# ﻿Introduction

# Braunstein, Alfredo, Anna Paola Muntoni, and Andrea Pagnani. “An Analytic Approximation of the Feasible Space of Metabolic Networks.” *Nature Communications* 8, no. 1 (April 6, 2017): 1–9. <https://doi.org/10.1038/ncomms14915>.

“﻿Assuming a steady-state condition within a cell, metabolic fluxes satisfy an underdetermined linear system of stoichiometric equations. Characterizing the space of fluxes that satisfy such equations along with given bounds (and possibly additional relevant constraints) is considered of utmost importance for the understanding of cellular metabolism. Extreme values for each individual flux can be computed with linear programming (as flux balance analysis), and their marginal distributions can be approximately computed with Monte Carlo sampling”

# Fernandez-de-Cossio-Diaz, Jorge, and Roberto Mulet. “Maximum Entropy and Population Heterogeneity in Continuous Cell Cultures.” *PLOS Computational Biology* 15, no. 2 (February 27, 2019): e1006823. <https://doi.org/10.1371/journal.pcbi.1006823>.

# Fernandez-de-Cossio-Diaz, Jorge, Kalet Leon, and Roberto Mulet. “Characterizing Steady States of Genome-Scale Metabolic Networks in Continuous Cell Cultures.” *PLoS Computational Biology* 13, no. 11 (2017): 1–22. <https://doi.org/10.1371/journal.pcbi.1005835>.

“Biotechnological products are obtained by treating cells as little factories that transform substrates into products of interest. There are three major modes of cell culture: batch, fed-batch and continuous. In batch, cells are grown with a fixed initial pool of nutrients until they starve, while in fed-batch the pool of nutrients is re-supplied at discrete time intervals. Cell cultures in the continuous mode are carried out with a constant flow carrying fresh medium replacing culture fluid, cells, unused nutrients and secreted metabolites, usually maintaining a constant culture volume.”

“﻿While at present most biotechnology industrial facilities adopt batch or fed-batch processes, the advantages of continuous processing have been vigorously defended in the literature1–5,and currently some predict its widespread adoption in the near future6.”

# Hädicke, O., V. Lohr, Y. Genzel, U. Reichl, and S. Klamt. “Evaluating Differences of Metabolic Performances: Statistical Methods and Their Application to Animal Cell Cultivations.” *Biotechnology and Bioengineering* 110, no. 10 (2013): 2633–42. <https://doi.org/10.1002/bit.24926>.

﻿“Mathematical modeling of animal cell growth and metabolism is essential for the understanding and improvement of the production of biopharmaceuticals. Models can explain the dynamic behavior of cell growth and product formation, support the identification of the most relevant parameters for process design, and significantly reduce the number of experiments to be performed for process optimization.”

# De Martino, Andrea, and Daniele De Martino. “An Introduction to the Maximum Entropy Approach and Its Application to Inference Problems in Biology.” *Heliyon* 4, no. 4 (April 2018): e00596. <https://doi.org/10.1016/j.heliyon.2018.e00596>.

“A cornerstone of statistical inference, the maximum entropy framework is being increasingly applied to construct descriptive and predictive models of biological systems, especially complex biological networks, from large experimental data sets. Both its broad applicability and the success it obtained in different contexts hinge upon its conceptual simplicity and mathematical soundness.”

“It is not unfair to say that the major drivers of biological discovery are currently found in increasingly accurate experimental techniques, now allowing to effectively probe systems over scales ranging from the intracellular environment to single cells to multi-cellular populations, and in increasingly efficient bioinformatic tools, by which intracellular components and their putative interactions can be mapped at genome and metabolome resolution. Yet, at least to some degree, these approaches still appear hard to integrate into quantitive predictive models of cellular behavior. In a sense this is not surprising. Even if we possessed detailed information about all sub-cellular parts and processes (including intracellular machines, their interaction partners, regulatory pathways, mechanisms controlling the exchange with the medium, etc.), it would be hard to build a comprehensive mechanistic model of a cell, and possibly even harder to infer deep organization principles from it. In large part, this is due to the fact that cells have an enormous number of degrees of freedom (e.g. protein levels, RNA levels, metabolite levels, reaction fluxes, etc.) which, collectively, can take on an intimidatingly large number of physicochemically viable states.”

Cell culture-derived products are a major part of the multi-billion biotechnological industry portfolio. Although advantages of continuous cultures have been commonly mentioned in the literature, the preferred use of these technics over batch or fed-batch struggle with the complexity displayed by these systems, i.e., hysteresis, multistability or sharp transitions between metabolic states. Mathematical models could be then used to suggest strategies to lead the system to the desire, more productive, state. In this sense, common approaches usually take strong assumptions such as cell population homogeneity, although the impact of heterogeneity in culture features is not well understood. To gain insight into this subject, in a recent work, the maximum entropy principle (MaxEnt) was used to model the phenotypic distribution of a heterogeneous population of cells in a chemostat (continuous culture device). The goal of the present work is to face this formalism to literature-available experimental data derived from chemostat cultivations. We compared the data with observables inferred from two models, one using Flux Balance Analysis (FBA), an industry-standard that assume culture homogeneity, and the MaxEnt approach. We used publicly available genome-scale metabolic networks (GEM) to model the metabolism of the cell line for each culture. Partial results, using data from Escherichia coli (EColi K-12) and a human-derived cell line (AGE1.HN.AAT) cultures, suggest better agreement between model and experiment for the MaxEnt framework, more evidently for the case of EColi K-12. Although we encountered important limitations, like the lack of context-specific GEMs for each cell line, the results could be an indication that the MaxEnt approach, and the inclusion of culture heterogeneity, is a powerful tool for modeling cell metabolism in continuous cultures.

Constraint-based technics as Flux balance Analysis (FBA)