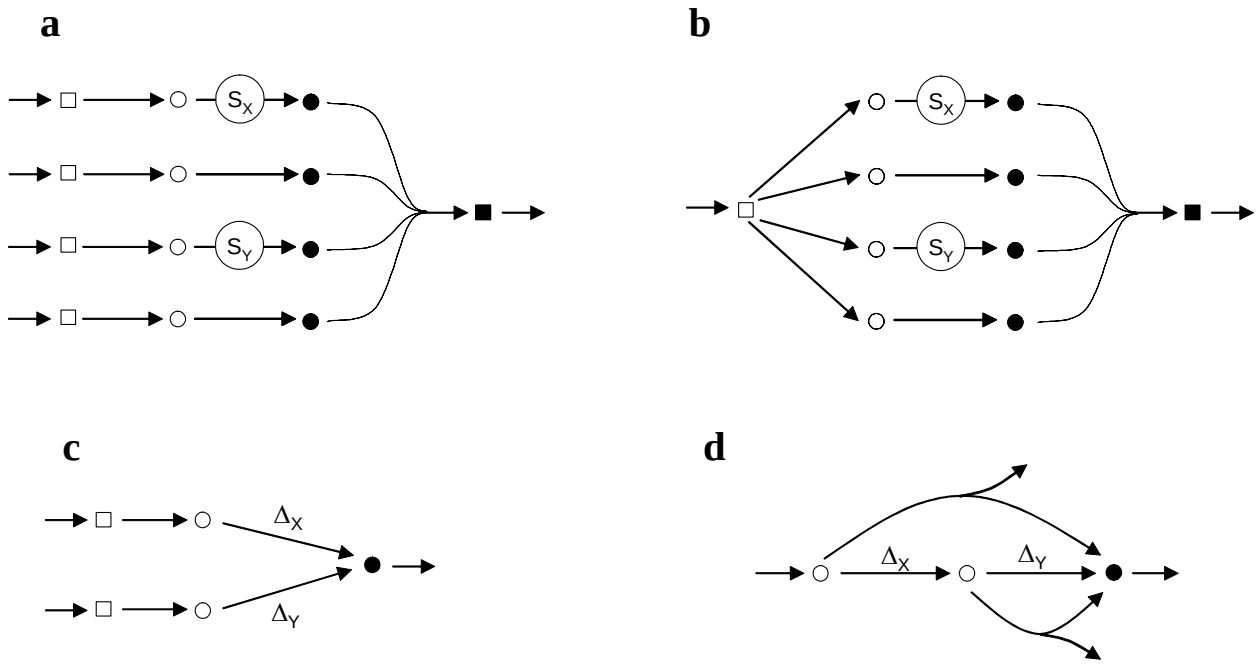
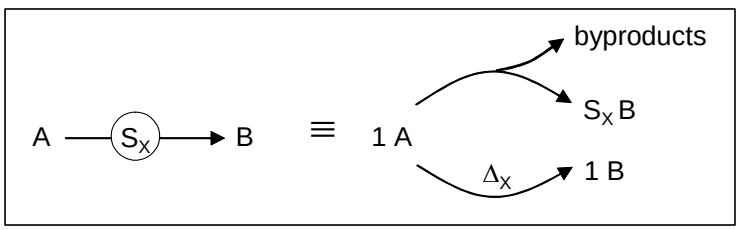
Supplementary Figure 2





Supplementary Fig. 2: Analysis of the effects of double deletions of metabolic enzyme genes in simple metabolic networks demonstrating examples of multiplicative, aggravating and buffering gene deletion interactions. Essential biomass components (full dots, representing for example amino acids or nucleotides) have to be synthesized in order for the cell to be able to grow. Flux Balance Analysis (FBA) assumes an optimal allocation of nutrients (e.g. glucose, empty squares), to reach maximal production of biomass (filled square), through metabolic intermediates (empty dots). The two enzyme deletions are represented by Δ_x and Δ_Y. **(a)** In this idealized case, all biomass components are derived from independent nutrient sources. A deletion (Δ_x) along a pathway is represented by an arrow labelled with the resulting reduced "effective stoichiometry" (S_X, see Box on bottom of the figure). For the depicted single mutants with effects S_X and S_Y, the fitness values (growth, normalized to wild type) can be computed to be simply $W_X=S_X$ and $W_Y=S_Y$. When both deletions are performed, the deletion that has the smaller effect on fitness will be buffered by the one having a larger effect, i.e. $W_{XY}=\min(W_X,W_Y)$. **(b)** A more realistic situation, where the parallel and mutually required pathways demand an optimal allocation of a common nutrient. In this case, the fitness values for the two single deletions and the double deletion can be computed to be: $W_X = 2\,S_X/(1+S_X)$, $W_Y = 2\,S_Y/(1+S_Y)$, and $W_{XY} = 2\,S_X\,S_Y/(S_X+S_Y)$, respectively. In case of small effects of the deletions, i.e. for $\delta = 1-S_X = 1-S_Y << 1$, one obtains an almost multiplicative effect $W_{XY} = (1+\delta^2/4)\,W_XW_Y$. This multiplicative result, helps understanding why mutations in different functional modules normally fall in the central peak of no interactions in Fig.1b. **(c)** A simplified example of synthetic lethality in a case where a single biomass component can be produced through two alternative routes. Disruption of both pathways obviously precludes growth ($W_{XY}=0$), while the single mutants would be viable. Since both pathways produce the same intermediate metabolite, these types of aggravating interactions are normally correlated with functional association between the mutated genes. **(d)** Example of a topology resulting in complete buffering, $W_{XY}=\min(W_X,W_Y)$, between mutations in the same pathway. This kind of topology explains the enrichment of functional association in buffering interactions.

Note that some of the epistatic interaction classes could be viewed in terms of coupling between the corresponding reaction fluxes[33].

(For references, see **Supplementary References** online)