

Conformational Dynamics of Complex Oligosaccharides



The operation and stability of many types of biomolecules in the extracellular matrix are influenced by the presence of attached carbohydrate residues. The specific function of these sugar components and thus their potential, e.g., for developing novel vaccines, are to a large extent unknown.

Systematic exploration of *glycans* is difficult in general. Experimental access is mostly limited to

solution NMR, and on the numerical side, the abundance of different types of glycosidic linkages inhibits the development of generalizable force fields suited for molecular dynamics (MD) simulations.

In this situation, *invariant* carbohydrate components may serve as a starting point for a more concise investigation. The backbone of the so-called Glycosylphosphatidylinositol (GPI) anchor that covalently binds many types of proteins to cell membranes, is an example of such a recurrent core structure.

Atomistic Modelling of the GPI backbone

The whole molecule linking a protein to the cell membrane consists of several parts, see **Fig. 1**. An ethanolamine residue links the protein to a backbone carbohydrate sequence which connects via an inositol ring and a phosphate group to a fatty acid immersed in a cell membrane **[1]**. The whole GPI anchor is expressed in a large number of variations, phosphate groups and/or further oligosaccharides being attached to the hydroxyl groups of the backbone. A prototypical question to ask here is: what kind of characteristic overall structure does the backbone maintain?

To model the backbone atomistically, we employ an all-atom bio-molecular force field particularly devoted to carbohydrates **[2]**. It is also sufficiently generalizable to adopt a strategy of independently considering various fragments of the backbone. This facilitates comparison with detailed NMR studies (2D-NOE spectra of di- and tri-saccharide fragments) and also allows us to run long simulation trajectories on a single fragment: conformational dynamics significantly slows down because of the surrounding aqueous solution which must be modeled explicitly, since many hydroxyl groups maintain a complicated network of hydrogen bonds with solvent water molecules.

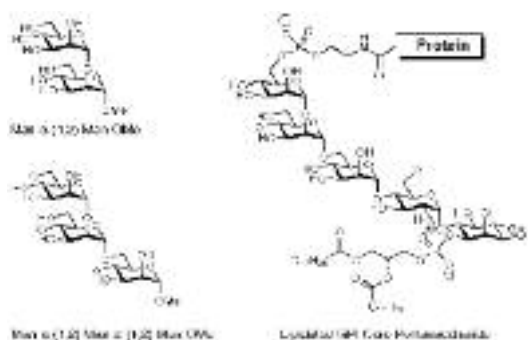


Fig. 1. GPI-core structure in its simplest configuration (right). The backbone consists of 4 sugars and one inositol ring. For detailed experimental (NMR) and theoretical (MD) analysis, the backbone can be decomposed into adequate subunits, two of which are to the left. The chemical structure formulas only indicate the molecule topology, not the actual geometric conformation.

Dynamics of Glycosidic Linkages

Because single sugar rings are relatively rigid, the conformational preferences of an oligosaccharide can be characterized in much the same way as torsion angles within a peptide backbone. In **Fig. 2**, the disaccharide 1-6-linkage, central to the backbone, is shown. The overall dihedral dynamics is revealed only with long (100ns-1 μ s) MD-simulations. The glycosidic angle trajectories reveal the different time scales involved. Whereas during periods up to several tenths of nanoseconds a conformation corresponding to one particular value of omega may appear very stable, on a time scale one order of magnitude larger the picture changes completely. On yet larger timescales, the molecule thus appears quite flexible. For the backbone, it might even turn out that it can rather be viewed as a flexible chord, particular conformational preferences losing their significance **[3]**.

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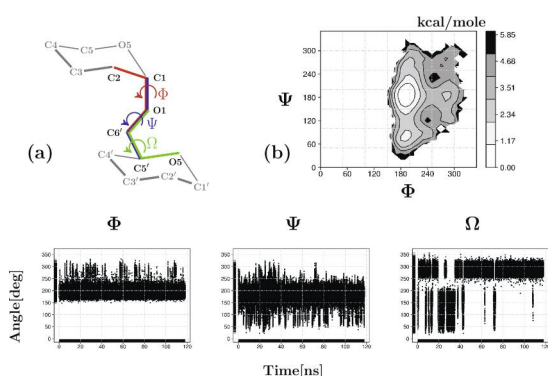


Fig. 2. (a) Definitions of dihedral angles for a 1-6 linkage between two mannoses (exocyclic groups omitted). Lower ring is the reducing end (primed). (b) Frequency distribution of Phi-Psi occurrences, converted to energy units (histogram assembled from 240.000 data points during a 120ns simulation). Lower panel: trajectories of the three glycosidic dihedral angles taken from the same simulation run.

Characterising Oligosaccharides on a Monomer Basis

Complementary to a “per linkage” point of view is to consider the oligosaccharide on a “per monomer” basis. One may ask which properties of a monosaccharide unit, apart from possible linkages to others, may favor its occurrence in a certain oligosaccharide. To tackle this question one may simply start with looking at differences across a stereo-chemical series. For a certain subset of aldohexoses the chemical formula is the same, yet they differ in orientation of their hydroxyl groups. In a preliminary studies we are considering mannose and glucose, deviating only in the orientation of their hydroxyl group at the C2 carbon atom. Within our atomistic model, we have used thermodynamic integration to grow glucose into mannose in solution as well as in vacuum, the differences of these values determining the variation in solvation free energy between the two molecules [4]. Especially in vacuum the analysis is complicated by the trapping of hydroxyl and hydroxy-methyl conformations, resulting in large and strongly varying error bars, see Fig. 3. No standard procedure is available to meet this situation. In our case one has to force the molecule to go along a smoother path between the initial and final state, a procedure that finally leads to a respectable difference of 1.8 kcal/mole between the two species.

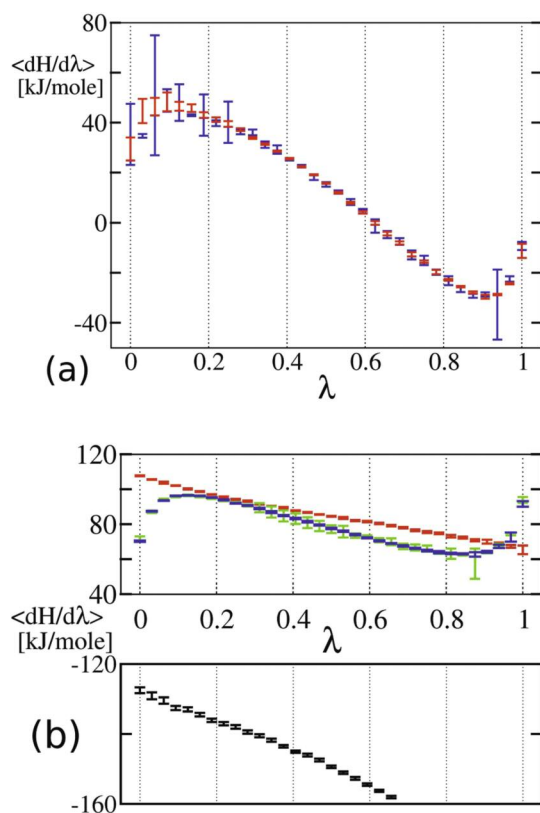


Fig. 3. Behavior of the derivative of the internal energy $\langle H(\lambda) \rangle$ with respect to the thermodynamic integration parameter λ , for two distinct paths leading from Glucose ($\lambda=0$) to Mannose ($\lambda=1$) in vacuum. (a) all parts of the molecular Hamiltonian vary simultaneously (blue: 1.6ns simulation time per data point, red: 16ns). (b) Before changing the molecular topology, charges on hydroxyl hydrogens are absorbed into their oxygen atoms (red). At least for the simulations with 16ns per data point (blue), we can achieve a smooth variation with relatively small error bars. The lower panel in (b) shows the contribution to the free energy change, when all remaining charges are restored (last part of the graph omitted for clarity).

The general type of difficulty met here is similar to the dynamical study of the backbone. Averages of any kind of observables are strongly influenced by a series of closely spaced conformers.

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- [3] Wehle, M., Lipowsky, R. and Santer, M.: Challenges in simulation studies of oligosaccharides. Is the GPI-backbone a hinge or a chord? In preparation
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