5-9-2017

# Of SUT and stainless

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# OF SUT and Stainless


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***www.ibet.pt***



***ECI Single-Use Technologies II***

**May 7-10, 2017**

**(Hotel dos Templários, TOMAR – PORTUGAL)**

# Single use devices: Ready to Process

# DRIVERS FOR SU - I

- Higher upstream titers (reducing volume)
- Shorter turn around times
- Reduce water & buffer consumptions
- Reduce foot print
- Reduce maintenance
- Reduce CAPEX & OPEX (?)

# DRIVERS FOR SU - II

- Assure Sterility (fool proof)
- Facilitate technology transfer
- Faster facility completion
- Reduce hazardous cleaning solutions
- Regulatory encouragement (?)
- Reduce risk for cross contamination

# CONCOMITANT TRENDS - I

- Higher upstream titers
- Continuous processes
- Integrated processes
- Need for production in emerging countries

# CONCOMITANT TRENDS - II

- Improved process robustness (PAT, QbD)
- Increase in Non-platform processes
- Increase in use of CDMO (diversity)
- Need for “fail-proof” processes (autologous CT)

# DISADVANTAGES (Perceptions…) - I

- Often single source supply
- No interoperability (“protect” suppliers control)
- Non “inert” contact materials
  (Extractables|leachables|particulates|adsorption)
- Full product validation/testing difficult (BPSA)
- Still costly – non “disposable” SU? (eg. membranes)

# DISADVANTAGES (Perceptions…) - II

- Sensors – disposable/reusable…
- Low nº of ports
- Limited to 2KL scale (? Sets options…)
- Material changes/logistics in “plastics” suppliers
- Aeration/agitation (Ex., microbial ferment.)

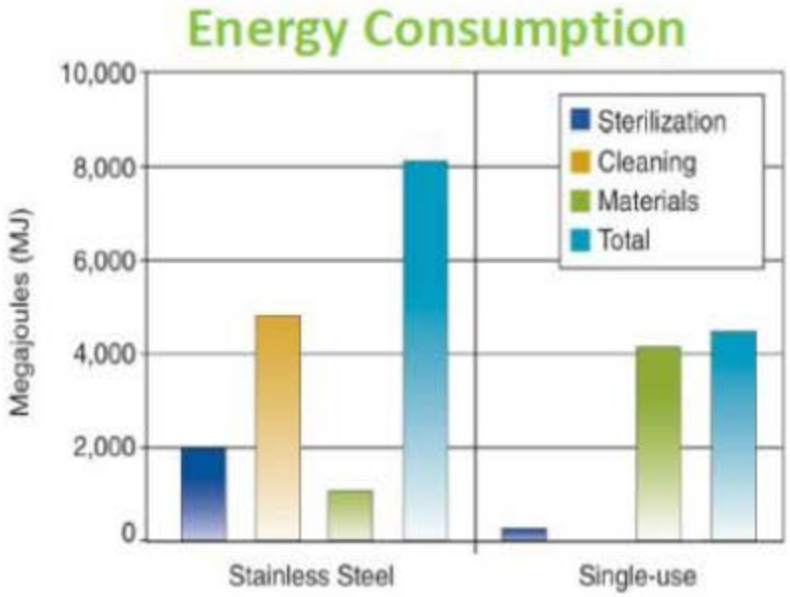
# Some Systems currently on the market

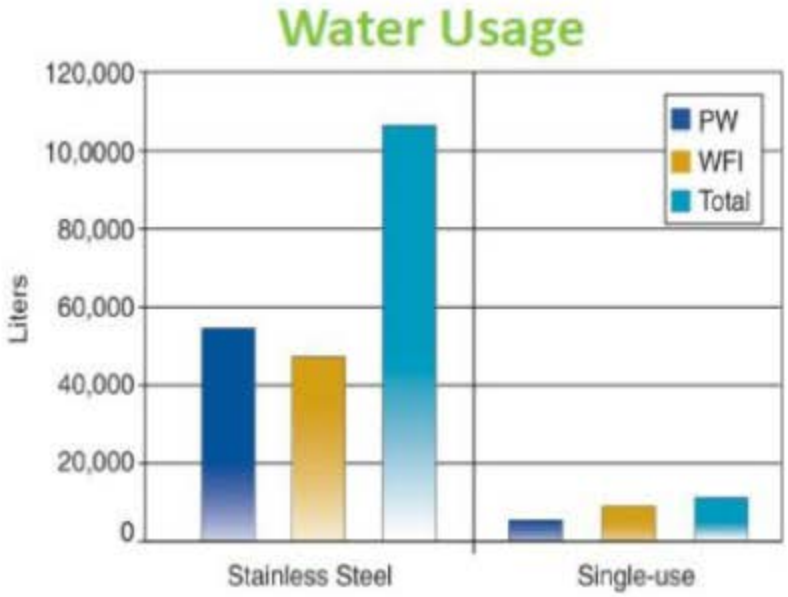
| Supplier/model | Lab scale (200L) | Pilot scale (2000L) |
|---|---|---|
| GE/Xcellerex |  |  |
| Sartorius/ BIOSTAT STR |  STR 200L |  STR 2000L |
| Merck Millipore |  |  |
| Thermo/Hyclone |  |  |

# Advantages and Disadvantages of single-use technologies in biopharmaceutical manufacturing

|  |  |
|---|---|
| • Flexible modular design-> replication in many locations | • Current processes are developed in/for non-single use |
| • No cleaning (-validation) | • New contact materials (see cell growth issues related to extrusion components) |
| • Fast product change-over | • Dependence on consumable supply from one source |
| • Easy tech transfer | • Harvest solutions currently limited |
| • Reduces risk of cross contamination | • Currently DSP solutions not fully available for SUS |
| • Smaller footprint-> cheaper facility ("Ball room" concept) | • Consumable costs |
| | • Limited to 2kL scale currently |

# Environmental Considerations




**Missing: Energy to incinerate the plastic waste**

*Ref: Sinclair A, et al. BioPharm Int. (2008), Rawlings B, Pora H. BioProcess Int. (2009), Noe, W, Antibody Development and Production (2011)*

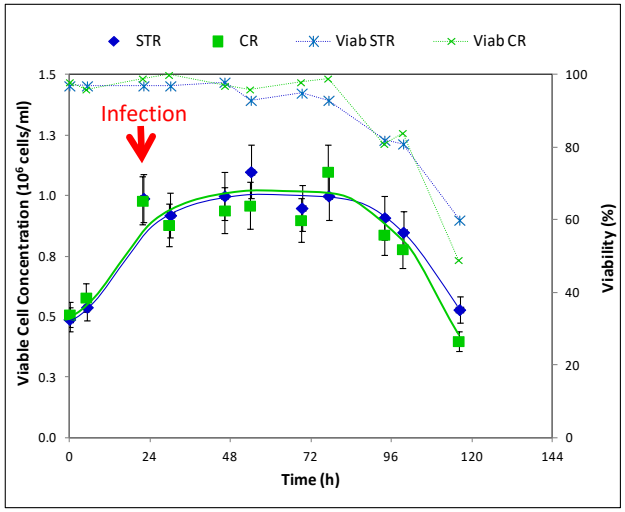
# - Single Use Bioreactors

# Production of RetroVLP-HCV in bioreactor using Sf9 cells

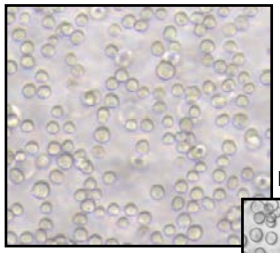
## Glass Stirred Tank Reactor (STR) *versus* disposable Mobius® CellReady (CR)

STR Conditions:
Sf9 cells (Life technologies)
SF900II medium (Life technologies)
27º C, $pO_2$ = 30%
Agitation = 70-250 rpm
Aeration rate: 0.01 vvm
Working volume = 2 L
CCI = 1 x $10^6$ cells/ml;
MOI 2 for each baculovirus

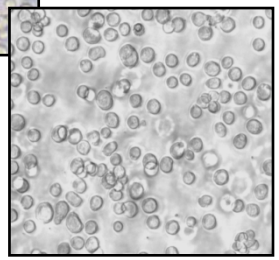
CR Conditions:
Sf9 cells (Life technologies)
SF900II medium (Life technologies)
27º C, $pO_2$ = 30%
Agitation = 150 rpm
Aeration rate: 0.01 vvm
Working volume = 2 L
CCI = 1 x $10^6$ cells/ml;
MOI 2 for each baculovirus




Infected Sf9 cells in STR



Infected Sf9 cells in CR

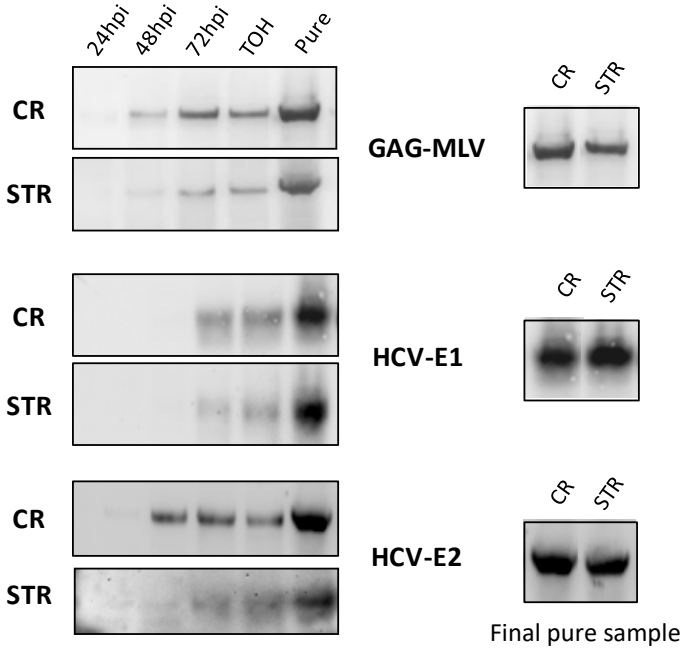


- Sf9 cells have similar growth profile in the two types of bioreactors

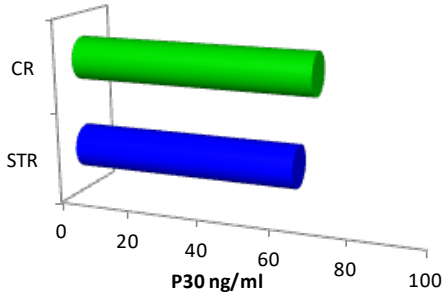
# Production of RetroVLP-HCV in bioreactor using Sf9 cells

## Glass Stirred Tank Reactor (STR) *versus* disposable Mobius® CellReady (CR)

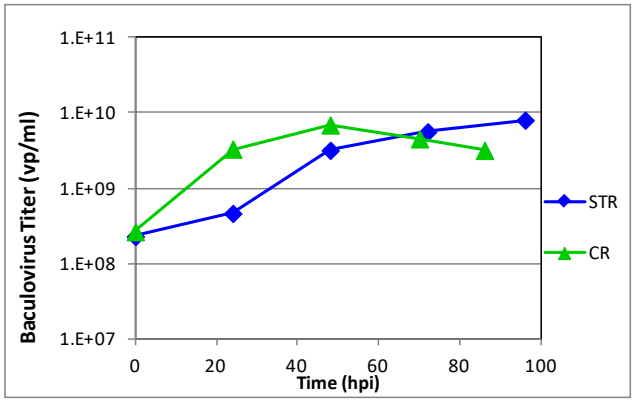
VLP-HCV production kinetics in the bioreactors




Gag-MLV titer (P30) in the bioreactors harvested bulk




Baculovirus replication kinetics in the bioreactors



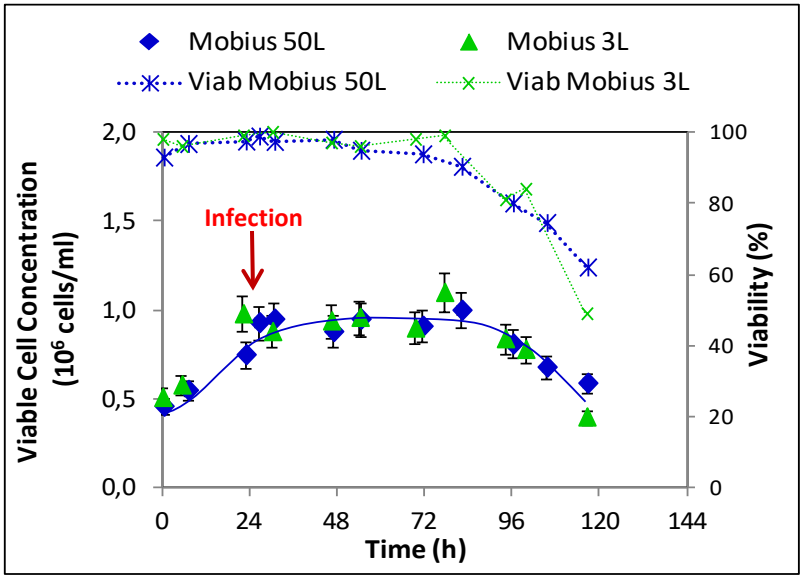

- **Similar production kinetics for VLP-HCV in the two bioreactors**
- **Similar final Gag productivity and baculovirus replication kinectis**

# Scale-up of RetroVLP-HCV production: 2 L to 50 L

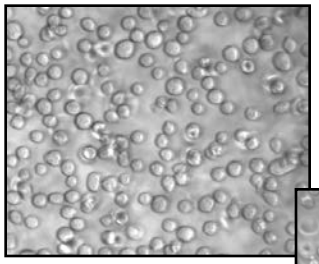
## Mobius® CellReady 3L *versus* Mobius® CellReady 50L

Mobius® 3L Conditions:
Sf9 cells (Life technologies)
SF900II medium (Life technologies)
27º C, $pO_2$ = 30%
Agitation = 150 rpm
Aeration rate: 0.01 vvm
Working volume = 2 L
CCI = 1 x $10^6$ cells/ml;
MOI 2 for each baculovirus

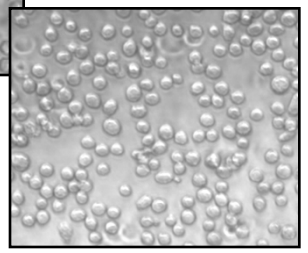

Mobius® 50L Conditions:
Sf9 cells (Life technologies)
SF900II medium (Life technologies)
27º C, $pO_2$ = 30%
Agitation = 110 rpm
Aeration rate: 0.01 vvm
Working volume = 50 L
CCI = 1 x $10^6$ cells/ml;
MOI 2 for each baculovirus




Infected Sf9 cells in M3L



Infected Sf9 cells in M50L

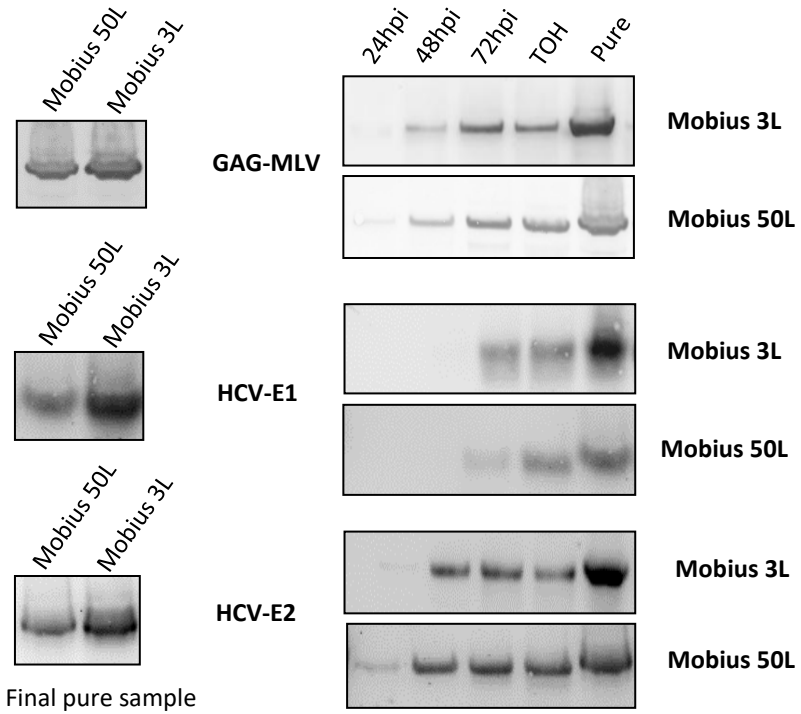


- **Sf9 cells have similar growth profile in the Mobius® 3L and Mobius® 50L Bioreactors**

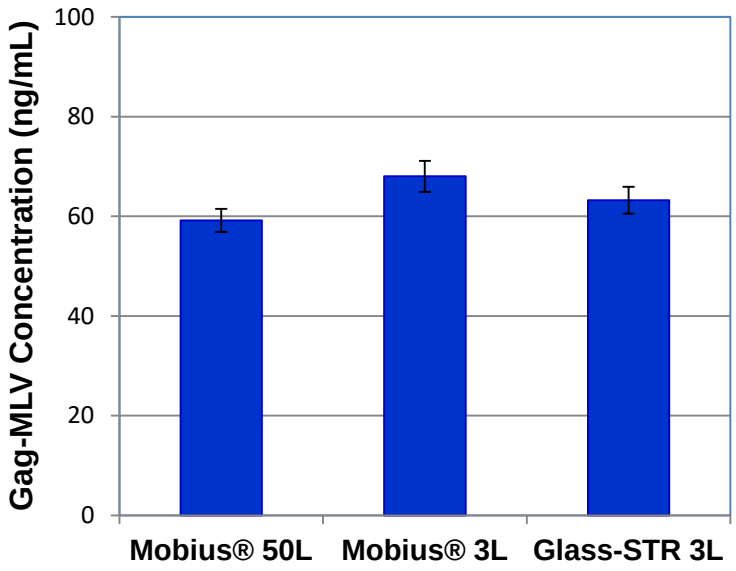
# Scale-up of RetroVLP-HCV production: 2 L to 50 L

## Mobius® CellReady 3L *versus* Mobius® CellReady 50L

VLP-HCV production kinetics in the bioreactors

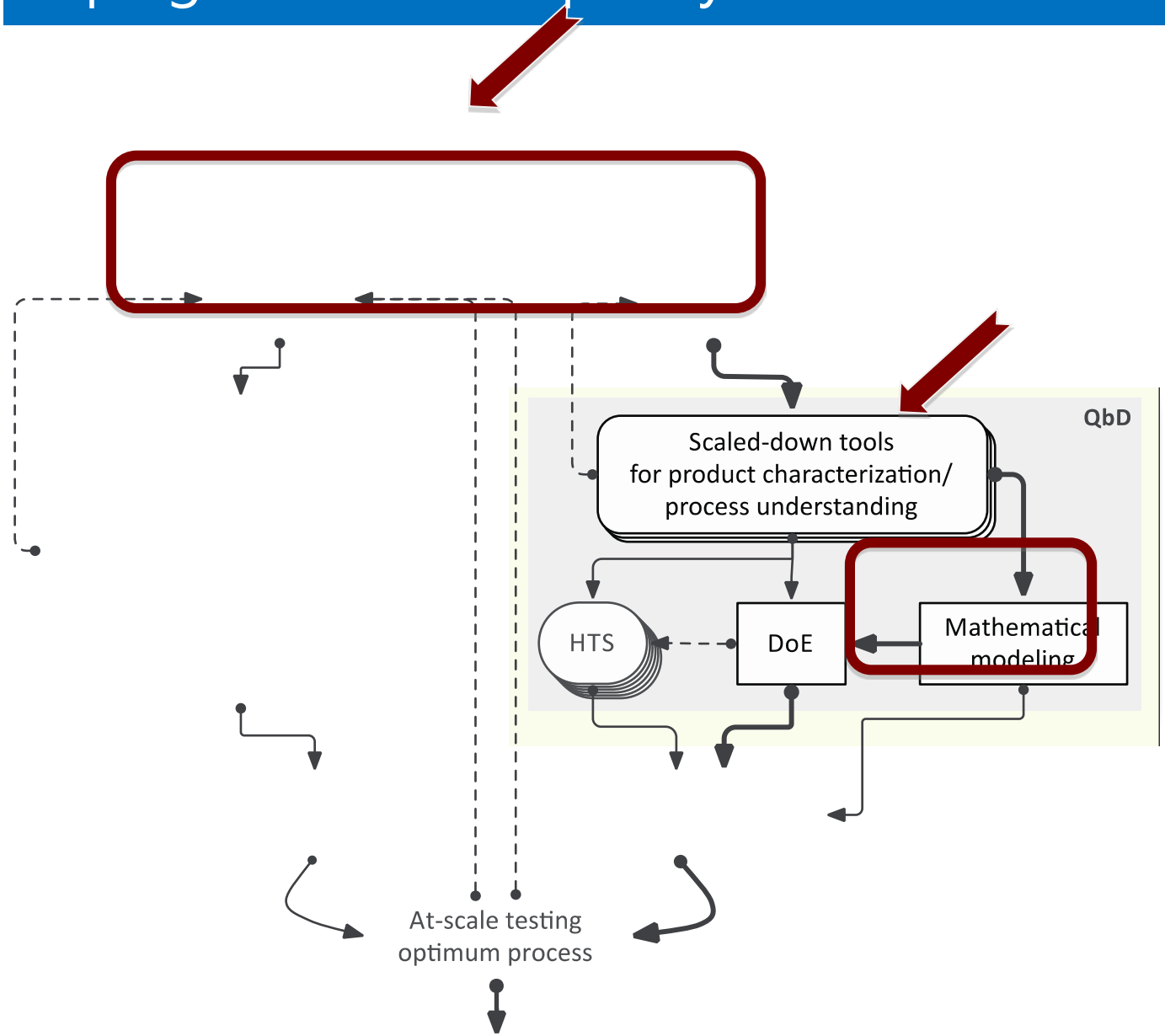



Gag-MLV titer (P30) in the bioreactors harvested bulk




- Similar production kinetics for VLP-HCV in the Mobius® 3L and Mobius® 50L Bioreactors
- Similar final Gag productivity

# Coping with the complexity of baculoviruses (rBVs)

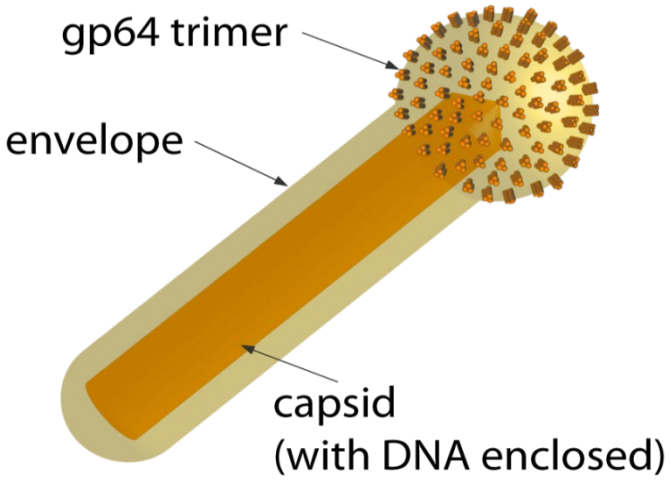


# Ion-exchange chromatography: an interaction-driven

- Measuring adsorption/electrostatics of the components of the bulk
- Predicting the behavior of both the product of interest and the main impurities
- Surface plasmon resonance (SPR) and dynamic light scattering (DLS) as potential tools

Baculovirus bulk divided into three cuts:

- Product:
  - Baculovirus, intact, purified, infective
- Product-derived impurities:
  - Baculovirus, naked envelope
  - Baculovirus capsid
  - gp64 protein isolated
  - Baculovirus, empty-capsids
- Process-derived impurities:
  - Host cell protein: BSA as model
  - DNA: cell nuclei DNA
  - Endotoxin: Lipopolysaccharide



# SPR (I): a scaled-down tool

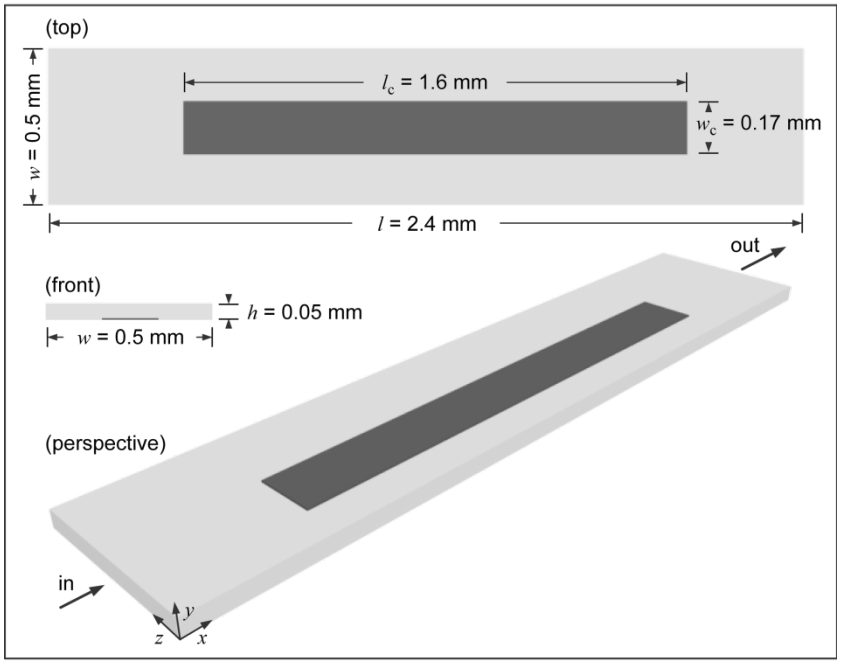
- SPR used as a tool to obtain real-time adsorption kinetics onto a customized DEAE anion-exchange sensor chip surface
- Opportunities:

✓ >1200-fold scale-down;

✓ adsorption isotherms at varied salt concentration

✓ product and impurities

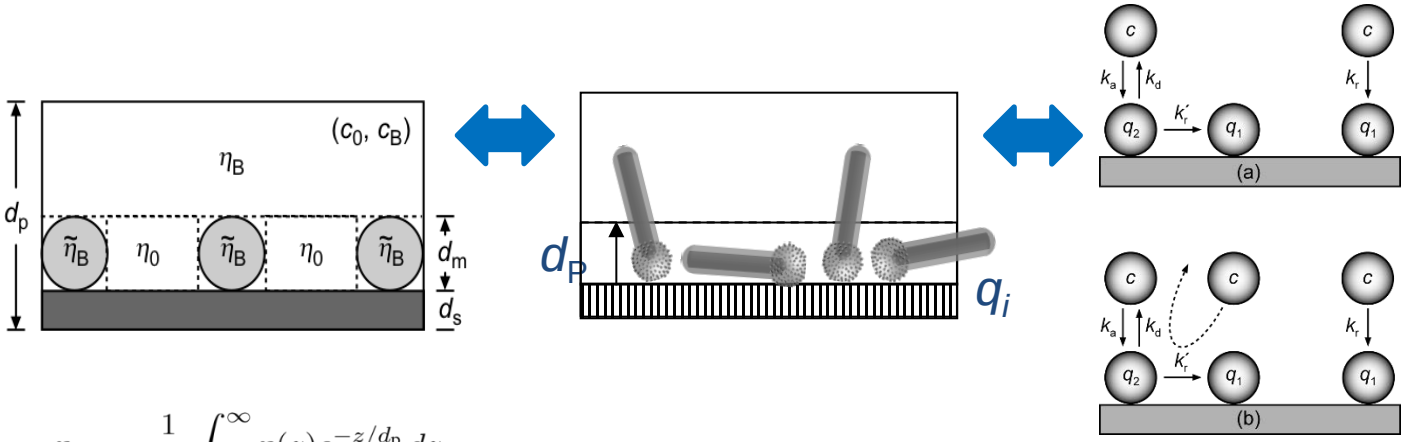
✗ model needed to express the readout into adsorption isotherms




Vicente *et al.* 2010, *Journal of Chromatography A*, 1217:2032-41.

# Surface Plasmon Resonance (SPR)

- Obtain real-time adsorption kinetics
- Measurement of adsorption isotherms at various load conditions
- Modeling IEX chromatography

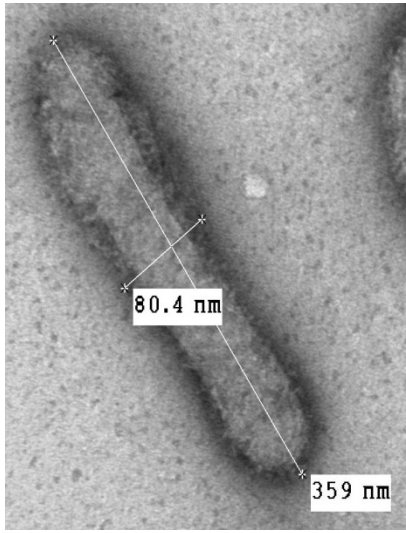



$$\eta_{\text{eff}} = \frac{1}{d_p}\int_0^{\infty} \eta(z) e^{-z/d_p}\, dz$$

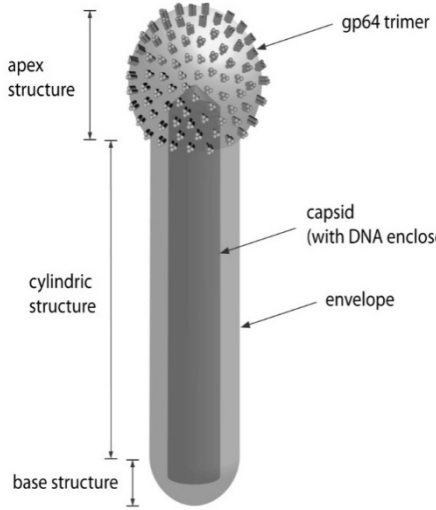
$$\Delta R = (m_B - m_0 c_0 \nu_B)\,[c_m + \phi_m(c_B - c_m)] + m_0(c_0 - c_{0,\text{ref}})$$

$$\frac{dq_1}{dt} = k_r c_B(\tilde{q}_1^{\infty} - q_1) + k_r' c_B q_2.$$

$$\frac{dq_2}{dt} = k_a c_B(\tilde{q}_2^{\infty} - q_2) - k_d q_2 - k_r' c_B q_2$$



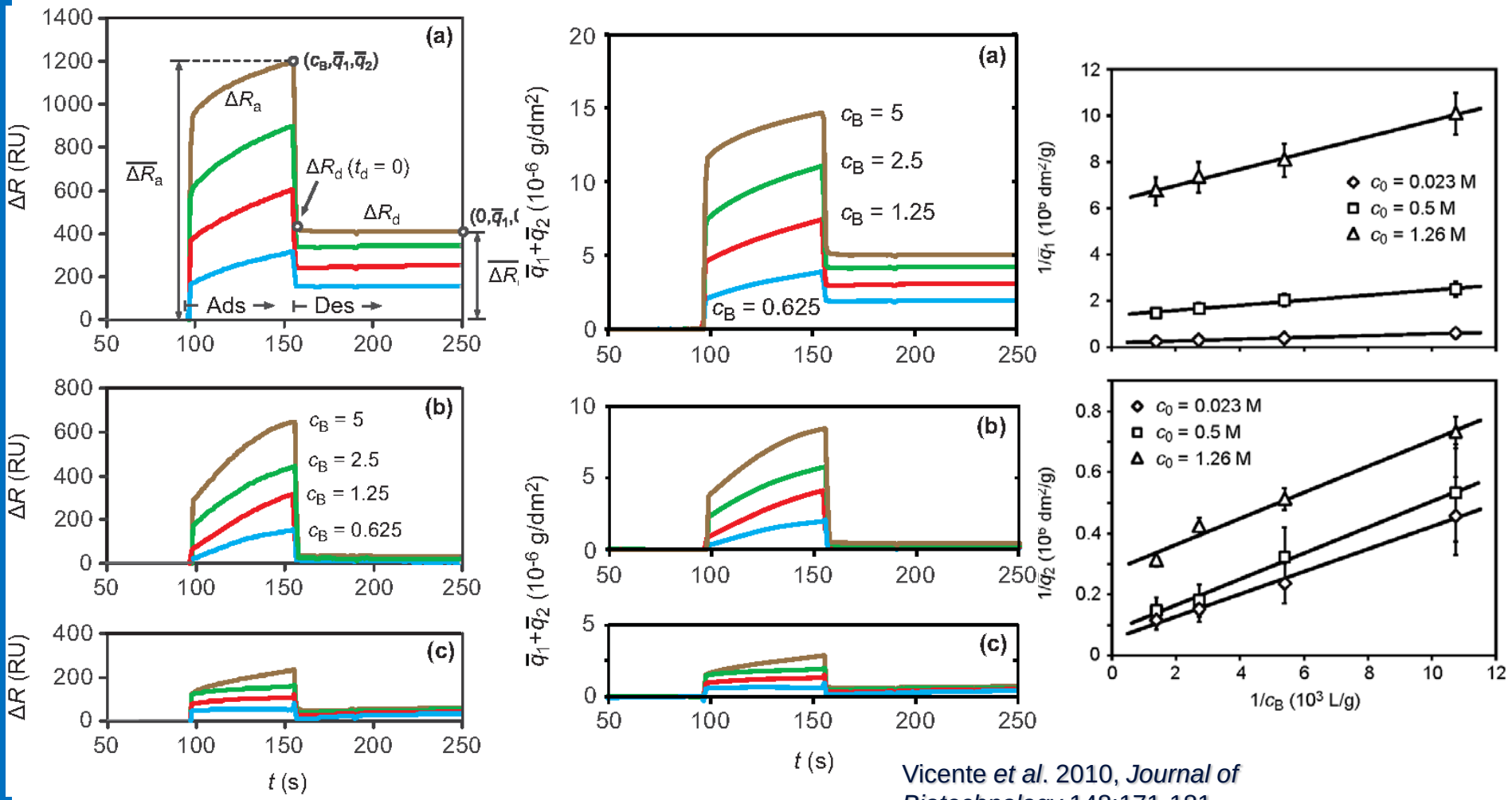

## Baculovirus






Vicente *et al.* 2010, *Journal of Chromatography A*, 1217:2032-41;
Vicente *et al*. 2010, *Journal of Biotechnology*,148:171-181

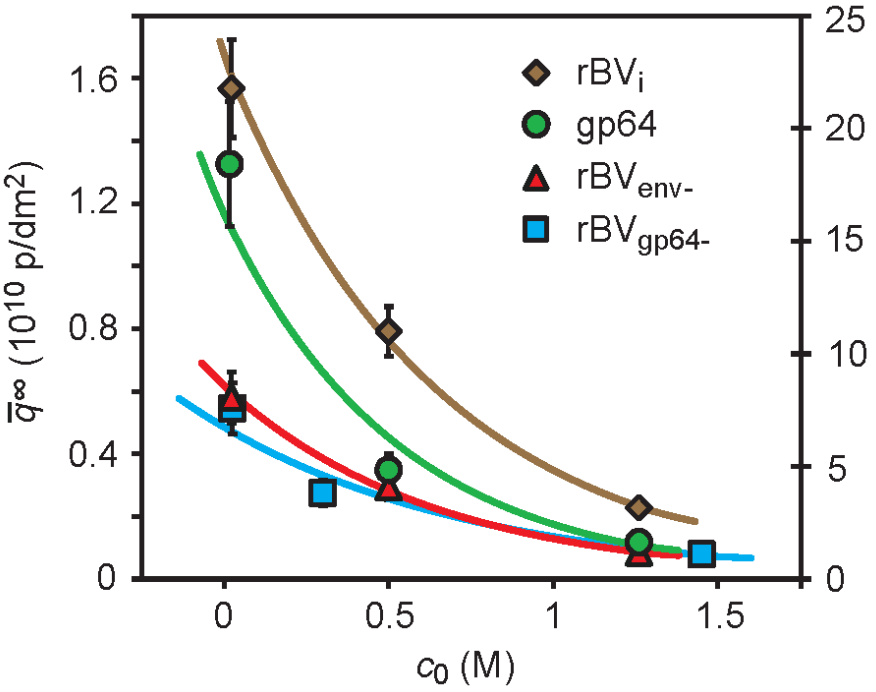
# SPR (III): deconvoluting SPR data into adsorption isotherms



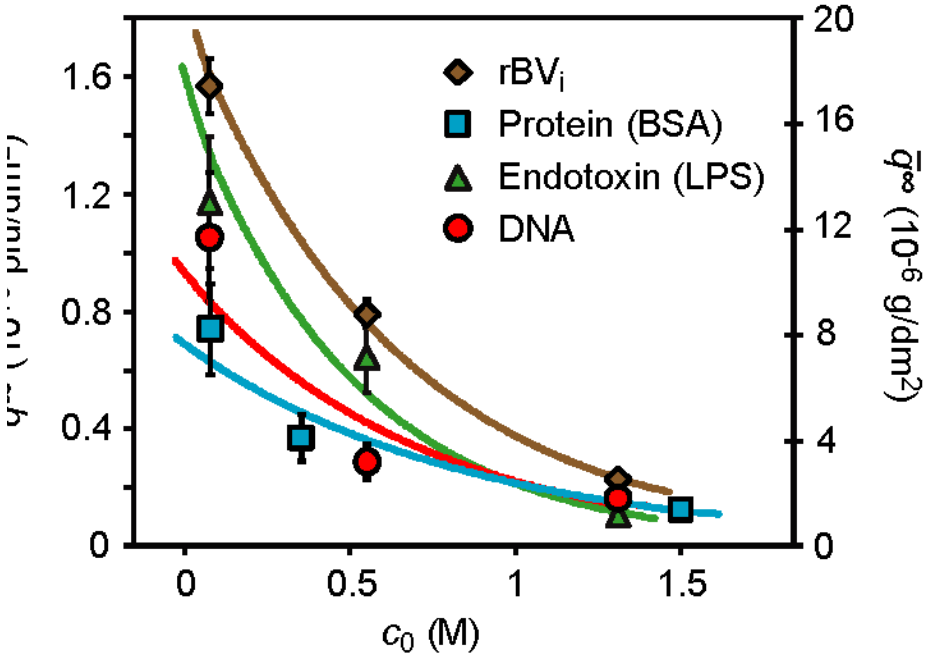

Vicente *et al*. 2010, *Journal of Biotechnology*,148:171-181

- Proof of concept: predicting adsorption isotherms for a model complex biological system: rBVs and impurities

# SPR (IV): comparing product and major impurities




Vicente *et al*. 2010, *Journal of Biotechnology*,148:171-181




- Prediction of binding capacity for product and impurities at broad salt concentration range

# DLS (I): …yet another scaled-down tool

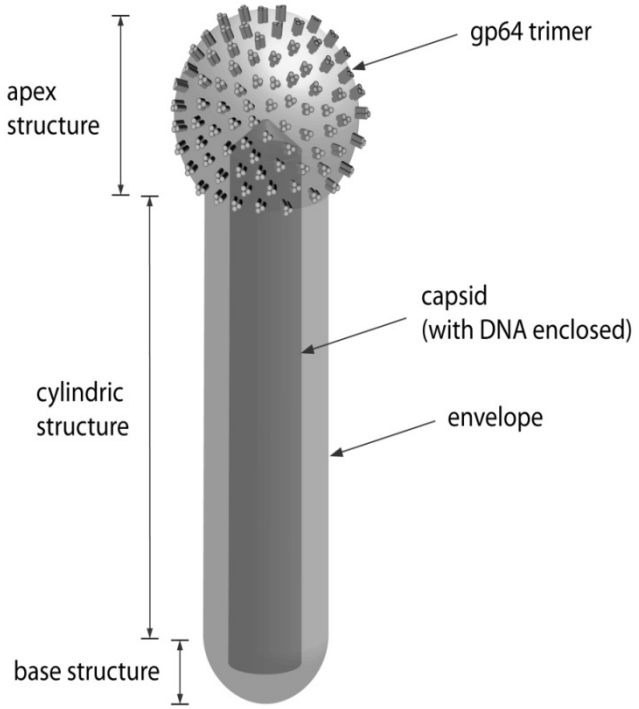
- Evaluation of the ζ-potential as a function of pH of the buffer
- Estimation of hydrodynamic size distributions of rBVs (in buffer conditions)
- Opportunities:

✓ prediction of interaction energies at varied salt concentration and pH;

✓ the above for the various components of the system: product and impurities

✗ not spherical-like

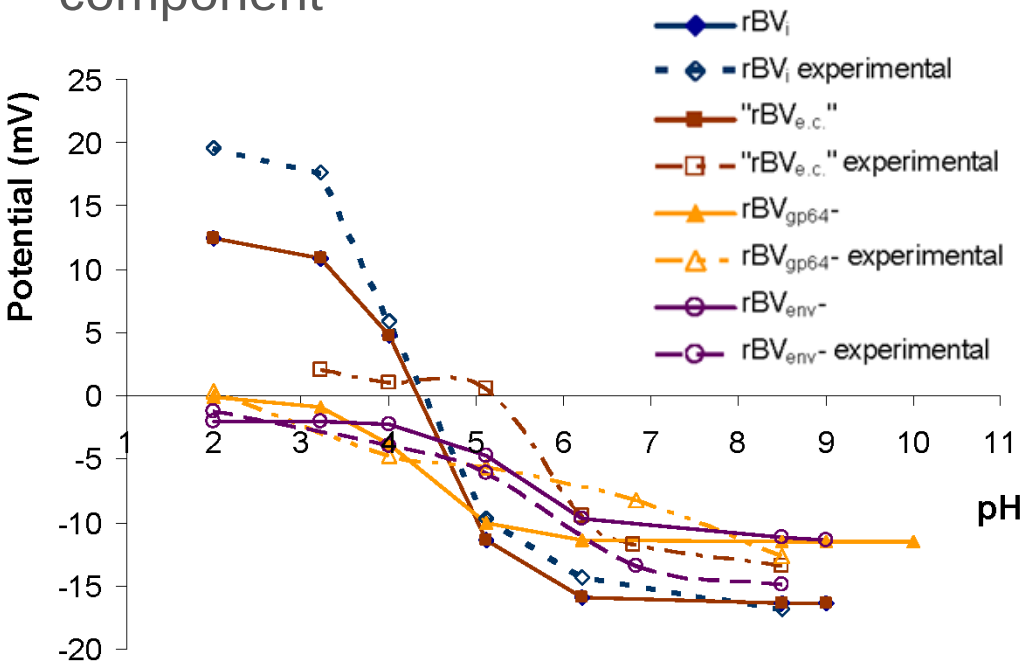
✗ charge density is not homogenous (gp64 localized in the baculovirus "head")

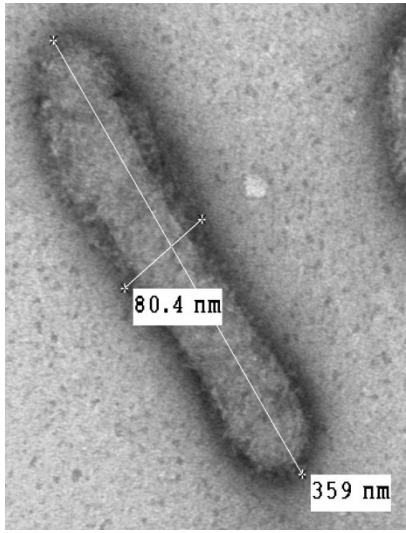



Vicente *et al*. 2010, *Journal of Chromatography A*, 1217:3754-3764

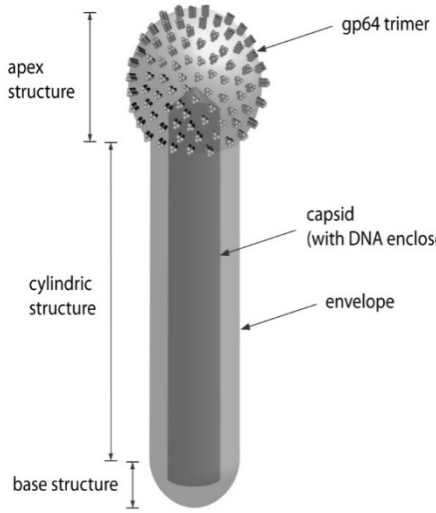
# Dynamic light scattering (DLS)

- Evaluation of the ζ-potential as a function of pH of the buffer
- Estimation of hydrodynamic size distributions of rBVs (in buffer conditions)
- Prediction of electro kinetic properties of the different BV component

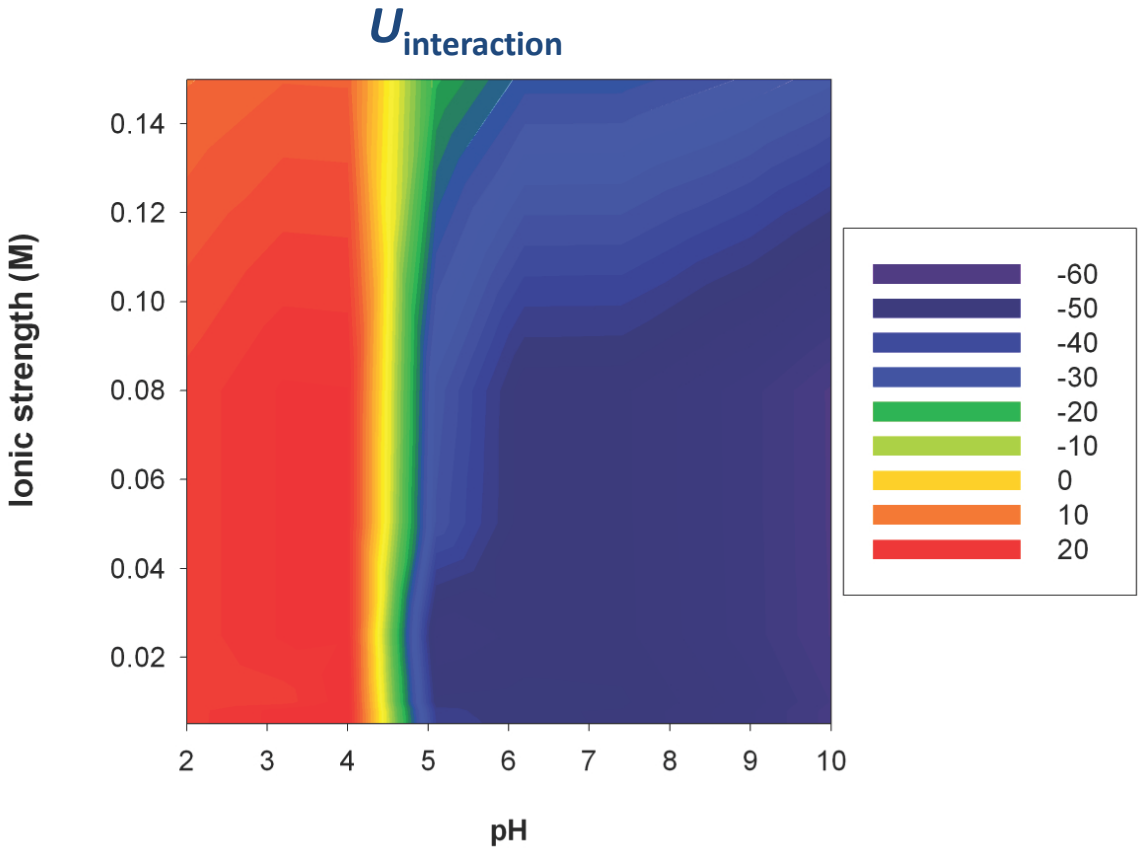


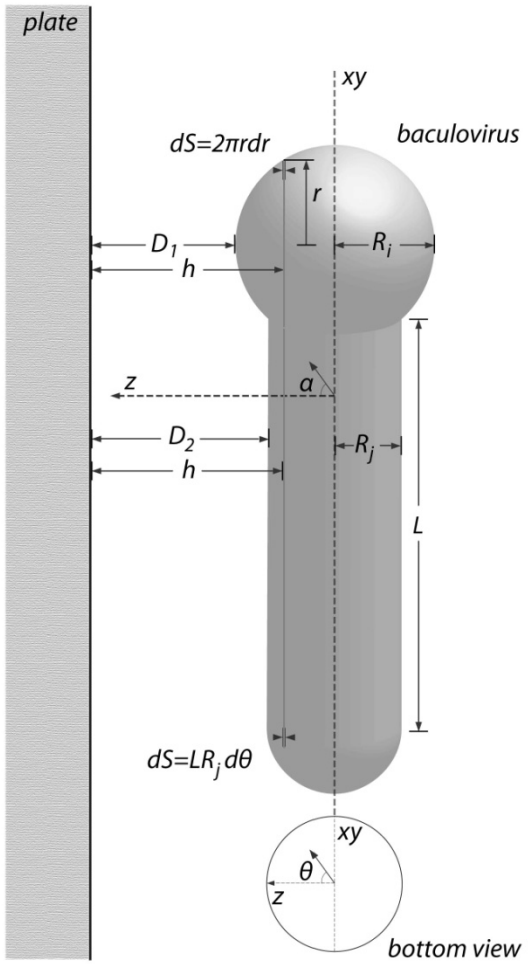




## Baculovirus



# DLS (III): from ζ-potential data to a predictive model

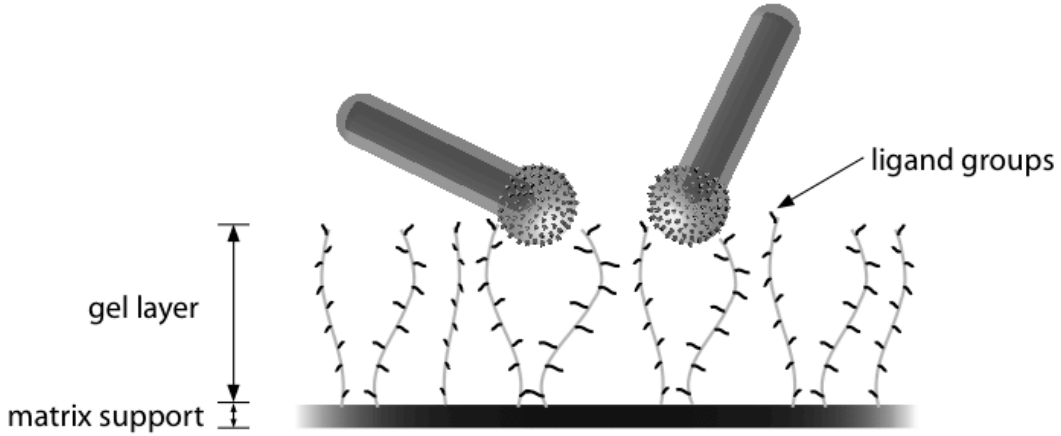



- Prediction of the interaction energies due to electric double layer at different conditions
- 6 < pH < 10
- rBV stability does matter!

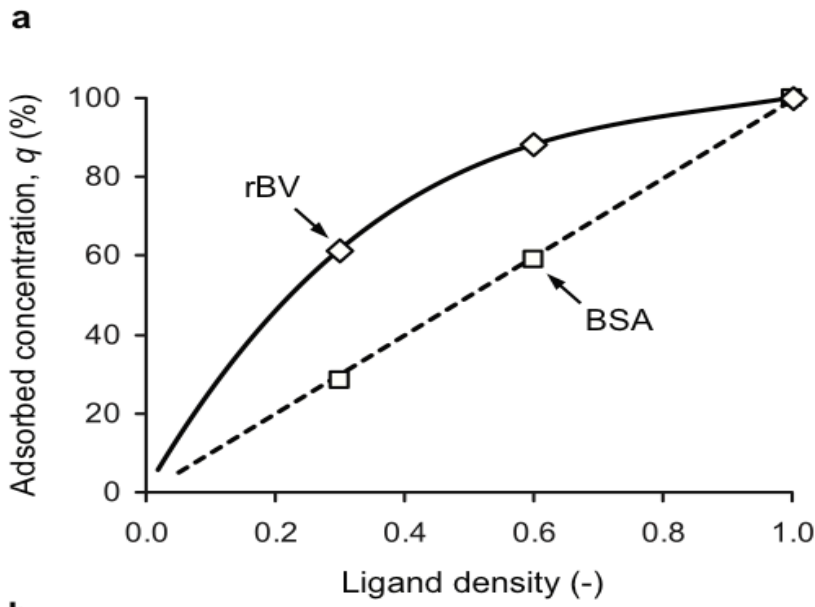
Vicente *et al*. 2010, *Journal of Chromatography A*, 1217:3754-3764

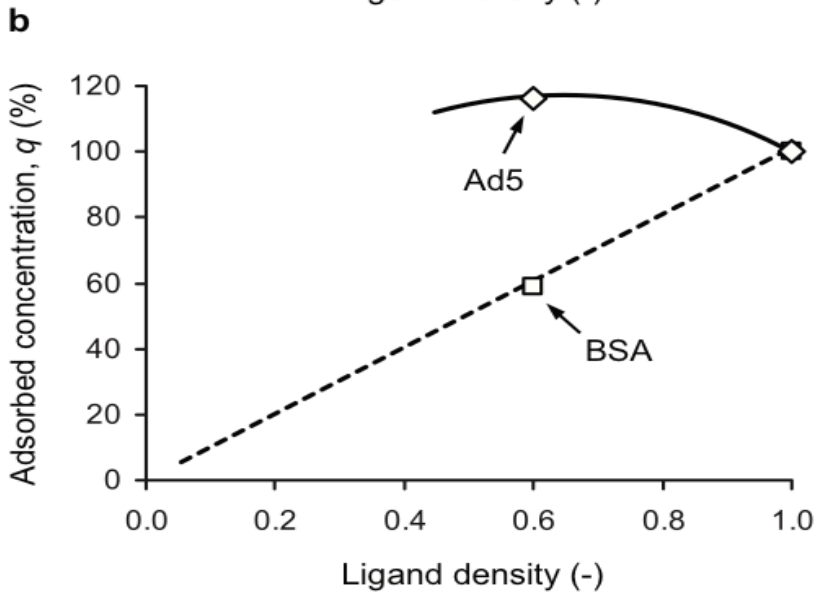
# Improving purification performance: assessing ligand density




Vicente *et al*. 2011, *Biotechnology and Bioengineering*, 108:6 1347-1359

- Decreasing ligand density does not affect virus binding as much as protein
- The large size of the viruses prevents them to access all ligand sites



# Improving purification performance: assessing membrane prototypes

**Table 1. Results of DoE-based study of the impact of ligand density (LD, $\mu$mol/cm$^2$), salt equilibration (SE, mM), and gradient length (GL, MV) on binding and elution of rBVs, measured in terms of recovery of infective viruses (IP, %), TV/IV ratio†, dsDNA (ng/10$^8$ IP), and total protein (HCP, mg/10$^8$ IP)**

| LD | SE | GL | IP | TP/IP | dsDNA | HCP |
|---|---|---|---|---|---|---|
| 2.2 | 30 | 40 | 73 | 4 | 5 | 61 |
| 2.2* | 30 | 40 | 47 | 6 | 15 | 121 |
| 2.2 | 30 | 40 | 82 | 4 | 8 | 35 |
| 2.2 | 45 | 120 | 73 | 4 | 9 | 66 |
| 2.2 | 60 | 40 | 83 | 5 | 7 | 37 |
| 2.2 | 60 | 120 | 75 | 5 | 7 | 31 |
| 5.0 | 50 | 40 | 70 | 6 | 8 | 55 |
| 5.0 | 50 | 80 | 64 | 6 | 4 | 35 |
| 5.0 | 50 | 80 | 56 | 9 | 14 | 51 |
| 5.0 | 50 | 120 | 59 | 8 | 8 | 45 |
| 6.1 | 30 | 40 | 52 | 24 | 120 | 101 |
| 6.1 | 30 | 120 | 28 | 11 | 168 | 231 |
| 6.1 | 60 | 40 | 59 | 2 | 105 | 101 |
| 6.1 | 60 | 120 | 50 | 6 | 143 | 151 |

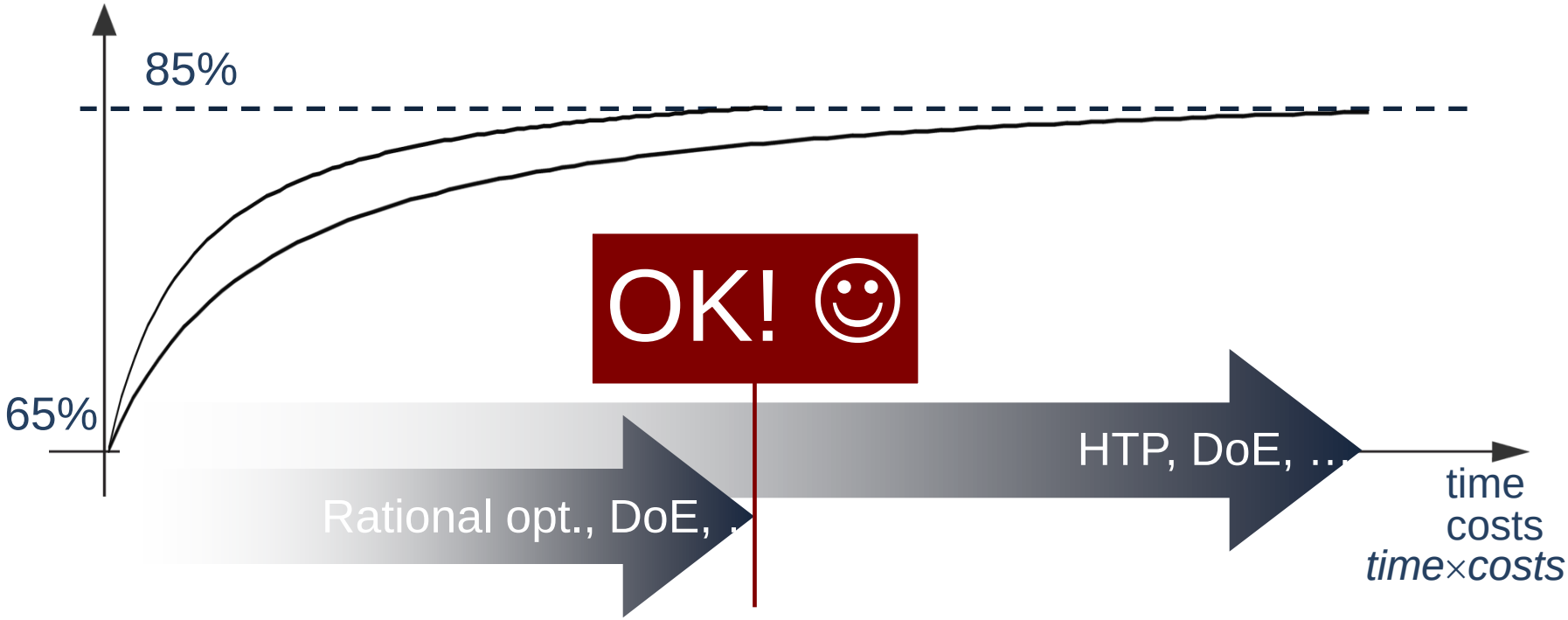
*An outlier experiment.

†The total to infective virus particle ratio, TP/IP, provides a means for estimating virus quality.

- ✓ SPR strategy set exploratory adsorption studies
- ✓ DLS strategy set operating conditions, pH 7.2, I = 30 – 60 mM NaCl

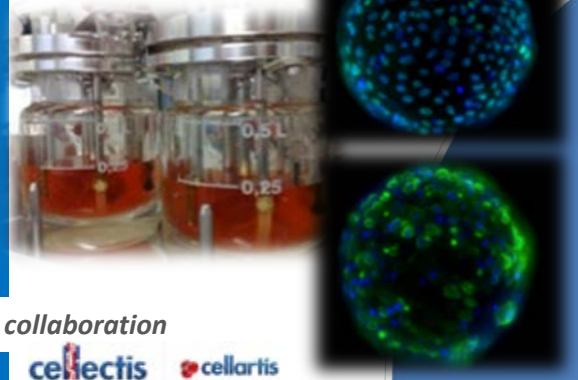
- 65% to 85% recovery yield improvement
- Lower TP/IP, HCP, dsDNA

*Product recovery yield*




a synergistic strategy

# BIOPROCESSING OF HUMAN STEM CELLS FOR CLINICAL APPLICATION @ iBET

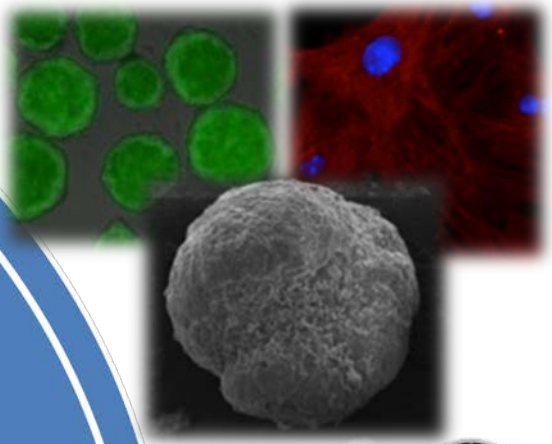


Clinical-grade expansion of **hESCs & hiPSC**

Production, Enrichment and Maturation of Cardiomyocytes derived from **hiPS cells**

Production & Characterization of **hCSC**

Up- and Down-stream processing of **hMSCs**



*collaboration*

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*collaboration*

Murdoch Childrens Research Institute

CARE Mi

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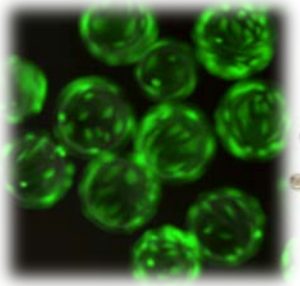
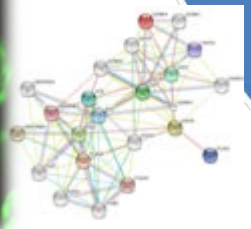
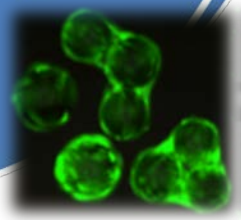
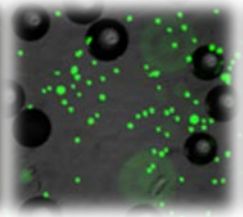
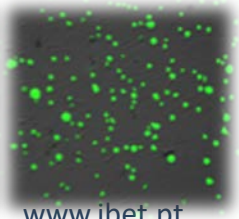










*collaboration*

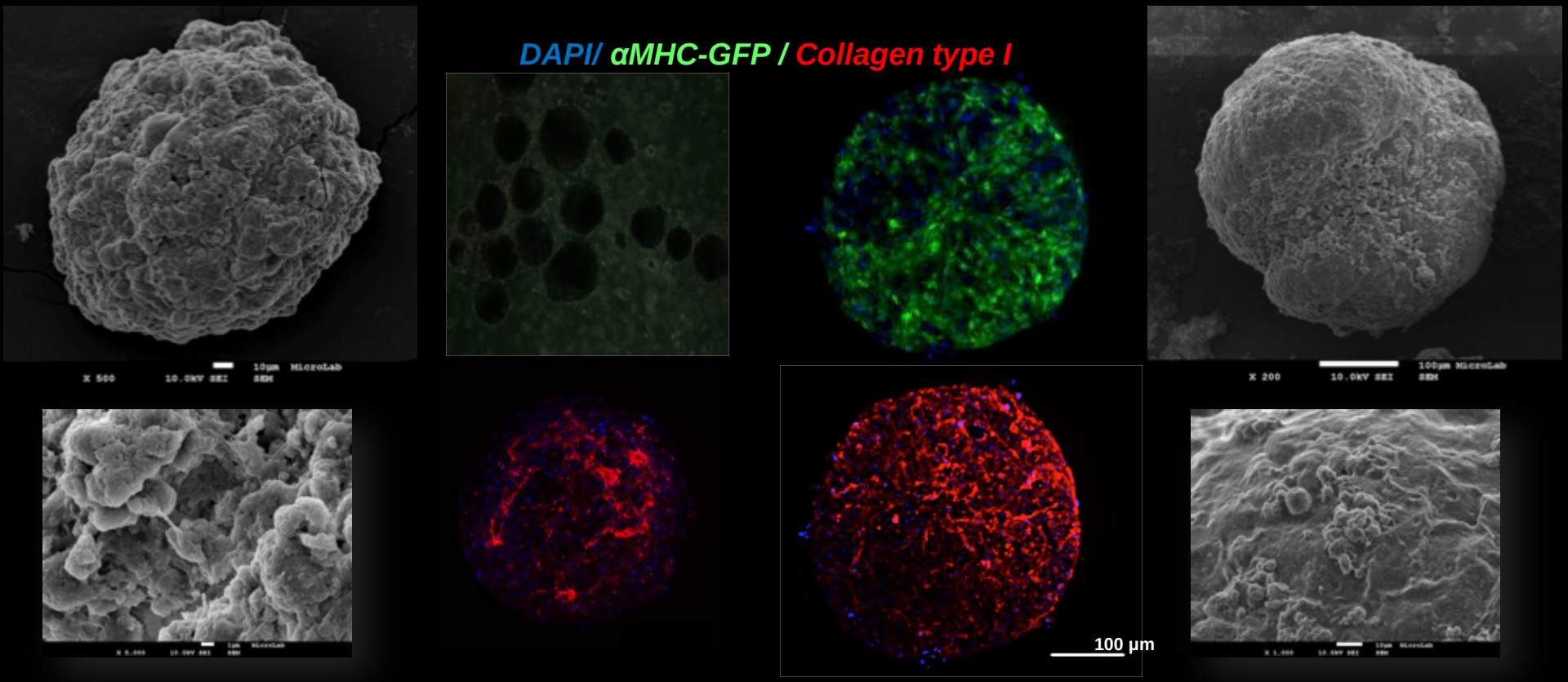
cnic ceretherapix

# Impact of the Agitation Type: Stirred Tank vs. Wave BR

## Scanning Electron Microscopy & Confocal Microscopy (day 9 – before Purification)

### Stirred Tank Bioreactor

### Wave Bioreactor




## Wave-induced Agitation Accelerates Differentiation of iPS cells into CMs

✓ Higher deposition of Collagen Type I

*Correia et al 2014, Stem Cell Rev Rep*

# PRODUCTION OF iPSC-CMs: RESULTS

- ***MURINE iPSC – EB-BASED DIFFERENTIATION***

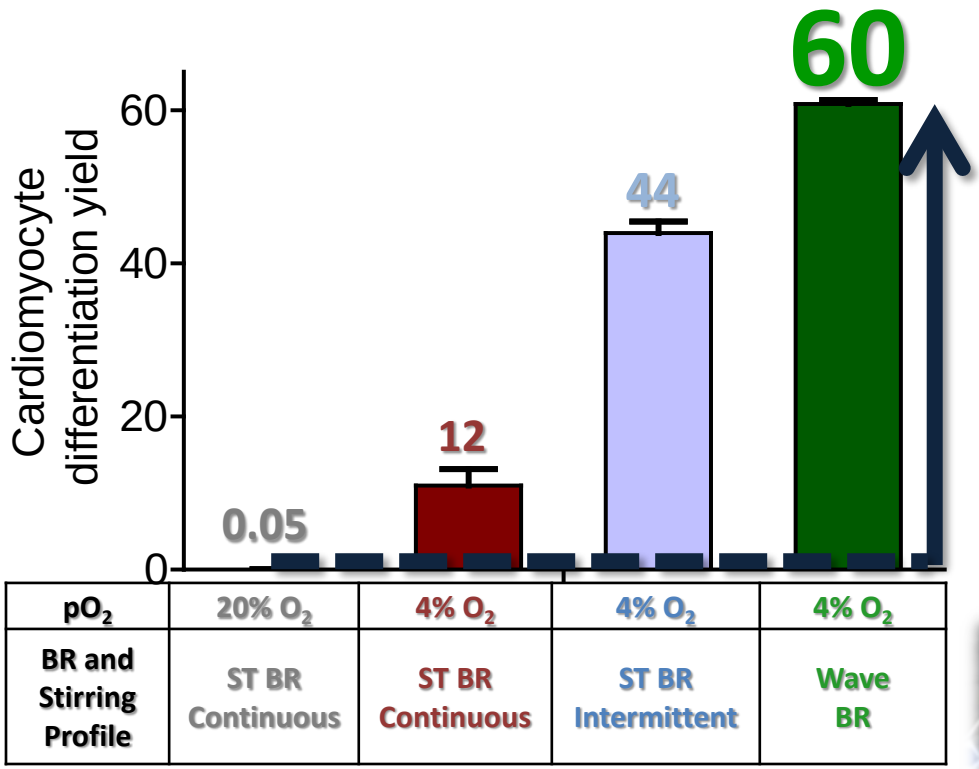
**The use of environmentally controlled bioreactors is critical to ensure *Efficient* and *Scalable* production of iPSC-derived CMs**

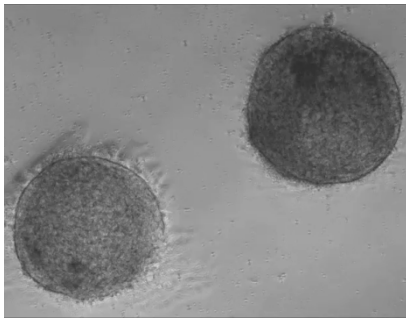
Stem Cell Rev and Rep (2014) 10:786–801
DOI 10.1007/s12015-014-9533-0

**Combining Hypoxia and Bioreactor Hydrodynamics Boosts Induced Pluripotent Stem Cell Differentiation Towards Cardiomyocytes**

Cláudia Correia · Margarida Serra · Nuno Espinha · Marcos Sousa · Catarina Brito · Karsten Burkert · Yunjie Zheng · Jürgen Hescheler · Manuel J. T. Carrondo · Tomo Šarić · Paula M. Alves

**Cardiomyocyte Differentiation Yield** = $\frac{\text{No. of CMs}}{\text{No. of iPS cells}}$



**2.3 x10$^9$ CMs**
*per 1L Wave bioreactor*

*Correia et al 2014, Stem Cell Rev Rep*

# - USP OF hMSC: CELL EXPANSION

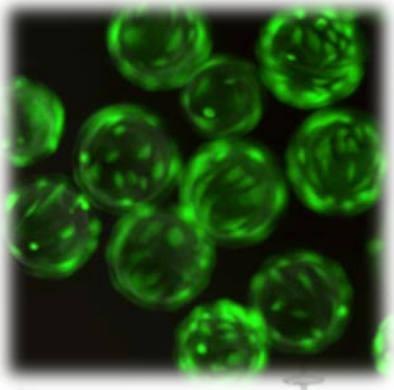
## Production of hMSCs (different sources: bone marrow, adipose tissue, umbilical cord tissue)

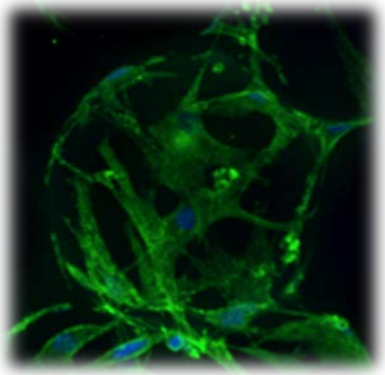
*(for Autologous and Allogeneic Therapies)*

apceth
applied cell therapy

## UPSTREAM BIOPROCESSING

AIM: Increase cell <u>volumetric productivities</u> (cell/mL) without compromising cell quality **(viability, identity and potency)**





## Bioprocess Development:

- Microcarrier type and concentration
- Culture operation mode (fed-batch, perfusion)
- Environmental conditions (e.g. $pO_2$)
- Establishment of cGMP compatible processes
- Process scale-up (from 100mL to 2L)

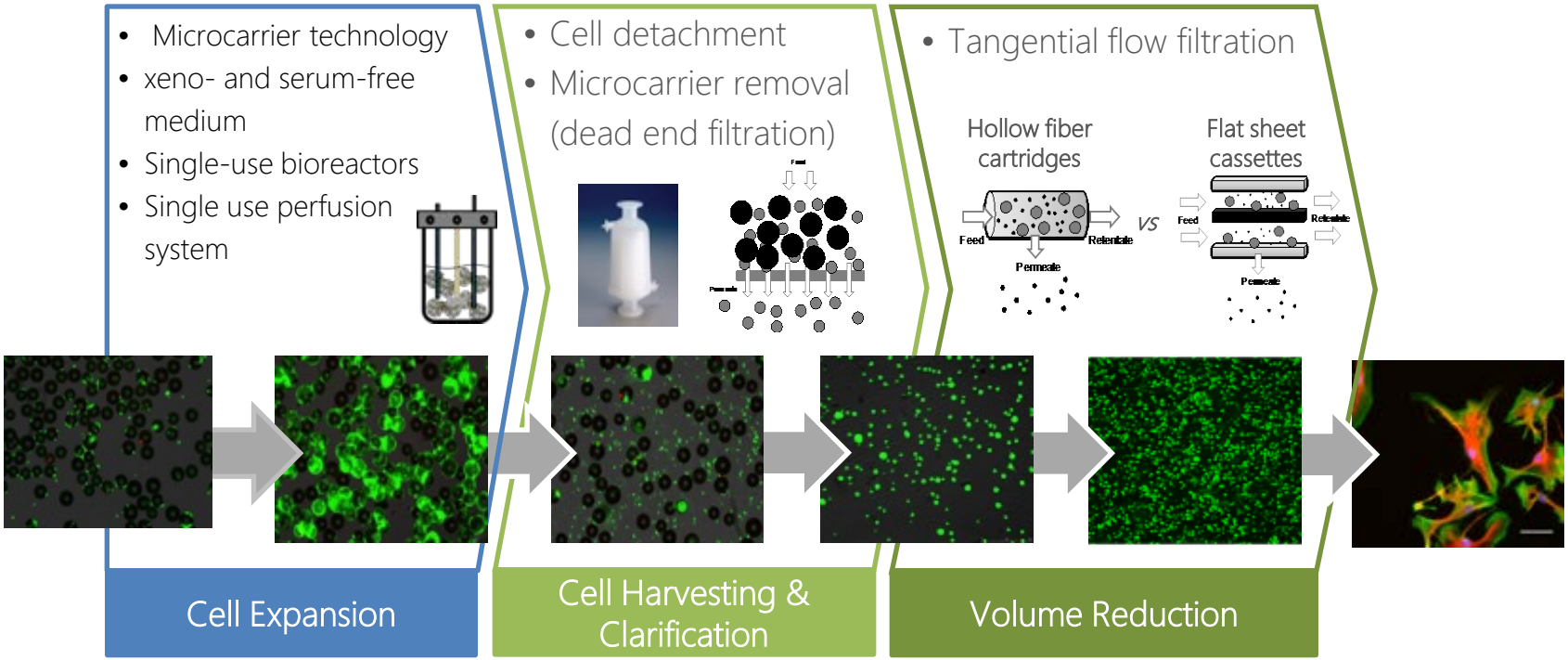
Refine TECHNOLOGY

PBS BIOTECH

*Cunha B et al 2015, Journal of Biotechnology*
*Sousa et al 2015, Biotechnology Progress*

# SINGLE USE AND CELL THERAPY

Develop and prove **scalability** of an **integrated** and streamlined (and cGMP-compatible) bioprocess comprising cell expansion, harvesting, clarification and volume reduction operations for hMSCs (hMSC-BM and hMSC-AT)




Evaluate the impact of critical process parameters on cell's viability, recovery and purity

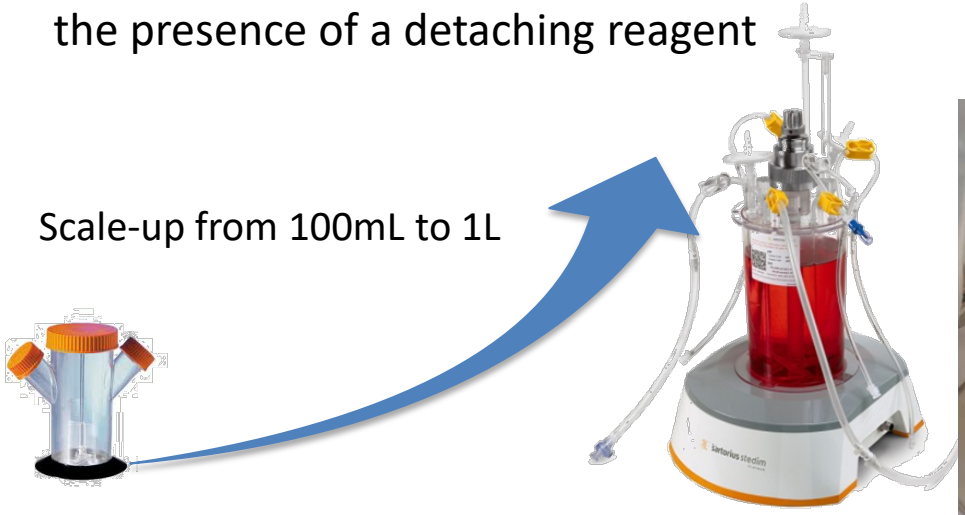
Establish a proteomics workflow based on Mass Spectrometry tools to characterize the impact of processing on cells' CQA

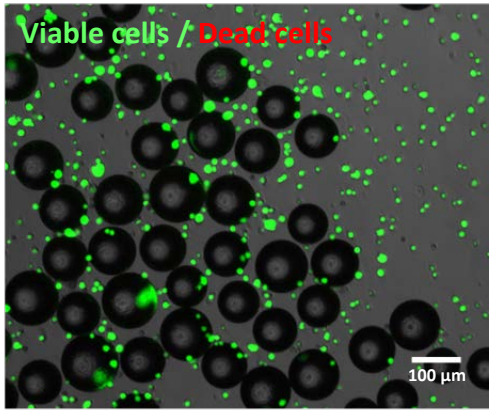
# Cell Harvesting: (1) Scale-up cell detachment step in Bioreactors

**Cell detachment from microcarriers in bioreactors :** short periods of intense agitation in the presence of a detaching reagent

*Protocol adapted from Nienow et al 2014*

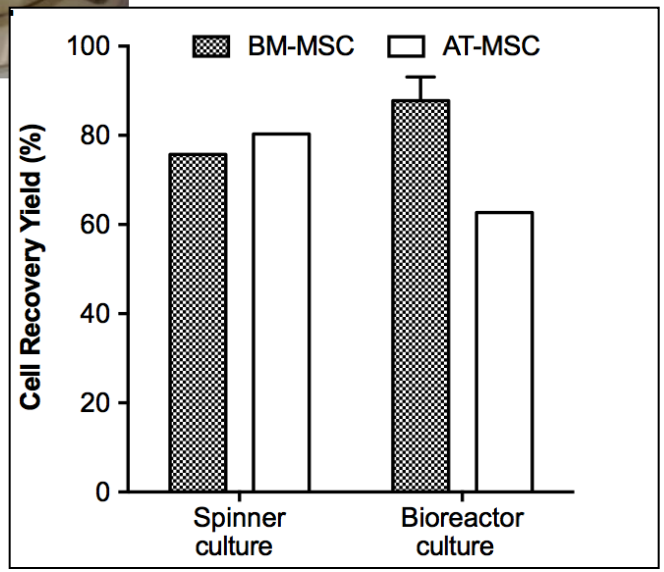
Scale-up from 100mL to 1L






**Scale-up criteria:** P/V (power transferred by the impeller per volume, W/m$^3$) constant

$$P_1 = P_2 \Leftrightarrow \left(\frac{N_p \rho N^3 D_i^5}{V}\right)_1 = \left(\frac{N_p \rho N^3 D_i^5}{V}\right)_2 \Leftrightarrow N_2 = \sqrt[3]{\left(\frac{N_p \rho N^3 D_i^5}{V}\right)_1 \left(\frac{V}{N_p \rho D_i^5}\right)_2}$$




hMSC-BM: similar cell recoveries were achieved after scale-up (cell viability > 90%)
hMSC-AT: lower cell recovery after scale-up

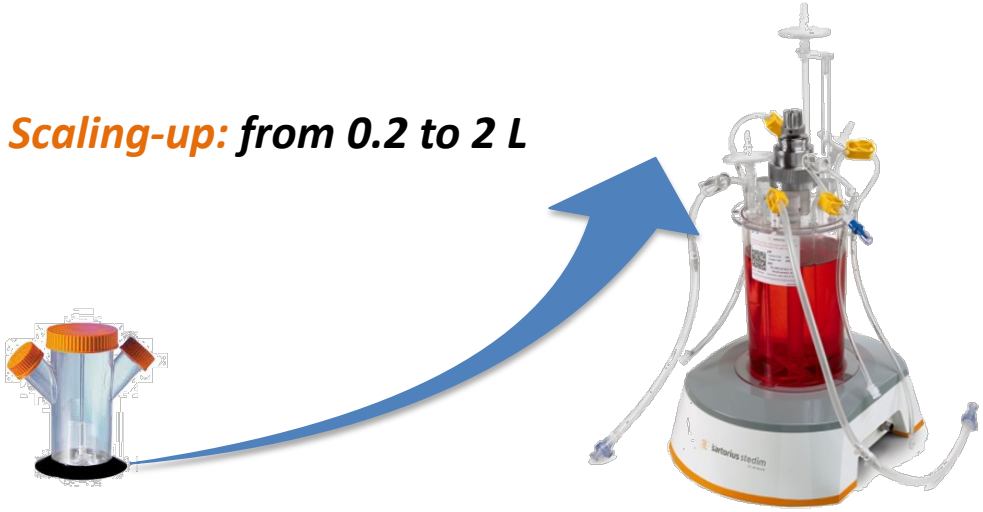


Cunha B et al, Journal of Biotechnology,213:97-108, 2015

# CELL CLARIFICATION: (3) SCALE-UP

## *Evaluation of scalable strategies for microcarrier removal*

***Scaling-up:*** ***from 0.2 to 2 L***



**OptiCap® XL 1 Capsules (EMD Millipore)**
(polypropylene, 100 µm pore size)

| | Process Scale | Cell Recovery (%) | Cell Viability (%) |
|---|---|---|---|
| BM-MSC | 0.2 L | > 90% | > 95% |
| | 2 L | 94% | 98% |
| AT-MSC | | 95% | 98% |

✓ **Cell recovery yields and viability** were maintained after scale-up

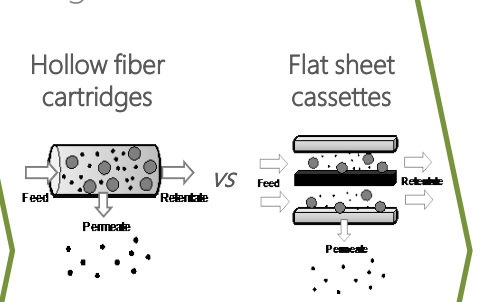


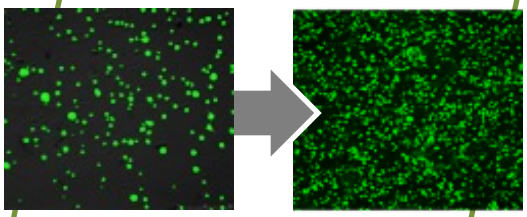
Cunha B et al 2015, Journal of Membrane Science, 478:117-129 | Cunha B et al (2013) BMC Proceedings, 7 (Suppl 6)

# VOLUME REDUCTION: AIMS

## Aims

- Tangential flow filtration



Volume Reduction

**1** Impact of **critical process parameters** (CPP) (ex: membrane material, pore size, shear rate, permeate flux) on cells' CQA and recovery yield

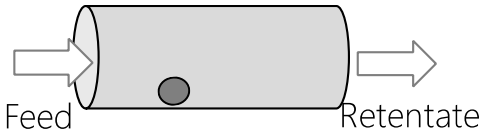
**2** Process **scale-up** (up to 2 L): impact **of TFF device** ( Hollow Fibers *vs* Flat Sheet Cassettes)

**3** Process **validation** with 2 hMSC (hMSC-BM and hMSC-AT)

**ENSURE HIGH VOLUME REDUCTION FACTORS ( up to 50X)**

# VOLUME REDUCTION: (1) IMPACT OF CRITICAL PROCESS PARAMETERS

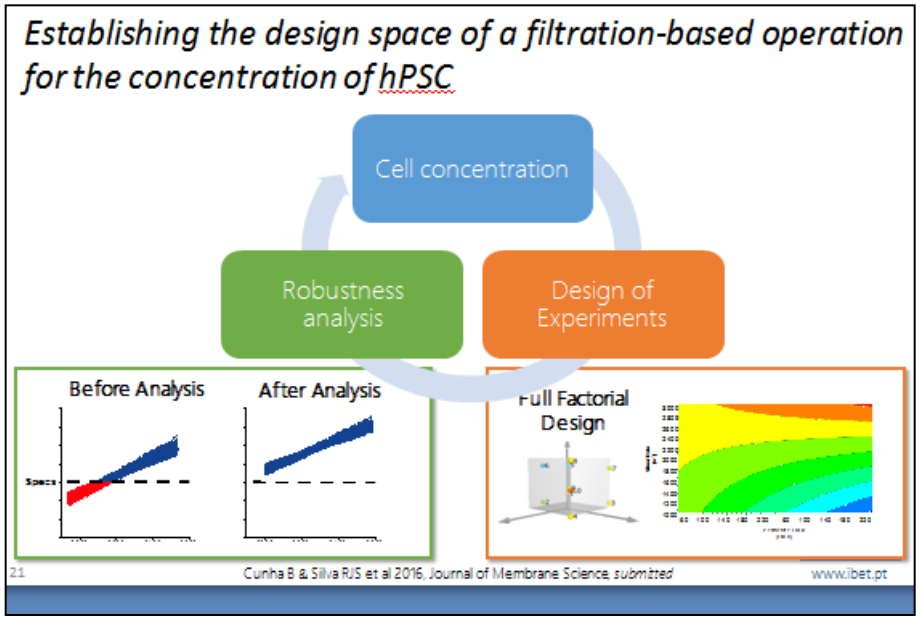
In *Cunha et al (2015) Journal of Membrane Science*:




TFF device: Hollow fibers

✓ Applicability of TFF to concentrate hMSC and hPSC (up to a VRF of 20);
✓ Impact of TFF's parameters on cell recovery yield and characteristics;

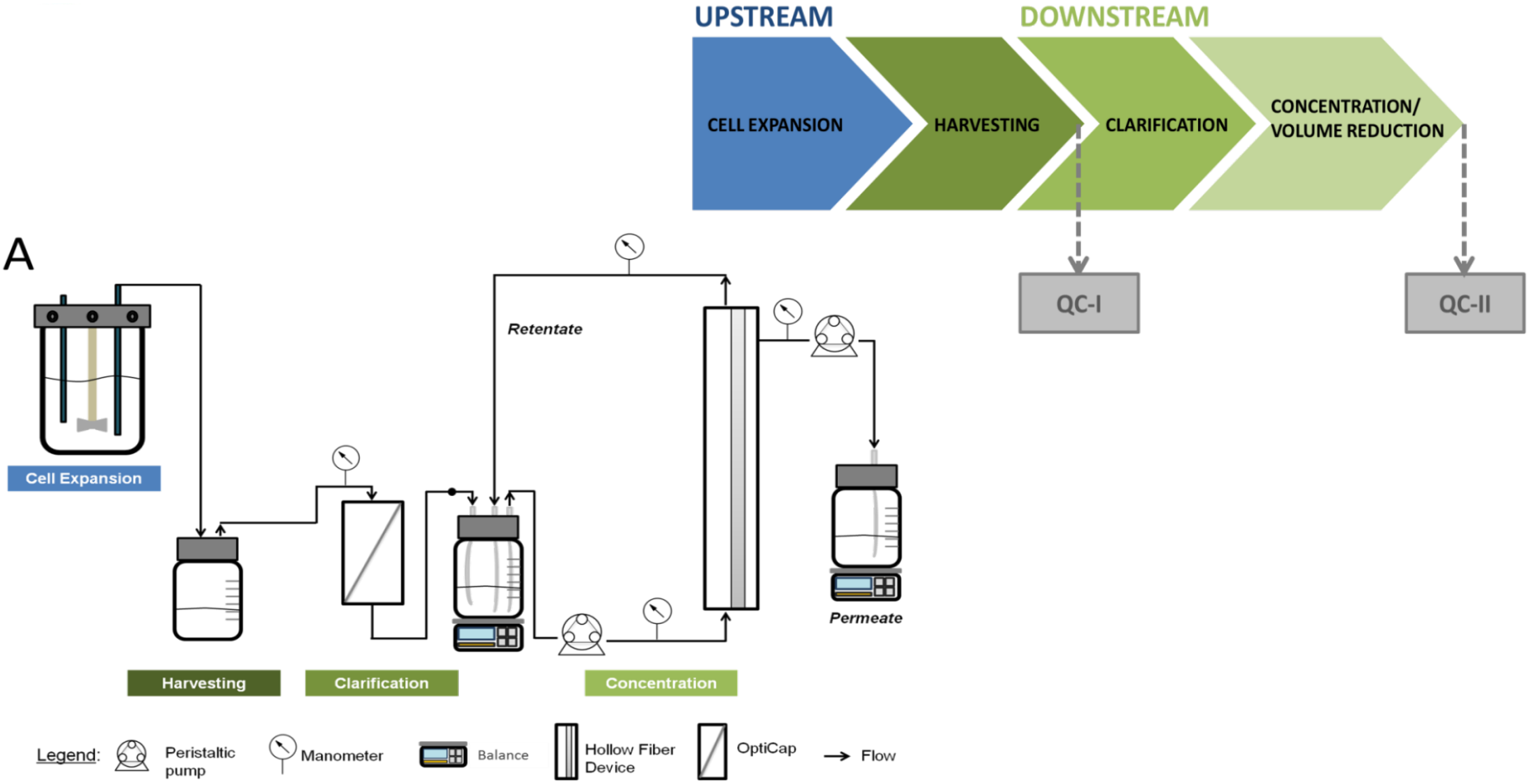
- **Membrane's material**
  Polysulfone
- **Pore size**
  > 0.45 µm
- **Initial cell concentration**
  > 2 x $10^5$ cell/mL
- **Shear rate**
  3000 $s^{-1}$
- **Permeate flux**
  250 LMH
- **Operation mode**
  Discontinuous TFF






Cunha B et al, Journal of Membrane Science, 478:117-129, 2015

# Process Integration




**Integration:**

Closed system, resulting in the elimination of hold steps and decreasing the equip. footprint

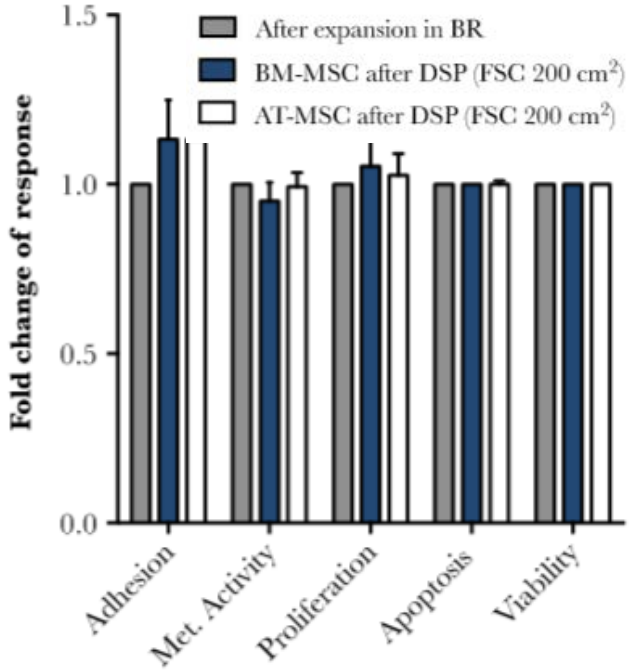


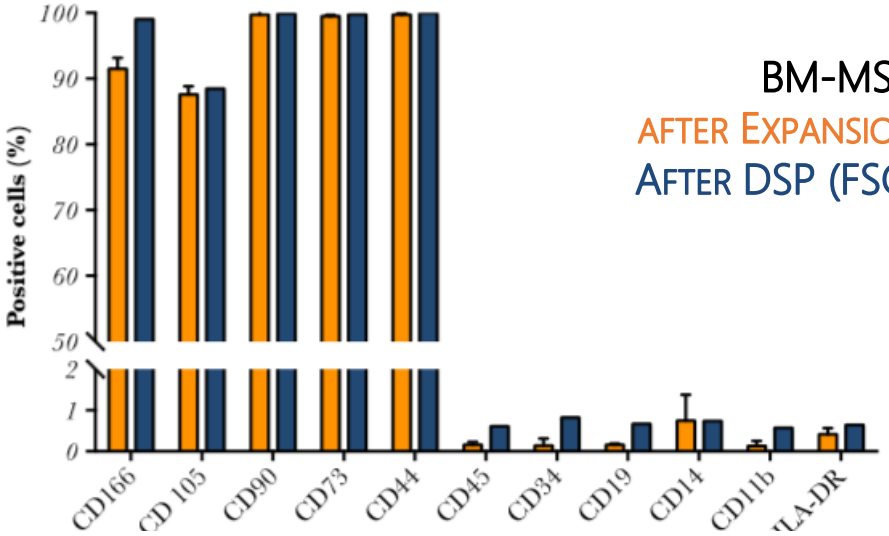
Cunha B et al 2015, Journal of Biotechnology, 213:97-108; Cunha B et al 2017, Journal of Biotechnology, *in press*

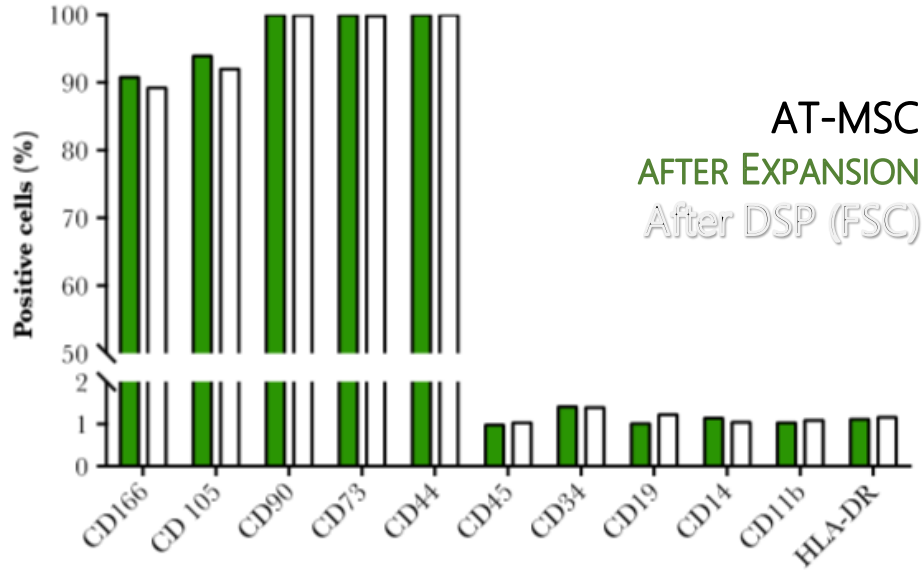
# Process Integration- Cell Characterization

## Cells maintain their quality attributes after DSP

*Apoptosis, adhesion, viability*




✓ hMSC maintained their immunophenotype and metabolic activity after processing;

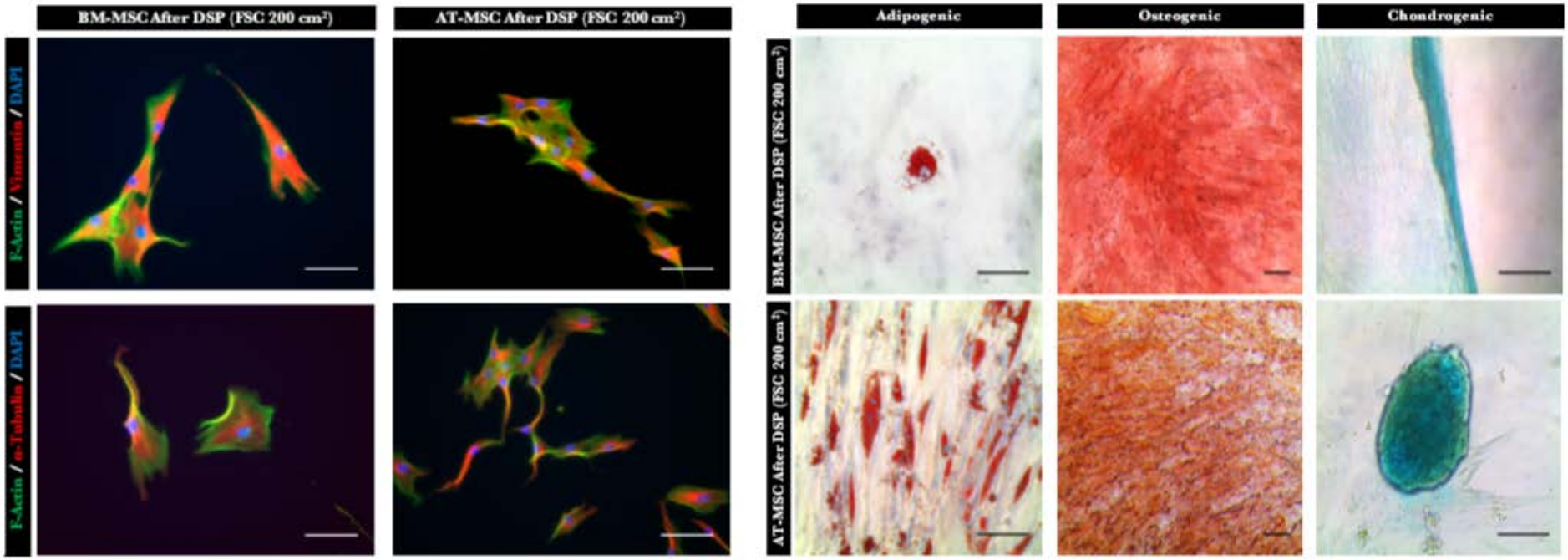
✓ Ability to adhere to plastic surfaces and proliferative capacity after re-plating



Cunha B et al 2015, Journal of Biotechnology, 213:97-108; Cunha B et al 2017, Journal of Biotechnology, *in press*

# Process Integration- Cell Characterization

## Cells maintain their critical quality attributes after DSP




**Morphology and adhesion: hMSC successfully re-acquire their typical spindle-like morphology with organized actin fibers**

**hMSC maintained their multilineage differentiation potential**



Cunha B et al 2015, Journal of Biotechnology, 213:97-108

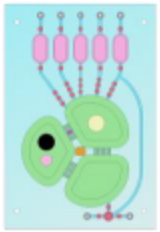
# Features of the Micro-bioreactor

Pall 24

SIM Cell

HTBR


Ambr (TAP)



µBR

| Capabilities | Pall 24 | SIM Cell | HTBR | Ambr | µBR |
|---|---|---|---|---|---|
| Comprehensive online sensors | No | No | No | No | Yes |
| Completely independent | No | No | No | No | Yes |
| Compatible with existing analysis tools | Yes | No | Yes | Yes | Yes |
| Independently sterile | No | No | Yes | No | Yes |
| Automated feeding | No | No | No | No | Yes |

## Micro-bioreactor features:

- Mimics environments of larger bioreactors
- Online monitoring of 4 parameters: pH, DO, DCO2 and OD
- Sufficient Volume for offline analysis & complete protein characterization

# Fluorescence-based bioprocess monitoring

**Metabolites with intrinsic fluorescence**

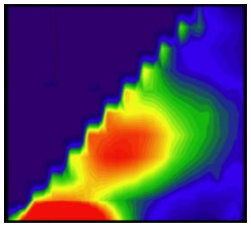
**Amino acids**: Tryptophan, Tyrosine, Phenylalanine

**Cofactors**: NADH, NADPH, FAD

**Vitamins**: pyridoxine, thiamine, riboflavin, folic acid

**Fluorescence Excitation-Emission Matrix**

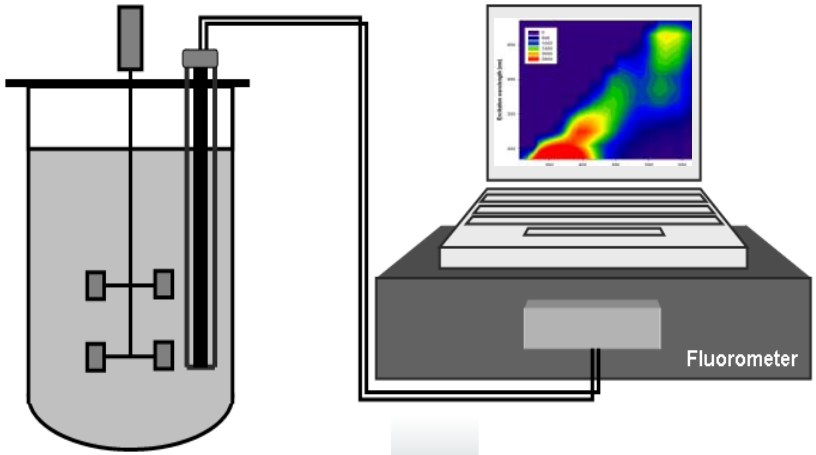
**or Fluorescence Map**



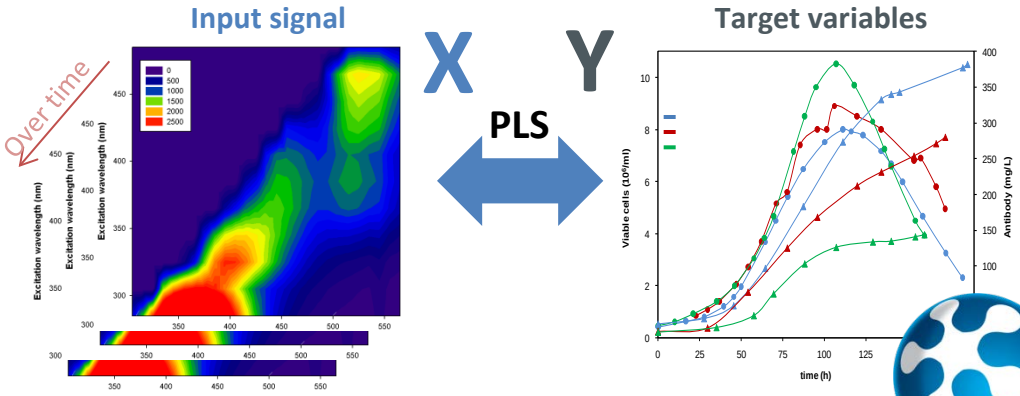
## Data collection over time

*Online fluorescence data*

*Off-line measurements of target variables*




## Multivariate Data Analysis




## Advantages

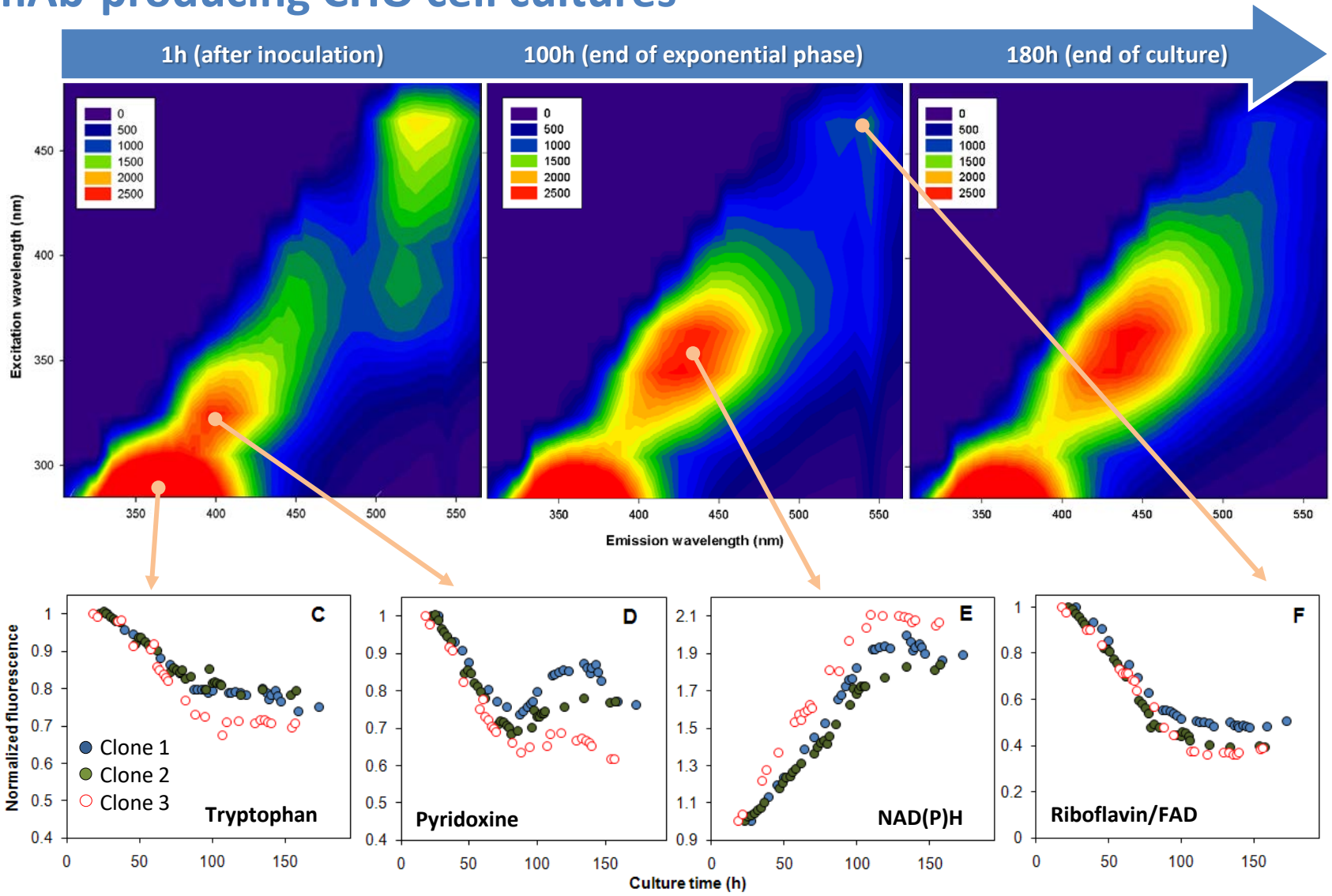
- ✓ Multi-component analysis
- ✓ Different orders of magnitude
- ✓ Non-destructive and non-invasive
- ✓ Remote monitoring


Teixeira *et al.* (2011) *Biotech Bioeng* 108: 1852-1861

Teixeira *et al.* (2011) *J Biotech* 151:255-260

# Fluorescence maps over time

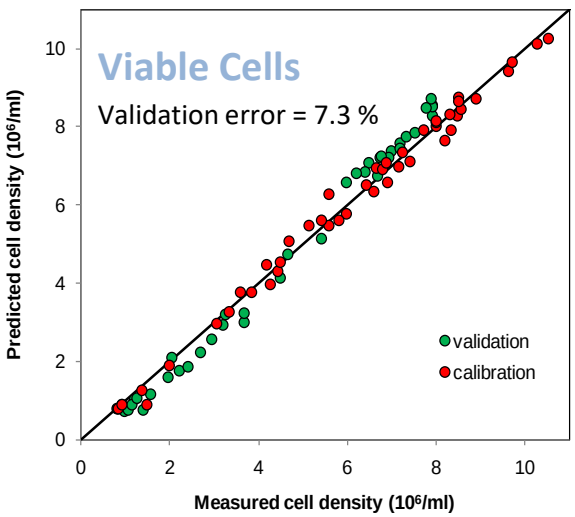
## mAb-producing CHO cell cultures

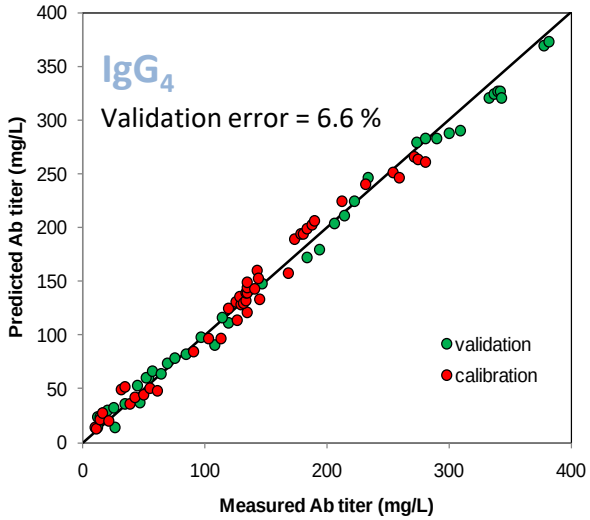



Teixeira *et al.* (2011) *Biotech Bioeng* 108: 1852-1861

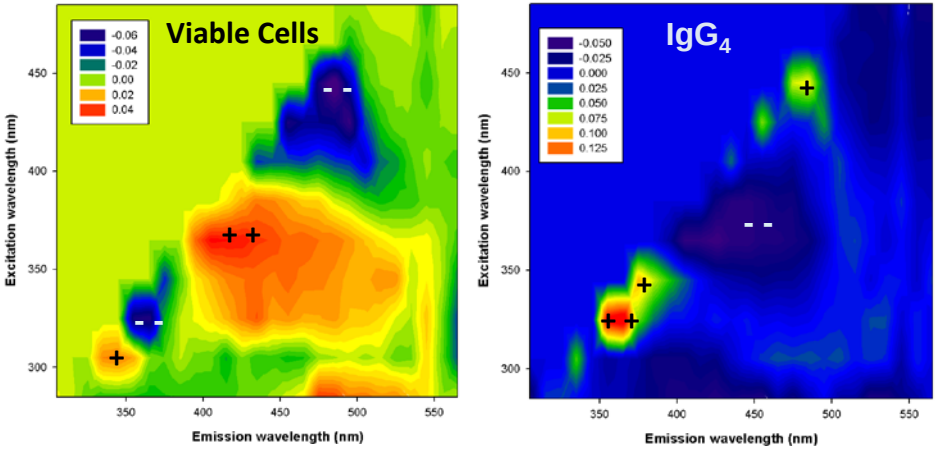
# Fluorescence-based predictors

## Model predictions




## Relevant $\lambda_{ex/em}$ pairs







**Good agreement** between model & offline measurements

✓ **2D fluorometry** suitable for real-time monitoring of cell and mAb concentration

Teixeira *et al.* (2011) *Biotech Bioeng* 108: 1852-1861

# - THE ANIMAL CELL TECHNOLOGY UNIT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# Thank you

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