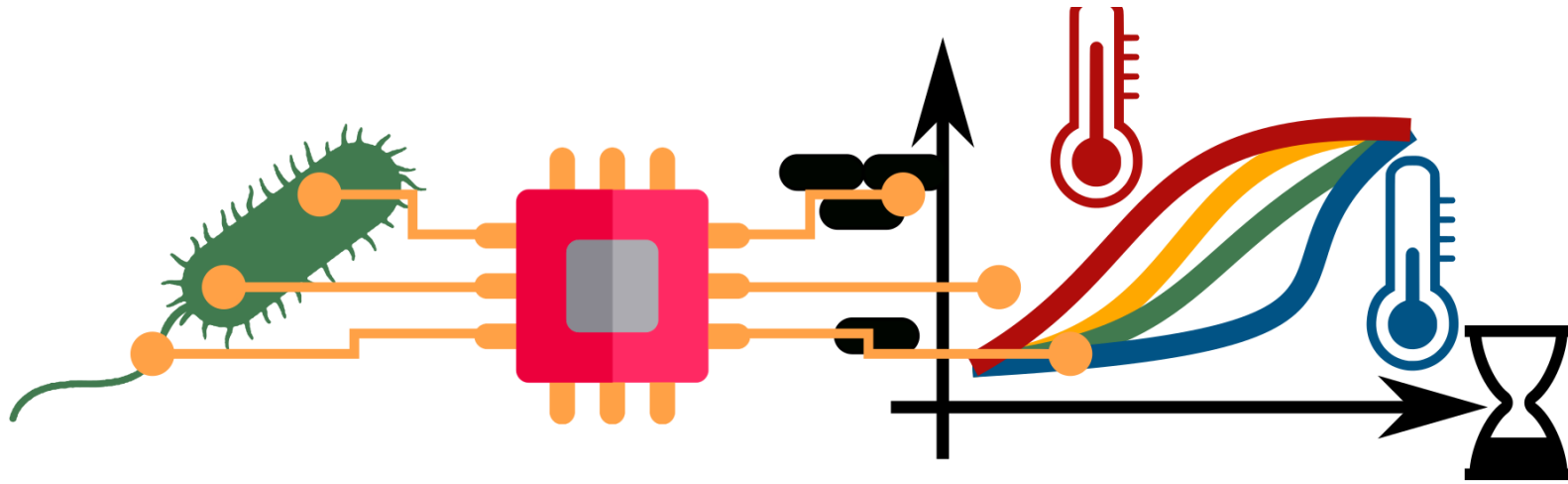# Recombinant Expression Simulation: Theoretical Background

- The BioLabSim.nrw consortium

Outline

1. **Theory**
   - Microbial Growth
     - » Growth Phenotypes
     - » Mathematical Models
     - » High-Throughput Measurements
     - » Biotechnological Relevance
   - Bacterial Gene Expression
     - » Expression factors
     - » Contribution to expression strength
   - » Expression Strength Prediction

2. **Simulation**
   - Life presentation of the simulation

Teaching Objectives

1. Distinguish a suitable growth law for data analysis.
   - ➢ Knowledge of growth types
   - ➢ Knowledge of equations and their variables

2. Identify factors of gene expression on a DNA sequence.
   - ➢ Knowledge of expression factors

3. Judge whether a phenomenon is better described by a mechanistic or statistic model.
   - ➢ Knowledge of modelling principles

Microbial Growth Phenotypes

- Environmental conditions determine growth rate:
  - ➢ Temperature, Substrate, pH, etc.
- Growth is separated in different phases
- In non-optimal conditions and environmental changes microorganisms adapt their growth.
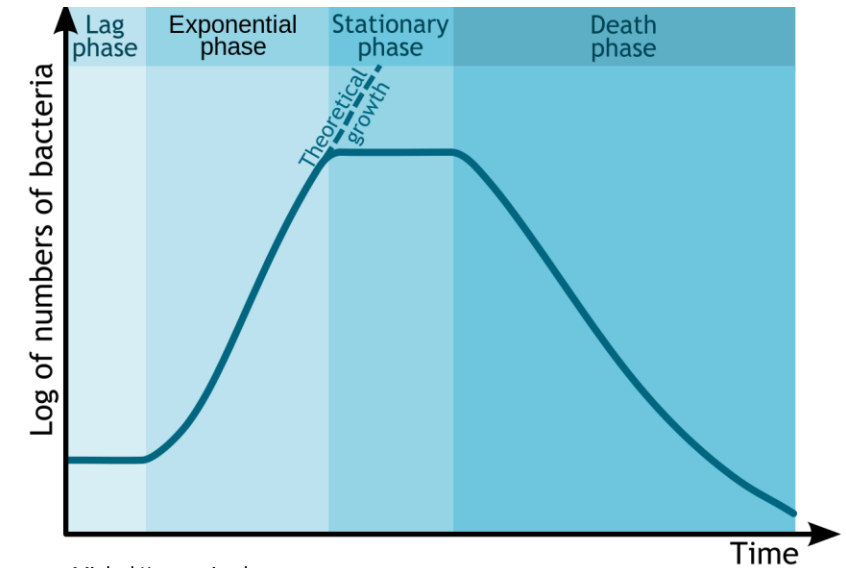
Michał Komorniczak,
https://en.wikipedia.org/wiki/Bacterial_growth#/media/File:Bacterial_growth_en.svg
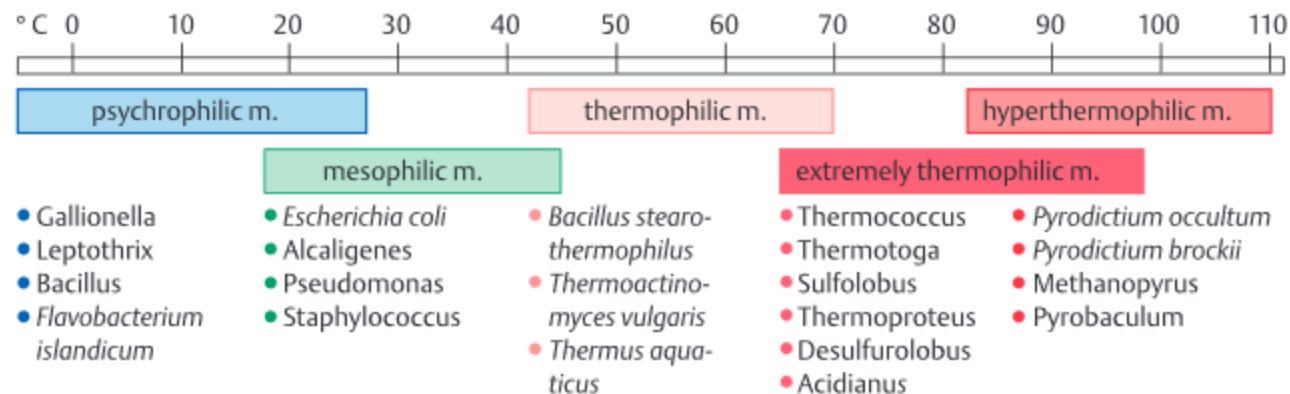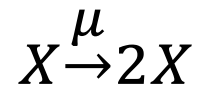

Schmid, Schmidt-Dannert, Hammelehle (2014) Biotechnology, An illustrated primer, ISBN-10: 9783527335152
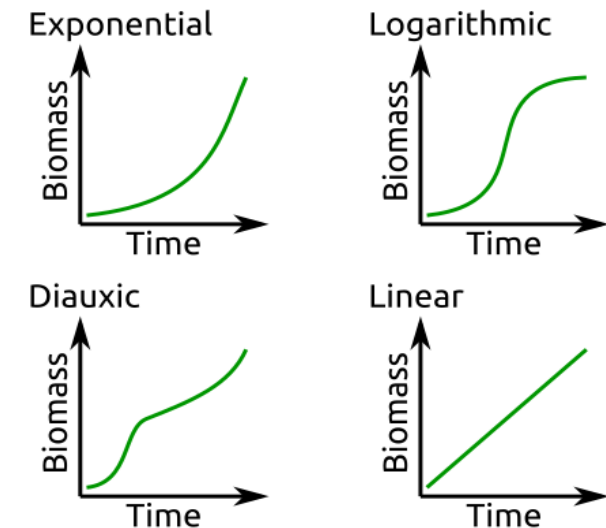
Growth Models: The Exponential Function

- In optimal conditions microorganisms growth exponentially
- Many processes initially follow exponential dynamics:
  - ➢ Covid19 spread, Computing power, etc.
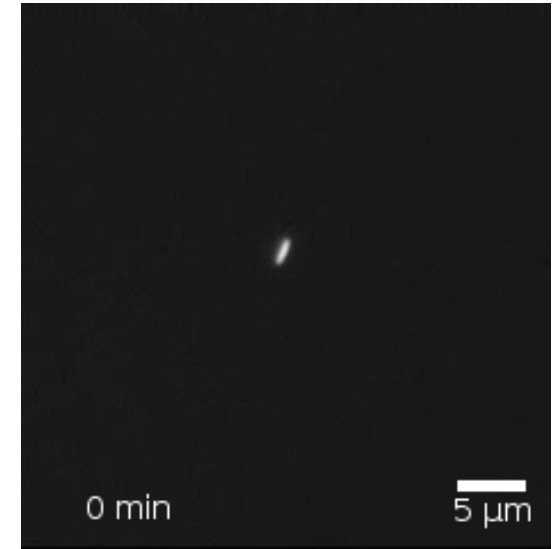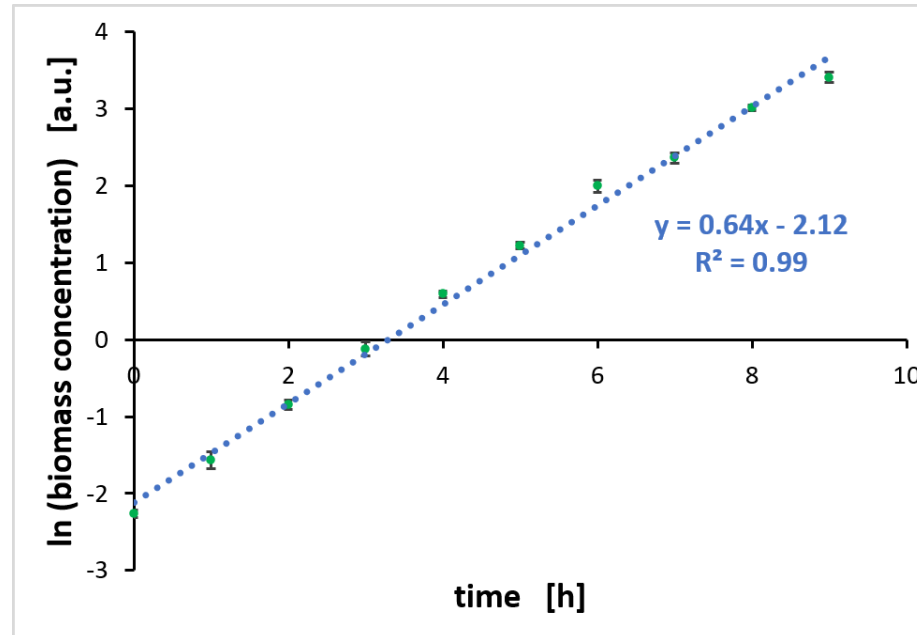- Growth rate remains constant.

Mechanistic Growth Model:

0 min   5 µm

$$X \overset{\mu}{\rightarrow} 2X$$

X: Biomass, gDW/L

μ: growth rate, /h

t: time, h

$$\frac{dX}{dt} = \mu X$$

$$X(t) = X_0 e^{\mu t}$$

$$\ln(X(t)) = \ln(X_0) + \mu(t - t_0)$$

y = 0.64x - 2.12
R² = 0.99

In (biomass concentration) [a.u.]

time [h]

Growth Models: The Logistic Function

- Environmental capacity limits cause transition from exponential to logistic growth.

- Capacity limits: substrates, space, waste accumulation, etc.

- Growth rate remains constant

$$\frac{dX}{dt} = \mu X \left(1 - \frac{X}{C}\right)$$
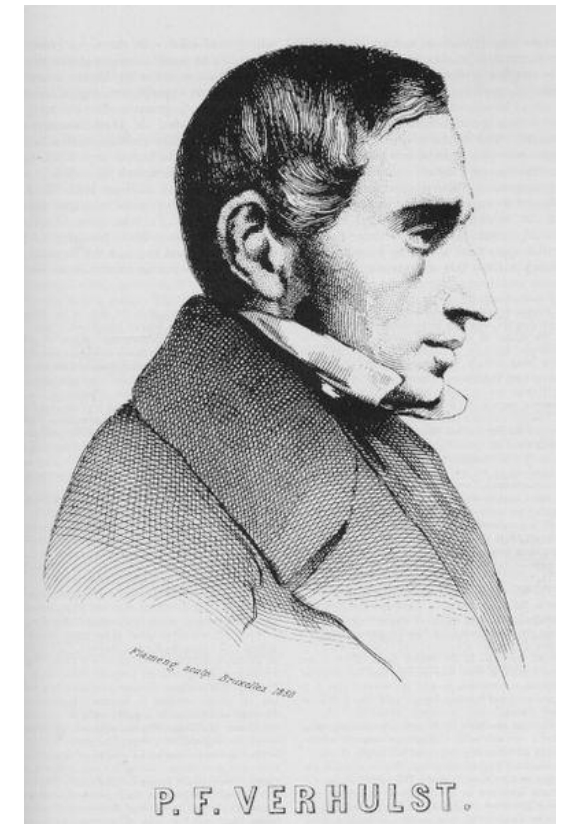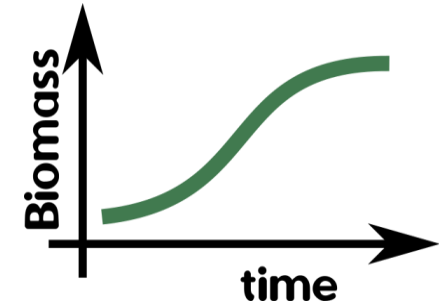
X: Biomass, gDW/L

μ: **growth rate, /h**

C: capacity limit, gDW/L

t: time, h

$$X(t) = \frac{C}{1 + \left(\frac{C - X_0}{X_0}\right)e^{-\mu t}}$$


P. F. VERHULST.

- Growth rate is estimated with parameter search to minimize error.

source: https://en.wikipedia.org/wiki/Logistic_function

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Growth Models: The Monod Equation

- Growth rate is variable and depends on environment, e.g. substrate level, temperature.
- Monod assumed that growth is substrate limited by enzymatic actions.
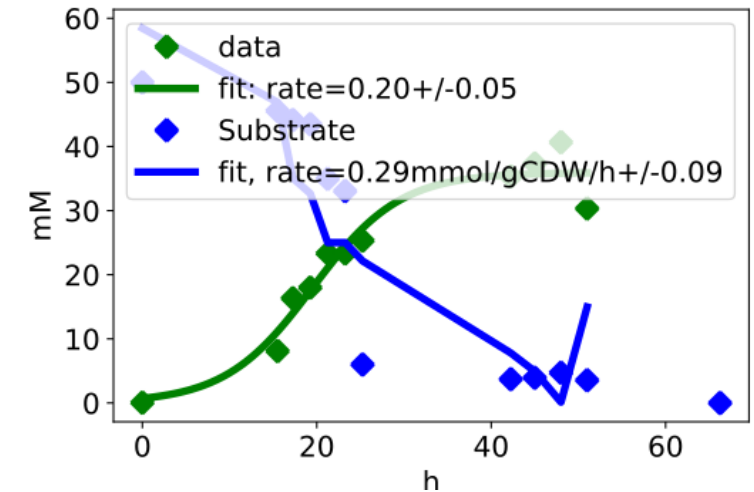- Similar to Michaelis-Menten equation.
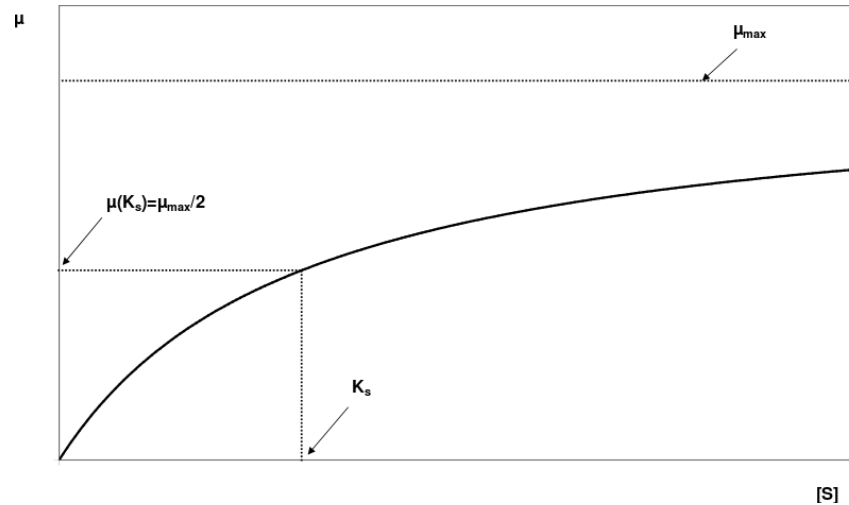
$$X+S \xrightarrow{\mu} 2X$$

$$\mu = \frac{\mu_{max}S}{K_S+S}$$
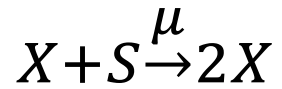
μ: growth rate, /h

μ_{max}: max rate, /h

S: substrate, mM

K_S: substrate affinity, mM

- Substrate measurements required for parameter estimation.
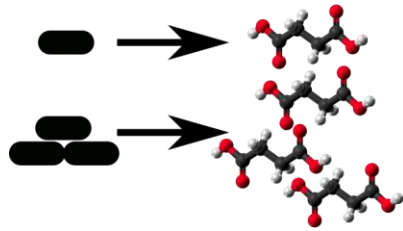
High-Throughput Measurements

- Organisms with high growth rates need to be selected based on mutations and medium.
- 96-well plate measurement of growth by Enzyscreen GrowthProfiler

- Data export as csv-files
- Automated analysis in Jupyter Notebooks

Source: https://www.enzyscreen.com/growth_profiler.htm
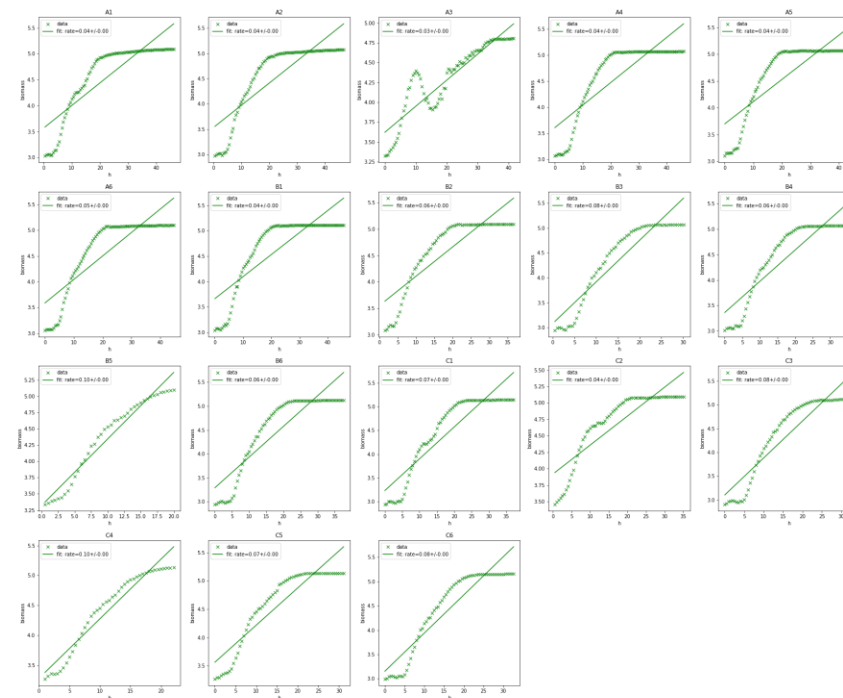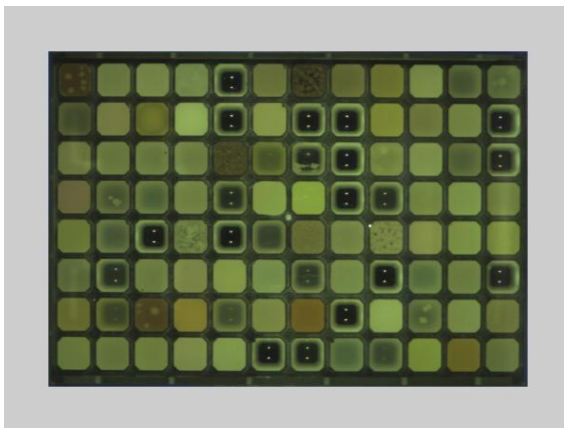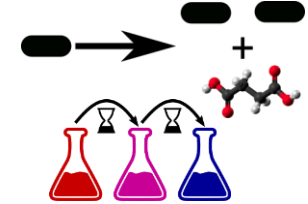
Biotechnological Relevance

High cell densities lead to high productivity.
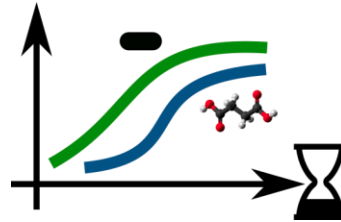
Target products produced in different growth phases.

Growth-coupled production enables optimization with ALE.
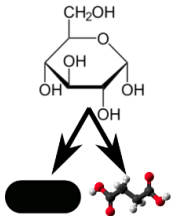
Efficiency tradeoff by substrate use in biomass and product.
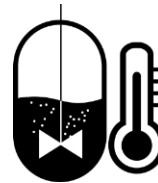
Optimal growth requires energy for heating/cooling.

Biomass waste disposal strategy for GMOs.

Growth Curve Data Analysis Example

Objective

Optimal Temperature

Growth Rate & Max Biomass

Procedure

Analysis

- Scrambled code-lines provided
- User rearranges line order for functional code

- Standard for quick laboratory data analysis.
- Users implement Excel linear regression.

Excel-basee Growth Rate Calculation

1. Determine mean value and standard deviation (only necessary for biological replicates)

2. Plot the Logarithm of biomass concentrations against time

3. Perform a linear regression with the linear range

→ The slope of the regression line is equal to the growth rate

$y = 0.64x - 2.12$
$R^2 = 0.99$
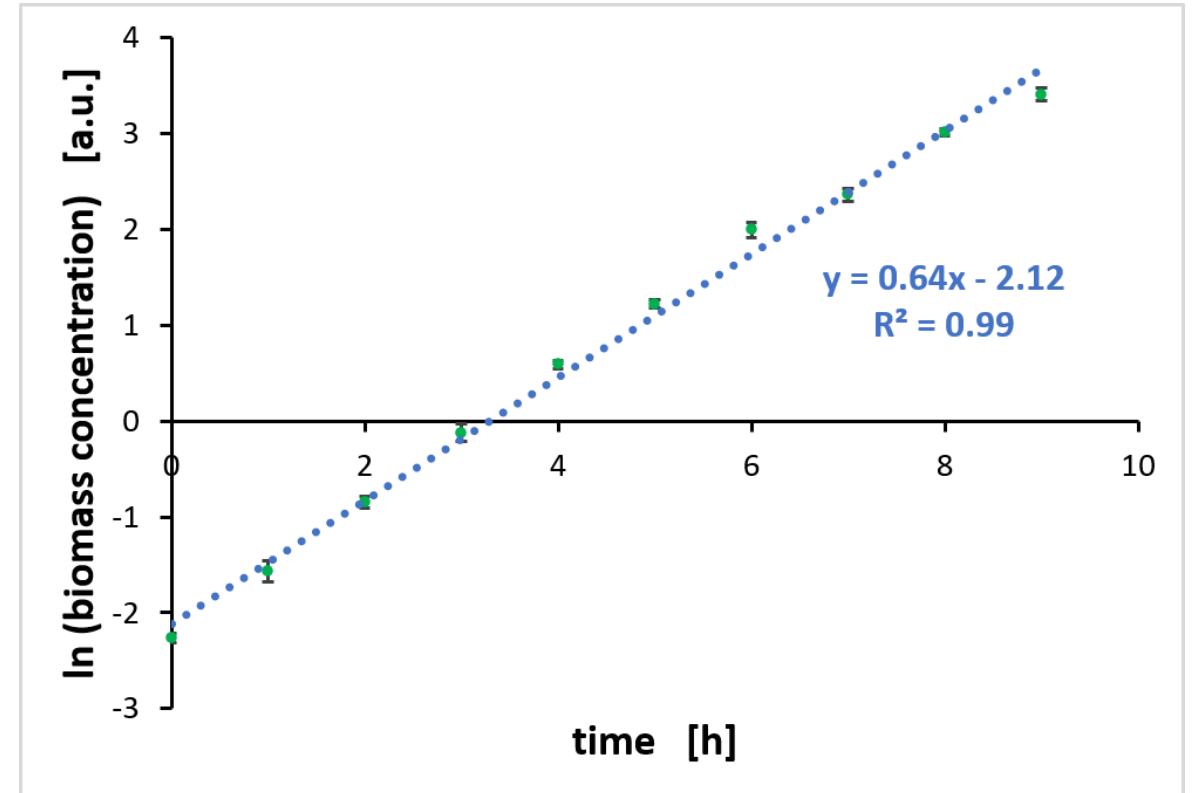
Example cultivation from BioLabSim at 35 °C.
Shown is the mean value and the standard deviation of three biological replicates.

Python-based Growth Rate Calculation

1. Rearrange code lines

```
# Rearrange the correct code sequence for plotting the growth curves with the logarithm of biomass.
Time, Biomass = my_data[:,0], my_data[:,1:]
DataFile = 'Strain_characterization_1.csv'
LnBiomass = np.log(Biomass)
plt.scatter(Time, X, label=Exp) for Exp,X in enumerate(LnBiomass.T)]
plt.legend(bbox_to_anchor=(1.05, 1), loc='upper left')
my_data = np.genfromtxt(DataFile, delimiter=',', skip_header=1)
```

2. Extract the highest growth slope for ln(biomass)
3. Extract the range of linear slope for ln(biomass)
4. Rearrange code lines for linear regression and max biomass extraction

Outline

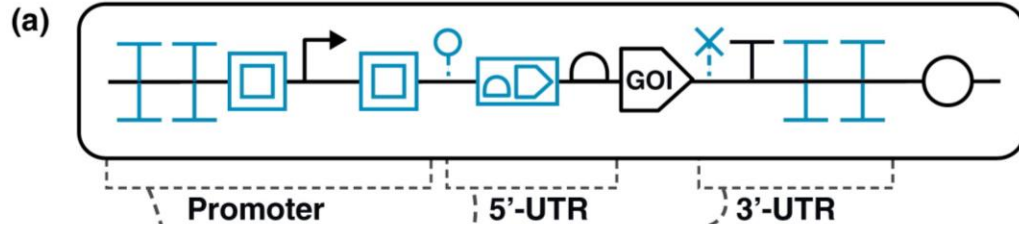1. **Theory**
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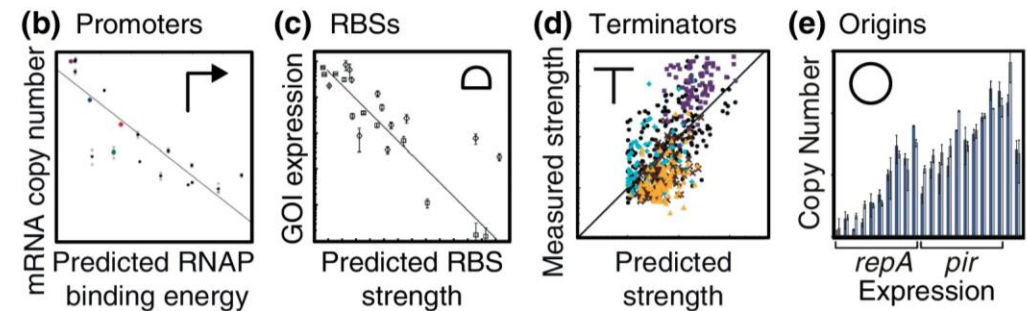   - » Expression Strength Prediction

2. **Simulation**
   - Life presentation of the simulation

Bacterial Gene Expression

- Three levels of gene expression regulation:
  - Transcription initiation by the promoter
  - Translation initiation by the 5' UTR
 - mRNA stability by the 3' UTR

Sigma Factors and Sequence Boxes

- The RNA-polymerase function is extending a nucleotide sequence based on homology.

- A σ-factor guides the RNA-polymerase to the promoter of a gene.

- Different σ-factors have different recognition sequences and expression efficiencies.

- A useful biotechnical used σ–factor in *E. coli* and *P. putida* is σ70.

- Important σ70 recognition sites are the boxes:
    - -35: TTGACA
    - -10: TATAAT
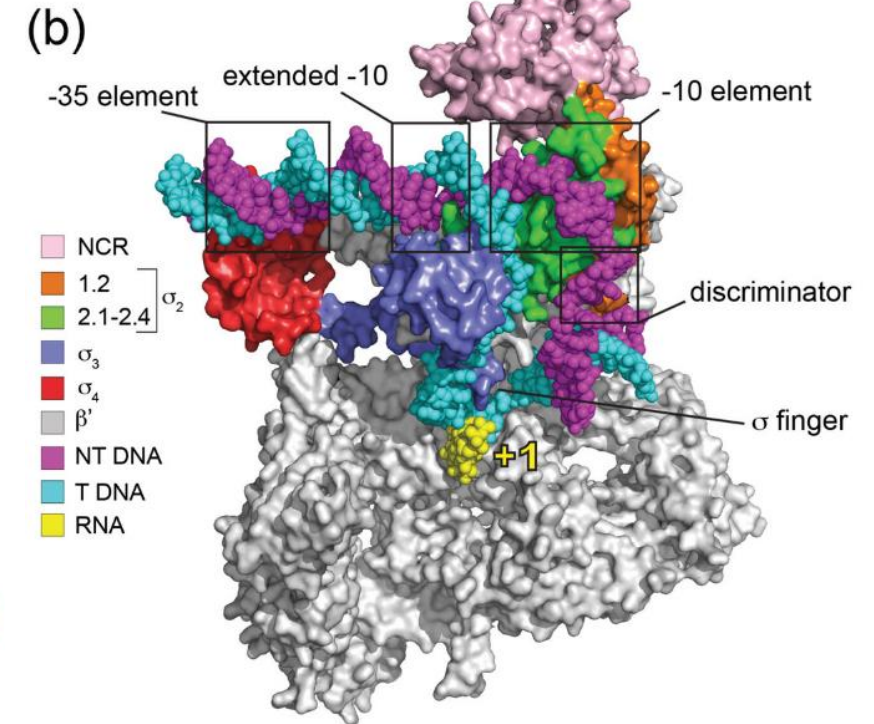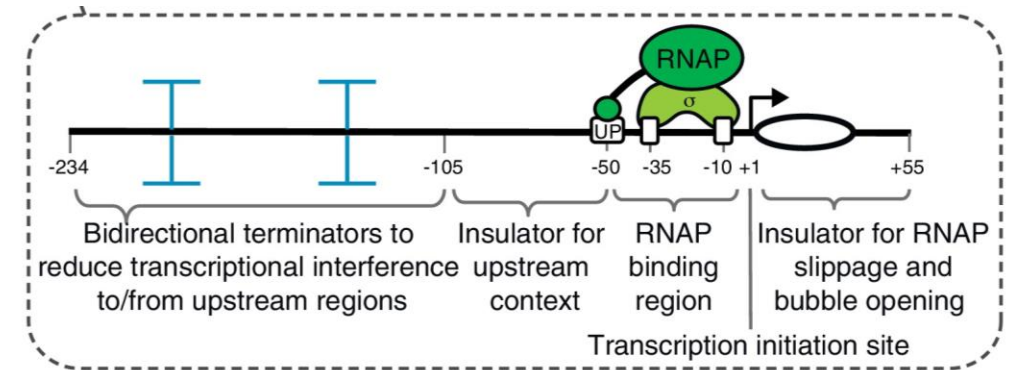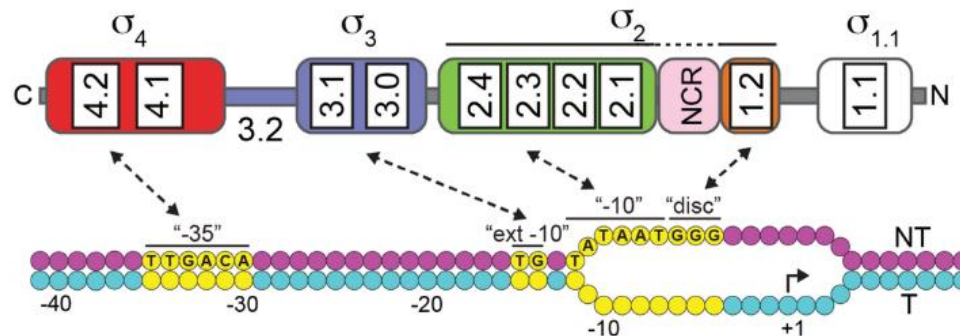
P. putida promoter library statistical analysis

Measured promoter library
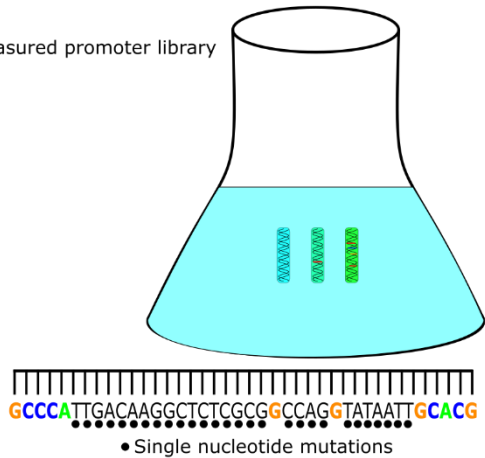
GCCCATTGACAAGGCTCTCGCGGCCAGGTATAATTGCACG
• Single nucleotide mutations
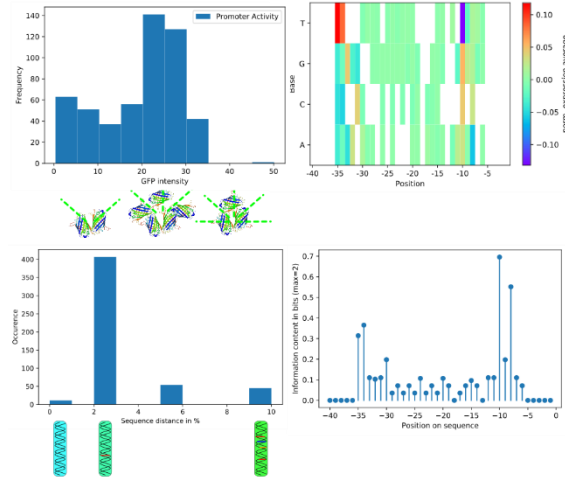
Promoter Library Analysis

1. Promoter library with mutations in promoter sequence and GFP activity.

2. RF-Learning with sequence as binary input, and additional variables, e.g., GC-content.

3. Output is GFP activity.

4. Prediction quality control with R^2 and RMSE

5. Variable importance: nucleotide contribution to the prediction.

6. Identification of novel strong promoters.

Promoter Activity Prediction with Random Forest

Synthetic Promoter Library:

- Promoter samples

- Nucleotide Positions (in our case 40)

- Base variations (A, C, G, T)

- Observation (GFP activity, real number)

Pos	1	2	3	GFP-Exp	GFP-Pred
Sample 1	A	T	T	10	10
Sample 2	A	G	T	11	10.5
Sample 3	G	G	T	2	6.5

Algorithm

1. Select sample subset.

2. Find nucleotide that predicts expression.

3. Average results of many trees.

Tree #1

Trained with Samples 1, 3

If: Pos1 == A

Yes   No

10   2
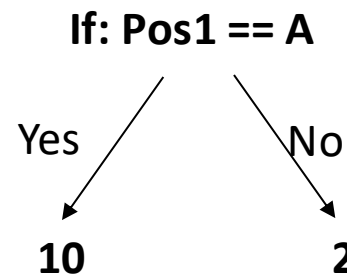
Tree #2

Trained with Samples 1, 2

If: Pos2 == G

Yes   No

11   10

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Principles of Regression

Variables

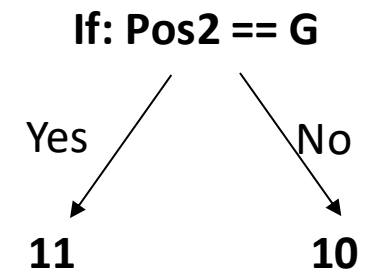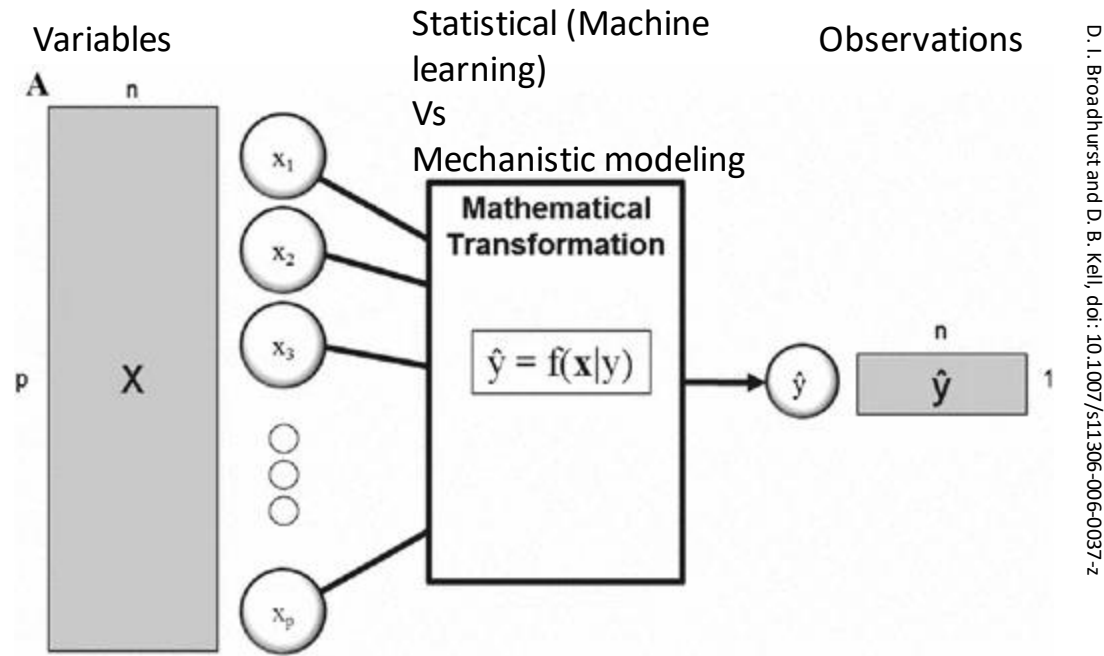Statistical (Machine learning)
Vs
Mechanistic modeling

Observations

A   n

p   X

x₁

x₂

x₃

xₚ

Mathematical Transformation

$$\hat{y} = f(\mathbf{x}|y)$$

$\hat{y}$

n

$\hat{y}$ 1

Regression:

- **Prediction** of continuous observations with variables.

Mathematical transformation:

- **Statistical modeling** with generic formula (machine learning, AI).

- **Mechanistic modeling** with biological interactions and laws.

Examples

Statistical Model

- X: Sequence Library with mutations in promoter region

- Y: Expression rate, measured with GFP

- Regression model: Random Forest

Mechanistic Modeling

- X: time of culture growth

- Y: Biomass

- Regression model: Logistic Function

Outline

1. **Theory**
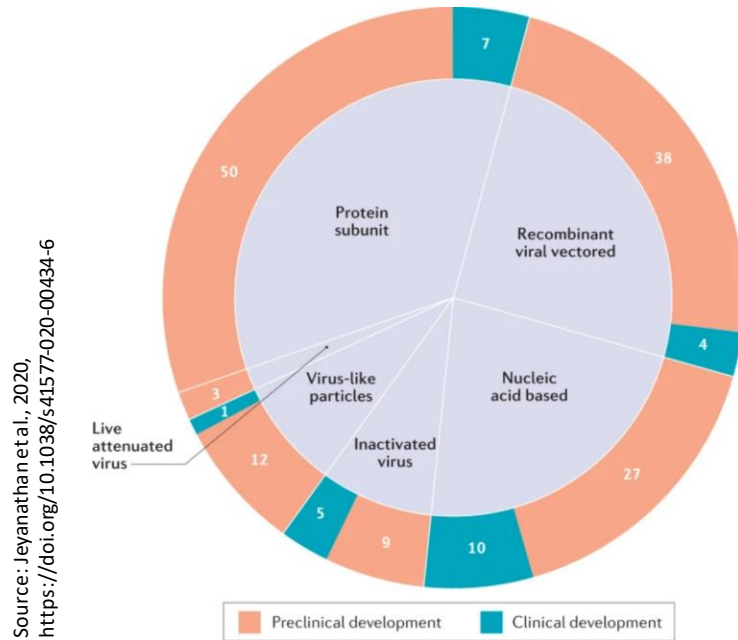   - Microbial Growth
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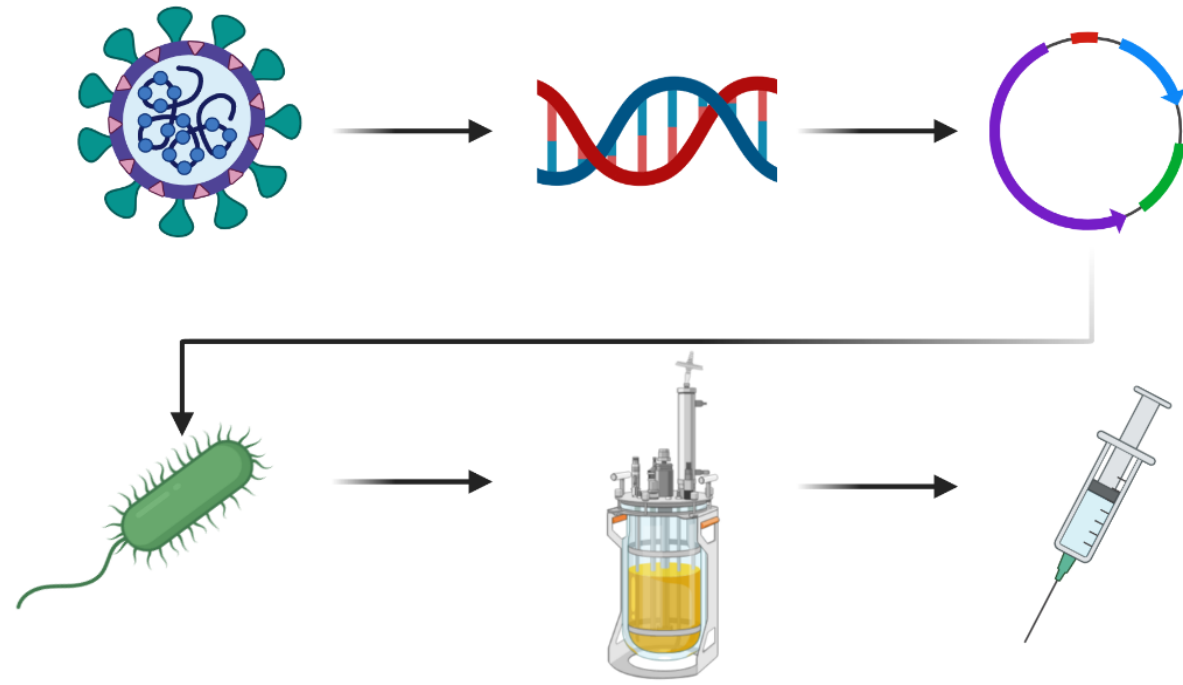   - Life presentation of the simulation

Simulation of Recombinant Expression

Develop an expression system for recombinant production of COVID19 protein subunits for vaccination.

Source: Jeyanathan et al., 2020,
https://doi.org/10.1038/s41577-020-00434-6

The majority of vaccine (candidates) is based on recombinant expression of protein subunits.

Your tasks:

1. Characterize two strains for their growth properties
2. Generate promoter sequences
3. Clone promoter sequences into strain
4. Measure expression rate
5. Conduct batch fermentation to harvest protein subunits

Authors and License

Check the BioLabSim.nrw members at:

https://git.rwth-aachen.de/ulf.liebal/biolabsim/-/blob/91dd40b2f40e6a24263af96c2d5b9817733f4a25/AUTHORS.md

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