# Popular summary in English

All bodily functions involve proteins in one way or another, for example movement, digestion and defence against infections. By studying how proteins interact with other proteins and molecules it is possible to get a deeper understanding of the bodily functions. Through this knowledge it is possible to design pharmaceuticals for when something goes wrong. One example of this is cancer where the body's own cells starts to grow uncontrollably. The creation of a new pharmaceutical is a long and expensive process which fail in a lot of stages during the development process. The reasons for failure can be for example a lack of uptake in the body, the drug can turn out to be toxic or it might not bind the target with sufficient strength.

All proteins in humans consists of 20 different amino acids which are linked into chains of different compositions and lengths. These chains in turn take on different folds depending on amino acid composition. It is the fold and the amino acid composition which gives the protein a certain function. One very important function of a protein is the ability to bind other molecules, so called ligands, which can be other proteins, DNA, small molecules or something else. One example of where ligand binding is important is the binding of oxygen to haemoglobin in the lungs to be transported in the blood to the rest of the body. Pharmaceuticals can also bind proteins to provide their healing powers. For example the antibiotic tetracyclin which binds the bacterial ribosome, which is the cells protein factory. Tetracyclin acts by binding and there by blocking the site where new amino acids enter to be added to the growing peptide chain. This leads to that the creation of the new protein is halted.

During my PhD studies I have investigated different aspects of ligand binding to a protein called Galectin-3. This is a protein involved in a multitude of cell functions in and around the cell, such as gene regulation and programmed cell death. Galectin-3 have also been indicated in a large number of diseases such as cancer and lunch fibrosis. This makes Galectin-3 an interesting target for drug interventions.

Isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) spectroscopy have been the two main techniques I have used to study the binding process. ITC is a techniques where small aliquoted of one of the interacting partners are repeatedly added to a of the other. During these additions the heat which is needed to keep the temperature constant

is measured. From the resulting titration curve the binding strength can be calculated alongside the thermodynamic profile of the binding. The Thermodynamics can be separated into two components, enthalpy and entropy. Enthalpy is the heat which is absorbed or released as the two partners bind and the entropy is a measure of the change in disorder of the system as the components bind.

In the second technique, NMR spectroscopy, the quantum mechanical phenomenon of nuclear magnetic spin is used. This phenomenon can be likened to that some atomic nuclei having a small bar magnet. When these are placed in a stong magnetic field the nuclear spins will align with the magnetic field. By manipulating the nuclear spin trough electromagnetic pulses the spins can get out of alignment with the magnetic field. Measuring the rate at which the spins realign with the magnetic field and through a mathematical model it is possible to calculate the amount of dynamics at individual sites in the protein which, in turn can be used to calculate the entropy. Today NMR is the only technique which can get an atomic resolution of the dynamics and entropy.

During my PhD time I have been involved in measuring ligand affinities with ITC for a number of ligands some of whom binds very strongly to Galectin-3. All of these ligands bind with a very favourable enthalpy but with a penalty in entropy. With the help of NMR I have also characterised the difference is dynamics and conformational entropy for a pair of ligands. Collaborators made simulations of the two complexes which indicates that the difference in entropy have a larger contribution from conformational entropy compared to solvation entropy. In another project I have tried to, in the same way as for the protein, calculate the dynamics and entropy for the ligand when it is bound to the protein. I have also looked at the on and off rates of different ligands to the Galectin-3 and correlated these rates to the binding strength. The results show that the ligand on rate is more or less independent of the binding strength but the off rate gets smaller when the binding affinity increases, so the ligand will stay bound for longer when the affinity increases. The last project have been to investigate the binding mechanisms of ligand binding to lactose. When a protein binds a ligand it is common that the protein changes the structure to accommodate the ligand. This leads to the question of which comes first the binding of the ligand or the conformational change? We have shown that for Galectin-3 binding lactose the dominant binding pathway is to first bind lactose and then undergo conformational change.

The hope is that the knowledge gathered in this thesis eventually will lead to a deeper understanding of the underlying driving forces and mechanisms in ligand binding to proteins. This in turn will hopefully lead to a efficient way of designing and develop pharmaceuticals with a high binding affinity, specificity and the desired effect.