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UCRL-CONF-212106

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May 9, 2005

230th ACS National Meeting
Washington, DC, United States
August 28, 2005 through September 1, 2005

QUANTITATIVE COMPOSITION ANALYSIS OF LIPID MEMBRANES BY HIGH-RESOLUTION SECONDARY ION MASS SPECTROMETRY

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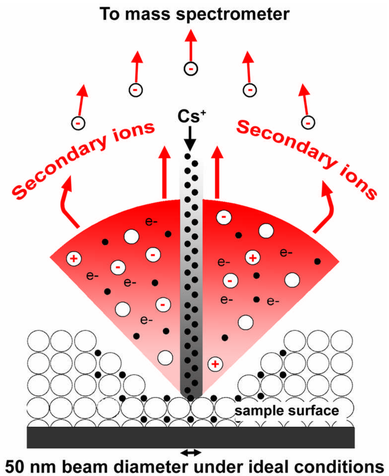
Overview

The lateral organization and interactions of lipid and protein components within biological membranes are essential for their functions. Investigations of the lateral organization within membranes hinge upon the ability to differentiate one component of interest from another. Typically, fluorophores are conjugated to specific components, and the organization is probed with fluorescence microscopy. However, bulky fluorophores may change the physical properties of the components they label, only the labeled components can be visualized, and the diffraction limit of light restricts the lateral resolution.

Here we present a method to image microdomains within supported lipid membranes using isotopic labels and high-resolution secondary ion mass spectrometry (SIMS) performed with the NanoSIMS 50 (Cameca). Lateral resolution of 100 nm is achieved with high sensitivity. Quantitative information on the lipid composition within each domain was determined using calibration curves constructed from homogeneous lipid bilayer samples that systematically varied in the isotopically labeled lipid content.

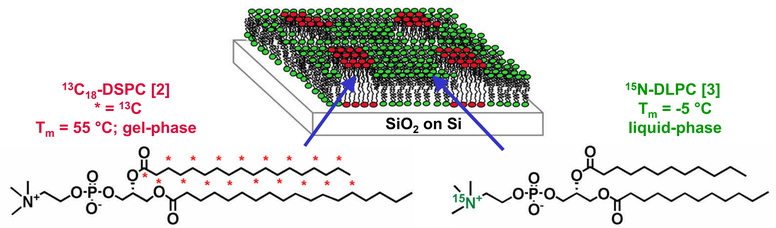
The NanoSIMS 50 Secondary Ion Mass Spectrometer



- Tightly focused Cs^+ beam is scanned across the sample, fragmenting membrane components.
- Ejected small negative atomic ions (i.e., $^{12}C^-$, $^{13}C^-$, $^{31}P^-$) and molecular ions (i.e., $^{12}C^1H^-$, $^{13}C^1H^-$, $^{12}C^{15}N^-$) are collected.
- Up to 5 different ions can be simultaneously detected.
- Location-specific signal intensity is used to create an image.

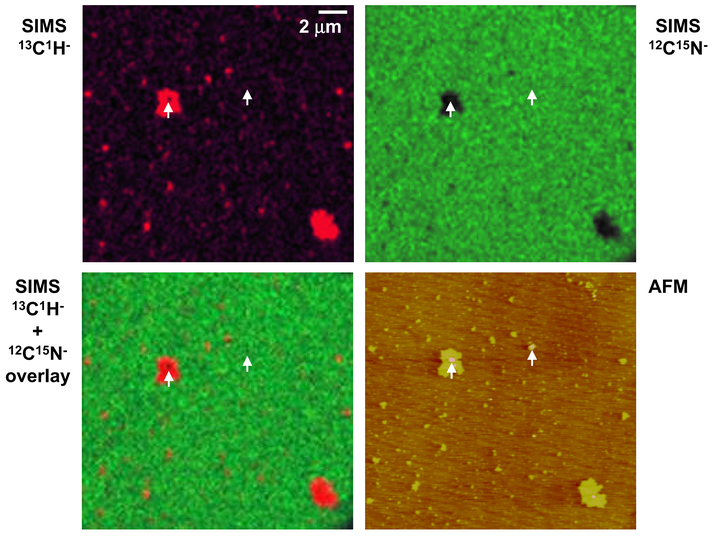
By incorporating stable isotopes into specific membrane components, the secondary ions that are exclusively produced by each component can be used for identification [1]

Gel-Phase Domains within Supported Lipid Bilayers



- Deposit vesicles ($^{13}C_{18}$-DSPC / ^{15}N-DLPC) onto oxidized silicon substrates patterned with chrome grids at a temperature > T_m
- Gel-phase domains form as the sample is slowly cooled to 22 °C
- Freeze-dry sample to preserve organization in the UHV environment of the NanoSIMS

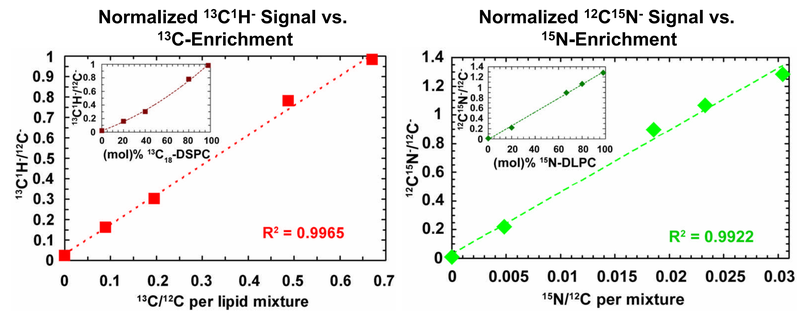
NanoSIMS and AFM Images of Lipid Bilayers



Domains with diameters as small as ~100 nm were detected by the NanoSIMS

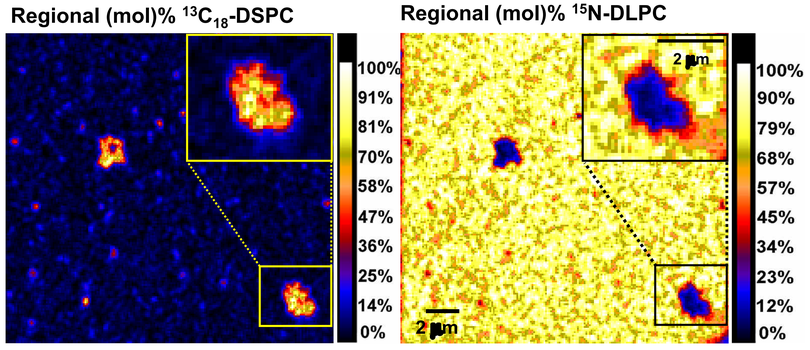
Arrows indicate features in the AFM image that are not domains, and their corresponding locations in the SIMS images
0.5 ms total dwell time, 100 nm spot size, 25 x 25 µm raster, 256 x 256 pixels, entire area rastered is not shown

Quantitative Composition Analysis of Microdomains



Error bars in SIMS data (two standard deviations) are smaller than the data points

- Calibration curves were created using NanoSIMS measurements made on sets of homogeneous bilayers that systematically varied in ^{15}N-DLPC or $^{13}C_{18}$-DSPC (mol)%
- Normalized signal intensities ($^{13}C^1H^-/^{12}C^-$ and $^{12}C^{15}N^-/^{12}C^-$) are linearly related to the isotopic enrichment ($^{13}C/^{12}C$ and $^{15}N/^{12}C$, respectively) within each lipid mixture
- The composition within the domain samples was determined using these calibrations



Summary

Microdomains within isotopically labeled supported lipid bilayers were imaged with the NanoSIMS 50. The lipid composition within the domains was determined using calibration curves constructed from sets of homogeneous bilayers that varied in lipid content. This approach might be adapted to investigate lateral organization within biological membranes.

UCRL-CONF-212106

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Publications / Abbreviations

[1] Marxer, C. G.; Kraft, M. L.; Weber, P. K.; Hutcheon, I. D.; Boxer S. G. *Biophys. J.* 2005, *88*, 2965-2975

[2] 1-stearoyl-2-stearoyl-$^{13}C_{18}$-*sn*-glycero-3-phosphocholine

[3] ^{15}N-dilauryl-*sn*-glycero-3-phosphocholine

Acknowledgments

M. L. Kraft is supported by an NIH NSRA fellowship

NSF Interdisciplinary Research Teams program (to M.L.L.)

Stanford Nanofabrication Facility (NSF ECS-9731293

NSF Biophysics program and NIH GM06930 (to S.G.B.)

NSF MRSEC CPIMA

Portions of this work were performed under the auspice of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract W-7405-Eng-48.