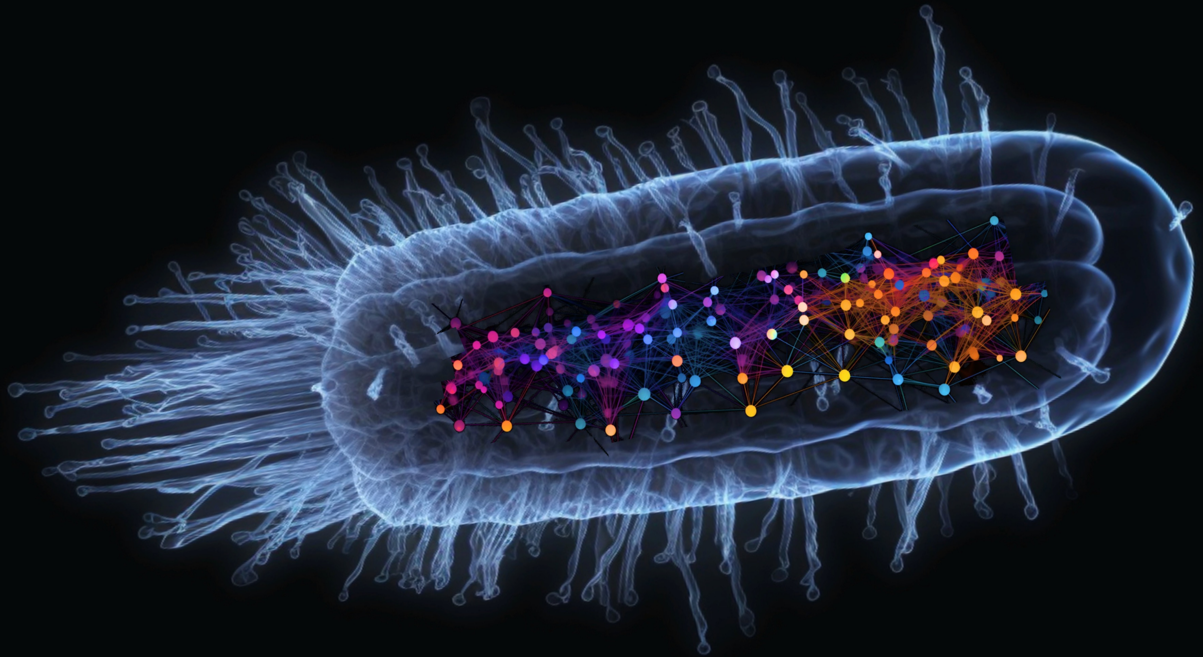

Machine learning models applied to Synthetic Biology

Jean-Loup Faulon – Jean-Loup.Faulon@inrae.fr – https://jfaulon.com/course-materials

MICALIS Institute

ACS Synthetic Biology

Key words: AI or Machine Learning

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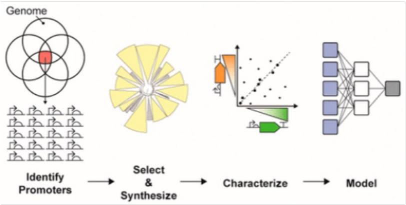

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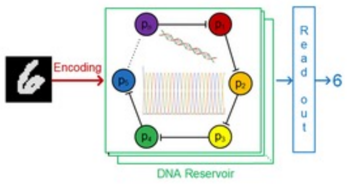

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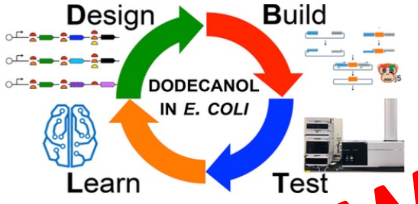

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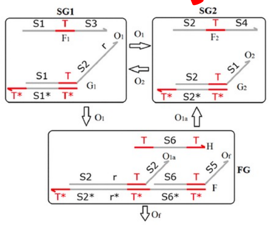

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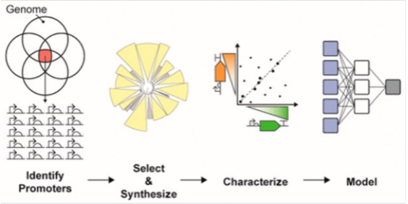
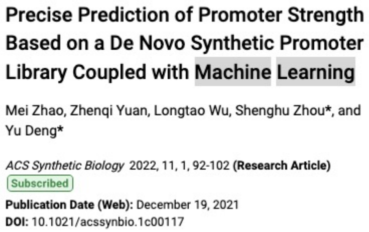
Supervised / Unsupervised Learning

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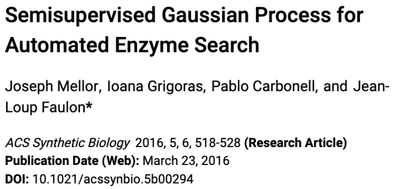
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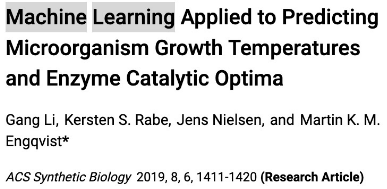
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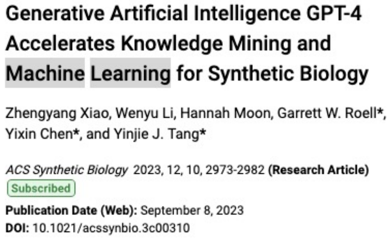
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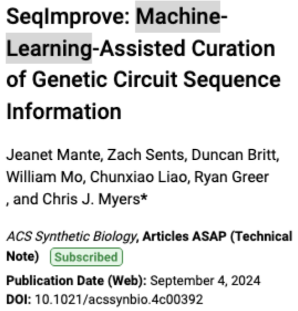
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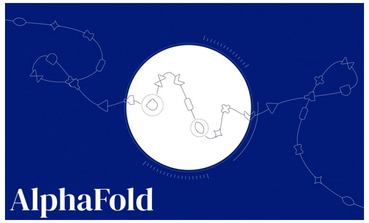
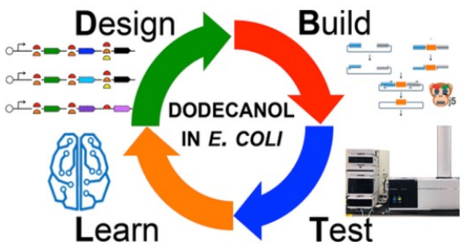
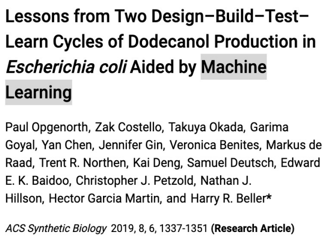


Active / Reinforcement Learning

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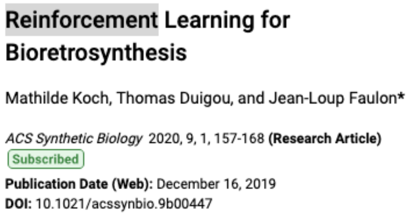
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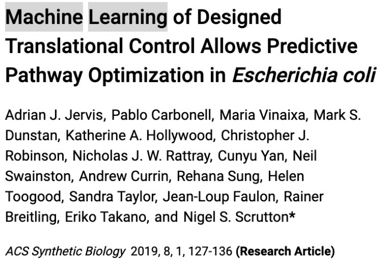
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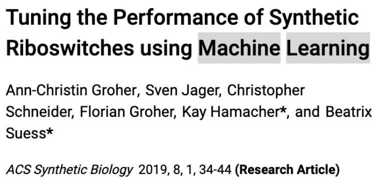
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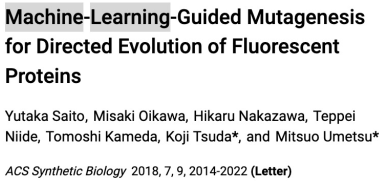
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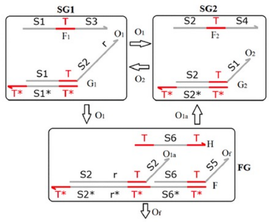
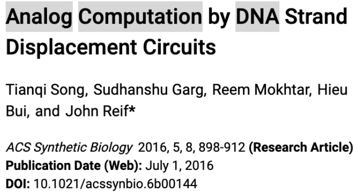
in vitro / in vivo Learning

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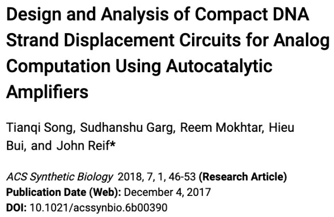
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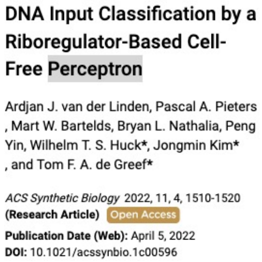
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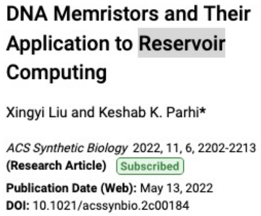
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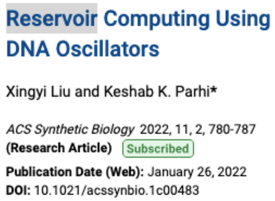
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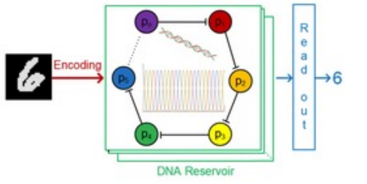
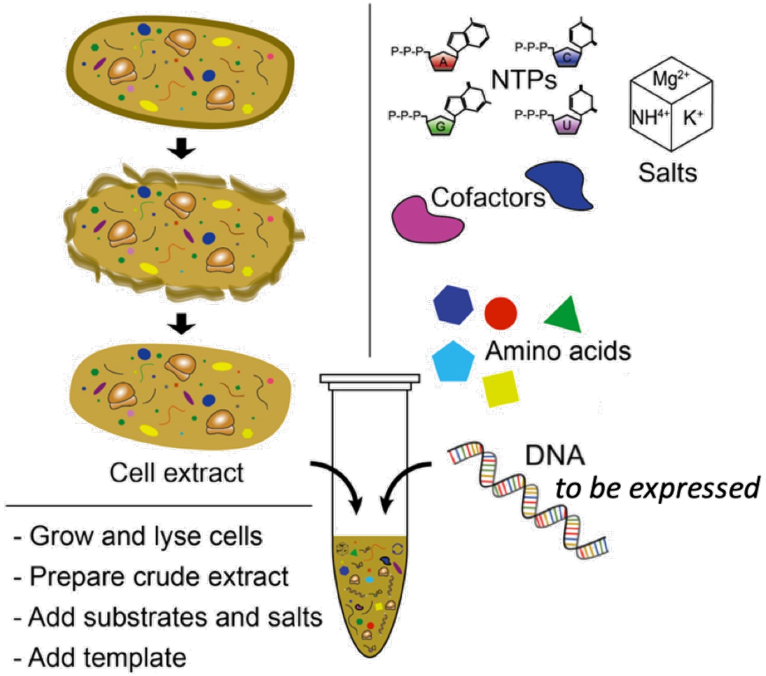


Active learning to optimize cell-free productivity

Lysate-based cell-free systems (TXTL)



- Grow and lyse cells
- Prepare crude extract
- Add substrates and salts
- Add template
- Incubate

Reference composition
Sun Z.Z. *et al. J. Vis. Exp.* 2013

Component	Concentration			
Mg-glutamate (mM)	0.4	1.2	2	4
K-glutamate (mM)	8	24	40	80
Amino Acid (mM)	0.15	0.45	0.75	1.5
tRNA (mg/ml)	0.02	0.06	0.1	0.2
CoA (mM)	0.026	0.078	0.13	0.26
NAD (mM)	0.033	0099	0.165	0.33
cAMP (mM)	0.075	0.225	0.375	0.75
Folinic Acid (mM)	0.0068	0.0204	0.034	0.068
Spermidine (mM)	0.1	0.3	0.5	1
3-PGA (mM)	3	9	15	30
NTP (mM)	0.15	0.45	0.75	1.5

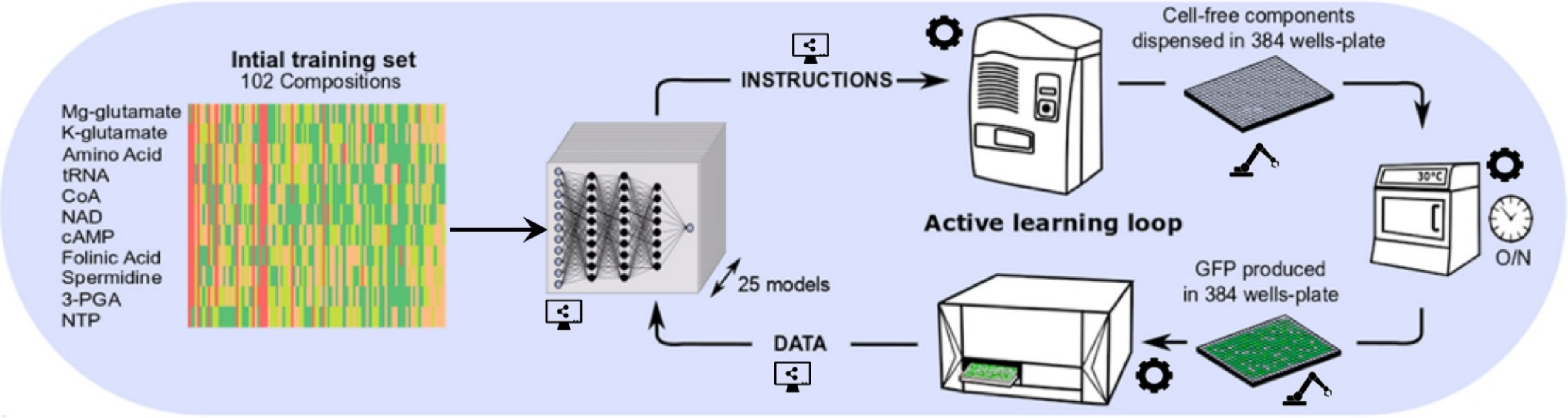
Combinatorial space = 4^{11}
= **4 194 304** compositions

⇓

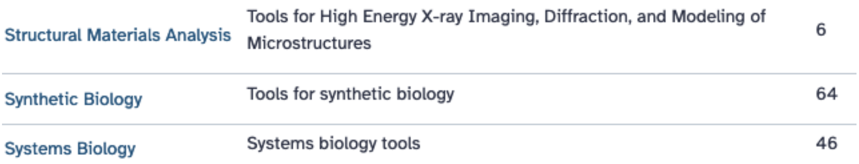
- Can we improve protein production without increasing the price of cell-free reaction?
- Can we provide efficient predictions of protein production *in vitro*?
- Can we highlight the critical parameters involve in protein production *in vitro*?

Active learning to optimize cell-free productivity

- **Set up an initial batch sampling the space of possible compositions**
- **Measure yield level though fluorescence**
- **Develop a Neural Network models predicting yield from composition**
- **Use the models to predict the yield for each composition not yet tested**
- **Select next batch of compositions to be measured based on exploitation vs. exploration**
- **Repeat**

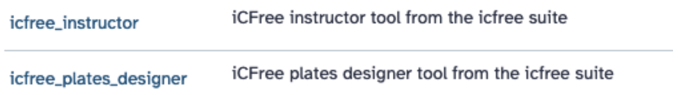


Galaxy Tool Shed →



Structural Materials Analysis	Tools for High Energy X-ray Imaging, Diffraction, and Modeling of Microstructures	6
Synthetic Biology	Tools for synthetic biology	64
Systems Biology	Systems biology tools	46

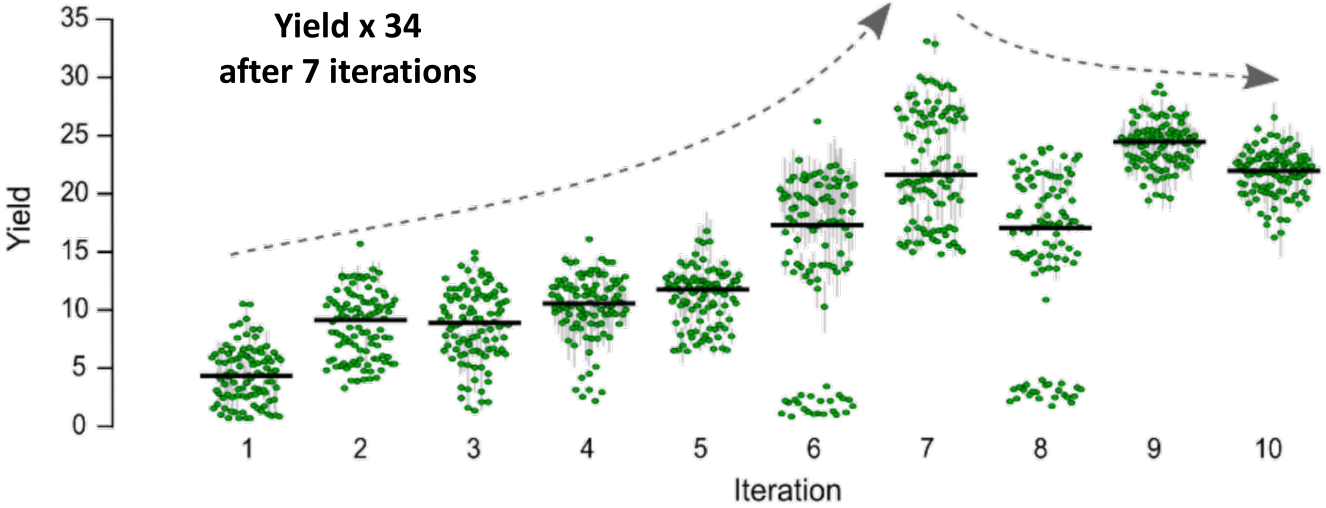
→

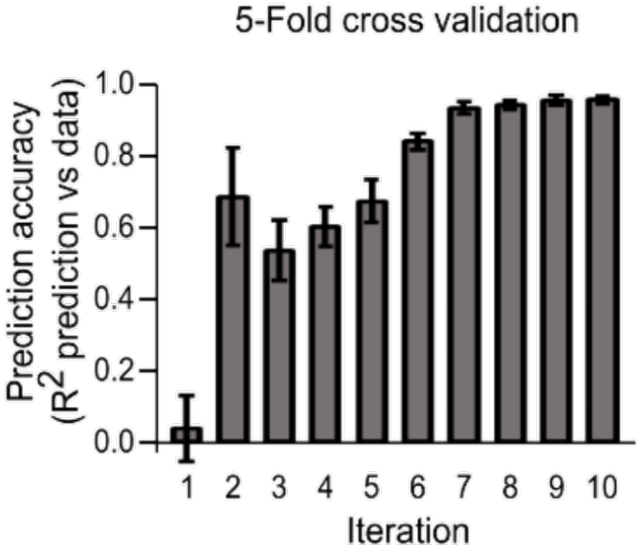


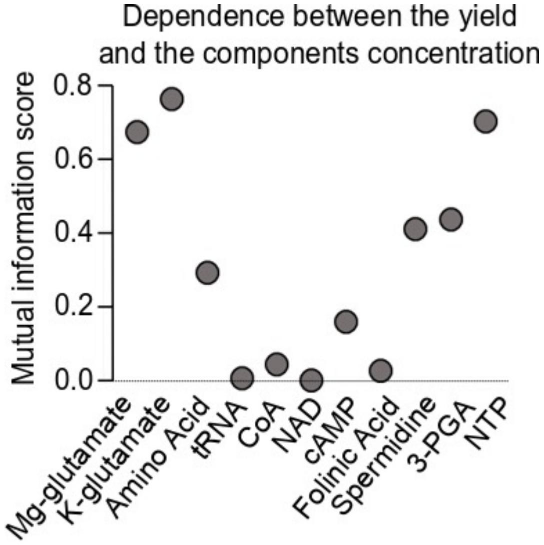
icfree_instructor	iCFree instructor tool from the icfree suite
icfree_plates_designer	iCFree plates designer tool from the icfree suite

- Herisson J, *et al. Nat Commun* **13**, 5082 (2022)

Active learning to optimize cell-free productivity

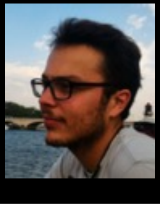


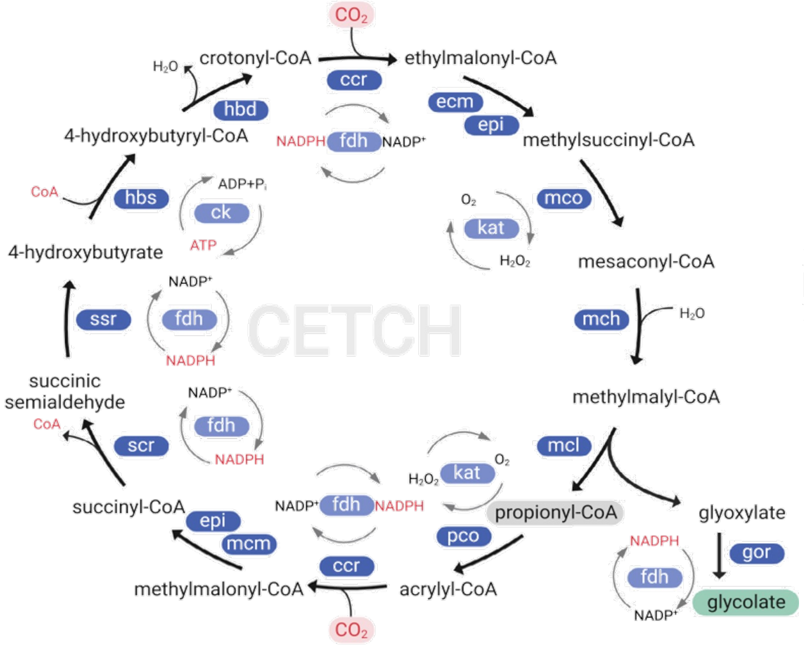


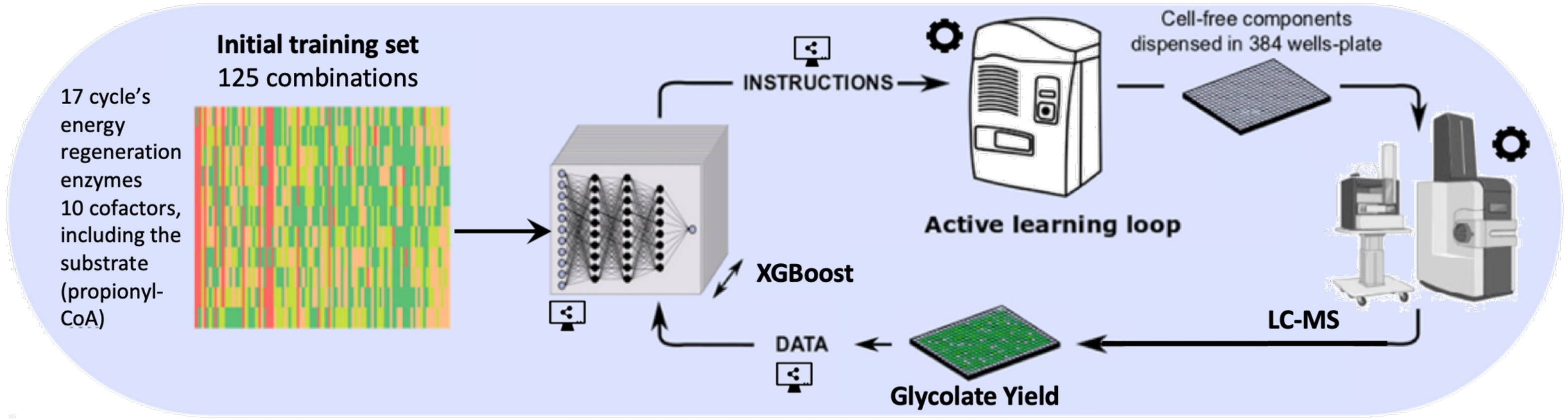


- Borkowski O, *et al. Nat Commun* **11,** 1872 (2020)

Active learning to optimize cell-free metabolic pathways



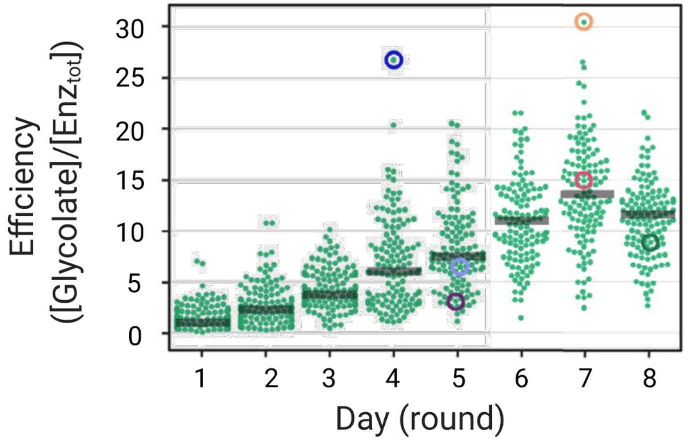


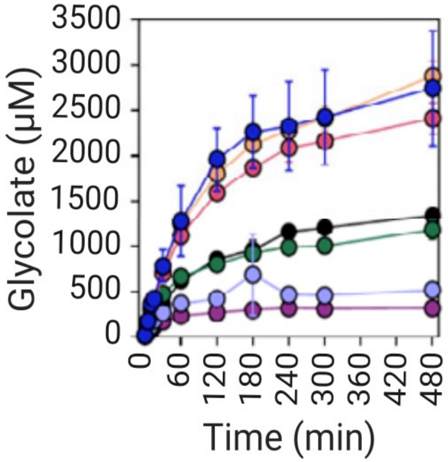


Initial training set
125 combinations

17 cycle's energy regeneration enzymes
10 cofactors, including the substrate (propionyl-CoA)

Cell-free components dispensed in 384 wells-plate





Top 3 best producers

Control (CETCH5.4)

Randomly selected producers

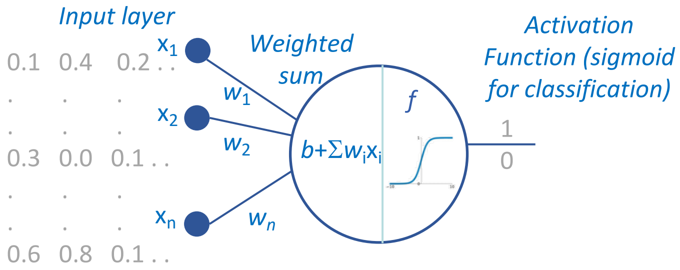
6x more efficient than the best in vitro CO_2-fixing system described to date (CETCH 5.4 , Schwander *et al. Science* 2016)

- Pandi A., *et al. Nat Commun* **13,** 3876 (2022)

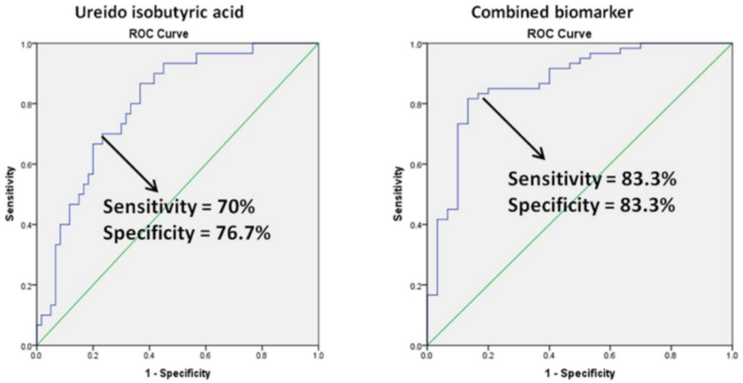
In vitro / in vivo learning: why?

TRAINING THE NETWORK

Perceptron weights (w_i) are learned to increase classifier accuracy



Prostate cancer $\text{PCa metabolic score} = b + \sum_{j=1}^{j} w_j x_{ij}$



- Zang, *et al.* ***PLoS One*** 2013 and ***J Proteome Res.*** 2014
- Shen B, et al. ***Cell***. 2020
- .....

USING THE TRAINED NETWORK

To perform a diagnostic:

- Quantify a panel of biomarkers (metabolites) on clinical samples (using metabolomics)
- Feed measured biomarkers concentrations (x_i) to

$$\boxed{f\,(b+\Sigma w_i x_i\,)}$$

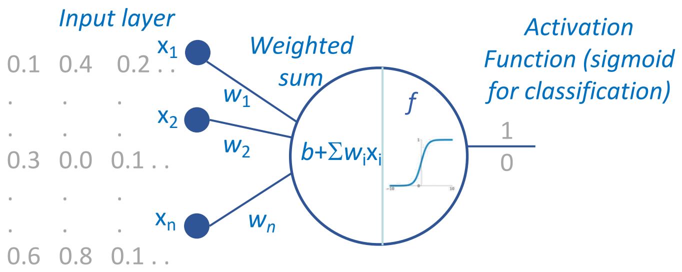
- **Is it possible to avoid biomarker concentration measurements?**

  ➢ **Engineer the trained network *in vitro* or *in vivo* and directly use it on clinical samples**

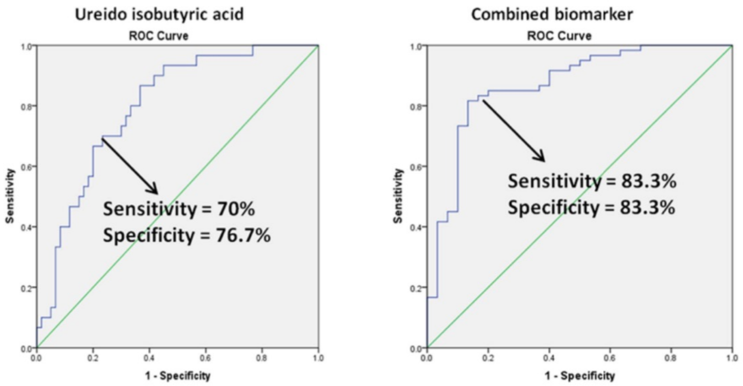
Engineering a neural metabolic network: the concept

TRAINING THE NETWORK

Perceptron weights (w_i) are learned to increase classifier accuracy



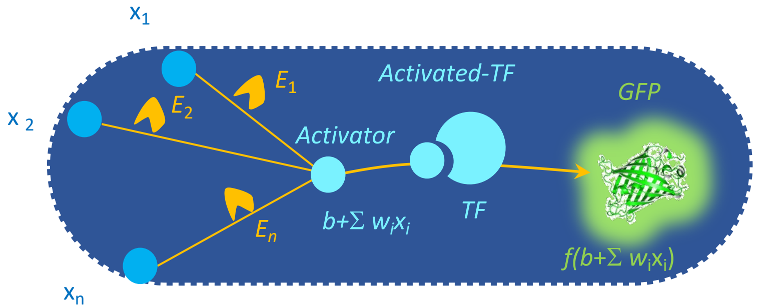
Prostate cancer $\quad \text{PCa metabolic score} = b + \sum_{j=1}^{j} w_j x_{ij}$



- Zang, *et al.* ***PLoS One*** 2013 and ***J Proteome Res.*** 2014
- Shen B, et al. ***Cell***. 2020
- …..

ENGINEERING THE TRAINED NETWORK

Need to actuate weighted sum and activation function

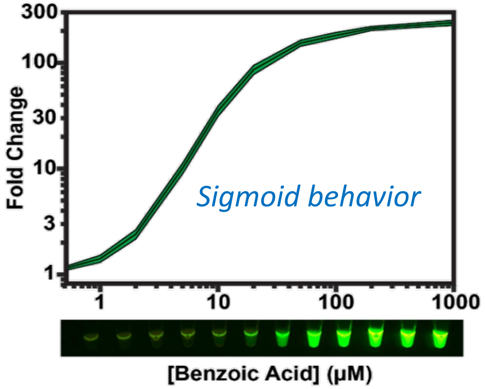


Enzymatic transformation *Reporter gene*

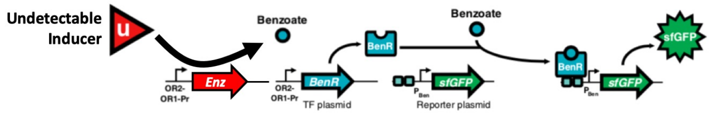
In theory (Michaelis-Menten) when $x_i << [E_i]$:

$$d[p] = \Sigma\, k_i[E_i]x_i\, dt$$

$w_i = k_i\, [E_i]$
where k_i is a kinetics constant



Engineering a neural metabolic network: the concept



$$\frac{dbenzoate}{dt} = enz * \frac{k_{cat} * inducer}{inducer + K_M}$$

$$\frac{dinducer}{dt} = -enz * \frac{k_{cat} * inducer}{inducer + K_M}$$

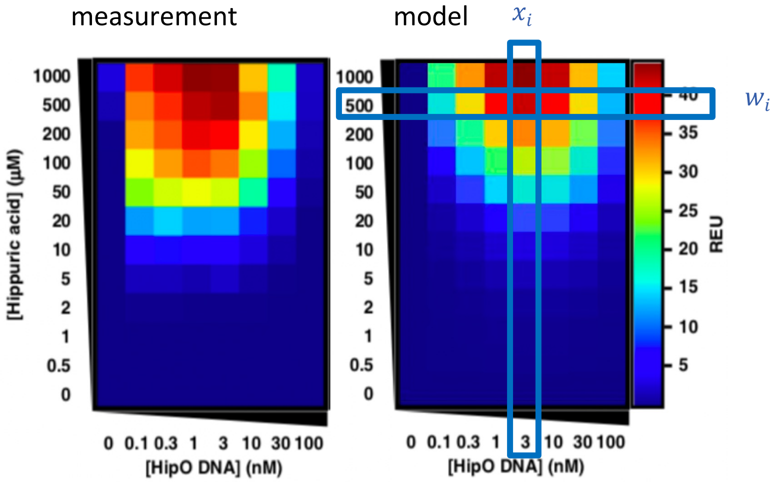
$$TF_{activated} = TF * \frac{benzoate}{benzoate + K_d^{inducer}} + 0.0005$$

$$\epsilon = \frac{TF_{activated}}{TF_{activated} + K_d^{activated}} \text{ for BenR}$$

$$\epsilon = 1 \text{ for constitutive expression}$$

$$\frac{dmRNA}{dt} = \gamma * n * \epsilon \frac{x}{x+\chi} * \frac{K_{tox}}{K_{tox} + tox} * \frac{R_{mRNA}}{R_{mRNA} + K_{mRNA}} - \delta * mRNA$$

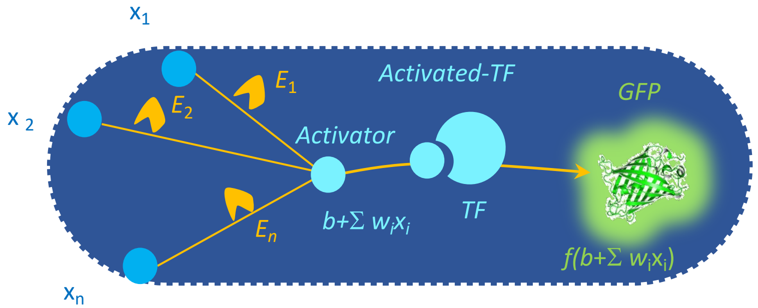
$$\frac{dprot}{dt} = \pi * mRNA * \frac{y}{y+k} * \frac{K_{tox}}{K_{tox} + tox} - \lambda * prot$$


measurement

model

ENGINEERING THE TRAINED NETWORK

Need to actuate weighted sum and activation function



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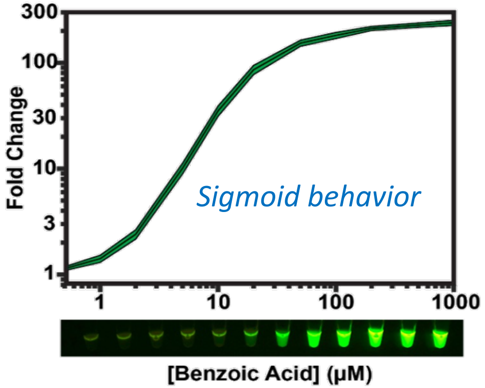


Sigmoid behavior

- Voyvodic, P.L., Pandi, A., Koch, M. *et al. Nat Commun* **10**, 1697 (2019).

Engineering a neural metabolic network in vitro

TARGETED BEHAVIOUR

	(−)			(+)												
Hippurate……………		●				●	●	●				●	●	●		●
Cocaine……………….			●			●			●		●	●	●		●	●
Benzamide………….				●			●		●	●		●		●	●	●
Biphenyl-2,3-diol…					●			●		●	●		●	●	●	●

$f(b+\sum w_i x_i) < 0.5$

$f(b+\sum w_i x_i) \geq 0.5$

- Kinetics model is used to compute the enzyme concentration for each weight

Logistic regression

$f(b+\sum w_i x_i)$

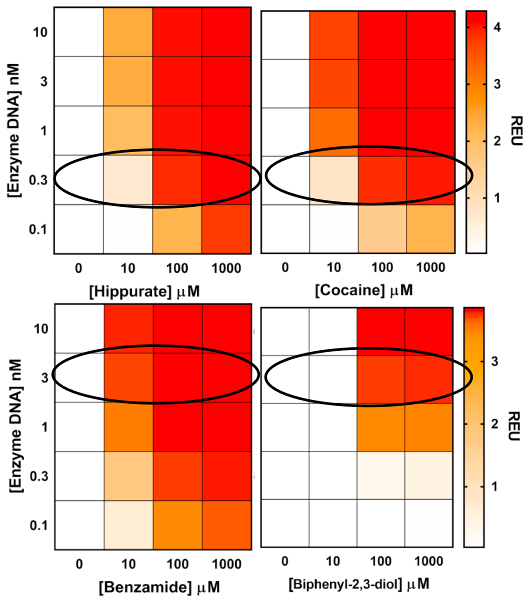
$b =$ - 0.50

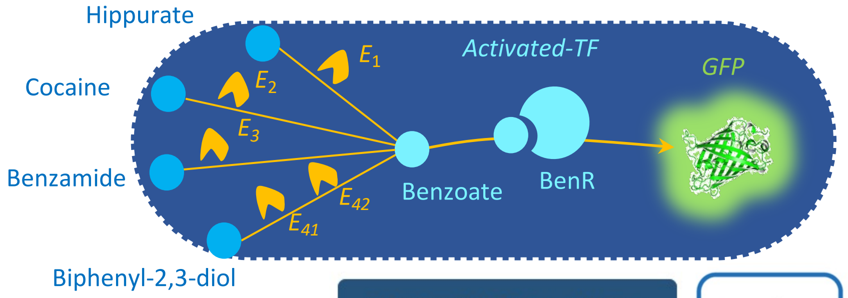
$w_1 =$ 0.25

$w_2 =$ 0.25

$w_3 =$ 0.50

$w_4 =$ 0.50

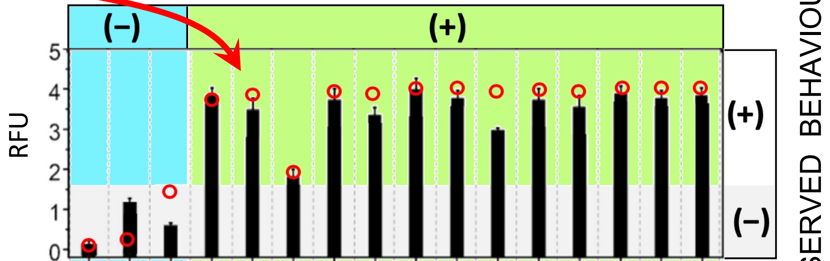




Retrosynthesis Workflow »

Galaxy

TARGETED BEHAVIOUR



Hippurate……………		●				●	●	●				●	●	●		●
Cocaine……………….			●			●			●		●	●	●		●	●
Benzamide………….				●			●		●	●		●		●	●	●
Biphenyl-2,3-diol…					●			●		●	●		●	●	●	●

- Pandi A., Koch M. *et al. Nat Commun* **10**, 3880 (2019)

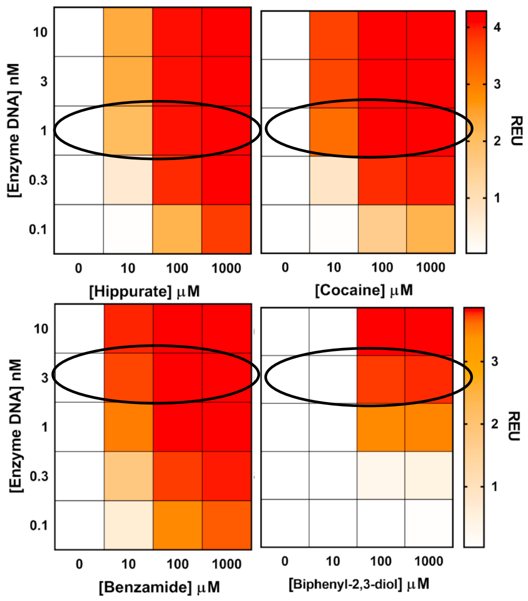
Engineering a neural metabolic network in vitro

TARGETED BEHAVIOUR

	(–)	(+)														
Hippurate…………….		●				●	●	●				●	●	●		●
Cocaine……………….			●			●			●		●	●	●		●	●
Benzamide………….				●			●		●	●		●		●	●	●
Biphenyl-2,3-diol…					●			●		●	●		●	●	●	●

$f(b+\sum w_i x_i) < 0.5$ $\quad$ $f(b+\sum w_i x_i) \geq 0.5$

- Kinetics model is used to compute the enzyme concentration for each weight



Logistic regression

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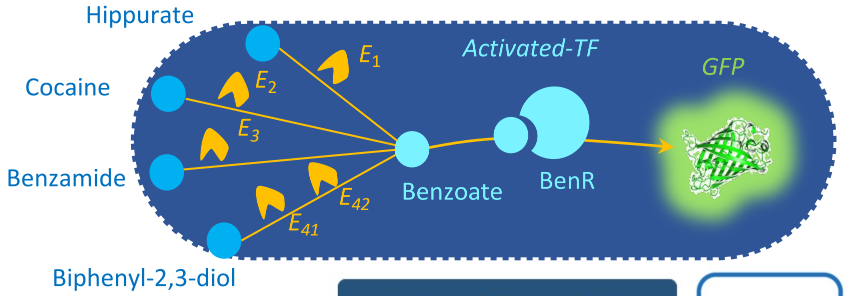
b = - 0.50

w_1 = ~~0.25~~ 0.50

w_2 = ~~0.25~~ 0.50

w_3 = ~~0.50~~ 0.50

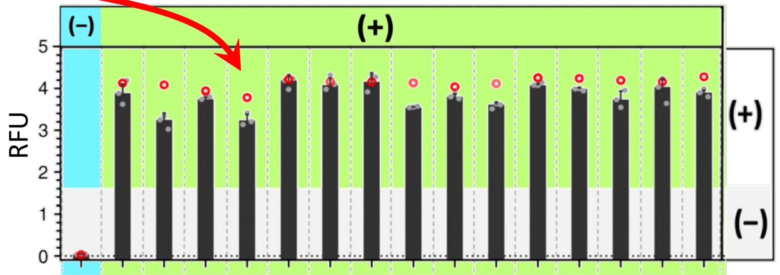
w_4 = ~~0.50~~ 0.50



Retrosynthesis Workflow »

Galaxy

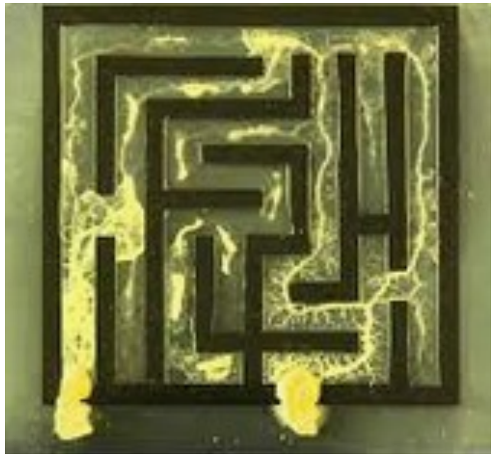
TARGETED BEHAVIOUR



	(–)															
Hippurate…………….		●				●	●	●				●	●	●		●
Cocaine……………….			●			●			●		●	●	●		●	●
Benzamide………….				●			●		●	●		●		●	●	●
Biphenyl-2,3-diol…					●			●		●	●		●	●	●	●

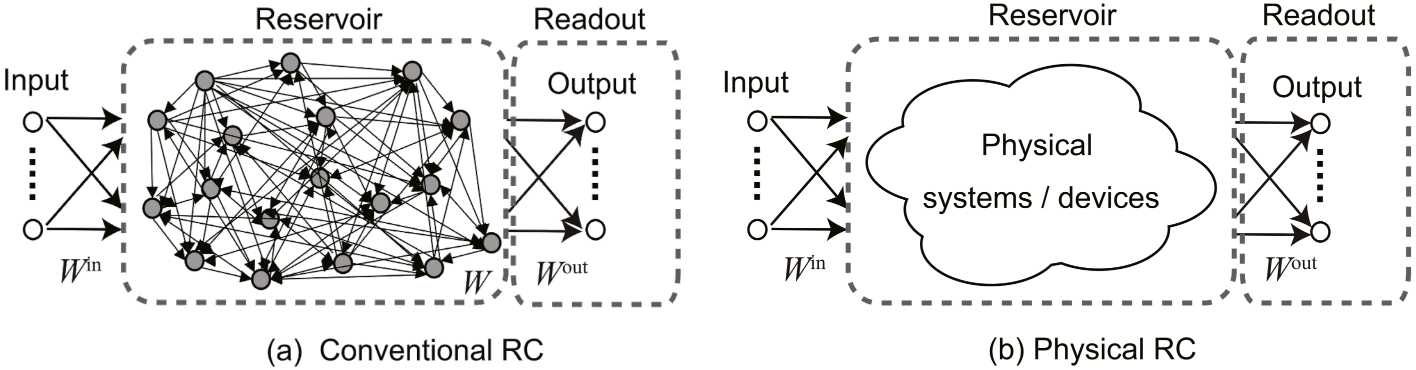
- Pandi A., Koch M. *et al. Nat Commun* **10**, 3880 (2019)

Engineering a neural metabolic network in vivo?



- Can we divert native metabolism to handle problems that are usually solved *in silico*?

- Ability of physical, chemical or biological devices to solve problems is studied in AI with Reservoir Computing (RC)



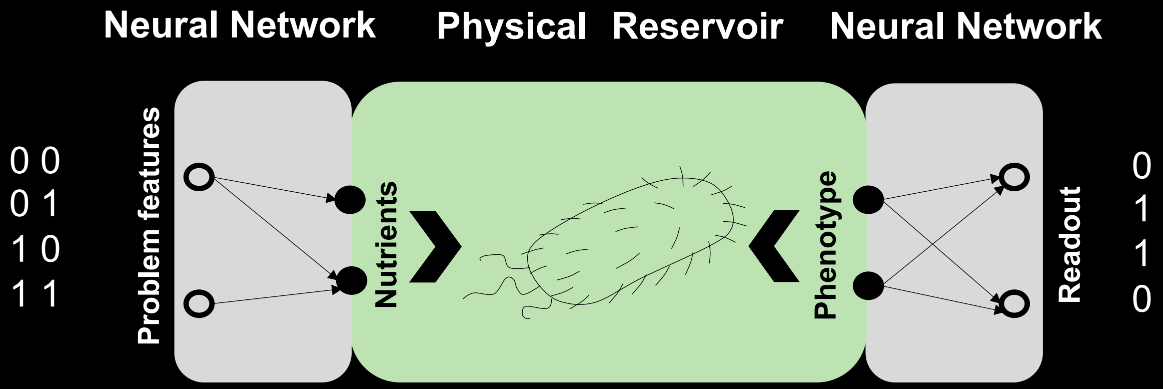
Tanaka G. et al. *Neural Networks* **115**, 100 (2019)

E. coli Reservoir Computer (E. coli RC)

Can we exploit *E. coli* native metabolism to build an *E. coli* RC to solve computational problems?

How complex a problem can *E. coli* RC solve?

Can we find practical uses of *E. coli* RC?



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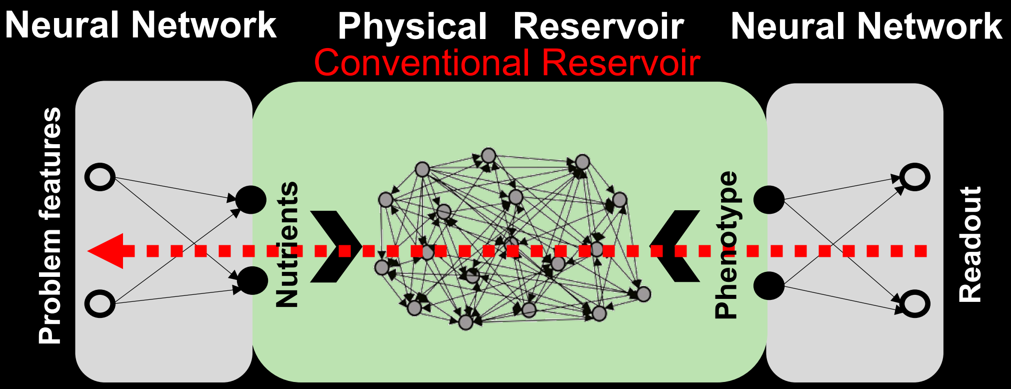


gradient backpropagation

Conventional Reservoir should:

- *accurately reproduce phenotype for different media composition*
- *enable gradient backpropagation*

The reservoir

GEnome-scale Metabolic Model (GEM/FBA)

Max $(v_{biomass})$

Subjected to contraints:

$S\,V = 0$

$0 \leq V \leq V_{in}$

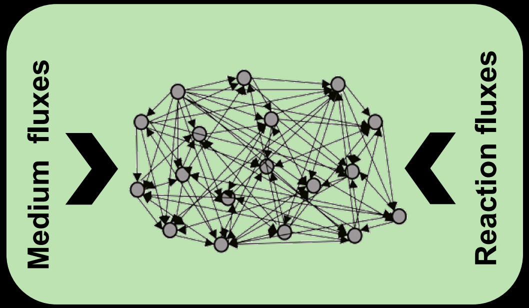
where

$-\ V\ =$ set of all reaction fluxes

$-\ \mathrm{S}\ =$ stochiometric matrix

$-V_{in} =$ uptake medium fluxes upper bounds

Conventional Reservoir



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$-\,S$ = stochiometric matrix

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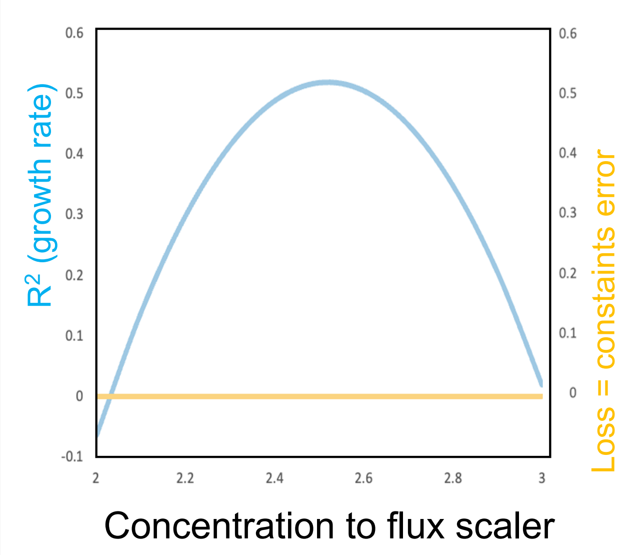
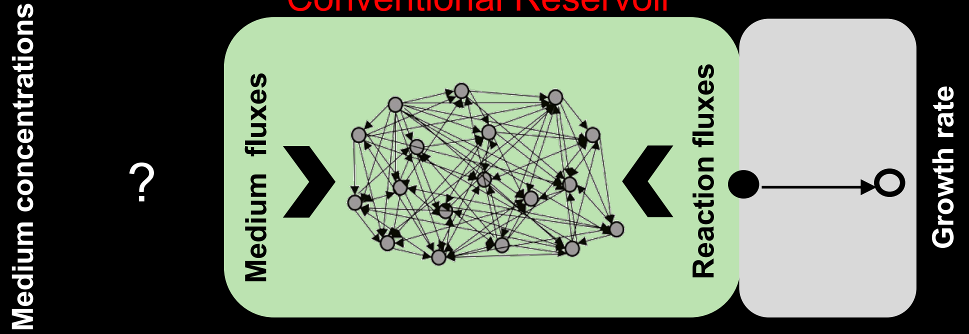
GEM/FBA growth rates vs. measured growth rate in E. coli MG1655 for 1 to 4 nutrients added to M9



Conventional Reservoir



Conventional Reservoir should:

- *accurately reproduce phenotype for different media composition*

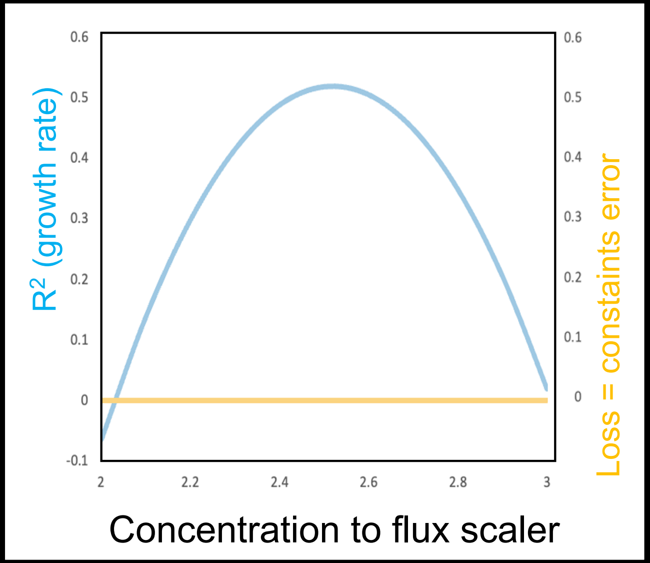
Building an E. coli RC to increase mechanistic model predictability

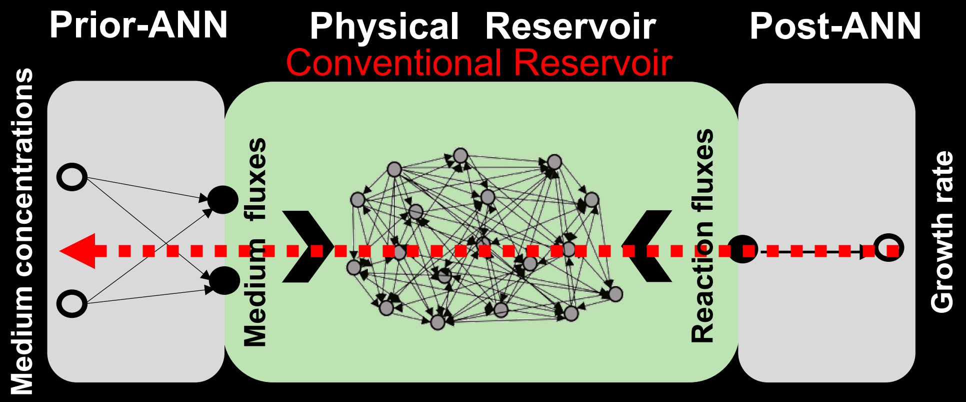
GEM/FBA is a Linear Program solved using Simplex algorithm not compatible with gradient propagation

GEnome-scale Metabolic Model (GEM/FBA)

$$\left\{\begin{array}{l} \text{Max } (v_{biomass}) \\ \\ \text{Subjected to contraints:} \\ \quad S\,V = 0 \\ \quad 0 \leq V \leq V_{in} \\ \\ \text{where} \\ - V = \text{set of all reaction fluxes} \\ - \text{S} \; = \text{stochiometric matrix} \\ - V_{in} = \text{uptake medium fluxes upper bounds} \end{array}\right.$$

GEM/FBA growth rates vs. measured growth rate in E. coli MG1655 for 1 to 4 nutrients added to M9



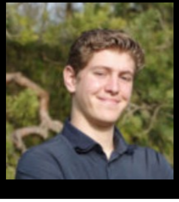


gradient backpropagation to find mapping between medium concentrations and uptake fluxes

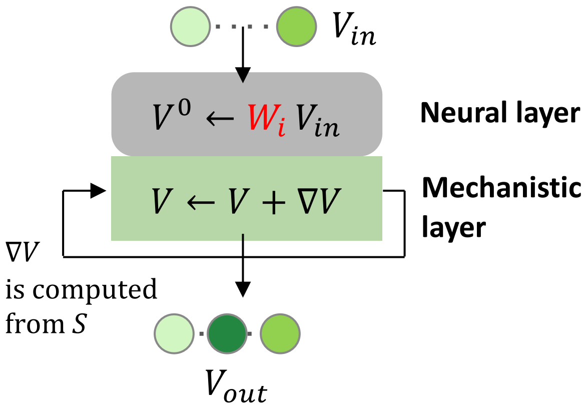
Conventional Reservoir should:

- *accurately reproduce phenotype for different media composition*
- *enable gradient backpropagation*

AMNs (Artificial Metabolic Networks): a gradient backpropagation compatible method surrogating classical mechanistic models



Physics informed neural network (PINN)



V_{in}

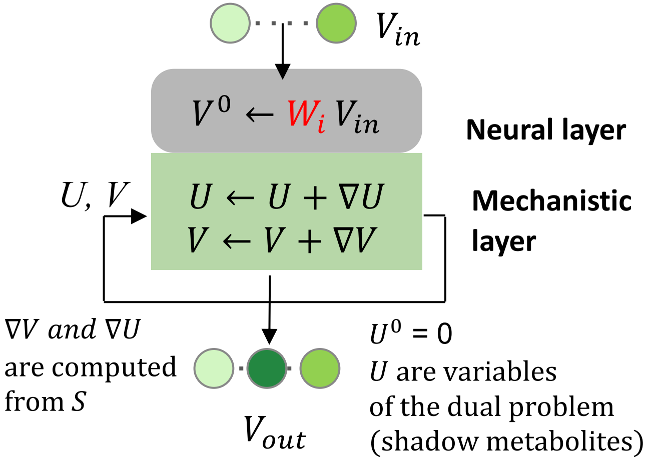
$V^0 \leftarrow W_i \, V_{in}$ Neural layer

$V \leftarrow V + \nabla V$ Mechanistic layer

∇V is computed from S

V_{out}

Hopfield's network



V_{in}

$V^0 \leftarrow W_i \, V_{in}$ Neural layer

$U, \, V$

$U \leftarrow U + \nabla U$

$V \leftarrow V + \nabla V$ Mechanistic layer

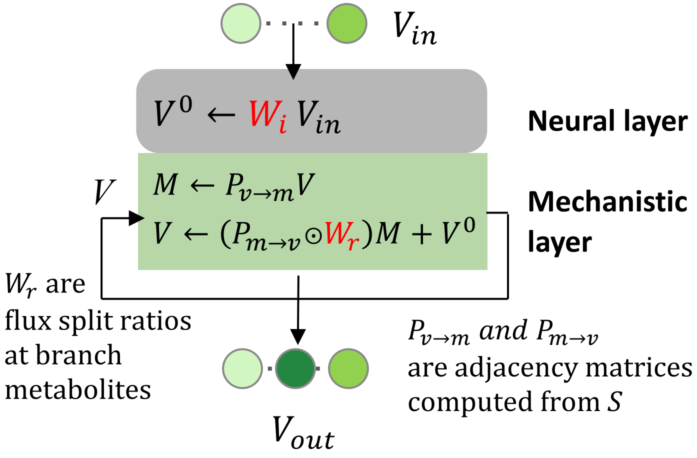
∇V *and* ∇U are computed from S

V_{out}

$U^0 = 0$

U are variables of the dual problem (shadow metabolites)

Graph neural network (GNN)



V_{in}

$V^0 \leftarrow W_i \, V_{in}$ Neural layer

V

$M \leftarrow P_{v \to m} V$

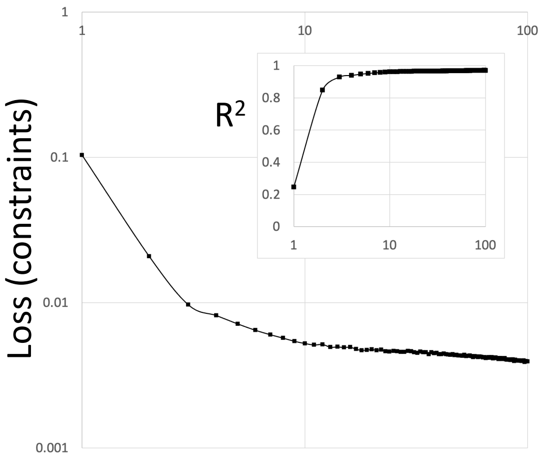
$V \leftarrow (P_{m \to v} \odot W_r) M + V^0$ Mechanistic layer

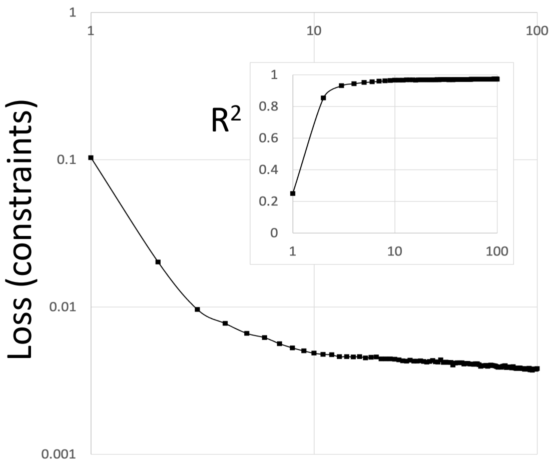
W_r are flux split ratios at branch metabolites

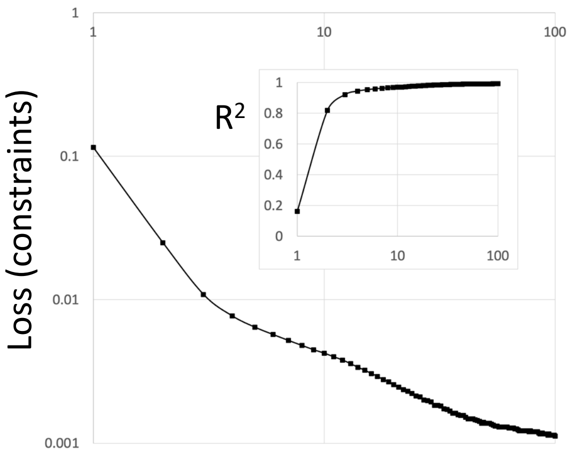
V_{out}

$P_{v \to m}$ *and* $P_{m \to v}$ are adjacency matrices computed from S

Trained on GEM/FBA (cobrapy) calculated growth rates with *E. coli* iML1515 model for 1000 different media (M9 + random combinations of nutrients among sugars, nucleotides, amino acids)

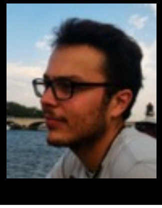


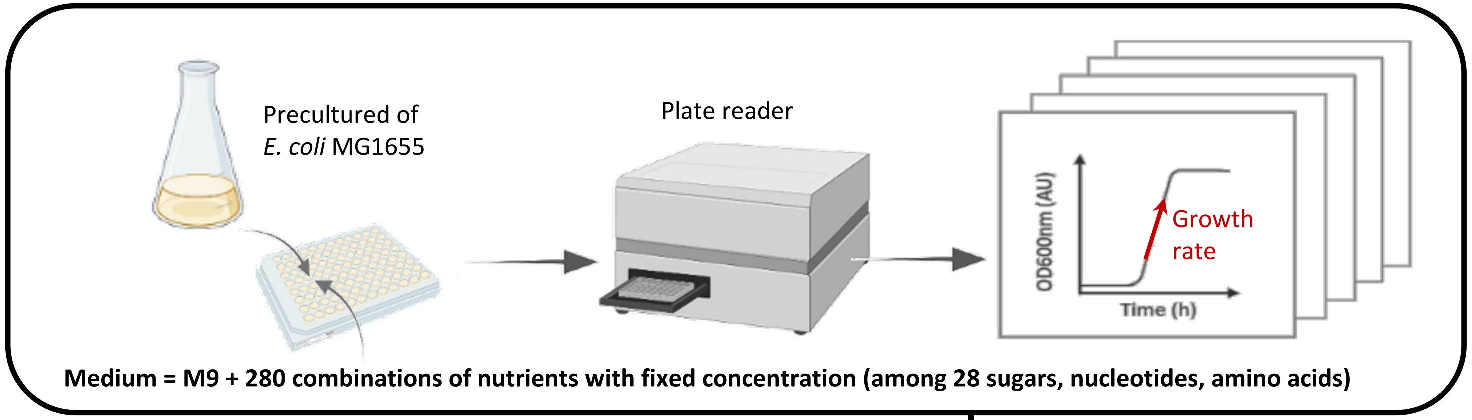




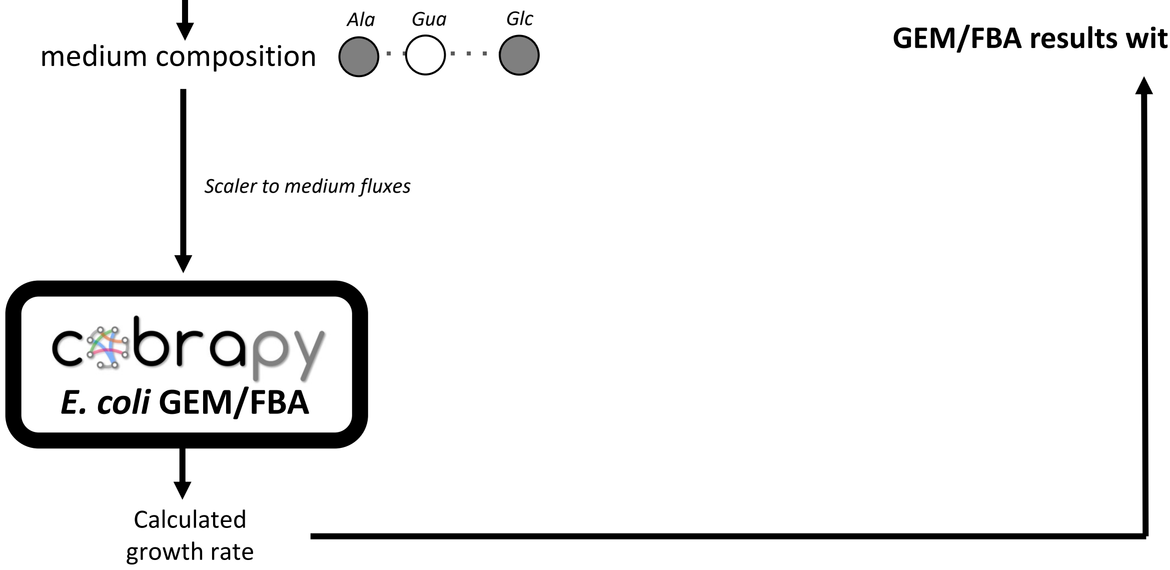
- Faure L. *et al*. *Nat Commun* **14**, 4669 (2023)

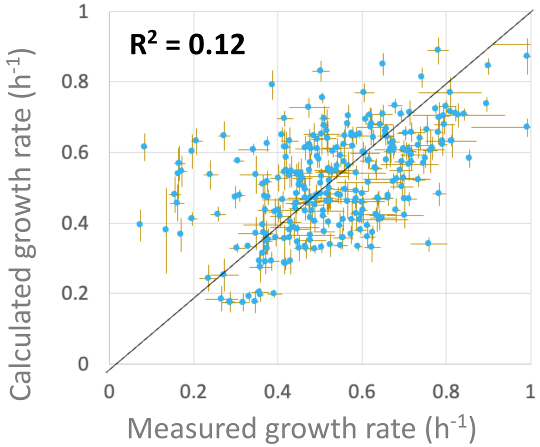
AMNs can be used as reservoir in RC to improve mechanistic model predictability





Medium = M9 + 280 combinations of nutrients with fixed concentration (among 28 sugars, nucleotides, amino acids)





GEM/FBA results with best scaled input

- Faure L. *et al*. *Nat Commun* **14**, 4669 (2023) & Faulon et al. *bioRxiv DOI: 10.1101/2024.09.12.612674* (2024)

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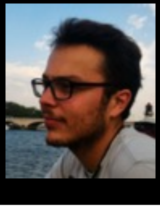
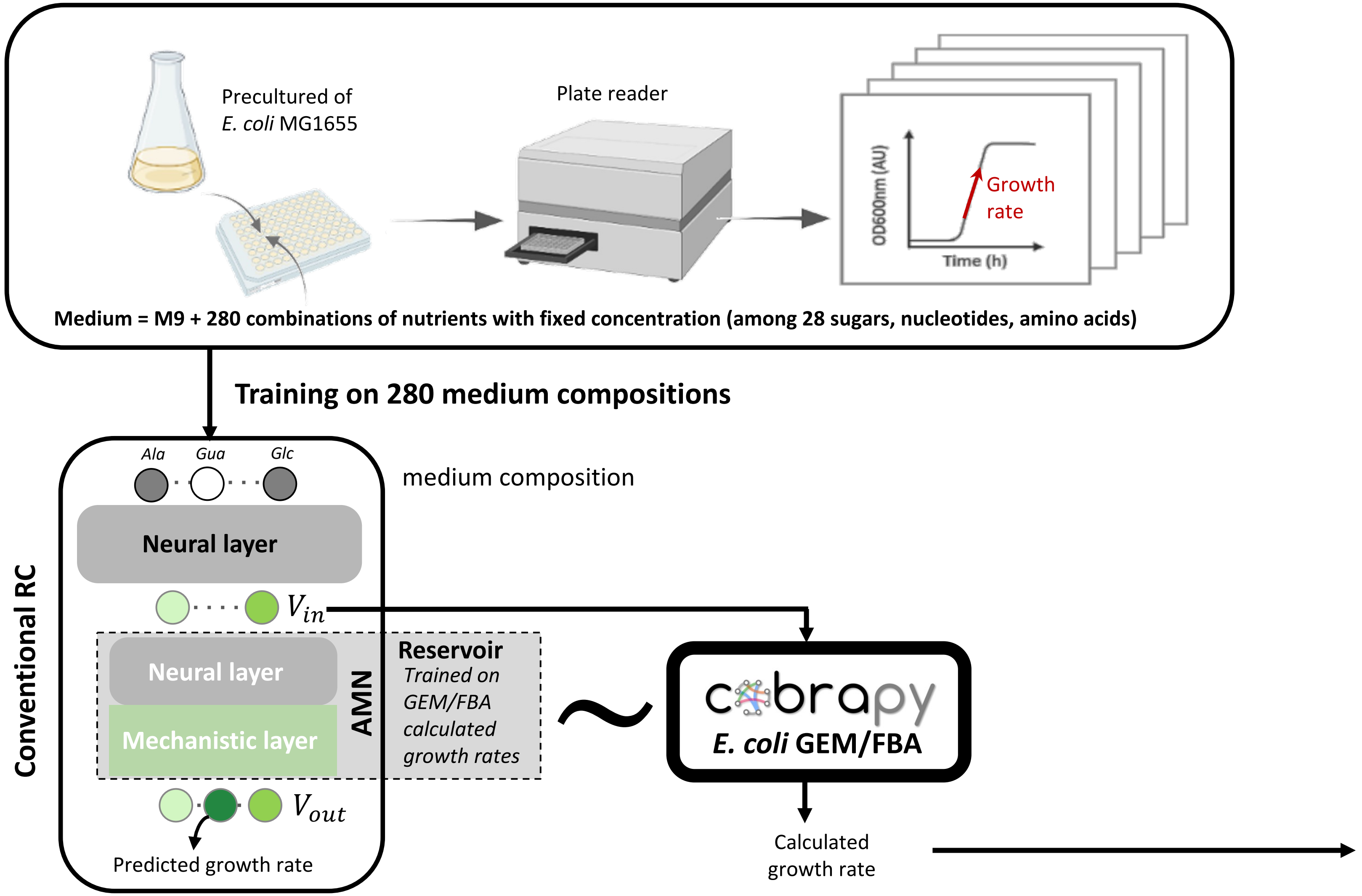
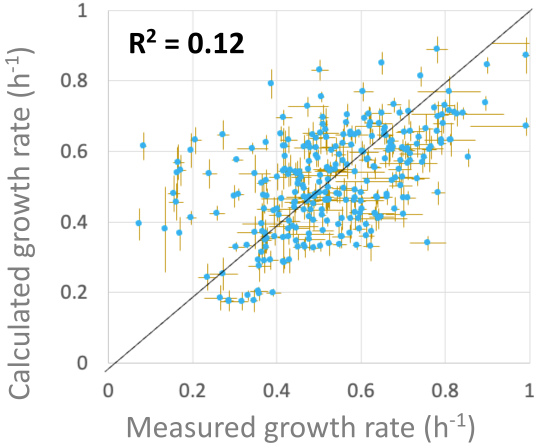


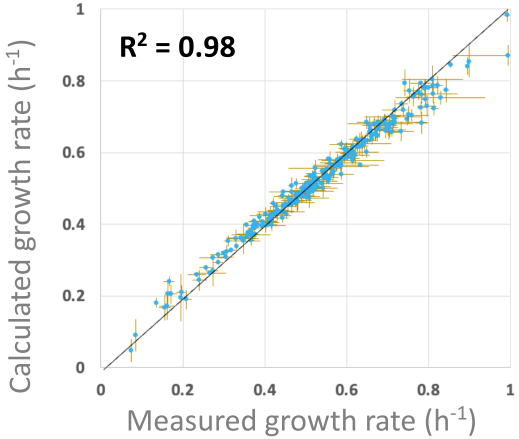


Medium = M9 + 280 combinations of nutrients with fixed concentration (among 28 sugars, nucleotides, amino acids)

Training on 280 medium compositions



GEM/FBA results with best scaled input



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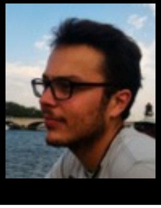
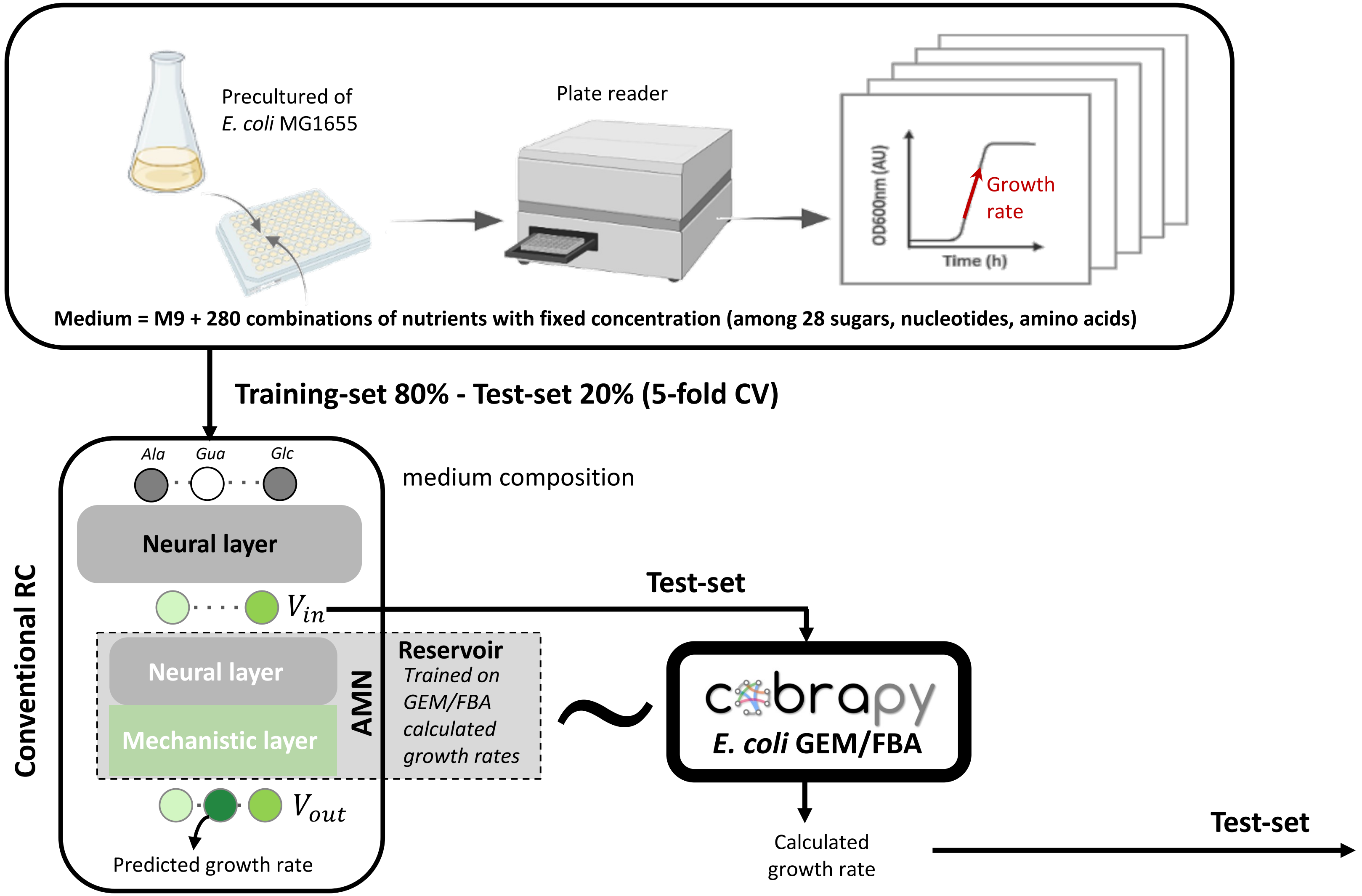




Precultured of *E. coli* MG1655

Plate reader

Medium = M9 + 280 combinations of nutrients with fixed concentration (among 28 sugars, nucleotides, amino acids)

Training-set 80% - Test-set 20% (5-fold CV)

medium composition

Neural layer

V_{in}

Test-set

Conventional RC

Neural layer

Mechanistic layer

AMN

Reservoir
Trained on GEM/FBA calculated growth rates

~

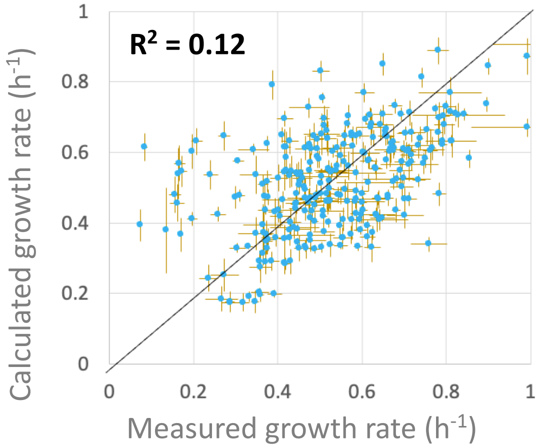
***E. coli* GEM/FBA**

V_{out}

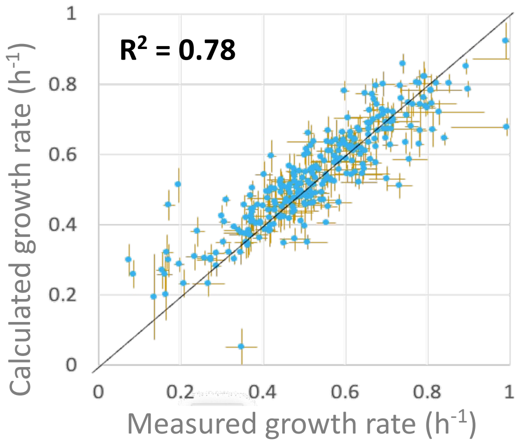
Predicted growth rate

Calculated growth rate

Test-set



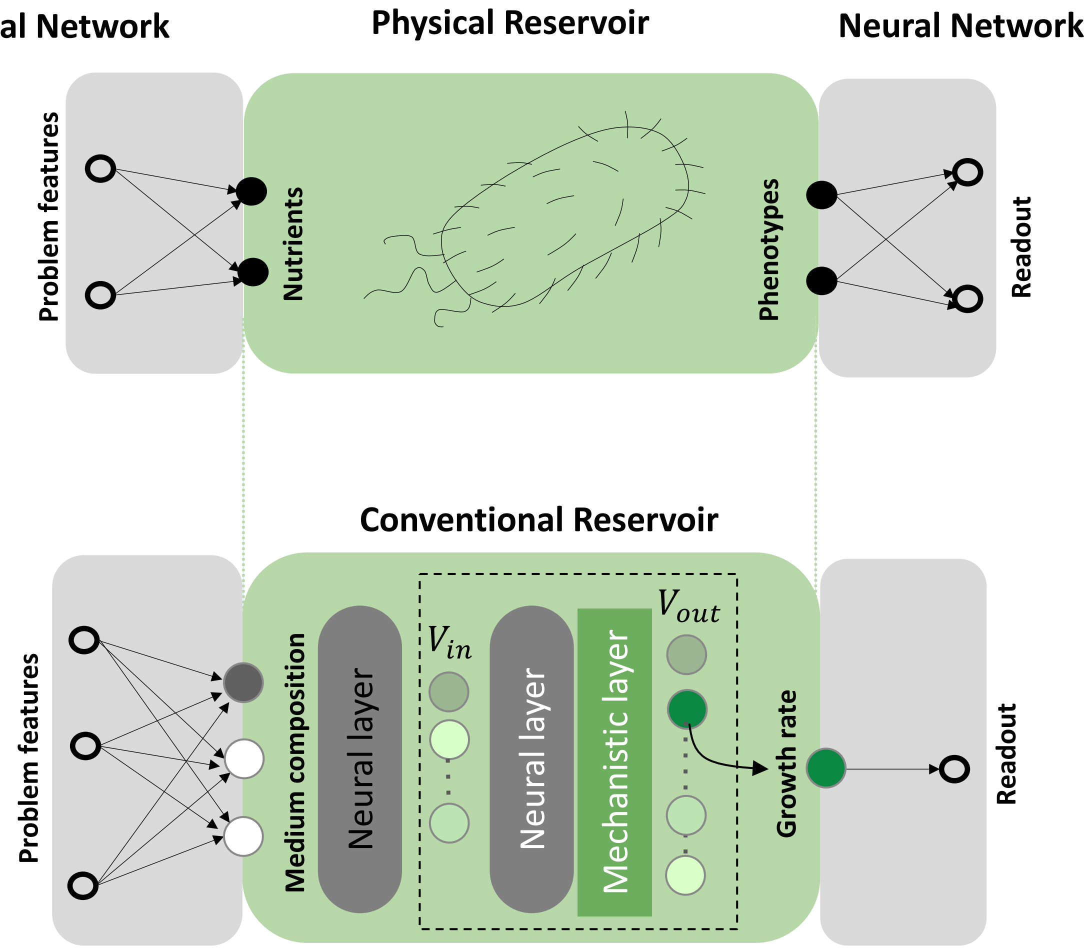
GEM/FBA results with best scaled input



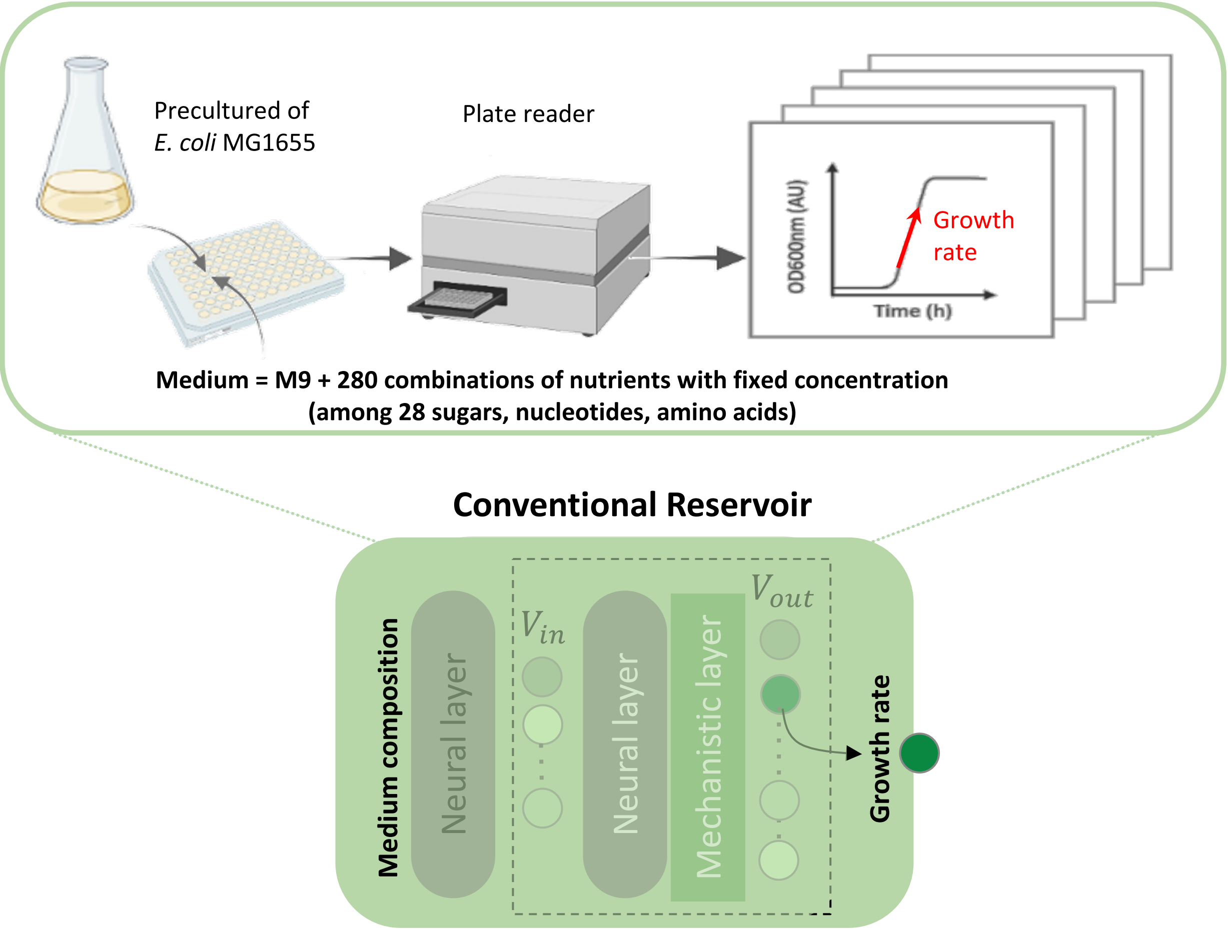
GEM/FBA results with reservoir inputs

- Faure L. *et al*. *Nat Commun* **14**, 4669 (2023) & Faulon et al. *bioRxiv DOI: 10.1101/2024.09.12.612674* (2024)

Can E. coli RC be used to solve a classical machine learning problem?



Building an E. coli RC to solve a regression problem



Precultured of
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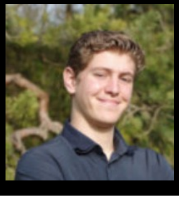
Conventional Reservoir

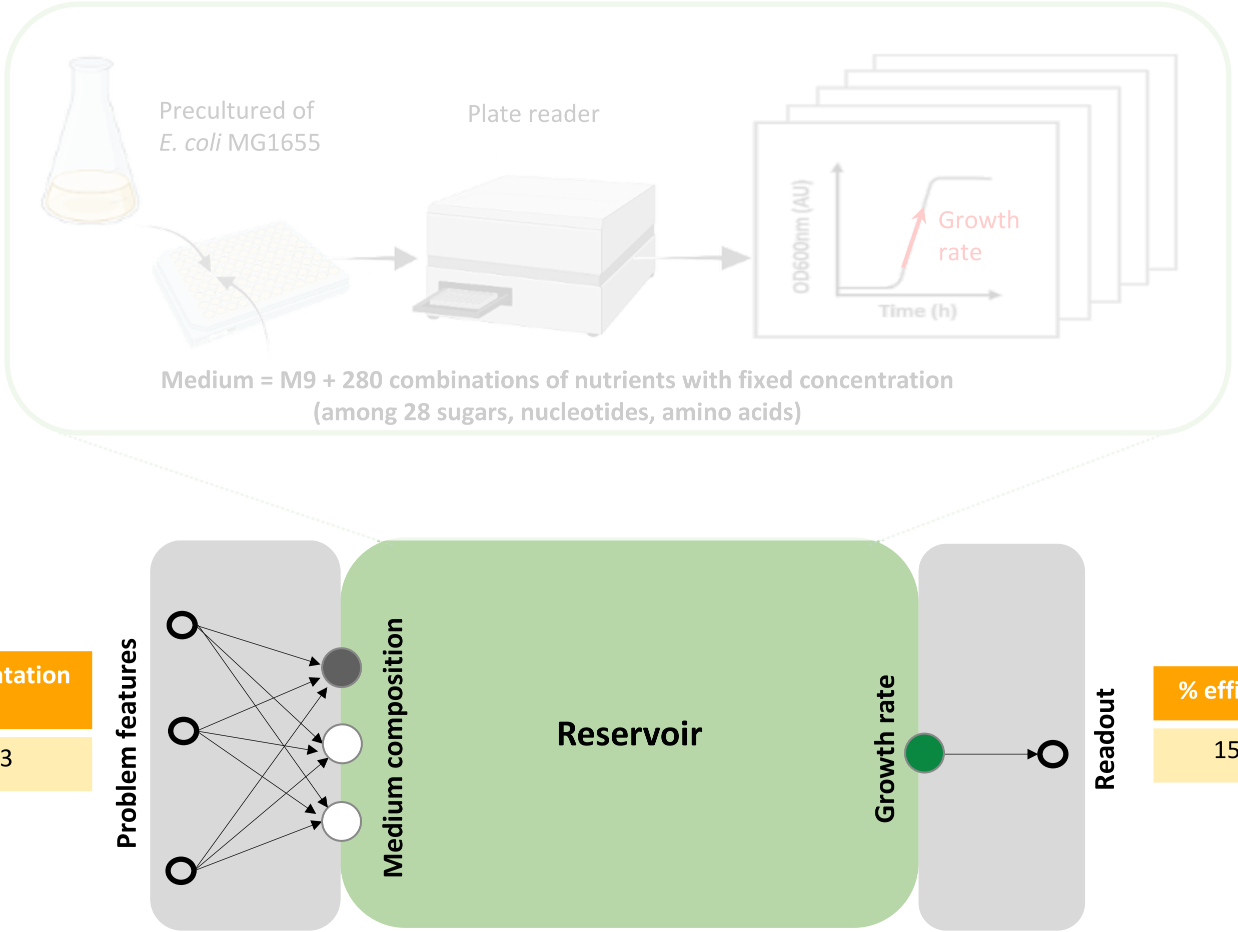
Using E. coli RC to solve a regression problem

OpenML
A worldwide machine learning lab



Example of regression problem : OpenML 'Energy Efficiency' dataset (768 instances, X = 8 features, y = % efficiency)

Compactness	Surface Area	Orientation
0.98	514.5	3



% efficiency
15.55

Glc	Xyl	Succ	Trp
1	0	1	1

µ
0.71

- Faulon et al. *bioRxiv DOI: 10.1101/2024.09.12.612674* (2024)

Using E. coli RC to solve a regression problem

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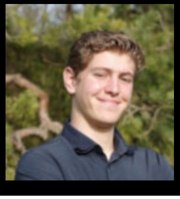

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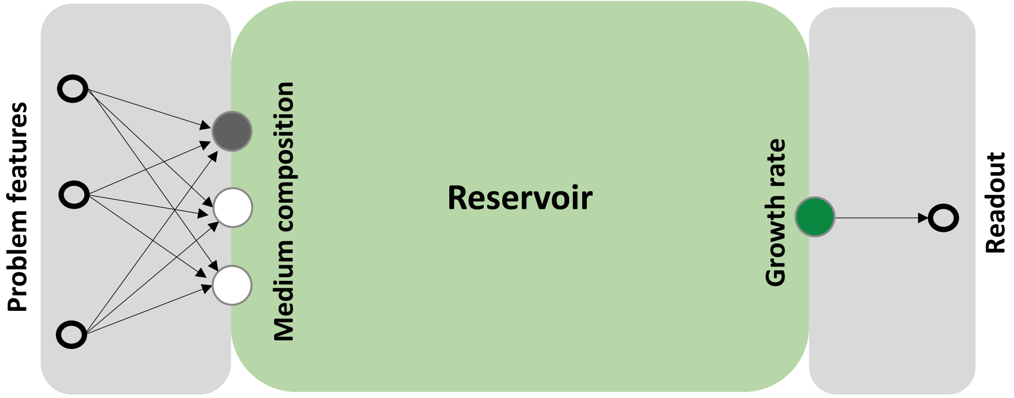


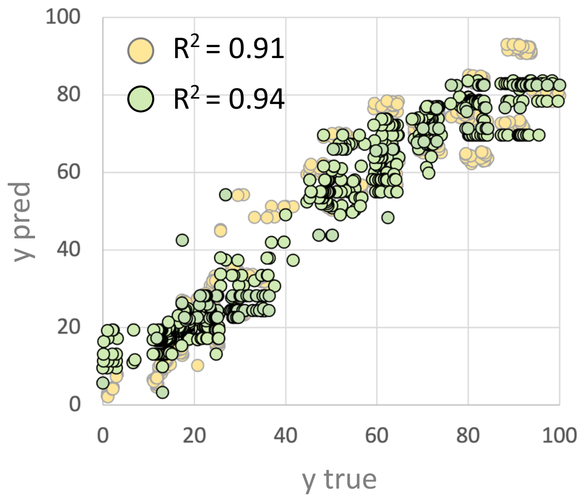
Glc	Xyl	Succ	Trp
1	0	1	1

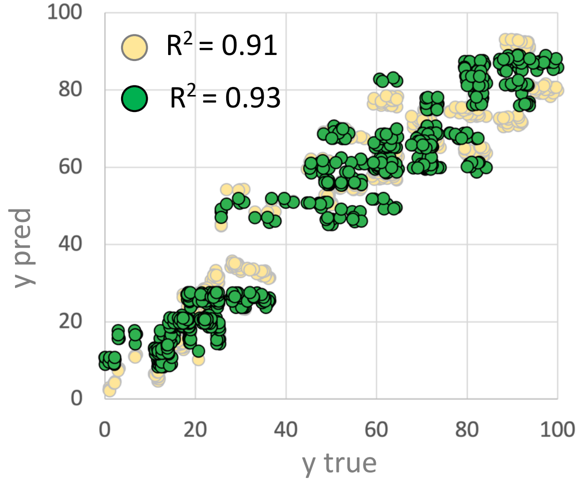
μ
0.71

% efficiency
15.55

○ MLR
○ Conventional RC
● Physical RC

5-fold CV





- Faulon et al. *bioRxiv DOI: 10.1101/2024.09.12.612674* (2024)

Using E. coli RC to solve many regression problems

OpenML
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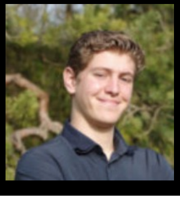
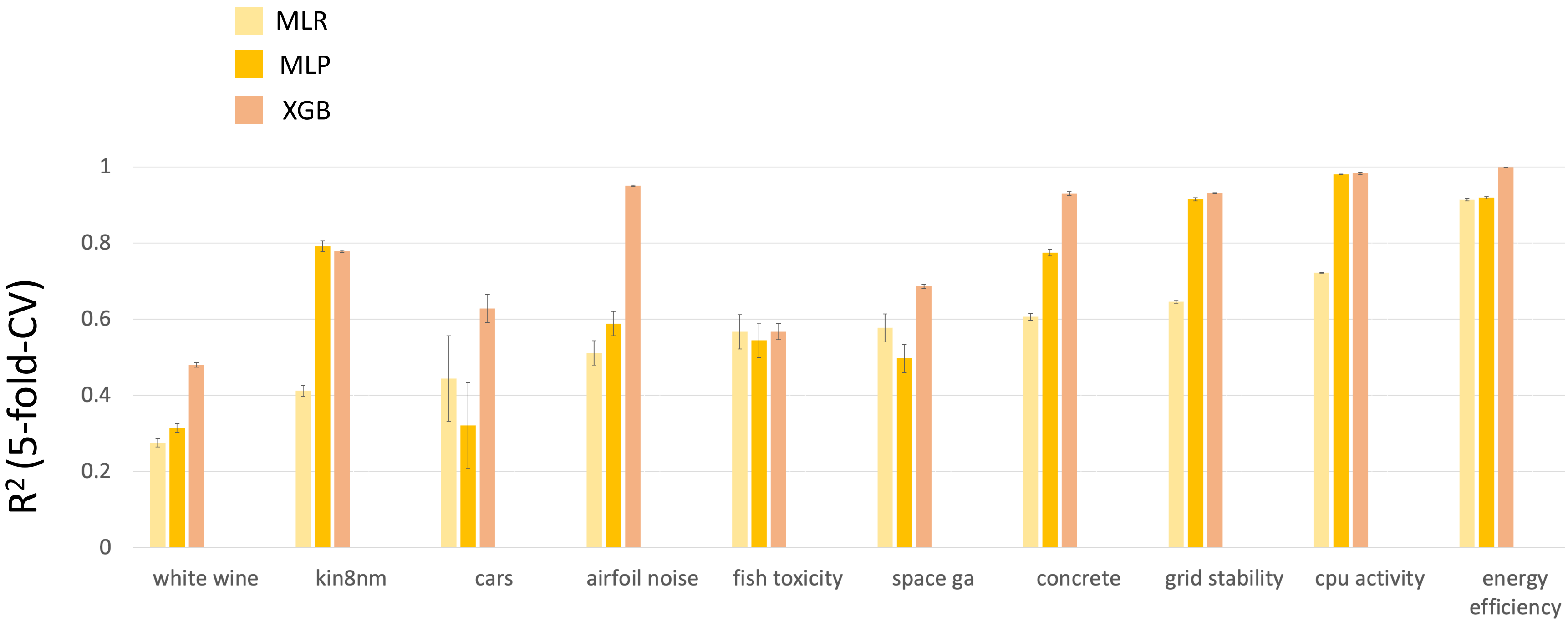


10 OpenML regression problems of increasing difficulty



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Using E. coli RC to solve many regression problems

OpenML

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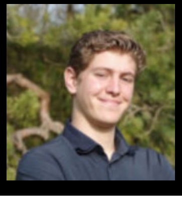

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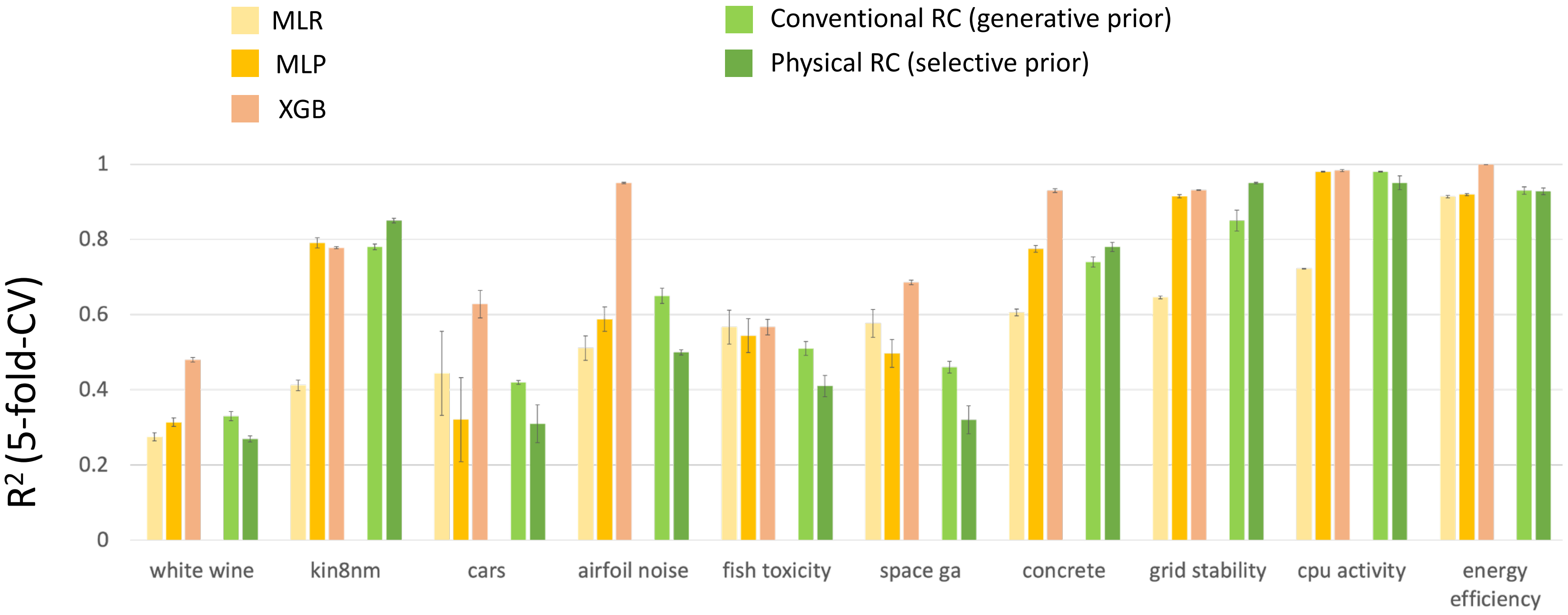
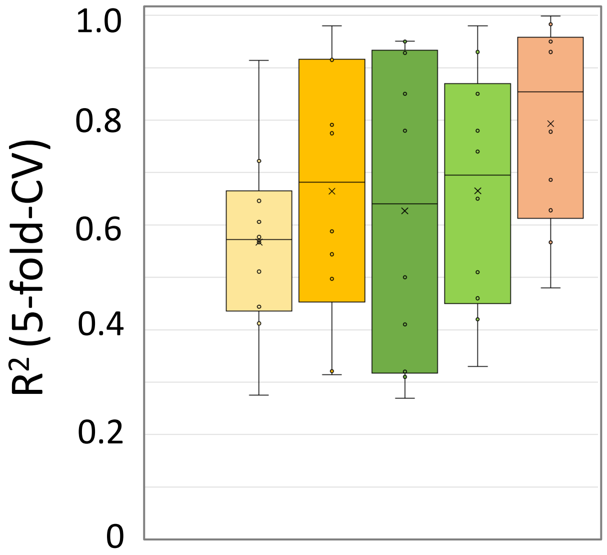
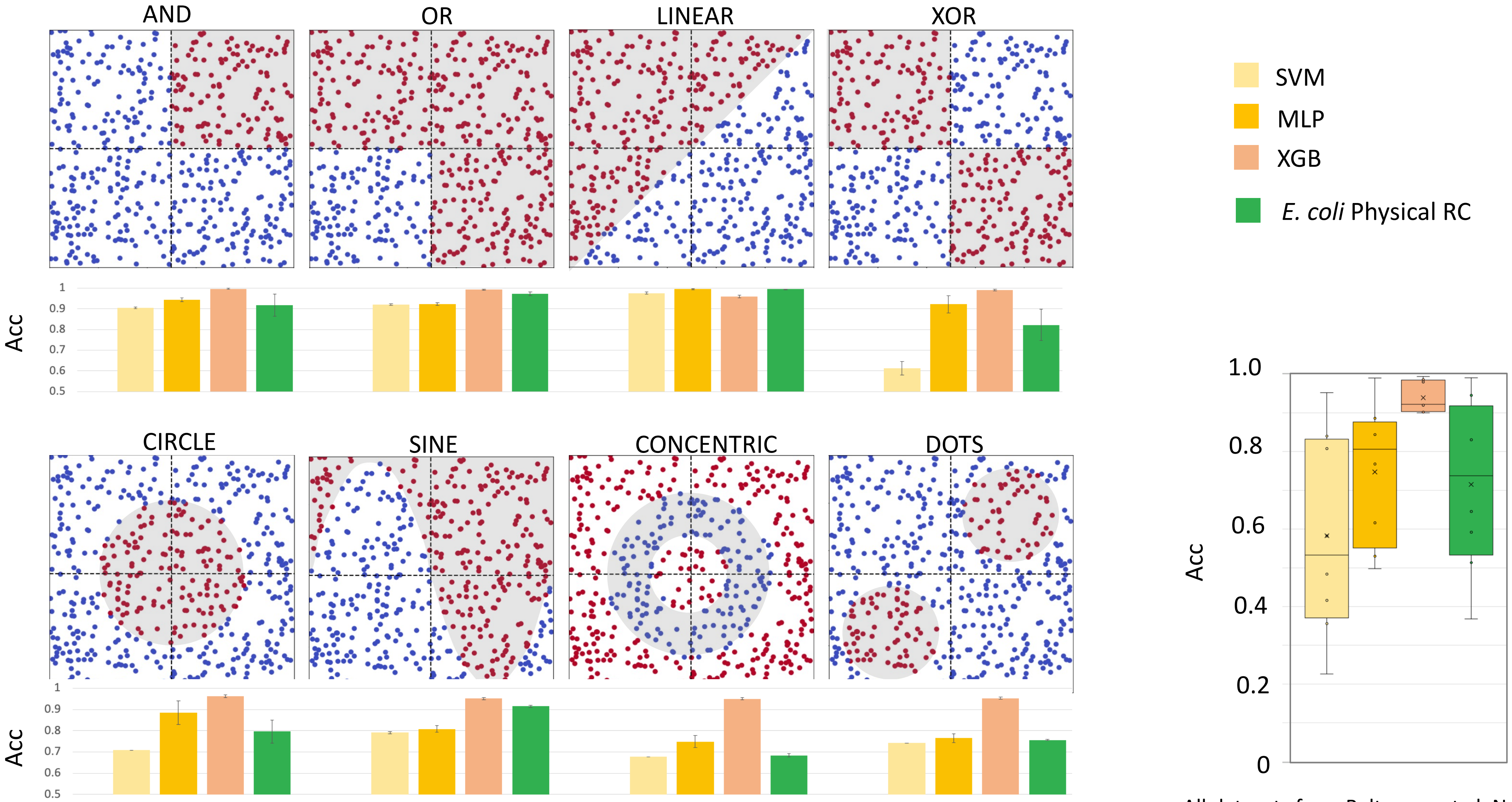




- Faulon et al. *bioRxiv DOI: 10.1101/2024.09.12.612674* (2024)

Using E. coli RC to solve classification problems



- All datasets from Baltussen et al. Nature 2024

- Faulon et al. *bioRxiv DOI: 10.1101/2024.09.12.612674* (2024)

Can E. coli RC solve a concrete classification problem ?

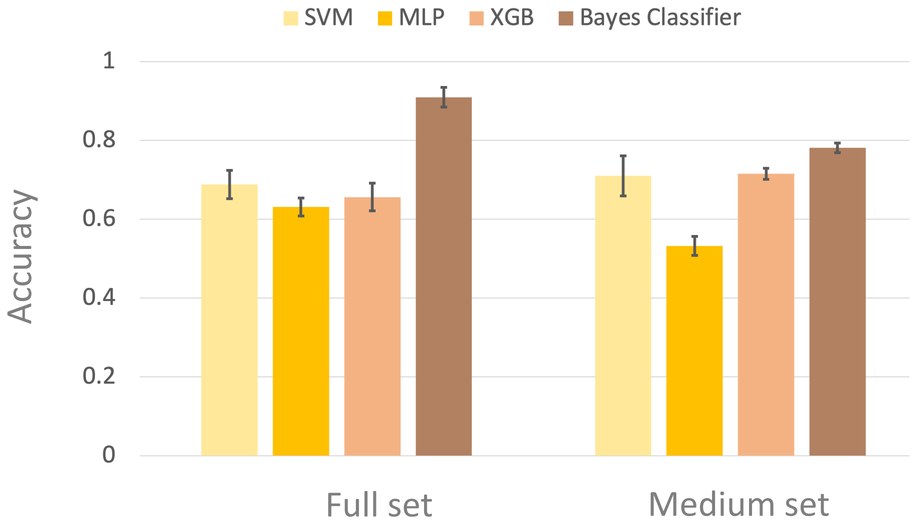
The problem:

- Blood sample are collected for Covid-19 patients once they enter the hospital
- Metabolomics analyses are carried out on the samples
- Can we predict from the analyses if the disease outcome will be severe or moderate?

CHU Grenoble-Alpes cohort (training set):

- 81 patients
- 624 molecules detected (56 *E. coli* medium molecules)
- severe (31) – moderate (50)

Classifier performances (20-fold CV results)



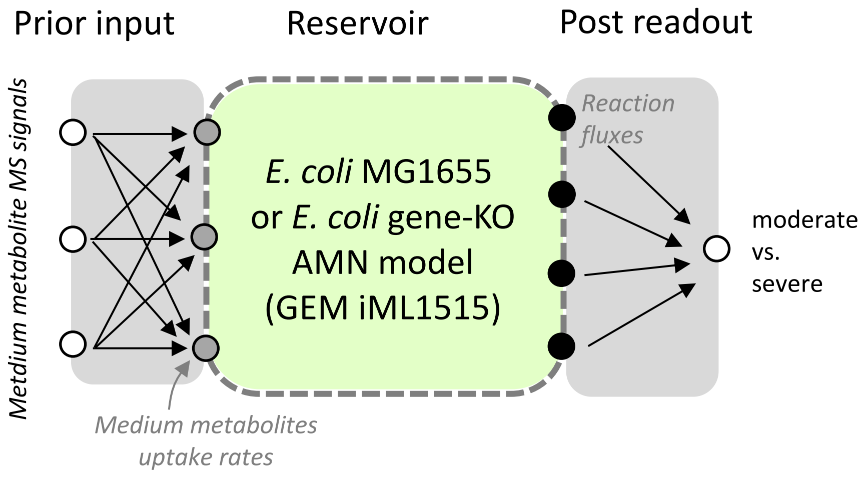
Accuracy = 0.84 in Shen et al. *Cell* 2020; 182(1): 59–72

Using E. coli RC for classification

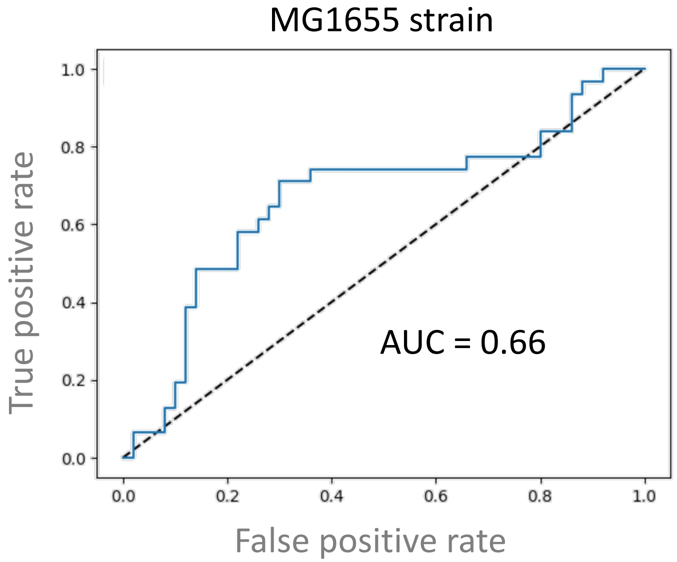
The problem:

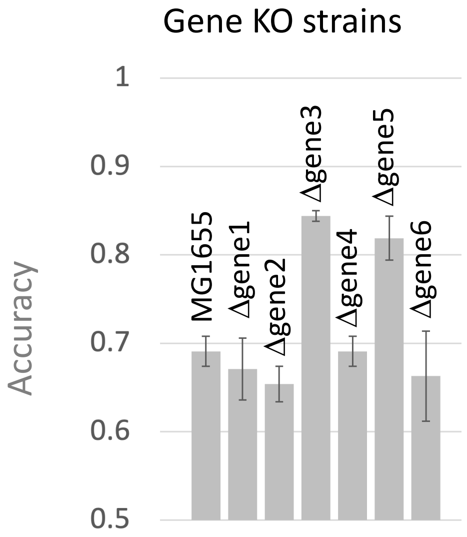
- Blood sample are collected for Covid-19 patients once they enter the hospital
- ~~Metabolomics analyses are carried out on the samples~~
- ~~Can we predict from the analyses if the disease outcome will be severe or moderate?~~
- Can we use an *E. coli* RC grown on the patient's sample to predict if the disease outcome will be severe or moderate ?

Conventional RC to predict disease outcome from phenotype

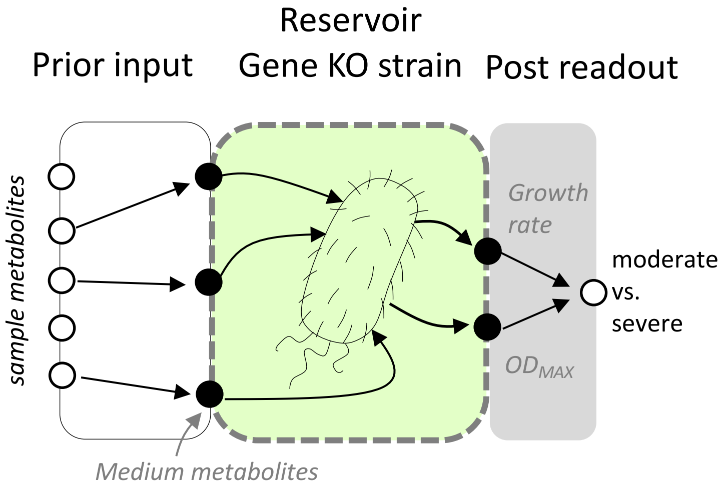


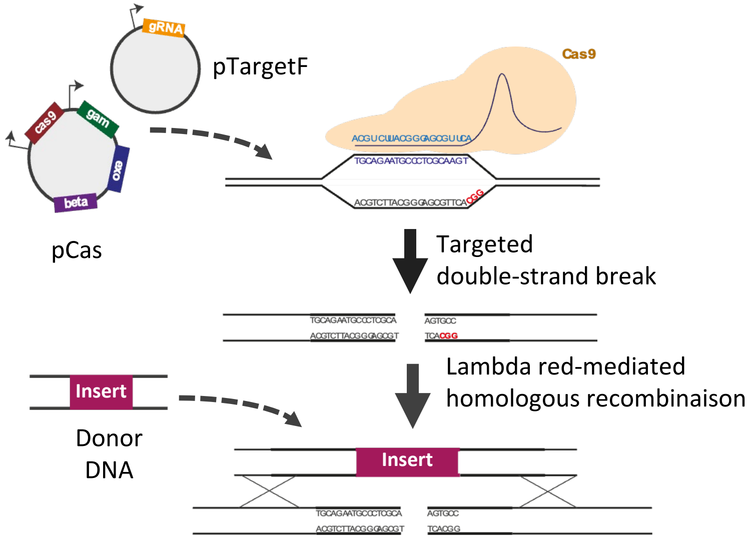
Prior input
Reservoir
Post readout
Metdium metabolite MS signals
E. coli MG1655 or *E. coli* gene-KO AMN model (GEM iML1515)
Reaction fluxes
moderate vs. severe
Medium metabolites uptake rates



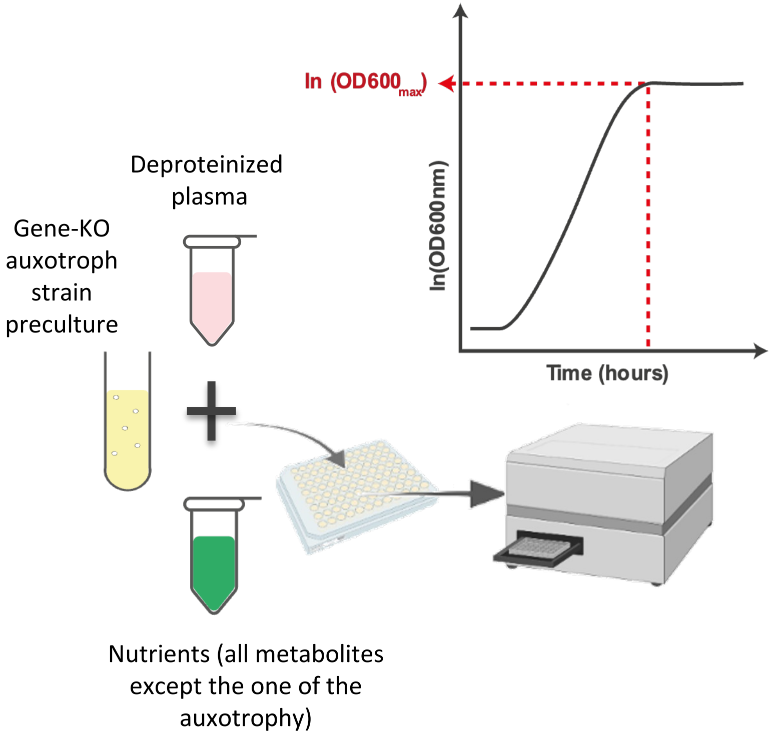


Building an *E. coli* physical RC for classification





gRNA
pTargetF
cas9
gam
exo
beta
pCas
Cas9
Targeted double-strand break
Lambda red-mediated homologous recombinaison
Insert
Donor DNA
Insert



ln (OD600max)
ln(OD600nm)
Time (hours)
Deproteinized plasma
Gene-KO auxotroph strain preculture
+
Nutrients (all metabolites except the one of the auxotrophy)

- Gene-KO *E. coli* Physical RC to predict disease outcome from growth rate and OD_{MAX}

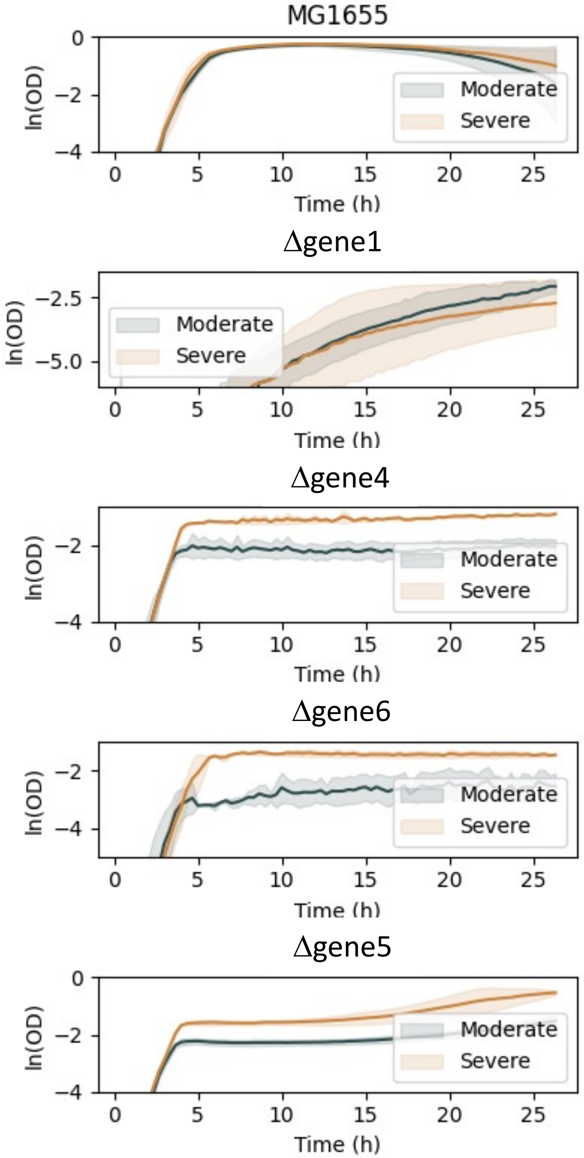
- CRISPR-Cas9/Lambda red system

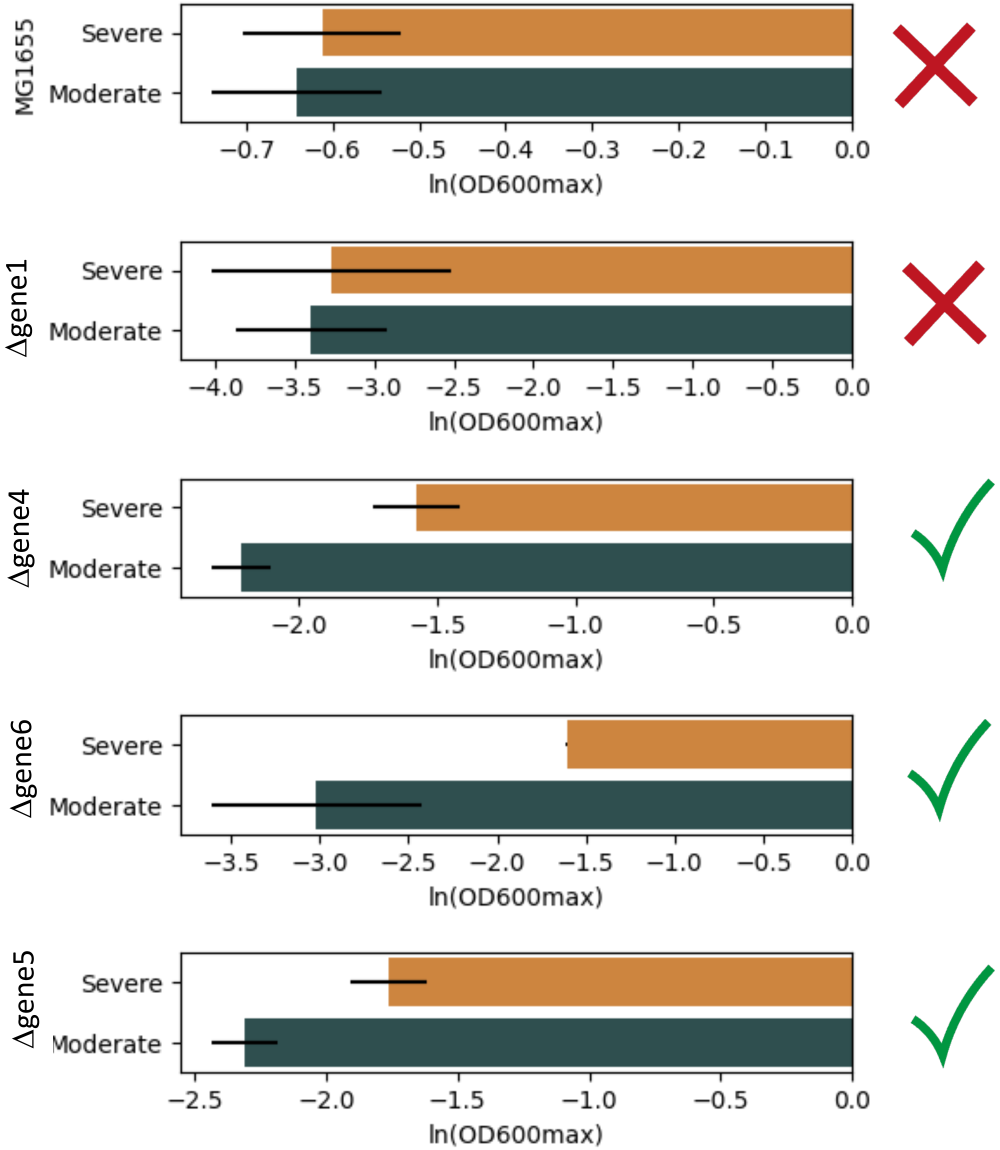
  - Jiang et al. , *Appl Environ Microbiol*, 2015
  - Scarless, Efficient, Multiplexable
  - ~80 KOs built

- Gene deletions force *E. coli* to collect specific nutrients from the plasma in order to grow

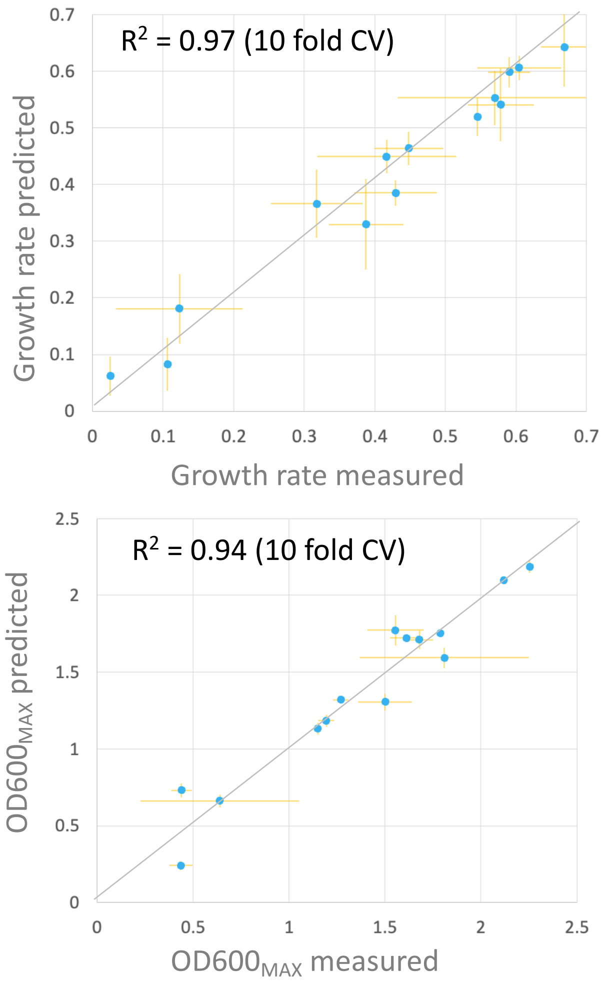
- According to conventional RC, differences of nutrients concentration in the plasma should result in different growth curve

Benchmarking E. coli physical RC for classification





Conventional reservoir predictions (8 moderate – 6 severe)



Perspectives

- Supervised Learning & Active learning
  - New generative AI models (transformer) for retro-(bio)synthesis and to generate sequences
  - LLMs (like GPT4) to drive biofoundries
  - Active Learning / Transfer Learning / Hybrid Learning to cope with small training set sizes

- *in vitro/in vivo* learning
  - Decades of research and development in Synthetic Biology to build bottom-up computing devices (digital, analog, neural,…)… but many difficulties
  - Most devices were inspired from natural biological networks: perhaps one should to consider building devices top-down, *i.e*. exploiting/modifying hosts rather than plugging orthogonal devices.

Acknowledgments

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université PARIS-SACLAY

INRAE

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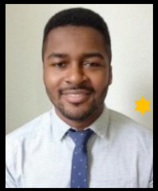
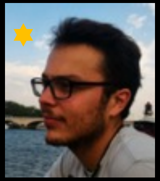

**Paul
Ahvi**

**An
Hoang**

**Bastien
Mollet**


**Léon
Faure**

**Amir
Pandi**

**Mathilde
Koch**

**Olivier
Borkowski**

**Anne
Giralt**

**Joan
Hérisson**

★ Molecular Biology
★ Computational Biology

Jérôme Bonnet’s group

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Tobby Erb’s group

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