Glycans as Universal Tools for Proteins and Lipids

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Carbohydrates are known to be important for cell-cell communication or in modifying the properties of proteins and lipids in the extracellular matrix. Still many of the possible biological roles of these oligosaccharides, or glycans, are yet to be elucidated. In addition to the specific recognition of many small saccharides by certain biomolecules, larger oligosaccharides have the potential to support a much broader, unspecific functionality, owing to their internal flexibility and overall diversity. For computational approaches complementing experimental studies, the latter aspects pose serious challenges, in particular at the atomistic level with respect to force field development and conformational sampling. To explore the behavior of glycans in a larger context when they are expected to fine-tune the interaction between biomolecules, a mapping onto reduced or effective models must be devised.

In our group, we currently pursue two long term case studies in order to establish a corresponding ladder of descriptions. The first study deals with the class of so-called Glycosylphosphatidylinositol(GPI)-Anchors emphasizing the interaction with lipids (with *D. Varon Silva* and *P.H. Seeberger*, Department of Biomolecular Systems, MPIKG; *C. Stefaniu* and *G. Brezesinski*, Department of Interfaces, MPIKG), and the second with specific carbohydrate-protein interactions (with *S. Barbirz*, University of Potsdam and *G. Widmalm*, University Stockholm) important for infections of gram-negative bacteria by bacteriophages.

GPI Anchors as Glycolipids

GPIs are glycans that covalently link proteins to the outer leaflet of cell membranes **[1]**. The carbohydrate part is in close proximity to both, a protein and a lipid component at the same time, see **Fig. 1**.

For the atomistic representation of the complete GPI, only the connection to the protein is available from force field databases. For the part comprising glucosamine, phosphoinsitol and the lipid (glucosamine -α1-6*myo*lno-1- phosphodistearylglycerol, highlighted by the red frame in **Fig. 1**), an adaption of the force field has been developed. For complex molecules such as this, there are few opportunities to validate the force field prediction against structural data from experiment. In a joint effort, we have investigated this molecule within Langmuir monolayers of crystalline order **[2]**.

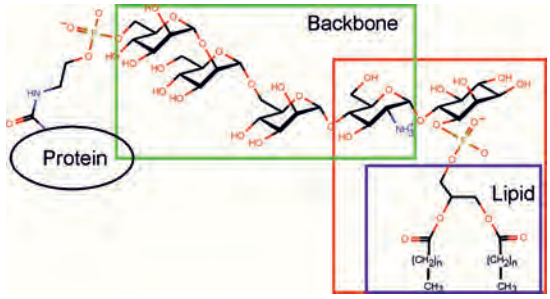

*Fig. 1.Chemical structure of a lipidated GPI molecule with protein and carbohydrate backbone. The part indicated by the red frame has been studied as a separate molecule in **[2]**.*

The Nature of the GPI Anchor Backbone

Apart from the established role as an anchoring device for proteins, there is only indirect evidence for many other possible functions of the GPI, such as being a mediator for the association of the attached protein with lipid rafts. One complication here is the heterogeneity of the molecule, its composition sensitively depends on the protein attached. For developing computational models, the invariant GPI backbone is a natural and convenient starting point. But even the seemingly basic and simple question whether this backbone is a rather flexible link or maintains a characteristic structure can only be answered comprehensively after a mapping of the atomistic to an adequate reduced model has been accomplished (**Fig. 2**). The different notions of the backbone -flexibility vs. rigidity-, can actually be reconciled by stating that the backbone assumes a rather rigid structure that can little be stretched, but is to some extent compressible by forces of physiological magnitude (starting at roughly 10pN) **[3]**.

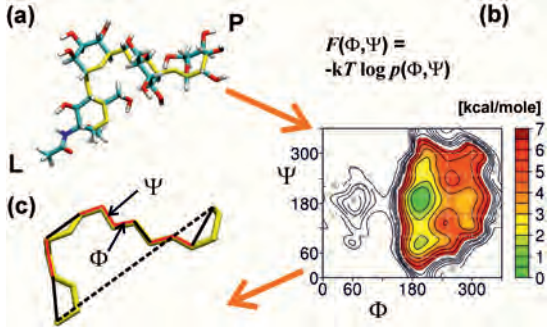

Fig. 2. (a) GPI backbone with four carbohydrate rings in stick representation. "L" and "P" indicate the direction towards the protein and the lipid, respectively. Highlighted atoms (yellow) are those retained in a reduced description. The data from all-atom MD simulations are projected onto the relevant degrees of freedom, the glycosidic torsions (c). They largely determine conformational characteristics such as the end-to-end distance (dashed line). Sugar rings are effectively represented by non-rotatable bonds (black). The free energy landscape of a corresponding pair of dihedral angles, obtained from their distribution function p, is shown in (b), reflecting the effective influence of all remaining degrees of freedom.

Do Bacteriophages Utilize the Protective Polysaccharide Coat of Gram-Negative Bacteria?

Similar questions as for the GPI emerge for certain lipopolysaccharides (LPS). Gram-negative bacteria protect themselves against invaders through a dense polysaccharide coat, which is also a target for the immune response of higher organisms invaded by these bacteria. The coat is formed by an LPS brush, the carbohydrate part of which (the O-Antigen) consists of repeating units (RU) of a tetrasaccharide building block (**Fig. 3**)

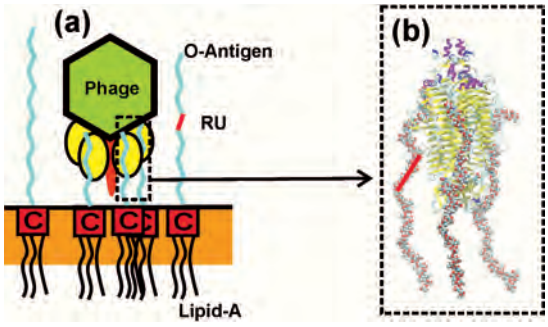

Fig. 3. (a) Schematic representation of a phage penetrating the lipopolysaccharide coat on the membrane of gram-negative bacteria. (b) snapshot of an MD simulation where three O-Antigens simultaneously attach to one tail spike protein.

A bacteriophage must, prior to infection, overcome the polysaccharide barrier before its DNA can be injected into the cell. It does so by recognizing a 2 RU epitode (an octasaccharide) with its so-called tail spike proteins (TSP), and cleaving the O-Antigen by hydrolysis at an active site on the corresponding protein scaffold. Little is known about important further aspects of the cleavage process e.g., whether it occurs continuously, is used as a means to orient the phage or to generate a force in order to push it against the membrane and initiate the DNA injection process.

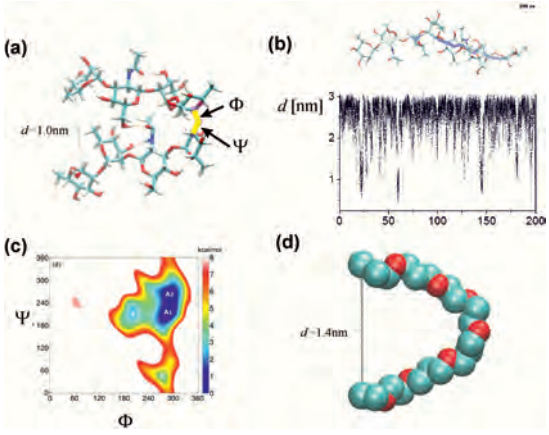

Fig. 4. (a) Hairpin-like extreme conformation of two repeat units of a serotype Y Shigella flexneri O-Antigen, the end-to-end distance of the octasaccharide can become as small as 1nm, but this event is relatively rare as shown in (b). Most of the time the molecule resided in an extended conformation as depicted above. The formation of the hairpin can be attributed to the metastable state B in the free energy landscape of the glycosidic angles, see contour plot in (c). (d) The same conformation is also observed in a reduced model similar to that in Fig. 2 (only backbone atoms are displayed).

A first clue to these questions is given by a comprehensive simulation study of short fragments (a few RU long) of the O-Antigens, see **Fig. 4**. They show the formation of hairpin-like conformations that can lead to significant temporal coiling of the otherwise stiff polysaccharide. This suggests a rich variety of carbohydrate-protein interactions, such as conformational selection of the O-Antigen by the TSP. Here we have an analogy to the interaction of a GPI anchor with a membrane. The time scales needed to characterize the problem appropriately, e.g., as transitions between many possible intermediate states, exceed the scope of atomistic simulations, and the mapping to reduced models is called for.

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References:

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