Non-perturbative, site-specific labeling of α-synuclein for studying amyloid disease and screening therapeutics

Brief Description
Fluorescently labeled α-synuclein to examine destabilization of synuclein fibrils by small molecules and therapeutics for neurodegenerative diseases

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Problem
Misfolding of the neuronal protein α-synuclein triggers oligomerization and the formation of amyloid fibrils that are the pathological hallmarks of Parkinson’s disease. The molecular mechanism of synuclein misfolding has not been elucidated and therefore screening of compounds that block or reverse aggregation can establish novel mechanisms of action. Previous efforts to employ small molecule probes, such as Thioflavin T and Congo Red, in drug screening experiments have been unfavorable, as the probes compete with candidate compounds for binding sites on synuclein. There is no cure for these progressive neurodegenerative diseases, although therapeutics and biologics are in development. A high-throughput screening method to examine fibril disaggregation as a measure of therapeutic efficacy would be advantageous to the pharmaceutical industry developing the next generation of therapeutics.

Solution
Researchers in the Petersson lab have developed a minimally-perturbative fluorescence polarization assay to monitor the local conformational dynamics of α-synuclein. Synuclein has been site-specifically labeled with the fluorophores, chromatographically purified, and characterized with mass spectrometry. The non-perturbing nature of the labels has been validated by in vitro kinetics experiments, quantification of the incorporated labeled protein, and TEM imaging of the synuclein fibrils. The labeled synuclein can be used to examine fibril formation and destabilization in in vitro and cellular models of neurodegenerative disease propagation and progression.