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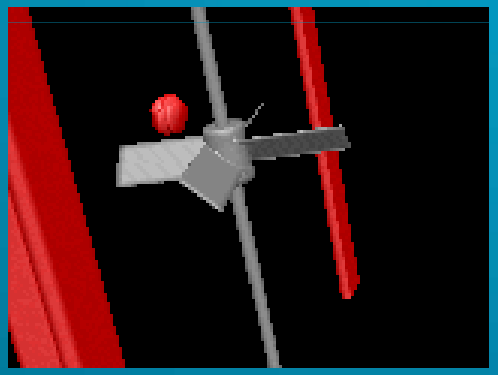
Bioprocesses scale-up

Interactions between physico-chemical and biological parameters

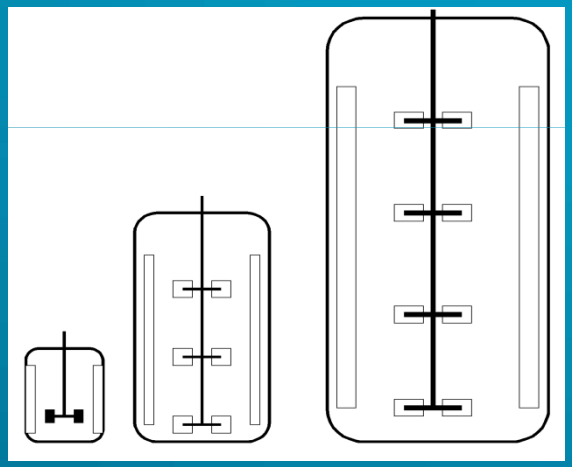


Frank DELVIGNE

ULg – Gembloux Agro Biotech
Centre wallon de Biologie industrielle

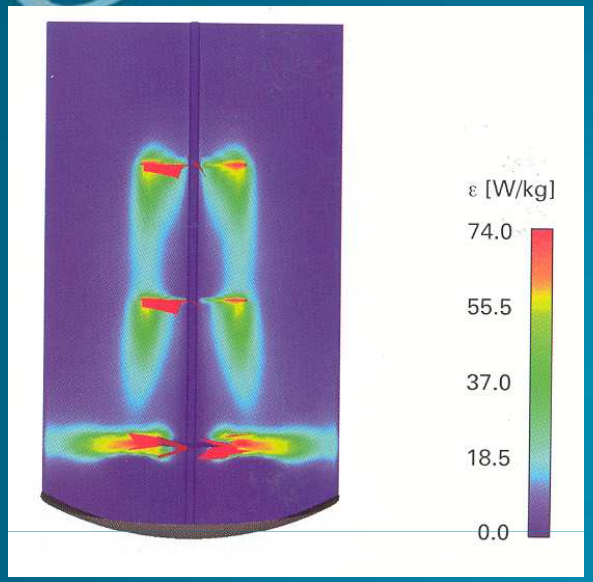
1. **Chemical engineering constraints** : f(D)
2. **Physiological constraints** : stress response

1. + 2. gives the process yield in function of D



Scale-up (D↗)

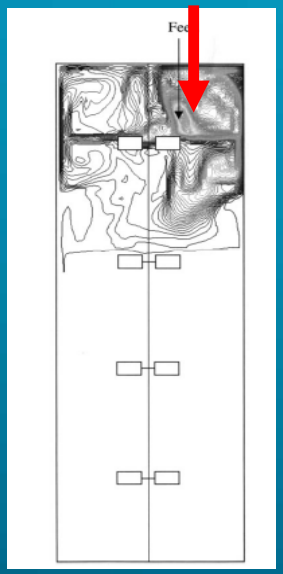
It is important to characterize the physico-biological interactions occuring in the bioreactor and their potential impact on the microbial physiology



Increasing the reacting volume : physical issues:

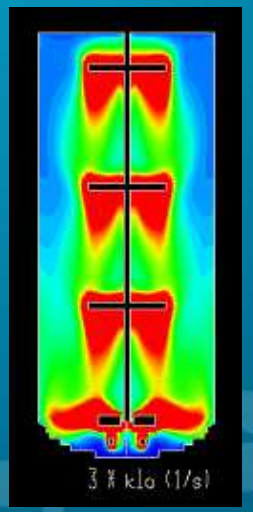
⇨ Segregation of the local dissipated power
⇨ Gradient formation

Substrate gradient



Enfors *et al.* [2001] Journal of biotechnology

Dissolved oxygen gradient



Bakker [2003] www.bakker.org

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During this talk :

- How to model the physical perturbations encountered by the micro-organisms in a heterogeneous bioreactor

- Methodology to track the physiological status of the cells exposed to process-related extracellular fluctuations

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1. Predictive tools for scale-up : hydrodynamic model

Several solutions, (in decreasing order of mathematical complexity)

- Computational fluid dynamics (CFD)
- Compartment modelling approach (CMA)
- Determination of the mixing time based on correlations

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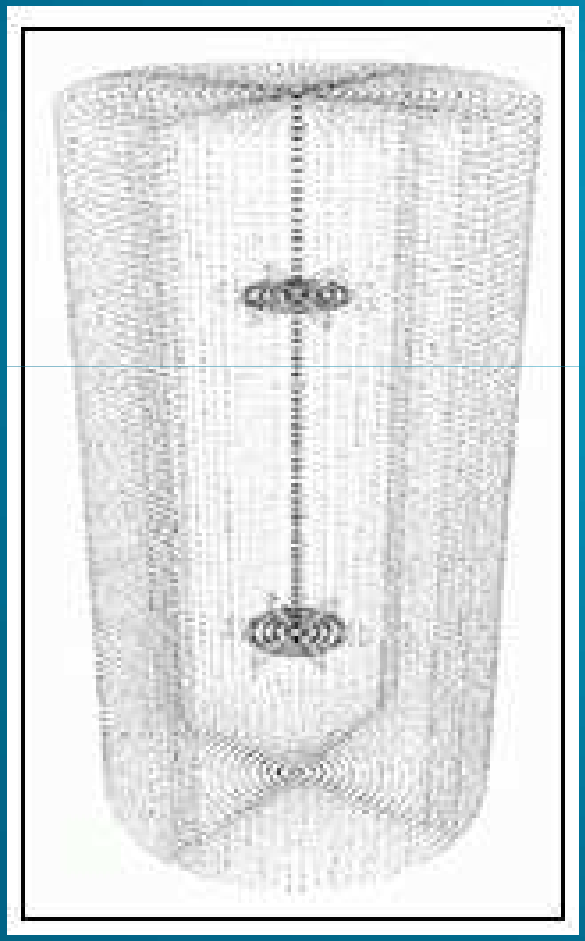
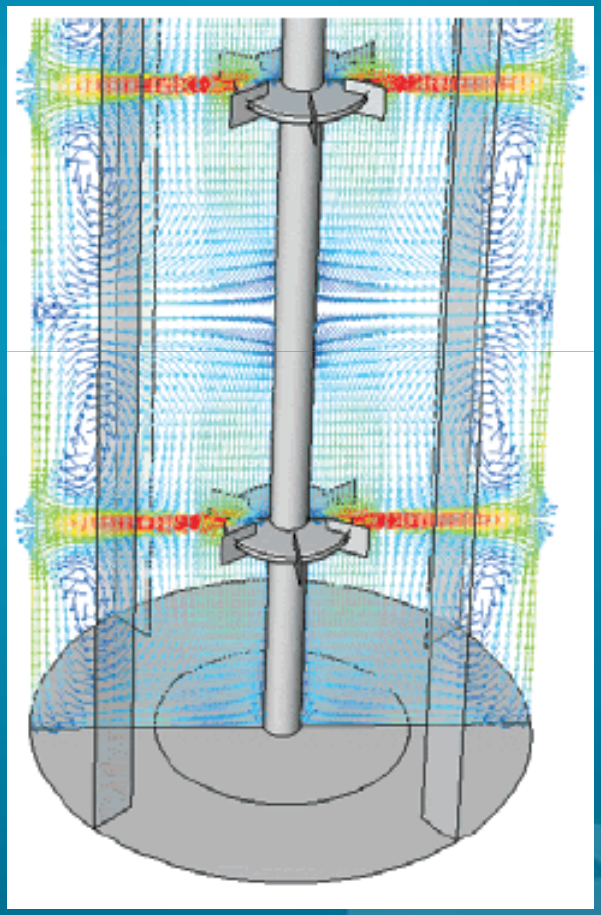
1.1. Application of CFD

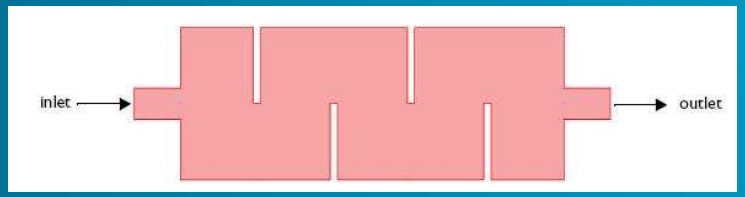




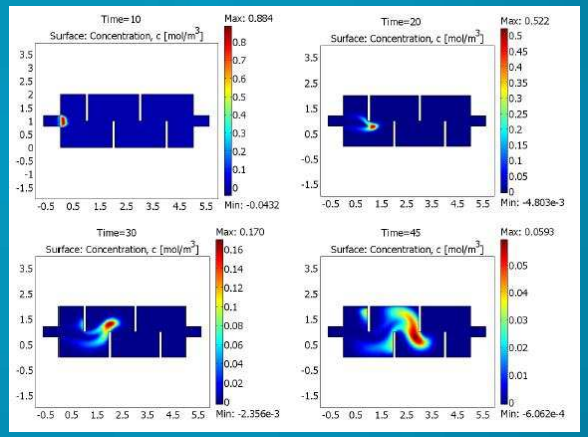
Wastewater engineering application of CFD

Example 1 : chloration bassin:

Source : Comsol Multiphysics



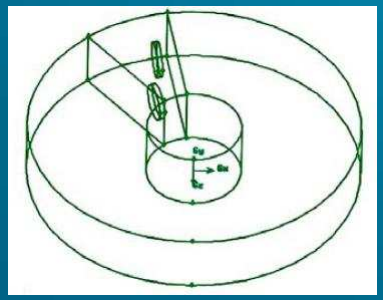
Géométrie du système



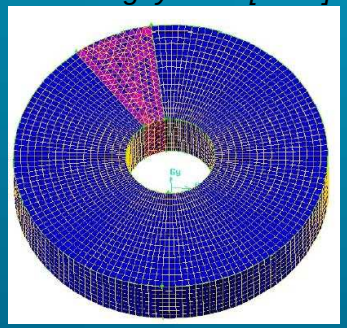
Simulation

Example 2 : oxydation ditch

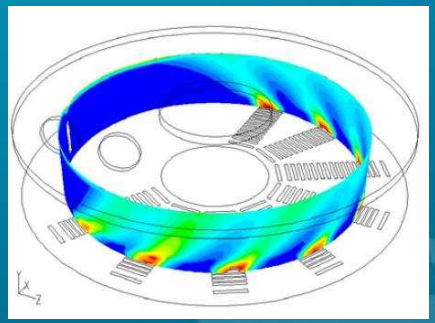
Tanguy et al. [2003]



Step 1 : geometry specification

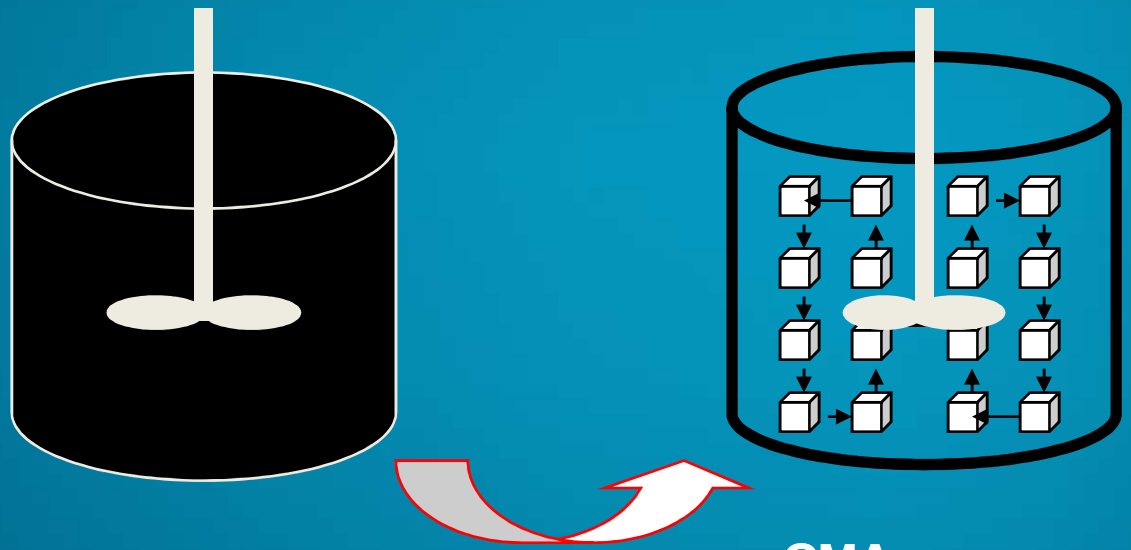


Step 2 : meshing



Step3 : simulation

1.2. Application of CMA



Unstructured model

« black box »

CMA

Vrabel *et al.* [2001] Chem. Eng. J.

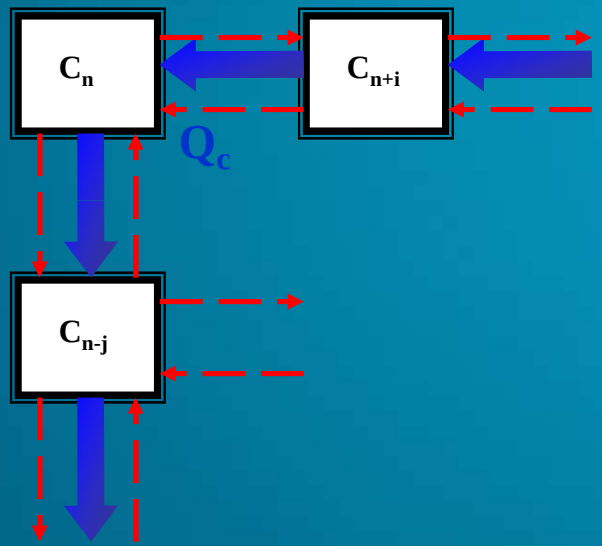
Zahradnik *et al.* [2000] Chem. Eng. Sci.

Cui *et al.* [1996] Trans. IChemE

Machon *et al.* [2000] Chem. Eng. Technol.

Mayr *et al.* [1994] Biotech. Bioeng.

Mass balance of what goes in and what goes out for each compartment : the resulting set differential allows to simulate the evolution of the concentration of a species for each compartment delimited in the reactig volume



Variation = In - Out

$$V \cdot \frac{dC_n}{dt} = Q_c \cdot (C_{n+i} - C_n) + Q_e \cdot (C_{n+i} + C_{n-j} - 2C_n)$$

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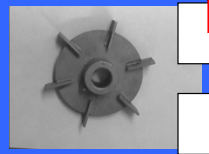
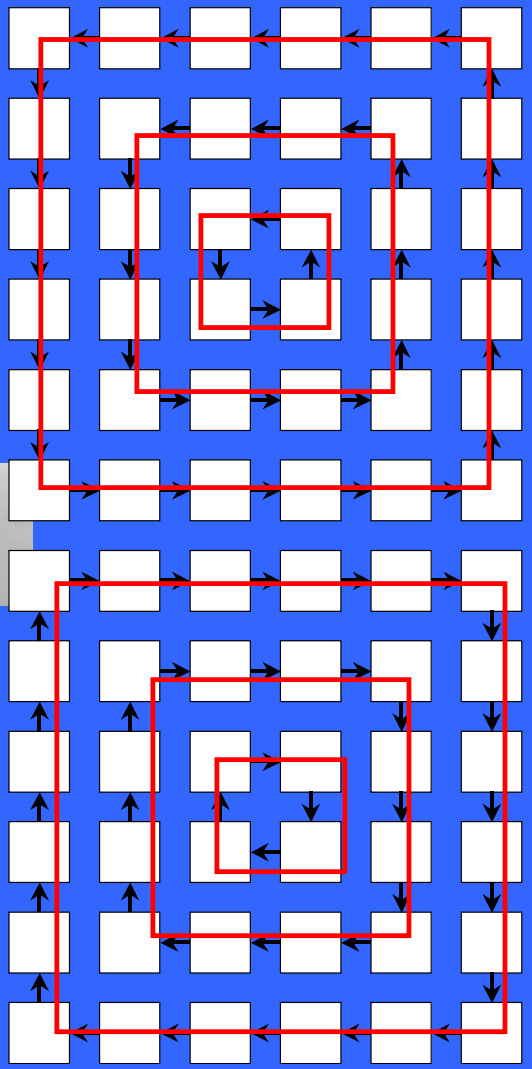
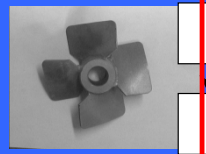
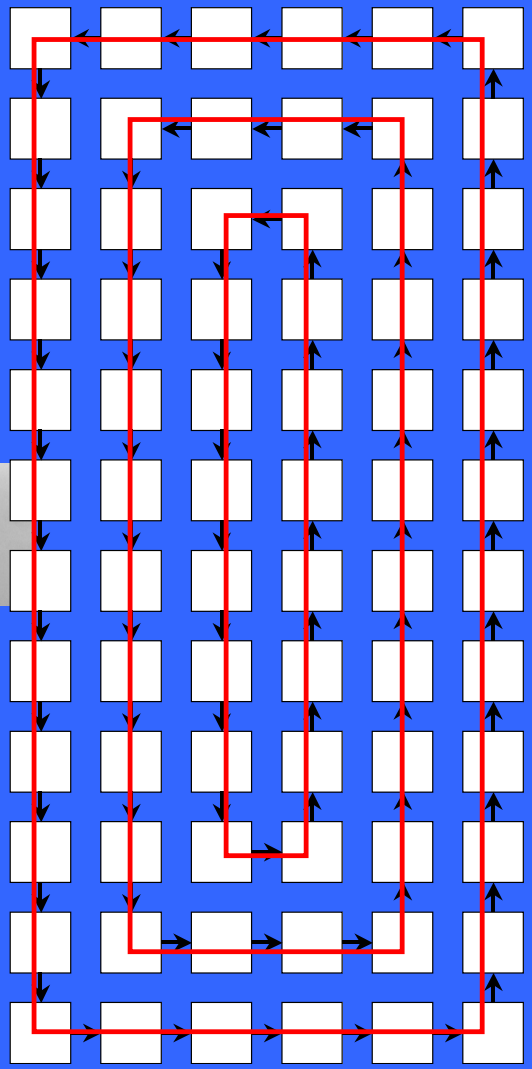
RADIAL





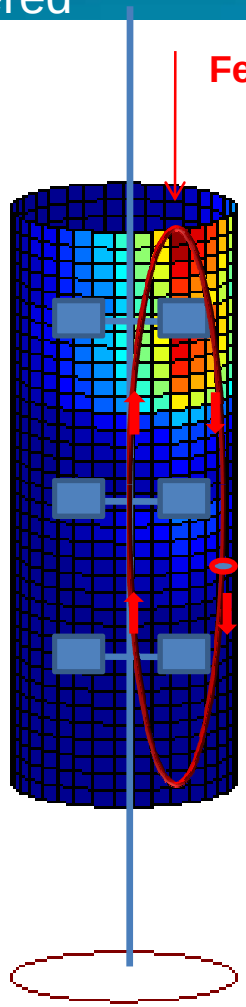
AXIAL





Example : simulation of the mixing of a tracer pulse in a three-staged bioreactor
2592 fluid zones considered

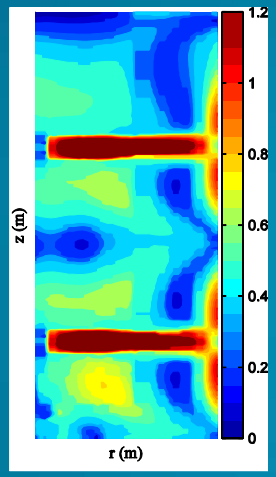
Simulation : gradient field 5 seconds after pulse addition



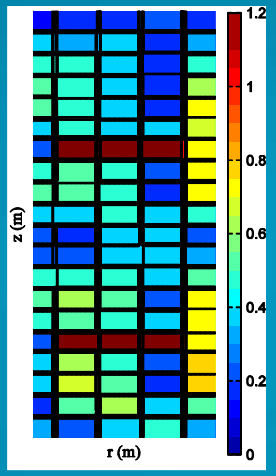
Considering the global circulation flow path

Delvigne *et al.* [2006] Chemical engineering journal

1.3. Hybrid approach (CMA based on CFD computation)

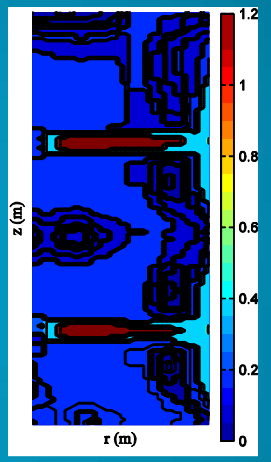


a. CFD



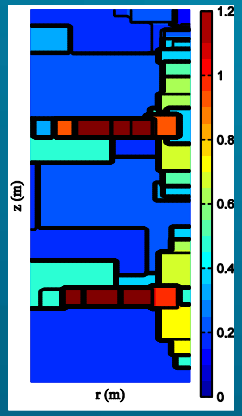
b. Manual zoning

(N_Z = 1800)



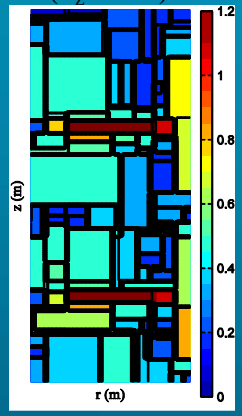
c. CBC zoning

(**P** = **U**, N_Z = 1784)



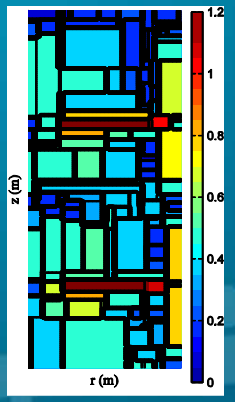
d. LBL1 zoning

(**P** = **U**, N_Z = 1023)



e. LBL2 zoning

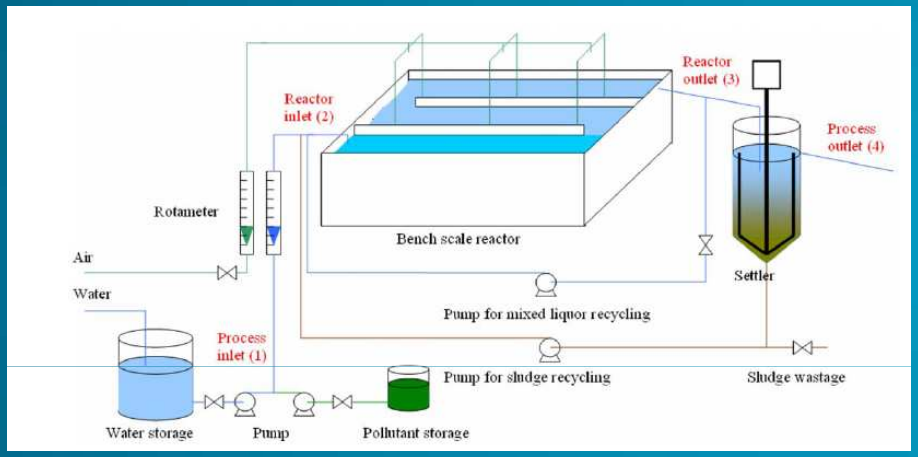
(**P** = **U**, N_Z = 3897)



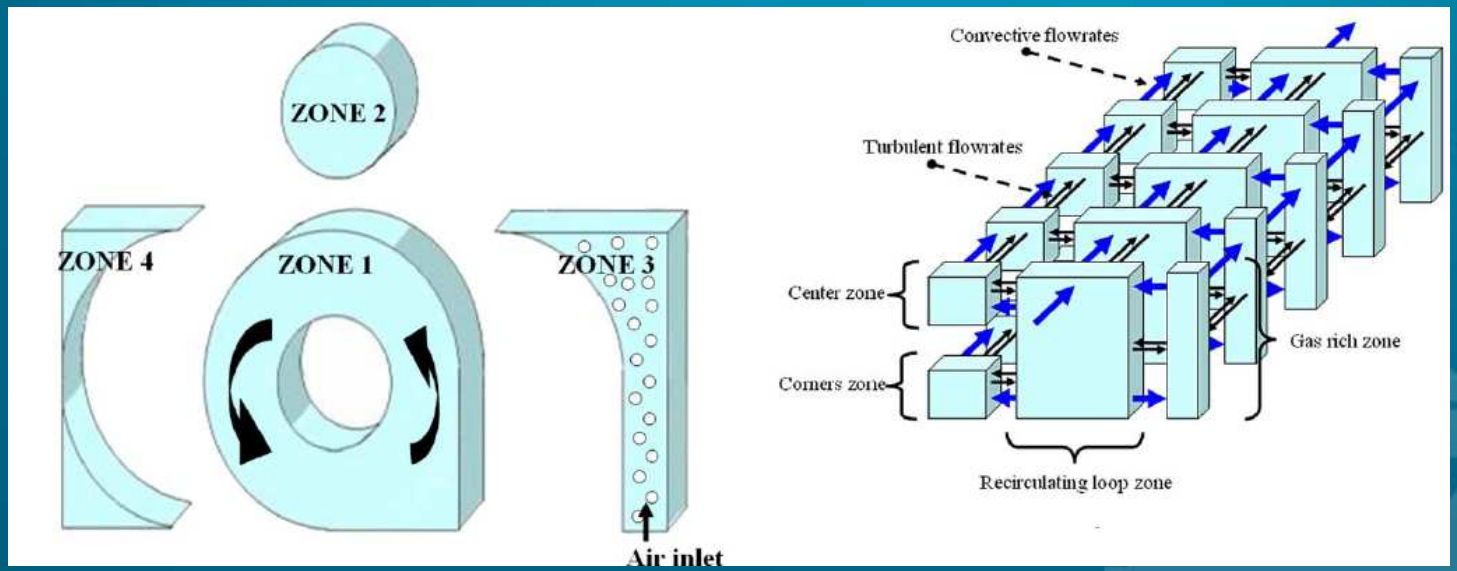
f. LBL2 zoning

(**P** = $\mathbf{U_i}$, N_Z = 4300)

Example of application of the hybrid in wastewater engineering



Le Moullec *et al.* [2010] Chemical engineering science **65**, 343-350



1.4. Stochastic model

Determinist formulation

$dC(t)/dt = Q.C(t)$

$C(t) = C_0 . \exp(Q.t)$ C being the concentration in a given compartment

Stochastic formulation

$dP(t)/dt = Q.P(t)$

$P(t) = P_0 . \exp(Q.t)$ P being the transition probabilities

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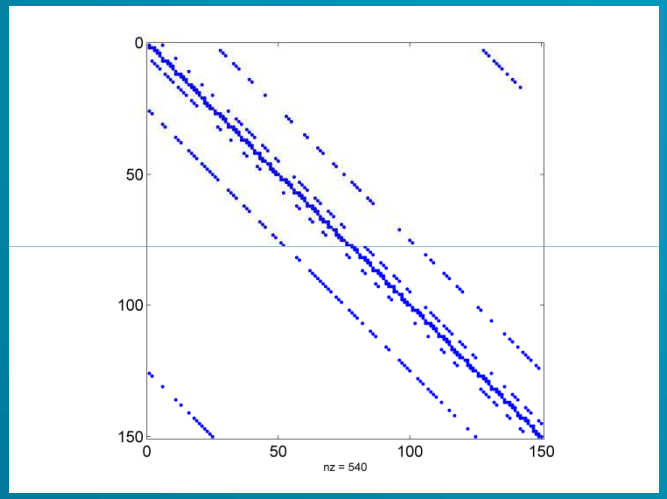
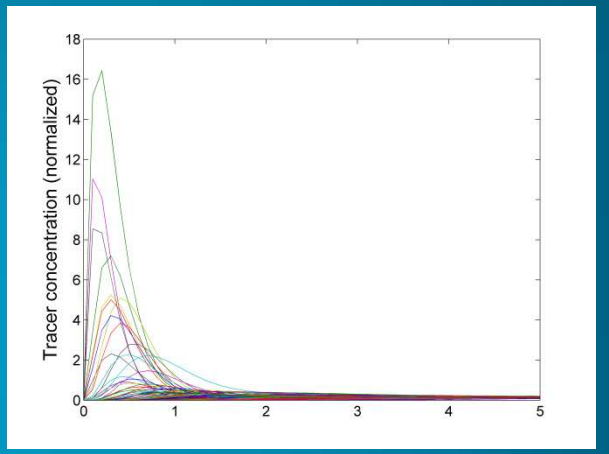
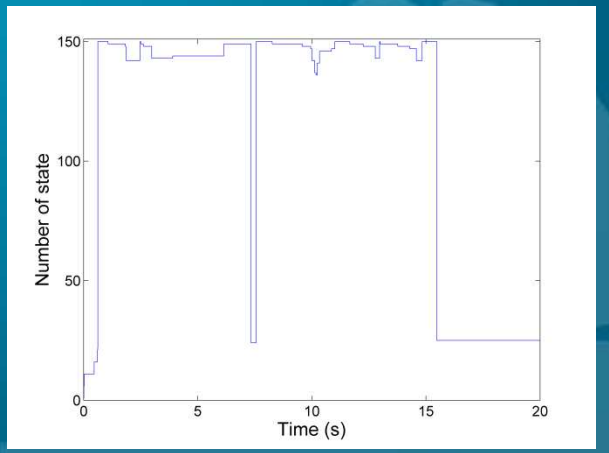
$dC(t)/dt = Q.C(t)$

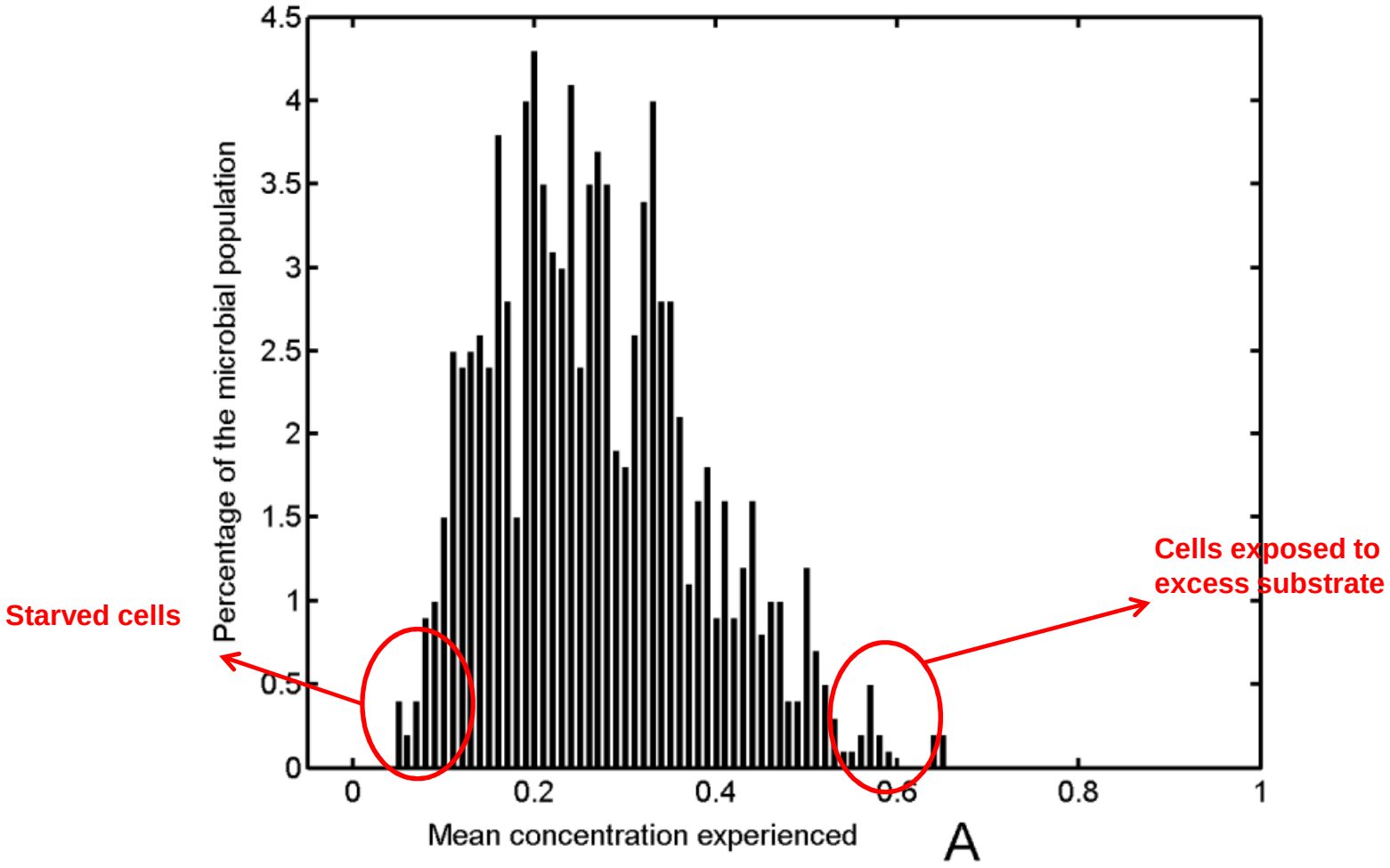
Same matrix structure

$dP(t)/dt = Q.P(t)$









Delvigne *et al.* [2006] Chemical engineering journal

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Process related stress:

- Heat shock
- Hypoxia
- pH shock
- Carbon limitation or starvation
- Carbon excess
- Nitrogen limitation
- Osmotic shock (*fed-batch*)
- High cell density (*fed-batch*)
- Turbohypobiosis (shear stress)

Physiological impact :

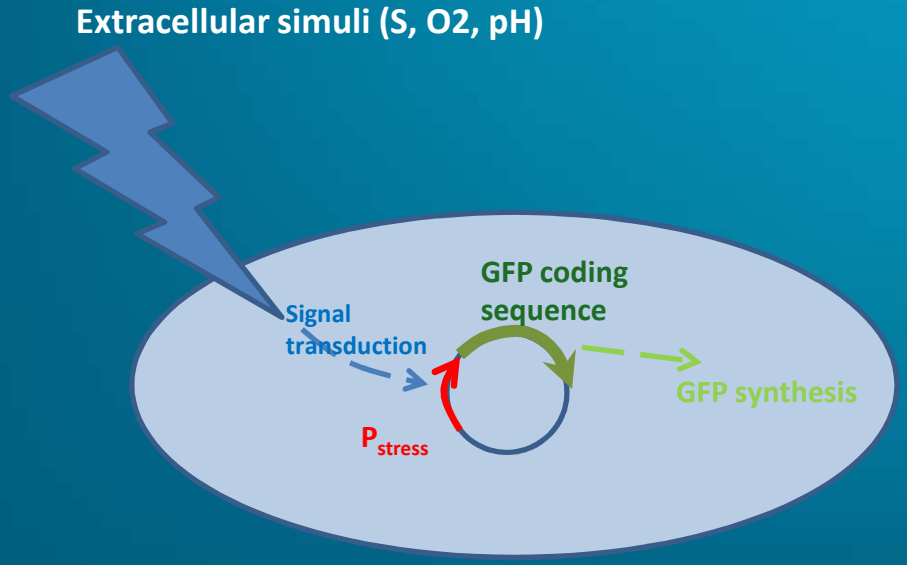
- **short-term** : metabolic shift
- **long-term** : gene induction/repression (genomic remodeling)

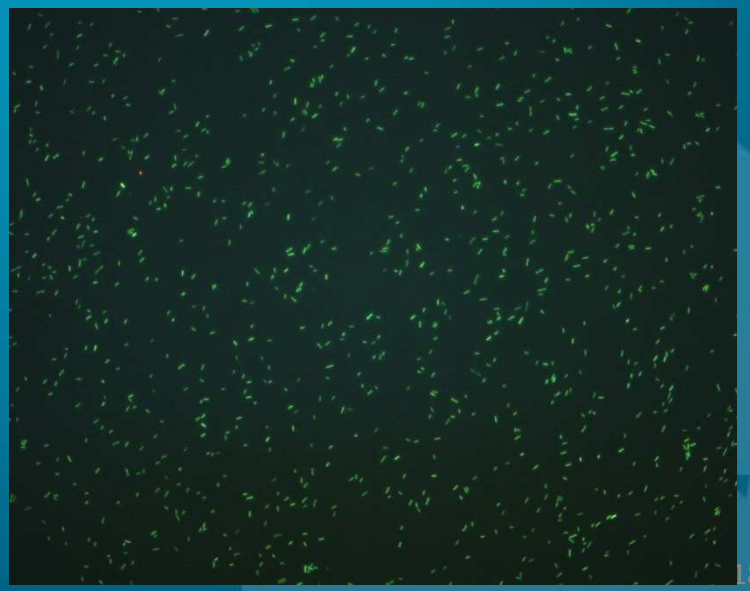
Case study : fed-batch culture of *E. coli*

The addition of glucose during the culture induces several process-related stresses

2. Biological response to extracellular fluctuations

Basic principle :
Using the microbial population as « physiological tracer » for the estimation of the bioreactor mixing and transfer efficiency (potentially capturing the stochasticity linked with the CTD)
This principle leads to the following of parameters representative of the phusiological complexity of the microorganisms (**direct parameter**)





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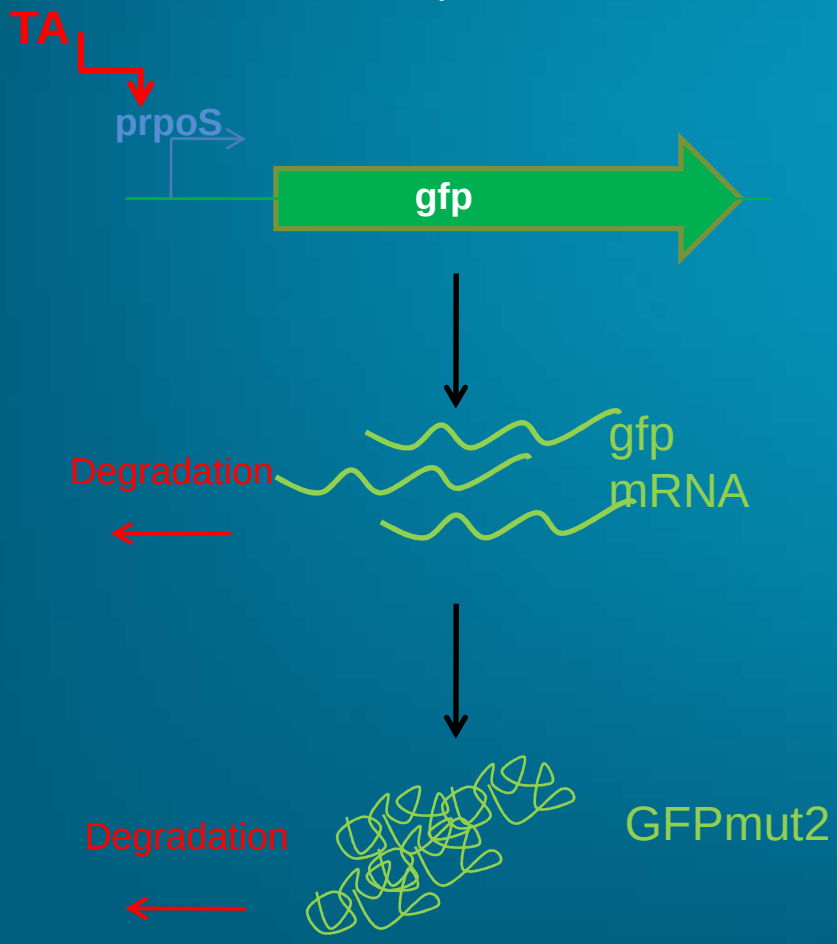
Complexity of the biological response

- Two sources of noise (extrinsic and intrinsic)
- Very different characteristic time constants (physical and biological pocesses)

→ A model is required



$$\xrightarrow{k_1} TA$$

$$TA + DNA \xrightarrow{k_2} TA\_DNA$$

$$TA\_DNA \xrightarrow{k_3} TA + DNA$$

$$TA \xrightarrow{k_4} \emptyset$$

$$TA\_DNA \xrightarrow{k_5} TA\_DNA + RNA$$

$$RNA \xrightarrow{k_6} RNA + GFP$$

$$RNA \xrightarrow{k_7} \emptyset$$

$$GFP \xrightarrow{k_8} \emptyset$$

Reaction scheme :

Exposure to glucose excess = $f(t_m, t_c)$

$$\xrightarrow{k_1} TA$$
$$TA + DNA \xrightarrow{k_2} TA\_DNA$$
$$TA\_DNA \xrightarrow{k_3} TA + DNA$$
$$TA \xrightarrow{k_4} \emptyset$$
$$TA\_DNA \xrightarrow{k_5} TA\_DNA + RNA$$
$$RNA \xrightarrow{k_6} RNA + GFP$$
$$RNA \xrightarrow{k_7} \emptyset$$
$$GFP \xrightarrow{k_8} \emptyset$$

Generation time : $k8 = \log(2)/t_g$

ODEs system :

$$\frac{dTA}{dt} = k_1 - k_2.TA.DNA - k_4.TA + k_3.TA\_DNA$$
$$\frac{dTA\_DNA}{dt} = k_2.TA.DNA - k_5.TA\_DNA - k_3.TA\_DNA$$
$$\frac{dDNA}{dt} = k_3.TA\_DNA - k_2.TA.DNA$$
$$\frac{dRNA}{dt} = k_5.TA\_DNA - k_6.RNA - k_7.RNA$$
$$\frac{dGFP}{dt} = k_6.RNA - k_8.GFP$$

$$GFP_{steady-state} = RNA_{steady-state}.\left(\frac{k_6}{k_8}\right)$$

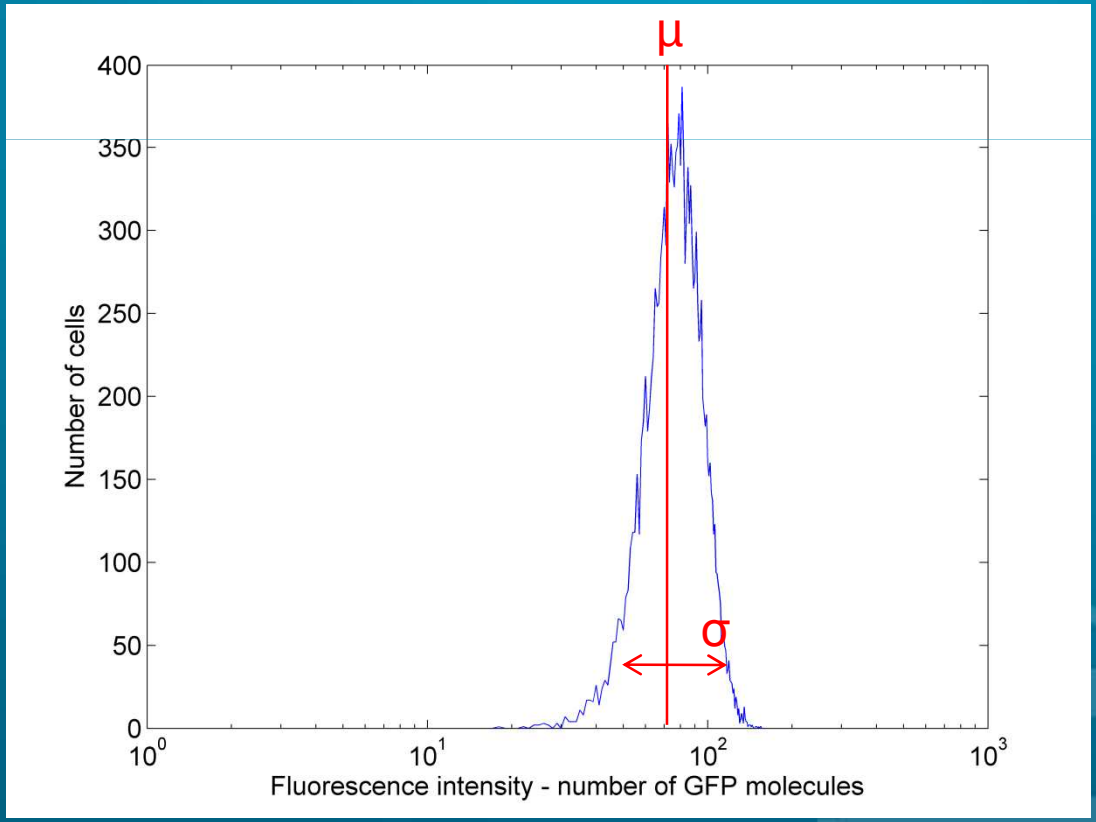
8 rates (including the characteristic time constants) to specify

These equations can be used in the classical deterministic formalism (ODEs solver), but more interestingly in the stochastic formalism :

Probablity that reaction μ occurs at time τ (Gillespie algorithm)

Gillespie [1977] J. of physical chemistry, **81**:2340-2361

Example : simulation of 30,000 cells after 6 hours of induction



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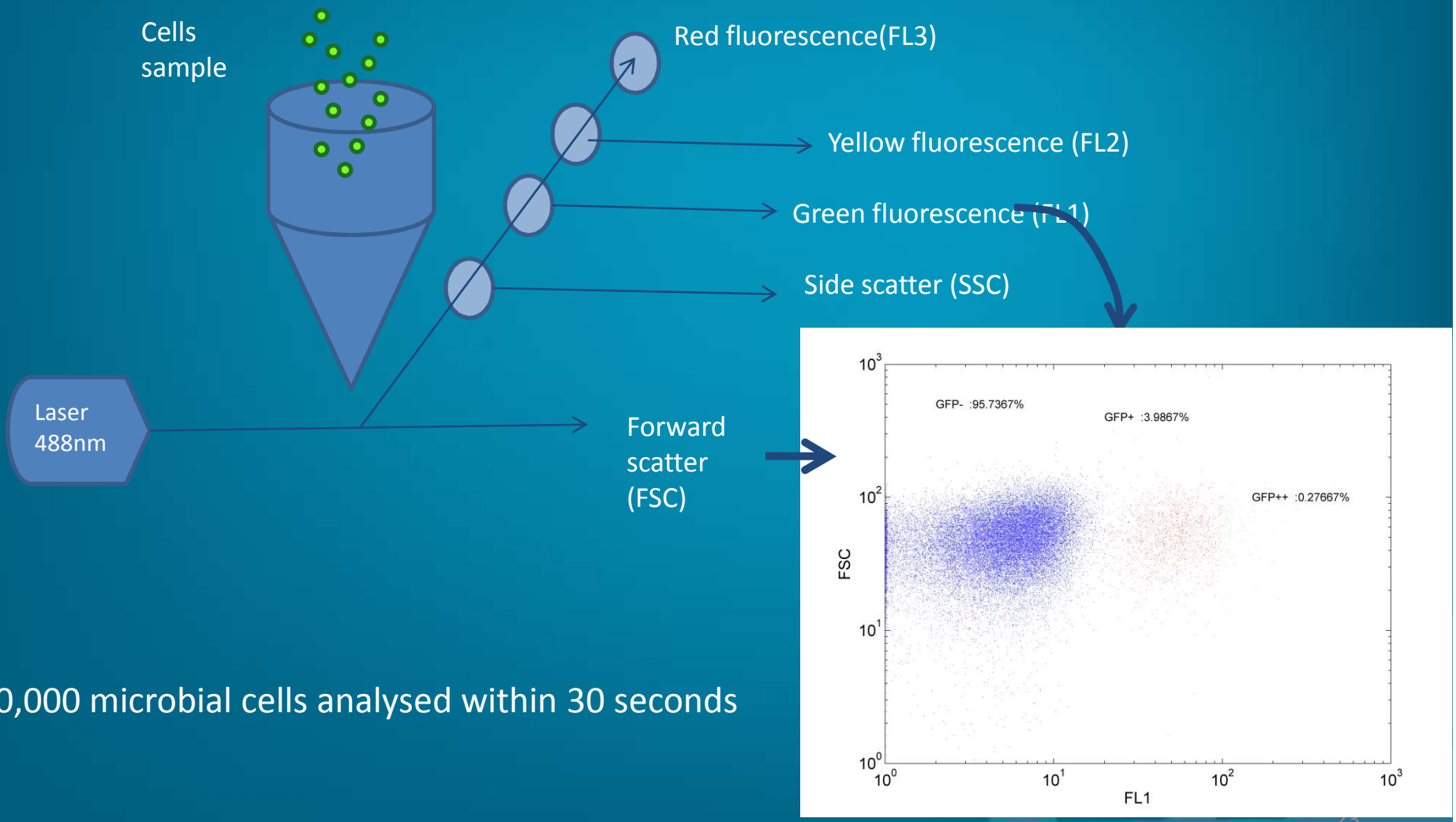
Gene expression is intrinsically NOISY
Difficulty to distinguish intrinsic noise from extrinsic noise



30,000 microbial cells analysed within 30 seconds

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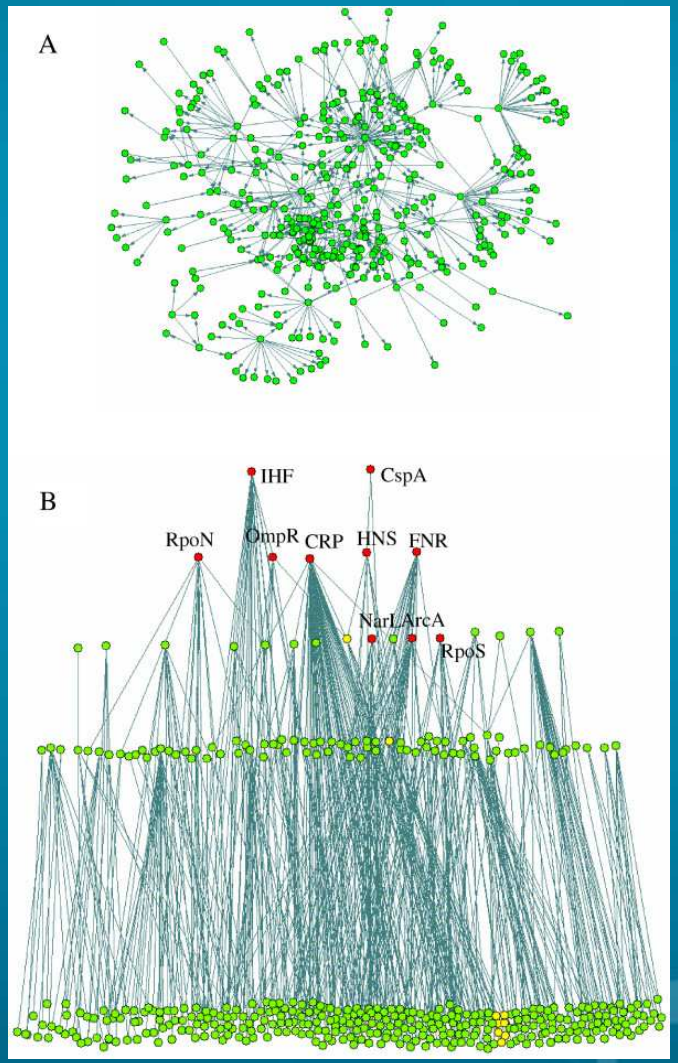
E. coli : about 4000 ORFs :

Transcriptional network

Transcriptional network – hierarchical classification



Ma *et al.* [2004] BMC Bioinformatics, **5**:199

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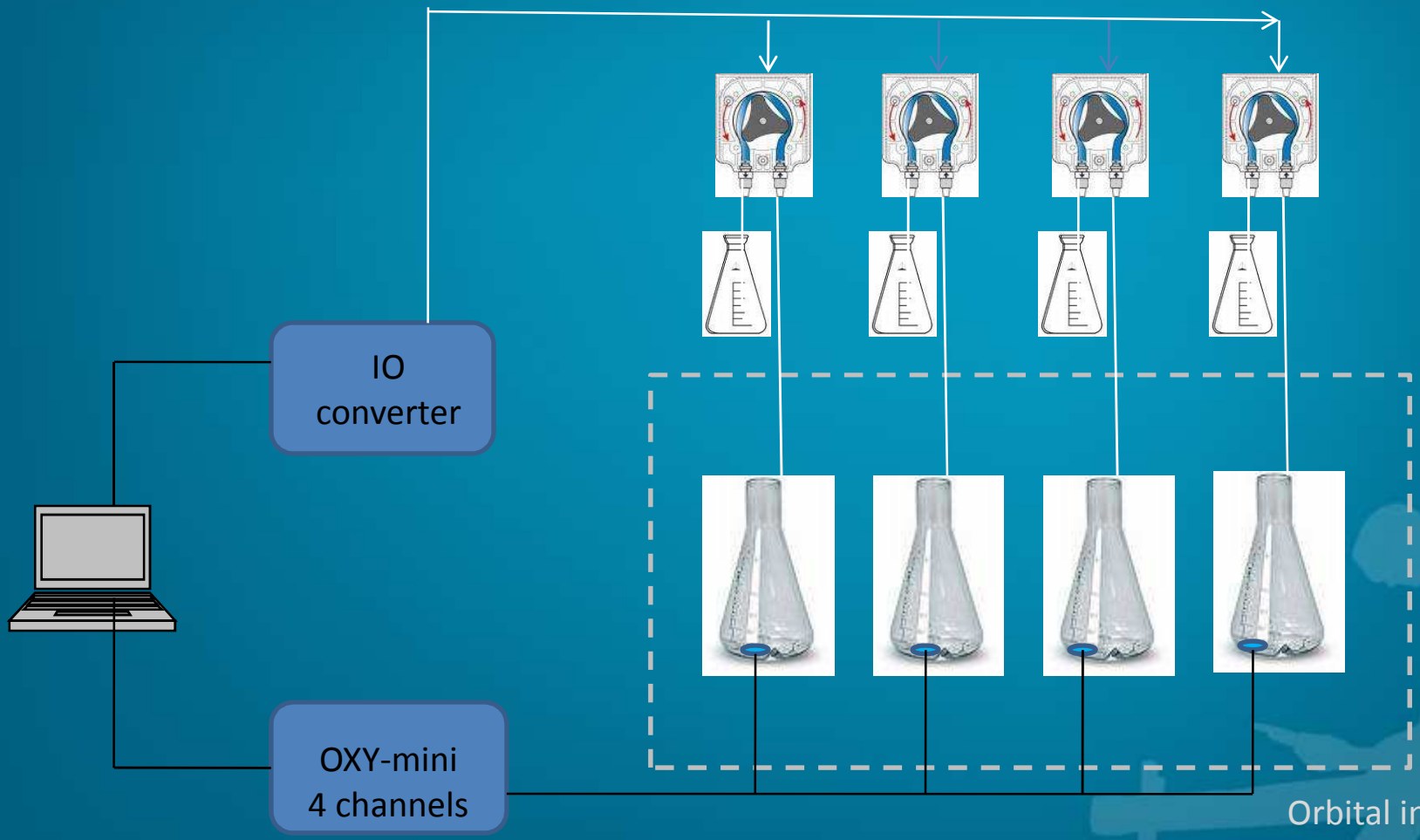
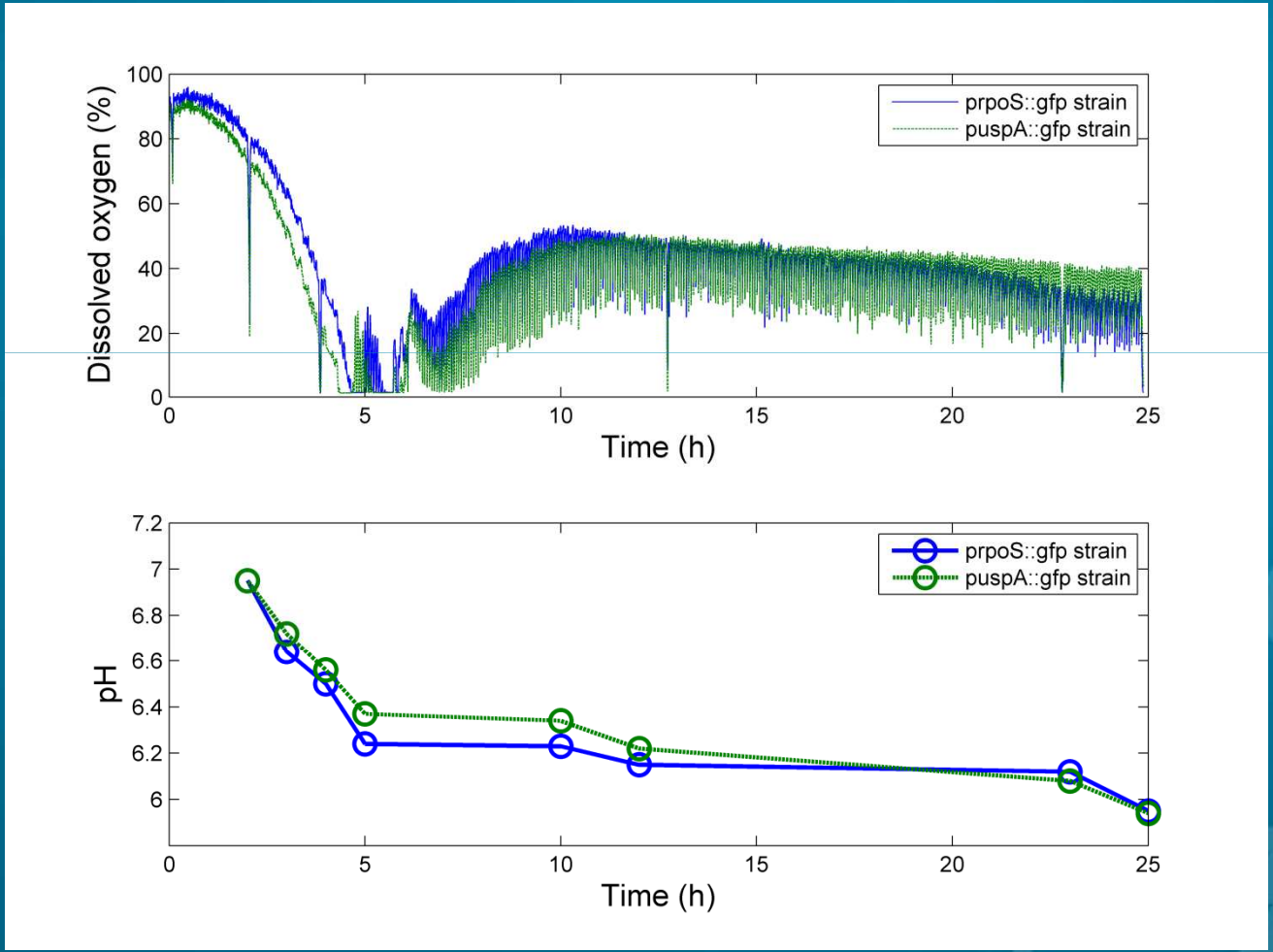
Intermittent feeding strategy

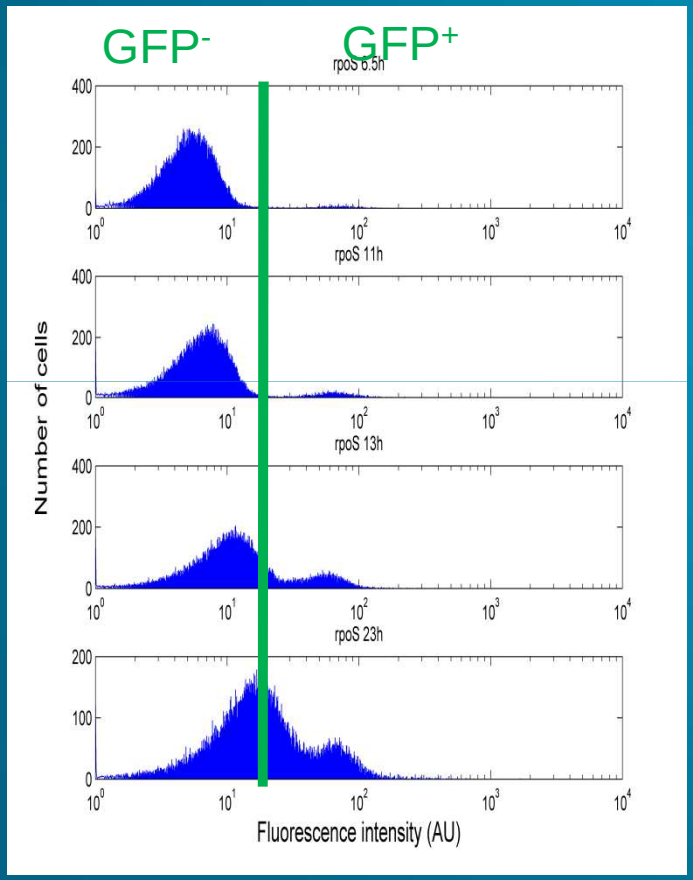


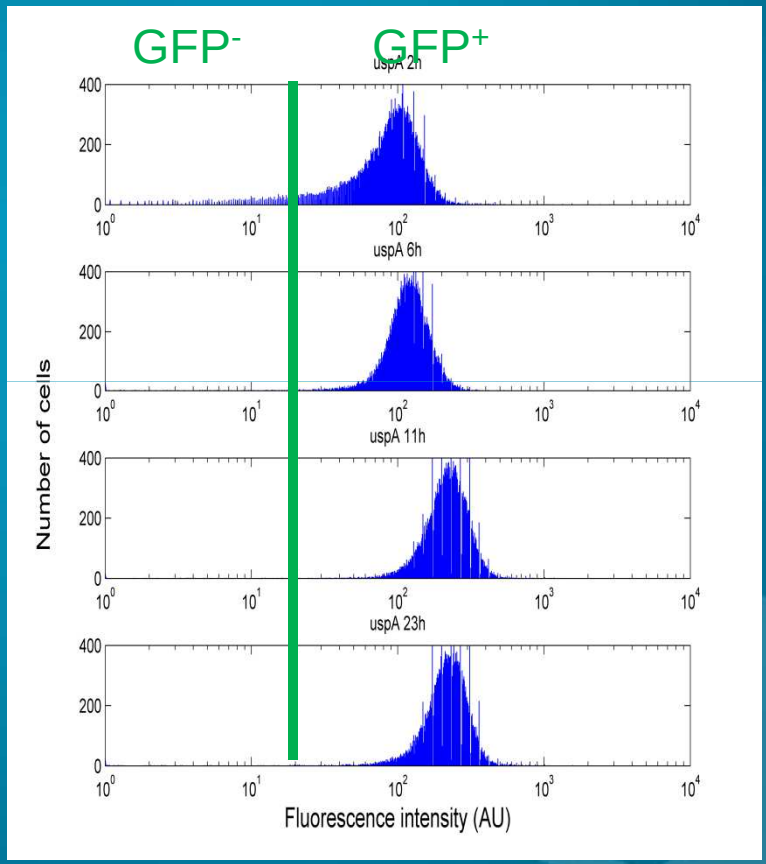
Orbital incubator
(T° and shaking frequency controls)

Cultures of GFP clones in shaken bioreactors (1L baffled shake flask : initial working volume : 200mL ; final working volume : 400 mL)



Growth inhibiting value : 4.5

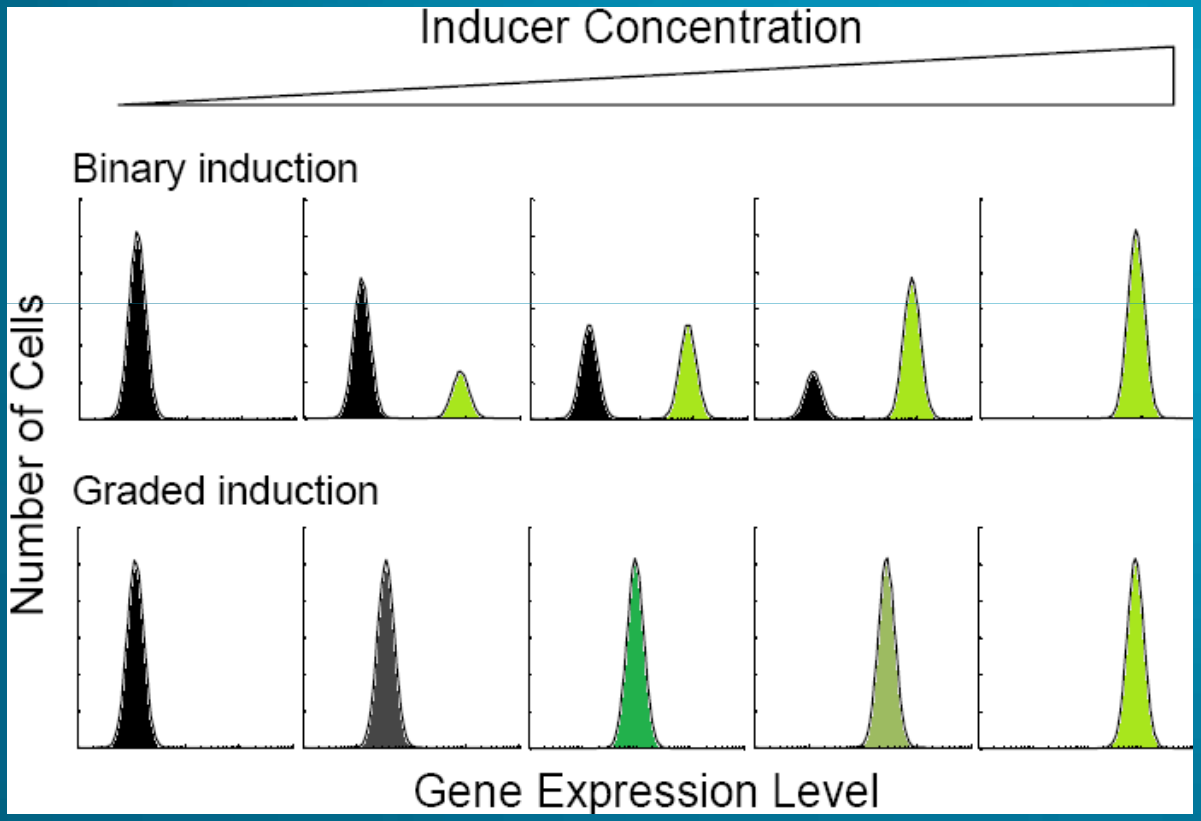




prpoS::gfp

puspA::gfp

Two modes of expression : binary or graded



→ rpoS

→ uspA
csiE
inaA
osmC
…

Zhang *et al.* (2006) Theoretical biology and medical modelling, **3**:18

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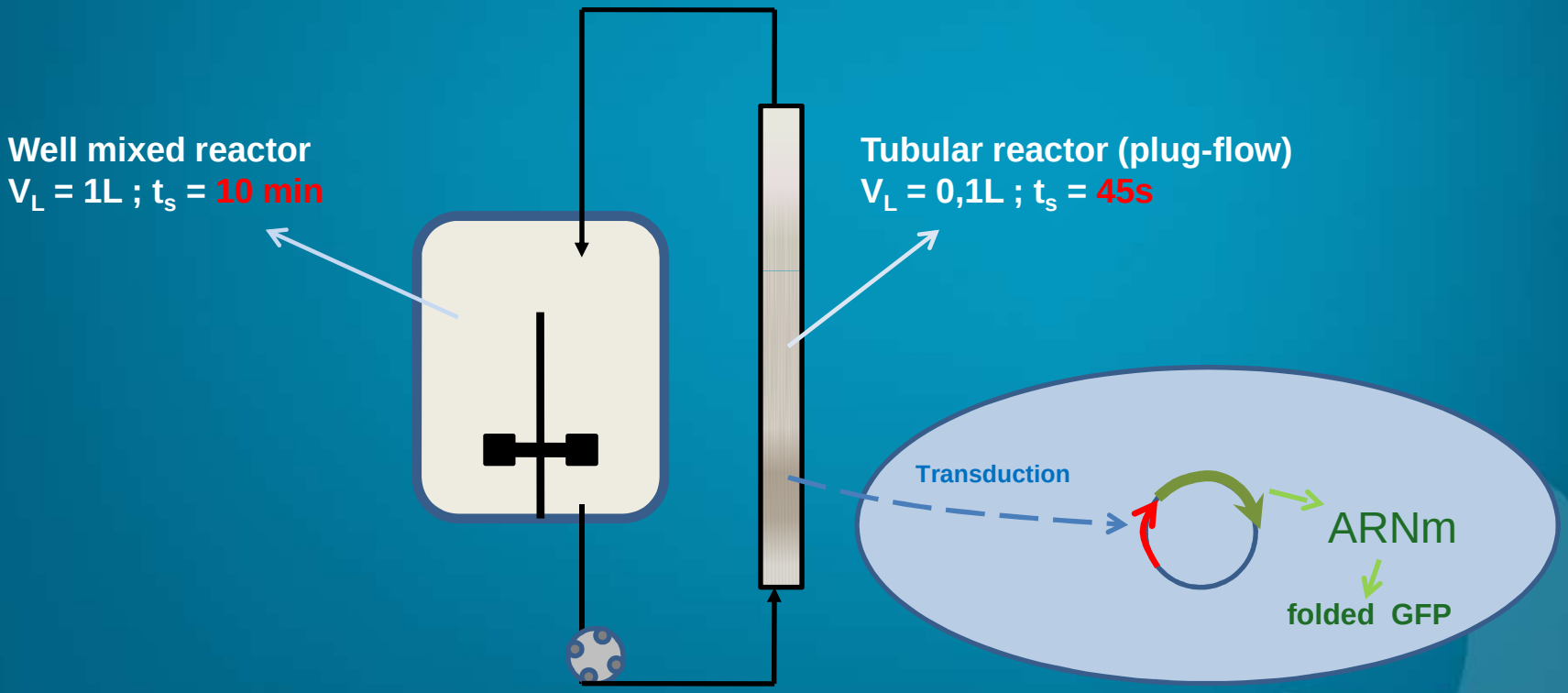
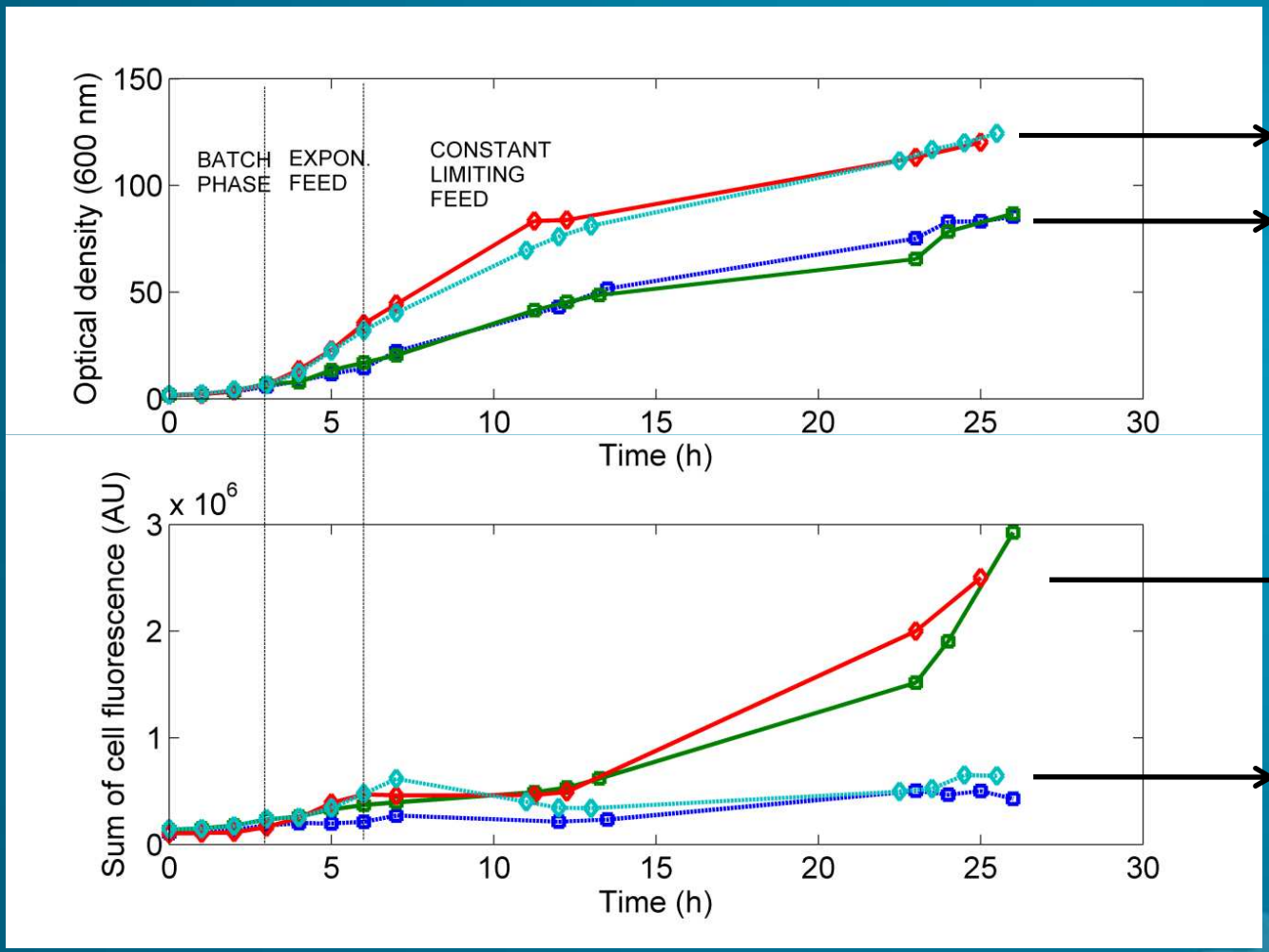
Binary mode of gene expression → sources :
-Short mRNA and protein half-lives
-High sensitivity for the detection of the reporter protein

Generally not observed for GFP reporter system considering the high protein stability of this system compared with β-galactosidase and luciferase reporters

This mechanism of gene induction give rise to differentially expressed phenotypes at the protein level. Can potentially be used to gain more sensitivity about the impact of extracellular fluctuations

Scale-down strategy : reproducing the local excess in glucose (mean exposure time in this case : 45 s)





Limitation at 10 g/h

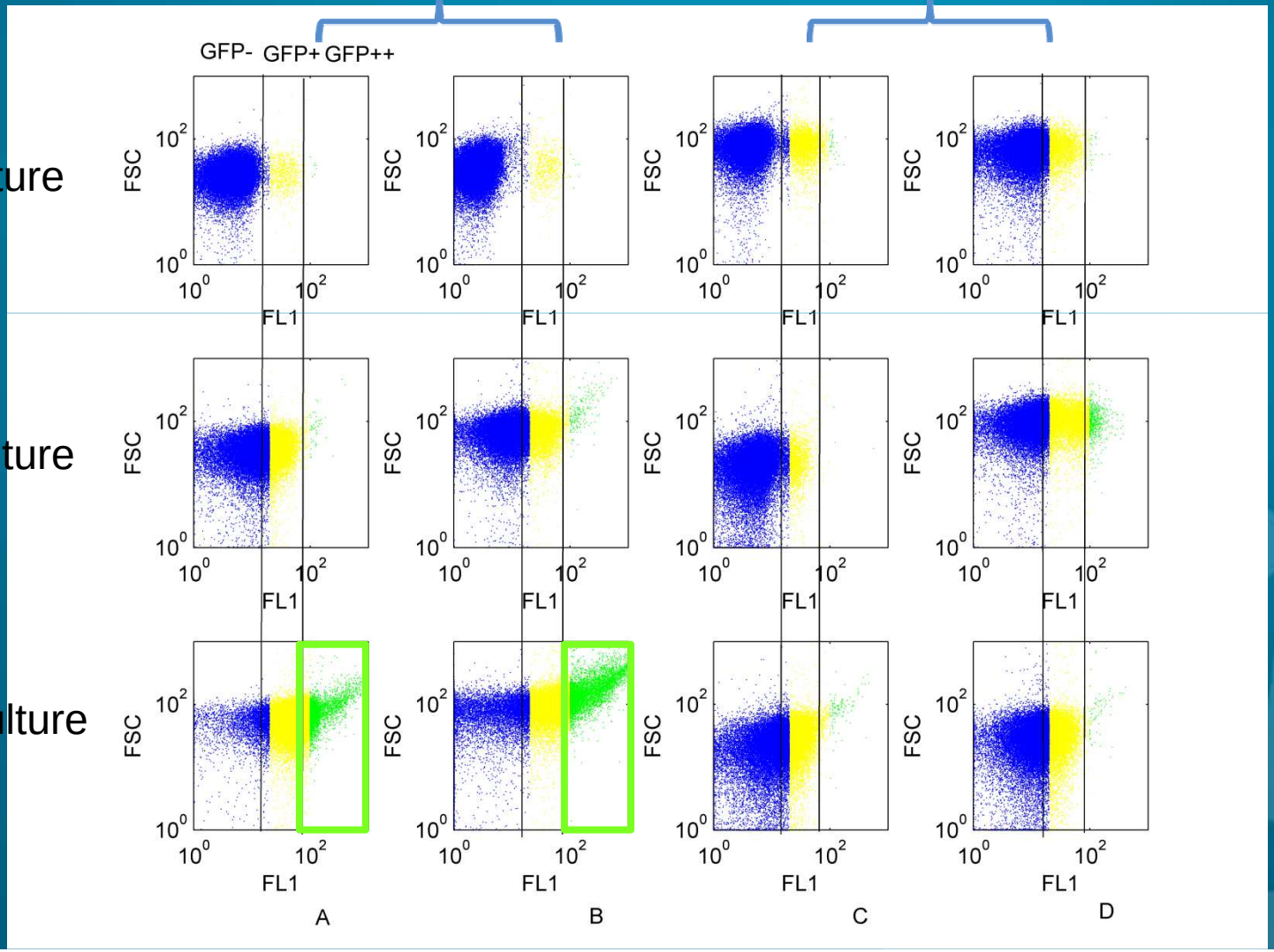
Limitation at 7 g/h

Reactor without recycle loop

Scale-down reactor

Reactor without recycle loop

Scale-down reactor



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Conclusion and perspectives

- Physical side of the problem resolved (improvements can be added)

- Biological side more complex. A methodology has been elaborated but several lacks have to be reported

These issues can be resolved by fearless PhD students (like you)

For more informations, please consult our last publications :

Delvigne F., Boxus M., Ingels S., Thonart P. [2009], *Microbial cell factories*, 8, 15

Delvigne F., Ingels S., Thonart P. [2010], *Process biochemistry*, in press

AND OF COURSE…

…thank you for your attention