Implementation of structural bioinformatics in thromboinflammation studies

Appendix IV: Impact paragraph

The objective of this thesis. In this thesis, we have applied various structural bioinformatics tools in combination with wet-lab experiments to investigate the mechanism of action of (selected) proteins involved in thromboinflammation. Also, we have employed these approaches for structure-based molecular design to develop bioactive compounds to modulate inflammation. Structural bioinformatics can bring insight into protein structures and protein-protein/ligand interactions at the atomic level. With the advancement of technologies and the increase in computer power, structural bioinformatics increasingly can help researchers to solve complex problems while saving considerable time and cost in biomedical science.

How does structural bioinformatics help researchers in modern drug discovery? Drug discovery is a very time- and money-consuming process. The concise workflow of drug discovery includes target identification, hit identification, lead optimization, preclinical trials and clinical trials, drug approvals. Structural bioinformatics have made key contributions to the early drug discovery process by offering a wide variety of different tools and technologies. Some of these tools were successfully used in this thesis. For instance, an homology modeling approach was used to build a three-dimensional (3D) model structure of the target protein based on knowledge of its primary sequences and of the 3D structure of a template protein. By taking advantages of available 3D structures and bioinformatic tools, researchers can explore the ligand binding site, the flexibility of a binding pocket and also protein-ligand interactions. Additionally, when crystalized or homology modelled 3D structure of target protein are available, researchers can perform a molecular docking approach to obtain protein-ligand/drug complexes and estimate binding affinity of ligands. However, the application of molecular docking is not only limited to predict possible ligand binding conformations. Docking is also widely conducted to screen for potential (drug) candidates binding to a specific target from a database containing large numbers of drug-like compounds. QSAR (Quantitative Structure and Activity Relationships) approach is applied to characterize and investigate a correlation between the physico-chemical features of molecules and their experimental biological activities. With the purpose of lead optimization, such relationship between molecular structure and activity can be employed to predict the activity of new designed molecules. Molecular dynamics (MD) simulation approach monitors protein motions in a semi-physiological environment by adding solvent and ions or membranes for transmembrane proteins to a protein structure in silico. By analyzing molecular dynamics trajectories, researchers can investigate the structural stability of a target protein, an allosteric pocket, and key residues between protein-protein/protein-ligand complex. Moreover, MD trajectories can also be utilized for binding free energy (BFE) calculations to predict and prioritize binding affinity of candidate compounds which can help to reduce the number of compounds that will be subjected to experimental testing, in order to save cost and time.

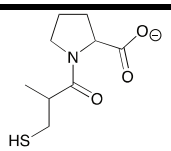
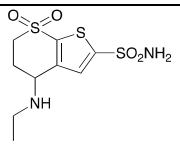
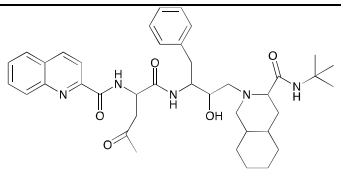
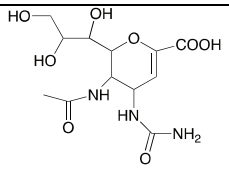
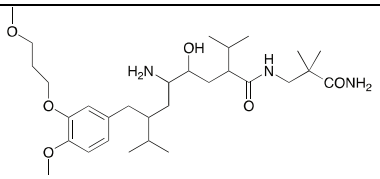
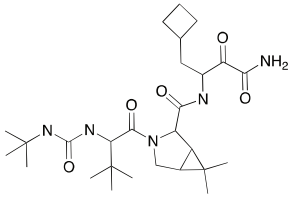
How do structural bioinformatics impact cardiovascular diseases (CVDs)? With the development of computational power, structural bioinformatic tools have been widely used in a broad range of researches, including recently emerging topics like COVID-19[1]. Here, we have chosen several potential therapeutic targets implicated in CVD, particularly to target proteins involved in thromboinflammation which are PAD4, coagulation factor V and CXCR4. The American Heart Association reported that ~48% of the total population in American adults have some type of cardiovascular disease and CVD is the number one cause of death for people in US and worldwide[2]. Although increasing biological pathways are revealed and characterized in cardiovascular diseases through disease biology, the detailed mechanisms of action of proteins involved in pathways is hardly disclosed by only relying on wet-lab techniques. Structural Bioinformatics can assist here and can guide and rationalize experimental studies. With the help of computational methods, it becomes achievable to uncover possible mechanisms and provide novel therapeutics towards specific disease.

For example, in Chapter 4, we studied whether autocitrullinated PAD4 has enzymatic activity by simulating PAD4 and autocitrullinated PAD4 structures using molecular dynamic (MD) simulations. The obtained computational results were consistent with obtained experimental results and further explained what has been displayed in experimental outcomes. Another example is the study of coagulation factor FVa target in human plasma (hFVa) and in the venom of pseudonaja textilis (ptFVa) described in Chapter 5. hFVa structure lost its activity after cleavage by activated protein C (APC); in contrast, the homologous FVa protein in the venom of pseudonaja textilis showed complete activity even after APC cleavage. Through wet-lab approaches, it was confirmed that none of the structural elements (disulfide bond across different domains, the number of cleavage sites) play a role in preserved activity in ptFVa after proteolysis by APC. However, it was challenging to further explore what structural features then determine the observed activity differences by only relying on wet-lab techniques. Therefore, we decided to employ computational methods to investigate APC cleaved hFVa and the ptFVa structure. MD simulations showed a trend towards A2 domain dissociation in hFVa but not in ptFVa, which is consistent with experimental results. Furthermore, the computational methods uncovered the presence of a key region which possibly plays a crucial role in the structural integrity of ptFVa. Details of this need to be verified by further experiments. In Chapter 6, we have applied computer- and structure-based methods to design CXCR4 agonists. By application of these approaches, we were able to select three peptides from more than trillion candidates available in chemical space for experimental testing of their agonistic activity in in vitro and in vivo experiments. Importantly, the most potent peptide exhibited therapeutic benefits to protect mice from myocardial death during acute-myocardial infarction. Obviously, as demonstrated in Chapter 6, we can accelerate the drug discovery process by use of structural bioinformatic methods to identify “lead” compounds which can be further improved, optimized and tested in different pre- and clinical phases and finally developed into drugs for CVDs.

Who gains benefits from structural bioinformatics tools practically? Increasingly pharmaceutical companies apply computational approaches into drug design and discovery pipelines. The pharmaceutical industry employs computational chemists to work with medicinal chemists to accelerate

the drug discovery process. Some examples[3] are listed in **Table 1** to show successful applications of computer-aided drug discovery. Additionally, pharmaceutical companies engage in more relationships with AI-derived biotech companies. For example, Sumitomo Dainippon Pharma announced collaboration with Exscientia and their research alliance resulted in the discovery of DSP-1181 which has entered Phase I clinical trial in 2020[4]. Another recent example is during COVID-19 period start-up company BenevolentAI screened approved drugs for treating COVID-19, several promising candidates were identified only within several days, with baricitinib (Eli Lilly's Olumiant) approved for treating COVID-19 in the USA and EU[5]. Conclusively, this trend is expected to be continued since a drug can be brought from its concept into clinical trials much more cost-effective and faster with the assistance of computational approaches. Not only pharmaceutical companies who gain profits from these approaches and methods. In the long run medical doctors, patients, and whole societies will also gain benefits (both economical and by improved public health) from an improved development of new potent drugs. These novel drugs can be used to save or improve patients' life which will help to reduce the number of patients admitted to hospital and save treatment cost.

Table 1. Examples of drugs[3] that are approved for use with the assistance of computer methods.

Drug	Structure	Target protein	Use	Company
Captopril		angiotensin converting enzyme inhibitor	hypertension and heart failure	Bristol Myers-Squibb
Dorzolamide		carbonic anhydrase inhibitor	glaucoma	Merck
Saquinavir		HIV-1 protease inhibitor	HIV-1	Roche
Zanamivir		sialic acid-analogue neuraminidase inhibitor	influenza A and B virus	Gilead Sciences
Aliskiren		active nonpeptide renin inhibitor	hypertension	Novartis
Boceprevir		hepatitis C virus NS3/4A protease inhibitor	chronic Hepatitis C genotype 1 infection	Schering-Plough

New developments. Inherently to the nature of computational methods, artificial intelligence (AI) is expected to have an impact on future structural bioinformatics and computational drug design. AI has already been widely used in virtual screening, QSAR, de novo molecular design and even clinical trial prediction. It is expected to be increasingly applied into more directions in drug discovery with more advanced algorithms and more powerful computing ability.

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