A High-Speed Assay to Aid in Early Diagnosis of Parkinson's Disease

Amprion uses the PlateCrane EX™ microplate handler to multiply misfolded protein assay throughput.

Background

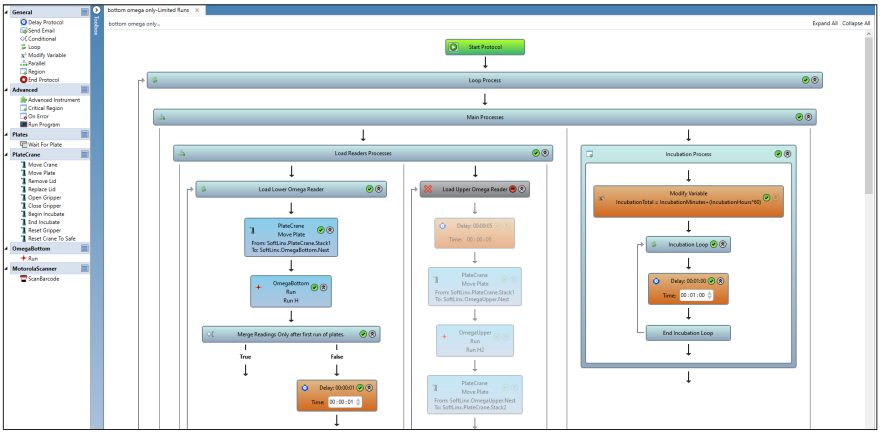
Parkinson's is a neurodegenerative disease that affects more than one million people in the U.S., where approximately 60,000 new cases are diagnosed each year.[1] There is no accepted method of early diagnosis. By the time the disease has progressed to the point where a differential diagnosis is possible, irreversible changes have occurred in the brain. The cause of the disease is still unknown, but the misfolding and subsequent accumulation of the protein α-synuclein is deeply associated to the etiology of Parkinson's disease. Misfolding, aggregation, and accumulation of α-synuclein in Parkinson's leads to cellular dysfunction, loss of synaptic connections, and brain damage, particularly in areas controlling movements. The misfolded α-synuclein aggregates are present in very low concentrations in biological fluids, making detection by conventional methods challenging. However, misfolded proteins are probable pathologic agents, and sensitive detection may represent a strategy for early diagnosis.

An Assay for Misfolded Proteins

Misfolded protein aggregates can self-propagate through seeding and spread pathological abnormalities.[2] The seeds accelerate the aggregation process by recruiting the soluble, normal protein into the growing aggregate. In the case of Parkinson's disease, misfolded α-synuclein aggregates interact with soluble, normal α-synuclein.

At Amprion, PMCA (Protein Misfolding Cyclic Amplification) is used to detect the abundance of misfolded protein seeds in CSF (cerebral spinal fluid).[3] In PMCA, minute amounts of misfolded oligomer are incubated with the recombinant α-synuclein protein substrate. The substrate is converted into more aggregates by direct interaction with the seed. The number of seeds available to induce misfolding is then multiplied by strong shaking, which fragments large aggregates into many smaller aggregates. The number of seeds increases exponentially after each cycle. In Amprion's assay, one cycle takes 30 minutes.

Misfolded proteins are detected by the fluorophore thioflavin T, which fluoresces in the presence of the characteristic β-sheet structure formed by misfolded protein aggregation.[4] The fluorescent signal is used to construct an aggregation curve over time. Samples with a greater number of initial seeds have steeper saturation curves that reach maximum fluorescence sooner than samples with a lesser number of seeds.



PlateCrane EX comes with SoftLinx™ lab automation scheduling software.

PlateCrane EX Multiplies PMCA Throughput

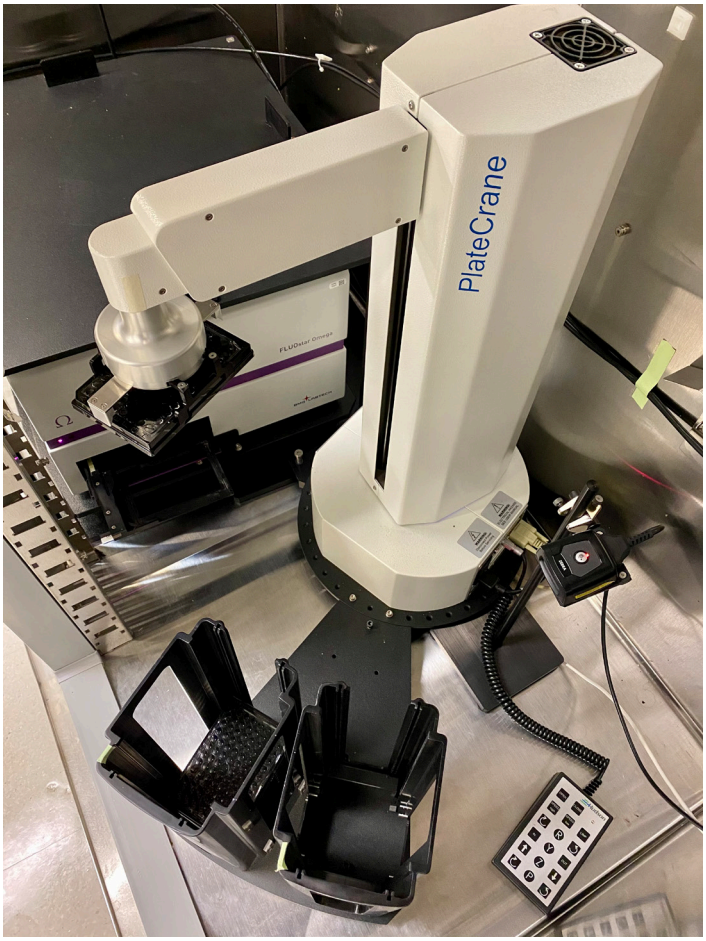
Through optimization, Amprion scientists reduced the assay time from three weeks to one week. A remaining limitation, however, was that microplate shaking, fluorophore quantification, and incubation of each individual plate were all taking place inside a single microplate reader. Increasing throughput, then, would require the purchase of many more microplate readers. Amprion determined that with an incubation chamber and automated microplate handling, plate incubation could take place outside the microplate reader, which would then be available to shake and read additional plates.

When reading each plate with 2 fluorescence gains, one FLUOstar Omega microplate reader can shake and read seven PMCA assay microplates for six days when paired with a PlateCrane EX microplate handler from Hudson Robotics. Incubation takes place outside the microplate reader, in the temperature-controlled environment that surrounds the reader, the stack of microplates, and the PlateCrane EX system. The PlateCrane EX system places and removes a microplate from the reader on a schedule that ensures that each microplate is read every 30 minutes, including time to re-arrange the microplates bottom-to-top so that they are queued in the correct order for the subsequent round of shaking and reading.

Amprion has entered into collaborations with many third parties that supply CSF samples, in quantity, to be read using Amprion's PMCA assay. Using a PlateCrane EX modification that slows down the microplate arm, scientists from Amprion can exchange an entire set of microplates after six days without stopping the system. In this manner, they have run PMCA assays for 90 days continuously.

The PMCA Assay is a "Breakthrough Device"

Using CSF and α-synuclein to aid in the diagnosis of Parkinson's disease recently received "Breakthrough Device" designation from US FDA (United States Food and Drug Administration). Amprion is working closely with FDA to fast-track development and review of α-synuclein PMCA tests toward final approval.



The addition of PlateCrane EX increased Amprion's PMCA assay throughput seven-fold.

PlateCrane EX Selection Criteria

In their search, Amprion found that there are many systems that can competently move a microplate from one place to another. However, for Amprion, integrating the system to meet the particular demands of the PMCA assay was paramount.

"We needed something more than what one person building a system in their garage could offer. We had other options, but they didn't have local representation that could help us with setting up the robotic arm."

Luis Concha Marambio, PhD
Associate Director of R&D, Amprion

"We've had several new ideas for making the assay better, and Hudson Robotics representatives have been able to implement them effectively. Anything we've thought of, they've been able to do."

Carly Farris
Senior Research Associate, Amprion

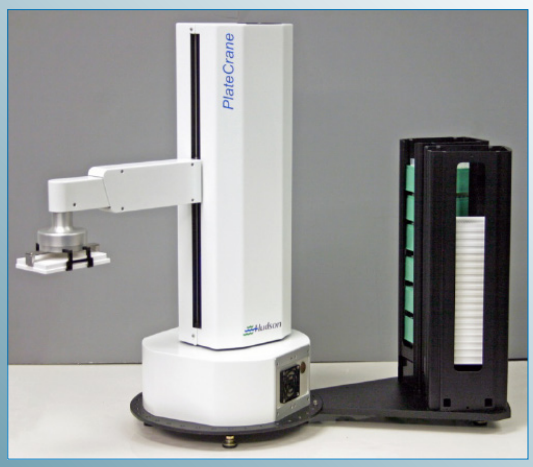
References

1. www.parkinsons.org. Accessed October 26, 2020.
2. Soto, C. Unfolding the role of protein misfolding in neurodegenerative diseases. *Nat. Rev. Neurosci.* **4**, 49-60 (2003).
3. Shahnawaz, M., Tokuda, T., and Waragai, M., et al. Development of a biochemical diagnosis of Parkinson Disease by detection of α-synuclein misfolded aggregates in cerebrospinal fluid. *JAMA Neurol.* doi:10.1001/jamaneurol.2016.4547
4. Gade Malmos, K., Blancas-Meija, LM., Weber, B., et al. THT 101: A primer on the use of thioflavin T to investigate amyloid formation. *Amyloid* **24**, (1): 1-16. 2017

For More Information

Find out more about the PlateCrane EX microplate handler and other laboratory robotics at
www.hudsonrobotics.com

Read more about Amprion, Inc. at
www.amprionme.com



The PlateCrane EX microplate handler.

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