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

Students' Annual Seminar

The bacterial plasmid-segregating protein ParM as a model system to understand nucleotide-dependent polymerisation in actin-like proteins

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Plasmid segregation is a vital step for bacteria's containing low copy number plasmid, as these plasmids provide them with certain antibiotic resistance. One such protein that drives the plasmid segregation by actin-like ATPase activity is ParM. Like actins, ParM responds to the presence/absence of different nucleotides which modulates the polymerisation and depolymerisation of the ParM filament. To understand the dynamics of actin-like proteins in their filamentous state (F-state) in response to different, nucleotides, their stabilization/destabilization by cofactors, and interaction with membranes, atomic level information is desired. This can in principle be achieved by solid-state NMR spectroscopy. ParM in particular fits to be an ideal model system to answer some of these questions using solid-state NMR spectroscopy.

ParM is a 37 kDa protein which makes it difficult to study by traditional ^{13}C-detected MAS NMR. We will be using fast magic-angle-spinning (40-100 kHz) ^{1}H-detected solid-state NMR, which will allow us to study this protein at the atomic level. I will be presenting our work that have enabled us to prepare uniformly labelled (either U-(^{2}H, ^{13}C, ^{15}N) or U-(^{13}C,^{15}N)) ParM in the F-state fibril formation using a non-hydrolyzable nucleotide analog. Conditions that allowed us to stabilize this protein for the duration of the lengthy NMR experiments will also be discussed. This has enabled us to to obtain well-resolved spectra and record multiple ^{1}H-detected 3D experiments for obtaining assignments of this protein. I will also present initial Rotational-echo double-resonance (REDOR) based experiments that give us insights into side chain dynamics in this protein. The study of ParM filaments in the ADP bound can also tell us about the dynamics in different nucleotide state, a brief discussion on the use of ParM(E148A) as an end cap to stabilize the filaments will also be addressed

Friday, Apr 28th 2023

10:00 AM (Tea / Coffee 09.45 AM)

Seminar Hall, TIFR-H