

## COMPUTER SIMULATIONS ON GLYCOSYLTRANSFERASES – BEAUTIES AND PITFALLS

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Glycosyltransferases catalyze the transfer of glycosyl moieties from a donor sugar to an acceptor. In most cases, the donor is a nucleoside phosphosugar and the acceptor is a hydroxyl group of another sugar, a lipid, or another component of glycoconjugates. The glycosyltransferases are classified as either retaining or inverting, depending on the stereochemical outcome of the reaction catalyzed. Even if a reasonable effort was put into the field, there are still several important unanswered questions concerning binding substrates and the reaction mechanism of these enzymes.

To address the above matter we have decided to run several molecular dynamics simulations on fully solvated glycosyltransferase LgtC that transfers alpha-D-galactose from UDP-galactose (UDP-Gal) to a terminal sugar unit from lipooligosaccharide with retaining configuration on the anomeric carbon. We have chosen molecular dynamics for our studies as it becomes a reliable and frequently used computer simulation method of today that may usefully complement experimental observations. It allows, for example, for very detailed analysis of binding energy and solvent behavior.

Since molecular dynamics works on atomic level, good initial 3D structure is required to perform simulations. In our case, recently solved crystallographic structures of LgtC in the presence of modified donor (2-deoxy-2-fluor-UDP-galactose) and acceptor (deoxylactose) were used. These two molecules are inhibitors of LgtC therefore they have to be, prior to simulations, modified to native substrates, UDP-Gal and lactose.

We will show that useful information about the enzyme structure and dynamics and substrate binding may be obtained from the simulations. This is very important as no crystal structure of free LgtC in the presence of its native substrates is available. For example, we may observe how LgtC behaves without any substrate, in the presence of only UDP-Gal, in the presence of two substrates and in the presence of UDP-Gal and deoxylactose. In the case of LgtC without any substrate, spontaneous opening of loops that cover UDP-Gal in active site was observed whereas in the presence of UDP-Gal these loops remain closed. This is in good agreement with experimental suggestion about order of substrate binding. Stable dynamics with deoxylactose (inhibitor) and lactose (acceptor) were also performed. In the later case, a possible role of water molecules on lactose binding was speculated. Finding specially ordered water molecules in the active site of the enzyme opens new look on possible mechanism.

We will also show in the lecture that one has to be careful running computer simulations because molecular dynamics is based on empirical models that can generate serious artefacts. Some of them will be shown as pitfalls of simulation methods.