Communicating Research to the General Public

The WISL Award for Communicating PhD Research to the Public launched in 2010, and since then over 100 Ph.D. degree recipients have successfully included a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance—as well as their excitement for and journey through their area of study—to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere, through the cooperation of PhD candidates, their mentors, and departments. WISL offers awards of $250 for UW-Madison Ph.D. candidates in science and engineering. Candidates from other institutions may participate, but are not eligible for the cash award. WISL strongly encourages other institutions to launch similar programs.

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Developing Mass Spectrometry-Based Strategies for the Enhanced Characterization of Biomolecules in the Nervous System

By
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Chapter for the General Public

Scientific research is generally viewed as a daunting undertaking to perform and an even more difficult task to understand the resulting conclusions and implications. Media tends to portray scientists as geniuses possessing knowledge and understanding that will never be reached by the average person. This is a major issue that leads to inaccessibility and potential distrust of the field. I deeply believe that as a community, the field of science should strive to improve communication of the scientific process and subsequent outcomes in a way that the general public can digest more easily. To help reach this goal and with the help of the Wisconsin Initiative for Science Literacy at the University of Wisconsin-Madison, I've decided to include a chapter in my PhD dissertation aimed at providing a glimpse into the research I've been performing over the past 4.5 years in a way that I hope everyone can understand. It is my desire that this chapter will help bridge the gap between fascinating science research and everyone's inherent desire to learn and know more.

During my graduate research, I studied neurons and neuronal signaling molecules. Specifically, I was drawn to the complexities of the interactions on a tiny molecular scale that we knew were present but were unable to see visually. I knew that tools existed to help us learn more about what exists, how much of it is there, and what it does, but I also knew that as a species, we were just at the beginning of developing techniques and methods to understand the human brain. The desire to help uncover the mysteries of neural communication eventually led me to my research at UW-Madison focused on developing and enhancing tools to probe different aspects of neurons.

Introduction

Neurons are a type of cell found in the brain and throughout your nervous system. They are important for all types of signaling and responses in the body (**Figure 1**). Think of neurons as a control center for a city. The neuron receives electrical impulses via receptors and communicates with the next neuron to lead to a downstream response or change, just as a control center dispatches and alerts police, fire department, child protective services, etc. All of this is contingent on properly receiving signals and sending out another signal correctly. Many things can go wrong from mishearing to misinterpreting, or not even receiving the call, leading to a cascade of issues where neurons downstream don't know how to respond.

One way to diagnose issues is based on observing the unintended outcomes to understand the root cause. An example would be in the case of a house burning down. A firetruck not arriving is a necessary observation to track its movements to know that traffic is causing a delay. Knowing this, a traffic control center can alter the traffic light pattern. Once a cause is understood fully, true solutions can be uncovered. This is the strategy that I pursued to understand differences in neuronal signaling. There are several types of biological molecules in the body, each with varying roles and comprised of different chemical compounds. Specifically, I study proteins and peptides, which are made of amino acids that are linked together to form

long chains, leading to a unique amino acid sequence. These proteins and peptides each have unique roles in the body. To probe the molecular mechanisms of neuron communication, one can look at the changes of different biomolecule categories, the neuropeptides. We can also examine the vehicles sent out by the "control center" neuron, that is, the proteins that enable the signal to be sent or received. Over the years, I've been conducting research that looks at both strategies by measuring changes in neuropeptides and proteins involved with these processes.

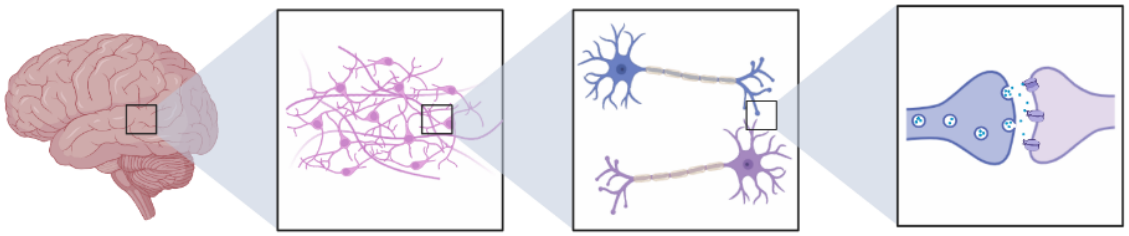

Figure 1. For the brain to communicate with itself and the rest of the body, it uses a network or neurons. A signal is transmitted from neuron to neuron in the form of neuropeptides and other biomolecules that are released by one neuron and received by the next.

The Technique

My lab does this using mass spectrometry, a technique that allows us to detect peptides and fragment them into smaller pieces, enabling us to determine the order of amino acids to identify the peptide. Some other techniques can only tell us the size of the peptide, and not what it is comprised of. This means they can't differentiate from peptides with different amino acid orders. For instance, if several people require assistance in a jewelry store, a photo can show how many people have entered the room, but only a video can show the order in which customers walked in to determine the order they should be helped. Mass spectrometry enables us to determine an exact peptide sequence order/identity, as demonstrated in **Figure 2**.

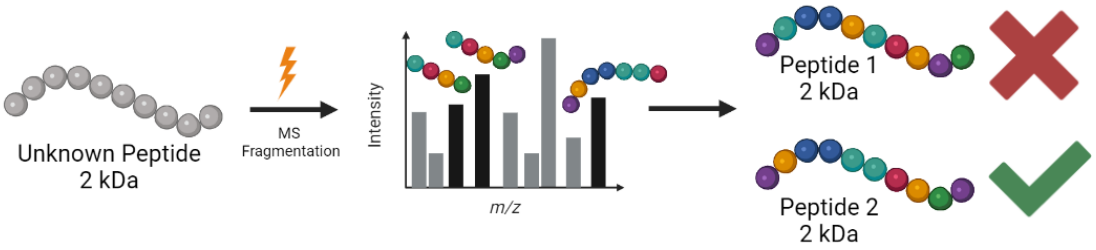

Figure 2. The mass of a peptide is detected through mass spectrometry. The peptide can then be fragmented into smaller pieces that are also essentially weighed. These fragments can be used to map an exact amino acid sequence of a peptide.

Proteins and peptides can be modified with a variety of post-translational modifications (PTMs). These are chemical changes to the amino acids, each of which can cause the protein to have different properties, such as changing its ability to move in and between cells, the duration of its lifetime, or its ability to bind to receptors. You can think of it as adding labels to items. Shipping labels will send them to different places, and different expiration dates will change how

long you keep them around. PTMs can cause a single peptide to have hundreds of different properties and roles. While the effects can be large, these peptide changes are not always predictable and can be difficult to detect. If a vehicle has a trailer attached, it can't drive as fast. In the end, the response is slower, but if you don't look at the trailer, you don't know why it is slower. There could also be no response received; if that trailer is too large to pass under a bridge, you wouldn't know how to adjust the route to enable it to reach the end.

Figure 3 demonstrates forms of glycosylation which is a common and complex PTM. Glycosylation describes the addition of several monosaccharides – sugar molecules that can be thought of like beads. These monosaccharides connect to form different glycans - like a bracelet made from different beads, in different orders - that can attach to a peptide. These glycans, or sugar chains, alter proteins, sometimes in a small way, but sometimes drastically. Like all PTMs, the effects of the addition of these "labels" vary widely due to the vast combinations that can exist. My research focuses on detecting and characterizing the neuropeptides and protein receptors that are glycosylated to better understand their roles.

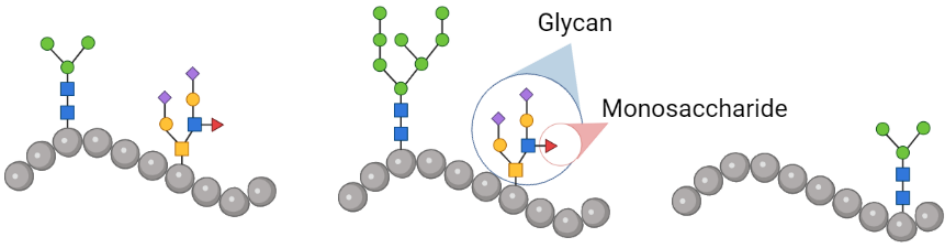

Figure 3. Glycopeptides are created when a peptide is modified by one or more glycans, each made up of monosaccharides in different combinations. The same peptide can be modified by different glycans attached to different amino acids, greatly increasing the peptides that can be formed from a single amino acid chain.

Neuropeptides: The Vehicles Sent Out

As I mentioned previously, it is crucial to identify the exact amino acid sequence order of a peptide to know what is there. This is especially important for neuropeptides, which tend to have similar amino acid compositions to other neuropeptides, due to conserved sequence motifs. These are certain amino acids that appear in a particular order or pattern that is shared with other neuropeptides of the same family. The uniform of a sports team could be considered a conserved sequence motif; it is shared across team members and can help indicate a relationship. While this is helpful to discover neuropeptides, this shared trait makes it harder to differentiate one from another. For instance, from far away, it can be hard to find a specific person out of the group running around in their identical uniforms.

Additional difficulty is added when considering glycosylated neuropeptides, which are not as abundant as many other biomolecules. This decreases our ability to detect and fragment them for identification. We can simplify our sample by removing molecules based on their chemical properties, such as their capabilities to interact with water. Glycans interact with water strongly, meaning we can trap glycopeptides on a stationary water layer and rinse off the non-

glycosylated peptides using an organic solvent, such as acetonitrile, which does not have the same interaction capabilities as water. This is done through a technique called hydrophilic interaction liquid chromatography (HILIC), shown in **Figure 4**.

Many non-modified bio molecules would rather interact with the acetonitrile and flow away, rather than stay on the water layer. With our simplified sample containing a higher percentage of glycopeptides, we can better find glyconeuropeptides. I combined this technique with a specific fragmentation mechanism in the mass spectrometer to break apart the peptide. Instead of one high energy fragmentation event, the glyconeuropeptide is fragmented once, then again by a different type of energy to produce more, and different, peptide fragments for identification. We can use this information to more confidently determine the identity of the peptide. By doing this, I was able to increase the number of known glyconeuropeptides in the crustacean model organism by 656%. This further enables this model organism to be used to better understand simpler molecular mechanisms before attempting to probe more complex systems such as the human body.

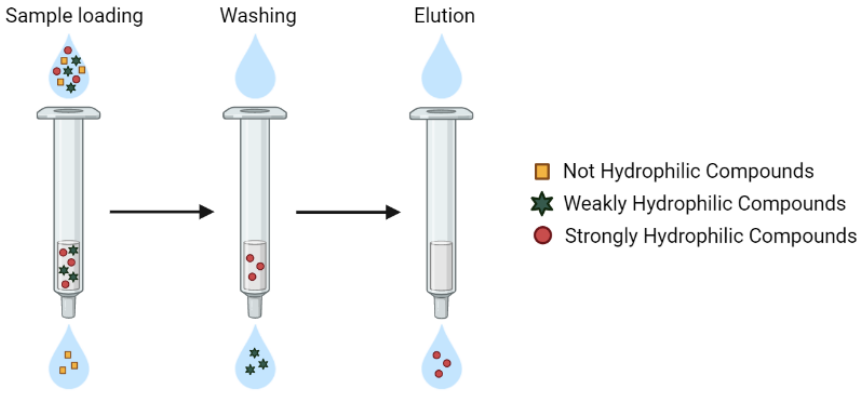

Figure 4. A complex mixture with several types of biomolecules in a low water content liquid can be simplified using solid phase extraction methods such as HILIC. Hydrophilic molecules, those that interact with water, bind to the solid material. Less hydrophilic compounds can be washed off as well by washing the material with a solution with slightly more water. The most hydrophilic compounds can then be collected with a solution containing mostly water.

Protein Receptors: The Machinery

Another part of my research focuses on how the protein receptors of neuropeptides are altered by glycosylation and what their downstream effects are. Recall the analogy where neuropeptides act as response vehicles that may have a trailer, or chemical modifications. It is equally important to know if the path to receive the signal is modified in a way that either enables or obstructs the vehicle's ability to arrive. To determine this, we can identify the glycoproteins with quantitative or qualitative changes in regulation in response to a condition or stimuli. This will tell us which glycoproteins are involved in the changes and should be studied further. To differentiate between glycoproteins involved in neuronal signaling and those that change due to other processes, we must separate them from glycoproteins that are present elsewhere on the non-signaling end of the neuron or on a different cell type.

We must also do this because it is possible for the same glycoprotein to be present in different types of cells and a change in one location may not be reflected overall. Global brain tissue investigations may obscure or only reveal a small change in glycoprotein expression relative to the total amount and would likely not appear as significant, even if there is a large change to the glycoprotein structure or its abundance in neurons specifically. This can also happen if there was a change, but the opposite change was observed elsewhere in the brain; the net change would be zero. For example, traffic in a large city must be assessed per city block division to reveal areas of high and low traffic. If the average travel time of all vehicles in the entire city is recorded, it would appear as the entire city experiences a normal level of traffic.

Unfortunately, glycosylated protein receptors of neuronal signaling molecules are difficult to fully purify as the brain is a complex organ with many sections and components and there is no inherent consistent identifier to separate glycoproteins based on their function or types of molecules that bind to them. However, special properties of the neuron enable the extraction of the portion of the neuron responsible for sending and receiving signals called the synaptosome (**Figure 5**).

This enables improved access to neuronal glycoprotein receptors by removing the many other glycoproteins present in the brain that are not directly involved in neuronal signaling. By using a solution that simulates the osmotic pressure, or salt composition, and pH of the biological environment, we can gently disrupt the cells to tear the neurons at the pre-synaptic dendrite, which then heals itself by reforming with the post-synaptic dendrite. The resulting synaptosome then exists in solution with other cellular structures, called organelles, or cellular debris which is cellular waste after cell death.

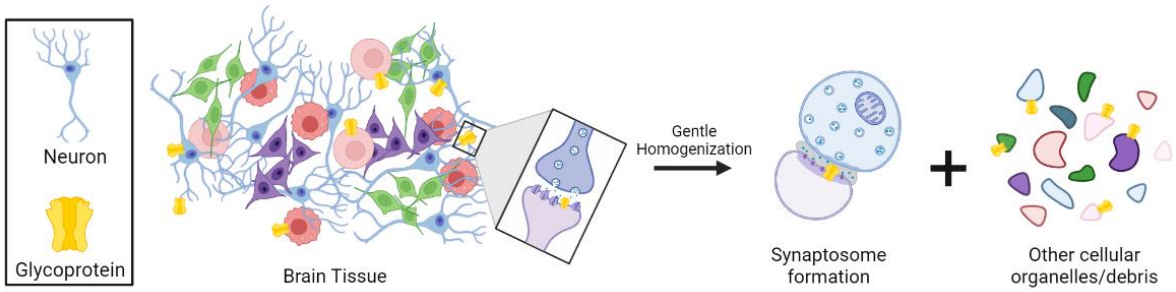

Figure 5. A specific glycoprotein can be found in different types of cells or different locations on the same cell. Synaptosomes can be formed during certain conditions and contain the parts of a neuron that send and receive signaling molecules. The synaptosomes can then be isolated from the other brain tissue components.

This can then be sorted using different centrifugation techniques where centrifugal force is created by spinning a sample at an angle to separate things based on size and density. Think of it like putting various objects into a pool. Many will sink, but each object will likely also differ in the time it takes to get to the bottom. While many other objects float, some can be pulled down with a little force, meanwhile a floaty would need a lot of force to be pulled down.

To ensure we collected the right portion which does not include other organelles, we look for proteins unique to a certain organelle. Think of it like describing a room in your house with the objects in it. To indicate the bathroom, you can't say the room with a faucet because the kitchen also has a faucet, you would need to look for a room with a toilet. The toilet would be called a bio marker because it is isolated to the bathroom. Looking for bio markers for synaptosomes and other organelles I didn't want showed me that the technique successfully isolated synaptosomes while removing many other organelles.

We can then take the glycoproteins isolated in the synaptosomes, and thus involved in neuronal signaling, and apply the same glycopeptide separation technique I described earlier in my work with glyconeuropeptides. Through this method, I have enabled the identification and quantification of glycosylated proteins involved specifically in neuronal signaling.

Conclusions

The major focus of my work during my Ph.D. has been to create new methods to uncover more information about the biological processes within the nervous system. These methods I've developed can be applied to study the regulation of glycoproteins and glyconeuropeptides in the body and their functions. Once we identify those that have increased or decreased production due to various neurological diseases or stressors (such as oxygen deprivation from a stroke), we can perform more targeted experiments. This can then lead to the creation of therapeutics and treatments for a variety of conditions or disorders. My next research endeavors will hopefully be benefiting this later stage in the drug development pipeline, working as a scientist at a pharmaceutical company.