0.1 Motivation and scope of this thesis

In the past two decades, several research groups worldwide tried to determine the first crystal structure of insulin bound to the IR. After many unsuccessful attempts, the first crystal of insulin bound to the primary site of the IR was released in 2013 by a group from Melbourne [1]. This breakthrough gave answers to many key questions that were unanswered for many years and revealed the critical role of the BC-CT in the activation process of insulin, which opens away from the B chain α-helix to expose the hydrophobic core of insulin. Moreover, it revealed the crucial role of several residues of insulin in its activation process, namely the aromatic triplet of the BC-CT, F24-F25-Y26, as well as, some critical interactions between insulin and the L1 domain and the αCT segment of the IR.

However, this breakthrough also created new critical questions, with many of them being impossible to be answered using experimental techniques. For example, there were several key questions about insulin activation, i.e. what is the mechanism that triggers insulin activation, what is the frequency of insulin activation etc.. Furthermore, the role of the critical residues of insulin, namely F24, F25 and Y26, was not clearly specified. To this end, MD simulations was thought to be the best method to answer all the previous questions, and also use the advantage of this computational method in revealing even more insights into insulin activation.

After examining insulin activation by investigating the activity, dynamics, energetics and conformations of insulin, we thought that it would be interesting to perform our computational analysis on the other insulin-family proteins, namely IGF-I and IGF-II. The motivation for this study was the release of a crystal structure of the IR/IGF-1R hybrid receptor in complex with IGF-I [2], showing that IGF-I attains the same conformation as insulin to bind to its receptor, and the fact that IGF-II can bind to the A isoform of the insulin receptor (IR-A), apart from binding to its own receptor. Thus, we performed MD simulations to reveal the activation mechanisms of the IGFs and compare them with that of insulin.

The follow-up objective was to create a comprehensive computational analysis using all the computational methods used in the previous two studies to examine and predict the effect of mutations of insulin on its activity, dynamics, energetics and conformations. This computational analysis was performed on two particular groups of insulin analogues, namely three mutant insulins that are associated with hyperinsulinemia and three mutant insulins that include a mutation on the critical residue B26 and exhibit

similar binding affinity to the insulin receptor. However, this computational analysis can be performed on any set of mutations of insulin, which in combination with experimental techniques can help for designing more effective therapeutic candidates for insulin.

Lastly, the role of an extended BC-CT was also examined due to a recent experimental study, where new properties introduced on insulin by extending the BC-CT by one up to four residues [3]. Interestingly, the part of the BC-CT from the residue B27 and onwards was considered negligible in insulin activation and binding until the release of this recent experimental study. To this end, we performed MD simulations of insulin with an extended BC-CT by one and two resides to examine its activity, dynamics, energetics and conformations compared to WT-insulin. Our study may also help for developing effective therapeutic candidates for insulin, by carefully extending the BC-CT, in comparison with most of the studies where mutations are performed mainly on critical residues of insulin.

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