4.B.5. A Step towards Understanding Raine syndrome: Structure investigation of Fam20C, a Golgi casein kinase

The broad aim of this research project was to investigate the structure and function of Fam20C, a secreted Golgi casein kinase, which was found to target multiple proteins with the same motif.

Fam20C is important, since its mutations can lead to osteoclerotic bone dysplasia in humans, also known as Raine syndrome. Raine syndrome is characterized by ectopic calcification which increases bone density and often causes death in neonatal stages. Studying the protein’s structure and specifically its ATP binding pocket will allow researchers to better understand its function, better control it for experimental purposes, and use that information for a later drug discovery project.

The approach of the project is to conduct a molecular dynamics simulation of Fam20C. The simulation of the unbound form was carried for 200 ns and set with FF99SB Amber force field. Several tools were used to analyze the structure of Fam20C once the simulation was complete: root mean square deviation and fluctuation (RMSD and RMSF) to study certain regions of the protein, bond lengths throughout the simulation to measure salt bridges in the binding pocket, VolMap VMD plug-in to calculate isovalue and water occupancy, GROMOS clustering, and FTMap and FTProd to analyze clusters of binding sites inside and outside of the pocket.

By measuring the fluctuation of bond lengths throughout the simulation, it was found there are two dominant salt bridges throughout the simulation: Lys192-Glu218 that exists 71.8% of the simulation time, and Lys178-Glu213 salt bridge interactions exists 66.1% of the time. The results of the above analysis are interesting since they show that alternate conformation is possible between these pairs and could be exploited for drug design. The above observation also means that the binding pocket will have fluctuations in its electrostatics depending on which conformation the salt bridge is.

The hinge region, Phe297 ring, that acts as a gate mechanism for the binding pocket was also analyzed.

The frequent movement of the hinge’s ring allows observation of how flexible it is for drug design purposes, allows estimation of the size of the pocket, illustrates its changes and suggests what can potentially bind to it, and shows the free energy contributions to binding that would need to be compensated.

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