

# Investigating the Roles of a Mitochondrial Thioredoxin in Baker's Yeast Identified to Potentially Regulate Branched Chain Amino Acid Synthesis Enzymes

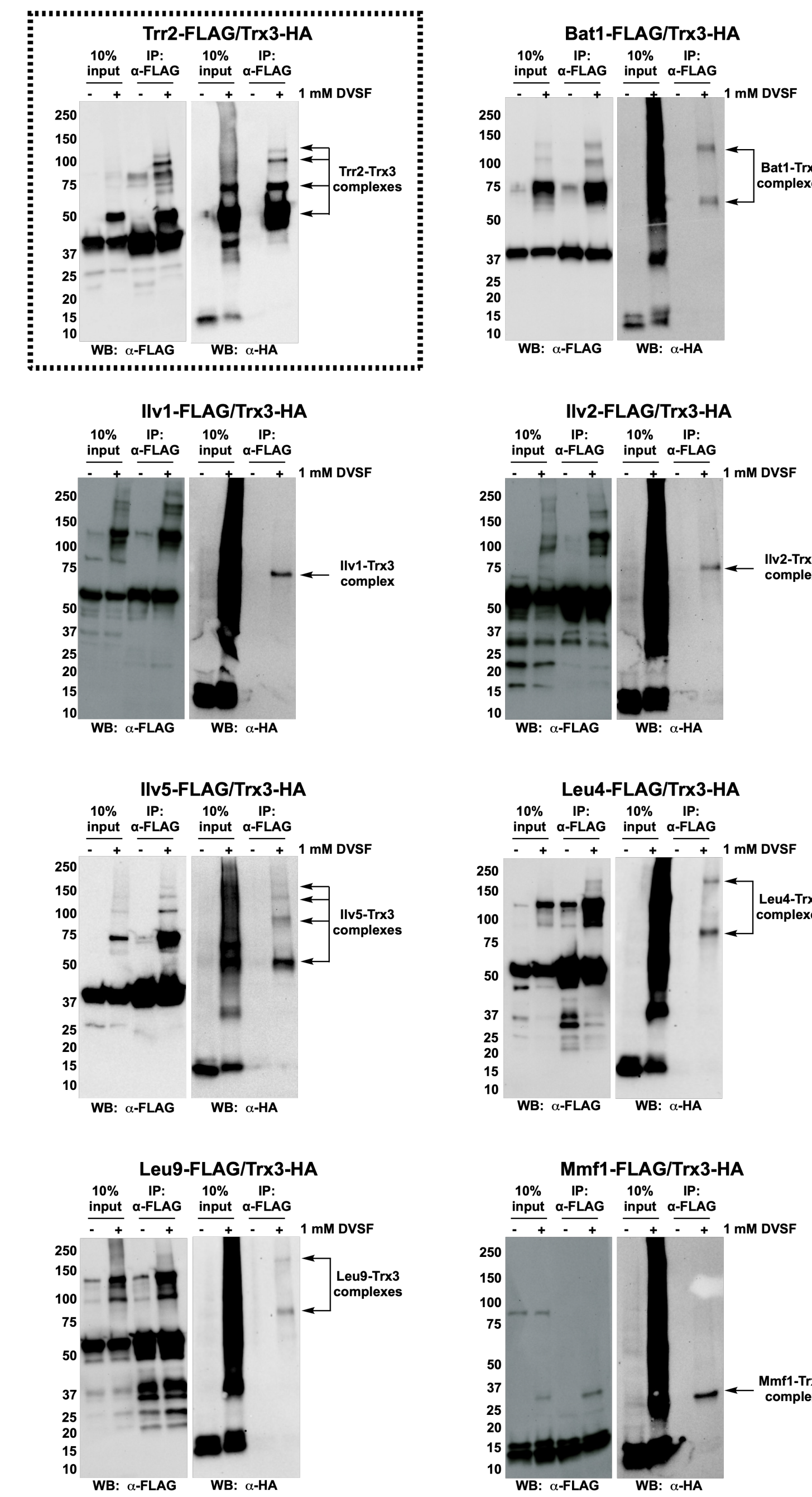
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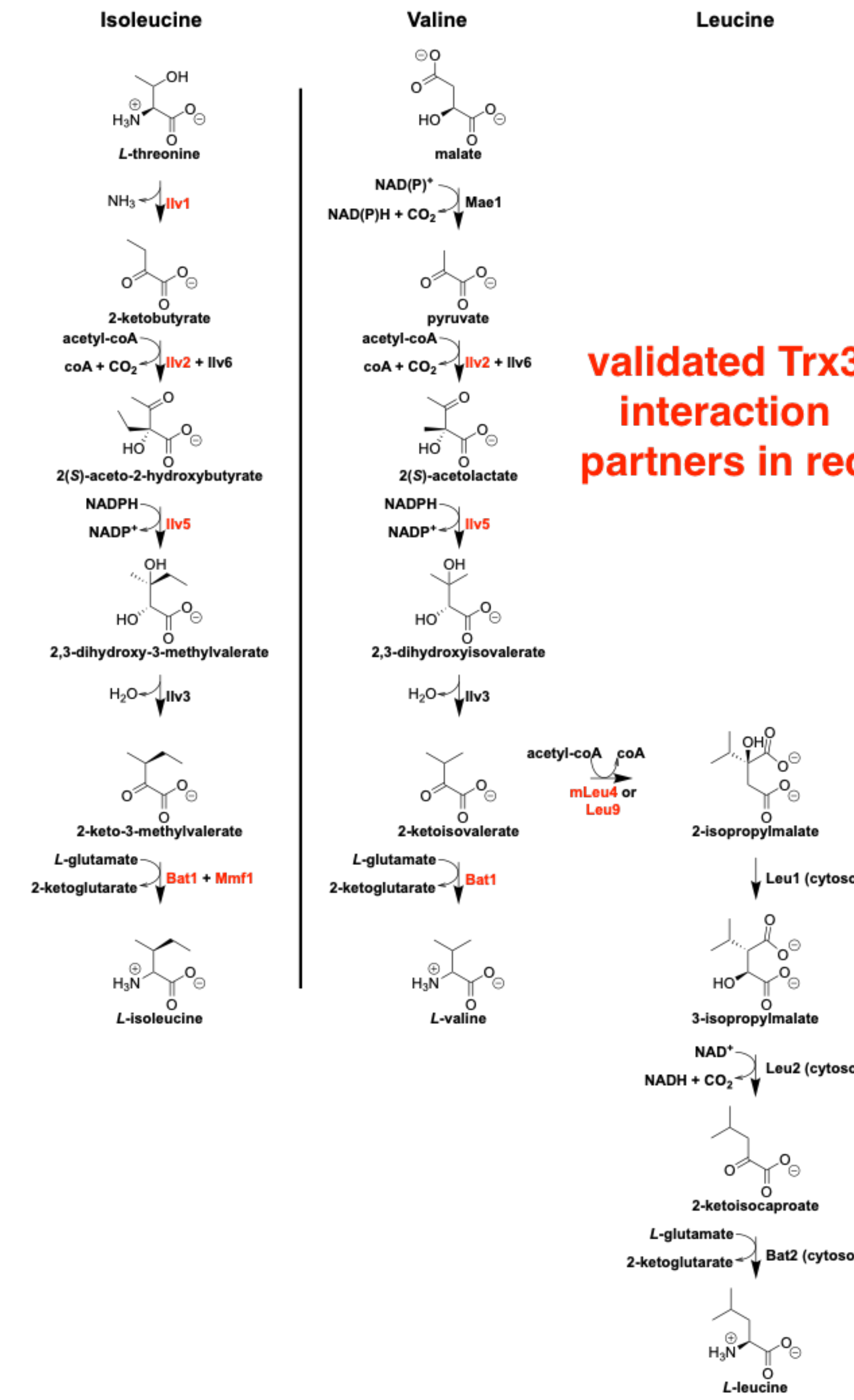
## Abstract

To mitigate the adverse impacts of oxidative protein damage caused by ROS, eukaryotes rely on compartmentalized thioredoxin systems that consist of the protein disulfide reductase thioredoxin and its partner enzyme thioredoxin reductase. Although cytoplasmic thioredoxins have been studied extensively, less is known about the mitochondrial thioredoxins, particularly with regard to their substrate proteins/redox partners. In a baker's yeast model, we identified potential redox partners of the mitochondrial thioredoxin Trx3 using the small, thiol-reactive cross-linker divinyl sulfone (DVSF). Our analysis revealed many proteins involved in amino acid biosynthesis, including members of the branched chain amino acid (BCAA) synthesis pathway, lysine synthesis pathway and the citric acid cycle. My project validated the interactions between Trx3 and key mitochondrial BCAA synthesis enzymes. Subsequent, phenotypic assays revealed that mutants lacking the glutathione reductase and mitochondrial thioredoxin system have an enhanced peroxide sensitivity on BCAA-deficient media. My research also found that the complete mitochondrial thioredoxin system knockout had a severe growth defect when grown on respiratory media. Due to Trx3's diverse array of potential interaction partners, I expanded upon our biological results using an in silico approach via flux balance analysis. These findings indicate that Trx3 could potentially function as a redox regulator of the citric acid cycle.

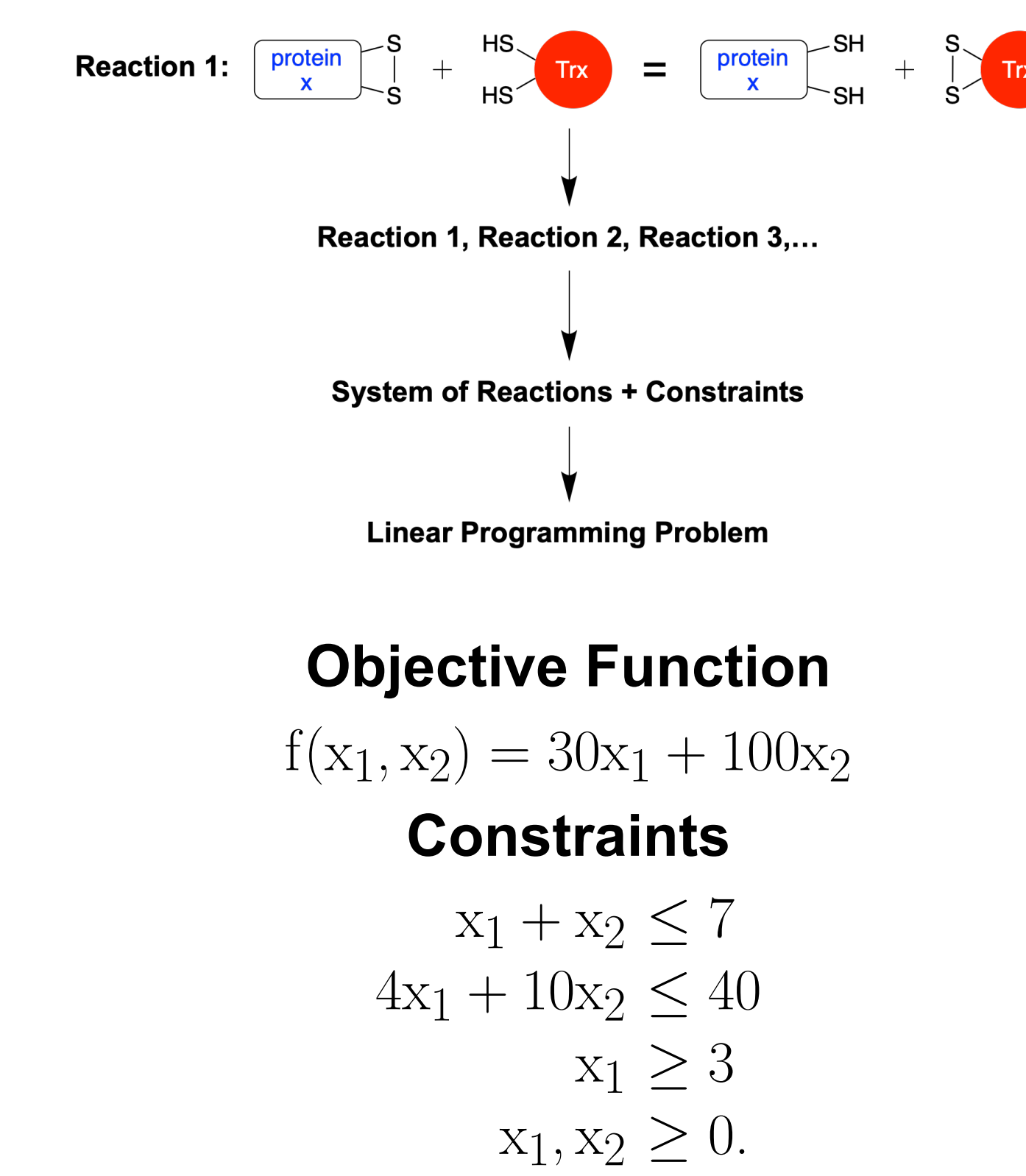
## Validating Trx3 Interactions with Branched Chain Amino Acid Biosynthesis Enzymes by Co-Immunoprecipitation



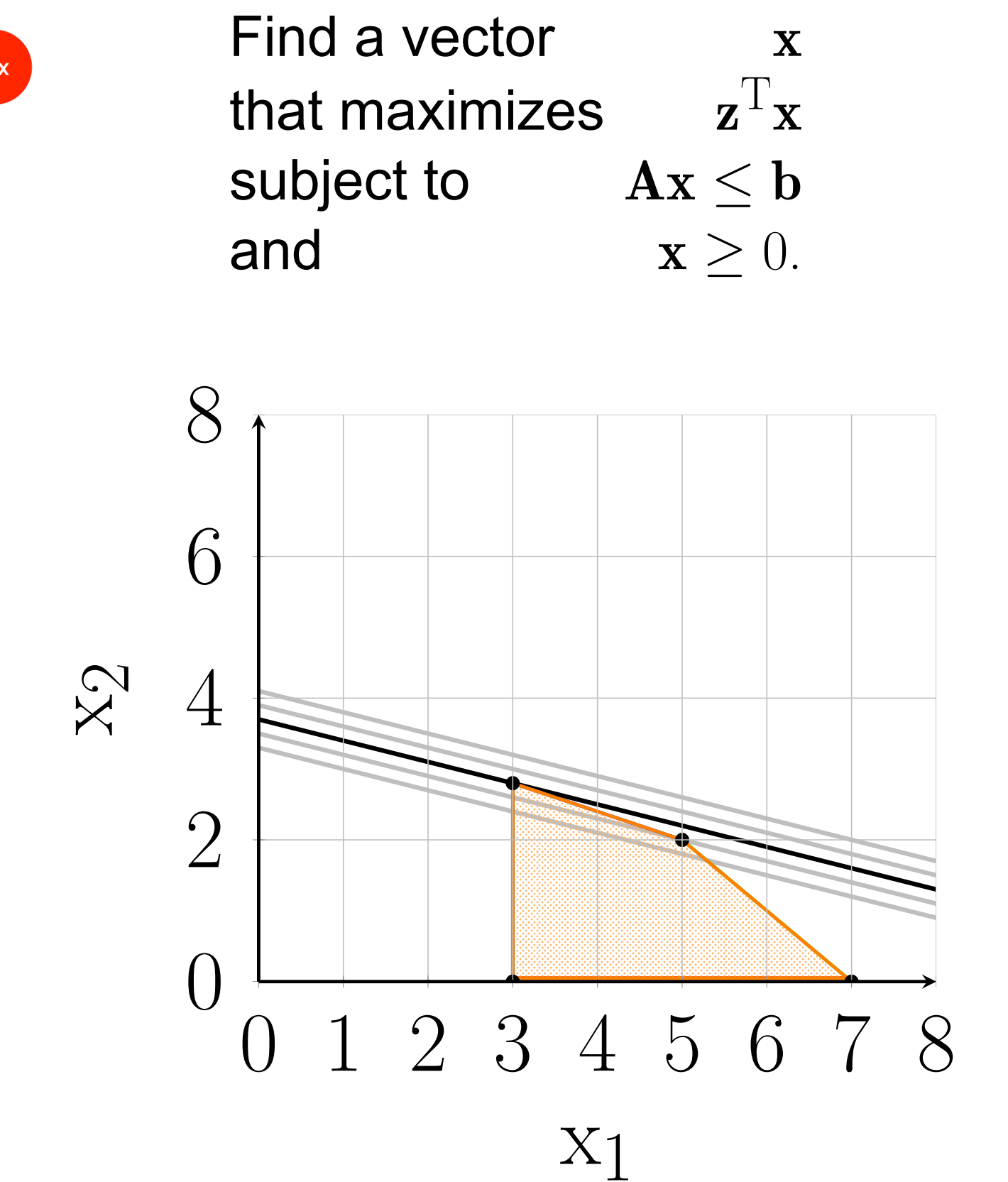
## Branched Chain Amino Acid Synthesis Pathway



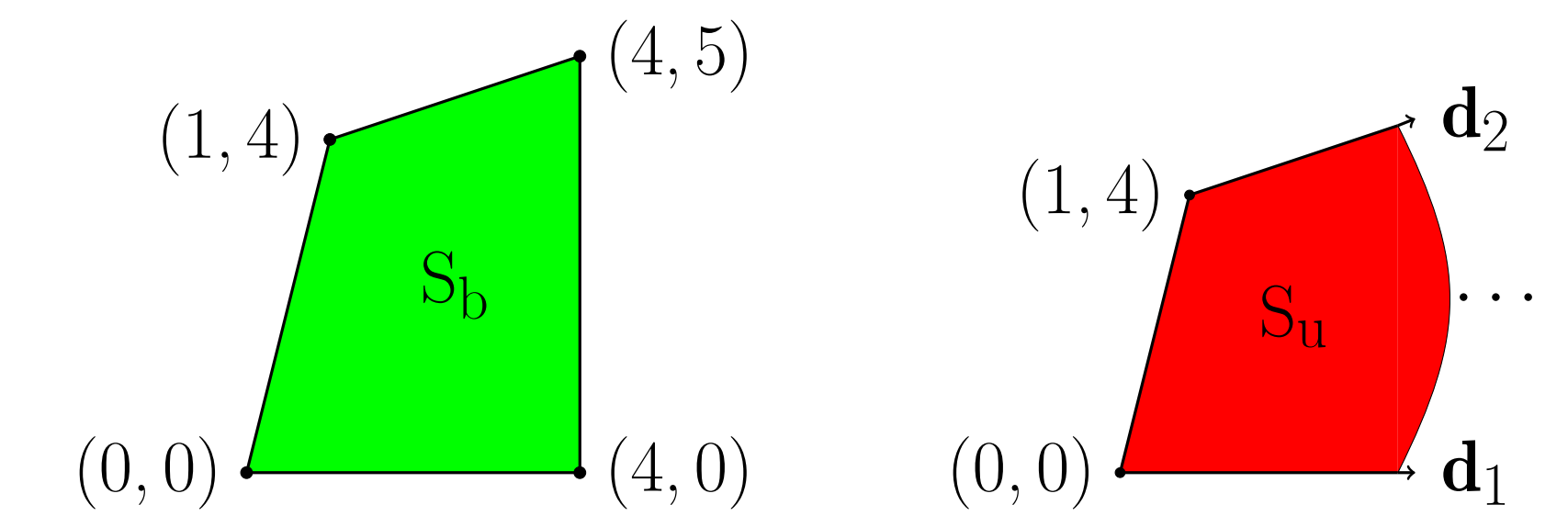
## Flux Balance Analysis



## Linear Programming Problem

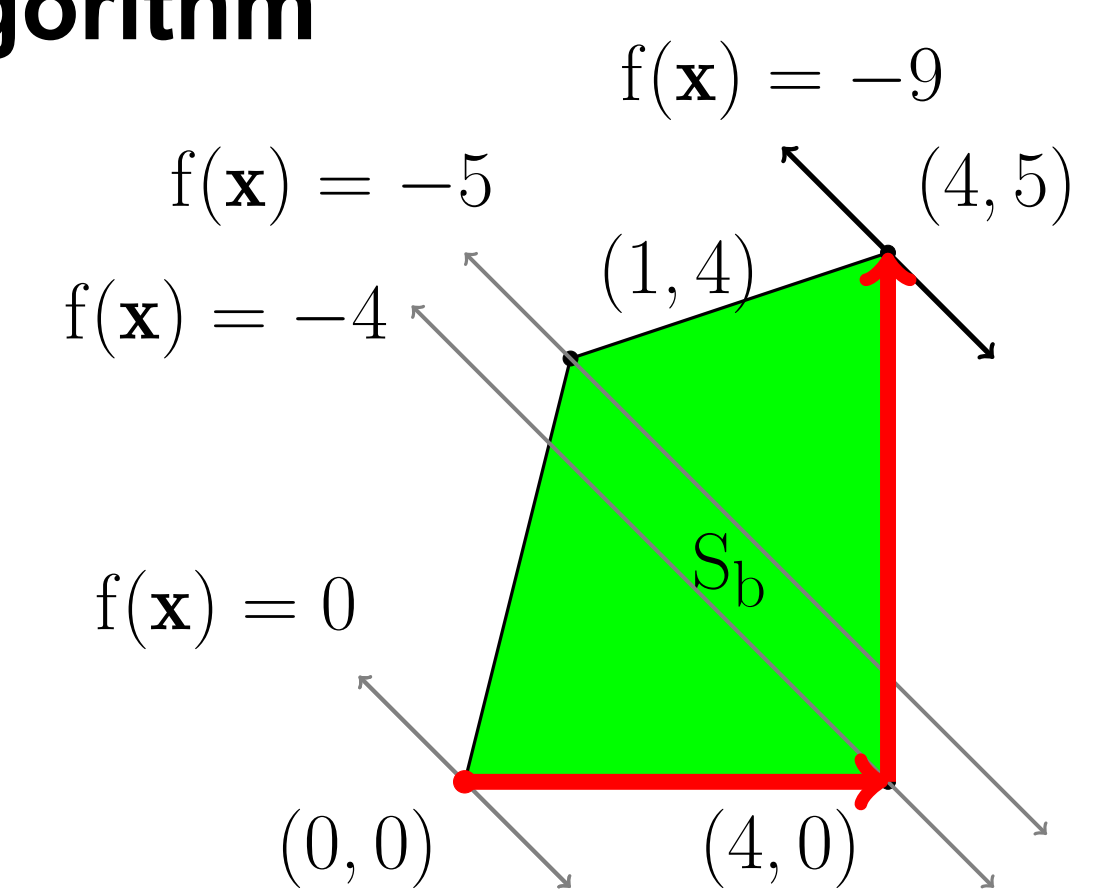


## The Feasible Region of a Linear Programming Problem forms a Polyhedral Set

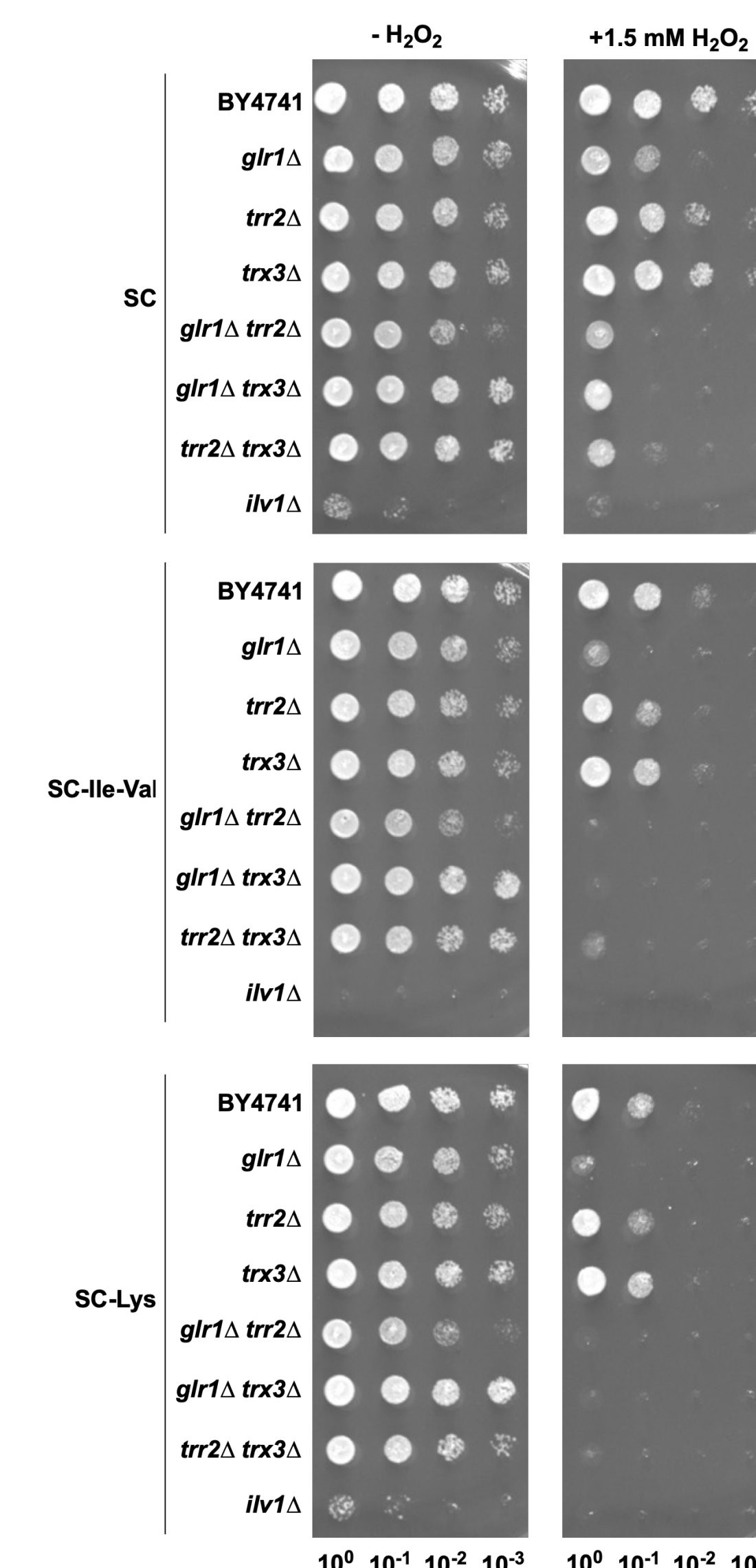


## Simplex Algorithm

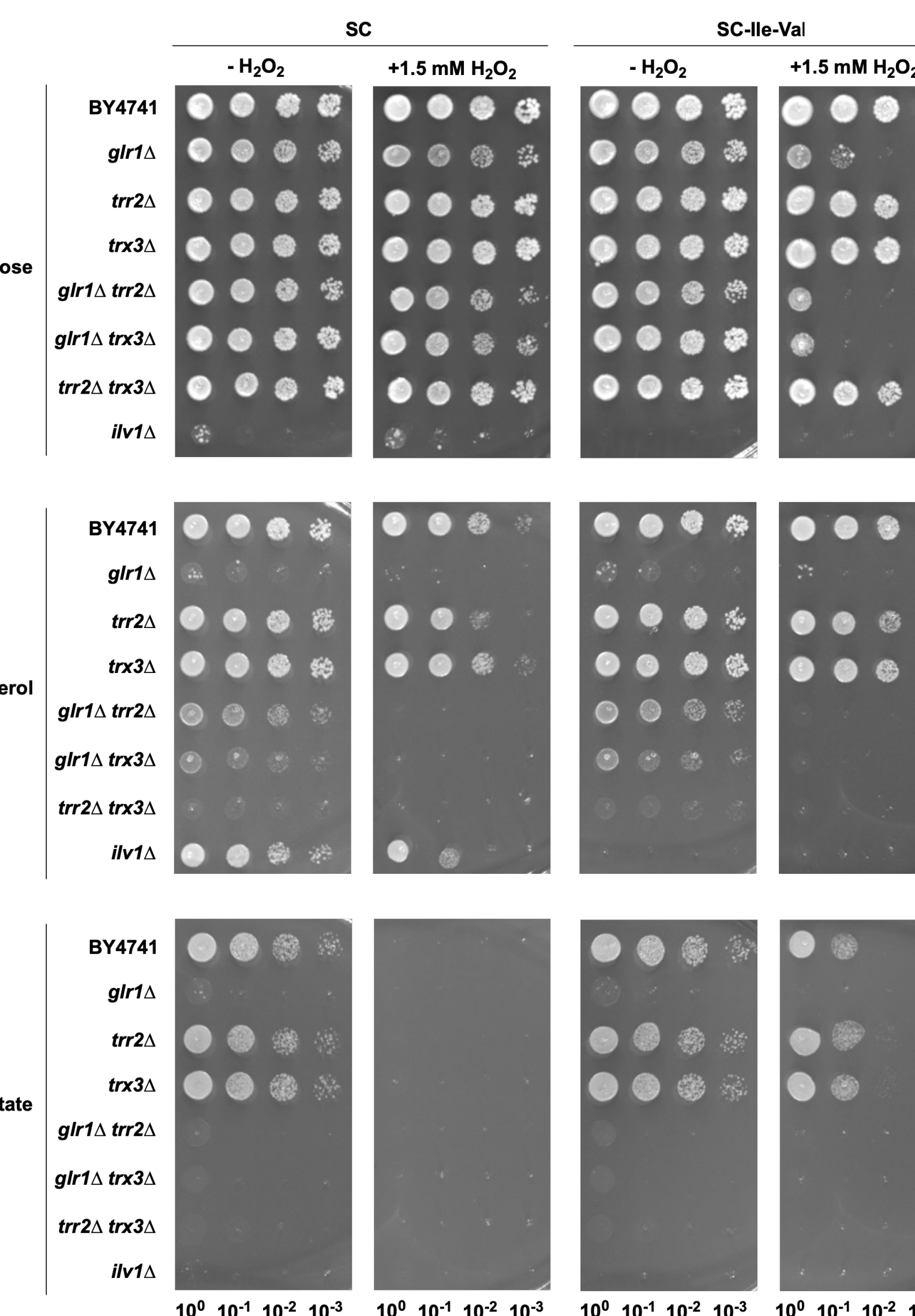
The simplex algorithm systematically moves from extreme point to extreme point to find an extreme point that optimizes the objective function.



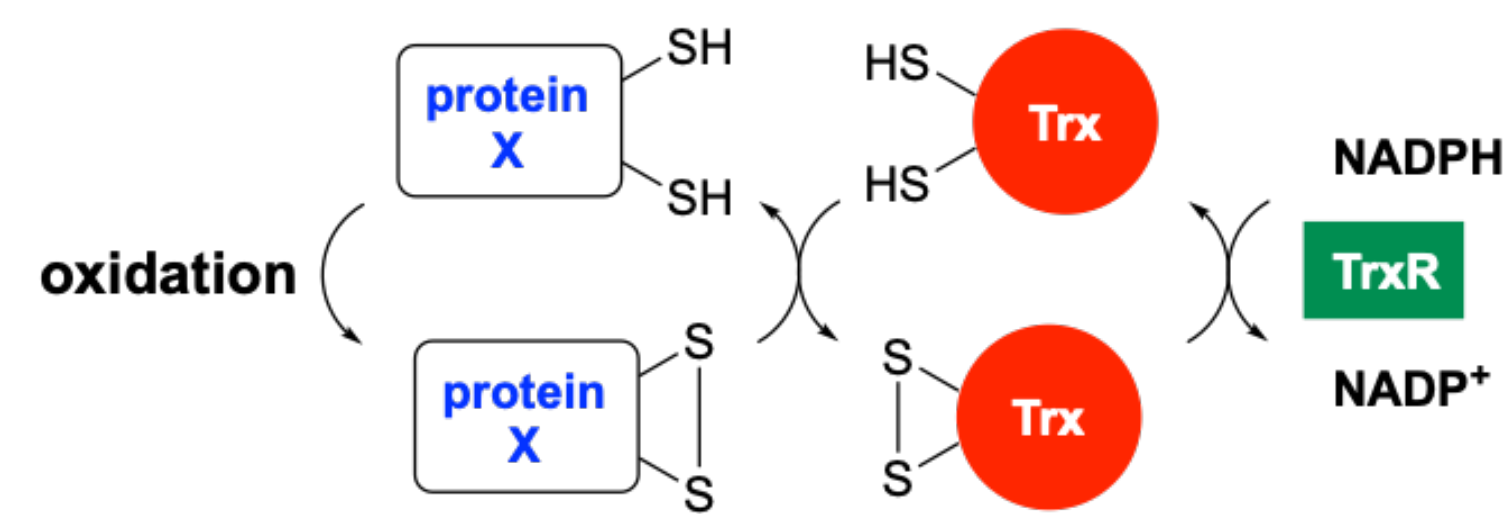
## Disulfide Reductase Mutants Exhibit Enhanced Sensitivity to Oxidative Stress on BCAA- and Lysine-deficient Media



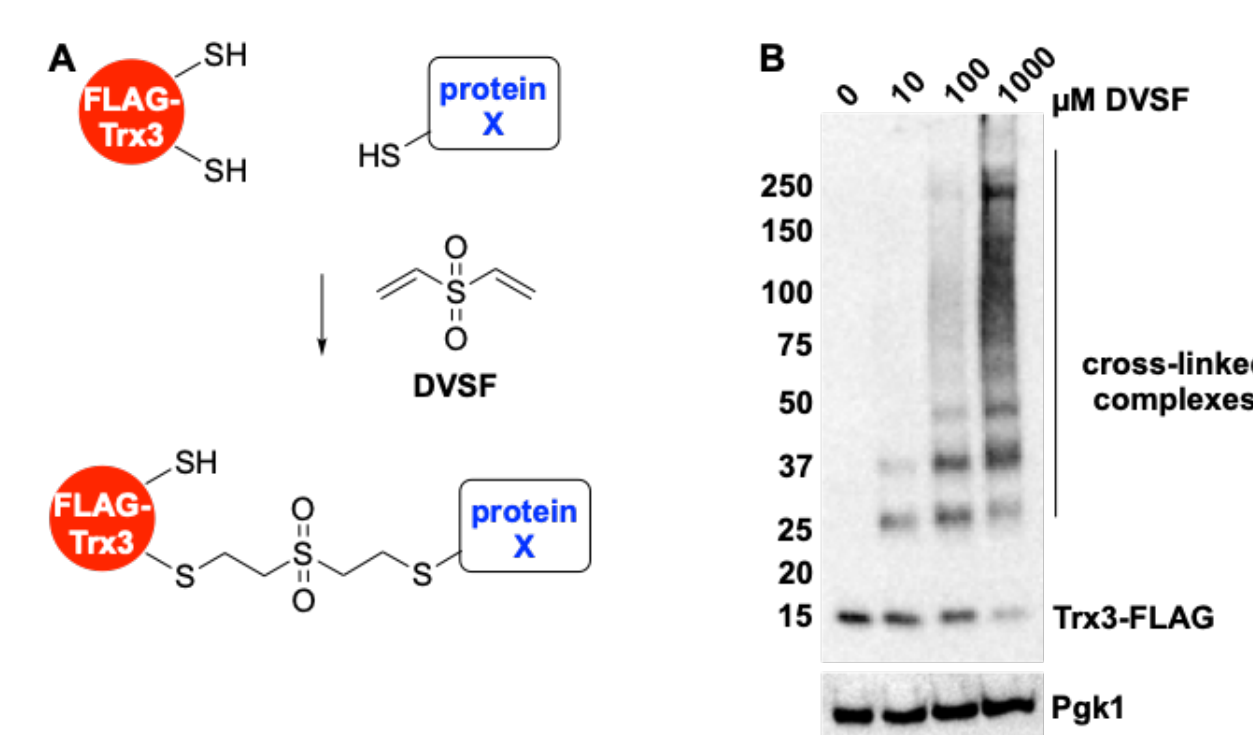
## Deletion of the mitochondrial thioredoxin system results in impaired growth on respiratory media



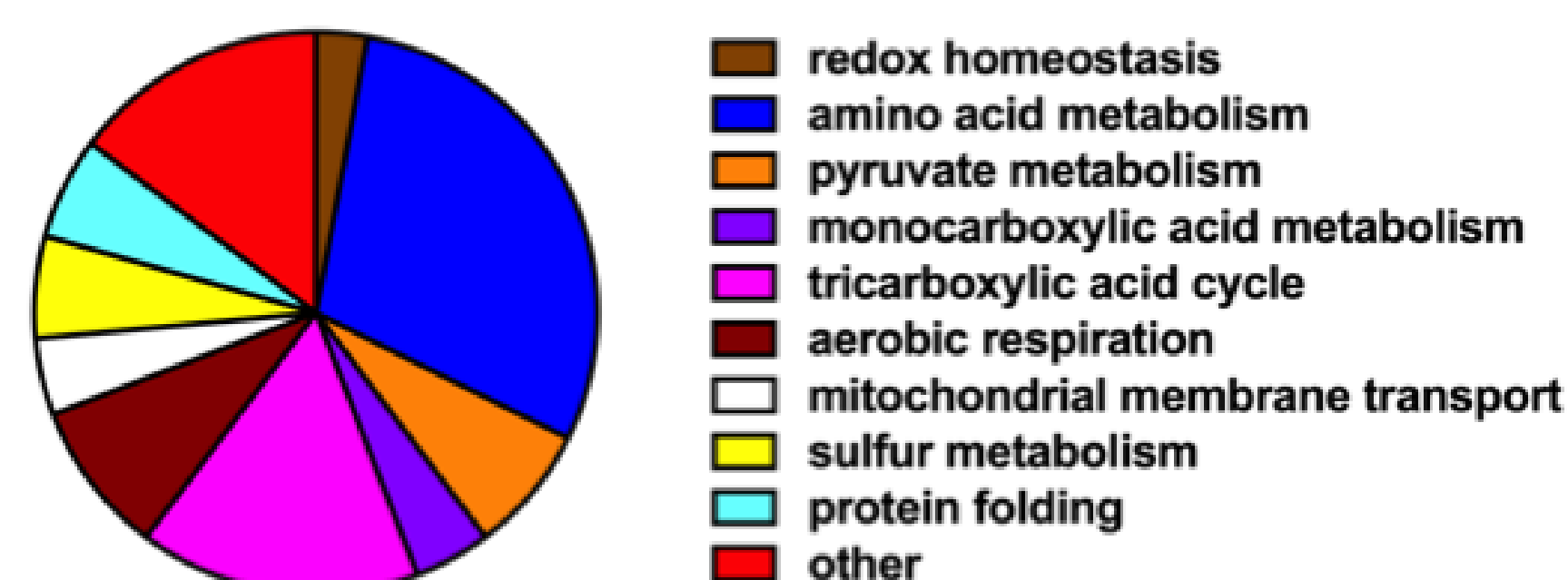
## Thioredoxins Reduce Disulfides in Substrate Proteins by Partnering with Thioredoxin Reductases



## The Yeast Mitochondrial Thioredoxin Trx3 Is a Target of the Thiol-Reactive Cross-Linker Divinyl Sulfone (DVSF)



## Proteins Immunoprecipitating with Trx3 from DVSF-Treated Cells



## Next Steps in Studying Connections between Trx3 and Citric Acid Cycle

- Screen for other phenotypes using *trr2Δtrx3Δ*. Specifically, can yeast transformed with Trx3 rescue the phenotype and if so what cysteine residues are required for this activity.
- Monitor if levels of key citric acid cycle metabolites are altered in yeast mutants lacking Trx3, Trr2, and other mitochondrial proteins involved in disulfide reduction.
- Determine if Trx3 can become glutathionylated and investigate the mitochondrial thioredoxin systems role in nicotinamide adenine dinucleotide metabolism/balance.

## Acknowledgments

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