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Tau proteoform assay performance using a single-molecule analysis platform

The microtubule binding protein Tau (MAPT) has been implicated as a driver of diverse progressive neurodegenerative diseases such as Alzheimer's disease. Six splicing isoforms of the Tau protein and over 70 different post-translational modifications (PTMs) have been identified on Tau that are believed to impact fibril formation and ultimately disease progression. Though the general prevalence of these alterations has been studied, there remains a significant knowledge gap with regards to which proteoforms (defined by splice variants and one or more PTMs) are most prevalent.

We have developed a single-molecule protein analysis platform. The platform immobilizes individual protein molecules on a hyper-dense flow cell and then iteratively probes those molecules with splice-variant specific or PTM-specific affinity reagents. Each protein molecule on the flow cell is assigned an estimated proteoform based the probes that were detected to have bound that protein.

For platform validation we first created reference proteoforms with recombinantly expressed proteins and mass spectrometric analysis was used to determine which sites and percent of Tau were modified. Several mixtures of these control proteoforms were then generated, with different ratios of components and analyzed on the platform. These studies showed both that mixtures of proteoforms could be accurately quantified. We additionally estimate the platform reproducibility. Finally, the quantitative sensitivity was measured for individual proteoforms over 3 logs of dynamic range. To demonstrate the biological relevance of the assay, we applied the assay to a number of neuronal tissue such as iNeurons, miBrains and human brain.

These studies demonstrate that the Nautilus platform is able to accurately and reproducibly quantify proteoform molecular heterogeneity in cell-based samples. We anticipate that assays like this will ultimately enable a deeper investigation into the role that proteoform heterogeneity plays in diseases like Alzheimers and may also potentially enable proteoform-based diagnostics with greater sensitivity than existing approaches.

User consent

yes

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