# OMICS Group International Pharmaceutical Conferences GMP Summit-2014

## Quality of Biosimilars produced in Developing Countries



Valencia, Spain

Day 2 September 26, 2014

Committee Room 5

Keynote Forum

10:25-10:50


**Wael Ebied**

Sr. Manager; Technical, Quality Assurance & EHS

SEDICO Management Representative & Qualified Person

# The Keynote Forum will:

- examine the requirements and difficulties in this therapeutic arena
- discuss precautions which should be taken in South Countries' home grown biotechnology
- explore the road forward.

# An overview of , Production, Technology and Regulatory aspects of Biosimilars/Bigenerics/FoPs/FoBs.

Biotech-enabled Healthcare Products for Management, Prevention and Diagnosis of Human and Animal Diseases.

Include: Therapeutic APIs, Vaccines/Sera, Diagnostics and Devices

Macromolecules of elaborate structure v simple chemicals/small molecules,

Molecular wt Aspirin; 180 versus hu insulin; 5808 Da.

Small molecules: synthesis pathway very well defined
interchangeability/Substitution – Brand by generic.

# An overview of , Production, Technology and Regulatory aspects of Biosimilars/Bigenerics/FoPs/FoBs.

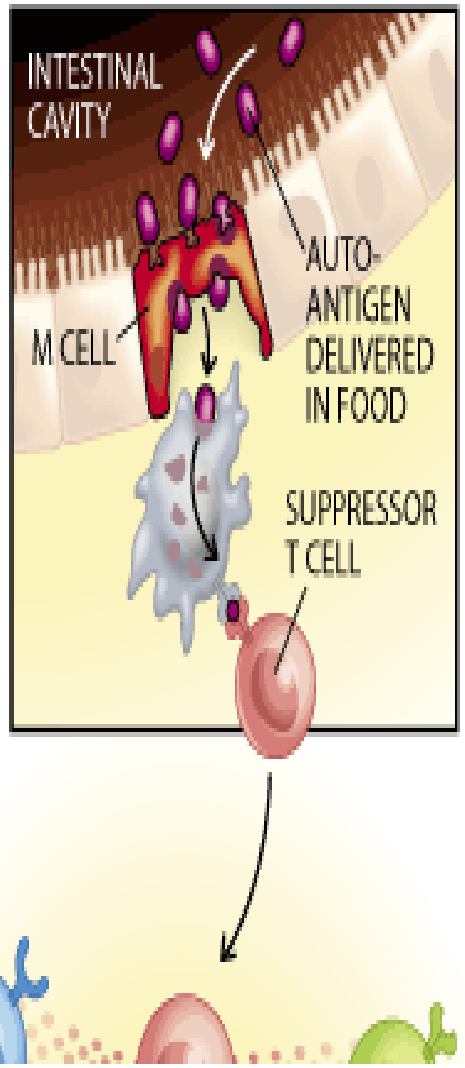
Biologics: Eq pathway in the making – Guidelines - ??? Re interchangeability – biogeneric term is not used, rather Biosmilar / 2nd generation / FoP / FoB – Biosimilars are Different – Issues of Sameness and Identity

Include: Proteins and NA fragments, None-protein Biologics, mAbs and Vaccines.

Produced in living organisms( Prokaryotes such as GE Bacteria and Eukaryotes such as GE Yeasts/Transgenic plants & animals "Pharming" ).

i.e not via simple chemical synthesis.

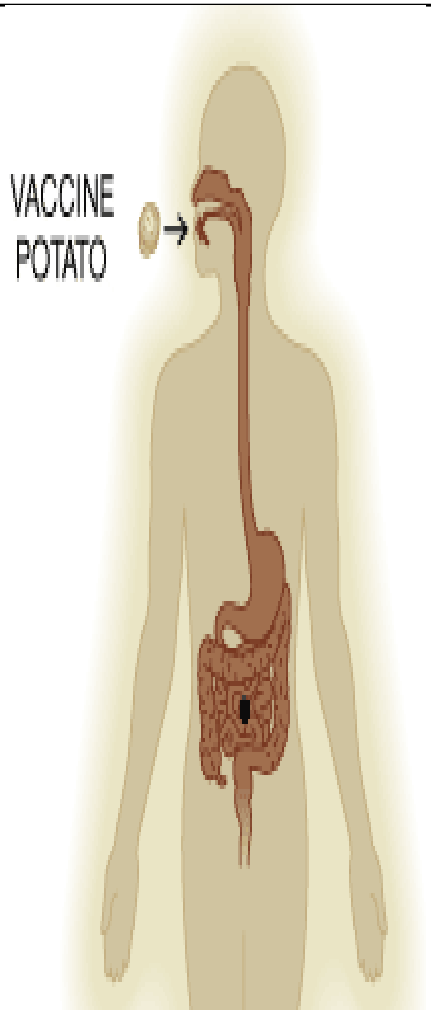
# Production in GE Plants



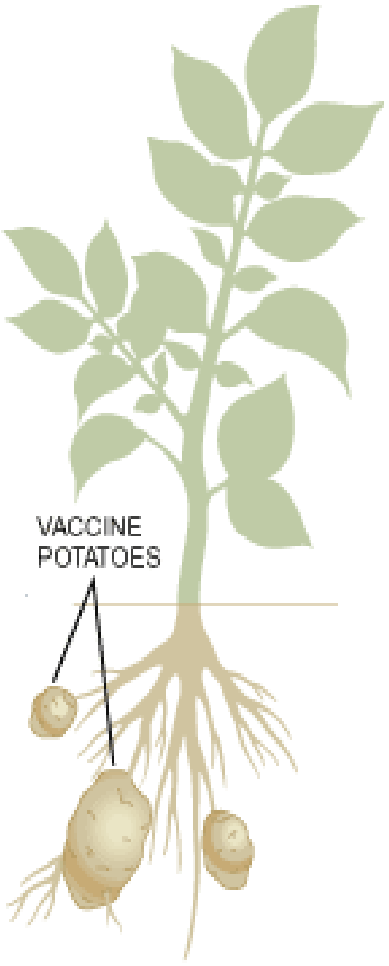

STOPPING AUTOIMMUNITY



MOVING AGAINST MALNUTRITION




HOW EDIBLE VACCINES PROVIDE PROTECTION

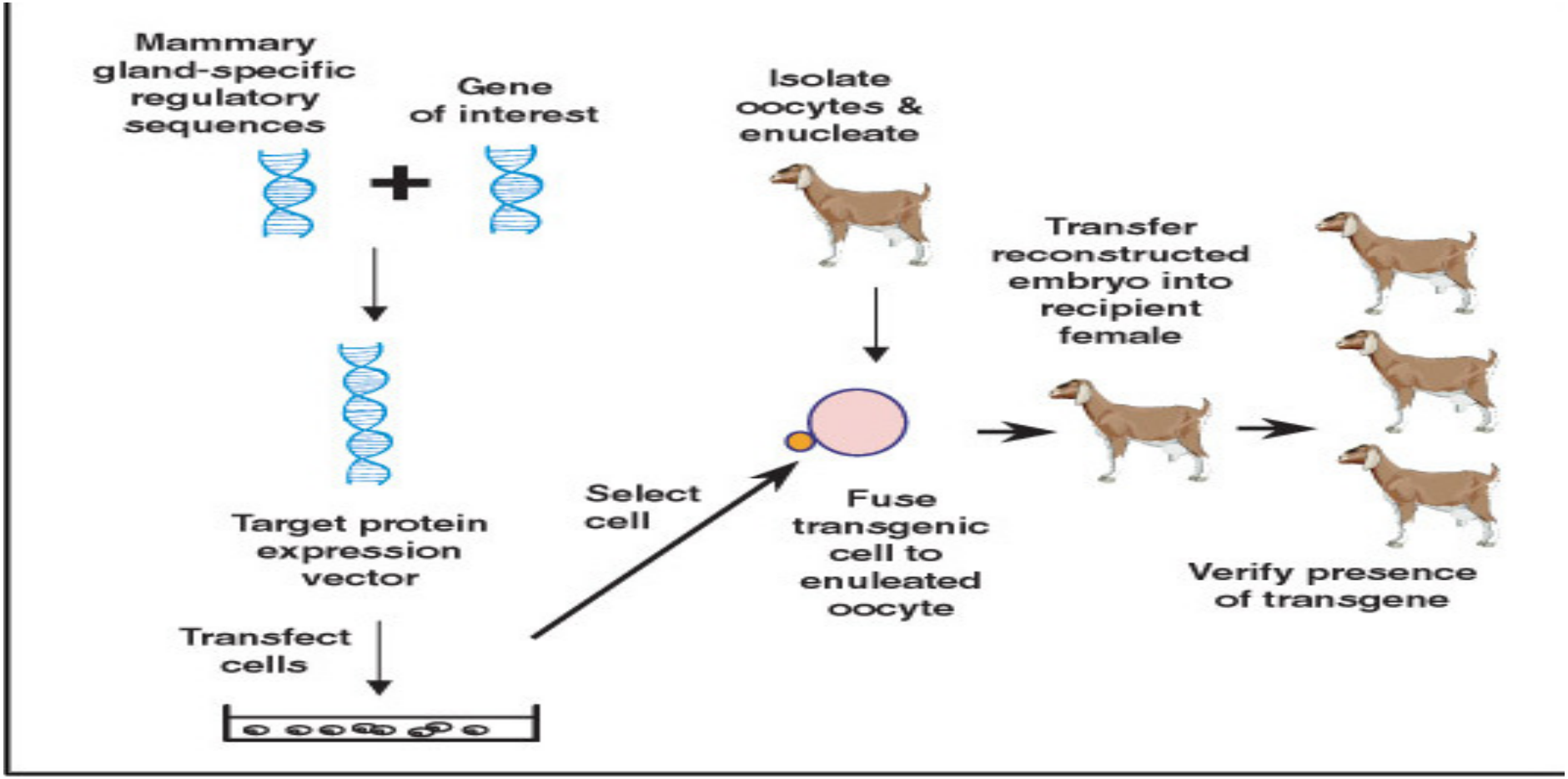



HOW TO MAKE AN EDIBLE VACCINE

Source: (Lengridge W 2000).

# Production in GE Animals

Production of Recombinant Therapeutic Proteins in the Milk of Transgenic Animals: Somatic Cell Nuclear Transfer



# Production in GE Animals

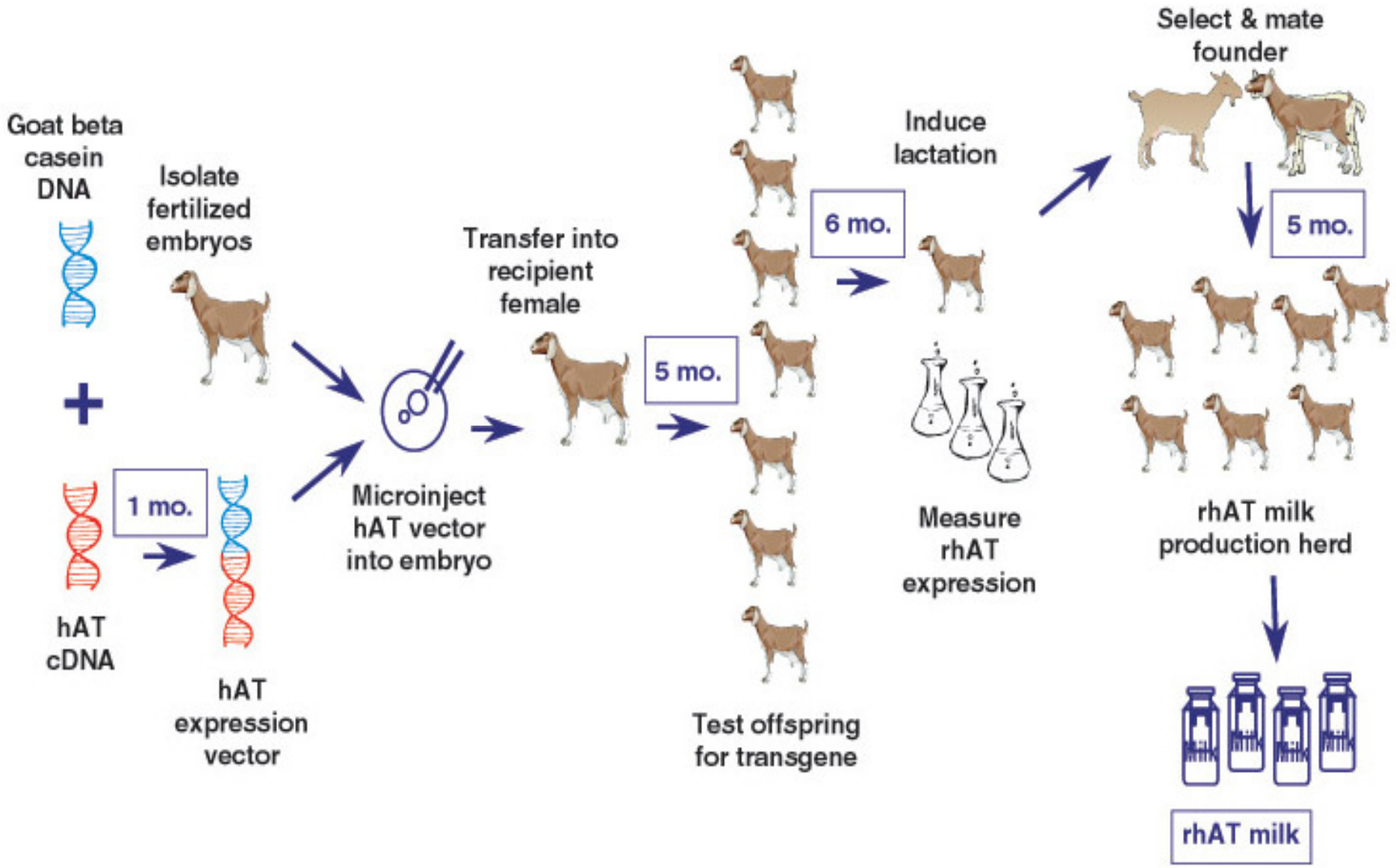
List of therapeutic proteins produced in the milk of transgenic animals that are currently in commercial development.

| Products | Companies | Production Animal | Developmental Stage |
|---|---|---|---|
| ATryn | GTC Biotherapeutics | Goats | EU: CHMP positive opinion<br>US: Phase 3 |
| C1 Esterase Inhibitor | Pharming | Rabbits | Phase 3 for HAE |
| MM-093 (AFP) | Merrimack and GTC Biotherapeutics | Goats | Phase 2 for RA |
| Alpha-Glucosidase | Pharming | Rabbits | Phase 2, on hold |
| Human growth hormone | BioSidus | Cows | Preclinical |
| Albumin | GTC Biotherapeutics | Cows | Preclinical |
| Fibrinogen | Pharming | Cows | Preclinical |
| Collagen | Pharming | Cows | Preclinical |
| Lactoferrin | Pharming | Cows | Preclinical |
| Alpha-1 Antitrypsin | GTC Biotherapeutics | Goats | Preclinical |
| Malaria vaccine | GTC Biotherapeutics | Goats | Preclinical |
| CD137 (4-1BB) MAb | GTC Biotherapeutics | Goats | Preclinical |

Abbreviations: HAE = hereditary angioedema, AFP = alpha-fetoprotein, MAb = monoclonal antibody

panel, Hyderabad, India

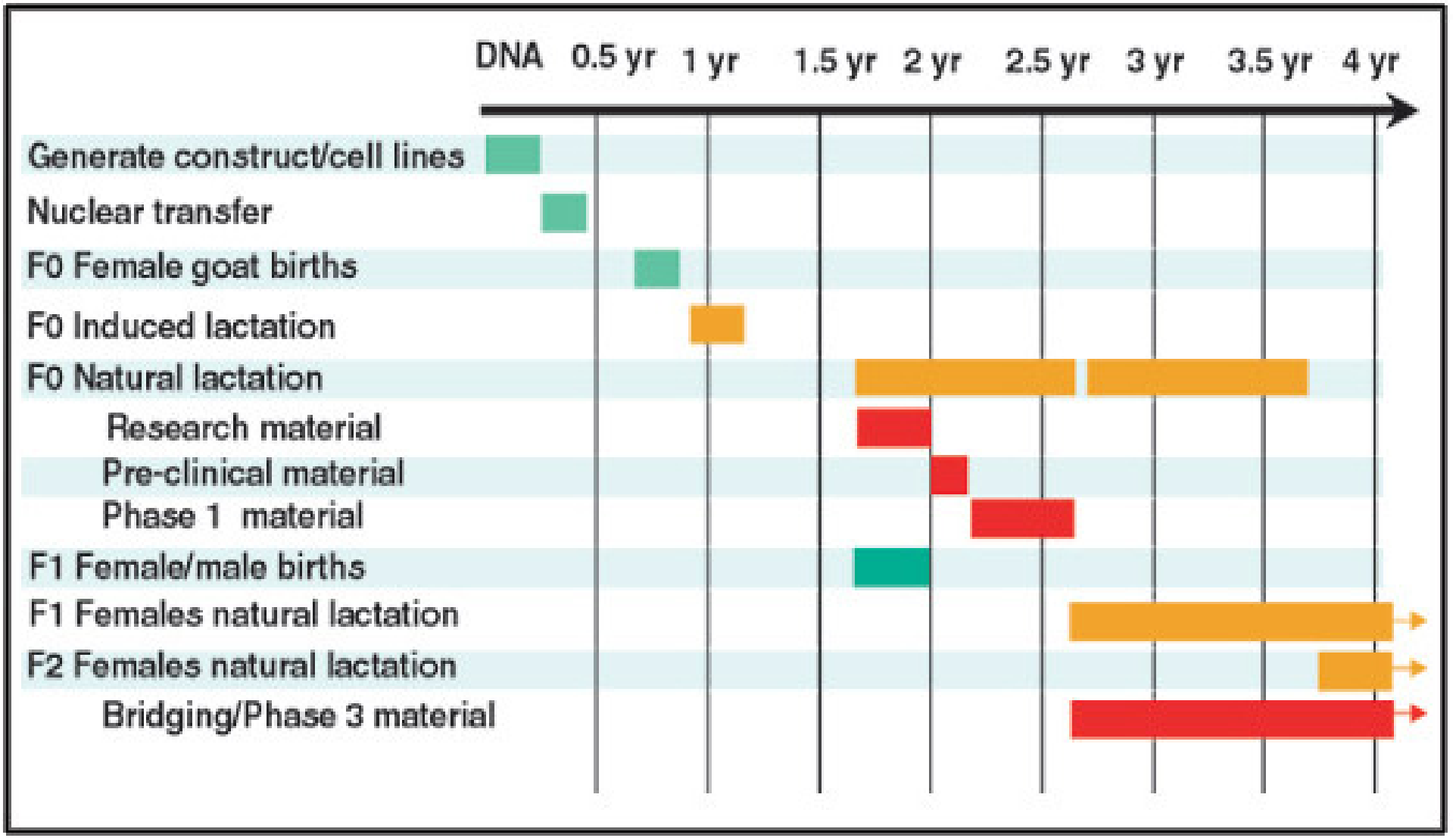
# Production in GE Animals




panel, Hyderabad, India

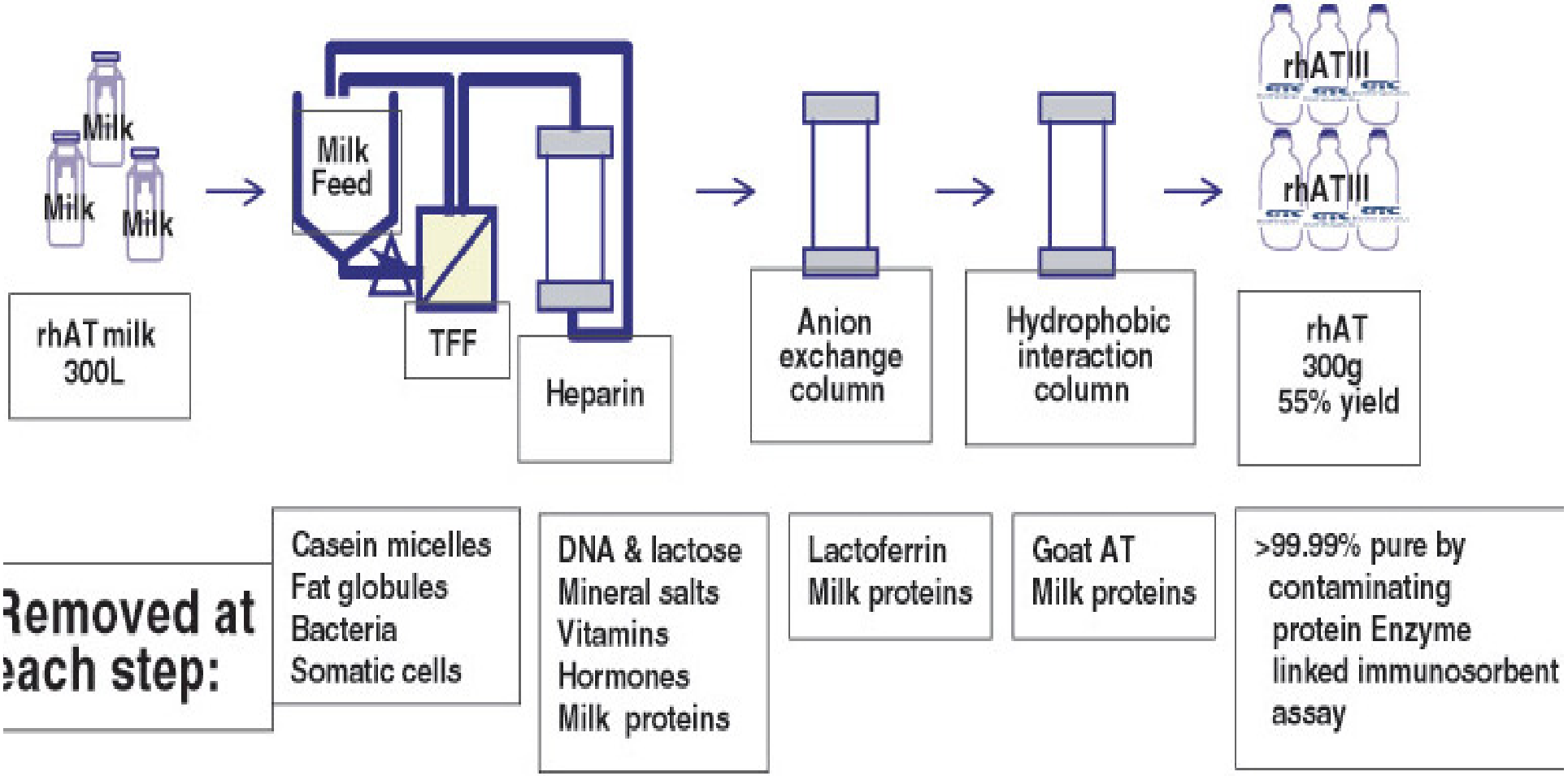
# Production in GE Animals

Timeline associated with the creation of a herd of transgenic goats producing recombinant proteins in their milk.



# Production in GE Animals

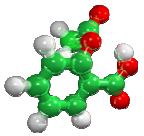
Schematic representation of the process used to purify ATryn from the milk of transgenic goats.




panel, Hyderabad, India

# Biologics are Highly Complex Molecules

- **Larger molecular size and weight (Aranesp darbepoetin alfa) than "small molecules" (Asprin) traditional pharmaceuticals**
- **Derived from living organisms**
- **Each cell line is unique**
- **Difficult to produce and replicate**



Aspirin®

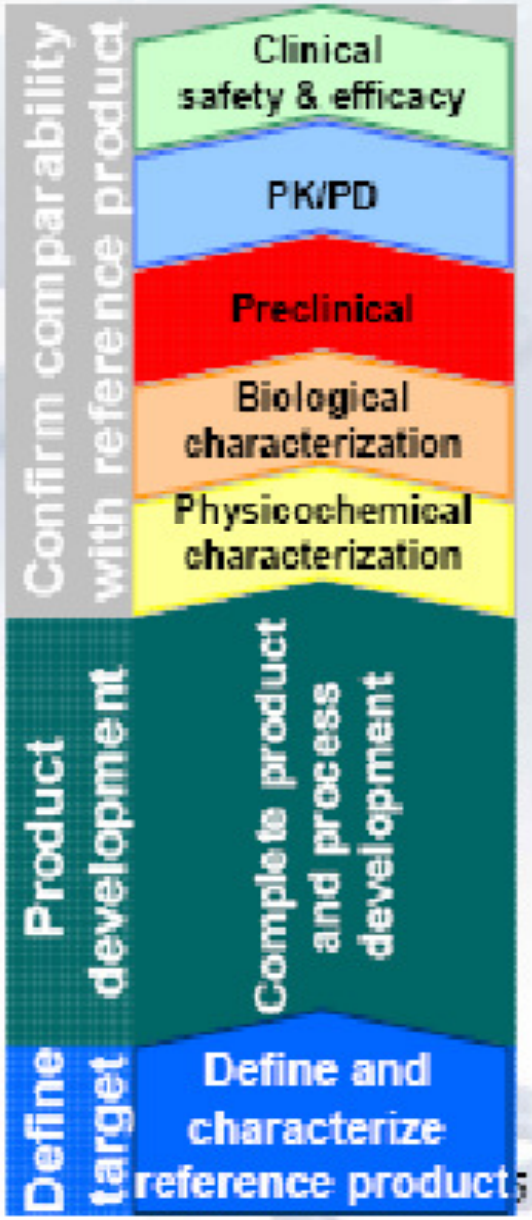


Aranesp®

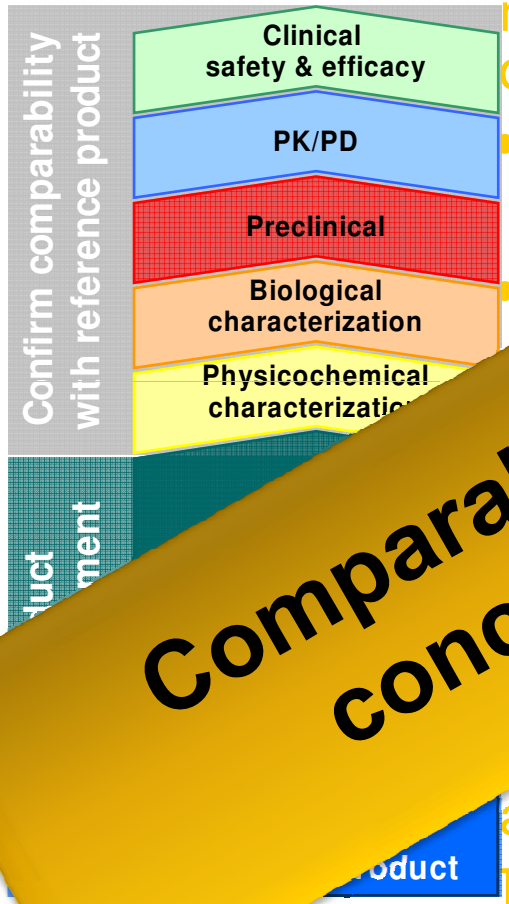
# Biosimilar Development

The development of a biosimilar product is targeted to match the reference medicinal product through the application of state-of-the-art science and technology in head-to-head studies.

The development is based on a complete stand-alone development of the production process taking all relevant guidelines into account



# The Comparability Exercise is fundamental to the Development of an EU Biosimilar Product




**Comparability is not easy, but the concept is well established**

The comparability/similarity wi… ct must be demonstrated at… development:

- Analytical co…

**… SIMILARITY**

- Fu… …, and shown by

**…ONFIRMING SIMILARITY**

…ity shown in Phase I and III

…milar product is designed to meet the criteria of …e reference product with regards to quality, safety and efficacy.

This rigorous comparability exercise qualifies Biosimilars for therapeutic interchange

# Subsequent Entry Biologic / Biosimilar regulatory Doc

## Chemistry/Manufacturing

- Drug substance
  - Manufacture
  - Characterisation
  - Control
  - Reference standard
  - Container
  - Stability
- Drug product
  - Description
  - Development
  - Manufacture
  - Control
  - Reference standard
  - Container
  - Stability
  - \+ Analytical comparison with reference product

## Nonclinical studies

- Pharmacology
  - 1ry pharm.
  - 2nd pharm.
  - Safety pharm.
  - Interactions
- Pharmacokinetics
  - ADME
  - Interactions
- Toxicology
  - Single dose
  - Repeat dose
  - Genotoxicity
  - Carcinogenicity
  - Reproduction
  - Local tolerance

## Clinical studies

- Pharmacology
- Pharmacokinetics/ Pharmacodynamics
  - Single dose
  - Repeat dose
  - Special populations
- Efficacy and safety
  - Dose finding
  - Schedule finding
  - Pivotal
    - Indication 1
    - Indication 2
    - Indication 3
    - Indication 4
  - \+ Clinical comparison with reference product
- Immunogenicity
- Risk Management Plan
  - Post-marketing studies

# Clinical Testing is needed to determine efficacy and patient safety

## Biosimilar A

*Vs Reference product*

- some patients developed antibodies in the first study
- Problem was residual host-cell protein
- Re-developed purification process
- Conducted a second phase 3 study
  - Antibody levels reduced

**APPROVED**

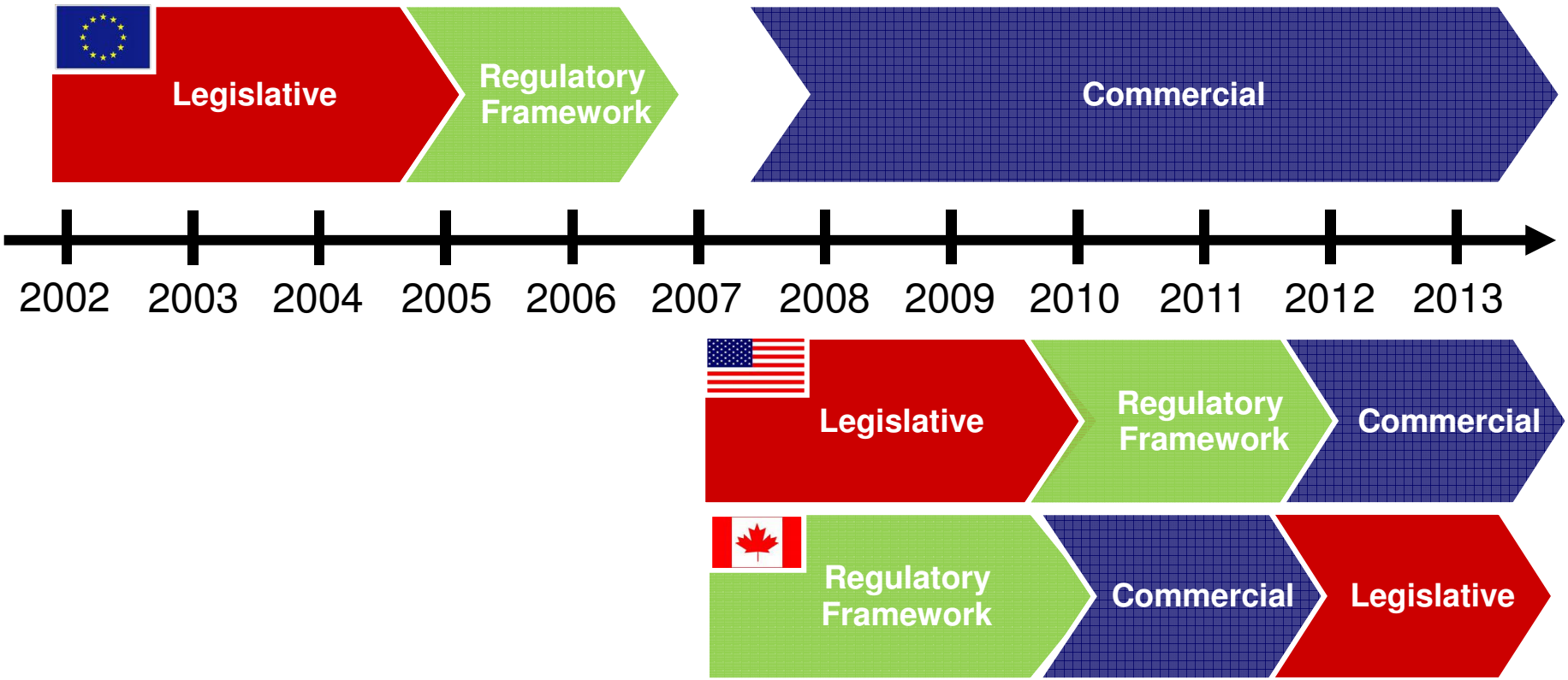
## Biosimilar B

*Vs Reference product*

- Lower quality
  - Not as pure as Ref.
- Lower efficacy than Ref.
  - More patients relapsed
- Safety profile worse than Ref.
  - More side-effects

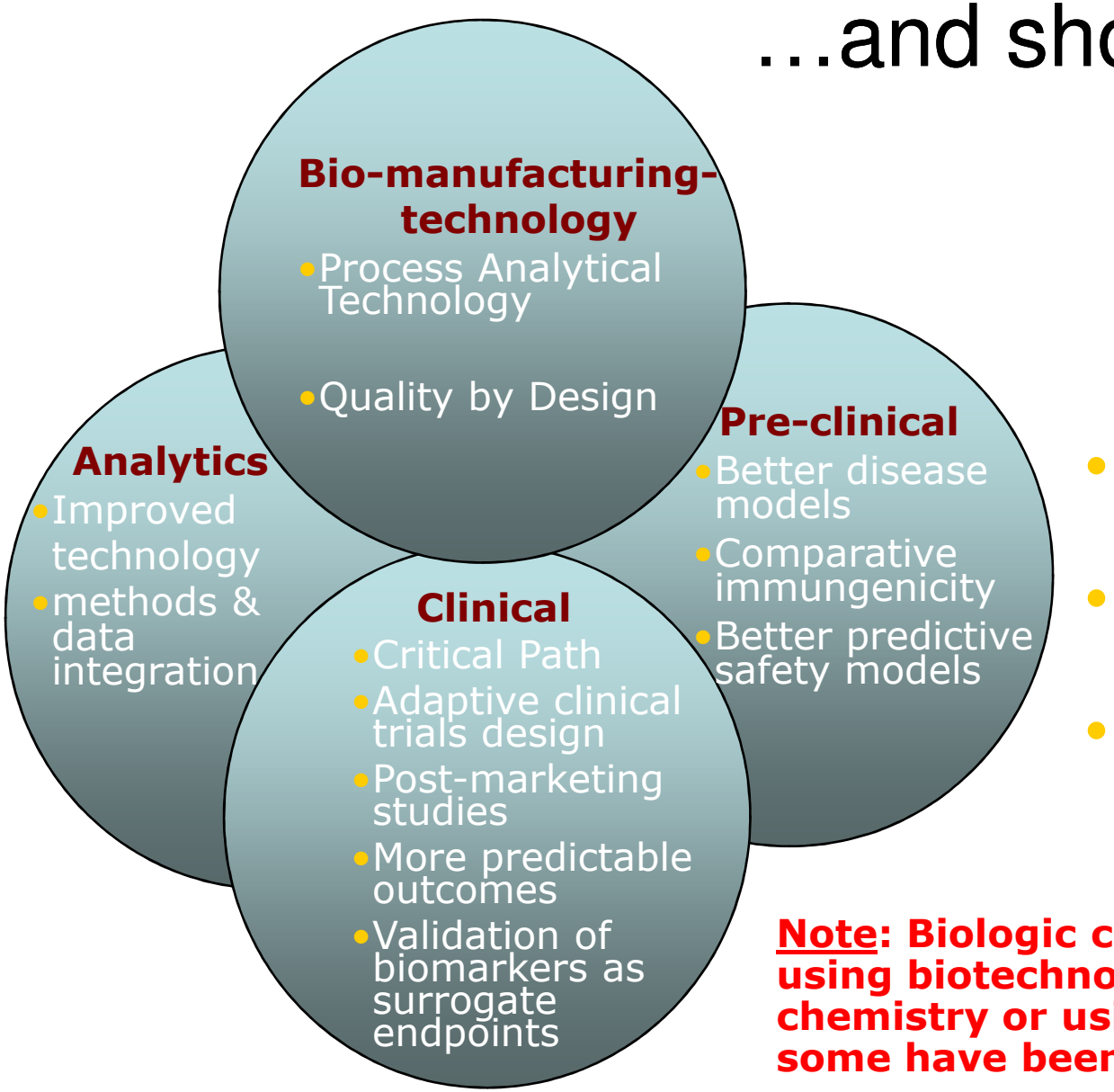
**REJECTED**

# Biosimilars worldwide for the past few years



# Progress in science and technology is a continuum …

# …and should be global

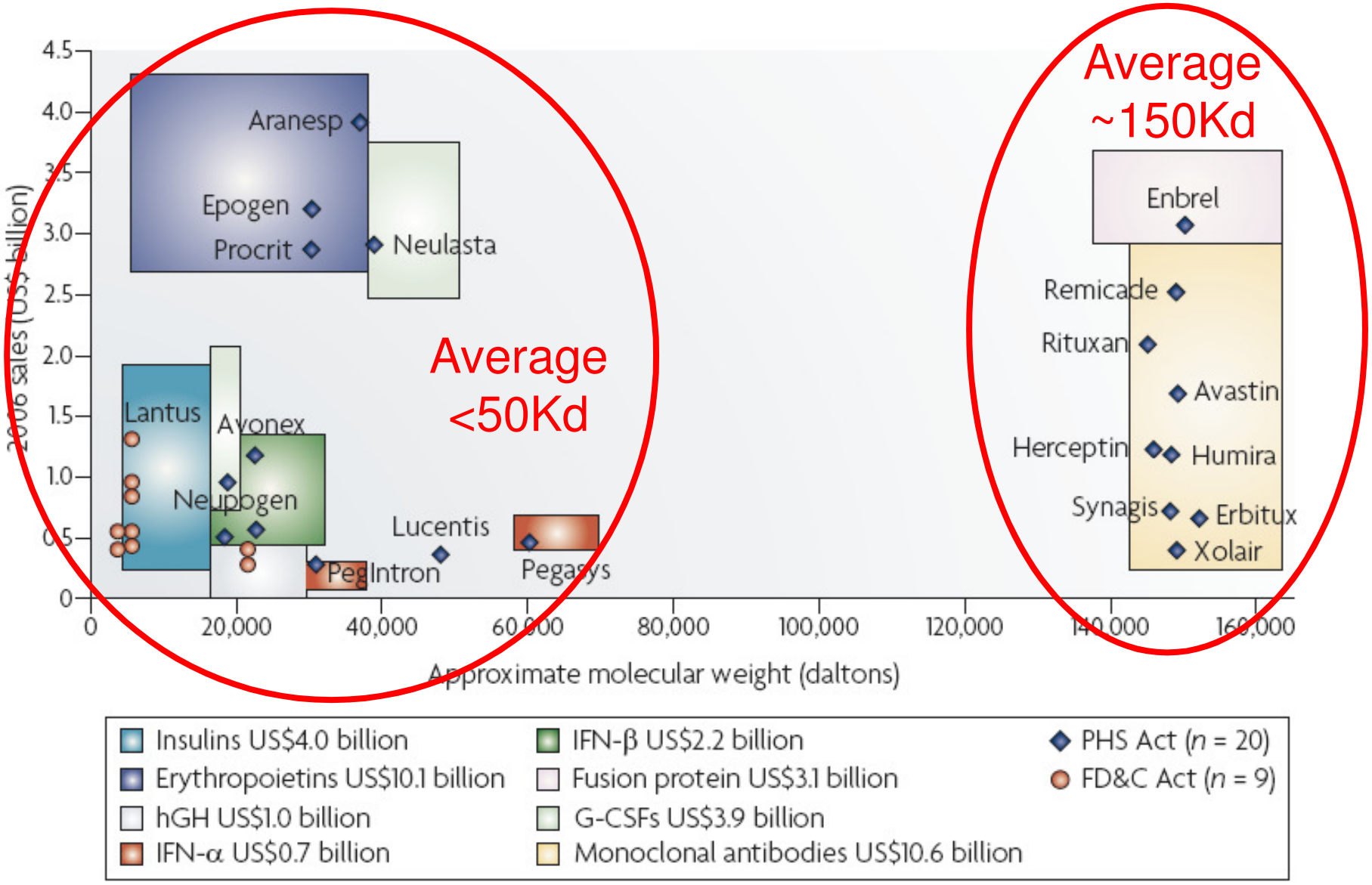



**Note: Biologic can be manufactured using biotechnology, synthetic chemistry or using natural sources; some have been made with all three**

# Patent expiration of biopharmaceuticals

| Pioneer company | Product | Indication(s) | EU patent/market exclusivity expires | USA patent/market exclusivity expires |
|---|---|---|---|---|
| Genentech | Nutropin® (somatropin) | Growth disorders | Expired | Expired |
| Abbott | Abbokinase® (eudurase urokinase) | Ischaemic events | Expired | Expired |
| Eli Lilly | Humulin® (recombinant insulin) | Diabetes | Expired | Expired |
| Genzyme | Ceredase® (alglucerase); Cerezyme® (imiglucerase) | Gaucher disease | Expired | Expired |
| AstraZeneca | Streptase® (streptokinase) | Ischaemic events | Expired | Expired |
| Biogen / Roche | Intron A® (IFN-alfa-2b) | Hepatitis B and C | Expired (France) 2007 (Italy) | Expired |
| Serono | Serostim® (somatropin) | AIDS wasting | NA | Expired |
| Eli Lilly | Humatrope® (somatropin) | Growth disorders | NA | Expired |
| Amgen | Epogen®, Procrit®, EPREX® (erythropoietin) | Anaemia | Expired | 2013 |
| Roche | NeoRecormon® (erythropoietin) | Anaemia | Expired | NA |
| Genentech | TNKase® (tenecteplase TNK-tPA) | Acute myocardial infarction | Expired | 2005 |
| InterMune | Actimmune® (IFN-gamma-Ib) | Chronic granulomatous disease (CGD), malignant osteopetrosis | Expired | 2005, 2006, 2012 |
| Genentech | Activase®, Alteplase® (tPA) | Acute myocardial infarction | Expired | 2005, 2010 |
| Chiron | Proleukin® (IL-2) | HIV | Expired | 2006, 2012 |
| Amgen | Neupogen® (filgrastim G-CSF) | Anaemia, leukaemia, neutropenia | Expired | 2015 |

# Selected Therapeutic Biologics by Product Class



# Terminology has been distracting…

- Biologic – is a prophylactic, in vivo diagnostic, or therapeutic substance that is made in a living system, and that, generally, has a large and complex molecular structure
- Follow-on Biologic (FOB) – a subsequent version of a biologic, independently developed and approved, but that shares the same mechanism of action as a previously approved product
- Second-generation biologic - subsequent versions of biologics that are independently developed and approved, share the same mechanism of action as a previously approved product but are explicitly different in some manner, e.g. inhaled. Sometimes called "evergreened".
- Biogeneric, or Generic Biologic Drugs – should only be applied for biologic drugs approved by FD&C Act. Federal Food, Drug, and Cosmetic Act enforced by the U.S. Food and Drug Administration which are interchangeable with their reference product

# Manufacturing differences between biopharmaceuticals and low molecular weight drugs

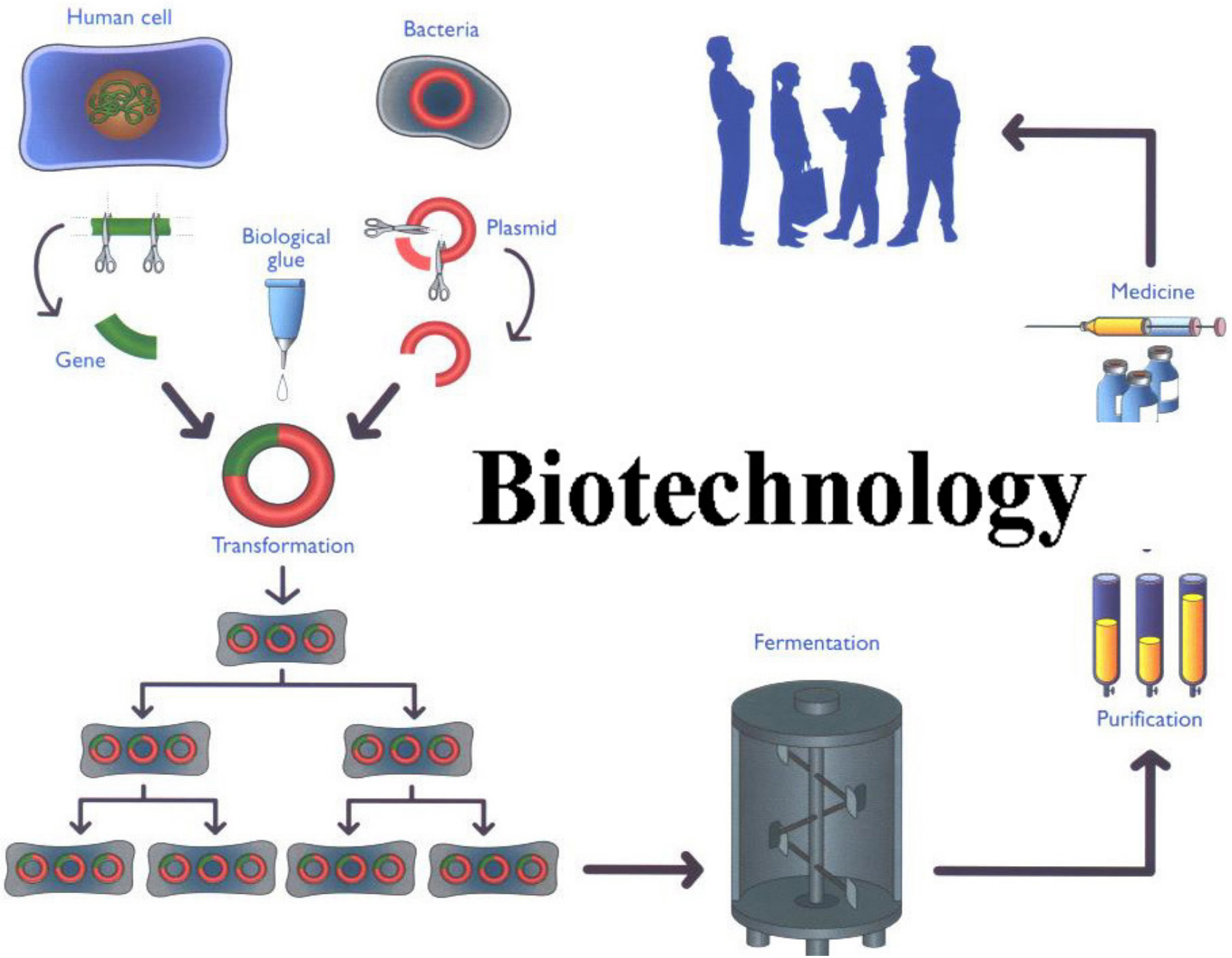
Low molecular weight drugs are made by adding and mixing together known chemicals and reagents, in a series of controlled and predictable chemical reactions. This is organic & inorganic chemistry

Biopharmaceuticals are made by harvesting the proteins that are produced and secreted by specially genetically engineered living cells

**This is genetic engineering**

# How biopharmaceuticals made?

1. Develop host cell
2. Establish a cell bank
3. Protein production
4. Purification
5. Analysis
6. Formulation
7. Storage and handling

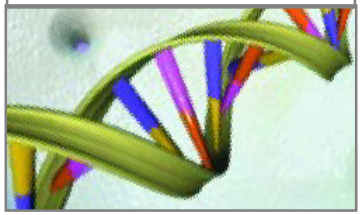

Human cell
Bacteria
Biological glue
Plasmid
Gene
Medicine
Transformation
Biotechnology
Fermentation
Purification

# Typical Protein Production Process

## "Different manufacturers will have different processes"

START

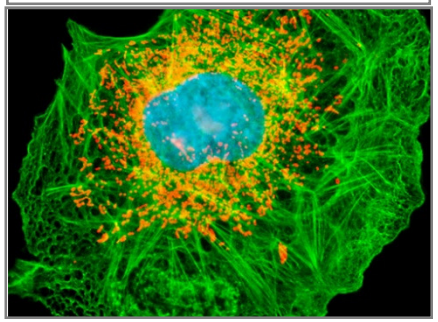
Probably same gene sequence



Different vector



Different host cell



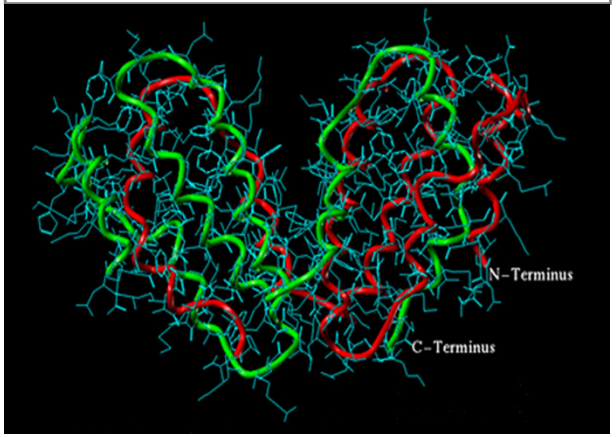
Different fermentation/culture conditions



Different downstream processing



Will result in different biophysical characteristics




END

# Develop host cell

- Identify the human DNA sequence for the desired protein
- Isolate the DNA sequence
- Select a vector to carry the gene
- Insert the gene into the genome of a host (a suitable bacterial or eukaryotic cell)

**The exact DNA sequence and the type of host cell used will significantly affect the characteristics of the product**

# Establish a cell bank

A cell bank is then established, using an iterative and elaborate cell screening and selection process, yielding a unique master cell bank.

**No two master cell banks are exactly alike**

# Protein production

- **The conditions under which cells are cultured can affect the nature of the end product.**

# Purification

- **Any change in the purification process can affect the clinical characteristics of the product.**

# Formulation

- Formulation is a key step in stabilizing the protein.
- The components of the formulation, and the process used, can significantly affect the product's behavior in patients.

# Analysis

- Protein molecules are analyzed for uniformity in terms of structure and potency.

- A wide variety of analytical tools is used to examine:

  3D structure / Aggregation /

  ISOform profile, including glycosylation patterns/ Heterogeneity / Potency.

- These tests remain limited in their ability to detect all product characteristics that may affect clinical efficacy and safety.

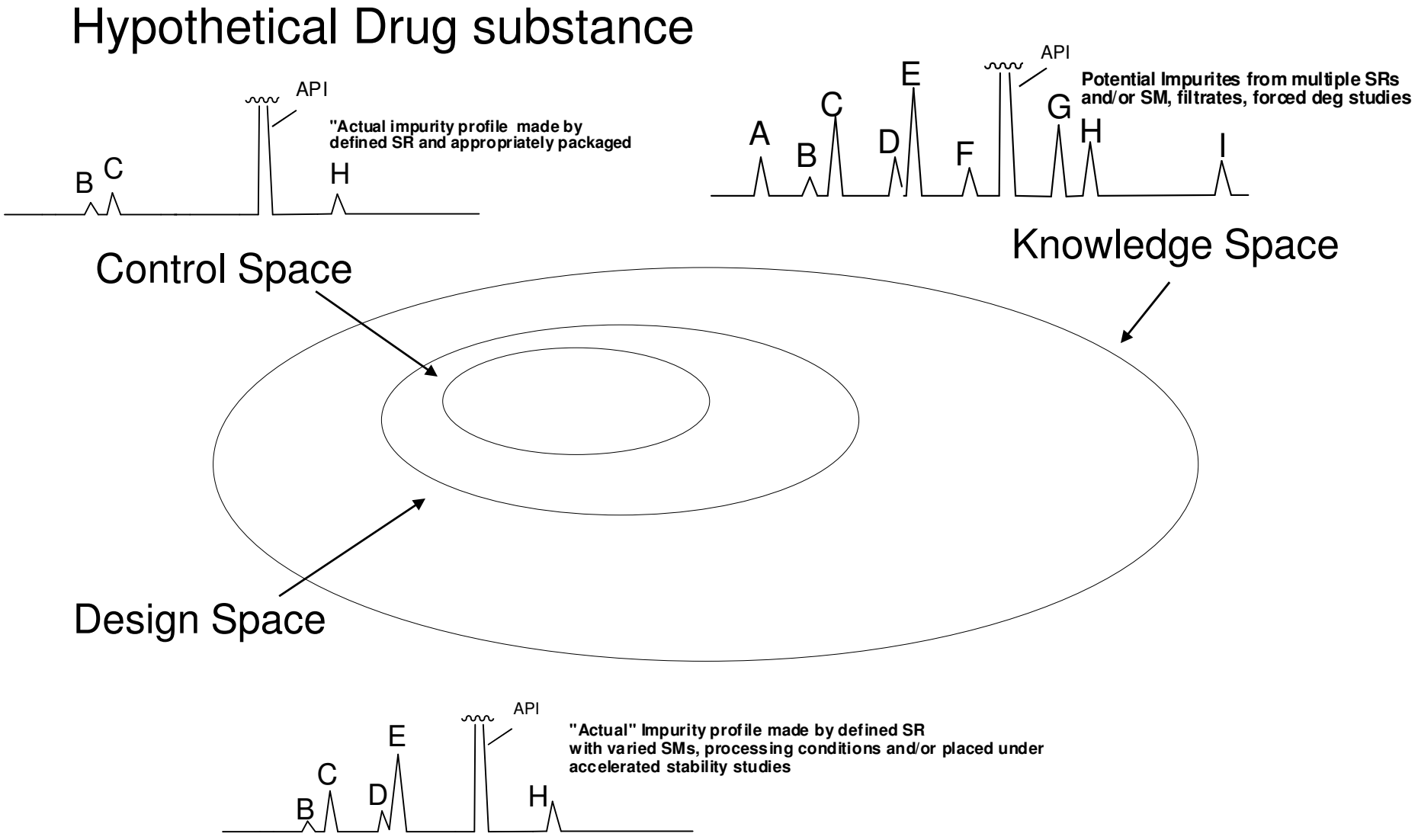
# Storage and handling

- Biopharmaceuticals are very sensitive to temperature changes and/or shaking.
- Strict storage and handling conditions are therefore essential for maintaining product integrity and stability.
- Poor adherence to (cold) storage requirements can affect clinical efficacy and safety.

# Quality of products

- Each of these stages can have a major influence on the characteristics of the biopharmaceutical end product.
- These differences clearly apply to biosimilars as well as to original biopharmaceuticals.

# Drug Substance Impurity Control in QbD Language – Process-Specific Impurities



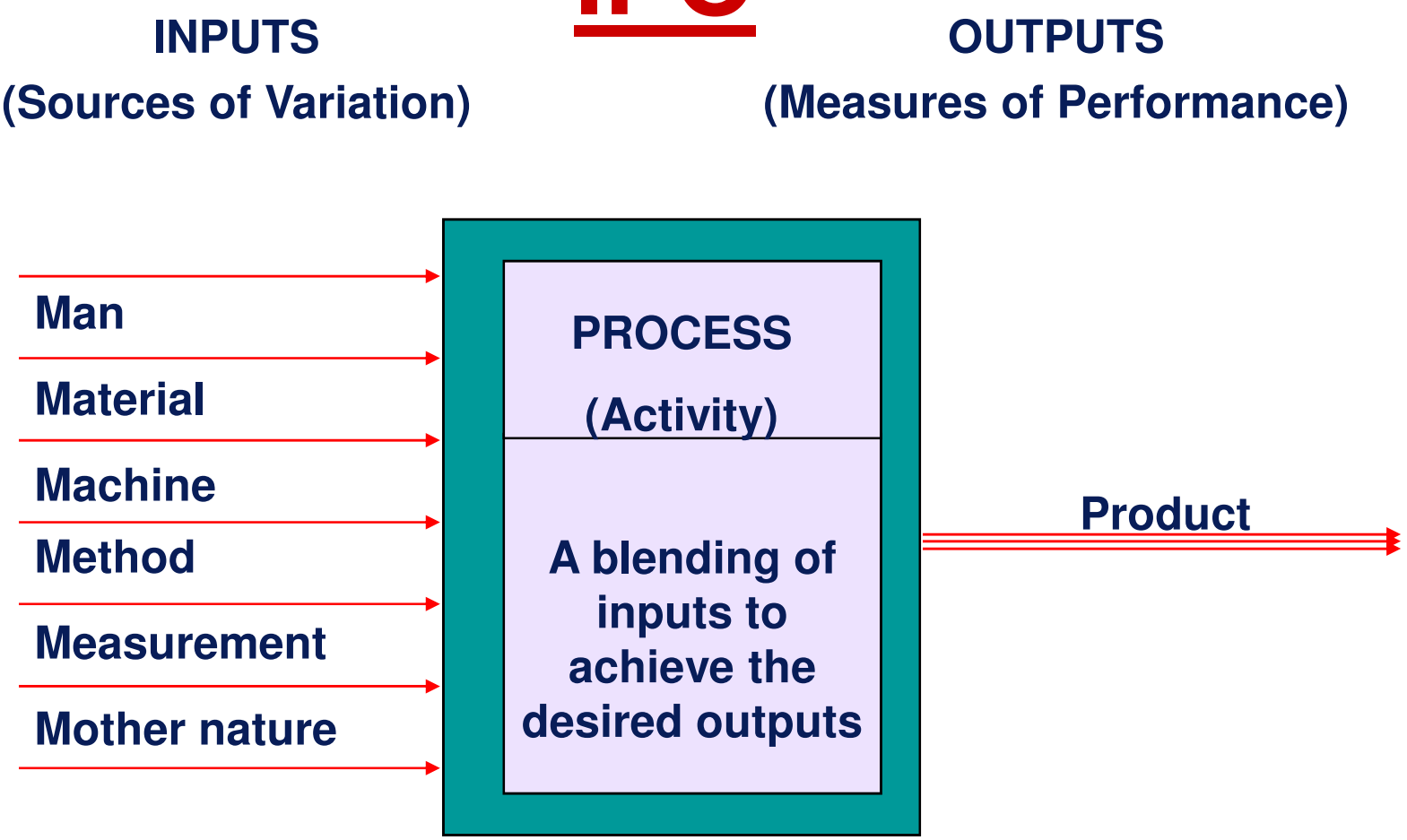
# Conclusions

- The manufacturing process for biopharmaceuticals (and biosimilars) is far more complex than for low molecular weight drugs (and generics).
- Any (minor) change made at any stage may have a critical effect on the clinical efficacy and safety.
- Manufacturing Majors include:
  - Start-up producing a biosimilar
  - opening/starting a new production site
  - scaling-up to meet market demands

**The process is the product**

# Definition of a Process (Activity)

## IPO



# Points to consider

- Can a new manufacturer produce a biosimilar that is similar enough to the original biopharmaceutical to be considered the same?
- How can the level of similarity be established?
- Are there risks associated with currently undetectable differences?

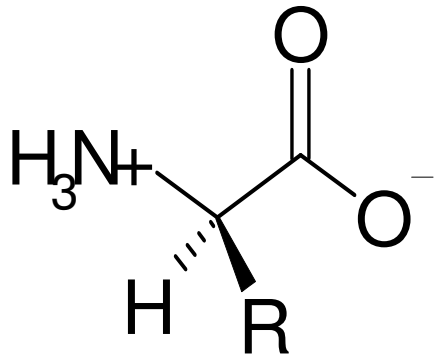
**How similar is similar enough?**

# Development of Biopharmaceuticals and Biosimilar

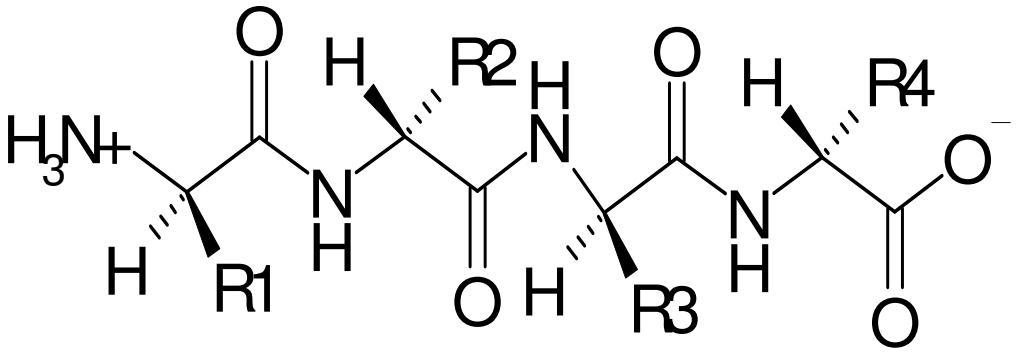
## Protein and peptide

- Proteins - Chains of amino acids, each joined together by a specific type of covalent bond
- Proteins formed by joining same 20 amino acids in many different combinations and sequences
- Protein > 50 amino acids
- peptide < 50 amino acids
- Function of a protein determined by its non-covalent 3D structure

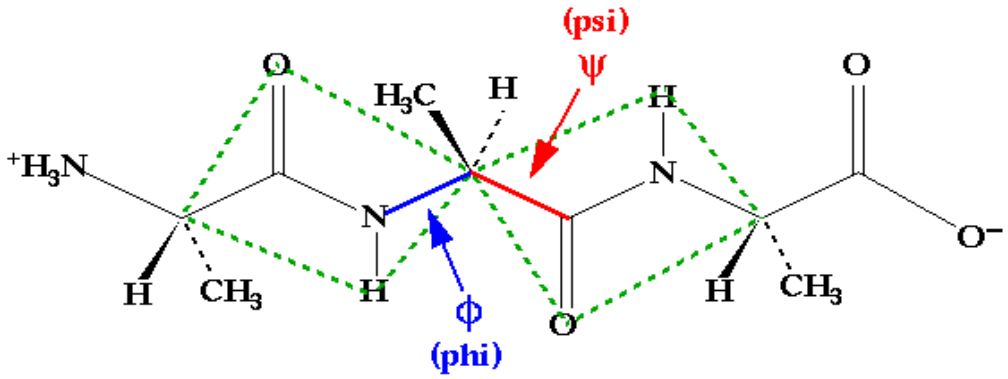
# Covalently linked Amino Acids




Amino Acids




Polypeptides



# Characteristics of therapeutic proteins

- Size
  - 100 – 500 times larger than classic drugs
  - Can not be completely characterized by physico-chemical methods
- Immunogenicity
- Structural heterogeneity
- Relatively high biological activity
- Relatively unstable

# Protein Structure



Lactate Dehydrogenase:
Mixed α / β

Immunoglobulin
Fold: β

Hemoglobin B
Chain: α

# Factors influencing activity of therapeutic proteins

- Gene and promotor
- Host cell
- Culture conditions
- Purification
- Formulation
- Storage and handling
- Unknown factors

# Protein Pharmaceuticals



- **Insulin (diabetes)**
- **Interferon β**
- **Interferon γ (granulomatous)**
- **TPA** Tissue plasminogen activator **(heart attack)**

# Challenges with Proteins

- Very large and unstable molecules
- Structure is held together by weak non-covalent forces
- Easily destroyed by relatively mild storage conditions
- Easily destroyed/eliminated by the body
- Hard to obtain in large quantities

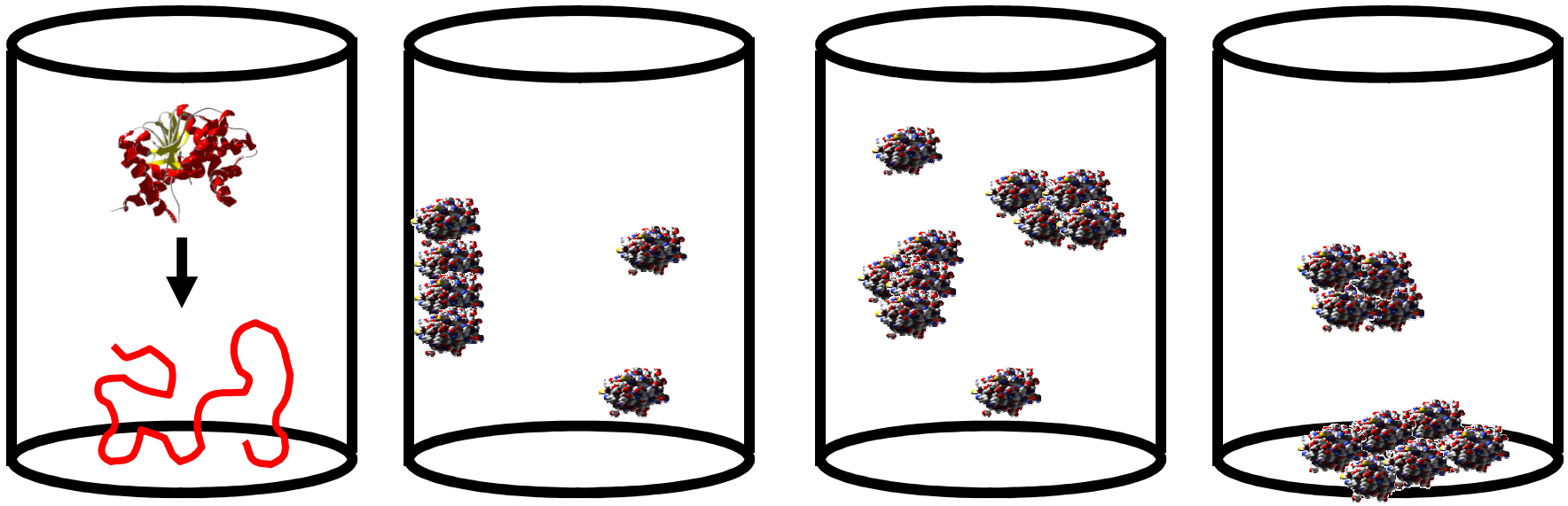
# Problem with Proteins
## (in vivo – in the body)

- Elimination by B and T cells
- Proteolysis by endo/exo peptidases
- Small proteins (< 30 kD) filtered out by the kidneys very quickly
- Unwanted allergic reactions may develop (even toxicity)
- Loss due to insolubility/adsorption

# Problem with Proteins
## (in vitro – in the bottle)

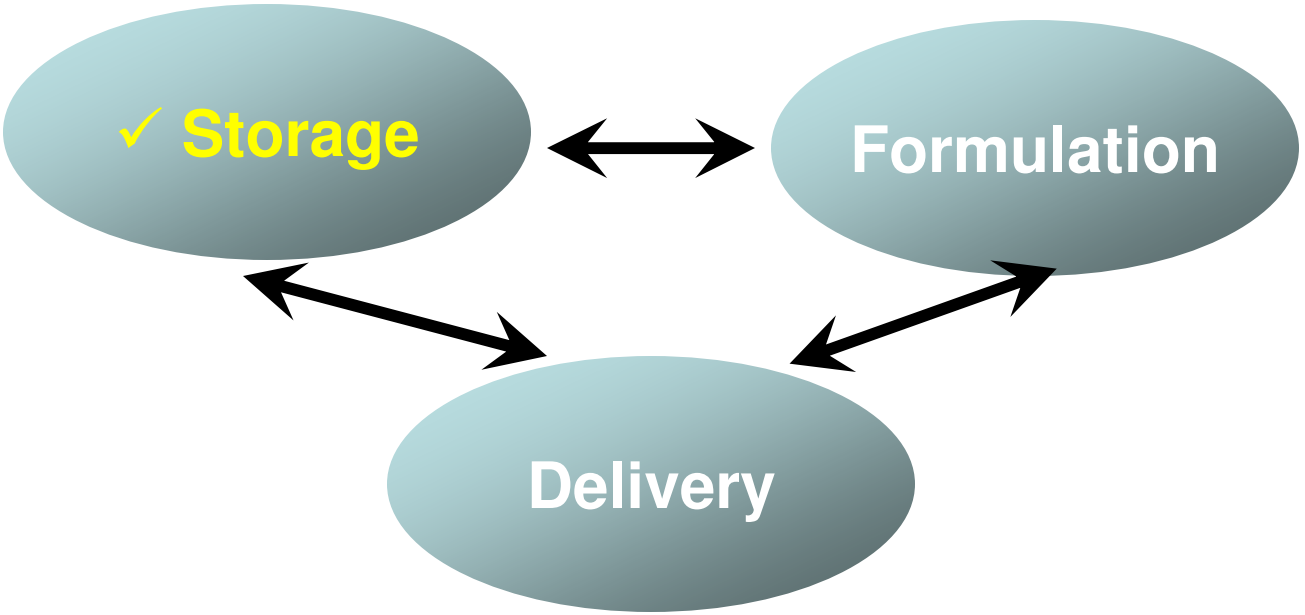
| Noncovalent | Covalent |
| --- | --- |
| - Denaturation | - Deamidation |
| - Aggregation | - Oxidation |
| - Precipitation | - Disulfide exchange |
| - Adsorption | - Proteolysis |

# Noncovalent Processes



Denaturation Adsorption Aggregation Precipitation

# How to Deal with These Problems



# Storage

- Refrigeration
- Packaging
- Additives
- Freeze-Drying

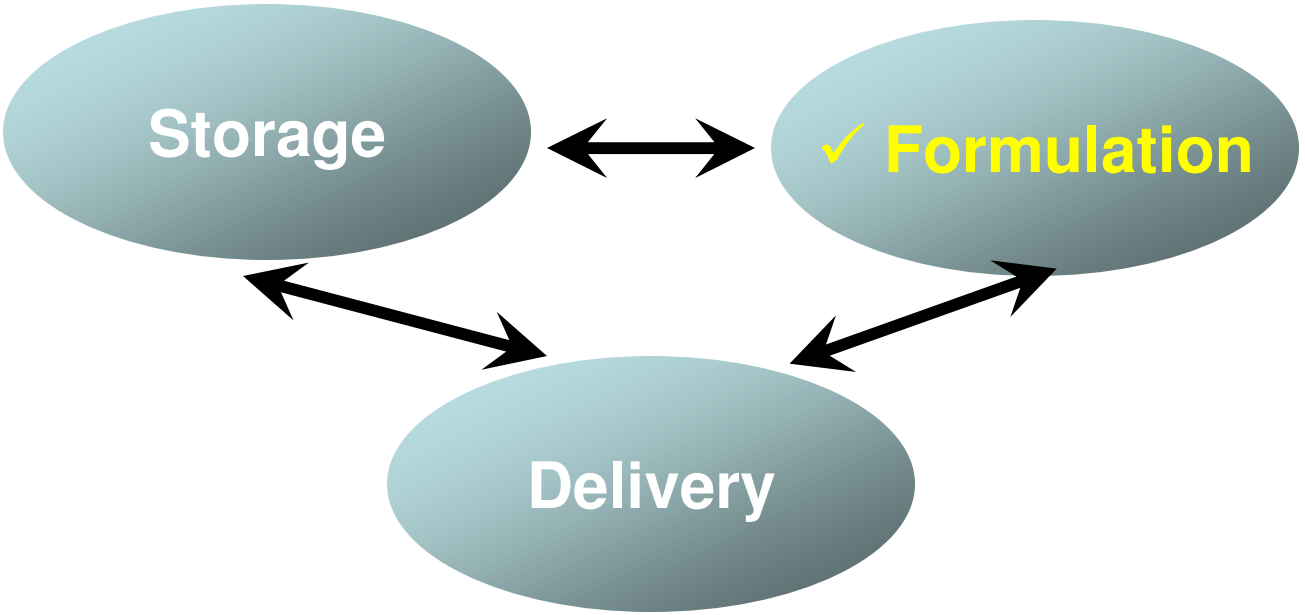
# **Storage** (additives)

- Addition of stabilizing salts or ions ($Zn^{+}$ for insulin, Albumin for EPO)
- Addition of polyols (glycerol and/or polyethylene glycol) to solubilize
- Addition of sugars or dextran to displace water or reduce microbe growth
- Use of surfactants to reduce adsorption and aggregation

# Storage
## Lyophilization (Freeze Drying)

- Freeze liquid sample in container
- Place under strong vacuum
- Solvent sublimates leaving only solid or nonvolatile compounds
- Reduces moisture content

# How to Deal with These Problems

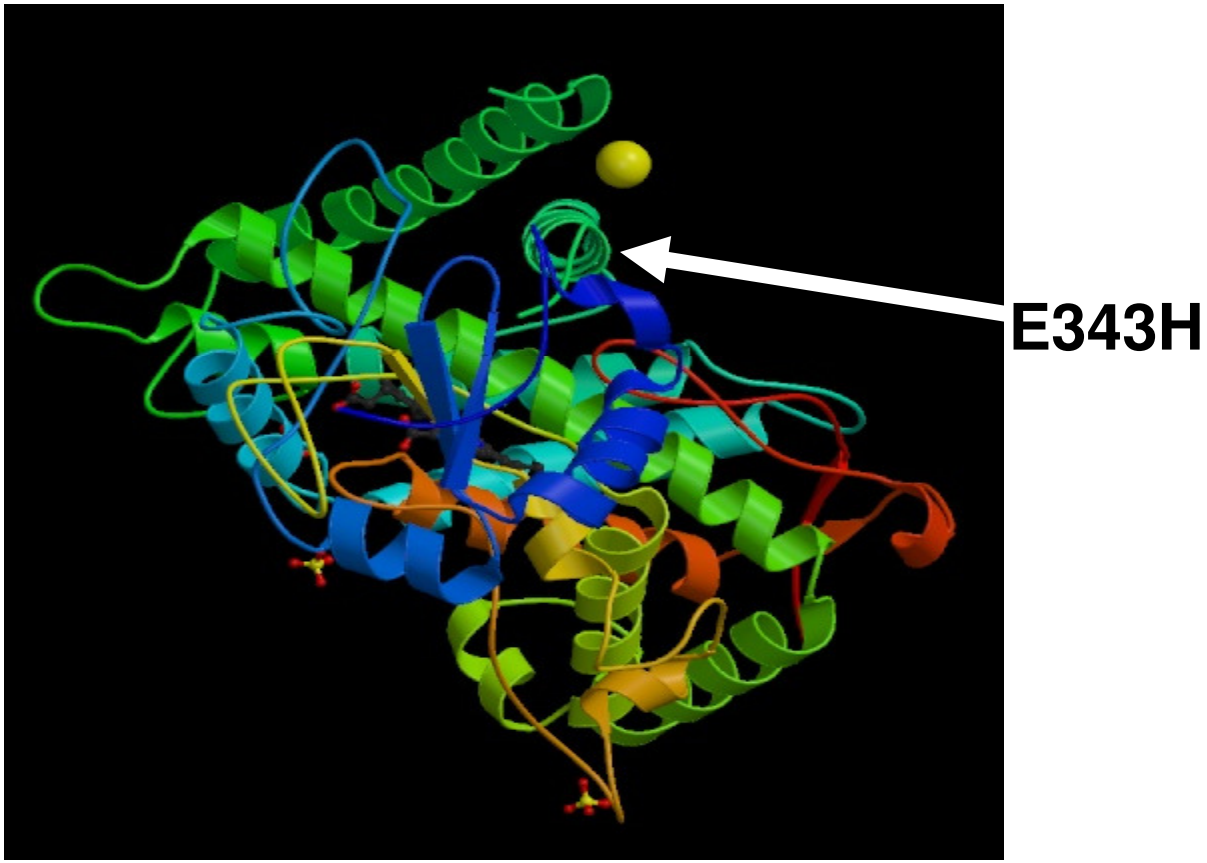


# Protein Formulation

- Protein sequence modification (site directed mutagenisis)
- PEGylation
- Proteinylation
- Peptide Micelles
- Formulating with permeabilizers

# Site Directed Mutagenesis

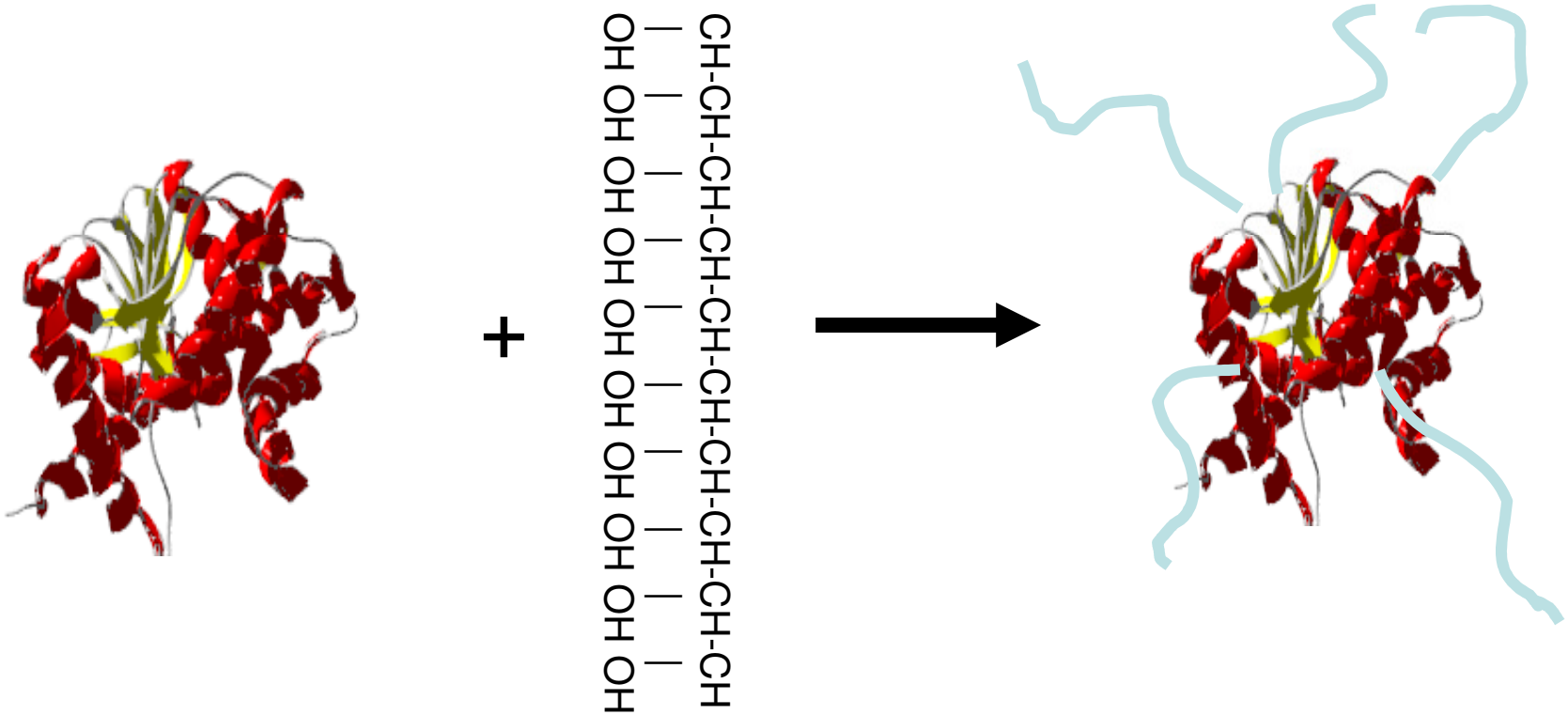
## Human ferrochelatase



# Site Directed Mutagenesis

- Allows amino acid substitutions at specific sites in a protein
- i.e. substituting a Met to a Leu will reduce likelihood of oxidation
- Strategic placement of cysteines to produce disulfides to increase Tm
- Protein engineering (size, shape, etc.)

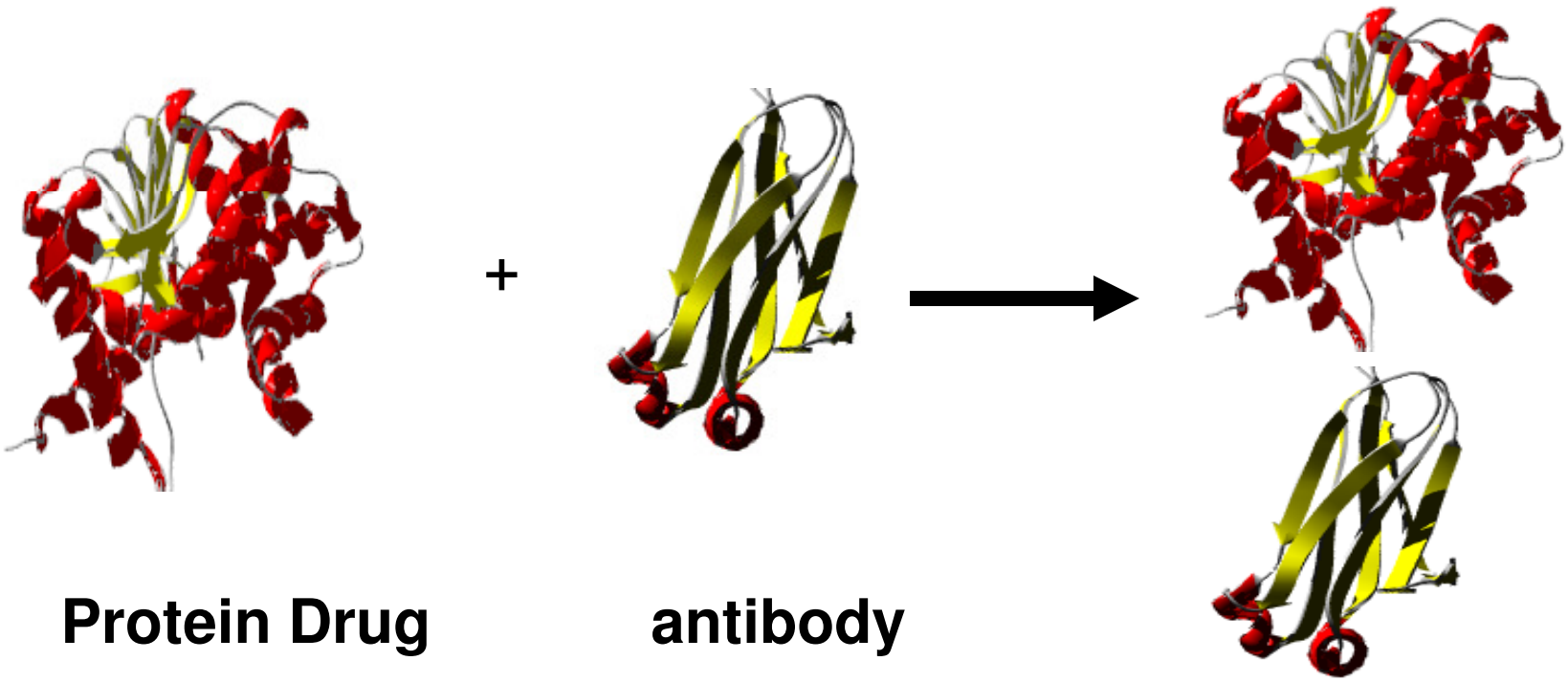
# PEGylation



# PEGylation

- PEG is a non-toxic, hydrophilic, FDA approved, uncharged polymer
- Increases in vivo half life (4-400X)
- Decreases immunogenicity
- Increases protease resistance
- Increases solubility & stability
- Reduces depot loss at injection sites

# Proteinylation



Protein Drug antibody

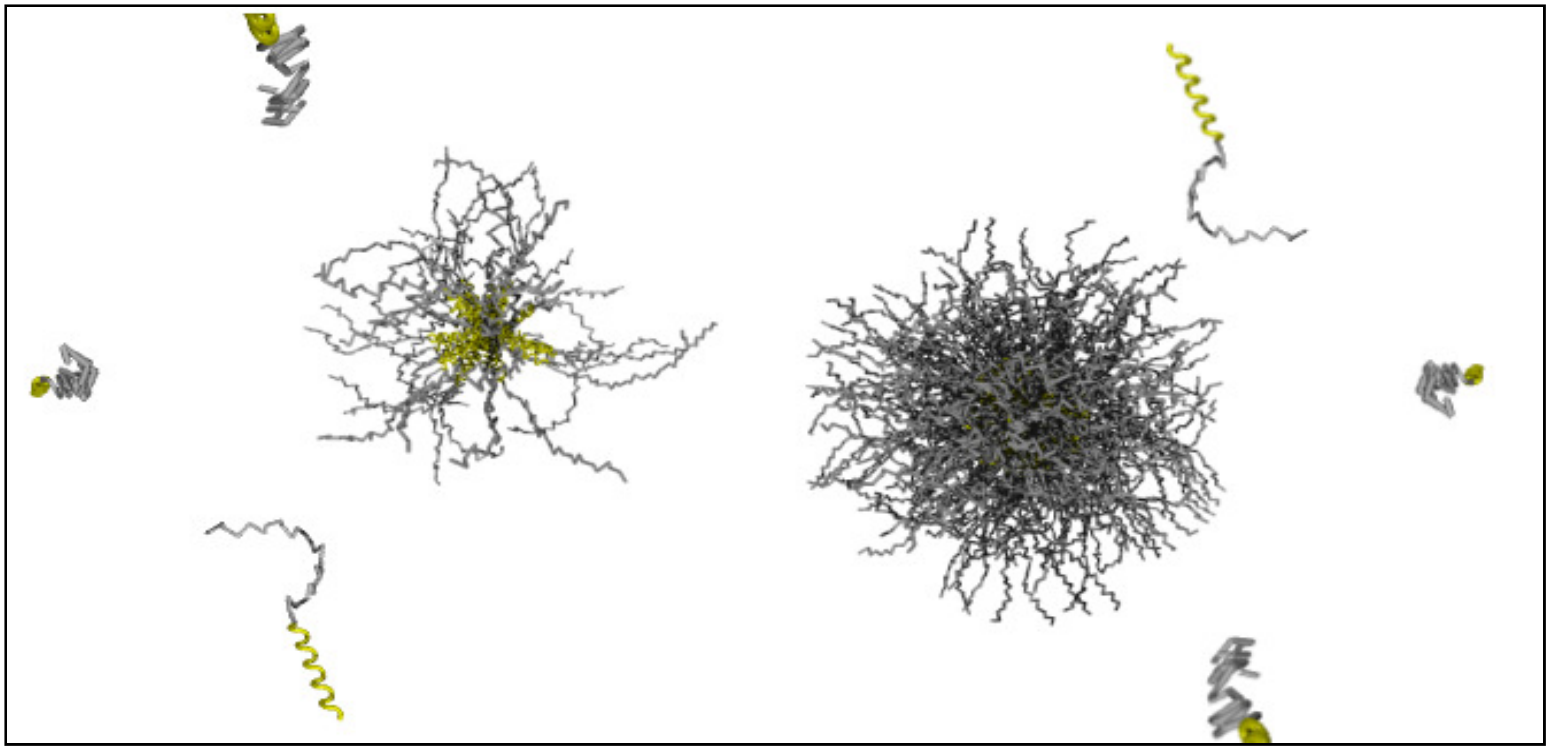
# Proteinylation

- Attachment of additional or secondary (nonimmunogenic) proteins for in vivo protection
  - Increases in vivo half life (10X)
- Cross-linking with Serum Albumin
- Cross-linking or connecting by protein engineering with antibody fragments

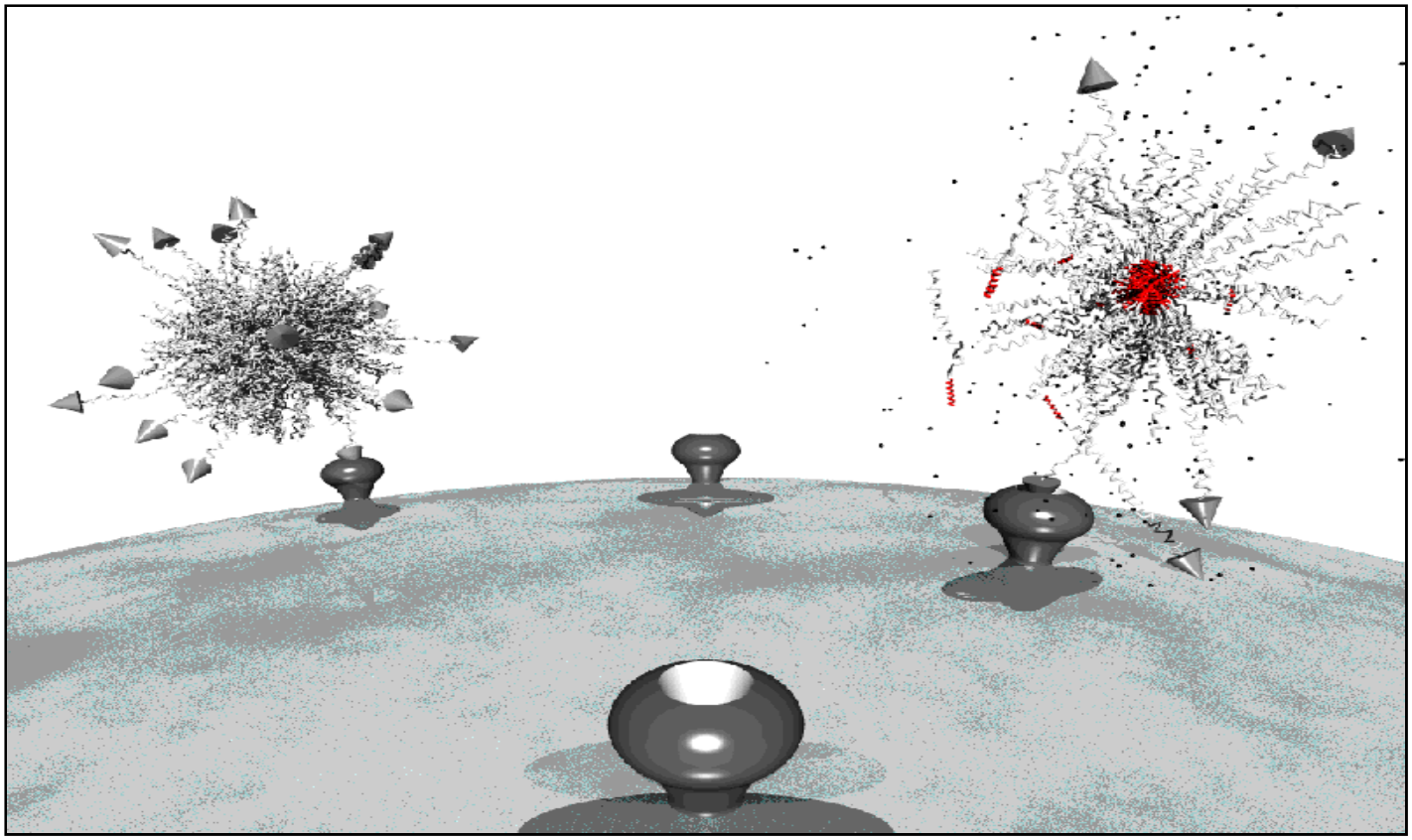
# Formulation with permeabilizers

- Salicylates (aspirin)
- Fatty acids
- Metal chelators (EDTA)
- Anything that is known to "punch holes" into the intestine or lumen

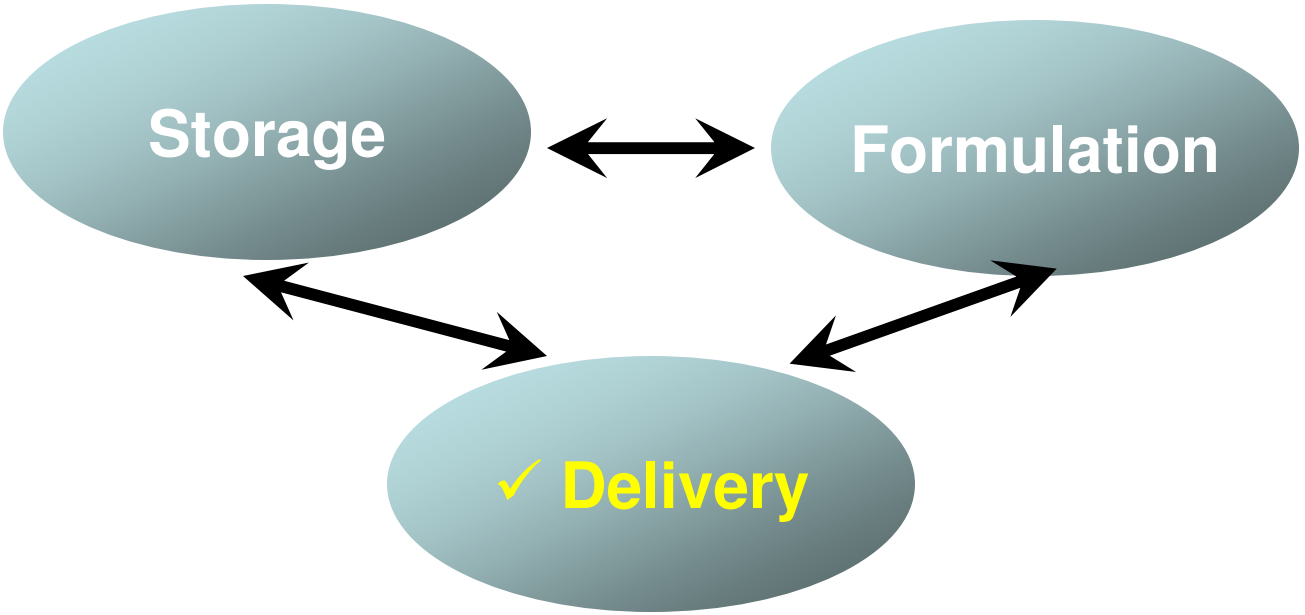
# Peptide Micelles



# Targeted Micelles



# How to Deal with These Problems



# Polymeric Drug Delivery

ADV:

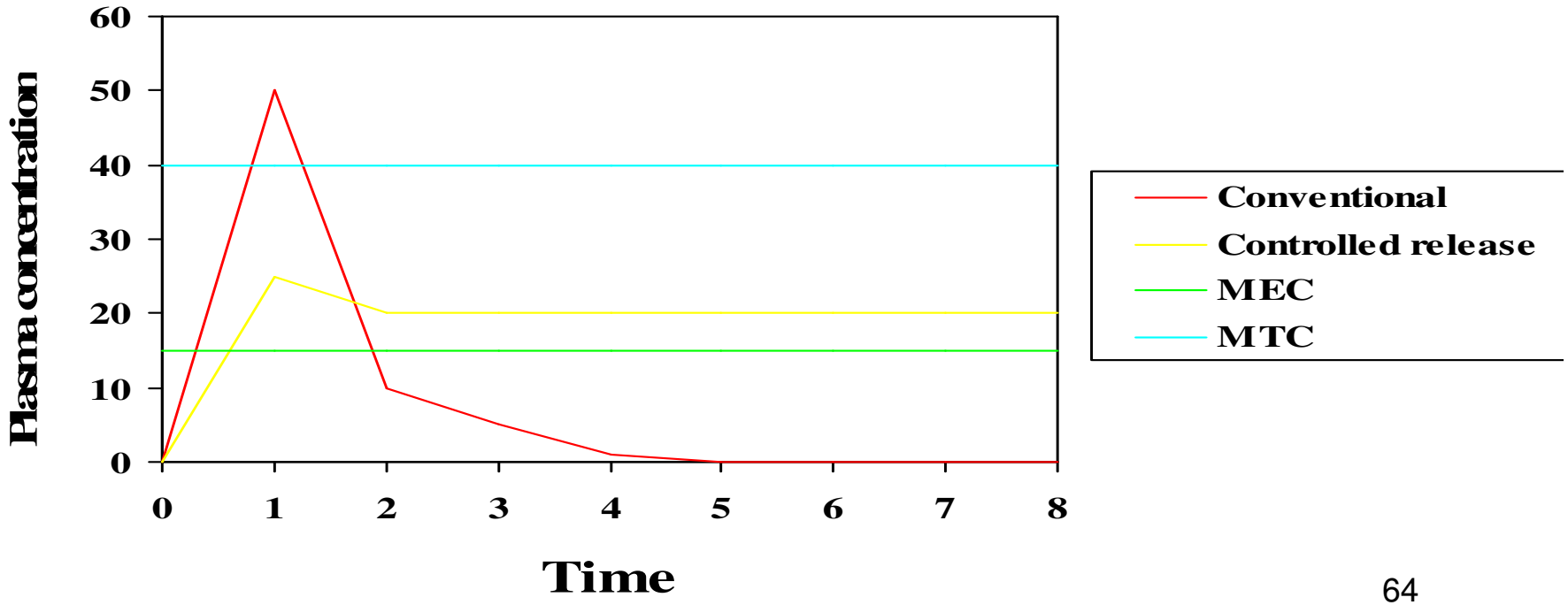
- Frequency of doses reduced
- Drug utilized more effectively
- Drug stabilized inside the polymer matrix
- Reduced side effects

DISADV:

- Possibility of dose-dumping
- De-activation of drug inside polymer

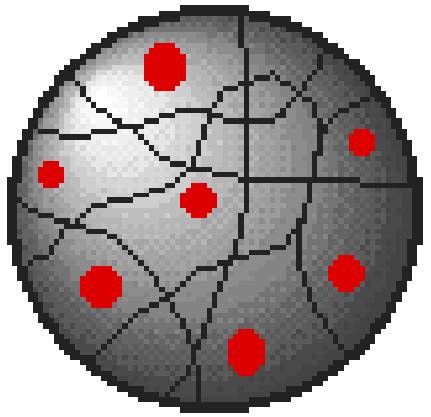
# Polymeric Drug Delivery

- Controlled Release of drugs



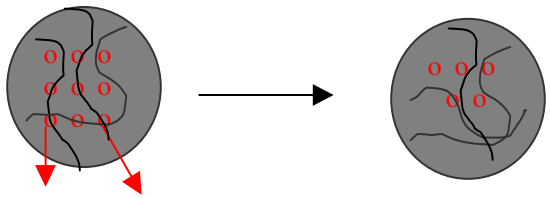
# Polymeric Drug Delivery

- Polymers should be:
  - Biodegradable
  - Bio-compatible
  - Non-toxic
- Examples:
  - Polylactides/glycolides
  - Polyanhydrides
  - Polyphosphoesters



# Polymeric Drug Delivery

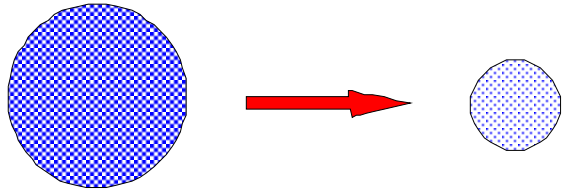
- Diffusion of drug out of the polymer
  - Governing equation: laws of diffusion
  - Drug release is concentration dependant



- Less applicable for large molecules

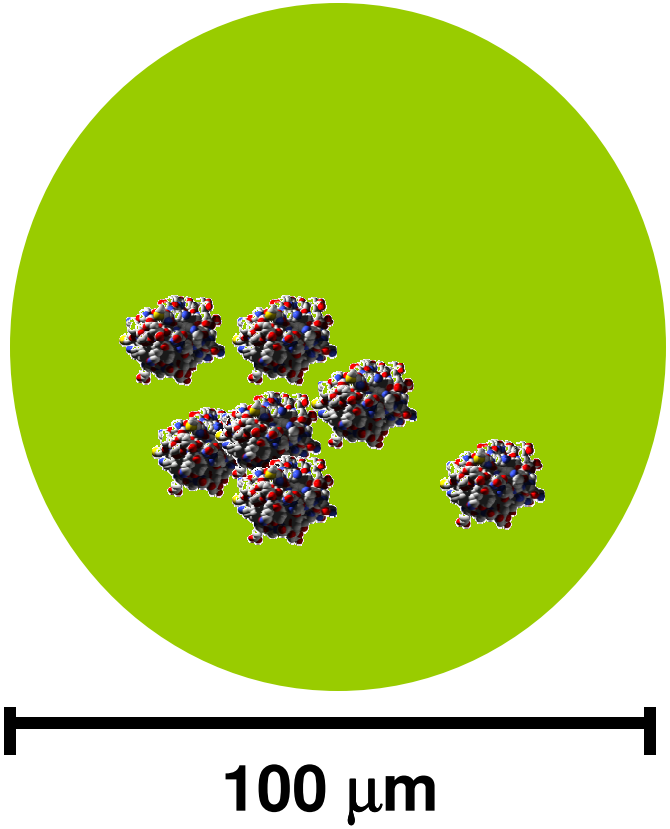
# Polymeric Drug Delivery

- Drug Release by Polymer Degradation



- Polymer degradation by:
  - Hydrolysis
  - Enzymatic (Phosphatases; Proteases etc.)

# Microsphere Encapsulation



# Encapsulation

- Process involves encapsulating protein or peptide drugs in small porous particles for protection from “insults” and for sustained release

- Two types of microspheres
  - nonbiodegradable
  - biodegradable

# Types of Microspheres

- Nonbiodegradable
  - ceramic particles
  - polyethylene co-vinyl acetate
  - polymethacrylic acid/PEG
- Biodegradable (preferred)
  - gelatin
  - polylactic-co-glycolic acid (PLGA)

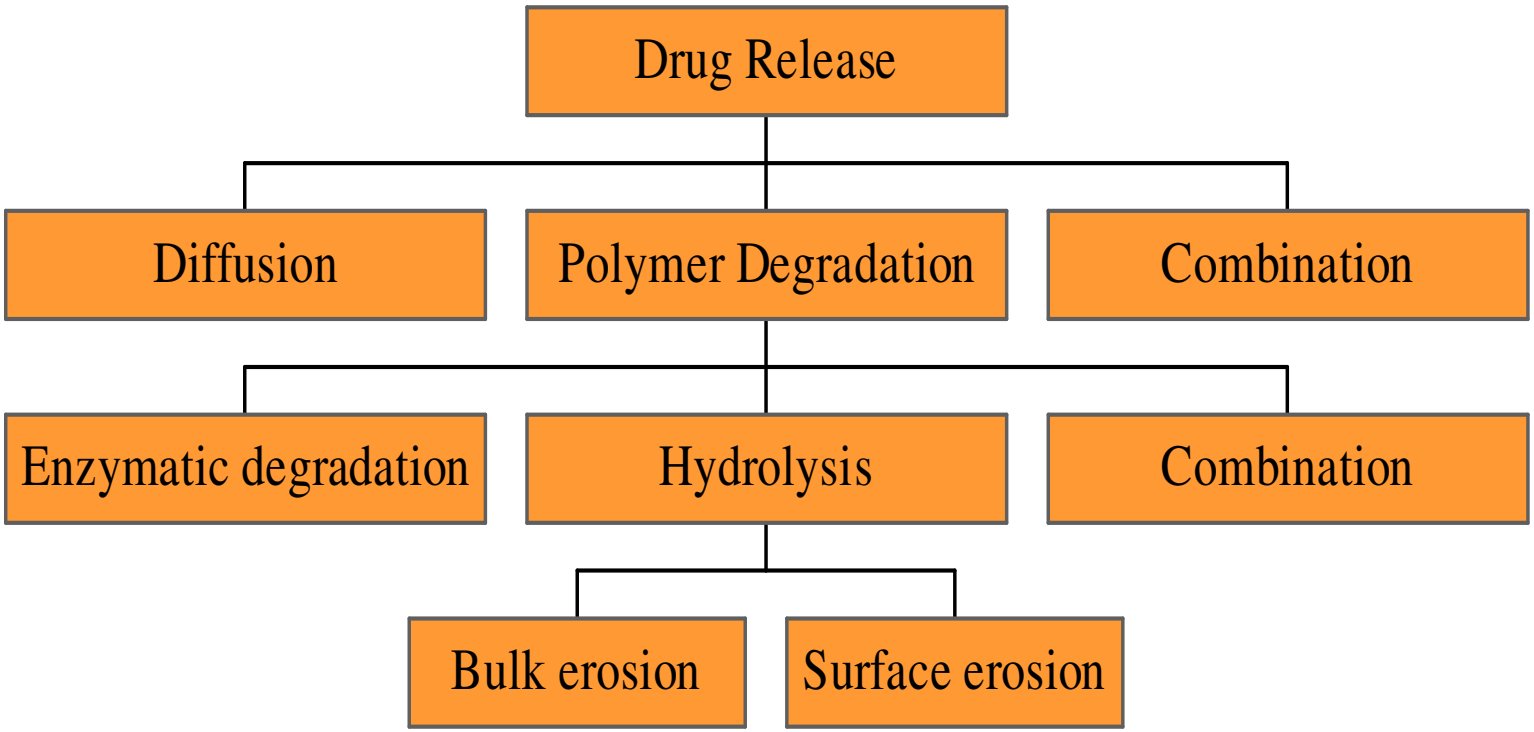
# Microsphere Release

- Hydrophilic (i.e. gelatin)
  - best for burst release
- Hydrophobic (i.e. PLGA)
  - good sustained release (esp. vaccines)
  - tends to denature proteins
- Hybrid (amphipathic)
  - good sustained release
  - keeps proteins native/active

# Polymer Scaffolds

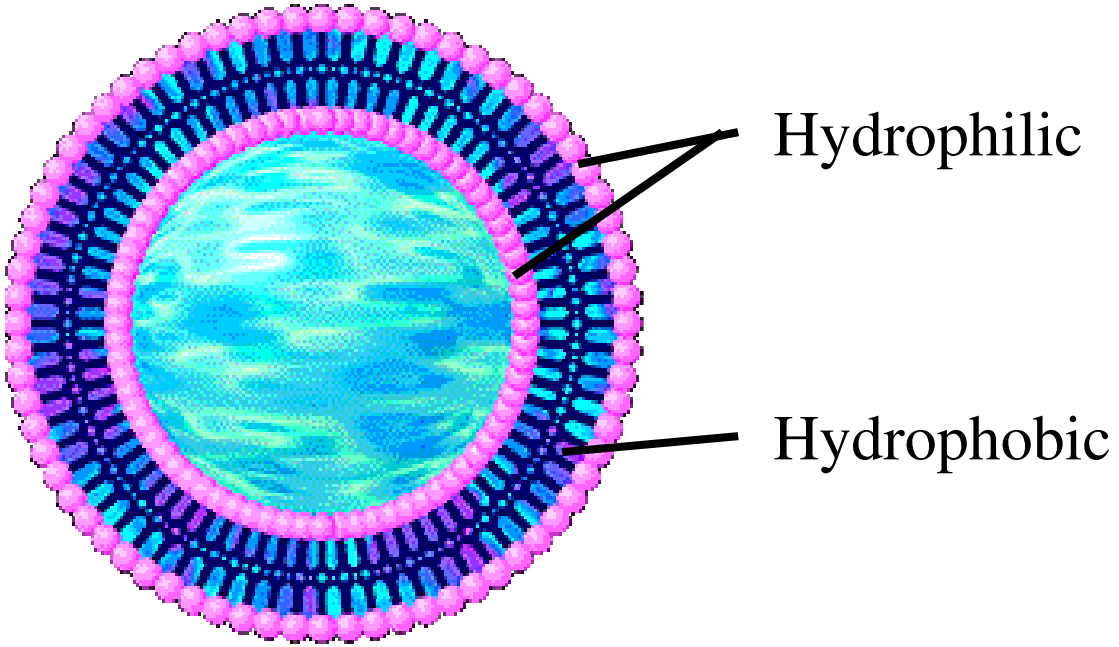
- Incorporate drug into polymeric matrix
- Protection of drug from enzymatic degradation – particularly
- Applicable to *peptide* and protein drugs
- Release drug at known rate over prolonged duration
- Drug dispersed or dissolved in suitable polymer
- Release
  - diffusion of drug through polymer
  - diffusion through pores in polymer structure
  - therefore different release profiles result (dissolved or dispersed)

# Release Mechanisms



# Liposomes

Spherical vesicles with a phospholipid bilayer

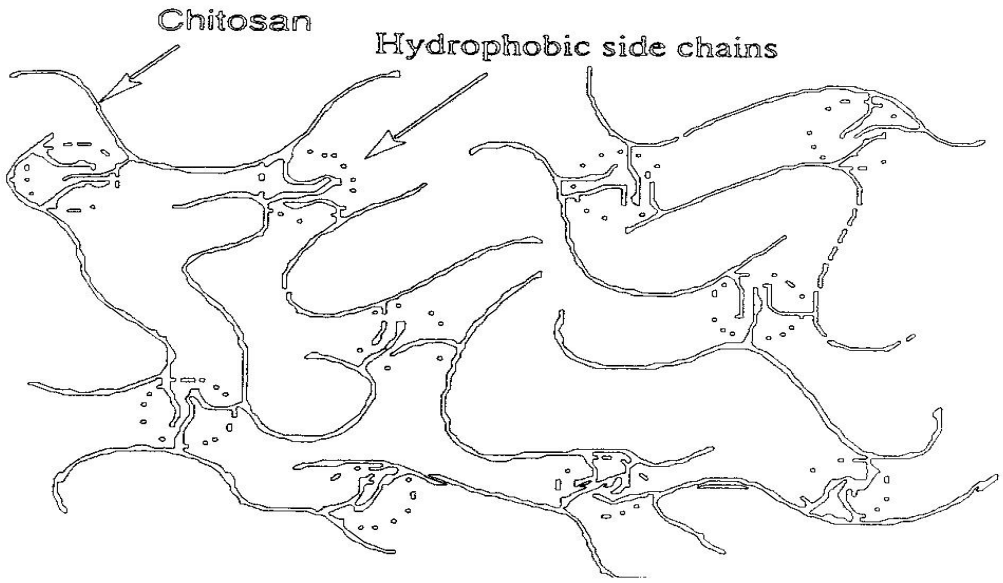


# Liposomes Drug Delivery

- Potential of liposomes in drug delivery has now realized
- Bloemycin encapsulated in thermosensitive liposomes enhanced antitumor activity and reduced normal tissue toxicity
- S.C injection of negatively charged liposomes produced a prolonged hypoglycemic effect in diabetic dogs
- Liposomes have recently been used successfully as vehicles for vaccines

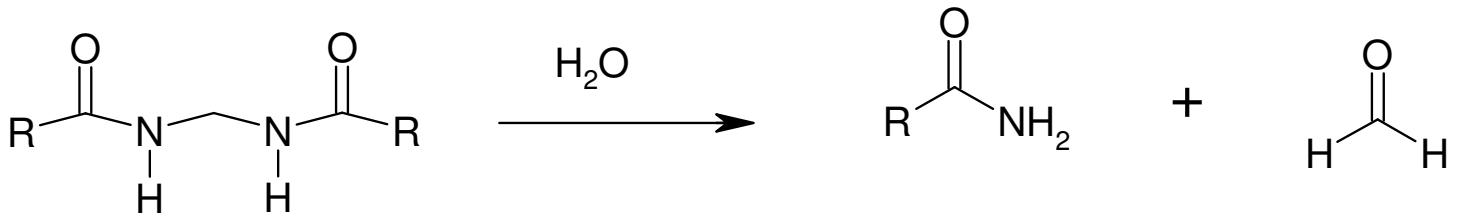
# Hydrogel Based Drug Delivery

**Hydrogels are three dimensional networks of hydrophilic polymers that are insoluble**



# Hydrogel Based Drug Delivery

Hydrogels can swell as a result of changes in pH, Temp., ionic strength, solvent composition, pressure and the application of electric fields




R = polymer backbone

Insulin has been one drug that has been incorporated in hydrogels and investigated by researchers extensively

# Conclusion

- **No two master cell banks are exactly alike**
- **The process is the product**
- **How similar is similar enough?**
- **These differences clearly apply to biosimilars as well as to original biopharmaceuticals**

# References


- http://www.emea.europa.eu
- USP, General Chapters, Anthony J. DeStefano, Ph.D. Vice President
- Schellekens H. Trends Biotechnol 2004;22:406-10
- NATURE REVIEW DRUG DISCOVERY Vol 7, Sept. 2008
- EGA, Ministry of Health HU, National Institute for Strategic Health Research
- Biosilmilars, Siriwan  Chaisomboonpan, Bureau of Drug and Narcotic, Dec 2008
- AMGEN, Montreal Forum Pharmaceutical Discussions, Apr 2010
- Engel & Novitt, LLP, 2nd Annual Biotech Supply Chain Academy, Oct 2009
- Brigham and Women's Hospital, Harvard Medical School
- Manufacturing differences between biopharmaceuticals and low molecular weight drugs, Basant Sharma, PhD Vice President, Pharmaceutical Technology Centocor Raritan, New Jersey, USA
- Development of Biopharmaceuticals and Biosimilar Drug Delivery, Dr. Basavaraj K. Nanjwade M.Pharm., Ph.D, KLE University's College of Pharmacy, Belgaum-590010

# Discussion

## Q&A

# Thank You!

Wael Ebied
Technical Quality Assurance Sr. Manager
Management Representative & Qualified Person
SEDICO Pharmaceuticals Co.
Egypt
www.sedico.net
wael.ebied@sedico.net
Wael.ebied@sqaservices.com