Meaningful Bioassays to Elucidate Structure-Function and Demonstrate Control

Colleen Santoro
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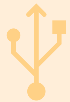
Bristol Myers Squibb™

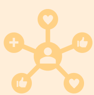
Outline

The role of potency methods on elucidating structure vs. function and considerations during process changes

A case study on supporting a process change with a slate of potency methods



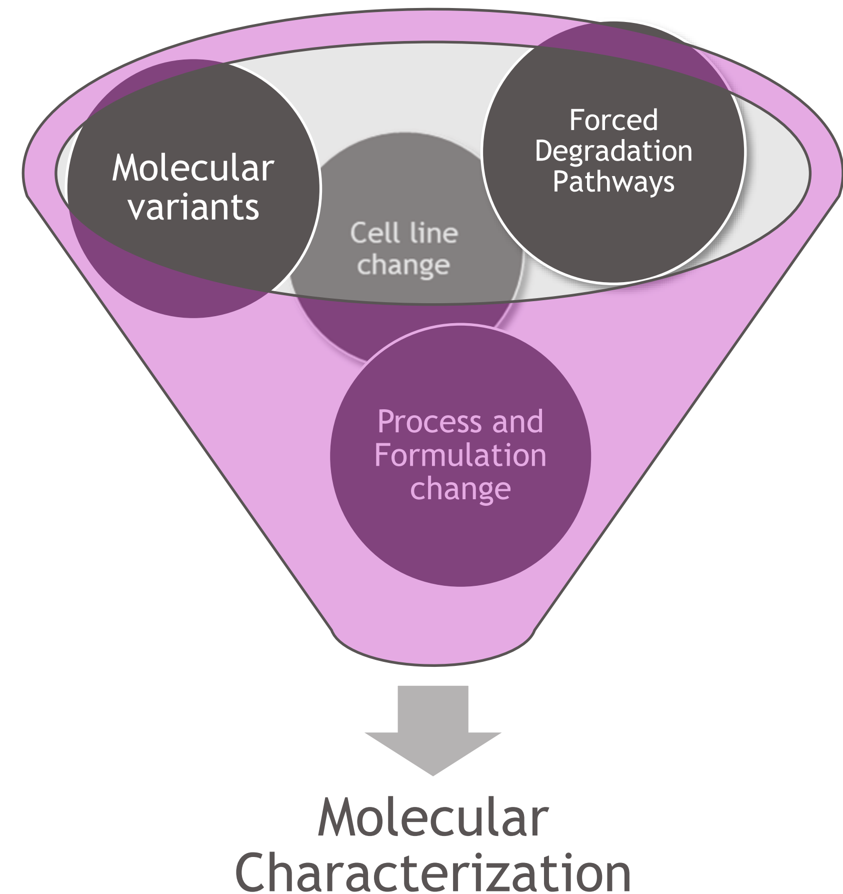
Phase-appropriate method replacement



Extended biological characterization to elucidate the implications of structural changes on product quality, potency, and FcRn binding

The Role of Potency Methods in Product Characterization

- Potency methods are used to understand degradation pathways and their impact
- Participate in forced degradation assessment to help set specification limits
- Aid in the development process
- Demonstrate full understanding of our molecule!
  - Where Fc effector function, C1q binding, or FcRn kinetics are not part of the MOA, these are still critical to understanding and characterizing all aspects of biological function.



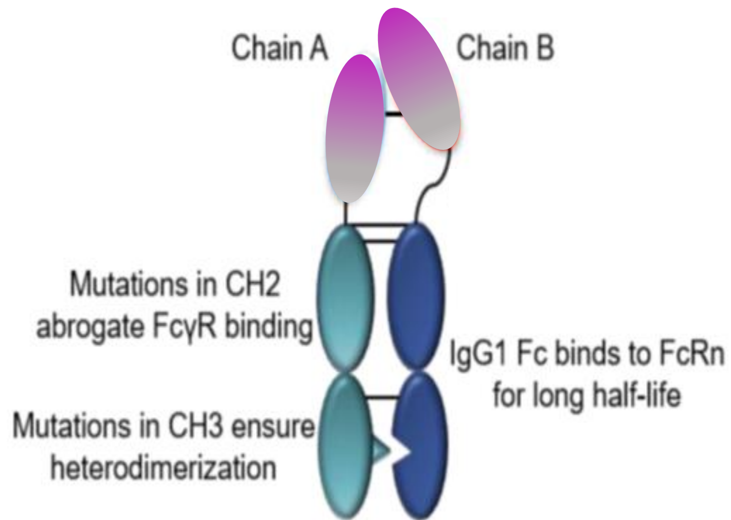
The Role of Potency Methods to Support Process Changes

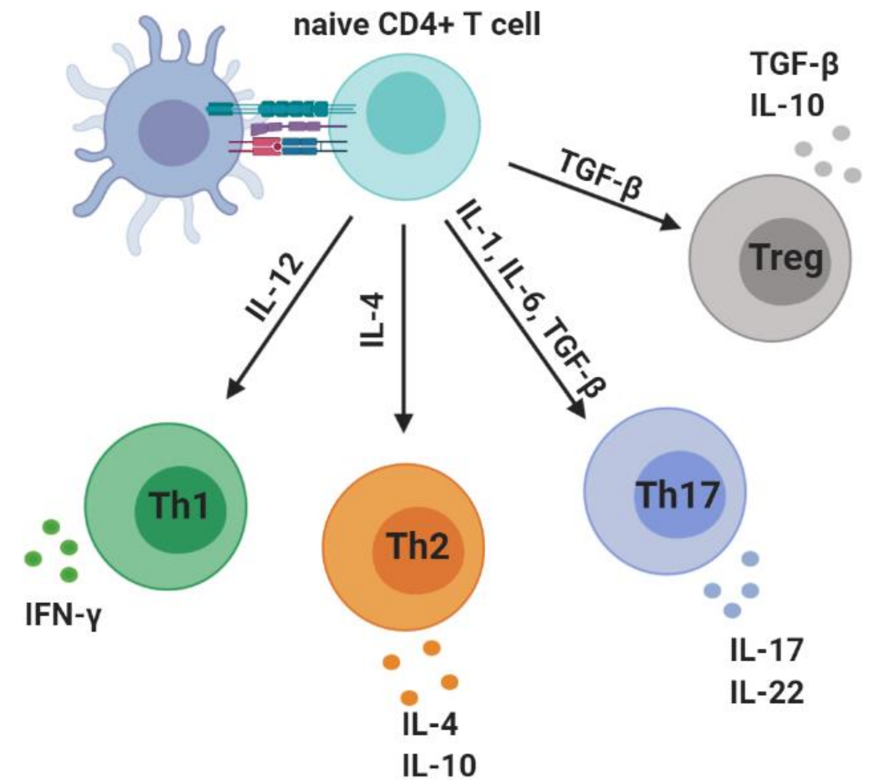
- ICH Q6B, “Potency (expressed in units) is the quantitative measure of biological activity based on the attribute of the product which is linked to the relevant biological properties...”
- Process Changes can vary, i.e. formulation buffer change, downstream process change, cell line change, etc. and may have impact on relevant biological properties.
- ICH Q5E, “… is to ascertain that pre- and post-change drug product are comparable in terms of quality, safety, and efficacy.”
  - Is it the material different?
  - Does it matter?
- Considerations:
  - Changes to how the functional MOA is assessed
  - Changes to FcRn binding kinetics, Fc effector function, or C1q binding properties

Critical quality attributes must be identified and controlled to support a process change

Case Study: Process Change for an Fc Fusion Protein

- **MOA**: Binding to Protein-X receptor stimulates proliferation of lymphocytes, and cytotoxicity of target cells by activated T cells and NK cells.
- **BMS Protein-X/Fc** was designed to provide better efficacy/safety due to longer $T_{1/2}$ and lower C_{max} versus Protein-X without an Fc conjugate.

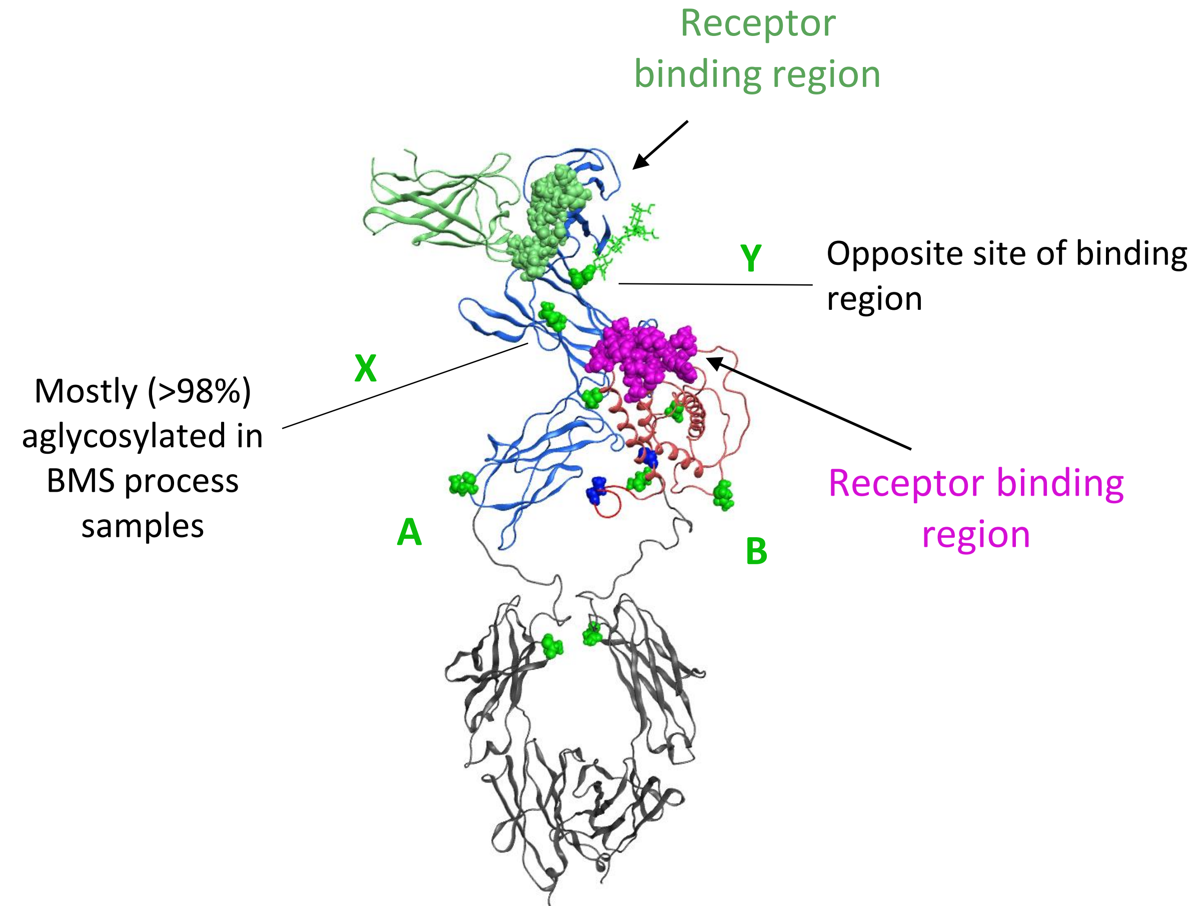




High Level Outcomes of BMS Protein-X/Fc Process B

- Optimized the legacy process to monoclonal cell line and optimized formulation.
- Post-translational modifications associated with process change resulted in increased glycosylation with sialic acid end-capping, which is advantageous to molecule pharmacokinetics.
- Increases in glycosylation were *distal* from the Protein-X receptor binding regions
- Other N-linked sites were modeled to be aglycosylated (X) or on rigid ß-sheet on opposite side of binding regions (Y).

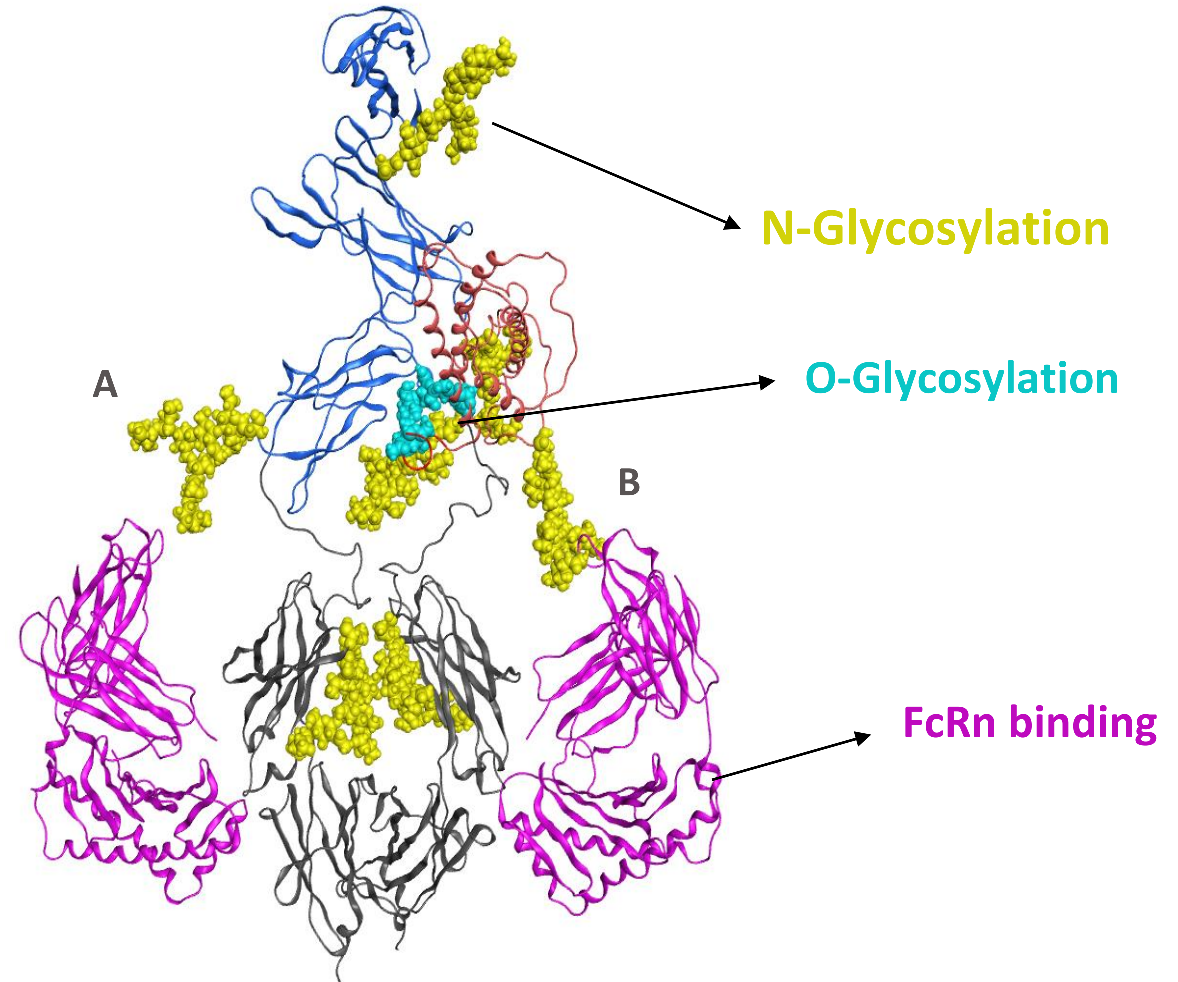
- Increased glycosylation of BMS Protein-X was not expected to impact biological activity.



Structural Impacts of Glycosylation on Potency

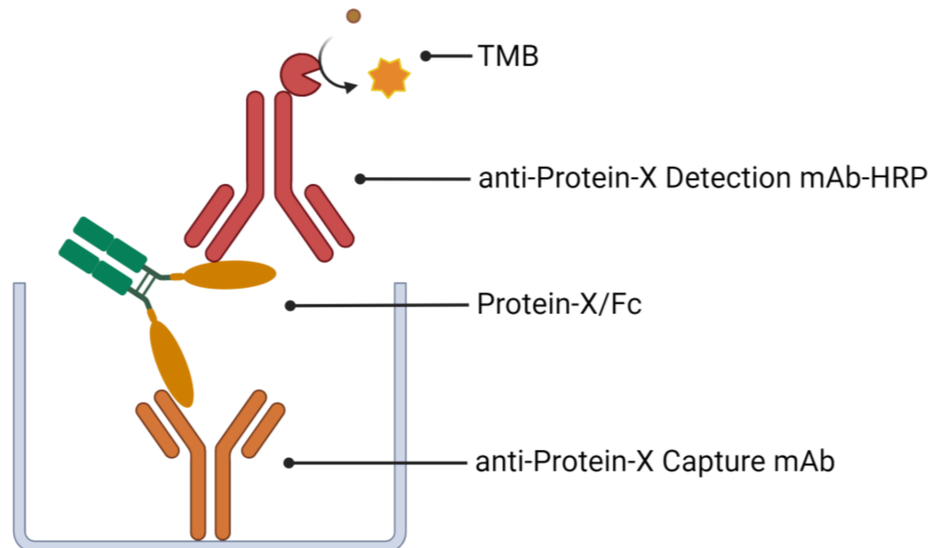
- In silico modeling identifies most glycosylation sites as either aglycosylated or rigid B sheets away from receptor binding domains.
- Glycosylations at A & B are not near receptor binding domains but are capable of forming long, flexible chains that have potential for steric hindrance

  - *Potency: Choosing the right tool to fully assess the attribute is imperative*

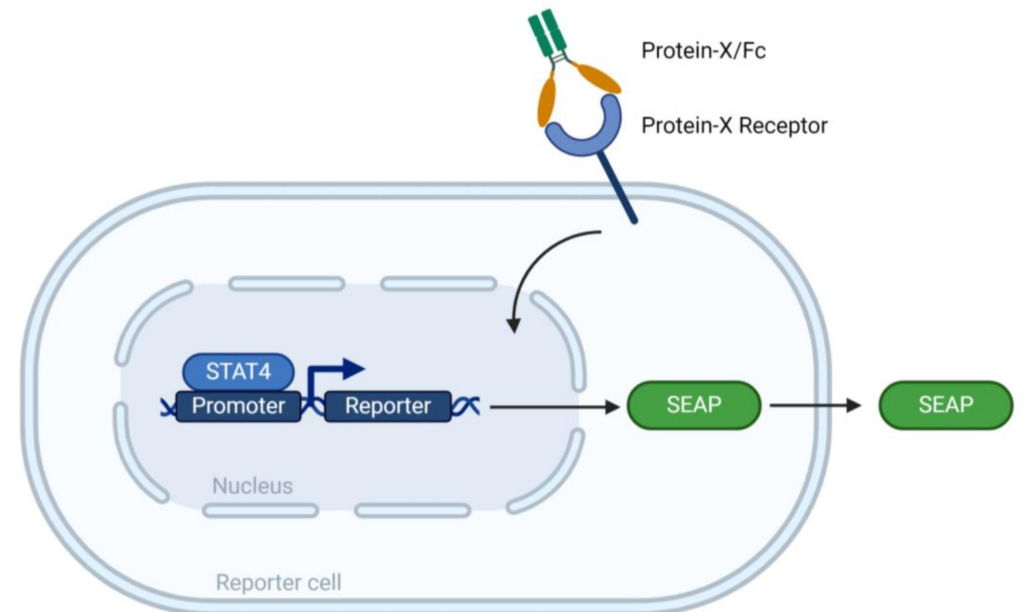


Potency Methods Available for Product Characterization

- Legacy ELISA Method (Release/Stability)
- Protein-X sandwich ELISA from an off-the-shelf commercial kit
  - Marketed primarily for detecting concentration of Protein-X in serum and plasma samples.
  - BMS Protein-X/Fc in the sample is captured and detected by two kit antibodies and does not demonstrate binding to the drug target (Protein-X receptor).

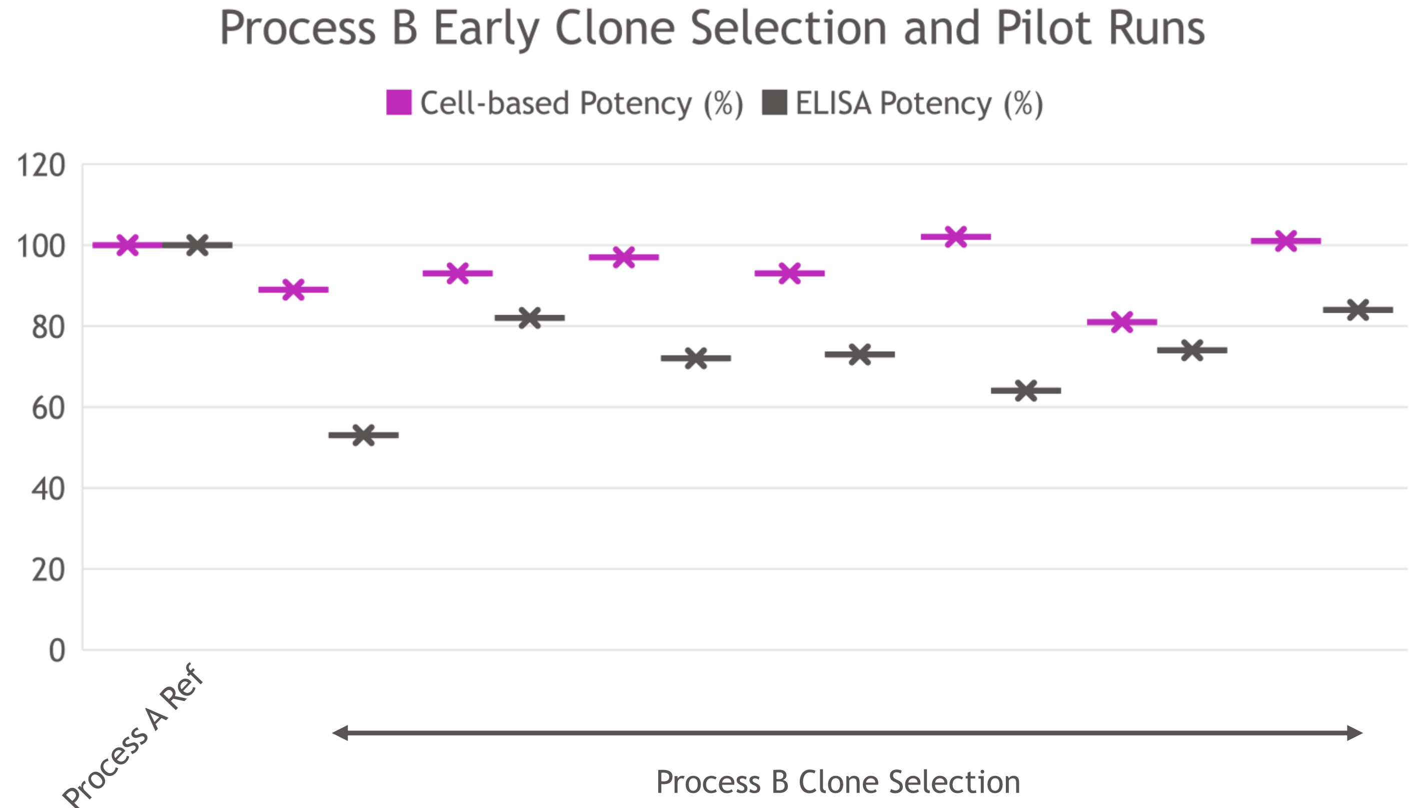


- Bioassay Method (Characterization)
- MOA-reflective cell-based Bioassay
  - HEK cell line transduced with gene for Protein-X receptor.
  - Activation of the Protein-X receptor is measured by a STAT4-inducible SEAP (secreted embryonic alkaline phosphatase) reporter protein.



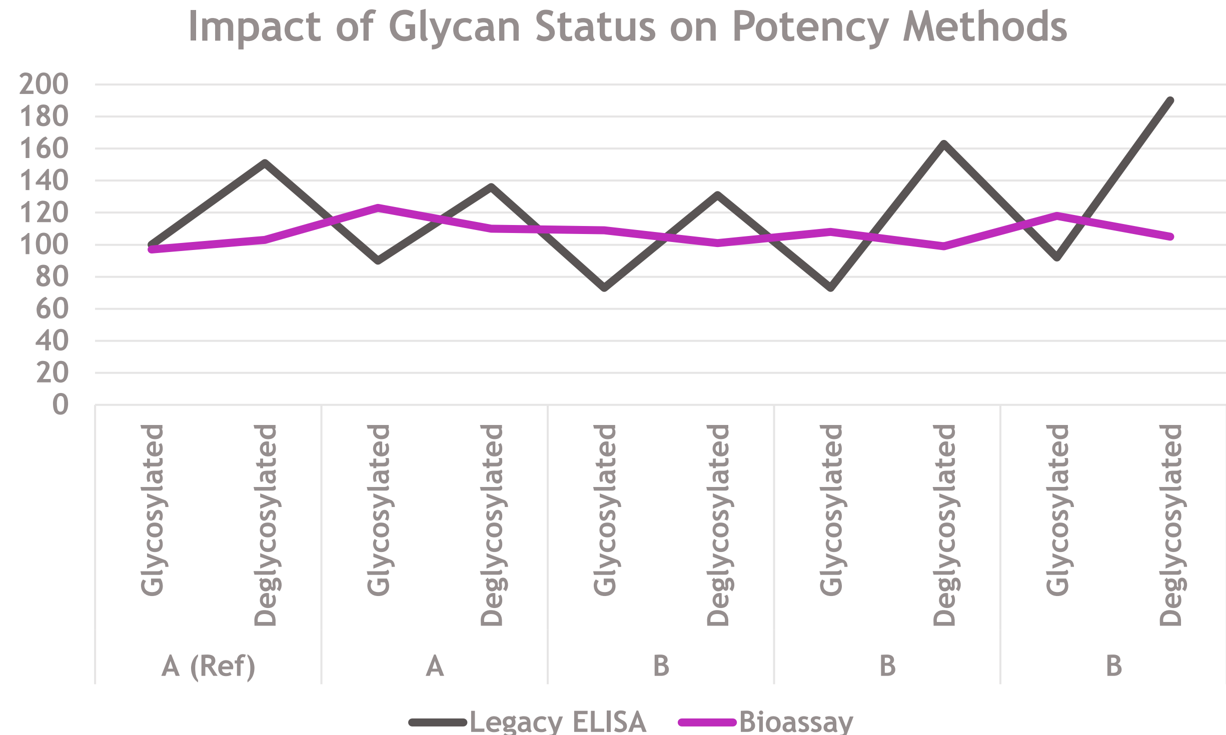
Appropriate Analytical Methods Establish Confidence in Evaluating Molecular Attributes

- The legacy ELISA shows reduced potency of Process B relative to Process A material.
- In MOA-reflective cell-based bioassay, Process B is comparable to Process A
- Its critical to choose the appropriate tool to evaluate molecular attributes, and bridging from one method to another must be justifiable.



Glycosylation Impacts: Understanding Method Detection vs. Potency

- Samples from Process A and B were deglycosylated to understand interactions in both methods
- When deglycosylated, ELISA values go above 100% potency and Bioassay values stay consistent around 100%.
  - Indicates even normal Process A glycosylation has reduced detection by ELISA compared to fully deglycosylated material.
- When deglycosylated, there is no difference between Process A and Process B by either method.
- The cell-based bioassay method is mechanistically reflective, stability indicating, and is insensitive to process-derived glycosylation changes outside of the sites of biological activity.

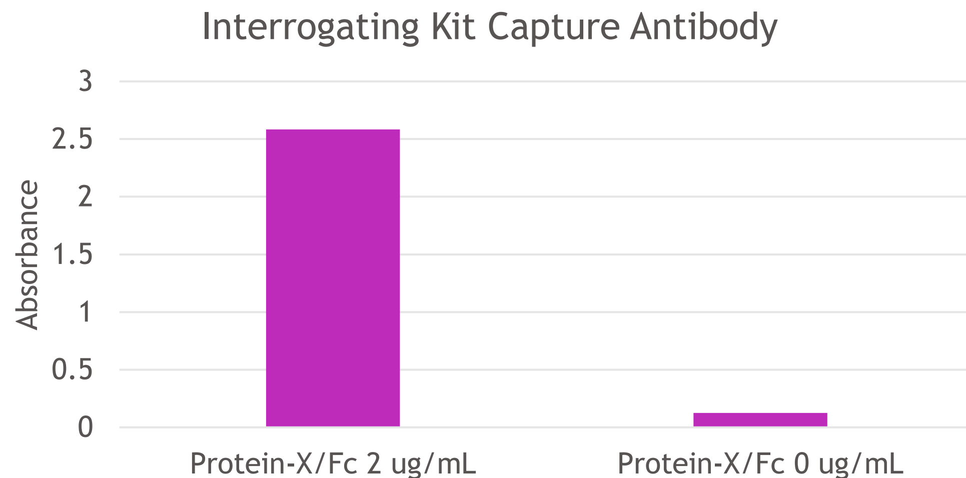


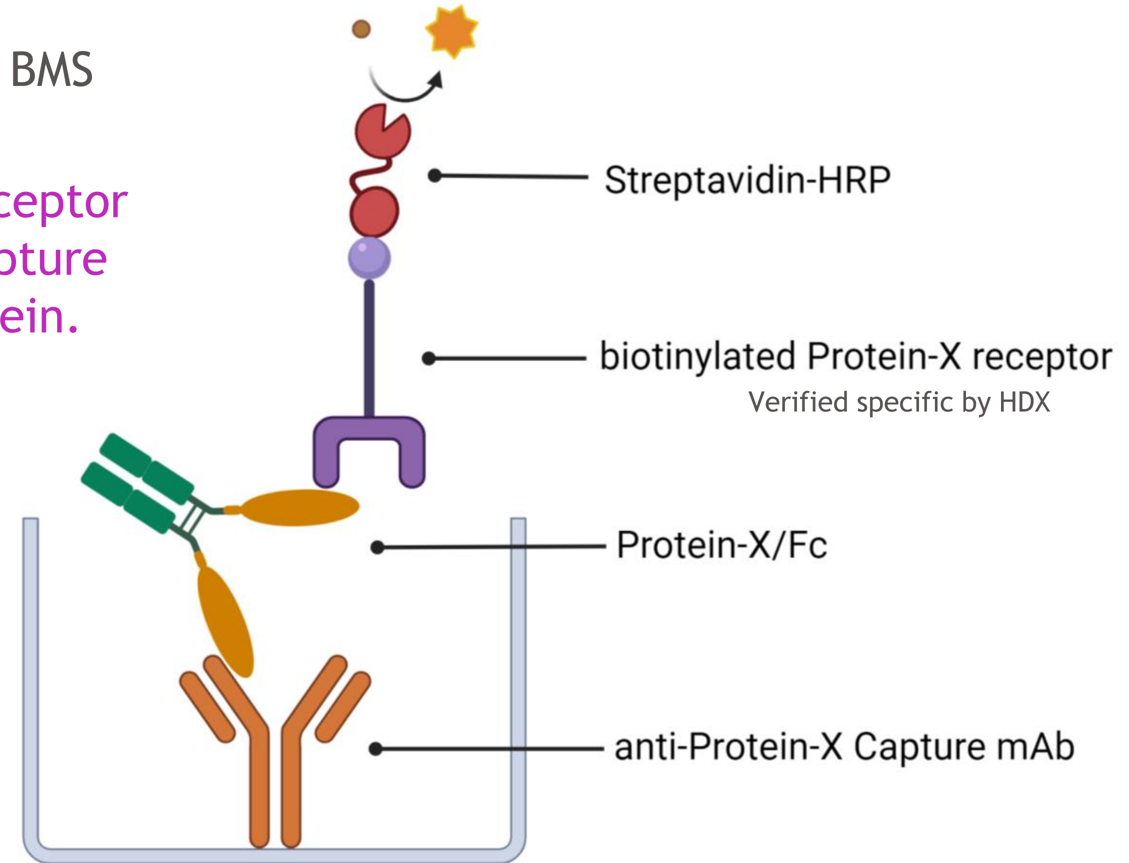
Legacy ELISA can *detect* the presence of BMS Protein-X/Fc in a dose-dependent manner, but is not a true reflection of Potency like the Cell-Based Bioassay

ELISA Interrogation Studies: Capture Antibody Specificity

- Justification for why the legacy ELISA is limited in its ability to specifically measure product potency required.

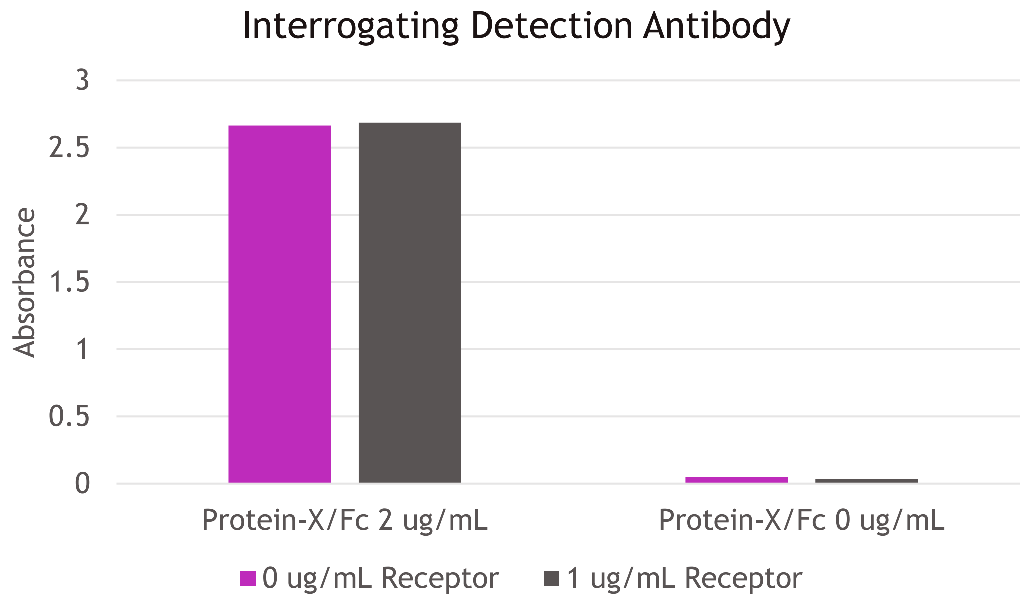
- Biotinylated Protein-X receptor binds to antibody-captured BMS Protein-X/Fc in a dose dependent manner.
- Capture antibody does not recognize/interfere with the receptor binding regions of BMS Protein-X/Fc, indicating that the capture mAb is not a suitable substitute for the specific target protein.

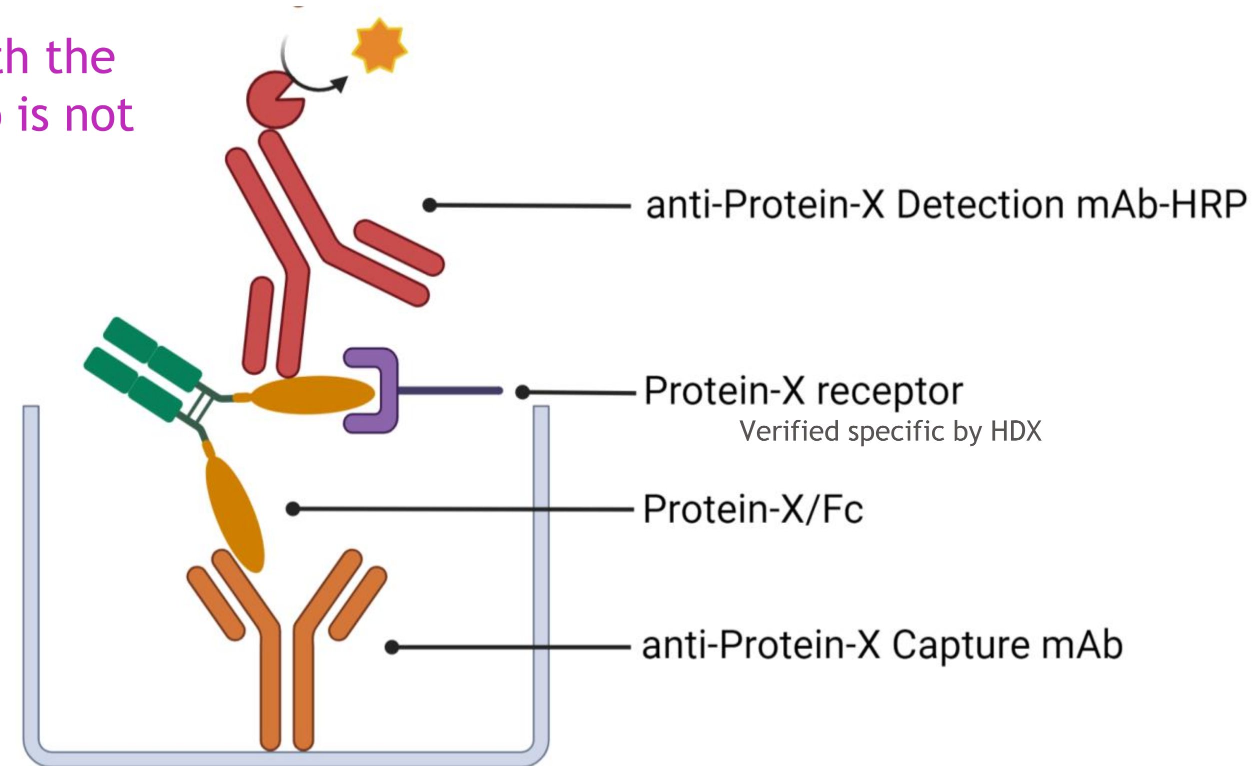




ELISA Interrogation Studies: Detection Antibody Specificity

- Addition of Protein-X receptor does not decrease the signal from the detection antibody.
- Detection antibody does not recognize/interfere with the receptor binding regions of BMS Protein-X/Fc; it also is not target-specific





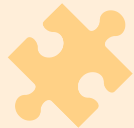
A potency method needs to be MOA-reflective

ELISA Method Interrogation Outcomes

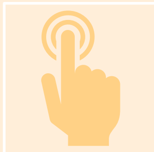
- General conclusion:
  - The two kit antibodies don’t bind to the target receptor binding domain epitopes of the Protein-X/Fc.
- Legacy ELISA Method Risks:
  - Misidentifying an attribute as critical simply because a change in potency is detected.
  - Lack of specificity to the Protein-X receptor binding region could result in missing a relevant critical attribute.
- Not only is the ELISA method not reflective of the mechanism of action, but it presents serious risks to understanding product potency, stability, and functional attributes.

BMS Protein-X/Fc Glycosylation: Potency Impact

Glycosylation interferes with the ability of legacy kit-based ELISA reagents to detect Protein-X/Fc, resulting in reduced ELISA potency.



Glycosylation does not impact the biological interaction of Protein-X/Fc with the Protein-X receptor, as shown in the MOA-reflective Bioassay method.



Glycosylation impact on the legacy ELISA potency was artifactual

Validation of the Cell-Based Reporter Gene Bioassay

- Method is developed to be a simple overnight assay with robust performance and control of critical reagents.
- Method was phase-appropriately validated following ICH guidelines.
- Method demonstrated to be:
  - Accurate, Precise, Specific, Stability-indicating, Fit-for-Purpose

Stability Condition	Cell-based Potency	ELISA Potency
65C 10 min	92%	96%
65C 30 min	~28%	~28%
65 C 1 hour	~3%	~3%

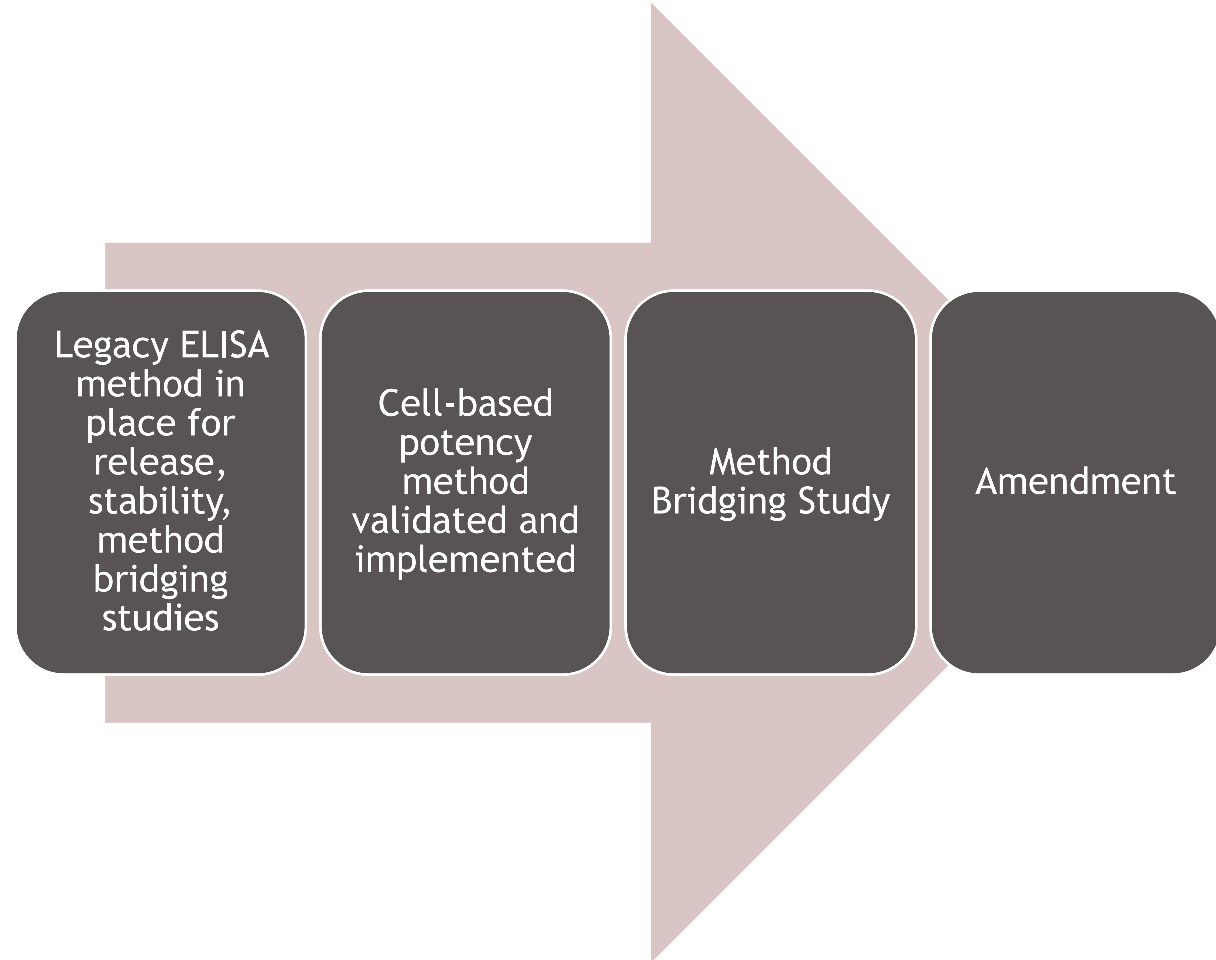
Target Potency (%)	40	70	100	130	160
% Recovery (Accuracy)	98	102	100	97	97
Acceptance Criterion % Recovery	≥ 80% and ≤120%				
% RSD (Intermediate Precision)	7				
Acceptance Criterion RSD (%)	≤ 20%				
Linearity (R^2)	1.00				
Acceptance Criteria	$R^2 \geq 0.97$				
Pass/Fail	Pass				

Mitigation Strategy: Fit-for-Purpose Method Replacement Process

- The control system method should adequately reflect the biological activity of the molecule.
  - The ELISA is not MOA-reflective and is influenced by irrelevant attributes raised in the cell line process change.

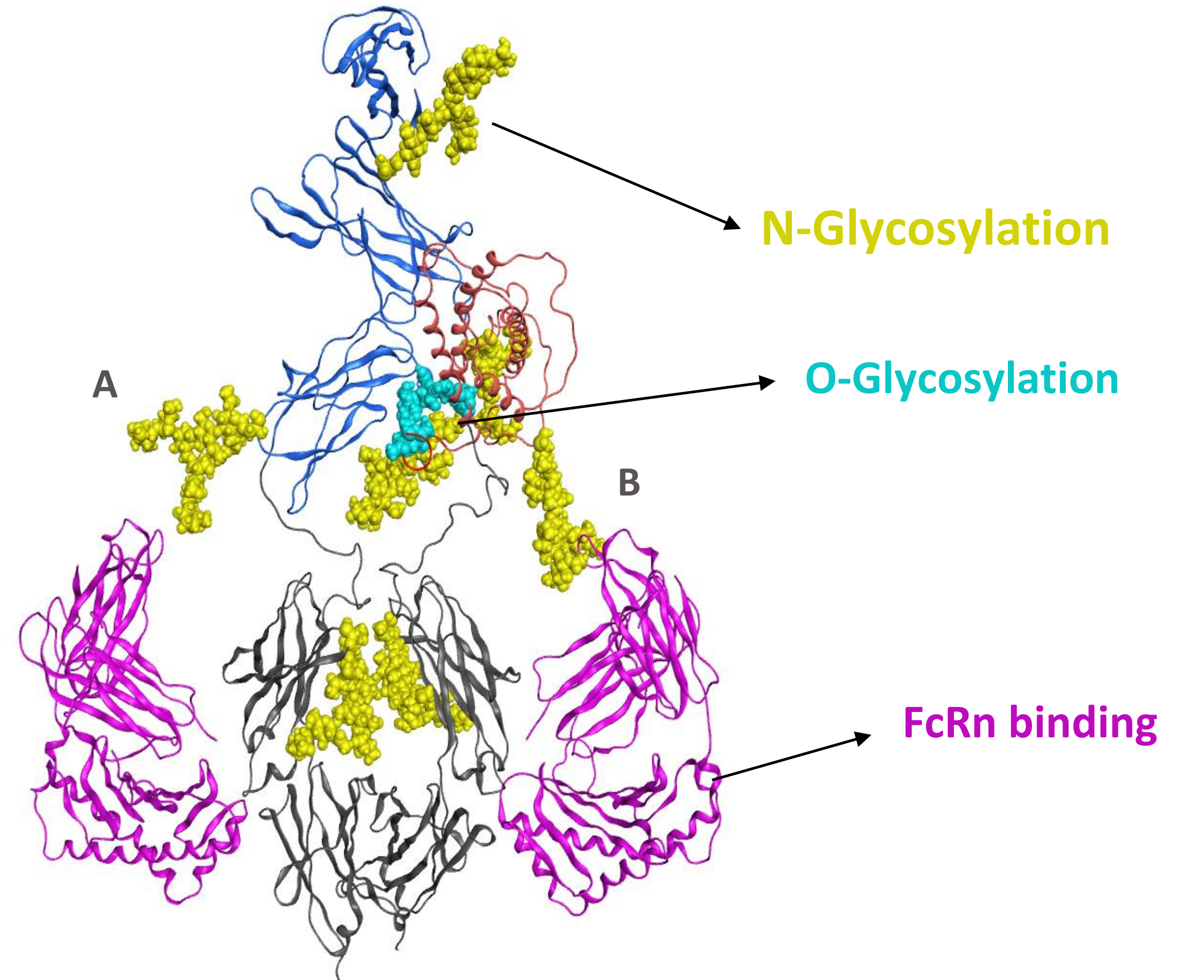
    → Move method to For-Information-Only on specification
  - The Cell-based method is MOA-reflective and insensitive to attributes outside of the site of biological activity.

    → Replace specification test method with functional method capable of product control



Structural Impacts of Glycosylation on FcRn Binding

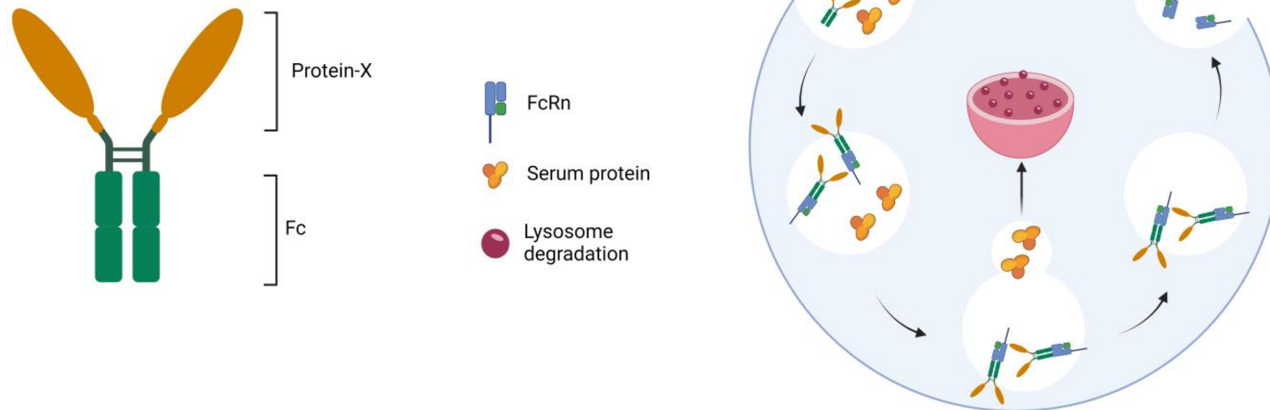
- Biological Characterization of the WHOLE molecule
- Additional glycosylation chains with sialic acid end capping were anticipated to be beneficial to product PK.
- Glycosylation moieties at A & B are capable of forming long, flexible chains that have potential for steric hindrance
  - *Fc Interactions: Do they interfere with binding to the Fc neonatal receptor?*



Biological Characterization of Structural Changes

- FcRn binding studies showed ~35% lower affinity for Process B material relative to Process A, an unexpected result based on molecular structure.

Process	%KD
A (Ref)	100.0
A	99.2
B	136.1
B	136.0
B	137.8



FcRn Binding: Deglycosylation Normalizes Binding Profiles

- Relative to deglycosylated Reference, the Process A and B deglycosylated batches have comparable FcRn binding.
- Undetermined if glycosylation changes create enough steric hindrance to reduce FcRn binding in vivo consistently. They are flexible.
- This is not an artifact of the method. Exploring requirements to bridge process changes will be important.

Relative to **Deglycosylated** Process A RM

Process	%KD
A (Ref) Glycosylated	163.2
A (Ref) Deglycosylated	100.0
A Deglycosylated	97.1
B Deglycosylated	111.5
B Deglycosylated	107.6

BMS Protein-X/Fc Glycosylation: FcRn Impact

Glycosylation reduces the ability of FcRn to bind the Fc of Protein-X/Fc in SPR analysis.

Depending on conformation, this may have the potential to decrease FcRn binding in vivo and further studies are warranted

Key Takeaways

The potency method should adequately reflect the biological activity of the molecule and translate the significance of structural attributes into measurable impacts to the MOA of the drug.

Ensuring consistent product quality is at the heart of biological control: a well-characterized method enables a trusted assessment of manufacturing consistency. This touches both MOA-related potency and a wholistic understanding of molecular critical quality attributes.

- The legacy ELISA is not MOA-reflective and is influenced by irrelevant attributes identified during the cell line process change.
- The Cell-based method is MOA-reflective and insensitive to non-relevant attributes.
- Increased glycosylation is not a CQA for potency but warrants further explorations for potential impact to PK/PD.
- Increased glycosylation is not a potency CQA but may be a PK CQA.

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Thank you

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