SHINING LIGHT ON AMYLOID PROTEIN AGGREGATES: Establishing the composition of alpha-synuclein oligomers using single-molecule photobleaching

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Growing evidence suggests that alpha-synuclein oligomeric aggregates are key players in the onset and progression of Parkinson's disease. However, very little is known about the molecular details of these aggregates.

For large protein aggregates, such as alpha-synuclein oligomers, it is very difficult to determine the number of monomers that form an oligomer using conventional techniques. We developed a method that uses sub-stoichiometric labeling, that is, only a fraction of the monomers contain a fluorescent label, in combination with single-molecule photobleaching to determine the number of monomers per oligomer [1]. The number of bleaching steps gives the number of fluorescent labels per oligomer. By using the exact label density, that is, the fraction of labeled monomers at the start of the aggregation, we can link the number of fluorescent labels per oligomer to the total number of monomers. Using this method, we can determine the composition, probe the distribution in the number of monomers per oligomer, and investigate the influence of the fluorescent label on the aggregation process.

We find that the composition of alpha-synuclein oligomers varies between different preparation protocols. For oligomers prepared using a high concentration of alpha-synuclein, we find no distribution in the number of monomers per oligomer and find a single, well-defined alpha-synuclein oligomeric species consisting of ~30 monomers per oligomer. For oligomers formed under the addition of dopamine however, we find multiple oligomeric species ranging from ~20 to ~35 monomers per oligomer.

[1] N. Zijlstra, C. Blum, I.M.J. Segers-Nolten, M.M.A.E. Claessens, V. Subramaniam, "Molecular composition of sub-stoichiometrically labeled α-synuclein oligomers determined by single-molecule photobleaching," *Angew Chem Int Ed* **51** (35), 8821–8824 (2012)