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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The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

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Liquid-liquid phase separation as a novel mechanism for synaptotagmins 1 and 7 oligomerization and deciphering calcium binding activity of synaptotagmin 1

by

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CHAPTER V: THE CONDUCTOR OF THE SYNAPTIC SYMPHONY

Summary

Science can often feel locked away in technical language, accessible only to specialists. However, the questions it seeks to answer - like the intricate workings of the human brain - belong to all of us. I wrote this chapter, "The Conductor of the Synaptic Symphony," to share the story of my doctoral research with a broad, non-specialist audience because I am passionate about making science accessible to everyone.

My work explores the microscopic "game of catch" that allows our brains to think, feel, and remember. The process of translating this complex molecular dance into an understandable narrative has not only been a rewarding challenge but has also sharpened my own perspective on the research. It has reinforced my belief that when we strive to share our work widely, we can inspire new imaginations and build a stronger scientific community.

I am immensely grateful to the Wisconsin Initiative for Science Literacy (WISL) at UW-Madison for creating this platform and actively encouraging scientists to communicate with the public. WISL reminds us of our responsibility to share the stories of our discoveries with the taxpayers and community members who make our work possible. I am immensely grateful to Professor Bassam Shakhashiri and Elizabeth Reynolds at WISL for helping me with this chapter of my thesis.

The Brain's Whispers

Have you ever wondered how a thought - a fleeting, ephemeral thing - flashes through your mind? Or how do your fingers know how to type a word before you've consciously finished thinking it? Or how you can recall the scent of your grandmother's kitchen from decades ago with perfect clarity? It all comes down to a conversation, an intricate and impossibly fast dialogue happening inside your head at every moment of your life.

This conversation is carried on by billions of specialized cells called neurons, the unsung heroes of our nervous system. They form a network of staggering complexity, a living web that creates who we are. As shown in Fig. 5-1, neurons talk to each other by passing chemical messages across tiny gaps that separate them, called synapses. Imagine it as a microscopic game of catch on a cosmic scale. One neuron packages a message into a tiny bubble, runs to the edge of the gap, and throws the bubble across to its neighbor, who catches it. This simple act, repeated trillions of times per second across your brain, creates the symphony of our consciousness, our memories, our emotions, and our every action.

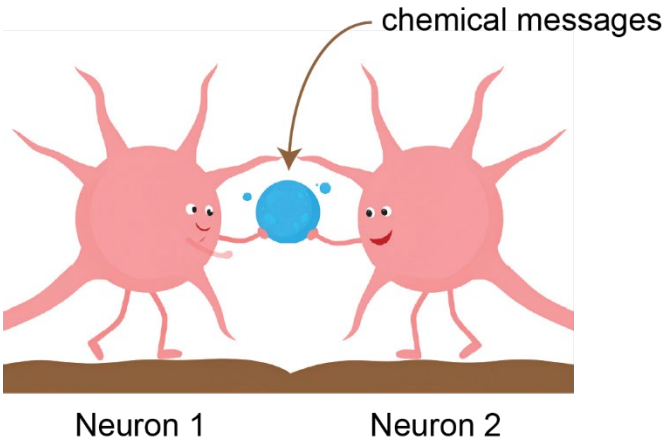

Figure 5-1: The Synaptic "Game of Catch"

Neurons 'talk' by throwing chemical messages (in vesicles) to each other across a tiny gap called a synapse. A cartoon showing two neurons. Neuron 1, on the left is "throwing" a small bubble-like vesicle, which looks like a ball, across a small gap. On the right, Neuron 2 is open, ready to catch the ball.

My journey into this hidden world began not with a grand vision, but with a simple, fundamental question: how does the “throwing” neuron know exactly when to release its chemical message? The timing has to be perfect. In this high-speed game of catch, a message sent too early or too late could disrupt the entire symphony, leading to a jumble of noise instead of a coherent thought.

The answer, we’ve known for a while, involves a rush of calcium ions (a charged atom). Think of calcium as the conductor's baton. An electrical signal, the nerve impulse, travels down the neuron like a wave. When it reaches the synapse, it throws open a gate, and calcium floods in. With a flourish of this calcium baton, the message is sent. But who is watching the conductor? What molecular player sees that rush of calcium and gives the final, split-millisecond "go" order (Fig. 5-2)? That's where my protagonist comes in: a protein called synaptotagmin 1, or as I’ve come to know it, syt1.

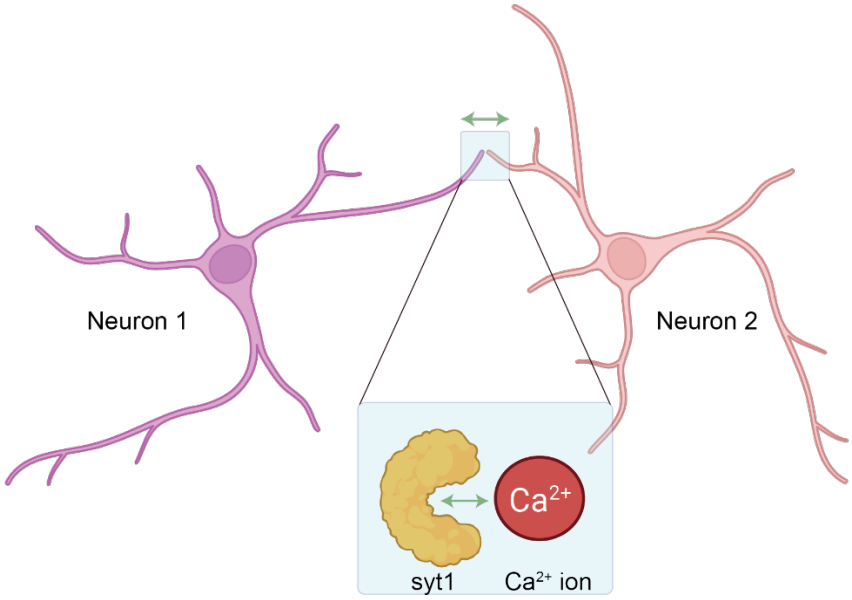

Figure 5-2: Who's the protagonist?

Depiction of communication between neurons guided by interaction of a protein and calcium ion. The fourth chapter in my thesis involves unraveling the role of this protein, syt1, senses calcium ions.

A Molecular Enigma

For years, scientists have known that syt1 is the primary calcium sensor (Fig. 5-3). It's a protein that sits on the surface of those little bubbles, or vesicles, that hold the neurotransmitter messages. When calcium rushes in, syt1 is supposed to grab it and, in a flash, trigger the vesicle to fuse with the neuron's edge, releasing its message. It's a job that requires breathtaking speed and precision, one of the fastest biological processes known.

But syt1 was a puzzle. For a protein with such a critical job, it was surprisingly enigmatic. When we tried to study it in the lab, different scientists came up with conflicting results about its behavior. Some scientific papers described it as a lone wolf, acting on its own. Others were convinced it worked in teams, forming groups or "oligomers" of various sizes—dimers, tetramers, even large ring-like structures. The data was all over the map, and it felt like we were missing a key piece of the story.

One part of the protein, in particular, was a black box: a long, floppy arm called the juxtamembrane linker. This linker connects the part of the protein anchored in the vesicle to the C2 domains—the "hands" that catch the calcium. For decades, to make the protein easier to work with in a test tube, most researchers simply cut this part off. It was technically difficult to produce the whole protein, and the floppy linker was presumed to be nothing more than a simple tether holding the working parts in place.

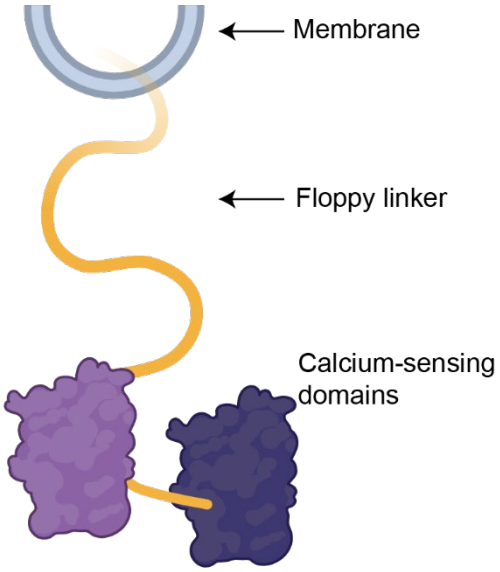

Figure 5-3: Syt1 - Protein with a Floppy Arm

Depiction of a cartoon of syt1 protein. It's anchored at the top to a membrane, followed by a long, wavy, flexible linker connecting the two calcium-binding domains. My thesis involves demystifying the role of this long floppy linker.

My curiosity was piqued by this neglected piece of the puzzle. Science often moves forward by questioning assumptions, and this felt like a big one. What if this linker wasn't just a rope? What if it was playing a more important role, one that could explain all the confusing data? This question became the driving force of my PhD research. I felt that to truly understand how syt1 performs its magic, we had to look at the whole protein, linker and all.

A Surprising Discovery in a Droplet

Working with the full-length syt1 protein was a challenge, but with some new purification tricks my lab had developed, we finally managed to get a clean, intact version to study. The first surprise came when we tested how well it binds to calcium. Counterintuitively, the full protein, with its linker intact, was worse at binding calcium than the truncated version everyone had been using! It was as if the linker was somehow getting in the way, dampening the protein's main ability. This was the opposite of what we expected and a huge clue that something much more interesting was going on.

The mystery deepened when we looked at how the linkers on different syt1 molecules interacted with each other. We had a hunch they might be responsible for the teamwork we suspected. And that’s when we stumbled upon the most exciting discovery of my research. To see the proteins, we tagged them with a molecule that glows green under a

microscope. When we put our syt1 into a solution that mimicked the crowded environment of a cell, something amazing happened. The linkers were causing the syt1 proteins to cluster together, but not in a rigid, fixed structure. Instead, they were forming tiny, dynamic liquid droplets that shimmered in the eyepiece (Fig. 5-4).

This phenomenon is called liquid-liquid phase separation, or LLPS. It's a bit like what happens when you mix oil and vinegar for a salad dressing. They stay separate, with the oil forming little droplets. In the crowded environment of a cell, some proteins and other molecules can do the same, separating out from the cellular soup to form membraneless compartments. This is a revolutionary new idea in cell biology, showing that cells are organized not just by membranes, but by these dynamic liquid condensates. And here it was, happening with our little brain protein. The floppy linker, rich in positively charged amino acids, was the key. It was acting as a kind of molecular glue, allowing syt1 molecules to condense into their own private, liquid club.

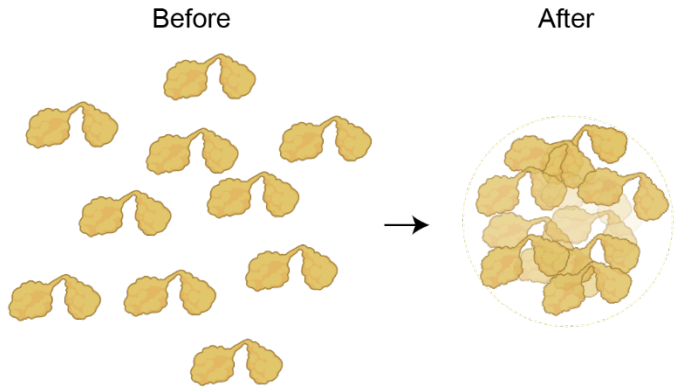

Figure 5-4: The Syt1 Droplet Experiment

The left panel shows many individual syt1 protein molecules scattered randomly. Under certain conditions, the same protein molecules cluster together into a single, large,

circular droplet. Like oil in vinegar, syt1 proteins use their linkers to separate from the cellular soup and form a liquid droplet.

The Joy of a New Story

Seeing those droplets for the first time under the microscope was a moment of pure scientific joy. It was a classic "aha!" moment where years of confusing data suddenly snap into a clear picture. The protein wasn't just a simple switch; it was a sophisticated regulator that could change its properties by entering this droplet state. This explained everything! The reason it bound less calcium was because inside the dense droplet, its "hands" were a bit shielded. The reason different researchers saw different-sized teams was because LLPS is a dynamic process, not a fixed structure.

This discovery opened up a whole new way of thinking about how neurotransmission is controlled. We realized that by forming these droplets, syt1 was creating a feedback loop. The droplet state made it a bit harder for syt1 to see the calcium, fine-tuning its sensitivity. But at the same time, the presence of calcium and the lipids in the vesicle membrane actually favored the droplets to form and fuse together. It's an exquisitely balanced system, designed for precise control.

The significance of this work goes beyond just understanding one protein. The machinery of our synapses is incredibly complex, and when it breaks down, it can lead to devastating neurological diseases like Alzheimer's, Parkinson's, and epilepsy. These are now sometimes called "synaptopathies," or diseases of the synapse. Our discovery that a key synaptic protein is regulated by LLPS provides a new angle from which to look at these conditions. Could it be that in some of these diseases, the normal, healthy phase

separation of proteins like syt1 goes awry? Perhaps the dynamic liquid droplets become too sticky, turning into the solid, toxic protein clumps that are the hallmark of neurodegeneration.

Looking back on this journey, I'm filled with a sense of awe. We started with a nagging question about a small, overlooked part of a protein and ended up uncovering a new layer of regulation at the very heart of how our brains work. It's a reminder that even in the most well-studied fields, there are still secrets hiding in plain sight, waiting for a curious mind to look a little closer. The symphony of the brain is more complex and beautiful than we ever imagined, and I feel incredibly privileged to have been able to listen in and decipher one of its hidden melodies. It's a story I'm excited to continue exploring, to see what other secrets syt1 and its molecular cousins are waiting to tell us.