Yeast cell factories for production of pharmaceuticals

In silico protein secretion model (pcSecYeast)

Metabolic engineering to improve yeast cell factories

Characterization of yeast cell factories

Production of pharmaceutical proteins

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In silico protein secretion model (pcSecYeast)
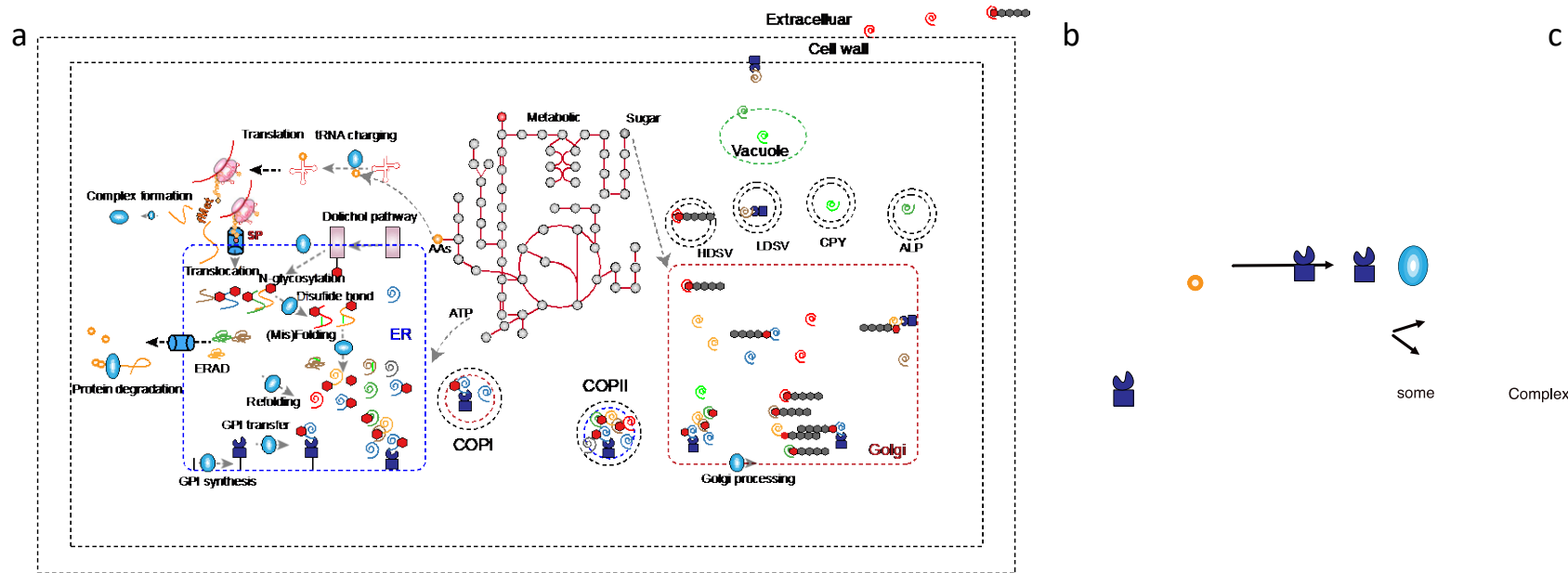
a. Overview of pcSecYeast for *S. cerevisiae*.   b. Main constraints existing in the model.   c. Model statistic feature.

Aim: Predict metabolic engineering targets to increase recombinant protein secretion.

❧ pcSecYeast model integrates genome-scale metabolic model with protein synthesis and secretion.

❧ pcSecYeast model enables systematically computation of energetic costs and machinery protein demands of each secreted protein.

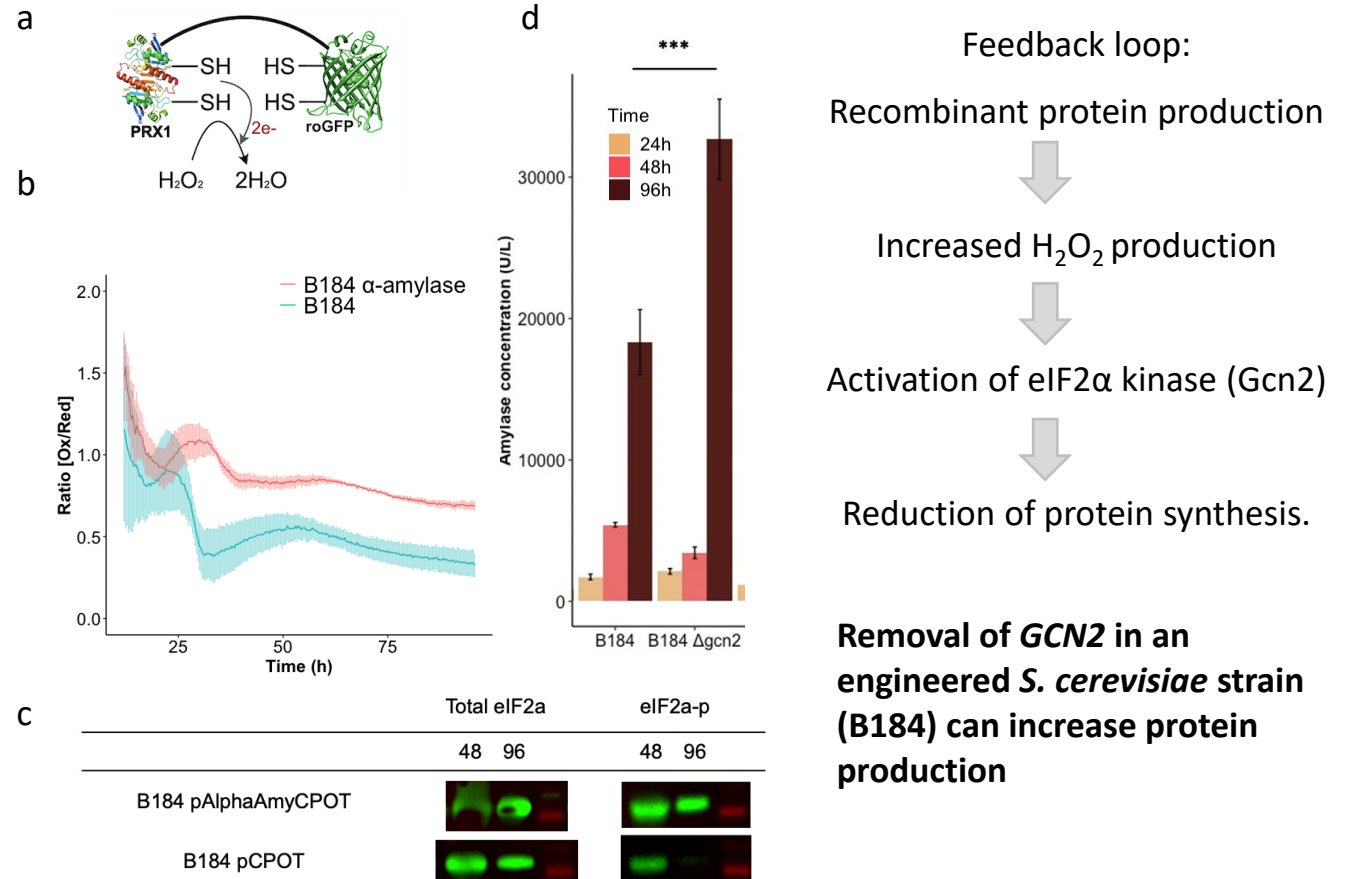# Metabolic engineering to improve yeast cell factories

Improved protein production using candidates from a yeast neurodegenerative model
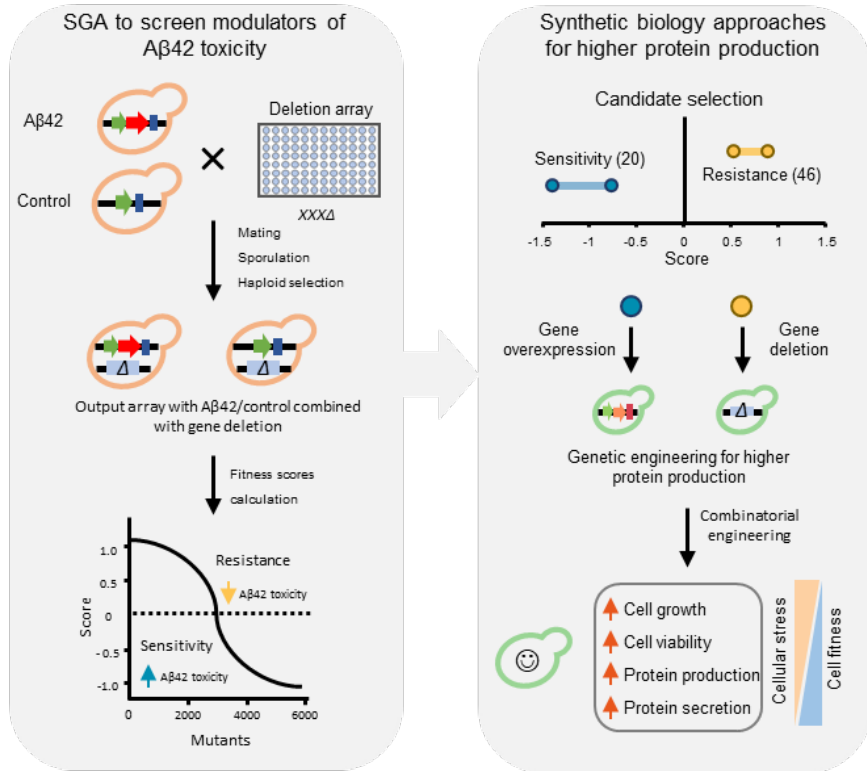
Schematic workflow for identifying novel candidates

☙ Both misfolded proteins (Aβ42) and recombinant protein expression result in ER stress and reactive oxygen species (ROS).
☙ Genetic alterations that protect cells against Aβ42 can improve recombinant protein production.

## Discovery of negative feedback loop within protein production pathway

Feedback loop:

Recombinant protein production
⬇
Increased H_2O_2 production
⬇
Activation of eIF2α kinase (Gcn2)
⬇
Reduction of protein synthesis.

Removal of *GCN2* in an engineered *S. cerevisiae* strain (B184) can increase protein production

a. Biosensors with Prx1 and GFP fusion construct which detects cytosolic H_2O_2. **b**. Level of cytosolic H_2O_2. **c**. eIF2α phoshorylation. **d**.Recombinant α-amylase production.

Production of pharmaceutical proteins

Production of Affibody molecules

❧ ABD domain is substrate of yeast proteinase A (Pep4) and carboxypeptidase Y (Prc1).

❧ Intact Zer31-ABD-Zer31 is secreted by *S. cerevisiae* with correct binding affinity and characteristics.
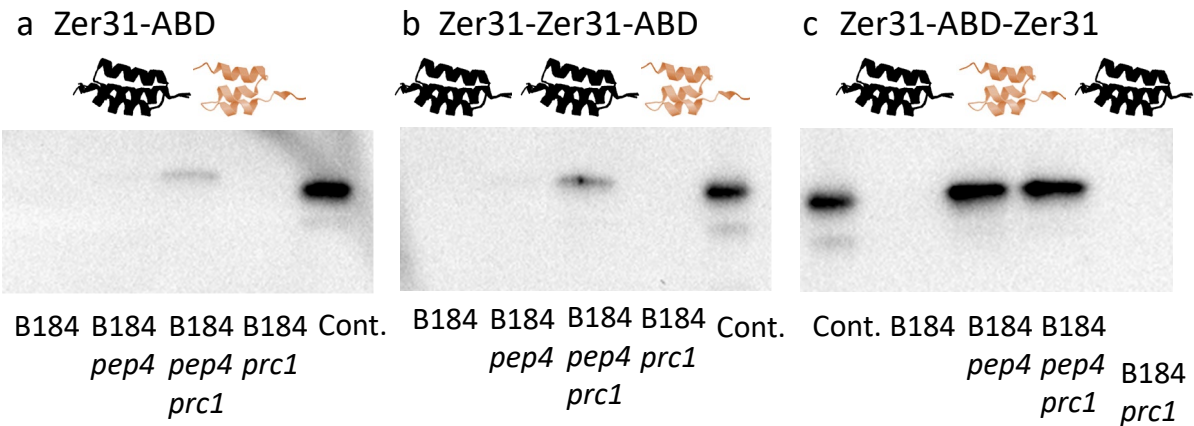
a. Anti-ABD Western blot of Zer31-ABD. **b**. Anti-ABD Western blot of Zer31-Zer31-ABD. **c**. Anti-ABD Western blot of Zer31-ABD -Zer31.

Production of human antibody fragments

Nanobody (single V-type domain), Pexelizumab (single-chain variable fragment) and Ranibizumab (antigen-binding fragment) are successfully expressed in *S. cerevisiae* with full biological activities.
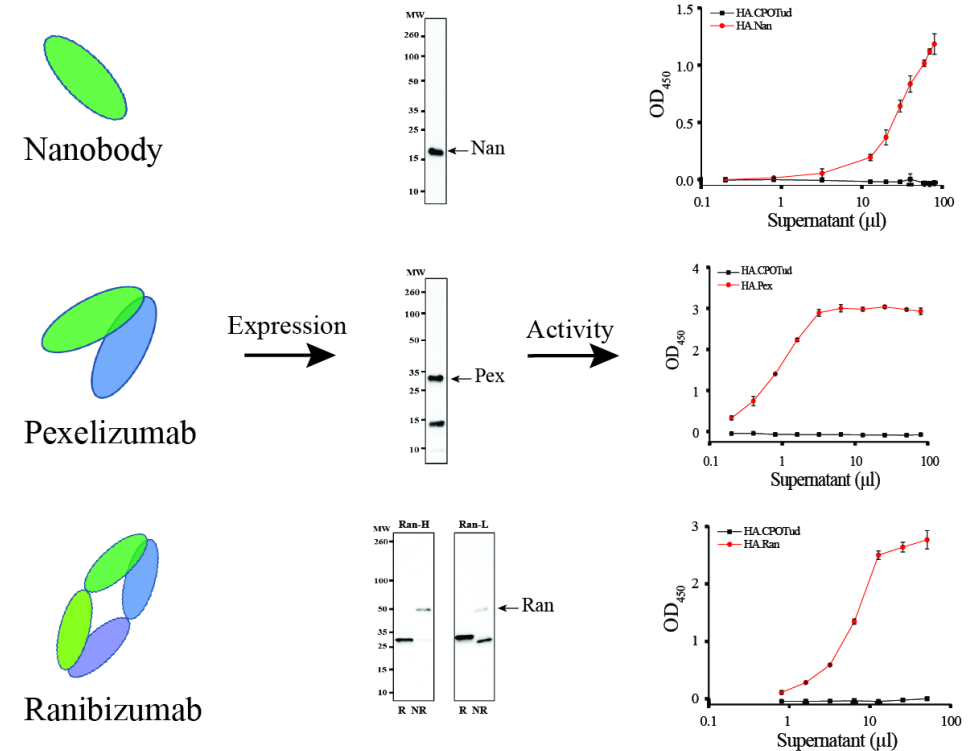
Characterization of yeast cell factories

ॐ Yeast cell factories require metabolic reprogramming to provide more amino acids and NADPH via the Gcn2p signaling pathway for improved recombinant protein production.

ॐ Both the protein folding precision and the protein folding capacity are key factors that affect the folding and protein production efficiency.

ॐ Cwh41p and Pdi1p play crucial roles in the folding precision control and the folding capacity control, respectively.