Fast Decoding of the First Steps of Protein Aggregation Using a Nanopipette

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B. Cressiot and J. Pelta

New nanopipette reservoirs and electrical sensors aim to detect the first steps of amyloid protein aggregation and open up new perspectives in early neurodegenerative disease diagnostics.

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I n this issue of *ACS Central Science*, Meyer et al. reports on the development of new nanopipette reservoirs to detect at the single-molecule level the first steps of amyloid protein aggregation related to neurodegenerative pathologies.[1] Many diseases are caused by misfolding of proteins, which often lead to aggregate formation.[2] These diseases reduce life quality and expectancy. In particular, protein aggregation is responsible for many neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and prion disease.[3,4] This aggregation process occurs with intrinsic-disordered conformations to highly ordered structures rich in β-sheets, called amyloids. Understanding the aggregate formation pathway implicated in human diseases remains a challenge at the interface of several disciplines. Moreover, the ability to detect the early stages of alpha-synuclein aggregation implicated in synucleinopathies at low concentrations is a considerable challenge for developing powerful diagnostic approaches. Conventional techniques do not address this challenge.

Up to now, the nanopore technique and electrical detection have been used to detect amyloid β oligomers and fibrils.[5−9] Most of these studies have used relatively high concentrations of proteins to detect the different oligomers. Additionally, the time needed to sense these oligomers is usually on the order of days. None of these studies have explored the idea of decreasing the long lag-time (several days) required to reach an aggregate concentration detectable by conventional techniques.

Meyer et al. pave the way to the early detection in vitro of α-synuclein (αS) assemblies with a nanopipette.[1] 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
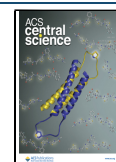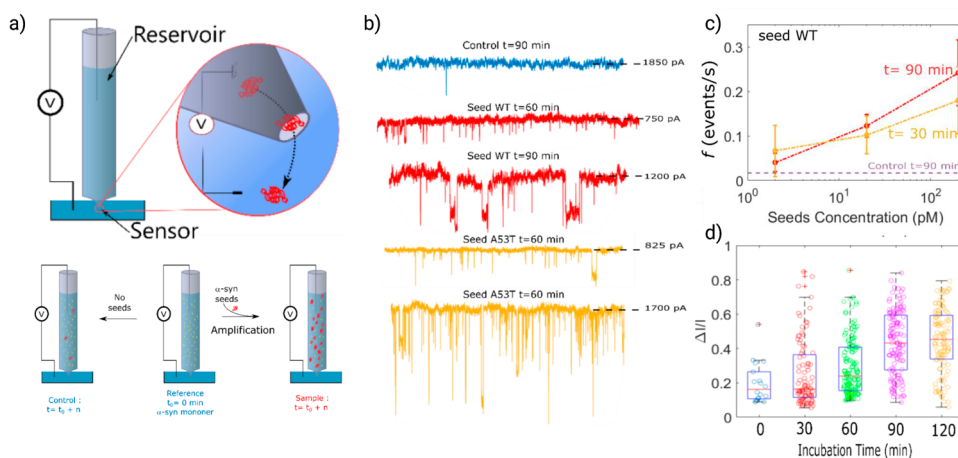
Figure 1. (a) Set-up for the detection of alpha-synuclein oligomers using a nanopipette. The sample can be seeded with either wt or A53T seeds. (b) Current traces for monomeric αS (blue), seeded with wt (red) and A53T seeds (yellow). (c) Frequency as a function of the initial αS WT seed concentration for incubation times of 30 min (yellow) and 90 min (red). (d) Distribution of the amplitude of the current blockade recorded for the sample seeded with 200 pM of α-synuclein A53T as a function of incubation time. Reproduced with permission from ref 1. Copyright 2022 The Authors. Published by American Chemical Society.

scale using electrical detection through 34 nm diameter nano-pipettes, 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-Courouronnes, France*; Email: juan.pelta@univ-evry.fr

Author
B. Cressiot − *CY Cergy Paris Université, 95000 Cergy, France*

Complete contact information is available at:
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Notes
The authors declare no competing financial interest.

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