General Discussion
CHAPTER VI

GENERAL DISCUSSION

Tubulin is a highly dynamic protein which forms the fundamental unit of microtubules assisting in series of functions like cell division, chromosomal segregation, motility and intracellular transportation. Of this, the critical role of this hetero-dimeric protein in cell division makes them a potential anticancer drug target. With three distinct sites of taxol, epothilone and vinca alkaloid β-tubulin is the target for various drugs which assists in stabilizing and destabilizing polymerization which ultimately controls cell division. Research is still going on in wet lab and in silico so as to identify the potential leads and to understand their dynamics.

In spite of all these, there are no details available for the 3D structure of human tubulin; residual deletions and their substitutions in β-tubulin; binding affinity between α-α in comparison with β-β; significance of residual substitution and drug resistance.

In this study we tried to address all these issues through in silico methods. To begin with the three dimensional structure of the human α- and β-tubulin was modeled and validated. As far as the homology modelling was concerned, the single template method provides a better structure for human β-tubulin which is again favored both from structural and energy perspective. For the generation of human α-tubulin multiple templates based method fared well with an average model because of the alignment of poorly resolved structure 1TUB-A chain with the well resolved 1JFF-A chain. But still, the generated model was exhibiting a better score due to the alignment of a short missing region from H1-S2. Overall residual core clustering was lesser for α tubulin in comparison with the β-tubulin. At the same time the outliers in the disallowed region was slightly higher in α-tubulin.

Next the lead search was initiated as many of the existing drugs showed increased side effects. The leads are virtually searched from marine seaweed secondary metabolites which are targeted against the taxol binding site of human β-tubulin. Molecular interactions shown by these compounds mimic the interactions of taxol against β-tubulin. In addition to this, the regions of contacts are with the H1-S2 loop, M-loops helix H7 and S-loop which are directly or indirectly involved in lateral contacts between two monomeric
tubulin subunits. Comparison of these compounds with existing drugs and those in the pipeline confirms better drug like properties from the perspective of their molecular weight, binding affinity and other related descriptors.

Next, we reported the significance of two glycine deletions from the H1-S2 loop and eight residual insert and their deletion in the S-loop. It could be a misnomer to address this as an insert because they are intact in the α-tubulin, but their deletion in the S-loop makes the loop quite smaller in comparison with the α-tubulin. Thus the S-loop of α-tubulin was quite longer than the β-tubulin. Residual bulkiness for both the protein sequences was carried out. Sequence based bulkiness analysis for α-tubulin reports less bulky H1-S2 and M-loops with a more bulky S-loop. Conversely in β-tubulin the residual bulkiness of H1-S2 loop and M-loop is higher, while the S-loop region maintains less bulkiness. But when we simulated the protein, we observed a considerable change in flexibility of the loop regions mainly involved in lateral interactions. The H1-S2 loop was highly flexible in α-tubulin which was quite rigid in β-tubulin. At the same time, the M-loop was rigid in α-tubulin which was flexible in β-tubulin. Thus α- and β- tubulin are contrasting each other regarding their flexibility and rigidness. So as to understand the reason behind the flexibility and rigidness, the interactions between the S-loop and the neighboring regions are investigated. Interestingly, the long S-loop with intact eight residues showed hydrogen bonds with M-loop without disturbing the H1-S2 in α-tubulin. This makes the H1-S2 loop very flexible, while the M-loop rigid. On the other hand, the short S-loop with their eight residual deletions from β-tubulin showed salt bridges between H1-S2 loop resulting in rigidness of the same. However, this does not disturb the M-loop thus resulting in increase in their flexibility.

But still one question that remains is the significance of such flexibility of the substructures that are involved in lateral interactions. The literature survey has already reported that α-α subunit has better monomeric interactions in comparison with β-β monomeric interactions. But as such no reasons are cited for the same. But when we subjected these monomeric subunits for protein-protein interaction wherein the regions actively involved in lateral interactions like H1-S2 and M-loop are considered for docking, we observed that the flexible H1-S2 loop exhibits a better monomeric interaction in α-tubulin. Whereas the rigid H1-S2 loop in β-tubulin showed a reduced
protein-protein interactions. Thus, here we report that H1-S2 is quite significant in assisting better protein-protein interactions. The rigidness of the H1-S2 loop due to the intra-monomeric interaction with S-loop ultimately brings in reduced binding energy between β-β subunits. With this restricted mobility of the H1-S2 loop in the β-tubulin there is a reduced binding affinity observed between β-β monomeric subunits.

As a result better understanding of the tubulin dynamics and residual substitution could assist in better drug designing. In addition to this, the drugs with their interactions with H1-S2 loop along with M-loop and S-loop could assist in better stability of lateral interactions which could ultimately arrest cell division. Such type of compounds can be potential drugs against cancer.

In addition to this, the substitutions and deletions of negatively charged Aspartic acids from the H1-S2 loop and S–loop respectively, from β-tubulin brings an overall change in the charge distribution in the pocket. In α-tubulin these regions are very much hydrophilic, but their deletion in the β-tubulin makes them quite hydrophobic.

Next, the residual substitution related to drug resistance when investigated through Amino Acid Substitution tools confirms the mutations to be deleterious when they are observed in the functionally significant regions like the region of lateral interaction and dimer interface which might ultimately bring in drug resistance. Of the eighteen substitutions reported, only ten are considered to be deleterious by three softwares. Their positions include V60A, P173A, D197N, F240I F270V, Q292E, R306C, K350N, A364T and Y422C. These regions occupy the functionally significant regions of the tubulin protein and any substitutions in these regions could ultimately results in structural instability.

Finally the selection of three mutants based on their deleterious effect and proximity to the taxol and epothilone binding site confirms that the substitution of Phe 270 with Val (mutant1) does not result in any increase in the binding affinity in comparison with the taxol-wild type. This is because of the conservative substitution of hydrophobic residues against hydrophobic residues. Further, there is an increased drug binding site developed due to the substitution. With respect to mutant2 the substitution of Ala364 with Thr brings a better interactions among few of the marine and higher plant
isolates. This is mainly due to the substitution of the non-reactive methyl group with the polar residues. Further, this also reduces the volume of the active site. Finally the substitution of Gln292 with Glu in mutant3 from epothilone binding site brings a remarkable change in drug binding in the mutant3 in comparison with the wild type due to the substitution of uncharged residue with the negatively charged one. But as such there is no change in overall topology of the active site observed before and after the residual substitution in mutant3.

Thus to conclude, better understanding of the sequence and structure of the tubulin protein and effect of residual substitutions could assist in developing better drugs against this dynamic protein. This could again prove to be a better way for treatment against cancer in the long run. Thorough this study we emphasized on the sequential and structural aspect of tubulin protein so as to identify the potential leads and docking of them in wild and mutant β-tubulin proteins. This gave a clue about the drugs which exhibits better interactions even in mutant β-tubulins. At last, the dynamics of α- and β-tubulin throws light on the role of loop flexibility in protein-protein interactions. Thus taking the clues from the dynamics status, residual positional specificities and mutations could assist in identifying better drugs for cancer treatment in the near future.

**Future Perspective**

A better understanding of the tubulin protein dynamics and residual substitutions could assist in better drug designing. This could ultimately reduce the drug resistivity for the pipeline drugs. In addition to this, the drugs with their interactions with H1-S2 loop along with M-loop and S-loop could assist in better stability of lateral interactions which could ultimately arrest cell division. Such type of compounds can be a considered here as a potential drugs against cancer. Next the identification of leads from seaweeds could be a better substitute for the existing drugs which exhibits severe side effects. Thus, we conclude that these lead compounds can be tested experimentally against human β-tubulin to obtain potential drug molecules.