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Conclusions

The main objective of this work was to target the protein aggregation process through designed nanoparticle based inhibitors. Important outcomes from this work are summarized below.

- In the first chapter, the process of amyloid formation of phenylalanine was studied and its effect on co aggregation and cross seeding on globular proteins was examined. It was observed that the phenylalanine-fibrils can effectively initiate an aggregation process in proteins under physiological conditions, converting native protein structures to β-sheet assembly. This work also showed that phenylalanine fibrils can initiate the aggregation of amino acids. Hemolysis studies showed that both phenylalanine fibrils and phenylalanine-induced mature fibrils of proteins can cause severe hemolysis that yielded a plethora of deformed erythrocytes, a condition that is highly relevant to the pathophysiology of phenylketonuria. Both experimental and computational data suggest that unique arrangement of zwitterionic phenylalanine molecules in their amyloid-like higher order entities is predicted to promote both hydrophobic and electrostatic interaction, sufficient enough to trap proteins and to preferentially interact with the membrane components of RBCs. The findings of this work on inherent property of phenylalanine to cause hemolysis and to drive protein aggregation have a direct relevance to phenylketonuria.

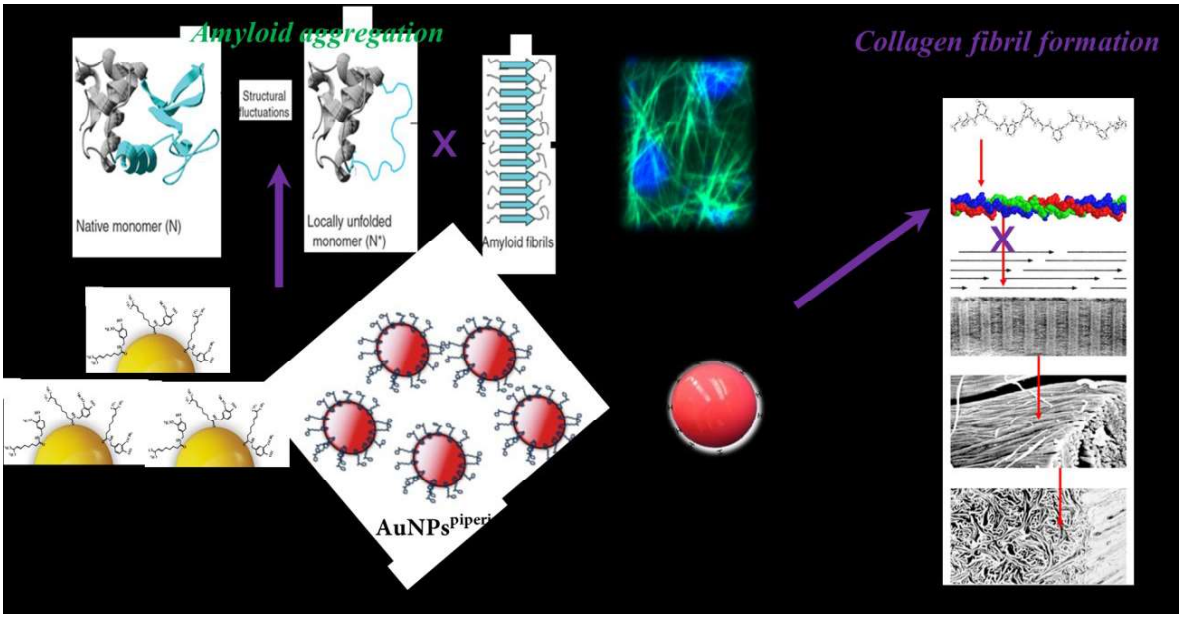

Figure7.1 Schematic representation of the thesis overview.

- In the next chapter, stable silver nanoparticles coated with capsaicin ($AgNPs^{Cap}$) have been successfully synthesized and their anti-amyloid activity against aggregation of serum albumin (BSA), has been tested. Strong inhibition of amyloid formation of BSA was observed in the presence of $AgNPs^{Cap}$ nanoparticles. However, isolated capsaicin and uncapped control nanoparticles did not show such inhibition effect. Bioinformatics analysis reveals CH-π and H-bonding interactions between capsaicin and BSA in the

formation of protein-ligand complex and it was also observed that the capsaicin molecule strongly binds to the aggregation prone residues of BSA. These results suggest the significance of surface functionalization of nanoparticles with capsaicin which probably enables capsaicin to effectively interact with the key residues of the amyloidogenic core of the protein.

- In the next chapter, an attempt was made to test whether surface functionalization of piperine molecules on gold nanoparticles would be effective against protein aggregation. Uniform polycrystalline, thermostable and hemocompatible piperine-coated gold nanoparticles were synthesized. Strong inhibition of insulin and BSA fibril assembly was observed in presence of piperine coated nanoparticles. Surface functionalization of piperine was found to be critical for the inhibition effect because isolated piperine and uncoated nanoparticles did not show any such inhibition effect. In addition to its inhibition effect on amyloid fibril formation of globular proteins, piperine coated nanoparticles were also able to inhibit collagen fibril formation. The findings of this work may help in the development of nanoparticle-based formulations to prevent medical problems linked to insulin aggregation and excess collagen fibril formation.

- Finally, in the last section of this thesis, stable gold nanoparticles surface functionalized with selected amino acids have been synthesized to target collagen fibril formation. Strong inhibition of fibril formation was observed in the presence of amino acid coated gold nanoparticles coated with aromatic residues such as phenylalanine and tryptophan. The nanoparticles coated with proline and hydroxyl proline also showed strong inhibition effect. Molecular docking studies suggested possible interactions between amino acids and collagen peptide. The results also confirmed that surface-functionalization of these amino acids was vital for the inhibition mechanism because isolated amino acids did not show any such inhibition effect. Additionally, it was also observed that all these nanoparticles are hemocompatible. Since excess collagen fibril formation has been linked to many medical problems, the current results may offer an attractive approach for both production and formulation of effective nanoparticle based inhibitors against collagen linked severities.

Overall, the results of this thesis establish the effectiveness of the designed nanoparticle based inhibitors to target aggregation of both amyloidogenic and collagen proteins (Figure 7.1).The foundational knowledge gained from this research work may inspire researchers working on cell models and animal models to test the efficacies of these nanoparticle based inhibitors.

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