**Mechanical aspect of human lamin A as revealed by Single Molecule Force Spectroscopy**

Manindra Bera\*, Ainavarapu SR#, Kaushik Sengupta\*

\*Biophysics and Structural Genomics Division, Saha Institute of Nuclear Physics, Sector-1, Block-AF, Bidhannagar, Kolkata-64, India, # Chemical Science Division, Tata Institute of Fundamental Research, Mumbai, India

Abstract: Lamin A is a type V nuclear intermediate filament protein which forms an elastic meshwork underlying inner nuclear membrane thereby providing mechanical strength and rigidity to the nucleus. For most vertebrates, nuclear lamins have been categorized into two types, A and B-type lamins As of all other intermediate filaments, nuclear lamins also have same tripartite structure consisting of an N-terminal head domain, central α-helical rod domain and C-terminal tail domain containing the conserved s-type immunoglobulin fold (Ig). Till date, more than 450 point mutations have been discovered in LMNA gene which produces diverse spectra of diseases in tissue specific manner, collectively coined as laminopathies which include Dilated Cardiomyopathy, Emery-Dreifuss Muscular Dystrophy etc. Structural and gene regulation hypothesis account in a mutually exclusive and/or inclusive manner to account for the pathogenesis of these diseases. Structural hypothesis suggests lamin mutations perturb the higher order structure formation resulting into fragile nuclei which is a major hallmark for the laminopathies. We have focussed on the structural hypothesis to understand the mechanoelastic behaviour of lamin A and how it modulates the nuclear rigidity. To establish this, we performed Single Molecule Force Spectroscopy (SMFS) with human lamin A protein. Three DCM causing mutations (K97E, E161K, R190W) from 1B domain, R377L from 2B domain and one EDMD causing mutation, R453W from immunoglobulin domain were selected to compare the mechanical unfolding events at single molecule level. We have also probed the interaction of emerin with the mutant Ig fold domain by SMFS. Particularly due to R453W mutation, lamin-emerin (a major constituent of nucleoskeleton-cytoskeleton coupling complexes) interactions were perturbed, as also evidenced earlier from cell biological studies. R453W mutant domain was unfolding at lower force compared to the wild type Ig domain whereas we found out that the coiled-coil 1B and 2B domain was unfolding in several pathways suggesting a very flexible coiled-coil formation.