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Studying the Effects of Familial Alzheimer's Disease Mutations on the Assembly of a Constrained β -Hairpin Peptide Derived from A β And Developing and Implementing Specifications Grading Systems in an Organic Chemistry Laboratory Course and a Chemistry ...

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Effects of Familial Alzheimer's Disease Mutations on the Assembly of a Constrained β -Hairpin
Peptide Derived from A β
And
Specifications Grading Systems in an Organic Chemistry Laboratory Course and a Chemistry
Writing Course

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Chemistry

by

Kate Joy McKnelly

Dissertation Committee:
Professor James S. Nowick, Chair
Professor Renée D. Link
Professor Elizabeth R. Jarvo
Professor Jennifer A. Prescher

2020

DEDICATION

To Evan. Thank you for standing with me on this journey.

and

To my parents. Thank you for instilling in me a sense of curiosity and wonder.

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VITA

Kate Joy McKnelly

EDUCATION

- University of California, Irvine: Department of Chemistry** Irvine, CA
Doctor of Philosophy Candidate, *Chemistry*, emphasis in *Chemical Biology* Jun 2020
Thesis Defense date: June 11, 2020
- University of Leeds: School of Chemistry** Leeds, UK
Master of Science, *Chemical Biology and Drug Design* Sept. 2015
Thesis Advisor: Dr. Andrew Wilson
- University of California, Berkeley: College of Chemistry** Berkeley, CA
Bachelor of Science, *Chemical Biology* May 2013

TEACHING EXPERIENCE

University of California, Irvine

- Instructor of Record – Writing for Chemists (Chem 101W)* Sept. 2019–Dec. 2019
- Instructor during fall quarter 2019.
 - Upper division lecture course for chemistry majors. Fulfills upper division writing (Ia) General Education Requirement at UCI.
 - Performs all duties required of instructor of record at UCI including, but not limited to: designing course curricula, using Canvas, developing lectures using specifications grading, and providing support to students.
- Instructor of Record – Introduction to Chemical Biology (Chem 128)* Aug. 2019–Sept. 2019
- Instructor during second summer session 2019.
 - Upper division lecture course for chemistry majors. Introduction course to the chemical biology field.
 - Performed all duties required of instructor of record at UCI including, but not limited to: designing course curricula, using Canvas, developing lectures incorporating active learning and a flipped class approach, and providing support to students.
- Instructor of Record – Chemistry Around Us (Chem 12)* June 2019–Aug. 2019
- Instructor during first summer session 2019.
 - Online chemistry survey course designed for non-STEM majors. Fulfills science and technology (II) and quantitative literacy (Va) General Education Requirements at UCI.
 - Performed all duties required of instructor of record at UCI including, but not limited to: designing course curricula, using Canvas, developing online discussions, and providing support to students.
- Teaching Assistant (TA) – Senior Thesis in Chemistry (Chem 180W)* Apr. 2019–Jun. 2019
- Upper division lecture TA with Dr. Mang during spring quarter 2019.
 - 21 students in lecture, with weekly 2 hour writing labs.
 - Designed writing assignments, gave lectures, held office hours and graded assignments.

Teaching Assistant (TA) – 2nd quarter Organic Chemistry lecture (Chem 51B) Jan. 2019–Mar. 2019

- Lecture TA with Dr. King during winter 2019 quarter.
- 380 students in lecture, 40–50 students per discussion section.
- Taught three hour-long discussion sections per week and held office hours every week.
- Organized and directed extra office hours and review sessions before midterms and finals.

Teaching Assistant – Writing for Chemists (Chem 101W) Sept. 2018–Dec. 2018

- Upper division lecture TA with Dr. Mang during fall quarter 2018.
- 24 students in lecture, with weekly 2 hour writing labs.
- Designed writing assignments, gave lectures, and graded assignments.

Instructor of Record – 1st quarter Organic Chemistry Lab (Chem 51LB) June 2018–Aug. 2018

- Instructor during first summer session 2018.
- 70 students in lecture.
- Performed all duties required of instructor of record at UCI including, but not limited to: designing course curricula, using Canvas, developing lectures incorporating active learning, and providing support to students.

Head Teaching Assistant – Organic Chemistry Lab Series (Chem 51LB/C/D) Sept. 2017–June 2018

- Yearlong (fall 2017, winter 2018, and spring 2018) head TA position with Dr. Link.
- Taught in-person and online lectures, 200-400 students per lecture.
- Trained first-year TAs, organized TA meetings, managed TAs for all Chem 51L series classes, scheduled TAs for beta-testing experiments, designed and beta-tested potential new experiments for future Chem 51L classes, and managed grade books.

Lecture Teaching Assistant – 2nd quarter Organic Chemistry Lecture (Chem 51C) Apr. 2017–June 2017

- Lecture TA with Dr. Wang during spring quarter 2017.
- 350 students in lecture, 40–50 students per discussion section.
- Taught five hour-long discussion sections per week and held office hours every week.
- Organized and directed review sessions before midterms and finals.

Head Teaching Assistant – Chemical Biology Lab (Chem 128L) Jan. 2017–Mar. 2017

- Head TA for upper division class with Dr. Prescher in winter quarter 2017.
- Beta-tested each experiment before students performed them in class, taught all TAs how to perform experiments before they taught their lab classes, maintained beta-test records for future head TAs, and prepared materials and reagents every week for lab classes.

Laboratory Teaching Assistant – 1st quarter General Chemistry Lab (Chem 1LC) Apr. 2016–June 2016

- Laboratory TA for with Dr. Edwards in Spring quarter 2016.
- 30-40 students per laboratory section.
- Taught two laboratory sections, each four hours long, and held office hours every week.

Lecture Teaching Assistant – 2nd quarter General Chemistry Jan. 2016–Mar. 2016
Lecture (Chem 1B)

- Lecture TA with Dr. Arasasingham in Winter quarter 2016.
- TA for two lecture courses, 350 students per lecture, 40–50 students per discussion section.
- Taught five hour-long discussion sections per week and held office hours every week.
- Organized and directed review sessions before midterms and finals.

Laboratory Teaching Assistant – 3rd quarter Organic Sept. 2015–Dec. 2015
Chemistry Lab (Chem 51LD)

- Laboratory TA with Dr. Link in Fall 2015.
- 30-40 students per laboratory section.
- Taught two laboratory sections, each four hours long, and held office hours every week.

RESEARCH EXPERIENCE

University of California, Irvine

Department of Chemistry

Graduate Student Researcher

Irvine, CA

Sept. 2015–Present

- Currently working with Dr. James Nowick and Dr. René Link.
- Designed, executed, and interpreted experiments and experimental results.
- Currently investigating the effects of familial mutations of Alzheimer's Disease on the supramolecular assembly of a macrocyclic β -sheet mimic derived from the amyloid- β protein with Dr. Nowick.
- Performing teaching as research projects exploring the effects of specifications grading on student class performance, sense of control over grade, and class-related anxiety with Dr. Link.

University of Leeds

Department of Chemistry

Graduate Student Researcher

Leeds, UK

Sept. 2014– Sept. 2015

- Worked with Dr. Andrew Wilson at the University of Leeds.
- Designed, executed, and interpreted experiments and experimental results.
- Masters research culminated in Masters Thesis and presentation of results concerning stapled α -helical peptides as inhibitors of protein–protein interactions.

University of California, San Francisco

Pathology Department

Laboratory Assistant

San Francisco, CA

May 2013–June 2014

- Worked with Dr. Stephen Nishimura, M. D., Professor in Residence.
- Researched mechanisms of lung fibrosis and methods to counter it to help find methods of slowing and/or halting the progression of diseases such as chronic obstructive pulmonary disease (COPD).

European Molecular Biology Laboratory

Mouse Biology Unit

Intern

Monterotondo, Italy

Jan. 2012–May 2012

- Worked with Dr. Paul Heppenstall, Team Leader European Molecular Biology Laboratory (EMBL), Mouse Biology Unit.
- Researched *Drosophila* embryonic cell calcium channels, and mouse potassium channels.

PUBLICATIONS

Manuscripts in Preparation:

McKnelly, K. J.; Howitz, W.; Yoo, S.; Kreutzer, A. G; Haduong, K.; Ashby, S.; Laayouni, M.; Hart, C.; Nowick, J. S. Effects of Familial Alzheimer's Disease Mutations on the Assembly of a Constrained β -Hairpin Peptide Derived from A β . *Manuscript in progress*, planned submission to *J. Am. Chem. Soc.*

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Published Papers:

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McKnelly, K. J.; Sokol, W.; Nowick, J. S. Anaphylaxis Induced by Peptide Coupling Agent: Lessons Learned from Repeated Exposure to HATU, HBTU, & HCTU. *J. Org. Chem.* **2019**, ASAP Article.

Kreutzer, A. G; Spencer, R. K.; **McKnelly, K. J.**; Yoo, S.; Hamza, I. L.; Salvesson, P. J.; Nowick, J. S. A Hexamer of a Peptide Derived from A β_{16-36} . *Biochemistry.* **2017**, *56*, 6061-6071.

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PROFESSIONAL DEVELOPMENT

Safety Fellow
Department of Chemistry
University of California, Irvine

Sept. 2019-Present

- Fellowship appointment for the 2019–2020 academic year.
- Duties include: leading the Department of Chemistry Graduate Safety Team (GST); designing and organizing safety seminars, workshops, and activities; organizing trips to local companies to observe industry safety standards; facilitating safety workshops in EH&S TANGO training and Chem 100S. Chem 100S is a one-day safety training course for all undergraduate students who enroll in upper division organic and inorganic chemistry lab courses.

Pedagogical Fellow

Jan. 2017–Present

Division of Teaching Excellence & Innovation (DTEI)

University of California, Irvine

- Trained in advanced pedagogy and professional development.
- Designed and implemented 2018 and 2019 TA Professional Development Program (TAPDP) for the Chemistry and Pharmaceutical Sciences Departments. TAPDP is a one and one half day course to train incoming TAs in: TA roles and responsibilities, educational technology, lesson planning, office hours, leading a class, active learning techniques, diversity and inclusion, teaching observation and feedback, and practice in giving a mini-lesson.

Certified CIRTL Associate

Dec. 2018

Center for the Integration of Research in Teaching and Learning (CIRTL)

University of California, Irvine

- Trained in implementing evidence-based teaching practices.

Teaching Apprenticeship Program in STEM (TAP-STEM) Trainee

Sept. 2018–Aug. 2018

(Now called STAP - Summer Teaching Apprenticeship Program)

GPS-BIOMED and DTEI

University of California, Irvine

- Attended lectures of 1st quarter Organic Chemistry Lab (Chem 51LB) to observe and receive mentoring and training in teaching from Dr. Renee Link.
- Guest lectured for twenty lectures of Chem 51LB during winter quarter 2018.
- Prepared teaching materials for and was the instructor of record for Chem 51LB in summer 2018.

Certificate in Teaching Excellence

Dec. 2018

Division of Teaching Excellence & Innovation (DTEI)

University of California, Irvine

- Trained in designing course lessons using evidence-based pedagogical principles, analyzing and assessing teaching practice, and facilitating learning.

MENTORSHIP

Chemistry Teaching Assistant Mentoring Program (CTAMP)

Aug. 2018–Present

University of California, Irvine

- Currently participating in the second year of the Teaching Assistant Mentor Program at UCI
- Participated in the first year of the Teaching Assistant Mentor Program at UCI.
- Helped train incoming TAs for their duties as laboratory TAs in the Chemistry Department.
- I had six first-year TA mentees the first year, and I currently have eight first-year TA mentees. I meet with them three times per quarter to help them establish teaching goals, review midterm and final TA evaluations from their students, and analyze and reassess their goals at the conclusion of every quarter.

Undergraduate Researchers Opportunities Program (UROP)

Sept. 2017–Present

University of California, Irvine

- Mentored undergraduates in Dr. Nowick's research lab.

- Instructed undergraduates in lab techniques, designed their projects, and supervised their research experiments.
- Undergraduates:
 - Mohamed Laayouni (Sept. 2017 – Jun. 2018): successfully attained funding award, the Undergraduate Researchers Opportunities Program Award in the 2017-2018 academic year and presented his research at the UCI Undergraduate Research Symposium in May 2018. graduated from UCI in June 2018 and is now working in industry.
 - Katelyn Haduong (July 2018 – present): currently working in lab and will graduate in June 2020.
 - Shareen Ashby (Sept. 2018 – present): currently working in lab and will graduate in June 2020.

PRESENTATIONS

24th Annual Green Chemistry & Engineering Conference Los Angeles, CA
Safety: A Pillar of Green Chemistry Symposium June 17, 2020

Invited Presenter for the Safety: A Pillar of Green Chemistry Symposium

- Abstract Title: Anaphylaxis Induced by Peptide Coupling Agent: Lessons Learned from Repeated Exposure to HATU, HBTU, & HCTU (control ID#: 3399379)
- Authors: **McKnelly, K. J.**; Sokol, W.; Nowick, J. S.
- Will present virtually, due to COVID-19 social distancing measures.

2020 Workshop on Laboratory Safety Los Angeles, CA
Advancing Safety in Teaching & Research May 2021

Invited Panelist

- Hosted by the University of California, Los Angeles
- Invited as a panelist for the Panel discussion on Student Safety Engagement
- Originally to be held in May 2020, but postponed due to COVID-19.

American Chemical Society (ACS) Fall 2019 San Diego, CA
National Meeting & Exposition Aug. 2019

Oral Presentation

- Abstract Title: Anaphylaxis Induced by Peptide Coupling Agent: Lessons Learned from Repeated Exposure to HATU, HBTU, & HCTU (final paper #: CHED 87)
- Authors: **McKnelly, K. J.**; Sokol, W.; Nowick, J. S.
- Division of Chemical Education, Academic Lab Safety Session

2019 Southern California PKAL Regional Network Meeting Claremont, CA
Oral Presentation Mar. 2019

- Abstract Title: Extraction on paper, an active learning technique to facilitate student understanding of liquid-liquid extraction
- Authors: **McKnelly, K. J.**; Link, R. D.
- Active Learning in Organic Chemistry Session

25th Biennial Conference on Chemical Education South Bend, IN
Oral Presentation July 2018

- Abstract Title: Extraction on paper, an active learning technique to facilitate student understanding of liquid-liquid extraction
- Authors: **McKnelly, K. J.**; Link, R. D.
- Active Learning in Organic Chemistry Session

255th American Chemical Society (ACS) New Orleans, LA
National Meeting & Exposition Mar. 2018

Poster Presentation

- Abstract Title: Effects of familial mutations on the structure and assembly of a peptide derived from A beta 16-36
- Authors: **McKnelly, K. J.**; Yoo, S.; Kreutzer, A. G; Laayouni, M.; Hart, C.; Nowick, J. S.
- Division of Biological Chemistry Current Topics Session

A W A R D S & G R A N T S

Outstanding Contributions to the Department Award May 2020
for the Department of Chemistry
Awarded by the School of Physical Sciences at UCI in recognition of a graduate student's time in the department spent contributing to the department's undergraduate educational program and demonstrates promise as a future teacher

Safety Fellowship Award Sept. 2019– June 2020
Fellowship award provides full funding for the entire academic year, provided by the UCI Department of Chemistry and UCI Environmental Health and Safety

Most Promising Future Teacher in Organic Chemistry Award May 2019
Awarded by the Department of Chemistry at UCI in recognition of Exemplary contributions to the chemistry program for that academic year, and demonstrates promise as a future faculty member.

Travel Grant from Physical Sciences Student Affairs, UCI Apr. 2018
Awarded for travel to the 255th ACS National Meeting & Exposition

Associated Graduate Students Winter Travel Grant , UCI Jan. 2018
Awarded for travel to the 255th ACS National Meeting & Exposition

Pedagogical Fellowship Award, UCI Jan. 2018
\$2,000 fellowship award for preparing future faculty

P R O F E S S I O N A L M E M B E R S H I P S

Professional and Organizational Development (POD) Network Oct. 2018–Present

American Chemical Society Nov. 2011–Present

ABSTRACT OF THE DISSERTATION

Studying the Effects of Familial Alzheimer's Disease Mutations on the Assembly of a
Constrained β -Hairpin Peptide Derived from A β
And
Developing and Implementing Specifications Grading Systems in an Organic Chemistry
Laboratory Course and a Chemistry Writing Course

by

Kate Joy McKnelly

Doctor of Philosophy in Chemistry

University of California, Irvine

2020

Professor James S. Nowick, Chair

The dissertation is composed of two main parts. The first half describes efforts to study amyloid peptides using macrocyclic peptide model systems, and the potential deleterious effects of uronium peptide coupling agents on human health. The second half focuses on my experience in chemistry teaching and the chemical education research I conducted.

Chapter 1 describes the design and synthesis of seven peptides and acetylated variants containing familial Alzheimer's disease mutations (FADs). All peptides are based on a macrocyclic β -hairpin peptide derived from amyloid beta (A β), residues 16-36. The supramolecular structure of A β aggregates remains unknown due to the heterogeneous and unstable nature of A β oligomer formation. Elucidating the structure of A β aggregates will lead to insights into the function of A β in the progression of AD and FAD. Peptides derived from A β assemble in a crystalline state as dimers, trimers, hexamers, and even dodecamers, which

recapitulates oligomeric species observed of full-length A β . In particular, the A β ₁₆₋₃₆ region is important for A β oligomer formation. Lactate dehydrogenase release assays and dye-release assays with lipid-bilayers indicate that the mutant peptides exhibit more toxicity towards neuronally derived SH-SY5Y cells and cause more membrane destabilization than the parent peptide. Sodium dodecyl sulfate - polyacrylamide gel electrophoresis reveals that mutant peptides assemble as higher-order oligomers, and the E22 mutant peptides all assemble to form hexamers, similar to the parent peptide. Size exclusion chromatography and circular dichroism indicate that similarly charged mutant peptides exhibit similar solution-phase behavior. X-ray crystallography reveals that the E22 mutant peptides assemble to form hexamers in the crystal state. These studies will aid in our understanding of how mutations of full-length A β perturb the biophysical characteristics and assembly of A β oligomers, which may give insight into A β 's mode of causing toxicity in AD and FAD.

Chapter 2 presents a case study of chemical sensitization. Anaphylactic reactions can occur from repeated exposure to peptide coupling agents. Many researchers in the peptide and protein chemistry fields recognize the peptide coupling agent DCC as an infamous immune sensitizer. Fewer researchers know that the uronium coupling agents HATU and HBTU are also sensitizers. Reports of sensitization caused by these peptide coupling agents have been published in allergy and immunology journals, but these sources are not generally read by researchers who use uronium coupling agents. In this chapter, I present my case study of anaphylaxis induced by three uronium agents: HATU, HBTU, and HCTU, as a cautionary note for researchers who handle peptide coupling agents frequently. I also include recommendations for handling coupling agents more safely in the research laboratory.

Chapter 3 overviews my journey towards becoming an effective instructor. I chronicle my experience as a TA, Head TA, and Instructor of Record. I also discuss my time as a Pedagogical Fellow, TAP-STEM trainee, TA Mentor, and the Safety Fellow for the Department of Chemistry. Each experience taught me something different that helped prepare me to pursue and obtain a teaching focused faculty position at Emory University starting in summer 2020.

Chapter 4 describes the design and implementation of specifications grading systems in organic chemistry laboratory courses and a “Writing for Chemists” course. I worked with Dr. Renée Link to convert her entire three-course organic chemistry laboratory series from a traditional, points-based grading system to a specifications grading system, first as a pilot study during a summer term course and then scaled up for the large (1,000+ students) course in winter 2020. I worked with Dr. Stephen Mang concurrently to redesign the “Writing for Chemists” course he started in 2017 using a specifications grading system and adapting assignments from a textbook on the practice of nonfiction writing. I taught the course in fall 2019 and used the redesigned course materials. In both courses, we collected surveys of students perceptions of the grading system, and in the organic chemistry laboratory courses we also collected feedback from the course TAs. Responses from students about the nature of the grading system in the laboratory course were mixed. Their perceptions indicate that initial buy-in and multiple reminders about the bigger picture of the grading system will be essential to the success of this grading system on a larger scale. After the writing course, students self-reported increased propensities to pre-write and edit, and several mentioned that they appreciated the transparency of rubrics and the control the specifications system gave them over their grades.

Chapter 5 describes the development of an educational activity I designed and implemented with Dr. Renée Link — the *Extraction on Paper Activity*. Undergraduate students often find it

difficult to understand the chemical principles underlying liquid-liquid extractions. Explanations on how extractions work at the molecular level in textbook and internet resources are plentiful, but students still do not seem able to grasp how extractions work before having to perform the technique in a laboratory course. To address student discomfort with conducting extractions, I developed an *Extraction on Paper Activity*. I envision this activity as a tool to help students understand and apply the chemical principles underlying liquid-liquid extractions outside of and before entering into a laboratory setting.

Chapter 1

Effects of Familial Alzheimer's Disease Mutations on the Assembly of a Constrained β -Hairpin Peptide Derived from $A\beta$

1.1 Introduction

Familial Alzheimer's Disease (FAD) causes early onset of Alzheimer's Disease (AD) as young as age 50; it is currently unpreventable and untreatable as the disease progression is not well understood at the molecular level.¹ A single amino acid point mutation in the β -amyloid ($A\beta$) region of the amyloid precursor protein (APP) leads to an increase in overall $A\beta$ production, which causes early onset of AD.^{2,3} The $A\beta$ peptide contributes to neuronal toxicity through the formation of higher order oligomeric species in AD and FAD patients.⁴ The increase in disease pathology associated with FAD likely stems from FAD mutations causing changes in

A β morphology, oligomerization propensity, and aggregation ability.⁵ The heterogeneous nature of the assemblies formed by A β makes it difficult to determine their mode of supramolecular assembly through high resolution techniques such as nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography. Devising model systems that can be studied using high resolution techniques is necessary to better understand how native A β and mutated versions of A β oligomerize and cause AD and FAD. This chapter describes one such model system I used to study point mutations involved in causing FAD, and how those mutations affected the supramolecular assembly and biophysical properties of the peptide sequences used in the model system.

1.1.1 The A β Peptide — A Pore-Forming Peptide

The A β peptide comprises part of the transmembrane, cell-anchoring, domain of APP. Various secretases cleave APP and release it from cell surfaces, but the purpose of APP cleavage and its downstream effects are poorly understood. If great amounts of APP are cleaved and A β concentrations increase unchecked, the peptide can have deleterious effects on surrounding cells and consequently tissues, contributing significantly to disease progression in AD and FAD. Increases in A β concentrations contribute significantly to disease progression in AD and FAD.⁶

AD and FAD pathology is characterized by plaque deposition in the brain. In 1906 Alois Alzheimer suggested that plaques in the brains of dementia patients are likely the cause of — what we now call — Alzheimer's Disease.⁷ These plaques are now known to be composed of protein fibrils, formed by A β , and protein tangles, formed by the microtubule-associated protein tau.^{8,9} Initially, these fibrils and tangles were thought to cause disease progression in AD.¹⁰

Further investigation into the molecular basis of AD led to a new hypothesis — that AD progression is instead induced by soluble oligomeric species of A β .^{11–13}

A β oligomers are formed from the aggregation of single molecules of the A β protein, termed A β monomers. Oligomers of A β are composed of a heterogeneous mixture of oligomers of various sizes, and are thus difficult to characterize. One could imagine an oligomer as being assembled from two, three, six, twelve, or more A β monomers. Despite the metastable nature of A β oligomers and resulting difficulty in determining their mode of cellular toxicity,^{14,15} there is growing support and consensus that A β oligomers do indeed cause the cellular toxicity and subsequent tissue damage observed in AD.^{16–18}

One mechanism by which A β oligomers are thought to cause toxicity is through pore formation in cell membranes. In 1993, Arispe et al. reported that soluble A β causes membrane destabilization in planar lipid bilayers and induces cation flux across the membrane. Their work suggests that this membrane destabilization occurs through the formation of A β channels, further supporting that A β may cause cytotoxicity through pore formation.¹⁹ It has since been determined that A β oligomers can form ion channels (i.e. membrane pores), causing an influx of calcium ions (Ca²⁺) from the extracellular space to the intracellular cytoplasm, and ultimately cause cytotoxicity.^{20–23} In 2018, Julien et al. found that A β oligomers cause cell membrane damage — akin to that of the known pore-forming toxin CRY5B protein — in a *C. elegans* model by inducing an increase in endosomal endocytosis in the worm's intestinal membrane, which occurs as a part of the process of membrane repair. The authors found that A β oligomers localized to endosomal membranes, suggesting that A β localizes to cell membranes to form pores. This observation of A β oligomer-induced pore formation is the first in an *in vivo* model.²⁴

In addition to evidence of the membrane-destabilization properties of A β , pores formed by A β oligomers — called annular protofibrils (APFs) — have also been observed. APFs of differing sizes — with inner diameters ranging from 1.5-2 nm and outer diameters from 7-25 nm have been observed with atomic force microscopy (AFM) and transmission electron microscopy (TEM).^{25–29} Regardless of the differences in A β oligomer size, all share an annular morphology and all seem to be modular in that smaller oligomers appear to comprise the annular pores. Additionally, APFs have been seen accumulating in neuronal processes and synapses in APP23 transgenic mice³⁰ and subsequently were isolated from brains of patients with AD through immunoprecipitation.³¹ The APFs were similar in size to those observed through AFM and TEM, having inner diameters ranging from 2.5-4 nm and outer diameters from 11-14 nm. In 2016, the Nowick lab reported an X-ray crystallographic structure of a synthetic macrocyclic peptide that contains A β ₁₇₋₃₆. This peptide assembles in the crystal lattice to form an annular pore composed of five dodecamers, which can be further subdivided into four triangular trimers of three antiparallel β -sheets. The annular pore has an inner diameter of about 2 nm and an outer diameter of 11-12 nm, which mirrors the pore sizes of synthetic and brain-isolated A β observed with AFM and TEM.^{25–29,32} These data, considered with other studies conducted on how A β oligomers assemble in membranes, indicate that pore-forming A β oligomers are likely composed of monomeric β -sheets of the A β peptide.³³

1.1.2 FAD Mutants of A β

Although approximately 97% of AD cases are spontaneous and generally occur in people over the age of 65, the remaining 3% of AD cases are caused by familial AD (FAD) and occur with near certainty around the age of 50.^{1,34} Point mutations in the A β peptide or other regions of

the APP have profound effects upon the progression of AD. A single mutation in a single copy of the *APP* gene that falls in the A β region leads to the certain onset of AD at about age 50 (Figure 1.1).^{2,3,35} These mutations, termed FAD mutations due to their prevalence in distinct family lines, cause changes in A β production, oligomerization propensity, and aggregation ability. Of the known FAD mutations that occur in the A β peptide, 66% are found between residues K16 and L34 (see Table 1.1 for a list of amino acid names and abbreviations). The K16N mutation, where K16 is substituted for an N, causes an increase in overall A β production and only exerts cytotoxicity in the presence of wild-type (WT) A β . The E22 Δ mutation — where Δ indicates a deletion of E22 — induces faster A β aggregation without up-regulating the production of A β . Other mutations, such as A21G, E22G, E22Q, E22K, D23N, and L34V, all cause early onset AD as well as cerebral amyloid angiopathy (CAA), which causes microhemorrhaging and premature death. The E22G, E22Q, and E22K mutations also have enhanced oligomerization, aggregation potential, and cytotoxicity behavior as compared to WT A β .³

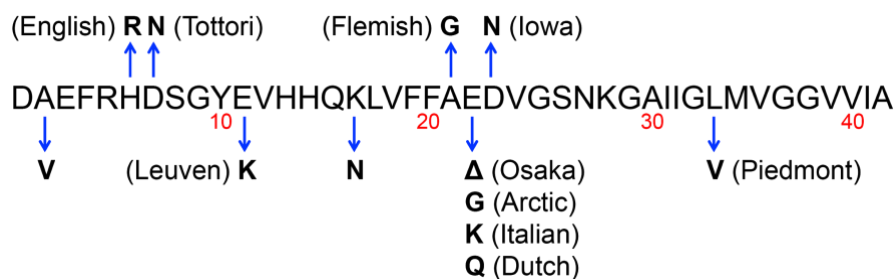


Figure 1.1. Amino acid sequence of A β with known early onset familial Alzheimer’s disease mutations. (Adapted from Weggen and Behr.³)

Table 1.1. Abbreviations of amino acid residues found in FAD mutants.

amino acid side chain	3-letter code	1-letter code
alanine	Ala	A
arginine	Arg	R
asparagine	Asn	N
aspartic acid	Asp	D
glutamic acid	Gln	E
glutamine	Glu	Q
glycine	Gly	G
histidine	His	H
isoleucine	Ile	I
leucine	Leu	L
lysine	Lys	K
methionine	Met	M
phenylalanine	Phe	F
valine	Lys	V

Many of the FAD mutations cause a change in the nominal charge of A β at physiological pH (~7.4) because of the varying charged states of amino acid residues present in the A β molecule (Table 1.1). These differences in charge are significant because the charged state of a peptide dictates how it can associate and destabilize cell membranes. A β has a nominal charge of ca. -2.9 at physiological pH. Six out of the twelve FAD mutations of A β (D7N, E11K, E22G, E22K, E22Q, D23N) involve the loss of an acidic residue in exchange for a neutral or basic residue. One FAD mutation, E22 Δ , is simply a deletion of an acidic residue without replacement. All of these mutations thus cause a one or two unit decrease in the nominal negative charge of the mutations of A β , from ca. -2.9 to ca. -1.9 or -0.9. Only two FAD mutations (H6R and K16N) occur at basic residues which are replaced with another basic residue or a neutral residue. The remaining three FAD mutations (A2V, A21G, L34V) involve the loss of a neutral residue in exchange for a neutral residue. Over half of the FAD mutations lead to a decrease in the nominal negative charge of the A β peptide, in other words they are trending towards neutral, which could increase their propensity to associate with cell membranes.

FAD mutations affect the propensity of A β to aggregate and cause the cytotoxicity observed in early onset AD. TEM images reveal that mutations K16N, A21G, E22 Δ , E22Q, E22K, and D23N form amorphous aggregates, suggesting that these mutations cause an increase in A β oligomer formation. A21G and E22K typically form very few fibrils, which are generally in short fibril bundles.³⁶ E22 Δ , E22Q, and L34V however induce more fibrilization to the mutant A β peptides than WT A β , as seen by ThT and TEM studies. The E22G, E22Q, and E22K mutations exhibit enhanced cytotoxicity as compared to WT A β , and the E22G and E22K mutations of full-length A β are thought to reinforce the occurrence of stable oligomers and protofibrils.^{37,38} While these studies show how the FAD mutations of A β form aggregates and enhance cytotoxicity, we still do not understand how FAD mutations affect the assembly of A β at the molecular level. Previous work in our laboratory has approached the lack of information about the supramolecular assembly of A β by studying model peptide systems. These model peptides contain regions of A β thought to be important in how A β can self-assemble, and will be discussed in detail subsequently.

1.1.3 Model Systems of A β

Solid-state NMR and X-ray crystallographic studies of A β fibrils and A β fragments have shown that A β packs to form networks of parallel β -sheets.^{39–48} Hoyer et al. demonstrated that the A β _{16–36} region may be important to the ability of A β to form toxic oligomers through NMR and toxicity assays.⁴⁹ Their NMR studies indicate that A β forms a β -hairpin in the A β _{16–36} region (Fig. 1.2A), and also show that upon affibody binding of this β -hairpin, there is a reduction in the ability of A β to cause cellular toxicity. Inspired by this work, our laboratory has shown that model peptides derived from A β assemble to form dimers, trimers, hexamers, and even

dodecamers and an annular pore by X-ray crystallography.^{32,50–55} We have studied the effect of β -sheet registration on peptide assembly and determined that different amino acid registrations on the top and bottom strands of a β -sheet can cause differences in their assembly. We have also learned that oligomers formed by different model peptides have different biological activities. Of all model peptides synthesized and tested to date in our group, the model peptide that contains the A β ₁₆₋₃₆ region (peptide **1**, Fig. 1.2B) is the only monomeric peptide to cause cytotoxicity to neuronally-derived SH-SY5Y cells. The cytotoxicity activity of peptide **1** recapitulates the biological activity of the full-length A β peptide. Peptide **1** has also been shown to self-assemble into discrete, tightly packed, hexamers that share other key biophysical characteristics with full-length A β oligomers.⁵⁴

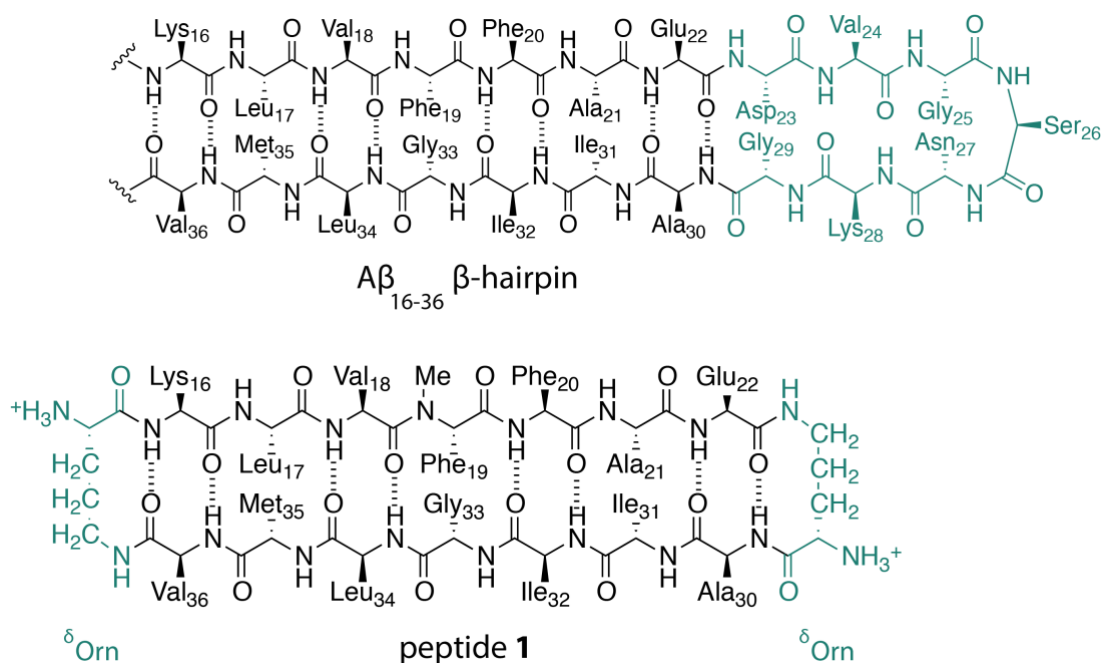


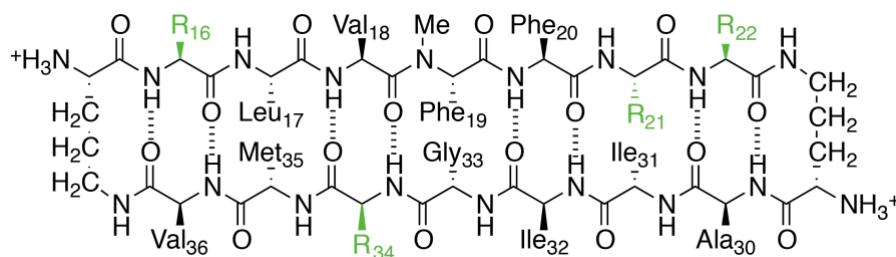
Figure 1.2. Design of macrocyclic peptides based on full length A β . β -Hairpin formed by A β ₁₆₋₃₆ and chemical structure of peptide **1**, a macrocyclic β -sheet peptide mimic based on A β ₁₆₋₃₆.

I chose peptide **1** as a model platform to study the effects of FAD point mutations on the biophysical properties and assembly of the A β ₁₆₋₃₆ region (Fig. 1.2). Peptide **1** is a model peptide comprising A β ₁₆₋₂₂ on the top strand, A β ₃₀₋₃₆ on the bottom strand, and has two δ -linked ornithine (δ Orn) turn units and an *N-methyl* amino acid.⁵⁶ The δ Orn turn unit that connects residues E22 and A30 mimics a β -hairpin turn and the δ Orn that connects K16 and V36 serves to constrain the β -sheet into a macrocycle, which in turn should template β -sheet formation. The *N-methyl* group on F19 provides a blocking group on one face of the β -sheet to prevent uncontrolled fibrillization and promote oligomerization.

In the crystal structure, peptide **1** forms a tightly packed trimer of dimers or dimer of trimers, also considered a hexamer.⁵⁴ By sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis, it appears that peptide **1** has a solution-phase assembly that corroborates its crystal structure, exhibiting a molecular weight consistent with that of a hexamer and that of a monomer or dimer. Cytotoxicity studies also indicate that peptide **1** is toxic to neuronal cells.⁵⁴ The assembly and solution-phase behavior of peptide **1** make it a suitable model system to study the effects of point mutations — derived from FAD mutations — on the supramolecular assembly of peptide **1**.

I incorporated key FAD mutations into peptide **1** and studied their effects upon peptide cytotoxicity, biophysical behavior, and crystallographic assembly. Peptide **1** accommodates FAD mutations K16N, A21G, E22 Δ , E22G, E22Q, E22K, and L34V (the E22 Δ mutation results in D23 in place of E22 and is thus designated E22D for the mutant peptide). I prepared peptides **1**_{K16N}, **1**_{A21G}, **1**_{E22D}, **1**_{E22G}, **1**_{E22K}, **1**_{E22Q}, and **1**_{L34V} to better understand the effects of these point mutations (Chart 1.1). To further probe how nominal charge changes peptide behavior, I also

prepared capped variants of the E22 mutant peptides (**2**, **3**, **4**, **2E22Q**, **3E22Q**, **4E22Q**, **2E22K**, **3E22K**, **4E22K**).



peptide	R ₁₆	R ₂₁	R ₂₂	R ₃₄	nominal charge at neutral pH
1	Lys	Ala	Glu	Leu	+2
1E22D	Lys	Ala	Asp	Leu	+2
1E22G	Lys	Ala	Gly	Leu	+3
1E22Q	Lys	Ala	Gln	Leu	+3
1E22K	Lys	Ala	Lys	Leu	+4
1K16N	Asn	Ala	Glu	Leu	+1
1A21G	Lys	Gly	Glu	Leu	+2
1L34V	Lys	Ala	Glu	Val	+2
2	Lys	Ala	Glu	Leu	+1
3	Lys	Ala	Glu	Leu	+1
4	Lys	Ala	Glu	Leu	0
2E22Q	Lys	Ala	Gln	Leu	+2
3E22Q	Lys	Ala	Gln	Leu	+2
4E22Q	Lys	Ala	Gln	Leu	+1
2E22K	Lys	Ala	Lys	Leu	+3
3E22K	Lys	Ala	Lys	Leu	+3
4E22K	Lys	Ala	Lys	Leu	+2

Chart 1.1. β -Hairpin peptides incorporating FAD mutations. Mutated residues are shown in green, R = residue.

1.2 Results & Discussion

I began my studies by synthesizing the mutant peptides in Chart 1.1 and determining the effect of mutations upon the cytotoxicities of the peptides (see Appendix for synthesis and experimental details). I then probed the effect of mutations on membrane destabilization. To further elucidate the assembly of the mutant peptides, I turned to sodium dodecyl sulfate-

polyacrylamide gel electrophoresis, circular dichroism spectroscopy, and size exclusion chromatography. Finally, I turned to X-ray crystallography to determine how the mutant peptides assemble in the crystal state. Here I describe the studies, first focusing on the E22 mutants (**1E22D**, **1E22G**, **1E22K**, **1E22Q**), then the other familial mutants that can be studied using peptide **1** (**1K16N**, **1A21G**, **1L34V**), and finally select capped variants of the E22 mutant peptides (**2**, **3**, **4**, **2E22Q**, **3E22Q**, **4E22Q**, **2E22K**, **3E22K**, **4E22K**).

1.2.1 E22 Mutant Peptides (1E22D, 1E22G, 1E22K, 1E22Q) Results

1.2.1.1 Cytotoxicity

Most FAD mutants are more toxic to neuronal cells than normal A β , particularly the E22 FAD mutations. To determine if mutant peptides **1E22D**, **1E22G**, **1E22K**, and **1E22Q** cause cytotoxicity like their full-length A β counterparts, I treated SH-SY5Y cells — derived from human neuroblastoma cells — with each peptide for 72 hours. I then utilized lactate dehydrogenase (LDH) release assays to determine the amount of LDH released upon treatment with peptide. LDH is a cytosolic enzyme that is upregulated in cells undergoing toxic stress, and LDH release assays provide a way to quantitatively measure a cell's LDH release spectrophotometrically. By comparing the percent LDH released for peptide treatment to that released for lysis buffer, I thus determined which mutant peptides cause more or less cell death than peptide **1**.^{54,57}

Peptides **1E22G**, **1E22K**, and **1E22Q** exhibit greater cytotoxicity towards SH-SY5Y cells than peptide **1**. All peptides elicit cytotoxicity at concentrations of 50 μ M (Figure 1.3A). Peptides **1** and **1E22D** are not toxic at concentrations below 50 μ M. In contrast, peptides **1E22G** and **1E22Q** exhibit greater cytotoxicity, causing cell death at concentrations as low as 25 μ M (Figure 1.3A and B). Peptide **1E22K** exhibits even greater cytotoxicity, at the lowest concentration of all E22

mutant peptides, 12.5 μ M (Figure 1.3B). All mutant peptides exhibit cytotoxicity propensities that are similar or more active than peptide **1**, which recapitulates the toxicity of full-length FAD mutants of A β compared to WT A β . The concentration at which the mutant peptides cause cytotoxicity decreased two-fold with increasing nominal positive charge at physiological pH: **1** (+2) \approx **1E22D** (+2) < **1E22G** (+3) \approx **1E22Q** (+3) < **1E22K** (+4) These results suggest that peptide nominal charge plays a pivotal role in causing cytotoxicity, and that greater positive charge causes greater cytotoxicity.

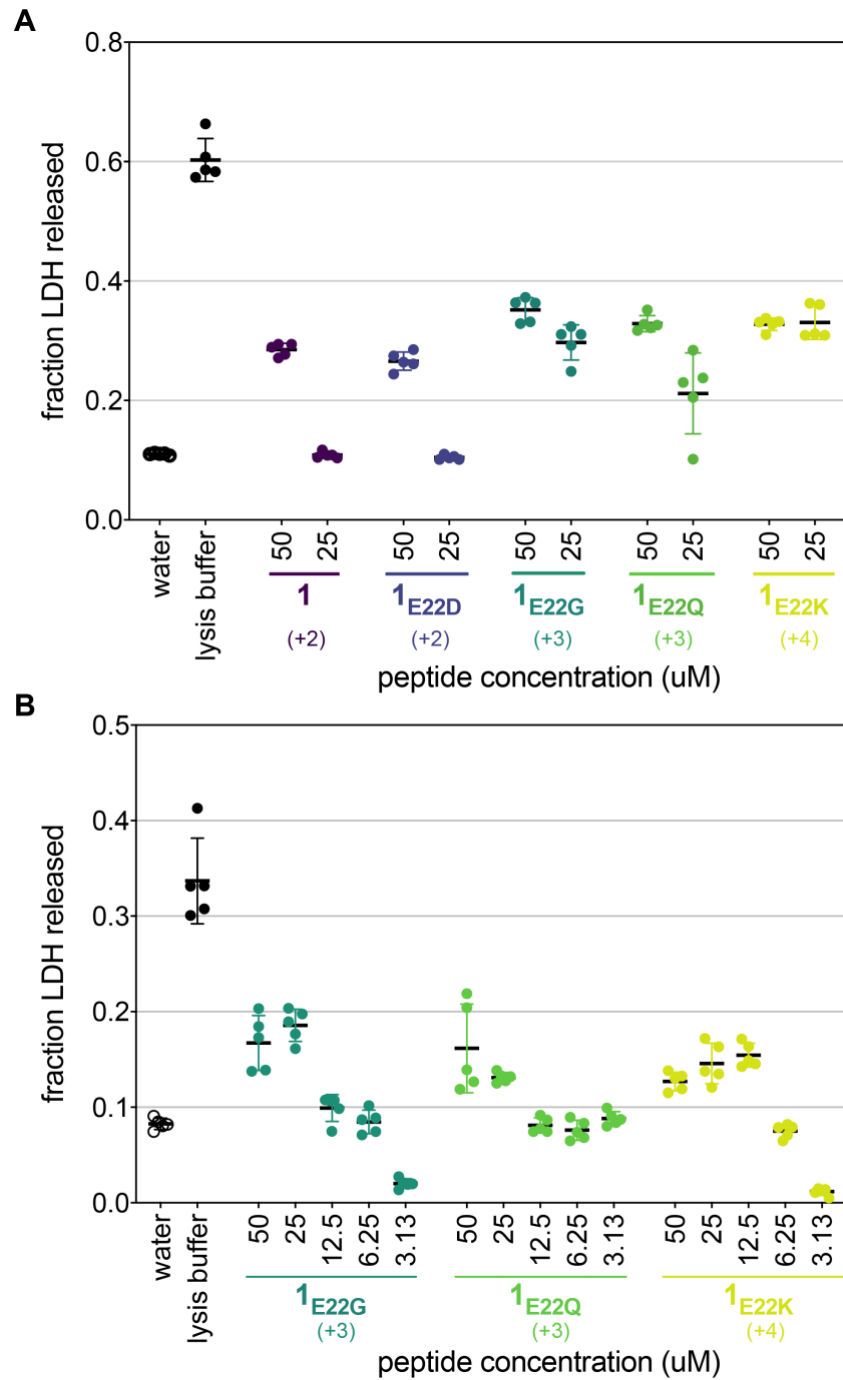


Figure 1.3. Cytotoxicity of mutant peptides as assessed by LDH release assays. Peptides **1**, **1E22D**, **1E22G**, **1E22Q**, and **1E22K** induce varying degrees of LDH release, and thus cytotoxicity to neuron-derived SH-SY5Y cells. Cells were incubated at 37°C for 72 h with the aforementioned concentrations of peptide before measuring supernatant absorbances at 490 nm and 680 nm. Data are plotted as values resulting from the difference 490 - 680 nm. Each dot represents a single data point of five technical replicates, the horizontal black bars represent means, and the colored error bars representing standard deviations. Water served as a negative, vehicle, control and lysis buffer was the positive control. (A) Toxicity profiles of peptides **1**, **1E22D**, **1E22G**,

1E22Q, and **1E22K** at 50 and 25 μM . (B) Toxicity profiles of peptides **1E22G**, **1E22Q**, and **1E22K** at 50, 25, 12.5, 6.25, and 3.13 μM .

1.2.1.2. Membrane Disruption

The correlation of positive charge with cytotoxicity suggests that peptide interactions with cell membranes and subsequent membrane destabilization may be important for the mutant peptides to cause cytotoxicity. To investigate the degree to which mutant peptides interact with membranes and destabilize them, I performed dye leakage assays.⁵⁸⁵⁹ In these assays, large unilamellar vesicles (LUVs) — prepared in buffer to encapsulate a fluorescent dye — are exposed to varying concentrations of mutant peptide. Spectrophotometric detection of an increase in fluorescence correlates to dye leakage from LUVs, which correlates to LUV membrane destabilization by mutant peptide.

I hypothesized that, at physiological pH, mutant peptides with nominal positive charges would interact more readily with negatively charged LUVs than with neutrally charged LUVs. I performed dye leakage assays with LUVs composed of either 1:1 phosphatidyl choline:phosphatidyl serine (PC:PS, negatively charged) lipids or only PC (neutrally charged) lipids — enclosing 70 mM calcein — to probe whether lipid surface charge affects mutant peptide association with and destabilization of lipid membranes. For each replicate LUV batch, each concentration of peptide was added to three separate wells of a 96-well black-walled, clear-bottom, plate to which LUVs were subsequently added. The amount of peptide-induced membrane destabilization was then detected by an increase in fluorescence. Each concentration was tested in triplicate and all data points were normalized to a positive lysis buffer control, which was defined as 100% dye-leakage. A negative vehicle control lacking added peptide,

water, established the baseline for 0% dye-leakage. Each concentration gradient curve represents a nonlinear regression curve-fit to the normalized data points (Figure 1.4).

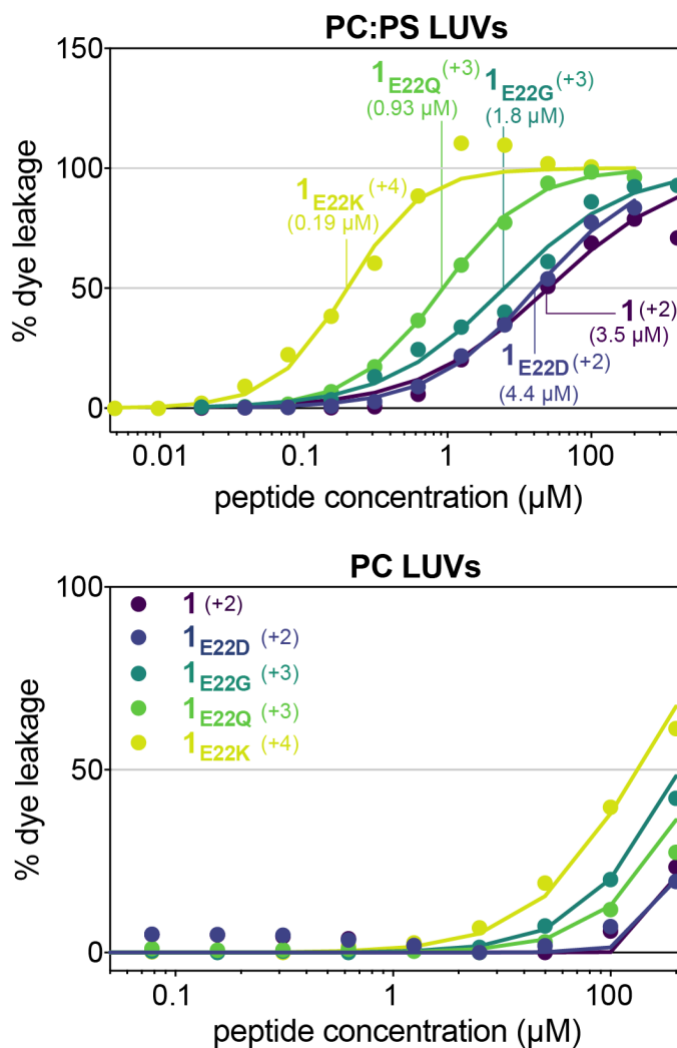


Figure 1.4. Membrane destabilization of mutant peptides as determined by dye-leakage assays. Peptides **1**, **1_{E22D}**, **1_{E22G}**, **1_{E22Q}**, and **1_{E22K}** cause membrane destabilization to large unilamellar vesicles. Peptides were prepared at various concentrations in Tris buffer (10 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA) and incubated with 70 mM calcein-encapsulated LUVs composed of either 1:1 PC:PS (negatively charged) or PC (neutrally charged) lipids. Fluorescence intensity was then measured at an emission wavelength 520 nm and an excitation wavelength of 490 nm. The data were normalized by setting the lysis buffer positive control to 100% dye leakage and the water negative control to 0% dye leakage. Dots represent averages of three replicate runs, error bars represent corresponding standard deviations (but are obscured by data points), and curves show nonlinear regression fits to the data.

Peptides **1**, **1E22D**, **1E22G**, **1E22Q**, and **1E22K** cause dye leakage from LUVs composed of negatively charged 1:1 PC:PS (Figure 1.4). Peptides **1** and **1E22D** cause 50% dye-leakage from LUVs at 3.50 μM and 4.41 μM respectively. Peptides **1E22G** and **1E22Q** cause 50% dye-leakage at 1.85 μM and 0.93 μM , concentrations about half those of **1** and **1E22D**. Peptide **1E22K** causes 50% dye-leakage at 0.20 μM , a concentration that is approximately one order of magnitude less than those of **1**, **1E22D**, **1E22G** and **1E22Q** (Figure 1.4). The membrane destabilization exhibited by the mutant peptides follows the same trend as the cytotoxicity they exhibit. An increase in membrane destabilization to negatively charged PC:PS LUVs increases with nominal positive charge at physiological pH: **1** (+2) \approx **1E22D** (+2) < **1E22G** (+3) \approx **1E22Q** (+3) < **1E22K** (+4).

Peptides **1**, **1E22D**, **1E22G**, **1E22Q**, and **1E22K** cause less dye-leakage to neutrally charged PC LUVs than to negatively charged PC:PS LUVs by one order of magnitude (Figure 1.4). Peptides **1**, **1E22D**, **1E22G**, **1E22Q**, and **1E22K** induce 50% dye-leakage to neutral PC LUVs at concentrations ranging between 13 – 27 μM , which are almost ten times higher than all mutant peptide concentrations which induce 50% dye-leakage to negatively charged PC:PS LUVs (Figure 1.4). These results suggest that charge-charge interactions facilitate nominal positively charged peptide association with negatively charged lipid bilayers. This association could aid in the mutant peptides abilities to insert into membranes and cause membrane destabilization and subsequent cytotoxicity.

The trend in dye-leakage activity of the mutant peptides correlates to their cytotoxicity by LDH release assays. Peptides **1** and **1E22D** cause the least amount of dye leakage and therefore the least amount of membrane destabilization. This behavior mirrors the cytotoxicity exhibited by peptides **1** and **1E22D** in LDH release assays because they cause the lowest observable cytotoxicity — at concentrations higher than the other mutant peptides. Similarly, peptides **1E22G**

and **1E22Q** induce more membrane destabilization and cause cytotoxicity at concentrations that are lower than those of peptides **1** and **1E22D**. Peptide **1E22K** causes the most membrane destabilization and cytotoxicity of all mutant peptides. This trend correlates to the charged state of the peptides at physiological pH; an increase in cytotoxicity to SH-SY5Y cells and membrane destabilization to negatively charged PC:PS LUVs increases with nominal positive charge at physiological pH: **1** (+2) \approx **1E22D** (+2) < **1E22G** (+3) \approx **1E22Q** (+3) < **1E22K** (+4).

1.2.1.3. SDS-PAGE

To further probe how the E22 mutants cause lipid membrane destabilization and cell cytotoxicity, I turned to Tricine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)⁶⁰ to determine how the mutant peptides assemble in a membrane-like environment. SDS-PAGE is a biophysical technique in which proteins are loaded onto a SDS-PAGE gel, an electric current is run through the gel, and the SDS-associated proteins travel through the gel at varying rates based on molecular weight (MW). Larger protein species — single proteins or oligomers — will travel slower through the gel, running higher similar to how amyloid oligomers generally run, and smaller ones will travel faster, running lower, and result in discrete protein bands. Running the gel with mutant peptides adjacent to a standard size ladder — composed of proteins of known MWs — facilitates the determination of peptide and protein size when visualized with a peptide-staining technique such as silver staining or Coomassie blue staining.⁶¹

Peptides **1**, **1E22D**, **1E22G**, **1E22K**, and **1E22Q** assemble to form apparent hexamers in the presence of SDS, with MWs of approximately 10.6 kDa (Figure 1.5). Peptides **1** and **1E22D** form comet-shaped bands around 10 kDa that streak downwards, and peptides **1E22G**, **1E22Q**, and **1E22K**

form sharper bands at MWs consistent with hexamers. The appearance of both comet-shaped bands and sharper bands suggests that the mutant peptides have varying propensities to form different MW oligomers in the presence of SDS. Peptides **1** and **1E22D** likely assemble as hexamers in equilibrium with lower-order oligomers, which causes the formation of comet-shaped bands. Peptides **1E22G**, **1E22Q**, and **1E22K** seem to assemble as oligomers where the equilibrium is shifted towards hexamer formation, causing visualization of sharper bands. The apparent oligomerization propensities of the mutant peptides in the presence of SDS implies that the hexamers formed by peptides **1** and **1E22D** are not as stable as those formed by peptides **1E22G**, **1E22Q**, and **1E22K**.

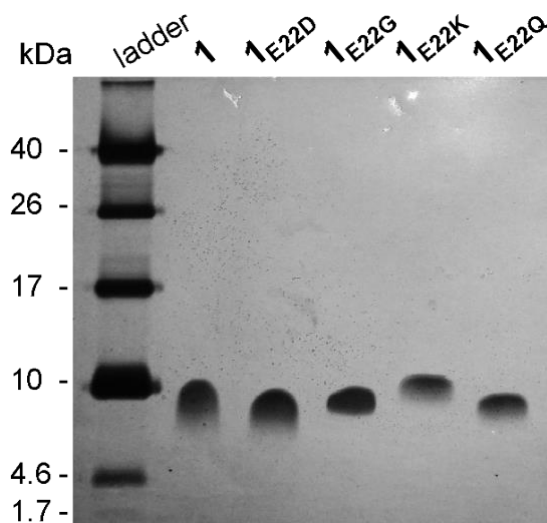


Figure 1.5. Peptides **1**, **1E22D**, **1E22G**, **1E22K**, and **1E22Q** assemble to form higher order oligomers in silver-stained SDS-PAGE gels. All mutant peptides assemble at molecular weights consistent with hexamers (~10.6 kDa). The gel was composed of 16% polyacrylamide, 0.1% SDS, 1M Tris, 0.33 M HCl, at pH 8.45. The cathode buffer contained 0.1 M Tris, 0.1 M Tricine, 0.1% SDS at pH 8.25, and the anode buffer contained 0.1 M Tris and 22.5 mM HCl at pH 8.9. Mutant peptides were prepared in 50 mM Tris buffer (pH 6.8) with 10% (w/v) glycerol and 2% (w/v) SDS to final concentrations of 0.2 mg/mL.

To qualitatively assess the oligomeric stability of peptides **1**, **1E22D**, **1E22G**, **1E22K**, and **1E22Q** to form stable hexamers in the presence of SDS, I ran the mutant peptides on an SDS-PAGE gel with concentration gradients (Figure 1.6). Peptides **1** and **1E22D** form bands that drift

down in a stepwise manner as peptide concentration decreases. The lowest-order oligomer appears for the peptides run at 0.05 mg/mL, at MWs right above the ladder marker of 4.6 kDa — consistent with a mutant peptide trimer MW of 5.3 kDa. (Figure 1.6A). Peptides **1E22G**, **1E22Q**, and **1E22K** all appear as bands that are close to 10 kDa in MW, thus remaining hexameric down to peptide concentrations of 0.05 mg/mL (Figure 1.6B and C). It is difficult to visualize bands from peptides **1E22G** and **1E22Q** when run at 0.05 mg/mL, possibly due to peptide diffusion out of the gel. Additionally, peptide **1E22K** remains a visible hexamer at concentrations as low as 0.025 mg/mL (Figure 1.6C). Peptides **1**, **1E22D**, **1E22G**, **1E22Q** are no longer visible by silver staining or Coomassie blue staining at concentrations below 0.05 mg/mL, so they are not shown here. These results suggest that peptides **1E22G**, **1E22Q**, and **1E22K** form hexamers that are more stable in the presence of SDS than peptides **1** and **1E22D**. As with cytotoxicity and membrane destabilization behaviors, a pattern emerges with mutant peptide nominal charge. An increase in nominal positive charge at physiological pH — **1** (+2) \approx **1E22D** (+2) < **1E22G** (+3) \approx **1E22Q** (+3) < **1E22K** (+4) — correlates to an increase in the appearance of SDS-stable oligomers, specifically hexamers, which suggests that these assemblies are relevant to how the peptides assemble in membrane environments.

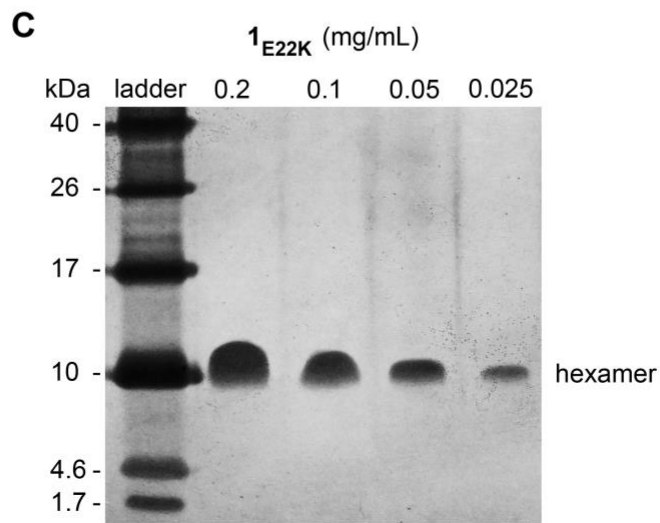
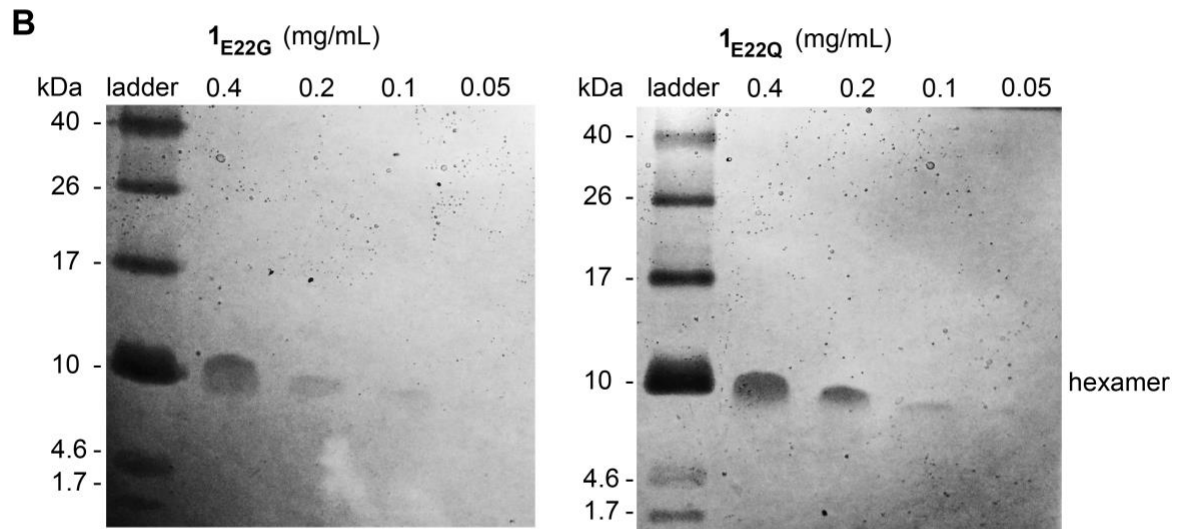
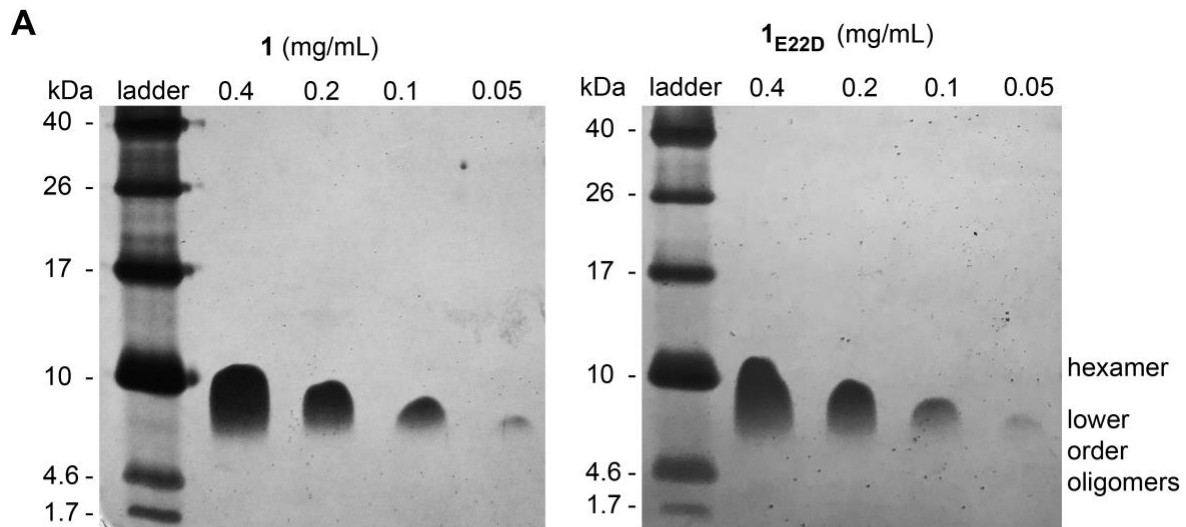


Figure 1.6. Oligomer stability of mutant peptides in the presence of SDS. Peptides **1**, **1E22D**, **1E22G**, **1E22Q**, and **1E22K** exhibit various levels of oligomer stability at decreasing concentrations in SDS-PAGE gels. Silver-stained SDS-PAGE with concentration gradients of mutant peptides is depicted. Each gel had the same running conditions described in Figure 1.5. (A) Peptides **1** and **1E22D** have bands that drift down significantly, with oligomers that appear to tend towards a trimer or other lower order oligomer at 0.05 mg/mL. Peptides were no longer visible at concentrations below 0.025 mg/mL. (B) Peptides **1E22G** and **1E22Q** have bands that drift down, however they appear to stay as hexamers down to 0.05 mg/mL. Peptides were no longer visible at concentrations below 0.025 mg/mL. (C) Peptide **1E22K** remains a hexamer down to 0.05 mg/mL.

1.2.1.4. Circular Dichroism Spectroscopy

I performed circular dichroism spectroscopy (CD) to further probe how peptides **1**, **1E22D**, **1E22G**, **1E22K**, and **1E22Q** behave in solution. CD reveals the secondary structure that a protein or peptide adopts in solution. To perform a CD experiment, mutant peptides are dissolved in buffer, placed in a cuvette, and exposed to circularly polarized light. The distinct mean residue ellipticity curve that a peptide yields can be correlated to specific protein secondary structures, e.g. α -helix, β -sheet, random coil.^{62,63} The mutant peptides are constrained into macrocycles to help template β -sheet formation, which should subsequently aid in oligomer assembly. If the mutant peptides exhibit β -sheet character in aqueous buffer, then they will likely form oligomers more readily.

CD spectra revealed that peptides **1**, **1E22D**, **1E22G**, **1E22Q**, and **1E22K** maintain some β -sheet character (Figure 1.7). Peptides **1** and **1E22D** have primarily canonical β -sheet character by CD, as shown by bands with minima at ~218 nm. However, both peptides **1** and **1E22D** have additional slight band dips at ~190 nm, indicating that the peptides also have partial random coil character. Peptides **1E22G** and **1E22Q** have bands with two dips, with local minima at ~218 nm and ~190-200 nm, revealing that the peptides have both β -sheet and random coil character. Peptide **1E22K** has a CD spectrum that appears to indicate β -sheet character, but the band is broadened at the local minima, from ~205 – 215 nm (Figure 1.7). The behavior of the E22 mutant peptides follows the

emergent pattern that peptides with the same nominal charge at physiological pH — **1** (+2) \approx **1E22D** (+2), **1E22G** (+3) \approx **1E22Q** (+3), **1E22K** (+4) is alone — have similar biophysical properties. By CD, the mutant peptides with the same nominal charge have similar folding patterns in solution, which could inform their cytotoxicity and membrane destabilizing behavior.

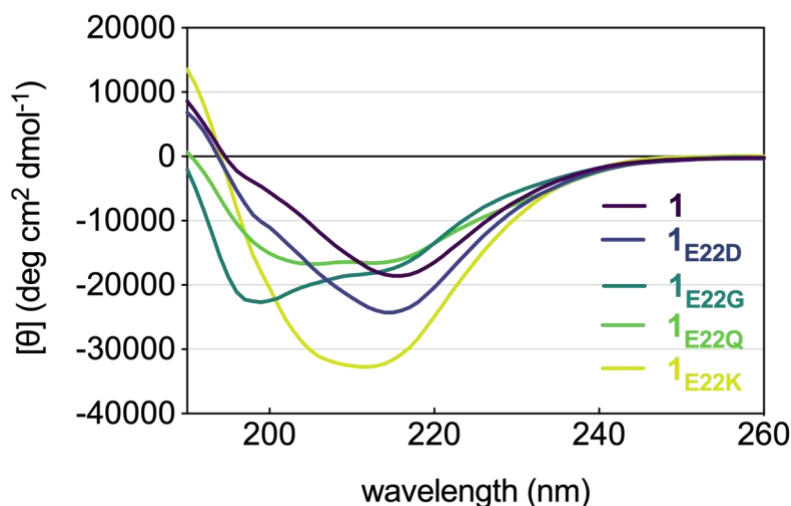


Figure 1.7. Peptides **1**, **1E22D**, **1E22G**, **1E22Q** and **1E22K** have β -sheet character by CD. Mutant peptides were prepared at 50 μ M concentrations in 10 mM phosphate buffer at pH 7.4. The spectrum was acquired at 25 $^{\circ}$ C, and data is shown as the mean residue ellipticity at varying wavelengths.

1.2.1.5. Size Exclusion Chromatography

To further probe how peptides **1**, **1E22D**, **1E22G**, **1E22K**, and **1E22Q** behave in solution, I turned to size exclusion chromatography (SEC). SEC is a separation technique that separates proteins and peptides based on molecular weight. An SEC column has pores through which only molecules of certain sizes or molecular weights can traverse. Larger molecules will not fit through the pores, so they will travel through the column and elute more quickly than smaller molecules. Smaller molecules will travel through the pores, so they will travel through the column and elute more slowly than larger molecules. This process allows the separation of

molecules of differing sizes or with different molecular weights. In the case discussed here, my aim was to separate mutant peptide oligomers of different sizes to identify which oligomeric species exist in solution.

SEC chromatograms indicated that mutant peptides with the same nominal charge at physiological pH behave in the same manner in solution (Figure 1.8). Cytochrome c, aprotinin, and vitamin B12 were included as molecular weight markers; each mutant peptide is around 1.8 kDa. Peptides **1** and **1E22D** (nominal charge of +2), appear to elute as monomers, dimers, and trimers. The SEC chromatograms of peptide **1** shows three peaks at 17, 18, and 19 mL that likely correspond to peptide **1** assembled as a trimer (MW of 5.3 kDa), dimer (MW of 3.5 kDa), and monomer (MW of 1.8 kDa) respectively. The SEC chromatogram of peptide **1E22D** has two peaks at 17.5 and 19.5 mL, which are consistent with MWs of a dimer or trimer and a monomer. Peptides **1E22G** and **1E22Q** (nominal charge of +3) both elute as monomers and dimers and stick to the column. The SEC chromatograms of peptides **1E22G** and **1E22Q** have three peaks each — at 18, 19, and 22 mL — and the first two are consistent with MWs of a dimer (3.5 kDa) and a monomer (1.8 kDa). The third peak, at 22 mL, is at a MW lower than the MW of the mutant peptides, suggesting that a major portion of the peptides stuck to the SEC column before eluting. Peptide **1E22K** (nominal charge of +4) only sticks to the column. The SEC chromatogram of peptide **1E22K** has only one peak that eluted after the smallest MW weight marker, vitamin B12, which suggests all of the mutant peptide stuck to the column. However, the peptides that share the same nominal charge behave similarly by SEC. Similar to behavior in cytotoxicity, membrane destabilization, SDS-PAGE, and CD, the mutant peptides SEC behavior can be characterized by nominal charge at physiological pH: **1** (+2) \approx **1E22D** (+2), **1E22G** (+3) \approx **1E22Q** (+3), **1E22K** (+4) is alone.

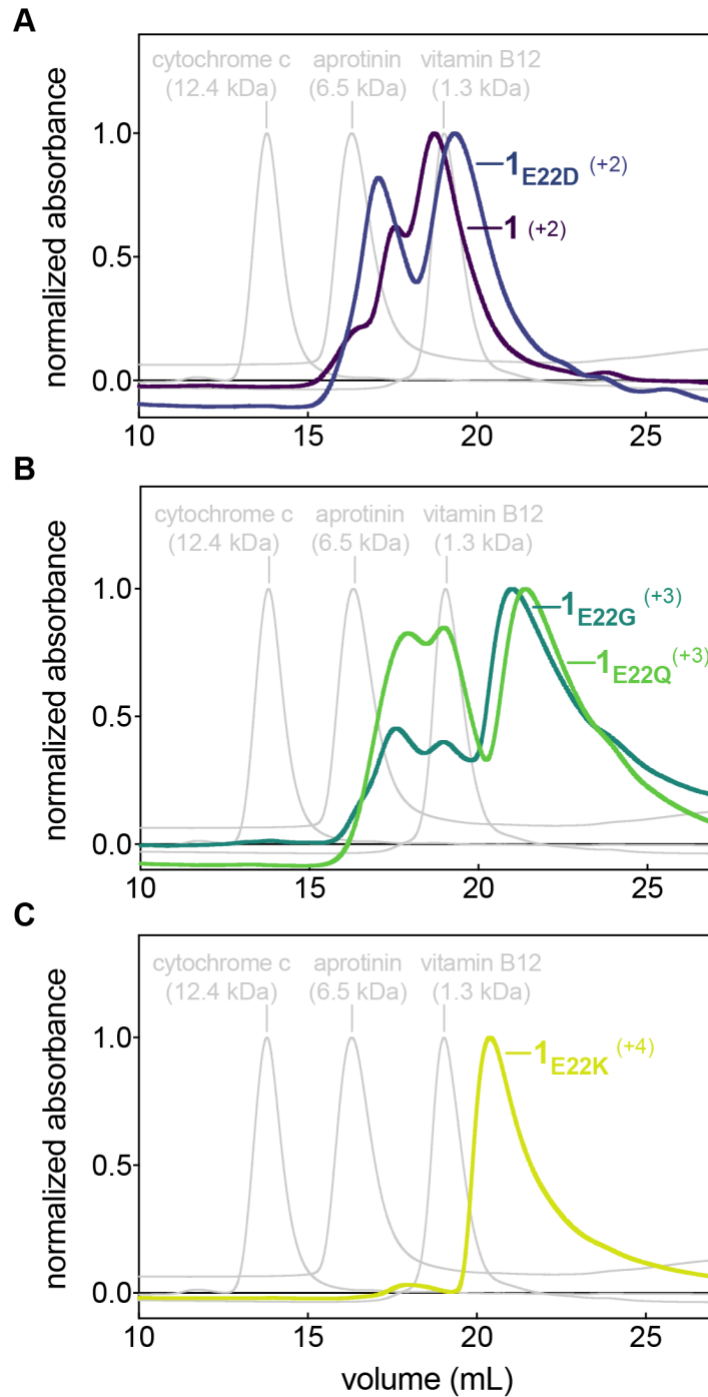


Figure 1.8. SEC reveals that mutant peptides **1**, **1E22D**, **1E22G**, **1E22Q** and **1E22K** assemble in solution. Peptides were prepared at 1.0 mg/mL in 50 mM Tris buffer (pH 7.4) with 150 mM NaCl and run on a Superdex 75 10/300 column. Absorbance was recorded at 215 nm, and each mutant peptide chromatogram was normalized. Cytochrome c, aprotinin, and vitamin B12 were included as molecular weight markers. Each mutant peptide is around 1.8 kDa. (A) SEC chromatograms of peptide **1** shows three peaks that are with the respective MWs of 1.8, 3.5, and 5.3 kDa, and that of peptide **1E22D** reveals two peaks that are consistent with MWs of 1.8 kDa (monomer) and between 3.5 - 5.3 kDa (dimer/trimer). (B) SEC chromatograms of peptides

1E22G and **1E22Q** have three consistent with MWs of 1.8 kDa (monomer), 3.5 kDa (dimer), and a peak that suggests the mutant peptides stuck to the column. (C) SEC chromatogram of peptide **1E22K** has one peak that eluted after vitamin B12; all of the mutant peptide stuck to the column.

The mutant peptides propensities to stick to the SEC column also seems to be correlated to peptide nominal charge. The SEC column used here was a Superdex 75 10/300 column that is composed of an agarose-dextran composite. This composite has many hydroxyl functional groups from the sugar rings that comprise the monomer units of the polymer, which renders the composite polar. The mutant peptides that have a greater nominal positive charge, i.e. peptides **1E22K** (+4), **1E22G** (+3), and **1E22Q** (+3) stick to the agarose-dextran column more readily, with peptide **1E22K** (+4) sticking completely. The mutant peptides that have a nominal positive charge of +2 — peptides **1** and **1E22D** — do not stick to the column at all. The SEC data support the emergent pattern of the mutant peptides, in this case specifically that an increase in nominal charge at physiological pH — **1** (+2) \approx **1E22D** (+2) < **1E22G** (+3) \approx **1E22Q** (+3) < **1E22K** (+4)— correlates to greater ion-dipole interactions between the mutant peptides and the SEC column.

1.2.1.6 X-ray Crystallography

I screened the E22 mutant peptides in over 200 crystallization conditions, found diffracting crystals of each peptide, and successfully solved the X-ray crystallographic structures of all E22 mutant peptides: **1E22D**, **1E22G**, **1E22K**, and **1E22Q**. The crystallographic structure of peptide **1** was previously elucidated by Kreutzer et al. to reveal a compact hexamer composed of dimers and trimers.⁵⁴ Crystallization conditions and statistics for all crystal structures are in the Appendix. I solved the X-ray crystallographic structure of peptide **1E22Q** using molecular replacement with the crystallographically observed trimer of peptide **1** as a search model. I could

not solve the crystallographic structures of peptide **1E22D**, **1E22G**, and **1E22K** using molecular replacement with peptide **1** or peptide **1E22Q**, so I soaked crystals of all three peptides in an aqueous solution of KI to attempt to incorporate a heavy atom into the crystal lattice, facilitating SAD phasing.⁶⁴ None of these attempts were successful, however. I next attempted to use triiodide (I_3^-) — prepared by combining solid I_2 and KI salt in water — as an alternative means of heavy atom incorporation into crystals of peptide **1E22D**, **1E22G**, and **1E22K**. I decided to try facilitating incorporation of I_3^- into the crystal lattice of **1E22K** because the crystallization conditions of **1E22K** include potassium thiocyanate (KSCN) and I hypothesized that I_3^- — as an isostere of SCN^- — could be incorporate into the crystal lattice of **1E22K**. The crystals of peptide **1E22K** turned purple, suggesting successful incorporation of I_3^- into the crystal lattice, but I was not able to successfully phase off of triiodide. I was successful, however, in using SAD phasing — from I_3^- incorporation into the crystal lattice of a crystal of peptide **1E22G** — to solve the crystallographic structure of peptide **1E22G**. I then used the monomer from the asymmetric unit of the peptide **1E22G** crystal structure as a search model to successfully perform molecular replacement of peptides **1E22D** and **1E22K** (Figure 1.9).

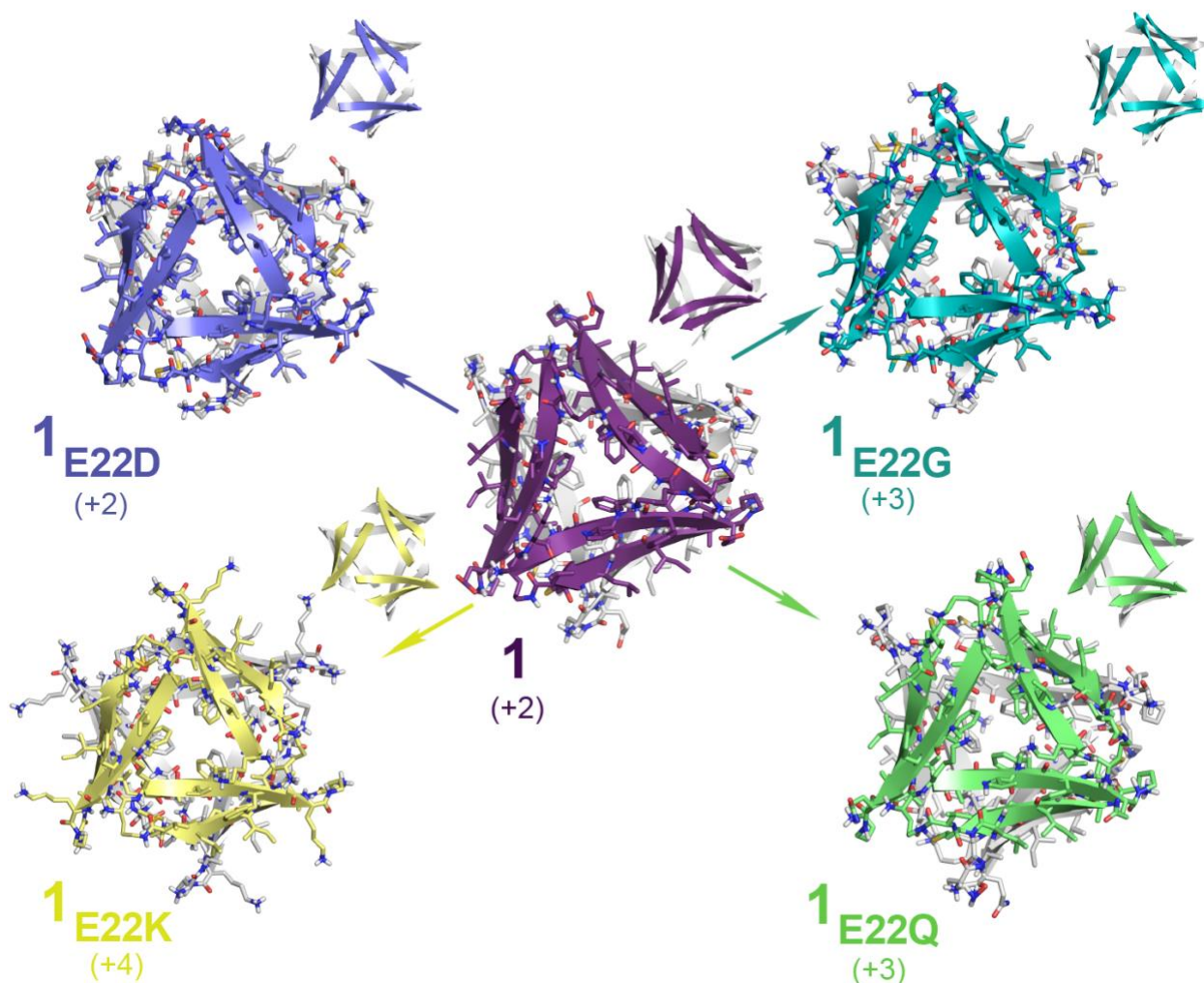


Figure 1.9. X-ray crystallographic hexameric structures of peptides **1**, **1_{E22D}**, **1_{E22G}**, **1_{E22Q}**, and **1_{E22K}**. Peptide **1** is depicted in the center in purple. Moving clockwise from top left: peptide **1_{E22D}** is shown in violet, peptide **1_{E22G}** is shown in teal, peptide **1_{E22Q}** is shown in light green, and peptide **1_{E22K}** is shown in yellow.

The X-ray crystallographic structures of peptides **1_{E22D}**, **1_{E22G}**, **1_{E22K}**, and **1_{E22Q}** all assemble to form compact hexamers that are nearly identical to the crystal structure of peptide **1**. This is surprising because all peptides have distinct cytotoxicity and biophysical characteristics, as discussed previously. The X-ray crystallographic structures of peptides **1_{E22D}**, **1_{E22G}**, **1_{E22K}**, and **1_{E22Q}** suggest that the hexamer unit formed by all mutant peptides is important to their cytotoxicity and biophysical behavior. These results also indicate that peptide **1** can

accommodate point mutations, at least at position E22, without majorly comprising how it assembles into a hexamer. However, there is something unique that each point mutation contributes to the peptides ability to cause cytotoxicity and assemble in solution. One major difference between the E22 mutant peptides is their charged state at physiological pH, which has thus far been discussed in detail and is summarized in Table 1.2. The mutant amino acid residues could contribute to the ability of the more active peptides (peptides **1E22G**, **1E22K**, and **1E22Q**) to self-assemble with higher affinity. The differences in nominal charge at physiological pH could also contribute to these differences.

Table 1.2. Summary of familial mutant peptide biophysical properties and assembly.

	1K16N	1A21G	1L34V	1	1E22D	1E22G	1E22Q	1E22K
formal nominal charge at pH 7.4	+1	+2	+2	+2	+2	+3	+3	+4
cell toxicity (uM)	>50	50	>50	37.5	37.5	25.0	25.0	12.5
PC:PS EC ₅₀ (LUVs, uM)	12.2	1.9	11.9	3.5	4.4	1.8	0.9	0.2
PC EC ₅₀ (LUVs, uM)	—	—	—	23	27	19	26	13
SDS assembly	t	h	h	h	h	h	h	h
SDS disassembly (conc. grad.)	—	—	—	yes	yes	no	no	no
CD	β, r	β	β	β	β	β, r	β, r	β?
SEC	—	m, d	—	m, d, t	m, d	m, d, stuck	m, d, stuck	stuck
X-ray crystal structure	—	—	—	hex	hex	hex	hex	hex

*m = monomer, d = dimer, t = trimer, hex = hexamer, β = beta-sheet, r = random coil, stuck = stuck on column

1.2.2 E22 Mutant Peptides (**1E22D**, **1E22G**, **1E22K**, **1E22Q**) Discussion

One proposed mechanism of full-length A β -induced cytotoxicity is through oligomer-induced pore formation (Arispe PNAS 1993).¹⁹ A β can assemble into β -barrel-like pores in the presence of membrane-like lipid environments;⁶⁵ the ability of A β to insert into a lipid bilayer is implicated in the capability of A β to cause cytotoxicity.⁶⁶ The insertion of A β into a lipid membrane causes membrane destabilization and calcium dysregulation, which are thought to be important factors that contribute to A β -induced cytotoxicity.^{67,68} Mutant peptides **1E22D**, **1E22G**, **1E22K**, and **1E22Q** cause membrane destabilization and cytotoxicity, possibly in a similar manner to full-length A β .

The X-ray crystallographic structures of peptides **1**, **1E22D**, **1E22G**, **1E22Q**, and **1E22K** reveal that the mutant peptides assemble as hexamers composed of β -sheet monomers, which could inform how these mutant peptides assemble as apparent hexamers in SDS-PAGE gels. CD and SEC experiments corroborate that all mutant peptides have β -sheet character in solution, and that peptides **1**, **1E22D**, **1E22G**, and **1E22Q** assemble to form dimers and trimers in solution. These dimers and trimers may be structurally similar to those found in the X-ray crystallographic structures of the mutant peptides.

Charge-charge interactions significantly contribute to the ability of WT A β to oligomerize and to interact with lipid membranes. Modulation of WT A β oligomerization can be tuned by varying the ionic strength of its solution.^{69,70} To determine the effect of electrostatic interactions between WT A β and lipid membranes, Osterlund et al. investigated the ability of WT A β to interact with different surfactants.⁷¹ Their results indicate that charge-charge interactions between WT A β and surfactant head-groups play an important role in the ability of WT A β to associate and assemble in a membrane-like environment. In particular, surfactants

with a negatively charged head group induce greater A β association and subsequent A β oligomerization.

In my study, I observed a similar dependence of charge on both the interactions between mutant peptides and lipid membranes, and the oligomeric stability of mutant peptides in SDS-PAGE gels. Both LUVs — composed of PC:PS lipids — and SDS have negatively charged head groups. Mutant peptides **1**, **1E22D**, **1E22G**, **1E22Q**, and **1E22K** cause increased membrane destabilization and self-assembly into stable hexamers in SDS with increasing nominal charge at physiological pH: **1** (+2) \approx **1E22D** (+2) < **1E22G** (+3) \approx **1E22Q** (+3) < **1E22K** (+4). These results mirror the ability of the mutant peptides to cause cytotoxicity. These studies suggest that peptides **1**, **1E22D**, **1E22G**, **1E22Q**, and **1E22K** interact with negatively charged membranes and that this interaction could induce mutant peptide membrane insertion, self-association into oligomers (potentially hexamers), and cytotoxicity — possibly in a similar manner to the WT A β molecule.

1.2.3 Other FAD Mutant Peptides (1K16N, 1A21G, 1L34V) Results

1.2.3.1. Cytotoxicity

Peptides **1K16N**, **1A21G**, and **1L34V** are not as cytotoxic as peptides **1**, **1E22D**, **1E22G**, **1E22K**, and **1E22Q**. I performed LDH release assays as described previously with peptides **1K16N**, **1A21G**, and **1L34V** and found that peptides **1K16N**, and **1L34V** are not toxic at 50 μ M (Figure 1.10). This lack of apparent toxicity could be a result of peptide precipitation at the concentrations needed to perform this assay. These peptides precipitate when prepared at 5 and 10 mg/mL in water — the stock concentrations used to prepare dilutions for the assay — likely resulting in less peptide added per cell treatment well. I attempted to mitigate the peptide insolubilities by preparing peptides **1K16N** and **1L34V** in DMSO. Both peptides did completely dissolve in DMSO, but when

cells were treated with peptides **1K16N** and **1L34V** in DMSO, observable toxicity was still not present (Figure 1.10B). At physiological pH, peptide **1K16N** has a nominal charge of +1 and peptide **1L34V** has a nominal charge of +2. The nominal charge of peptide **1K16N** is less positive than peptides **1** (+2), **1E22D** (+2), **1E22G** (+3), **1E22K** (+4), and **1E22Q** (+3), and this +1 nominal charge could contribute to the insolubility and therefore the inability of peptide **1K16N** to cause cellular toxicity. The nominal charge of peptide **1L34V** (+2) is the same as those of peptides **1** and **1E22D** (+2). The point mutation that results in a change from L34 to V34 could contribute to the increased aggregation of peptide **1L34V**, resulting in increased peptide insolubility.

Peptide **1A21G** exhibits some cytotoxicity at concentrations as low as 50 μ M, which is consistent with the toxicity levels elicited by peptides **1** and **1E22D**. These similarities in the abilities of peptides **1A21G**, **1**, and **1E22D** to cause cellular toxicity correlates with the nominal charge of +2 that each mutant peptide has at physiological pH, further supporting the observation that the nominal charge of mutant peptides is relevant to the ability of mutant peptides to cause cytotoxicity.

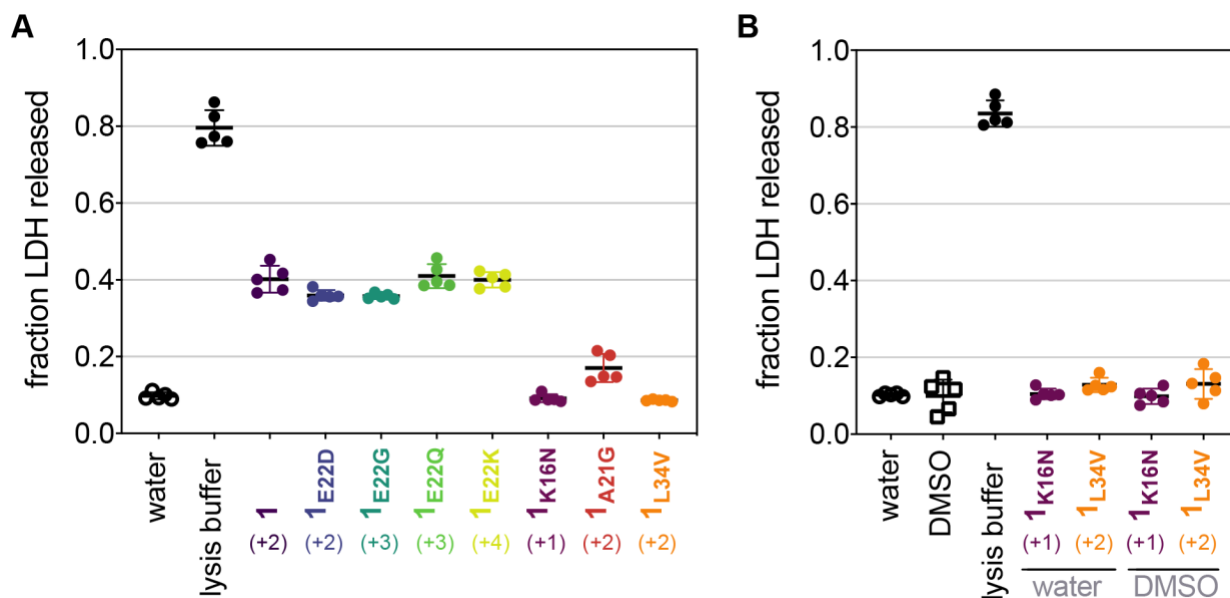


Figure 1.10. Peptides **1K16N**, **1A21G**, and **1L34V** are not cytotoxic to SH-SY5Y cells. LDH release assays and data analyses were performed using the same conditions and procedures as those described in Figure 1.3. (A) Toxicity profiles of peptides **1**, **1E22D**, **1E22G**, **1E22Q**, **1E22K**, **1K16N**, **1A21G**, and **1L34V** at 50 μM . (B) Toxicity profiles of peptides **1K16N** and **1L34V** at 50 μM , dissolved in either water or DMSO prior to treatment on cells.

1.2.3.2. Membrane Disruption

Peptides **1K16N** and **1L34V** do not cause lipid bilayer membrane destabilization as readily as peptides **1**, **1E22D**, **1E22G**, **1E22K**, and **1E22Q** — but peptide **1A21G** does. I conducted dye-leakage assays with peptides **1K16N**, **1L34V**, and **1A21G** using the procedure described previously to test the ability of these peptides to cause membrane destabilization to LUVs composed of negatively-charged 1:1 PC:PS and neutrally-charged PC lipids. I experienced the same aforementioned challenge of obtaining precipitated peptides **1K16N** and **1L34V** when preparing stock solutions for the assays. When the LUVs composed of negatively-charged 1:1 PC:PS lipids were treated with the mutant peptides, the peptides induced 50% dye-leakage at the following concentrations: **1K16N** - 12.2 μM , **1L34V** - 11.9 μM , and **1A21G** - 1.85 μM (Figure 1.4C and D). The membrane destabilization caused by peptides **1K16N** and **1L34V** mirrors the ability of the peptides to cause

cytotoxicity. Peptides **1**_{K16N} and **1**_{L34V} do not cause cytotoxicity, nor do they cause significant membrane destabilization to negatively-charged LUVs — causing 50% dye-leakage at concentrations on the order of those exhibited by peptides **1**, **1**_{E22D}, **1**_{E22G}, **1**_{E22K}, and **1**_{E22Q} to neutrally-charged LUVs (13 – 27 μ M).

The membrane destabilization caused by peptide **1**_{A21G} is on the order of magnitude exhibited by peptides **1** and **1**_{E22D}. Peptide **1**_{A21G} causes 50% dye-leakage at 1.85 μ M, which is in a similar range to the concentrations of peptides **1** and **1**_{E22D} that cause 50% dye-leakage, 4.4 μ M and 3.5 μ M respectively. Peptides **1**_{K16N}, **1**_{L34V}, and **1**_{A21G} were not tested for their ability to cause membrane destabilization in neutrally-charged LUVs because I became allergic to uronium-based peptide coupling agents in the lab before I could run those experiments. These experiments will be conducted by my colleague, William Howitz, once social-distancing restrictions from COVID-19 are relaxed or lifted.

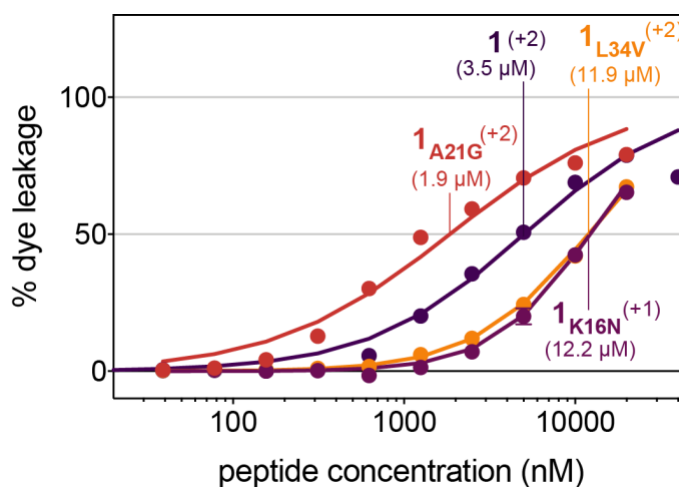


Figure 1.11. Peptides **1**_{K16N}, **1**_{A21G}, and **1**_{L34V} cause membrane destabilization to negatively charged LUVs. Dye leakage assays and data analyses were performed using the same conditions and procedures as those described in Figure 1.4.

1.2.3.3. SDS-PAGE

Peptides **1A21G**, **1K16N**, and **1L34V** assemble to form apparent trimers and hexamers in the presence of SDS. SDS-PAGE gels were run and stained under the same conditions as gel experiments previously discussed. Peptides **1A21G** and **1K16N** run as comet-shaped bands at MWs consistent with a trimer (~5.3 kDa) when loaded on the gel at concentrations of 2.0 mg/mL (Figure 1.12). Peptide **1L34V** also runs as a comet-shaped band, but at a MW consistent with a hexamer (~10.6 kDa) at a concentration of 2.0 mg/mL. Increasing the concentration of peptide **1A21G** to 4.0 mg/mL raises the position of the comet-shaped band to run at a MW just under 10 kDa, suggesting that the increase in peptide concentration shifted the oligomer equilibrium slightly towards hexamer formation (Figure 1.12). Peptide **1A21G** has a nominal charge of +2 at physiological pH. As seen with other mutant peptides that have a nominal charge of +2 (peptides **1** and **1E22D**), peptide **1A21G** also runs as bands that decrease in MW in a stepwise manner upon decrease in peptide concentration, from 4.0 mg/mL to 2.0, 1.0, and 0.05 mg/mL. Similar to the dye-leakage experiments, concentration gradients for peptides **1K16N** and **1L34V** were not run on SDS-PAGE gels before I became allergic to uronium-based peptide coupling agents, so they will be completed by my colleague, William Howitz.

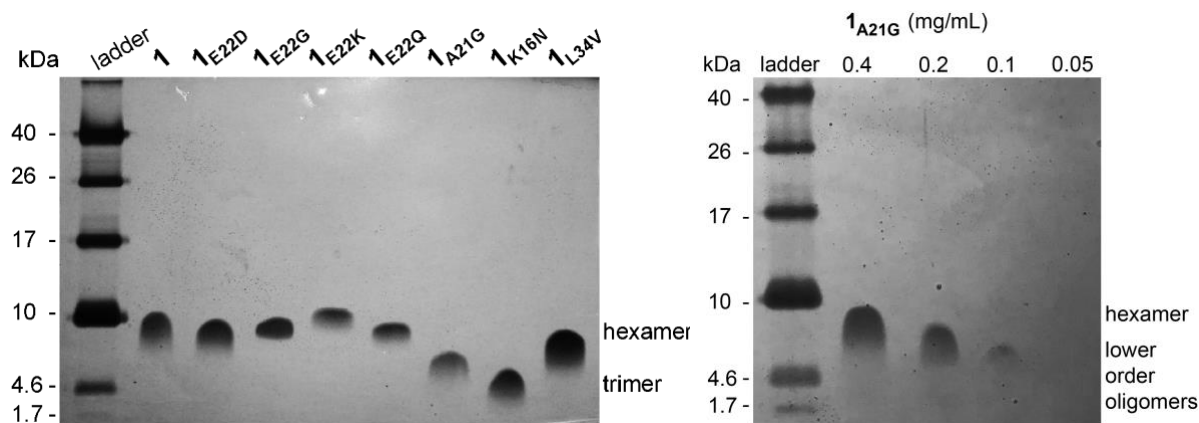


Figure 1.12. Peptides **1_{K16N}**, **1_{A21G}**, and **1_{L34V}** assemble to form higher order oligomers in silver-stained SDS-PAGE gels. SDS-PAGE conditions are the same as those described in Figure 1.5. All mutant peptides run in the gel on the left were run at 0.2 mg/mL. Peptides **1_{K16N}**, **1_{A21G}**, and **1_{L34V}** have comet-shaped bands at MWs consistent with trimers and a hexamer. When run at decreasing concentrations, peptide **1_{A21G}** has bands that drift down — from an apparent hexamer at 0.4 mg/mL to an apparent trimer at 0.1 mg/mL. The mutant peptide band is not visible at 0.05 mg/mL.

1.2.3.4. Circular Dichroism Spectroscopy & Size Exclusion Chromatography

CD spectra reveal that peptides **1_{A21G}**, **1_{L34V}**, and **1_{K16N}** all have β -sheet character and SEC chromatograms indicate that peptide **1_{A21G}** assembles to form monomers, dimers, and trimers in solution. CD and SEC experiments were conducted using the same parameters and conditions as described previously.

Peptides **1_{A21G}**, **1_{L34V}**, and **1_{K16N}** have β -sheet character by CD, as all mutant peptide spectra have bands with minima at ~ 218 nm (Figure 1.13). Peptide **1_{L34V}** appears to have relatively canonical β -sheet character, as the spectra has only one band at ~ 218 nm. Peptides **1_{A21G}** and **1_{K16N}**, however, both have additional band dips at ~ 190 nm, indicating that the peptides also have partial random coil character.

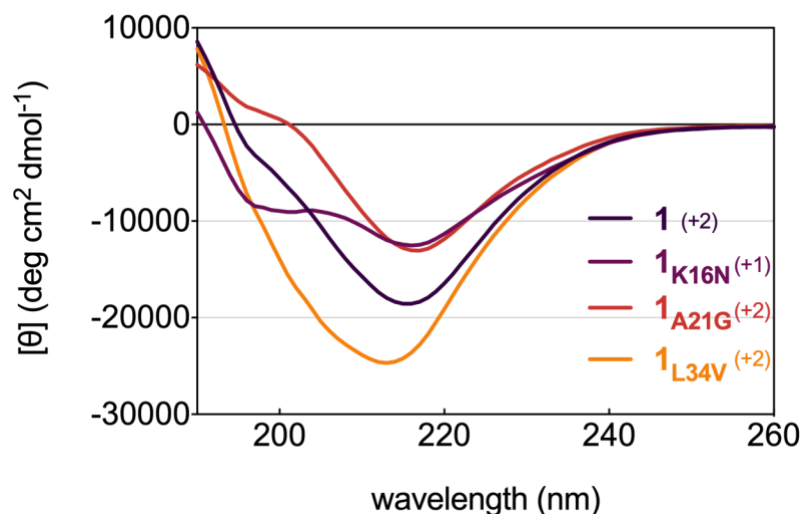


Figure 1.13. Peptides **1_{K16N}**, **1_{A21G}**, and **1_{L34V}** have β -sheet character by CD. Mutant peptides were prepared at 50 μ M concentrations in 10 mM phosphate buffer at pH 7.4. The spectrum was acquired at 25 $^{\circ}$ C, and data is shown as the mean residue ellipticity at varying wavelengths.

The SEC chromatograph of peptide **1_{A21G}** reveals that it assembles in solution as dimers or trimers and monomers (Figure 1.14). Peptide **1_{A21G}** elutes as two peaks, at 17.5 mL and 18.5 mL. The peak at 17.5 mL likely correspond to trimers (MW of 5.3 kDa), dimers (MW of 3.5 kDa), or a combination of dimers and trimers. The peak at 18.5 mL likely corresponds to monomers of peptide **1_{A21G}** (MW of 1.8 kDa). The elution pattern of peptide **1_{A21G}** mirrors those of peptides **1** and **1_{E22D}**, which suggests that the similarity in solution assembly is because these three mutant peptides all have a nominal charge of +2 at physiological pH. Peptides **1_{K16N}** and **1_{L34V}** could not be analyzed with SEC because both mutant peptides precipitated out of the buffer solution and thus could not be injected onto the SEC column.

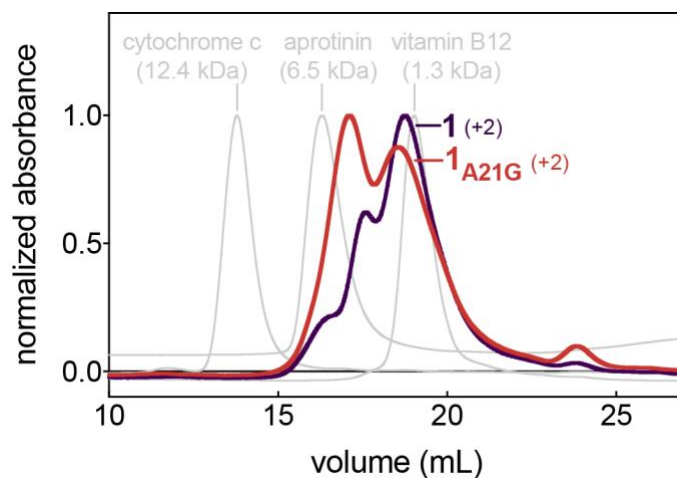


Figure 1.14. SEC reveals that mutant peptide **1_{A21G}** assembles in solution. The SEC conditions used are the same as those described in Figure 1.7. The SEC chromatogram of peptide **1_{A21G}** shows that it assembles as a monomer and either a dimer or a trimer — revealing peaks that could be consistent with the respective MWs of 1.8 and 3.5 - 5.3 kDa.

1.2.4 Other FAD Mutant Peptides (**1_{K16N}**, **1_{A21G}**, **1_{L34V}**) Discussion

To study all FAD mutations that could be incorporated into the macrocyclic peptide model system peptide **1**, I designed and studied peptides **1_{K16N}**, **1_{A21G}**, and **1_{L34V}** in addition to peptides **1**, **1_{E22D}**, **1_{E22G}**, **1_{E22Q}**, and **1_{E22K}**. Peptides **1_{K16N}**, **1_{A21G}**, and **1_{L34V}** were screened for crystallization conditions, but no viable crystals grew. The biophysical activity of peptide **1_{A21G}** in cytotoxicity assays, dye leakage assays, SDS-PAGE gels, CD, and SEC is similar to the biophysical activities of peptides **1** and **1_{E22D}**. These similarities may have emerged because peptides **1**, **1_{E22D}**, and **1_{A21G}** have nominal charges of +2 at physiological pH. Thus far, the nominal charge of mutant peptides correlates with peptide behavior in cytotoxicity assays, dye leakage assays, SDS-PAGE gels, CD, and SEC.

The lack of aqueous solubility of peptides **1_{K16N}** and **1_{L34V}** rendered these mutant peptides difficult to characterize. These two mutant peptides exhibited no appreciable cytotoxicity by

LDH release assays or membrane destabilization by dye leakage assays, which could be because the mutant peptides precipitate out of solution. Precipitation of peptides **1K16N** and **1L34V** also made SEC experiments untenable. CD and SDS-PAGE experiments indicate that peptides **1K16N** and **1L34V** do indeed fold as β -sheets and form higher-order oligomers, suggesting that they self-assemble like the other mutant peptides. The propensity of peptides **1K16N** and **1L34V** to self-assemble may be stronger than those of peptides **1**, **1E22D**, **1E22G**, **1E22Q**, **1E22K**, and **1A21G**, which could cause greater propensity to aggregate into insoluble oligomers. The increased aggregation potential of peptides **1K16N** and **1L34V** mirrors the K16N and L34V FAD mutations of the WT A β molecule in that K16N causes an increase in oligomer formation and L34V induces enhanced fibrilization. The aggregation-prone behavior of peptides **1K16N** and **1L34V** likely contributes to them not following the same emergent charge-dependent pattern of peptides **1**, **1E22D**, **1E22G**, **1E22Q**, **1E22K**, and **1A21G**.

1.2.5. Capped Variants of Select E22 Mutant Peptides (2, 3, 4, 2E22Q, 3E22Q, 4E22Q, 2E22K, 3E22K, 4E22K) Results

To further probe the effect of mutant peptide nominal charge at physiological pH on mutant peptide biophysical properties — cytotoxicity, membrane destabilization, SDS-assembly — peptides **1**, **1E22Q**, and **1E22K** were acetylated, or capped, at the N-terminus of the ornithine macrocyclic turn-units (Table 1.3, Figure 1.14). I chose peptides **1**, **1E22Q**, and **1E22K** as representative mutant peptides with nominal charges of +2, +3, and +4 respectively. Each peptide was systematically capped at the following positions: (1) on the N-terminus of the ornithine turn unit of the top strand, to yield peptides **2**, **2E22Q**, and **2E22K**, (2) on the N-terminus of the ornithine turn unit of the bottom strand, to yield peptides **3**, **3E22Q**, and **3E22K**, and (3) on

the N-termini of the ornithine turn units of both the top and bottom strands, to yield peptides **4**, **4E22Q**, and **4E22K**. My goal was to synthesize — for each parent mutant peptide **1**, **1E22Q**, and **1E22K** — a series of capped peptides with decreasing nominal positive charges (Table 1.3): (A) the capped series for peptide **1** with nominal charges would yield peptides **1 (+2)**, **2 (+1)**, **3 (+1)**, and **4 (0)**, (B) the capped series for peptide **1E22Q** with nominal charges would yield peptides **1E22Q (+3)**, **2E22Q (+2)**, **3E22Q (+2)**, and **4E22Q (+1)**, and (C) the capped series for peptide **1E22K** with nominal charges would yield peptides **1E22K (+4)**, **2E22K (+3)**, **3E22K (+3)**, and **4E22K (+2)**. Each series of peptides would then be analyzed for changes in ability to cause cellular toxicity and membrane destabilization and changes in assembly in the presence of SDS.

Table 1.3. Series of capped mutant peptides.

capped series	peptide	capped position	nominal charge at neutral pH
A	1	—	+2
	2	top strand	+1
	3	bottom strand	+1
	4	top and bottom strands	0
B	1E22Q	—	+3
	2E22Q	top strand	+2
	3E22Q	bottom strand	+2
	4E22Q	top and bottom strands	+1
C	1E22K	—	+4
	2E22K	top strand	+3
	3E22K	bottom strand	+3
	4E22K	top and bottom strands	+2

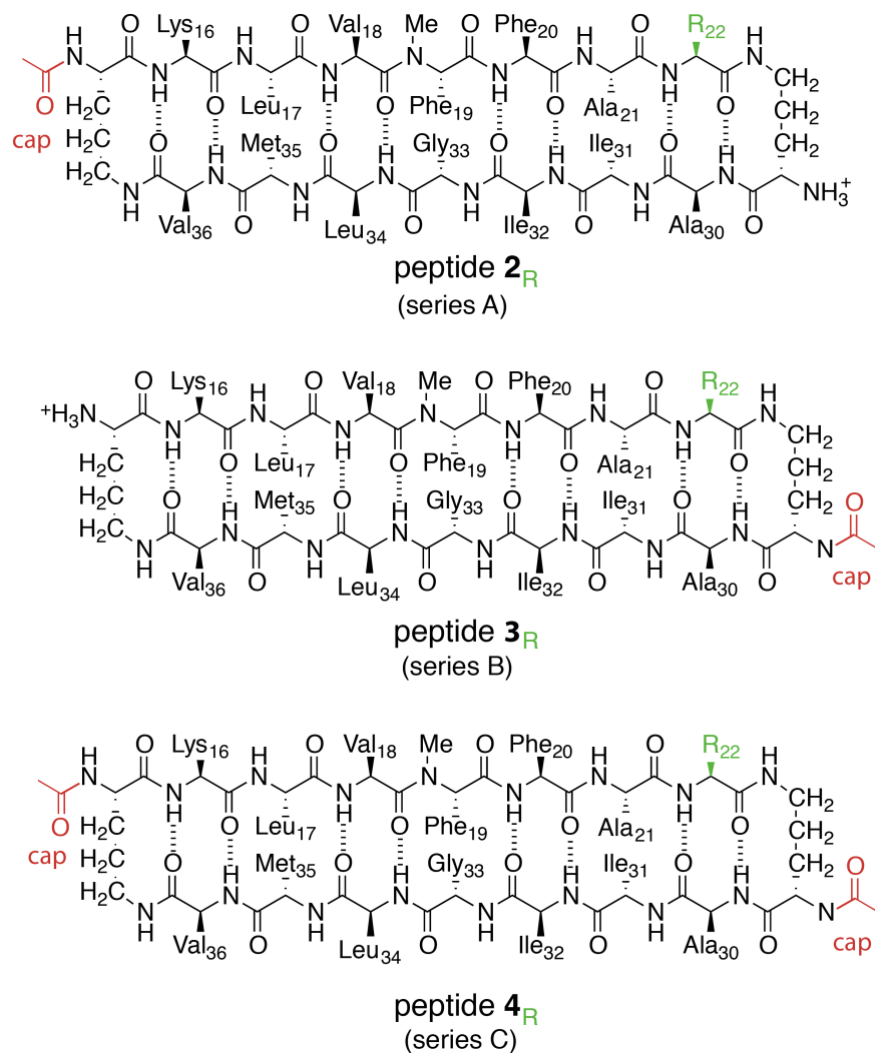


Figure 1.15. Capped series of macrocyclic peptides based peptide **1** and mutant peptides. Acetyl caps are shown in red. R = nothing for peptides based on peptide **1**, E22Q for peptides based on peptide **1_{E22Q}**, and E22K for peptides based on peptide **1_{E22K}**.

Before I became allergic to uronium-based peptide coupling agents — an account that I cover in detail in Chapter 2 of this dissertation — I synthesized all but the double-capped mutant peptides and collected preliminary information about their ability to cause cytotoxicity and assemble in SDS-PAGE gels. By the time I was so sensitized that I could no longer work in the lab, I had not yet completed the capped series of peptide **1_{E22Q}**, nor was I able to complete all of the double-capped mutants for the capped series of peptides **1** and **1_{E22K}**. I turned to colleague

William Howitz to finish synthesizing and testing the capped series of mutant peptides. He has successfully synthesized the capped mutant peptides and run LDH-release assays and SDS-PAGE gels for the capped series of peptides **1** and **1E22K**. I have included this data as follows. He has not yet been able to complete the remainder of the planned experiments because of the necessary stay-at-home order that arose in response to the coronavirus pandemic in mid-March 2020. Once research laboratories re-open, he plans to complete the remainder of the aforementioned experiments, and we will complete and submit a manuscript of this work for publication.

1.2.5.1. Cytotoxicity

Peptides **2**, **3**, and **4**, are not cytotoxic; peptides **2E22K**, **3E22K**, and **4E22K** are cytotoxic. The capped mutant peptides were treated on SH-SY5Y cells to perform LDH release assays as previously described. Peptides **2**, **3**, and **4** have nominal charges of +1, +1, and 0 respectively, and do not cause observable LDH release at concentrations between 50 - 6.25 uM (Figure 1.15A). Peptides **2E22K** and **3E22K** have nominal charges of +3 at physiological pH, and cause LDH release at concentrations as low as 25 uM (Figure 1.15B). This behavior mirrors that of peptides **1E22G** and **1E22Q**, which also have nominal charges of +3 and cause LDH release at concentrations as low as 25 uM (Figure 1.3). Peptide **4E22K** has a nominal charge of +2 and induces cytotoxicity at a concentration of 50 uM, and not lower (Figure 1.15B). This behavior also mirrors that of other peptides with a nominal charge of +2, peptides **1**, **1E22D**, and **1A21G**, which induce cytotoxicity at concentrations of 50 uM, but not at lower concentrations (Figure 1.3). These results suggest that mutant peptide nominal charge supersedes amino acid residue composition in regards to a mutant peptides cytotoxicity. An increase in mutant peptide nominal

charge at physiological pH — (+1) < (+2) < (+3) < (+4) — correlates to an increase in cytotoxicity.

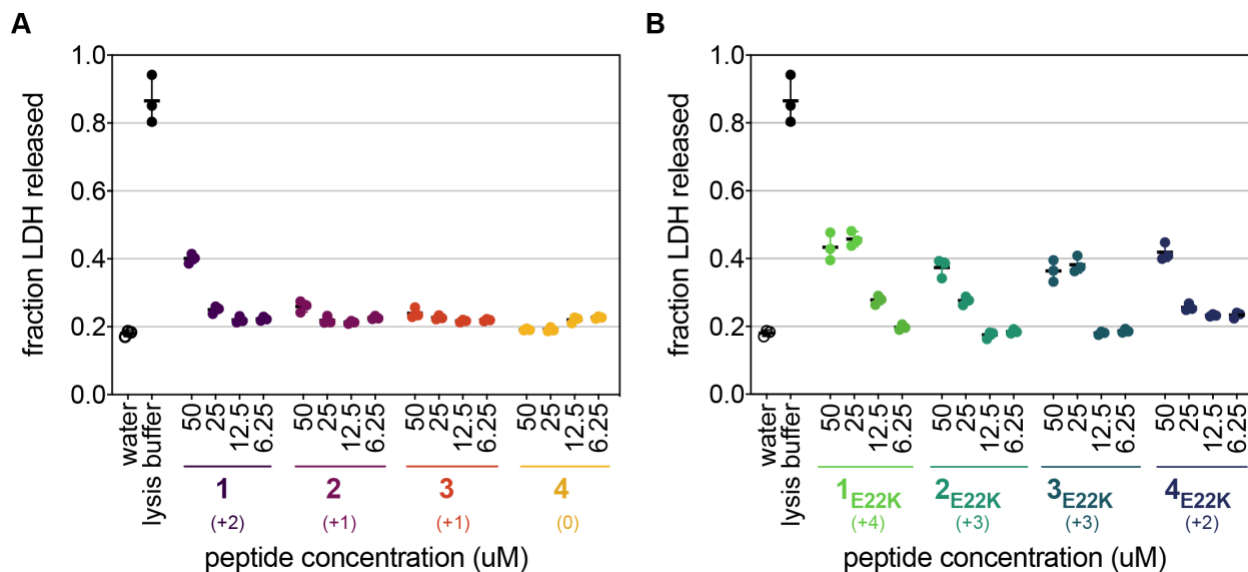


Figure 1.16. Peptides **2**, **3**, and **4** do not cause cytotoxicity to SH-SY5Y cells, but peptides **2E22K**, **3E22K**, and **4E22K** do. The LDH assay conditions and data analyses are the same as those explained in Figure 1.3. (A) Toxicity profiles of peptides **1**, **2**, **3**, and **4** at various concentrations. (B) Toxicity profiles of peptides **1E22K**, **2E22K**, **3E22K**, and **4E22K** at various concentrations.

1.2.5.2. SDS-PAGE

The capped mutant peptides assemble in SDS to form oligomers. SDS-PAGE gels were run using the same conditions as discussed previously and all mutant peptides were run at a concentration of 2.0 mg/mL. Peptide **2** runs as a comet-shaped band at an apparent molecular weight of a hexamer (~10.6 kDa). Peptide **3** also runs as a comet-shaped band, but at a lower molecular weight than peptide **2**, an apparent trimer (~5.3 kDa). Peptide **4** is not visible, which may be a result of precipitation upon peptide **4** sample preparation, which is unsurprising because it has a nominal charge of 0 and is not soluble in aqueous solution. Peptides **2** and **3** have nominal charges of +1. Thus far only one other mutant peptide, **1K16N**, has a nominal charge

of +1, and it runs as a trimers in SDS. Peptides **2** and **3** also run as higher order oligomers — hexamers and trimers.

Peptides **2_{E22K}** and **3_{E22K}** run as sharper bands at molecular weights consistent with hexamers (~10.6 kDa). How peptides **2_{E22K}** and **3_{E22K}** assemble in the presence of SDS mirrors how peptides **1_{E22G}** and **1_{E22Q}** assemble (Figure 1.5), which is unsurprising because all of these peptides have nominal charges of +3 at physiological pH. Peptide **4_{E22K}** also runs as an apparent hexamer, but it appears as a comet-shaped band, suggesting that it is in equilibrium with lower order oligomers. This behavior is also not surprising, as peptide **4_{E22K}** has a nominal charge of +2, and the observed behavior in the presence of SDS mirrors that of peptides **1** and **1_{E22D}** which also have nominal charges of +2 (Figure 1.5). The oligomerization of the capped peptides in the presence of SDS further suggests that mutant peptide nominal charge supersedes amino acid residue composition in regards to the propensity of mutant peptides to oligomerization. An increase in mutant peptide nominal charge at physiological pH — (+1) < (+2) < (+3) < (+4) — correlates to an increase in hexamer formation in the presence of SDS. Peptide **2** is the exception because it runs as a hexamer in SDS gels, which suggests that the cap on the top of peptide **1** strand facilitates oligomer formation more readily than the cap on the bottom strand.

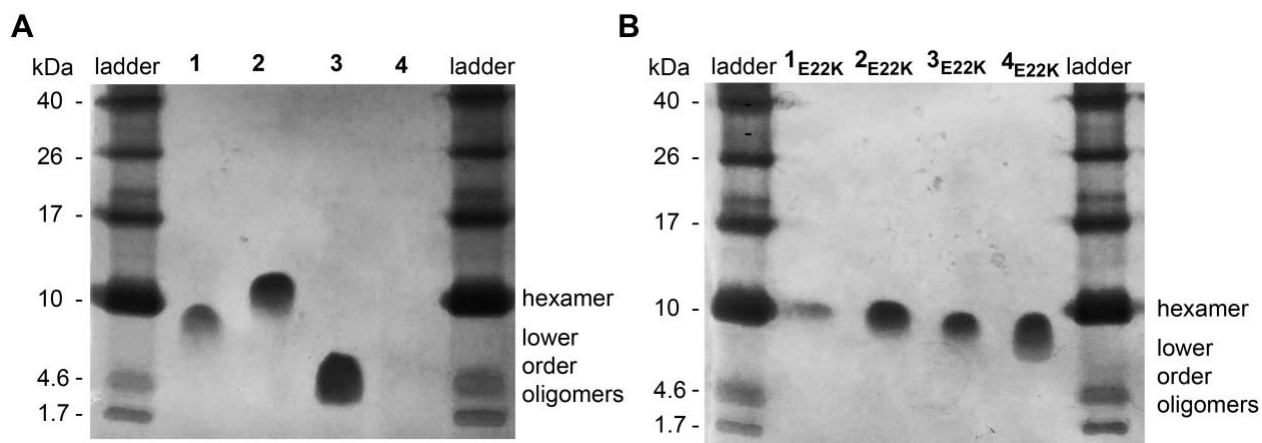


Figure 1.17. Peptides **2**, **3**, **4**, **2_{E22K}**, **3_{E22K}**, and **4_{E22K}** assemble as oligomers in SDS-PAGE gels. The conditions used for SDS-PAGE are the same as those described in Figure 1.5. All mutant peptides were run at a concentration of 2.0 mg/mL.

1.2.6. Capped Variants of Select E22 Mutant Peptides (2, 3, 4, 2E22Q, 3E22Q, 4E22Q, 2E22K, 3E22K, 4E22K) Discussion

Cytotoxicity and SDS-PAGE experiments suggest that the nominal charge of mutant peptides plays a significant role in their ability to cause cytotoxicity and assemble as oligomers. The charge trends discussed at length for peptides **1**, **1E22D**, **1E22G**, **1E22Q**, and **1E22K** remain the same in peptides **1**, **2**, **3**, **4**, and **1E22K**, **2E22K**, **3E22K**, **4E22K** even though each capped series has the same molecular composition except for one or two additional acetyl groups (Table 1.3). These charge trends suggest that mutant peptide nominal charge is more important to peptide cytotoxicity and assembly than individual amino acid residue identities, at least in the context of the peptide **1** model system. Once the COVID-19 stay-at-home orders are relaxed, dye leakage assays, CD, and SEC experiments will be completed on the three capped series of mutant peptides to determine if the charged trends continue.

Table 1.4. Summary of acetylated mutant peptides biophysical properties and assembly.

peptide	4	3	2	4E22Q	1	3E22Q	2E22Q	4E22K	1E22Q	3E22K	2E22K	1E22K
nominal charge at pH 7.4	0	+1	+1	+1	+2	+2	+2	+2	+3	+3	+3	+4
cytotoxicity (uM)	>50	>50	>50	—	50	—	—	50	25	25	25	12.5
SDS assembly	t	t	>h	—	h	—	—	h	—	h	h	h

*m = monomer, d = dimer, t = trimer, h = hexamer, β = beta-sheet, r = random coil, stuck = stuck on column

1.3 Conclusion

I incorporated FAD mutations of A β into a macrocyclic model system — peptide **1** — that can facilitate the study of FAD point mutations K16N, A21G, E22 Δ , E22G, E22Q, E22K, and L34V. I included these FAD mutations in the mutant peptides **1K16N**, **1A21G**, **1E22D**, **1E22G**, **1E22Q**, **1E22K**, and **1L34V**, and I synthesized the additional capped series of mutant peptides **2**, **3**, **4**, **2E22Q**, **3E22Q**, **4E22Q**, **2E22K**, **3E22K**, and **4E22K** to further probe the effects of nominal charge on the aqueous behavior, assembly, membrane destabilization, and cytotoxicity of mutant peptides. I also elucidated the X-ray crystallographic structures of the four E22 mutant peptides — **1E22D**, **1E22G**, **1E22Q**, and **1E22K** — which give insight into mutant peptide assembly at the molecular level.

CD and SEC studies yield insight into the aqueous behaviour of mutant peptides, SDS-PAGE gels reveal the assembled-state of mutant peptide oligomers, and dye leakage and cytotoxicity assays indicate the membrane destabilization and cytotoxicity propensities of mutant peptides. CD experiments indicate that mutant peptides (**1K16N**, **1A21G**, **1E22D**, **1E22G**, **1E22Q**, **1E22K**, and **1L34V**) have β -sheet character, suggesting that they all fold as β -sheets in aqueous solution. SEC chromatograms of peptides **1A21G**, **1E22D**, **1E22G**, **1E22Q**, and **1E22K** show that peptide oligomerization into dimers and trimers and propensity to stick to the agarose-dextran column correlates to peptide nominal charge, with higher nominally charged (+3 and +4) peptides sticking more to the column. SDS-PAGE experiments reveal that the 13 mutant peptides studied assemble as trimers or hexamers, with peptides that have increased nominal charge (+3 and +4) assembling as more stable hexamers. Membrane destabilization and cytotoxicity studies of 13 mutant peptides indicate that an increase in nominal charge — from +1 to +2 to +3 to +4 —

correlates to an increase in the propensity of mutant peptides to both destabilize membranes and cause cellular cytotoxicity.

The trend in mutant peptide behavior emerged as intrinsically tied to mutant peptide nominal charge, which made the assembly of peptides **1E22D**, **1E22G**, **1E22Q**, and **1E22K** at the molecular level surprising. Initially, I hypothesized that changes in nominal charge would lead to differences in mutant peptide assembly, but the X-ray crystallographic structures of peptides **1E22D**, **1E22G**, **1E22Q**, and **1E22K** reveal that they assemble to form the same hexamer. These assemblies may form in solution as well, so the hexamers may assemble further to form pore-like assemblies in cell membranes, subsequently causing membrane destabilization and cytotoxicity. No other mutant peptides crystallized, so I could not determine their X-ray crystallographic structures. However, since the peptide **1** hexamer can facilitate point mutations at the E22 position, the hexamer may also facilitate point mutations at the other locations studied, which could mean that the trimers and hexamers formed by the other mutant peptides in SDS-PAGE may assemble in a similar manner to those formed by peptides **1**, **1E22D**, **1E22G**, **1E22Q**, and **1E22K** in the crystal state.

The nominal charge of mutant peptides is intrinsically linked to aqueous behavior, assembly, membrane destabilization, and cytotoxicity. The emergence of the trend in increased nominal charge correlating to mutant peptide characteristics suggests that this trend may be relevant to the FAD mutations of WT A β .

1.4 Appendix

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General information

Unless otherwise stated, all materials and reagents were used as received. *N,N*-Dimethylformamide (DMF), amine-free and anhydrous, was bought from Alfa Aesar. Methylene chloride (CH_2Cl_2) was dried with alumina under nitrogen before use. Nano-pure, deionized water was obtained from a Barnstead NANOpure Diamond water purification system set to a minimum resistance of 18 M Ω . A Beckman Gold Series P or a Rainin Model SD-200 instrument with Agilent Zorbax SB-C18 columns were utilized to perform preparative reversed phase high performance liquid chromatography (RP-HPLC) purifications of all peptides. An Agilent 1200 or with a Phenomenex Aeris PEPTIDE 2.6u XB-C18 column was used to conduct analytical RP-HPLC analyses. Both preparative and analytical HPLC methods were performed using HPLC grade acetonitrile (CH_3CN) and nano-pure water containing 0.1% trifluoroacetic acid (TFA) each. Electrospray ionization mass spectrometry (ESI-MS) analyses were performed on a Micromass QTOF2 instrument. Pure peptides were utilized as the trifluoroacetate salts and were expected to have one molecule of TFA per amine group per molecule of peptide.

Peptide Synthesis

a. *Resin Loading.* All peptide analogues were made with 2-chlorotrityl chloride resin in order to yield carboxy termini for subsequent macrolactamization. Resin was placed in a 10 mL Bio-Rad PolyPrep chromatography column. Nine milliliters of dry CH_2Cl_2 were used to swell 300 mg 2-chlorotrityl chloride resin. Methylene chloride was drained from 2-chlorotrityl resin after swelling. Boc-Orn(Fmoc)-OH (0.5 equiv., 82 mg, 0.18 mmol) with 6% (v/v) 2,4,6-collidine was prepared as a solution in 8 mL dry CH_2Cl_2 , and added directly to the swelled 2-chlorotrityl resin. The resin-filled column was rocked gently for 12 h, followed by draining and washing with dry CH_2Cl_2 (3 x 9 mL). A 10 mL solution of CH_2Cl_2 :MeOH:*N,N*-

diisopropylethylamine (DIPEA) (17:2:1) was subsequently added to the column and left rocking for one hour in order to cap unreacted positions on the 2-chlorotrityl resin. The resin was then washed with dry CH₂Cl₂ (3 x 9 mL) followed by dry DMF (3 x 9 mL) before transferring to an automated peptide synthesizer reaction vessel.

b. *Peptide coupling.* All peptide synthesis steps, including peptide couplings, washings, and deprotections were performed on either a CEM Liberty 1 Automated Microwave Peptide Synthesizer or a Protein Technologies PS3 synthesizer. Linear peptides were constructed from C- to N-terminus using Fmoc-protected amino acids (AAs), with each coupling step comprising the following; 1) addition of 20% (v/v) piperidine in DMF to remove Fmoc from protected N-terminal amines (2 x 2 min.), 2) DMF washes (3 x 9 mL), 3) addition of Fmoc-AA-OH (0.75 mmol, 5.0 equiv.), *O*-(6-chlorobenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HCTU) (0.68 mmol, 4.5 equiv.), and DIPEA (0.9 mmol, 6.0 equiv.) in DMF for peptide coupling (20 min. each), 4) repeat cycles from steps 2–4. Once the final AAs were coupled, one last round of Fmoc deprotection was conducted as described above. The resin was then removed from the automated synthesizer reaction vessel and transferred back to a Bio-Rad Poly-Prep chromatography column.

c. *Acetylation of Orn N-terminal amine.* After Fmoc-Orn(Dde)-OH was incorporated into the peptide chain, the Fmoc group was deprotected with 20% (v/v) piperidine, followed by washing 3x with DMF. Acetylation of the free N-terminal amine of Orn was done by adding acetic anhydride:pyridine (3:2) to the coupling vessel and leaving for 30 min. The resin was then washed 5x with DMF before proceeding to hydrazine deprotection of Dde.

d. *Hydrazine deprotection of Dde.* After Fmoc deprotection and acetylation of the Orn N-terminal amine with piperidine and washing 3x with DMF, 10% hydrazine in DMF was

added to the resin for 20 min at ambient temperature, bubbling under nitrogen, to remove the Dde protecting group from the Orn side-chain amine. The resin was subsequently washed with DMF 8x before proceeding with normal peptide coupling of the rest of the peptide chain.

e. *Peptide cleavage from resin.* Linear peptides from 2-chlorotrityl chloride resin were cleaved without removing side-chain protecting groups. 20% 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) in CH₂Cl₂ (10 mL) was added to the resin in a PolyPrep column and left to rock for one hour. The filtrate was drained into a 250 mL round-bottom flask, and an additional 10 mL of HFIP cleavage cocktail was added to resin and left to rock for 30 minutes. The solution was then drained into the same round-bottom flask. The HFIP filtrates were subjected to rotary evaporation to yield white solids. Linear peptides were dried by vacuum pump followed directly by macrolactamization.

f. *Linear peptide macrolactamization.* Side-chain protected peptides were dissolved in 125 mL DMF before addition of *O*-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) (320 mg, 0.75 mmol, 5 equiv.), 1-hydroxybenzotriazole (HOBT) (110 mg, 0.75 mmol, 5 equiv.) and DIPEA (0.33 mL, 1.8 mmol, 12 equiv.). The reaction mixture was then stirred under nitrogen for 48 h. Upon completion of macrolactamization, rotary evaporation was utilized to remove excess solvent and yield yellow-brown solids.

g. *Cyclic peptide global deprotection.* Cyclic peptides were globally deprotected with TFA:TIPS:H₂O (18:1:1). The solution was added to the Poly-Prep column and left rocking for one hour. The supernatant was added directly to the round-bottom flask with the cyclized peptide. The reaction mixture was stirred for 1.5 h, followed by rotary evaporation to remove excess solvent. The yellow cyclic peptides were then purified by RP-HPLC (described below).

h. *Purification of peptides using reversed phase HPLC.* Peptides were dissolved in 20% aqueous acetonitrile (CH₃CN), and the resulting clear yellow solutions were purified by RP-HPLC. Peptides were injected at 20% aqueous CH₃CN (with 0.1% TFA) and eluted with a 20–40% gradient of CH₃CN over 30 min. All peptides containing two disulfide bridges eluted from 32-35% CH₃CN, while peptides containing one disulfide bridge eluted from 25-30% CH₃CN. Upon complete elution of desired peptides, column was washed with 95% CH₃CN to ensure all peptidic material was removed from the column. Fractions were analyzed with analytical HPLC and ESI-MS, pure fractions were combined, and excess solvent was removed utilizing rotary evaporation. The concentrated peptides were re-dissolved in filtered nano-pure water and lyophilized to yield white fluffy powders.

Cytotoxicity Assays

LDH assays were conducted as previously described.^{54,57} Into a 96-well plate, 100 μ L of 1:1 DMEM/F12 media — supplemented with 10% FBS, 100 U/mL penicillin and 100 μ g/mL streptomycin at pH 7.4 — containing 15,000 SH-SY5Y cells was placed into each well and left in an incubator at 37°C with 5% CO₂ atmosphere for 24 h. To prepare for treatment with peptides, the medium on the plate was aspirated and replaced with 90 μ L of serum-free, phenol-red free 1:1 DMEM/F12 media. Peptides were prepared gravimetrically as stock solutions in nanopure water at 10 mg/mL. These stocks were used to prepare samples that were 10X concentrated than the test concentrations of the plate. Final dilutions were prepared by adding 10 μ L of the 10X samples into the wells to bring their total volume up to 100 μ L. Each concentration had five technical replicates, and the cells were left to incubate with peptide for 72 h in the aforementioned incubator.

A Thermo Scientific LDH assay was performed on the plate after 72 h. Cell supernatant (50 μ L) was transferred to a new clean, clear-bottom 96-well plate and analyzed for the presence of LDH through spectrophotometric detection according to the manufacturer's instructions.

Dye Leakage Assays

Large unilamellar vesicles (LUVs) composed of PC or PC:PS lipids were prepared as previously described.⁷² Chicken egg-derived L- α -phosphatidylcholine (PC, product number: 840051C) and porcine brain-derived L- α -phosphatidylserine (PS, product number: 840032) were obtained from Avanti Polar Lipids as 10 mg/mL solutions in chloroform. LUV preparation was done by using 2.6 μ mol lipids — only PC for neutral LUVs or 1:1 molar ratio of PC:PS for negatively charged LUVs.

Lipids for LUVs were left under a stream of nitrogen to remove chloroform. Lipids were then placed under vacuum in the absence of light for 12 h. The lipid cake was then hydrated with leakage buffer (10 mM Tris, pH 7.4, 150 mM NaCl and 1 mM EDTA) supplemented by the addition of 70 mM calcein, freeze-thawed with a dry-ice/acetone bath three times, and then extruded through 100 nm filters. The LUVs were then purified from the free calcein remaining in buffer through separation with a Sephadex G-50 column. Upon elution of yellow fractions that did not fluoresce under long-wave UV light, a successful purification was achieved. The concentrations of the purified LUVs was then determined using a modified phosphorus assay,⁵⁸ as previously described.⁵⁹

LUVs were prepared to a final concentration of 11 μ M. Peptides were prepared at 10X working concentration from 10 mg/mL solutions in nanopure water. The 10X peptide solutions were transferred to the wells of a 96-well, black side, clear bottom, plate in 20 μ L aliquots. Lysis buffer was also added in the same volume as peptide as the 100% leakage control, and nanopure

water was used as the 0% leakage control. Each sample was run in three technical replicates. Once each well had sample, 180 μ L of the LUV suspension was added to the plate using a multi-channel pipette. Fluorescence was recorded immediately on a ThermoFisher Varioskan LUX fluorescent plate reader, with an excitation wavelength of 490 nm and emission recording of 520 nm. Each set of replicates was averaged and data was plotted as follows:

$$\% \text{ dye leakage} = 100 \times (F_{\text{peptide}} - F_{\text{water}}) / (F_{\text{lysis buffer}} - F_{\text{water}})$$

F_{peptide} = average fluorescence of peptide wells

F_{water} = average fluorescence of water wells

$F_{\text{lysis buffer}}$ = average fluorescence of lysis buffer wells

SDS-PAGE

Tricine SDS-PAGE was employed to study the oligomerization of the mutant peptides. All reagents and gels were prepared according to previously established methods.⁶⁰ Every peptide was prepared to 10 mg/mL in deionized water. These solutions were diluted with deionized water to yield 4.0 mg/mL aliquots. These were then diluted again with 4x LDS loading buffer to give final concentrations of 2.0 mg/mL working concentrations of all peptides. Aliquots of 5.0 μ L of 2.0 mg/mL solutions were loaded onto gels containing 16% polyacrylamide gels with 4% stacking polyacrylamide gels. The gels were then run at 80 volts at room temperature. Silver nitrate staining was used to stain all peptide bands on the SDS-PAGE gel. All materials were prepared according to previously established procedures.⁶¹ The silver stain developing was stopped once the ideal amount of staining was obtained.

Circular Dichroism Spectroscopy

A Jasco J-810 Circular Dichroism Spectropolarimeter was used to obtain all circular dichroism (CD) spectra. Mutant peptides were prepared as 50 μ M solutions in 10 mM potassium phosphate buffer at pH 7.4. Data was averaged over 10 accumulations that were acquired at 0.5 nm intervals from 190 to 260 nm and graphed as mean residue ellipticity. Mean residue ellipticity, $[\Theta]$, is calculated as follows:^{62,63}

$$[\Theta] = \text{millidegrees}/(\text{path length}(\text{mm}) \times [\text{peptide}] (\text{M}) \times \text{number of residues})$$

Size Exclusion Chromatography

SEC was performed on mutant peptides using an AKTA Explorer 10 FPLC with a GE Superdex 75 10/300 column at ambient temperature. Mutant peptides and standards were prepared at a 10 mg/mL in deionized water and then diluted to 1.0 mg/mL in TBS (50 mM Tris buffer, pH 7.5, and 100 mM NaCl) to a final volume of 700 μ L. Mutant peptide and standard solutions were centrifuged at 12,000 RPM for two minutes, then the soluble material in the supernatant was loaded onto the column at 0.5 mL/min for 1 min. Once loaded, mutant peptide and standard samples were run at 1.0 mL/min in TBS buffer. SEC chromatograms were collected at 214 nm and normalized to the highest absorbance value.

X-ray Crystallography

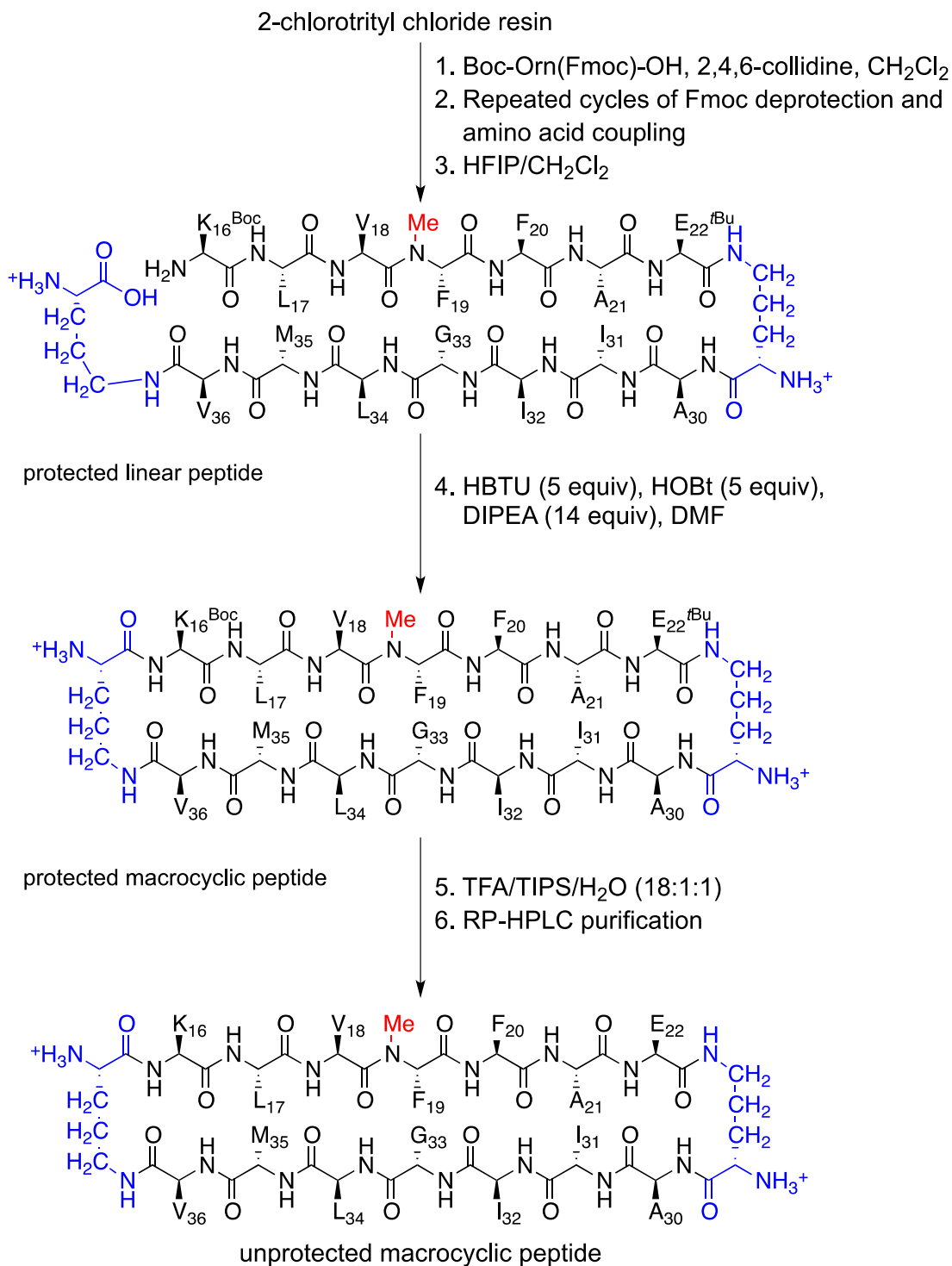
Crystal Screens

Peptides were screened for crystal growth in 96-well plates utilizing the hanging-drop vapor-diffusion method. Crystal Screen, Index, and PEG/ION kits from Hampton Research were

employed to set up 288 crystallization conditions per peptide. All conditions were present at 100 μ L volumes in every screen. A TPP Labtech Mosquito robot was used to set up the three hanging drops in each plate, with each drop being formed by combining 150 nL of a 10 mg/mL aqueous peptide stock solution with 150 nL of well solution. Optimization of wells in which peptide crystals grew was conducted in a 4 x 6 well plate (Hampton VDX 24-well plate). Optimizations were carried out by varying pH and concentration of cryoprotectant, and the hanging drops were placed on glass slides in 1:1, 1:2 and 2:1 peptide to well solution ratios.

X-ray Diffraction Data Collection, Processing, and Structure Determination

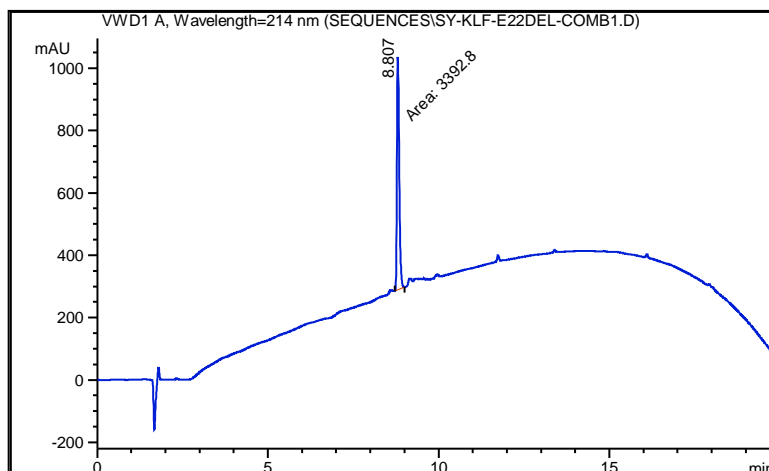
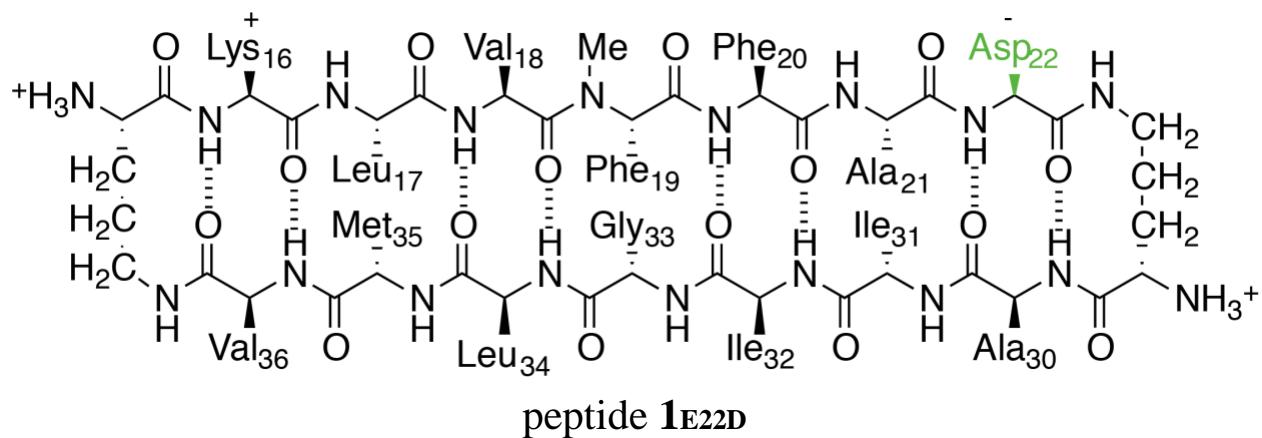
Diffraction data for peptides **1E22D**, **1E22G**, **1E22Q**, and **1E22K** was collected on a Rigaku Micromax-007HF X-ray diffractometer containing a rotating copper anode at wavelength of 1.54 Å and 0.5° oscillation. Crystal Clear software was employed to collect diffraction data and iMosflm scaled and merged the diffraction data for peptide **1E22Q**.⁷³ Diffraction data for peptides **1E22D**, **1E22G**, and **1E22K** were scaled and merged using XDS.⁷⁴ Coordinates for the anomalous signals were determined by HySS in the Phenix software suite 1.10.1.⁶⁴ Electron density maps were generated using anomalous coordinates determined by HySS as initial positions in Autosol. The electron density map for peptide **1E22G** was generated through single-wavelength anomalous diffraction (S-SAD) using the anomalous signal from the three iodide atoms in the I₃⁻ that was incorporate into the crystal lattice through soaking with KI and I₂ using HuSS in the Phenix software suite 1.10.1. The coordinates from the X-ray crystal structure of peptide **1** were used in molecular replacement to generate the electron density map of peptide **1E22Q**.⁶⁴ Coordinates from the X-ray crystal structure of peptide **1E22G** were used in molecular replacement to generate the electron density maps of peptides **1E22D** and **1E22K**. Model molecular manipulations were conducted with Coot,⁷⁴ and phenix.refine was used to refine coordinates.

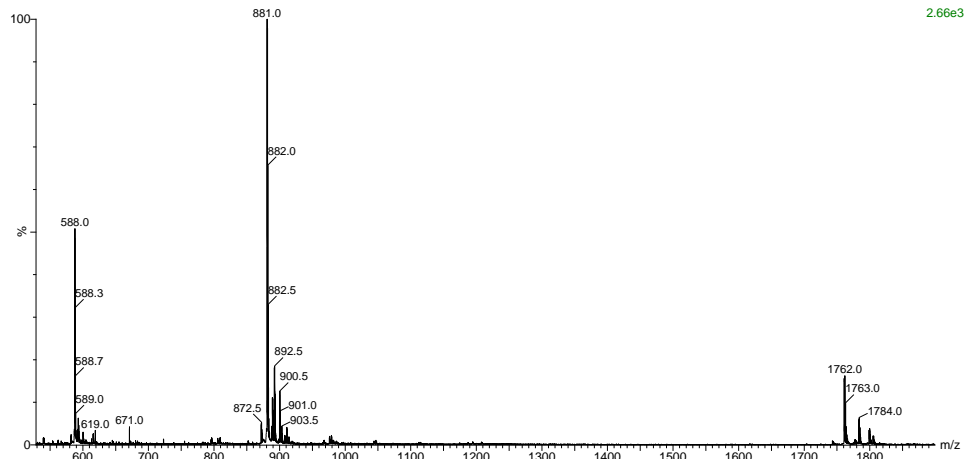


Scheme 1.1. Representative synthetic scheme of macrocyclic peptide **1** and mutant peptides.

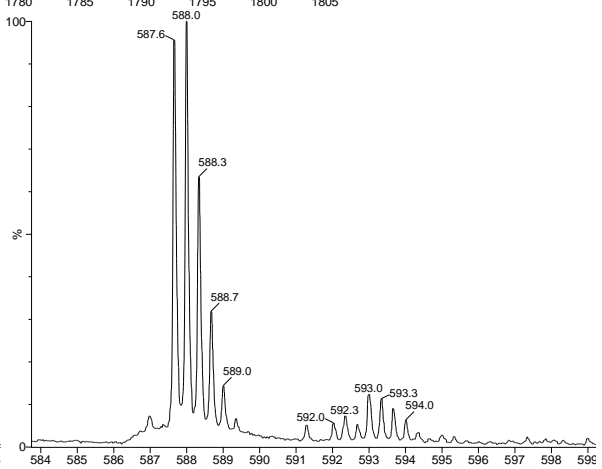
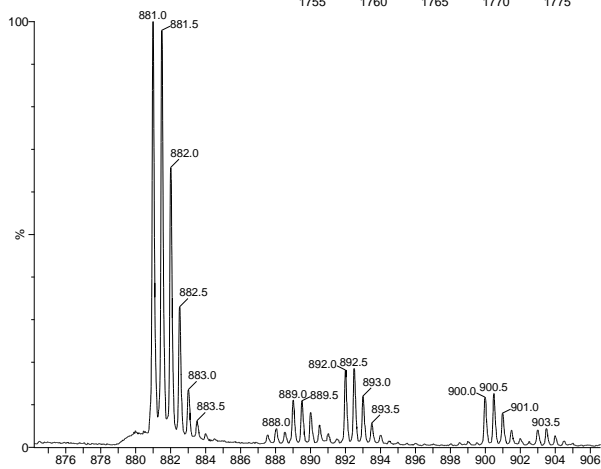
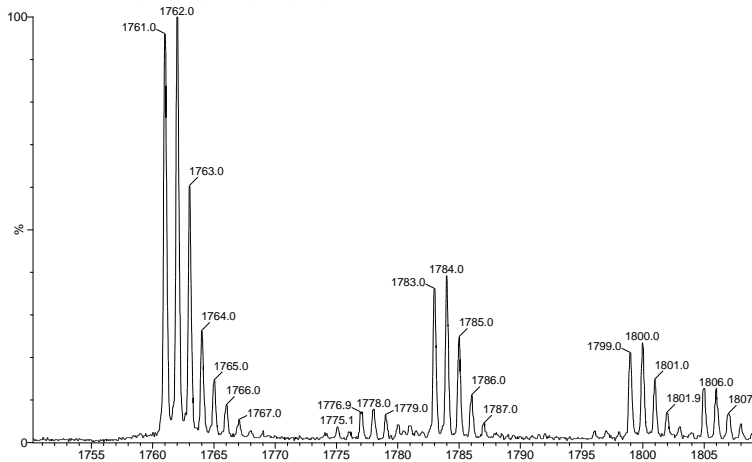
Characterization Data

Characterization of peptide **1E22D**



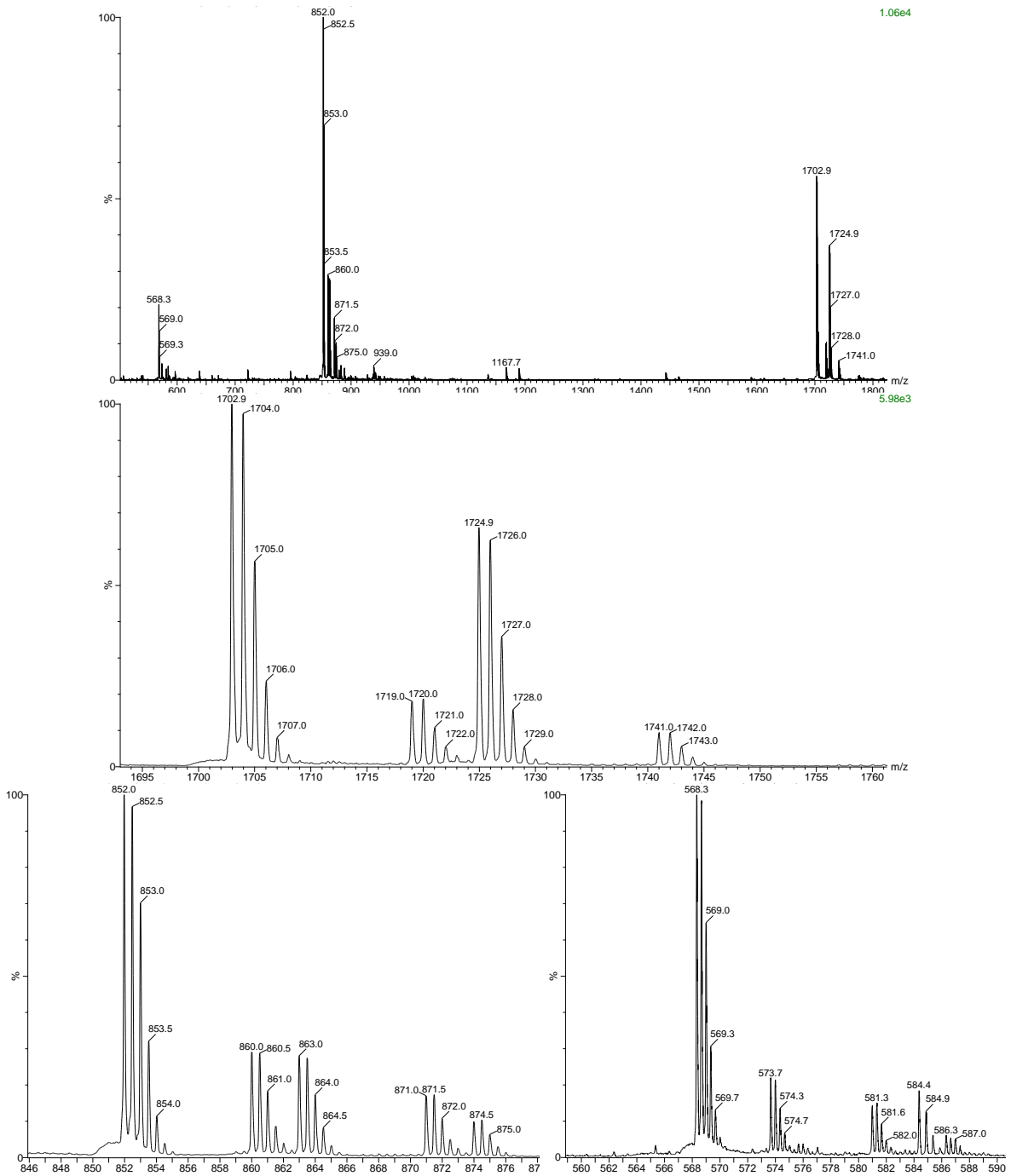


2.66e3



Calculated mass for peptide peptide **1E22D**:
1760.04 g/mol

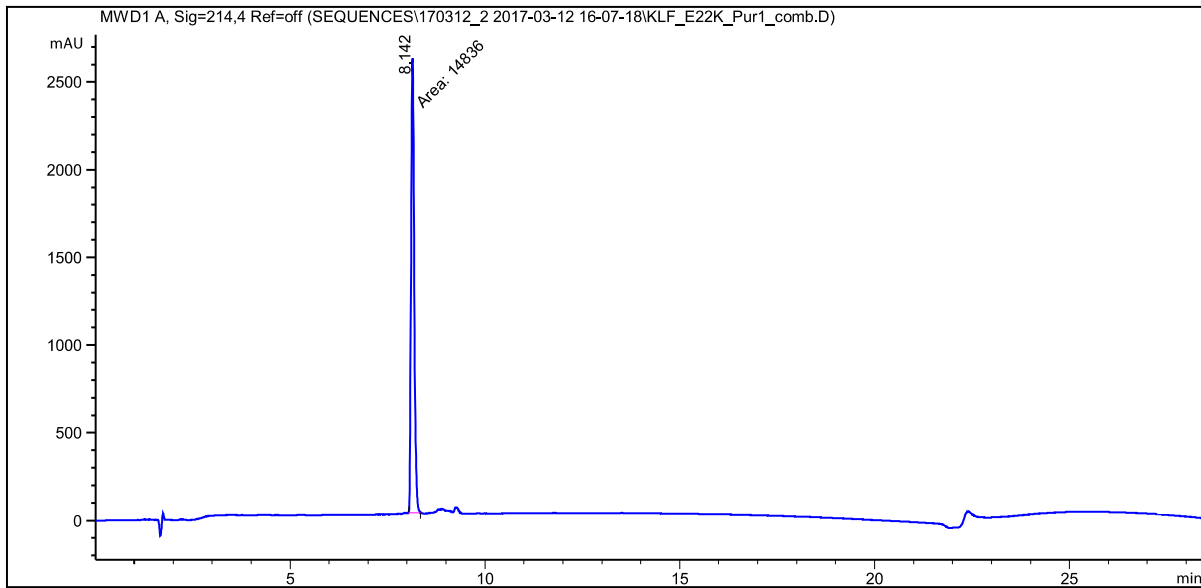
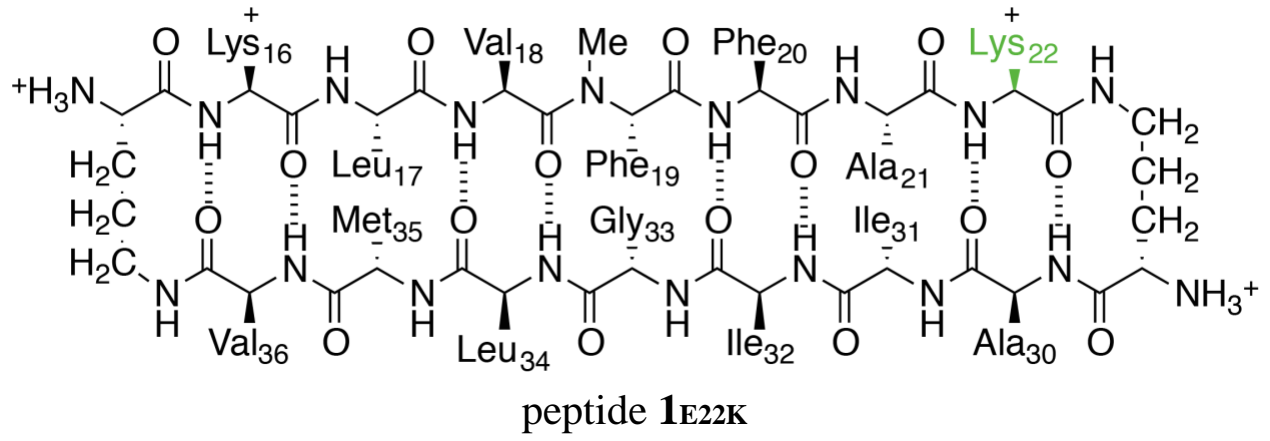
$[M+1H]^+ = 1761.0$
 $[M+1Na]^+ = 1783.0$
 $[M+2H]^{2+} = 881.0$
 $[M+H+Na]^{2+} = 892.0$
 $[M+3H]^{3+} = 587.6$

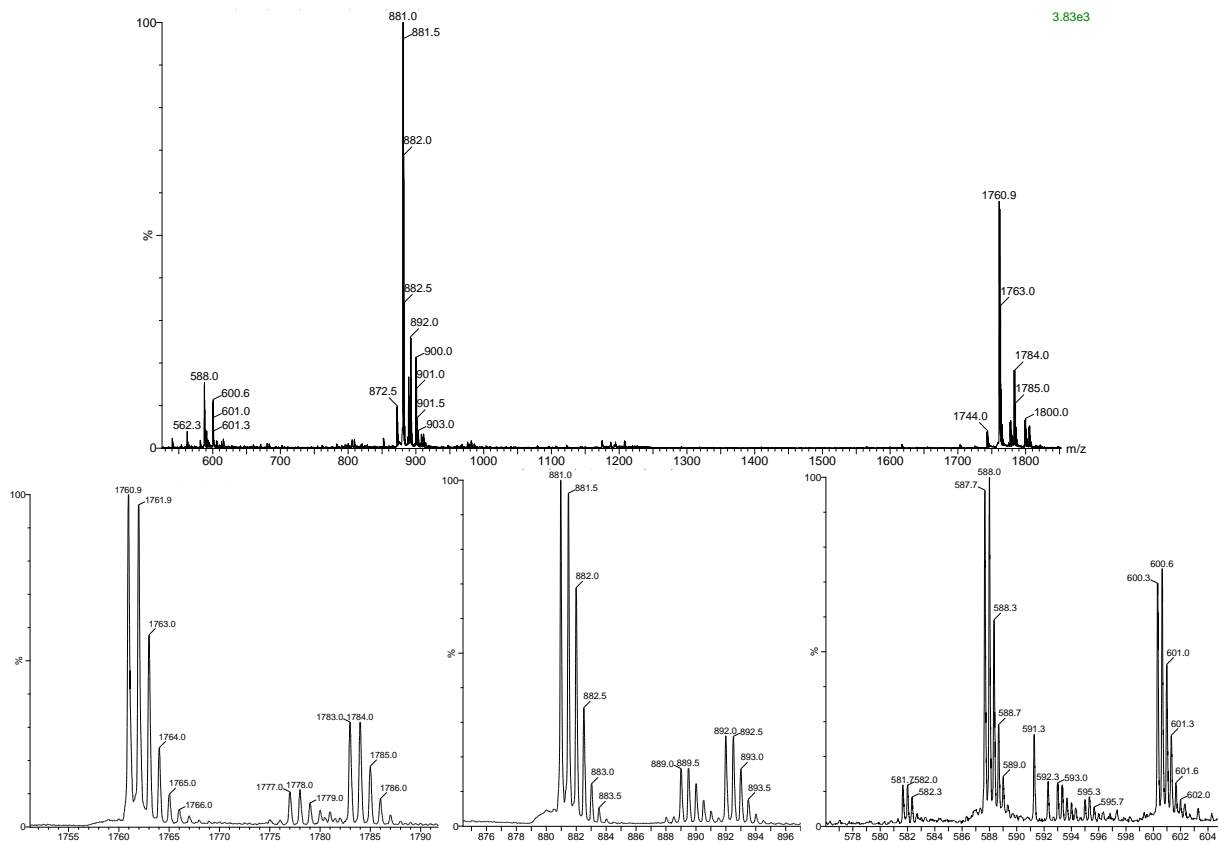


Calculated mass for peptide peptide **1E22G**:
 1702.04 g/mol

$[M+1H_+]_+ = 1702.9$
 $[M+1Na_+]_+ = 1724.9$
 $[M+2H_+]_{2+} = 852.0$
 $[M+H_++Na+]_{2+} = 863.0$
 $[M+3H_+]_{3+} = 568.3$

Characterization of peptide **1E22K**





Calculated mass for peptide peptide 1E22K:

1759.11 g/mol

$[M+1H]^+ = 1760.9$

$[M+1Na]^+ = 1783.0$

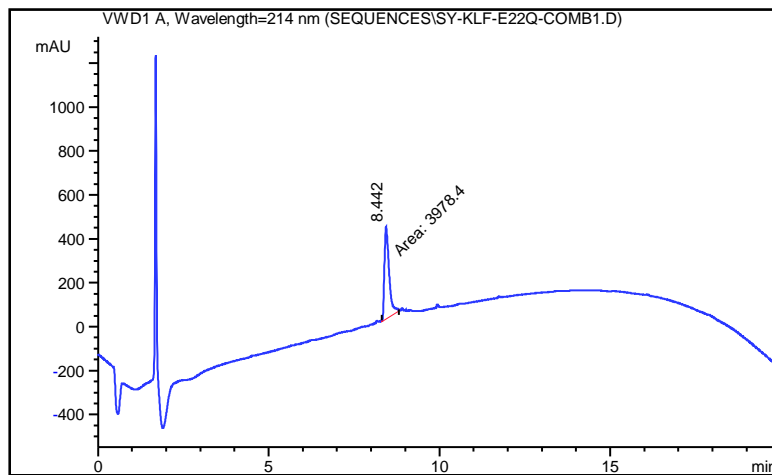
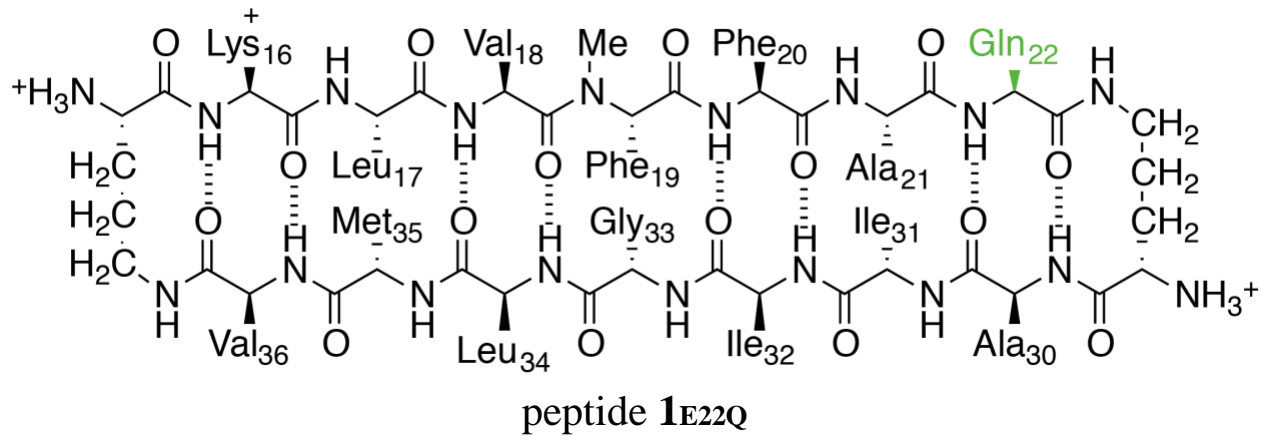
$[M+2H]^{2+} = 881.0$

$[M+H+Na]^{2+} = 892.0$

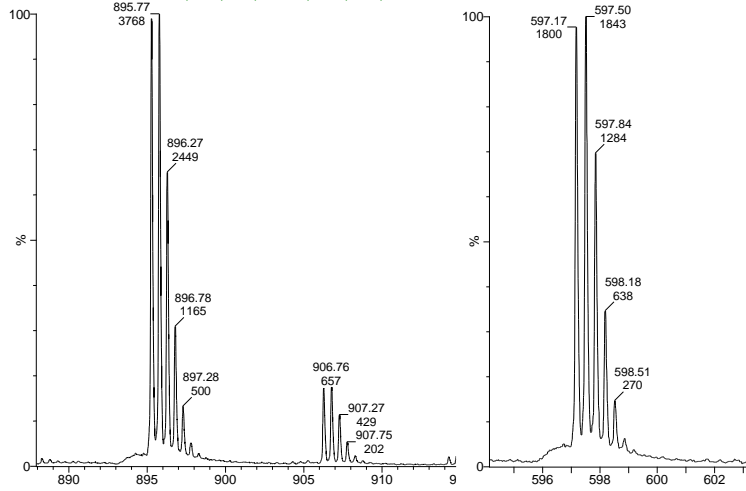
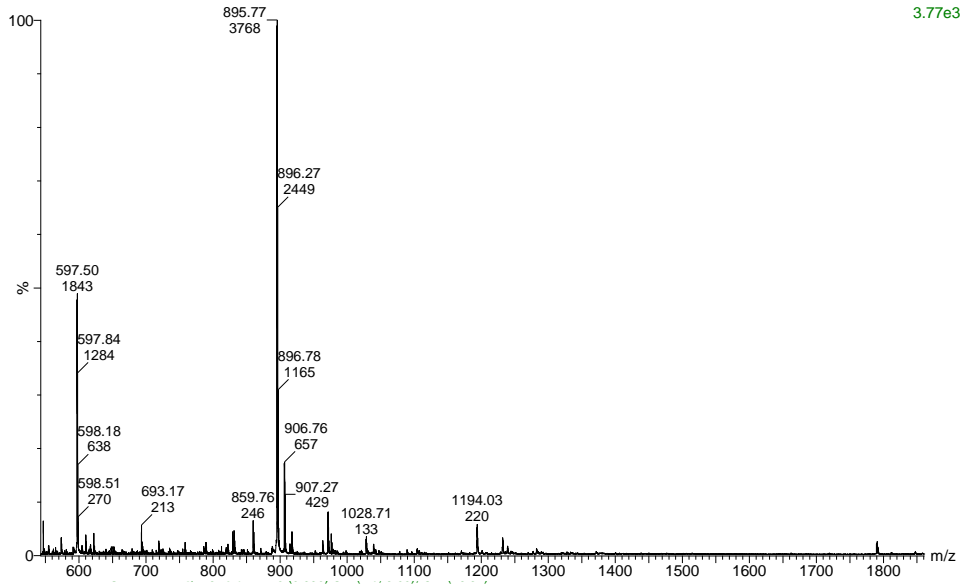
$[M+3H]^{3+} = 587.7$

$[M+2H+Na]^{3+} = 600.3$

Characterization of peptide **1E22Q**



3.77e3



Calculated mass for peptide peptide **1E22Q**:
1773.07 g/mol

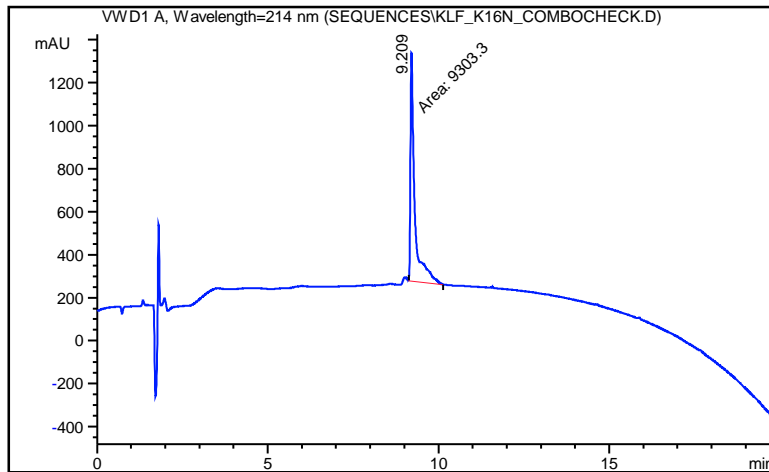
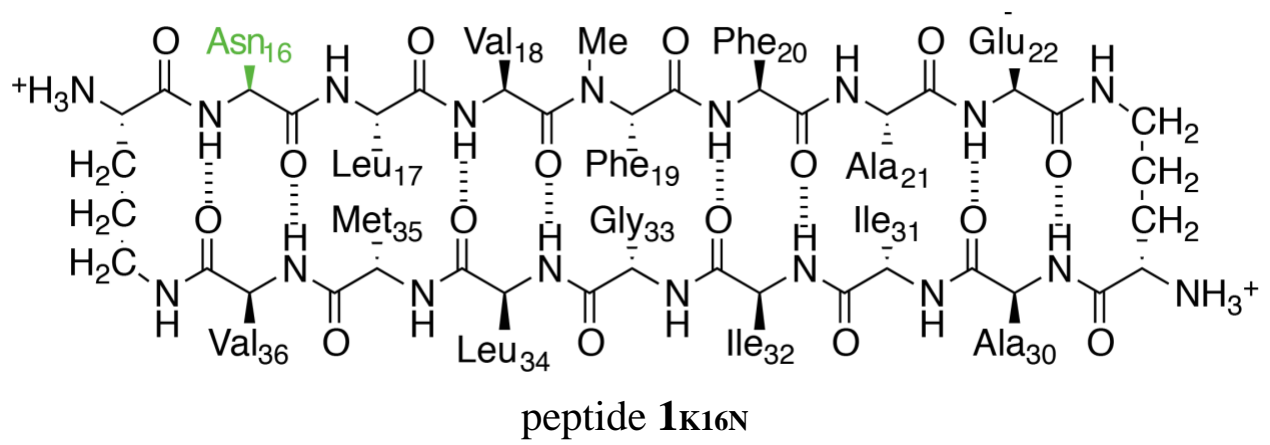
$[2M+3H_+]_{3+} = 1194.3$

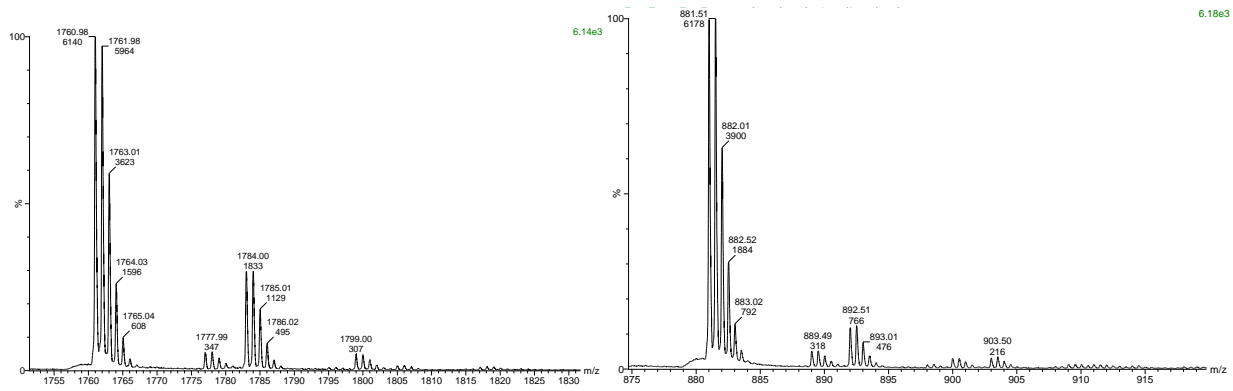
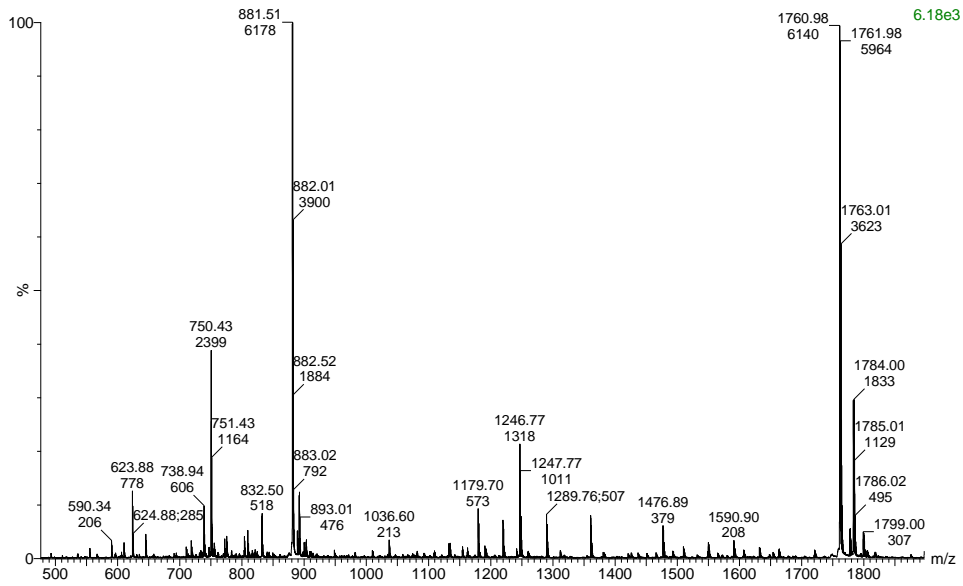
$[M+2H_+]_{2+} = 895.$

$[M+H_++Na+]_{2+} = 906.8$

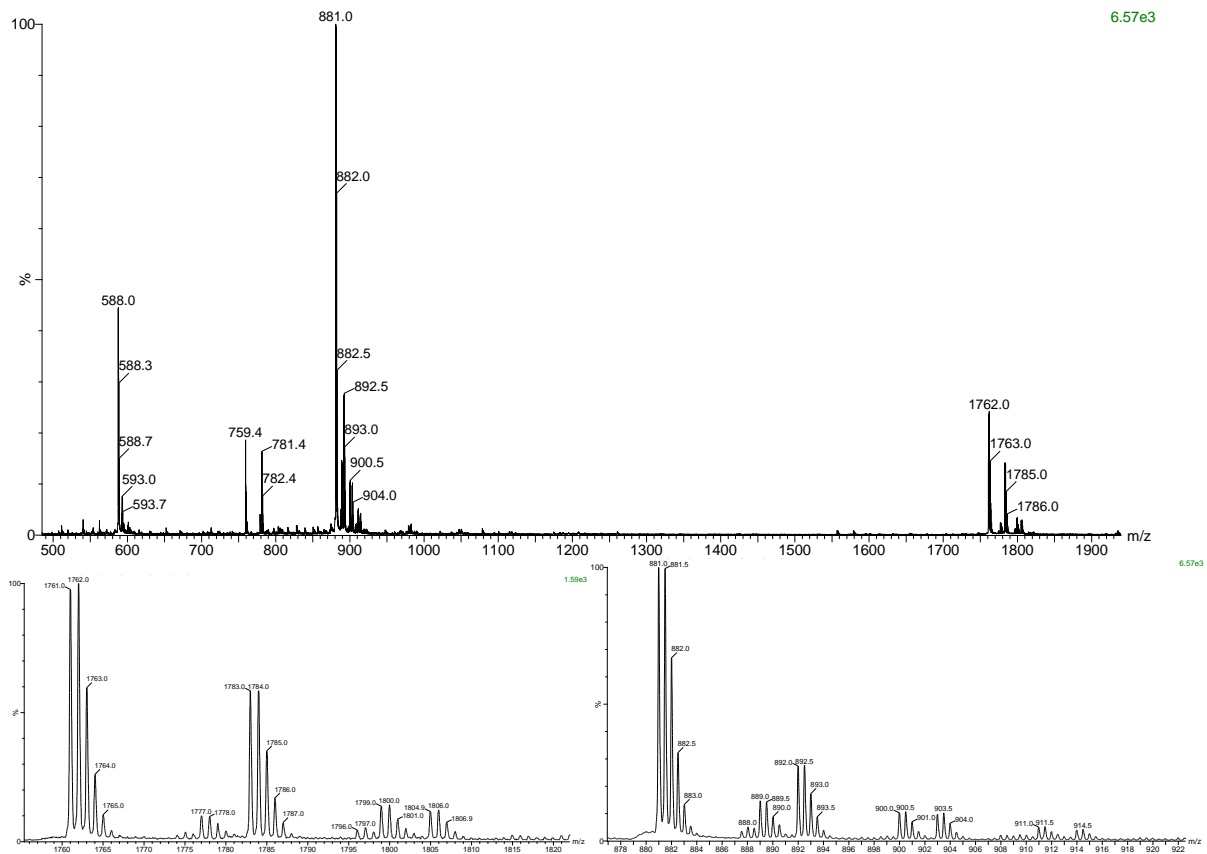
$[M+3H_+]_{3+} = 597.2$

Characterization of peptide **1K16N**



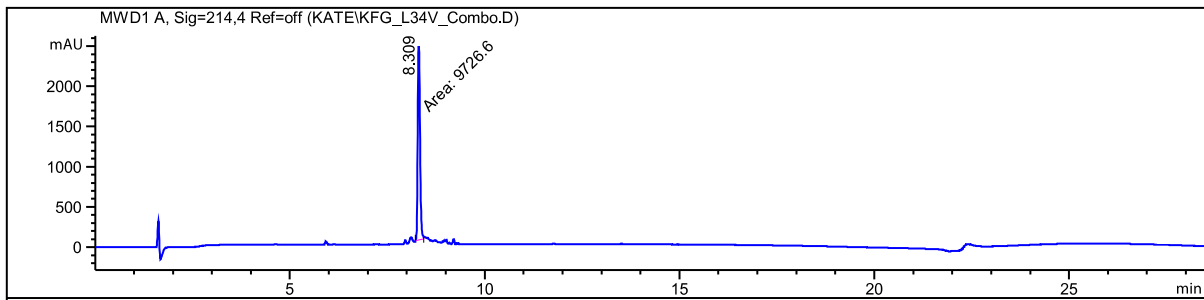
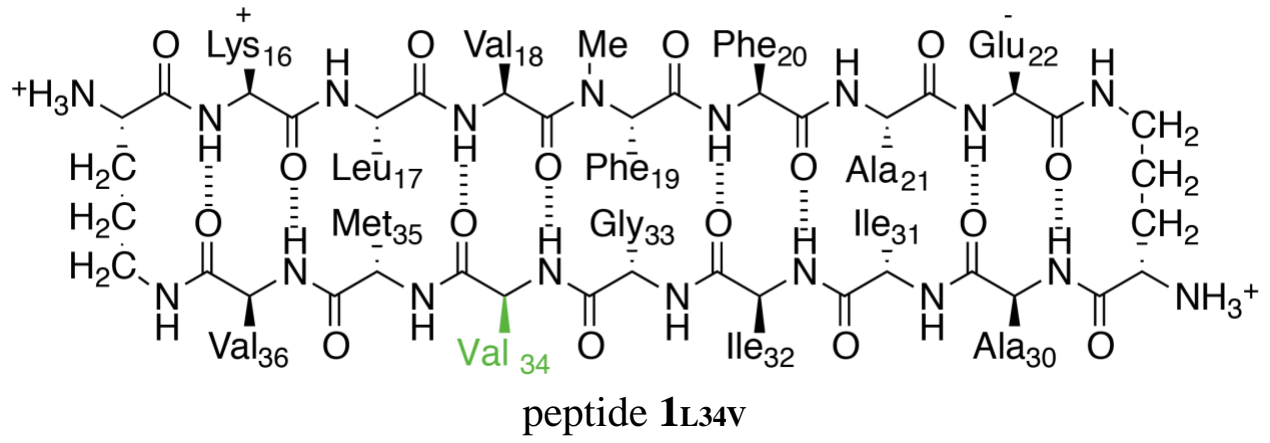


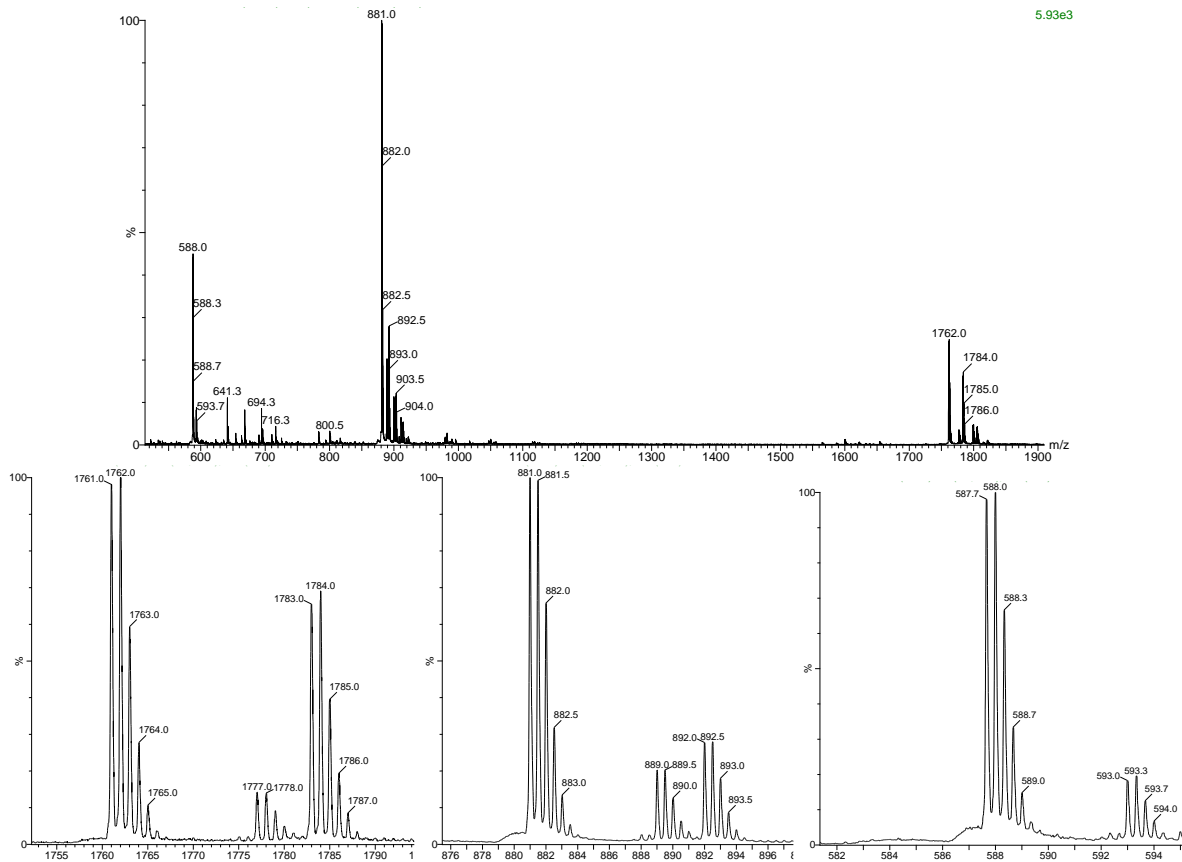
<p>Calculated mass for peptide 1K16N: 1760.01 g/mol</p> <p>$[M+1H^+]_+ = 1761.0$</p> <p>$[M+1Na^+]_+ = 1784.0$</p> <p>$[M+2H^+]_{2+} = 881.5$</p> <p>$[M+H^++Na^+]_{2+} = 892.5$</p>
--



<p>Calculated mass for peptide peptide 1A21G: 1760.04 g/mol</p> <p>$[M+1H_+]_+ = 1761.0$ $[M+1Na+]_+ = 1783.0$ $[M+2H_+]_{2+} = 881.0$ $[M+H_++Na+]_{2+} = 892.0$ $[M+3H_+]_{3+} = 588.0$</p>
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Characterization of peptide **1L34V**





Calculated mass for peptide peptide **1L34V**:
1760.04 g/mol

$[M+1H_+]_+ = 1761.0$

$[M+1Na_+]_+ = 1783.0$

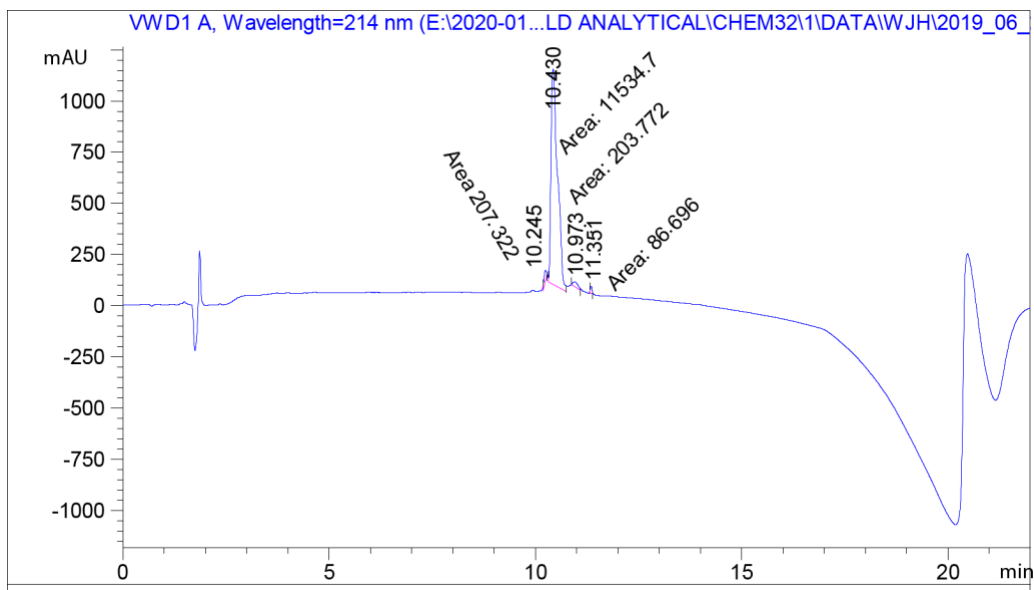
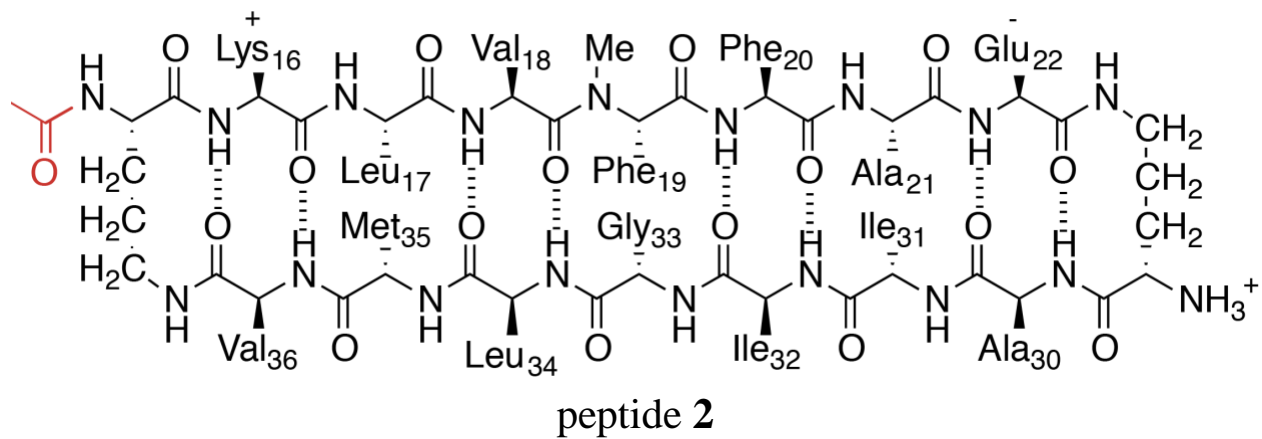
$[M+2H_+]_{2+} = 881.0$

$[M+H_++Na_+]_{2+} = 892.0$

$[M+3H_+]_{3+} = 587.7$

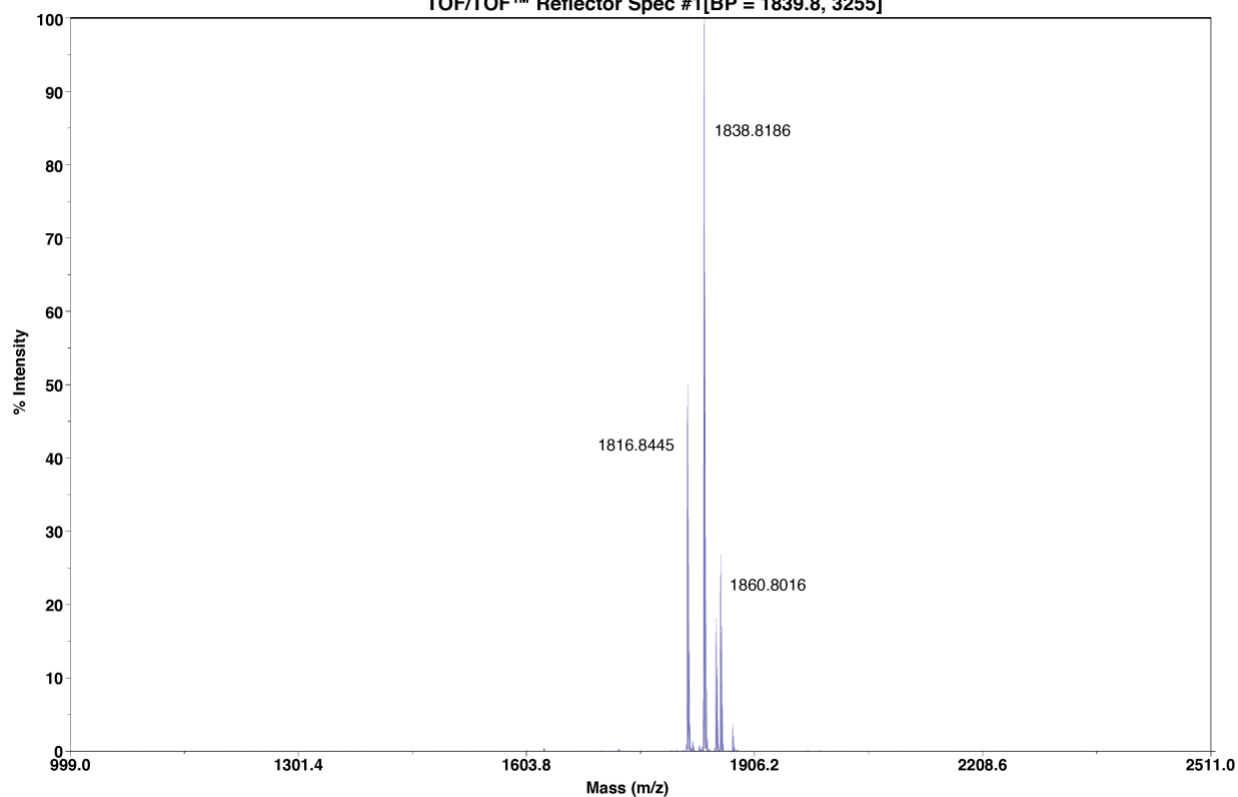
$[M+2H_++Na_+]_{3+} = 593.0$

Characterization of peptide 2

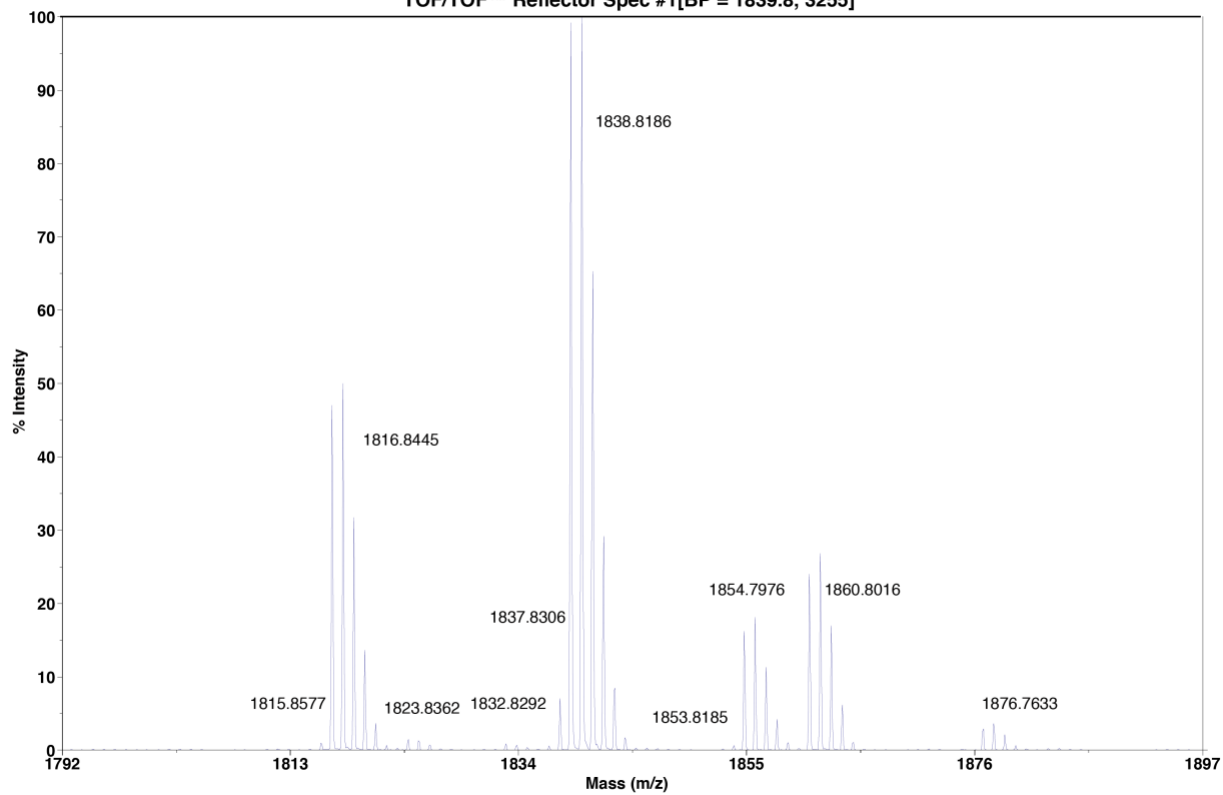


Calculated mass for peptide peptide 2: 1817.10 g/mol
[M+H ₊] ₊ = 1818.10
[M+Na ₊] ₊ = 1840.10
[M+K ₊] ₊ = 1856.1

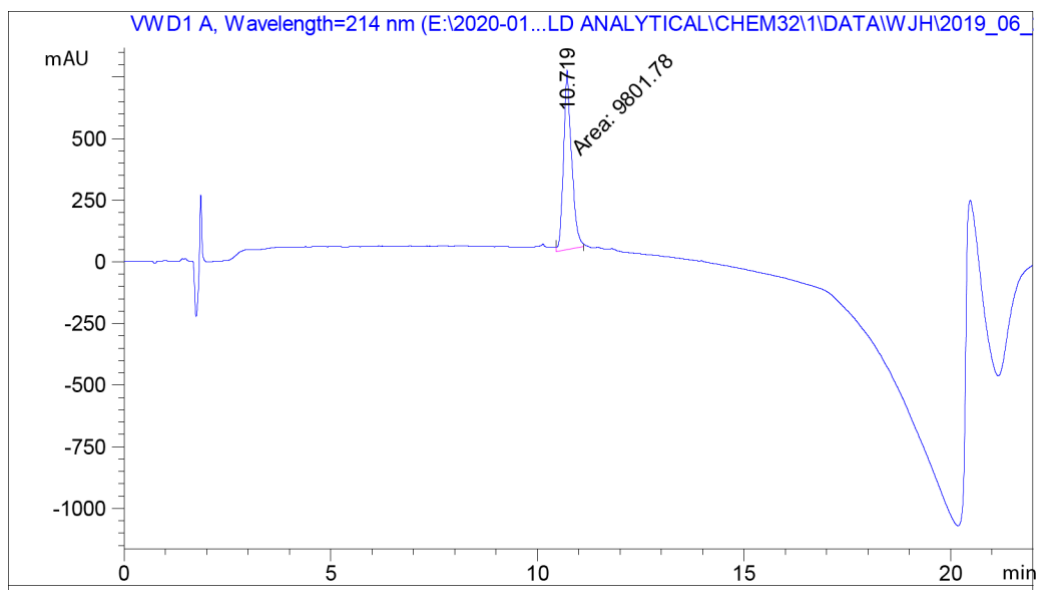
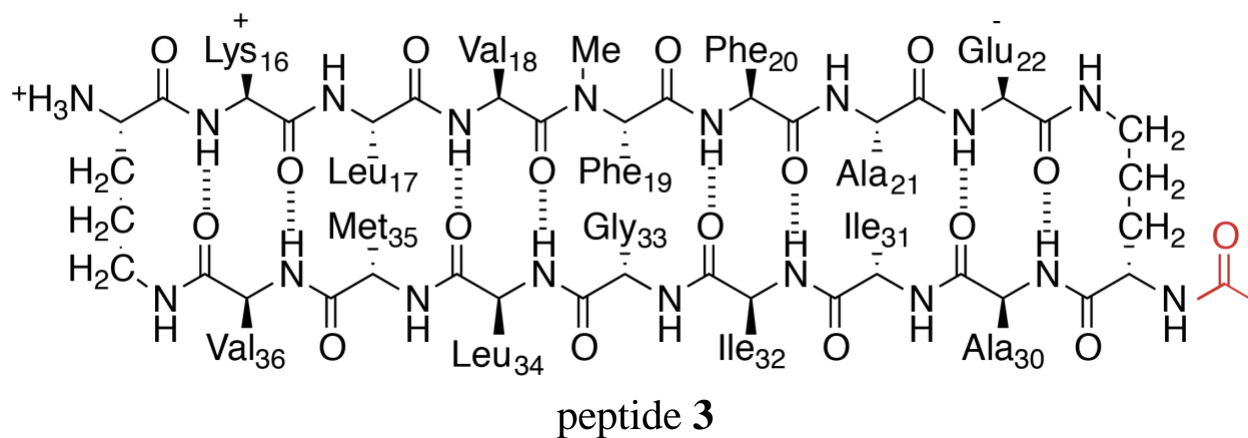
Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1839.8, 3255]



Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1839.8, 3255]

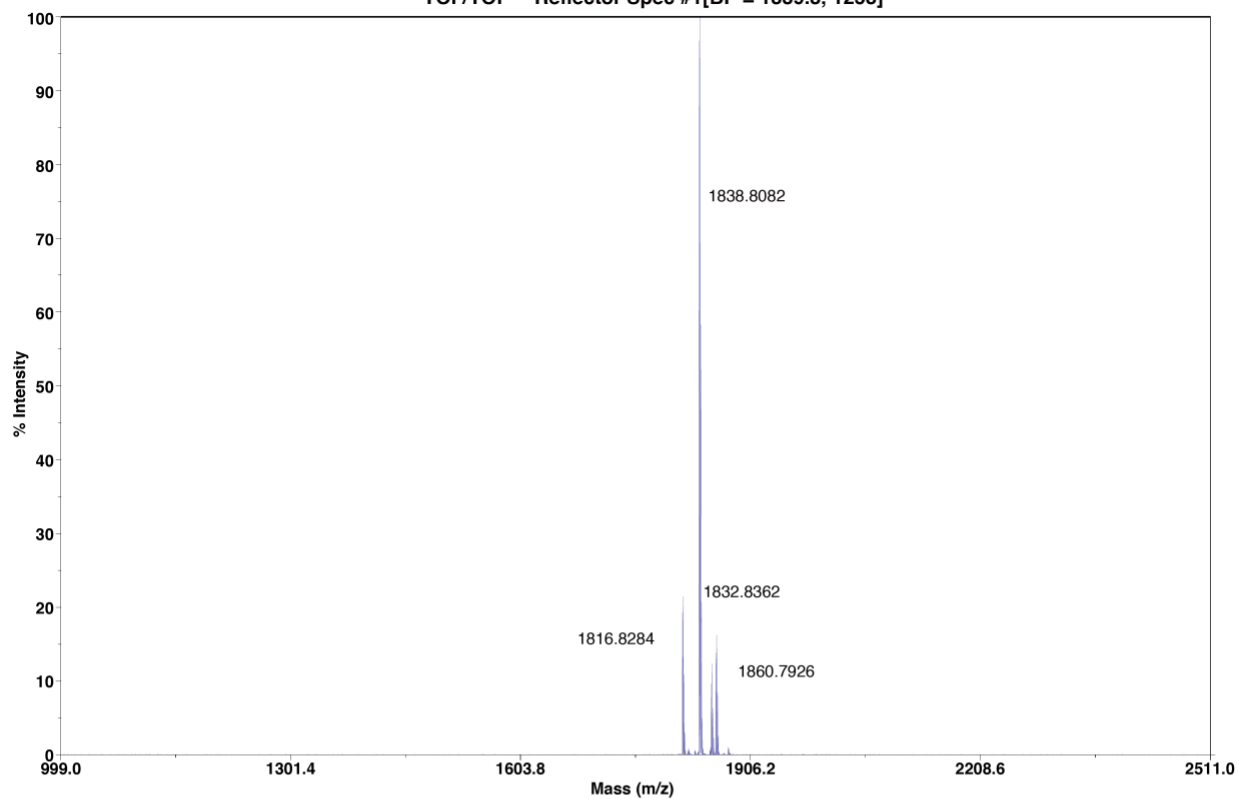


Characterization of peptide 3

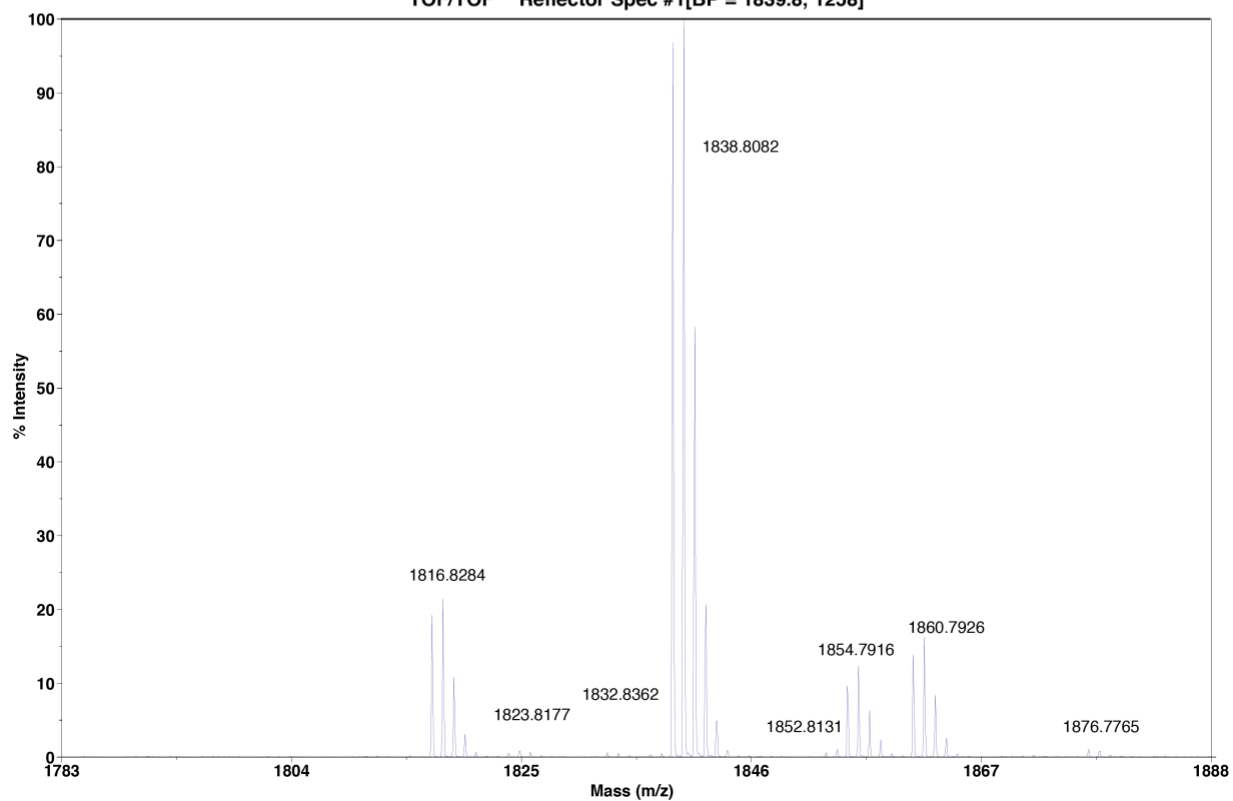


Calculated mass for peptide peptide 3: 1817.10 g/mol
[M+H ₊] ₊ = 1818.10
[M+Na ₊] ₊ = 1840.10
[M+K ₊] ₊ = 1856.1

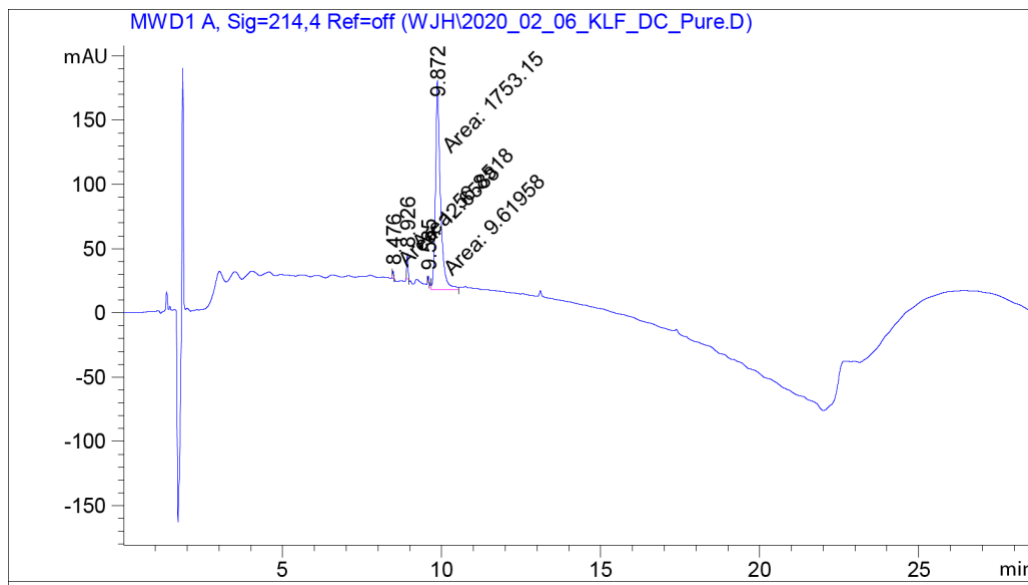
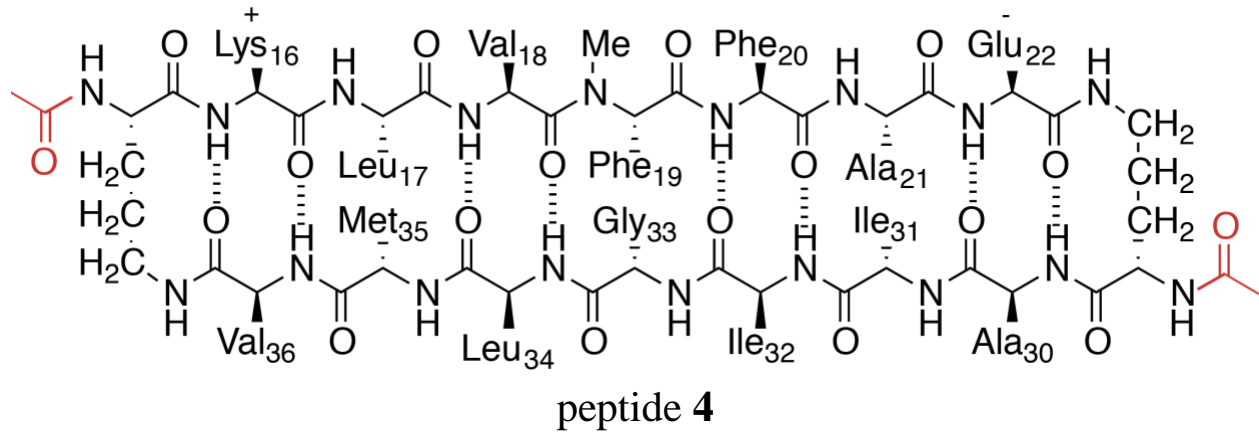
Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1839.8, 1258]



Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1839.8, 1258]

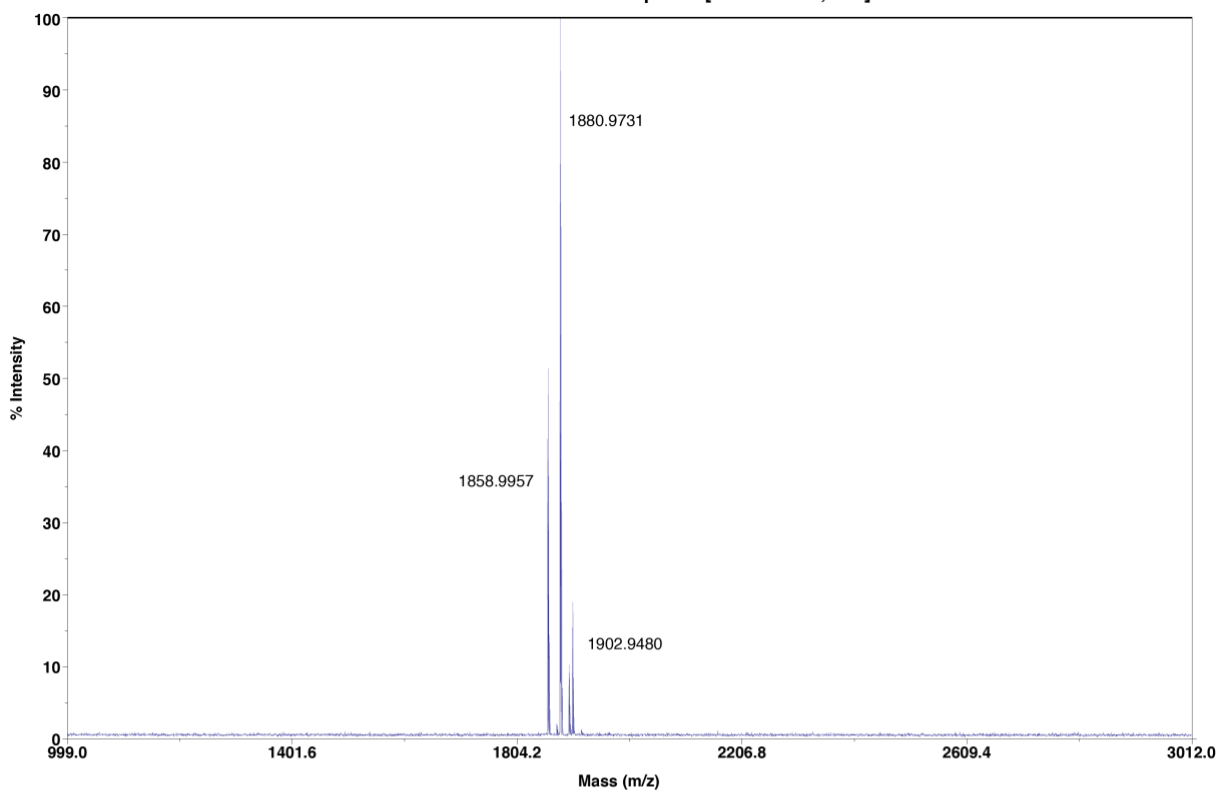


Characterization of peptide 4

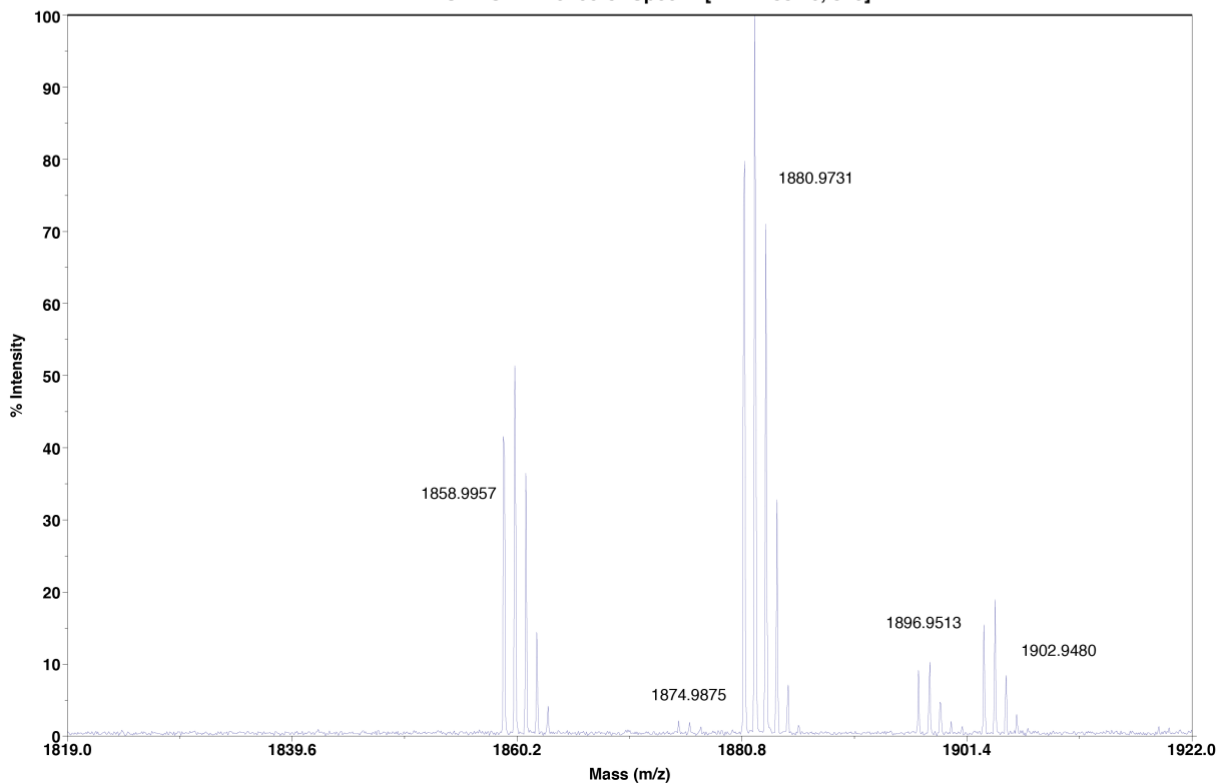


Calculated mass for peptide peptide 4: 1860.15 g/mol
[M+H ⁺] ₊ = 1861.15
[M+Na ⁺] ₊ = 1883.15
[M+K ⁺] ₊ = 1899.15

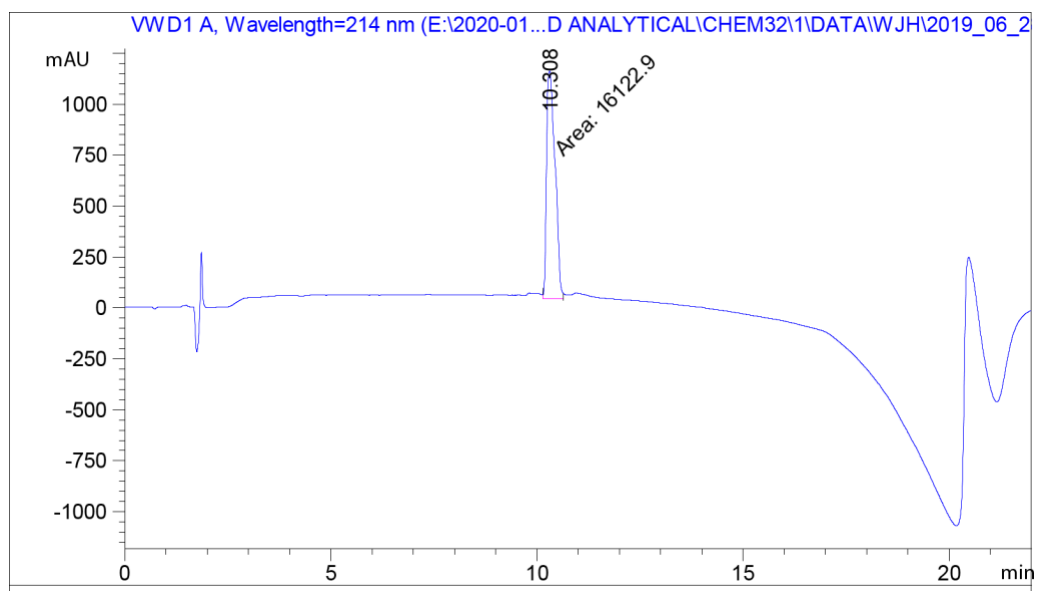
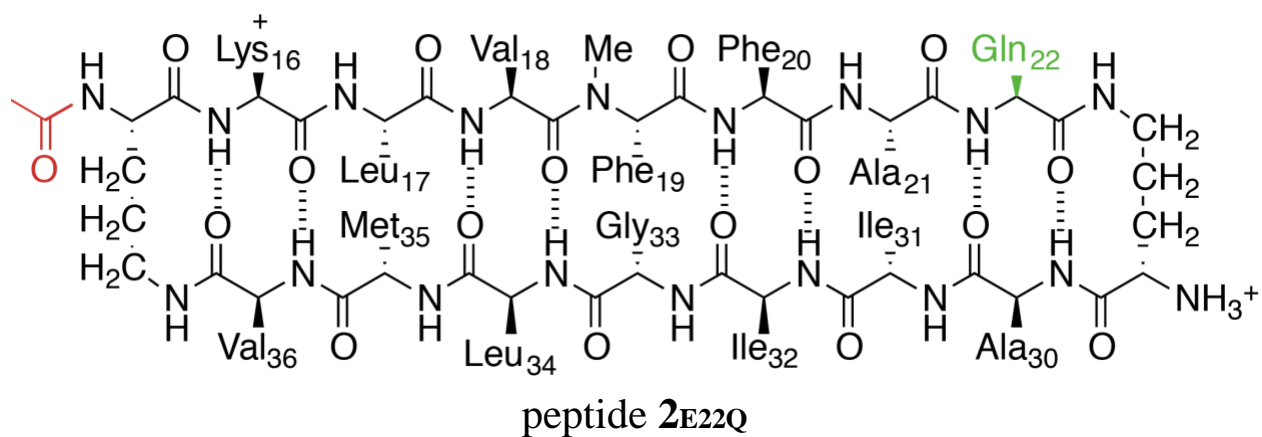
Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1882.0, 375]



Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1882.0, 375]



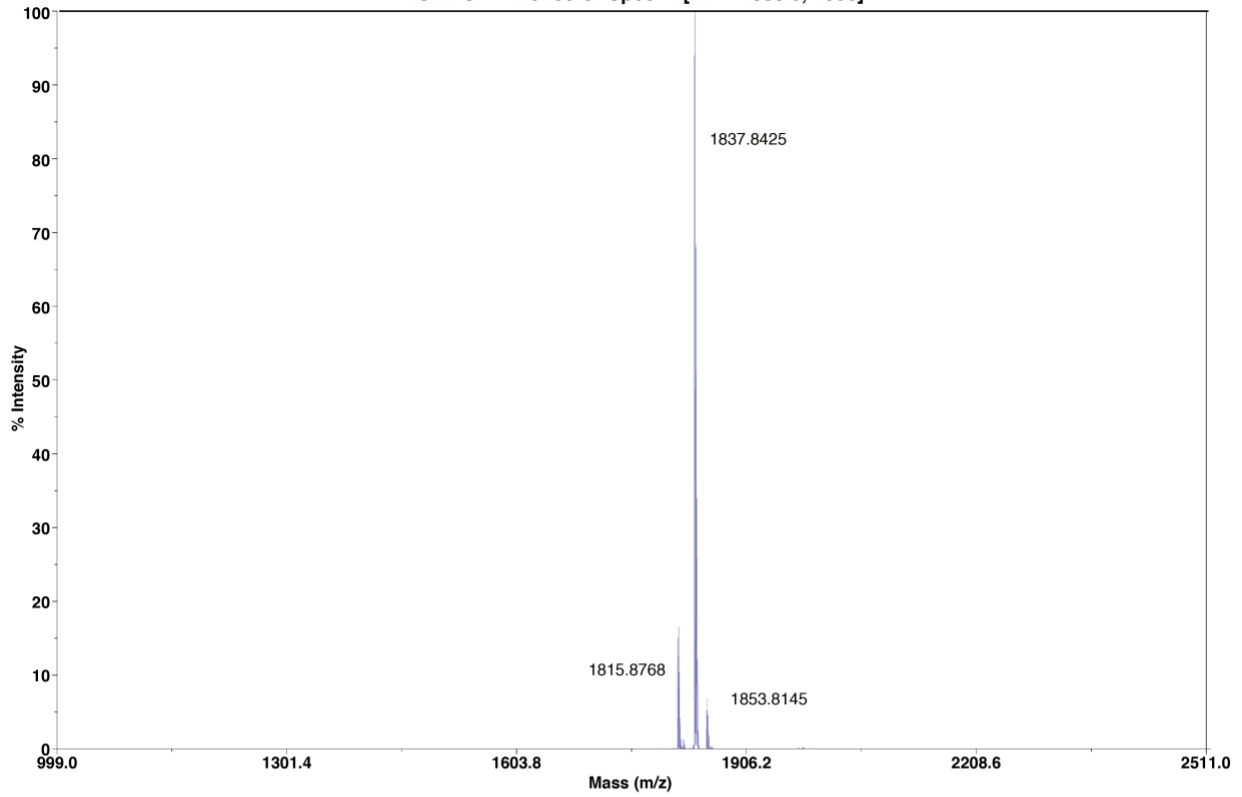
Characterization of peptide 2E22Q



Calculated mass for peptide peptide 2E22Q: 1816.12 g/mol
[M+H ₊] ₊ = 1817.12
[M+Na ₊] ₊ = 1839.12
[M+K ₊] ₊ = 1855.12

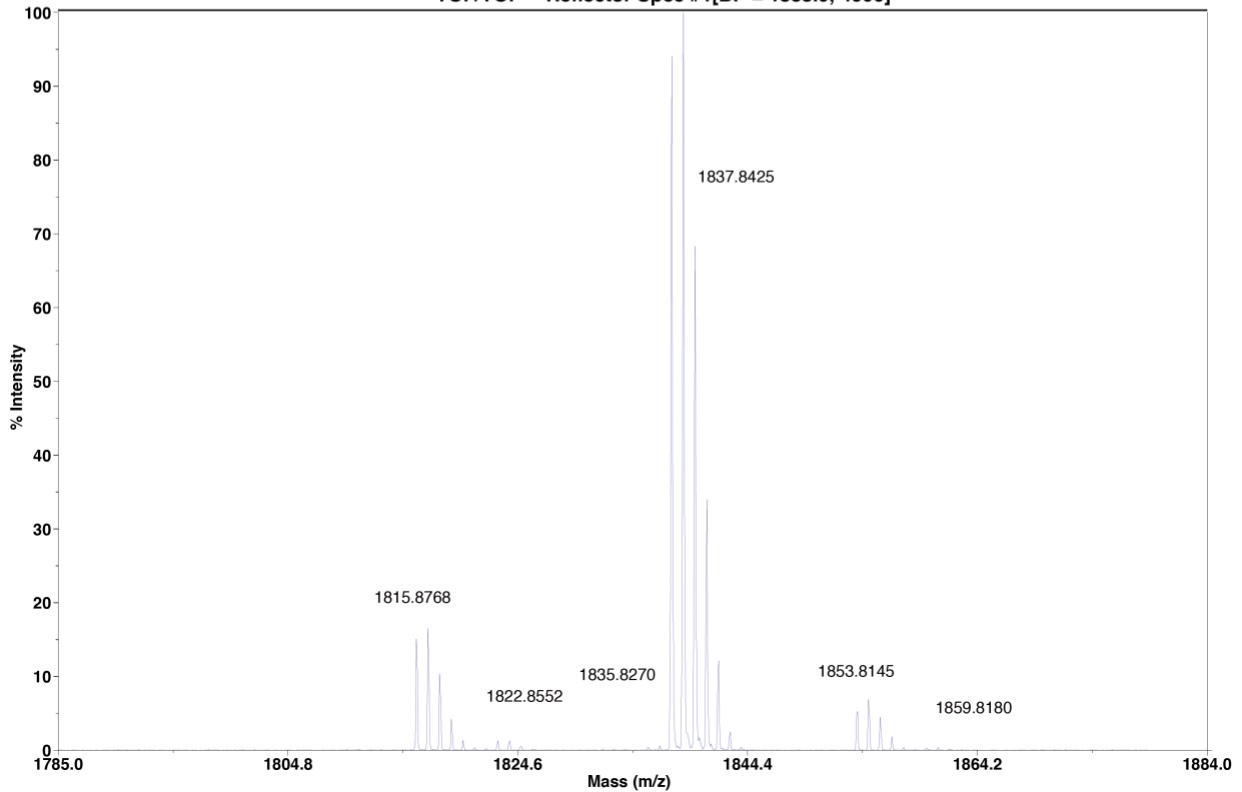
Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039

TOF/TOF™ Reflector Spec #1[BP = 1838.9, 4096]

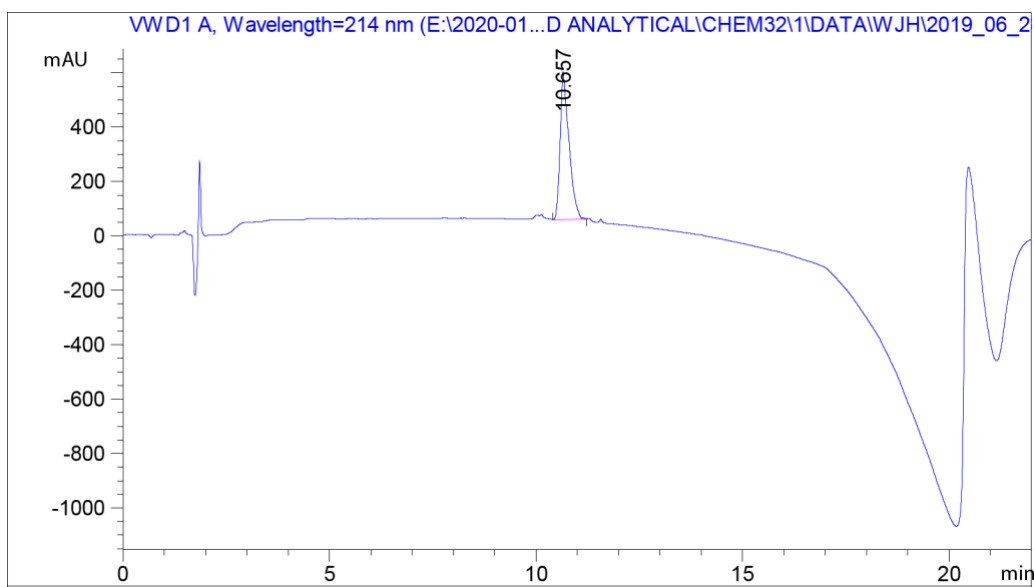
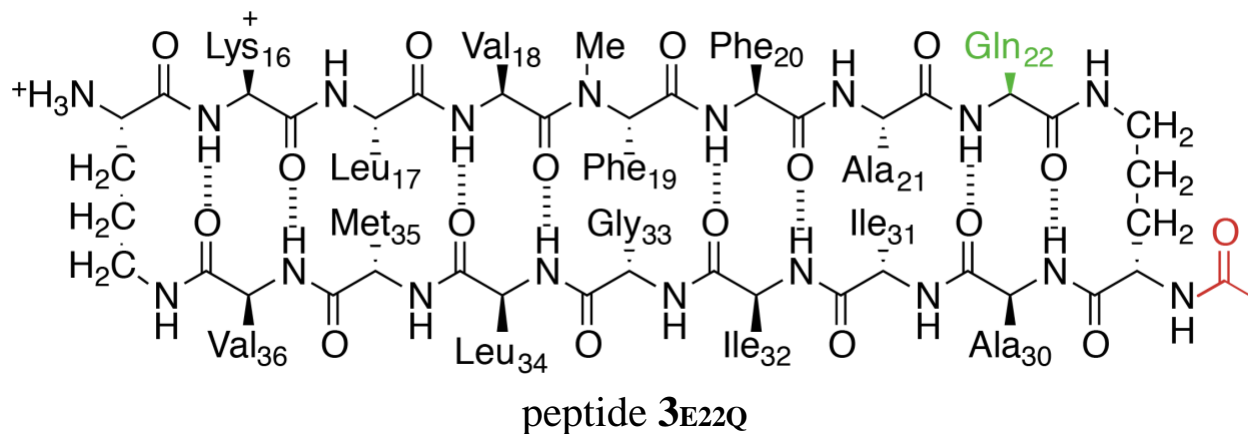


Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039

TOF/TOF™ Reflector Spec #1[BP = 1838.9, 4096]

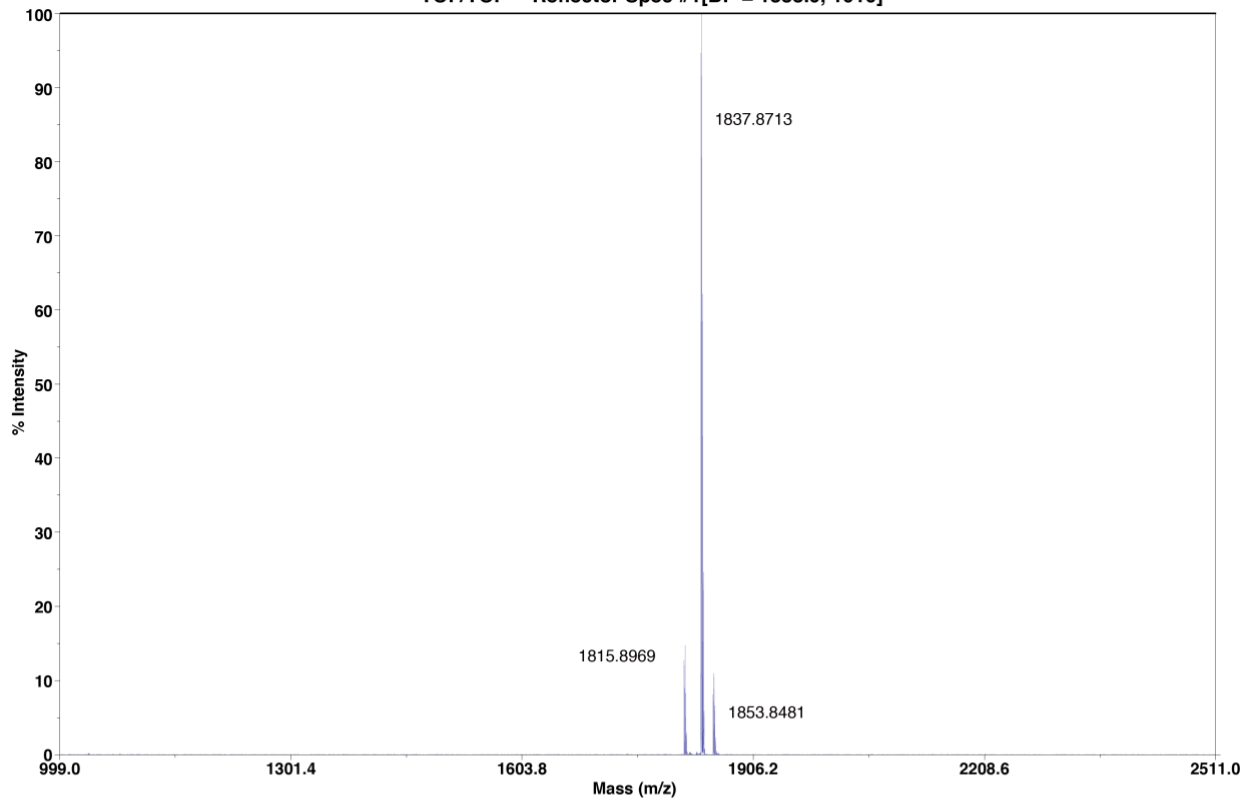


Characterization of peptide 3E22Q

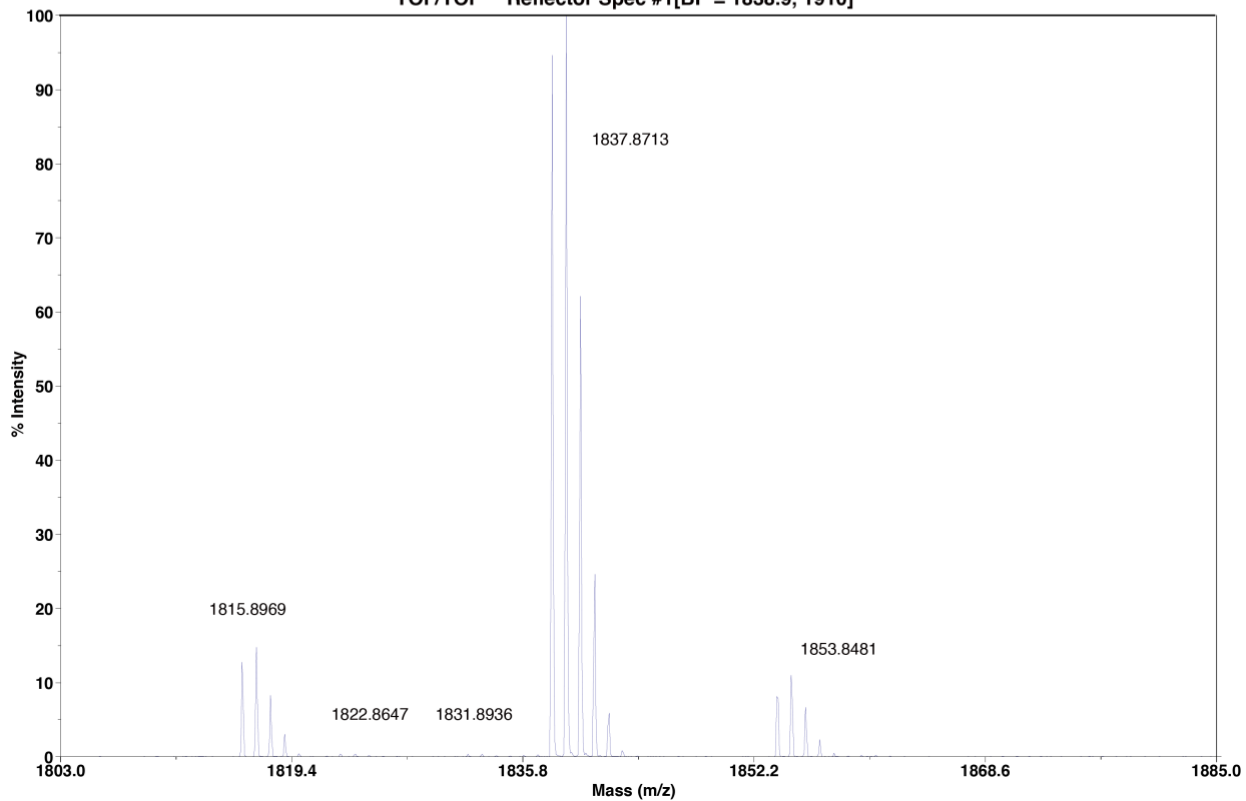


Calculated mass for peptide peptide 3E22Q: 1816.12 g/mol
[M+H ⁺] ⁺ = 1817.12
[M+Na ⁺] ⁺ = 1839.12
[M+K ⁺] ⁺ = 1855.12

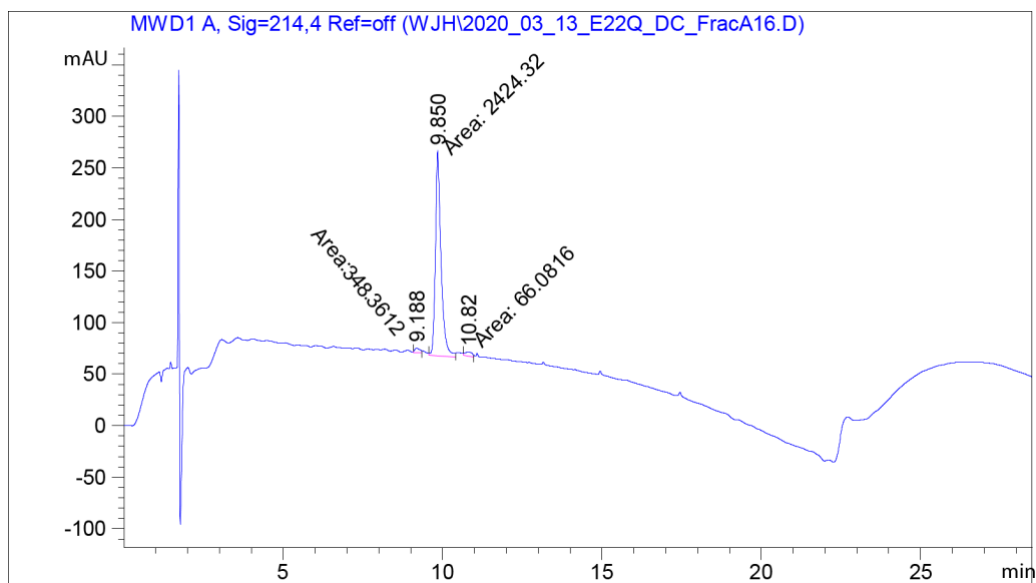
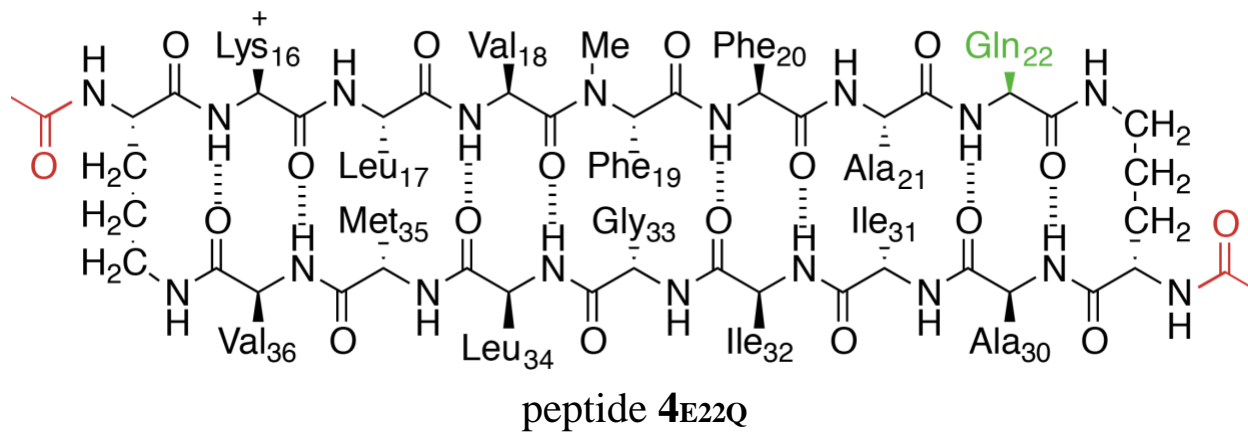
Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1838.9, 1910]



Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1838.9, 1910]



Characterization of peptide **4E22Q**



Calculated mass for peptide peptide **4E22Q**:

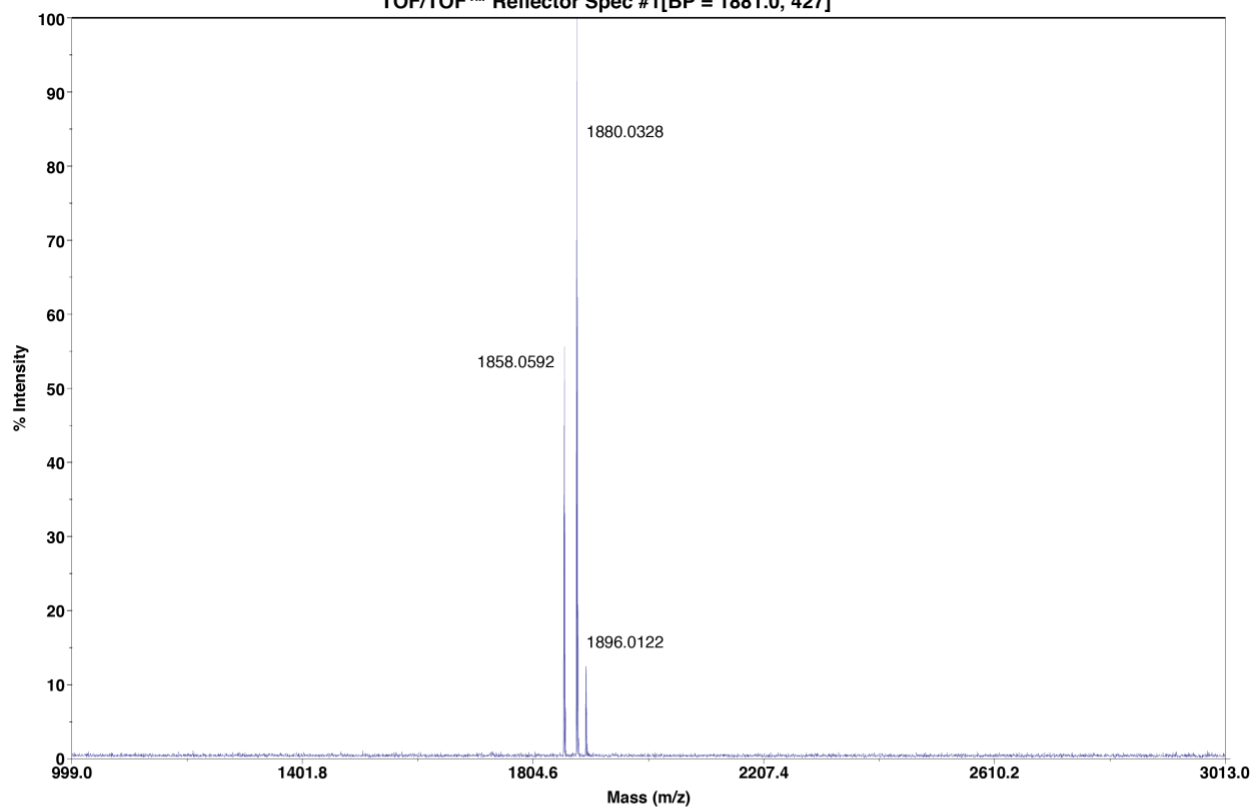
1859.17 g/mol

[M+H⁺]₊ = 1860.17

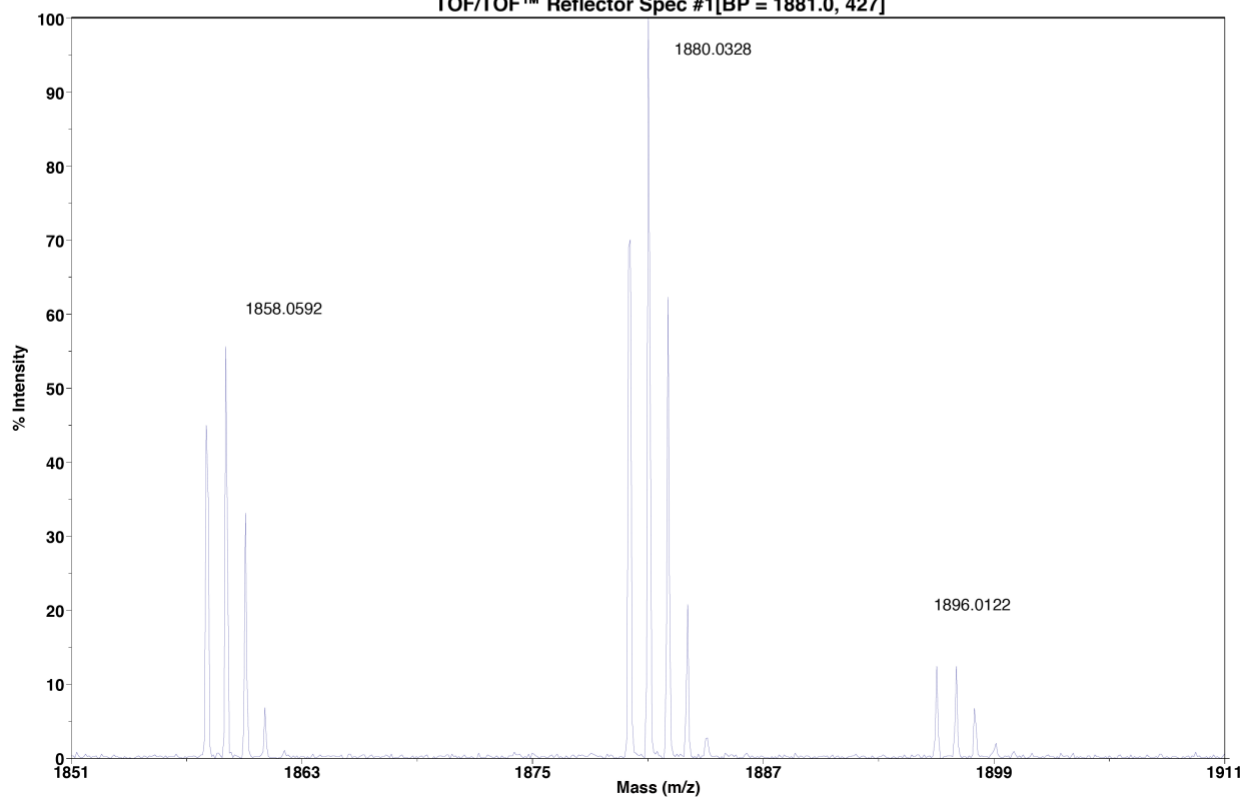
[M+Na⁺]₊ = 1882.17

[M+K⁺]₊ = 1898.17

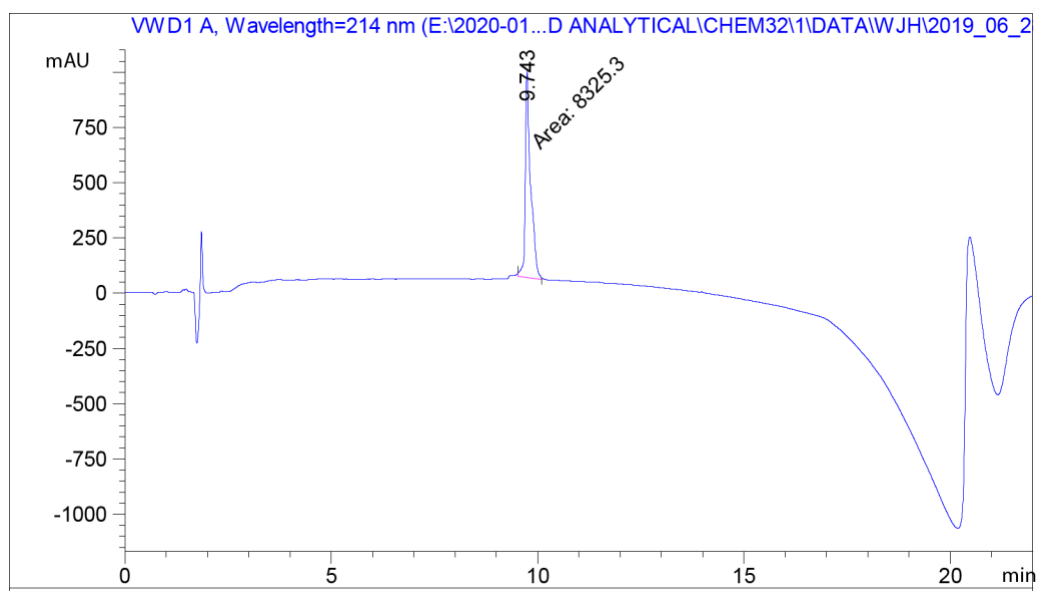
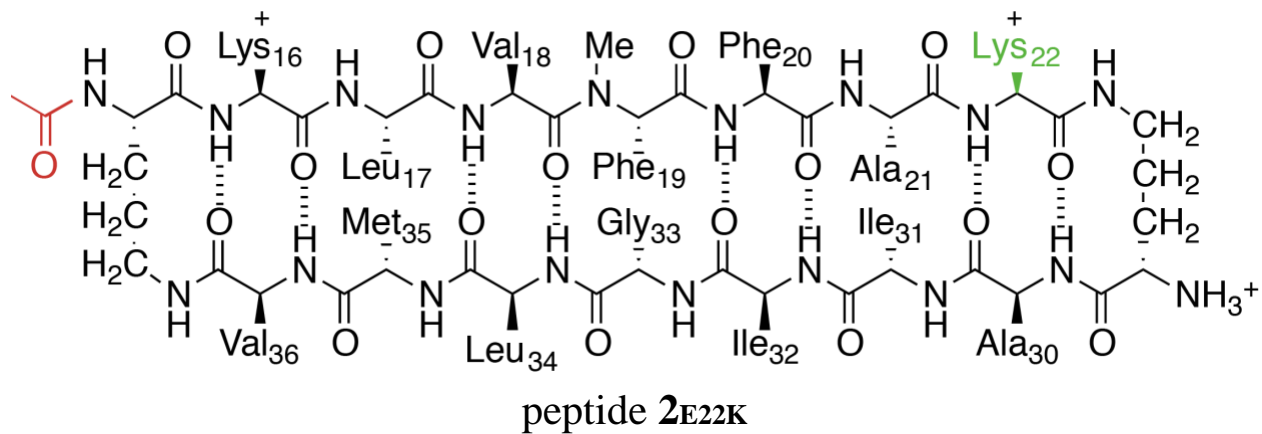
Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1881.0, 427]



Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1881.0, 427]



Characterization of peptide 2E22K



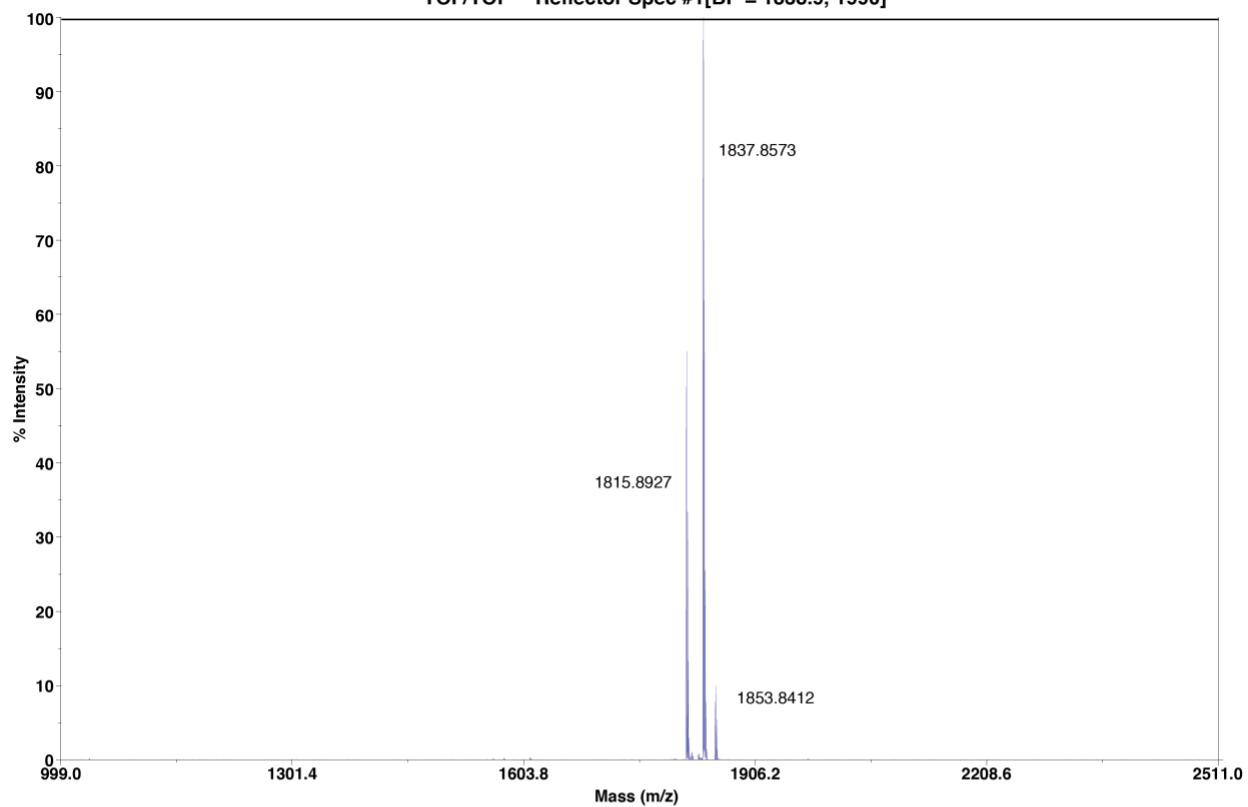
Calculated mass for peptide peptide 2E22K:
1818.12 g/mol

[M+H⁺]⁺ = 1819.12

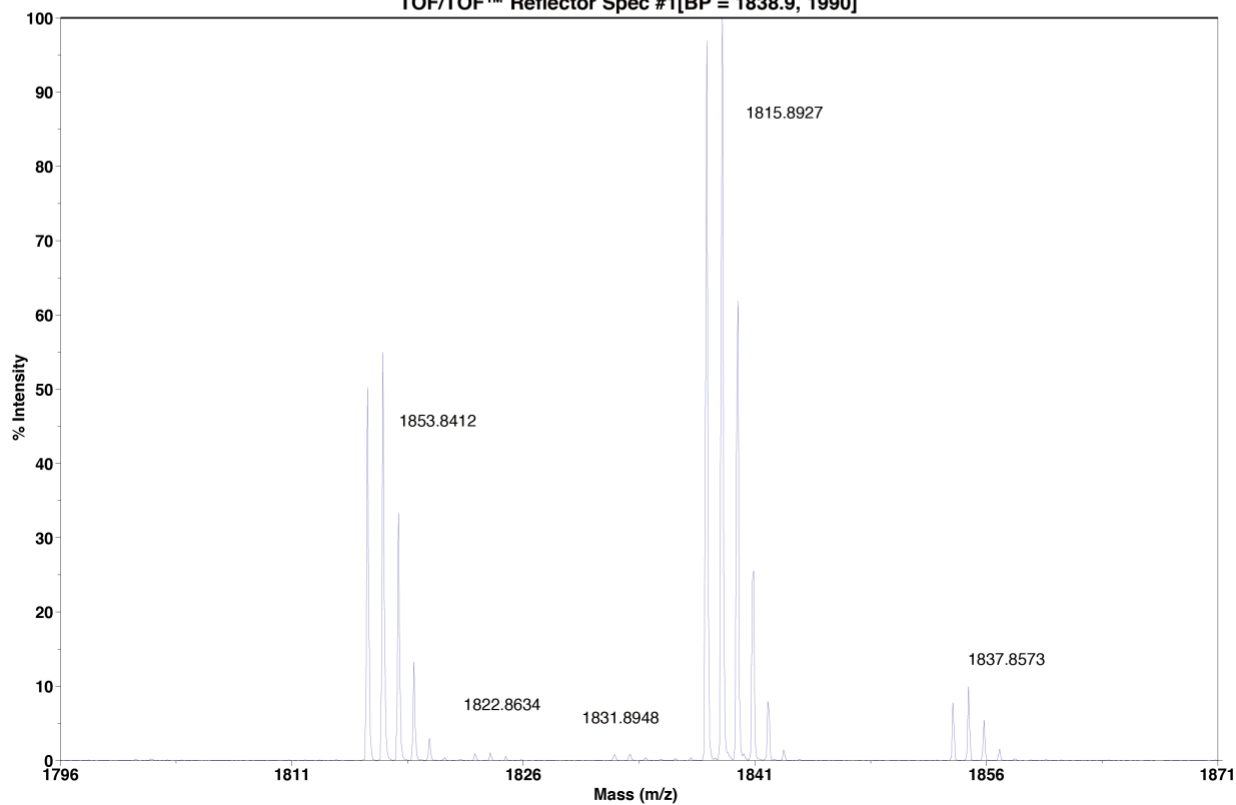
[M+Na⁺]⁺ = 1841.12

[M+K⁺]⁺ = 1857.12

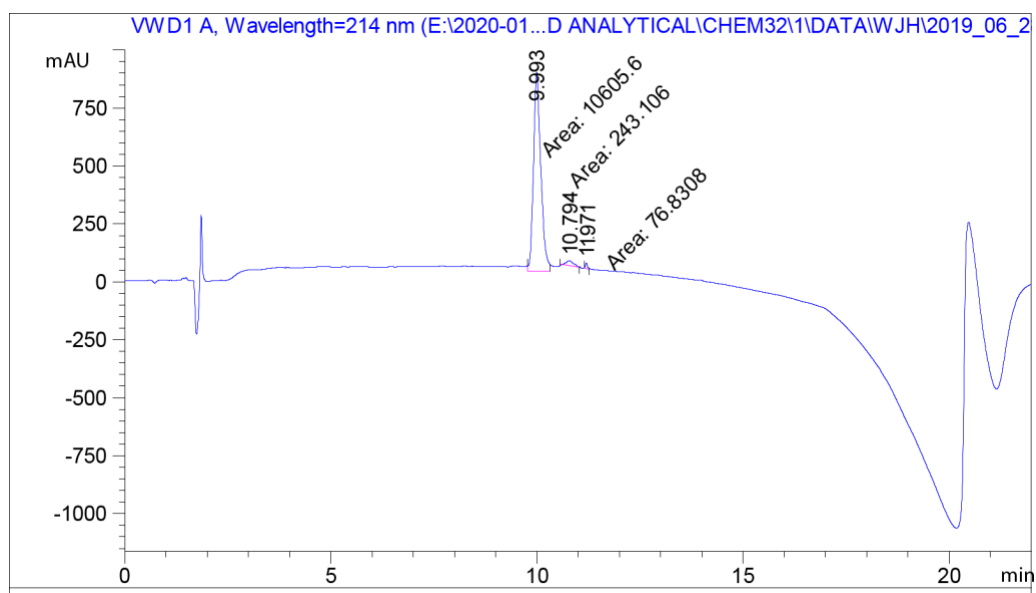
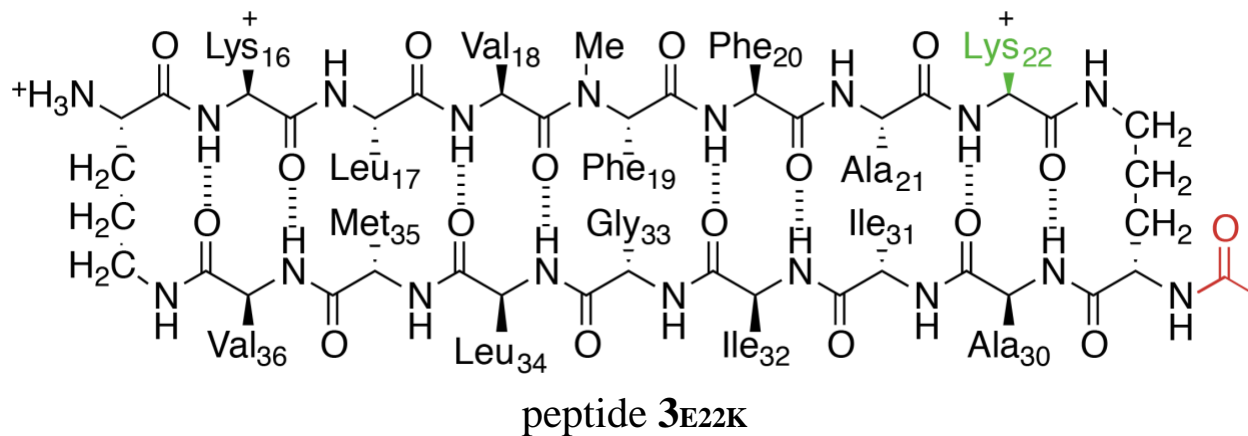
Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1838.9, 1990]



Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1838.9, 1990]

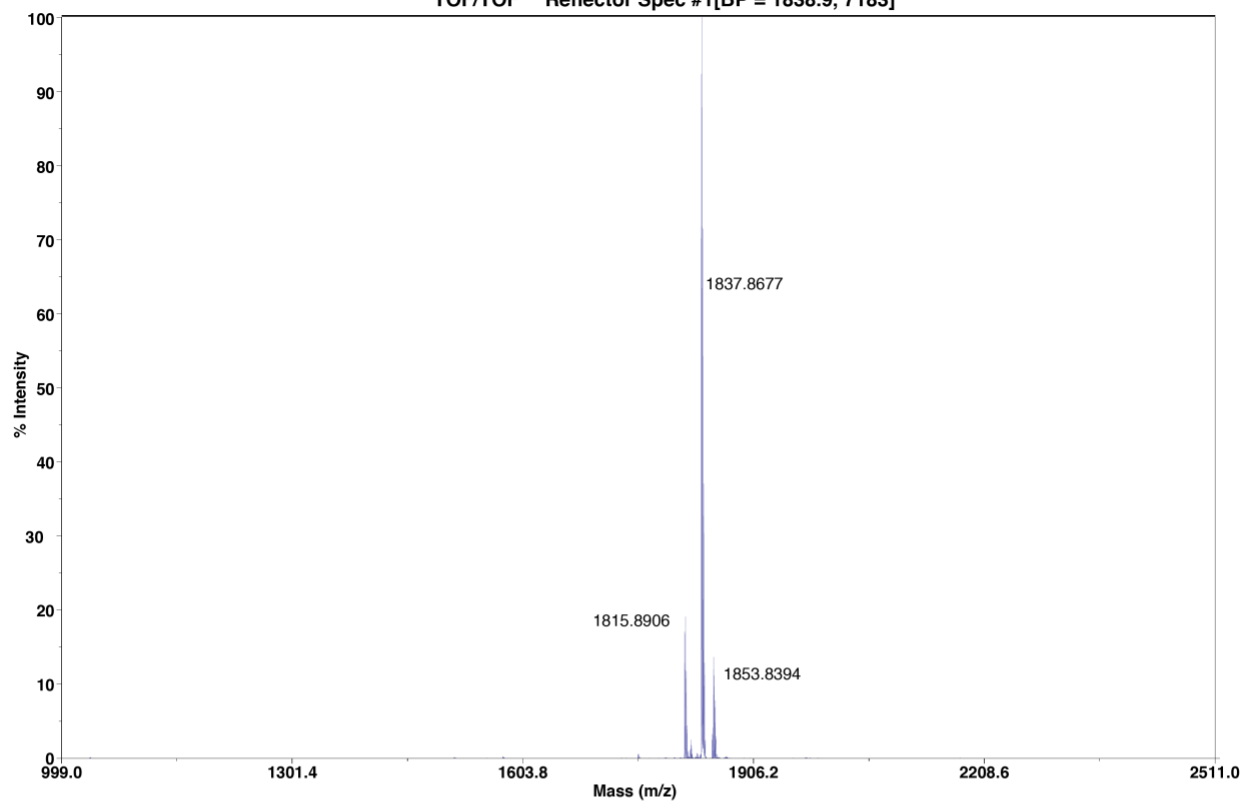


Characterization of peptide 3E22K

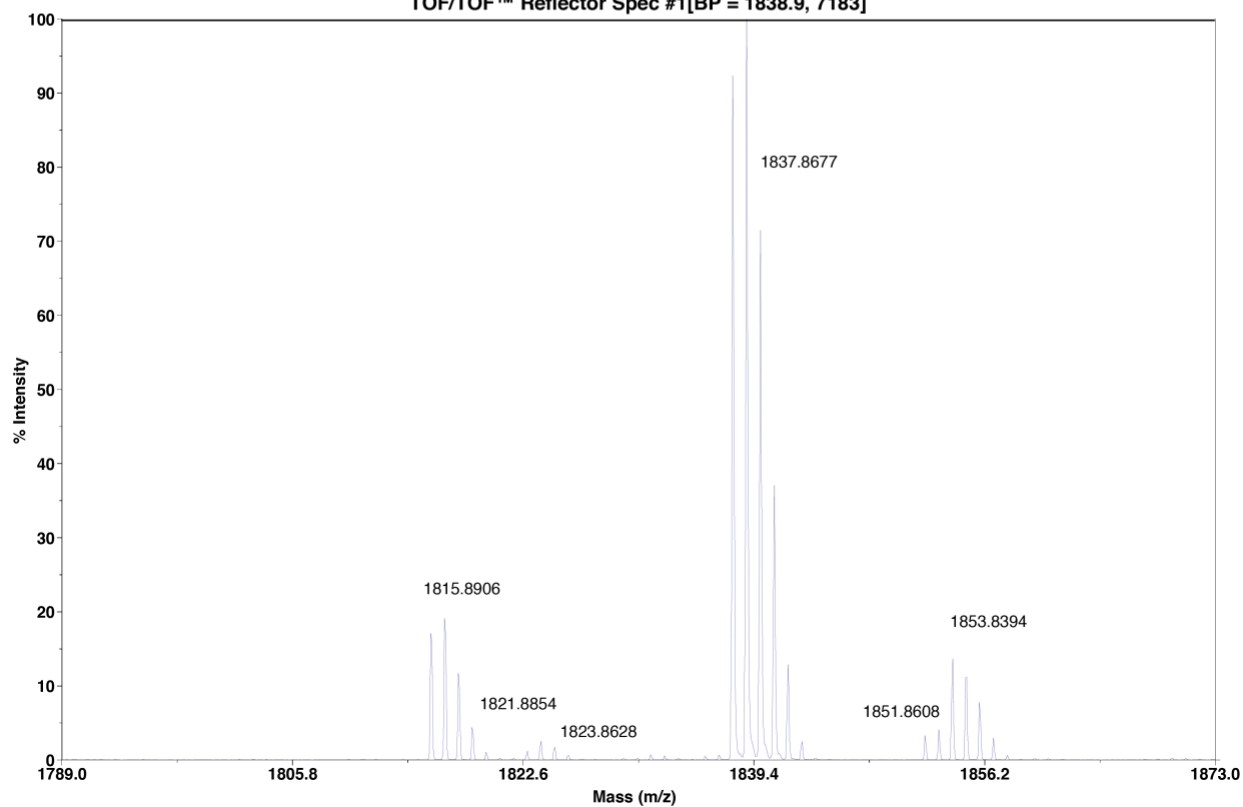


Calculated mass for peptide peptide 3E22K: 1818.12 g/mol
[M+H ⁺] ⁺ = 1819.12
[M+Na ⁺] ⁺ = 1841.12
[M+K ⁺] ⁺ = 1857.12

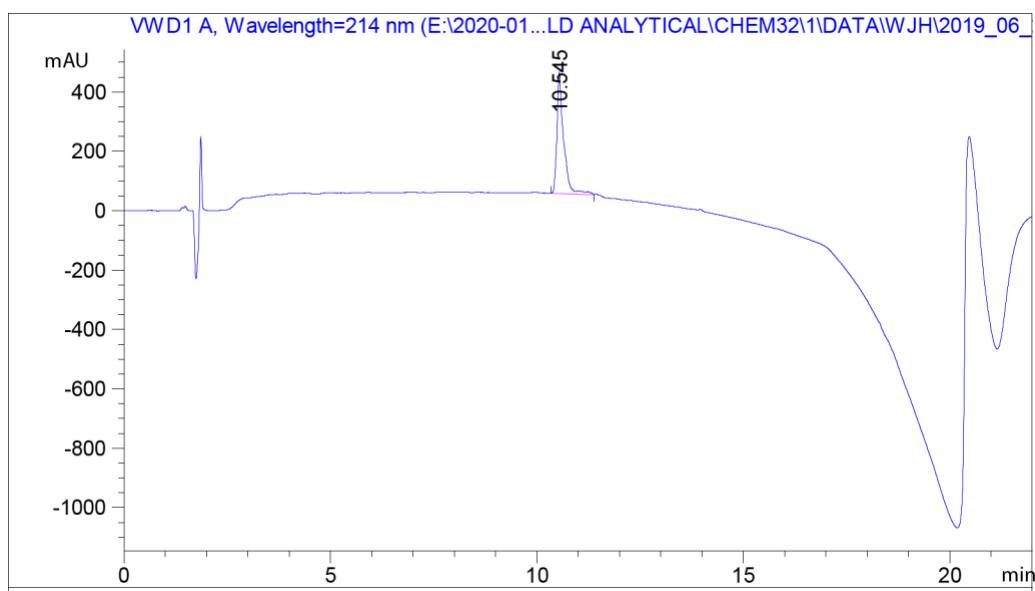
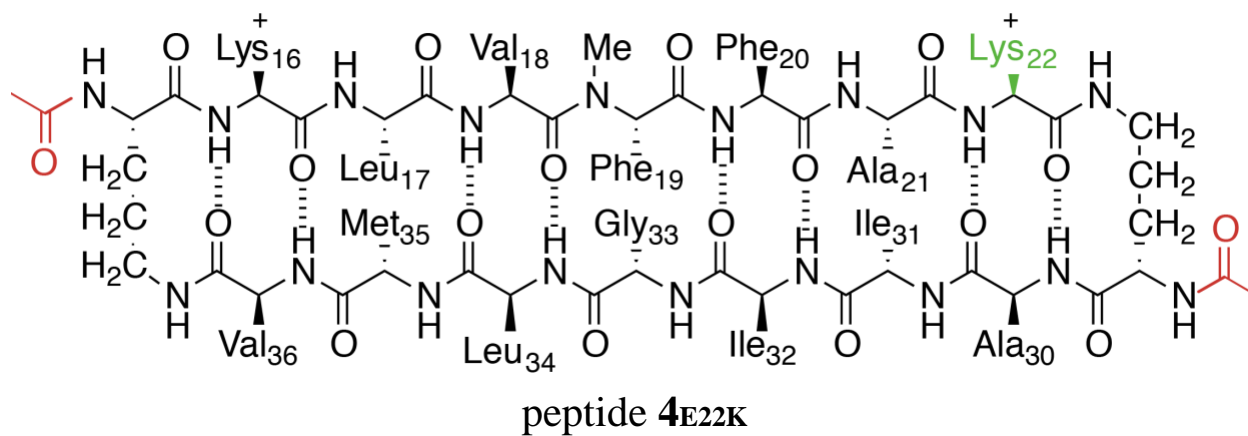
Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1838.9, 7183]



Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039
TOF/TOF™ Reflector Spec #1[BP = 1838.9, 7183]



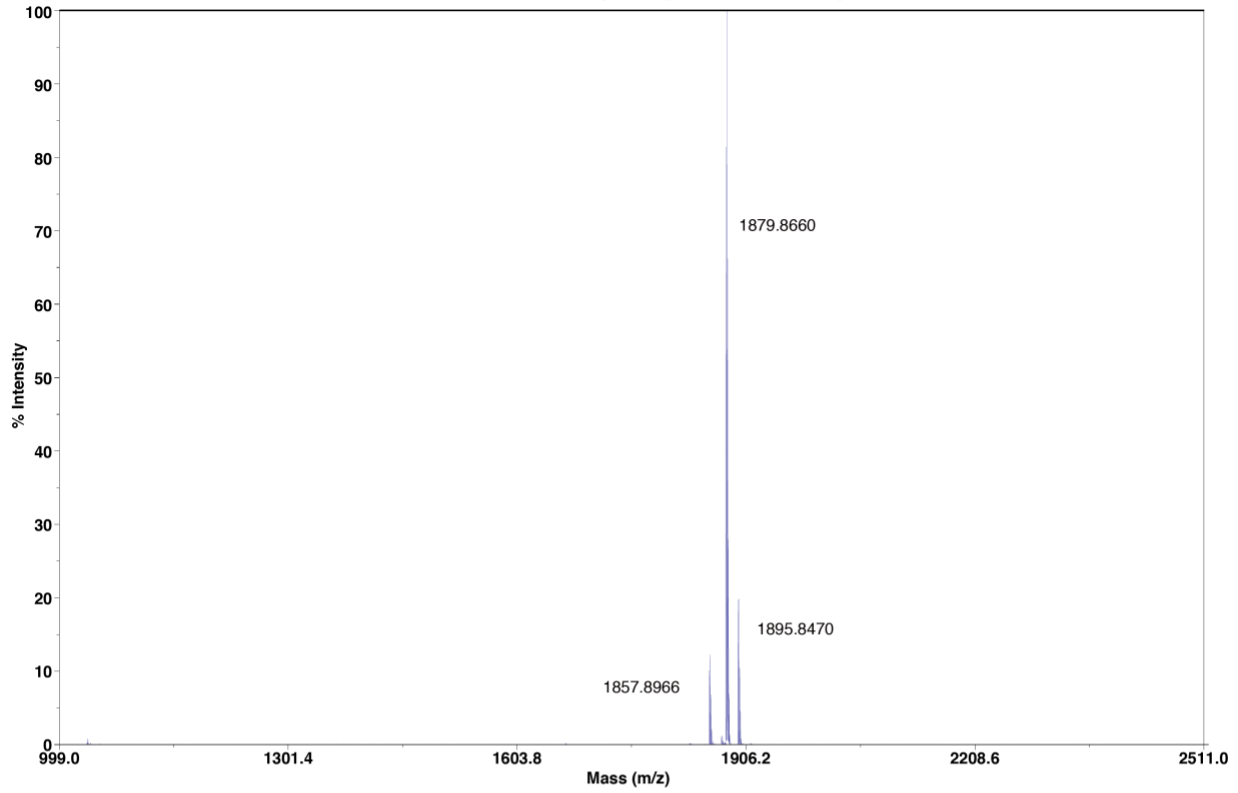
Characterization of peptide 4E22K



Calculated mass for peptide peptide 4E22K: 1861.17 g/mol
[M+H] ⁺ = 1862.17
[M+Na] ⁺ = 1884.17
[M+K] ⁺ = 1900.17

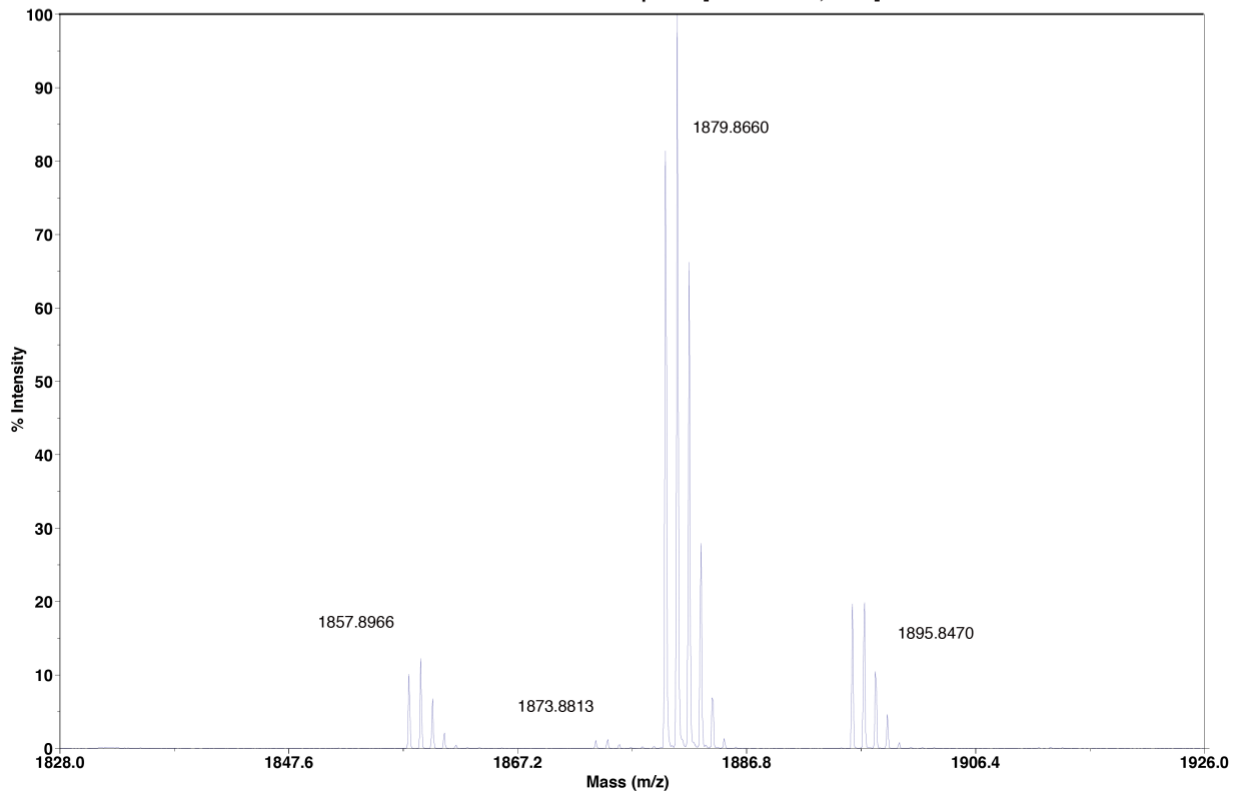
Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039

TOF/TOF™ Reflector Spec #1[BP = 1880.9, 2589]



Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039

TOF/TOF™ Reflector Spec #1[BP = 1880.9, 2589]



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Chapter 2

Anaphylaxis Induced by Peptide Coupling Agents: Lessons Learned from Repeated Exposure to HATU, HBTU, & HCTU₁

2.1 Background & Introduction

After working for years with peptide coupling agents HATU, HBTU, and HCTU, I developed a life-threatening anaphylactic reaction. I began working with the aforementioned peptide coupling agents in May 2015. During the next few years, I worked heavily with these uronium² peptide coupling agents. In March 2016, I began developing allergy symptoms of sneezing, coughing, and a runny nose. During the next couple of years, my symptoms progressed to the point of anaphylaxis. These coupling agents are especially insidious because a severe allergy

developed slowly over the course of three and a half years of exposure to the point of a life-threatening incident.

About one and a half years after beginning to work with these coupling agents, I noticed I had allergy symptoms when weighing out coupling agents and Fmoc-protected amino acids for use in solid-phase peptide synthesis. In July 2018, I began suspecting that I was becoming allergic to coupling agents because I experienced sneezing and a runny nose immediately after spilling HCTU onto my glove. It was not until September 2018 that I experienced my first brush with allergy-induced anaphylaxis. I was at the weekly research group meeting, in a seminar room down the corridor from the laboratory, and I began wheezing slightly. The wheezing was fleeting and went away after group meeting, when I left the building. A couple weeks later, I started wheezing as I drove two labmates home. This time, the wheezing was louder — my lab mates could also hear it — so I took the antihistamine diphenhydramine (generic Benadryl) to stop the reaction. Within twenty minutes, I could no longer hear wheezing.

Finally, in late October 2018, I sat down at my desk in the lab, and almost immediately began coughing, sneezing, feeling tightness in my throat, and subsequently wheezing. I attempted to remove myself from whatever I was exposed to in the lab, and moved down the hallway to an office outside the lab. Once there, I continued reacting, and the wheezing progressed until I could hear a rattling wheezing sound when breathing through my nose. I immediately left the lab to obtain diphenhydramine. As I exited the building, my symptoms stopped progressing. An hour after taking diphenhydramine, the wheezing subsided completely. In hindsight, I should have called 911 for emergency medical help, because a throat-closing anaphylactic reaction can occur quickly, sometimes so quickly that there is barely enough time to avoid fatality.

How did this happen? How could this have been prevented? Our laboratory has been tackling these questions since the incident occurred. I provide this case study as a cautionary note about the potential hazards from chemical exposure that can develop over time and sneak up on a researcher. I first sought to determine what caused this anaphylactic reaction to occur. I then adjusted how peptide coupling agents were handled in the lab to minimize exposure and attempt to prevent other researchers from becoming sensitized as well. In sharing my experience here, I hope to contribute to the widespread implementation of standard operating procedures for peptide coupling agents and protect others who work with them.

2.2 Literature Search

I first scoured the literature for information on sensitization by peptide coupling agents HATU, HBTU, and HCTU (Figure 2.1) and Fmoc-protected amino acids. Information regarding sensitization varied amongst chemical supplier material safety data sheets (MSDS's). HATU is reported to cause skin, eye, or respiratory irritation, and is denoted by an exclamation mark hazard symbol.³⁻⁵ HBTU is reported to cause respiratory sensitization.⁶⁻⁸ HCTU is not reported to have known toxic effects.⁹⁻¹¹ I found only nine published cases of sensitization by the uronium² coupling agents HATU and HBTU, and none by HCTU nor by Fmoc-protected amino acids. The first reported case implicating uronium coupling agents as chemical sensitizers came in 2003. Yung et al. described a researcher at a university that first developed eye irritation, a runny nose, and coughing (rhinitis) after weighing out HBTU. Her symptoms progressed over the course of two weeks, developing into chest tightness, a cough, and skin rashes (urticaria) and culminating in sore, red itchy eyes, coughing and sneezing and urticaria within one hour of being in the laboratory.¹² The researcher was tested with skin prick tests for allergies to HATU, HBTU, and HCTU since all chemicals were present in the lab. She tested positive for sensitivity to HATU and

HBTU, but negative for HCTU and various Fmoc-protected amino acids. Since the researcher did not exhibit sensitivity to HCTU, the authors suggested that this uronium coupling agent may be a safer alternative for widespread use. Other publications report that HCTU is nontoxic and nonirritating.¹³⁻¹⁴

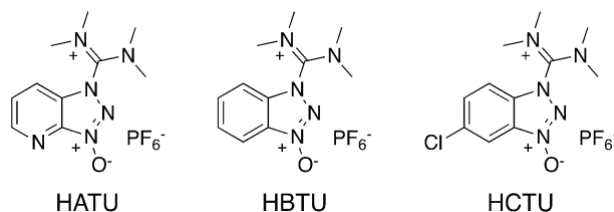


Figure 2.1. Chemical structures of uronium² coupling agents HATU, HBTU, and HCTU.

The other published instances of chemical sensitization to uronium coupling agents have involved HBTU exclusively and are summarized here. In 2003, another researcher, this time in a pharmaceutical plant, developed occupational rhinitis and bronchial asthma from HBTU and TBTU, which is identical to HBTU except for the counterion. The allergies were confirmed by positive skin prick and nasal challenge tests.¹⁵ In 2005, Bousquet et al. reported a chemistry researcher who developed allergic rhinitis and dermatitis on the hands and fingers which then progressed over the course of a year to include his face, upper back, neck, elbows, and ankles. The authors confirmed the researchers' sensitivity to HBTU through patch testing, and found he was not allergic to dimethylformamide, dichloromethane, acetonitrile, triisopropylsilane, HATU, or BOP.¹⁶ From 2006 to 2010, six more instances of chemical sensitization from HBTU were reported, with similar respiratory and skin reactions.¹⁷⁻¹⁹ One example, in 2006, involved a university researcher developing an anaphylactic response to HBTU over the course of three years, similar to the case reported in this paper.¹⁷ All of these examples were published in allergy and immunology journals, which are not generally read by researchers who use peptide coupling agents.

2.3 Experimental Confirmation²⁰

We suspected that peptide coupling agents caused my allergic reactions. An allergist and clinical immunologist (WS) tested me for allergies to a panel of over 60 allergens by skin prick tests to determine if common environmental allergens accounted for my anaphylaxis. I was only slightly allergic to two environmental allergens, but not so allergic that they would cause anaphylaxis. Skin prick tests were then performed to determine if I was allergic to HATU, HBTU, HCTU, DCC, Fmoc-leucine-OH, Fmoc-phenylalanine-OH, and Fmoc-asparagine(Trt)-OH (Figures 2.1 & 2.2). I worked with most of the canonical amino acids in their Fmoc-protected forms, so three were chosen as representative amino acids. DCC was included as a control, because it is a notorious sensitizer that I had never previously worked with.

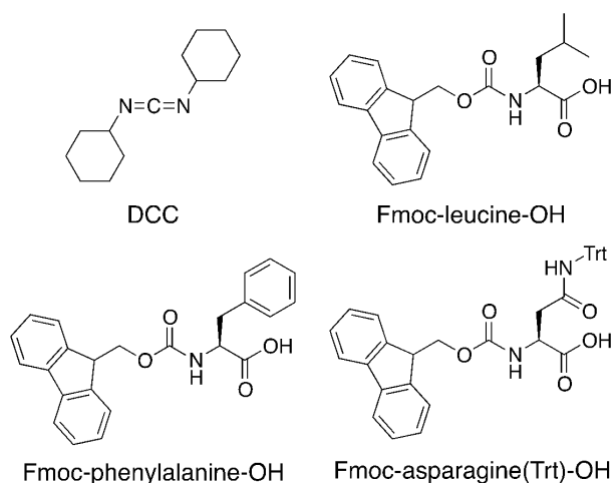


Figure 2.2. Chemical structures of additional compounds chosen for allergy tests. (Trt indicates a trityl protecting group.)

As hypothesized, I had severe positive allergic reactions to uronium peptide coupling agents, but only mild responses to Fmoc-protected amino acids. The coupling agents HATU, HBTU, and HCTU all caused the formation of large hives, comparable in size to those formed by the histamine positive control (Figure 2.3). DCC did not cause any reaction, which is not surprising as I was never previously exposed to DCC. Fmoc-leucine-OH, Fmoc-phenylalanine-OH, and

Fmoc-asparagine(Trt)-OH all elicited minor reactions and produced hives much smaller in size than the histamine positive control. The lack of a strong reaction to the Fmoc-protected amino acids is not surprising, as they are not known chemical sensitizers.



Figure 2.3. Allergic hives which formed within 20 minutes after skin prick tests. HATU, HBTU, and HCTU hives are approximately the same size as the histamine positive control. The Fmoc-protected amino acid hives are much smaller. DCC caused no hive formation.

2.4 Discussion

This paper serves as the first reported case of chemical sensitization resulting in anaphylaxis from three common uronium coupling agents; HATU, HBTU, and HCTU. I can no longer work in my research lab. I cannot go into the building where the lab exists; the hallways, rooms, and common spaces all cause me to react, first with a runny nose and throat tightness and then with wheezing. My allergic response is so severe that I risk anaphylaxis whenever exposed

to these coupling agents, and I now must carry an epinephrine auto-injector (generic EpiPen) as a safety precaution whenever I am near researchers actively working with peptide coupling agents. I have become sensitive to colleagues who have been in our research laboratory, and must be careful to ask them to change their clothes and in some cases wash or cover their hair to prevent my exposure to the pervasive coupling agents. These events prompted the research group as a whole to re-evaluate how the group handles peptide coupling agents and to change their standard operating procedures to prevent other group members from becoming sensitized to coupling agents.

Chemical sensitization causes an immune response in the form of reactions as mild as seasonal allergy symptoms, like rhinitis, and as severe as dermatitis and anaphylaxis. Many chemical sensitizers are chemicals that can modify human proteins. All reactive compounds that can modify proteins should be treated as potential sensitizers, unless they are known with certainty to be safe. In spite of this hazard, most researchers do not treat compounds that can react with proteins with proper precautions. Peptide coupling agents are prime examples.

Peptide coupling agents induce the formation of an amide bond from the reaction of a carboxylic acid group with an amine group. The coupling agents react with the carboxylic acid and activate it for subsequent attack by a nucleophilic amine. After the amine reacts with the activated carboxylic acid, an amide bond forms.²¹ Human proteins display multiple carboxylic acid groups (e.g., glutamic acid and aspartic acid) and amine-containing groups (e.g., lysine) in the form of amino acid residues at protein surfaces. The reactivity of coupling agents toward amino acid residues primes them to cause sensitization by modifying proteins in the human body.²²⁻²⁴

The carbodiimide coupling agent DCC (dicyclohexylcarbodiimide) is a notorious chemical sensitizer, with a long history of causing sensitization. DCC was first reported as a peptide

coupling agent by Sheehan and Hess in 1955.²⁵ It quickly grew in popularity due to the ease with which it induced the formation of peptide bonds. Soon after its introduction, a publication reported that DCC caused three cases of allergy-induced skin rashes (contact dermatitis), in 1959.²⁶ Zschunke and Folesky subsequently reported seven cases of DCC-induced contact dermatitis in a pharmaceutical plant, in 1975.²⁶ In 1979, two independent cases of DCC sensitivities were published in the journal *Contact Dermatitis*. In one case, a lab worker developed a blistering eruption rash on his hands and forearms,²⁷ and in the second case a research chemist developed a rash over nearly his entire body that persisted for five days before he was hospitalized.²⁸ Since 1979, eleven more cases were reported of DCC causing similar skin contact allergic reactions.²⁹⁻³⁴ In one of these cases, the researcher also developed sensitivity to diisopropylcarbodiimide (DIC) and suffered a vesiculopapular rash on his cheeks and the backs of his hands from both DCC and DIC.²⁹ The authors of each of these reported cases confirmed sensitization with skin patch tests.

The many reports of DCC sensitization lead to toxicology testing to confirm the hazard it poses to human health. DCC and DIC were nominated for testing by the National Toxicology Program in 1993.³⁵ Hayes et al. then tested DCC and DIC on the skin of mice for their potential as sensitizers, and in 1998 reported sensitization at concentrations as low as 0.006% (w/v) for DCC and 0.3% (w/v) for DIC.³⁶ Another report, in 2002, confirmed DCC and DIC as sensitizers to mice when examining the mechanism of DCC- and DIC-induced chemical sensitization.³⁷ In 2011, Surh et al. further characterized DCC and DIC for toxicity and carcinogenicity and determined that both DCC and DIC caused skin sensitivity in rats and mice, but only DCC exhibited carcinogenicity.³⁸ The detrimental health effects of the peptide coupling agents DCC and DIC are worrisome for anyone who handles them.

HATU, HBTU, and HCTU were developed between the late 1970's and the early 2000's and are now widely used as coupling agents in peptide synthesis.^{13,39-43} Despite being implicated as sensitizers in at least ten reported cases, including the current one, they have not been rigorously tested for their immunogenic and toxicological properties.

2.5 Laboratory Action Plan

In response to my sensitization, our lab developed standard operating procedures to handle HATU, HBTU, and HCTU more safely. We found guidelines for handling sensitizers, which recommended never opening sensitizers outside of a fume hood and minimizing exposure if handling them outside of a fume hood.⁴⁴ Our lab dedicated a portion of a fume hood to weighing out coupling agents and amino acids and placed a balance in the hood. A waste container was placed in this fume hood as a receptacle for weighing paper and other materials contaminated by coupling agents or Fmoc-protected amino acids. Coupling agents and amino acids are transferred into sealable containers before removal to individual researchers' fume hoods. As with other standard operating procedures for handling hazardous chemicals, personal protective equipment (PPE) in the form of a lab coat, eye protection, and disposable gloves should be worn at all times when handling coupling agents. We anticipate that these procedures will reduce the risk of other researchers becoming sensitized in the future.

Any research lab that performs peptide synthesis should take extra precautions to avoid exposing researchers to coupling agents. Section 2.7 provides a standard operating procedure to handle peptide coupling agents more safely in the research laboratory by minimizing exposure.

2.6 Conclusion

Peptide coupling agents — regardless of whether they are carbodiimide reagents, uronium reagents, phosphonium reagents, etc. — all perform the same chemical function of facilitating amide bond formation, and therefore can all covalently modify human proteins. If a chemical can modify human proteins, it is a prime candidate as an immune sensitizer, even if it is not a known sensitizer. We hope that our laboratory's experience of the hazards of HATU, HBTU, and HCTU will serve as a cautionary note to those working with any peptide coupling agents.

2.7 Additional Information

Standard Operating Procedure (SOP): How to Safely Handle Peptide Coupling Agents

Hazards: Peptide coupling agents are potent immune sensitizers. They have caused cases of both skin and respiratory sensitization in the form of rashes and lesions (dermatitis) and coughing, sneezing, and throat-closing (anaphylaxis) reactions. Peptide coupling agents can modify human proteins, which is the most likely mechanism through which they cause immune sensitization. Researchers should take care to avoid exposure to them as much as possible. As with other standard operating procedures for handling hazardous chemicals, personal protective equipment (PPE) in the form of a lab coat, eye protection, and disposable gloves should be worn at all times when handling coupling agents.

Engineering Precautions: Every research laboratory using peptide coupling agents should have a fume hood with a balance dedicated to weighing out peptide coupling agents and other sensitizing agents. The fume hood and balance should be free of debris and clutter, and any spilled reagents should be promptly cleaned up and removed. The fume hood should also be equipped with a waste container dedicated to contaminated weighing paper.

Procedure

1. Obtain a closed bottle of peptide coupling agent and a sealable container (such as a glass flask with a screw-on lid) and transport both to a fume hood equipped with a balance.
2. Open the bottle of peptide coupling agent completely inside the fume hood. Do not open the bottle outside the fume hood or you will risk exposure to the peptide coupling agent.
3. Weigh out the desired amount of peptide coupling agent on weighing paper and transfer it to the sealable container, or weigh it directly into the sealable container.
4. While still working in the fume hood, seal the sealable container with the coupling agent inside the container.

5. Dispose of the contaminated weighing paper in the waste container within the fume hood. Do not dispose the weighing paper in a trash can outside of the fume hood, as this will cause exposure to peptide coupling agents. (Note: If you spill peptide coupling agents on the balance, fume hood floor, your gloves, etc., make sure to clean up your spill and place your contaminated gloves in the waste container in the fume hood before removing your hands from the fume hood.)
6. Transport your sealable container with coupling agent to your own fume hood or to the automated peptide synthesizer.
7. Continue performing your reactions as normal, always inside a fume hood if possible. If using an automated peptide synthesizer that is not in a fume hood, open all reagent containers as little as possible outside of a fume hood.

2.8 References

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Chapter 3

Towards a Career in Chemical Education

3.1 Introduction

I love teaching. University level teaching is inherently challenging, as instructors not only must have vast knowledge in their discipline, but must also be adept in the art of effective teaching. New graduate students enter Ph.D. programs after exiting the undergraduate, novice, stage in their discipline and subsequently develop into expert Ph.D.s. This transformation is nurtured and developed through the practice of research and guidance from instructors, peers, and research advisors. The transformation from novice to expert instructor is also an invaluable part of the Ph.D. experience. The major method through which teacher training occurs is through teaching

assistantships. There are other professional development opportunities at most universities, and I am fortunate that UCI had many. Throughout my graduate school career, I pursued numerous professional development opportunities that have helped me become an effective instructor.

I began developing as an instructor my first quarter at UCI, and continued until my final quarter. As is usual in the Department of Chemistry, my first experience leading a class was as a Teaching Assistant (TA) for an undergraduate chemistry course. I continued to practice my teaching through my second year, when I decided to pursue more formalized preparation in best teaching, or pedagogical practices. I applied for and was accepted into the Pedagogical Fellows (PF) Program during my third year. Simultaneously, I secured a year-long Head TA position for Dr. Renee Link's organic chemistry laboratory course series, and worked with her as my mentor for the Teaching Apprenticeship in STEM (TAP-STEM) program that I applied to and was accepted into. TAP-STEM included the opportunity to teach a course as instructor of record during the summer that marks the end of the year-long program. Because of my pedagogical training and experience, I then had the opportunity to continue honing my teaching ability in three additional courses as the instructor of record the summer after my fourth year and fall of my fifth year. To round out my professional development experiences as an aspiring university instructor, I served as a mentor in the TA Mentor Program during my fourth and fifth years and as the Safety Fellow for the Department of Chemistry during my fifth year.

The knowledge, experience, and expertise I have gained over the past five years have been invaluable to my development and ability as an instructor. Without the opportunities I pursued as a graduate student, I would be less skilled as a teacher and underprepared to be an effective instructor. I am fortunate to have pursued and obtained the pedagogical and professional development opportunities previously mentioned. I am beyond happy and excited that they have

helped me successfully secure a career in teaching as a Lecture-Track Professor at Emory University starting in fall 2020. Here, I share my experience and reflections on my teaching journey — from bright-eyed novice to wizened “expert” — with the hope of providing guidance and inspiration to other graduate students pursuing teaching-focused careers after their Ph.D.s.

3.2 TA Experience

I began my TA experience much like others who first enter grad school — excited, anxious, and terrified. After two weeks of orientation activities, three days of which were TA training, I was itching to get my Ph.D. started and a little overwhelmed by the amount of responsibility placed on first year graduate students in Chemistry. My first fall quarter at UCI, in 2015, was completely filled from taking classes, rotating in research groups, and — of course — teaching.

Being thrust into a TA position without teaching experience or a formalized mentorship system was challenging, and would have been paralyzing had it not been for those few days of TA training at the beginning of the quarter. The Chemistry Department runs a laboratory specific day-and-a-half-long TA training, which is followed by the UCI Division of Teaching Excellence and Innovation’s (DTEI’s) general teaching day-and-a-half-long TA Professional Development Program (TAPDP) training. Both experiences provided a valuable introduction into how to run a laboratory or discussion section in an undergraduate course. We learned basic teaching strategies, such as how to generally prepare to teach, structure a teaching presentation, and grade effectively and efficiently.

I used the teaching strategies I learned to get through the classes I helped teach as a TA during the three quarters of my first year. I taught for three different courses: organic chemistry lab, general chemistry lecture, and general chemistry lab. A different instructor taught each course,

which gave me insight into various instructor styles and ways courses could be run. I enjoyed teaching, and I learned much about student-instructor dynamics during my first year, but I still felt nervous walking into a classroom even with my preliminary experience. I knew I needed more training in best teaching practices so I could gain the necessary skills to help me feel comfortable in the classroom.

Near the end of my first year, I rediscovered a program at UCI called the Pedagogical Fellows (PF) Program. I learned that the PF Program, run through the DTEI, trains PFs in advanced pedagogy and prepares them to run the TAPDP program for incoming TAs. I remember thinking that being a PF would be a great way for me to learn more about how to teach better. Part of the PF Program application prerequisites was successful advancement to candidacy, so I put a pin in my goal of becoming a PF until after passing my oral exam.

After my first year, I was a TA for almost every quarter up until my fourth year in graduate school. I was fortunate to have taught a wide breadth of courses as a TA for general and organic chemistry labs and lectures, and as a Head TA for organic chemistry labs, chemical biology lab, writing for chemists, and undergraduate thesis writing. My teaching experience and stint as a PF prepared me well to be an instructor of record.

3.3 My Time as a Pedagogical Fellow

After teaching as a TA for two years, realizing I enjoyed teaching and wanted to learn more about how to do it effectively, and successfully advancing to candidacy, I was able to apply to the Pedagogical Fellows (PF) Program during the fall quarter of my third year. The PF Program is a competitive program run by the Division of Teaching Excellence and Innovation (DTEI). PF positions are open to every graduate student at UCI with TA experience after they have

successfully advanced to candidacy in their department. In addition to submitting a formal application, graduate students must take a course in Developing Teaching Excellence — which introduces best teaching strategies and practices in higher education — and undergo three peer teaching evaluations to receive feedback on their strengths and weaknesses as an instructor. Graduate students with strong application packages are invited to interview with a panel of current PFs and the DTEI Director at the end of fall quarter. New PFs are chosen and begin their PF appointment at the beginning of winter quarter.

The PF Program consists of three courses — University Studies 390A, B, and C — and designing and implementing the TA Professional Development Program (TAPDP). The three courses run sequentially in winter, spring, and fall quarters. The winter and spring courses train PFs in advanced pedagogy, not only to help them develop as more effective instructors, but also to help them prepare for TAPDP at the beginning of the impending academic year's fall quarter.

PFs begin preparing and workshoping material for TAPDP throughout the winter, spring, and summer leading up to fall. TAPDP runs for one full and one half working day, and covers the following topics: TA Roles and Responsibilities, Lesson Planning, Active Learning and Leading a Class, Diversity and Inclusion in the Classroom, Grading Effectively, Holding Office Hours, Giving Students Feedback, Using Teaching Technology, and Microteaching Demo. The instruction PFs receive during the winter and spring courses takes a deep dive into each of the aforementioned topics to prepare PFs to design their own workshops for TAPDP.

Designing and planning the TAPDP workshops taught me much about the amount of work and thought that must go into teaching — both for teaching students and teaching teachers. Every aspect of a course, from overarching student learning objectives to assignments and individual class meetings, needs to be designed with purpose. This fact may seem obvious, but it may not be

something a novice instructor thinks about without being made aware. Each assignment, each assessment, each lecture, each in-class activity must meet and reinforce course student learning objectives.

The last course of the PF program, given the fall quarter after PFs run TAPDP, focuses on preparing PFs for their careers after graduate school. PFs are introduced to the types of academic teaching positions that are available and what each job type entails. For example, there are many different institutions of higher education — research, primarily undergraduate, private, public, liberal arts, community college, etc. There are also a wide range of professor types which have varying job descriptions. Some professor positions are research track, which involves research, teaching, and service, whereas others are teaching track, which involves primarily teaching and service, and sometimes research. In the course, PFs also prepared job application materials such as sample cover letters, CVs, teaching statements, research statements, and diversity and inclusion statements. This course was excellent preparation for my entry into the academic job market.

The pedagogy training I received as a PF was invaluable to my emergence as a more thoughtful and effective instructor. I learned many active learning techniques and how to effectively and seamlessly incorporate them into my class design. I practiced course design and instructing through TAPDP. I prepared for future job applications by preparing application materials. Without my PF experience, I would not have been as prepared to run a course as an instructor of record at UCI and secure a teaching-focused faculty position starting at the conclusion of my Ph.D.

3.4 TAP-STEM Trainee

The summer after I advanced to candidacy was both liberating and daunting. I no longer had the greatest anxiety-inducing exam of my Ph.D. career looming over my head, but I also had no other hard deadlines, papers, or exams road-blocking my path to finishing my degree. I felt great, all I had left was finishing my research, publishing papers and writing my thesis. But with the sudden weightlessness, there also came a feeling of foreboding — what would I do next to develop my teaching? I was fortunate that I had secured a year-long Head TA position for Dr. Link's organic chemistry lab series during my third year, and I was planning to apply to the PF program in the fall. In my email, I came across a call for applications to the Teaching Apprenticeship in STEM (TAP-STEM) program, now called Summer Teaching Apprenticeship Program (STAP), and decided to apply.

The TAP-STEM program aimed to give graduate students and postdocs the opportunity to teach a course as the instructor of record under the guidance of a mentor faculty member. A grad student or postdoc mentee would shadow their mentor as they teach a course. The mentee would then teach that same course the following summer. The mentor-mentee pair would meet once a month to discuss teaching goals and strategies, and the mentee would guest-lecture at least once for the mentor's course.

The TAP-STEM program was housed in the School of Biological Sciences when I applied (it has since been run through the Division of Teaching Excellence and Innovation (DTEI)), so the course options were primarily in BioSci courses. I was interested in being a TAP-STEM trainee for a biochemistry course because my field is chemical biology. I received permission from my advisor to apply and I applied to the program.

Logic suggests that the next thing I write is that I was accepted into the program... but this is not the case. I was not accepted into the program.

Wait, what?! Where have I been going with this, I set up a nice background about what the program is and why I'm interested in it, and now I have subverted your expectations in an unsatisfying way. Wait for it. Keep reading.

I received an email from a BioSci faculty member informing me that I was not accepted into the program, but I was a great candidate who would have been considered had there been fewer qualified postdocs and if I had any questions or wanted to chat about teaching, they would be happy to meet with me. Now, at this point, I was disappointed that my young academic status essentially precluded me from being a competitive applicant, but I was touched that the professor was willing to talk with me about teaching. I took them up on their offer because I knew I should learn as much as I could while in graduate school, so if an accomplished, acclaimed, teaching professor offered to share their wisdom, I would be remiss to decline the invitation.

I met with the professor, who was kind and excited to talk to a budding instructor. While discussing methods to keep students engaged in course material, a colleague of the professor stopped by the office and was happily surprised to learn about my interest in the TAP-STEM Program. The colleague had missed the deadline to sign up as a mentor for TAP-STEM, but they were happy to jump into a mentor role if there was an interested grad student. We spoke about my position in the chemistry department and they encouraged me to ask Dr. Link if she would be interested in being a mentor for the TAP-STEM program. It was currently housed in BioSci, but it was a program for STEM courses in general, so chemistry would be fine. I spoke with Dr. Link, she agreed to be a mentor, so I ended up as a TAP-STEM trainee under her tutelage during my third year. This played out particularly well because I was also her Head TA for the year.

Dr. Link helped shape me into a competent and confident instructor. I met with Dr. Link formally for the TAP-STEM program a couple times a month, but I also interfaced with her on a weekly basis as her Head TA. We decided I would teach her Chem 51LB course — first quarter organic chemistry lab — during the first summer session of 2018, so we planned to have me guest lecture for her course twice a week during her large course offering in winter 2018. The course had around 1,100 students enrolled, which necessitated offering the same one hour lab lecture course four times a week. Each lecture covered the same material, which perfectly facilitated observation and practice. During the first couple weeks of the quarter, Dr. Link would teach the first two lectures, I would observe her, and then I would teach the last two lectures. After a couple weeks, I graduated to running the first and second lectures during the week and Dr. Link would run the latter two. This process of teaching observation and immediate practice helped build my confidence and ability to run a lecture class with as little as a hundred students and as many as four hundred.

I felt extremely prepared to run my own course as an instructor of record (IOR) that summer. I'm happy to report that it went off without a hitch.

My experience with this program may seem like a series of fortunate events that would possibly not happen for most, but I learned an extremely important lesson from this experience: be proactive. Opportunities may or may not be presented, but either way I should always take opportunities to learn, to grow, and to network. I hope I don't sound too cliché, but if you don't take risks and put yourself out there, you won't reap any benefits. If an opportunity does not come your way, try to make your own. In this case, if you want to be an instructor of record (IOR), but there aren't any formalized programs to help you practice by being an IOR yourself, see if you can find a faculty mentor who will let you practice lecturing once or twice with their course. The TAP-

STEM program gave me extensive practice in front of a lecture hall full of students, and hands-on experience handling the logistics of a course. I would not have shed my stage-fright and anxiety about my own competence leading a course if I had not participated in this program.

3.5 Instructor of Record Positions

I have been an instructor of record (IOR) at UCI for four different courses. The TAP-STEM program provided my first independent teaching opportunity after my third year, in summer session I 2018. I taught Chem 51LB, which is the first in an organic chemistry laboratory course series. I was competent and successful in this position, so during my fourth year I was approached by the department and offered the positions of IOR for Chem 12 — Chemistry Around Us — and Chem 128 — Introduction to Chemical Biology — in summer sessions I and II 2019, and Chem 101W — Writing for Chemists — in my fifth year, fall 2019. Each IOR position challenged me in a different way and taught me much about the thought, work, and logistics that must go into planning different types of courses.

My first IOR position for Chem 51LB was a lovely first independent teaching experience. Dr. Link mentored me the entire academic year prior to teaching it in summer session, and I guest lectured for her extensively during Chem 51LB's offering in winter quarter 2018. As this was a lab course, I lectured for one lab lecture every week and managed TAs who taught students in the lab sections. This lab course was a great way to ease into independent IOR teaching because there were less lectures to prepare for every week than lecture courses. I found that my preparation as a PF, TAP-STEM trainee and Head TA were keys to my success in running Chem 51LB. I very much enjoyed teaching, and knew I wanted to pursue a teaching-focused career after grad school.

After successfully teaching my first course as IOR, the Department of Chemistry asked if I was also willing to teach courses the following summer and fall, in 2019. I was ecstatic that the department had confidence in my ability to run a course completely independently. I accepted, and began preparing to teach the following year.

My second IOR position for Chem 12: Chemistry Around Us was an unpredictable challenge. Chem 12 is an online course, is always run in summer session, and fulfills two university-wide general education requirements. It is not a majors course, and when I was planning to teach it, it had not been run the summer prior because it had been under-enrolled. I was interested in teaching it because — in an ever-evolving technology age — I wanted experience running an online course. I did not have a mentor coaching me in how to teach an online course effectively, but I was given teacher training-wheels in the form of the prior instructor, Dr. Link's Canvas course. *Having access to a well thought-out online course that had pedagogically sound design helped me learn how online courses must be engaging through short videos and readings, rather than in-person lectures.* The major challenge I faced with this course was in actually getting summer session to run the course. I was unaware that courses needed to meet a minimum enrollment for the course to be offered. In retrospect, this course offering fluidity makes sense, but it also makes sense that I would be unaware because I had never before faced this issue as a graduate student. I had to advertise my course by identifying potential students — non-STEM majors — and posting flyers by contacting departments to post my flyers on department screens in building hallways. My course was almost cancelled a couple times, which was very nerve-racking. Luckily, the enrollment hit the magic number, 13, and I was able to teach it. Luckily, it prepared me well to teach remotely during the COVID-19 pandemic. I was, *ironically*, prepared.

When I began preparing for my third IOR position for Chem 128: Introduction to Chemical Biology, I felt ready to tackle a completely new course design plan. Little did I realize the monumental/colossal task ahead of me that was designing a course from scratch. A month before Chem 128 began, I decided to take a course design refresher by enrolling in the Course Design Certificate Program offered through the DTEI. It had been over a year since I was an active Pedagogical Fellow. I am extremely grateful I did this because it helped me better use backwards course design to ensure my assignments, activities, and assessments aligned with my course learning objectives. Chem 128 is an upper-division course generally taken by Chemistry Majors, and had twice as many course meetings during the accelerated summer session. I took a risk by partially flipping my course, which meant students built base content knowledge at home through completing readings and reading guides, and then in-class time was spent emphasizing difficult core concepts and practicing problem solving. This approach required much more content-prep than I had anticipated; I spent hours everyday designing course materials, in addition to the two weeks of prep I did ahead of time building the Canvas course space and designing the overall course. I learned a valuable lesson about the amount of time it takes to design a “good,” effective course.

My fourth, and final IOR position at UCI, for Chem 101W: Writing for Chemists served as my capstone teaching experience. I worked with original course instructor, Dr. Mang, to change the course assessment from traditional, points-based, grading to specifications grading. We determined the overall Course Student Learning Objectives, designed specifications grading rubrics, and decided which assignments to adapt from John Warner’s *The Writer’s Practice* to chemistry and general science-themed assignments. I then built all assignments in Canvas. Re-

designing a course to make it more student-centered was a gratifying challenge that should help students better learn chemistry writing.

Taking teaching baby-steps was vital in my progression from nervous-TA to able-IOR. Becoming an effective, independent, IOR takes time, effort, and practice. This is the best way to prepare for a future career in chemistry education teaching.

3.6 What is a TA Mentor?

Entering graduate school and finding myself in the dual role of Ph.D. student and undergraduate teacher was both exciting and overwhelming. I am glad I had TA training from the chemistry department and the DTEI, but I always felt that the transition would have been smoother had I been able to ask a more experienced TA for guidance. During my third year, I had conversations with my peers in which they expressed similar sentiments. A colleague and I mentioned our reflections to Dr. Link, and we were pleased to discover that she had been interested in starting a support program for TAs ever since she had heard of a TA mentorship program at Stanford University. She approached the chemistry department about doing something similar, obtained approval, and initiated the department's first formal Chemistry TA Mentoring Program, now called CTAMP.

CTAMP pairs a first year chemistry graduate student with a post-candidacy graduate student that has extensive experience as a TA. The TA Mentor meets with their mentee a couple times per quarter for their entire first year to help them set achievable teaching goals, reflect on their teaching ability through student evaluations and teaching observations, and work with them to help them become better TAs. Fall quarter meetings focus on helping mentees adjust to being a TA, teaching them to set and reassess personal goals, understand and learn from student

evaluations, observing their teaching ability to help them identify early how to improve, and provide general support as they transition to graduate school. In winter quarter, Mentors again help mentees set personal teaching goals for the quarter and assess mid-quarter and final student evaluations. In addition, TAs observe a more senior TA teach a course to learn more effective teaching strategies. Finally in spring quarter, TA Mentors continue providing support and guidance about student evaluations, but they take a more hands-off approach to help TAs transition to their second year as independent TAs.

I was a TA Mentor for the first two years of CTAMP. Before the call for applications was released for the first time, I helped Dr. Link design a flier to send out to the department. We received eleven responses, and had twelve total mentors for the first year. We had one and one half day of training on how to be a mentor and how we were expected to mentor our mentees. I had the opportunity to mentor six incoming TAs during the first year I was a TA Mentor and eight during the second. It was enlightening to see what first year graduate fears and frustrations are, and it helped me better understand how to guide and mentor students in general.

I will carry the knowledge and experience I obtained from being a TA Mentor with me as I embark on my future voyage as a chemistry instructor. I feel much more prepared to advise undergraduate and graduate students should they seek out my mentorship. Because student mentorship is an integral service component of university faculty, I believe all graduate students should seek out mentorship practice so they are better prepared as they graduate with their Ph.D..

3.7 Striving to Improve Safety Culture as the Safety Fellow

After I experienced a personal laboratory safety incident, I was motivated to actively advocate for better safety culture in research labs. In October of my fourth year, my lungs had an anaphylactic reaction to the peptide coupling agent compounds in my lab. I chronicled my allergy progression and clinical confirmation in a *Journal of Organic Chemistry* lessons learned note. Shortly after my close encounter with hazardous chemicals, I joined the Department of Chemistry's Graduate Safety Team (GST). I then served as the Safety Fellow for the GST for my fifth and final year at UCI.

As Safety Fellow, I was dedicated to helping change safety culture in the department. The Safety Fellow leads the GST in supporting best safety practices amongst graduate students and postdocs in research labs. Together, the entire team runs seminars, workshops, lab safety walkthroughs, and safety outreach events. Early in my time as Safety Fellow, I realized there was a disconnect in communication between the stakeholders in safety (lab Safety Representatives and research group members) and those in charge of enforcing safety (EH&S and PIs). My main goal as the Safety Fellow was to help bridge that gap to facilitate better safety cooperation amongst all research lab players.

I proposed that the GST start opening a line of dialogue between research lab members and PIs/EH&S by running round-table discussions. I got this idea when attending a Safety Workshop at the ACS San Diego meeting in August 2019. A graduate student at the University of Connecticut started round-table discussions in her department to hear about research lab safety concerns. The GST agreed that running these types of discussions, with the anonymity and safety of a panel of graduate students, devoid of the presence of EH&S staff and faculty-members, would give SRs a

safe space to voice their concerns and frustrations about the state of safety in their labs and management by EH&S and PIs.

The first round-table meetings were held in winter quarter 2020 and were regarded with great positivity. SRs appreciated being heard, and voiced challenges they faced such as PIs not providing enough support or feeling extra policed by EH&S. The GST gained valuable information on how to best help the department by acting as intermediaries between stakeholders in safety and the bosses and enforcers. The GST plans to run these more often in the future. I am currently chronicling this experience in a manuscript that is in preparation for *J. Chem. Ed.* about the safety standards in place at UCI — from undergraduate teaching labs to research labs to safety interventions from the GST.

My time as the Safety Fellow taught me the importance of listening and communication. There is a disconnect in academia between how research labs are expected to behave in terms of safety, and how that behavior is enforced or supported. The current system makes research labs feel targeted and policed by the powers that be. Some labs, instead, feel supported and heard. The difference seems to be that labs who clearly communicate with EH&S representatives and PIs feel supported because these powers work with them to make their labs as safe as possible. In contrast, those labs that don't initiate an open line of dialogue with EH&S and PIs feel targeted and are less motivated to enforce best safety practices. They feel the powers are unreasonable and are unwilling to compromise, but from their descriptions, they seem like they never react to safety violations with openness and compromise, instead they react with defensiveness and stubbornness about changing practices in their labs. Safety guidelines are in place for a reason. Many lab practices are not black and white, there are often many ways to amend practices to make something more safe.

I feel immensely prepared to approach safety concerns and facilitate open communication and compromise as a future instructor.

3.8 Concluding Reflections

Looking back at my time as a graduate student, I remember being told to pursue my interests and professional development opportunities as a young Ph.D. candidate, but I did not truly grasp what those opportunities were until I could look back with clarity. I did, indeed, pursue opportunities that interested me and that — I determined later — were perfect professional development opportunities that helped me get to where I am today.

I now realize that my path towards becoming an independent, effective, instructor did indeed prepare me nicely for a career as a university professor. Not only do faculty have to teach and conduct research, but they also need to mentor their students and TAs and perform service to their departments and schools. My path began when I was an interning instructor — a TA. I prepared to be a future faculty member by training through multiple pedagogical fellowship opportunities. I then continued to practice and hone my pedagogical expertise through multiple instructor of record positions. I finally dipped into other pre-faculty roles through formal mentoring and service opportunities. Graduate school is so much more than a time to take classes, teach classes, and perform research. Graduate school is an incubator for nurturing and cultivating future leaders in STEM, especially leaders in teaching and learning. Pursue passions, pursue professional development opportunities, and make the most out of the grad school experience.

Chapter 4

A Turn Towards Chemical Education Research

4.1 Introduction

After discovering my severe peptide coupling agent allergy and realizing I could no longer conduct research in a laboratory setting, I expanded my Ph.D. focus to include performing research in chemistry education. I worked with Dr. Renée Link, and colleague William Howitz, to convert her lower division organic chemistry laboratory course series (Chem 51LB, 51LC, and 51LD) from a traditional, points-based, grading system to a specifications grading system. We three worked collaboratively to study how students and teaching assistants perceived the new grading system. We wrote and submitted a manuscript describing our pilot study to the Journal of Chemical

Education, and we will submit a manuscript on our scaled-up study in the near future. These writings are included here in section 4.2 of this dissertation. I also worked with Dr. Stephen Mang to design and implement a specifications grading system for his upper division "Writing for Chemists" course (Chem 101W). We have also written a manuscript collaboratively, which is included in section 4.3 of this dissertation.

Grading, a fundamental component of assessment in higher education, is intended to reflect student achievement of course learning outcomes. Finding an objective way to assess qualitative work is challenging and has depended traditionally on points-based grading systems.^{1,2} This approach to grading in the college classroom is not ideal as it places emphasis on the extrinsic motivational factor of accumulating points rather than the intrinsic motivation of learning and meeting course learning outcomes.³⁻⁵ The education community has demonstrated awareness of flaws in the traditional, points-based, grading systems it employs, as evidenced by the continuous development of methods to improve the grading process.^{1,2,6-9} Specifications grading — popularized by Linda Nilson in 2014 — represents a new grading system that moves away from a reliance on points and has the potential to make substantial positive changes in student learning.¹⁰ In this chapter, I first discuss the origins of the specifications grading system, outline the potential benefits of adopting it for large university science, technology, engineering, and mathematics (STEM) programs, and describe the first implementation and the outcomes of this grading system in an organic chemistry laboratory course (section 4.2). I then discuss how we implemented a specifications grading system in a redesigned upper division "Writing for Chemists" course and what the outcomes were.

4.1.1 Evolution of Alternative Grading Systems

Specifications grading evolved from three previous grading systems: mastery learning, competency-based grading, and contract grading, and has been adopted in a variety of college-level courses (Figure 4.1).^{10–22} Mastery learning, coined by Benjamin Bloom in 1968, requires that students meet an instructor's established performance standards on one course topic before advancing to subsequent topics.²³ To meet students' individual learning needs, Bloom advocated for variation in teaching methods and flexibility in time allotted for students to complete course topics. While mastery learning approaches are effective, the challenges of providing individualized instructional strategies and having sufficient time to ensure all students achieve the same level of learning make the mastery learning approach daunting for instructors to implement.²⁴

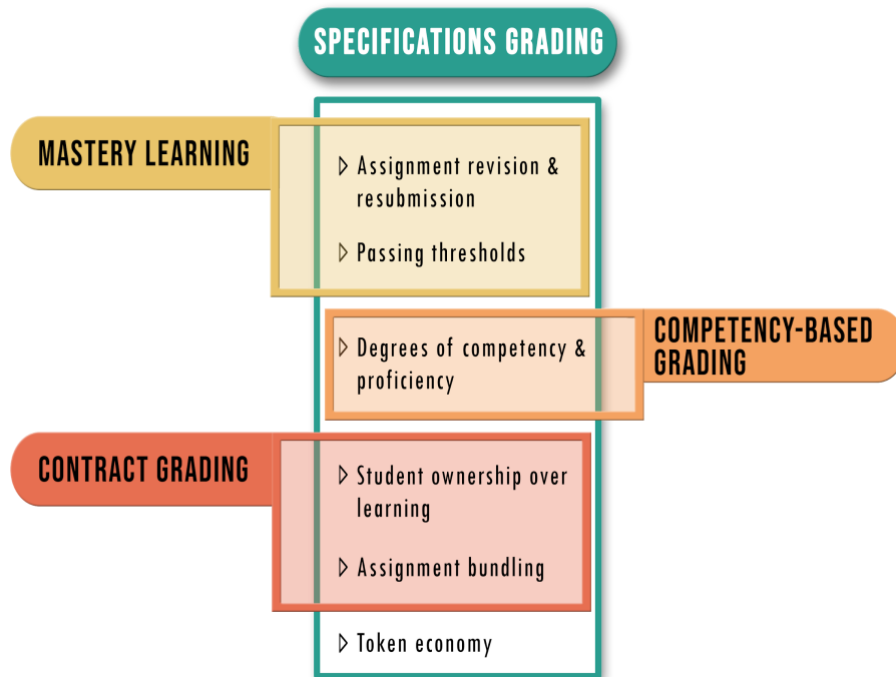


Figure 4.1. Specifications grading evolved from mastery learning, competency-based grading, and contract grading. Graphic design by K.M., W.H., and R.L., and graphic construction by Denise Bui.

Technological advancements, such as the internet, enabled the development of competency-based grading — an extension of mastery learning. Competency-based grading similarly uses instructor-defined passing thresholds on assessments, but these thresholds are differentiated into multiple categories based on a student's level of competency. This approach empowers students to take greater control over their own learning by providing them the option to demonstrate proficiency on an assessment above the minimum level.^{11,25–28} The use of technology in this grading approach allows for personalized and immediate feedback as students move through course material at their own pace. A drawback of the technology-driven, competency-based interpretation of mastery learning is its de-emphasis on student engagement with instructors and peers, which are important for student retention of course material.^{29–32}

Contract-based grading gives even greater control to students over their learning than competency-based grading. In this system, students negotiate a contract with the instructor to define which assignments they want to complete for a predetermined grade in the course.^{33,34} If students meet the level of performance expected, the instructor awards the student the predetermined letter grade. This system retains the student-instructor and student-student engagement that may be lost in competency-based approaches, while also giving students more ownership over their learning. This ownership is valuable because it increases student motivation to learn the course material.^{35,36} This system also has the added benefit of eliminating competition between students as each student's grade is independent of their peers' grades. However, this method of grading has been criticized for potentially allowing students to easily earn higher grades while putting in less effort than a traditional grading system.^{10,37} Another drawback — similar to the original version of mastery learning — is that contract-based grading requires extensive

amounts of instructor time because each student has to develop their own contract which is then instructor-approved.

The benefits and drawbacks of each of these three grading systems informed the design and development of the specifications grading system.¹⁰ To keep course workload manageable for instructors in specifications grading, the instructor, rather than the student, defines the contract options that are tied to specific letter grades. Students still retain a degree of ownership over their learning by choosing to complete the bundle of assignments — that is, the contract — for the letter grade they want to earn in the course. The instructor defines passing thresholds for each assignment in the contract that students must meet to achieve proficiency, which ties back to the core idea of competency-based education. Because each bundle of assignments is developed with the course learning outcomes in mind, the learning outcomes a student has met will be evident based on their satisfactory completion of the associated bundle. In addition, the specifications grading system includes a token system, which provides students with limited options to revise and resubmit work that does not meet the criteria set to reach a satisfactory level. Limiting options for resubmitting work is necessary to keep the time needed for grading manageable. The token system incorporates a mastery learning element and gives students increased ownership over their learning in the specifications grading system, as students can choose which work they will revise and resubmit in exchange for using one of their tokens.

4.2 Developing and Implementing a Specifications Grading System in an Organic Chemistry Laboratory Course

4.2.1 Background: Grading Challenges in Large, Multi-section Laboratory Courses

The high-enrollment, multi-section laboratory courses that predominate in most large college and university STEM programs present particular grading challenges. The traditional, points-based systems typically used in these courses do not always accurately reflect student achievement of course learning outcomes. In addition, having multiple graders leads to grading inconsistencies which necessitates grade standardizations, often in the form of student score standardization and curving. These standardizations prevent students from accurately tracking their grade throughout the course. In addition, grade standardizations place students in competition with one another as their final grades depend on how their assignment scores compare to their peers' scores. Specifications grading can minimize, and potentially resolve, these issues.

Grading should evaluate student success based on achievement of competency in one or more course learning outcomes. Under traditional, points-based, grading systems, this is not always the case. If points are not clearly allotted for specific course learning outcomes, students may earn enough cumulative points to pass the course without clearly meeting any of the course learning outcomes.^{10,12,18} The structure of specifications grading resolves this issue because each bundle of assignments tied to the final course grade is developed with the course learning outcomes in mind. Therefore, a student demonstrates competency in course learning outcomes by satisfactorily completing the associated assignment bundle.

Another drawback of the points-based system is the focus students place on receiving points rather than meeting course learning outcomes. Students may view points as a transaction

for effort put into work submitted rather than credit to be earned for demonstration of understanding course material. By removing the distribution of points on assignments and using a binary satisfactory/unsatisfactory approach, specifications grading shifts student focus to understanding course concepts and demonstrating skills.^{10,19}

At the University of California, Irvine, as many as 35 teaching assistants (TAs) are responsible for grading assignments from over 1,000 students spread across 60 or more organic chemistry laboratory sections for a single course. Because grading systems using point-based rubrics can lead to significant variations in how individual TAs grade students' work, the scale of our courses at UCI requires final grade standardizations to account for the large number of TAs and associated laboratory sections.^{19,38} In our experience we have found that TAs generally agree on the quality of student work, but not the point values they assign as partial credit. Students also disagree with TAs about the number of partial credit points they are awarded per assignment. Removing points from the grading system could reduce these grading inconsistencies because specifications grading uses a binary satisfactory/unsatisfactory approach. TAs only need to identify one threshold per rubric item as opposed to the spectrum of thresholds contained within point-based rubrics. The specifications grading system could also have the advantage of reducing time spent on grading because less grader energy is put into deciding between a satisfactory or unsatisfactory assessment compared to having to select a score along a spectrum.

In a points-based system, students are generally unsure of their final course grade because they are unable to anticipate how the score standardization and course curve will change their unstandardized scores. Variation in TA grading requires final grades to be normalized and curved because each laboratory section can have drastically different section averages. Without standardization, students with less critical TAs would be rewarded with higher course letter grades

while students with more critical TAs would be punished with lower course letter grades. This uncertainty in grade standing not only contributes to student anxiety, but is also contrary to a cooperative and collaborative learning environment because this grading system perpetuates a student culture of competition.^{39–41} Each student feels that they are competing against other students for each point so they can have a higher point total at the end of the course. The higher their point total, the better their chance of benefiting from the curve when final letter grades are determined. Under a specifications grading system, the need for standardization and a curve is eliminated. Each student knows exactly what they must accomplish to earn their desired course grade, and each student's grade is solely dependent on the work they produce, rather than being partially dependent on the performance of other students.

4.2.2 Designing a Scalable Specifications Grading System for a Laboratory Course

Specifications grading has been used in various STEM courses, including chemistry lecture courses, but has not yet been reported in a chemistry laboratory course.^{14–17,19,21} With an end-goal of scaling up specifications grading to our larger, 1,000 plus student, on-sequence courses, we chose to pilot a specifications grading system in the final course in the organic chemistry laboratory sequence. We specifically chose to pilot specifications grading in the accelerated summer session course because it has the smallest enrollment — about 40 students. Each week in this course, students attend two 50-minute laboratory lectures taught by the instructor and two four-hour laboratory sections taught by a graduate student TA.

To transition the organic chemistry laboratory course grading system, we began by defining criteria students must meet to achieve specific grade levels: A, B, C, D, or F (Table 4.1).⁴² These criteria were designed to reflect the Student Learning Outcomes (SLO's) for the course and

encompassed all previously graded components of the course: online pre-laboratory homework, pre-laboratory video quizzes, laboratory notebook assignments, post-laboratory assignments, laboratory lecture participation, and practical exams. Students were given a Student Grade Tracker as a checklist tool to track progress towards earning their desired grade (shown in Appendix A, section 4.4.2). Rubrics for course laboratory notebook and post-laboratory assignments were adjusted to a binary satisfactory/unsatisfactory form, consistent with the specifications grading system. To incorporate a mastery learning aspect into the system, we also instituted a token system where students could redeem a token for the opportunity to resubmit an assignment that was assessed as unsatisfactory. We also divided the practical exam into components and specified which components students needed to complete to earn their desired letter grade.

Table 4.1. Comparison of letter grade requirements under the previous, points-based grading system and the specifications grading system.

Course Requirements	Criteria from Points-Based Grading System		Criteria from Specifications Grading System	
	Items Students Must Complete	Final Grade Weight	Course Grade Level*	Set of Criteria Completed
Online Pre-laboratory Homework Assignments	1 every week	28 points	A B C D	90 - 100 % complete 80 - 100 % complete 70 - 100% complete < 70% complete
Pre-Lab Video Quizzes	1 every week	18 points	A B C D	85 - 100 % complete 80 - 100 % complete 75 - 100% complete < 75% complete
Laboratory Notebook Assignments	8	15 points / day	A B C D	7 Satisfactory 6 - 7 Satisfactory 5 - 6 Satisfactory 4 Satisfactory
Post-laboratory Assignments	4	20-110 points	A B C D	5 Satisfactory + 1 full written laboratory report 4 Satisfactory 3 Satisfactory 2 Satisfactory
Lab Lecture Participation	Must participate	18 points	A B C D	7 required 6 required 4 - 5 required < 4 required
Practical Exam	1 final exam	205 points	A B C D	Pass Mastery Final Pass Knowledge Check w/S Passed 3 Lab Techniques Passed 4/6 safety questions Pass Mastery Final Pass Knowledge Check w/S Passed 2 Lab Techniques Passed 4/6 safety questions Pass Knowledge Check w/S Passed 1 Lab Technique Passed 4/6 safety questions < above criteria

*Students who do not meet the minimum criteria for D grade earn an F in the course.

Course letter grade bundles were defined and included on the Student Grade Tracker, and students earned the highest grade for which they met all of the criteria. For example, to earn a C-level grade, a student must have achieved at least 70% of the points for the online pre-laboratory homework, 75% of the points for the pre-laboratory video quizzes, five or more satisfactory laboratory notebook assignments, three or more satisfactory post-laboratory assignments, have attended four or more laboratory lectures, and have passed the required practical exam components. The course letter grade bundles were designed to align with the following course SLO's:

1. Perform fundamental organic chemistry techniques in the context of laboratory experiments.
2. Demonstrate understanding of concepts underlying fundamental techniques by proposing solutions to actual or potential problems encountered during an experiment.
3. Accurately draw reaction mechanisms for reactions conducted in laboratory sessions.
4. Use spectroscopy data to determine structures of unknown molecules.
5. Use data collected from an experiment to make claims supported by evidence.
6. Identify safe and unsafe practices related to techniques used in laboratory sessions.

Students could earn higher grades by achieving requirements for higher grade bundles, and ultimately would earn the highest grade for which they met all of the criteria in a given bundle. Higher grade bundles required higher levels of performance as demonstrated through higher percentages on homework/video quizzes, completing more laboratory assignments as satisfactory, and passing additional exam components.

4.2.2.1 Specifications Grading Assignment Rubrics

Under the specifications grading system, expectations for satisfactory work on assignments must be provided clearly. To communicate these expectations, we adjusted the assignment rubrics from the points-based rubrics — which allowed for partial credit in addition to full credit — to binary satisfactory/unsatisfactory-based rubrics. In this new rubric design, students either earned credit for a rubric item or they did not; no partial credit was awarded. This redesign necessitated revision of the points-based rubrics to better separate elements that had been grouped together into defined, separate, rubric items. For example, we parsed the singular theory rubric item of an experiment's post-laboratory assignment under the old grading system into four individual rubric items under the specifications grading system (Table 4.2, see Appendix A for a more detailed example). Satisfactory thresholds for assignments were set to approximately 80% of the total rubric items. These thresholds were chosen to ensure that students who earned credit for an assignment achieved proficiency.

Table 4.2. Comparison of a section of a points-based rubric and a specifications rubric for a post-laboratory assignment.

Criteria from Points-Based Rubric	Points	Criteria from Specifications Rubric	Satisfactory	Unsatisfactory
Theory (Full Credit): Student discusses fundamentals of column chromatography and relates the technique to TLC, noting similarities and differences and how a successful separation is achieved.	7	Theory 1a: Clearly describes the chemical principle(s) that govern how compounds are separated using column chromatography. Note: Be sure to include the importance of solvent choice.	<input type="checkbox"/>	<input type="checkbox"/>
		Theory 1b: Clearly compares and contrasts column chromatography to TLC.	<input type="checkbox"/>	<input type="checkbox"/>
		Theory 1c: Clearly describes what procedural steps must be taken to achieve a successful separation using column chromatography.	<input type="checkbox"/>	<input type="checkbox"/>
		Theory 1d: Clearly explains how separation is monitored in real time, and how this allows the determination of whether the separation was successful or not.	<input type="checkbox"/>	<input type="checkbox"/>

Restructuring the rubrics may provide the added benefit of simplifying grading for the TAs. While grading, TAs only need to view one criterion, or rubric item, at a time and decide whether the student's work meets the criterion or not. This system is intended to reduce the time TAs need to spend deciding what score — on the spectrum of each rubric criterion from the original rubrics — a student's report should earn.

For a competency-based approach to function under the specifications grading framework, students need to be given opportunities to learn from their mistakes and to be reassessed. Any students whose work does not meet the satisfactory threshold established for an assignment does

not earn any credit for that assignment. The token system provides students with a limited number of opportunities to revise and resubmit work for credit that would overwrite their previous grade. This structure not only allows students to incorporate feedback to pass assignments they initially did not, but it also permits students to choose if and when to resubmit work. The token system provides the additional benefit of acting as a safety net for students when unexpected events temporarily hinder their ability to complete coursework.

We were inspired by Blackstone *et al.*'s token system, and used it as a model for our own.¹⁸ To earn an initial four tokens, students completed a short, self-regulatory learning assignment at the beginning of the course.⁴³ Students could earn an additional, limited number of tokens throughout the course for completing additional tasks such as participating in midterm and end-of-term course feedback surveys. In addition to using tokens for assignment revisions, students could also redeem tokens in other course contexts, such as extending assignment deadlines or attending a make-up laboratory if a laboratory section was missed. This flexibility eliminated the need for students to provide explanations and to request exemptions for late work and absences. Token redemptions were tracked through the use of a Google form and a placeholder assignment in the course learning management system (LMS) that listed each student's current token count.⁴⁴

4.2.2.2 Specifications Grading Exams

Converting our course to a specifications grading system also necessitated a restructuring of the laboratory practical exam. Under the previous points-based grading system, students completed a laboratory practical during the last week of the term; this exam consisted of a wet laboratory portion, where students performed an organic chemistry laboratory technique (e.g. TLC, recrystallization, extraction, or melting point), and a dry laboratory portion. For the dry laboratory

portion, students answered a critical thinking question, performed experiment-based calculations (e.g. theoretical yield, unit conversions, etc), drew an accurate reaction mechanism for a reaction covered during the course, used provided spectra to identify an unknown organic compound, and answered multiple-choice laboratory safety questions.

Under the specifications grading system, we defined four components of the laboratory practical exam. The first three components — a safety final, a knowledge check final, and a technique final — represent the core competencies a student needed to demonstrate to pass the course and were required to earn a C-level or higher grade (Figure 4.2). Students who aimed for a higher letter grade were required to complete additional laboratory techniques and to complete the fourth component of the practical exam — the mastery final.

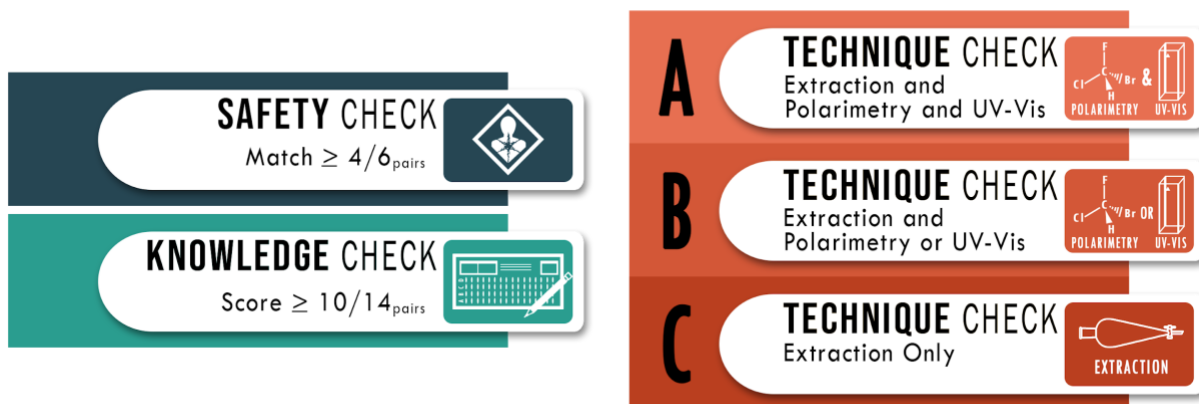


Figure 4.2. Required components of the laboratory practical exam under the specifications grading system. Students must perform the safety check, knowledge check, and the technique check. Depending on the final grade the student aims for, they can choose to complete only the extraction technique for a C-level grade, both the extraction technique and polarimetry or UV-Vis technique for a B-level grade, or all three techniques for the technique check portion of the exam. (Graphic design by K.J.M, W.J.H, and R.D.L, and graphic construction by Denise Bui.)

The safety final component was included to determine if students had achieved competency in determining best safety practices in the lab; it consisted of a collection of six images illustrating

unsafe laboratory practices (e.g. glass waste in the trash can, an open chemical container sitting out on the bench top, etc.). Students matched each image to the appropriate unsafe practice chosen from an answer bank. To pass the safety component, four of the six unsafe practices had to be matched correctly (Figure 4.2).

The knowledge check component was included as a multiple-choice exam with fourteen questions to assess if students achieved competency in fundamental course concepts and skills. The exam included conceptual questions on each laboratory technique taught that term, stoichiometry and limiting reagent calculations, identification of GHS hazard symbols, matching a ¹H NMR spectrum to a molecular structure, and recognizing the correctly drawn reaction mechanism for a reaction conducted that term. To pass the knowledge check component, ten of the fourteen questions must have been answered correctly (Figure 4.2). If students did not pass this exam component on their first attempt, they were given a second chance to pass by taking a different version of the exam. This final retake option was included to incorporate a mastery learning component to the final exam structure, where students are given an opportunity to learn from their mistakes and be reassessed.

The technique final component, designed to test students' ability to perform a fundamental laboratory skill, retained the format from the wet laboratory portion of the previous version of the laboratory practical exam. All students aiming for a C-level grade or higher had to perform and pass one technique exam chosen by the instructor (Figure 4.2). Liquid-liquid extraction was selected as the required technique for a C-level grade because it was the laboratory technique used most frequently throughout the course. Students aiming for a B-level grade had to perform and pass one additional technique; they could choose between polarimetry and absorbance

spectroscopy. Those students aiming for an A-level grade had to perform and pass both of the additional techniques.

The mastery final component provided students an opportunity to demonstrate a level of ability greater than competency, i.e. mastery, over the course content. This exam component consisted of three main question categories: conceptual critical thinking, experimental calculation critical thinking, and spectroscopy (Figure 4.3). Two questions were provided in each category and students were given the option of choosing to complete one or both questions in each category. Each question was given a partial pass threshold (0.5) and a full pass threshold (1). The following cumulative pass thresholds were needed to achieve the corresponding letter grade: 3 for an A, 2.5 for an A-, 2 for a B+, 1.5 for a B, and 1 for a B-. The students would only earn these final letter grades if they also met all other criteria specified for that letter grade (Table 4.1).

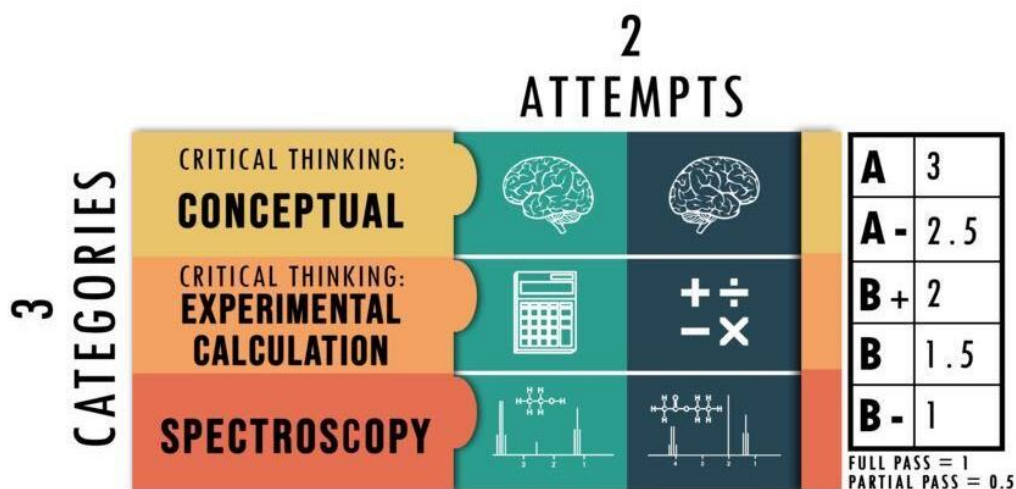


Figure 4.3. Categories of the mastery final portion of the laboratory practical exam under the specifications grading system. Students must complete the mastery final component if they wish to aim for an A-level or B-level final grade in the course. There are two questions per category, with a total of six questions on the mastery final. Students can attempt any of the six questions, and earn the depicted number of full or partial passes to be eligible to earn the corresponding letter grades. (Graphic design by K.M., W.H., and R.L., and graphic construction by Denise Bui.)

4.2.3 Pilot Implementation Outcomes

In this specifications grading pilot implementation, we endeavored to trial the new specifications grading system and to determine how students and TAs perceived it. The implementation of this system in the small organic chemistry laboratory course allowed us to assess whether the grading system could be viable in a large laboratory course setting. We surveyed students midway through the course, and we asked for both student and TA feedback at the conclusion of the course to determine their perceptions of what worked well and what needed improvement. To determine if student perceptions correlated to their performance in the course, we also compared students' course letter grades from this course to a previous course offering which did not use a specifications grading system. We also administered survey questions from the Motivated Strategies for Learning Questionnaire (MSLQ) and the Chemistry Laboratory Anxiety Instrument (CLAI) before and after the course to determine if student learning strategies, academic motivation, and laboratory anxieties changed after taking the course with a specifications grading system.

4.2.3.1 Teaching Assistant Perceptions

The two course TAs — who have taught two or more organic chemistry laboratory courses in previous terms — were asked for feedback on the specifications grading system at the conclusion of the course. Their perceptions of the grading system were strongly positive, and both described grading student work with the new rubrics as simpler and faster compared to using traditional rubrics with partial credit options:

“I think this grading and overall system is a lot easier to use and it makes the workload for TAs less intensive and time consuming.”

“I liked that it was in binary.”

“I think it makes it a lot easier to grade and gets rid of the uncertainty about meeting the rubric criteria.”

In addition to efficiency, the TAs also reported spending more time discussing student understanding of course material, over email and in person, than discussing complaints over assignment grading (Appendix A). This report contrasts with anecdotes from previous TAs, who taught in iterations of the course where traditional points-based rubrics were used. The TAs stated that students generally contacted them in an attempt to negotiate for more points.

4.2.3.2 Student Perceptions

Students were surveyed twice in this course to determine their perceptions of specifications grading. Anonymous surveys were administered midway through the course and at the end of the course. Student attitudes toward the specifications grading system were mixed, and changed from more negative during the course to more positive after the course concluded.

Of the 37 students enrolled in the course, five responded to the midterm survey, and four to the post-course survey. Although the response rate was low, recorded perceptions matched what students reported anecdotally through in-person interactions. In the midterm survey, students commented that the “all-or-nothing” aspect of the assignment grading made the class more stressful for them (Table 4.3). Although students praised the token system, they did not like that a token was necessary to revise and resubmit an assignment that had missed the “satisfactory” cutoff by only one rubric item. Several students commented that they felt it was unfair that they did not receive any credit for turning in work even though it did not meet the “satisfactory” criteria. Students also commented that the new rubrics were far less detailed than previous versions.

However, TAs had the opposite perception of the rubrics, describing the new rubrics as more detailed and clear.

Table 4.3. Student feedback themes during and after the course.

Midterm Feedback (n = 5)	Post-Course Feedback (n = 4)
Grading is stressful because of the “all-or-nothing” approach	Grading is less stressful because students could track their grade
Liked tokens in general	Grading is less stressful because of the option to revise for credit
Did not like that a token was required to revise if only short one rubric item	Perceived TA grading as more standardized
Perceived rubrics as less detailed	Wanted partial credit Satisfactory thresholds set too high

The student feedback from the post-course survey was more positive than the midterm feedback (Appendix A, section 4.4.6). Three students commented that the grading was less stressful because they always knew where they stood and because the system allowed them to try again when needed. Two of these three students also felt that the grading between TAs was more standardized with the all-or-nothing rubric items. Of the remaining two students who provided feedback, one had a more negative view of the specifications grading system. The student commented that partial credit from the old grading system was better because at least they could get some credit for an assignment, whereas in the specifications grading system, missing a requirement for a grade in any one category could ruin their chances of earning that grade. The

other student felt that the cutoffs for earning a satisfactory on assignments was too high for undergraduates and that the cutoff should be set at a C-level, requiring only 70% of rubric items.

4.2.3.3 Comparison of Grade Distributions

Although students voiced concerns that the lack of partial credit opportunities would hurt their grades, students in the specifications graded course earned higher letter grades than students in a previous course offering with points-based grading. Final letter grades for students in the specifications graded course (n = 37) were compared to those from a traditionally graded version of the same course taught by the same instructor in a prior year (n = 68). In the specifications graded course 43% of students earned A-level grades, 46% of students earned B-level grades, and 11% of students earned C-level grades (Figure 4.4).⁴⁵⁻⁴⁷ These grades represent a shift toward higher overall grades when compared to the traditionally graded version of the course where students earned 34% A-level grades, 43% B-level grades, 22% C-level grades and 1% D-level grades. No F grades were recorded for either course, and there were no withdrawals because university rules do not permit students to withdraw after the conclusion of the second week of the term.

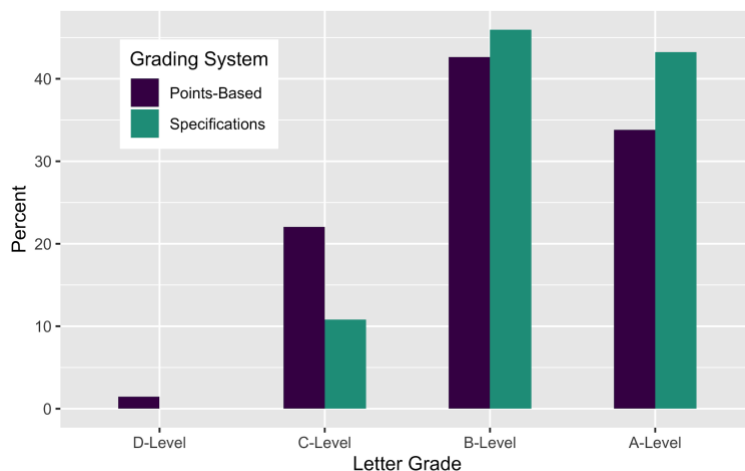


Figure 4.4. Grade distributions of a previous iteration of the course using a points-based system and the current course with the specifications grading system. See Appendix A, section 4.4.7, for the distribution including +/- grades.

4.2.3.4 MSLQ & CLAI Survey Data

To determine if student learning strategies, academic motivation, and laboratory anxieties changed after taking this course with a specifications grading system, we turned to the Motivated Strategies for Learning Questionnaire (MSLQ) and the Chemistry Laboratory Anxiety Instrument (CLAI). The MSLQ is a survey instrument used to determine how students at the undergraduate level employ learning strategies and find motivation academically.⁴⁸⁻⁵³ The CLAI is a survey instrument that measures student anxieties that they experience in chemistry laboratory courses at the undergraduate level.⁵⁴ We administered the survey at the beginning of the course and at the end of the course to see if student motivations increased and anxieties decreased.

To determine if students changed their learning strategies and had shifts in their academic motivations as a result of the specifications grading system, we used the following categories of the MSLQ: Control of Learning Beliefs (CLB), Help Seeking (HS), Individual Goal Orientation (IGO), Metacognitive Self-Regulation (MSR), Peer Learning (PL), and Self Efficacy for Learning and Performance (SELP) (Appendix A). The survey was administered on a seven point Likert

scale with the following responses: (1) very untrue of me, (2) untrue of me, (3) somewhat untrue of me, (4) neutral, (5) somewhat true of me, (6) true of me, and (7) very true of me. Each student's response is assigned a number value between 1 and 7, as indicated in the list of the previous sentence. A segment score was then calculated for each student by summing each of their response values for the set of questions in each MSLQ category. The student segment scores from the beginning (pre) and the end (post) of the course are shown in Figure 4.5. An increase in segment score represents an increase in students reporting that a statement is more true of them, and a decrease in segment represents that a statement is less true of them (Appendix A). There were no statistically significant differences in the survey responses from the beginning and end of the course, which suggests that the specifications grading system did not significantly change students' learning strategies or their academic motivations (Figure 4.5).

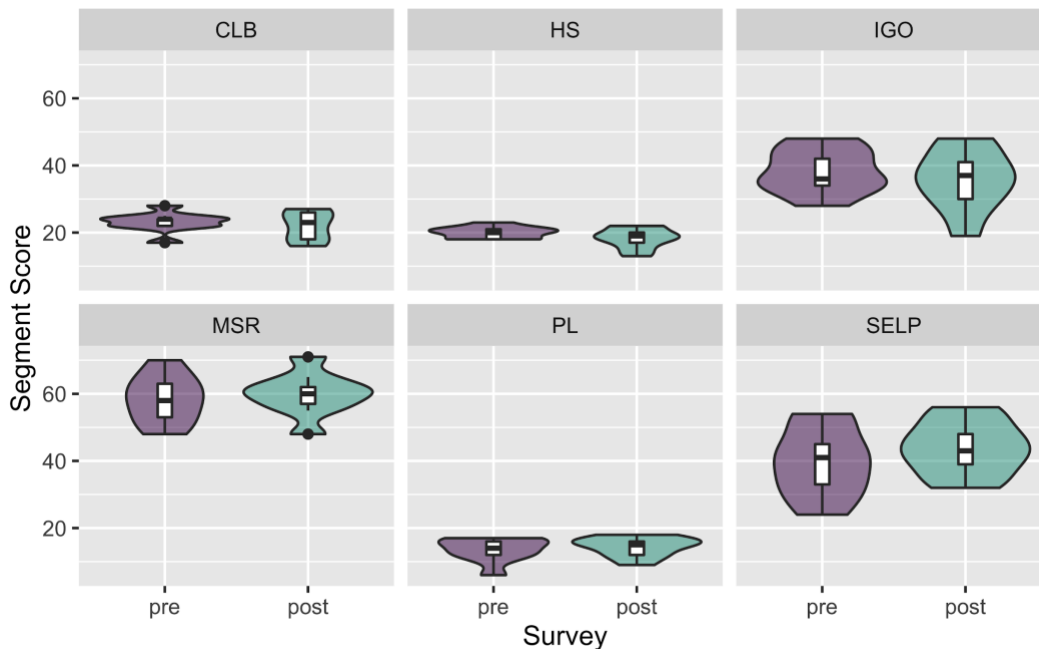


Figure 4.5. MSLQ results from surveys administered at the beginning of the course (pre) and at the end of the summer session 2019 course (post). MSLQ categories: Control of Learning Beliefs (CLB), Help Seeking (HS), Individual Goal Orientation (IGO), Metacognitive Self-Regulation (MSR), Peer Learning (PL), and Self Efficacy for Learning and Performance (SELP). To obtain a segment score, each students responses to questions in a given category are given a number, from 1 (very untrue of me) to 7 (very true of me). These numbers are then summed as a segment score. The violin plots show the distribution of student segment scores. The box plot-portions within the violin plots show the median as a horizontal black line, the first and third quartiles as the vertical boundaries of the white box, the inner quartile range as the black vertical lines above and below the white box, and outliers as black dots.

To determine if student anxieties about working in a chemistry laboratory environment decreased as a result of the specifications grading system used in this course, we used the CLAI survey (Appendix A). Similar to the MSLQ survey, the CLAI survey was administered on a Likert scale, but with five points: (1) strongly disagree, (2) disagree, (3) neither agree nor disagree, (4) agree, and (5) strongly agree. The CLAI score was calculated in the same manner as the MSLQ segment score, but by summing values from 1 to 5. A higher CLAI score indicates more student anxiety and discomfort in a chemistry laboratory; a lower CLAI score indicates less student anxiety and more comfort in a chemistry laboratory. Like the MSLQ survey results, there were no

statistically significant differences in student responses from the beginning and end of the course (Figure 4.6). However, the distribution of CLAI scores shifted toward less student anxiety and more comfort in a chemistry laboratory at the end of the course (post), indicating that students who felt most anxious about a chemistry laboratory setting at the beginning of the course felt less anxious by the end of the course. These results suggest that while the specifications grading system didn't cause statistically significant changes in overall student anxieties about learning in a chemistry laboratory setting, it did help make the most anxious students feel more comfortable. However, this result could also be caused by students feeling more comfortable at the end of the laboratory course, after they have had practice in a laboratory setting over the time-span of the course. This course was the last in the organic chemistry laboratory series, however, so students should have gained comfort in a laboratory setting in the first two courses of the series, again suggesting that the specifications grading system may have had a minor effect on student comfort.

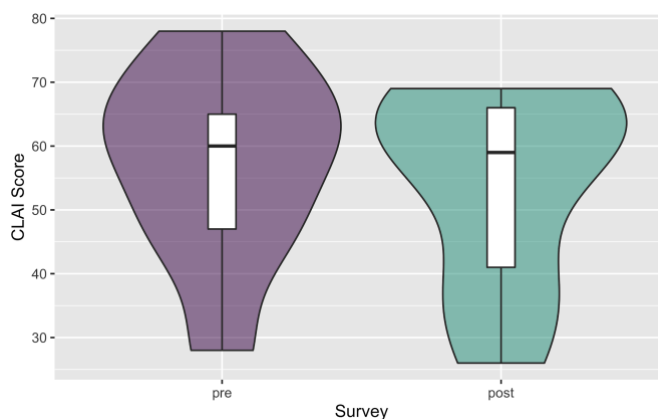


Figure 4.6. CLAI results from surveys administered at the beginning of the course (pre) and at the end of the summer session 2019 course (post). CLAI scores are determined using the same method as described for MSLQ segment scores.

4.2.4 Considerations for Scaled-Up Course Implementations

The primary goal of implementing a specifications grading system in this course was to test how the new system would work on a small scale. By identifying and resolving concerns in this smaller course, we would be better prepared to implement specifications grading on a larger scale in on-sequence lab course offerings. We were concerned that the time spent on managing the token system and grading revised assignment submissions would prove laborious, but we found that this aspect of the grading system should indeed be scalable. We also learned that establishing student buy-in to the new grading system was especially important to prevent student misconceptions about their course grade standing.

Contrary to our initial concerns, the time required to implement the token system and grade revised student work was not onerous. The instructor checked the Google form and updated students' token balances by changing the "score" in the placeholder assignment in the course LMS — a process that required approximately 10 minutes per day, on average. Most token trade requests were for assignment revisions, and by viewing the marked assignment rubric in the course LMS, students could identify items for which they did not earn a satisfactory assessment. If students chose to use a token to revise and resubmit an assignment, they only had to revise the unsatisfactory sections. Even with assignment revision requests, TAs reported that the time commitment was not burdensome. These considerations suggest that the specifications grading system should also be manageable in other STEM laboratory courses.

To prevent student misconceptions about their course grade standing, which can result in an overwhelming number of complaints in a larger course, it will be essential to establish buy-in and consistently provide reminders about the big picture of the grading system. Throughout the first half of the course, students were focused on the perceived higher stakes for individual

assignments. Students had access to a Student Grade Tracker, but they seemed unaware of how individual assignments related to the requirements for each letter grade. Students indicated they were stressed about not earning satisfactory scores for post-laboratory assignments, but they did not realize that they could earn an unsatisfactory score on one post-laboratory assignment and still earn an A-level grade in the course. After the mid quarter survey, we realized students were misinterpreting the Student Grade Tracker, so we devoted a small amount of class time to reviewing the tracker.

To address student misconceptions, we will provide more information at the beginning of the next course offering to establish greater student buy-in. Students will explicitly be told to shift their focus from individual category achievement to their overall grade standing in the course. Our goal is to ensure students realize that missing one category from the Student Grade Tracker will not cause them to fail the course. We will also reframe the binary satisfactory or unsatisfactory grading system as satisfactory or needs revision, and emphasize that students can resubmit assignments using the token system. These adjustments should result in less student concerns about stress related to earning a satisfactory score on all assignments.

Despite students' concerns about "all-or-nothing" grading, final course grades were higher overall for the course when offered with specifications grading than with points-based grading. This discrepancy could indicate that students are better able to meet course outcomes, or possibly that we must adjust our final grade requirements so they are more stringent. We will explore these possibilities in future studies.

4.2.5 Designing the Scaled Specifications Grading System for a Large Laboratory Course

After piloting the specifications grading system in the 2019 smaller summer term course, we implemented the grading system in the winter 2020 larger organic chemistry laboratory course — the first in the sequence, with 1,041 students. In the large course, we used similar materials to those we developed for the smaller course, i.e. a Student Grade Tracker, specifications grading rubrics modified specifically for the content in the scaled-up course, and a token system. The course also contained the same graded components: online pre-laboratory homework, pre-laboratory video quizzes, laboratory notebook assignments, post-laboratory assignments, laboratory lecture participation, and practical exams (Table 4.1). The differences between the small course and larger, scaled-up, course are shown in Table 4.4.

Table 4.4. Differences between pilot course and larger, scaled-up, course.

	Pilot Course	Larger, Scaled-Up, Course
Place in Course Sequence	3 rd	1 st
Students Enrolled	37	1,041
TAs for Course	2*	34
Lab Lecture Sections	1	4
Individual Lab Sections	2*	68
Exam Versions Needed		
<i>Knowledge Check</i>	3	10
<i>Safety</i>	6	6
<i>Technique</i>	3**	3**
<i>Mastery</i>	4	12

*Each TA teaches 2 lab sections per week during the summer term, so the number of sections TAs teach is different than during the normal academic year term.

**There are three versions of exam documents provided, but the quantities of reagents for the practical are varied and thus result in more exam variations.

We considered what we learned from the smaller course and applied those considerations to the larger course. To prevent student misconceptions about how final course grades are calculated, we established student buy-in by reviewing the Student Grade Tracker in detail on the first day of the class meetings. We also reviewed the tracker with TAs during the first weekly TA meeting. To allay student fears about the “all-or-nothing” grading aspect of the specifications grading system, we also began introducing rubric items as satisfactory or needs revision rather than satisfactory or unsatisfactory. We emphasized that students could use tokens to resubmit assignments if they did not achieve a satisfactory assessment. To facilitate student practice on critical thinking questions that would be seen on the Mastery-portion of the practical exam, we also incorporated example problems during the weekly 50-minute laboratory lecture class, posted extra practice problems to the course LMS, and provided solutions to those practice problems with explicit satisfactory criteria listed. We also reminded students about how to determine their final course grade at various points throughout the course in lab lecture and through announcements on the LMS because we anticipated student and TA confusion with the new specifications grading system.

4.2.6 Large Course Implementation Outcomes

In the implementation of the specifications grading system in the larger, scaled-up, course, we aimed to address the following:

1. Determine how students and TAs perceived the new grading system.
2. Compare how students performed in an iteration of the course using a points-based grading system versus the course using a specifications grading system.
3. Compare the quality of student assignments in a points-based grading system to the quality of student assignments in a specifications grading system.
4. Determine if student learning strategies, academic motivation, and laboratory anxieties changed after taking the course with a specifications grading system.

We endeavored to trial the new specifications grading system and to determine how students and TAs perceived it. The implementation of this system in the small organic chemistry laboratory course allowed us to assess whether the grading system could be viable in a large laboratory course setting. We surveyed students midway through the course, and we asked for both student and TA feedback at the conclusion of the course to determine their perceptions of what worked well and what needed improvement. To determine if student perceptions correlated to their performance in the course, we also compared students' course letter grades from this course to a previous course offering which did not use a specifications grading system. We also administered survey questions from the Motivated Strategies for Learning Questionnaire (MSLQ) and the Chemistry Laboratory Anxiety Instrument (CLAI) before and after the course to determine if student learning strategies, academic motivation, and laboratory anxieties changed after taking the course with a specifications grading system.

4.2.6.1 Teaching Assistant Perceptions

We surveyed TAs from both the 2019 points-based course and the 2020 specifications grading system course at the end of each course for feedback on the specifications grading system.

Of the following questions, we included 1-8 on the 2019 survey and 1-12 on the 2020 survey:

1. How many emails did you receive about a student disagreeing about whether they met the minimum criteria for a satisfactory score on rubric items in general? (*For the points-based course, the question read: How many emails did you receive about a student disagreeing about partial credit points in general?*)
2. How many emails did you receive about student understanding in general?
3. How many emails did you receive regarding questions like "why is this my letter grade"?
4. How many interactions did you have with students in-person about students disagreeing about whether they met the minimum criteria for a satisfactory score on rubric items in general? (*For the points-based course, the question read: How many interactions did you have with students in-person about students disagreeing about partial credit points in general?*)
5. How many interactions did you have with students in-person about student understanding?
6. On average, how long did it take you each week to grade notebook (pre-lab) pages?
7. On average, how long did it take you each week to grade post-labs?
8. About how much time did you spend per week agonizing over whether a student met the minimum criteria for a satisfactory score on rubric items? (*For the points-based course, the question read: About how much time did you spend per week agonizing over whether a student should be assigned partial credit or not on an assignment?*)
9. What did you think about the grading method used in this course?
10. What aspects of the grading did you think worked well?
11. What aspects of the grading would you change? Please be specific about the change you would make and why you think this is a beneficial change.
12. Use this space to provide any additional feedback.

The differences in the amount of time TAs spent answering student emails about student grades varied slightly between the points-based and specifications grading course offerings (Figure 4.7, Q1, Q2, and Q3). The amount of student emails that TAs received regarding grades did not change between the points-based and specifications grading courses (Figure 4.7, Q1 and Q3). However, the amount of emails TAs received about students inquiring for help understanding course material increased in the specifications grading course (Figure 4.7, Q2). For question 2, the TA response distribution is shifted lower, with a median of 5 emails received in the points-based course and 6 received in the specifications grading course.

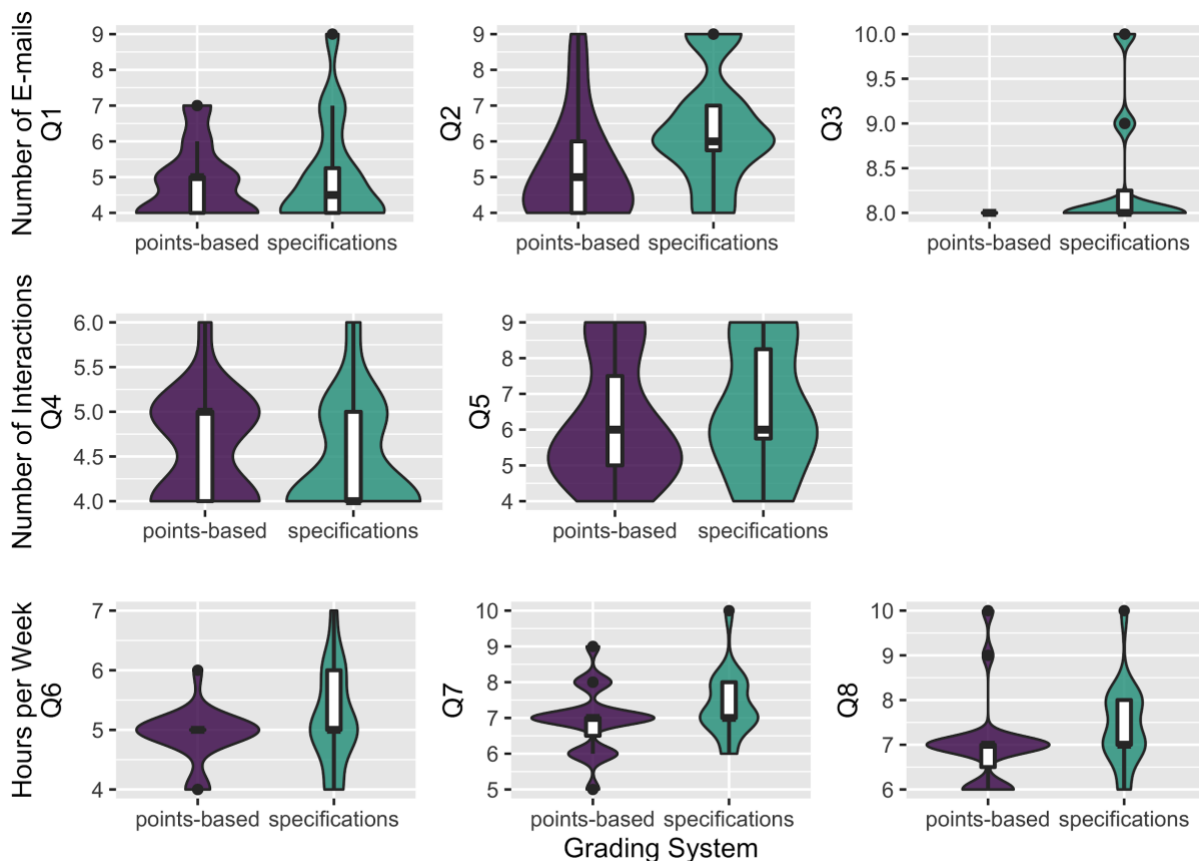


Figure 4.7. TA survey responses about time spent answering student emails, interacting with students, and time spent grading in a points-based grading version of the course versus a specifications grading version of the course. Q1-Q8 refer to the questions listed in beginning of section 4.2.6.1 (n = 33 for point-based course, n = 34 for specifications course).

The differences in TA time spent interacting with students in person about student grades and course understanding also varied slightly between the two course offerings (Figure 4.7, Q4 and Q5). In the points-based grading course, TAs reported spending more time interacting with students in person who disagreed with the amount of partial credit points they received on assignments. The distribution in TA responses has a median of 5 interactions and is relatively spread over 4-5 interactions (Figure 4.7, Q4). In the specifications grading course, there is a shift in TA responses towards 4 interactions, with a median of 4 interactions. While this is not a statistically significant shift, there is a shift in overall TA response distributions. In both course iterations, TAs indicated having similar in-person interactions about discussing student understanding of course material (Figure 4.7, Q5).

When asked about how much time TAs spent grading and agonizing over points each week, TA responses from the points-based course were similar to those from the specifications grading course (Figure 4.7, Q6, Q7, and Q8). The amount of time TAs reported spending on grading pre-laboratory and post-laboratory assignments is more spread in the specifications grading course than the points-based grading course, but the medians are the same, 5 and 7 hours respectively (Figure 4.7, Q6 and Q7). TAs reported spending similar amounts of time agonizing over points in both course iterations, with a median of 7 hours, but again the responses are more spread in the specifications grading course than the points-based grading course (Figure 4.7, Q8).

In the specifications grading course, TA perceptions about the specifications grading system were contradictory (see Appendix A for a complete list of responses). Some TAs found the grading system was more efficient and liked the ease with which the binary grading system facilitated consistent and fair grading. Other TAs did not like how the grading system seemed to disincentivize student effort and penalized students who only missed a couple rubric items to earn

a satisfactory assessment on an assignment. Some representative quotes from the TA feedback are as follows:

“Very easy and fast. Makes life easy.”

“I think it's good, I like the binary selection: either they met the criteria or they didn't.”

“Students only care about meeting the minimum requirements for regrade, they don't really care about fixing all the mistakes.”

“I didn't like it, it made me upset that some students who tried but just got some things wrong would get the same grade as someone who turned in nothing.”

When asked which aspects of the specifications grading system worked well, which aspects should be changed, and if there was any additional feedback, TAs provided mixed perceptions. TAs reported that the grading system was very clear and organized, provided ample opportunities for students to improve their unsatisfactory assessments through revision, and seemed to be less stressful for students since they didn't have to earn a satisfactory on each assignment to achieve a good grade in the class.

“I liked how organized the rubric is. It simplified the grading process, and made it easier to look for certain aspects of the student's writing that was or was not correct.”

“It was straightforward and there were less gray areas for points.”

“Revisions were a nice way to allow students to understand more.”

TA responses regarding aspects of the class they think should change focused on desiring more specific rubrics that would further break down each grading criteria, reverting back to a points-based rubric, and providing less time for students to resubmit assignments through trading in a token. In additional feedback, TAs expressed concern about the seemingly large amount of time they spent grading assignments and discussing the course grading scheme with students — rather than discussing a student's understanding of course material. However, as discussed previously,

the amount of time TAs spent grading assignments and discussing course grading with students in both the points-based and specifications grading courses was reported to be nearly the same (Figure 4.7).

4.2.6.2 Student Perceptions

We administered surveys at the end of the course to determine student perceptions of the specifications grading system. We asked students what they thought about the grading system, what they think worked well, and what they thought should be changed. Similar to TA perceptions, student attitudes toward the specifications grading system were mixed.

We analyzed student responses and categorized them into categories as generally positive, negative, neutral, mixed, or uncategorized. Positive and negative responses expressed general satisfied or unsatisfied sentiments. Neutral responses were considered comments that simply expressed observations of the grading system, without expression of how students felt. Mixed responses were student comments that expressed both positive and negative sentiments, and uncategorized comments were those that fell into none of the other categories. Of the 768 students who responded to the survey, 289 had positive responses, 241 had negative responses, 47 were neutral, 179 were mixed, and 13 were uncategorized.

Student perceptions of the specifications grading system were mixed (Table 4.5). Students generally appreciated the transparency of the grading system, and they thought it was overall more equitable. Similar to the pilot study course, students liked the token system and valued the opportunity to resubmit assignments. Students also recognized that their grade was no longer dependent on others because of the necessity of a final curve. On the negative side of student perceptions, students felt that there was a high degree of TA variability in grading, which is the

opposite of what students felt in the pilot course — that TA grading was more standardized. Students also felt that too much weight was placed on the final exams, the course was too “cut-throat” as one unsatisfactory assessment could ruin their chance for a desired final grade, the course was more work than their other courses, and that they should be rewarded for effort.

Table 4.5. Comparison of student feedback themes from small course and large course.

Small, Pilot Study, Course Feedback (n = 4)	Large, Scaled-Up, Course Feedback (n = 768)
Grading is less stressful because students could track their grade	Liked how they knew how to achieve the grades they wanted
Liked tokens in general	Liked tokens in general
Grading is less stressful because of the option to revise for credit	Liked the transparency/felt it was more equitable
Perceived TA grading as more standardized	Appreciated that their grade wasn't dependent on other students
Satisfactory thresholds set too high	Enjoyed not having to worry about a curve
Perceived rubrics as less detailed	Did not like the variability in TA grading
Wanted partial credit	Did not like how much weight was placed on the practical exams Did not like “cut-throat” nature of course, missing one assignments prevented students from achieving certain grades Felt that class was more work than other courses Felt like they should be awarded for effort

4.2.6.3 Comparison of Grade Distributions

Similar to the pilot course, students in the specifications graded course earned higher letter grades than students in a points-based grading course. A comparison of final letter grades in the specifications graded course ($n = 1,040$) to those from a points-based grading course ($n = 1,189$) — taught by the same instructor the year prior — showed that in the specifications graded course, the following percentages of students earned the corresponding grades: 42% A-level grades, 45% B-level grades, 7% C-level grades, 4.5% D-level grades, 3% failing grades (Figure 4.8). Overall, these grades are shifted higher than the grades from the points-based grading version of the course where 14% of students earned A-level grades, 43% B-level grades, 32% C-level grades, 9% D-level grades, and 3% failing grades.

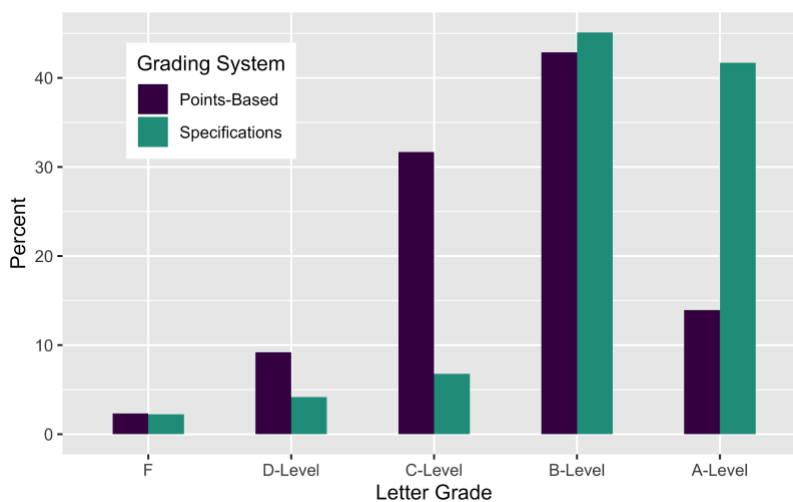


Figure 4.8. Grade distributions of the course using a points-based system (previous course) and the current course with the specifications grading system. See Appendix A, section 4.4.7, for the distribution including +/- grades.

4.2.6.4 Grading Comparison

To compare the quality of student assignments in a points-based grading system to the quality of student assignments in a specifications grading system, we graded student assignments from each course iteration. We randomly selected 30 student laboratory reports for the acid-base

extraction experiment that ran in both a course iteration that used points-based grading and the course that used specifications grading, with a total of 60 reports. We then solicited help from TAs to grade the reports using a points-based rubric. To prevent unconscious bias, the graders were unaware of whether the reports they graded were from students in the points-based course or from students in the specifications grading course. We gave each TA an equal number of student reports from the points-based grading course and specifications grading course. Each student report was graded by two TAs to ensure points were allotted consistently. We chose to grade all reports with a points-based rubric because student reports from the points-based grading system would not receive scores that were comparable to student reports from the specifications grading system, as the specifications grading rubrics are more detailed.

The results of the grading comparison indicate that there is not a significant difference in the quality of student laboratory reports from a course that used a points-based grading system to the same course that instead used a specifications grading system (Figure 4.9). The median scores for the student assignments from the points-based course and those from the specifications grading course are both 25 out of 40 points total. The only difference in the scores between the two course iterations is in their distributions. In the specifications grading course, the score distribution is not as spread, possibly indicating that the specifications grading rubrics provide a framework for students to complete assignments that are of a more consistent quality than with the points-based rubrics.

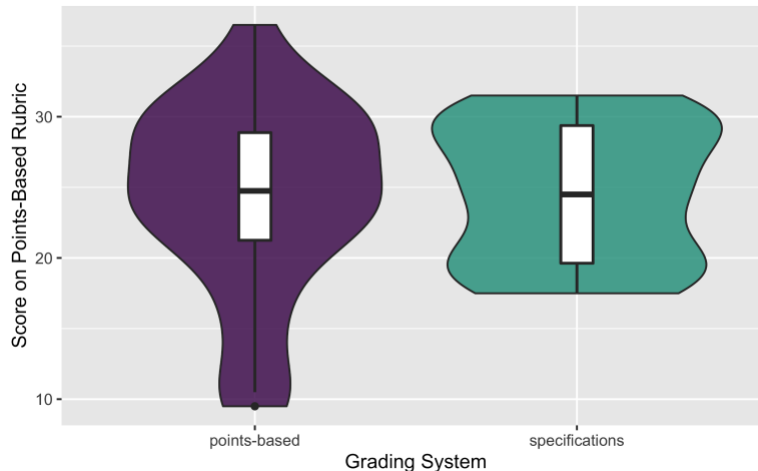


Figure 4.9. Grading comparison of student acid-base extraction laboratory reports submitted in a points-based grading course ($n = 30$) and the specifications grading course ($n = 30$). All reports were graded with a points-based rubric because the specifications grading rubric added additional criteria, which would render grading of reports completed with a points-based rubric a poor comparison.

4.2.6.5 MSLQ & CLAI Survey Data

We administered MSLQ and CLAI surveys in the scaled-up course, just as we did with the pilot course implementation. Both surveys were implemented in the same manner as described for the pilot course. The survey results from the scaled-up course mirror the results obtained for the pilot course in that no statistically significant changes emerged between the surveys administered at the beginning of the course (pre) and the end of the course (post) (Figure 4.10 and 4.11). Unlike the pilot course, we also administered the surveys in the prior year course that used a points-based grading system. Comparison of the survey results from the points-based course to the specifications grading course also yields no statistically significant differences.

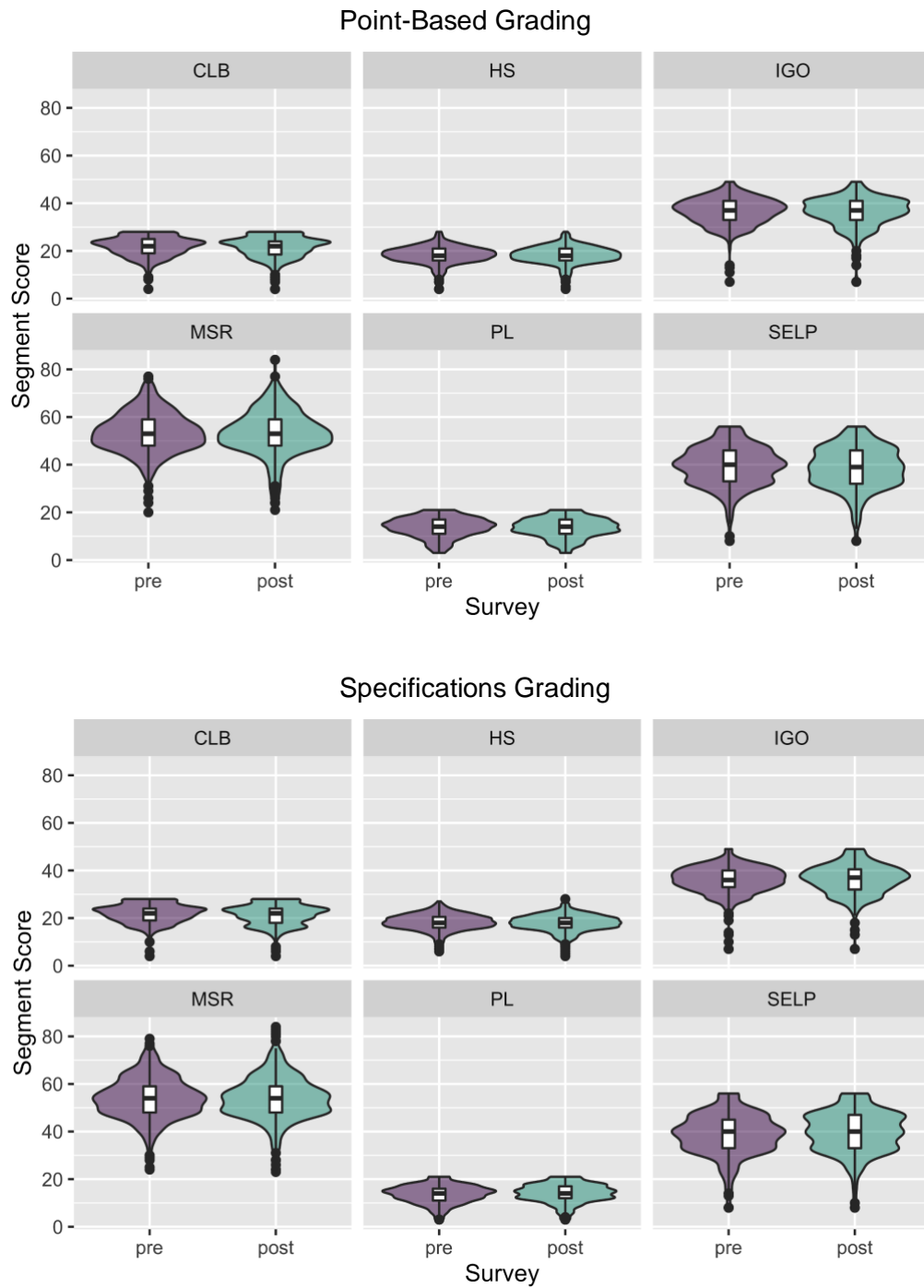


Figure 4.10. MSLQ survey results from two iterations of the course, one that used a points-based grading system and one that used a specifications grading system. The points-based grading system was used in winter 2019 and the specifications grading system was used in winter 2020. In each course, the surveys were administered at the beginning of the course (pre) and at the end of the course (post). The segment scores were calculated as described previously.

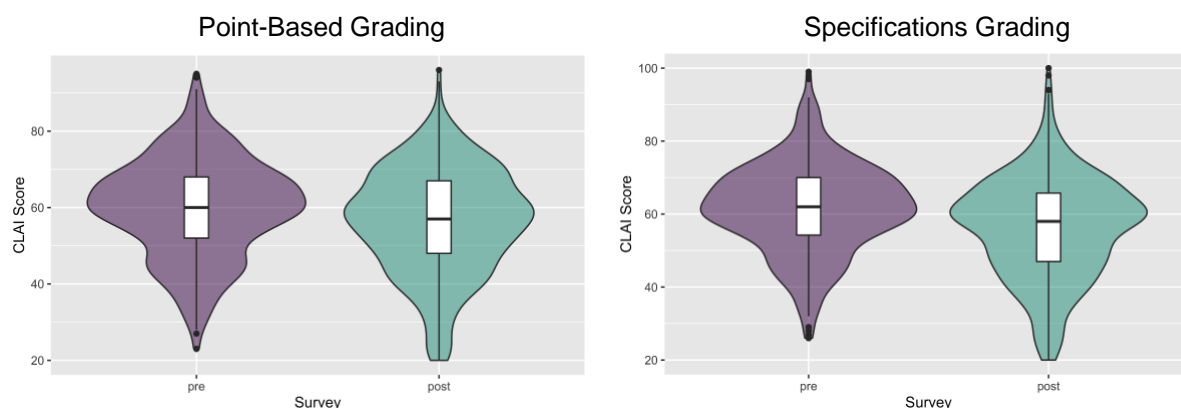


Figure 4.11. CLAI survey results from two iterations of the course, one that used a points-based grading system and one that used a specifications grading system. The points-based grading system was used in winter 2019 and the specifications grading system was used in winter 2020. In each course, the surveys were administered at the beginning of the course (pre) and at the end of the course (post). The CLIA scores were calculated as described previously.

4.2.7 Large Course Implementation Conclusions

We successfully scaled the specifications grading system from the pilot study course with 37 students to the large course with 1,041 students. While we applied the lessons we learned from the pilot course to the larger course, there were challenges that we did not anticipate. We found that scaling the grading system to a course this size limits the effectiveness with which we can communicate with our students and TAs. Similar to the pilot course, there were misconceptions by students and TAs. We also found that there were no significant changes in the quality of student reports from a course iteration that used a points-based system to the specifications grading system, student learning strategies, academic motivation, or laboratory anxieties.

Of the 50% of course TAs that responded to the survey, their perspectives of the specifications grading system were polarized, with about half of the respondents reflecting positively on the system and the other half reflecting negatively. We are pleased to report that some TAs reflected on aspects of the class that were designed for specific purposes — such as making grading more straightforward for TAs. Some TAs felt they spent more time grading than

in a course with a points-based system, but TAs self-reported time spent in the points-based course versus the specifications grading course were virtually identical (Table 4.7). Some TA sentiments, such as those feeling bad for penalizing students who tried, did not reflect the fact that student effort cannot be measured. These reflections mirror the comments we observed from students in the pilot study course and the large course and could reflect the fact that most of these TAs were first-year graduate students who possibly still view courses through a student lens rather than an instructor lens. TA comments also expressed desire for more detailed rubrics, which was also a desire of TAs in the course that used points-based rubrics. We attempted to make the rubrics more detailed with the specifications grading rubrics, but TAs still wanted more detail. The previous, points-based, rubrics were much less detailed (see Appendix A), but the TAs likely did not realize this as they were new graduate students, unfamiliar with past course grading systems.

Students had similar misconceptions about the specifications grading system. Like some TAs, students felt that they should be rewarded for the extensive effort they put into the class. It is difficult to measure student effort, and students are likely not putting in more effort than what is expected of a university level course. Students also felt that too much course weight was placed on the final exams and that the course was too cut-throat since getting an unsatisfactory on one course assignment could make or break their grade. Many university level courses place a significant amount of course weight on the final exam, so it is not unusual that this course places similar weight on the final exams. There are also many instances in courses where a student's final grade can be made or broken by a single assignment, so again this course is no different.

Specifications grading is not widely used, so students in our course are likely encountering it for the first time. Extra care and effort must be taken in future iterations of the course using the specifications grading system to establish buy-in for both students and TAs. The instructor should

be as transparent as possible and indicate the similarities — in addition to the differences — between the specifications grading system and points-based systems. Efforts to measure the amount of student time spent on coursework should also be taken.

4.3 Redesigning a “Writing for Chemists” Course Using Specifications Grading

4.3.1 Background

In the Fall of 2017, the UCI Department of Chemistry created an upper-division writing class (Chemistry 101W, “Writing for Chemists”) to allow junior- and senior-level chemistry majors to fulfill the university’s upper division writing requirement in their home department. The goals of the course were to introduce students to the discipline-specific writing conventions used in chemistry and to train them in searching and reading the chemical literature. The first two offerings of Chemistry 101W were taken exclusively by seniors, so the class focused on developing writing skills that could be applied to a variety of chemistry writing tasks instead of simply learning to write lab reports. These skills included communicating results in writing and with figures, writing proper sentences and paragraphs, and supporting arguments with evidence. We believed that the amount of preparation undergraduates received prior to enrolling in our course would be adequate for an advanced chemistry writing course. During these first two offerings of the class we observed that this was not a good assumption. Many students struggled with the communication of discipline-specific material, mainly because they lacked mastery of fundamental writing skills — such as using correct standard English grammar, maintaining a consistent tense, writing with the audience in mind, developing thesis statements, and transitioning clearly between sentences and paragraphs.

Chemistry majors beginning their upper-division studies are often several years removed from their most recent lower-division writing course, which was likely not focused on scientific writing. Any training in chemistry writing they have received was in lower-division lab courses.

As a result, students entering an upper-division chemistry writing course may be trying to learn discipline-specific writing conventions while simultaneously trying to connect the writing they are doing with the experiences they had previously. This situation leaves them susceptible to cognitive overload^{55,56} — a common occurrence in novices who are trying to learn the foundations of writing while simultaneously attempting to synthesize their knowledge of writing to produce novel pieces — potentially reducing the quality of work that they are capable of. During the first two offerings of Chemistry 101W, we observed that the majority of our students were not making connections between the formal writing skills they had learned in their lower-division writing courses and the writing experiences they were having in our class, resulting in assignments that were not completed at the level we expected. To address this problem, we created new assignments that asked students to make this connection and developed specifications grading rubrics so that the accomplishments we expected them to achieve were clear.¹⁰ We hoped that redesigning the course using a specifications grading framework could help our students translate their existing writing skills to a course focused on chemistry writing.

In Fall 2019, we created a new version of Chemistry 101W that was designed to reinforce writing skills and habits while introducing discipline-specific writing conventions, which we have now offered twice. Since specifications grading has been used successfully in writing-intensive courses in a variety of disciplines from political science to math,^{10–12,18,19,57–59} we created a set of assignments with specifications grading rubrics in this redesigned course. To emphasize writing skills and practices, we adapted these assignments from a textbook on the practice of nonfiction, formal writing.⁶⁰ We collected student attitude surveys before and after the class to determine if the new structure had any effect on their attitudes about writing and the practices they employed in their writing. While few statistically significant changes in attitudes were observed, general

trends regarding the practices students used in their writing and the way they thought about their writing emerged from students' survey responses and free-response comments.

4.3.2 Course Design

To refocus our course on general writing skills and conventions used in chemistry writing conventions, we reorganized the course using a specifications grading system. We designed our specifications grading approach on a high-pass, low-pass, unsatisfactory system predicated on whether students meet a certain number of criteria for each grading level. Using criteria instead of points to assess student achievement of Student Learning Outcomes (SLOs) made it easier for students and instructors to accurately measure student learning. We gave students standardized rubrics and a student grade-tracker to clarify the relationship between meeting criteria and earning final course grades. Because we wanted the course to reinforce general writing skills, we adapted writing exercises from a book on general nonfiction writing to focus more on science and topics specific to chemistry.⁶⁰

The following course goals guided the specifications grading redesign of the course. We aimed:

1. to increase transparency in student grade achievement of SLO's
2. to provide consistent and clear grading rubrics
3. to give constructive feedback and frequent revision opportunities
4. to provide a framework for students to master general writing skills in addition to discipline-specific conventions.

These course goals informed our choice to use specifications grading. The specifications grading rubrics are transparent and straightforward, without the subjectivity of a traditional, points-based, rubric with different levels of achievement for the same criteria. Grading with a specifications rubric is faster for the instructor, allowing more instructor feedback and revision opportunities to

be offered over the course of an academic term. In addition, the clarity of the criteria that the students must meet lets them know not only what they are being evaluated on, but also where they need to improve to master the skills needed for a given assignment.

4.3.2.1 Student Learning Outcomes for Chem 101W

For a course at UC Irvine to fulfill the upper-division writing requirement — that is, to be designated by the university as an upper-division writing course — it has to meet several criteria. Students who complete the course must demonstrate proficiency in discipline-specific research methods, genres, and formal conventions. Students must develop information literacy skills appropriate to the discipline, and they must produce a final work of edited, revised writing for an appropriate audience (academic, public, or professional). Guided by these expectations, we developed a set of Student Learning Outcomes (SLOs, see Table 4.6) for the course. To facilitate course transparency, we also included course modules on the course learning management system (LMS) that aligned with the SLOs (Table 4.6).

Table 4.6. Course modules and associated SLOs.

Course Student Learning Outcomes

After successful completion of this course, students will be able to:

1. create professional papers, proposals, reports, and other forms of scientific writing
2. efficiently search the chemical literature and other sources relevant to chemistry researchers
3. communicate the results of experiments and the meaning of data in both written and oral formats

Course Modules	Associated SLO's
Professional Skills	1, 2
Engaging with the Chemical Literature	1, 2
Writing Mechanics	1, 3
Scientific Ethics	1, 2, 3
Presentations	2, 3

4.3.2.2 Specifications Grading Rubric Design

The new specifications grading rubric design aimed to address the four course goals. Two template rubrics — one for small assignments and one for large assignments — were constructed to provide a consistent grading framework for students (Table 4.7). These templates were used for the majority of course assignments and were adjusted as needed to better assess assignments that did not fit well in either template, such as presentation rubrics (see Appendix B). Each template was divided into three main categories — Sentences, Paragraphs, and Assignment Content — that were common to all assignments. We defined the number of criteria students had to meet to achieve a High Pass, Low Pass or Unsatisfactory on each assignment; the numbers varied by rubric type (Table 4.7).

Table 4.7. Specifications grading rubrics for small and large writing assignments.

Small Rubric Criteria	Met	Not Met	Large Rubric Criteria	Met	Not Met
High Pass:	6/7		High Pass:	8/10	
Low Pass:	5/7		Low Pass:	6/10	
Unsatisfactory:	≤ 4/7		Unsatisfactory:	≤ 5/10	
<u>Sentences</u> : The writing is grammatically correct according to the rules of Standard Edited Written English.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Sentences</u> : The writing is grammatically correct according to the rules of Standard Edited Written English.	<input type="checkbox"/>	<input type="checkbox"/>
<u>Sentences</u> : Words are spelled and used correctly.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Sentences</u> : Words are spelled and used correctly.	<input type="checkbox"/>	<input type="checkbox"/>
<u>Sentences</u> : Sentences are constructed correctly according to the rules of Standard Edited Written English.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Sentences</u> : Sentences are constructed correctly according to the rules of Standard Edited Written English.	<input type="checkbox"/>	<input type="checkbox"/>
<u>Paragraphs</u> : Each paragraph has a clear and coherent topic sentence.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Paragraphs</u> : Each paragraph has a clear and coherent topic sentence.	<input type="checkbox"/>	<input type="checkbox"/>
<u>Paragraphs</u> : Each paragraph has one clear and coherent main idea that relates to the thesis of the piece of writing.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Paragraphs</u> : Each paragraph has one clear and coherent main idea that relates to the thesis of the piece of writing.	<input type="checkbox"/>	<input type="checkbox"/>
<u>Assignment Content</u> : The writer clearly addresses the intended audience.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Paragraphs</u> : The order and flow of paragraphs is clear and logical.	<input type="checkbox"/>	<input type="checkbox"/>
<u>Assignment Content</u> : The author adequately responds to all parts of the assignment.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Assignment Content</u> : The writer clearly addresses the intended audience.	<input type="checkbox"/>	<input type="checkbox"/>
			<u>Assignment Content</u> : The thesis of the work is supported by the rest of the paper.	<input type="checkbox"/>	<input type="checkbox"/>
			<u>Assignment Content</u> : The writer clearly supports all assertions with evidence.	<input type="checkbox"/>	<input type="checkbox"/>
			<u>Assignment Content</u> : The writer has constructed a consistent and coherent narrative.	<input type="checkbox"/>	<input type="checkbox"/>

4.3.2.3 Assignment Design

We designed most of the course small writing assignments by adapting writing experiences from John Warner's *The Writer's Practice: Building Confidence in Your Nonfiction Writing*. While the experiences were not specific to scientific writing, we adapted them to focus on science and chemistry topics. Warner designed his book as a guide to practice the art of writing, with particular emphasis on purpose and target audience. We felt this framework lent itself well to scientific writing, as communicators of science must consider their purpose and audience carefully to write a coherent, logical, and engaging piece. This way of thinking about writing instruction also agrees with the UCI requirements for an upper-division writing course, and addresses the frequently observed difficulty that undergraduate students have in structuring a paper and identifying the appropriate audience when writing about chemistry.⁶¹⁻⁷¹

Each writing experience is generally divided into a series of steps (*Audience, Process, Reflect, and Remix*) to teach students the cognitive and practical steps they must take when writing for any genre or discipline. This process is particularly important and applicable to scientific writing, where students must be able to plan, draft, revise, edit, polish, and reflect on their own pieces. Scaffolding course assignments in this way — where students have to perform these steps for each assignment — addresses our fourth course goal: to provide a framework for students to master general writing skills and apply these skills to chemistry writing.

As an example, one of the first writing experiences in *The Writer's Practice* is called “How do I...?”, in which the student is guided through the steps of writing instructions for completing a task that they know well (Table 4.8). In the unmodified/original assignment, the student is first asked to consider the background of the person who will read the instructions (the *Audience*), to identify what that reader wants to know, and to determine how they can best communicate their

knowledge to that reader. The student is then introduced to the *Process* of writing their instructions. As part of the *Process*, the writing student is advised to read models of the type of instructions they want to give and to create a draft that can be tested to make sure it communicates the appropriate information in a way that helps the reader accomplish their task. The final steps of the *Process* consist of revision and editing of the draft based on any feedback, and finally polishing the edited draft to eliminate remaining errors. Finally, they are asked to *Reflect* on their writing by considering other approaches that may have been more effective, and invited to *Remix* their writing by, for example, adapting it for another medium such as social media. We did not use these last two steps in our assignments. To adapt this exercise for our course, we asked students to choose an experimental procedure from their undergraduate research or a previous laboratory course. The students used the *Process* steps in the “How do I...?” writing experience to construct an outline for an experimental procedure that was later adapted into one of the four 1000-word large writing assignments. For this exercise, students were required to submit a document in which they wrote about each of the *Process* steps (see Table 4.8) and the resulting outline. See Appendix B for more examples of adapted writing experiences (Tables 4.13, 4.14, 4.15, 4.16).

Table 4.8. Adapting the Process steps of *The Writer’s Practice* “How Do I...?” writing experience for a chemistry writing exercise.

Writing Experience Process Step	<i>The Writer’s Practice</i>	Chemistry 101W*
1. Select Subject	What one skill do you think best lends itself to this particular writing-related problem? Why have you chosen that skill?	Same questions, with the stipulation that students had to write about a chemistry experiment.
2. Plan	A good way of preparing to write the solution to this writing-related problem is to do the action itself while taking careful notes along the way.	Students don’t have to answer this one, but they are prompted to think about it.
3. Audience Analysis	Who is your audience? What might their attitudes be toward this task? What is their background knowledge, and what background knowledge is required?	Same questions.
4. Find and Analyze Models	Look for models that serve similar purposes. Stay away from ones too closely related to your own task to prevent unintentional copying. How are these models formatted and structured? How do they begin? How is the information conveyed?	Same questions; in addition, students must provide at least one citation to a peer-reviewed journal article that works as a model.
5. Draft	Doing your best to meet your audience’s needs, draft your document.	Instead of a full draft, students write a detailed outline of their experimental procedure.

*Our purpose for adapting this exercise was to help students start thinking about how to craft an Experimental section that they would include in a thesis or article submission.

The adapted assignment fulfills two major roles in meeting the course SLOs. First, the assignment walks students through the important steps in writing the Experimental section of a lab report or journal article by asking them to consider the important experimental details and the expectations of the reader (SLOs 1 and 3, Table 4.6) . Second, it requires them to briefly search the scientific literature to find examples of how other authors have accomplished the same task (SLO 2, Table 4.6). The required changes to the writing experience in *The Writer’s Practice* are minimal, as was the case for many of the assignments that we adapted.

4.3.2.4 Specifications Grading Course Scheme

In addition to rubrics, we defined passing thresholds for earning course letter grades (Table 4.9), including plus and minus grades. For simplicity, Table 4.9 only shows whole letter grades. To earn a specific letter grade, students must pass each Evaluation Category at a defined threshold. Categories were defined as formative and summative assessments we deemed necessary for the students to achieve course SLO's; the participation category is the only Evaluation Category that does not fall under a formative or summative assessment type. During the course, we provided a Student Grade Tracker for students so they could monitor their course grade throughout the academic term (see Appendix B for Student Grade Tracker).

As the course title "Writing for Chemists" suggests, a primary goal of this course was to train students in scientific writing — specifically for the chemistry discipline. The major categories of both formative and summative assessments were small, large, and complete/incomplete assignments. A significant component of learning how to write better is through reading, so we also included reading completion and reflection as a formative assessment category. Lecture and presentation participation categories were tracked because we used a partial active learning approach, which only benefited students if they came to class meetings and participated. We also wanted to train students not only in written communication, but also in oral communication and active listening, which is why we had presentations and final presentation formative and summative assessment categories. Table 4.9 shows the percentage or total number of high or low passes students must earn in each evaluation category to achieve a specific final letter grade in the course.

Table 4.9. Course letter grade requirements in the original grading system and the new specifications grading system.

Original Grading System		Specifications Grading System		
Assignment Type	% of Final Grade	Evaluation Category	Final Course Grade	Set of Criteria Completed
In-Class Writing Skills Assignments	15	Small Writing Assignments	A	90 - 100 % High Pass
			B	75 - 100 % High Pass
			C	OR 70% High Pass w/25% Low Pass
			D	60 - 100% High Pass OR 80 - 100% Low Pass < 80% Low Pass
Journal Article Reading & Writing Assignments	10	1000-Word (Large) Writing Assignments	A	2 High Pass + 2 Low Pass
			B	4 Low Pass to 3 Low Pass + 1 High Pass
			C	3-4 Low Pass
			D	< 3 Low Pass
Peer Review	5	Reading Completion	A	95 - 100% Complete
			B	90 - 100% Complete
			C	80 - 100% Complete
			D	< 80% Complete
Presentations	10	Complete/ Incomplete Assignment Completion	A	90 - 100% Complete
			B	80 - 100% Complete
			C	70 - 100% Complete
			D	< 70% Complete
Final Paper Scaffolding Assignments	20	Presentations	A	2 High Pass + 2Low Pass
			B	4 Low Pass
			C	2-3 Low Pass
			D	< 2 Low Pass
Final Paper	40	Presentation Participation	A	4 Complete
			B	4 Complete
			C	3 Complete
			D	< 3 Complete
		Lecture Participation	A	5 Complete
			B	4 Complete
			C	2 - 3 Complete
			D	< 2 Complete
		Final Presentation	A	High Pass
			B	Low - High Pass
			C	Low Pass
			D	Unsatisfactory

	Final Paper	A	High Pass
		B	Low - High Pass
		C	Low Pass
		D	Unsatisfactory

We anticipated that students would resist the new grading scheme if they felt their early assignment mistakes would make it challenging to earn their desired course grade. To avoid student resistance and allow students additional opportunities to revise and resubmit writing assignments, we implemented an intangible token economy for the course.^{10,72} Students could earn tokens by completing simple tasks such as filling out surveys. There were also performance-based opportunities to earn tokens by revising and resubmitting several small assignments or keeping a reading log of journal articles. Tokens could be redeemed for a chance to submit an assignment late, an opportunity to resubmit an assignment, or an increase from a low-pass mark to a high-pass — according to a set of rules given to students at the beginning of the quarter.

4.3.3 Student Perceptions

We used the specifications grading system in two course offerings of Chemistry 101W, in fall 2019 (taught by K.J.M.) and winter 2020 (taught by M.A.M.). To determine if the course significantly impacted students' perceptions about the practice of writing, we administered a survey both before and after the course. The survey included open-ended free response questions (see Appendix B for questions). Between the two course offerings, 39 students responded to the pre-course survey and 32 to the post-course survey, and we observed statistically significant changes in students' attitudes from before or after the course on only a few questions. However, students' comments from free response questions allowed us to identify trends in student understanding and writing practice.

The MSLQ-based survey that we administered before and after the class was composed of statements chosen to determine student attitudes about writing, as well as other aspects of learning (see Appendix B). Students could indicate their agreement on a Likert scale that went from 1 (very untrue of me) to 7 (very true of me). Responses to the pre-class and post-class surveys were grouped together for the Fall 2019 and Winter 2020 classes, resulting in sets with $N = 39$ for pre-class and $N = 32$ for post-class surveys. The responses were weighted according to their Likert scale number and a two-sample t-test was performed on the results to establish whether any statistically significant changes had been observed. Of the responses to the statements about writing, only one statement having to do with effort regulation showed a significant increase ($p < 0.05$) between pre-class and post-class surveys (Figure 4.12). Several other responses related to effort regulation and metacognition in the writing process increased, but not at a statistically significant level. None of the statements related to writing showed a significant decrease in average Likert score.

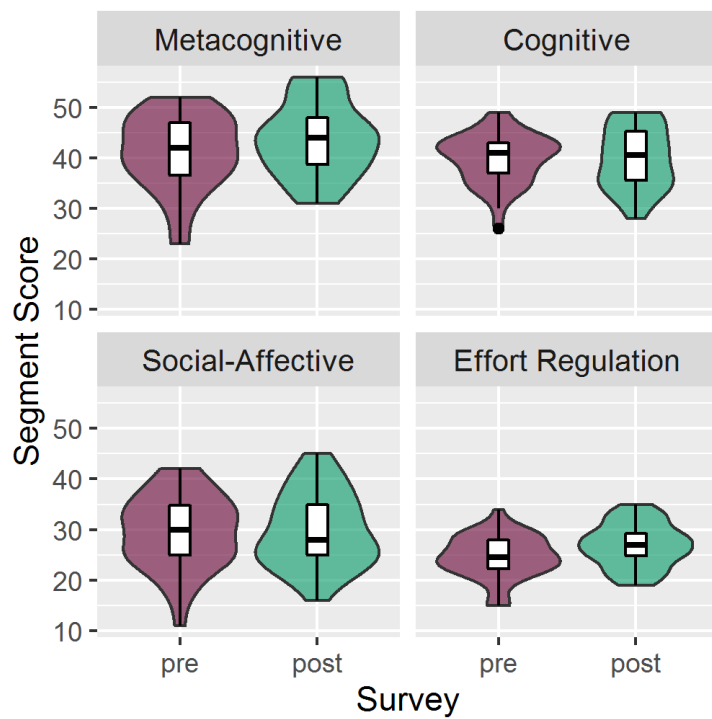
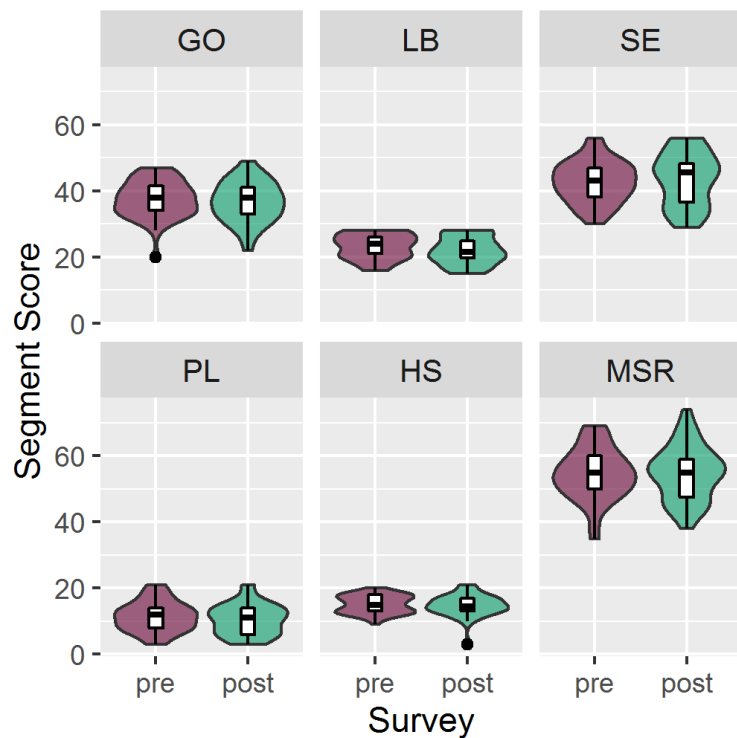


Figure 4.12. MSLQ survey results from Chem 101W run in fall 2019 and winter 2020.

Despite the lack of significance in the increase in average scores, we saw that in many cases students were more likely to report that statements about metacognition in the writing process were very true of them. This was especially true of the statement, “I organize my ideas prior to writing”; in the two classes there were no students who said that this statement was “very true of me” before taking the class, but 15% of respondents answered in this way after completion of the course.

Students self-reported that the specifications grading system made them more aware of course assignments expectations, which was one of our original course goals. The following quotes are from students responding to the survey questions: *What did you think about the grading method used in this course?* and *What aspects of the grading did you think worked well?*

“I liked that it was very clear about what was expected to earn a grade.”

“I liked the grading method because then my grade is not dependent upon others.”

“I really liked knowing exactly what you had to do to receive the grade you wanted.”

“I feel more in control of my grade because I know what I have to get on assignments to get the grade that I want.”

“I think that the grading worked well at making me stop thinking about the numbers aspect of grading: getting to see that I did well on an assignment and looking toward the rubric and comments instead of a percentage was refreshing.”

“The clarity of the grading system and the tokens as a safety system.”

We intentionally designed the course to facilitate writing revision and editing, and students self-reported that they did indeed find the process of revising useful. Students were forced to revise and resubmit some assignments, and they could redeem tokens to resubmit assignments for which they received a low-pass or unsatisfactory assessment. These course attributes provided increased student editing and revising opportunities. As we would expect after seeing the response trends in

Figure 1, students reported that the course helped them develop their writing because they could edit, polish, and resubmit multiple drafts. Students commented:

“I really enjoyed this class, and thought it really helped me develop as a writer.”

“This course was great for me to develop my writing skills.”

“Submitting a second draft for the opportunity to raise your past grade. I think that reflects well a student's ability to take input, edit and polish their draft, and then improve their writing in the process.”

“I have to face unsatisfactory assignments and polish them again.”

While many student comments positively reflected on the specifications grading system, some common student concerns also emerged (Table 4.10). Students indicated that the system was stressful because of the ease with which their course letter grades could drop. For some evaluation categories, a single unsatisfactory or low-pass could lower a student's grade. The token system was in place to help buffer this effect on students, but some of them did not make this connection. In-class exercises where students calculated their grade at different points in the quarter helped to alleviate this confusion, and will be done earlier in future course offerings. Students also felt that passing thresholds were set too high and that the grading system did not adequately reward student effort and time spent on assignments. While effort is important to a student's success in any course, it is challenging to measure said effort.

Table 4.10. Trends from student feedback to free-response questions.

Student Response Trends
Final grade expectations were clear and specific, students knew what to do to get the grade they wanted
Grading helped students identify how to improve their writing
Having the opportunity to submit multiple drafts was helpful
Found token system helpful
System is nerve-wracking because it's easy to be dropped a grade-level
Passing thresholds set too high
<u>Grading system doesn't adequately reward effort / time spent</u>

Regardless of student concerns about the course specifications grading system, some students who voiced those concerns also reflected that the course made them better writers. One student commented, “I personally dislike this grading method but I admit that it forces me to improve my writing skills.”

Our perceptions about the effectiveness of the specifications grading system mirrored those of the students who saw improvement in their writing ability. Throughout each course offering, we saw significant improvements in students' use of standard written English grammar, spelling, sentence structure, and paragraph construction. We also observed that students were more mindful about their audience and the purpose behind each piece of writing.

4.3.4 Conclusion

We redesigned an upper division "Writing for Chemists" course to focus on developing student writing skills and practices in addition to chemistry discipline-specific writing conventions. We designed a specifications grading system and adapted writing experiences from *The Writer's Practice* to support the redesign of the course and to provide students with a scaffold to hone and master writing in chemistry. Student responses to end-of-term survey questions indicated that we were successfully transparent about how students could obtain specific grades and achievement of SLOs, and that we provided consistent and fair grading rubrics. Students were able to practice and refine their writing through multiple opportunities of instructor and teaching assistant feedback and assignment resubmission. At the end of the class, students tended to report that they were more likely to spend time to improve their writing, to monitor their writing process, and to revise and edit work before submitting it.

Specifications grading proved particularly useful for grading student writing. Beyond grading standard written English language and grammar conventions, assessing writing quality can be subjective. Using standardized specifications grading rubrics allowed us to more objectively assess student writing, and eliminated the need for a course curve. We believe these types of rubrics would be useful in other chemistry writing-heavy courses, such as those which have many laboratory reports.

4.4 Appendix A

IRB Statement

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

Specifications Grading Course Student Grade Tracker

CHEM 51LD STUDENT GRADE TRACKER

MINIMUM To Earn C-

Sapling: 70% or higher class total <input type="checkbox"/>
Video Quizzes: 75% or higher class total <input type="checkbox"/>
Pre/In-Lab: 5 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Post-Lab Scaffolds: 3 Satisfactory Required Lab Skills: <input type="checkbox"/> Others: <input type="checkbox"/> <input type="checkbox"/>
Lab Lecture Participation: 4 Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Safety Final : 4/6 questions on safety portion during Week 10 exam <input type="checkbox"/>
Final - Lab Techniques: 1 required* Instructor assigned <input type="checkbox"/>
Knowledge Check Final: Pass at S level <input type="checkbox"/>

*Students not trying for an A-level or B-level grade do not need to complete Mastery Final

MINIMUM To Earn C

Sapling: 70% or higher class total <input type="checkbox"/>
Video Quizzes: 80% or higher class total <input type="checkbox"/>
Pre/In-Lab: 5 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Post-Lab Scaffolds: 3 Satisfactory Required Lab Skills: <input type="checkbox"/> Others: <input type="checkbox"/> <input type="checkbox"/>
Lab Lecture Participation: 5 Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Safety Final : 4/6 questions on safety portion during Week 10 exam <input type="checkbox"/>

Final - Lab Techniques: 1 required* Instructor assigned

Knowledge Check Final: Pass at S level

*Students not trying for an A-level or B-level grade do not need to complete Mastery Final

MINIMUM To Earn C+

Sapling: 80% or higher class total

Video Quizzes: 80% or higher class total

Pre/In-Lab: 6 Satisfactory Required

Post-Lab Scaffolds: 3 Satisfactory Required **Lab Skills:** Others:

Lab Lecture Participation: 5 Required

Safety Final : 4/6 questions on safety portion during Week 10 exam

Final - Lab Techniques: 1 required* Instructor assigned

Knowledge Check Final: Pass at S level

*Students not trying for an A-level or B-level grade do not need to complete Mastery Final

MINIMUM To Earn B-

Sapling: 80% or higher class total

Video Quizzes: 80% or higher class total

Pre/In-Lab: 6 Satisfactory Required

Post-Lab Scaffolds: 4 Satisfactory Required **Lab Skills:** Others:

Lab Lecture Participation: 6 Required

Safety Final : 4/6 questions on safety portion during Week 10 in lab exam

Final - Lab Techniques: 2 required** Instructor assigned Student choice

Knowledge Check Final: Pass at S level

Mastery Final: Pass at B Level or above (minimum partial on one question in each of three categories OR full credit in one question a category, partial in one another category)

*Students trying for A-range or B-range grades must complete additional lab technique test during Week 9 (end of Week 5 for summer session classes). Sign-ups will be available 1 week prior and will close on Friday before the testing week begins.**3 Tokens may be exchanged for a pass on the student choice lab technique.

MINIMUM To Earn B

Sapling: 80% or higher class total <input type="checkbox"/>
Video Quizzes: 85% or higher class total <input type="checkbox"/>
Pre/In-Lab: 6 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Post-Lab Scaffolds: 4 Satisfactory Required Lab Skills: <input type="checkbox"/> Others: <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Lab Lecture Participation: 6 Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Safety Final : 4/6 questions on safety portion during Week 10 in lab exam <input type="checkbox"/>
Final - Lab Techniques: 2 required** Instructor assigned <input type="checkbox"/> Student choice <input type="checkbox"/>
Knowledge Check Final: Pass at S level <input type="checkbox"/>
Mastery Final: Pass at the B Level or above <input type="checkbox"/> (minimum 1 question pass in one of three categories, a total of 2 partial in remaining two categories)

*Students trying for A-range or B-range grades must complete additional lab technique test during Week 9 (end of Week 5 for summer session classes). Sign-ups will be available 1 week prior and will close on Friday before the testing week begins.**3 Tokens may be exchanged for a pass on the student choice lab technique.

MINIMUM To Earn B+

Sapling: 80% or higher class total <input type="checkbox"/>
Video Quizzes: 85% or higher class total <input type="checkbox"/>
Pre/In-Lab: 7 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Post-Lab Scaffolds: 4 Satisfactory Required Lab Skills: <input type="checkbox"/> Others: <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Lab Lecture Participation: 6 Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Safety Final : 4/6 questions on safety portion during Week 10 in lab exam <input type="checkbox"/>
Final - Lab Techniques: 2 required** Instructor assigned <input type="checkbox"/> Student choice <input type="checkbox"/>
Knowledge Check Final: Pass at S level <input type="checkbox"/>
Mastery Final: Pass at B+ Level or above <input type="checkbox"/> (minimum 1 question pass in two of three categories)

*Students trying for A-range or B-range grades must complete additional lab technique test during Week 9 (end of Week 5 for summer session classes). Sign-ups will be available 1 week prior and will close on Friday before the testing week begins.**3 Tokens may be exchanged for a pass on the student choice lab technique.

MINIMUM To Earn A-

Sapling: 90% or higher class total <input type="checkbox"/>
Video Quizzes: 85% or higher class total <input type="checkbox"/>
Pre/In-Lab: 7 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Post-Lab Scaffolds: 5 Satisfactory Required Lab Skills: <input type="checkbox"/> Others: <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Full Written Lab Report: 1 Required - Student may choose <input type="checkbox"/>
Lab Lecture Participation: 7 Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Safety Final : 4/6 questions on safety portion during Week 10 exam <input type="checkbox"/>
Final - Lab Techniques: 3 required* <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Knowledge Check Final: Pass at S level <input type="checkbox"/>
Mastery Final: Pass at A- Level or above <input type="checkbox"/> (minimum 1 question pass in two of three categories, 1 partial in the remaining category)

*Students trying for A-range or B-range grades must complete additional lab technique test during Week 9 (end of Week 5 for summer session classes). Sign-ups will be available 1 week prior and will close on Friday before the testing week begins.**3 Tokens may be exchanged for a pass on one student choice lab technique.

MINIMUM To Earn A

Sapling: 90% or higher class total <input type="checkbox"/>
Video Quizzes: 85% or higher class total <input type="checkbox"/>
Pre/In-Lab: 7 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Post-Lab Scaffolds: 5 Satisfactory Required Lab Skills: <input type="checkbox"/> Others: <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Full Written Lab Report: 1 Required - Student may choose <input type="checkbox"/>
Lab Lecture Participation: 7 Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Safety Final : 5/6 questions on safety portion during Week 10 exam <input type="checkbox"/>
Final - Lab Techniques: 3 required* <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Knowledge Check Final: Pass at S level <input type="checkbox"/>
Mastery Final: Pass at A Level <input type="checkbox"/> (minimum 1 question pass in each of three categories)

*Students trying for A must complete at least 1 lab technique test during Week 9 (end of Week 5 for summer session classes). Sign-ups will be available 1 week prior and will close on Friday before the testing week begins. *3 Tokens may be exchanged for a pass on one student choice lab technique.

Completing all A requirements AND scoring above A level on Mastery Final = A+

FAQ

What happens if...

...I meet most of the requirements for a grade, but not all of them?

You earn the highest grade for which you meet ALL of the minimum requirements.

...I don't meet the minimum requirements for a C-?

For a D, you need a minimum of 4 satisfactory pre/in-lab assignments and 2 satisfactory post-lab assignments. There are no minimums in any other category. If you fall below this minimum threshold, your grade will be F. There are no D+ or D- grades for this class.

Example of a Traditional Course Rubric

The colored rubric sections below correspond to the colored, expanded, rubric items in the specifications grading example course rubric (Section 4.4.4).

Introduction	2 pts	1 pts	0 pts		
	Student recognizes the purpose of the project is to compare separation methods.	Student states some purposes of the lab but not all.	Absent or stated information is irrelevant.		
Theory	7 pts	4 pts	1 pts	0 pts	
	Student discusses fundamentals of column chromatography and relates the technique to TLC, noting similarities and differences and how a successful separation is achieved.	Student discusses some fundamentals of chromatography, but important ideas or relationship to TLC are missing.	Student mentions some facts about chromatography, but description of theory is severely lacking.	No chromatography theory provided.	
Results 1	2 pts	1 pts	0 pts		
	Table contains all necessary results in an organized manner.	Table contains only some results or is disorganized.	Table is absent.		
Results 2	2 pts	1 pts	0 pts		
	Student correctly calculates masses, recoveries, and Rf values.	Student is capable of explaining some of the above with the goal of the project with minor mistakes and/or omissions.	Student mentions some of above points but provides little or incorrect explanation.		
Discussion 1	10 pts	7 pts	4 pts	1 pts	0 pts
	Student discusses results of each technique used and ties together results in coherent manner.	Student capably discusses most points with only minor errors or omissions.	Student has difficulty rationalizing the results and/or has major omissions.	Student states facts but provides little to no analysis of the results.	Absent or student's discussion is

					incoherent /irrelevant.
Discussion 2	4 pts	2 pts	1 pts	0 pts	
	Student provides detailed and valid explanations for problems encountered during the experiment and proposes reasonable ways to improve the experiment.	Student provides detailed and valid explanations for problems encountered during the experiment but proposes ways to improve the experiment that clearly will be ineffective or have no value.	Student provides some explanation for problems encountered during the experiment but proposes no methods to improve the experiment.	Absent or student's discussion is incoherent /irrelevant.	
Conclusion	2 pts	1 pts	0 pts		
	Student capably summarizes the result of the experiment and draws conclusions that mesh well with the results.	Student merely summarizes the results.	Absent or student's discussion is incoherent /irrelevant.		
Writing	2 pts	1 pts	0 pts		
	Student adheres to the required formatting and the document possesses only minor writing mistakes.	Student does not fully adhere to the required formatting or the document possesses substantial writing errors.	Student ignores required formatting or writing errors are so severe so as to make report unreadable.		
Quality	4 pts	2 pts	1 pts	0 pts	
	Excellent report.	Average report.	Below average report	Unacceptable report	

Example of a Specifications Grading Course Rubric

The colored rubric sections below are derived from the corresponding colored rubric items in the traditional grading example course rubric (Section 4.4.3).

Introduction	Satisfactory
	Clearly states the overall goal of the experiment, including specific compounds and techniques used.
Theory 1a	Satisfactory
	Clearly describes the chemical principle(s) that govern how compounds are separated using column chromatography. Note: Be sure to include the importance of solvent choice.
Theory 1b	Satisfactory
	Clearly compares and contrasts column chromatography to TLC.
Theory 1c	Satisfactory
	Clearly describes what procedural precautions must be taken to achieve a successful separation using column chromatography.
Theory 1d	Satisfactory
	Clearly explains how separation is monitored in real time, and how this allows the determination of whether the separation was successful or not.
Results: Body	Satisfactory
	All of the following data from extraction are presented in well-organized table(s): 1) Recovered mass of extract, 2) percent recovery of extract, 3) Rf value(s) for the extract, the original mixture and the standards, 4) melting range of the extract and the standards, 5) mixed melting range of the extract with biphenyl, and 6) mixed melting range of the extract with naphthalene.
Results: Body	Satisfactory
	All of the following data from column chromatography are presented in well-organized table(s): 1) Recovered mass of extract, 2) percent recovery of extract, 3) Rf value(s) for the extract, the original mixture and the standards, 4) melting range of the extract and the standards, 5) mixed melting range of the extract with biphenyl, and 6) mixed melting range of the extract with naphthalene.
Results: Appendix	Satisfactory
	Clearly and correctly calculates percent recovery and Rf values for the extraction data. Work is clearly shown for all calculations.
Results: Appendix	Satisfactory
	Clearly and correctly calculates percent recovery and Rf values for the column chromatography data. Work is clearly shown for all calculations.

Discussion 1a: Extraction	Satisfactory
	1) Clearly and explicitly integrates data from results to support claims made in discussion. 2) Clearly states a position on the EFFICACY of separating the compounds in the given mixture by extraction. 3) Clearly proposes an argument to support the stated position.
Discussion 1b: Extraction	Satisfactory
	1) Clearly and explicitly integrates data from results to support claims made in discussion. 2) Clearly states a position on the EFFICIENCY of separating the compounds in the given mixture by extraction. 3) Clearly proposes an argument to support the stated position.
Discussion 1c: Extraction	Satisfactory
	1) Clearly and explicitly integrates data from results to support claims made in discussion. 2) Clearly states the identity of the unknown that was isolated following extraction and justifies the identification using evidence-based argument(s). If the determination is not clear, provides possible identities, evidence for possible identities, and an explanation for why a determination cannot be made.
Discussion 1d: Column Chromatography	Satisfactory
	1) Clearly and explicitly integrates data from results to support claims made in discussion. 2) Clearly states a position on the EFFICACY of separating the compounds in the given mixture by column chromatography. 3) Clearly proposes an argument to support the stated position.
Discussion 1e: Column Chromatography	Satisfactory
	1) Clearly and explicitly integrates data from results to support claims made in discussion. 2) Clearly states a position on the EFFICIENCY of separating the compounds in the given mixture by column chromatography. 3) Clearly proposes an argument to support the stated position.
Discussion 1f: Column Chromatography	Satisfactory
	1) Clearly and explicitly integrates data from results to support claims made in discussion. 2) Clearly states the identity of the unknown that was isolated following column chromatography and justifies the identification using evidence-based argument(s). If the determination is not clear, provides possible identities, evidence for possible identities, and an explanation for why a determination cannot be made.

Discussion 1g: Comparison of Techniques	Satisfactory
	1) Clearly and explicitly integrates data from results to support claims made in discussion. 2) Clearly compares and contrasts the efficacy and efficiency of the two separation techniques. 3) Provides a clear position on which was better overall and provides evidence-based argument(s) to justify the position.
Discussion 2a: Error Analysis	Satisfactory
	1) Clearly identifies probable error(s) that did occur or could have occurred when separating the mixture. 2) Clearly explains (in 1-2 sentences) why resolving the error is relevant to the experiment.
Discussion 2b: Error Analysis	Satisfactory
	1) Clearly hypothesizes how the error occurred. Human and equipment error are not acceptable. 2) Clearly proposes a method or use of an analytical technique that would allow you to support or refute the error hypothesis.
Conclusion	Satisfactory
	1) Clearly summarizes the results in the context of the objective(s). 2) Clearly states whether the objective(s) was/were met or not, and includes 1-2 sentences that support that statement. 3) Clearly proposes at least one future experiment that builds upon the results of the experiment, with 1-2 sentences of justification based on chemical principles.

TA Open-Ended Questions and Responses

Chem 51L TA Student Email Survey - Summer 2019, Winter 2019, & Winter 2020

Please answer the following questions as best as you can. Unless specified in the question, assume that each question refers to the amount of emails or interactions over the course of the entire quarter. If you have all student emails from this quarter, we would greatly appreciate it if you could go back and count when answering the following questions. If you don't have the emails, no worries, please estimate as best as you can.

1. How many emails did you receive about a student disagreeing about whether they met the minimum criteria for a satisfactory score on rubric items in general?
2. How many emails did you receive about student understanding in general?
3. How many emails did you receive regarding questions like "why is this my letter grade"?
4. How many interactions did you have with students in-person about student disagreeing about whether they met the minimum criteria for a satisfactory score on rubric items in general?
5. How many interactions did you have with students in-person about student understanding?
6. On average, how long did it take you each week to grade notebook (pre-lab) pages?
7. On average, how long did it take you each week to grade post-labs?
8. About how much time did you spend per week agonizing over whether a student met the minimum criteria for a satisfactory score on rubric items?
9. What did you think about the grading method used in this course?
10. What aspects of the grading did you think worked well?
11. What aspects of the grading would you change? Please be specific about the change you would make and why you think this is a beneficial change.
12. Use this space to provide any additional feedback.

Table 4.11. TA responses to student perception survey. One TA, out of two, provided a response for Summer Session 2019.

Question #	Specifications Grading Course (Summer Session 2019)
1	0 to 5
2	26 or more
3	0 to 5
4	0 to 5
5	6 to 10
6	less than 1 hour
7	3 - 5 hours
8	less than 15 minutes
9	I think it makes it a lot easier to grade and gets rid of the uncertainty about meeting the rubric criteria
10	I liked that it was in binary. I didn't get a lot of complaints and the students seemed more at ease over the grading and assignments
11	I think there were certain parts of the rubric for post labs that could be interpreted differently between TAs. I think a more specific key for the TAs would be better. I think the rubric does a good job for the students but for grading I think there can be misinterpretation between TAs if they are not communicating.
12	I think this grading and overall system is a lot easier to use and it makes the workload for TAs less intensive and time consuming.

TA Responses to Open-ended Questions

Chem 51LB TA Student Email Survey - Winter 2020

Please answer the following questions as best as you can. Unless specified in the question, assume that each question refers to the amount of emails or interactions over the course of the entire quarter. If you have all student emails from this quarter, we would greatly appreciate it if you could go back and count when answering the following questions. If you don't have the emails, no worries, please estimate as best as you can.

1. What did you think about the grading method used in this course?
 - Students only care about meeting the minimum requirements for regrade, they don't really care about fixing all the mistakes. Some students only finish half of the report on first try and actually finish the report for regrade. The students seem to misunderstand the rubric items especially for error analysis, I myself sometimes misunderstand it too. How I interpreted the rubric is sometimes different from how the grading calibration wanted it to be. I think they need to be more clear. I had quite a bit of students not knowing where to see comments on the lab report (they usually see the comments on rubric, but not the ones on the actual lab report-its hard to find), and I didn't realize that until the end of the quarter when a student came up to me and asked me why she missed some of the questions. I don't like regrades, the students all have false expectations that they can get an A, so they kept arguing over getting a S on the lab report so they can get an A. The first real lab report should not be two weeks long, I couldn't get the grading back in time before they turn in the next one.
 - I didn't like it, it made me upset that some students who tried but just got some things wrong would get the same grade as someone who turned in nothing. It was also irritating to have to fill out a whole rubric just to give someone a 1 or 0; a rubric is a lot of work just to determine whether someone met a threshold. If a rubric needs to be used, I think it needs to be far less rubric items.
 - I think this grading method is efficient
 - Very easy and fast. Makes life easy
 - While it makes it easier and faster for TAs to grade, students are often upset about the black and white nature of the rubric
 - Although I think it's good for students to have a baseline standard level of understanding required to pass the class, I think this grading method encourages students to only put in the bare minimum to pass each assignment. I've noticed students strategically ignoring some parts of the rubric, especially the theory, error analysis, mechanisms on the postlabs/prelabs, when these sections were especially good practice for their week 10 practicals/exams.

- I disagree that students can mess up a single exam (out of 5 exams) and score maximum a C in the course. I think the token system was too lenient however. Students could turn in a junk report on time and wait like a week to get an unsatisfactory grade, then revise the assignment a week later than the original deadline for a single token.
 - I like it because it's less time spent on our part trying to decide whether or not the student gets the point or not
 - Not a fan.
 - I think it's good, I like the binary selection: either they met the criteria or they didn't.
 - A lot more straightforward than GenChem, which is very nice for us, but it tends to make the students a little more on edge because if they get one small thing wrong, I need to mark the whole thing wrong.
 - I really disliked it. I found it incredibly challenging to determine what answers merited a passing score, and found myself having to create my own rubrics. My students were often confused about the requirements and hated that the rubrics were so vague. I got many emails asking for a single point back, and many emails asking for clarification. It was stressful.
 - Penalizes students more for missing small things that I would ordinarily only take a few points for.
 - I think a binary system is sensible but in practice it's reasonable to expect many more people pass than fail.
2. What aspects of the grading did you think worked well?
- I like the rubric where it is just 1 point or no point for each rubric items, it makes it easy to give out points.
 - I think this works because there is less room for argument over the grade and requirements
 - Very clear and dry
 - The concept of specifications grading is great. It makes it very clear cut for TAs to grade.
 - I think in general, it was less stressful for many students because they don't need to be perfect to get an A as all they needed to do was pass.
 - It was nice giving clear satisfactory or not. Revisions were a nice way to allow students to understand more.
 - it's either all or nothing (either have everything rubric item asks for or no points) and i think the students also understand that
 - I think prelab grading was simplified, which is good.
 - I liked how organized the rubric is. It simplified the grading process, and made it easier to look for certain aspects of the student's writing that was or was not correct.
 - It's procedurally simple for TA's because the questions are all-or-nothing.
 - It saved time on pre-labs

- It was straightforward and there were less gray areas for points
 - Opportunities for students to change their grade
 - The concept.
3. What aspects of the grading would you change? Please be specific about the change you would make and why you think this is a beneficial change.
- All of the redoes meant that the time required to grade was extra
 - I'd probably allow regrades after 72 hours for two tokens in the beginning rather than at the end.
 - Not sure. Most kids report feeling bad when getting a 0 even though they worked hard. Feels bad giving a 0 when the kid is close
 - Rubrics could be a little more specific/tailored to specific assignments so that students have a better idea of what specifically is expected of them in each assignment. They seem to think rubrics are too vague/general at times.
 - I think that the grading for the postlab should be divided into small subsections such as theory, mechanisms, error analysis, etc... and that each subsection should have a minimum score in order to pass that subsection of the lab report. All subsections must have a satisfactory score in order to get a satisfactory score on the lab report. Maybe this is a step towards forcing students to not completely ignore some sections of the lab report??
 - I would revert to a point system or a percentage based system. I like the idea of revisions, but the 1 or zero combined with the strict grading scale allowed students with great records to get bad grades overall. I think a point based grading scale gives students with a better record an easier time to get a decent score in the course.
 - I would revert to the old style of grading since it requires significantly less explanation to students and TAs.
 - I think the binary selection still feels arbitrary: either the student met the requirements or they didn't, but sometimes they would miss one requirement and there would be disagreement between TAs on whether or not that would constitute a 0 or a 1. I think it either needs to be enforced as all or nothing in order to promote uniformity across grading so that the grading scale is fair to all undergraduates.
 - Some parts of the rubric are difficult to stretch onto what the students have written and I tried to be understanding, but no matter how many times I told my student to follow the rubric, some of them still refused to. I think for purposes of grading, it might be useful to tell students precisely what their grades will be based off of (I.e. the canvas rubrics, mores than the set of instructions on scaffold requirements provided separately).
 - I would create detailed rubrics for the TAs of specific bullet items needed to be included in the introduction, theory question 1, etc. This way, when we are grading, we look for those three or four things that need to be mentioned so we can be more consistent in our grading and actually have an idea of what a good answer is. The

grading calibration doesn't really work well to emphasize this. I also feel that it would help calibrate among TAs because even with the token system, some students run out of tokens much faster and then don't have the option to bump their score at the end. It also makes for a much more extrinsically motivated education process because the students want to earn points and care about the grade and getting that extra point to get a satisfactory. I think they get much more caught up in earning tokens and less in learning

- Clearer rubric items would be helpful ie specific concepts they should mention in theory so that grading is more consistent
 - Shift threshold of standard to meeting most of the requirements for that specification. Limit the number of resubmissions/the reasons tokens can be used. Streamline email confirmations for tokens. Make rubric items shorter; some of them literally span the entire page when grading, making it difficult to scroll quickly
 - In practice there were specifics that instructors were looking for that aren't well-discerned by a binary system. In this way, a points-based system would be more effective.
4. Use this space to provide any additional feedback.
- I did not like this grading system
 - Easier on me, even with Regrades. Worst thing is giving students 0s when they work hard. Maybe there could be like a 0.5 for getting a little below the minimum for a 1 and you need 5 points to get an A, 4 to get a B and so forth
 - I think it's a great idea on paper but in practice I hate this. I know you guys put a lot of time into this and you've clearly worked very hard getting this into production but I really don't think this is the best way forward. I spend almost 40% of my interactions with students discussing this grading style instead of talking about actual chemistry. TAing is a difficult job to begin with and this makes it significantly worse since it is more time consuming and frustrating to grade when I have around 30 boxes to check on the rubric instead of 5-10 items. I'm not sure how many TAs will fill this out but I've heard a lot of TAs complaining about the way things are set up, and I guessing these complaints and frustration do not reach the instructor or head TA. Again, I understand that a lot of time and effort has gone into coming up with more effective teaching practices and I appreciate that. But I think you should take the TAs honestly about how to improve the system and make it work better for all parties involved.
 - Thanks for all of your hard work!
 - For some reason, the volume of grading was particularly difficult to keep up with for those of us who were taking 3 courses concurrently with teaching. I don't know if this will be an issue next year, but I often found myself not being able to complete things on schedule and get it back to my students within a reasonable amount of

time. One possible solution to this might be to heavily condense theory sections since they are typically the most time-intensive sections to grade (at least for me). Most of my students provided long-winded (and often wrong) responses, which made them difficult to grade. If they are challenged with explaining a "theory" section in less than 5 sentences, this will probably make it easier and faster for us to grade and they'll get some practice with being accurate and concise like they would need to be if they were publishing.

- Students have a hard time realizing that it is okay to get a 0 and tend to obsess over their tokens
- I'm pretty sure I spent more time grading w/ students overall receiving worse grades/passing fewer assignments. Like, I literally looked for reasons to give students an extra point or two even though I don't think they "deserve" it because I didn't feel like going back and grading another time, ESPECIALLY on assignments that had already been resubmitted.
- The token system is more trouble than it's worth - do away with it entirely.

Student Open-Ended Questions and Responses

1. What did you think about the grading method used in this course?

- This was a terrible idea especially since this was done during a 6 week period. The old grading system was better than this.
- An improvement over the traditional system.
- Earning an A required more effort than before. In the past, writing decent lab reports was enough to get a good grade. The system was also a little nerve-racking. Waiting to see if you passed or not because there was no in between. Sometimes the passing cut off was just missed.
- It was stressful but put me on top of my work. I do like the S/U part of the postlabs because the points can get annoying when you and your TA disagree with how much points your answer is worth.
- Much better than the previous grading method. Removed a lot of stress.

2. What aspects of the grading did you think worked well?

- The tokens
- I think the grading system has a very strong base in the uniformity throughout the TA's and what constitutes as satisfactory or unsatisfactory. The binary system for grading was very clear and easy to understand. Grade tracker was a great addition and allowed students to track there grades so they didn't have to wait until transcripts rolled out. Token system was also a great addition.
- The system made it easy to know your grade at the end of the quarter. You knew what you needed to do to get the grade you wanted. This was a good system for someone who was looking to pass with the minimum amount of work. The tokens were also a good backup.
- less work for those that put in effort throughout the quarter
- Having either a 1 or a 0 allows for the grading to be more standardized across TAs. The several final exams lessens stress as well. Having a grade tracker helped a lot.

3. What aspects of the grading would you change? Please be specific about the change you would make and why you think this is a beneficial change.

- Assignments should have different weight from one another, your grade shouldn't be ruined based off one factor from a list. If you are going to do satisfactory level grades, C level grades should be considered satisfactory. It is more beneficial to have points because you actually have a chance to still get an A/B grade still if you mess up on something.
- The amount of satisfactory or "green boxes" a student had to achieve on a post lab was way too high. Most of them required a B+ or higher to achieve a satisfactory, when you divided the green boxes by the total achievable and in my opinion undergraduate students should be graded on the standard scale of a C is

satisfactory or even a C+ at maximum. We aren't grad-students and we shouldn't be held to there satisfactory grade stated in the grading policies of UCI.

- A curve would have been beneficial. This would make A level grades possible without passing all the lab reports. Point based categories rather than full marks or no marks would be more reflective of the quality of a paragraph. Points could be tallied in the end and a passing threshold could be set. This way if some detail is missing from an explanation points could still be received.
- I think to revise a postlab where you were one category away from an S should not require a token. I think everyone should be given a chance to revise their work for a better grade and it just encourages them to do a better job. If tokens were involved, they would ration the amount of token and decide if they should take the U or waste a token.

4. Use this space to provide any additional feedback.

- Overall the new system is a leap in a forward direction compared to the older system of grading. It just needs some minor tweaks and changes, there is more that I would change but is difficult to explain through this form. Attendance, rubric changes, page limit grading and so on.
- The way the system was introduced made it seem that the only reason for it was so that students knew their grades immediately at the end of the quarter.
- I think we could spend class time going over some of the mastery final material (we only had 1 class period to go over the critical thinking questions). Although we should be prepared for all the questions from taking so many labs before this class, a refresher or practice set would've been a good supplement in addition to the powerpoint slides. Having a key after doing a practice set, like the multiple choice practice, was helpful for me to see what I really understood and what I don't know. I wished the notes on the slides were published because I think I missed key points in getting a B vs A level answer.
- Great course and very fun.

Full Letter Grades Graph & Table Including +/- Grades

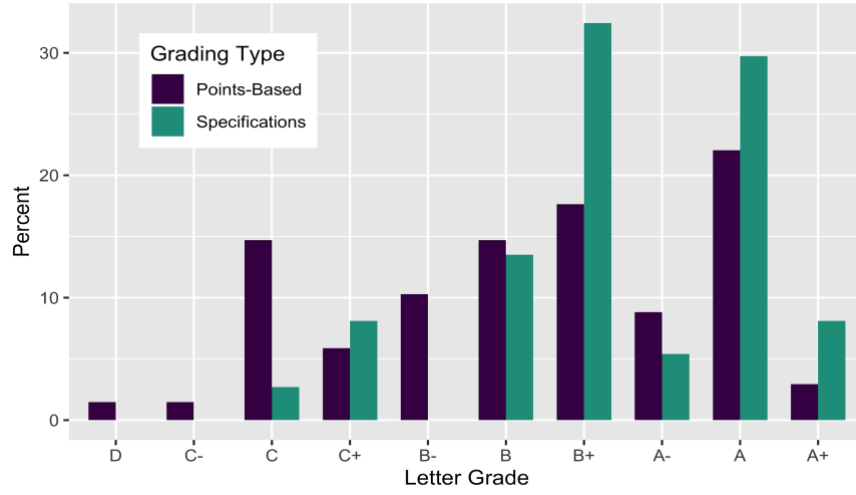


Figure 4.13. Pilot Course Implementation: Full letter grade distributions of a previous iteration of the course using a points-based system and the current course with the specifications grading system.

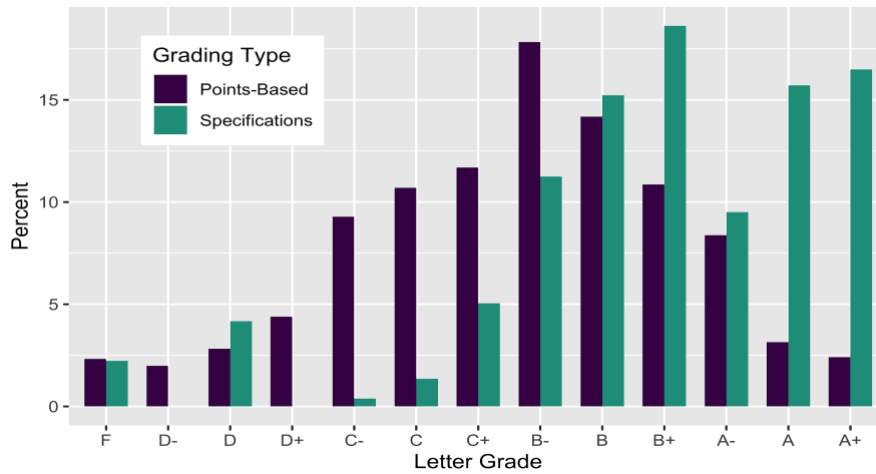


Figure 4.14. Scaled Course Implementation: Full letter grade distributions of a previous iteration of the course using a points-based system and the current course with the specifications grading system.

MSLQ and CLAI Instrument Questions

- **MSLQ Goal Orientation:** Please indicate the extent to which each of the following statements are true of you.
 - a. In a class like this, I prefer course material that really challenges me so I can learn new things.
 - b. In a class like this, I prefer course material that arouses my curiosity, even if it is difficult to learn.
 - c. The most satisfying thing for me in this course is trying to understand the content as thoroughly as possible.
 - d. When I have the opportunity in this class, I choose course assignments that I can learn from even if they don't guarantee a good grade.
 - e. Getting a good grade in this class is the most satisfying thing for me right now.
 - f. The most important thing for me right now is improving my overall grade point average, so my main concern in this class is getting a good grade.
 - g. If I can, I want to get better grades in this class than most of the other students.
- **MSLQ Control of Learning Beliefs:** Please indicate the extent to which each of the following statements are true of you.
 - a. If I study in appropriate ways, then I will be able to learn the material in this course.
 - b. It is my own fault if I don't learn the material in this course.
 - c. If I try hard enough, then I will understand the course material.
 - d. If I don't understand the course material, it is because I didn't try hard enough.
- **MSLQ Self Efficacy for Learning and Performance:** Please indicate the extent to which each of the following statements are true of you.
 - a. I believe I will receive an excellent grade in this class.
 - b. I'm certain I can understand the most difficult material presented in the readings for this course.
 - c. I'm confident I can learn the basic concepts taught in this course.
 - d. I'm confident I can understand the most complex material presented by the instructor in this course.
 - e. I'm confident I can do an excellent job on the assignments and tests in this course.
 - f. I expect to do well in this class.
 - g. I'm certain I can master the skills being taught in this class.
 - h. Considering the difficulty of this course, the teacher, and my skills, I think I will do well in this class.
- **MSLQ Peer Learning:** Please indicate the extent to which each of the following statements are true of you.
 - a. When studying for this course, I often try to explain the material to a classmate or friend.

- b. I try to work with other students from this class to complete the course assignments.
- c. When studying for this course, I often set aside time to discuss course material with a group of students from the class.
- **MSLQ Help Seeking:** Please indicate the extent to which each of the following statements are true of you.
 - a. Even if I have trouble learning the material in this class, I try to do the work on my own, without help from anyone.
 - b. I ask the instructor to clarify concepts I don't understand well.
 - c. When I can't understand the material in this course, I ask another student in this class for help.
 - d. I try to identify students in this class whom I can ask for help if necessary.
- **CLAI Lab Comfort 1:** Please indicate the extent to which each of the following statements are true of you.
 - a. I am anxious when I use chemicals during lab.
 - b. When I work in the chemistry lab, I feel at ease using the equipment.
 - c. When getting ready for chemistry lab, I get concerned about the lab procedures we will use.
 - d. When my chemistry lab is over, I am relieved to be done recording data.
 - e. When I work in the chemistry lab, I feel nervous working with other students.
 - f. I worry about whether I have enough time to complete the lab.
 - g. When my chemistry lab is over, I am relieved to be finished working with other students.
 - h. When working in the chemistry lab, I feel at ease doing the lab procedures.
 - i. When I get ready for lab, I get concerned about recording the data we will generate.
 - j. When my chemistry lab is over, I am relieved to be away from the chemicals.
- **CLAI Lab Comfort 2:** Please indicate the extent to which each of the following statements are true of you.
 - a. When I work in the chemistry lab, I feel nervous being around the equipment.
 - b. When working in the lab, I am nervous about the time it will take.
 - c. When working in the chemistry lab, I feel nervous about recording the data I will need.
 - d. When preparing for lab, I am concerned about the time available for doing the experiment.
 - e. When I get ready for chemistry lab, I get concerned about the chemicals we will use.
 - f. When my chemistry lab is over, I am relieved to be away from the equipment.
 - g. When working in the chemistry lab, I feel nervous carrying out the lab procedures.

- h. I am relieved when I complete the lab in the time available.
- i. When working in the chemistry lab, I feel nervous being around the chemicals.
- j. I feel comfortable working with other students when I am in lab.
- **CLAI Lab Comfort 3:** Please indicate the extent to which each of the following statements are true of you.
 - a. When getting ready for chemistry lab, I get concerned about the equipment we will use.
 - b. When my chemistry lab is over, I am relieved to be finished doing the lab procedures.
 - c. I feel anxious when I work with other students during lab.
 - d. I am comfortable being near chemicals when I am in lab.
 - e. I am anxious when I record data during lab.
 - f. I am comfortable with the amount of time available for doing the lab.
 - g. When I get ready for chemistry lab, I get concerned about working with other students.
 - h. I am anxious when I carry out a lab procedure.
 - i. When working in the chemistry lab, I feel at ease recording the necessary data.
 - j. I feel anxious when I use equipment during lab.
- **MSLQ Metacognitive Self-Regulation:** Please indicate the extent to which each of the following statements are true of you.
 - a. During class time I often miss important points because I'm thinking of other things.
 - b. When reading for this course, I make up questions to help focus my reading.
 - c. When I become confused about something I'm reading for this class, I go back and try to figure it out.
 - d. If course readings are difficult to understand, I change the way I read the material.
 - e. Before I study new course material thoroughly, I often skim it to see how it is organized.
 - f. I ask myself questions to make sure I understand the material I have been studying in this class.
 - g. I try to change the way I study in order to fit the course requirements and the instructor's teaching style.
 - h. I often find that I have been reading for this class but don't know what it was all about.
 - i. I try to think through a topic and decide what I am supposed to learn from it rather than just reading it over when studying for this course.
 - j. When studying for this course I try to determine which concepts I don't understand well.
 - k. When I study for this class, I set goals for myself in order to direct my activities in each study period.

1. If I get confused taking notes in class, I make sure I sort it out afterwards.

4.5 Appendix B

IRB Statement

As a student in this course, you are being asked to participate in a research study that measures the effectiveness of different teaching strategies. This survey is a part of the study.

This survey will take less than 15 minutes to complete.

Participation in this study is voluntary. Your instructor is offering extra credit to complete this survey, but you can earn the maximum extra credit in this course through other opportunities if you choose not to participate in this study.

Your responses to the survey will be identified with your name, but your instructors **WILL NOT** have any access to identified survey results. Your instructors will not see any survey results until the end of the session (after fall quarter final grade deadline). When survey results are shared with your instructors, only aggregate results will be shared. Your instructors will not see your name connected with your responses.

Participation in this study is voluntary. You may refuse to participate or discontinue your involvement at any time without penalty. You are free to withdraw from this study at any time before final grades are distributed. If you decide to withdraw from this study you should notify the Teaching and Learning Research Center (kdenaro@uci.edu) immediately. Use your UCI email and provide your name and the following text: "I would like my classroom and survey data from course code _____ to be removed from the research project."

Please see the course Canvas site (in the Course Logistics Module) for the full Study Information Sheet for this research study.

Example of how Small Specifications Grading Rubric was Amended for Other Required Rubric Types

Table 4.12. Specifications grading rubrics for presentations and figures and table assignment.

Who (What) Are They? [Figures & Tables] Rubric Criteria*	Met	Not Met	Presentation Rubric Criteria	Met	Not Met
High Pass:	6/7		High Pass:	8/9	
Low Pass:	5/7		Low Pass:	6/9	
Unsatisfactory:	≤ 4/7		Unsatisfactory:	≤ 5/9	
<u>Sentences</u> : The writing is grammatically correct according to the rules of Standard Edited Written English.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Spoken language</u> : The language is grammatically correct according to the rules of Standard Spoken English.	<input type="checkbox"/>	<input type="checkbox"/>
<u>Sentences</u> : Words are spelled and used correctly.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Spoken language</u> : The language does not contain any slang or colloquialism not appropriate for an academic presentation.	<input type="checkbox"/>	<input type="checkbox"/>
<u>Sentences</u> : Sentences are constructed correctly according to the rules of Standard Edited Written English.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Speaking Style</u> : Presentation is given in a clear and coherent manner (i.e. the content is understandable).	<input type="checkbox"/>	<input type="checkbox"/>
<u>Assignment Content</u> : The writer adequately analyzes the figure and table in sufficient detail.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Speaking Style</u> : Presentation is given in an engaging manner that captivates the audience's attention.	<input type="checkbox"/>	<input type="checkbox"/>
<u>Assignment Content</u> : The author adequately responds to all parts of the assignment.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Assignment Content</u> : The presenter clearly addresses the intended audience at an appropriate level.	<input type="checkbox"/>	<input type="checkbox"/>
<u>Formatting</u> : The constructed table is formatted in a clear and logical fashion.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Assignment Content</u> : The thesis of the presentation is clearly articulated and supported by the presentation.	<input type="checkbox"/>	<input type="checkbox"/>
<u>Formatting</u> : The constructed table includes all necessary and appropriate information.	<input type="checkbox"/>	<input type="checkbox"/>	<u>Assignment Content</u> : The speaker clearly supports all assertions with evidence.	<input type="checkbox"/>	<input type="checkbox"/>
			<u>Assignment Content</u> : The presenter adequately responds to all parts of the assignment.	<input type="checkbox"/>	<input type="checkbox"/>

	<u>Visual Content:</u> The presenter's visual aids are clear and easy to see/read.	□	□
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*Some course assignments did not match well to the general small writing assignments rubric, so we amended them accordingly. This assignment is one example, and the adapted assignment is shown in Table 4.15.

Examples of Chemistry 101W Assignments Adapted from *The Writer's Practice*

The following tables show how four additional writing experiences from *The Writer's Practice* were adapted for Chemistry 101W assignments.

Table 4.13. Adapting the Process steps of *The Writer's Practice* “Should I...?” writing experience for a chemistry writing exercise.

Writing Experience Process Step	<i>The Writer's Practice</i>	Chemistry 101W*
1. Select Subject	Choose a subject (e.g. go to a movie, buy an app, attend a concert, etc.) to review that will help your audience make a decision about whether or not that subject is any good or if they should attend or buy something.	Rather than thinking of any subject, the student's subject is: Should I read primary literature articles about chemistry research (whether I am in a research lab or not)?
2. Find and Analyze Models	Find models, study the models, and experience the subject. Find examples of writing solutions to this writing-related problem. Determine what kinds of information and background the models share, including how these examples help an audience make a decision. Take notes.	Same questions, but students should pay particular attention to finding examples of articles about why people should read scientific articles.
3. Audience Analysis	What does your audience know about the subject? What will they need to know from your review, and when in the piece will they need to know it?	The audience will be other undergraduate chemistry majors. What does the audience need from the student's piece of writing (i.e. what do they need to know about reading journal articles)? What kinds of attitudes will the audience have as they start to read the writing (i.e. will they feel like they need to read journal articles)? What does the audience know, or think they know, about reading journal articles?
4. Draft	Use your process to draft your piece. Keep your audience and purpose in	Same directions.

	mind.	
5. Revise, Edit, & Polish	Revise the review, addressing areas where you missed audience needs. Could the audience identify the purpose of the review, why or why not? Did the audience receive your recommendation message? Would the audience recall your most important evidence and arguments? What could you do differently to highlight what matters most? Have you addressed potential audience questions? Should you add anything to the review? Read out loud to do final edits and polishing.	Same directions.

*Our purpose for including this exercise was to help students start thinking about why reading scientific articles, especially in the field of chemistry, will help them and their peers develop as scientists. The assignment also helps them practice writing persuasively to a specific audience.

Table 4.14. Adapting the Process steps of *The Writer's Practice* "Who (What) Are They? [Figures and Tables]" writing experience for a chemistry writing exercise.

Writing Experience Process Step	<i>The Writer's Practice</i>	Chemistry 101W*
1. Audience	A person interested in the insights gained from your analysis of a set of keys.	The audience will be other undergraduate chemistry majors interested in the insights gained from an analysis of one example figure and one table.
2. Observe	The book provides an image of a set of car keys. Write down observable facts about the set of car keys. Do not write down judgements, simply facts. Also consider what may be missing from the set of keys.	Students were provided with examples of a figure and a table. In addition to the book prompts, students must also discuss what sorts of things should go in figures or tables that aren't depicted.
3. Draw Inferences	What conclusions can you draw based on your observations? Who is the owner of the keys and what do they like? What do they do with their time, and what are their beliefs?	Students were instructed to determine what conclusions they can make about the data shown in the figure and table. They were also asked to discuss what the figure and table were meant to describe. What information was easily understandable?
4. Extend Inferences	Based on those initial inferences, what other conclusions can you draw? What does this person do for fun, who are their associates? Make speculations grounded in observation and inferences.	Students were asked to draw conclusions. In addition, students were also to discuss if any information was NOT easily understandable from the figure and table. What prior knowledge are they assuming their audience knows? Are there any problems with the figure, or its caption, and the table? What about them is unclear to the audience?
5. Report Findings	Deliver the information in a way that will be useful to your audience. Be mindful of connecting your inferences to your observations so the audience can appreciate your evidence. How much confidence do you have in your conclusions? What do you know? What do you suspect? Ground all of your findings in specific	In addition to the questions posed, students must also describe the figure and table to their peers, assuming they have not read the journal articles where the figure and table are from. To apply what students have learned about depicting data visually and effectively, they also had to report the following data in a properly-formatted, clear, table — keeping in mind the principles talked about in lecture about

observations.

constructing effective figures and tables:

The pressure in a vessel containing liquid water was measured at several temperatures in order to study the temperature dependence of vapor pressure. Each pressure measurement was made five times so that an average and standard deviation could be calculated. The data are as follows:

At a temperature of 35 °C, the pressures were 41.9, 42.2, 42.3, 41.8 and 42.0 torr

At a temperature of 45 °C, the pressures were 71.5, 71.8, 71.9, 71.6 and 71.9 torr

At a temperature of 55 °C, the pressures were 117, 118.2, 117.5, 117.7 and 117.4 torr

At a temperature of 65 °C, the pressures were 187.5, 187.1, 188.3, 187.2 and 187.9 torr

*Our purpose for including this exercise was to help students think about why figures and tables in scientific articles must be clear and easily understandable. Students must consider how their audience will read and interpret any figures and tables they produce.

Table 4.15. Adapting the Process steps of The Writer's Practice "Huh? Say What? (Research Translation)" writing experience for a chemistry writing exercise.

Writing Experience Process Step	<i>The Writer's Practice</i>	Chemistry 101W*
1. Choose a Journal and an Article	Choose an article you find interesting.	Students must choose an article that is in the chemistry realm. It could be from a journal that publishes research in organic, inorganic, physical, theoretical, atmospheric, instrumental, or computational chemistry, or chemical biology.
2. Digest Article	Read the article with your purpose in mind. Focus on learning what you need to know to meet your objective — translating the article for a non-academic audience. Concentrate on the findings, evidence, and implications. Read other sources if you need to help you understand your chosen article.	Same directions.
3. Translate Article	Focusing on your audience's needs, attitudes, and knowledge, tell them what the research article means. Think about how to hook their interest and then satisfy their questions and curiosity once their interest is hooked.	Same directions.
4. Test Translation	Test your translation on an audience and ask them to rate their interest in repeating your message to someone else on a scale from 1-10. Ask them to repeat what they believe they've learned.	Same directions, except the test audience was conducted as a peer-review in class.
5. Revise, Edit, Polish, Title	Use the audience feedback, and your own reflections on how well your draft engages your audience and purpose. Revise your translation accordingly.	Same directions, using the peer review suggestions.
6. Reflect	How long did it take you to digest the article? Do you feel more confident in your ability to interact with this kind of specialized research and writing? If so, what technique or skill you employed will be most useful going forward? If not, what do you think you need to work on it in the future to increase your confidence?	Same directions.

*The purpose of this assignment was the same as that stated in *The Writer's Practice*: "1. Get (students) working with academic research in order to up (their) comfort level with texts that are often complex and foreign, and 2. Do a favor for the academics who publish their research by 'translating' their findings for a more general audience." As future chemists, students enrolled in Chemistry 101W must learn how to interpret academic findings for a non-academic audience.

Table 4.16. Adapting the Process steps of The Writer's Practice "Why Should I Trust This? (Understanding Sources)" writing experience for a chemistry writing exercise.

Writing Experience	<i>The Writer's Practice</i>	Chemistry 101W
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Process Step		
1. Finding a Source to Check	Imagine someone has approached you with a source or fact, and they don't know if it's true or not. You will help them investigate. Find a dubious source.	Students need to find a "scientific source or fact" specifically that seems dubious.
2. Examine the Questionable Claim	Use internet resources to investigate the source of fact. Check for previous fact-checking work, go upstream from the source, and read laterally. See what others say about the source of the claim. Look for as much background information as possible to determine if the source or fact is true or not.	Same directions. Find primary literature sources to support or refute the "scientific source or fact" if possible.
3. Plan Your Case	Your job is to report your findings. This involves informing your audience as to what you set out to do, and then walking them through what you did, and finishing with your conclusions. Track your progress clearly and provide sources for your own claims and conclusions.	Same directions. Be sure to plan how you will bring sources in to substantiate your claims.
4. Write Your Report	Write a report that meets your audience's needs while attending to their attributes and knowledge — which may be especially important. You may consider mentioning why the sources you cite are trustworthy.	Same directions.
5. Revise, Edit, and Polish Report	Revise your work until your audience would find it convincing. Edit and polish.	Same directions.

*Our purpose for adapting this writing experience for Chemistry 101W was to help students think about how to be ethical scientists when explaining why a scientific "source" or "fact" is — or is not — scientifically sound. As an ethical scientist, students need to back up their explanations with evidence.

Specifications Grading Course Student Grade Tracker

MINIMUM To Earn C-

Small Writing Assignments (including rough drafts): 60% w/high-pass <input type="checkbox"/> OR 80% low-pass <input type="checkbox"/>
1000-Word Writing Assignments: 3 w/low-pass class total <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Presentations: 2 w/low-pass <input type="checkbox"/> <input type="checkbox"/>
Presentation Participation: 3 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Lecture Participation: 2 Required <input type="checkbox"/> <input type="checkbox"/>
Reading Completion: 80% completed <input type="checkbox"/>
Complete/Incomplete Assignment Completion : 70% completed <input type="checkbox"/>
Final Presentation: 1 required low-pass <input type="checkbox"/>
Final Paper: Pass at low-pass level <input type="checkbox"/>

MINIMUM To Earn C

Small Writing Assignments (including rough drafts): 65% w/high-pass <input type="checkbox"/> OR 85% low-pass <input type="checkbox"/>
1000-Word Writing Assignments: 3 with low-pass class total <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Presentations: 3 w/low-pass <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Presentation Participation: 3 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Lecture Participation: 2 Required <input type="checkbox"/> <input type="checkbox"/>
Reading Completion: 80% completed <input type="checkbox"/>
Complete/Incomplete Assignment Completion : 75% completed <input type="checkbox"/>
Final Presentation: 1 required low-pass <input type="checkbox"/>
Final Paper: Pass at low-pass level <input type="checkbox"/>

MINIMUM To Earn C+

Small Writing Assignments (including rough drafts): 70% w/high-pass <input type="checkbox"/> OR 90% low-pass <input type="checkbox"/>
1000-Word Writing Assignments: 3 with low-pass class total <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

Presentations: 3 w/low-pass <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Presentation Participation: 3 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Lecture Participation: 3 Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Reading Completion: 80% completed <input type="checkbox"/>
Complete/Incomplete Assignment Completion : 80% completed <input type="checkbox"/>
Final Presentation: 1 required low-pass <input type="checkbox"/>
Final Paper: Pass at low-pass level <input type="checkbox"/>

MINIMUM To Earn B-

Small Writing Assignments (including rough drafts): 75% w/high-pass <input type="checkbox"/> OR 70% w/high-pass + 25% low-pass <input type="checkbox"/>
1000-Word Writing Assignments: 4 with low-pass class total <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Presentations: 4 w/low-pass <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Presentation Participation: 4 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Lecture Participation: 4 Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Reading Completion: 90% completed <input type="checkbox"/>
Complete/Incomplete Assignment Completion : 80% completed <input type="checkbox"/>
Final Presentation: 1 required low-pass <input type="checkbox"/>
Final Paper: Pass at low-pass level <input type="checkbox"/>

MINIMUM To Earn B

Small Writing Assignments (including rough drafts): 80% w/high-pass <input type="checkbox"/> OR 75% w/high-pass + 20% low-pass <input type="checkbox"/>
1000-Word Writing Assignments: 4 with min. low-pass <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> w/1 high-pass <input type="checkbox"/>
Presentations: 3 w/low-pass <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> , 1 w/high-pass <input type="checkbox"/>

Presentation Participation: 4 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Lecture Participation: 4 Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Reading Completion: 90% completed <input type="checkbox"/>
Complete/Incomplete Assignment Completion : 85% completed <input type="checkbox"/>
Final Presentation: 1 required low-pass <input type="checkbox"/>
Final Paper: Pass at high-pass level <input type="checkbox"/>

MINIMUM To Earn B+

Small Writing Assignments (including rough drafts): 85% w/high-pass <input type="checkbox"/> OR 80% w/high-pass <input type="checkbox"/> OR 75% w/low-pass <input type="checkbox"/>
1000-Word Writing Assignments: 3 low-pass <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> w/1 high-pass <input type="checkbox"/>
Presentations: 2 w/low-pass <input type="checkbox"/> <input type="checkbox"/> , 2 w/high-pass <input type="checkbox"/> <input type="checkbox"/>
Presentation Participation: 4 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Lecture Participation: 4 Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Reading Completion: 90% completed <input type="checkbox"/>
Complete/Incomplete Assignment Completion : 85% completed <input type="checkbox"/>
Final Presentation: 1 required high-pass <input type="checkbox"/>
Final Paper: Pass at high-pass level <input type="checkbox"/>

MINIMUM To Earn A-

Small Writing Assignments (including rough drafts): 90% w/high-pass <input type="checkbox"/>
1000-Word Writing Assignments: 4 with min. low-pass <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> w/2 high-pass <input type="checkbox"/> <input type="checkbox"/>
Presentations: 2 w/low-pass <input type="checkbox"/> <input type="checkbox"/> , 2 w/high-pass <input type="checkbox"/> <input type="checkbox"/>
Presentation Participation: 4 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

Lecture Participation: 5 Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Reading Completion: 95% completed <input type="checkbox"/>
Complete/Incomplete Assignment Completion : 90% completed <input type="checkbox"/>
Final Presentation: 1 required high-pass <input type="checkbox"/>
Final Paper: Pass at high-pass level <input type="checkbox"/>

MINIMUM To Earn A

Small Writing Assignments (including rough drafts): 90% w/high-pass <input type="checkbox"/> + 5% low-pass <input type="checkbox"/>
1000-Word Writing Assignments: 4 with high-pass <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Presentations: 1 w/low-pass <input type="checkbox"/> , 3 w/high-pass <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Presentation Participation: 4 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Lecture Participation: 5 Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Reading Completion: 95% completed <input type="checkbox"/>
Complete/Incomplete Assignment Completion : 90% completed <input type="checkbox"/>
Final Presentation: 1 required high-pass <input type="checkbox"/>
Final Paper: Pass at high-pass level <input type="checkbox"/>

MINIMUM To Earn A+

Small Writing Assignments (including rough drafts): 90% w/high-pass <input type="checkbox"/> + 5% low-pass <input type="checkbox"/>
1000-Word Writing Assignments: 4 with high-pass <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Presentations: 4 w/high-pass <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Presentation Participation: 4 Satisfactory Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Lecture Participation: 5 Required <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
Reading Completion: 95% completed <input type="checkbox"/>
Complete/Incomplete Assignment Completion : 95% completed <input type="checkbox"/>
Final Presentation: 1 required high-pass <input type="checkbox"/>

Final Paper: Pass at high-pass level □

Frequently Asked Questions

What happens if I meet most of the requirements for a grade, but not all of them?

You earn the highest grade for which you meet ALL of the minimum requirements.

What happens if I don't meet the minimum requirements for a C-?

For a D, you need a minimum of 65% low-pass for small assignments and 3 low-pass long assignments. There are no minimums in any other category. If you fall below this minimum threshold, your grade will be F. There are no D+ or D- grades for this class.

Pre- and Post-Class Survey Questions

Please indicate the extent to which each of the following statements are true of you. (Very Untrue of Me, Untrue of Me, Somewhat Untrue of Me, Neutral, Somewhat True of Me, True of Me, Very True of Me)

1. In a class like this, I prefer course material that really challenges me so I can learn new things.
2. In a class like this, I prefer course material that arouses my curiosity, even if it is difficult to learn.
3. The most satisfying thing for me in this course is trying to understand the content as thoroughly as possible.
4. When I have the opportunity in this class, I choose course assignments that I can learn from even if they don't guarantee a good grade.
5. Getting a good grade in this class is the most satisfying thing for me right now.
6. The most important thing for me right now is improving my overall grade point average, so my main concern in this class is getting a good grade.
7. If I can, I want to get better grades in this class than most of the other students.
8. If I study in appropriate ways, then I will be able to learn the material in this course.
9. It is my own fault if I don't learn the material in this course.
10. If I try hard enough, then I will understand the course material.
11. If I don't understand the course material, it is because I didn't try hard enough.
12. I believe I will receive an excellent grade in this class.
13. I'm certain I can understand the most difficult material presented in the readings for this course.
14. I'm confident I can learn the basic concepts taught in this course.
15. I'm confident I can understand the most complex material presented by the instructor in this course.
16. I'm confident I can do an excellent job on the assignments and tests in this course.
17. I expect to do well in this class.
18. I'm certain I can master the skills being taught in this class.
19. Considering the difficulty of this course, the teacher, and my skills, I think I will do well in this class.
20. When studying for this course, I often try to explain the material to a classmate or friend.
21. I try to work with other students from this class to complete the course assignments.
22. When studying for this course, I often set aside time to discuss course material with a group of students from the class.
23. Even if I have trouble learning the material in this class, I try to do the work on my own, without help from anyone.
24. I ask the instructor to clarify concepts I don't understand well.
25. When I can't understand the material in this course, I ask another student in this class for help.
26. I try to identify students in this class whom I can ask for help if necessary.

Please indicate the extent to which each of the following statements are true of you. (Strongly Disagree, Disagree, Neither Agree nor Disagree, Agree, Strongly Agree)

27. I am anxious when I use chemicals during lab.
28. When I work in the chemistry lab, I feel at ease using the equipment.
29. When getting ready for chemistry lab, I get concerned about the lab procedures we will use.
30. When my chemistry lab is over, I am relieved to be done recording data.
31. When I work in the chemistry lab, I feel nervous working with other students.
32. I worry about whether I have enough time to complete the lab.
33. When my chemistry lab is over, I am relieved to be finished working with other students.
34. When working in the chemistry lab, I feel at ease doing the lab procedures.
35. When I get ready for lab, I get concerned about recording the data we will generate.
36. When my chemistry lab is over, I am relieved to be away from the chemicals.
37. When I work in the chemistry lab, I feel nervous being around the equipment.
38. When working in the lab, I am nervous about the time it will take.
39. When working in the chemistry lab, I feel nervous about recording the data I will need.
40. When preparing for lab, I am concerned about the time available for doing the experiment.
41. When I get ready for chemistry lab, I get concerned about the chemicals we will use.
42. When my chemistry lab is over, I am relieved to be away from the equipment.
43. When working in the chemistry lab, I feel nervous carrying out the lab procedures.
44. I am relieved when I complete the lab in the time available.
45. When working in the chemistry lab, I feel nervous being around the chemicals.
46. I feel comfortable working with other students when I am in lab.
47. When getting ready for chemistry lab, I get concerned about the equipment we will use.
48. When my chemistry lab is over, I am relieved to be finished doing the lab procedures.
49. I feel anxious when I work with other students during lab.
50. I am comfortable being near chemicals when I am in lab.
51. I am anxious when I record data during lab.
52. I am comfortable with the amount of time available for doing the lab.
53. When I get ready for chemistry lab, I get concerned about working with other students.
54. I am anxious when I carry out a lab procedure.
55. When working in the chemistry lab, I feel at ease recording the necessary data.
56. I feel anxious when I use equipment during lab.

Please indicate the extent to which each of the following statements are true of you. (Very Untrue of Me, Untrue of Me, Somewhat Untrue of Me, Neutral, Somewhat True of Me, True of Me, Very True of Me)

57. During class time I often miss important points because I'm thinking of other things.
58. When reading for this course, I make up questions to help focus my reading.
59. When I become confused about something I'm reading for this class, I go back and try to figure it out.

60. If course readings are difficult to understand, I change the way I read the material.
61. Before I study new course material thoroughly, I often skim it to see how it is organized.
62. I ask myself questions to make sure I understand the material I have been studying in this class.
63. I try to change the way I study in order to fit the course requirements and the instructor's teaching style.
64. I often find that I have been reading for this class but don't know what it was all about.
65. I try to think through a topic and decide what I am supposed to learn from it rather than just reading it over when studying for this course.
66. When studying for this course I try to determine which concepts I don't understand well.
67. When I study for this class, I set goals for myself in order to direct my activities in each study period.
68. If I get confused taking notes in class, I make sure I sort it out afterwards.

Browser Meta Info (Very Untrue of Me, Untrue of Me, Somewhat Untrue of Me, Neutral, Somewhat True of Me, True of Me, Very True of Me)

69. I organize my ideas prior to writing.
70. I revise my writing to make sure that it includes everything I want to discuss.
71. I check my spelling before submitting an assignment
72. I check my writing to make sure it is grammatically correct before submitting an assignment.
73. I evaluate and re-evaluate the ideas in my writing before submitting an assignment.
74. I monitor and evaluate my progress in writing.
75. I revise and edit an essay two or more times before final submission.
76. I go through the planning, drafting, revising, and editing stages in my writing.

77. I write a lot to develop my writing skills.
78. I often work hard to do well in my writing even if I don't enjoy the writing task.
79. Even if they writing activities are difficult, I try to engage in them rather than giving up.
80. I concentrate as hard as I can when doing a writing task.
81. I spend a lot of time and energy on making sure my writing assignments are good.
82. I use newly-learned vocabulary in my sentences.
83. I brainstorm in order to generate ideas for my writing.
84. I use different words that have the same meaning.
85. I use my experiences and knowledge in my writing.
86. I try to use effective linking words to ensure clear logical relationships between sentences and paragraphs.
87. I understand what plagiarism is.
88. I understand how to avoid plagiarism in my writing assignments.

89. In order to generate ideas for my writing, I usually discuss the writing topic with a friend or classmate.
90. After revising or editing my essay thoroughly, I ask a friend or classmate to read and comment on it.
91. I try to identify friends or classmates who I can ask for help with my writing.
92. When I have trouble with a writing assignment, I try to do it with classmates or friends.
93. I complete in-class writing assignments with confidence and ease.
94. I try to relax whenever I feel afraid of writing.
95. I encourage myself to write even when I am afraid of making mistakes.

Open-Ended Post-Class Survey Questions

1. What did you think about the grading method used in this course?
2. What aspects of the grading did you think worked well?
3. What aspects of the grading would you change? Please be specific about the change you would make and why you think this is a beneficial change.
4. Use this space to provide any additional feedback.

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Chapter 5

Extraction on Paper: an Active Learning Technique to Facilitate Student Understanding of Liquid-Liquid Extraction

5.1 Introduction

Liquid-liquid extraction is a ubiquitous technique that has long been taught in undergraduate chemistry laboratory courses.^{1,2} Undergraduate students have resources in the form of lectures, textbooks, video podcasts, laboratory manuals, demonstrations, etc. to help them understand and apply the chemical principles behind liquid-liquid extraction.³⁻⁸ In the general and organic chemistry laboratory courses at the University of California, Irvine (UCI) in particular, students have access to all of the aforementioned items. Despite this abundance of available resources, students continue to struggle with connecting the conceptual aspects of how liquid-

liquid extractions — including acid-base extractions — work at the molecular level to the physical process of extraction.⁹

I developed an active learning technique — the *Extraction on Paper Activity* — to help students feel more comfortable when conducting liquid-liquid extractions. This Activity has been used in large lecture settings, specifically in Dr. Link's large organic chemistry laboratory courses, and can be used in any classroom or laboratory setting. Dr. Link helped me refine and implement this activity, and we submitted this account as a manuscript that was recently accepted for publication in the *Journal of Chemical Education*.

One barrier to student understanding in laboratory courses is the high cognitive load required to perform laboratory processes. Lower order cognitive processes, such as manipulating glassware, are not yet automated for novices like they are for experts. Novices developing these lower order cognitive skills are not able to engage simultaneously in the higher order cognitive skills necessary to conceptualize how molecular-level events occur.^{10–14} Providing pre-laboratory activities to visualize molecular processes and to help students become familiar with macroscopic laboratory procedures may increase student understanding of, and comfort with, laboratory work.^{9,11–17} These pre-laboratory activities provide an opportunity for students to engage in higher order cognitive processes outside of the laboratory setting with an expert available for guidance.

Active learning has proven to be an effective learning technique that engages students and helps them better conceptualize difficult material. Many researchers in science, technology, engineering, and mathematics (STEM) education disciplines have demonstrated that incorporating active learning in lecture and discussion settings greatly improves student performance on challenging course material.¹⁸ Active learning can take many forms, from think-pair-share and group work to clicker questions and physical activities that students can manipulate. Chemistry

educators have often turned to hands-on active learning techniques, which allow students to interact with a chemistry laboratory technique in a physical or virtual form.^{9,19–23} The goal of these active learning techniques is to help students perform in-lab experiments with greater comfort and expertise.

Two recent active learning techniques were developed as guided laboratory extraction activities.^{15–17,24,25} The activities were meant to help students understand extraction at the molecular level, including principles of immiscibility, solubility, acid-base reactions in the context of extraction, mixture equilibrium, and planning how to execute an extraction. While these activities are useful and effective in a laboratory setting, they are not amenable to lectures, discussions, and other learning environments outside of a laboratory due to the use of hazardous chemicals that would not be appropriate in these settings.

Hill and McGurran developed a model to help students visualize the molecular interactions that occur in a liquid-liquid extraction, particularly how polar molecules interact with the more polar solvent and how nonpolar molecules interact with the more nonpolar solvent.²⁶ They used cardboard covered with two different pieces of colored paper to represent two immiscible layers, and styrofoam balls matching each color of the “layers” to represent polar and nonpolar molecules. Hill and McGurran’s activity was tactile and allowed students to attach the molecules with different polarities to their respective layers using velcro. This simple model only illustrated principles of solubility and miscibility in the context of a liquid-liquid extraction. The *Extraction on Paper Activity* we present in this paper incorporates principles of polarity and extends the concepts illustrated to include how acid-base extraction works at the molecular level. We also provide a means for students to “separate” the immiscible layers and continue the extraction purification to the point of isolating the desired compound.

Our approach to aiding student comfort with the molecular basis of liquid-liquid extraction is to provide students with a physical kit that facilitates the performance of a dry extraction on paper. We developed an *Extraction on Paper Activity* kit containing a laminated worksheet that can be used in any learning environment. The kits are designed so that an instructor or teaching assistant can modify the Activity to best fit their needs to guide a course, or a student can work independently, through the *Extraction on Paper Activity*.

5.2 *Extraction on Paper Activity* Design

The *Extraction on Paper Activity* kits were designed to provide students with a tactile learning experience that guides them step-by-step through a liquid-liquid extraction process. The Activity provides students with an opportunity to conceptualize the extraction process, either a simple liquid-liquid or acid-base extraction, on the molecular level. Images of glassware also provide a simple introduction to the general procedure before students use the equipment in the laboratory.

We used the Activity in lecture during the first quarter organic chemistry laboratory. Prior to the lecture, students were expected to complete pre-class readings and to watch a pre-class video reviewing fundamental extraction and acid-base concepts. The students were already introduced to the concepts of extraction because they performed a simple extraction in a prior general chemistry laboratory course. The Activity worksheet depicts an image of a separatory funnel with boxes labeled as the top or bottom layer (Figure 5.1). Spaces are included for students to label the layers with lower or higher density. The back of the worksheet has general instructions for using the Activity and an image of two beakers labeled aqueous layer and organic layer. Once printed, the worksheets were laminated and velcro or magnets were pasted onto the blank spaces of the worksheet. We chose this design to facilitate Activity use in large lecture halls where students only

have table space in the form of tablet-arm desks. The corresponding labels were also laminated and affixed with either velcro or magnets. The Activity kits were then assembled with the complete worksheet, labels, a dry-erase marker and a felt eraser. Full worksheets and labels are provided in the Supporting Information.

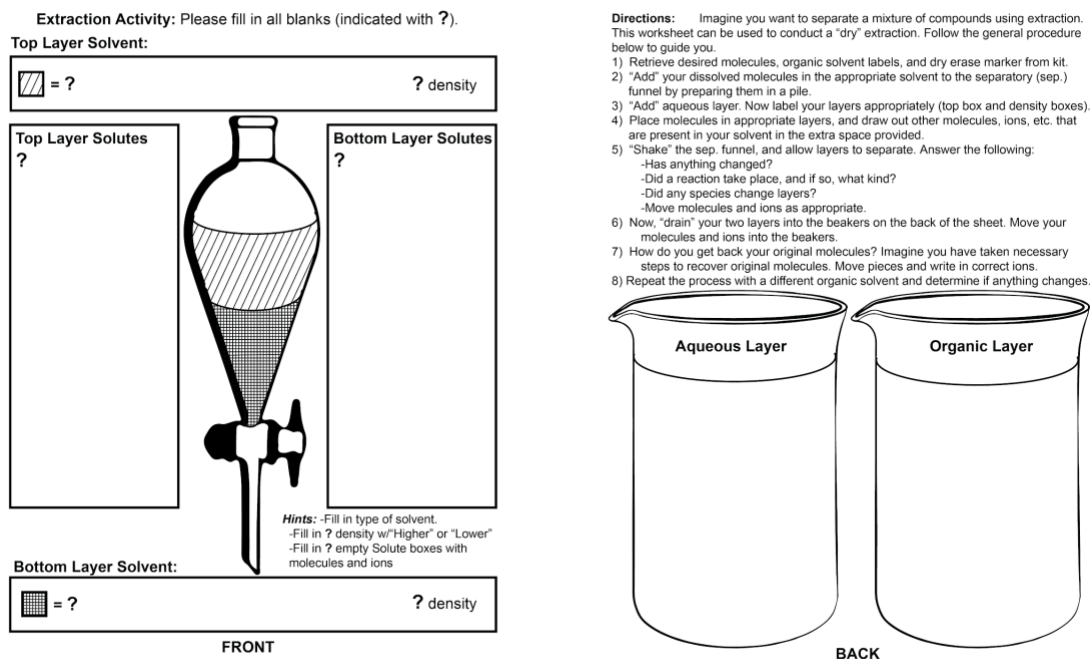


Figure 5.1. Diagram of *Extraction on Paper Activity* worksheet.

Each part of the *Extraction on Paper Activity* worksheet was designed to illustrate important concepts and chemical principles underlying liquid-liquid extraction. Labels corresponding to the top and bottom layer solvent identities were included to show that the two solvents chosen for an extraction should be immiscible. Spaces next to the top and bottom layer solvent identities should be filled with either the label "Higher" or the label "Lower" to indicate where the higher or lower density liquids will be located. Students must locate the densities of the two solvents they are using in any appropriate reference material such as a laboratory manual or

safety data sheet file to determine which solvent will be in which layer. The provided dry-erase markers are useful to write the density next to each density label.

Acid-base chemistry is also amenable to demonstration with the *Extraction on Paper Activity* (Figure 5.2). A stepwise procedure for the Activity was designed to help students feel more comfortable with the chemical principles that allow a liquid-liquid extraction to work at the molecular level and to connect these principles to each step of extraction they perform in a laboratory setting. We describe use of the Activity in reference to the labeled molecular species (A-D) that can be physically manipulated on the worksheet. The instructions are also projected on the front lecture screens when the Activity is in use so that students who wish to move ahead of the class may do so. In our example, we have the students imagine they are in a laboratory and they want to separate benzoic acid (A-B) from a compound mixture containing benzoic acid (A-B), methyl benzoate (C) and fluorene (D). The only way to separate benzoic acid from this mixture is to exploit it as an acid. We designed a label with a benzoic acid molecule (A) that has a removable proton (B). After “dissolving” our mixture in an organic solvent such as dichloromethane or diethyl ether, we “add” aqueous sodium hydroxide (NaOH) solution to the separatory funnel on the front of the *Extraction on Paper Activity*. Before mixing, we tell the students to label both layers and place all molecules in the correct Solutes box (before Top Layer Solutes in Figure 5.2). We then instruct the students to “shake” the separatory funnel, and describe what happens. Are the solvents interacting more? Can a chemical reaction now take place? We guide the students through an acid-base reaction between NaOH and benzoic acid by instructing them to remove the proton (B) from the acid (A) and then transferring the charged carboxylate to the aqueous layer Solutes box (Bottom Layer Solutes in Figure 5.2).

Extraction Activity: Please fill in all blanks (indicated with ?).

Top Layer Solvent:
 [diagonal lines] = ? **diethyl ether** [Lower] ? density

Top Layer Solutes
 ?
 A c1ccccc1C(=O)O B c1ccccc1C(=O)O C c1ccccc1C(=O)OC
 D c1ccc2ccccc2c1

Bottom Layer Solutes
 ?
 ?
 ?

Bottom Layer Solvent:
 [grid] = ? **water** [Higher] ? density

Hints: -Fill in type of solvent.
 -Fill in ? density w/ "Higher" or "Lower"
 -Fill in ? empty Solute boxes with molecules and ions

FRONT

Extraction Activity: Please fill in all blanks (indicated with ?).

Top Layer Solvent:
 [diagonal lines] = ? **diethyl ether** [Lower] ? density

Top Layer Solutes
 ?
 C c1ccccc1C(=O)OC
 D c1ccc2ccccc2c1

Bottom Layer Solutes
 ?
 A c1ccccc1C(=O)O
 Na⁺
 -OH
 H₂O

Bottom Layer Solvent:
 [grid] = ? **water** [Higher] ? density

Hints: -Fill in type of solvent.
 -Fill in ? density w/ "Higher" or "Lower"
 -Fill in ? empty Solute boxes with molecules and ions

FRONT

Figure 5.2. Demonstration of the *Extraction on Paper Activity* depicting acid-base extraction before (left image) and after (right image) mixing with aqueous base.

After completing the “separation” of benzoic acid with the compound mixture, we then ask the students to flip the *Extraction on Paper Activity* to the BACK side (Figure 5.1). Students are then instructed to “drain” the layers into the appropriately labeled beakers and transfer all molecules from the FRONT of the worksheet to the BACK beakers. To complete the process of extracting on paper, students must recover the original desired molecule, benzoic acid. Students must “add” aqueous hydrochloric acid solution to re-protonate the carboxylate anion, and recover the benzoic acid through “precipitation.” Images of these steps are provided in the Supporting Information.

5.3 Results and Discussion

The *Extraction on Paper Activity* was used in the laboratory lecture component of the first course in a series of organic chemistry laboratory courses, having as few as 71 students and as many as 442 students in a large lecture hall setting. At UCI, the regular academic year courses have multiple large lab lectures taught by the same instructor and they are graded together as a single course. The Activity has been used in all of them, so the survey numbers reflect the full enrollment of the course. The Activity has been used in the laboratory lecture component of the same course in four different academic terms, including an accelerated summer term. Because the Activity was designed for use during laboratory lecture, 200 kits were constructed to allow students to work in groups of two or three.

The presentation of the original Activity was transformed to the version presented here through two rounds of refinement following suggestions from student feedback (Table 5.1). During the first iteration, we allotted ten to fifteen minutes of lecture to guide students through one example extraction — using velcro versions of the Activity worksheet. In their feedback, students indicated that they wanted more time to work through the Activity and more examples to practice. The second time we ran the Activity the following year, we allotted 35-40 minutes. We also included an extra extraction example that the students worked on in pairs after completing the initial example with the entire class. After the second iteration, we again received student feedback indicating they would like to spend more time on the Activity, although the amount of these requests was reduced. Students also complained that the velcro activities were distractingly loud in a four-hundred person lecture hall, and that some found the velcro sound severely unpleasant. Based on this feedback, we decided to redesign the activities to have magnets. Activities with

velcro are being phased out as velcro pieces inevitably become lost, and they are being replaced with magnetic versions.

Table 5.1. *Extraction on Paper Activity Refinement*

Iteration 1	Iteration 2	Iteration 3
1. Designed Activity 2. Used Activity for 5-10 mins. in laboratory lecture 3. Collected student feedback a. Requested more time	1. Refined Activity a. Added extra example for independent student work 2. Used Activity for 35-40 mins. in laboratory lecture 3. Collected student feedback a. Requested no velcro	1. Refined Activity a. Swapped velcro for magnets 2. Used Activity in laboratory lecture

After the *Extraction on Paper Activity* was refined, two surveys (Pre- and Post-) were administered to determine if students felt more comfortable with the molecular principles of extraction after performing the Activity. The survey was first given as a Pre-Activity survey at the beginning of lecture — before the Activity was executed — and then it was given again as a Post-Activity survey at the end of completing the Activity. Both surveys consisted of the same three questions (Q1, Q2, Q3) — shown in Table 5.2 — with response choices on a Likert scale, labeled: (1) extremely uncomfortable, (2) moderately uncomfortable, (3) slightly uncomfortable, (4) neither comfortable nor uncomfortable, (5) slightly comfortable, (6) moderately comfortable, and (7) extremely comfortable.

Table 5.2. Pre- and Post-Survey Questions

Questions

Q1. How comfortable do you feel if asked to explain **HOW** acid-base extraction works at the molecular level?

Q2. How comfortable do you feel if asked to explain **WHY** taking advantage of acid-base chemistry makes acid-base extraction possible?

Q3. If your lab was today and you needed to do an acid-base extraction, how comfortable would you feel doing that extraction?

Students self-reported having greater comfort in their ability both to explain how and why extraction works at the molecular level and to do an extraction in the laboratory after performing the Activity (Figure 3).²⁷⁻³⁰ A total of 714 students completed both the Pre and Post surveys. Before the Activity, the Pre-responses showed a wide spread of student comfort, with the median student response being neutral (4) neither comfortable nor uncomfortable, across all survey questions. After the Activity, the median student response shifted towards a higher level of comfort — (5) slightly comfortable for both Q1 and Q2, and (6) moderately comfortable for Q3. Indeed, all student responses after the Activity, with the exception of outliers, shifted to fall between (4) neither comfortable nor uncomfortable and (7) extremely comfortable. High self-reported confidence in performing an extraction may reduce the technical and analytical errors made by students in the laboratory even if the self-reported confidence level may not be reliable.^{11,17,31}

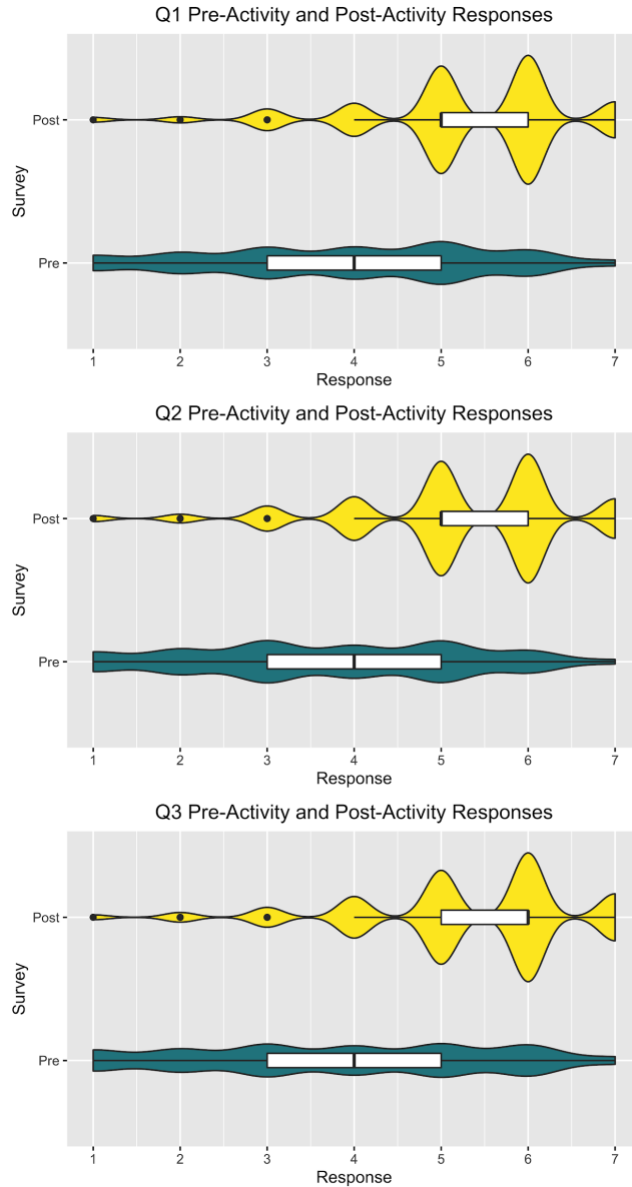


Figure 5.3. Student Self-Reported Comfort Level Before (Pre) and After (Post) Activity (n=714). Responses range from 1) extremely uncomfortable to 7) extremely comfortable. Yellow and teal curves of the violin plot³² show the distribution (similar to a histogram) of student responses. White bars with the black horizontal lines are box plots with five number summaries — depicting the minimum, maximum, and median with quartiles. The black dots are outliers.

5.4 Conclusion

The *Extraction on Paper Activity* allows students to walk through the thought processes required to conduct a liquid-liquid extraction in a laboratory, but in a lower stress setting. The Activity highlights principles of immiscibility, polarity, acid-base reactivity, and compound isolation in the context of an extraction. The *Extraction on Paper Activity* is amenable to demonstrating simple liquid-liquid extractions and acid-base extractions, and can be used with any compound labels to demonstrate separation. The Activity can also be used in a variety of different learning settings, including small and large lectures, discussions, office hours, tutoring sessions, and laboratories. Students self-reported having an increased level of comfort in understanding how and why extraction works at the molecular level and in performing an extraction in a laboratory setting. An increased level of comfort in understanding extraction after using this Activity could lead to an increase in comfort level with performing an extraction in a laboratory setting, and impact on lab performance could be assessed in future work.

5.5 Example Script for Instructor Use

Use this script as a guide for developing the *Extraction on Paper Activity* for use in a class setting. This activity can be used to demonstrate a liquid-liquid extraction or an acid-base liquid-liquid extraction.

1. “Today we are going to do an *Extraction on Paper Activity* to help you learn how an acid-base liquid-liquid extraction works at the molecular level. Please take out your kits and separate the molecules from the word labels.”
2. “Imagine you have a compound mixture that contains benzoic acid, methyl benzoate and fluorene (*or insert the molecules you wish to use here*). You dissolve the compound mixture in diethyl ether (*or insert solvent you wish you use here*), and you place your solution into a separatory funnel.”
3. “You add (but do not mix) aqueous sodium hydroxide—NaOH (*or insert solvent you wish you use here*) and see two separate layers.”
4. “Label which solvent is in your Top Layer Solvent box, with the appropriate ‘Higher’ or ‘Lower’ density label, and label which solvent is in your Bot Layer Solvent box, with the appropriate ‘Higher’ or ‘Lower’ density label.”
5. “Place your the compounds in your compound mixture in the appropriately labeled Solutes Box depending on whether they are in the Top or Bottom Layer (keep in mind this is BEFORE you shake or swirl your separatory funnel, so all molecules should still be in your organic solvent layer).” *The worksheet should now look like Step 1 below.*
6. “Now ‘shake’ or ‘swirl’ your separatory funnel. What happens at the molecular level? Are there any reactions that can now occur due to the closer proximity of reactive species? (*In our case, when doing an acid-base extraction, the NaOH can react through an acid-base reaction with the benzoic acid. The benzoic acid will be deprotonated, negatively charged, and now the benzoate anion will be more soluble in the aqueous layer.*) Move any compounds to their appropriate layer and write in all species present in the aqueous layer.” *The worksheet should now look like Step 2 below. Don’t forget to have your students remove the proton from benzoic acid if you are demonstrating an acid-base extraction.*
7. “We have successfully separated our compounds in the separatory funnel! Let your two layers separate and ‘drain’ your layers into the appropriately labeled beakers on the BACK of the worksheet.” *The worksheet should now look like Step 3 below.*
8. *If you are not doing an acid-base extraction, you can now talk about how you would isolate your desired compound. If you are doing an acid-base extraction, ask your students how you would get the benzoic acid back. The students should respond that you need to add acid to*

*re-protonate the benzoate, which will crash out of solution. You can then filter the product. Tell the students to 'add' aqueous HCl to re-form benzoic acid. The worksheet should now look like **Step 3** below.*

9. "You have now successfully completed a dry Extraction on Paper."

5.6 Example steps to use *Extraction on Paper Activity*

Step 1: Label Top and Bottom Layer Solvent with 'Higher' and 'Lower' density labels, and appropriate solvent identifications (i.e. water, dichloromethane, diethyl ether, etc.) on the FRONT side of the worksheet.

Place molecules in mixture in appropriate box according to whether they are Top Layer Solutes or Bottom Layer Solutes.

Step 2: 'Add' aqueous sodium hydroxide (NaOH) or other aqueous solvent of your choice. 'Shake' the separatory funnel, and indicate the transfer of appropriate solutes from one solvent layer to the other.

Step 3: 'Drain' the layers in the separatory funnel into the two beakers on the BACK of the worksheet by transferring the labels from the front of the worksheet into their appropriately labeled beakers on the back of the worksheet.

Step 4: If one product needs to be isolated (as in our example), add the appropriate solution (aqueous hydrochloric acid - HCl) to precipitate the desired product.

5.7 Example images to use *Extraction on Paper Activity*

Step 1

Extraction Activity: Please fill in all blanks (indicated with ?).

Top Layer Solvent:
 [diagonal lines] = ? **diethyl ether** [solid black] **Lower** ? density

Top Layer Solutes
 A OC(=O)c1ccccc1 B OC(=O)c1ccccc1 C CC(=O)Oc1ccccc1
 D c1ccc2ccccc2c1

Bottom Layer Solutes
 ?

Bottom Layer Solvent:
 [checkered] = ? **water** [solid black] **Higher** ? density

Hints: -Fill in type of solvent.
 -Fill in ? density w/ "Higher" or "Lower"
 -Fill in ? empty Solute boxes with molecules and ions

FRONT

Step 2

Extraction Activity: Please fill in all blanks (indicated with ?).

Top Layer Solvent:
 [diagonal lines] = ? **diethyl ether** [solid black] **Lower** ? density

Top Layer Solutes
 ? C CC(=O)Oc1ccccc1 D c1ccc2ccccc2c1

Bottom Layer Solutes
 ? B OC(=O)c1ccccc1 A OC(=O)c1ccccc1
 H⁺
 Na⁺
 -OH
 H₂O

Bottom Layer Solvent:
 [checkered] = ? **water** [solid black] **Higher** ? density

Hints: -Fill in type of solvent.
 -Fill in ? density w/ "Higher" or "Lower"
 -Fill in ? empty Solute boxes with molecules and ions

FRONT

Step 3

Directions: Imagine you want to separate a mixture of compounds using extraction. This worksheet can be used to conduct a "dry" extraction. Follow the general procedure below to guide you.

- Retrieve desired molecules, organic solvent labels, and dry erase marker from kit.
- "Add" your dissolved molecules in the appropriate solvent to the separatory (sep.) funnel by preparing them in a pile.
- "Add" aqueous layer. Now label your layers appropriately (top box and density boxes).
- Place molecules in appropriate layers, and draw out other molecules, ions, etc. that are present in your solvent in the extra space provided.
- "Shake" the sep. funnel, and allow layers to separate. Answer the following:
 - Has anything changed?
 - Did a reaction take place, and if so, what kind?
 - Did any species change layers?
 - Move molecules and ions as appropriate.
- Now, "drain" your two layers into the beakers on the back of the sheet. Move your molecules and ions into the beakers.
- How do you get back your original molecules? Imagine you have taken necessary steps to recover original molecules. Move pieces and write in correct ions.
- Repeat the process with a different organic solvent and determine if anything changes.

Aqueous Layer
 water
 A OC(=O)c1ccccc1 B OC(=O)c1ccccc1
 H₂O
 Na⁺
 -OH

Organic Layer
 diethyl ether
 C CC(=O)Oc1ccccc1 D c1ccc2ccccc2c1

BACK

Step 4

Directions: Imagine you want to separate a mixture of compounds using extraction. This worksheet can be used to conduct a "dry" extraction. Follow the general procedure below to guide you.

- Retrieve desired molecules, organic solvent labels, and dry erase marker from kit.
- "Add" your dissolved molecules in the appropriate solvent to the separatory (sep.) funnel by preparing them in a pile.
- "Add" aqueous layer. Now label your layers appropriately (top box and density boxes).
- Place molecules in appropriate layers, and draw out other molecules, ions, etc. that are present in your solvent in the extra space provided.
- "Shake" the sep. funnel, and allow layers to separate. Answer the following:
 - Has anything changed?
 - Did a reaction take place, and if so, what kind?
 - Did any species change layers?
 - Move molecules and ions as appropriate.
- Now, "drain" your two layers into the beakers on the back of the sheet. Move your molecules and ions into the beakers.
- How do you get back your original molecules? Imagine you have taken necessary steps to recover original molecules. Move pieces and write in correct ions.
- Repeat the process with a different organic solvent and determine if anything changes.

Aqueous Layer
 water
 A OC(=O)c1ccccc1 B OC(=O)c1ccccc1
 H₂O
 Na⁺
 H₃O⁺
 Cl⁻

Organic Layer
 diethyl ether
 C CC(=O)Oc1ccccc1 D c1ccc2ccccc2c1

BACK

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