Thermodynamic principles governing metabolic operation: inference, analysis, and prediction
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Conclusions and future perspectives

Bastian Niebel
This thesis investigated how thermodynamic principles govern metabolic operations. To this end, we developed a thermodynamic metabolic network model for the yeast *Saccharomyces cerevisiae*, which encompasses ~250 metabolic processes, and a comprehensive thermodynamic description for metabolic and growth processes (Chapter 2). Notably, we did not assign any a-priori directionalities on these metabolic processes, but rather constrained their directionalities by the second law of thermodynamics. Then we analyzed with this model experimental data of *S. cerevisiae*, which has been determined from chemostat cultures at different glucose uptake rates (83). Here we inferred the cellular entropy production rate with the thermodynamic metabolic network model from this data (Chapter 2, Fig. 1). We identified that the cells reach a maximum entropy production rate, which, to our surprise, happened at the same critical glucose uptake rate as the onset fermentation.

Based on this finding, we speculated that this limitation might be the cause for the counterintuitive reshuffling in the metabolic operation from respiration at low (with a high ATP yield) and fermentation at high glucose uptake rates (with a low ATP yield). To test this hypothesis, we performed, for different glucose uptake rates, flux balance analysis (FBA)—using the growth rate as an cellular objective—with the thermodynamic metabolic network model and a maximum limit in the cellular entropy production rate. FBA with our model and the maximum limit in the entropy production rate correctly predicted the physiology of *S. cerevisiae* including aerobic-fermentation at high glucose uptake rates, and also predicted the maximum growth rate (Chapter 2, Fig. 2). When we repeated the same optimizations without the limitation in the entropy production rate, the model did not predict aerobic-fermentation and also did not predict a maximum growth rate (Chapter 2, Fig. 2). Besides, exploring the physiological impact of the maximum limit in the cellular entropy production rate, we were also interested in its constraining effect on the intracellular behavior. To this end, we determined the entropy fluxes produced by the different metabolic processes. We found a major reshuffling of the entropy production from respiratory to fermentative pathways, when the maximum limit in the cellular entropy production rate was reached (Chapter 2, Fig. 3). From these analyses, we concluded that the maximum entropy production rate is indeed the cause for the switch between respiration and fermentation and explains maximum growth rates. Further, we concluded that *S. cerevisiae* indeed maximizes its growth rate.

In the future, we plan to show the existence of this limitation and its constraining effect on a multitude of different organisms. To this end, we developed a computational workflow (Chapter 3, Fig. 1) which allows to construct thermodynamic metabolic network models, similar to the one developed for *S. cerevisiae* in Chapter 2. This workflow builds on existing genome scale metabolic reconstructions that are publicly available for a wide array of different organisms (131,132), ranging from bacteria, fungi, mammalian cells, to plants.

Another open question is the underlying mechanistic reason for the maximum cellular entropy production rate. We speculate that the respective cause is linked to the cellular morphology. More specifically the surface to volume ratio of the cell might serve as a limiting factor. We will test this in future by using thermodynamic network models that will be developed for different organisms and determine their maximum entropy production rates. Then we determine for each of these organism the cellular surface to volume ratios. From this comparison, we will be a step further to gain fully insight into the mechanistic principles behind the
limitation in the entropy production rate.

Further, we used the thermodynamic metabolic network model to investigate the relationship between thermodynamic principles and enzyme kinetics. To investigate this relationship, we inferred intracellular metabolic fluxes and backward rates by combining the thermodynamic network model with isotopomer balances in a novel statistical method (Chapter 4, Fig. 1). This method allows to infer metabolic fluxes based on a multitude of different experimental data, i.e. extracellular rates, metabolomics data, standard Gibbs energies of reactions, and isotopomer patterns from $^{13}$C-labeling. Besides inferring intracellular metabolic rates for *S. cerevisiae*, we used our method and inferred for 34 reactions their Gibbs energies and their ratio of the forward over the backward enzymatic rates (Chapter 4. Fig. 4). By comparing the experimentally inferred correlation between the Gibbs energies and these ratios with a thermodynamic theory, we could identify possible enzymatic regulatory control.

To test this enzymatic control, future research should aim at identifying additional ratios between the forward and the backward rate by increasing the quantity of the measure isotopomer patterns using advanced analytical techniques (158). Thereby we will establish a correlation between these ratios and the Gibbs energy of reactions for a large amount of different enzymes. Then, we will determine these correlations for the different yeast strains at different glucose uptake rates (156) and integrating proteome data. From this multi-strain comparison, we will be able to identify the *in vivo* enzymatic control (by inferring kinetic parameters from the complete data, i.e. forward/backward rates, metabolite and protein levels).

Overall, in this thesis we investigated and showed the importance of thermodynamic principles on metabolic operations. We developed statistical methods to infer model parameters and intracellular quantities from experimental data, to analyze intracellular behaviors using these statistical methods, and to predict new metabolic behaviors with high unprecedented accuracy. This excellent predictive capabilities will not only facilitate fundamental research of metabolism, but also impact the development of new cell factories in biotechnology industries.