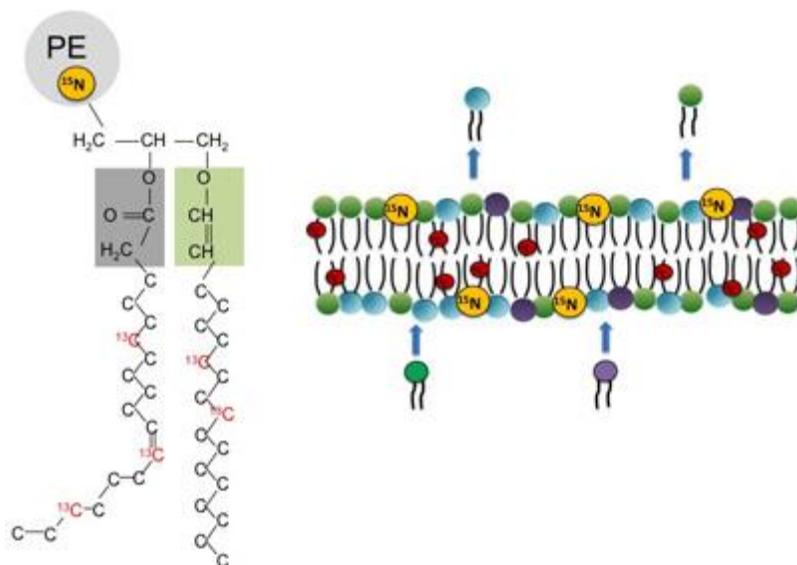


Cleaning out the membrane each day

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Model of heavy isotope labeling strategies for monitoring membrane lipid dynamics.

Image provided by Dr. Carissa Olsen.

Cell membranes are made up of a lipid bilayer that is constantly changing due to the flux of material in and out of the cell. The staggering variety of lipid species that make up the membrane must be constantly maintained in order for the cell to respond to environmental cues or simply grow and divide normally. Aberrant membrane composition is observed in numerous diseases such as neurodegenerative diseases and cancer. Understanding membrane composition and its regulation is an important step toward developing effective therapeutics to target and penetrate the abnormal membrane of cancer cells.

There are challenges to studying lipid dynamics in living organisms. The membrane lipids are tremendously diverse and they are not genetically encoded. Therefore, the ideal way to investigate how a membrane is maintained over time is to manipulate a relatively simple model organism such as the worm, *Caenorhabditis elegans*. Another feature of membrane lipids is that they are in constant flux; however, there are no established methods to quantify the dynamics of each individual population. Researchers in the lab of Dr. Carissa Olsen (Basic Sciences Division) are able to track lipid dynamics in great detail using a method they developed in which worms are fed a diet enriched for heavy carbon, ^{13}C , and then, following a defined growth period, lipids are isolated for identification by mass-spectrometry. Previous work from the lab has demonstrated that the ^{13}C label

can be used to detect all major species of fatty acids, the tails of lipid molecules. In recent work, published in *PLoS One*, they have extended their analysis of the lipidome—all of the lipids in the cell—and outline a new method that uses heavy nitrogen (^{15}N) to track intact phospholipids.

Using heavy carbon labeling and gas chromatography-mass spectrometry (GS-MS), the authors were able to quantitatively track how much of each lipid species in the membrane is replaced over time. The rates they measured for each fatty acid predicted that the majority of the fatty acids in the membrane are replaced within twenty-four hours. They found good agreement between their predictions and the actual measurements of replenishment for most fatty acid species, however some fatty acids were not completely replaced in twenty-four hours. This demonstrates that some fatty acids are protected, either actively or inactively, from removal.

Fatty acid tails are found in polar phospholipids that make up the bilayer and are also found in neutral fat-storage lipids such as the common triacylglycerols. In order to track whether these two groups of lipids have different dynamics, the authors physically separated them before analysis of each group by GC-MS. Strikingly, they found that phospholipid maintenance consumes more dietary resources than neutral lipids, which are, on average, replaced roughly half as quickly. This observation underscores the importance of preserving membranes over maintaining fat stores in adult animals.

Labeled carbon can come from two sources, either the fully labeled fatty acids from the diet or from *de novo* synthesis, which generates fatty acids with a mixture of labeled to unlabeled carbon. After mathematically separating these two groups during their analysis, the authors found that in general most of the new fatty acids incorporated into the membrane come directly from dietary sources, although synthesis did account for a significant portion of the population.

Because the fatty acids in the cell membrane are attached to a phospho-glycerol backbone, directly measuring intact phospholipids rather than their isolated fatty acid tails would give a better picture of membrane dynamics. In order to do this, the authors turned to high-performance liquid chromatography (HPLC) and mass spectrometry. The substantial amount of variation in phospholipid species prompted the researchers to label only a single atom, the nitrogen in the polar head group with a heavy isotope, ^{15}N . They analyzed the most common phospholipid classes in the worm membrane, phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn), and found that PtdEtn phospholipids are exchanged nearly three times the rate of the PtdCho group. This disparity in turnover rates highlights the importance of monitoring the dynamics of individual populations and how they are differentially regulated. Indeed, the methods put forth in this research

provide a new framework for beginning to dissect the genetics and biochemistry of membrane dynamics.

[Dancy BC*, Chen SW*, Drechsler R, Gafken PR, Olsen CP](#). 2015. “¹³C- and ¹⁵N-Labeling strategies combined with mass spectrometry comprehensively quantify phospholipid dynamics in *C. elegans*. *PLoS One*. 10(11): e0141850.

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