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Information processing circuits in cell-free systems: beyond gene expression regulation

Manish Kushwaha, Pandi Amir, Koch Mathilde, Soudier Paul, Batista Angelo Cardoso, Levrier Antoine, Voyvodic Peter L., Zuniga Ana, Thomas Duigou, Bazi-Kabbaj Kenza, et al.

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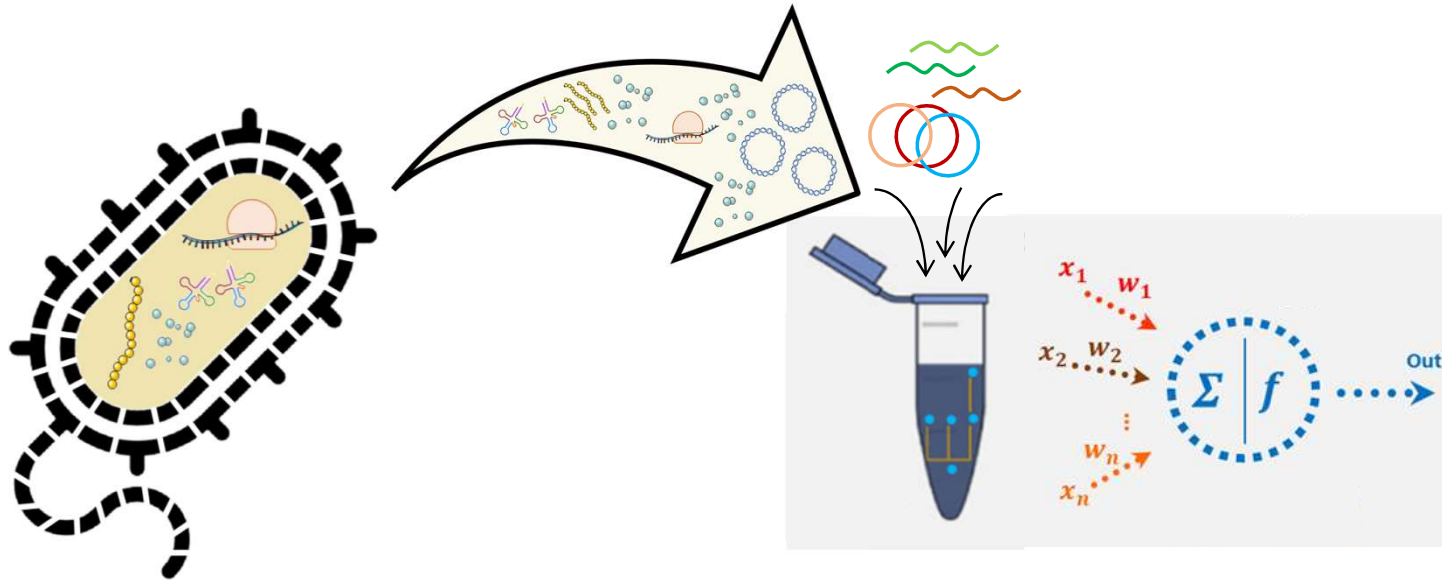
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Information processing circuits in cell-free systems: beyond gene expression regulation




SYNCELL2023



23 May 2023

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AgroParisTech 

Micalis
Microbiologie de l'Alimentation au service de la Santé

université
PARIS-SACLAY

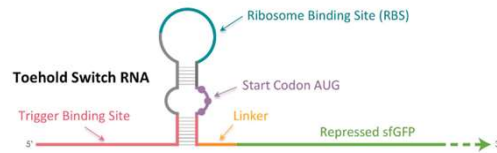
Research Interests

- Cell-free biosensors

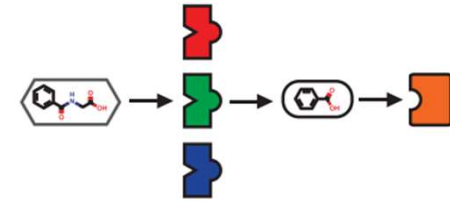
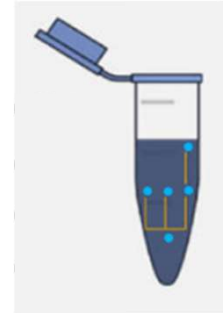


Rosewood

Evry Paris-Saclay
2020 iGEM team

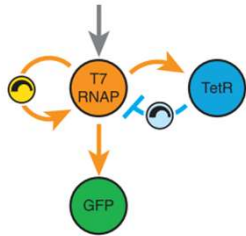


(Soudier *et al.*, 2022.
Synthetic and Systems Biotech.)

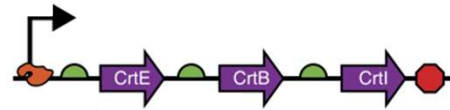


(Batista *et al.* *ACS Syn Bio.* 2022)

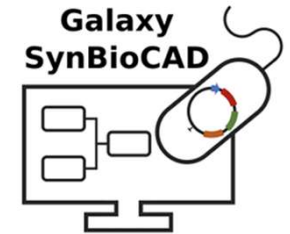
- Cellular genetic circuits and metabolic pathways



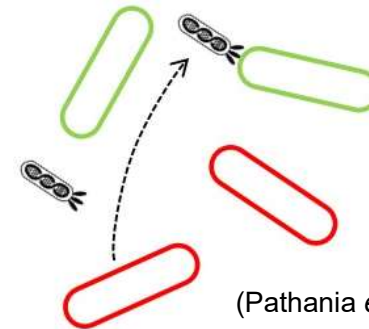
(Kushwaha & Salis, 2015. *Nat. Comm.*)



(Herisson *et al.*,
2022. *Nat. Comm.*)

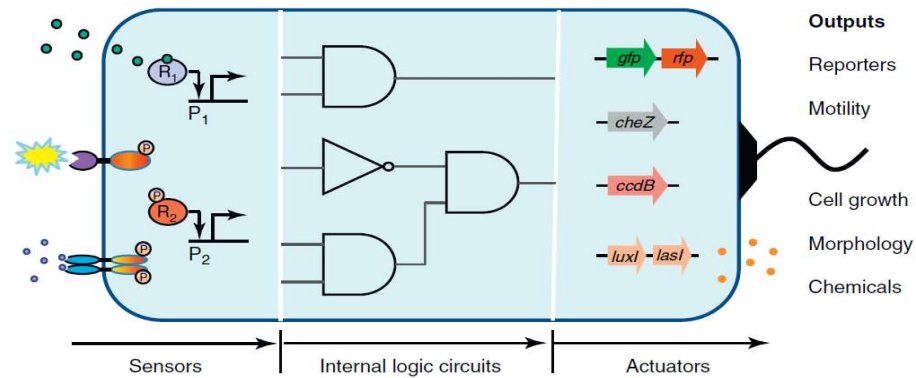


- Distributed circuits in co-cultures



(Pathania *et al.*, 2022. *bioRxiv.*)

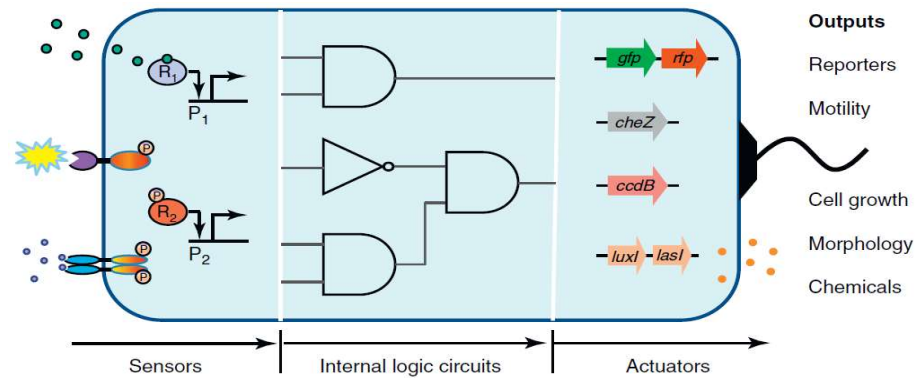
Information processing in whole-cell and cell-free systems



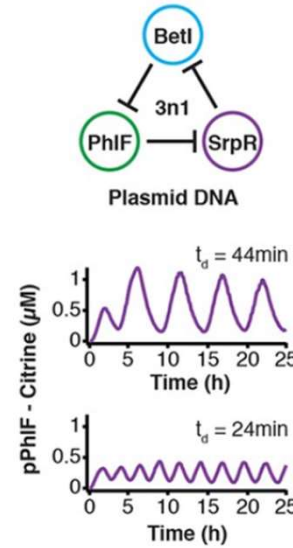
(Wang & Buck, 2012. Trends in Microbiology.)

- Cellular and cell-free systems can be engineered to process a wide variety of information

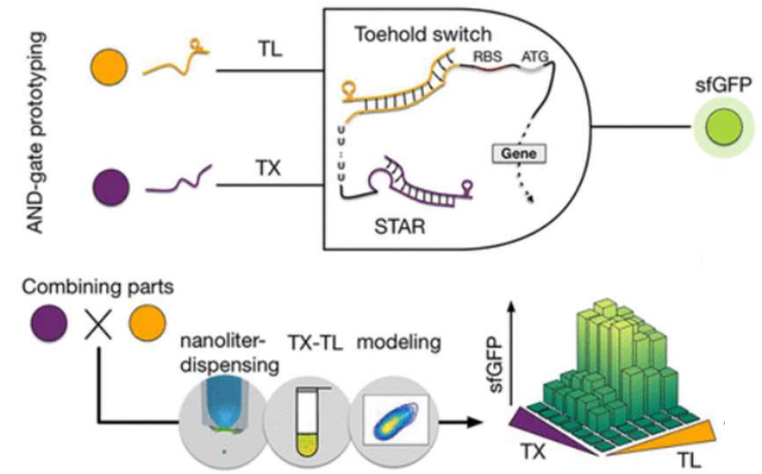
Information processing in whole-cell and cell-free systems



(Wang & Buck, 2012. Trends in Microbiology.)



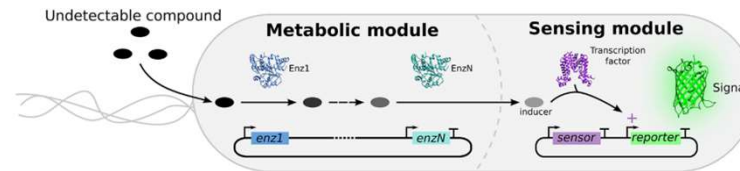
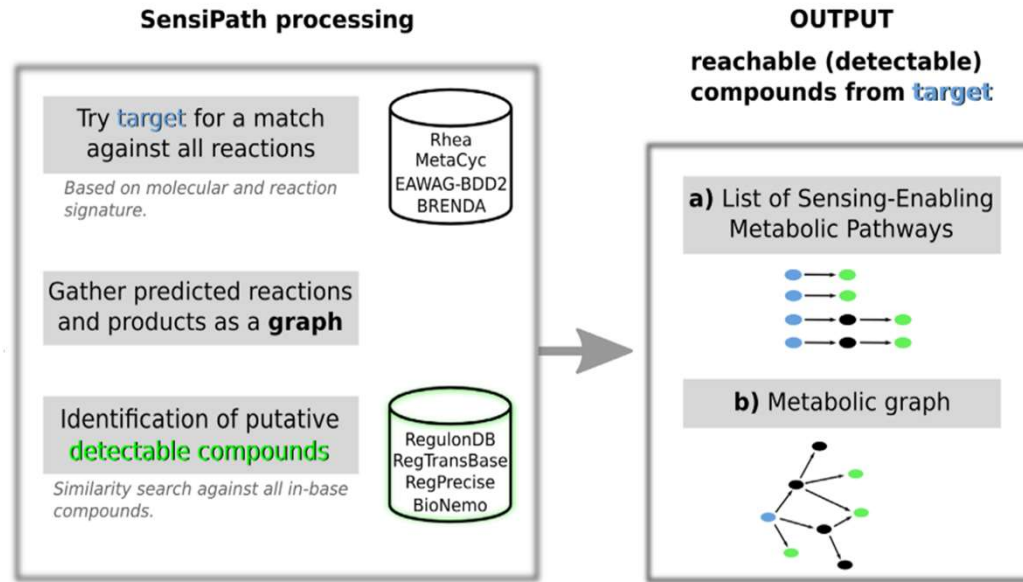
(Niederholtmeyer *et al.*, 2015. eLife.)



(Lehr *et al.*, 2019. ACS Syn Bio.)

- Cellular and cell-free systems can be engineered to process a wide variety of information

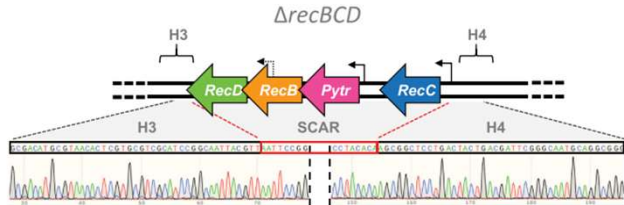
Sensing-enabling metabolic pathways (SEMP)



(Delepine *et al.*, 2016. NAR.
Libis *et al.*, 2016. ACS Synth. Biol.)

- Sensing-enabling metabolic pathways (SEMP) enable indirect sensing of metabolites via “transducer” enzymes

Rapid screening of Transducer enzymes



(Batista *et al.*, 2022. ACS Syn. Biol.)



Angelo Cardoso Batista

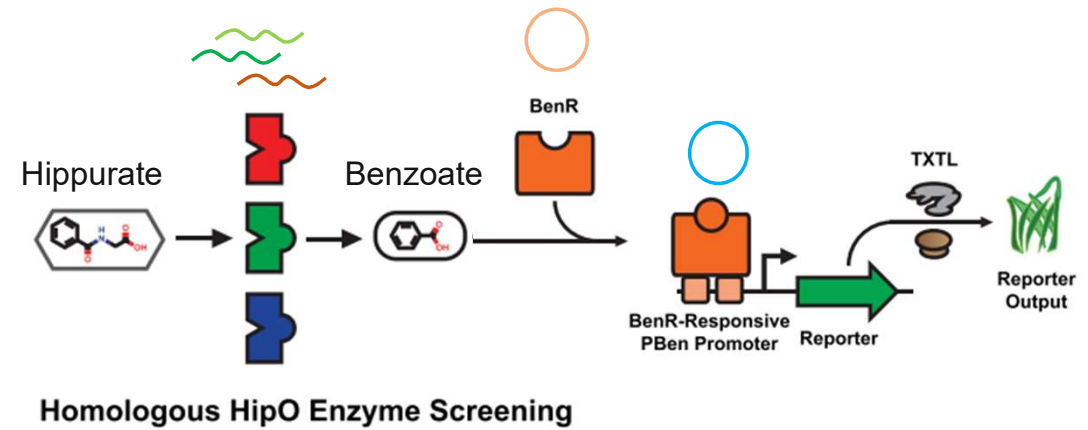
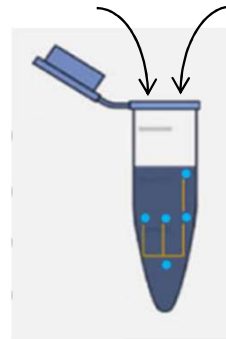
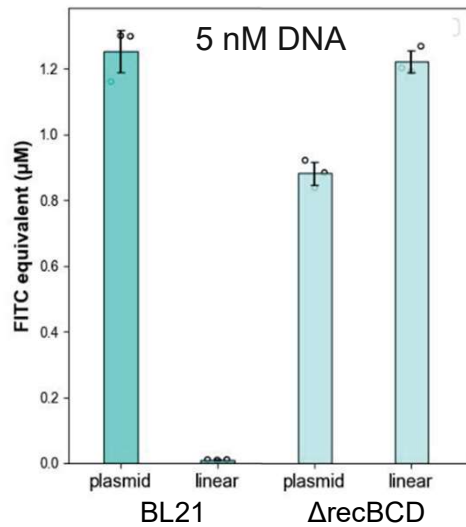


Paul Soudier



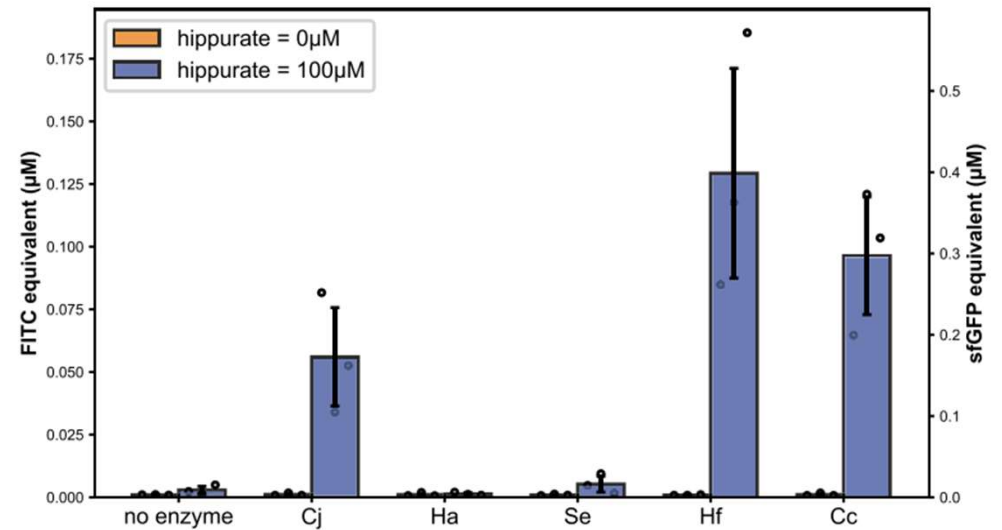
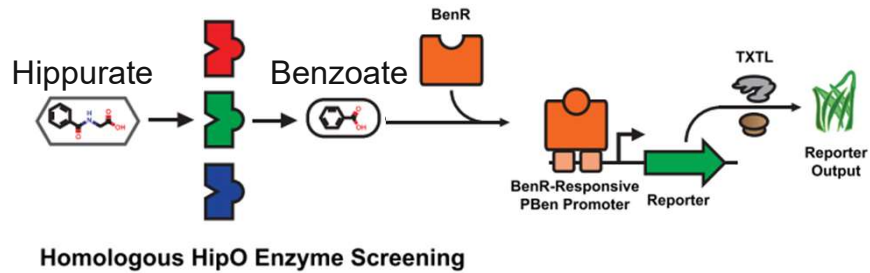
Antoine Lévrier
(Bonnet lab)

Rosetta 2



- Linear DNA templates enable rapid screening of enzyme variants
- A “transducer” enzyme converts an undetectable metabolite to a TF-detectable one

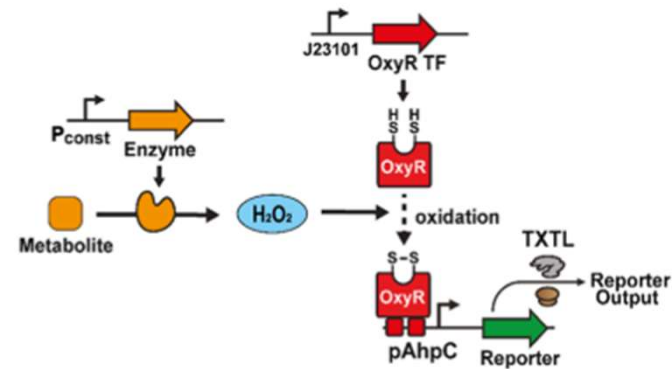
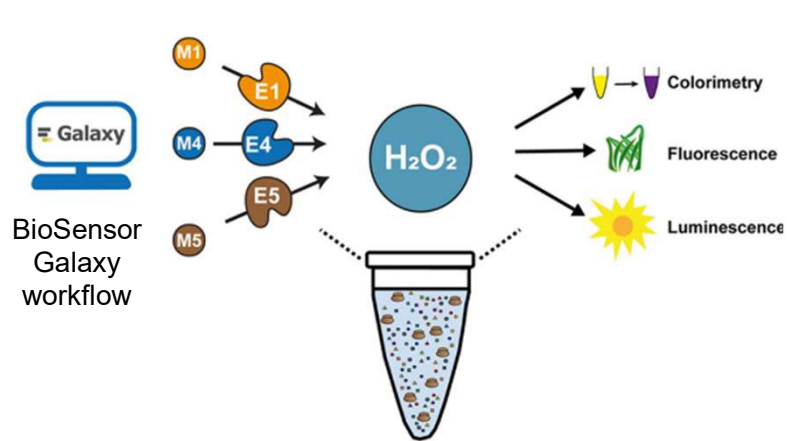
Rapid screening of Transducer enzymes



(Batista *et al.*, 2022. ACS Syn. Biol.)

- 5 homologous HipO enzyme sequences were tested
- *Helicobacter felis* and *Campylobacter coli* HipO enzymes exhibited best hippurate detection

A modular cell-free biosensing platform

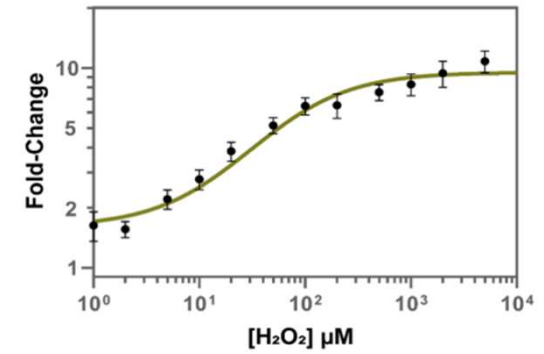
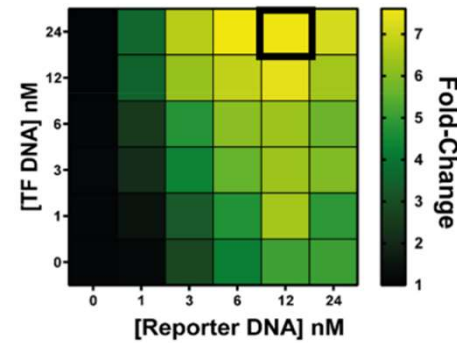
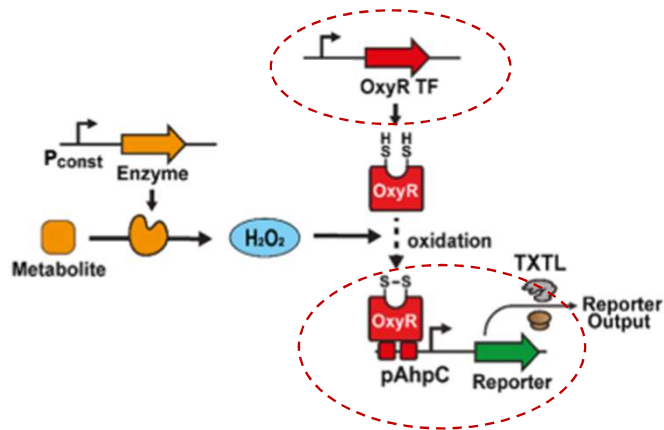


Paul Soudier

(Soudier *et al.*, 2022. ACS Syn. Biol.)

- PeroxiHUB enables detection of several disease-associated metabolites via H_2O_2 intermediate
- 89% of the 2490 HMDB molecules can be converted to H_2O_2 in 1 or 2 enzymatic steps

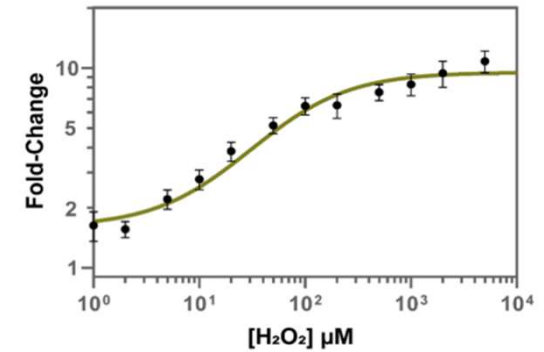
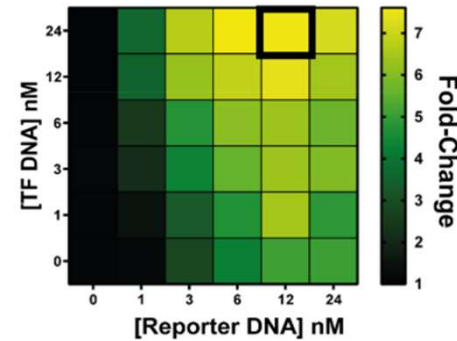
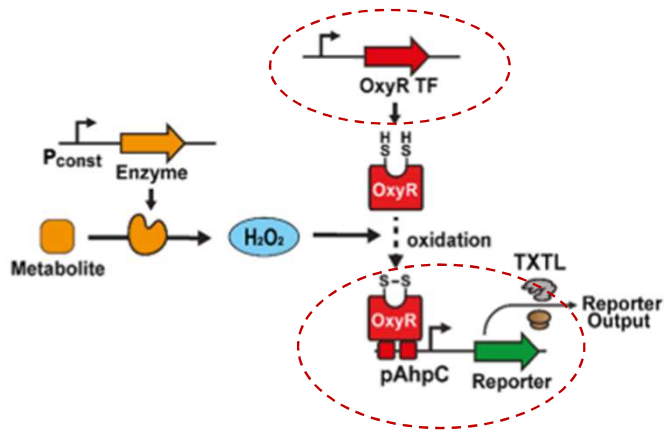
A modular cell-free biosensing platform



(Soudier *et al.*, 2022. ACS Syn. Biol.)

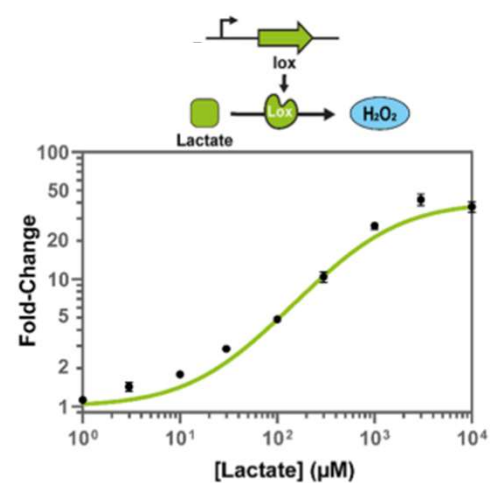
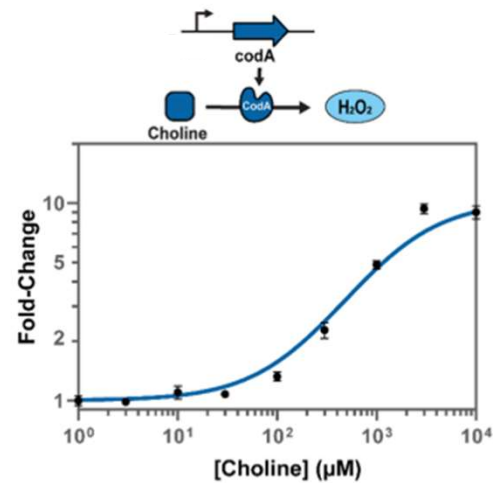
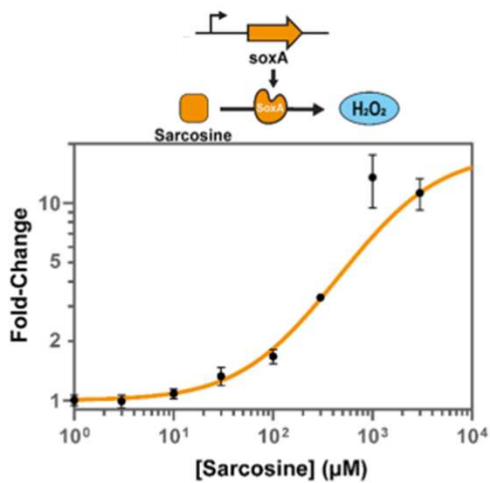
- Tuning DNA concentrations to optimize fold-change

A modular cell-free biosensing platform



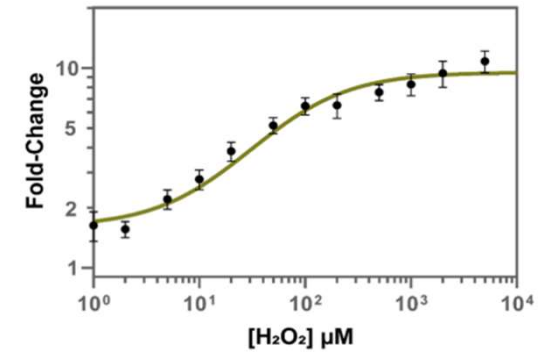
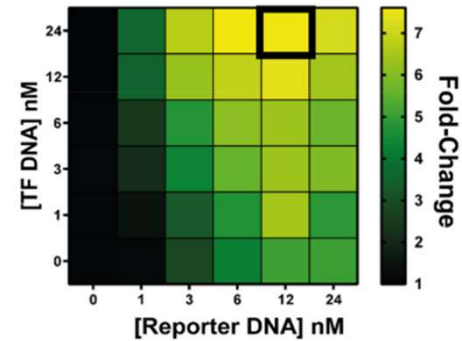
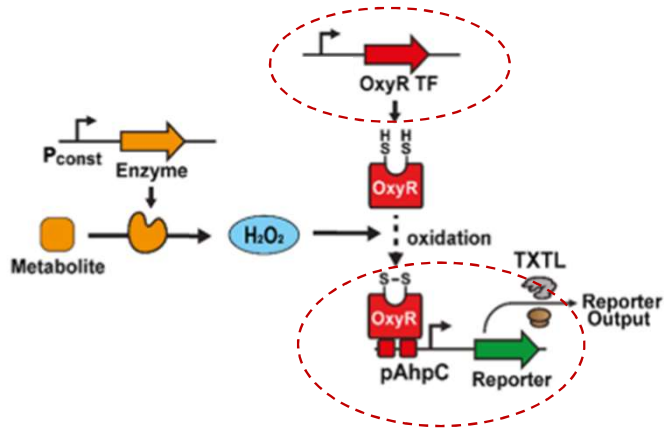
(Soudier *et al.*, 2022. ACS Syn. Biol.)

- Tuning DNA concentrations to optimize fold-change



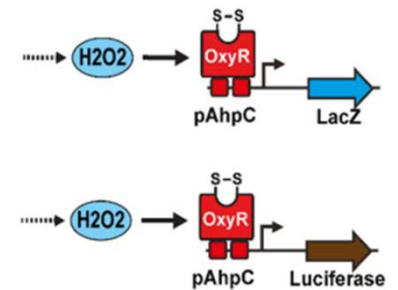
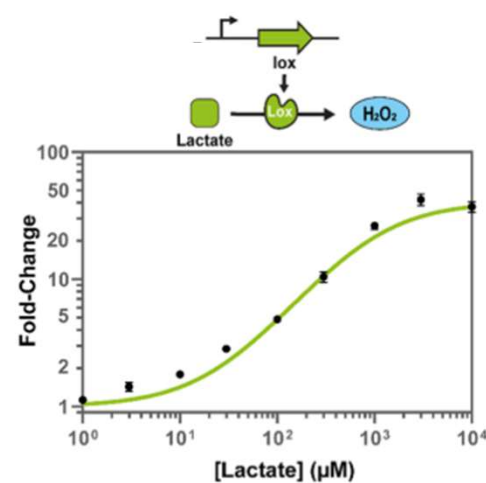
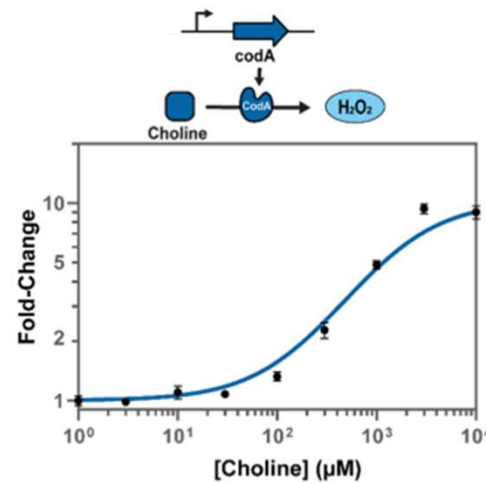
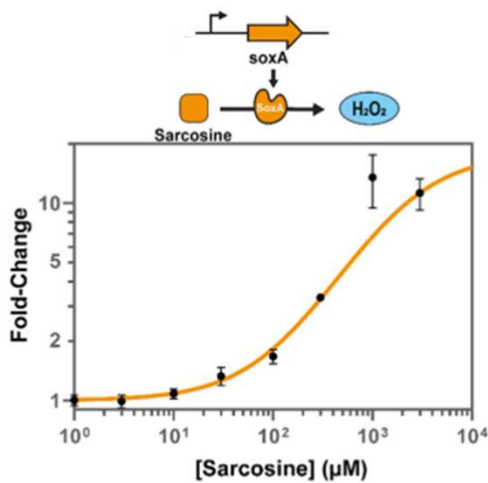
- Detection of several disease-associated metabolites

A modular cell-free biosensing platform



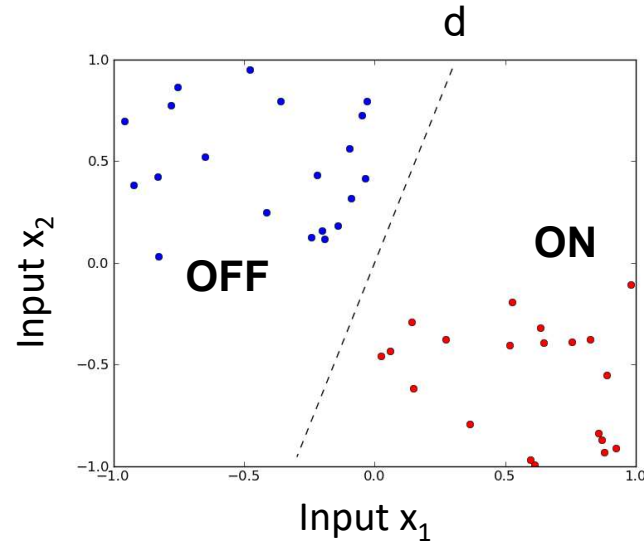
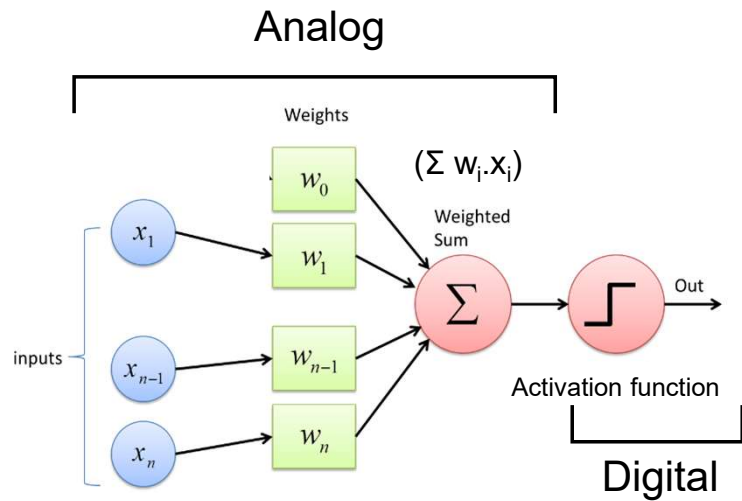
(Soudier *et al.*, 2022. ACS Syn. Biol.)

- Tuning DNA concentrations to optimize fold-change



- Detection of several disease-associated metabolites

A Perceptron for multi-input hybrid logic

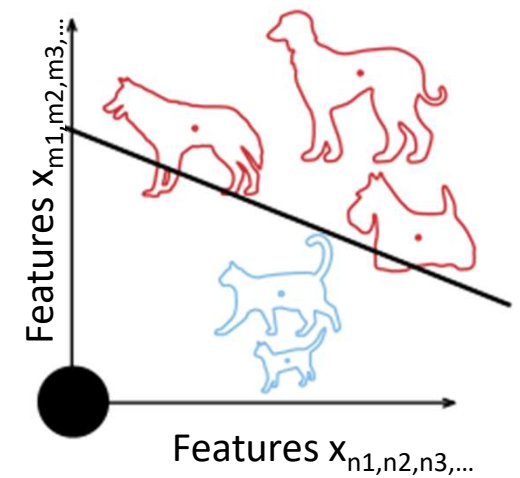
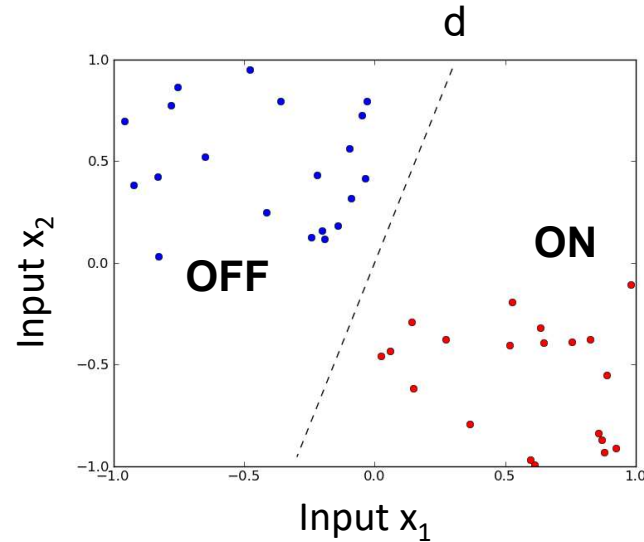
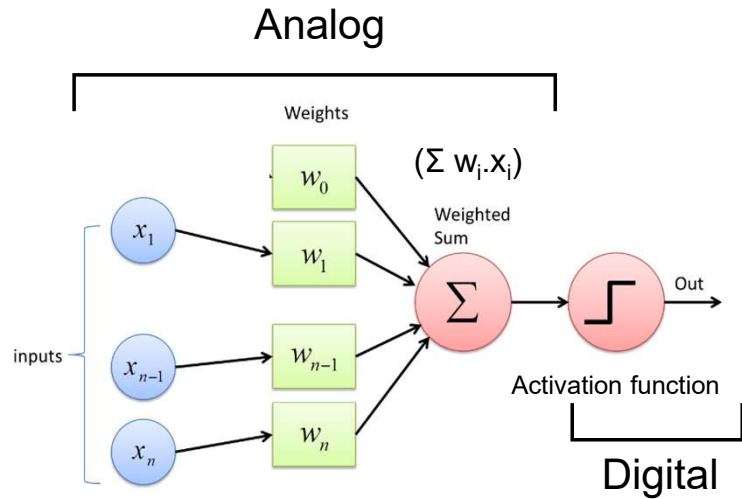


If $(\sum w_i \cdot x_i) > d$, ON

If $(\sum w_i \cdot x_i) \leq d$, OFF

- The perceptron is a basic block of artificial neural networks
- It mimics the neuron's ability to process information

A Perceptron for multi-input hybrid logic

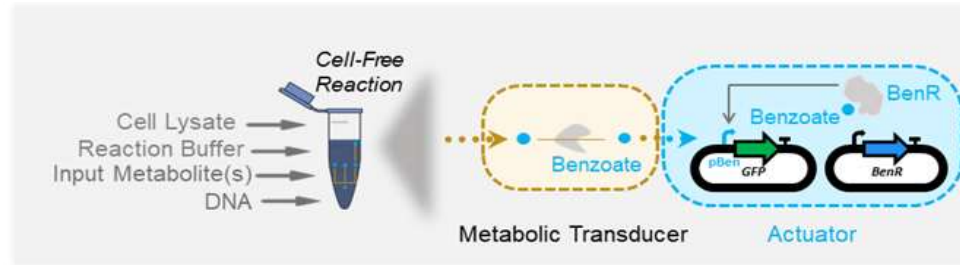


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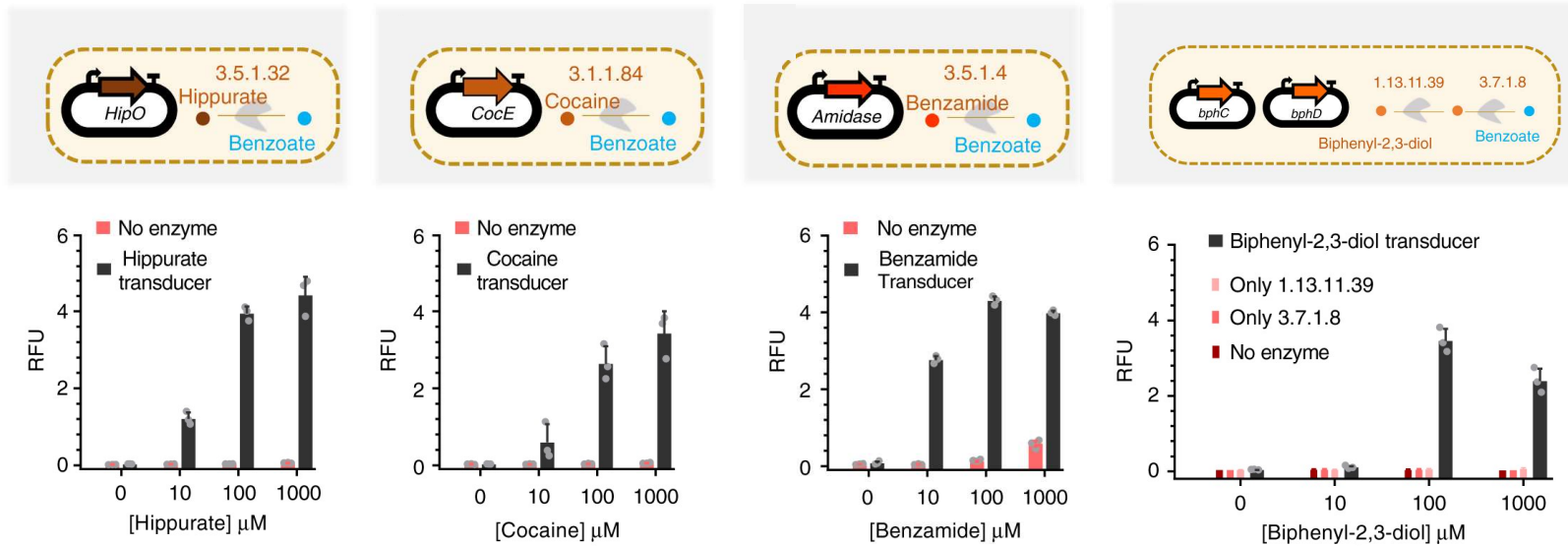
Transducer enzymes enable indirect sensing



Amir Pandi



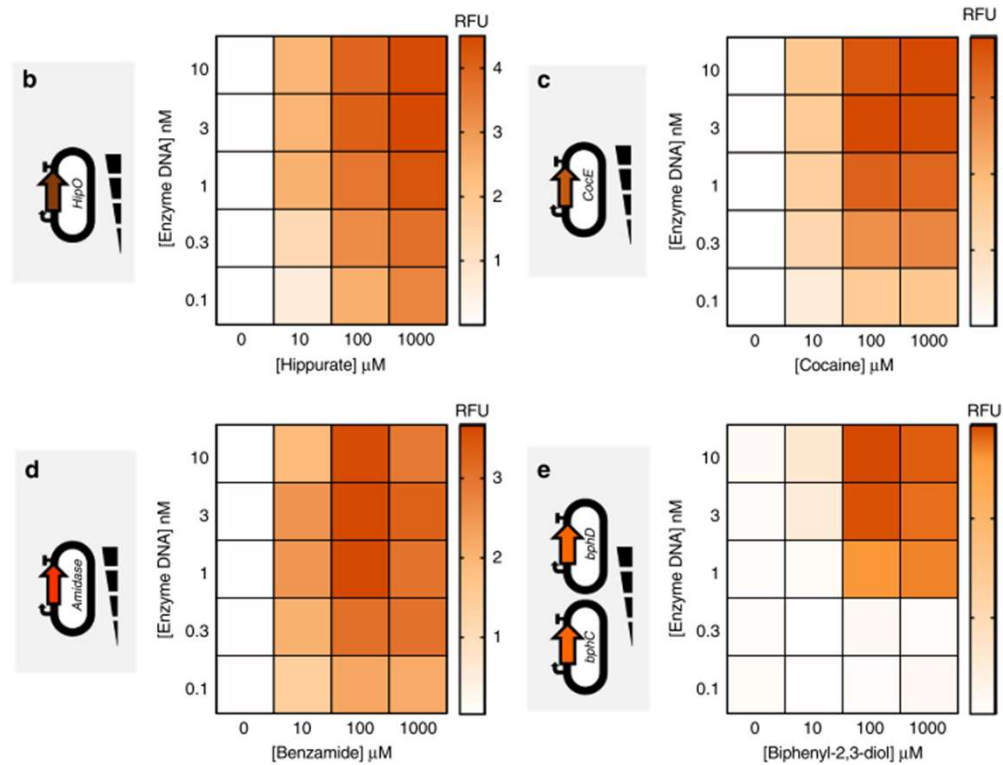
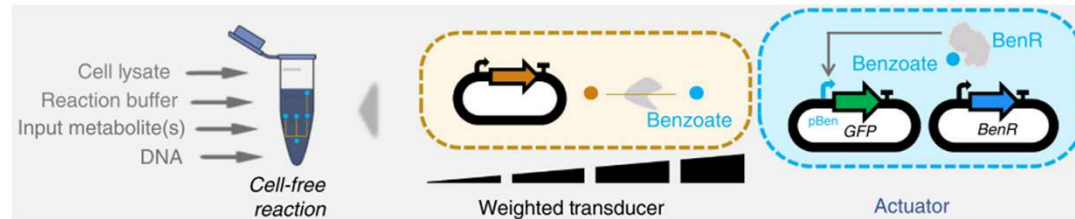
Mathilde Koch



(Pandi *et al.*, 2019. Nat Comm.)

- A “transducer” enzyme converts an undetectable metabolite to a TF-detectable one

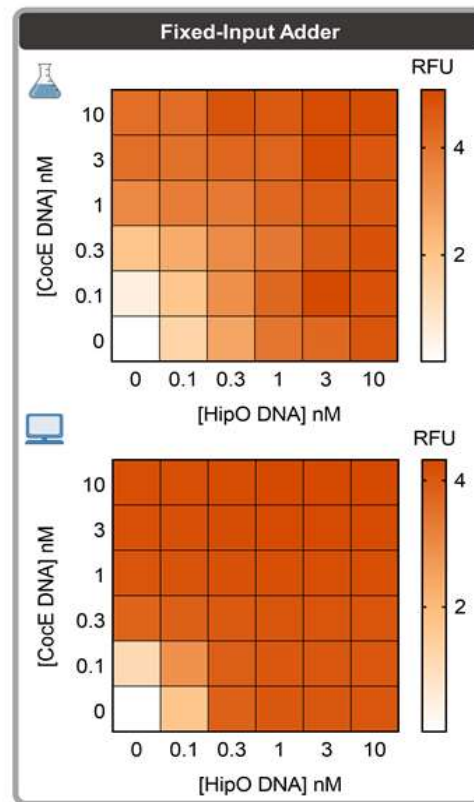
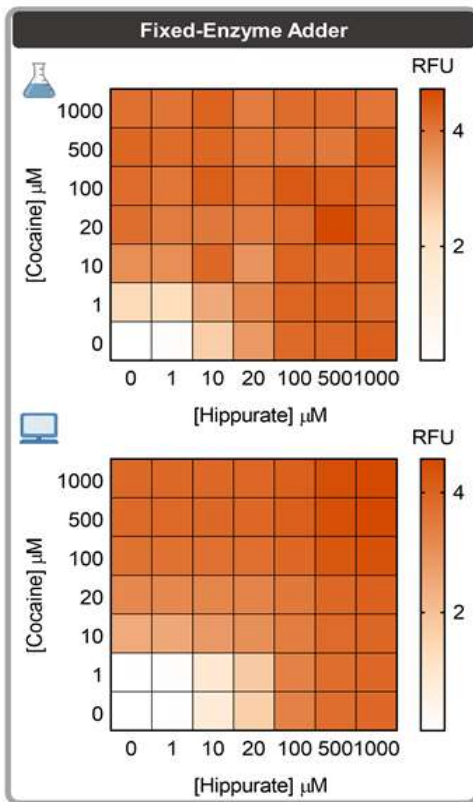
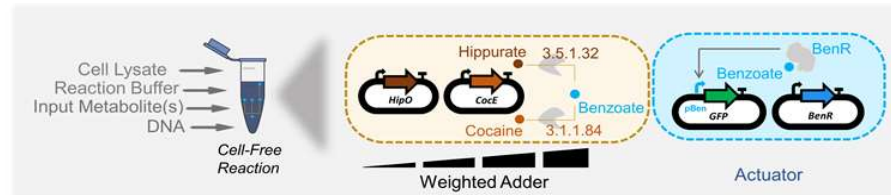
Building the cell-free weighted transducers



(Pandi *et al.*, 2019. Nat Comm.)

- The weight of a transducer can be tuned by changing the amount of transducer DNA added

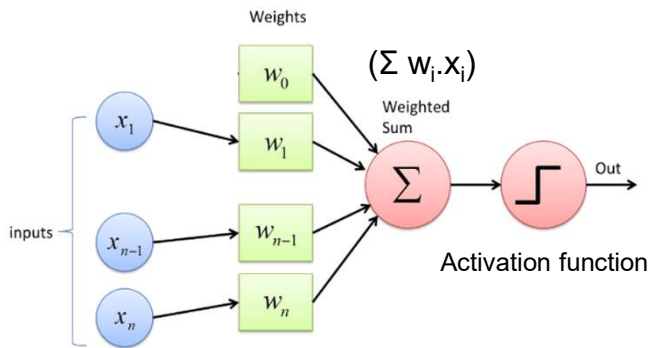
Characterizing and modeling the metabolic adders



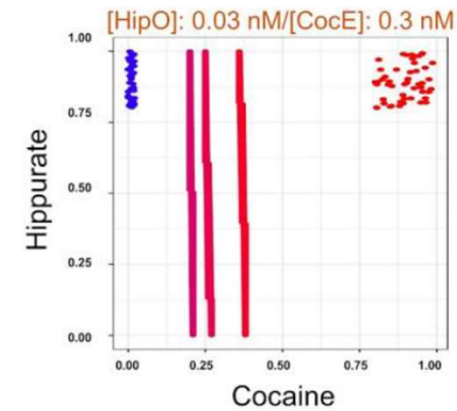
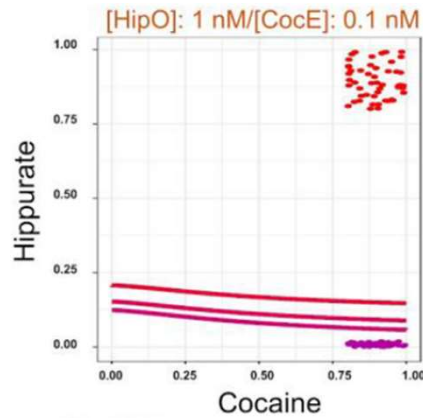
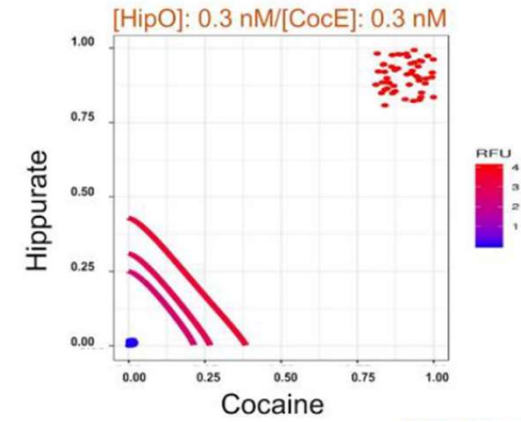
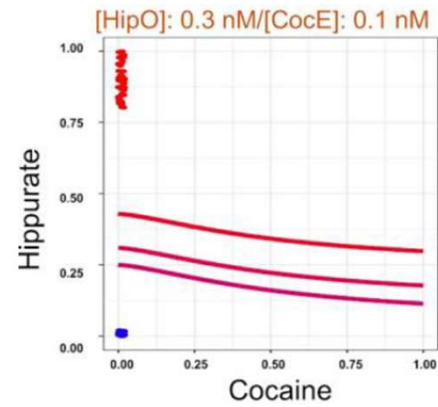
- Cell-free weighted adders with:
 - (1) varying metabolite concentrations
 - (2) varying DNA concentrations

(Pandi *et al.*, 2019. Nat Comm.)

Perceptrons for binary classification

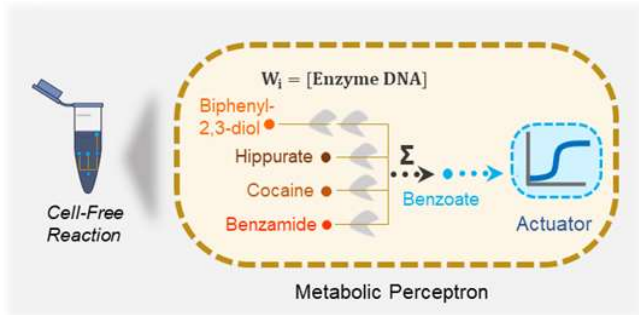
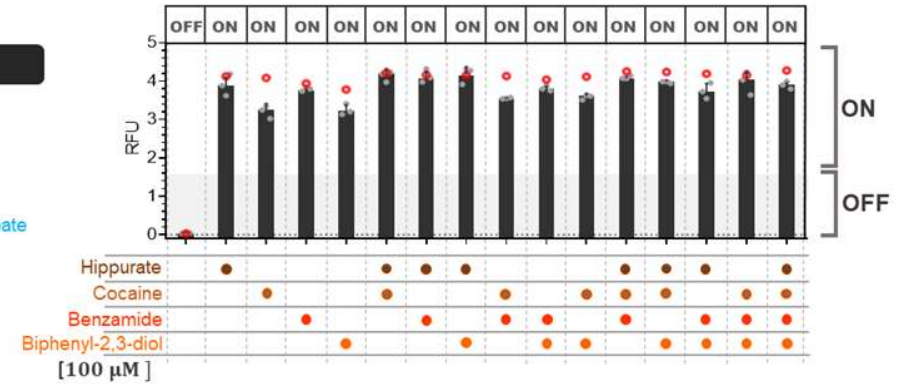
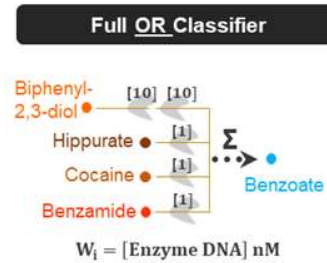
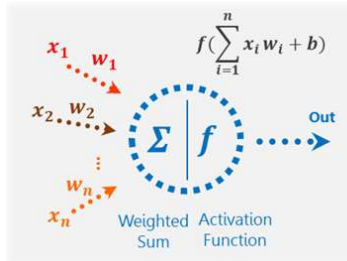


(Pandi *et al.*, 2019. Nat Comm.)



- Model-guided construction of a binary classifier based on 2-input combinations

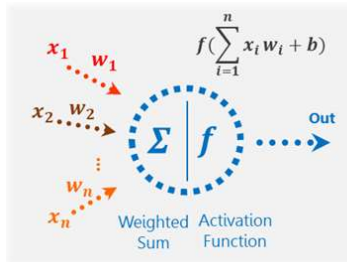
Four-input metabolic perceptrons



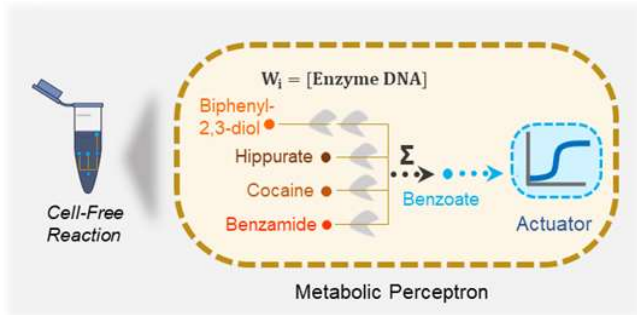
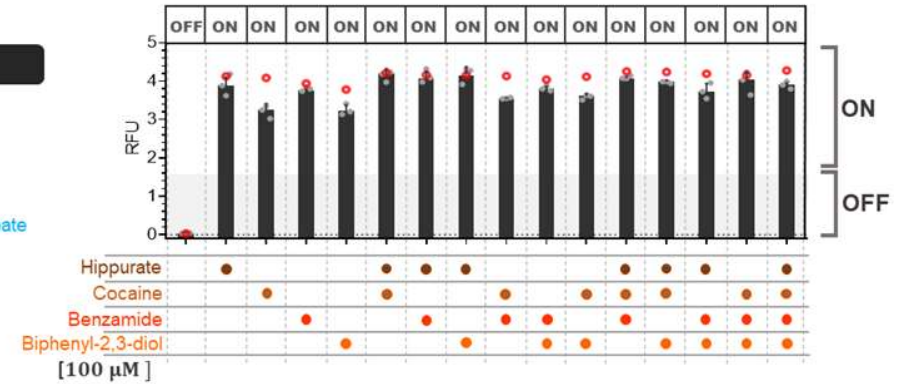
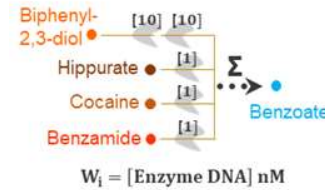
(Pandi *et al.*, 2019. Nat Comm.)

- Model-guided construction of a binary classifier based on 4-input combinations
- The same metabolic circuit has different behaviors when used with different weights

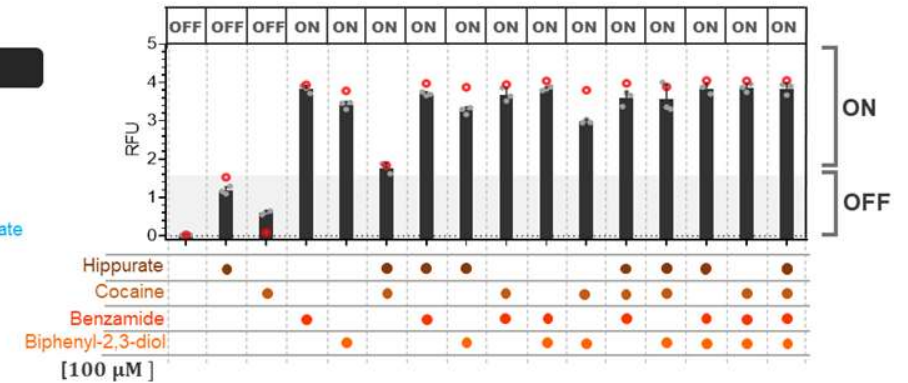
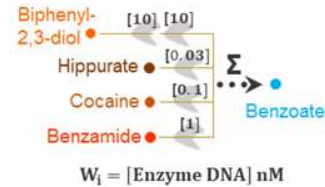
Four-input metabolic perceptrons



Full OR Classifier



(C AND H) OR B OR F



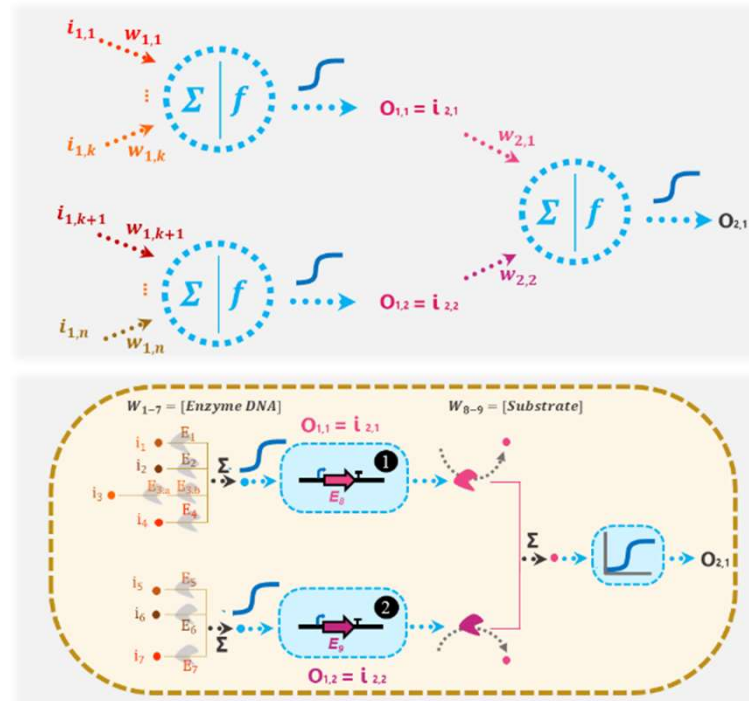
(Pandi *et al.*, 2019. Nat Comm.)

- Model-guided construction of a binary classifier based on 4-input combinations
- The same metabolic circuit has different behaviors when used with different weights

Multiplexing biosensors

Metabolites
Nucleic acids (DNA, RNA)
Proteins

Multiple classes of input molecules

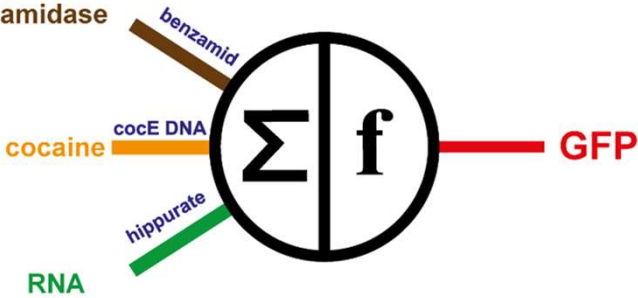
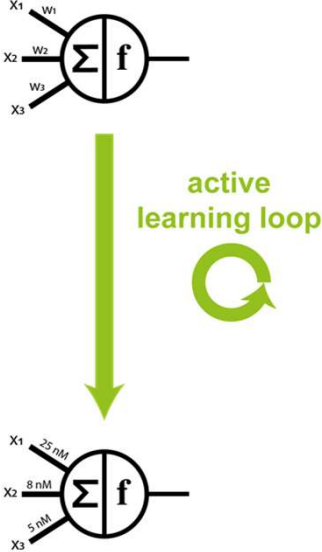


(Pandi *et al.*, 2019. Nat Comm.)

- By combining different types of inputs, diagnostic approaches can be dramatically improved
- Multi-layered perceptrons can classify complex input patterns

Multi-modal perceptrons

Experimental training



Paul Soudier

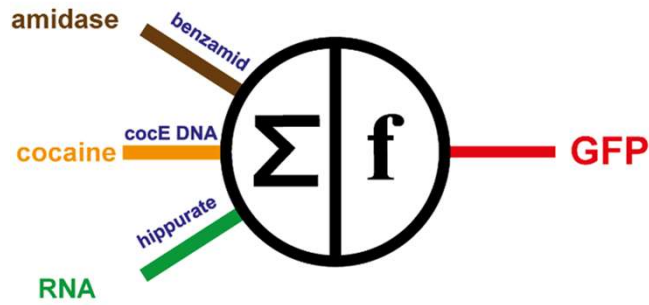
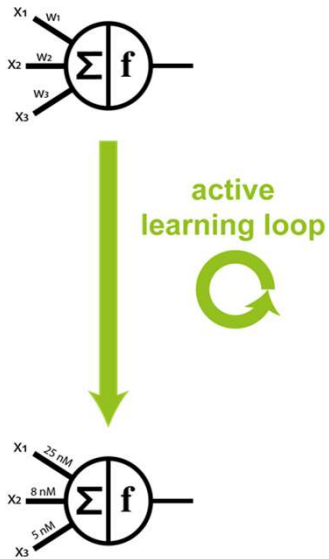


Mahnaz Sabeti Azad

- Multi-modal perceptrons can sense protein, metabolite, and RNA

Multi-modal perceptrons

Experimental training

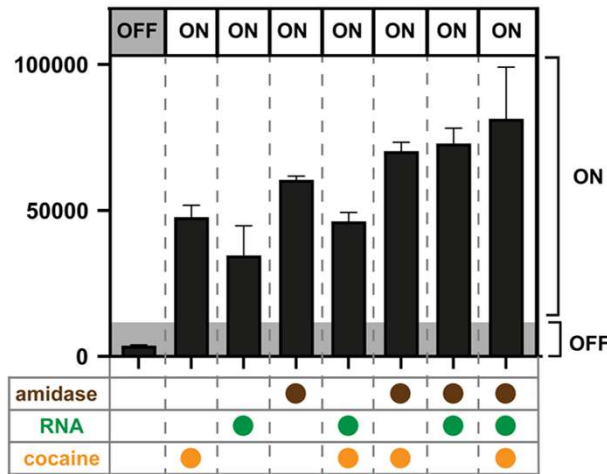


Paul Soudier

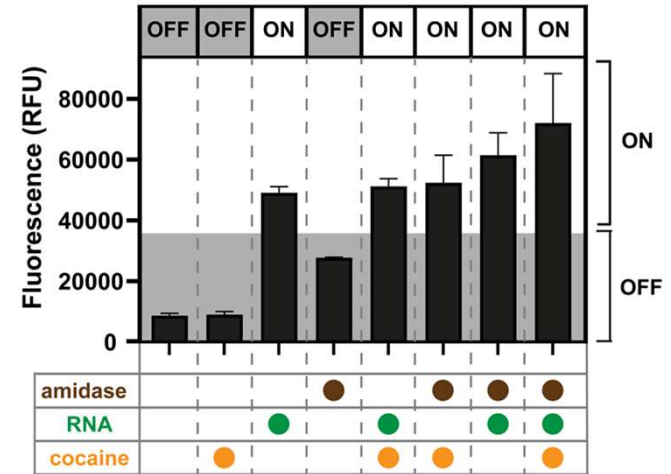


Mahnaz Sabeti Azad

enzyme OR RNA OR metabolite Gate



(metabolite AND enzyme) OR RNA Gate



(Soudier, Azad, unpublished.)

- Multi-modal perceptrons can sense protein, metabolite, and RNA

Summary

- Sensing-enabling metabolic pathways (SEMPs) enable indirect sensing of “undetectable” metabolites
- Cell-free expression systems can be engineered to sense a wide range of biomarkers: protein, metabolites, RNA
- By using analog-digital hybrid logic (perceptron), sensing of complex input patterns is possible

Acknowledgements

Team BioRetrosynth

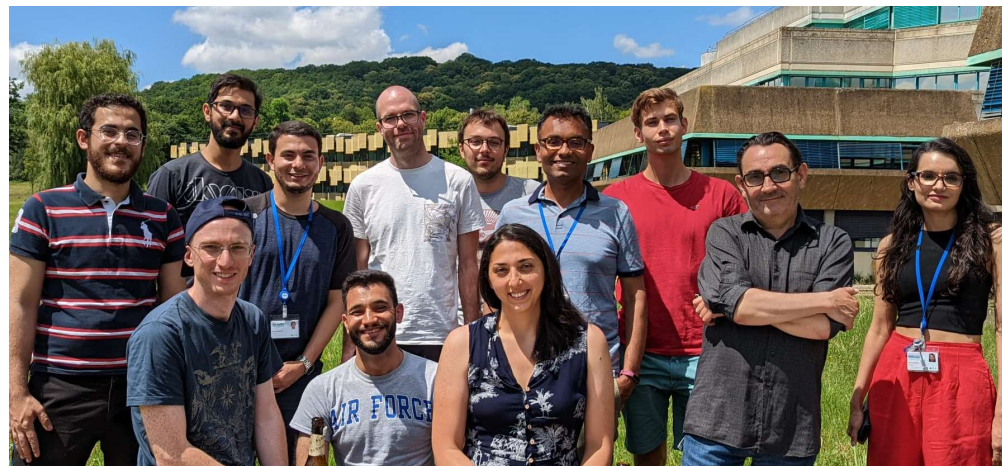
Jean-Loup Faulon
Amir Pandi
Mathilde Koch
Paul Soudier
Mahnaz Sabeti Azad
Thomas Duigou
Angelo Cardoso Batista
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Thank you

