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CALCIUM BRIDGING BETWEEN CARBOHYDRATES AND LECTINS: A DIFFICULT CASE FOR CLASSICAL MOLECULAR DYNAMICS

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The developments of additive carbohydrate force fields increased the reliability of molecular dynamics (MD) simulations of protein-carbohydrate complexes [1]. The presence of bridging Ca^{2+} ions can, however, pose problems for structural and energetic description due to quantum effects, such as polarisation and charge transfer [2, 3]. To overcome this limitation, we had developed Ca^{2+} parameters with effective electronic polarisation for use with additive force fields [2] and applied them to a calcium-dependent lectin/carbohydrate complex whose structure we have determined crystallographically (Fig. 1) [4]. Such a treatment improved the structural description of the binding site ($Ca^{2+}\cdots Ca^{2+}$ distance) in submicrosecond MD but an extension to 1.4 μs showed instabilities in protein/carbohydrate interactions. Thus, a systematic testing of charge-group scaling and use of various water models was launched to determine a universal protocol to describe reliably lectin – calcium –carbohydrate complexes by MD. We propose that such protocols will be transferable to simulations of other charged biomolecular systems.

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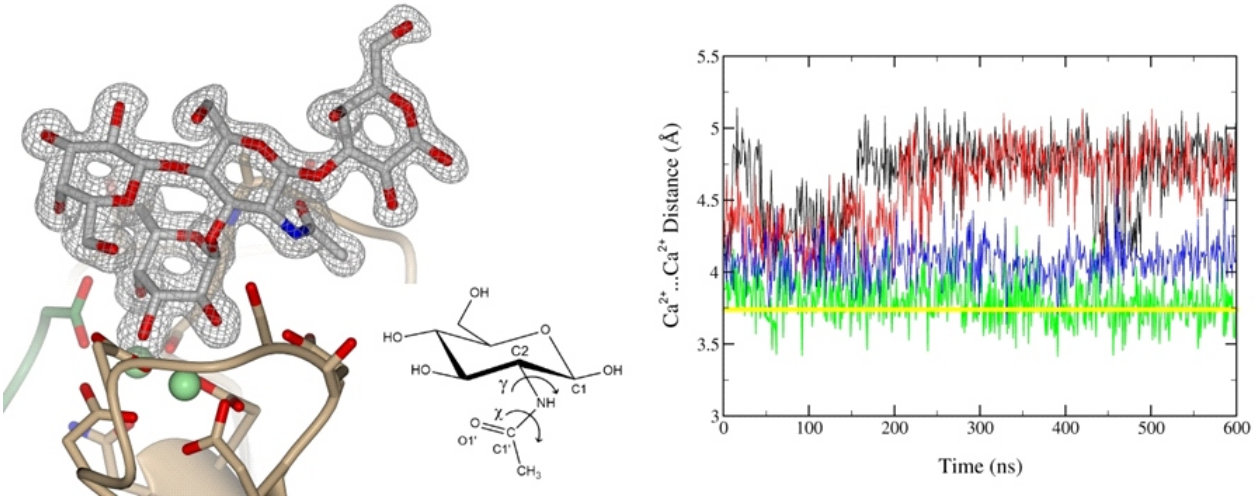

Figure 1. The binding mode of Lexis x tetrasaccharide complexed to two calcium ions in the binding site of LecB lectin of *Pseudomonas aeruginosa*. Dihedral angles ă and ÷ of N-acetyl glucosamine which are monitored during MD are shown. $Ca^{2+}\cdots Ca^{2+}$ distances in several MD setups (classical Ca^{2+} parameters - black, red; scaled Ca^{2+} parameters – blue, green) are compared with the crystallographic value (yellow).

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ELUCIDATING PHOSPHOLIPID MEMBRANE BINDING OF DEP DOMAIN IN THE WNT SIGNALING PATHWAY

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Signal transduction represents the mechanism by which cells communicate and interact with the extracellular environment and each other. Wnt signaling pathway is arguably one of the most studied and dissected pathway, due to its high level of conservation among species and involvement in a variety of (patho-)physiological processes [1]. Yet, the molecular events underlying the pathway activation and progression remain mostly elusive. It has been suggested that Dishevelled protein (DVL) plays the key role of a signaling hub for both canonical and non-canonical Wnt signaling branches [2,3]. In particular, a single DVL domain, called DEP (DVL, Egl-10, Pleckstrin), was shown to interact with phospholipid bilayers [4] and Wnt transmembrane receptor, Frizzled [5], triggering DVL recruitment to the plasma membrane (PM) and cytoplasmic signaling activation. By means of all-atom Molecular Dynamics simulations, we elucidated the structural details responsible for DEP-PM interaction and how this event is modulated by membrane lipid composition and the domain post-translational modifications (PTMs). Our results suggest that the recently identified phosphorylation sites on DEP domain [6] do not act as simple electrostatic modulators but rather make the interaction environment dependent.

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ATOMISTIC SIMULATION OF CARBOHYDRATE-PROTEIN COMPLEX FORMATION: HEVEIN-32 DOMAIN

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Interactions between proteins and their small molecule ligands are of great importance for the process of drug design. Here we present our recent study, an unbiased molecular dynamics simulation of systems containing hevein domain (HEV32) with N-acetylglucosamine mono-, di- or trisaccharide [1]. Carbohydrate molecules were placed outside the binding site. Three of six simulations (each 2 ěs) led to binding of a carbohydrate ligand into the binding mode in agreement with the experimentally determined structure. Unbinding and another binding was observed in one simulation (monosaccharide). There were no remarkable intermediates of binding for mono and disaccharide. Trisaccharide binding was initiated by formation of carbohydrate-aromatic CH/ interactions. Our results indicate that binding of ligands followed the model of conformational selection because the conformation of the protein ready for ligand binding was observed before the binding. Results of essential dynamics also support the model of conformational selection. This study extends the concept of docking by dynamics on carbohydrate-protein interactions.

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ELECTRON TRANSFER KINETICS IN CYTOCHROME C

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Electron transfer (ET) in biological system such as redox proteins usually undergoes by incoherent electron-hopping mechanism described by Marcus theory. Within this theoretical framework the free energy surfaces of the initial and final states are parabolic and the free energy barrier for ET is fully determined by the driving force G and the reorganization free energy . The theory is valid as far as distribution of the vertical energy gap E fluctuations is Gaussian and the phase space is sampled with Boltzmann distribution on the time scale of the ET process, meaning that the system must be ergodic.

However, it has been suggested in literature that heme-containing cytochrome c, due to its slow molecular motions and large anisotropy in polarizability of the its active site, operates in ergodicity-breaking regime violating the Marcus theory. This would lead to large imbalance between Stokes-shift reorganization free energy st and variational reorganization free energy ^{var} related to thermal fluctuations of the vertical energy gap, and, as a result, to considerably lower free energy barrier for ET. Yet, by applying various sampling techniques involving high-level QM/MM calculations with the whole cytochrome active site treated by DFT with electrostatic embedding, we were not able to reproduce such behavior.

By detail analyses of extensive molecular dynamics (MD) trajectories and decomposition of reorganization free energy to its potential and electric-field contributions we show that due to the alignment of intrinsic electric-field vector to direction of the largest polarizability, the calculations are extremely sensitive to the field distribution. However, both st and ^{var} converge to the same value in accord with the Marcus theory. Therefore, the free energy barrier lowering leading to fast ET kinetics observed experimentally is more-likely caused by electronic polarization of the environment, rather than ergodicity breaking.

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