EPFL minor in Biotechnology

Module ChE-437
Part 3: Downstream Processing

Simon Crelier, HES-SO Valais – Sion

simon.crelier@hevs.ch

+41 (0)27 606 86 65

Course calendar, spring semester 2025

Date	Lecturer	Topic
18.02.2025	M. Zinn	
25.02.2025	M. Zinn	
04.03.2025	M. Zinn	
11.03.2025	M. Zinn	
18.03.2025	M. Zinn	
25.03.2025	K. Eyer	
01.04.2025	K. Eyer	
08.04.2025	K. Eyer	
15.04.2025	K. Eyer	
22.04.2025	Easter break	
29.04.2025	S. Crelier	
06.05.2025	S. Crelier	
13.05.2025	S. Crelier	
20.05.2025	S. Crelier	
27.05.2025	S. Crelier	

Exam session

June 16 to July 5, 2025

Written exam

30.06.2025

09:15 . 12:15 am

Room CM 1 121

Lecture plan

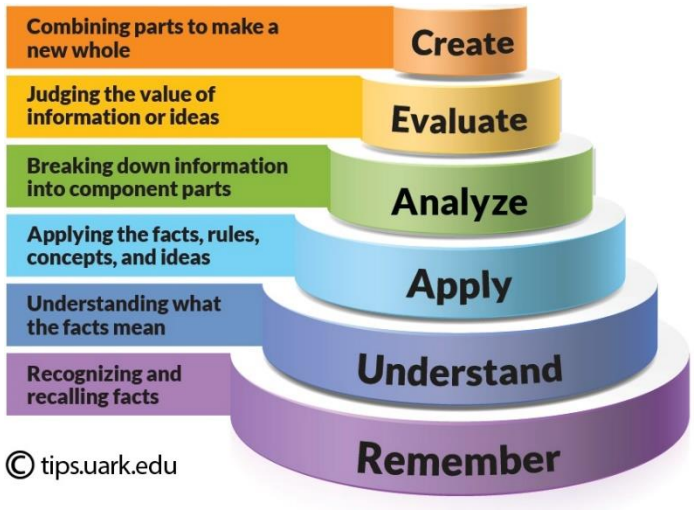
- **Lecture 1:** Anatomy of a bioprocess, overview of biotech products and DSP in biotechnology; Analytical aspects. Production of mAbs. Purification platforms
- **Lecture 2:** Clarification, L/S separation: centrifugation, filtration
- **Lecture 3:** Membrane separation, chromatography part 1
- **Lecture 4:** Chromatography part 2, viral clearance
- **Lecture 5:** Reserve time (and possibly: continuous biomanufacturing, precipitation, crystallization, stability assessment)

Lecture 1

1.1 DSP & Bioprocesses
1.2 Bioproducts at large
1.3 Production of antibodies
1.4 Purification of antibodies

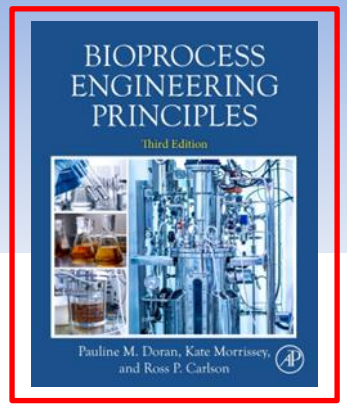
Learning objectives for the DSP section

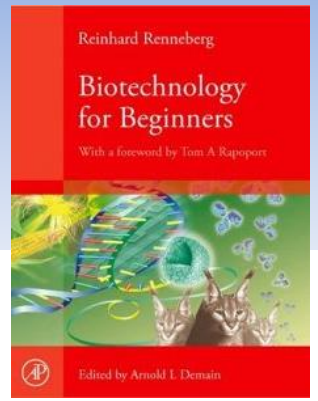
- Name and explain the relevance of DSP in bioprocess developments
- Propose a sequence of unit operations for a specific purification
- Calculate/size unit operations to achieve a required capacity
- Discuss case studies in various contexts of applications
- Evaluate the suitability of a given purification strategy
- Name some recent developments and trends



The Bloom taxonomy ©: University of Arkansas

Bibliography





Biotechnology at large

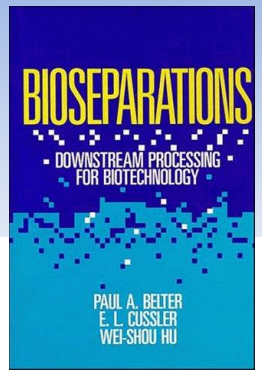
- Reinhard Renneberg. Biotechnology for beginners. Academic Press, London, 2007
- Rolf D. Schmid. Pocket guide to biotechnology and genetic engineering. Wiley VCH, Weinheim, 2003

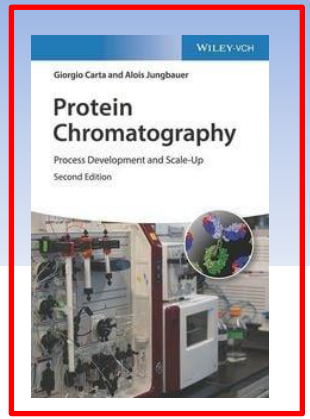
Best value for your money!
960 excellent pages for about CHF 100.-

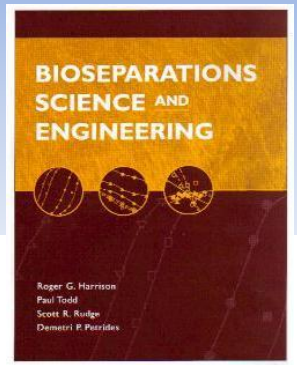
Bioprocess engineering

- Pauline M. Doran, Kate Morrissey, Ross P. Carlsen. Bioprocess engineering principles. Academic Press, London, 3rd edition, 2024 (On sale August 15, 2024)
- Michael L. Shuler & Fikret Kargi. Bioprocess engineering. 2nd edition, Prentice Hall,Upper Saddle River, 2002
- Shigeo Katoh & Fumitake Yoshida. Biochemical – a textbook for engineers, chemists and biologists. Wiley VCH, Weinheim, 2009

Bibliography (contd.)





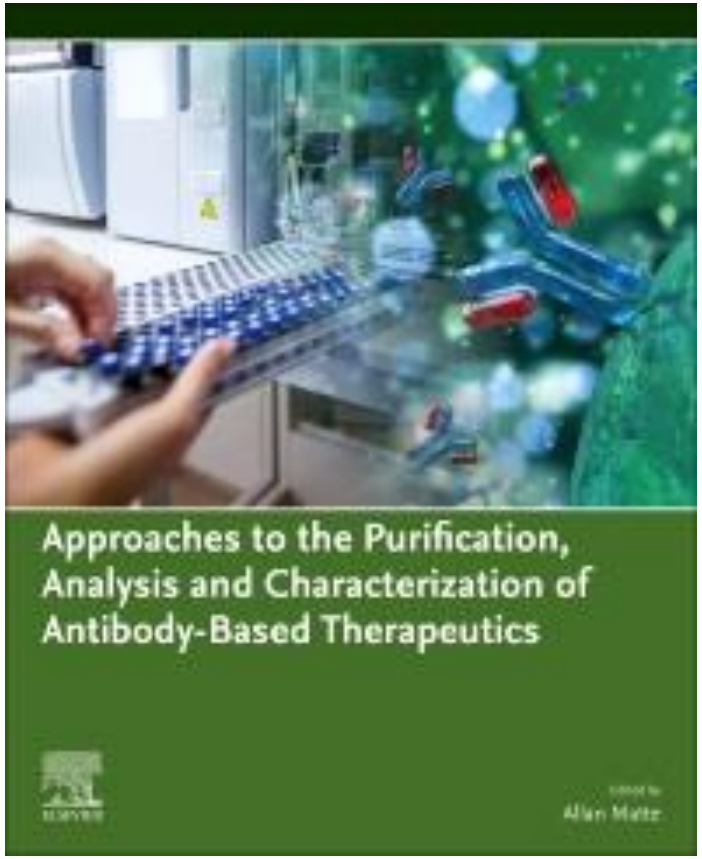


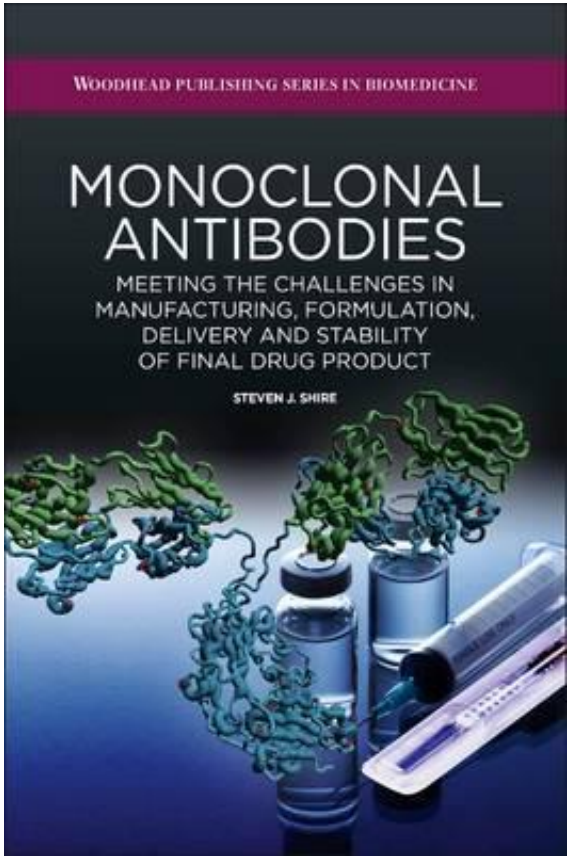
Downstream processing

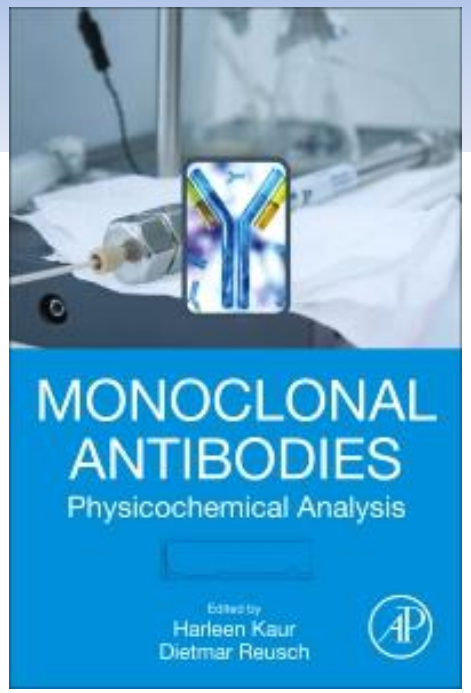
- J. D. Seader & E. J. Henley. Separation process principles. 2nd edition, John Wiley & Sons, New York, 2006
- P. A. Belter, E. L. Cussler, W.-S. Hu. Bioseparations – downstream processing for biotechnology. John Wiley & Sons, New York, 1988.
- R. G. Harrison, P. Todd, S. R. Rudge, D. P. Petrides. Bioseparations science and engineering. Oxford University Press, New York, 2003
- A. A. Shukla, M. R. Etzel, S. Gadam editors. Process scale bioseparations for the biopharmaceutical industry. CRC Press, Boca raton, 2007
- G. Carta & Alois Jungbauer. Protein chromatography – process development and scale-up. 2nd edition, Wiley VCH, Weinheim, 2020

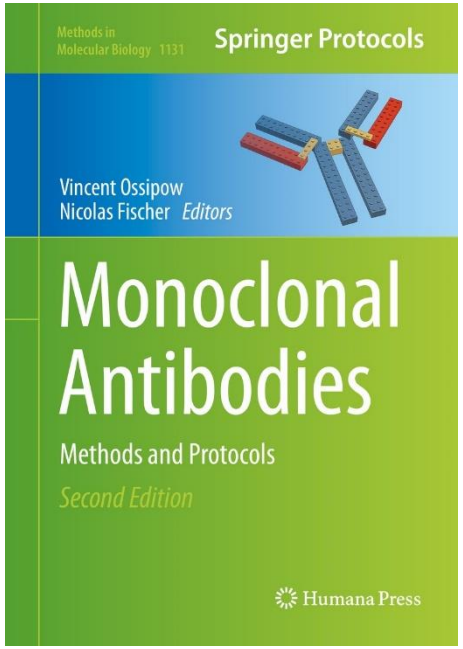
Great reference for industrial chromatographic purification! Price around CHF 130.-

Bibliography (contd.)



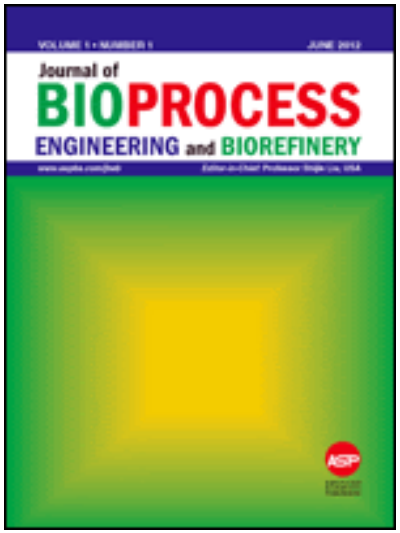


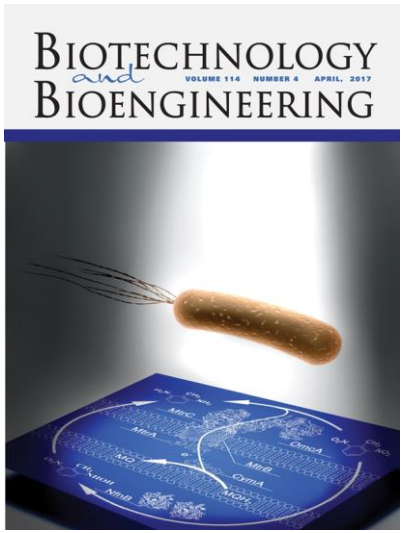


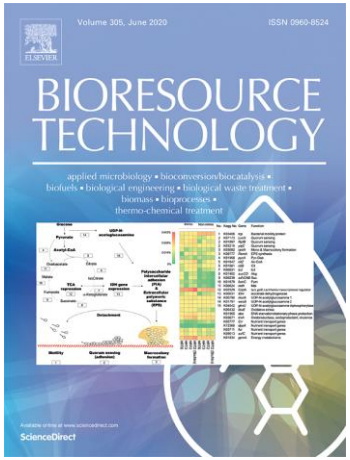


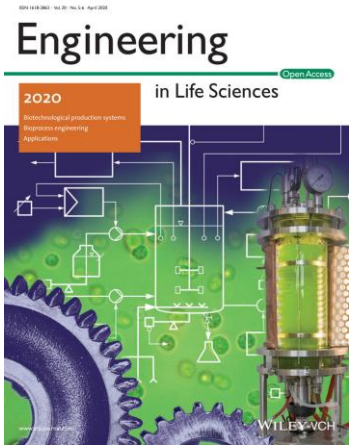
A focus on monoclonal antibodies production and purification

A selection of biotech/bioprocess-dedicated journals

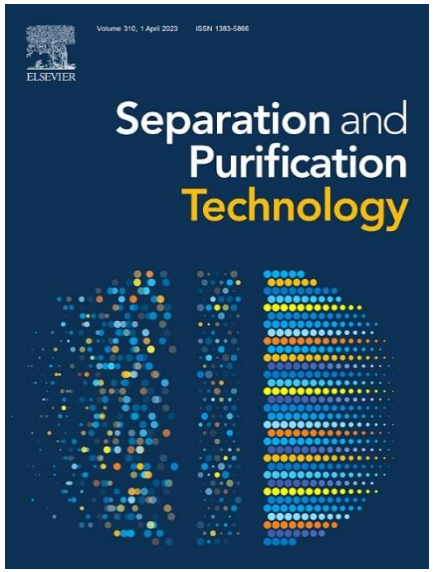


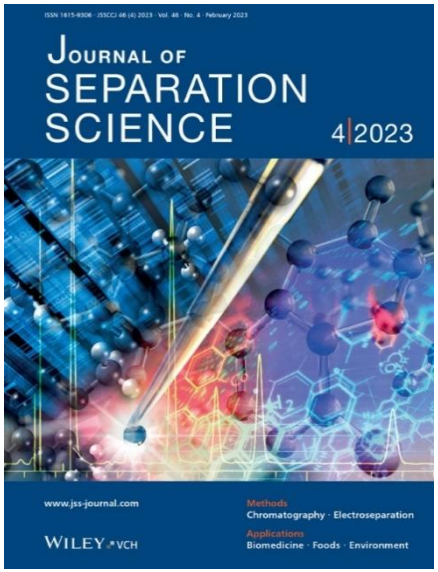


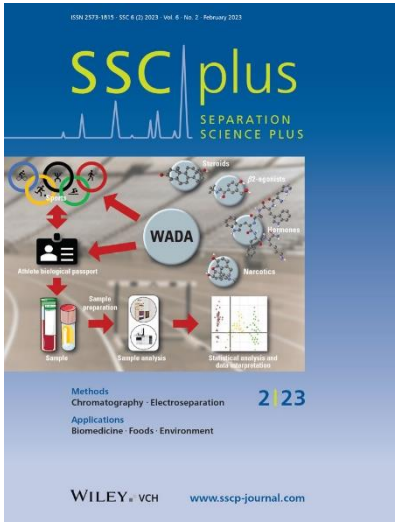


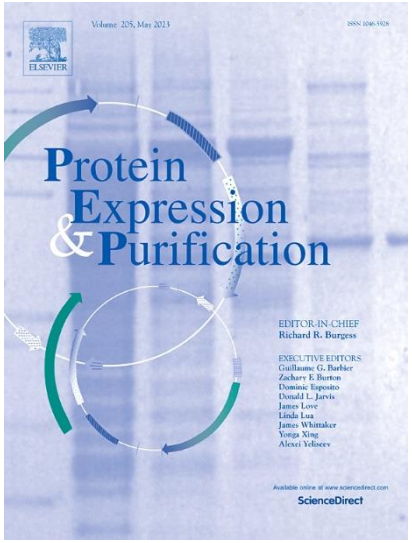


Some journals focusing more on DSP





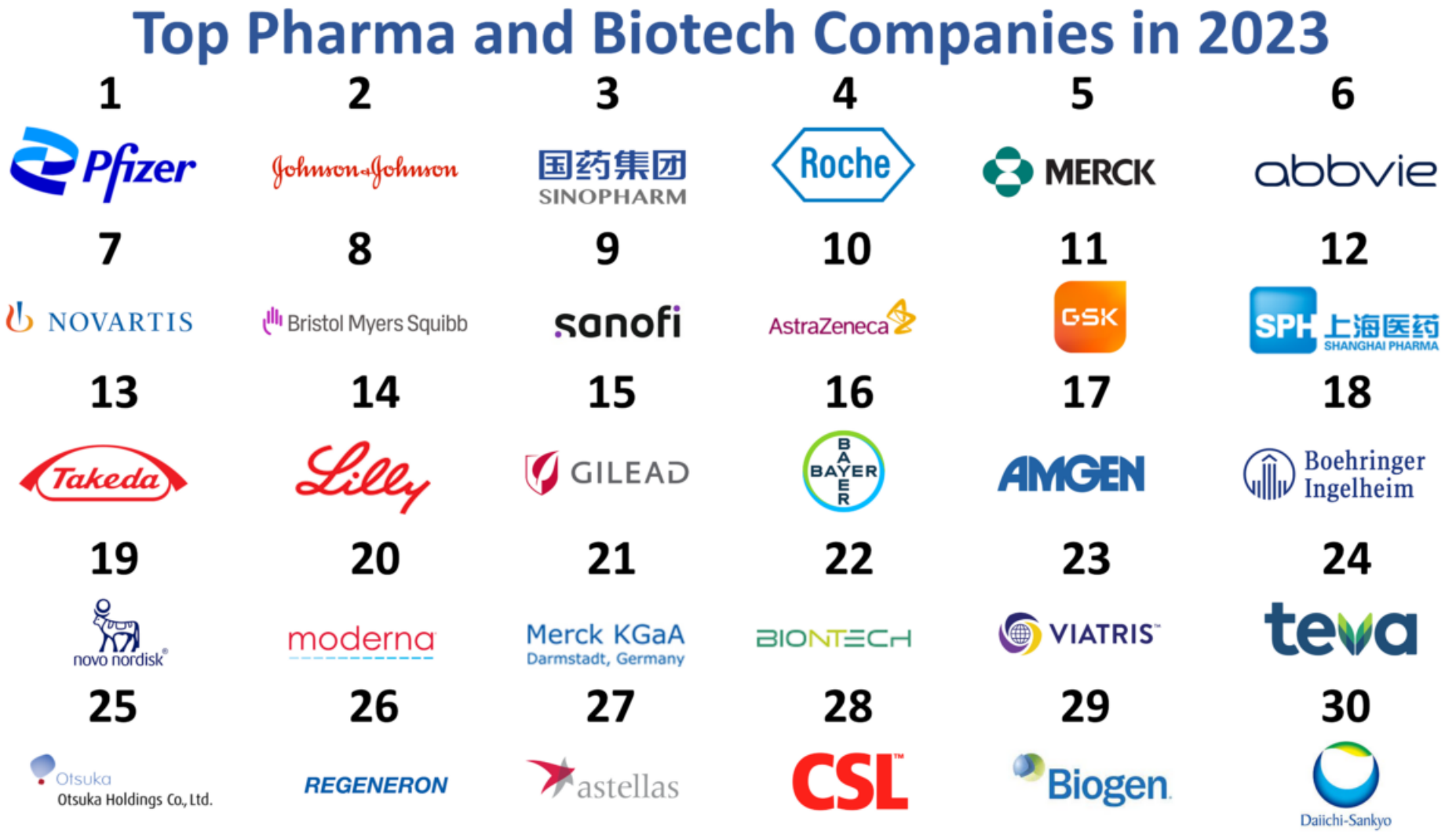




Don’t neglect the professional publications, though!

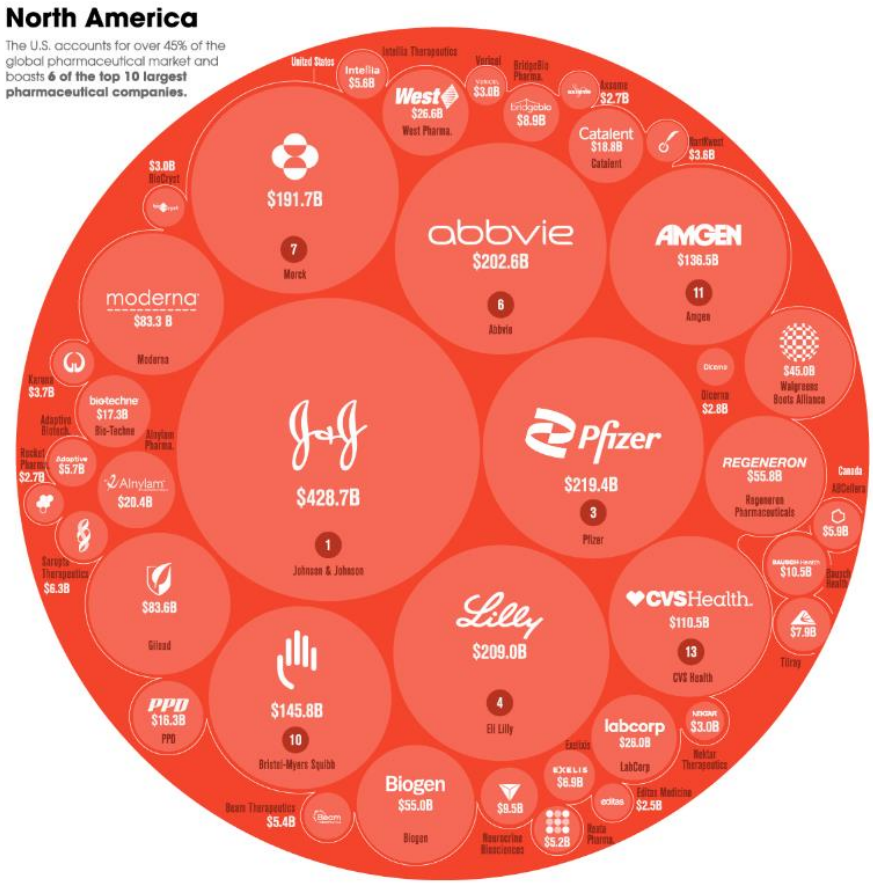
- Not necessarily peer-reviewed, can often be consulted on-line for free (lot of advertisement)
- Besides scientific articles they publish sponsored content, inform on the latest news in the biopharma field (new products & technologies, mergings & acquisitions, FDA approvals etc …)
- Biomanufacturing companies as well as equipment suppliers are the main contributors

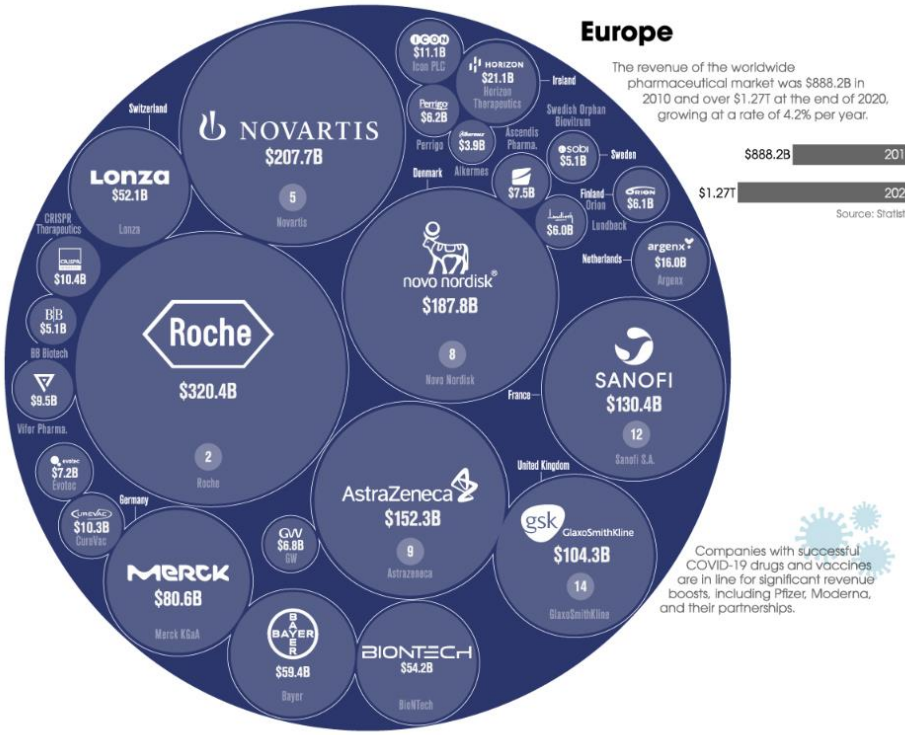
Introducing … your future employer?



https://xtalks.com/top-30-pharma-and-biotech-companies-in-2023-statistics-and-trends-3482/

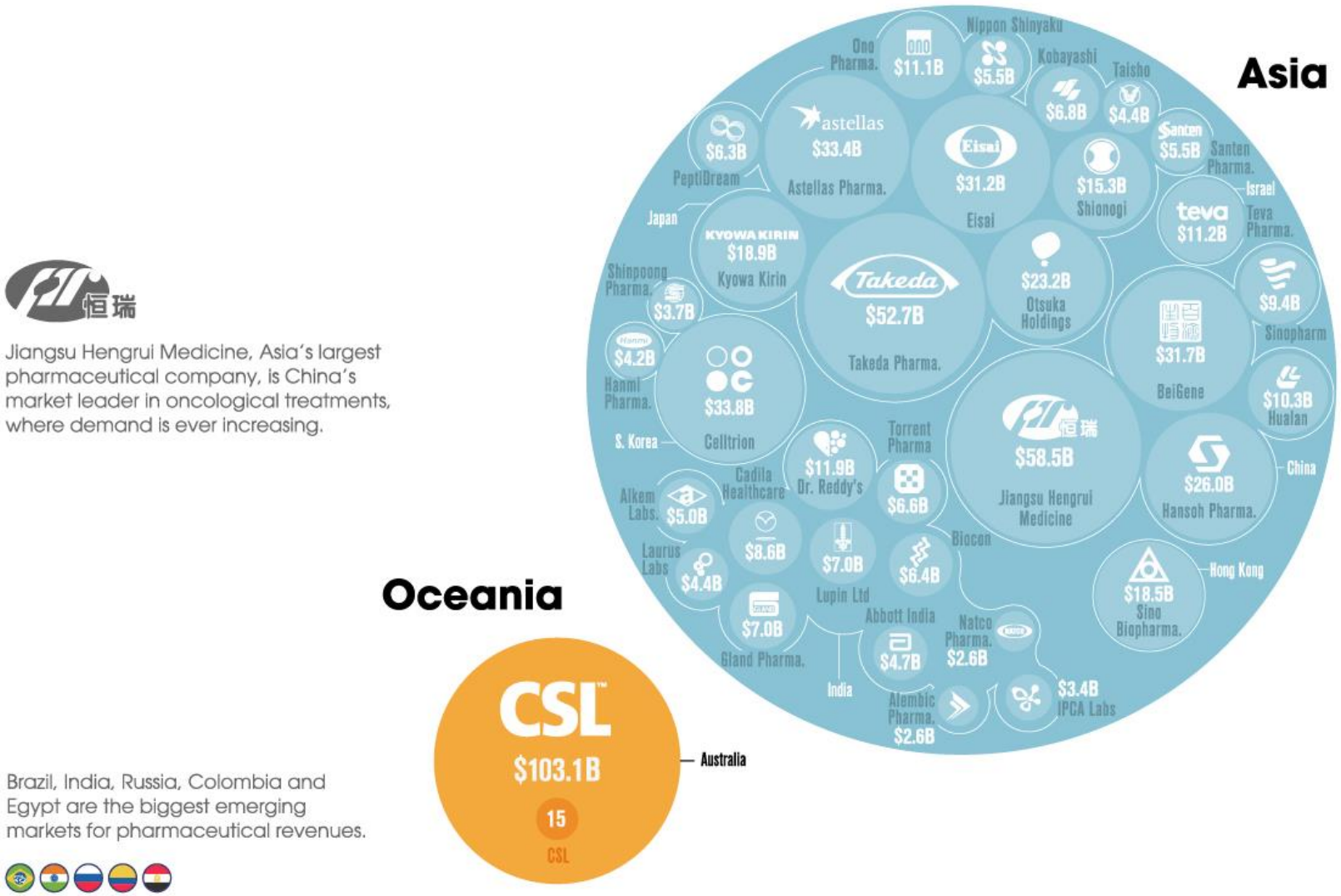
The same, geographically sorted out (1/2)





https://www.visualcapitalist.com/worlds-biggest-pharmaceutical-companies/

The same, geographically sorted out (2/2)



恒瑞

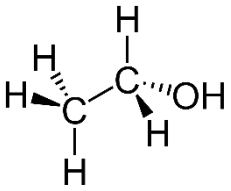
Jiangsu Hengrui Medicine, Asia's largest pharmaceutical company, is China's market leader in oncological treatments, where demand is ever increasing.

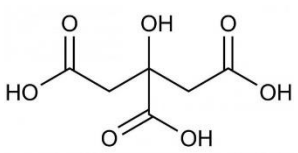
Brazil, India, Russia, Colombia and Egypt are the biggest emerging markets for pharmaceutical revenues.

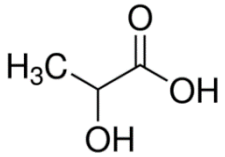
Source: Companies Market Cap. All data as of 23rd June 2021

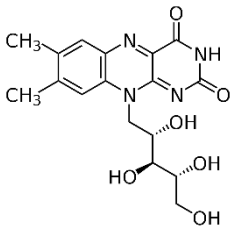
https://www.visualcapitalist.com/worlds-biggest-pharmaceutical-companies/

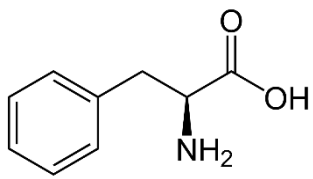
Biotech products and how they are purified

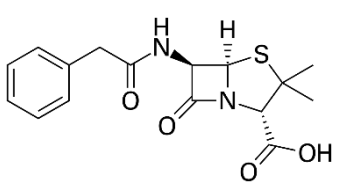


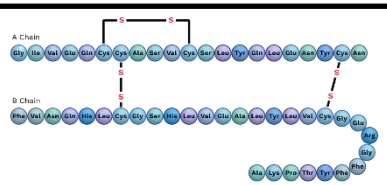


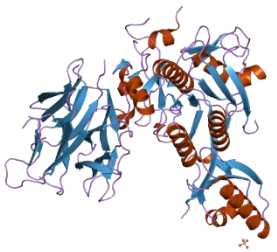


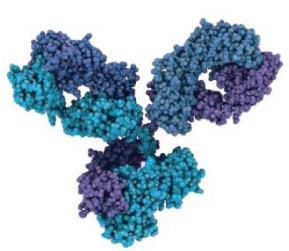




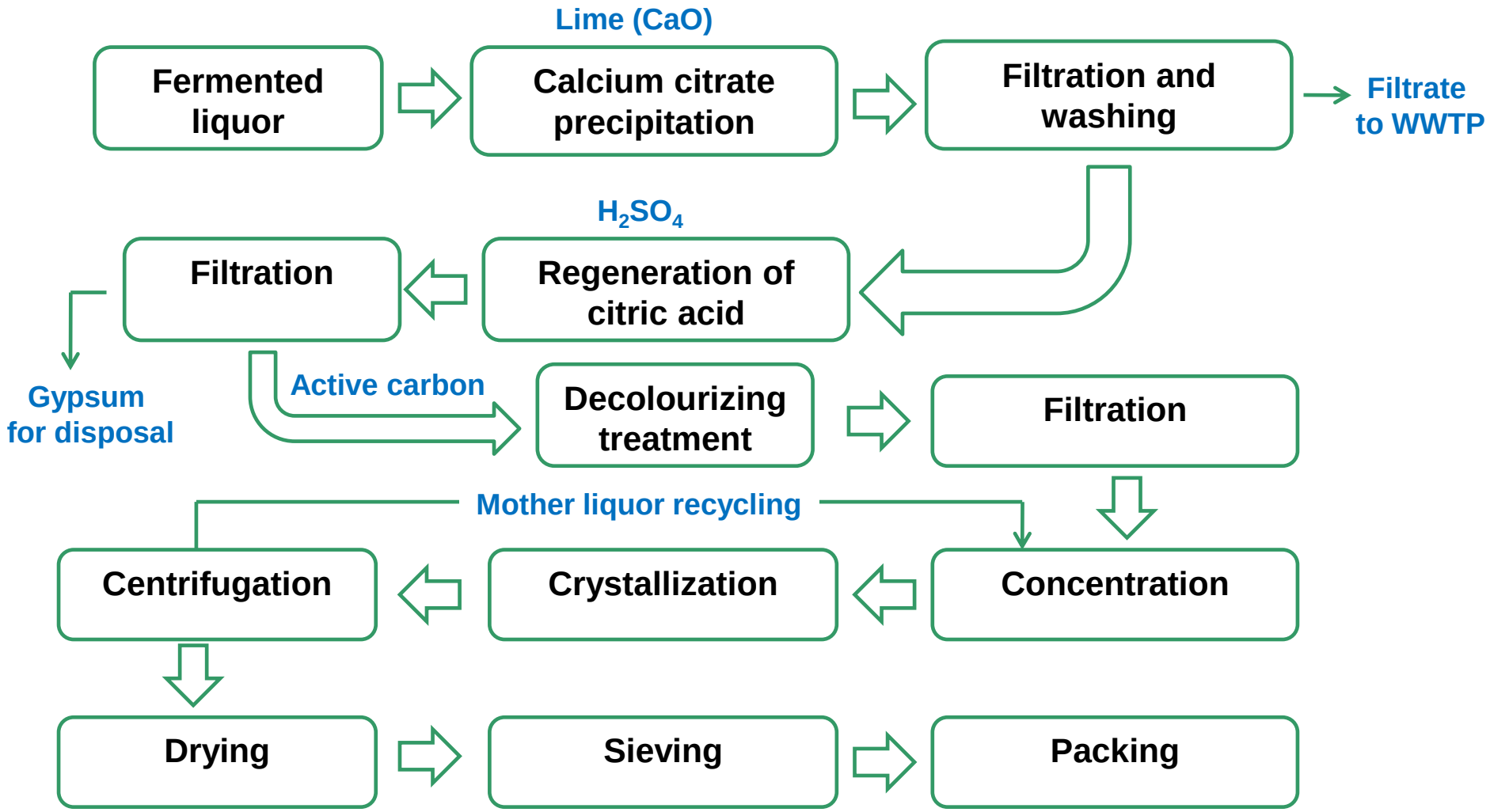




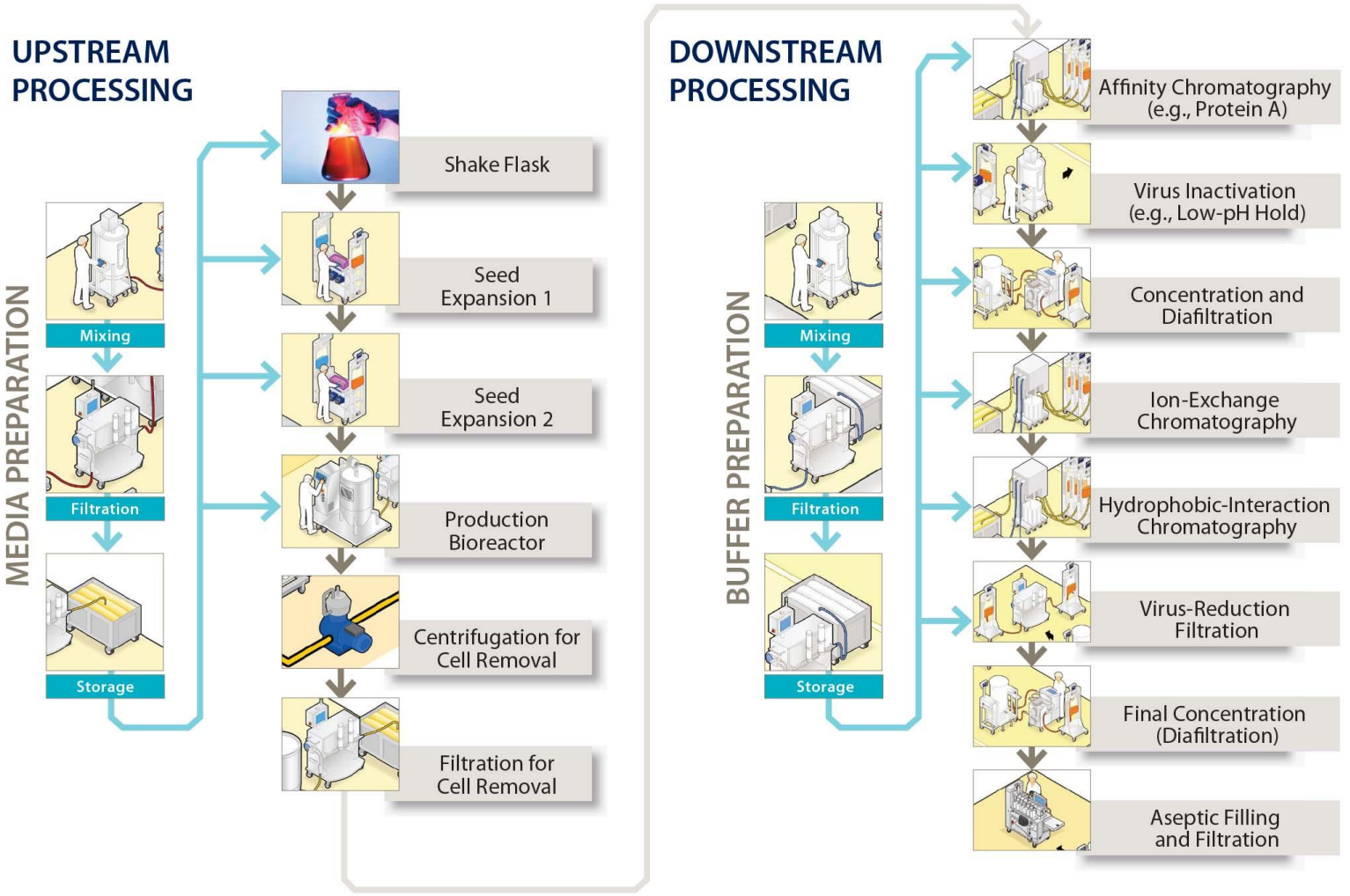




White biotech: Citric acid production (> $2.5 \cdot 10^6$ tons/year)



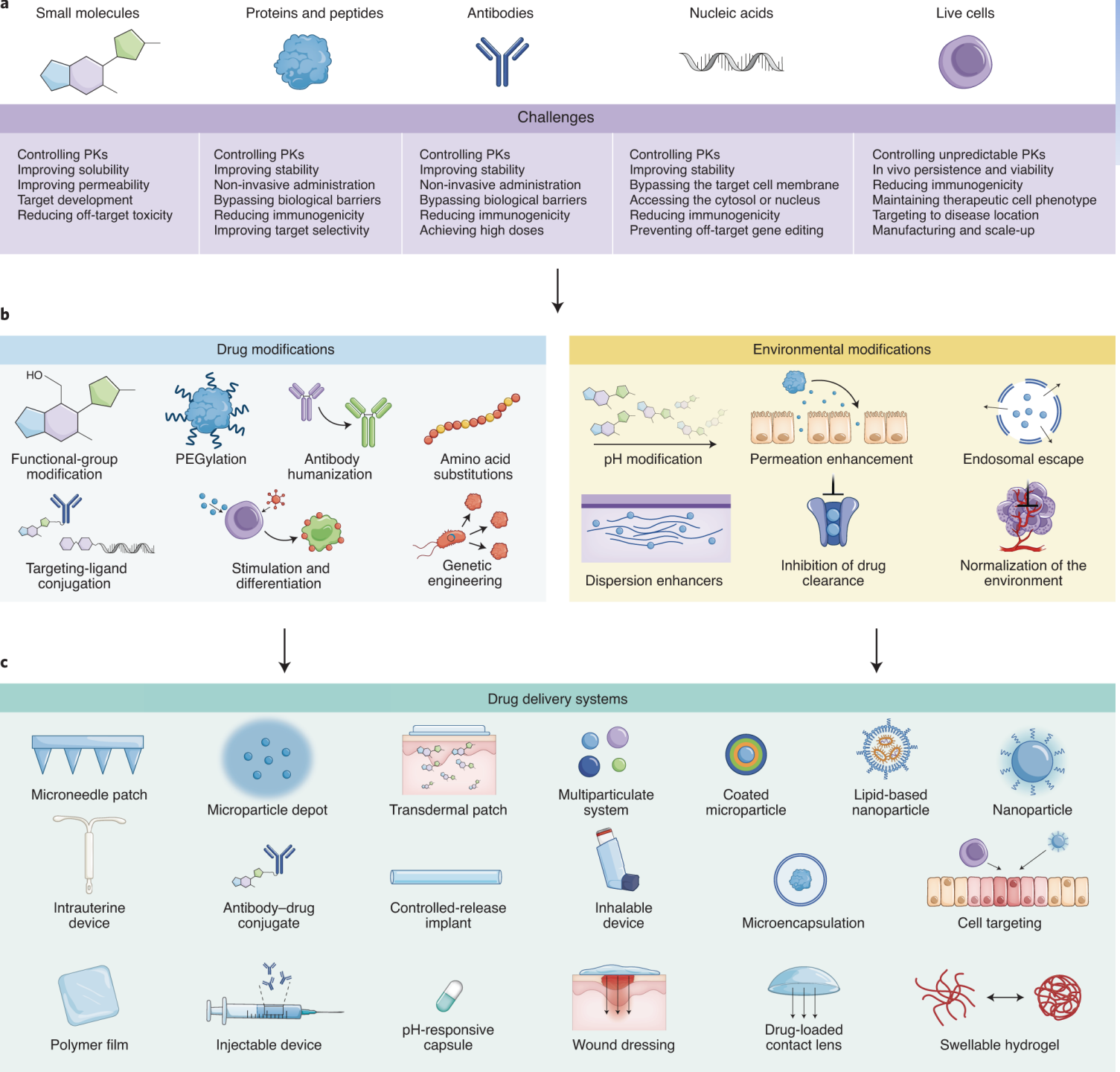
Production of a monoclonal antibody (a few tons/year)



Process diagram for the production and purification of a monoclonal antibody © Bio Process International, 2015

Meet the big family of biotech products

A. M. Vargason et al., Nature Biomedical Engineering 5, 951-967 (2021)



DSP: where does it start, where does it end?

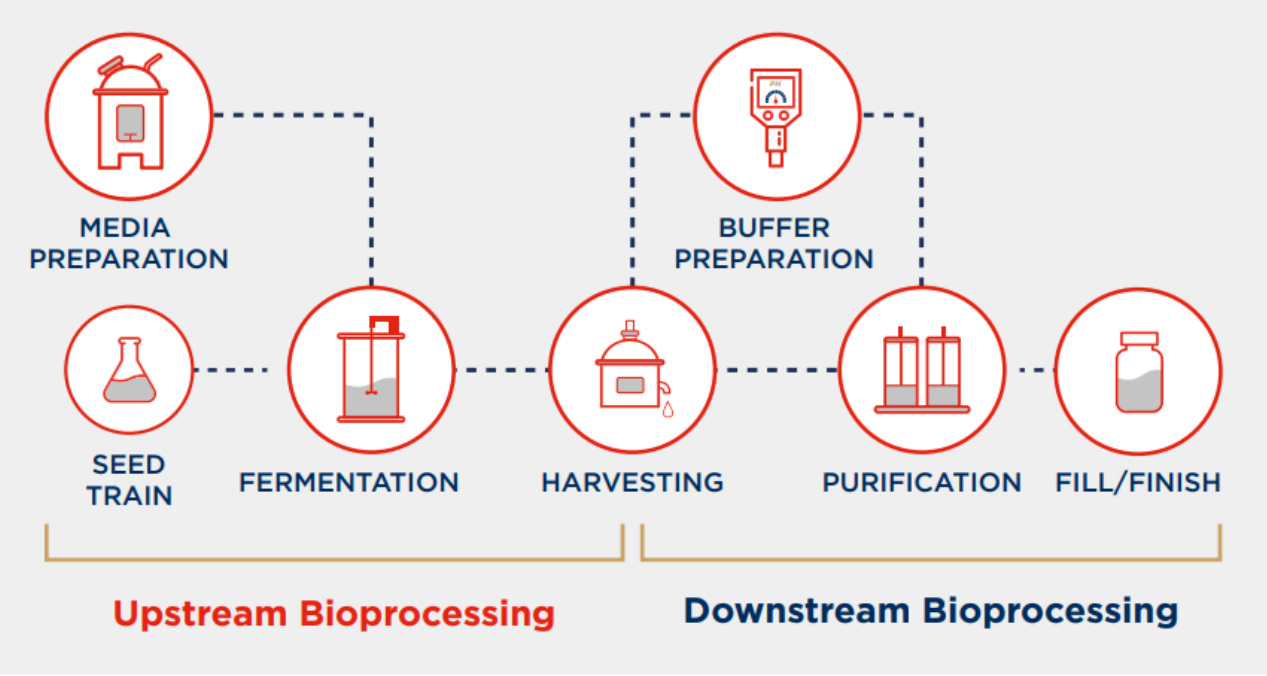
There are various processes included in bioprocessing, but generally two main stages are identified:

1. Upstream

At this initial stage, naturally sourced living material such as cells, bacteria or microbes used for a specific purpose are isolated and cultivated in bioreactors (fermenters).

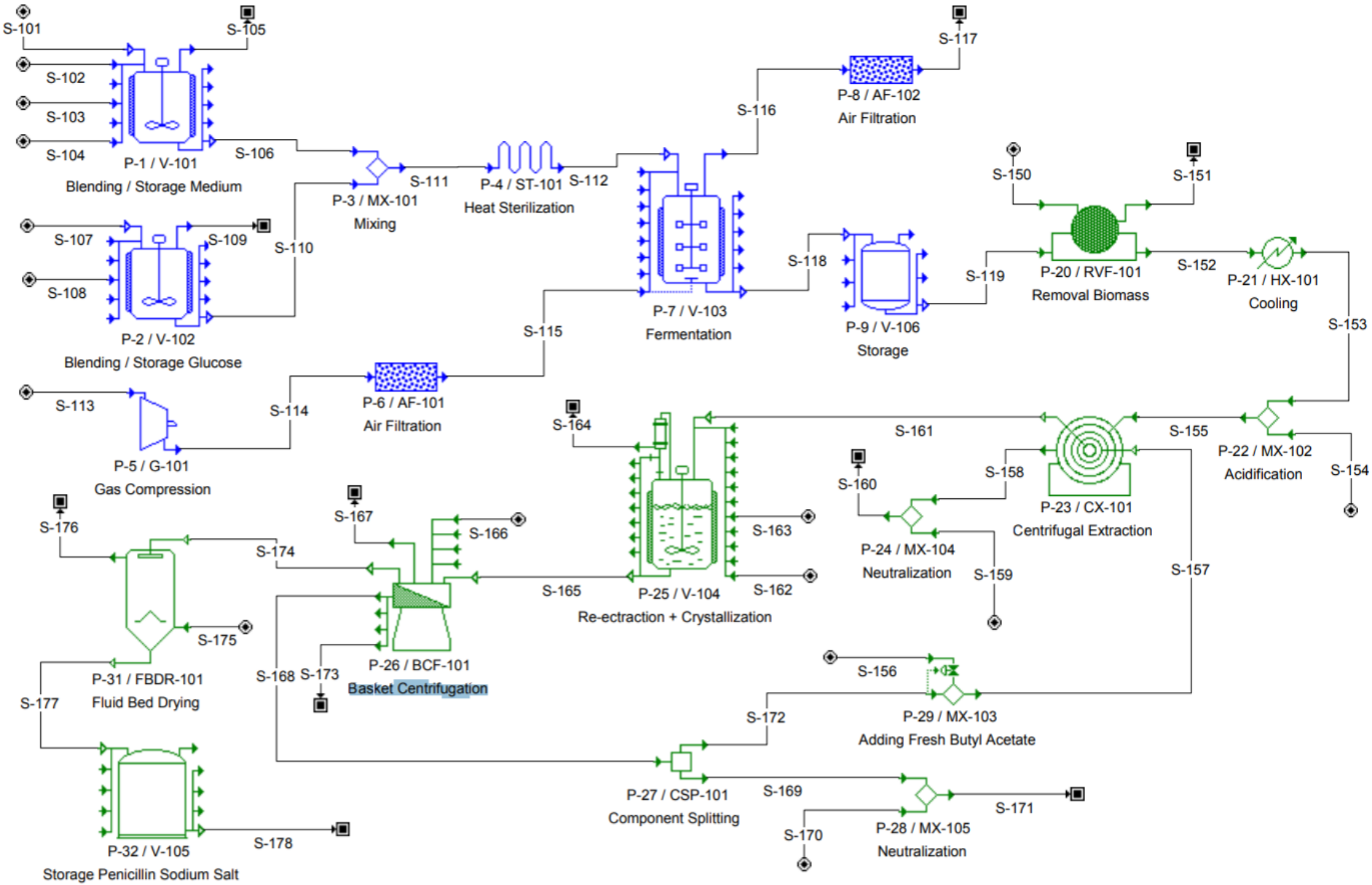
2. Downstream

At this stage, the resulting cell mass grown in the upstream stage is retrieved, processed and placed in a fermentation broth. Here, it is purified, and then formulated into the final product fit for use.

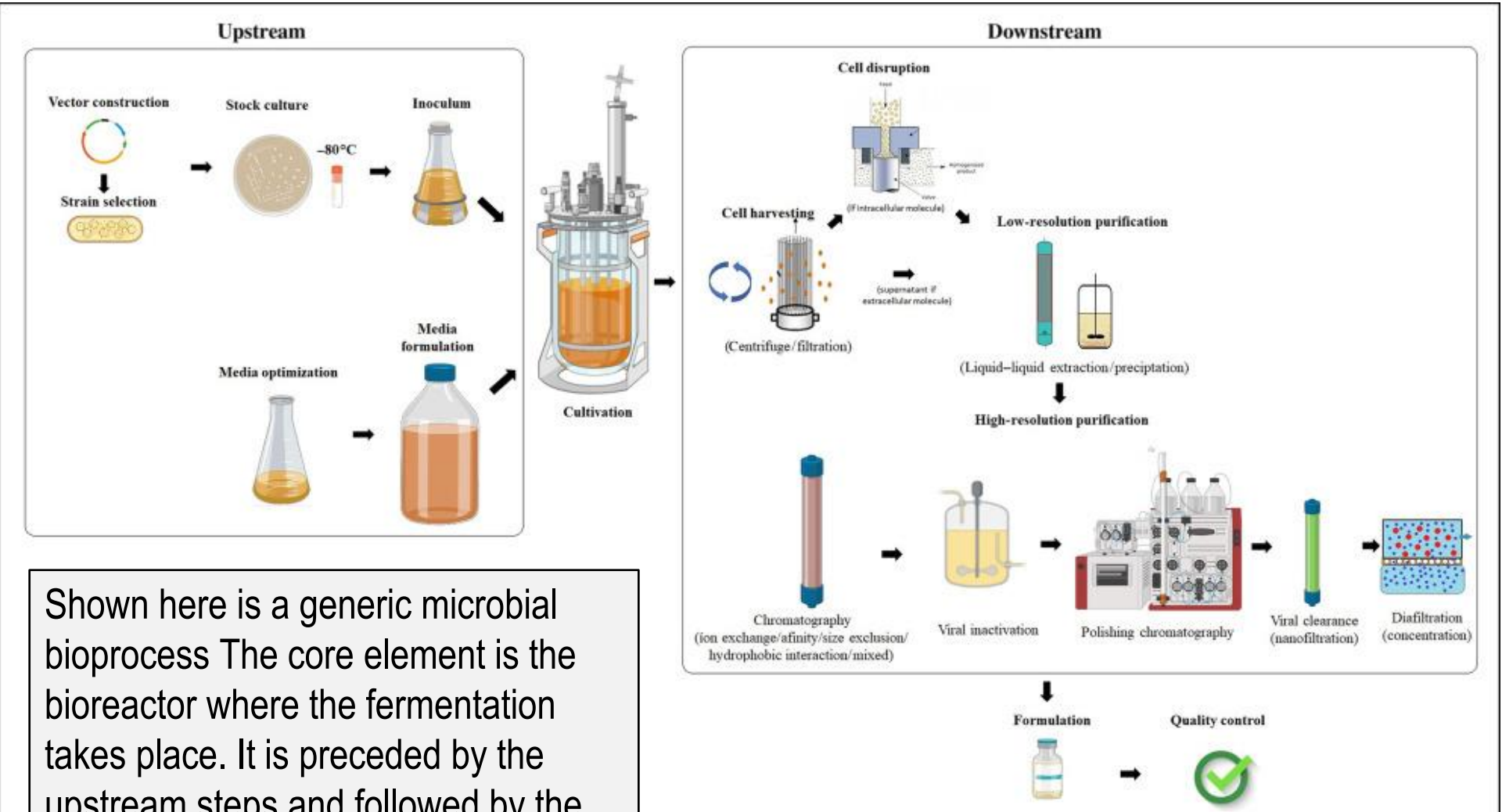


Source: www.thomaspumps.com

Process Flow Diagram: Penicillin



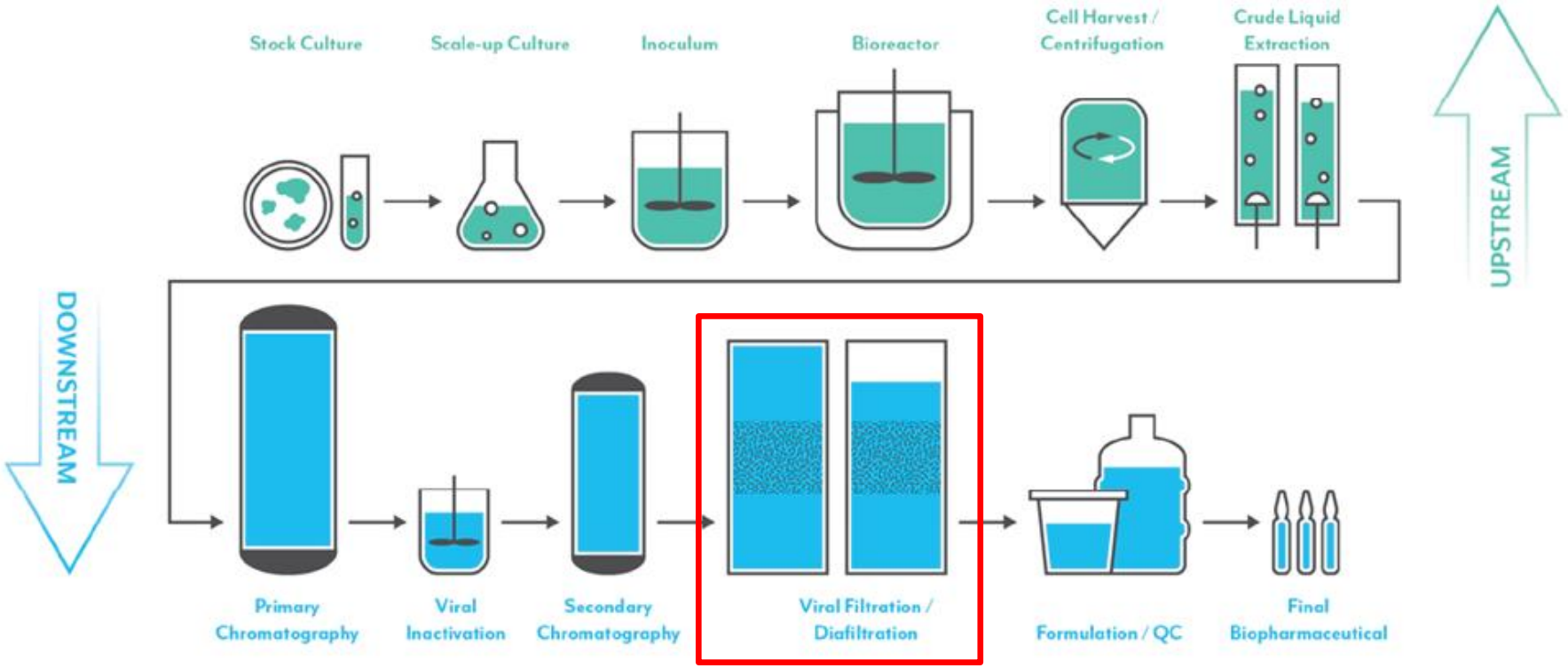
Anatomy of a bioprocess (1/3)



Shown here is a generic microbial bioprocess The core element is the bioreactor where the fermentation takes place. It is preceded by the upstream steps and followed by the downstream processing

Source: B. Benyahia et al., 2020

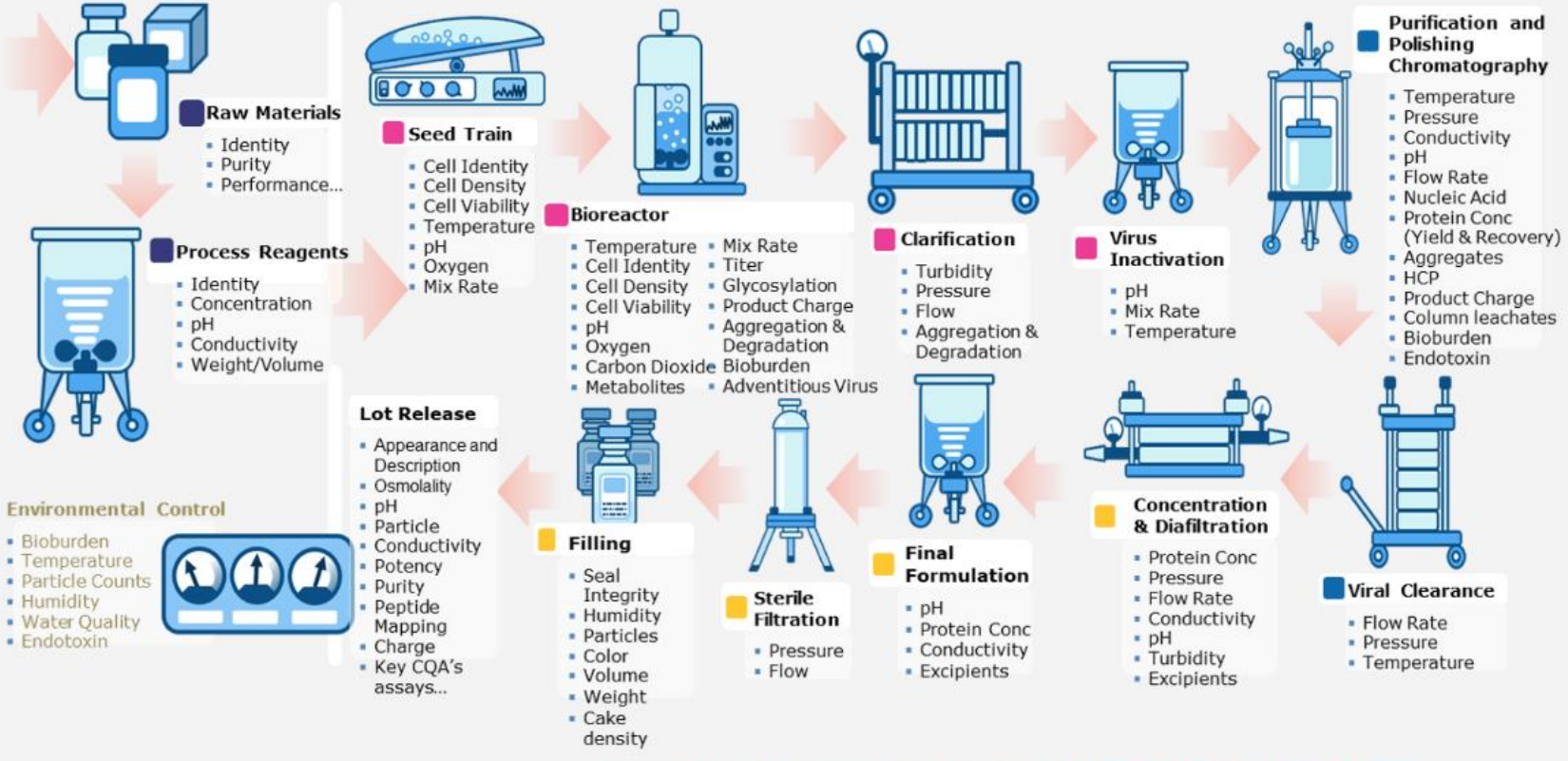
Anatomy of a bioprocess (2/3)



Source: www.spectrumchemicals.com

This flow diagram more specifically depicts a mammalian cell bioprocess, with the scale up culture(s) prior to entering the production bioreactor. Please note the viral inactivation steps featured in the downstream processing part.

Anatomy of a bioprocess (3/3)

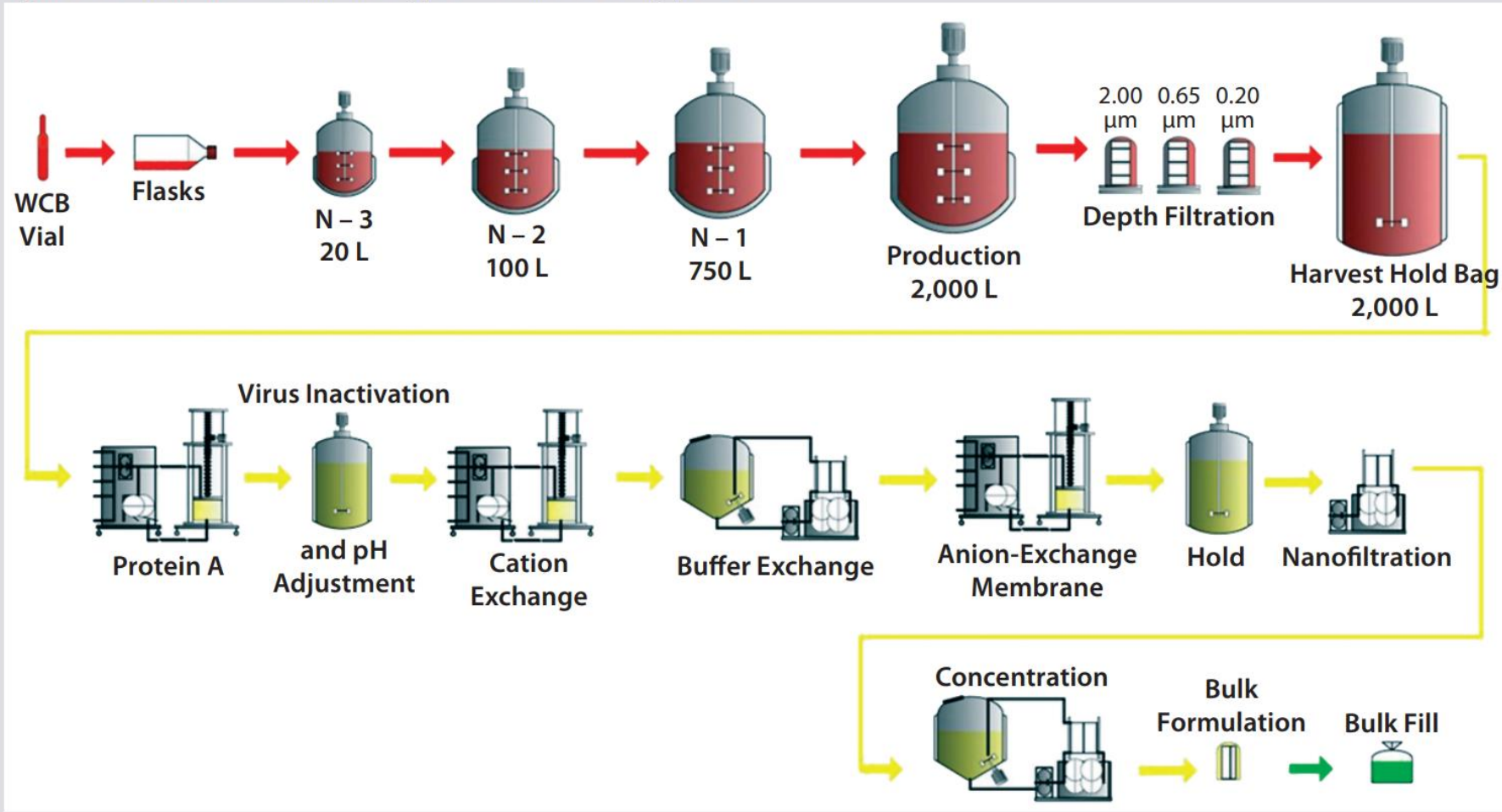


Colors on labels for process steps- **Purple: Raw Materials**, Magenta: Upstream Process, Blue: Downstream Process, Orange: Fill-Finish

Image courtesy of BioPhorum Operations Group from the BioPhorum Technology Roadmap for the Biopharmaceutical Manufacturing Industry.

A typical mammalilan cell bioprocess

Figure 1: Standard monoclonal antibody platform manufacturing process




Merck
our upstream ecosystem
mAb & Recombinant
Cell Lines & Expression Technologies
Cell Line Development
CELL LINE & PROCESS DEVELOPMENT
Cloning & Banking
Expansion
Cryopreservation
UPSTREAM DEVELOPMENT EXPERTISE
Cell Line Development, Analytical Development, Media Development, Process Development, Tech Transfer, GMP Clinical Supply
Product Characterization Cell Line Characterization
MEDIA DEVELOPMENT
Biopharma Raw Materials & Supplements
Custom Media (GMP and small non-GMP)
Catalog Media ("Fed Batch" and "Perfusion" Media)
Media Mixing
Virus Filtration
Bioburden Reduction
PRODUCTION
Bioreactor (Fed Batch or Perfusion Mode)
Raw Material Testing
ANALYTICS & BIOSAFETY
Sensors & Software
Lot Release Testing Product Characterization
Millipore.
Preparation, Separation, Filtration & Monitoring Solutions
SAFC.
Pharma & Biopharma Raw Material Solutions
BioReliance.
Pharma & Biopharma Manufacturing & Testing Services
www.MerckMillipore.com/right-path-upstream
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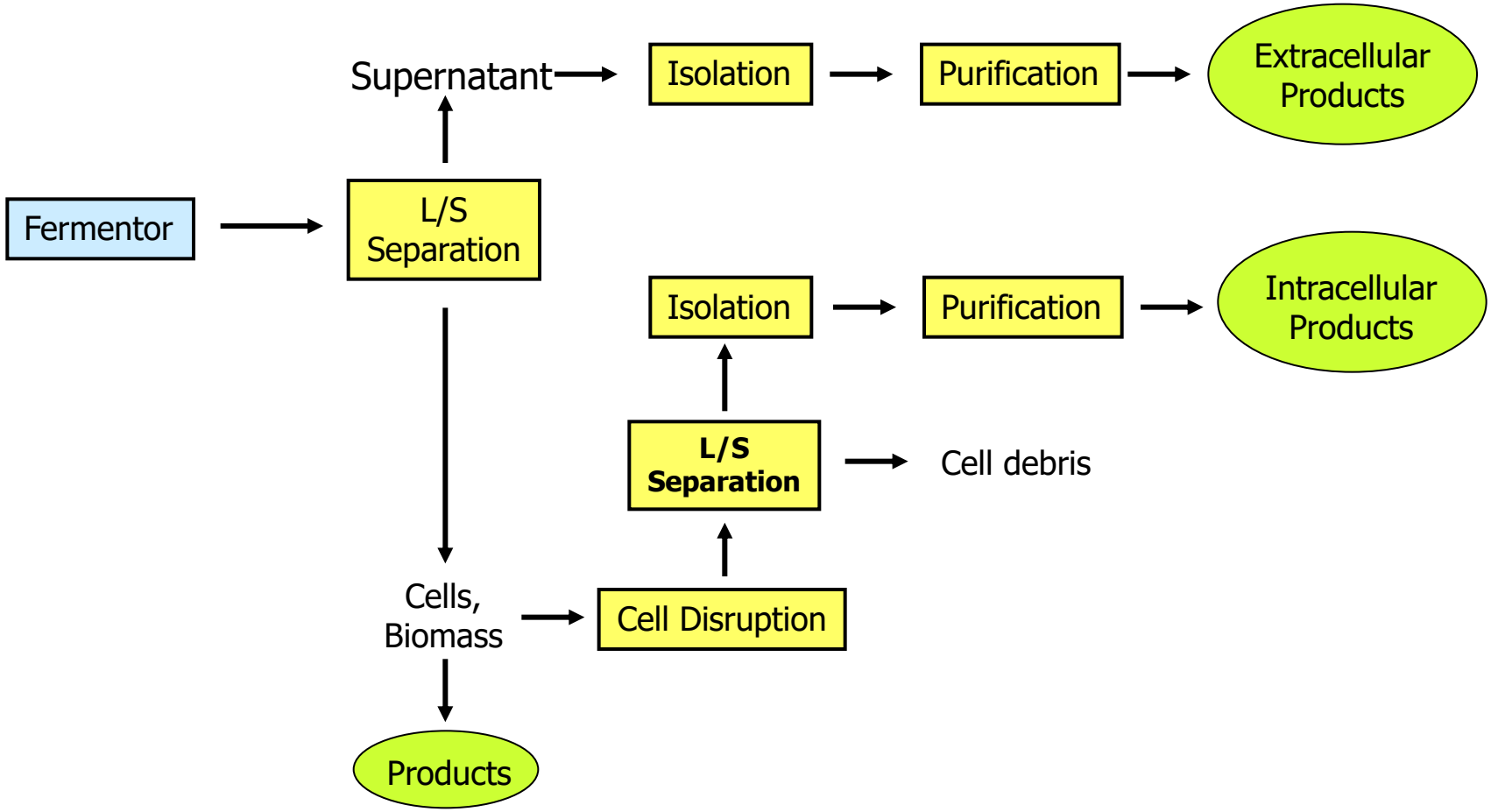
Common pathways for the isolation of products

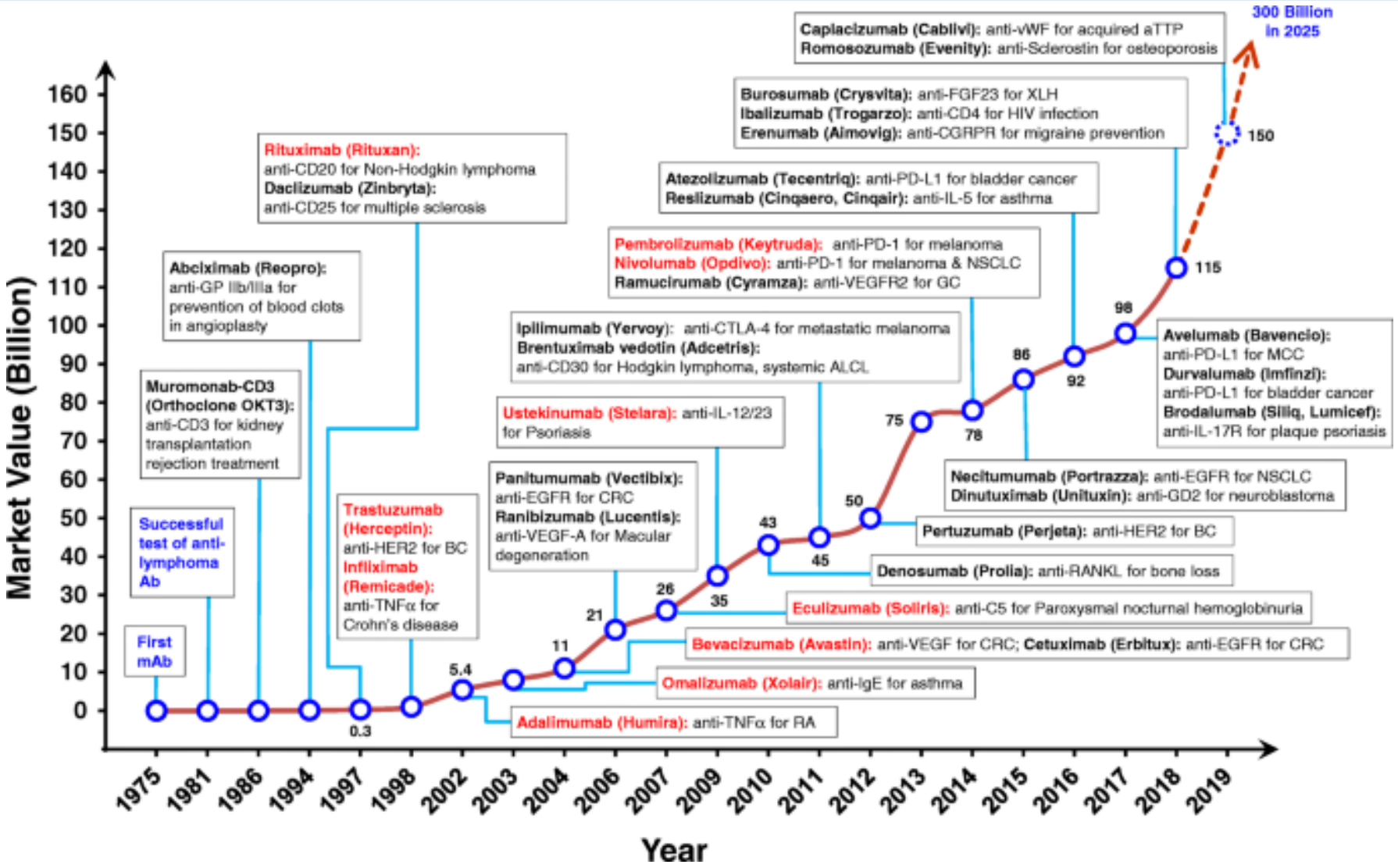


Here, focus will be placed on antibodies

(With a few short excursions)

- Monoclonal antibodies (mAbs) include some of the most potent (and profitable) biopharmaceutical drugs
- They are produced mostly in cultures of mammalian cells
- These cells grow at a slower pace than yeast or bacteria, but they are able to glycosylate the proteins they produce
- Glycosylation is key ot the bioactivity of the antibodies
- Mammalian cells, on the other hand, are more sensitive to mechanical stress (from agitation or aeration) and require specially designed reactors

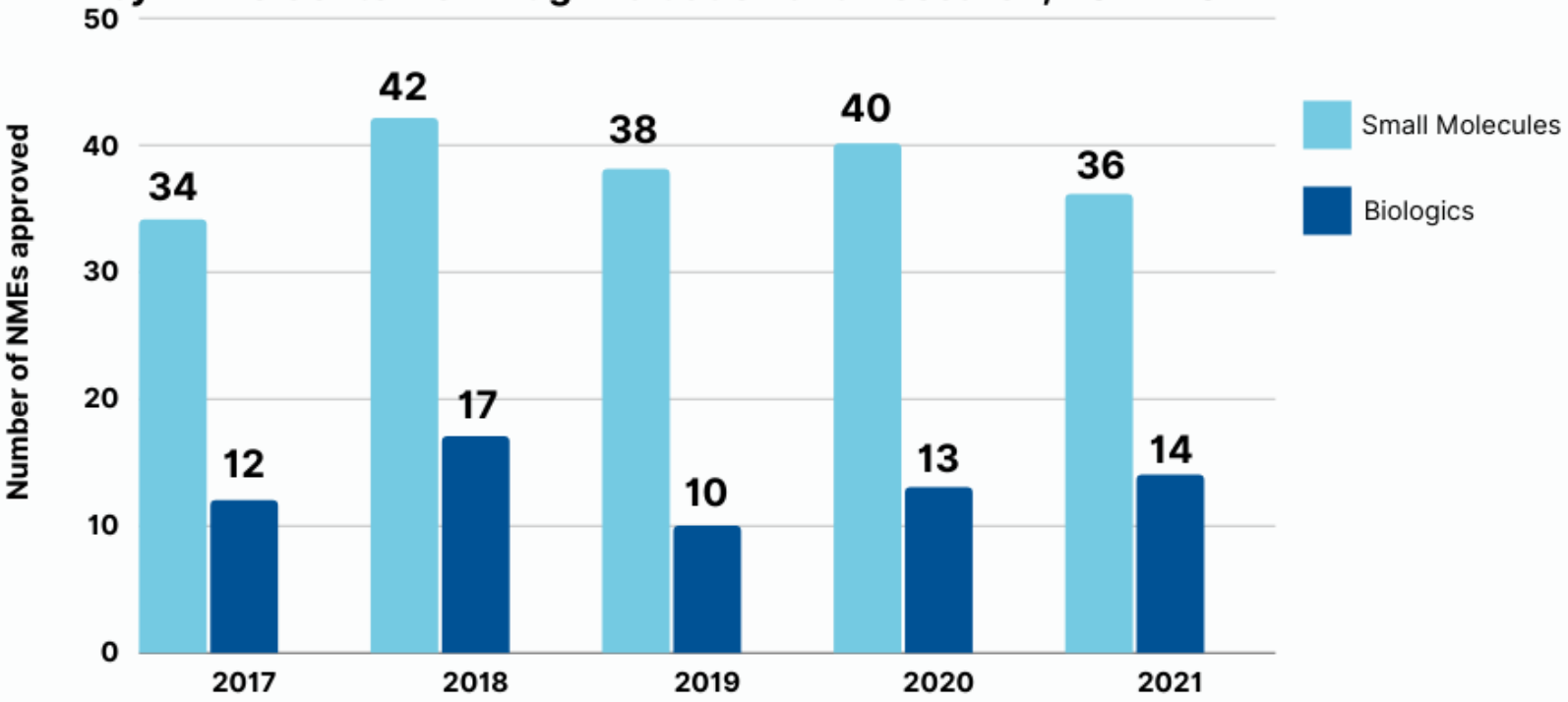
Monoclonal antibodies: timeline and market value



R. M. Lu et al., Journal of Biomedical Science 27 (1), 2020 https://doi.org/10.1186/s12929-019-0592-z

Biologics are outnumbered by synthetic drugs …

Figure 3: Number of Small Molecules and Biologics Approved as New Molecular Entities by FDA's Center for Drug Evaluation and Research, 2017-2021

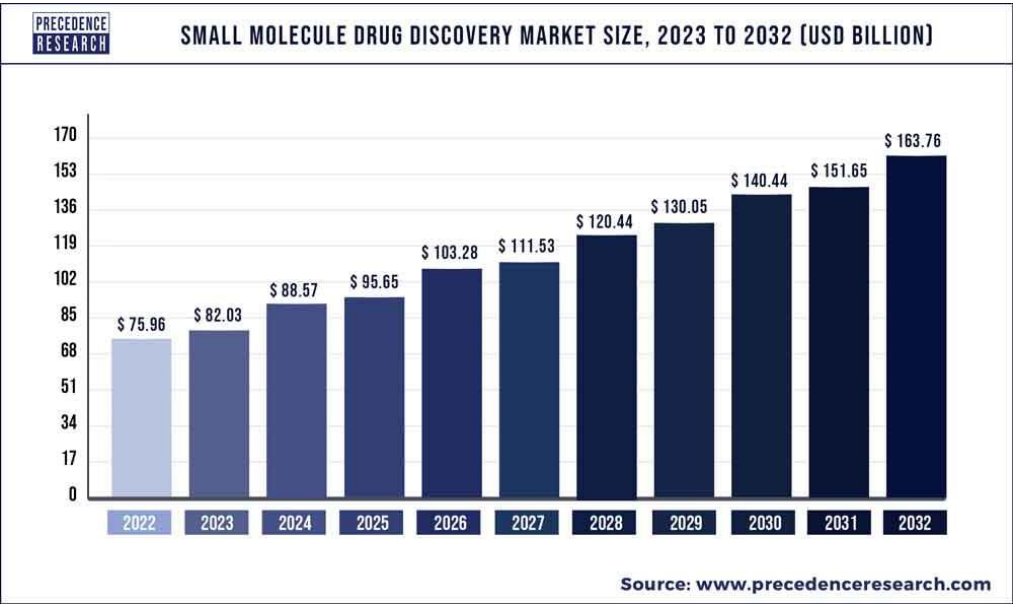


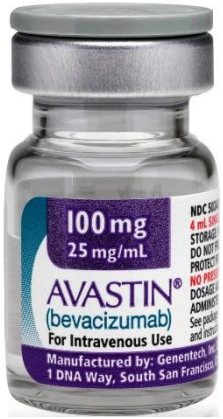
NME is new molecular entity

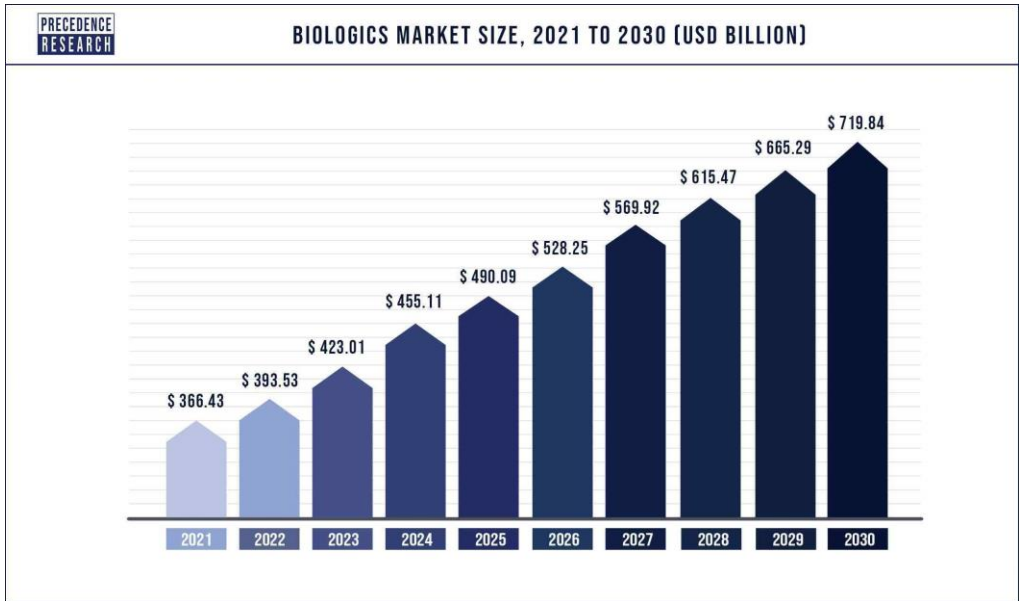
Source: Center for Drug Evaluation and Research, US Food and Drug Administration

Source: DCAT Value Chain Insights

… but they represent a much larger market







Projected sales and indications of mAbs

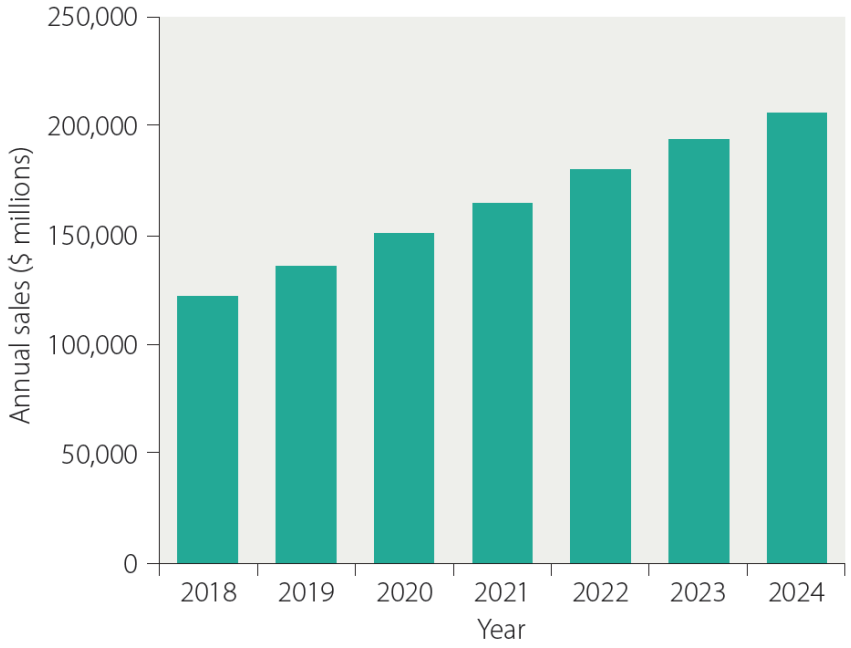


Fig. 1 | Growth in the global sales of monoclonal antibodies from 2018 to 2024. Source: EvaluatePharma, July 2019.

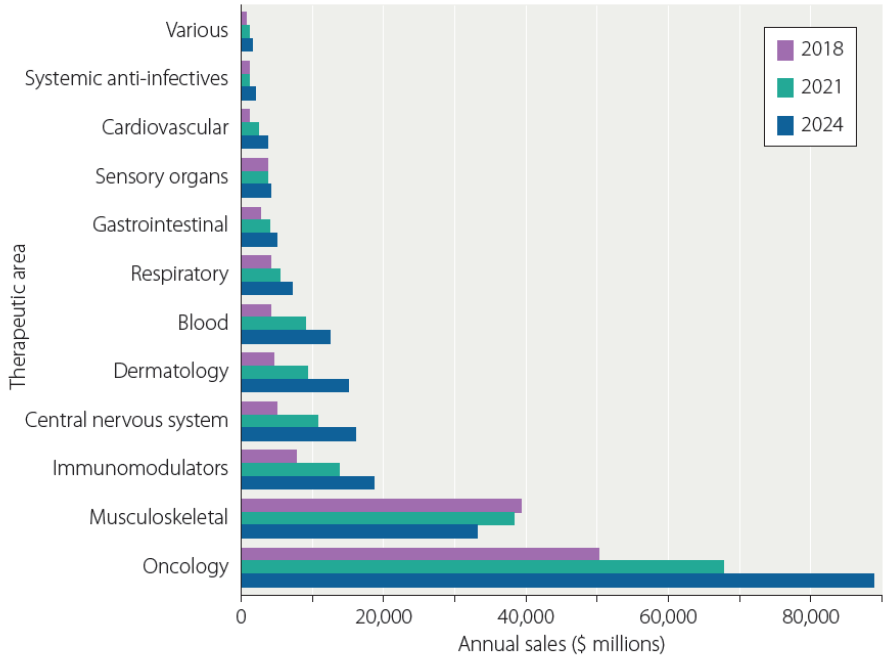


Fig. 2 | Trends in monoclonal antibody sales by therapy area. Source: EvaluatePharma, July 2019.

- In 2018, seven out of the ten best selling drugs were biologics
- > 80 mAbs approved for a variety of indications/domains
- The two dominant ones are oncology and immune disorders

Introducing the top sellers and some newcomers

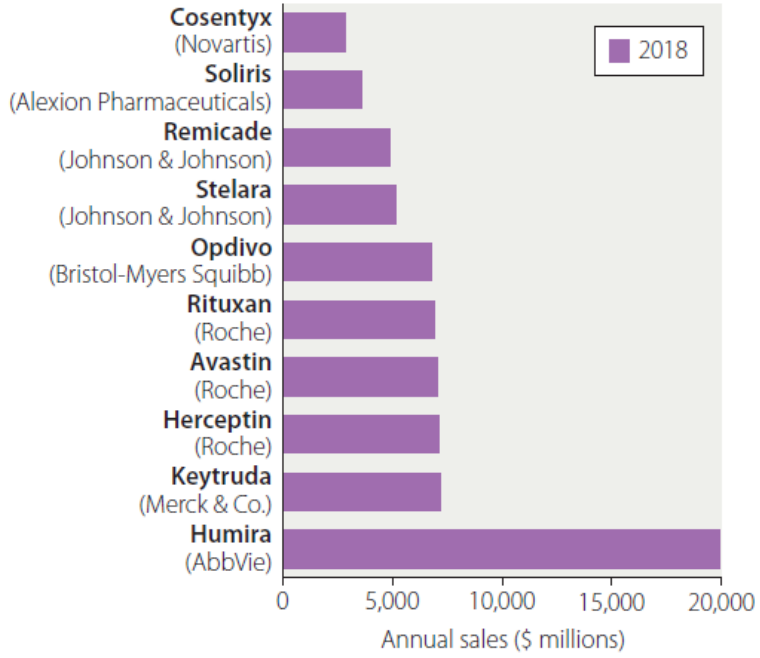


Fig. 3 | Top ten monoclonal antibodies by sales in 2018.
Source: EvaluatePharma, July 2019.

Table 1 | FDA approvals of monoclonal antibodies in 2018

Drug (brand name)	Developer(s)	Indication
Ibalizumab (Trogarzo)	TaiMed Biologics and Theratechnologies	HIV
Tildrakizumab (Ilumya)	Sun Pharma	Plaque psoriasis
Burosumab (Crysvita)	Ultragenyx Pharmaceutical and Kyowa Hakko Kirin	X-linked hypophosphatemia
Erenumab (Aimovig)	Amgen and Novartis	Migraine
Mogamulizumab (Poteligeo)	Kyowa Hakko Kirin	Mycosis fungoides and Sezary syndrome
Moxetumomab pasudotox (Lumoxiti)	AstraZeneca	Hairy cell leukemia
Fremanezumab (Ajovy)	Teva	Migraine
Galcanezumab (Emgality)	Eli Lilly	Migraine
Cemiplimab (Libtayo)	Regeneron and Sanofi	Cutaneous squamous cell carcinoma
Emapalumab (Gamifant)	Novimmune	Primary hemophagyocytic lymphohistiocytosis
Ravulizumab (Ultomiris)	Alexion Pharmaceuticals	Paroxysmal nocturnal hemoglobinuria

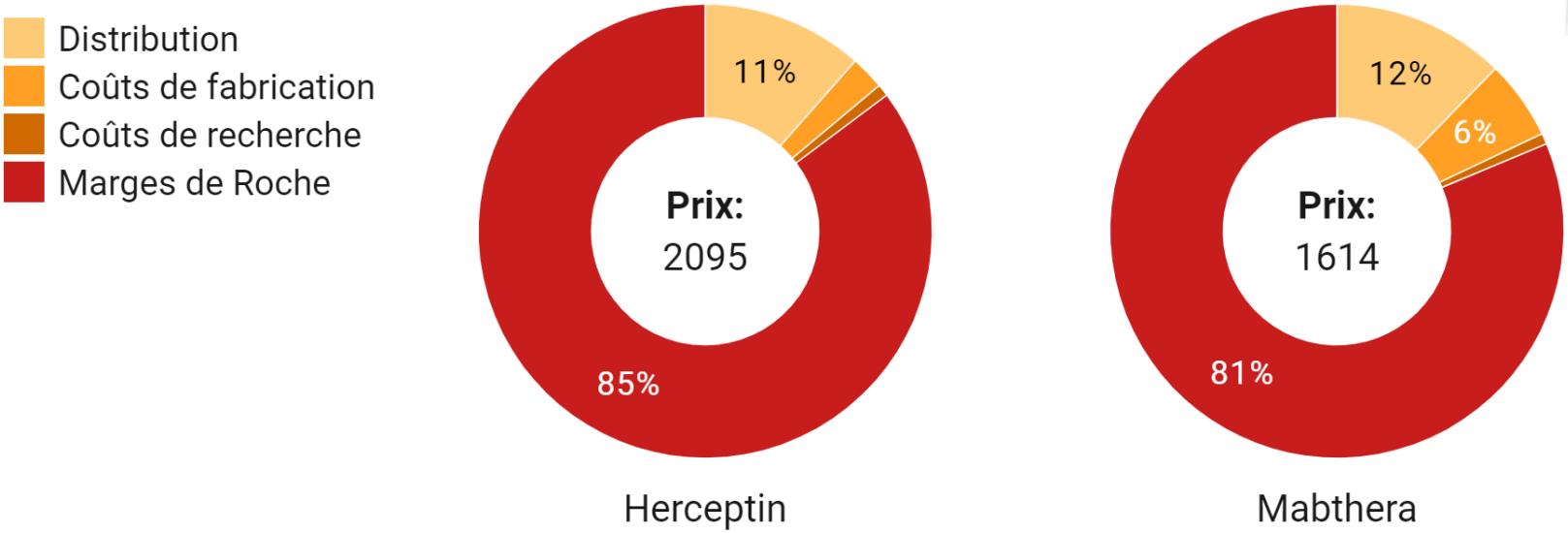
FDA, US Food and Drug Administration.

- Humira is by far the best selling drug ever
- The arrival of **biosimilars** will modify this ranking

mAbs are also a (highly) profitable market

Example of Trastuzumab / Herceptin
CHF 2095.- for 440 mg, with 85% margin for Roche
© RTS, Emission Mise au Point (17.02.2019)

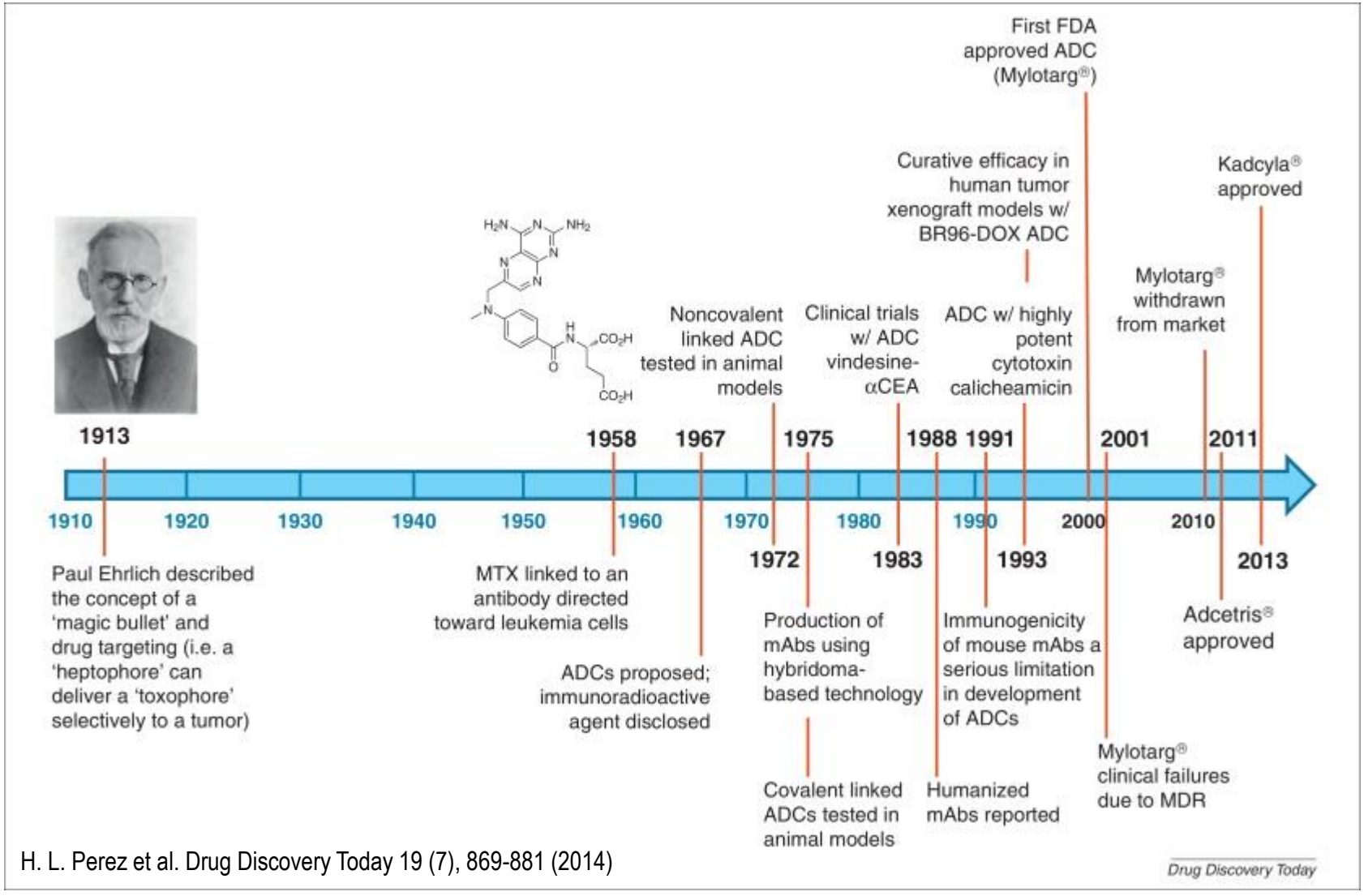
Estimation des marges de deux anti-cancéreux de Roche



La TVA (2,5% pour les médicaments) et les dépenses marketing n'ont pas été prises en compte.

ADC: at the interface of biotech and synthetic drugs

(ADC = Antibody-Drug Conjugate)

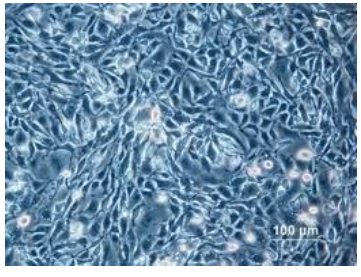


H. L. Perez et al. Drug Discovery Today 19 (7), 869-881 (2014)

Mammalian cells

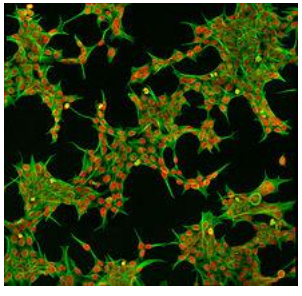
CHO (chinese hamster ovary cells)

- CHO cells are the most commonly used mammalian hosts for industrial production of recombinant protein therapeutics.
- They are able to produce proteins with complex glycosylation, post-translational modifications (PTMs) similar to those produced in humans.
- They are easily growable in large-scale cultures and have great viability, which is why they are ideal for GMP protein production.
- Further, they are tolerant to variations in parameters, be it oxygen levels, pH-value, temperature or cell density.

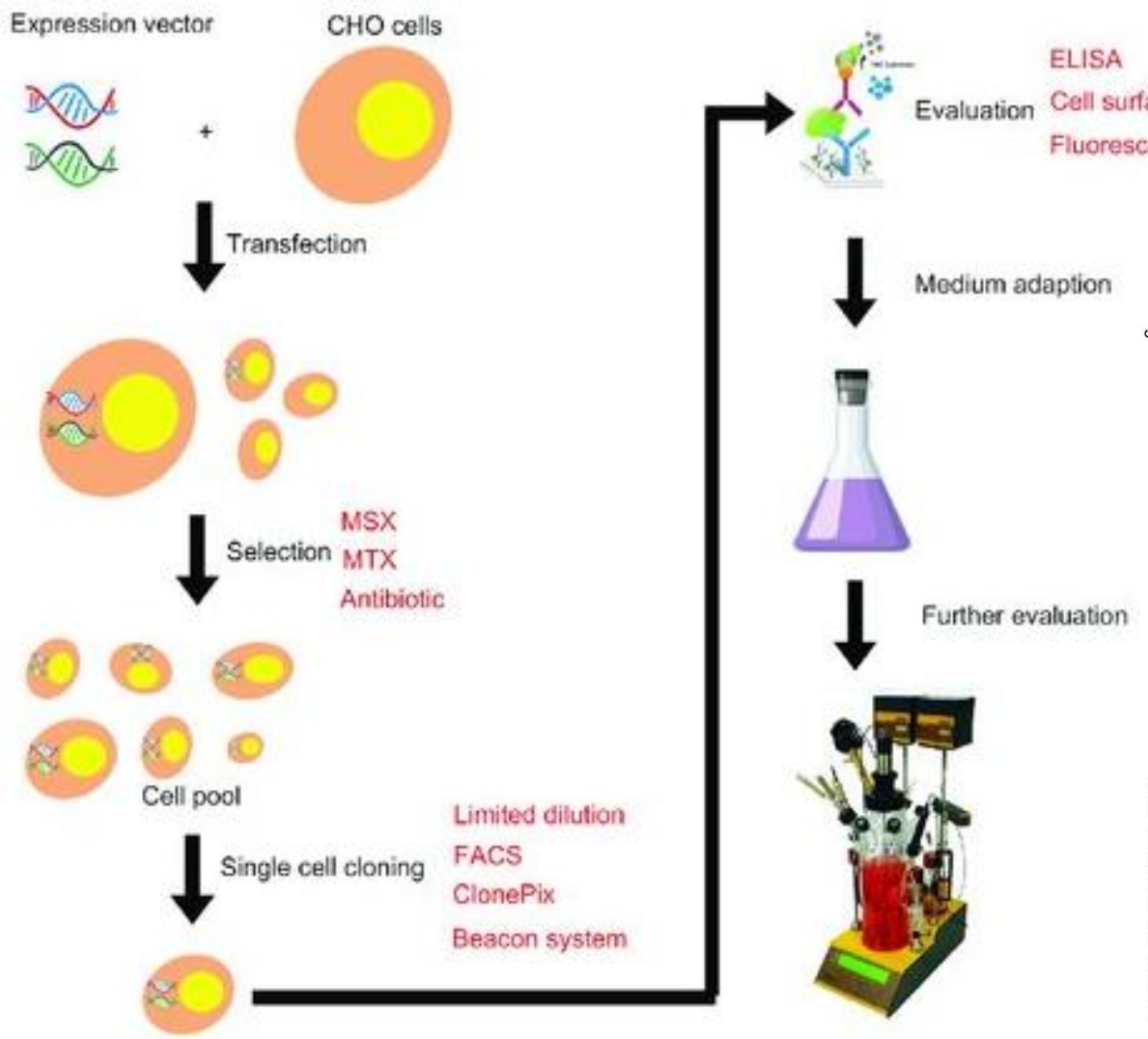


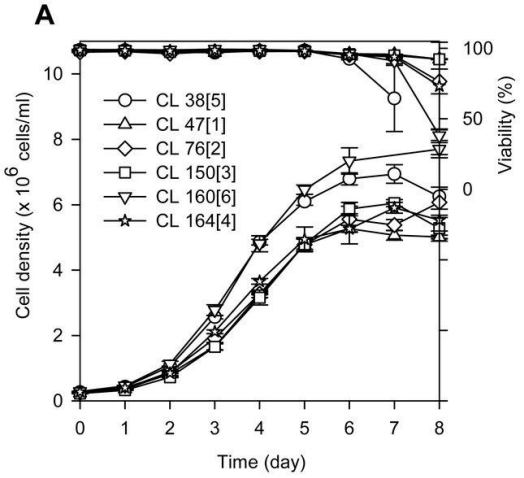
HEK 293 (human embryonic kidney cells)

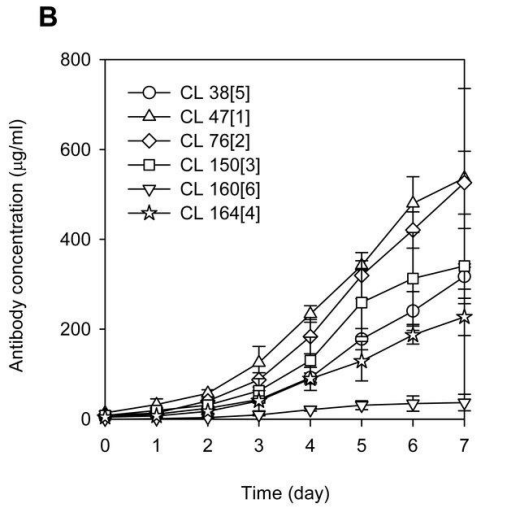
- They are a specific immortalised cell line derived from a spontaneously miscarried or aborted fetus
- HEK 293 cells are straightforward to grow in culture and to transfect. They have been used as hosts for gene expression.
- Typically, these experiments involve transfecting in a gene (or combination of genes) of interest, and then analyzing the expressed protein.
- The widespread use of this cell line is due to its easy and efficient transfectability by various techniques



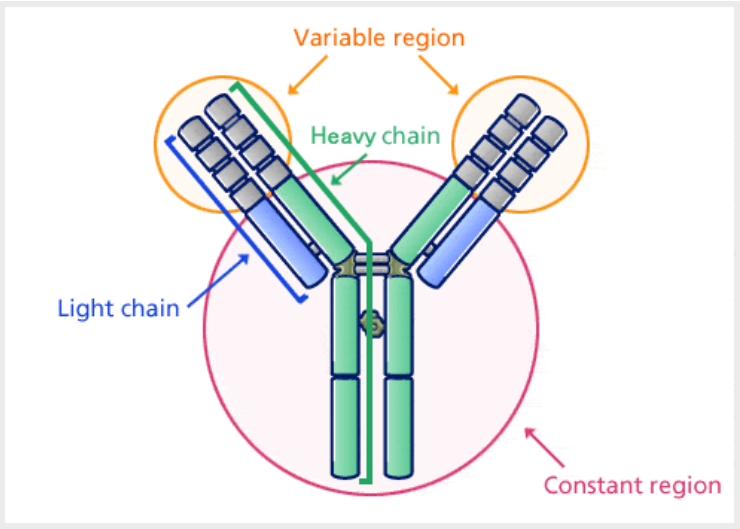
typical development of a mammalian cell line for recombinant protein manufacturing.

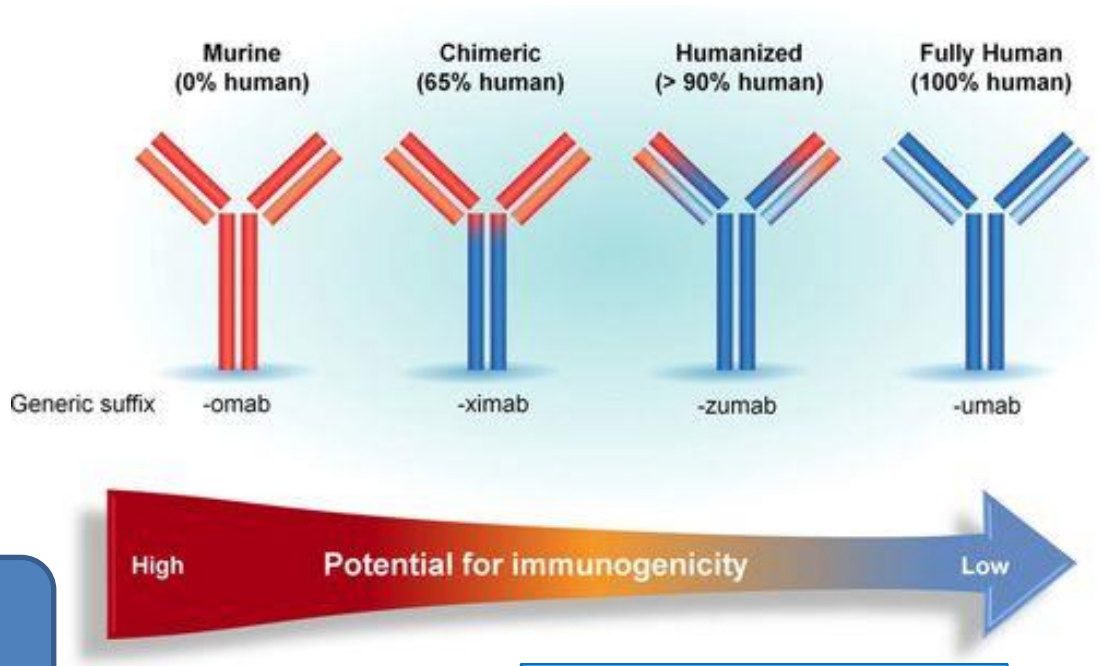




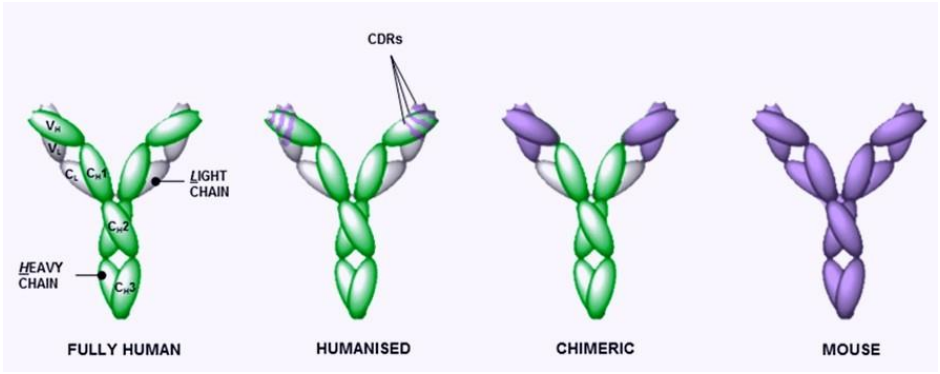


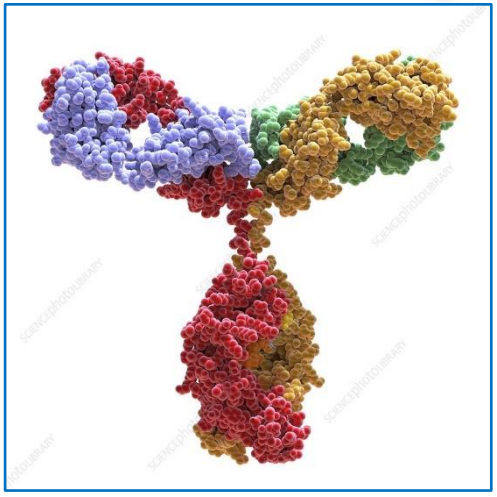
A few things about (monoclonal) antibodies?



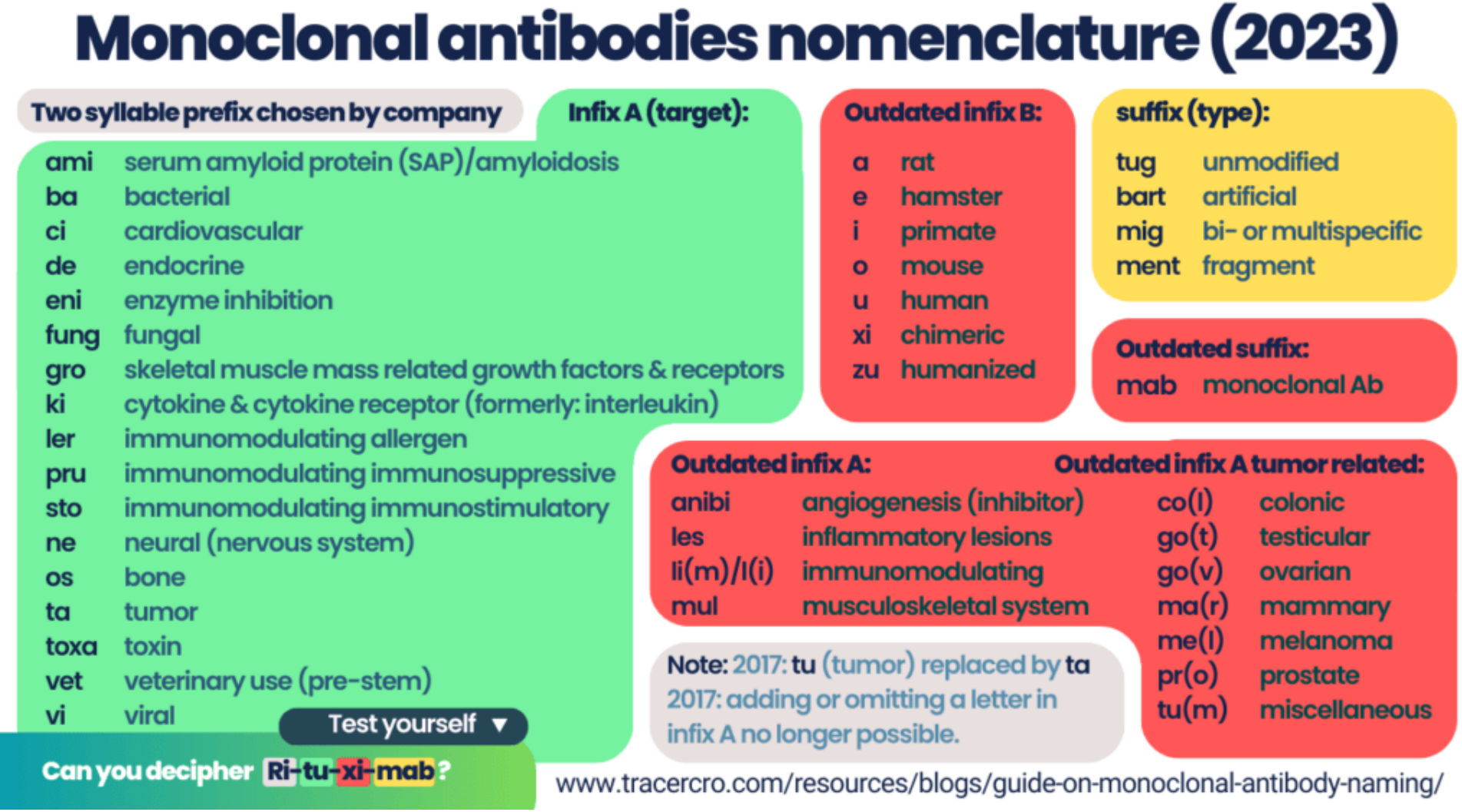


What can you say about their main physico-chemical characteristics?





mAbs: strange code names deciphered



https://www.tracercro.com/resources/blogs/guide-on-monoclonal-antibody-naming/

Herceptin (Roche)

https://www.cancerhealth.com/drug/herceptin-trastuzumab

- The active molecule in Roche's Herceptin® is Trastuzumab
- What does the name tell you?

Analytical aspects: a short doc search

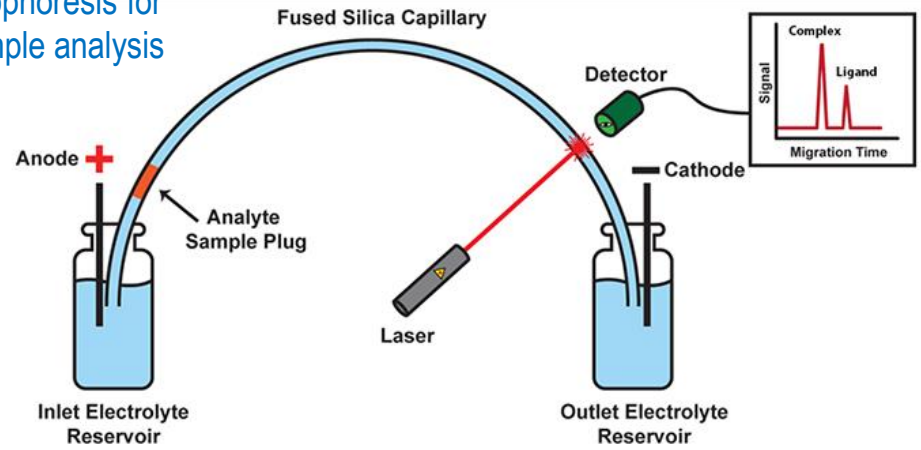
- Search the various analytical techniques that can be used to determine the concentration of the antibody?
- Which parameters are commonly measured to assess the quality of the produced antibody?
- How can you monitor the progress of its purification?

Monitoring purification: the analytics

- Total protein concentration: A_{280}, A_{260}/A_{280}, Bradford assay, Lowry assay, Qubit, variable pathlength spectrometer, Nanodrop, Variable pathlength devices
- Product (mAb) concentration: Qubit, ELISA, HPLC-Protein A, HPLC-SEC, HPLC-HIC
- Global overview: SDS-PAGE, Capillary electrophoresis

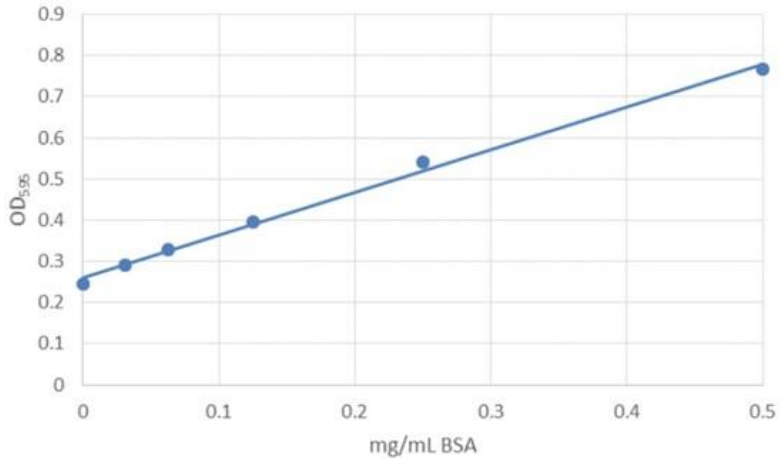
Variable pathlength spectrometer

Principle of capillary electrophoresis for (among others) protein sample analysis

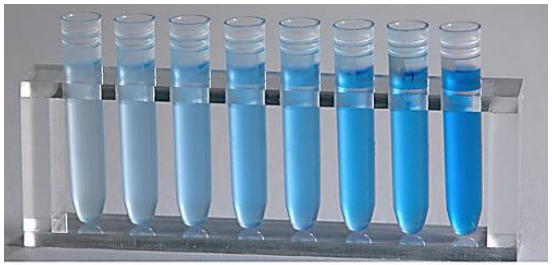


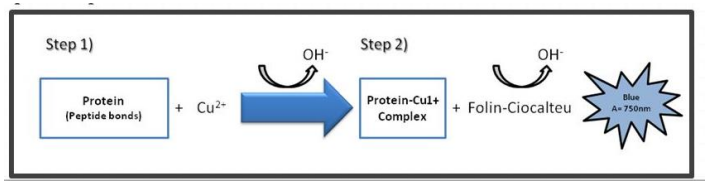
Bradford & Lowry assays

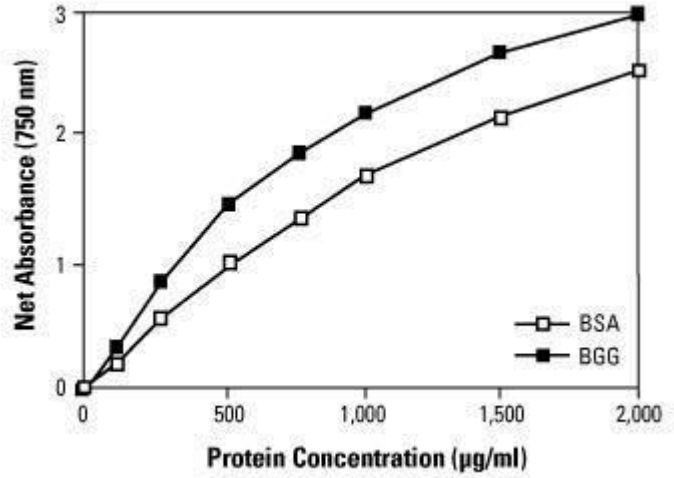
Bradford



Lowry

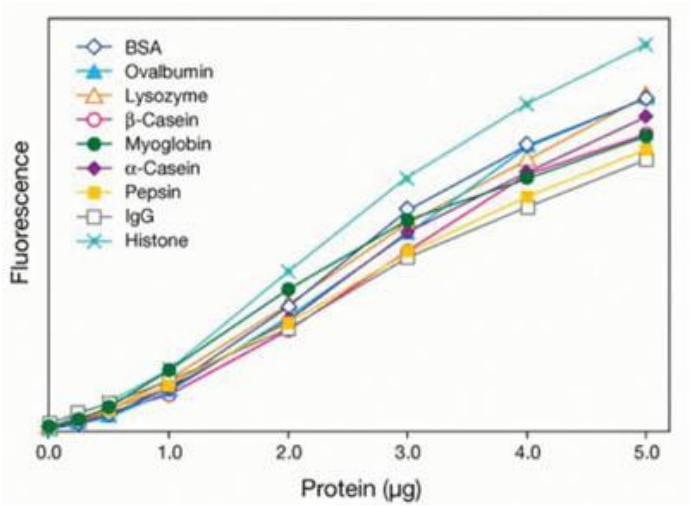




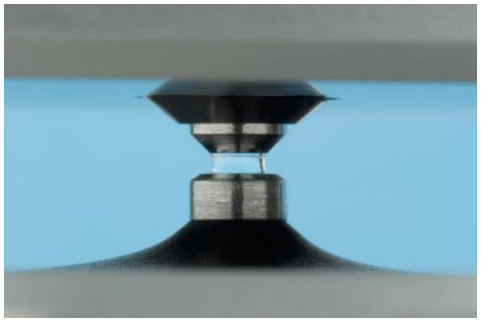


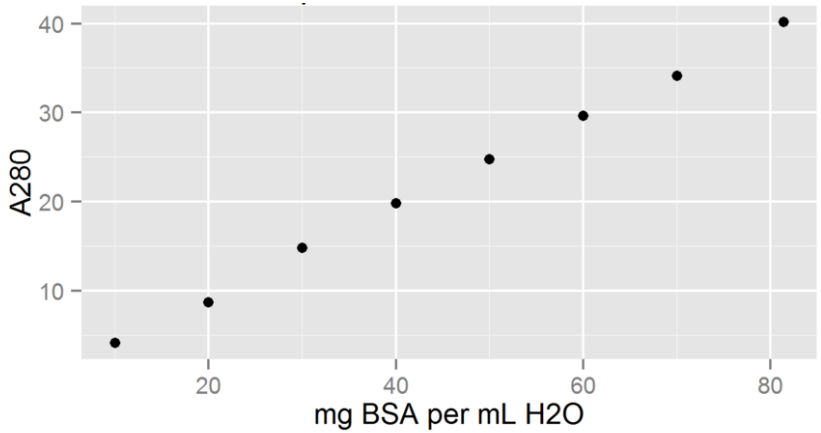
Qubit & Nanodrop

Qubit fluorometer



Nanodrop





Quality control for monoclonal antibodies

- SDS-PAGE
- Charge variants
- Glycosylation pattern(s)
- HCP = Host Cell Proteins (ELISA assay)
- DNA, RNA: A_{260}/A_{280} ratio
- Bioburden
- Mycoplasma
- Virus

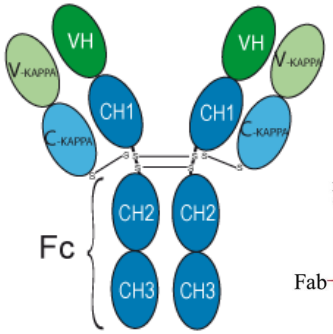
Characterization of the product (1/2)

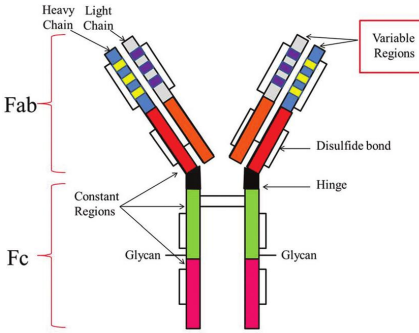
- Molecule: mAbs, Fc-fusion proteins, biosimilars, vaccine, viral vector, cell therapy
- Biochemical & Biophysical property: Size, pI, post-translation modification
- Host: Mammalian, microbial, insect, human

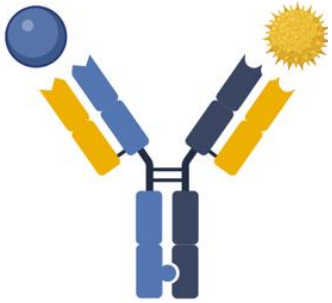
Raw Materials:
- Resin: ProA, IEX, HIC, SEC, memetic ligand
- Chemical: Tris, sodium acetate, sodium phosphate
- Water: deionized water, WFI
- Filter: depth filter, viral filter, 0.22 µm filter
- Medbrane: TFF membrane, Sartobind Q membrane
- Chemical Grade: Multicompendial, USP, EP, JP, BP, ACS, NF

- QA: ensure quality requirement met (e.g., documentation)
- Operation, CMC compliance, deviation investigation, CAPA
- QC: Testing and releasing for DS/DP and manufacturing support
- RA: ensure PD/MFG/clinical trail and filling activities met regulatory requirement (e.g., ICH 5A, 5E, 7, 8 , 9, 10, 11)

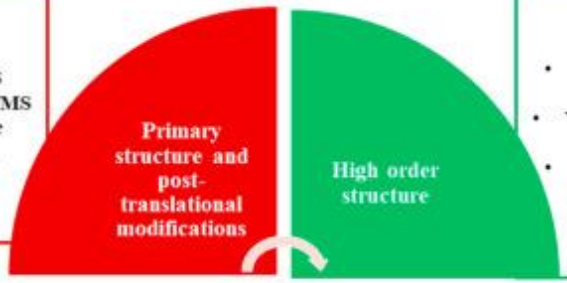
- PD: Process development (study design, protocol, report, viral clearance study, tech transfer, scale-up)
- Equipment: bioprocess skid, pump, tank, floor scale, pH/conductivity meters, Solo VPE
- Facility: ISO 7/5, Physical and air recirculation segregation, terminal HEPA filtration, positive pressure cascade system





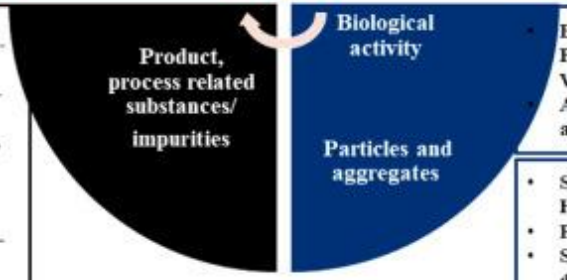


- Amino acid analysis (sequence) - LC-MS/MS
- N-linked glycan-HILIC/MS
- Molecular mass, peptide mapping (reduced, non-reduced) – LC/MS



- Secondary structure – FTIR, far UV CD
- Tertiary structure - Near UV CD, IFS
- Melting temperature - DSC

- Size variants -SEC-HPLC, nrCE-SDS, rCE-SDS
- Charge variants – CEX-HPLC
- Residual HCP – ELISA, LC-MS, 2D-DIGE
- Residual protein A analysis - ELISA
- Residual DNA analysis – qPCR

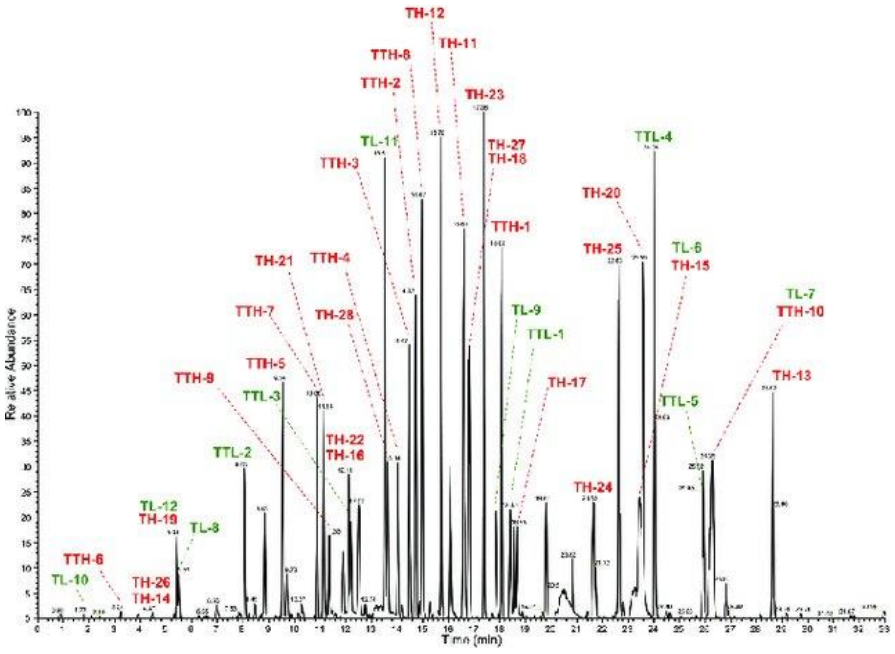


- Binding assay (FcγR, FcRn, TNF, LTa3, C1q, VEGF-A)
- Assay (CDC, ADCC, apoptosis)

- Subvisible particles – HIAC, MFI, FFF
- Particles - DLS
- Submicron size distribution- AUC-SV

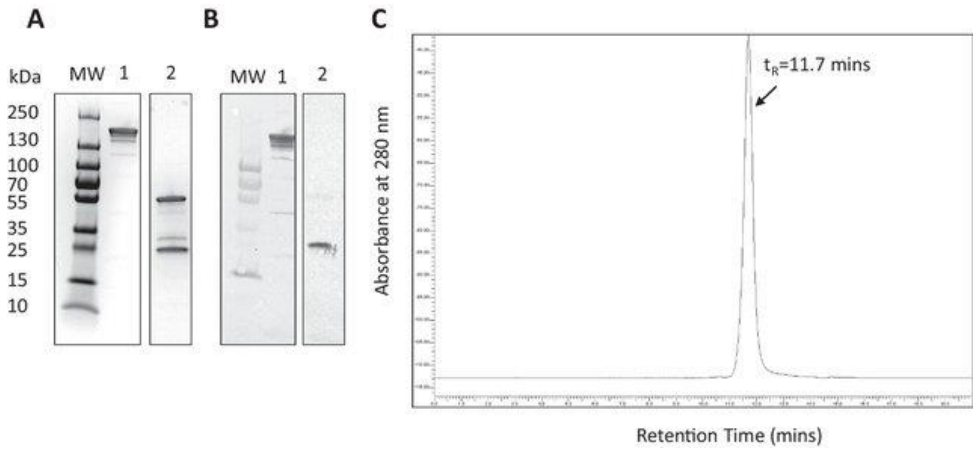
Characterization of the product (2/2)

Base peak chromatogram of tryptic digests from Trastuzumab prepared by our proposed sample preparation method. TH and TL were the peptides from the constant region of Trastuzumab. TTH and TTL were the peptides containing the CDR region of Trastuzumab



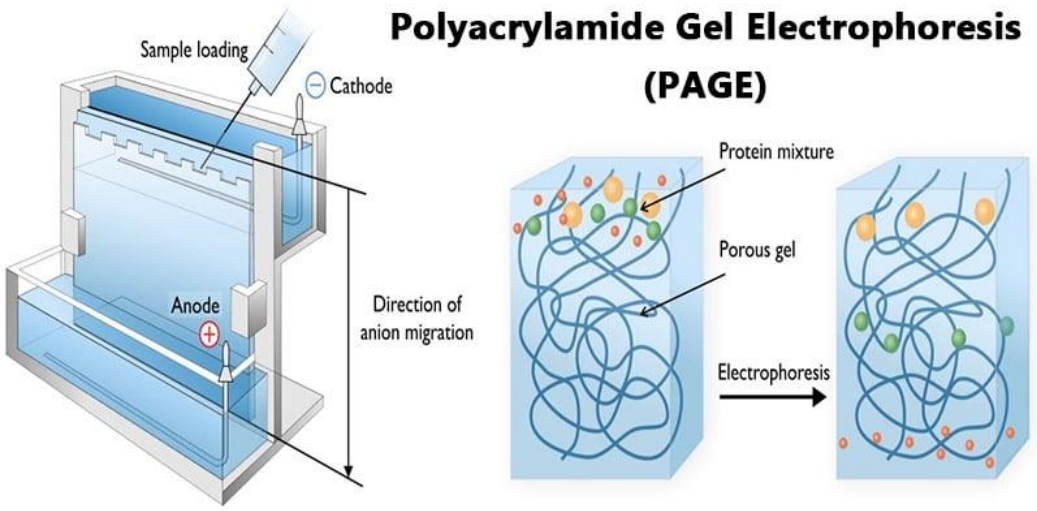
M. Tajiri-Tsukada **et al.** Bioengineered 11 (1), 984–1000 (2020) https://doi.org/10.1080/21655979.2020.1814683

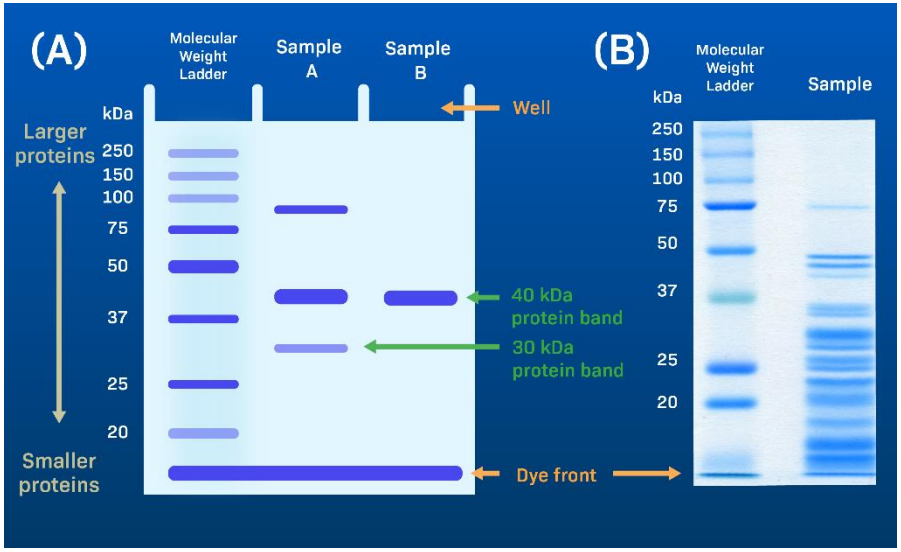
C. Chan et al. EJNMMI Radiopharmacy and Chemistry (2022) 7:33 https://doi.org/10.1186/s41181-022-00186-9

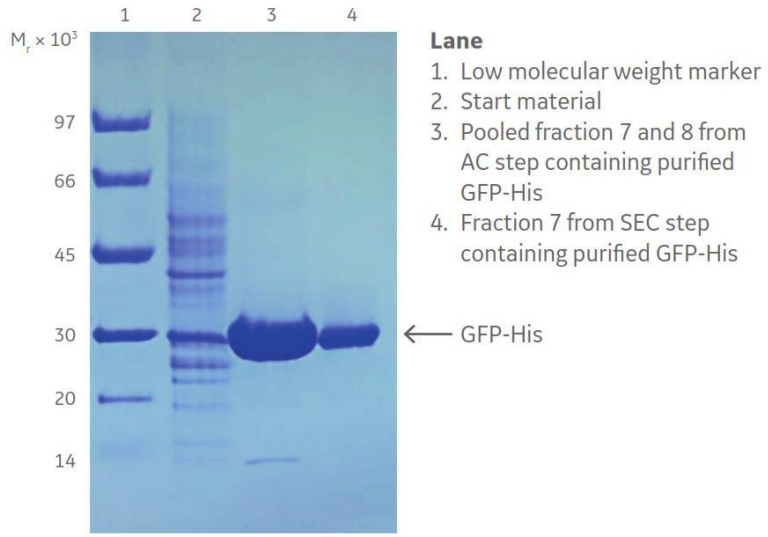


A SDS-PAGE analysis of Trastuzumab under non-reducing conditions (lane 1) or reducing conditions (DTT, lane 2) on a 4–20% Tris HCl gradient mini-gel. MW: broad range molecular weight standards. The gel was stained with Coomassie R-250 Brilliant Blue. B Corresponding Western blot under non-reducing (lane 1) or reducing (lane 2) conditions showing immunopositivity with goat anti-human Fab specific IgG-horseradish peroxidase (HRP) immunoconjugates. C SE-HPLC analysis of trastuzumab on a BioSep SEC-s4000 column (Phenomenex, Torrance, CA, USA) eluted with 100 mM NaH2PO4 buffer, pH 7.0 at a flow rate of 0.8 mL/min with UV detection at 280 nm containing the CDR region of Trastuzumab

SDS-PAGE


Polyacrylamide Gel Electrophoresis
(PAGE)
Sample loading
Cathode
Anode
Direction of
anion migration
Protein mixture
Porous gel
Electrophoresis


(A)
Molecular Weight Ladder
Sample A
Sample B
kDa
Larger proteins
250
150
100
75
50
37
25
20
Smaller proteins
Well
40 kDa protein band
30 kDa protein band
Dye front
(B)
Molecular Weight Ladder
Sample
kDa
250
150
100
75
50
37
25
20


1
2
3
4
$M_r \times 10^3$
97
66
45
30
20
14
GFP-His
Lane
1. Low molecular weight marker
2. Start material
3. Pooled fraction 7 and 8 from AC step containing purified GFP-His
4. Fraction 7 from SEC step containing purified GFP-His

Downstream processing in short

Source: Hochschule Biberach

DSP

Source: Roche

STARTING MATERIAL

- **Complex, heterogenous mixtures**
- **Mostly aqueous**
- **Low product concentrations**
- **Large volumes to be handled**
- **Molecules are sensitive/labile**
- **High risk of contamination**

SHOULD BE:

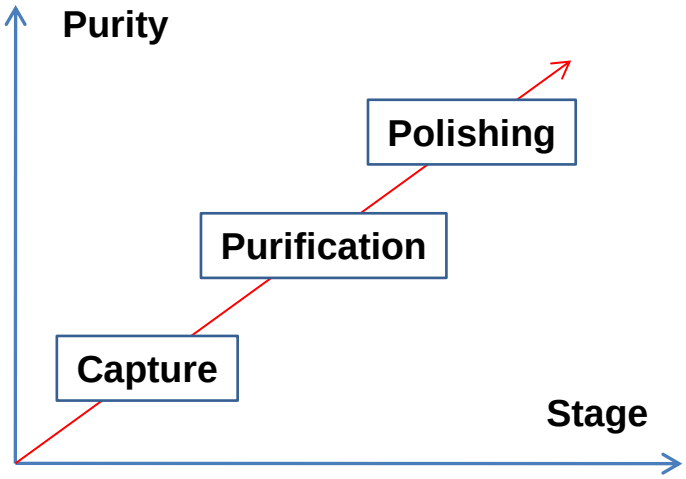
Short
Efficient
« Gentle »
Cheap

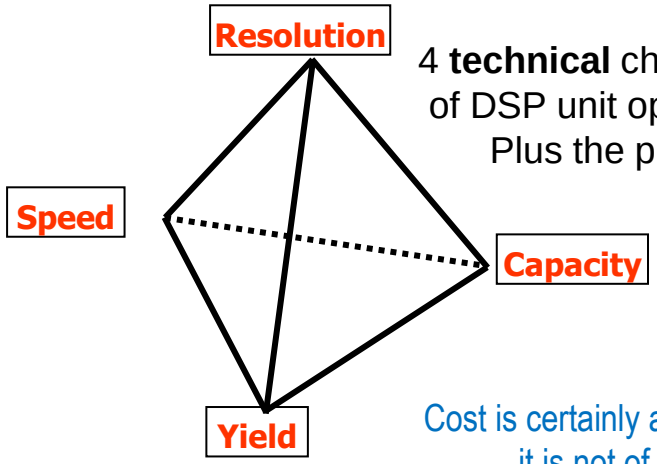
Working on it!

PRODUCT HAS TO BE:

Pure
Active
Safe
Well-formulated
Stable

Key elements of DSP (in a nutshell)

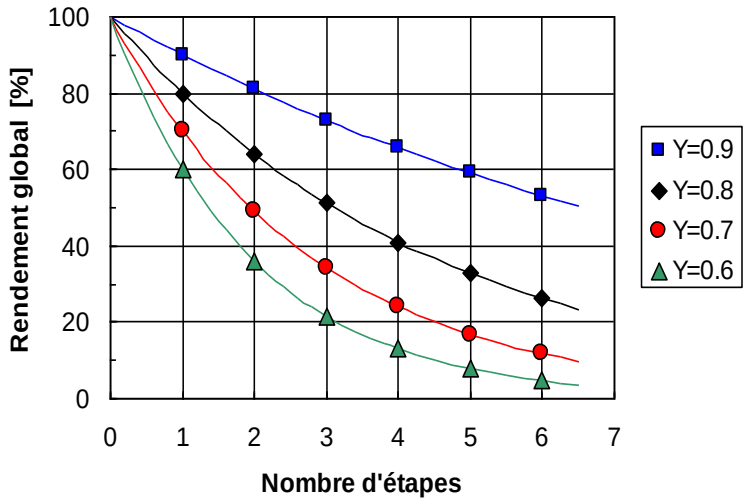




4 **technical** characteristics of DSP unit operations …
Plus the price tag!

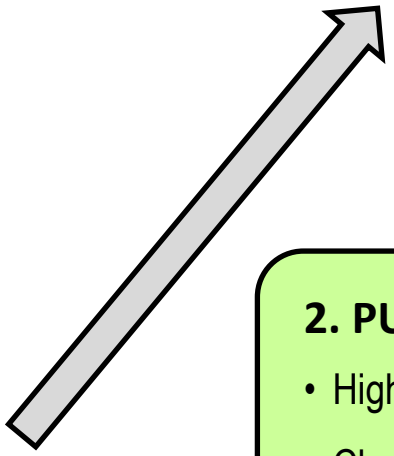
Cost is certainly an iomportant aspect, but it is not of a technical nature

- DSP development can rely on a large selection of techniques based on different separation principles
- Depending on their features they might be applied at different stages of purification
- Most of them are the same in chemical engineering and in bioprocesses
- However, the working conditions need to account for the lability of biomolecules
- By the way, which DSP techniques do you know?



Wanted: few steps with high recovery yields

Three steps, from fermentor to product



3. POLISHING

- Aims at removing the last impurities (aggregates, fragments)
- Refolding and crystallisation very often used at this stage
- Drying, stabilization and formulation are also important

2. PURIFICATION

- High selectivity, removes impurities similar to product
- Chromatography is THE purification technique (but alternatives are being sought)

1. CAPTURE / ISOLATION

- Aims at reducing the volumes/quantities to be handled
- Not very specific, but significant increase in product concentration and purity
- Adsorption, precipitation, and liquid/liquid extraction are most often used

Sorting out the catalog of techniques

Physical techniques

- Sedimentation
- Centrifugation
- Filtration
- Ultrafiltration
- Nanofiltration
- Reverse osmosis
- Size exclusion chromatography

Thermal techniques

- Precipitation
- Crystallization
- Destillation
- Sublimation
- L/L extraction
- Absorption
- Adsorption
- Chromatographies: IEX, HIC, RPC, Affinity

There is a driving force behind every type of separation !!!

Thermal separation techniques are driven by thermodynamics

These techniques are characterized by a **partition** or **distribution of the molecules between phases**

Thermodynamics determines the concentrations of the molecules in each phases **at equilibrium**

An important question for engineers is «how long until I reach equilibrium»? That brings in the notion of **mass transfer kinetics**

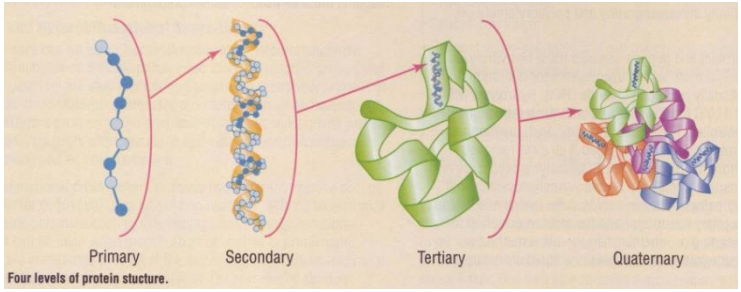
This **kinetics in influenced by many parameters** such as temperature, viscosity, agitation rate, particle size, diffusion rate etc …

NB: **Cell lysis** rightfully belongs to DSP, although it is not – *stricto sensu* - a purification technique

White biotech, red biotech, from low- to high-tech?

- Citric acid
- Lactic acid
- Antibiotics
- Biopolymers
- Ethanol, biofuels
- Industrial enzymes

Purification involves a large range of « standard » unit operations such as precipitation, Liquid-liquid extraction, high pressure homogenization



Four levels of protein stucture.

- Recombinant proteins
- Monoclonal antibodies
- Hormones

DSP relying on techniques more specific to biotechnology: centrifugation, membrane filtration, affinity chromatography ..

Biopharma relies on a smaller selection of techniques

Textbook DSP

- Cell/medium separation
  - Sedimentation
  - Filtration
  - Centrifugation
- Cell lysis
  - Bead mill
  - HP homogenizer
  - Ultrasound
- L/L Extraction
- Precipitation
- Adsorption
- Chromatography
- Membrane filtration
- etc …

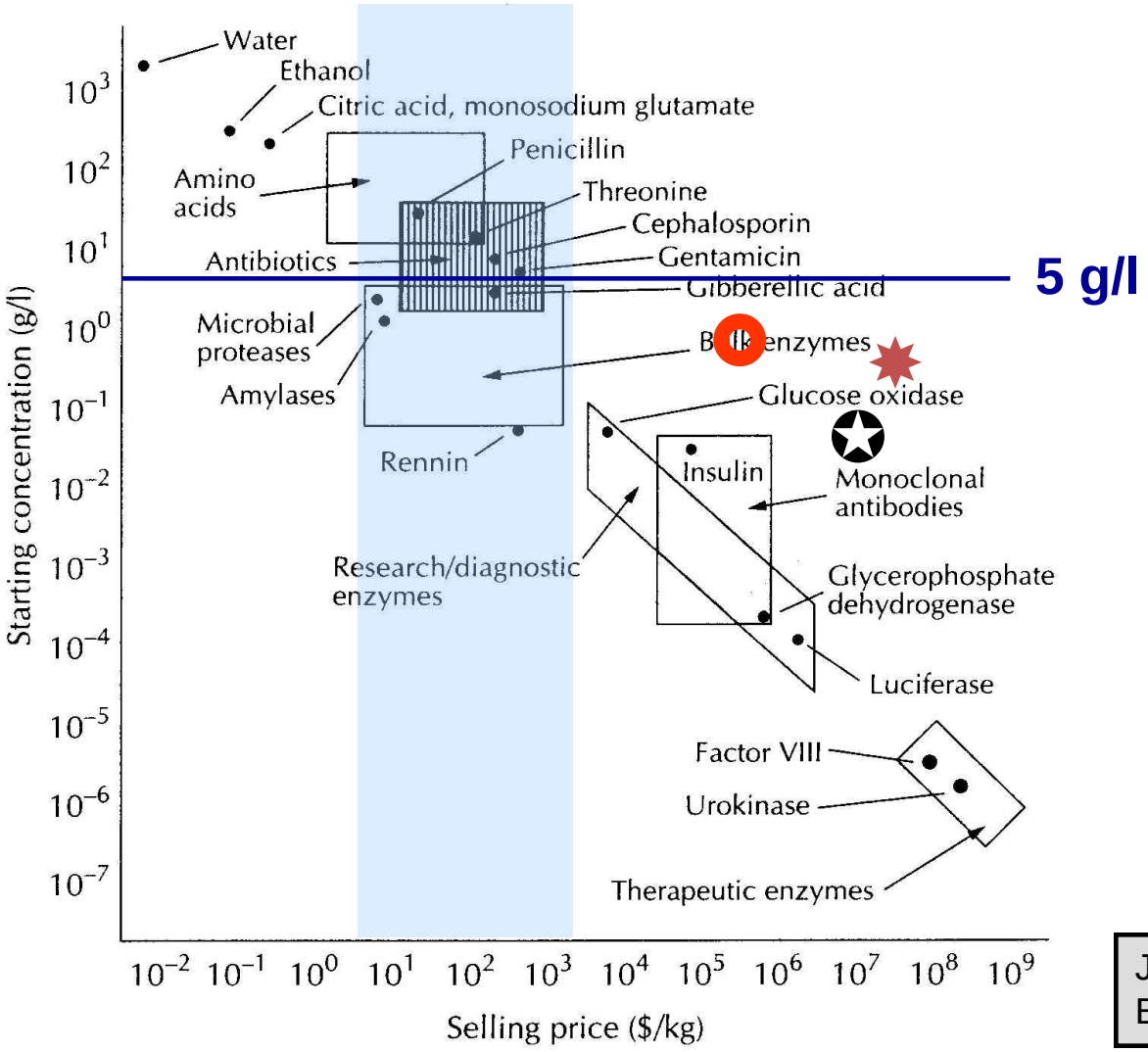
DSP in the biopharma

- Centrifugation
- Micro- & Ultrafiltration
- Chromatography

Are we teaching the right things?

Do we place our focus on the correct topic(s)?

Take home message: the more dilute the product, the more expensive its purification



5 g/l

Human Growth Hormone (1985)

Tissue Plasminogen Activator (1987)

Hepatitis B Vaccine (1986)

H. W. Blanch, D. S. Clark
Biochemical Engineering, Dekker, 1997

J. L. Dwyer
Bio/Technology 2 (11), pp 957, [1984]