Introduction

Chinese Hamster Ovary (CHO) cells are typically grown on both glucose and glutamine as the main carbon sources. In a previous publication [1], the authors used FACS-assisted evolution to establish a CHO-K1 cell line growing on glucose as the sole carbon source. This new cell line grows at a comparable rate to the original one and apparently compensates for the loss of glutamine by increased uptake of glutamate and aspartate. Now, we are interested in identifying the changes in CHO metabolism that occurred due to the adaptation to glutamine-free media. The high connectivity of metabolism requires system-wide analyses to identify such changes.

Methods

The concentrations of major medium components were measured and uptake / secretion rates calculated for cells adapted to different concentrations of glutamine (Fig. 1). We used exponential-phase transcriptomics data, the recently created genome-scale metabolic model iCHO1766 [2] and the GIMME (Gene Inactivity Moderated by Metabolism and Expression) algorithm [3]:

\[
\text{minimize:} \quad \sum c_i |v_i| \\
\text{subject to:} \quad S_V = 0 \\
\quad \quad a_i < v_i < b_i \\
\text{with} \quad c_i = x_{\text{cutoff}} - x_i, \quad \text{for} \quad x_{\text{cutoff}} > x_i \\
\quad \quad c_i = 0, \quad \text{otherwise}
\]

The cut-off for the gene expression values, \( x_i \), was set to the 25th percentile. Furthermore, biomass production was required to be at least 90% of the value obtained via standard FBA.

Flux Distributions

Figure 1: Uptake and secretion rates of cells adapted to different concentrations of glutamine. Lack of glutamine is compensated by a higher uptake of glutamate and aspartate.

Figure 2: Flux distribution in the TCA in cells grown in medium without glutamine (top) and in medium containing 8mM glutamine (bottom). Note that for clarity of the graphical representation some reactions were lumped and some cofactors omitted.

Preliminary Results

For cells grown without glutamine, the citrate cycle is fed via pyruvate (PCm) and acetyl-CoA (CSm). When glutamine is provided, it enters the TCA via alpha-ketoglutarate, as expected. The route, however, is not via glutamate (which also yields two molecules of ammonia), but rather via 2-oxoglutaramate, which produces one molecule of alanine from pyruvate and one molecule of ammonia. It is not clear yet whether this is an error in the model or due to the lack of regulation. In both scenarios, fumarate and malate form a cycle with conversion reactions in opposite directions in the cytosol and mitochondria, respectively; balancing via an antiporter (r0822) leads to a comparatively small net flux.