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Biopharmaceutical Attributes and Considerations for Manufacturing of the Future

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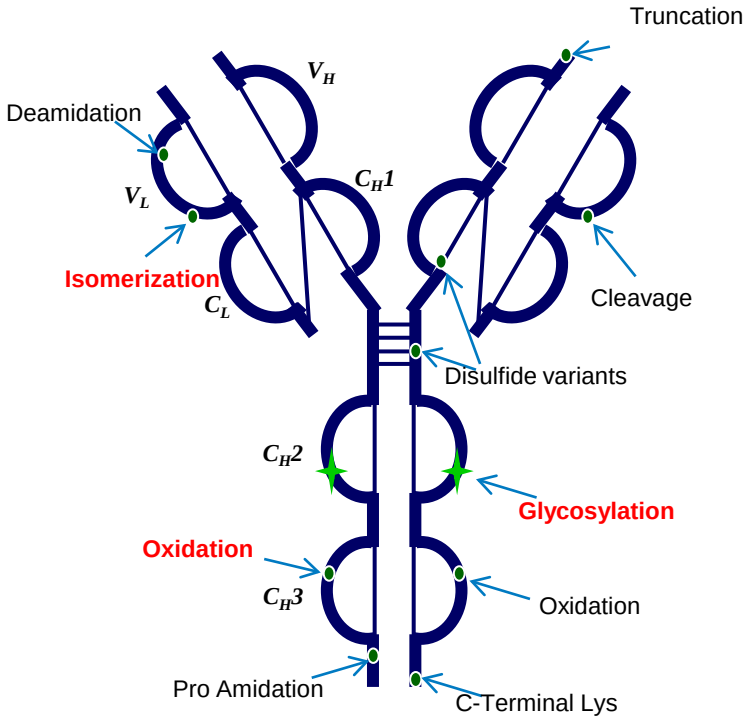
Overview

- Where are we today?
- What are the attributes we “care” about? Which attributes control product performance?
- How can the processes be made to bend to the product quality needs (q b d). Adaptive processing.
- Advances in analytical technology and our understanding of biology, allow us to focus on the attributes we care about. We develop processes and control them in a “data-rich” environment

Where are we today?

- We have had a lot of reliance on engineering and repeatability to establish control.
- A controlled process, plus release testing insures consistent product.
- “The Process is the Product”

The Process is NOT the Product



The Product is.

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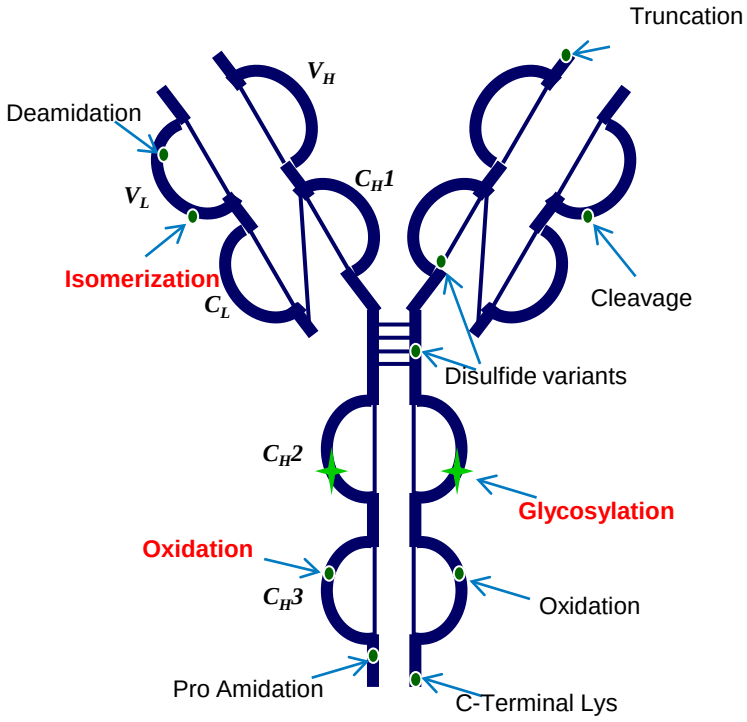
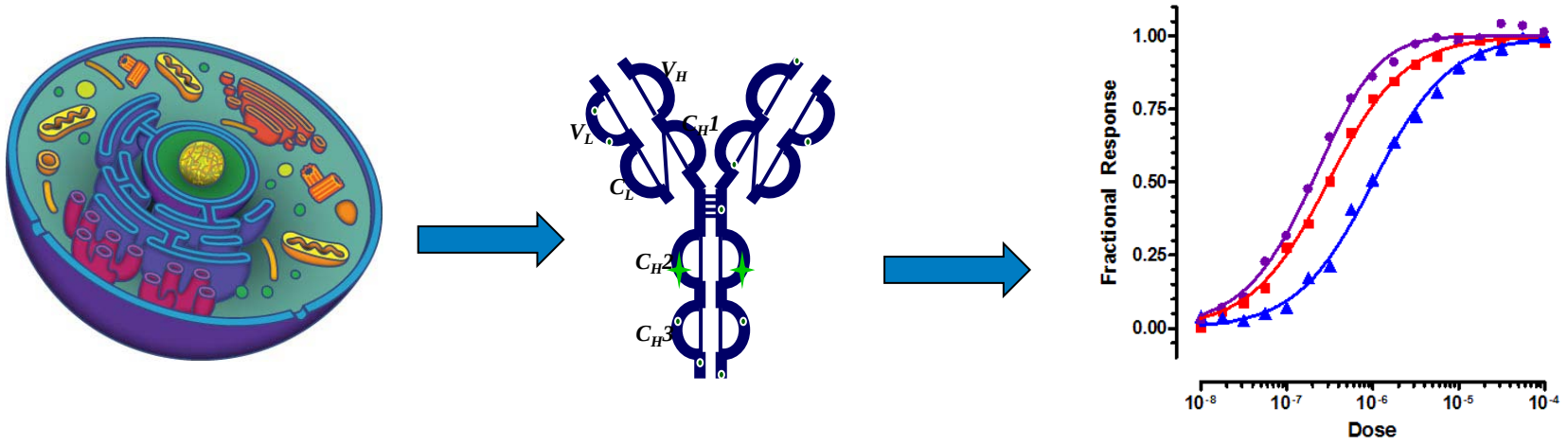
The Attributes Define the Product

- We want to focus on critical quality attributes that control product performance
- We want to achieve product attribute control through the design of our processes
- We want to actively control our attributes based on understanding of the process relationship to the product attributes

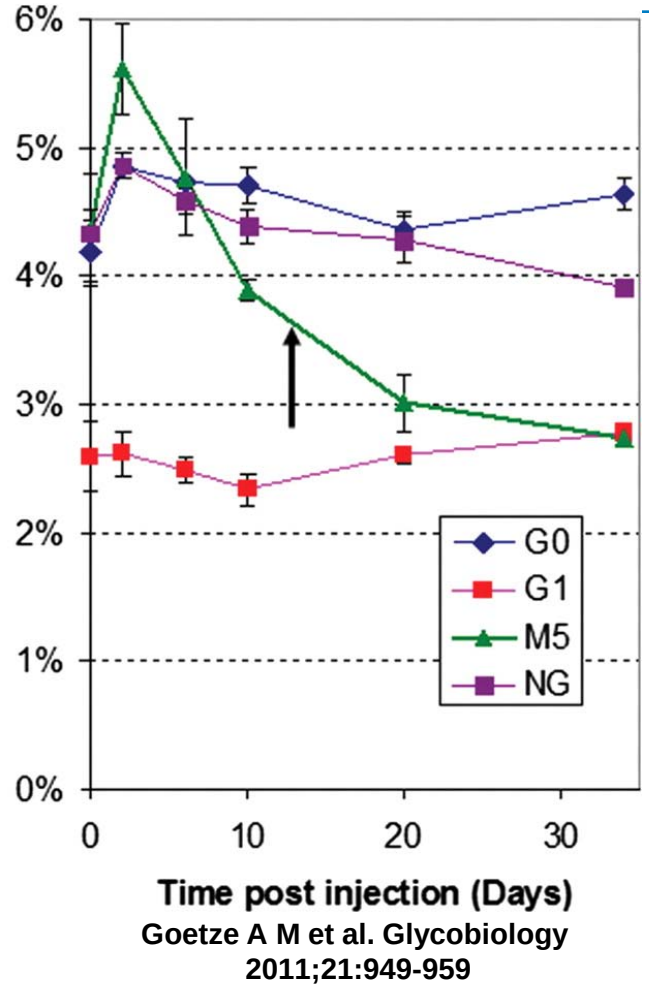


Shift in focus from Engineering to Biology

- Engineering and control are still important
- Need to develop a deeper understanding of the biology that impacts the PQA's
  - Needs to be at the attribute level for both the relations to the process and its biologic relevance



Establish CQA's by Understanding the Biological relevance of Product Quality Attributes



Goetze A M et al. Glycobiology 2011;21:949-959

- One way is to look at attributes in vivo
- Does High Mannose have a higher clearance?
- Analyze samples from patient blood over a timecourse
- Yes it clears faster for this drug, so it is a critical attribute to control

- As an industry, we need to find out what the critical quality attributes are.

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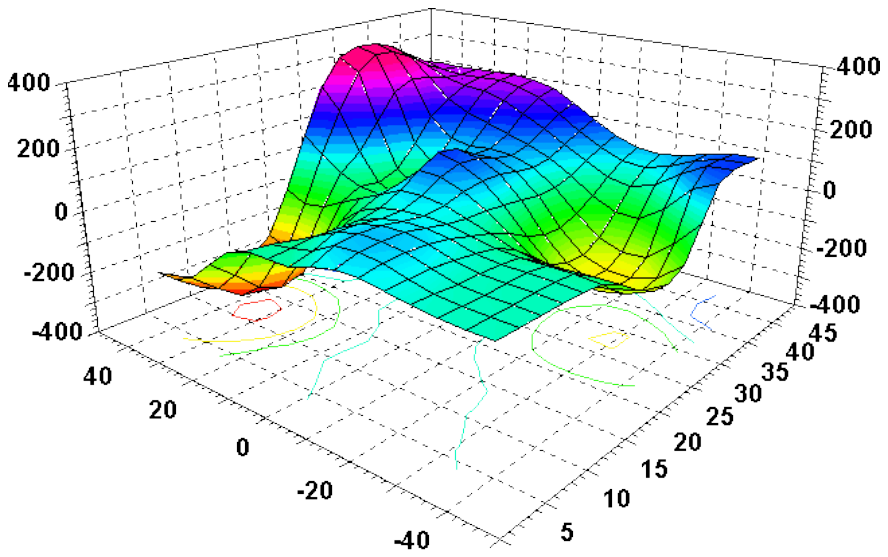
Differentiate Product Quality Attribute, from *Critical* Quality Attributes

- Generally regarded as PQAs
  - C-terminal lysine
  - N-terminal cyclization
- Product-specific PQAs; Establish that these are low immunogenicity, safety or efficacy risk
  - Glycation
  - N-linked glycosylation changes (eg terminal galactose)
  - Disulfide Variants
- If sufficient data exists to establish non-criticality, why test for them?

quality by design; lower case

- QbD is inclusive of quality systems and development
- quality by design, is the direct link between our understanding of the attributes, and their connection to the process
- In some cases, that link between product and process is active; meaning we are directly controlling attributes of interest
- Benefits
  - Deeper understanding of the link between attributes and the process: data-rich process development
  - Methodologies to do release testing for the attributes that we actually care about: data-rich release

But you have to do the development to understand the levers to control the attributes: “Enhanced Process Understanding”



- DOE are well established for upstream development
- Typically the focus is on the process; eg titer, growth viability
- Looking for process “edges”
- Increase the power of the DOE by incorporating many attributes to see the process and product link
- “Data-rich” process development

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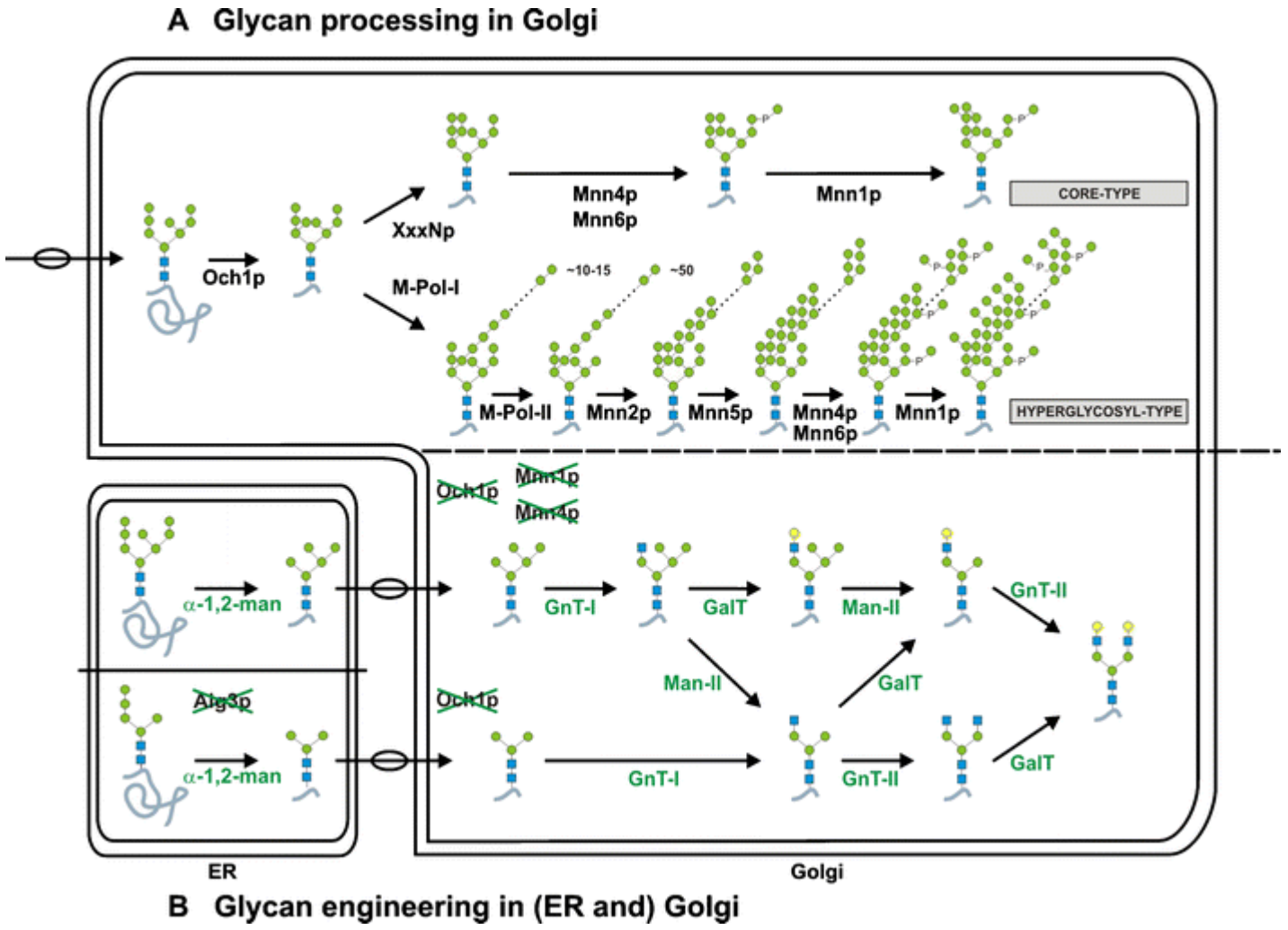
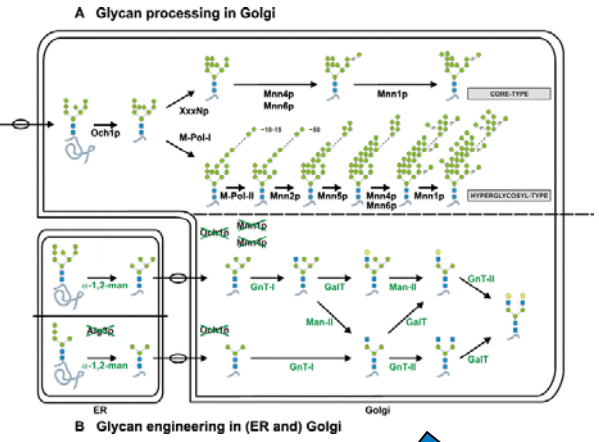
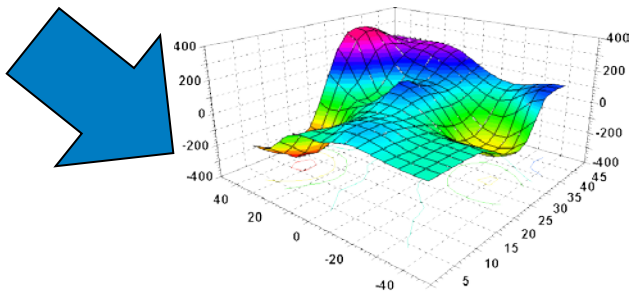
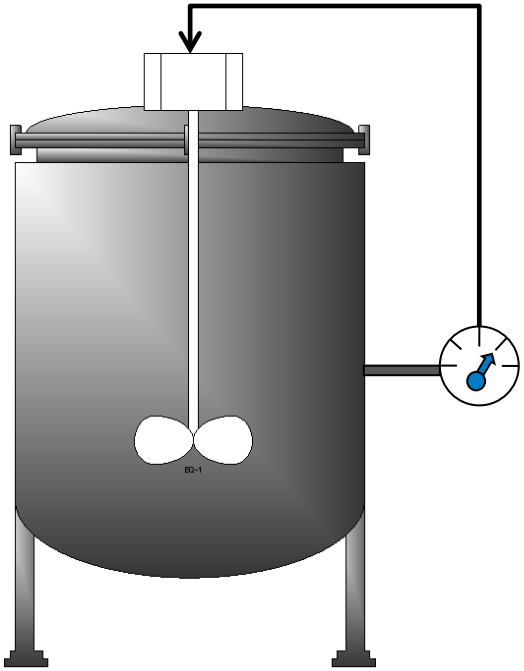
And maybe even before that you really have to understand the biology of the cells producing your product



Understanding of the biology to create an improved “control strategy”







PQA
Clips
Charge Variants
Glycosylation
Identity
Oxidation
Isomerization
Disulfide Variants

Fundamental Understanding

Process to Product Link

Product Attribute Control, in some cases active

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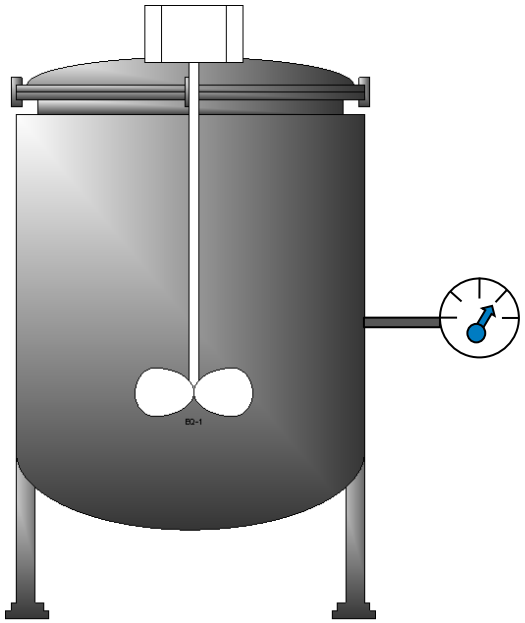
The Future is Data-Rich Process Development and Release Testing



Process Monitoring

pH
Temp.
Osmo
Lactate
Viability

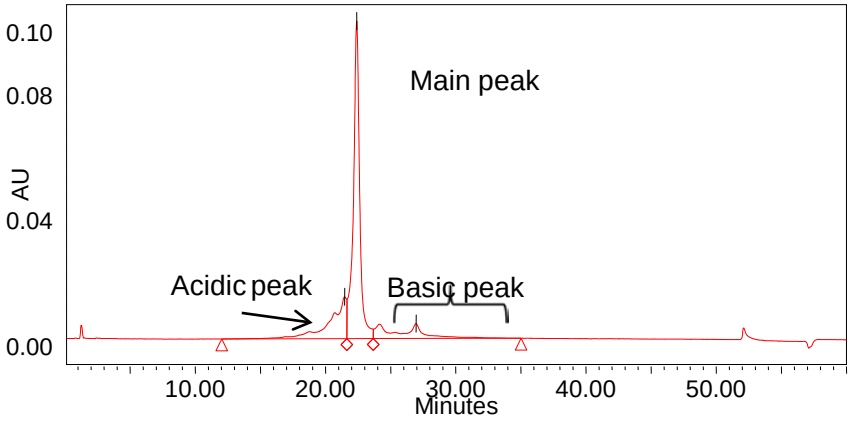
Attribute Monitoring

Glycosylation
Aggregate
Deamidation
Oxidation
Fragmentation

By measuring the attribute of interest, we produce more valuable data

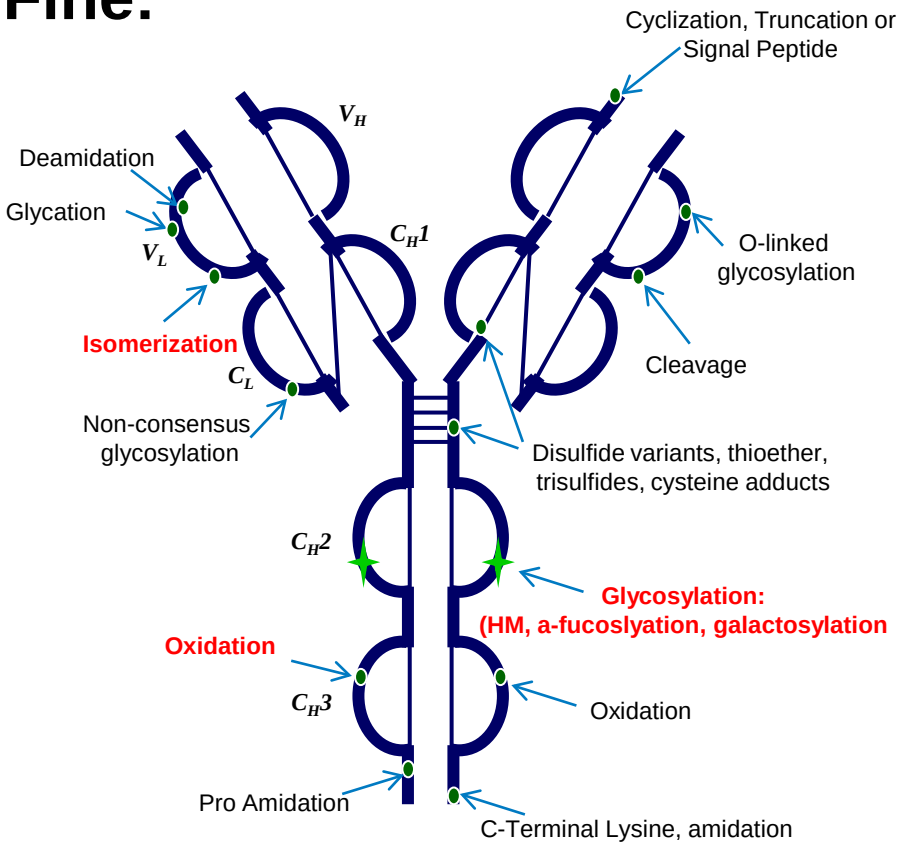
Surrogate Versus Direct Testing of PQAs

Coarse:



Sample	Acidic	Main	Basic
Ref Std	17%	76%	7%
Team Supply #4	16%	77%	7%

Fine:



CEX can establish consistency, but is too coarse to link process changes to specific CQAs we want to follow

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Data-Rich Process Future Enabled by better Analytical Techniques

Antibody PQA	Pep Map-MS
Aggregate Assessment	no
Deamidation (Isomerization) Assessment	yes
Disulfide Isoform Assessment	maybe
Glycation Assessment	yes
High Mannose Assessment	yes
Methionine Oxidation Assessment	yes
Signal Peptide Assessment	yes
Unusual Glycosylation Assessment	yes
CDR Tryptophan Degradation Assessment	yes
Non-consensus Glycosylation Assessment	no
N-terminal pyroGlutamate Assessment	yes
C-terminal Lysine Assessment	yes
Galactosylation Assessment	yes
Dimer Assessment	no
Fragmentation (peptide bond) Assessment	maybe
Disulfide Reduction (DS Fragmentation) Assessment	maybe
Host Cell Protein Assessment	yes
Mutations/Misincorporations Assessment	yes
Hydroxylysine Assessment	yes
Thioether Assessment	yes
Trisulfide Assessment	maybe
Non-glycosylated Heavy Chain	yes
DNA Assessment	no
Cysteine Adducts Assessment	maybe
C-terminal Amidation Assessment	yes
CDR Conformers (HIC Isoform) Assessment	no
O-linked glycans Assessment	maybe
Fucosylation Assessment	yes
Residual Protein A	yes
Identity	yes

Cost of high resolution mass spectrometers is dropping

Reliability of instruments increasing rapidly

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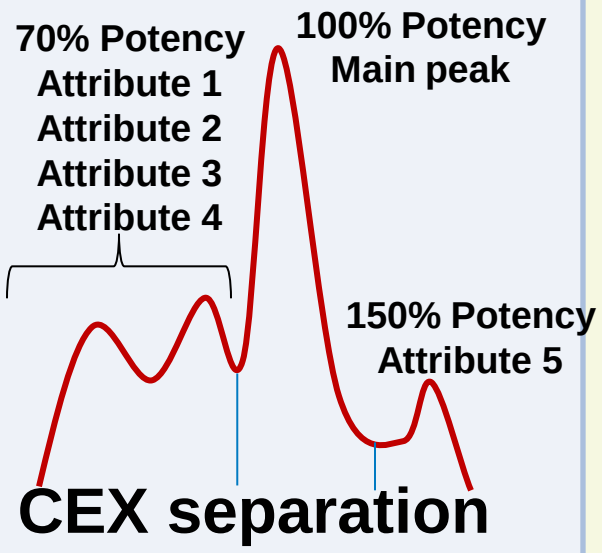
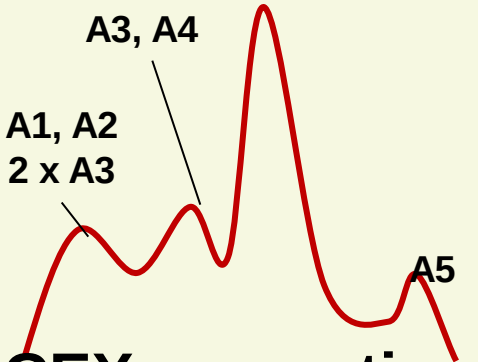
Replace non-attribute specific assays with methods capable of specifically detecting and measuring critical attributes.

Current Release Method



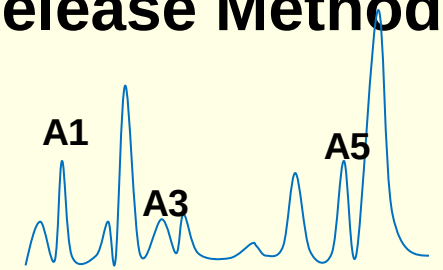
Product Understanding



Sub-fraction & BRQA

Attribute	Potency *	Safety
Main peak	100%	1
A1	50%	3
A2	110%	3
A3	95%	7
A4	102%	1
A5	150%	5

Future Release Method



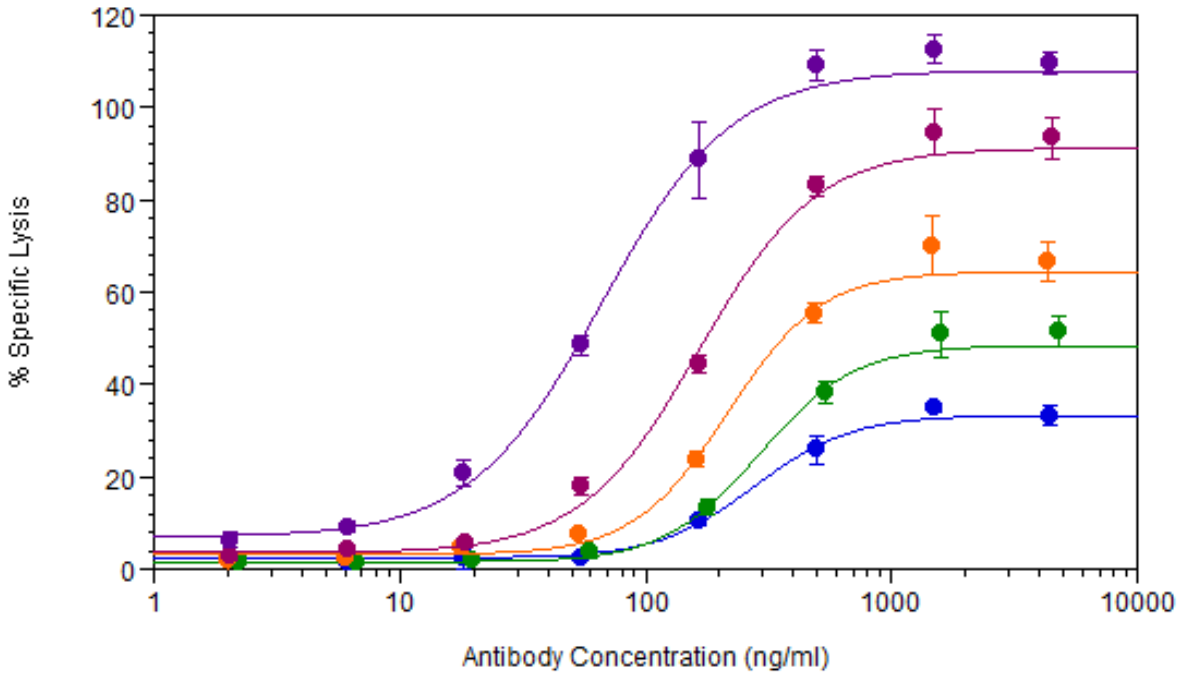
MS Based method

<u>Regulatory Argument:</u>
Replacing CEX monitoring of pre-peaks with more *specific* method monitoring *relevant* attributes:

- **A1 (efficacy)**
- **A3 (safety)**
- **A5 (safety and efficacy)**

Example #1: IgG1 Core Fucosylation Criticality

Antibody-Dependent Cellular Cytotoxicity (ADCC) is Very Sensitive to Levels of Afucosylation



Afucosylation Level

87.5 %

23.0 %

11.2 %

5.7 %

2.8 %

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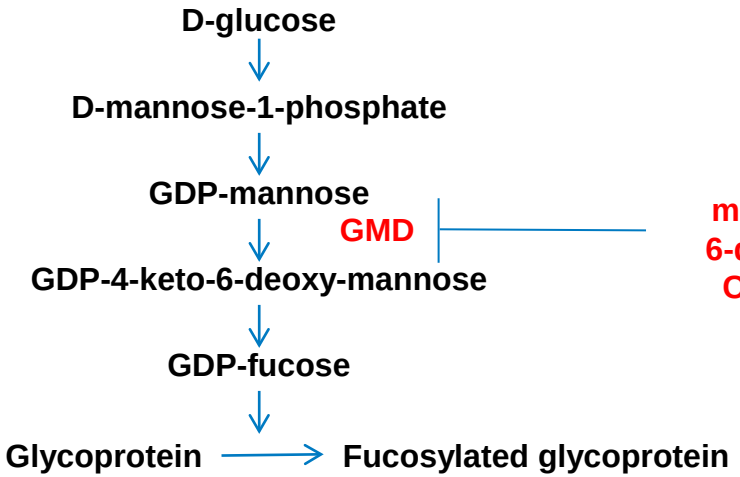
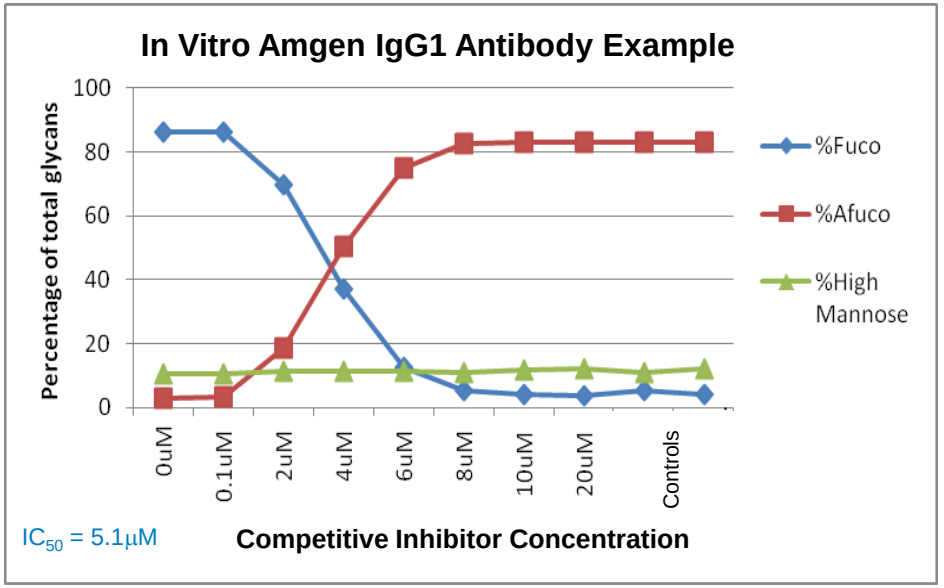
Example #1: Inhibition of Core Fucosylation with a Small Molecule Inhibitor

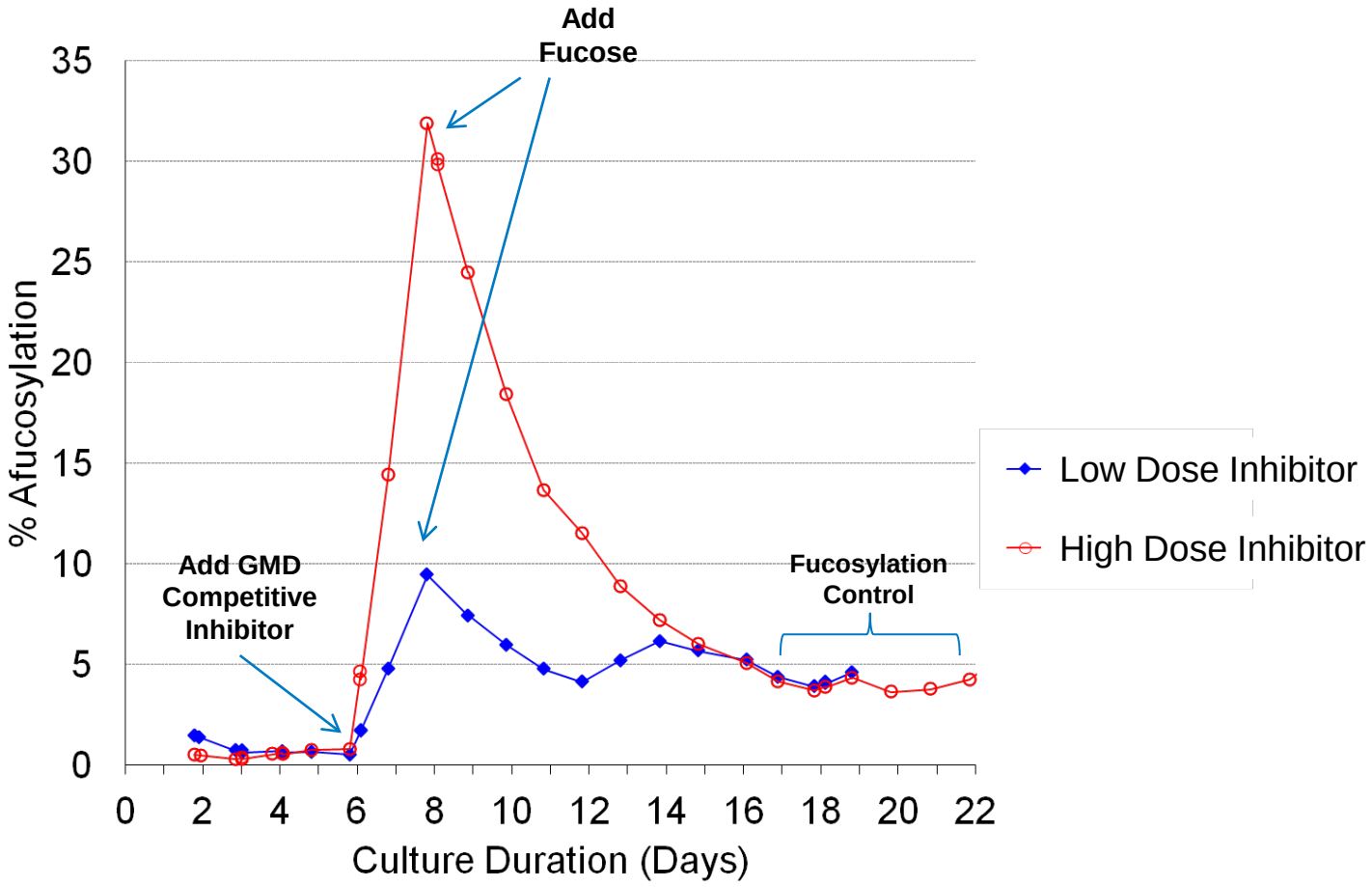


GDP-D-mannose e-4, 6-dehydratase Competitive Inhibitor

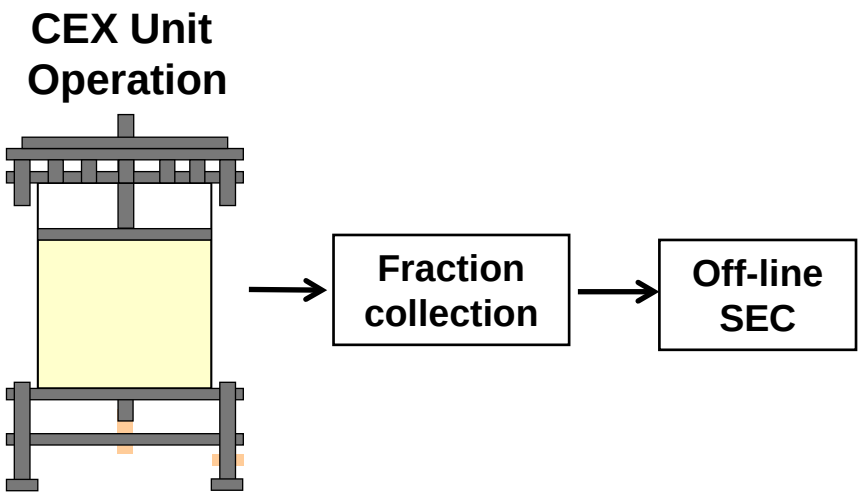


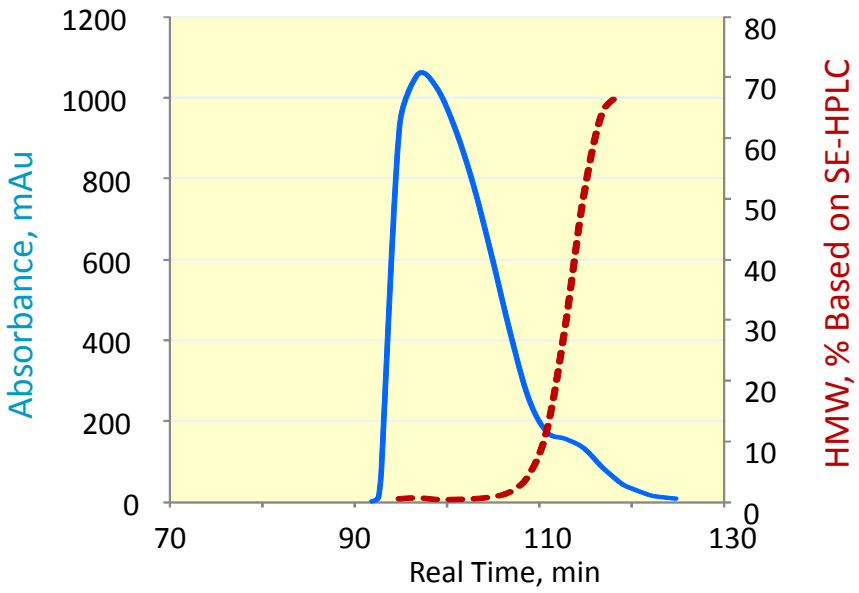
Example #1: Control of Core Fucosylation

Utilize PAT approach to target afucosylation level = 4-5%



Example #2: Relationship between CEX Process Step and High Molecular Weight (HMW) Species





Off-line characterization of the CEX profile showed HMW species elute on the descending side of the chromatogram

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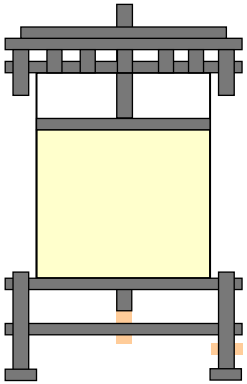
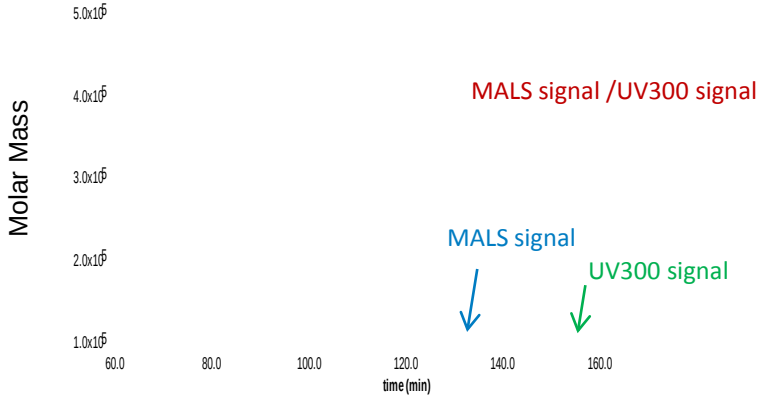
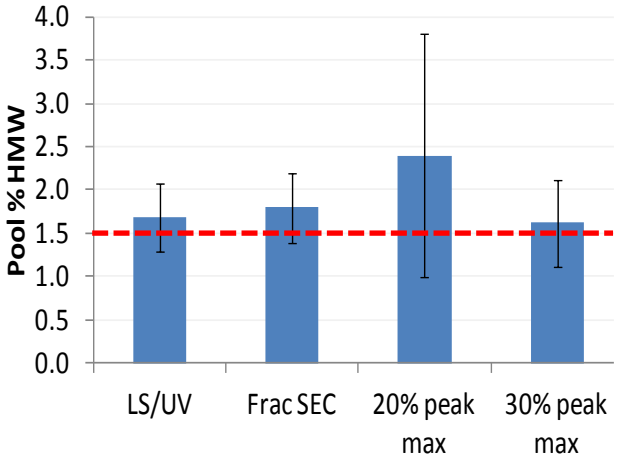
Example #2: On-line Multi-Angle Light Scattering (MALS) with Downstream Processing Step



On-Line

Multi Angle Light Scattering (MALS)





Better control of HMW species using on-line MALS LS/UV signal

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What are We Trying to Achieve Through This Approach to Process Development?

Reduce the Cost of Development over the Long Run

- Eliminate elements of process characterization
- Eliminate the cost of comparability failures

Reduce the Cost of Quality

- Move to implementation of real time release (RTR)
- Eliminate in-process testing and elements of release testing for drug substance

Generate Reproducible Product Quality Attributes

- Patient safety
- Product efficacy

Reduce the Risk of Process and Raw Material Variability

- Process Analytical Technology

Implement "quality by design" Concepts

- Significantly improve our process and product knowledge
- Develop protein therapeutics based on desired attributes

Acknowledgements

- Bob Bailey
- Dean Pettit
- Jim Thomas