Gelsolin, a six-domain actin severing, nucleation and capping protein, exists as a compact globular structure under Ca^{2+} free conditions at physiological pH and attains an open functionally active structure in the presence of 1 mM Ca^{2+} or at lower pH (Burtnick, Koepf et al. 1997; Ashish, Paine et al. 2007; Garg, Peddada et al. 2011). Despite the continuous efforts from crystallographers, no high resolution crystal structure of the activated full length plasma gelsolin is available till date, suggesting the flexibility of the system in active state. Our group, for the first time, has solved the solution structures of the fully activated and the intermediate states of full length plasma gelsolin in calcium-activation pathway. We showed the role played by the flexible linker regions in the opening of the molecule and differential orientation of the G1 domain in actin bound and the unbound state (Ashish, Paine et al. 2007). Structural studies mainly from the crystal structures of G1-G3/actin (PDB:1RGI) and G4-G6/actin (PDB:1HIV) in the presence of Ca^{2+}, Small angle X-ray Scattering (SAXS) studies (Ashish et al, 2007) and the radiolytic foot printing studies (ref) together combined with some biochemical studies have explained the complete mechanism of Ca^{2+} activation of gelsolin *in vivo*. Besides calcium, low pH has also been shown to activate the severing and nucleation functions of the gelsolin (Lamb, Allen et al. 1993). However, no structural evidence of the pH activation of the gelsolin has been documented yet except an increase in the hydrodynamic radius of the molecule upon lowering the buffer pH values. With prior experience in hand, we made an effort in this thesis to track the global structural changes in full length gelsolin associated with pH activation by using SAXS.

Back scattering data from the DLS experiments in the buffers of different pH values suggested that the molecular mobility of gelsolin decreases by ~1.6 fold upon lowering the pH of buffers from 9 to 5. Structural parameters deduced from the SAXS data also suggested a gradual increment in the size of the molecule upon lowering the pH of the buffers. The sigmoidal growth curve fitted on the R_G and Dmax suggested that 50% change happened at pI values of the protein and the pattern of the pH induced changes can be confidently fitted to a two state model, unlike the three state model proposed for the calcium activation of gelsolin. The models generated within the shape constraints of the SAXS profiles of the samples at pH 9, 8, 7, 6 and 5 showed that gelsolin remains as a tight compact globular structure in the buffers of pH 9, 8, 7, but starts opening progressively from pH 6 to 5. Upon manual overlaying of the open crystal structures of N- and C-terminal halves of gelsolin resolved as

Ca^{2+} activated forms bound to actin within the SAXS volume of gelsolin at pH 5 revealed that the N-terminal G1 domain extends away from the rest of the molecule through extended g1g2 linker region while g3g4 linker remains unopened. Overall, our SAXS data has suggested that the low pH induces only a partial opening of the gelsolin but not fully. To confirm the partial opening of the gelsolin at low pH conditions, we have made dT GSN (which requires less amount of calcium for full activation), and looked at the structural changes induced in it atdifferent pH values. Structural parameters of this truncated protein at pH 5 were comparable with those derived for full length gelsolin at pH 8 in 1 mM Ca^{2+}. To further demonstrate the partial opening of gelsolin at low pH, pre-equilibrated full length gelsolin in different pH buffers was supplemented with different amounts of free calcium. A supplementation with just 40 nM of free calcium was capable of completely opening the molecule at pH 5, which compared very well with the shape at 1 mM calcium at pH 8. Overall our studies have revealed two important facts of pH activation of gelsolin i) opening of the N-terminal half i.e., opened g1g2 linker ii) compact shaped C-terminal half i.e., unopened g3-g4 linker. We have provided the structural insight into how low pH alone can open the g1g2 linker, which is essential for F-actin severing but the mechanism of severing still remains to be fully understood.

Due to its ability of F-actin severing, plasma gelsolin is emerging as a therapeutic molecule in different diseases. It necessitates the need for minimal versions with better efficacy (Biobetters), which can replace the full length protein in a cost and dosage effective manner. Knowing the fact that the severing function of the full length gelsolin resides in its N-terminal half (G1-G3), we have rationally designed several gelsolin constructs retaining the necessary components required for actin binding/severing function. Depolymerization assay of F-actin with different truncated gelsolin variants [G1-G3 G1-G2, G2-G6 , G4-G6, dT-GSN, 1-161, 25-161, 28-161, 30-161, 32-161, 36-161, 40-161, 42-161 and 56-161] showed that 30-161 is the smallest gelsolin which can sever the F-actin with equal efficacy as the full length protein, while deleting any residues from either side (N-terminal [32-161, 36-161, 42-161 and 56-161] or C-terminal [28-158, 28-156]) resulted in a loss of the function and these fragments became inactive. Thus here we have redefined the minimal gelsolin i.e. 30-161, which can depolymerize the F-actin in a calcium and pH dependent manner. Interestingly, although G1-G3 severs the F-actin in calcium independent manner, the complete dependency of truncates

;1-G3 [G1-G2, 1-161, 25-161, 28-161] on Ca^{2+} or Low pH for activation remained ling to us. Ab *intio* modeling of the SAXS data collected for the truncated gelsolins (G1-G1-G2, 28-161) in the buffers containing either EGTA or 1 mM Ca^{2+} at pH 8 and EGTA H 5 showed that G1-G3 adopted an open shape in all the conditions, where as G1-G2 and 61 existed as collapsed state under Ca^{2+} free conditions, but attained an open shape by er supplementation of 1 mM Ca^{2+} or low pH. To gain an insight into the calcium pendent severing activity of the G1-G3, we have made some site directed mutants of the ;3 linker based on this linker region of the severin, a homolog of gelsolin, which severs F-n in a calcium dependent manner. Severing data combined with the SAXS data ıonstrated that Mut2 through Mut4-G1-G3 became calcium dependent for their severing vity. Structure reconstruction of these mutants further revealed that while the three ıains of Mut1-G1-G3 adopt an open-shape similar to G1-G3 under EGTA conditions at pH Mut2 through Mut4-G1-G3 prefer a collapsed state under these conditions. Their severing ı combined with the SAXS data thus demonstrated a role played by the g2-g3 linker in ıorting the calcium insensitivity to wild type G1-G3 for its severing activity.

Finally, the *in vivo* experiments using murine sepsis model have established the tective nature of these minimal gelsolins (G1-G3, 28-161). The infusion of LPS reduced endogenous plasma gelsolin levels to 47% of the control value and the exogenous letion with 2 mg of the full length, G1-G3, or 28-161 proteins was able to bring these els back to the normal levels within 24 hrs of the administration and also significantly proved the survivability of the septic mice (G1-G3, upto 50% survival, $p<0.007$ *versus* PBS ıtrol, 28-161, 75% survival, $p<0.001$ *versus* PBS control respectively). The treatment with : exogenous proteins also shifted the cytokine profiling of septic mice from pro lammatory to anti inflammatory state, indicating the anti-inflammatory role of the minimal rsion like the full length protein shown previously (Lee, Waxman et al. 2007).

Overall, this thesis has put forward two novel findings into the field of gelsolin ;earch. First, we have shown how low pH alone is able to activate the full length gelsolin ıder calcium free conditions and second, we have made biobetters for the gelsolin placement therapy, which can rescue the mice from septic shock. Eventually, gelsolin placement therapy in future could consist of formulations of some of these biobetters instead the full length protein.