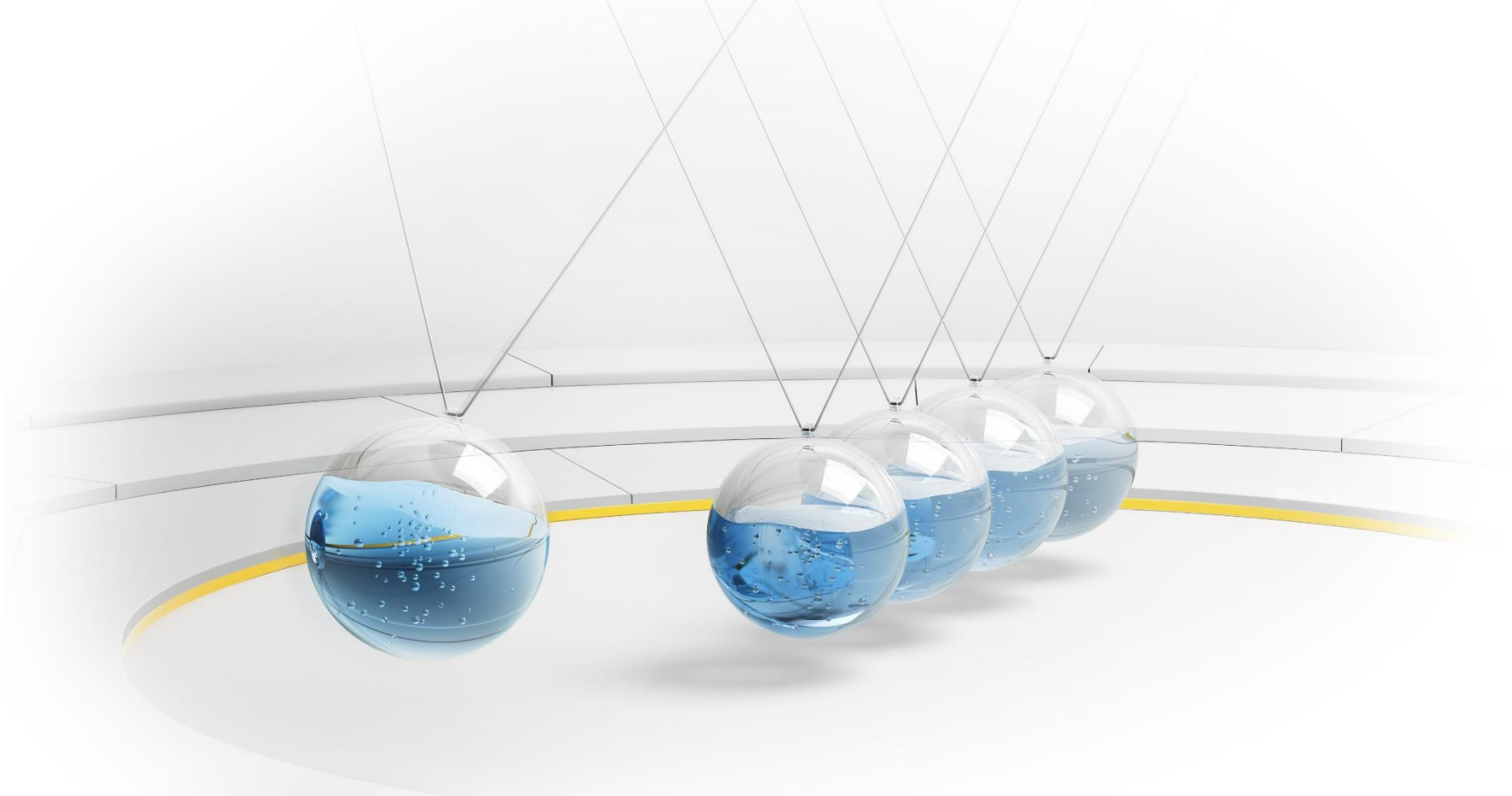

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Generation Change in Biopharmaceuticals Production
From Fed-Batch to Hybrid Processes

Key Facts About the Global Pharmaceutical Industry in 2013

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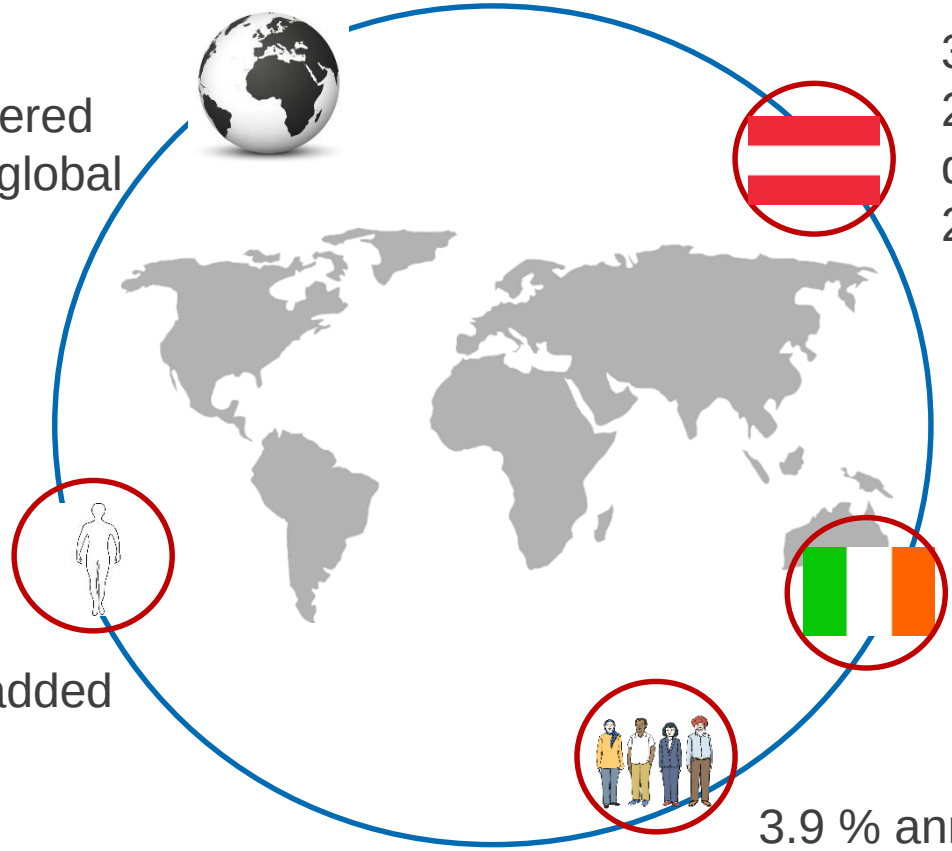
Pharmaceutical industry covered 3.6 % of the global share of global gross value added of the manufacturing industry.



330 Mrd. € gross value addd in 2013 – equivalent to the gross domestic product of Austria in 2014.

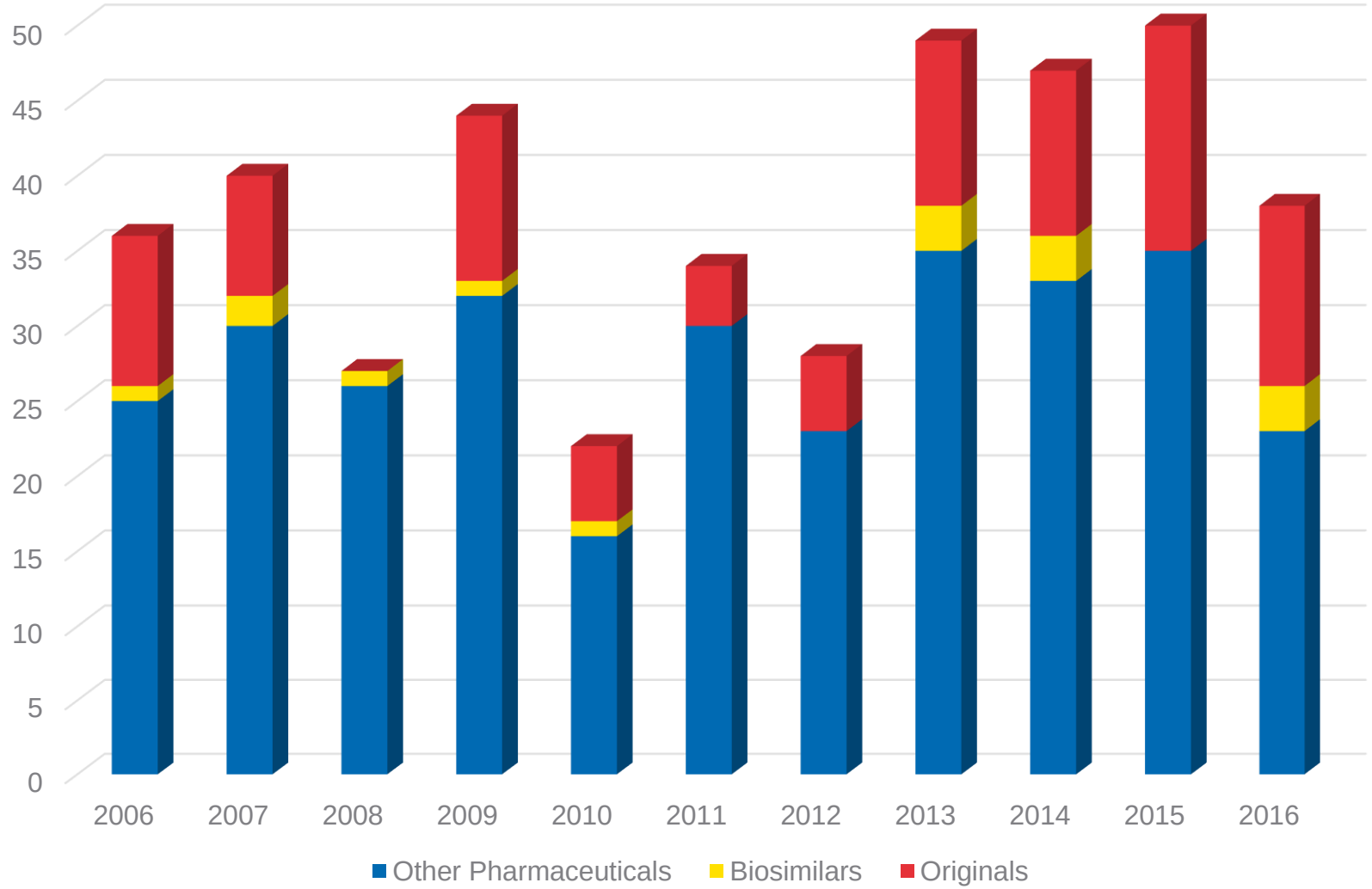
4.8 million employees – equivalent to the polulation of Ireland.

68500 € gross value added per employee.

3.9 % annual growth rate of employment from 2005 bis 2013.

Data taken from The World Bank Group, 2015 ; WifOR calculation; WifOR illustration

Approvals for Biopharmaceuticals on the EU Are Higher than Ever



More than 250 biopharmaceuticals are on the market.

Germany‘s Biopharmaceutical Pipeline Is Well-Filled

2/3 of active ingredients are monoclonal antibodies

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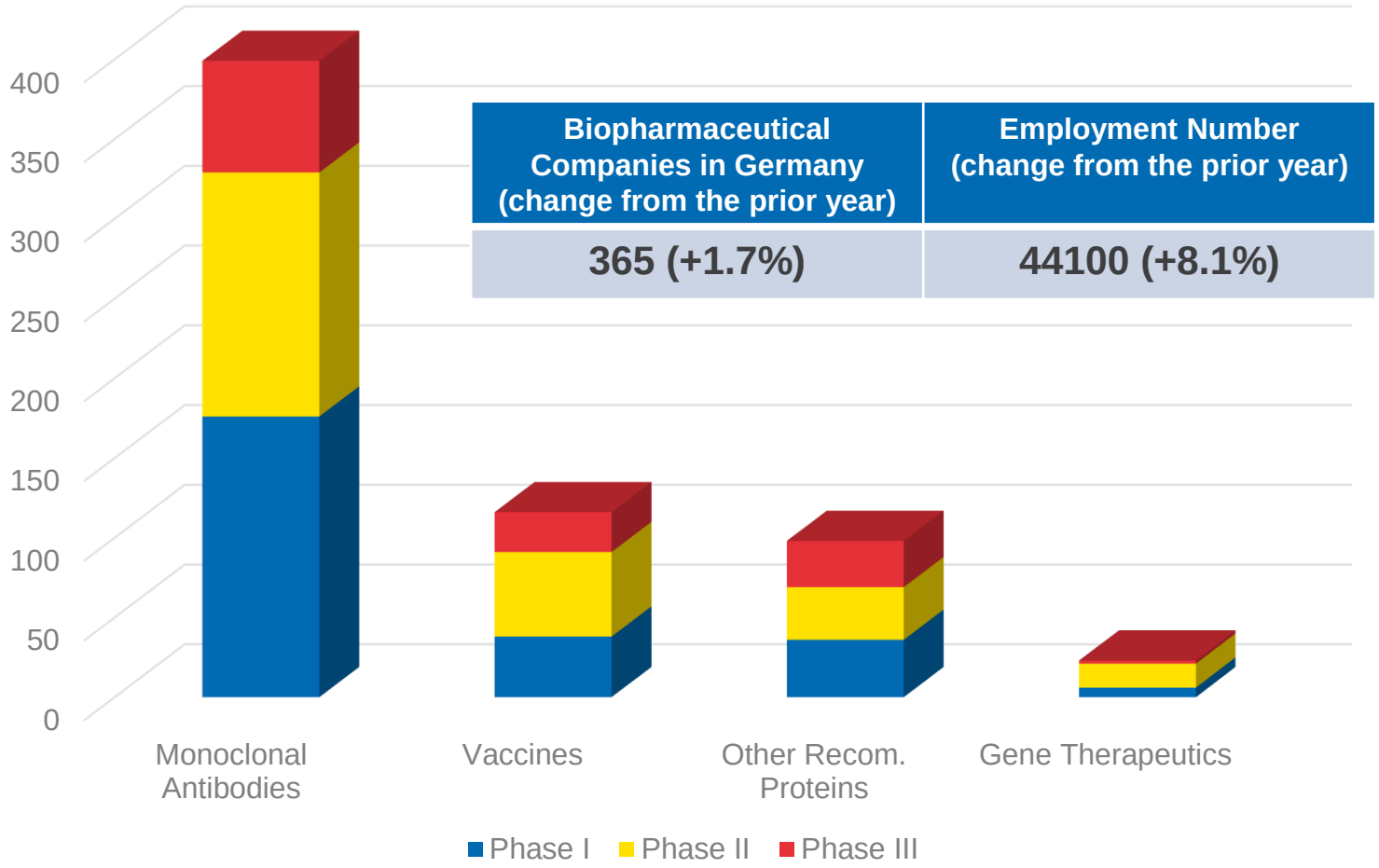
Change from the prior year	+3%	+1%	-6%	+10%	2016
Total amount	399	116	98	23	



Biopharmaceutical Companies in Germany (change from the prior year)	Employment Number (change from the prior year)
365 (+1.7%)	44100 (+8.1%)

Pharmaceutical Location Germany

The biggest in Europe (EU28 + Switzerland, 2015)

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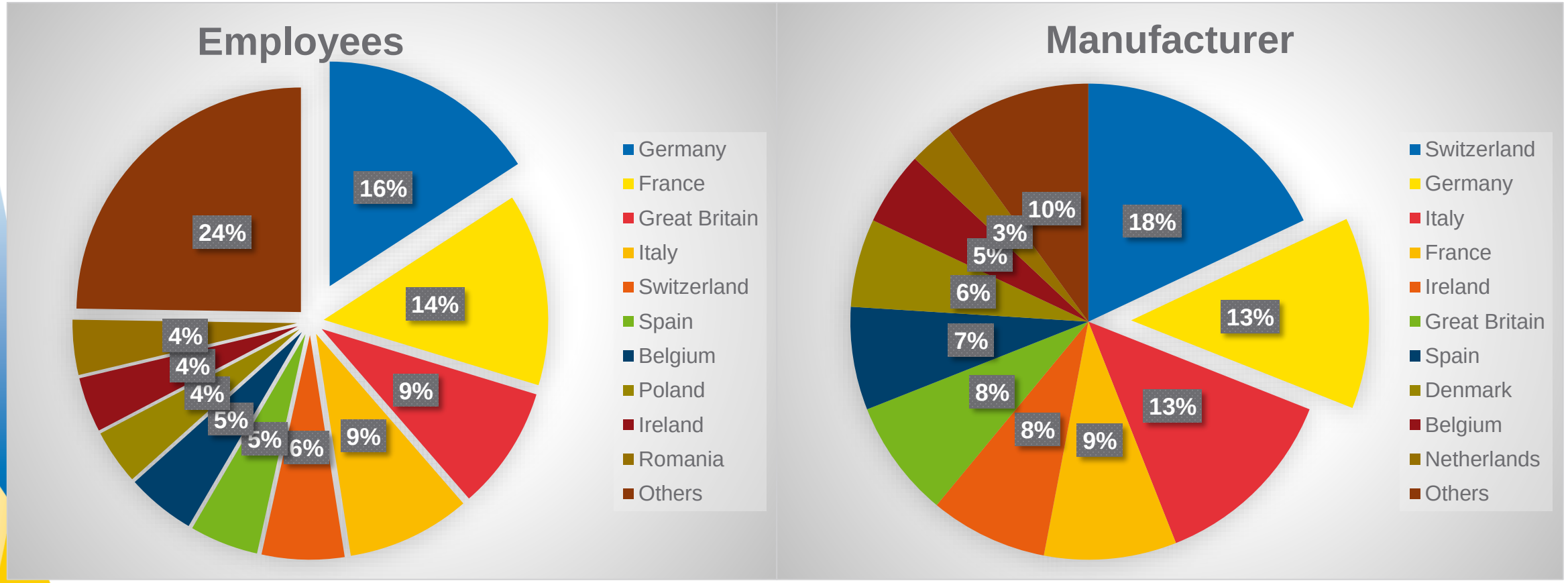
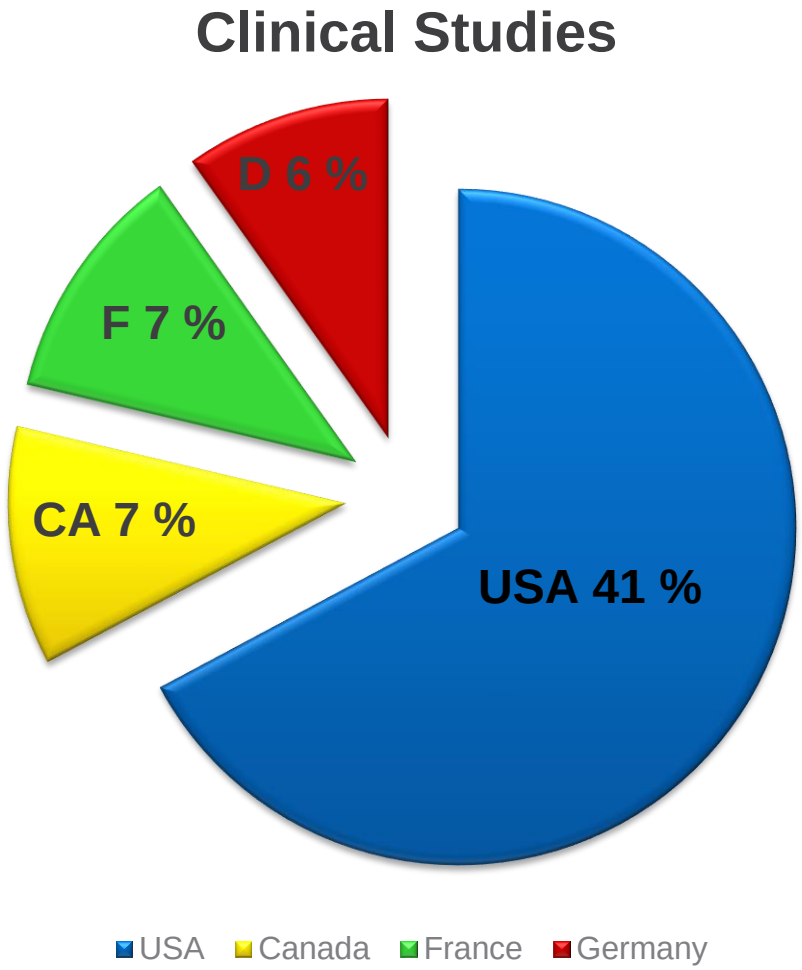


EFPIA, IW Köln

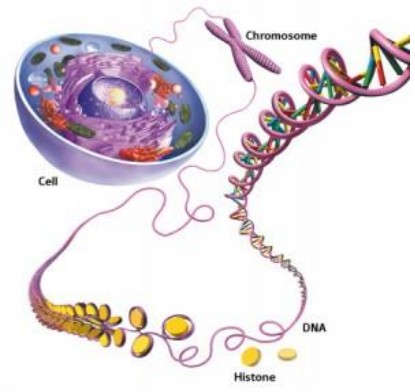
Number 2 in Europe, Number 4 in the World

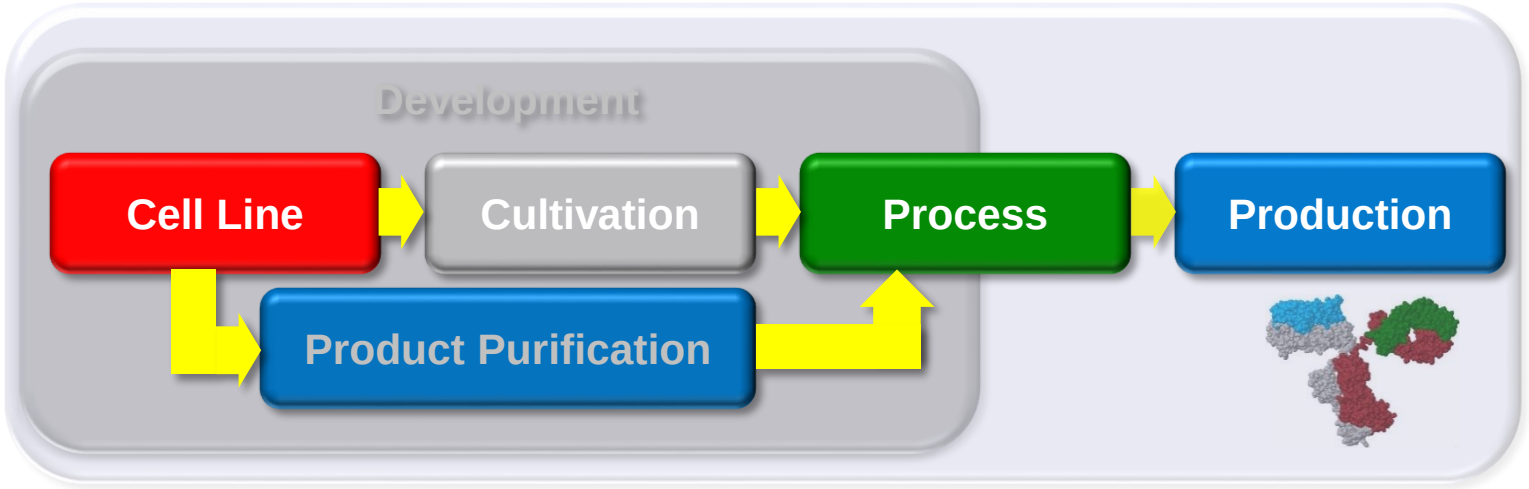


vfa

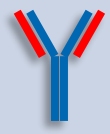
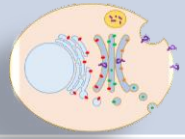
A Seemingly Easy Way from Gene to Therapy

Rentschler Biopharma



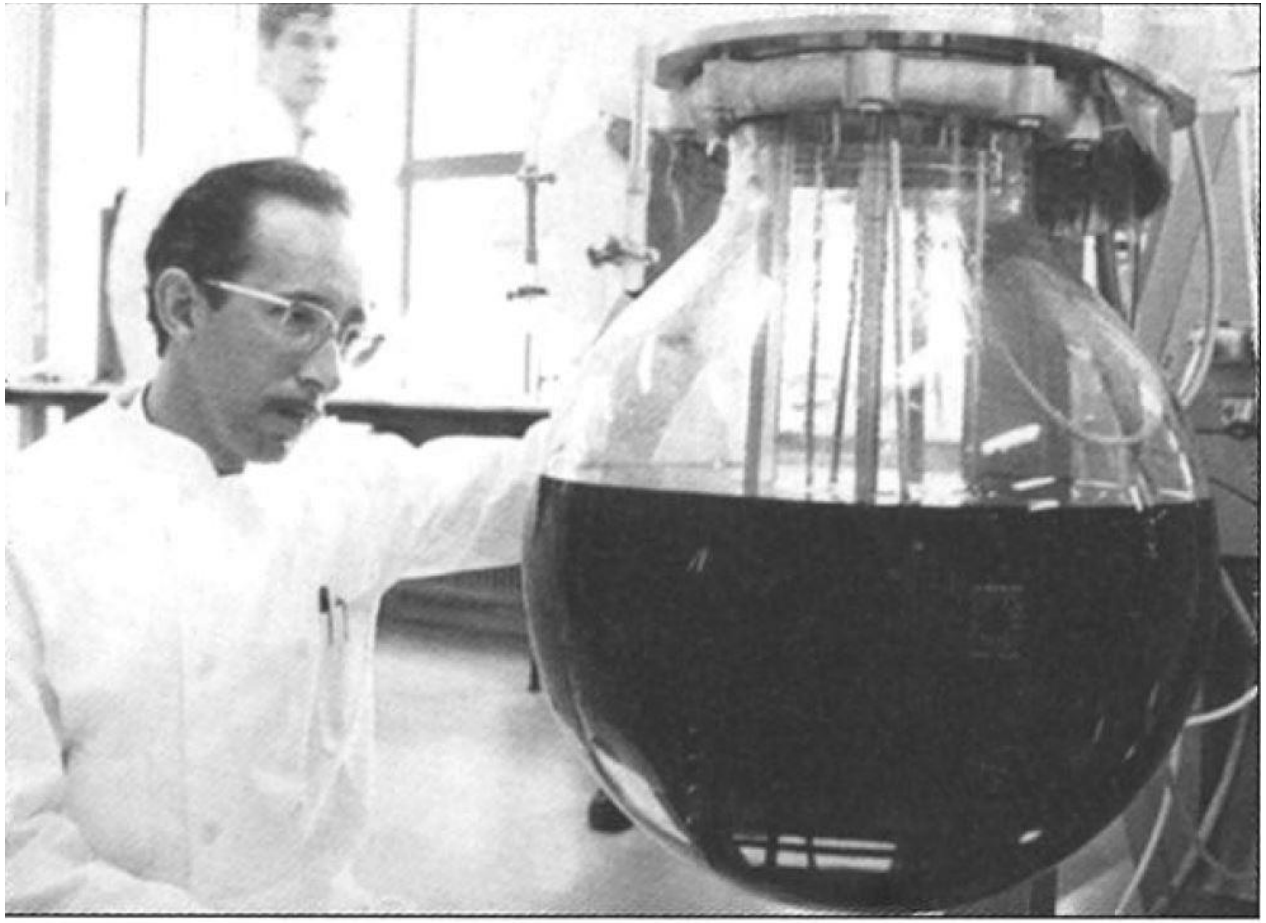


From Gene to Bedside

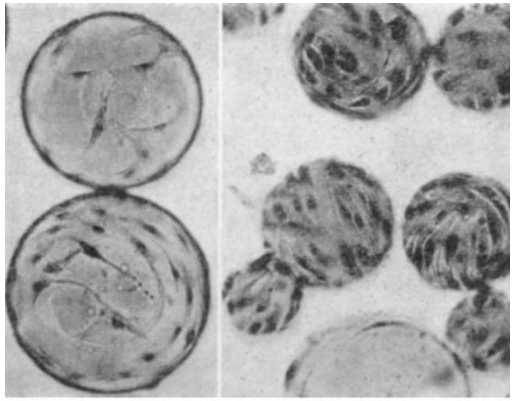
Molecule design	Host cell selection	Upstream process	Downstream process	Fill finish
				
• Sequence • Hydrophobicity	• Proteases • Oxidative stress • Media components • Posttranslational modifications	• Stirring • Temperature • Trace metals • pH • Air/liquid interface • Osmolality	• Pressure • Shear forces • Mixing • pH • Air/liquid interface • Light	• Agitation • Shear forces • Excipients • Oxidation • Freeze-thaw • Light

Factors affecting protein stability

Pioneering Cell Culture Bioprocesses in 1967



Paul van Hemert with the so-called “Bilthoven Unit” with a Polio virus vaccine production bioreactor in 1967.



Filled with cells grown on microcarriers (Anton van Wezel. 1967. Nature 216: 64).

From Basic Pilot Research to Manufacturing Sciences

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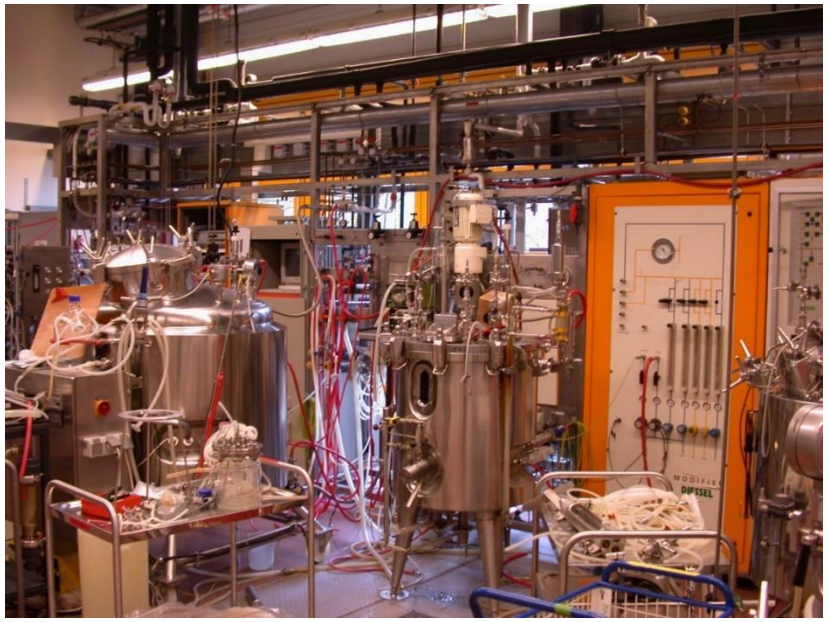
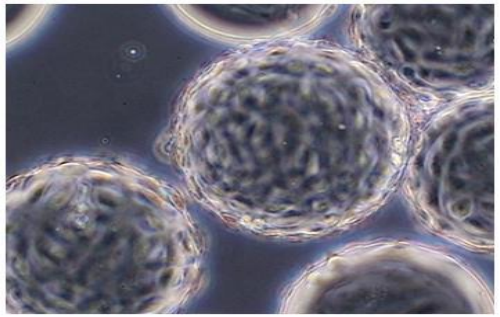
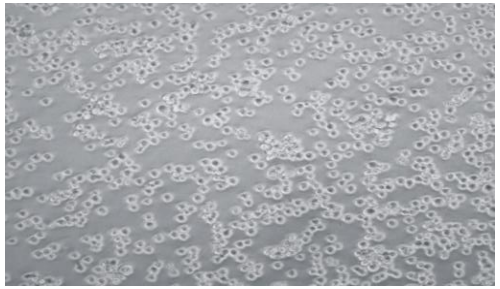


1990: GBF's 100 l Pilot Plant for cell culture-based bioprocess research

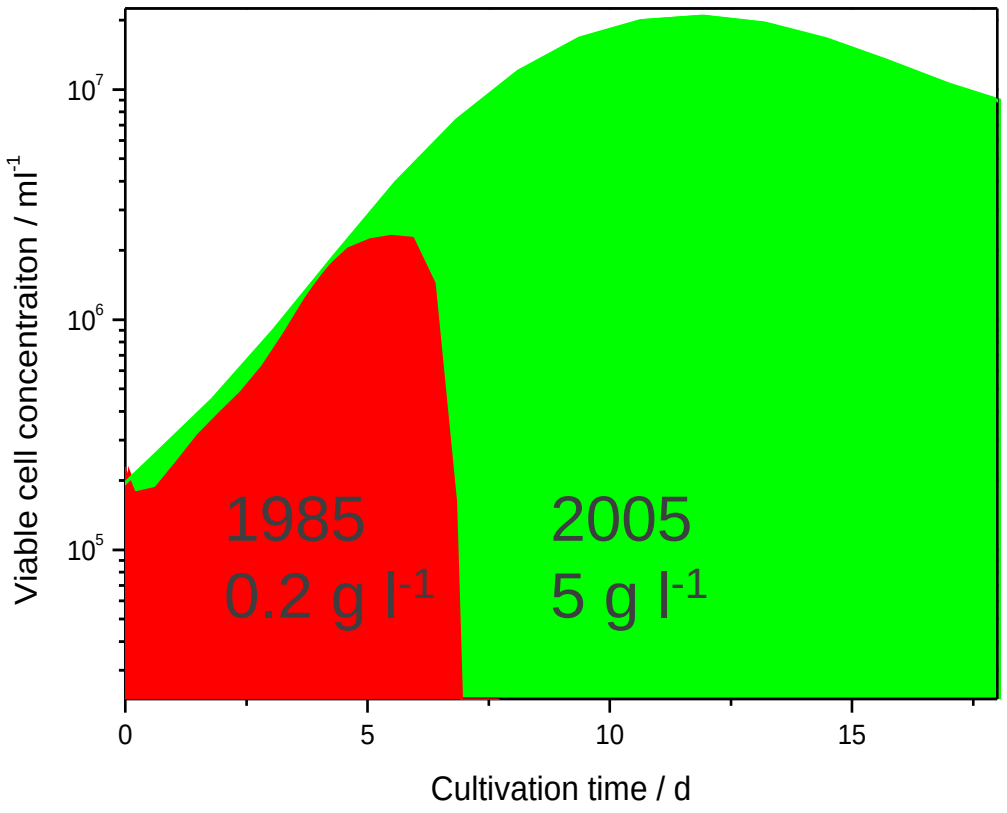
2015: Rentschler's 2× 3000 l TWIN Facility for cell culture-based biopharmaceutical manufacturing





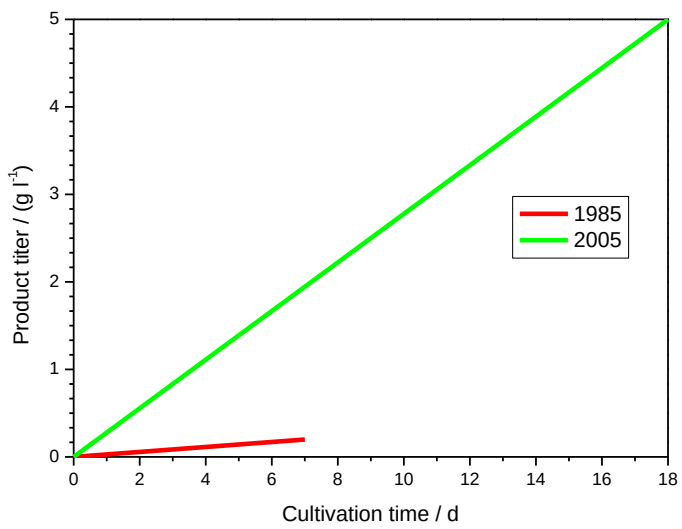
Celldays Increased Within 20 Years By a Factor of 10
Titer Increased Within 20 Years by a Factor of 20

Rentschler Biopharma



20 × 10^6 ml^{-1}

2 × 10^6 ml^{-1}



5 g l^{-1}

0.2 g l^{-1}

High-End Cell Culture Processes Are Approaching Natural Blood Cell Concentrations

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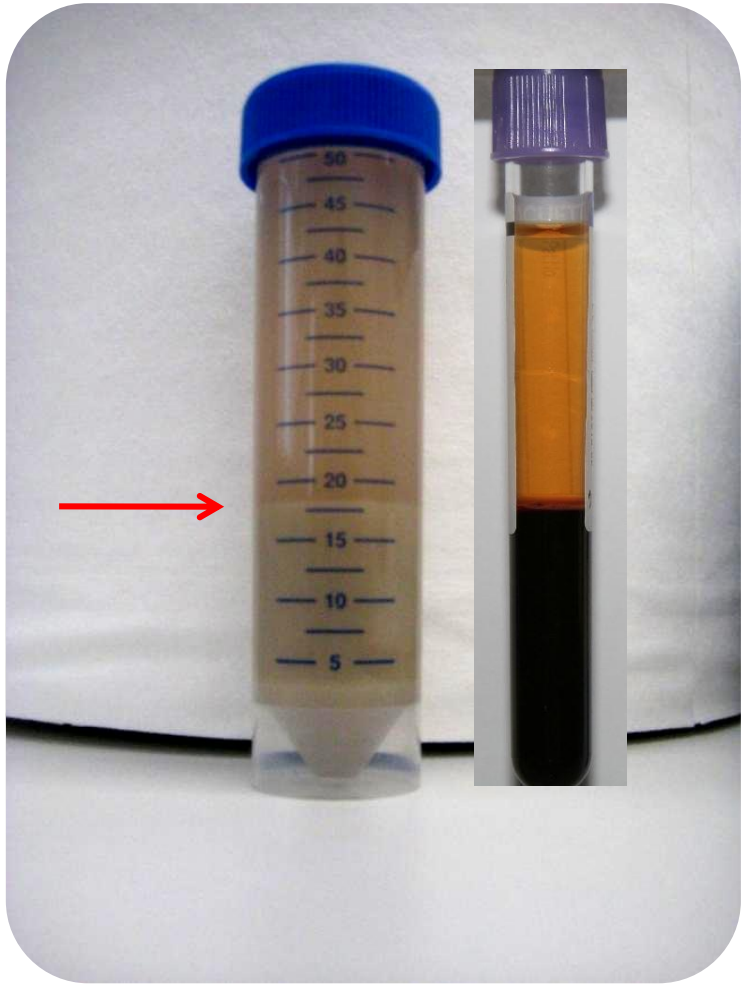
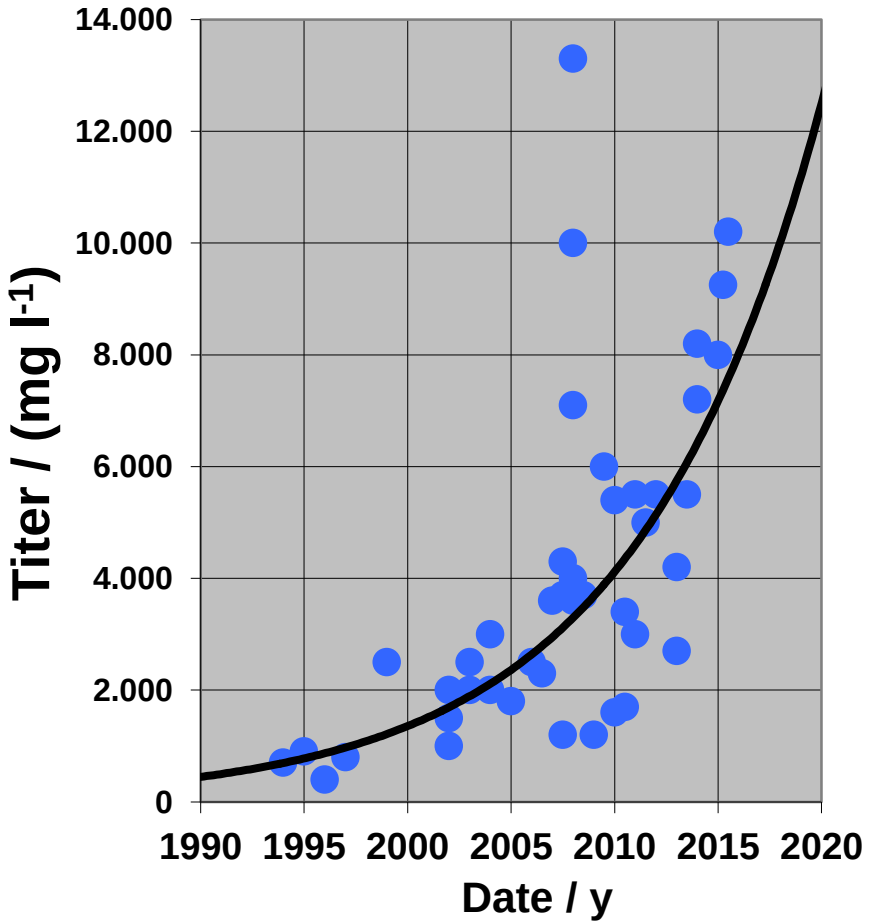
Cell concentration in bioreactor:
1.25×10^8 ml^{-1}
25 % packed cell volume
Ø = 10-20 µm

Cell concentration in blood:
$5\text{-}10 \times 10^9$ ml^{-1}
50 % packed cell volume
Ø = 7.5 µm



Boost Performed by Cell Culture Technologists

A fantastic job over the last 20 years



According to Thomas Ryll, 25th ESACT Meeting, Lausanne, 2017

- Drivers are
  - **Improving Safety** to patients (CD media)
  - Improved **Control & Consistency**
  - Development **Time Line Acceleration**
- In principle for antibodies and Fc-fusion proteins

Improved Cell Culture Production is Driven by

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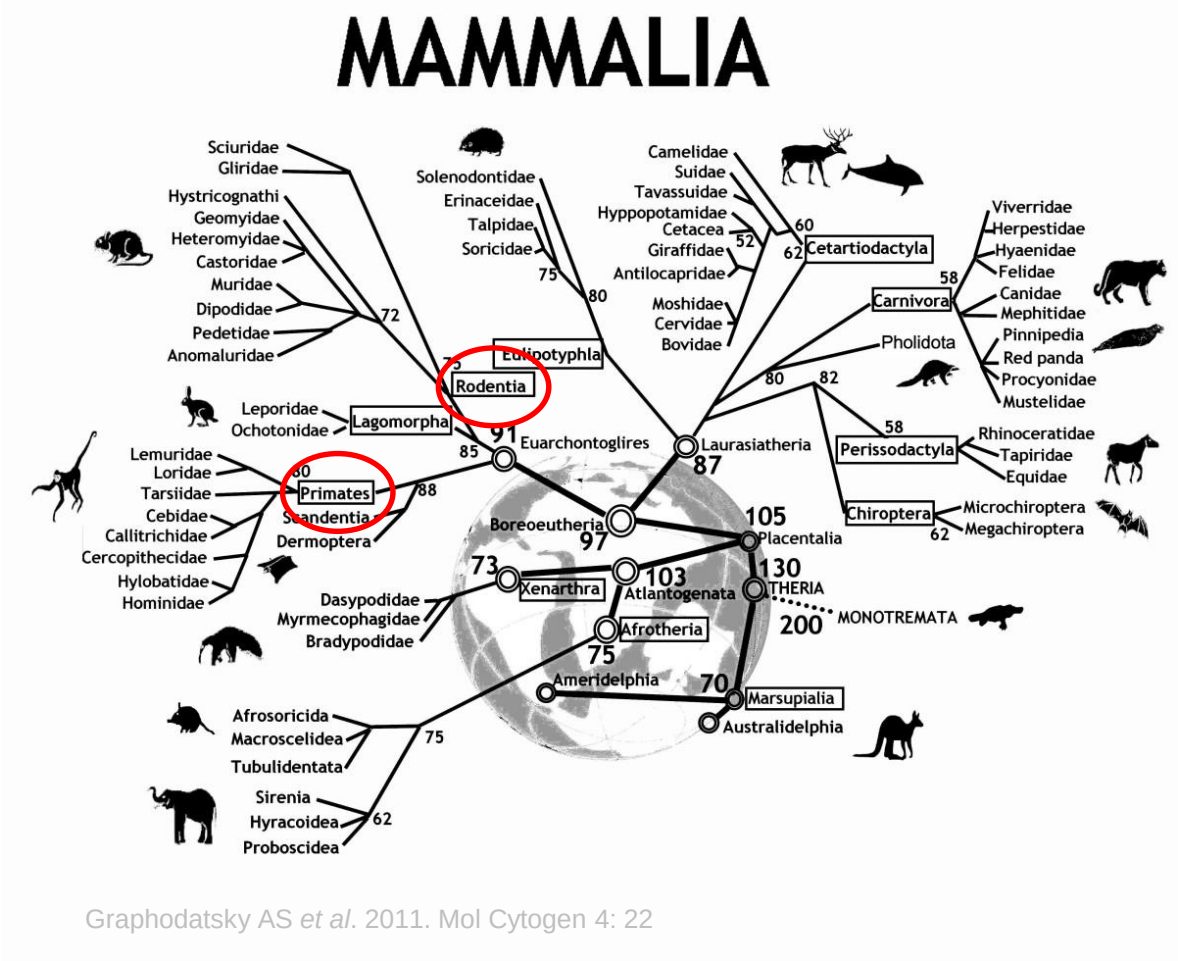
- **Safety, Control and Robustness**
  - Enhanced patient **safety**
  - Improved process **robustness** and consistency
  - Tailor-made product **quality** (comparability & similarity)

- **Efficiency, Potency and Cost**
  - Enhanced **productivity** and facility output (titer and volumetric productivity)
  - Excellent **pharmacokinetics**
  - Reduced processing time
  - Reduced process scale
  - Ease downstream operation
  - Ease technology transfer
  - Overall **cost** reduction (cost per gram of product produced)

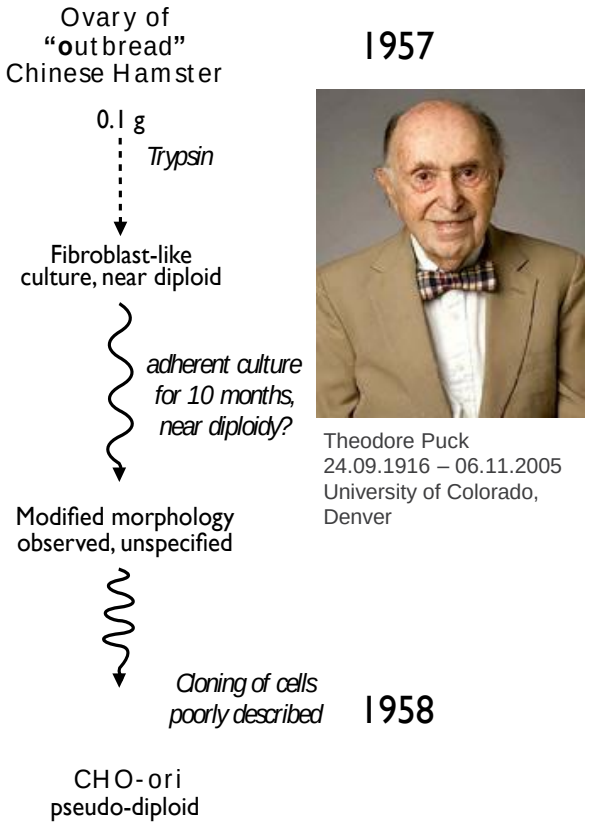
Only 3 % of the Genes Are Different in Humans and Rodents



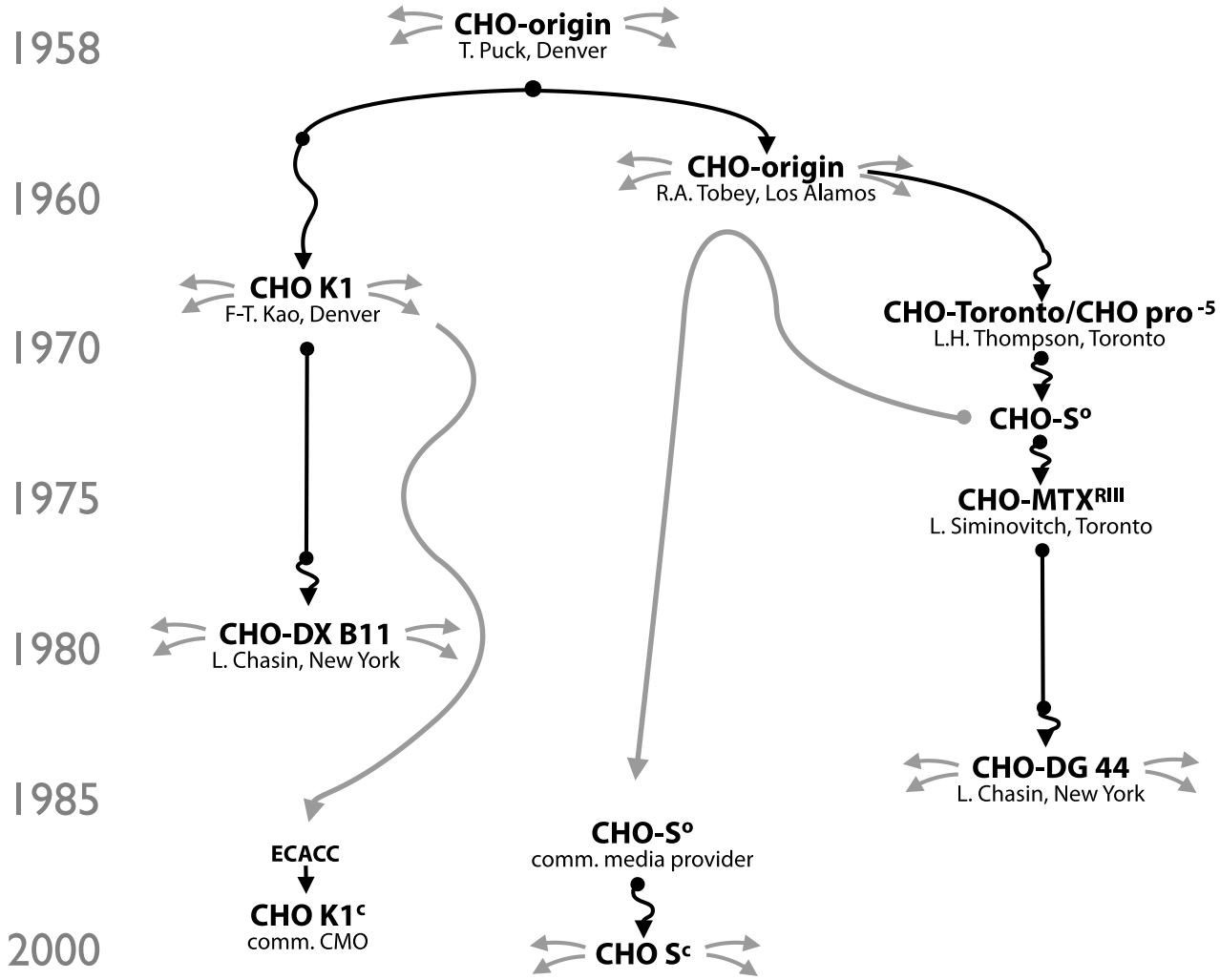
Graphodatsky AS *et al*. 2011. Mol Cytogen 4: 22

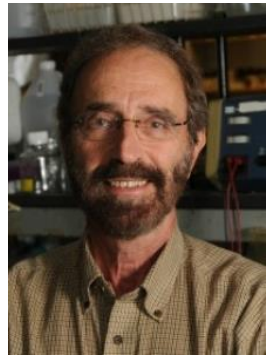
Chinese hamster *Cricetulus griseus*
Industrial cell line provider

The **CHO**dyssee



Theodore Puck
24.09.1916 – 06.11.2005
University of Colorado, Denver

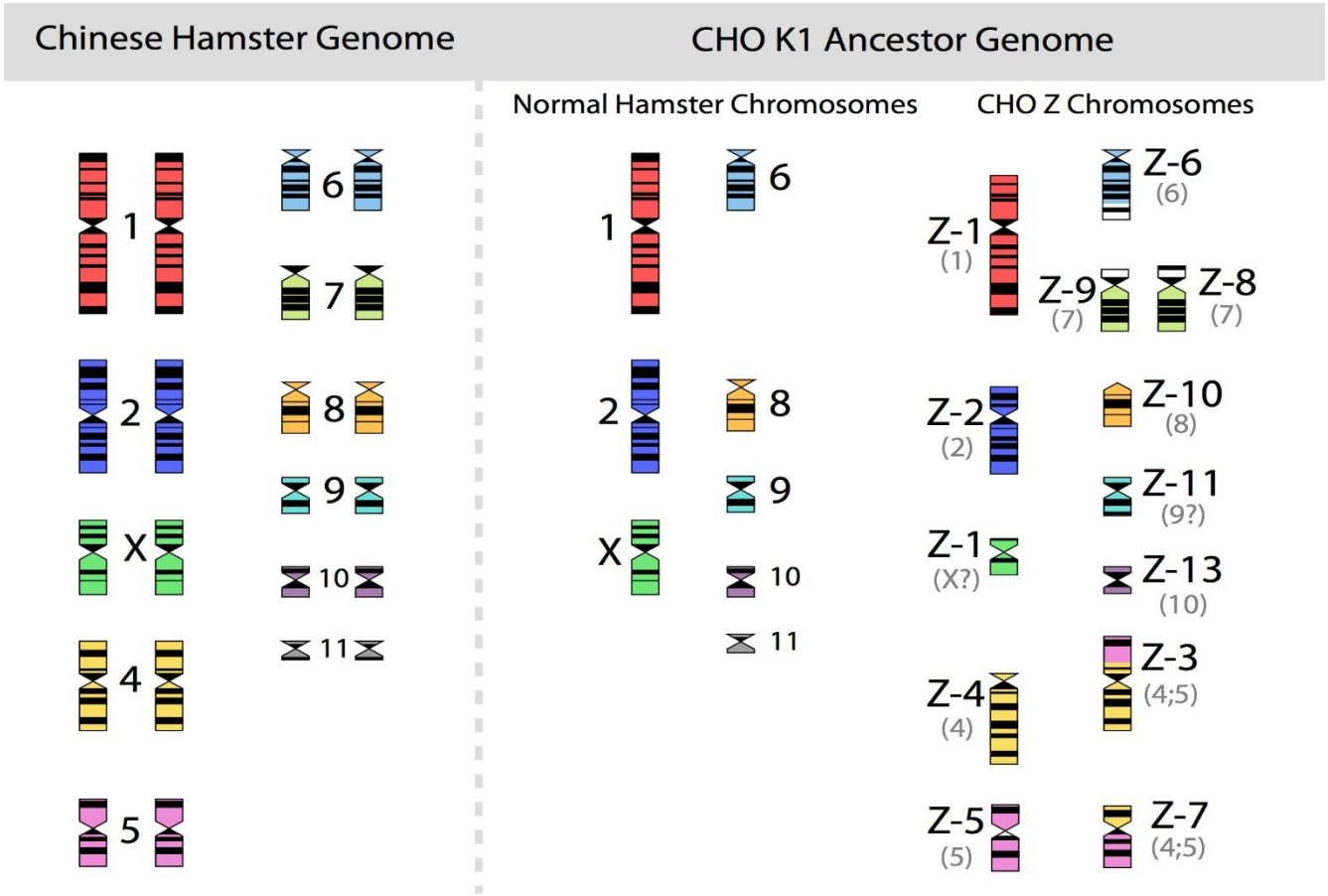




Lawrence Chasin
Columbia University, NY

The **CHrO**mosomes

The world of quasispecies



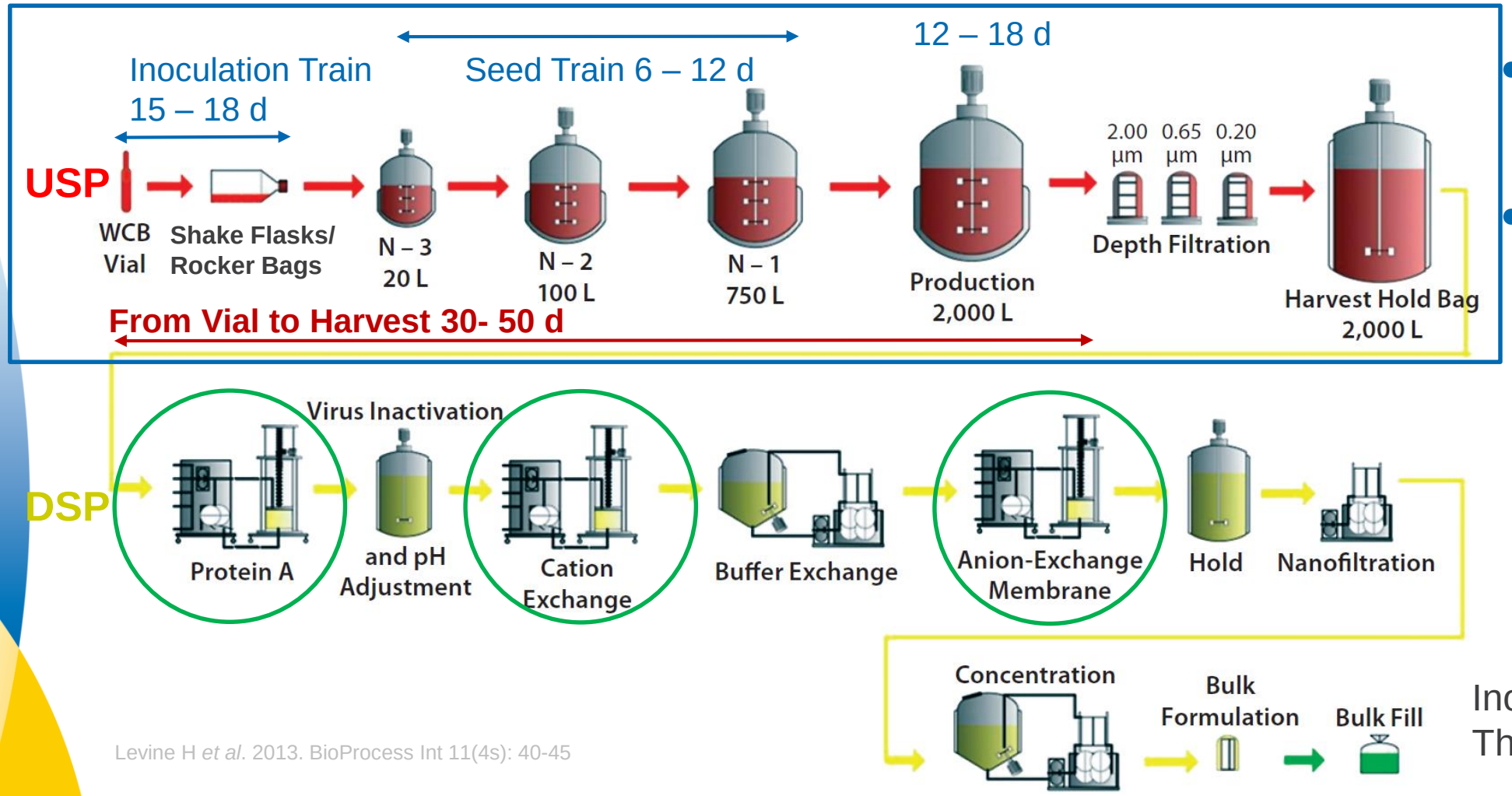
Cricetulus griseus
Starts in 1919 for typing pneumcococci

† 1957
Lifespan: 2-4 years

2n = 22 Chromosomen
predestinate it for research of
radiation cytogenetics

Wurm F. 2015. In Hauser H, Wagner R. Animal Cell Biotechnology in Biologics Production. WdG, Berlin

Typical Antibody Manufacturing Process Over the Last 20 Years



- Challenge: Overall Process Intensification
- Focus areas for improvements
  - Cell Banks
  - Seed Expansion
  - N-1 Cell Mass
  - Production Culture

Inoculation Train: Thaw and expand cells

Levine H *et al*. 2013. BioProcess Int 11(4s): 40-45

Cell Banks and Seeding Intensification

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- Increasing starting cell mass and reducing initial expansion steps
  - Rolling seed train
  - High cell concentration in vials or bags
  - Frozen seed train intermediates in bags

Basic Strategy	Advantage	Disadvantage

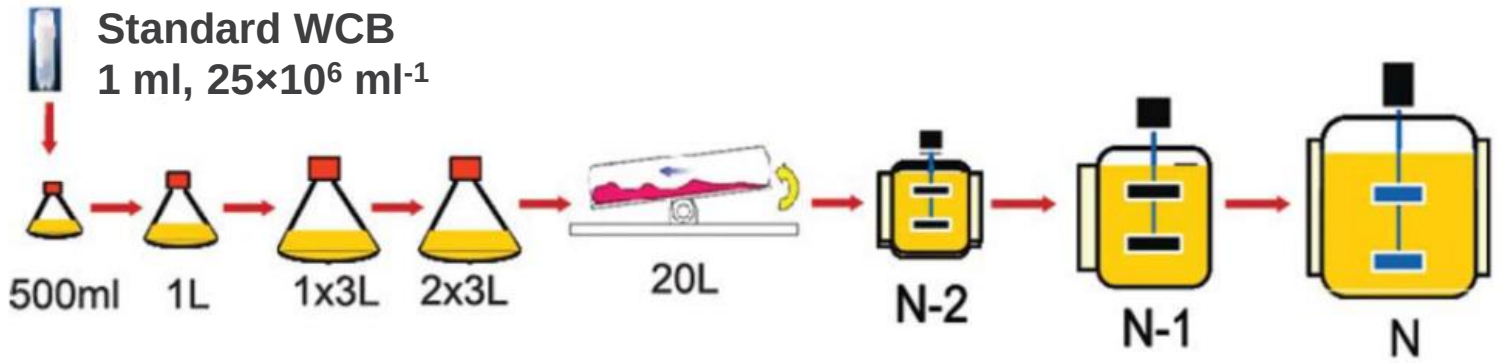
One Vial Only Batch	• Batch to batch identical seed train
	  • **Identical population** doublings
	  • Identical cell age
	
	SLOW
		• More WCB vials used
		• More labor intensive
		
		RELIABLE

Rolling Seed Train (small scale bioreactors)	• Less labor intensive
	• Less WCB vials used
	• Reduced number of seed train stages for each production run
	
	FAST
		• Each batch has a **different seed train history**
		  • Each batch has different cell age
		  • Cell line stability limits
		
		• **FAILURE-PRONE**

Heidemann R *et al.* 2002. Cytotechnology 38: 99-108; Heidemann R *et al*. 2010. Biotechnol Prog 26: 1154-1163; Tao YW *et al*. 2011. Biotechnol Prog 27: 824-829; Seth G *et al*. 2013. Biotechnol Bioeng 110: 1376-1385; Clincke MF *et al.* 2013. Biotechnol Prog 29: 768 - 777

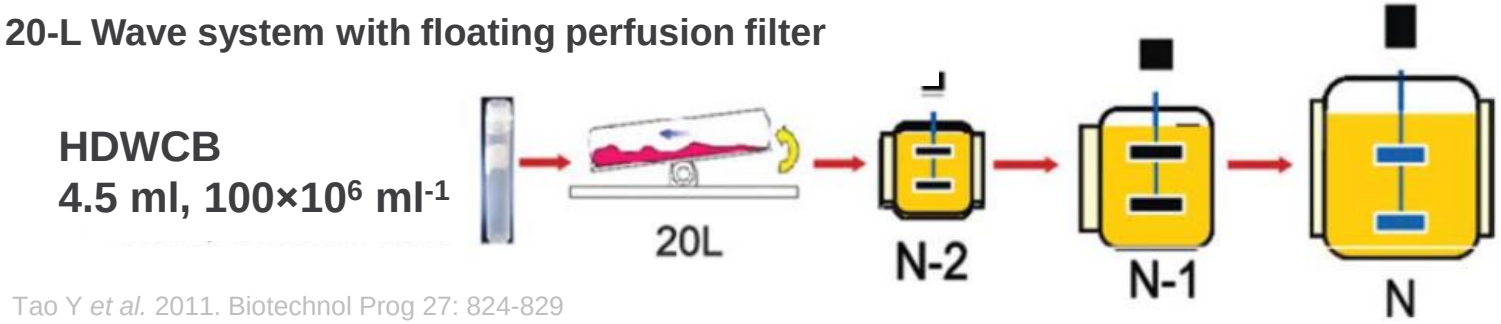
Cell Banks and Seed Intensification

High cell number working cell bank



Standard Vial Master Cell Bank + High Density Working Cell Bank

20-L Wave system with floating perfusion filter



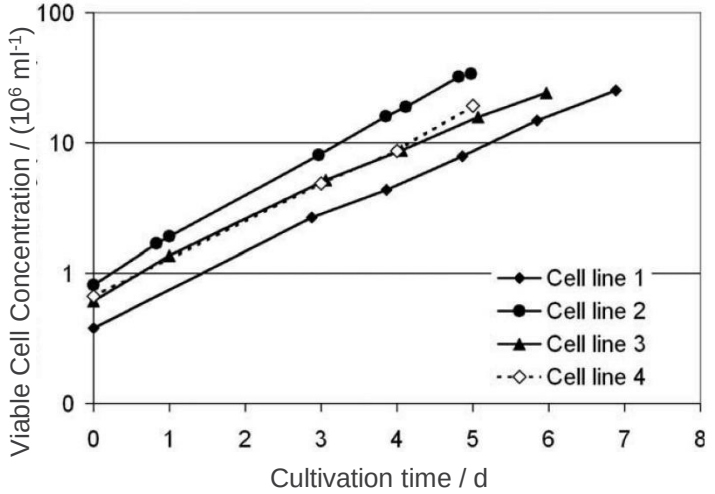
Tao Y *et al.* 2011. Biotechnol Prog 27: 824-829

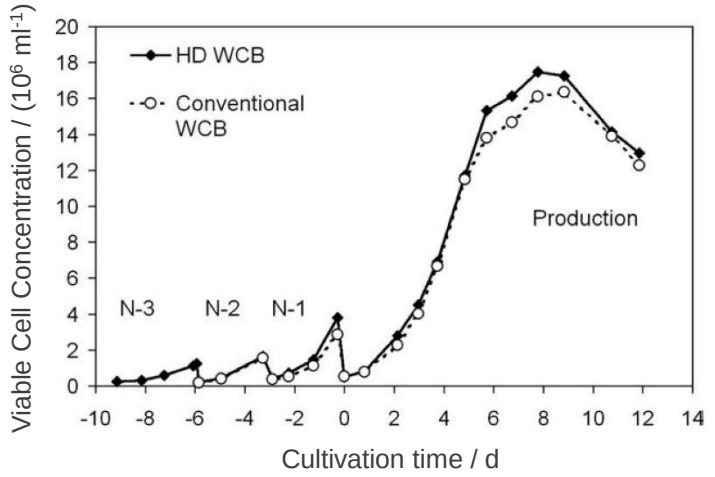
Thaw directly into Wave from 5-ml CryoTube vial (cell conc.100×10⁶ ml⁻¹)

Elimination of more variable seed expansion stages

9 days facility time saving after change over

20-L Wave system with floating perfusion filter





Production Culture Intensification

From batch to hybrid processes

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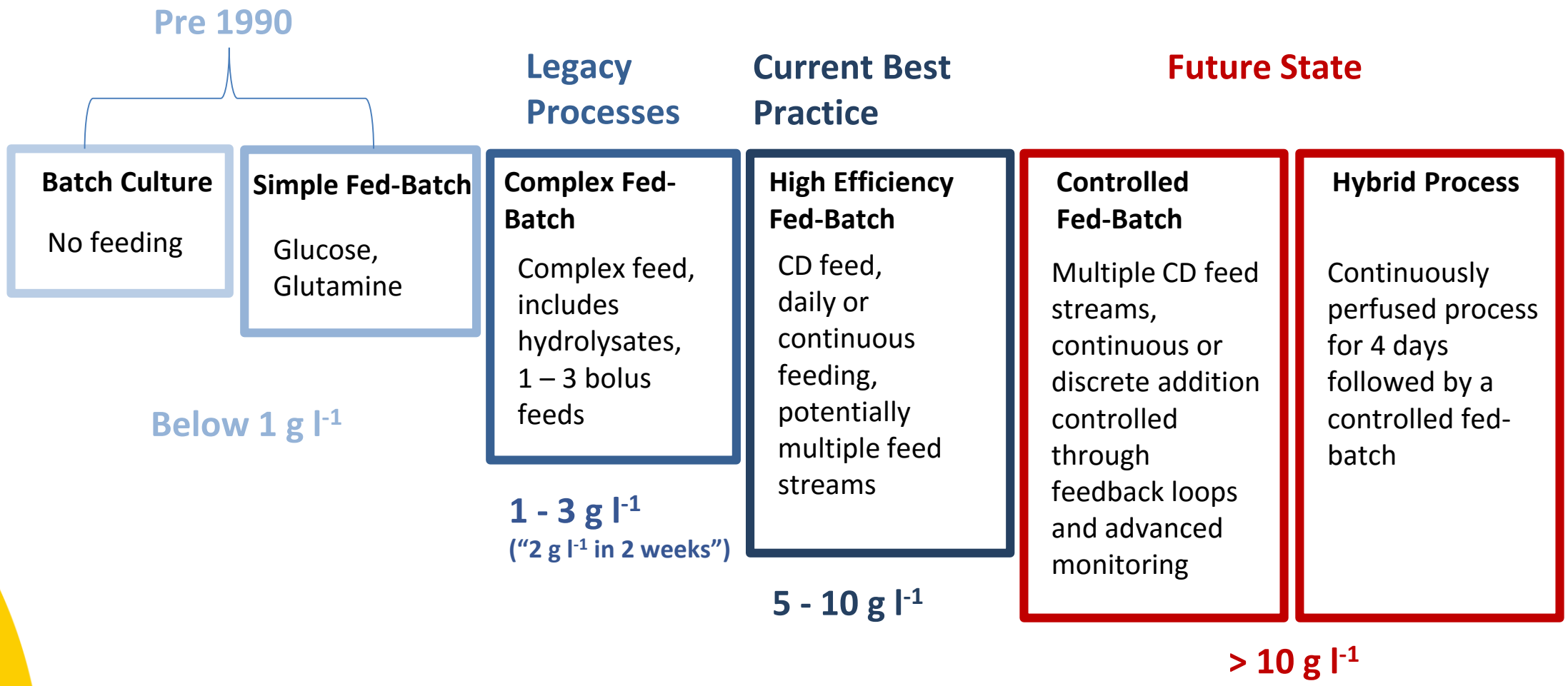
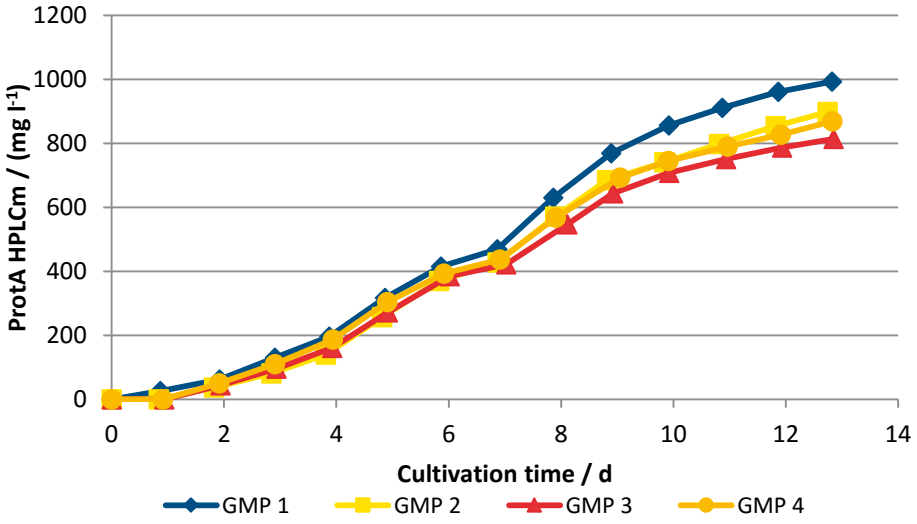
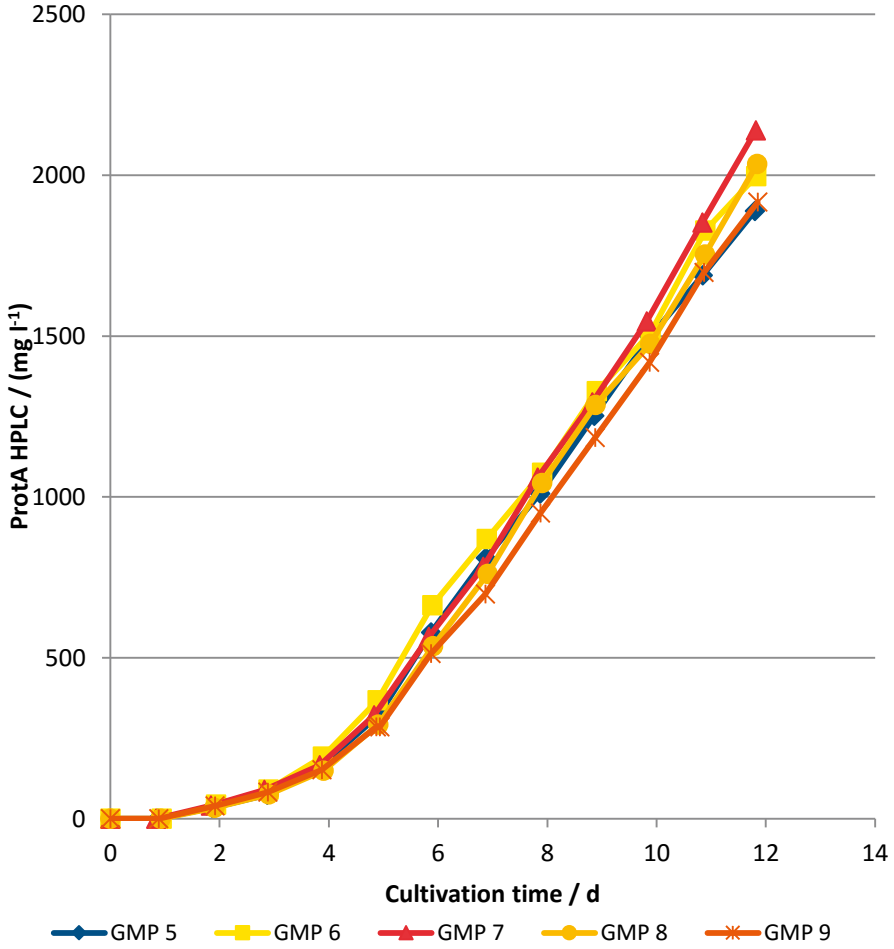


Effects of Accelerated Feed Strategy

- Shortened process time by 1 day
- Comparable cell conc. (<10×10^6 ml^{-1}) & viability
- Doubled protein titer at harvest





Fed-Batch Production Improvements Over the Last 20 Years
Iso-Titer Lines in Efficiency / Intensity Space

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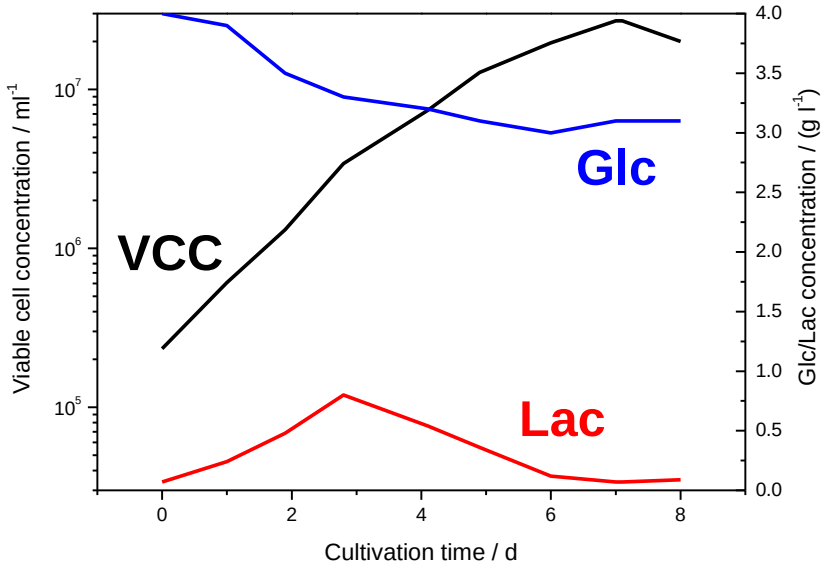
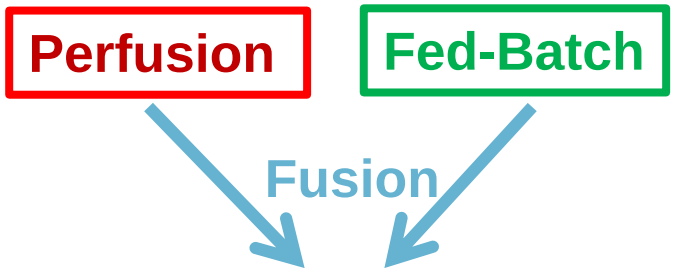
Cellsp. Productivity [pg d^{-1}]	Integral Cell Mass [10^6 ml^{-1}]	Titer [g l^{-1}]	Year
20	20	1	1990
30	70	2.5	- 2005
50	100	5	- 2010
50	200	10	> 2015
50	300	15	> 2017

Perfusion Applications:
- Perfusion supported Fed-Batch
- Concentrated Fed-Batch
- High Density Perfusion Culture

- Media / Feed Optimization
  - CD formulas, balanced feeding
- Host Cell Line that grows to high cell mass
  - Adapted to process materials and format
- Improved Process Control
  - Feedback loop driven feeding: Feed per cell, Glc / Lac control
  - Lactogenesis: HIPDOG, HIPCOP



Combining Benefits of Fed-Batch and Perfusion to Hybrid Processes



N-1 Perfusion
Seed culture Perfusion followed by Fed-Batch

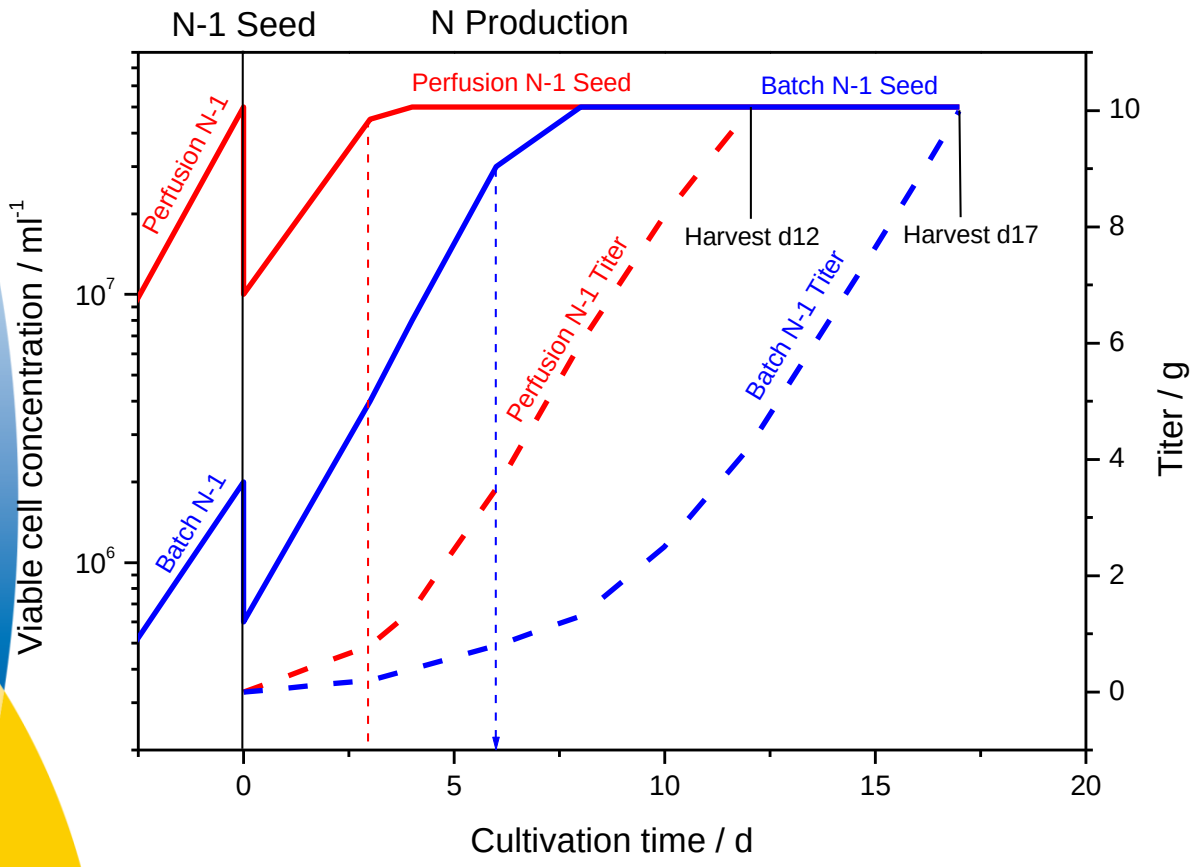
Hybrid Perfusion - Fed-Batch
Perfusion during Fed-Batch growth phase to boost cell mass

Concentrated Fed-Batch
Perfusion during Fed-Batch production phase using UF membrane to concentrate the product

Process Feature	Titer	Media Consumption	Complexity	Available Manufacturing Capacity	Cell Mass	Volumetric Productivity	Product Residence Time
Benefit	Fed-Batch	Fed-Batch	Fed-Batch	Fed-Batch	Perfusion	Perfusion	Perfusion

1st Concept: The N-1 Perfusion Production

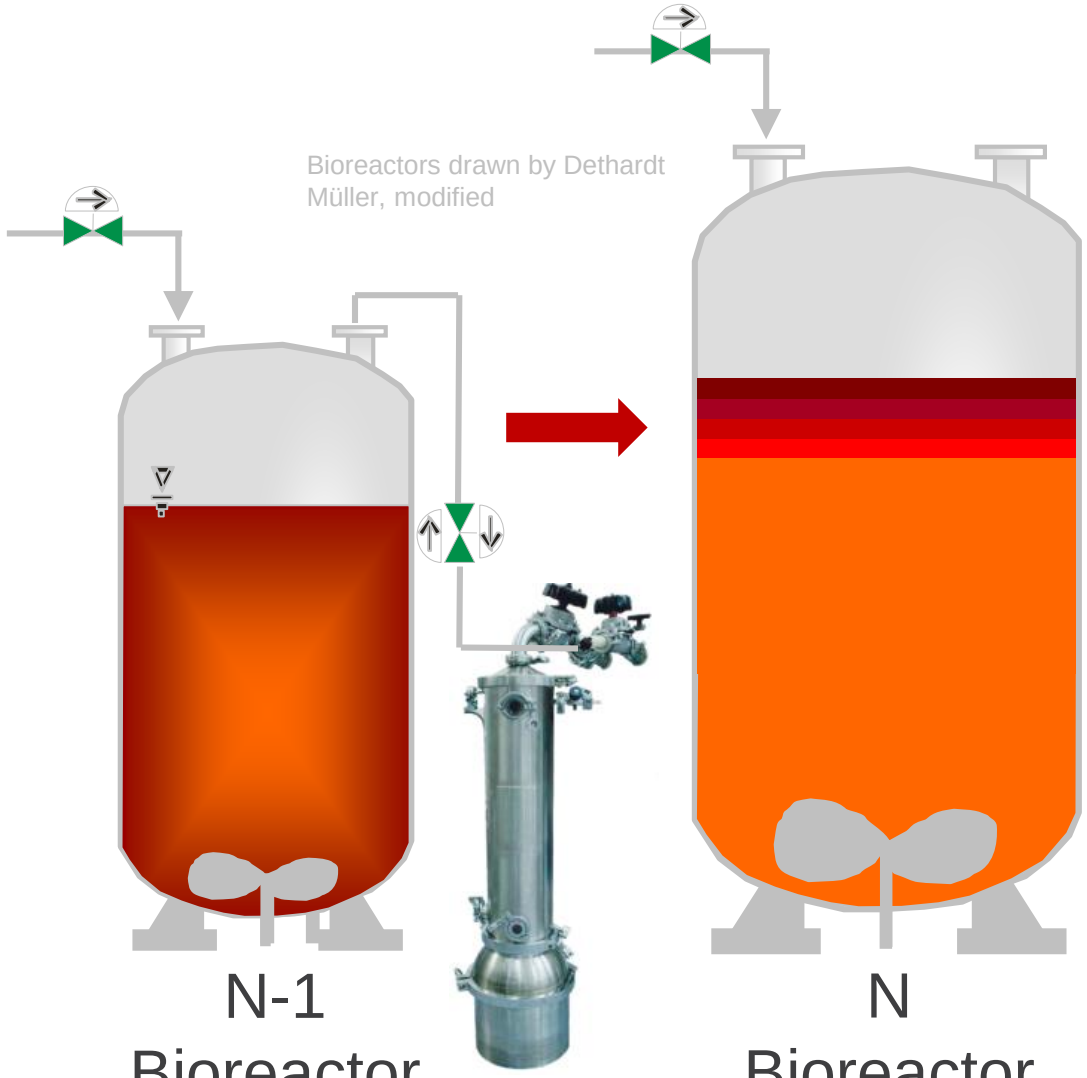
Perfusion by Microfiltration



Referring to Ryll T. 2017. 25th ESACT Meeting

Reduced production time & simultaneous increase of volumetric productivity

Bioreactors drawn by Dethardt Müller, modified

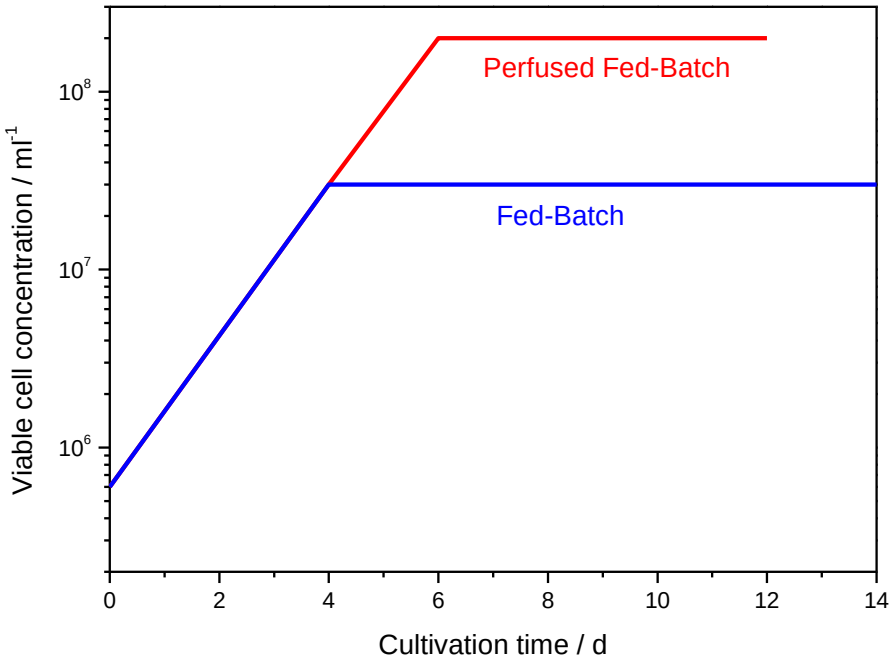


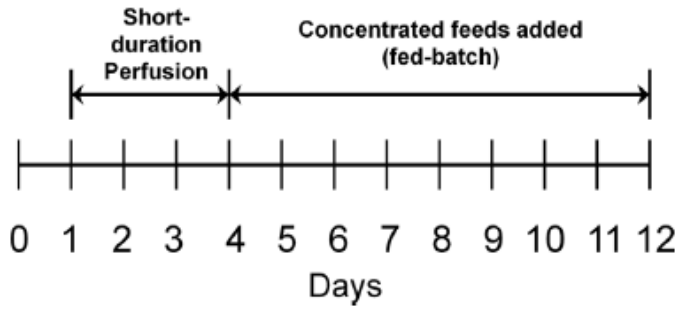
N-1 Bioreactor

N Bioreactor

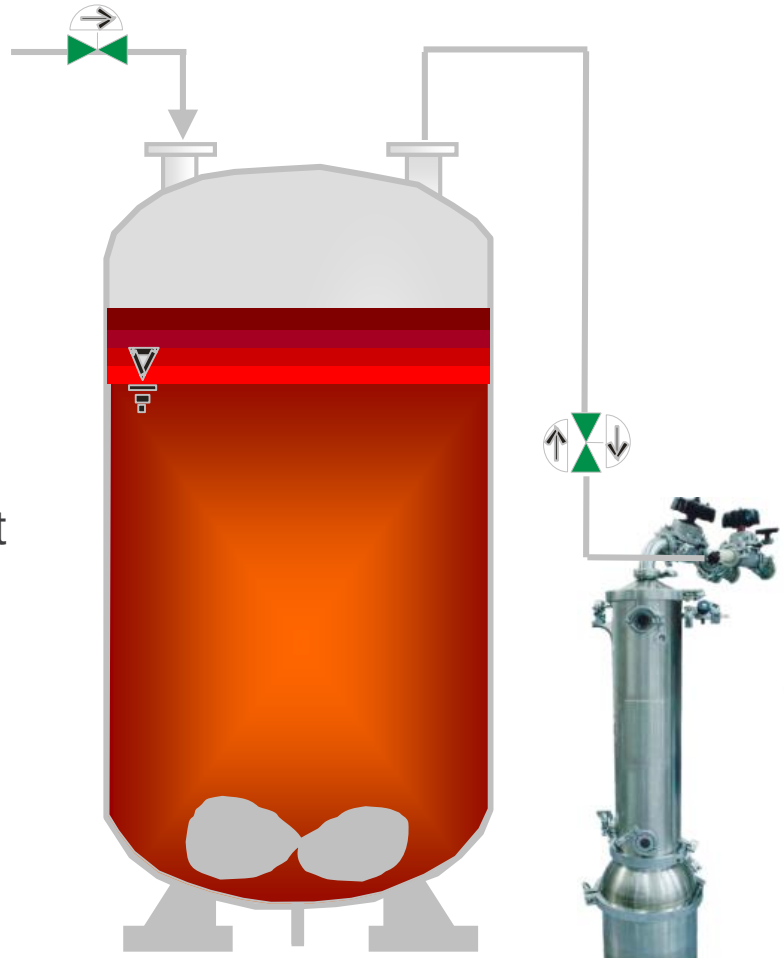
2nd Concept: The Hybrid Perfusion

Perfusion by Microfiltration





- **Perfusion** during initial growth phase **followed by fed-batch** culture at very high cell concentrations
- HIPCOP: High-end pH control of perfusion
- Increased celldays result in highest titers
- Titers **beyond 10 g l^{-1}** were achieved during 12 days run
- Volumetric productivities range at **1 g l^{-1} d^{-1}** for multiple cell lines with a moderate cellular productivity

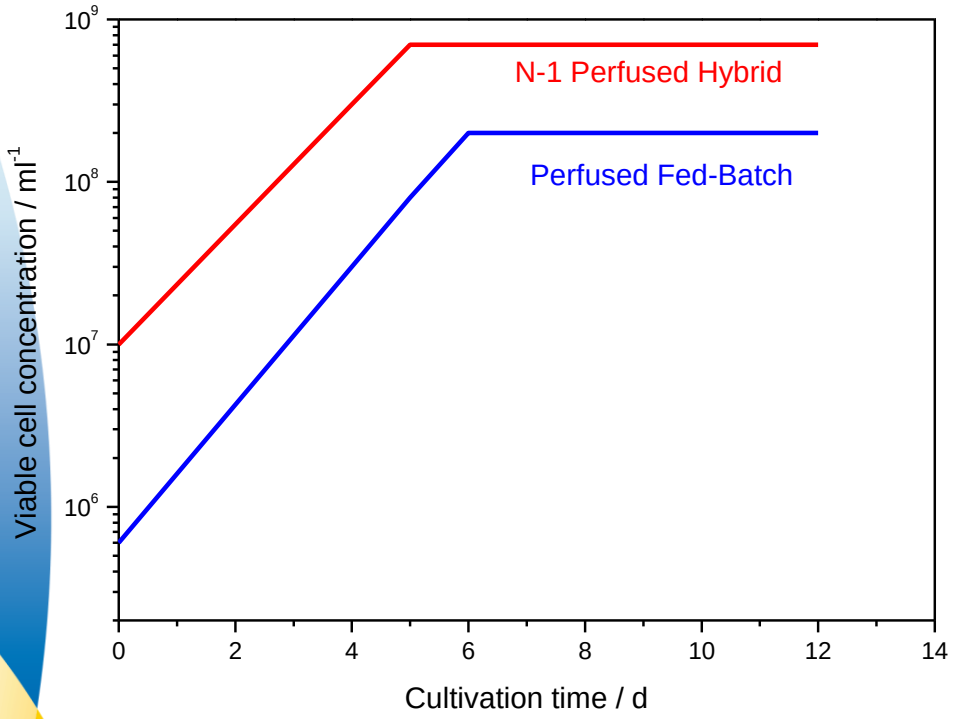


N Bioreactor

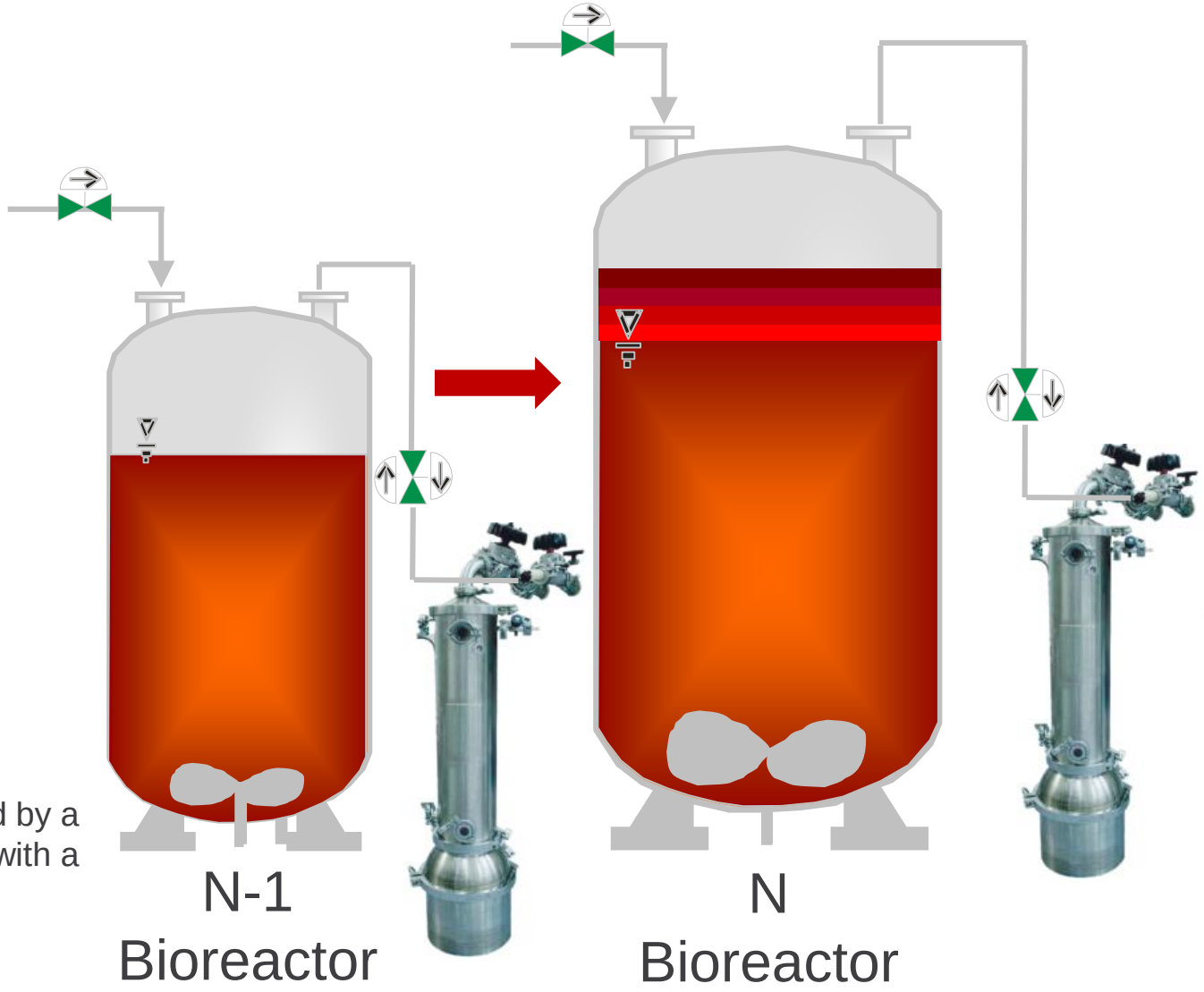
Bioreactor drawn by Dethardt Müller, modified

3rd Concept: The N-1 Perfusion Hybrid Process

Perfusion by Microfiltration



- Start perfusion at the N-1 seed bioreactor state followed by a short perfusion period at production scale and finished with a fed-batch process
- Reduction of process time by simultaneous increase of productivity

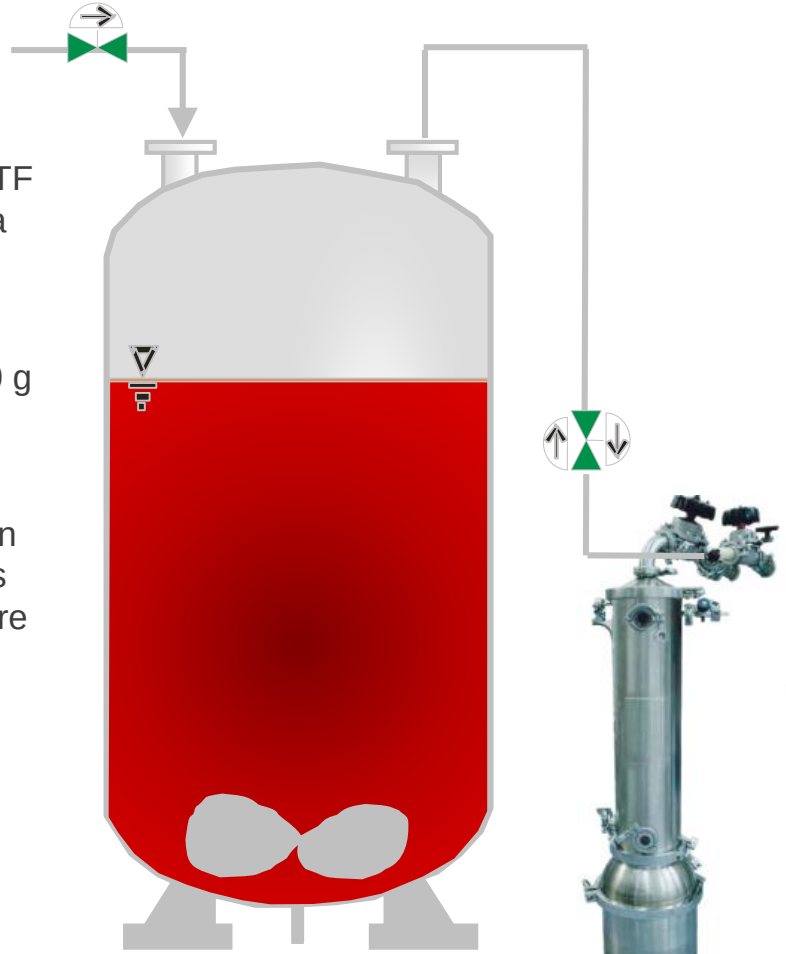


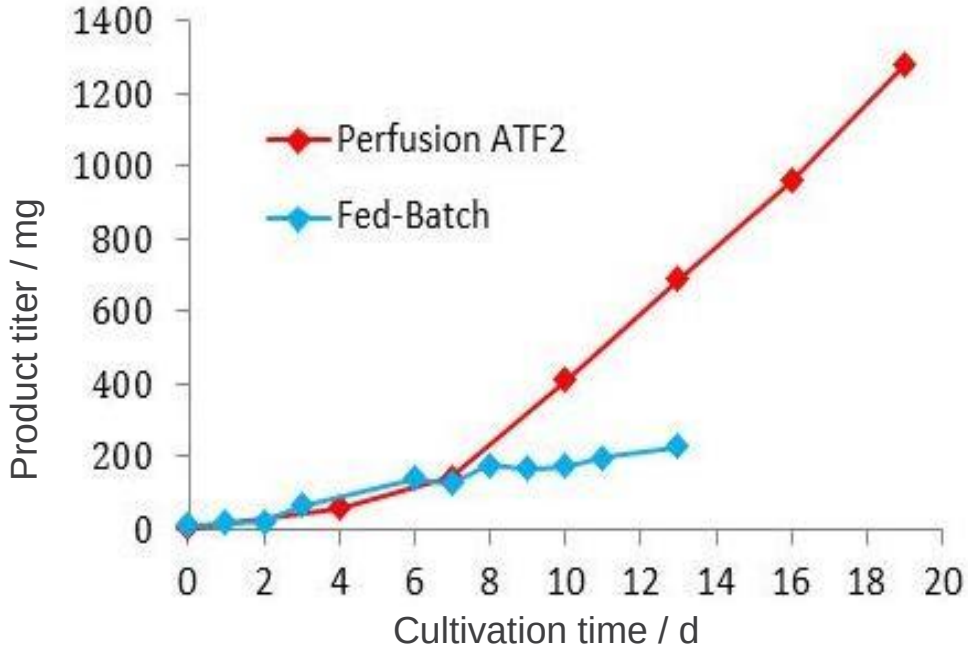
Bioreactors drawn by Dethardt Müller, modified

Go the Extra Mile: The Concentrated Fed-Batch Concept

Perfusion by Ultrafiltration

- Ultrafiltration using the XCell™ ATF module consisting of a 30-50 kDa membrane
- Retains cells and proteins
- Concentrates proteins beyond 20 g l^{-1} (XD® Process, DSM)
- Permeate allows waste removel
- Total cumulative protein content in XCell™ ATF perfusion is ~6 times higher than in the fed-batch culture

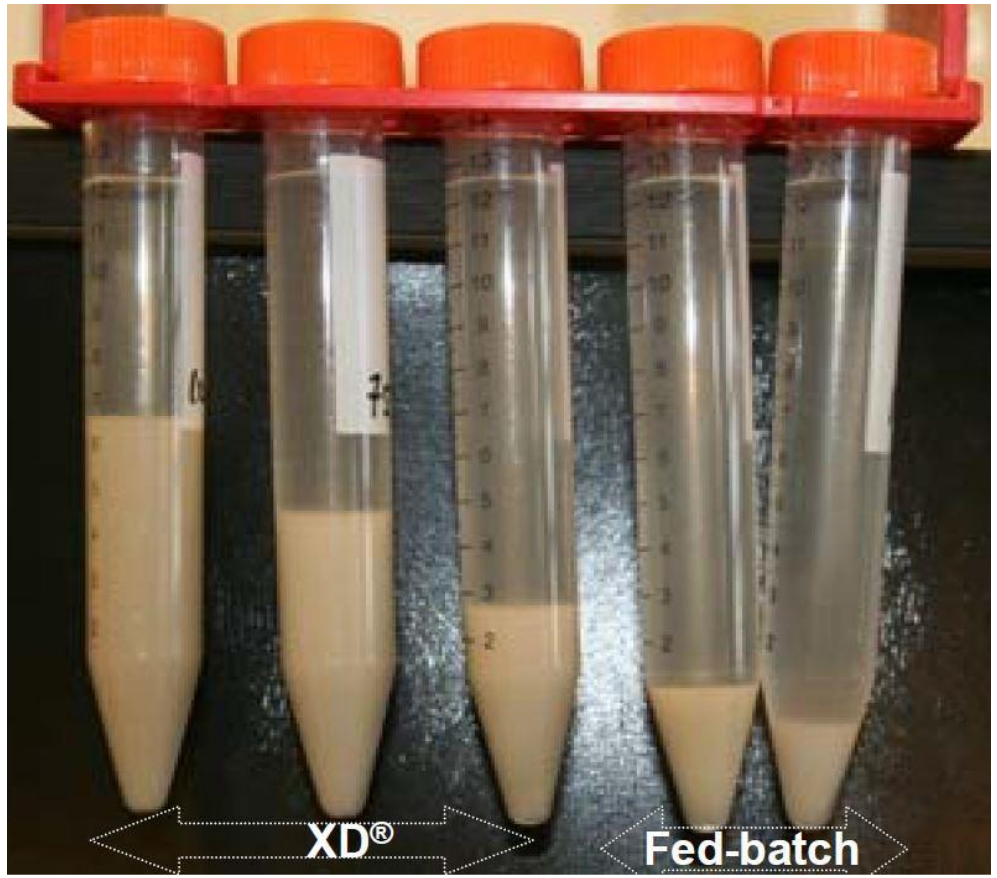




Genzel Y, MPI, Magdeburg, Germany

The Concentrated Fed-Batch Concept

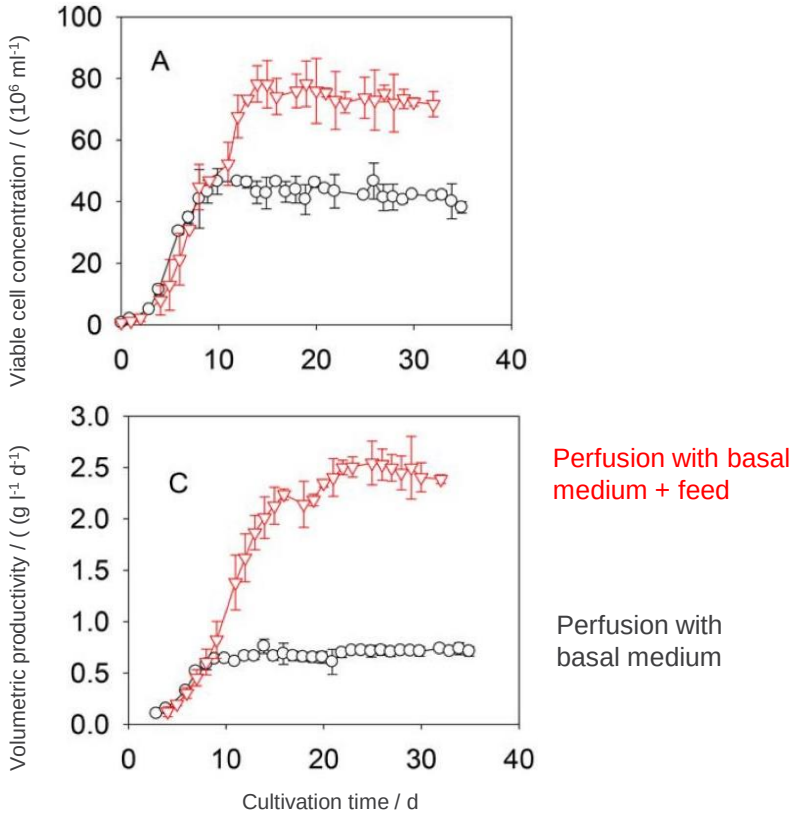
Process	Cell concentration [ml^{-1}]	Titer [$g\ l^{-1}$]	Volumetric productivity [$mg\ l^{-1}\ d^{-1}$]	Reference
Fed-Batch	5×10^7	8	430	Yang WC *et al.* 2016. J Biotechnol 217:1-11
Concentrated Fed-Batch	18×10^7	25	790	Yang WC *et al.* 2016. J Biotechnol 217:1-11
XD® Process	15×10^7	27	1800	Douwenga R. 2013. Bioproc Intl. Industry Yearbook: 99
Concentrated Fed-Batch	9×10^7	37	2500	Xu S *et al.* 2017. Biotechnol Prog 33: 867-878



Linz F. 2011. DSM

Impact of Continuously Perfused High Density Cultures

Iso-Volumetric Productivity Lines in Efficiency / Intensity Space

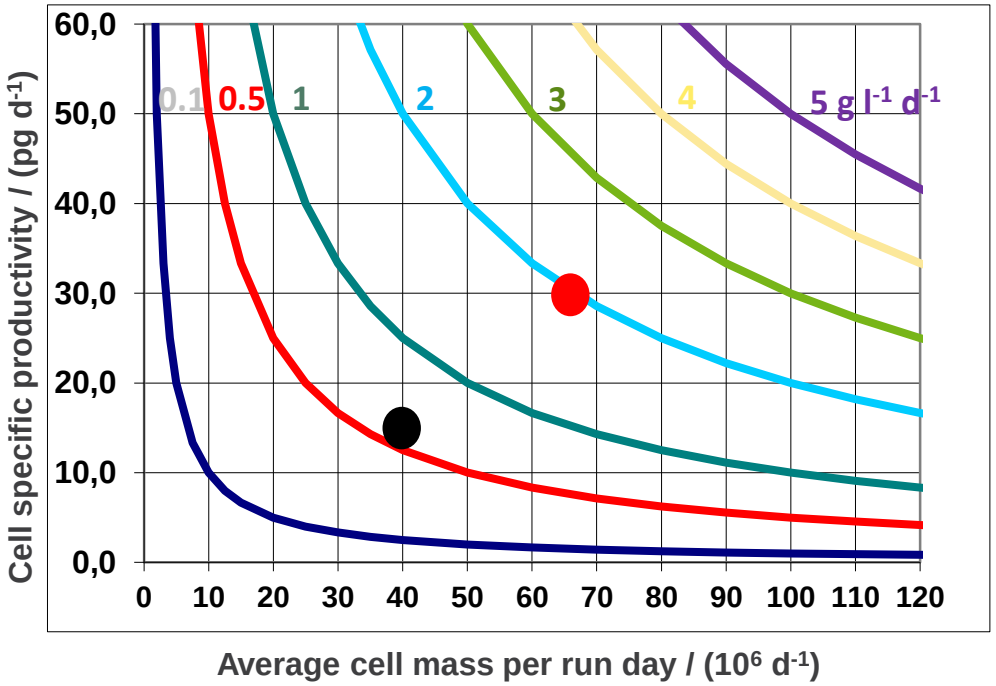


Perfusion with basal medium + feed

Perfusion with basal medium

Xu S *et al.* 2017. Biotechnol Prog 33: 867-878

Efficiency



Intensity

Ryll T. 2017. 25th ESACT Meeting, Lausanne

Supply of an Antibody Market - Economy Determines the Process

Rentschler Biopharma

- Case study to supply a large market (e.g. Alzheimer - a worse case)
  - Potential patients: >10 million next 30 years.
  - 50 % market penetration: 5 million patients
  - Yearly demand per patient: 24 g
  - 5 million patients: 120 000 kg
  - 2500 kg with 20 000-l bioreactor per year
  - 10 – 50× 20-k bioreactors required

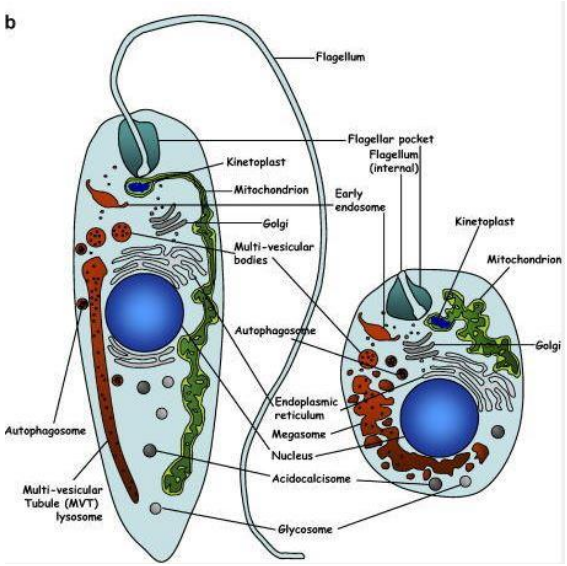
Not a problem even with todays technology

One Step Further

Cricetulus griseus

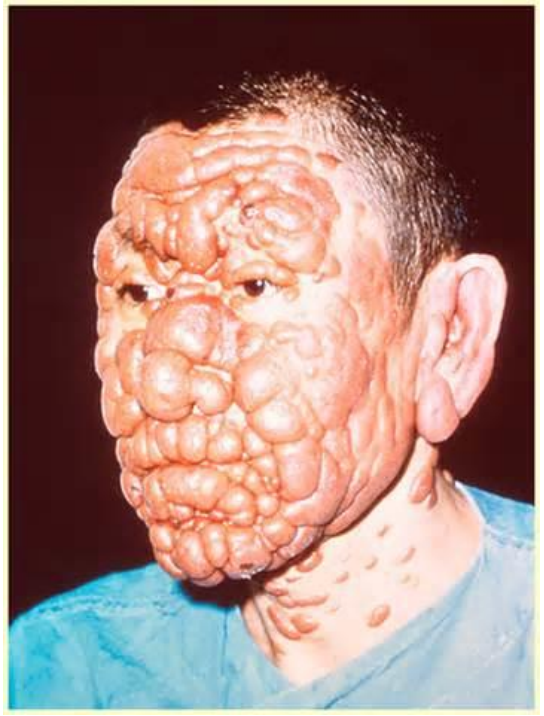
† 1957
2n = 22 Chromosomes
CHO cell size: 15×15 µm
Max. cell concentration 8×10^{8} ml^{-1}
Doubling time: 20 h
Culture temperature: 37 °C

Leishmania tarentolae



Will be born
2n = 36 Chromosomes
Cell size: 15×5 µm
Max. cell concentration: 5×10^{9} ml^{-1}
Doubling time: 6 h
Culture temperature: 26 °C

Leishmaniasis Hosts

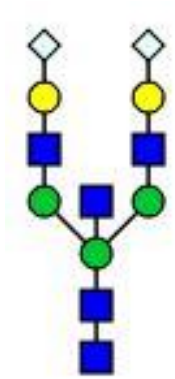


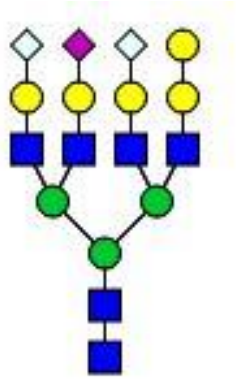
Man *Homo sapiens*
Kala Azar from *Leishmania donovani*

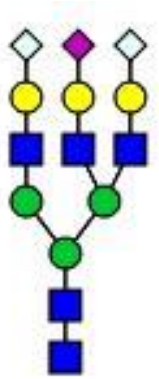
Phlebotomus
Carries promastigotes.
Poikilothermic insects have an average temperature of 26 °C.

White-Spotted Wall Gecko *Tarentula annularis*
Leihmaniasis from *Leishmania tarentulae*

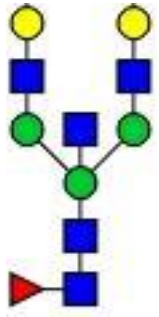
Comparison of N-Glycan Patterns







CHO



Leishmania

- Complex type N-glycans with α-linked galactose and fucose residues.
- Very homogeneous.
- Only biantennary asialo N-glycans.
- *In vitro* sialylation possible.

Next Challenges Just Around the Corner

Rentschler Biopharma

- **Artificial Cells**
  - Cell specific productivity
  - Stable cell lines for continuous processing
- **Process Intensification**
  - Highly concentrated media and feeds
  - Harvest process
- **Drug Safety and Potency**
  - Quality control
  - ADC
- **Processes for Gene & Cell Therapeutics**
  - Viral vectors
  - Cells as products