Tata Institute of Fundamental Research
Survey No. 36/P, Gopanpally Village, Serilingampally, Ranga Reddy Dist., Hyderabad – 500 046

Internal Webinar

Segmental Isotopic Labelling and Formation of Fibril Polymorphs of α-Synuclein

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The aggregation of proteins into amyloid fibrils is implicated in several neurodegenerative diseases. Various Synucleopathies are caused by aggregation/deposition of the α-Synuclein protein in the cytoplasm of neurons. α-Synuclein is a 140 residue intrinsically disordered protein abundant in the brain. α-Synuclein oligomers seem to be more cytotoxic and play a crucial role in defining the polymorphism associated with its aggregation. Solid-state NMR spectroscopy can be used to characterize the different oligomers structurally. However, oligomers are structurally very heterogeneous, giving rise to various local environments and compromising the spectral resolution. To improve the spectral resolution of α-Synuclein oligomers, we employed segmental isotopic labelling. For this, a hybrid approach combining recombinant protein production with chemical ligation was used. Various C-terminal and N-terminal fragments of α-Synuclein were combinatorially labelled and ligated in vitro to create the full-length protein. The segmentally labelled α-Synuclein was confirmed by multiple biophysical techniques, including NMR spectroscopy. I discuss the challenges and the outcomes of this approach.

I will also discuss fibril polymorphism arising from the incubation of α-Synuclein protein under identical conditions. We incubated solubilized α-Synuclein protein prepared in three different ways for fibril formation. Though the monomer being a predominant species in the three protein preparations, the resulting fibrils appear to be very different. Using various biophysical characterization techniques, we found that these polymorphs have different fibril-core structures and differ in their kinetics of fibril formation. Site-specific structural differences between these fibrils resulting from three different α-Synuclein preparations are currently being investigated using multi-dimensional solid-state NMR spectroscopy.

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