Microbial heterogeneity affects bioprocess robustness

Future of dynamic single-cell analysis for large-scale bioprocess control

Frank Delvigne
Université de Liège, Gembloux Agro Bio Tech
Passage des Déportés, 2
5030 Gembloux BELGIUM
F.Delvigne@ulg.ac.be

Outline

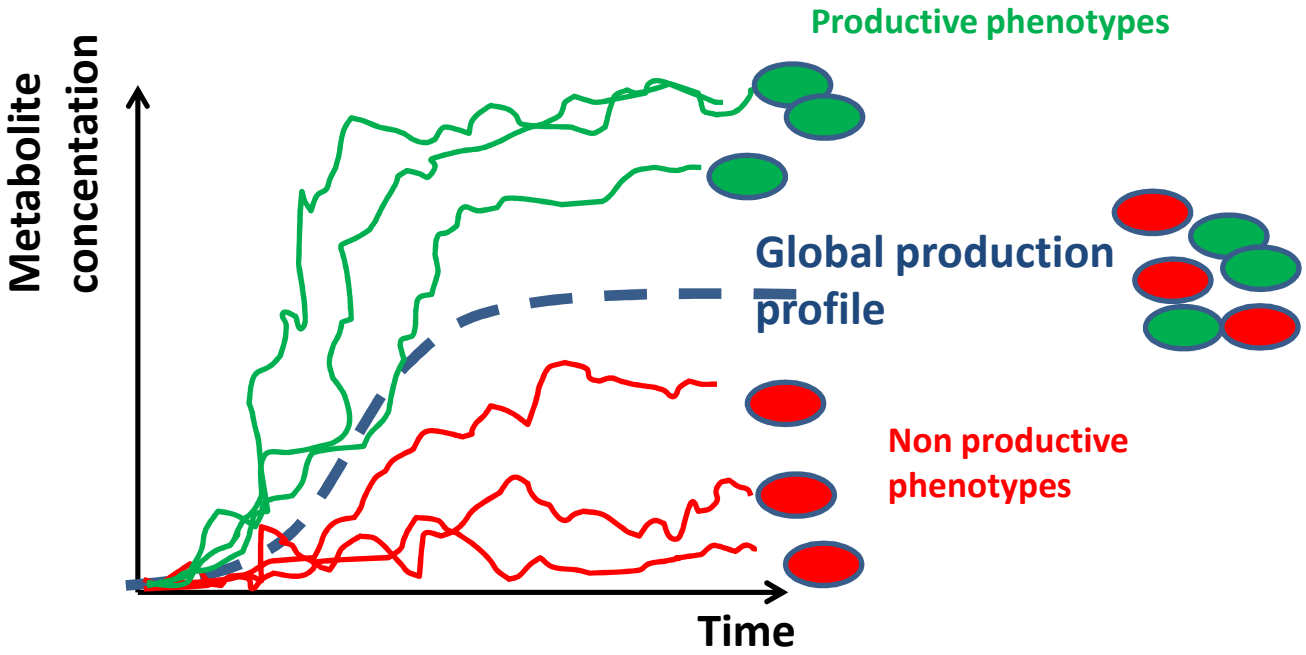
1. Introduction : microbial phenotypic heterogeneity, two fields, two views
2. Single cell analysis in process conditions : *rpoS* response
3. Single cell analysis in process conditions : membrane permeability and protein leakage
4. What's next?

Introduction

Microbial phenotypic heterogeneity, two fields, two views

Impact of microbial phenotypic heterogeneity on process productivity

Accepted picture : only a fraction of the population (non productive phenotypes) affects the global production profile



Single cell studies are of great importance both from a fundamental and from an applied perspective:

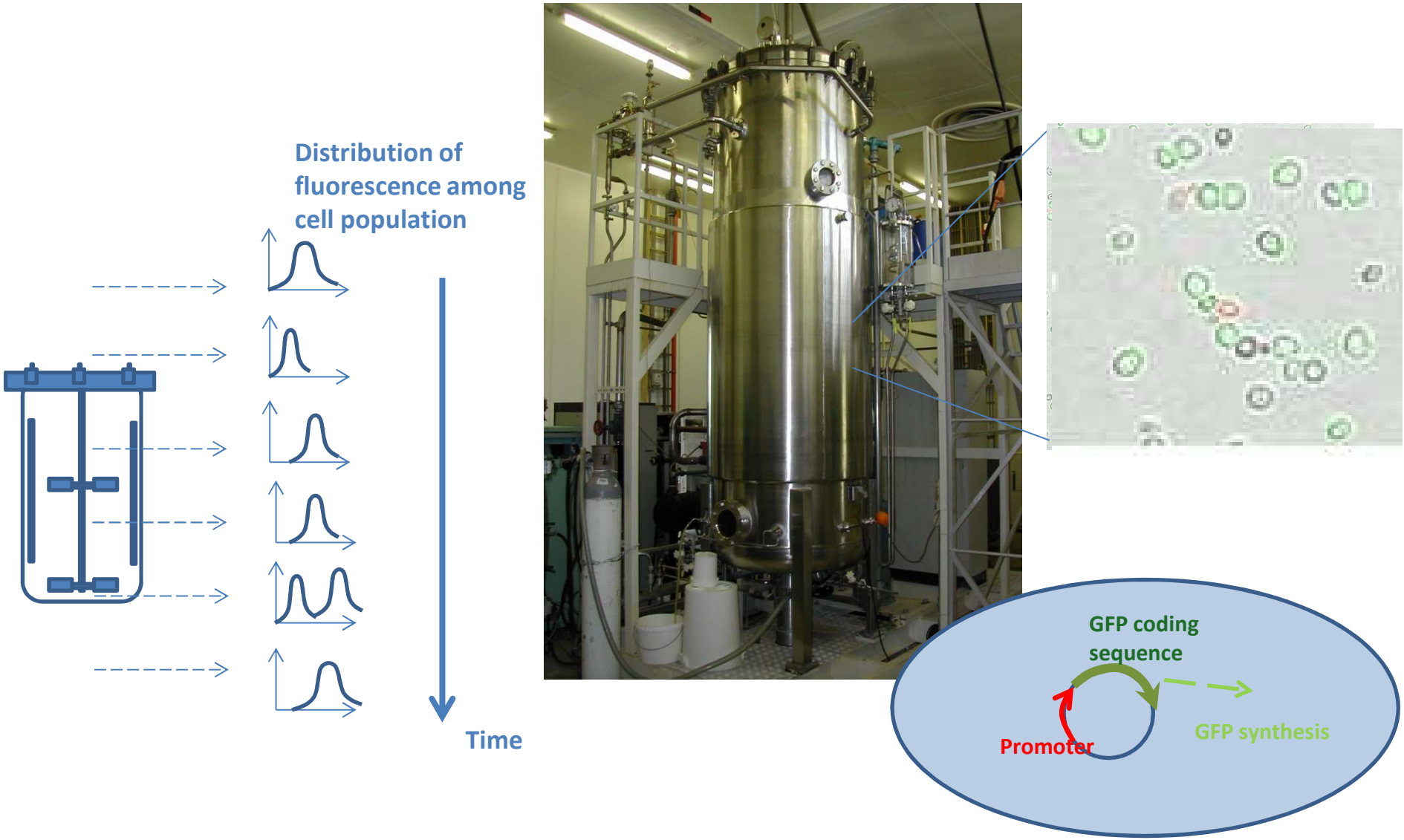
Two fields with two distinct perceptions of microbial phenotypic heterogeneity :

1. **Chemical engineering** : focused on the effect of external perturbations on biological noise

2. **System biology** : focused on the intrinsic and extrinsic source of noise

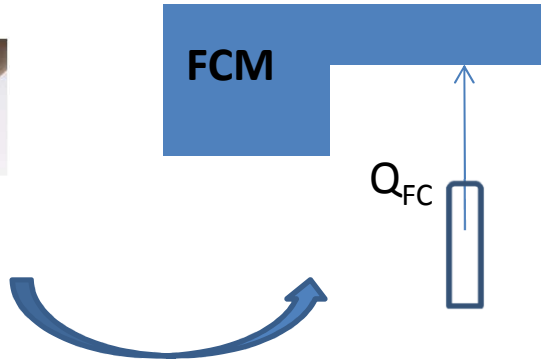
Each discipline integrates only a fraction of the knowledge and have evolved by considering distincts (cultivation and analytical) tools for the determination of microbial phenotypic heterogeneity

(Bio)chemical engineering approach :

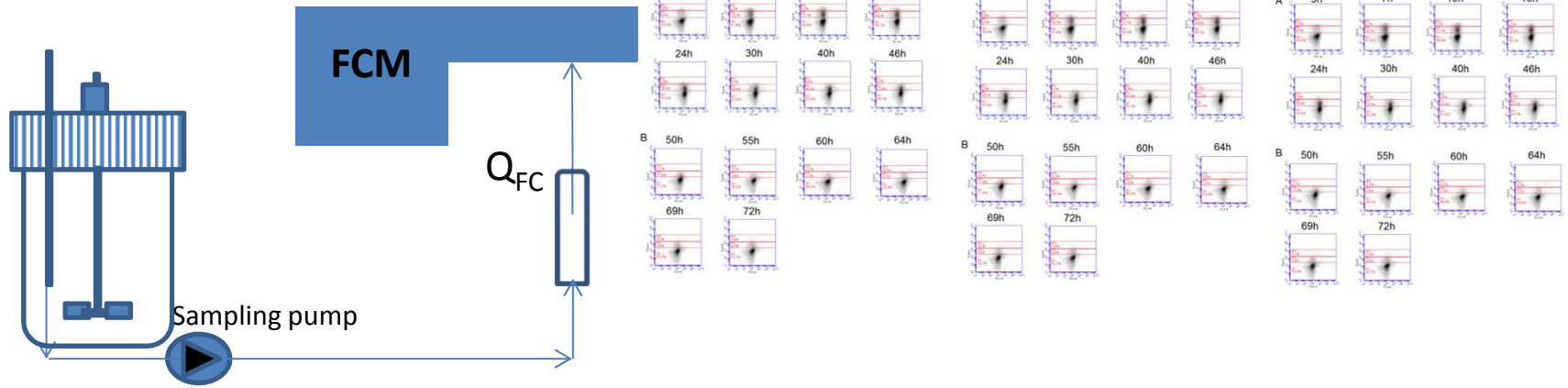


Flow cytometry (FCM) : different modes of operation

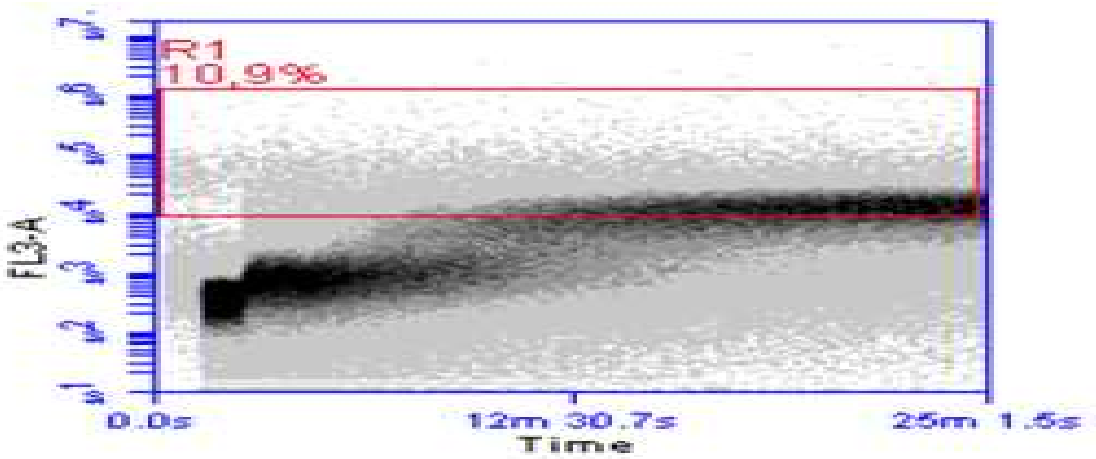
Manual sampling



On-line

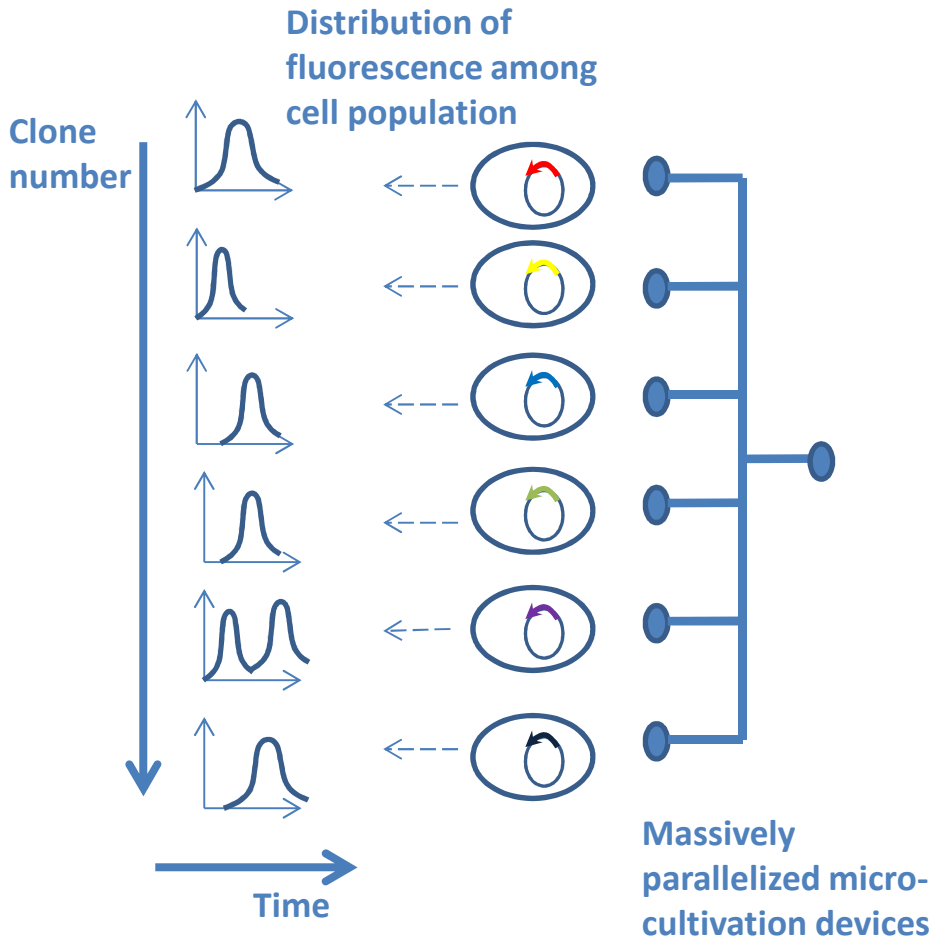


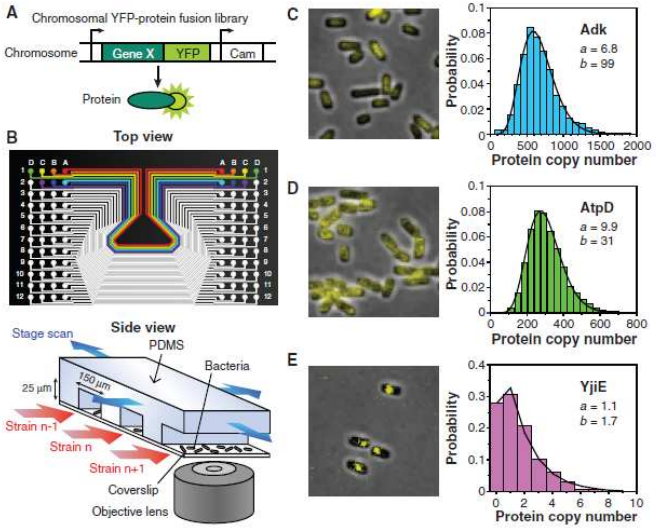
Real time



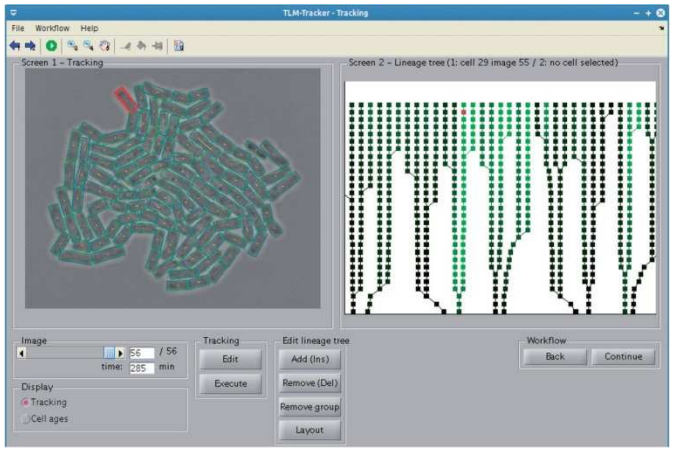
Alison Brognaux (L20)

System biology approach :





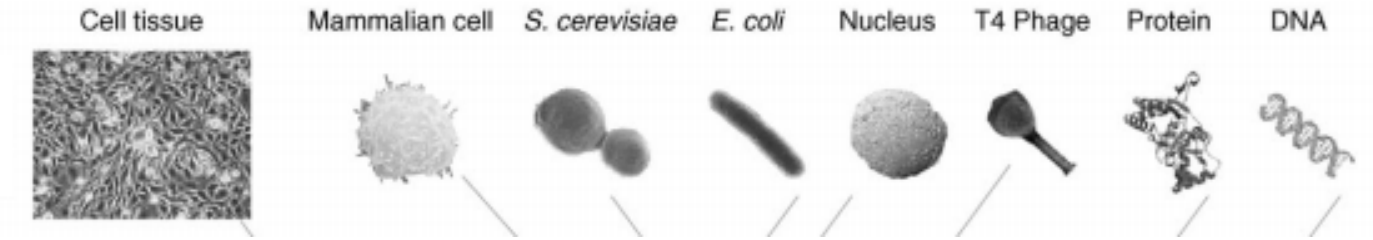
Taniguchi Y, Choi PJ, Li GW, Chen H, Babu M, Hearn J, Emili A and Xie XS, Quantifying E. coli proteome and transcriptome with single-molecule sensitivity in single cells. *Science* **329**: 533-538 (2010).



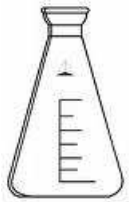
Klein J, Leupold S, Biegler I, Biedendieck R, Munch R and Jahn D, TLM-Tracker: software for cell segmentation, tracking and lineage analysis in time-lapse microscopy movies. *Bioinformatics* **28**: 2276-2277 (2012).

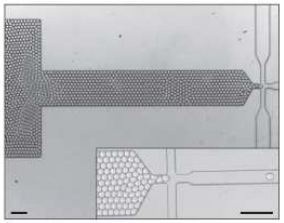
Inter-scale comparison : biology vs chemical engineering

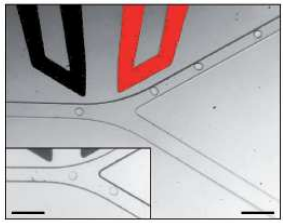
Biological systems…

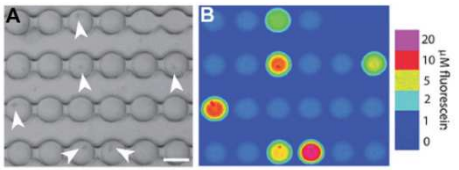


1m 1 cm 1mm 100µm 10µm 1µm 100nm 10nm 1nm





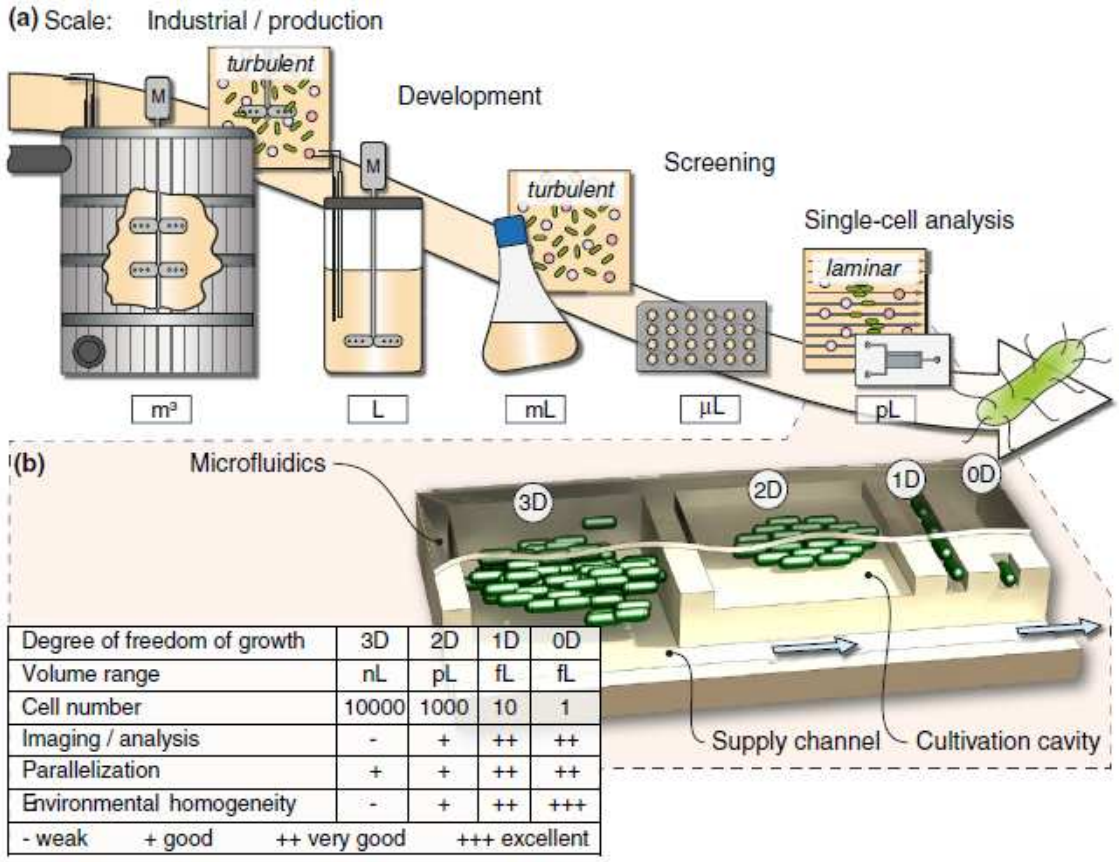




… cultivation devices

1m 1 cm 1mm 100µm 10µm 1µm 100nm 10nm 1nm

Integrating mcirofluidics (and single cell microfluidics) in the scale-up/down loop ?



Degree of freedom of growth	3D	2D	1D	0D
Volume range	nL	pL	fL	fL
Cell number	10000	1000	10	1
Imaging / analysis	-	+	++	++
Parallelization	+	+	++	++
Environmental homogeneity	-	+	++	+++
- weak + good ++ very good +++ excellent				

Grunberger A, Wiechert, W., Kohlheyer, D.,, Single-cell microfluidics: opportunity for bioprocess development. *Current opinion in biotechnology* **29**: 15-23 (2014).

BUT, flow regime is not the only difference between microfluidic-based micro-bioreactors and large-scale bioreactors

Some examples reported from the litterature :

- Cell density effect (Quorum sensing)

  Boedicker J, Vincent, ME, Ismagilov, RF., Microfluidic confinement of single cells of bacteria in small volumes initiates high-density behavior of quorum sensing and growth and reveals its variability. *Angewandte chemie* 48: 5908-5911 (2009)

- Competition for metabolically efficient phenotypes

  Bachmann H, Fischlechner, M, Rabbers, I, Barfa, N, Branco dos Santos, F, Molenaar, D, Teusink, B.,, Availability of public goods shapes the evolution of competing metabolic strategies. *Proc Natl Acad Sci U S A* 110: 14302-14307 (2013)

- Differences in growth rate

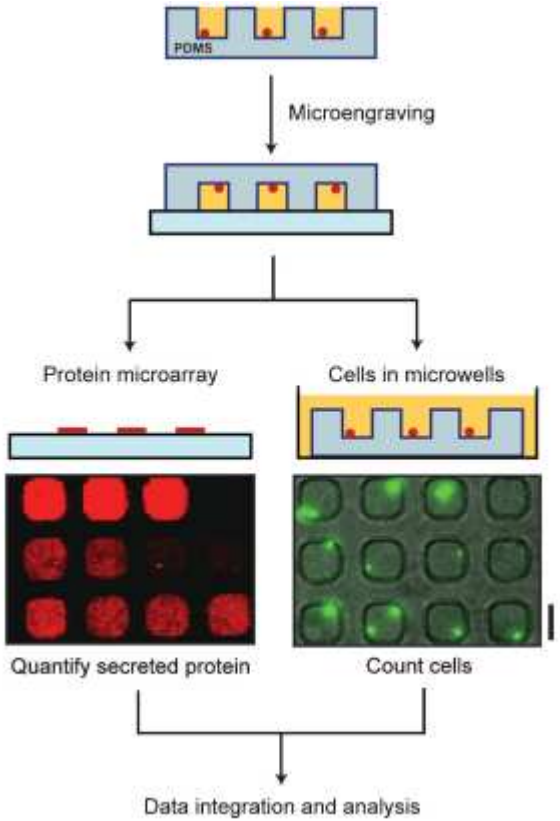
  Dusny C, Fritzsch, F.S.O., Frick, O., Schmid, A., Isolated Microbial Single Cells and Resulting Micropopulations Grow Faster in Controlled Environments. *Applied and environmental microbiology* 78: 7132-7136 (2012).

- Transition to « solid-culture » phenotypes (biofilm, floculation)

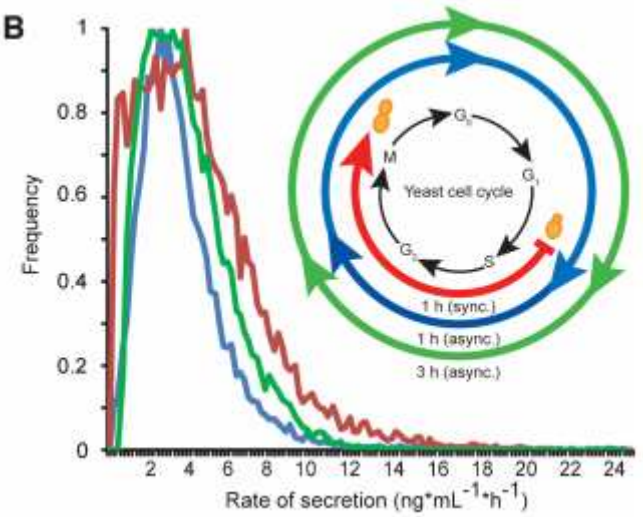
  Kortmann H, Blank, LM, Schmid, A.,, Single cell analysis reveals unexpected growth phenotype of S. cerevisiae. *Cytometry Part A* 75: 130-139 (2009).

- …

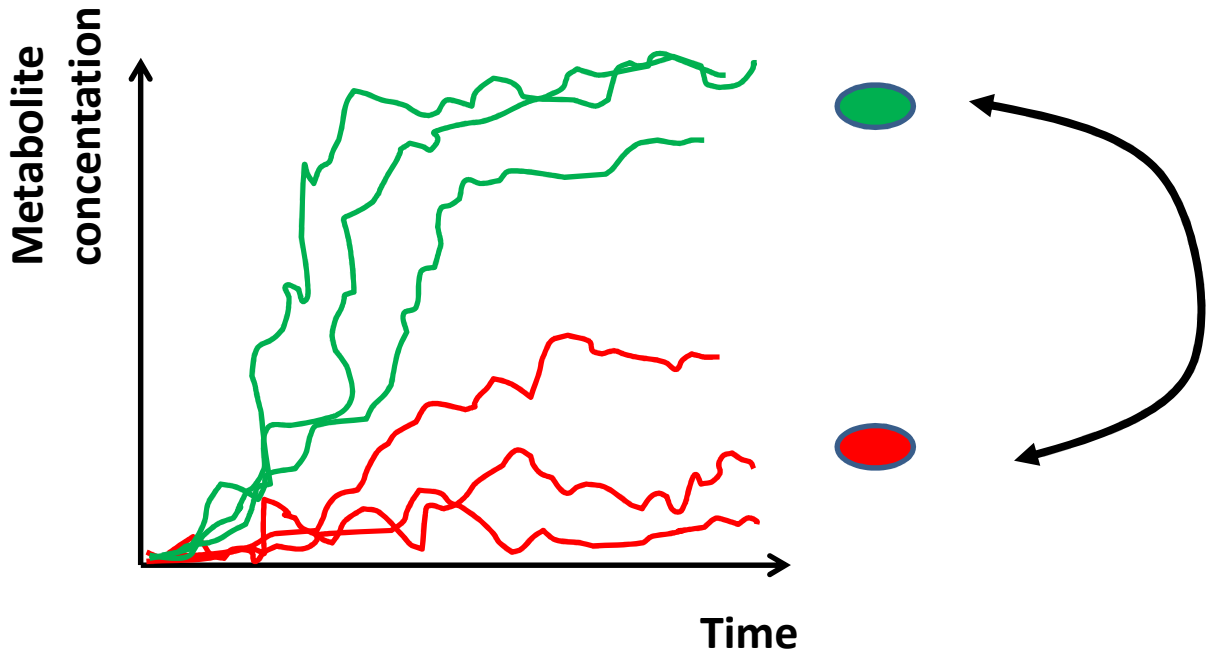
But gives usefull informations at the level of history dependent mechanisms



Recombinant protein secretion is cell-cycle dependent (swith between producer/non producer state)



Love K, Panagiotou, V, Jiang, B, Stadheim, TA, Love, JC., Integrated single-cell analysis shows Pichia pastoris secretes protein stochastically. *Biotechnology and bioengineering* **106**: 319-325 (2010)

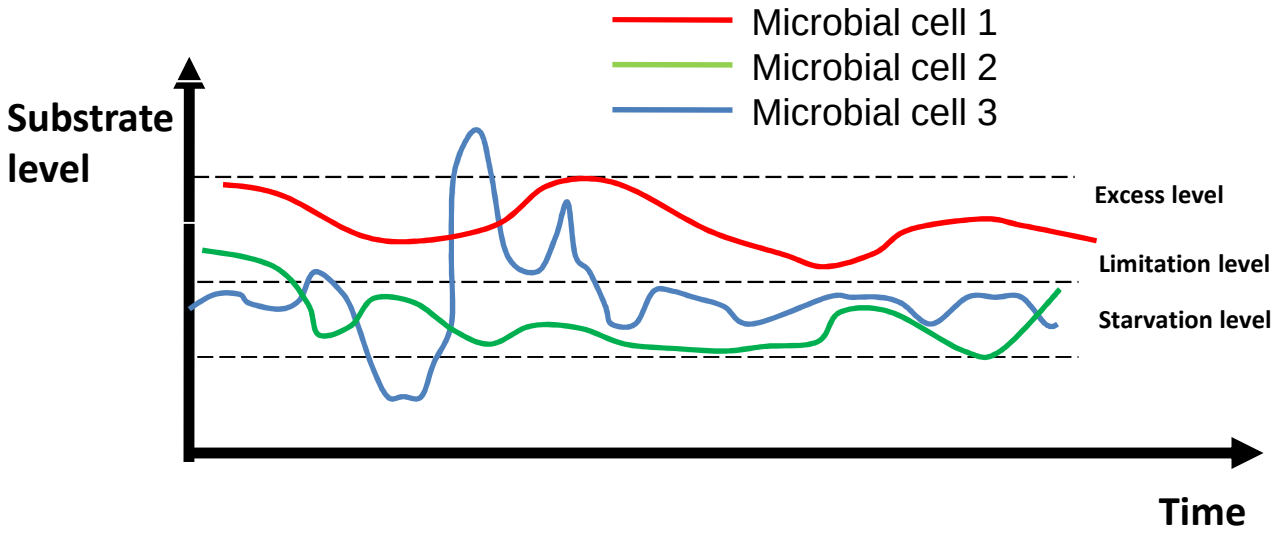


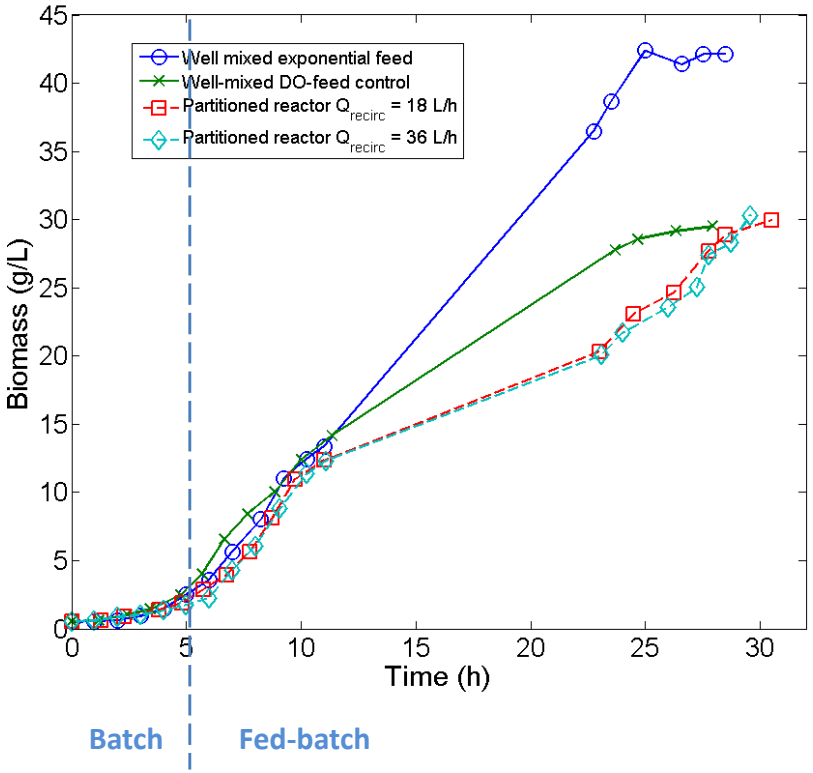
Impossible to point out this phenomenon in process conditions (history independent techniques)

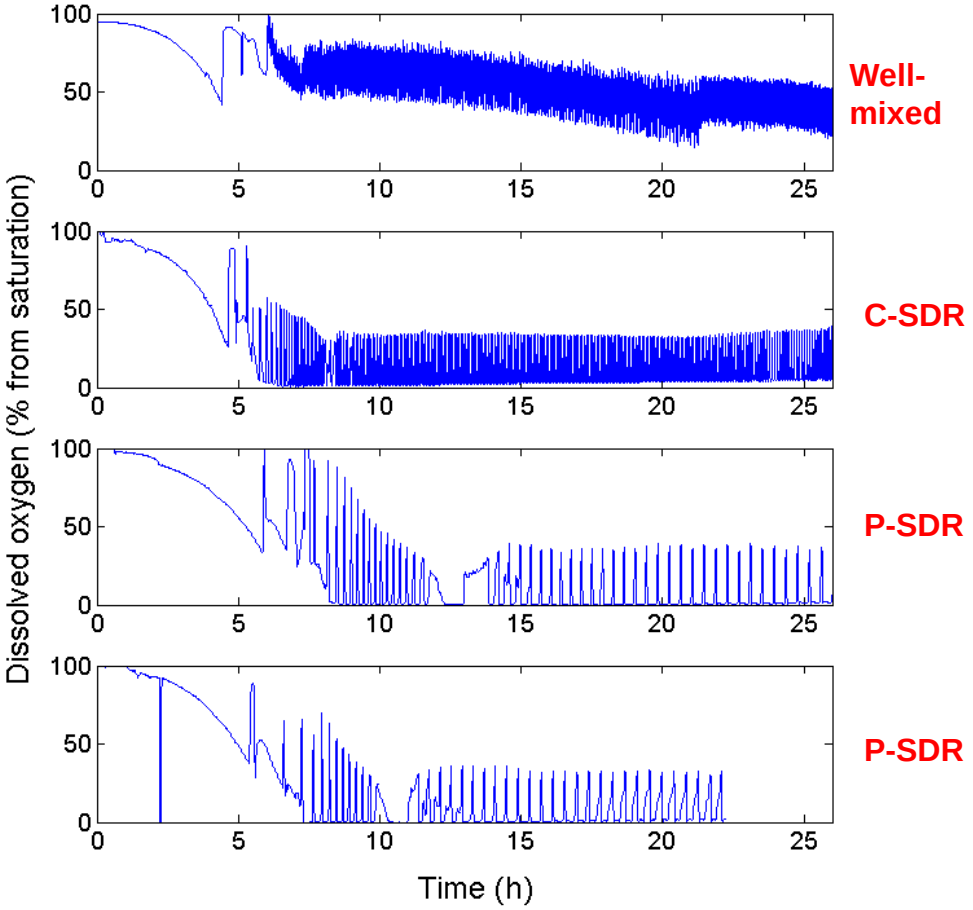
Single cell analysis in process conditions

E. coli K12 MG1655 with fluorescent reporters

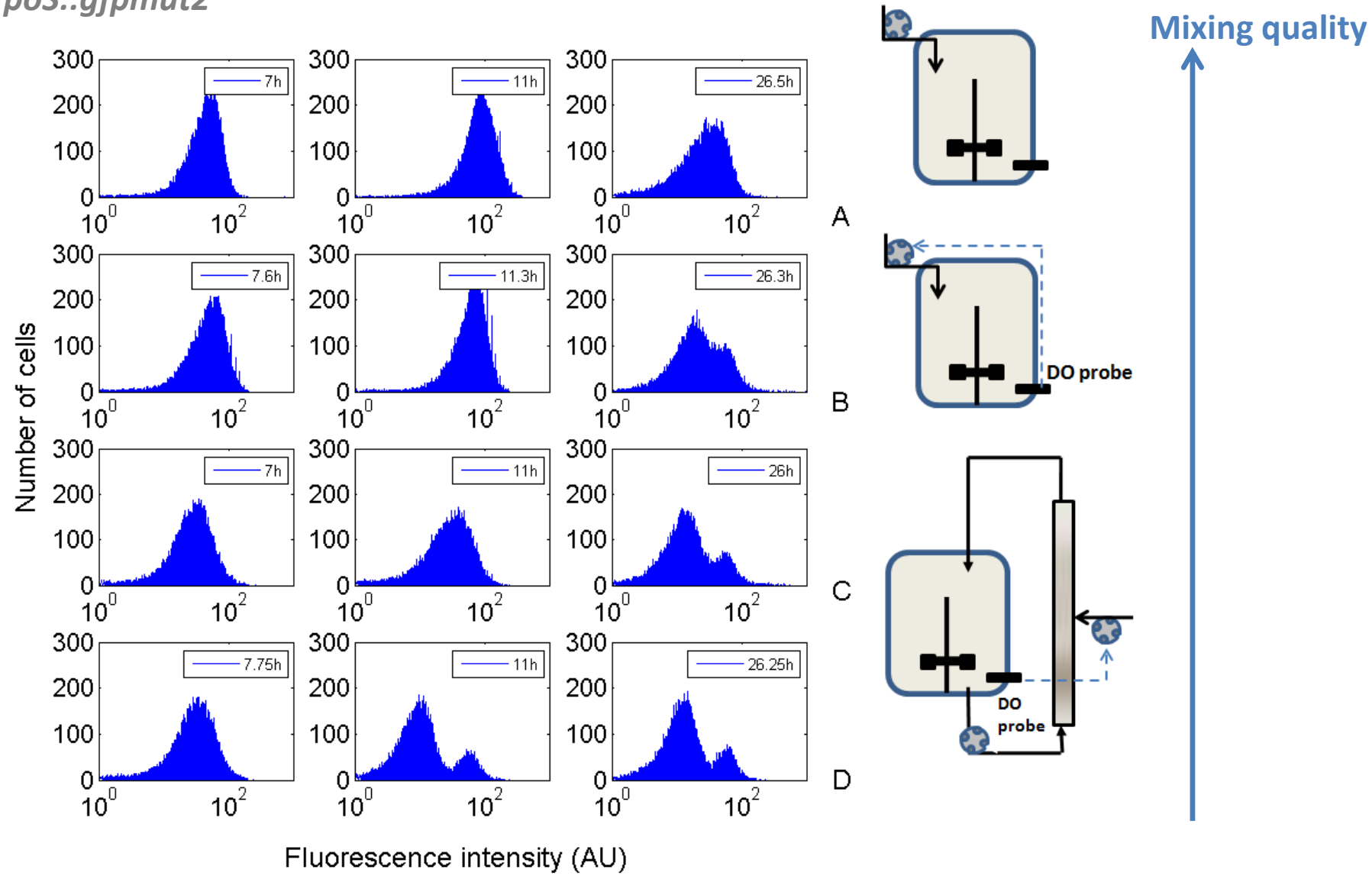
Well-mixed **vs** **Scale-down**




Well mixed exponential feed
Well-mixed DO-feed control
Partitioned reactor Q_recirc = 18 L/h
Partitioned reactor Q_recirc = 36 L/h
Biomass (g/L)
Time (h)
Batch
Fed-batch


Dissolved oxygen (% from saturation)
Time (h)
Well-mixed
C-SDR
P-SDR
P-SDR

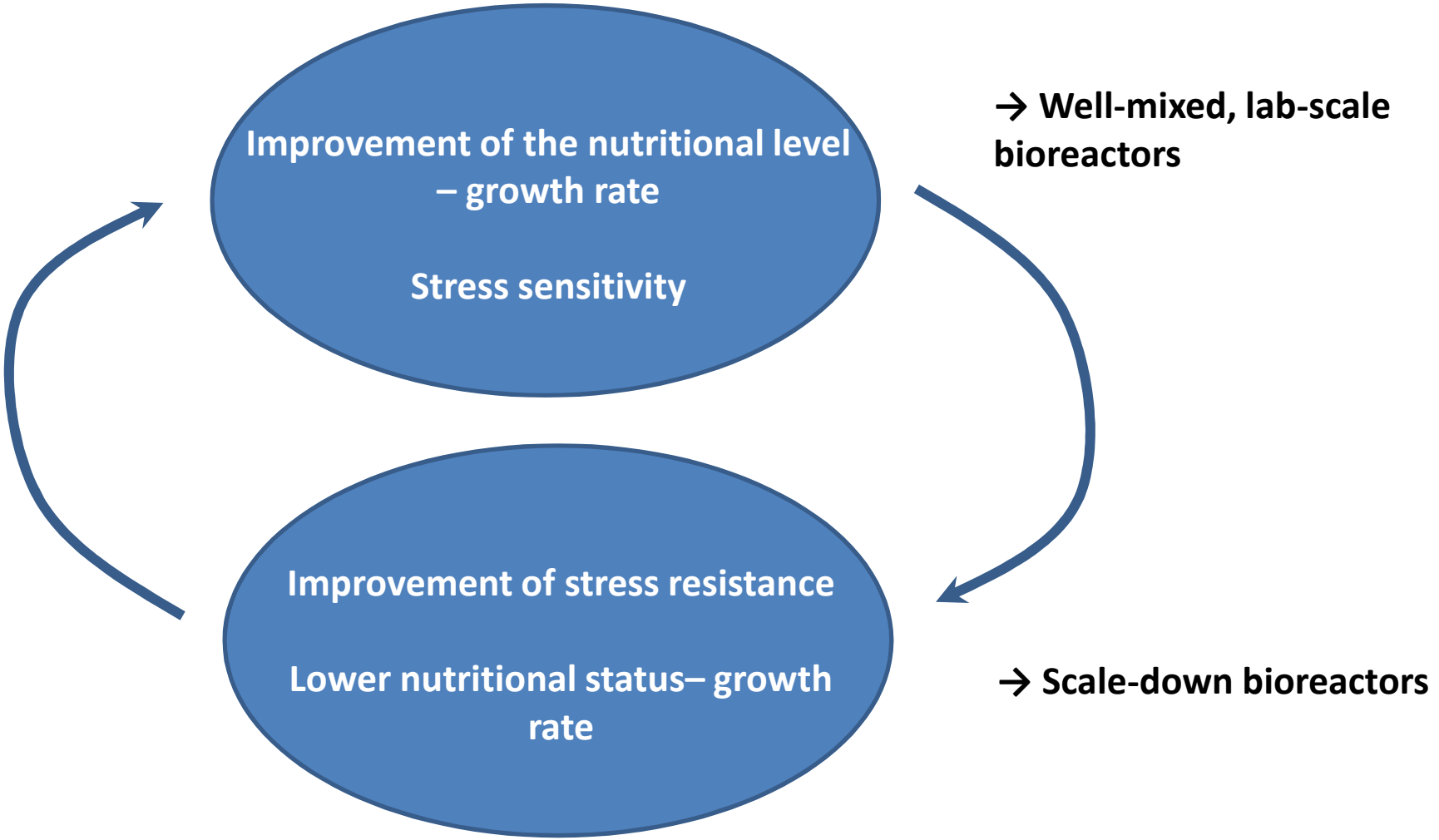
prpoS::gfpmut2



Delvigne F, Boxus, M., Ingels, S., Thonart, P., Bioreactor mixing efficiency modulates the activity of a *prpoS::GFP* reporter gene in E. coli. *Microbial cell factories* 8: 15 (2009)

Link between mixing quality and population segregation at the level of the *rpoS* response :

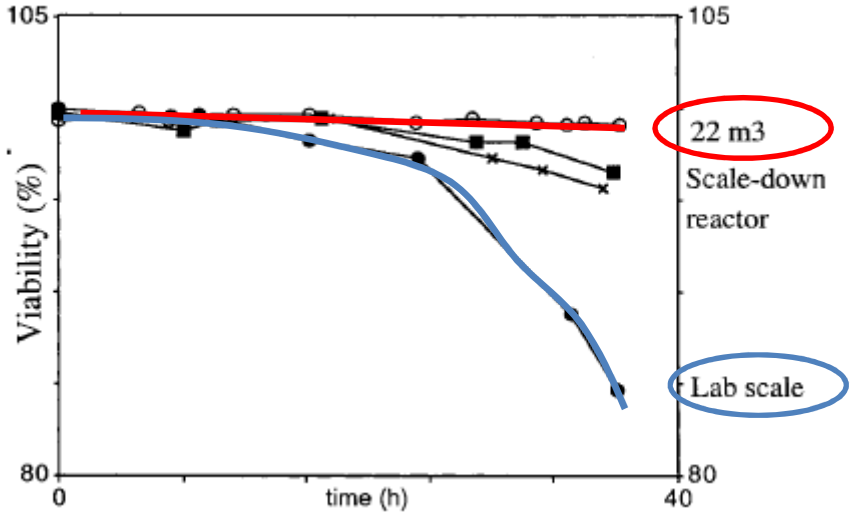
Trade-off effect - microbial cells have to choose between :



Single cell analysis in process conditions

Membrane permeability and protein leakage

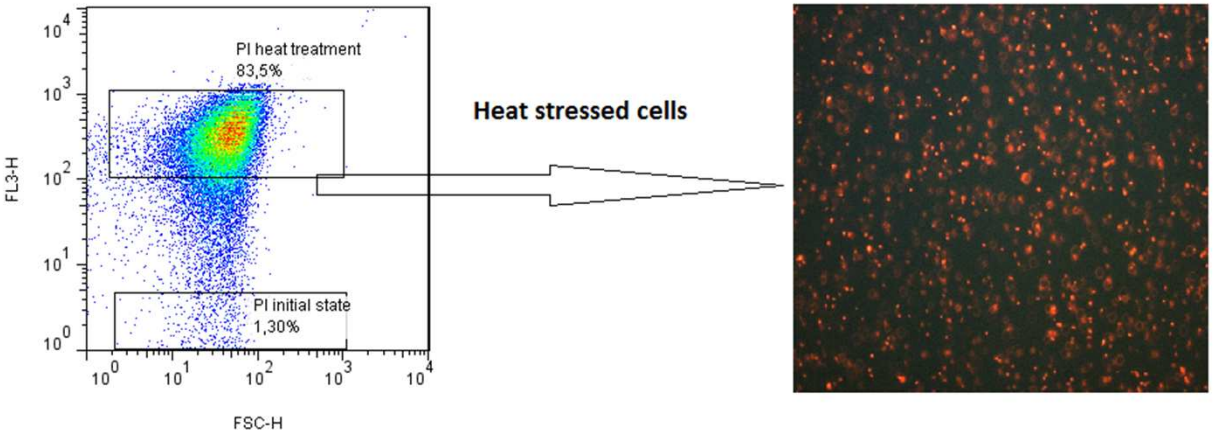
Extracellular perturbations increases cell viability

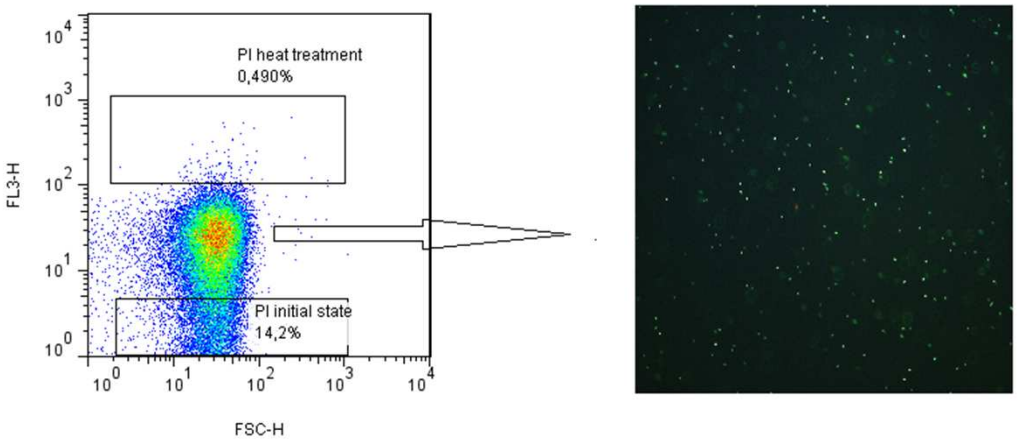


Enfors SO, *et al.* Physiological responses to mixing in large scale bioreactors. *Journal of biotechnology* **85**: 175-185 (2001)

Analysis performed by flow cytometry with propidium iodide exclusion test and thus related to cell membrane permeability

Intermediate PI uptake :

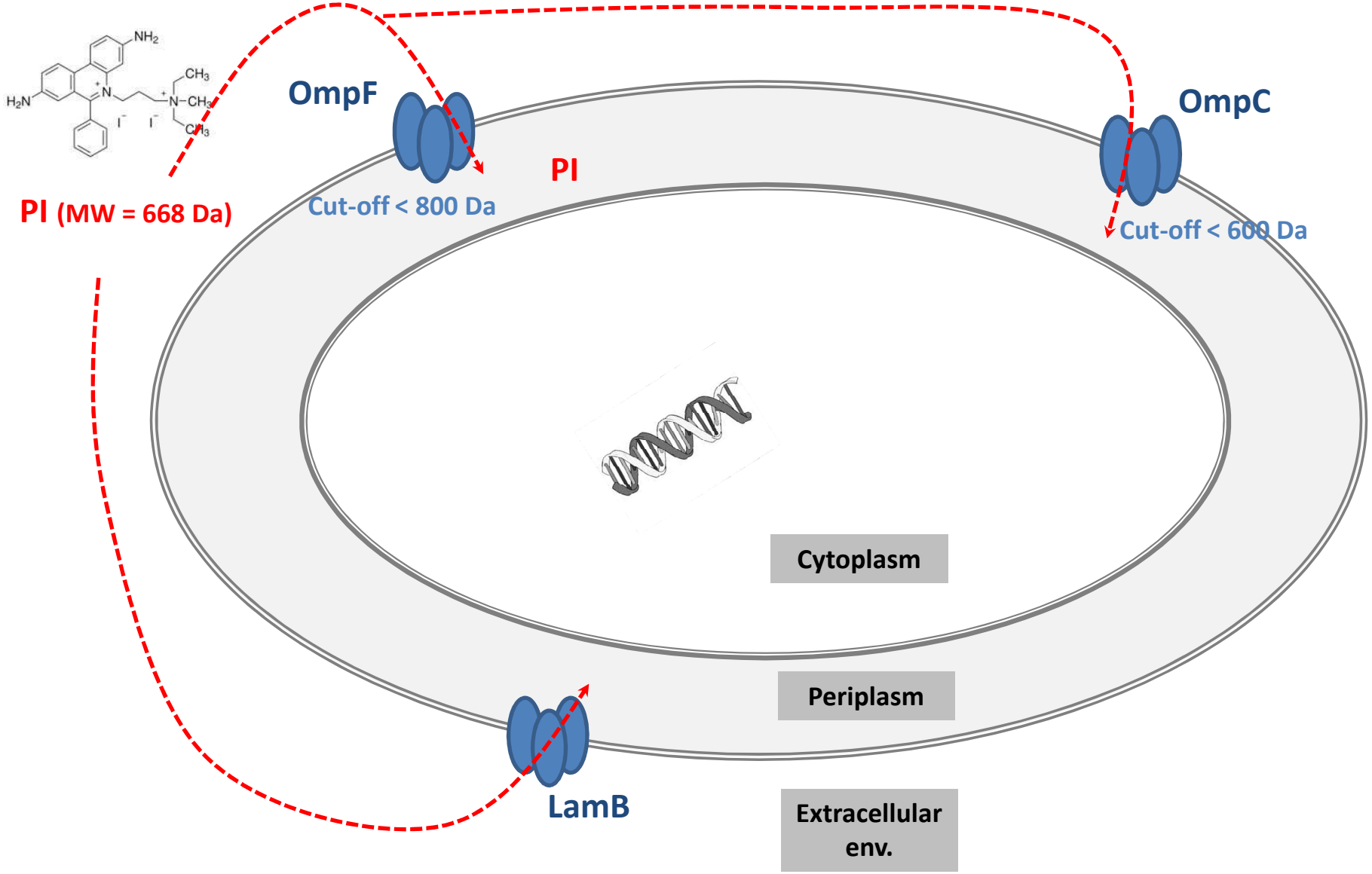




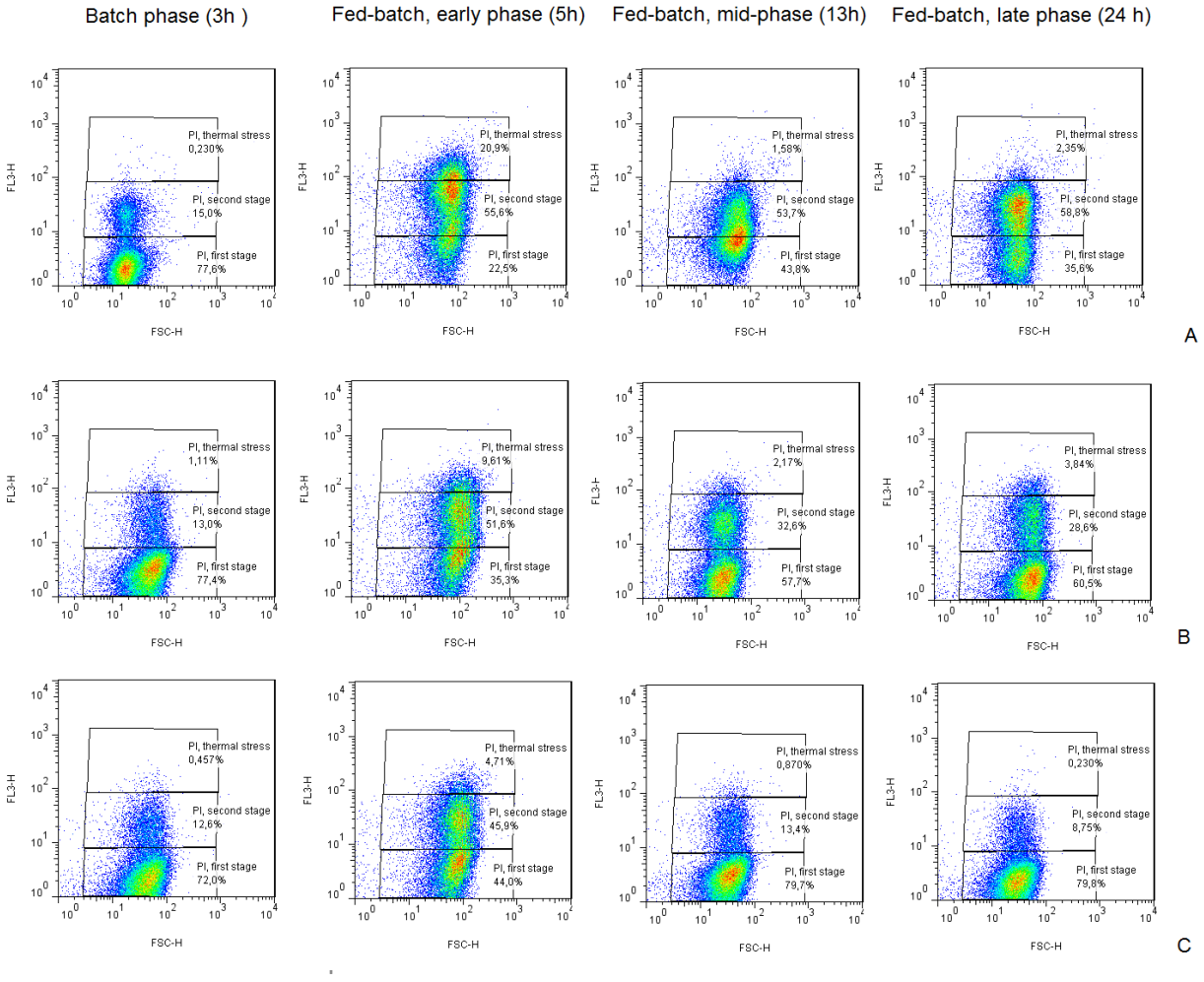
Fed-batch, well-mixed reactor

Propidium iodide (PI) uptake and appearance of an intermediate subpopulation with reduced red fluorescence

Hypothesis : accumulation of PI in the periplasm when cells are exposed to substrate limitation (increased expression of porins)

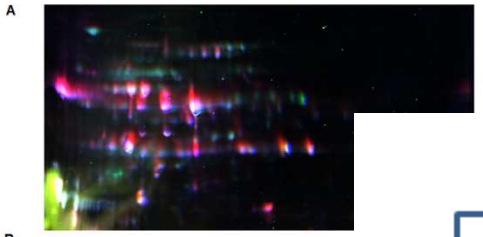


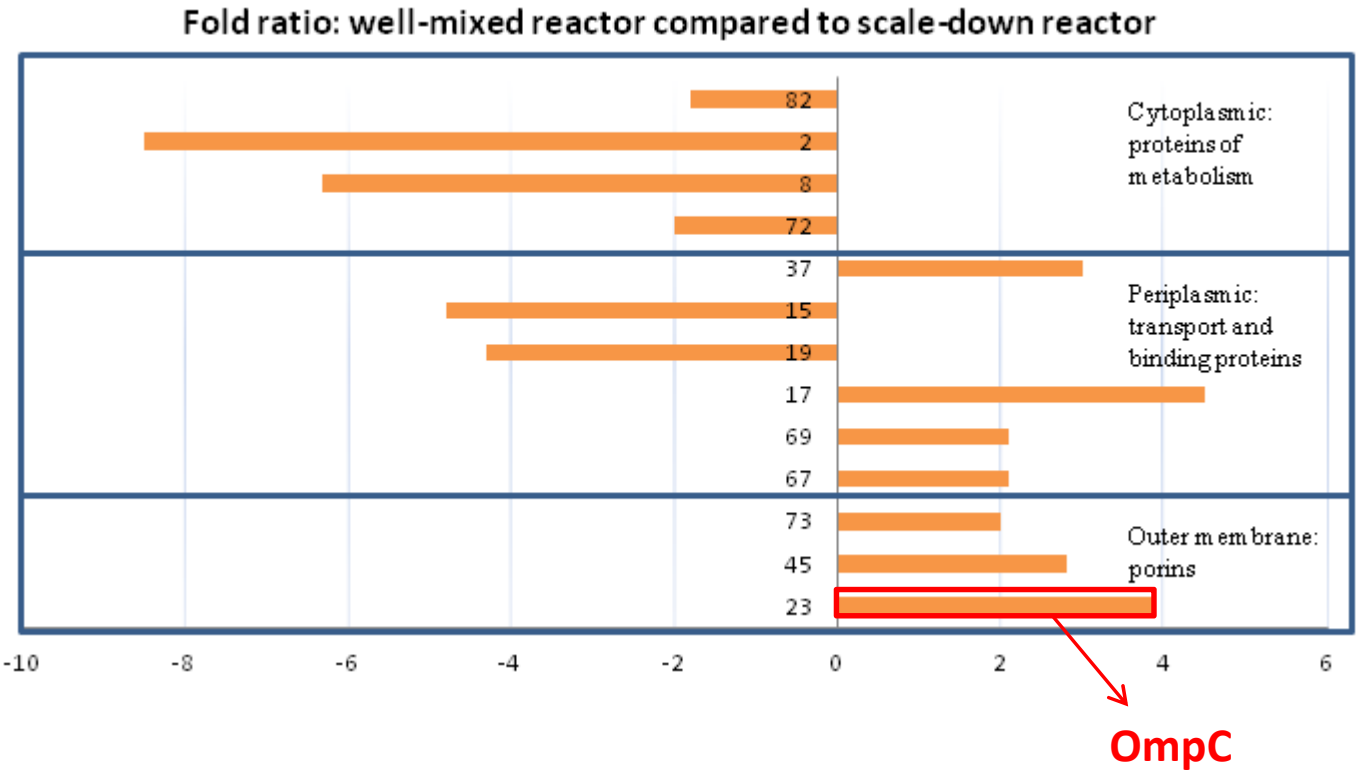
PI exclusion test



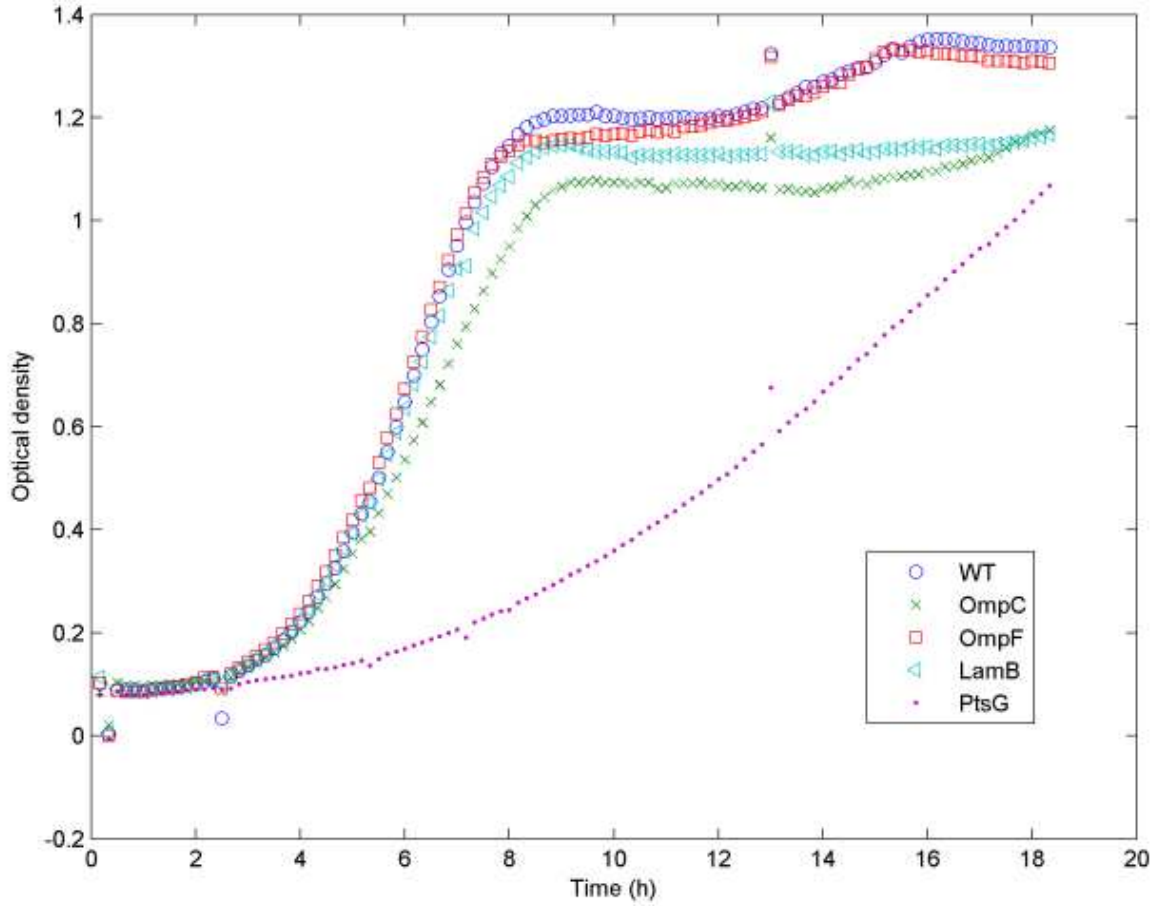
Extracellular proteome analysis of the impact of bioreactor performances

Well-mixed/scale-down comparative analysis : 2-D differential in-gel electrophoresis

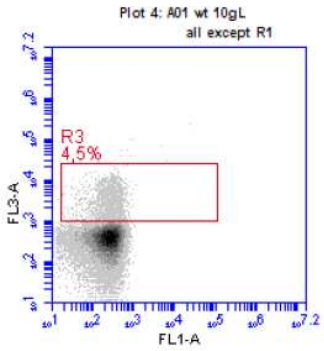




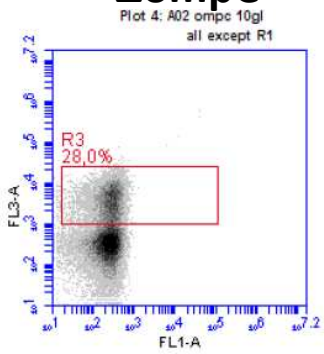
Brognaux A, Francis F, Twizere JC, Thonart P and Delvigne F, Scale-down effect on the extracellular proteome of Escherichia coli: correlation with membrane permeability and modulation according to substrate heterogeneities. *Bioprocess Biosyst Eng* **14**: 14 (2014)



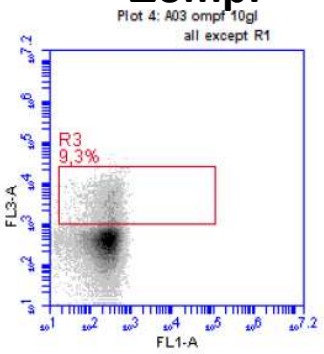
WT



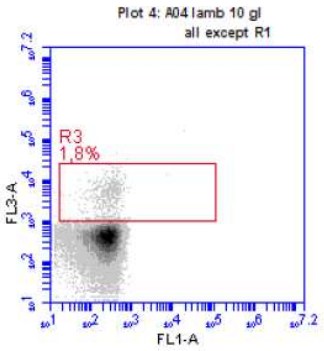
ΔompC



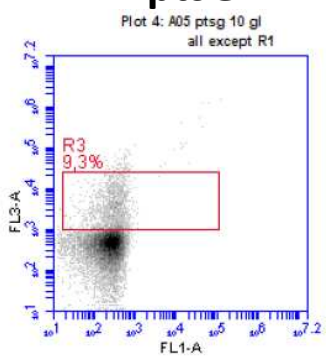
ΔompF

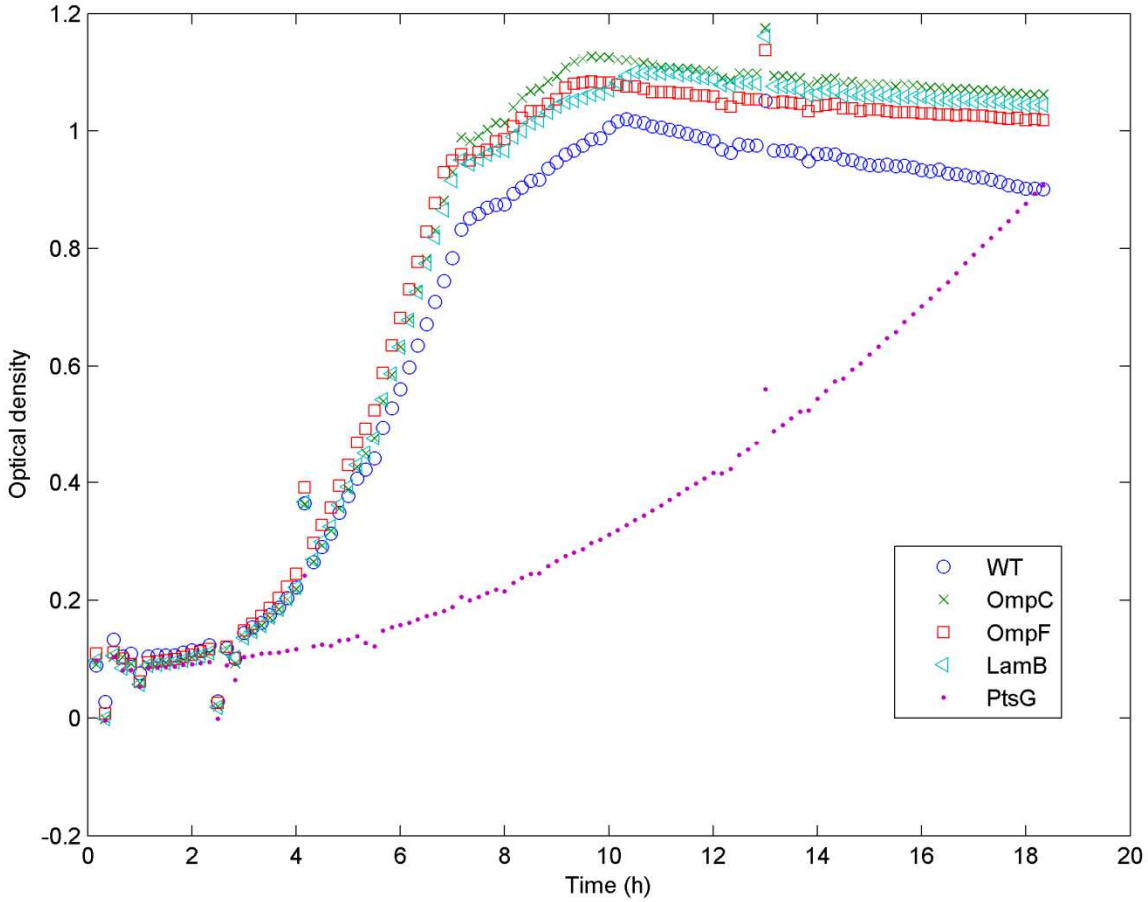


ΔlamB

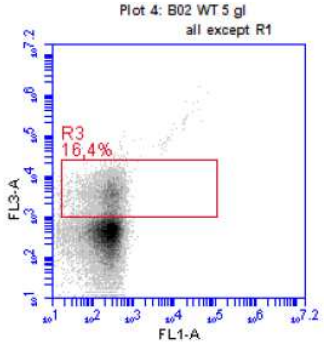


ΔptsG

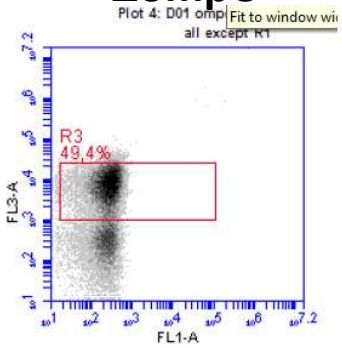




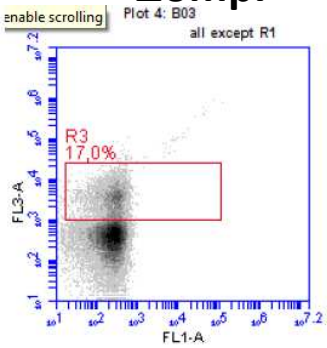
WT



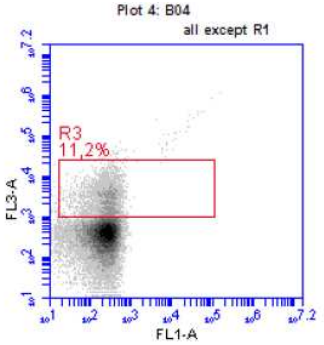
ΔompC



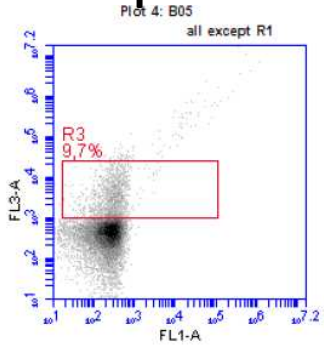
ΔompF



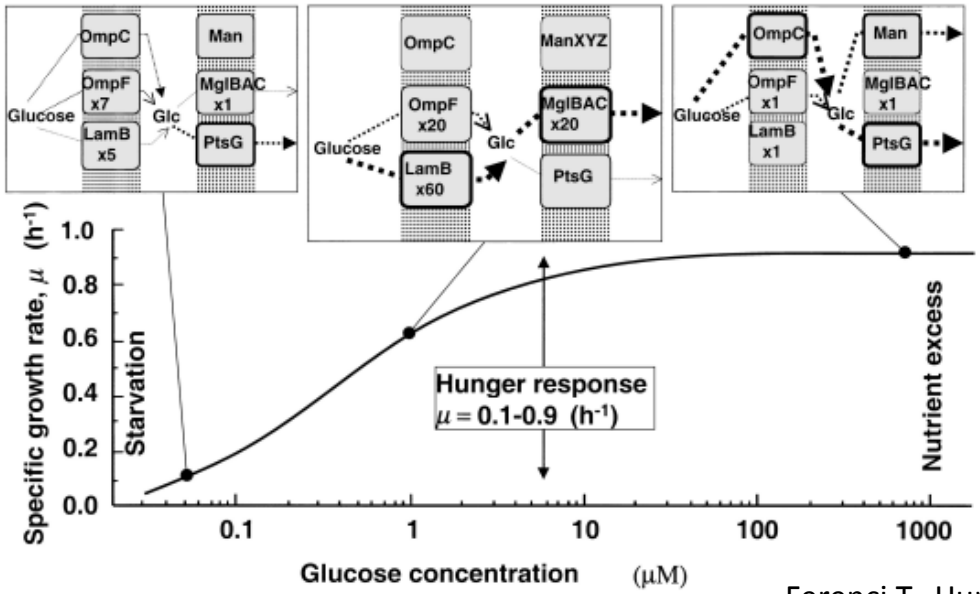
ΔlamB



ΔptsG

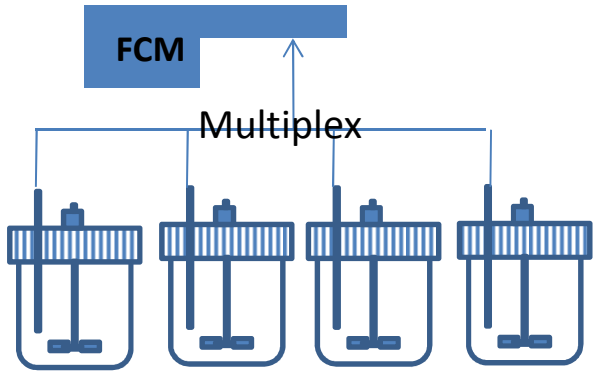


Interesting results but without any control at the level of the substrate limitation and growth (batch)

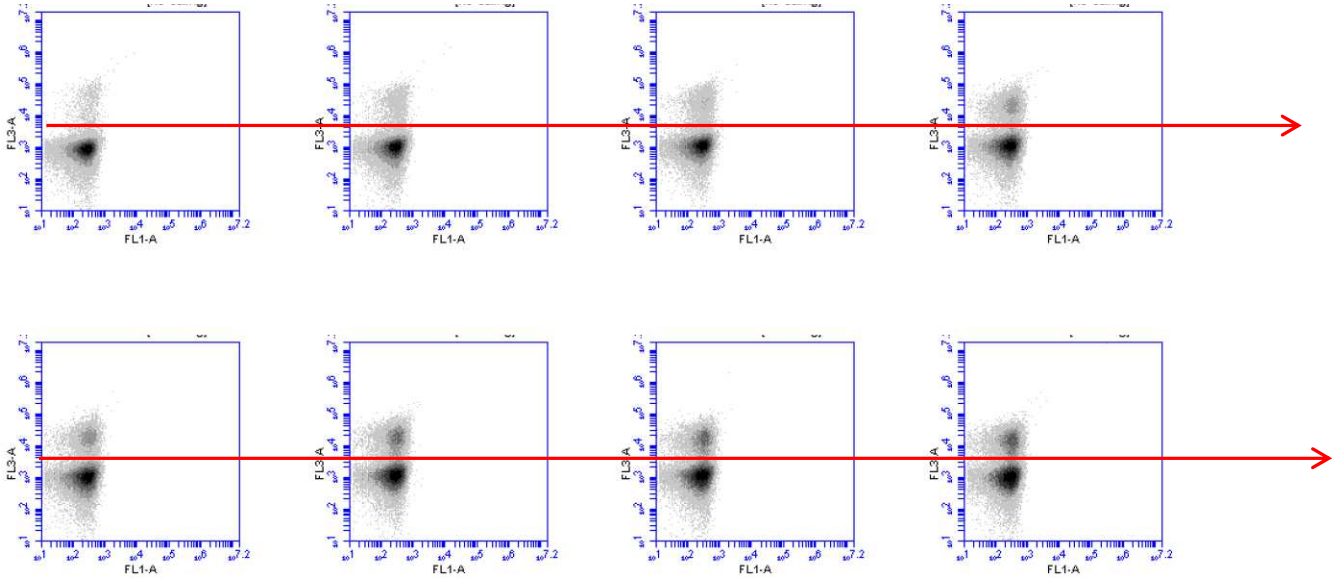


Ferenci T., Hungry bacteria - definition and properties of a nutritional state. *Environmental microbiology* **3**: 605-611 (2001)

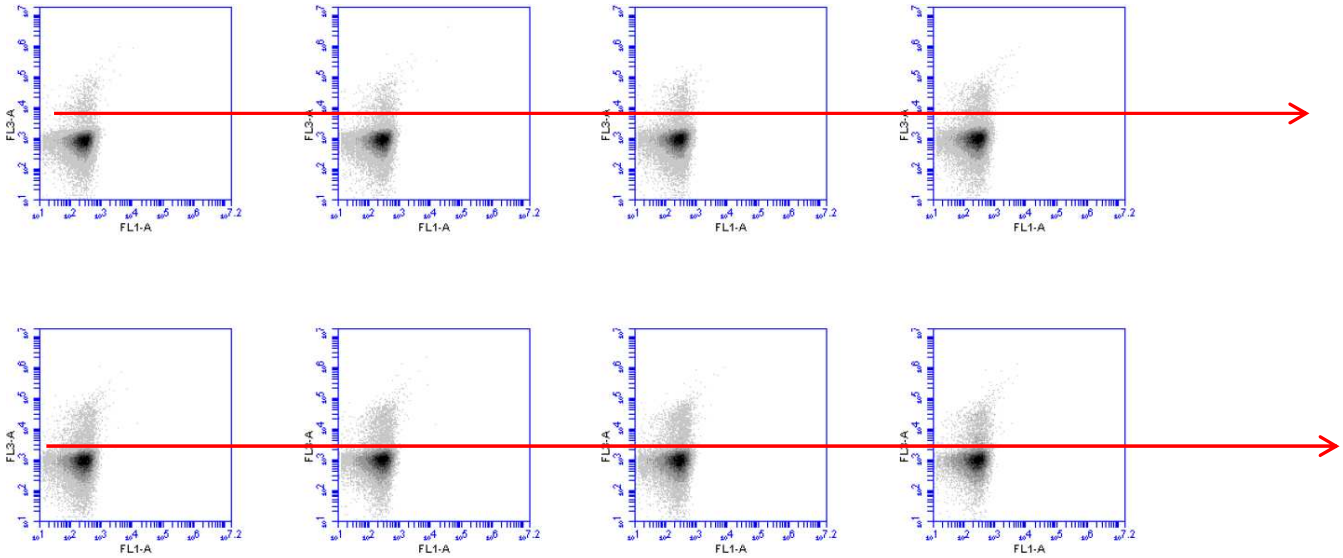
Validation is needed (chemostat)



ΔompC



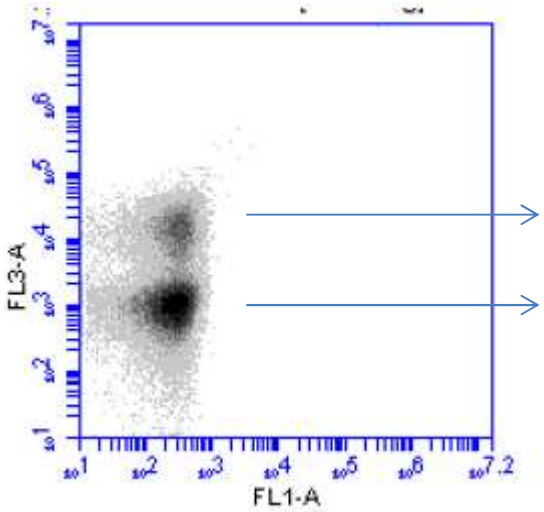
ΔlamB



What's next

Limitation of the actual fluorescent reporter system : global view of physiology. There is a need to be closer to the metabolism :

- By appropriately choosing promoter sequences
- By using new generation of promoter independent fluorescent reporter
- Subpopulations omics



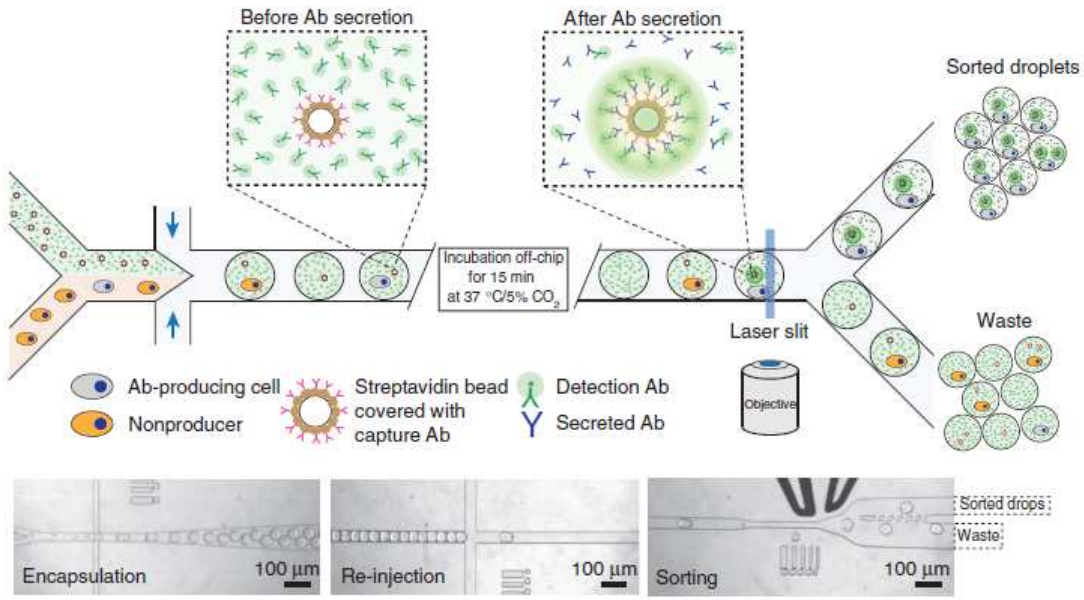
Subpopulations → Metabolically relevant ?
Switch between subpopulations ?

Challenges :

Going beyond the need of fluorescent tags : single cell omics technologies

Metabolic engineering aiming at controlling microbial phenotypic heterogeneity

Insertion of microfluidics biroeactor in the scaling-up/down loop



Mazutis L, Gilbert J, Ung WL, Weitz DA, Griffiths AD and Heyman JA, Single-cell analysis and sorting using droplet-based microfluidics. *Nat Protoc* **8**: 870-891 (2013)

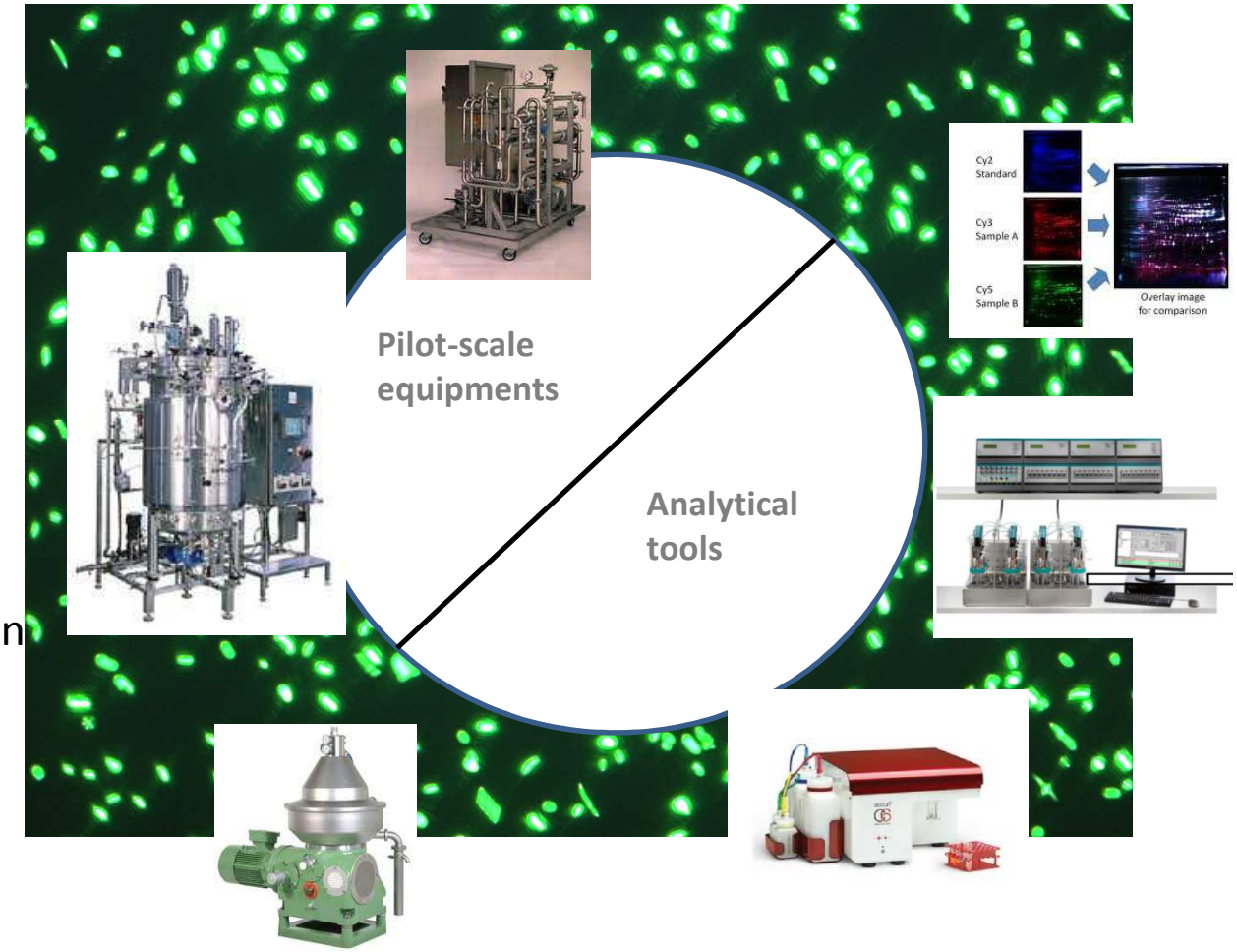
Technical staff
Samuel Telek
Benoît Massaux

Post-docs
Alison Brognaux
Tambi Kar

PhD students
Quentin Zune
Jonathan Baert
Romain Kinet
Michel Musoni
Thanh Huu Nguyen
Naila Boudjelal
Annick Lejeune

Collaborations :
INSA Toulouse
Tech Univ Denmark
Copenhagen Univ
UNA-Cuajimalpa
Leiden Univ

Lunchtime ! But don't forget to ask for some questions
Thank you



GSK biologicals
Puratos/Beldem
Sequip