Experimental report

03/07/2019

Proposal: 8-02-807 **Council:** 4/2017

Title: Flexible Modulation of AnisotropicMechanical Properties of Membrane Stacks Separated by Hydrogels: Off-Specular Neutron Scattering Study

Research area: Biology

This proposal is a new proposal

Main proposer: Akihisa YAMAMOTO

Experimental team: Judith THOMA
Esther KIMMLE
Lukas SCHEIDERER
Yusuke SAKAMAKI
Akihisa YAMAMOTO

Local contacts: Bruno DEME

Samples: acrylamide
dodecyl gryceryl itaconate

Instrument	Requested days	Allocated days	From	To
D16	7	7	28/06/2018	05/07/2018

Abstract:

To physically model how cell-cell contacts establish a finite distance with aid of biopolymers, we design a well defined model based on planar bilayer stacks whose periodicity can be flexibly tuned by the mixing ratio of amphiphilic DGI and hydrophilic AAm monomers. An anisotropic hydrogel can be fabricated under shear stress, showing a highly uniform lamellar stacks of DGI whose periodicity can reach even up to hundreds of nm. The goal of the proposed experiment is to quantitatively unravel how the anisotropic mechanical parameters of membranes, such as bending rigidity and vertical compression modulus, are modulated by changing the DGI/AAm ratio in the presence and absence of external osmotic pressure. To gain specular/off-specular scattering signals from such lamellar systems possessing the periodicity of about 10 nm, we utilize D16 membrane diffractometer equipped with a high-precision humidity control system.

Background

Biological cells are filled with 50 - 85 wt% water, but their mode of deformation is clearly different from "balls" made out of synthetic hydrogels. For example, cell membranes share common features with smectic liquid crystals, whose deformability is known to be highly anisotropic. In nature, the contact between neighboring cells is mediated via hydrated layers of biopolymers, such as glycocalyx and extracellular matrix [1]. To date, planar stacks of synthetic and native glycolipid membranes have been utilized as the model of saccharide-mediated, cell-cell contacts. Via full calculation of two-dimensional scattering functions, the influence of molecular structures [2,3], genetic mutations [4], and environmental factors [5] on the structural correlation and anisotropic mechanics of cell membrane models have been quantified using off-specular neutron scattering at D16. Here, we focus on the anisotropic lamellar structures developed in material science, which can tune its intermembrane distance as a function of degree of hydration (Fig. 1) [6, 7]. They exhibit highly anisotropic physical properties, e.g., swelling ratio and Young's modulus parallel and perpendicular to the membrane planes are largely different. Since the anisotropic physical properties can be flexibly adjusted (Fig. 1b), such systems offer a unique advantage to physically model cell-cell interfaces. However, there has been no systematic study shedding light on the influence of swelling ratio and density of polymer interlayers on the membrane mechanics of such complex structures.

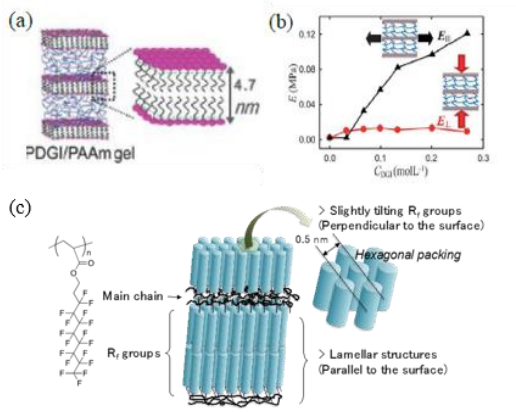

Fig. 1. (a) Schematic illustration of the lamellar hydrogels. (b) Young's modulus of the lamellar gels is highly elastic and anisotropic. (c) Schematic illustration of lamellar structure of perfluoroalkyl polymers.

Our previous accounts [2-5] 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. 2) 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 this study is to quantitatively characterize the anisotropic physical property of (A) lammelar hydrogel and (B) perfluoroalkyl polymer stack. The lamellar hydrogels based on DGI (dodecyl glycerol itaconate) and AAm (acrylamide) was fabricated by the group of Profs. Gong and Nakajima (Hokkaido University, Japan) under shear stress [9], where DGI was deuterated in order to

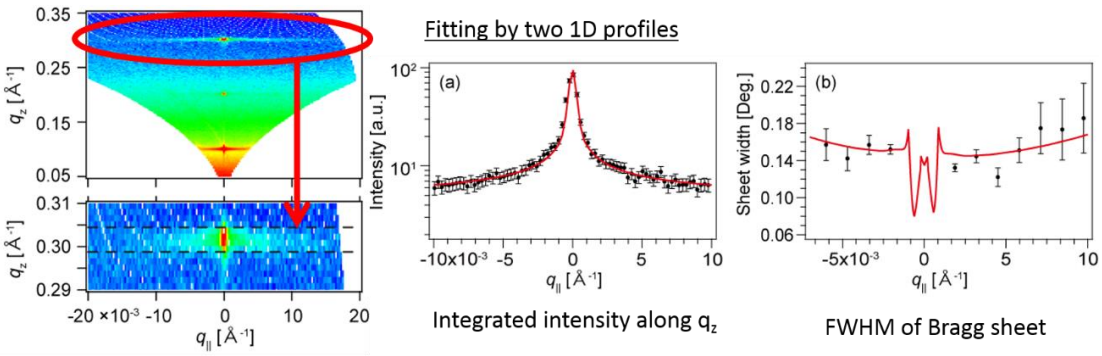

Fig. 2. Scattering profile in reciprocal space map obtained from off-specular scattering on pure Gb3 glycolipid multilayer. Measured data are represented in two 1D profiles along the Bragg sheet ((a) Intensity profile and (b) width of Bragg sheet) to fit scattering function $S(q_z, q_{\|})$ (solid lines). Compression modulus B and bending modulus κ are obtained from the best matching simulation between experimental data and theoretical profile.

enhance the contrast. Poly(fluoroalkyl acrylate) with different kinds of side chains such as 2-(perfluorooctyl)ethyl acrylate (PFA-C8) and 2-[(perfluorooctylethyl)carbamate]ethyl acrylate (PFAUr-C8) were synthesized by the group of Profs. Takahara and Higaki (Kyushu University, Japan).

Results of Experiments

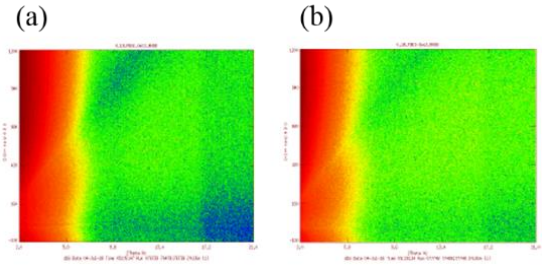

Fig 3. Scattering signal of PDGI-AAm gels ([d-DGI] = 0.1 M) at high relative humidity (RH > 95 %) presented in an angular coordinate. Glycerol concentration was varied from (a) 0 wt% to (b) 3 wt% to control the volume of AAm gel.

(A) lamellar hydrogel: The experiments were carried out with different feeding concentration of d-DGI ([d-DGI] = 0.1 M and 0.14 M), and different mixing concentration of glycerol (0, 0.5, 0.8, 1.2, 1.5, 2.0, and 3.0 wt%). To ensure that membranes are in $L\alpha$ phase samples were kept at 25 °C in the humidity chamber. To control the osmotic pressure, samples were kept in the humidified air with low (~ 30 %) and high (> 95 %) relative humidity or in bulk buffer and pre-incubated for 2 hour prior to the measurement. As show in Fig. 3, scattering signals did not show very clear specular signals. The reproducibility of results was confirmed from the measurements of same sample conditions with two days of incubation time to ensure the sample quality. This result indicates that the lamellar structure of DGI membranes are not well ordered under all the measured conditions. This could be attributed to the high concentration of DGI, which is more than 10-folds compared to previous study [8]. Formation protocol such as the strength of shear stress has to be further optimized.

(B) Fluorinated polymer: The experiments were carried out with different side chains of polymers, and different osmotic pressure (RH = 30, 60, 80, 95% and bulk water). Temperature of sample chamber was also varied below and above the temperature for the isotropic transition T_i. We obtained strong scattering signals around Bragg sheet under all the conditions below T_i (Fig. 4), which allows us not only to determine the equilibrium intermembrane distance but also to fully characterize mechanical

parameters B and κ. Intermembrane distance showed clear tendency of the increase as increasing relative humidity, indicating the hydration of lamellar stack. It was also found that PGA-C8 membranes show very strong lateral correlation compared to PFAUr-C8, which appears in the intensity decay of Bragg sheets in the horizontal direction. Furthermore, similar scattering patterns were obtained from PFA-C8 even above T_i, suggesting that the lamellar structure was kept stable above the phase transition temperature. The full calculation of scattering functions for each condition and detailed analysis in order to determine bending modulus and compression modulus are in progress.

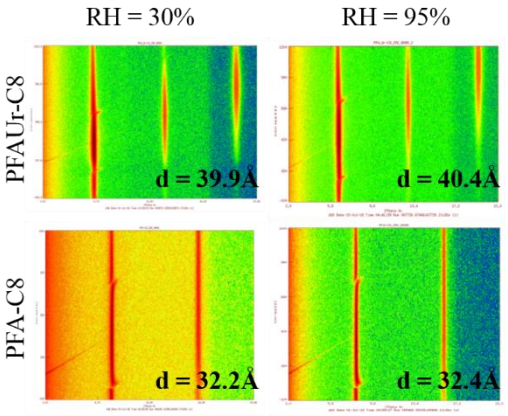

Fig. 4. Scattering signal of fluorinated polymers (PFAUr-C8 and PFA-C8) at low and high relative humidity (RH = 30% and 95%, respectively) in 25 °C.

Summary and Prospects

The allocated beam time allowed us to systematically investigate how the structure of anisotropic lamellar material is influenced by the chemical structure and hydration degree. Measured scattering profiles showed excellent quality which enables us to fully calculate compression modulus B and bending rigidity κ. Further detailed analysis is undergoing for revealing the interaction between lamellar structures and water molecules, and resulting change in mechanical properties of lamellar structures.

References

[1] M Tanaka, *Curr. Opin. Colloid Interf. Sci.* **18**, 432 (2013).
[2] E. Schneck, F. Rehfeldt, R. G. Oliveira, C. Gege, B. Deme, and M. Tanaka, *Phys. Rev. E* **78**, 061924 (2008).
[3] A. Yamamoto, W. Abuillan, A. S. Burk, A. Körner, A. Ries, D. B. Werz, B. Demé, and M. Tanaka, *J. Chem. Phys.* **142**, 154907 (2015).
[4] E. Schneck, R. G. Oliveira, F. Rehfeldt, B. Deme, K. Brandenburg, U. Seydel, and M. Tanaka, *Phys. Rev. E* **80**, 041929 (2009).
[5] E. Schneck, B. Deme, C. Gege, and M. Tanaka, *Biophys. J.* **100,** 2151 (2011).
[6] K. Tsujii, M. Hayakawa, T. Onda, and T. Tanaka, *Macromolecules* **30**, 7397 (1997).
[7] Y. Liu, Y. Higaki, M. Mukai, N. Ohta, T. Kabe, and A. Takahara, *Polymer Journal* **51**, 189 (2019).
[8] S. K. Sinha, *Phys. III* **4**, 1543 (1994)
[9] M. A. Haque, T. Kurokawa, G. Kamita, Y. Yue, and J. P. Gong, *Chem. Mater.* (2011).