Supporting Information for:

Sphingomyelin/Phosphatidylcholine and Cholesterol Interactions Studied by Imaging Mass Spectrometry

Leiliang Zheng, Carolyn M. McQuaw[†], Andrew G. Ewing, Nicholas Winograd[*]

Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802.

*Corresponding Author's Email: nxw@psu.edu

†Current Address: Vollum Institute and Department of Molecular Microbiology and Immunology, Oregon Health and Science University, Portland, Oregon 97220.

Sample Preparation

Substrate preparation and Langmuir-Blodgett film formation are described in detail by McQuaw et al.[1] The substrates are self-assembled monolayers (SAM) of 16-mercaptohexadecanoic acid on gold. The gold was deposited onto single crystal (100) silicon wafers that were first cleaned via piranha etch (3:1 H_2SO_4:H_2O_2) in order to ensure a uniform SiO_2 surface. (Extreme caution must be exercised when using piranha etch. An explosion-proof hood should be used.) The resulting substrate is hydrophilic because of the acid termination. The LB monolayer of lipid has its hydrophilic headgroup facing the substrate and the hydrophobic tail group facing the air. Thus the final film is hydrophobic with no water molecules involved. Gold deposition, SAM self-assembly, and LB film deposition were confirmed with a single wavelength (632.8 nm, 1 mm spot size, 70° incidence angle) Stokes ellipsometer (Gaertner Scientific Corporation, Skokie, IL; Model LSE). All the films are transferred at low surface pressure. Films drawn at high surface pressure were not studied since the domain size is expected to be too small to be observed by SIMS imaging.

Instrumentation and Data Analysis

All the mass spectra and images were taken by a home-built time-of-flight secondary ion mass spectrometry (TOF-SIMS) equipped with a 15 KeV Ga^+ liquid metal ion gun. The detail of the TOF-SIMS instrument is described by Braun et al.[2] Spectra were acquired without the need for charge compensation. All images are acquired with either 20 or 40 shots/pixel, both of which have an ion dose less than 10^{12} ions/cm^2. Normally a smaller value of shots/pixel is preferred. A larger value is sometimes used in order to achieve sufficient signal-to-noise ratio for mapping. The primary ion beam was rastered across the sample surface and mass spectra were acquired at each pixel for total ion images (256 x 256 pixels). Molecular-specific images were obtained by mapping the ions of interest (256 x256 pixels) or by converting to 128 x 128 pixel images. The conversion is performed by summing the adjacent 4 pixels and using the one larger pixel to represent the original 4 smaller pixels. Thus the field-of-view is the same after conversion but the size of one pixel is 4 times larger.

Quantitative Issues

The signal intensity in SIMS imaging is determined by many experimental parameters, including the primary ion flux, the detector efficiency on any particular day, and by the ionization efficiency of the selected mass ion. In our case, for example, the relative sensitivity factor (RSF) for CH is more than 50 times larger than for SM and 6.6 times larger than for PC as noted in reference 1. These factors are largely responsible for the intensity differences noted between the images on the left column and the ones on the right, shown in Figure 2 of the manuscript. The intensity variations between the domains in a specific image are, however, directly related to the concentration of the selected mass ion, at least to within a factor of 2. This factor arises since the RSF of these specific ions can change by up to a factor of two, depending upon their chemical environment. As noted in reference 1, for example, the RSF of CH decreases when it co-localizes with PC or SM, while the RSF for PC and SM increases when co-localized with CH. (*Note there is an error in Table 2 of reference 1. The entry for the 2:1 SM/CH under the SM heading should read 0.023 ± 0.001, rather than 0.23 ±0.001*.) Since PC and SM respond similarly to CH, the intensity in those domains should directly reflect the relative concentrations. With this in mind, it is clear that the intensity changes observed in Figure 2 are sufficient to support the conclusion that the hydrocarbon saturation level of SM acyl chains is the important driving force for SM co-localization with CH.

A measure of the degree of intensity variation is given by the line scans shown at the bottom of Figure 2. These line scans are constructed by measuring the intensity from a 5x5 pixel square as it moves along the yellow arrow superimposed on the images. The start point and the end point of the yellow arrow correspond to the start point and the end point of the x-axis of the line scan graph, although the thickness of the yellow arrow has no significance. Note also, that a value of zero in the line scan plot does not necessarily mean that no molecules are present in this area – only that the number of molecules is below the detection limit of the SIMS technique.

References

1. McQuaw, C. M.; Zheng, L.; Ewing, A. G.; and Winograd, N. *Langmuir* **2007,** *23*, 5645-5650.
2. Braun, R. M.; Blenkinsopp, P.; Mullock, S. J.; Corlett, C.; Willey, K. F.;Vickerman, J. C.; Winograd, N. *Rapid Commun. Mass Spectrom.* **1998,** *12*, 1246-1252.

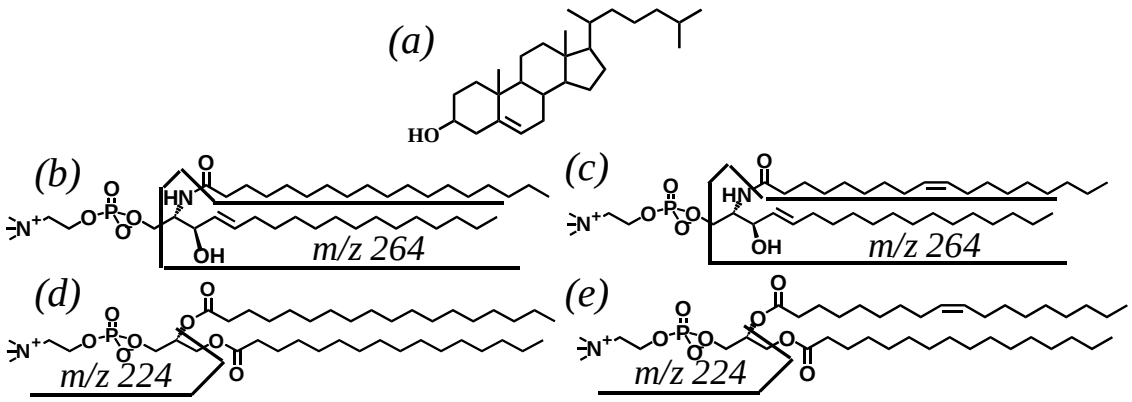

Figure S1. Molecular structures of (a) CH, (b) SSM, (c) OSM, (d) PSPC, and (e) POPC.

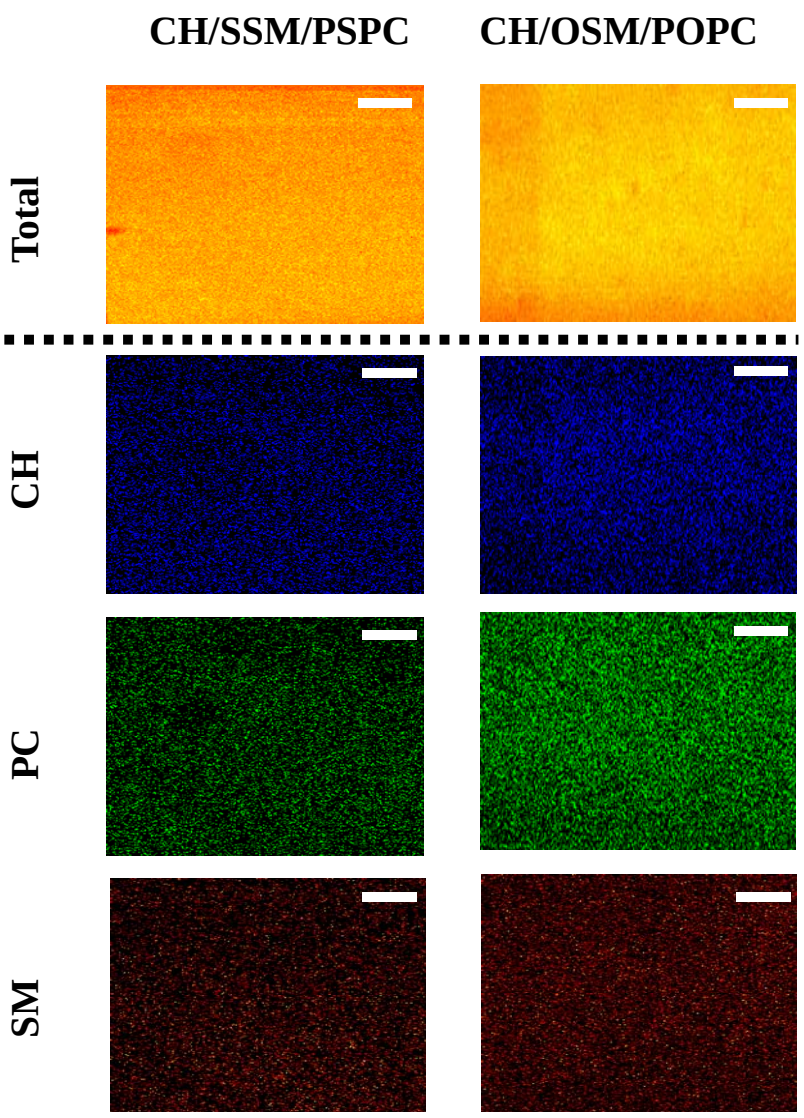

Figure S2. TOF-SIMS images of CH/SSM/PSPC and CH/OSM/POPC LB films. The scale bar represents 100 μm. All the images are 256 x 256 pixels, with 20 shots/pixel. CH (blue), PC (green), and SM (red) are represented by m/z 369, m/z 224, and m/z 264 respectively.

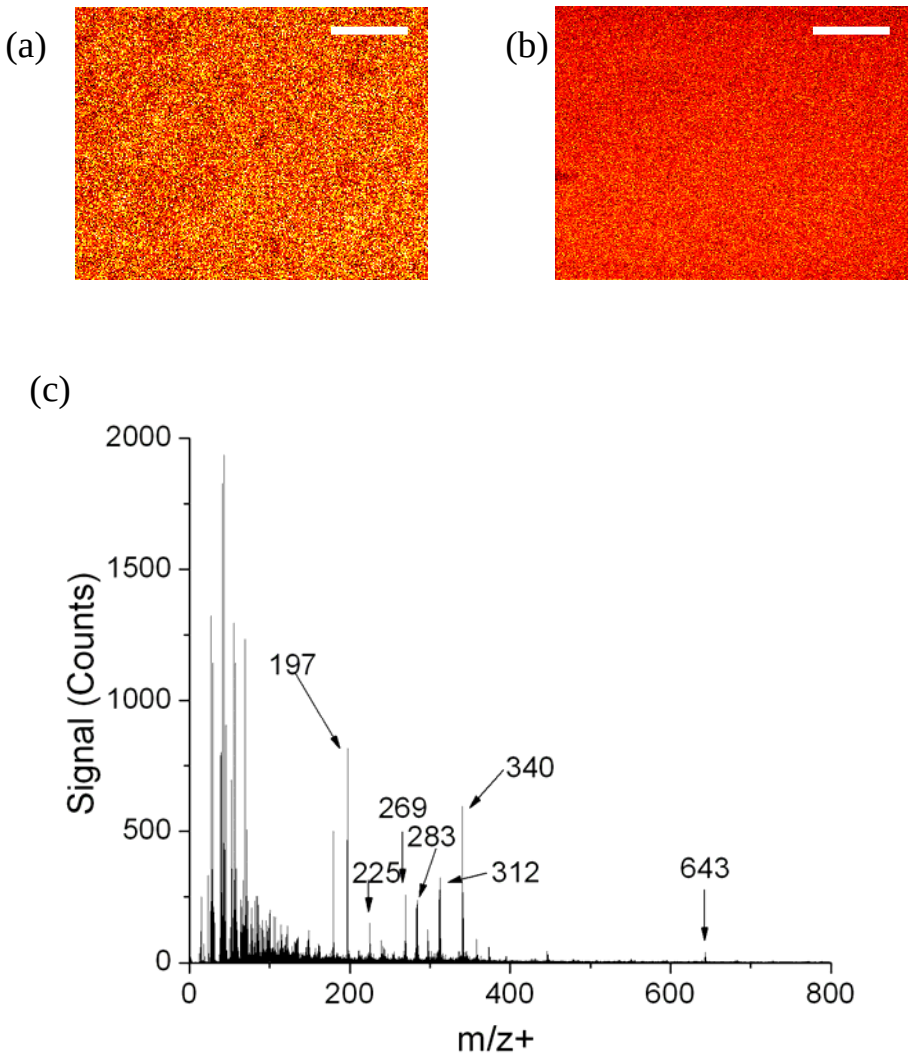

Figure S3. TOF-SIMS spectrum and images of the substrate: self-assembled monolayer (SAM) of 16-mercaptohexadecanoic acid on Au. The scale bar represents 100 µm. The images are 256 x 256 pixels with 20 shots/pixel. (a) SIMS image of Au (m/z 197), (b) SIMS image of SAM (m/z 340), (c) Mass spectrum of the substrate.

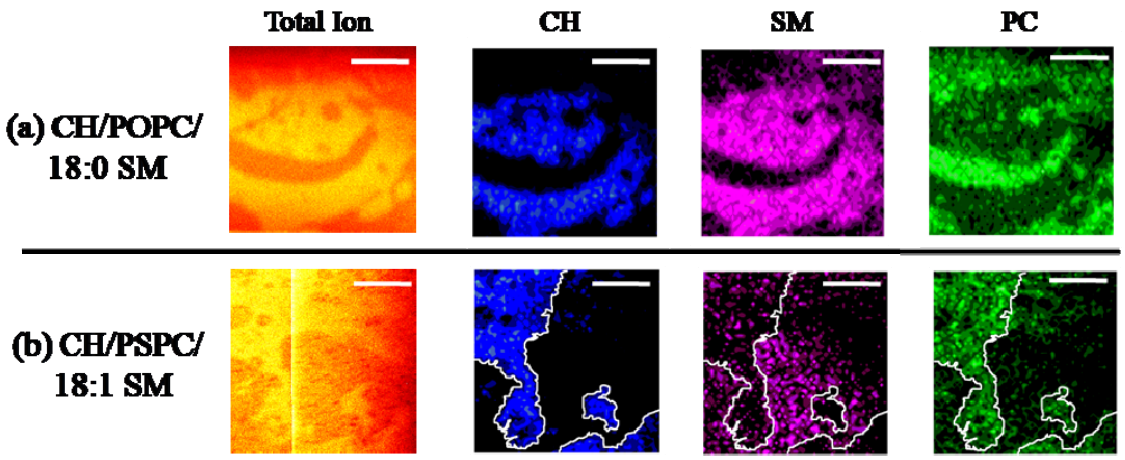

Figure S4. Another set of TOF-SIMS images of the 2 supported lipid 3-component LB films. The lipid content of these 2 films are the same as those shown in Figure 2. The field of view for both images is 300 x 300 μm^2. The scale bar represent 100 µm. The total ion images are 256 x 256 pixels and the molecular specific images are 128 x 128 pixels, with 40 shots/pixel. CH (blue), PC (green), and SM (pink) are represented by m/z 369, m/z 224, and m/z264 respectively.