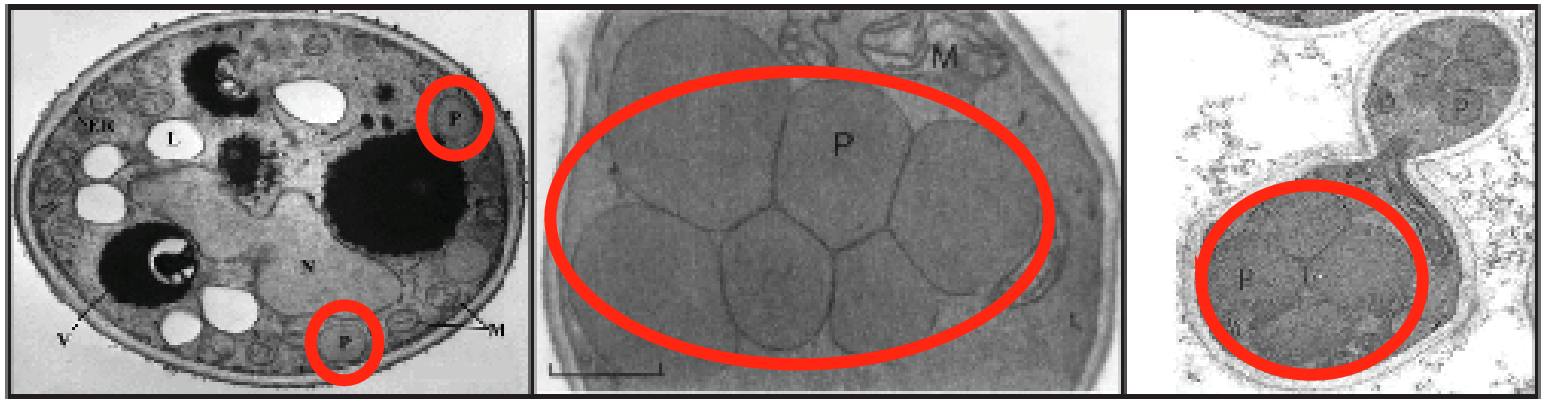
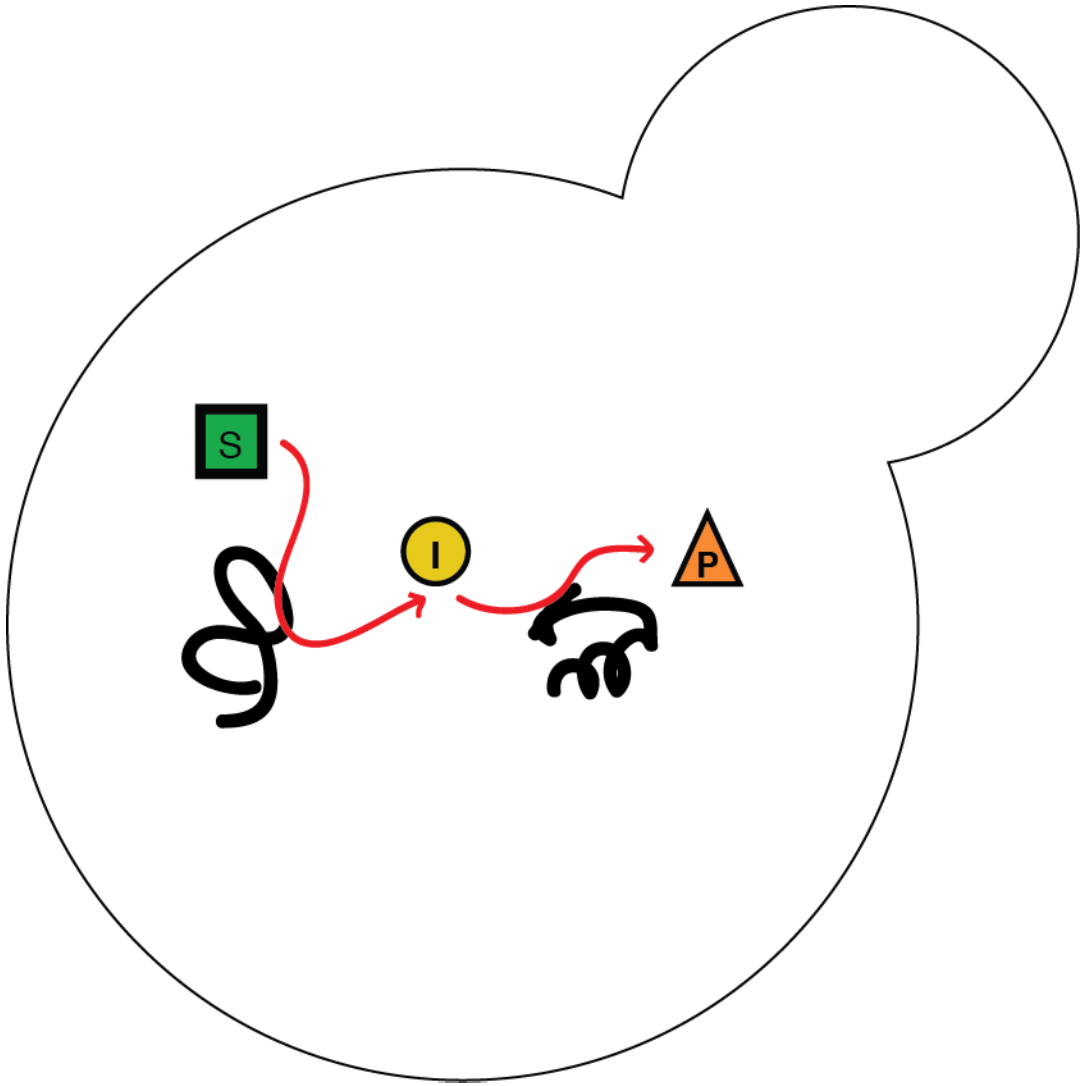


S. cerevisiae *P. pastoris* *H. polymorpha*

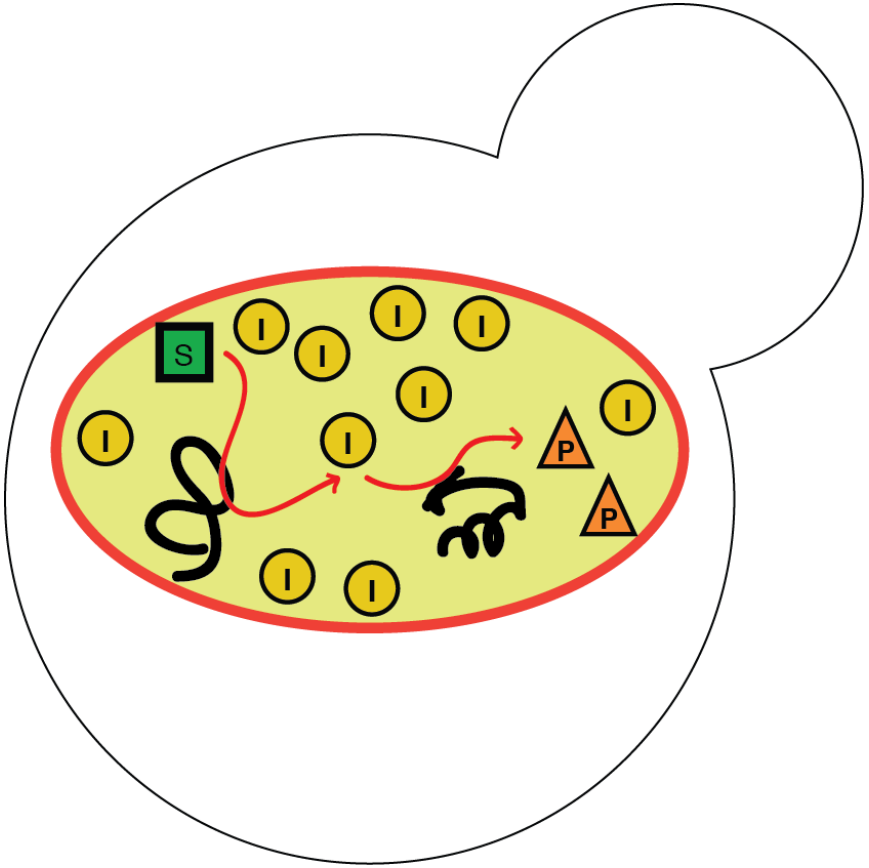
Repurposing the Yeast Peroxisome for Compartmentalized Metabolic Pathways

John Dueber, Dept. of Bioengineering, U.C. Berkeley

Metabolic Engineering Motivation



Insulating Engineered Pathways



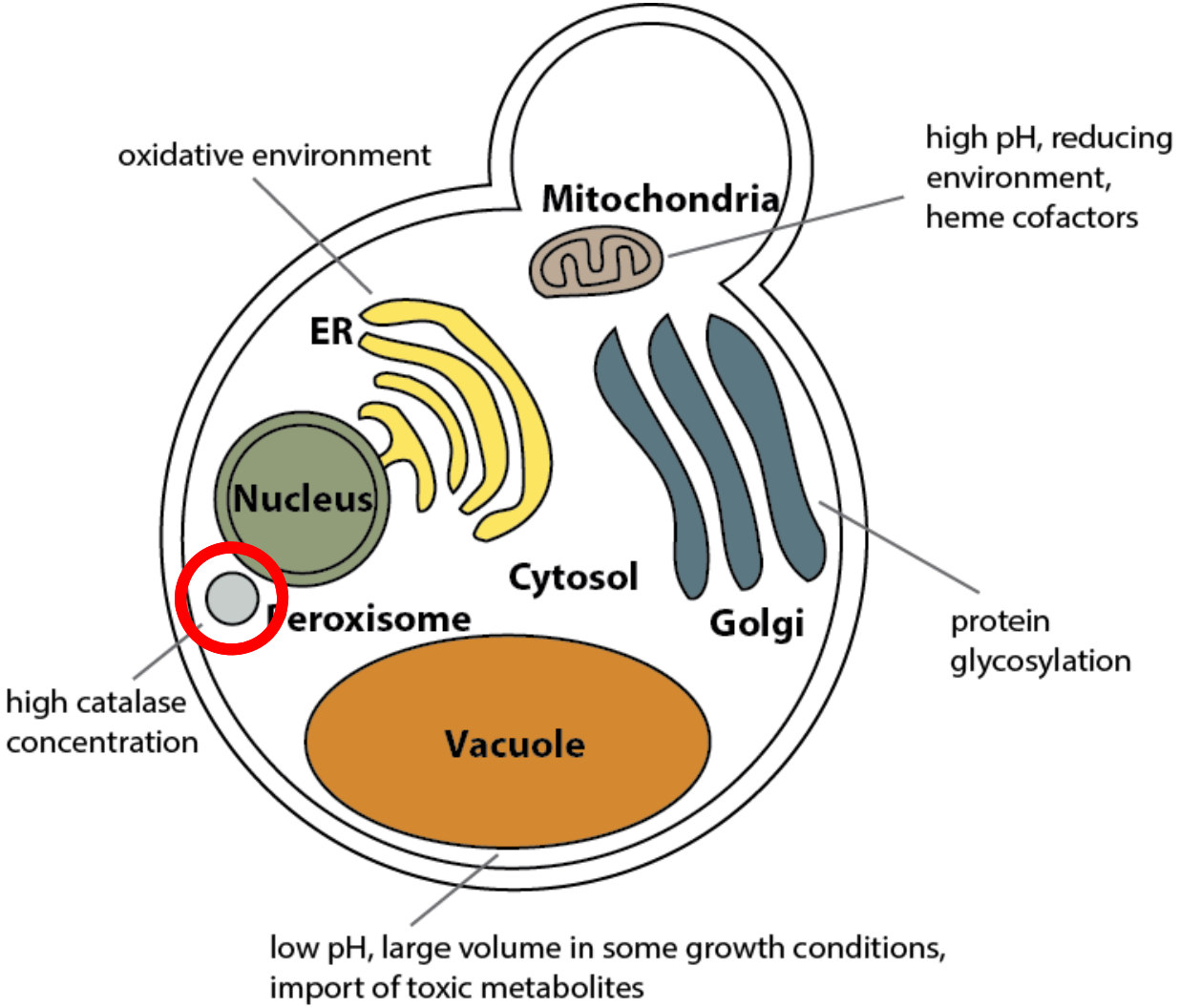
Short-term goals:

Pathway insulation to prevent cross-talk and/or toxicity

Long-term goals:

Alter chemical environment

Organelles are Specialized Compartments



DeLoache. 2013. NBT. Perspective.

Why the Peroxisome?

Not Required by *S. cerevisiae*

Wild-type

Protein Import Mutant

Why the Peroxisome?

Not Required by *S. cerevisiae*

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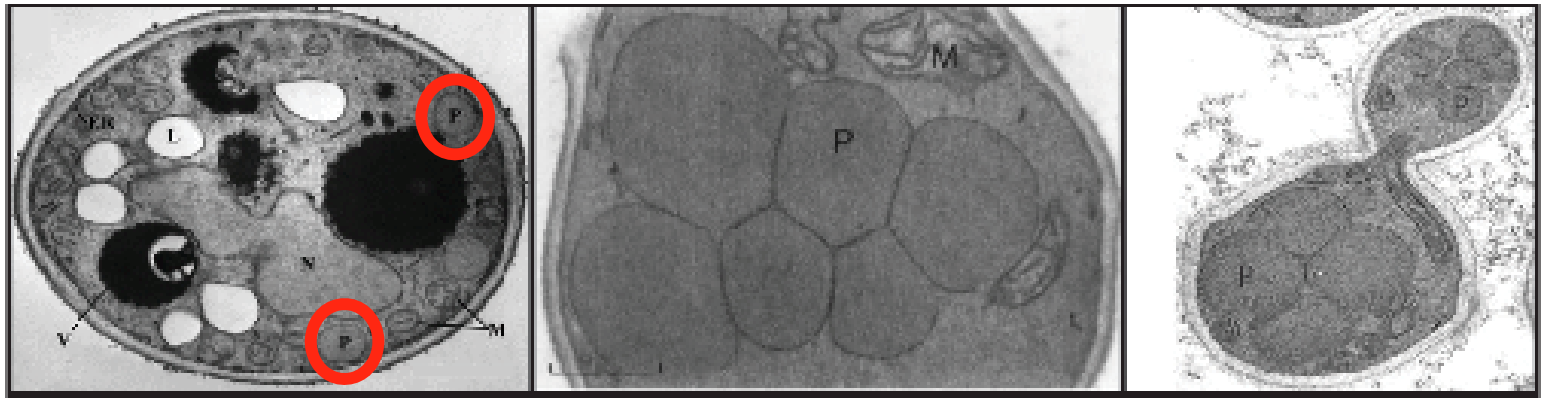
Organelle size varies greatly across fungi



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Purdue, P.E. & Lazarow, P.B. Annu Rev Cell Dev Biol 17, 701–752 (2001).
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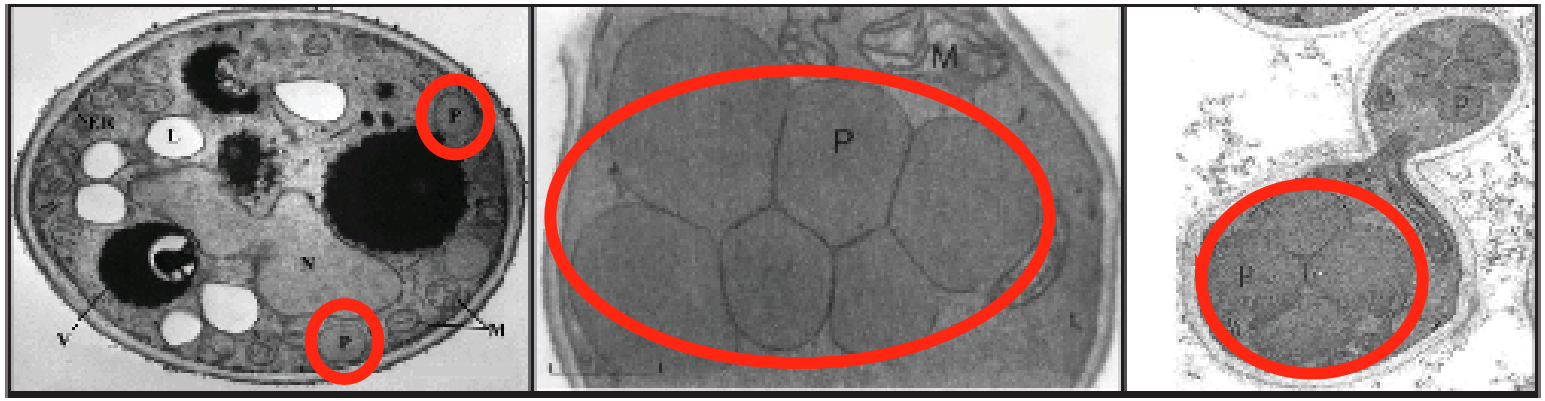

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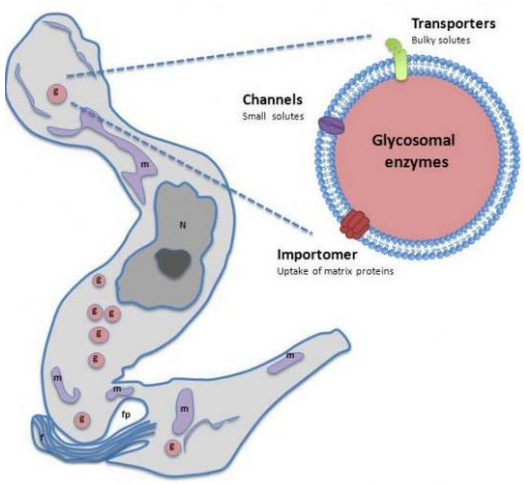
Gellissen, G. et al. FEMS Yeast Research 5, 1079–1096 (2005).

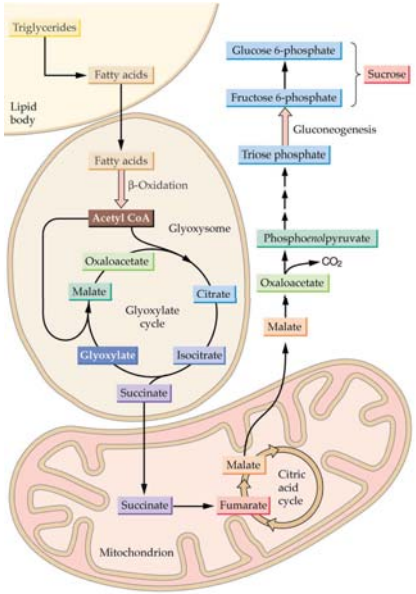
Peroxisome has been Naturally Specialized

glycosome (glycolysis)



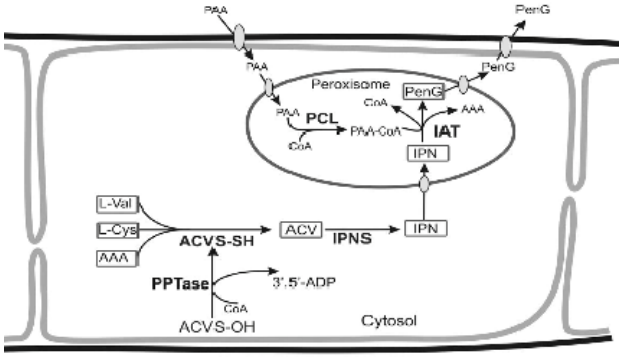
T. brucei

glyoxysome (fat to sugar)



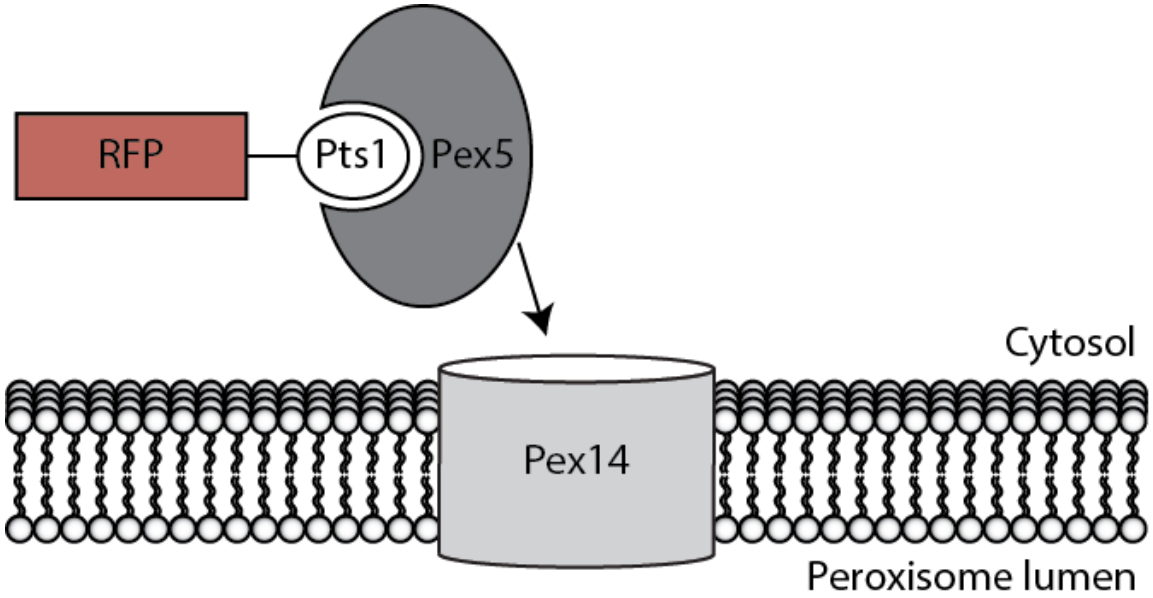
plants and filamentous fungi

pencillin (2 enzymes)

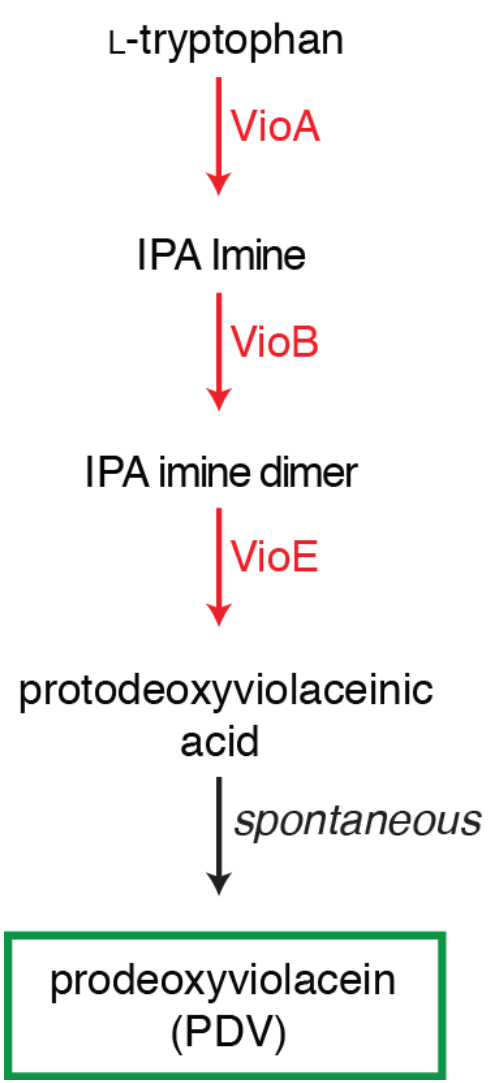


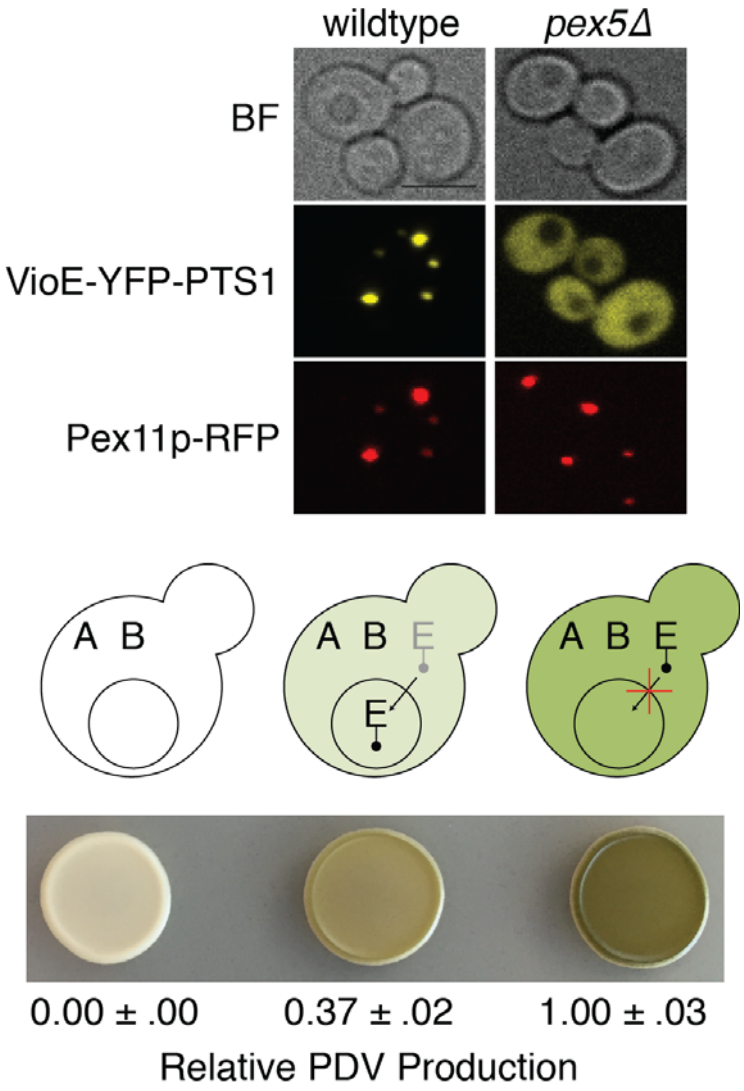
P. chrysogenum

Control of Folded Protein Transport

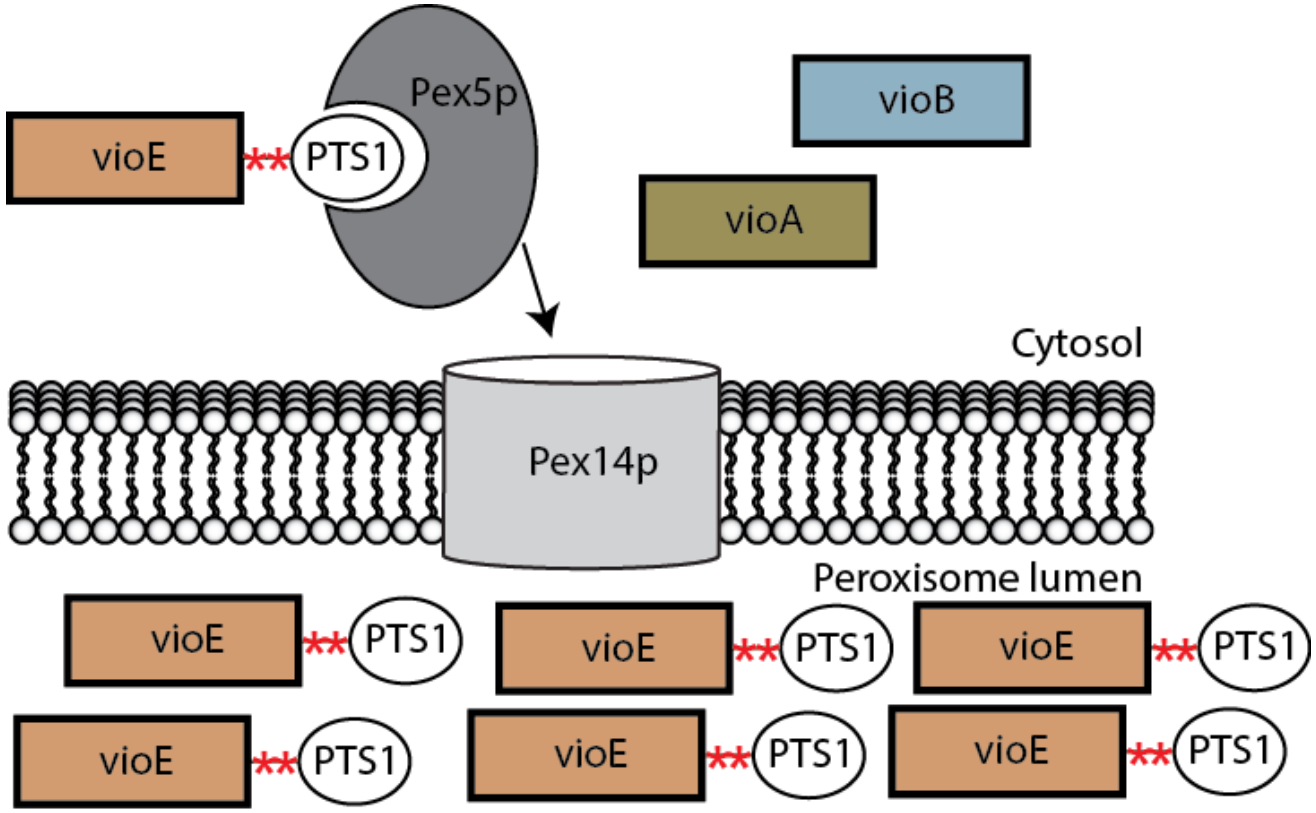


Metabolic Enzyme Sequestration Assay

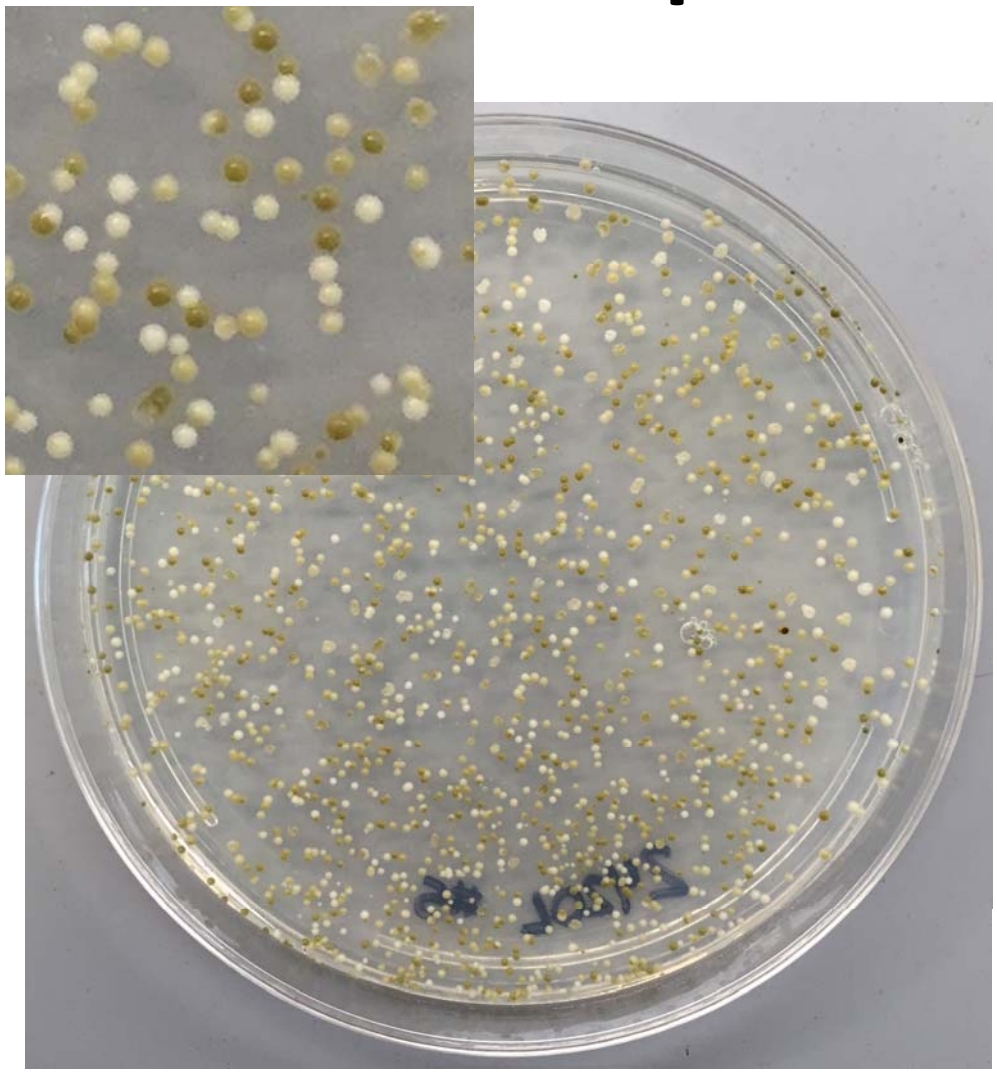


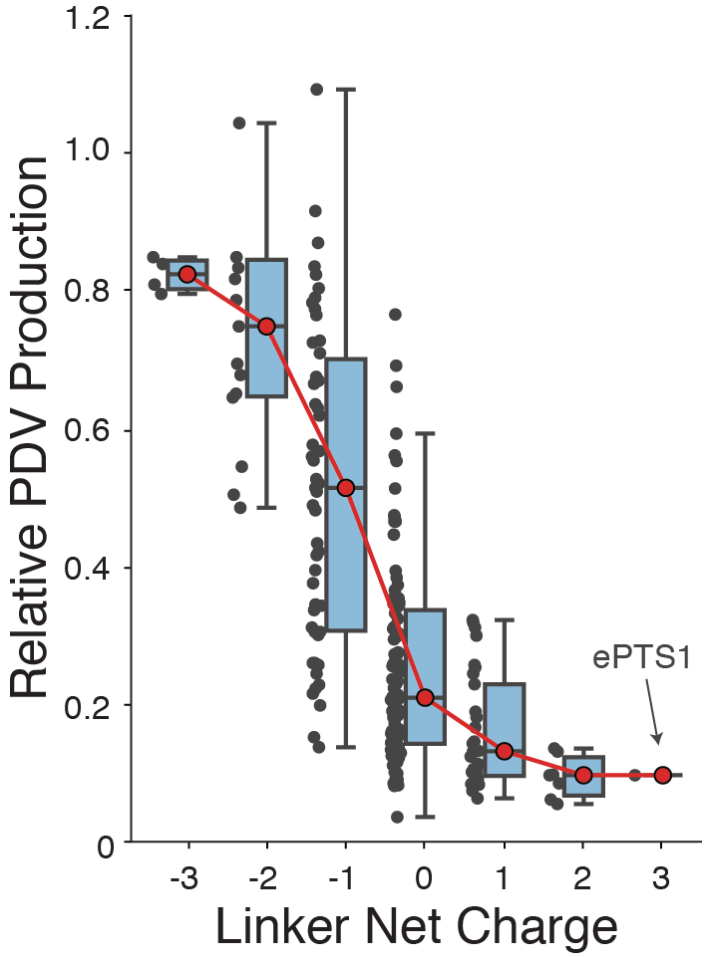


Linker Mutagenesis Assay for an Enhanced PTS1 Tag



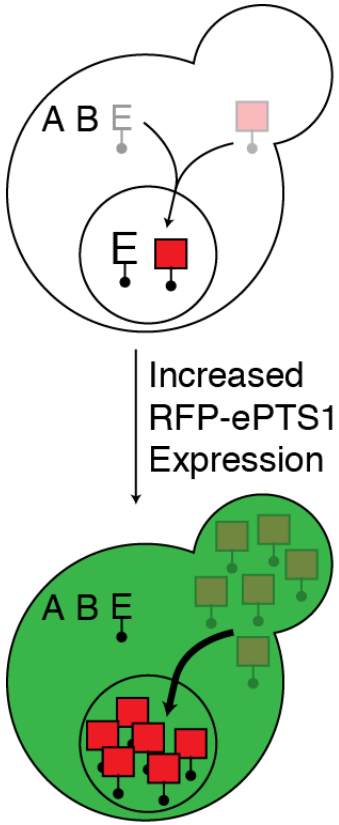
Linker Library Yielded Varying Import Efficiencies

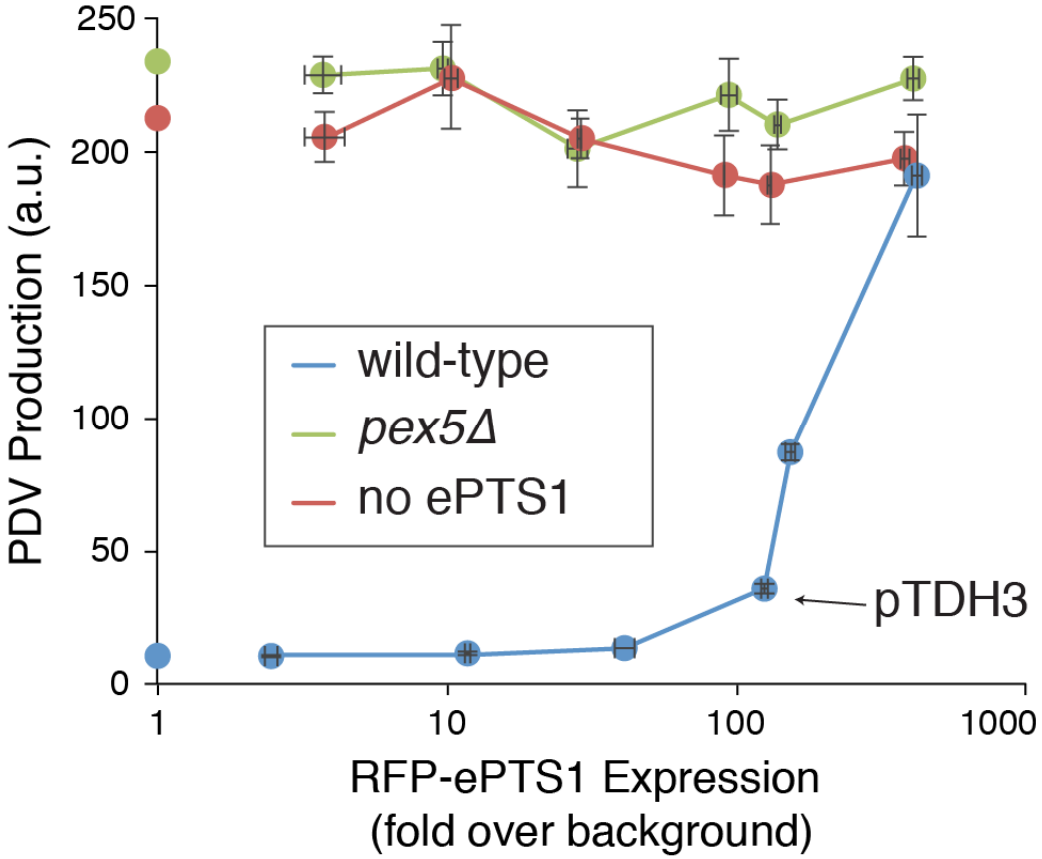




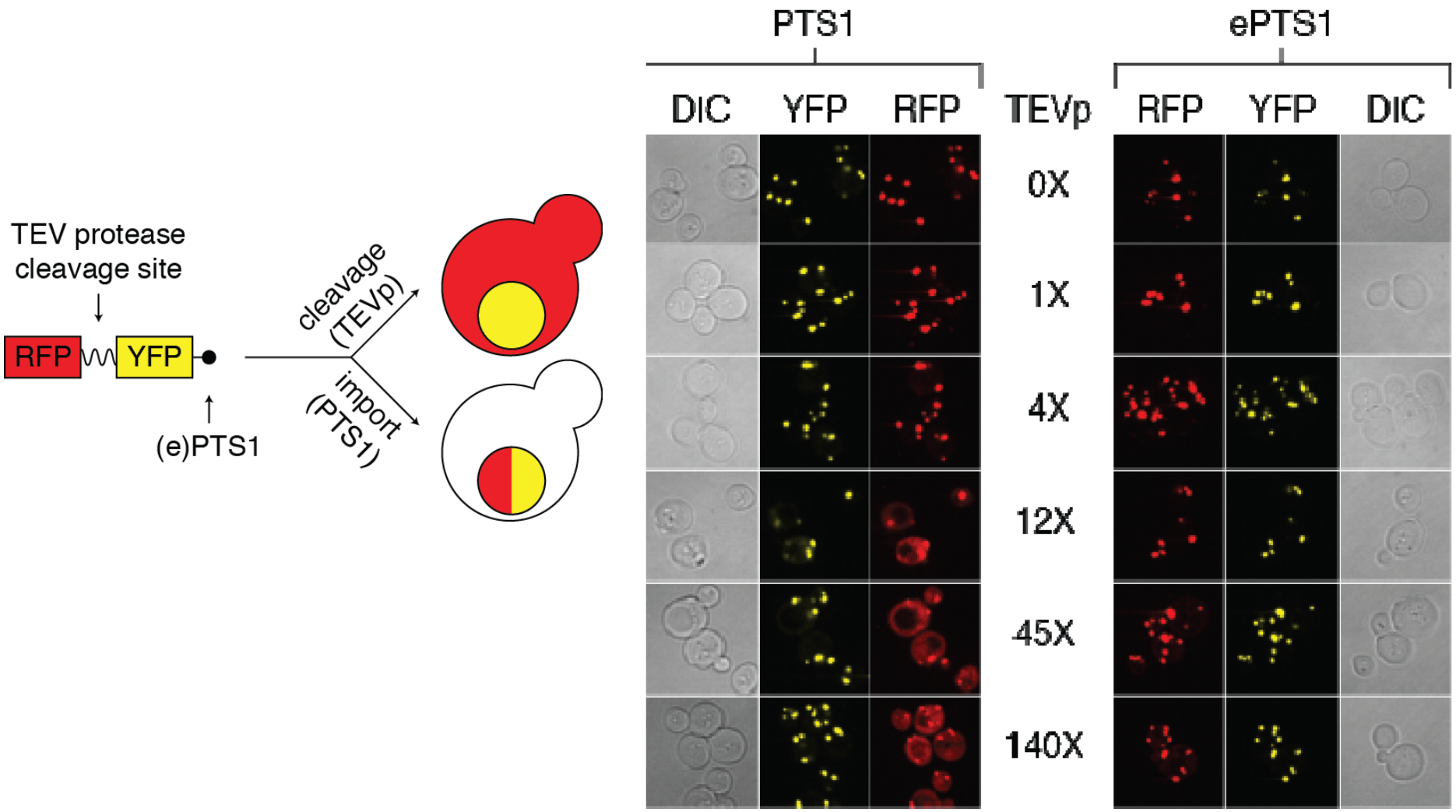
Peroxisomes Naturally have High Capacity

Express constant amount of VioE-ePTS1 and vary levels of RFP-ePTS1

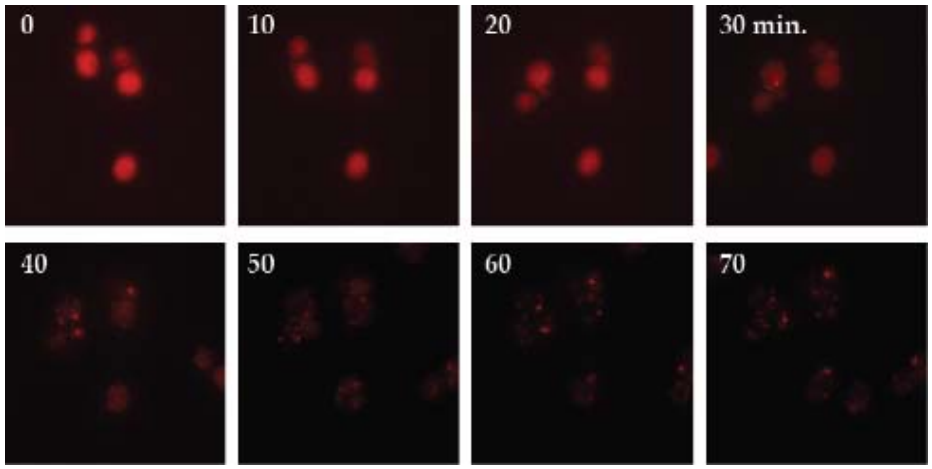




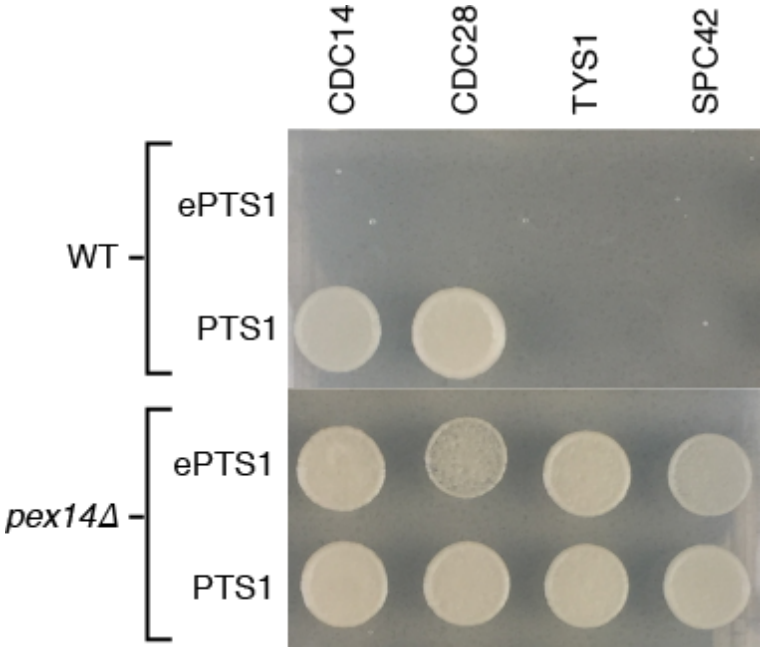
Improved Cargo Import Rate



Pex5 Induction of Cargo Sequestration



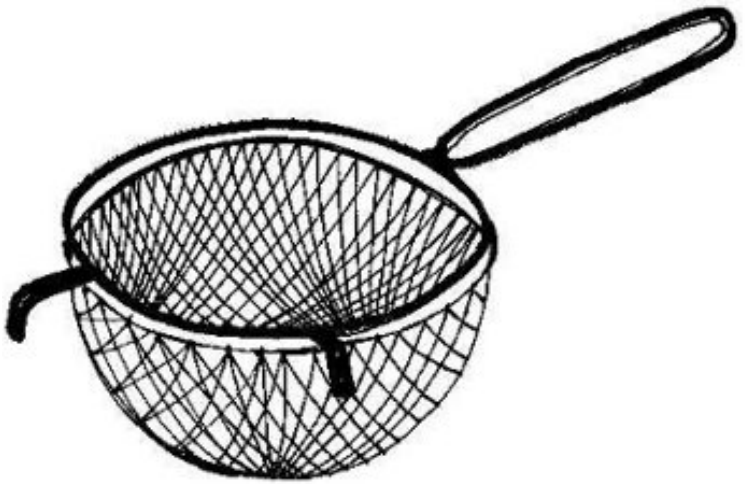
Growth Assay with Induced Pex5



The Enhanced Peroxisome Targeting Tag Appears to be Modular

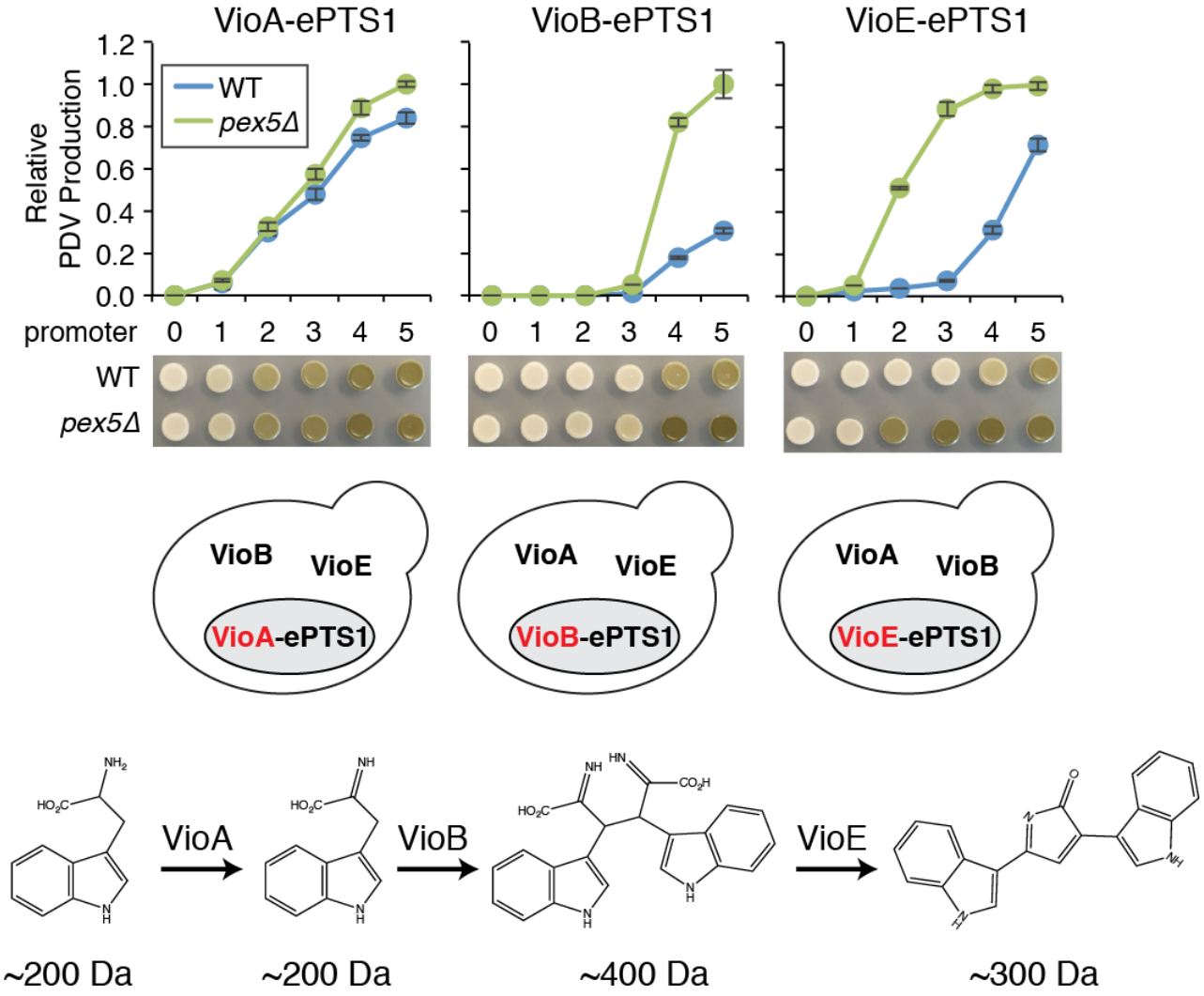
Peroxisome Membrane Permeability: a 50-Year Debate

Camp 1: Peroxisomes are permeable up to ~700 Da (cutoff just below NADH cofactors that have transport shuttles)

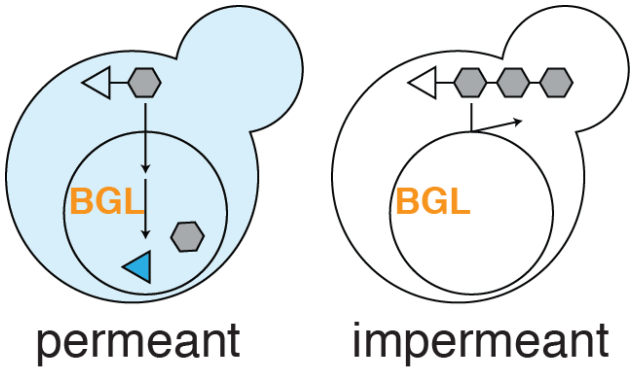


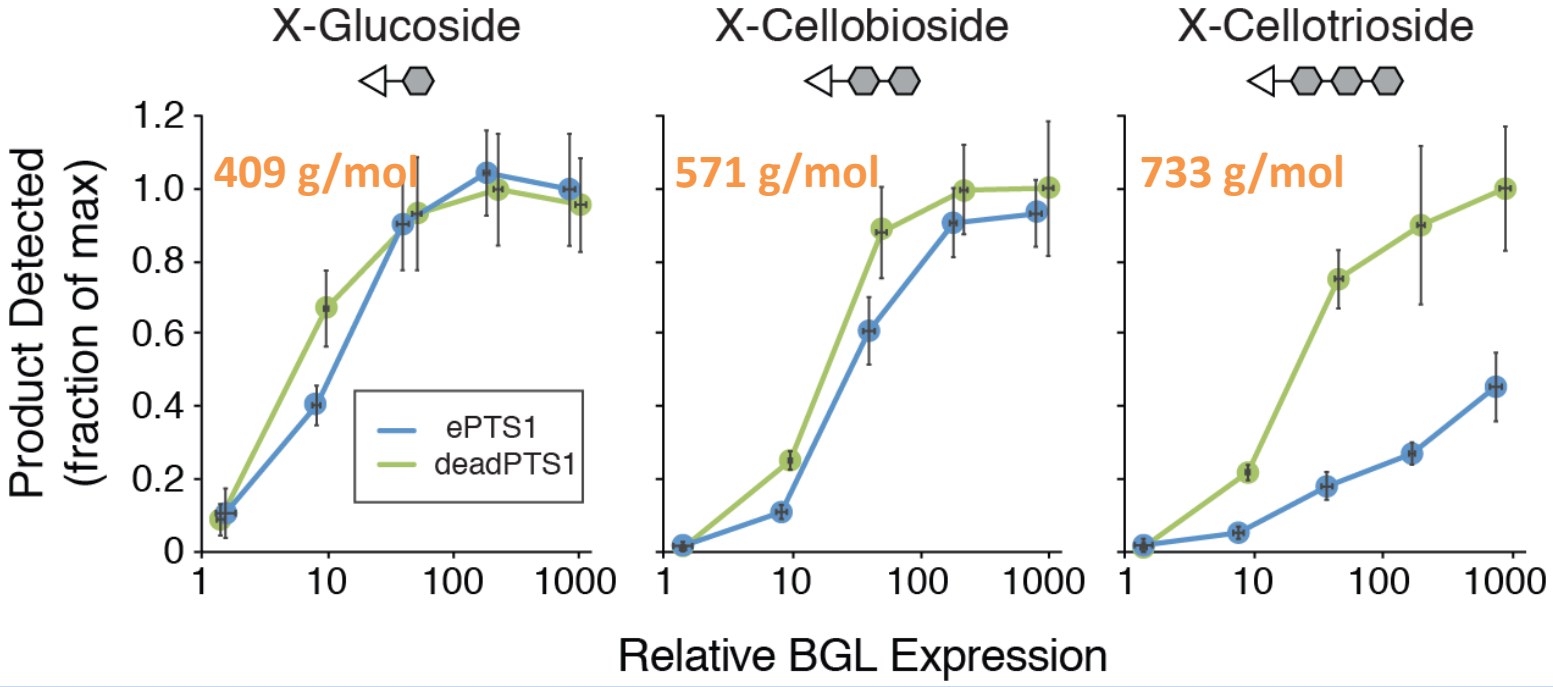
Camp 2: Camp 1 unintentionally lyses their peroxisomes during the purification process.

Peroxisome Membrane Appears to have a Permeability Size Cutoff

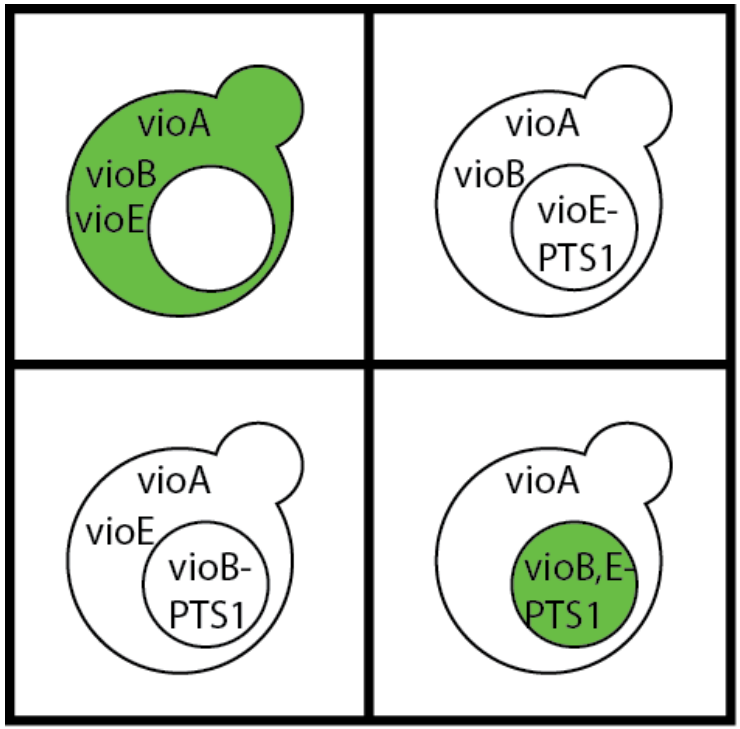


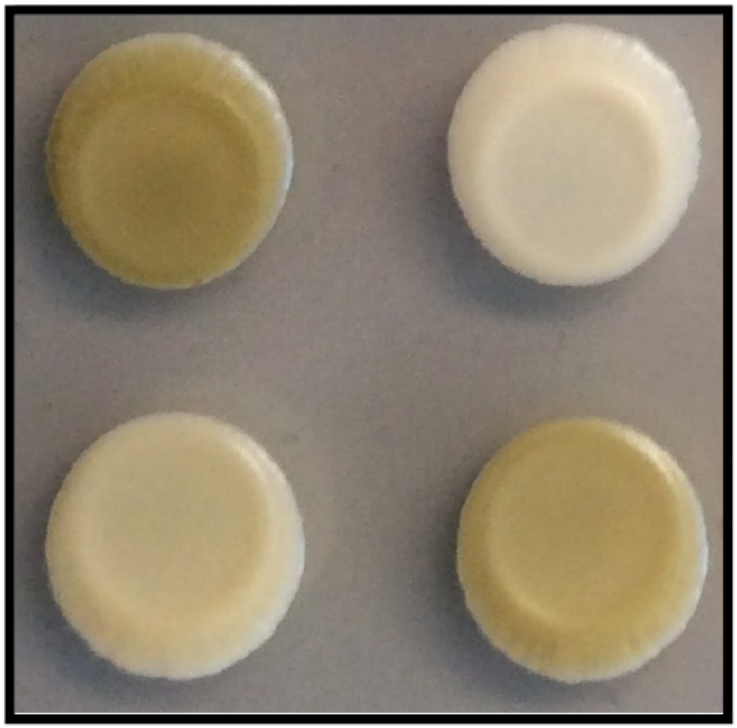
Further Evidence for a Size Limit to Membrane Permeability



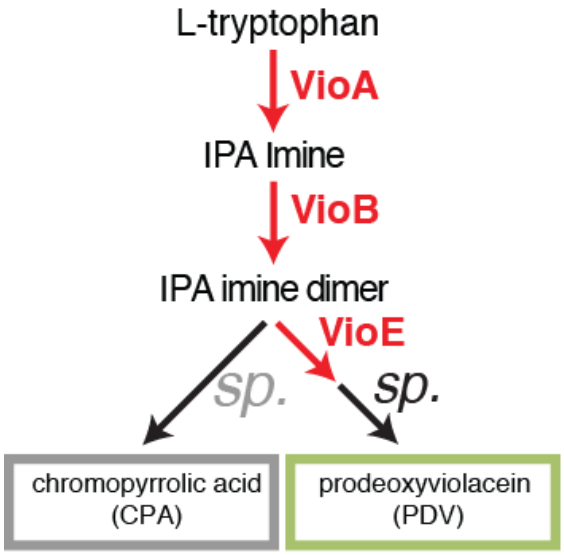


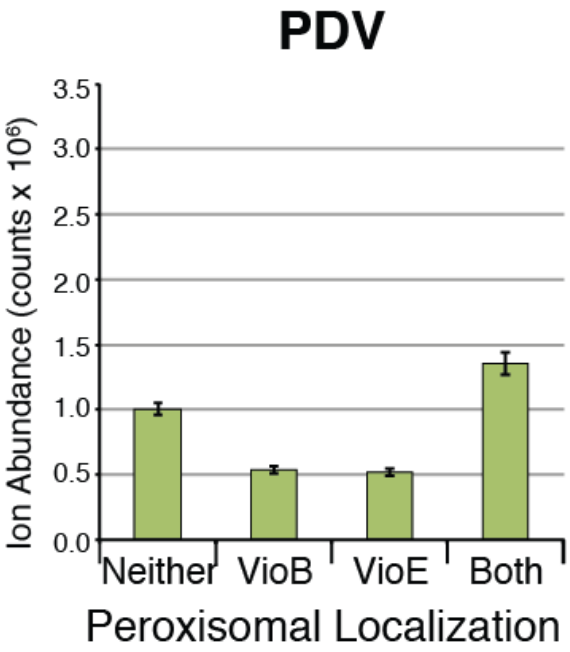
PDV Pathway Enzymes Can be Functionally Compartmentalized

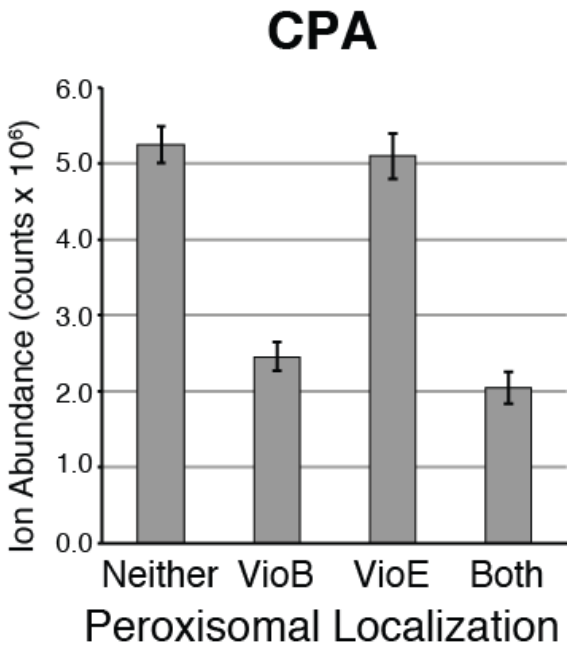




Compartmentalization of VioE-limited Pathway May Substrate Channel







Early Career Award – Multiplier Effect

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ARTICLE

Received 11 Nov 2015 | Accepted 23 Feb 2016 | Published 30 Mar 2016

DOI: 10.1038/ncomms11152 OPEN

Towards repurposing the yeast peroxisome for compartmentalizing heterologous metabolic pathways

William C. DeLoache[1,2,*], Zachary N. Russ[1,2,*] & John E. Dueber[2]

nature chemical biology

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PUBLISHED ONLINE: 18 MAY 2015 | DOI: 10.1038/NCHEMBIO.1816

An enzyme-coupled biosensor enables (*S*)-reticuline production in yeast from glucose

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