Metabolic flux inference with a combined stoichiometric-thermodynamic model and classical ^{13}C-MFA

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Quantification of cell metabolism is essential to understand cellular behaviour. Metabolic fluxes cannot be experimentally measured, thus need to be inferred from experimental data by means of mathematical models. However, ^{13}C metabolic flux analysis (^{13}C-MFA) [1], as state-of-the-art method, has limitations regarding the size of the solvable metabolic networks and heuristic assumptions on reaction directions [1]. In this work we present a new powerful method for steady- state metabolic flux inference based on thermodynamic constraint-based modelling and classical ^{13}C- MFA.

First, we constrain the metabolic flux space by fitting a Thermodynamic-Stoichiometric Metabolic model (TSM) with measurements on extracellular fluxes and metabolite concentrations. Next, we determine the bounds of the space defined by TSM and experiments through variability analysis. Here we found 78% of reactions to be constrained to one direction. Then, we sample the space through a novel method for uniform sampling of fluxes and Gibbs free energies based on scaled Hit- and-Run [2] and Expectation Propagation [3]. The sampled flux distributions gave interesting insight into differences between two growth conditions in yeast. Finally, we pass the flux samples through a ^{13}C-MFA model to fit with and evaluate against ^{13}C-labelling measurements. Here, we found that similar fitting scores were widely spread across the sampled space, indicating that the labelling did not harbour additional information to further constrain this space.

Thus our work provides a promising method for solving the challenging problem of accurate estimation of metabolic fluxes in large metabolic networks without bias from *a priori* definition of reaction direction.