Title:
Monitoring lipid localization and molecular identity in cellular transport processes

Introduction:
Lipids are crucial to cellular function – as building blocks of membranes, for cell signaling and in energy metabolism and storage. Despite their obvious importance, we know very little about their cellular dynamics – both with regard to trafficking and localized metabolism. There are two reasons for this lack of knowledge: Lipids are difficult to visualize as bulky molecular tags disrupt their function. Furthermore, there is a huge variety of chemically distinct lipid structures in biology, with recent estimates putting the number of lipid species in the typical mammalian cell well into the 4-digit range.

Methods:
Clearly, strategies are needed to closely monitor lipid transport and metabolism on the level of individual lipid species. We addressed this by generating a library of bifunctional (clickable and photo-crosslinkable) phospholipids to serve as chemical probes for monitoring lipid localization and chemical identity. The subcellular localization of such probes can be visualized via click chemistry after photochemical crosslinking and cell fixation.
Using a novel lipid delivery protocol, we were able to place comparable amounts of lipid probes into the outer plasma membrane leaflet of living cells. This gave us a well-defined starting point for a comparative analysis of transport processes for individual lipid species representing different lipid classes, saturation degrees and chain lengths.

Results & Conclusion
We determined the subcellular distribution of each lipid probe by counterstaining for the major organelle types in four-color fluorescence microscopy experiments and complemented our imaging data with whole-lipidome high-resolution mass-spectrometric analysis at each time point, covering both native and chemically modified lipid species. The combination of both approaches enabled us to generate a comprehensive dataset of both spatial and metabolic lipid flux of most major phospholipid classes. Our data enable us to derive the general rules that govern intracellular lipid transport, we can now readily distinguish between vesicular and non-vesicular lipid trafficking pipelines for individual lipids, determine the dynamics of lipid transport and outline which chemical features within the lipid structure are most influential with regard to their intracellular dynamics. Ultimately, we aim to convert our datasets into an open-source, searchable lipid transport atlas, which would serve as a resource for all membrane biology adjacent fields.