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Very high cell density perfusion of CHO cells in disposable bioreactor, challenge or reality

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Perfusion in industry for animal cell

- Majority of fed-batch processes world-wise
- Perfusion used 'traditionally'
  - for unstable proteins
  - in companies or lab's with 'culture' perfusion, i.e. where knowledge, competence and PEOPLE are present
- Perfusion
  - more technically challenging → higher risk of failure, higher risk of contamination
  - smaller cultivation vessel
  - less process development
  - constant cellular environment is beneficial for cell metabolism and product quality
- Perfusion equipment robust and disposable
  - robust equipment → ~~higher risk of failure~~
  - disposable equipment → ~~higher risk of contamination~~

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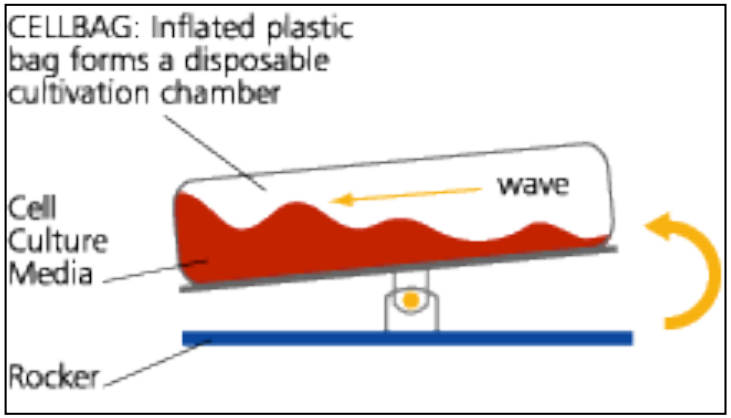
Three systems studied at CETEG (KTH)

	Collaborations
• WAVE Bioreactor™ equipped with ATF	GE Healthcare (Sweden, USA)
• WAVE Bioreactor™ equipped with TFF	GE Healthcare (Sweden, USA)
• CellTank™	CerCell (Denmark), Belach (Sweden)

WAVE Bioreactor™ in perfusion with ATF or TFF

- Goal
  - Evaluation of disposable WAVE Bioreactor™ in perfusion mode
  - Evaluation of two types of cell separation based on hollow fiber filtration:
    - Alternating Tangential Flow filtration
    - Tangential flow filtration
  - Evaluation of the limits of the system



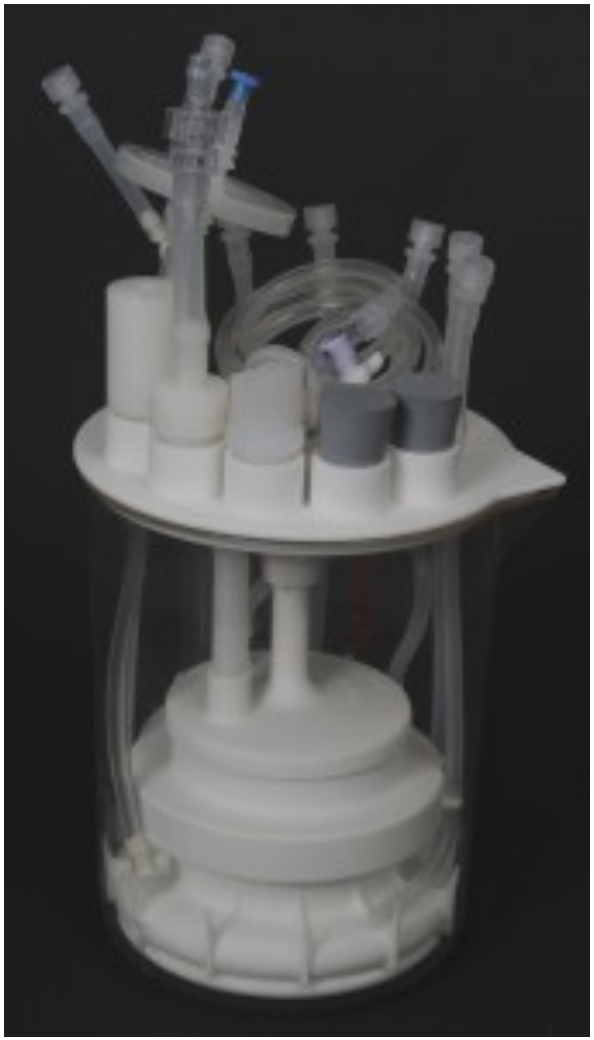
WAVE Bioreactor™
source: http://www.gelifesciences.com

- Strategy
  - Cell line #1 = IgG producing Chinese Hamster Ovary cells
  - Study of perfusion → learning phase and study of the equipment
  - Study of perfusion → study of the limits of the system
  - Evaluation for application of IgG production and comparison with fed-batch
  - Evaluation for application of cryopreservation / cell banking

CellTank™ in perfusion mode

- Goal
  - Evaluation of disposable CellTank™ in perfusion mode (CerCell, Denmark)
  - Evaluation of the system

- Strategy
  - Cell line #2 = IgG producing Chinese Hamster Ovary cells
  - Study of perfusion → trouble shooting / learning phase and study of the equipment
  - Study of perfusion →
    - Evaluation of the system at very high cell density
    - Evaluation of effect of temperature decrease

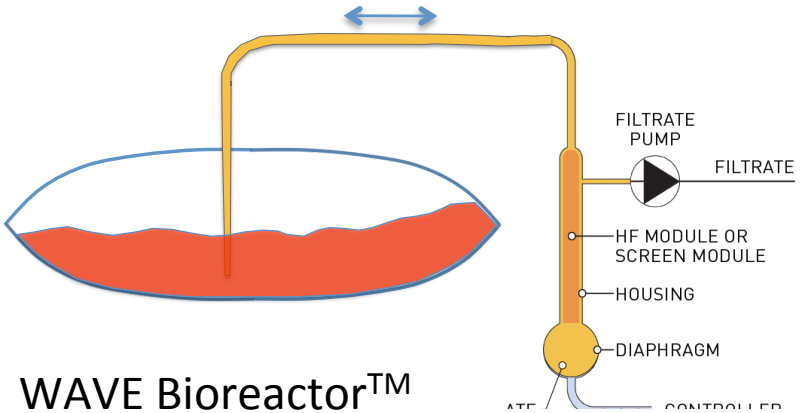


Introduction
and
System set-up

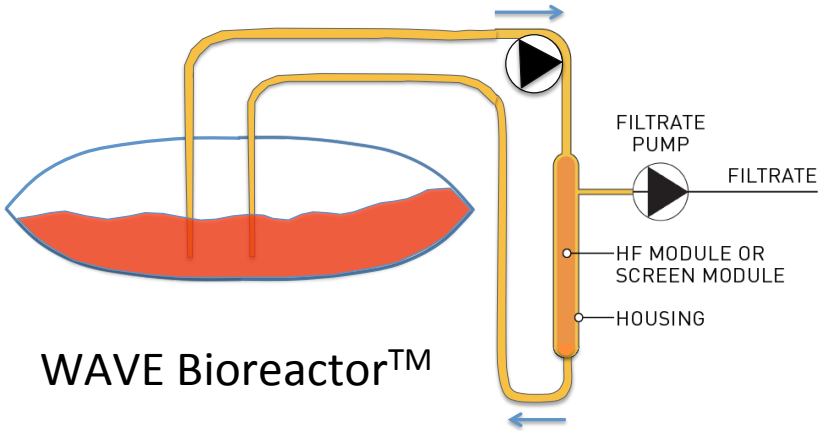
Perfusion devices connected to WAVE Bioreactor™

ATF (REFINE Technology)
Alternating
Tangential
Flow
with
ReadyToProcess™ filter
GE Healthcare

TFF
Tangential
Flow
Filtration
with
ReadyToProcess™ filter
GE Healthcare

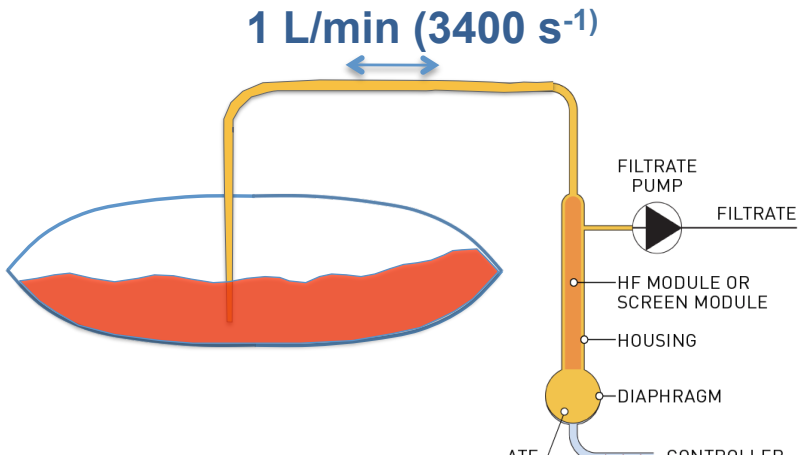


WAVE Bioreactor™

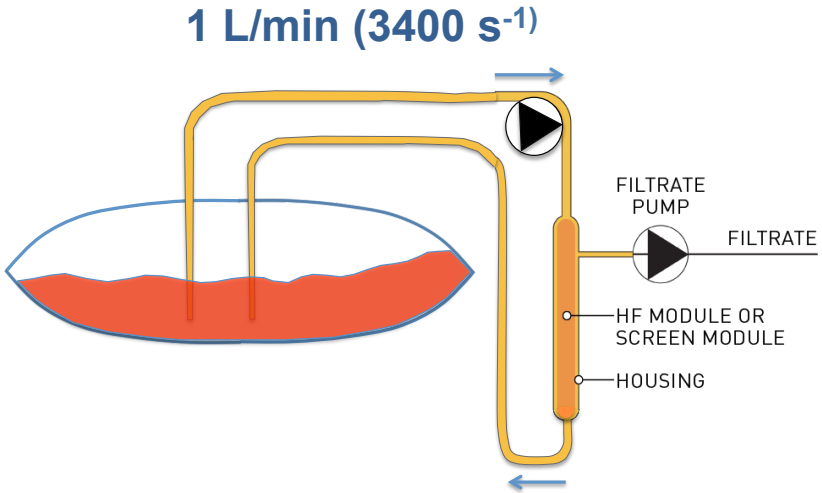


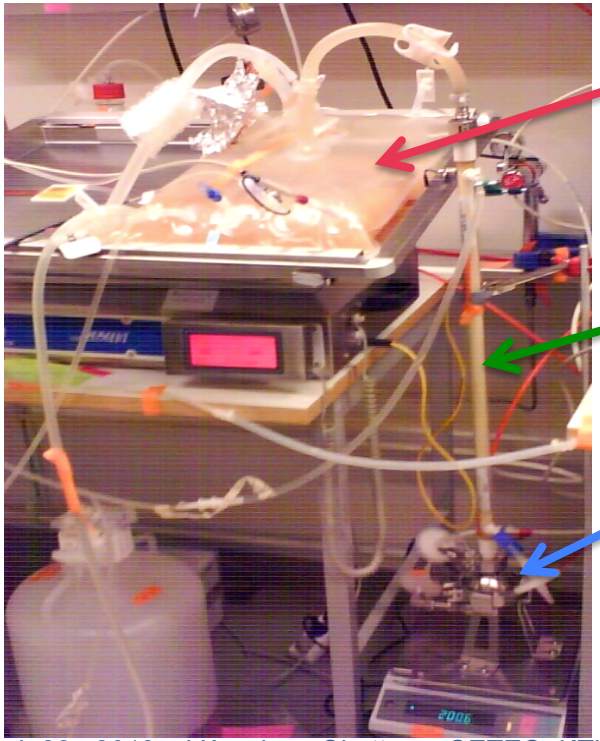
WAVE Bioreactor™

ATF & WAVE Bioreactor™



TFF & WAVE Bioreactor™





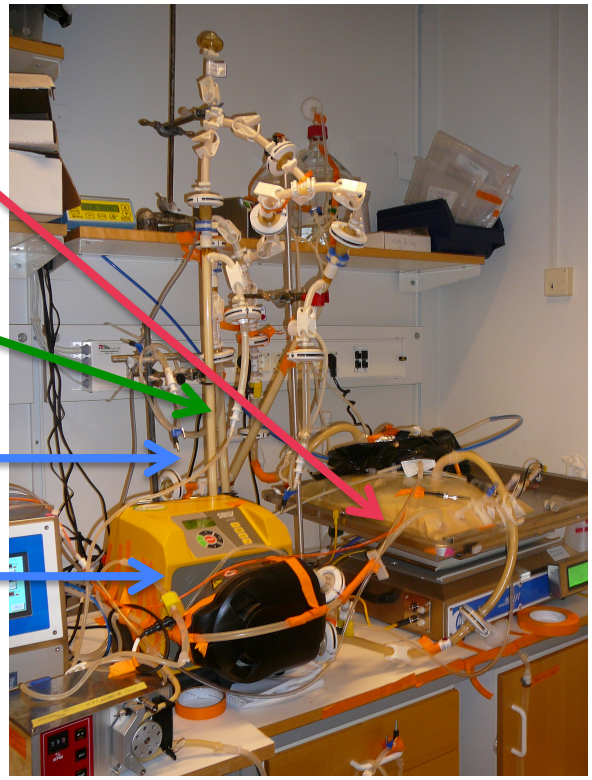
WAVE™ Cellbag 10 L

Hollow fiber filter

ATF-2

TFF

Pump Watson Marlow 620S



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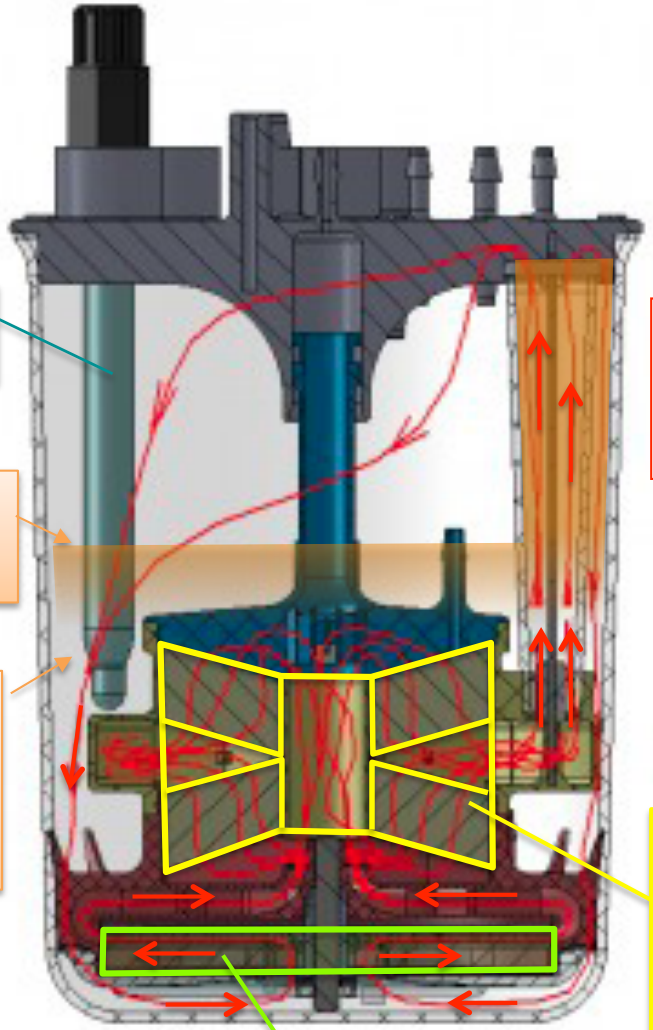
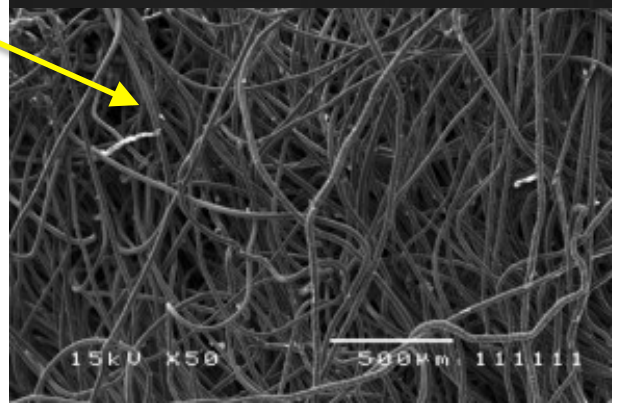
CellTank (CerCell)



→ Red arrows = fluid circulation

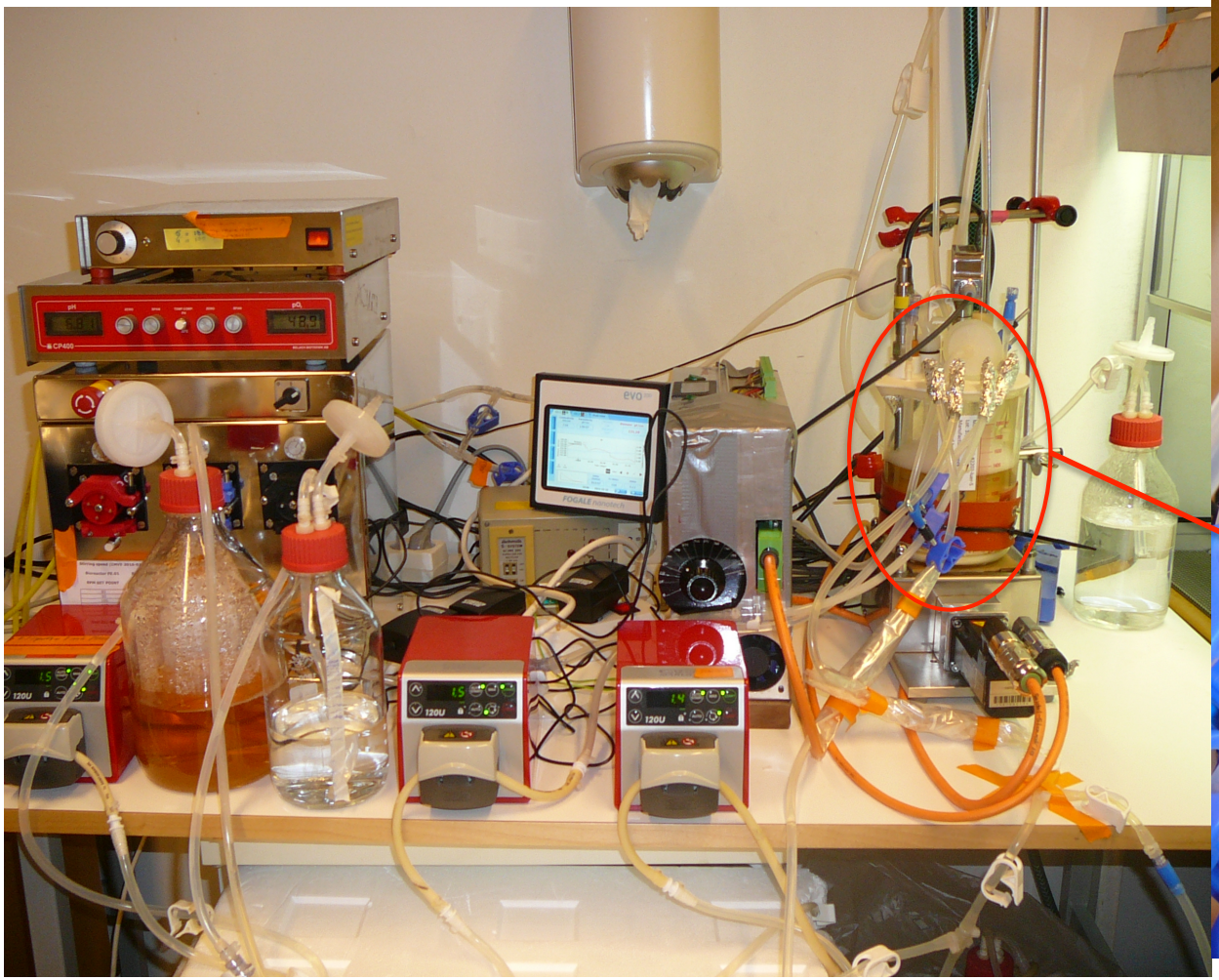
Matrix where cells are cultivated entrapped

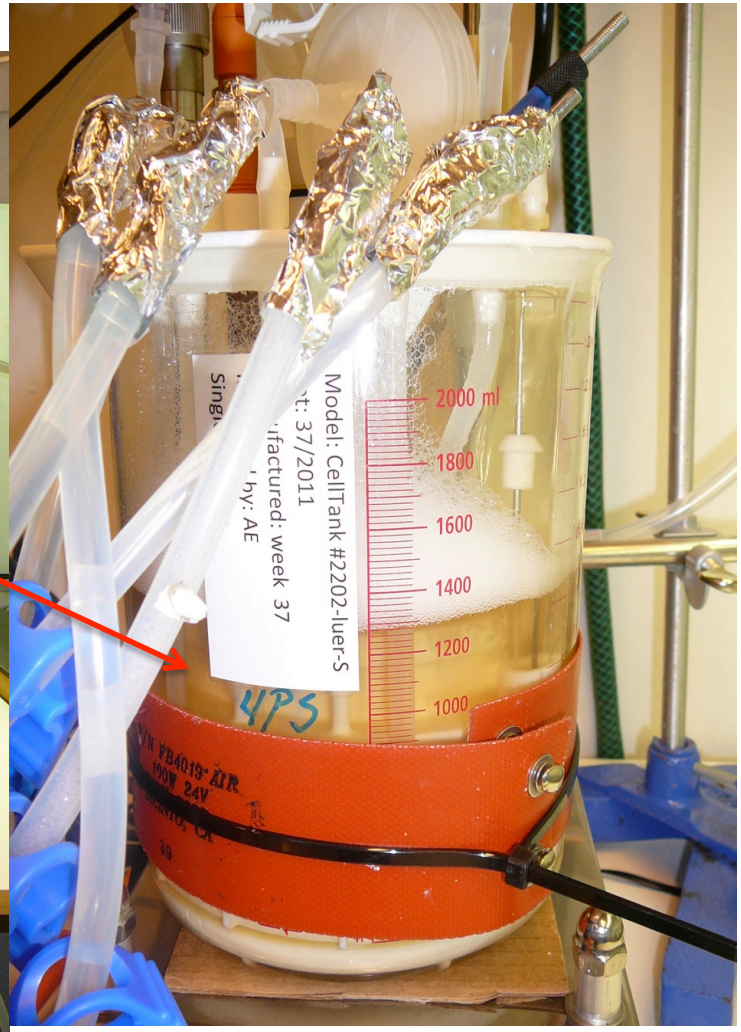
CellTank (CerCell) photo



Source http://cercell.com/

CellTank 2202 system at CETEG





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Experimental set-up TTF and ATF runs with Wave Bioreactor™

Cell line	mAb producing DHFR$^-$ CHO #1					
Bioreactor	10 L WAVE Cellbag™ with two dip tubes (GE Healthcare)					
Working volume	4 L					
Cell separation device	ATF-2 (Refine Technology) & ReadyToProcess™ filter					
	TFF ReadyToProcess™ filter via Watson Marlow 620S pump					
Hollow fiber filters (HF)	ReadyToProcess™ filter polysulfone RTPCFP-2-E-4X2MS (GE Healthcare)					
	pore size	0.2 µm	lumen	1 mm	filter area	850 cm^2
Recirculation flow rate*	(0.7 or) 1 L/min → shear rate 3400 s^{-1}					
Cell density specific perfusion rate	0.05 Reactor Volume/(day x 10^6 cells/mL)					
pH	7 control by adding 0.5 M Na_2CO_3 or pulsing CO_2 into headspace					
Temperature	37°C					
DO	35 % control by varying the agitation, O_2 addition into headspace (20-100%)					
Agitation rate / rocking angle	ATF 20-26 rpm / 6-7°					
	TFF 20-27 rpm / 6-8°					
Cultivation medium	animal-component free IS CHO CD XP medium with hydrolysate (Irvine Scientific) + 3 % of IS-CHO Feed-CD XP (Irvine Scientific)					
Supplementations of glucose and glutamine	according to cell consumption					
Addition of Antifoam C (SAFC)	up to 50 ppm concentration (boost addition or by continuous pumping)					
Analyses by Nova Bioprofile FLEX	cell density, viability, cell diameter, pH, pCO2, osmolality, concentrations of glucose, glutamine, lactate and ammonia					
Analysis of mAb concentration	protein A HPLC					

* or pressure rising flow (ATF) and exhaust flow (ATF)

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Experimental set-up CellTank™

Cell line	mAb producing DHFR$^-$ CHO (DP12)
Bioreactor and cell separation device	CellTank™ (CerCell) with matrix (~12 grams non-woven polyester matrix) @ ~ 3.6 m^2 matrix surface
Working volume	150 mL
Recirculation flow rate*	1 & 1.6 L/min
Cell density specific perfusion rate	≥ 0.05 nL/cell/day (or 1 Reactor Volume/day for 20 x 10^6/mL)
pH	7 & 7.1 control by adding 0.5 M Na_2CO_3 or pulsing CO_2 into headspace
Temperature	37°C & 29 to 32˚C
DO	40 & 45 % control by O_2 sparging
Cultivation medium	animal-component free IS CHO CD XP medium with hydrolysate (Irvine Scientific) + 3 % of IS-CHO Feed-CD XP (Irvine Scientific)
Supplementations of glucose and glutamine	according to cell consumption
Addition of Antifoam C (SAFC)	/
Analyses by Nova Bioprofile FLEX	cell density, viability, cell diameter, pH, pCO2, osmolality, concentrations of glucose, glutamine, lactate and ammonia
Analysis of mAb concentration	protein A HPLC

Results
Perfusion using ATF or TFF in Wave Bioreactor™

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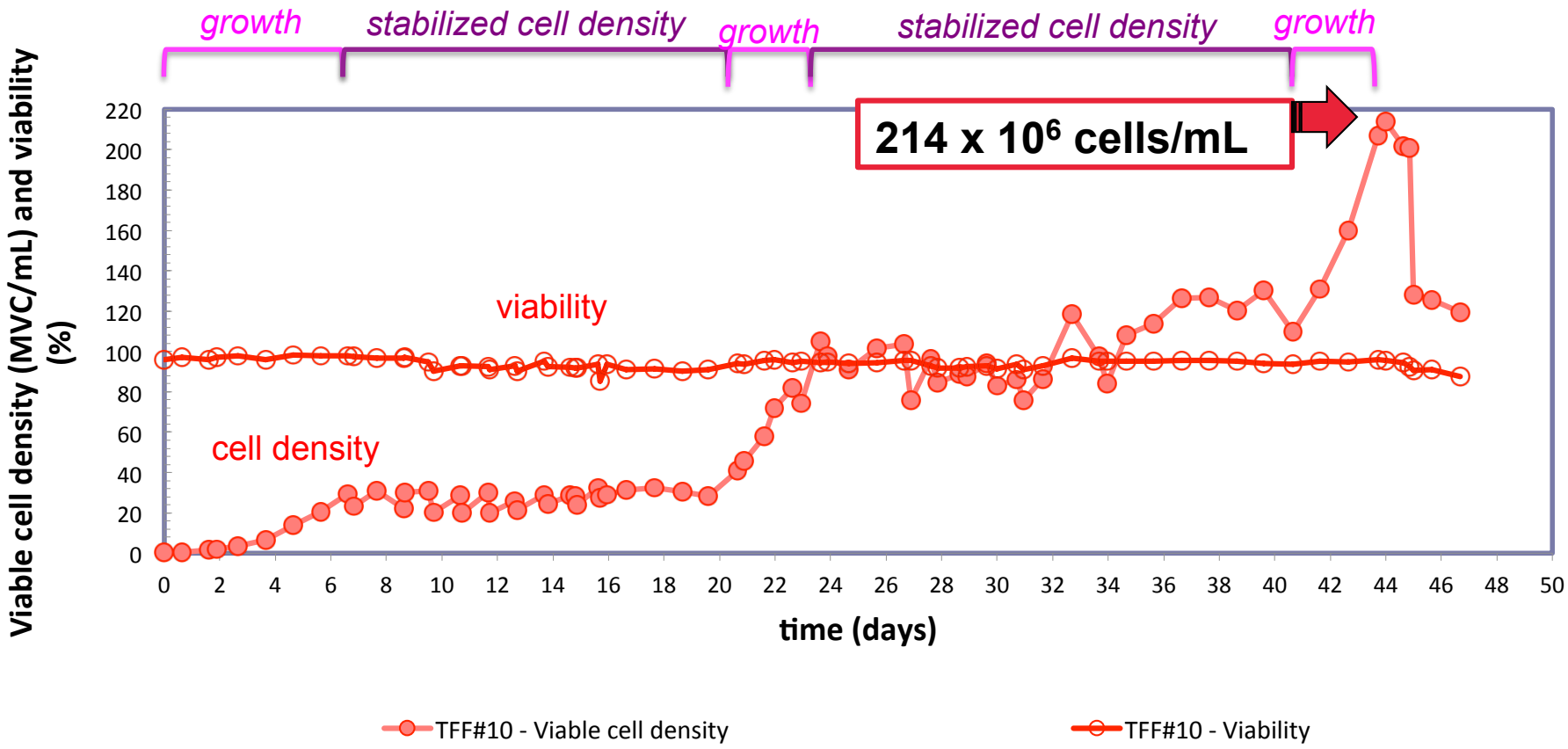
Continuation of run using TFF at very high cell density



- Cell density stabilized at 100 x 10^6 and 120 x 10^6 cells/mL by daily cell bleeds during > 2 weeks
- Cell densities ≥ 200 x 10^6 cells/mL (2 days) → Max cell density = 214 x 10^6 cells/mL
- Cell density limit due to limitations of membrane capacity for the encountered high viscosity (pressure = 1 bar), oxygenation and CO_2 level (31 kPa)

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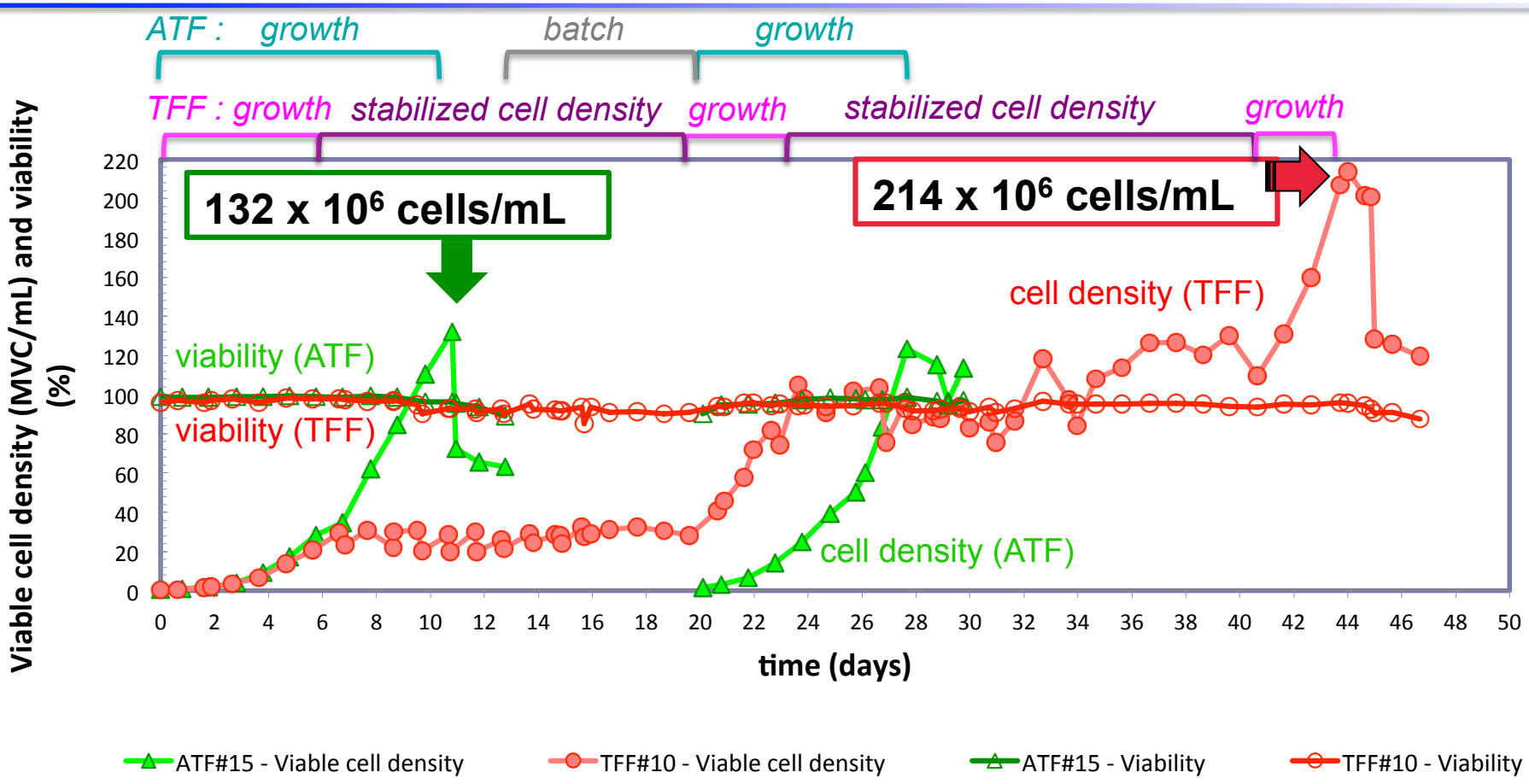
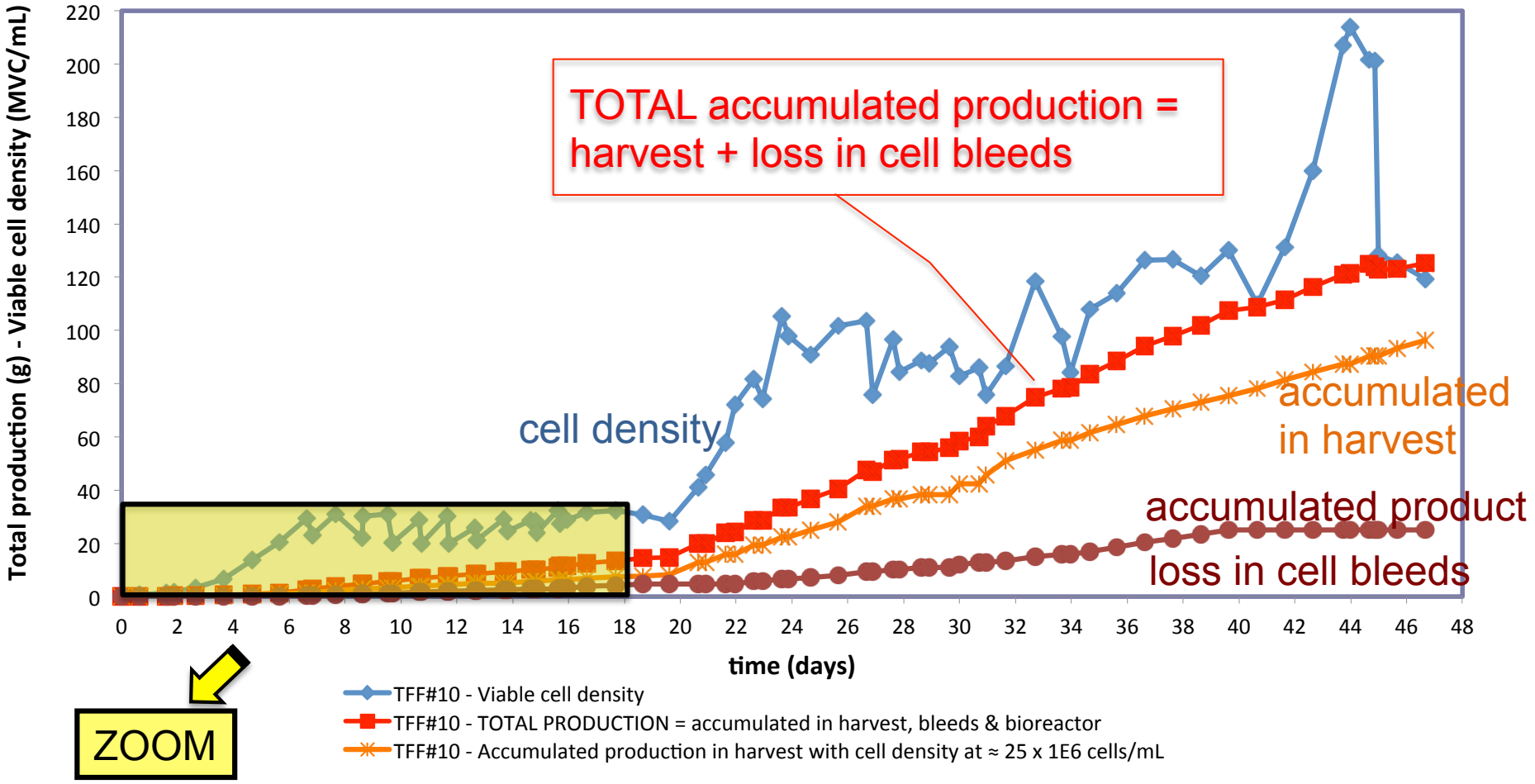
Perfusion using ATF or TFF at very high cell densities



- Max cell density = 132 x 10^6 cells/mL using ATF
- After maximum reached → cell density maintained at ≈ 100 x 10^6 cells/mL using ATF

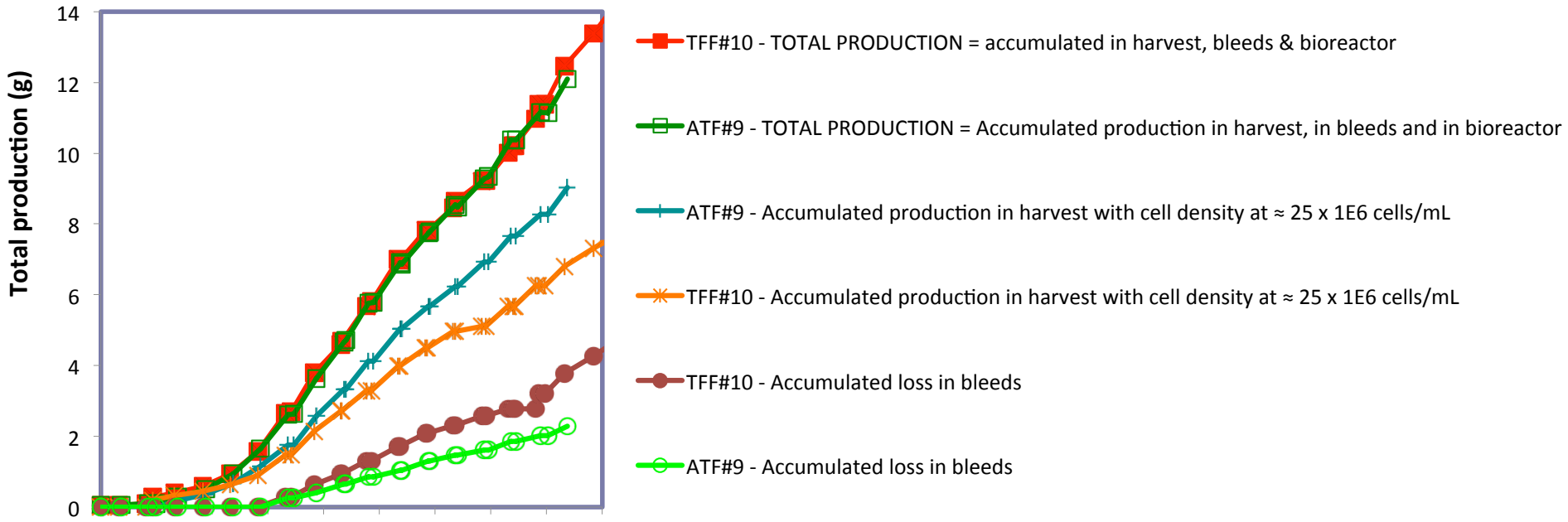
→ Cell density limit due to pressure limitation to push highly viscous fluid using non-pressurisable disposable bioreactor

Total accumulated antibody production in the harvest, the cell bleed, the bioreactor and cell density using TFF



- Observation of partial IgG retention by the hollow fiber filter
- Calculation exercise in this study run
  - 17 days at ≈ 20-30 x 10^6 cells/mL → ≈ 7 g
  - 47 days at ≈ 25 x 10^6 cells/mL and ≈ 110 x 10^6 cells/mL → ≈ 96 g

Total accumulated antibody production in the harvest, the cell bleed, the bioreactor and cell density using TFF or ATF after 17 days at ≈ 25 x 10^6 cells/mL



	ATF	TFF
• Partial retention of IgG by hollow fiber filter	yes	yes
• Cell specific productivity (pg/cell/day)	**10-15**	**10-15**
• Total accumulated production	**12**	**12**
• Accumulated production in harvest (g)	**9**	**7**
• Total removal of mAb in cell bleeds/total production (w/w in %)	**19**	**30**
• Yield (production in harvest/total production) (w/w in %)	**75**	**55**
• Residual mAb mass in bioreactor/total production (w/w in %)	**6**	**15**

Results

Perfusion using CellTank™

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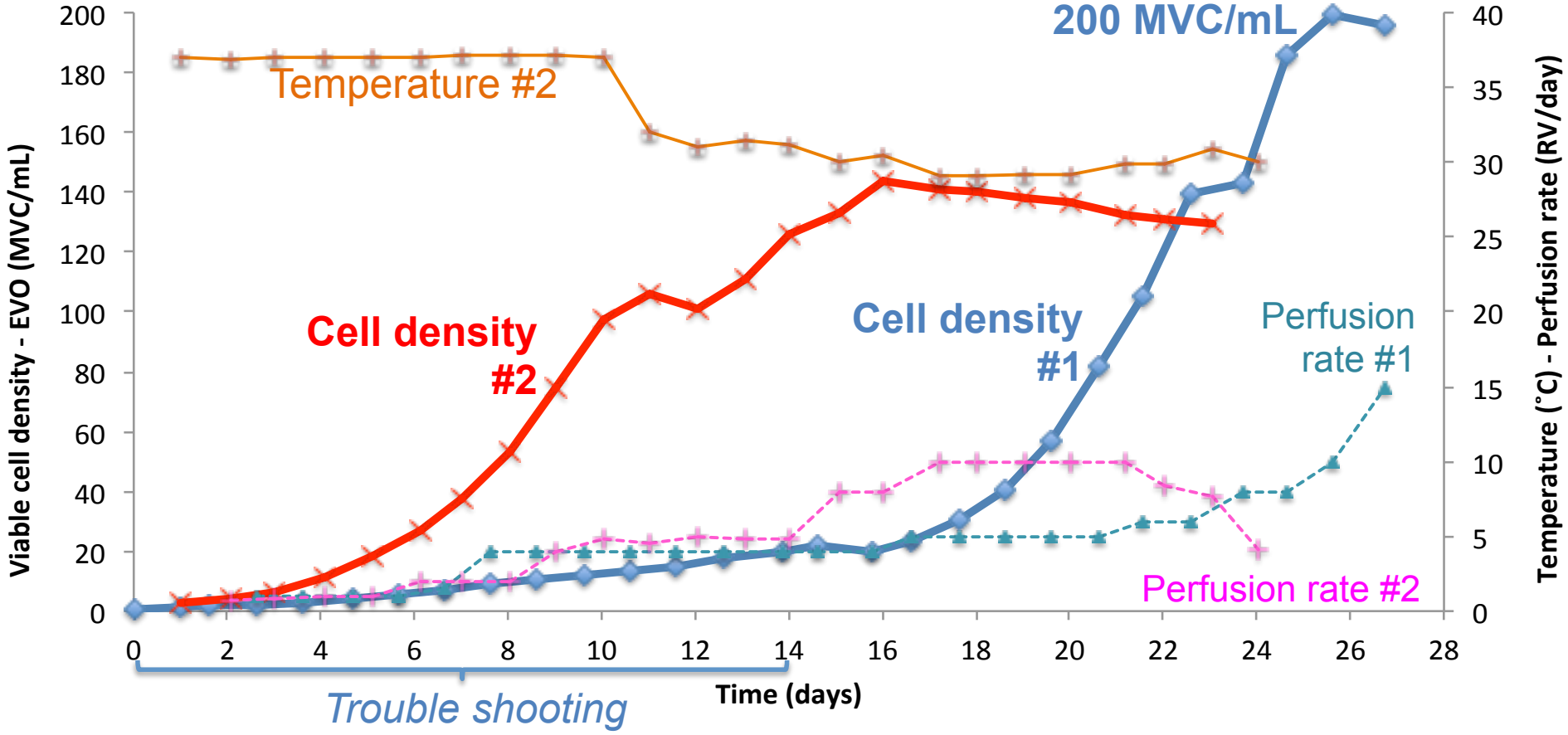
Cell density and perfusion rate in CellTank runs (BOL#1, BOL#2)

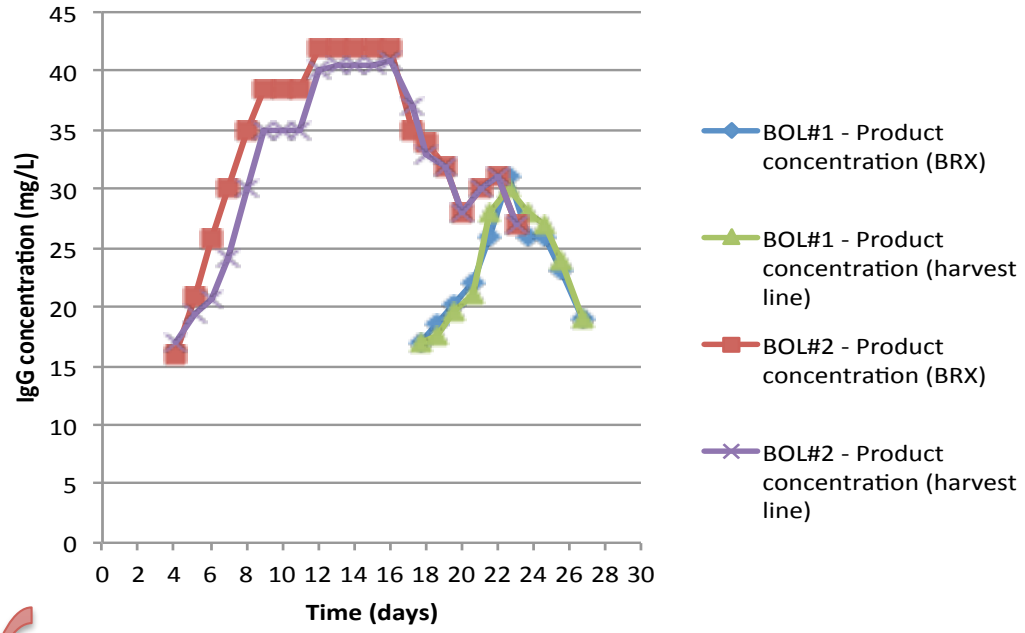


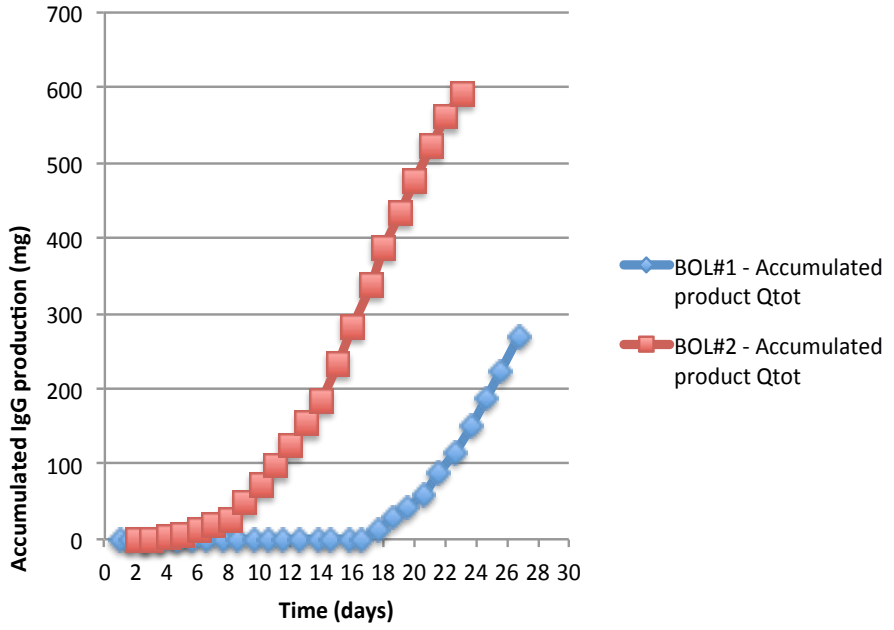
BOL#1: Fast growth after day 14 (after trouble shooting (1st run)) → up to **200 x 10^6/mL**

BOL#2: Cell density kept ≈ **130 x 10^6/mL** at perfusion rate of 8-10 RV/day for over 10 days

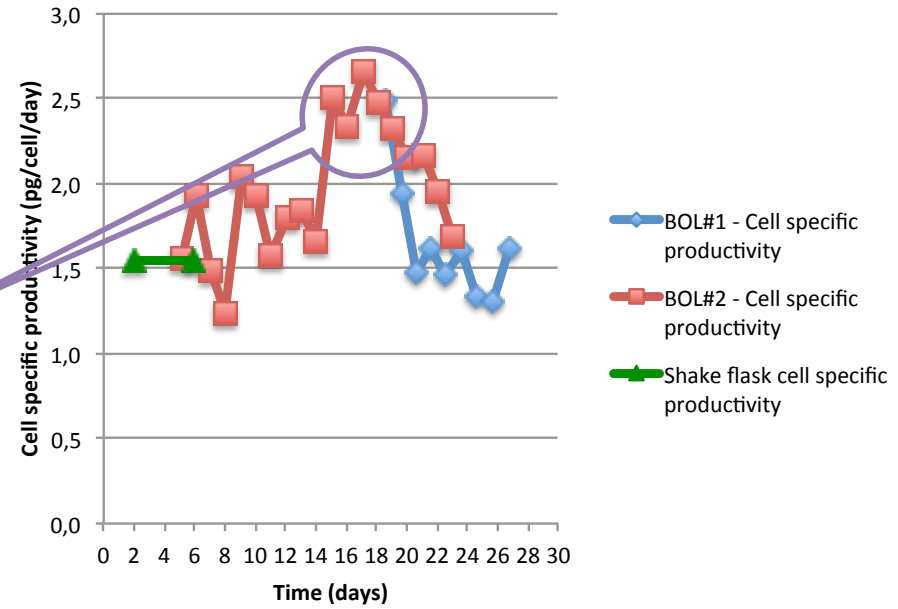
Temperature lowered from 37˚C to 32˚C/31˚C/30˚C/29˚C on day 16 → cell growth arrest

IgG production in CellTank™ runs





- Product accumulated with time and increasing cell density (after day 14 for BOL#1)
- Cell specific productivity in perfusion mode comparable to shake flask productivity except at 30°C where it was ≈ 40 % higher
- No retention of IgG in the polymer matrix.



Results
Fed-batch versus perfusion using ATF or TFF

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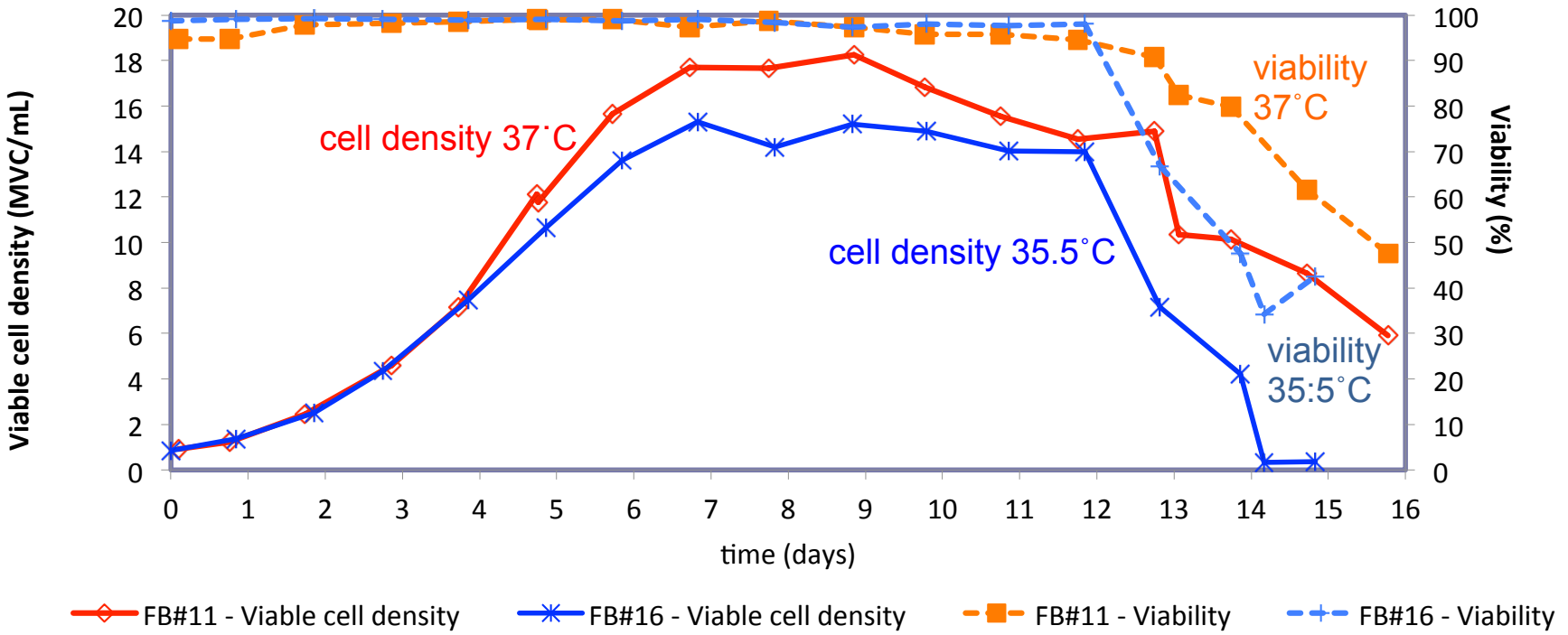
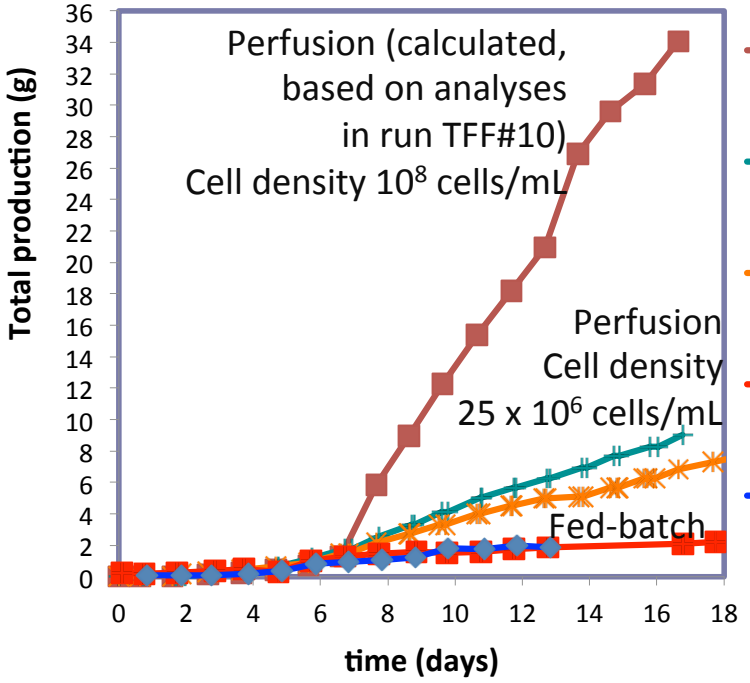
Comparison with fed-batch process

Experimental set-up

- Initial → final volume = 2 L → 4 L
- Same set-points of DO, pH
- Temperature → 37˚C (run FB#11) and 35.5˚C from day 7 (run FB#16)
- Base medium = IS CHO CD XP medium with hydrolysate (Irvine Scientific)
- Feed medium = base medium + feed concentrate Efficient Feed A & B (InVitrogen)



Production in perfusion or fed-batch 4 L WAVE Bioreactor™



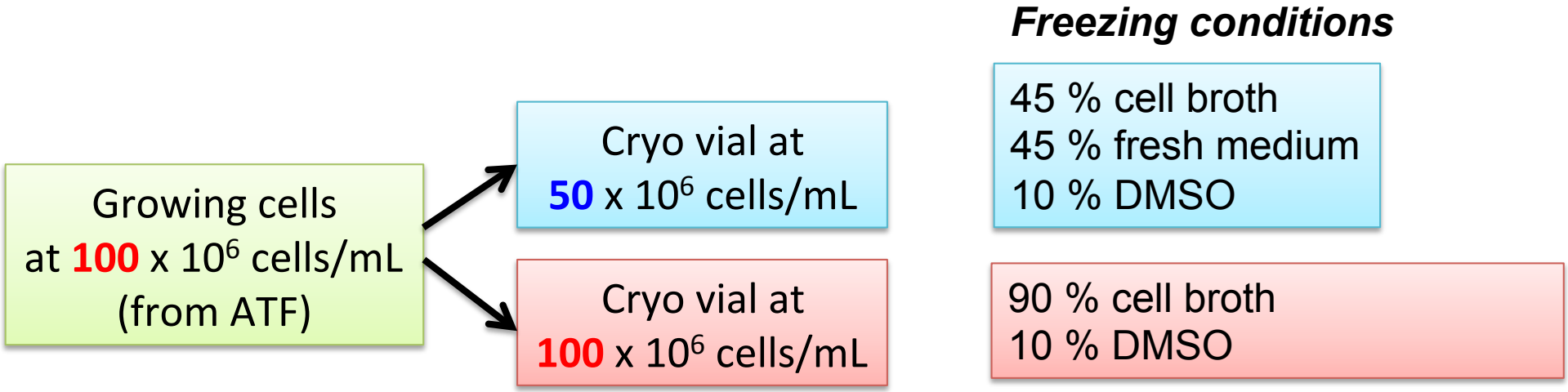
- TFF#10 - CALCULATED Accumulated production in harvest with cell density at ≈ 100 x 1E6 cells/mL
- ATF#9 - Accumulated production in harvest with cell density at ≈ 25 x 1E6 cells/mL
- TFF#10 - Accumulated production in harvest with cell density at ≈ 25 x 1E6 cells/mL
- FB#11 - Product in bioreactor
- FB#16 - Product in bioreactor

	Fed-batch	Perfusion (ATF or TFF) with cell density at ≈ 25 x 10^6 cells/mL	Perfusion (calculated from analyses in run TFF#10) with cell density at ≈ 100 x 10^6 cells/mL
mAb production after 17 days run	≈ 2 g	≈ 7 to 9 g	≈ 34 g (Rm: ≈ 22 g/week produced at 10^8 cells/mL)

Results

Cryopreservation from very high cell density perfusion

Cryopreservation study: set-ups



After cryopreservation (N2 tank)

→ Cell thaw in shake flasks at 10^6 cells/mL

→ Study of cell revival and mAb production

Rm: freezing single experiment

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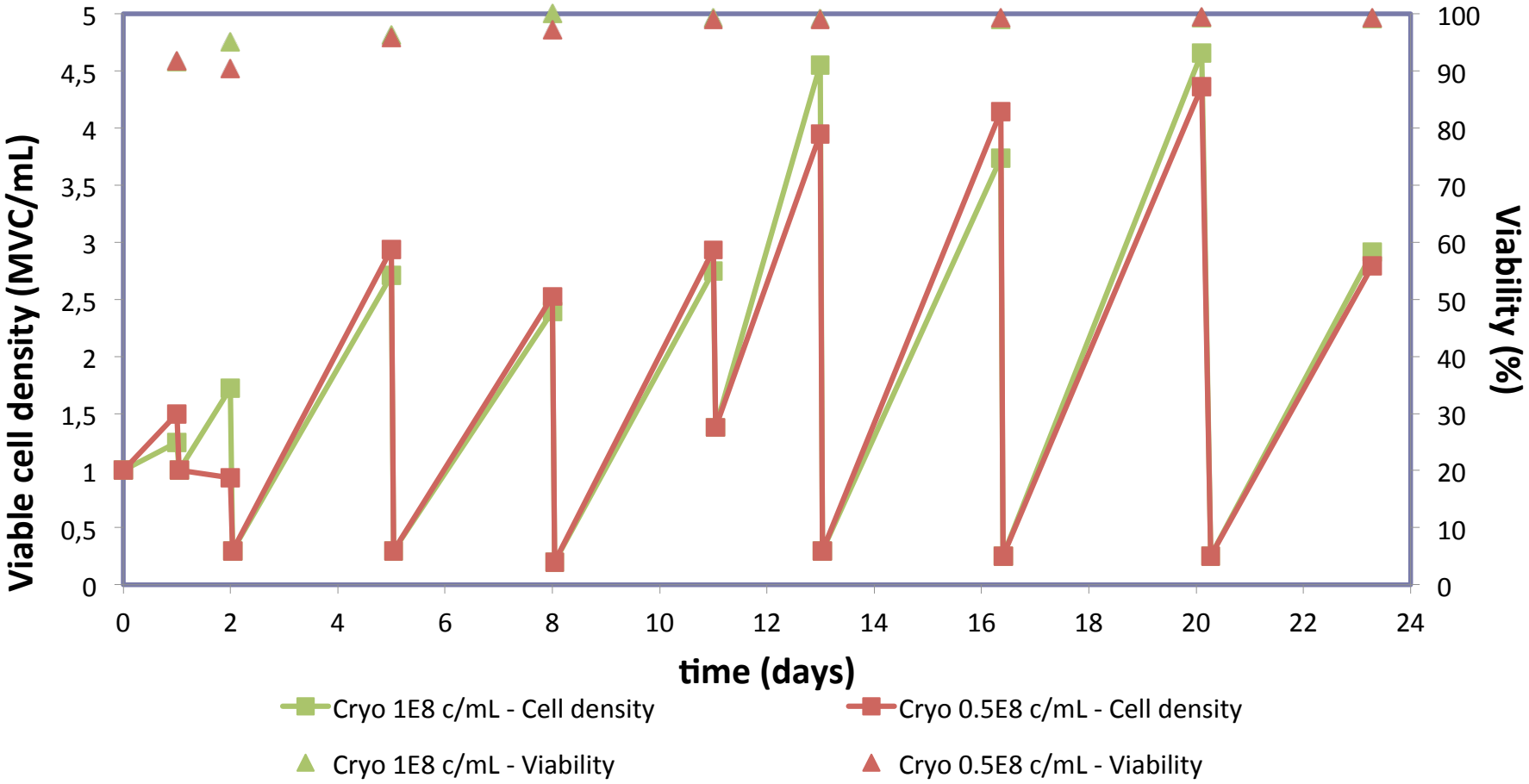
Cell thaw after cryopreservation from high cell density culture



- Cryopreservation from 100 x 10^6 cells/mL in vials of 100 or 50 x 10^6 cells/mL
  → excellent cell resuscitation

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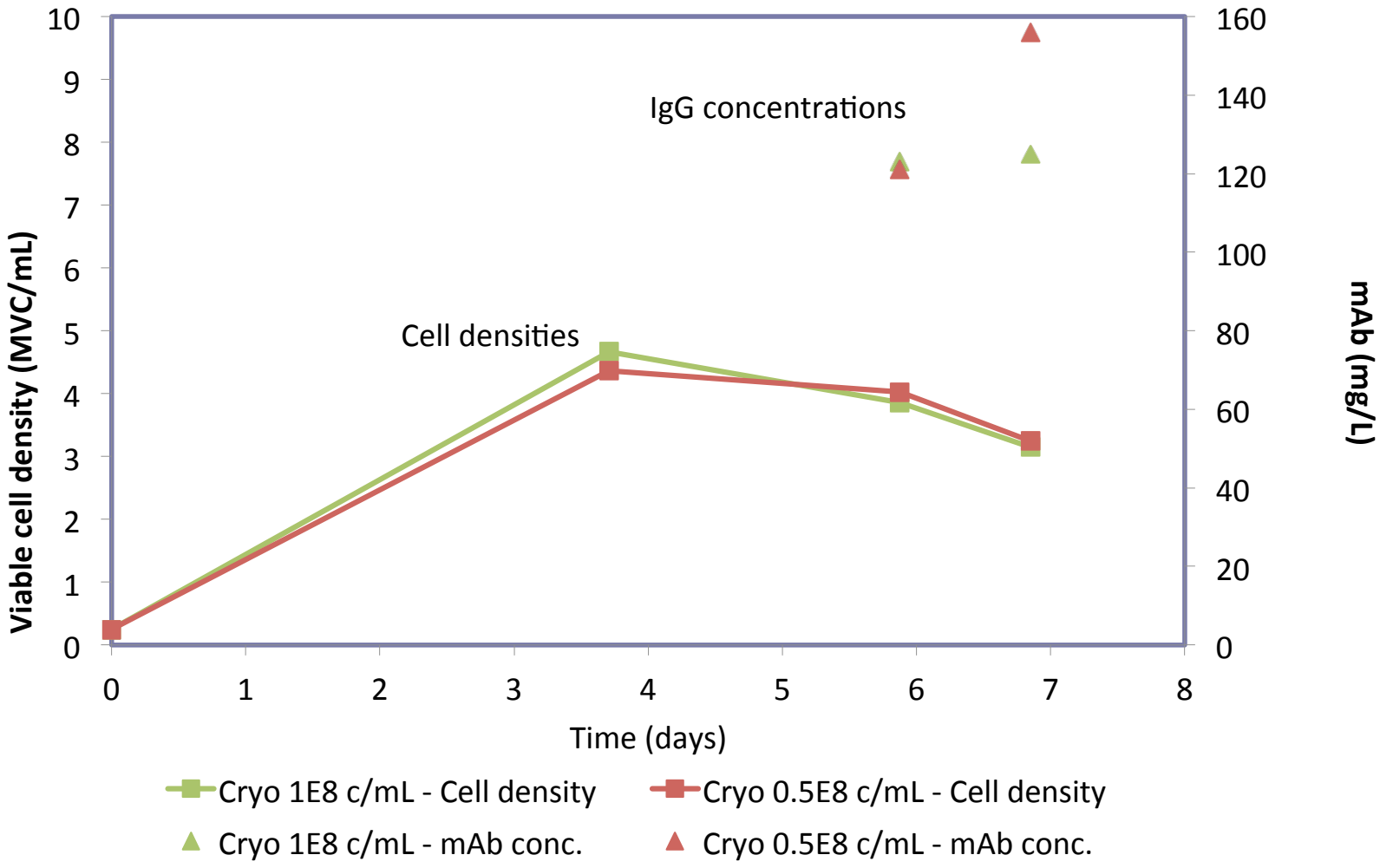
Cryopreservation: Production test in shake flasks 2 weeks after thaw

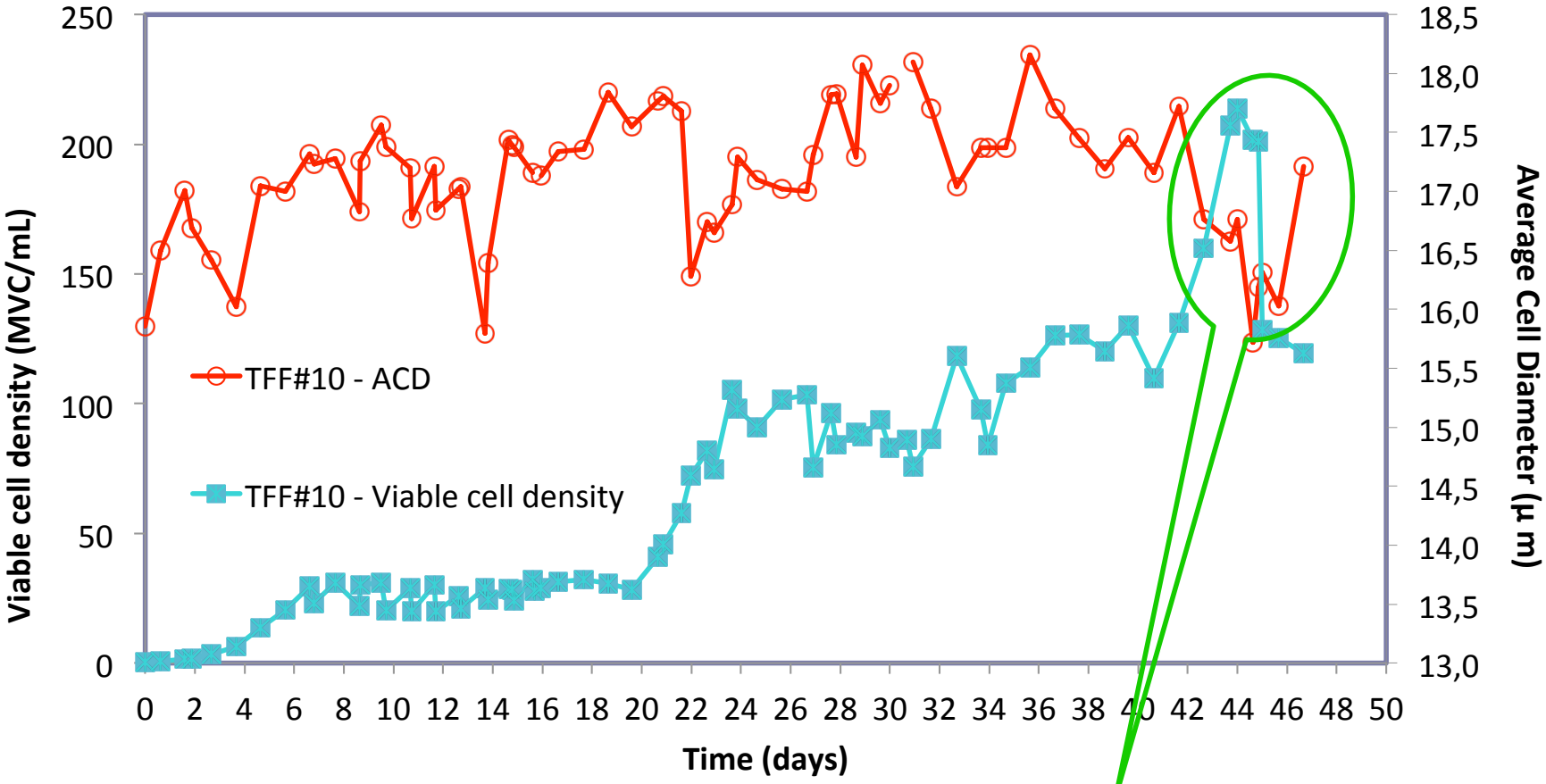


- Normal mAb production 2 weeks after thaw

Cells at very high cell density

Cell diameter at very high cell density

1. TTF perfusion run in Wave Bioreactor™

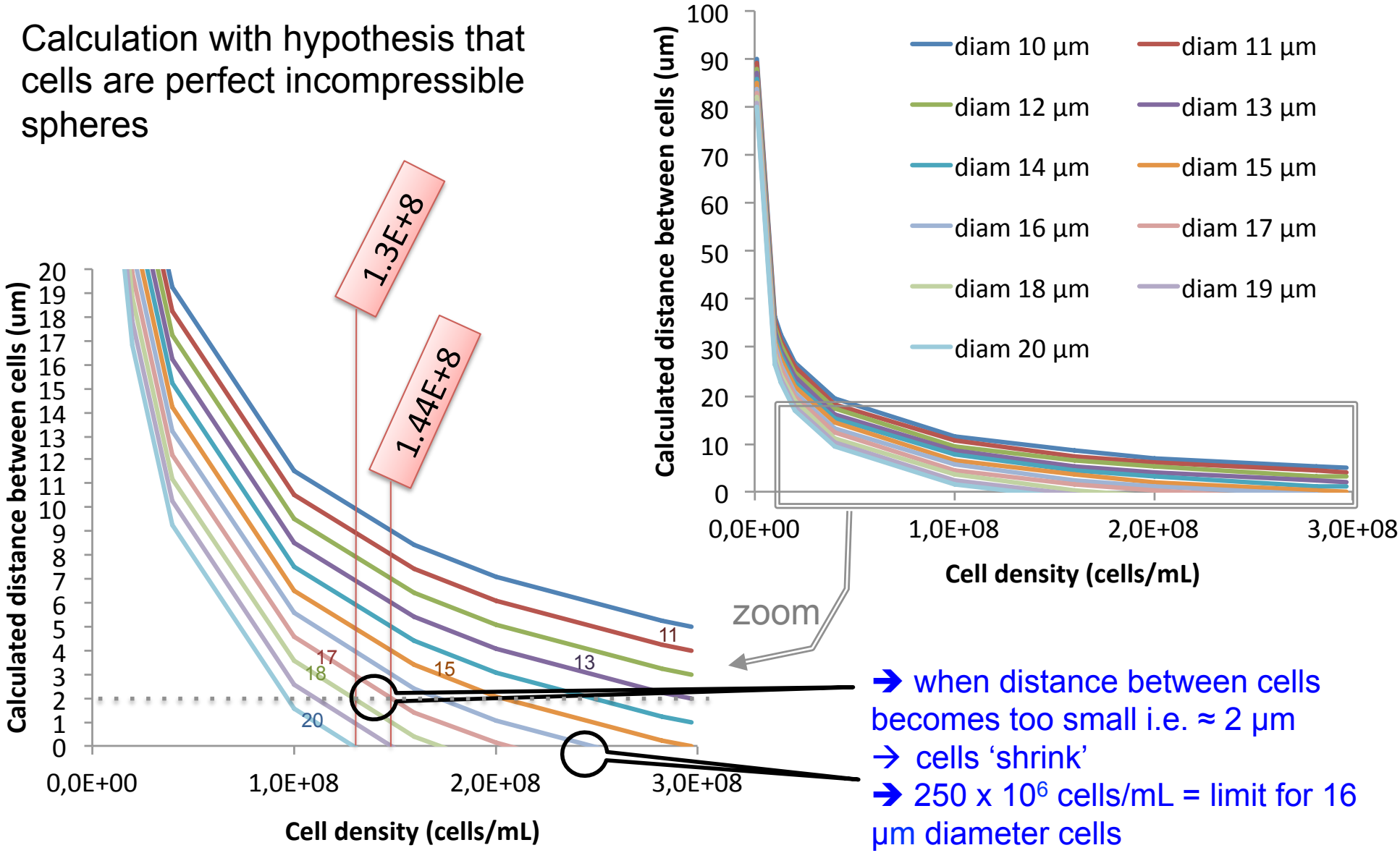


Smaller cell diameter when cell density > **131 x 10^6** cells/mL

2. Perfusion run in CellTank™ → cell diameter smaller when cell density ≥ **144 x 10^6** cells/mL (Fogale cell density probe - multi-frequency signal)

Distance between cells for different cell diameters

Calculation with hypothesis that cells are perfect incompressible spheres



➔ when distance between cells becomes too small i.e. ≈ 2 µm
→ cells 'shrink'
➔ 250 x 10^6 cells/mL = limit for 16 µm diameter cells

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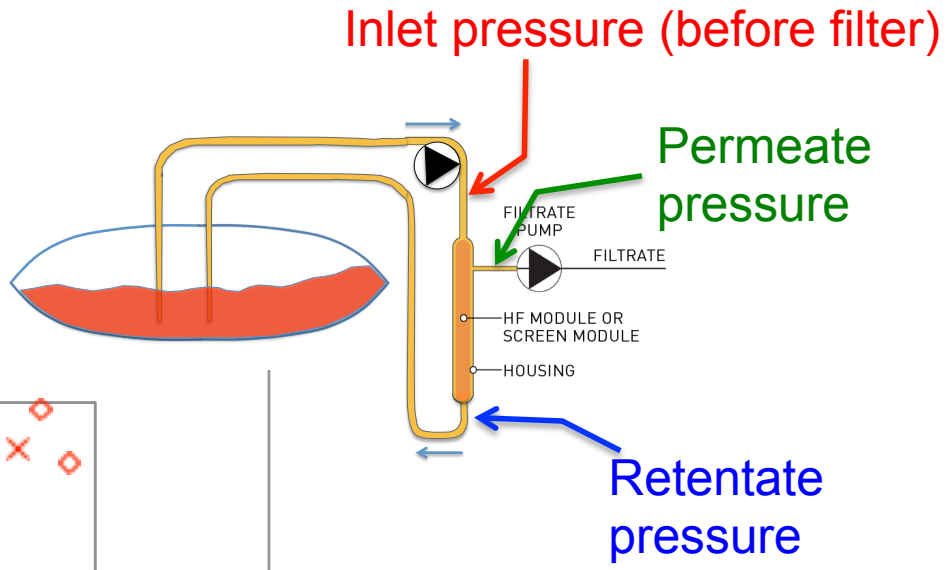
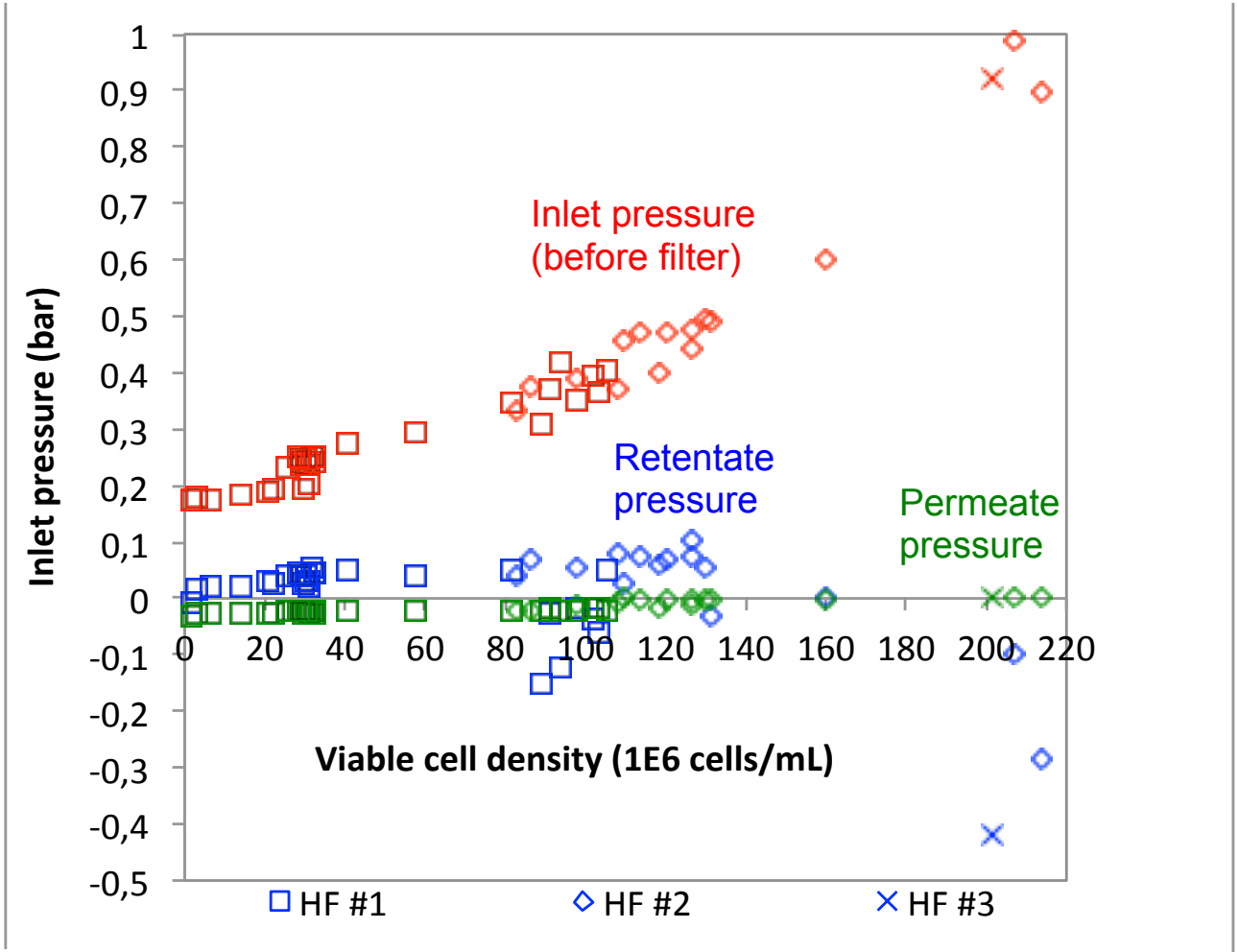
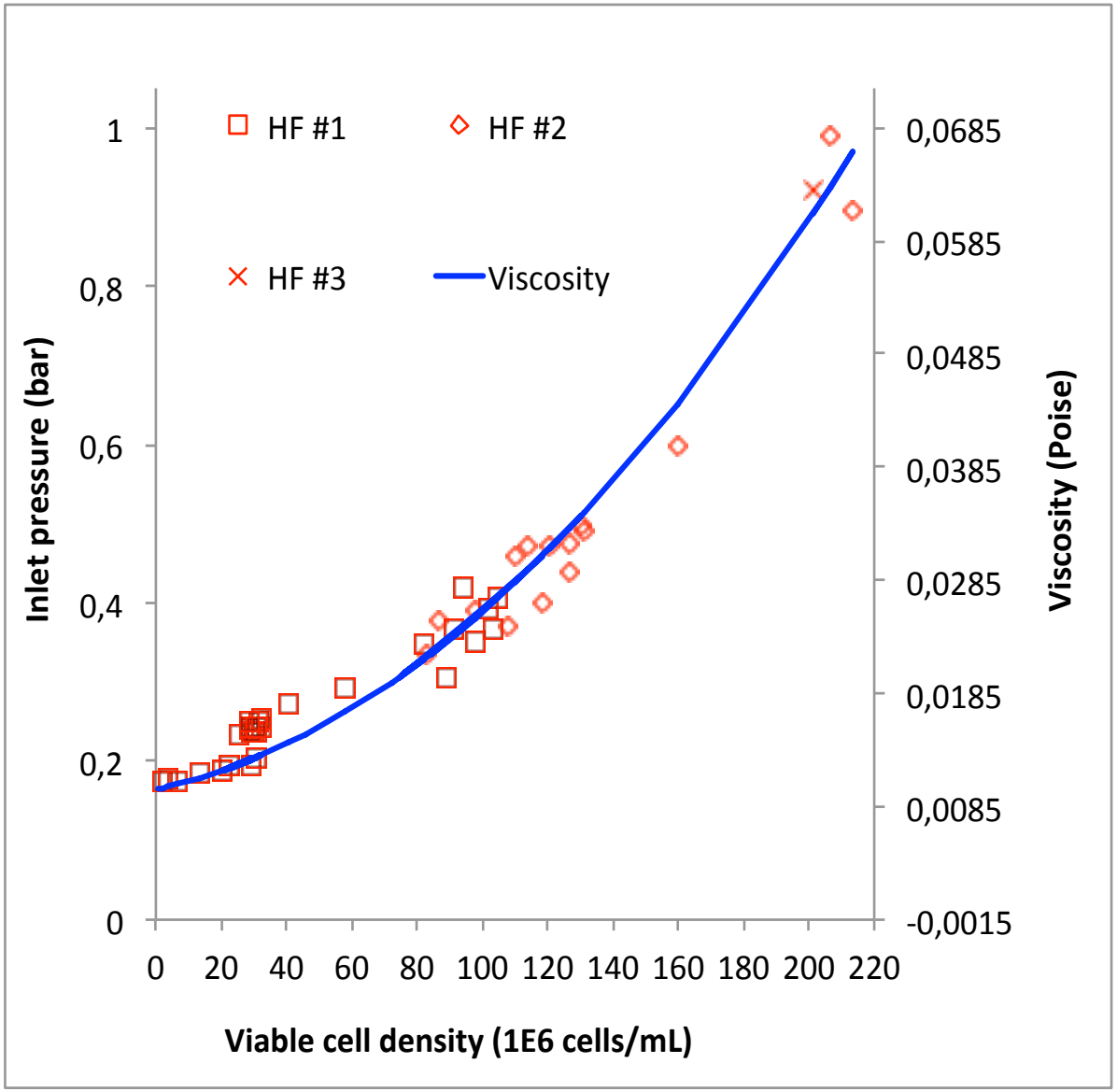
Pressures during TTF perfusion





Viscosity very well correlated with cell density



Viscosity = $0.01 (1 + 2.5 \Phi + 14.1 \Phi^2)$

Φ = volume fraction of cells in the mixture

Calculation for cell diameter 17 µm of viscosity of slurry according to Thomas D. G. 1965 J. Colloid Science 20:267

➔ Increased viscosity due to increased cell density

➔ Increased pressure due to increased viscosity in a constricted filter section

➔ Calculation of filter fiber numbers (or filter section) can be done given target cell density, allowed inlet pressure

Conclusions

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Conclusions – Cell density

- Very high cell density of 100 x 10^6 cells/mL stably maintained in growing phase and at high viability by cell bleeds in a perfused WAVE Bioreactor™ using TFF or ATF cell separations

- Very high cell density of 200 x 10^6 cells/mL achieved in CellTank™
- Very high cell density of 130 x 10^6 cells/mL during 11 days with lower temperature in CellTank

- With present settings → maximal cell density = 214 x 10^6 cells/mL with TFF
  → maximal cell density = 132 x 10^6 cells/mL with ATF

- TFF → cell density limited by membrane capacity (for the encountered high viscosity), oxygenation and CO_2 level

- ATF → cell density limited by insufficient pressure to push highly viscous fluid when using non-pressurisable disposable bioreactor

➔ TFF and CellTank™ allow reaching higher cell densities than ATF with present settings

- First time, CHO cell density 200 x 10^6 cells/mL in a wave-agitated bioreactor
- First time, CHO cell density 200 x 10^6 cells/mL in CellTank

Conclusions – Cell density limit?

- Upper limit of cell density for suspension
  - depends of cell diameter
  - calculated theoretically for perfect spheres
  - for CHO cells (diameter 16 µm) → 250 x 10^6 cells/mL
  - smaller limit than tissue cells or adherent cells in absence of contact inhibition

- Applicable limit of cell density for suspension
  - depends of cell diameter
  - depends of equipment
  - impact of cell shrinking?
    - perhaps recommended to avoid shrinking
    - limit of 130 x 10^6 cells/mL for CHO cells
    - theoretical smallest distance between cells with unchanged diameter = 2 µm

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Conclusions – mAb production

- No retention of mAb in CellTank™ matrix using cell line #2
- Retention using cell line #1 in hollow fibre filter: Higher retentions of mAb by hollow fibre filter using TFF than ATF using
- In perfusion, major effect of this retention = loss of mAb in the cell bleeds

➔ CellTank™ the most favourable for production according to this study

➔ ATF more favourable for production at stable cell density maintained by cell bleeds

- Potential production per bioreactor volume by perfusion much larger than fed-batch

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Conclusions – Operation

- Recommended to apply cell specific perfusion rate
- No cell sample today in CellTank™ (under development)
- Short residence time ≈ 20-30 sec for TFF and ATF
- Short 'residence time' ≈ 6-9 sec for CellTank™
- Shear rate of 3400 s^{-1} well tolerated for TFF and ATF
- TFF ReadyToProcess disposable, easy to put in place and easy to put a new hollow fiber filter during cultivation → easier operation than ATF (autoclavable)
- Operation using CellTank™ easy and handy with robust integrated perfusion device
- Operation during 24 and 27 days could have been continued longer
- Absence of sparging in Wave Bioreactor™ and in CellTank™ in small scale → ADVANTAGEOUS
- The use of a single-use bioreactor equipped with robust cell separation device offers a solution alleviating technical and sterility challenges occurring in perfusion processes

Conclusions – Hollow fiber operation

- Present study give mathematical tools to select / design hollow fibers
  - lumen size and / or number of fibers → important for high cell density
  - recommendation of larger lumen or larger number of fibers
  - recommended to use larger number of fibers than 50 fibers (present study) using ATF for cell density ≥ 100 - 120 x 10^6 cells/mL in disposable bioreactor
  - filter area → impact on fouling

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Perspectives - Applications

High or very high cell densities of CHO cells, i.e. 50 to 130 x 10^6 cells/mL, are applicable for

	Wave Bioreactor™ & TFF or ATF	CellTank™ (1)
Seed bioreactor	X	
Production bioreactor – instable protein – non mAb (where fed-batch platform not straight applicable) – small company lacking fed-batch platform	X	X
Rapid, non optimized production of protein (e.g. explorative research) – compensation of low titer by very high cell density	X	X
Cell expansion for cell banking – cryopreservation from culture at 100 x 10^6 cells/mL – good cell resuscitation and normal mAb production – allows significant time cuts in cell banking and cell expansion	X	

(1) Cell detachment is in development

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