# Analysis of short-term dynamic *in vivo* response of *E. coli* to alternative substrate perturbations

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Microorganisms have been used as 'cell factories' in biotechnological industry for centuries. In the last two decades research fronted to improve industrial bioprocesses with metabolic engineering strategies. This approach can be fastened with the help of genome scale kinetic models allowing the identification of premium gene targets for metabolic pathway engineering, before actually performing the tedious genetic engineering work. Kinetic modeling of metabolism requires experimental information on *in vivo* kinetics that means information on fluxes, enzyme levels and metabolite concentrations under a number of different conditions. In fast short-term dynamic experiments (< 200 s) enzyme levels can be assumed constant and only the measurements of concentrations of intracellular metabolites and fluxes are required for kinetic modeling purposes. In the present work rich information was gathered on *in vivo* kinetics of central metabolism of *Escherichia coli* required for future kinetic modeling. For this purpose aerobic glucose-limited *E. coli* K12 MG1655 culture grown at dilution rate of 0.1 $h^{-1}$ in a chemostat was perturbed with different carbon sources in the BioScope (adapted for *E. coli*), which is a mini plug-flow reactor for carrying out pulse response experiments outside the reactor, to observe the metabolite concentration dynamics. The pulse substrates were selected in a way that the carbon source would be uptaken from top (glucose), middle (pyruvate) and bottom (succinate) of the central carbon metabolism. During the transient possible oxygen-limitation was avoided by using enriched air. Responses of wide range of intracellular metabolites were measured with a newly developed differential method which avoids metabolite leakage during quenching and subsequent sample treatment. Following, the kinetic mechanisms and equilibrium relationships permitting the sudden switch of *E. coli* to each different condition are discussed.