

## Lipid rafts inflate at the trans-Golgi

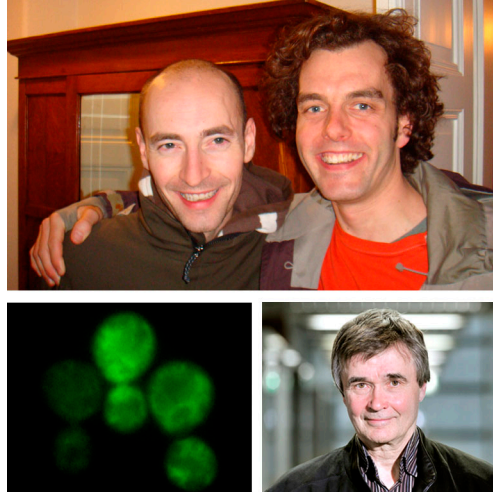
A new method to characterize the lipid content of vesicles suggests how cells sort secretory cargo.

**H**ow do cells sort proteins at the trans-Golgi network (TGN) and package them into vesicles for delivery to their correct cellular destination? Surprisingly, examining the lipid content of those vesicles is helping researchers answer this fundamental question (1).

Newly synthesized proteins traffic through the secretory pathway and are sorted at the trans-face of the Golgi into vesicles for delivery to the plasma membrane or endocytic system. Membrane lipids also show different distributions within the cell. For example, the apical membranes of polarized epithelial cells are highly enriched in cholesterol and sphingolipids. The transport of proteins and lipids to the cell surface are intimately connected by lipid rafts: small dynamic assemblies within membranes that contain sterols and sphingolipids, as well as many apical membrane proteins (2). Rafts might sort proteins and lipids by clustering together at the TGN into bigger patches, which would then pinch off as vesicles for delivery to the membrane (3).

Kai Simons and his group reasoned that if raft clustering does drive the packaging of membrane proteins at the TGN, then lipid sorting should also occur. The lipid composition of vesicles containing raft proteins should therefore be very different from the trans-Golgi membranes from which they derive. Measuring the lipid content of these different compartments, however, was no simple task.

Working in yeast, where the trafficking of lipid rafts is conserved, the different membranes had to be isolated with very high purity—higher than ordinary organelle fractionation techniques would provide. “This was the big challenge,” says Simons. “We had to use all the tricks that yeast allow.” The team engineered bait proteins that could bind



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to beads via an affinity tag before being specifically released by protease cleavage. Bait proteins localizing to either the TGN or to post-Golgi vesicles were then used to immunoprecipitate each of these compartments from yeast cells—the first time that vesicles have been obtained with such high purity from living cells.

The next step also required a new method. In collaboration with Andrej Shevchenko (4), Simons’s group developed a novel shotgun mass spectrometry technique to analyze the lipid composition of the purified organelles. The new technique enabled the researchers to work with smaller amounts of material and, most importantly, quantify each individual lipid species rather than just measure the total amount of each lipid class. “It’s the first time that the full, quantitative lipidome of an organelle has been acquired,” says Simons. “We could measure the whole spectrum of lipids.”

Klemm et al. quantified 83 lipid species (covering 12 different classes) in both the purified vesicles and in the TGN. Sure enough, the vesicle membranes were enriched in ergosterol (the yeast

equivalent of cholesterol) and in sphingolipids, suggesting that lipid rafts do coalesce and concentrate into vesicles budding from the TGN.

So what triggers the clustering of rafts at the TGN? “That’s the next question!” explains Simons. “Our working model is that there’s a protein that nucleates raft clustering by binding to either the sphingolipids or the raft proteins. We’re using yeast proteomics and genetics to define the network of interactions involved in post-Golgi sorting.”

This network will include both lipid–protein and lipid–lipid interactions, and Simons believes that the contribution of lipids to membrane trafficking has been neglected for too long. “In our model, the membrane lipids themselves are functionally involved in the sorting process. There are a few thousand genes involved in lipid synthesis, metabolism, and regulation in mammalian cells. The diversity of lipid species is there for a reason.”

### FOCAL POINT

Lipid rafts—specialized membrane patches with a unique lipid and protein composition—may cluster at the trans-Golgi network (TGN) to mediate the sorting of lipids, as well as proteins, into secretory vesicles. Christer Ejsing, Robin Klemm, Kai Simons (pictured clockwise from top left), and coworkers developed new techniques to purify post-Golgi vesicles containing raft proteins (green) and analyze their lipid content. Their data reveal that raft lipids are also sorted and enriched at this stage of the secretory pathway, supporting the clustering model.

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2. Simons, K., and E. Ikonen. 1997. *Nature.* 387:569–572.
3. Schuck, S., and K. Simons. 2004. *J. Cell Sci.* 117:5955–5964.
4. Ejsing, C.S., et al. 2009. *Proc. Natl. Acad. Sci. USA.* 106:2136–2141.