CHAPTER 10

SUMMARY AND FUTURE PROSPECTS

Summary and Future Prospects

10.1. Overview of Results:

The main theme of this thesis involves studying the inhibition strategies for the prevention of aggregation of the causative agents of AD and PD, i.e. $A\beta_{1-42}$ peptide and αS respectively. The thesis has been divided into two major parts. The first part deals with studying the inhibition approaches for prevention of aggregation of the $A\beta_{1-42}$ peptide. The second part deals studying the inhibition approaches for prevention of aggregation of the αS protein.

Within the first part of the thesis, three different inhibition approaches were discussed that affects $A\beta_{1-42}$ peptide aggregation. These inhibition approaches were effect of ionic strength, effect of dimerization of peptides and role of a small molecule, Resveratrol (a polyphenol). All these inhibition approaches discussed in the **chapters 4, 5 and 6** have highlighted their effect on the aggregation of the $A\beta_{1-42}$ peptide.

The first inhibitory study for $A\beta_{1-42}$ peptide was to understand the effect of ionic strength of the solution on the aggregation propensity of $A\beta_{1-42}$ peptide, using computational approaches. From the MD trajectory analysis, it was observed that with increase in the ionic strength of the solution, $A\beta_{1-42}$ peptide monomer showed lesser tendency to undergo aggregation. From RMSD and SASA analysis, it was noticed that the $A\beta_{1-42}$ peptide monomer to undergo rapid change in conformation with increase in the ionic strength of the solution. In addition, from the radius of gyration (R_g) analysis, it was observed that the $A\beta_{1-42}$ peptide monomer to be more compact at moderate ionic strength of the solution. The $A\beta_{1-42}$ peptide was also found to hold its helical secondary structure at moderate and higher ionic strength of the solution. The diffusion coefficient of $A\beta_{1-42}$ peptide monomer was also found to vary with the ionic strength of the solution. Relatively higher diffusion coefficient value was observed for $A\beta_{1-42}$ peptide at moderate ionic strength of the solution. In summary, the findings from this computational study highlighted the marked effect of ionic strength of the solution on the conformational dynamics and aggregation propensity of $A\beta_{1-42}$ peptide monomer.

In this second study of the inhibition of $A\beta_{1-42}$ peptide aggregation, the association and dissociation between the CTerm of Human Albumin (peptide) and the $A\beta_{1-42}$ peptide (Heterodimer) and the two units of $A\beta_{1-42}$ peptide (Homodimer) was studied. In this

work, using Potential of mean force (PMF) and binding free energy (BFE) calculations, we have demonstrated the effect of CTerm of HA on the dimerization of $A\beta_{1-42}$ peptide. From the PMF profile, we noticed $A\beta_{1-42}$-CTerm Heterodimer (10.99 kcal mol^{-1}) complex to have higher disassociation energy than $A\beta_{1-42}$-$A\beta_{1-42}$ Homodimer (2.23 kcal mol^{-1}) complex. And also from the BFE calculations, we found that the binding affinity between $A\beta_{1-42}$ peptide and CTerm (ΔG_{bind} = -32.27 kcal mol^{-1} from MM-GBSA and ΔG_{bind}= -2.83 kcal $mol^{-1}$from MM-PBSA) to be stronger than the $A\beta_{1-42}$ peptide and another $A\beta_{1-42}$ peptide (ΔG_{bind} = -16.20 kcal mol^{-1} from MM-GBSA and ΔG_{bind} = -1.95 kcal $mol^{-1}$from MM-PBSA). In this study, our findings from PMF and BFE analysis of the two complexes, provides salient structural, binding and unbinding features and thermodynamics that support the ability of CTerm of HA in affecting the dimerization of $A\beta_{1-42}$.

The third inhibitory research for $A\beta_{1-42}$ peptide studied the role of Resveratrol (RSV) on the $A\beta_{1-42}$ peptide aggregation. The secondary structure and the conformational analysis obtained from MD trajectories showed that the binding of RSV with $A\beta_{1-42}$ peptide monomer caused an increase in the helical content in the structure of the $A\beta_{1-42}$ peptide. The BFE and PRED results showed a high binding affinity (GB_{total}=-11.07 kcal mol^{-1}; PB_{total}= -1.82 kcal mol^{-1}) of RSV with $A\beta_{1-42}$ peptide. Also we found the RSV to interact with crucial residues (Asp 23 and Lys 28) of $A\beta_{1-42}$ peptide. These residues are known to play a significant role in facilitating the formation of toxic amyloid oligomers and amyloid fibrils. The salt bridge interaction between these residues D23–K28 was found to be destabilized in the$A\beta_{1-42}$ peptide when it is complexes with RSV. In summary, it can be concluded that the prevention of the $A\beta_{1-42}$ peptide aggregation is greatly aided by RSV and therefore it can be considered as a possible drug candidate for therapeutic strategies of AD.

In the second part of this thesis, three different inhibition approaches that affects αS aggregation were discussed. These inhibition approaches were effect of macromolecular crowding, effect of peptides and role of a small molecule, Oleuropein aglycone (a polyphenol). All these inhibition approaches discussed in the **chapters 7, 8 and 9** have highlighted their role in preventing the aggregation of the αS protein.

In the first inhibition study for αS protein, the αS protein structural and conformational changes were studied in the presence of crowding agent, Polyethylene glycol (PEG). The trajectories resulting from the MD simulation were used to analyze

the effects induced by PEG in the conformational and structural dynamics of αS during the simulation time. The conformational snapshots from the two crowded environments compared with the control showed that the amount of α-helices in the secondary structure of αS decreased as simulation time progressed. The RMSD of the three systems showed that the stability of the αS protein is more in the 10PEG-α-Synuclein system than in the 5PEG-α-Synuclein system when compared with the control. It was also observed that the amount of anti-parallel β-sheets is highest in the 10PEG-α-Synuclein system than in the 5PEG-α-Synuclein system and the control. This study had thus helped us to understand that αS protein was sensitive towards an inert crowding environment and hence this crowding environment affected the structural and conformational properties of the protein.

In the second inhibition approach for αS protein, the interaction sites of K84s and K102s with αS using computational tools was studied. The binding free energies between the αS protein and the two peptides were found to be indeed high. The (α-Synuclein-K84s) and (α-Synuclein-K102s) complexes have binding free energies of -33.61 kcal mol^{-1} and -40.88 kcal mol^{-1} respectively. Using PDBsum server analysis, in the (α-synuclein-K84s) complex, the residues GLY 25, ALA 29, VAL 49, LEU 38, VAL 40, GLU 28, GLY 47, LYS 32, GLU 35, GLY 36, TYR 39, VAL 48 and VAL 26 (from αS) and SER 23, LEU 7, ILE 12, HIS 25, PHE 1, HIS 18, CYS 6, ARG 24, PRO 21 and ARG 8 (from K84s peptide) were identified to be present at the interface.In the (α-Synuclein-K102s) complex, the residues VAL 40, GLY 36, GLU 35, TYR 39, LYS 45, LEU 38, LYS 43, VAL 37, THR 44, VAL 49, VAL 48 and GLU 46 (from αS) and ARG 10, GLY 12, GLY 18, SER 15, THR 13, SER 19, TRP 11, ALA 14, CYS 16, ARG 7, ARG 4 and GLY 17 (from K102s peptide) were identified to be present at the interface. The PRED analysis using MM-GBSA algorithm showed that their intermolecular interaction can be credited to the residues PHE 1, LEU 7, ILE 12, LEU 2, VAL 3, GLY 5 and PRO 21 of K84s peptide and residues VAL 48, ALA 29, VAL 40, TYR 39, VAL 49, VAL 26and GLY 36 present in the αS of (α-Synuclein-K84s) complex. In case of the (α-Synuclein-K102s) complex, the inter-molecular interaction can be credited to the residues ARG 4, ARG 10, TRP 11, ALA 14, SER 15, CYS 16 and SER 19 of K102s peptide and residues GLU 46, LYS 45, VAL 49, GLU 35, VAL 48, TYR 39 and VAL 40 of αS. It has also been noticed that the αS has retained the helical content to a significant extent when it is in complex form with the K84s and K102s peptides. Taken together the

data suggest that the two novel peptides studied here could be suitable candidates for future therapeutic development against αS aggregation.

In the third inhibition study for αS protein, we have investigated the effect of Oleuropein aglycone (OleA) on the conformational dynamics and the aggregation propensity of αS using molecular dynamics simulation. From MD trajectory analysis, we noticed that when OleA is bound to αS, the intra-molecular distance between non-amyloid-β component (NAC) domain and C-terminal domain of αS was increased, while long-range hydrophobic interactions between the two region was reduced. OleA was found to interact with the N-terminal domain of αS, making this region unavailable for interaction with membranes and lipids for the formation of cellular toxic aggregates. From the binding free energy (BFE) analysis, we found binding affinity between αS and OleA to be indeed high (ΔG_{bind} = -12.56 kcal mol^{-1} from MM-PBSA and ΔG_{bind} = -27.41 kcal mol^{-1} from MM-GBSA). Our findings in this study thus substantiate the effect of OleA on the structure and stabilization of αS monomer that subsequently favors growth of stable and non-toxic aggregates.

10.2. Future Prospects:

This thesis gives molecular level information about the inhibition strategies for prevention of the aggregation of $A\beta_{1-42}$ peptide and αS using *in silico* approaches such as molecular docking, and molecular dynamics simulation. This work on inhibition strategies for prevention of aggregation of the intrinsically disordered proteins, $A\beta_{1-42}$ peptide and αS protein has further possibilities to be worked upon, which comprise of:

a. Study and design of the C-terminal of Human Albumin with mutations/alterations as inhibitory drug target for the prevention of aggregation of $A\beta_{1-42}$ peptide and αS.

b. Use of Oleuropein aglycone and Resveratrol (natural compounds) as potential drug targets with further refinement according to drug design protocols.

c. Study of the direct interaction between $A\beta_{1-42}$ peptide and αS using computational methods.

d. Design of a common inhibitor for the regulation of the aggregation of $A\beta_{1-42}$ peptide and αS using computational tools. Inhibitors can be either some small molecule ligands, or peptides, or circular RNAs (circRNAs), or microRNAs (miRNAs).