

Towards Repurposing the Yeast Peroxisome for Compartmentalizing Heterologous Metabolic Pathways

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Our long-term goal is to repurpose the peroxisome to be a synthetic organelle that can insulate engineered multi-enzyme pathways from undesired cross-talk with the rest of the cell production host. Ultimately, we endeavor to control the chemical environment of the organelle such that catalysis can be conducted that would not be feasible in the cytoplasm. Towards this long-term goal, we aim to learn about natural peroxisome biology, increase the efficiency of protein cargo import, and characterize small molecule permeability.

Compartmentalization of enzymes into organelles is a promising strategy for limiting metabolic crosstalk and improving pathway efficiency, but improved tools and design rules are needed to make this strategy available to more engineered pathways. Here we focus on the *Saccharomyces cerevisiae* peroxisome and develop a sensitive high-throughput assay for peroxisomal cargo import. We identify an enhanced peroxisomal targeting signal type 1 (PTS1) for rapidly sequestering non-native cargo proteins. Additionally, we perform the first systematic *in vivo* measurements of nonspecific metabolite permeability across the peroxisomal membrane using a polymer exclusion assay. Finally, we apply these new insights to compartmentalize a two-enzyme pathway in the peroxisome and characterize the expression regimes where compartmentalization leads to improved product titer. This work builds a foundation for using the peroxisome as a synthetic organelle, highlighting both promise and future challenges on the way to realizing this goal.

Publications

1. DeLoache, WC, Russ, ZN, and Dueber JE. Towards Repurposing the Yeast Peroxisome for Compartmentalizing Heterologous Metabolic Pathways. *Nature Communications*. 2016. In press.
2. DeLoache, W. C., Russ, Z. N., Narcross, L., Gonzales, A. M., Martin, V. J. J., & Dueber, J. (2015). An enzyme-coupled biosensor enables (S)-reticuline production in yeast from glucose. *Nature Chemical Biology*, 11(7), 465–471. <http://doi.org/10.1038/nchembio.1816>