Understanding flux re-routing in metabolic networks through an analysis of synthetic lethal pairs

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Robustness in the face of environmental perturbations is essential for microorganisms. At an individual level, this robustness can be attributed to the presence of multiple alternate pathways that have identical metabolic functions. The redundancy introduced by the alternate pathways comprise a large fraction of most metabolic networks. The redundancy in reaction pathways show surprising variance in their distribution. Some of these alternate pathways are very simple, arising due to a gene duplication, while the other alternate pathways could be extremely complex, with compensating reactions spanning different metabolic submodules. A straightforward method of studying these alternate pathways is to identify the higher-order lethals in a metabolic model.

Higher-order synthetic lethals consist of a set of reactions, which when simultaneously deleted result in cell death. However, the deletion of individual reactions does not affect the viability of the cell. When a single reaction is deleted, a complex rerouting of fluxes occurs in the network. Very little is known about how these organisms reroute their fluxes. How do the set of reactions evolve to contribute to one another? How do reactions that are present in different metabolic submodules, compensate for each other?

In order to identify the minimal reaction set, we propose a novel approach, MINREROUTING. We use a $p-norm$ solution approach, where we simultaneously solve for flux distributions that satisfy the stoichiometric constraints, maximize the biomass constraint (with a slack of δ) and also minimize the number of reactions with varying metabolic flux values. Three different MINREROUTING sets were obtained based on the norms employed: sparse (l_0), linear (l_1) and quadratic (l_2). The results obtained were compared across norms and models.

A MINREROUTING set is defined as the minimal reaction set comprising of all the reactions that have a modified flux distribution after deletion of synthetic double lethal reactions. This can be seen in context in Figure 1, which represents a network comprising 9 reactions and 8 metabolites.

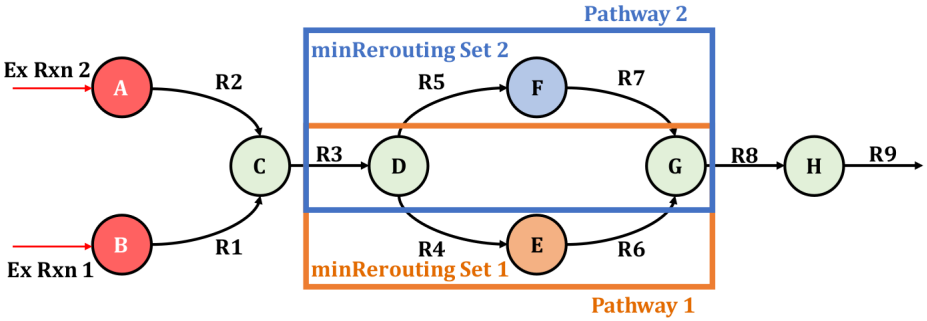

Figure 1: MINREROUTING in a sample metabolic subnetwork. Here, the nodes are metabolites and the edges are reactions.

The network consists of multiple double lethal pairs - (R1, R2), (R4, R5), (R4, R7), (R5, R6), and (R6, R7). Taking the reaction pair (R4, R5) into consideration, we can see that when reaction R4 is active and R5 is deleted

or inactive, all the fluxes will be routed through reactions R4 and R6. Similarly, when reaction R5 is active and R4 is deleted or inactive, all the fluxes will be routed through reactions R5 and R7. In addition to these changes, the fluxes routed through the remaining reactions could vary based on which pathway is chosen. For instance, the flux through reaction R3 could be significantly higher when the R4 pathway is used than when the R5 pathway is used.

Hence, when reaction R4 is active and reaction R5 is inactive or deleted, the rerouting set becomes $\{R3, R4, R6\}$. When reaction R5 is active and reaction R4 is inactive or deleted, the rerouting set becomes $\{R3, R5, R7\}$. The common rerouting set for the double lethal pair $(R4, R5)$ is $\{R3\}$ and the complete rerouting set for the double lethal is $\{R3, R4, R5, R6, R7\}$.

The MINREROUTING set obtained using $l_2(\text{mostly} > 300)$ optimization are the largest in size, while the cluster sizes obtained for $l_0(\text{mostly} < 25)$ and $l_1(\text{mostly} < 60)$ are much smaller in comparison. The common rerouting set sizes for each double lethal pair obtained using l_0 and l_1 is also found to significantly lower (mostly < 5 in l_0) and (mostly < 10 in l_1) than the rerouting set size obtained using l_2 optimization (mostly > 50).

Robustness that is consequential of double lethals have been studied previously. It has been proposed that double lethal pair robustness in an organism can be ascribed to two classes of reaction pairs - Plastic Synthetic Lethals (PSL) and Redundant Synthetic Lethals (RSL) [1]. PSL pairs are reaction pairs where only one reaction is active, while the other reaction is inactive. The second reaction becomes active only when the first reaction is inactive. RSL pairs are reaction pairs where both the reactions are active simultaneously. It has also been shown that these classes are conserved even across different nutrient conditions. The very presence of two distinct reaction pair classes calls for us to analyze the cause behind the selective activation. Are the inactive reactions more "metabolically costly" than the active ones? What kind of reactions make up the RSL pairs, especially when they are both simultaneously active?

In order to investigate the above question, we make use of parsimonious Flux Balance Analysis (pFBA) [2]. In our study, we make use of the reaction classes obtained from pFBA. The possible reaction classes are - Enzymatically Less Efficient (ELE), Metabolically Less Efficient (MLE), Zero Flux, Blocked and pFBA Optimal Reactions. We obtain the classes of each reaction in the double lethals, analyze the reaction distribution in the PSL and RSL classes. We found that 60% of the models under consideration had $> 90\%$ of PSL pairs belonging to (pFBA Optimal, pFBA Optimal) pairs, of which PSL pairs of 40% models were 100% (pFBA Optimal, pFBA Optimal) pairs. As $\mathrm{pFBAOptimal}$ reactions are considered crucial for the growth of an organism, than when compared to MLE and ELE reactions, our hypothesis was validated.

Through our study we show that MINREROUTING can be used to understand the complex metabolic reroutings. This would be particularly helpful to study the rerouting which occurs in several diseases. Especially, 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

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