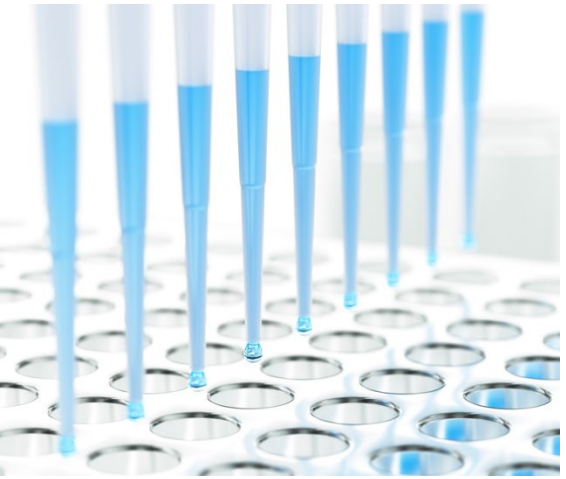

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BIOLOGICS

IPAC-RS
International Pharmaceutical Aerosol Consortium on Regulation & Science

IPAC-RS Workshop:
Inhaled Biologics: Preparing for a Future Beyond Small Molecules

September 4-5, 2024

Testing Requirements for Protein Biologics Therapies

WAI LAM W. LING, PH.D.
VP, SCIENTIFIC ADVISORY

SEPTEMBER 4, 2024
IPAC-RS BIOLOGICS WORKSHOP

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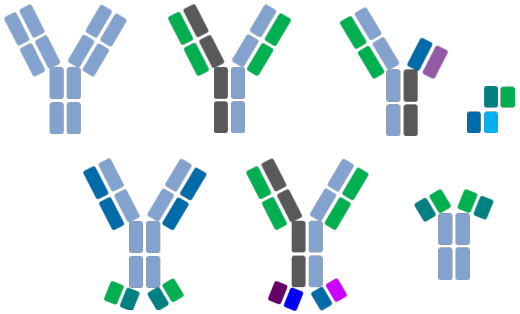
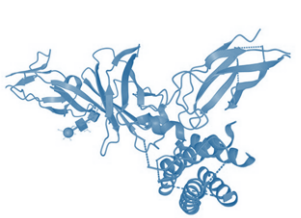
Growing Diversity of Biologics

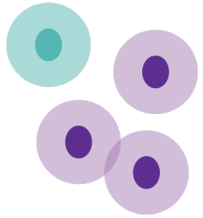


mAb, Multi-specific Ab, BiTE, etc.

ADCs

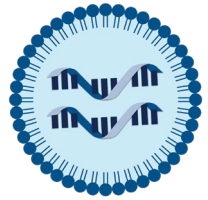


Cytokines, recombinant proteins



Cell therapy products

Gene therapy products



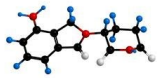
mRNA products

Blood products

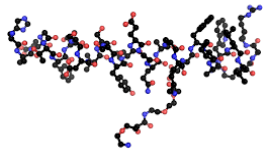
Catalent BIOLOGICS

1. Structural complexity of large molecules
2. Regulatory landscape of QbD
3. CMC workflow for protein biologics
4. Developability of protein biologics
5. Summary

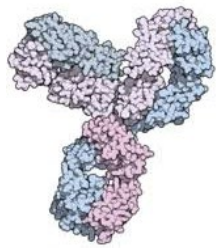
Complexity In Therapeutic Modalities



Lenalidomide



Semaglutide



Pembrolizumab

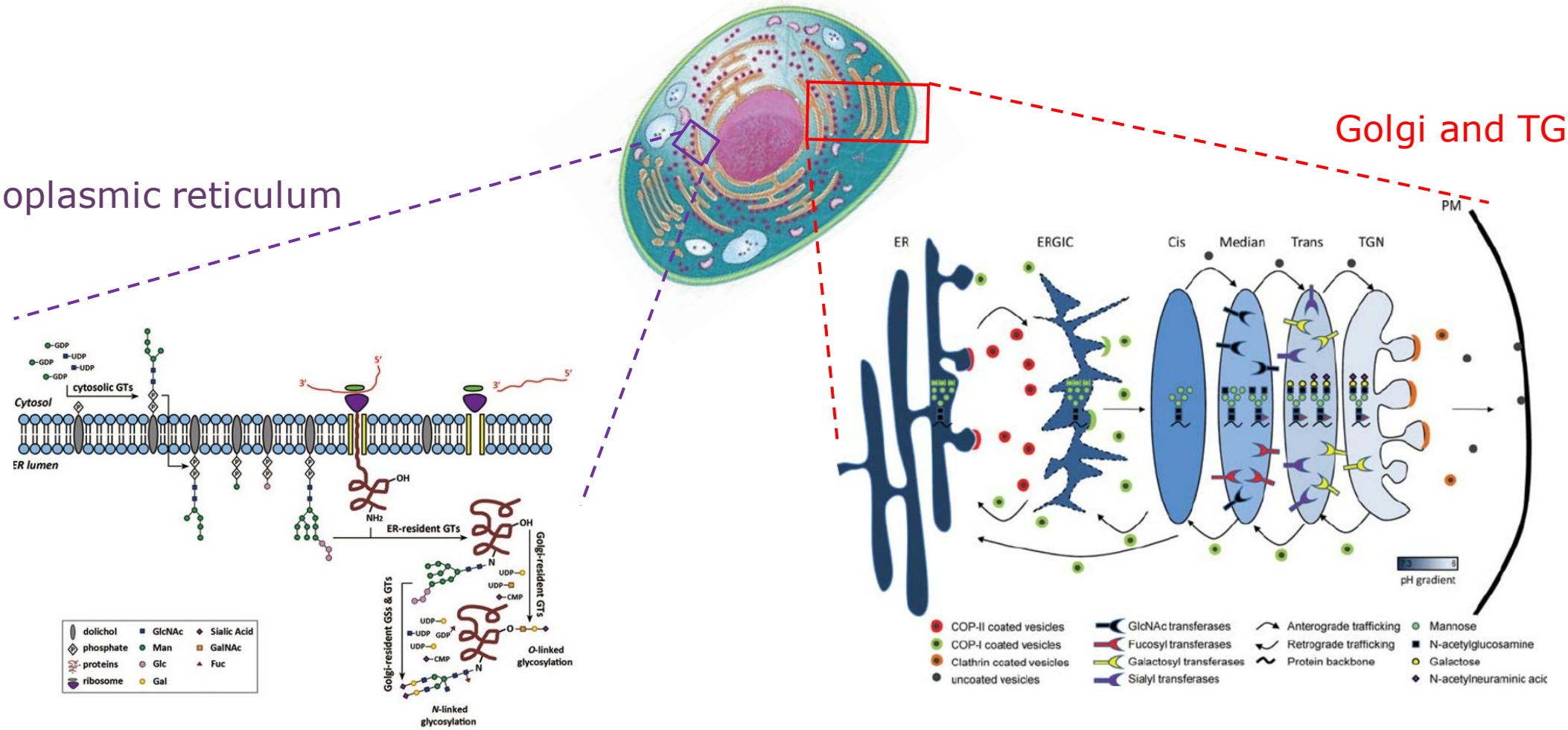
Characteristic	Small Molecules	Biologics
Molecular Weight	Low (80-100 atoms)	High (hundreds to thousands of atoms)
Stability	Stable at RT	Unstable at RT
Structural Complexity	Simple	Complex
Administration Route	Often oral	Usually injection or infusion
Cell Membrane Permeability	High	Low
Distribution	Widely distributed via circulation	Limited to circulation & lymphatics
Immunogenic Potential	Low	Higher possibility
Cost of Treatment	Generally low	Generally high

Adapted from 2023 Singh et al. Frontiers | Drug discovery and development: introduction to the general public and patient groups (frontiersin.org)

Intracellular Protein Processing And Trafficking

Endoplasmic reticulum

Golgi and TGN



2014 Wang et al. Protein post-translational modifications and regulation of pluripotency in human stem cells | Cell Research (nature.com)

2011 Reynders et al. How Golgi glycosylation meets and needs trafficking: the case of the COG complex | Glycobiology | Oxford Academic (oup.com)

Nanodevice placed inside cell shows how cells change with time - The Week

Post-translational Modifications Affect Product Functions And Efficacy

Influencing factors

pH


Light

Temperature


Oxidative stress


Agitation


Process related

Post-translational Modifications

Chemical Modifications

- Aspartate isomerization
- N-terminal glutamate to pyroglutamate
- C-terminal amidation
- Acetylation
- Ubiquitination

Oxidative Modifications

- Methionine oxidation
- Tryptophan oxidation

Structural Modifications

- Disulfide shuffling
- Cysteine pairing
- Aggregation

Glycosylation-related Modifications

- Glycosylation
- Glycation

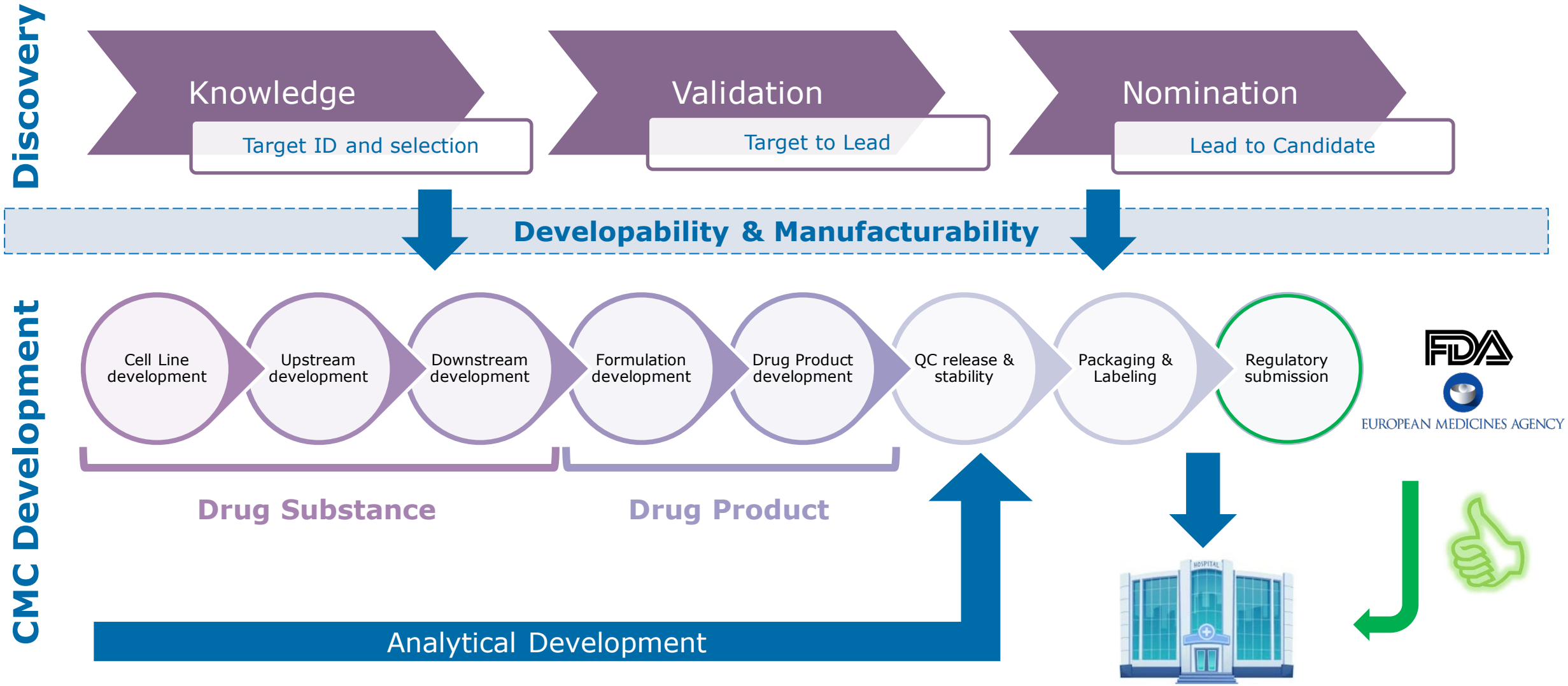
Attributes affected

! Product stability | ! Product variability | ! Structural heterogeneity | ! Charge variants | ! Color change

Functional impacts

! Potency | ! Immunogenicity | ! PK/PD | ! CDC, ADCC | ! Safety

Biologics CMC Development: Ph1 IND Enabling



Glycosylation On Bioactivity

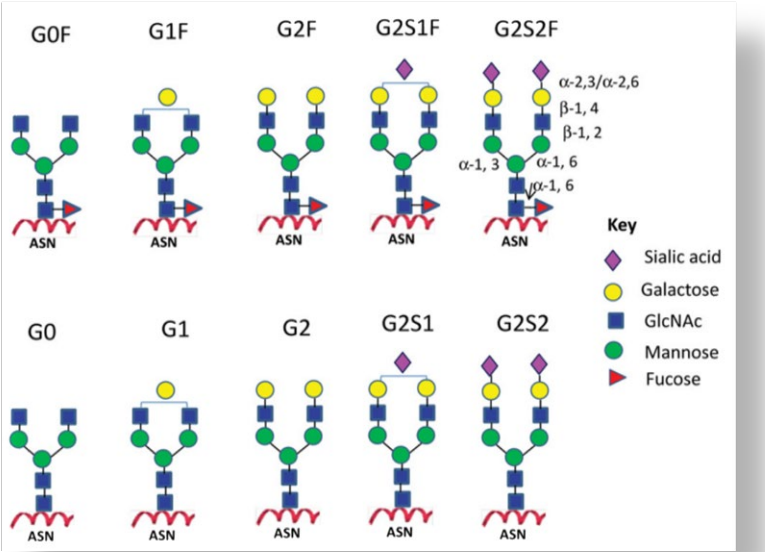
Glycosylation can be N-linked and O-linked

- N-linked motif: N of Asn-X-Ser/Thr (X=any AA except Pro)
- O-linked motif: O of Ser, Thr, Tyr, hydroxy-Lys, hydroxyl-Pro

Effect on bioactivity

- Fucose removal enhances ADCC activity
- Bisecting GlcNAc enhances ADCC activity
- Mannose enhances mAb clearance
- Galactose enhances mAb clearance
- Sialic acid NANA reduces clearance
- Sialic acid NGNA is immunogenic
- α-galactosylation mAb are immunogenic

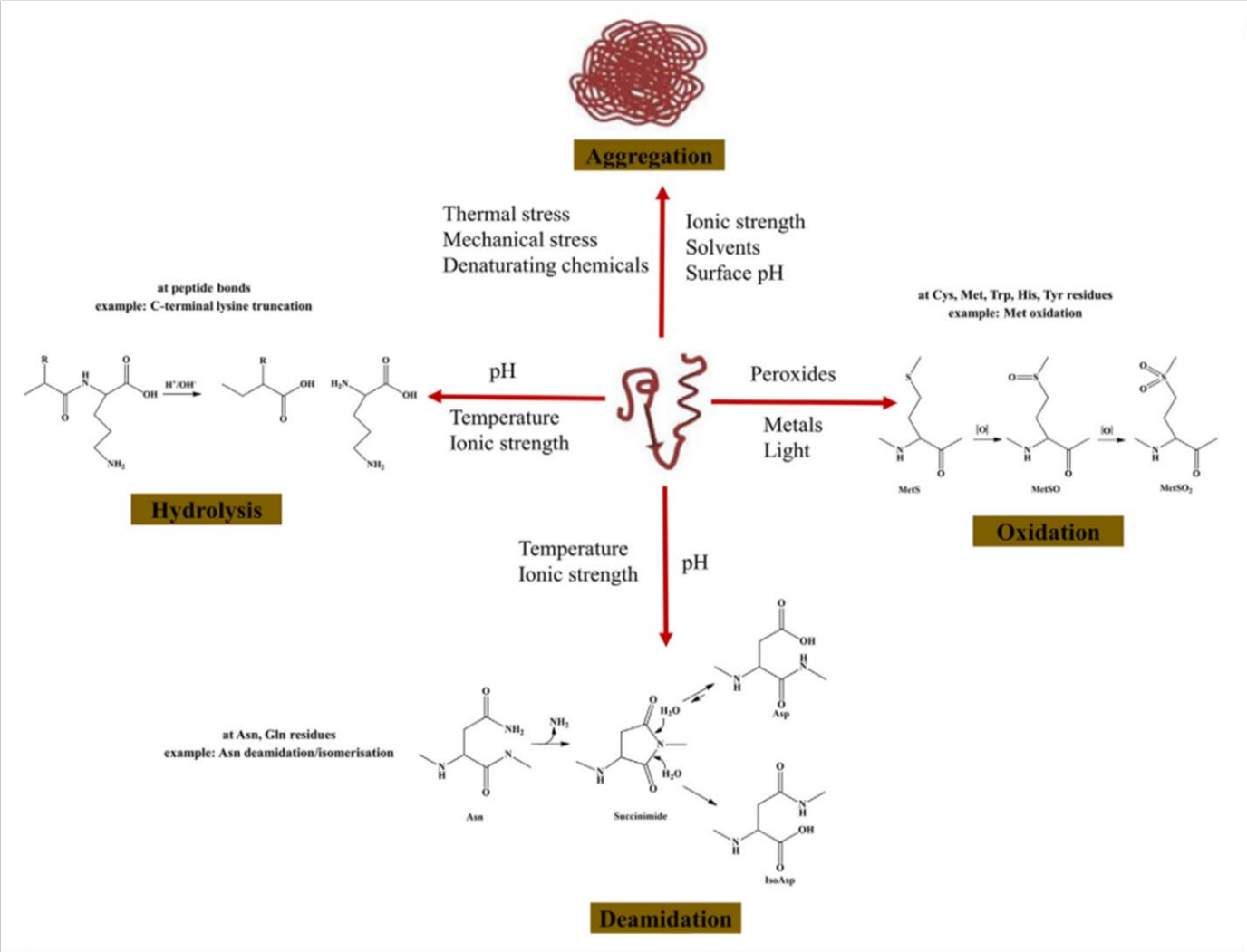
Common glycoforms in mAb



Receptors	Expression	Monomeric IgG			
		IgG1	IgG2	IgG3	IgG4
FcγRI	Monocytes, macrophages	+	−	++	++
$FcγRIIa_{H131}$	Monocytes, macrophages, dendritic cells neutrophiles, platelets, eosinophiles	−	−	−	−
$FcγRIIa_{R131}$	Same as FcγRIIaR131	−	−	−	−
FcγRIIb	B cells, monocytes, macrophages, dendritic cells, neutrophiles	−	−	−	−
FcγRIIc	NK cells, B cells	−	−	−	−
$FcγRIIIa_{F158}$	NK cells, monocytes	−	−	+/−	−
$FcγRIIIa_{V158}$	NK cells, monocytes	−	−	++	
FcγRIIIbNA1	Neutrophiles, eosinophiles	−	−	−	−
FcγRIIIbNA2	Neutrophiles, eosinophiles	−	−	−	−
FcγRIIIbSH	Neutrophiles, eosinophiles	−	−	−	−
FcRn	Cells of endothelial/epitherial origin, monocyte, dendritic cells, kidney podocytes	+++	+++	++	+++

2015 Liu. Antibody Glycosylation and Its Impact on the Pharmacokinetics and Pharmacodynamics of Monoclonal Antibodies and Fc-Fusion Proteins - Journal of Pharmaceutical Sciences (jpharmsci.org)

Effects Of Process Factors On Protein Quality



2021 Butreddy et al. Instability of therapeutic proteins — An overview of stresses, stabilization mechanisms and analytical techniques involved in lyophilized proteins - ScienceDirect

Effects Of Process Factors On Protein Aggregation

Factors	Protein/antibodies	Condition of protein aggregation
Temperature	β-Lactoglobulin	Rapid increase in aggregates from 30 °C to 50 °C.
pH	Bovine-serum albumin, β-galactosidase glucagon-like peptide-1	pH 7 to 1 caused freezing-induced aggregation (20 to −25 °C).
	β-Lactoglobulin	pH 8.2 to 7.5 resulted in the formation of amyloid-like fibrils. Building blocks vary at pH 2 and 3.5.
Ionic strength	Soy protein	Decrease in surface charge and hydrophobicity when strength was increased from 0–500 mM.
Ligands	Insulin	Small peptide prevented the early formation of a critical nucleus.
Cosolutes	Lysozyme, insulin	Trehalose inhibits aggregation.
Salt type	Myofibrillar protein	$MgCl_2$ and $CaCl_2$ induced higher disulphide bonding than NaCl.
Salt concentration	Potato protein	Increase in NaCl affects surface dilatational elasticity and surface activity; also facilitates protein–protein interactions within surface film.
Pumping	Intravenous Ig	Electrostatic interactions between pump surfaces (negatively charged) and antibodies (positively charged).
Agitation	Whey protein	At 5% protein concentration, aggregation increased at a high shear rate.
Drying	Blood plasma	Sugar addition checks freeze-drying-induced aggregation.
Light exposure	α-Lactalbumin	Disulphide-mediated aggregation on exposure to UV-B (0–24 h).

2021 Rajan et al. Review of the current state of protein aggregation inhibition from a materials chemistry perspective: special focus on polymeric materials - Materials Advances (RSC Publishing)

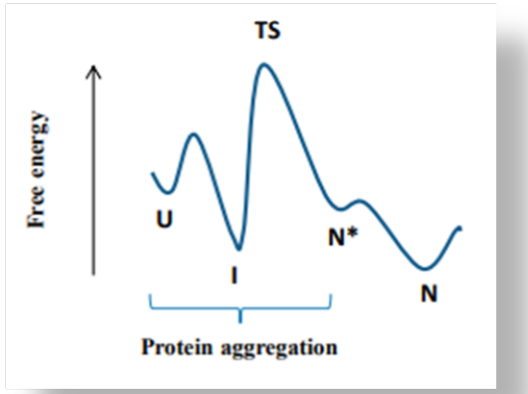
Protein Aggregates On Immunogenicity

≤10 μm impact product stability & immunogenicity

≥10 μm occlude blood flow

≤50-100 μm consider SubVis particles

≥150 μm detectable visually



USP<788> limit for ≤100 mL

- **6000 particles ≥10 μm**
- **600 particles ≥25 μm**

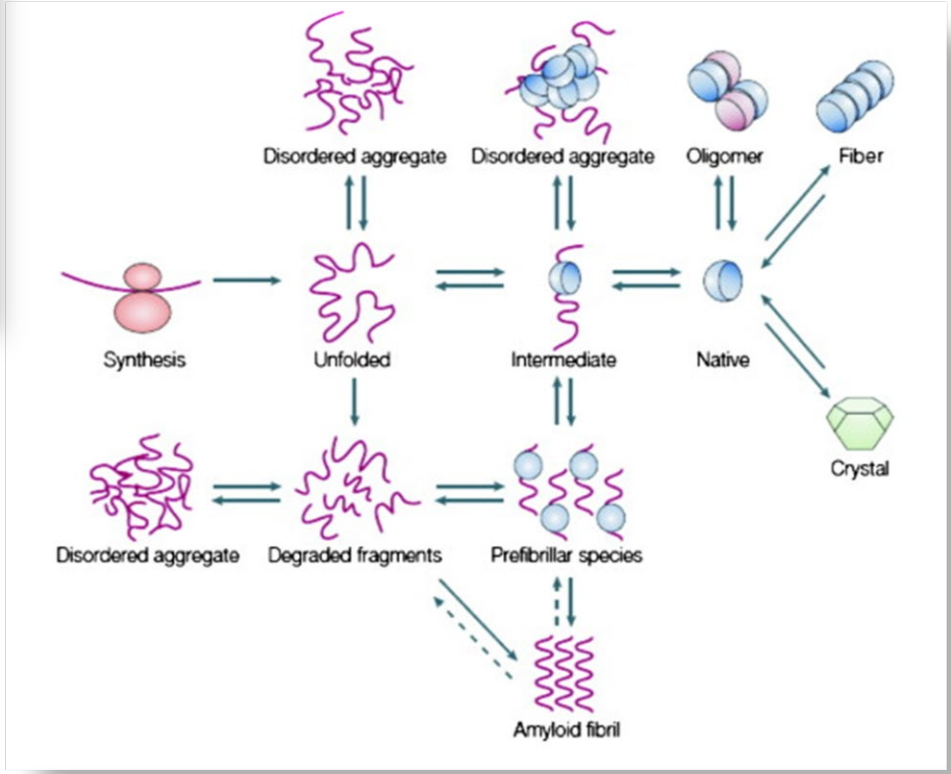
Factors in aggregate formation

- **Temperature (i.e. freeze/thaw)**
- **Ionic strength**
- **Interfacial exposure (solid-liquid, liquid-liquid, gas-liquid)**
- **Chemical degradation (Tyr oxidation, deamidation, S-S shuffle)**
- **Physical degradation (adsorption to interfaces, protein unfolding)**

Aggregate control

- **Good mixing**
- **Conformational and colloidal stability**

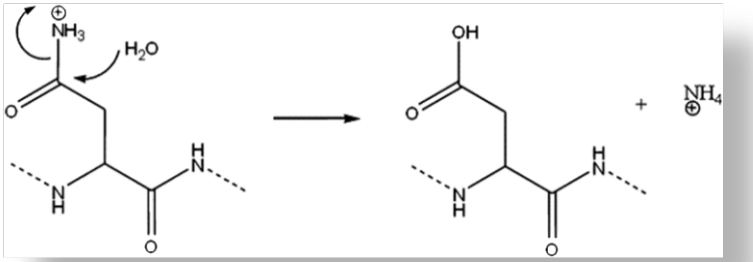
Protein aggregation can be reversible (soluble) and irreversible (insoluble)



2005 Frokjer & Otzen. Protein drug stability: a formulation challenge | Nature Reviews Drug Discovery
2016 Li et al. Antibodies | Free Full-Text | Antibody Aggregation: Insights from Sequence and Structure (mdpi.com)
FDA guidance, Immunogenicity Assessment for Therapeutic Protein Products, 2014

Protein Deamidation On Product Quality

Deamidation: conversion of amide functional group to carboxylic acid



- **Usually occur on Asn and Gln**
- **Deamidation rate increases with temperature and ionic strength**
- **Has a role in protein folding, degradation and biological activities**

Effect on protein quality

- **Charge heterogeneity**
- **Purity**
- **Stability**
- **Antigenicity**
- **Protein half-life**

2022 Jin et al. Mass spectrometric analysis of protein deamidation – A focus on top-down and middle-down mass spectrometry - ScienceDirect

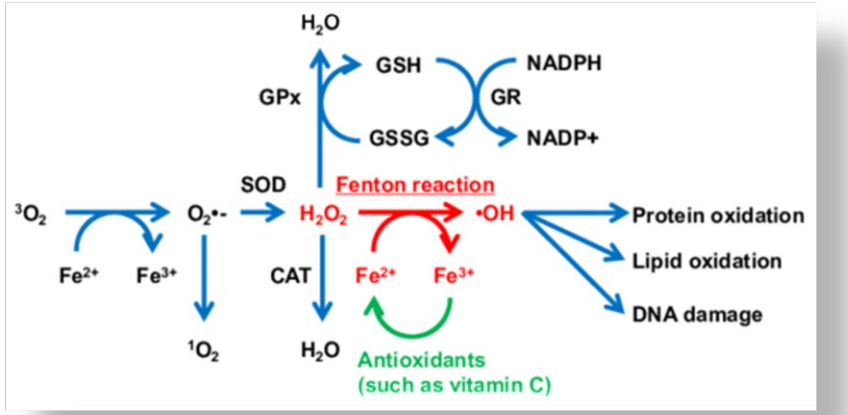
Protein Oxidation On Product Quality

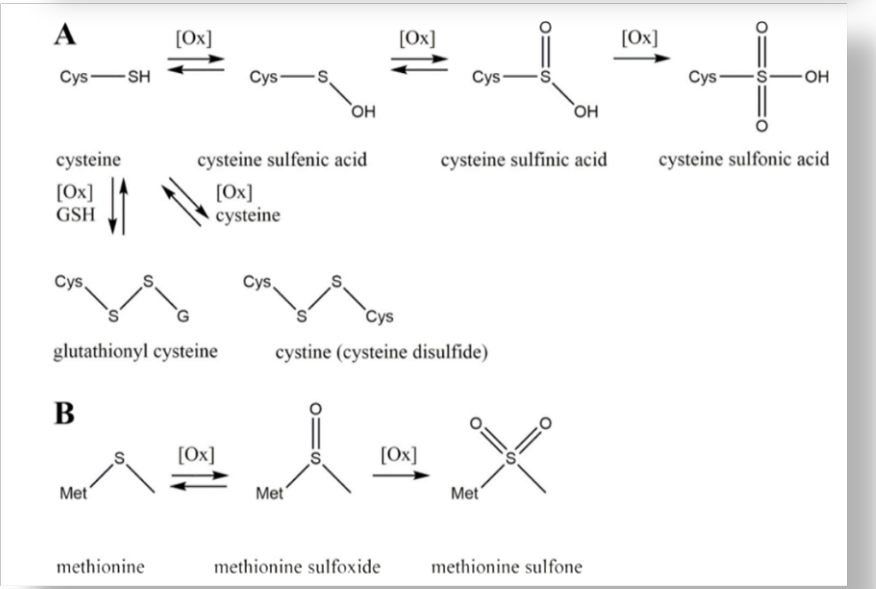
Oxidation: modification of a protein by ROS or by other oxidative stress

- **Usually occur on S-group of Cys and thio-group of Met**
- **Less often but also occur on aromatic amino acids (Tyr, Phe, Trp, His)**
- **Degrade lipids and carbohydrates to reactive intermediates**

Effect on protein quality

- **Stability**
- **Biological activity, i.e. CDC, ADCC**
- **Color**
- **Binding affinity to Protein A and FcRn (for mAb)**



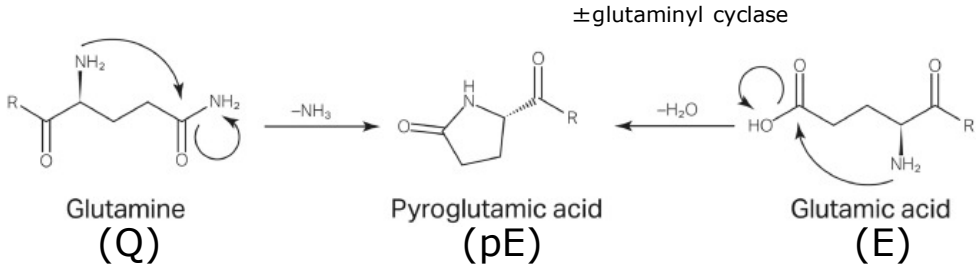


2022 Abe et al. Molecules | Free Full-Text | Current Use of Fenton Reaction in Drugs and Food (mdpi.com)
2022 Jin et al. Mass spectrometric analysis of protein deamidation – A focus on top-down and middle-down mass spectrometry - ScienceDirect

N-terminal Glu Cyclization On Product Quality

PyroGlutamate generation: N-terminal cyclization

- **Very common**
- **Occur on N-terminal Gln and Glu**
- **Rate of conversion: Q to pE > E to pE**

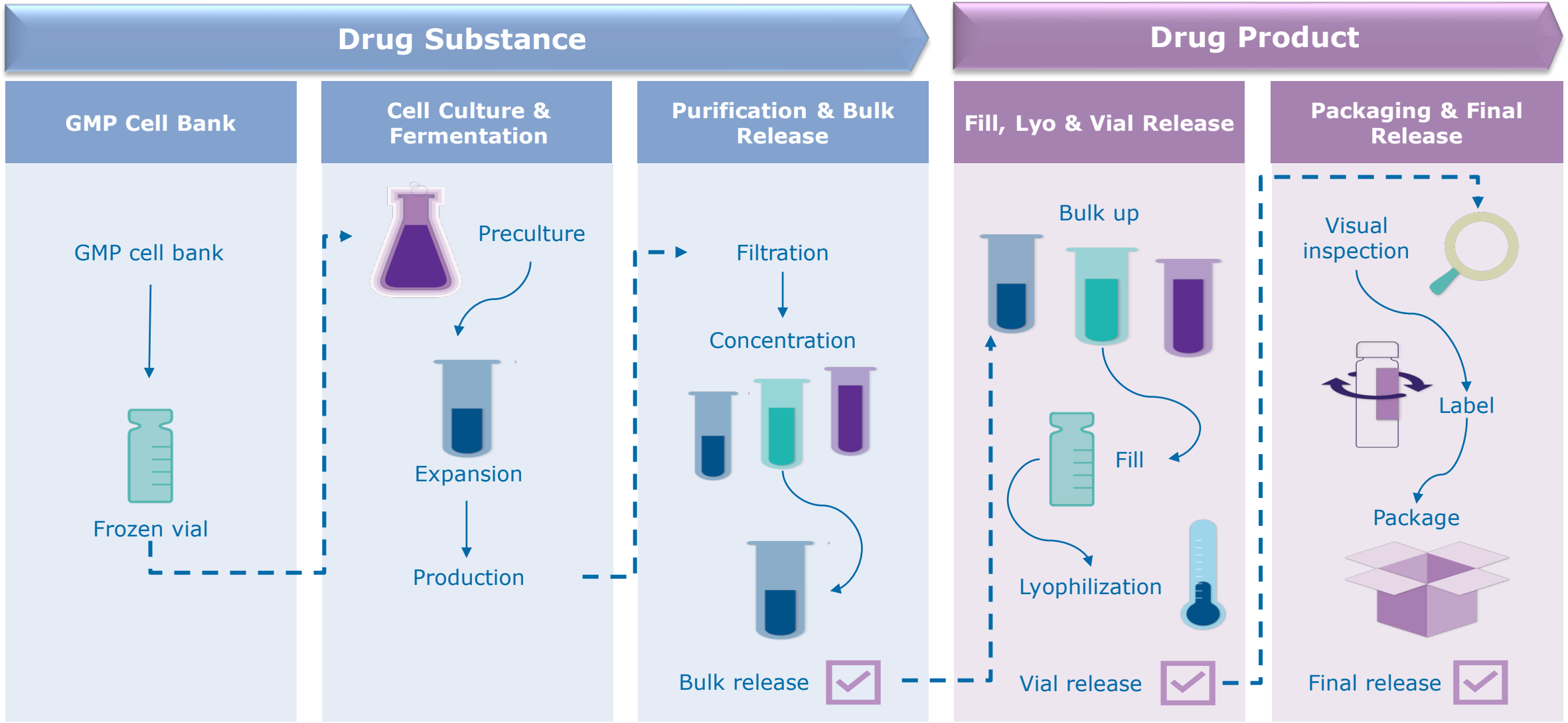


Effect on protein quality

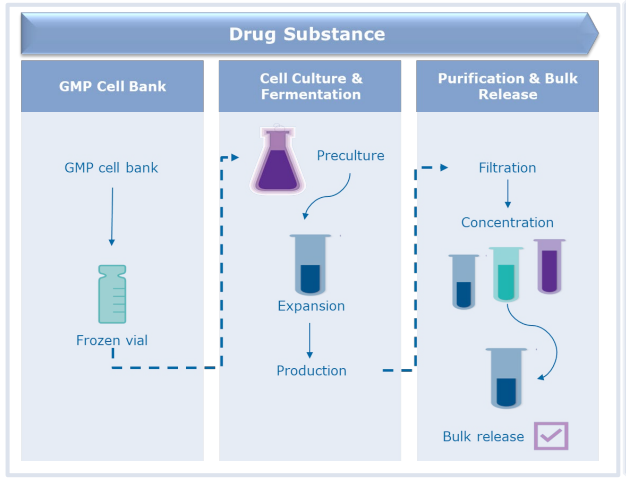
- **More acidic leading to charge heterogeneity**

2022 Cao et al. Characterization of N-Terminal Glutamate Cyclization in Monoclonal Antibody and Bispecific Antibody Using Charge Heterogeneity Assays and Hydrophobic Interaction Chromatography - Journal of Pharmaceutical Sciences (jpharmsci.org)

Protein Biologic Development & Manufacturing



Protein Biologic Development & Manufacturing – Documents



Drug Substance

GMP Cell Bank

- ❑ Traceability
- ❑ Safety
- ❑ Identity
- ❑ Clonality
- ❑ Stability
- ❑ 2-tiered cell banking

Catalent BIOLOGICS GPEx® LIGHTNING

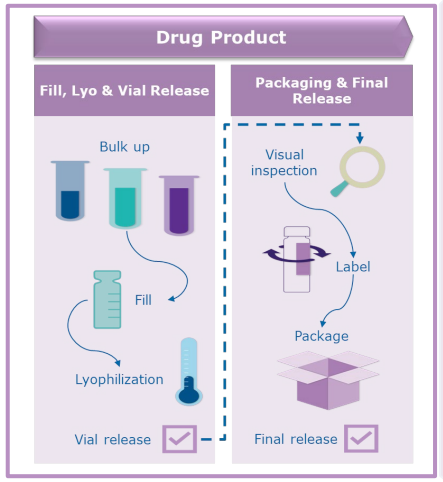
Cell Culture & Fermentation

- ❑ Scalable process
- ❑ Reproducible attributes
- ❑ Product quality profile

Purification & Bulk Release

- ❑ Scalable process
- ❑ UPB
- ❑ Viral clearance
- ❑ Reproducible attributes
- ❑ Product quality profile
- ❑ Impurity profile

Protein Biologic Development & Manufacturing – Documents



Drug Product

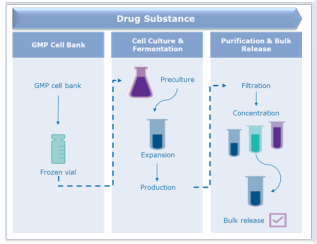
Fill, Lyo & Vial Release

- ❑ **Robust formulation**
- ❑ **Scalable process**
- ❑ **Stability profile & expiry**
- ❑ **Drug delivery formats**
- ❑ **Product quality profile**
- ❑ **Impurity profile**
- ❑ ***In-use stability & compatibility studies***

Packaging & Final Release

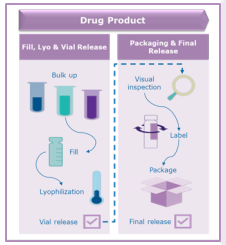
- ❑ **Inspection**
- ❑ **Labeling**
- ❑ **Packaging**
- ❑ **QP release**
- ❑ **Final release**

Protein Biologic Development & Manufacturing – Documents



Drug Substance

GMP Cell Bank	Cell Culture & Fermentation	Purification & Bulk Release
❑Traceability ❑Safety ❑Identity ❑Clonality ❑Stability ❑2-tiered cell banking	❑Scalable process ❑Reproducible attributes ❑Product quality profile	❑Scalable process ❑UPB ❑Viral clearance ❑Reproducible attributes ❑Product quality profile ❑Impurity profile



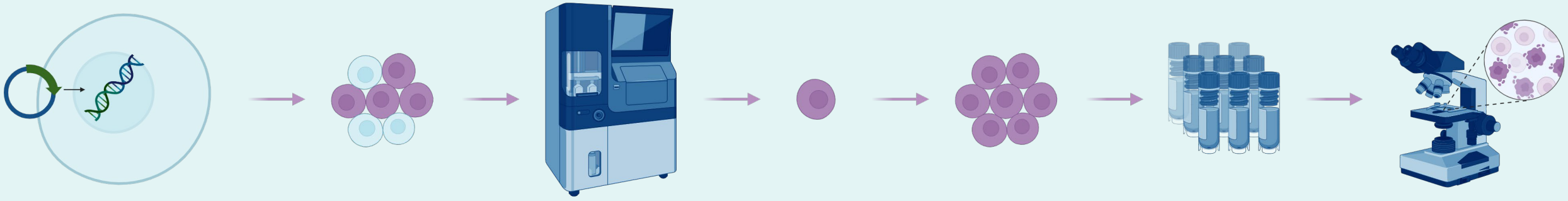
Drug Product

Fill, Lyo & Vial Release	Packaging & Final Release
❑Robust formulation ❑Scalable process ❑Stability profile & expiry ❑Drug delivery formats ❑Product quality profile ❑Impurity profile ❑*In-use stability & compatibility studies*	❑Inspection ❑Labeling ❑Packaging ❑QP release ❑Final release

Analytical

- **Raw material, in-process testing and release of product batches**
- **Stability-indicating methods/assays for product stability**
- **Characterization methods/assays for product reproducibility and comparability**
- **IND-enabling and formal stability studies for expiry**
- **Reference standards and bridging**
- **Qualify and/or validate methods/assays**

DS: Cell Line Development



1 Stable transfection

2 Pool generation

3 Clone selection

4 Single cell cloning

5 Cell expansion

6 MCB generation

7 MCB testing

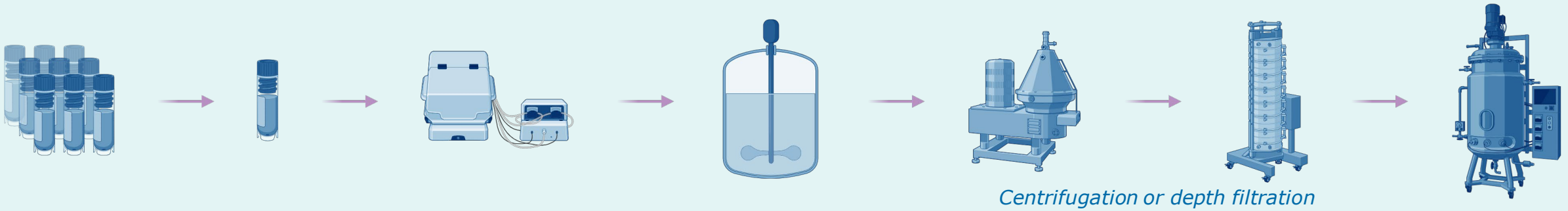
Monoclonality

Identity

Microbial contamination

Virus testing

DS: Cell Culture Development & Manufacturing



1 MCB

2 Cell bank vial thaw

3 Cell expansion

4 Production

5 Clarification

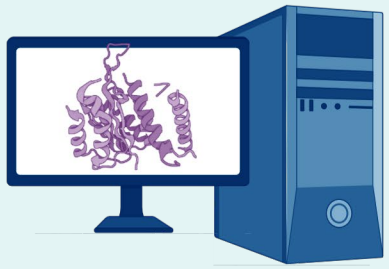
5 Clarified Bulk

Unprocessed bulk testing
Microbial contamination
Virus testing

Product yield
Turbidity

A-Mab a case study in bioprocess development, 2009

DP: Formulation Development



2 *In silico* modeling

- 3D model construction
- Surface batches
- Post translational modifications
- Hotspot assessment

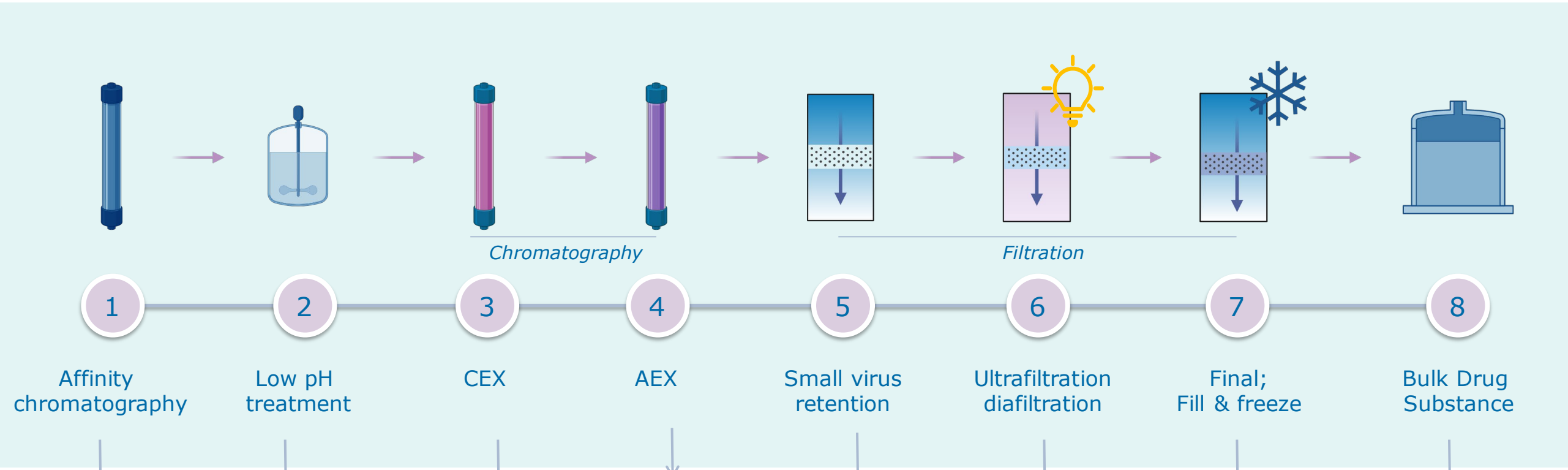
4 Development

- Colloidal stability
- Conformational stability
- Physical stability
- Chemical stability

5 Decision

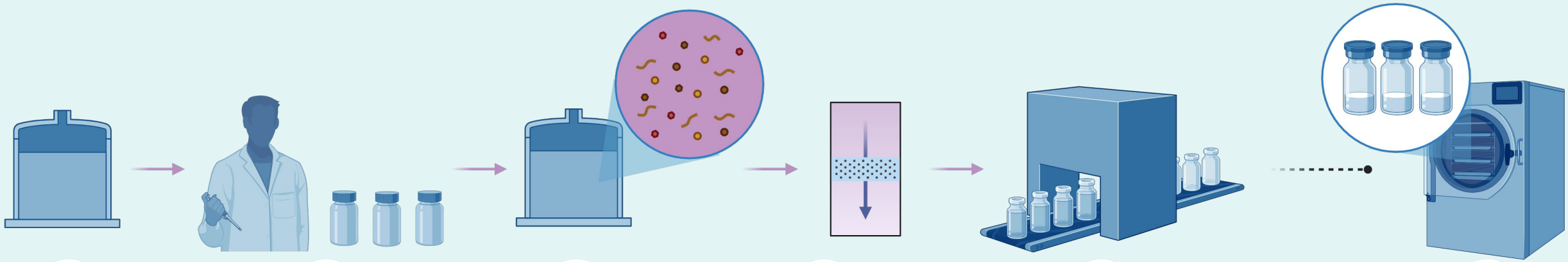
- Product quality profile

DS: Purification Development & Manufacturing



A-Mab a case study in bioprocess development, 2009

DP: Development & Manufacturing



1 DS preparation & handling

2 Component preparation

3 Compounding

4 Sterile filtration

5 Filling, stoppering & capping

- Physical stress
- Chemical stress
- Solution homogeneity

6 Lyophilization

- Moisture content
- Reconstitution time

A-Mab a case study in bioprocess development, 2009

Typical DS And DP Release Panels For Mab

Attributes	Test	DS	DP
Safety	Bioburden	X	
	Endotoxin	X	X
	Sterility	X	X
General	Appearance (color/clarity)	X	X
	pH	X	X
	Concentration	X	X
Identity	Peptide mapping (LC-UV)	X	X
Identity (purity, charge species)	IEX-HPLC/IEF/cIEF/CZE	X	X
Purity (monomer, aggregate)	R/NR CE-SDS	X	X
	SEC-HPLC	X	X
Potency	Binding ELISA	X	X
	Cell-based assay	X	X
	Effector functions*	X	X
Glycosylation (glycan species)	NP-HPLC	X	X
Impurities	HCP	X	
	Residual DNA	X	
	Residual Protein A	X	
Particulate	HIAC		X
Moisture content**			X

ICH Q6B
2017 Ambrogelly et al. Full article: Analytical comparability study of recombinant monoclonal antibody therapeutics (tandfonline.com)

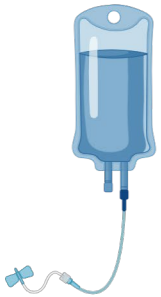
*Related to MoA
**For lyophilized DP

Developability Assessment

Property	mAb 1	mAb 2	mAb 3	mAb 4
Calculated pI				
Conformational Stability				
Colloidal Stability				
Hydrophobicity				
Aggregation & Purification experience				
PTMs assessment (in silico + MS)				
Charge Variants				
Solubility				
Serum compatibility				
Viscosity (incl. excipient test)				
Degradation / Truncation				
CHO Expression				
In vivo fitness				
Pre-Formulation (thermal stress)				
Pre-Formulation (mechanical stress)				
Cumulative Risk				

Developability Assessment of Biologics by Integrated Biologics Profiling | American Pharmaceutical Review - The Review of American Pharmaceutical Business & Technology

Concentrations



IV

SC

Inhaled

Stability

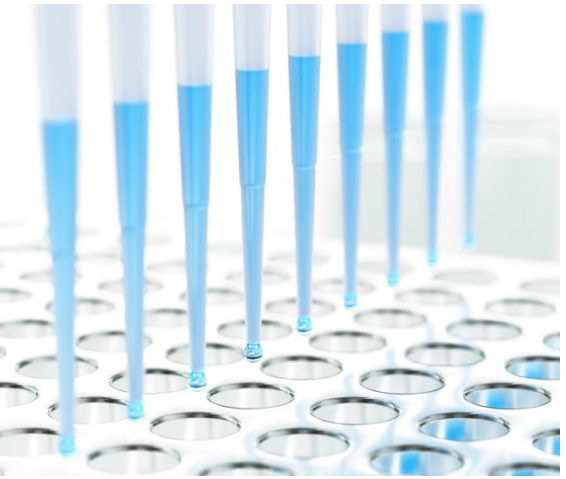
Liquid

Lyophilized

Spray dry?

Summary

- Biologics are sensitive to temperature, pH, light, agitation and enzymatic degradation.
- PTMs for biologics can affect potency and stability.
- PTMs are sensitive to RM and process conditions.
- In-process tests are critical to product consistency.



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BIOLOGICS

THANK YOU!

more products. better treatments. reliably supplied.™