



evropský
sociální
fond v ČR



EVROPSKÁ UNIE



MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY



OP Vzdělávání
pro konkurenceschopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Rekombinantní biotechnologie

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Training Outline

Differences between biologics and synthetic drugs

Manufacturing of rFVIII

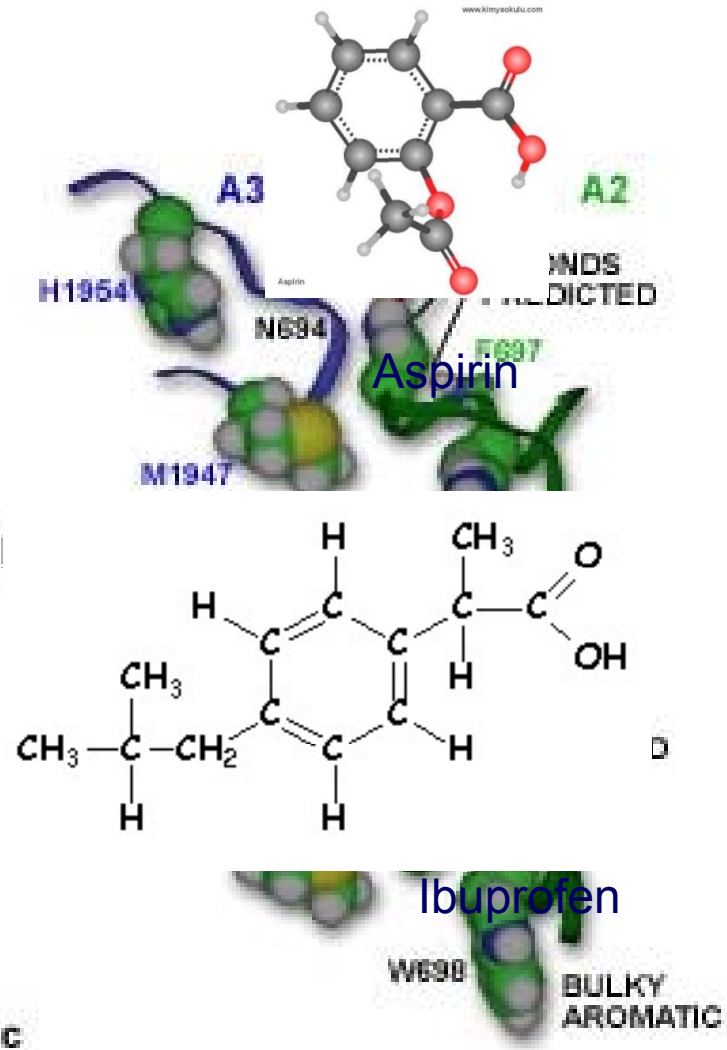
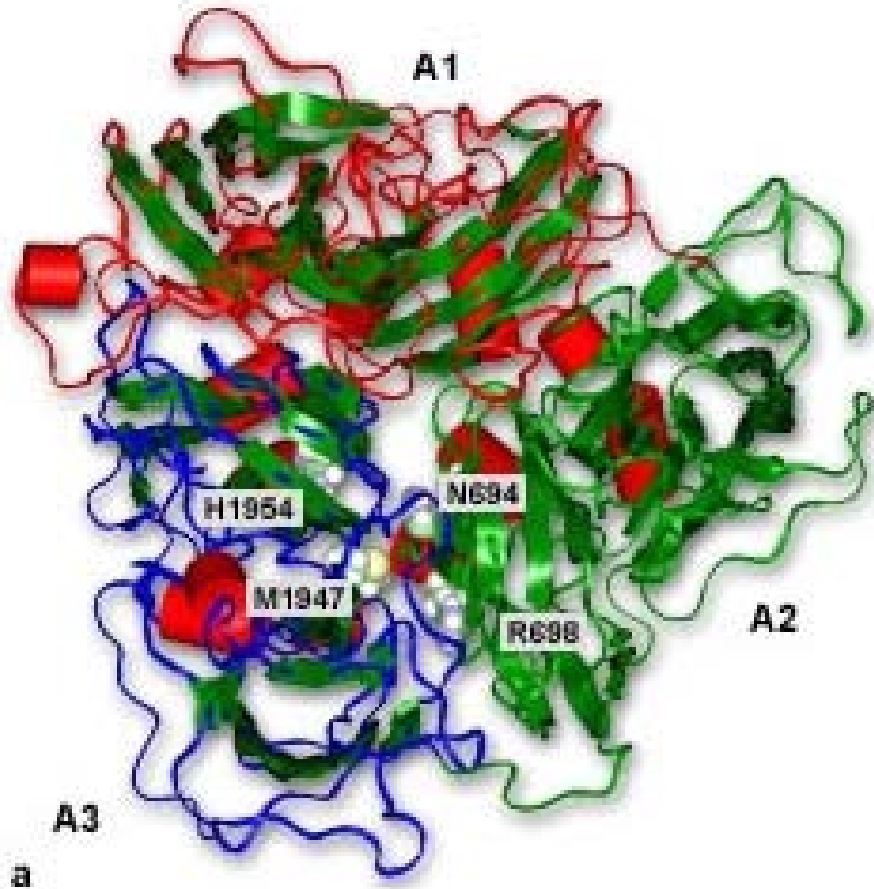
- Cell lines and cell culture media
- Master and working cell banks
- Fermentation processes
- Purification
- Final formulation

Biologics versus conventional synthetic drugs

FVIII: Glycoprotein with 6 domains

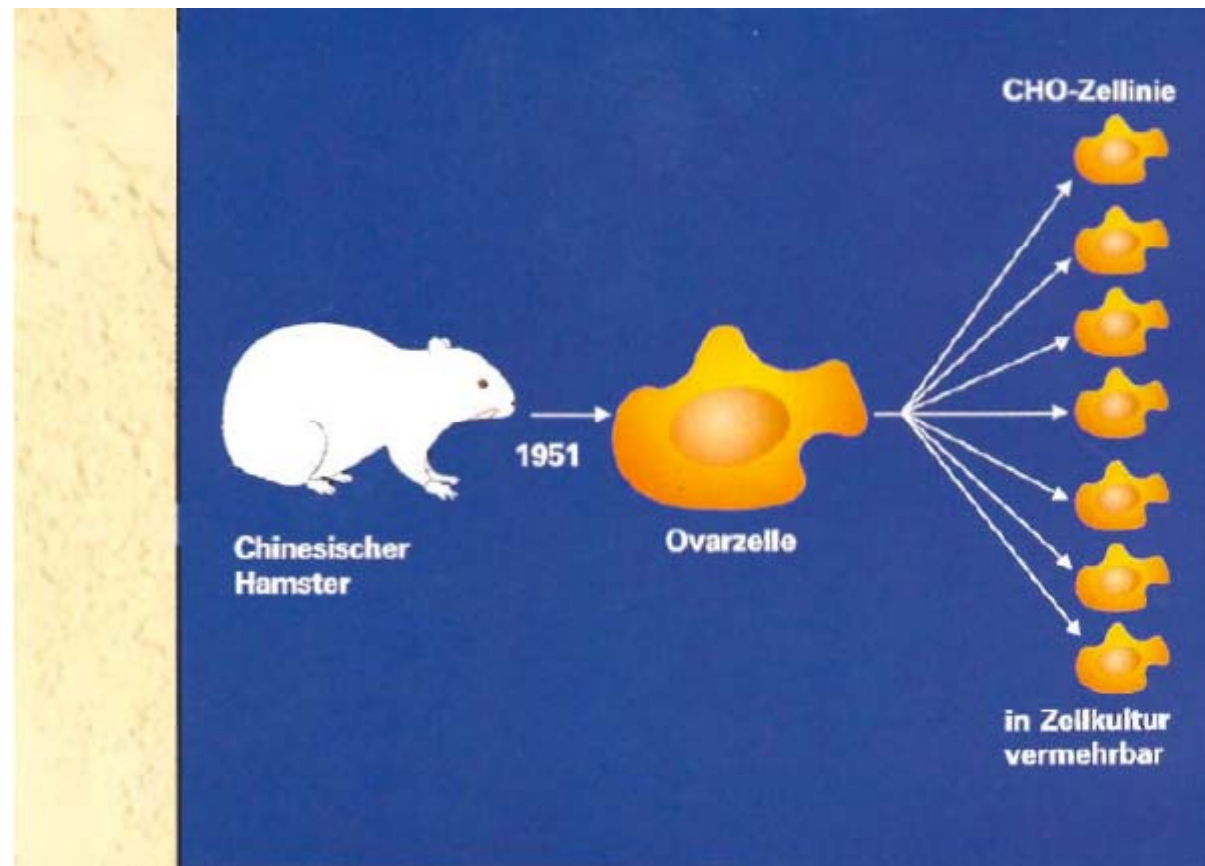
2,351 amino acids

MW 270,000 Dalton



Biological cell systems are needed to synthesize complex biologics such as human FVIII

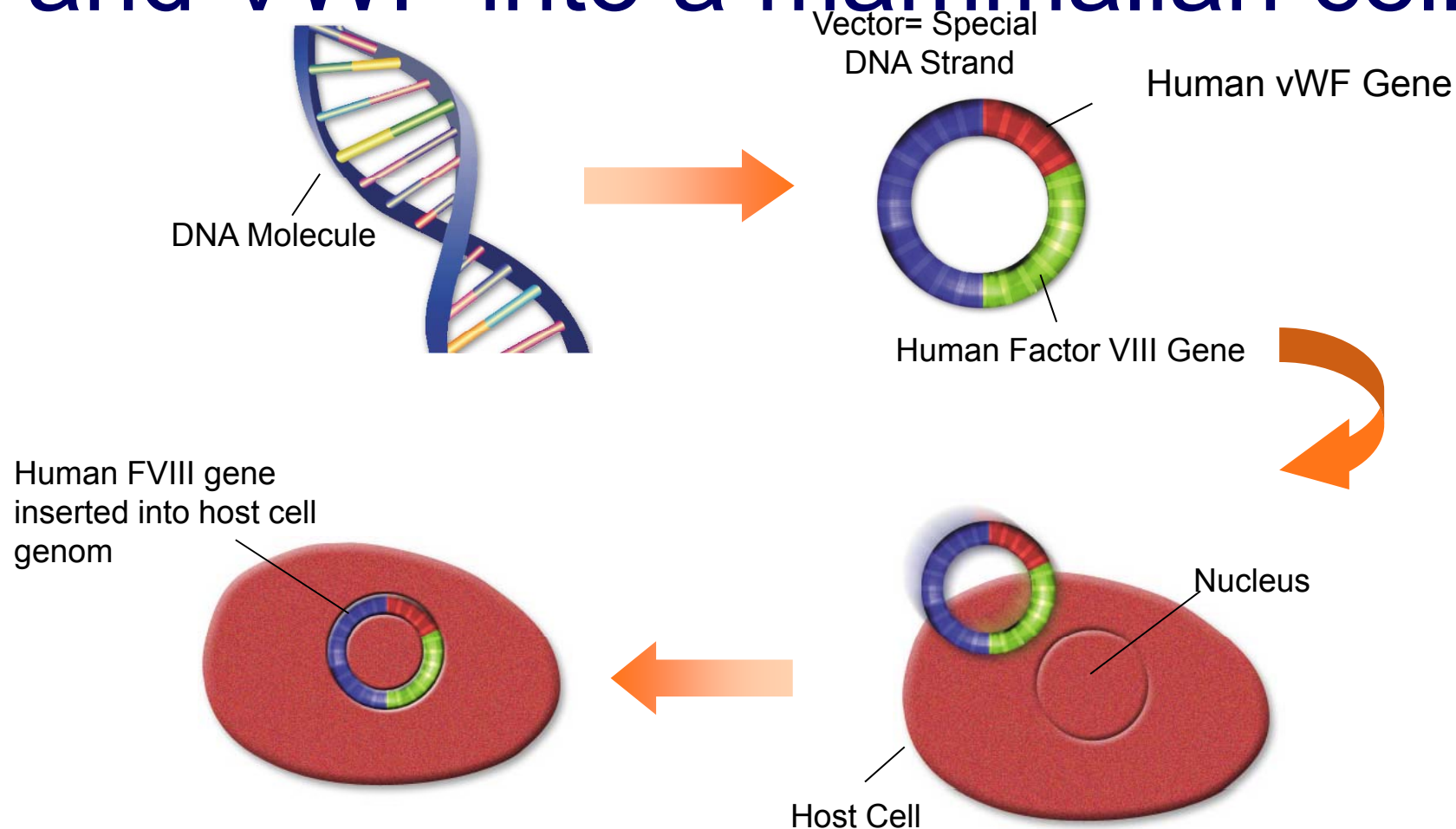
1. Step: Development of a mammalian cell line



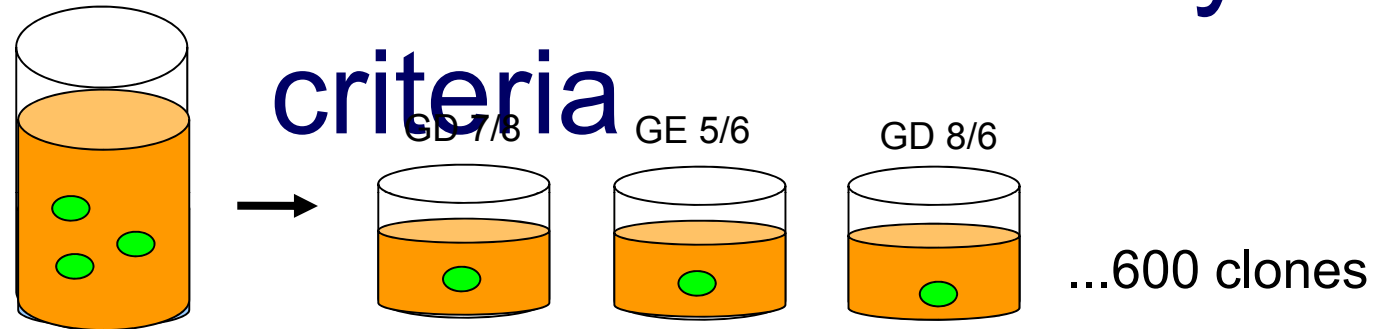
The Chinese Hamster Ovary (CHO) cell line

- Extensively studied and well characterized cell line (not generated from a tumor cell line)
- Used to produce many recombinant proteins
 - EPO (anemia), t-PA (acute myocardial infarction),
Interferon- β 1a (relapsing multiple sclerosis), follitropin- β
(infertility), Dnase (cystic fibrosis)
- Capable of all post-translational modifications in Factor VIII
- Resistant to infection by most human viruses
(Corona, Measles, Influenza, Herpes, Rhino viruses...etc.)
- In production, scaleable to large volumes

Transfer of the human factor VIII and VWF into a mammalian cell



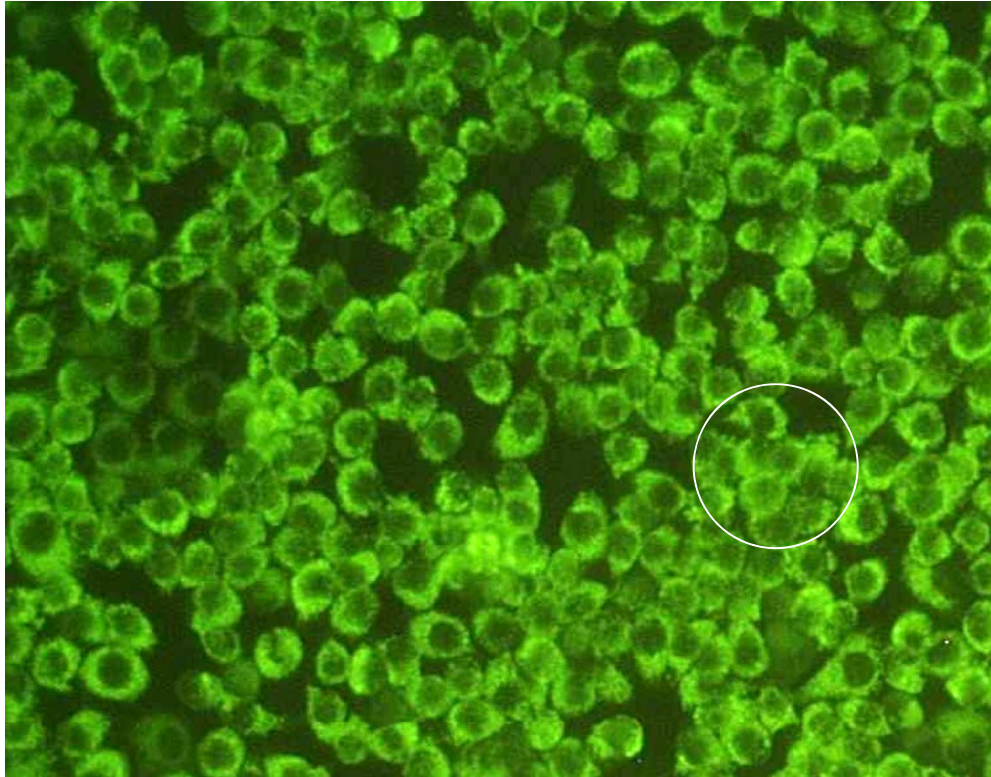
Selection of a FVIII producing cell clone based on certain key



Clone test program

- Homogeneity of cell population
- Robust Factor VIII and vWF production
- Western blot profile

FVIII producing CHO cells



FVIII Immunofluorescence

Cell culture medium

Nutrient components to keep cells alive

Sugars

Salt

Amino acids

Vitamins

Additives that support cell growth

fetal calf serum

1950s

Insulin, albumin, aprotinin, transferrin (bovine)

1990s

Human albumin and recombinant insulin

late 1990s

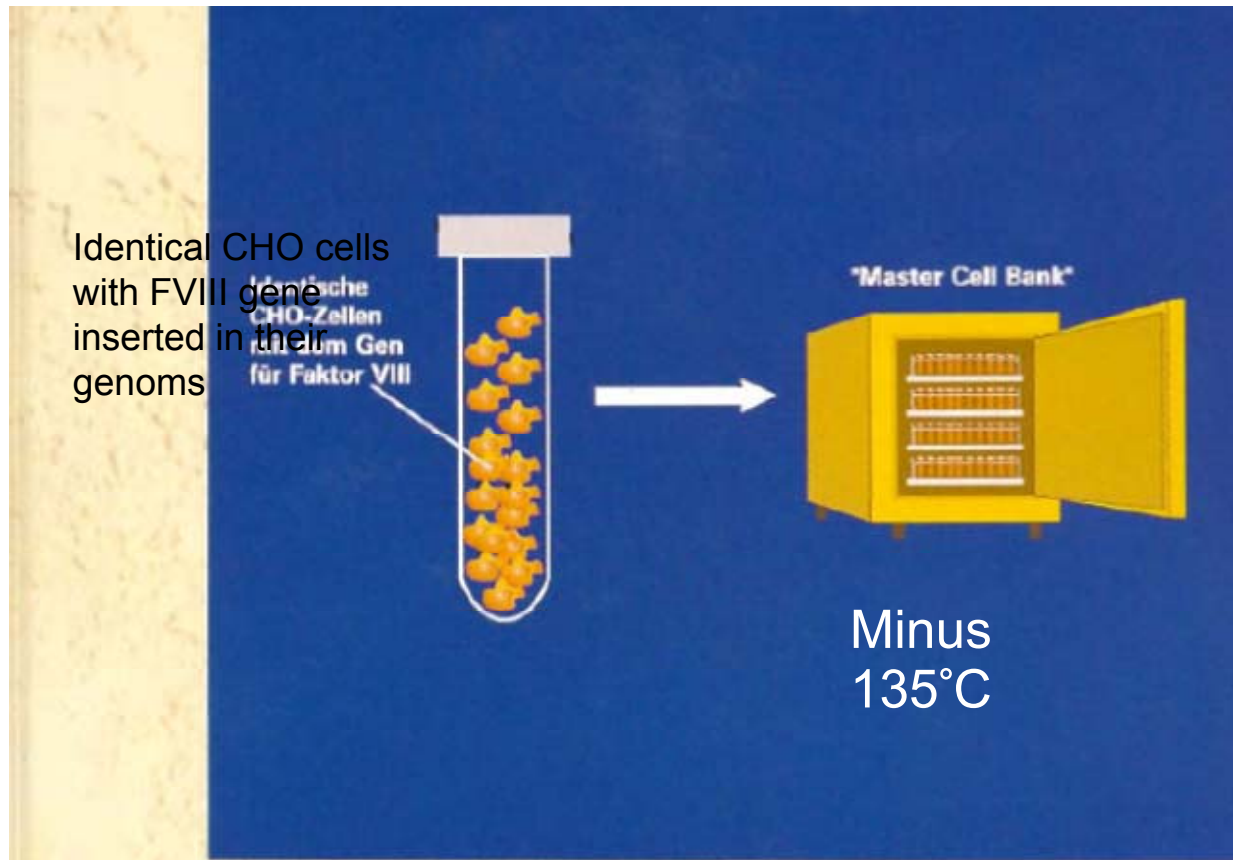
Vegetarian protein free medium (ADVATE)

2004

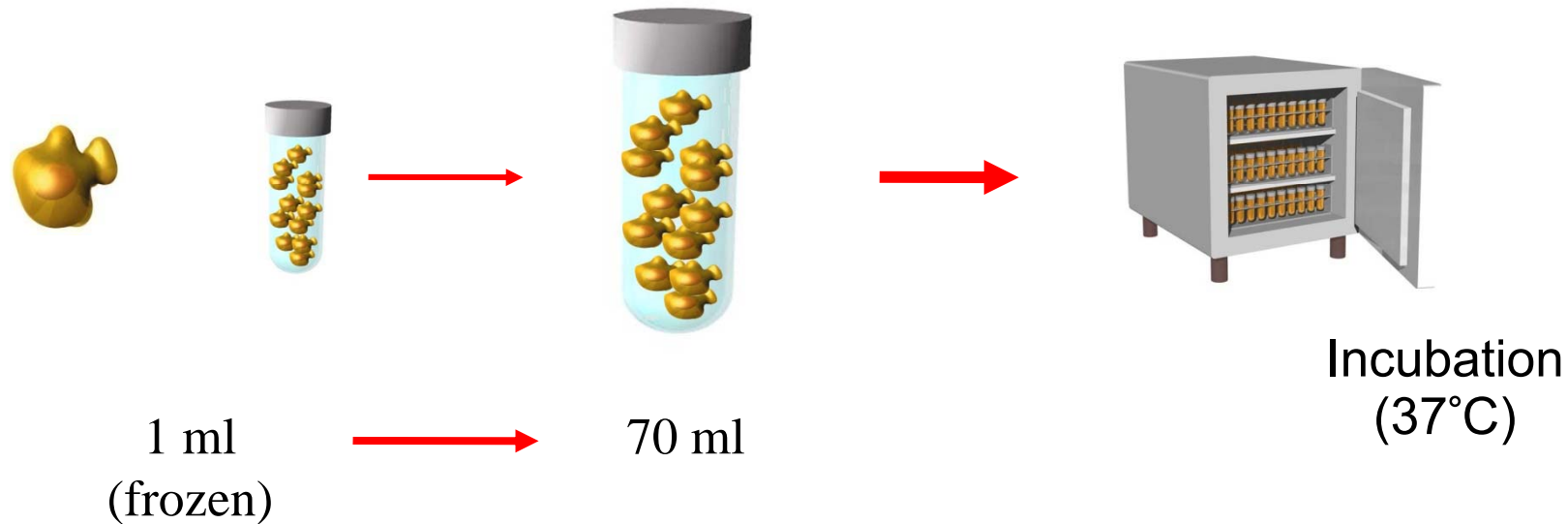
Evolution



Development of master and working cell banks



Every fermentation run starts with the re-animation of cells from the working cell bank



Cell solution preparation



Cell solution incubation at 37°C



FVIII fermentation process

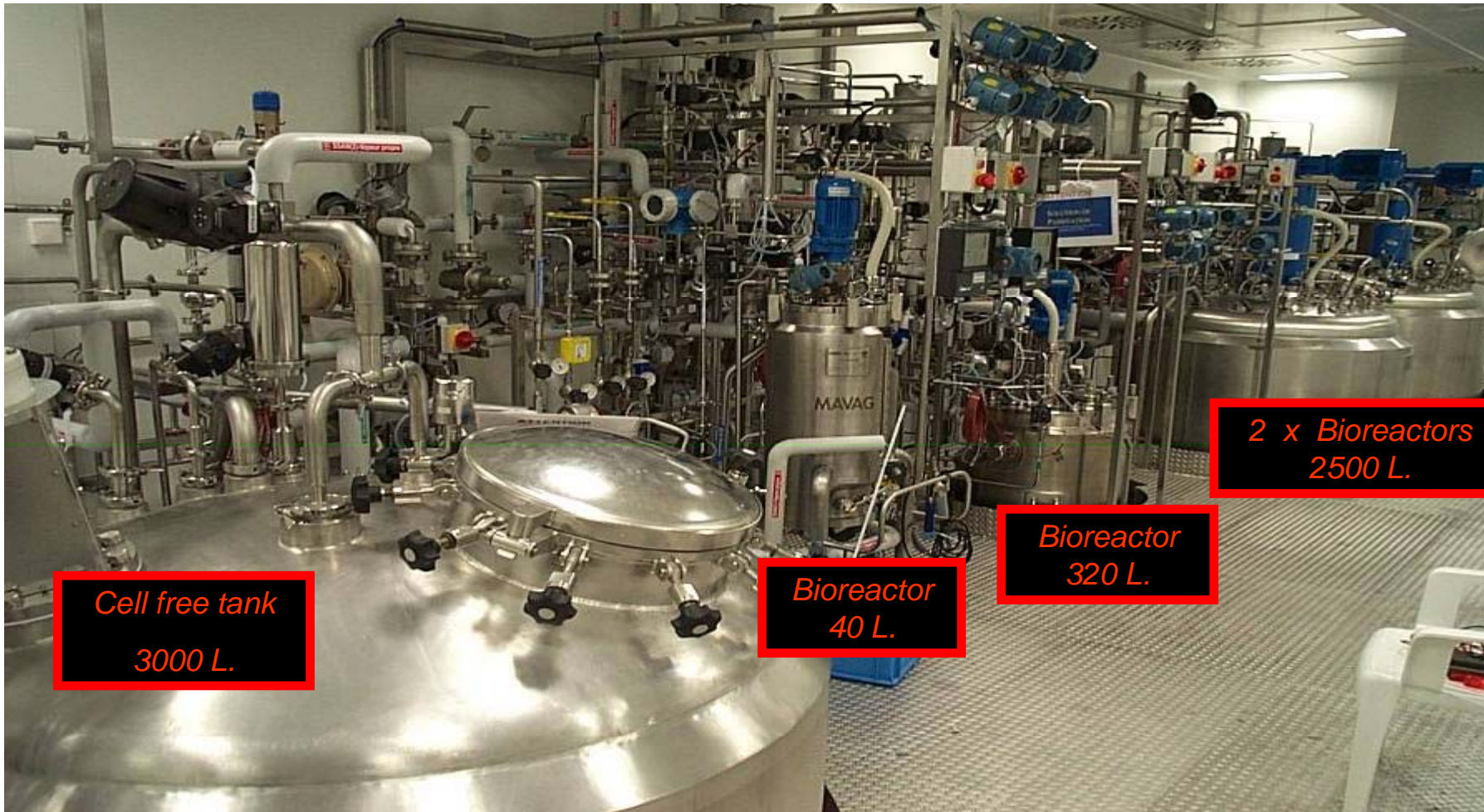
- Roller bottle cell cultures grown to a defined cell density are pooled and used to start fermentation run in bioreactors

- 3 fermentation steps in bioreactors
 1. Bioreactor 40 L
 2. Bioreactor 320 L
 3. Bioreactor 2500 L (2 X)

Expanded cell solution incubation in roller bottles at 37°C



Bioreactors



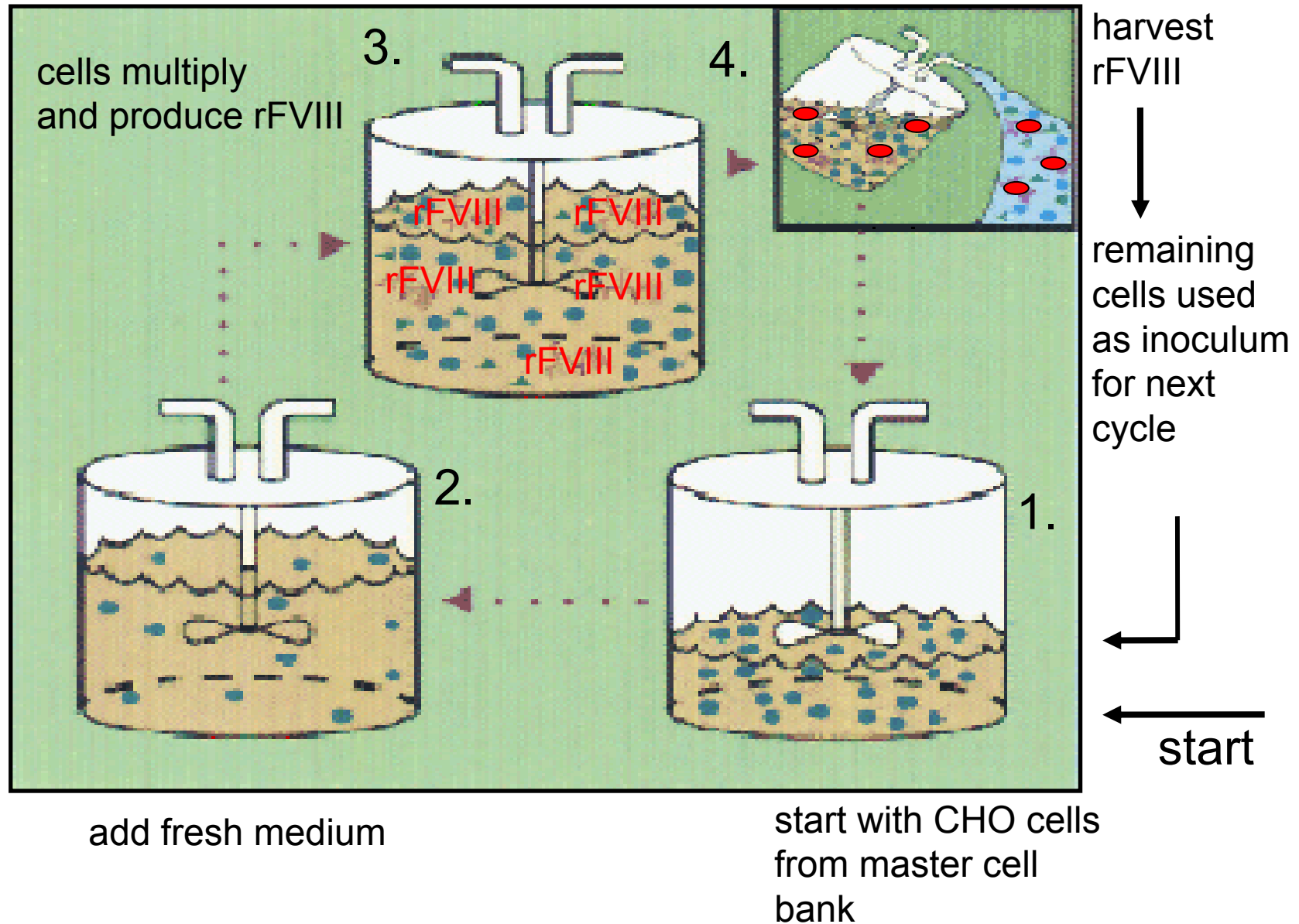
*Cell free tank
3000 L.*

*Bioreactor
40 L.*

*Bioreactor
320 L.*

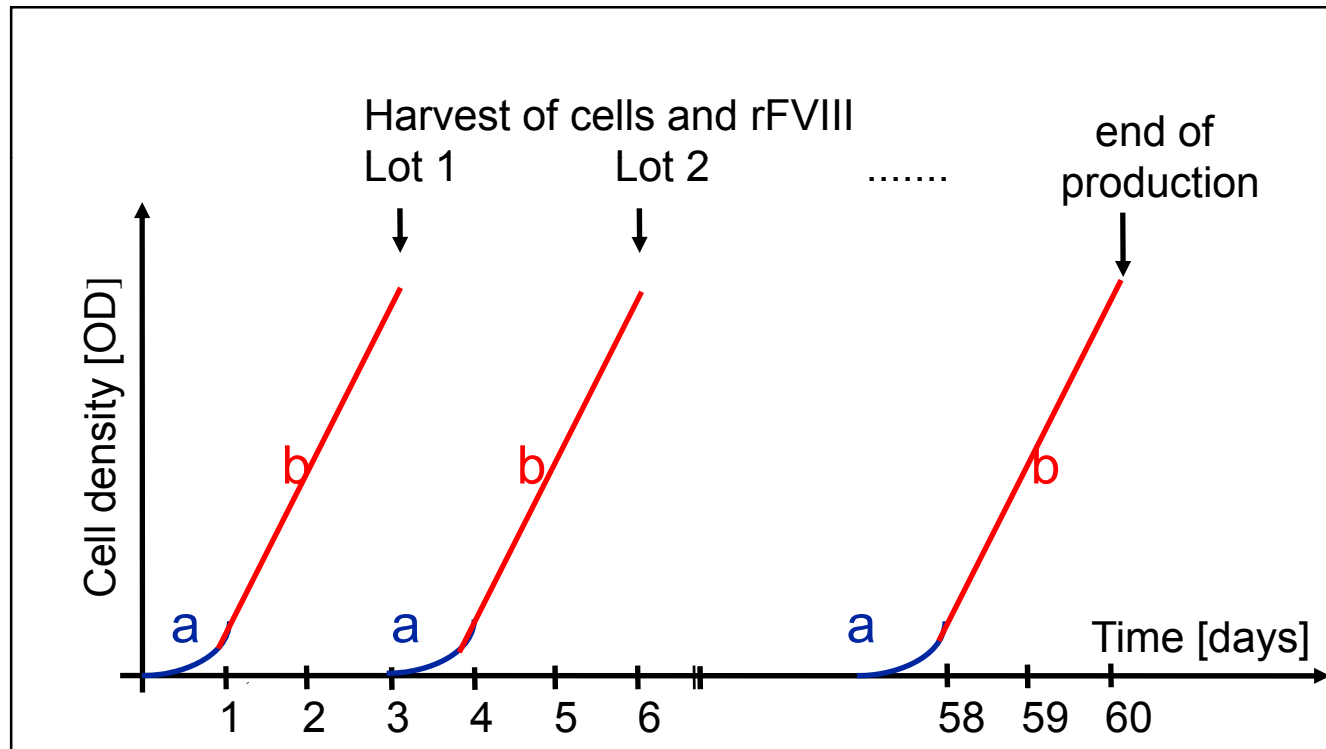
*2 x Bioreactors
2500 L.*

The batch refeed fermentation process (20 cycles)



Batch-refeed fermentation process

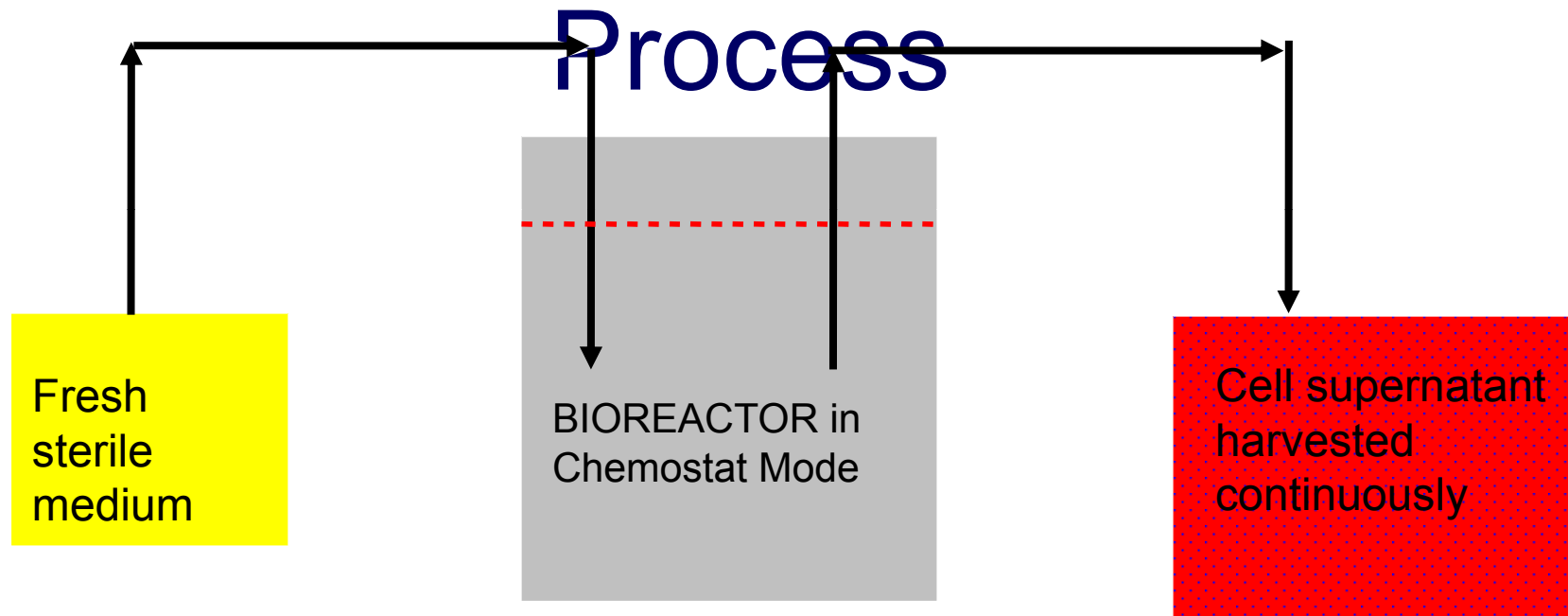
Recombinebate



a lag-phase

b log-phase

CHEMOSTAT Fermentation



Permanent monitoring of:

- cell density
- cell viability
- pH
- Oxygen use
- Stirring

The manufacturing process of rFVIII consists of 3 main steps

1. Biosynthesis of FVIII by a cell culture in bioreactors
2. Purification of rFVIII by chromatography steps
3. Final formulation, filling and freeze drying

Purification of rFVIII by chromatography steps (Immunoaffinity and ion exchange chromatography)



Purified rFVIII pooling



rFVIII Bulk is
then stored at –
80°C until final
formulation

Aseptic filling



Freeze dryers



Capping machine

