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N&B and Cross-N&B Analysis Detect Oligomerization of Huntingtin in Live Cells

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Aggregation of misfolded proteins is a hallmark of several neurodegenerative diseases such as Huntington's disease (HD). HD is caused by a mutation of Huntingtin caused by an elongation of a polyglutamine (polyQ) sequence in the protein. Here we describe the application of the recently developed Number and molecular Brightness method (N&B) to monitor the aggregation process of Huntingtin exon1 (Httex1). N&B measures the molecular brightness of the protein aggregates in the entire cell non-invasively based on the fluctuation dynamics at each pixel of an image. This analysis provides a map of aggregation with pixel resolution.

We observed the behavior of Httex1-97QP-EGFP this is a construct with 97 polyQ repeats corresponding to Juvenile onset of the disease.

We preformed experiments in ST14A cells transfected with Httex1-97QP-EGFP. We establish that the process of nucleation leading to inclusion formation has four phases: i) Initially only monomers are present; ii) Following an increase in protein concentration (~1 μM), due to protein accumulation, small oligomers (8-15 proteins) form throughout the cell; iii) At higher protein concentrations, an inclusion is formed in the cytoplasm; iv) The inclusion recruits most of the Httex1 protein in the cell, including those in the nucleus, leaving only monomers at very low concentration.

We also performed cross-N&B analysis to measure the size of the oligomeric species. Cross-N&B recovers the stoichiometry of the complexes from the simultaneous fluctuations of the fluorescence intensity in two image channels. The experiments were done on ST14A cells co-transfected with Httex1-97QP-EGFP and Httex1-97QP-mCherry. These experiments confirmed the mechanism of aggregation observed by N&B and the range of size of the oligomers.

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