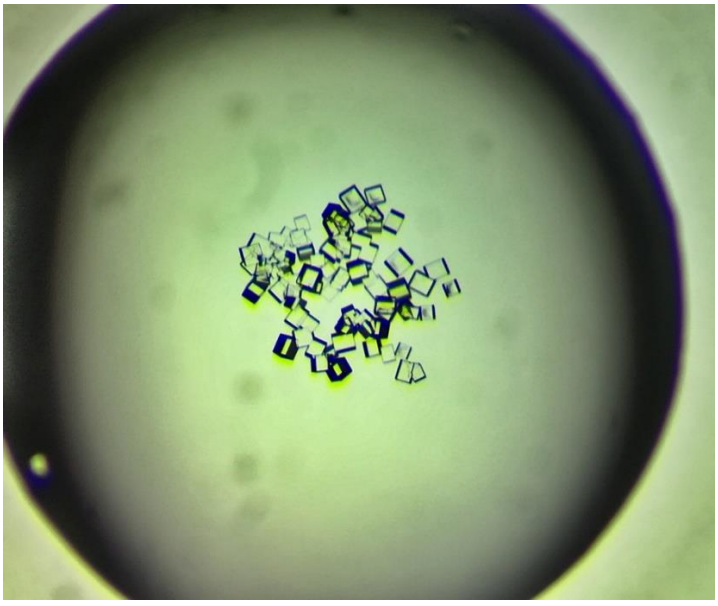


Figure 2.9. Crystals of peptide **1** within a crystallization drop.

2.6.2 General Information[51]

All Fmoc-protected amino acids including the unnatural amino acid, Boc-ornithine(Fmoc)-OH were purchased from Chem-Impex or Anaspec. 2-Chlorotrityl chloride resin was purchased from Chem-Impex. Trifluoroacetic acid (TFA), and HPLC grade acetonitrile (MeCN) were purchased from Fischer Scientific. Water was purified to 18 MΩ with a ThermoFischer GenPure Pro water purification system. All other solvents and chemicals were purchased from Alfa Aesar and Sigma Aldrich. All amino acids, resins, solvents, and chemicals were used as received, with the exception that dichloromethane (DCM) and *N*,*N*-dimethylformamide (DMF) were dried by passage through dry alumina under argon. Analytical HPLC chromatograms were obtained using an Agilent 1260 Infinity II HPLC equipped with Phenomenex bioZen C18 column (150 mm × 4.6 mm, 2.6 μm particle size). A 1 mL/min flow rate was used with peak detection at 214 nm, all monitored using the provided HPLC OpenLAB software. Preparative-scale purification of peptides was done using an Agilent Zorbax SB-C18 PrepHT column (21.2 mm x 250 mm, 7 μm particle size) on a Rainin Dynamax HPLC with a flow rate of 7.0 mL/min, monitored at 214 nm with the accompanying DA Rainin HPLC software. MALDI mass spectrometry was performed using an Applied Biosystems SCIEX TOF/TOF under reflector positive ion mode using 2,5-dihydroxybenzoic acid matrix. Spectra were analyzed using the accompanying TOF/TOF Series Explorer software.

2.6.3 Abbreviations

DCM	dichloromethane
DIPEA	diisopropylethylamine
DMF	*N*,*N*-dimethylformamide
HATU	*N*,*N*,*N'*,*N'*-tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate

HBTU	*N*,*N*,*N'*,*N'*-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate
HCTU	*N*,*N*,*N'*,*N'*-tetramethyl-O-(6-chlorobenzotriazol-1-yl)uranium hexafluorophosphate
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
HPLC	high-performance liquid chromatography
HOBt	hydroxybenzotriazole
MALDI	matrix-assisted laser desorption ionization
MeOH	methanol
MeCN	acetonitrile
TFA	trifluoroacetic acid
TIPS	triisopropylsilane

2.6.4 Synthesis of Macrocyclic Peptide[52]

The synthesis of macrocyclic β-sheet 1 involved the following sequence of operations: (1) resin loading, (2) solid-phase amino acid couplings, (3) cleavage of the linear peptide from the resin, (4) solution-phase cyclization of the linear peptide, (5) global deprotection of acid-labile protecting groups, and (6) purification with preparative reverse-phase HPLC. The purified peptide was characterized by analytical HPLC and MALDI mass spectrometry.

2.6.5 Resin Loading

2-Chlorotrityl chloride resin (0.300 g, 1.6 mmol/g, 100-200 mesh) was added to a 10-mL Bio-Rad Poly-Prep chromatography column (8 mm x 40 mm). The resin was suspended in dry DCM (8 mL) and allowed to swell undisturbed for 30 min. The solution was drained from the resin using nitrogen and a solution of Boc-ornithine(Fmoc)-OH (150.0 mg, 0.33 mmol) in 20% (v/v) 2,4,6-collidine in dry DCM (8 mL) was added immediately. The suspension was gently agitated

for 24 h. The solution was then drained using nitrogen and washed with dry DCM (3x). After washing, a mixture of DCM/MeOH/DIPEA (8.5:1:0.5, 8 mL) was added immediately. The suspension was gently agitated for 30 min to cap any unreacted sites on the resin. The resin was washed with DMF (3x) and dried by passing nitrogen through the chromatography column. The resin loading was determined to be 0.61 mmol/g based on UV analysis (290 nm) of the Fmoc cleavage product.

2.6.6 Solid-Phase Peptide Synthesis

The Boc-ornithine(Fmoc) loaded resin was transferred to a solid-phase peptide synthesizer reaction vessel designed for the Protein Technologies PS3 automated peptide synthesizer. The resin was subjected to cycles of automated amino acid couplings using Fmoc-protected amino acid building blocks. The linear peptide was synthesized from the C-terminus to the N-terminus. Each coupling consisted of: (1) Fmoc deprotection with 20% (v/v) piperidine in DMF for 5 min (2x); (2) resin washing with DMF (3x); (3) activation of the Fmoc protected amino acid (4 equiv) with 20% (v/v) 2,4,6-collidine in DMF (8 mL) in the presence of HCTU (4 equiv); (4) coupling of the activated Fmoc-protected amino acid; (5) resin washing with DMF (3x). All amino acid couplings took 20 min except for the tryptophan that followed the *N*-methyl-leucine. The tryptophan that followed the *N*-methyl-leucine (4 equiv) was coupled twice for 1 h each with HATU (4 equiv) in 20% (v/v) 2,4,6-collidine in DMF (8 mL), with no Fmoc deprotection in between the two coupling reactions. [This modification ensured complete amino acid coupling onto a more sterically hindered, secondary amine.] After the last amino acid was coupled, and its Fmoc protecting group deprotected, the resin was transferred from the peptide synthesizer reaction vessel to a new Bio-Rad Poly-Prep chromatography column. The resin was washed with DCM (3x), and dried by passing nitrogen through the column.

2.6.7 Protected Cleavage of the Linear Peptide

The protected linear peptide derivative of macrocyclic β-sheet 1 was cleaved from the resin by subjecting the resin to a cleavage solution of 20% (v/v) HFIP in DCM (8 mL). The resin and the suspension immediately turned red. The suspension was gently agitated for 1 h. The suspension was filtered, and the filtrate was collected in a 250-mL round-bottom flask. The resin was washed with additional cleavage solution (8 mL) and agitated for another 30 min. The suspension was filtered and the filtrate collected into the same round-bottom flask as the previous filtrate. The suspension was then washed with DCM (3 x 2 mL) until the resin was no longer red with all washes collected into the round-bottom flask. The combined filtrates were concentrated under reduced pressure to give a clear film. The protected linear peptide derivative of macrocyclic β-sheet 1 was then cyclized without prior purification.

2.6.8 Cyclization

The protected linear peptide derivative of macrocyclic β-sheet 1 was dissolved in dry DMF (125 mL) in the same 250-mL round-bottom flask as the previous step. HOBt (0.150 g, 1.11 mmol, 6.2 equiv) and HBTU (0.300 g, 0.79 mmol, 4.4 equiv) were dissolved in 8 mL of dry DMF in a test tube to which 300 μL of 2,4,6-collidine was added and the solution mixed until homogenous. The solution was then added to the flask and the mixture was stirred under nitrogen at room temperature for 96 h. The reaction mixture was concentrated under reduced pressure to give a white solid. The crude product was immediately subjected to global deprotection.

2.6.9 Global Deprotection of Acid-Labile Protecting Groups and Ether Precipitation

The protected cyclic peptide derivative of macrocyclic β-sheet 1 was dissolved in a mixture of TFA/TIPS/H_2O (9.0:0.5:0.5, 10 mL) in the same 250-mL round-bottom flask as the previous

step. The reaction mixture was then stirred under nitrogen at room temperature for 1 h. During the 1 h deprotection, two 50-mL conical tubes containing 40 mL of dry ether each were chilled on dry ice. Following the 1 h time, the peptide solution was split between the two conical tubes of ether. The tubes were then centrifuged at 500 *x g* for 15 min. The ether supernatant was poured off and the pelleted peptides dried under nitrogen for 15-20 min.

2.6.10 Purification of Macrocyclic Peptide

The crude peptide pellets were dissolved in 15% MeCN/H_2O (4 mL), and the solution was centrifuged at 500 *x g* for 5 min to pellet any insoluble material. The supernatant was then filtered through a 0.2 µm nylon syringe filter. The crude peptide was purified by reverse-phase HPLC following a gradient whereby the percentage of MeCN in the mobile phase was increased at a rate of 0.2%/min. Elution occurred at ca. 28% MeCN/H_2O. The pure fractions were lyophilized to afford 52.6 mg (13.9 % based on resin loading) of peptide 1 as the trifluoroacetate salt as a white powder.

2.6.11 Crystal Screening[53]

A 10 mg/mL stock solution of peptide was prepared using 18 MΩ water. Two 96-well screening kits (Index and Crystal) from Hampton Research were used to screen crystallization conditions. The peptide was screened against 576 crystal growing conditions with a nanoliter liquid handling instrument (TTP Labtech Mosquito) using the default "three drops" method to facilitate nanoliter-scaled crystallization (2:1, 1:1, and 1:2 peptide to well solution with a total volume of 150 nL). Crystal growing conditions of 0.02 M calcium chloride dihydrate, 0.1 M sodium acetate trihydrate pH 4.6, and 30% v/v (+/-)-2-methyl-2,4-pentanediol were identified (Crystal, A1). Optimization of the crystal growing conditions using Hampton VDX 24-well plates

and varying the pH of the sodium acetate and the concentration of the (+/-)-2-methyl-2,4-pentanediol resulted in larger crystal growth in 0.02 M calcium chloride dihydrate, 0.1 M sodium acetate trihydrate pH 6.5, and 30% v/v (+/-)-2-methyl-2,4-pentanediol. The best X-ray diffracting crystals grew as cubes. To facilitate crystallographic phasing, crystals were also grown in 0.02 M barium chloride, 0.1 M sodium acetate trihydrate pH 6.0, and 30% v/v (+/-)-2-methyl-2,4-pentanediol to afford the same cubic crystals.

2.6.12 X-Ray Crystallography

Diffraction data for the barium salt of peptide 1 were collected at the Advanced Light Source at Lawrence Berkeley National Laboratory beamline 8.2.2. The dataset was indexed and integrated with XDS,[54] scaled and merged with pointless and aimless in CCP4.[55] The crystallographic phase determination was done with Autosol.[56] The structure was refined using phenix.refine,[57] with manipulation of the model performed using Coot.[58]

Diffraction data for the calcium salt of peptide 1 were collected using a Bruker Microstar APEX II CCD diffractometer equipped with Cu-K_α source (1.54178 Å) up to a resolution of 1.32 Å. The dataset was processed with SAINT (Bruker), and scaled using SADABS. The structure was solved through isomorphous replacement using the previously refined structure of the barium salt, and refined with phenix.refine. Data collection and refinement statistics are shown below in Supplementary Table 1.

2.6.13 Supplementary Tables

Table 2.2. Crystallographic properties, crystallization conditions, data collection, and model refinement statistics for the calcium salt of peptide **1**.

peptide	**1**
PDB ID	7JRH
Wavelength / Å	1.54178
Resolution range / Å	21.24 - 1.321 (1.368 - 1.321)
Space group	I23
Unit cell parameters	42.486 42.486 42.486 90 90 90
Total reflections	51865 (2021)
Unique reflections	3117 (315)
Multiplicity	16.6 (6.4)
Completeness / %	99.74 (99.83)
Mean I/σ(I)	30.81 (3.32)
Wilson B-factor	15.04
R_{merge}	0.04568 (0.3153)
R_{meas}	0.04648 (0.3423)
R_{pim}	0.008085 (0.1275)
$CC_{1/2}$	1.000 (0.925)
CC*	1.000 (0.980)
Reflections used in refinement	3117 (315)
Reflections used for R_{free}	311 (35)
R_{work}	0.1779 (0.3195)
R_{free}	0.2106 (0.2877)
$CC_{(work)}$	0.987 (0.659)
$CC_{(free)}$	0.937 (0.695)
Number of non-hydrogen atoms	143
macromolecules (peptide)	130
ligands (Ca^{2+})	2
solvent (H_2O)	11
Protein residues	16
RMS(bonds)	0.011
RMS(angles)	1.62
Ramachandran favored (%)	100.00
Ramachandran allowed (%)	0.00
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.00
Clashscore	7.97
Average B-factor	18.59
macromolecules	18.04
ligands	22.62
solvent	24.31

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Chapter 3: A Macrocyclic Peptide Derived from the WW Domain of IQGAP1 that Inhibits the Binding of p110α to Native IQGAP1

3.1 Preface

During my fifth year of the Ph.D. program I had the opportunity to collaborate on a project brought to the Nowick lab by Dr. Jane Bardwell and Professor Lee Bardwell. The Bardwells were interested in designing a peptide derived from a scaffolding protein called IQGAP1 to determine if it could be used as a cancer therapeutic. They came to the Nowick lab because the part of the IQGAP1 scaffold protein that wanted to mimic naturally folds as a β-hairpin and our lab specializes in studying peptides that adopt this conformation. I had the opportunity to work on this project given the breadth of my experience synthesizing and characterizing β-hairpin peptides derived from a variety of other peptides and proteins including Aβ and medin, Two undergraduate students that I trained and mentored, Denise Bui and Shareen Ashby, assisted me with the synthesis, purification, and characterization of the peptides in this study. I performed the homology modeling and collected the circular dichroism spectra. The co-sedimentation assays were performed by Dr. Jane Bardwell. Xingyue Li, another member of the Nowick lab will be taking over this project from me. He will will collect NMR and X-ray crystallographic data of the peptide to assess how it is folding in solution and in the crystal lattice.

3.2 Glossary

Scaffold Protein	A protein that binds two or more protein binding partners to facilitate signal transduction.
WW Domain	A domain within scaffold proteins that binds to protein partners with proline-rich sequences. It is named for the two tryptophans contained in its amino acid sequence that are approximately 20-23 residues apart.
PPXY	One of four common binding motifs recognized by WW domains, consisting of two prolines followed by another amino acid and a tyrosine.
PPLP	One of four common binding motifs recognized by WW domains, consisting of two prolines followed by a leucine and then another proline.
PR	One of four common binding motifs recognized by WW domains, consisting of a proline followed by an arginine.
(*po*S/*po*T)P	One of four common binding motifs recognized by WW domains, consisting of a phosphoserine or phosphothreonine followed by a proline.
IQGAP1	A scaffold protein that mediates both the MAPK and PI(3)K-Akt cell signaling pathways.
CHD	The calponin homology domain, one of several domains within the IQGAP1 scaffold protein.
IR Region	The internal repeat region, one of several domains within the IQGAP1 scaffold protein.
IQ Domain	The isoleucine glutamine domain, one of several domains within the IQGAP1 scaffold protein. Its name comes from the first two most common amino acids in its sequence.

GRD	The GAP-related domain, one of several domains within the IQGAP1 scaffold protein.
RGCT	The Ras GAP C-terminus domain, one of several domains within the IQGAP1 scaffold protein.
MAPK Pathway	A signaling pathway known for its regulation of cell proliferation composed of a G-protein (Ras) and three kinases (Raf, MEK, and ERK).
Ras	A G-protein that in its GTP-bound form binds to and activates Raf.
Raf	A kinase that phosphorylates and activates MEK. Its activity is enhanced when it is in proximity to MEK on IQGAP1.
MEK	A kinase that phosphorylates and activates ERK. Its activity is enhanced when it is in proximity to ERK on IQGAP1.
ERK	A kinase that phosphorylates and activates transcription factors to increase the expression of genes involved in cell proliferation.
PI(3)K-Akt Pathway	A signaling pathway known for its regulation of cell proliferation and cell survival that depends on the kinases, PIPKIα and PI(3)K.
PIPKIα	A kinase that associates with IQGAP1 and phosphorylates phosphatidylinositol-4-phosphate (PtdIns(4)P) to make phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2).
PI(3)K	A kinase that associates with IQGAP1 and phosphorylates phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) to make phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3). It is a dimer composed of p110 and p85.

3.3 Introduction

Protein-protein interactions govern many molecular processes including transcription, translation, cell motility, and cell proliferation.[1-5] To mediate these interactions, a number of signaling proteins contain specialized domains that will recognize and bind short peptide motifs in the binding partners.[6] One class of these specialized domains are WW domains.

WW domains are roughly 40 amino acids long and contain two conserved tryptophan residues, from which the domain gets its name, that are approximately 20-23 residues apart.[7] These domains fold as triple-stranded, antiparallel β-sheets. The folding structure of the WW domain was first elucidated by NMR for the hYAP65 WW domain 1996; its crystal structure was determined in 2015 (Figure 3.1).[8,9] The binding of the WW domain to protein partners occurs through proline-rich or phosphoserine/phosphothreonine-containing sequences such as PPXY, PPLP, PR, and (*po*S/*po*T)P.[10] Approximately 50 human proteins have been identified that contain a WW domain.[1]

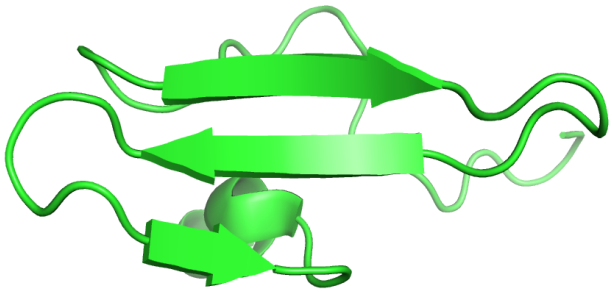


Figure 3.1. Crystal structure of hYAP65 WW domain. PDB 4REX.[9]

IQ Motif Containing GTPase Activating Protein 1 (IQGAP1) is one of these 50 human proteins. IQGAP1 is a scaffold protein (Figure 3.2) that mediates the MAP kinase (Ras-Raf-MEK-ERK) signaling pathway and in addition to having a WW domain, also has a calponin

homology domain (CHD), internal repeat (IR) region, an IQ domain composed of four isoleucine and glutamine-containing motifs, a GAP-related domain (GRD), and a Ras GAP C-terminus domain (RGCT).[11] Because the MAP kinase pathway is upregulated in approximately 30% of cancers, it is a promising target for cancer treatment.[12] Although inhibition of the kinases is not tolerated, *Iqgap1* knockout mice are viable and fertile, suggesting the scaffold protein is not essential for life.[13]

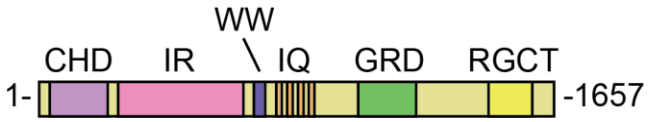


Figure 3.2. Structural domains of the human IQGAP1 scaffold protein. Calponin homology domain (CHD); internal repeat (IR) region; GAP-related domain (GRD); Ras GAP C-terminus domain (RGCT). The WW domain spans residues 681-710 and the IQ domain spans residues 745-864. Image adapted from Bardwell et al., 2017.[14]

Jameson et al. hypothesized that blocking the scaffolding function of IQGAP1 would have a selective, negative affect on cancer cells "addicted" to the scaffolded pathway.[15] Roy *et al*. have reported that the MAPK pathway protein ERK2 binds to the WW domain of IQGAP1 (Figure 3.3).[16] To disrupt this interaction, Jameson et al. treated melanoma cell lines with a WW domain peptide linked to a cell-penetrating peptide. These researchers found that this treatment reduced the viability of the melanoma cells to a level similar to the positive control treatment, vemurafinib, a Raf inhibitor. They also tested their exogenous peptide on mice bearing SK-Mel-28 melanoma tumors and mice bearing MDA-MB-468 breast tumors. The treatment impaired tumorigenesis in both cases. Administration of the peptide to non-cancerous cell lines showed no significant loss of viability, supporting their hypothesis that blocking the scaffolding function of IQGAP1 would only adversely affect cancer cells.

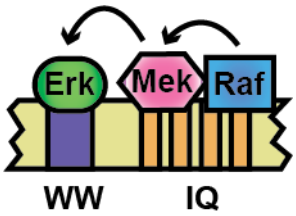


Figure 3.3. Model of the MAPK pathway binding partners of the IQ and WW domains of IQGAP1 proposed by Roy and co-workers. Raf and Mek bind the IQ domain and Erk binds to the WW domain. Raf phosphorylates Mek which then phosphorylates Erk. The black arrows represent phosphorylation of one kinase by the one before it. Image adapted from Bardwell et al., 2017.[14]

In spite of the success of targeting the WW domain of IQGAP1 as a cancer treatment, Bardwell et al. recently reported that ERK2 might not be the natural binding partner of the WW domain.[14] Using the same binding assay as Roy et al., the Bardwell group found instead that ERK2 binds the IQ domain (Figure 3.4). The only difference between the Bardwell study and the Roy study was that Bardwell et al. assessed the binding of truncated variants of the IQGAP1 domain as opposed to the internal deletion mutants employed by Roy et al. Bardwell et al. speculated that using deletion mutants affects the natural folding of the scaffold protein and that instead of ERK2 the WW domain has a different, undiscovered binding partner.

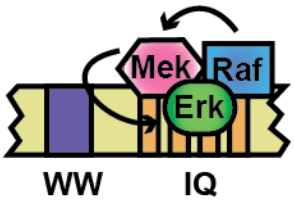


Figure 3.4. Alternative model of the MAPK pathway binding partners of the IQ and WW domains of IQGAP1 proposed by Bardwell and co-workers. Raf, Mek, and Erk all bind the IQ domain. Raf phosphorylates Mek which then phosphorylates Erk. The black arrows represent phosphorylation of one kinase by the one before it. The WW domain is not involved with these members of the signaling pathway. Image adapted from Bardwell et al., 2017.[14]

Although IQGAP1's role in the MAPK pathway is the best characterized, IQGAP1 also regulates a number of other signaling pathways, including the PI(3)K-Akt pathway. Choi et al. identified that modulation of the PI(3)K-Akt pathway could be achieved through the binding of

PIPKIα and PI(3)K to IQGAP1 (Figure 3.5).[17] These researchers found that PIPKIα binds the IQ domain and PI(3)K, a dimer composed of p85α and p110α, binds both the IQ domain and WW domain. They also determined that generation of the downstream signaling molecule, PtdIns(3,4,5)P_3 was enhanced by the proximity of PIPKIα and PI(3)K on IQGAP1. As the PI(3)K-Akt pathway is also upregulated in many types of cancers, Choi et al. took inspiration from Jameson et al. and administered IQ and WW domains of IQGAP1 linked to cell-penetrating peptides to breast cancer cell lines. Choi et al. found that these treatments reduced the viability of the cancer cells. Treatments administered to noncancerous lines showed no significant toxicity. These findings suggest that the cancer treatment of Jameson et al. was successful because it interfered with the PI(3)K-Akt pathway rather than the MAPK pathway.

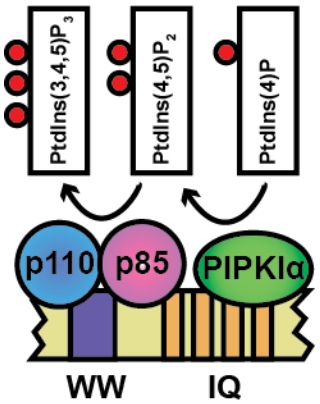


Figure 3.5. Model of the PI(3)K pathway binding partners of the IQ and WW domains of IQGAP1 proposed by Choi and co-workers. PIPKIα binds the IQ domain while PI(3)K, composed of p85 and p110, bind both the IQ and WW domains. PIPKIα phosphorylates PtdIns(4)P to make PtdIns(4,5)P_2 and PI(3)K phosphorylates PtdIns(4,5)P_2 to make PtdIns(3,4,5)P_3. Red dots represent phosphate groups.

Using truncations of IQGAP1, the Bardwell group found that it is the p110α subunit of PI(3)K that is the specific binding partner of the IQGAP1 WW domain.[18] Based on the work of the Bardwell group and Choi et al.'s previous work, only 14 of the 41 residues of the WW domain (residues 684-697) appear to be necessary for binding to p110α. The Bardwell group

hypothesized that if these 14 residues of the WW domain could be constrained into the β-hairpin conformation found in native WW domains, the constrained peptide should be able to retain the capacity to bind p110α and serve as a competitive inhibitor with the native IQGAP1 WW domain.

3.4 Results and Discussion

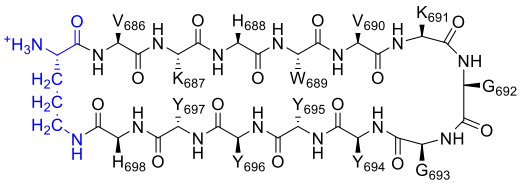
3.4.1 Homology Modeling

To design a constrained version of the 14-residue WW domain, I turned to homology modeling. Because a crystal structure of the WW domain of IQGAP1 does not exist, I used the crystal structure of the human YAP65 WW domain as a substitute because it is one of the only human WW domains ever crystallized, and it recognizes the most common WW domain binding motif, PPXY.[9] In the hYAP65 WW domain, residues 177 to 182 and 186 to 191 adopt β-strand configurations, leaving a three-residue unstructured loop between them (Figure 3.6).[8,9]

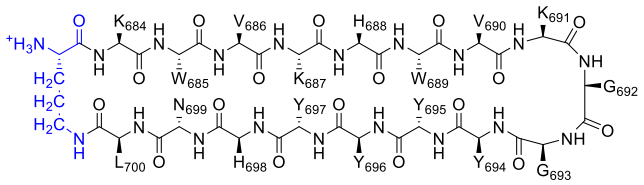
Figure 3.6. Sequence alignment of human YAP65 with human IQGAP1. Conserved residues are highlighted in red and β-strand regions of the hYAP65 sequence are underlined.

Based on a sequence alignment between hYAP65 and IQGAP1, I expected that residues 685-690 and 694-699 of IQGAP1 would adopt β-strands, leaving residues 691-693 as a three-residue unstructured loop between them. To connect the two β-strands, I proposed incorporating a δ-linked ornithine turn unit (blue) which has been used previously by our lab to favor β-hairpin conformations in cyclic peptides.[19,20] Because the geometry of the ornithine requires each strand to be composed of an odd number of residues, I proposed two initial macrocyclic peptide

models: one with pentapeptide strands (peptide **1**) and one with heptapeptide strands (peptide **2**). Peptide **1** includes residues 686-690 and 694-698, omitting one residue from each strand predicted by homology to be a part of the β-strand. Peptide **2** includes residues 684-690 and 694-700, having an additional residue in each strand outside of the predicted β-strand region.



peptide **1**



peptide **2**

To determine if the expected β-hairpin conformation would be retained in peptides **1** and **2**, I used PyMOL. I first mutated the hYAP65 WW domain residues to those from IQGAP1's, adjusting the rotamer of each side chain to minimize steric interactions. Then I built in the δ-linked ornithine turn and minimized the entire sequence, inspecting it closely to see if the β-hairpin motif and proper geometry of the ornithine were retained. I found that both peptide **1** and peptide **2** maintained the desired geometry and secondary structure (Figure 3.7).

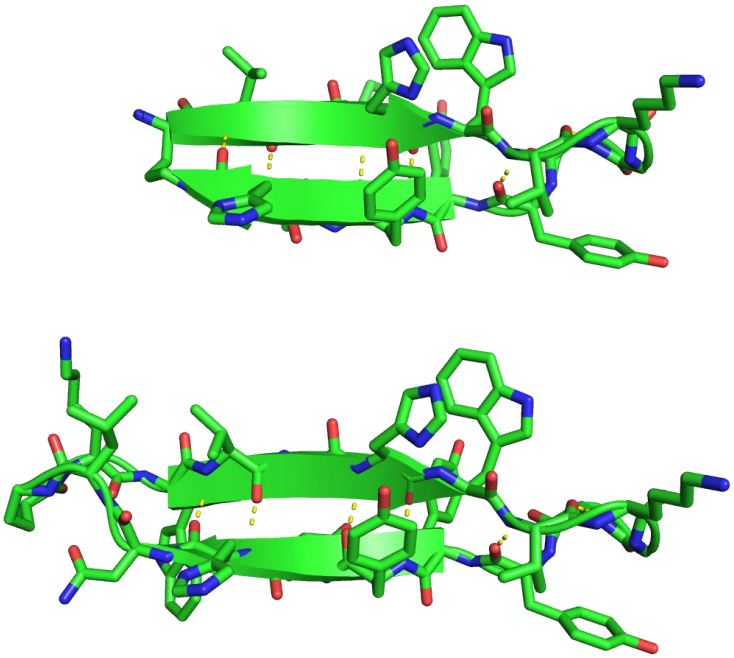


Figure 3.7. IQGAP1 WW domain homology models. Top, peptide **1**; bottom, peptide **2**.

3.4.2. Synthesis

Peptides **1** and **2** were synthesized by solid-phase peptide synthesis on chlorotrityl resin using standard Fmoc-based chemistry. The linear peptides were cleaved from the resin and cyclized on the δ-ornithine to produce the macrocycles. Following cyclization, the side chain protecting groups were cleaved and the peptides purified by preparative high-performance liquid chromatography. Following purification, 14.71 mg (3.45 % based on resin loading) of peptide **1** and 10.81 mg (1.99 % based on resin loading) of peptide **2** were obtained. Analytical HPLC and mass spectrometric analysis confirmed the identity and purity (> 95%) of the peptides.

3.4.3. Co-Sedimentation Assay

To assess the ability of peptides **1** and **2** to compete with native IQGAP1 WW domain for p110α, a co-sedimentation assay was performed.[21] In this competition experiment, p110α is introduced to a solution containing the peptide and an IQGAP1 WW domain-glutathione-S-

transferase fusion protein (WW-GST) bound to Sepharose beads. Following incubation of the mixture, the beads are centrifuged and the amount of bound p110α is analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The WW-GST fusion protein was expressed in *E. coli* and purified by adsorption to glutathione-Sepharose beads. The p110α was produced via *in vitro* translation in the presence of radiolabeled ^{35}S methionine which allowed for its detection using autoradiography.

Based on the results of the co-sedimentation assay, peptide **1** at concentrations of 10 and 100 μM minimally inhibited the binding of p110α to WW-GST (Figure 3.10). In contrast, peptide **2** inhibited binding substantially at both 10 and 100 μM. For both peptide **1** and peptide **2** there was little difference in the degree of inhibition between the two concentrations, indicating a lack of dose-response. Based on this preliminary result, I moved forward with peptide **2** as the lead compound in this study with two immediate aims. The first was to conduct the co-sedimentation assay with more replicates to obtain error bars. The second was to evaluate more concentrations of peptide **2** to construct a dose-response curve.

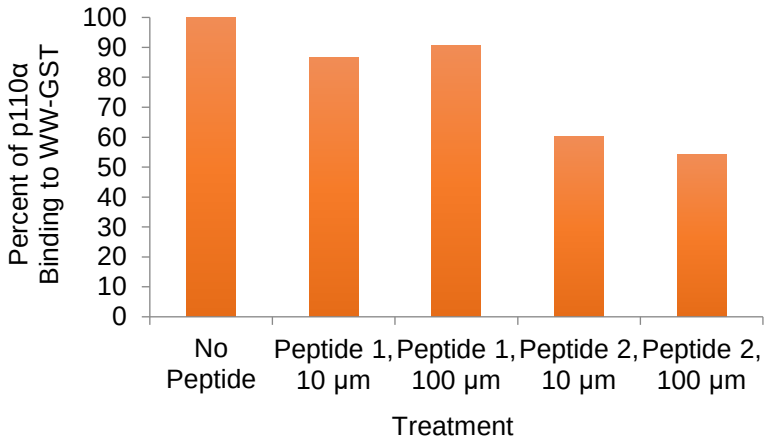
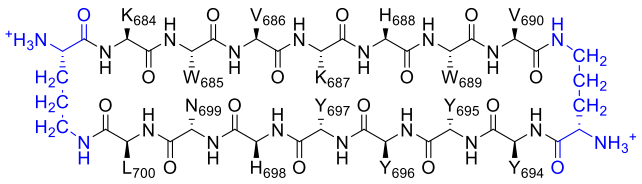


Figure 3.8. Binding propensities of peptides **1** and **2**. The no peptide treatment is set to 100% and all other treatments are listed relative to the no peptide treatment. Each treatment was run once so there are no error bars.

3.4.4 Dose-Response

While pursuing the aims described above, I also sought to synthesize a peptide that could better inhibit the binding of p110α to WW-GST than peptide **2**. I hypothesized that the KGG turn in peptide **2** could be replaced with a second δ-linked ornithine to make peptide **3**. The incorporation of the additional δ-ornithine turn was predicted to possibly tighten the β-hairpin conformation which may be essential for binding while also leaving the net charge of the peptide unchanged.



peptide **3**

The co-sedimentation assay revealed that peptide 3 had a similar degree of inhibition as peptide **2** (Figure 3.11). Dose-response curves for peptides **2** and **3** show 50% inhibition at ca. 10 μM. As was observed in the preliminary co-sedimentation results for peptide **2**, there is no substantial inhibition of p110α binding at concentrations of peptide above 10 μM. In contrast, peptide **3** does appear to exhibit dose-response behavior as the binding of p110α to WW-GST decreased from ca. 50% at 10 μM to ca. 25% at 30 μM.

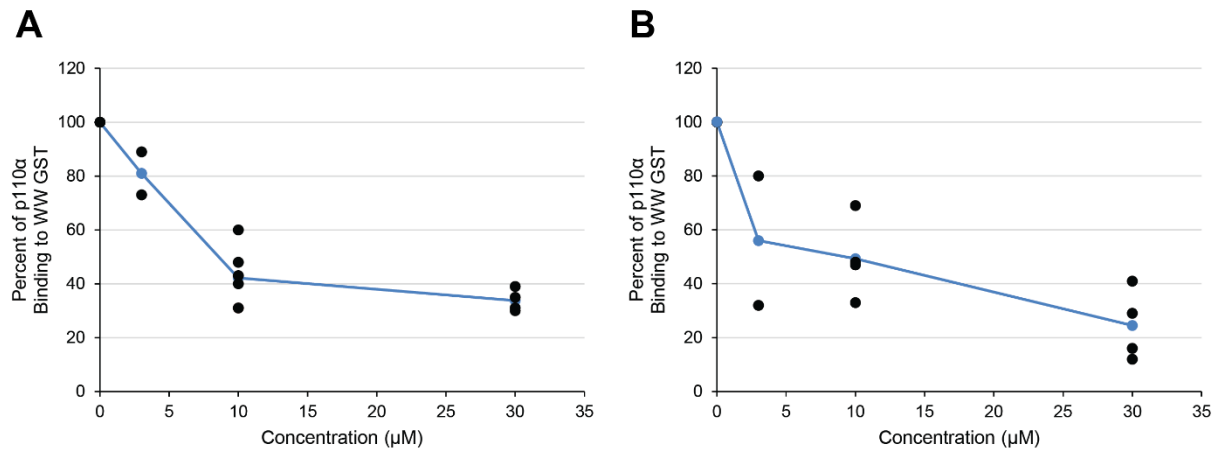
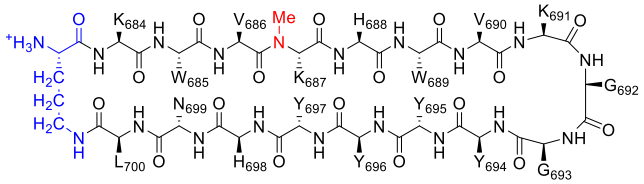


Figure 3.9. Dose-response curves of peptides **2** and **3**. Black dots represent independent measurements. Blue dots represent averages of measurements at the indicated concentrations. The blue line connects the averages. A) Peptide **2**. B) Peptide **3**.

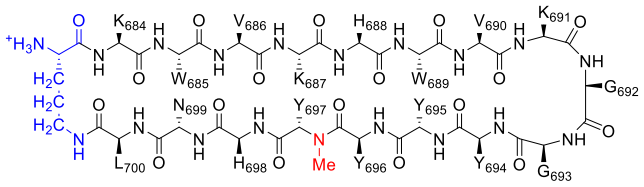
3.5 Conclusions and Future Directions

We have successfully designed, synthesized, and characterized two peptides derived from the WW domain of IQGAP1 that are able to inhibit the binding of p110α to the native IQGAP1 WW domain as determined using a co-sedimentation assay. Peptide **2** and peptide **3** are able to inhibit 50% of p110α binding at concentrations of ca. 10 μM.

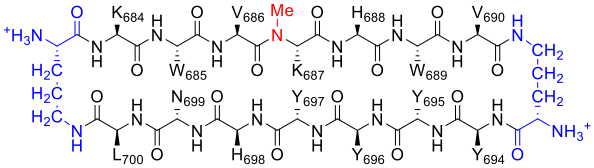
To provide further evidence that peptides **2** and **3** adopt β-hairpin conformations, CD spectra and NMR and X-ray crystallographic structures of the peptides need to be determined. Attempts to crystallize peptides **2** and **3** have been unsuccessful due to the insolubility of the peptide in nearly all of the crystallization conditions. Because *N*-methylation of the peptide backbone is a strategy that has been used in our lab to successfully crystallize peptides previously, I proposed and synthesized *N*-methylated (red) variants of peptide **2** (peptides **4** and **5**) and peptide **3** (peptides **6** and **7**).[22,23] Screening efforts are ongoing. NMR spectra will be collected and solved by fellow lab member Xingyue Li who has more experience with peptide NMR.



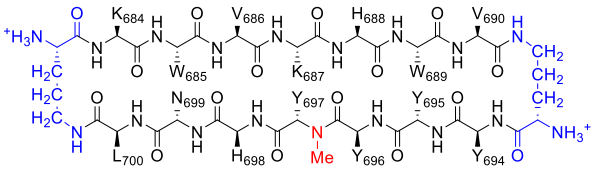
peptide **4**



peptide **5**

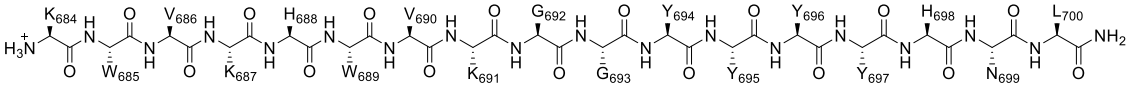


peptide **6**

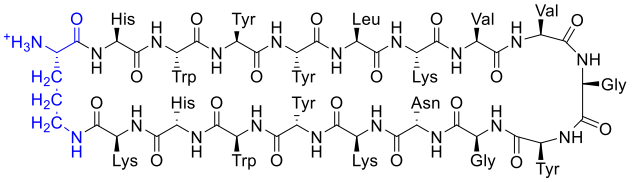


peptide **7**

To assess whether the β-hairpin structure and the amino acid sequence of the peptide macrocycles are critical for the inhibitory activities of peptides **2** and **3**, additional controls need to be conducted. To verify that the β-hairpin conformation of peptide **2** is critical for its observed inhibition of p110α binding to WW-GST, I synthesized peptide **8**, a linear variant of peptide **2** lacking the δ-ornithine turn unit. Peptide **8** was prepared using Rink resin to give an amidated C-terminus, ensuring it would have the same net charge as peptide **2**. To assess the importance of amino acid sequence, I synthesized peptide **9**, a scrambled peptide. Peptide **5** contains the same number and type of residues as peptide **2**, but in a different order, chosen randomly. These peptides will be used in co-sedimentation assays performed by Dr. Jane Bardwell in the near future.



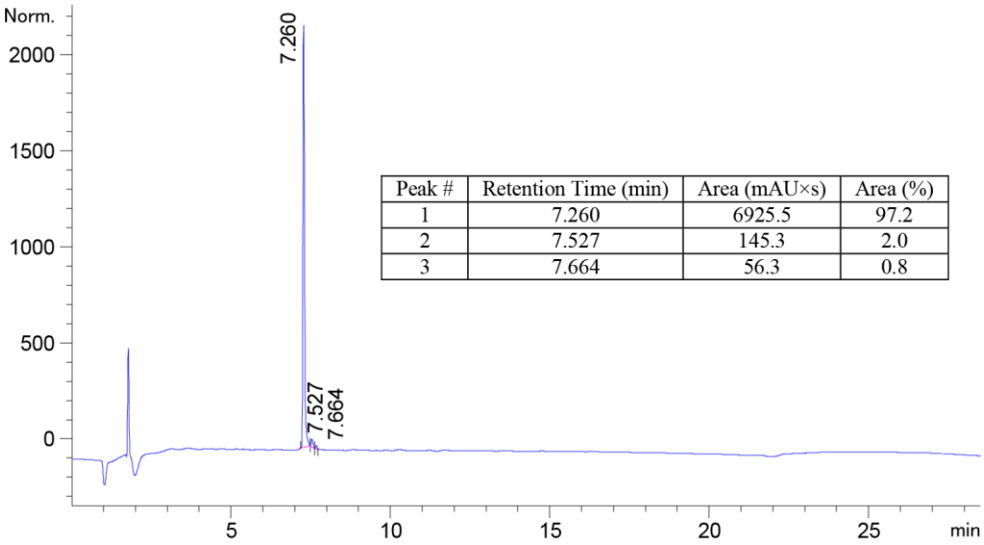
peptide **8**



peptide **9**

3.6 Appendix

3.6.1 Supplementary Figures



Peak #	Retention Time (min)	Area (mAU×s)	Area (%)
1	7.260	6925.5	97.2
2	7.527	145.3	2.0
3	7.664	56.3	0.8

Figure 3.10. Analytical HPLC trace for peptide **1**.

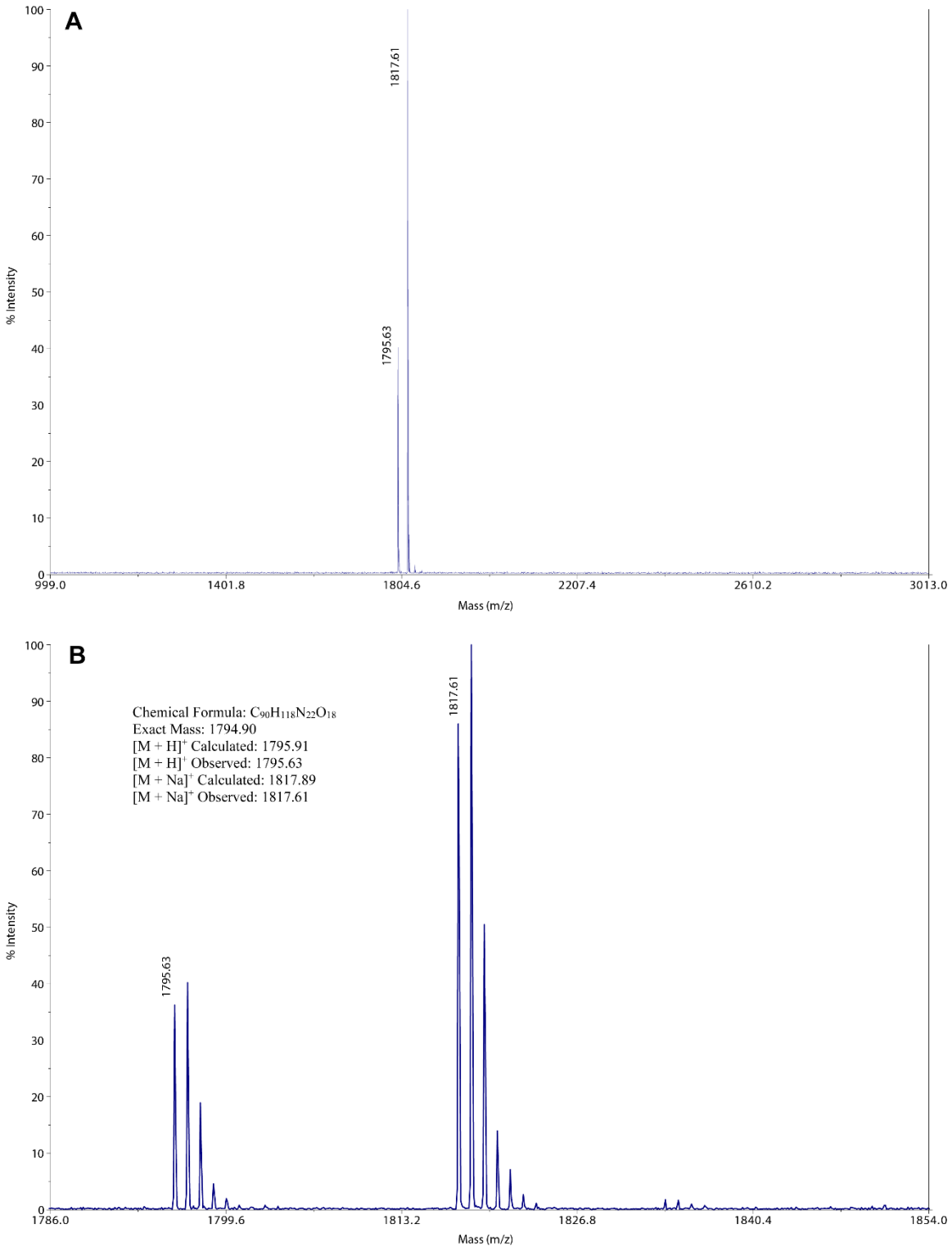
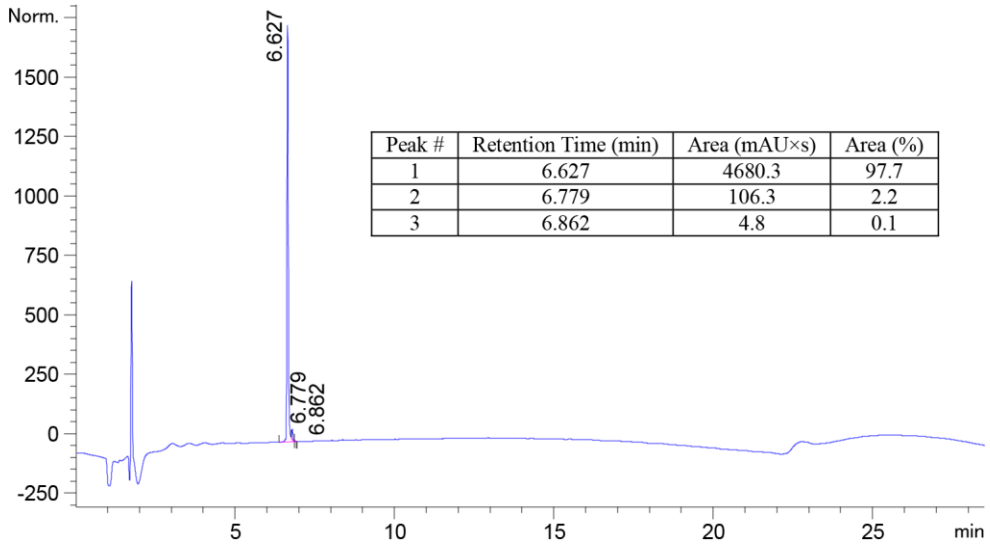


Figure 3.11. MALDI-TOF mass spectrum of peptide **1**. A) Full spectrum. B) Zoomed in spectrum.



Peak #	Retention Time (min)	Area (mAU×s)	Area (%)
1	6.627	4680.3	97.7
2	6.779	106.3	2.2
3	6.862	4.8	0.1

Figure 3.12. Analytical HPLC trace for peptide **2**.

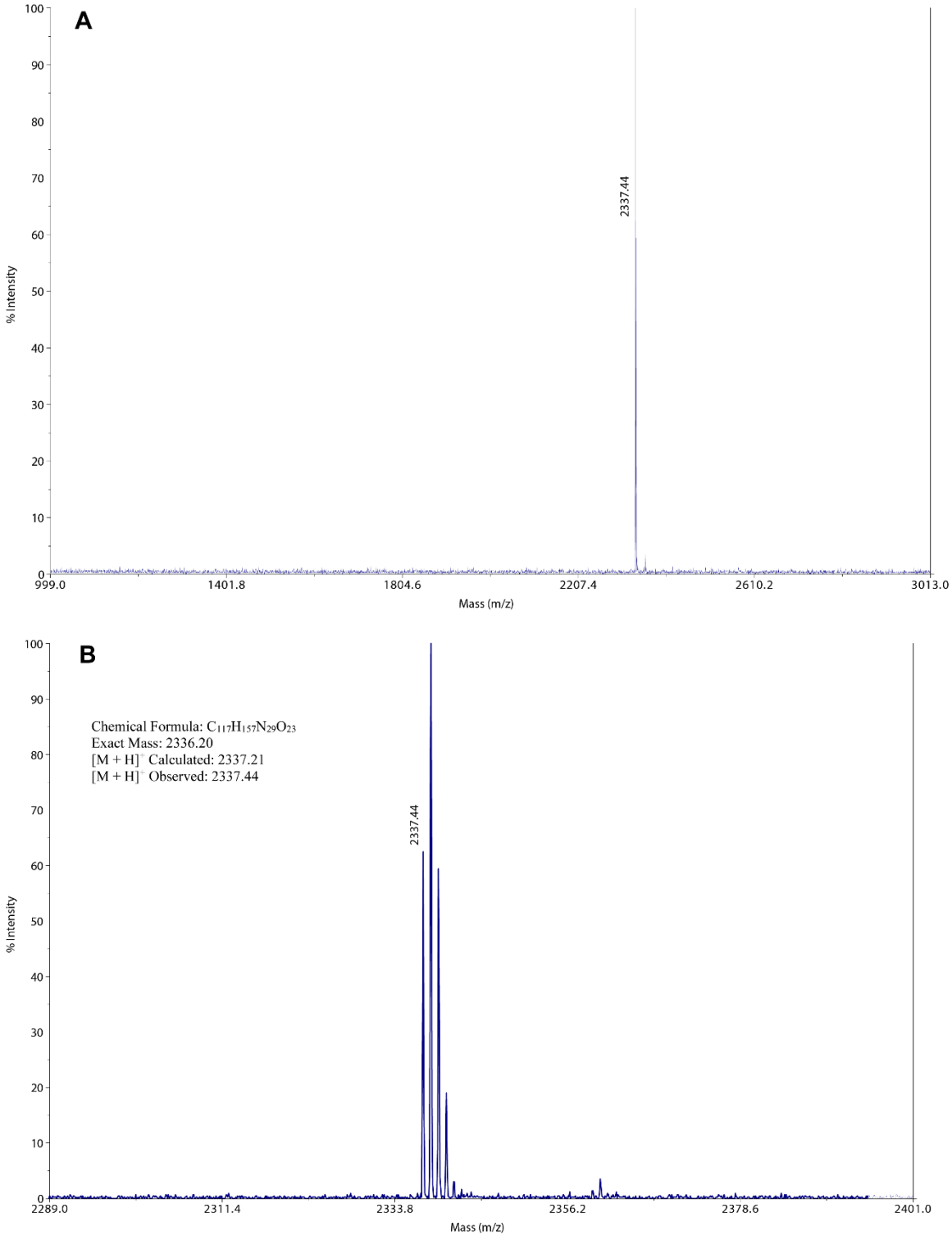
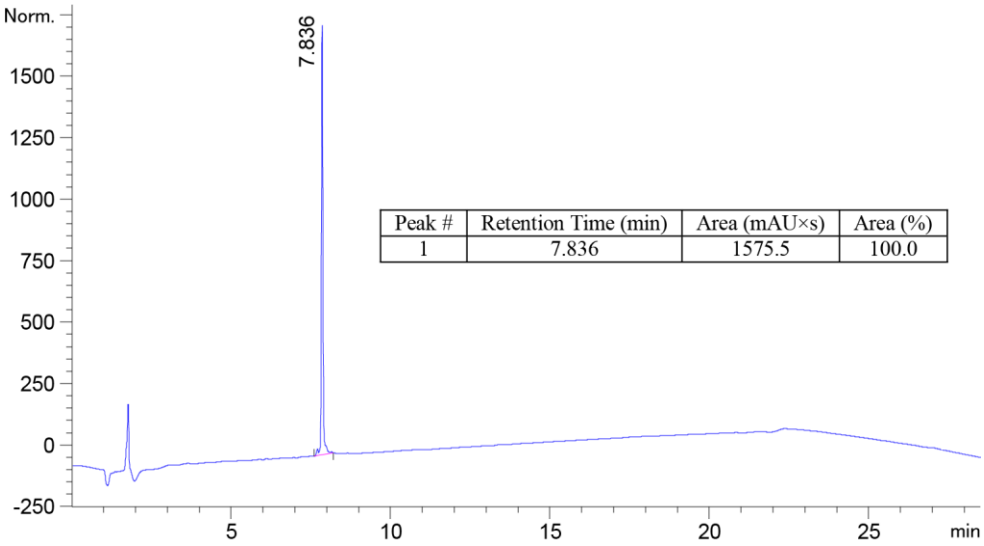


Figure 3.13. MALDI-TOF mass spectra of peptide **2**. A) Full spectrum. B) Zoomed in spectrum.



Peak #	Retention Time (min)	Area (mAU×s)	Area (%)
1	7.836	1575.5	100.0

Figure 3.14. Analytical HPLC trace for peptide **3**.

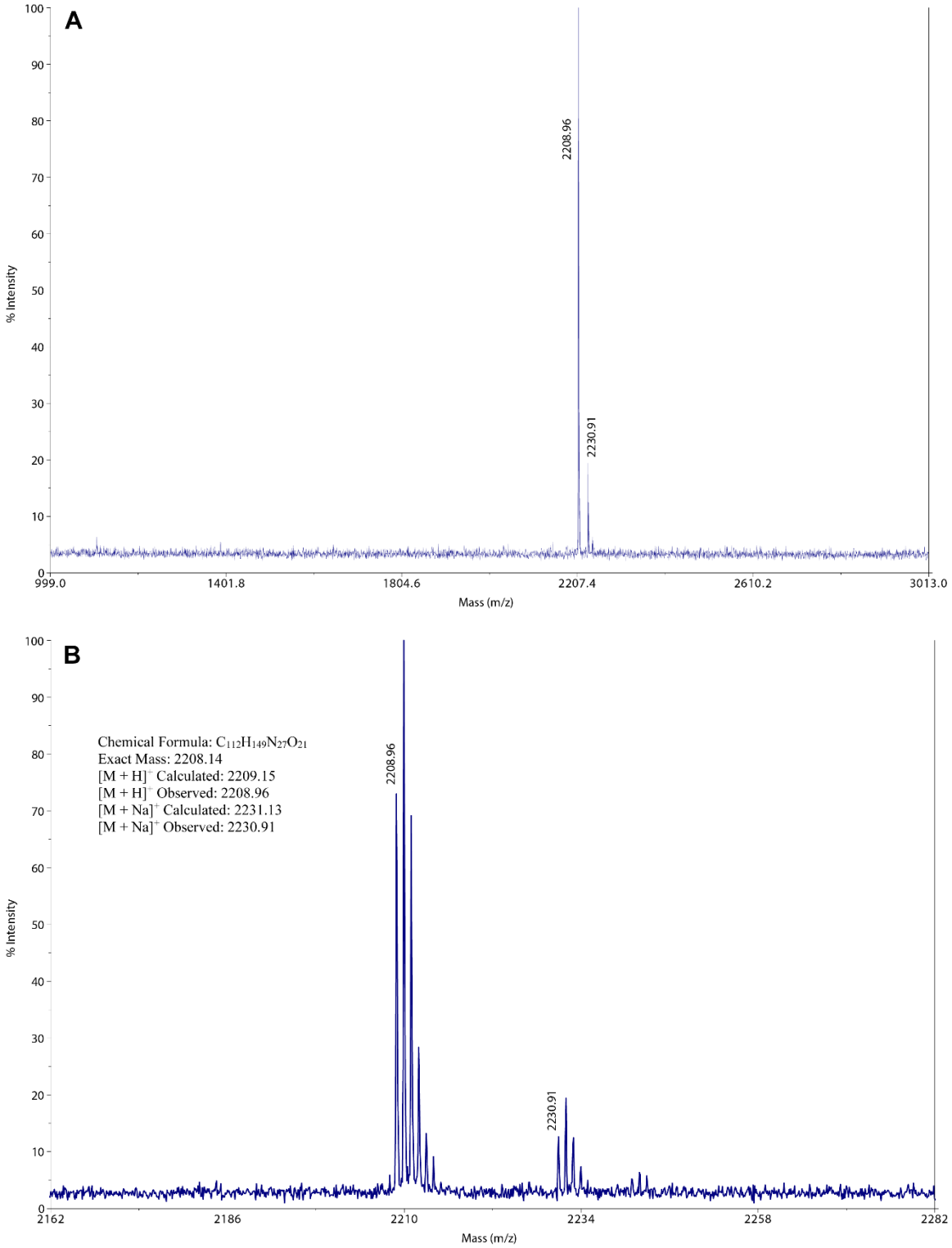
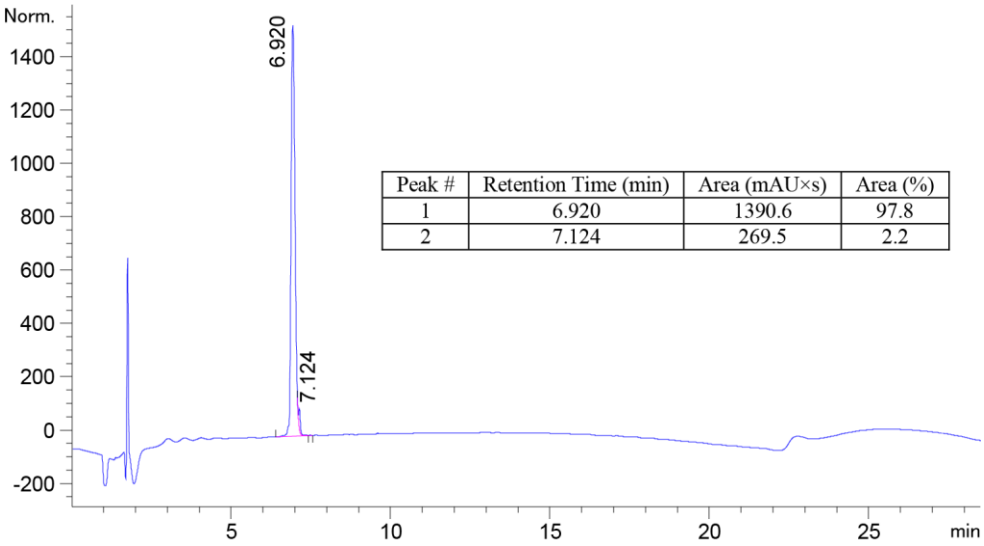


Figure 3.15. MALDI-TOF mass spectra of peptide **3**. A) Full spetrum. B) Zoomed in spectrum.



Peak #	Retention Time (min)	Area (mAU×s)	Area (%)
1	6.920	1390.6	97.8
2	7.124	269.5	2.2

Figure 3.16. Analytical HPLC trace for peptide **5**.

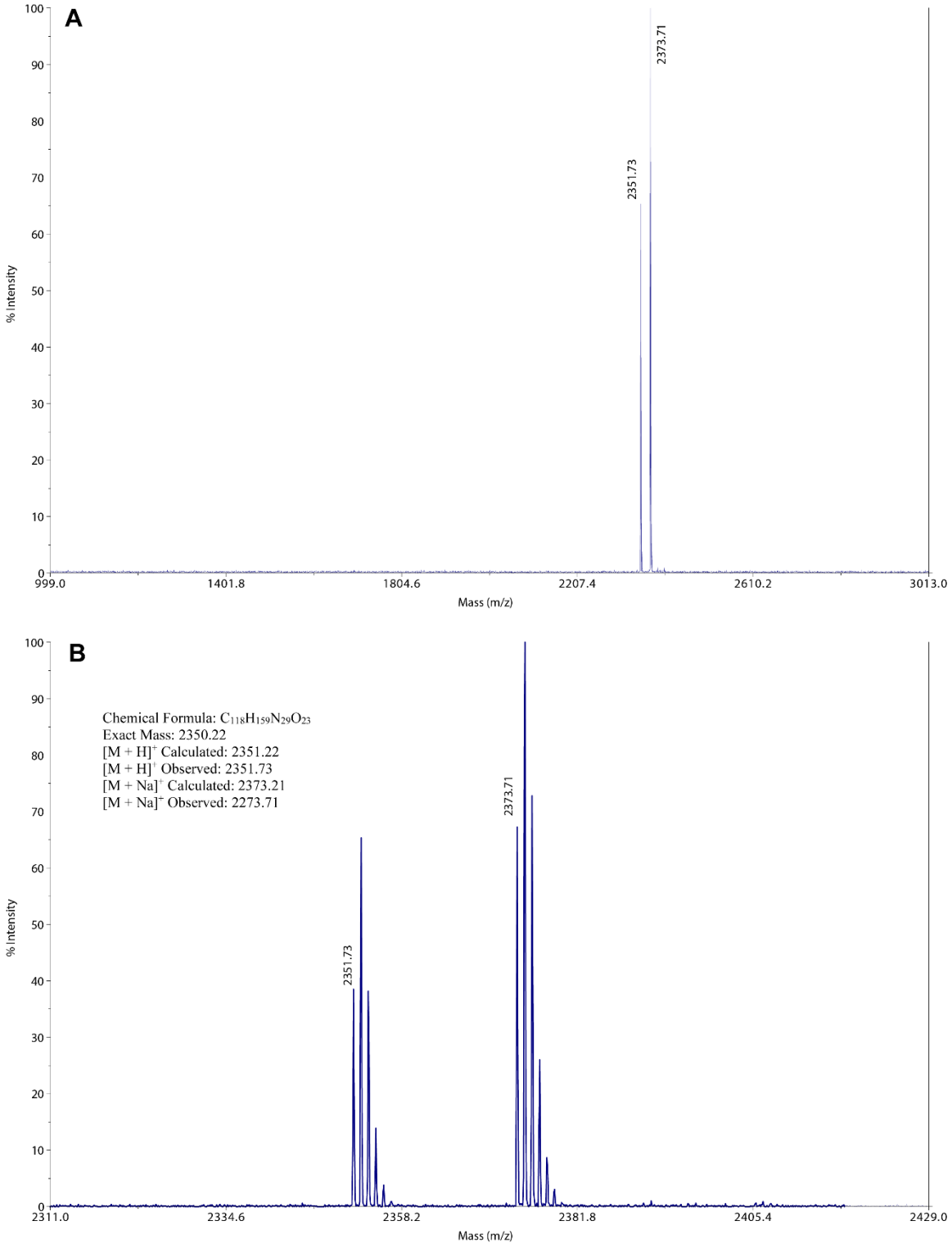
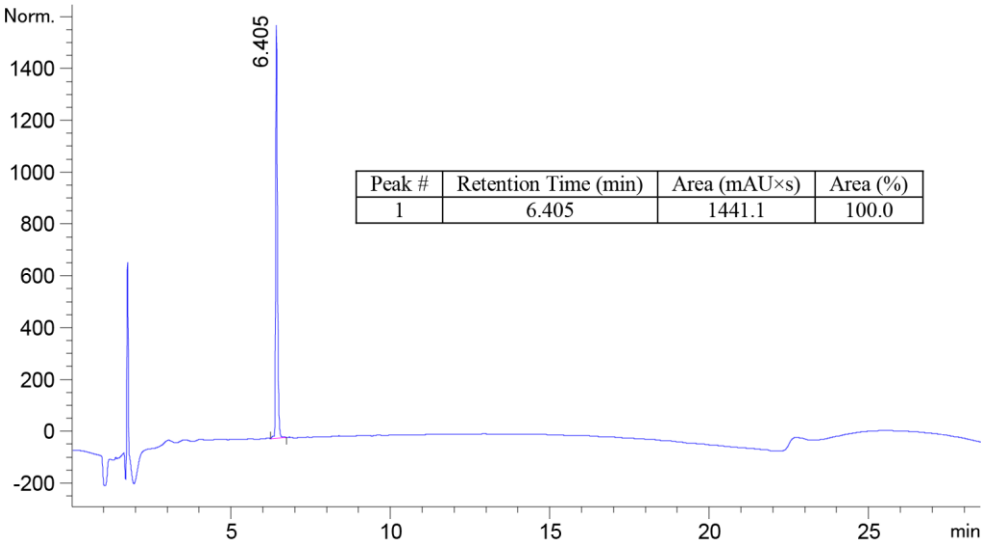


Figure 3.17. MALDI-TOF mass spectrum of peptide **5**. A) Full spectrum. B) Zoomed in spectrum.



Peak #	Retention Time (min)	Area (mAU×s)	Area (%)
1	6.405	1441.1	100.0

Figure 3.18. Analytical HPLC trace for peptide **6**.

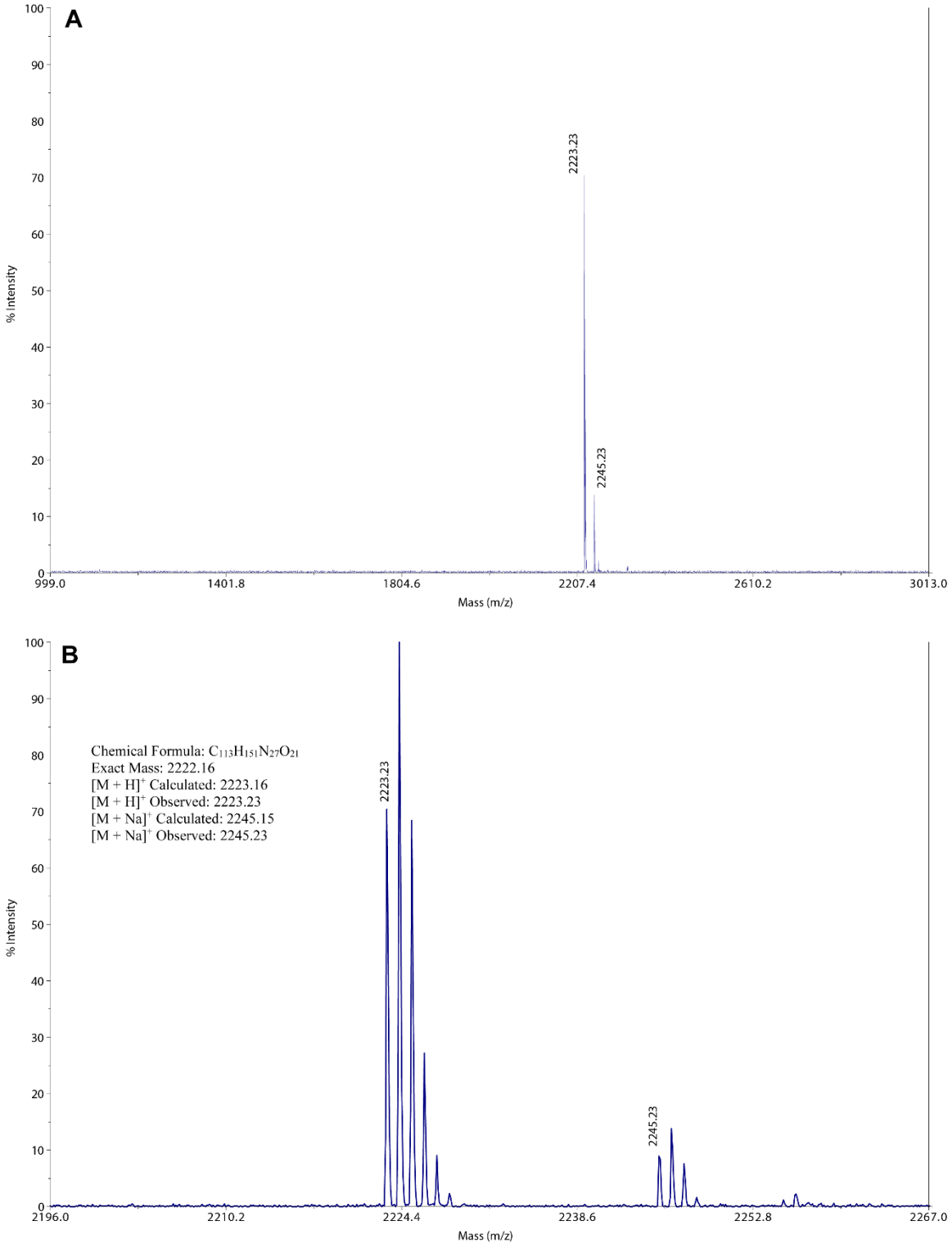


Figure 3.19. MALDI-TOF mass spectrum of peptide **6**. A) Full spectrum. B) Zoomed in spectrum.

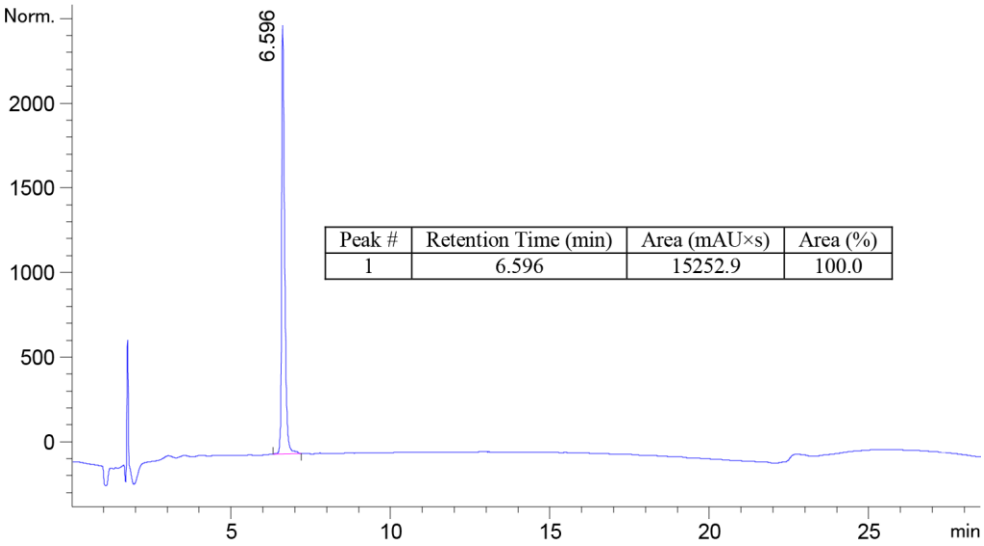


Figure 3.20. Analytical HPLC trace for peptide **7**.

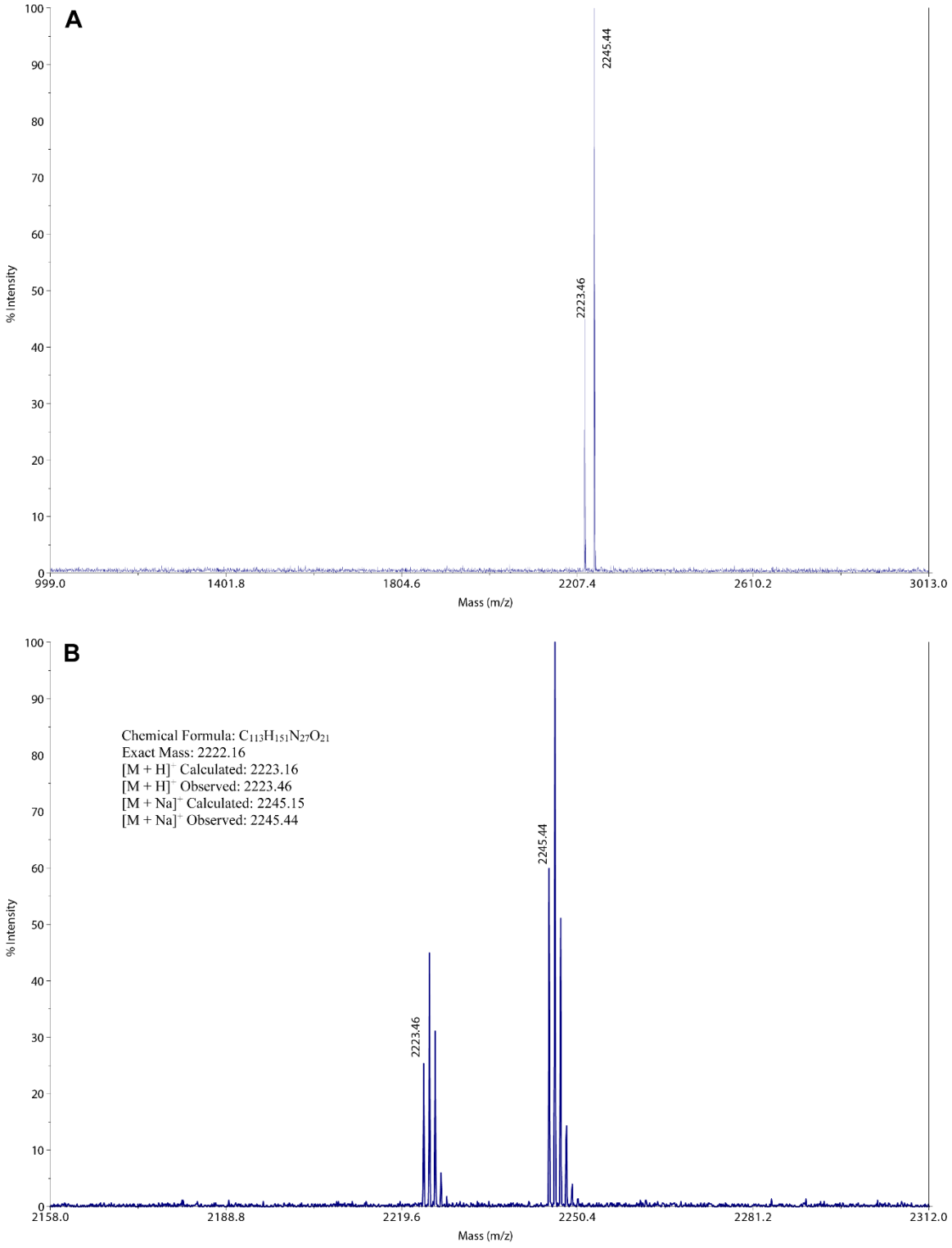
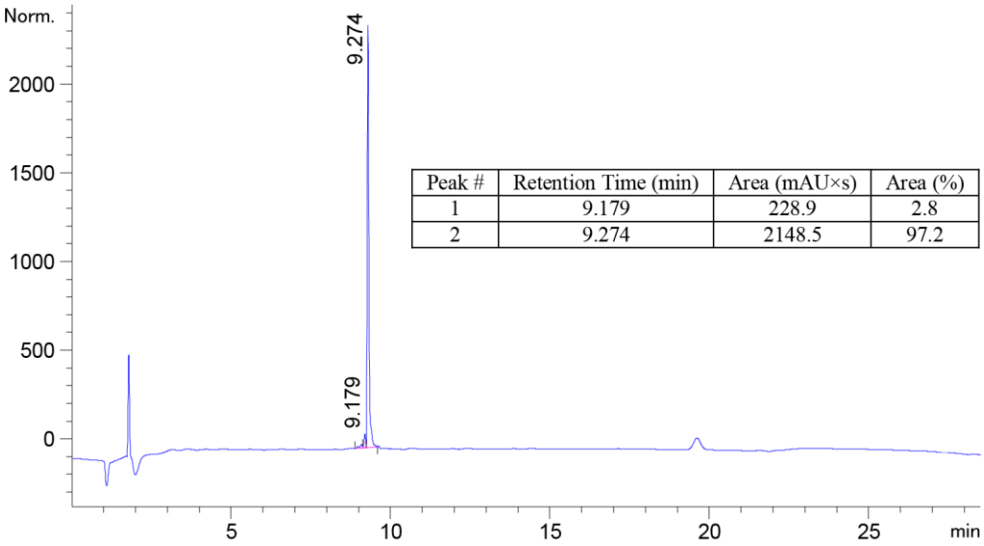


Figure 3.21. MALDI-TOF mass spectrum of peptide **7**. A) Full spectrum. B) Zoomed in spectrum.



Peak #	Retention Time (min)	Area (mAU×s)	Area (%)
1	9.179	228.9	2.8
2	9.274	2148.5	97.2

Figure 3.22. Analytical HPLC trace for peptide **8**.

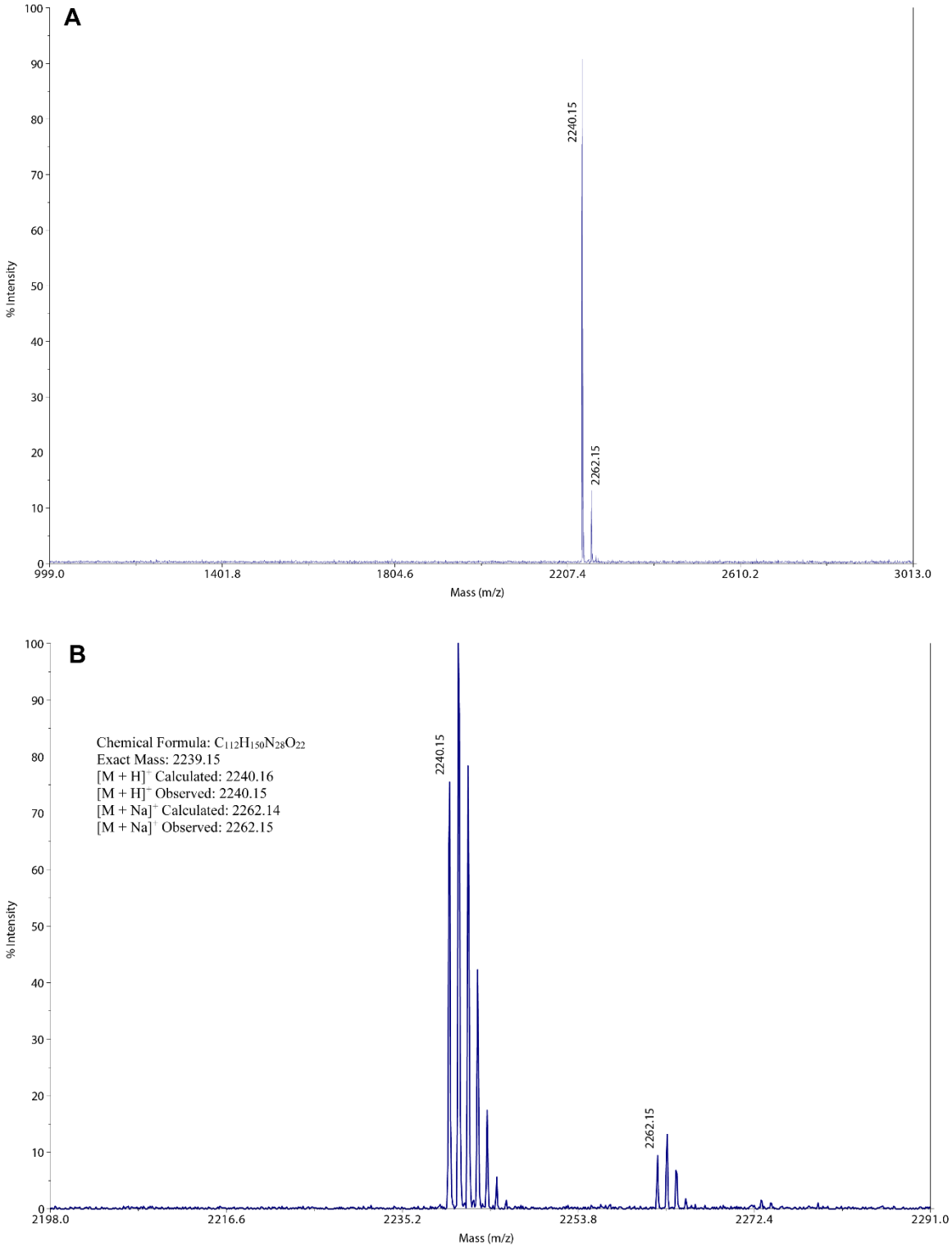
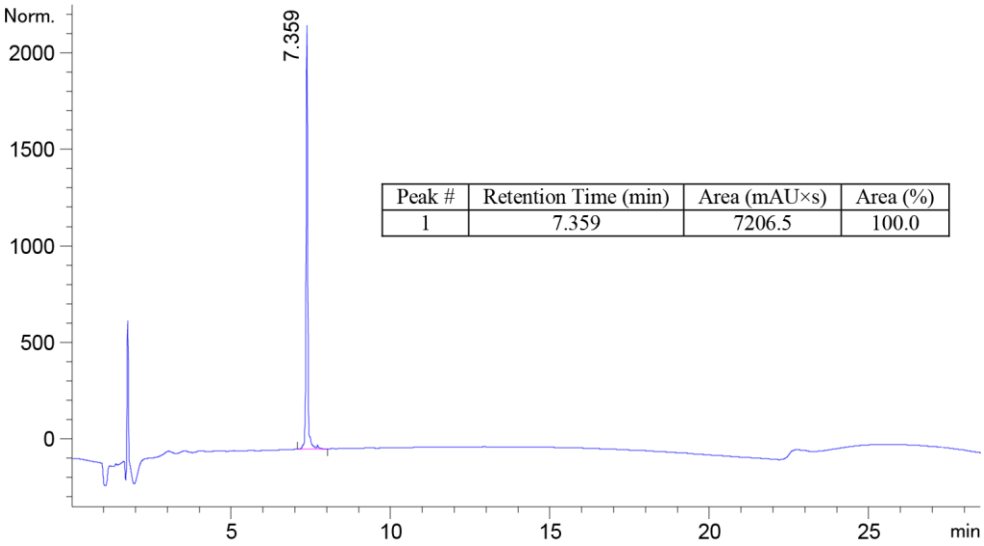


Figure 3.23. MALDI-TOF mass spectrum of peptide **8**. A) Full spectrum. B) Zoomed in spectrum.



Peak #	Retention Time (min)	Area (mAU×s)	Area (%)
1	7.359	7206.5	100.0

Figure 3.24. Analytical HPLC trace for peptide **9**.

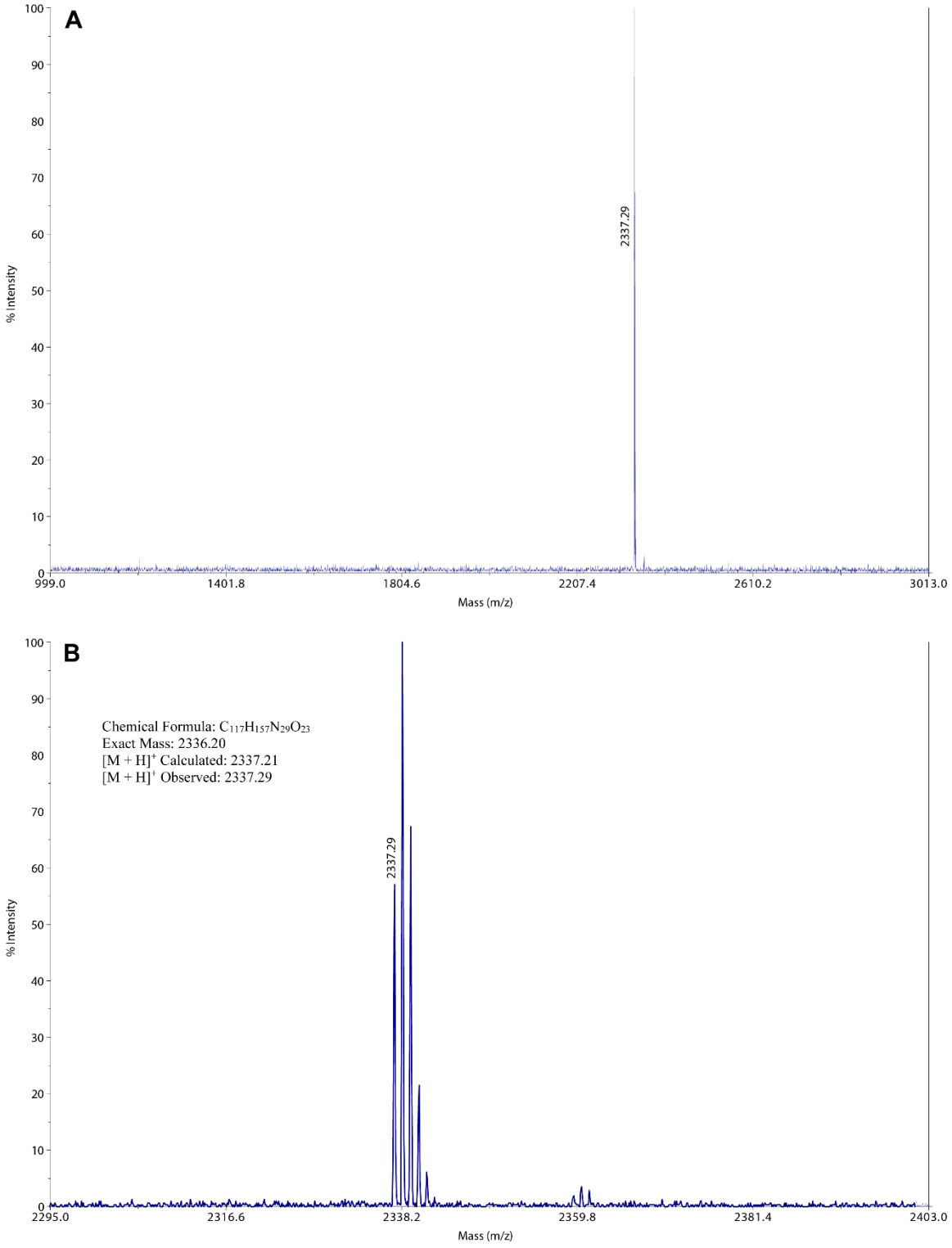


Figure 3.25. MALDI-TOF mass spectrum of peptide **9**. A) Full spectrum. B) Zoomed in spectrum.

3.6.2 General Information[24]

All Fmoc-protected amino acids including the unnatural amino acid, Boc-ornithine(Fmoc)-OH were purchased from Chem-Impex or Anaspec. 2-Chlorotrityl chloride and Rink amide resins were purchased from Chem-Impex. Trifluoroacetic acid (TFA), and HPLC grade acetonitrile (MeCN) were purchased from Fischer Scientific. Water was purified to 18 MΩ with a ThermoFischer GenPure Pro water purification system. All other solvents and chemicals were purchased from Alfa Aesar and Sigma Aldrich. All amino acids, resins, solvents, and chemicals were used as received, with the exception that dichloromethane (DCM) and *N*,*N*-dimethylformamide (DMF) were dried by passage through dry alumina under argon. Analytical HPLC chromatograms were obtained using an Agilent 1260 Infinity II HPLC equipped with Phenomenex bioZen C18 column (150 mm × 4.6 mm, 2.6 μm particle size). A 1 mL/min flow rate was used with peak detection at 214 nm, all monitored using the provided HPLC OpenLAB software. Preparative-scale purification of peptides was done using an Agilent Zorbax SB-C18 PrepHT column (21.2 mm x 250 mm, 7 μm particle size) on a Rainin Dynamax HPLC with a flow rate of 7.0 mL/min, monitored at 214 nm with the accompanying DA Rainin HPLC software. MALDI mass spectrometry was performed using an Applied Biosystems SCIEX TOF/TOF under reflector positive ion mode using either 2,5-dihydroxybenzoic acid matrix or α-cyano-4-hydroxycinnamic acid matrix. Spectra were analyzed using the accompanying TOF/TOF Series Explorer software.

3.6.3 Abbreviations

DCM	dichloromethane
DIPEA	diisopropylethylamine
DMF	*N*,*N*-dimethylformamide

HATU	*N*,*N*,*N'*,*N'*-tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate
HBTU	*N*,*N*,*N'*,*N'*-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate
HCTU	*N*,*N*,*N'*,*N'*-tetramethyl-O-(6-chlorobenzotriazol-1-yl)uroniumhexafluorophosphate
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
HPLC	high-performance liquid chromatography
HOBt	hydroxybenzotriazole
MALDI	matrix-assisted laser desorption ionization
MeOH	methanol
MeCN	acetonitrile
TFA	trifluoroacetic acid
TIPS	triisopropylsilane

3.6.4 Synthesis of Macrocyclic Peptides[25]

The synthesis of all peptides involved the following sequence of operations: (1) resin loading, (2) solid-phase amino acid couplings, (3) cleavage of the linear peptide from the resin, (4) solution-phase cyclization of the linear peptide, (5) global deprotection of acid-labile protecting groups, and (6) purification with preparative reverse-phase HPLC. The purified peptides were characterized by analytical HPLC and MALDI mass spectrometry.

3.6.5. Resin Loading

2-Chlorotrityl chloride resin (0.300 g, 1.6 mmol/g, 100-200 mesh) was added to a 10-mL Bio-Rad Poly-Prep chromatography column (8 mm x 40 mm). The resin was suspended in dry DCM (8 mL) and allowed to swell undisturbed for 30 min. The solution was drained from the resin using nitrogen and a solution of Boc-ornithine(Fmoc)-OH (150.0 mg, 0.33 mmol) in 20%

(v/v) 2,4,6-collidine in dry DCM (8 mL) was added immediately. The suspension was gently agitated for 24 h. The solution was then drained using nitrogen and washed with dry DCM (3x). After washing, a mixture of DCM/MeOH/DIPEA (8.5:1:0.5, 8 mL) was added immediately. The suspension was gently agitated for 30 min to cap any unreacted sites on the resin. The resin was washed with DMF (3x) and dried by passing nitrogen through the chromatography column. Each batch of resin loading was determined to be between 0.60 and 0.65 mmol/g based on UV analysis (290 nm) of the Fmoc cleavage product.

3.6.6 Solid-Phase Peptide Synthesis

The Boc-ornithine(Fmoc) loaded resin was added to a Chemglass solid phase peptide synthesis vessel and subjected to cycles of amino acid couplings using Fmoc-protected amino acid building blocks. The linear peptides were synthesized from the C-terminus to the N-terminus. Each coupling consisted of: (1) Fmoc deprotection with 20% (v/v) piperidine in DMF for 5 min (2x); (2) resin washing with DMF (3x); (3) activation of the Fmoc protected amino acid (4 equiv) with 20% (v/v) 2,4,6-collidine in DMF (8 mL) in the presence of HCTU (4 equiv); (4) coupling of the activated Fmoc-protected amino acid; (5) resin washing with DMF (3x). All amino acid couplings took 20 min except for the tyrosine that followed the *N*-methyl-tyrosine in peptides **5** and **7** and for the valine that followed the *N*-methyl-lysine in peptides **4** and **6**. The tyrosine that followed the *N*-methyl-tyrosine (4 equiv) and the valine that followed the *N*-methyl-lysine (4 equiv) were coupled twice for 1 h each with HATU (4 equiv) in 20% (v/v) 2,4,6-collidine in DMF (8 mL), with no Fmoc deprotection in between the two coupling reactions. This modification ensured complete amino acid coupling onto a more sterically hindered, secondary amine. After the last amino acid was coupled, and its Fmoc protecting group deprotected, the resin was transferred from the coupling vessel to a new Bio-Rad Poly-Prep

chromatography column. The resin was washed with DCM (3x), and dried by passing nitrogen through the column.

3.6.7 Protected Cleavage of the Linear Peptide

The protected linear peptide was cleaved from the resin by subjecting the resin to a cleavage solution of 20% (v/v) HFIP in DCM (8 mL). The resin and the suspension immediately turned red. The suspension was gently agitated for 1 h. The suspension was filtered, and the filtrate was collected in a 250-mL round-bottom flask. The resin was washed with additional cleavage solution (8 mL) and agitated for another 30 min. The suspension was filtered and the filtrate collected into the same round-bottom flask as the previous filtrate. The suspension was then washed with DCM (3 x 2 mL) until the resin was no longer red with all washes collected into the round-bottom flask. The combined filtrates were concentrated under reduced pressure and then the protected linear peptide was cyclized without prior purification.

3.6.8 Cyclization

The protected linear peptide was dissolved in dry DMF (125 mL) in the same 250-mL round-bottom flask as the previous step. HOBt (0.150 g, 1.11 mmol) and HBTU (0.300 g, 0.79 mmol) were dissolved in 8 mL of dry DMF in a test tube to which 300 μL of 2,4,6-collidine was added and the solution mixed until homogenous. The solution was then added to the flask and the mixture was stirred under nitrogen at room temperature for 96 h. The reaction mixture was concentrated under reduced pressure and then the crude product was immediately subjected to global deprotection.

3.6.9 Global Deprotection of Acid-Labile Protecting Groups and Ether Precipitation

The protected cyclic peptide was dissolved in a mixture of TFA/TIPS/H_2O (9.0:0.5:0.5, 10 mL) in the same 250-mL round-bottom flask as the previous step. The reaction mixture was then stirred under nitrogen at room temperature for 1 h. During the 1 h deprotection, two 50-mL conical tubes containing 40 mL of dry ether each were chilled on dry ice. Following the 1 h time, the peptide solution was split between the two conical tubes of ether. The tubes were then centrifuged at 500 *x g* for 15 min. The ether supernatant was poured off and the pelleted peptides dried under nitrogen for 15-20 min.

3.6.10 Purification of Macrocyclic Peptides

The crude peptide pellets were dissolved in 10% MeCN/H_2O (4 mL), and the solution was initially purified using a Biotage® Isolera One. Fractions containing the desired peptide were identified by mass spectrometry, combined, and reduced under pressure to a volume less than 5 mL. That solution was then purified by reverse-phase HPLC following a gradient whereby the percentage of MeCN in the mobile phase was increased at a rate of 0.2%/min. Elution of all occurred within the range of 15-25% MeCN/H_2O. The pure fractions were lyophilized to afford 14.71 mg of peptide **1** (3.45% based on resin loading), 10.81 mg of peptide **2** (1.99% based on resin loading), 8.00 mg of peptide **3** (1.54% based on resin loading), 4.38 mg of peptide **5** (0.80% based on resin loading), 1.87 mg of peptide **6** (0.36% based on resin loading), 2.54 mg of peptide **7** (0.49% based on resin loading), 2.08 mg of peptide **8** (0.41% based on resin loading), and 10.16 mg of peptide **9** (1.87% based on resin loading). All peptides were obtained as the trifluoroacetate salts and were white powders.

3.6.11 Homology Modeling in PyMOL

The crystal structure of the WW domain of hYAP65 was used as the basis for modeling the IQGAP1 WW domain. To model peptide 1, residues 178-190 of hYAP65 were mutated to residues 686-698 from IQGAP1. To model peptide 2, residues 176-192 of hYAP65 were mutated to residues 684-700 from IQGAP1. The residue mutations were achieved in PyMOL using the Mutagenesis tool under the Wizard menu. The rotamers of each amino acid side chain were selected to minimize steric interactions with neighboring amino acid side chains. To install the δ-linked ornithine turn unit, residues 177 and 191 (for peptide 1) or residues 175 and 193 (for peptide 2), were mutated to alanine and the alanine side chains linked together using the Create bond option in the Builder menu. The carboxy group of alanine 191 (peptide 1) or alanine 193 (peptide 2) were removed from the structure using the Delete bonds and atoms feature within the Builder menu. The α-amino group of the newly created ornithine was made into an ammonium group by changing the charge of the nitrogen atom to +1 using the charge option in the Builder menu. To assess whether the mutations and installation of the δ-ornithine turn unit had any deleterious impact on the β-hairpin structure, the overall structures were energy-minimized using the Clean function within the Builder menu.

3.6.12 Fusion Protein Expression and Purification

Expression of the WW-GST fusion protein was performed by Dr. Jane Bardwell.[18] Purification of the fusion protein was conducted following a previously published protocol.[21]

3.6.13 *In Vitro* Translation

In vitro translation and quantification of p110α was performed by Dr. Jane Bardwell.[18] The procedure is based on a previously published protocol.[26]

3.6.14 Co-Sedimentation Assay

The co-sedimentation assay was performed by Dr. Jane Bardwell.[18] The procedure is based on a previously published protocol.[21]

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24. The chemicals and instruments required for the synthesis of the peptides in this study are similar to those used in our laboratory's previous publications. This information was either adapted from or taken verbatim from Chen, K. H.; Corro, K. A.; Le, S. P.; Nowick, J. S. X-ray Crystallographic Structure of a Giant Double-Walled Peptide Nanotube

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25. The peptides used in these studies were synthesized following a protocol similar to those published previously by our laboratory. The procedures were either adapted from or taken verbatim from: Spencer, R.; Chen, K. H.; Manuel, G.; Nowick, J. S. Recipe for β-Sheets: Foldamers Containing Amyloidogenic Peptide Sequences. *Eur. J. Org. Chem.* **2013**, *17*, 3523–3528.

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Chapter 4: Converting an Organic Chemistry Course to an Online Format in Two Weeks: Design, Implementation, and Reflection

4.1 Preface

Toward the end of winter quarter 2020, a meeting was held between the teaching faculty, stockroom managers, and the chair of the department concerning how best to approach teaching courses remotely for spring quarter. I was invited to this meeting because I was a senior graduate student who had received a large amount of pedagogy training and had several instructor-of-record experiences. During that meeting, it was determined that I would take on a larger TA role within the department. In addition to completing my work as a Head TA for the general chemistry laboratory, I served as a development TA for Professor Susan King's and Professor Chris Vanderwal's organic chemistry lecture courses. This chapter describes the process of converting Professor King's course to an online format and discusses the student perceptions of these changes. I took Professor King's previously recorded lecture videos, parsed them into digestible segments, assisted with captioning them, and crafted two-question quizzes to accompany each video. Professor King taught the synchronous components of the organic chemistry course and developed the discussion section content that was taught by four TAs. Gretchen Guaglianone, one of the TAs, provided crucial insights into how the discussion sections were taught, student perceptions of the discussion sections, and the online office hour experience. All three of us collaborated on the design and administration of exams and student surveys.

4.2 Introduction

Spring 2020 brought unprecedented challenges to universities worldwide as shelter-in-place orders were given to prevent transmission of the SARS-CoV-2 virus. At the University of California, Irvine, we were in the last week of the winter quarter when it was determined that final exams and all spring quarter classes would be moved online. Panic ensued, as students were forced to move out of on-campus housing at the same time as they were studying for final exams. Professors implemented various approaches to final exams, including canceling them, making them open-book and un-proctored, or making them optional. But final exams were a small challenge compared to preparing for an upcoming quarter where every class would need to be online in less than two weeks. In order to convert our face-to-face (F2F) spring quarter organic chemistry class to a remotely delivered version, we relied on a combination of existing online infrastructure and insights gained from previous teaching experience.

4.3 Traditional Face-to-Face Course

UCI operates on the quarter system, with the academic year composed of three 10-week quarters. The third quarter of the organic chemistry lecture series occurs during the spring quarter, and it covers aromatic, carbonyl, and enolate chemistries as well as the chemistry of amines. Enrollment is typically between 300 and 350 students. In the F2F course, students attend three 50-minute lectures and one 50-minute discussion section each week. During lectures, the instructor uses a tablet-stylus combination to present lecture content, using accompanying notes to serve as a template and outline (see appendix for sample outline).[1-3] Students can purchase the bound lecture notes from the bookstore or access web-posted PDFs. The lecture notes contain descriptive information and selected pre-drawn structures, leaving space for students to draw and write as they

watch the lecture.[4,5] Lectures are held in an active learning lecture hall with a 400-student capacity containing parallel desks and rotating seats to allow students to work together during in-class activities. The lecture content is supplemented with eight short lecture videos curated from the OpenChem branch of UCI's Open Education Consortium (OEC) site to allow time for active-learning problem-solving activities.[6,7] The instructor's lecture videos from a F2F organic chemistry class were recorded five years ago for OpenChem, which contains open access video lectures for most of the chemistry classes needed to complete an undergraduate chemistry degree. In discussion sections, led by the instructor and TAs, students gather in groups of approximately 15-90 students to solve weekly problem sets with the help of learning assistants (LAs). Graded online homework, administered through Sapling Learning, consists of seven assignments, one for each chapter, which are due at the end of each chapter.[4,8,9] Two midterms, a final exam, Sapling online homework, and class participation in active learning assignments during lecture are used to assess each student's final course grade.

4.4 Transition to an Online Course

There is a great deal of literature on the relative merits of online (OL) versus F2F courses,[10-17] as well as analyses of the types of students who thrive in an OL learning environment.[18-23] There are also guidebooks for creating effective OL classes.[24] In the field of organic chemistry, there are examples of flipped and partially flipped classes,[25-30] online organic chemistry preparatory classes,[31-33] Massive Open Online Courses (MOOCs),[34] and a partially online chemistry course that was developed under similar time constraints.[35] With less than two weeks before the start of spring quarter, however, there was little time to consult these resources, much less develop a model online course experience. Although it was not possible to follow literature precedent in the design of this course, the instructor and TAs were able to draw upon previous experience, as the instructor

had previously developed an online organic chemistry preparatory course, and the head TA was an experienced Pedagogical Fellow (a senior graduate student with advanced pedagogical training).

The course was designed with a high structure format consisting of low-stakes assessments and regular due dates to hold students accountable for asynchronous work.[36-44] A combination of asynchronous and synchronous meetings were implemented to help guide students through the novel online course experience, and to maximize the sense of community, providing students a chance to interface with the instructor, the TA's and their peers.[45,46]

4.4.1 Lecture

In the transition to OL teaching, the F2F course structure was maintained as much as possible. Students can purchase the same bound lecture notes as the F2F class or access web-posted pdfs. The entire set of the instructor's OpenChem lecture videos for the third quarter of organic chemistry were uploaded into the video content management platform YuJa, and, to make the lecture videos more digestible for students, the original videos were segmented by topic so that each video was no more than around 20 minutes.[46-51] For this OL course, 27 original lecture videos were segmented into a total of 86 topic videos. Each video was followed by a two-question quiz which students had to complete before moving on to the next video.[41] The video quizzes were newly created for the OL course to assess student comprehension of the lecture topic content. Links to the videos were incorporated into weekly modules in the Canvas Learning Management System (LMS)[52]. Every video was titled with the topic name, the length of the video, and the chapter and section(s) of the textbook to which the video content referred. All videos were captioned for student accessibility.[53] Video quizzes were due at the end of every week to incentivize students to keep up with the lecture material.[54,55]

With all of the lecture content provided asynchronously, the regularly scheduled lecture times remained available for other activities. The Monday lecture time was used for a Monday check-in Zoom meeting to provide students with a weekly opportunity to interface with the instructor and their peers in an informal manner about their new online struggles and triumphs.[56,57] On Fridays, the instructor went over solutions to weekly homework problems (discussed in more detail below).

4.4.2 Discussion

Discussion sections were held over Zoom and formatted in the same way as the F2F discussions, with similar enrollment in sections scheduled throughout the week.[58] A weekly problem set (same as F2F problem set – see appendix for an example) was distributed prior to the discussion section, giving students ample time to review and attempt the problems individually before discussion with their classmates. To encourage student participation, TAs posed questions to students using Zoom's poll feature and students were randomly assigned into small groups of 4-5 in breakout rooms to solve problems. In the breakout rooms, students were given approximately 20 minutes to discuss the answers to the worksheet problems and were encouraged to use Zoom's screen share feature. TAs and one or more LAs monitored the breakout rooms. They refrained from screen sharing, but instead encouraged students to take turns sharing their own work. Students could request the assistance of their TA if they had an unresolved question using Zoom's hand-raise feature. After breakout rooms closed, the TA — with constructive input from students — went over the solutions. The LAs monitored the chat window, bringing student questions to the attention of the TA. For more information about discussion sections, see the appendix.

4.4.3. Office Hours and Discussion Board

Each TA held one office hour per week, and the instructor held three. Office hours were conducted over Zoom, and the instructor used an iPad and the annotation software Notability to draw structures during office hours.[59] Students were able to share their screens if they had questions that necessitated drawing structures. Links to all office hours were provided in the course LMS for ease of student access. A Piazza discussion board provided an additional forum for students to ask questions about course content anonymously.[60,61]

4.4.4. Homework

In contrast to the F2F course, in which online homework assignments were due at the end of each chapter, the Sapling homework assignments for the OL course were due at the end of each week.[54,55] In addition to Sapling, students had a graded "Synthesis and mechanism of the week" (SMW) assignment that was due each Thursday. The ability to draw mechanisms and structures is essential to learning organic chemistry, but these are challenging skills to assess in an online exam, where students are limited to the content that can be entered in a rich text box. Therefore, it was necessary to give them practice in drawing mechanisms outside of exams. In the SMW assignments, which were similar in content to the active learning assignments in the F2F class, students were given a four-hour window to independently draw a mechanism and a multistep synthesis and then upload it into Gradescope, an assignment submission and grading platform, which was integrated into the LMS.[62] The time window was chosen to be maximally accessible to students in different time zones. Solutions to these problems were discussed during the synchronous Friday Zoom session. The session was recorded and captioned so students who were unable to attend could have complete access to the session. A sample SMW assignment is included in the appendix.

Academic dishonesty became a significant hurdle in the assessment of the weekly SMW assignment. Some students used sites like Chegg and Course Hero to post the weekly SMW assignment questions.[63,64] Because of the four-hour window for the assignments, there was plenty of time for students to wait for an answer to be posted to these websites, and more than one student copied the posted answers. Scheduling multiple Zoom meetings with these students to discuss academic dishonesty charges was time consuming and disheartening. The surest way to circumvent this problem would be to narrow the window in which students could complete the assignment, but this would disadvantage students who were in vastly different time zones. In order to more equitably address the academic dishonesty issues, the graded SMW assignment was converted mid-quarter into an optional assignment that was not worth any points. Students were asked to voluntarily post their best attempt at each problem so that the instructor would be able to find common mistakes and better address them during the synchronous and recorded Friday Zoom meeting. Over half the class chose to voluntarily post their answers, providing ample material for constructive feedback.

4.4.5 Exams

As with the F2F course, two midterms and a final exam were administered. Each online exam was built in the LMS and required the use of the Respondus LockDown Browser (details below) to minimize cases of academic dishonesty.[65] Multiple dropdowns, multiple-choice, and fill-in-the-blank style questions were used to assess comprehension of chemical trends, understanding of product prediction, and identification of correctly drawn mechanisms. These questions were auto-graded by the course LMS. Multistep synthesis questions were answered using the essay format and were graded manually.

In constructing the OL exams, it became apparent that some questions from F2F exams could easily be adapted into an OL format, while others did not translate well and had to be compromised to fit within the constraints of the LMS software. For example, chemical trends questions were similar in both OL and F2F exams. Predicting products questions, however, were much easier for students in an OL exam because they were multiple choice. Mechanism questions were also easier for students in OL exams; in F2F exams, students were required to draw multistep mechanisms, whereas in OL exams, students were asked to choose the correct order of pre-drawn steps. Multistep synthesis questions were equally challenging in both F2F and OL exams, but care was necessary to make sure that every reagent could be typed in condensed form or easily named using common or International Union of Pure and Applied Chemistry (IUPAC) nomenclature. Grading these problems was complicated by students' uneven ability to draw understandable condensed structures. After the first midterm, a handout providing guidelines for naming and drawing condensed structures was provided to students, and it measurably improved performance on the subsequent exams. This handout and sample exam questions in both the F2F and online exams are included in the appendix.

Another challenge of adapting F2F exams to an online format was determining how to best mitigate instances of academic dishonesty. One resource made available to instructors was the Respondus LockDown Browser which was adopted by the University of California, Irvine. The Respondus LockDown Browser is a fully automated system that uses a student's webcam to record and analyze a student's exam session. The Browser prevents students from accessing websites to look up test answers. Analytics are used to detect suspicious behaviors and video recordings are flagged when suspicious behavior is detected. In the startup sequence before each exam, students show their ID, say their name, move their camera to show their exam environment, show both

sides of an allowed scratch sheet of paper, and show their phones, which they are asked to place three feet behind them. The Respondus LockDown Browser minimizes cheating, but does not prevent it entirely. For example, students have the ability to hide cheat sheets or access a second phone or another laptop that is off-camera.

The additional technology requirements of taking an online exam are also significant and required forethought. Students need a microphone, a webcam, a stable wifi source, and a quiet place to take their exam. They need to download the Respondus Browser software well in advance of the exam because it can take more than one hour to download. If the environment is noisy, or if someone else enters the room while a student is taking an exam, their video can get flagged. Respondus warns students when this is occurring and this creates a lot of additional anxiety for students, who are already nervous in a testing environment. The LMS keeps an exam log while every student takes their exam. This log keeps track of exam progress and will indicate if there have been any wifi connection lapses. Viewing these logs, it is clear that wifi lapses are extremely common, but most lapses are brief and do not interfere with a student's ability to complete an OL exam. Although most students did not have a problem, there were a few students on every exam that had serious wifi issues or power outages. Because of these factors, especially when students are using Respondus for the first time, it is critical to give a low stakes practice exam a few days in advance of the actual exam. A practice exam gives students a chance to get accustomed to the Respondus start-up procedure, to see if their wifi and camera are working properly, and to determine if they are able to view the embedded images in the exam.[66] For the relatively few students who are unable to view images embedded in the OL exam, an embedded PDF containing images for every question was provided at the end of the exam as an auto-open inline preview.

In spite of the technological issues and the reported additional stress of taking an online exam, OL exam averages were significantly higher than F2F exams, in part, no doubt, because some question types were easier to solve in a multiple-choice format. The role of academic dishonesty in contributing to higher scores is less certain.

4.4.6 Grading

Flexibility was built into the grading policy because of the uncertainty of the online course environment and the new testing modality.[67,68] Students were told at the start of the OL course that the instructor would meet with a statistician to calculate their grades five different ways: varying the weights of the midterms, the final, the video quizzes, the SMW assignments, and participation. Students would receive the highest grade of the five. This greatly eased student anxiety and provided unexpected benefits towards the end of the quarter, when the social upheaval in response to the incidents of police brutality and racial injustice added an enormous amount of stress to students who were already overwhelmed by shelter-in-place restrictions. University-wide, professors were encouraged to provide accommodations for the final, and students were encouraged to request them. The instructor of this online course told students that the final would not be optional, but a calculation that minimized the weight of the final would be included with the other five methods as an additional calculation for final grades. There was minimal pushback. In contrast, other professors who had optional finals or who changed their grading strategy at the end of the quarter reported severe pushback from their students, many of whom expressed fears regarding how this novel method of conducting exams would impact their grades. A number of students also found the decision of whether or not to take an optional final extremely stressful.

4.5 Outcomes and Lessons Learned

To determine how students perceived the new online course structure, mid-quarter and end-of-quarter surveys were administered using the Qualtrics survey tool.[69] Of the 300 students enrolled, 260 completed the mid-quarter survey (87%) and 257 completed the end-of-quarter survey (86%). A full list of questions from each survey is included in the appendix. Student perceptions were mostly positive, with praise given for the flexibility provided by the lecture format, the incorporation of video quizzes as checkpoints for the comprehension of lecture material, and the weekly homework deadlines. The most common concerns students had pertained to the reduced social interactions with their peers due to the OL format.

4.5.1 Student Perceptions of the Benefits of the Online Course

When surveyed about which components of the online course structure supported their learning, students agreed that every component was valuable (Table 4.1). The lecture videos, associated video quizzes, online homework, and weekly deadlines for video quizzes and online homework all had a median response of agree (4) on a five point Likert scale, which contained the options: strongly disagree (1), disagree (2), neither agree nor disagree (3), agree (4), strongly agree (5).

Table 4.1. Student perceptions of course structure (N = 260).

Survey Question	Mean Score	Standard Deviation	Minimum Score	Q_1	Median	Q_3	Maximum Score
Q1. The weekly lecture videos helped me to understand the course material better.	4.19	0.82	1	4	4	5	5
Q2. The lecture videos were appropriate lengths to hold my attention.	4.23	0.82	1	4	4	5	5
Q3. The quizzes at the end of each lecture video helped me learn the course material better.	4.00	0.98	1	4	4	5	5
Q4. Having regular, weekly deadlines for video quizzes helped me to keep up with the lecture content.	4.41	0.73	1	4	5	5	5
Q5. Sapling assignments helped me to understand the course material better.	3.97	1.01	1	3	4	5	5
Q6. Having regular, weekly deadlines for the Sapling assignments helped me keep up with the class.	4.15	0.96	1	4	4	5	5

In responding to an open-ended survey question asking students what they liked most about the OL course, students commended the choice of an asynchronous format for the lecture content, comparing the format favorably to the previous F2F format. Because all of the week's lecture videos and associated assignments were provided at the start of each week, students had the opportunity to space out their coverage of the weekly content as they liked, subject to work, family, and other coursework obligations. Many students expressed that they preferred to view the lecture content prior to attending a discussion section so they could be more prepared to go over applications of the lecture material. As a result, most students chose to attend discussion sections towards the latter part of the week (see appendix). Another benefit of the lecture video format was that students had the option to rewatch videos as needed. One student expressed, "I like how we have lecture videos…since we can pause or replay the videos multiple times to better understand the mechanism because it is hard in class to be able to copy down all the notes along with

understanding them." Review of lecture material was also made easier because the videos were divided by topic instead of by the duration of a typical in-person lecture. One student expressed, "The sectioning of the lectures into topics... really helps keep my attention and helps organize what I learn by material." This organization was cited by students as a benefit when studying for exams because they could easily find the topic they wanted to review.

Although there were initial concerns about how students would react to having to take 86 video quizzes accompanying the lecture videos, it was gratifying to find that students saw them as valuable resources. Students appreciated receiving feedback on their understanding of the lecture content they had just watched. One student said, "I liked the post lecture video quizzes because they helped me apply what I just learned and it made learning a lot more interactive and helped me retain the material better." In addition to using the video quizzes as comprehension checks, students also used them to identify which lecture topics to review when studying for the exam. As exams covered more conceptual questions than usual due to the online format, students saw the video quizzes as good preparation for the new exam format. Students also appreciated the balance between flexibility and structure afforded by these video quizzes, as they could watch the videos and take the quizzes at any time during the week, but they were kept on schedule by the quizzes' weekly due dates. Students who had experienced the previous online homework due date structure from the F2F course favored the new weekly format, as it better spaced out the course workload and reinforced the weekly video quiz due dates.

Students were also surveyed to see if they felt the overall structure of the course and the educational resources provided were better than what had been offered in the F2F format (Table 4.2, Figure 4.1). The median response of neither agree nor disagree (3) likely indicates that students found the online course comparable to a F2F course.

Table 4.2. Survey questions addressing course type.

Survey Question
Q1. This course had more structure than previous in-person courses.
Q2. This course had more available educational resources to support my learning than previous in-person courses.

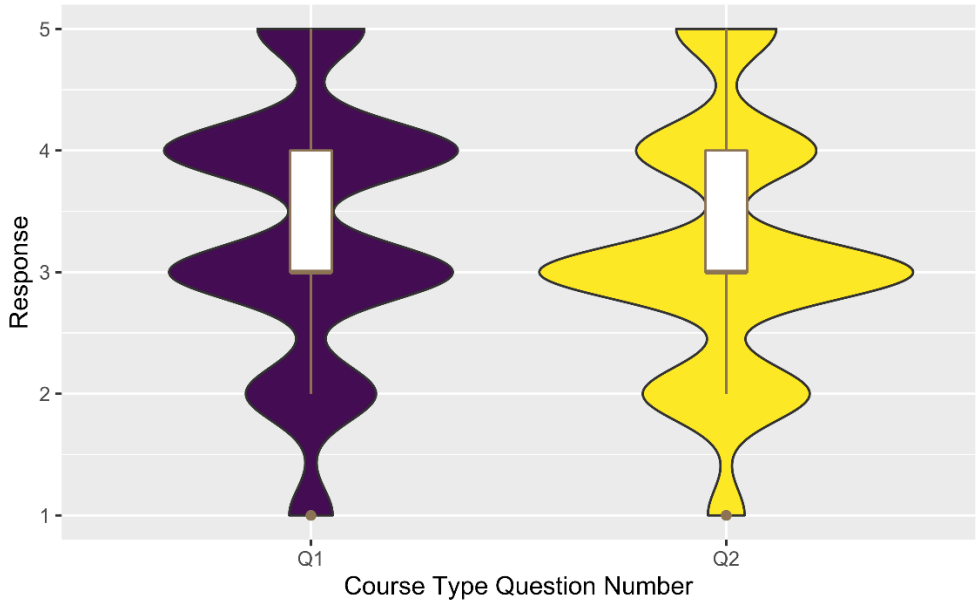


Figure 4.1. Student perceptions of course type (N = 257). Responses to the questions were submitted on a 5-point Likert scale ranging from strongly disagree (1) to strongly agree (5). The colored curves of the violin plot[70] show the distribution (similar to a histogram) of student responses. White bars with the brown horizontal lines are box plots with five number summaries — depicting the minimum, maximum, and median values with quartiles. The brown dots are outliers.

4.5.2 Student Perceptions of the Challenges of the Online Course

Despite the merits of the course structure, students had mixed feelings about the opportunities for collaborative social interactions, as they felt that they had ample opportunities to connect with their instructor, but fewer opportunities to connect with their peers. When surveyed about the extent to which the instructor supported not just their learning but also their well-being, the response from students was exceptionally positive (Table 4.3). The positive responses appear to stem from the empathetic social environment of the Monday check-ins. The informal nature of

the check-in was a welcome relief for students looking for a way to connect with their peers and the instructor. One student summed up the sentiment well, "I like how the Monday-Check in allows us to talk about non-business-related topics and just to connect with each other. It boosts my confidence and let's [sic] me know everyone is going through the same thing right now, not just me. I really like getting to know other people in the class and the professor."

Table 4.3. Student perceptions of instructor support (N = 257).

Survey Question	Mean Score	Standard Deviation	Minimum Score	Q_1	Median	Q_3	Maximum Score
Q1. The instructor was invested in my learning.	4.38	0.72	2	4	4	5	5
Q2. The instructor made reasonable accommodations to support student learning in this remote environment.	4.23	0.88	1	4	4	5	5
Q3. The instructor was receptive to student concerns.	4.26	0.93	1	4	4	5	5
Q4. The instructor made reasonable accommodations to support student well-being.	4.11	0.96	1	4	4	5	5

The importance of these check-ins became even more apparent at the end of the quarter, when students were contending with stresses stemming from the pandemic as well as from the tremendous civil unrest in response to incidents of police brutality and racial injustice. Weekly check-ins gave students a stable place to talk about what was going on, and they allowed the instructor to help give students guidance as they entered finals week. Student responses to open-ended survey questions and anecdotal comments during the Monday check-in discussions revealed how much they appreciated the instructor's transparency about how accommodations would be made to support them. This seemed to provide students with a sense of relief.

Despite the positive social interaction students experienced during the Monday check-ins, most students indicated that they felt less connected to their peers in an OL course compared to F2F courses, especially in the context of discussion sections. Survey questions addressing student interactions with their peers and their TAs in their discussion sections are included in Table 4.4. Students reported that contributions from their peers in breakout rooms varied.[71] One student said, "Sometimes I would be in break out rooms where everyone was muted and didn't answer when I talked and it make [sic] me feel uncomfortable participating myself." Students also expressed some discomfort about asking their TA for assistance during discussion. Because student responses to Q1-4 in Table 4 were positive (Figure 4.2), the discomfort appears to stem from the anxiety students had about speaking up in the presence of their peers because there was no opportunity in the Zoom discussion format to have a one-on-one conversation with the TA.

Table 4.4. Survey questions addressing interactions during discussion sections.

Survey Question
Q1. My discussion leader helps me to understand the course material.
Q2. I feel that my discussion leader is invested in my learning.
Q3. My discussion leader gives me chances to interact with my classmates.
Q4. My discussion leader creates a fair and open learning environment.
Q5. I feel comfortable contributing to small group discussions in breakout rooms.
Q6. I am comfortable asking my discussion leader for help.

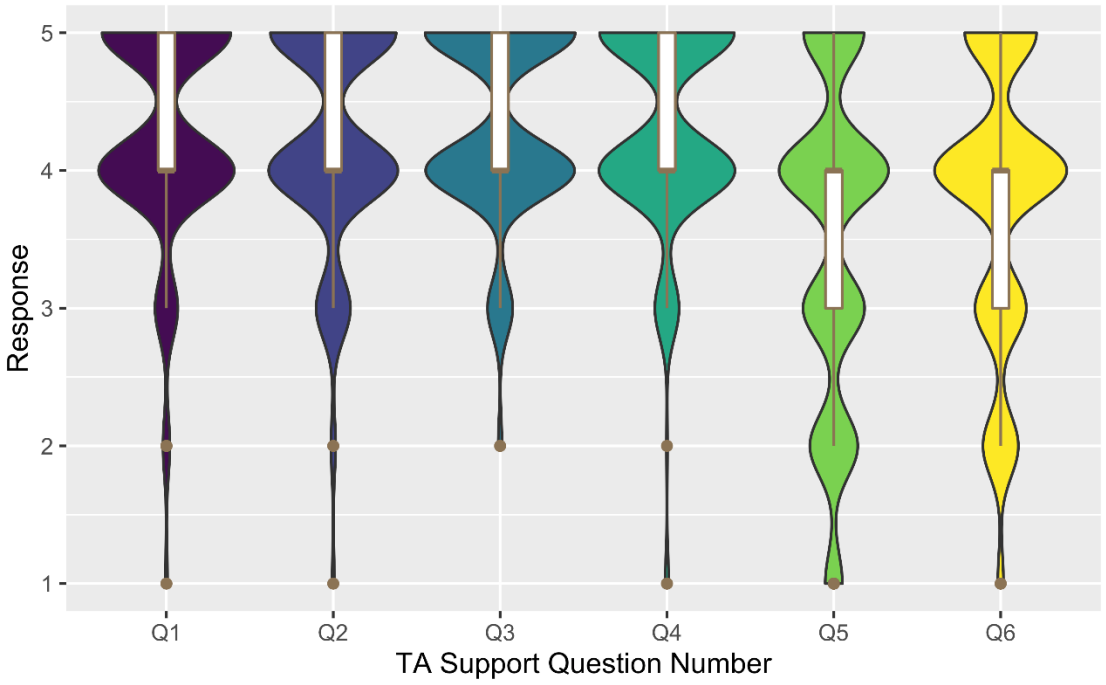


Figure 4.2. Student perceptions of interactions with TAs and peers during discussion sections (N = 260). Responses to the questions were submitted on a 5-point Likert scale ranging from strongly disagree (1) to strongly agree (5). The colored curves of the violin plot[70] show the distribution (similar to a histogram) of student responses. White bars with the brown horizontal lines are box plots with five number summaries — depicting the minimum, maximum, and median values with quartiles. The brown dots are outliers.

To determine if the random nature of the group assignments was adversely affecting the comfort students had in contributing to group discussions, we asked students to report if they would prefer to keep the randomized groups or switch to having assigned groups. Unexpectedly, 158 of 260 students (60.8%) preferred the randomized groups. Based on student responses to open-ended survey questions, the preference for this option appears to come from a desire to maintain anonymity.[72] Some students commented that they felt anxious when asked by the TA to contribute during the small group discussions and instead preferred to listen to the TA's explanations of the answers to the problems. Despite the majority favoring randomized groups, the most requested change by the students was for the TAs to put students in assigned groups so they could get to

know one another better and feel more comfortable engaging in conversations about the course material.

4.6 Conclusions and Future Directions

Although it is not possible to directly compare student performance between previous F2F classes and this OL course, is clear that students were able to learn a great deal in this novel OL environment. The structure of the course, with its combination of regular deadlines and flexible scheduling, provided students with valuable opportunities for self-directed learning.[73] The responses of the students likewise testify to the success of the OL course: they responded positively to the course as a whole and indicated that the resources made available to support their learning were comparable to those offered in previous F2F courses. As a result, many of the structural elements of this OL course, including the asynchronous lecture videos, associated video quizzes, regular weekly due dates, SMW assignment, and the Monday check-in, will be reused in future iterations of online organic chemistry classes as long as the pandemic prevents in-person instruction.

To account for the challenges students faced in the OL course, especially concerning community building, key changes to discussion sections and SMW assignments will be implemented in future OL courses. To encourage more student-to-student interactions in discussions sections, students will be assigned to the same breakout room every week, in the hope of facilitating more active and engaged conversations by allowing students to become more comfortable working with one another.[74-76] To promote participation and accountability, every student in a breakout room will be assigned a role (facilitator, note-taker, timekeeper, etc). Students will be required to post their answers to a shared Google slide which will allow for easy sharing of answers from different groups with the whole class.[77-79] Students will be encouraged to change

roles every week and to share contact information with one another so they can communicate outside of discussion sections. At the end of each discussion section, students will be asked to complete a survey addressing the "muddiest point" (the most difficult or confusing part of the lesson).[80] Incorporating this brief activity will provide the TAs and instructor with regular feedback concerning the topics with which students are struggling. This is important because students do not get the same opportunities to ask questions about confusing topics due to the asynchronous nature of the lecture content. The muddiest points will then be addressed in subsequent Zoom meetings.

The SMW assignment will also be modified. For eight of the assignments, students will be encouraged to work together and online submissions will be voluntary or worth participation points. On midterm weeks, when the Friday midterm exam takes the place of the Friday synchronous SMW meeting, students will be asked to work individually and upload a graded assignment during a one-hour window on Wednesday. This assignment will be part of the total midterm grade, and it will include problems that require hand-drawing that cannot be tested on an online exam, such as drawing chair conformations, drawing mechanisms, drawing resonance structures, using curvy arrows to move between resonance structures, and drawing resonance hybrids. Although this SMW assignment will not be proctored (the Respondus LockDown Browser does not allow students to upload assignments) multiple versions of the assignment and a small assignment window will hopefully minimize opportunities for academic dishonesty. With these future course enhancements, we hope to have an even more robust online organic chemistry course in the upcoming quarter.

4.7 Appendix

4.7.1 Supplementary Figures

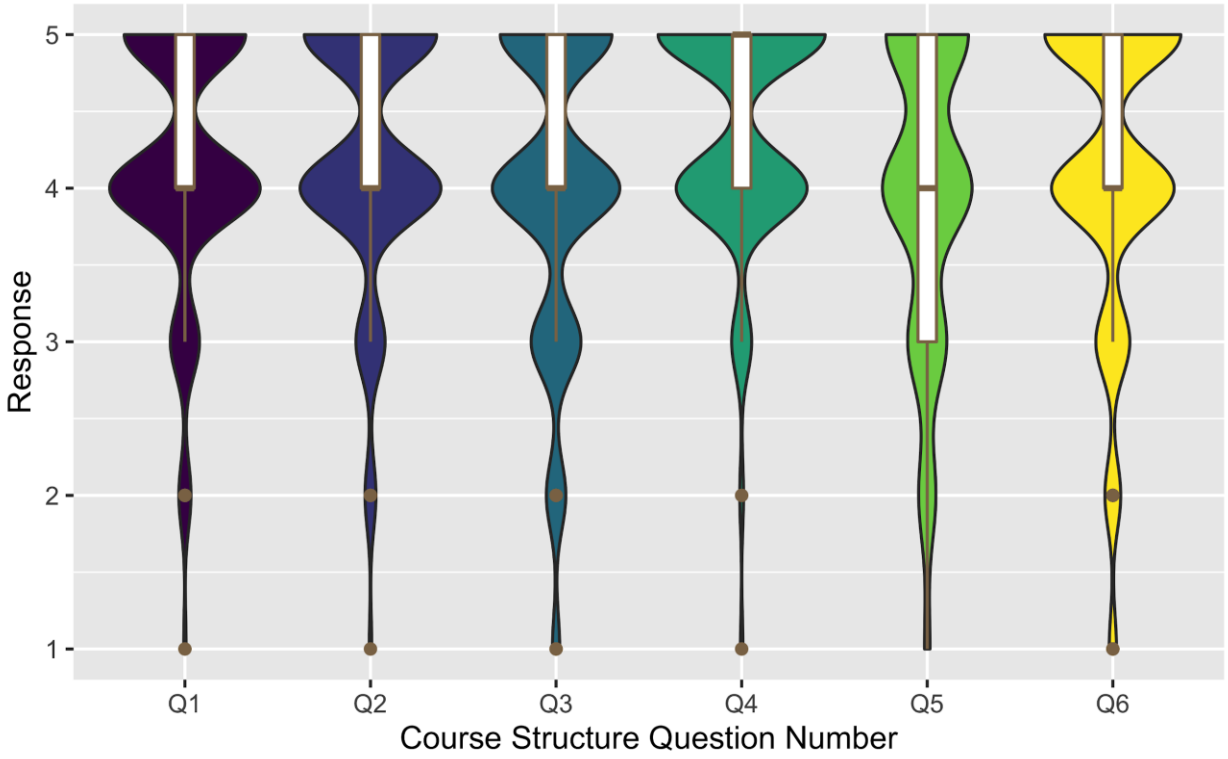


Figure 4.3. Student perceptions of course structure (N = 260). Responses to the questions were submitted on a 5-point Likert scale ranging from strongly disagree (1) to strongly agree (5). The colored curves of the violin plot show the distribution (similar to a histogram) of student responses. White bars with the brown horizontal lines are box plots with five number summaries — depicting the minimum, maximum, and median with quartiles. The brown dots are outliers.

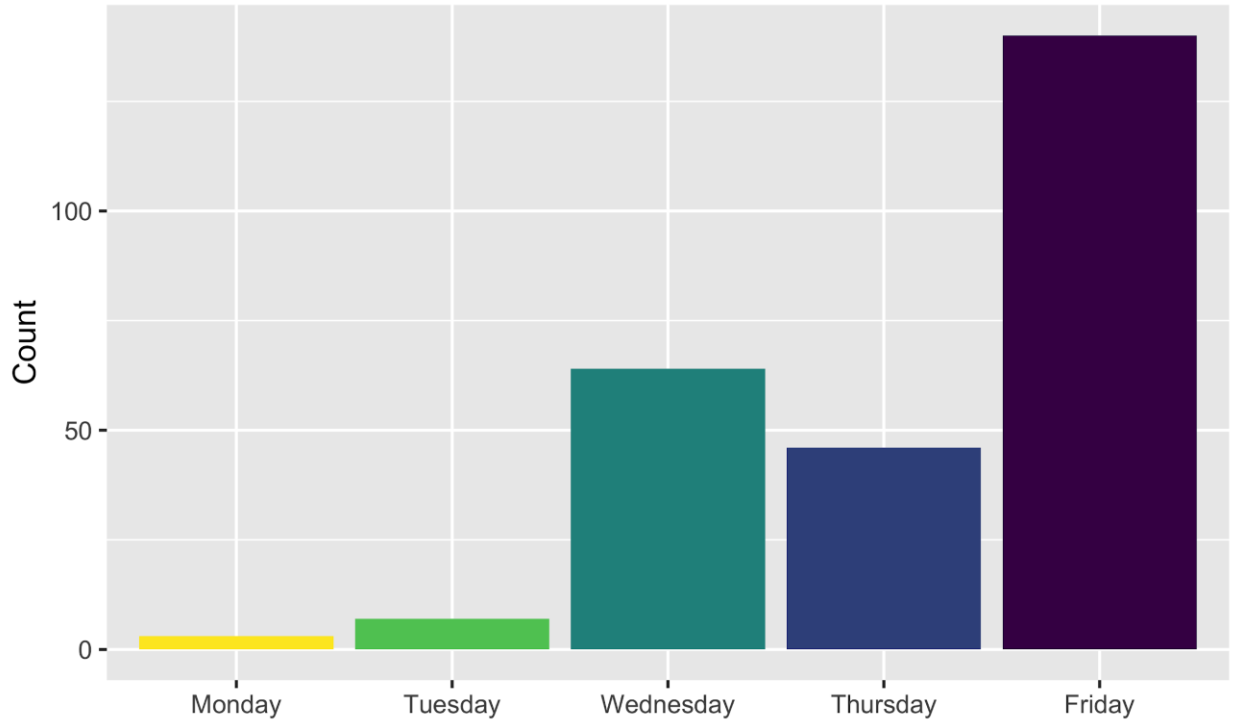


Figure 4.4. Discussion attendance by day of the week (N = 260). Monday (3), Tuesday (7), Wednesday (64), Thursday (46), Friday (140).

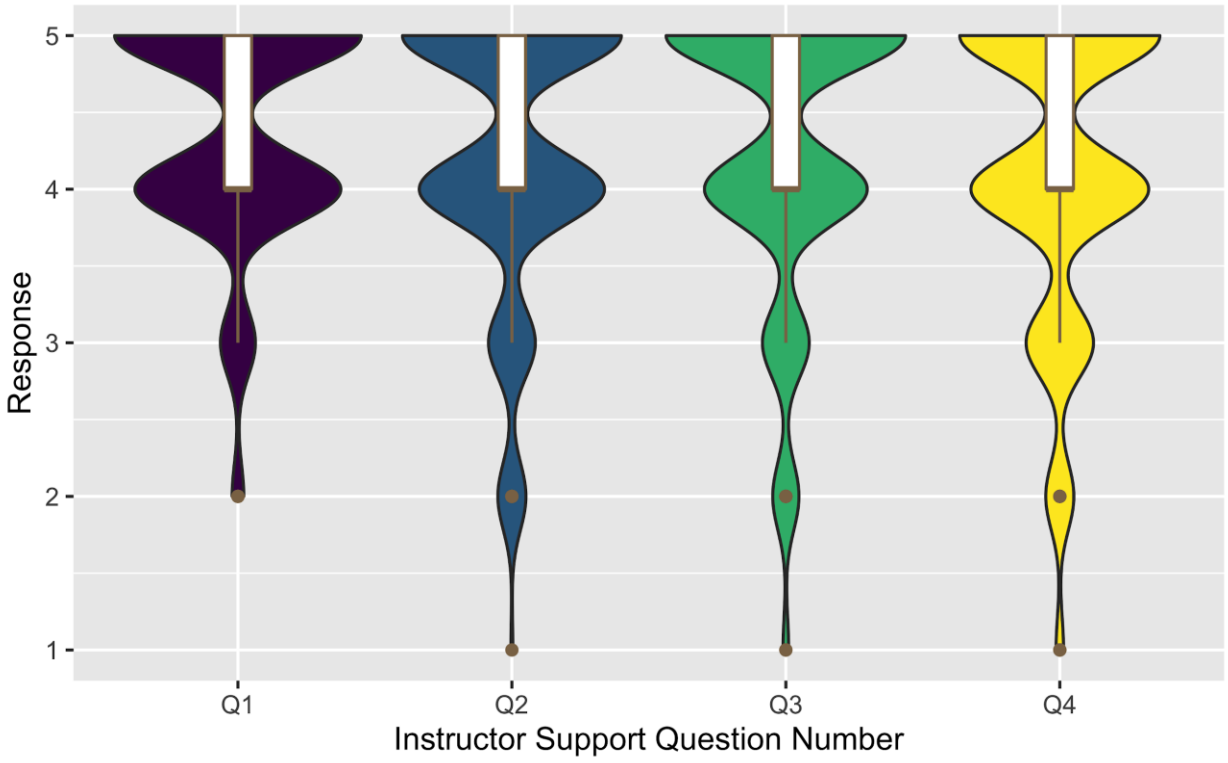


Figure 4.5. Student perceptions of instructor support (N = 257). Responses to the questions were submitted on a 5-point Likert scale ranging from strongly disagree (1) to strongly agree (5). The colored curves of the violin plot show the distribution (similar to a histogram) of student responses. White bars with the brown horizontal lines are box plots with five number summaries — depicting the minimum, maximum, and median with quartiles. The brown dots are outliers.

4.7.2 IRB Statement

This study was approved by the University of California, Irvine, Institutional Review Board as exempt (IRB 2018-4661) including FERPA compliance.

4.7.3 Mid-Quarter Survey Questions

1. 5-Point Likert Scale--Indicate the extent to which you agree with the following statements:
    a. I have communicated with my classmates about learning the course material through different electronic means, such as email, discussion boards, social media, instant messaging, etc.
    b. I feel comfortable volunteering ideas/opinions to my classmates (i.e. Zoom chats, Piazza, etc).
    c. I rarely communicate with my classmates outside of discussion.
    d. It is hard to get to know other students in this online course.

    e. If I did not understand something, I asked the instructor or TA for help.
    f. I have used Chegg, Course Hero, Koofers, Khan Academy, or other online resources to help me learn the course material.
2. Drop-down Menu--Which discussion section do you attend?
    a. Monday (4-5 pm)
    b. Tuesday (9-10 am)
    c. Tuesday (12-1 pm)
    d. Wednesday (11-12 pm)
    e. Wednesday (1-2 pm)
    f. Thursday (10-11 am)
    g. Thursday (11-12 pm)
    h. Thursday (12-1 pm)
    i. Friday (11-12 pm)
    j. Friday (1-2 pm)
3. 5-Point Likert Scale--Indicate the extent to which you agree with the following statements:
    a. My discussion leader helps me to understand the course material.
    b. My discussion leader gives me chances to interact with my classmates.
    c. My discussion leader creates a fair and open learning environment.
    d. I feel comfortable contributing to small group discussions in breakout rooms.
    e. I am comfortable asking my discussion leader for help.
    f. I am comfortable asking the learning assistants for help.
    g. I feel that my discussion leader is invested in my learning.
4. Drop-down Menu--Which of the following would you prefer?
    a. Randomly assigned groups in breakout rooms each discussion section.
    b. The same assigned groups in breakout rooms each discussion section.
5. 5-Point Likert Scale--Indicate the extent to which you agree with the following statements:
    a. The weekly lecture videos helped me to understand the course material better.
    b. The lecture videos were appropriate lengths to hold my attention.
    c. The quizzes at the end of each lecture video helped me learn the course material better.
    d. Having regular, weekly deadlines for video quizzes helped me to keep up with the lecture content.
    e. Sapling assignments helped me to understand the course material better.
    f. Having regular, weekly deadlines for the Sapling assignments helped me keep up with the class.
    g. I have used the textbook to learn the 51C course material more than I did in 51B.
    h. The Smith end-of-chapter problems help me to understand the course material better.
    i. The synthesis and mechanism of the week helped me to learn the course material better.
    j. The Monday check-in made me feel more comfortable in this online course.
    k. I believe there is more academic dishonesty occurring in this online course than there would be if it were held in person.

6. Open-Ended Response--We want to know how to make the learning experience better! Do you have any innovative ideas, or have you done things in some of your other classes that help build community, relationships, and connections in this online environment?
7. Open-Ended Response--Is there anything that you like better this quarter when compared to an in-person class? Why?
8. Open-Ended Response--What has surprised you most this quarter?

Table 4.5. Summary of statistics for questions 1, 3, and 5 from the mid-quarter survey.

Survey Question	Mean Score	Standard Deviation	Minimum Score	Q_1	Median	Q_3	Maximum Score
Q1a	3.32	1.29	1	2	4	4	5
Q1b	3.11	1.11	1	2	3	4	5
Q1c	3.50	1.25	1	2	4	5	5
Q1d	4.39	0.79	1	4	5	5	5
Q1e	3.26	1.08	1	2	3	4	5
Q1f	2.91	1.39	1	2	3	4	5
Q3a	4.29	0.77	1	4	4	5	5
Q3b	4.36	0.67	2	4	4	5	5
Q3c	4.33	0.74	1	4	4	5	5
Q3d	3.50	1.15	1	3	4	4	5
Q3e	3.77	1.01	1	3	4	4	5
Q3f	3.83	1.03	1	3	4	5	5
Q3g	4.25	0.78	1	4	4	5	5
Q5a	4.19	0.82	1	4	4	5	5
Q5b	4.23	0.82	1	4	4	5	5
Q5c	4.00	0.98	1	4	4	5	5
Q5d	4.41	0.73	1	4	5	5	5
Q5e	3.96	1.03	1	3	4	5	5
Q5f	4.15	0.96	1	4	4	5	5
Q5g	2.70	1.33	1	2	3	4	5
Q5h	3.37	1.16	1	3	3	4	5
Q5i	3.75	1.10	1	3	4	4.25	5
Q5j	3.60	0.96	1	3	4	4	5
Q5k	3.53	1.06	1	3	4	4	5

Table 4.6. Distribution of student responses to questions 1, 3, and 5 from the mid-quarter survey.

Survey Question	Responses of Strongly Disagree (#, %)	Responses of Disagree (#, %)	Responses of Neither Agree Nor Disagree (#, %)	Responses of Agree (#, %)	Responses of Strongly Agree (#, %)
Q1a	33, 13	44, 17	35, 13	102, 39	46, 18
Q1b	22, 8	59, 23	69, 27	88, 34	22, 8
Q1c	13, 5	61, 23	38, 15	78, 30	70, 27
Q1d	1, 0	8, 3	19, 7	92, 35	140, 54
Q1e	16, 6	52, 20	68, 26	97, 37	27, 10
Q1f	59, 23	45, 17	49, 19	69, 27	36, 14
Q3a	2, 1	6, 2	20, 8	119, 46	113, 43
Q3b	0, 0	3, 1	20, 8	118, 45	119, 46
Q3c	3, 1	1, 0	21, 8	118, 45	117, 45
Q3d	15, 6	42, 16	54, 21	96, 37	53, 20
Q3e	5, 2	31, 12	45, 17	116, 45	63, 24
Q3f	8, 3	26, 10	43, 17	112, 43	71, 27
Q3g	2, 1	4, 2	30, 12	113, 43	110, 42
Q5a	2, 1	10, 4	24, 9	124, 48	100, 38
Q5b	2, 1	9, 3	24, 9	116, 45	109, 42
Q5c	6, 2	15, 6	41, 16	105, 40	92, 35
Q5d	2, 1	3, 1	16	105, 40	134, 52
Q5e	7, 3	19, 7	44, 17	99, 38	91, 35
Q5f	6, 2	13, 5	28, 11	101, 39	112, 43
Q5g	60, 23	68, 26	54, 21	46, 18	32, 12
Q5h	22, 8	30, 12	84, 32	77, 30	47, 18
Q5i	16, 6	18, 7	46, 18	115, 44	65, 25
Q5j	6, 2	23, 9	86, 33	99, 38	46, 18
Q5k	14, 5	19, 7	95, 57	80, 31	52, 20

Table 4.7. Distribution of student responses to question 2 from the mid-quarter survey.

Survey Option	Responses (#, %)
Monday (4-5 pm)	3, 1
Tuesday (9-10 am)	2, 1
Tuesday (12-1 pm)	5, 2
Wednesday (11-12 pm)	30, 12
Wednesday (1-2 pm)	34, 13
Thursday (10-11 am)	14, 5
Thursday (11-12 pm)	11, 4
Thursday (12-1 pm)	21, 8
Friday (11-12 pm)	31, 12
Friday (1-2 pm)	109, 42

Table 4.8. Distribution of student responses to question 4 from the mid-quarter survey.

Survey Option	Responses (#, %)
Randomly assigned groups in breakout rooms each discussion section.	158, 61
The same assigned groups in breakout rooms each discussion section.	102, 39

Table 4.9. Representative student responses to question 6 from the mid-quarter survey.

Responses
“I’m not sure about anything “new” that we could do but I feel the best thing is to take advantage of the somewhat smaller environments during office hours with the professor and TAs to get to know them and the classmates who attend them better.”
“I can't think of anything other than more accountability in breakout rooms during discussion. Although people show up, every single week it's only been me and 1 or 2 other students discussing when there's like 6 of us in the room. Everyone else just mutes there [sic] mic and I find that unfair and unhelpful.”
“One thing would be to start off with an ice breaker during new breakout rooms to stimulate participation among peers.”
“Having the same people in breakout rooms will help with meeting new people for this course. With meeting every week individuals will start to feel more comfortable with each other.”
“Having mechanism/synthesis of the week that did not count towards my grade actually expanded my learning more rather having it graded because I’m more stressed out figuring out the right answer than actually learning the material when it’s graded.”

Table 4.10. Representative student responses to question 7 from the mid-quarter survey.

Responses
"I really enjoy watching videos at my own pace. While in in class lecture, I can NEVER focus for long periods of time. I've gone through college learning nothing in lecture because I can never seem to focus my mind wonders elsewhere. I always have to go home and reteach myself everything because I couldn't focus in class. Now, when I feel my mind start to wonder, I can pause the video, get my thoughts together and refocus, and then play the video. I've never been so caught up in a lecture before."
"I like how the sapling assignments are weekly instead of the whole chapter because they are less overwhelming now and it's better to keep up with the content."
"I really enjoyed the Monday check-ins with Dr. King where it's mostly a heart-to-heart conversation, as it is a time where we all bond over the struggles of the pandemic."
"I think its [sic] a lot easier and convenient to ask questions. Because the professor and TA monitor the chat during meetings, I can usually get answers pretty quickly. Its [sic] also easy to just pop into office hours just to ask a question or two, while it may be a bit difficult and more time consuming to go to office hours in person."
"I definitely like the video quizzes assigned after each video section. They are a good way to offer students additional points to help them out. Also, I liked how the modules were separated in a clear and easily recognizable way. Another thing I liked was testing. For me, I am a bit of an anxious test taker, especially when there are others around me. When I'm home alone in my room, I definitely feel less anxious."

Table 4.11. Representative student responses to question 8 from the mid-quarter survey.

Responses
"The transition to online was near seamless, I think Dr. King and the TA's did a phenomenal job, but there were inevitable problems like mechanisms on tests. This remote learning could work, but it takes out the social aspect of being in a classroom and working with peers."
"Testing and online classes where you have to participate because you can't get the kind of privacy on reduced noise level where you can focus when you live at home."
"How distant I would feel when I'm not in a class full of peers. I work better when i [sic] am forced (more like advised) to openly discuss with others during lecture. With the remote learning, i [sic] feel that it's difficult for me to reach out to others outside class to study together."
"I was surprised by the amount of accessibility there was in the class still. It was still possible to communicate with the professor and the TAs through Zoom effectively. Though, even though it is not the same as in person it is still surprising."
"I was surprised by how difficult it is to study at home. I am accustomed to studying in on-campus libraries, and to have a different environment that isn't quiet or considerate, makes the learning experience that much more difficult."

4.7.4 End-of-Quarter Survey Questions

1. 5-Point Likert Scale--Indicate the extent to which you agree with the following statements:
    a. Doing well in this class was important to me.
    b. I was able to master the skills taught in this course.
    c. Compared to 51B, I had a much harder time learning organic chemistry this quarter.
    d. Compared to 51B, I had a harder time getting help when I didn't understand something.
    e. Compared to 51B, I was more anxious taking exams.
    f. Worrying about Respondus issues affected my performance this quarter.
    g. Worrying about Wifi (and other related technological) issues affected my performance this quarter.
    h. I had a harder time keeping up with the class without regular lectures 3 times a week.
    i. I worked harder this quarter than I ever did in 51A or 51B.
    j. I learned more chemistry this quarter than I did in 51A or 51B.
    k. This course had more structure than previous in-person courses.
    l. This course had more available educational resources to support my learning than in previous in-person courses.
    m. This course contributed to my educational development.
2. 5-Point Likert Scale--Indicate the extent to which you agree with the following statements:
    a. The instructor was invested in my learning.
    b. The instructor made reasonable accommodations to support student learning in this remote environment.
    c. The instructor was receptive to student concerns.
    d. The instructor made reasonable accommodations to support student well-being.
3. Drop-down Menu--Did your study environment affect your learning because of the noise within your home and/or the noise in the surrounding area?
    a. Frequently
    b. Occasionally
    c. Rarely
4. Drop-down Menu--Did your study environment affect your learning because of the lack of privacy?
    a. Frequently
    b. Occasionally
    c. Rarely
5. Drop-down Menu--Did you make use of the captions on any of the videos in this course?
    a. Frequently
    b. Occasionally
    c. Rarely
6. Drop-down Menu--Of the three ways the synthesis/mechanism of the week was conducted, which did you prefer?
    a. Graded based on accuracy with individual feedback (Weeks 2, 3, 5, 6)

b. Not graded, but with individual feedback (Week 7)
c. Graded based on completion with no feedback (Week 9)

7. Open-Ended Response--What have you found beneficial about taking this course remotely?
8. Open-Ended Response--What have you found challenging about taking this course remotely?

Table 4.12. Summary of statistics for questions 1 and 2 from the end-of-quarter survey.

Survey Question	Mean Score	Standard Deviation	Minimum Score	Q_1	Median	Q_3	Maximum Score
Q1a	4.60	0.59	1	4	5	5	5
Q1b	3.67	0.88	1	3	4	4	5
Q1c	3.63	1.14	1	3	4	5	5
Q1d	3.29	1.06	1	3	3	4	5
Q1e	3.87	1.11	1	3	4	5	5
Q1f	3.64	1.18	1	3	4	5	5
Q1g	3.77	1.13	1	3	4	5	5
Q1h	3.10	1.32	1	2	3	4	5
Q1i	3.82	1.01	1	3	4	5	5
Q1j	3.48	1.06	1	3	4	4	5
Q1k	3.34	1.04	1	3	3	4	5
Q1l	3.16	1.01	1	3	3	4	5
Q1m	3.83	0.90	1	3	4	4	5
Q2a	4.38	0.72	2	4	4	5	5
Q2b	4.23	0.88	1	4	4	5	5
Q2c	4.26	0.93	1	4	4	5	5
Q2d	4.11	0.96	1	4	4	5	5

Table 4.13. Distribution of student responses to questions 1 and 2 from the end-of-quarter survey.

Survey Question	Responses of Strongly Disagree (#, %)	Responses of Disagree (#, %)	Responses of Neither Agree Nor Disagree (#, %)	Responses of Agree (#, %)	Responses of Strongly Agree (#, %)
Q1a	1, 0	0, 0	8, 3	84, 33	164, 64
Q1b	1, 0	26, 10	71, 28	118, 46	41, 16
Q1c	4, 2	50, 19	57, 22	71, 28	75, 29
Q1d	8, 3	52, 20	95, 37	61, 24	41, 16
Q1e	3, 1	38, 15	45, 18	74, 29	97, 38
Q1f	15, 6	33, 13	51, 20	86, 33	71, 28
Q1g	10, 4	33, 13	41, 16	94, 37	79, 31
Q1h	30, 12	68, 26	57, 22	50, 19	52, 20
Q1i	4, 2	23, 9	65, 25	89, 35	76, 30
Q1j	9, 4	36, 14	83, 32	80, 31	49, 19
Q1k	13, 5	39, 15	85, 33	88, 34	32, 12
Q1l	11, 4	50, 19	112, 44	54, 21	30, 12
Q1m	6, 2	15, 6	47, 18	137, 53	52, 20
Q2a	0, 0	6, 2	18, 7	106, 41	127, 49
Q2b	1, 0	15, 6	25, 10	99, 39	117, 46
Q2c	3, 1	14, 5	25, 10	87, 34	128, 50
Q2d	4, 2	15, 6	36, 14	95, 37	107, 42

Table 4.14. Distribution of student responses to questions 3, 4, and 5 from the end-of-quarter survey.

Survey Question	Responses of Rarely (#, %)	Responses of Occasionally (#, %)	Responses of Frequently (#, %)
Q3	34, 13	113, 43	110, 42
Q4	76, 29	95, 37	86, 33
Q5	132, 51	56, 22	68, 26

Table 4.15. Distribution of student responses to question 6 from the end-of-quarter survey.

Survey Option	Responses (#, %)
Graded based on accuracy with individual feedback (Weeks 2, 3, 5, 6)	71, 27
Not graded, but with individual feedback (Week 7)	125, 48
Graded based on completion with no feedback (Week 9)	61, 23

Table 4.16. Representative student responses to question 7 on the end-of-quarter survey.

Responses
“Having much greater access to office hours and discussion sections.”
“Strangely enough, I found it a bit more personable than an in person class because there were so many opportunities for smaller group interactions with the Monday check in and Friday synthesis. By using the chat, I felt like it made it more fun and others were also more engaged. Since this was also kind of extra times [sic], everyone would go off topic a bit but we’d learn things about each other which made learning the subject more fun. I also thought it was nice that I could go at my own pace with the lectures and homework.”
“I am able to learn at my own pace. I really like having close [sic] caption because sometimes I can't hear what the professor is saying. I also like weekly check-ins because it helps me plan out my week according to the weekly agenda.”
“I can watch all lectures for the week in one sitting if I wanted to, which is often what I did at the beginning of the week. This way, I can start the sapling problems early and attempt the discussion worksheet on my own first, before asking questions during discussion.”
“The online format that Dr. King adopted was very good. Breaking lecture videos into individual concepts was extremely helpful when studying and reviewing.”
“Having weekly videos and quizzes and having them due on Sunday. It gives the student freedom to do them whenever they had time, but having them due at the end of the week made them still be on track within the class.”
“The way the videos were broken up into mostly less than 20 minute videos helped a lot in my attention and focus during lectures. I actually felt that I was able to complete more lectures compared to my other classes where we had more than 50 minutes of lecture at a time (recorded). I was also able to spend time on each lecture and the post lecture video quizzes and had more flexibility in which discussion to attend.”
“I think it was very useful to be able to take the week's content at my own pace. I felt like I was able to learn more by spacing out the material when I didn't feel like I could focus or doing a large chunk at once when I was in the zone. Receiving feedback after each video with quizzes really helped me to stay engaged in make [sic] sure I was picking up the details. More frequent and regular practice problems with instructor feedback was also a significant plus to keep me on top of the material. Honestly, this was my favorite quarter of OChem, and I feel like the online model Dr. King was superior to the in-person course layouts and the online layouts used my other ochem professors this quarter.”
“It was a lot easier to participate during discussions through the chat as it generated far less anxiety about doing anything embarrassing. The chat also made it far easier to share my thoughts as well as working with my peers when voice chat was not a viable option.”
“The sapling assignments being due regularly made it easier to keep up with them as it was easier to forget them before. Being able to do the lectures at your own pace was also very helpful.”

Table 4.17. Representative student responses to question 8 on the end-of-quarter survey.

Responses
“I think the most challenging part is just making sure that you stay disciplined enough to stay on track in the course. But, Dr. King really helped with this aspect with the weekly assignments (Synthesis and Mechanism of the Week, Podcast Quizzes, and Sapling) and with the weekly check-in meetings. These things really encouraged me to stay on track, and if she had not structured the class this way, I think I would have fallen behind much more easily.”
“Not being able to talk to peers during lecture or ask questions made the learning more difficult. And discussion sections were useful but sometimes not all students would participate so it would be me and maybe one other student speaking with each other while the rest of the room was silent.”
“It's been challenging mentally to remain 100% focused because I really struggle with working in a non-classroom environment sometimes. I guess it's more of a "inside my head" type of thing, but taking this course remotely emphasized how much I have to be responsible and accountable for myself when pacing my work.”
“The challenging aspect of taking this course remotely was how there were not enough interactions among the students; however, that was accommodated through discussion sections in which we participated or ask [sic] questions to the TA's or the professor.”
“Taking online Ochem exams has been challenging because I’m not used to that format.”
“It was difficult given that I had poor wifi which added a lot more stress when it came to exams. Also being able to access the videos due to slow internet was frustrating.”
“The main thing that I found challenging from taking this course remotely was that I felt I wasn't getting the same experience as I would have if I took it in person. Whenever I don't necessarily understand something, I would ask my peers in lecture or those sitting around me, but being remote, I did not have this same opportunity, so I had to adjust to this change.”
“Taking this course has been challenging in terms of having to express certain answers that are best presented by drawing difficult.”
“I found it harder to not get distracted while working on the course since I am at home. I also found it harder to be motivated some days since there wasn't a large external factor forcing me to learn.”
“I have found it challenging to keep myself on track sometimes with the lack of a typical three day in-person lecture schedule. I found it necessary to structure my viewing of the lecture material in a similar three day format.”

4.7.5 Additional Lessons Learned

Have a short practice exam before the real thing. It is critical to give a low stakes practice exam a few days in advance of the actual exam before conducting an online exam for the first time. This is especially true when the exam is being monitored by a proctoring service, when students are required to have a webcam, and/or when students are required to have a lockdown

browser. A practice exam gives students a chance to see if their wifi and camera are working properly and to determine if they are able to view the embedded images in the exam.

Cheating is easier in an online environment - be proactive. A few students will engage in academic dishonesty no matter what the circumstances, but it is easier to cheat in an OL atmosphere. Clearly state in your syllabus that academic dishonesty will not be tolerated, and be prepared to spend some time policing your class on any graded assignment. Proctoring services for all exams are recommended.

Everyone needs to be on the same page. Because the lecture podcasts were available asynchronously, only the students in the class were watching them. Not all of the LAs or the TAs were watching them. This presented problems during grading, especially in one case when a TA graded multistep synthesis problems on an exam incorrectly. It took a great deal of time and effort to regrade these problems accurately. I highly recommend that LAs and TAs watch the videos. I will require this in the upcoming fall quarter.

Discussion section details: Size of breakout rooms matter! We typically have around 15 to 90 students per discussion section (with a total of 10 discussion sections). Discussion sizes are set by the registrar at a maximum size of 40. We do, however allow students to attend whichever discussion they want. TAs (four total) conduct two discussions each, and the instructor also conducts two discussions. The instructor's two discussions, as a result, have very large enrollments (as high as 90), especially the Friday, 1 pm discussion in both the OL and F2F class. There are one to two LAs that attend each discussion section. Each discussion section covers the same content each week and there is no incentive for students to share information between discussion sections because there is no grade associated with discussion aside from attendance.

On exam weeks, the midterm is on Friday at 12 noon, so the Friday 1 pm discussion is cancelled, and those 90 students typically move to discussions earlier in the week. Overall attendance at discussion sections is fairly consistent throughout the quarter, but does dip during the exam weeks. These trends remained consistent in the OL course.

The number of breakout rooms used in discussion sections in the OL course varied depending on how many problems were on the worksheet, with each breakout room working on a different problem. At the start of the OL class, we had approximately 10 breakout rooms, which created group sizes that were too large for the larger discussion sections of nearly 90 students (9 students per breakout room). We shifted to 4-5 students per breakout room, with some rooms working on the same problem, and this was more ideal.

In the F2F discussion, students are given a weekly worksheet and broken in small groups in an active learning classroom. Each group is assigned a problem. After about fifteen minutes, playing cards are passed to each student in the group, and whoever has the highest or lowest card must come to the board and write the answer. After everyone has written answers, the instructor goes over the answers, pointing out common mistakes, and pitfalls. In the OL discussion, an attempt was made to use the same format, except that students were given the worksheet at the beginning of the week and encouraged to work problems on their own. In breakout rooms, students were still assigned a problem to work on, but they were not required to submit answers. Some students were able to initiate engagement in their breakout rooms, but all too often, we found no one was talking in breakout rooms. There was no video and no sound, and it was uncomfortable entering the breakout room. As a result, in the upcoming fall quarter, we have decided to shift gears and utilize proven strategies for engaging breakout room members. Each student will have a role in the breakout room and students will be expected to vary their roles

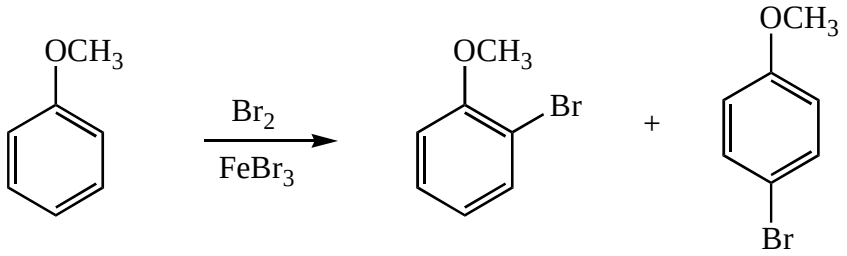
every week (we will keep track of this)! Roles will include: Facilitator, Note-taker (screen sharer), Reporter, Timekeeper, and Accuracy-checker. Students will also need to report their answer in a Google slide. The use of Google slides over a Google document is because individual groups do not have to share the same writing space. In a Google document, multiple editors working at the same time can cause the text in the document to shift as you are trying to work on it. In Google slides, each group works on their own slide and so multiple editors from different groups do not affect one another's work in the same way. Students have the option to draw their answer on paper and take a picture with their phone to upload into the Google slide, or they can take a screenshot of a drawing on tablet or computer. Because the breakout room has to produce an answer that everyone agrees with, we hope that students will be more engaged.

4.7.6 Sample Discussion Worksheet

Weekly worksheets are the same in the F2F and OL class. The following page shows a sample weekly worksheet from the first week of discussion in spring quarter:

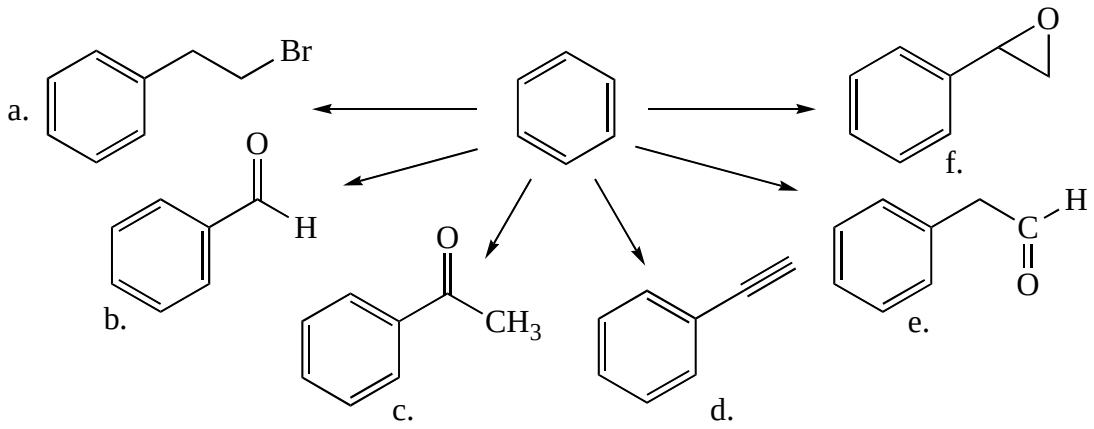
Discussion Worksheet
Week 1

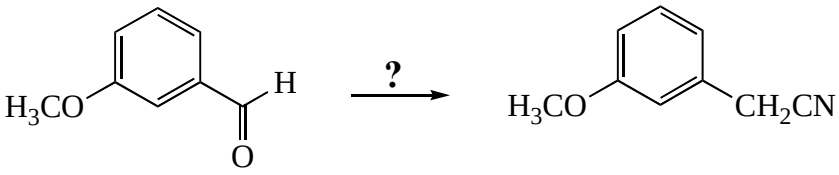
1. Anisole *(methoxybenzene)* undergoes bromination much faster than benzene, and directs incoming substituents into the *ortho, para* positions. Use resonance structures the carbocation intermediate formed for addition to the ortho, meta, and para positions to explain for the accelerated rate.

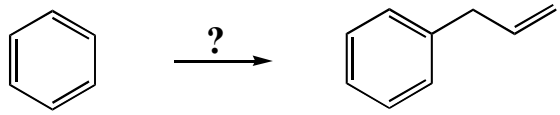


2. Provide a synthesis of the following products from the given starting materials:

Hints:
#1: In chapter 18, we learn how to do simple bromination, nitration, Friedel Crafts acylation, and Friedel Crafts alkylation (see page 1 of notes). If you have to incorporate anything more complicated than these groups, then put the simple group on first, and then do functional group manipulation using techniques from chapters 9, 10, 11, 12, 15 & 16 (<u>only 2c below can be put on in one step</u>).



g. 

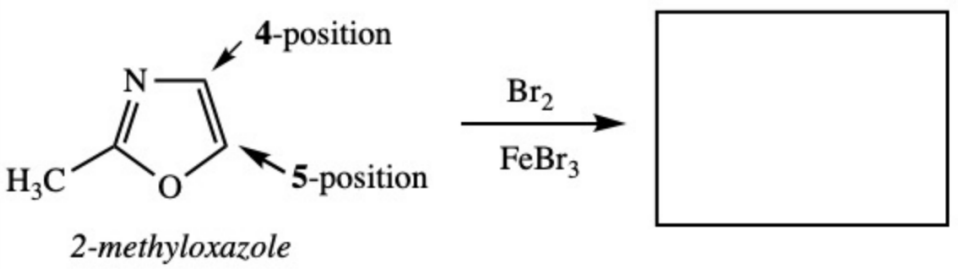
h. 

4.7.7 Sample SMW Assignment

Q1 Mechanism of the Week

18 Points

Aromatic heterocycles also undergo electrophilic aromatic substitution! When 2-methyloxazole is treated with Br2 and FeBr3, there are two possible places the bromine can go, in the 4-position, or in the 5-position. Show a complete mechanism for this bromination reaction. Write all contributing resonance structures for the carbocation intermediate formed when bromine goes in the 4-position and then do the same for the 5-position. Show complete resonance structures, including all charges and non-bonding electrons. From examination of the carbocation intermediates for the 4-position and the 5-position of 2-methyloxazole, choose the correct position for the bromine, and give a brief explanation why this position is favored.



No files uploaded

Q2 Synthesis of the Week

5 Points

Provide reagents for the following transformation. More than one step is required, so please number individual steps.

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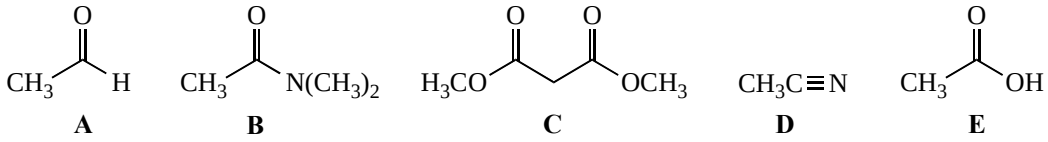
4.7.8 Comparison of Question Types Used in F2F and OL Exams

F2F version:

1. For the following set, write in the box a compound letter that correctly answers the questions that follow:



a. Which compound is the most acidic compound? ☐

b. Which compound is the least acidic? ☐

c. Which compound is an active methylene compound? ☐

d. Which compound does *not* have an enolizable hydrogen? ☐

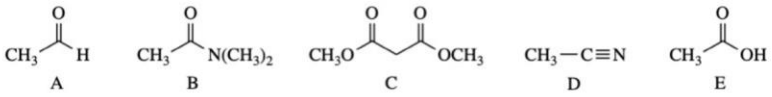
e. Which compound would form the greatest amount of enol at equilibrium? ☐

f. Which compound would undergo an aldol reaction when treated with NaOEt in ethanol? ☐

OL version (same as F2F):

Question 1 12 pts

For the following set, write the compound letter that correctly answers the questions that follow:



a. Which compound is the most acidic compound?

b. Which compound is the least acidic?

c. Which compound is an active methylene compound?

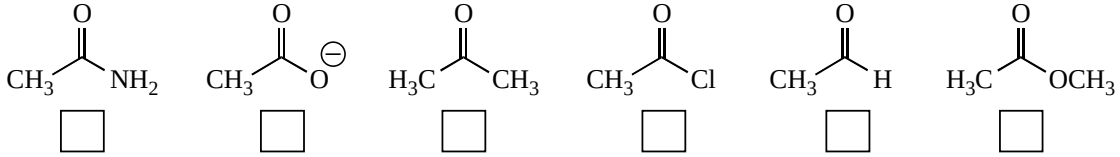
d. Which compound does *not* have an enolizable hydrogen?

e. Which compound would form the greatest amount of enol at equilibrium?

f. Which compound would undergo aldol reaction when treated with NaOEt in ethanol?

F2F version:

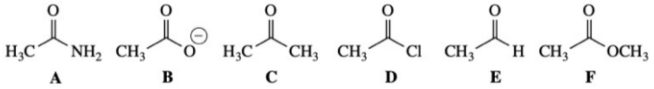
3. Rank in order of *decreasing* electrophilicity (1 = best electrophile):



OL version (same as F2F):

Question 3 6 pts

Rank in order of *decreasing* electrophilicity (1 = best electrophile):



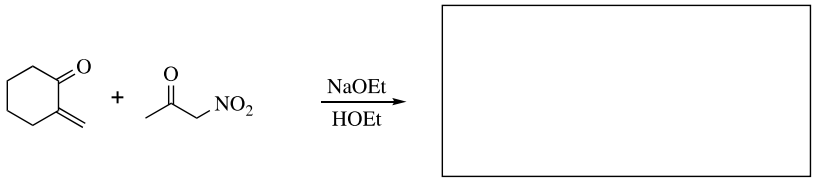
Most electrophilic = 1

1.
2.
3.
4.
5.
6.

Least electrophilic = 6

F2F version:

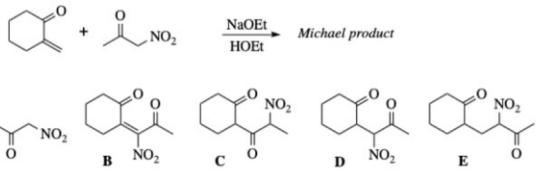
15. Predict the product in the following Michael reaction:



OL version (easier because students have four possible products to choose from, and therefore a 25% chance of getting it right just by a guess, and 50% chance of getting it right if the student recognizes that a Michael product gives a 1,5-dicarbonyl):

Question 15 4 pts

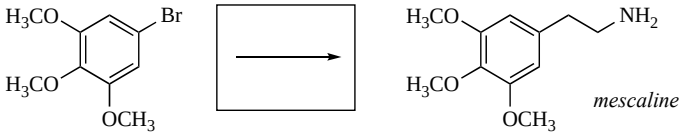
Predict the product in the following Michael reaction:



- C
- A
- E
- D
- B

F2F version:

25. Mescaline is a hallucinogenic drug that occurs naturally in certain cacti plants and has been used by Native Americans for thousands of years in religious ceremonies and for medicinal purposes. Provide a synthesis of mescaline from the given starting material. Draw numbered steps in the box provided.



OL version (same as F2F):

Question 21 5 pts

Mescaline is a hallucinogenic drug that occurs naturally in certain cacti plants and it has been used by Native Americans for thousands of years in religious ceremonies and for medicinal purposes. Provide a synthesis of mescaline from the given starting material. Draw numbered steps in the box provided.

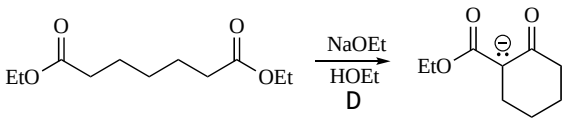
HTML Editor

12pt Paragraph

0 words

F2F version:

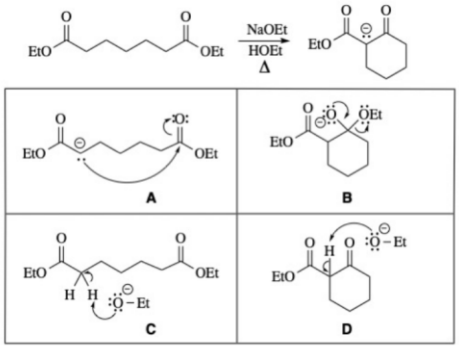
25. Provide a detailed mechanism for the following Claisen condensation. Include all lone pairs and formal charges, and do not combine two steps into one.



OL version (much, much easier. >95% Of students got the OL version completely correct):

Question 25 8 pts

Determine the stepwise mechanism for the following Claisen condensation by indicating the correct order of lettered steps in the boxes below:



Step 1:

Step 2:

Step 3:

Step 4:

4.7.9 Sapling Due Dates Comparison

F2F Spring 2019 (start of quarter on March 27):

Chapter 18:	April 10
Chapter 19, 20:	April 24
Chapter 21:	May 7
Chapter 22:	May 15
Chapter 23:	May 22
Chapter 24:	June 3
Chapter 25:	June 8

OL Spring 2020 (start of quarter on March 30):

Week 1:	April 5
Week 2:	April 12
Week 3:	April 19
Week 4:	April 26
Week 5:	May 3
Week 6:	May 10
Week 7:	May 17
Week 8:	May 24
Week 9:	May 31
Week 10:	June 8

Although the same Sapling Problems are in both the F2F and OL classes, the weekly assignments have a more even distribution of questions (4 assignments in April, 4 assignments in May, and two assignments in June). We will continue to use weekly assignments even when courses are F2F.

4.7.10 Nomenclature Handouts

The following pages contain handouts given before the second midterm and the final to correct mistakes make in multistep synthesis problems:

Some abbreviations, condensed structures & common names that may be useful for exams!

1. You can write simple aldehydes and ketones using common names, IUPAC names, or condensed structures:

   Formaldehyde: $H_2C{=}O$
   Acetaldehyde: $CH_3(C{=}O)H = CH_3CHO$
   Acetone: $CH_3(C{=}O)CH_3$

** Technically the (C=O) does not have to be drawn. In other words, acetone can be written as CH_3COCH_3 instead of $CH_3(C{=}O)CH_3$. I like to include the carbonyl in parentheses because there is a tendency for students to mistakenly interpret CH_3COCH_3 as an ether rather than a ketone!

2. Carboxylic acids, esters, amides, and acid chlorides can all be written as common name, IUPAC names, or condensed structures:

   Acetic acid: $CH_3CO_2H = CH_3COOH$
   Ethyl acetate: $CH_3CO_2CH_2CH_3$
   Amides: $CH_3(C{=}O)NH_2$
   Acid Chloride: $CH_3(C{=}O)Cl$

3. Epoxides. There's no good way to draw these condensed, so it is best to use the name. Two to know:

   (Epoxides are named based on the alkene they come from)

   ethylene oxide

   propylene oxide

4. Grignards, organolithium reagents, & cuprates as well as many other common reagents are all easily typed.

5. A good one to know for MT 2 if you are synthesizing a barbiturate:

   Urea: can write urea or $NH_2(C{=}O)NH_2$
   Diethyl malonate or $CH_2(CO_2Et)_2$

6. Don't forget how to draw branched chain alkyl halides! Examples:

   Isoproypl bromide: $(CH_3)_3CHBr$
   2-methyl-1-bromobutane: $CH_3CH_2CH(CH_3)CH_2Br$

Rules for Drawing Condensed Structures

I am teaching 51A in the fall, and I will have to heavily emphasize condensed structures since all exams will be online. For this class, here's a reminder of the rules for drawing condensed structures so you ***don't miss unnecessary points on the final***.

All atoms are drawn in (*including hydrogens*), but bond lines are generally omitted. It can be helpful to use an equal sign for a double bond:

CH_2CHCH_2MgBr (allyl magnesium bromide), or better: $CH_2{=}CHCH_2MgBr$

*(*Can you see how the second structure reads better even though are accepted ways to draw allyl magnesium bromide)?*

Atoms are drawn next to the atoms to which they are bonded:

Don't draw like this: CH_2CHCH_2BrMg *or* $MgBr\text{-}C(CH_3)_3$

*(*The magnesium is bonded to the carbon not the bromine*).

It is $[(CH_3)_3C]_2CuLi$ ***not*** $CuLiC(CH_3)_3$

(*Two problems with this one: Copper is bonded to the carbon, not Li, and there are two tert-butyl groups not one*)!

<u>Use capitals for atoms, and subscripts for numbers</u>.

Don't draw like this: Ch2ChCh2MgBr *or* Ch2ChCh2MGBR or lic(ch3)3

Please use brackets around parentheses if you have multiple sets of parentheses like I've done with the cuprate above. $[(CH_3)_3C]_2CuLi$ *not* $((CH_3)_3C)_2CuLi$

Parentheses are used around similar groups bonded to the same atom or for branches.

$CH_3(CH_2)_3CH_2Br$ *is the same as* $CH_3CH_2CH_2CH_2CH_2Br$

$CH_3CH(CH_3)CH_2CH_2Br$ (*The methyl in the parentheses is a branch and it needs to be in parentheses*).

Common mistakes on MT 2:

1. Missing a Carbon.

$CH_3)_3Br$ (*That should be a tert-butyl group, not bromine bonded to three methyls*). **This is a ridiculously easy mistake to make – please be careful**.

2. Too many hydrogens or not drawing hydrogens at all.

C-C-C-C-Br (*not a valid condensed structure*)

$(CH_3)_3CHMgBr$ (*the carbon bonded to magnesium has five bonds!*)

3. Miscellaneous mistakes from MT2

Cl-C=O-CH(CH3)3, AlCl3

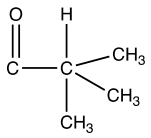
(*If you are going to draw the Carbon-oxygen double bond, please place it in parentheses, otherwise it looks like oxygen is bonded to the CH, rather than the carbonyl carbon*).

lic(ch3)3

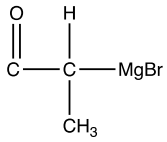
(*This looks more like a word not a condensed structure. Use capitals for atoms, not lower case, unless it is a two-letter atom like Br*).

These last three fall into the ***Huh?*** Category:

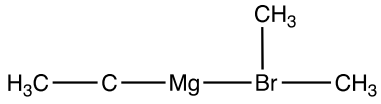
(C=O)CH(CH3)3: *this means*:



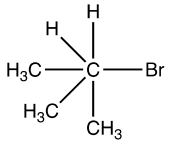
C=O)CH(CH3)Mg-Br: *this means:*



CH3CMgBr(CH3)CH3: *this means:*



CH2(CH3)3Br: *this means:*



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Chapter 5: Online in No Time: Design and Implementation of a Remote Learning First Quarter General Chemistry Laboratory and Second Quarter Organic Chemistry Laboratory

5.1 Preface

Toward the end of winter quarter 2020, a meeting was held between the teaching faculty, stockroom managers, and the chair of the department concerning how best to approach teaching courses remotely for spring quarter. I was invited to this meeting because I was a senior graduate student who had received a large amount of pedagogy training and had several instructor-of-record experiences. At that meeting it was determined that in addition to taking on additional TA duties that I would continue to serve as the Head TA for the general chemistry laboratory courses, a responsibility I had been assigned for the 2019-2020 academic year. Due to the challenge associated with converting a general chemistry laboratory course from an in-person to an online format, a second Head TA was appointed and a curriculum development team was formed to provide much needed assistance. The same approach was taken for the organic chemistry laboratory course. This chapter describes the process of converting the first quarter general chemistry laboratory course and second quarter organic chemistry laboratory course from in-person to online formats and discusses the student perceptions of the online courses. Taylor Frey and I served as the Head TAs for the general chemistry laboratory course and were involved in the scripting and directing of experiment videos. We also constructed the course schedule, scaffolded the electronic laboratory notebook, developed video quizzes to accompany the experiment videos, and designed the surveys. For the general chemistry laboratory course, Chase Tretbar was responsible for the filming and editing of the experiment videos, Peter Tieu contributed to the

scripting of the experiment videos, Melanie Nguyen provided the chemicals and equipment needed for the TAs to conduct the experiments that were filmed, Joe Gonzales managed the Piazza messageboard, and Dan Seith created the BigBrother computer script which identified students with incomplete online homework. Taylor Thane and Sarah Wang served as the Head TAs for the organic chemistry laboratory course and were involved in the scripting and directing of experiment videos. They also developed video quizzes to accompany the experiment videos and developed the mastery projects. For the organic chemistry laboratory course, Simon Lam provided the chemicals and equipment needed for the TAs to conduct the experiments that were filmed and Shannon Saluga scaffolded the electronic laboratory notebook.

5.2 Introduction

The onset of the global COVID-19 pandemic forced chemistry laboratory courses to rapidly shift from hands-on, experiential learning courses to remotely delivered courses.[1] For the lower division laboratory courses at the University of California, Irvine (UCI), this emergency pivot to remote instruction occurred at the end of our winter term, requiring us to create a full quarter of chemistry laboratory courses for more than one thousand students in two weeks. Unlike schools on semester terms where instructors and students already had an established relationship, the students in our largest laboratory course (general chemistry) began their laboratory experience in this new remote format. We leveraged our existing course infrastructures, including extensive online tools, to create remote learning experiences as similar to our hands-on courses as possible. Both courses took very similar approaches, deviating only where needed to account for differing student needs.

5.2.1 In-Person Course Structure

UCI is a quarter-system school with three, 10-week terms per academic year. Chemistry laboratory courses for non-chemistry majors at UCI are offered in quarters offset from lecture courses (Table 5.1).[2] The total approximate enrollment for these laboratory courses is 1,400 students for General Chemistry Lab I (GCL-I) and 1,000 students for Organic Chemistry Laboratory II (OCL-II). These students are spread across laboratory sections consisting of 20–24 students supervised by graduate student teaching assistants (TAs). Students attend their assigned laboratory section for a single 3 hour 50 minute session each week in the first eight weeks of the term and laboratory practical exams are given in the last two weeks of the term. The general chemistry laboratory courses contain weekly instructor lecture videos but no in-person lecture component. In contrast, the organic chemistry laboratory courses include both prelaboratory

lecture videos and a 50-minute weekly interactive laboratory lecture taught by the instructor and offered in multiple sections of approximately 200-400 students.

Table 5.1. Structure of general and organic chemistry courses.

Year	Fall Quarter	Winter Quarter	Spring Quarter
First Year	General Chemistry Lecture I	General Chemistry Lecture II	General Chemistry Lecture III
	No laboratory course	No laboratory course	General Chemistry Laboratory I (GCL-I)
Second Year	Organic Chemistry Lecture I	Organic Chemistry Lecture II	Organic Chemistry Lecture III
	General Chemistry Laboratory II	Organic Chemistry Laboratory I	Organic Chemistry Laboratory II (OCL-II)

GCL-I is the first college laboratory course taken by undergraduate science majors, primarily from biological sciences, public health, pharmaceutical sciences, and engineering. For undergraduate students, the first laboratory course can be a difficult transition as this may be their first experience with four-hour laboratory sections, electronic laboratory notebooks (ELNs), new laboratory techniques, and weekly reports. The large enrollment of these inexperienced students is challenging under normal circumstances. In a remote environment where instructional content was developed and implemented right before use, a large instructional team of seven development TAs and three learning assistants (LAs) was needed in addition to the 28 section TAs that would normally be assigned to the course. The development TAs supported the instructor by developing course material, while the LAs provided additional support for students through message boards and office hours.

OCL-II is the last chemistry laboratory course many students complete, typically at the end of their second year. They have already completed three chemistry laboratory courses and are

familiar with the rigor, course policies, and technology requirements. Students enrolled in this laboratory course are also familiar with the instructor from a previous laboratory course experience. While the enrollment for this course typically approaches 1,000 students, a smaller offering with only 104 students was required in this scenario because the instructor (RDL) was also supporting colleagues who were converting organic chemistry lecture courses into an emergency remote format. A smaller instructional team of five development TAs and four instructional TAs was needed for this course.

5.2.2 Laboratory Course Objectives and Existing Online Infrastructure

In designing our emergency remote delivery course structures, we focused on maintaining as many of our existing course objectives as possible (Table 5.2). The objective of students performing techniques with chemicals, glassware, equipment, and instrumentation could not be achieved.[3,4] Therefore, we focused on other objectives typically assessed throughout a laboratory course by laboratory reports and during laboratory final exams: data interpretation and calculation, theory behind experiments, conceptual understanding of techniques/procedures, and laboratory safety.[5]

Table 5.2. Course learning objectives for general and organic chemistry laboratory courses.

General Chemistry Laboratory	Organic Chemistry Laboratory
1. Prepare solutions using volumetric glassware and calculate solution concentration. Use burette to perform titrations. Demonstrate understanding of the procedures and calculations associated with these techniques.	1. Perform fundamental organic chemistry techniques in the context of laboratory experiments.
2. Operate temperature, conductivity and voltage probes, a simple visible spectrometer, and digital balance to acquire data.	*2. Demonstrate understanding of concepts underlying fundamental techniques by proposing solutions to actual or potential problems encountered during an experiment.
*3. Proficiently use an electronic laboratory notebook to record qualitative observations in detail and quantitative data with the correct number of significant figures.	*3. Accurately draw reaction mechanisms for reactions conducted in laboratory sessions.
*4. Interpret experimental data and calculate results to develop scientifically sound conclusions.	*4. Use spectroscopy data to determine structures of unknown molecules.
*5. Employ basic computational chemistry to explain resonance, acid strength, and reaction coordinate diagrams.	*5. Use data collected from an experiment to make claims supported by evidence.
*6. Demonstrate understanding of basic safety symbols, safety data sheets, corrosives, handling of chemical waste, fire safety, and chemical spill response.	*6. Identify safe and unsafe practices related to techniques used in laboratory sessions.

*Course learning objectives prioritized in designing the remote delivery format.

We were fortunate that our courses were well positioned for the remote environment because we had already built the necessary internet-accessible framework of curriculum and instructional tools.[6] Manuals, technique videos, readings, and instructor videos were already embedded in the ELN, LabArchives, and/or the learning management system, Canvas.[7–22] Prelaboratory work consisted of online homework and completion of select portions of the ELN.[10,13,23,24] During the laboratory session, students also utilized the ELN to enter procedures, observations, and data. Rubrics for grading on Canvas were already built, and Gradescope, an assignment submission and grading platform, had been used for laboratory practical exam grading

for two years.[25] Additionally, we had an existing means of communication with students through the message board, Piazza.[13,26–29] Finally, we recognized we could compile authentic experimental data from the student ELNs of previous iterations of the courses.

5.2.3 Determining Our Emergency Pivot Approach

When converting our courses to a remote delivery format, both instructional teams were guided by principles grounded in the existing chemistry and STEM education literature. Courses were designed in a highly structured format to provide students with accountability for asynchronous coursework and regular formative assessments.[30–37] A combination of asynchronous work and synchronous meetings were included to provide students with a connection to the instructor and TAs while also accommodating their rapidly changing schedules.[38–41] We aimed to keep the course workload similar to the previous course format (or lighter if possible) for students and TAs.

In considering the best approach to transition our courses into an online format, we evaluated known replacements for experimental work. While simulations exist for general chemistry laboratory courses, we determined that we could not develop a rich online framework around such simulations comparable to the existing curriculum of GCL-I. Furthermore, we could not find simulations to cover half of the topics within GCL-I (Table 3). Vendor-supplied kits for home experiments were not considered because of their cost and the lead time required to customize kits.[42] Far fewer resources exist for virtual organic chemistry laboratories. The resources that do exist focus mainly on introducing laboratory techniques typically covered in a first-term course[43] or incorporate verification experiments at odds with our standard curriculum (Table 5.3).[44] For both courses, we felt that the instructional tools present in our current electronic course content (i.e., lecture videos, online homework, computational studies) were essential for student

understanding of the content of whatever modality we chose to replace in-lab experimentation.[6] We also felt that developing supporting curriculum and summative assessments for new content would add significant effort to an already challenging quarter. We concluded the more expedient and pedagogically appropriate choice was to film experiments and use previously obtained data for both laboratory courses.[18–20] Access to a public Google Drive folder containing our instructional materials and experiment videos is available in the appendix.

Table 5.3. Descriptive summaries of GCL-I & OCL-II.

GCL-I Experiments	OCL-II Experiments
1. Enthalpy of Formation: Coffee cup calorimetry and Hess' Law used to find the enthalpy of reaction.	1. Clove Oil Steam Distillation: Eugenol is distilled from cloves. Purity is assessed by TLC and ^{1}H NMR.
2. Equilibrium and Visible Spectroscopy: Equilibrium constant of iron thiocyanate found using visible spectroscopy and LeChatelier's principle.	2. Electrophilic Aromatic Substitution: Relative reactivities determined by bromination of aromatic rings bearing various substituents.
3. Computational Study of the Thiocyanate Ion: Spartan is used to investigate the actual structure of thiocyanate by looking at bond lengths and orbitals. Diatomic molecular orbitals are determined in the process	3. Wittig: Students select a variable to explore (Wittig salt, aldehyde, or base). Students identify any trend in how variable affects E/Z selectivity of products using ^{1}H NMR and address how this trend corresponds with their initial hypothesis.
4. Dissolution Thermodynamics: The enthalpy and entropy change for the dissolution of borax is determined by acid-base titration of borate ion samples taken at different temperatures.	4. Oxidation and Reduction: Oxidation of 4-t-butylcyclohexanol to 4-t-butylcyclohexanone. Reductions of 4-t-butylcyclohexanone to 4-t-butylcyclohexanol using sodium borohydride and Meerwein-Pondorff-Verley conditions. Analysis requires explaining the differences in product mixtures assessed using ^{1}H NMR.
5. Electrical Conduction of Solutions: The conduction of electrolytes is measured as a function of increasing atomic mass, acid strength, and increasing concentration. The equivalence point of a double displacement reaction is determined by conductometric titration.	5. Determining Absolute Configuration: Students qualitatively and quantitatively determine which reaction proceeds faster in a matched and mismatched case of acetylation of an alcohol with a chiral catalyst and determine absolute configuration of unknown chiral alcohol.
6. Acid-Base Buffers: Preparation and the investigation of the effects of acid or base addition and buffer dilution on pH. Spartan investigation of dissociation as a function of acid strength.	6. Aldol Condensation: Double aldol condensation with unknown aldehyde and ketone. Differentiate aldehyde and ketone by IR spectroscopy. Determine structures of unknowns by first determining structure of product by ^{1}H and ^{13}C NMR.
7. Electrochemical Cells: Measurement of cell potentials, creation of reduction potential table, and investigation of the effect of concentration on cell potential.	
8. Rate Law Determination and Visible Spectroscopy: Visible spectroscopy is used to measure the disappearance of crystal violet as a result of hydroxylation. The rate law, rate constant, and half-life are determined. Spartan investigation of a reaction coordinate diagram.	

5.2.4 Creating Video Versions of Our Existing Experiments

Both the GCL-I and OCL-II course teams filmed video content during spring break and the beginning of the spring quarter while following all public health guidelines. The GCL-I videos were filmed and edited by the development TAs, whereas the OCL-II videos were filmed and edited by the university media team. TAs wrote the scripts and served as actors in the videos. During the editing process, videos were segmented into approximately 15 minute portions to maintain student attention and increase comprehension.[45] Automatically graded Canvas video quizzes promoted student accountability and engagement.[46,47] TAs in the general chemistry videos narrated their actions in detail to guide less experienced students through the basic techniques and data collection performed. In contrast, the more advanced students in OCL-II were already familiar with fundamental laboratory techniques, so video narration required less detail.

5.3 Structuring the Remote Versions of the First-Term General Chemistry and Second-Term Organic Chemistry Laboratory

5.3.1. Scheduling

To achieve a high structure format for the remote versions of both GCL-I and OCL-II, modifications from the in-person versions of the courses were required. Many of the structural similarities between the two courses allowed for equivalent alterations to scheduling and ELN use. The typical experiment schedule for both courses was delayed by one week to expand the time available for curriculum development and provide students with a structured introduction to the online laboratory format. In the GCL-I course, the first week of the quarter introduced students to the online tools required for the course (i.e. Zoom video conferencing tool, Piazza message boards, ELN, Canvas, Spartan computational software, and Sapling Learning online homework) through webinars.[21,22,26,48–50] Because most students in the OCL-II course were already familiar with the

online tools, the first week was devoted to a writing workshop in which students critiqued and revised one of their laboratory reports from a previous course. Delaying experimental work also allowed us to support the technological needs of students and TAs. Laptops with cameras were loaned to students from our teaching laboratory stockroom. Writing tablets, webcams, and smartphone holders were distributed to TAs to enable remote teaching.

For the in-person version of both courses, assignment due dates were scheduled to correlate to the day and time of a student's laboratory section. To provide a clearer course structure for students enrolled in the remote courses, the availability of weekly content and assignment due dates were made the same for all students, regardless of the day and time of their scheduled laboratory section (Figure 5.1).[47,51] To provide additional clarity, both courses utilized the announcement function of Canvas on a weekly basis to connect due dates to assignment expectations.[29,52]

Week	Monday	Tuesday	Wednesday	Thursday	Friday
3	GCL-I SAPLING DUE 11:59 PM	GCL-I EXP 2 VIDEO AND CANVAS QUIZ OPEN 12 PM		GCL-I EXP 1 POST LAB DUE 11:59 PM	GCL-I EXP 2 PRE/IN-LAB AND CANVAS QUIZ DUE 11:59 PM
	OCL-II EXP 2 VIDEO OPEN 10 PM	OCL-II PRE-LAB VIDEO QUIZZES, SAPLING, AND EXP 2 PRE-LAB DUE 1 PM		OCL-II EXP 1 POST LAB DUE 11:59 PM	OCL-II EXP 2 IN-LAB AND CANVAS QUIZ DUE 11:59 PM
4	GCL-I SAPLING DUE 11:59 PM	GCL-I EXP 3 VIDEO AND CANVAS QUIZ OPEN 12 PM		GCL-I EXP 2 POST LAB DUE 11:59 PM	GCL-I EXP 3 PRE/IN-LAB AND CANVAS QUIZ DUE 11:59 PM
	OCL-II EXP 3 VIDEO OPEN 10 PM	OCL-II PRE-LAB VIDEO QUIZZES, SAPLING, AND EXP 3 PRE-LAB DUE 1 PM		OCL-II EXP 2 POST LAB DUE 11:59 PM	OCL-II EXP 2 IN-LAB AND CANVAS QUIZ DUE 11:59 PM

Figure 5.1. Representative two-week schedule for GCL-I (green) and OCL-II (blue).

5.3.2 Electronic Laboratory Notebook and Data Sets

In previous quarters, students completed all sections of a blank ELN page weekly. To account for the lack of in-person communication this quarter, scaffolding was added to the ELN page and gradually reduced as the course progressed. In initial experiments, prefilled sections were added as examples for students to reference in later weeks when the scaffold was removed. This modification was included in both courses to ease the ELN learning curve for the new laboratory students in GCL-I and provide added direction in OCL-II.

Student ELN entries from previous iterations of the course were also leveraged to provide unique data sets to minimize academic dishonesty. These data sets were distributed to each section at the end of the Canvas video quizzes (see appendix for data delivery instructions). The two instructional teams had different goals when selecting data. The general chemistry team provided "good" data that approximated ideal results to ease new students into the teaching laboratory course environment. The organic chemistry team, however, provided their more experienced students with imperfect data to provide opportunities for rich discussion around limitations of experiments and their outcomes.

5.3.3 Staff Meetings, Office Hours, and Class Meetings

Although both courses retained similar course structures, differences in enrollment and student demographics required course-specific approaches to laboratory lectures, teaching staff meetings, office hours, and online homework. Some synchronous class meetings were held for each course, although the approach to these meetings differed. During weeks when challenging concepts were introduced in GCL-I (e.g. graphing, calculations with significant figures, etc.), multiple live webinars were held to supplement instructor lecture videos. Students in OCL-II attended one 50-minute weekly, interactive laboratory lecture on Zoom. Students engaged with

material using PollEverywhere to earn participation credit and communicated using Zoom's chat feature.[53] The laboratory lecture was recorded and provided along with a make-up assignment on Canvas for students who could not attend. A similar online lecture format has been offered in previous years. This experience enabled us to easily shift to a fully online laboratory lecture and expand existing instructional techniques in OCL-II.

Management of the TAs in both courses was handled using a weekly one-hour staff meeting on Zoom. During previous in-person meetings, a group of 3–4 TAs who performed the current week's experiment beforehand would present various procedural tips and tricks which they believed would help fellow TAs in the laboratory. For GCL-I this quarter, these TAs could not perform the experiments, so presentations focused on contextualizing the laboratory material and explaining the theory underlying the laboratory techniques and instrumentation. Because OCL-II had fewer TAs due to lower enrollment, staff meetings required less structure.

The GCL-I TAs held one, two-hour office hour weekly over Zoom in pairs. Each pair's office hours were scheduled on the same day and time as their assigned laboratory section to ensure students could meet with their designated TA. Student attendance was encouraged, but not required. More experienced TAs were strategically paired with less experienced TAs. Within the pair, one TA responded to questions by speaking while the other TA responded to questions by typing into the chat window.

The OCL-II TAs met with students online during the first half of their regularly scheduled laboratory section and held two, one-hour office hours each week. Like GCL-I, student attendance at these scheduled meetings was encouraged, but not required. TAs began synchronous class meetings by giving a short summary of the experiment. Then TAs played the in-laboratory videos using screen share and stopped the video at strategic points to engage students in a discussion of

key steps or concepts. Class meetings ended with a question and answer session. In addition to the class meeting, OCL-II TAs held two unstructured office hour meetings. Pairing TAs was unnecessary for office hours as there were fewer attendees. Office hours for the organic chemistry laboratory course were also held over Zoom using the Canvas integration.[54]

5.3.4 Monitoring Online Homework

Although both courses use online prelaboratory homework, the GCL-I team developed a computer script, titled BigBrother, to streamline TA responsibilities. In a typical academic term, TAs would log into Sapling and manually check for incomplete assignments. In GCL-I, a course with 28 TAs for 56 lab sections, BigBrother identified students with incomplete online homework and sent a student list by section directly to the appropriate TA's account in a messaging platform (Slack).[55] The annotated code is provided in the appendix.

5.4 Replacing the In-Person Laboratory Practical Exams

Both the GCL-I and OCL-II courses typically conclude with a practical exam.[56–59] Different approaches were taken by each course to replace these exams because GCL-I uses a traditional points-based grading system, whereas OCL-II uses a specifications grading system.[60,61] However, the widespread social uprising that occurred in late May and early June of 2020 in response to the deaths of Ahmaud Arbery, Breonna Taylor, George Floyd, and others necessitated alterations to our plans. Many of our students were directly impacted by the widespread protests and media reporting. We include both the intended exam replacement plans and our emergency adjustments here for clarity and discussion.

The traditional, in-person format of the GCL-I practical exam consisted of students performing two short wet-laboratory exercises taken directly from experiments conducted during

the quarter, analyzing data collected from a computational study, and answering multiple-choice questions pertaining to safe laboratory practices. In the remote version of the course, the new exam consisted of two parts: a Canvas quiz, requiring the Respondus LockDown browser and Monitor AI, and two "take-home" essays submitted to Gradescope.[62] The Canvas quiz assessed the understanding of chemical theory and data analysis. The goal of the essays was to encourage students to demonstrate conceptual understanding of two general chemistry laboratory techniques.[63–65] Students selected and responded to two of six possible essay prompts. They then researched and described the procedures of their two chosen laboratory techniques in detail. A table of essay prompts, response rates, and averages can be found in the appendix.

GCL-I final exams began Monday, June 1, 2020. However, campus guidance for changes to final examinations was announced two days later as administrators attempted to respond to the evolving social uprising and its impacts on our students. Because the exams had already started for about half of the 1,403 students, the alteration of exam content or conditions could be perceived as unfair by those who had already taken the exam. However, students needing accommodations throughout the week were allowed to take the online exam or turn in the essays at later dates.

The in-person final exam structure for OCL-II consisted of three required assignments for all students to earn passing grades and additional assessments to achieve an A or B letter grade. The initial plan for final assessments in this remote format retained all of the in-person components with two adjustments. Two of the mandatory assessments, a safety exam and an exam covering concepts and data analysis, would be administered as automatically graded Canvas quizzes. These quizzes were intended to evaluate understanding of laboratory safety and overall understanding of course content. A third mandatory assessment on thin layer chromatography would be converted

from a hands-on activity to an online quiz using both Canvas and Chemix, a chemistry diagramming software.[66]

To earn more than a passing grade in the course, students would have completed additional technique assessments using Canvas and Chemix on liquid-liquid extraction and recrystallization (Figure 5.2). Students would also have completed a mastery project where they develop a hypothesis and analyze experimental data related to a previously studied reaction. The project, designed to replace open-ended questions on typical practical exams, could either be presented as a lab report for a B grade or as a journal-style article or research poster for an A grade.[67,68]

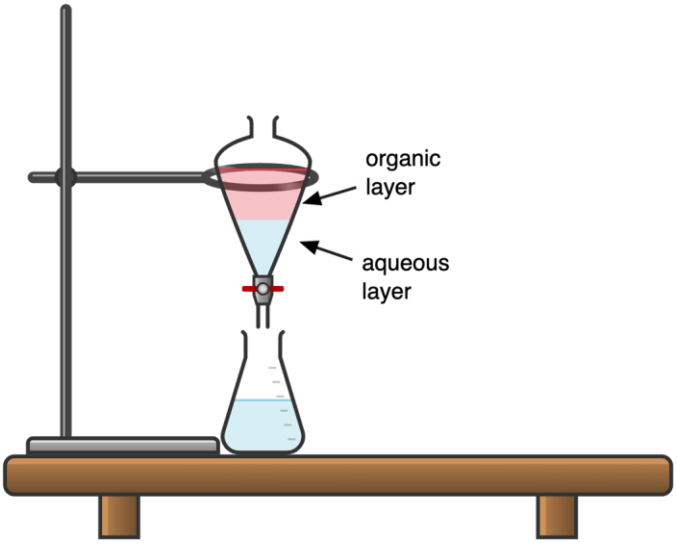

Figure 5.2. Chemix drawing of liquid-liquid extraction.

Although we created a comprehensive set of exam replacements, the social upheaval that impacted our students necessitated a rapid change in plans. The following adjustments were made to the final examination for the organic chemistry laboratory: technique tests required for A and B grades were already completed, but the remainder of the planned assessments were cancelled. A set of alternative assignments were introduced. All students chose from one of the three following options: 1) complete the mastery project they had already started, 2) write advice to students

attempting to study during times of great trauma, or 3) create a multimedia presentation of their choice connecting chemistry to something they were experiencing. All three assessment options were graded on a complete/incomplete basis with credit awarded for any good faith effort. Students appreciated the accommodations, and several welcomed them as a safe space to express their struggles in dealing with traumatic experiences.

5.5 Student and TA Feedback

Surveys were administered to determine how students and TAs perceived the remote course structure. The GCL-I team administered two surveys: a mid-quarter and a post-quarter. Of the 1,403 students and 28 TAs in the GCL-I course, 79% of students and 64% of TAs responded to the mid-quarter survey, respectively (Table 5.4). A total of 67% of students and 82% of TAs completed the post-quarter survey. The OCL-II team administered one student survey late in the quarter. Of the 104 students in the OCL-II course, 84% of students responded. Because this survey was completed later in the quarter than the corresponding survey in the GCL-I course, a final student survey was not conducted. Informal feedback was collected from TAs weekly, and a TA survey was conducted at the conclusion of the term. Survey questions are included in the appendix.

Table 5.4. Feedback collection methods and response rates for remote delivery GCL-I and OCL-II.

Feedback Collection	GCL-I Response Rate	OCL-II Response Rate
Student mid-quarter	1,101 (79%)	87 (84%)
TA mid-quarter*	18 (64%)	4 (100%)
Student post-quarter	943 (67%)	NA
TA post-quarter	23 (82%)	4 (100%)

*Feedback from OCL-II TAs was solicited through conversations in weekly staff meetings.
Students: N = 1,403 for GCL-1, N = 104 for OCL-II, TAs: N = 28 for GCL-I, N = 4 for OCL-II.

5.5.1 GCL-I Student Feedback

Student responses to the mid-quarter and post-quarter surveys were mostly positive. Students valued Canvas, citing its modular set-up and summary of assignment due dates. They also appreciated the video demonstrations of the experiments and taking associated quizzes. Perceptions of the ELN and Piazza in the mid-quarter survey were mixed. Actions were taken to address these concerns and post-quarter survey responses indicated the changes made were well received.

While students liked the scaffolding of the ELN, they wanted more direction for its use. We subsequently recorded an instructional video describing the use of the ELN functionalities, especially how to properly download and submit the page for grading to aid students navigating the ELN for the first time.[69,70] Students also expressed frustration and anxiety about the time consuming nature of filling out the ELN, a sentiment which is not unique to this remote course. Students are often surprised by the workload in their first laboratory course. The instructor and two development TAs filmed short videos addressing these and other student concerns from the mid-quarter survey which was intended to enhance student perceptions of instructor presence in the course.[18,40]

Students also identified the Piazza message board as a source of anxiety. The number of message board posts was ten times higher than the previous year (Table 5.5). Many students felt they needed to read all responses to ensure understanding of assignment expectations. Conversely, the redundant questions indicated many other students were not reviewing answered posts before making their own. This behavior was partially encouraged by a faster average response time compared to the prior year.

Table 5.5. GCL-I Piazza statistics for 2019 and 2020.

Comparison of In-Person & Remote Instruction	Spring 2019	Spring 2020
Questions asked	903	7,131
Posts, responses, edits, follow ups, comments	2,615	29,806
Average response time	33 minutes	6 minutes
Percentage of students with at least one contribution	32%	62%

To reduce the number of posts and student anxiety, many question-by-question responses were curtailed. The most commonly asked questions each week were compiled and answered in a single announcement. This reduced the overall number of posts and provided the TAs with a set of talking points to address during office hours. Immediately following the first announcement, the number of posts was almost cut in half, but the number of users (viewers) remained very high (Figure 5.3).[71,72] In the final course survey, students indicated the changes to Piazza reduced anxiety by making answers easy to find.

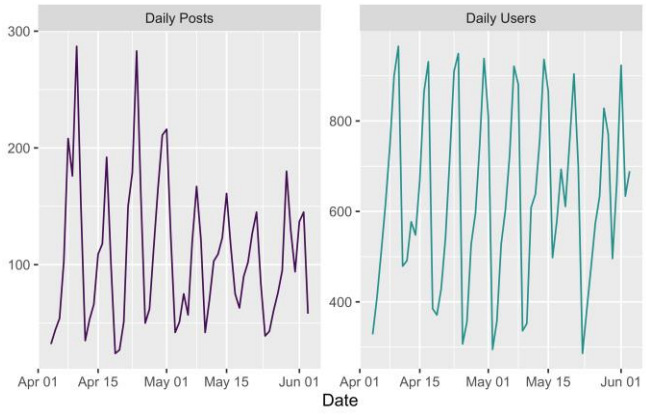

Figure 5.3. GCL-I remote instruction Piazza messageboard users and posts.

5.5.1 GCL-I TA Feedback

GCL-I TAs overwhelmingly agreed that the most positive moments they experienced with students were running office hours through Zoom. TAs noted having a partner to split work between vocal and written (chat) response was an optimal arrangement. However, TAs did indicate occasional difficulty fielding a large volume of student questions through the chat function. TAs appreciated the ability to screen share to guide students through online tools and subject matter questions. They also noted the regular attendance of a sizable number of motivated students, in contrast to much lower attendance of typically 5-10 students for in-person office hours in previous quarters. Weekly attendance at each office hour started at over 100 students on average and then dropped to about 25 students toward the end of the quarter (Figure 5.4). While TAs commented positively about the use of Zoom, they also voiced concerns about the lack of connection to their students because of the absence of face-to-face contact.

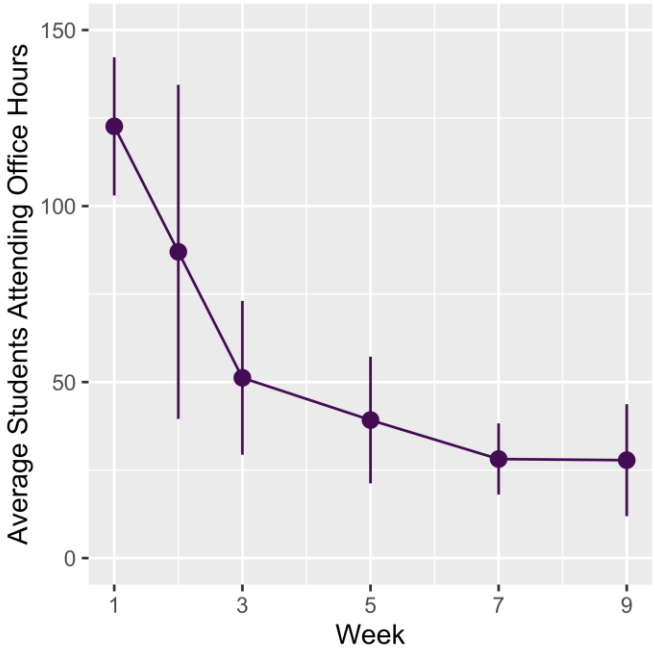

Figure 5.4. Average GCL-I office hour attendance by week. Error bars are ± standard deviation.

The changes to in-person staff meetings that were adopted for the remote setting were described by TAs as insufficient preparation for teaching during office hours. Most of the student questions pertained to assignment rubrics and grading rather than the theory underlying the laboratory techniques and instrumentation. TAs expressed that going over the rubrics during the staff meeting would be better preparation for their office hours. This change was made following the mid-quarter survey and was received positively based on TA responses to the post-quarter survey. TAs voiced that the change lessened the time spent on grading overall so it was easier to meet the weekly grading deadlines set by the instructor. TAs also unanimously praised the integration of the BigBrother code with Sapling and Slack, commenting that the code lightened their workload because it simplified checking the Sapling prelaboratory requirements, which was done manually in previous quarters.

5.5.2. OCL-II Student Feedback

Students in OCL-II were surveyed once in the latter part of the term before final exams. No immediate course changes were made because the survey was administered after the final experiment week concluded. We planned a post-course survey to gather student feedback on the exam components, but this survey was abandoned when exams were cancelled. When asked what they liked best about the online lab sections, many students remarked they could more easily ask questions in this format. Students attributed this difference to greater ease of getting the TA's attention and a lack of time pressure to complete laboratory work. Many students valued watching the videos together with their TA and classmates. However, they suggested that the TAs should have more structure in guiding the class discussion around what was happening in the experiment videos. Students appreciated the overall structure and organization of the course, especially the consistent weekly deadlines. Most comments on improvement for the course organization

addressed issues of Canvas structure that cannot be altered. Students felt that the lab lecture component of the course was helpful, and those who had experienced the in-person laboratory lecture in previous courses thought the online version was similar. Based on student comments, we succeeded in establishing a sense of connection between the students and the instructors in this new course format, but many students felt disconnected from their classmates in the online environment.

5.5.3. OCL-II TA Feedback

At the conclusion of the course, the OCL-II TAs completed a survey comparing the remote teaching experience to their prior in person experience. TAs felt that the remote lab required a smaller time commitment due to the lack of in-person, four-hour lab periods. An average of 13 students attended weekly "in-lab" meetings where TAs led viewing and discussion of the video with students, and office hours attendance was less than for in-person courses they had taught previously. Typically, students needed more guidance when interpreting data and performing error analysis as compared to TAs' previous experiences.

OCL-II TAs also commented on some of the benefits and challenges associated with remote learning. TAs cited an increased focus during the remote "in-lab" meetings on theory and concepts associated with the experiment in comparison with prior in-person teaching experiences. This change may have resulted from a decreased cognitive load required to watch experiment videos instead carrying out experimental procedures.[73,74] The increased flexibility of the online format also allowed students to contact their TAs more easily compared to attending in-person office hours. In contrast, TAs felt the biggest challenges, aside from lack of hands-on experience, were associated with TA-student interactions. Although some students were more engaged, this

was not true for all students. TAs perceived an overall decrease in student participation and struggled to assess the gaps in students' knowledge.

5.6 Lessons Learned and Planned Changes for Future Iterations

Despite the limited time frame to enact our emergency pivot to a new remote delivery environment for the GCL-I and OCL-II courses, the students and instructional teams for both courses felt the endeavor was a success. The positive student response to our emergency remote laboratory courses will inform the creation of additional laboratory courses while the global pandemic necessitates continued remote learning. Future courses, currently in development, will retain the same overall structure, consistent due dates, and synchronous class meetings with asynchronous options for students experiencing scheduling challenges.

The instructional teams of the general and organic chemistry laboratories historically have worked together, adopting many of the same web-based tools that served us well during this pandemic. Our similar approaches allow us to address the challenges we encountered during the first quarter of remote instruction in ways that will improve future iterations of both laboratory series. Based on the GCL-I team's experience, staff meetings will be restructured to help TAs focus on student needs in the remote delivery of the course. Because the experimental videos are now complete, TAs will be required to watch the experiment video and fill out an electronic survey before each meeting. This survey will have two goals: (1) to actively engage the TAs in video experiments and, (2) to generate talking points for office hours with students. A group of TAs will also be assigned to lead a discussion of the survey responses and rubrics for the experiment running that week. All large-scale courses will manage message boards with daily instructor posts. Because course content has now been prepared, we plan to open modules earlier in each term to allow students greater flexibility in managing their weekly workload. Finally, we endeavor to create

more connections between students during Zoom lab sessions by strategically employing tools such as polling and emoticon use to encourage full participation, using the new Live Chat function in Piazza to structure discussion, and instituting group work where applicable in break out rooms.[75–78]

Regardless of the successes we have had in creating online laboratory courses, we still strongly assert that this emergency replacement does not meet the primary objective of any laboratory course — performing fundamental laboratory techniques. To enhance all aspects of learning chemistry, hands-on interaction with chemicals and laboratory instruments are essential.[3] While we were able to challenge our students with assignments that required conceptual understanding and critical analysis, we could not assess their ability to manipulate laboratory glassware or use laboratory instrumentation.[5] We look forward to the return of in-person laboratory courses.

5.7 Appendix

5.7.1 IRB Statement

This study was approved by the University of California, Irvine, Institutional Review Board as exempt (IRB 2018-4661) including FERPA compliance.

FERPA compliance.

5.7.2 GCL-I Mid-Quarter Survey Questions for Students

1. What times zone are you in? *short answer*
2. Who is your TA? *drop down*
3. Are lab reports returned in a timely manner and graded fairly? Do they contain useful feedback? Provide specific examples. *paragraph*
4. How are office hours? What have TAs done well during this time? What could be done better? Please share any suggestions you have. *paragraph*
5. Does your TA respond in a timely manner to email? Is the response helpful? *paragraph*
6. My TA's expectations are clear and the enforcement of those expectations is consistent. *5-point Likert scale: strongly agree to strongly disagree*
7. My TA is well organized and provides clear explanations. *5-point Likert scale: strongly agree to strongly disagree*
8. Please use this space to provide any other comments about your TA that you would like to include. *paragraph*
9. What has been the most positive moment for you in this course so far and why? *paragraph*
10. What has been the most challenging moment for you in this course so far and why? *paragraph*
11. What is your primary source of information in this course? How can it be improved? *paragraph*
12. What technology challenges have you had? If there is something you think we could do to help, please describe your issue. *paragraph*
13. What do you like about the LabArchives ELN? What could be better? Is the prompting on the Pre/In lab pages helpful? *paragraph*
14. What do you like about the course content in Canvas? What could be better? *paragraph*
15. Please use this space to provide any other comments about the course that you would like to include. *paragraph*

5.7.3 GCL-I Mid-Quarter Survey Questions for TAs

1. What time zone are you in? *short answer*
2. What year of your program are you in? *short answer*
3. Have you taught a general chemistry lab before? *multiple-choice: yes or no*

4. Select the classes you have taught before. *multiple answer*
5. Describe what worked well on Zoom. *paragraph*
6. What has been the most positive moment for you in this course thus far? *paragraph*
7. What has been the most challenging moment for you in this course thus far and why? *paragraph*
8. What would you change about this course and why? *paragraph*
9. How would you change this course? *paragraph*
10. Please use this space to provide any other comments about the course that you would like to include. *paragraph*

5.7.4 OCL-II Mid-Quarter Survey Questions for Students

1. Are you attending the online equivalent of lab section time with your TA on Zoom? *Likert: never-always*
2. What is helpful about attending the online equivalent of lab time? *paragraph*
3. What suggestions do you have for improving the online equivalent of lab time? *paragraph*
4. Are lab reports returned in a timely manner? *Likert: never-always*
5. Do graded lab reports contain useful feedback? *Likert: never-always*
6. Please provide specific examples about timeliness of graded work and/or feedback on graded work. *paragraph*
7. Do you attend your TA's office hours? *Likert: never-always*
8. Describe something that is working well in your TA's online office hours. *paragraph*
9. Describe any suggestions you have to improve office hours. *paragraph*
10. Does your TA respond to email in a timely manner? *Likert: never-always*
11. Are email responses from your TA helpful? *Likert: never-always*
12. Please use this space to provide any other comments about how your TA is supporting your learning. Remember to be specific and professional with your feedback. *paragraph*
13. How do you normally attend lab lecture? Choose the option you use most often. *Multiple choice*
14. What aspects of lab lecture do you feel have worked well for you? *paragraph*
15. What aspects of lab lecture would you change? How would you change them? Please be specific. *paragraph*
16. What has been the most positive moment for you in this course so far and why? *paragraph*
17. What has been the most challenging moment for you in this course so far and why? *paragraph*
18. What is your primary source of information in the course? How can it be improved? *paragraph*
19. What technology challenges have you had? If there is something you think we could do to help, please describe your issue. (Note: Results from this survey are anonymized. If you need help with a specific issue, please reach out to Dr. Link so we can help you find a solution!) *paragraph*
20. What do you like about the LabArchives ELN? *paragraph*
21. What about the ELN could be better? *paragraph*
22. Was the template for the Pre/in lab pages in the early experiments useful? *Likert: not at all useful - extremely useful*

23. What is helpful about the class organization in Canvas? *paragraph*
24. What aspects of the class organization on Canvas could be improved? Please provide specific suggestions. *paragraph*
25. How often do you use the captions provided with the in-lab videos? *Likert: never-always*
26. Choose the most recent Chem 51L class you took before this one. *Multiple choice*
27. If you have taken a previous Chem 51L class at UCI, how does the workload in the remote learning format compare to your previous class experience? *Likert: much lower - much higher*
28. Please tell us about any new challenges/responsibilities you have taken on during the pandemic (if you are comfortable sharing). This might include changes to job situation, new or changed responsibilities in caring for children or other family members, food or housing insecurity, or any other major change that impacts your ability to complete your class work. (Reminder: These survey responses are NOT connected with your name. We are using this question to get a sense of what challenges our students are dealing with.) *paragraph*
29. Please use this space to provide any additional feedback. *Paragraph*

5.7.5 GCL-I Post-Quarter Survey Questions for Students

1. Who is your TA? *drop down*
2. What, if anything, did your TA do differently in the way they conducted their office hour after the mid-quarter survey? *paragraph*
3. If your TA changed what they were doing, did you like the change? Why or why not? *paragraph*
4. Describe TA-led activities that worked well during office hours. *paragraph*
5. Did grading change after the mid-quarter survey? If so, how? *paragraph*
6. What could be done differently to make grading expectations clearer? *paragraph*
7. Please use this space to provide any other comments about TA grading or office hours that you would like to include. *paragraph*
8. What aspects/topics/techniques of general chemistry laboratory do you feel the MOST confident about after taking this course? *paragraph*
9. What aspects/topics/techniques of general chemistry laboratory do you feel the LEAST confident about after taking this course? *paragraph*
10. What have you found beneficial about taking this lab remotely? *paragraph*
11. What has been challenging about taking this lab remotely? Propose ways you think might help change the experience for future students. *paragraph*
12. How did the changes made to Piazza after the mid-quarter survey affect your use of the message board? *paragraph*
13. What was the easiest electronic tool to use (Canvas, ELN, Spartan, Sapling, Zoom, or Piazza)? *paragraph*
14. What was the hardest electronic tool to use (Canvas, ELN, Spartan, Sapling, Zoom, or Piazza)? *paragraph*
15. How many times this quarter have you encountered technological problems which adversely affected your work in the course? *multiple choice: 0 - 10*
16. Did you use the captioning of the experiment videos? If so, why? *paragraph*

17. Please use this space to provide any other comments about the course that you would like to include. *paragraph*

5.7.6 GCL-I Post-Quarter Survey Questions for TAs

1. What year are you in the Ph.D. program? *drop down*
2. Which general chemistry laboratory course(s) have you taught previously? Please select all that apply. *multiple answer*
3. Please estimate the average number of students that would come to your in-person office hours when you taught a general chemistry laboratory class previously. *drop down*
4. Indicate the extent to which you agree with the following. *5-point Likert scale: strongly agree to strongly disagree*
    a. I feel that the structure of the experiment videos with associated video quizzes supported student learning of the laboratory content.
    b. I feel that the due dates set for student assignments were appropriate.
    c. I feel that the due dates by which assignments had to be graded helped me stay up to date with grading.
    d. I feel that the due dates set for when assignment grading had to be completed were fair.
    e. I feel that the instructor digest posts on Piazza were helpful.
    f. I feel that the instructor digest posts on Piazza were an improvement over the way Piazza posts were previously answered.
    g. I feel that the structural changes made to the TA meeting since mid-quarter were beneficial.
    h. I feel that checking Sapling pre-lab completion was easier using BigBrother integrated with Slack than it would have been without it.
5. Did you like the changes made to the TA meeting format in the second half of the quarter? Why or why not? *paragraph*
6. What would you change about the TA meeting format in the future, if anything, and why? *paragraph*
7. How did you feel about BigBrother? What would you like to see changed about how sapling pre-lab completion is assessed in the future and why? *paragraph*
8. What is better about teaching remotely compared to your previous in-person experience and why? *paragraph*
9. What is more challenging about teaching remotely compared to your previous in-person experience and why? *paragraph*
10. What recommendations do you have for improving the future? *Paragraph*

5.7.7 OCL-II Post-Quarter Survey Questions for TAs

1. Compared to previous quarters, was this class more/less work than holding this TA position in person? *paragraph*
2. On average, how many students show up to your “in lab – video watch session” each week? *paragraph*

3. On average, how many students show up to your office hours each week? *paragraph*
4. Did your students need more/less guidance in interpreting data and understanding techniques? *paragraph*
5. What do you feel are the benefits of a remote lab experience? *paragraph*
6. What do you feel are the downfalls of a remote lab experience? *paragraph*
7. Did you borrow technology from the department? If so, what did you need? *paragraph*
8. In your opinion, what was the biggest challenge teaching remotely? *paragraph*
9. Use this space for anything else you want to include. *paragraph*

5.7.8 GCL-I Guide to Filming

Equipment:

The general chemistry labs used simple consumer-grade video cameras to record videos. Videos were recorded and acted out by TAs assigned to the course. The combined cost of the equipment was less than $1,000:

- Sony alpha 6100 mirrorless camera
- 16-50 mm lens included with camera
- Several 64gb SanDisk Extreme memory cards
- Manfrotto tiltable tripod

The camera used in our experiments supports 4k video recording, however recording at such high definition has the downside of consuming more storage space and being difficult to edit on most computers. As most students do not have 4k displays and most educators do not have the computer processing power to edit 4k, footage was shot on 1080p in a properly angled shot. The dedicated zoom feature was useful when getting shots where the tripod could not get close enough. Note that the digital zoom used on phone cameras, action cameras, etc. is not a substitute for a dedicated zoom lens. When choosing a videography equipment, it was important that the video cameras accept memory cards as opposed to cell phones/tablets that do not have the internal storage to film a complete experiment.

Production:

As the videos were a large part of the student's education, it was important to tell a complete story that brings together the most important aspects of the experiment. Without a well thought out plan, the videos may seem disjointed, incomplete, or completely unusable requiring a re-shoot. Before shooting experiments, a director's script was written which ensured that the story was well thought out and there were no missing segments. The director's script included all necessary reagents, correct experimental values, and side notes for when to introduce new chemicals/equipment.

In most experiments, the TA was facing the camera and the equipment was in front of the TA. If a fume hood was required, the video was recorded at an angle so the viewer could clearly observe the regent handling. The TA was asked to use the furthest arm from the camera to perform the bulk of the work so that they are not blocking the shot. The TA stood in an "open" position where the front of their body faced the camera as much as possible. This gave a warmer feeling to the video as opposed to a TA that had their back to the camera.

When filming, the camera needed to be close enough to see the details, ensuring that everything was in the shot with no unused space. If there was unused space, the camera was brought closer and the equipment rearranged to make it fit within the shot. Once the equipment was set up, strips of tape were placed around the workspace so that the TA knew the edges of the shot.

The videos were almost exclusively shot using a tripod. It was beneficial to have a tripod tall enough to rest on the ground and film into the fume hood. We encourage using a tripod as it is easily reproducible, will reduce shakiness, and will make post production a cleaner process. The automatic video settings were used on the camera and resulted in fairly consistent video

quality. Consistency in both style and quality were important for maintaining the student's expectations.

Post-Production:

We used Apple iMovie® to edit the videos due to its ease of use and native integration in Apple laptops. Videos were broken down into the following sections: Title, Introduction, Safety, Chemicals & Equipment, Part A, B, C, etc, Waste and Equipment Cleanup, and Final Notes for a report. While editing, title slides were used to clearly separate the important sections of a video. Subtitles were added at the bottom of the screen where applicable.

The first video in the series began by panning around the laboratory to introduce the students to the university's chemistry labs. After showing the whole lab, the safety features were pointed out to familiarize the students with the locations of the fire extinguishers, eye wash stations, and safety showers. Each experimental video started by going over safety, and ended by showing the TAs cleaning glassware and returning it to storage. Along with the main videos, a supplemental 'Safety Moment' video was recorded. Less than two minutes long, the safety moment detailed small aspects of chemical safety and hygiene that the students would normally pickup from attending the lab in person such as the contents of a spill kit, how to dispose of gloves, solvent safety, etc.

A photograph of a white board detailing the main concepts, formulas, and equations was added at the beginning of each video along with a voice-over narration for the experiment's introduction. Then, highlighted arrows were added post-production to emphasize talking points. The voice-over narration followed a pre-written script that highlighted the important aspects of the video. As this was the first general chemistry laboratory for some students, the narration was key in describing the techniques used for the experiments. When repeating similar processes

such as weighing samples, pipetting liquids, or titrating solutions a fast forward effect was used to speed up the shot and reduce overall video time. Each video was targeted to be less than 20 minutes to maintain student attention. Videos longer than 30 minutes were broken into multiple shorter videos. *Crossblur* transitions were used in between different conceptual shots. If there wasn't a conceptual change, no transition was used. The completed videos were sent to the head TAs and instructor for final edits and then exported in 1080p high quality compression.

5.7.9 OCL-II Guide to Filming

Equipment:

If possible, employ a two-camera system for filming, one on a stable wide shot, and one that is your mobile close-up camera. This can be achieved with only one camera operator, as the wide only needs to have the record button pressed at the beginning of filming. Having two cameras, one recording the whole experiment and one recording a close angle ensures that no part of the process will be missed. Using two cameras also meant we never had to pause the experiment to adjust the camera zoom or angle/ the experiment could be carried out in real time as it would in the classroom. Our main camera was a cinema-grade camera with xlr inputs so that audio could be run through it/monitored through headphones, and our close-up was a dslr for portability.

- Canon C200 (used for wide) w/24mm lens
- Nikon D500 (used for close-up) w/24-70mm lens
- Lectrosonic wireless Lavalier (with XLR inputs run through C200)
- 2 Manfrotto tripods
- 2 sd cards (each 128g due to the file size of 4k footage)

Our videos were filmed in 4k. While this does require a large amount of storage space, having the ability to punch in to certain aspects of the experiment (such as TLC plates drying)

without breaching the fume hood was important. Using 4k footage downscaled to 1080p meant that we could zoom in much more than our standard zoom lens allowed, and gave the greatest overall picture quality.

Production:

We found it beneficial to film each video with two TAs, one to perform the experiment, and one to narrate what was happening. A script was written prior to filming, and the TA reading from the script would be positioned at a distance where they could still see the experiment, but far enough away to eliminate as much noise from the fume hood as possible. If the fume hood proved to be too noisy at any point, portions of the script would be read after the experiment and synced up in post-production to the point in the video where the original lines were read. Having someone read the script allowed the person performing the experiment to concentrate on the experiment to execute it properly.

The wide and close up camera would start recording at the same time, and the camera operator would operate the close up camera, adjusting for angles, making sure to capture labels and markings on beakers and vials, etc. We used a higher angle for the wide shot, and a low angle for the close up camera, as it needed to be able to film underneath the fume hood.

Cameras were cut during processes that would take long amounts of time that weren't necessary to be shown (ex., a solution stirring for 15 min+, condensation processes, etc.) This saved us storage space on the SD cards and helped eliminate portions we would have had to cut down in post-production. The TAs would keep the camera operator well-informed about stages of the process, key images to be captured, etc. Insert shots would sometimes be filmed during down time to later be added to the final video.

Post-Production:

All videos were edited in Premiere Pro. We would cut between the wide shot and the close-up so students could routinely see the entire experiment and extremely detailed shots of steps being carried out- much clearer than they would with the naked eye if they were observing in the classroom. Video would be color corrected, audio would be mastered to eliminate as much background noise as possible, and a light, instrumental music track was placed on the video to keep some audio running during otherwise silent parts of the experiment. We chose meditative music to be calming and non-disruptive, while keeping the video from becoming boring if there were long stretches without narration.

The most common editing techniques we employed, though, were to either fast-forward during certain processes or cross-dissolve between two shots. For example, we would fast-forward when TLC plates were in solution, so students would see the plate absorb the solution in a matter of seconds. If a process would take a lot of time, such as stirring a solution or waiting for a solution to boil, we would film short segments every 10-15 minutes, and dissolve between them so that students could see the change in the experiment but not take up large amounts of time. This means an experiment that would take up to 4 hours in the lab could be fully seen and demonstrated in a 20-minute video. We would overlay text on the video when time would pass to let the students be aware of the time taken in the experiment.

Depending on the length of the experiment, we also chose to chunk up the full video into several parts (each about 10 minutes each). If the experiment had two main components, we would create a video of each component to make viewing easier for the students. The end of the experiment would include a sign off from the TA and a reminder to clean up workspaces, so students would know they were at the last video in the experiment.

5.7.10 GCL-I and OCL-II Guide to Post-Lab Data Distribution

1. Create a practice quiz that does not count towards the students' grades in Canvas.
2. Upload PDFs of the requisite data to the Files section of Canvas. Once uploaded, change the access settings so that only students *with the link* are able to access the file.
3. Create a question in the practice quiz asking students to choose their lab section.
    a. GCL-I: The files were named by experiment title and course ID number. Ex. Electrochemical Cells 40202.
    b. OCL-II: The files were named by course ID number, scheduled time, and TA name. Ex. 40700 Tuesday 8AM, Jane Smith.
4. Enter answer choices.
5. Click the three horizontal dots below an answer choice. The alt-text is: Click to enter comments for the student if they choose this answer. This opens a rich text editor.
6. Click into the rich text editor and then scroll up until you see the Links toolbar on the right side of the page. Click to the Files tab and find the data PDFs you previously uploaded. Click on the relevant PDF you wish to attach.
7. Your comment should now contain a blue hyperlink to download the relevant PDF.
8. Update the question.
9. Save the quiz.
10. Preview the quiz.

5.7.11 GCL-I Essay Response Statistics

Table 5.6 GCL-I essay response statistics.

Essay Prompts	Number of Students	Average out of 50 pts (±Std Dev)
Group I		
Acid Dilution: Describe the procedural steps for dilution of 3.20 M HCl(aq) to make 100 mL of 0.800 M HCl(aq).	1152	36.36 (±5.11)
Filtration: Describe the procedural steps for collecting solid $PbSO_4$ precipitate from a liquid mixture contained in an Erlenmeyer flask and how an accurate mass of the precipitate is obtained afterward.	151	36.33 (±5.39)
Visible Spectroscopy: Describe the procedural steps for obtaining an absorbance spectrum of a crystal violet solution.	71	40.49 (±6.14)
Group II		
Electrochemistry: Describe the procedural steps for setting up a copper / zinc electrochemical cell based on the standard reduction potentials.	537	43.62 (±4.71)
Solution Formation: Describe the procedural steps for the formation of 10 mL of 0.200 M KSCN(aq) solution from solid KSCN.	448	38.83 (±5.39)
Titration: Describe the procedural steps for the titration of a 5 mL borax solution, sampled at 55℃, with a 0.52 M HCl solution. (Pictures of initial and final volumes in burette given.)	384	36.89 (±6.89)

5.7.12 Link to Public Google Folder

The following link (https://bit.ly/2YOnw8J) will take you to a public Google folder containing the following:

1. A readme directory file.
2. Full class schedules for GCL-I and OCL-II.
3. Copies of all experiment handouts for GCL-I and OCL-II.
4. Links to all experiment videos from GCL-1 and OCL-II.
5. Sample video quizzes used in GCL-I and OCL-II.
6. Sample lab final Canvas quiz from GCL-I
7. Sample “take-home” essay prompt from GCL-I.
8. Sample concept and data analysis exam from OCL-II.
9. Sample safety exam from OCL-II.
10. Sample technique exam from OCL-II.
11. Mastery project instructions from OCL-II.
12. Sample poster and journal article mastery projects from OCL-II.

13. Code for the BigBrother Python script as a .py file. (The most recent version can be located in the script author's GitHub Repository.

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