FAST DECODING OF THE FIRST STEPS OF PROTEIN AGGREGATION USING A NANOPIPETTE

Meyer et al. pave the way to the early detection in vitro of α-synuclein (αS) assemblies with a nanopipette. The team developed a real-time fast amyloid seeding and translocation (RT-FAST) technique to detect and quantify this neural presynaptic biomarker involved in Parkinson’s disease using nanopipettes and electrical detection. The study shows the detection of α-synuclein seeding in real time over 90 min, much faster than the traditional methods proposed (days scale). The experimental principle consists of injecting an αS monomer solution into the nanopipette reservoir (Figure 1a). Samples mixed with WT, or A53T seeds that promote aggregation associated with early onset Parkinson’s disease, are compared with a control condition that contains only αS monomers, with the reference being the control at t = 0 min. Every 30 min, the current is recorded for 10 min to electrically detect αS (Figure 1b). The nanopipette geometry accelerates the reaction with a large surface/volume ratio favoring the amyloid seeding reaction through protein adsorption, conformational change, and desorption process. α-Synuclein seeding is then detected at the single-molecule...
scale using electrical detection through 34 nm diameter nanopipettes, giving a positive/negative response for aggregate presence. Noticeably, the assay developed is ultrasensitive to the initial seed concentration, from 2 pM to 200 pM (Figure 1c). As expected for the A53T, which promotes aggregation, the authors observe an increase of the normalized current blockade as a function of time. In other words, the size of the oligomers sensed increases with time (Figure 1d).

This study shows the detection of α-synuclein seeding in real time over 90 min, much faster than the traditional methods proposed (days scale).

Using very low recombinant protein concentrations, this proof of concept for fast detection of the first steps of protein aggregation with a micropipette and an electrical signal is an exciting development with colossal potential. While this study is qualitative, interesting future studies might probe the reversibility of the phenomenon at the first step of aggregation. What are the aggregate sizes and the number of proteins composing each aggregate? What is the role of diffusion and the mechanism of diffusion during aggregation formation? What is the best resolution using dwell time and current blockades to separate the different aggregate sizes present in the sample? Furthermore, it would be interesting in the future to use this nanopipette to probe potential drugs that prevent or decrease the kinetics of the aggregation process. Moreover, the key milestone will be the ability to directly detect aggregates from body fluids of patients as a potential diagnostic for neurodegenerative diseases.

**Author Information**

**Corresponding Author**

J. Pelta – Université Paris-Saclay, 91025 Evry-Courcouronnes, France; Email: juan.pelta@univ-evry.fr

**Author**

B. Cressiot – CY Cergy Paris Université, 95000 Cergy, France

Complete contact information is available at: https://pubs.acs.org/10.1021/acscentsci.2c00267

**Notes**

The authors declare no competing financial interest.

**REFERENCES**

(1) Meyer, N.; Janot, J.-M.; Torrent, J.; Balme, S. Real-Time Fast Amyloid Seeding and Translocation of α-Synuclein with a Nanopipette. *Acs Central Sci.* 2022, DOI: 10.1021/acscentsci.1c01404.

(2) Dobson, C. M. Protein folding and misfolding. *Nature* 2003, 426, 884—890.

(3) Ross, C. A.; Poirier, M. A. Protein aggregation and neurodegenerative disease. *Nat. Med.* 2004, 10, S10—S17.

(4) Soto, C. Unfolding the role of protein misfolding in neurodegenerative diseases. *Nat. Rev. Neurosci* 2003, 4, 49—60.

(5) Yukio, E. C.; et al. Single-particle characterization of Aβ oligomers in solution. *ACS Nano* 2012, 6, 5909—19.

(6) Yu, R.-J.; et al. Single molecule sensing of amyloid-β aggregation by confined glass nanofluid. *Chem. Sci.* 2019, 10, 10728—10732.

(7) Wang, H.-Y.; Ying, Y.-L.; Li, Y.; Kraatz, H.-B.; Long, Y.-T. Nanopore Analysis of β-Amyloid Peptide Aggregation Transition Induced by Small Molecules. *Anal. Chem.* 2011, 83, 1746—1752.

(8) Li, X.; et al. Label-free detection of early oligomerization of α-synuclein and its mutants A30P/E46K through solid-state nanopores. *Nanoscale* 2019, 11, 6480—6488.

(9) Houghtaling, J.; List, J.; Mayer, M. Nanopore-Based, Rapid Characterization of Individual Amyloid Particles in Solution: Concepts, Challenges, and Prospects. *Small* 2018, 14, e1802412.