Unique Physics of Carbohydrate Complexes at the Interface

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Surfaces of plasma membranes are rendered with glycocalix, which are oligo- and polysaccharide chains adjacent to glycolipids, peptidoglycans, and glycoproteins. They are serving as stabilizers to retain plasma membrane structures as well as "repellers" to keep a certain distance between neighboring cells. They take stable conformation via relatively weak (generic) forces, such as electrostatic interaction, hydrogen bonding, and long-range van der Waals interaction. In the last several decades, increasing number of studies have also been conducted to investigate more specific functions of glycoconjugates.

In the first part of this article, I will describe about several physical methods to study the impact of molecular structures in their generic interactions in various length scales (phase transition, 2D/3D structures, viscoelasticity) at the interface. In the second part, I will introduce our recent challenge toward the control of bio-specific functions through controlled self-assemblies of functional carbohydrate clusters.

1) Generic models of cell surface glycocalix

As model "building blocks", we chose synthetic glycolipids, which consist of carbohydrate head groups and hydrophobic lipid anchors. Sophisticated stereo-selective synthesis enables us to study the impact of molecular structures (hydrophobic/hydrophilic balance, junction between saccharides, structural asymmetry, etc.) on cooperativity in two and three dimensions (Figure 1).

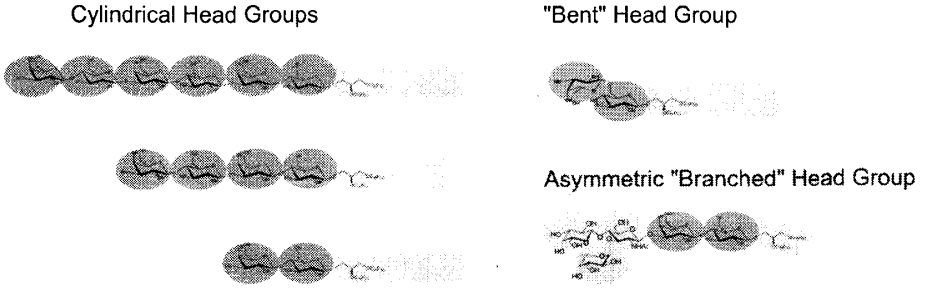

Figure 1: Glycolipids used in this study.

1a) Structural characterizations in two and three dimensions

Here we study the lateral (in-plane) cooperativity in insoluble glycolipid monolayers at air water interface under precise control of thermodynamic parameters using a Langmuir type film balance. Classical pressure-area isotherm measurements and fluorescence imaging allows us to quantify thermodynamic parameters (latent heat, phase transition entropy, critical pressure/temperature, etc.) as well as to observe phase separation and domain formations in μm scale. [1]

To understand molecular correlations in shorter length scales (several Å to 100 nm), we also applied surface diffraction techniques, such as grazing incidence x-ray diffraction (GIXD). [2] As schematically illustrated in Figure 2, a monochromatic beam from the synchrotron source strikes the glycolipid monolayer on a Langmuir trough. Besides the specular reflected light, the diffracted ones from the evanescent beam can be detected as a function of vertical scattering vector component.

$$Q_{xy} \approx \frac{2\pi}{\lambda}\sqrt{1+\cos^2\alpha_i - 2\cos\alpha_i\cos(2\Theta)} \cong \frac{4\pi}{\lambda}\sin\frac{2\Theta}{2}, \text{ and } Q_z \approx \frac{2\pi}{\lambda}\sin\alpha_f$$

By rotating the entire detector, one can collect the diffracted beam at different angle 2Θ and obtain in-plane (Qxy) and out-of-plane (Qz) components of the diffracted beam. From the iso-intensity profile vs. Qxy and Qz (contour plot), one can gain quantitative information about the ordering of lipid anchors (in air), such as lattice parameter, tilt angle/azimuth, and (signed) lattice distortion. When carbohydrate head groups are correlated via strong hydrogen bonding (i.e. squeezing out of free water), one can even obtain the weak but distinct peak from the carbohydrate-carbohydrate correlation.

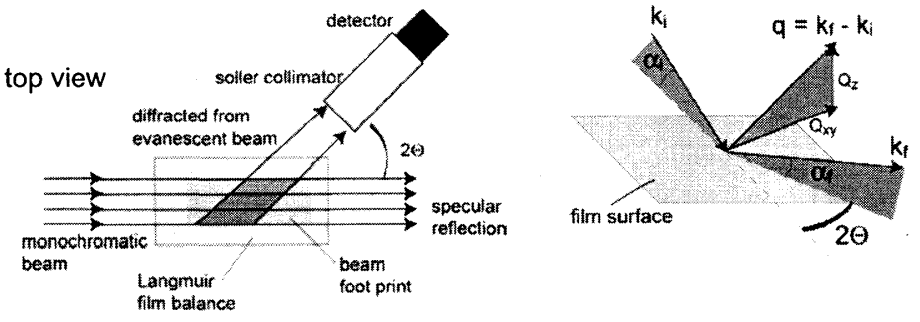

Figure 2: Top view of GIXD and scattering genmetry.

1b) Structural characterizations in two and three dimensions

To model interplays of intra-plane and inter-plane cooperativity, we also studied thermotropic phase behaviors of glycolipid lamellae using differential scanning calorimetry (DSC) and small- and wide-angle x-ray scattering (SAXS and WAXS) measurements. [3] Thermotropic

phase transition parameters (ΔH, ΔS, T_m) showed a clear dependence in hydrophobic/hydrophilic balance and head group conformations. Indeed, we even found that lipids with long, unicylindrical head groups can form "frozen" lamellae, where the lipid anchors take "frozen" (hexatic) lattice that has been enforced by dehydration of head groups.

1c) Mechanical (rheological) characterizations in two dimensions

Such structural features of carbohydrate conjugate layers can complimentarily be related to the mechanical properties by the use of an interfacial stress rheometer (ISR) coupled to a film balance (Figure 3). A thin (Ø = 0.1 – 0.2 μm, L = 50 mm) magnetic rod resides at the air/water interface, whose position is confined between two glass walls at a distinct distance W. AC magnetic field is applied to oscillate the magnetic rod, and its displacement can be recorded using linear diode arrays.

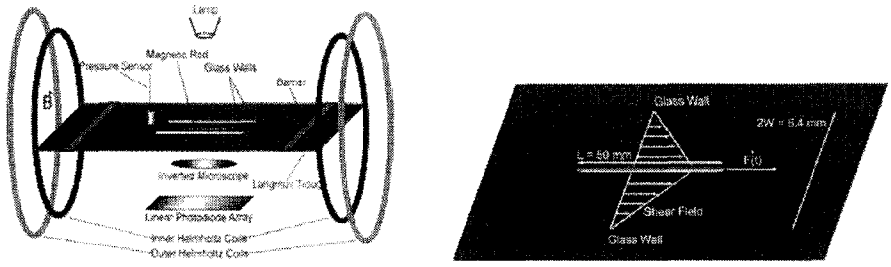

Figure 3: Schematic views of an ISR and the applied shear field.

Storage (G') and loss (G") modulus of the monolayer can be measured at various frequency and amplitude and be represented in a complex plane like force impedance spectroscopy:

$$G^*(\omega) + iG''(\omega) = \frac{\sigma_0}{\gamma_0}\exp(i\delta(\omega)) \text{ and } \frac{G''(\omega)}{G'(\omega)} = \tan\delta(\omega),$$

while the amplitudes of stress and strain can be qualified from the experimental measures:

$\sigma_0 = F_0/2L$, and $\gamma_0 = x_0/W$.

F_0 and x_0 correspond to force and displacement amplitudes, respectively.

This techniques allows for the simultaneous measurement of both G' and G" at a high sensitivity (approximately 10 times than conventional rotating disk devices) under defined thermodynamic conditions. Namely, the viscoelastic information obtained from this technique is fully complimentary to those from structural characterization. More recently, we reported interesting rheological transitions such as viscous-to elastic and isotropic-to-nematic transitions, which could be related to the molecular structures and in-plane cooperativity. [4]

2) Control of bio-specific carbohydrate functions

On cell/tissue surfaces, carbohydrates take distinct conformations (stabilized by generic interaction forces mentioned above), and can be recognized specifically with the specific counter part receptors and even with the complimentary carbohydrates. Such "lock-and-key" recognition mediated by "stronger" interactions (amounted often above the order k_BT) play key roles in cell-cell and cell-tissue interactions. For example, extravasation of leukocytes at the inflamation points are triggered by docking of blood group antigens (sialyl Leiws ^{X}) to their specific receptors (E-selectins) expressed on endotherial vessel walls. It should be noted that typical dissociation constants gained by in-vitro experiments are far too small in comparison to those from in-vivo experiments, implying the multivalency of the ligand-receptor pairs. In the late 90's, Simons and Ikonen proposed glycolipid "raft" models and postulated that they are playing key roles in cell adhesion and signal transduction

Recently, we designed artificial "rafts" of sialyl Leiws ^{X} using strong de-mixing between alkyl ($-CH_2-$) lipid anchors and fluoro-alkyl ($-CF_2-$) lipid anchors. We confirmed that size and distribution of micro-domains can systematically be controlled by mixing ratio, rather independent from the head group functions. Dynamic adhesion experiments of CHO cells over-expressing E-selectins (Figure 4a) demonstrated that the de-mixing of fluoro-alkyl ligands and alkyl matrix (and *vice versa*) can both reduce and enhance their bio-functionalities. [5] More recently, we accomplished the reconstitution of blood group antigens in "phantom cells (giant lipid vesicles)", which can be used as physical models of leukocytes (Figure 4b).

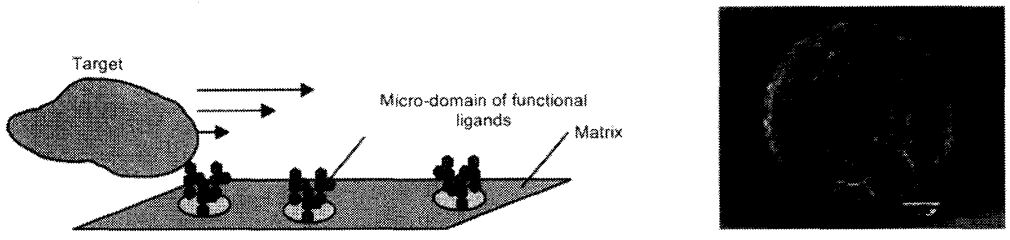

Figure 4: (a) Dynamic cell adhesion onto artificial glycolipid "rafts".
(b) "Artificial leukocyte" expressing μ-domains of blood group antigens.

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