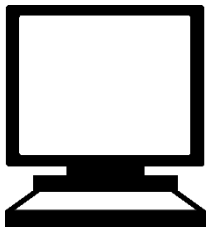
A Shared Framework for Protein Analytics; Bioassays Enhancing Drug Development

Bioassays 2020:
Scientific Approaches & Regulatory Strategies
April 30, 2020
Virtual Meeting

Steven Kozlowski, Director, Office of Biotechnology Products,
OPQ, CDER, FDA

Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.



Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.

Drugs are no different.

FDA
Patients expect safe and effective medicine with every dose they take.

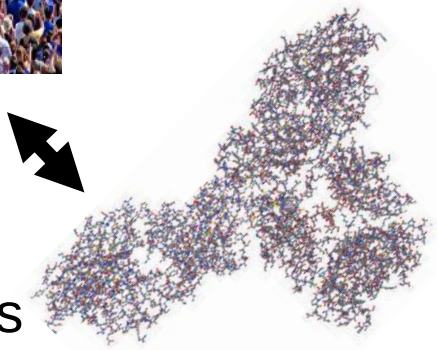
Pharmaceutical quality is

assuring *every* dose is safe and effective, free of contamination and defects.

It is what gives patients confidence in their *next* dose of medicine.

Quality Evolves with Analytics

Past Mantra: The Product is the Process



Quality Attributes

- Product Quality
  - Control Strategies
- Comparability
  - Enable Manufacturing Changes
    - Response to changes in demand
    - Development times
- Efficiency, lower overall costs
- Biosimilars--Access

- Better understand variability
  - Kim et al. mAbs, 2017, 9:704; Schiestl et al. Nature Biotechnology, 2011, 29(4)
- Better mitigate risks
  - Variable clinical performance, Adverse events; Limited availability, Shortage

Many Analytical Tools to Evaluate Proteins

Amino acid sequence and modifications: Mass spectrometry (MS), peptide mapping, chromatographic separations

Folding: S-S bonding, calorimetry, HDX and ion mobility MS, NMR, dyes, circular dichroism, Fourier transform spectroscopy, fluorescence

Subunit interactions: chromatography, ion mobility MS

Heterogeneity of size, charge, hydrophobicity:
Chromatography resins; gel & capillary electrophoresis, light scatter, IM-MS

Glycosylation
Anion exchange, enzymatic digestion, peptide mapping, CE, MS

PEGylation & isomers: chromatography, peptide mapping

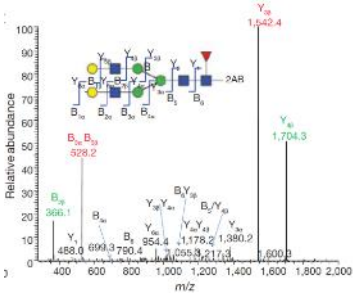
Bioactivity: cellular and animal bioassays; ligand & receptor binding (ELISA, surface plasmon resonance), signal transduction

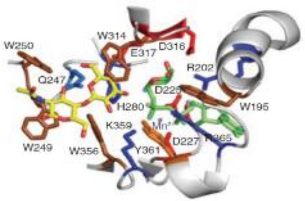
Aggregation: Analytical ultracentrifugation, size-exclusion chromatography, field flow fractionation, light scatter, microscopy

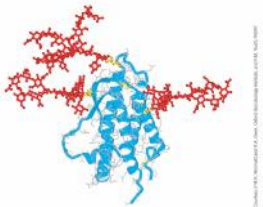
Proteolysis: electrophoresis, chromatography, MS

Impurities: proteomics, immunoassays, metal & solvents analysis

Adventitious Agents: sterility, qPCR, bioassays, clearance

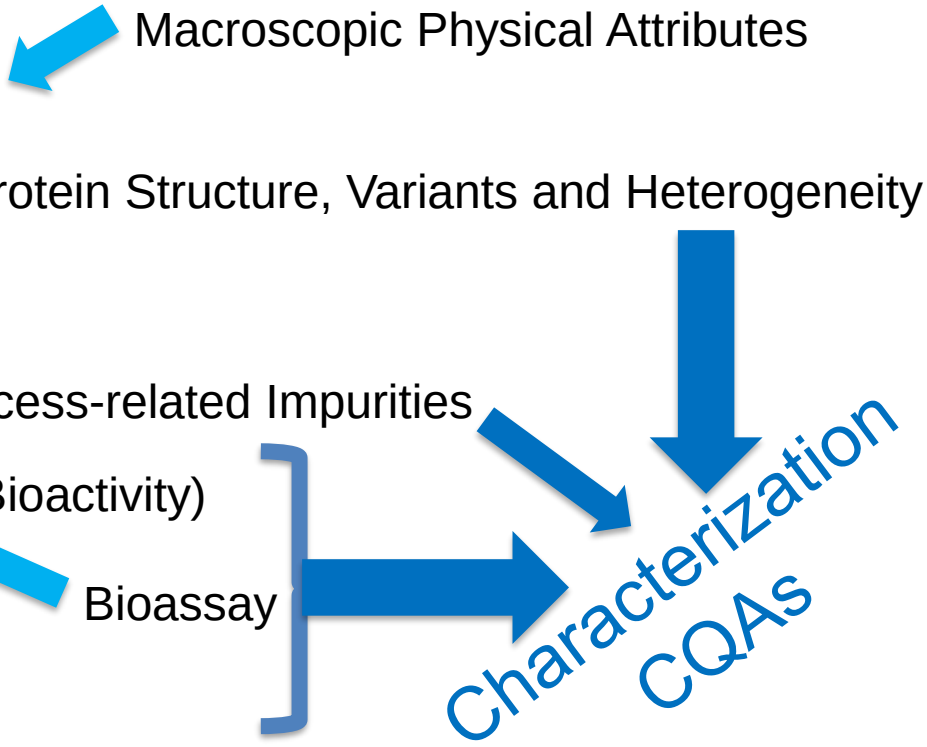






Specifications & Attributes

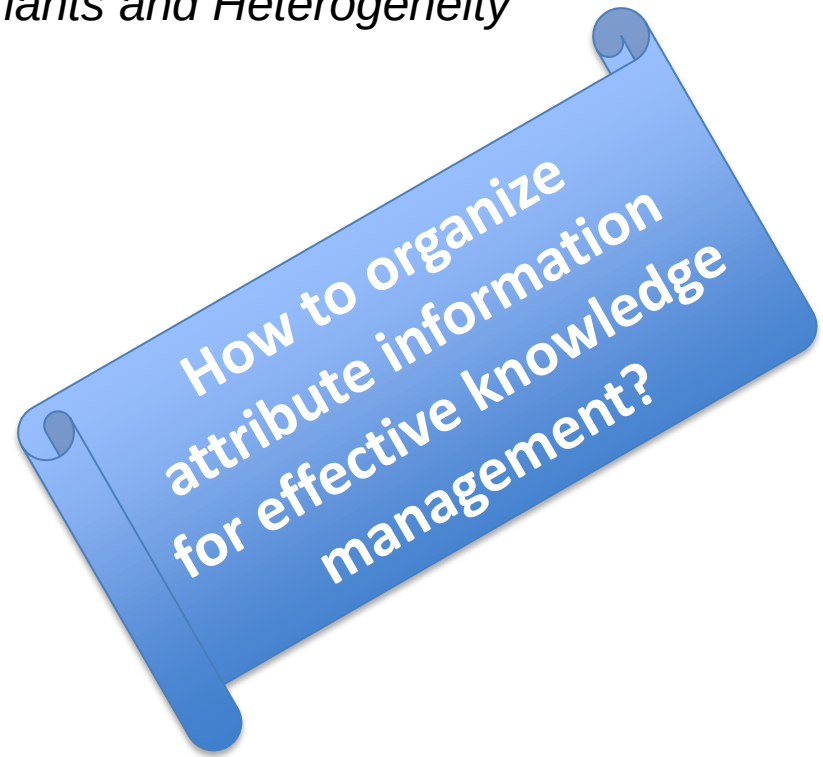
- Appearance & Description
- Identity
- Purity & Impurities
- Potency
- Quantity

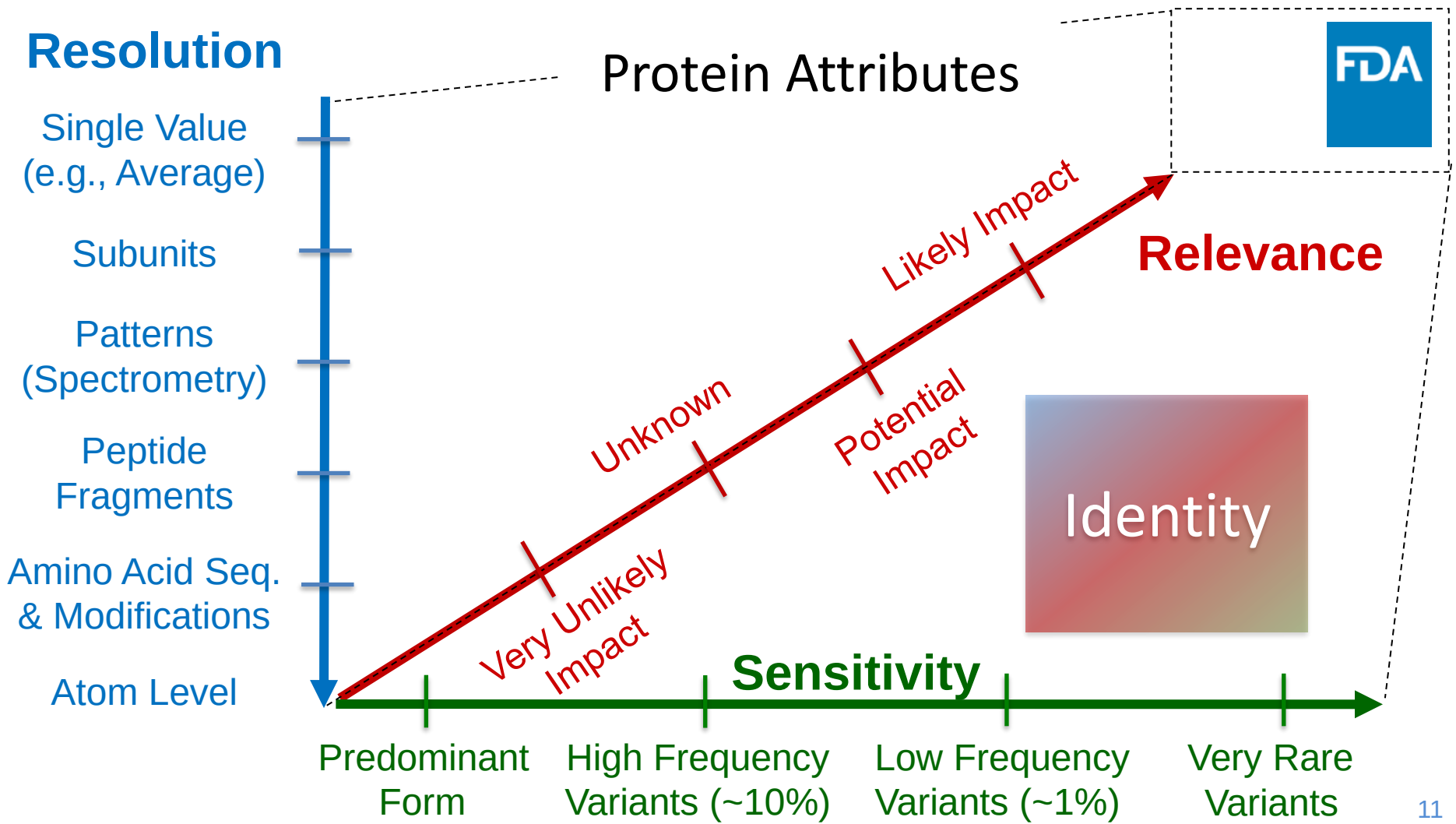


Protein Attributes

Protein Structure, Variants and Heterogeneity

- Size (mass)
- Charge (charge distribution)
- Hydrophobicity
- Higher order structure
- Primary sequence
- Modifications
- Polymer modifications
  - glycosylation, PEGylation




Resolution
Protein Attributes
FDA
Single Value (e.g., Average)
Subunits
Patterns (Spectrometry)
Peptide Fragments
Amino Acid Seq. & Modifications
Atom Level
Likely Impact
Relevance
Unknown
Potential Impact
Very Unlikely Impact
Identity
Sensitivity
Predominant Form
High Frequency Variants (~10%)
Low Frequency Variants (~1%)
Very Rare Variants

Disclaimer

- The examples of methods, resolution, sensitivity and relevance are for illustrative purposes only.
- No actual method reputations should be injured by the following slides.
- Whatever method **you are** an expert on may have much better resolution and sensitivity than indicated and be the best approach.

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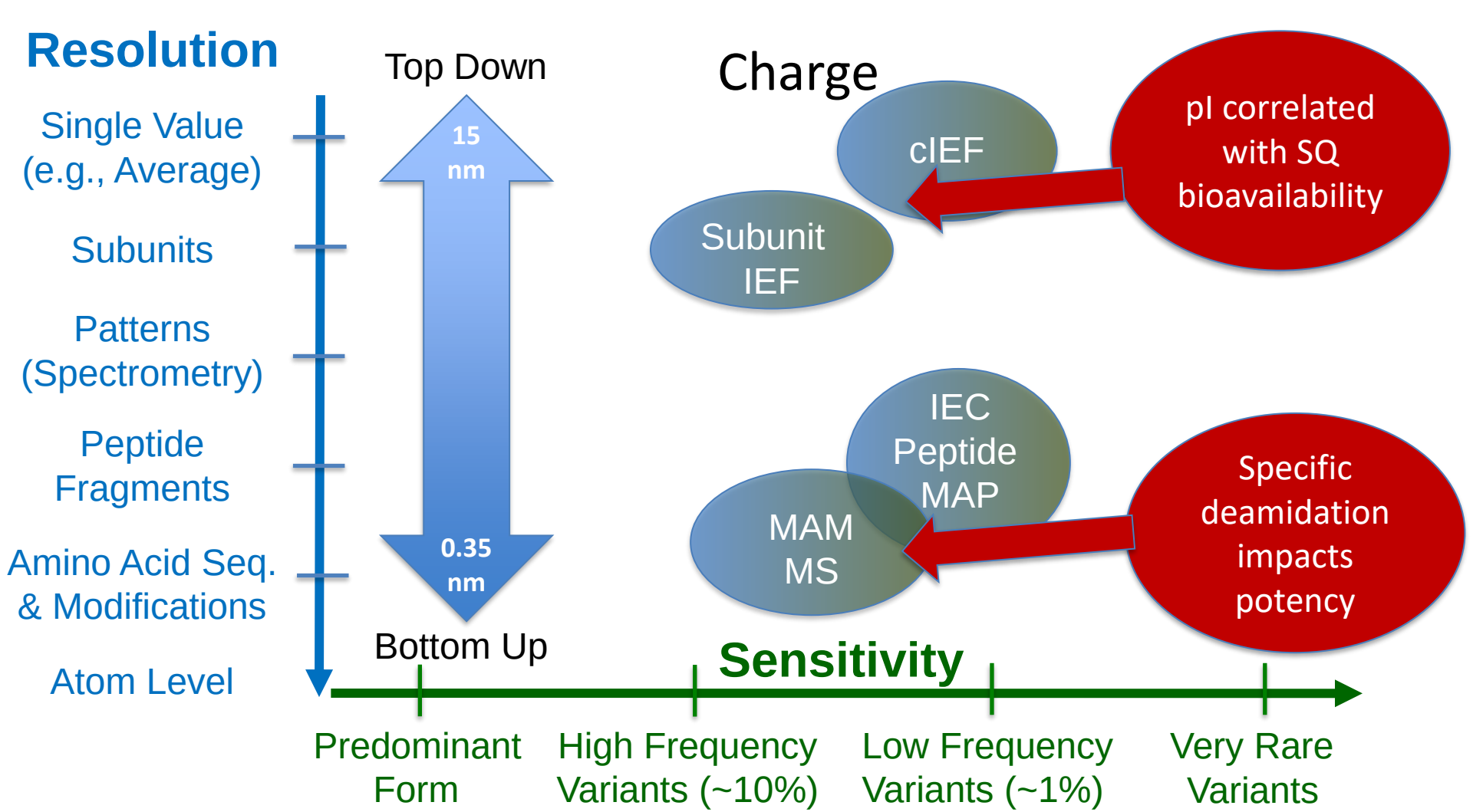
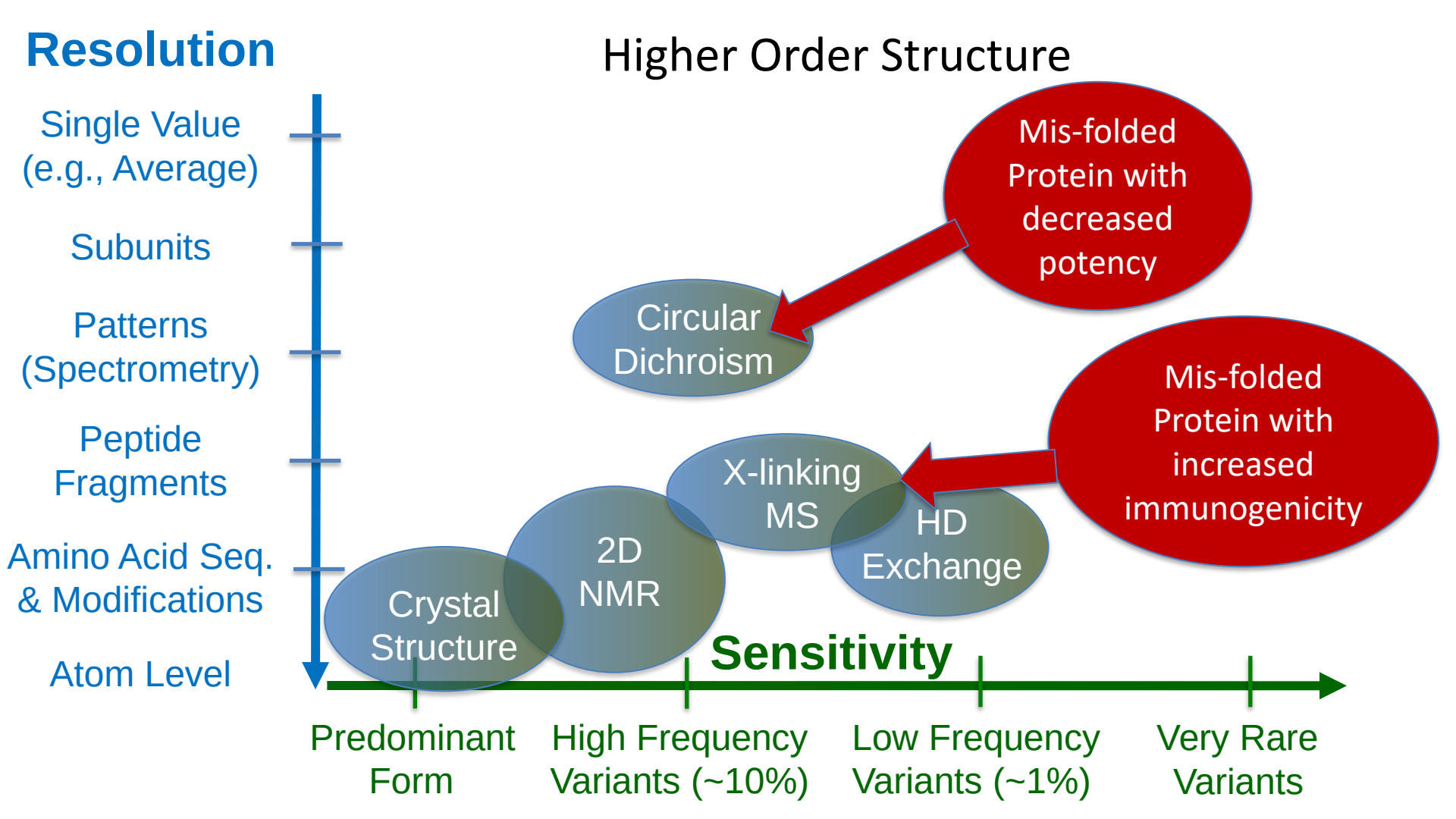
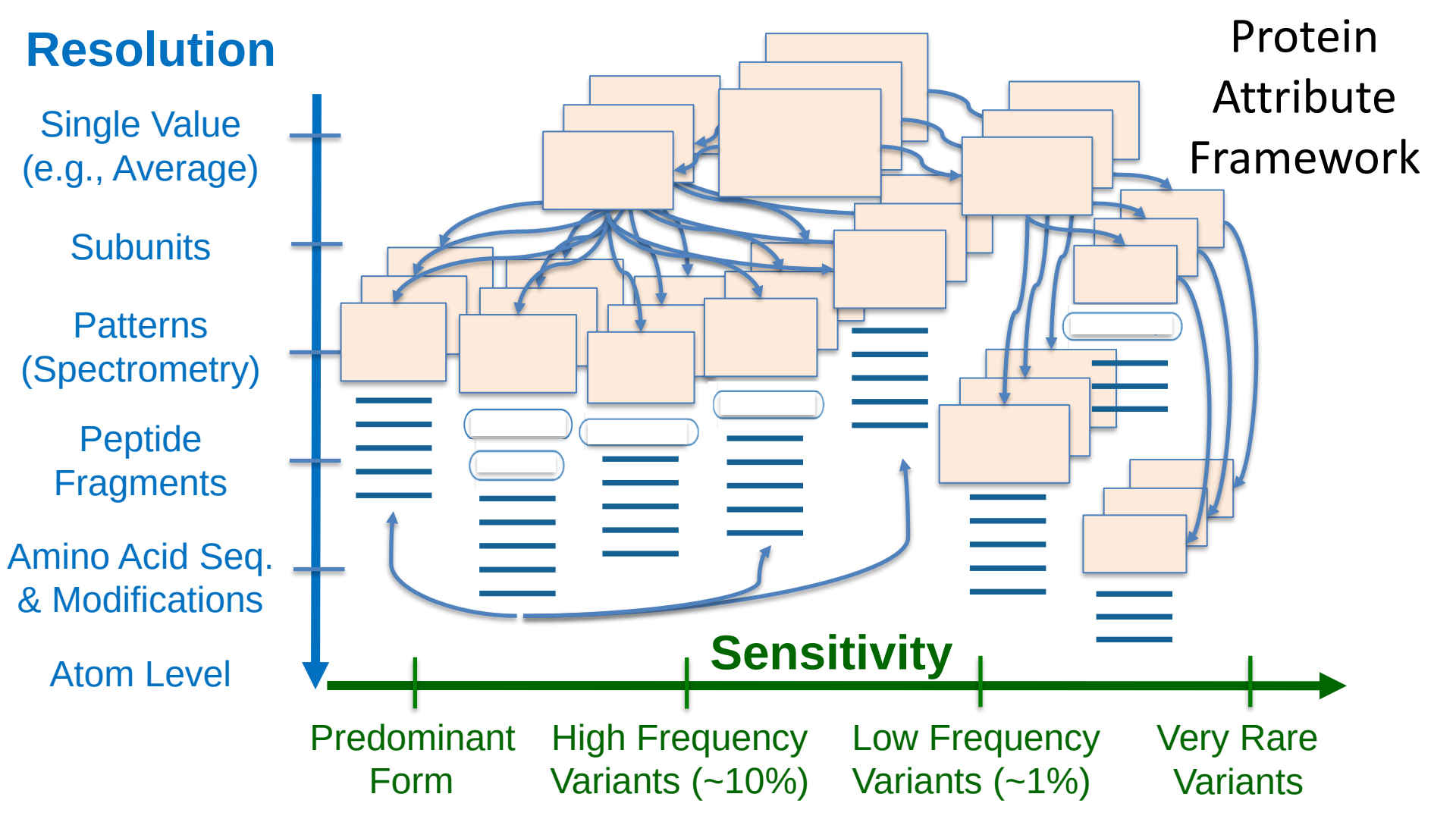
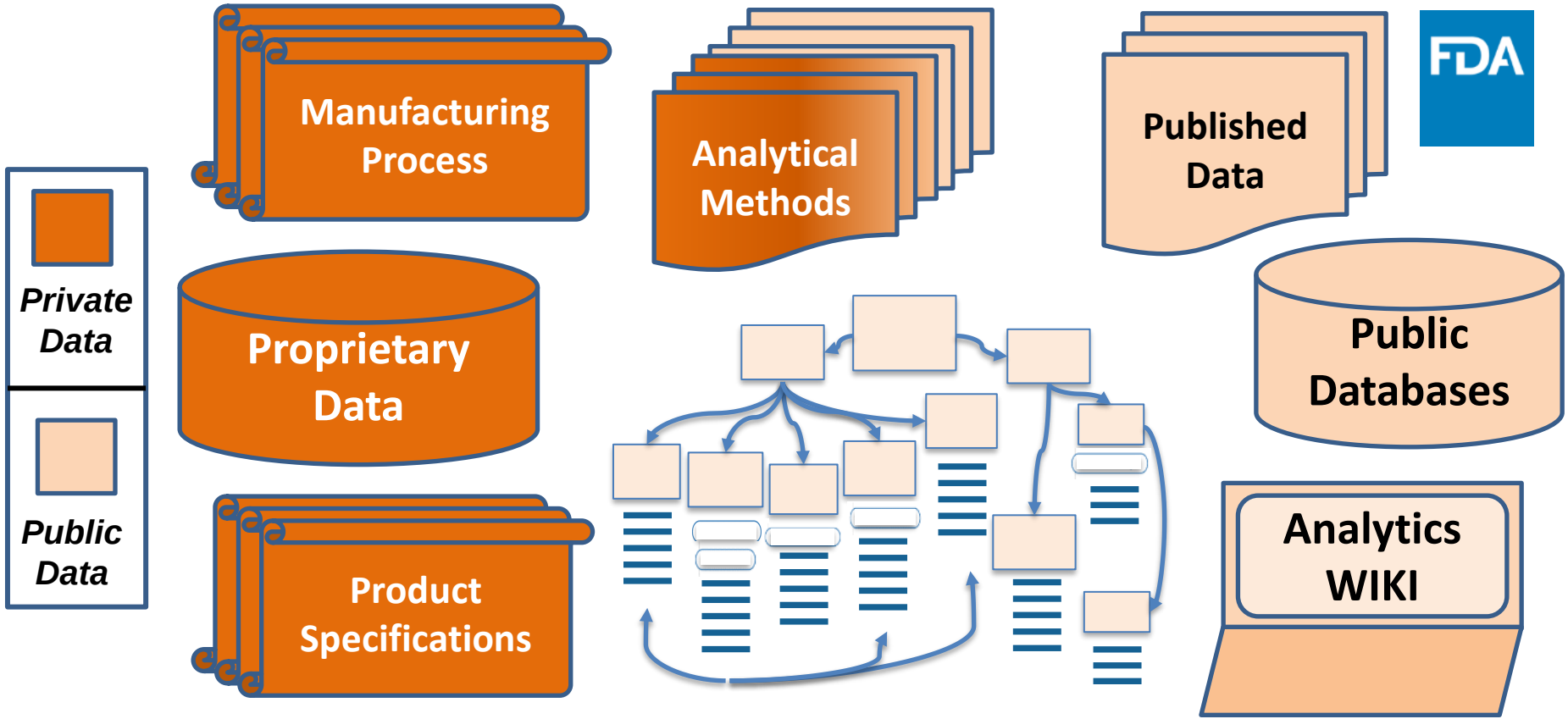

Resolution
Top Down
Single Value (e.g., Average)
Subunits
Patterns (Spectrometry)
Peptide Fragments
Amino Acid Seq. & Modifications
Atom Level
15 nm
0.35 nm
Bottom Up
Charge
cIEF
Subunit IEF
pI correlated with SQ bioavailability
IEC Peptide MAP
MAM MS
Specific deamidation impacts potency
Sensitivity
Predominant Form
High Frequency Variants (~10%)
Low Frequency Variants (~1%)
Very Rare Variants

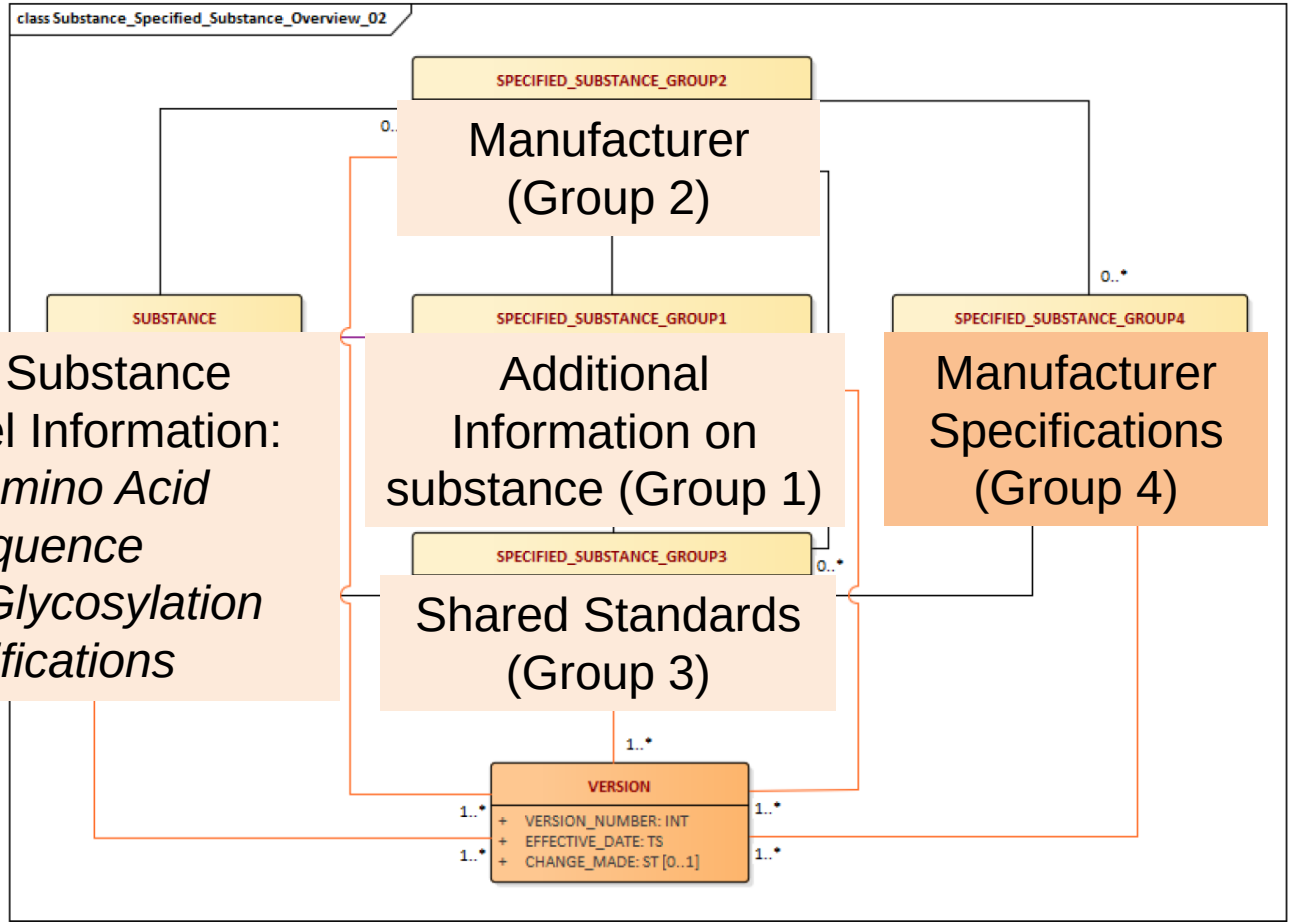

Resolution
Higher Order Structure
Single Value (e.g., Average)
Subunits
Patterns (Spectrometry)
Peptide Fragments
Amino Acid Seq. & Modifications
Atom Level
Mis-folded Protein with decreased potency
Circular Dichroism
Mis-folded Protein with increased immunogenicity
X-linking MS
HD Exchange
2D NMR
Crystal Structure
Sensitivity
Predominant Form
High Frequency Variants (~10%)
Low Frequency Variants (~1%)
Very Rare Variants


Resolution
Single Value (e.g., Average)
Subunits
Patterns (Spectrometry)
Peptide Fragments
Amino Acid Seq. & Modifications
Atom Level
Protein Attribute Framework
Sensitivity
Predominant Form
High Frequency Variants (~10%)
Low Frequency Variants (~1%)
Very Rare Variants



Dashboard for Knowledge Aided Assessment

ISO Specified Substance



Regulatory Education for Industry (REdI) Webinar: Identification of Medicinal Products (IDMP) – June 13, 2019

Overview of Global Substance Registration System (GSRS), IDMP
Larry Callahan, PhD

FDA

- **G**lobal **In**gredient **A**rchival **S**ystem (Ginas)
  - **Un**ique **I**ngredient **I**dentifiers (UNII)
- **G**lobal **S**ubstance **R**egistration **S**ystem (G-SRS)
  - ISO compliant system in development at NIH
  - FDA's Health Informatics program
    - Assigns unique identifiers (UNIIs) to substances and defines them
    - Collaborates with internal and external stakeholders worldwide to define requirements
    - Provides content for G-SRS

SILTUXIMAB

T4H8FMA7IM

- Overview
- Names 10
- Classification 7
- Identifiers 11
- Subunits 4
- Disulfide Links 16
- Glycosylation 2
- Active Moiety 1
- Characteristic Attributes 1
- Notes 1
- Audit Info
- References 23

Subunits

Protein Substance
High Level Information:
e.g., Amino Acid Sequence
Type of Glycosylation
Modifications

G-SRS

FDA

Subunit 1

```
>SUBUNIT_1
EVQLVESGGKLL                                    YYY
PDTVTGRFTISR                                    SA
STKGPSVFPLAP                                    SG
LYSLSSVVTVPS                                    GP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK
```

Disulfide Links

From	To
1_22	1_96
1_146	1_202

etc.

Glycosylation

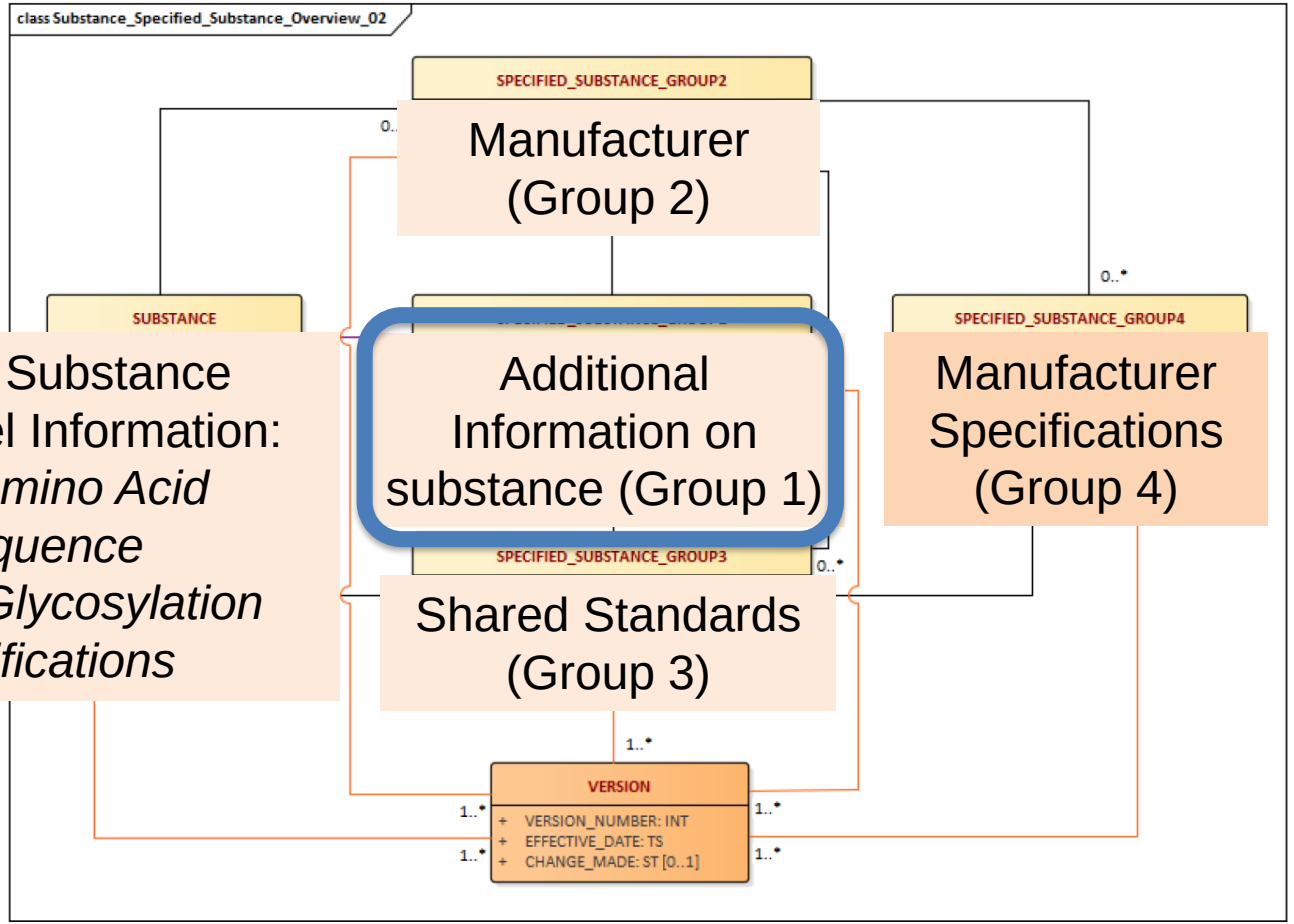
Glycosylation Link Type	Site
N	1_299
N	2_299

Characteristic Attributes

Properties

Name	Property Type	Amount
MOL_WEIGHT:NUMBER(CALCULATED)	CHEMICAL	ESTIMATED 145000 Da (average)

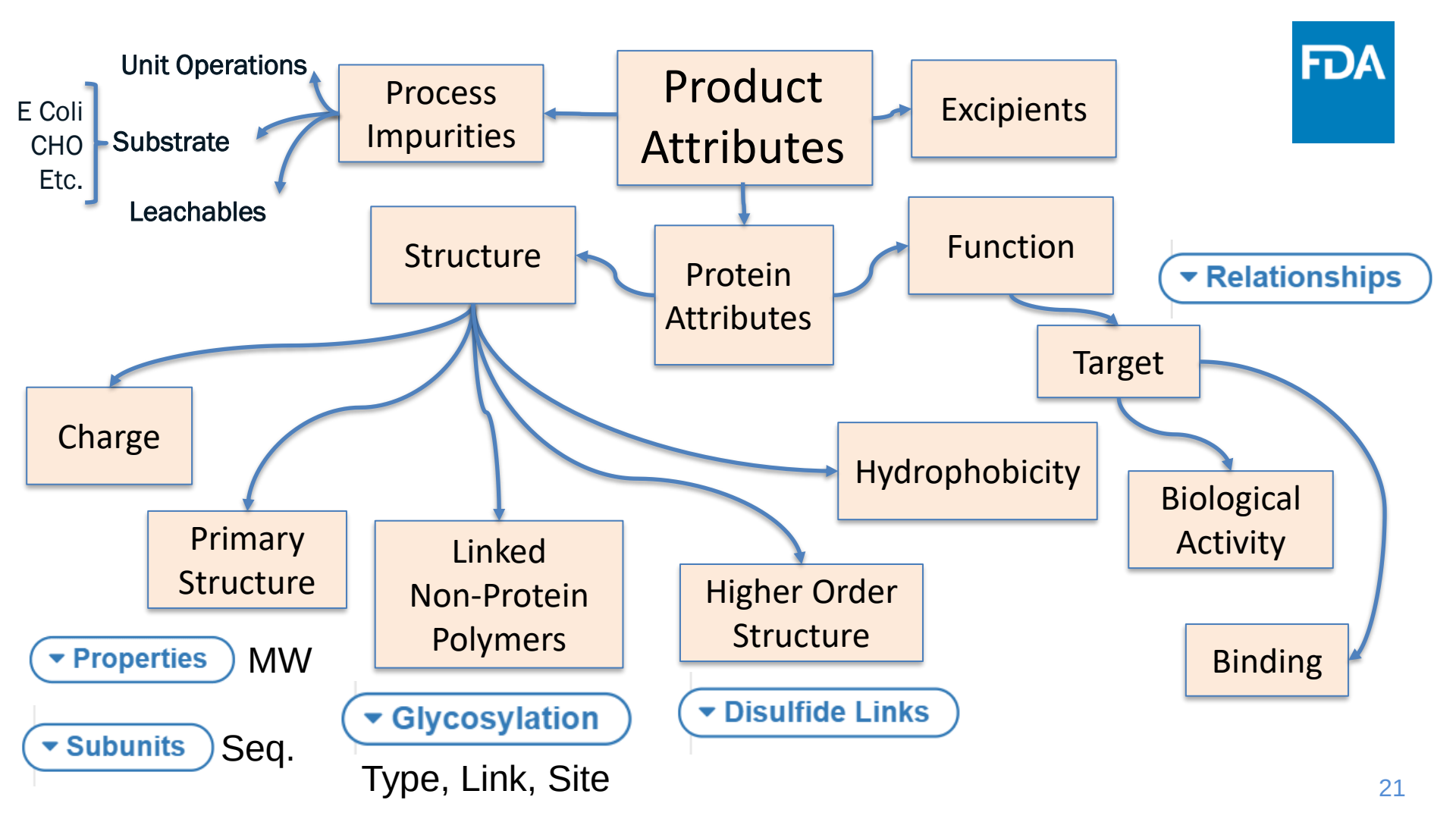
ISO Specified Substance

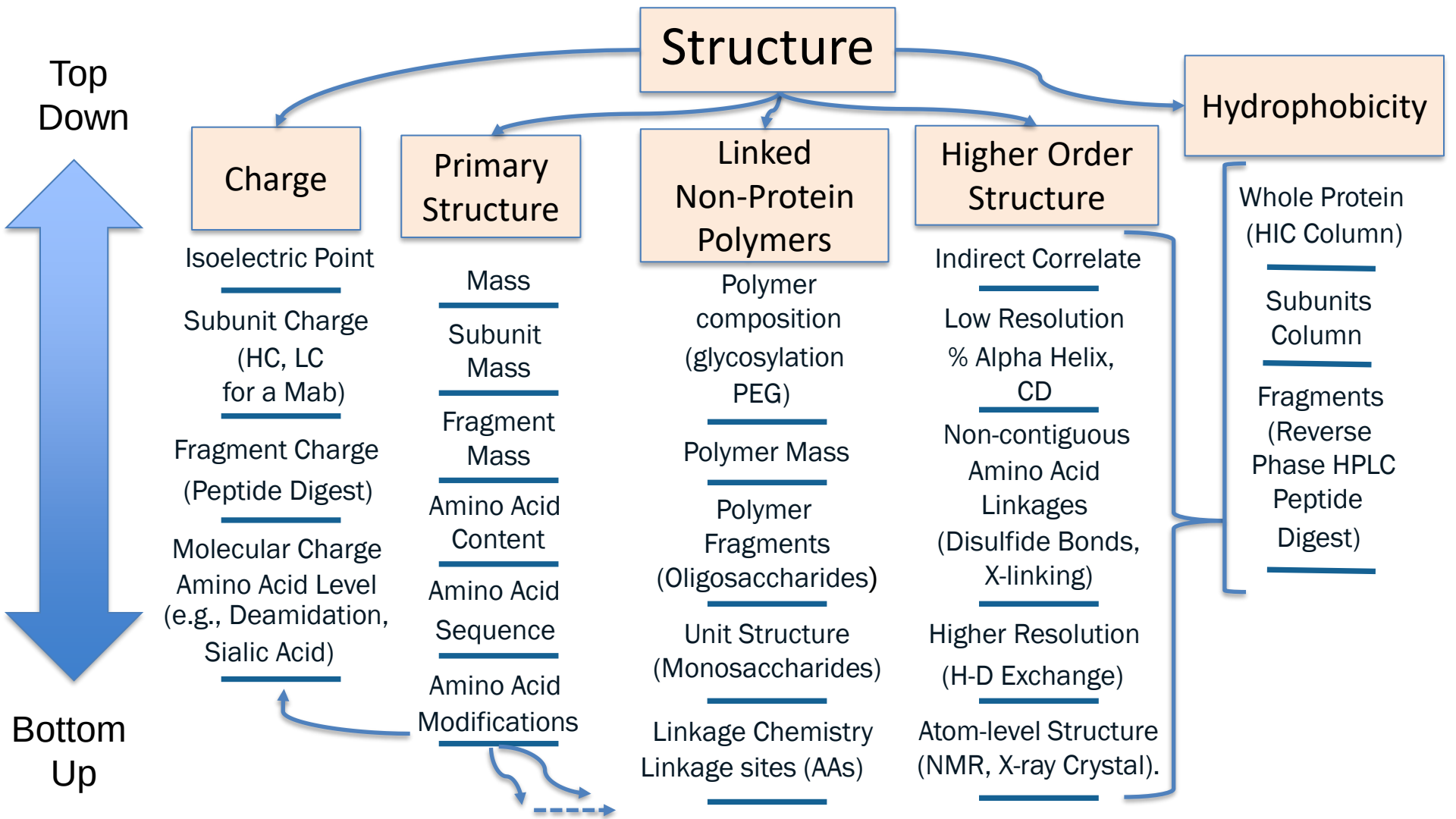


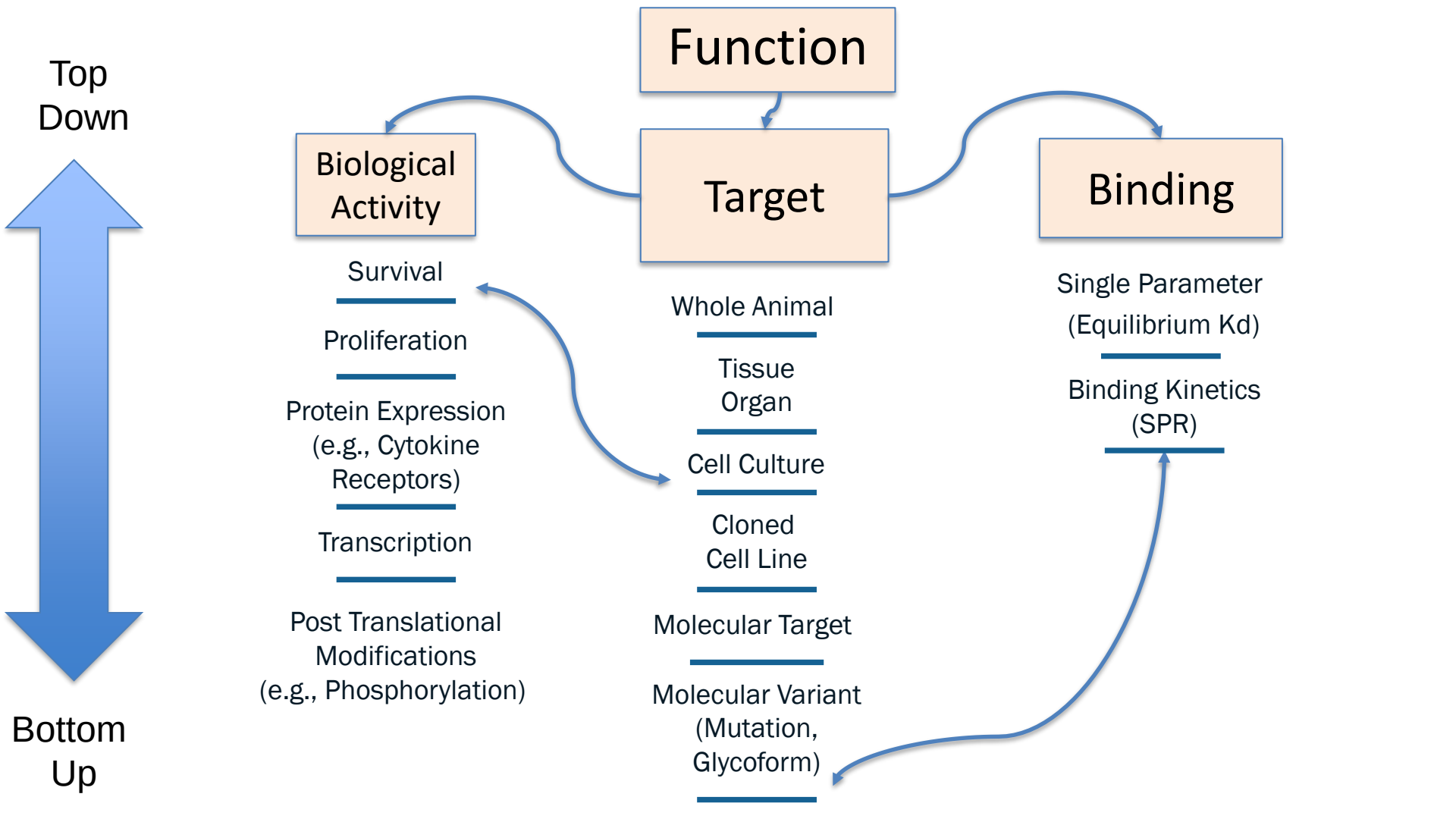
Protein Substance High Level Information:
e.g., Amino Acid Sequence
Type of Glycosylation
Modifications

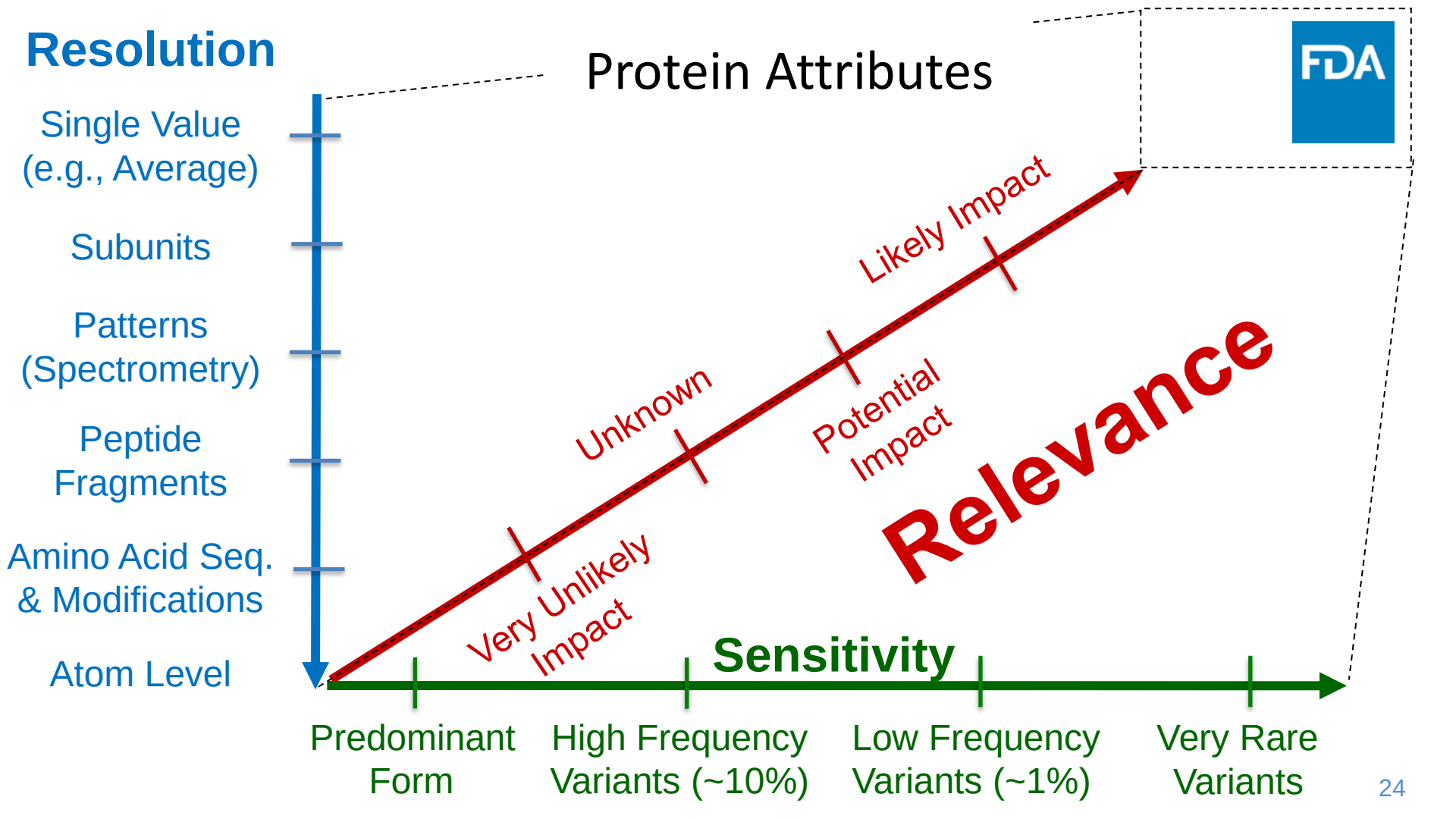
Regulatory Education for Industry (REdI) Webinar: Identification of Medicinal Products (IDMP) – June 13, 2019

Overview of Global Substance Registration System (GSRS), IDMP
Larry Callahan, PhD


Unit Operations
E Coli
CHO
Etc.
Substrate
Leachables
Process Impurities
Product Attributes
Excipients
Structure
Protein Attributes
Function
Relationships
Target
Charge
Primary Structure
Linked Non-Protein Polymers
Higher Order Structure
Hydrophobicity
Biological Activity
Binding
Properties
MW
Subunits
Seq.
Glycosylation
Type, Link, Site
Disulfide Links


Top
Down
Bottom
Up
Structure
Charge
Primary Structure
Linked Non-Protein Polymers
Higher Order Structure
Hydrophobicity
Isoelectric Point
Subunit Charge (HC, LC for a Mab)
Fragment Charge (Peptide Digest)
Molecular Charge Amino Acid Level (e.g., Deamidation, Sialic Acid)
Mass
Subunit Mass
Fragment Mass
Amino Acid Content
Amino Acid Sequence
Amino Acid Modifications
Polymer composition (glycosylation PEG)
Polymer Mass
Polymer Fragments (Oligosaccharides)
Unit Structure (Monosaccharides)
Linkage Chemistry Linkage sites (AAs)
Indirect Correlate
Low Resolution % Alpha Helix, CD
Non-contiguous Amino Acid Linkages (Disulfide Bonds, X-linking)
Higher Resolution (H-D Exchange)
Atom-level Structure (NMR, X-ray Crystal).
Whole Protein (HIC Column)
Subunits Column
Fragments (Reverse Phase HPLC Peptide Digest)


Top
Down
Bottom
Up
Function
Biological Activity
Target
Binding
Survival
Proliferation
Protein Expression (e.g., Cytokine Receptors)
Transcription
Post Translational Modifications (e.g., Phosphorylation)
Whole Animal
Tissue
Organ
Cell Culture
Cloned
Cell Line
Molecular Target
Molecular Variant (Mutation, Glycoform)
Single Parameter (Equilibrium Kd)
Binding Kinetics (SPR)


Resolution
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Amino Acid Seq. & Modifications
Atom Level
Likely Impact
Unknown
Potential Impact
Very Unlikely Impact
Relevance
Sensitivity
Predominant Form
High Frequency Variants (~10%)
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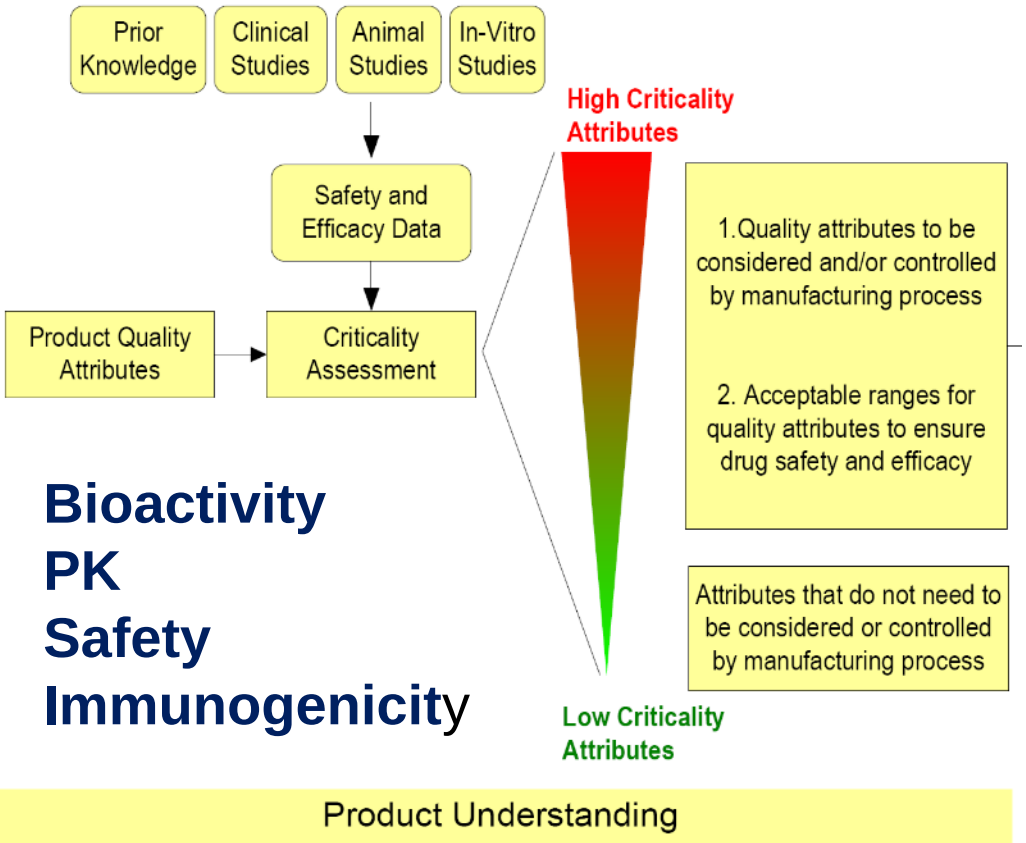
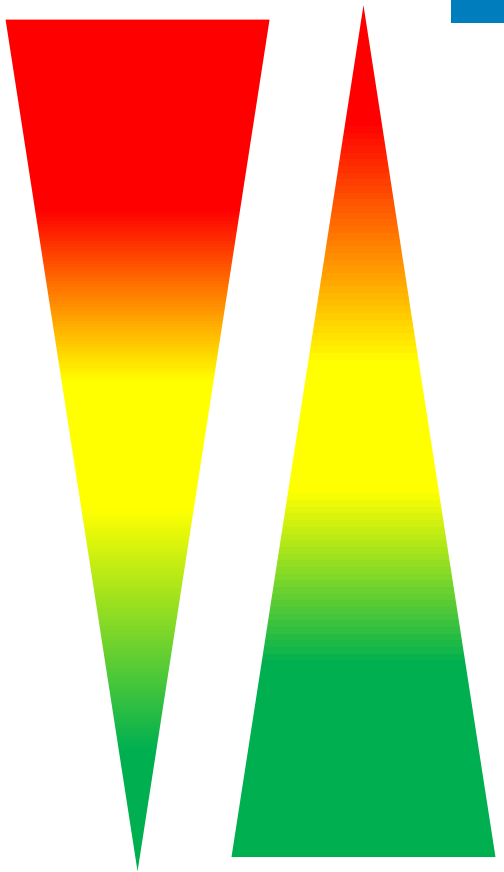
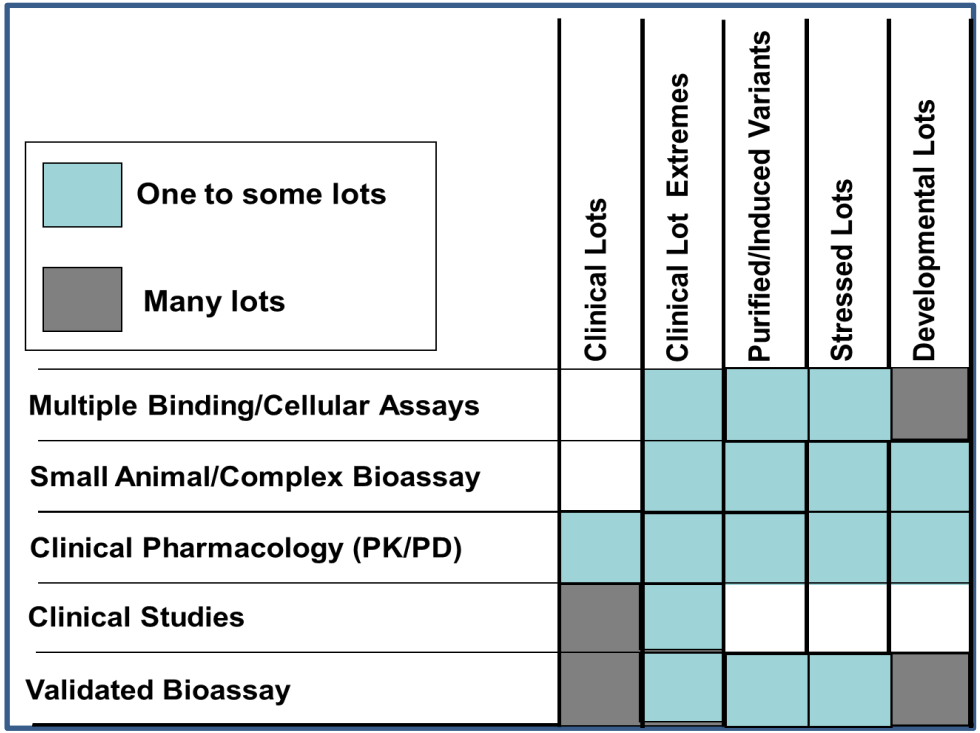
A-Mab Risk Ranking of Quality Attributes





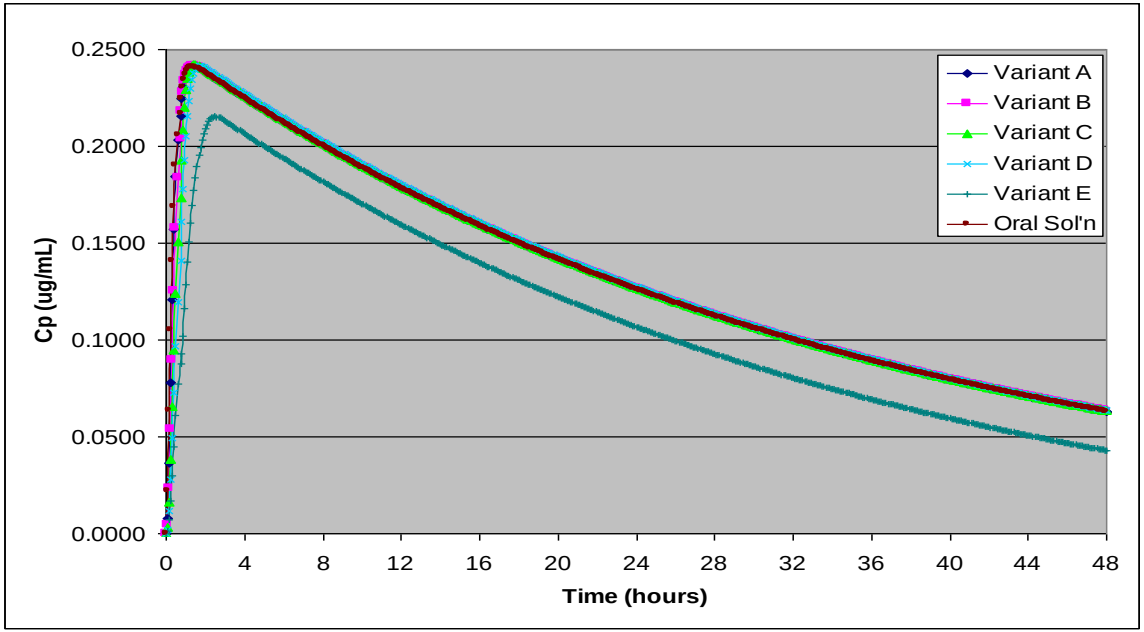
Attribute Ranges-Decision Making Matrix

- Consider all product and related product data
  - Knowledge Management
  - Appropriate weighting
- Clinical Data
  - Limited role in to supporting quality
    - Cost--if only allocated to quality
    - Exposing patients to variants or less purified material



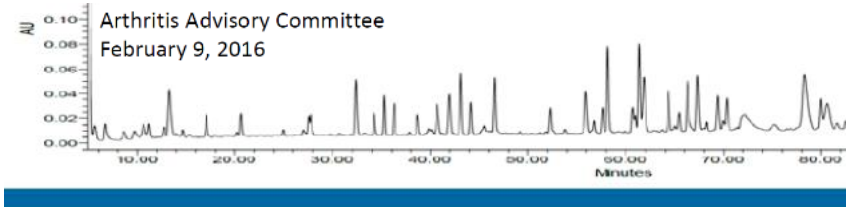
ACE Hypothetical QbD Case Study

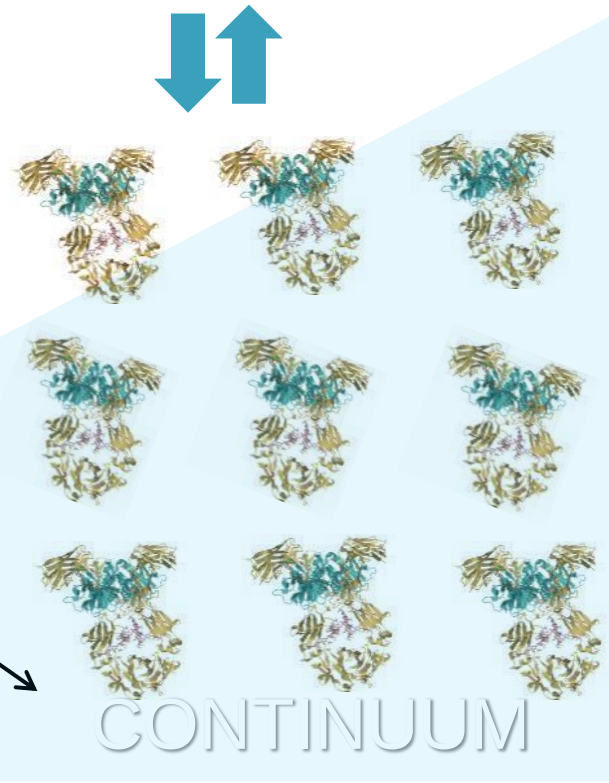
- Five variants were taken into a biopharmaceutics study to evaluate *in vivo* effects of process and formulation variation.
  - API particle size
  - Ribbon density
  - Lubricant levels
- IVIVC
  - dissolution



ACE Case Study
CMC-IM Working Group
Conformia 2008

Paraphrasing Barry Karger---
Looking at Molecules not Attributes



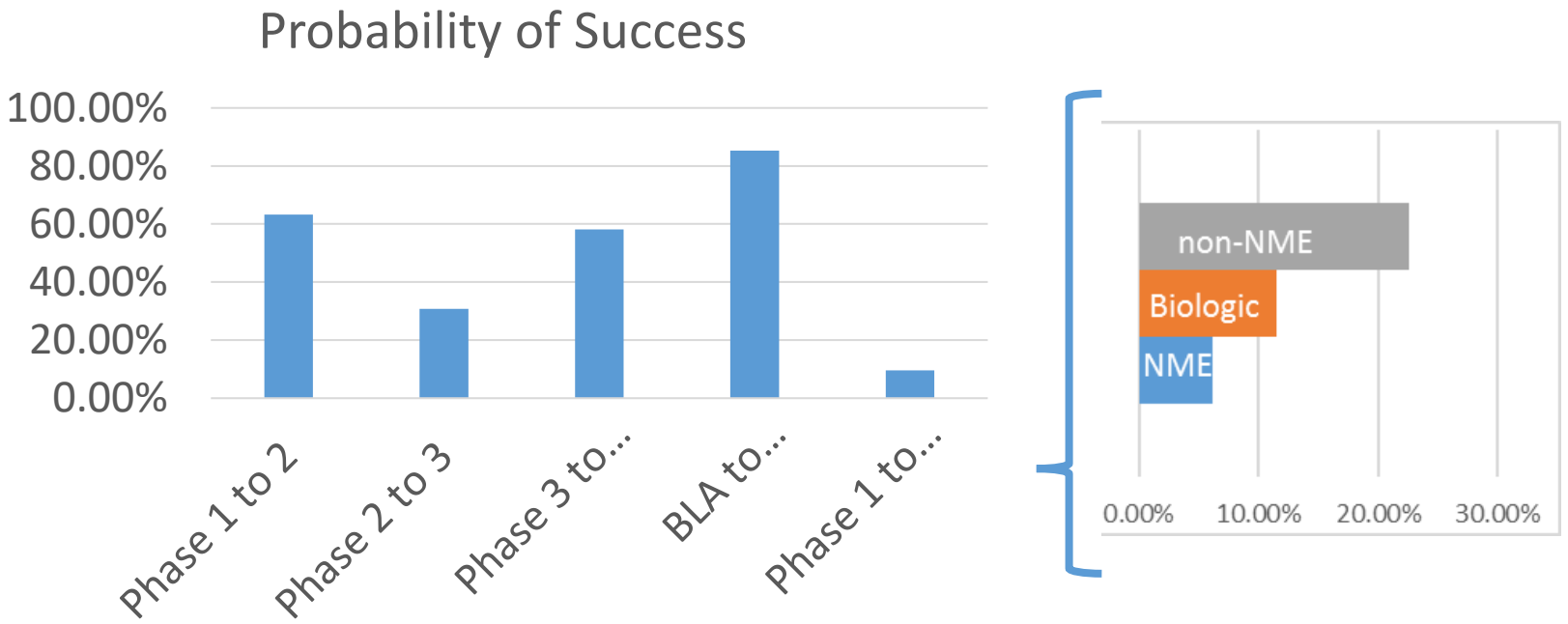


Meaningful– Does it matter to the patient? How can we set ranges?

Drug Development Failures
Tools for Success

Clinical Development Success Rates 2006-2015

BIO, Biomedtracker, Amplicon

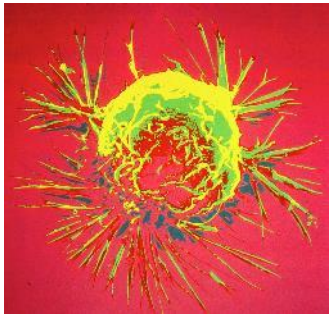


Efficacy issues dominated both Phase 2 and Phase 3 Failures—More than 50%
Tufts CSDD in Applied Clinical Trials

Clinical Development Success Rates 2006-2015

Phase 1 to Approval

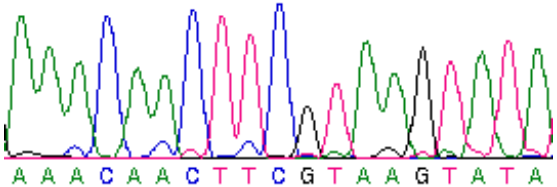
Indication	Probability of Success
Oncology	5.10%
non-Oncology	11.90%

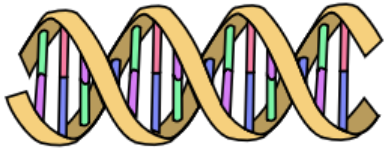


BIO, Biomedtracker, Amplicon

Disease Prevalance	Probability of Success
All Diseases	9.60%
Rare Disease	25.30%

Selection Biomarkers	Probability of Success
No	8.40%
Yes	25.90%





Failed agents often show only **borderline activity** in early-phase clinical trials, and it is **rare** for such trials **to** include studies to **ensure** that new agents **inhibit** their putative **molecular target**.

Seruga et al. Clin Cancer Res; 21(20); 4552–60

https://upload.wikimedia.org/wikipedia/commons/a/a3/Zebra_ribbon.svg By David Richfield

https://upload.wikimedia.org/wikipedia/commons/5/59/Breast_cancer_cell_%281%29.jpg NCI

Right target

Target Validation

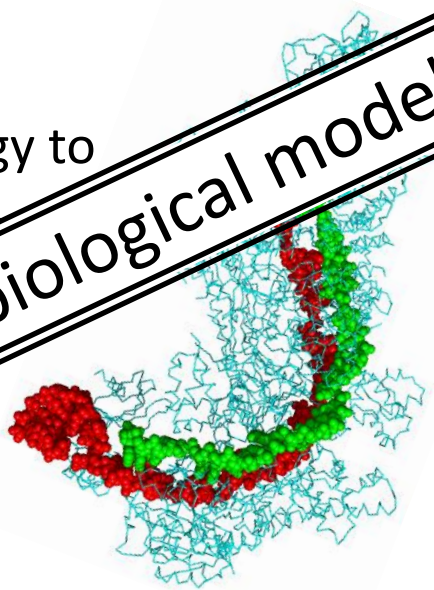
FDA

"genome editing with CRISPR and TALEN technology to create more relevant cellular and animal models…

Libraries of CRISPR reagents can also be used … to validate or invalidate targets and pat…

Morgan et al. Nature R… Drug Discovery 2…

Complex in silico, in vitro and in vivo biological models



By Boghog - Own work, CC BY-SA … https://commons.wikimedia.org/… x.php?curid=68597347

"drug m… …th direct genetic support increases…. from 2.0% at the pre… … to 8.2% among… approved drugs…

e… …e that selecting genetically supported targets could double the success rate in clinical development."

Nelson et al. Nature Genetics 2015 47:856

Linking Structure to Product Survival

Three Pillars of Survival

Morgan et al. Drug Discovery Today 2012 Volume 17 :419

i. **Exposure at the target**

- *Structure to Pharmacokinetics*
- *Structure to Localization*

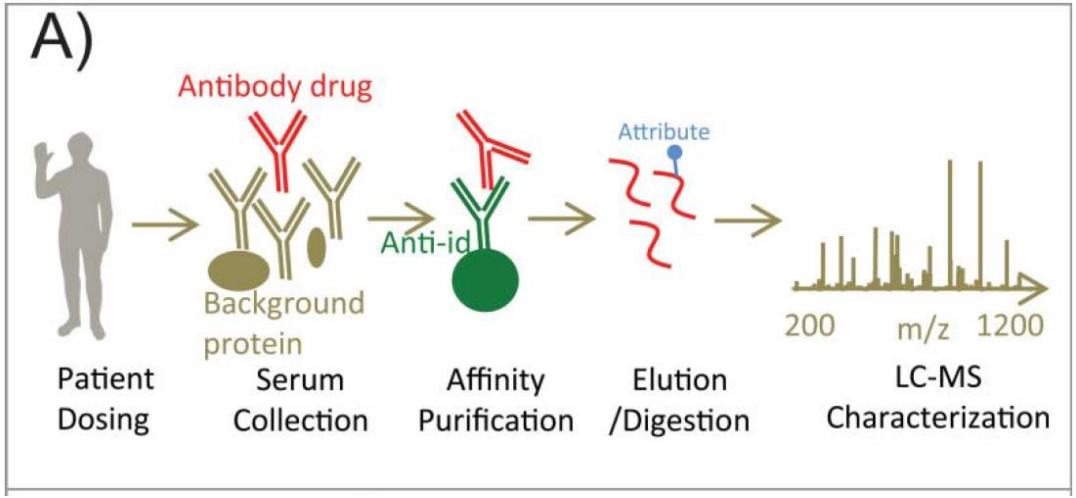
ii. **Binding to the target**

- *Structure to Binding (in vitro, in vivo, pharmacodynamics)*

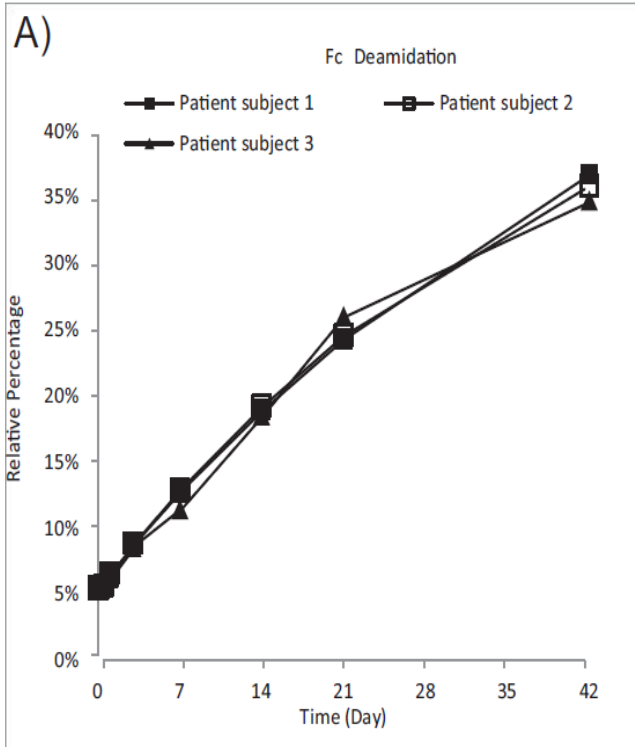
iii. **Expression of pharmacological activity**

- *Structure to Function (in vitro, in vivo, pharmacodynamics)*

Structure to Pharmacokinetics



Quantitation and pharmacokinetic modeling of therapeutic antibody quality attributes in human studies
Yinyin Li et al. MABS 2015, VOL. 8, NO. 6, 1079–1087



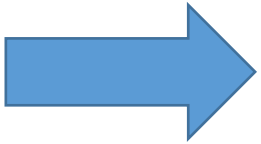
PK Studies that Assess Variants

The effect of Fc glycan forms on Human IgG2 antibody clearance In humans

Chen, Liu, and Flynn, Glycobiology, 2009 Mar; 19 (3):240-9

High-mannose glycans on the Fc region of therapeutic IgG antibodies increase serum clearance in humans

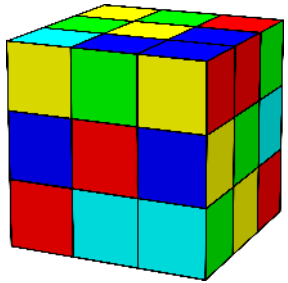
Goetze at al. Glycobiology,2011 Mar21(7);949–59



- *Elimination rate of M5 glycan containing antibodies from 60 -153% higher than baseline*
- *Elimination rate of non-glycosylated antibodies 15-23% higher than baseline*

Antibody PK Puzzles

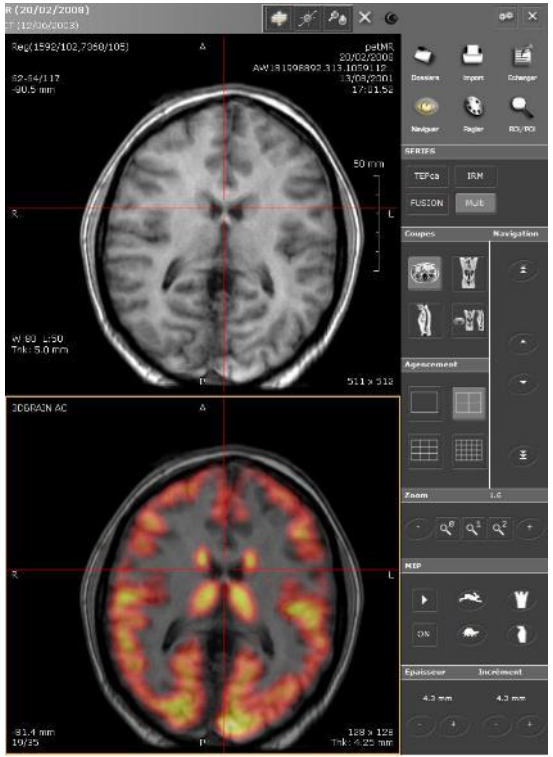
- Palivizumab anti-RSV variants: A4b4 with increased *in vitro* neutralization; howev decreased lung bio-distribution and PK
  - Associated with increased non-specific tissue binding and k_{on} increasing mutations
  - Wu et al. J. Mol. Biol. 2007, 368:652–665
- Anti-IL21 antibodies that differ by 4 aa in CDR3 have very different PK profiles in mice, rats and cynomolgus monkeys
  - Vugmeyster et al. mAbs 2010, 2(3):335
- Engineering CDRs of IgG4 antibodies can alter PK--- high pI--- high clearance.
  - Igawa et al. Protein Engineering, Design & Selection 2010, 23(5):385—392
- Anti-amyloid beta with rapid clearance in non-human primates cross-reacts with fibrinogen
  - Vugmeyster et al. Pharm Res 2011, 28:1696-1706
- Non-specific binding and other PK de-risking tools
  - Dostalek et al. mAbs 9(5):756-766
  - Hoetzel et al. mAbs 4(6):753-760
- Where do these rapidly clearing antibodies go?
- Does glycosylation impact localization?



Localization

Technique	Labels	Signal	Cost	Sensitivity (moles)	Resolution
PET	Radiolabelled	Positrons	High	10^{-15}	1–2 mm
SPECT	Radiolabelled (multiple labels)	γ-rays	High	10^{-14}	1–2 mm
MRI	Isotope-labelled (limited #)	Magnetic fields	High	10^{-9} to 10^{-6}	50 µm
Optical	bioluminescent and flourescent	Light --IR Light	Low	10^{-12}	1–2 mm
Photoacoustic	absorb light and create	Sound	Low	10^{-12}	50 µm

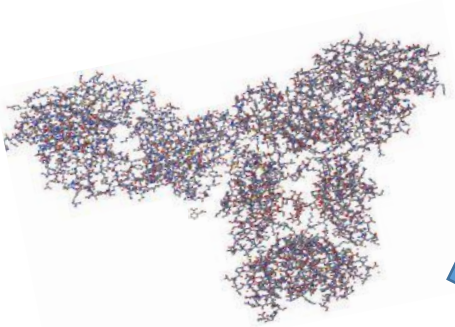
Baker The whole picture 2010 Nature 463: 978



By Mco44 [Public domain], from Wikimedia Commons https://upload.wikimedia.org/wikipedia/commons/6/6b/PET-IRM-cabeza-Keosys.JPG

- Theranostics
  - 14 clinical imaging studies with labeled trastuzumab
  - Labeled anti PD-1 and anti PD-L1 Imaging studies
    - Moek et al. J Nucl Med 2017 58:83S

Wis Wishful Imaging ng



PET label

Stable Isotope Label

PET Imaging
CT or MRI (may verify isotope location)

Directed biopsy

PD Markers

Gene expression
Genomics
Immunohistochemistry

Sample Prep

Isotope Gated MS/MS

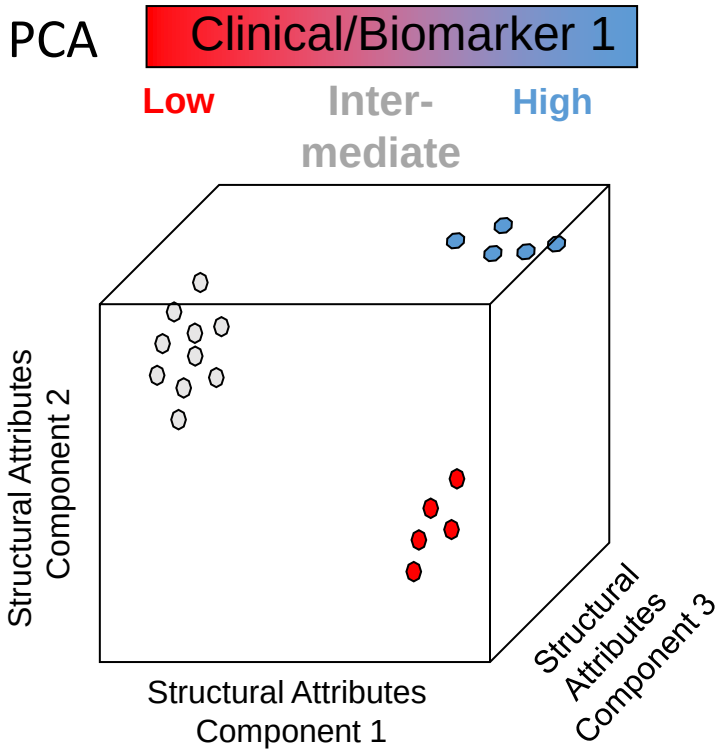
Structural information on in situ product colocalized with PD marker evaluation

All the Pillars Together

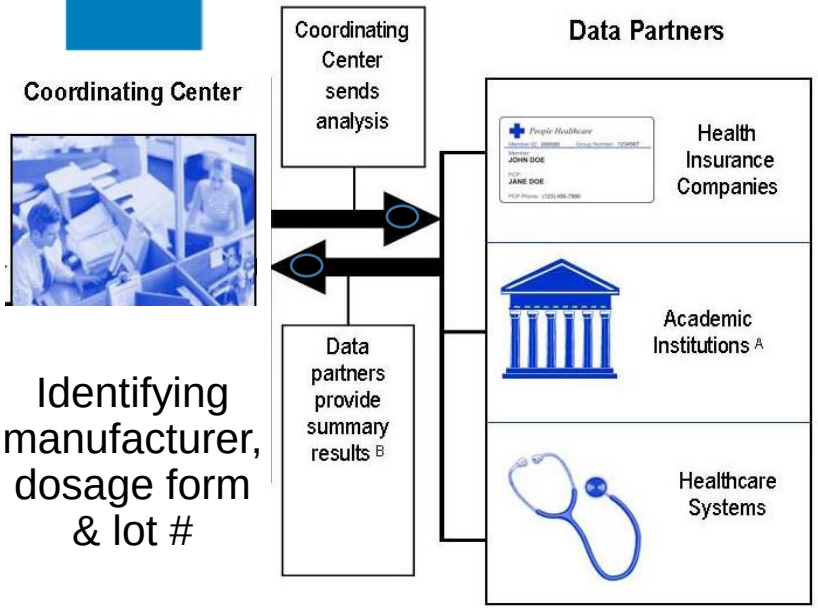
Myo Han. Whole body PETCT scan with diffuse mets.

Linking Analytics to Clinical Performance Later in Development & Post-Market

- Multivariate Statistical Analysis
- PCA



The Sentinel Initiative



Wishful Thinking

Knowledge for Repurposing & Development

Access to sites of acti

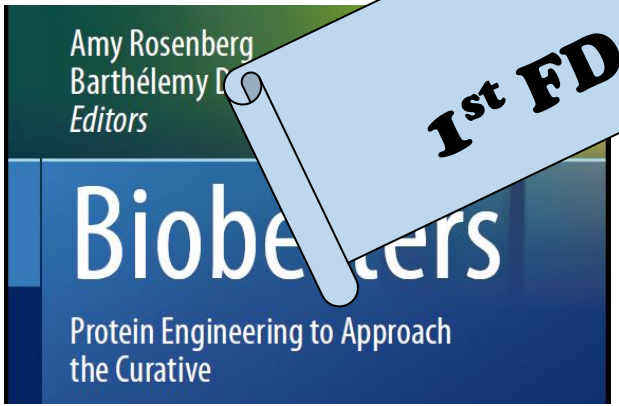
- Acid α-Glucosi
  - rem

ke

04 279:50336-50341

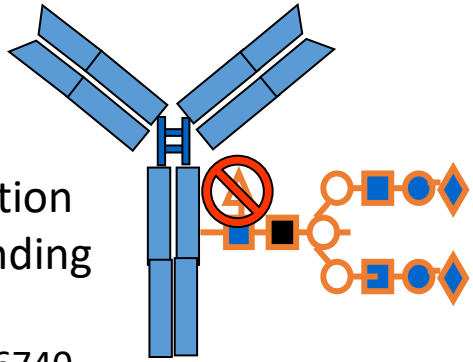
e meant to
approaches
as judgments or
endorsements

obinutuzumab:
1st FDA breakthrough approval



ity at site of action(s)

- Monoclonal Antibodies
  - altered glycoform- defucosylation
  - 50-fold increase in FcRγIIIa binding
  - Increased ADCC
    - J Biol Chem. 2002 277:26733-26740



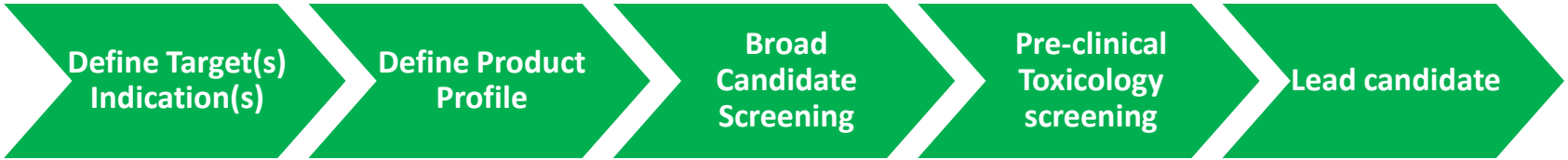
Drug Development & Decisions

Pre-clinical Development

- In silico, in vitro and in vivo biological models
- Disease mechanisms & natural history

IND Enabling

- Toxicology Studies Short term



- Protein selection
- Biological assay development
- Early purification studies

- Limited structural characterization
- Preliminary biological characterization
- Limited viral clearance
- Limited stability
- Immuno-assay based lot release
- Early CQA

Clinical Development

- Dose ranging
- Safety

- Dose ranging
- Safety, Efficacy
- Proof of Concept
- Mechanism

- Toxicology Studies
- Pharmacokinetics (PD)

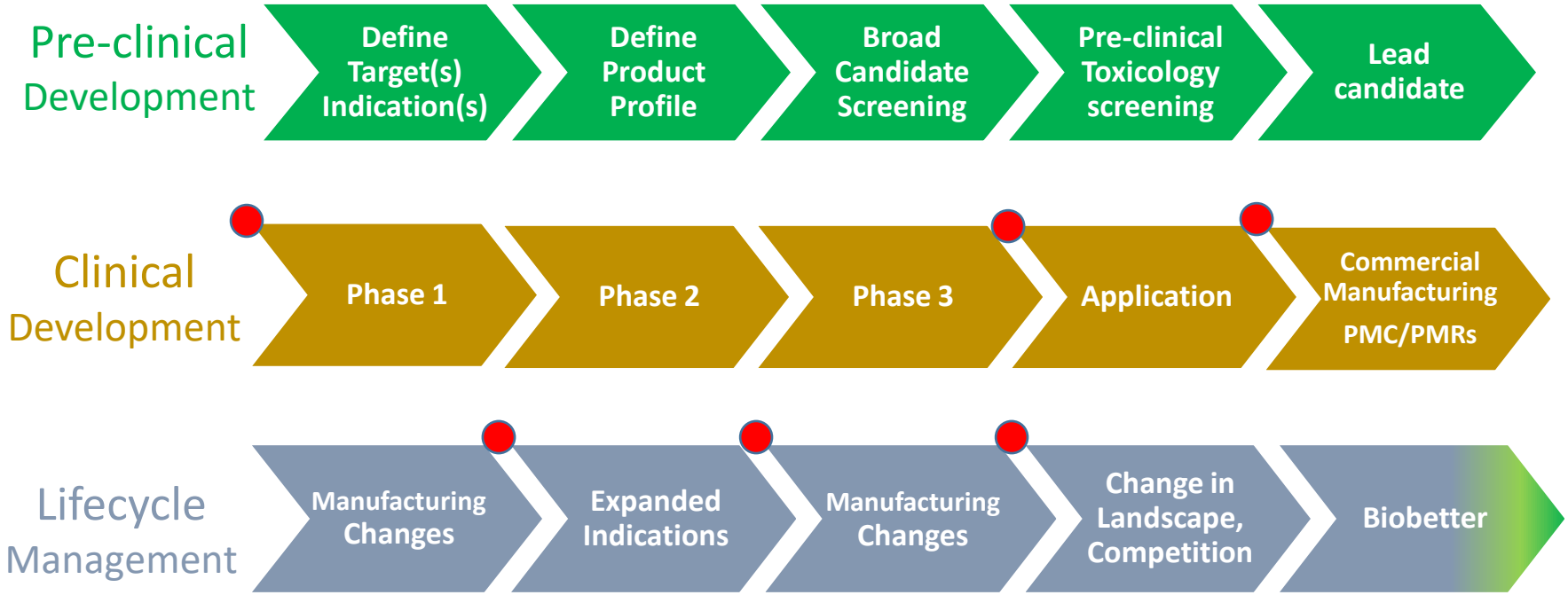
- Efficacy
- Safety

- In depth characterization
- Assay development
- CQAs
- Specification setting
- Manufacturing scale up
- Stability
- Viral Clearance

- Process validation
- Validated lot release assays
- Protocols
- Design space
- Established conditions

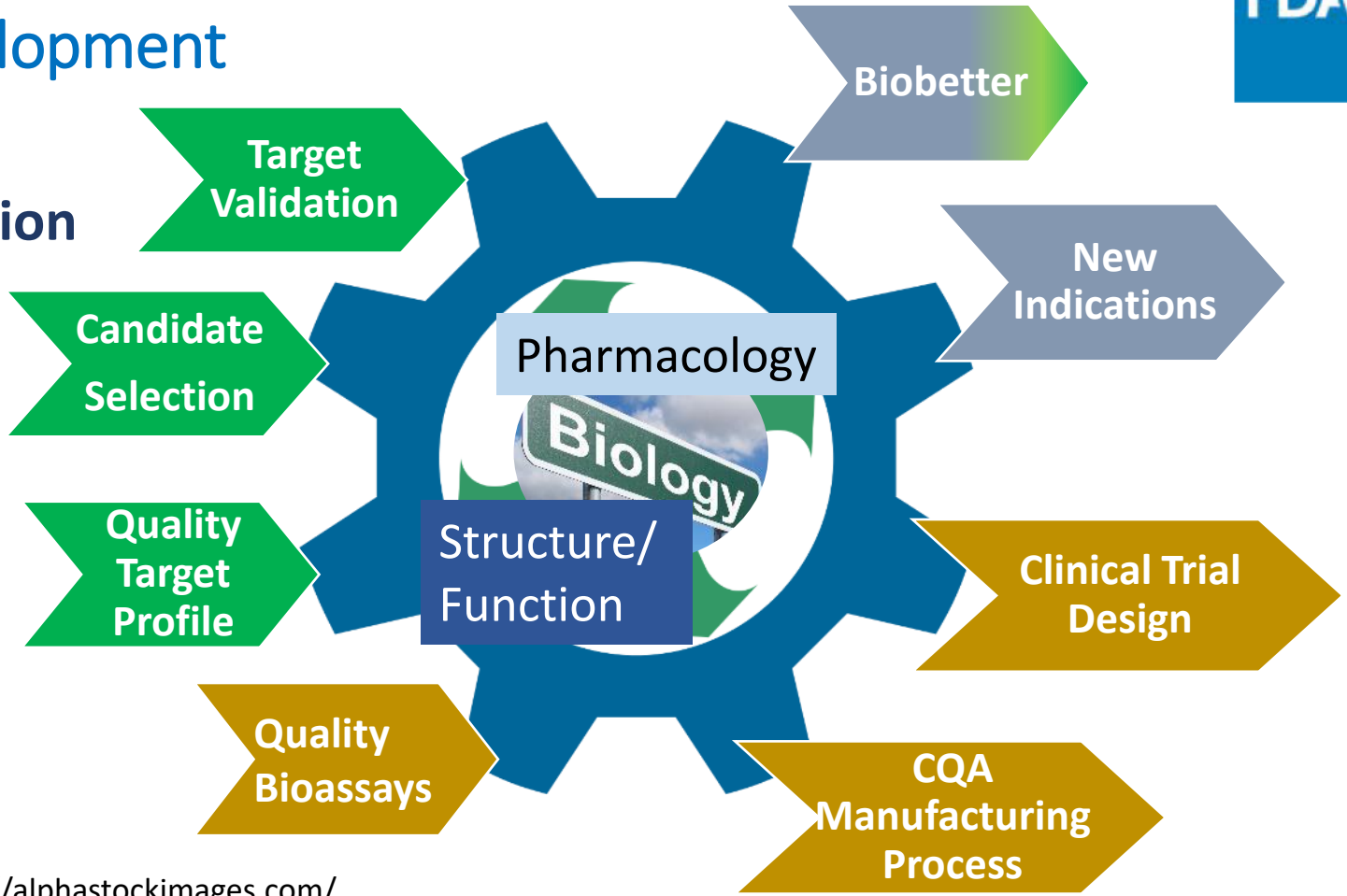
- Lot release
- Cont. process verification
- Post-marketing surveillance
- Stability

Product Lifecycle



Biology is at the Center of Drug Development

Analytical Characterization



Alpha Stock Images - http://alphastockimages.com/

Business Model Innovation

Four Paths to Business Model Innovation
by Karan Girotra and Serguei Netessine
July–August 2014 issue of Harvard Business Review.

- ***What*** your offerings [or focus] will be
- ***When*** decisions are made
- ***Who*** makes them, and
- ***Why*** they are made.

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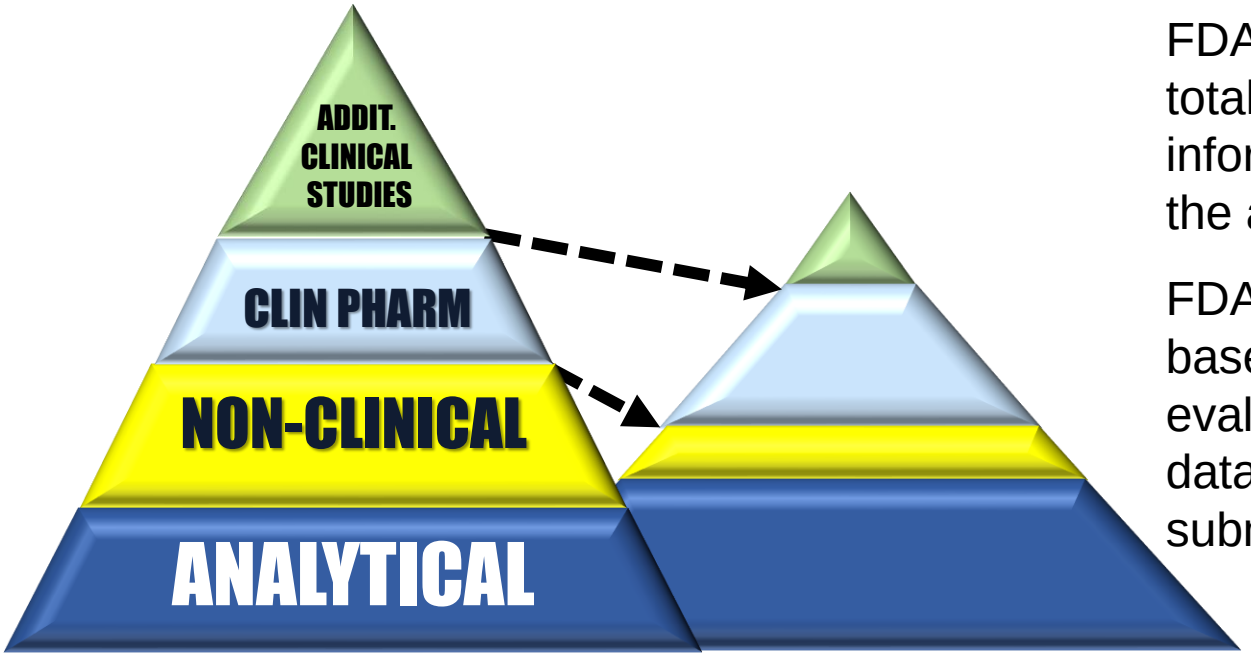
Business Model Innovation - Drug Development

Cutting edge biology needs to be linked to analytical characterization and clinical development decisions

- **What**: Integrated hub of biology, analytics, structure/function & pharmacology
- **When**: Frontload first in human trials with analytics and imaging; defer lead candidate selection
- **Who**: Role of Biology Hub (Structure/Function--Analytics, Pharmacology) in decisions and in oversight over failed development--- opportunities for biobetters
- **Why**: Cost accounting and incentives should recognize clinical development benefits of analytics

FDA: Analytics impacting both Quality and Clinical Disciplines

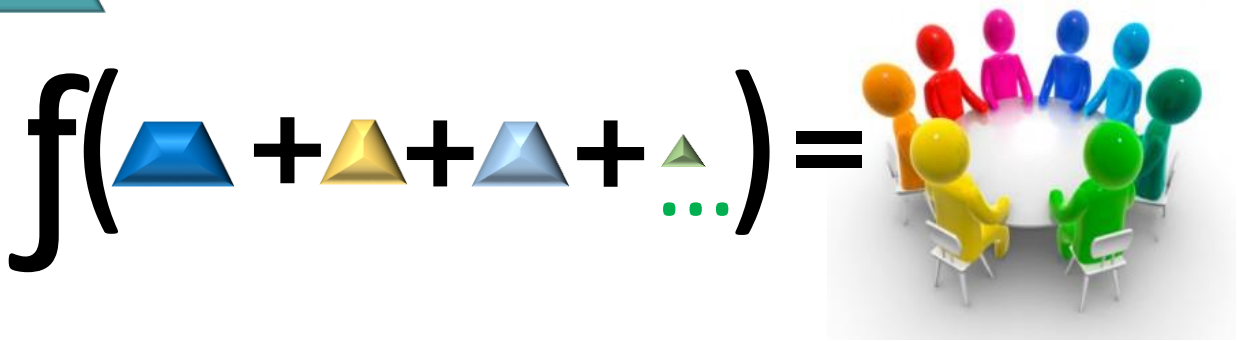
Biosimilars: Totality of Evidence



FDA will consider the totality of the data and information submitted in the application...

FDA intends to use a risk-based approach to evaluate all available data and information submitted

Biosimilars: Totality of Evidence



- Office of Therapeutic Biologics and Biosimilars (OTBB): Ensure consistency in regulatory approach and in advice provided to sponsors
- CDER's Biosimilar Review Committee (BRC)
- Collaborative review team
  - The **relationship** between quality attributes and the clinical safety & efficacy profile aids ability to determine **residual uncertainty** about biosimilarity and to predict expected "clinical similarity" from the quality data.

CDER OPQ

U.S. FOOD & DRUG ADMINISTRATION

CENTERS OF EXCELLENCE

Advancing public health through collaboration: Connecting research, policy and practice

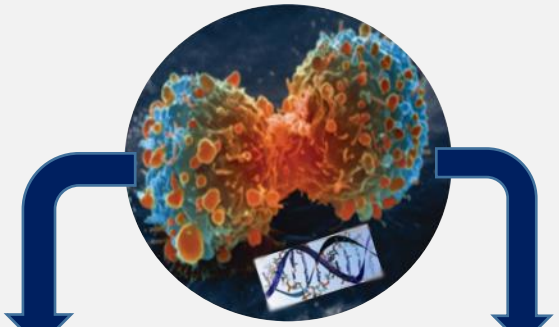
The Centers of Excellence (COEs) provide a platform for research collaboration and communication within the agency, across OPQ, CDER, FDA as well as in the broader public domain. Their collaborative research responds to and anticipates regulatory challenges to further pharmaceutical quality.

Who the COEs are and what they do

- Immunology
- Infectious Disease & Inflammation (IDI)
- Pharmaceutical Analysis & Characterization (PAC)
- Manufacturing Science & Innovation (MS&I)
- Tumor Biology

What the COEs do

- Collaboration/ Coordination
- Research Management
- Knowledge Sharing



Quality Bioassay

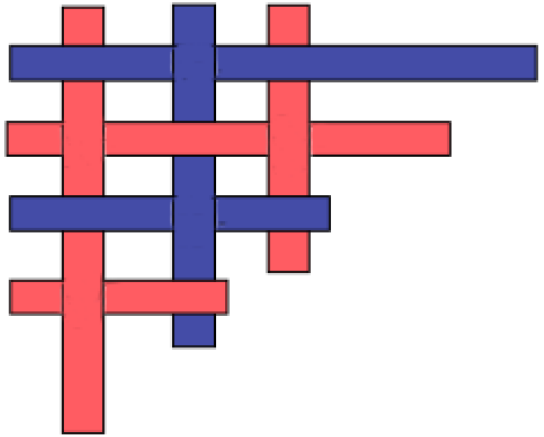
PD Biomarker

Outcomes

- ✓ Advance OPQ's core research areas and strategic priorities
- ✓ Advance the FDA's mission through regulatory-impacting research

Summary

- Shared Framework for Protein Attributes
- Enable connections across analytical methods
  - Specifications
  - Control Strategy
  - Characterization
  - Comparability & Similarity



- Integrated Biology Hub
- Informs Quality
- Links to all of Drug Development & Lifecycle

Questions