PDM
Pharmacokinetics, Dynamics and Metabolism

Pfizer

Biotherapeutics Drug Development

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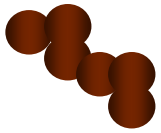
Outline

- Background
- Therapeutic Modalities
- Examples of Challenges for Biotherapeutics
- Bioanalytical Overview
- Summary

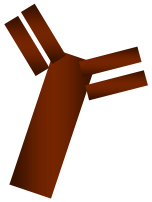
What are Biotherapeutics

- The term Biotherapeutics usually refers to therapeutic materials produced using biological means, including recombinant DNA technology.

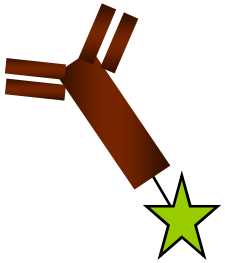
Types of Biopharmaceutical "Modalities"



Recombinant protein



Antibodies



Antibody-conjugates

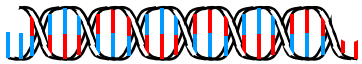
FC fusion proteins

Other engineered molecules

Vaccines

Antibody fragments 

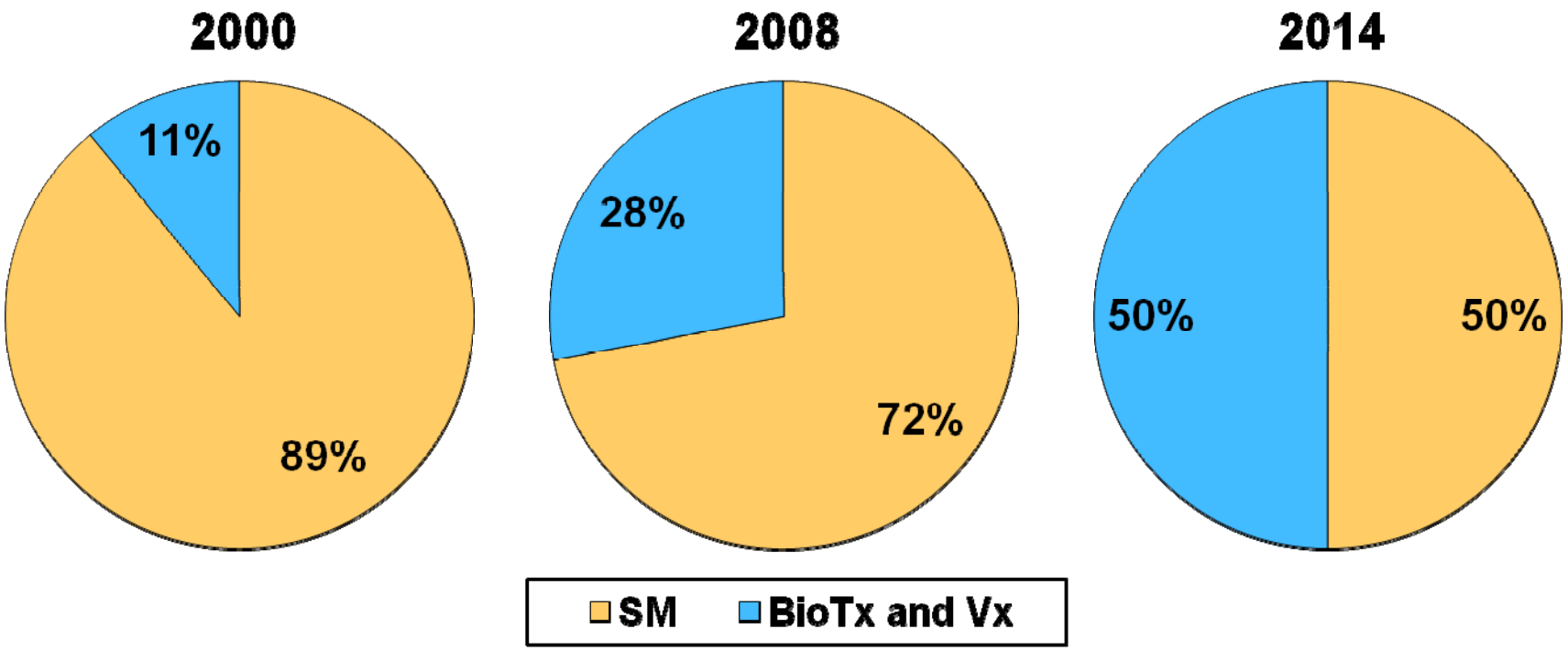
Peptides



Oligonucleotides

Slide modified from Bonnie Rup

Biotherapeutics (including vaccines) are predicted to compromise 50% of Top 100 products by 2014



Slide Courtesy of Arin Bose

Source: EvaluatePharma

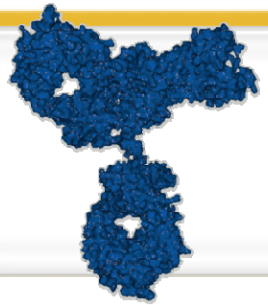
Advantages To Biotherapeutics

- Favorable attrition rate
- Inherently highly specific for the target
- Minimum risk for non-mechanism based toxicity and safety issues
- Can augment bodies normal growth factors, hormones, and enzymes
- Able to modulate protein/protein interactions intractable to small molecules
- Applicable in multiple therapeutic areas and for a variety of targets

Slide Courtesy of Arin Bose

Background:
How are biotherapeutics different?

Small-Molecule Pharmaceuticals



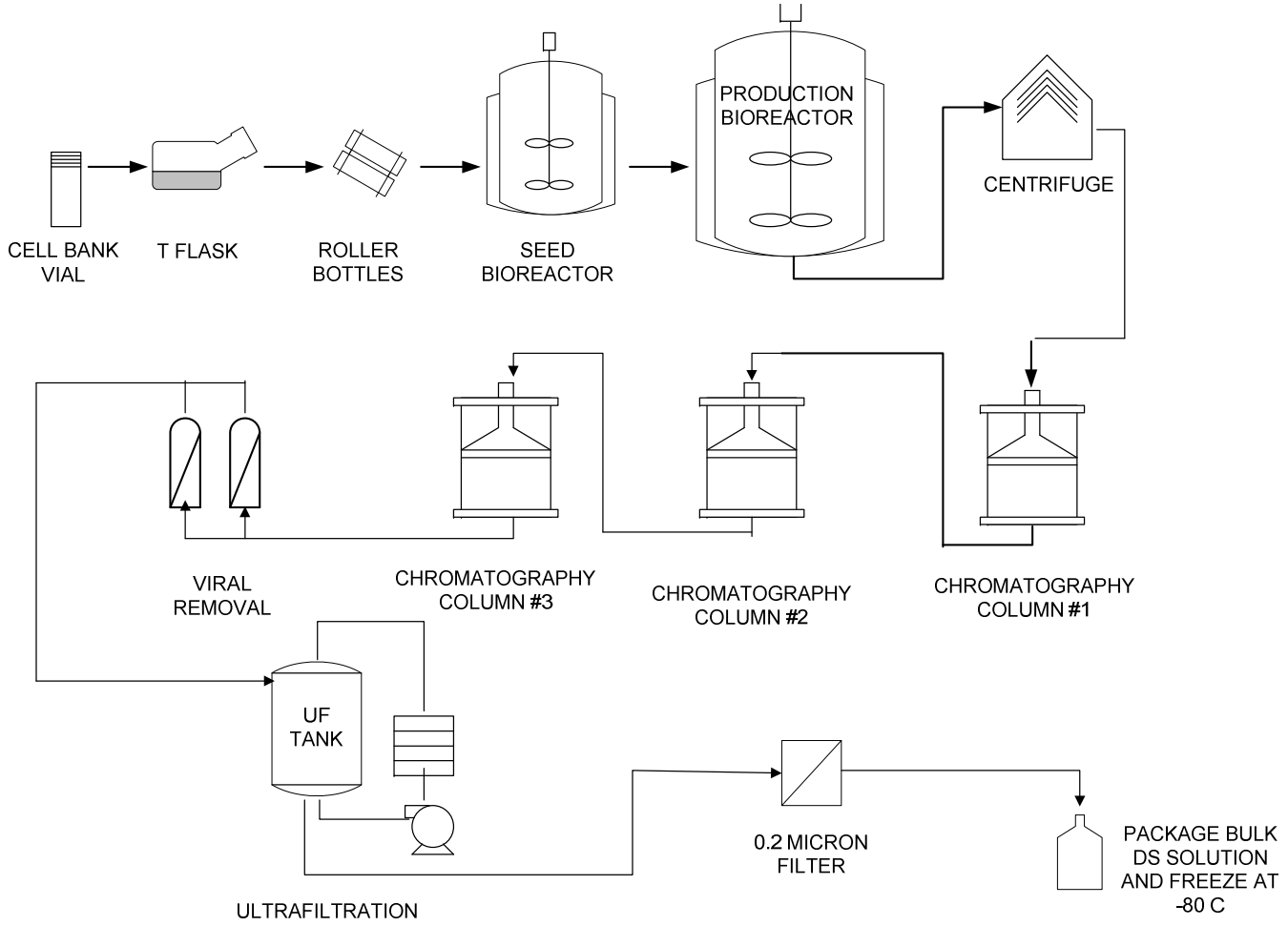
Large-Molecule Therapeutics

- Small molecule drugs are organic or metallic compounds which bind with proteins in the body, thereby altering their function and their role in disease
- Size is <600 Da
- Typically made utilizing chemistry synthesis
- Work intra-cellularly
- Less specificity
  - Can inhibit multiple targets in family
  - Unspecific off-target toxicities
- Easier to deliver (often oral, e.g., aspirin, antibiotics)
- Generally cheap to manufacture (low COGS), and easy to replicate after patent expiration (the high cost is the initial development)

- Large molecule therapeutics treat diseases using biological matter, e.g., proteins, monoclonal antibodies, peptides, RNA, cells, vaccines, etc.
- Size is ~150,000 Da
- Typically grown and extracted from living cells
- Work extra-cellularly
- High specificity limits toxicities
- Technology IP restrictions often resulting in royalties to companies which have patented means of producing biologics
- Difficult to deliver (usually must be injected)
  - Difficult to affect targets inside the cell
- Generally expensive to manufacture

Slide Courtesy of Arin Bose

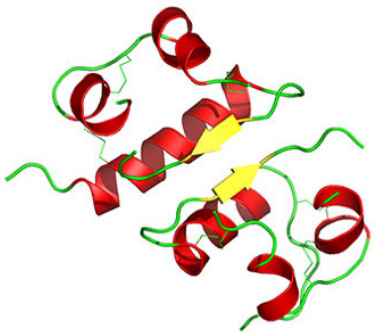
Typical Biologics Manufacturing Process: Drug Substance



Slide Courtesy of Arin Bose

Recombinant Proteins

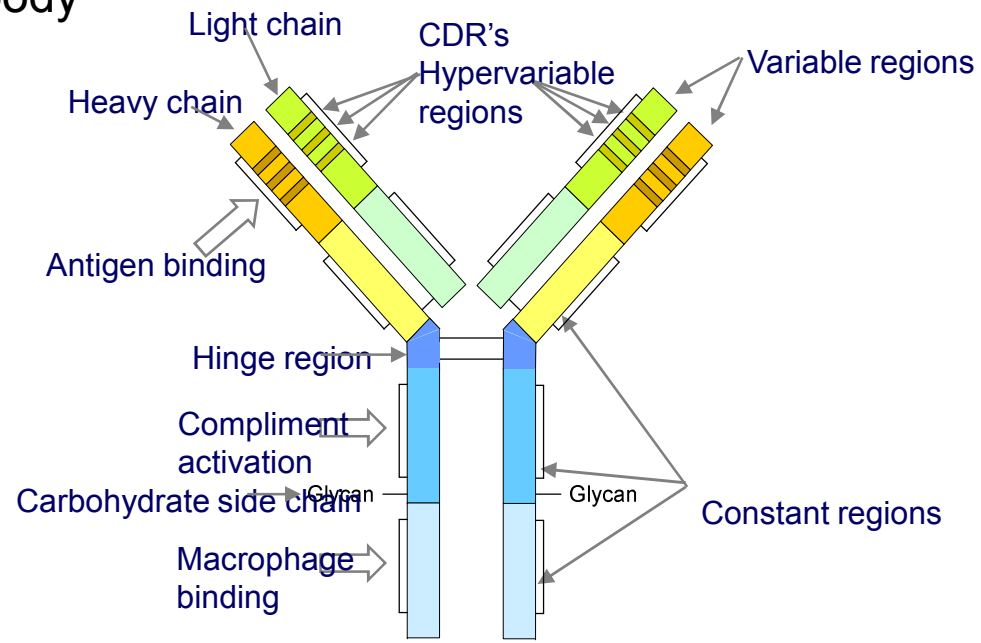
- First products (human insulin, growth hormone) approved in the early 80's
- Initial products were recombinant versions of natural proteins; recent shift to altered forms as well as to highly engineered proteins
- Very diverse set of products with dissimilar properties and modes of action
- Technologies vary from product to product though there are some aspects of a "platform"



Monoclonal Antibodies

- Designed to bind very specifically to a target
- Can function via several different mechanisms of action
  - Bind to target and stimulate cell mediated immune response
  - Block protein ligand binding to its receptor

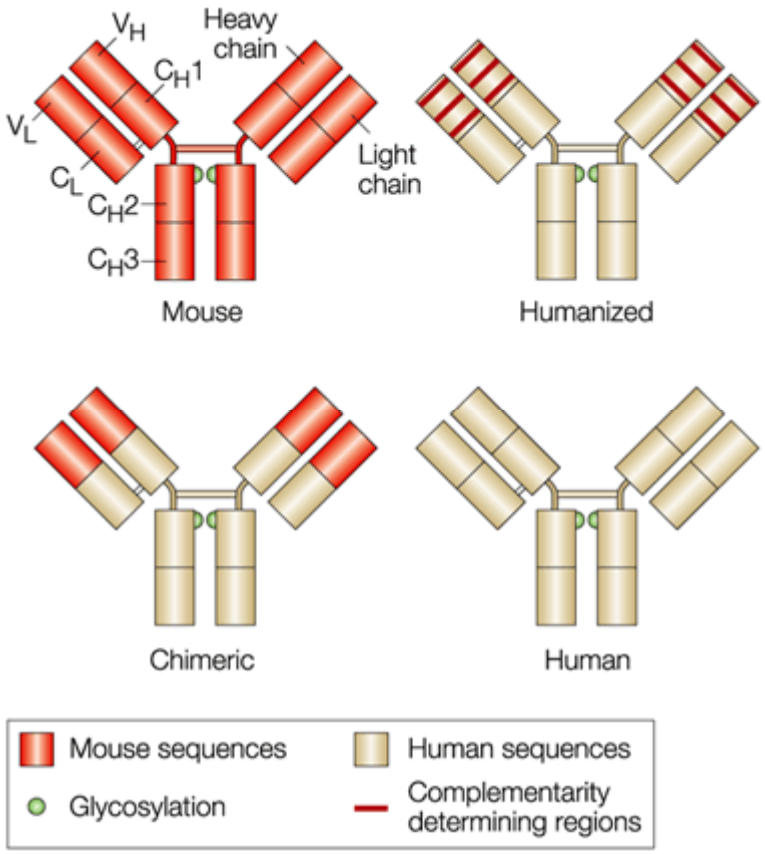
IgG2 Monoclonal Antibody



Monoclonal Antibodies

- Different sub-classes: IgG1, IgG2, IgG4
- Different frames: Murine, Chimeric, Humanized, Fully human
- Can be directed against a soluble or membrane bound target
- Mechanism include direct binding to the target or ADCC (Antibody Dependent Cellular Cytotoxicity: where antibodies coat target cells making them vulnerable to immune response)
- Can be either an antagonist or agonist

Antibody Frames



Nature Reviews | Cancer

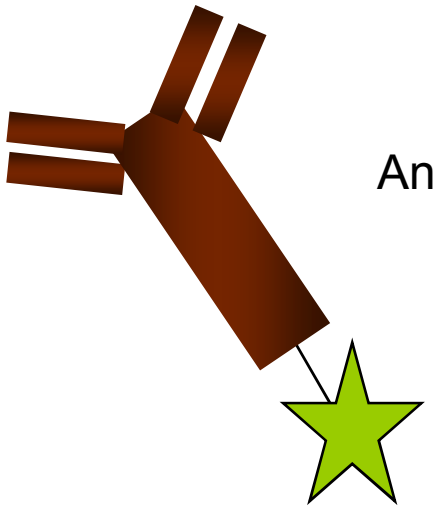
FROM THE FOLLOWING ARTICLE:

Improving the efficacy of antibody-based cancer therapies

Paul Carter

Nature Reviews Cancer **1**, 118-129 (November 2001)

Antibody-Drug Conjugates (ADCs)

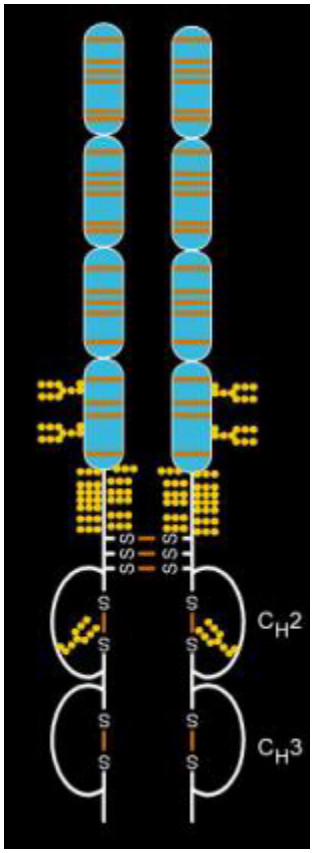


An antibody carrier attached to a payload via a linker

- ADCs can be thought of as:
  - Prodrugs
  - Sophisticated delivery systems for Payload drug delivery

FC Fusion Proteins

Fusion proteins also called **chimeric proteins** are proteins created through the fusing of two or more genes which originally coded for separate proteins.

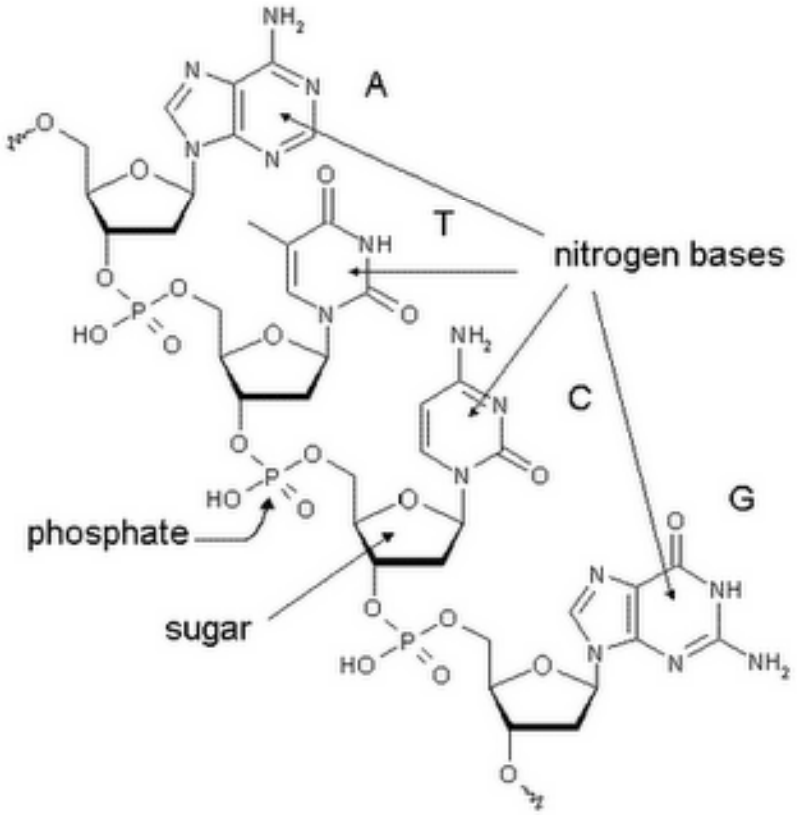


Ex. Enbrel: TNF receptor 2 fused to an Fc component of IgG1

Oligonucleotides?

- Are short nucleic acid polymers, typically with 50 or fewer bases
- Each monomer is made up of a sugar, phosphate, and heterocyclic base
- Can be chemically synthesized

Base
Adenine (A)
Cytosine (C)
Guanine (G)
Thymine (T)
Uracil (U)



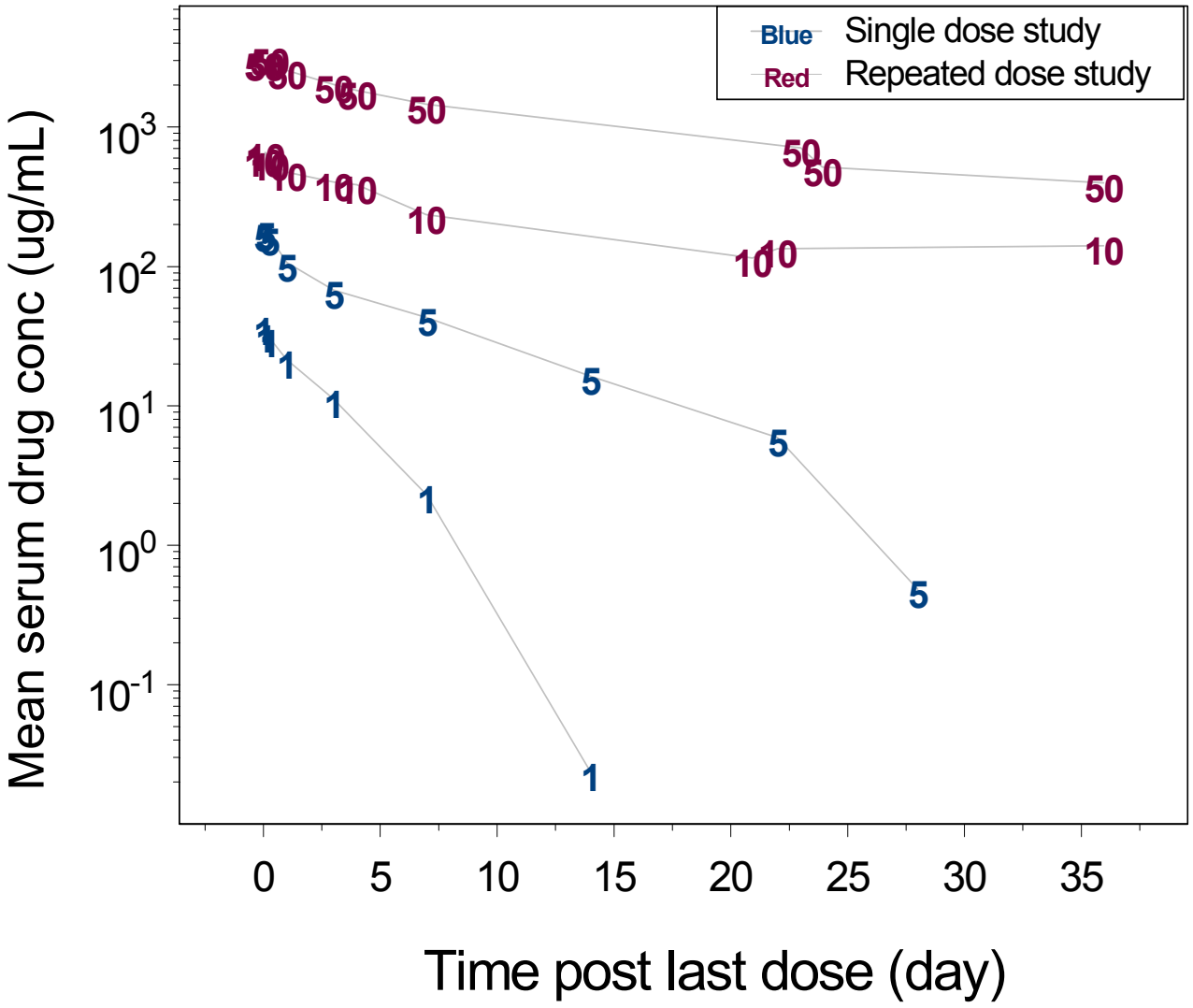
Additional Challenges For Biotherapeutics

- Species specificity may limit standard preclinical models for safety testing
- Delivery options are currently limited (main routes are intravenous and subcutaneous)
- Target Mediated Disposition may lead to **Nonlinearity**
- Manufacturing is significantly more complex and a critical factor in safety and efficacy thus manufacturing changes have to be carefully assessed (**Comparability**).
- Products can lead to **immunogenicity** where the body mounts an immune response to the product. This is especially true for products that contain other species components (i.e. giving human protein to animals for safety studies).

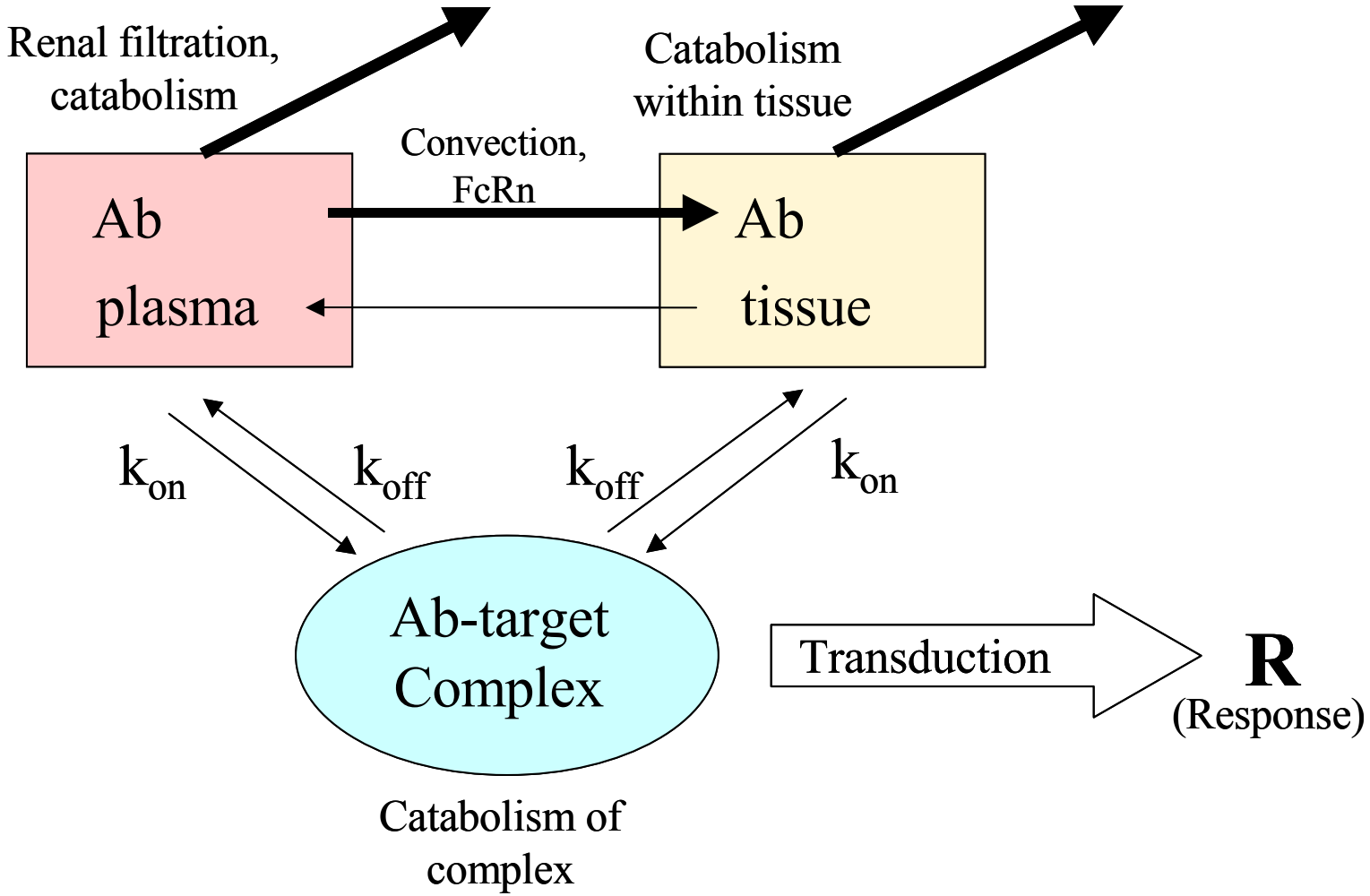
Examples of Special Biotherapeutics Considerations

- Nonlinearity
- Comparability
- Immunogenicity

Nonlinearity

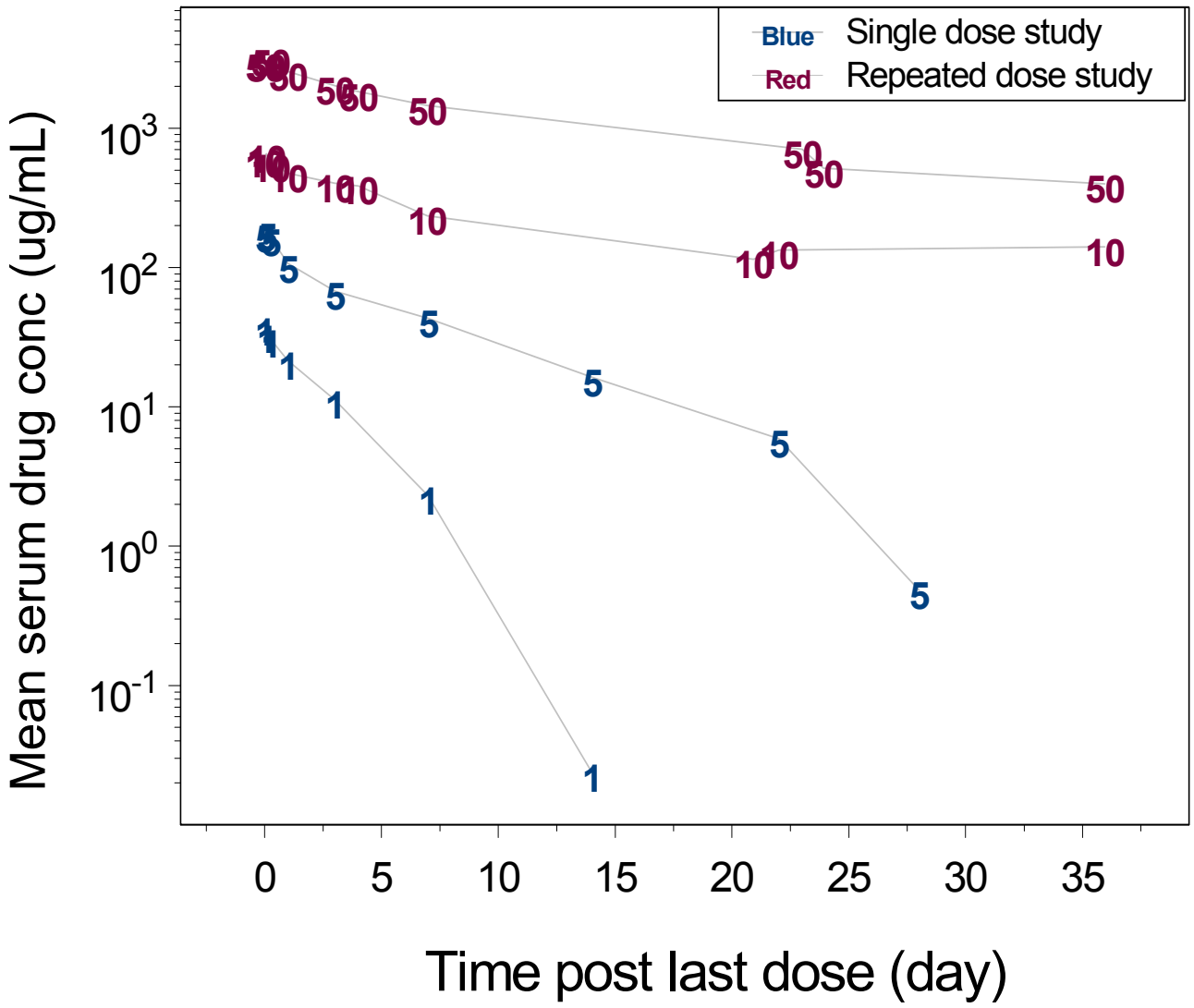


Antibody PK and PD: General Scheme



Lobo et. al. ***J. Pharmaceutical Sci.*** Vol 93. No. 11 pp 2645 - 2668

Nonlinearity: Target Mediated Disposition



Comparability

Understanding potential changes in a product due to manufacturing changes and the subsequent impact on the product

Comparability: Definition

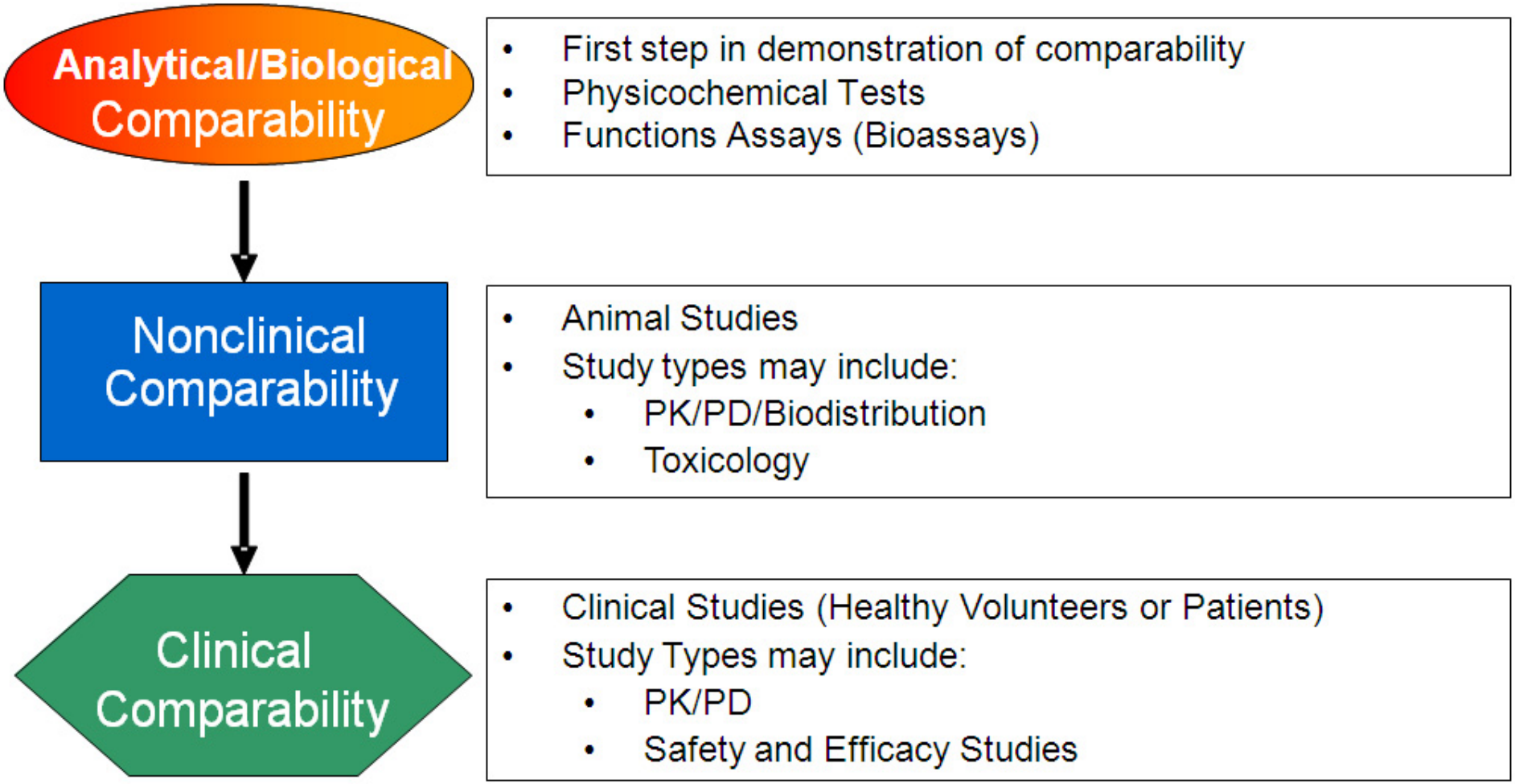
- Comparable means “highly similar” not necessarily “identical”
- ICH Q5E
  - “The demonstration of comparability does not necessarily mean that the quality attributes of the pre-change and post-change products are identical; but that they are highly similar and that the existing knowledge is sufficiently predictive to ensure that any differences in quality attributes have no adverse impact upon safety and efficacy of the drug product”.

Triggers for Comparability Studies

Potential changes to manufacturing process

1. Expression system
   - Master cell bank
   - Working cell bank
2. Fermentation/culture process
   - Raw materials, cell culture conditions, scale, equipment, site change
3. Purification process
   - Column/resin, reagents, scale, site, equipment
4. Formulation and filling
   - Excipient, liquid to lyophilized or *vice versa,* equipment, change in manufacturing protocol, strength, scale, site change, shipping
5. Drug product
   - Batch definition, shelf-life, container/closure, shipping, storage.

Comparability: Hierarchical Process



Analytical Comparability

A typical manufacturing comparability program will assess the major aspects of biotherapeutic production and testing:

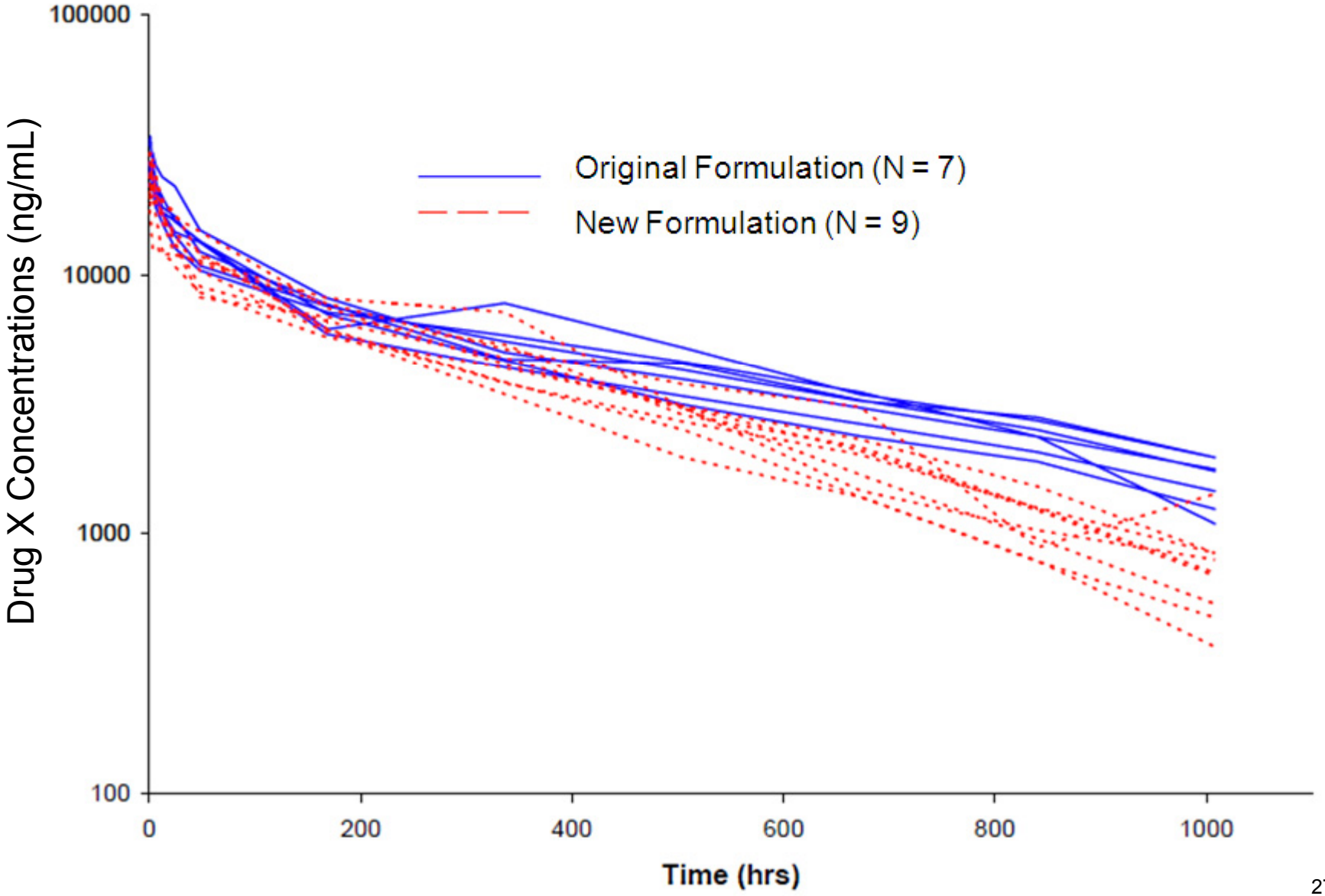
- Characterization testing
  - cell bank and drug substance, as appropriate
- Release testing
  - drug substance and drug product, as appropriate
- Stability testing
  - drug substance and drug product, as appropriate
- In-Process testing
  - cell bank performance, bulk harvest and process parameters, as appropriate

In Vivo Testing (preclinical or Clinical)

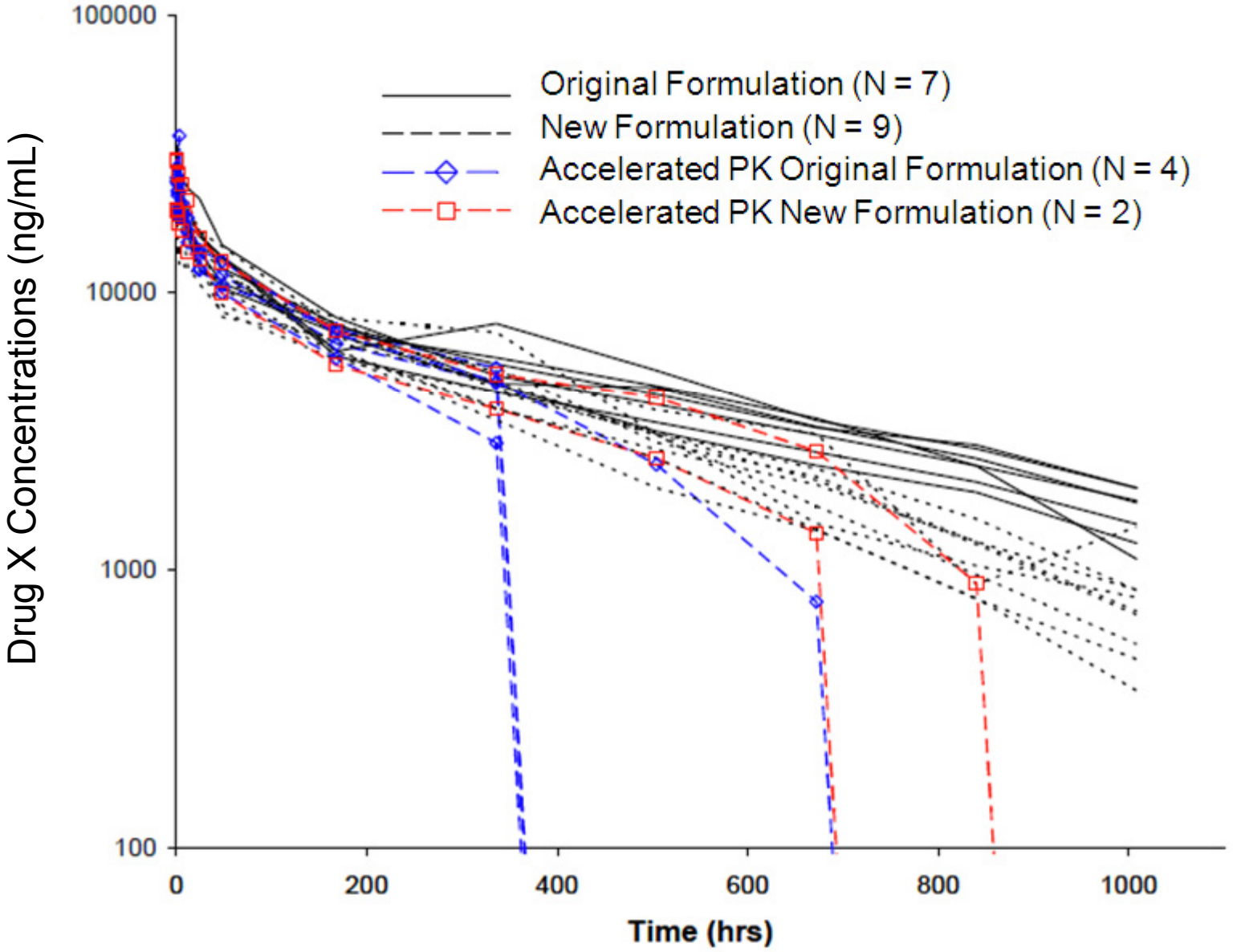
The extent of in vivo testing needed as part of the comparability exercise depends upon the:

- Product risk assessment
- Type of Manufacturing changes
- Potential for the process change to impact product characteristics
- Stage of development when the change is introduced
- Understanding of the relationship between critical product quality attributes, and safety/efficacy, both pre-clinically and clinically

Individual Plasma Drug X Concentrations Over Time in animals without immunogenicity



Individual Plasma Drug X Concentrations Over Time in all animals



Bioanalytical Concerns

- The variability of the PK assay should be considered when deciding the comparability acceptance criteria.
- The PK and immunogencity assays should be cross-validated using both the old and new material
  - If a difference is noted analytically for the two materials this is considered a flag regarding potential comparability issues even if the material passed the Manufacturing analytical analysis.
  - Reagent conjugation variability could add additional challenges for ADA bridge assay formats
- The PK assay ideally should be set up for simultaneous analysis (i.e. both sets of material qualified against a common lot).
- All parameters should be considered to determine whether there is evidence of difference in immunogenicity
  - e.g. incidence, time of onset, titers, transience

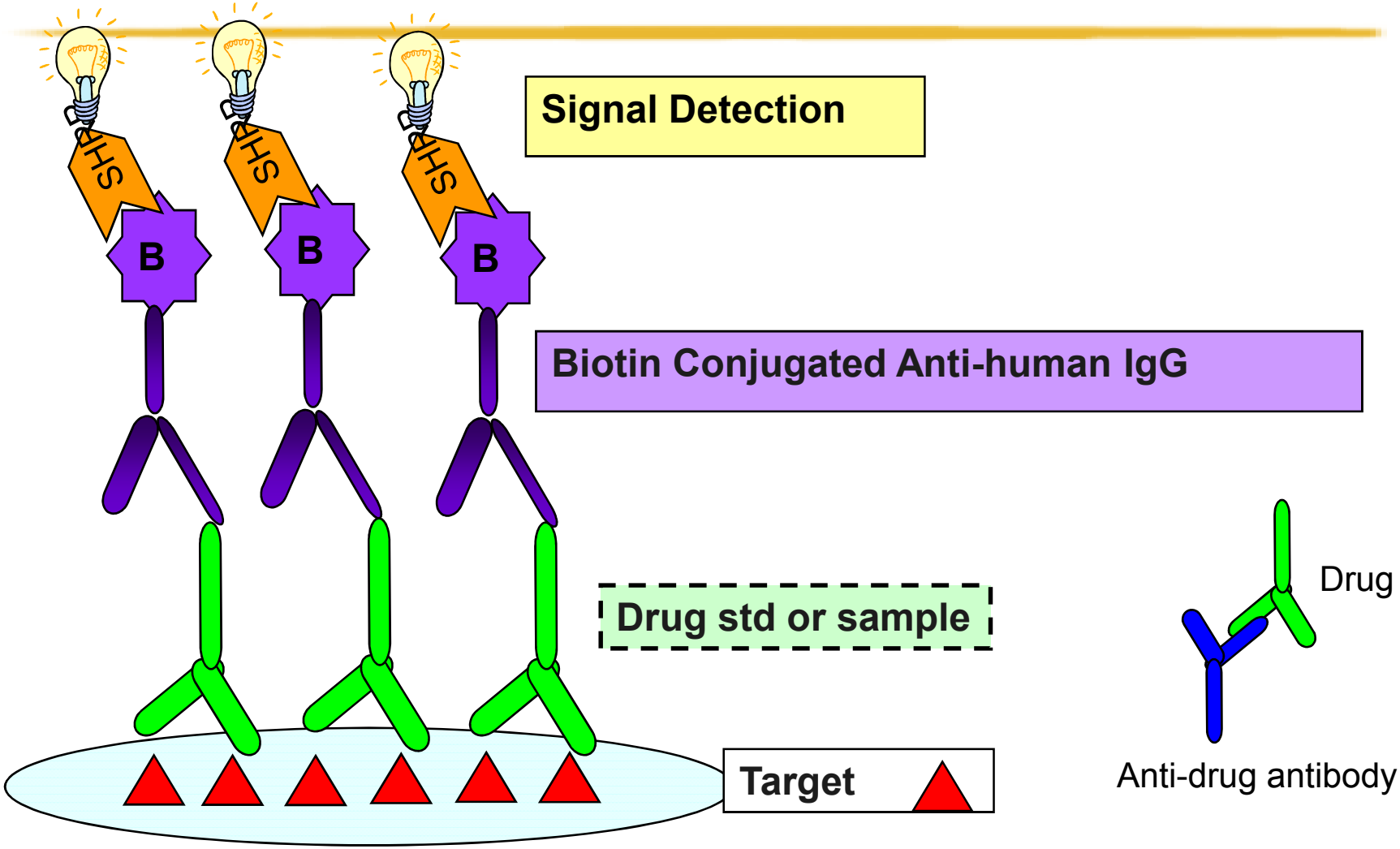
Immunogenicity

- Biological therapeutics have the potential to elicit anti-drug antibodies (ADAs) and generate unwanted immunogenicity
  - Non-neutralizing antibodies (NNab)
  - Neutralizing antibodies (Nab)
- In preclinical studies, ADA can affect drug exposure, affecting the interpretation of the toxicity, pharmacokinetic and pharmacodynamic data
- Potential to induce ADAs is a safety issue that is an important consideration in the development of biologics and a critical aspect of regulatory filing
- Immunogenicity testing is a key component in the demonstration of clinical safety and efficacy
- Areas of concern for Nab
  - **Safety**
    - Cross react with endogenous protein to induce adverse affects
  - **Efficacy**
    - Effect bioavailability, alter PK/PD (increased or decreased rate of clearance)
    - Neutralize biological effects and compromise further therapy

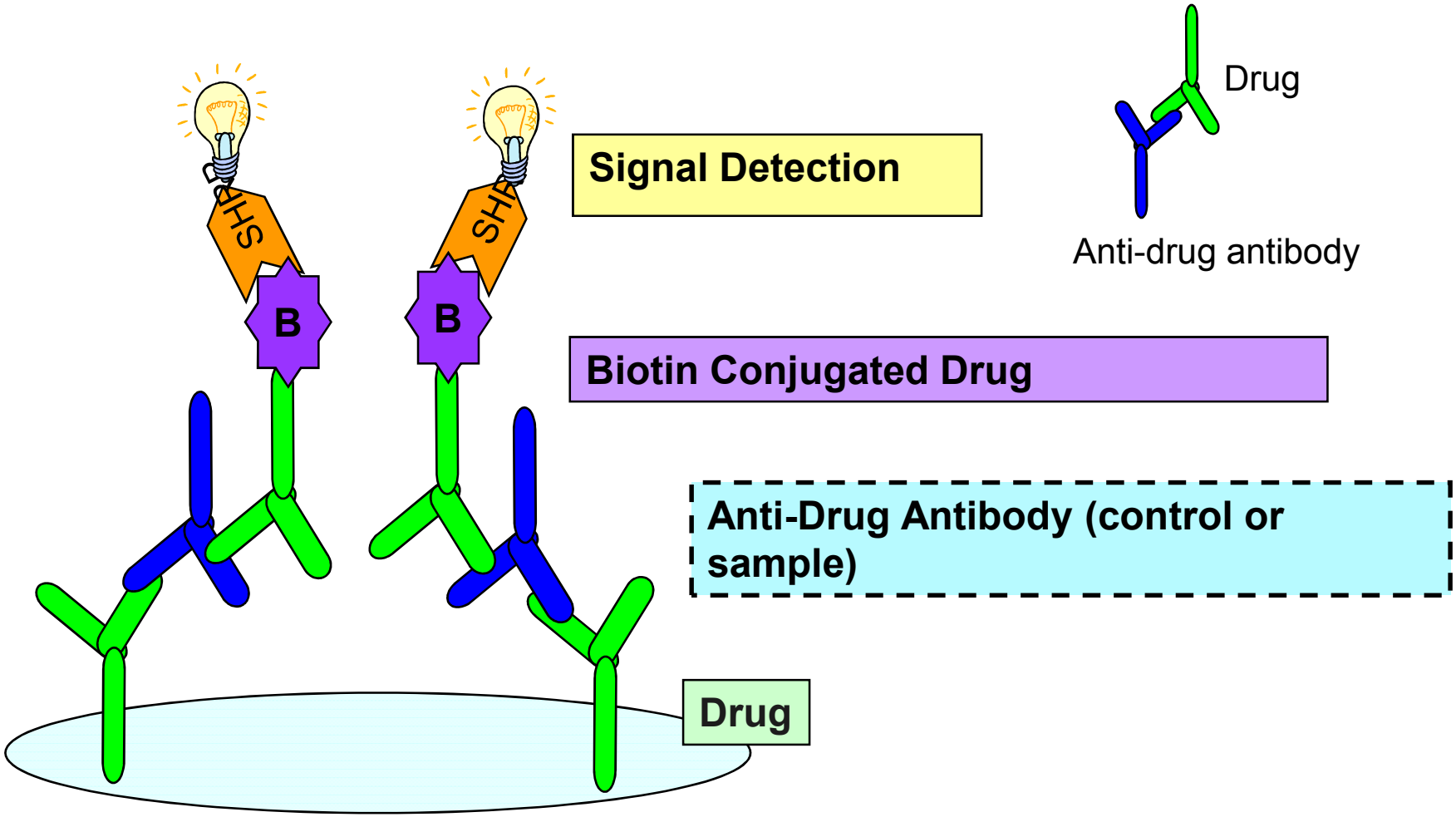
Slide Courtesy of *Corinna Krinos-Fiorotti*

Bioanalytical Overview

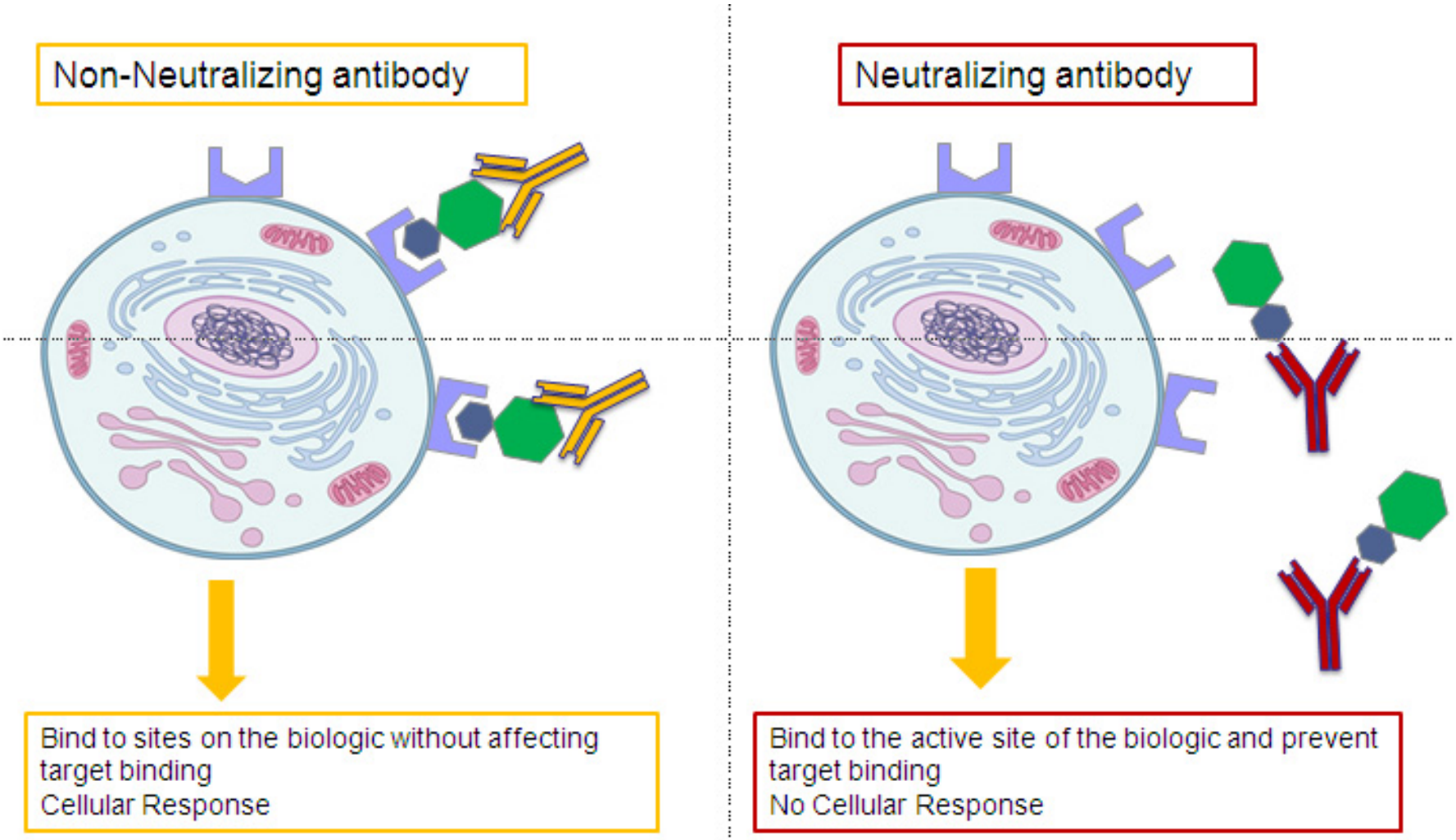
Basic ELISA: Drug Assay



Basic Sandwich Anti-Drug Antibody Assay

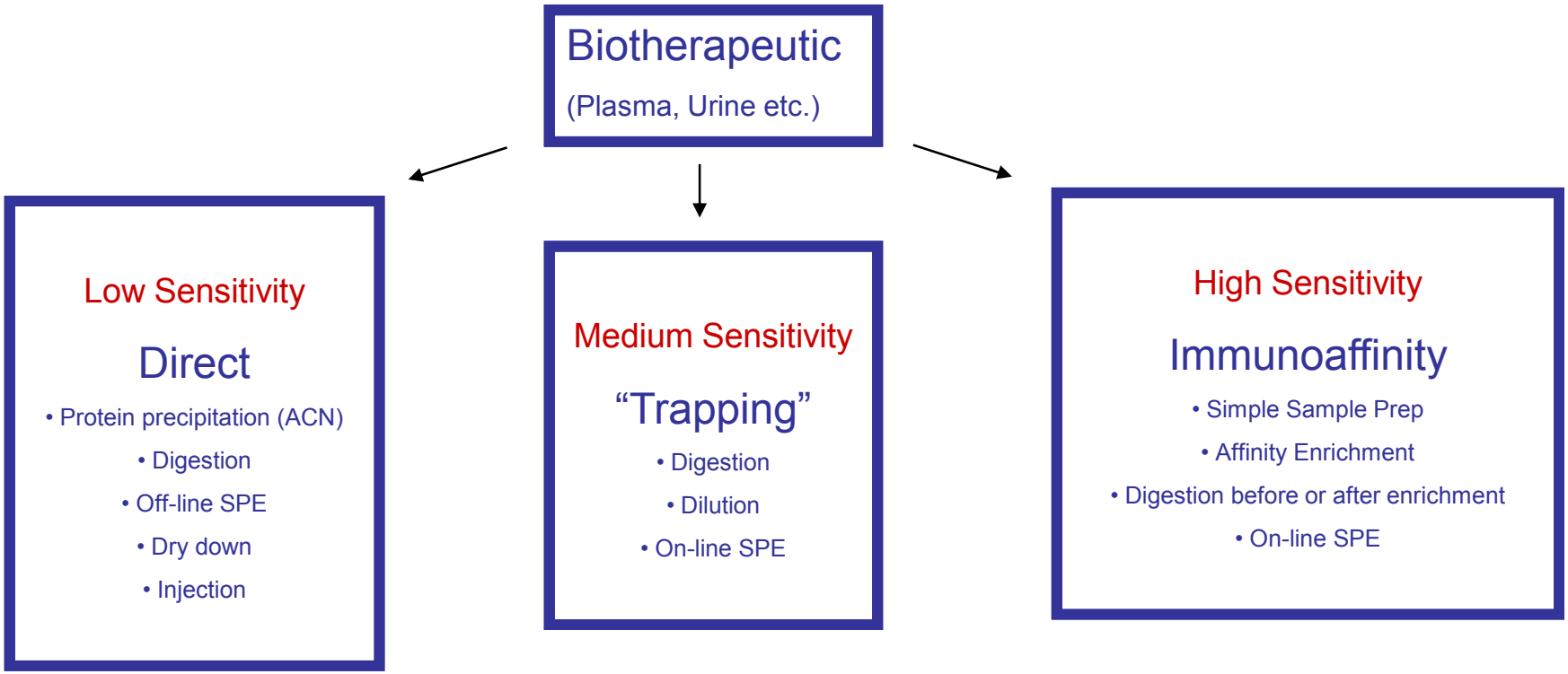


Neutralizing Antibody Assays



Slide Courtesy of *Corinna Krinos-Fiorotti*

Protein Quantification by Mass Spectrometry



- 1 Step
  - LC/MS/MS
- 2 Step
  - Trap Enrichment
    - Pre-concentration
  - LC/MS/MS
- 3 Step
  - Affinity Enrichment
  - Trap Enrichment
  - LC/MS/MS

SPE – solid phase extraction

Slide Courtesy of Dawn R. Dufield

Comparison of Analytical Assays for Protein Quantitation

ELISA's

- Pros
  - Sensitivity (pg/mL)
  - High Throughput
  - Automated
  - Often commercial kits are available
- Cons
  - Limited dynamic range
  - Matrix Effects
  - Not suitable for metabolic studies
  - Susceptible to cross-reactivity
  - Long development for multiple Ab's - mAb

Mass Spectrometric Methods

- Pros
  - Comparable sensitivity (pg/mL – ug/mL)
  - Increased specificity
  - Large dynamic range
  - Ability to measure metabolites and degradation products
- Cons
  - Need Internal standard
  - Lower Throughput
  - Cost of Instrumentation
  - MW Limitation – need digestion

Comparison of Analytical Assays for Protein Quantitation

Table 2. Comparison of immunoassays and LC–MS for the bioanalysis of therapeutic antibodies in biological fluids.

Variable	Immunoassay	Direct LC–MS	Immunocapture LC–MS
Limit of quantification	5–200 ng/ml	500–1000 ng/ml	20 ng/ml
Target concentration	Mixture of free and bound forms	Total	Mixture of free and bound forms
Precision	Medium, no internal standard possible	High, with addition of internal standard	High, with addition of internal standard
Time for development	~8 months (monoclonal antibody)	Less than 1 month	~4 months (polyclonal antibody)
Throughput	High	High	Low/medium, possibility of automation (robot)
Cost	Low	High	High

Ezan *et al* Bioanalysis, 2009, 1(8), 1375-1388

When to use LC/MS/MS

- First Choice
  - Peptides
  - Oligonucleotides in tissues
  - Early discovery (when no reagents are available)
  - Antibody drug conjugates (ex. tracking the loss of the payload)

- LC/MS/MS as a compliment to ligand binding assays (ex. ELISA)
  - When there are issues with unresolved interference.
  - Characterization – to understand what the ligand binding assay is measuring

Summary

- Biotherapeutics are becoming an increasingly important part of drug development
- There are multiple types of biotherapeutics each with their own distinct characteristics and drug development challenges
- Biotherapeutics have unique challenges for drug development when compared to small molecules including (but not limited to) target mediated disposition, comparability, and immunogenicity
- A key factor for biotherapeutics is the development of fit for purpose bioanalytical assays with the appropriate validations.