Application of irreversible thermodynamics to determine the influence of cell mimicking conditions on the kinetics of equilibrium reactions of the glycolysis

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One of the most investigated biochemical pathways is the glycolysis that consists of 10 consecutive enzyme catalyzed reactions. In these investigations, the model of Michaelis-Menten is most frequently used, which describes the reaction with a simplistic mechanism that neglects the back-reaction of product to the enzyme-substrate complex. However, the glycolysis pathway contains several equilibrium reactions that cannot be evaluated with the Michaelis-Menten model. In addition, most data were measured under standard conditions, which do not correspond to conditions in the cell. Therefore, it is necessary to generate new kinetic data for these reactions.

In our work several equilibrium reactions of the glycolysis were calorimetrically investigated under cell mimicking instead of standard conditions. In addition, a new thermokinetic approach based on irreversible thermodynamics was used, overcoming the weaknesses of the conventional Michaelis-Menten kinetics. Other models overcoming this weakness also exist (e.g. the Hoh and Cord-Ruwisch approach or the reversible Michaelis-Menten mechanism), but our approach needs fewer fitting parameters and better reflects the natural cell conditions. The influence of different cell mimicking conditions (e.g. temperature, pH-value, ionic strength, concentration of magnesium, and crowding agents) on the thermodynamic and kinetic parameters was determined in detail. Our results show that the biggest influence originates from the crowding effect that dramatically slows the reaction due to the size exclusion effect, while all other parameters also have an impact.

The long term goal of this project is to apply the determined thermodynamic and kinetic data to a new thermodynamic feasibility analysis to explore the predictive potential of thermodynamics for systems biology.