### Introduction

**Chinese Hamster Ovary (CHO) cells** are the pharmaceutical industry’s major working horse for the production of therapeutic proteins, mainly due to their ability to perform human-like post-translational modifications. In order to gain a deeper understanding and identify potential bottlenecks of CHO metabolism, we are currently working on refining a recently established genome-scale metabolic model of CHO\(^1\). One important input for accurate predictions\(^2\) is the **maintenance energy**, which is the **non-growth associated energy** needed to sustain intracellular processes that are independent of growth and generation of the product of interest (e.g. protein turnover)\(^3\). It is an important input into the metabolic model, because it is necessary to know how much of the resources the cell needs just to stay alive and how much can be used for growth and protein production.

In microbial systems, the maintenance energy can be determined by measuring the steady-state uptake of glucose and oxygen and secretion of CO\(_2\) at different growth rates. From these measurements the ATP turnover can be inferred directly and extrapolating to a hypothetical growth rate of zero yields the maintenance energy. Since CHO cells grow on nutrient-rich media and use **multiple carbon sources**, this method is not directly applicable.

Instead, we will calculate the ATP turnover at each growth rate using the metabolic model. This requires the determination of uptake and secretion rates for all major nutrients and waste products, such as glucose, amino acids, lactate and ammonia.

### Methods

CHO-K1 cells are grown in chemically defined CD-CHO medium supplemented 0.2% Anti-clumping agent and 8 mM glutamine (CHO-K8). During exponential phase, feeding is initiated and a constant volume of 270 mL is set. The culture is left to equilibrate for at least 5 volume exchanges. We will take samples at five different dilution rates, expressed as volume exchange per day (VEX/day).

**Sampling includes:**

- Cell growth measured 3x/day using Vicell
- Amino acid samples 2x/day
- Multiple metabolites 3x/day via Bioprofile
- RNaseq 2x/dilution rate

The feed medium is exchanged every 5 days due to glutamine degradation observed in previous lab-internal studies.

### Conclusions and Outlook

- **Continuous fermentation of CHO-K8 with viabilities >90% was completed for 0.7 and 0.55 VEX/day. A second process to replicate 0.7 VEX/day and provide data for anew dilution rate of 0.42 VEX/day is currently running.**
- **Statistical analysis is going to be performed do determine if steady state was reached.**
- **Ongoing research encompassing 2-3 more dilution rates will enable the calculation of maintenance energy.**
- **We aim to compare host- (K1) and producer cell lines (DUXB11 expressing EpoFc) using different glutamine supplementation (0 mM/8 mM) in order to get a comprehensive overview. This will improve model predictions for the growth rate as well as protein production.**

### References