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Title of the Thesis: Structure function analysis of Neuroserpin for its role in human disease

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Conformational diseases are a newly defined group of disorders whose underlying cause is the accumulation of pathogenic conformers of a host protein in the affected organ. Point mutations in the conserved residues of a protein may lead to its deficiency in the cell resulting in its reduced activity and causing several diseases. Moreover, misfolded proteins have been observed in the endoplasmic reticulum (ER) or cytosol and are associated with a variety of clinical disorders called protein misfolding diseases. Serine protease inhibitors (serpins) are a widely distributed superfamily of proteins that are ubiquitous in nature and perform vital array of functions in diverse organisms. Serpins use unique suicide substrate-like inhibitory mechanism for inhibiting their target proteases and the region responsible for interaction with target protease is the Reactive Centre Loop (RCL).

Neuroserpin (NS) is a brain specific 46 KDa serpin involved in brain development, memory and synapse formation and it has been shown to inhibit tissue plasminogen activator (tPA). Natural variants of NS form ordered polymers that accumulate within the ER of neurons. These mutations are the underlying cause of an autosomal dominant dementia called as Familial Encephalopathy with Neuroserpin Inclusion Bodies (FENIB). In NS, helix F and helix B are present on either side of β-sheet A and they serve to accommodate the incoming RCL, but the exact molecular details of the conformational changes during strand 4A incorporation are still not well understood.

In order to understand the serpin related diseases, it is imperative that we first understand the conformational changes involved in the folding and unfolding of serpins. One of the probable key players in determining conformational aspects of serpins is helix F. We initiated our study with multiple sequence alignment and found that Trp 154 is highly conserved among both NS orthologs and paralogs in helix F. To check the importance of this residue in maintaining structure-function of NS, it was substituted to Ala (W154A) and Pro (W154P) through site-directed mutagenesis. Before getting into *in-vitro* experiments, we performed *in-silico* studies to understand the nature of hydrophobic interactions. For the first time we identified, a previously unknown conserved hydrophobic patch at the interface between helix F and β-sheet A centered

around Trp 154 which has the potential to influence helix F conformational transition during inhibition mechanism.

Molecular Dynamics simulations of these variants revealed marked changes in helix F region, strands 1A and 2A. Evaluation of W154A and W154P variants for structure-function analysis showed decreased fluorescence intensity with W154P showing a red-shift indicating partial unfolding and a more hydrophilic environment. An increase in surface hydrophobicity along with marginal increased α-helical content was observed in W154P but not in W154A and WT NS. These results supported that both the variants have different conformations. The inhibition analysis of the W154P variant demonstrated a significant decrease in association rates and absence of tPA-NS complex formation and appearance of cleaved NS.

The residues of shutter region in serpin are highly conserved and plays crucial role in the accommodation of the incoming RCL upon cleavage by protease during normal inhibition mechanism. Helix B and strand 6 B (s6B) are part of shutter region, to understand the role of this region, similar studies with helix B and s6B residues Leu 55, Ala 54, Ile 46 and Phe 48 revealed that substitution of Leu 55 to Pro lead to distortion of helix B as visible in the biophysical and biochemical analyses i.e. fluorescence, circular dichroism, thermal denaturation, heat induced polymerization and complex formation assays. And behaviour of I46D and F48S variants of s6B were very similar to L55P. In contrast, the other helix B variants L55A and A54F behaved like wild-type NS. The data indicates that these residues of shutter region are important in maintaining the metastable conformation of NS and any mutation at these positions will cause an imbalance in the normal functioning of NS leading to polymerization and uncontrolled activity of tPA.

Overall this is the first study where a novel hydrophobic patch has been identified surrounding Trp 154 in helix F and surrounding Leu 55 in helix B. We have shown that partial deformation of helix F shifts it in a synchronized manner with strands 1A and 2A occurring along with the relaxation in the loop connecting strand 1A. This coordinated movement occurs important for not allowing a gap to form between the strands of β-sheet A before inhibition process for insertion of the strand 4A. In case of helix B and s6B, the substitution at conserved position leads to alteration in the structure of variant causing polymerization and/or aggregation. Further studies needs to be done using variants of conserved hydrophobic residues identified in this study to better understand the mechanistic aspects of NS in disease onset and development.