5. Summary and conclusion

The two main aims of this work were to understand the effect of the phosphorylation state of the bound nucleotide on the conformation of actin and discern the mechanism of F-actin nucleation by gelsolin. As these problems are not amenable to commonly used techniques like X-ray diffraction, Nuclear magnetic resonance, electron microscopy etc. due to reasons described earlier, we used small angle X-ray scattering at high brilliance synchrotron sources. Owing to the extremely high flux of X-rays, we were able to study the global shapes of the proteins-ligand and protein-protein complexes in more "native-like" conditions, at lesser concentrations and in relatively smaller durations of time.

The small angle X-ray scattering studies of actin bound to ATP and AMP-PNP at different time points revealed that the radius of gyration and maximum linear dimension of actin increased with time. As such an increase in the dimensions of actin was not observed in case of AMP-PNP, which is a non-hydrolysable analog of ATP, we concluded that actin adopted a more relaxed and open state as the nucleotide bound to it got hydrolyzed. This study provided the first direct evidence for the existence of an open state of α-actin in complex with ADP analogous to other ATPases like hexokinase.

Before proceeding for the second major aim of this experiment i.e. understanding the global shapes of gelsolin and actin, we achieved the relatively larger scale expression of recombinant gelsolin at the scale of 3 L bioreactor. In order to simplify the process of purification and avoid the formation of inclusion bodies, we engineered a construct of gelsolin which drives its secretion into the culture media. After standardizing the conditions like media composition, time of induction etc., we were able to achieve $O.D_{600}$ of ~60. The yield of gelsolin after ion exchange chromatography was ~600mg/L. We, then, studied the complexes of gelsolin with one, two and

four actin molecules using small angle X-ray and neutron scattering. We were able to derive the global shape parameters the above mentioned complexes and reconstruct three dimensional models. Our SAXS data showed that the binary complex of gelsolin and actin (GA) is formed by the binding of an actin molecule to the N-terminal half of gelsolin. The SAXS and SANS data of the ternary complex of gelsolin with two actin molecules (GA_2) revealed that the two actin molecules in the ternary complex are not in a "filament-like" arrangement. This observation provided a structural insight into the inefficiency of the GA_2 complex to nucleate the formation of F-actin filaments. Our model of the GA_2 complex, in which, the actin molecules bound to gelsolin are in an arrangement unfavorable for F-actin formation, also provides a mechanism by which gelsolin may perform its physiological role in the plasma i.e. to depolymerize the actin filaments and prevent them from reassembling. However, as gelsolin is able to nucleate the formation of F-actin filaments, the actin molecules bound to gelsolin must adopt an arrangement conducive to nucleation, probably at the level of a higher order complex. The SAXS data of the complex of gelsolin with four actin molecules showed that the actin molecules adopted a "filament-like" arrangement in this complex. This model agrees with the experimental observation that short actin filaments capped with gelsolin on one end are able to nucleate the formation of F-actin. In conclusion, our SAXS and SANS data show that the two actin molecules bound to gelsolin in the GA_2 complex are in a "non-filament" like arrangement which are brought into a "filament-like" arrangement by the addition of more actin molecules.

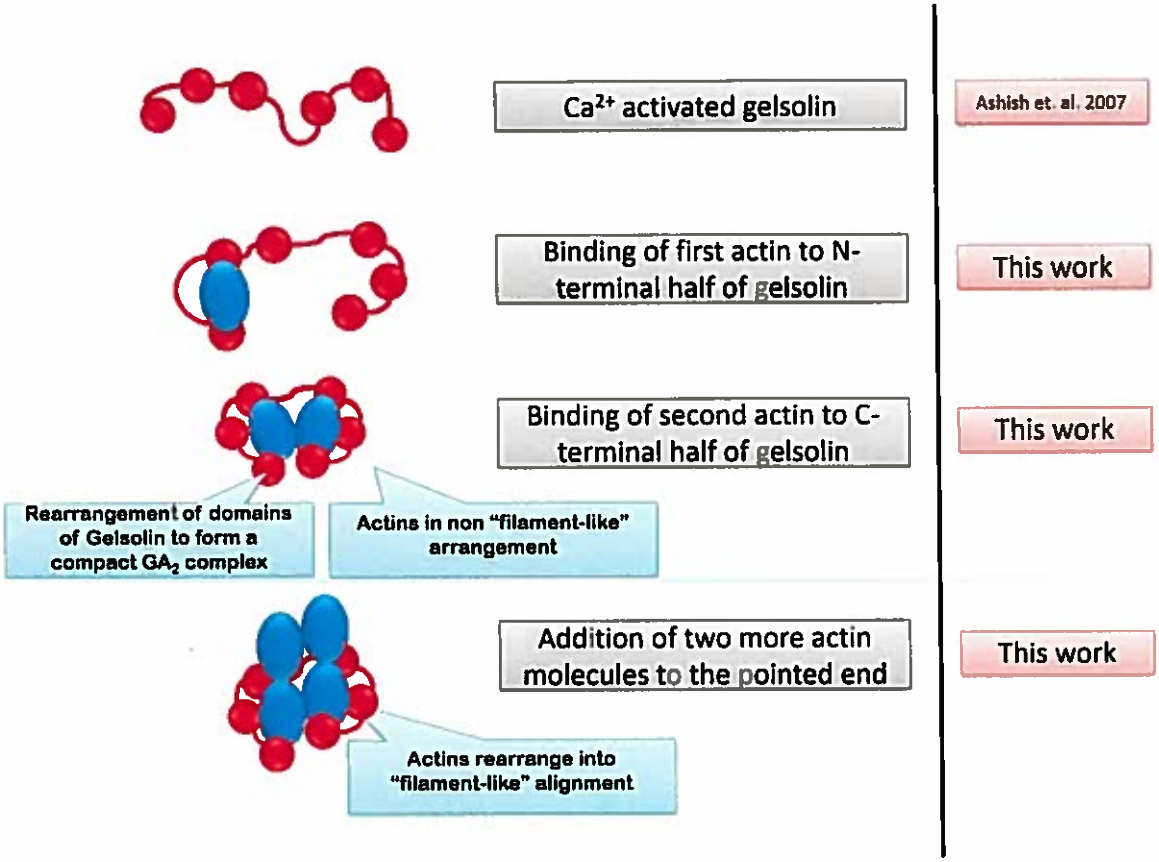

Figure 5.1: Schematic representation of the mechanism of gelsolin-actin complex formation leading to nucleation of filament formation.