Unraveling the secrets of neurodegenerative diseases, one protein at a time

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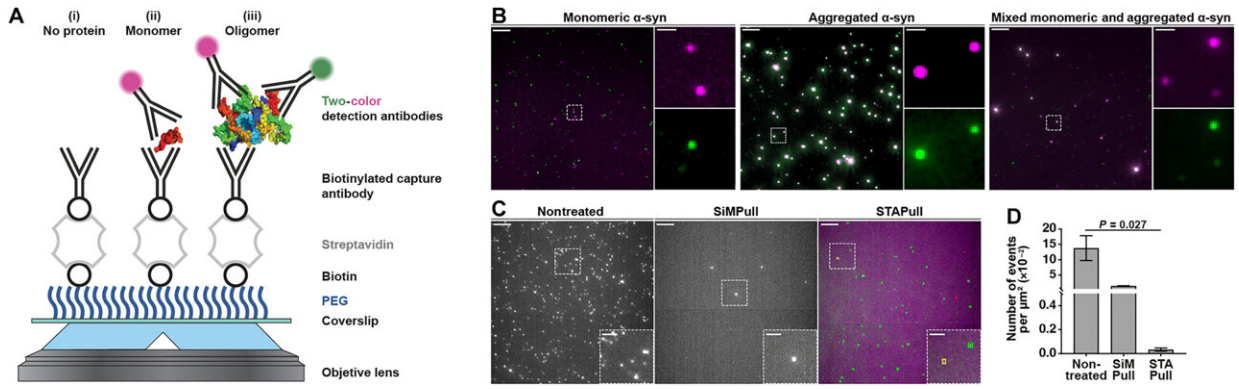

STAPull concept.(**A**) Glass coverslip is silanized and conjugated to PEG, of which 5% is biotinylated, and treated sequentially with streptavidin, biotinylated capture antibody, and the target biomolecule to surface-immobilize. Following incubation with the sample, pulled-down protein is probed using a mixture of AF488- and AF647-labeled monoclonal detection antibody such that (i) no bound protein produces no signal, (ii) monomeric protein produces single-color signal, and (iii) oligomeric protein produces two-color signal via TIRF microscopy. (**B**) Demonstrative composite STAPull images for 45 nM α-syn monomer (left), 5 nM α-syn aggregates (center), or both (right). Monomer is visible as single-color puncta (green or magenta) and oligomers as two-color (white). Single-channel enlargements of the indicated regions shown. Scale bars, 5 μm; crops, 1 μm. (**C**) Representative images of the background signal resulting from adsorption of detection antibody in the absence of target protein to clean nontreated (left), SiMPull (center), or STAPull (right) surfaces. In the latter case, the channel of local maxima are indicated with colored boxes to highlight whether single-channel (green and red) or two-channel (yellow). Scale bars, 5 μm; insets, 2 μm. (**D**) Mean density and SD of detections in (C) (n = 3, 16

technical repeats), statistical significance determined using a Kruskal-Wallis test (refer to table S2 for Dunn's post hoc pairwise means comparison analysis).

Proteins misfolding and clumping together, a process known as aggregation, is a key feature seen in several neurological conditions, including Alzheimer's and Parkinson's diseases.

These disorders involve the formation of small, potentially harmful structures called oligomers, which could serve as valuable indicators for early diagnosis. They are incredibly small, however, and much rarer than the healthy non-aggregated proteins. This makes it hard to detect and measure them accurately.

In collaboration with UCB Biopharma, researchers from the University of Edinburgh's Horrocks group have come up with an innovative solution called "single-molecule two-color aggregate pull-down," or STAPull for short.

This cutting-edge technique works by examining proteins that have been immobilized (held in place), and labeled with different colors using specific detection antibodies. By carefully analyzing signals where these colors overlap using sensitive microscopes, researchers can distinguish and quantify the aggregated proteins, while excluding the individual, non-aggregated ones.

To put it to the test, scientists used alpha-synuclein, the protein associated with Parkinson's disease, and found that STAPull could detect these aggregates at physiologically relevant concentrations. Furthermore, STAPull isn't limited to a specific type of sample, but can be applied to a wide range of samples, including biofluids from humans. This versatility makes it a valuable tool in the study of protein aggregates associated

with various disorders.

By enabling researchers to detect and quantify protein aggregates, STAPull opens up new possibilities for identifying biomarkers that can be used to diagnose these debilitating conditions early on, which could be crucial in the fight against these diseases.

Lead author, Dr. Rebecca Saleeb, Lady Edith Wolfson Research Fellow, School of Chemistry, University of Edinburgh, said, "Currently, patients are diagnosed with neurodegenerative disease based on their symptoms, which appear when the disease is advanced and irreversible cell damage has already occurred.

"In this work we present an alternative technology, STAPull, that can detect neurodegenerative disease in human biofluids. We are excited to continue developing this technology and explore if it can aid pre-symptomatic diagnosis."

Lead author, Dr. Ji-Eun Lee, Postdoctoral Research Associate, School of Chemistry, University of Edinburgh, said, "Early diagnosis of neurodegenerative diseases is a key to an increased range of treatment options, improved long-term survival with independence and improved quality of life. Our new technique, STAPull, improved the detection, especially for early stage oligomeric species, which are potentially more harmful but couldn't be detected with current methods. We are excited to apply this tool to assisting early diagnosis of neurodegenerative diseases in a wide range of samples, including biofluids from humans."

Senior author, Dr. Mathew Horrocks, Senior Lecturer in Biophysical Chemistry and Horrocks Lab team leader, University of Edinburgh, said, "This paper is the result of a fantastic collaboration with UCB Biopharma, who have provided our team with expertise and a range of highly specific antibodies. Using this approach, we're now able to

directly visualize aggregates, and also identify the proteins that they are composed of. This is a game-changer for future diagnostic approaches, and takes advantage of the ability to detect individual molecules."

The research is published in the journal *Science Advances*.

More information: Rebecca S. Saleeb et al, Two-color coincidence single-molecule pulldown for the specific detection of disease-associated protein aggregates, *Science Advances* (2023). DOI: 10.1126/sciadv.adi7359

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