Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include 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 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.

Over 40 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of $250 for UW-Madison chemistry Ph.D. candidates.

Wisconsin Initiative for Science Literacy

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.

UW-Madison Department of Chemistry
1101 University Avenue
Madison, WI 53706-1396
Contact: Prof. Bassam Z. Shakhashiri
bassam@chem.wisc.edu
www.scifun.org

Fluorescence Anisotropy Measurements of Single Molecules to Probe Solution-Phase Conformational Heterogeneity of Intrinsically Disordered Proteins

By
Lydia H. Manger

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The dissertation is approved by the following members of the Final Oral Committee:

Randall Goldsmith, Assistant Professor, Chemistry
James Weisshaar, Professor, Chemistry
Aaron Hoskins, Assistant Professor, Biochemistry
John Wright, Professor, Chemistry

Chapter 1

1. An Introduction to Tau Protein and the Anti-Brownian Electrokinetic (ABEL) Trap for Non-Specialists: for the Wisconsin Initiative for Science Literacy

1.1 Protein Function

Proteins are the building blocks of life. Their unique sequence of amino acids determines their structure and function. Proteins come in various shapes, sizes, and function: antibodies are proteins that fight infections; hormones are proteins that coordinate bodily processes; structural proteins provide support to connective tissues; transport proteins move molecules around the body. These are just a few of the examples of proteins that the human body relies on to perform essential functions.

Since proteins are essential for life, what, then, happens when proteins malfunction? And what are the different ways that they can malfunction? These two questions have important ramifications for understanding disease and finding treatments. In my research, I have been concerned with tau protein. Tau protein is implicated in Alzheimer's disease and other neurodegenerative diseases. Even though the cause of these diseases is not well understood, the brains of patients with these neurodegenerative diseases often contain a buildup of aggregates of tau protein. Why the tau protein aggregates and why it no longer performs its intended function is an active area of research.

1.2 Tau Protein

Everyone has tau protein – it is an essential component of healthy brains. Tau protein is known as an intrinsically disordered protein or IDP because in its healthy (native) state it does not have a well-defined structure as some other proteins do. This means that tau protein is free to explore many different structures: sometimes it might be folded like a paperclip, sometimes it might be folded like an "S" (Figure 1-1). In the middle of the protein is a region known as the microtubule-binding region.

To understand tau protein, and its importance, imagine a railroad track that connects one station to the next. Different trains can move along these tracks carrying a variety of cargo. But when the railroad ties are torn up, the structural integrity of the tracks crumbles and they are no longer able to transport their cargo. A similar process happens in the brains of people with Alzheimer's disease.

Brains are primarily composed of neurons, special cells that contain a cell body, axon and dendrites (Figure 1-2). Microtubules function as the railroad tracks. They run along the axons of neurons and provide structural support and a mechanism for transport. Upon closer inspection, tau protein is found bound to

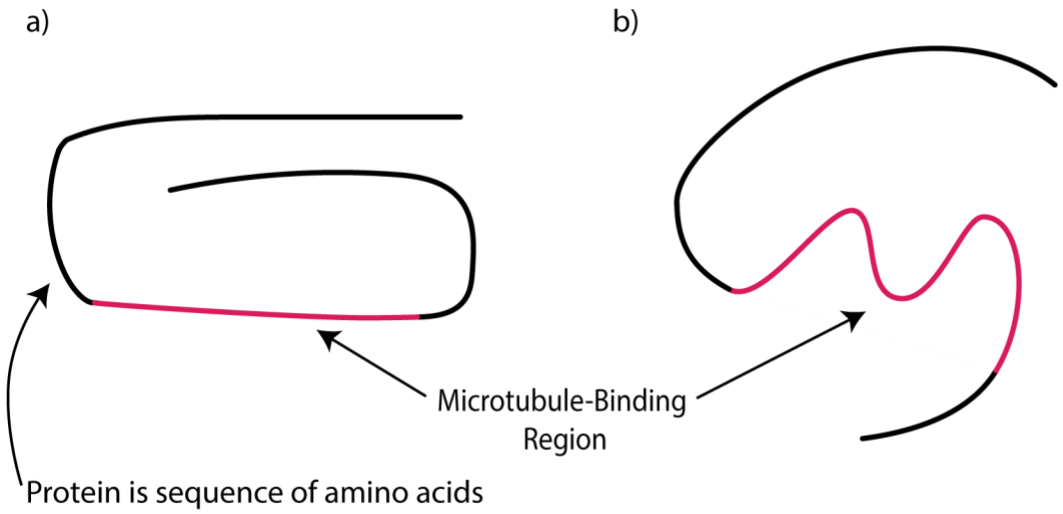

Figure 1-1. Possible structures of tau protein.

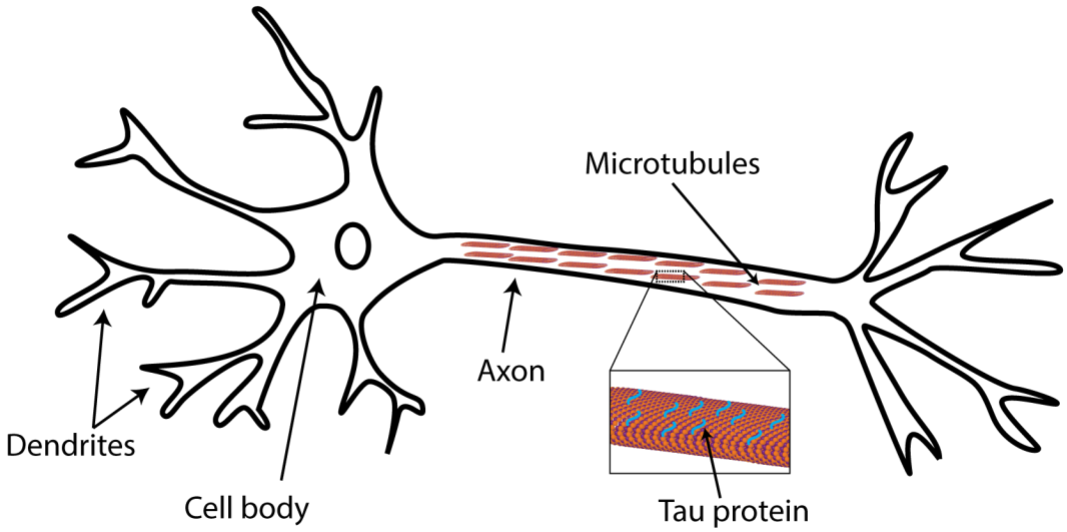

Figure 1-2. Schematic of neuron showing microtubules in the axon.

microtubules, stabilizing the tracks (i.e. railroad ties) (Figure 1-2, inset). But in the neurons of Alzheimer's patients, tau protein is no longer found attached to microtubules, but rather found in tangled clumps.

We are interested in learning more about the many structures that this protein adopts and how it behaves in solution so that we can understand how the molecule exists in the aqueous environment of the cell. We are also interested in learning about this molecule at the single-molecule level so that we can understand the heterogeneity, or mixture, of behavior before it begins to be harmful.

1.3 Single-Molecule Studies

Studying single molecules is a fairly recent path of experimentation. Traditional methods for studying a sample of protein, for example, have relied on looking at millions of molecules at a time. But when we look at millions of molecules at once, we miss information from the individual molecules. Imagine looking at a crowd of people at a concert and you are asked to gauge the energy of the crowd. People are moving and dancing, so you conclude that the energy level is high. But if you could take the time to look at each individual person, you might find that some people are dancing much more than others; your original

assessment of the energy level is not uniform across the crowd. The different energy of each person in the crowd cannot be understood by the ensemble measurement of looking out over the crowd, but a single-person measurement of looking at each individual could give a more detailed picture. Understanding the heterogeneity of a sample of molecules is similar to this: we want to know how each molecule moves independently so that we can learn more about properties that would otherwise be hidden.

Since we want to learn about individual molecules, and we want to learn about them in a relevant situation, we study proteins in a solution. Unfortunately, a protein in solution will not stand still while being observed on a microscope because it experiences Brownian motion. Brownian motion is a term to describe the natural, random movement of particles in solution. One way to overcome this phenomenon is to tether a molecule to the surface, essentially putting a leash on the molecule so that it does not float away. But putting a leash on a protein like tau protein, which does not have a defined shape, could force it into an unnatural configuration, thus distorting the data and making the experiment irrelevant to the true motion of the molecule. On the other hand, the molecule can be left to float freely in solution so that we only see it when it is in the field of view of the microscope. Even though we only get to see it for 0.001 seconds, its conformation is not being distorted. A third option would allow for a molecule to freely diffuse, but also cancel its natural, random motion so that it can be observed for a longer period of time. This third option is made possible by the anti-Brownian electrokinetic (ABEL) trap.

1.4 Anti-Brownian Electrokinetic (ABEL) Trap

The anti-Brownian electrokinetic trap, or ABEL trap, was developed at Stanford University in California, and is used in only a handful of labs around the world. The ABEL trap consists of a microfluidic device, a rotating laser spot, and feedback voltages (Figure 1-3).

A microfluidic device is used to hold and control small volumes of liquid, typically less than ~1/5th of one teaspoon (or 1 milliliter). The ABEL trap microfluidic holds ~1/5000th of one teaspoon (or 1

microliter). Even with this small volume, there are still approximately 602,000 individual protein molecules in the trap. If approximately 40,000 of these protein molecules were to lie end-to-end, they would span the width of a typical human hair. There are 4 reservoirs in the microfluidic, each containing an electrode that will be used to apply feedback to the molecule of interest.

The laser travels around a grid of 32 spots, spending ~800 nanoseconds (0.00000008 seconds) at each spot (Figure 1-3b). Only one of the 32 spots are illuminated at a given time. When the location of the laser overlaps with the location of the protein, a fluorophore attached to the protein is illuminated and excited. A fluorophore is a special type of molecule that, when excited, will emit fluorescence, similar to materials that glow in the dark. Think of the molecule as a person in a dark room. Without light, the person cannot be located. But if the person turns on a flashlight, then it becomes easier to estimate where we expect the person to be standing. In this analogy the flashlight is the fluorophore, and the person is the molecule we are interested in.

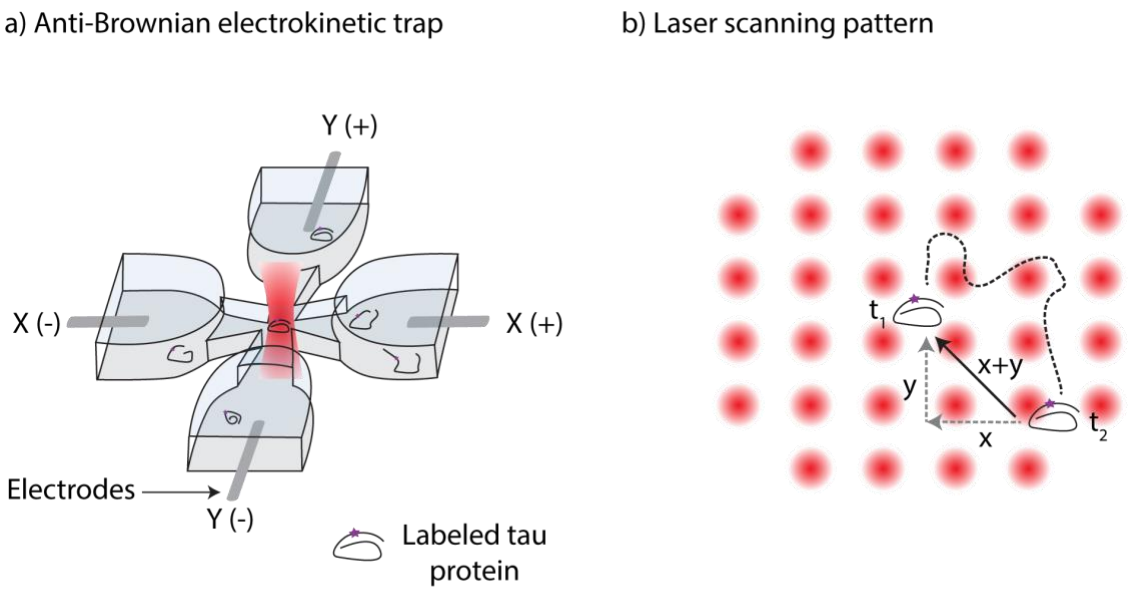

Figure 1-3. Anti-Brownian electrokinetic (ABEL) trap design. (a) Microfluidic device showing tau protein in the trap and 4 electrodes. (b) A molecule moves from its initial position at t_1 to a position at t_2 via Brownian motion along the dashed line. The electrodes then exert a force in the x and y directions to push the molecule back to center.

Once we know where the protein is, it is time to apply feedback voltages. These bursts of electricity will cause the solution in the microfluidic to flow in a particular direction, dragging the molecule with it.

Imagine a molecule initially at t_1 in Figure 1-3b. This is the ideal position for the molecule — right in the center. But as detailed earlier, the molecule will move away from this spot via Brownian motion, as indicated by the black dashed line, to a new position at t_2. The system now finds the molecule at this new position and calculates the electricity to apply to push the molecule back to its original position. There are two sets of electrodes that will be used to apply the feedback in the x- and y-directions (gray dashed lines). Combining a force in the x-direction and a force in the y-direction will give an overall force that moves the molecule diagonally (black arrow).

1.5 Fluorescence anisotropy

Now that we have a molecule of interest trapped in our ABEL trap, we want to learn as much information as possible. We will use the fluorophore, the flashlight, attached to the molecule as a proxy for understanding what conformations the molecule is adopting since we cannot see the molecule directly. One way to do this is to measure anisotropy. Anisotropy is a measure of the extent of polarized emission when a molecule is excited with polarized light. Let's look at this a little more closely to find an intuitive understanding of this phenomenon and why it is helpful for our experiments.

Light has polarization. You may have experienced this polarization when you wear polarized sunglasses — the world looks different if you hold your head upright vs. tilted to one side. If you looked at a computer screen with perfectly polarized light, in one orientation you would be able to see something on the screen while wearing your polarized glasses, and then by tilting your head, the screen would appear completely dark because no light is able to pass through your polarized lenses.

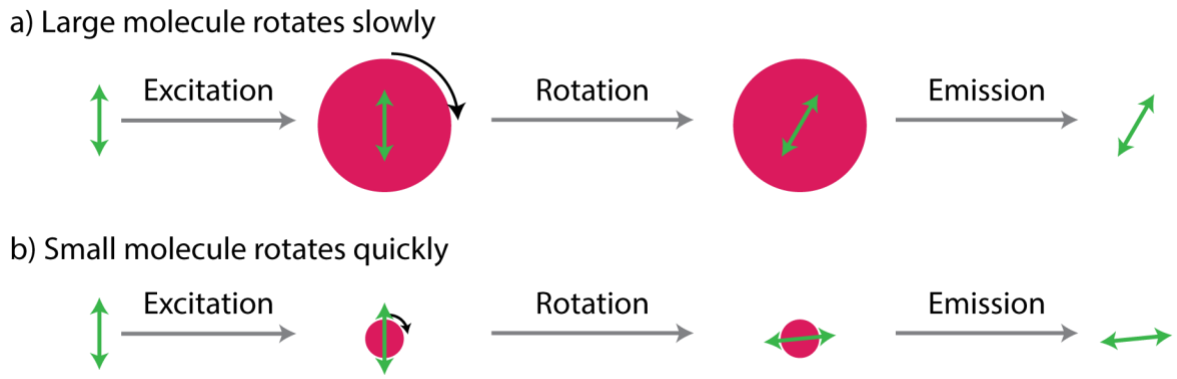

Figure 1-4. Anisotropy measurement shows how much the molecule has rotated. A photon (green double-headed arrow) interacts with a molecule (pink circle) and the molecule is excited. The molecule then moves and rotates, and the orientation of the photon is also changed so that when the photon is emitted, the photon is in a different orientation than when it excited the molecule. (a) A large molecule rotates slowly and (b) a small molecule rotates quickly so that the orientation of the emitted green arrow is different.

We will be exciting our molecules with polarized light so that all the molecules absorb light with a specific orientation. A molecule absorbs a "photon" of light (a term for a bundle of light energy) when the light is oriented in one direction, say vertically (Figure 1-4). But when the molecule releases that light, the molecule has moved and rotated in solution and will release the light in a different orientation, say diagonally. Based on the polarization of this emission light (orientation of green arrow in Figure 1-4 on right), we can determine how much the molecule rotated while it was "holding onto" the light. The extent that the molecule has rotated during the period of time the molecule holds onto the light will provide information about the conformation of the protein — a person swimming in a pool will somersault slower when not holding her legs in tightly than when curled tightly into a ball. This measurement, therefore, allows us to understand how compact the protein is while it rotates and moves through the solution.

1.6 What We Have Learned

Using this method, we have studied the single-molecule motions of tau protein in solution. We used anisotropy as a way to describe how much these molecules rotated in solution, which we then used to describe the variety of conformations that the protein adopts. When we plotted a distribution of these

anisotropy values, we found that there were two distinct peaks, indicating that there were some molecules that tumbled faster (more compact structures), and some that tumbled slower (less compact structures). This work contributes to the needed understanding of the conformations of tau protein since the conformation of tau protein may be important for determining disease pathology. One possible interpretation of this finding is that one conformation is more prone to the pathological aggregation seen in neurodegenerative diseases, but more studies are needed to fully characterize the biological ramifications. From here, studying the anisotropy of this same protein in the presence of factors that induce or hinder aggregation of the protein can provide information about the changes in conformation experienced under such conditions, which could have relevance to understanding disease progression and potential therapeutic treatments.