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Understanding glycobiology through multiscale molecular dynamics simulations: From basic principles to case studies

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Abstract

Recent advancements in high performance computing (HPC) have allowed molecular simulation methods not only to play a more substantial role in supporting experiments, but to transcend such mandate to guide experimental design and to lead autonomously scientific discovery. In this review we will present the basic foundations multiscale molecular simulations methods, from atomistic to coarse-grained, used in complex carbohydrates research, with some key example of successful applications that significantly contributed to our knowledge and mechanistic understanding of different processes of fundamental importance in glycobiology.

1. Introduction

Complex carbohydrates (or glycans) are among the most chemically complex post-translational modifications in nature. As polymers of simple sugars, or monosaccharides, unlike proteins and nucleic acids, carbohydrates can be branched, resulting in a combinatorial explosion of possible sequences and structures. Moreover, because of differences in anomeric configurations (α or β), stereochemistry (D or L), and the variety of possible linkages (regiochemistry), the resulting number of different polysaccharides with the same sequence is staggering. Interestingly, not all possible sequences occur naturally, strongly indicating that specific glycoforms are linked to precise biological functions¹. Because glycans are not template-driven and glycosylation patterns are only indirectly related to the genome, determining these links, also dubbed as “deciphering the glycode”², is a monumental task.

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Furthermore, understanding the structure-to-function paradigm in glycobiology is also enormously complicated by the glycans' intrinsic flexibility, which makes their characterization by experimental structural biology methods rather difficult. Molecular simulation techniques have reached nowadays the sampling power and a sufficient level of sophistication to provide not only the missing structural insight necessary to interpret or support experiments, but also to be the primary tool of scientific discovery. Indeed, computer simulations are becoming an indispensable tool to understand at the molecular level the driving forces underlying the structural and dynamical features of carbohydrates and glycoconjugates within complex environments³⁻⁵. As such, molecular simulations can be viewed as “computational microscopy” that can complement or lead experimental design. In this chapter we will briefly overview the fundamental concepts and background behind atomistic and coarse-grained molecular dynamics (MD) simulations of free glycans and glycoconjugates and the impact and contribution of these studies in the discovery of the different fundamental roles that glycans play in the biology of health and disease.

2.0 Computational Background

2.1 All-Atom MD Simulations. Within the approximation that molecular structure and dynamics can be described sufficiently accurately by the laws of classical mechanics, atoms can be represented as hard, impenetrable spheres, characterized by a mass, size (van der Waals radius) and electrostatic point charge, different for each specific atom type. Within the molecular mechanics (MM) formalism, the potential energy of a molecule can be defined by an empirical force field, which general functional form is given in **Eq. 1**.

$$\begin{aligned}
 V(r) = & \sum_{bonds} k_b (r - r_0)^2 + \sum_{angles} k_a (\theta - \theta_0)^2 \\
 & + \sum_{torsions} \sum_n \frac{1}{2} V_n [1 + \cos(n\omega - \gamma)] \\
 & + \sum_{j=1}^{N-1} \sum_{i=j+1}^N f_{ij} \left\{ 4\epsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^6 \right] + \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}} \right\}
 \end{aligned} \tag{Eq. 1}$$

Within the MM force field approximation, the molecule's potential energy $V(r)$ is a function of the positions of the N atoms that make-up the system. The first three terms in **Eq. 1**

correspond to the potential energy contributions from bonded interactions, such as covalent bonds, bond angles and torsions. These are all represented by Hooke-type potentials, where bond vibrations, angle bending and dihedral angle potentials are regulated by classical spring constants ($k_{b/a}$) that modulate the stretch from an equilibrium distance (r_0) and an equilibrium angle (θ_0). Torsion potentials are generally represented, as in **Eq. 1**, through a Fourier series. The last term in **Eq. 1** is the contribution to the total potential energy from non-bonded (or non-covalent) interactions, namely dispersion and electrostatic interactions, represented by a Lennard-Jones (LJ) and by Coulomb potentials, respectively. In the LJ term, ϵ is the energy well's depth at the equilibrium distance σ , while in the Coulomb term q values indicate the partial charges on the atoms i and j and ϵ_0 is the permittivity of vacuum. The treatment of dispersion interactions with alternative formalisms to the LJ potential, such as the Buckingham potential⁶, are also possible in some software packages and can be used, provided that *ad hoc* parameters are available. Nevertheless, for most applications in biomolecular structure and dynamics, the LJ potential is sufficiently accurate and also generally preferred to the Buckingham more rigorous treatment of dispersion for the sake of computational speed. Long range electrostatic forces are treated within periodic boundary conditions⁷ within the framework of the Particle-Mesh Ewald (PME) approach. PME electrostatics eliminates artefacts due to the abrupt truncation of the Coulomb potential through the introduction of cut-off values and/or effects due to a finite size of the simulation box⁸. According to **Eq. 1**, the force field parameters values determine the molecule's potential energy corresponding to each specific configuration, therefore the choice of a carefully developed and thoroughly tested force field is at the very core of the validity of an MD simulation. In the case of complex carbohydrates the choice of all-atom empirical force fields nowadays rests between two types of parameter sets, namely Amber/GLYCAM⁹ and CHARMM^{10, 11}, which are both fairly complete, in terms of the ability to cover the vast majority of common monosaccharides and to treat different glycosidic linkages and branching complexity³. To note also the recent advancements in the development of the GROMOS 53A6(GLYC) parameter set for the simulations of carbohydrates within a united-atoms framework¹²⁻¹⁴ and also the availability of Drude polarization models in CHARMM^{11, 15, 16}.

Once the potential energy of the system is set by the choice of force field, the conformational space accessible to the glycan can be studied over a time coordinate through MD simulations.

Within the context of MD simulations, the dynamic evolution of the system is obtained through a deterministic approach, namely by solving Newton's equations of motion, see **Eq. 2**, through numerical integration.

$$m\vec{a} = -\frac{dV(\vec{r})}{d\vec{r}} \quad \text{Eq. 2}$$

The integration time step is an infinitesimal δt to guarantee the conservation of energy. In practical terms, the δt value should be chosen as one order of magnitude lower than the fastest bond vibration, which is approximately of 10 fs for a C-H bond stretching mode⁸. Constraining the distance of all bonds to hydrogen atoms, including water molecules, with schemes such as SHAKE¹⁷ or LINCS¹⁸ allows to increase the time step to 2 fs, thus to increase the sampling capability. There are a number of different numerical integrators that can be used for all-atom deterministic simulations and such choice determines the reproducibility of the data collected, more specifically the reversibility of the integration discrete steps, and ultimately the conservation of the total energy. For an overview of the different types of integrators and temperature coupling schemes available, we refer the reader to a recent comprehensive and very insightful review on molecular simulation methods⁸ and to references therein. The same work includes an exhaustive overview of commonly used pressure coupling schemes, if the simulations are done in an isothermal-isobaric (NPT) ensemble.

Once the mechanistic framework for the propagation of the system's dynamics is set, the question we often ask, or should ask, ourselves about our simulation is "how long is long enough?". Deciding on the optimal length of MD sampling is a complex, highly system size-dependent problem that is generally limited by the computational resources available to the researcher. Because most glycans are extremely flexible and dynamic, when unbound they can potentially occupy a very high number of conformational states with non-negligible populations. Therefore, to understand their molecular recognition by different receptors is important to determine the correct statistical distribution of the different 3D structures they can adopt, which requires exhaustive sampling of the conformational space. Because transition between different free (unbound/unlinked) glycan conformers is generally not energetically hindered at room temperature (300 K), conventional MD schemes are quite capable of delivering the required sampling. Nevertheless, it is highly advisable to run

different MD simulations of the same glycan started from all potentially relevant conformations, especially if highly flexible (1/2-6) linkages are present. Meanwhile, when studying the dynamics of glycans linked to glycoproteins or bound to lectins/enzymes, it is of paramount importance to realize that the starting conformation may be biasing sampling due to direct protein-carbohydrate interactions that may prevent transition to other relevant conformations. This should be especially concerning in (very common) cases when the starting conformation of the bound/linked glycan is built from scratch, due to lack of experimental information. In these cases the use of accelerated MD schemes, such as temperature replica exchange MD¹⁹ (REMD), Hamiltonian REMD²⁰, swarm MD²¹, or metadynamics²², is highly advisable as it ensures a more exhaustive coverage of the conformational energy hypersurface.

2.2 Coarse-Grained Simulations. MD simulations scale linearly with the number of atoms in the system and despite the enormous advancements in high-performance computing (HPC) technologies²³⁻²⁶, the study of very large molecular systems with a number of atoms exceeding the million, is still prohibitive for routine simulations. The huge computational costs are imposed by the large number of degrees of freedom that, as discussed previously, require complex sampling schemes. In these cases, a coarse-grained (CG) description of molecules, offering a progressive way of augmenting the spatial and temporal scale of simulations, may be suitable and highly informative. Within a CG representation, the reduction of the degrees of freedom is achieved by merging (or graining) multiple atoms together into “pseudo-atoms” or “beads”, which, as represented graphically in **Figure 1**, contributes to smooth the potential energy landscape and thus accelerates sampling²⁷⁻²⁹. The merging schemes vary depending on the specific approach used and on the level of precision required³⁰⁻³³. The least invasive approach is to group the non-polar hydrogen atoms with the heavy atoms they are bound to, a framework called “united atoms”, which yields up to a ten-fold reduction of the computational effort³⁰. Merging schemes of several heavy (non-hydrogen) atoms can accelerate simulations by three to four orders of magnitude in comparison with classical all-atom MD simulations^{27, 30, 34}.

In CG simulations the potential energy is determined by a force field defined through a set of equations analogous to those used in all-atom force fields, shown in **Eq.1**. More specifically, a CG force field also contains terms for covalent interactions, namely bonds, angles and torsions between pseudo-atoms, and also non-covalent terms that account for dispersion

interactions through a LJ term, and electrostatic interactions through a Coulomb term. To represent covalent interactions between beads, the potential energy in function of the bond length, angle and torsion, is fitted by an harmonic function³⁵. Owing to the merging of atoms into beads, the description of the energy of CG models requires additional terms beyond the classic empirical force field representation. Internal correlations between groups of atoms are introduced explicitly in the form of multi-body terms, which are computationally expensive³⁶.

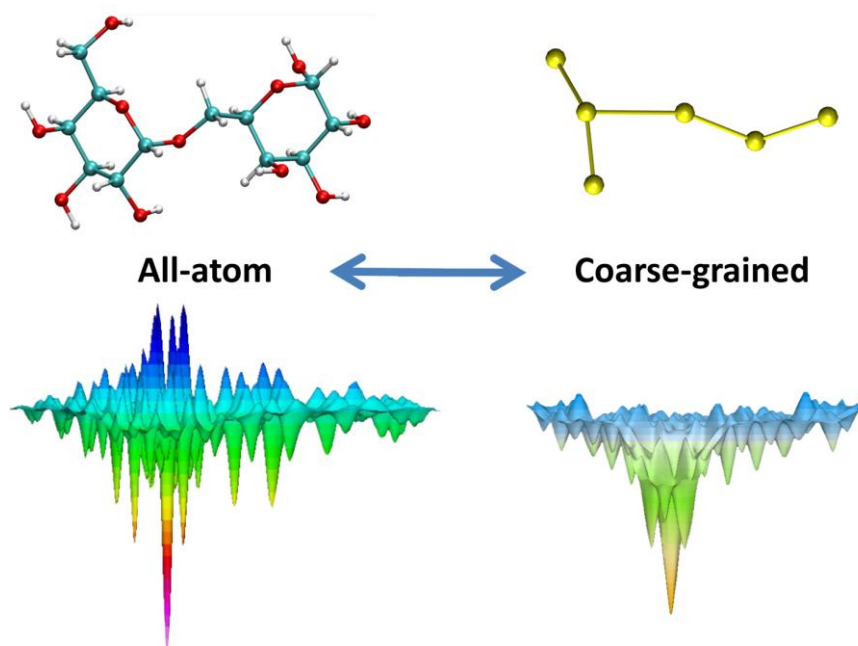


Figure 1. Schematic view of the different all-atom and coarse-grained (CG) representations of a carbohydrate fragment and the corresponding potential energy surface. Note: lowest energies are shown in red and highest energies in blue.

Most approaches keep the distinction between local energy terms and so-called contact potentials. The local energy terms describe spatial correlations between pseudo-bond vectors, which no longer follow classical harmonic behaviour, and are often expressed by arbitrarily chosen functions. A single formula describes the non-bonded terms with proper formulation depending on the type of interacting atoms their distance and sometimes their mutual orientation and local neighbourhood. Due to the diffused nature of a spherical cloud representing a group of physical atoms, these interactions tend to be softer and the 12-6 exponents in the LJ potential are parametrized accordingly. Finally, in CG simulations the solvent, most commonly water, can be represented implicitly³⁷, through continuum electrostatics, further reducing the complexity of the system, or explicitly³⁸.

The MARTINI Force Field^{32, 39} is widely used for simulation of glycolipid membranes in conjunction with both monotopic and transmembrane proteins and also an extension for carbohydrates⁴⁰. The philosophy behind MARTINI is to use universal modular building blocks, which makes relatively straightforward and effective to change the representation from all-atom to coarse-grain (CG) in a wide range of biological systems. The MARTINI force field uses a one-to-four mapping approach, where a single bead represents a group of four heavy atoms. Within this framework, one bead represents four water molecules. Small ring-like fragments, e.g. aromatic amino acid side chains, sugar residues, cholesterol, etc., are mapped with a slightly higher resolution of up to two heavy atoms per bead. Overall, four main types of coarse-grained particles are defined according to their chemical properties, namely, polar (P), nonpolar (N), apolar (C), and charged (Q). They are divided into subtypes based on hydrogen-bonding capabilities, i.e. donor, acceptor, both or none, and polarity, ranging from 1 to 5. Their combination gives a total of 18 unique “building blocks”. Interactions between CG particles are modelled according to a classical mechanics approach with an interaction potential equivalent to the one used in atomistic simulations. Bonded interaction parameters are derived based on atomistic structural data or from iterative fitting to atomistic MD simulations of corresponding atom groups. Meanwhile, non-bonded interactions are described by a Lennard-Jones (LJ) 12-6 potential for dispersion and by a Coulomb potential for electrostatics, with parameters adjusted to reproduce experimental thermodynamics data of the free energy of hydration, free energy of vaporization and partition free energies between water and several organic phases for each of the 18 types of coarse-grained particles^{32, 39}. Within this framework, the development of a MARTINI force field is based on the iterative fitting of the CG parameters that define bonded interaction potentials to atomistic trajectory data, while non-bonded terms are derived to reproduce experimental data. Thus, MARTINI combines both, structure-based (or top-down) and thermodynamic-based (or bottom-up) parameterization strategies.

In regards to the solvent, within the MARTINI representation, water can be treated explicitly at the same level of coarse-graining as all other molecules, namely with four water molecules *per* bead. These water beads, just as many other CG water models, do not bear charges and consequently are blind to electrostatic fields and polarization effects. To compensate for the neglect of explicit polarization, screening of electrostatic interactions is done implicitly, assuming a uniform relative dielectric constant. While this is a reasonable approximation for bulk water, problems arise at the interfaces between water and other phases and in the

vicinity of charged particles. Because of the implicit screening, the interaction strength of polar substances is underestimated in non-polarizable solvents. A MARTINI-type polarizable water model was introduced in by Yesilevskyy et al.⁴¹, where a three-bead model representing four water molecules, accounts for reorientation of water molecules due to the polarization effects. Ions are represented by charged particles, referred to as Q type. In the case of single atom ions, such as sodium and chloride ions, the first hydration shell is considered to be included in the CG representation, to which the full charge is assigned. The coordination numbers for ion pairs and ion solvent remain in reasonable agreement with atomistic data. Keeping in mind the difficulty of modeling of ions already with all-atoms force fields, the CG ion force field is only qualitatively accurate.

Among the general limitations of CG models, we should highlight the loss of directionality of hydrogen bonding interactions, which prevents CG and MARTINI to be used in protein folding studies. Also, depending on the level of graining, the loss of atomistic resolution and dihedral structure within the CG bead, which prevents obtaining information on stereoisomers and of puckering of the carbohydrate ring. However, providing a cautious CG parametrization, CG models can capture time-averaged properties and conformational space of atomistic systems of interest⁴². Nevertheless, careful parameterization protocols are of the essence as, for example, non-physical aggregation of glycans represented by MARTINI can be caused by an imbalance of the non-bonded solute-solute, solute-water, and water-water interactions⁴³. The latter can be fixed by reducing the depth of the potential well of solute-solute LJ interactions. The efficient scaling relies on the experimental values of the second virial coefficient of osmotic pressure, which describes the deviation from ideal behavior of a solution with solute molar concentration⁴³.

It is noteworthy to underline that coarse-graining affects the thermodynamic properties of a modeled system and in particular the balance between enthalpic and entropic contributions. The reduction of the degrees of freedom inherent in a CG model affects the entropy of the simulated system, which is compensated by a reduction of the enthalpic terms. In turn, a CG model may accurately reproduce free energy differences, but contributing enthalpy and entropy values may be inaccurate. The main advantage of a CG model rests in the flexibility of the representation; in other words, in the number of beads chosen *per* carbohydrate unit. Going to a coarser representation, one sacrifices the more detailed features of the modeled process and regulate the thermodynamic contributions by appropriate scaling of the non-

bonded interaction term. However, the transferability of the CG model to a wide range of concentrations and of molecule types, i.e. glycolipids and glycoproteins, to describe diverse and biologically relevant systems is important. Up till now, with exception of the generic MARTINI model, none of the CG force fields schemes can describe the precise arrangements of real biosystems^{44, 45}. MARTINI provides an intrinsically consistent CG approach, which can be extended to including different lipid types, sterols, sugars, peptides and polymers. This flexibility especially important when dealing with complex sugar-based systems as functionalized glycomaterials and glycoconjugates or to explore the protein-carbohydrate interactions.

3.0 Applications of multiscale MD simulations in glycobiology

3.1. Structure and function of complex carbohydrates through atomistic simulations.

Glycosylation is the most common and complex post-translational modification of biomolecules¹. In the specific case of protein glycosylation, the glycans that decorate proteins surfaces can be extremely different, in terms of size, branching complexity and monosaccharides sequence. Overwhelming evidence gathered through glycobiology research over the past 30 years shows that specific types of glycosylation are essential for correct and efficient protein function and the alteration of such motifs modulates protein structure and activity⁴⁶⁻⁵⁰. Unfortunately, the molecular basis underlying the different roles that specific glycoforms play is often unknown, while having such information would be a remarkable step forward in understanding the glycobiology of health and disease states. To this end atomistic MD simulations provide unique insight, giving an accurate description of the 3D structure and real dynamics of glycan motifs at the actual timescale when molecular events take place. Below we will briefly discuss a few examples of selected all-atom MD simulation studies that contributed to our understanding of difficult problems in glycobiology, such as carbohydrate recognition, of how specific N-glycosylation patterns modulate protein function, structure and dynamics and the structure and function of the human immunodeficiency virus 1 (HIV-1) glycan shield.

Case 1) MD Simulations Insight into Carbohydrate Recognition. Computational simulations are probably the most powerful method we have nowadays to obtain atomistic-level information on the molecular recognition of highly dynamic systems, such as complex carbohydrates. According to our current understanding, the recognition pathway can follow

two diametrically opposed processes described as “conformational selection” and “induced fit”⁵¹. According to the conformational selection theory, the receptor will bind selectively only the conformers that correspond to the final, bound conformation, which in practice defines an effective concentration of the substrate. The induced fit theory explains that recognition occurs regardless of the substrate’s specific 3D conformation and that the substrate will fold in-place to match the spatial constraints of the receptor binding site. Borrowing from the intrinsically disordered proteins field, an intermediate case scenario describes that the substrate can form local 3D motifs, known as molecular recognition features (MoRFs)⁵², that are recognized by the receptor. MoRFs act as nucleation sites initiating a folding in-place process⁵². Because of their highly dynamic architecture, carbohydrates are intrinsically disordered biomolecules. Consequently, it is rather difficult if not impossible to determine experimentally how they are recognized by lectins or by glycan-processing enzymes. A very challenging case was presented by the solution of the crystal structure of a β -propeller lectin from *Ralstonia sanacearum* (RSL) in complex with a rare “open” conformation of the Lewis X (LeX) trisaccharide⁵³. Such conformation has not been previously observed for the unbound LeX⁵⁴⁻⁵⁶, see **Figure 2 panel a**. The crystallographic analysis of the open LeX complex⁵³ was accompanied by conventional (non-accelerated) MD simulations of two LeX-RSL complexes, of 1 and 0.85 μ s production, and by the umbrella sampling (US) analysis⁵⁹ of the binding and unbinding pathways. Additionally, the conformational equilibrium of the LeX unbound (free) in solution was studied through a set of 30, 1 μ s trajectories run in parallel, started from uncorrelated conformations⁵³. The MD results confirmed the stability of the LeX open conformation in the complex and highlighted the specific interactions with the binding site residues that stabilise it. Additionally, the MD simulations of the free LeX were extensive enough to capture opening events, thus to define a potential opening pathway that involves a concerted conformational change of the two glycosidic linkages and of the GlcNAc ring pucker, and also confirmed that the LeX structure opening does indeed happen in solution, but is too much of a rare occurrence to be captured by NMR. In summary, the extensive MD work⁵³ suggested that RSL binds LeX through an induced-fit mechanism, where the nature of the interactions and architecture of the binding site compensate for the enthalpic cost of opening, namely 10.6 kJ/mol from sampling. Within this context, a notable contribution from Alibay et al⁶⁰ shows how their enhanced MD scheme, namely a multidimensional variant of the swarm-enhanced sampling MD (msesMD) method, compares to conventional (unbiased) MD sampling, i.e. 3,

10 μ s trajectories, to US and to accelerated MD (aMD)⁶¹ for characterizing exhaustively the LeX/A and sialyl-LeX/A (sLeX/A) conformational spaces. This study shows that enhanced sampling schemes such as the msesMD provide the same structural, dynamics and energetic insight obtained from the microsecond-long multiple unbiased trajectories, at a fraction of the time. Important to note here is that, while exhaustive sampling can be reached in the case of tri- and tetrasaccharides, even if it requires high-energy transitions, it becomes quickly unachievable as the number of atoms and the complexity of the system increases.

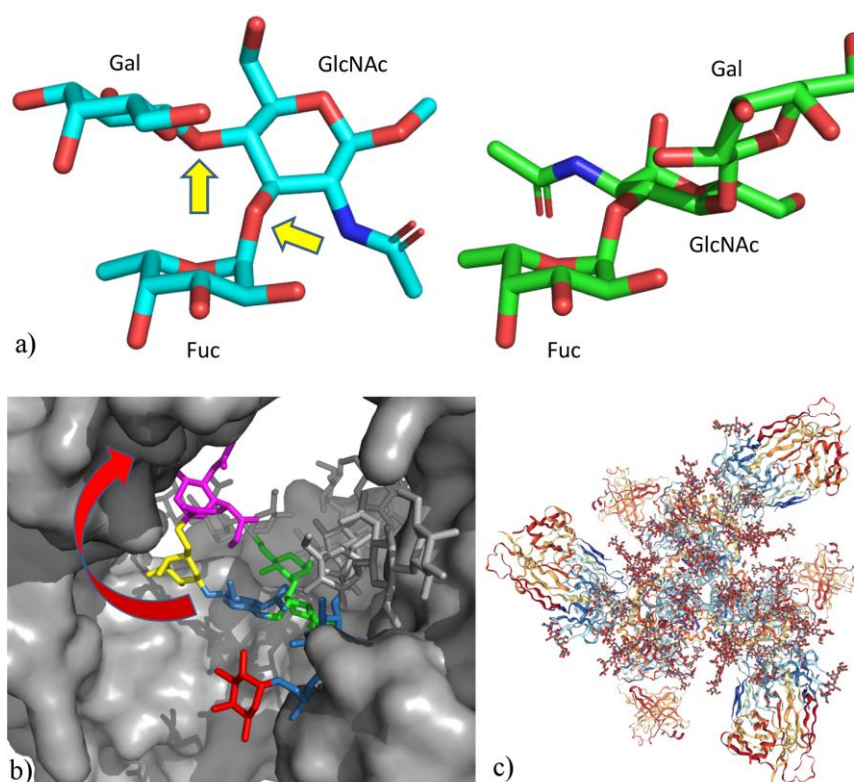


Figure 2. Panel a) The most stable (closed) 3D structure of the trisaccharide Lewis X (LeX) is shown on the left-hand side with C atoms in cyan, the less stable (open) 3D structure identified in the complex between LeX and the β propeller RSL (PDBid 5AJC) is shown on the right-hand side with C atoms in green. The glycosidic linkages which torsion allows for the transition between the two conformers are highlighted with arrows. **Panel b)** Snapshot from the REMD study⁵⁷ showing the bending of the $\alpha(1-3)$ of one of the two Fc N-glycans in function of the core fucosylation of the symmetrically opposed N-glycan. **Panel c)** Crystal structure of the trimeric HIV01-Env structure and glycan shield from clades A, B and G⁵⁸ (PDBids 1FYL/K/J).

Case 2) How Core Fucosylation Affects Structure and ADCC Function of IgG1s. A

glycobiology research area where atomistic simulations have great potential for discovery is in understanding how glycans' sequence affects the glycoprotein function. This is a particularly thorny problem for classic experimental structural biology approaches as glycans are highly dynamic when unbound. This conformational disorder is retained by protein-

linked glycans that do not form extensive interactions with the protein surface. As a result, experimental data obtained from highly dynamic glycans are often sparse and underdetermined and as a consequence the corresponding glycan 3D structures can be rather speculative, with highly questionable ring and glycosidic linkages conformations. Additionally, the very nature and degree of the glycans conformational dynamics depends on their sequence⁶² and that is an aspect that atomistic MD simulations can address quite exhaustively. One emblematic case where glycan sequence affects protein function is in immunoglobulins G1 (IgG1), where core fucosylation of the fragment crystallizable (Fc) N-glycans results in a dramatic quenching of the antibody-dependent cellular cytotoxicity (ADCC) with a consequent reduction of the antibody therapeutic efficacy⁶³⁻⁶⁶. In recent work⁶² from one of our labs, we determined the role of sequence in unbound complex N-glycans 3D structure through extensive sampling with a cumulative simulation time exceeding 64 μ s. More specifically, this work shows that while core fucose does not affect the glycans' intrinsic dynamics, galactosylation of the $\alpha(1-6)$ arm does promote folding of the arm over the chitobiose core. This explains the known higher difficulty of sialylating the $\alpha(1-6)$ relative to the $\alpha(1-3)$ arm in free glycans and the differential recognition of positional isomers in glycan arrays⁶⁷. Based on these results, it was clear that the effect of core fucose of ADCC quenching was dependent on how the architecture of the Fc glycosylated region affects the glycans dynamics and their availability for interactions with the Fc γ RIII receptors. Earlier crystallographic work⁶⁸ shows that core fucose weakens glycan-glycan interactions in the complex between the IgG Fc and Fc γ RIII. Recent work based on accelerated sampling through temperature replica exchange MD (REMD) simulations of Fc-linked complex N-glycans show that, not only core fucosylation alters dramatically the dynamics and exposure to the solvent of the sialylated $\alpha(1-3)$ arms, see **Figure 2 panel b**, but also that in doing so it enhances the overall Fc region dynamics⁵⁷. Furthermore, the simulations show that core fucose resides in position within the Fc that in the complex with the Fc γ RIII is occupied by the Fc γ RIII N162 glycan^{68, 69}. This suggests an increased binding free energy required to clear the steric hindrance and displace the fucose upon Fc γ RIII binding⁵⁷, in agreement with earlier crystallographic work showing a clash free IgG1 in complex with a non-glycosylated Fc γ RIII⁷⁰.

Case 3) Structure and Dynamics of the HIV-1-Env Glycan Shield. The ability of HIV to escape the humoral immune response is likely due to the high density glycan cover of the

trimeric envelope (Env) proteins complex that masks the virus from recognition⁷¹, see **Figure 2 panel c**. Over 90 high-mannose N-glycans decorate the complex surface, an amount that corresponds to about half of the molecular weight of the whole glycoprotein⁷². The majority of these N-glycans are Man-5 and Man-8/9, with a small population of Man-6/7⁵⁸. The crystal structure of the trimeric HIV-1 Env shows that these N-glycans outstretch from the gp120 surface through their chitobiose core, while the $\alpha(1-3)$ and $\alpha(1-6)$ arms extend perpendicularly interacting with neighbouring N-glycans and forming a dense glycan-glycan interaction network that effectively shields the protein surface⁵⁸. This seminal crystallographic work⁵⁸ was complemented by canonical MD simulations, more specifically by three 500 ns trajectories. The complexity of the system in terms of its sheer size and of the intricacy of the N-glycans interaction network makes the discovery potential of these simulations rather limited, mostly because of lack of sampling. For this reason, the structural and dynamic insight provided is not substantially incremental to the analysis of the crystallographic data. These canonical MD simulations were extended to 2 μ s in later work from the same group⁷³, which delivered more complete information on the collective dynamics of the shield and of protein surface accessibility. Notable in this particular context is the excellent work by Yang et al⁷⁴ that shows how enhanced sampling MD achieved through Hamiltonian Replica Exchange (HREX) is a much more informative approach. Indeed, the HREX simulations reveal a more complete image of the N-glycans conformational space and its heterogeneity, providing important insight on the pre-structuring of the N-glycans for recognition and binding by broadly neutralizing antibodies (bNAbs) and of their accessibility, as well as the accessibility of the protein surface to CD4 receptors and to CCR5 and CXCR4 co-receptors.

3.2 Coarse-Grained simulations of glycoconjugates

As a result of their structural diversity and a high degree of conformational freedom, carbohydrates represent a very challenging class of biomolecules in terms of CG modeling. Consequently, current CG carbohydrate models are currently developed to simulate very specific systems, such as oligosaccharides, polysaccharides, plant cell-wall polysaccharides, chitin and chitosan, glycosaminoglycans, glycoconjugates, N- and O-linked glycans, glycolipids and lipopolysaccharide membranes. Below we give a few examples of recent successful application of CG simulations in glycobiology.

Case 4) Structure, function and dynamics of glycolipids in plasma membrane.

Glycolipids are key components of cellular membranes. They are structured with an hydrophobic lipid tail and one or more hydrophilic carbohydrate groups linked by a glycosidic bond. The basic structure of glycolipids consists of a mono- or oligosaccharide moiety attached to a sphingolipid or a glycerol group with one or two fatty acids. These are defined as glycosphingolipids and glyco glycerolipids, respectively. Glyceroglycolipids are characterized by acetylated or non-acetylated glycerol groups with at least one fatty acid as ceramide as the lipid complex. The acyl group of ceramides is a long chain saturated or monounsaturated fatty acids. The specific nature of the carbohydrate moiety defines further classes and subclasses, namely cerebroside (galactocerebroside, glucocerebrosides, sulfatides), gangliosides, globosides, glycosphosphosphingolipids and glycosphosphatidylinositols. The carbohydrate moiety is the most exposed structures on glycolipids, sitting on the extracellular surface of cells. Their flexible structure with numerous binding positions, makes them highly suitable for recognition and cell signalling. Since the lipid moiety is buried within the membrane, carbohydrate-carbohydrate interactions are the predominant interactions that occur between glycolipids. Because of their biophysical properties, glycolipids are also essential for stabilizing the membrane bilayer.

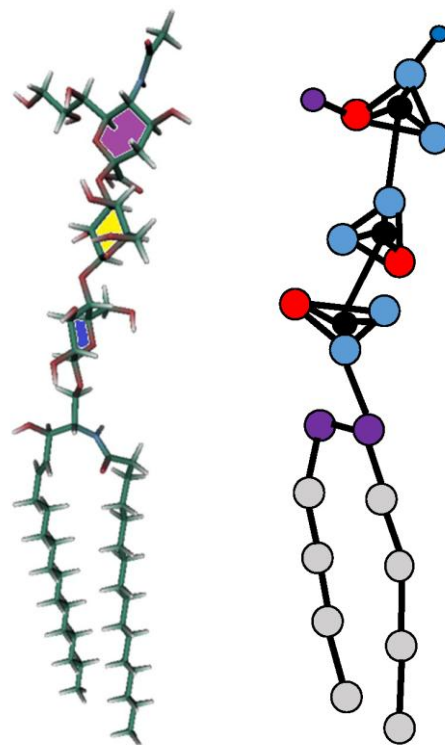


Figure 3. Atomistic (on the left) and CG (on the right) representations of the GM3 ganglioside.

Because of the reduced complexity inherent to the model, CG computer simulations have the potential to bridge the gap between experiment and all-atom calculations for systems of high complexity, more specifically they are well-suited to study the dynamic nano-clustering of glycolipids in which the carbohydrate-carbohydrate interactions play a key role. In a seminal work Ingólfsson et al.²⁸, studied an idealized mammalian plasma membrane comprising 63 different lipid species, combining 14 types of headgroups and 11 types of tails asymmetrically distributed across the two leaflets. The 40 μ s CG-MD trajectory, obtained based on the MARTINI representation, showed a general non-ideal lateral mixing of the different species. Glycolipids, monosialotetrahexosylganglioside (GM1) and monosialodihexosylganglioside (GM3), shown in **Figure 3**, also referred to as gangliosides, showed the highest non-ideal mixing behaviour, consistent with both *in vitro* and *in vivo* experimental data. The size of nanodomains in the CG model had a broad distribution of up to approximately 50 glycolipids, which were highly dynamic with individual clusters breaking apart and reforming at the microsecond time scale. Another group⁷⁵ reported that the formation of such GM3 nanoscale clusters correlated with spontaneous curvature of the membrane. The interactions between the gangliosides GM1 and GM3 were further studied using equilibrium MD simulations at both CG and atomistic levels⁷⁶. In this work the MARTINI ganglioside force field was re-parameterized to reduce self-interactions of GMs and to bring the radial distribution function (RDF) closer to the atomistic results. For more information on mammalian cell membrane CG models, we refer the reader to a mini-review⁷⁷.

The lipopolysaccharides (LPS) in the bacterial outer membrane present the most extensive and complex chemical diversity. Ma et al.⁴² developed an LPS parameter set for a MARTINI representation that was benchmarked against available experimental data and atomistic simulations. This model was based on several approximations concerning primarily the oligosaccharide domain of the LPS molecule, which contains anomeric centres. Due to the loss of atomistic resolution within the CG bead, this model does not provide information on stereoisomers. The model was applied to more than 27 different membrane compositions for a cumulative simulation time of 100 μ s. Increasing the LPS to 1,2-dipalmitoyl-3-phosphatidyl-ethanolamine (DPPE) composition in the outer leaflet contributes to decrease the phase transition values from 346 to 290 K, indicating a less ordered packing of the LPS molecules and increased area per lipid for the membrane. Such simulation outlines how chemical heterogeneity in membrane composition may result in significant variation in

membrane properties such as fluidity and phase transition temperatures, which in experimental systems may vary by ± 15 degrees. Also to note, the response to the mechanical stress of the *E. coli* outer membrane was investigated by Jefferies et al.⁷⁸. The authors found that the different packing of terminal O-antigen chains affected lipid mobility and the mechanical strength of the Gram-negative membrane models. For a more detailed overview of recent progress in the understanding of bacterial cell envelope from CG modelling the reader is referred to a comprehensive perspective by Khalid et al.⁷⁹. Notably, the authors underline that the motions of LPS molecules are highly correlated with each other and also with the outer proteins embedded within the membrane.

4.0 Conclusions

In this review we have presented the fundamental theoretical background of atomistic and CG levels for the simulations of complex carbohydrates and their complexity within realistic representation of glycoproteins and glycoconjugates in isolation and within the cell membranes. Through the illustration and discussion of recent applications we have shown the high potentials and limitations of each method, with the aim of presenting to the reader a complete and informative framework for their use in glycobiology research and discovery.

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