(α-FeO) and maghemite (γ-FeO). These results are in accord with MER Mössbauer results that show nanophase oxides in bright soils at both landing sites. The widespread nature of these materials and the lack of evidence for hydration suggest that they formed primarily under dry, oxidizing conditions that have prevailed for much of martian history.

The results from OMEGA and MER suggest that surface water may have led to production of phyllosilicates early in martian history, and perhaps somewhat later to deposition of hydrated sulfates. Because phyllosilicates and sulfates are found in different parts of the planet, the extent to which their formation might have overlapped in time is an important subject for future work. The acidity that promoted sulfate precipitation likely resulted from sulfur outgassing during volcanic activity. Both the roughly neutral pH suggested by phyllosilicates and the lower pH suggested by sulfates could have produced habitable surface environments; the former may have been more suitable for the origin of life. A future mission to phyllosilicate-rich terrains, followed by sample return from whichever terrain type shows the best overall potential for preservation of biosignatures, could be a good strategy for future Mars exploration. The combined results of the OMEGA and MER investigations illustrate how important international collaboration and associated synergistic analyses could be in such a venture.

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CHEMISTRY

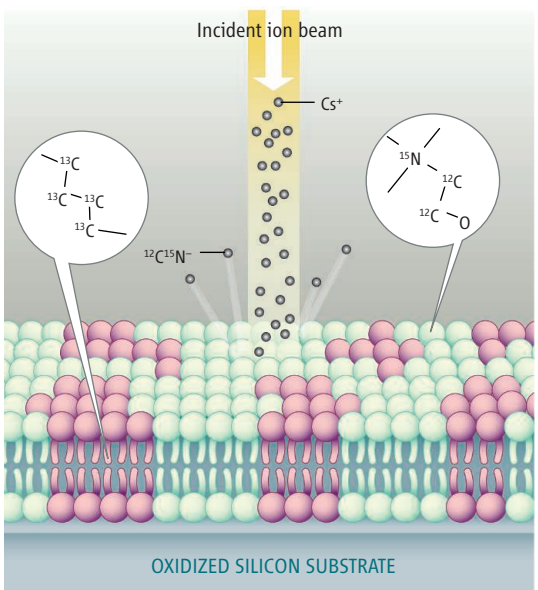
Unveiling the Membrane Domains

A new form of mass spectrometry can determine a membrane's chemical composition with a resolution of less than 100 nanometers.

Jay T. Groves

Cell membranes consist of a richly heterogeneous fluid mosaic of lipids and proteins. Molecular complexes, from tens to hundreds of nanometers in size, dynamically assemble and dissolve while performing the biochemical functions of life. But the spatial organization of the cell membrane and its role in the regulation of biochemical processes remain little understood, because current imaging technologies cannot resolve the most important features. On page 1948 of this issue, Kraft *et al.* take the next step in biomembrane imaging by using a form of secondary ion mass spectrometry (NanoSIMS) to map the chemical composition of a lipid membrane with 70 to 100 nm resolution (*1*).

Present knowledge of the organization of living cells comes mostly from fluorescence and electron microscopy. Over the past 20 years, fluorescence microscopy has flourished, due in part to the introduction of an extensive array of fluorescent probe molecules. By synthetically or genetically—such as with green fluorescent protein—coupling a fluorescent probe to the protein of interest, the ambiguity of what is being observed is broken. The ability to track a specific protein in living cells has revealed a tremendous wealth of information about the inner workings of biological systems. However, the spatial resolution of optical imaging techniques is generally restricted to a few hundred nanometers. Electron microscopy can be used to view cellular structures down to molecular length scales, allowing the bilayer structure of lipid membranes to be directly imaged. However, the lateral organization within the membrane has been more difficult to resolve. The need for imaging biomembrane organization at length scales of 10 to 300 nm thus remains largely unmet.



A detailed look at membrane composition. In the NanoSIMS experiments reported by Kraft *et al.*, a focused beam of ions bombards the sample, releasing a barrage of small ions that are analyzed with mass spectrometry. By scanning over the bilayer membrane sample, a high-resolution (about 70 to 100 nm) image of its chemical composition is generated.

Imaging mass spectrometry has the potential to step into this resolution gap. In recent years, this technique, which offers unparalleled chemical specificity, has been increasingly used to study biological systems (*2*, *3*). There are several ways in which mass spectrometry may be performed in a spatially resolved manner. In matrix-assisted laser desorption ionization (MALDI), a focused laser spot is scanned over a sample that has been prepared in a chemical matrix. The method produces relatively large molecular ions and enables direct identification of peptides and proteins without the need for specific labeling. Imaging MALDI has been successfully applied to biological tissue samples and has been used as a bioanalytical tool in array-based protein assays (*4*–*6*). However, the spatial resolution is typically limited by the laser spot size (about 1 μm).

Secondary ion mass spectrometry (SIMS) provides an alternative strategy. In this method, the sample is bombarded with an incident ion or molecular beam. The beam locally vaporizes the sample into secondary molecular and atomic ions. In time-of-flight SIMS, the incident ion beam is pulsed, and the secondary ion mass-to-charge ratio (m/z), and hence its identity, is determined by the time it takes these sec-

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ondary ions to reach the ion detector. Imaging time-of-flight SIMS can be used to map the chemical composition of cell membranes. For example, using an ion beam with a diameter of 200 nm, Ostrowski *et al*. were able to resolve the heterogeneous distribution of lipids at highly curved intercellular fusion pores with a spatial resolution of about 250 nm (*7*). An acyl chain fragment ($C_5H_9^+$), which is a secondary ion produced from most membrane lipids, served as a general membrane marker. The specific identity of the lipids was determined from molecular fragments of their chemically distinct phosphate head groups.

In the NanoSIMS imaging experiments reported by Kraft *et al.*, a tightly focused beam of Cs^+ ions scans the sample. The resulting secondary ions, which are primarily mono- and diatomic, are identified by a conventional high-resolution mass spectrometer (see the figure). Direct determination of chemical composition from these data is essentially impossible, and isotopic labeling must be used. Kraft *et al.* examined a phase-separating binary mixture of lipids. In this mixture, one lipid is labeled with ^{13}C, yielding $^{13}CH^-$ secondary ions, and the other lipid is labeled with ^{15}N, yielding $^{12}C^{15}N^-$ secondary ions (*1*). Simultaneous monitoring of signals due to both secondary ions, which provide unique signatures from each of the two lipid types, enables quantitative mapping of the membrane chemical composition with a spatial resolution of about 70 to 100 nm. This observation sits squarely in the current blind spot with respect to biomembrane structure imaging.

Kraft *et al.* have corroborated the NanoSIMS images of membrane phase separation against atomic force microscopy (AFM) images of the same samples. AFM has previously been used to image lateral structures in lipid membranes deposited onto flat substrates. For simple model membrane systems, AFM can reveal nanometer-scale domain patterns with striking clarity (*8*). Kraft *et al.* found precise agreement between the AFM and NanoSIMS images of membrane domain structures. However, the NanoSIMS image also provided chemical specificity, which is lacking in AFM and most other forms of microscopy.

In biomembrane imaging, knowledge of chemical composition is critically important. The existence of heterogeneous structures in membranes is not in doubt; it is their chemical composition that is hotly debated. Do protein interactions nucleate membrane domains rich in saturated lipids and cholesterol (rafts)? Or does lipid phase separation sort the proteins into raft domains? Perhaps both mechanisms occur; if so, then how many types of domains are there? These questions have proven very difficult to address with current imaging technologies. Even in simple binary mixtures, the two phases rarely consist of pure components; the entropic cost is too large. Precise determination of phase composition—for example, with NanoSIMS—can reveal the strength of the driving force for phase separation and is a key ingredient in developing a physical picture of how membrane organization is governed.

NanoSIMS may also be used to image membrane-associated proteins in model systems or in whole cell membranes. Antibodies or small-molecule binders labeled isotopically or atomically (for example, with fluorine) could be used to mark the proteins of interest and map their positions. The repertoire of distinguishable secondary ions allows for a full spectrum of "colors" to identify numerous different molecules. Although imaging mass spectrometry is still in its infancy, it is emerging as a powerful technique that uniquely accesses a strategic gap in our knowledge of cell membrane structure.

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IMMUNOLOGY

Restless T Cells Sniff and Go

How a key immunological regulator prevents autoimmune disease has been unclear. Live imaging shows that it may prevent immune cells from lingering too long in the lymph nodes.

Tomas Mustelin

A long-standing puzzle in immunology has found a dynamic new explanation—quite literally. On page 1972 of this issue (*1*), Schneider and colleagues uncover a new paradigm for how a protein expressed on the surface of T lymphocytes suppresses excessive immune responses and prevents autoimmune disease. It turns out that T cell motility is the key.

T cells normally transit rapidly through the lymph node (at velocities of up to 25 μm/min), while continuously adhering to other cells, scanning their surfaces for antigens, those molecules that stimulate an immune response (*2*, *3*). Most immunologists had not expected T cells to move as fast or act as deliberately in their search for antigen.

Foreign antigens, such as those expressed by microorganisms or transplanted organs, are ingested and digested by dendritic cells, the professional antigen-presenting cells of the immune system. These antigen-loaded cells travel to their regional lymph node, where they present short peptides derived from antigens on their surface in the context of major histocompatibility (MHC) molecules. Dendritic cells also present ligands, accessory molecules, and adhesion molecules that are surveyed by T cells. When recognized, this suite of molecules can cause a transiting T cell to change its locomotive behavior (*2*, *3*). It slows down, moves around more carefully, and eventually forms a conjugate with the cell that presents an antigen recognized by the T cell receptor (see the figure). This contact, which can last for several hours, develops a highly organized molecular architecture called the "immunological synapse" (*4*) that acts as a hub for intracellular signaling cascades. These signals are delicately balanced between a forward urge to initiate T cell activation leading to a full immune response and negative influences to abort the mission. Numerous cell surface glycoproteins and signaling molecules recruited by the immunological synapse serve these opposing functions (*5*).

One of the best-studied negative regulators of T cell activation is cytotoxic T lymphocyte antigen–4 (CTLA-4) (*6*), a cell surface receptor for the B7 (CD80/CD86) molecule on dendritic and other antigen-presenting cells. CTLA-4 is a 41- to 43-kD dimeric transmembrane glycoprotein, and a

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