Vibrational Spectroscopic Characterization of Model Biomembranes

NIST is developing infrared spectroscopy methods to analyze dynamic features of lipid bilayers that make up the membranes of living cells and their organelles. This probe-free, label-free method avoids the formation of artifacts in membranes, observed with other methods. Most drugs are targeted at protein receptors that span lipid bilayer membranes on the surface of cells. The measurement and detection of various lipid structures in membranes is therefore critical to the understanding of how drugs work, and is expected to promote new innovations in healthcare.

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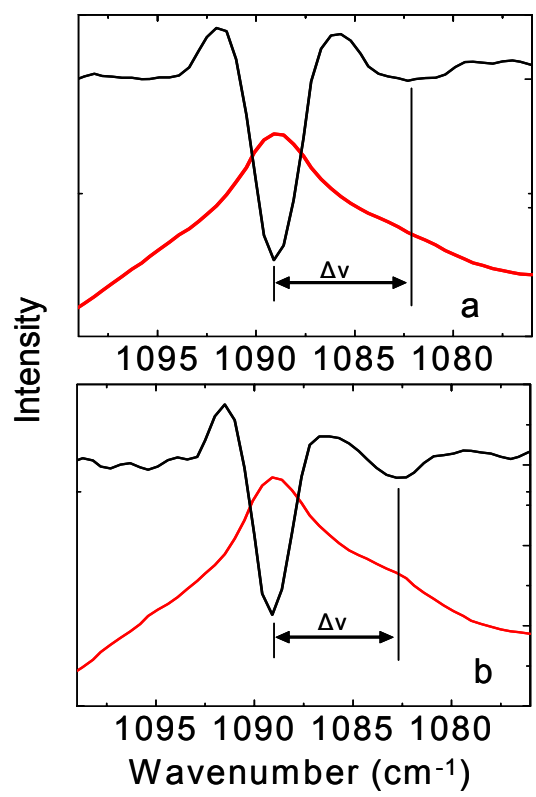
The behavior of lipids in biological membranes is important in a variety of biological functions. For example, how lipids organize permits transport across the membrane and the relationship between lipids and proteins is involved in signaling and recognition. Numerous lipids are found in cellular membranes, and the distribution of these lipids in the membrane is somewhat controversial. The existence of heterogeneous lipid microdomains in cell membranes, also called lipid rafts, was initially hypothesized to explain detergent insoluble portions of cell membranes. Skepticism of these rafts is often associated with large disparities in size reported for these raft domains, ranging from a few nm to µm.

Lipid bilayer membranes are critical structures in life processes. NIST's new method of analyzing membranes will enable a better understanding of how drugs work.

One problem in characterizing microdomains is the potential for fluorophores and other exogenous probes to induce aggregation. Infrared spectroscopy offers a label free method to determine the size of these raft domains from splitting in the CH_2 scissor mode. Lipids, reported to form rafts in biological membranes, are combined into aqueous dispersions, homogenized and allowed to self assemble into lamellar bilayer stacks. The stacks can be conceptualized as a series of bilayers stacked on top of each other with water between. By rapidly cooling the stacks, the lipid distributions that form at elevated temperatures can be trapped in the gel phase. Furthermore, at lower temperatures the lipids can be annealed into an orthorhombic packing configuration where correlation field splitting is evident. These stacks increase the number of molecules being investigated compared to single bilayers, facilitating measurement for weak vibrational signals like the CH_2 scissor mode. To distinguish between the different lipids in the dispersion, a raft marker is chosen with a saturated, perdeuterated chain. In our experiments we use perdeuterated galactocerebroside as our raft marker. Galactocerebroside is a glycosphingolipid, a class of molecules associated with rafts in vivo. Isotopic substitution permits discrimination of the field correlation splitting associated with the raft marker from other vibrational signals. The field correlation splitting is dependent on the number of chains in the cluster. The formation and size of these domains is then evaluated with respect to changing the components of the model membrane.

To date we have successfully measured band splitting associated with the CD_2 scissor mode of the perdeuterated galactocerebroside (GalCer) in two different lipid matrices: dipalmitoylphospatidylcholine (DPPC) and DPPC with cholesterol. Shown in Figure 1 are the CD_2 scissor bands and the second derivatives obtained from GalCer and DPPC (Fig. 1a) and DPPC-Cholesterol (Fig. 1b) matrices.



The second derivative is used for determining peak separation of the overlapping bands. For a 1:1 mixture of GalCer and DPPC (Fig. 1a), a splitting $\Delta\nu = 7.1\ cm^{-1} \pm 0.1\ cm^{-1}$ is observed. The addition of 0.6 mol fraction Cholesterol (Fig. 1b) to the sample results in a splitting of $\Delta\nu = 6.6\ cm^{-1} \pm 0.1\ cm^{-1}$.

$$N = \left[\frac{\pi}{\sqrt{2\left(1 - \Delta\nu / \Delta\nu_0\right)}} - 1 \right]^2$$

Using the equation above, and referencing to the splitting ($\Delta\nu_0 = 7.5\ cm^{-1} \pm 0.1\ cm^{-1}$) obtained for a pure layer of the perdeuterated chain (d70-distearoylphosphatidylcholine, DSPC), the number of interacting chains is calculated to be 74 in the GalCer-DPPC mixture and decreases to 29 in the presence of 0.6 mol fraction cholesterol. This model assumes two chains per lipid, but since our raft marker has only one perdeuterated chain, the number of lipids in each aggregate is doubled, giving 148 and 58, respectively. Assuming a head group size of 5 nm and a square geometry, this supports aggregates 85 and 50 nm in diameter respectively. This is approximation is in agreement with small rafts predicted from fluoresence measurements. Interestingly, our results support previous reports by others that the addition of cholesterol decreases the size of lipid rafts.

Future Plans: This methodology provides the basis for exploring more complex systems associated with cellular membranes. The incorporation of membrane spanning proteins and the inclusion of unsaturated lipids will help elucidate fundamental interactions of biomembranes. Using model systems to understand basic interactions will be helpful in explaining distributions and behavior observed in intact cellular membranes.

This work is a first step in using vibrational spectroscopy as a label-free method of investigating biological membranes.

Future efforts will include more complex systems and imaging experiments on intact membranes.

Figure 1. Infrared a
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Galactocerebroside s
Galactocerebroside-C
drawn to guide the e
splitting.