CHAPTER-7

*Future perspective*
The current study has set up foundation for exploring several intriguing questions:

1. The residue level detailed information obtained from HPV18 E2 and procaspase-8 interaction analyses has shed new lights on role of E2 in apoptosis. The conservation of the interface residues across different HPV types suggests similar mechanism of apoptosis induction by E2 proteins. It will be therefore interesting to perform analogous studies with E2 protein from other viruses (including both low and high risk types) particularly targeting the key residues identified in the present study, and attempt to develop a model for E2-induced apoptosis.

2. Knowledge of the structure and dynamics of proteins and protein assemblies is critical both for understanding the molecular basis of physiological and patho-physiological processes and for guiding drug design. Structural analysis of a novel E2-procaspase-8 interaction in atomic detail therefore becomes imperative. Owing to the poor solution behavior of these proteins, a high resolution structure of the full length complex is however impractical. Strikingly, the information on minimal binding regions now could be utilized to determine the structure and dynamics of the E2-procaspase-8 complex. These atomic details could be utilized to design E2 analogs with enhanced proapoptotic properties that might have potential for disease intervention.

3. Evidence from the current study suggests that initiation of apoptosis by E2 and FADD-mediated procaspase-8 activation occur due to oligomerization of caspase-8 induced through distinct mechanisms. It would be thus interesting to determine whether E2 induced cell death can co-operate with FADD to enhance caspase activation. Furthermore, it would be important to address how the activation of procaspase-8 is regulated in the DED chain. Establishing a direct link between these pathways would be necessary to understand the effect of the protein-protein interactions on DISC
formation and its overall function with an aim at targeting the extrinsic cell death pathway from a different angle.

4. Multidomain proteins due to their structural complexity require different levels of regulatory mechanisms for executing cellular functions efficiently within a specified time period. Allosteric modulation is one such way which often helps a protein such as an enzyme to regulate functional behavior. Although we could provide the first evidence of the complex allosteric regulation of HtrA2, the mechanism of allosteric propagation and the underlying conformational plasticity needs to be delineated. Identification of the control switch regulating the protease activity will provide mechanistic details of its association in binding to myriads of cellular substrate and their cleavage. Furthermore, it will provide an idea of how HtrA2 might be activated in vivo in presence of different stimuli. These advances in our understanding of HtrA2 mechanism and function will help design allosteric modulators to manipulate its functions with desired characteristics.