Chapter 6

Conclusion

Over the last few years, there has been a burst of activity towards the development of efficient drugs, because of the increasing resistance of dangerous viruses including Influenza A. In the present study, we employed molecular docking and molecular dynamics approach, to understand the mechanism of influenza drug resistance due to H274Y, N294S, R292K and S31N mutations. We have also proposed some novel candidate molecules especially against drug resistant influenza strain type with the aid of virtual screening technique.

Firstly, we studied the resistance mechanism of H274Y mutation in influenza virus. The $<R^2>$ data showed that the fluctuation behavior of binding site residues in the MT was different from WT. Docking studies suggested that H274Y mutation significantly affect the prevalence of intermolecular interaction which in turn results the decrease in binding free energy ($\Delta$G) in the complex structure. Furthermore our results indicate that two Arginine residues, R-152 and R-156 are presumably needed to make interactions with the oseltamivir molecule, thus being important residues for effective binding and the stability of NA-oseltamivir complex. In addition the potential energy and the RMSD data obtained by means of molecular dynamic simulation certainly helpful in the understating of stability of NA-oseltamivir complexes. Finally the prevalence of the intermolecular hydrogen bonds during MD simulation also confirms that H274Y significantly alter the binding free energy of NA-oseltamivir complexes and leads to drug resistance.

Secondly, we reported the mechanism of N294S mutation in influenza virus. Normal mode based docking analysis clearly indicates that N294S mutation significantly decrease the binding affinity of mutant type NA-oseltamivir than native type NA-oseltamivir. Furthermore, the $<R^2>$ value obtained from normal mode analysis shows that
the fluctuation behavior of binding residues in the mutant structure is slightly different from the native structure; however the RMSF data obtained from molecular dynamics study revealed that this slight variation may be highly significant in causing drug resistance. In addition, the increase in number of hydrogen bond observed during MD simulation results obviously indicates that the N294S mutation significantly stabilizes the tertiary structure of the mutant type NA (PDB Code: 3CL2). Further, it is understood that amino acid E276 rotate and forming a pocket for side chain of oseltamivir by bonding with R224 and this N294S mutation inhibits the pocket formation. These observations emphasize the justification for influenza viruses to cumulate decreased drug sensitivity and preserve good fitness to maintain virulence and transmissibility.

Thirdly, we have also studied the molecular and structural properties of the oseltamivir binding to both native and mutant (R292K) type of NA using the molecular docking and molecular dynamics simulation approach. The difference in binding affinity observed in the docking study clearly signifies that oseltamivir is less effective in the treatment of patients with R292K variant. The intermolecular interaction analysis shows that the disruption of important H-bond interactions in mutant structure affects the flexibility around the mutational site, which may affect the complex structure stability and function. Molecular dynamics simulations have been performed to provide insights into the structural stability of complexed and uncomplexed structures. The RMSD value of both R292K and native type exceeded 0.2 nm, indicating the conformational changes have taken place during the simulations in both R292K and native type structure. The radial distribution function provided the potent evidence to the loss of hydrogen bond interactions between arginine residues and oseltamivir. Analysis of the prevalence of intermolecular hydrogen bonds during the MD simulation makes possible to evaluate whether the intermolecular interaction is kept during the trajectory or not. Many intermolecular hydrogen bonds observed in the crystallographic structure are not conserved in a MD simulation. The highest permanence of intermolecular hydrogen bond in the complex structure especially in native type than R292K variant during the MD indicates the importance of this interaction for ligand-binding affinity. Furthermore, Salt bridge analysis, Radius of gyration and Solvent accessible surface area in the MD
simulation indicates that mutation significantly alter the conformation of the binding residues, thus affecting the stability and also its functions. Furthermore, the PMF data obtained from the MD simulations followed by the subsequent ΔG analysis results also confirms that R292K significantly alter the binding free energy and leads to drug resistance.

Furthermore, the binding property of peramivir with native and mutant (R292K) type of NA was also studied by computational techniques. The reduced binding affinity of peramivir in the mutant (R292K) type of NA clearly indicates that R292K mutation decrease the drug efficacy of peramivir. Further the interatomic interactions analysis showed the disruption of hydrogen bond in the mutated region (i.e. K292) in the mutant structure. RMSD data obtained from molecular dynamic simulation revealed structural stability of the native type NA-peramivir complex. Furthermore RMSF and number of hydrogen bonds analysis during the MD simulation confer stable binding of peramivir with native type of NA than mutant type NA.

Finally, we have examined the rimantadine resistance mechanism of M2 proton channel using insilico approaches. The results from CUPSAT and FoldX shown that the overall stability of the mutant type is found to be increased when compared to native type. The docking studies revealed that S31N mutation significantly alter the binding pattern of the drug with the loss of intermolecular interactions. Furthermore, our results indicates that D-44 hydrogen bond interaction is presumably needed for tight packing of helices, thus being important residue for effective binding and the stability of M2-rimantadine complex.

To overcome these drug resistant problems, we have made an attempt to screen lead compounds that can bind efficiently to both native and drug resistant targets of influenza virus. Initially pubchem database was used for our analysis. Our approach demonstrated that CID 25145634 binds to influenza virus more tightly than oseltamivir. We have also predicted that CID 25145634 can bind to not only native-type but also resistant mutants of A/H5N1. The MOLINSPIRATION calculation undoubtedly
indicates that CID 25145634 was found to express zero violations to the rule of five, hence an indication of favorable bioavailability based on drug-likeness. The considerable number of hydrogen donor/acceptor atoms incurred significant hydrophilic character into the drug. The structural comparison clearly indicates that CID 25145634 is a deuterium incorporated oseltamivir molecule. Deuterium (D or 2H) is a naturally occurring, stable, non-radioactive isotope of hydrogen. It is believed that in favorable condition, the drug molecule with appropriate level of deuterium has the unique effect of retaining the biochemical potency and selectivity. The insilico toxicity profiles, drug-likeness, drug score, and molecular simulation data of CID 25145634 makes that this could be a promising leads for future development of safe and efficient antiviral agents. Finally, the results of RMSD, NHbond, and radial distribution function data obtained from the molecular dynamics simulation studies undoubtedly indicates the stable binding CID 25145634 with native- and mutant-type of NA.

We have also extended our study with aid of Traditional Chinese medicinal database (TCMD). Neoglucobrassicin is a compound extracted from TCMD was predicted to be more potent inhibitor of NA than the existing drugs such as Oseltamivir. Favorable binding interactions with native and mutant type of NA have been observed through an extensive docking study. Furthermore, RMSD analysis observed during MD simulation results certainly indicates the stable binding of neoglucobrassicin with NA structures. Moreover, neoglucobrassicin compound is based on natural product metabolites and are not expected to have undesirable side effects as the traditional drugs. This is also confirmed from our ADME and toxicity studies.

Finally, we have used DrugBank to find novel compounds against M2 proton channel. Memantine is a drug screened from DrugBank database was predicted to be more potent inhibitor than rimantadine that targets M2 proton channel. The ADME and drug score of the Memantine is predicted to have good bioavailability. Furthermore, the estimated free energy of binding (ΔG) of this drug is high when compared to rimantadine. Additionally molecular dynamics simulation revealed the stable binding of memantine with the target. Finally -pLD50 value obtained from CORAL software suggested the
memantine is less toxic and having good safety profiles when compared to rimantadine. We certainly hope that these results presented here can be more crucial in developing new drugs that can be more effective against native and mutant types of M2 proton channel.

Virtual screening has become an integral part of contemporary drug research. A variety of computational tools are being developed and refined to effectively employ fast screening methods to yield potent hits. The last few years have witnessed an explosive growth in the successful applications employing a wide ranging methods, spanning similarity analysis, scoring, fast docking, pharmacophore based search, graph theoretical approaches, machine learning tools, etc. Efforts are also being made to employ the drug likeliness of a given compound. There seem to be a lot of issues related to pharmacokinetic, pharmaco-dynamic and toxicity aspects which may have to be considered in the virtual screening approaches. The interplay between computational modeling and experimental research seem to have reached a decisive stage where the inputs from each of these disciplines are essential for their mutual growth. While computations in fact does not replace the experimental research it has been very clear that an effective interplay between the experimental and computational approaches is noticeably important to guide the prospective experimentalists in the synthesis and screening of compounds in a more rational way. Virtual screening of compound libraries offers new opportunities to prioritize a few compounds for experimental evaluation. The tremendous increase in the number crunching power of the computers and the design and development of faster computational methods and algorithms are providing a good basis for the growth of this field.

Hence in summary, new drugs are desperately needed for the treatment drug-resistant Influenza virus and innovative approaches are needed to identify new lead compounds that can enter the pipeline of lead optimization and therapeutic testing. We sincerely hope that the ingenuity and success of the computational approach discussed above bode well for the future prospects of finding new therapeutics which could results into massive reductions in therapeutics development time, which would provide us a
hefty head-start against drug resistant viral adversaries. Further, investigation of these molecules using experimental approaches would be an interesting future direction.