State Seminar 2025 II

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Overview

1. Parameter Estimation

2. PioReactor feat. Jannis Bergmann

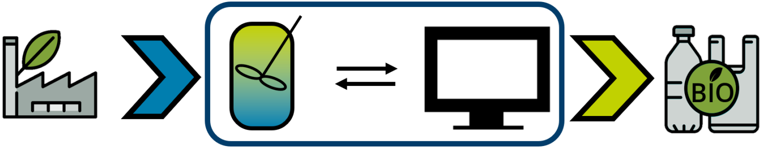
Previously on "State Seminar 2025"

AUFBRUCH:

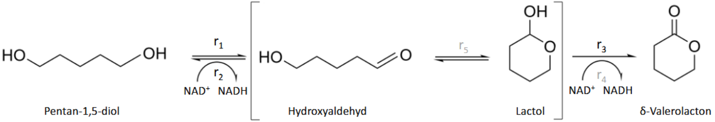
stateSeminar2_25/figure/Aufbruch_Zusammenfassung.png

Previously on "State Seminar 2025"

AUFBRUCH:



Enzyme cascade:



How good are my parameters?

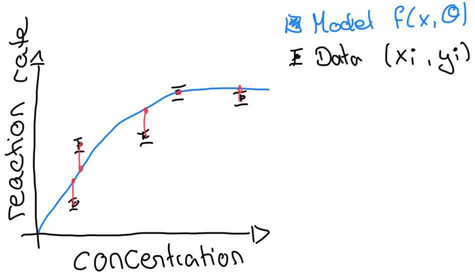
Given

- Chemical drawing of enzyme cascade
- Model equations
- Estimated parameters and standard deviation
- Experimental data

Question

How "good" are these estimated parameters?

Estimate Parameters from Kinetic Data



Fitting parameters

Assumption:

$$y_i = f(x_i, \theta)$$

Find θ such that

$$\min_{\theta} \sum_i (f(x_i, \theta) - y_i)^2$$

Michaelis-Menten Kinetics

Enzyme-Substrate Reaction Mechanism

$$S + E \underset{}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P.$$

Michaelis-Menten Equation

$$r = \frac{V_{\max} \cdot [\mathrm{S}]}{K_m + [\mathrm{S}]}$$

Assumptions

- Steady-state approximation:
  $\frac{d[\mathrm{ES}]}{dt} = 0$

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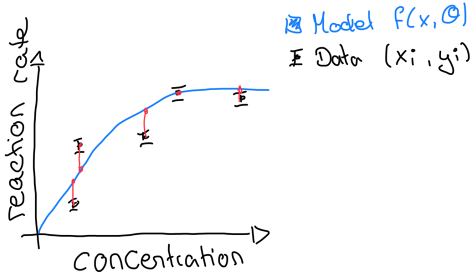
Estimate Parameters from Kinetic Data



Fitting parameters

Assumption:

$$y_i = f(x_i, \theta) + \varepsilon_i \text{ with } \varepsilon_i \sim N(0, \sigma_i^2).$$

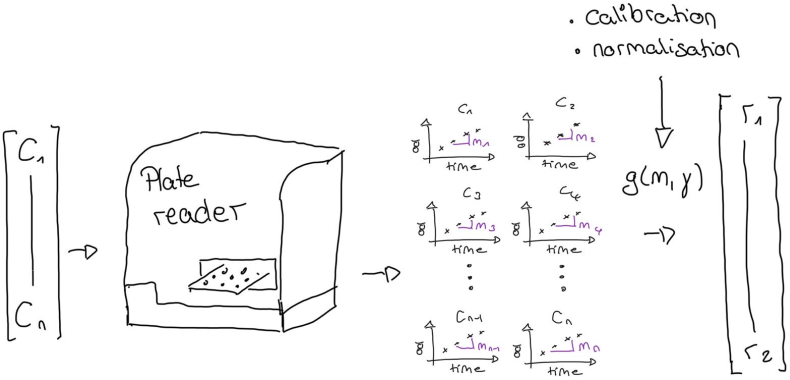
Find θ such that

$$\min_{\theta} \sum_i ((f(x_i, \theta) - y_i)/\sigma_i)^2$$

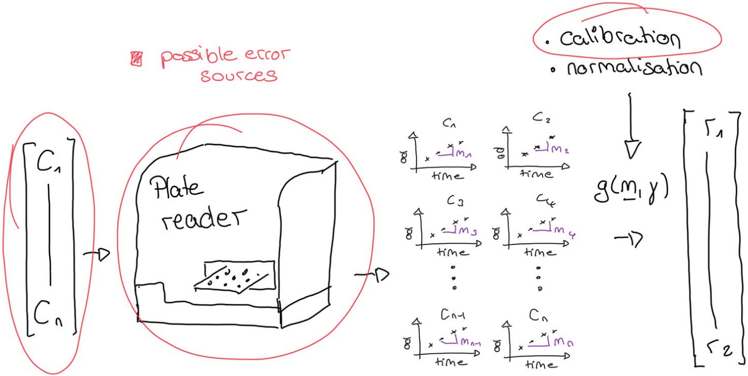
Problem

- We can not assume that the errors ε_i are normally distributed.
- $y_i - f(x_i, \theta)$ is not the direct measurement error since y_i is determined in an experimental workflow.

Calculating reaction rates from plate reader data



Calculating reaction rates from plate reader data



Approach

Implement parameter estimation script that accounts for different types of error sources

Monte Carlo Bootstrap

Source of error

- Calibration errors $\rightarrow$ errors in calculating reaction rates
- Pipetting errors $\rightarrow$ concentration errors $c_i = c_{true} + \epsilon c_i$
- **Measurement errors** in plate reader $\rightarrow$ fluorescence errors $o_i = o_{true} + \epsilon o_i$

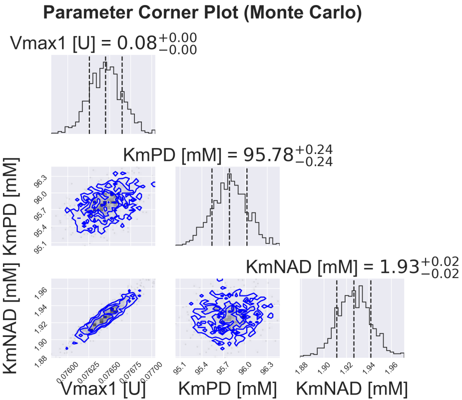
Monte Carlo Bootstrap Algorithm

1. For $i = 1, \ldots, N$ bootstrap samples:
   1. Sample errors: $\epsilon_{o_k} \sim N(0, \sigma^2_{o_k})$
   2. Calculate perturbed values: $o_k^{(i)} = o_k + \epsilon_{o_k}$
   3. Compute reaction rates with perturbed data
   4. Fit parameters $\theta^{(i)}$ to perturbed data
2. Get parameter distribution from $\{\theta^{(1)}, \ldots, \theta^{(N)}\}$
3. Calculate confidence intervals and parameter uncertainties, and correlations

Results for reaction 1 with noisy Plate Reader data

$$r_1 = \frac{V_{\max,1} \cdot [\mathrm{NAD}] \cdot [\mathrm{PD}]}{(K_{m,\mathrm{PD}} + [\mathrm{PD}]) \cdot (K_{m,\mathrm{NAD}} + [\mathrm{NAD}])}$$

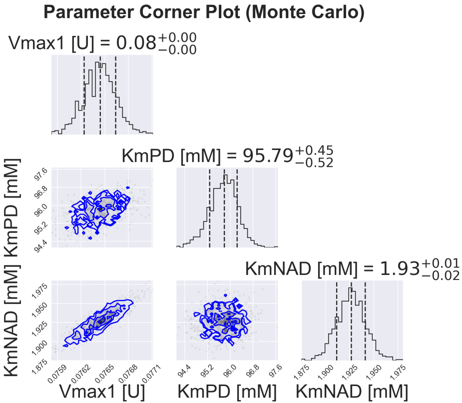
Parameter	Value $\pm$ σ
$V_{\max,1}$	0.0765 ± 0.0002
$K_{m,\mathrm{PD}}$	95.78 ± 0.24
$K_{m,\mathrm{NAD}}$	1.927 ± 0.015



Results for reaction 1 with noisy processed Data

$$r_1 = \frac{V_{\mathrm{max},1} \cdot [\mathrm{NAD}] \cdot [\mathrm{PD}]}{(K_{m,\mathrm{PD}} + [\mathrm{PD}]) \cdot (K_{m,\mathrm{NAD}} + [\mathrm{NAD}])}$$

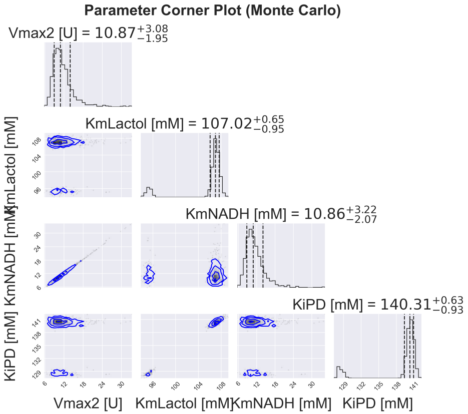
Parameter	Value $\pm$ σ
$V_{\mathrm{max},1}$	0.0764 ± 0.0002
$K_{m,\mathrm{PD}}$	95.7672 ± 0.4926
$K_{m,\mathrm{NAD}}$	1.9265 ± 0.0150



Results for reaction 2 with noisy Plate Reader data

$$r_2 = \frac{V_{\max,2} \cdot [\text{Lactol}] \cdot [\text{NADH}]}{\left(K_{m,\text{Lactol}}\left(1 + \frac{[\text{PD}]}{K_{i,\text{PD}}}\right) + [\text{Lactol}]\right) \cdot (K_{m,\text{NADH}} + [\text{NADH}])}$$

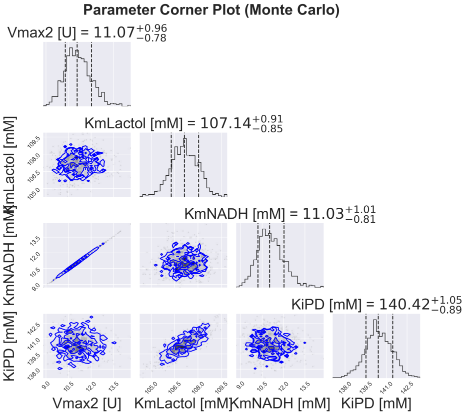
Parameter	Value $\pm$ σ
$V_{\max,2}$	11.57 ± 3.32
$K_{m,\text{Lactol}}$	105.6 ± 4.04
$K_{m,\text{NADH}}$	11.60 ± 3.50
$K_{i,\text{PD}}$	138.85 ± 4.06



Results for reaction 2 with noisy processed Data

$$r_2 = \frac{V_{\text{max},2} \cdot [\text{Lactol}] \cdot [\text{NADH}]}{\left(K_{m,\text{Lactol}}\left(1 + \frac{[\text{PD}]}{K_{i,\text{PD}}}\right) + [\text{Lactol}]\right) \cdot (K_{m,\text{NADH}} + [\text{NADH}])}$$

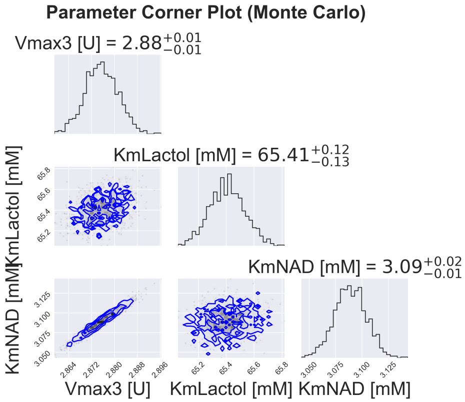
Parameter	Value $\pm\ \sigma$
$V_{\text{max},2}$	11.15 ± 0.88
$K_{m,\text{Lactol}}$	107.14 ± 0.87
$K_{m,\text{NADH}}$	11.10 ± 0.92
$K_{i,\text{PD}}$	140.45 ± 0.97



Results for reaction 3 with noisy Plate Reader data

$$r_3 = \frac{V_{\max,3} \cdot [\text{Lactol}] \cdot [\text{NAD}]}{(K_{m,\text{Lactol}} + [\text{Lactol}]) \cdot (K_{m,\text{NAD}} + [\text{NAD}])}$$

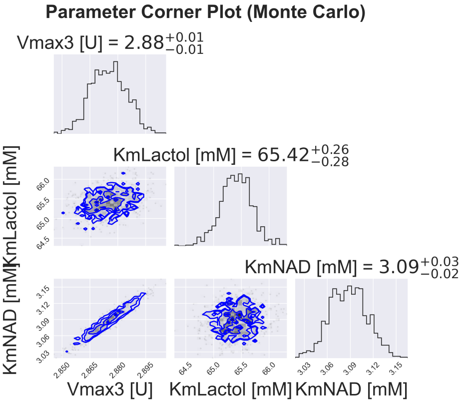
Parameter	Value $\pm\ \sigma^2$
$V_{\max,3}$	2.8755 ± 0.0059
$K_{m,\text{Lactol}}$	65.4094 ± 0.1227
$K_{m,\text{NAD}}$	3.0888 ± 0.0157



Results for reaction 3 with noisy processed data

$$r_3 = \frac{V_{\max,3} \cdot [\text{Lactol}] \cdot [\text{NAD}]}{(K_{m,\text{Lactol}} + [\text{Lactol}]) \cdot (K_{m,\text{NAD}} + [\text{NAD}])}$$

Parameter	Value $\pm$ σ
$V_{\max,3}$	2.8753 ± 0.0095
$K_{m,\text{Lactol}}$	65.4097 ± 0.29514
$K_{m,\text{NAD}}$	3.0885 ± 0.0240



Summary and Outlook

Key Findings

- Reaction 1: Very low uncertainties
- Reaction 2: Higher uncertainties, especially for $V_{\max,2}$ and $K_{m,\mathrm{NADH}}$
- Reaction 3: Mixed results - $K_{m,\mathrm{NADH}}$ shows high uncertainty
- Correlation results vary depending on the error source (plate reader vs processed data)

Outlook

- Investigate **experimental design optimization** to reduce parameter correlations
- Optimize **uncertainty quantification pipeline**
- Simulate **cascade simulation with additional reactions** with CADET
- Keep learning

Part II

Motivation

- Extending CADET
  - Simulates biotechnical processes
  - Runs simulations "offline"
- Development of a Digital Twin

stateSeminar2_25/figure/cadet_offline.

CADET-Live*

- New module to extend CADET's features
- Running CADET during bio-processes
- Change process environment based on simulations

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CADET-Live* (Features)

- Written in C/C++
- Interfaces with CADET-Core
- Build Modular e.g. different methods of data input
- Efficient
- Open Source

Proof-of-concept with Pioreactor

- Open Source mini bio-reactor
- Based on RaspberryPi
- Can be used standalone or in cluster
- Uses MQTT for communication

stateSeminar2_25//figure/pioreacto

Figure: Pioreactor 40ml

Current State

- Pioreactor build
- Software setup completed
- Self-tests run successfully
- Getting familiar with software for CADET-Core and PioReactor

The End - Thank you for your attention!

Summary

- Parameter estimation and error propagation
  - Example of estimating kinetic parameters from experimental data
  - Where and how you assume measurement error matters.
- PioReactor
  - Small bio process which is easy to control
  - Staring point to get familiar with feedback systems