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Carbohydrate self-assembly at surfaces: STM imaging of sucrose conformation and ordering on Cu(100)

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Abstract: Saccharides are ubiquitous biomolecules but little is known about their interaction with and assembly at surfaces. Combining preparative mass spectrometry with scanning tunneling microscopy, we have been able to address the conformation and self-assembly of the disaccharide sucrose on the Cu(100) surface with subunit-level imaging. By combining a multi-stage modelling approach and experimental data, we can rationalize the conformation on the surface as well as the interactions between the sucrose molecules, yielding models of the observed self-assembled patterns on the surface.

Carbohydrates (also called saccharides) serve as the key structural component of plants, making up the majority of biomass, and are a source of chemical energy for living organisms.^[1] Although these molecules are highly flexible in solution, they can form stable crystals due to the many hydrogen bonds formed between the hydroxyl groups at their periphery. The 3D crystal structure can be determined by diffraction,^[2] exploiting the symmetry of the crystal, which enables averaging over many molecules. The conformations as well as assembly motifs of saccharides in environments of reduced symmetry, e.g. on surfaces and in contact with few/other molecules, are essentially unknown. The former is of paramount importance in biological signaling,^[3] and the interactions with solid substrates are of relevance, e.g. in the technological use of saccharides in

cement hydration^[4] or for medical applications such as biomineralization.^[5] In both domains, however, the conformation and interactions matter on the level of the individual molecule. Gaining this knowledge at the molecular level presents an enormous analytical challenge chemically and structurally, because monosaccharides are generally structural isomers, whose specific function lies in the subtle arrangement of functional groups, and furthermore on the details of their conformations that are constrained by the way the monosaccharides are connected.

Scanning probe microscopy is a suitable technique to gain information about adsorbed molecules because it allows the direct imaging of individual molecules with sub-molecular resolution.^[6] Here we present the study of sucrose molecules arranged in a molecular network, which forms on a Cu(100) surface after deposition. The structural complexity of saccharides is already reflected in the behavior of sucrose as this consists of two monosaccharides that can exhibit different adsorption conformations linked by a glycosidic bond. Employing a new approach combining direct imaging of the monosaccharide building blocks of sucrose by scanning tunneling microscopy (STM) and multi-stage modelling, we gain insight into the conformation of individual sucrose molecules on a surface and can infer intra- and intermolecular interactions from the patterns observed for the assemblies.

Sucrose (α -D-glucopyranosyl-(1 \rightarrow 2)- β -D-fructofuranoside), chosen for the study presented herein, is an abundant disaccharide that crystallizes in anhydrous form in a monoclinic 3D structure.^[2] Sucrose is non-reducing, and thus remains stable in aqueous solution because no isomerism due to ring opening reactions is expected. Due to its non-volatility, we employ soft-landing electrospray ion beam deposition (ES-IBD)^[7] in order to deposit the deprotonated molecule as a negatively charged ion on Cu(100). This enables us to employ mass spectrometry and mass filtering as well as to decelerate the ion beam for soft-landing on the surface, ensuring the intact and highly pure deposition of the selected species, hence making sure that imaging is performed on the intact, e. g. non-fragmented or degraded molecular species (dehydrogenated sucrose).^[8] (see also supplementary information, SI-A).

After deposition, the deprotonated sucrose molecules most likely lose their charge to yield dehydrogenated sucrose molecules. These are mobile on the Cu(100) surface at room temperature, as we do not observe stable aggregates by STM.

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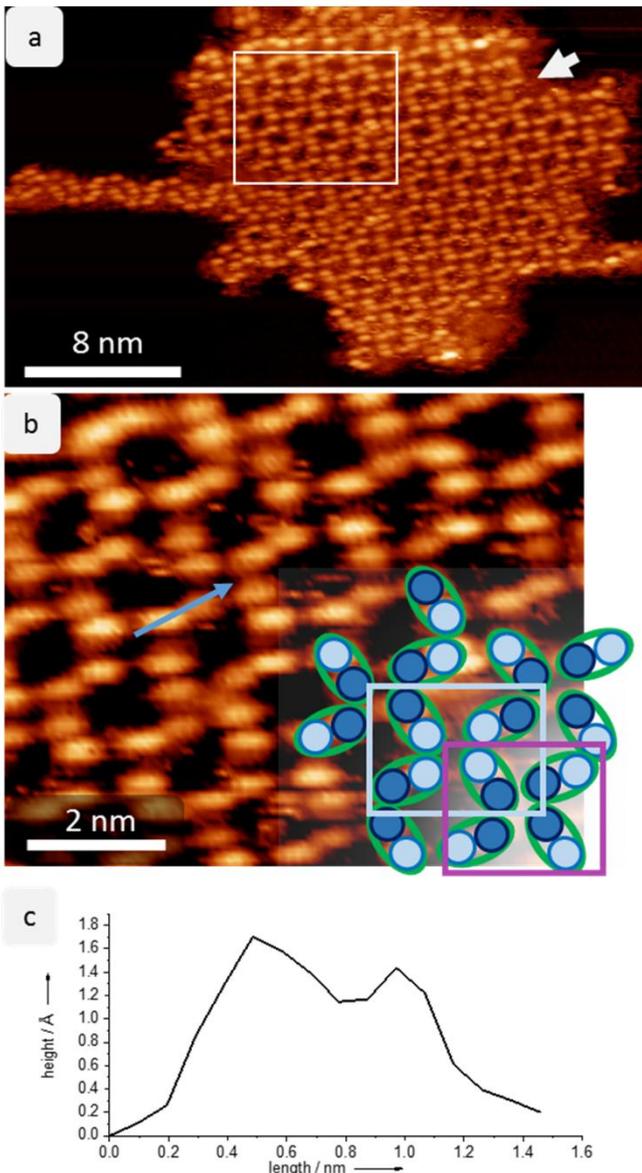
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Figure 1. Sucrose self-assembly on Cu(100) at 40K. a) STM image of one



island of sucrose on Cu(100) showing the periodic network. The molecules are seen as oval lobes; a white arrow indicates a domain boundary that divides the whole island. An additional structure is found on the left side of the island. The white rectangle marks the area of the zoom-in. b) Magnification of the periodic network. The sucrose molecule is imaged as double lobes with two features of different intensity. In the lower right corner, a cartoon is overlaid, picturing the assembly. Two motifs are formed having either the bright features (light blue rectangle) or the lower-intensity features (violet rectangle) together in a node. c) The line profile on a sucrose molecule shows the height of the two different features.

After cooling to 40 K, we observe nearly all molecules assembled in 2D islands arranged into a periodic porous network that exhibits frayed ends. (as shown in Fig. 1; for an overview image, see SI Fig. S2). The apparent building blocks of this network are elongated double lobes, four of which come together to form a node. We assign each (double) lobe to a single molecule because the dimension of the lobes of 1.0 ± 0.2 nm in length and 0.5 nm in width fit the size expected of a sucrose molecule.

At high magnification (presented in Fig. 1b), the entity representing the molecule (i.e. each double lobe) is resolved as

two distinct, round features that differ by 0.3 \AA in apparent height. Note that apparent height in an STM measurement is a convolution of actual topographic height and electron density. We assign these features to the two different monosaccharide building blocks of sucrose. In the overlaid cartoon on the right side of the STM image in Fig. 1b, the molecule is sketched as a green oval with two circles of different color, corresponding to the different intensities observed in the STM. In the 2D network, the molecules assemble in such a way that each node is associated with exclusively one kind of feature, high or low, intermittently: either all bright features come together to form a node (light blue rectangle) or all dark features (violet rectangle). Moreover, these nodes also differ in compactness: the node associated with the dark features measures 0.9 ± 0.2 nm along the diagonal, whereas the bright feature node measures 1.1 ± 0.2 nm diagonally across the node. Thus, there are two different nodes, which are formed exclusively either by the glucose or the fructose building block. However, an unambiguous assignment of the glucose or fructose unit to the bright or dark features is not possible based on the subunit-resolved STM data alone.

In order to shed light on the molecular conformation and interactions in the observed assemblies, we modelled the structures at the atomic level. In contrast to the experiment, we considered neutral molecules without dehydrogenation in the calculations. We applied this approximation because we have no information regarding the position of the deprotonation within the molecule on the surface. Nevertheless, we assumed that the overall conformation of the sucrose molecule on the copper surface is only slightly affected by the dehydrogenation. This point is further discussed in the SI-B. We have to take into account the inherent flexibility of the molecule, which enables different adsorption configurations and intermolecular interactions (vdW- and H-bonds). High-accuracy calculations are complicated and not feasible due to the large surface area that the assembly occupies. Thus, we employed a multi-stage procedure to accommodate all these needs (c.f. SI-B), as often done in such a case.[9]

In a first step, we applied an iterative global exploration and local optimization (IGLOO) scheme, using an empirical potential, to explore the energy landscape of a single sucrose molecule on the surface aiming to find suitable candidate configurations (for details c.f. SI-B). Subsequently, we further optimized these candidate conformations using dispersion-corrected DFT. By relaxing the constraints on bond lengths and bond angles imposed for the global exploration, the optimized conformations improve the molecule-surface interactions as well as the intramolecular interactions by stronger hydrogen bonds. In a last step, we employed the single molecule conformations to obtain models of the assemblies according to constraints defined by the STM images, such as dimensions and symmetry, including chirality. It is important to note that only constrained translations of the molecule by the Cu-unit cell parameter as well as rotation of 90 , 180 and 270° are possible in order to keep the molecule on the surface in its optimized state. With these restrictions, only a very limited number of patterns can be constructed. For more details on the simulation procedure, we refer to the supplementary information (SI-B).

Figure 2 shows representative conformations of a single sucrose molecule on Cu(100) of the three lowest energy basins found by the global exploration using the IGLOO method, referred to as s-min_A^{IGLOO}, s-min_B^{IGLOO} and s-min_C^{IGLOO} (see

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Figure 2 left). The subsequent DFT optimization of the single sucrose molecules produced only minor deformations (see Figure 2 right), the most significant being a modification of the ring puckering of the fructose moiety. The planar conformation in the model considered for the global search moves to a C3'-endo conformation after DFT minimization. These rather small structural changes upon switching from empirical potential to ab initio energies lend confidence to the expectation that we have identified the most relevant molecule-surface conformations during the global searches.

These three low-energy conformations are quite different from each other with respect to the position and orientation on the surface as well as in their overall molecular conformation, as seen in the side view (right panel). In $s\text{-min}_1^{\text{DFT}}$ the fructose ring is standing upright, while the glucose ring is almost flat on the surface, leading to a global shape in which one side is higher than the other one by 2.3 Å. In $s\text{-min}_2^{\text{DFT}}$, the molecule is bent and exhibits both rings pointing to the surface. This bowl-like structure has a maximum height in its center part. Finally, $s\text{-min}_3^{\text{DFT}}$ is characterized by a tilted glucose unit, in such a way that the C4-C6 atoms point towards the surface. Consequently, the bridging oxygen atom also points towards the surface while the fructose unit is nearly standing up-right so that the ring oxygen and the C6 are pointing away from the surface, yielding a structure that exhibits a height differences of 1.4 Å between the two subunits. While the conformations of these three low-energy structures differ strongly, the IGLOO and DFT energy rankings are similar, the total energy difference with respect to $s\text{-min}_1^{\text{DFT}}$ being 12.6 kJ/mol for $s\text{-min}_2^{\text{DFT}}$ and 22.2 kJ/mol for $s\text{-min}_3^{\text{DFT}}$, which is in the order of a hydrogen bond.^[11]

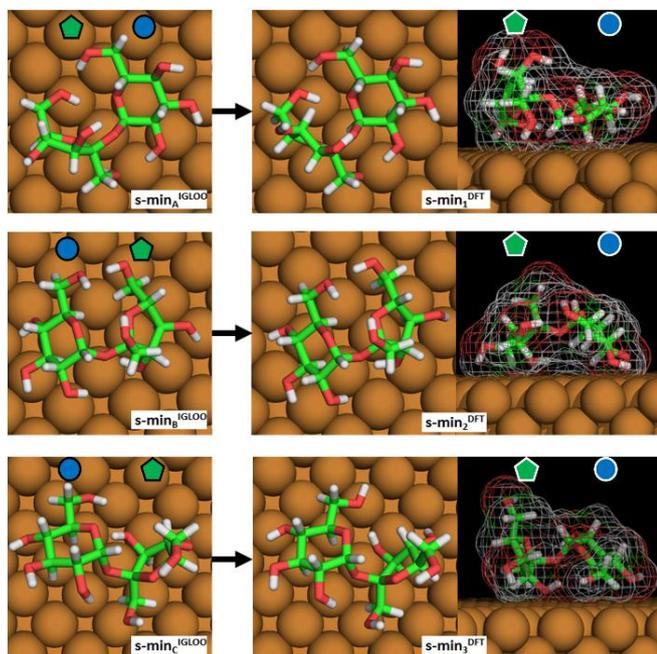


Figure 2: The three lowest-energy conformations of sucrose on Cu(100) obtained by the IGLOO algorithm (left images) and after DFT minimization (right images). The glycan notation is used to indicate the glucose and fructose monosaccharide unit.

Due to these small differences in energy, we cannot unambiguously determine which of the conformations forms the assemblies, based solely on these single-molecule models and their energy ranking. Thus, we tested all three molecular conformations as possible building blocks for the formation of the assembly pattern, as described above. The possible assembly patterns are discussed in detail in SI-C. The assembly pattern that best reflects the dimensions and symmetry of the observed 2D network is obtained from $s\text{-min}_3^{\text{DFT}}$. We note that the conformation better fitting the assembly is not the lowest-energy conformation for the isolated individual molecule on the surface. However, as intermolecular interactions might slightly change the conformation and thus the energies, it is reasonable that $s\text{-min}_3^{\text{DFT}}$ is the most favourable conformation as part of the network, i.e.: a slightly higher energy at the individual molecule level can be compensated by a stronger interaction between molecules. It is also possible that the deprotonation of the molecule during the ESI workflow and the subsequent dehydrogenation on the surface incurs small energy changes, which might reverse the energetic ranking.

The model of the periodic 2D network exhibits two different types of nodes, in which only glucose or fructose units bind, respectively. In the glucose node, the molecules are rather flat, bending downwards at the end of the molecule, and can thus interact with each other forming three hydrogen bonds to adjacent molecules. This node is formed on a hollow site of the Cu(100) surface. The distance between two glucose units diagonally across the node is 1.1 nm, while the distance across the node of the four fructose building blocks is approximately 0.9 nm. However, due to the up-right conformation of the fructose ring, no H-bonds are formed between the molecules at this site. The absence of H-bonds at the junction of fructose units is a general trend for all conformations and assemblies tested. As the O4 atom points towards the surface and the O6 atom is oriented towards the molecule itself, there are no possibilities for H-bond formation.

The simulated STM image of this model, as shown in Fig. 3, agrees very well with the experimental data, thus validating the model. The intensity distribution on the single molecule is very characteristic: the two monosaccharide building blocks are clearly distinguishable by a depression border between them, and the brightest feature is found on the fructose building block due to the upstanding hydroxymethyl chain. Therefore, we can assign the bright feature in the STM image to the fructose building block, while the low-intensity feature corresponds to the glucose unit.

In this context, we note that these 2D-periodic networks do not appear to be related (e.g. as a cut-out or cross section) to the three-dimensional arrangements of sucrose molecules in the known molecular crystal modification of sucrose.

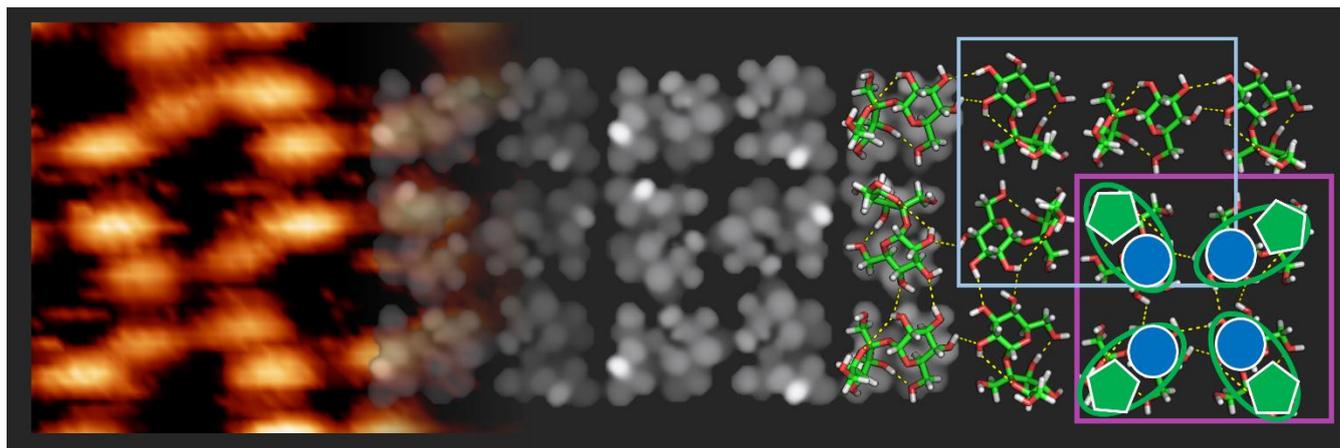


Figure 3: Model of the sucrose assembly pattern. Comparison of the measured STM image (left side) with the simulated STM image (center) demonstrating good qualitative agreement of the observed features, such as a porous structure and different intensities between the glucose (darker) and fructose (brighter) building block. On the right side, the molecular structures are presented, hydrogen bonds are indicated as yellow dotted lines.

When considering the (energetic) driving force behind the formation of this periodic structure, four main contributions can be identified: molecule-surface interactions, intramolecular forces, and intermolecular hydrogen bonds and dispersion forces. While the variation of the molecule-surface interaction and the internal energy of the molecule upon formation of the assembly need to be taken into account to judge the stability of the assemblies, the large number of intermolecular hydrogen bonds that are present in the proposed pattern suggests that they are central to the formation of the molecular assembly. Nevertheless, the molecules remain mobile at room temperature, indicating low barriers against lateral movement, likely promoted by the molecule's flexibility in conjunction with a large number of available binding sites on the metallic surface, as suggested by the observation of many similar, energetically closely spaced conformations found in the potential landscape searches. Thus, the specific type of periodic network arrangement is defined by the distribution and orientation of the hydrogen bonds, while the intermolecular dispersion (van der Waals) interactions produce an overall tendency to pack tightly.

In summary, we have imaged for the first time the disaccharide sucrose at submolecular resolution by STM, allowing the intuitive recognition of its subunits. This high-resolution imaging was enabled by electrospray ion beam deposition, which can be used as an universal method to generate intact molecular adsorbates from nonvolatile species. In spite of the large degree of flexibility of the sucrose molecule, we find a stable periodic arrangement at low temperatures. By combination of simulation and imaging, we can identify the conformation of the disaccharide on the surface. The specific knowledge about the adsorption geometry of sucrose can help to understand the influence of sucrose on e.g. hydration properties. Moreover, at this level of resolution, STM - in combination with state-of-the-art multistage simulation methods - is a very promising approach to characterize the structure of complex carbohydrates, e.g. by identifying branching points. Thus, by employing a new tool for the single molecule characterization, the present study lays the groundwork of understanding polysaccharide conformation on the submolecular level.

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Keywords: Disaccharide • scanning tunnelling microscopy • electrospray ion-beam deposition • self-assembly • multi-stage modelling

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