XENOBIOTIC DETOXIFICATION IN *CAENORHABDITIS ELEGANS* AS A CONTEXT FOR CAREER DEVELOPMENT OF POSTGRADUATE RESEARCHERS

by

MUHAMMAD ZAKA ASIF

(Under the Direction of Erin L. Dolan & Arthur S. Edison)

ABSTRACT

STEM graduate students go through various stages in their development as researchers. Specifically, Ph.D. students are expected to complete coursework, work on their research, help with projects in the lab they are working in, maintain equipment, mentor other researchers, and fulfill departmental requirements. In this dissertation, I have explored three different aspects of a Ph.D. After joining a Ph.D. program, a student must first transition into the department, find a research lab and settle into a new city or country if they have moved geographically. I studied the transition experiences of South Asian international students in life science doctoral programs in the United States. Then, a student has to work on their research to make discoveries and develop their research expertise. I accomplished this by studying xenobiotic detoxification in *C. elegans,* focusing on 1-hydroxyphenazine (1-HP) detoxification. Finally, students can solidify and share their knowledge and develop their mentoring skills by mentoring junior researchers. I describe this process in my chapter on postgraduate perspectives on mentoring undergraduate

researchers serves that purpose. Altogether, this dissertation outlines postgraduate researchers' career development while providing insights into detoxification in *C. elegans.*

INDEX WORDS: South Asian international students, PhD transitions, DBER, mentoring, postgraduates, undergraduate research, vertically integrated projects, qualitative content analysis, *Caenorhabditis elegans,* UGTs, UGT-23, UGT-49, 1-hydroxyphenazine, glycosylation, natural products, detoxification

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DEDICATION

To my grandparents, who sacrificed so much all their lives to build up our family after Partition. I hope to make you proud and never let your sacrifices go to waste. Especially my nani, Jahan Ara Begum, wife of Muhammad Usman Farooqui, who very recently passed away and who taught me what it means to care for others and showered me with her love and wisdom. May Allah have mercy on her soul and grant her Jannah. Ameen.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Why did I Study the Transition Experiences of South Asian International Students?

My interest in studying transition experiences into doctoral programs started with my experience in the ILS program. I joined in August 2017 and rotated through my first two labs without being sure which lab I would join. Then I had a negative experience in my third rotation and ended up without a lab at the end of the rotation period. If not for Dr. Dolan taking a chance with me, I would have had to leave the Ph.D. program. It took me over a year to fully settle, find my wet lab, and be on track for a Ph.D. This experience and conversations with some of my South Asian (Desi) peers made me want to learn more about transition experiences.

Surprisingly, I found some research on transition experiences into doctoral programs, but very little of this research focused on international students (Cemalcilar & Falbo, 2008; Prescott & Hellsten, 2005), of the research that included international students even less focused on Asian students (Huang, 2012; Zhang, 2016). Furthermore, there was almost no research on the experiences of South Asian international students. While there was some research on their acculturation experiences (Frey & Roysircar, 2006; Rahman & Rollock, 2004), none focused on their transition experiences.

As a South Asian student, it troubled me that Asian students were being studied as a monolith and that experiences and problems that could be unique to us were ignored by research in the field. This spurred me to make studying the transition experiences of South Asian international students a central part of my Ph.D. and the main project I worked on for

the DBER part of my Ph.D. In order to study transition experiences and identify unique experiences for South Asian international students, we must first understand what we already know about transition experiences for students in general, which is what I will summarize in the next section

1.2 Transitioning to a Ph.D. Program: What do we know?

As the number of students receiving doctorates has increased, there has been more of an interest in the experiences of doctoral students, translating to research on different aspects of their experiences, including the experience of admission to a doctoral program, transition to candidacy and then post candidacy to the dissertation (Gardner, 2008). Despite all this research, little agreement exists on what period constitutes a transition. Different researchers have defined this period to range from the period of coursework to the first year of the Ph.D. program (Cornwall et al., 2019; Gardner, 2008; Levecque, Anseel, De Beuckelaer, Van der Heyden, & Gislef, 2017; Sverdlik & Hall, 2020; Thomson & Esses, 2016). Prior research has found that doctoral students experience numerous stressors as they transition to Ph.D. programs, including time pressure, uncertainty about doctoral processes, financial pressures, lack of a sense of belonging in scholarly communities, isolation, difficulties in the mentoring relationship (Cornwall et al., 2019; Gardner, 2008). Many of these issues are exacerbated for historically minoritized communities (Nettles, 1990).

Research on transition experiences, especially of international students, is frequently examined through acculturation literature (Bashir, Brinkman, Biemans, & Khalid, 2021; Guzel & Glazer, 2019). As a result, to gain a detailed understanding of international student experiences, we must learn about the acculturation of international students, which I will introduce next.

1.3 Acculturation Experiences of International Students.

When international students enter doctoral programs in a new country, they not only have to transition into the doctoral program, but they are simultaneously going through a transition to living in a new country and culture. As a result, they have been a focus of research in the acculturation space (Lyken-Segosebe, 2017; Ma, Pitner, Sakamoto, & Park, 2020).

Before we can review what is known about international student acculturation, we must first understand what acculturation is. Acculturation is the psychological changes that occur when an individual raised in one cultural context adapts to another cultural context due to migration, colonization, or other intercultural contacts. At a cultural level, acculturation is the interplay as cultures interact, such as during colonization (Berry, 1990). For this dissertation, we will focus on psychological acculturation.

Another notion that is important for understanding acculturation is adaptation. Adaptation is the experience of learning how to survive in a new cultural setting during acculturation (Berry, 2005). There are two different forms of adaptation: psychological and sociocultural. Psychological adaptation entails personal well-being and good mental health, while sociocultural adaptation refers to the individual's social competence in managing their daily life in an intercultural setting (Berry, Phinney, Sam, & Vedder, 2006).

International students are an exciting subsection of sojourners to study acculturation as they are often temporary visitors to a new cultural environment. Prior research has shown that international students face more distress during the first six months of their Ph.D. programs than domestic students (Hechanova-Alampay, Beehr, Christiansen, & Van Horn, 2002).

International students from South and East Asia also experience more perceived discrimination than other groups (Frey & Roysircar, 2006). Furthermore, there were differences

between these two groups and how much they utilized help resources (Frey & Roysircar, 2006). East Asian students were also shown to suffer from lower self-efficacy due to language and cultural barriers (Lyken-Segosebe, 2017). When they arrived in a new country, international students with high communication effectiveness reported ease of acculturation, indicating that cultural proximity and ease of communication are essential factors in helping international students acculturate to new surroundings (Ramelli, Florack, Kosic, & Rohmann, 2013).

There is little literature on South Asian students' acculturation experiences (Bashir et al., 2021; Rahman & Rollock, 2004). Rahman and Rollock (2004) found that South Asian students in the US reported higher levels of depressive symptoms due to high levels of prejudice, decreased perceived competence in their work, and decreased levels of social efficacy and ability to interact intraculturally. Some of these symptoms are mediated by gender. Specifically, women scored lower on their sample's perceived acceptance and self-efficacy scales (Rahman & Rollock, 2004). A more recent study by Bashir et al. (2021) on Pakistani students and their acculturation experiences in the Netherlands found that seven basic themes affected students' ability to acculturate: cultural disparity, linguistic challenges, limited interaction with host nationals, discrimination, difficulty in religious practice, attitudes towards acculturation, and participant coping strategies (Bashir et al., 2021). Many of these findings are similar to my research in Chapter 2 and suggest that these issues should be taken seriously for South Asian students studying in Western countries, including the US.

This body of research suggests that international students from non-western regions and regions where English is not the native language are likely to struggle to acculturate to life in the US, at least initially. My research in Chapter 2 describes these and other difficulties that affect

South Asian international student transition experiences, adding to the literature and hopefully impacting how graduate programs accommodate South Asian international students.

1.4 Interpretative Phenomenological Analysis (IPA)

Before I end my introduction to my study on the transition experiences of South Asian international students, I think it is worth discussing IPA, the methodology used for this study. Although IPA is popular in the health sciences, it has only recently started being used in education research (Emery & Anderman, 2020), and it is even rarer in DBER. Due to the novelty of this methodology for DBER research, I will introduce this study, its principles, and the markers that make for a good IPA in the following paragraphs.

IPA has three broad elements: an epistemological position, a set of guidelines for conducting research, and a body of empirical research (J.A. Smith, 2008). In terms of theoretical position, IPA is a form of phenomenological research in that it is concerned with the personal lived experience of participants and how the participant makes sense of their experiences. It differs from traditional phenomenology in its emphasis on the central role of the researcher. An IPA is how the researcher makes sense of how the participants make sense of their lived experiences. By explicitly acknowledging the researcher in this process, IPA allows the researcher's voice to be seen in emergent findings (J. A. Smith, 1996; J.A. Smith, 2008).

There are three characteristic features of IPA; IPA is idiographic, inductive, and interrogative. I describe each of these features next. An IPA has an idiographic because it does not aim to produce generalizable results but rather to understand how a particular person in a particular situation makes sense of that situation. Thus, a researcher has to thoroughly examine a case before starting with a second case, and then thoroughly examine that case before moving to the next case, and so on. Due to its strong idiographic focus, cases can only be cross-examined to

build themes once individually evaluated. This idiographic focus makes the analysis very laborious, thus limiting sample sizes. Usually, IPA is performed for 5-10 participants, although it is possible to have only one participant in an IPA (Emery & Anderman, 2020; J.A. Smith, 2008).

The second characteristic of IPA is its inductive nature. Chronologically, IPA starts off as idiographic in that the researcher identifies and studies each case in detail. Then, the researcher cross-examines cases to generate themes, which is where IPA's inductive nature comes in, meaning that the themes are generated from the data itself and not from pre-existing theories in the literature (Emery & Anderman, 2020; J.A. Smith, 2008).

Finally, IPA is interrogative. This means that findings that arise from a study should inform the existing literature in the field, either by supporting ideas that already exist or adding new ideas, or by strengthening, expanding, limiting, or undermining prior theories. This last step requires situating themes generated from individual cases within the broader literature in the field.

Along with these central characteristics, there are four markers, or qualities, of a quality IPA. An IPA study should construct a compelling, unfolding narrative, develop a vigorous experiential and existential account, close analytic reading of participant's words, and attend to convergence and divergence (Nizza, Farr, & Smith, 2021). First, a quality IPA should convey a story, both within each theme and across themes. Second, a quality IPA should adequately convey the existential meaning participants place on events described in the study. This means that it is incumbent upon the researcher to convey the ideas relayed by participants properly. Third, researchers should analyze the words of the participants instead of leaving quotes without analysis for the reader, reflecting the interpretative element of IPA. Finally, a quality IPA attends to convergence and divergence. This means that the researcher should point out the

commonalities between the different cases that make up the study while pointing out the individual characteristics of each case.

1.5 My Interest in Discipline-Based Education Research (DBER) and Mentoring Research.

This chapter will introduce all the salient topics necessary to understand my dissertation. However, the nature of this dissertation is such that, at first glance, it seems like a disparate set of topics. The things that unite all these topics are my interests and experiences during the Ph.D. So I will introduce each topic by describing how my interest in studying these topics began. A large chunk of my Ph.D. can be categorized as DBER. I was introduced to DBER during my rotations when I joined the Integrated Life Sciences (ILS) program in the fall of 2017.

I did my second rotation in the Andrews lab, where I was tasked with performing qualitative content analysis on a subsection of a larger dataset that had been collected. In my subset, I analyzed qualitative data on how people identified as scientists. I was fascinated by the variety of responses and how, despite the variety, the qualitative method allowed us to group ideas into themes that rang true for the whole breadth of experiences observed in the dataset. While I did not end up joining Andrews' lab, the experience in her lab meant that I was open to an opportunity in a DBER lab, which Dr. Erin Dolan later offered me.

In the Dolan lab, I was introduced to mentoring research. I worked with a postdoctoral researcher in her lab, Dr. Lisa Limeri, on a project characterizing graduate student motivations behind being involved in mentoring undergraduate researchers. I not only learned about the different reasons why graduate students mentor but also learned about their experiences mentoring while reading the transcripts.

Next, I worked with Dr. Limeri and a team of undergraduate researchers to characterize negative mentoring in undergraduate research. This allowed me to learn what not to do from the

undergraduate perspective and made me want to put my learnings into practice. I got my opportunity in the fall of 2018 when I learned that Dr. Arthur Edison was seeking help managing his Vertically Integrated Projects (VIP) undergraduate research teams. By the fall of 2021, I realized that my experiences, coupled with the dataset I worked on with Dr. Limeri to characterize the motivations of graduate student mentors, would make for an exciting research topic. It would contribute to understanding how graduate students understand their role as mentors and provide an example of how to put those ideas into practice in team-based research. This is how Chapter 3 of this dissertation was born. In the next section, I will set the stage for my research on postgraduate perspectives on mentoring by first laying out what we already know from the literature about the role of mentoring in undergraduate research in science.

1.6 Role of Mentoring in Undergraduate Research in Science

There has been an increased emphasis recently on undergraduate retention in scientific careers. (National Academies of Sciences et al., 2017). Research has shown that culturally relevant mentoring increases the inclusion and retention of underrepresented minorities (URMs) in STEM careers, primarily due to increased science identity and value alignment (Estrada, Hernandez, & Schultz, 2018; Haeger & Fresquez, 2016). The question that arises is what about mentoring makes it so that it positively influences student retention.

Most undergraduate research is structured in an apprenticeship model, and interactions with mentors influence the outcomes undergraduates realize from research experiences (E. Dolan & Johnson, 2009; E. L. Dolan & Johnson, 2010; Joshi, Aikens, & Dolan, 2019). Effective mentoring relationships can engage a broader group of students, increasing diversity and access across the student body. Research carried out by a diverse body is more likely to produce higher impact output as people with different ways of thinking tackle problems collaboratively. For

instance, the cultural similarity between student mentees and undergraduate mentors allows mentoring dyads to build closer relationships and allows them to have more influence as well (Estrada et al., 2022; Pedersen et al., 2022). This does not mean those dyads that are not culturally similar cannot have close relationships. Mentoring research has been able to identify specific practices and behaviors that allow mentors to promote mentee growth regardless of ethnic background (Byars-Winston, Dahlberg, & National Academies of Sciences Engineering and Medicine (U.S.), 2019)

While many studies suggest the positive effects of mentoring on student retention, that is not always the case. For instance, both undergraduate and graduate researchers experience negative interactions with mentors. Behaviors that could be classified as the absence of positive mentoring or actively harmful mentoring have both been described in the literature (Limeri et al., 2019), and that shows that while mentoring relationships are essential for undergraduate research, they can have both positive and detrimental effects (Limeri et al., 2019). Specifically, Limeri et al. found seven ways undergraduate researchers experienced negative mentoring: absenteeism, abuse of power, interpersonal mismatch, lack of career support, lack of psychosocial support, misaligned expectations, and unequal treatment (Limeri et al., 2019). Graduate students have also reported a wide range of ways they experience negative mentoring ranging from interpersonal differences and poor relationship quality to issues at the research group, departmental, institutional, and discipline levels (Tuma, Adams, Hultquist, & Dolan, 2021)

As a result, I chose to study postgraduate perspectives on mentoring to understand the range of factors that influence mentoring relationships and the behaviors carried out by the mentors themselves. As postgraduates do the majority of hands-on mentoring of undergrads in large,

research-intensive institutions, we must fully understand how they go about mentoring. Chapter 3 sheds light on postgraduate perspectives on what constitutes effective mentoring and allows us to understand how these perspectives may align with the existing literature on effective mentoring and where we need to provide more mentoring professional development to educate mentors on what works and what does not.

1.7 Different Types of Undergraduate Research Experiences (UREs) and the Benefits of Team-based Group Undergraduate Research.

While there is great emphasis on undergraduate research, what these UREs may look like varies between traditional apprentice-style research experiences and team-based research experiences. Traditional research experiences revolve around an undergraduate working one on one with a postgraduate or faculty member in an apprentice-style experience (E. L. Dolan, 2017). While these sorts of UREs have their benefits, such as increased access to the mentor, they also make it so that the research enterprise is accessible to a much smaller part of the broader STEM undergraduate community (Byars-Winston et al., 2019; Linn, Palmer, Baranger, Gerard, & Stone, 2015). This introduces issues of inequity, especially around the inclusion of historically minoritized communities (Krim et al., 2019).

One way to increase inclusivity in access to research opportunities is to provide course-based undergraduate research opportunities. Bangera and Brownell (2014) argue that there are several barriers to entry for undergraduates in traditional UREs. Barriers from the undergraduate side include a lack of awareness of research opportunities, a lack of awareness of the benefits of research, a lack of knowledge on how to reach out to professors for research, or an inability or unwillingness to talk to faculty about research opportunities, and the opportunity cost of missing out on paid opportunities for students from lower socioeconomic backgrounds. From the faculty

side, barriers include a preference for the 'best' students, high grades, and unconscious societal biases, most often towards people of color (Bangera & Brownell, 2014). It is believed that by introducing research into the classroom, not only can we introduce students who would otherwise not have access to research to the field, but also, by aligning learning objectives to mimic a 'true' lab setting closely, we can prepare students to be able to access traditional UREs in the future (Bangera & Brownell, 2014).

There exists significant variability in what a CURE could look like. However, other options exist for creating a team-based research environment that increases access for more students to undergraduate research. One such example is Vertically Integrated Projects (VIP). The VIP program is a research education program that started in engineering education. The basic tenets are undergraduates joining teams of students working in different research and development fields for academic credit. These teams assist graduate students and faculty members in their research. The teams are meant to be multidisciplinary, meaning students from different majors work together on a common problem; vertically integrated, meaning that students from Ph.D. students to sophomores all participate in the team; and long-term, with undergraduates able to participate up to seven semesters (Coyle, Allebach, & Krueger, 2006; Marshall et al., 2014). This allows teams to work on real research problems outside of the classroom setting. At the same time, vertical integration allows senior members to train junior members meaning the responsibility of training students is shared instead of falling on one individual. This allows for the research to be scaled up and gives access to more students to research than in a traditional research setting, which allows mentors to engage in the talent development of more students.

1.8 Talent Development.

Talent Development is the steady transformation of 'gifts' into 'talents' (Gagné, 2021). Gifts or giftedness can be thought of as people, usually children, who possess above-average ability in a particular field for their age group, often described as being in the top 10% (Baccasino & Pinnelli, 2023; Gagné, 2021). Conversely, talent can be defined as having developed mastery in a particular field (Gagné, 2021). As we can see, while giftedness can be thought of as almost an innate ability that requires some sort of predisposition, talent is precisely something that can be improved with practice and guidance, hence the scope for talent development.

There are different frameworks for talent development; however, I opted to use Talent-Development-in-Achievement-Domains (TAD) framework for my research. The TAD framework articulates levels of talent development, each of which differs in its importance at different stages in a person's life. The first stage is aptitude, which refers to variations in an individual's psychological traits that predict positive future development (Preckel et al., 2020). An example of this is the differences in people's spatial ability. As a psychological variable, this is less amenable to talent development; however, an individual may manifest their aptitude later in life, and a mentor can help them to do so. The next level is competence which can be understood as the cluster of systematically developed abilities, knowledge, and skill sets that enable an individual to act effectively in a situation (Preckel et al., 2020). This can be thought of as the knowledge gained through years of schooling in different fields, including STEM. Another example of this is the different instruments one may learn to use during a research experience. When individuals with aptitude engage in a learning activity, they build competence.

According to the TAD framework, the next level of talent development is expertise. Expertise can be understood as a high level of constant achievement in a subject. An expert

should have a firm grasp of a subject so that they can solve problems related to the field (Subotnik, Olszewski-Kubilius, & Worrell, 2019). The time taken to reach expertise can vary from field to field, but also within the field expertise can vary based on what exactly one wants to gain mastery in.

The final level is defined as a transformational achievement. This level refers to an achievement that goes beyond expertise in any one field and generates creative responses that break down domain boundaries (Preckel et al., 2020). While this level perhaps says more about an individual's innate ability and less about talent development, harnessing this ability and honing it requires experience, practice, and effective mentorship.

1.9 Why did I choose to study xenobiotic metabolism in *C. elegans*?

After I got admission into the ILS program, and as I have described earlier in this chapter, after some initial troubles, I landed upon doing DBER with Dr. Dolan. A few months into my Ph.D., I realized that as much as I enjoyed DBER, I also missed being on the bench. Around this time, I was introduced to the VIP program in the Edison Lab by a graduate student in the Edison lab who also wanted to do DBER.

Initially, this would be an opportunity to get some bench experience and help manage the VIP students while I focused on my work in the Dolan Lab. After meeting with Art and hearing him describe the research in his lab, I realized there was potential for a lot more than this here. While applying to Ph.D. programs, ILS was the only non-chemistry program I had applied to. I had wanted to learn more chemistry techniques but had foregone that when I joined the ILS. In the Edison Lab, I realized I could have a chance to learn the analytical chemistry techniques I had wanted to learn initially.

Furthermore, I learned that I would have the opportunity to work with C. *elegans*, which I had learned about in undergrad. All this while, I would get to practice what I was learning from my research in the Dolan lab by mentoring the students in the VIP program in the Edison Lab. I did not quite have a project yet, but I knew then that a worm project would be a big part of my Ph.D.

I was first exposed to *C. elegans* during my undergraduate studies. I took a course called Advanced Cell Biology, where our final project involved working with *C. elegans.* My lab partner and I performed a lifespan assay on *daf-2, daf-16,* and double knockout mutants of *C. elegans.* I do not think I remember our results, but I enjoy working with the worms and am fascinated by the literature available about them. We often take for granted that *C. elegans* really are an ideal model organism. However, for a new researcher like me, at that moment, I was fascinated by the fact that we had completely mapped their cell lineage, that we could generate fluorescent worms, and the wealth of genomic information on these tiny worms (Nonet, 1999; J. Sulston et al., 1992; J. E. Sulston, Schierenberg, White, & Thomson, 1983).

The final part of this puzzle fell into place in the spring of 2019. After initially being introduced to the Edison Lab and the VIP team in the fall, in the spring I started working in the lab, mentoring undergraduate students and learning basic worm husbandry. During this time, I learned about prior work in the lab with 1-HP and how there was an idea for a project to study the role of *UDP-glycosyl transferases (ugt)* genes in 1-HP detoxification, but it just was not taking off. Immediately, here was a project that I thought would allow me to learn all the techniques I wanted to, and was amenable to involving undergraduate students. I immediately started developing protocols for screening different gene knockouts with the initial guidance of a

graduate student in the lab, Bif, and the VIP students. This project later formed the thrust of my bench research and chapter 4 and 5 of my Ph.D.

1.10 The Innate Immune system of *C. elegans.*

Xenobiotic metabolism and detoxification in *C. elegans* is carried out as a part of the innate immune response of *C. elegans.* As a result, in order to understand detoxification, we must first have a primer on how the *C. elegans* innate immune system works. This is by no means meant to be an exhaustive review, but rather provide enough background to set the stage for us to understand xenobiotic metabolism and the role of *ugt* genes, the main focus of my work.

C. elegans are bacterivores in soil and decaying matter (Barriere & Felix, 2006; Frezal & Felix, 2015). As they feed on all different kinds of bacteria, they are exposed to pathogens in their environment (Schulenburg & Felix, 2017). To combat pathogen exposure, worms have developed three main strategies for defense. One is avoidance, where they can sense potentially hostile environments and avoid going to them (Pradel et al., 2007; Schulenburg & Ewbank, 2007). Another is the presence of a strong cuticle and pharyngeal grinder to physically prevent pathogens from entering the worm (Engelmann & Pujol, 2010). Finally, if pathogens can enter the worm, a number of mechanisms are activated, which constitute the innate immune response.

In most organisms, pathogens are detected by conserved structures not present on host cells, known as microbe-associated molecular patterns (MAMPs), which are recognized by pattern recognition receptors (PRRs) (Akira, 2013; Akira, Uematsu, & Takeuchi, 2006; Nosratabadi, Alavian, Zare-Bidaki, Shahrokhi, & Arababadi, 2017). *C. elegans* surprisingly do not possess most canonical receptor proteins except for TOL-1 (Irazoqui, Urbach, & Ausubel, 2010; Pujol & Ewbank, 2022; Pujol et al., 2006). However, TOL-1 has primarily been implicated in avoidance behavior instead of immune response (Brandt & Ringstad, 2015; Garcia-Sanchez, Ewbank, &

Visvikis, 2021; Pradel et al., 2007). This means that *C. elegans* possesses many non-canonical PRRs, such as G-Protein coupled receptors (GPCRs) that act as PRRs (Reboul & Ewbank, 2016; Venkatesh & Singh, 2021; Zugasti et al., 2014). Furthermore, this leads to worms monitoring perturbations in their chemical homeostasis as markers for infection, often due to the ubiquitination of specific proteins due to infection (Garcia-Sanchez et al., 2021).

After pathogen recognition, the next step is the activation of signaling pathways that help combat pathogen infection. One such pathway is the Mitogen-Activated Protein Kinase (MAPK) pathway. It is activated in response to bacterial and fungal pathogens (Aballay, Drenkard, Hilbun, & Ausubel, 2003; Begun et al., 2007; Pujol et al., 2008; Sifri, Begun, Ausubel, & Calderwood, 2003). This pathway also neutralizes toxins produced by pathogens (Huffman et al., 2004). Other pathways involved are the DAF-2/Insulin-like Receptor pathway, the unfolded protein response pathway, transforming growth factor b (TGFb) pathway, and the apoptosis, necrosis, and autophagy pathways, but these pathways have not been associated with toxin neutralization (Engelmann & Pujol, 2010)

C. elegans also possess several effector molecules in the immune response. These include antimicrobial peptides such as ABF (Engelmann & Pujol, 2010; Kato et al., 2002) and caenopores (Roeder et al., 2010). Other effector molecules include lysozymes and lectins (Engelmann & Pujol, 2010). Finally, *C. elegans* can also produce bactericidal reactive oxygen species which kill cells in a rather unspecific manner (Chavez, Mohri-Shiomi, & Garsin, 2009; Chavez, Mohri-Shiomi, Maadani, Vega, & Garsin, 2007). Along with pathogen response, *C. elegans'* innate immune system is also activated upon exposure to xenobiotics, which will be the focus of the next section.

1.11 Xenobiotic Metabolism in *C. elegans.*

Xenobiotics are defined as substances foreign to a body or ecological system. In nature, *C. elegans* feed on a wide variety of bacteria, many of whom produce toxic compounds for the worms. As a result, *C. elegans* have developed a wide array of detoxification enzymes, more effective than some other parasitic nematodes (Stasiuk et al., 2019). Xenobiotic metabolism is canonically divided into three phases (Williams, 1959). Phase I is the addition of reactive moieties, such as hydroxyl groups, to the parent xenobiotic. Phase II is the conjugation of either the phase I modified or parent xenobiotic to a large, water-soluble molecule to facilitate excretion. Phase III is the transport of these metabolized compounds out of the cell (Hartman et al., 2021).

Phase I reactions are broadly grouped into oxidation, reduction and hydrolysis (Hartman et al., 2021). In *C. elegans,* all three categories of reactions can be seen, and enzymes responsible for these reactions can be found in somatic cells, intestinal cells, and neurons (Lindblom & Dodd, 2006). Oxidative reactions are carried out mainly by a family of proteins called cytochrome P450. However, some reactions are also carried out by flavin-containing monooxygenases (FMOs), alcohol and aldehyde dehydrogenases, monoamine oxidases, and peroxidases (Harlow, Perry, Stevens, & Flemming, 2018; Hartman et al., 2021).

The *C. elegans* genome contains 86 genes for cytochrome P450s, compared to 60 in humans (Menzel, Bogaert, & Achazi, 2001). A large number of P450 genes and the limited knowledge of the exact catalytic activity of these genes suggest that the function of these genes might be different from their human isoforms, and there might even be the possibility of redundancy in terms of gene function (Abbass, Chen, Arlt, & Sturzenbaum, 2021; J. B. Harris et al., 2020).

Much less research is available that functionally categorizes activity for FMOs (Hirani, Westenberg, Seed, Petalcorin, & Dolphin, 2016). As far as alcohol and aldehyde dehydrogenases are concerned, research in *C. elegans* suggests that their alcohol dehydrogenases work in much the same way as human isoforms; however, worms have several aldehyde dehydrogenases that have not been functionally characterized (Alaimo et al., 2012; Williamson, Long, & Theodoris, 1991). Finally, *C. elegans* have a single ortholog of monoamine oxidase, which has been shown to metabolize serotonin (Schmid et al., 2015; Wang et al., 2017).

Hydrolysis reactions use water to break chemical bonds. In *C. elegans,* only epoxide hydrolases are studied in detail, with *C. elegans* possessing two isoforms (T. R. Harris et al., 2008). Further studies should be conducted to establish both isoforms' substrate specificity.
In addition to these phase I enzymes, there are three significant categories of phase II enzymes in *C. elegans.* These are UDP-glucuronosyltransferase (UGTs), sulfotransferases (SULTs), and glutathione S-transferases (GSTs). In this section, I will briefly introduce SULTs and GSTs. I will introduce UGTs in more detail in the next section.

SULTs catalyze the conjugation of a sulfonate group from a donor molecule, typically 3-phosphoadenosine-5'-phosphosulfate (PAPS), to a substrate, usually at a hydroxyl or amine functional group (Hartman et al., 2021). There is a single cytosolic sulfotransferase enzyme in *C. elegans, ssu-1,* which has been shown to sulfonate hydroxylated compounds (Hattori, Inoue, Inoue, Arai, & Tamura, 2006).

GSTs conjugate glutathione to various endogenous and xenobiotic substrates and are divided into membrane-bound and soluble family members (Hartman et al., 2021). There are 56 GST genes in *C. elegans.* They have been reported to have been upregulated upon xenobiotic exposure, with *gst-24* being upregulated upon BaP exposure and *gst-5, gst-6, and gst-33* being

upregulated upon indole exposure (Lee et al., 2017; Wu et al., 2015). Ursolic acid has also been shown to upregulate *gst-7* and exogenous heme was shown to upregulate *gst-19, gst-7,* and *gst-5* (Negi, Saikia, Kanaujia, Jaiswal, & Pandey, 2017; Perally, Lacourse, Campbell, & Brophy, 2008)

There are two significant categories of phase III enzymes in C. elegans, ATP-binding cassette (ABC) transporters and solute carriers (SLCs) (Hoglund, Nordstrom, Schioth, & Fredriksson, 2011; Sheps, Ralph, Zhao, Baillie, & Ling, 2004). ABC transporters bind and hydrolyze ATP to move substrates across membranes (Rees, Johnson, & Lewinson, 2009), while SLCs are membrane-bound, ATP-independent transporter proteins. They include vesicular and mitochondrial transporters, passive transporters, coupled transporters, and exchangers (Hediger, Clemencon, Burrier, & Bruford, 2013). Knockouts of several ABC transporters have been examined and have shown increased sensitivity to heavy metals, xenobiotics, and bacterial toxins (Broeks, Gerrard, Allikmets, Dean, & Plasterk, 1996; Broeks, Janssen, Calafat, & Plasterk, 1995; Figueiredo et al., 2018; Mahajan-Miklos, Tan, Rahme, & Ausubel, 1999; Stupp et al., 2013). SLCs, on the other hand, are relatively understudied in *C. elegans.* While their role in neuronal development, apoptosis, and other bodily functions has been studied, their exact role in xenobiotic metabolism remains understudied (Gallo et al., 2011; Hartman et al., 2021; Serrano-Saiz et al., 2013). There are also several transcription factors responsible for activating the genes that code for these proteins, but they fall outside the scope of this section. For a very detailed understanding of xenobiotic metabolism in *C.elegans* one can refer to the review by Hartman et al, 2021.

1.12 Role of UGTs in xenobiotic metabolism in *C. elegans*

UGTs are enzymes that catalyze the conjugation of xenobiotics to a sugar group, usually glucuronic acid or glucose. They localize in the endoplasmic reticulum. The site of metabolism for UGTs is usually at the hydroxyl, carboxyl, or amine group (Burchell, Brierley, Monaghan, & Clarke, 1998). *C. elegans* possess 66 *ugt* genes classified into families based on sequence homology. Most *C. elegans* UGTs are homologous to multiple human isoforms (Hartman et al., 2021).

UGTs have been implicated in response to different chemical exposures. Research has shown that exposure to allyl isothiocyanate induced the expression of *ugt-13* (Hartman et al., 2021; Hasegawa, Miwa, Tsutsumiuchi, & Miwa, 2010). The mycotoxin deoxynivalenol has been shown to induce the expression of *ugt-26* and *ugt-28* (Di, Zhang, & Lawton, 2018). Albendazole is another compound that has been studied extensively regarding the role of UGTs. Prior research has found that *ugt-16, ugt-22,* and *ugt-63* are upregulated upon albendazole exposure and that *ugt-22* upregulation is downstream of the transcription factor *skn-1* (Fontaine & Choe, 2018; Laing et al., 2010). Fontaine and Choe also found that mutating *ugt-22* protects against albendazole-mediated toxicity. Several *ugt* genes were also shown to be upregulated upon acrylamide exposure (Hasegawa et al., 2008). Finally, transcriptional studies have also shown several UGT isoforms, including *ugt-13* and *ugt-63* to be upregulated upon exposure to chemical inducers like fluoranthene and b-naphthoflavone (Taubert, Hansen, Van Gilst, Cooper, & Yamamoto, 2008).

Researchers have postulated for over 20 years that UGTs in *C. elegans* should be considered glycosyltransferases, not glucoronysyl transferases, as they are more likely to catalyze the transfer of glucose or galactose (Kapitonov & Yu, 1999). Worms exposed to

albendazole have been shown to produce albendazole-glucose metabolites, further backing up this idea (Laing et al., 2010). Indole has also been shown to be glycosylated by *C. elegans* (Stupp et al., 2013). Another compound that has been shown to be glycosylated when worms are exposed to it is 1-hydroxyphenazine (1-HP) (Stupp et al., 2013). Since 1-HP has been the focus of my dissertation research, I will provide some information about this compound in the next section.

1.13 1-HP and its Effects on *C. elegans*

1-HP was first isolated from *Pseudomonas aeruginosa* culture and found to inhibit oxygen uptake in mouse liver mitochondria culture (Armstrong, Stewart-Tull, & Roberts, 1971). Initial research also showed that 1-HP affected mammalian cell respiration (Stewart-Tull & Armstrong, 1972). In the 1980s, 1-HP was purified, its structure was analyzed, and its effect on human cilia (where *pseudomonas* most often causes infection in patients with cystic fibrosis) was elucidated (Watson, Macdermot, Wilson, Cole, & Taylor, 1986; Wilson et al., 1987). Since then, there has been a wealth of research on 1-HP and its effects, not only in humans but on other organisms as well, including *C. elegans* (Ambreetha & Balachandar, 2022; Cezairliyan et al., 2013; Dormehl, Ras, Taylor, & Hugo, 1991; Kerr et al., 1999; Mahajan-Miklos et al., 1999; Muller & Sorrell, 1995; Pastells, Pascual, Sanchez-Baeza, & Marco, 2016).

In *C. elegans,* 1-HP, along with pyocyanin and phenazine 1-carboxylic acid, is toxic, with pyocyanin and phenazine 1-carboxylic acid being considered responsible for most of their toxicity as they are produced in higher concentrations than 1-HP (Cezairliyan et al., 2013; Stupp et al., 2013). Of all three toxins, 1-HP can function at the broadest pH range (Cezairliyan et al., 2013). It is thought that 1-HP produces reactive oxygen species in the worms, causing a-synuclein and polyglutamine-induced protein misfolding, exacerbating dopaminergic

neurodegeneration in *C. elegans.* Adding anti-oxidants cannot attenuate this phenotype (Ray, Rentas, Caldwell, & Caldwell, 2015). Worms have been shown to detoxify 1-HP by adding one, two, or three glucose molecules (Stupp et al., 2013).

1.14 Methods for Structural Elucidation

1.14.1 HPLC-UV

This section is not meant to be a detailed introduction to instrumentation for structural characterization. Instead, it is meant to be a brief introduction to nuclear magnetic resonance (NMR), high-performance liquid chromatography – ultraviolet spectroscopy (HPLC-UV), and liquid chromatography–mass spectrometry (LC-MS), as I used them for my research.

In my research, the first analytical platform that we used was HPLC-UV. HPLC is a powerful chromatographic tool for separating natural products in complex matrices. It is a very commonly used method, and sample preparation is convenient (Miller & Neuss, 1978). Columns with different phase chemistry permit the separation of almost any type of natural product (Nguyen, Guillarme, Rudaz, & Veuthey, 2006). Different solvent systems can be used based on the analyte's column chemistry and chemical properties of interest. However, in our case, we used a MeOH-H2O system spiked with phosphate for HPLC-UV and a pure MeOH-H2O system for LC-MS, which we will discuss later.

HPLC is often coupled with a detection method. In our case, we used UV with photodiode array detection (DAD). DAD provides UV spectra directly online and is very useful for detecting natural products with characteristic chromophores, in our case 1-HP (Wolfender, 2009). DAD-UV spectral libraries can be built, but one must use the same HPLC conditions, as the composition of the mobile phase can affect the UV bands. Because we can acquire data at several different wavelengths, we can acquire several UV spectra for a given LC peak to assess

peak purity (Wolfender, 2009). UV is considered to have the best combination of sensitivity, linearity, versatility, and reliability of all LC detectors (Wolfender, 2009).

In UV detection, the relationship between the intensity of light transmitted through the detector and the solute concentration is given by Beer's law (Vial & Jardy, 1999). In addition, detector sensitivity is controlled by the magnitude of the extinction coefficient of the analyte of interest at a given wavelength and the path length passing through the UV cell. Sensitivity increases with increased path length, but increased cell volume causes peak dispersion, so an empirical middle ground is generally used by manufacturers (Wolfender, 2009).

For our research, we used two different gradients to perform a gradient elution to separate 1-HP from its glycosylated products. We used a modifier (phosphate buffer) for our experiments. Modifiers can cause baseline drift at low wavelengths, but that did not affect our experiments (Wolfender, 2009). Using a DAD over a fixed wavelength detector, we optimized the wavelength whose absorbance intensity we eventually used to determine the relative amounts of glycosylated product. We were also able to track when our solvent DMSO came off the column because we could track multiple wavelengths simultaneously.

From the UV data, we could identify 1-HP derivatives and understand some chemical properties based on their retention time in our C18 reverse phase column. However, data derived from UV absorbance is insufficient for structural characterization. As a result, we used LC-MS, LC-MS/MS, and NMR analysis for final structural elucidation.

1.14.2 NMR Spectroscopy

NMR has been used for structure determination and quantification of small molecules since the 1970s (Wishart et al., 2022). Data collection for NMR is nondestructive; sample prep is easy, and results are reproducible, making it very attractive for our purposes as we could pool

fractions collected at different times. The signature differences in spectral features for compounds with the same molecular weight meant that NMR would allow us to differentiate the sugar modifications we were interested in. To illustrate how I used NMR for structural elucidation, I will use publicly available L-Tryptophan data obtained from the Biological Magnetic Resonance Data Bank (Hoch et al., 2022) as an example.

The primary purpose of an NMR experiment is to determine the structure of molecules. For our purposes, that was done by using high-resolution NMR spectra recorded in D2O. Any liquid with a low viscosity can be used as a solvent, and even solids can be used for solid-state NMR, but I will not go into detail about that in this section. This section will focus on some fundamental proton (1H) and carbon-13 (13C) experiments. I will also not go into detail with nuclide properties that allow for NMR experiments such as nuclear angular momentum *P*, nor will I explain concepts such as chemical shift or magnetic anisotropy. For more details on the theory behind basic NMR experiments, I refer to a book by Horst Friebolin called Basic One- and Two-Dimensional NMR Spectroscopy.

Seven different types of information can be obtained from NMR spectra. These are chemical shifts d, intensities *I*, indirect spin-spin coupling constants *J*, spectral type, relaxation times *T1* and *T2*, scalar and dipolar coupling between neighboring nuclei, and line shape. Since no single spectrum can provide all that information, we often conduct different experiments to obtain multiple spectra to gather more information. In this study, I will not go into detail with all these information types. Instead, I will explain things as we work through the example of tryptophan.

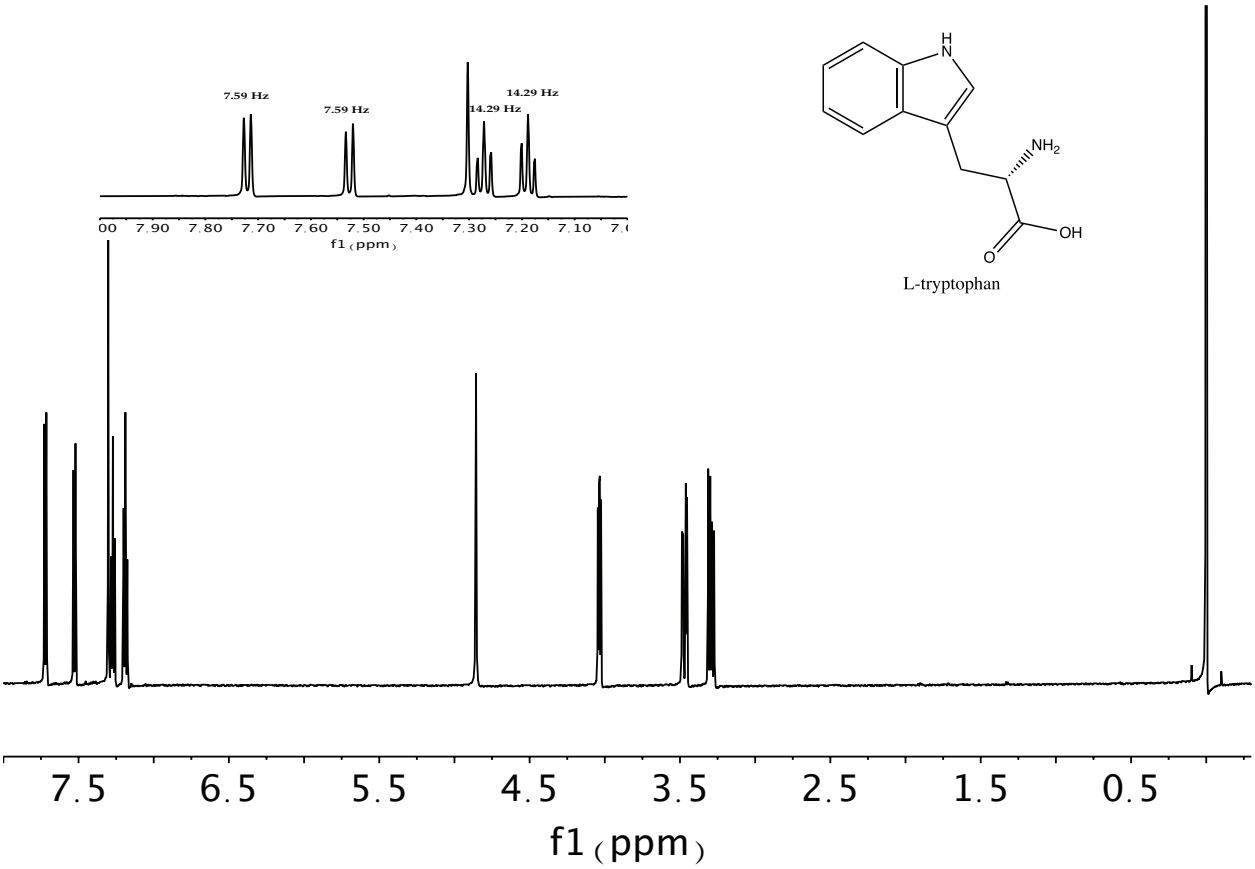

Figure 1. ^{1}H proton spectrum of L-tryptophan taken from BMRB.

We can glean much information from this spectrum. For starters, the chemical shift data help us understand what the compound could be. Usually, for amino acids, peaks are in between the 1-3 range; however, in the case of tryptophan, we can see in the structure that there is an electronegative ring structure. Proximity to this ring structure causes the amino acid peaks to be shifted to the 3 - 4.5 range. We can also notice the peaks in the 7 - 8 range. These can be associated with the protons connected to the carbons in the ring structure. To provide an example of detailed analysis that can be performed on proton spectra, I have provided a magnified inset of the region from 7-8. We can see the peaks around 7.5 and 7.7 Hz are split into a doublet. That indicates that they have one other proton in their chemical environment, i.e., on the adjacent carbon. The peak at 7.3 Hz is a singlet indicating no protons on the adjacent molecule. The other

two peaks are split into a triplet, indicating two protons on the adjacent molecule. For an example of how inferences can be made, looking at the structure, the peak at 7.3 could be the proton on the carbon next to the nitrogen on the five-membered ring.

In the inset, I have also shown the indirect spin-spin coupling constants. Indirect spin-spin coupling, also known as indirect dipole-dipole interactions or *J*-coupling, is a magnetic interaction between individual nuclear spins transmitted by the bonding electrons through which the nuclear spins are indirectly connected (Friebolin, 2005). This measures the magnetic interaction between the nuclear spin (a property of nuclei) within a spin system without going into too much detail. A spin system includes all nuclei between which spin-spin interactions exist. The value of these spin-spin couplings can indicate the proximity of the protons, causing the splitting patterns mentioned earlier. The magnitude of this value decreases with increasing numbers of bonds between coupled nuclei. The magnitude of these values can help determine whether 'weak' or 'strong' coupling is taking place. The values can also indicate how line intensities and line positions in a multiplet will be altered. These spin-spin couplings are affected by the atoms' hybridization, the bond angles, dihedral angles, C – C bond length, and substituent effects (electronegativity, neighboring p-bond, and lone pair effects) (Friebolin, 2005). In the case of tryptophan, the relatively high *J*-coupling indicates a 'strong' bonding between the protons, causing multiplicity. These values are also affected by the nature of the compound with the ring structure etc. We can also judge the relative amount of each nucleus by the intensity of the peaks. Since the ratio between different peaks should remain constant in an NMR experiment, we can tell how many protons are represented by each peak if we can identify a peak that represents one proton. All other peaks can be scaled accordingly in a process called integration.

The rest of the experiments I will describe here are 2D NMR experiments. There are two types of 2D experiments. One has chemical shifts on one of the axes and coupling constants on the other. This 2D experiment is not very useful to us, so I will not go into detail about it. The other type of experiment shows 1H vs. 1H or 1H vs. 13C chemical shift correlations. These are most relevant to my work from a practical standpoint. I will elaborate on them further. Two-dimensional experiments are based on couplings between nuclear dipoles. These may be scalar couplings or coupling through space, such as in the Overhauser Effect. There are also two-dimensional experiments based on magnetization transfer by chemical exchange. Unlike standard one-dimensional experiments, two-dimensional experiments have a preparation, evolution, and mixing phase between the initial pulse sequence and data acquisition.

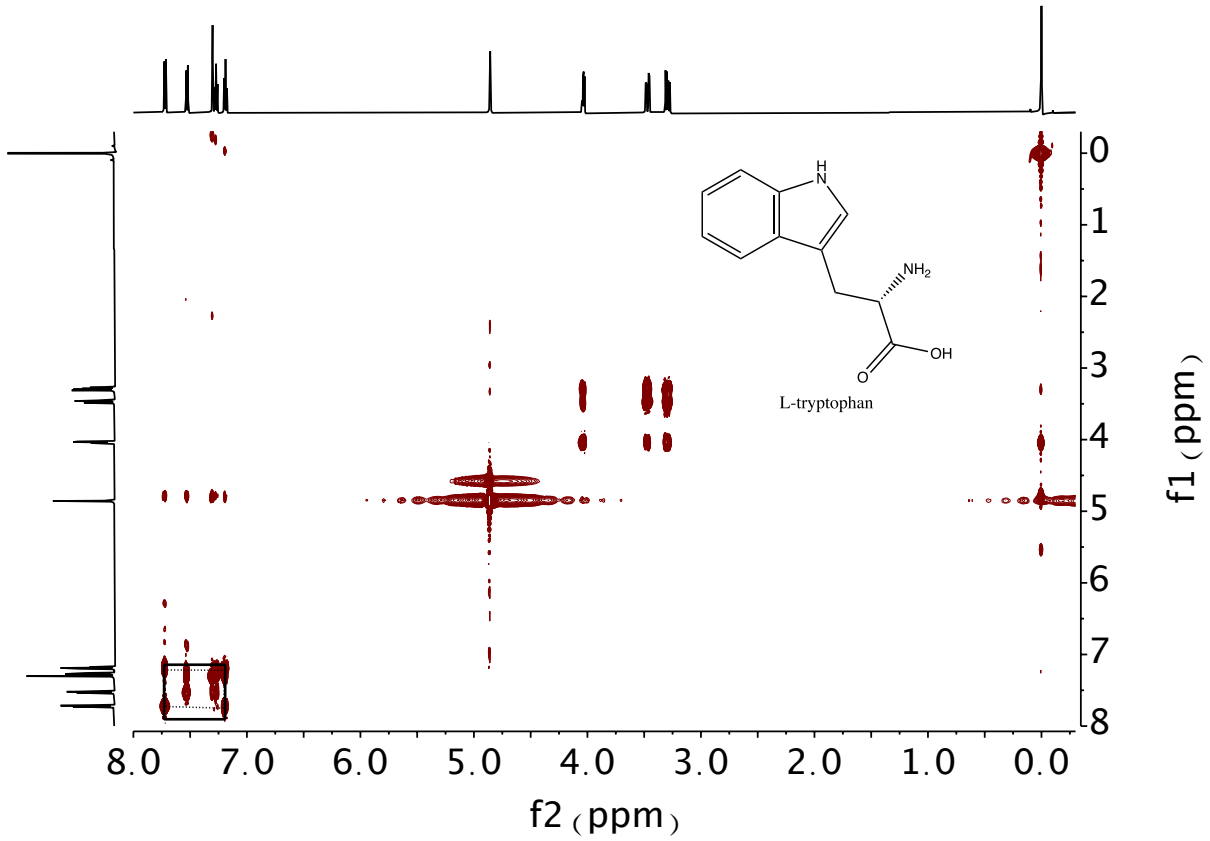

Figure 2. dqf Correlated Spectroscopy (COSY) spectrum for L-tryptophan from BMRB

The first experiment I will describe is the homonuclear correlated NMR Spectroscopy experiment (COSY). This experiment yields NMR spectra in which 1H chemical shifts along both frequency axes are correlated (Aue, Bartholdi, & Ernst, 1975). We can observe short and sometimes long-range coupling between protons in a COZY experiment. The peaks that show up between coupled protons are the ones that can be seen along the same vertical or horizontal lane, as pointed out by the square in Figure 2 between the peak at 7.8 PPM and 7.2 PPM. We can see that a straight line connects peaks in the square horizontally and vertically; this layout indicates scalar coupling between the peaks. The separation in each dimension between two adjacent

signals gives us the coupling constant, and therefore, the projection of this spectrum on either axis corresponds to a 1H NMR spectrum (Friebolin, 2005). Usually, however, when we look at a COSY spectrum, we are most interested in seeing correlations between peaks and not determining coupling constants.

In the COSY experiment shown in Figure 2, we can observe the correlations between the protons associated with the ring structure in the 7-8 PPM region and the protons associated with the amino acid backbone in the 3-4 PPM region. We can see a correlation between the protons at 7.3 and 7.5 PPM and 7.2 and 7.8. We also see correlations between all the peaks in the 3-4 PPM region but not between those in the 3-4 PPM region and the 7-8 PPM region. There are other types of COSY experiments where we can see more long-range coupling by introducing a fixed delay time, but I will not be going into detail with those experiments.

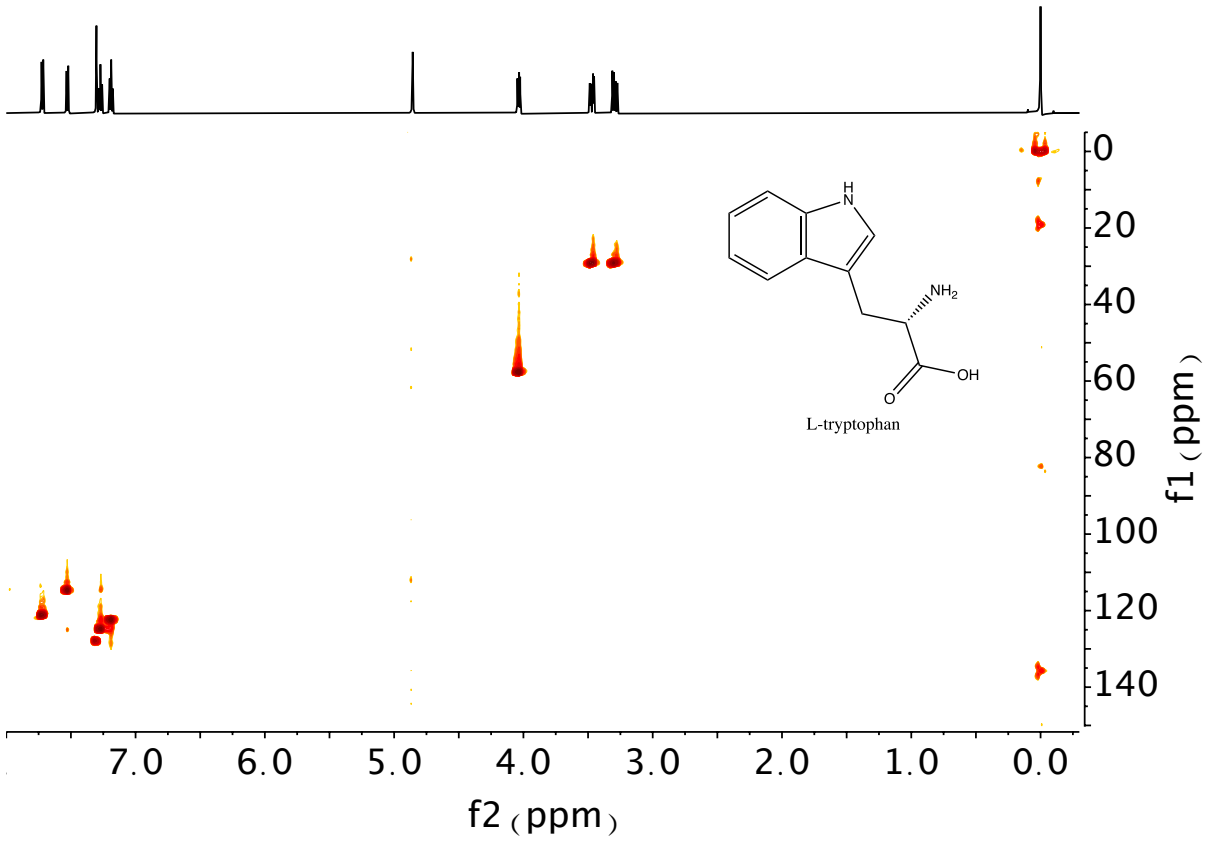

Figure 3. Heteronuclear single quantum coherence (HSQC) spectrum of L-tryptophan taken from BMRB.

The following experiment I will describe is a reverse two-dimensional heteronuclear correlated NMR spectroscopy experiment called heteronuclear single quantum coherence (HSQC) (Bodenhausen & Ruben, 1980). The strategy of this experiment is to transfer the 1H polarization corresponding to its magnetization to the 13C nuclei by way of an INEPT pulse sequence. The details of pulse sequences are outside the scope of this section, so I will not be going into detail here. After this, the magnetization vector for carbon is allowed to develop for a time *t1* which is changed incrementally. Finally, a reverse INEPT sequence transfers the resulting polarization back to the protons, and the 1H resonance is recorded (Friebolin, 2005).

This experiment reveals the correlations between directly bonded carbon and hydrogen atoms. It differs from most other heteronuclear experiments because it takes less time than other experiments (Friebolin, 2005).

In our example experiment in Figure 3 with tryptophan, we can immediately assign carbon peaks based on the proton peaks we already know. Without going into detail with each assignment, which is not the purpose of this section, we can get an example of this by following the case of the singlet we identified in Figure 1 at 7.3 PPM. We were able to assign that proton with some degree of confidence, and since we were able to do that, we can identify the carbon bonded to that proton, in this case, the carbon at 130 PPM marked by a circle, as the carbon bonded to that proton. In this way, we can make confident carbon assignments using this experiment, except for quaternary carbons, which do not have protons bonded to them.

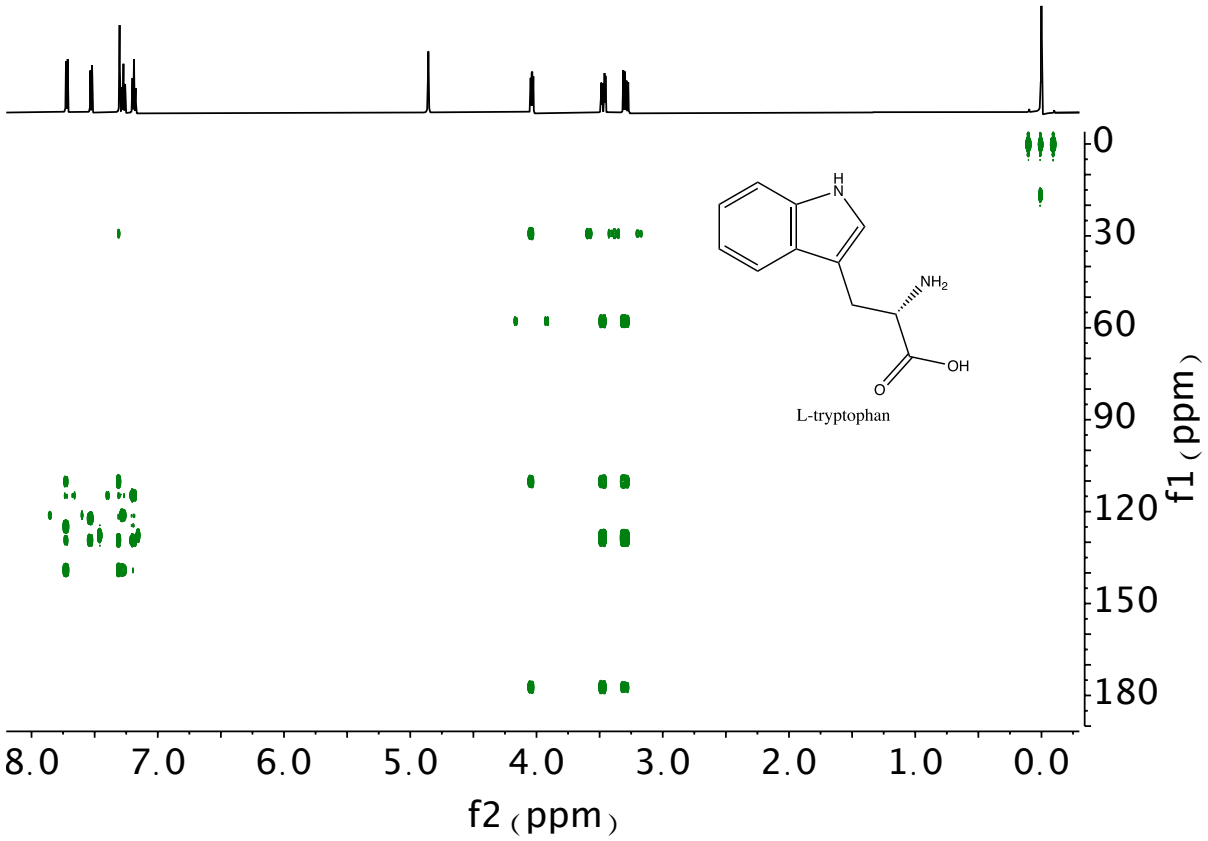

Figure 4. Heteronuclear multiple bond correlation (HMBC) Spectrum of L-tryptophan taken from BMRB.

As mentioned in the last paragraph, the one area for improvement with an HSQC experiment is that we cannot observe and annotate quaternary carbons. In order to address this issue, I will describe another experiment called the heteronuclear multiple bond correlation (HMBC) experiments (Bax & Summers, 1986). This experiment allows us to make assignments where 13C and 1H nuclei are coupled through two or more bonds, thus having smaller *J*-values.

As shown in Figure 4, an HMBC looks somewhat like an HSQC, with cross peaks that allow us to see correlations between 13C and 1H nuclei. However, we can see many new 13C peaks that we did not see in the HSQC. We can also see cross peaks with 13C nuclei that we

could not see earlier. If we combine the data from both experiments, we can assign not only the quaternary carbons but gain further evidence for assignments made from proton and HSQC experiments. For example, with tryptophan, we identified a carbon peak at about 115PPM, which correlated with the proton at 7.2 PPM in the HSQC. The exact peak in the HMBC can be seen to have cross peaks with protons in the 3-4 PPM range. This shows that while that carbon is not directly bonded to the protons in the amino acid backbone, it is close enough to form a 'weak' coupling with those protons. Furthermore, we can identify quaternary carbons, such as the carbon at 180 PPM. These peaks do not appear in the HSQC, indicating no protons directly bonded. However, they do have cross peaks with the protons in the 3-4 PPM range, indicating that this is the quaternary carbon bonded to the ethyl group bonded to the amino acid backbone.

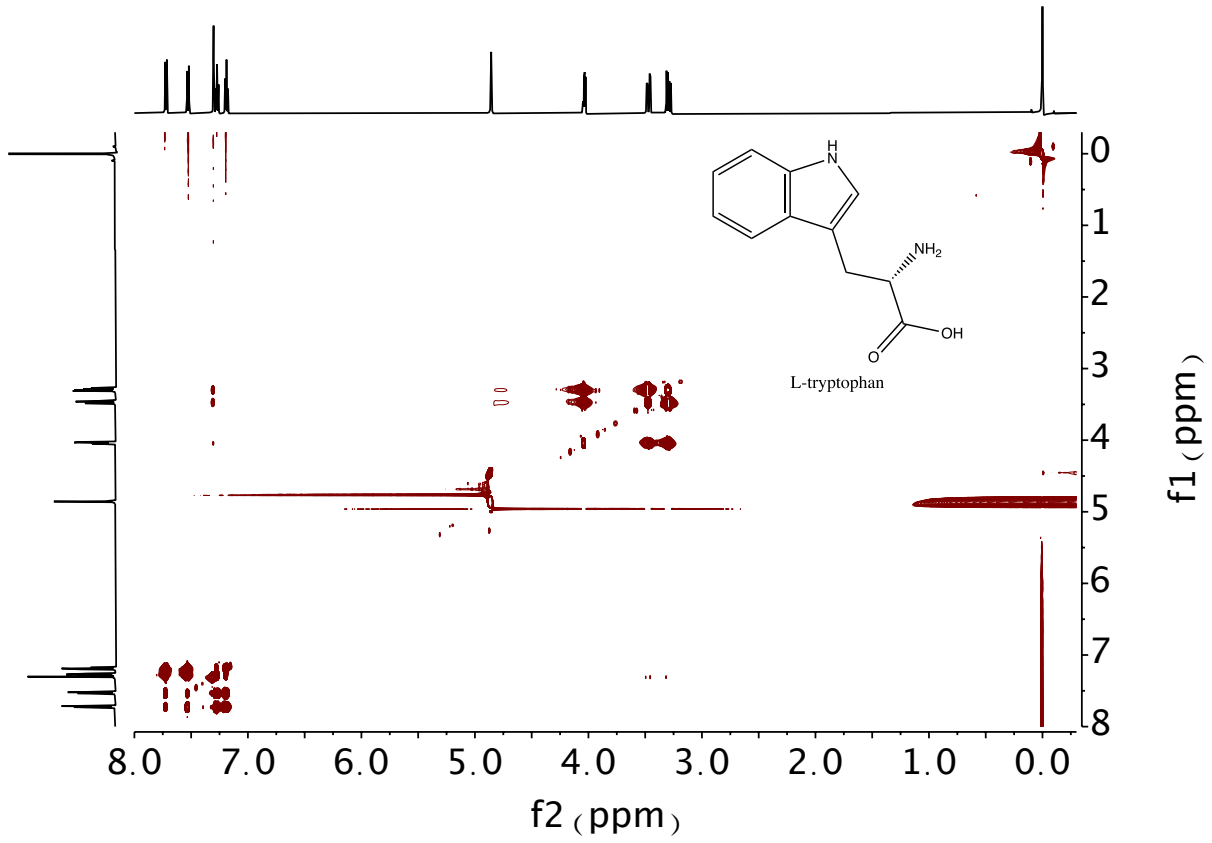

Figure 5. Total Correlation Spectroscopy **(**TOCSY) Spectrum for L-tryptophan obtained from BMRB.

Figure 5 describes a two-dimensional NMR experiment called total correlation spectroscopy or TOCSY. It was initially introduced by Braunschweiler and Ernst (Braunschweiler & Ernst, 1983). TOCSY works similarly to a COSY in that it has similar pulse sequences, except that the second 90o pulse is replaced by a spin-lock stage (Friebolin, 2005).

Like a COSY, in a TOCSY, the diagonal peaks correspond to the standard 1D spectrum, while the off-diagonal peaks show correlations. The difference here is that in a TOCSY, one should be able to see all the protons in a spin system correlated. In Figure 5, we can see more cross peaks in the 7-8 PPM range than in the COSY, and all the peaks have cross peaks indicating they are from the same spin system. Combining Figure 5 and Figure 2 can make assignments for each spin system in this molecule. It is also important to mention that we can perform 1D selective TOCSY experiments here. A 90o pulse is tuned to the resonance frequency of a particular proton. After that, we perform a normal TOCSY, a spin-lock followed by a transfer of magnetization that allows us to see all the protons in a 1D spectrum for the particular proton we selected earlier.

Before I end my section on NMR, I briefly want to introduce two-dimensional exchange NMR spectroscopy. There are two kinds of such experiments. One kind of experiment allows magnetization to be transferred to us through space based on the nuclear Overhauser effect (Friebolin, 2005). The other is magnetization transfer through chemical exchange processes. For my dissertation, I used the rotating frame Overhauser enhancement spectroscopy (ROESY), and in this section, I will briefly introduce ROESY along with another similar experiment, the

nuclear Overhauser enhancement spectroscopy (NOESY) (Bax & Davis, 1985; Bothnerby, Stephens, Lee, Warren, & Jeanloz, 1984; Jeener, Meier, Bachmann, & Ernst, 1979). These experiments are responsible for giving evidence for the spatial proximity of nuclei. These kinds of experiments helped me connect spin systems in my research. Since the NOESY and ROESY depend on the nuclear Overhauser effect, which falls off with distance (inverse proportion to $r6$), we can only observe peaks of protons within a certain distance from each other, in the case of a ROESY, about 5 Ao. The main difference between these two experiments is that in a ROESY, magnetization occurs through transverse magnetization components instead of longitudinal magnetization components (Bax & Summers, 1986). For more detailed information on these and all other NMR experiments mentioned in this section, please refer to the book Basic One- and two-dimensional NMR Spectroscopy by Horst Friebolin.

1.14.3 Liquid Chromatography-Mass Spectrometry

To avoid redundancy in this section, I will avoid going into too much detail with the liquid chromatography, as all salient chromatography details have been covered in the HPLC-UV section. Instead, this section will briefly introduce what mass spectra look like, explain the ionization strategies used in this dissertation, and write about how the data collected helped with the analysis.

Simply put, a mass spectrum shows the mass of a molecule and the mass of the pieces (fragments) derived from it. Usually, we observe a spectrum in bar graph form where the abscissa (x-axis) indicates the m/z (the ratio of mass to the number of charges on the ion employed), and the ordinate (y-axis) indicates relative intensity (McLafferty & Tureček, 1993). In our case, the ion employed was 1H, so the m/z value was equal to the mass of the fragment.

There are also small mass peaks above what we expect for a specific molecular weight. These are less-abundant isotopes, and modern software such as MZmine® allows for deisotoping during data processing. A raw spectrum also contains additional peaks, considered "background" due to the instrument (McLafferty & Tureček, 1993). These background ions come from compounds desorbing from the instrument's walls, which is why it is recommended to run a number of blanks to identify such peaks.

We used two different ionization strategies in this dissertation. One of the methods is called electrospray ionization (ESI). This technique was pioneered by Dole et al. For this technique, a solution of the sample is sprayed at atmospheric pressure through a several-kilovolt potential difference toward the differentially pumped entrance to the mass spectrometer (Dole, Cox, & Gieniec, 1971; Ogorzalek Loo, Udseth, & Smith, 1992). The resulting droplets are electrostatically charged, and as the solvent evaporates, the electrostatic repulsions produce smaller and smaller charges until the macromolecule is 'saturated' with charges (Fenn, Mann, Meng, Wong, & Whitehouse, 1989). The data generated from positive ESI in my work was then compared to known compounds and used to identify three of the four 1-HP compounds.

I also used tandem mass spectrometry (MS/MS) data. There are two major applications of MS/MS. One uses the first mass spectrometer (MS-I) to separate mixtures. This is followed by soft ionization to yield dissociation product ions that can be separated in MS-II. This method can then be used for structural determination (McLafferty & Bryce, 1967; McLafferty & Tureček, 1993). The second application involves hard ionization (EI)mass spectra, measuring MS-II mass spectra of fragments ions to characterize their structures (Shannon & McLafferty, 1966). This second application was more amenable to my research needs, and the data generated allowed us to identify fragments that confirmed our identification from the ESI data generated earlier.

Furthermore, MS/MS data helped identify a hexNAc unit in one of the unidentified compounds, which we could clarify further using NMR.

1.15 Dissertation Layout

This dissertation is split into two parts. The first part will outline my work on career development of postgraduate researchers. The second part will highlight my work on xenobiotic detoxification in *C. elegans*. I will conclude with comments on the future directions of these works

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PART 1

CAREER DEVELOPMENT OF POSTGRADUATE RESEARCHERS

CHAPTER 2

UNDERSTANDING THE UNIQUE FACTORS AFFECTING SOUTH ASIAN INTERNATIONAL (SAI) STUDENT TRANSITIONS INTO PHD PROGRAMS IN THE US: AN INTERPRETATIVE PHENOMENOLOGICAL ANALYSIS

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[1] Asif, M.Z., Jain, C., Dolan, E.L., Understanding The Unique Factors Affecting South Asian International (SAI) Student Transitions Into PhD Programs In The US: An Interpretative Phenomenological Analysis. Submitted to SAGE Open, expected acceptance 2023.

Foreword

Chapter 2 is reprinted with permission from Muhammad Zaka Asif, Chaitya Jain, Erin L. Dolan, Understanding the unique factors affecting South Asian international (SAI) student transitions into PhD programs in the US: An interpretative phenomenological analysis, submitted after revisions for publication in SAGE Open. The motivation behind this project was driven primarily by my interest in studying the experiences of South Asian international students. I was responsible for conceptualizing the project with guidance from Erin L. Dolan. I designed the interview questions and screening survey, developed the IRB protocol and associated materials, and invited participants to the study with support from Erin L. Dolan. I performed the interviews to collect data, and analyzed the transcripts with support from Chaitya Jain and Erin L. Dolan. I wrote the manuscript as well with input from Chaitya Jain and Erin L. Dolan, and was responsible for reviewing and editing the manuscript as well. Funds for this research were provided by the Georgia Athletic Association.

Abstract

International students comprise over 50% of the graduate student population in the life sciences in the US, over 70% of whom are Asian. Research that aims to understand international students' experiences has often treated Asian students as a monolith, discounting significant cultural and historical differences between regions in Asia that may affect students' motivations for pursuing graduate degrees, their experiences in graduate school, and their identities as scientists in training. To begin to understand the experiences of SAI students as they transition to PhD programs in the sciences, we conducted an exploratory study in which we interviewed 10 SAI students and 12 US native students during the first six months of their doctoral programs. We performed content analysis of the interview data with the aim of identifying factors that shaped students' doctoral transitions. We then selected factors that were distinctive to SAI students. Finally, we carried out interpretative phenomenological analysis to understand and describe the following factors that SAI students experienced as influencing their doctoral transitions: prior exposure to research; opportunities for networking; challenges with and affordances for acculturation; attitudes toward and understanding of mental health issues; financial affordances and constraints of pursuing a PhD, and barriers to communication. The results of this work have the potential to be useful to graduate programs seeking to ease SAI students' transition to doctoral programs.

Keywords: IPA, South Asians, International Students, Graduate education, Mental Health, Research training.

Introduction

Graduate education helps to create a highly skilled and innovative workforce to solve today's pressing issues. In recent years, the United States (US) has seen an increase in graduate enrollment, with the National Center for Education Statistics reporting 3.1 million students attending graduate school in 2019, with 33.9% of those being international students (Arbeit & Yamaner, 2021). There has been significant research on issues faced by international students, such as the stress associated with living in a new or different culture and the challenges of developing language fluency (Akram, Kamran, & Ahmad, 2020; A. Li & Gasser, 2005; S. Li & Zizzi, 2018; Ravichandran, Kretovics, Kirby, & Ghosh, 2017). Little if any of this research aims to understand how graduate students' experiences are shaped by their national or cultural origins (Alsahafi & Shin, 2017), which can lead to one-size fits all approaches to providing support. The sparse literature that does exist suggests that students from non-western countries can face more barriers when moving to the US than students from western countries (Leong, 2015).

Although individuals from across the globe seek graduate training in the US, over 65% of international graduate students are from the Asian continent (IIE Open Doors, 2021). Furthermore, South Asian students make up a sizable portion of this population, with Indian students alone making up over 17% of all international students (Arbeit & Yamaner, 2021). Prior research has explored issues experienced by Asian international students as a group; these issues include language barriers, difficulty with acculturation, and lack of social support (Bastien, Seifen-Adkins, & Johnson, 2018; A. Li & Gasser, 2005). Much of this research treats Asian international students as a monolith, despite their differing economic, social, and historical experiences (Frey & Roysircar, 2006; Rahman & Rollock, 2004).

In this study, we aim to elucidate the unique aspects of South Asian International (SAI) students' transitions to natural science doctoral programs. We have chosen to study natural science doctoral programs given that their structures are similar to each other, but different from other programs in other disciplinary domains such as humanities and social sciences. Furthermore, the demographic make-up of the graduate student population is also relatively similar and so they naturally lend themselves to being studied as a group (IIE Open Doors, 2021).

South Asia is a geographically and ethnically diverse region, encompassing modern-day Afghanistan, Pakistan, India, Nepal, Bhutan, Bangladesh, Sri Lanka, and the Maldives. However, there are commonalities that motivate our interest in considering South Asians as a group. First, the languages used in the region (i.e., Hindi and Urdu) are mutually intelligible and have similar grammatical structure, likely because they both emerged from the now defunct language Hindustani (Shackle & Snell, 1990). Second, South Asian countries have been heavily influenced by British colonialism (Bose & Jalal, 2017) and thus are home to many English speakers and similar school systems characterized by English instruction. Indeed, previous research indicates that South Asians experience fewer language issues than other Asian populations (Kainth, 2021; Lyken-Segosebe, 2017; Xiong & Yang, 2021), yet they still struggle with acculturation (Frey & Roysircar, 2006). Acculturation, in this case, is defined as "the dual process of cultural and psychological change that takes place as a result of contact between two or more cultural groups and their individual members" (Berry, 2005; R. A. Smith & Khawaja, 2011). Indeed, research suggests that SAI students experience unique issues with loss of identity due to changes to their socioeconomic standing and reduced contact with loved ones (Amin & Pant, 2018). In addition, SAI students are the least likely among international students to access

support resources (Rahman & Rollock, 2004). These results combined with low attrition rates for SAI students (Crede & Borrego, 2014; Espinosa, Turk, Taylor, & Chessman, 2021) led us to ask: what unique challenges do SAI students face in transitioning into PhD programs in the natural sciences, which may have been overlooked in the literature?

To answer this question, we studied a group of SAI students during their first six months of entering a science PhD program. We defined this as the "transition" period in which graduate students join research groups and begin the process of defining their dissertation research. Research indicates that the first several months of an international sojourner's experience in their new country and culture are crucial to how well they ultimately adapt to their new environment (Ramelli, Florack, Kosic, & Rohmann, 2013) – in this case, their graduate degree programs. There are understood to be two different forms of adaptation: psychological and sociocultural. Psychological adaptation entails personal well-being and good mental health, while sociocultural adaptation refers to the individual's social competence in managing their daily life in an intercultural setting (Berry, 2005; Berry, Phinney, Sam, & Vedder, 2006, p. 306; Pacheco, 2020). The sociocultural adaptation process is two-pronged, requiring SAI to overcome both cultural distance and logistical difficulties in order to acculturate.

Specifically, we interviewed 10 SAI students and 12 US native students during the first six months of their doctoral programs. While previous research has differed on how to define the period of transition, ranging from the period of coursework to the first year of a doctoral program (Cornwall et al., 2019; Levecque, Anseel, De Beuckelaer, Van der Heyden, & Gislef, 2017; Sverdlik & Hall, 2020), we chose to focus on the first six months as this is the period in which most students join and settle into their research groups. We had initially intended to interview participants throughout their first two years of their PhD program, which would have allowed us

to gain some empirical understanding of what should constitute a transition period. However, due to the COVID-19 pandemic, we realized that the situation had changed so dramatically that perhaps our results would not be reflective of what would be considered a "normal" transition experience. Hence, we decided to focus this study on the first six months.

Native students were included to provide insights into the factors that reflect graduate students' transition experiences more generally, thus enabling us to identify experiences unique to SAI students. For instance, prior research has shown that native students experience numerous stressors as they transition to PhD programs, including time pressure, uncertainty about doctoral processes, financial pressures, and lack of a sense of belonging in scholarly communities (Cornwall et al., 2019). Furthermore, stresses associated with doctoral students' transition experiences negatively affect students' mental health (Jackman, Jacobs, Hawkins, & Sisson, 2021). To explore whether similar or distinct factors were experienced by SAI students transitioning to science PhD programs, we first performed qualitative content analysis of the interview data. We then selected factors that were reported by SAI students alone, which we then characterized using interpretative phenomenological analysis to understand and describe the distinctive ways that SAI students experienced their doctoral transitions. To our knowledge, this is the first qualitative study focused on the transition experiences of SAI students in the US. We report our findings here.

Methods

Research Design

This study was designed to explore the factors that affect the unique lived experiences of South Asian International Students during their transition to a PhD program. We selected Interpretative Phenomenological Analysis (IPA) as our methodology in order to make meaning

of events and experiences from the perspective of the person experiencing the events. Where IPA differs from traditional phenomenology is in its idiographic focus, meaning its emphasis on the individuality of the participant, and its double hermeneutics, through which the researcher makes sense of the participant making sense of their personal and social world (J. A. Smith, 1996, 2004). Thus, IPA affords authors an active role in understanding and interpreting participants' descriptions of their lived experience. In the context of this study, IPA helps us recognize that, while we are presenting the unique experiences of SAI students, all of our findings are filtered through the lens of the experiences of the authors analyzing the transcripts.

We interviewed both native and international students because we believed the experiences of the native students would help us understand the uniqueness of SAI experiences. We realized that most of the analysis was done by foreign students and there was a potential blind spot about native student experiences due to which it would be difficult to differentiate which SAI experiences are unique to them and which of the issues are more general issues faced by the larger graduate student population. By including native student experiences, we were able to solve this potential issue in our analysis. This study was reviewed and determined to be exempt by the University's Institutional Review Board (protocol #STUDY00000979).

Participant Recruitment and Selection

We recruited respondents by emailing points-of-contact for doctoral programs in the natural sciences from five universities (3 urban, 2 rural) in different regions of the US (Northeast, Midwest, South, West Coast) that varied in their enrollment of international students, as we reasoned that those institutions with large international student populations would have more support mechanisms in place than institutions with fewer international students. We asked these individuals to distribute study information, including a screening questionnaire (see

Supplemental Material), to first-year PhD students in their programs. Participants provided written informed consent when agreeing to participate in the screening questionnaire. We also asked our respondents to share the study information with others they knew who met the selection criteria for the study (i.e., first-year doctoral student in the natural sciences).

We selected 22 respondents from seven universities (five public, two private) to interview from the 35 who completed the survey. The screening survey was designed to only allow completion by participants who were in the first six months of their doctoral program. Complete survey responses were manually inspected by the first author and participants were selected to maximize representation from different nationalities and ethnic/racial backgrounds within two groups: South Asian International and native (i.e., US born and raised) (see Table 1). Participants were selected in the order in which they responded, while accounting for their ethnic and national groups. Participants were asked to share study information with friends and classmates who fit the study criteria in order to increase the numbers of participants.

Table 1

Demographic Information of Interview Participants.

	Male	Female
South Asian International Students	**4**	**6**
Bangladesh	0	2
India	3	3
Pakistan	1	1
Native US Students	**5**	**7**
South Asian	3	2
Hispanic	0	2
White	2	3

Data Collection

We collected data using a single, 30-60 minute, in-person or video-conference interview of each respondent to gain detailed insight into their transition experiences; interviews are useful for understanding complex social processes or interactions that have yet to be uninvestigated (Fontana & Frey, 2000). We used a semi-structured approach to elicit relevant information from our respondents in a consistent manner while following the natural course of conversation and allowing for digressions when appropriate to collect additional relevant information. We constructed the questions to gain an in-depth understanding of each respondent's motivations and expectations for their doctoral programs because we expected motivations and expectations to influence students' transition experiences (see the Supplemental Material for interview

questions). For instance, we asked respondents about their research interests, how their interests developed, and why they chose to pursue a PhD. For international students, we also asked them about their decision to move to the US. We then questioned students about the positive and negatives factors they perceived as affecting their transitions. We also queried respondents about any potential effects of their transition, positive or negative, on their mental health and the extent to which socioeconomic or religious factors such as family monetary support for the former and access to religious community for the latter may have affected their transition.

We queried students on the effects to their mental health due to the mental health crisis known to exist in graduate students in the US (Allen et al., 2020; Evans, Bira, Gastelum, Weiss, & Vanderford, 2018). We wanted to see what effects the transition experience might be having on students' mental health. We queried students about their access to religious community as we recognized that most South Asian students were likely to belong to minority religions in the US and this could be a factor that affected their ability to find like-minded people to build community.

The first author conducted all interviews for consistency and to ensure that any cultural references made by South Asian students could be understood because he is a South Asian international graduate student. Each interview was then transcribed verbatim for analysis by the transcription service Rev.com. All participants chose to do their interviews in English but two of them switched to Urdu or Hindi to clarify certain ideas and those interviews were transcribed by the authors themselves. All names used here are pseudonyms chosen to reflect the ethnic origin of the respondent. All participants received a $25 gift card upon completion of the interview.

Data Analysis

We performed three rounds of analysis on the interview data to understand each participant's transition experience and identify the unique transition experiences of SAI students. For our first cycle coding, we employed in vivo coding as described by Saldaña (2013). Specifically, the first author began by carefully reading and identifying distinctive codes in the first six transcripts, creating an emergent system of codes that reflected the motivations, expectations, and factors affecting the transition of SAI students. The second author separately coded the same transcripts and generated his own system of codes. The first and second author discussed their coding of sets of 2-3 transcripts with the aim of coming to consensus and refining the codebook. After a unified codebook emerged through discussion, it was used by both coders to code all of the transcripts (i.e., structural coding). After coding each transcript separately, the first and second author discussed the coding until they reached consensus. The first author also created analytic memos, which were revised after coding each transcript to briefly define each code based on the corresponding quotes.

During our second round of analysis, we further analyzed the analytic memos to group codes into themes and identify codes unique to each of our populations: SAI and native students. We did this by performing pattern coding, a form of second cycle coding (Saldaña, 2013). For example, codes that reflected students' motivations to enter a PhD program were grouped and separated from codes that reflected students' expectations of their PhD experience. This allowed us to form distinct themes that could be analyzed further. After coding was completed, all coded segments were reviewed to ensure they related to the overarching research goal of understanding SAI students' transition experiences. For example, there was a code where a SAI student explained that her mental health had been affected by the lack of sunlight in her room. While this

was something that had only affected an SAI student in her sample, it was conceivable that this was an issue any student could have been affected by, regardless of their national origin.

We followed that by further fine-grained analysis of the quotes associated with codes unique to each population from the perspective of how the researcher understood the data according to the guidelines of IPA (J. A. Smith, 2004). This was possible since the first author was also a South Asian International graduate student who did his undergraduate in the US. His unique experience enabled him to interpret and analyze responses from both our population sets (Hechanova-Alampay, Beehr, Christiansen, & Van Horn, 2002). In conjunction with the second author, who had also lived in both South Asia and the US, the authors were able to draw on their personal experience in navigating cultural boundaries between South Asia and the US by discussing how they understood the experiences described by the respondents and find analogies with their personal experiences to be able to gain a nuanced understanding of the perspectives of the respondents in order to analyze the data. The first and second author analyzed the themes generated from the second round of coding, sorted similar themes together into "factors," and ultimately identified six factors that were unique to SAI students, which comprise the results.

Results

We identified six factors that SAI students described as influencing their transition experiences: prior experience doing research, opportunities for networking, financial affordances and constraints of pursuing a PhD, barriers to communication, attitudes toward and understanding of mental health issues, and challenges with and affordances for acculturation. Examples of how we derived these themes from the initial coding are shown in Table 2 and

detailed descriptions of each factor and how it affected SAI students as they transitioned into their PhD programs is described underneath.

Table 2

Example codes and quotes for each factor SAI students described as influencing their experiences transitioning into science PhD programs.

Factor	Code	Quote	Source*
Prior Experience Doing Research	Undergrad Research Experience	I started doing research as an undergraduate my freshman year … The graduate student who was overseeing my work gave me a lot of responsibility and I got really, really excited about the project. ... I was able to transition into leading an undergraduate team. - Allison	Native Student
	Access to facilities	This lab is really big with few people, but it's lots of facilities…I always wanted to be in a big lab with everything that I made. - Tabassum	International Student
Opportunities for Networking	Masters Experience	It was a one-year professional course. I couldn't get a lot of hands-on research experience, but I was interested in research, so I decided to pursue my PhD. - Rahini	International Student

	Proximity to Family	I was looking mostly at programs near where my fiancé was going to be. He started his PhD at [Institution Name2] which is in [City1], so I was like, "Okay, I like [State3], [State2], maybe the coast. That's kind of far… [Then] he told me that his lab was going to move to the [Institution Name3], and I was like, "Okay, I might as well look at programs down there, too." - Aadishree	Native Student
Challenges with and Affordances for Acculturation	Advisor Factors	Finding an advisor is probably one of the more stressful things of graduate school. - Sathwik	Native Student
	Discrimination	The other day I was in downtown and some... I have some scary experience because a white dude called me dirty Indian. - Usra	International Student
Financial Affordances and Constraints	Healthcare System	We have to pay like very large amount of insurance every month. Even when you have to claim your bills, it takes like million efforts of yours to claim your money back - Jhoomer	International Student
	Lab Environment	When I was working in my master's degree after I graduated for that year between PhD application cycles, they would routinely make me work 15 or 16-hour days, multiple a week, and wouldn't pay me correctly… treated me like I was an expendable worker,	Native Student

		which I was. I was doing these things because I didn't have a family to go home and feed. I was living alone. - Aadishree	
Barriers to Communication	Lack of Language Barrier	My second choice was going to Germany, but I have not had any experience of learning German language, and I did not want to invest a year or year and a half in learning German. - Rahul	International Student
	Culture of Aloofness	people [in the US], they are separated, you know, each person don't care about others. - Saleem	
Attitude Towards and Understanding of Mental Health Issues	Cultural Differences	Even if we talk with Americans, they have different interests… They have different festivals and we don't understand what they were trying to say sometimes. I really don't get most of the jokes. - Anika	International Student
	Peer Support	Older graduate students were really helpful in telling me what they experienced - Melanie	

* Note: Each theme contains a quote from a native and an international student to help compare and contrast how the two groups in our sample experienced similar issues

Prior Experience Doing Research

SAI students in our study experienced limited availability of research opportunities and research infrastructure, especially at the undergraduate level. Anila elaborated by explaining the extent of the difference in infrastructure between her home country and the US.

> *The state of education in our region is far from optimal… We have different constraints, we have limited resources, we have limited funds. The working conditions of our labs are not conducive to productivity… Even [though] I am a teacher [lecturer], sometimes I had to show only the videos to the students that this kind of apparatus is used for such work. I could not afford that apparatus to buy and show the students. But when you come here and I see that the undergrad students, they easily access all the apparatuses, [I find it amazing].*

As Anila talked about the limitations of research infrastructure between her home country and the US, she was struck by the extent to which native undergraduates had access to all of the equipment and resources to do high-level research. When asked about how she transitioned into her PhD program given her limited experience, Anila expressed surprise and delight that she had flexible access to a lab where she could gain the technical expertise necessary for success in her PhD, noting, "*They have given* [me] *the keys for the lab, I can go whenever I want to… [I have access to] Displays with the devices, … the instruments … [and] apparatuses in my spare time. So it's really a good thing.*" She further explained that this access allowed her "*the opportunities… for independent work,*" which eased her transition. For Anila and other SAI students with limited prior research experience, having additional, flexible time early on in their

degree plans – time that was not otherwise committed to seminars, assignments, or meetings – was especially important for getting up to speed technically.

In contrast, the native students in our study all had research experience prior to starting their doctoral degrees. In fact, they often cited undergraduate research experiences as important motivators for pursuing research careers and described how they benefited from the skills they developed during these experiences, as Adrianna describes here:

> *I was fortunate enough … to do undergraduate research. That's how I actually landed in [PI]'s lab, because he was one of the few molecular toxicology labs that was allowing undergraduate researchers to enter. From there I realized – the more I conducted research, I was like, "You know what? This is for me. This is what I want to do.*

As Adrianna explained, she was not necessarily considering a research career initially as an undergraduate student. The idea of prior research experience as a motivation to pursue a PhD was absent from international students' experiences because their prior research experience was much more limited. This raises a question of whether the transition experiences of SAI students should be designed to allow for more skill building and research exposure than is needed by native students with prior research experience.

Opportunities for Networking

Both SAI and native students tapped academic networks they had in place prior to starting their PhD programs in order to successfully transition. While native students were able to create this network during their undergraduate education, including their undergraduate research experiences, SAI students often, but not always, relied solely on gaining this experience by doing

a masters in the US before pursuing a PhD. SAI students in our sample focused on the benefits of a master's experience, especially in the US, for building professional networks with

potential PhD advisors, which eased their transition. SAI student, Jhoomer, explained, "*You go to conferences, you get to know other professors, they get to know you on the basis of talks you give or presentations or posters you present... That helped me a lot."* In contrast, native students relied on networks they built this network through their undergraduate education, as Sathwik explains, "*The biggest factors that have helped [are] talking to older grad students, both at [Institution Name1], as well as some of the ones from my undergrad who are ... in science graduate programs a couple years ahead of me.*"

Both SAI and native students required nonacademic support during their transition. Native students relied on family support to get settled, as Wyatt describes, "W*hen I first moved in, my dad came down and helped market scour Craigslist for furniture because this place had nothing whatsoever. I was sleeping on a yoga mat for my first week."* In contrast, SAI students often relied on academic networks such as their advisors for this support. SAI students with international advisors mentioned that their advisors helped them through transition by not just mentoring them in academics, but also helping them with non-academic matters, as Saleem describes, *"When the supervisor met me, he took me to the market, and he gave me general advice like what I should do here in the US and what should I avoid."* SAI students in our sample also relied on existing networks of other international students, especially other SAI students, to learn how to settle in to their new environment. Jhoomer described her role in such a network:

> *I know that there are a lot of students from my own [undergraduate] university who want to come here for their master's or PhD. They always contact me [asking], "What should*

we do? What's the procedure? To which professors should I apply?" I feel that if there is one person already in, he or she always leaves a path for the rest of the people.

Jhoomer clearly had a feeling of responsibility as a SAI student to be a source of guidance and support for others who come to the US for graduate school.

Challenges with and Affordances for Acculturation

The SAI students in our sample mentioned several other cultural factors that affected their transition, including culture shock, cultural expectations, discrimination, alcohol consumption, and perceived isolation from native students, all of which they felt affected their transition experience. South Asian students, both international and domestic, mentioned that they faced familial pressures due to cultural differences which also caused stress and affected their transition experience. There were also logistical issues such as access to ethnic grocery stores and access to a car that affected their experience.

SAI students reported being treated equitably by their PIs, other faculty, and institutional administration. For instance, Saleem felt both he and his international student peers were treated fairly, yet he felt like his peers who were native students tended to avoid him or not include him. Saleem had trouble adapting to the individualistic culture in the US – he felt that native students had an social obligation to reach out to international students and welcome them. He was surprised when this didn't occur, as he explains here: *In my opinion, people here, particularly those who are resident of the US or, [city] or[state]. If they have strangers [foreigners], they should get involved. Strangers [foreigners] are the newcomers in the cultural activity and social interaction.*

Saleem also struggled to pinpoint whether the lack of welcome was a general phenomenon in the US or particular to his locality. This meant he struggled to figure out whether he should be frustrated with his native peers for not making more of an effort to include him and

other international students, or whether this was a cultural difference that he needed to accept. This idea is further illustrated by Tabassum, who explains:

> *[I am in a] large cohort with 60 other students... I don't think that I'm accepted or I think they are not used to mixing with international students that much … At first I thought that something's wrong from my end because …. I'm not approachable enough or I'm not talking to people myself. Then I started doing it, but then at this age I know when people are interested in talking to me and how people are taking my conversation. I will start a conversation with someone in my class and they'll say yes or no, I will see that they are not interested in talking to me.*

Tabassum starts out by thinking that she is not being approachable enough. After making some effort, she concludes that native students who do not want to mingle with international students like her. In these instances, if these students had more prior knowledge of the idea of American individualism (Gelfand & Christakopoulou, 1999; He, Muhlert, & Elliott, 2021; Rhee, Uleman, Roman, & Lee, 1995), it might be easier for them to accept this behavior and not let it affect them as much.

Naturally, many international students felt a degree of culture shock due to the cultural differences alluded to in the previous point. While there were various aspects of American culture that stood out to different participants, every one of the participants reported negative feelings associated with the culture shock they experienced, as expressed by Anika:

And also being in a new environment and different people, ... I think it definitely affects all of the Indian ... I mean, all of the people coming to US for the first time. ... I think everyone has ... everyone will feel that culture shock and a kind of ... low feeling.

Anika identifies a low feeling associated with the culture shock she experienced. It is interesting to note that she claims that this negative feeling is something not unique to her, but something experienced by all Indian international students and even others while they acculturate to life in the US. This indicates her perception that her experience resonates with other international students she has talked to and that she has to some extent normalized her negative experience to cope with it and continue with her education.

International students in our sample reported logistical issues that further exacerbated their feelings of culture shock and homesickness. Logistical issues included not being able to access South Asian grocery stores, as Jhoomer reported when asked about issues she had faced initially upon coming to graduate school. There were also difficulties in mobility as many international students did not have cars immediately after arriving. Nevertheless, SAI students reported strong camaraderie from their compatriots and other South Asians in general which helped them overcome these logistical issues, as Anila described:

They [Pakistani community] guided me a lot. Also the other people, ... which are in my [graduate] class. Most of the people have similar culture, which I have. For example, the Nepalis, the Indians or Bangladesh[is], they have a similar culture. So they had same feelings which I had.

Anila's comments are noteworthy because they illustrate how she sought advice for issues she faced from other South Asian students and that she felt her South Asian classmates shared

similar feelings about their transition experience. This shared experience coupled with cultural similarity helps build a common ground to bring the South Asian community together in the US despite the animosity that exists between these countries on a political level.

Other cultural differences between US and South Asian affected the transition experiences of SAI students in our sample. For instance, Tabassum explains that she has noticed that in American culture people seem to want to only socialize with people when there is alcohol involved. She feels that because she does not drink, she gets excluded from social gatherings although she does not have a problem with people drinking around her. As a practicing Muslim, she believes she is perceived to be intolerant towards people drinking alcohol.

> *I think it's cultural thing. I don't like just being negative, meaning I think American people will get together just to have a drink. I will not spend my time with someone who I don't know. That's the basic thing, but I think people here doesn't mind mixing with other people [as long as] they have a drink or stuff like that. … I don't mind getting the alcohol, but that's the thing, they [native students] think that as I don't drink, I will not like to hang out with them or something that.*

Tabassum also noticed discrimination and felt like she experienced racism, as she describes below:

> *it is kind of like they [Americans] grouped people based on, I will have to say this word, based on color or how they are speaking, depending on their accent. I think that it's based on appearance because they don't know, not every one of them know that I am from Bangladesh, not every one of them know that I am Muslim, I don't drink or stuff like*

that. They're just not interested in doing this stuff they do all together. So I would say it's based on appearance.

This combination of experiences affected her transition, as she describes here: *"It's not allowing me to live like I wanted to live here. I think being in a grad school you need to have friends. You need to have a network that can help you."* Similar to other SAI students in our sample, Tabassum recognizes that building a community is essential for a successful transition, but she feels like, due to her background, she has not been able to build that community with her peers in her program. While she has been able to build community with compatriots, she is the only person from her country in her program and so she feels she needs to build relationships with natives for success and she has struggled with that.

These acculturation challenges were exacerbated SAI students' feelings of pressure, as described by Usra here: *"if I don't get a result, … my fund will be canceled and I have to go home. This is a huge pressure."* The SAI students in our sample were coming from institutions where they didn't have the sort of opportunities that are available in US institutions, so many of them perceived their graduate experiences in the US as their only chance for education and the only path to successful careers.

Financial Affordances and Constraints of Pursuing a PhD

Financial affordances and constraints affected students' transitions in multiple ways. Two main issues arose for SAI students: their lack of awareness of costs and their ineligibility for certain types of funding. Regarding costs, graduate students often have to pay fees that are not clearly disclosed prior to enrollment. SAI students were unaware of these fees and thus had not budgeted for them. SAI students also were unaware of the expenses associated with the American healthcare system and these expenses increased stress during the transition.

Specifically, SAI students struggled with the costs of healthcare in the US and found the US health system hard to navigate, as explained by Usra:

I … [got] sick here … [I had] medical [expenses]. Even the university health insurance cost a lot … The doctor gave me some tests and I … [turned out to be] autoimmune positive. So that doctor … suggest[ed] [a doctor outside the health center] and the

> *outside doctor charged me a huge bill. It is very different… in India or Bangladesh it is not so costly.*

For native students, the cost of the healthcare system was more familiar, including differences in costs depending on which facilities or physicians are used. While some native students in our sample mentioned that they were struggling with the exorbitant costs of student health insurance, they at least expressed awareness this issue might arise. For SAI students, the cost was not only difficult to afford, but also they were unaware that healthcare would be costly and that cost could differ depending on the source of the care.

SAI students also realized early in their transition they were ineligible for many types of grant or other financial support because of their immigration status, which they felt was unfair. For instance, Aakash explained that *"international students do not get any travel awards. I find it kind of unfair."* Other SAI students in our sample noted ineligibility for assistantships or fellowships offered by the federal government, which are only available to US citizens or permanent residents. This was problematic in two ways. First, SAI students had more limited options for financial support, which exacerbated their financial stress. Second, their sense of unfairness led to feelings of discrimination, which made their transition more challenging. Native students also noted financial factors in their decisions to pursue PhDs, but these differed from the factors noted by SAI students. For instance, native students appreciated that they would

get paid to go to school if they opted to pursue a PhD. Melanie explained, *"I decided on a PhD. It began as a financial decision, honestly. I [knew] I can't pay for a master's."* Other native students put off pursuing a PhD because of debt they had incurred as student loans from their undergraduate education. For example, Adrianna described how she *"did not start graduate school immediately after my undergraduate education… I'm a first-generation student and I had a lot of education expenses, I [had] to take out loans, and I had to pay off those loans."* None of the SAI students in our sample had been delayed in their graduate education due to student loans. Although Adriana felt this delay made her lose momentum in terms of taking classes and studying, which she felt hindered her transition, she used this time to work industry in multiple fields, which allowed her to gain exposure to different areas of research and hone her research interests and technical skills. The SAI students in our sample did not have this experience, and thus did not experience the associated benefits or challenges during their transition.

Barriers to Communication

The SAI students in our sample explained that one of the reasons they chose to pursue a PhD in the US was because it was an English-speaking country. Yet, they reported difficulties in communication due to their unfamiliarity with American accents as well as accents of other international students and faculty, which affected their transition. For instance, Anila described that she was fluent in English, but this "*kind of English was not the English which I learned in my whole life."* This led to stress as she was never really sure if she had misunderstood what her peers and faculty were telling her or if they would understand how she described issues she might be facing if she reached out to them.

Attitude Towards and Understanding of Mental Health Issues

Although both SAI and native students in our sample mentioned mental health issues that affected their transition experience, SAI described and responded to these issues differently. SAI students described all of their mental health issues generically as "stress," as Rahini describes here:

> *I think it [grad school] did affect my mental health when I was in [University 1] … Because of how my PI [behaved] and because of the weather also. Especially in the winters because it is like a very small town and there's like absolutely no one to talk to because most of the grad students are stressed. … and it's difficult to make friends.*

Native students tended to have a richer vocabulary when describing mental health issues, drawing on terms such as anxiety, burnout, cognitive dissonance, uncertainty, and stress. It was unclear whether SAI students simply had a more limited vocabulary for describing mental health issues they faced or whether they were uncomfortable opening up about their specific mental health challenges.

Only one of the SAI students in our sample sought resources for mental health (Usra), and she felt there was a cultural gap between her and her therapists, as she described here: *I also visited the university health center and because, because of the culture difference, I don't think white [American] people can understand my psychological problems.* While Usra perceived the therapist lacked cultural competence needed to provide support, it is also possible she was unable to explain why whatever issues she faced were affecting her as they were, as she admitted that she did have some trouble communicating in English.

Limitations

Due to the nature of this study's design, we did not have a large enough sample size to be able to generalize our findings to all SAI students. While this study does get at many different aspects of SAI experiences, there may yet be other factors that affect their transition experience that were not reported by our participants. Furthermore, the study was not designed to offer insights into the prevalence of the issues identified here are among the larger SAI student community in the US. Prevalence could be examined by surveying a larger and more diverse sample of SAI students in science PhD programs.

Interpretive phenomenological analysis affords insights into participants' lived experiences as understood by the authors (Emery & Anderman, 2020). Thus, other authors carrying out a similar study might have interpreted the data differently. To account for this limitation, we involved multiple South Asian authors in the study and we made transparent our own national, ethnic, and science PhD experiences. This methodology is more grounded in nature, thus limiting potential for theoretical connections. Our findings indicate that theories related to acculturation, communication, motivation, social capital, and social networking could be useful for further understanding the transition experiences of SAI students into science PhD programs. For instance, expectancy-value-cost model of motivation could be useful for understanding how SAI students weigh the benefits (e.g., financial affordances, access to facilities) and costs (e.g., financial constraints, distance from family) of continuing in and completing a science PhD in the US (Barron & Hulleman, 2015). Social capital or social network theories could be used to identify the social resources that facilitate SAI students' successful transitions into science PhD programs, or to compare the networks that native vs. SAI students tap as they transition into science PhD programs (Burt, 2000). It would also be interesting to

understand how the acculturation of SAI students, as partially described in this paper, may vary from other SAI immigrants or international students from other regions.

Language may be another factor that limited our results. All SAI students in our sample were non-native English speakers; it is possible that they were not able to convey all aspects of their experience. Although we offered to interview in Urdu and Hindi, for many SAI students, these languages are their second or third language. We were not able to interview the students in their first language and so that affects how much of their experience was actually relayed to us in this study. Future studies focused on people from specific regions within South Asia where those languages are spoken and with authors who speak the language of the participants could help solve that. Even then, it would be very hard to find one set of authors who would be fluent in all the languages that participants may speak. Something more feasible would be to work with people who could translate for specific participants who might have more of a language issue.

Finally, our original intention was to follow up with our participants after the initial six-month period, as noted above, but were unable to do so because COVID caused significant shutdowns and people could no longer have a "typical" transition experience. A longitudinal study designed to follow participants through the course of their PhD might help just understand which of the issues identified in this study persisted beyond the initial transition experience, and which of the issues faded away with time. Unfortunately, the time during which this study was carried out would have meant that if we had followed our students, the data we would get would not be representative of an average student's experience because of the special situations around the early stages of the Pandemic.

Discussion

Collectively, our results indicate that SAI students experience unique challenges during their transition to graduate education in STEM. These challenges are influenced by their prior educational experiences, their status as international students, and cultural differences between South Asia and the US. Through the interpretative process, we generated a conceptual model of the factors SAI students indicated as affecting their transitions, depicted in figure 1.

The SAI students in our sample had minimal research experience and often had not worked with the tools and resources that were readily available to native students in our sample. In addition, SAI students also differed in the networks they tapped to facilitate their transition, relying on advisors and peers at their new institution, especially other SAI students, because they often had no local family networks. In figure 1, we conceptualized these factors as antecedents because they preceded the start of SAI students' PhD experiences. Students with more research experiences felt they were more adept at performing research tasks and had larger supports network to help them with their research, which allowed them to settle in to their doctoral programs more easily. To address these issues, graduate programs could consider establishing summer bridge programs, which have been shown to support students in transitioning successfully into novel academic environments (Ghazzawi, Pattison, & Horn, 2021). For instance, international students could arrive during the summer preceding the start of their doctoral training to become familiar with their new environments and begin to develop knowledge and skills to be successful in doctoral training. Studies of bridge programs for historically minoritized communities in the United States show participants grow in their research self-efficacy and in their research productivity compared with similar students who

matriculate directly into PhD programs (Born & Brock, 2022; Rudolph, Holley-Bockelmann, & Posselt, 2019).

Because of communication barriers and cultural differences, SAI students relied on other SAI students for emotional support (see Transition Experience in figure 1). They were also heavily dependent on their advisors and other faculty for academic support, guidance, and networking as they were newcomers to the academic community in the US. Institutions could support SAI students in identifying such support by establishing mentoring programs that pair more experienced SAI students with new students going through the transition process. Indeed, near-peer mentoring programs have been shown to improve sociocultural adaptation and reduce acculturative stress amongst international students in pilot programs implemented in Canada (Thomson & Esses, 2016).

The SAI students in our study encountered both logistical and cultural differences that made acculturation difficult (see Transition Experience in figure 1). The logistical issues were exacerbated by the fact that, due to the differences in purchasing power between South Asia and the US, even students who had savings realized that their funds did not amount to much and they did not have economic support from their families back home. Cultural issues included discrimination and isolation, as well as preconceived notions about their religious identity. Language as well as financial affordances and constraints also affected transition experiences (see Transition Experience in figure 1). While higher education in South Asia is mostly in English, participants in our study felt the way English is learned and spoken in their home countries was very different from how people speak in the US. Indeed, there is an argument to be made that South Asian English is its own dialect, separate from the English spoken in other English-speaking countries (Gargesh, 2020). As depicted in figure 1, we considered barriers to

communication and financial affordances and constraints as factors students experienced during their transition rather than being shaped by their own prior experiences because they were beyond the students' control and because different students experienced these factors differently depending on their experience during the transition.

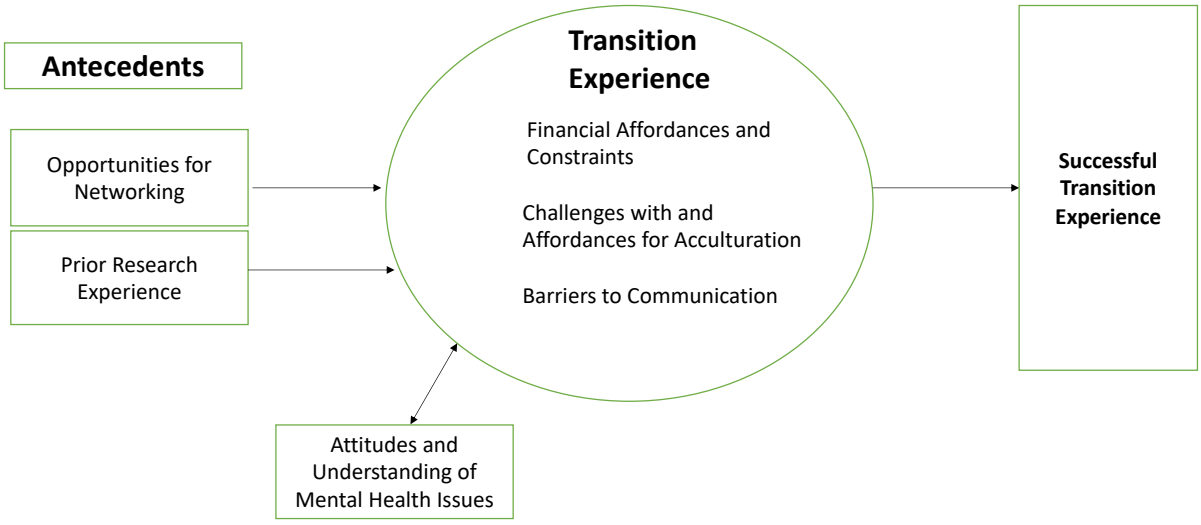
The difference is perhaps more pronounced for SAI students then speakers from most other English-speaking countries as although they do speak English, English is not the native language in any part of South Asia and so often they would use words from their native language to fill in gaps in vocabulary which they are unable to do when speaking to native English speakers, making it hard for them to communicate. At the same time, financial affordances in the form of a stipend and tuition waiver made it so that students could afford to study here without loans actually making the transition experience easier for SAI students.

The final factor that affected SAI students' transition was their attitude toward and understanding of mental health issues. Maintaining mental health is a documented challenge for graduate students, especially international graduate students of color (Anandavalli, Borders, & Kniffin, 2021). Discussing mental health issues and seeking help to maintain mental health is rarer in South Asian cultures, where individuals experiencing mental health issues are likely to hide it (Arafat et al., 2022; Marrow & Luhrmann, 2012). In figure 1, we describe attitudes toward mental health as preceding the transition experience and also being exacerbated by it. For instance, SAI students seemed hesitant to elaborate on mental health issues they may have faced, often describing mental health challenges using the more generic idea of "stress." This inability or unwillingness to talk about mental health appeared to make some students' transition experiences more difficult. In the few instances where a participant spoke about mental health, they suggested that there was a lack of culturally competent therapists that prevented them

getting the help they needed. This was a factor that students faced during their transition rather than because of any preceding notions about mental health. Institutions could help promote conversations around mental health issues among SAI student communities by having regular seminars with culturally competent therapists and providing access to creative mental health solutions, such as online therapy.

Figure 1

Conceptual Framework Describing Factors That Lead to a Successful Transition



Note: Students come in with prior research experiences that provide technical know-how which helps them transitions and networks that support them during their transition based on the opportunities they have had to network before entering their doctoral program. These factors combine with financial affordances and constraints they face during their graduate experience, challenges and affordances with relation to acculturating to graduate school and the US, and barriers that inhibit communication between them and their peers and faculty to make their transition experience. Their transition experience is also affected by their attitude and understanding of mental health issues coming into the program. At the same time, the nature of their transition experience can affect those very same attitudes as well. All of these factors combine to create a successful transition experience.

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CHAPTER 3

POSTGRADUATE PERSPECTIVES ON MENTORING UNDERGRADUATE RESEARCHERS FOR TALENT DEVELOPMENT

[1]Asif, M.Z.; Edison, A.S.; Dolan, E.L. *NY Annals of the Sciences* **2023**. Reprinted here with permission from the publisher

Foreword

Chapter 3 is reprinted with permission from Muhammad Zaka Asif, Arthur S. Edison, Erin L. Dolan, Postgraduate perspectives on mentoring undergraduate researchers for talent development, *NY Annals of the Sciences,* 2023. Available at https://doi.org/10.1111/nyas.14966. The motivation for this project came from my work with the Vertically Integrated Projects (VIP) team in the Edison lab and a dataset collected earlier by Dr. Lisa Limeri for a project on which I collaborated (Limeri et al., 2019). In the research presented here, I was responsible for designing the study along with Erin L. Dolan. I collated the data and carried out data analysis along with Erin L. Dolan. I worked alongside Arthur S. Edison to implement the factors identified in this study in the VIP team. I co-wrote the initial draft with Erin L. Dolan and it was reviewed and edited by all authors. Funding for this research came from the Georgia Athletic Association and the Georgia Research Alliance.

Abstract

Undergraduate Research Experiences (UREs) are critical for talent development of the STEM research workforce, and research mentors play an influential role in this process. Given the many life science majors seeking research experiences at universities, graduate and postdoctoral researchers (i.e., postgraduates) provide much of the daily mentoring of undergraduate researchers. Yet, there remains little research on how postgraduates contribute to talent development amongst undergraduate researchers. To begin to address this knowledge gap, we conducted an exploratory study of the experiences of 32 postgraduates who mentored life science undergraduate researchers. We identified four factors that they perceived as enabling undergraduate researcher talent development: undergraduate researcher characteristics, research project characteristics, and mentoring implementation as well as outcomes for both the postgraduate and undergraduate. We then describe a team-based approach to postgraduate mentoring of undergraduate researchers that attends to these factors to provide an example that practitioners can adapt or adopt for their own research groups.

Keywords: Undergraduate research, Talent development, Postgraduate, Mentorship, *Caenorhabditis elegans*

Introduction

There is an increasing emphasis on involving undergraduates in research to support their talent development and career decision-making and foster their persistence in STEM careers.[1, 2] Undergraduate research experiences (UREs) are typically structured as apprenticeships with more experienced researchers, who provide mentorship that shapes undergraduates' personal and professional growth.[3-5] Research on talent development indicates that the functions of mentors depend on the developmental stage of the student mentee.[6] At the undergraduate level, mentors can function as role models to their mentees and provide insider knowledge and connections to information and resources that support undergraduates in developing their research skills .

According to Gagné, talent development is defined as the outstanding mastery of systematically developed competencies in any occupation, which places the person among top 10% of their peers.[7] The Talent Development in Achievement Domains (TAD) framework includes four successive levels of talent development, three of which involve mentorship.[8] First is aptitude, which represents the psychological and dispositional factors that predict an individual's potential for development and future performance. Second is competence, which refers to systemically developed knowledge, skills, and abilities that enable an individual to act effectively. Mentors play a role in this level by helping their mentees gain access to resources that they can use to develop their knowledge, skills, and abilities. Third is expertise, which refers to consistent superior achievement. Fourth is transformational achievement, which refers to accomplishments that involve generating creative responses by drawing on multiple domains of expertise.[8] UREs can play an important role as contexts for achieving these levels of talent development, providing a space for mentors to nurture talent. In addition, prior research indicates

that postgraduate mentoring supports undergraduate researchers' growth in their abilities to think and work like a scientist, which reflects progress toward transformational achievement.[3, 4]

Although there is professional development available for postgraduates to develop their mentoring competence, most postgraduates are not required to complete formal mentoring training.[9-11] Thus, postgraduates' perceptions of successful undergraduate mentoring relationships may be the most influential factor in how they go about developing undergraduate researcher talent. In this study, we sought to characterize postgraduates' perceptions of successful undergraduate research mentoring relationships to understand how they might enhance or inhibit undergraduate talent development. We collected and qualitatively analyzed interview data from 32 postgraduates on their definitions of successful undergraduate research mentoring relationships and the elements necessary for creating such a relationship. We then interpret and discuss the alignment of these elements with factors known to support or constrain undergraduate talent development. We conclude by illustrating how to develop talent of a larger and more diverse group of undergraduate researchers using a team-based approach, which has developed recently as an alternative to the apprenticeship structure and is understudied as an approach.[12]

Methods

This research was designed to achieve two goals: (1) identify postgraduate perceptions of the characteristics of successful undergraduate research mentorship, and (2) apply these characteristics in the context of a mentored undergraduate research team. The study was reviewed and determined to be exempt by the University of Georgia Institutional Review Board (IRB determination #STUDY00005150).

Participants and Data Collection

This study is part of a larger interview study on postgraduate mentorship. Our recruitment, selection, and data collection methods are detailed elsewhere and described briefly here as context for the current study.[13] We recruited participants by emailing invitations to postgraduates listed on public websites of life science departments in U.S. research universities to identify postgraduates likely to be mentoring undergraduates in research in various subdisciplines (i.e., bench or wet lab, field, and computational or theoretical research). We also used snowball recruiting by asking participants to share information about the study with fellow postgraduates. We did not collect demographic information from our participants, but approximately half of our participants presented as women (18 apparent women, 14 apparent men). Most participants were graduate students (25 graduate students, seven postdoctoral associates). Participants spanned 10 different public and private research universities across the U.S., and five noted that they were international students during their interviews.

For this study, we focused on postgraduates' responses to the following interview questions: (1) What were your reasons for working for an undergraduate researcher? (2) Describe a typical work week with undergraduate researcher. (3) What did you do to help the undergraduate researcher? (4) What did the undergraduate researcher do to help you? (5) How, if at all, was working with the undergraduate researcher a hindrance to you? (6) How, if at all, was working with the undergraduate researcher beneficial to you? Interviews were conducted using a semi-structured format, meaning questions were asked in a similar order while allowing for the natural flow of the conversation and follow-up, unscripted questions to clarify the participants thoughts and experiences as needed. Here we focus on reporting results related to postgraduates' ideas of what constitutes a successful mentoring relationship.

Data Analysis

All interviews were transcribed verbatim and analyzed inductively using qualitative content analysis with the aim of identifying and characterizing the variety of factors postgraduates viewed as important to a successful undergraduate research mentoring experience.[14] The first author MZA initiated the analytic process by carrying out descriptive open coding. He read through all of the responses to interview question 3 and drafted codes to capture the main ideas in the postgraduates' responses. Then MZA carried out axial coding, re-reading all of the coded segments and categorizing them based on similarity of the ideas. For instance, postgraduates commented on how undergraduate researchers' interests, including whether they were interested in the particular project, what their academic interests were, and what their career interests were. MZA grouped these codes into a larger category of undergraduate interests. Then, last author ELD reviewed the categories and proposed four larger themes that represented the relationships among and distinctions between categories while representing the full scope of the data. These four themes and illustrative quotes are the main findings of this study.

Results and Discussion

Based on our interviews, we found that postgraduates had certain factors that they used to judge whether they had a successful undergraduate research mentoring relationship. These factors fit four overarching themes: (1) undergraduate researcher characteristics, (2) research project characteristics, (3) implementation of the mentored research experience, and (4) outcomes of the research experience. We describe and discuss each of these themes along with

the specific factors that postgraduates emphasized for each. We also discuss how these factors relate to those known to afford or constrain undergraduate researcher development.

Undergraduate Researcher Characteristics

Postgraduates in our study described a number of characteristics they valued in an undergraduate researcher, which is consistent with the first – or aptitude – level of the Talent Development in Achievement Domains (TAD) framework. The aptitudes that were most valued by postgraduates were more dispositional in nature. For instance, postgraduates emphasized the importance of undergraduates being interested in the research, rather than conducting research simply because it was a degree requirement or necessary for admissions to professional schools. An undergraduate's interest, engagement, and dependability mattered more than indicators of achievement, such as grades. One postgraduate remarked that they were successful in graduate school even though their own undergraduate grades had not been stellar. Instead, postgraduates gauged interest and engagement based on the questions undergraduates asked. These findings indicate postgraduates may be more inclusive in their development of undergraduate researcher talent by devaluing grades or test scores that are not predictive of success in research.[15] Yet, postgraduates might also not hamper undergraduate talent development by failing to recognize that undergraduates with different backgrounds and experiences may approach their engagement in research differently.[16]

Multiple postgraduates emphasized the importance of undergraduates having potential to get work done, either because they had a "work ethic" or they were committed to doing research for multiple terms as noted by this postgraduate:

> *The obvious benefit of having an undergrad… who has worked with you for a considerable amount of time is that they actually get work done… my PI and I ask*

them, "Do you plan to do it for one semester or more than that?" We prefer those who at least plan. They might change their plan, but at least in their plan they think of doing it more than a semester. As I said earlier, all three of mine, they stayed at least for two or three semesters. All three of them contributed in my research. That's why two of them are on my paper.

In addition, postgraduates sought undergraduate researchers they could come to trust because they were reliable – showing up on time and getting the planned work done. Postgraduates described trusting their undergraduate mentees not only because of their work ethic, but also because they were conscientious with good attention to detail, and thus trustworthy for carrying out protocols and making reagents accurately.

While some postgraduates were wary about mentoring undergraduates with healthcare career aspirations, others remarked that career interests didn't matter as long as the undergraduate was committed to being engaged and making progress in research, as this postgraduate explained:

My second student was very upfront that she was super interested in all of this, and she ended up going to med school… She was super upfront that, "I have a lot of stressful classes that I'm taking, and there's gonna be times where it's stressful, but when my other class-load isn't so crazy, I'm gonna dedicate as much as I can to lab", and it worked out great… She's a co-author on a paper that we had.

Postgraduates also described characteristics of undergraduate researchers' circumstances that could enhance or constrain their talent development. For instance, postgraduates noted that undergraduates who could commit to doing research for multiple terms would have time and experience to become more competent (i.e., second level of the TAD framework), and be better

positioned to make research contributions. Several postgraduates had expectations that undergraduates would begin their research experiences with some baseline level of technical and professional competence, such as being able to pipet accurately and reliably and being able to write abstracts, solve problems, and think critically. For one postgraduate in a computational lab, they expected undergraduates to start with some coding experience, and even asked for sample code before agreeing to mentor them. These postgraduates may be hindering talent development by assuming current competence of an undergraduate researcher is an indicator of their potential rather than their prior preparation.

Notably, postgraduates also described the importance of good interpersonal fit with their undergraduate mentee. This appeared to matter most to postgraduates who anticipated needing to work closely side-by-side with their mentee, as noted by this postgraduate:

> *When we're in the field, it's an unusual situation. You're around someone all the time. Not only are you working with them, but you're living with them, and so part of it is that you want your personalities to mesh well together.*

This finding indicates that the TAD framework might benefit from explicit attention to interpersonal match between mentors and mentees because mentors who perceive their mentees as a similar to them may provide more guidance and resources that support their mentees' competence development. Indeed, prior research has shown the importance of deep-level similarity, meaning shared values and culture, for promoting high quality STEM mentoring relationships.[17-19]

Research project characteristics

Postgraduates described the importance of selecting research projects that suited the undergraduate's skill level and background knowledge (i.e., current competence), as well as the

available time, including availability on a weekly basis, the duration of the time that the undergraduate anticipated spending on the research (e.g., one summer, two semesters, etc.), and the time the postgraduate could dedicate to mentoring. Other postgraduates noted the value of starting all undergraduate researchers on a particular type of project. They valued such projects as contexts for undergraduates to develop confidence, learn skills, and demonstrate desired competencies before taking on other research, as this postgraduate explains: *I definitely introduce undergraduates in the lab to experiments that play to their strengths. Then we transition into things that are outside their comfort zone more.* These approaches are consistent with theories of motivation that indicate people are more motivated when they already feel competent or have the potential to be so.[20, 21] Other postgraduates selected projects by first learning about the undergraduate and their interests and aspirations and then selecting a project that fit. One postgraduate described re-designing a project midstream to better fit the students' interests and thus be more motivating. This perception is consistent with the notion of intrinsic motivation – namely that individuals are more motivated to engage in a task when they find the task interesting to them.[22]

Other postgraduates approached selecting research projects in ways that run counter to research on learning and development. For instance, some postgraduates selected projects for which was feasible to generate results because, as one postgraduate remarked, "*I'd never want them to fail*." This perception contradicts at least one study that indicated experiencing research failure does not prevent realization of the benefits of undergraduate research.[23] Another postgraduate described selecting research projects that served more of a gatekeeping function, explaining:

We start all undergrads on [a particular type of project], because it is both boring and annoyingly difficult. [To be successful] you have to have repeatable skills with pipettes and good hands, and good calculation skills. Not the most difficult thing in the lab, but it's difficult in the worst possible way… We always have them start on that so that we can gauge, are they going to be any good in the lab?

This gatekeeping behavior can be experienced negatively by mentees, prompting them to consider educational and career pursuits outside of research.[24]

Multiple postgraduates also considered the cost of the research materials undergraduates would be using. These postgraduates noted that undergraduates were learning and that it was important to provide an experimental space for them to learn and develop their skills before conducting techniques or experiments that required the use of fragile or sensitive equipment and expensive or limited reagents. This tailored, cost-mindful approach to research project selection may be another way to prompt undergraduate motivation and talent development by accounting for both benefits (e.g., undergraduate competence development) and costs (e.g., financial constraints of doing the work).[25]

Implementation characteristics

Postgraduates described ways they carried out their mentorship of undergraduate researchers to develop their competence and expertise. Specifically, postgraduates worked to set and adjust expectations over time, maintain open lines of communication, provide both technical and psychosocial support, and set boundaries to protect themselves and foster their mentees' independence over time. These approaches to implementation are consistent with research on effective research mentoring.[11, 26, 27]

Multiple postgraduates emphasized the importance of clearly communicating expectations from the outset for the undergraduate to become more technically competent and integrated into the research group. Postgraduates described articulating expectations regarding when, where, and how to communicate (e.g., email, in person, how regularly) and how to carry out lab work (e.g., showing up on time, taking care and being attentive in research, if/when/how to seek help). Some postgraduates documented expectations in writing the form of a mentoring agreement or compact. Others were proactive in communicating about when expectations were not being met before they became frustrated. Postgraduates explained that setting and aligning expectations over time required maintaining open lines of communication. Communicating openly allowed postgraduates to make sure they were tailoring the experience to meet their mentees' needs and interests, and also to make mid-course corrections when things were not going as planned.

Postgraduates explained that they made special efforts to ensure undergraduate mentees had the support they needed, including experiencing the research group as a welcoming environment and building their confidence especially when the research is not progressing as anticipated (i.e., psychosocial support). Postgraduates also emphasized their role in helping undergraduate mentees develop technical competence so they could operate more independently and add value to the research. Both forms of support are thought to be critical to effective mentorship.[28, 29] Postgraduates described providing enough independence for undergraduates to take responsibility, make mistakes, and learn from them, along with providing sufficient structure by setting clear expectations, articulating milestones, and making adjustments as needed. One postgraduate who had two research advisors (i.e., principal investigators or PIs)

explained that they sought advice from both to improve their mentoring approach and better develop undergraduate researcher talent:

> *I have found conversations with my two different PIs to be constructive. Both have encouraged me to be less stressed about carefully sculpting each student I work with and instead to let the mentoring relationship develop a bit more organically, in response to the interests and needs of the student. Both PIs have also helped me to deal with the problem of undergrads often doing mundane, repetitive tasks instead of independent science. I worry a lot about this, but one encouraged me to try to stop the worrying - a lot of aspects of science are boring, and students should be exposed to that. The other has helped me to think of ways to make the process of doing mundane, repetitive tasks a positive learning experience for students and to identify students who are ready to "graduate" to more independent tasks and assist them in doing so.*

While some postgraduates made a point of seeking feedback on their mentoring as they were learning, other postgraduates described improving their mentoring through trial and error, reflecting on their own experiences and reminding themselves that:

> *students are beginners, they don't understand the systems as well as I do….*
> *(being) strict when I need to be strict, but give them a little bit of time to learn and guide them through that process of learning, which is not easy. I go back to my undergraduate, I didn't know anything… I have to put myself in their shoes, have patience, and work with them on a one-on-one basis*.

Although the postgraduates in our study described the need to maintain open lines of communication, they balanced this with the notion of maintaining boundaries. By setting

boundaries regarding when, where, and how an undergraduate researcher could seek help, postgraduate mentors felt they were better able to protect their own time, get their own work done, and foster their mentees' independence. For instance, one postgraduate explained:

> *If I'm working on the weekend, it's because I have way too much work and I can barely come above water. I need to have some dedicated time where that work is all for my high-priority work and not for my students… It's really hard to be totally on call to three people all the time, and I think that it's reasonable to say, "I will always be available to you during the workday, but outside of that…You should learn to solve problems on your own too."*

Indeed, postgraduates aimed to develop undergraduates' technical competence and capacity to work independently so that they could make research contributions, as this postgraduate explains:

> *Depending on the undergrad, it's a time sink. Especially in the beginning. It takes longer to do things if you're teaching someone instead of just doing it (yourself). That balances out differently depending on the undergrad and how quickly they take off. It's trying to get them independent quickly enough that the balance comes out positive.*

Postgraduate and Undergraduate Outcomes

Consistent with the TAD framework, postgraduates viewed a mentored research experience as successful if undergraduate mentees gained knowledge and developed technical and thinking skills. One postgraduate emphasized that undergraduate researchers should develop knowledge about what they were doing in research and why they were doing it. Postgraduates described skill development as valuable both in the undergraduate's current research and in their

future endeavors. Some postgraduates recognized that undergraduates' talent development needed to apply beyond particular career paths, given that many life science undergraduate researchers had career aspirations outside of research careers. One avenue for accomplishing this was to focus on developing thinking skills, as this postgraduate explained:

> *Nobody's ever asked them to think before. Nobody's ever asked them to synthesize. Nobody's ever asked them to produce something, without a bullet list of instructions. [There is this] transition between the expectation of regurgitation, transitioning into the expectation of producing and creating.*

The postgraduates in our study whose undergraduates successfully made this transition from "regurgitating" to "producing and creating" reported that they were able to accomplish more or different research than they would have accomplished working alone. In some instances, this resulted in publications that included undergraduates as coauthors. The postgraduates who had these experiences described them as a stepping stone to expertise development through pursuit of further education and research training, as this postgraduate described:

> *The first [undergraduate] I mentioned, we wrote a publication together. I didn't think about it at the time, but it apparently helped her get into grad school to have had the publication, and let her pick the lab she wanted. I wrote a paper with another student, and then he got into a grad program at [university]. It was something that he was really proud of, and so it really made me think about things differently too, in terms of what the science output could be.*

Postgraduates aimed for the URE to be worthwhile not only for their mentees but also for themselves, as one postgraduate described, *"day one I tell them usually this is going to be a symbiotic relationship. We're going to mutually benefit from each other, that's my goal in the*

end." Yet, several postgraduates noted that this potential was never realized for them because undergraduates fell short of reaching the desired level of competence or independence, as this postgraduate explained:

> *[the undergraduate] took a lot of my time as a mentor, and then I never got anything out of it. I was really frustrated by it. It would have been nice to have help with my project… It's like sort of a fake incentive to graduate students to mentor undergrads, right? They want undergrads because they want help, but then you put a ton of work into it, and then you don't get help.*

Application of Factors in a Vertically Integrated Project Team

Next, we describe the adaptation of a team-based approach to undergraduate research by postgraduate MZA and faculty member ASE to illustrate how a postgraduate mentor can feasibly attend to undergraduate researcher characteristics, research project characteristics, implementation, and outcomes to support more inclusive undergraduate researcher talent development. Typically, UREs select for students who are already inclined to pursue and persist science careers; this was evident in some of the ideas expressed by postgraduates regarding what makes for successful undergraduate research mentoring, such as their incoming level of competence.[1] The team-based approach, known as a Vertically Integrated Project, is distinctive because it makes use of open enrollment rather than selection based on achievement metrics such as GPAs or test scores.[30, 31] We aimed for our team to involve 12-15 students per semester, expanding the potential talent pool beyond what is possible through traditional one-on-one apprenticeships. We used open recruitment, inviting students to join the team as long as they were interested with no regard for their GPAs or specific career interests. We also encouraged team members to invite their peers to join the team, which avoided limiting involvement to

undergraduates who had insider knowledge about how to get access to research experiences.[16] We actively recruited students through programs that reached undergraduates from marginalized and minorities programs, further diversifying the team membership in terms and race and ethnicity. Consistent with the VIP model, team members earned small amounts of credit, which offers greater latitude to fit the schedules of undergraduates who may have other obligations (e.g., jobs, family commitments). Team members continued for multiple terms, earning credits that count toward their degree, helping to train new team members, and ensuring research continuity over time.

We selected a research project that provided multiple research directions to allow undergraduates to pursue the work that best fit their interests, facilitated undergraduates' development of technical competence, and fit the typical constraints of an undergraduates' academic schedule. Specifically, our team carried out research to understand mechanisms of chemical detoxification in the model organism, *Caenorhabditis elegans*. This organism is ideal for undergraduate investigation because it is simple and low-cost to maintain, it is widely studied and thus has a wealth of experimental resources and information that can inform investigations, and it does not require specialized animal approval or training.[32-34] We organized the team into 3-5 student sub-teams, each of which focused on a particular methodology. Students could choose their sub-team and switch projects to meet their interests. The sub-teams further diversified the VIP team by enabling research in areas including laboratory benchtop experiments, analytical chemistry, computational bioinformatics, and field collections. This structure brings together students with a variety of majors and interests. MZA was responsible for primary mentoring and organizing student involvement.

We employed several implementation strategies to set and adjust expectations over time, maintain open lines of communication, provide both technical and psychosocial support, and set boundaries to protect MZA's time and foster team members' independence. We established a common training regime for each sub-team so that they could develop technical competence with relatively modest guidance from MZA. Other postgraduates assisted by providing technical guidance as needed and experienced undergraduates helped to train new team members. We met weekly in sub-teams and as a whole team with ASE to discuss weekly progress, set weekly goals, and shift responsibilities and workload as needed to accommodate students' academic and personal demands. These meetings also served as a forum for undergraduates to develop their communication and presentation skills, ask questions, and clarify confusions while allowing MZA to protect his time. We also made use of the group messaging platform, Slack™, as a safe, or low stakes, environment for students to ask questions, communicate about issues, and get advice on problems. Slack™ has several features that made it especially useful for our team: channels can be used to organize information by topic and enable team members to help each other (i.e., not just one-on-one messaging), previous posts are visible to new members, various resources can be shared (e.g., journal articles, protocols, comments, photos), and posts can be made and accessed at any time (i.e., not just synchronously).

Our VIP team experienced multiple positive outcomes. First, the team was able to accomplish more as a group than could be accomplished by individual undergraduate researchers. Second, the team took on projects and completed complex, multi-day protocols that the postgraduate could not have accomplished alone. Undergraduates presented their research at a campus research symposium and at the International *C. elegans* Conference. The team published results of their work and have additional manuscripts in preparation.[35] Finally, we

have invited team alumni back to discuss where they are now so that current team members can build awareness of the educational and career paths they can pursue.

Conclusions, Limitations, and Future Directions

Here we report postgraduates' views of successful undergraduate research mentoring experiences, and interpret them in light of current knowledge about UREs, mentorship, motivation, and talent development. We found that postgraduates who had not formally trained to be mentors usually approached their mentoring of undergraduate researchers in ways that are consistent with effective mentoring (e.g., setting and adjusting expectations, maintaining open communication) and student development (e.g., selecting and implementing research training in ways that are motivating). We also observed that postgraduates might mentor undergraduates in ways that may be demotivating, for example by deciding an undergraduate's potential based on their engagement at the outset of the research or starting them on challenging and boring projects as a "test." By describing our design and implementation of the VIP team, we provided a practical example of a successful undergraduate research mentoring experience that is inclusive and relevant to postgraduates, who are responsible for much of the daily mentoring of undergraduate researchers at research universities.[36]

The work also has several limitations. The sample of postgraduates was modest and does not likely represent the diversity of postgraduates' experiences mentoring undergraduate researchers. We characterized postgraduates' views and compared them to related empirical work, but we did not directly examine the outcomes of the undergraduates they mentored. We described our design and implementation of the VIP team, including its scientific research

outcomes, but we did not study the outcomes of the team members or whether their outcomes differ from undergraduates who engage in apprenticeship-style UREs. We also did not directly examine the experiences of peers mentoring one another, which is integral to the VIP approach. Future research should more directly compare the experiences and outcomes of both undergraduates and their mentors in the apprenticeship vs. team-based approach and explore the unique contributions of peer mentoring in VIP teams.

Our results also suggest that postgraduates may benefit from professional development about motivation, especially why and how to consider undergraduate competence and interest, and about the "hidden curriculum," such as why asking questions, especially early on in a research experience, may not be a good indicator of a student's engagement or potential.[16] Thus, a fruitful next step would be to design and implement postgraduate-focused professional development on these topics and evaluate its effect on postgraduates' views, mentoring behaviors, and mentee talent development.

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Competing Interest Statement

The authors have no competing interests to declare.

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PART 2

XENOBIOTIC DETOXIFICATION IN *CAENORHABDTIS ELEGANS*

CHAPTER 4

A PLATE BASED ASSAY FOR DETERMINATION OF THE MEDIAN LETHAL DOSE OF 1-HYDROXYPHENAZINE IN *CAENORHABDTIS ELEGANS*

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[1]Asif, M.Z.*; Van der Gaag, V.L.*; Guo, J.; Nocilla, K.A.; Muzio, C.J.; Edison, A.S. A Plate Based Assay for Determination of the Median Lethal Dose of 1-Hydroxyphenazine in *Caenorhabditis elegans. Micropublication Biology,* 2021. Jan 13;2021: 10.17912/micropub.biology.000352. doi: 10.17912/micropub.biology.000352. PMID: 33458604; PMCID: PMC7807258. Reprinted here with permission from publisher (* indicates authors contributed equally to this work).

Foreword

Chapter 4 is reprinted from Muhammad Zaka Asif*, Victoria L. Van der Gaag*, Jane Guo, Kelsey A. Nocilla, Cole J. Muzio and Arthur S. Edison, A plate based assay for determination of the median lethal dose of 1-hydroxyphenazine in *Caenorhabditis elegans, Micropublication Biology,* 2021, Jan 13;2021: 10.17912/micropub.biology.000352. doi: 10.17912/micropub.biology.000352. PMID: 33458604; PMCID: PMC7807258. Reprinted here with permission from publisher (* indicates authors contributed equally to this work). My contributions to this project were (i) conceptualization of the project along with co-authors Victoria van der Gaag, Jane Guo, and Arthur Edison, (ii) data curation along with co-author Victoria Van der Gaag, (iii) formal analysis along with Victoria van der Gaag, (iv) investigation along with Victoria van der Gaag, Jane Guo, Kelsey Nocilla and Cole Muzio, (v) development of methodology along with Jane Guo, (vi) project administration, (vii) writing of the original draft with Victoria van der Gaag, Kelsey Nocilla, and Cole Muzio, and (viii) reviewing and editing the writing along with all other authors. Besides my role in this project, visualization was done by Victoria van der Gaag, and funding acquisition, resources and supervision were provided by Arthur Edison. Funding for this project was provided by the Georgia Research Alliance.

Abstract

Caenorhabditis elegans is an ideal model organism for studying the xenobiotic detoxification pathways of various natural and synthetic toxins. We developed a new workflow to study the effects of 1-hydroxyphenazine (1-HP), a toxin produced by the bacterium *Pseudomonas aeruginosa*, on the survival of *C. elegans*. Prior research has demonstrated that *C. elegans* can detoxify 1-HP through the general mechanism of O-glycosylation. As part of the Vertically Integrated Projects (VIP) undergraduate research team, we have developed a workflow for a plate-based toxicity assay to determine the median lethal dose (LD50) of 1-HP. This was achieved through a toxin exposure method in which the worms were exposed to varying concentrations of 1-HP. The death rates were measured using a fluorescent bead assay. This workflow can be used to test *C. elegans* responses to different toxins and also the response of different mutant strains to a toxin of interest.

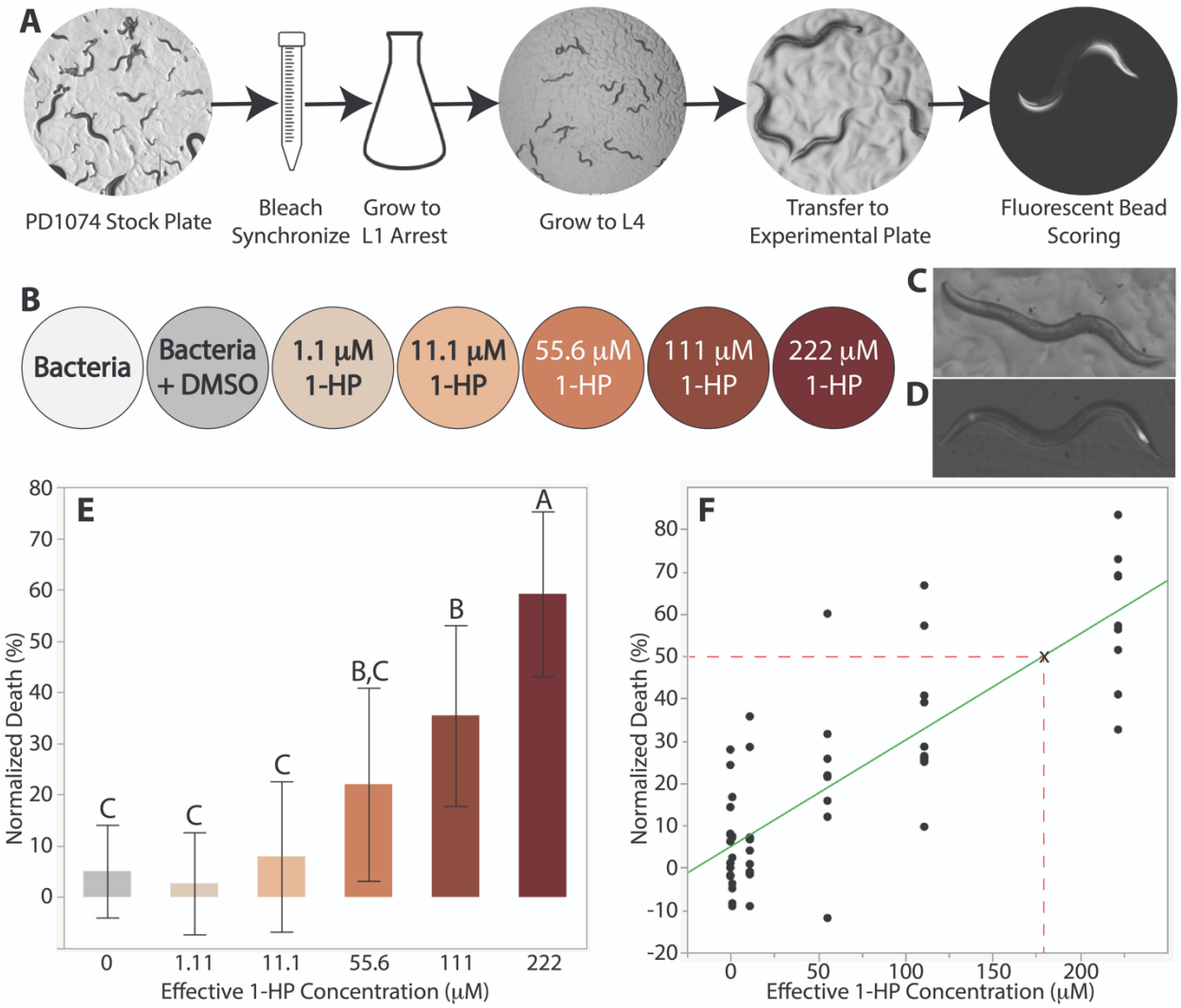

Figure 1. Determining the Median Lethal Dose for 1-Hydroxyphenazine in *C. elegans*: A) A stock plate of PD1074 worms at varying life stages was washed with M9 and transferred to a 15 mL conical tube. The worms were bleach synchronized and the eggs were resuspended and left to grow in M9 for 24 hours. The L1 arrested worms were transferred to 10 cm plates and left to grow to L4 for 35-42 hours. Approximately 15 L4 worms were transferred onto prepared experimental plates and incubated for 7 hours. Using a fluorescent bead assay, the worms were scored as dead or alive. **B)** Each replicate consisted of 7 plates, 2 controls and 5 toxin plates of varying concentrations. The applied concentrations of 1-HP were 90 x greater than the effective concentrations, which were corrected by the volume of the agar. **C)** A gravid adult PD1074 worm without exposure to fluorescent beads. **D)** A gravid adult worm following 5 min exposure to fluorescent beads. **E)** Mean normalized death (y-axis) plotted against effective 1-HP concentration (x-axis). Data labeled by the same letter (A, B, or C) are not significantly different, while those labeled by different letters are statistically different with a p-value < 0.05. **F)** A linear fit curve applied to the mean normalized death vs. effective 1-HP concentration yielded the following equation: y=0.25x+5.23. Using this equation, the effective LD50 concentration is 179 µM.

Description

1-Hydroxyphenazine (1-HP) is a small molecule produced by *Pseudomonas aeruginosa,* a bacterium that is used for pathogenesis models in *C. elegans* (Cezairliyan *et al.*, 2013; Mahajan-Miklos, Tan, Rahme, & Ausubel, 1999). 1-HP is an especially interesting toxin to study as it has been shown to interact with human cells causing ciliary-slowing associated with dyskinesia and ciliostasis (Wilson *et al.*, 1987). Prior research in our lab has shown that this molecule is toxic to *C. elegans*, with an LD50 between 150 and 200 μM, but *C. elegans* can glycosylate 1-HP, which detoxifies the molecule (Stupp *et al.*, 2013).

We have developed a modified, plate-based assay to determine the LD50 in the PD1074 strain of *C. elegans.* PD1074 is the recommended strain for genomics and genetic experiments that will utilize the new reference genome VC2010 (Yoshimura *et al.*, 2019). Importantly, PD1074 minimizes ambiguity caused by highly divergent N2 strains (Gems & Riddle, 2000; Sterken, Snoek, Kammenga, & Andersen, 2015; Vergara *et al.*, 2009).

To ensure that our results were not affected by differences in life stage, a mixed-stage population of PD1074 was synchronized and then allowed to grow to L1 arrest (Porta-de-la-Riva, Fontrodona, Villanueva, & Ceron, 2012) (Figure 1A). The worms remained in M9 media for over 24 hours to ensure that eggs had hatched and then were transferred to 10 cm plates with an OP50 *E. coli* lawn and allowed to grow for 36-39 hours until they reached the L4 stage (Figure 1A). The worms were then washed off the plates with M9 and an average of 15 worms were pipetted onto 6 cm plates. All control and experimental plates in a replicate were seeded by the same synchronized batch. Each of these plates corresponded to one of five toxin concentrations or one of two controls and the worms were left to incubate for 7 hours (Figure1B). This time

optimized the number of worms killed while avoiding developmental progression to young adult. Our toxin concentration range was derived from prior work done on N2 (Stupp *et al.*, 2013).

After incubation with the toxin, we used a fluorescent bead solution to visualize the worms under a fluorescent microscope (Kiyama, Miyahara, & Ohshima, 2012; Nika, Gibson, Konkus, & Karp, 2016). This was done in order to determine the number of worms that died during the incubation period. Worms with any observed fluorescence in their pharynx and anus were counted as alive while those that did not fluoresce were counted as dead (Figure 1C/D). Fluorescence, instead of movement, was used as a marker for alive worms as the effects of 1-HP on *C. elegans* motility are still not fully understood, so it is possible that the toxin could cause partial or complete paralysis. Indeed, we observed very little movement amongst worms exposed to relatively high (>55.6 μM) concentrations of 1-HP.

Our data show that at 1-HP experimental concentrations of 222 μM, 111 μM, and 55.6 μM, mortality rates are approximately 60%, 40%, and 20%, respectively. The difference of mortality rates between the control and lower concentrations were statistically insignificant (Figure 1E). We performed a Tukey's honestly significant difference (HSD) test using JMP statistical software that showed that the death at 222 μM was statistically significant compared to all other toxin concentrations in our range ($p<0.0001$) (Figure 1E). We also found that the death rate at 111 μM was statistically significant when compared to the death rates at the other toxin concentrations and controls except for 55.6 μM ($p<0.003$) (Figure1E). The data show that 1-HP is toxic to PD1074 at similar concentration ranges on plates as it was to N2 in liquid (Stupp *et al.*, 2013). Furthermore, by providing results which correspond with prior literature, we have shown that our plate-based assay is a reliable workflow for LD50 determination. We performed a

regression analysis on our data in order to calculate the LD50 for PD1074 and found the LD50 to be 179 μM (Figure 1F). This is consistent with the value reported for N2 (between 150 and 200 μM) (Stupp *et al.*, 2013). This suggests that 1-HP has a similar toxicity to PD1074 as it does to N2, which is to be expected due to the genetic similarities between the two strains.

This study not only provides evidence that PD1074 has a similar response to 1-HP as N2, it also provides a novel method for a plate-based assay to determine the LD50 in *C. elegans.* Using this technique, in the future we will screen mutants in order to help determine which genes are associated with 1-HP glycosylation.

Methods

Request a detailed protocol

***Strain, Growth, and Media*:** The PD1074 strain of *C. elegans* was grown and maintained on 10 cm NGM agar plates seeded with an LB-cultured, OP50 strain of *Escherichia coli* at 22° C. The worms were chunked onto new 10 cm seeded plates once a week. Additionally, a 10 cm stock plate of PD1074 *C. elegans* was stored at 15° C. Every three to four weeks, a new set of 10 cm plates were chunked from the stock plate in order to maintain a homogeneous experimental population and prevent significant genetic drift.

Experimental Plate Preparation: A stock concentration of 1-HP was diluted using DMSO in order to produce the following five experimental concentrations: 100 μM, 1 mM, 5 mM, 10 mM, and 20 mM. The concentrations at plate surface are the effective concentrations and were corrected by the ratio of the volume of the agar to the volume of DMSO (90 x). 100 μL of each toxin concentration was distributed onto the total surface area of 6 cm NGM plates using a cell

spreader and left to dry for 30 min. Then, each toxin plate was seeded with 25 µL of OP50 bacteria and spread to create a small lawn at the center of the plate. After an additional 30 minutes of drying, the plates were placed at 22°C in an insulated container overnight.

Bleach Synchronization: A plate of *C. elegans* was washed with 3 mL of M9 and pipetted into a 15 mL falcon tube. A second wash of the plate was performed with an additional 3 mL of M9 and left to sit for 5 min to ensure removal of any remaining worms. After 5 min, the contents were pipetted into the falcon tube. The worm/M9 mixture was centrifuged for 1 min at 2000 rpm. A worm pellet was formed at the bottom of the tube and isolated through aspiration. The pellet was then mixed with 2.5 mL of the bleach solution (Porta-de-la-Riva *et al.*, 2012). The tube was shaken gently to disperse the pellet throughout the solution. Every 30 sec, 5 µL of the worm solution was aliquoted onto a glass slide and observed under a microscope to check the state of the worms. Once eggs could be seen breaking out of the worm bodies, the bleaching process was stopped by adding 12.5 mL of M9 and quickly inverting the tube to ensure dilution. The solution was centrifuged for 1 min at 2000 rpm and the supernatant was aspirated. The pellet underwent 2 additional washes and centrifugations at 1200 rpm for 3 min. The egg pellet was resuspended in 5 mL of M9 and then transferred to a 25 mL Erlenmeyer flask and covered with foil. The flask was left to shake at 300 rpm in a 20°C shaker for 24 hours until the worms reached L1 arrest life-stage.

Experimental Worm Plating: After the worms reached the L1 stage, the worms were transferred to a 15 mL falcon tube. The liquid culture was centrifuged for 3 min at 1200 rpm and the supernatant was aspirated to leave a volume of about 300 µL in the tube. The tube was gently shaken to disperse the worm pellet and the 300 µL mixture was pipetted on the outer edge of a

seeded 10 cm plate. The plate of L1 worms were incubated at 22° C for about 35-42 hours until they reached the L4 life-stage. Then the worms were washed with 3 mL M9 and pipetted into a 15 mL falcon tube, twice. An average of 15 L4 worms were aliquoted onto each experimental toxin plate. This was done by aliquoting 10 µL of the worm and M9 mixture onto a glass slide to determine the average amount of worms present per µL of M9. Based on this the volume of M9 to be aliquoted onto the experimental plate was determined. After the M9 dried, the experimental plates were placed in an incubator set at 22° C for an exposure time of 7 hours.

Fluorescent Bead Assay and Data Collection: A fluorescent bead assay was used to distinguish between living and dead worms following toxin exposure. The solution was prepared with Fluoresbrite® Polychromatic Red Microspheres 0.5µm, according to a preexisting protocol (Kiyama *et al.*, 2012). After the 7-hour exposure period, the bead solution was dropped in 2 µL aliquots onto each worm on the experimental plates. The worms were left to feed on the bead solution for 5 minutes, and then observed under a fluorescent microscope with a Texas Red Filter. If there was visible fluorescence in the pharynx or anus, the worms were scored as alive. If there was no visible fluorescence, then the worms were scored as dead. Each plate was scored twice, by separate researchers, and the counts were averaged to determine the final count.

Statistical Analysis: The statistical test performed was a Tukey-Kramer HSD test. This test has three assumptions: observations being tested are independent within and among the groups, the groups associated with each mean in the test are normally distributed, and there is homogeneity of variance (Montgomery, 2013). Our data met these three assumptions thus we chose to carry out this test. The Tukey HSD test was performed using a publicly available software (Pro, 2015).

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CHAPTER 5

ROLE OF *UGT* GENES IN GLYCOSYLATION AND DETOXIFICATION OF 1-HYDROXYPHENAZINE IN *CAENORHABDTIS ELEGANS*

Asif, M.Z.; Nocilla, K.A.; Ngo, L.T.; Shah, M.K.; Smadi, Y.; Hafeez, Z.A.; Glushka, J.; Leach III, F.E.; Edison, A.S. Role of *ugt* Genes in Glycosylation and Detoxification of 1-Hydroxyphenazine in *Caenorhabditis elegans*. To be submitted to Cell Chemical Biology, anticipated acceptance 2023.

Foreword

Chapter 5 is printed with permission from the authors. The motivation from this work comes from prior work in the lab carried out by Gregory Stupp characterizing the glycosylation products of 1-hydroxyphenazine and related work carried out in the lab with *ugt* genes as part of the CIDC worm team. My inclusion in this project started out when I was introduced to this initial idea by an older graduate student, Bif. After that, I discussed this idea with Arthur Edison, and over the course of the next three to four years this initial idea came to fruition, with some very surprising results. My role in this project was to lead and train the VIP undergraduate students on preparing worm samples for the mortality screen. I also taught the students how to culture worms in liquid culture for large scale HPLC-UV assays. I also maintained all the different strains of C. elegans used in this study, collected phenotypic data, and taught some of the VIP students how to extract the 1-HP derivatives from the supernatant of the worms grown in liquid culture. Furthermore, I performed chromatography to separate the 1-HP derivatives, designed methods, maintained columns, and helped maintain the instrument. I also prepared samples for NMR spectroscopy, and ran NMR experiments on the instrument with help from John Glushka. I analyzed and interpreted all the data collected in this study with guidance from Arthur Edison. I also wrote the manuscript, made all the figures and reviewed and edited the writing along with other authors. Other author contributions are as follows: Kelsey Nocilla prepared worm samples and collected data for the mortality assays. Li Ngo performed chemical extractions and prepared samples for HPLC-UV. Man Shah helped prepare worm samples for the mortality assays and grew worms in liquid culture. Yosef Smadi helped prepare NMR samples and helped with collecting fractions of glycosylated 1-HP derivatives. Zaki Hafeez helped maintain worm strains for mortality assays. John Glushka helped design pulse sequences for

NMR experiments and helped with NMR analysis. Franklin Leach collected LC-MS data and performed MS/MS analysis. Arthur Edison supervised the entire project, got funding acquisition, provided resources and guided the data analysis and interpretation. All authors participated in reviewing and editing the manuscript. Kelsey, Man, Li, Yosef, and Zaki were all VIP students in Edison Lab trained as part of the VIP program. Funding for this research came from the Georgia Research Alliance.

ABSTRACT: *Caenorhabditis elegans* is an ideal model organism to study the xenobiotic detoxification pathways of various natural and synthetic toxins. One such toxin that has been shown to cause death in *C. elegans* is 1-hydroxyphenazine (1-HP), a molecule produced by the bacterium *Pseudomonas aeruginosa.* Prior research in our lab has shown the median lethal dose (LD50) for 1-HP in *C elegans* is 179 μM in PD1074 and between 150-200 μM in N2 (*C. elegans* lab strain). Prior research has also shown that *C. elegans* detoxifies 1-HP by glycosylation with one, two, or three glucose molecules in N2 worms. In this study, we wanted to study whether UDP-glycosyltransferase (*ugt)* genes play a role in 1-HP detoxification. We show that *ugt-23* and *ugt*-49 knockout mutants have higher sensitivity to 1-HP. Our data also show that *ugt-23* knockout mutants produce reduced amounts of the trisaccharide sugars, while the *ugt-49* knockout mutants produce reduced amounts of all 1-HP derivatives except for the glucopyranosyl product. We have also characterized the structure of the trisaccharide sugar phenazine structures produced by *C. elegans* and show that one of the sugar modifications contains a N-acetylglucosamine (GlcNAc) in place of a glucose. This implies broad specificity in terms of UGT function and the role of genes other than *ogt-1* in the addition of GlcNAc, at least in small molecule detoxification.

1-Hydroxyphenazine (1-HP) is a small molecule produced by many *Pseudomonas* species including *Pseudomonas aeruginosa.*[1-5] 1-HP is one of three related metabolites, along with pyocyanin and phenazine-1-carboxylic acid, produced by *Pseudomonas aeruginosa* that are toxic to *C. elegans.*[6] 1-HP is thought to act in *C. elegans* by causing a-synuclein and polyglutamine-induced protein misfolding and the exacerbation of a-synuclein-induced dopaminergic neurodegeneration.[7] Prior research has shown that *C. elegans* modifies 1-HP through the addition of either one, two, or three glucose moieties, with phosphorylation also observed in the endo-metabolome.[8] In this study, we sought to better characterize the metabolized 1-HP derivatives produced by *C. elegans* and begin to understand what gene family might be responsible for these modifications.

Uridine 5'-diphospho-glycosyltransferases (UGTs) are a family of enzymes critical for homeostatic regulation of endogenous metabolites and xenobiotic detoxification in several organisms including humans and *C. elegans*.[9, 10] UGTs are the primary protein family responsible for the addition of glucose moieties during phase-II xenobiotic detoxification in *C. elegans.*[11] Loss or modification of UGTs have been implicated in drug hypersensitivity.[12, 13] They have also been shown to be upregulated upon exposure to metals, pathogenic toxins, anthelmintics, and other small molecules.[13-18]

Table 1. Information on strains used in this study. All strains were obtained from Caenorhabditis Genetics Center (CGC).

Strain Name	**Genotype**	**Reference**	**Time from egg to L4 (hr)**
N2	-	a	~ 42
PD1074	-	b	~ 42
RB2055	*ugt-1*	c	~ 44
VC4207	*ugt-6*	d	~ 42
VC3950	*ugt-9*	-	~ 42
RB2550	*ugt-23*	c	~ 42
RB2607	*ugt-49*	c	~ 44
VC2512	*ugt-60*	e	~ 50
RB2011	*ugt-62*	c	~ 46
VC4339	*ugt-66*	d	~ 42
RB1342	*ogt-1*	c	~ 43

a [19] The genetics of *Caenorhabditis elegans* (domesticated laboratory strain of *C. elegans)*

b [20] Recompleting the *Caenorhabditis elegans* genome (A newer standardized version of the laboratory strain of *C. elegans)*

c *C. elegans* Gene Knockout Project at the Oklahoma Medical Research Foundation

d [21] CRISPR/Cas9 Methodology for the Generation of Knockout Deletions in *Caenorhabditis elegans*.

e *C. elegans* Reverse Genetics Core Facility at the University of British Columbia, international *C. elegans* Gene Knockout Consortium

Note: ogt-1 is included in this table as this strain was used for toxicity experiments as well based on results described in Figure 2.

In this study, we tested available *ugt* mutants for their involvement in 1-HP modification and susceptibility (Table 1, Supplemental Table 1). We analyzed the phylogeny of these *ugt* genes and found that they covered most of the clades in the UGT family (Supplemental Figure 7). We then adapted a plate-based mortality screen from our previous work to discover strains with modified sensitivity to 1-HP exposure at the LD50 (179 mM) concentration (Figure 1A).[22] All strains were paired with N2 and PD1074 replicates to recognize batch effects. We found that all strains had higher mortality when exposed to 179 mM 1-HP as opposed to the bacteria control. 1.1 % DMSO, the solvent used for 1-HP assays, had no effect on worm mortality (Supplementary Figure 1B). The *ugt-23* and *ugt-49* mutants had higher mortality compared to N2. This suggests that these genes might have a role in the glycosylation of 1-HP.

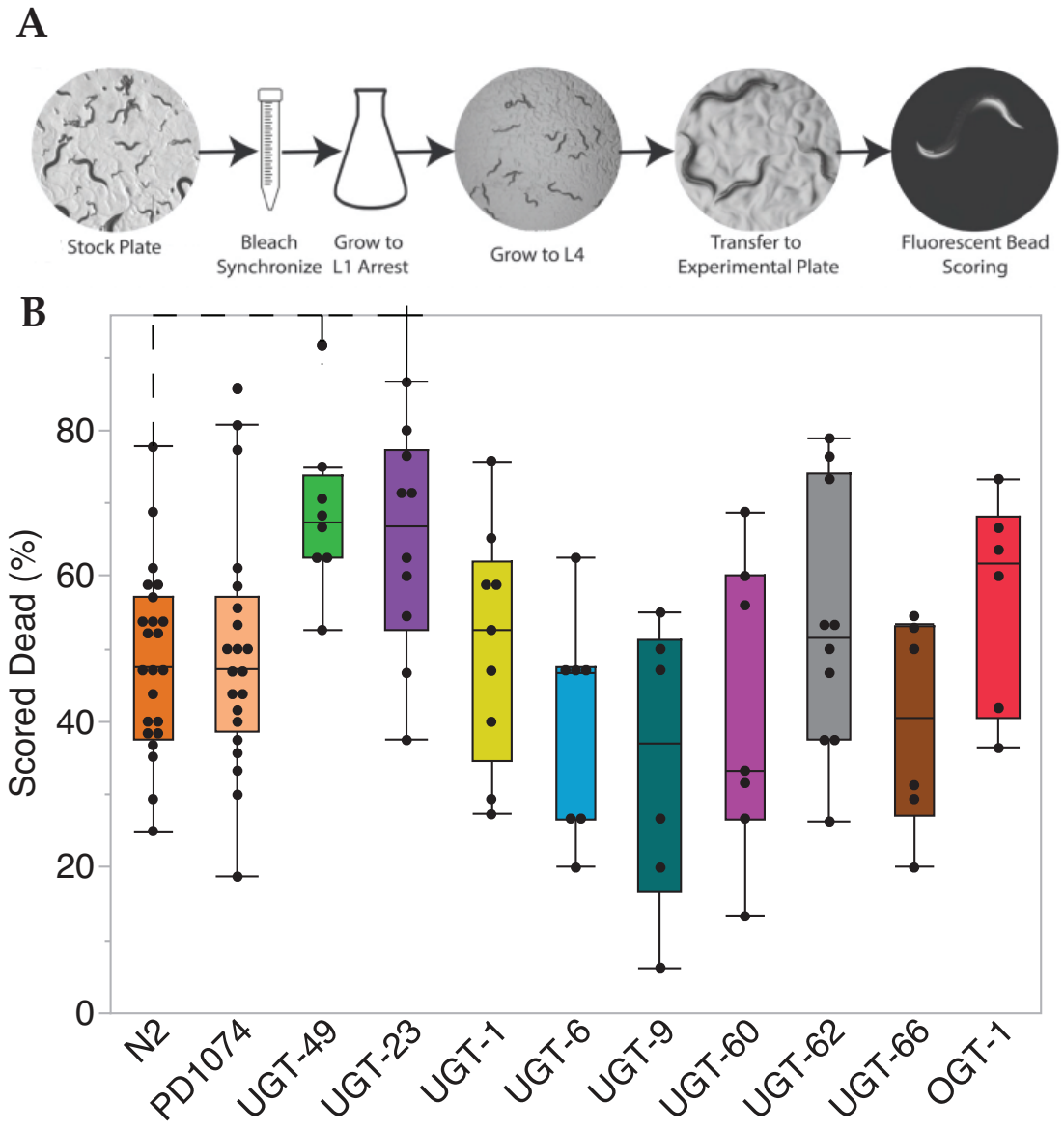
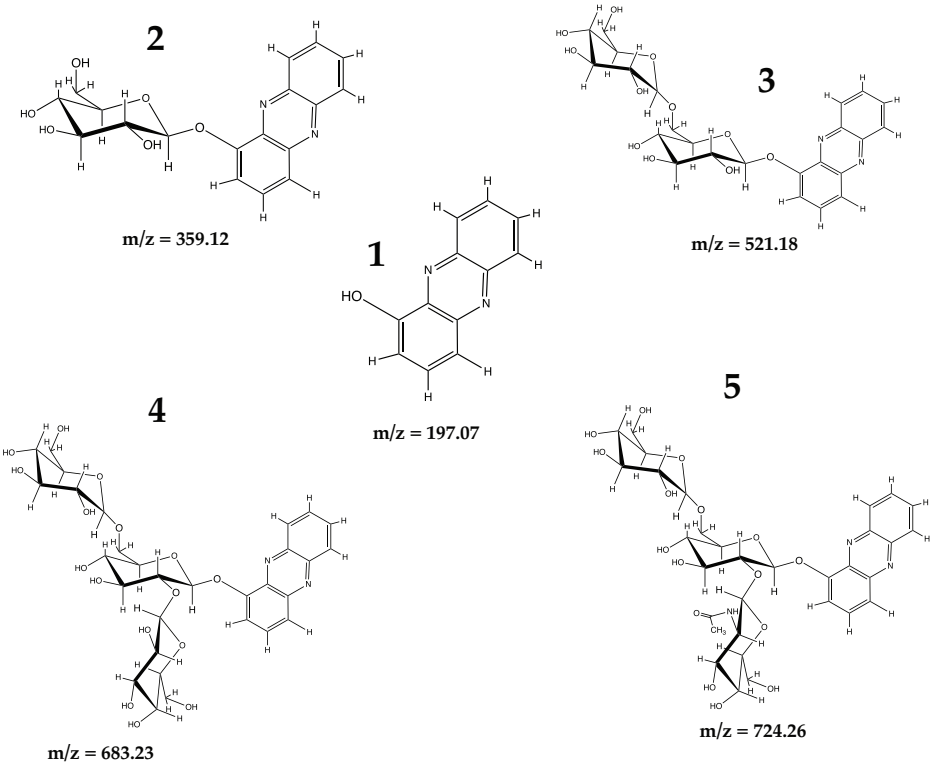


Figure 1. Plate based screen for susceptibility to 1-HP. (A) Schematic describing the method for the plate-based assay. Worms were incubated for 7 h with a minimum of 6 replicates per strain. (B) Box and whiskers plot showing mortality of various strains at 179 mM 1-HP. Dashed lines indicate significantly increased mortality compared to N2 with an a of 0.1 after a Wilcoxon Pairwise comparison followed by a Benjamini-Hochberg Correction.

We then explored whether *ugt-23* and *ugt-49* produced the same 1-HP glycosylated products identified previously in N2 .[8] We exposed L4 worms in large scale liquid culture for 24 hrs followed by HPLC-UV analysis of the worm media. We found that using the LD50 concentration of 1-HP resulted in more glycosylated product than a lower concentration of 1-HP due to the increased mortality. Empirical evidence suggested that a 22.3 μM concentration allowed a large enough number of worms to survive for 24 hours to accumulate sufficient modified 1-HP. We performed the analysis and observed 4 unique peaks that were present in all strains (Figure 2B). The peaks were isolated using semi-preparative HPLC and then analyzed by NMR and LC-MS (Figure 2A, Supplemental Figures 2-5). We identified compounds (**2**) and (**3**) which had also been identified in prior literature (Figure 2A).[8] However, we also identified two branch-chained trisaccharides, one with three glucoses (**4**) and one with two glucoses and a N-acetylglucosamine (GlcNAc) modification (**5**) (Figure 2A). Because (**5**) contained a GlcNAc, we then evaluated the GlcNAc transferase *ogt-1* with the same assays described above. *ogt-1* has previously been shown to modulate the immune response for *S. aureus* but not *P. aeruginosa*.[23] Consistent with those findings, the *ogt-1* knockout had no statistically significant difference in susceptibility to 1-HP compared with N2 and PD1074 (Figure 1B). LC-MS analysis of worm media conditioned by the *ogt-1* knockout mutant challenged with 1-HP showed that (**5**) was still produced (Supplemental Figure 6).

A


2
m/z = 359.12
1
m/z = 197.07
3
m/z = 521.18
4
m/z = 683.23
5
m/z = 724.26

B

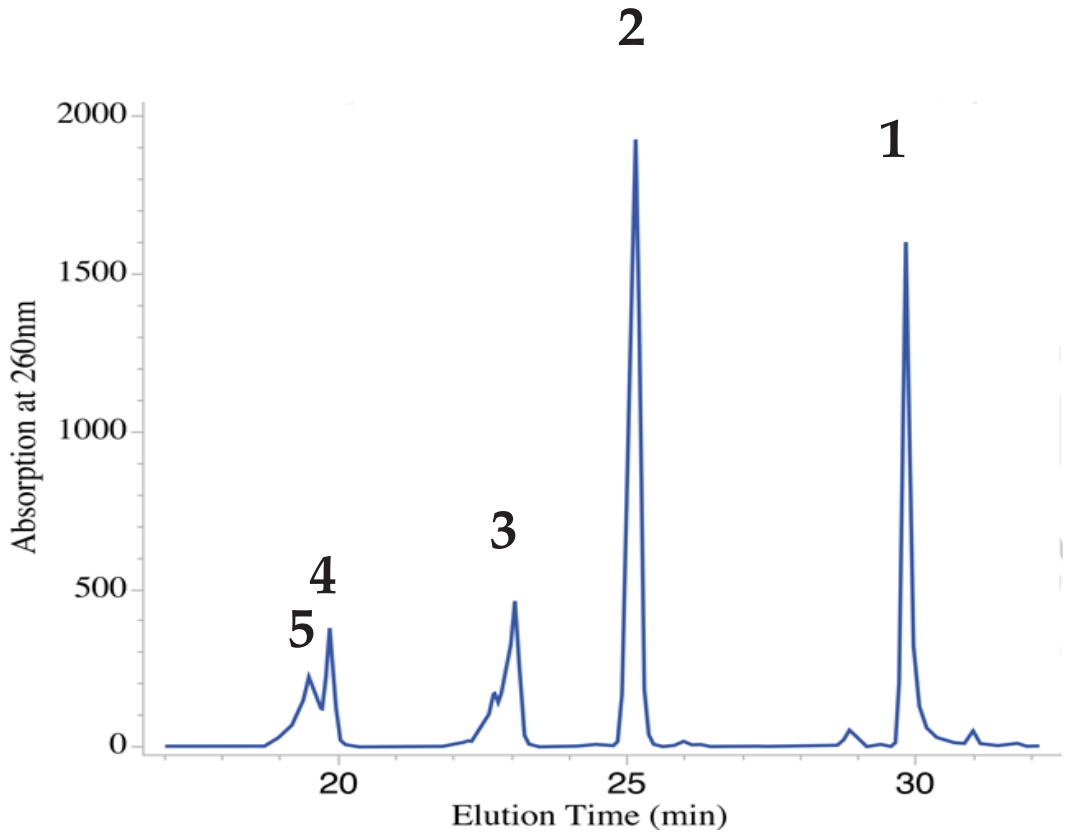

2
1
3
4
5
2000
1500
1000
500
0
Absorption at 260nm
20
25
30
Elution Time (min)

Figure 2. PD1074 exposed to 1-HP. (A) 1-HP and its glycosylated derivatives with their corresponding m/z values. (**1**) 1-HP, (**2**) b-D-glucopyranosyl-phenazine (**3**) b-D-glucopyranosyl (1-6)-b-D-glucopyranosyl-phenazine, (**4**) b-D-glucopyranosyl (1-6)- [b-D-glucopyranosyl (1-2)]-b-D-glucopyranosyl-phenazine, and (**5)** b-D-glucopyranosyl (1-6)- [b-D-N-acetylglucosamine-pyranose (1-2)]-b-D-glucopyranosyl-phenazine. The m/z values were obtained from high resolution MS data acquired using positive-ion electrospray ionization (ESI) (Supplemental Table 2). (B) Representative UV chromatogram of PD1074 exposed to 22.3 mM 1-HP for 24 hrs. in S-basal medium with 2% *E. coli*. Each peak corresponds to either 1-HP or one of its glycosylated derivatives The peak at 30 min. is (**1**), the peak at 25 min is (**2**), the peak at 23 min is (**3**), the peak at 19.8 min is (**4**), and the peak at 19.4 min is (**5**).

We then quantified the HPLC-UV data to observe if there was a reduction in the amounts of 1-HP derivatives for the *ugt-23* and *ugt-49* knockout mutants. We normalized the data to the sum of all the 1-HP related compounds for each replicate. This ensured that the ratio obtained was independent of any variation due to amount of 1-HP the worms were exposed to or the number of worms per replicate. We found that the *ugt-23* knockout mutant produced decreased amounts of both trisaccharide sugars (**4**) and (**5**), while the *ugt-49* knockout mutant produced decreased amounts of compounds (**3**), (**4**), and (**5**) (Figure 3).

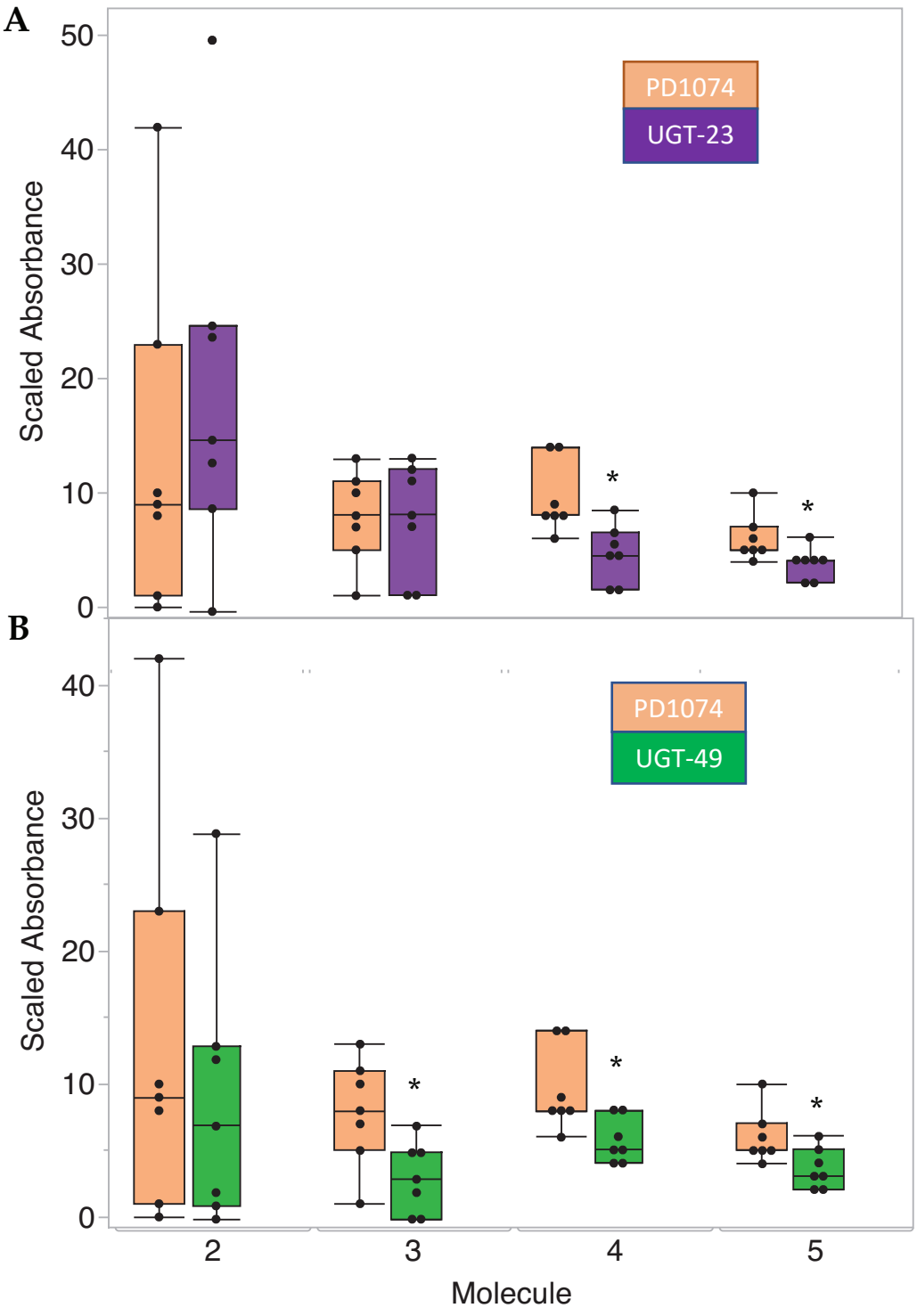

A
B
Scaled Absorbance
Molecule
PD1074
UGT-23
UGT-49
0
10
20
30
40
50
2
3
4
5
*

Figure 3. Box and whiskers plot showing the relative amounts of 1-HP and its derivatives after a 24 hrs. incubation at 22.3 mM 1-HP with 2% *E. coli* based on UV absorbance data (n=7). All replicates were paired and data were normalized by dividing the absorbance for each compound at 260 nm by the sum of the absorbances of 1-HP and all its derivatives for each run (abs *x*/[abs *z* + abs *y* + abs *x* + abs *w* + abs *v*]). * Indicates significant difference in relative amounts of compound compared to the relative amount of the same compound in PD1074 after Wilcoxon pairwise analysis (a = 0.05). (A) Relative amounts of glycosylated 1-HP derivatives for the *ugt-23* mutant compared to PD1074. Compounds **4** and **5** are reduced in this strain. (B) Relative amounts of glycosylated 1-HP derivatives for the *ugt-49* mutant compared to PD1074. Compounds **3**, **4**, and **5** are reduced in this strain.

These results show the involvement of *ugt* genes in 1-HP detoxification, suggest that they have broad specificity, and that multiple *ugt* genes are involved in detoxification of a xenobiotic in *C. elegans*. Prior research has implicated multiple *ugt* genes for being responsible for glycosylation of other small molecule toxins such as indole as well.[24] The workflow outlined in this study can be used to test the role of *ugt* genes in modification of other small molecule xenobiotics and future studies could validate whether broad specificity is seen in response to xenobiotics in general or whether this is a phenomenon specific to 1-HP.

Our data also suggests that genes other than *ogt-1* are responsible for the addition of GlcNAc in the 1-HP glycosylation pathway. Furthermore, our results implicate the addition of GlcNAc in detoxification in *C. elegans,* a result which, to our knowledge, has not been previously observed. This might be due to the broad specificity of *ugt* genes or perhaps that GlcNAc serves a particular purpose in detoxification. Using this workflow, it would be interesting to see if GlcNAc modified products are observed for other xenobiotics as well.

METHODS

Mortality Assay. All 11 strains of *C. elegans* were grown and maintained on 10 cm NGM agar plates seeded with an LB-cultured, OP50 strain of *E. coli* at 22° C. Knockout mutants were

paired with an N2 and PD1074 replicate for each strain. 10 cm NGM plates with *C. elegans* were bleached and grown to L1 arrest and then L1 arrested worms were transferred to new 10 cm plates and allowed to grow to L4. Upon reaching L4, ~ 15 worms were transferred either to control 6 cm plates or 6 cm plates with NGM and 179 µM 1-HP, the LD50 value of PD1074.[22] Worms were incubated on experimental plates for 7 hours. After 7 hours, fluorescent beads were added to the worms and the uptake of these beads was used as a marker to differentiate between alive and dead worms. [25]

Lifespan Timing Assay. Worms were observed to determine how long they took to go from egg to L4 in two different ways. The time to L4 for N2, PD1074, and the *ugt-1, ugt-23, ugt-49, ugt-60,* and *ugt-62* knockout mutants was measured by initially spot bleaching a single adult and following a single egg, observing them until they reached L4. The time to L4 for the *ugt-6, ugt-9, ugt-66, and ogt-1* knockout mutants was measured by bleach synchronizing a plate of worms and placing the resulting eggs on a 10 cm plate. The plates were observed every four to eight hr. until the majority of the population on the plate could reliably be identified as L4.

Generation of phylogenetic tree. Different UGT amino acid sequences were collected from the CAZy database and wormbase. These sequences were then aligned using the multiple sequence alignment (MAFFT) tool. Sequences for 79 UGTs were collected of which two were removed due to poor alignment. Using these sequences, a phylogenetic tree was generated using iqtree. Finally, the phylogenetic tree was visualized using an online tool called Interactive Tree of Life (IToL).

Large Scale Growth of *C. elegans*. Worms were grown on large scale culture plates (LSCPs) to generate worms for subsequent experiments. LSCPs were poured according to previously described protocols.[26] Poured plates were seeded with the HTS115 strain of *E. coli* prepared in K-media at a concentration of 0.5 g mL^{-1} bacteria generated according to the IBAT method.[27] Worms were chunked onto the LSCPs and then grown for seven to ten days depending on strain before being washed with M9 for subsequent experiments. After washing, worms were bleach synchronized and then grown to L1 arrest in M9. Upon reaching L1 arrest, they were transferred to S-basal medium (~30,000 worms mL^{-1}) and incubated with 2 % *E. coli* OP50 until they reached L4. After the worms had reached L4, they were incubated with either 1.1 % DMSO or 22.3 µM 1-HP. Worms were incubated for 24 hours and then centrifuged. The supernatant was collected for subsequent experiments.

Glucoside Collection and Analysis (HPLC-UV). After the supernatant was separated from the worms, it was centrifuged again at 20,800 RCF for 10 minutes to separate the bacteria from the supernatant. The resultant volume was lyophilized and extracted in an appropriate volume of methanol (200-600 µL depending on the starting volume of the supernatant). It was then centrifuged at 20,800 RCF for 30 minutes. Following centrifugation, the supernatant was concentrated to ~100 µL with 90 µL injected into the HPLC-UV and 10 µL separated for LC-MS.

The supernatant was analyzed on an Agilent 1200 Series HPLC system with a diode array collector and fractions were collected manually upon the observance of a peak. Absorbance was measured at 260nm. For worm media separation, 5% methanol (A) and 95 % 5 mM Phosphate

buffer pH 7.2 (B) were held isocratic for four minutes, increasing to 95 % A and 5 % B over 30 minutes and then held for five minutes, followed by a re-equilibration of the column. The separation was carried out at a flow rate of 2 mL min^{-1} in an Agilent SB C-18 column (9.4 mm x 250 mm, 5µM).

After initial fractionation, further separation of the fraction containing compounds 4 and 5 was carried out. For that separation, 5% methanol (A) and 95% 5 mM Phosphate buffer pH 7.2 (B) were held isocratic for four minutes, increasing to 50 % A and 50 % B over 17 minutes. The gradient was slowed and the ratio increased to 67% A and 33% B by 28 minutes before ramping it up to 95% A and 5% B by 30 minutes and then holding constant for five minutes. This was followed by a re-equilibration of the column. The column used for this separation was the same as the column used for the initial worm media separation.

Glucoside Analysis (LC-MS/MS). Samples aliquoted during glucoside collection were analyzed using a Thermo Fisher Scientific Q Exactive HF Orbitrap mass spectrometry coupled to a Vanquish UPLC with inline UV detection. Chromatographic separation was performed with a C18 column over 30 minutes starting with 95 % H_2O (A) and 5% methanol (B) held isocratic for 2.5 minutes then increased to 70% B by 22 minutes and 100% B at 22.5 minutes and held for 4 minutes before re-equilibration at 5% B for 3 minutes prior the next injection. The sample queue was randomized with injection blanks included to monitor for sample carryover. All samples were analyzed by positive mode electrospray ionization (ESI). Full MS scans were performed at a specified resolution of 30,000 (m/z 200) from 150 to 2,000 m/z with an AGC target of 3e6 and maximum IT of 200 ms. Corresponding UV traces were collected at 260 nm.

Target compounds were isolated with a 4.0 m/z quadrupole window to perform structural elucidation by higher-energy collisional dissociation (HCD). A normalized collision energy (NCE) of 15 V was applied, and fragment ions were detected with a specified resolution of 15,000, AGC target of 2e5, and maximum of IT 100 ms. MS data was analyzed with Thermo Qual Browser and manually interpreted.

Glucoside Analysis (NMR). Pooled fractions were dried, resuspended in 60 μL D_2O with 0.15 mM DSS as an internal standard, dried with a speed vac and resuspended twice in 60 μL D_2O in order to perform buffer exchange to remove excess H_2O before being transferred into 1.7 mm NMR tubes. 1D ^1H, 2D COSY, 2D TOCSY, selective 1D TOCSY, and selective 1D ROESY spectra were collected where appropriate on a Bruker 800 MHz NEO spectrometer using a 1.7 mm cryoprobe. Spectra were processed and analyzed with MestReNova 14.1.2 (Mestrelab Research).

Statistical Analysis. Analysis was performed using JMP®, a publicly available statistical software. A Wilcoxon test, followed by a Wilcoxon pairwise analysis and a Benjamini-Hochberg correction were performed for the mortality assays to determine significance between strains. Tukey's HSD test were performed to determine significance within each strain for 1-HP exposure. A Wilcoxon test followed by Wilcoxon pairwise analysis was performed on the scaled absorbance data.

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CHAPTER 6

COMPUTATIONAL ANALYSIS OF VARIATION IN *C. ELEGANS UGT*S

Asif, M.Z.*, Benveniste, M.*, Chism, K.*, Levin, A.*, Lanier, D., Watkins, R., Taujale, R., Tucker, N., Edison, A.S. Computational Analysis of Variation in *C. elegans ugt*s. (* Indicates authors contributed equally). Submitted to Micropublication Biology. Anticipated Acceptance 2023

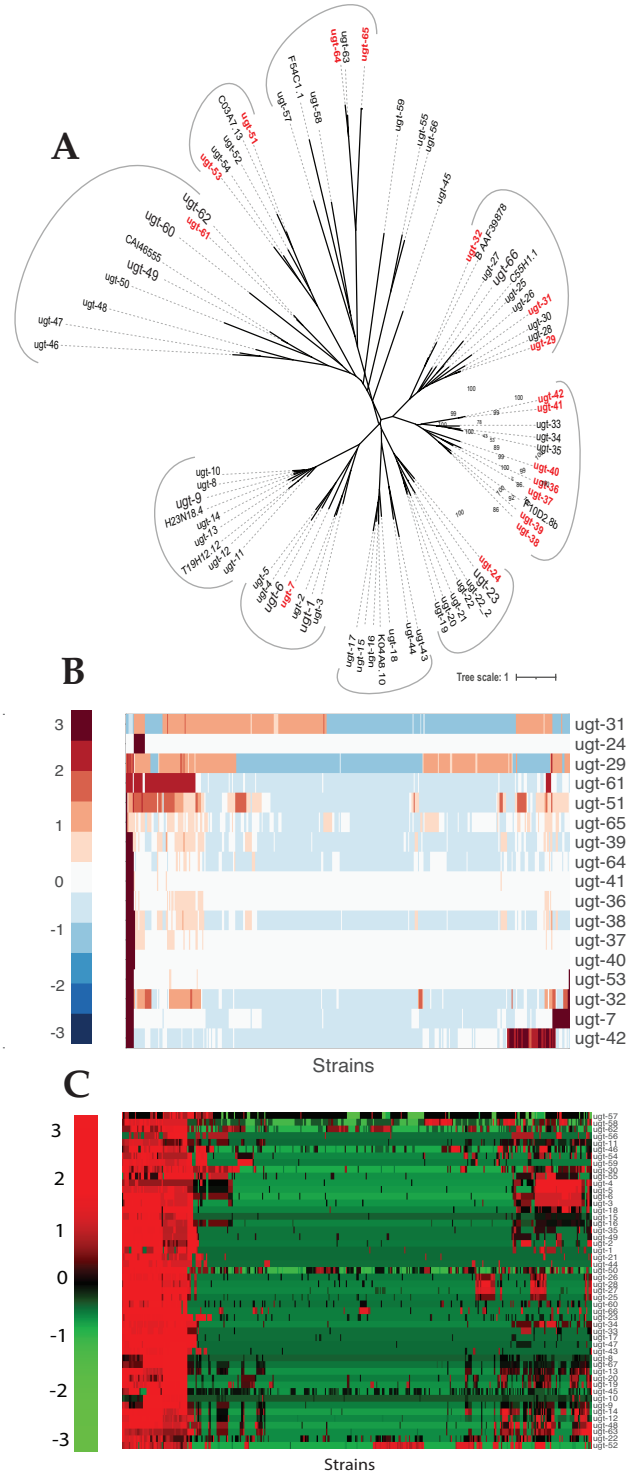
Foreword

Chapter 6 is printed here with permission from the authors. The motivation for this work came from a combination of my interest in *ugt* genes stemming from my research with 1-HP and the need to involve undergraduate students in computational research as part of the VIP program in the Edison Lab during the Covid pandemic. Added to this, there was prior research on a different branch of glycosyltransferases by co-author Rahil that helped develop this study further. My role in this project was to conceptualize the study along with Rahil Taujale and Arthur Edison. I was also involved in formal analysis along with Ari Levin, Maci Benveniste, Kyra Chism, Rahil Taujale, Rockford Watkins, Deanna Lanier, and Niyelle Tucker. I was also involved in the investigation along with Ari Levin, Maci Benveniste, Kyra Chism, Rockford Watkins, Rahil Taujale, Deanna Lanier, and Niyelle Tucker. I also designed the methodology, along with Ari Levin, Maci Benvensite, Kyra Chism, Rockford Watkins, Rahil Taujale, Deanna Lanier, and Niyelle Tucker. I administered the project with some help from Rahil Taujale and was involved in writing the original draft along with Maci Benveniste and Kyra Chism. I, along with all the authors, reviewed and edited the manuscript. Other authors contributions were as follows: Ari Levin was involved in data curation, formal analysis, investigation, methodology, and writing reviewing and editing. Maci Benveniste was involved in data curation, formal analysis, investigation, methodology, writing the original draft and reviewing the writing. Kyra Chism was involved in data curation, formal analysis, investigation, methodology, writing the original draft and reviewing the manuscript. Rockford Watkins was involved in formal analysis, investigation, methodology and reviewing the manuscript. Rahil Taujale was involved in conceptualization, data curation, formal analysis, investigation, methodology, project administration, and reviewing the manuscript. Deanna Lanier was involved in data curation,

formal analysis, investigation, methodology and reviewing the manuscript. Niyelle Tucker was involved in data curation, formal analysis, investigation, methodology and reviewing the manuscript. Arthur S. Edison was involved in conceptualization, funding acquisition, providing resources, supervision and reviewing the manuscript. Funding for this research came from the Georgia Research Alliance.

Abstract

Caenorhabditis elegans are free-living nematodes with a relatively short life cycle and a wealth of genomic information across multiple databases. Uridine diphosphate-glycosyltransferases (UGTs) are a family of enzymes involved in Phase II modification of xenobiotics in *C. elegans*, which is the addition of a large water-soluble molecule to a xenobiotic to allow for its excretion out of a cell. Little is known about the variation in UGTs across wild isolates and how that might affect their innate immune response. We analyzed the diversity in *ugt* genes across *C. elegans* isolates from different geographical locations from the *Caenorhabditis elegans* Natural Diversity Resource (CeNDR) database. This was accomplished using whole genome data and data that identified regions of the genome as hyperdivergent for each isotype. We implemented two steps to identify *ugt* genes and make inferences based on their variation. First, we created a catalog of UGTs in the N2 reference strain and used them to create a phylogenetic tree that depicts the relationships between the UGT protein sequences. We also quantified *ugt* variation using the strains from the CeNDR database and used the hyper-divergent file from the CeNDR database to remove hyperdivergent *ugt* genes. Of the 67 *ugt* genes analyzed, 17 were hyper-divergent. This research will help improve our understanding of *ugt* variation in *C. elegans*.


A
ugt-65
ugt-63
ugt-64
F54C1.1
ugt-58
ugt-57
ugt-59
ugt-55
ugt-56
ugt-45
C03A7.13
ugt-51
ugt-52
ugt-54
ugt-53
ugt-62
ugt-61
ugt-60
CAI46555
ugt-49
ugt-50
ugt-48
ugt-47
ugt-46
ugt-32
B.AAF.39878
ugt-27
UGT-66
C05H1.1
ugt-25
ugt-26
ugt-31
ugt-30
ugt-28
ugt-29
ugt-42
ugt-41
ugt-33
ugt-34
ugt-35
ugt-40
ugt38
ugt-37
R10D2.86
ugt-39
ugt-36
ugt-10
ugt-8
ugt-9
H23N18.4
ugt-14
ugt-13
T19H12.12
ugt-12
ugt-11
ugt-5
ugt-4
UGT-6
ugt-7
ugt-2
ugt-1
ugt-3
ugt-24
ugt-23
ugt-22_2
ugt-22
ugt-21
ugt-20
ugt-19
ugt-17
ugt-15
9H-16n
K04A8.10
ugt-18
ugt-44
ugt-43
Tree scale: 1
B
3
2
1
0
-1
-2
-3
ugt-31
ugt-24
ugt-29
ugt-61
ugt-51
ugt-65
ugt-39
ugt-64
ugt-41
ugt-36
ugt-38
ugt-37
ugt-40
ugt-53
ugt-32
ugt-7
ugt-42
Strains
C
3
2
1
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Strains

Figure 1: Visualizing *ugt* variation across *C. elegans*

A: Phylogenetic tree of the known UGTs in *C. elegans*. The hyper-divergent UGTs are enlarged and highlighted in red. **B**: Hierarchical cluster of the known hyper-divergent *ugt* genes. The mean (M) was set to zero which was equal to 9 base pair mutations with a standard deviation of 25. **C**: Hierarchical cluster of the non-hyper-divergent *ugt* genes. The mean (M) was set to zero which was equal to 2 base pair mutations with a standard deviation of 4.

Description

C. elegans has about 250 glycosyltransferases[1], and the *ugt* family of 77 genes is responsible for glycosylation of small molecule xenobiotics[2-5]. We quantified the variation in 67 *ugt* genes across *C. elegans* isotypes using N2 as the reference strain[6]. Regions with higher-than-average concentrated genomic variation compared to N2 are considered to be hyper-divergent.[7]. According to Lee at al, regions with higher-than-average genomic variation were those with nine consecutive bins of over 1kb that are equal to 16 SNVs/indels or have lower then 35% read depth to the genome wide average[7]. These hyper-divergent regions in their respective UGTs were identified and removed from our analysis.

We used the Multiple Alignment using Fast Fourier Transform (MAFFT) tool to align amino acid sequences to generate a phylogenetic tree via iqtree tool (Fig.1A). The UGTs were statistically clustered with their amino acid sequences to create clades representing similar sequences. Furthermore, genes that were found to be hyper-divergent were highlighted in red. In

total, ten clades were identified. Seven of them had at least one *ugt* that was hyper-divergent and the rest did not.

Figure 1B shows hyper-divergent genes that were removed from our analysis. This figure was created using a script embedded with the cluster gram function in MATLAB. The script contained a function to show both the mean and standard deviation. The mean was 9 and the standard deviation was 25. In the color bar (red-blue), the zero correlates to the mean (9) and each increase are one standard deviation above the mean (ie:1=36 and 2=61). The maximum number of mutations for a strain is also provided. This was 261 in ECA701 for *ugt-65*. ECA701 is also the most varied isotype in our analysis of hyper-divergent *ugts* with a total number of 1658 base-pair mutations across the 17 hyper-divergent genes. ECA701 is located in Hawaii, USA.

Figure 1C is the cluster gram for the non-hyper-divergent genes, created as described above. The red-green colormap was chosen to contrast with Figure 1B of the hyper-divergent regions. The mean was 2 and the standard deviation was 4. The color bar indicates the mean at 0 and each increase representing one standard deviation set (i.e., 1=6 and 2=10). The maximum number of base-pair mutations was 60 and this was found in strains ECA1228 and ECA 1293. Both of these strains were in *ugt-12,* the gene with the highest variations amongst non-hyper-divergent *ugts*. The isotype with the highest number of mutations for the non-hyper-divergent strains is ECA 722 with 436 base-pair mutations. ECA722 is located in Hawaii, USA. Regions of high variability were also identified, and their strains were located on the cluster gram.

Non-hyper-divergent *ugts* are an area of interest for future studies. Given the quantified genomic variation across isotypes from many locations, our results suggest there may be multiple environmental factors affecting variation such as climate, bacteria, pathogens, environmental toxins, etc.

Methods:

Generating Phylogenetic Tree: We collected 79 UGT amino acid sequences from the publicly available CAZy and Wormbase databases. Next, we used the Multiple Alignment using Fast Fourier Transform (MAFFT Alignment) tool to align the amino acid sequences for the UGTs. From this, two of the UGTs were removed due to poor alignment. Then, we generated the phylogenetic tree using the iqtree tool. We visualized the phylogenetic tree using the online tool called the Interactive Tree of Life (iTOL) (Fig. 1A). Null genes were removed to bring the total number of *ugts* to 67.

Identifying Genomic Variation of CENDR Strains Compared to N2: Using the information gathered above, we generated a Python script in Jupyter Notebook™ to parse CENDR's hard filtered variants vcf file ***WI.20200815.impute.isotype.vcf.gz*** (released 20200815) and extracted the number of variants and location of mutations in UGT regions for 403 isotypes compared to the N2 reference genome. The genomes of the isotypes were aligned and compared to the N2 genome.

Removal of Hyperdivergent Regions from Analysis: Using the CENDR hyper-divergent region data file (20220216.bed), we created a python script using Jupyter Notebook™ to

determine which UGTs had hyper-divergent isotypes. Our data included regions that partially fell in a hyper-divergent range, or a full overlap. A table was created with our data which we further separated into two excel files which contained both non-hyper-divergent and hyper-divergent strains and UGTs with the number of base pair mutations across isotypes. If a gene from an isotype partially or fully fell into a hyper-divergent region, it was considered hyper-divergent for analysis purposes. All others were considered non-hyperdivergent.

Creation of Heatmap: Once the hyper-divergent regions were identified, a spreadsheet was created for both hyper-divergent and non-hyper-divergent genes. Both files contained the *ugt* names on the rows and the strain names in the columns. The total number of nucleotide variations in each strain for each *ugt* was also added. The non-hyper-divergent and hyper-divergent spreadsheet files were added to a working MATLAB® script. They were then used to create two cluster grams to help visualize variation trends, if any. (Fig. 1B and Fig. 1C). Figure 1B was given a red-blue color map whereas 1C was given a red-green to help differentiate between the data. All scripts are available on GitHub.

Acknowledgements:

We would like to thank Prof. Erik Andersen, Orr Shalev, Jacob Salomon, Joshua Eli Mermelstein, Jacob “Slav” Slavkin, Benjamin Surasky, Megan McElroy, Sheza Mehdi, Eli Benveniste, Aleya Johnson, Bailey Nicolas, and Hao Nguyen for their assistance in our project. We would also like to acknowledge the Vertically Integrated Projects (VIP) Team under the Edison Lab from The University of Georgia.

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CHAPTER 7

DISCUSSION AND FUTURE DIRECTIONS

I have shown my work elucidating mechanisms of xenobiotic detoxification in *C. elegans* using 1-HP as an example. I have shown the role of *ugt* genes in this process and the great diversity in this gene family. I have also shown how, in this context, I have researched the career development of postgraduate researchers, specifically transitioning into doctoral programs and mentoring undergraduate researchers. In this final chapter, I will discuss potential avenues for continuing research on these topics and the possible implications of my findings.

7.1 Future Directions and Implications for Research on South Asian International (SAI) Student Transitions.

My research on SAI students shows that certain challenges this population faces during their transition to doctoral programs are unique to them. SAI students have suboptimal research opportunities in their home countries and need more contacts in their network in the US. As a result, in the initial phases of their doctoral education, they rely on support from other South Asians and their research mentors for research and non-research-related issues. One approach to smooth the transition for South Asian international students is bridge programming. Bridge programs have already been shown to be successful for students, especially historically minoritized communities in individual institutions (Ghazzawi, Pattison, & Horn, 2021; Rudolph, Holley-Bockelmann, & Posselt, 2019). Bridge programs should focus on international student issues, such as introducing them to basic lab techniques and helping students with writing workshops to improve their formal communication skills.

Furthermore, SAI students rely heavily on peers for research and personal support. Programs could introduce near-peer mentoring programs, specifically amongst international students nationally, and not just focused at an institutional level to facilitate these interactions. Near-peer mentoring programs have been shown to be successful, and scaling up these support systems that already exist in some form or another in most institutions will help alleviate acculturative stress (Thomson & Esses, 2016). Another action where institutions have some limited agency to act is hiring culturally competent therapists and promoting online therapy to support international students with mental health issues that arise from graduate school. However, I believe that tackling this problem also requires assessing ways to improve the graduate school experience to reduce the levels of stress and anxiety that students of all backgrounds tend to experience (Anandavalli, Borders, & Kniffin, 2021; Posselt, 2021). There also needs to be an evaluation of the attitudes of South Asian communities toward mental health to understand how to promote these communities' use of mental health services.

Future research on this topic should explore the extent to which the issues identified in my research are experiences within the broader SAI student community. I suspect that issues may vary based on whether students are based in larger urban centers with immigrant communities or more rural, less diverse areas. Issues might also vary based on religious and national background within South Asia. While my research is a good starting point, more research at a granular level and larger samples would benefit these students and improve student outcomes in the long run.

7.2 Future Directions and Implications for Research on Postgraduate Mentoring of Undergraduates for Talent Development.

My research on postgraduate mentoring of undergraduate mentors attempted to bring novelty to this field in two ways. Firstly, it connected the field of mentoring research with the field of talent development. Research in both fields focuses on student development and has grown in recent years (Acevedo-Duque et al., 2022; Byars-Winston, Dahlberg, & National Academies of Sciences Engineering and Medicine (U.S.), 2019; Carlsson-Wall, DeMott, & Ali, 2023; Pedersen et al., 2022), yet these fields remain largely separated. Talent development is a broader field within psychology with implications ranging from undergraduate research to youth sports teams. This research also brought novelty to the field by providing a guide to implementing team-based research informed by the literature on mentoring. Again, team-based research is not a new concept, with vertically integrated projects (VIP) programs have been around since 2006 and course-based undergraduate research experiences (CUREs) having been around for a similar period (Coyle, Allebach, & Krueger, 2006; Dolan, 2016). Unfortunately, much of the research on CUREs has developed separately from the research on VIP programs, which is spearheaded by the engineering community. Research on both in the same context is relatively limited, and my research adds to it. My research is also an attempt at bridging and bringing these related research areas together.

If I was to continue doing research in this field, I would like to see the implementation of our strategies in other labs and then compare outcomes with students in other CUREs and other undergraduate research settings in general. This would allow us to comment on the effectiveness of our proposed methods and validate our work further.

7.3 Future Directions and Implications for Research on Role of *ugt* Genes in Xenobiotic Detoxification in *Caenorhabditis elegans.*

My research on xenobiotic detoxification started as a means to show the involvement of *ugt* genes in 1-HP detoxification. However, our results have opened up lots of exciting avenues of research. Firstly, we were able to show that more than one *ugt* gene is involved in the detoxification of 1-HP. This means there is either a case for sequential glycosylation with different *ugt* involved in different aspects of the detoxification process. Alternatively, there is some degree of redundancy with more than one gene able to perform the same function. While we do not have definitive evidence for either, we plan to test mutants with attenuated function for our genes of interest, *ugt-23* and *ugt-49.* The results from mortality and UV assays with that mutant should shed some light on this matter. In prior research on worm exposure to indole, we have seen that multiple *ugt* genes are upregulated upon indole exposure (Lee et al., 2017). This is despite the fact that *C. elegans* only produce one glycosylated indole product (Stupp et al., 2013). These results suggest that determining the glycosylation mechanism will significantly enhance our understanding of xenobiotic detoxification and should be a future study area.

A second interesting finding from my research is the modification of 1-HP with N-acetylglucosamine (glcNAc). GlcNAc modification has previously been observed in proteins, where glcNAc has been shown to be added to serine or threonine residues by the enzyme *ogt-1* (Ma, Wu, & Hart, 2021). While glcNAc is a known important component of glycans linked with several different essential functions such as skeletal muscle regulation, diabetes, neurological disorders, and immunity (Lambert, Claeyssen, Bastide, & Cieniewski-Bernard, 2020; Ma et al., 2021; Saha & Fernandez-Tejada, 2023), we have shown glcNAc's role in modifying xenobiotics, which to my knowledge has not been shown elsewhere.

GlcNAcylation is controlled by the *ogt-1* gene, a lack of which is lethal in most organisms but surprisingly not in *C. elegans.* This gene has been shown to have a non-catalytic role in *C. elegans* function, such as fertility (Konzman, Fukushige, Dagnachew, Krause, & Hanover, 2022). However, our discovery is unique in that we have implicated glcNAcylation in small molecule xenobiotics, which, to my knowledge, has not been observed before. What is more, is that this research has also implicated genes other than *ogt-1* in glcNAcylation. This leads to important questions that are possible future directions for research. Firstly, we should test other xenobiotics, such as albendazole and indole, for possible glcNAcylated derivatives. It would also be interesting to investigate the role glcNAcylation plays in the immune response. Is it to detoxify compounds by adding a polar residue to make it easier to excrete the xenobiotic, or perhaps could there be a role in signaling to other cells about the presence of the toxin. Finally, this research implicates *ugt* genes in adding glcNAc in *C. elegans.* Further experiments to verify this could be performed. Considering that *C. elegans* is one of the only organisms known to survive without the *ogt-1* gene, the role of other genes in glcNAcylation seems plausible.

We have also investigated the diversity of *ugt* genes across *C. elegans* wild isotypes. This research has allowed us to catalog genes in this family with or without hyper-divergent regions, which isotypes the hyper-divergent regions exist in, the number of mutations for each gene across isotypes, and what those mutations are. This research can be the basis for future studies identifying mutations that repeat in different isotypes and identifying their impact on the structure of the particular *ugt* using protein structure simulators like AlphaFold®. Of course, we have to remember the possible impact of these mutations on splice sites and the unknown impact of intron mutations. However, this avenue of research could potentially open up our

understanding of how different worm isotypes have evolved to adapt to different chemical and environmental cues.

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SUPPLEMENTAL MATERIAL FOR CHAPTER 2

Supplemental online materials for

Understanding the Unique Factors Affecting South Asian International (SAI) Student Transitions into PhD Programs in the US: An Interpretative Phenomenological Analysis

This supplement contains the following:

Appendix 1: Screening Questionnaire

Graduate Transition Study

University of Georgia

Thank you for your interest in our research! Before responding to any of these questions, please take a moment to reflect on your experience in graduate school so far.

1) When did you start graduate school? (Select one)
   - Fall 2019
   - Summer 2019
   - Before Summer 2019

   If the response is before summer 2019 take participant to the end of the survey. If not, continue to next question.

2) What department/program are you enrolled in? (Open response)

3) What University are you enrolled in? (Open response)

4) Can you briefly describe the nature of your research? (Select one)
   - Mostly wet lab/bench research
   - Mostly theoretical, informatic or computational research
   - Mostly research conducted in the field or outdoor research
   - Mostly research conducted in clinical settings

5) What is your Nationality? (Select one)
   - Afghan
   - American (US)

- Bangladeshi
- Bhutanese
- Indian
- Maldivian
- Nepali
- Pakistani
- Sri Lankan
- Other

If the response is American, proceed to question 6, otherwise proceed to question 7.

6) With which race(s) or ethnicity/ies do you most closely identify? Please choose all that apply.
    - American Indian, Alaskan Native, or Native American
    - Black or African American
    - East Asian (e.g., China, Japan, Korea)
    - Hispanic or Latino/Latina
    - Native Hawaiian or Pacific Islander
    - North African or Middle Eastern
    - South Asian (e.g., India, Sri Lanka, etc.)
    - White
    - Other: Please Explain
    - Prefer not to respond

7) What gender do you most closely identify with?
    - Male
    - Female
    - Other: Please Explain

Thank you for completing this survey! We may want to contact you for multiple interviews to help us understand the process of transition into graduate school for South Asian international and native students. If you choose to participate, you will receive a $25 Amazon/Walmart (whichever you prefer) gift card per interview.

If you are willing, please enter your name and email address below. Please note that we will keep your information confidential but we need your name and contact information in order to schedule an interview.

First and Last Name:

Email Address:

Thank you for participating!

Appendix 2: Interview Questions

Two interview protocols were used: one for native (US) students and one for South Asian International (SAI) students. The SAI student protocol included questions about their experience in the US, which were not included in the protocol for native students.

South Asian International Students

1) I would like to start out by getting to know you a little better. Would you like to tell me about your research interests? How did they develop?
2) How did you decide to go to graduate school? How did you decide to pursue a PhD?
3) How did you decide that the US was the right place to further your education?
4) I'd like to learn more about your experience in graduate school so far. Please rate your experience thus far.

Graduate school has

5= fully met my expectations

4= met most of my expectations

3= met some of my expectations

2= fallen short of most of my expectations

1= not met any of my expectations

Please explain your rating

Follow up with: In what ways has graduate school lived up to your expectations? In what ways has it not met your expectations?

5) How has your experience in the US been so far? Please rate your experience thus far. Being in the US has:

5= fully met my expectations

4= met most of my expectations

3= met some of my expectations

2= fallen short of most of my expectations

1= not met any of my expectations

Please explain your rating

Follow up with: In what ways has being in the US lived up to your expectations? In what ways has being in the US not met your expectations?

6) I am interested in the experience of transitioning into graduate school. What has helped you transition into graduate school? Who has helped you make the transition? What has made it difficult for you to transition into graduate school?
7) If I understand correctly, your transition to graduate school also involved a transition to living in the U.S. What has helped you transition to the U.S? What has made it difficult for you to transition to the U.S.?
8) As you might be aware, there has been a lot of research documenting a mental health crisis amongst graduate students. In what ways has your experience during your

graduate school transition benefitted your mental health? In what ways has the experience made it worse?

9) In what ways has your experience during your transition to life in the U.S. benefitted your mental health? In what ways has the experience made it worse?
10) Tell me about the process for selecting a research lab in the program. How long does each rotation last if you have any? How many rotations do you have? By when do you have to select a research lab?
11) Do you feel as though your experience in finding rotations (if you had rotations) has been easier or harder than your peers? Please explain.
12) I am particularly interested in your experience as a South Asian International student. Do you have a sense that being from (nation) has affected your transition to grad school? Why or why not?
13) Has anything about your cultural background affected your transition into graduate school? Why or why not? Are you religious? If so, has anything about your religious background affected your transition into graduate school? Why or why not?
14) Has anything about your cultural background affected your transition into the U.S.? Why or why not? Are you religious? If so, has anything about your religious background affected your transition into the U.S.? Why or why not?
15) Is there anything else you would like to tell us?

Native students

1) I would like to start out by getting to know you a little better. Would you like to tell me about your research interests? How did they develop?
2) How did you decide to go to graduate school? How did you decide to pursue a PhD?
3) I'd like to learn more about your experience in graduate school so far. Please rate your experience thus far.

   Graduate school has

   5= fully met my expectations

   4= met most of my expectations

   3= met some of my expectations

   2= fallen short of most of my expectations

   1= not met any of my expectations

   Please explain your rating

   Follow up with: In what ways has graduate school lived up to your expectations? In what ways has it not met your expectations?

4) I am interested in the experience of transitioning into graduate school. What has helped you transition into graduate school? Who has helped you make the transition? What has made it difficult for you to transition into graduate school?
5) As you might be aware, there has been a lot of research documenting a mental health crisis amongst graduate students. In what ways has your experience during your graduate

school transition benefitted your mental health? In what ways has the experience made it worse?

6) Tell me about the process for selecting a research lab in the program. How long does each rotation last if you have any? How many rotations do you have? By when do you have to select a research lab?
7) Do you feel as though your experience in finding rotations (if you had rotations) has been easier or harder than your peers? Please explain.
8) Has anything about your cultural background affected your transition into graduate school? Why or why not? Are you religious? If so, has anything about your religious background affected your transition into graduate school? Why or why not?
9) Is there anything else you would like to tell us?

SUPPLEMENTAL MATERIAL FOR CHAPTER 5

Strain Name	Genotype	Gene Sequence	Description	Reference Article	Hours from egg to L4
N2	-	-	-	(Brenner, 1974)	~ 42 hours
PD1074	-	-	-	(Yoshimura et al., 2019)	~ 42 hours
RB2055	ugt-1(ok2718) V.	AC3.7	Homozygous. Outer Left Sequence: AGCCATGAGGACAAAGTTCG. Outer Right Sequence: TGTTGAAAATGCTTTGCCAG. Inner Left Sequence: TTGCTCTTCCATGTCTCGAA. Inner Right Sequence: TCGTCTAGATTCCGCCATTT. Inner Primer PCR Length: 1255 bp. Deletion Size: 787 bp. Deletion left flank: TTGCATTTCCAACACCATCACT TCCAAAAC. Deletion right flank: TTTGTTCAGTACTACATGTTAG ATGCTTTT.	C. *elegans* Gene Knockout Project at the Oklahoma Medical Research Foundation, International C. *elegans* Gene Knockout Consortium	~ 44 hours
VC4207	ugt-6(gk5292[loxP + myo-2p::GFP::unc-54 3' UTR + rps-27p::neoR::unc-54 3' UTR + loxP]) V.	ZC455.4	Homozygous viable. Deletion of 1469 bp with Calarco/Colaiacovo selection cassette conferring myo-2 GFP and G418 resistance inserted at break. Left flanking sequence: GCGTGTTTTACCATCAGATCAG TTGGCGTG ; Right flanking sequence: GATGGAATATGCTGCCTTCCAA GTTCATCA.	(Au et al., 2019)	~ 42 hours
VC3950	ugt-9(gk5024[loxP + myo-2p::GFP::unc-54 3' UTR + rps-27p::neoR::unc-54 3' UTR + loxP]) V.	T19H12.1	Homozygous viable. Deletion of 1266 bp with Calarco/Colaiacovo selection cassette conferring myo-2 GFP and G418 resistance inserted at break. Left flanking sequence: AAGTTTTCGGAAGGCTTTCTGT AGGGTGAA; Right flanking sequence: GGAGGTGCTGTTGCGTACGACA AATTTGAT.	-	~ 42 hours

RB2550	ugt-23(ok3541) X.	C17G1.3	Homozygous. Outer Left Sequence: cgtgacgctttagcatttca. Outer Right Sequence: tcattgatgccgatgaagaa. Inner Left Sequence: ttgatcagcgaatattggga. Inner Right Sequence: atgcacattctcatcttgcg. Inner Primer PCR Length: 1197. Estimated Deletion Size: about 300 bp.	C. *elegans* Gene Knockout Project at the Oklahoma Medical Research Foundation, International C. *elegans* Gene Knockout Consortium	~ 42 hours
RB2607	ugt-49(ok3633) V.	AC3.2	Homozygous. Outer Left Sequence: cgtgtgatggtgacaagacc. Outer Right Sequence: agaacagcaacgaacacgaa. Inner Left Sequence: acgtggcattcagtgaacaa. Inner Right Sequence: ggacaaaagcaataacatcaaga. Inner Primer PCR Length: 1279. Estimated Deletion Size: about 400 bp.	C. *elegans* Gene Knockout Project at the Oklahoma Medical Research Foundation, International C. *elegans* Gene Knockout Consortium	~ 44 hours
VC2512	ugt-60(ok3248) III/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III).	C07A9.6	Homozygous lethal deletion chromosome balanced by bli-4- and GFP-marked translocation. Heterozygotes are WT with pharyngeal GFP signal, and segregate WT GFP, arrested hT2 aneuploids, and non-GFP ok3248 homozygotes (probable early larval arrest). Homozygous hT2[bli-4 let-? qIs48] inviable. Pick WT GFP and check for correct segregation of progeny to maintain. External left primer: GAAGGTTTCGGACTTGTTGC. External right primer: CGCATCCACTTTCTTCAGGT. Internal left primer: CTGAGAGCATCGCGGATAGT. Internal right primer:	C. *elegans* Reverse Genetics Core Facility at the University of British Columbia, international C. *elegans* Gene Knockout Consortium	~ 50 hours

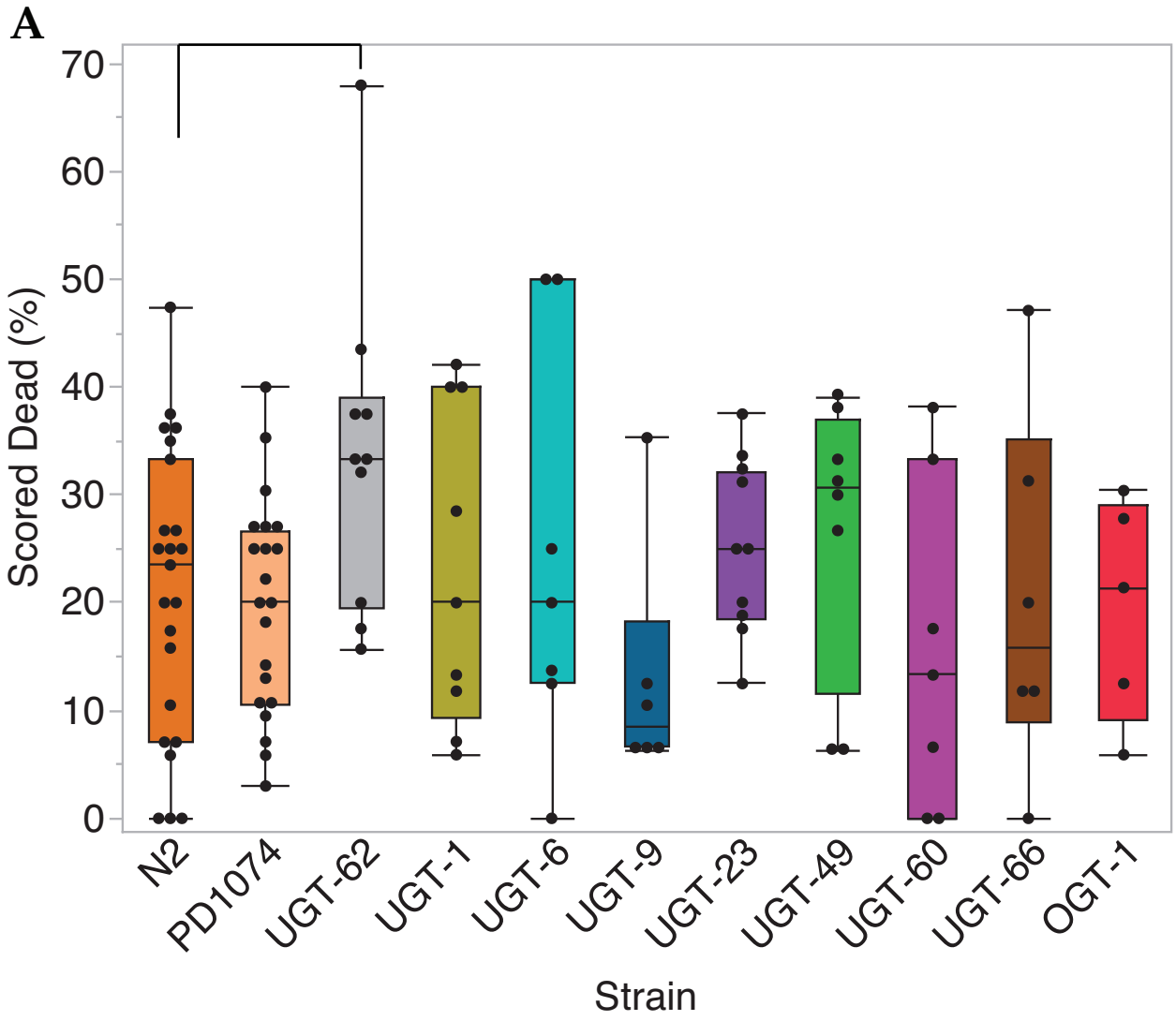
			TGACGCGTCTAGCTCAATTTT. Internal WT amplicon: 1354 bp. Deletion size: 525 bp. Deletion left flank: TATAGCCTCCATGTGCAATCATTAATTTCA. Deletion right flank: AACCTCGATAGAACAAATTCTCGTCAACGA.		
RB2011	ugt-62(ok2663) III.	M88.1	Homozygous. Outer Left Sequence: AAACATGGTTCCCGACATTC. Outer Right Sequence: AATTCGGTGCATTTGGAAAA. Inner Left Sequence: GCAACTTTGGAATTTTTGGG. Inner Right Sequence: ATCAGATTTCCTGCGCAACT. Inner Primer PCR Length: 3024 bp. Deletion Size: 1367 bp. Deletion left flank: ACGCTAAATTGTTTTAATACATTTTAAAGT. Deletion right flank: ATGAAATATTTCTCGATTAAAGTTTCTCAG.	C. *elegans* Gene Knockout Project at the Oklahoma Medical Research Foundation, International C. *elegans* Gene Knockout Consortium	~ 46 hours
VC4339	ugt-66(gk5422[loxP + myo-2p::GFP::unc-54 3' UTR + rps-27p::neoR::unc-54 3' UTR + loxP]) III.	C23G10.6	Deletion of 2473 bp with Calarco/Colaiacovo selection cassette conferring myo-2::GFP and G418 resistance inserted at break. Left flanking sequence: AAAATTTCAAAATATTAAATGAAGCCGTTG; Right flanking sequence: CAGGGAGGTGTCACAATTATTTGTGTCCTG.	(Au et al., 2019)	~ 42 hours
RB1342	ogt-1(ok1474) III.	K04G7.3	Homozygous. Outer Left Sequence: gccaaagaattgatttcgga. Outer Right Sequence: tgctcttgcaccacaaccta. Inner Left Sequence: acctgtccgagaccattctg. Inner Right Sequence: ccaacgctattgctcctctc. Inner Primer PCR Length: 2730. Estimated Deletion Size: about 1300 bp.	C. *elegans* Gene Knockout Project at the Oklahoma Medical Research Foundation, International C. *elegans*	~ 43 hours

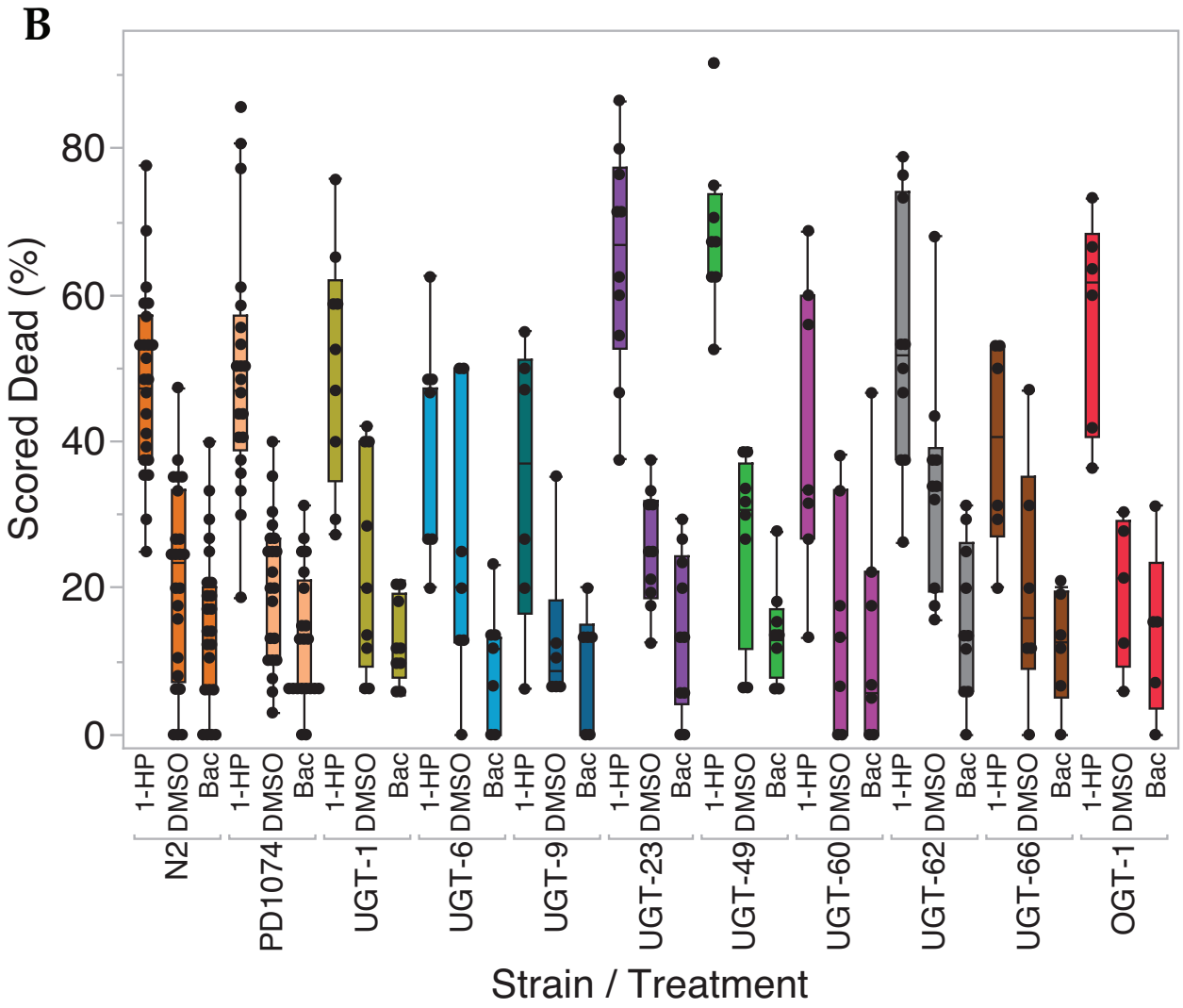
				Gene Knockout Consortium	

Supplemental Table 1. Detailed information on strains used in this study, including strain name, genotype affected, gene sequence, deletion strategy, reference article where strain is first mentioned, and time each strain takes to grow to L4. All strains were obtained from Caenorhabditis Genetics Center (CGC).

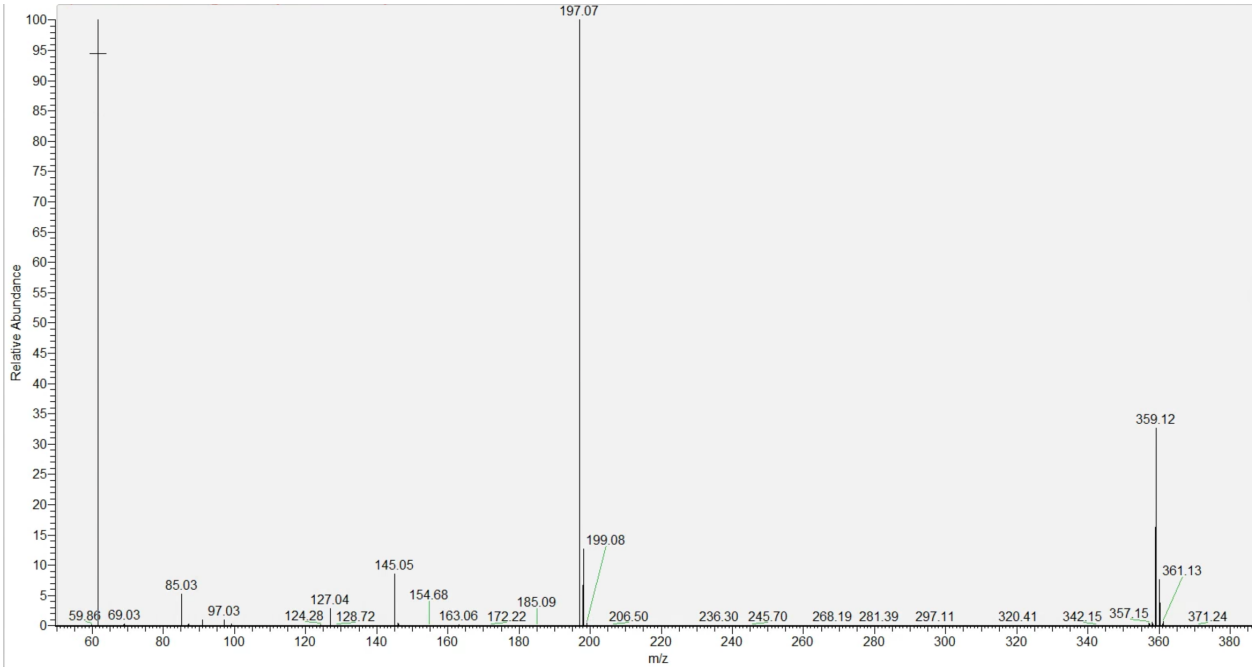
Compound	**Ion**	**Fraction Number**	**Ion Formula**	**Calculated *m/z***	**Observed *m/z***
b-D-glucopyranosyl-phenazine	$[M+H^+]$	3	$C_{18}H_{19}N_2O_6^+$	359.124	359.1240
b-D-glucopyranosyl (1-6)-b-D-glucopyranosyl-phenazine	$[M+H^+]$	2	$C_{24}H_{29}N_2O_{11}^+$	521.177	521.177
b-D-glucopyranosyl (1-6)- [b-D-glucopyranosyl (1-2)]-b-D-glucopyranosyl-phenazine	$[M+H^+]$	1	$C_{30}H_{39}N_2O_{16}^+$	683.229	683.23
b-D-glucopyranosyl (1-6)- [b-D-N-acetylglucosamine-pyranose (1-2)]-b-D-glucopyranosyl-phenazine.	$[M+H^+]$	1	$C_{32}H_{42}N_3O_{15}^+$	724.3532	724.257

Supplemental Table 2. High-resolution MS data for 1-HP derived C. elegans metabolites acquired using positive-ion electrospray ionization (ESI). Representative *m/z* is shown for N2.

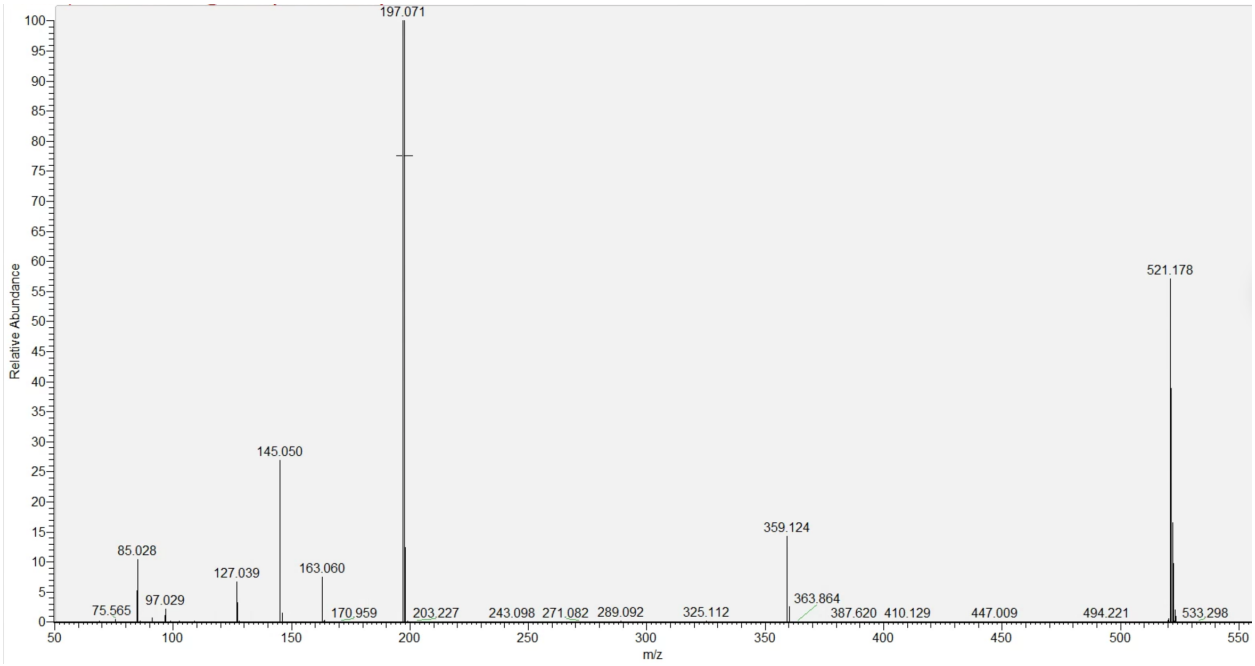

A
Scored Dead (%)
70
60
50
40
30
20
10
0
N2
PD1074
UGT-62
UGT-1
UGT-6
UGT-9
UGT-23
UGT-49
UGT-60
UGT-66
OGT-1
Strain



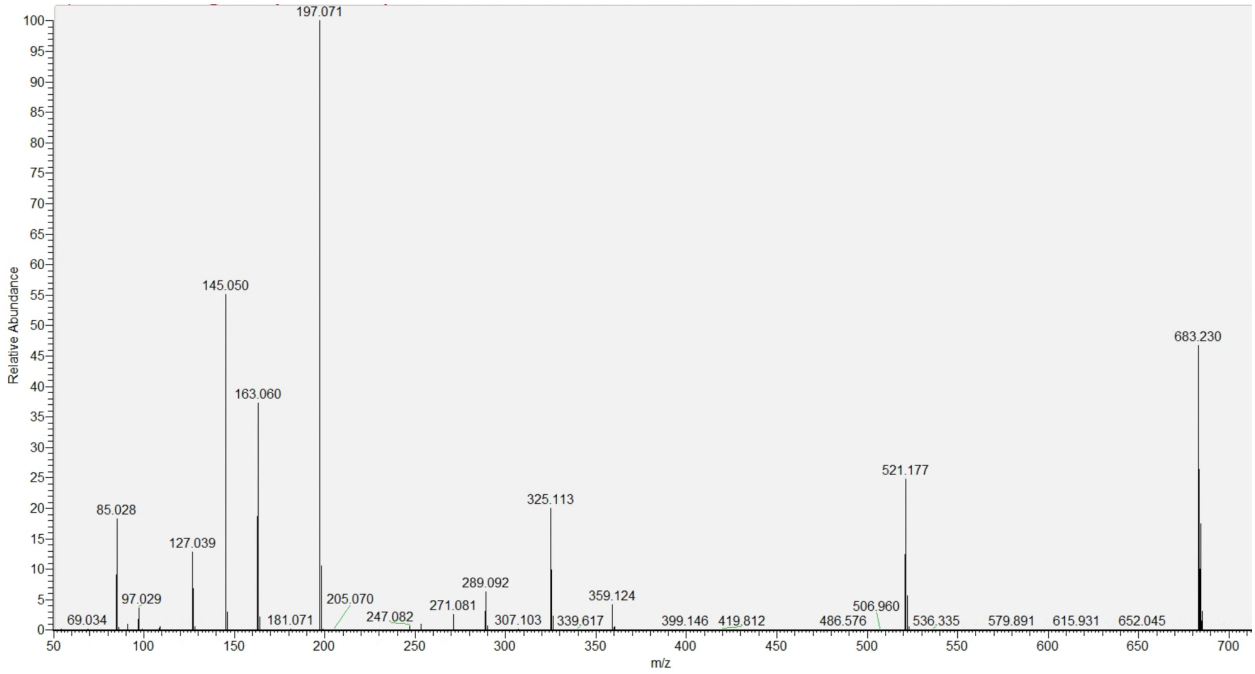
Supplemental Figure 1. Percentage of worms scored in plate-based mortality screen after 7 h incubation. (A) Box and whiskers plot showing mortality of various strains to 1.1% DMSO for 7h (n >/= 6). Line indicates significant mortality compared to N2 and PD1074 ($\alpha = 0.05$) after a Wilcoxon Pairwise comparison. (B) Box and whiskers plot showing combined data of exposure to 179µM 1-HP, 1.1% DMSO and bacteria control showing increased mortality upon 1-HP exposure for each strain compared to controls after Tukey's HSD test ($\alpha = 0.05$).



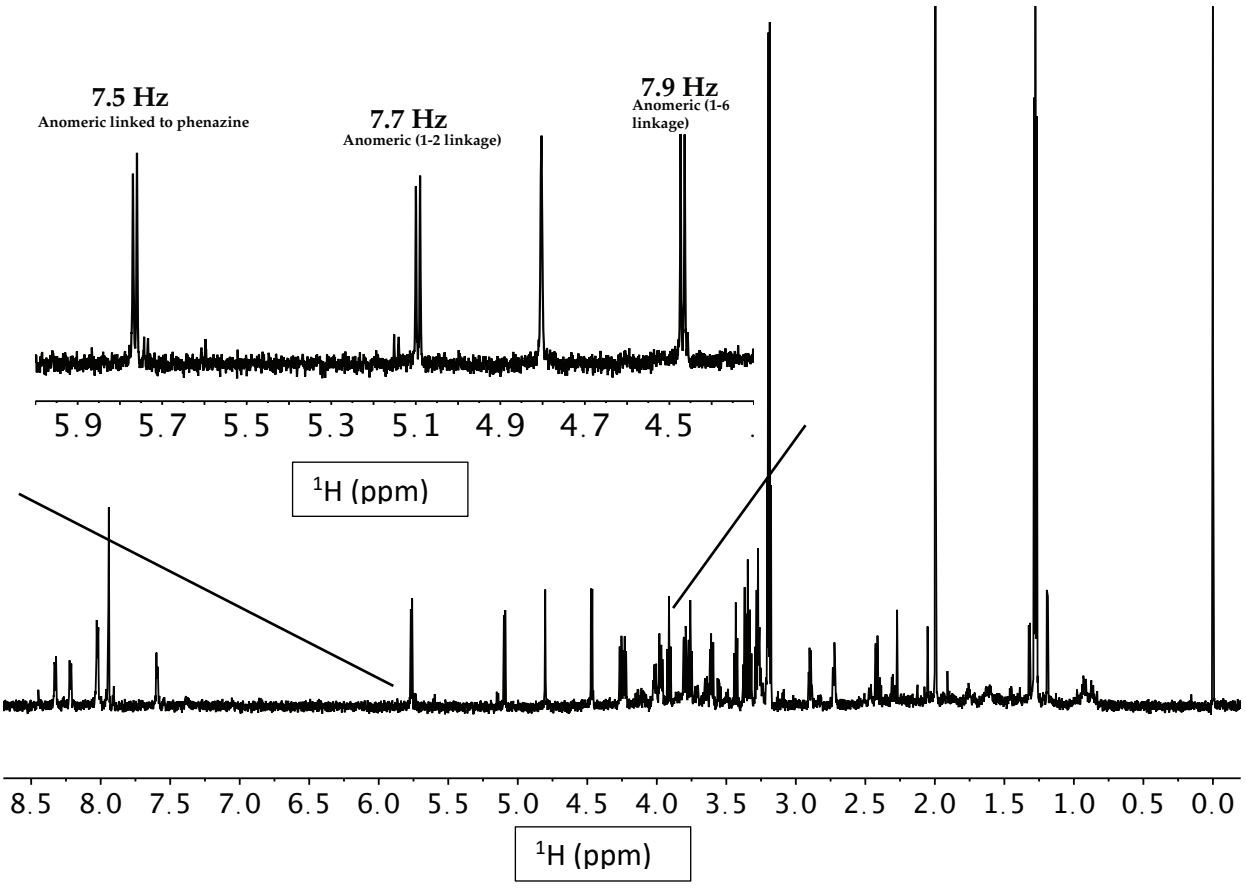
Supplemental Figure 2. MS/MS data for (2).



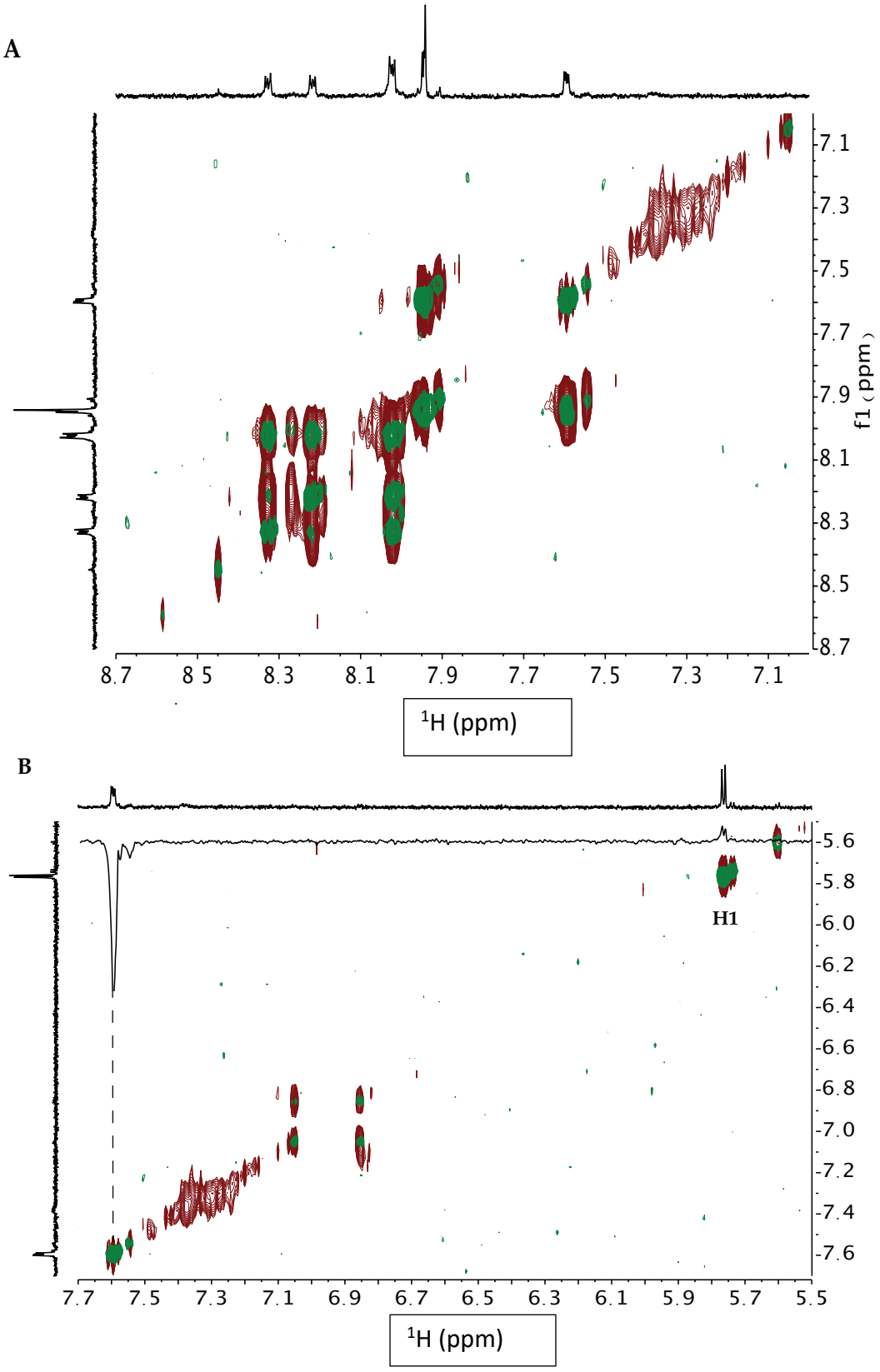
Supplemental Figure 3. MS/MS data for (3).

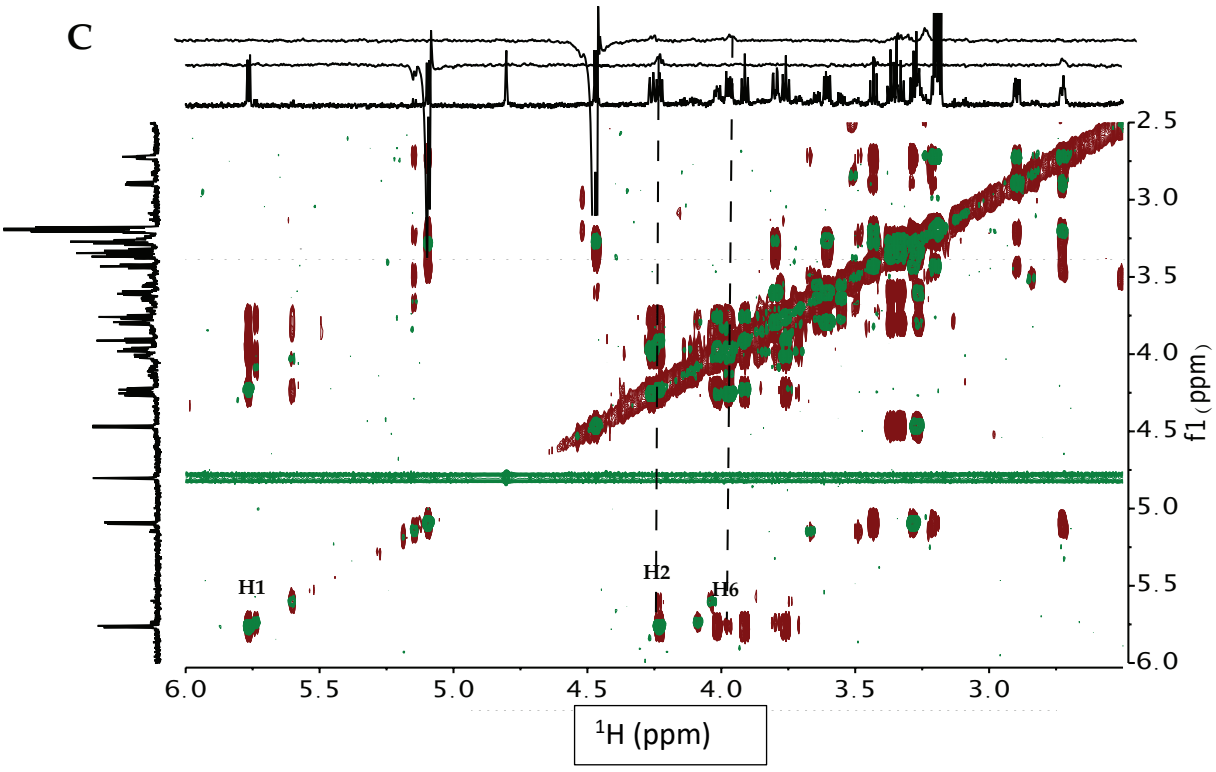


Supplemental Figure 4.1. MS/MS data for (4).

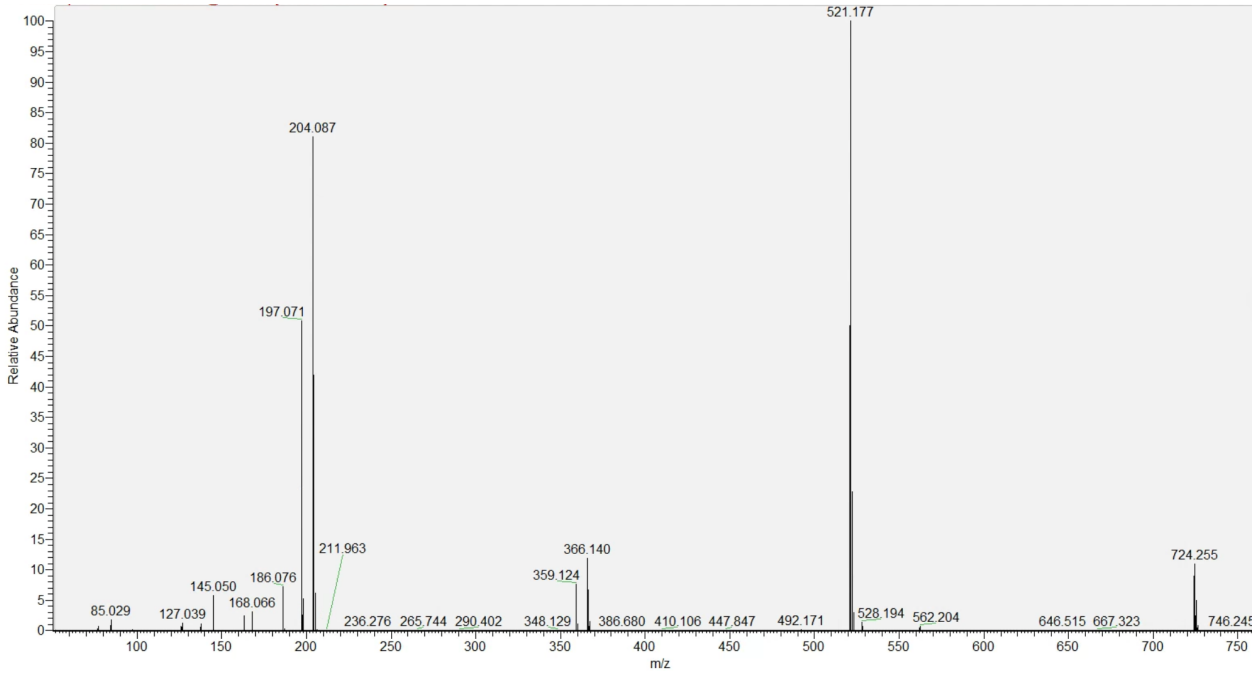


Supplemental Figure 4.2. ^{1}H NMR spectrum (800 MHz, D_2O) of (4). Expansion shows the anomeric protons with coupling constants above the peaks.

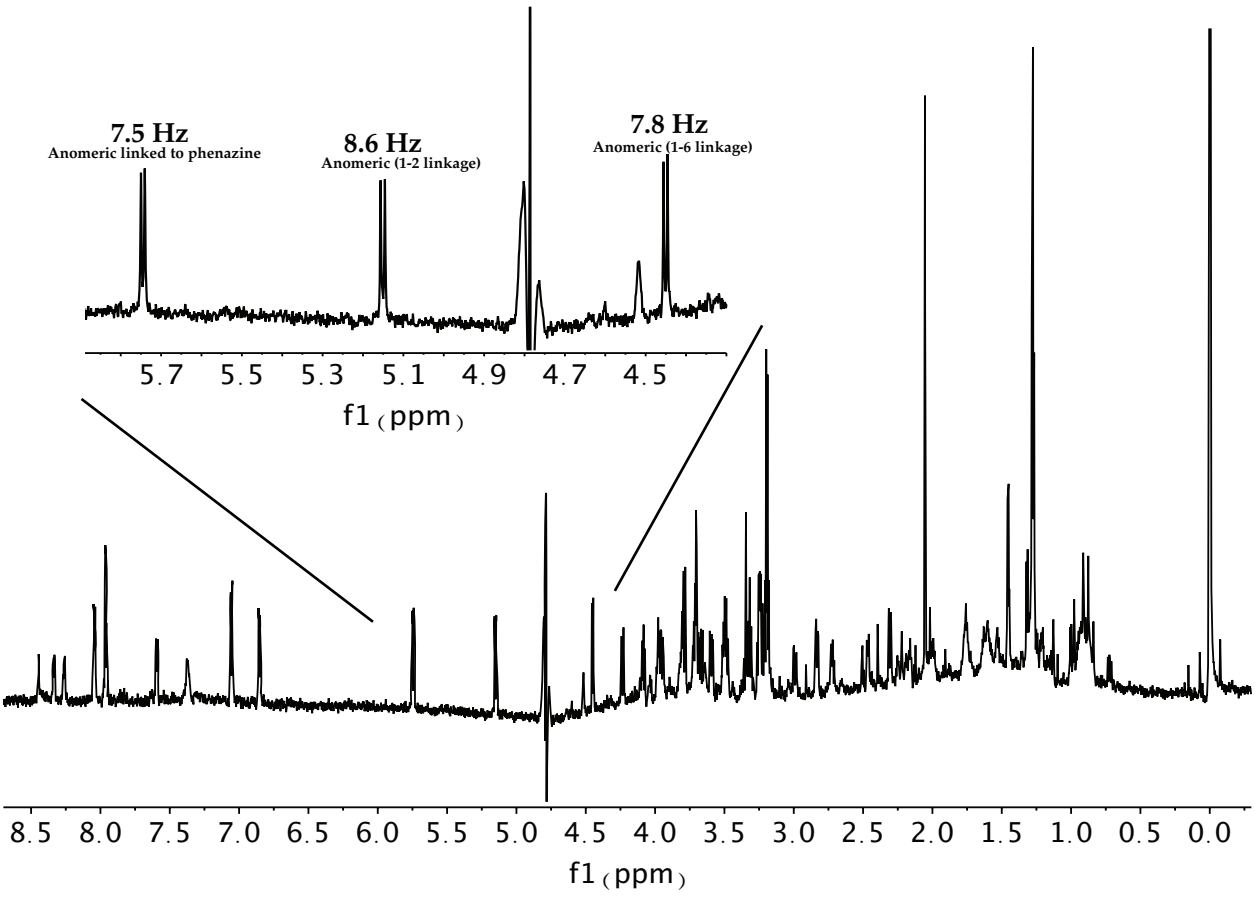




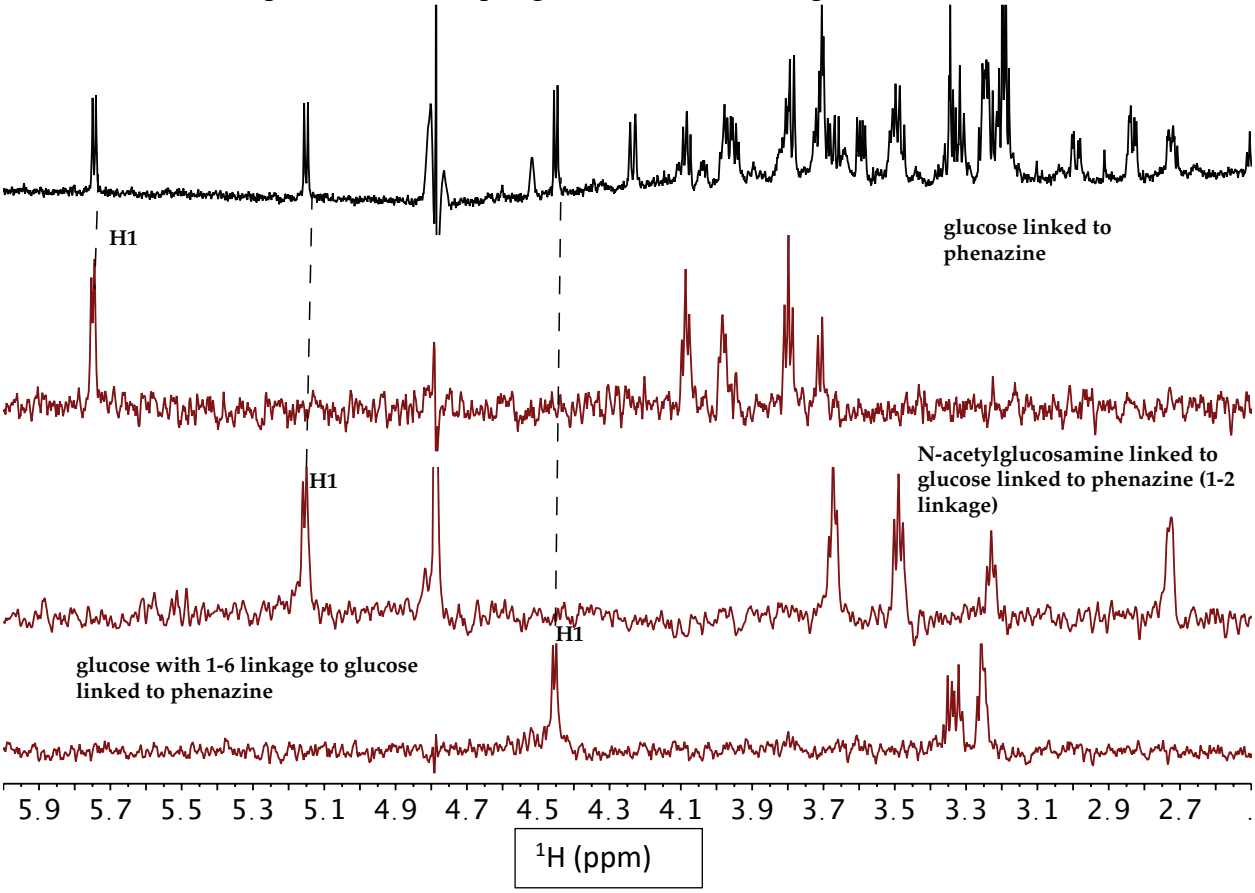
Supplemental Figure 4.3. 800MHz TOCSY Spectra (red) overlayed with dqfCOSY spectra (green), and relevant selective 1D ROESY traces shown for compound **4**. (A) shows the phenazine region. (B) shows the phenazine linkage with the anomeric at ~5.7 PPM. (C) shows the sugar region with selective 1D ROESY showing the 1-2 and 1-6 glucose linkage.



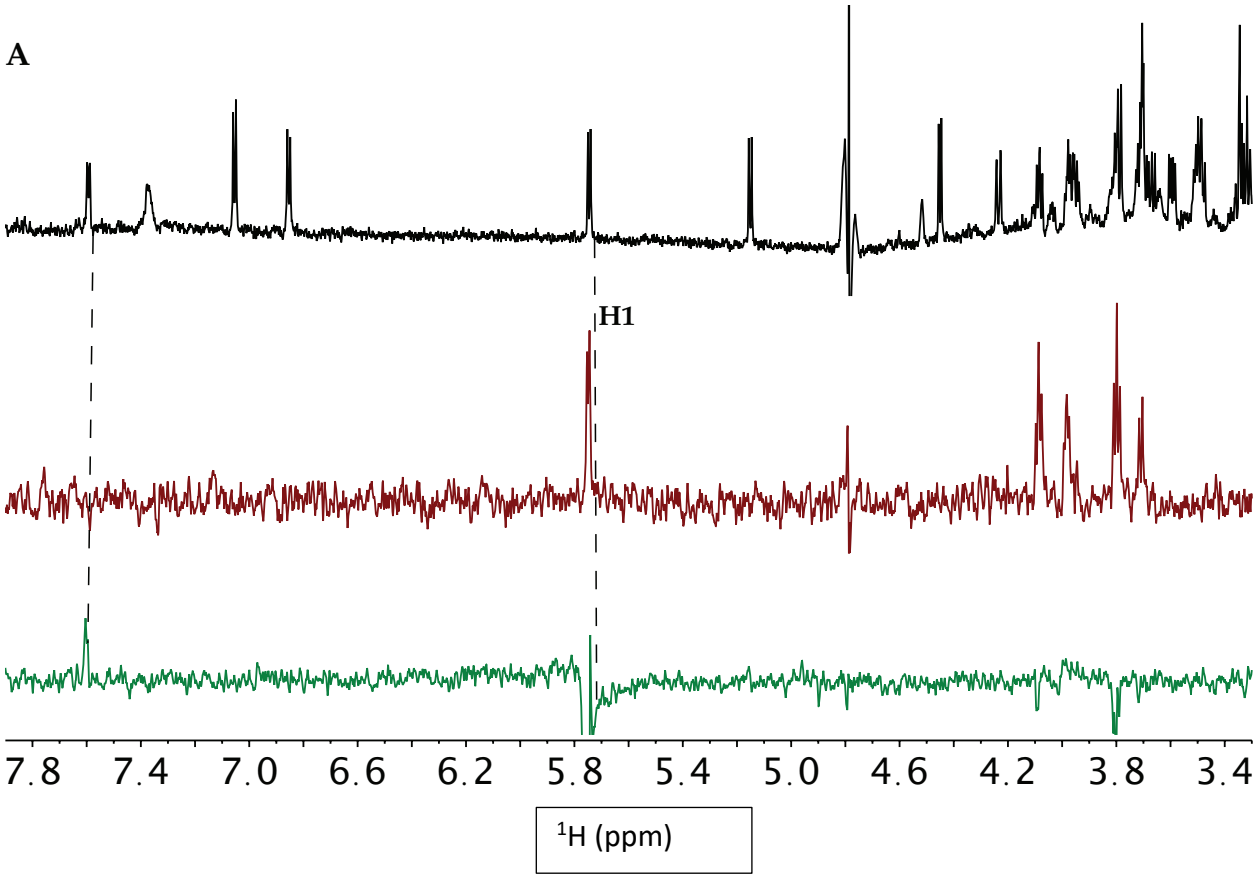
Supplemental Figure 5.1. MS/MS data for (5).

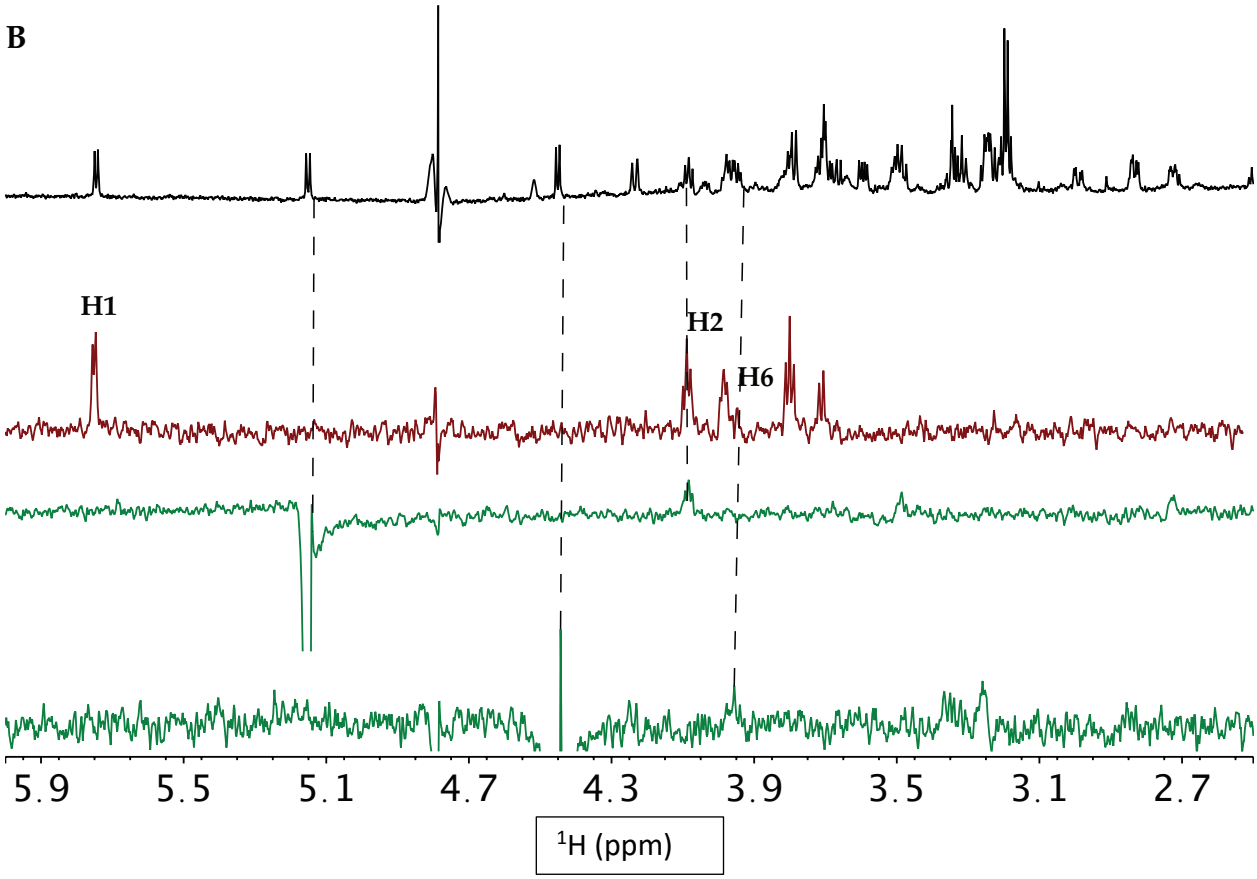


Supplemental Figure 5.2. ^{1}H NMR spectrum (800 MHz, D_2O) of compound **5**. Expansion shows the anomeric protons with coupling constants above the peaks.

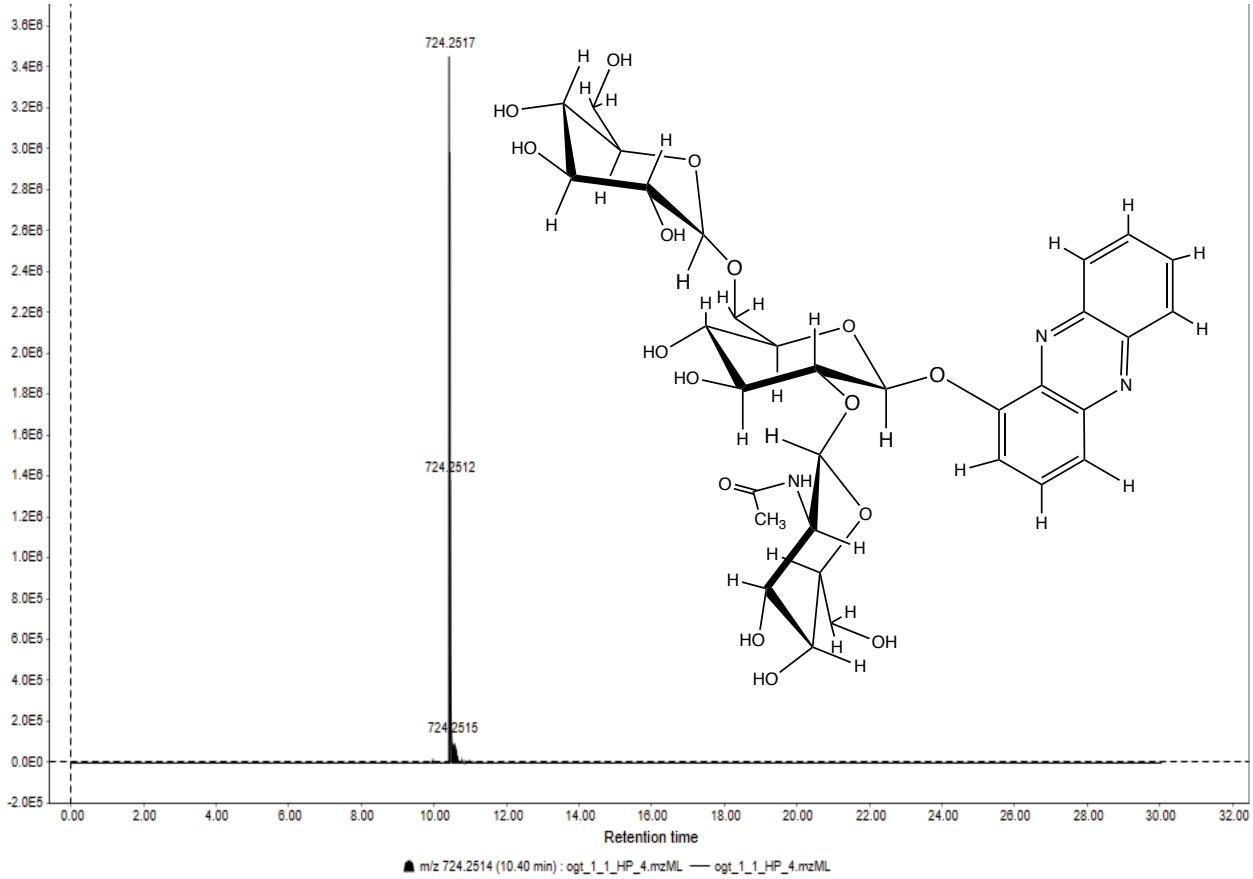


Supplemental Figure 5.3. ^{1}H NMR spectrum with selective 1D TOCSY (maroon) (800 MHz, D_2O) for each anomeric of (**5**).

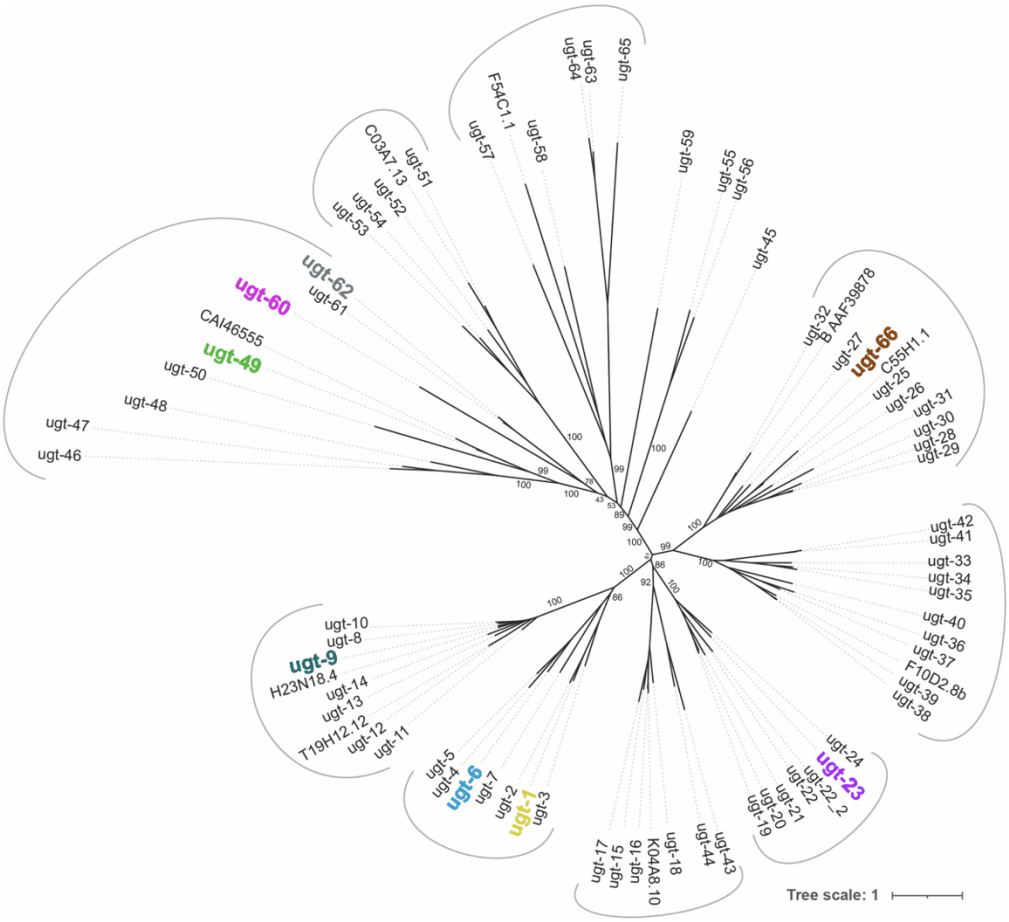

A
H1
7.8
7.4
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
^{1}H (ppm)



Supplemental Figure 5.4. ^{1}H NMR, selective 1D TOCSY (maroon) and selective 1D ROESY (green) spectra (800 MHz, D_2O) for (**5**). (A) spectra showing the phenazine region connecting with the anomeric at ~5.7ppm. (B) spectra showing the 1-2 and 1-6 linkage on the sugar linked to the phenazine.



Supplemental Figure 6. Feature spread for the m/z for (**5**) in the *ogt-1* knockout mutant exposed to 22.3 µM 1-HP.



Supplemental Figure 7. Phylogenetic tree highlighting *ugt* genes that have been used in this study.