French Identification group

Gaussian process modeling of bioprocesses: application to chinese ovary hamster cells cultivated in bioreactors

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November 25, 2021

Context of the study

- Monoclonal antibodies (mAb): inhibition of viral infection.

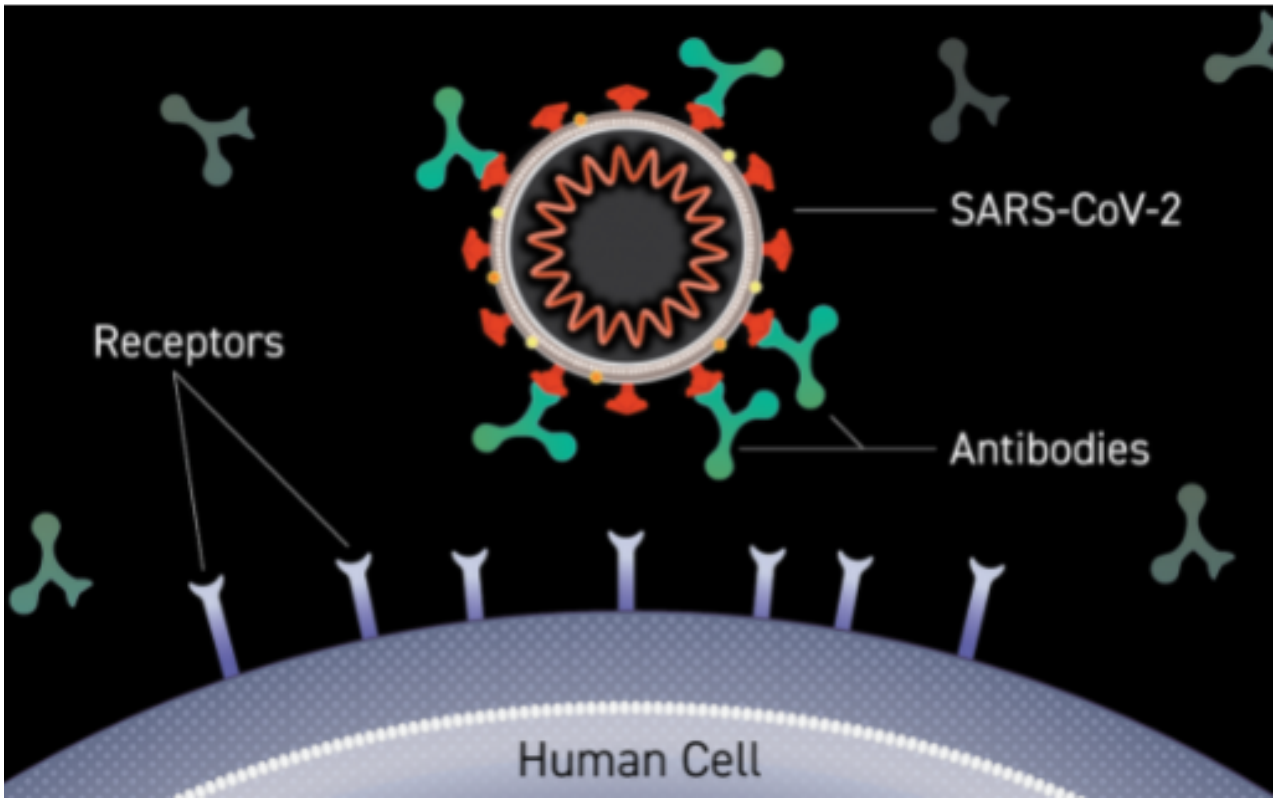
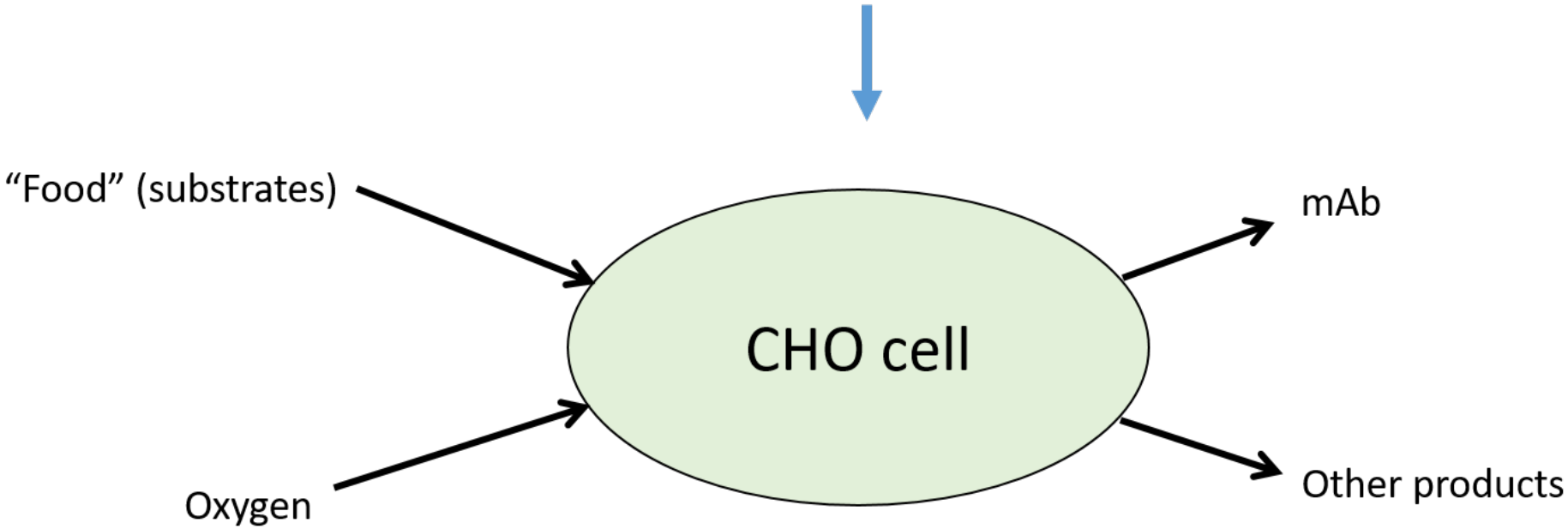


Figure: Scheme of mAb against COVID-19[1]

[1] Source: website of National Institutes of Health (https://www.nih.gov/)

Context of the study

- In industry, mAb is produced by Chinese Hamster Ovary (CHO) cells.



Context of the study

- Chinese Hamster Ovary (CHO) cells cultivated in large-scale bioreactors.

Figure: Picture of a bioreactor[2]

[2] https://www.engr.colostate.edu/CBE101/topics/bioreactors.html

Context of the study

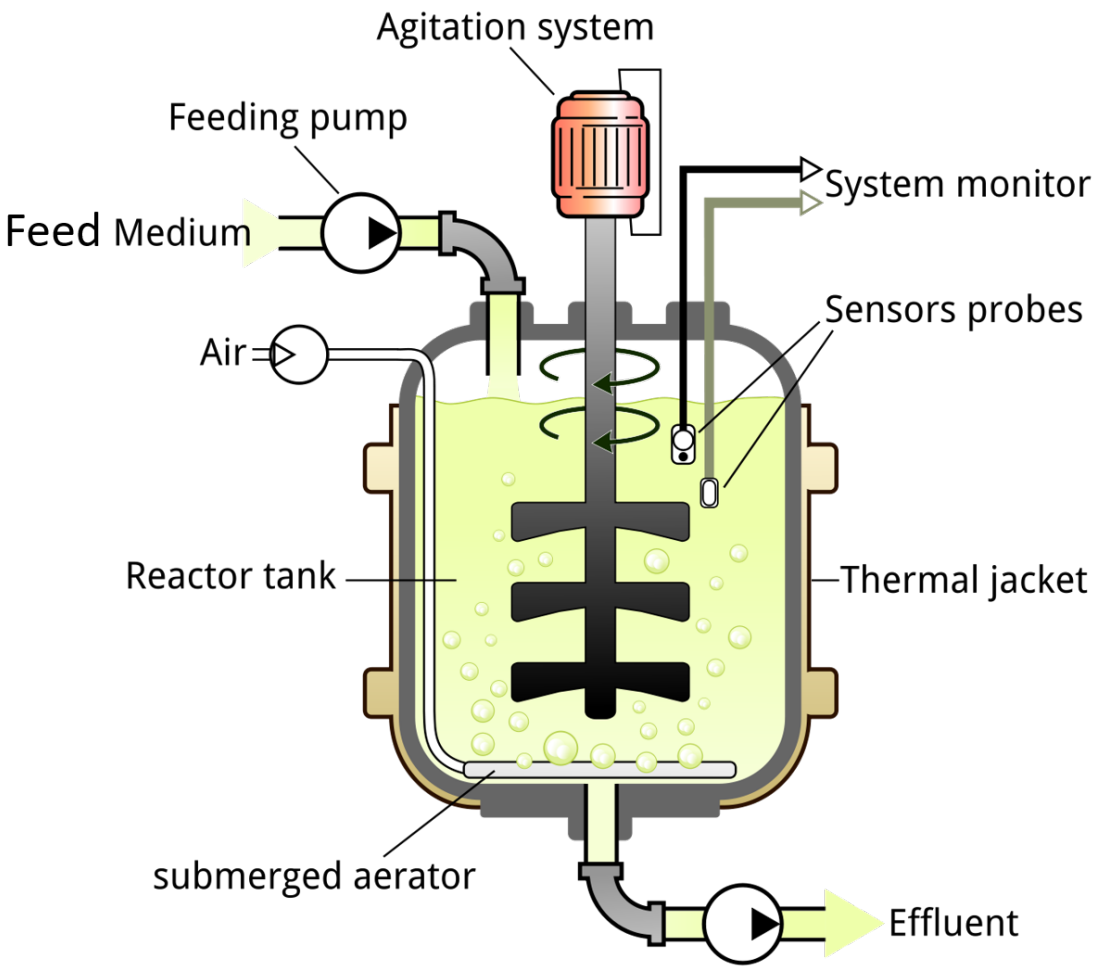


Figure: Scheme of a bioreactor[3]

[3] https://en.wikipedia.org/wiki/Bioreactor/media/File:Bioreactor_principle.svg

Context of the study

- Producing mAb is expensive (and so are the treatments)!
- Ronapreve approved by EU commission against COVID-19 at 1.700 EUR[4]
- **Goal**: maximization of the yield of mAb produced by CHO cells.
- Which variable should we change for the maximization?

[4] https://www.leparisien.fr/societe/sante/ronapreve-regkirona-ce-que-lon-sait-des-deux-traitements-approuves-par-lema-11-11-2021-U64NC7CAQJE2XGVSVW2QF7FIBM.php

Context of the study

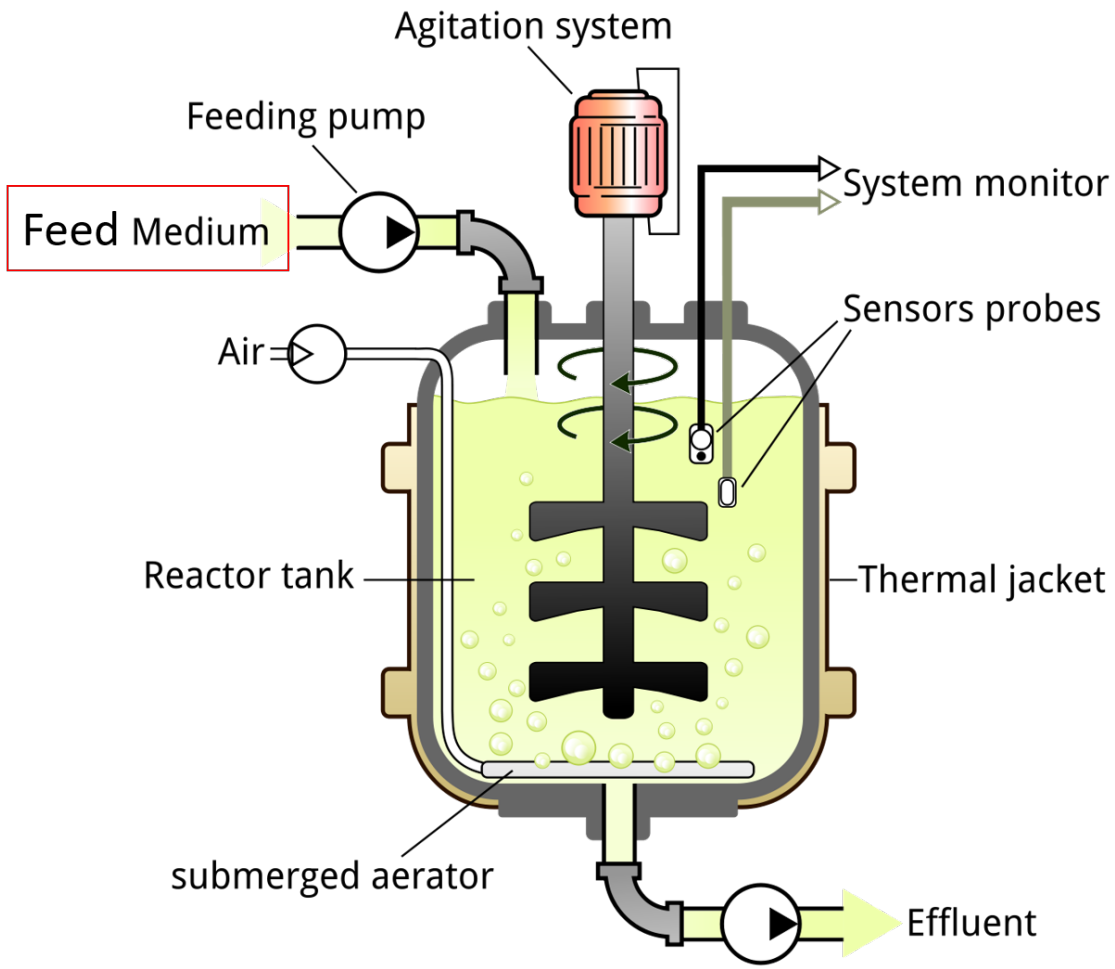
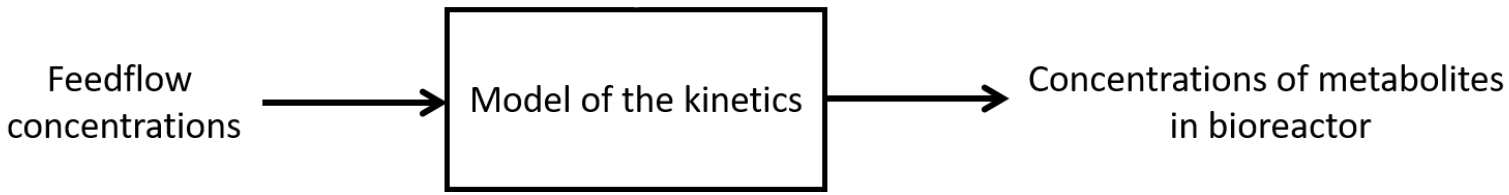


Figure: Scheme of a bioreactor

How to optimize the feed medium?

- Optimize experimentally? No, too long and expensive!
- Solution: model-based optimization.
- What to model? Kinetic evolution of the concentrations in the bioreactor w.r.t. variations of the feed medium concentrations.



Some first principles?

- The model should satisfy some first principles such as mass-balance equation

$$\begin{array}{c}\text{Evolution}\\ \text{of concentration}\end{array} = \begin{array}{c}\text{What the cells}\\ \text{consume/produce}\end{array} + \begin{array}{c}\text{What the feed}\\ \text{medium brings}\end{array} - \begin{array}{c}\text{What the effluent}\\ \text{takes out}\end{array}$$

- With mathematics (x = concentration vector)

$$\frac{dx}{dt}(t) = q(x(t)) + F_{in}(t)x_{feed}(t) - F_{out}(t)x(t)$$

where

- q: uptake/secretion rate (quantity of metabolites consumed/produced by the cells during one day)
- F_{in}: flow rate of feed medium.
- F_{out}: flow rate of effluent.
- x_{feed}: concentration of metabolites in the feed medium.

Some first principles?

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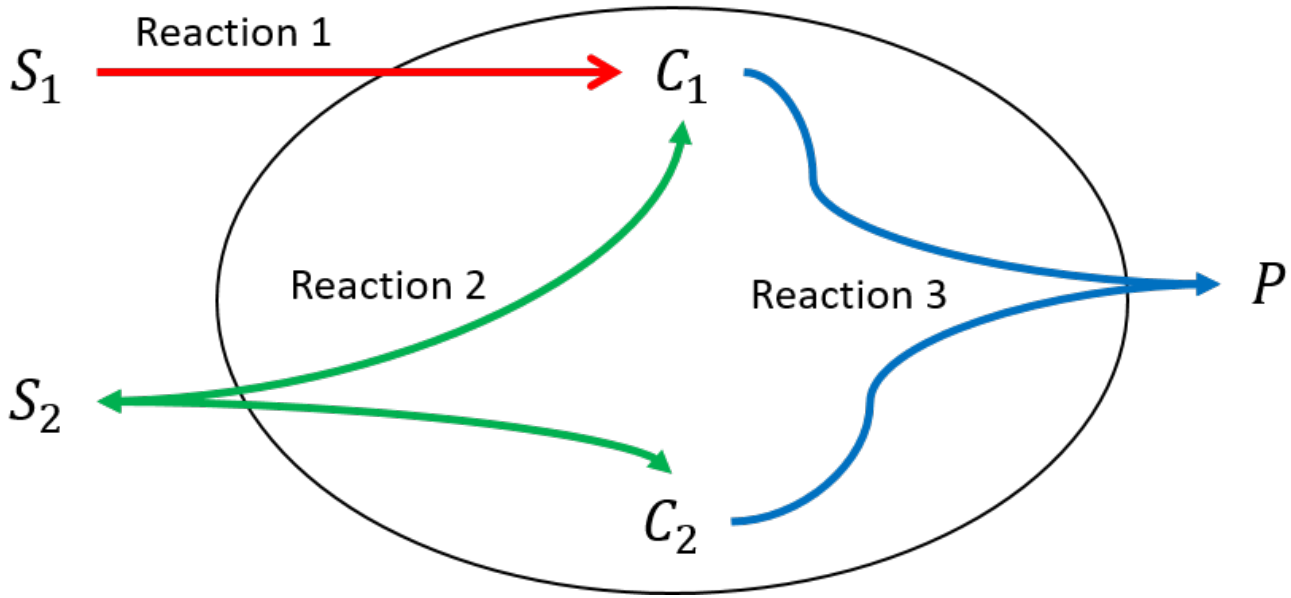
- F_{out}: flow rate of effluent.
- x_{feed}: concentration of metabolites in the medium feed.

How to model $q(x(t))$?

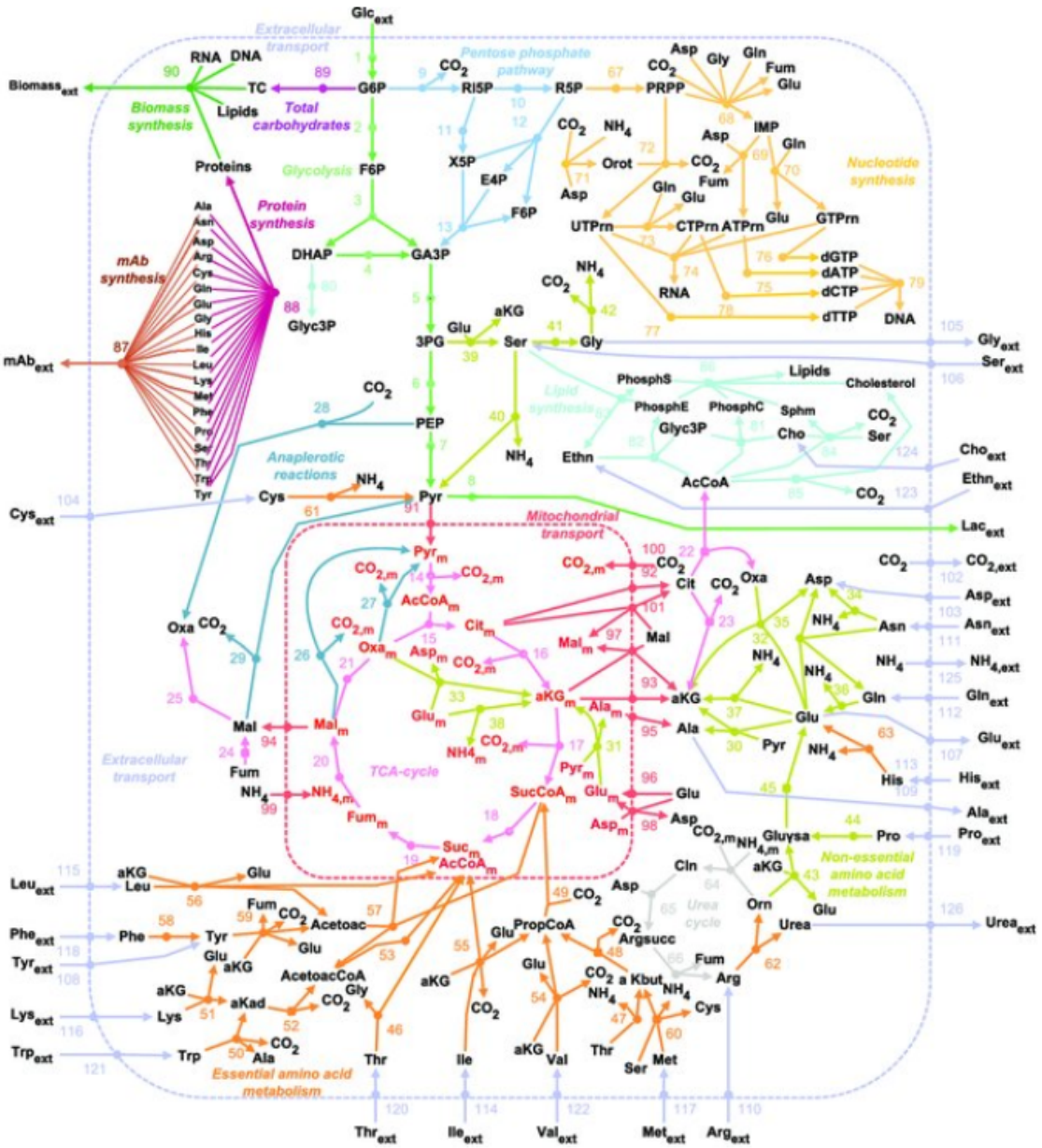
- The vector $q(x(t))$ describes the kinetics of the chemical reactions inside the cells.

- To model $q(x(t))$, we need 3 ingredients
  - Connections between the metabolites (metabolic network).
  - Stoichiometric coefficients of the reactions (stoichiometric matrix)
  - Rate (speed) of all the chemical reactions (kinetic expression)

Ingredient 1: metabolic network



REAL metabolic network



Ingredient 2: Stoichiometric matrix

- Stoichiometric coefficients:
  - Reaction 1: $S_1 \rightarrow 2C_1$
  - Reaction 2: $S_2 \rightarrow C_1 + C_2$
  - Reaction 3: $C_1 + 3C_2 \rightarrow P$

$$\mathbf{A} = \left[\begin{array}{c|ccc} & \text{Reaction 1} & \text{Reaction 2} & \text{Reaction 3} \\ \hline S_1 & -1 & 0 & 0 \\ S_2 & 0 & -1 & 0 \\ C_1 & 2 & 1 & -1 \\ C_2 & 0 & 1 & -3 \\ P & 0 & 0 & 1 \end{array}\right]$$

- Then,

$$q(x) = \mathbf{A}y(x)$$

Ingredient 3: Kinetic expression of the rates

- **Parametric** expression for the rate vector $y(x)$.

- For biochemical reactions: Monod kinetics

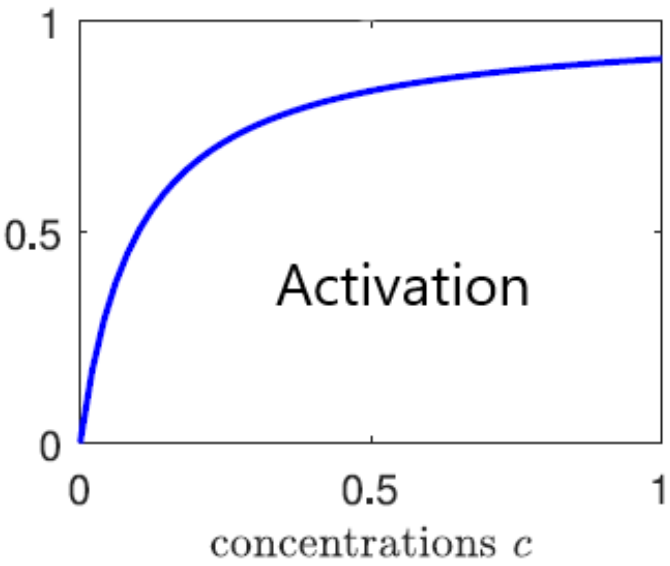
$$\text{Rate of Reaction i} = \prod_{j=1}^{n} \text{Modulation function}(x_j, \eta_{ij})$$

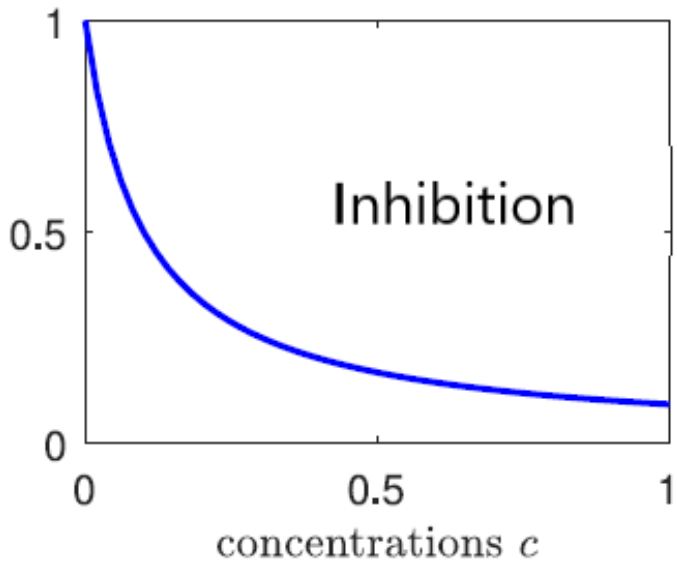
- 4 types of modulation functions:

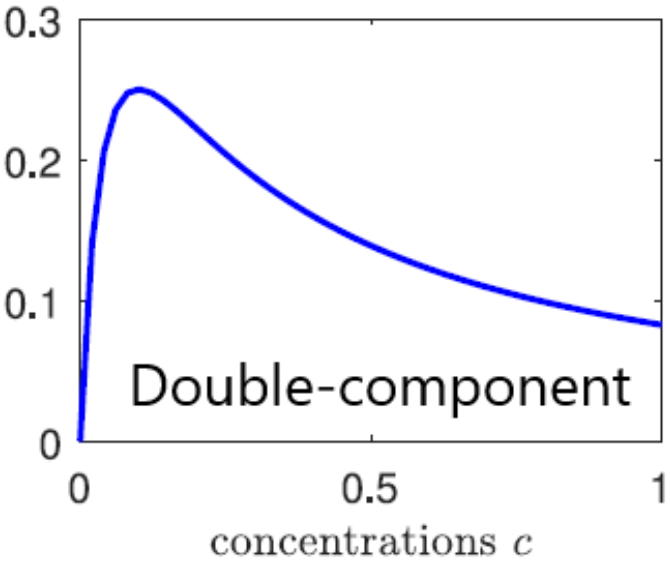
$$\text{Activation :} \quad \frac{x_j}{x_j + \theta_{ij}} \qquad \text{Inhibition :} \quad \frac{1}{1 + \mu_{ij} x_j}$$

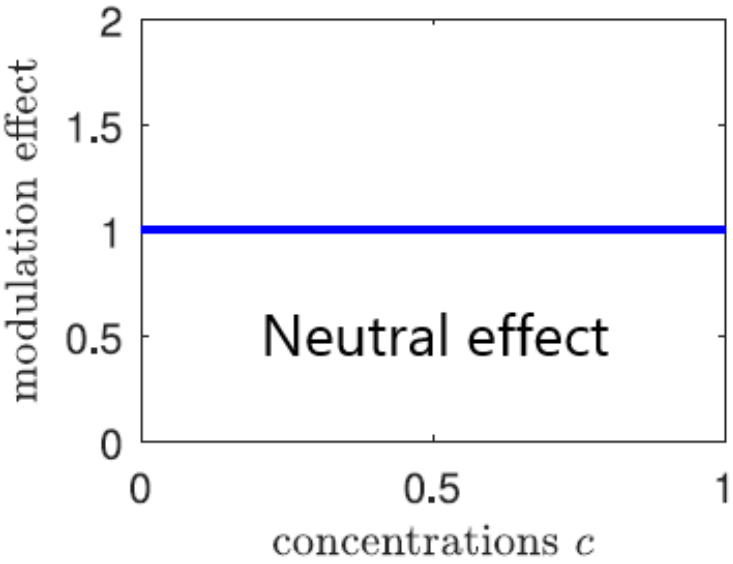
$$\text{Double-component :} \quad \frac{x_j}{x_j + \theta_{ij}} \frac{1}{1 + \mu_{ij} x_j} \qquad \text{Neutral effect :} \quad 1$$

Ingredient 3: Kinetic expression of the rates









Final parametrized dynamic model

- Final mass balance-equation with kinetics

$$\frac{dx}{dt}(t) = \mathbf{A}y(x(t), \eta) + F_{in}(t)x_{feed}(t) - F_{out}(t)x(t)$$

- Goal: identify η.

- Everything is measured/known except $y(x(t), \eta)$. We can estimate the data of the rates!

From dynamic to static map modeling

- Data: $\{y(t), x(t)\}_{t=1}^{N}$.

- The identification problem for the i-th rate is

$$\eta_i^* = \text{arg min} \sum_{t=1}^{N} ||y_i(t) - y_{m,i}(x(t), \eta_i)||^2$$

where

$$y_{m,i}(x(t), \eta_i) = \prod_{j=1}^{n} \frac{x_j(t)}{(x_j(t) + \theta_{ij})(1 + \mu_{ij} x_j(t))}$$

- We have transformed the nonlinear dynamic identification problem into a nonlinear static map identification.

Design of a kernel for Gausian process regression of Monod functions

The identification problem

- Focus on one rate.

- The identification problem is

$$\eta^* = \text{arg min} \sum_{t=1}^{N} ||y(t) - y_m(x(t), \eta)||^2$$

where

$$y_m(x(t), \eta) = \prod_{j=1}^{n} \frac{x_j(t)}{(x_j(t) + \theta_j)(1 + \mu_j x_j(t))}$$

The identification problem

- Local minima: we need a good initialization of the parameters.

- Main idea: perform a GP regression of **each** modulation function as intermediate modeling method for the initialization.

Step 1: Gaussian process modeling

- Assume additive noise:

$$y(t) = \prod_{j=1}^{n} h_j(x_j(t), \eta_j) + \epsilon(t)$$

where ϵ is a white Gaussian noise of variance σ_e^2.

- Each function h_j is modeled as a zero-mean Gaussian process

$$h_j \sim \mathcal{GP}(0, k_j)$$

- We need an appropriate kernel function for the modeling of the 4 types of Monod functions.

Kernel in the literature

- Kernel proposed in the literature[5]

$$k_j(x_j(t), x_j(t'), \Theta_j) = \gamma_j \left(\frac{x_j(t)}{x_j(t')} \right)^{-\delta_j \log\left(\frac{x_j(t)}{x_j(t')} \right)}$$

[5] Wang, M., Risuleo, R. S., Jacobsen, E. W., Chotteau, V., Hjalmarsson, H. (2020). Identification of nonlinear kinetics of macroscopic bio-reactions using multilinear Gaussian processes. Computers Chemical Engineering, 133, 106671

Kernel in the literature

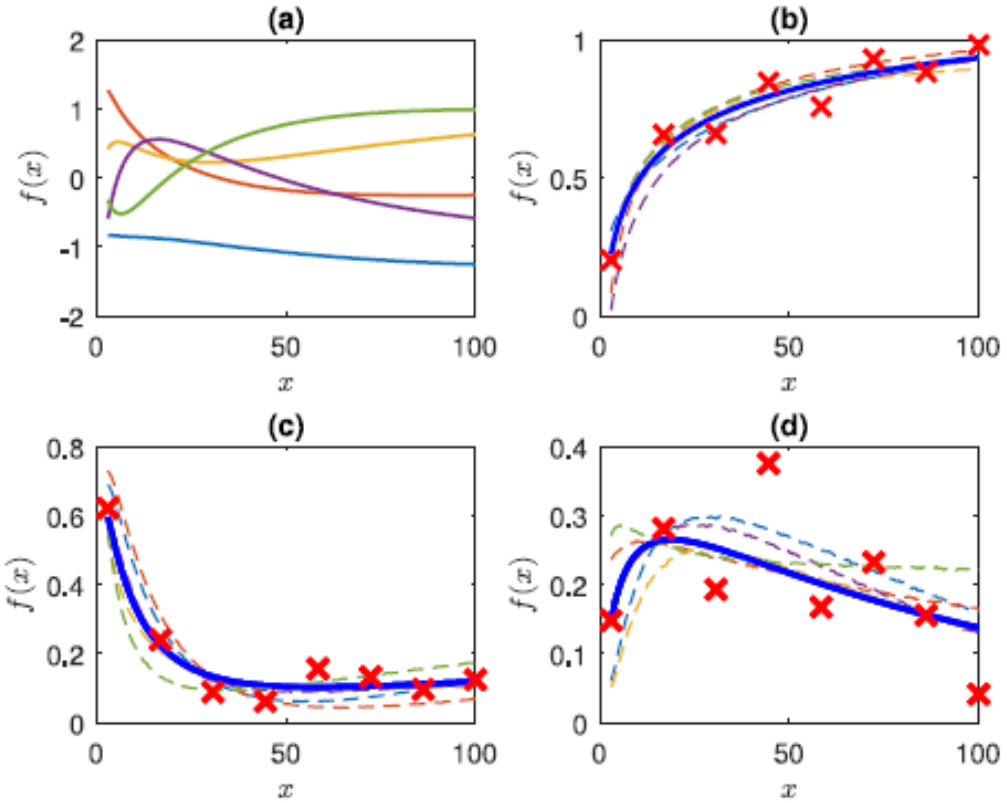


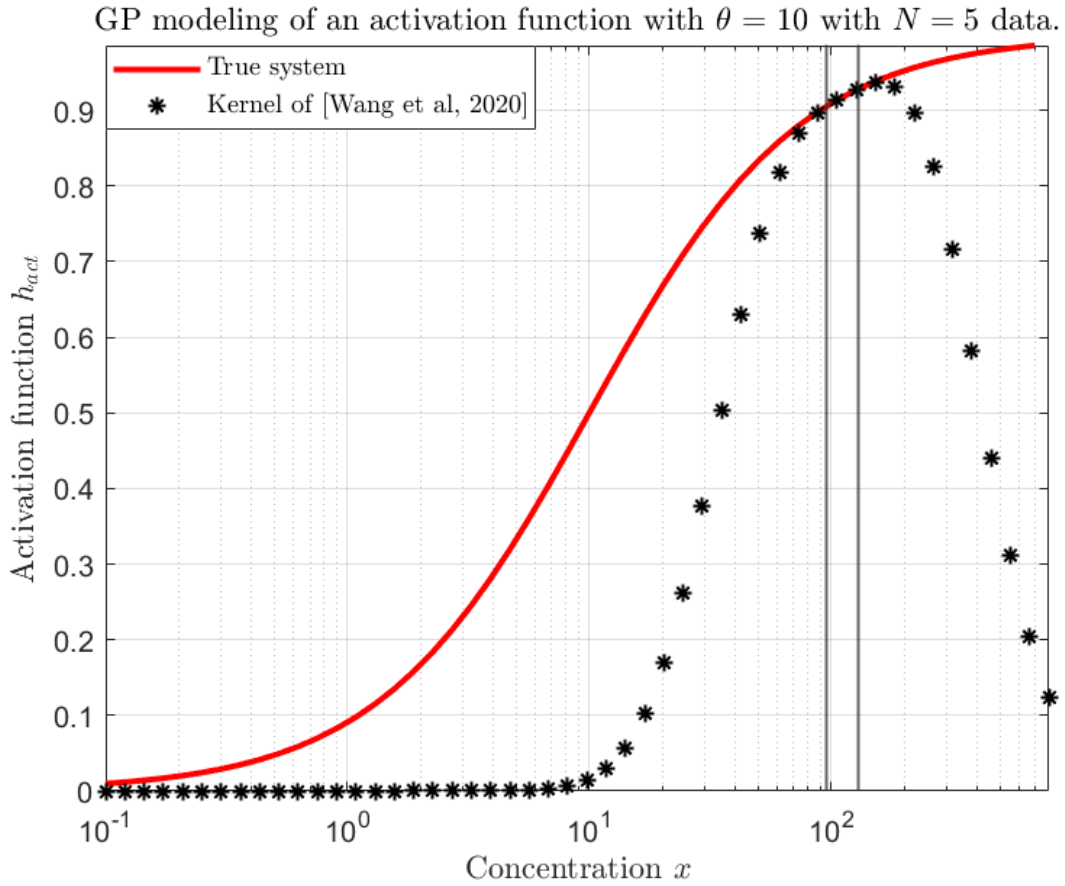
Figure: Some examples of posterior means[6]

[6]Wang, M., Risuleo, R. S., Jacobsen, E. W., Chotteau, V., Hjalmarsson, H. (2020). Identification of nonlinear kinetics of macroscopic bio-reactions using multilinear Gaussian processes. Computers Chemical Engineering, 133, 106671

Kernel in the literature

• However, it can yield inaccurate estimates for small data set and/or poorly distributed in the input space ($\approx$ bioreactor data).

• Example with $y = x/(x+10)$ with $N = 5$ noiseless data randomly chosen in the interval $[80, 130]$



Research problem

- What to do?

- Idea 1: experiment design (but it is costly!).

- Idea 2: incorporate priors in the kernel design.

Research problem

Design better-tailored kernel function for the modeling of the Monod functions h_j.

- Idea: incorporate the **structure** of the Monod functions in the design.

Design of a better kernel: activation

- Consider again a simple activation function for the true system

$$y(x(t)) = h_{act}(x(t), \theta) + \epsilon$$

where $h_{act}(x(t)) = x(t)/(x(t) + \theta)$.

- The covariance between two output data $y(x(t))$ and $y(x(t'))$ is equal to

$$E[y(x(t))y(x(t'))] = h_{act}(x(t), \theta)h_{act}(x(t'), \theta) + E[\epsilon(t)\epsilon(t')]$$

- Ideal kernel function for modeling of h_{act} is then

$$k_{act,ideal}(x(t), x(t'), \theta) = h_{act}(x(t), \theta)h_{act}(x(t'), \theta)$$

- But (i) this kernel is invalid (covariance matrix never positive definite) and (ii) it depends on the unknown θ

Design of a better kernel: activation

- Idea: integrate w.r.t. θ between two bounds θ^- and θ^+!

$$k_{act}(x, x') = \int_{\theta^-}^{\theta^+} h_{act}(x, \theta) h_{act}(x', \theta) d\theta$$

$$= \begin{cases} x^2 \left(\frac{1}{\theta^- + x} - \frac{1}{\theta^+ + x} \right) & \text{if } x = x' \\ \frac{xx'}{x - x'} \log \left(\frac{\theta^+ + x'}{\theta^+ + x} . \frac{\theta^- + x}{\theta^- + x'} \right) & \text{elsewhere} \end{cases}$$

- We obtain a valid kernel constructed directly from activation function (prior added in the design)!

- But, two additional hyperparameters (θ^- and θ^+)!

Step 2: kinetic modeling

- How to choose θ^- and θ^+?

- If no prior on kinetic parameters θ, we choose "wide bounds" but still biologically realistic (e.g., $\theta^- = 0.01$ and $\theta^+ = 100$).

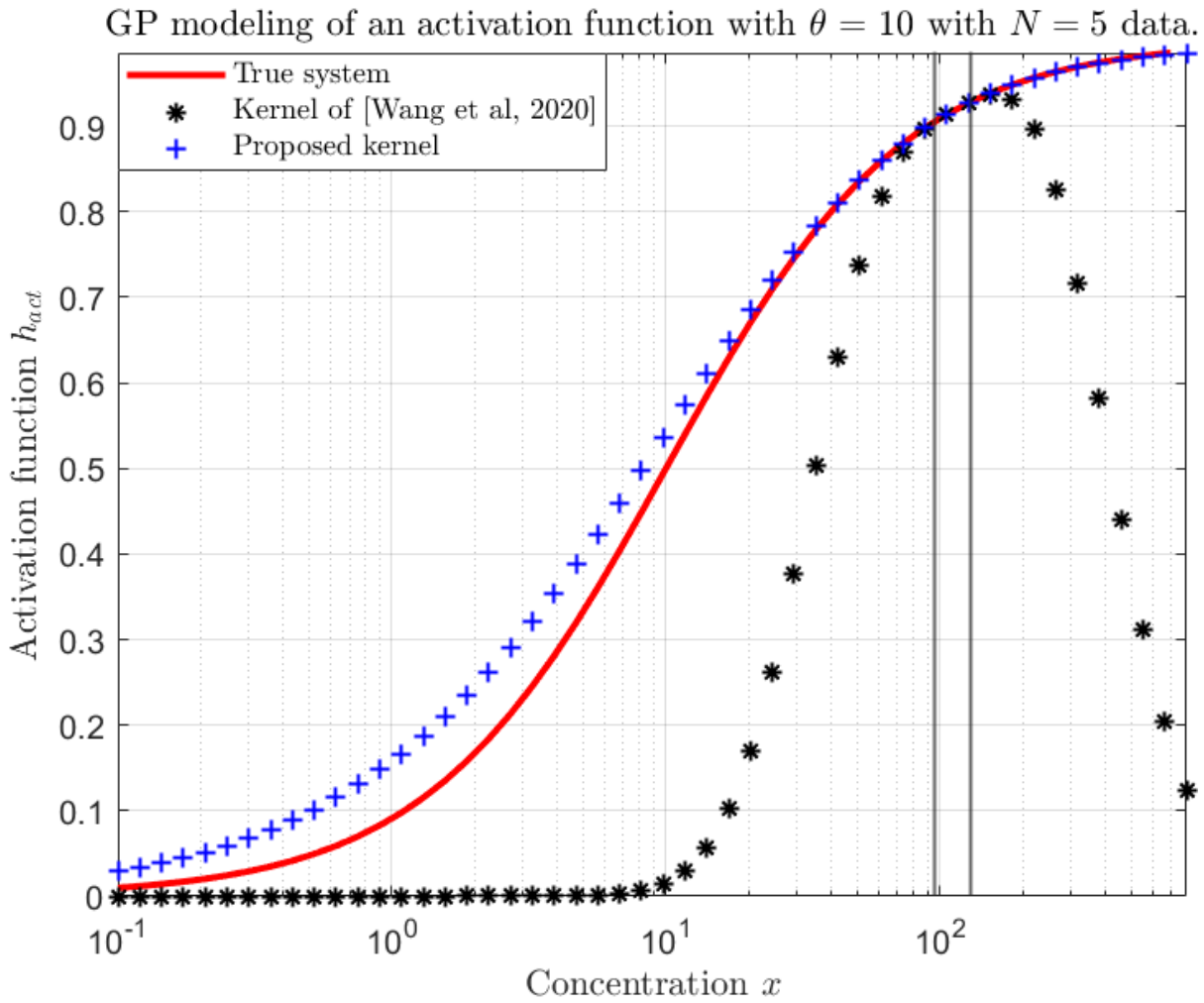
- However, if we have priors on θ in the form of uncertainty intervals

$$\theta \in [\Theta^-, \Theta^+]$$

then we choose $\theta^- = \Theta^-$ and $\theta^+ = \Theta^+$.

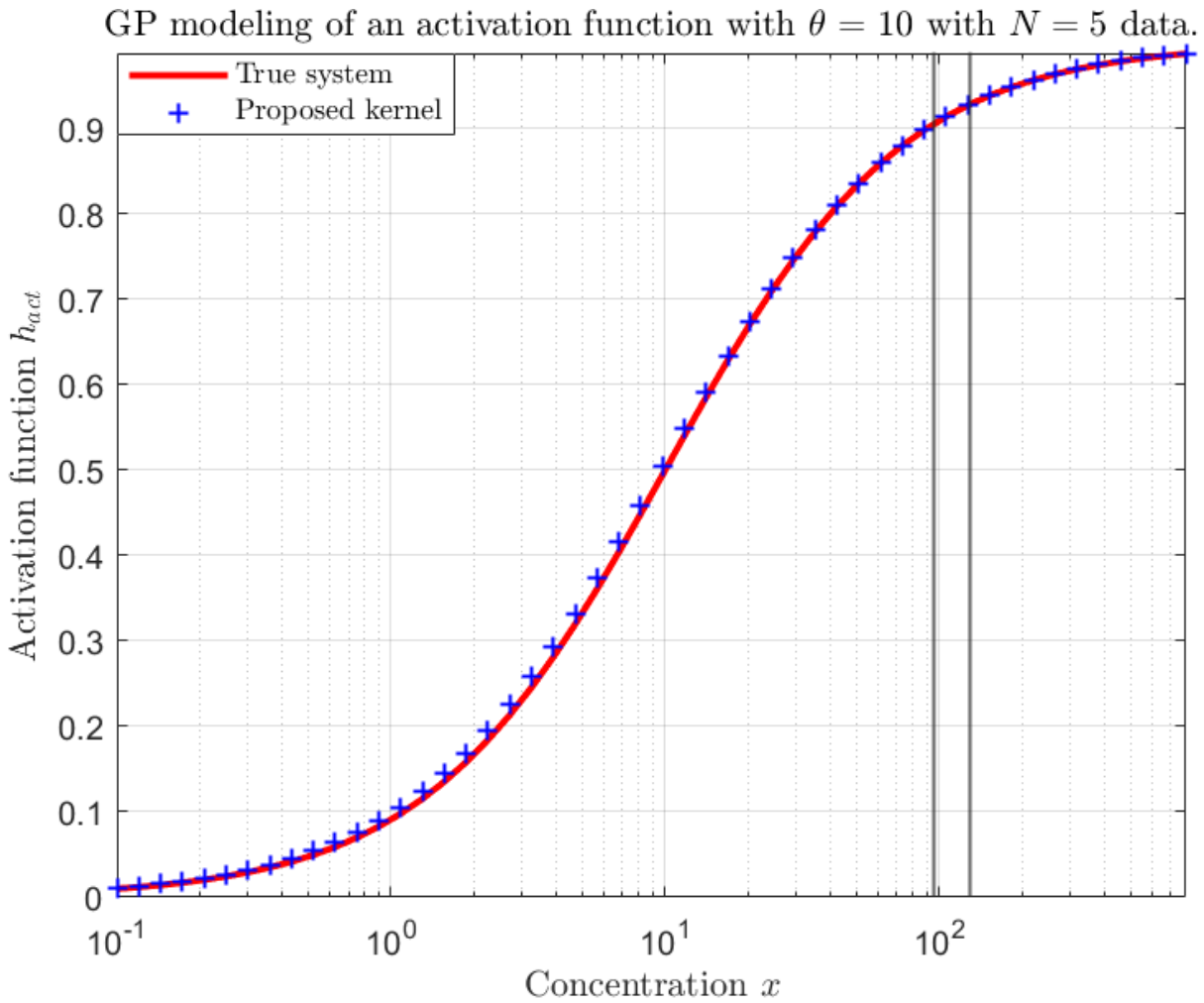
Design of a better kernel: activation

- Back to the example with bounds $\theta^- = 0.01$ and $\theta^+ = 100$.



Design of a better kernel: activation

- Assume that we know that $\theta \in [5, 20]$. Chosen bounds: $\theta^- = 5$, $\theta^+ = 20$.



Design of a better kernel: inhibition

- Inhibition function

$$h_{inh}(x) = 1/(\mu x + 1)$$

- We can similarly construct a kernel with integration:

$$k_{inh}(x, x') = \int_{\mu^-}^{\mu^+} h_{inh}(x, \mu) h_{inh}(x', \mu) d\mu$$

$$= \begin{cases} \dfrac{1}{x}\left(\dfrac{1}{\mu^- x + 1} - \dfrac{1}{\mu^+ x + 1}\right) & \text{if } x = x' \\ \dfrac{1}{x - x'} \log\left(\dfrac{\mu^+ x + 1}{\mu^+ x' + 1} . \dfrac{\mu^- x + 1}{\mu^- x' + 1}\right) & \text{elsewhere} \end{cases}$$

Design of a better kernel: double-component

- Double-component

$$h_{dc}(x) = h_{act}(x, \theta) h_{inh}(x, \mu)$$

- New kernel:

$$k_{dc}(x, x') = k_{act}(x, x') k_{inh}(x, x')$$

Design of a better kernel: neutral effect

- Neutral effect

$$h_{ne}(x) = 1$$

- We should consider a mean function and a zero covariance.

- Instead of this, we approximate for neutral function by

$$h_{ne}(x) = 1 + \varepsilon x$$

with $\varepsilon << 1$ (10^{-3}).

- Then, $k_{ne}(x, x') = (1 + \varepsilon x)(1 + \varepsilon x')$.

Final kernel

• We have a kernel for each type of kinetics. For each modulation function, we have to select the best kernel.

• BUT, we do not know the type of kinetic beforehand.

• Final kernel:

$$k_j(x_j, x_j') = \beta_{act,j} k_{act}(x_j, x_j') + \beta_{inh,j} k_{inh}(x_j, x_j') + \beta_{dc,j} k_{dc}(x_j, x_j') + \beta_{ne,j} k_{ne}(x_j, x_j')$$

• By tuning the hyperparameters $\beta_j = (\beta_{act,j}, \beta_{inh,j}, \beta_{dc,j}, \beta_{ne,j})^T$, we can select different types of kinetics.

• With $\beta_j = (1, 0, 0, 0)^T$, we will consider activation functions for h_j.

Tuning the hyperparameters with Empirical Bayes

Hyperparameter estimation

- With n different metabolites, we have n hyperparameter vectors β_j to estimate.

- We have data

$$\boldsymbol{y} = \begin{pmatrix} y(1) \\ \vdots \\ y(N) \end{pmatrix}$$

- Empirical Bayes: we want to estimate the hyperparameter vector $\beta = (\beta_1^T, \cdots, \beta_n)^T$ such that we maximize the likelihood $p(\boldsymbol{y}|\beta)$.

- BUT

$$y(t) = \prod_{j=1}^{n} h_j(x_j(t)) + \epsilon(t)$$

$$h_j \sim \mathcal{GP}(0, k_j(\beta_j))$$

$\rightarrow$ Likelihood intractable!

Hyperparameter estimation

• How to circumvent the problem?

• Solution: introduce latent variables $\boldsymbol{h}_j = \{h_j(t)\}_{t=1}^N$ for all $j = 1, \cdots, n$ ($\rightarrow$ $N \times n$ latent variables).

• How to estimate? Iterative estimation of the hyperparameters and latent variables as follows

- From $\beta^{(k)}$, we sample the latent variables $\boldsymbol{h}_j$ from the posterior $p(\boldsymbol{h}_1, \cdots, \boldsymbol{h}_n | \boldsymbol{y}, \beta^{(k)}) \Longrightarrow \hat{\boldsymbol{h}}_j^{(k)}$.
- From $\hat{\boldsymbol{h}}_j^{(k)}$, we estimate β which maximizes $p(\boldsymbol{h}^{(k)}|\beta) \Longrightarrow \beta^{(k+1)}$
- $k \rightarrow k+1$

• Expectation maximization + sampling (heuristic convergence to global optimum for β).

Hyperparameter estimation

- How to sample from joint posterior $p(\boldsymbol{h}_1, \cdots, \boldsymbol{h}_n | \boldsymbol{y}, \beta^{(k)})$?

- Solution: Gibbs sampling, i.e., iterative sampling of the conditional posterior distributions.

$$\widehat{\boldsymbol{h}}_1 \rightarrow \widehat{\boldsymbol{h}}_2 \rightarrow \cdots \rightarrow \widehat{\boldsymbol{h}}_n \rightarrow \widehat{\boldsymbol{h}}_1 \rightarrow \cdots$$

Hyperparameter estimation

- Hyperparameter optimization?

- Solve

$$\beta_j^* = \underset{\beta_j}{\arg\max} \; -\log\det(K(\beta_j)) - \hat{\boldsymbol{h}}_j^T K^{-1}(\beta_j)\hat{\boldsymbol{h}}_j \tag{1}$$

where

$$K(\beta_j) = \beta_{act,j}K_{act} + \beta_{inh,j}K_{inh} + \beta_{dc,j}K_{dc} + \beta_{ne,j}K_{ne} \tag{2}$$

- Nonconvex optimization:
  - Bruteforce optimization.
  - Combinatorial optomization.

Numerical example

Numerical example

- Toy example with $n = 6$ metabolites and $\sigma_\epsilon^2 = 10^{-3}$.

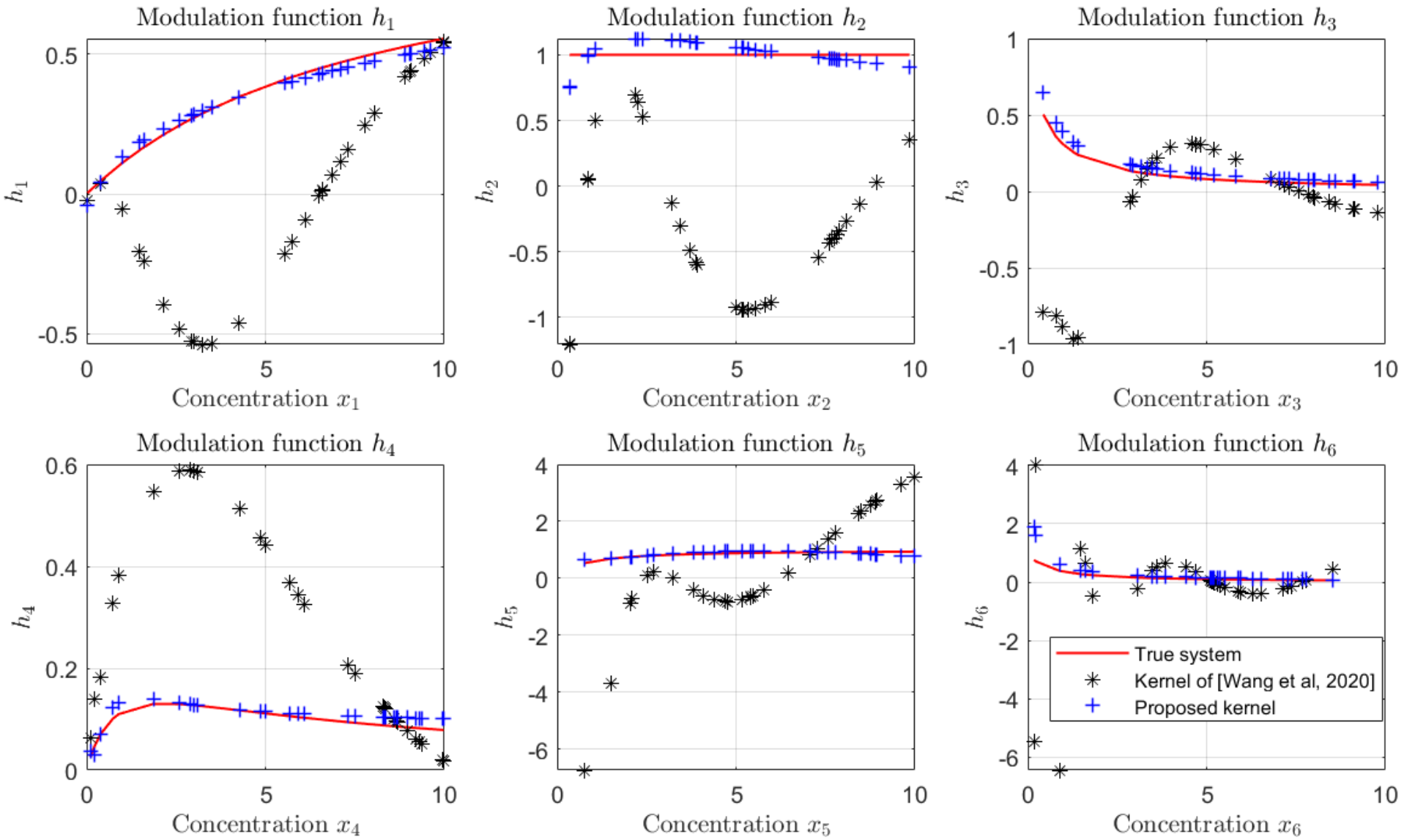
$$y = h_1 \times h_2 \times h_3 \times h_4 \times h_5 \times h_6 + \epsilon$$

Modulation function	Type of kinetic	θ_j	μ_j
h_1	Activation	8.01	-
h_2	Neutral	-	-
h_3	Inhibition	-	2.27
h_4	Double Component	6.81	0.82
h_5	Activation	0.67	–
h_6	Inhibition	–	1.8

Table: True type of kinetics and parameters for the 6 modulation functions h_i.

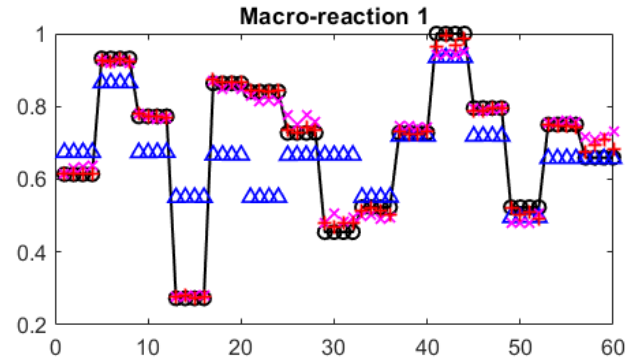
- $N = 30$ concentration data chosen randomly in interval $[0, 10]$.

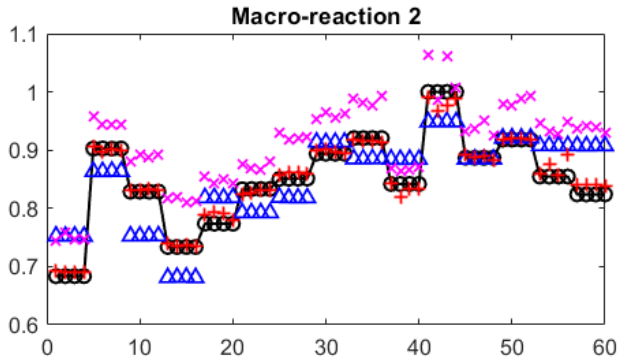
Numerical example

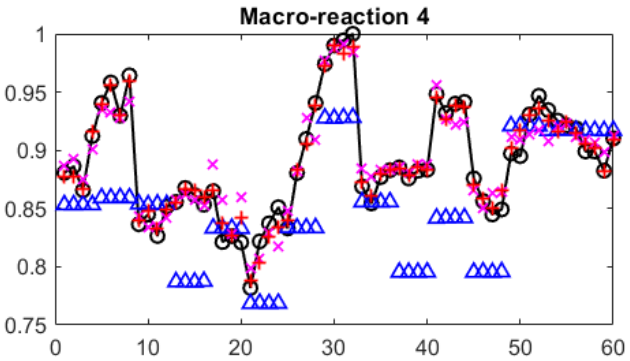


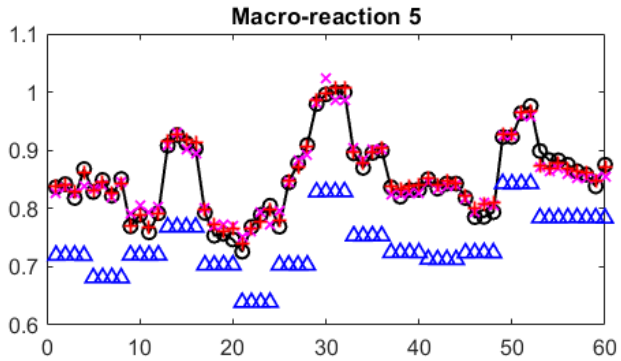
Real-life data with 4 metabolites in kinetic modeling

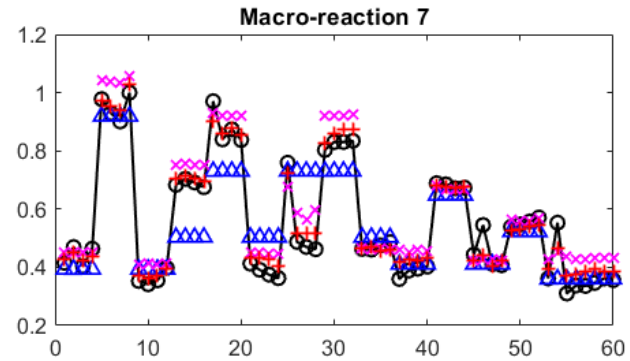
- Black: data, Blue: kernel from literature, Red and magenta: new kernel

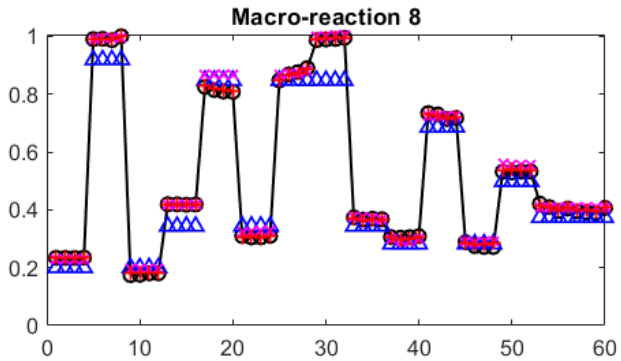












Conclusion and possible extension

Conclusion

- Design of a better-tailored kernel for the modeling of Monod functions.

- Better results than kernel in the literature.

- Main idea: incorporate the structure of the functions to be modeled in the kernel design.

Future work and possible extension

- Test on real-life data.

- First possible generalization of the study

$$\int_{\theta^-}^{\theta^+} h_{act}(x,\theta)h_{act}(x',\theta)d\theta \rightarrow \int_{-\infty}^{\infty} h_{act}(x,\theta)h_{act}(x',\theta)p(\theta)d\theta$$

Future work and possible extension

• Second possible extension: assume that we have a static map to be modeled of this form

$$y(x) = \sum_{j=1}^{n} \phi_j(x, \theta_j) + \epsilon$$

where $\phi_j(x, \theta_j)$ are parametrized nonlinear basis functions (structure known, parameter θ_j unknown).

• Nonlinear least-square optimization $\rightarrow$ local minimum.

• Idea: GP regression with a kernel equal to a linear combination of kernels designed as

$$k_j(x, x') = \int_{-\infty}^{\infty} \phi_j(x, \theta_j)\phi_j(x', \theta_j)p(\theta_j)d\theta_j$$

Thank you for your attention!