Experimental report

14/11/2017

Proposal: 8-02-760 **Council:** 4/2016

Title: Quantifying Physical Communications between Model Membranes of Pluripotent Stem Cells via Surface Saccharides

Research area: Physics

This proposal is a new proposal

Main proposer: Akihisa YAMAMOTO

Experimental team: Salomé MIELKE
Wasim ABUILLAN
Koichiro SADAKANE
Akihisa YAMAMOTO

Local contacts: Bruno DEME

Samples: DOPC
SSEA3 glycolipids
SSEA4 glycolipids

Instrument	Requested days	Allocated days	From	To
D16	7	6	17/11/2016	23/11/2016

Abstract:

During embryonic development, several kinds of saccharides, SSEA (stage-specific embryonic antigen) marker on cellular surfaces, are expressed and used for a pluripotency marker. In spite of an increasing knowledge on unique expression pattern of saccharide antigens displayed on pluripotent stem cell surfaces, no quantitative studies have been performed for pluripotent stem cells due to complex chemistry of surface saccharides and difficulties in measuring weak binding between saccharides. To understand how pluripotent stem cells communicate with the neighboring stem cells and their surrounding environments, it is essential to understand the functions of characteristic moieties expressed on their surfaces. The aim of the proposed experiment is to quantitatively understand how the SSEA moieties on phospholipid membranes control the mechanical properties of membranes. Compression rigidity and bending rigidity, which represent the vertical interaction potential between membranes and intra-membrane potential of membranes, respectively, will be obtained from scattering intensity profiles.

Background

Saccharide chains in glycolipids, glycoproteins and proteoglycans on animal cell surfaces form complexes so called *glycocalyx*. This structure is essential to serve not only as a hydrophilic "cushion" to maintain intercellular spacing but also as a selective ligand for complementary receptors. During embryonic development, several kinds of saccharides on cellular surfaces, SSEA (stage-specific embryonic antigen) markers, are expressed and used for a pluripotency marker. Recent reports demonstrated that pluripotent stem cells alter the characteristic expression patterns of SSEA markers, suggesting their vital roles in cellular communications during the fate decision [1]. In spite of an increasing knowledge on unique expression pattern of saccharide antigens displayed on pluripotent stem cell surfaces [2], no quantitative studies have been performed for pluripotent stem cells due to complex chemistry of surface saccharides and difficulties in measuring weak binding between saccharides [3]. In order to understand how pluripotent stem cells communicate with the neighboring stem cells and their surrounding environments, it is essential to understand the functions of characteristic moieties expressed on their surfaces.

Our previous accounts [4-7] have demonstrated that off-specular scattering is a powerful technique to fully calculate two principal mechanical parameters, the compression modulus B and bending modulus κ, by calculating the theoretical scattering function $S(q_z, q_{||})$ (Fig. 1) by preparing the membrane multilayers deposited on a planar substrate as a sample:

$$S(q_z, q_{||}) \propto \frac{1}{{q_z}^2}\left[N\int \exp\left(\frac{-{q_z}^2 g_0(r)}{2}\right)\exp(-iq_{||}r)\,dr + 2\sum_{k=1}^{N}(N-k)\cos(kq_z d)\int \exp\left(\frac{-{q_z}^2 g_k(r)}{2}\right)\exp(-iq_{||}r)\,dr\right],$$

where $g_k(r)$ is membrane-membrane height correlation function [8].

Aim of the Proposed Project

The primary aim of the proposed experiment is to quantitatively understand how the SSEA moieties on phospholipid membranes control the mechanical properties of membranes. D16 perfectly meets our purpose, since the planar geometry of the sample enables us to discriminate the momentum transfer parallel and perpendicular to the membrane surface and thus the structural ordering and mechanics. We plan to respectively dope SSEA3 and SSEA4 glycolipids to matrix lipids to investigate the drastic change of mechanical properties of membrane (Fig.2). Compression rigidity and bending rigidity, which represent the vertical interaction potential between membranes and intra-membrane potential of membranes, respectively, will be obtained from scattering intensity profiles. This approach will overcome the problem to measure the weak

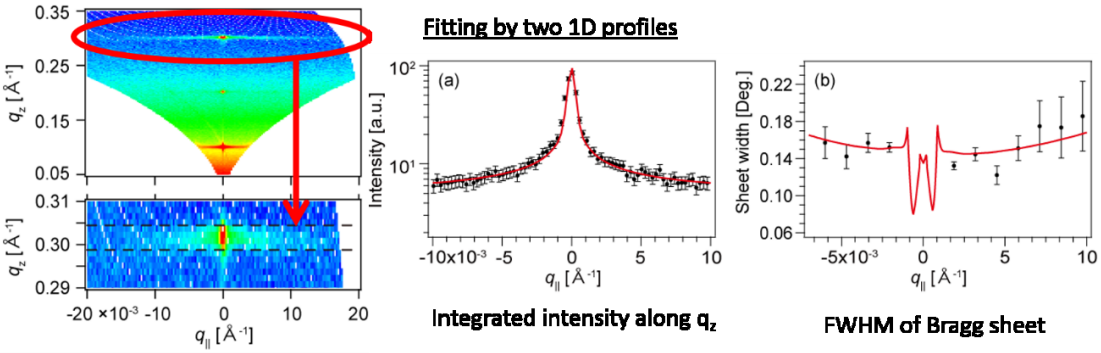

Fig.1 Scattering profile in reciprocal space map obtained from off-specular scattering on pure Gb3 glycolipid multilayer. Measured data are represented in two 1D profiles along the Bragg sheet ((a) Intensity profile and (b) width of Bragg sheet) to fit scattering function $S(q_z, q_{||})$ (solid lines). Compression modulus B and bending modulus κ are obtained from the best matching simulation between experimental data and theoretical profile.

interaction of a single pair of saccharide (in the order of k_BT [J]), and will give a new insight to answer how pluripotent cells in embryo are communicating via surface saccharides by dynamically controlling SSEA expression patterns.

Results of Experiments

The experiments were carried out with different molar ratio (SSEA3/DPPC = 0:100, 2:98, 10:90, and 25:75, SSEA4/DPPC = 0:100, 2:98, 10:90, and 25:75). To ensure that membranes are in Lα phase samples were kept at 60 °C in the humidity chamber. To control the osmotic pressure, samples were kept in the humidified air with low (~ 50 %) and high (> 95 %) relative humidity or in bulk buffer and pre-incubated for 2 hour prior to the measurement.

We obtained strong scattering signals around Bragg sheet under low and high humidity conditions, which allows us to fully characterize mechanical parameters B and κ, while bulk condition resulted in less intense scattering profiles from some conditions of mixing ratio of lipids, suggesting that multilayers are not stably kept (Fig. 3). Nevertheless, Bragg peak positions are clearly appeared from all the conditions, which allows to determine the equilibrium intermembrane distance. Interestingly, repetition length calculated from specular signals increases from low humidity to bulk condition, but 2mM Ca buffer condition makes it shorter, suggesting that sugar-sugar interaction is associated with Ca ion.

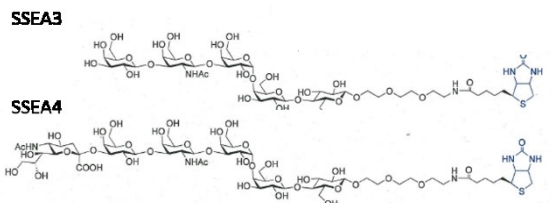

Fig. 2. Molecular structure of synthesized SSEA3 and SSEA4 saccharide chains.

(a) (b) (c) (d)

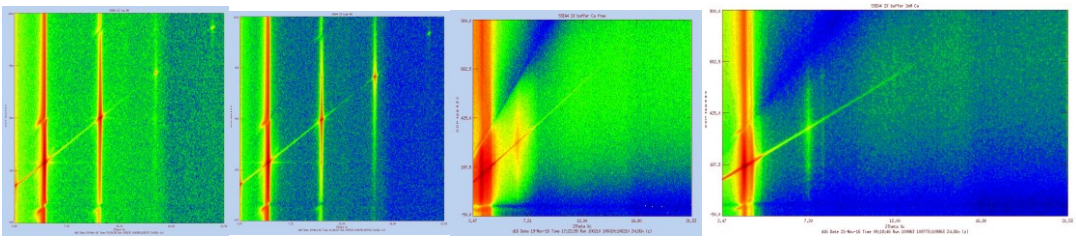

Fig.3 Scattering profile of SSEA4/DPPC = 10: 90 mol% in (a) low RH, (b) high RH, (c) Ca-free buffer, and (d) 2mM Ca buffer condition at 60 °C. Clear Bragg sheets as well as specular profile are obtained, indicating the existence of roughness in multilayer structures. Weaker intensity from buffer condition suggests that multilayers are not stably kept due to the weaker attractive force between membranes. Repetition length d varies from 51.1 Å, 65.1 Å, 81.5 Å, and 72.4 Å, respectively.

The full calculation of scattering functions for each condition and detailed analysis in order to determine bending modulus and compression modulus are in progress.

Summary and Prospects

The allocated beam time allowed us to systematically investigate how the structure of multilayer membranes is influenced by the saccharide moieties specific on embryonic stem cell surfaces and its mixing ratio to matrix lipids. Measured scattering profiles showed excellent quality which enables us to fully calculate compression modulus B and bending rigidity κ. Obtained results suggests a unique behavior of change in mechanical parameters as a function of lipid mixing ratio. Further detailed analysis is undergoing for revealing homophilic inter- and intra-membrane interaction of SSEA glycolipids.

References

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