# Quality by Design & Protein Characterization

IsrAnalytica
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OPS, CDER, FDA


# Outline

- QbD for Biotech Products
- Characterization
- Quality Attributes, Safety & Efficacy

*Some of the content is speculative and this talk should not be used in lieu of regulations, published FDA guidances and direct discussions with the agency*

# 1902 Biologics Control Act

- The "Virus-Toxin Law,“
  - Regulatory authority over the processes used to make biological products, or biologics
  - Responsibility to ensure their safety
- Drivers for Law
  - A horse named Jim whose tetanus-contaminated serum was used to produce a diphtheria antitoxin that caused the deaths of thirteen children in St. Louis, Missouri.
  - Contaminated smallpox vaccine which killed nine children in Camden, New Jersey.

# 1938 Federal Food Drug & Cosmetic Act

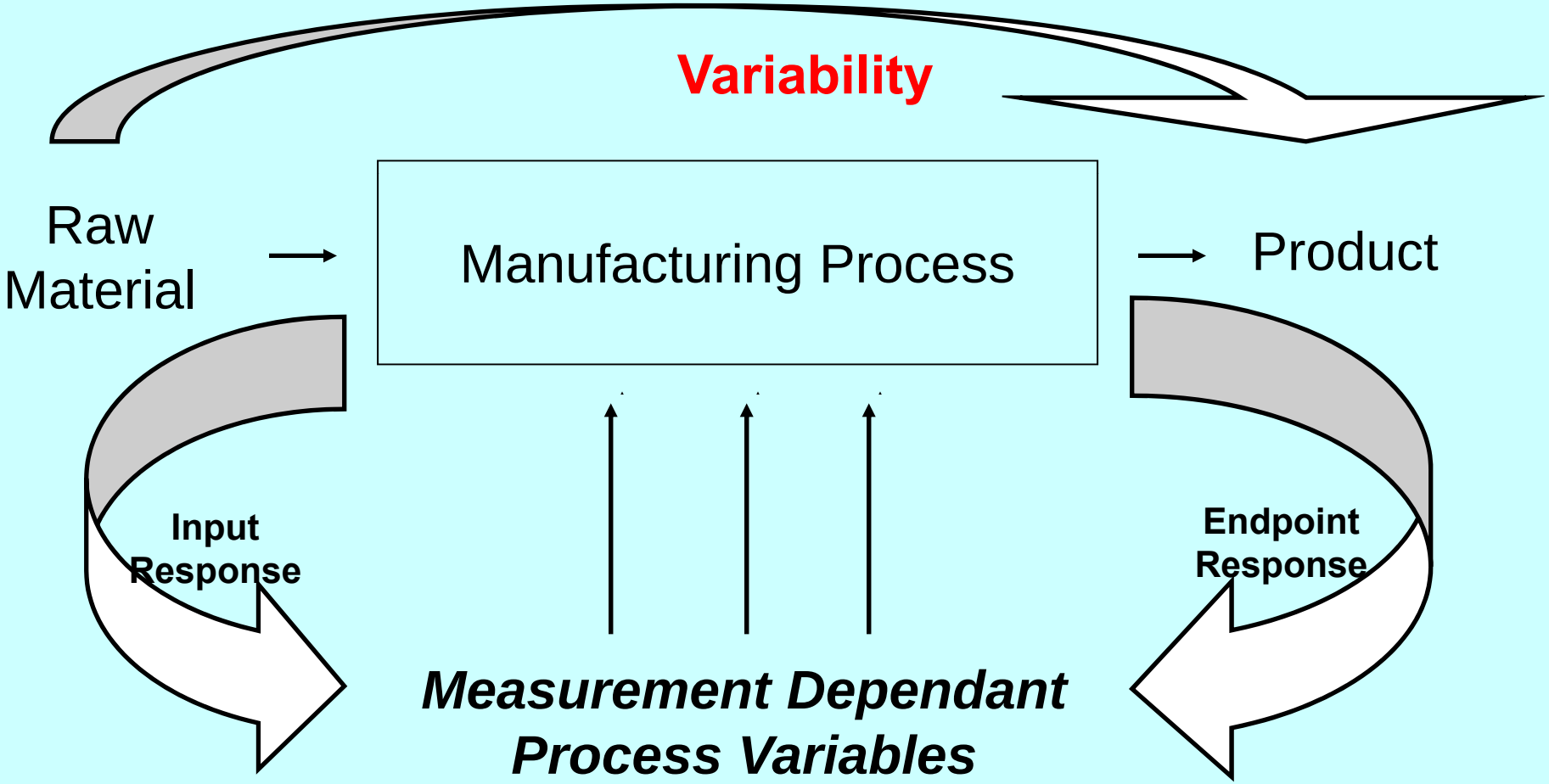
- New Drug Application (NDA)
  - Drug composition, manufacturing & quality
  - Report on safety
- Driver of Law
  - In 1937, S. E. Massengill Co., a pharmaceutical manufacturer, created a preparation of sulfanilamide using diethylene glycol (DEG) as a solvent, and called the preparation "Elixir Sulfanilamide
  - More than 100 deaths
  - Under Pure Food & Drug Act of 1906
    - Just misbranded “Elixir”

# Quality in the 21st Century

- In 2002, FDA identified a series of ongoing problems in pharmaceutical manufacturing problems
  - High costs regulatory compliance
  - Poor innovation & efficiency / Lack of agility
- Quality by Design (QbD)
  - A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management (ICH Q8R2)

# Manufacturing Process

*Biologics: The Process is the Product*

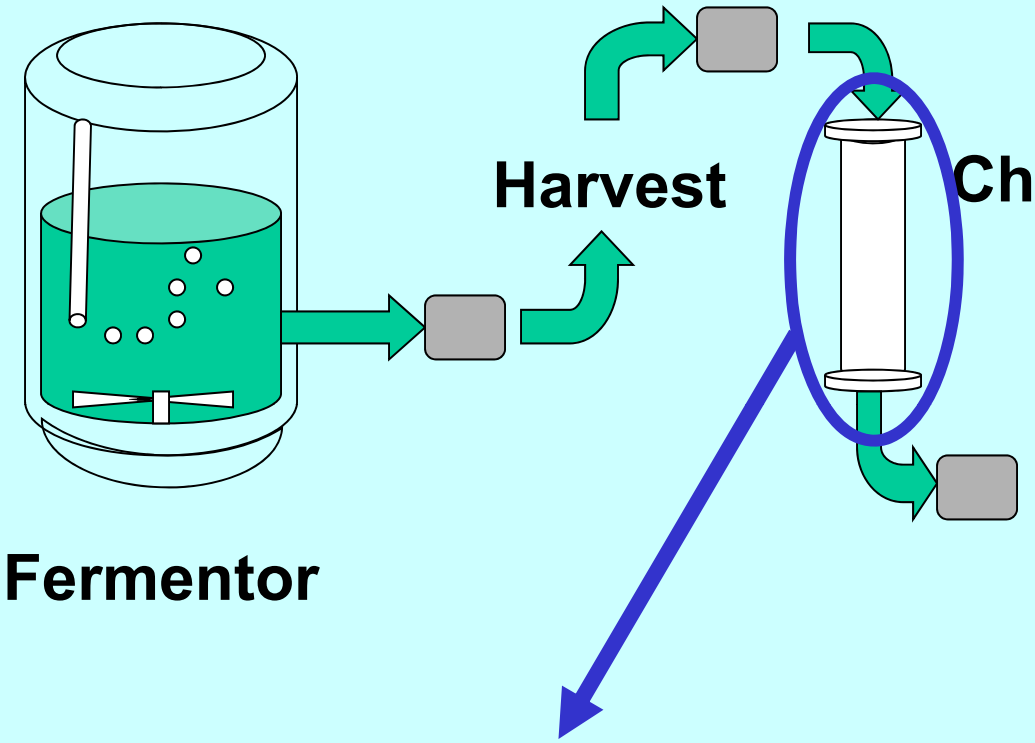



**Jon Clark**

# ICH Q8R2: Design Space

- Definition
  - The multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality
- Regulatory Flexibility
  - Working within the design space is not considered a change
- Important to Notice
  - Design space is proposed by the applicant and is subject to regulatory assessment and approval

# Biotechnology Manufacturing Process



**Harvest**

**Chromatography Columns**

**Proteolytic Steps**

**Conjugation**

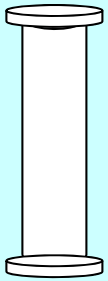
**Diafilt./Conc.**

**Formulation**

**Lyophilization**

**Fermentor**

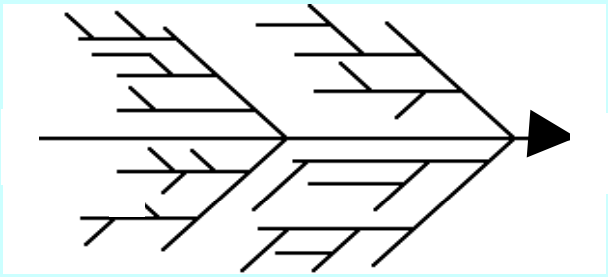
Flow through ion exchange column



Removes

- Sub-potent charge variants
- Impurities

# From Attributes to Spaces



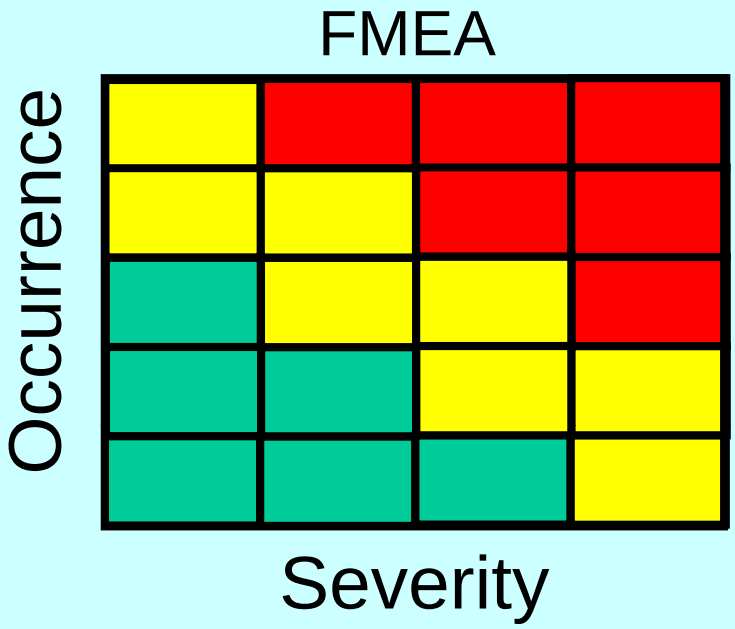
- Assign relative risk for each factor

  = [severity]

  x [occurrence]

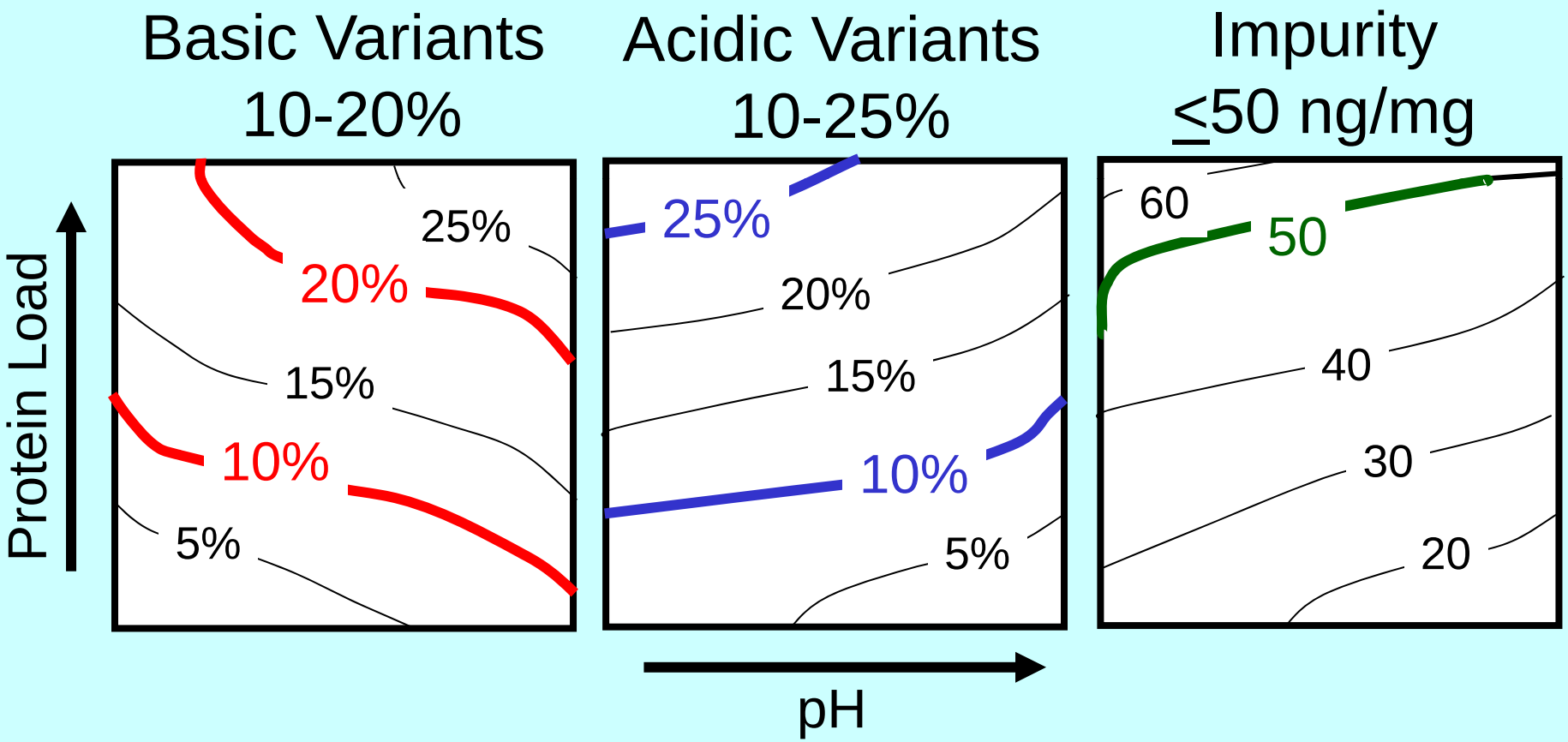
  x [detectability]

*Risk assessment includes process develop., manufact., QC staff, etc. & trained facilitator*

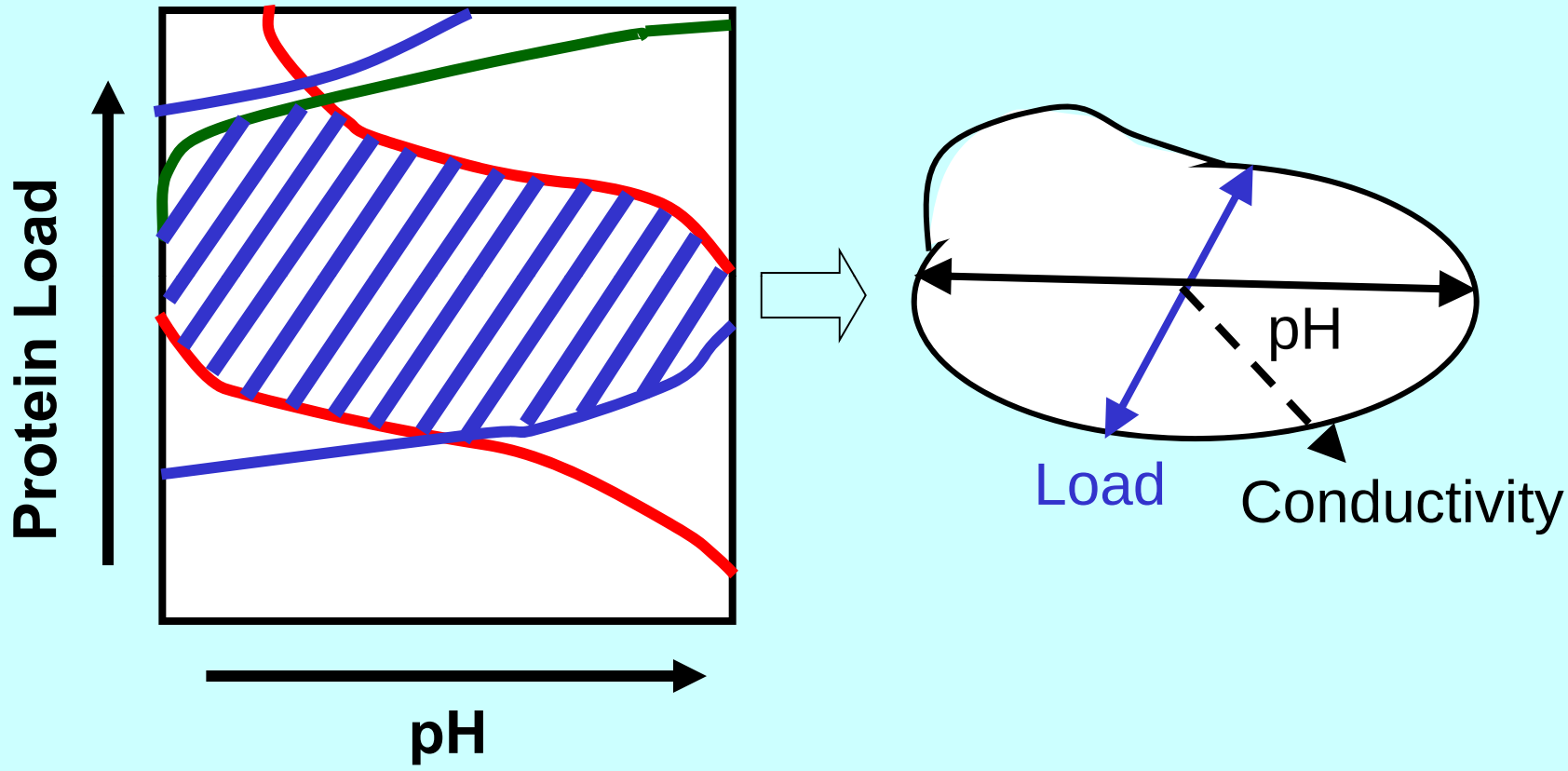



- Risk Ranking
- Screening DOE
- Optimization
- Process Characterization

# Surface Responses of Important Product Attributes

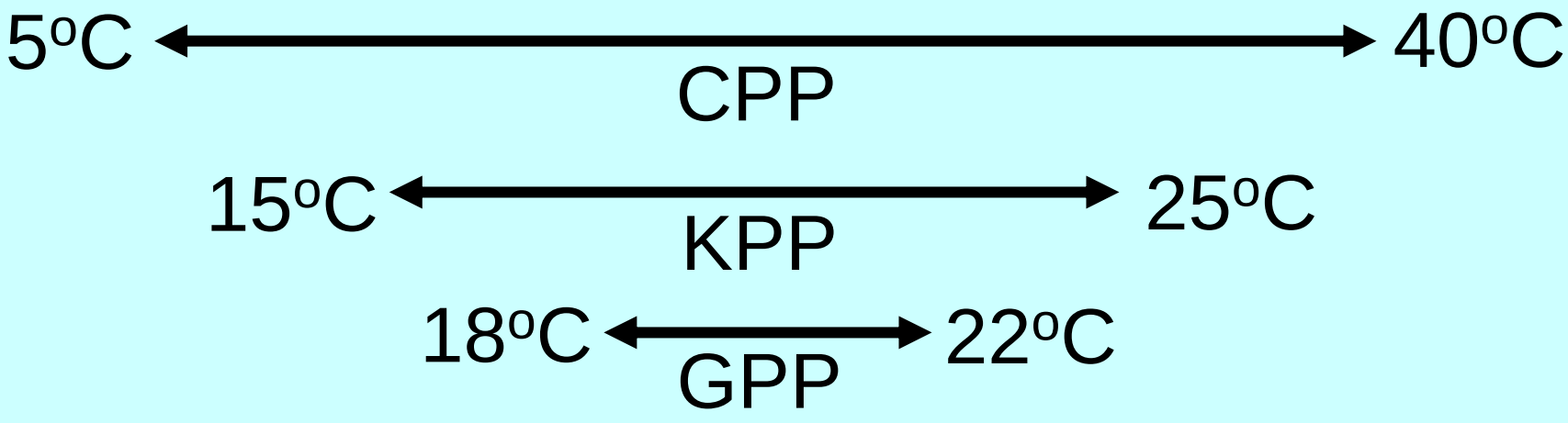


# Overlay of Response Surfaces into an Initial Design Space



# What is in the Design Space?

- **Critical Process Parameter (CPP):** A process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality. (ICH Q8R)
- How do we deal with uncertainty in impact?
- A CPP is a function of the range evaluated



# Initial Design Space Weaknesses

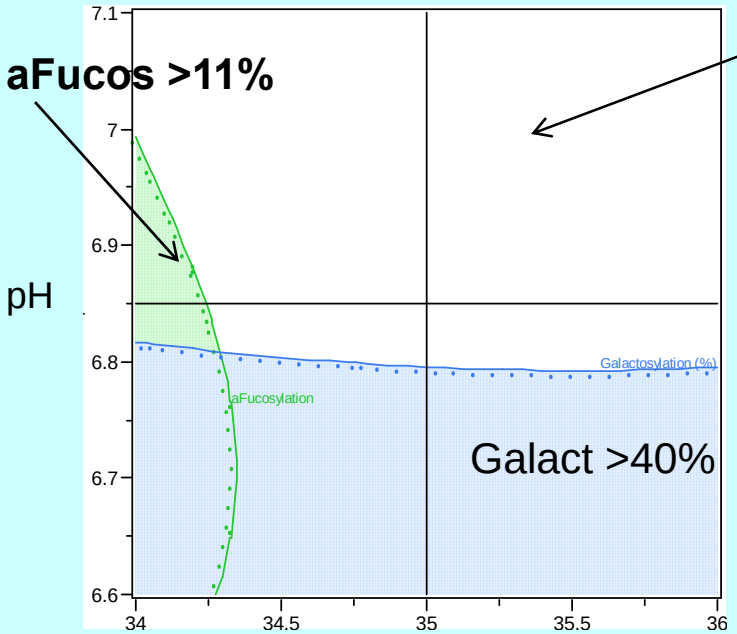
- Based on model (DOE)
  - Predictions are extrapolations
    - inside as well as outside explored space
- Missed factors
- Missed interactions at screening
  - Each factor alone has little impact
  - Larger risk with complex processes
  - A-Mab interaction risk score is of value
- Missed important responses
  - Larger risk with complex products
  - Interactions between responses
- Experiments done at lab scale

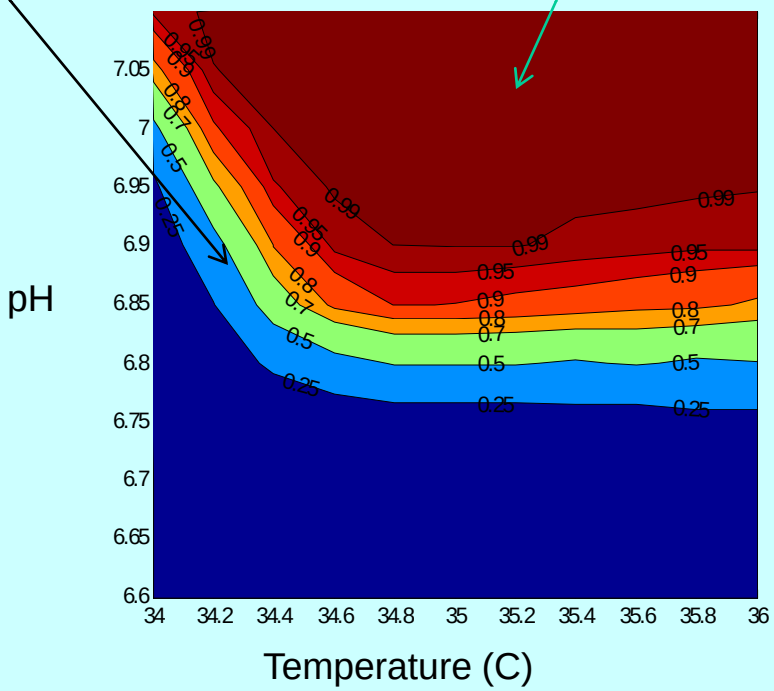
# A-Mab Design Space Based on Process Capability Bayesian Reliability

Example: Day 15, Osmo=360 mOsm and pCO2=40 mmHg

50% contour approximates “white” region” in contour plot

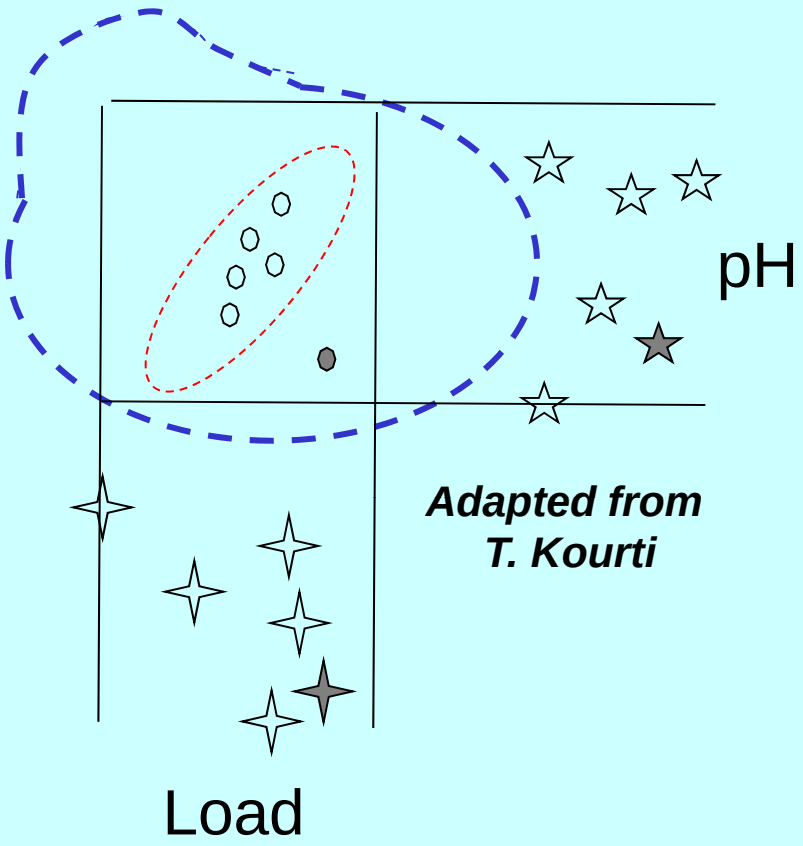
>99% confidence of satisfying all CQAs



# Lifecycle Approach

- Managing uncertainty
  - **Complex products**
  - **Complex processes**
  - **1st Prin. Models rare**
- Multivariate SPC
  - **Facilitates moving across scales**




Dimensionless Variables

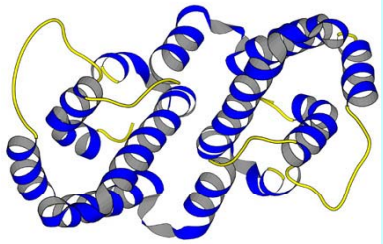
Engineering Approaches To Modeling

# QbD Implementation

- Link to small-molecule learnings
  - ONDQA pilot and application experience
- ICH IWG, Q8R, Q9, Q10
- ICH Q11
- OBP staff participating in conferences, forums and training on QbD
  - Design of Experiments Training
- **OBP Pilot**
- **Mock Case Studies**

# Outline

- QbD for Biotech Products
- **Characterization**
- Quality Attributes, Safety & Efficacy

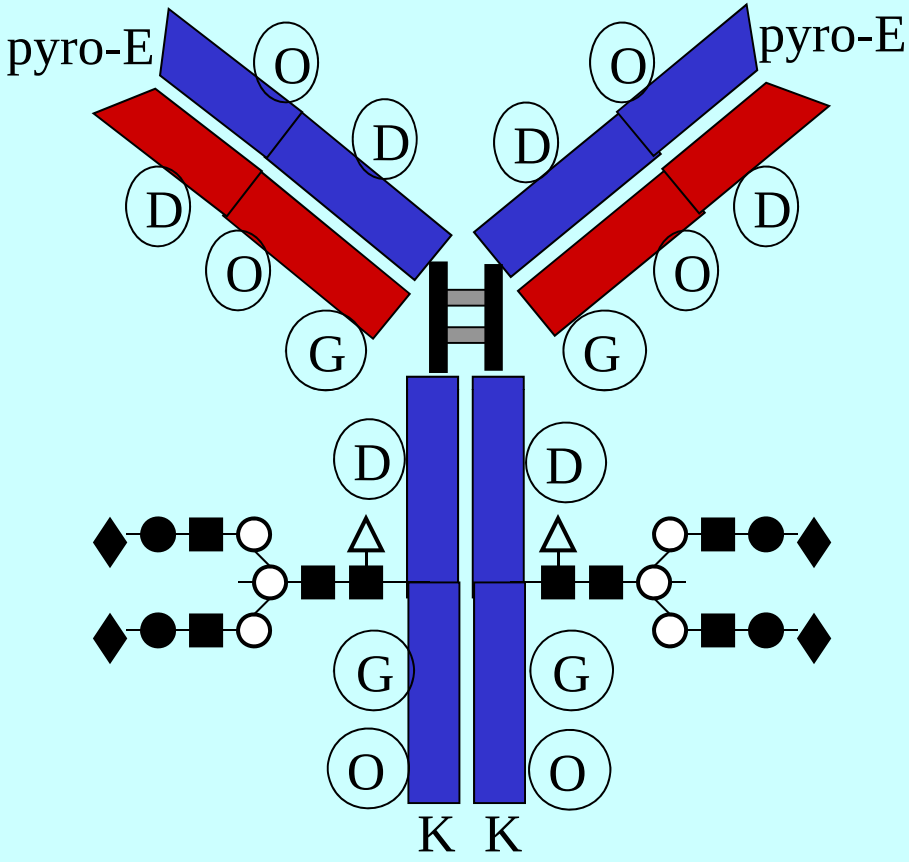


# Protein Heterogeneity



- Amino Acid Substitution
- Truncation
- Folding
- Mismatched S-S bonds
- N- and C-terminal mods
- Aggregation
- Multimer Dissociation
- Denaturation
- Acetylation
- Fatty acylation
- Deamidation
- Oxidation
- Carbamylation
- Carboxylation
- Formylation
- $\gamma$-Carboxyglutamic acid
- O-linked Glycosylation
- N-linked Glycosylation
- Methylation
- Phosphorylation
- Sulphation
- Glycation

# Attributes & Combinatorics



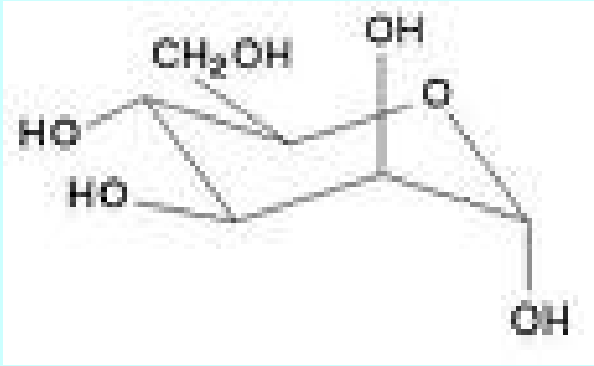

- Pyro-Glu (2)
- Deamidation (3 x 2)
- Methionine oxidation (3 x 2)
- Glycation (2 x 2)
- High mannose, Fucosylation G0, G1, G1, G2 (10)
- Sialylation (+5)
- C-term Lys (2)

- **$(8460)^2 \approx$ 75 million**
- **2 x 6 x 6 x 4 x (10+5) x 2 = 8460**

# Questions

- Do these matter?
- How much matters?
- Do combinations matter (interactions)?
- How independent are these attributes?

# Glycosylation (N or O)




Mannose ○

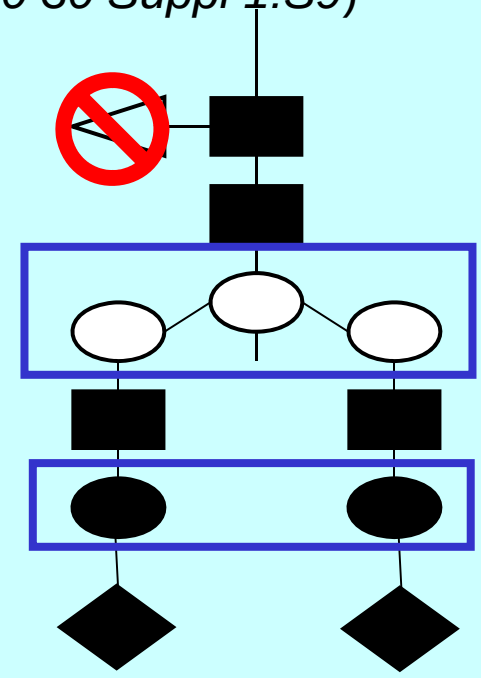
Fucose △ Galactose ●

N-Acetyl-Glucosamine ■

Sialic Acid ◆

# Antibody Glycosylation Matters

- In general the more galactose the more CDC activity. *(Boyd et al. Molec Imm 1995 32:1311)*
- Terminal sialic acid residues decrease binding to activating FcR (*Kaneko et al. Science 2006 313:670*)
- Sialylated IgG ($\alpha$2-6 linkage) increases inhibitory FcR expression. (*Anthony et al. J Clin Imm 2010 30 Suppl 1:S9*)
- Some antibodies with very high mannose forms were found to have a shorter *in vivo* half-life. *(Wright et al. Glycobiology 2000 10:1347)*
- Lack of fucose on huIgG1 improves binding to activation FcR and ADCC. (*Shields et al. JBC 2002 277: 26733*)



# Antibody Glycosylation Effects

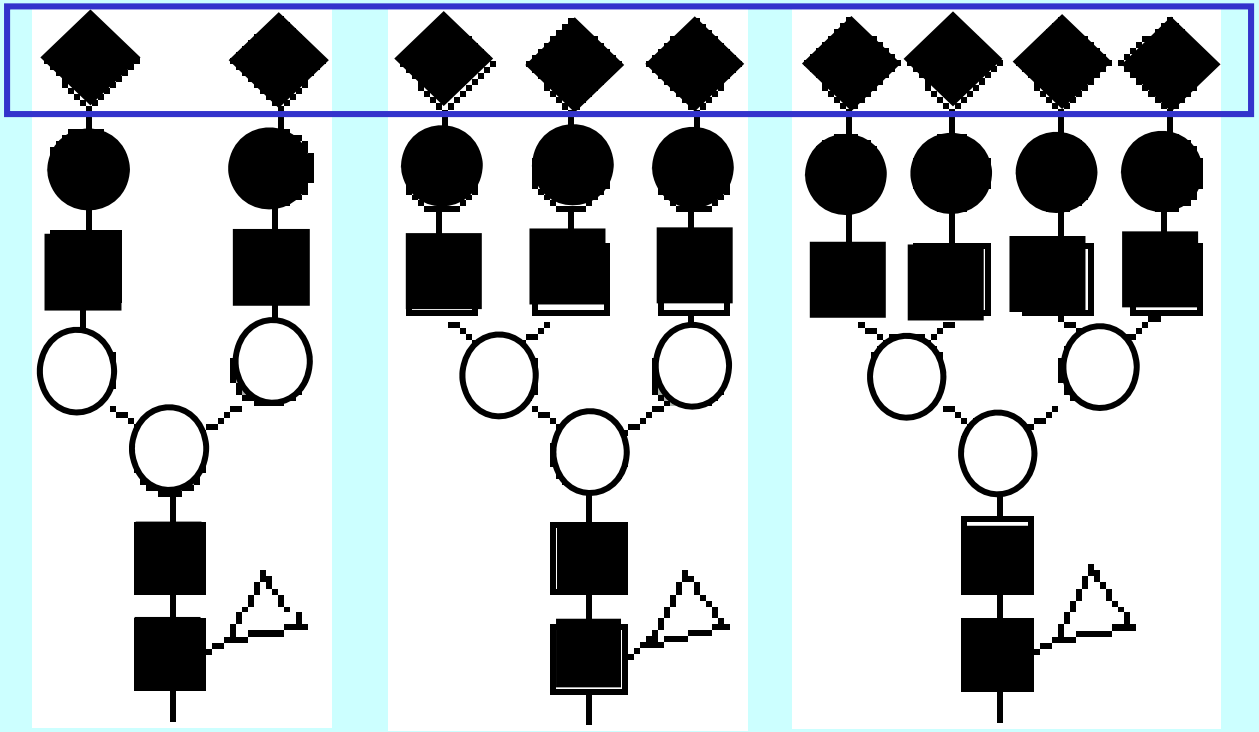
- Small amounts can matter
  - Defucosylation
  - 50-fold increase in FcR$\gamma$IIIa binding
  - Large increase in ADCC
- Interactions
  - Evaluation of defucosylation with three different oligosaccharide backbones
    - Glycobiology 2006 17:104
  - ADCC highest- Defucosyl-complex
  - ADCC intermediate- Defucosyl-hybrid, Fucosyl-complex, High mannose
  - ADCC lower- Fucosyl-hybrid

# Antibody Glycosylation Questions

- Sialylation & defucosylation together?
  - Ipsilateral
  - Opposite half antibodies
- Dual versus single sialylation on each biantennary glycoform?
- Sialylation or defucosylation on both half antibody biantennary glycoforms?
- Location $\alpha$1-6 versus $\alpha$1-2 chains of biantennary complex?
- Approaches?
  - Modeling
  - Linking half antibody glycoforms together
  - Purification strategies

# Protein Complex N-linked Glycans

- Tetraantennary species appear to contribute more to overall bioavailability (increased half life) of a protein product then tri- antennary structures ….

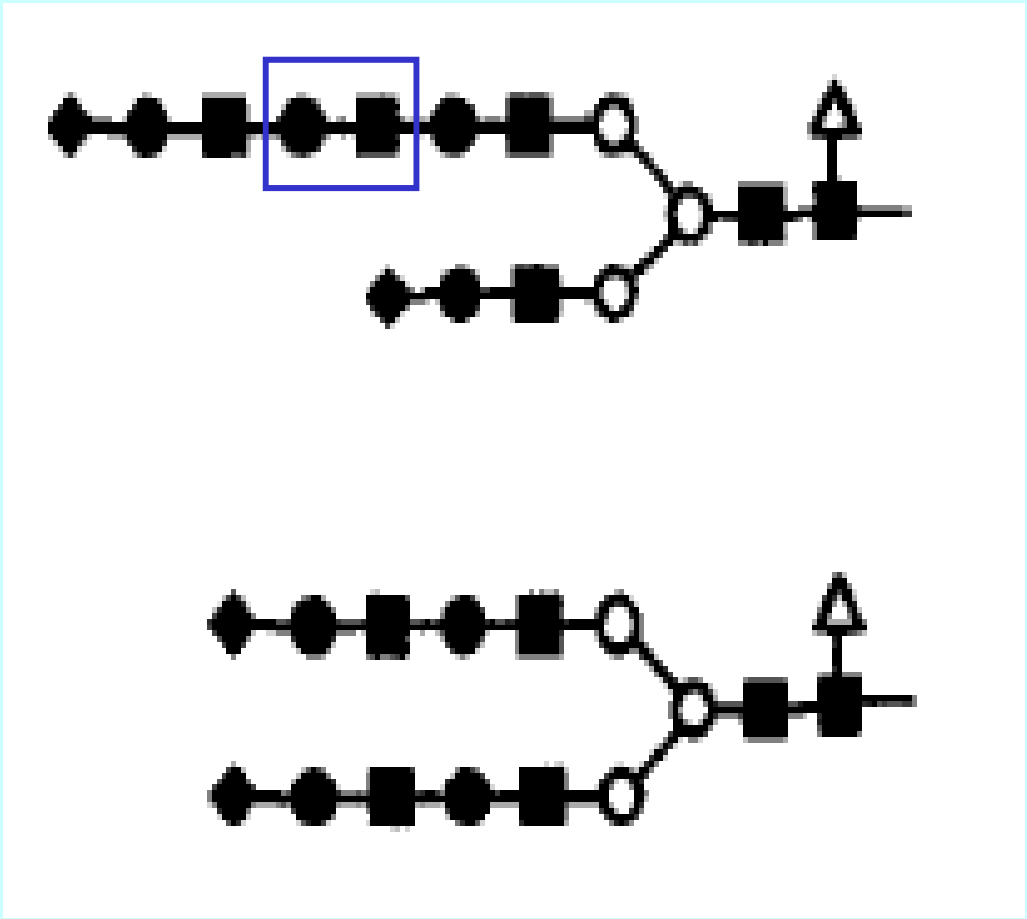


Terminal Sialic Acid (SA) prevents binding to asialo-glycoprotein R, enhancing availability at other sites

- Small changes in tetraantennary content and the presence or absence of terminal SA can have a significant impact on bioavailability (potentially S & E)

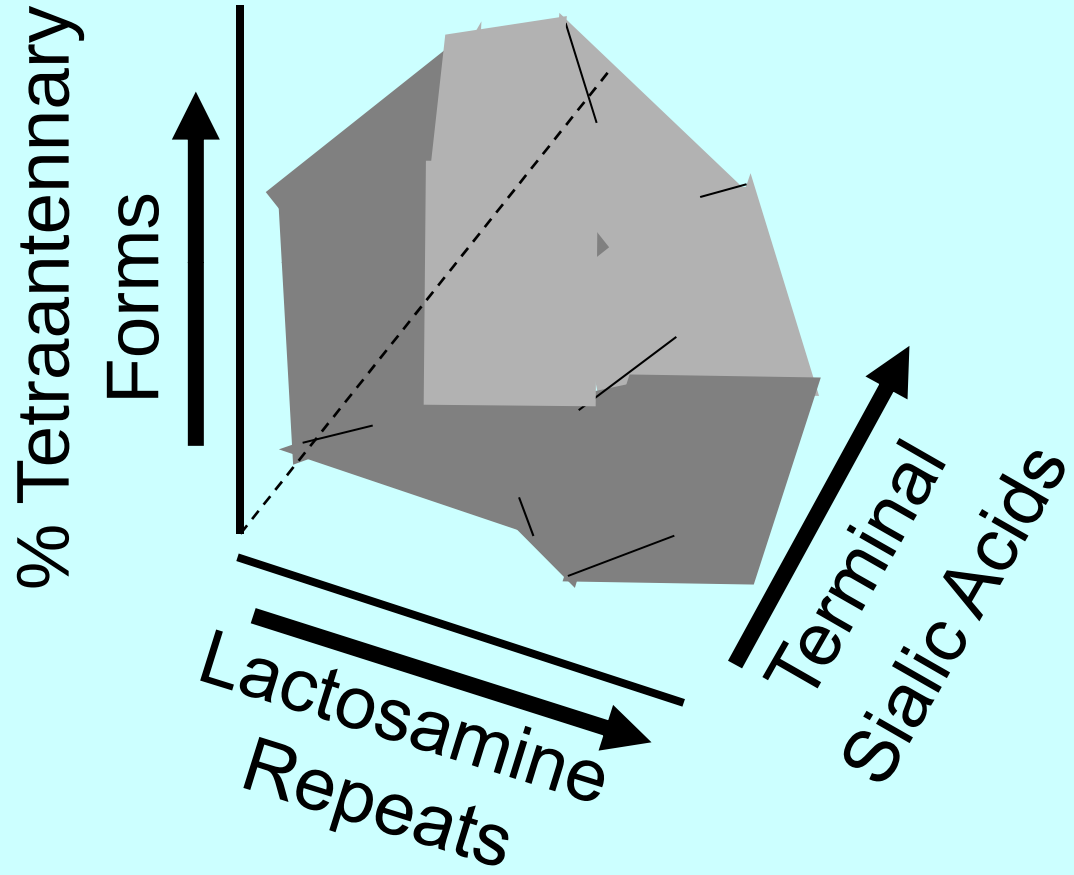
# Complex N-linked Glycans

- Repeating units of N-acetyl glucosamine and galactose
- Elongation of the oligosaccharide leading to greater hydrodynamic radius
- Associated with increase in *in vivo* potency presumably via increased bioavailability



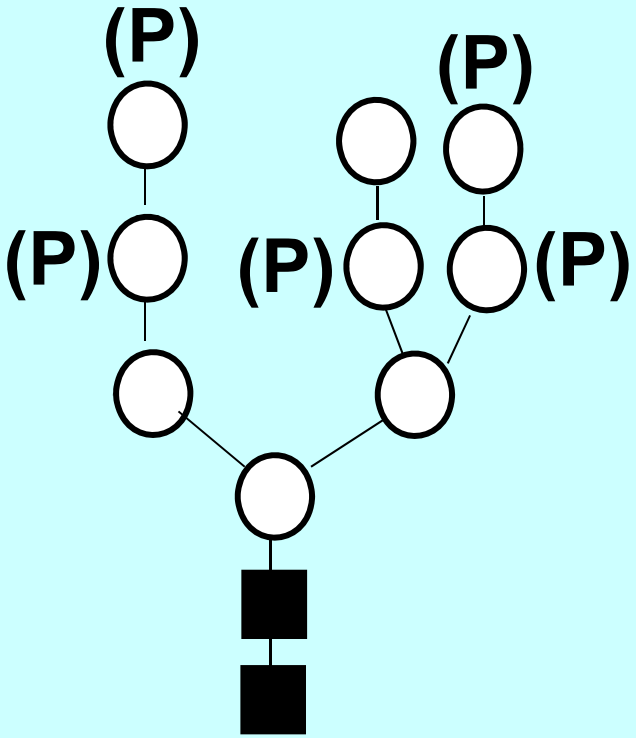
This & prior slide from Barry Cherney

# Attribute Space




- Bioactivity
- Clearance

# Lysosomal Enzymes



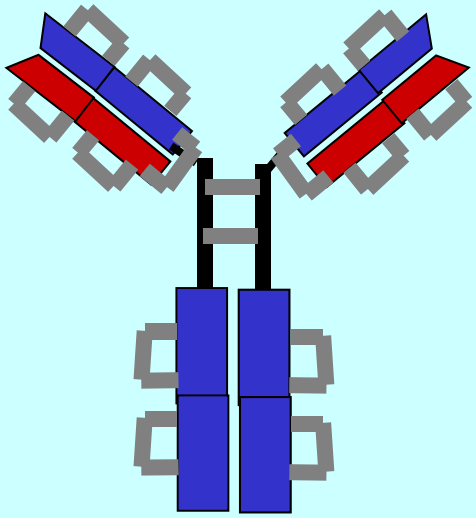

- Carbohydrate-remodeled acid alpha-glucosidase… demonstrates improved delivery to muscles of Pompe mice.
  - Biochem J. 2005 Aug 1;389(Pt 3):619-28.
- remodeled-rhGAA was internalized approx. 20-fold more efficiently
- Small amounts could matter

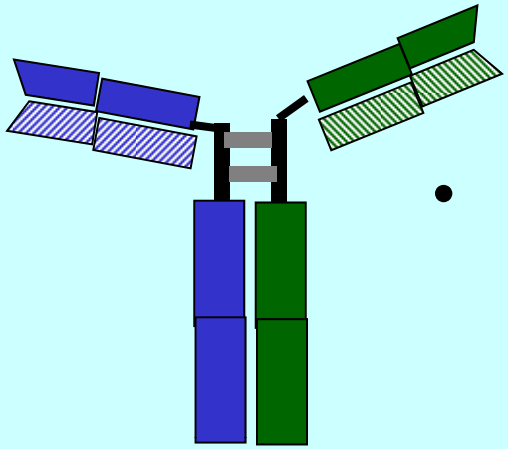
Interaction with other attributes that may impact activity?

# Other Changes

- Gal-α1-3-galactose
  - Cetuximab made in murine cells have this glycosylation-link to anaphylaxis (NEJM 2008 358:1109)
  - CHO cells can produce this glycosylation (Nat Biotech 2010 28:1153)
- Labile changes: O-GlcNAc
  - Cytoplasmic proteins: Some therapeutic proteins? Other labile changes?
- Total PEG content unchanged but a change in PEG distribution across sites changed PK > 2 fold

# Antibodies & Disulfides





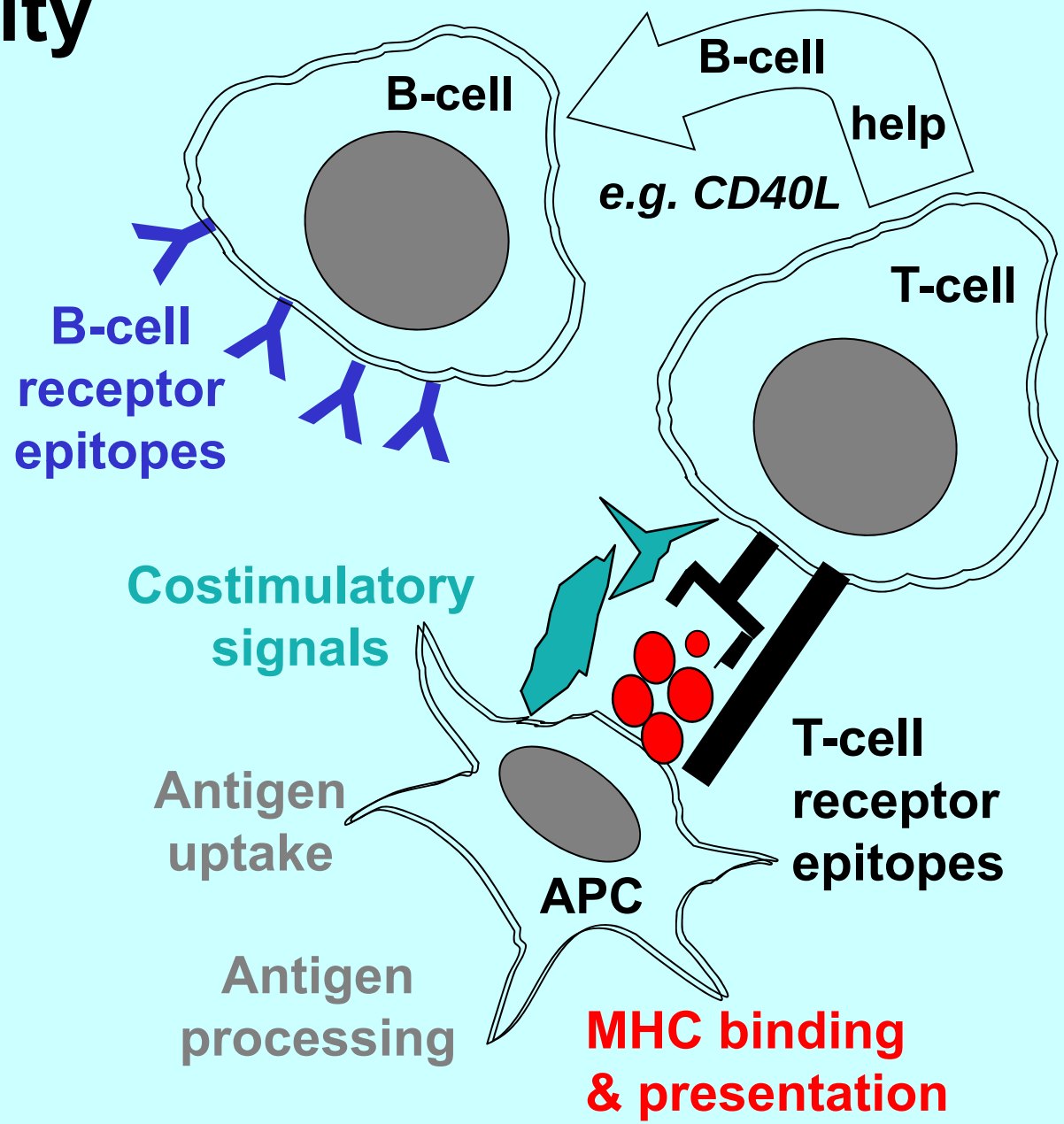
- IGG4 short hinge
  - Labile inter-chain disulfide bonds with half-antibody exchange
  - *Immunology 2002 105:19*

- IGG1 has 16 disulfide bonds with differential susceptibility to reduction
  - Can impact on stability & effector functions
  - *Anal. Chem. 2010 82:5219*
- Sensitive to manufacturing process
  - *Biotechnology & Bioengineering 2010 106:452*

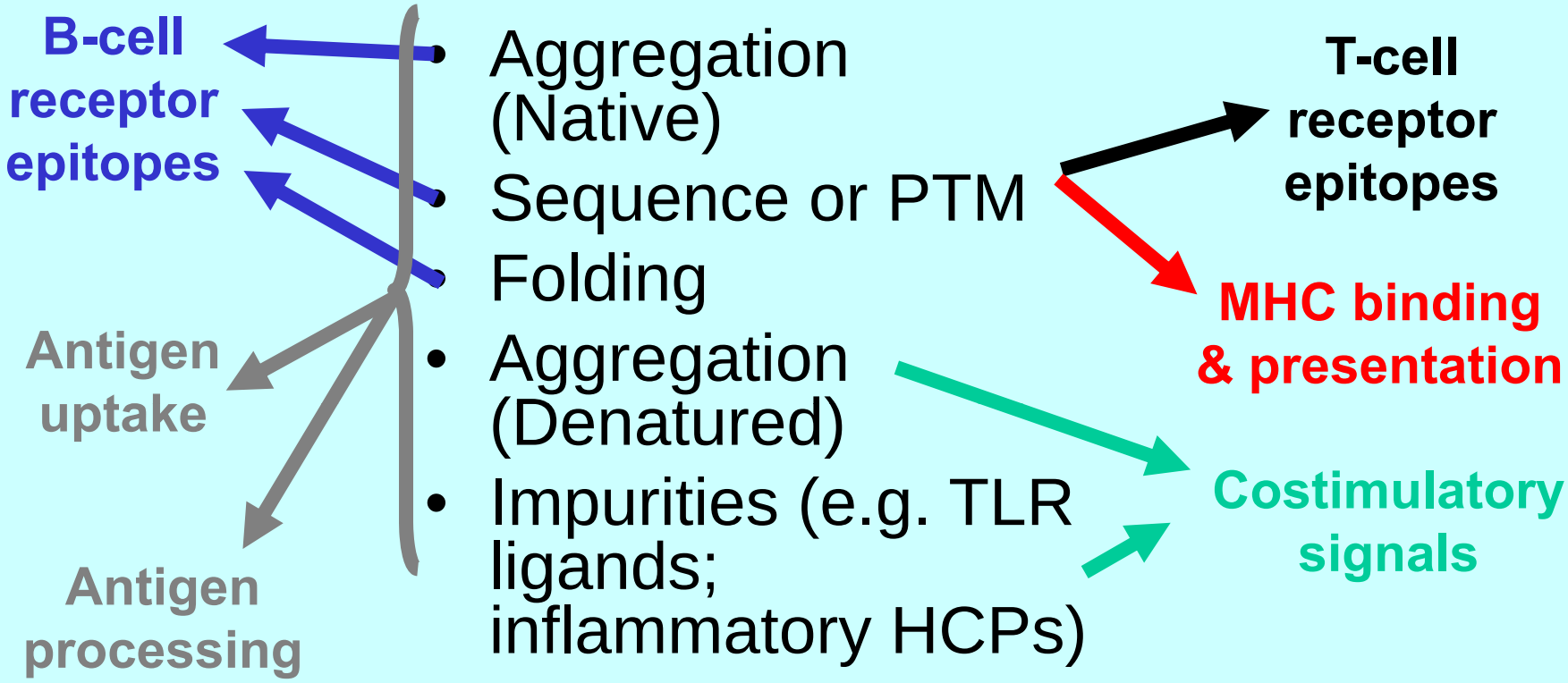
- IGG2-Disulfide form A converts to form B over time
  - *JBC 2008 283:29266*
- IGG2-Disulfide form B appears less potent than form A for some cell surface targets
  - Less effective bivalent binding?
  - *JBC 2008 283:16212*
- There is an IGG2 Disulfide form A/B
- Trisulfide bonds
  - *Anal. Chem. 2009 81:6148*

# Immunogenicity

- There are many aspects to development of an immune response.
- These events often need to interact in a multi-dimensional way
- Protein attributes may impact one or many of these



# Immunogenicity



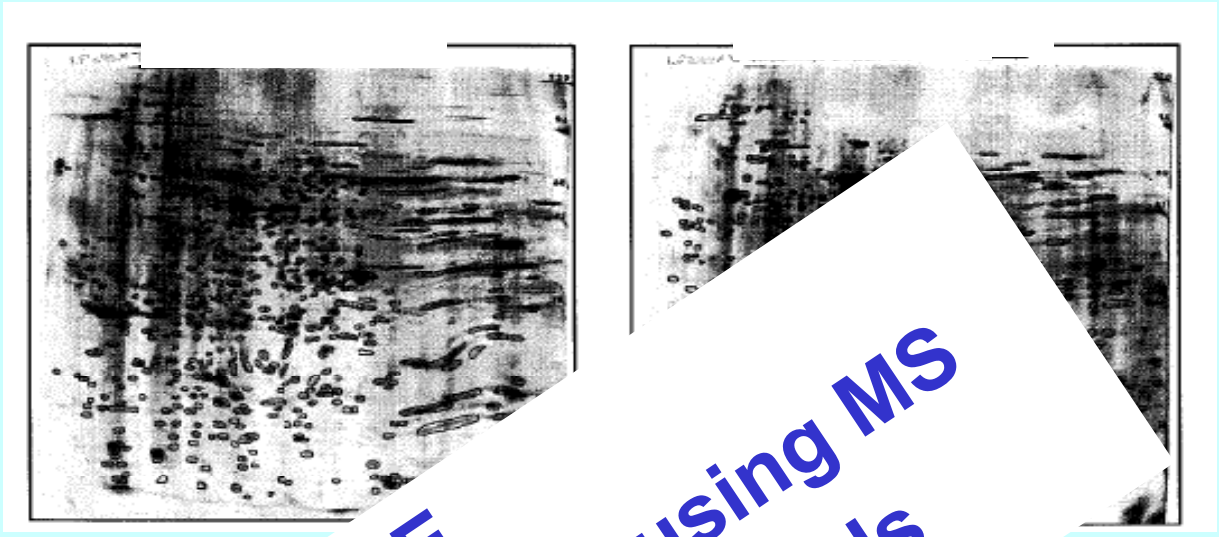

- *Citrullinated MBP digested at a greater rate by Cathepsin D*
- *Deamidation: linkage connecting isoaspartic acid residue and its C-terminus neighbor are not recognized by most proteases*
- *Truncation: revelation of cryptic sites*

Adapted from Amy Rosenberg

# Immunogenicity

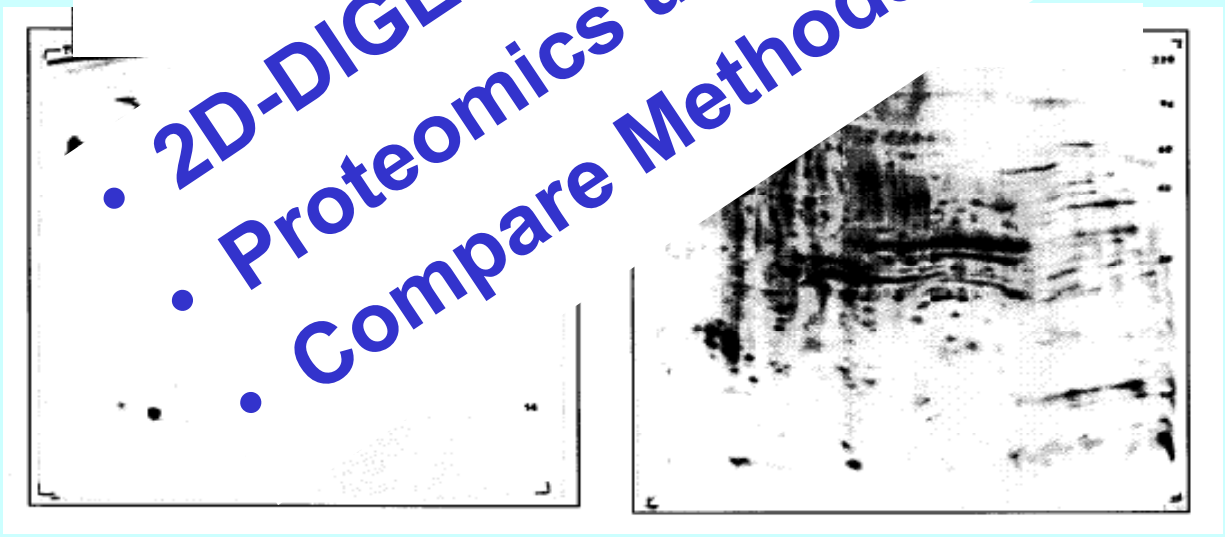
- An immune response usually involves multiple steps
  - Thus different attributes may interact in immunogenicity
- Erythropoetin PRCA increase associated with multiple changes
  - Changed formulation and primary packaging
  - HSA to polysorbate: syringes & non-coated rubber
- Immune responses can be generated with very small amounts of antigen
  - A low level of an immunogenic variant can matter

# Impurities: Host Cell Proteins



Silver stain of HCP assay standards

- 2D-DIGE
- Proteomics using MS
- Compare Methods



Western blots

Commercial HCP Assay Antibody

Specific HCP Assay Antibody

From Barbara Rellahan

# Charge Variants

- Bennett talk Aug 2008 regarding efalizumab change in PK
  - Increase C-term processing & acidic forms
  - Increase galactosylation
- IgG1 charge variants do not impact IV or SC PK in rats (MAbs 2010 15: Epub)
  - Combinatoric effect? Better model?
- Another MAb: Lower potency in basic variant

# Size Variants

- Tissue-Type Plasminogen Activator
  - Native sc-tPA occurs as two glycoforms. Type I sc-tPA is fully glycosylated, while type II lacks glycosylation at Asn-184. Type II is cleaved faster and has higher levels of activity
- Protein internal cleavage that lowers potency
- MAb pre-monomer peak
  - During the CHO manufacturing process an extra sequence of light chain can be translated (panitumumab EMA EPAR)
- Metal ions leaching from packaging leading to metalloprotease cleavage of product
  - Impact on stability

# Large Size Variants

- Aggregates
  - *BioPharm Int 2008 21(11)*
- Shape
- Interaction
- Composition
- Conformation
- Functionality

- Size
  - *J Pharm Sci 2009 98:1201*
  - *J Pharm Sci 2010 99:3302*

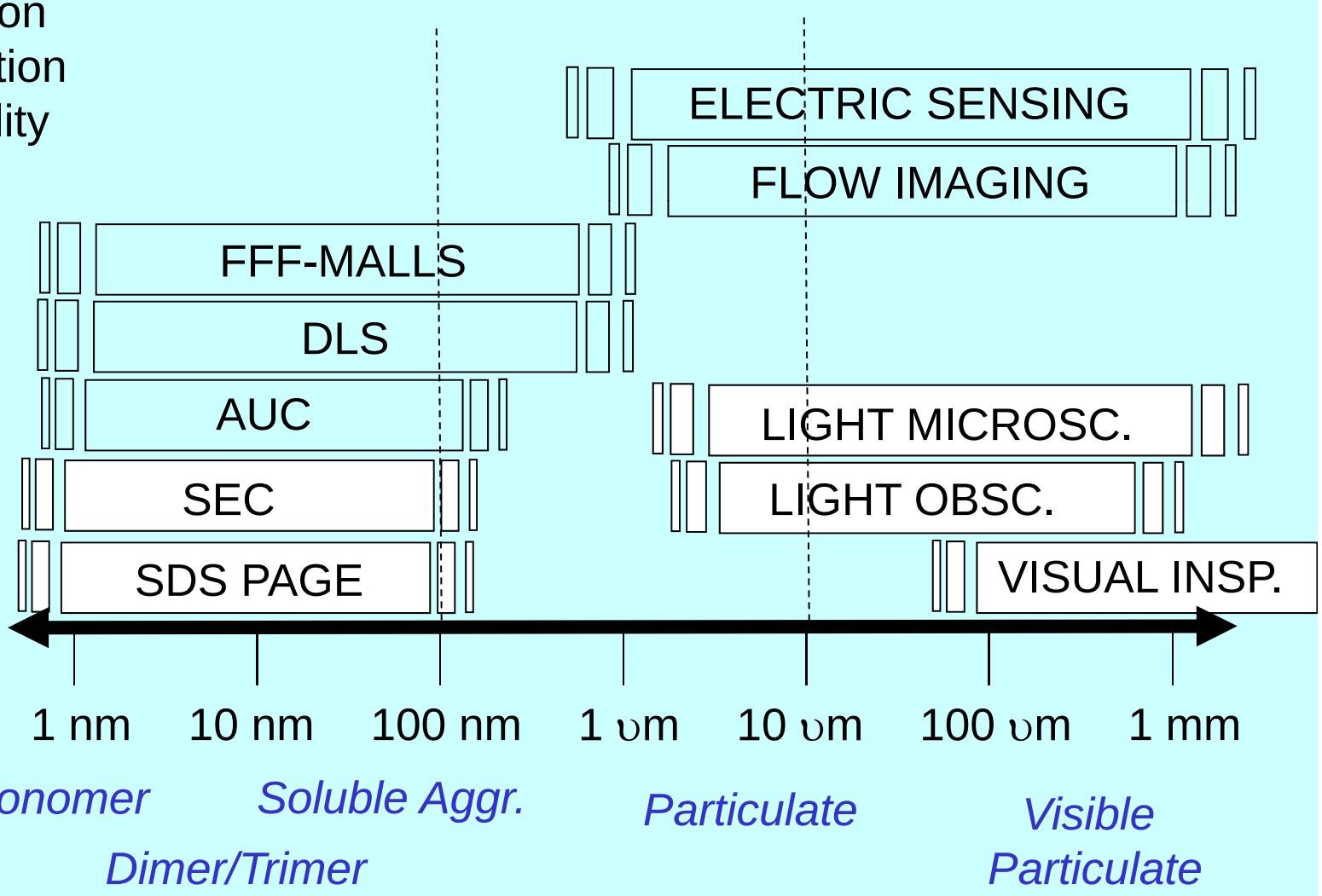
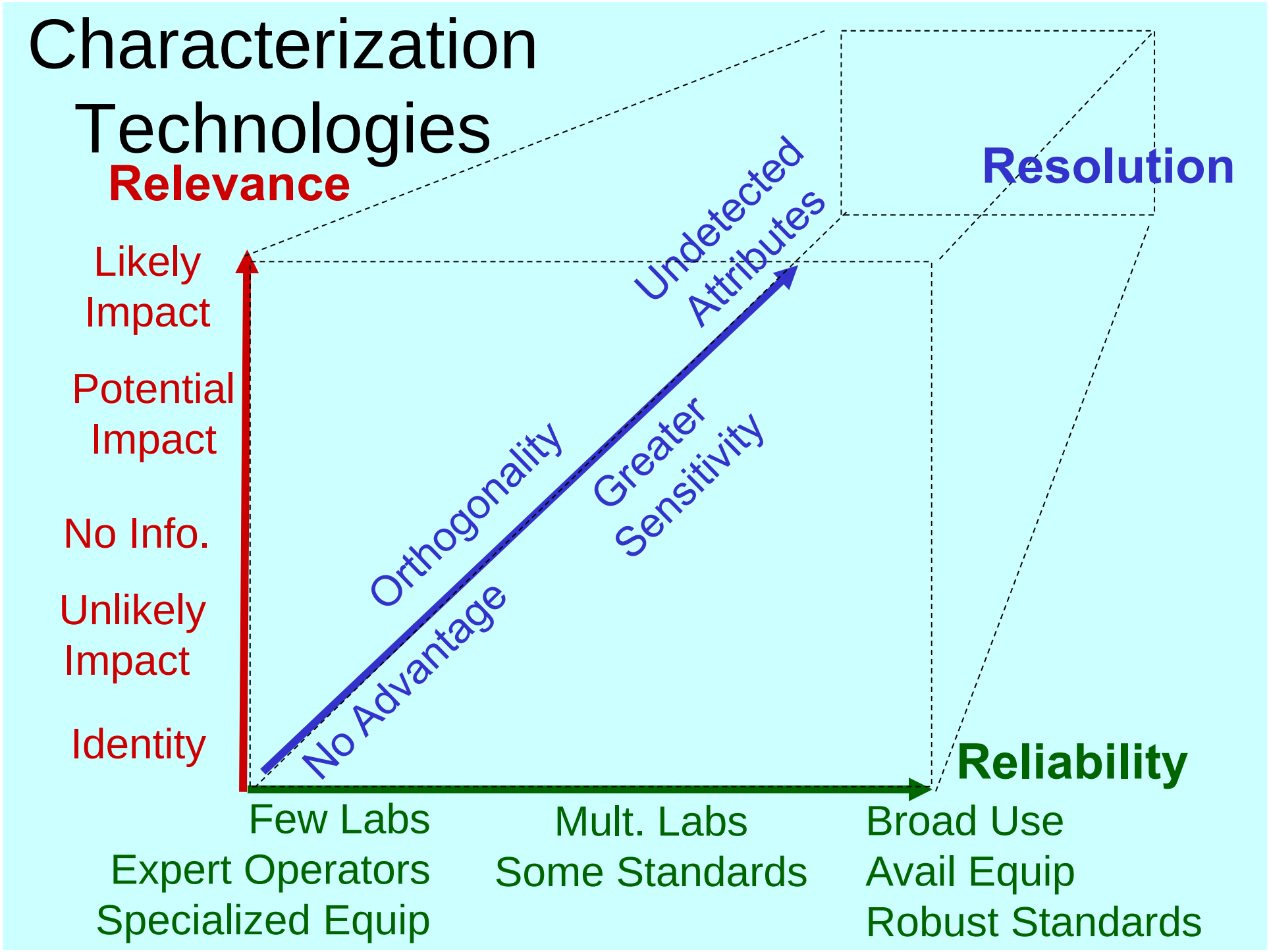



Fig adapted from J Pharm Sci 2010 99:3302 & Curr Biopharm Tech 2009 10:373

# Characterization

- Setting an unachievable bar?
  - Nat Biotechnol, 2005. **23**(9): p. 1054-8
- No!
- Advances in characterization are opportunities
  - Important to understand what attributes are present and how they can vary
- Methods to use
- How to deal with greater information
  - Need a balance between flexibility & uncertainty
  - Links to clinical performance

# Characterization Technologies



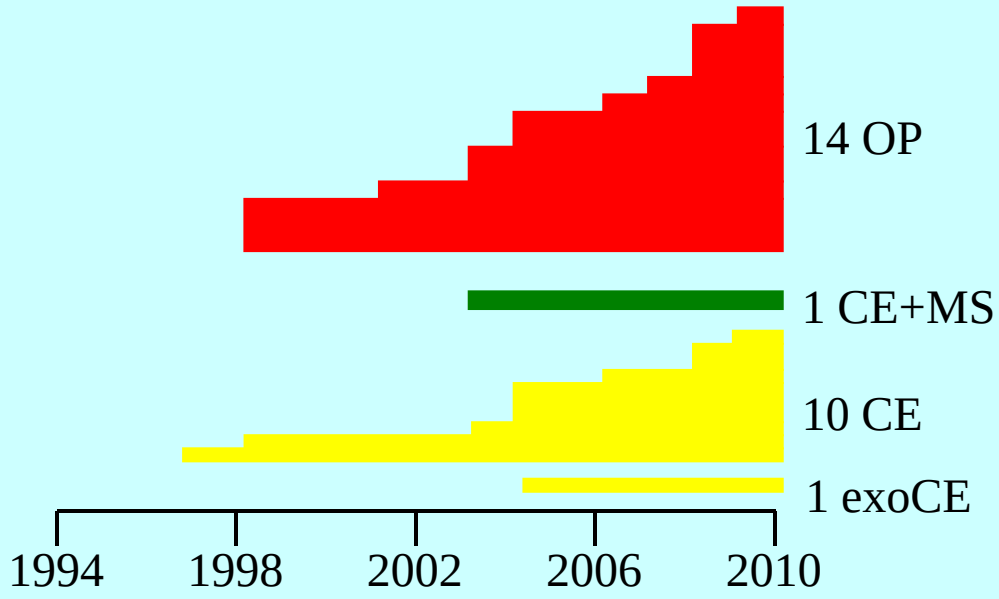
# CE Applications for Biologics (from Wassim Nashabeh, GNE)

| | |
|---|---|
| 1981-1983 | Initial Publication of "Zone Electrophoresis in Open Tubular Glass Capillaries" in Analytical Chemistry (81), followed by a paper in "Science" (83)—both widely credited with the launch of modern CE |
| 1983-1988 | Increased use in academic labs and few characterization or feasibility studies in industry (often in collaboration with academic labs) |
| 1989 | First international symposium HPCE (high performance capillary electrophoresis) held in Boston with the introduction of first commercial CE instruments, indicating growing use within academic centers—First conference was chaired by Prof Barry Karger |
| 1997 | Submission and approval by the FDA of two CE methods to be used as part of the control system QC release for a MAB—cIEF (identity) and Glycan analysis |
| 1999 | Launch of "CE in the Biotech and Pharmaceutical Industry" Symposium, reflecting acceptance and growing use in Pharma—Symposium is currently in its 12$^{th}$ year with international attendance and regulators on Organizing Committee; Also first mention of "CE" in ICH Q6B in appendix 6.1.2 (c) |
| 2001-2005 | Advances in instrumentation continued with significant expansion in applications (including CE-MS for Characterization), imaged cIEF and the introduction of platform methods |
| 2006-present | Method becomes routine, with general chapters being developed in pharmacopeias |
| 2010 | ICH Q4B—Global Harmonization of the General Chapter on CE in USP, EP, JP |

# CE for Evaluating Glycosylation

*Database: Glyco-assays over last 16 years*
*Read, Park & Brorson, Submitted*

- Other glyco-analytic method
  - OP= oligosaccharide profiling



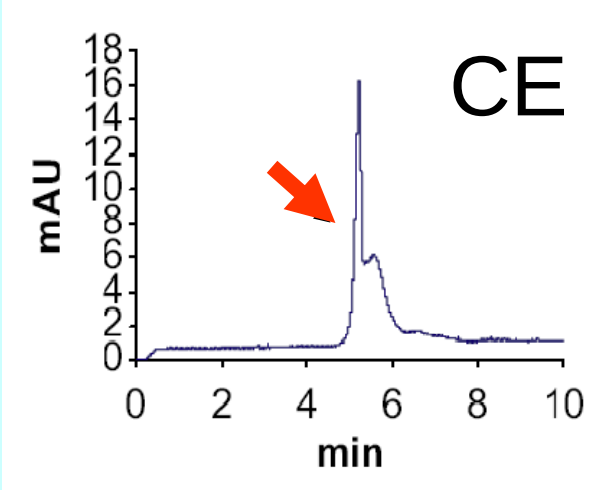

Year of assay implementation

- 23 BLAs
- Most BLA applications contain glyco-analytic data
  - More for product characterization vs. lot release
- CE= Capillary electrophoresis
- Many other modalities not covered here
  - MS, combinations

# Heparin Adverse Events

- Oversulfated chondroitin sulfate is a contaminant in heparin (Nat Biotechnol. 2008 Jun;26(6):669-75)
- CE is a routine assay for current complex product
  - would have picked up contaminant in crude, API or finished product

Biomaterials. 2008 (36):4808-14.




Update Analytical Methods

Regulatory agencies should have a transparent approach to newly discovered pre-existing variablity

# WCBP 2011 Assay Modernization

*– A Good Idea, But Not as Easy as It Sounds*

- Implementation of rapid microbiological methods for sterility testing
  - Three alternative methods evaluated only one comparable in sensitivity
    - Rajesh Gupta, *OCBQ, CBER, FDA*
- Use of NMR to identify polysaccharides in a polyvalent vaccine
  - NMR data on solvents showed LOD method was inaccurate
  - Thus the weight-based concentration of polysaccharide components was inaccurate
    - Robert Sitrin, *Merck & Co, Inc.*
- NMR method to assess OSCS contaminant
  - Also detected other variants- acetylated heparin
    - Edward Chess, *Baxter Healthcare Corporation*

# Outline

- QbD for Biotech Products
- Characterization
- **Quality Attributes, Safety & Efficacy**

# Critical Quality Attributes

Critical Quality Attribute (CQA): *A physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality* [Q8R]

- Biological Studies
  - In vitro
  - Animal
  - Clinical
- Prior Knowledge
  - Platform
  - Related products

*Uncertainties: A continuum*

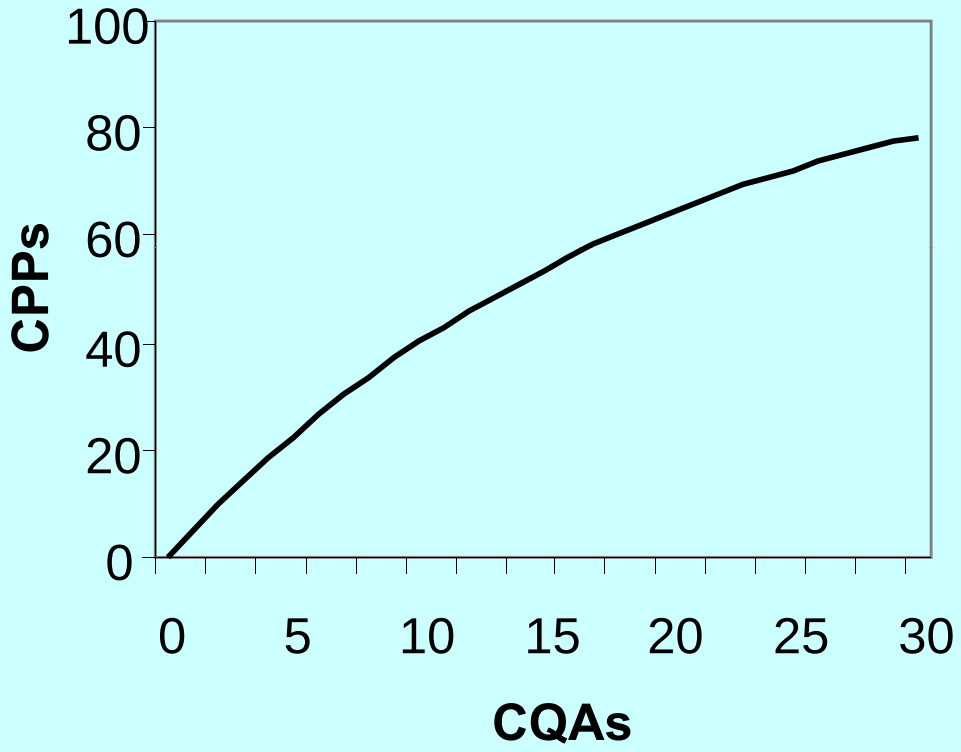
Risk Assessment & Ranking Approaches

Good Science; Consider Interactions

# CQAs & Process Limitations

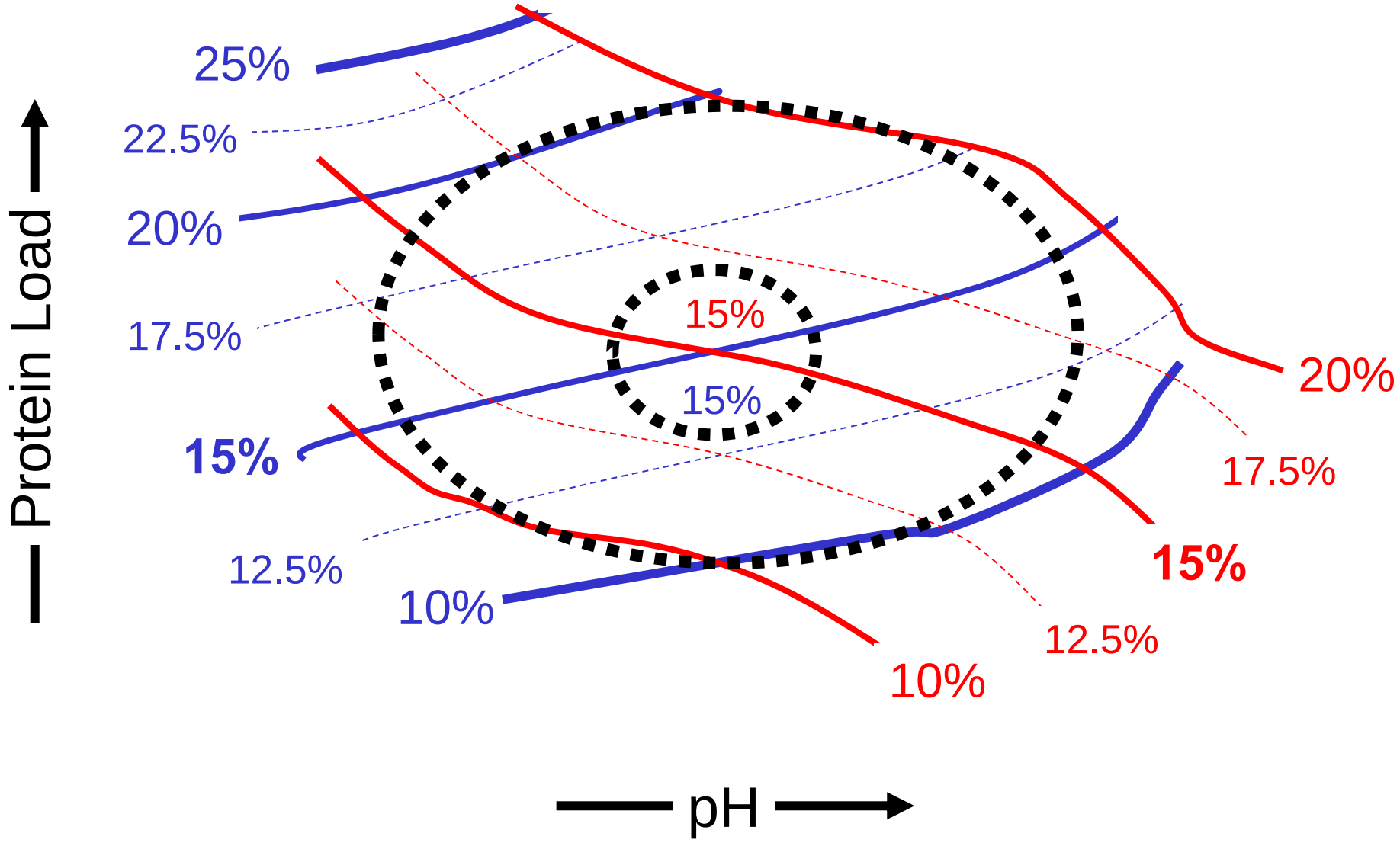
Assumptions

- 100 potentially important parameters
- 5% chance a parameter impacts a CQA
- CQAs are independent (big assumption)

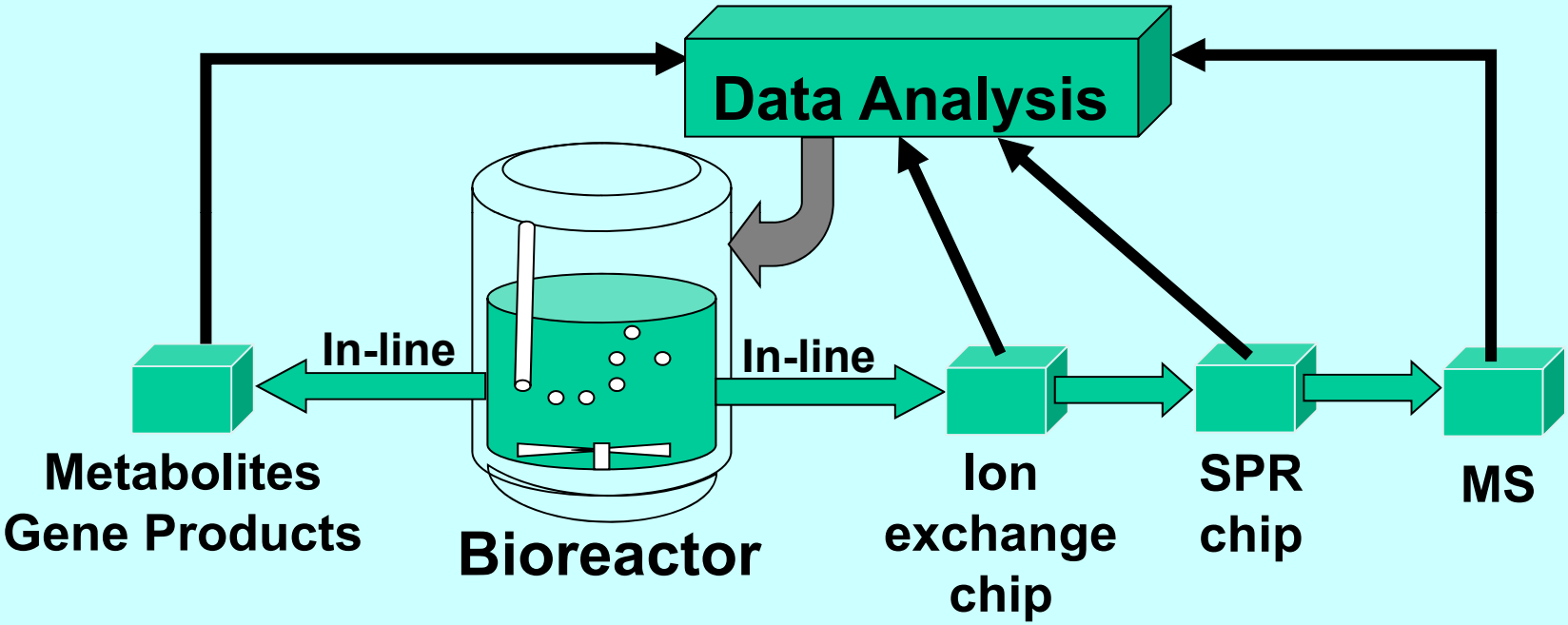



Acceptable ranges for CQAs

# Delivering Targeted Product Attributes



# Advanced Analytics & Manufacturing

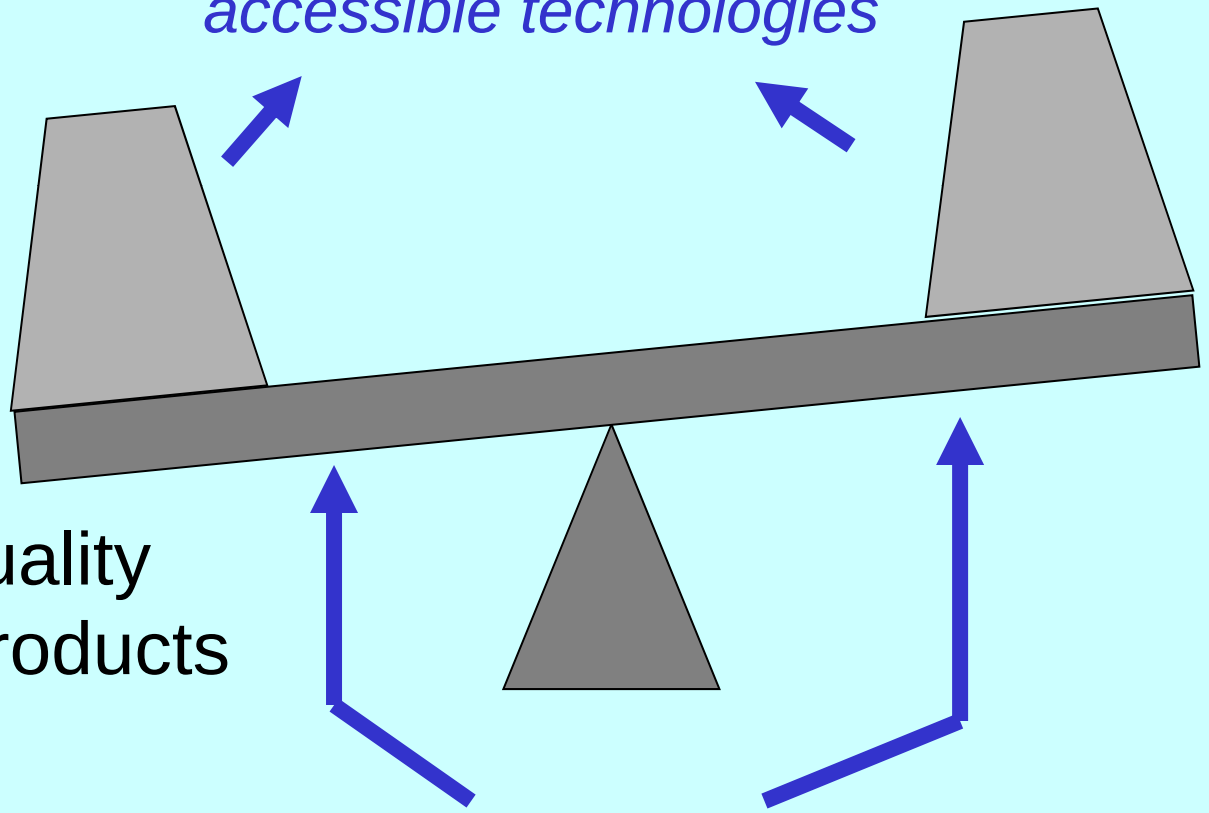



Better cell line selection & engineering

# Balance

efficient, agile, flexible development & manufacturing

*Better and more accessible technologies*



high quality drug products

*Better knowledge regarding clinical importance*

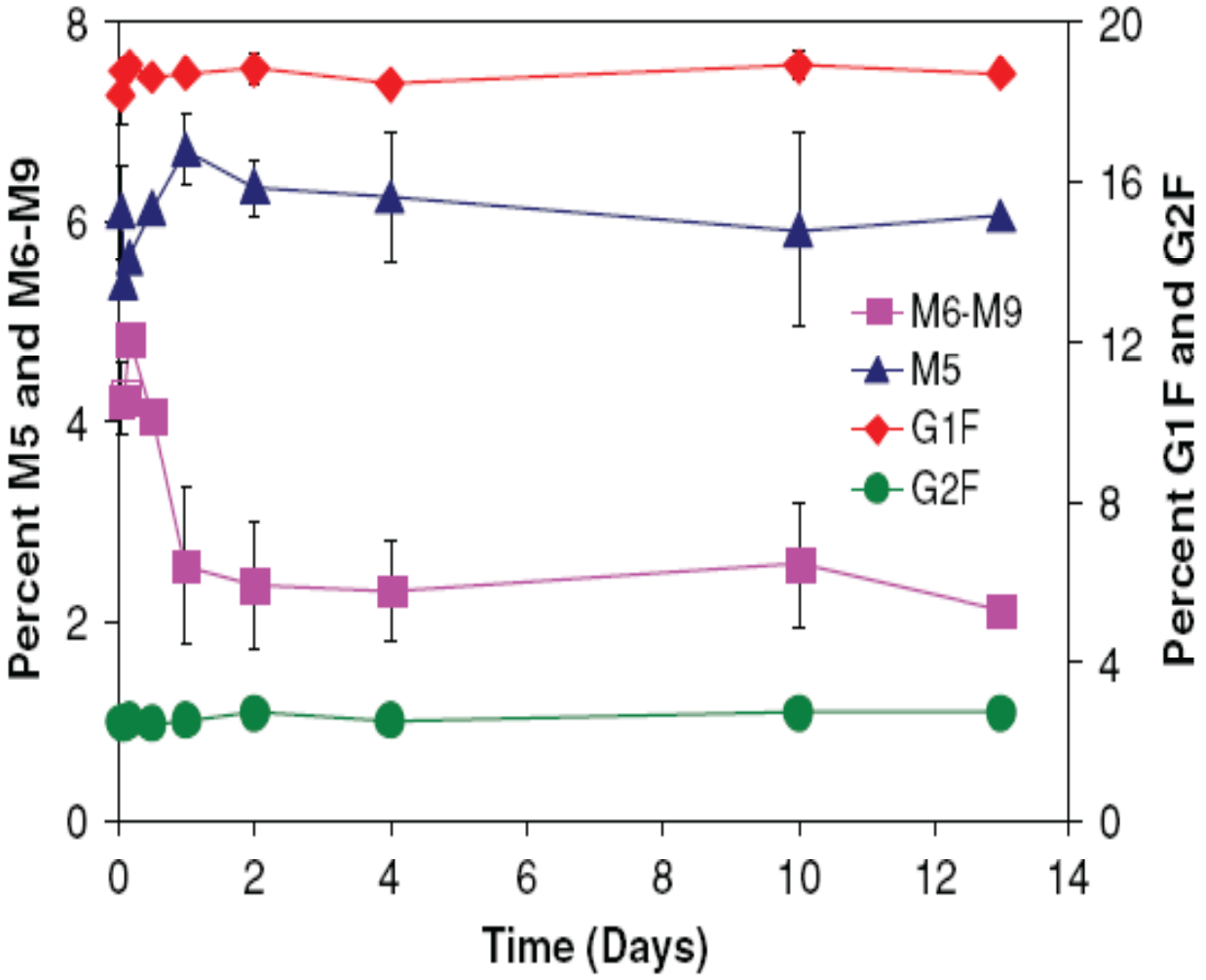
# Clinical Data to Assess Attributes

- High exposure to variants (with differing attributes) in dose escalation studies
  - Very small numbers
- *Bio-distribution*
  - *Labeled proteins and imaging (F18-PET?)*
- PK/PD of variants
- Analysis of randomized clinical trail data
  - Lot to lot variability
  - Stability
- Post market surveillance data linked to lot#

# A Single PK Study to 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

**…human IgG2 was affinity purified From human PK samples**



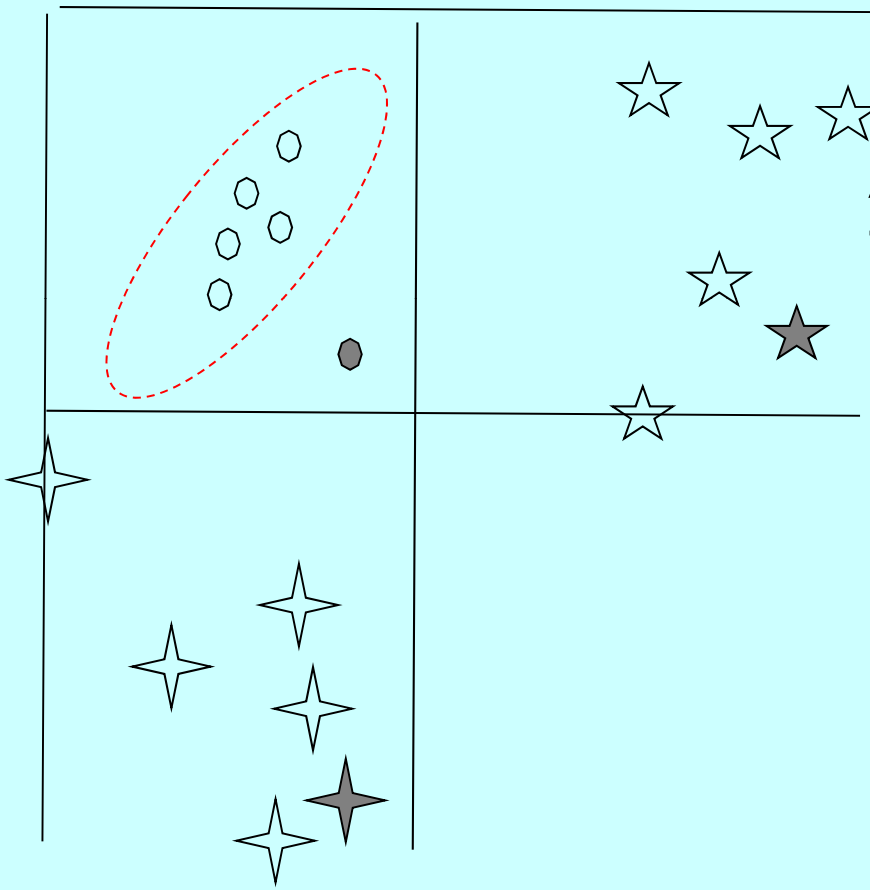

*Imagine if variants could be imaged in vivo?*

# Analysis of Clinical Trail Data

## *Lot to lot variability*

**Challenges**

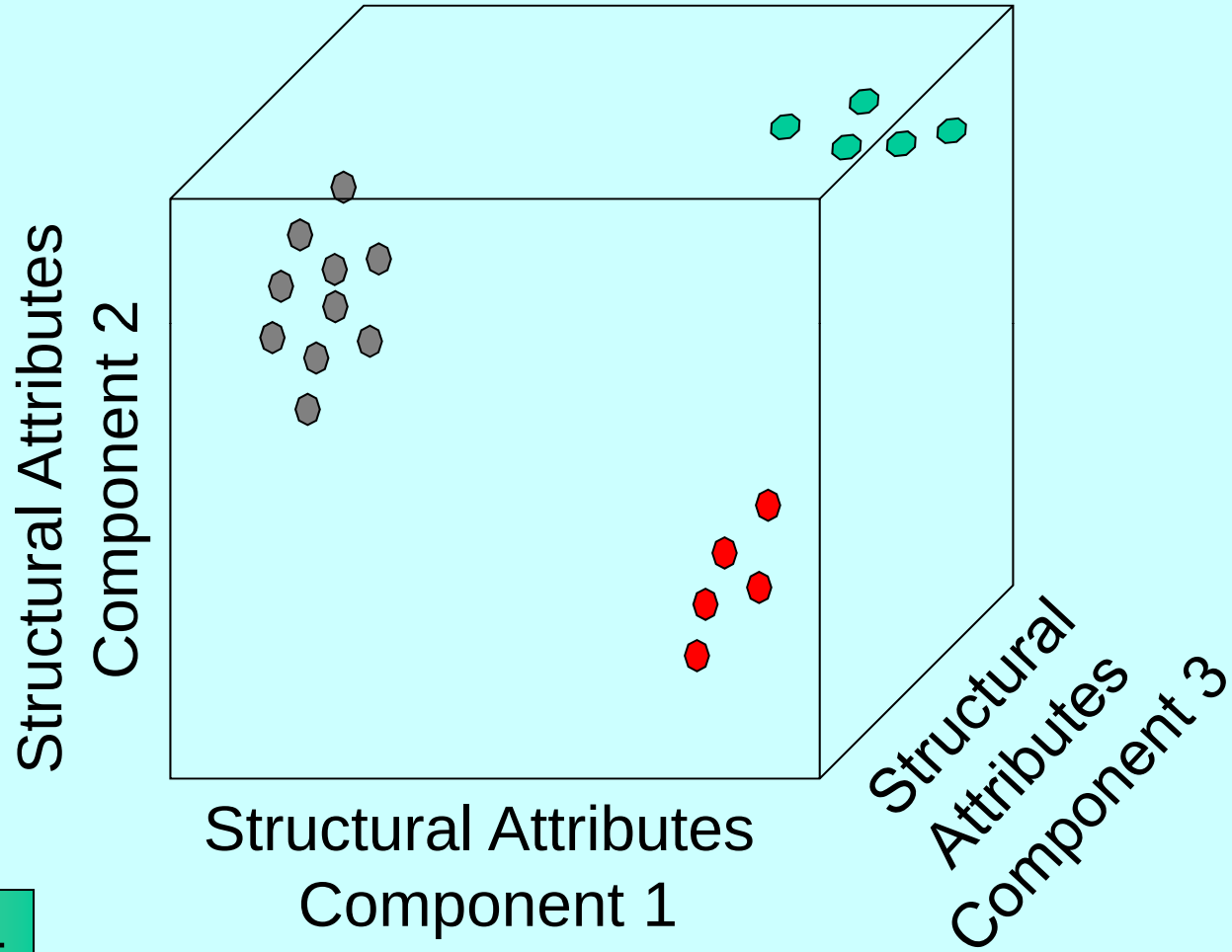
- Few lots may be used
- Lots may have very similar attributes
  - Clinical trialists not enthusiastic about “dirty” lots
- For trials with repeat dosing, each patients will be exposed to multiple lots




***Adapted from T. Kourti***

# Multivariate Approaches to Product Attributes in Humans

- Many, many attributes
- Many outcomes
- Multivariate Statistical Analysis
- PCA




Clinical/Biomarker 1

**Low** **Inter-mediate** **High**

*Wishful Thinking*

# Post-market Data and Quality

- Identifying brand, dosage form & lot#
- Capturing unusual conditions
  - Storage
  - Damaged containers
  - Microwaving prior to use
- Evaluating data capture on inspection
- Bar-coding
- Not just for AERs but many databases
  - Sentinel
- *Impact of systems biology & personalized medicine*
  - *Quality impacts more noticeable as expectation of safety and effectiveness increases*

# Closing

- Quality by Design approaches can improve manufacturing efficiency and product quality
- Characterize, characterize, characterize
  - Need to understand what can vary before evaluating impact
  - Need to understand relationships between attributes and potential attribute interactions
- Science based updating of analytics
  - Regulatory approaches to facilitate
- Science & Risk-based linkages of Q, S & E
  - Apply science to understand the impact of quality
  - Use of clinical data
  - Capture quality in passive & active surveillance

# Credits

- Patrick Swann
- Barry Cherney
- Amy Rosenberg
- Barbara Rellahan
- Emily Shacter
- Kathleen Clouse
- Susan Kirshner
- Moheb Nasr
- Ali Al-Hakim
- Christine Moore
- Keith Webber
- Wassim Nashabeh
- Eric Read
- Kurt Brorson