Understanding Flux Re-routing in Metabolic Networks Through an Analysis of Synthetic Lethal Pairs

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Introduction

Motivation

The main motivation of our study:

- Robustness
- Redundancies - Alternate Pathways
- Higher Order Synthetic Lethals
- Previous studies on Double Lethals (DL) [1]
- Presence of two classes of reaction pairs:
  - Plastic Synthetic Lethals (PSL)
    PSL are double lethals where only one reaction is active. The second reaction becomes active only when the first reaction becomes inactive.
  - Redundant Synthetic Lethals (RSL)
    RSL are double lethals where both the reactions are simultaneously active. Flux rerouting occurs when either reaction becomes inactive.

QUESTIONS

The main questions that are addressed in this study:

1. How do reactions that are present in different metabolic submodules compensate for each other?
2. Can we understand the complex flux reroutings that underlie synthetic double lethals?
3. Can we identify the minimal set of reactions through which flux should be rerouted?
4. Are the inactive reactions more metabolically inefficient than the active ones?
5. What kind of reactions make up the RSL pairs, especially since they are both simultaneously active?

Flux Rerouting

Flux Rerouting in Networks

How does Flux Rerouting occur in Networks?

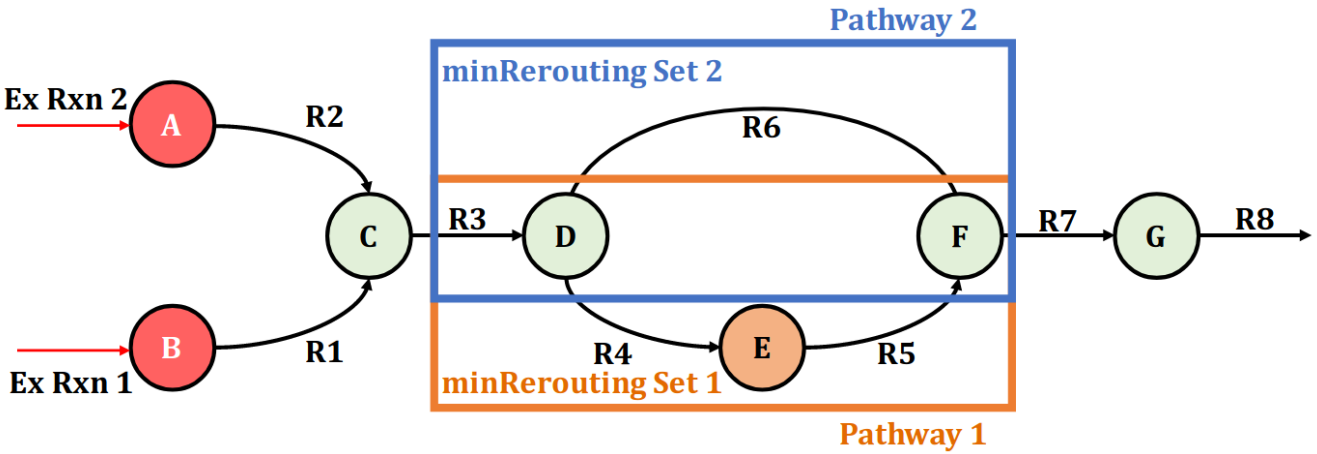

Figure 1: MINREROUTING in a sample metabolic subnetwork. Here, the nodes are metabolites and the edges are reactions.

Rerouting Sets:

- Exclusive for $R4$: $R4$ and $R5$
- Exclusive for $R6$: $R6$
- Common Rerouting Reaction: $R3$

Net Rerouting Set for the Reaction Pair $(R4, R6)$: $R3, R4, R5, R6$

Algorithm

To study the rerouting, minimize the reaction set through which fluxes are rerouted, we propose a constraint based optimization approach - minRerouting.

The mathematical formulation is as follows:

- Solves for flux distributions that satisfy the stoichiometric constraints, maximize the biomass constraint (with a slack of δ)
- Minimizes the number of reactions with varying metabolic flux values.

minRerouting

The generalized p-norm formulation for obtaining the minRerouting of a model, for a given lethal pair is as follows:

1. Step 1: MOMA-like optimization is performed to obtain the optimal flux distributions $v_{\Delta R1}$ and $v_{\Delta R2}$ with minimal flux distance between them.

$$\min \quad \|v_{\Delta R1} - v_{\Delta R2}\|_p \tag{1a}$$

$$s.t \;\; Sv_{\Delta R1} = 0; \;\; Sv_{\Delta R2} = 0; \tag{1b}$$

$$v_{LB} \leq v_{\Delta R1} \leq v_{UB}; \;\; v_{LB} \leq v_{\Delta R2} \leq v_{UB}; \tag{1c}$$

$$v_{\Delta R1,R1} = 0; \;\; v_{\Delta R2,R2} = 0; \tag{1d}$$

$$v_{\Delta R1,bio} \geq (1-\gamma)v^*_{\Delta R1,bio}; \;\; v_{\Delta R2,bio} \geq (1-\gamma)v^*_{\Delta R2,bio}; \tag{1e}$$

2. Step 2: The flux distributions $v_{\Delta R1}$ and $v_{\Delta R2}$, obtained from Equation 1 is analyzed. The reactions that have different flux values in $v_{\Delta R1}$ and $v_{\Delta R2}$ are identified as the rerouting set. The size of the rerouting set, size of the common rerouting set and the total flux difference are analyzed.

minRerouting Results

Our algorithm, minRerouting was used to identify the minimal rerouting set for 11 Genome Scale Metabolic Models (GSMMs). The main take away from the results are as follows:

- 0-norm optimization identified the minimal rerouting set of least size, followed by 1 and 2-norm.
- 0 and 1-norm optimizations resulted in approximately the same range of common minimal rerouting set size.

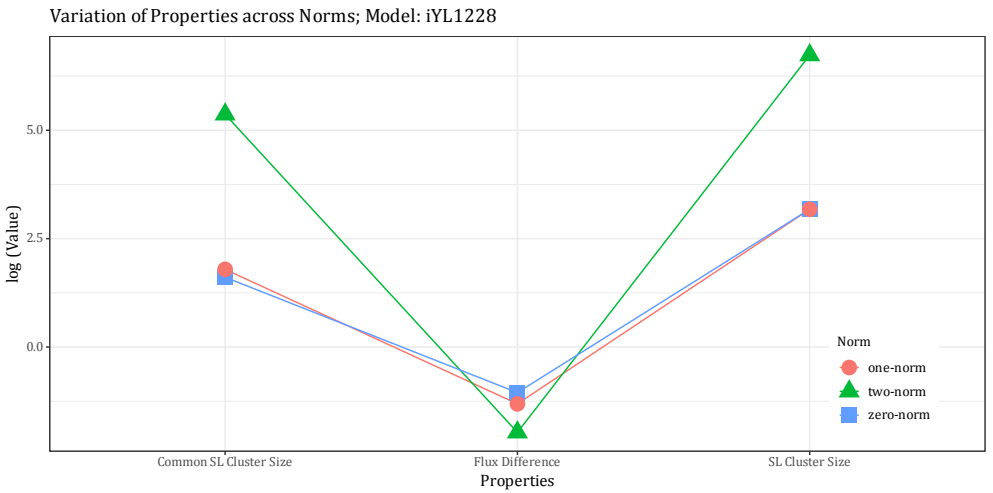

Figure 2: Variation of properties such as minRerouting Cluster Size, Common Cluster Size and Flux Difference. The values presented are in the log scale. Result obtained for iYL1228 (*Klebsiella pneumoniae*)

- While the cluster sizes returned by 2-norm optimization are much higher, the absolute flux difference between the rerouting sets is smallest for 2-norm, followed by 1 and 0-norm.

PSL/RSL activity

Proposed method to classify Synthetic Double Lethals

Using the maximum and minimum fluxes, we can identify reaction pairs where, one reaction can carry Zero Flux, while the other is active. Here, we use the conditional FVA approach:

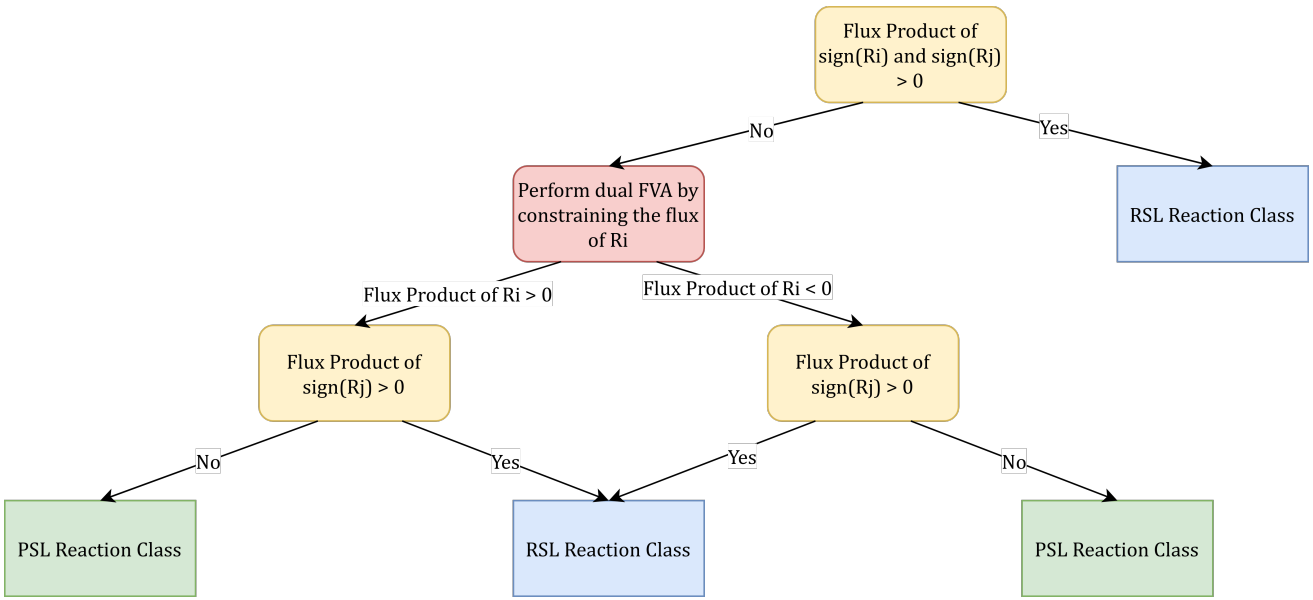

Figure 3: PSL, RSL Classification Procedure Flowcharts. Here, product refers to the product of the signs of the min and max flux values for the reactions.

pFBA and Metabolic Cost

Following the classification, the reaction classes from pFBA were used to identify the metabolic efficiency of individual reactions that comprise a DL. The results obtained for iYL1228 (*Klebsiella pneumoniae*) is as follows:

- 80% of the models had > 90% of RSL pairs belonging to (pFBA Optimal, pFBA Optimal) pairs.

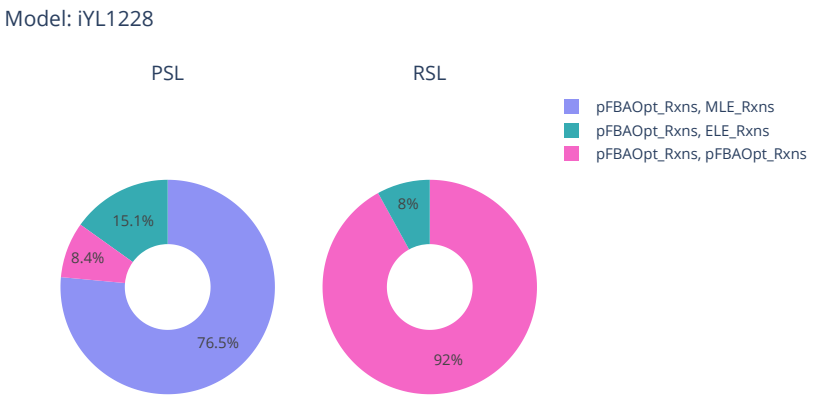

Figure 4: Reaction pair distribution between the RSL and PSL classes.

As pFBA Optimal reactions are considered more metabolically active, than when compared to MLE and ELE reactions, our hypothesis was validated.

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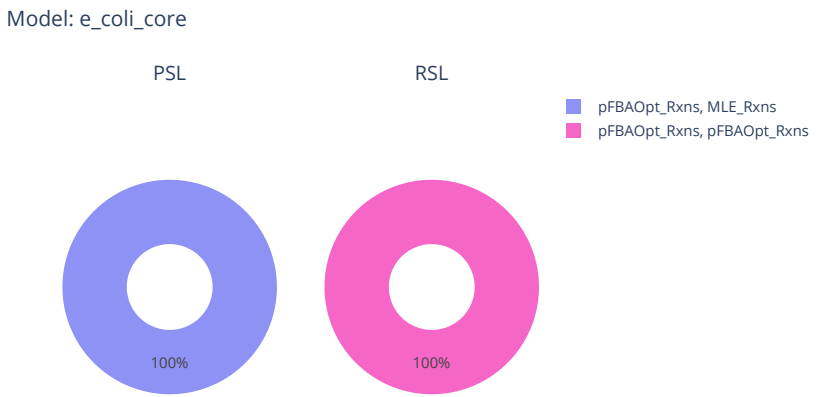

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Discussion

Importance of studying flux rerouting

- minRerouting can also be used to understand the complex metabolic reroutings that occur in several diseases.
- Particularly, in the case of cancer, where the cell reprogram their metabolic activities, rerouting fluxes in such a way that they can continue to proliferate and maintain their malignant properties.
- minRerouting can help us understand these reroutings and perhaps help in finding better therapeutic cures.

References

References

O. Güell, F. Sagués, and M. Á. Serrano, "Essential plasticity and redundancy of metabolism unveiled by synthetic lethality analysis," *PLoS computational biology*, vol. 10, no. 5, p. e1003637, 2014.