Imperial College London
Sargent Centre for Process Systems Engineering

Soft sensing of intracellular states in bioprocessing with Ensemble Kalman Filters

18th international EnKF workshop (2023)
Norwegian Centre for Data Assimilation

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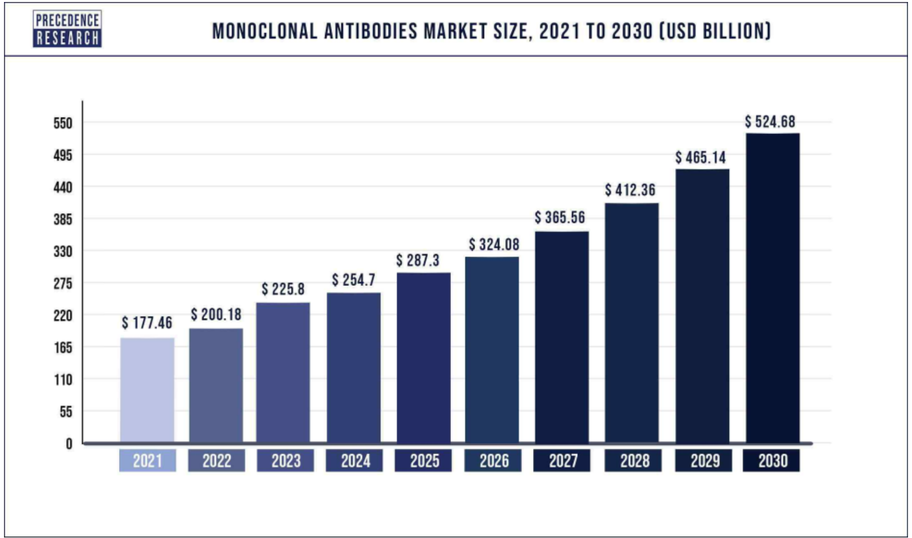
Overview

- Introduction of biotherapeutics and bioprocessing
- Manufacturing challenges
- Technical challenge of accessing intracellular states
- Hybrid approach – motivation for using EnKF
- Design & Implementation of EnKF for soft sensing in bioprocessing

Background: Therapeutic proteins

- Biologically derived drugs
- The most common example is monoclonal antibody(mAb)
- Can be used to treat many diseases including cancer, autoimmune diseases, inflammatory diseases and infectious diseases, approved by US FDA
- Explosive market growth of mAb



Bioprocessing of therapeutic proteins in mammalian cells

- Industrial production of therapeutic proteins, rely on living cells
- Mammalian cells are favoured due to compatibility to human bodies
- Nearly 70% of therapeutic proteins are produced in Chinese Hamster Ovary (CHO) cells.

Monoclonal Antibodies Market - Global Industry Analysis, Size, Share, Growth, Trends, Regional Outlook, and Forecast 2022 – 2030. Precedence Research

Manufacturing Challenges

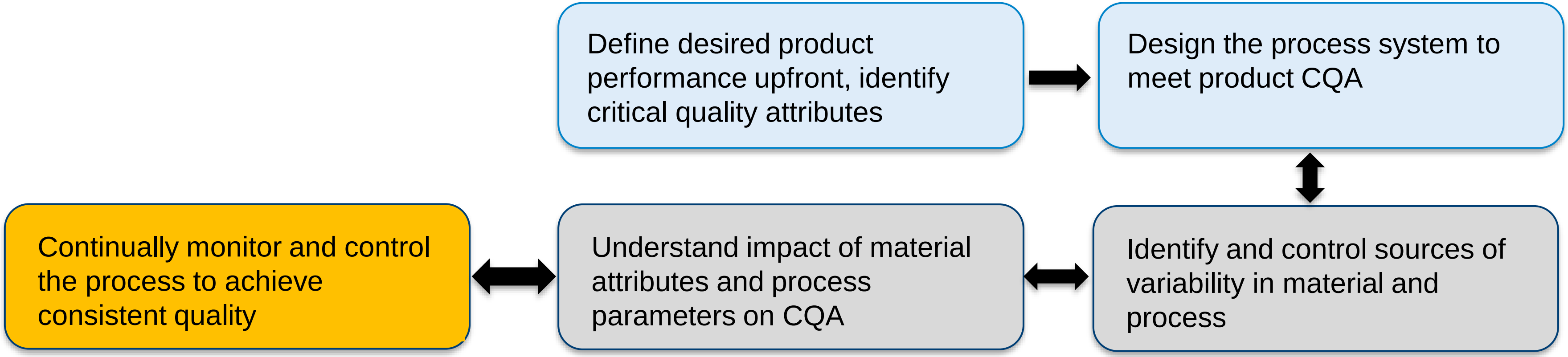
- Low yield (but high demand)

- Biotic phase is a black box

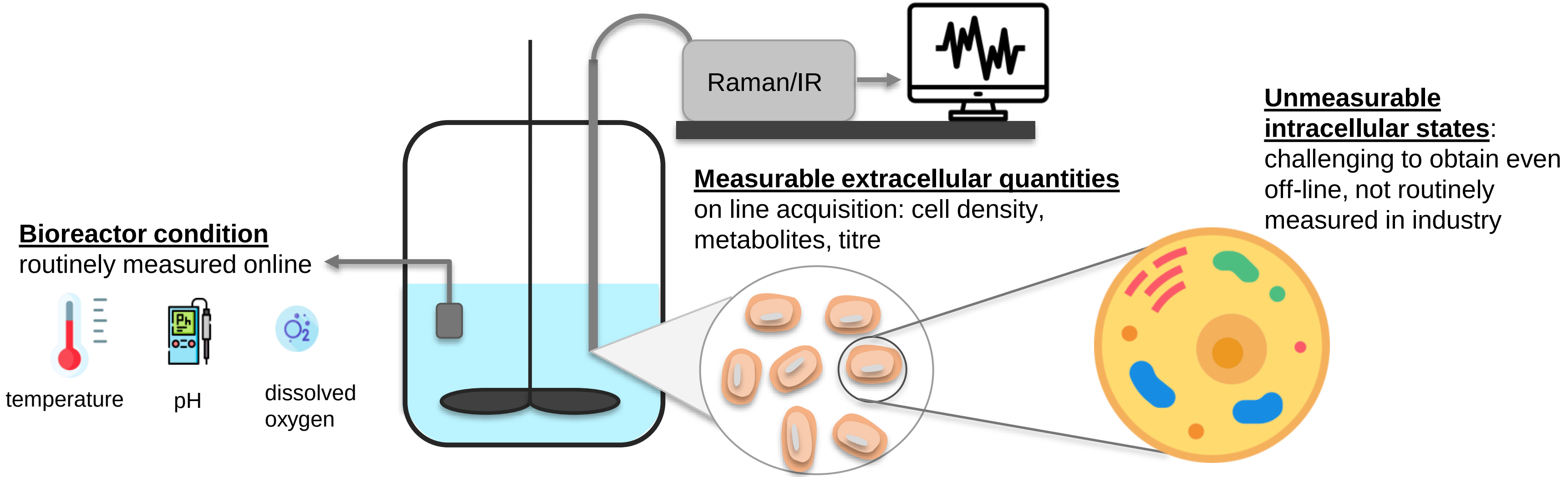
- Lack of comprehensive Process Analytical Technologies
  - ➢ Limited online measurements
  - ➢ No intracellular information

- Poor controllability: Major problem in a highly regulated industry

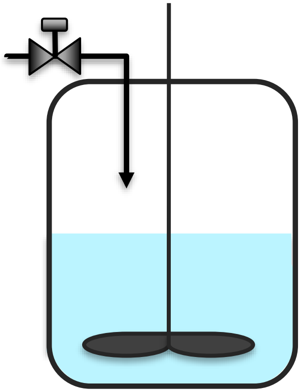
Product quality assurance - Quality by Design (QbD)



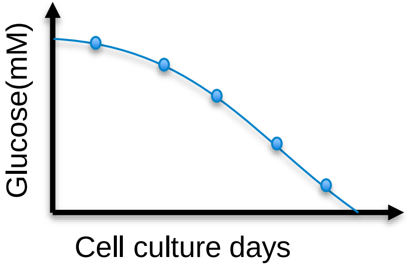
Online monitoring and control in bioprocessing

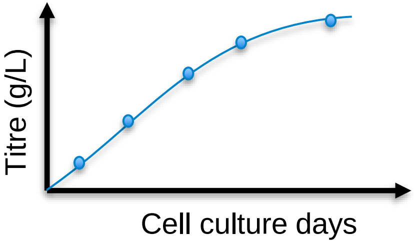


Mechanistic model development



Data Collection





Model Development

$$\mu = \mu_{max} \prod_{i=1}^{N.C.} \left(\frac{C_i}{K_{M,i} + C_i} \right)$$

$$q_i = \alpha_i \left(\frac{C_i}{K_{M,i} + C_i} \right)$$

$$q_p = \alpha_p \sum_{i=1}^{N.C.} \alpha_i \, q_i$$

Monod Kinetics

$$\frac{d(VX_V)}{dt} = \mu V X_V - Q_{out} X_V$$

$$\frac{d(VC_{i,in})}{dt} = Q_{in} C_{i,in} - Q_{out} C_i + q_i V X_V$$

$$\frac{d(VP)}{dt} = q_p V X_V - Q_{out} P$$

Material Balances

Apostolos Tsopanoglou, Ioscani Jiménez del Val, Moving towards an era of hybrid modelling: advantages and challenges of coupling mechanistic and data-driven models for upstream pharmaceutical bioprocesses, Current Opinion in Chemical Engineering,Volume 32,2021,100691,.

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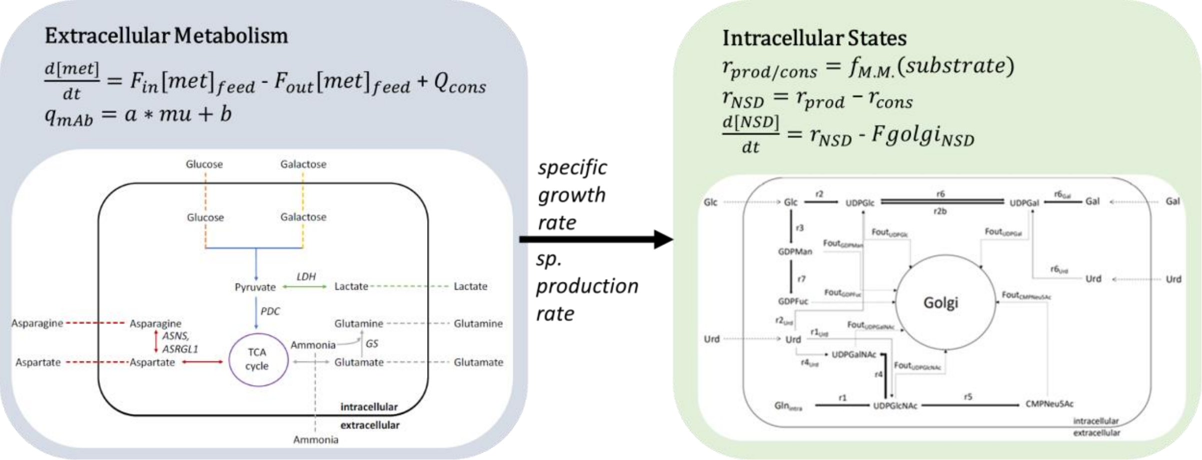
Previous Efforts – Mechanistic Models



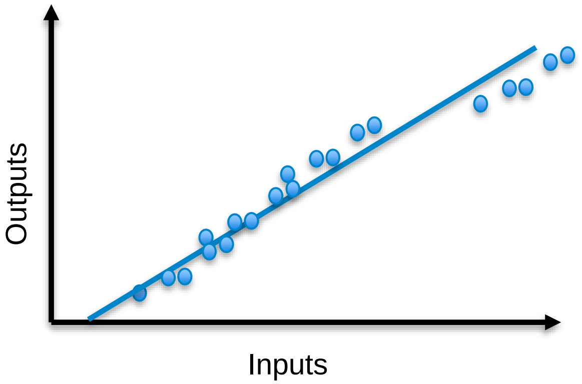
Challenges:

- Dynamic mechanistic models are highly specific to experimental conditions, cell line or product.
- Intracellular states are difficult to measure experimentally, therefore not routinely tracked.

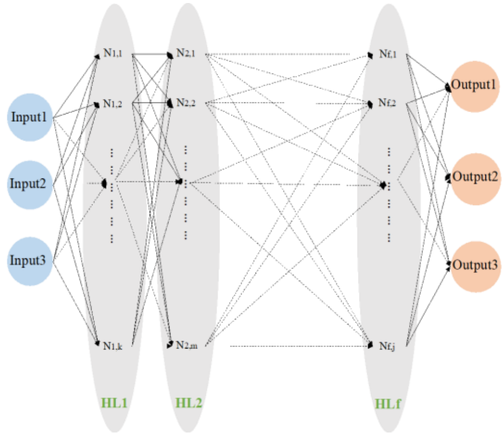
KOTIDIS, P., JEDRZEJEWSKI, P., SOU, S. N., SELLICK, C., POLIZZI, K., DEL VAL, I. J. & KONTORAVDI, C. 2019. *Biotechnol Bioeng*, 116, 1612-1626.

Previous efforts: Data-driven models

Partial Least Squares



Artificial Neural Networks



Data Driven Approaches

- No need to reparametrize the model kinetic parameters
- Model trained with data, not specially designed for the system

However,

- Large dataset required
- No system understanding embedded within the model

KOTIDIS, P., JEDRZEJEWSKI, P., SOU, S. N., SELLICK, C., POLIZZI, K., DEL VAL, I. J. & KONTORAVDI, C. 2019. *Biotechnol Bioeng*, 116, 1612-1626.

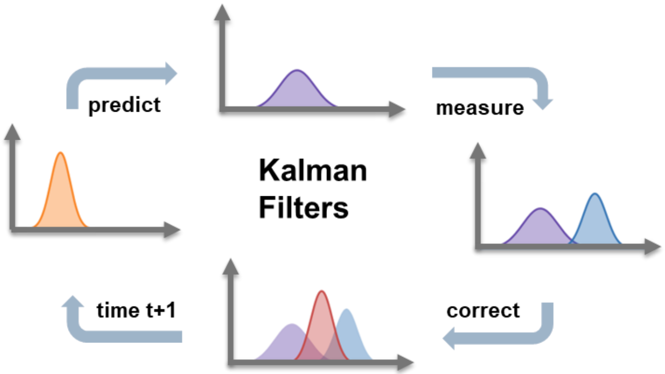
Ensemble Kalman Filter (EnKF) as soft sensor

Takes benefits from both mechanistic models and data-driven models for estimation of unmeasurable intracellular states

- Contains biological information, troubleshoot and inform process decisions
- Avoid reparameterization of kinetic parameters
- More flexible in adapting into different conditions without a large dataset, not bound to fixed parameters

Data Assimilation: Ensemble Kalman Filtering (EnKF)

- State estimation for unmeasurable intracellular states by model inference through extracellular metabolites



Experimental Setup

- A fed-batch CHO cell culture process producing an IgG antibody
- Control experiment: Glucose and amino acid nutrients added every two days
- Feeding experiment (10G5U): Additional 10mM galactose and 5mM uridine fed on Day 4 and Day 8
    - in order to change the quality profile of the product
- Daily measurements were taken for relevant extracellular metabolites and intracellular states

JEDRZEJEWSKI, P. M. 2015. A platform for the optimisation of metabolic pathways for glycosylation to achieve a narrow and targeted glycoform distribution. *PhD Thesis*, Imperial College London.
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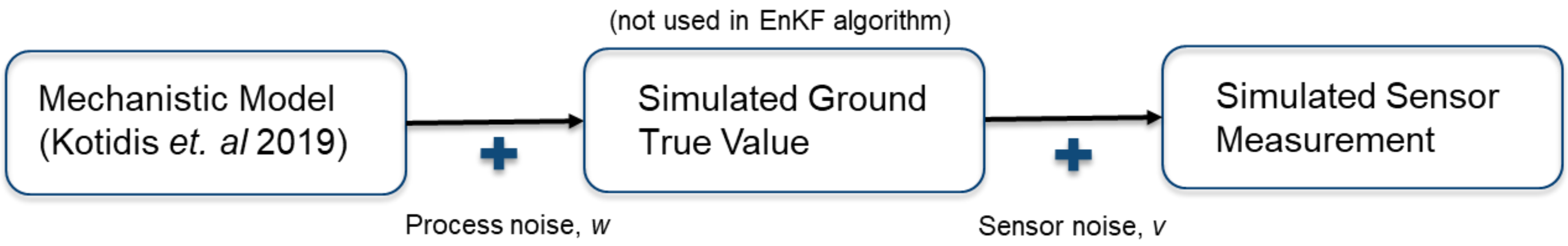
Data Augmentation



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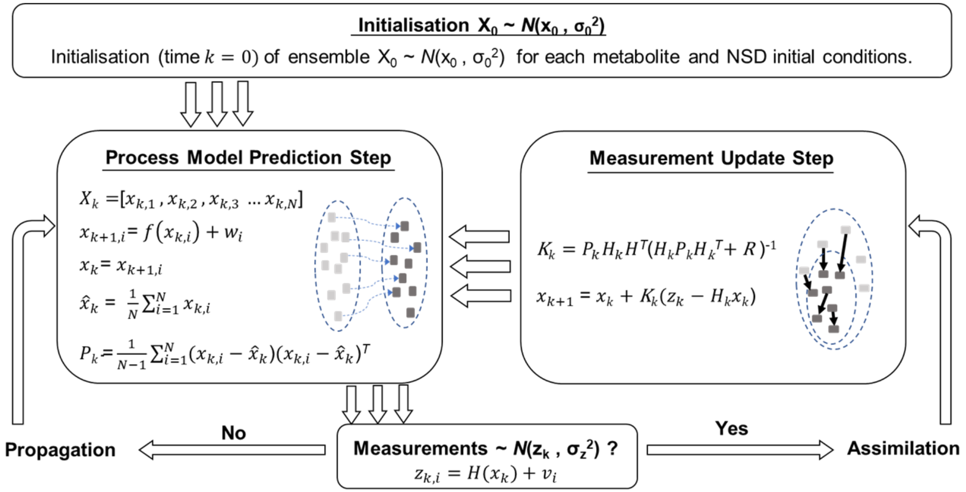
Ensemble Kalman Filter (EnKF) Algorithm

Notations:

- X, sampled ensemble;
- x, process states;
- f, process model;
- w, white Gaussian noise of process;
- P, state covariance;
- K, Kalman gain;
- H, measurement function;
- R, measurement noise covariance;
- z, measurement;
- v, white Gaussian noise of measurement



- **EnKF iteratively updating the forecast as new information becomes available**

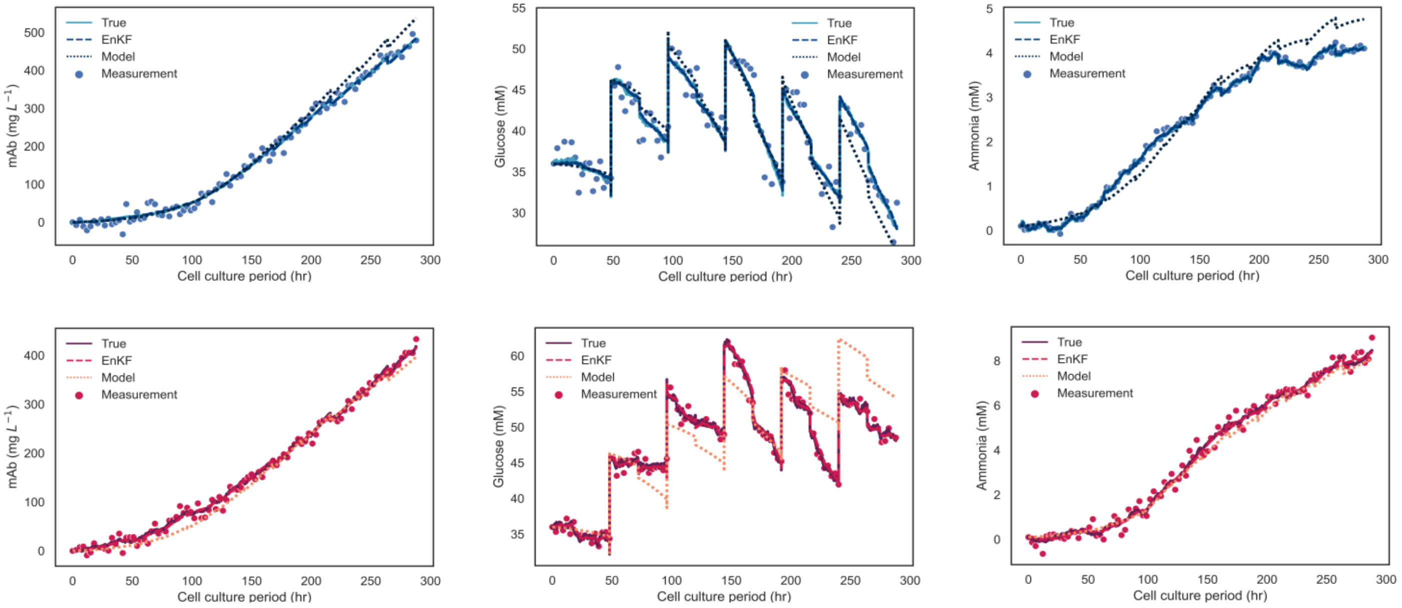
The Ensemble Kalman Filter algorithm, adapted from Pellenq and Boulet (2004).

Results 1 Extracellular Metabolites

**Control Experiment,
NO ADDITIONAL FEEDING**

**10mM GAL & 5mM URD
FED ON DAY 4 and 8**

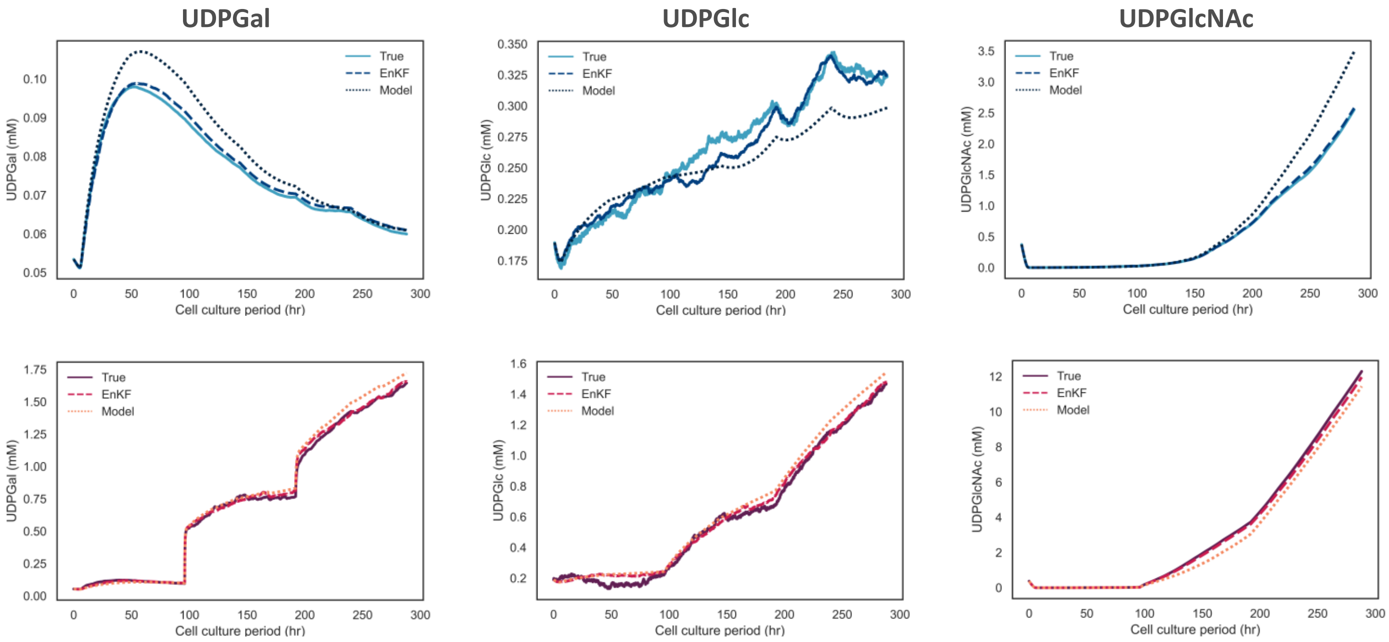
mAb Titre | Glucose | Ammonia



Results 2 Intracellular States

Control Experiment,
NO ADDITIONAL FEEDING

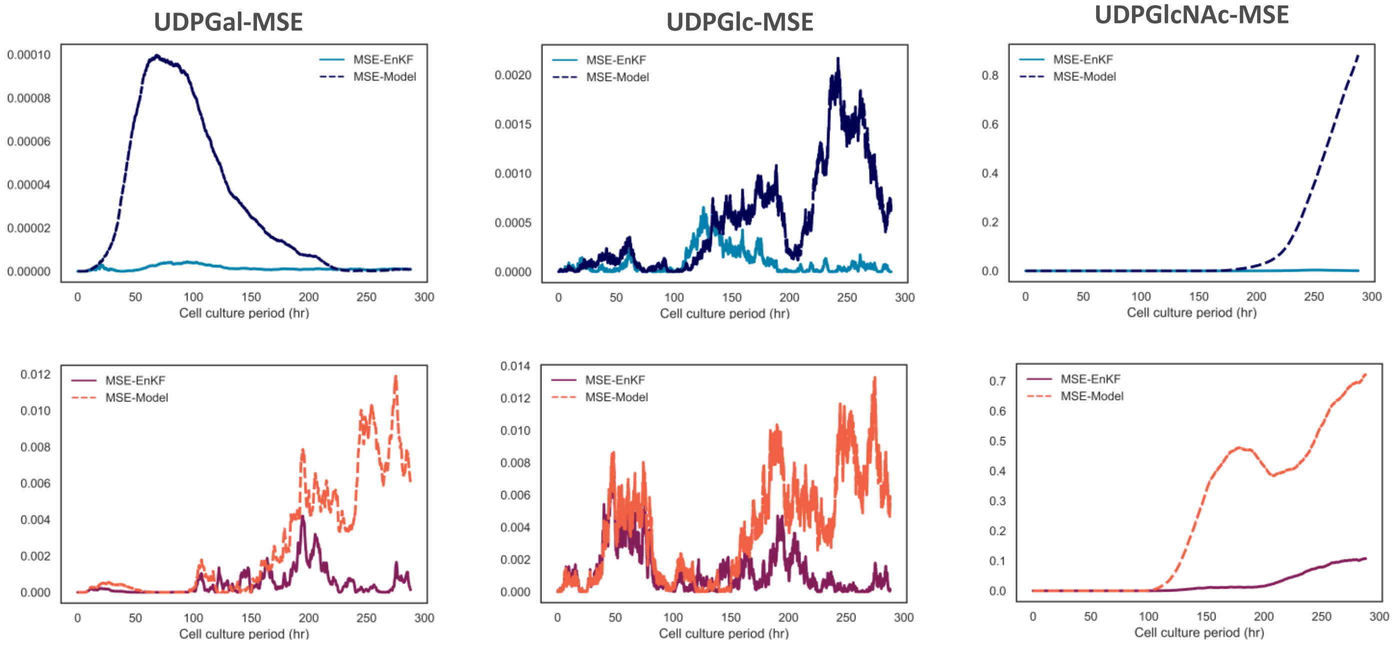
10mM GAL & 5mM URD
FED ON DAY 4 and 8



Results 2 MSE Comparison with True Values

Control Experiment,
NO ADDITIONAL FEEDING

10mM GAL & 5mM URD
FED ON DAY 4 and 8



Conclusion & Outlook

- EnKF takes advantages from both the mechanistic model and discrete sensor observations
- EnKF reduces the sensor noise for measurable extracellular metabolites
- EnKF as soft sensor for estimating unmeasurable intracellular states, which can be used to ensure product quality during manufacturing
- Enables more informed process control strategies
- Experimental validation
- Potentially transfer the framework to other process conditions, cell line or product

Thank you