Weak carbohydrate–carbohydrate interactions in membrane adhesion are fuzzy and generic

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Carbohydrates such as the trisaccharide motif LeX are key constituents of cell surfaces. Despite intense research, the interactions between carbohydrates of apposing cells or membranes are not well understood. In this article, we investigate carbohydrate–carbohydrate interactions in membrane adhesion as well as in solution with extensive atomistic molecular dynamics simulations that exceed the simulation times of previous studies by orders of magnitude. For LeX, we obtain association constants of soluble carbohydrates, adhesion energies of lipid-anchored carbohydrates, and maximally sustained forces of carbohydrate complexes in membrane adhesion that are in good agreement with experimental results in the literature. Our simulations thus appear to provide a realistic, detailed picture of LeX–LeX interactions in solution and during membrane adhesion. In this picture, the LeX–LeX interactions are fuzzy, i.e. LeX pairs interact in a large variety of short-lived, bound conformations. For the synthetic tetrasaccharide Lac 2, which is composed of two lactose units, we observe similarly fuzzy interactions and obtain association constants of both soluble and lipid-anchored variants that are comparable to the corresponding association constants of LeX. The fuzzy, weak carbohydrate–carbohydrate interactions quantified in our simulations thus appear to be a generic feature of small, neutral carbohydrates such as LeX and Lac 2.

Introduction

Carbohydrates are omnipresent at cell surfaces as constituents of glycolipids and glycoproteins. During cell adhesion, these carbohydrates come into contact with proteins and carbohydrates on apposing cell surfaces. While specific interactions between carbohydrates and proteins are known to play important roles in cell adhesion events, the role of carbohydrate–carbohydrate interactions in these events is less clear. About three decades ago, homophilic carbohydrate–carbohydrate interactions of the trisaccharide Lewis (LeX) have been reported to be involved in embryonal cell compaction and aggregation, and interactions between long carbohydrate chains have been linked to the species-specific aggregation of marine sponges. In the following decades, carbohydrate–carbohydrate interactions in adhesion have been investigated in a variety of reconstituted or synthetic systems including nanoparticles and surfaces functionalized with carbohydrates, atomic force microscopy setups, reconstituted vesicles or membranes containing glycolipids. While some carbohydrate–carbohydrate interactions have been reported to be strong interactions of small, neutral carbohydrates are typically considered to be weak. However, the binding association constants, in particular at membrane interfaces, and the structural binding mechanisms are often not known.

In this article, we present detailed results from atomistic molecular dynamics simulations of carbohydrate–carbohydrate interactions in membrane adhesion and in solution for LeX and the synthetic saccharide Lac 2, which is composed of two lactose units (see Fig. 1). Our simulations employ a recent carbohydrate force field that allows a more faithful representation of carbohydrate–carbohydrate interactions and exceed the times and system sizes in previous simulation studies of carbohydrate–carbohydrate interactions in solution by orders of magnitude. LeX has been investigated extensively as a model system for carbohydrate–carbohydrate interactions, and experimental data available from these investigations are central to corroborate our simulation results. In our LeX glycolipids, the LeX trisaccharide is connected via a lactose disaccharide and a glycerol linker to lipids tails (see Fig. 1). In our Lac 2 glycolipids, the LeX tri-saccharide is replaced by another lactose disaccharide, which allows to compare the carbohydrate–carbohydrate interactions...
of LeX to those of the common saccharide lactose. From simulations of soluble pairs of LeX and Lac 2, we obtain association constants $K_a$ of the order of $10^{-1}$, which agrees with a $K_a$ value of LeX derived from weak affinity chromatography experiments. From simulations of pairs of LeX and Lac 2 glycolipids at apposing membrane surfaces, we obtain comparable association constants $K_{\text{trans}}$ for the LeX and Lac 2 glycolipids that strongly decrease with increasing membrane separation. For the membrane separation and thermal roughness of membrane multilayers with 10 mol% LeX glycolipids measured in neutron scattering experiments, we determine an adhesion energy per area of the order of $10^{-2}$ μJ m$^{-2}$ from our $K_{\text{trans}}$ values, in agreement with the adhesion energy per area reported for vesicles that contain 10 mol% of LeX glycolipids. The average force on bound LeX glycolipid complexes determined in our simulations increases with increasing membrane separation up to a maximum value of about 20 pN, which agrees with the LeX–LeX unbinding force obtained from atomic force microscopy experiments. The agreement with experimental results indicates that our simulations provide a realistic, detailed picture of weak carbohydrate–carbohydrate interactions in solution as well as in membrane adhesion. A striking feature is that the carbohydrate–carbohydrate interactions are fuzzy, i.e. both soluble and lipid-anchored variants of LeX and Lac 2 interact in our simulations via a large variety of diverse, bound conformations.

Results

Interactions of soluble carbohydrates

We first consider the interaction of two LeX trisaccharides, which are composed of two lactose units (see Fig. 1). Standard carbohydrate force fields lead to osmotic pressures for solutions of neutral carbohydrates that are systematically too low compared to experimental values. This underestimation of the osmotic pressure of the carbohydrate solutions results from an overestimation of attractive carbohydrate–carbohydrate interactions. To avoid unrealistically attractive carbohydrate–carbohydrate interactions, we have used the GLYCAM06TIP5P force field, in which the van der Waals parameters for saccharide–saccharide interactions of the standard force field GLYCAM06 have been reparametrized to correctly reproduce experimentally measured osmotic pressures. The GLYCAM06TIP5P force field employs the TIP5P water model because this water model leads to more reliable carbohydrate–carbohydrate interactions in GLYCAM06, compared to the standard TIP3P water model. Using graphics processing units (GPUs) and the software AMBER GPU, we have generated 50 simulation trajectories with a length of 2.0 μs for two LeX molecules in a periodic simulation box of volume $V = 131.5$ nm$^3$, and 40 trajectories with a length of 1 μs or close to 1 μs for two Lac 2 molecules in a simulation box of volume $V = 260.5$ nm$^3$, at the simulation temperature $30 \, ^\circ\text{C}$. Our total simulation times are $100$ μs for the LeX pair and $39.5$ μs for the Lac 2 pair, which greatly exceed the total simulation times up to 40 ns in previous simulation studies of LeX–LeX pair interactions in solution and the total simulation time of a few ns for pair interactions of trisaccharide epitopes from marine sponges.

In our simulations, we observe thousands of interaction events in which the two LeX molecules or the two Lac 2 molecules are in contact. These interaction events are separated by longer or shorter trajectory parts in which the two molecules are not in contact. Fig. 2(a) and (b) display pair conformations...
of LeX and Lac 2 in which the two molecules exhibit at least 20 or 50 contacts of non-hydrogen atoms, respectively. The shown pair conformations are randomly selected from the simulation frames of our trajectories. One of the carbohydrate molecules is aligned in the pair conformations and represented in blue colors, while the other molecule is represented in red/yellow colors. In the aligned LeX molecules, fucose is represented in dark blue, galactose in light blue, and N-acetylglucosamine in cyan. In the other LeX molecules, these monosaccharide units are represented in red, orange, and yellow, respectively. In the aligned Lac 2 molecules, the terminal galactose is represented in dark blue, the adjacent glucose in light blue, and the remaining galactose and glucose in cyan. In the other Lac 2 molecules, these monosaccharides are shown in red, orange, and yellow. (c) Probability distributions of the number of contacts between non-hydrogen atoms obtained from our simulations of two soluble LeX or two soluble Lac 2 molecules. (d) Average lifetime of interaction events as a function of the maximum number of contacts of the interaction events. Interaction events are consecutive stretches of simulation frames at intervals of 0.1 ns with nonzero contacts of the two molecules. The error bars represent the standard deviations of the observed lifetimes. (e) Radial distribution functions $g(r)$ of two soluble LeX or Lac 2 molecules with center-of-mass distance $r$. 

Fig. 2 (a) and (b) Randomly selected pair conformations of two LeX and two Lac 2 molecules with at least 20 or at least 50 contacts between non-hydrogen atoms within a distance less than 0.45 nm, respectively. One of the molecules is aligned in the 50 pair conformations and represented in blue colors, while the other molecule is represented in red/yellow colors. In the aligned LeX molecules, fucose is represented in dark blue, galactose in light blue, and N-acetylglucosamine in cyan. In the other LeX molecules, these monosaccharide units are represented in red, orange, and yellow, respectively. In the aligned Lac 2 molecules, the terminal galactose is represented in dark blue, the adjacent glucose in light blue, and the remaining galactose and glucose in cyan. In the other Lac 2 molecules, these monosaccharides are shown in red, orange, and yellow. (c) Probability distributions of the number of contacts between non-hydrogen atoms obtained from our simulations of two soluble LeX or two soluble Lac 2 molecules. (d) Average lifetime of interaction events as a function of the maximum number of contacts of the interaction events. Interaction events are consecutive stretches of simulation frames at intervals of 0.1 ns with nonzero contacts of the two molecules. The error bars represent the standard deviations of the observed lifetimes. (e) Radial distribution functions $g(r)$ of two soluble LeX or Lac 2 molecules with center-of-mass distance $r$. 

Quantifying the attractive interactions of the two LeX or two Lac 2 molecules requires distinguishing bound and unbound states. This distinction is somewhat arbitrary because of the fuzzy interactions of the carbohydrates. The probability distributions of carbohydrate–carbohydrate contact numbers in Fig. 2(c) are monotonously decreasing and, thus, not bimodal as required for a clear distinction of two states. Table 1 presents association constants of two LeX or two Lac 2 molecules calculated for different cutoffs $n_c$ of the maximum number of contacts of interaction events. In these calculations, only interaction events with a maximum number of contacts larger or
equal to the cutoff \( n_c \) are taken to be binding events. The probability \( P_b \) that the two \( \text{Le}^X \) or two \( \text{Lac}^2 \) molecules have been bound is calculated from the total duration of the binding events, and the association constants from \( K_a = \frac{P_b}{P_u} \) where \( P_u = 1 - P_b \) is the probability that the molecules are unbound, and \( V \) is the volume of the simulation box. The \( K_a \) values in Table 1 slightly decrease with increasing contact cutoff \( n_c \) for binding events. For \( \text{Le}^X \), a \( K_a \) value of 10 M\(^{-1}\) has been obtained from weak affinity chromatography experiments,\(^{36} \) which is of the same order of magnitude as the values derived from our simulations.

**Interactions of lipid-anchored carbohydrates**

To investigate the interactions of two lipid-anchored \( \text{Le}^X \) or two lipid-anchored \( \text{Lac}^2 \) molecules, we have performed simulations of \( \text{Le}^X \) and \( \text{Lac}^2 \) glycolipids embedded in POPC lipid membranes. Our \( \text{Le}^X \) and \( \text{Lac}^2 \) glycolipids have the same lipid tails as POPC, and carbohydrate tips that are connected to these lipid tails by a glycerol linker group (see Fig. 1). The carbohydrate tip of the \( \text{Le}^X \) glycolipid consists of the \( \text{Le}^X \) trisaccharide and an additional lactose disaccharide as spacer between \( \text{Le}^X \) and the glycerol linker. The \( \text{Lac}^2 \) glycolipid has the linear \( \text{Lac}^2 \) tetrasaccharide as carbohydrate tip. The force field of our simulations combines the GLYCAM06mP4D\(^{14} \) carbohydrate force field\(^{42} \) for the TIP3P water model with the AMBER Lipid14 force field\(^{43} \) for lipid membranes. Because simulations of AMBER Lipid14 POPC membranes in TIP5P water lead to an unreasonably small area per lipid, we have rescaled the Lennard-Jones interactions between the TIP5P water molecules and the lipid headgroup atoms to obtain the same area per lipid as in standard AMBER Lipid14 simulations with the TIP3P water model (see Methods).

We quantify the interactions of two \( \text{Le}^X \) or two \( \text{Lac}^2 \) glycolipids at apposing membrane surfaces in a system that consists of a single lipid bilayer with one glycolipid anchored in each monolayer (see Fig. 3). In this system, the two glycolipids in the different monolayers interact according to the periodic boundary conditions of the simulation box, and the separation of the membrane monolayers can be adjusted by varying the number of water molecules in the simulation box. The values for the membrane separation \( l \) given in Fig. 3 correspond to the separation from membrane midplane to membrane midplane and, thus, to the height of the simulation box. At each membrane separation, we have generated 10 trajectories with a length of 3 \( \mu \)s for the \( \text{Le}^X \) system and a length of 1 \( \mu \)s for the \( \text{Lac}^2 \) system at the temperature 30 \( ^\circ \)C. The total simulation times at each membrane separation thus are 30 \( \mu \)s and 10 \( \mu \)s for the \( \text{Le}^X \) and \( \text{Lac}^2 \) systems, respectively. The membranes contain in each monolayer 35 lipids besides the single glycolipid and have an area \( A \) of 23.3 nm\(^2\). The height of the simulation box \( l \) increases with the number of water molecules \( n_w \) as \( l = 3.8 \text{ nm} + 0.013n_w \text{ nm} \). The thickness of the water layer in the simulations thus is about \( l = 3.8 \text{ nm} \).

The interactions of the glycolipids strongly depend on the membrane separation. For the membrane separations \( l = 5.5, 6.0, 6.5, \) and \( 7.0 \text{ nm} \), 50 randomly selected complexes of the \( \text{Le}^X \) glycolipid tips with at least 10 contacts of non-hydrogen atoms are displayed at the bottom of Fig. 3. The carbohydrate tip of the lower \( \text{Le}^X \) glycolipid is aligned in the 50 complexes and represented in blue colors, while the carbohydrate tip of the upper glycolipid is represented in red/yellow colors. The clouds of red/yellow carbohydrates illustrate that the interactions of lipid-anchored \( \text{Le}^X \) are fuzzy, similar to soluble \( \text{Le}^X \) and \( \text{Lac}^2 \) (see Fig. 2). The overlap of the cloud of the upper, red/yellow carbohydrates with the lower, blue carbohydrate decreases with increasing membrane separation. At the membrane separation 5.5 nm, the \( \text{Le}^X \) glycolipids interact via their entire carbohydrate tips. At the separation 6.0 nm, the interactions are limited to the \( \text{Le}^X \) trisaccharide of the glycolipid tip, and at the membrane separations 6.5 nm and 7.0 nm, the interactions are further restricted to the galactose and fucose monosaccharides at the branched end of the \( \text{Le}^X \) glycolipid. The decrease of interactions with increasing separation is also reflected in the probability distributions of contact numbers shown in Fig. 4(a) and in the average lifetime of the interaction events for different maximum numbers of contacts in Fig. 4(b). At the smallest membrane separation 5.5 nm, complexes of \( \text{Le}^X \) glycolipids can exhibit up to 60 and more contacts of non-hydrogen atoms (see Fig. 4(a)), and average lifetimes up to 50 ns for interaction events with a maximum number of 60 contacts (see inset of Fig. 4(b)), which are about one order of magnitude larger than the average lifetimes for interaction events of soluble \( \text{Le}^X \) molecules with the same maximum number of contacts. At the membrane separations 6.0 and 6.5 nm, the overall contact numbers and lifetimes of interaction events are significantly smaller.

Analogous to soluble carbohydrates, the binding association constants \( K_{\text{trans}} = A P_b/(1 - P_b) \) of the glycolipids in the different membrane monolayers can be determined from the probability \( P_b \) that the two \( \text{Le}^X \) or two \( \text{Lac}^2 \) glycolipids are bound. The binding constants shown in Fig. 5 are calculated for binding events with a maximum number of at least \( n_c = 5 \) contacts of non-hydrogen atoms. For the larger binding cutoff \( n_c = 10 \), the \( K_{\text{trans}} \) values of the two \( \text{Le}^X \) glycolipids are about 10% smaller than the values in Fig. 5 at the membrane separations 5.5 and 6.0 nm, and the values of the \( \text{Lac}^2 \) glycolipids are about 15% smaller at these separations. The \( K_{\text{trans}} \) values decrease with increasing membrane separation. For membrane separations larger than about 7.5 nm, the glycolipids cannot form contacts.

The binding constant \( K_{\text{trans}} \) can be related to membrane adhesion energies, which have been measured for membrane vesicles that contain 10 mol% of \( \text{Le}^X \) glycolipids.\(^{20,27} \) For two apposing, large membrane surfaces of area \( A \) that contain a

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**Table 1** Association constants \( K_a \) in units of M\(^{-1}\) for different cutoffs \( n_c \) for the contact number of binding events

|          | \( n_c = 5 \) | \( n_c = 10 \) | \( n_c = 20 \) |
|----------|--------------|--------------|--------------|
| \( \text{Le}^X \) | 6.4 ± 0.3    | 5.7 ± 0.3    | 4.5 ± 0.3    |
| \( \text{Lac}^2 \) | 13.2 ± 1.0   | 12.3 ± 1.0   | 10.7 ± 0.9   |

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total number of $N_t$ glycolipids, the free energy difference for forming the $n$th bond of the glycolipids is (see Methods)

$$\Delta G_n = -k_B T \ln[K_{\text{trans}}(N_t - n + 1)^2/nA] \quad (1)$$

The free energy differences $\Delta G_n$ are negative and, thus, favourable, from bond 1 until the equilibrium number $n_{eq}$ of bonds. For bond numbers $n > n_{eq}$, the free energy difference $\Delta G_n$ is positive and, thus, unfavorable for binding. The adhesion free energy $g_{ad}$ per area now can be calculated by summing up the free energy differences $\Delta G_n$ from bond 1 to bond $n_{eq}$:

$$g_{ad} = \sum_{n=1}^{n_{eq}} \frac{\Delta G_n}{A} \quad (2)$$

For an area per lipid of 0.65 nm$^2$ measured in our simulations, the area of a membrane surface that contains $N_t$ glycolipids at a concentration of 10 mol% is $A \approx 6.5N_t$ nm$^2$. From eqn (1) and (2) and the values of $K_{\text{trans}}$ for the LeX glycolipids in Fig. 5, we obtain the adhesion free energies $g_{ad} = 320 \pm 60$, $150 \pm 20$, $28 \pm 5$, and $5 \pm 2$ μJ m$^{-2}$ at the membrane separations $l = 5.5$, 6.0, 6.5, and 7.0 nm respectively. For lipid vesicles that contain 10 mol% of LeX glycolipids, an adhesion free energy per area of $27 \pm 2$ μJ m$^{-2}$ has been reported,$^{20}$ which is comparable to the adhesion free energy obtained from our simulations with membrane separation 6.5 nm.

**Forces on lipid-anchored carbohydrates in trans-direction**

The binding of glycolipids in our simulations is associated with deviations of the glycolipids relative to the surrounding...
lipids. These deviations in the trans-direction perpendicular to the membrane surface result from forces on bound glycolipid complexes. Fig. 6(a) illustrates distributions of trans-deviations between the center of mass of a LeX glycolipid (see Fig. 1) and the center of mass of all lipid head groups in the same monolayer as the glycolipid. The trans-deviations \( d \) are calculated from the simulation frames of our trajectories at intervals of 0.1 ns. We obtain two values of \( d \) per simulation frame for the two glycolipids relative to the monolayer in which they are embedded. An increase in \( d \) indicates glycolipid motion away from the membrane midplane. With increasing membrane separation, the distributions for bound LeX glycolipids deviate more and more from the distribution for unbound LeX, which reflects increasing forces. The distribution of trans-deviations \( d \) of unbound LeX glycolipids shown in Fig. 6(a) is calculated from our simulation trajectories at the membrane separation 8.0 nm, at which LeX bonds do not occur, and can be approximated by a Gaussian distribution \( \exp\left(-V(d)/k_B T\right) \) with \( V(d) = k \left(d - d_0\right)^2 \). The trans-deviations \( d \) of unbound LeX glycolipids thus can be described by a harmonic potential \( V(d) \) with force constant \( k \) and mean extension.
$d_u$, which can be determined from the standard deviation $\sigma$ and mean $\bar{d}$ of the Gaussian as $k = k_B T / \sigma^2 = 94 \pm 4 \text{ pN nm}^{-1}$ and $d_u = \bar{d} - 0.31 \pm 0.10 \text{ nm}$. The distributions of trans-deviations of bound Le$^X$ glycolipids in Fig. 6(a) are calculated from our simulation trajectories at the membrane separations 5.5, 6.0, 6.5, and 7.0 nm, for binding events with a maximum number of at least $n_c = 5$ contacts of non-hydrogen atoms. The average force $f = k(d_u - d_a)$ on bound Le$^X$ glycolipids at the membrane separations $l = 5.5, 6.0, 6.5, \text{ and } 7.0 \text{ nm}$ then can be calculated from the difference between the mean trans-deviations $d_a = -0.26 \pm 0.01, -0.22 \pm 0.01, -0.16 \pm 0.01, \text{ and } -0.08 \pm 0.02 \text{ nm}$ of the bound glycolipids at these membrane separations and the mean trans-deviation $d_u$ of the unbound glycolipids. The force $f$ on bound Le$^X$ glycolipids increases with increasing membrane separation up to a value of $21.7 \pm 2.4 \text{ pN}$ at the separation 7.0 nm (see Fig. 6(b)). This maximal force value agrees with the unbinding force $20 \pm 4 \text{ pN}$ of two Le$^X$ molecules obtained from atomic force microscopy experiments. For bound Lac 2 glycolipids, we obtain a maximal force of $14.7 \pm 3.5 \text{ pN}$ at the separation 7.0 nm, which is about of the same magnitude as the maximal force sustained by the Le$^X$ complexes.

The forces on bound Le$^X$ glycolipids lead to an adhesion pressure between the membranes. Fig. 7 illustrates the adhesion pressure $\rho$ of membranes that contain 10 mol% of Le$^X$ glycolipids as a function of the membrane separation. The adhesion pressure is estimated as $\rho = P_0 f / A$ where $P_0$ is the probability that a Le$^X$ glycolipid is bound at the concentration 10 mol%. $f$ is the average force on the bound glycolipid, and $A \approx 6.5 \text{ nm}^2$ is the average membrane area of membrane patch with a single glycolipid at this concentration (see above). The negative pressure values for membrane separations $l$ of 7.0 nm and smaller, at which the glycolipids can bind, indicate membrane attraction. From integration of the pressure profile along the dashed interpolation line shown in Fig. 7, we obtain adhesion energies $g_{ad} = \int_0^l \rho(l') dl' \approx 140 \mu \text{J m}^{-2}$ for $l = 6.0 \text{ nm}$ and $g_{ad} \approx 30 \mu \text{J m}^{-2}$ for $l = 6.5 \text{ nm}$. These adhesion energies per area agree with values $g_{ad} = 150 \pm 20 \mu \text{J m}^{-2}$ and $28 \pm 5 \mu \text{J m}^{-2}$ obtained directly from the binding constants $K_{trans}$ at the membrane separations $l = 6.0$ and 6.5 nm (see above), which indicates that average forces $f$ on bound Le$^X$ glycolipids of Fig. 6(b) are consistent with the binding constants $K_{trans}$ shown in Fig. 5.

### Discussion and conclusions

The membranes in our simulation systems are essentially planar because of the small size of the membranes, and because the glycolipid in one monolayer interacts with the glycolipid in the other monolayer across the periodic boundary of the simulation box. In larger, experimental systems, in contrast, the membranes exhibit thermally excited shape fluctuations, which lead to a steric repulsion between adjacent membranes. During membrane adhesion, this steric repulsion needs to be overcome by attractive interactions. The average separation and thermal roughness of the adhering membranes is determined by the interplay of the attractive interactions and the steric repulsion. From neutron scattering experiments of DPPC membrane multilayers that contain 10 mol% of Le$^X$ glycolipids, an average membrane separation of $l = 7.7 \pm 0.1 \text{ nm}$ and a relative membrane roughness of $\xi_{\perp} = 0.73 \pm 0.03 \text{ nm}$ has been obtained. Because of the periodicity of the membrane multilayers, the distribution of the local membrane separations $l$ between adjacent membranes can be approximated by the symmetric Gaussian distribution $P(l) \approx \exp[-(l-l)²/2\xi_{\perp}²]/(\sqrt{2\pi}\xi_{\perp})$ with mean $l$ and standard deviation $\xi_{\perp}$. The average membrane separation $l$ obtained from neutron scattering is larger than the membrane separations at which the Le$^X$ glycolipids interact in our simulations. Trans-binding of the glycolipids therefore requires local membrane separations of the fluctuating membranes that are smaller than the average separation of the membranes. The average adhesion energy per area of adjacent membranes can be estimated as $\bar{g}_{ad} = \int g_{ad}(l) P(l) dl$, where $g_{ad}(l)$ is the adhesion energy as a function of the local membrane separation $l$. From the four values of $g_{ad}(l)$ at the membrane separations $l = 5.5, 6.0, 6.5, \text{ and } 7.0 \text{ nm}$ determined in the section “Interactions of lipid-anchored carbohydrates”, we obtain the estimate $\bar{g}_{ad} = 7 \pm 3 \mu \text{J m}^{-2}$ for the average separation $l$ and relative membrane roughness $\xi_{\perp}$ of the neutron scattering experiments. This estimate of the average adhesion energy per area is comparable in magnitude to the adhesion free energy per area of $27 \pm 2 \mu \text{J m}^{-2}$ reported for adhering membrane vesicles that contain 10 mol% of Le$^X$ glycolipids. The Le$^X$ glycolipids embedded in the vesicles have the same carbohydrate tip as the Le$^X$ glycolipids of the neutron scattering experiments and of our simulations. However, the carbohydrate tip of the vesicle system is

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**Fig. 7** Adhesion pressure $\rho$ of membranes with 10 mol% of Le$^X$ glycolipids obtained for the force values $f$ on bound Le$^X$ of Fig. 6(b). The dashed interpolation line is added as a guide for the eye and used to estimate adhesion energies via integration (see text). In this integration, the pressure $\rho$ is taken to be zero at separations $l \geq 7.5 \text{ nm}$.

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†The relative membrane roughness follows from eqn (2) of ref. 23 as $\xi_{\perp} = \sqrt{g_{ad}(0)}$ with parameter values given in Table 2. Here, $g_{ad}(r)$ is the membrane displacement correlation function of adjacent membranes in the multilayer.
connected to a ceramide, which contains a different linker between the carbohydrate tip and the lipid tails. Another difference is that the neutron scattering experiments have been performed at the temperature 60 °C to ensure that the DPPC membranes in these experiments are fluid. The LeX glycolipids of our simulations differ from those of the neutron scattering experiments only in the lipid tails. We have focused on POPC membranes and corresponding glycolipid tails to be able to run simulations of fluid membranes at the temperature 30 °C, which is close to the calibration temperature of the force fields. In principle, membrane tension suppresses shape fluctuations of the membranes and can lead to stronger adhesion. However, the suppression of fluctuations occurs only on lateral length scales larger than the characteristic length $\sqrt{\kappa/\sigma}$, which adopts values between 100 and 400 nm for typical membrane tensions $\sigma$ of a few μN m$^{-1}$ (ref. 48–50) and typical membrane bending rigidities $\kappa$ between 10 and 40 k$\text{J m}^{-2}$. These values are significantly larger than the lateral correlation length $\xi_{\parallel}$ of membranes adhering via LeX glycolipids, which is only a few nanometers for a relative membrane roughness $\xi_{\perp}$ of about 0.7 nm. On these small length scales, the membrane shape fluctuations are dominated by the bending energy of the membranes and the adhesion energies of the glycolipids, and are not affected by membrane tension.

The fuzzy interactions and comparable magnitude of the association constants of LeX and Lac 2 obtained in our simulations indicate that the interactions of small, neutral carbohydrates such as LeX and Lac 2 are rather generic and not dependent on specific structural aspects of the carbohydrates. The good agreement to experimental results for the association constant of soluble LeX and Lac 2 in water. Initial structures of the LeX trisaccharides and Lac 2 tetrasaccharides were created with the Glycam Carbohydrate Builder program and solvated in truncated octahedral simulation boxes with 4287 TIP5P water molecules for the LeX pair and with 8504 TIP5P water molecules for the Lac 2 pair. In the initial conformations, the two saccharides were placed in the simulation boxes such that they were not in contact. We have subsequently minimized the simulation systems in 5000 minimization steps of steepest decent and additional 5000 steps of the conjugate gradient algorithm. The systems were then heated from the temperature 0 K to 303 K at constant volume in 50,000 integration time steps of 2 fs with temperature control by a Langevin thermostat with collision frequency $\gamma = 1.0 \text{ ps}^{-1}$.

**Production runs.** After equilibration for 2 ns at 303 K, we have generated 50 independent trajectories for the LeX pair and 40 trajectories for the Lac 2 pair with a 2 fs integration step in AMBER 14 and 16 GPU using the Monte Carlo barostat and a Langevin thermostat with collision frequency $\gamma = 1.0 \text{ ps}^{-1}$ to keep the temperature at 303 K and the pressure at 1 bar. On these trajectories, the lengths of bonds that contain hydrogens were restrained with the SHAKE algorithm, non-bonded interactions were truncated at a cutoff value of 1 nm, and the Particle Mesh Ewald algorithm (PME) was used to treat all electrostatic interactions. The 50 simulation trajectories for the LeX pair have a length of 2.0 μs, and the 40 trajectories for the Lac 2 pair have a length of 1 μs or close to 1 μs. The total simulation times of these trajectories are 100 μs for the LeX system and 39.5 μs for the Lac 2 system.

**Analysis of trajectories.** We have identified interactions events of the two LeX or two Lac 2 molecules along the simulation trajectories as consecutive stretches of simulation frames at intervals of 0.1 ns with nonzero contacts of the molecules. These interaction events are separated by stretches of simulation frames with zero contacts and can be characterized by their lifetime and by the maximum number of contacts during the events. The contacts are defined as contacts between non-hydrogen atoms of the two molecules within a distance of less than 0.45 nm. We consider interaction events with a maximum number of contacts that is larger or equal to a cutoff number $n_c$ as binding events. For the cutoff numbers
Simulations of lipid-anchored saccharides

System setup. We have generated the initial structures of the POPC lipid membranes with the CHARMM-GUI program. For our simulations with glycolipids, one lipid in each monolayer has been replaced by a LeX or Lac 2 glycolipid, which have the same lipids tails as POPC (see Fig. 1). Following ref. 43, we have performed the initial minimization and equilibration steps of all membrane systems as follows: we have first performed a minimization of the water molecules for fixed lipids and glycolipids in 2500 minimization steps of steepest descent and subsequent 2500 steps of the conjugent gradient algorithm. The lipids and glycolipids have been fixed by harmonic constraints with a force constant of 500 kcal mol$^{-1}$ Å$^{-1}$ in this minimization. We have next removed the harmonic constraints, and have repeated the same minimization steps for the complete systems. The subsequent heating of the systems has been performed in three steps: (1) heating from 0 K to 100 K at constant volume with harmonic constraints on lipids and glycolipids with a force constant of 20 kcal mol$^{-1}$ Å$^{-1}$; (2) heating from 100 K to 200 K with a reduced force constant of 10 kcal mol$^{-1}$ Å$^{-1}$ of the harmonic constraints on lipids and glycolipids; and (3) heating from 200 K to 303 K at constant pressure and a membrane tension of zero with the same harmonic constraints as in the second step using a semi-isotropic pressure coupling and the Berendsen barostat with a pressure relaxation time of 3 ps. Each heating step consist of 10 000 MD integration steps of length 2 fs with temperature control by a Langevin thermostat with a collision frequency of 5.0 ps$^{-1}$.

Rescaling of Lennard-Jones interactions between water and lipid headgroups. We have used the GLYCAM06\textsuperscript{TIP3P} carbohydrate force field\textsuperscript{10,42} for the carbohydrates and the AMBER Lipid14 force field\textsuperscript{43} for the lipids of our MD simulations of POPC membranes with glycolipids. Simulations of AMBER Lipid14 POPC membranes in TIP5P water lead to an unreasonably small area per lipid of 0.514 ± 0.002 nm$^2$ (see Fig. 8) and to density profiles that deviate significantly from profiles obtained from simulations in the standard TIP3P water model (see Fig. 9), which has been used in the parametrization of the AMBER Lipid14 force field.\textsuperscript{43} We have therefore rescaled the well depth of the Lennard-Jones interactions between the TIP5P water molecules and the Lipid14 lipid headgroup atoms by a scaling factor $\alpha$ in order to obtain the same area per lipid as in simulations of POPC membranes with TIP3P water. We chose to rescale the Lennard-Jones interactions between water and lipid headgroups because the density profiles of AMBER Lipid14 POPC membranes in TIP5P water show a smaller overlap between the water and lipid head group regions, compared to TIP3P water (see Fig. 9). This smaller overlap likely
results from weaker Lennard-Jones interactions, and not from different atom sizes, because the Lennard-Jones radius 3.502 Å of the TIP5P oxygen atom is in fact smaller than the radius 3.53 Å of the TIP3P oxygen atom. Therefore, we have only rescaled the Lennard-Jones well-depth $\epsilon$ for the interaction between TIP5P water and the lipid head group atoms by a scaling factor $\alpha$.

Fig. 8 illustrates simulation results for the area per lipid as a function of the scaling factor $\alpha$. The membranes in these simulations consists of 128 POPC lipids, and the number of water molecules is 6400. For each value of $\alpha$, we have generated 10 independent trajectories of length 150 ns with semi-isotropic pressure coupling at a membrane tension of zero and a temperature of 303 K using the same barostat and thermostat settings as in the last heating step of the system setup (see above).

We have determined the area per lipid from the last 100 ns of these trajectories, with errors calculated as error of the mean of the values for the individual trajectories. The value $\alpha = 1.4$ leads to an area per lipid in TIP5P simulations that is close to the area per lipid both in TIP3P simulations and in experiments (see Fig. 8). We have therefore used $\alpha = 1.4$ in our simulations of lipid-anchored saccharides. For this value of $\alpha$, the density profile of AMBER Lipid14 POPC membranes in TIP5P water (not shown) is practically identical to density profile in TIP3P water, and the membrane thickness $d_m$ and lateral diffusion coefficient $D$ of the lipids are identical within errors or close to the values obtained in TIP3P simulations (see Table 2). We have determined the bilayer thickness as the distance between the electron density peaks of the lipid head groups, and the lateral diffusion coefficient $D$ of the lipids are identical within errors or close to the values obtained in TIP3P simulations (see Fig. 8). We have therefore used $\alpha = 1.4$ in our simulations of lipid-anchored saccharides. For this value of $\alpha$, the density profile of AMBER Lipid14 POPC membranes in TIP5P water (not shown) is practically identical to density profile in TIP3P water, and the membrane thickness $d_m$ and lateral diffusion coefficient $D$ of the lipids are identical within errors or close to the values obtained in TIP3P simulations (see Table 2). We have determined the bilayer thickness as the distance between the electron density peaks of the lipid head groups, and the lateral diffusion coefficient $D$ of the lipids are identical within errors or close to the values obtained in TIP3P simulations (see Table 2).

**Analysis of trajectories.** We have identified interactions events between the carbohydrate tips of the two Le$^X$ or two Lac 2 glycolipids in the same way as described above for the soluble saccharides. For two Le$^X$ glycolipids, we have obtained 1490, 1609, 588, 141 binding events with a maximum contact number of at least $n_c = 5$ on the trajectories at the membrane separations 5.5, 6.0, 6.5, and 7.0 nm, respectively. For two Lac 2 glycolipids, we have obtained 609, 413, 183, and 34 such binding events on the trajectories at the corresponding membrane separations.

To ensure independence from the initial conformation of the trajectories, we have discarded the first 10% of each trajectory in our calculations of the binding probability $P_b$ of the two molecules. In analogy to soluble carbohydrates, we have determined $P_b$ and its error as mean and error of the mean of the values for the 10 trajectories at a given membrane separation. The binding constant then follows as $K_{trans} = A P_b (1 - P_b)$ where $A$ is the membrane area. We have calculated the errors of the probability distributions in Fig. 4(a) and 6(a) and of the forces in Fig. 6(b) as error of the mean of the corresponding quantities for the individual trajectories.

| Table 2 | Membrane thickness $d_m$ and lipid diffusion coefficient $D$ from simulations with TIP5P water for different values of the scaling factor $\alpha$, from simulations with TIP3P water, and from experiments on POPC lipid membranes |
|---------|------------------|------------------|
| $\alpha$ | $d_m$ [nm]       | $D$ [$\mu m^2 s^{-1}$] |
| 1.2     | 3.82 ± 0.03      | 3.27 ± 0.10      |
| 1.3     | 3.67 ± 0.01      | 4.15 ± 0.08      |
| 1.4     | 3.51 ± 0.01      | 5.57 ± 0.16      |
| 1.45    | 3.43 ± 0.01      | 5.85 ± 0.15      |
| 1.5     | 3.36 ± 0.01      | 6.77 ± 0.11      |
| 1.55    | 3.31 ± 0.01      | 7.31 ± 0.19      |
| TIP3P   | 3.54 ± 0.01      | 5.45 ± 0.19      |
| Exp.    | 3.68 (ref. 68)   | 10.7 (ref. 69)   |

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Calculation of adhesion free energies from trans-binding constants of membrane-anchored molecules

The binding constant $K_{\text{trans}}$ of molecules anchored to two apposing membrane surfaces 1 and 2 of area $A$ is related to the on- and off-rate constants of these molecules via

$$K_{\text{trans}} = k_{\text{on}} / k_{\text{off}}$$

(3)

If the total numbers of the molecules at the two surfaces are $N_1$ and $N_2$, up to $n \leq \min(N_1,N_2)$ trans-bonds can be formed. The effective rate for going from a state with $n - 1$ trans-bonds to a state with $n$ bonds is

$$k_+ = k_{\text{on}} (N_1 - n + 1)(N_2 - n + 1)/A$$

and the effective rate for going back from $n$ bonds to $n - 1$ is

$$k_- = nk_{\text{off}}$$

(5)

The condition of detailed balance implies

$$P_{n-1}k_+ = k_- P_n$$

(6)

where $P_n$ is the equilibrium probability of the state with $n$ trans-bonds. The free-energy difference $\Delta G_n$ between the states with $n$ and $n - 1$ bonds is related to the equilibrium probabilities via

$$\exp[-\Delta G_n k_B T] = P_n / P_{n-1}$$

From these equations, we obtain

$$\Delta G_n = -k_B T \ln \left[ \frac{K_{\text{trans}} (N_1 - n + 1)(N_2 - n + 1)}{nA} \right]$$

(8)

The adhesion free energy $\Delta G_{ad}$ per area then can be calculated by summing up the free energy differences $\Delta G_n$ from bond 1 to bond $n_{eq}$ where $n_{eq}$ is the equilibrium number of bonds at which $\Delta G_n$ changes sign (see eqn (2)).

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Financial support of the International Max Planck Research School (IMPRS) on Multiscale Bio-Systems and by the German Research Foundation (DFG) via Emmy Noether grant SCHN 1396/1 is gratefully acknowledged. We would like to thank Mark Santer for helpful discussions. Open Access funding provided by the Max Planck Society.

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