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Tools of the Trade

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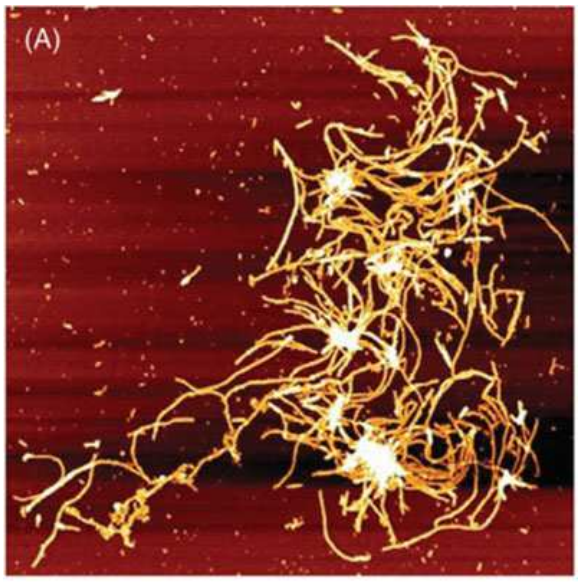
Neurodegenerative diseases such as Alzheimer's and Parkinson's are caused by toxic oligomers too small to be studied with optical microscopy. Now, new single molecule methods are resolving these protein aggregates, offering new insights into these diseases. Read more...

At two years old, my sister couldn't keep her hands away from the string of pearls around my great-grandmother's neck. After a few minutes, Grandma passed the baby to someone nearby, removed the pearls, and carried them into the kitchen where she hid them in a teacup high in the cupboard. When she returned, she only said, "We may need them for the apples."

She had Alzheimer's disease. By this time, the disease had claimed not only her short-term memories of ongoing conversations, but memories of many years of her life. She believed herself to be newly married and living in the early 1930s, a time when she regularly traded many of her valuables for apples or other food just to get by.



AFM image of amyloid aggregates. Source: Zoya Leonenko

Alzheimer's disease is thought to be caused by improper folding of the protein amyloid-β, leading to aggregation, and leaving long amyloid fibrils and plaques attached to neuronal membranes. These fibrils were thought to claim not only memories, but also the lives of the neurons and eventually the individuals themselves.

However, new evidence suggests that Alzheimer's disease actually results from early aggregates of just a few proteins —small soluble amyloid oligomers—and that those long fibers and plaques may actually play a protective role by sequestering the toxic small oligomers.

"It's very difficult to address and understand the disease unless you go to the very beginning and look at the single molecule interactions. What goes wrong, and why do they form these toxic oligomers?" said Zoya Leonenko, associate professor at the University of Waterloo (Ontario, Canada).

The Record Player

Advances in several techniques that explore small molecules and their interactions have allowed researchers to resolve even the tiniest of structures. In Leonenko's case, the method of choice is atomic force microscopy (AFM).

"AFM is the perfect instrument for this because we can achieve high resolution images on a single molecule level. We can resolve peptides, we can resolve single molecules of DNA, and we can even resolve these oligomers," she explained.

To achieve this resolution, AFM relies on a small needle, called the AFM probe, to come in close contact with a surface, similar to how a blind person touches a surface with their fingers or a record player with a sharp needle touches the vinyl and reads the topography of what is written underneath, according to

Leonenko. In the case of amyloid proteins, these are first allowed to aggregate in solution, and then they are placed on mica, a surface so flat even unbound amyloid-β monomers are distinguishable from the roughness of the slide.

Zoya Leonenko

While Leonenko's team has used AFM to study the molecular mechanisms of amyloid fibril formation and toxicity, they also plan to identify compounds for pharmaceutical development. To that end, they recently developed a new method for screening potential drug compounds using AFM in combination with atomic force spectroscopy (AFS) (1).

For AFS, the study authors layered single amyloid-β proteins on the mica slides and then attached another amyloid-β monomer to the AFM probe. As this probe sets out to read the surface, the monomer carried on the end might contact another monomer on the slide surface, an event that would be reported as a repulsive force sensed by the AFM tip. When this happens, the researchers can retract the tip, pulling the two bound proteins apart, while at the same time measuring the strength of their interaction, or binding force, until separated.

"If we add a drug that prevents binding, we can measure, at the single molecule level, how effectively this drug prevents the binding of two peptides," noted Leonenko. She added that this approach significantly reduces cost and effort compared to other drug screening methods since far less drug is needed for single molecule testing, and hundreds of AFS experiments with varied conditions and cofactors can be performed in a matter of hours.

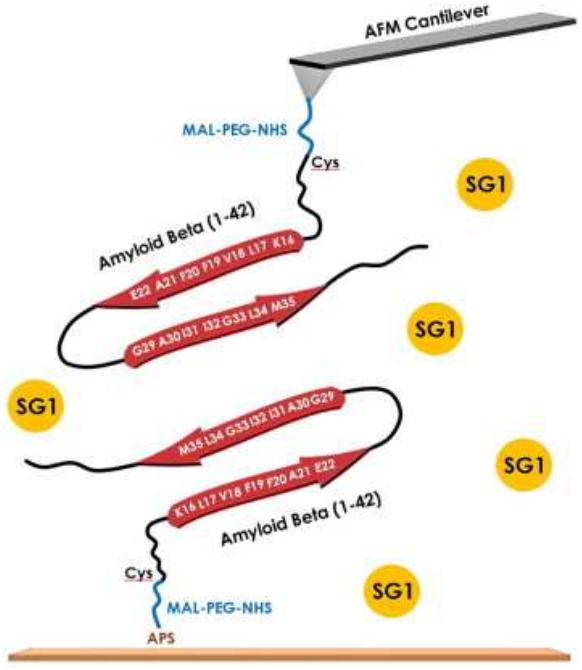


Diagram of the AFS experiment. Source: Leonenko lab

The Sewing Machine

While AFM and AFS are proving highly valuable in studying oligomer aggregation, Oleg Kolosov of Lancaster University turned to another method, ultrasonic force microscopy (UFM), for his studies on Alzheimer's disease.

Like AFM, UFM also uses a probe. But rather than scanning the probe over the sample, UFM avoids friction by using a high frequency vibration to move the probe while allowing the sample to slide underneath. "This very high frequency lets the tip detach from the surface on a very small timescale, like a microsecond. While it's detached, the sample can slide sideways. If you imagine a sewing machine, and you try to pull your textile, you will break either the needle or the textile. But if the sewing machine is going in and out, in and out, so effectively, you can sew as it goes. The probe is going up and down like a sewing needle. That is caused by ultrasonic vibration."

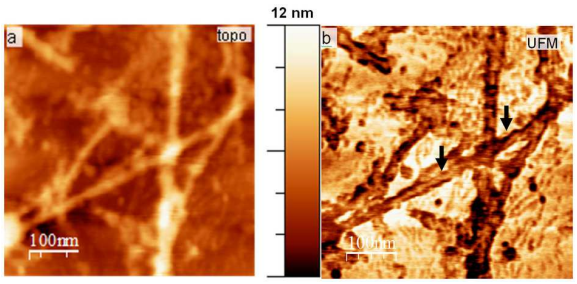
Oleg Kolosov

To adapt UFM for imaging amyloid-β aggregates, Kolosov needed contrast. In electron microscopy, this comes from staining the samples, but with UFM, the image is created by differences in nanomechanical properties. So Kolsov decided to coat his slides with poly-L-lysine (PLL), which not only provided a background protein for distinguishing the nanomechanics of the amyloid-β aggregates, but also solved potential difficulties in keeping the proteins stuck to the slide during bouts of ultrasonic vibration.

Kolosov used modified UFM methods to follow amyloid-β aggregation over time and found that toxic oligomers remain even after fibril formation, suggesting that there are rogue populations of oligomers that don't follow the known processes of aggregation (2). Moving ahead, Kolosov intends to refine this approach for liquid measurements, so aggregation can be monitored in a more physiological environment.

The Tweezers

While some researchers focus on formation of aggregates and fibrils, Michael Woodside from the University of Alberta (Edmonton, Canada) decided to look at the early oligomer formation of α-synuclein, the causative agent of Parkinson's disease. For this, Woodside and his team of researchers turned to optical tweezers and single molecule force spectroscopy (3).



AFM (left) and UFM images of amyloid fibers. Source: Oleg Kolosov

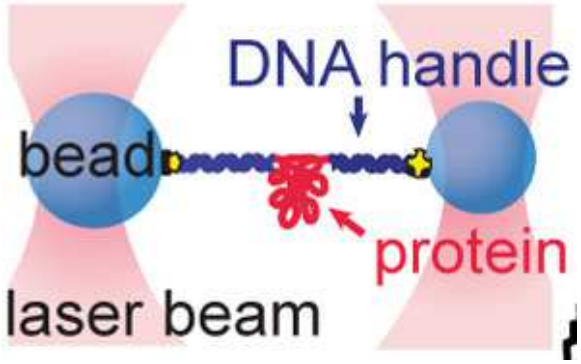


Diagram of a DNA-protein dumbbell held with optical tweezers. Source: Michael Woodside

Woodside's group started by creating constructs carrying one, two, and four copies of α-synuclein, all separated by linkers. At the ends of the constructs, they attached DNA handles produced by PCR, one end labeled with digoxigenin and the other labeled with biotin. The resulting proteins flanked by DNA handles could then be bound to anti-digoxigenin- or avidin-labeled polystyrene beads to create dumbbells.

Next, the researchers turned to a powerful single molecule technique called optical tweezers. When using optical tweezers, highly focused laser beams trap beads in place, explained Krishna Neupane, first author of the paper. "We make two traps ... then pull apart the laser beams, which will apply force on the protein." By measuring the positions of the beads, Neupane can determine the change in end-to-end extension as the protein or aggregate is separated.

By repeatedly pulling the same protein, the authors noticed a diversity in structural behavior, suggesting a variety of possible conformations. In fact, Woodside's group was able to resolve many more structures than previous work using AFM because of the sensitivity of the trap. The authors emphasize that these data represent a range of possible protein folds; whether all form in vivo is a question for further study.

Neupane is now applying this method to study proteins carrying mutations associated with familial Parkinson’s disease. He is also building hexamer and octamer constructs to look at the behavior of larger aggregates.

These studies offer the earliest glimpses of what exactly happens in the beginning of amyloid fibril formation. In time, a full picture of how oligomers and fibrils form and the mechanism of their toxicity will emerge. But Neupane found that the single molecule data he acquired already provides a solid foundation for advancing the field. Like Leonenko and Kolosov, he has already established a collaboration to begin looking for drugs that can alter oligomerization and aggregation.

"This is very challenging research that requires a lot of preparation and a lot of advanced techniques," Leonenko said. "It is important at this stage to link clinical and biomedical research to biophysics and single molecule studies because it may provide a better understanding and may result in a big improvement in terms of how we can cure and prevent this disease."

References

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Keywords: atomic force microscopy (AFM) atomic force spectroscopy (AFS) ultrasonic force microscopy (UFM) single molecule force spectroscopy (SMFS) optical tweezers Alzheimer's disease Parkinson's disease

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