



Microbial robustness 101: tools and applications

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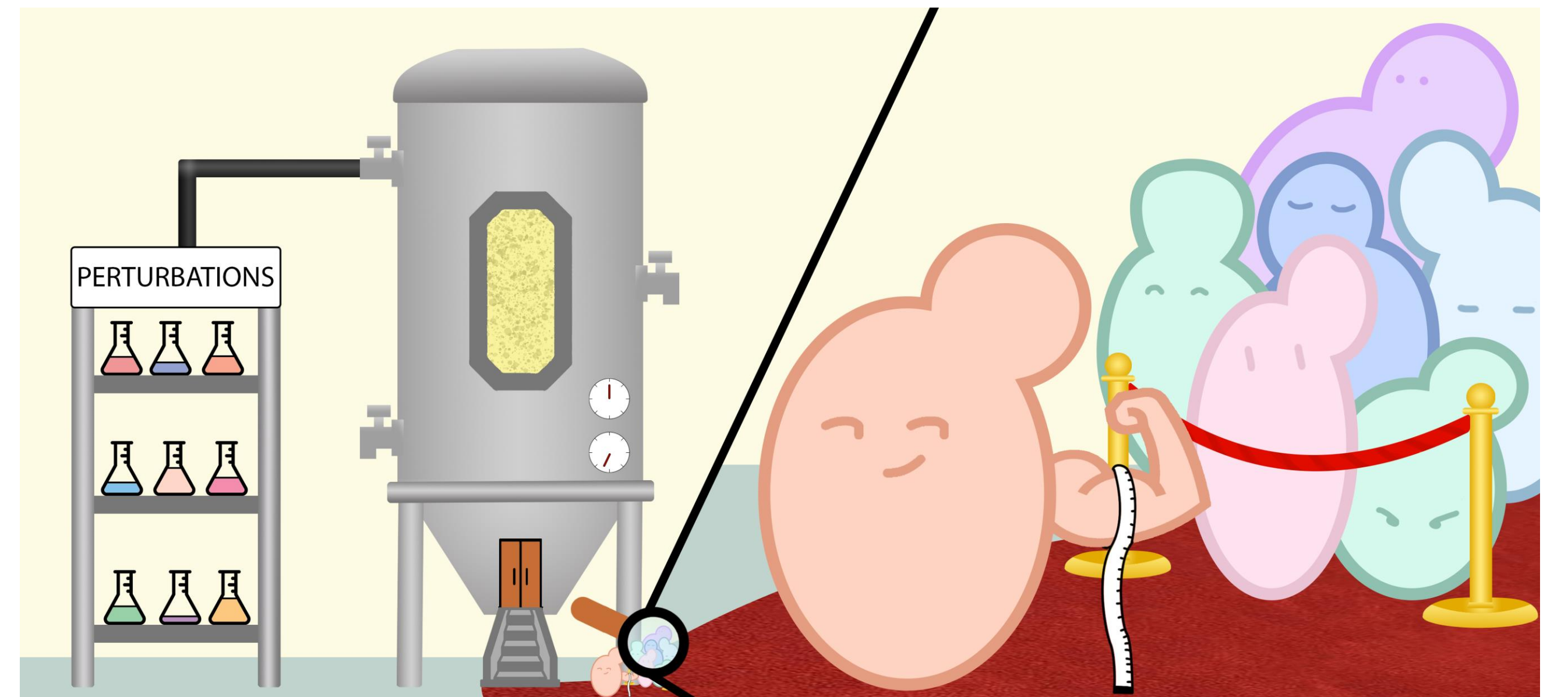
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Microbial Robustness



Check the review!

- A broad range of predictable and stochastic perturbations affects the performance and productivity of microorganisms in bioprocesses.
- **Microbial robustness** describes the stability of a phenotype (cellular function) when a system is challenged by different perturbations.
- It **differs** from **tolerance**, which relates to the survival of the cells or growth in the face of perturbations.
- **Lack of tools** to quantify microbial robustness and investigate the physiological traits associated with it under bioprocess-relevant perturbations.



Quantification



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R : robustness

$$R_{i,S,P} = -\frac{\text{Fano factor}}{\text{mean}} = -\frac{\sigma^2}{\bar{x}} \cdot \frac{1}{m}$$

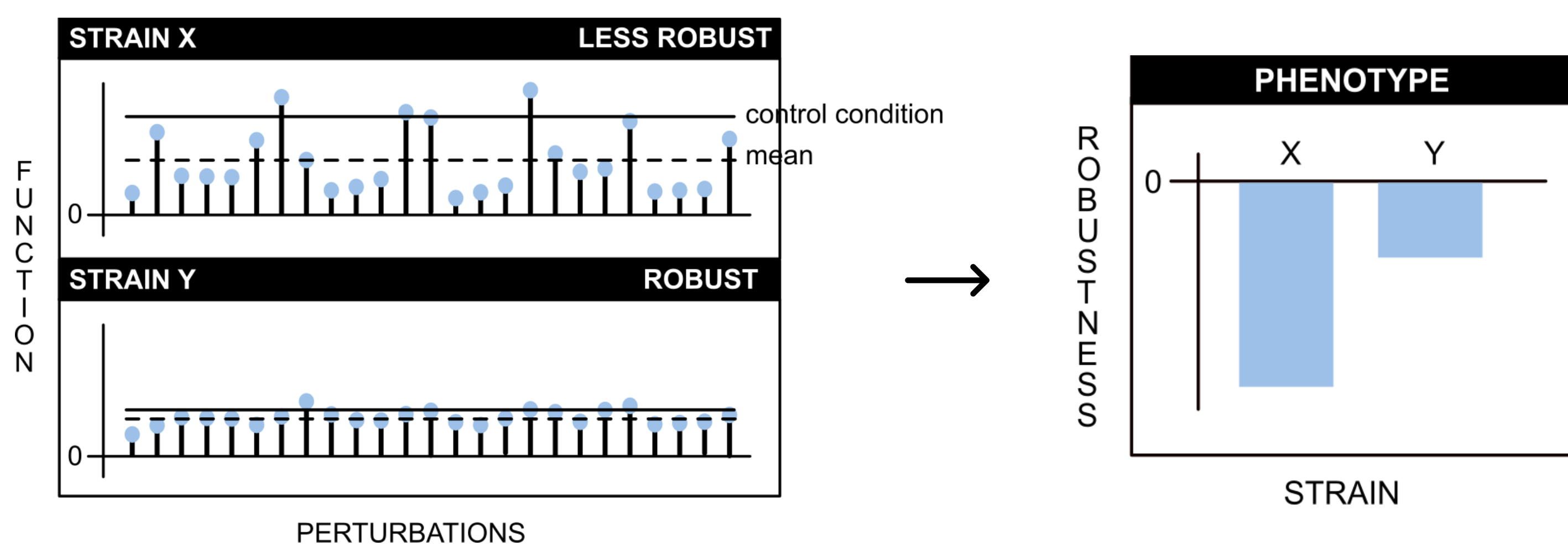
i : cellular function

S : system

P : perturbation space

$\bar{x}$: mean across perturbations

m : mean across strains



This quantification strategy is:

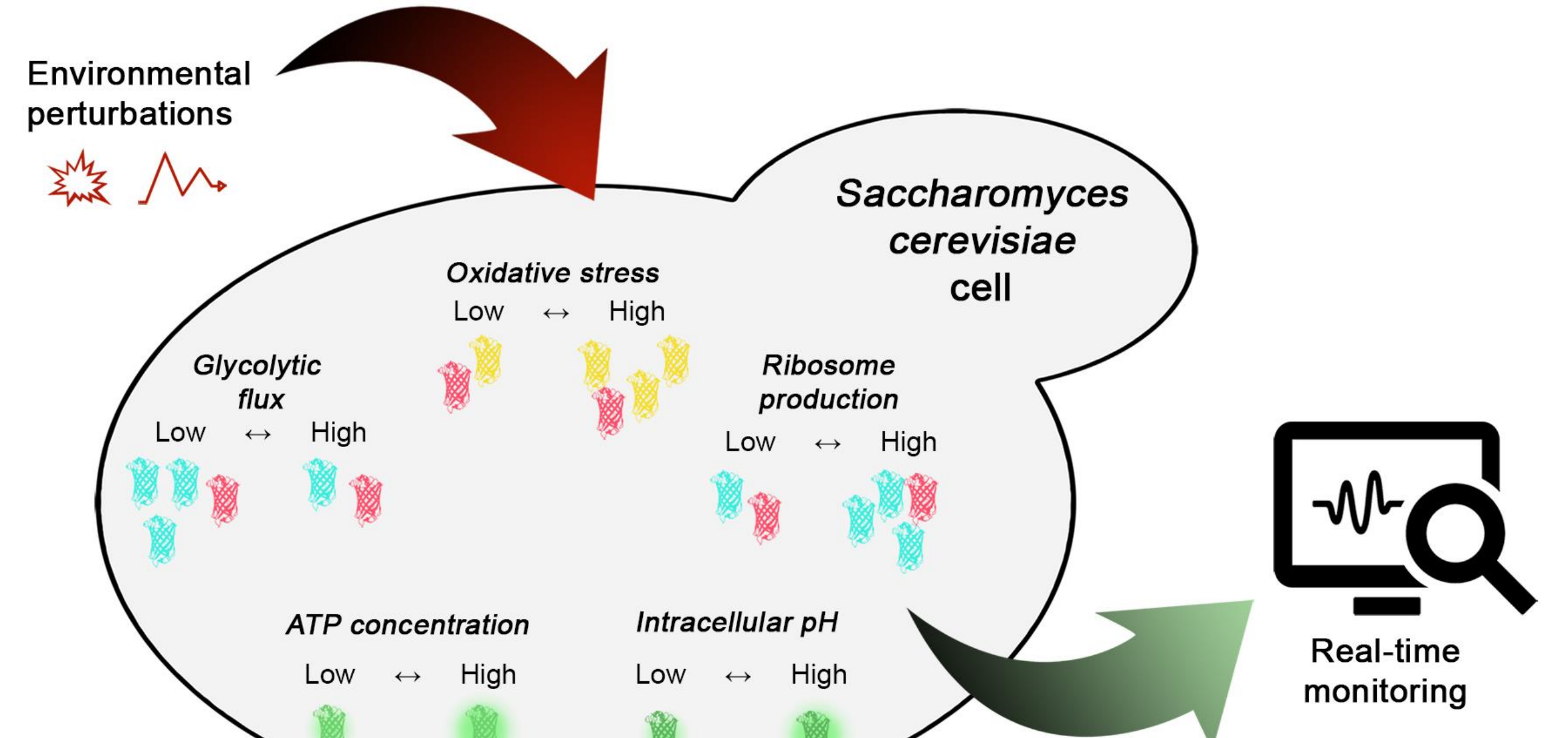
- (1) dimensionless (2) free from arbitrary control conditions (3) frequency independent

Toolkit



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- **Versatile toolbox** for investigating the intracellular environment in yeast *Saccharomyces cerevisiae* using genetically-encoded **fluorescent biosensors**.
- **Features:** easy to use, to implement with new biosensors, efficient marker-free genome integration, allowing for both population and single-cell studies.



S. cerevisiae

	CENPK	EthRed	PE-2
CDW (g/L)	■	■	■
lag phase (h)	■	■	■
Ethanol Yield (g/g)	■	■	■
Biomass Yield (g/g)	■	■	■
μ_{max} (1/h)	■	■	■

□ Robustness ○ Performance

❖ Case study for **bioethanol production from lignocellulosic materials**.

❖ R was calculated for each function and strain with the Fano factor-based method.

❖ **EthanolRed** exhibited the **highest values for robustness and performance**.

❖ **Trade-offs** were identified **between robustness and performance**, for example in the case of the ethanol yield of EthanolRed.

❖ **Possible to apply in other fields and organisms**.

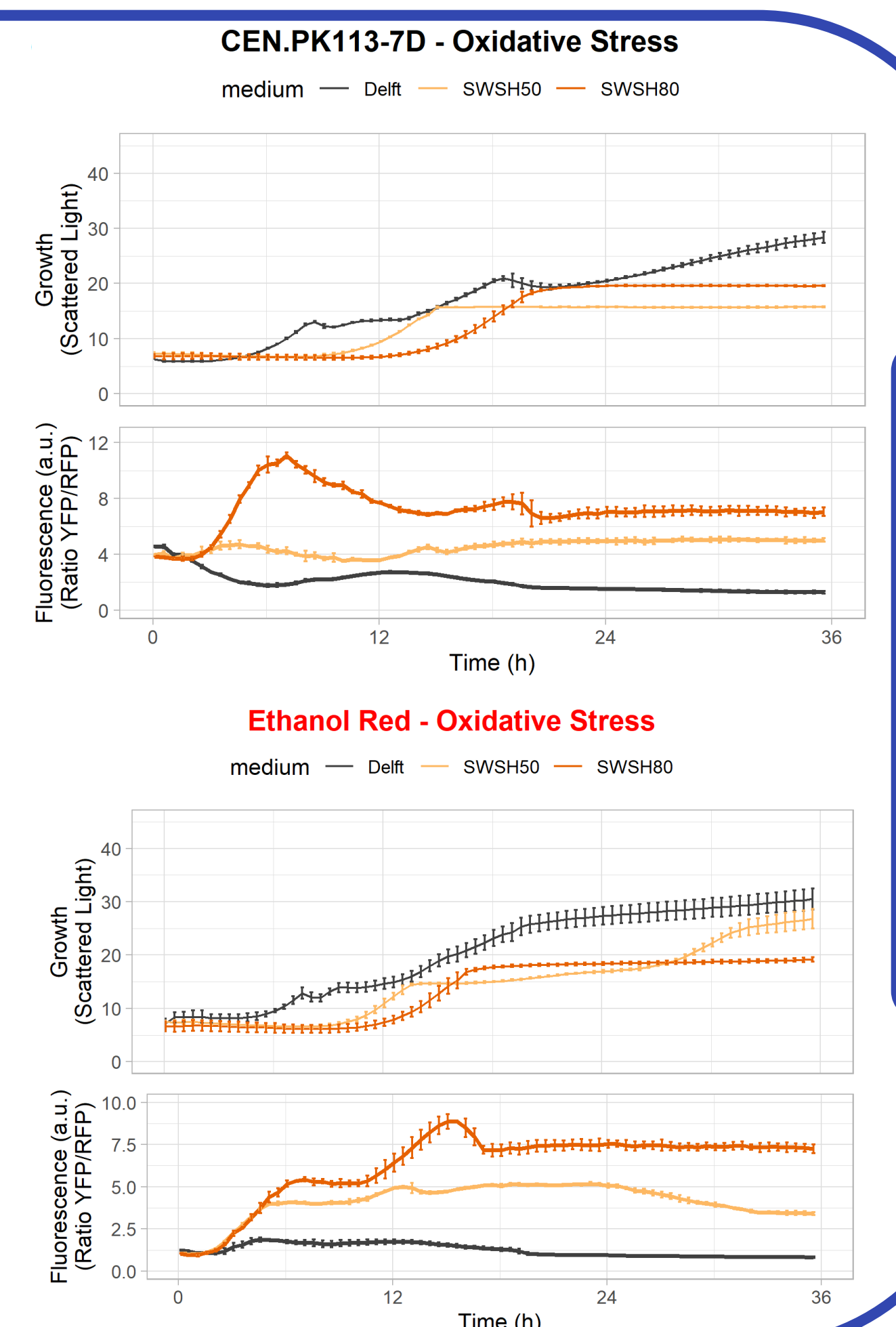
❖ **Investigation of the physiological response** of laboratory and industrial *S. cerevisiae* strains in a high-throughput setup.

❖ Implementation of **new sensors for metabolic statuses**. (ONGOING)

❖ Study at the **single-cell level** the cell **physiology in changing environments** in microfluidics chips. (ONGOING)

❖ Exploration of **subpopulation formation** and population heterogeneity in **large-scale fermentations**. (ONGOING)

❖ **Combination and correlations** with Robustness quantification.



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