Quality Control in Biotechnology

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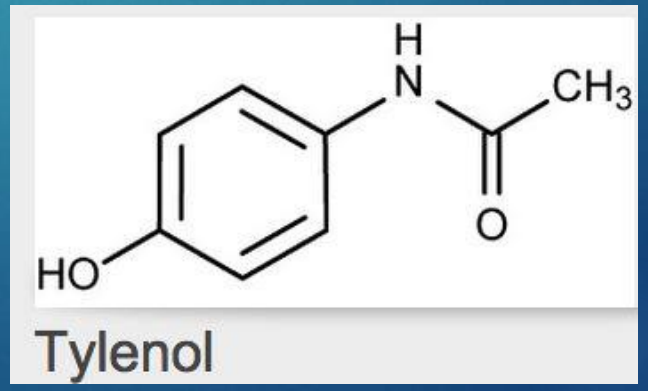
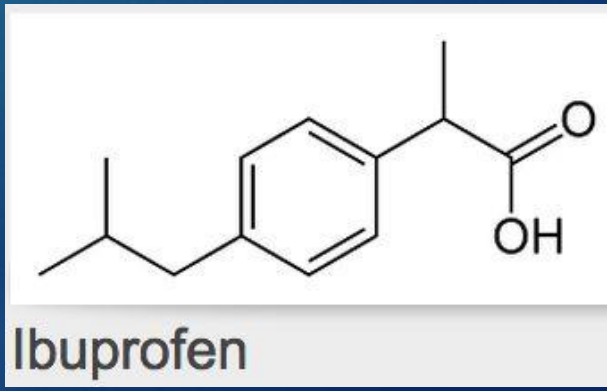
Chemical drugs vs Biologicals

- ***Chemical drugs*** can be precisely defined
  - Physical chemical characterization
    - NMR- structure
    - Mass Spectrometry- molecular weight
    - Chromatography- purity, quantity
  - Potency
  - Formulation

- Relatively easy to create "generics"



Tylenol



Ibuprofen

Chemical drugs vs Biologicals

- ***Biologicals*** are produced by living cells
- Impossible to
  - control every variable
  - completely characterize
  - Precisely replicate
- Traditionally, biologicals are defined by "product by process"
  - Product is defined by the manufacturing process
  - Quality is compliance-driven
- Goal is a "well-characterized biological"

current Good Manufacturing Process
cGMP

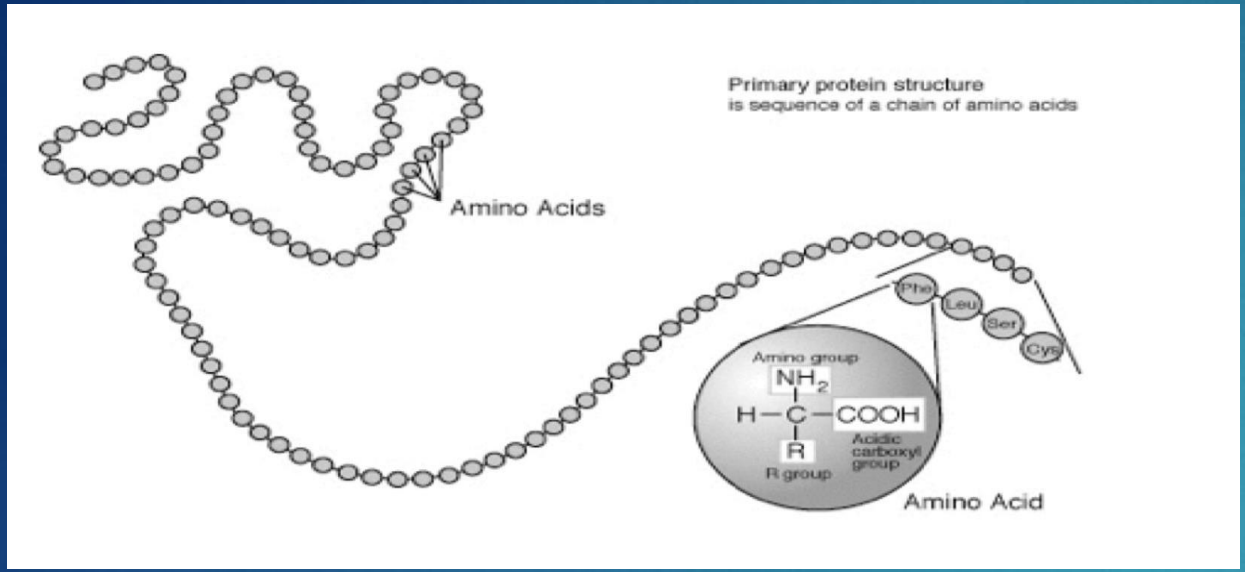
- Doing what you said you were going to do
- Proving that you did what you said
- Documenting that you did it.
- Following SOPs
- Validated methods

Examples of Biologicals

- Insulin
- EPO
- Interferons
- Toxins
- Antibody
- Conjugates
  - Antibody-drug conjugates
  - Fusion proteins

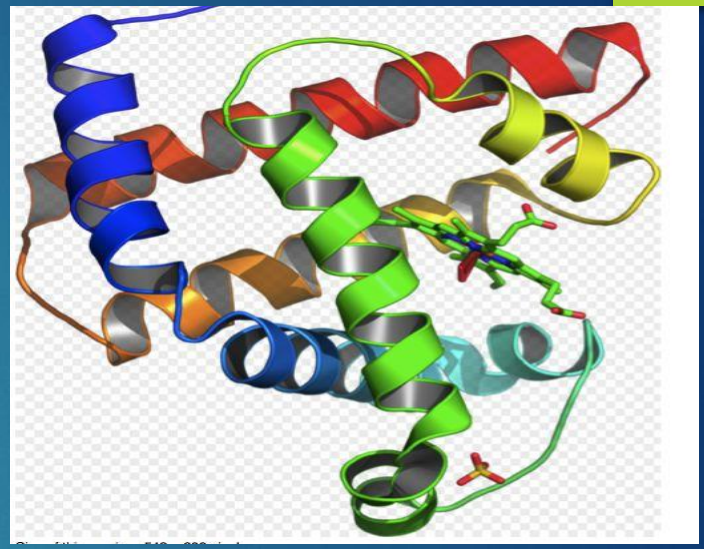
Protein Structure

Primary Structure:
Amino acid sequence



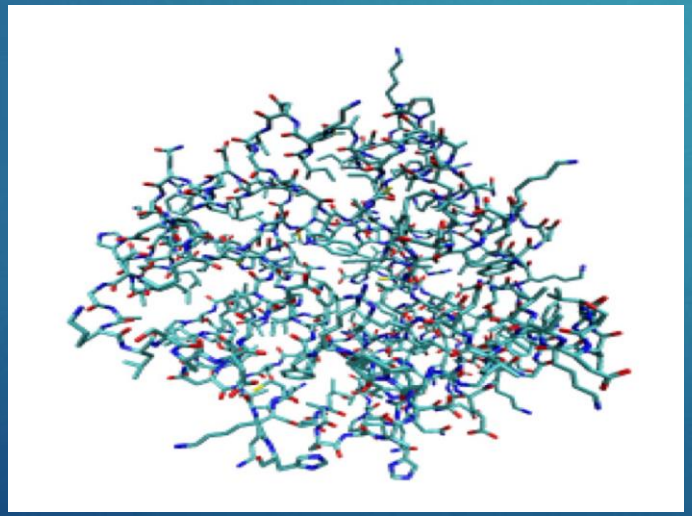
Secondary Structure:

3-dimensional structure of segments



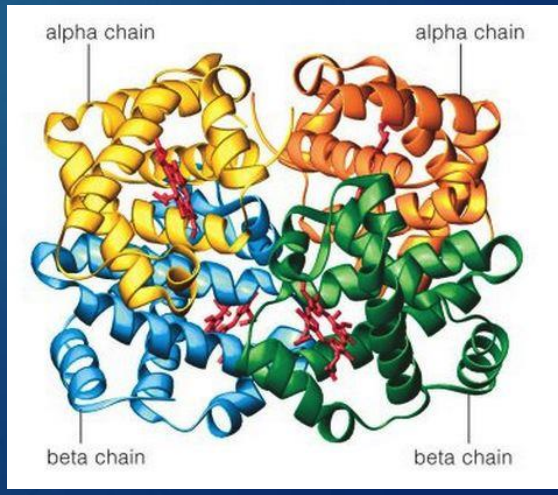
Tertiary structure:

Final specific geometric shape that a protein assumes

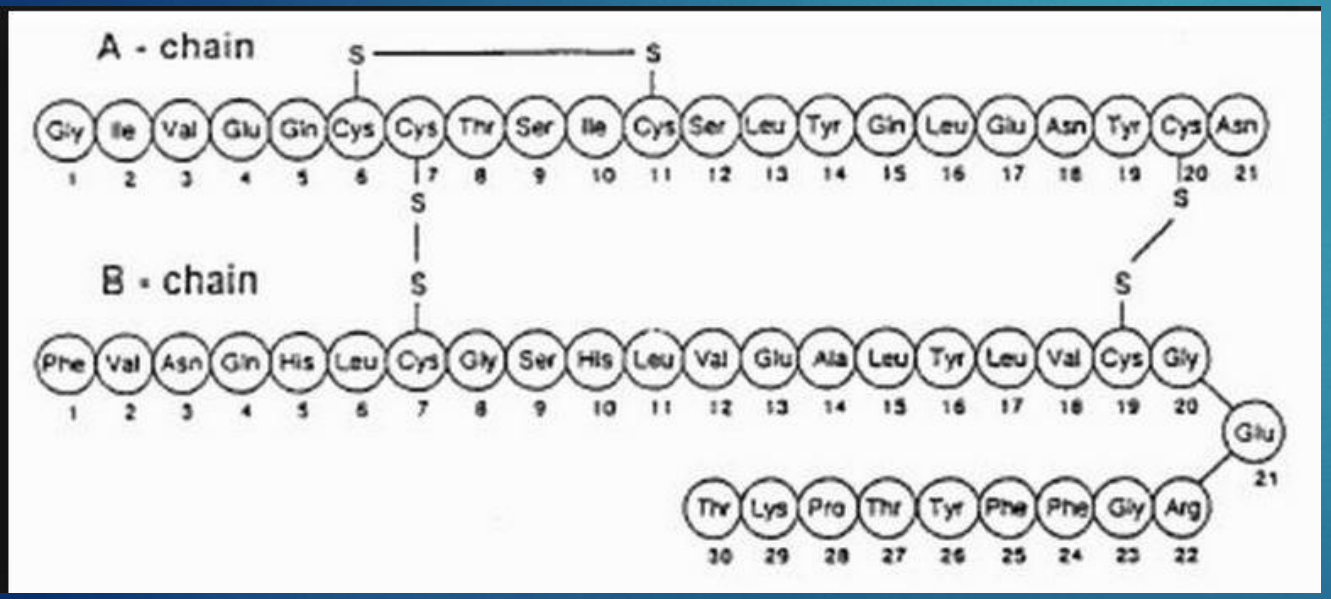


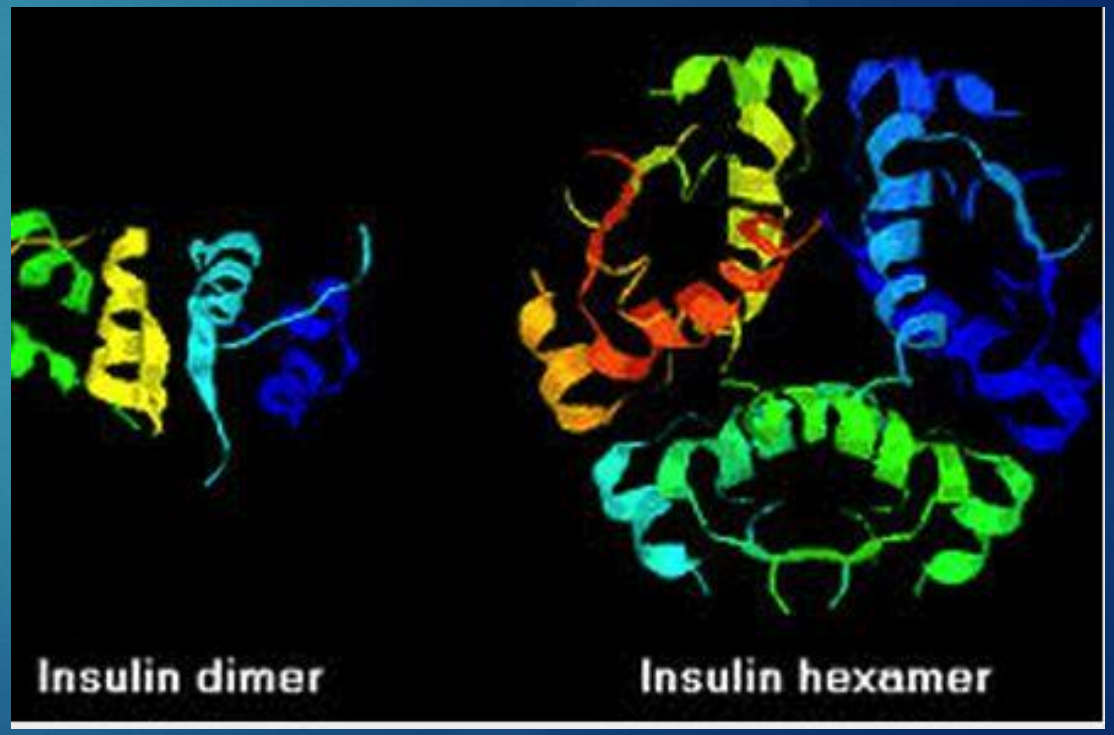
Quaternary structure:

Arrangement of multiple folded protein or coiling protein molecules in a multi-subunit complex.

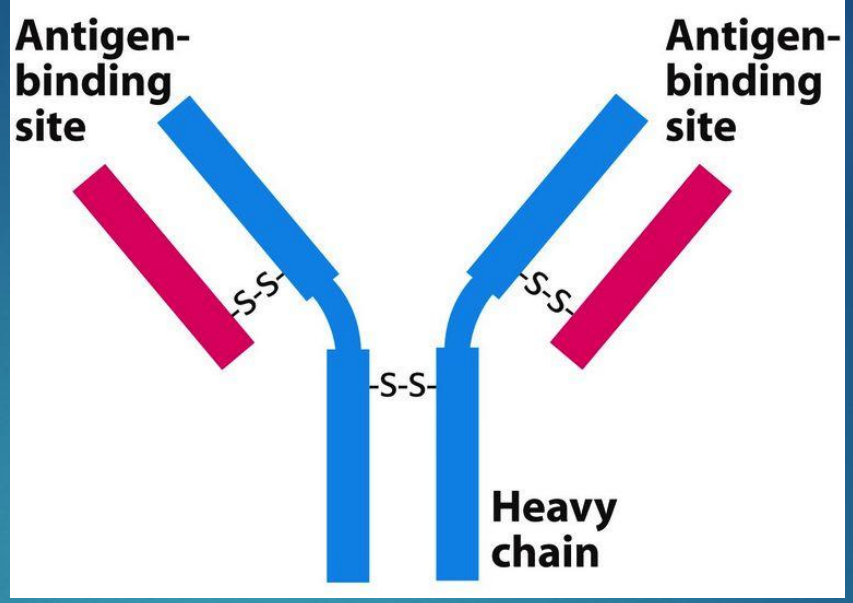


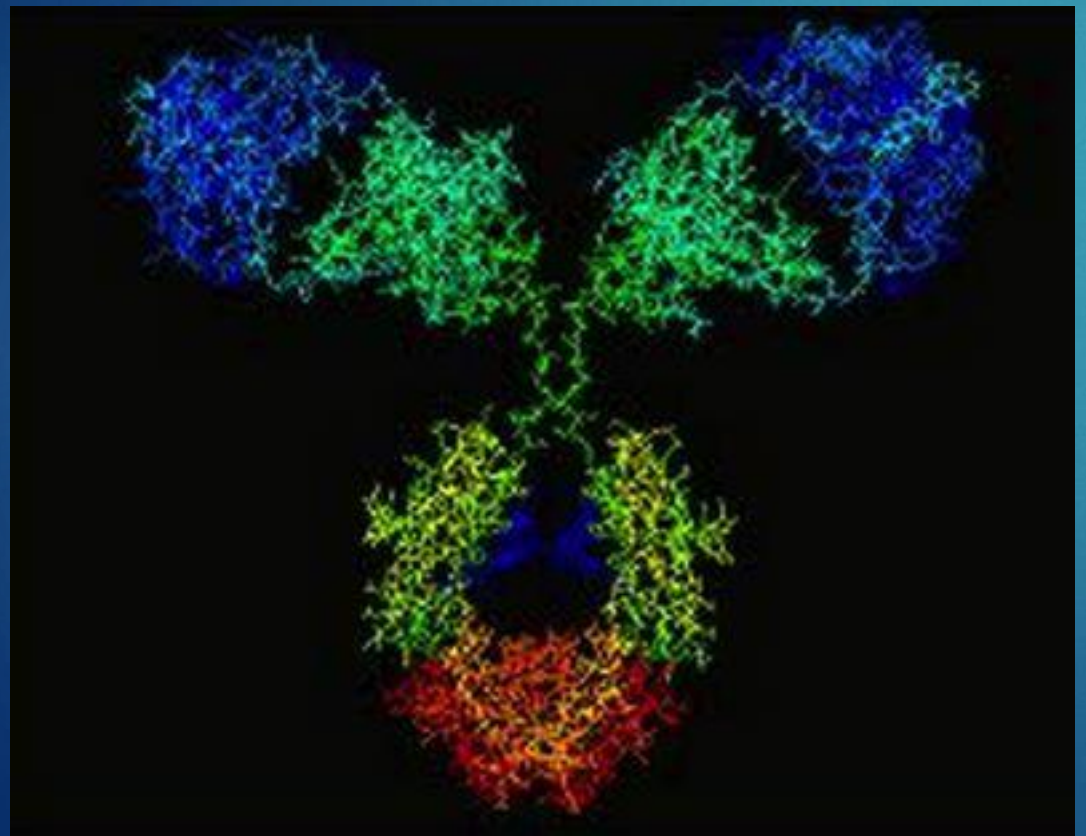
Insulin

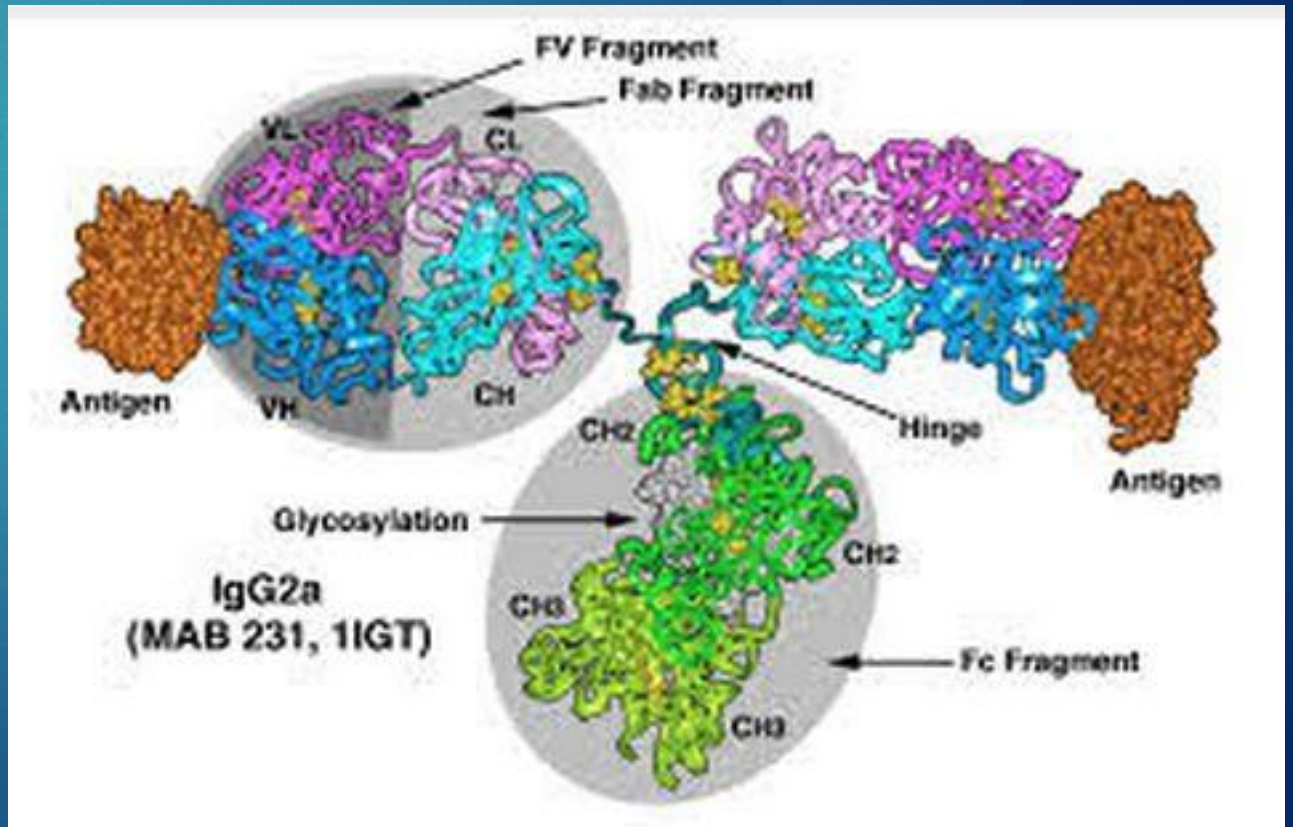

A - chain
S
S
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
S
S
B - chain
S
S
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Glu
21
Thr Lys Pro Thr Tyr Phe Phe Gly Arg
30 29 28 27 26 25 24 23 22


Insulin dimer
Insulin hexamer

Antibody


Antigen-binding site
Antigen-binding site
-S-S-
-S-S-
-S-S-
Heavy chain

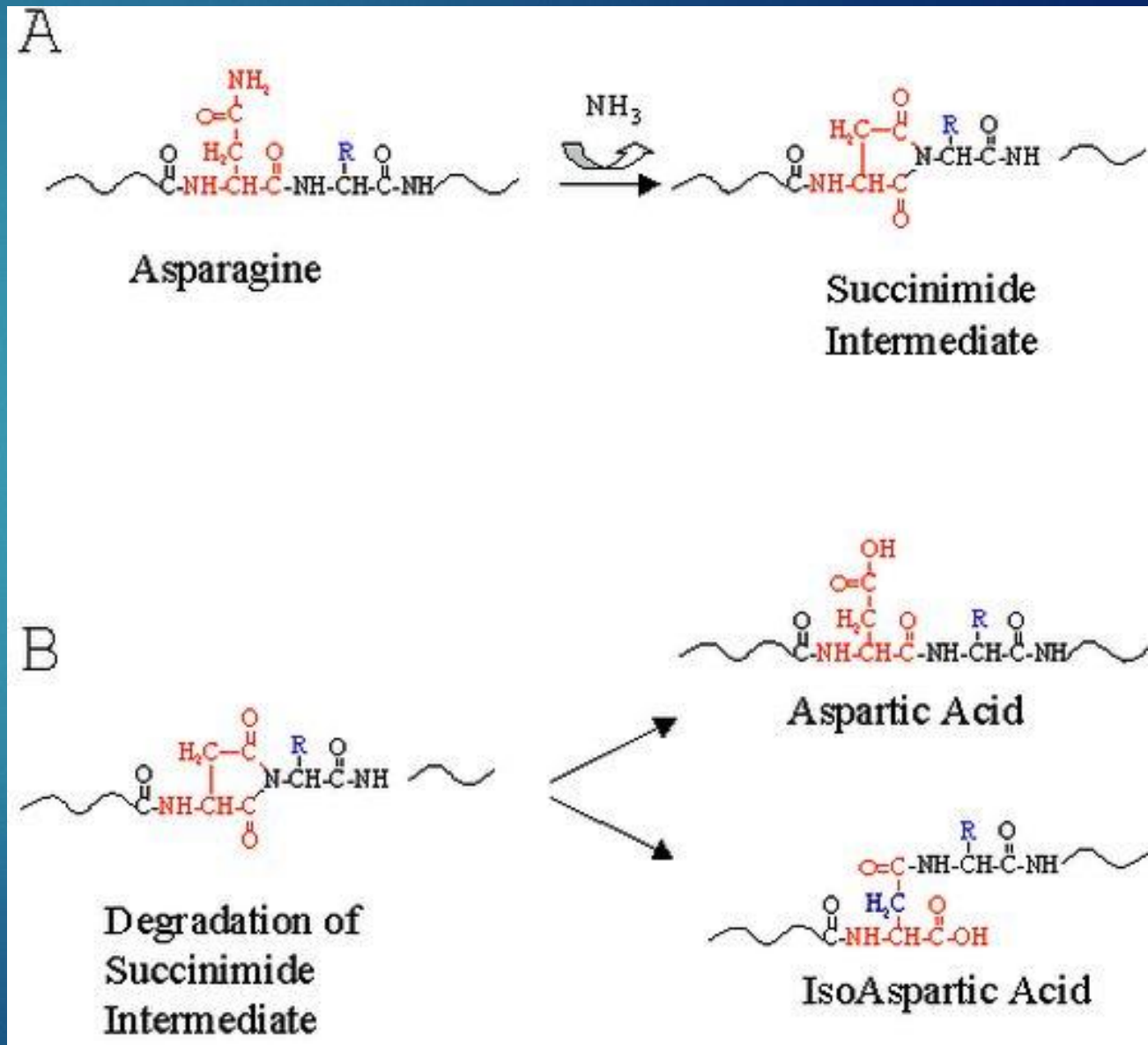



FV Fragment
Fab Fragment
VL
CL
Antigen
VH
CH
Hinge
CH2
Antigen
Glycosylation
IgG2a
(MAB 231, 1IGT)
CH2
CH3
Fc Fragment
CH3

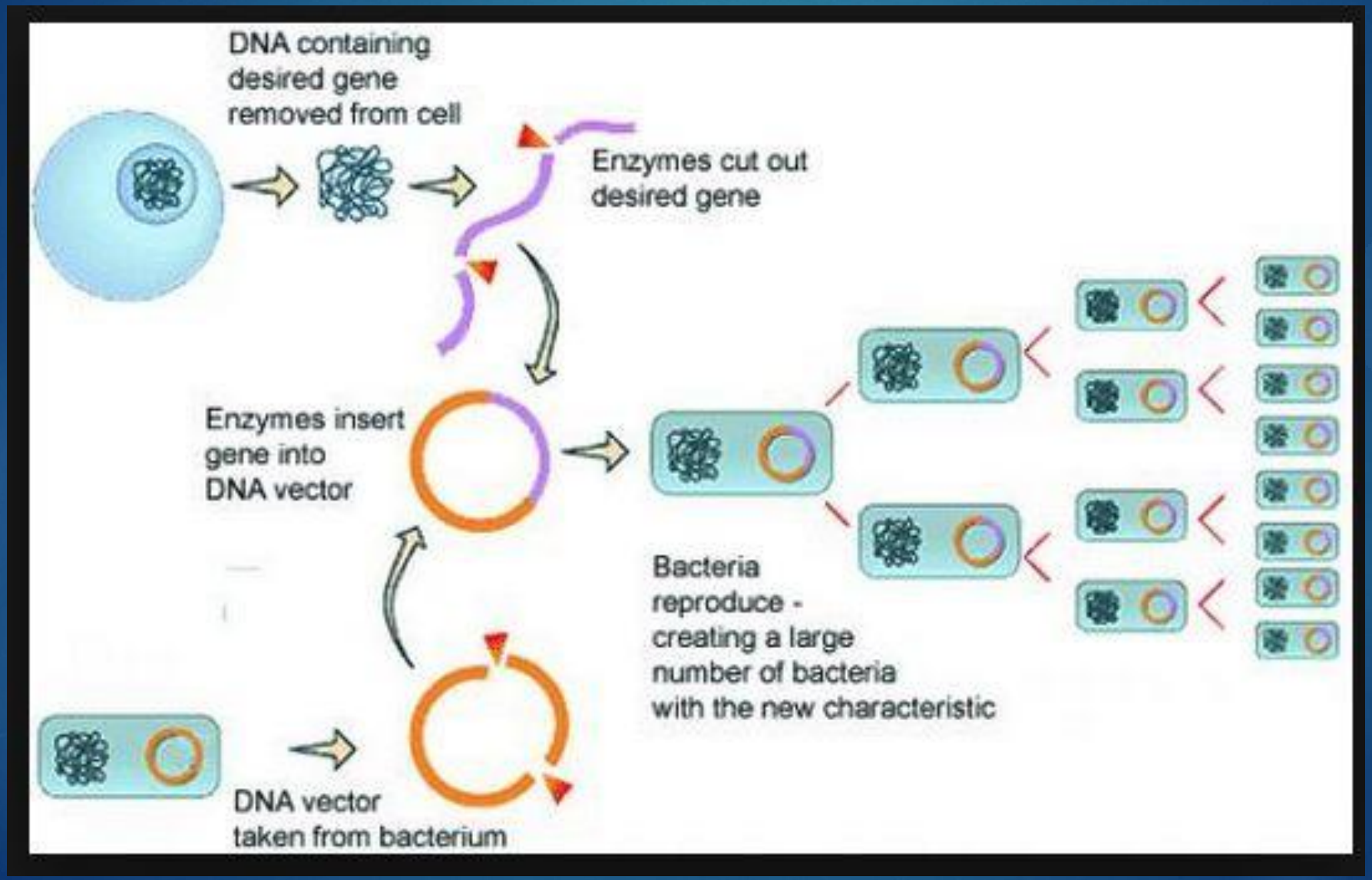
Modifications

- ***Post-translation modifications***
  - Chemical changes not directly coded in DNA
    - Glycosylation
    - Phosphorylation
    - Lipidation

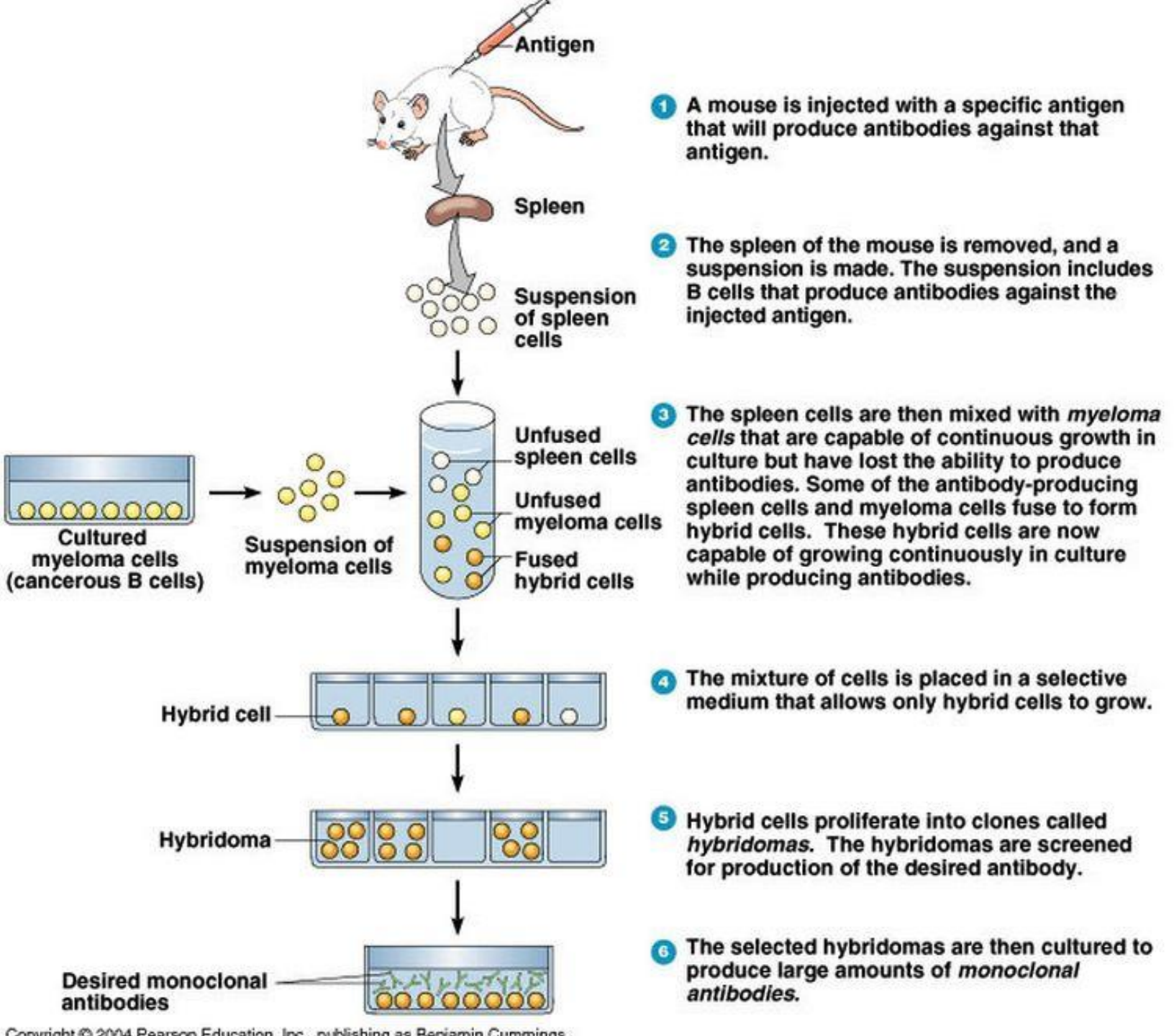
- ***Chemical degradations***
  - Oxidations
  - De-amidation
  - Rearrangements



Genetic Engineering (bacterial)



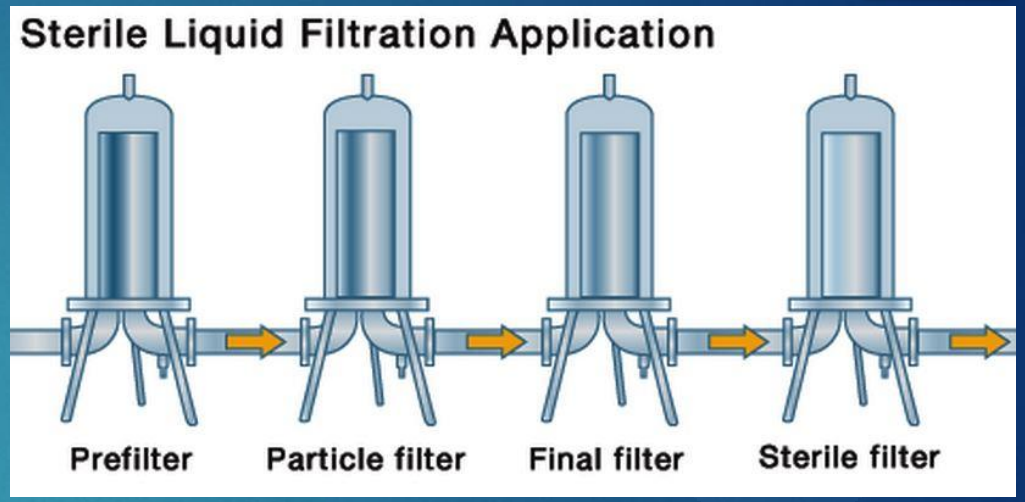
Monoclonal Antibodies



Bioprocessing Unit Operations

Fermentation

Centrifugation

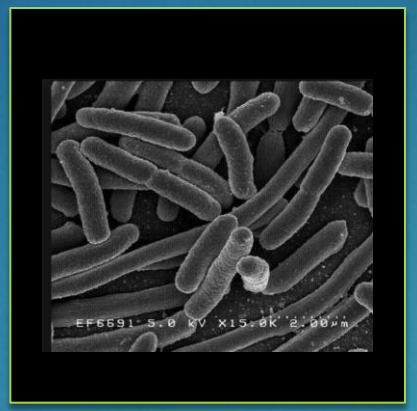


Chromatography

Variable inputs

Biologically derived
Chemically derived

Inherent heterogeneity
Stochastic processes



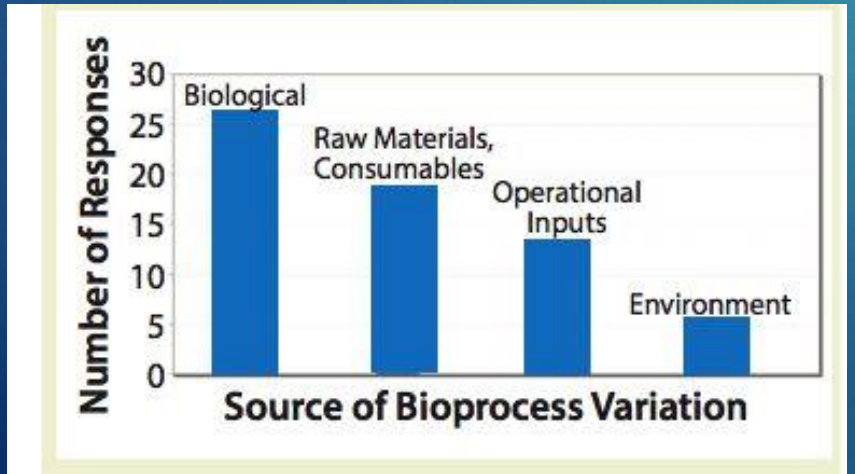
Cells
"black box"

Process variables

Variable
crude product

Heterogeneous
product

Formulation

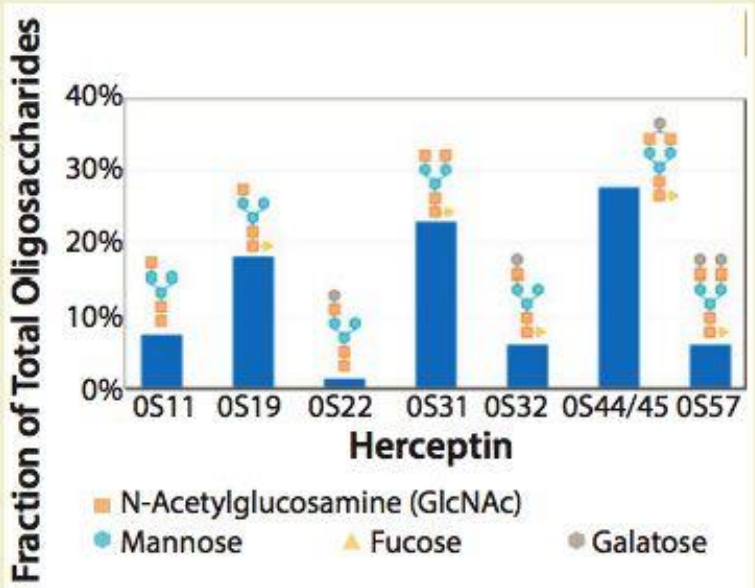


Herceptin

(anti-cancer antibody)

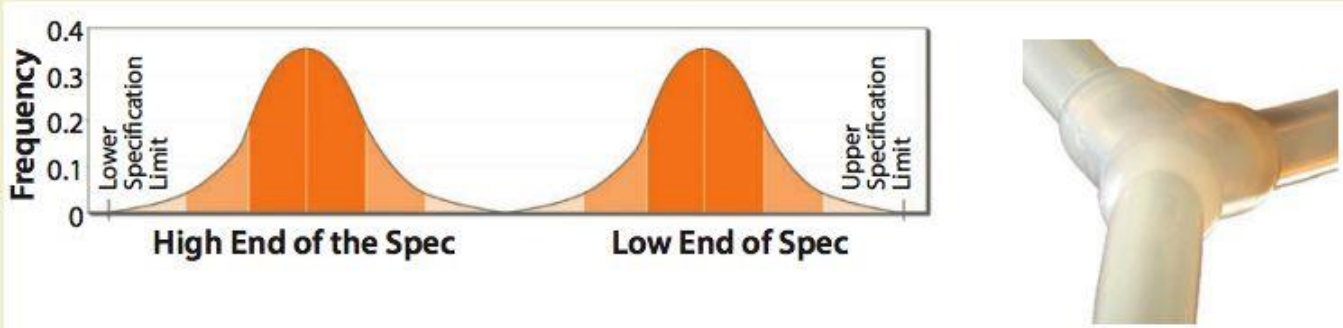
Seven different glycoforms, each with different levels of biological acitivity.

Figure 1: Experimental oligosaccharide profiles for Herceptin (recreated from "Application of Quality by Design Paradigm to the Manufacture of Protein Therapeutics," by del Val et al.)



Variability of materials

Figure 3: Original tubing diameter populations provided by the supplier for manufacturing overmolded manifolds (from Parker dominick hunter)



Product by process

Lot release criteria- pass/fail
Very difficult to make process improvements

Quality by Design (QbD)

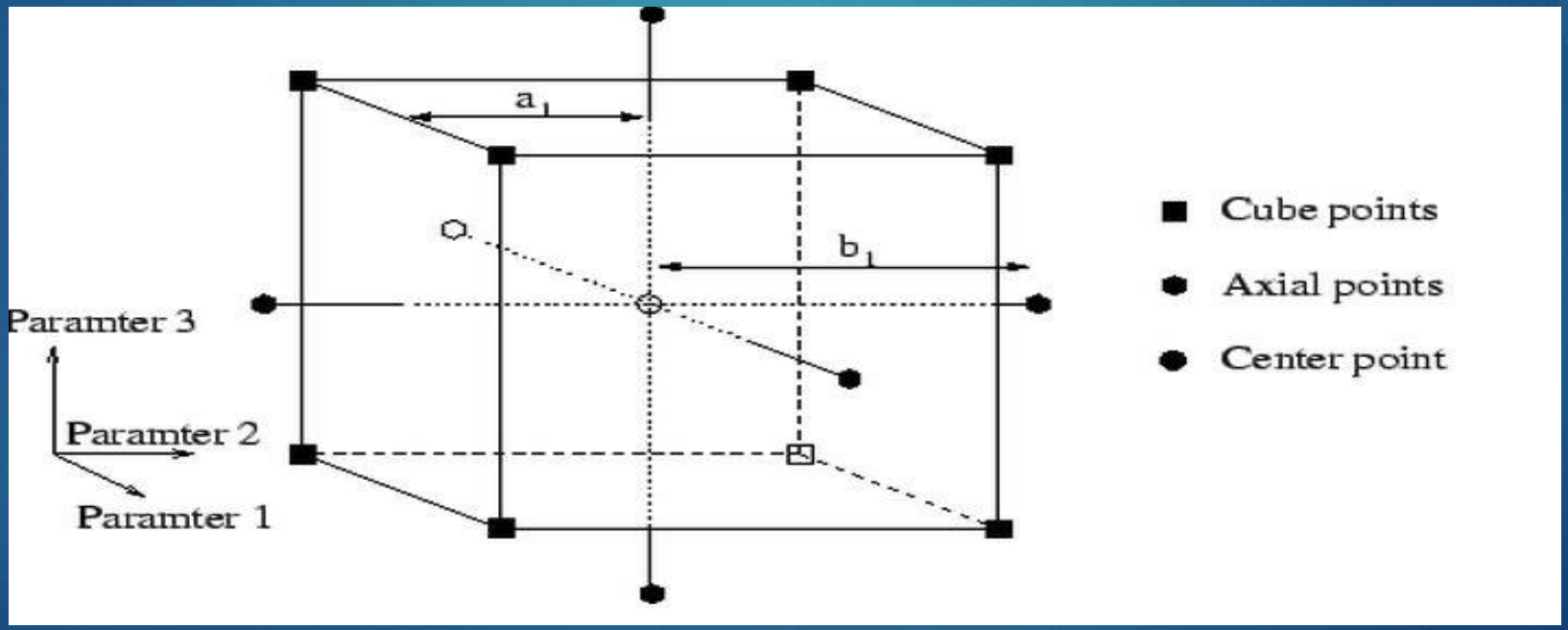
Operate within a specified design space
Target Product Profile
Critical Quality Attributes
Critical Product Attributes

Better understanding of process and product
Defining design space
Allows for more variability

Process Analytical Technologies
Real time monitoring & feedback.

Understanding the operating space

Design of Experiment



Design of Experiment (DOE)

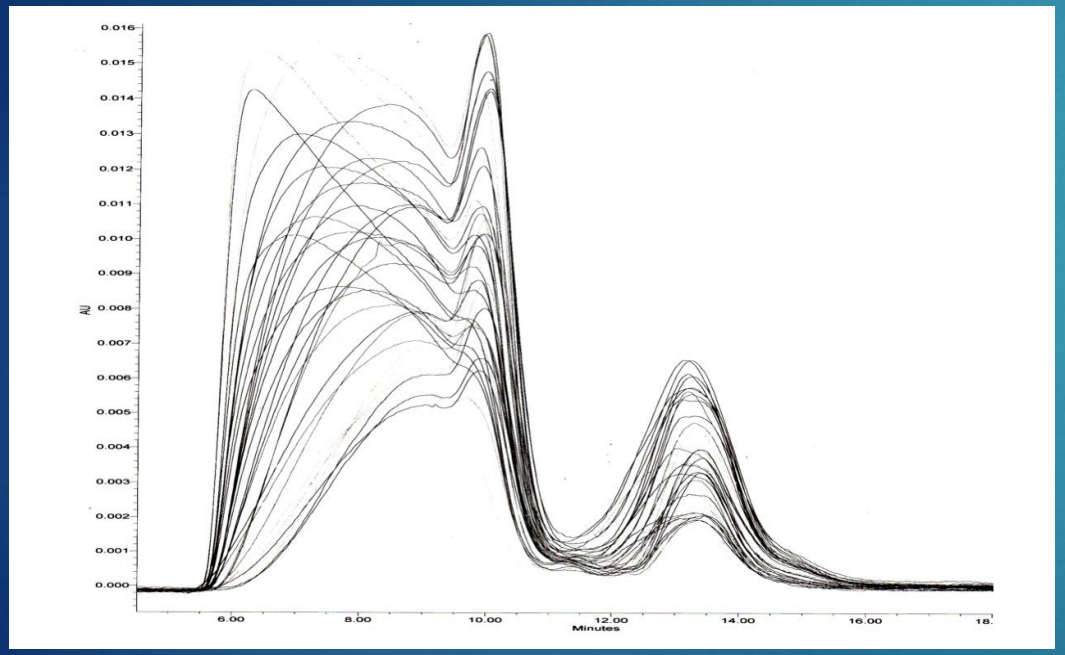
- *A statistical method to model a process*
- *Many fewer experiments than "one factor at a time"*
- *Allows for modeling interactions*
- *Useful to determine critical parameters*
- *Critical for "Quality by Design"*

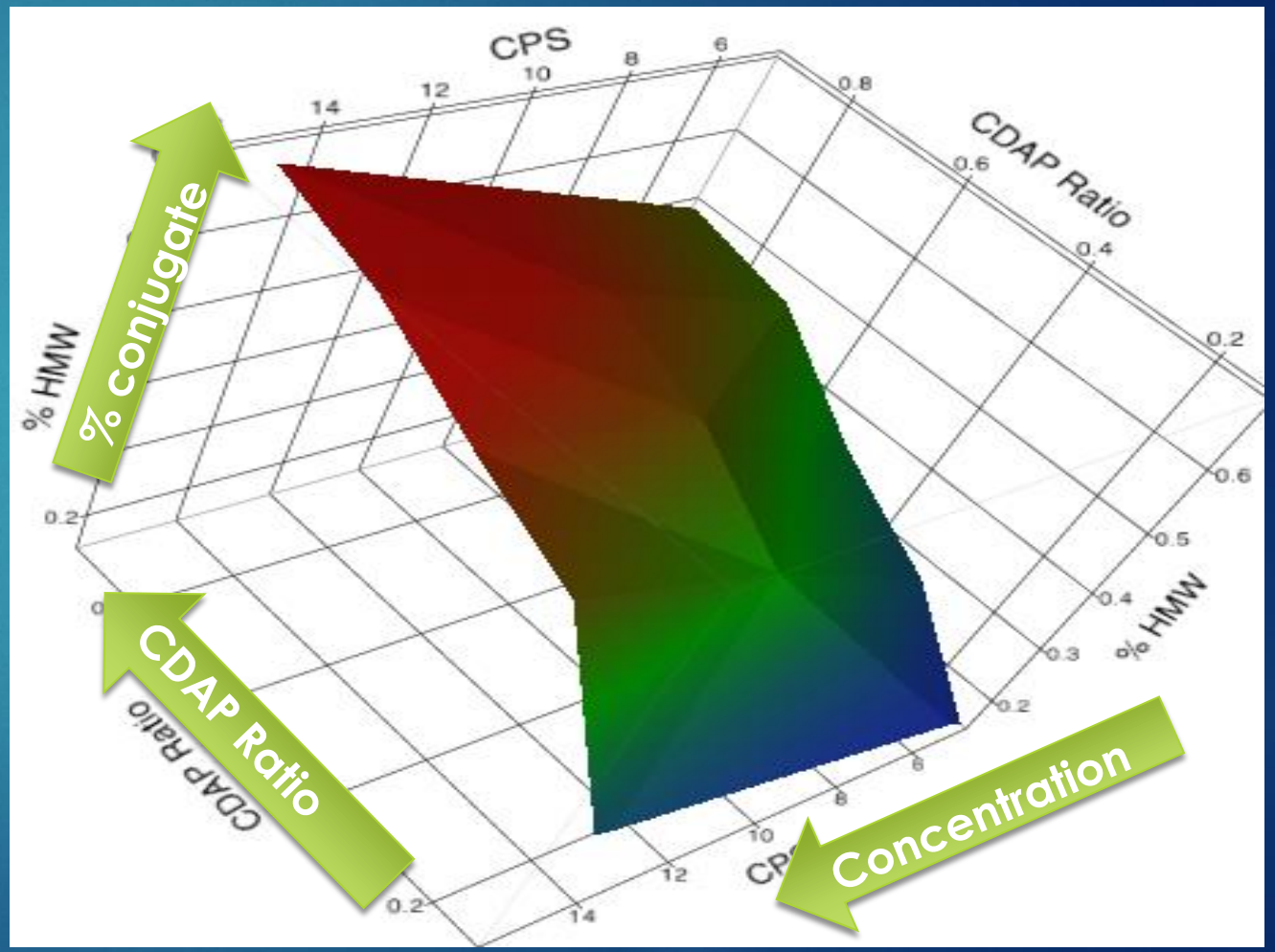
Run	CDAP	pH	CPS	Pro/PS
1	+	0	+	+
2	+	0	+	0
3	+	0	+	-
4	+	0	0	+
5	+	0	0	0
6	+	0	0	-
7	+	0	-	+
8	+	0	-	0
9	+	0	-	-
10	0	0	+	+
11	0	0	+	0
12	0	0	+	-
13	0	0	0	+
14	0	0	0	0
15	0	0	0	-
16	0	0	-	+
17	0	0	-	0
18	0	0	-	-
19	0	++	+	+
21	0	++	+	-
25	0	++	-	+
27	0	++	-	-
28	-	0	+	+

Run	CDAP	pH	CPS	Pro/PS
29	-	0	+	0
30	-	0	+	-
31	-	0	0	+
32	-	0	0	0
33	-	0	0	-
34	-	0	-	+
35	-	0	-	0
36	-	0	-	-
37	0	--	+	+
38	0	--	+	-
39	0	--	-	+
40	0	--	-	-
41	0	-	+	+
42	0	-	+	-
43	0	-	-	+
44	0	-	-	-
45	++	0	+	+
46	++	0	+	-
47	++	0	-	+
48	++	0	-	-
49	--	0	+	+
50	--	0	+	-
51	--	0	-	+
52	--	0	-	-
53	0	+	+	+
54	0	+	+	-
55	0	+	-	+
56	0	+	-	-

Parameter	++	+	0	-	--	Units
CDAP Ratio	0.9	0.7	0.5	0.3	0.1	mg/mg
CPS	-	15	10	5	-	mg/mL
Pro/PS	-	1.25	1	0.75	-	mg/mg
pH	9.5	9.25	9	8.75	8.5	pH

Design of Experiment

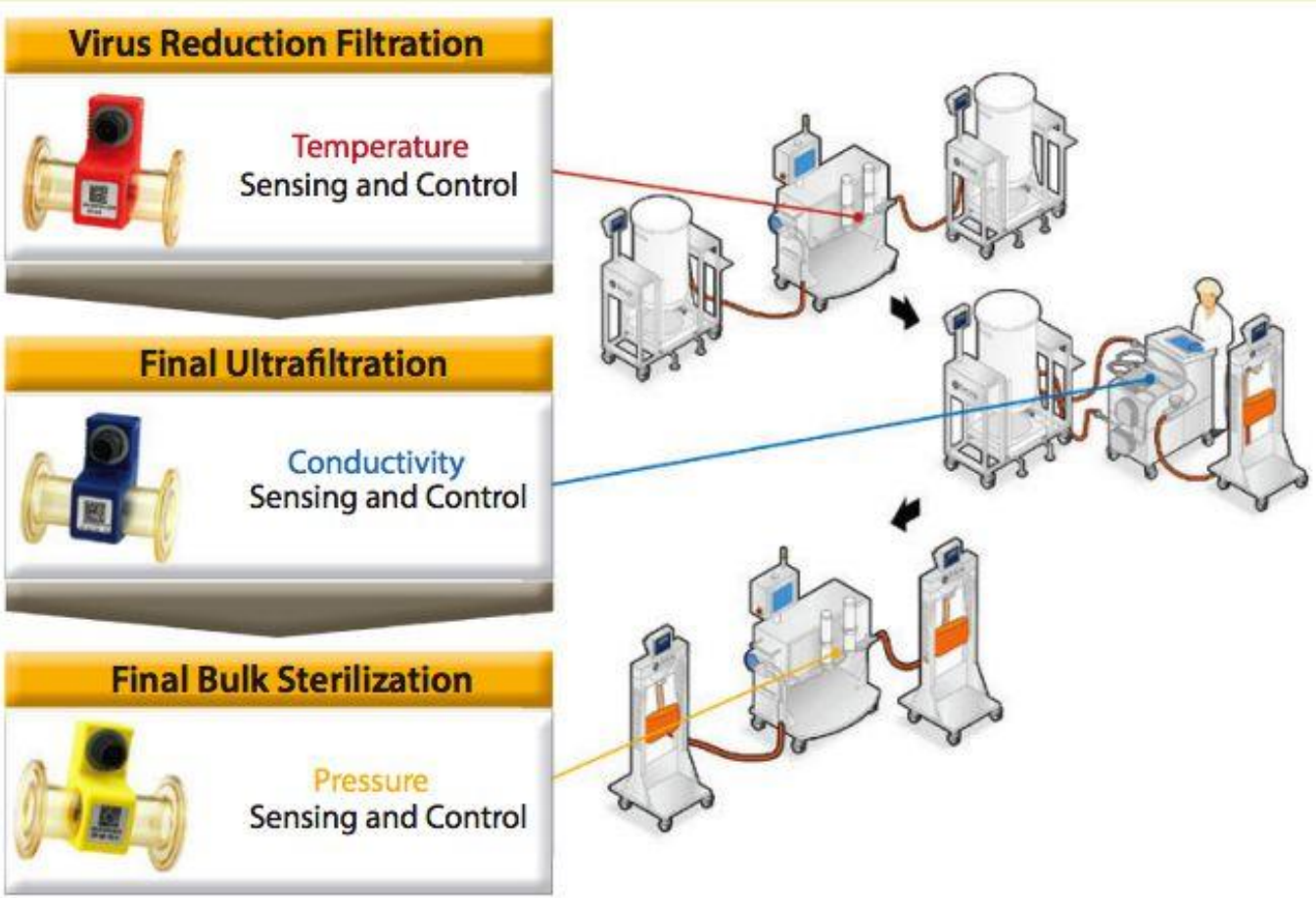




Process Analytical Technologies (PAT)

Real time feedback to control process

Figure 5: The role of process analytical technologies in controlling three unit operations used in biopharmaceutical purification



Types of Vaccines

- ***Subunit (protein) vaccines***
  - Tetanus toxoid
  - Diptheria toxoid
  - Pneumococcal (PneumoVax)

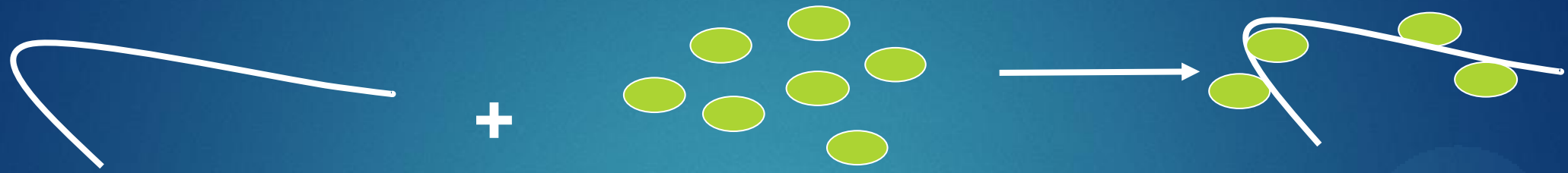
- ***Killed vaccines***
  - Rubella, Measles,
  - Polio (Salk)
  - Flu
  - Hepatitis A

- ***Conjugate vaccines***
  - Pneumococcal (Prevnar)
  - Meningicoccal (Menactra)
  - Haemophilus b (Hib)

- ***Live attenuated vaccines***
  - Polio (Sabin)
  - Flu (Flumist)
  - Rotavirus (Rotarix)
- ***Virus Like Particles (VLP)***
  - HPV (Gardisil)
- ***New Generation Vaccines***
  - DNA vaccines

Polysaccharide Vaccine

Conjugate Vaccine



Polysaccharide

Protein

- Poorly immunogenic in infants
- No boosting or memory
- No class switching
- No affinity maturation

- Immunogenic in infants
- Boosting and memory
- Class switching
- Affinity maturation

Conjugate Vaccines are Effective

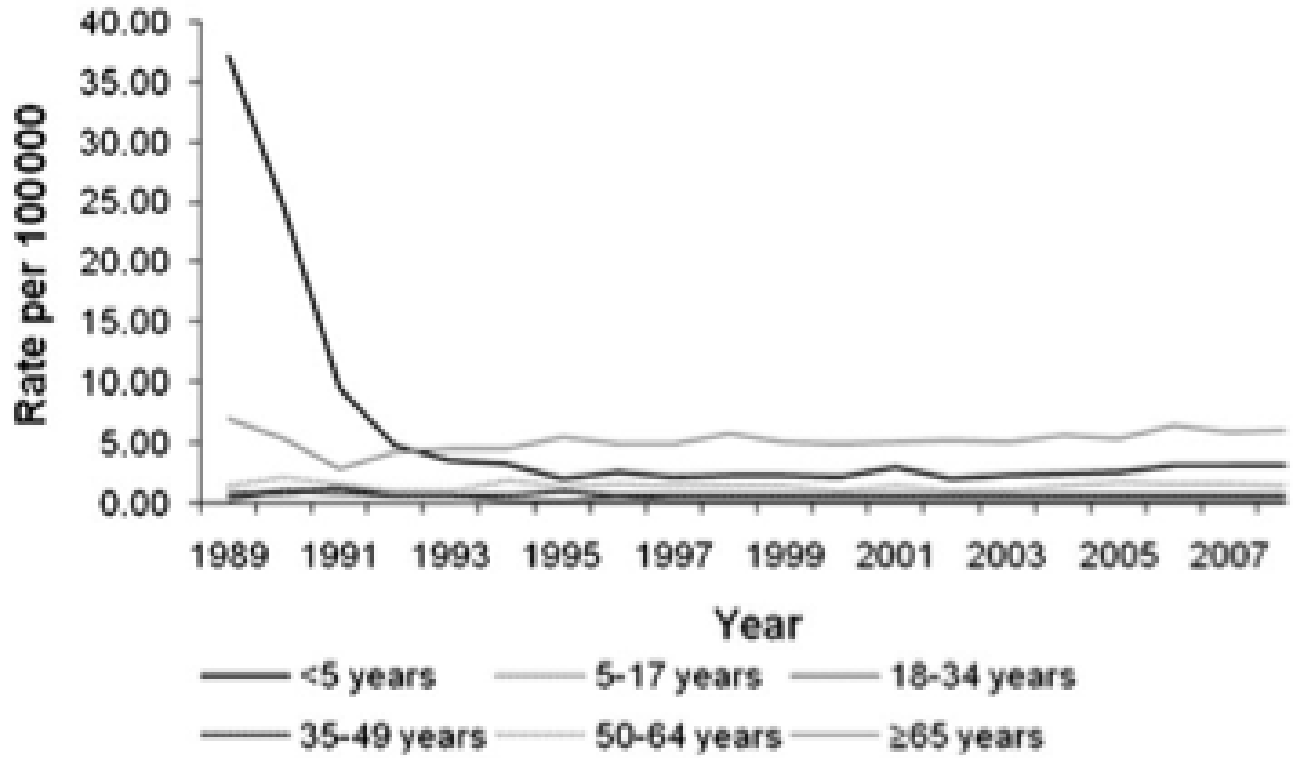


Figure 1. Trends in incidence of invasive *Haemophilus influenzae* disease, by age group—United States, 1989–2008.

Why Conjugate Vaccines?

Haemophilus influenzae b (Hib)

Neisseria meningiditis

Streptococcus pneumoniae

Salmonella typhi

Expensive

Challenging to manufacture

Many serotypes

TETANUS TOXOID PRODUCTION

Cl. tetani

HARVARD STRAIN

Revival On ATG medium

48 hours at 37 °C

INTERMEDIATE SEED HIGB (18 -32 hrs)

Inculcation into production

Medium (Muller &Miller) (PH – 7.5 ± 0.1)

FERMENTOR 35 ± 5°C ANAEROBIC CULTURE

5th Day sample collection for SMEAR, pH, growth lysis

CULTURE HARVEST 6\7 days

- SMEAR
- PH
- LF/ML

Millipore Filter Clarification

Sterilization

STERTILE TOXIN

- Sterility test
- LF/ML
- MLD
- MTV
- Antigenic purity

Detoxification with 0.5 % Formalin

4 days at Room temperature & 4 weeks at 35 °C

CRUDE TOXOID SUBLOT

- LF/ML
- Detoxification test in mice

Millipore Filter [30 KDa] 0.2 to 0.45 micron

CONCENTRATED TOXOID

- LF Test

pooling

POOLED CONCETRATE (1-2 SUBLOTS)

Centrifugation (4000 rpm @45 minutes) Precipitation with ammonium Sulphate – I st salting (10% - 12%)

- LF Test

SUPERNATANT

Precipitate with high ammonium sulphate (22% - 24%)

Centrifuge 4000 rpm @ 1 hour

PRECIPITATION

Dialysis

0.2 micron bag pore size

PURIFIED TOXOID

Final Filtration (Cartridge Filter)

STERILE FILTRATION

BULK PURIFIED TETANUS TOXOID

- Sterility test
- Antigenic purity
- LF/ML
- Specific Toxicity
- Irreversibility test

Released for Blending

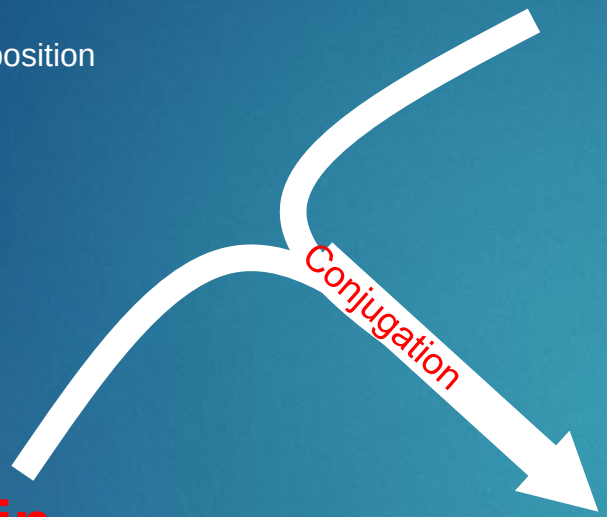
Synthesis of Conjugate Vaccines

Polysaccharide

1. Identity
2. Polysaccharide composition
3. Moisture content
4. Protein impurity
5. Nucleic acid impurity
6. Pyrogen content
7. Molecular size distribution

Activated saccharide

1. Extent of activation
2. Molecular size distribution



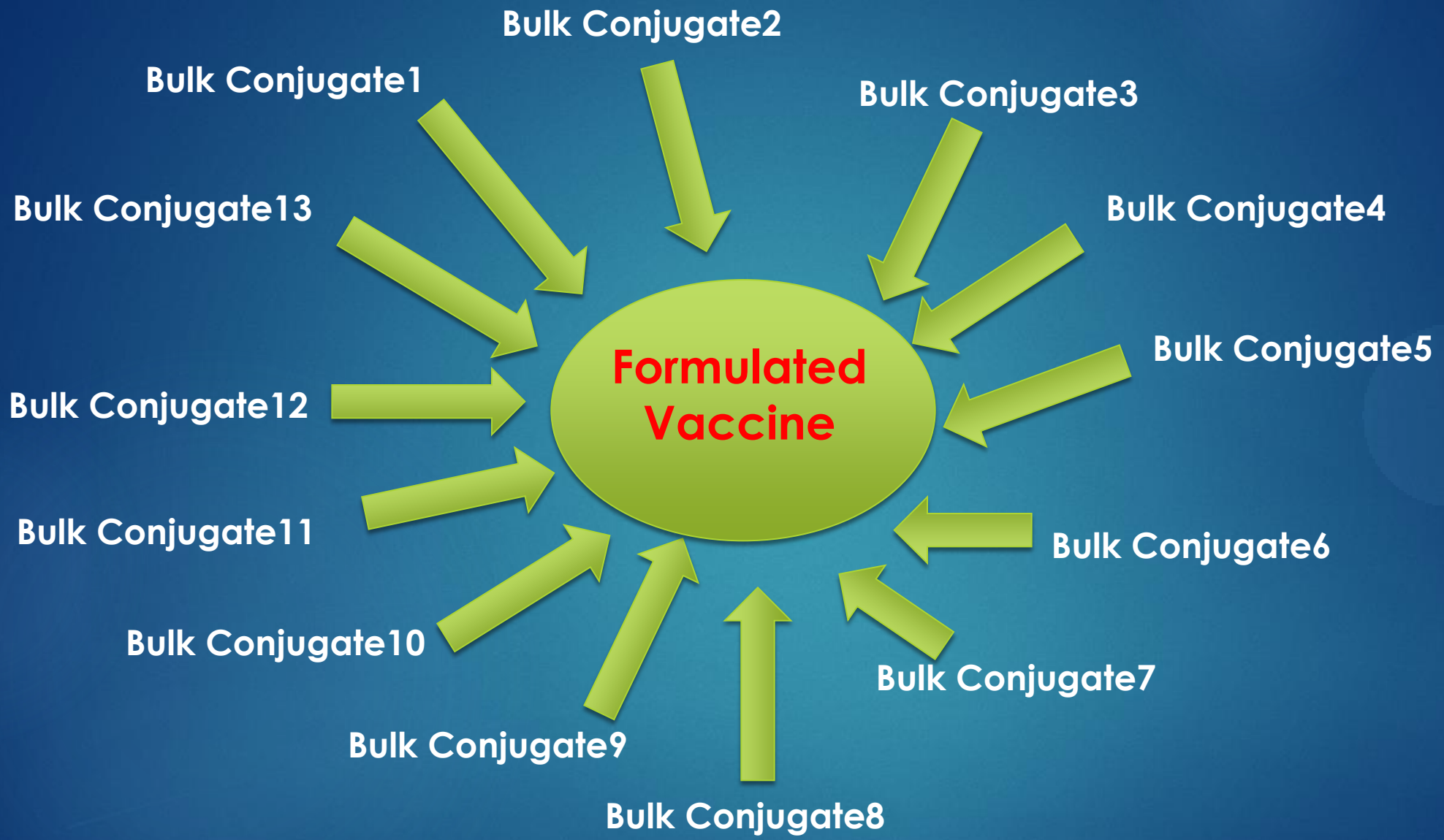
Carrier Protein

1. Identity
2. Purity
3. Toxicity
4. Extent of derivatisation (if appropriate) NR

Bulk Conjugate

1. Identity
2. Residual reagents
3. Saccharide:protein ratio & conjugation markers
4. Capping markers
5. Saccharide content NR
6. Conjugated v. free saccharide
7. Protein content
8. Molecular size distribution
9. Sterility
10. Specific toxicity of carrier (if appropriate)
11. Endotoxin content

WHO Recommendations for the production and control of pneumococcal conjugate vaccines, ECBS, October 2003. Updated 2009.



Multivalent pneumococcal conjugate vaccine

Control testing of Pn conjugates

Polysaccharide

1. Identity
2. Polysaccharide composition
3. Moisture content
4. Protein impurity
5. Nucleic acid impurity
6. Pyrogen content
7. Molecular size distribution

Activated saccharide

1. Extent of activation
2. Molecular size distribution

Conjugation

Carrier Protein

1. Identity
2. Purity
3. Toxicity
4. Extent of derivatisation (if appropriate) NR

Bulk Conjugate

1. Identity
2. Residual reagents
3. Saccharide:protein ratio & conjugation markers
4. Capping markers
5. Saccharide content NR
6. Conjugated v. free saccharide
7. Protein content
8. Molecular size distribution
9. Sterility
10. Specific toxicity of carrier (if appropriate)
11. Endotoxin content

Formulation

Final Vaccine

1. Identity
2. Sterility
3. Saccharide content (of each)
4. Residual moisture
5. Endotoxin content
6. Adjuvant content (if used)
7. Preservataive content (if used)
8. General safety test
9. pH
10. Inspection

WHO Recommendations for the production and control of pneumococcal conjugate vaccines, ECBS, October 2003. Updated 2009.

Complexity of Supply Chain & Quality Control

- >300 GMP steps for Prevnar13
- Managing supply chain & supply chain quality
- Each ingredient must be ready at the right time
- QA/QC for bulk and formulated vaccine

Thank You!

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