3D Structure of Biologics in a Convenient Immunoassay Format

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Topics Covered Today

- Why New Technologies?
- Technology Development.
- Case Studies for Novel and Biosimilar mAbs.
- Conclusions.

1. Why a New Technology for Protein Conformational Analysis?

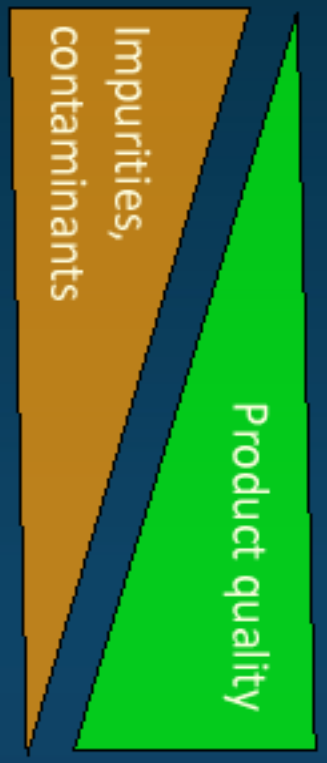
Studies Demonstrating the Importance of 3-D Structure and Its Stability for Immunogenicity

- James LC. et al. 2003. Antibody multispecificity mediated by conformational diversity. Science 299:1362-1367.
- Nobeli, I et al. 2009. Protein promiscuity and its implications for biotechnology. Nature Biotechnology 27(2):157-167.
- Halimi, H et al. 2005. Closed and open conformations of the lid domain induce different patters of human pancreatic lipase antigenicity and immunogenicity. Biochim. Biophys. Acta. 1753:247-256.
- So, T. et al. 2001. Contribution of conformational stability of hen lysozyme to induction of type 2 T-helper immune response. Immunology. 104:259-268.
- Schlellekens, H. 2005. Factors influencing the immunogenicity of therapeutic proteins. Nephrol Dial Transplant. 20:3-9.
- Laat, B. et al. 2011. Immune responses against domain I of β2-glycoprotein I are driven by conformational changes. Arthritis & Rheumatism. 63(12)3960-3968.
- Ohhuri, T. et al. 2010. A protein's conformational stability is an immunologically dominant factor: evidence that free-energy barriers for protein unfolding limit the immunogenicity of foreign proteins. J. Immunology. 185:4199-4205.
- Sharma, B. 2007. Immunogenicity of therapeutic proteins. Part 1: Impact of product handling. Biotechnology Advances. 25:310-317.
- Porter, S. 2001. Human immune response to recombinant human proteins. J. Pharmaceutical Sciences. 90(1):1-11.
- Kromminga, A. et al. 2005. Antibodies against erythropoietin and other protein-based therapeutics. Ann. N.Y. Acad. Sci. 1050:257-265.

History of medical use and immunogenicity of proteins

Practically all therapeutic proteins are immunogenic

- Proteins of animal origin (> 1920s)
  (e.g. equine antisera, porcine/bovine insulin)
- Human derived proteins (> 1950s)
  (e.g. growth hormone, factor VIII)
- Recombinant human proteins (> 1980s)
  (e.g. insulin, interferons, GM-CSF, epo)



TODAY:

- Reduced impurity levels, improved product quality
- Still, more immunogenicity concerns than ever before

From Wim Jiskoot, NBC, Seattle, 2009

Consequences of anti-drug antibodies

Loss of efficacy
- Insulin
- Streptokinase
- Staphylokinase
- ADA
- Calcitonin
- Factor VIII
- Interferon alfa 2
- Interferon beta
- Interleukin-2
- GnRH
- TNFR55/IgG1
- Denileukin diftitox
- HCG
- GM-CSF/IL3
- Various monoclonals

Enhancement of efficacy
- Growth hormone

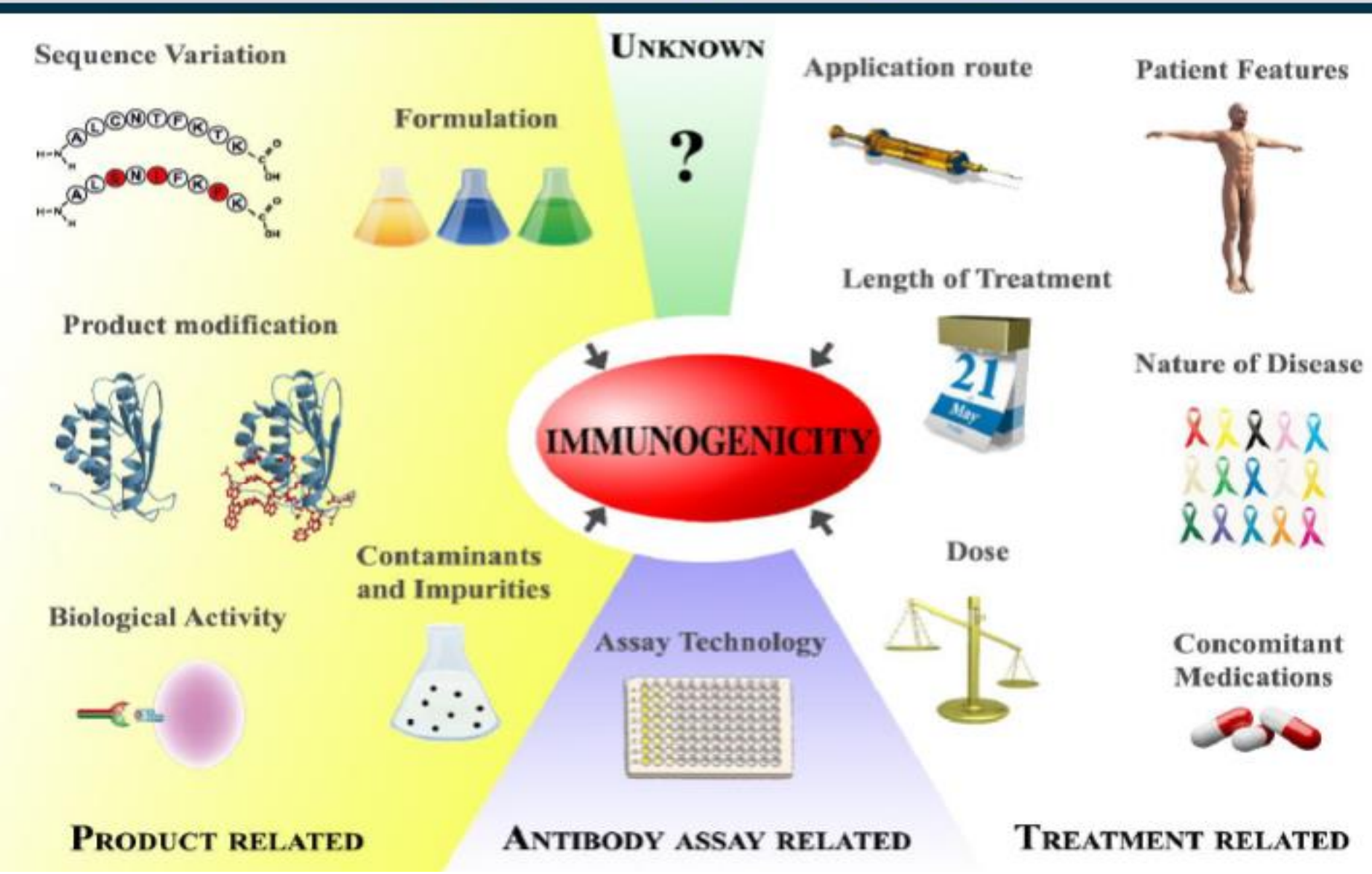
Neutralization of endogenous protein
- Epoetin
- Megakaryocyte-derived growth factor (MDGF)

General immune effects
- Allergy
- Anaphylaxis
- Serum sickness, etc

None

From Wim Jiskoot, NBC, Seattle, 2009

Factors influencing immunogenicity of proteins



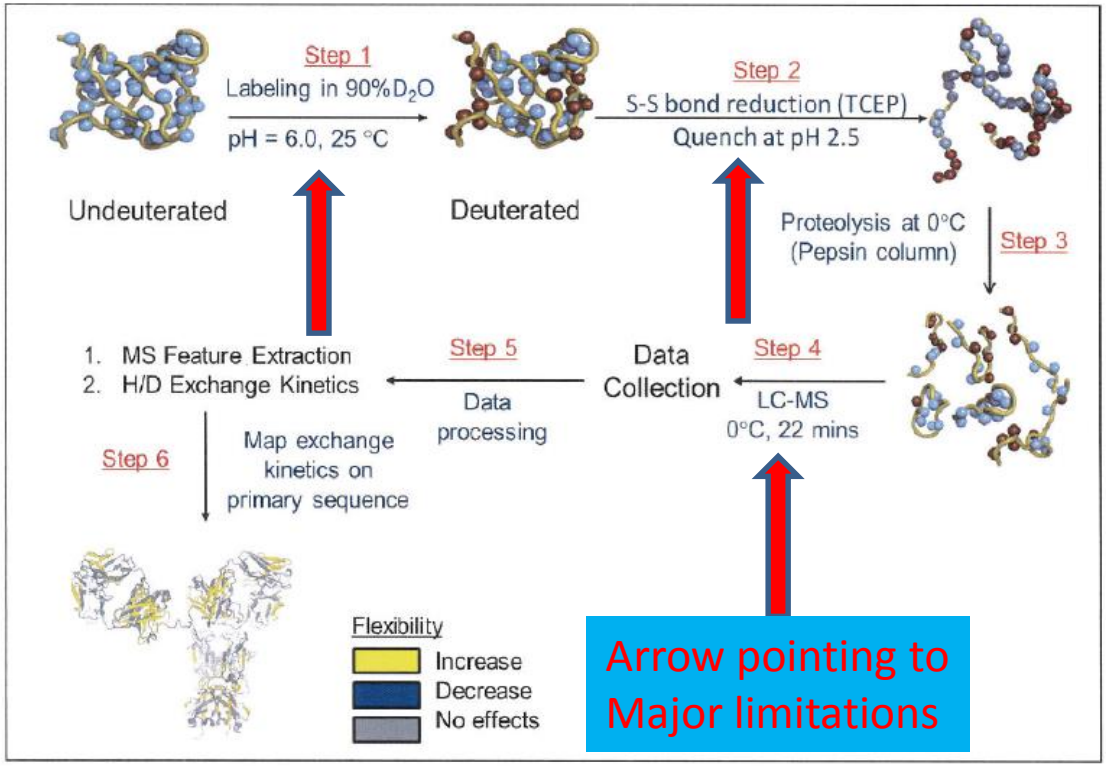
From Wim Jiskoot, NBC, Seattle, 2009

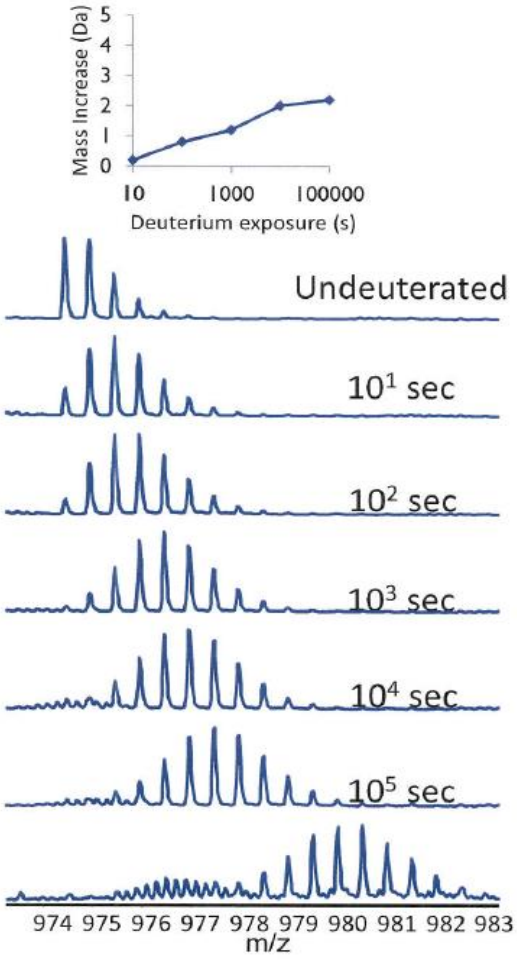
FDA Guidance on Biosimilars, 2012

The FDA guidance further stated that "The three dimensional conformation of a protein is an important factor in its biological function. Protein generally exhibit complex three-dimensional conformations (tertiary structure and, in some cases, quaternary structure) due to their large size and the rotational characteristics of protein alpha carbons. The resulting flexibility enables dynamic, but subtle, changes in protein conformation over time, some of which may be absolutely required for functional activity." "..... at the same time, a protein's three-dimensional conformation can often be difficult to define precisely using current physiochemical analytical technology."

Limitations of Current Technologies

Optimized Workflow of H/D Exchange-MS with mAb-B



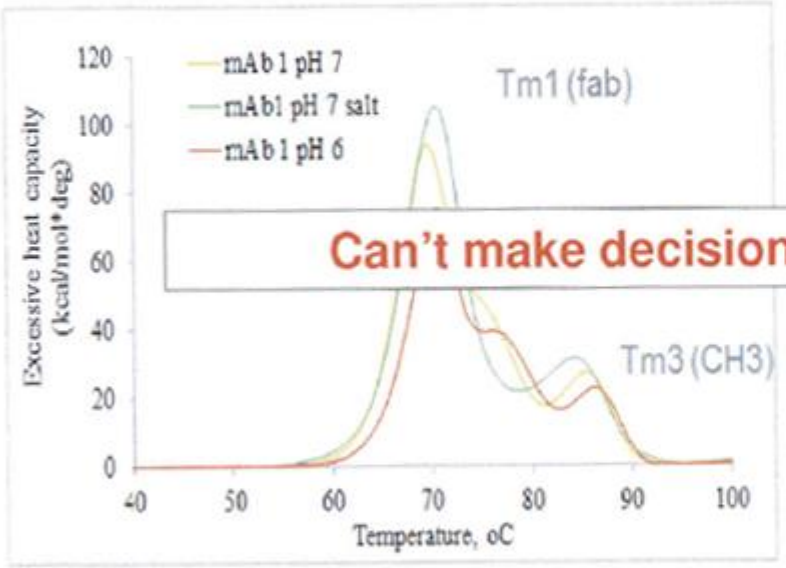


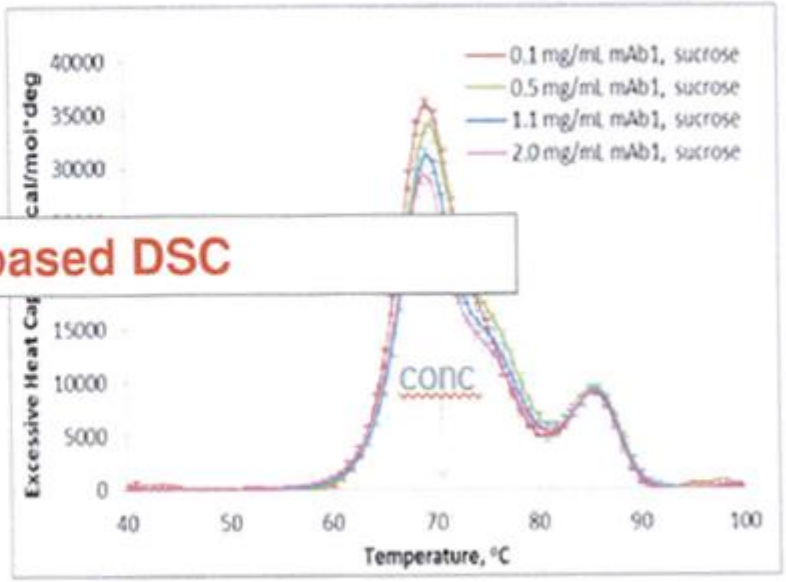
Majumdar et al (2012)
***J Am Soc Mass Spectrum* 23: 2140**

Limitations of Current Technologies

CASE STUDY- IgG1 mAb (mAb 1)

Preformulation: horrible viscosity, bad solubility, strange DSC.





Can't make decision based DSC

- Not much difference in 1st thermal transition
- 2nd transition usually not relevant

- Concentration-dependent inconsistency in Tm2/AUC

Biogen mAb HOS Studies

Current Technologies for Conformational Analysis

- Near UV CD Spectrum
- Size Exclusion Chromatography (SEC)
- Analytical Ultracentrifugation (AUC)
- Non-denaturing Electrophoresis
- Bioassays
- NMR
- Hydrogen/deuterium exchange (HDX)

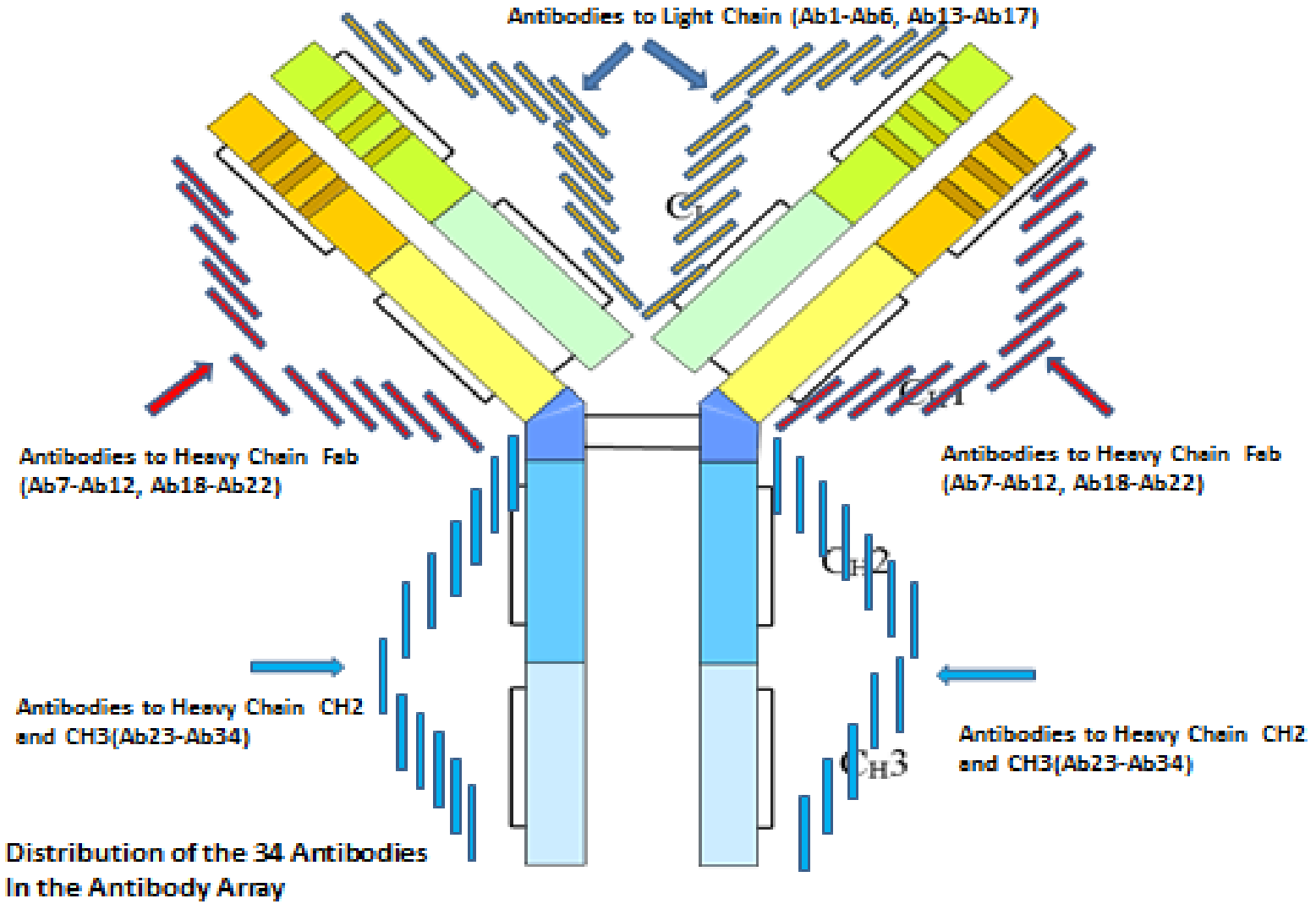
A more sensitive and high throughput technology is desirable to investigate protein conformational changes, especially for monoclonal antibodies.

Comparison of Antibody Array and H/DX-MS Technology

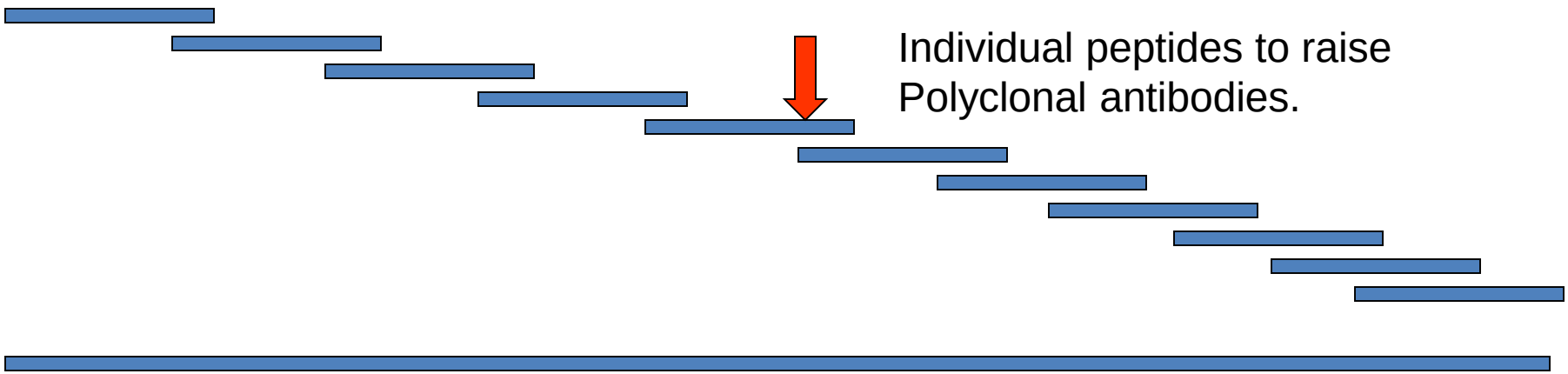
	Antibody Array	H/DX-MS
Principle	Epitope Distribution on the surface of proteins recognized by antibodies	Hydrogen-Deuterium Exchange for the amide group on the surface of proteins
Format	ELISA	Mass Spectrometry
Quantitation	0.1% epitope change with <15% RSD from ELISA	Sample comparison involving protein digestion, liquid chromatography and MS analysis
Throughput	>12 samples/day	Several days for 2 samples
Cost	$900/2 samples	>$25,000/2 samples

2. Technology Development

Diagram For Protein Conformational Array



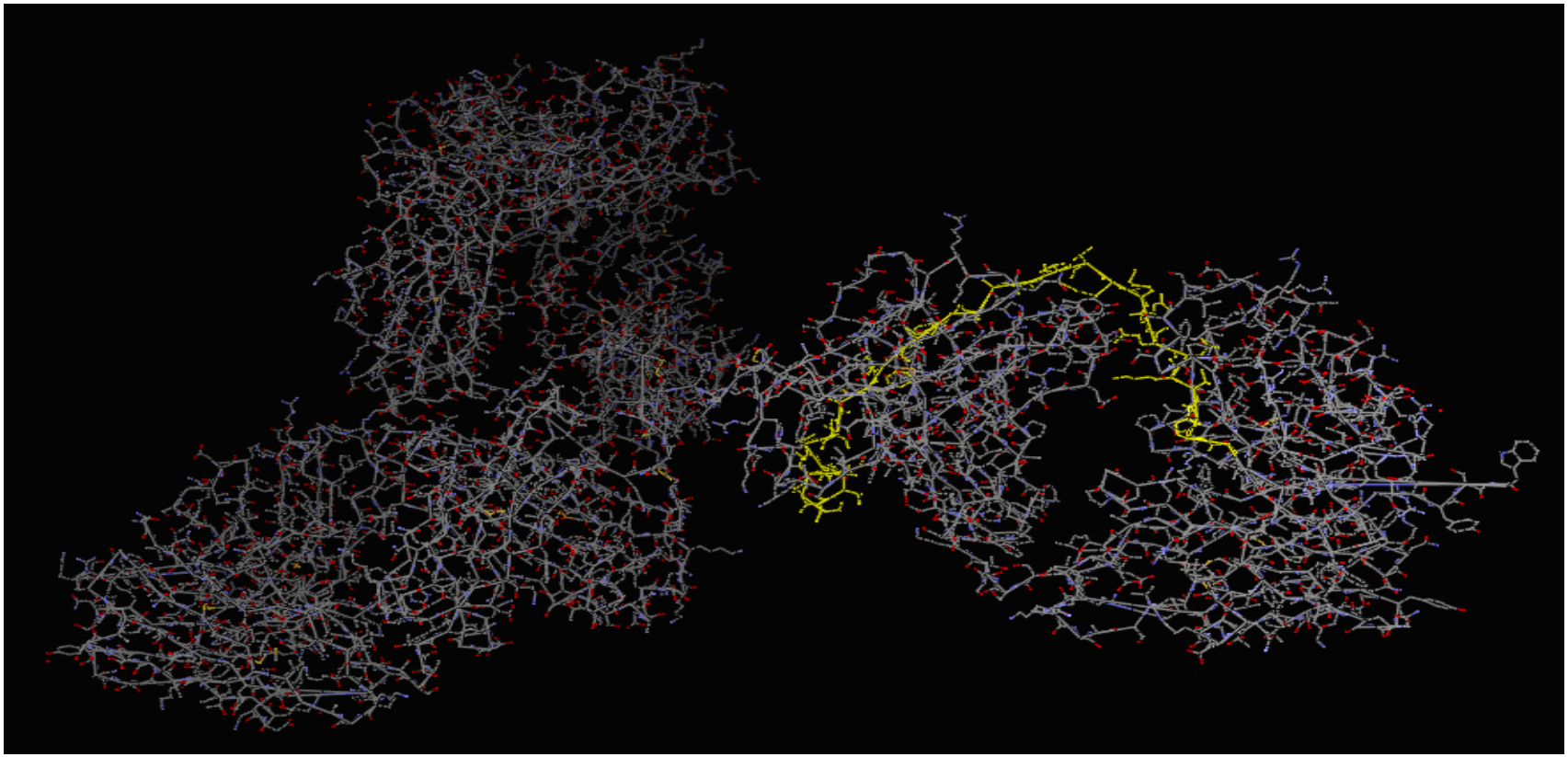
Technology Development



mAb Amino Acid Sequence

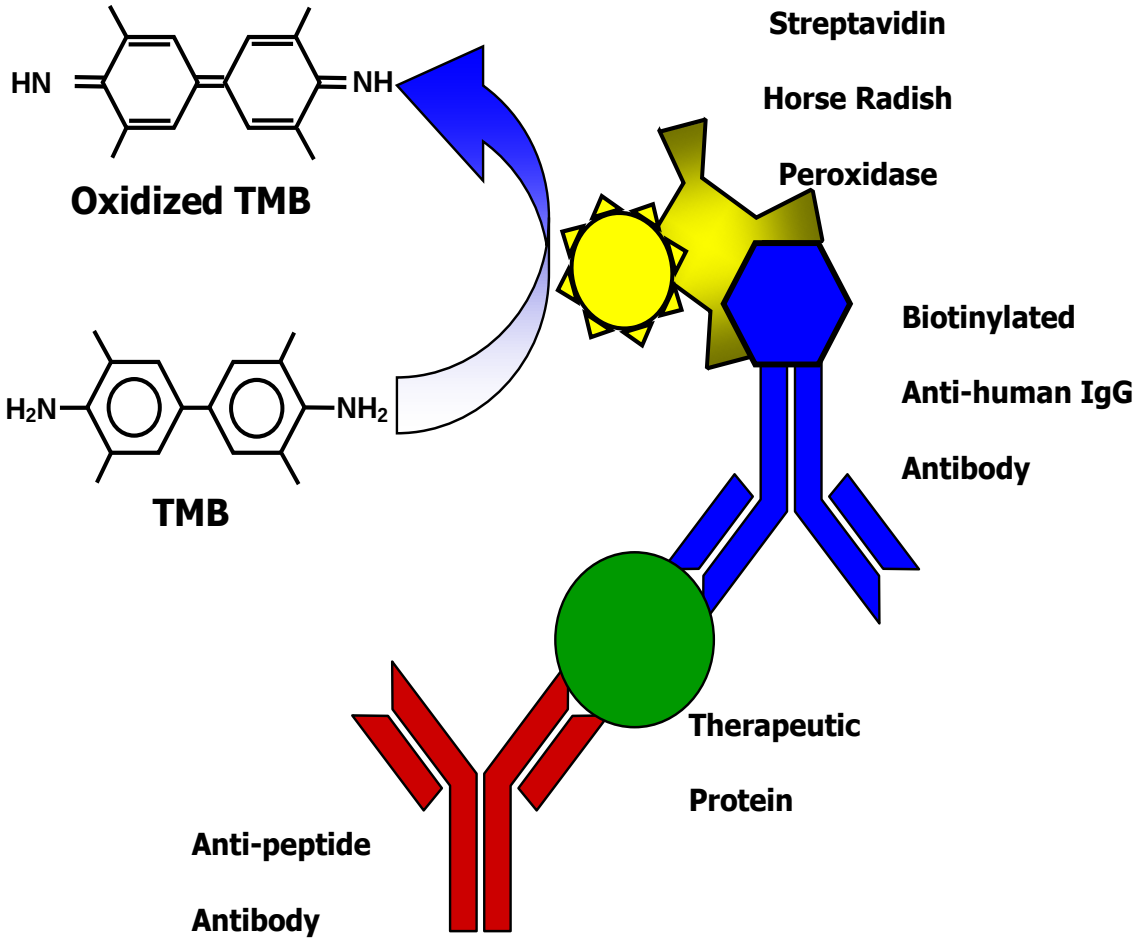
Antibody amino acid sequence is used to design the antibody array with overlapping regions to cover the whole mAb molecule

Detailed Structural Information

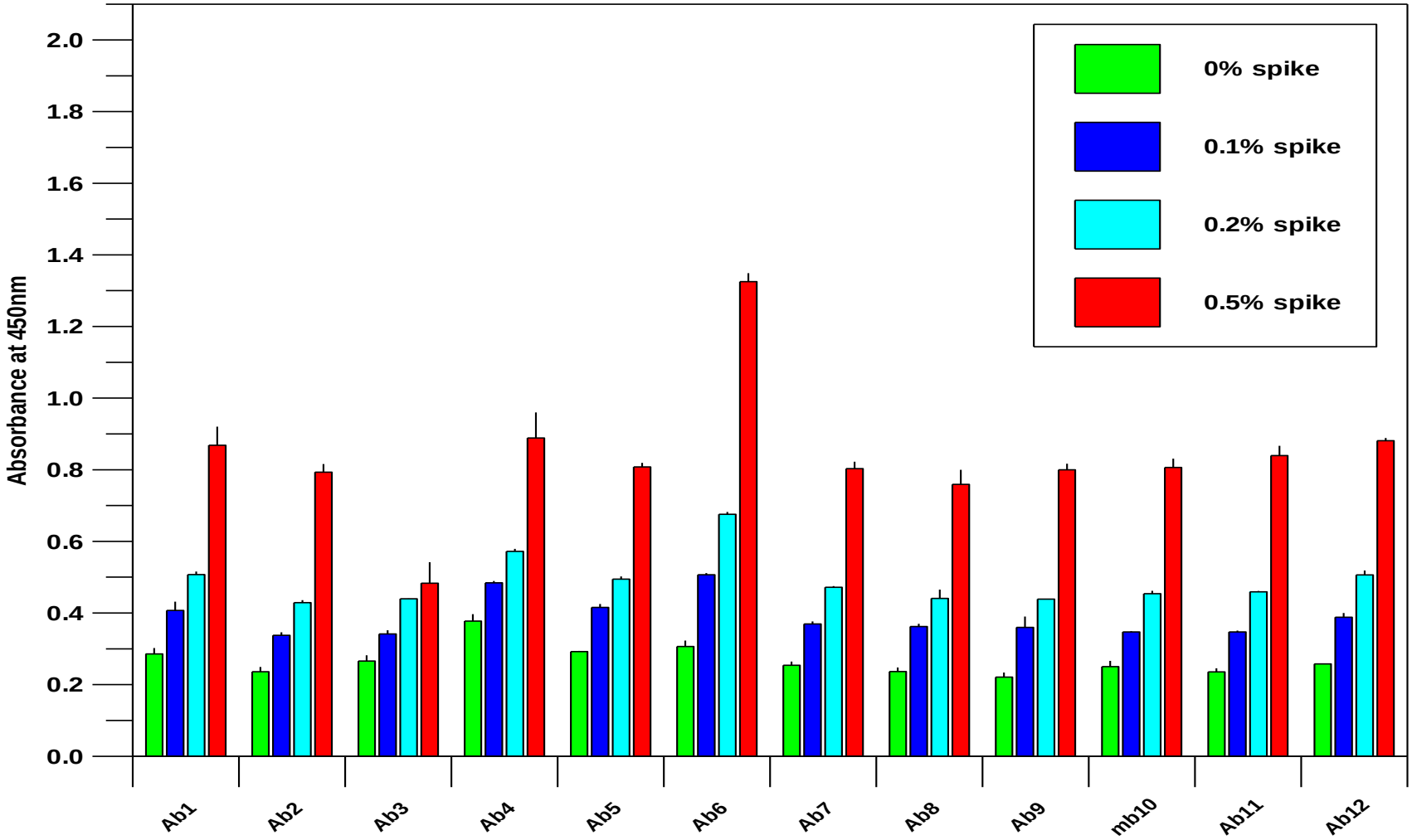


This antibody array technology measures the mAb surface **Linear Epitope** Exposure and Some **Secondary Structure-derived Epitope** Exposure, providing a signature epitope distribution that is unique to each mAb

Diagram of the Sandwich ELISA



0.1% New Epitope Exposures can be Quantified with the ELISA (QL=0.1%)



Spike Testing for the mAb Variable Region with InnoBridge ELISA

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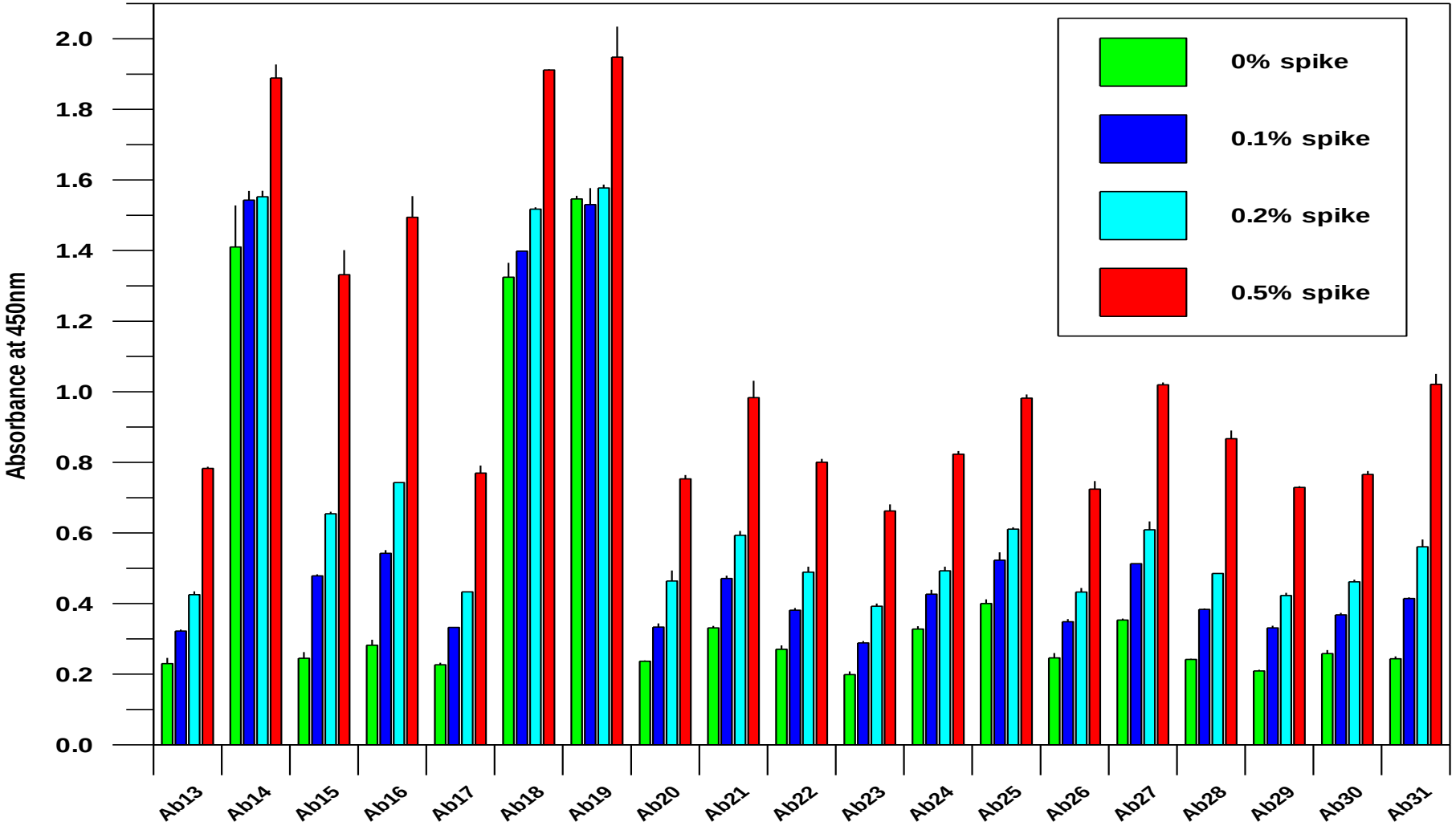


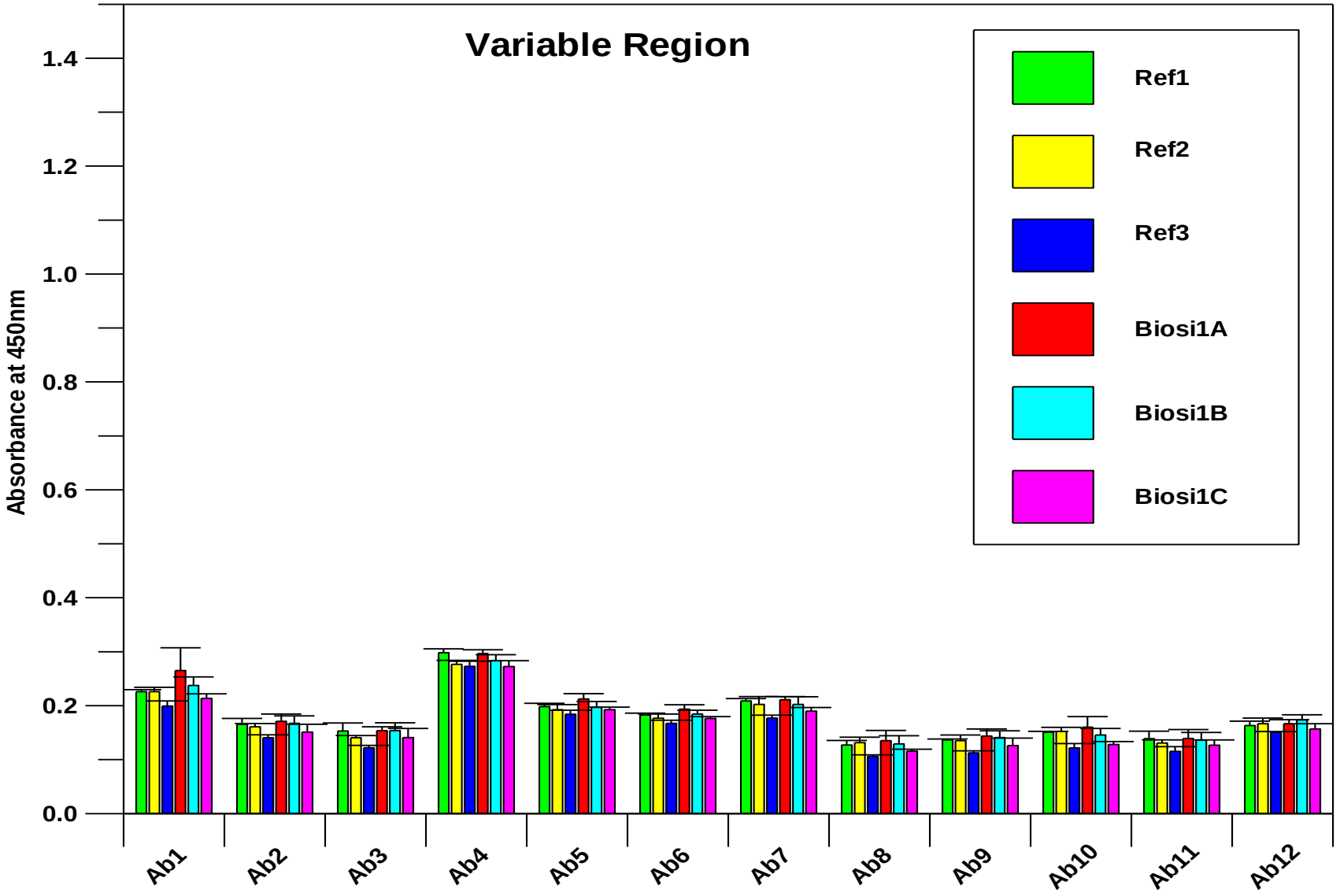
Spike Testing for the mAb Constant Region with InnoBridge ELISA

3. Case Studies in mAb Conformational Analysis

Outcomes of Biosimilar and Novel mAb Testing with Protein Conformational Arrays

1. Similar, with minor differences.
2. Highly similar, no noticeable difference.
3. Significantly different.

Case 1: Minor Differences in Conformation(Biosimilar)



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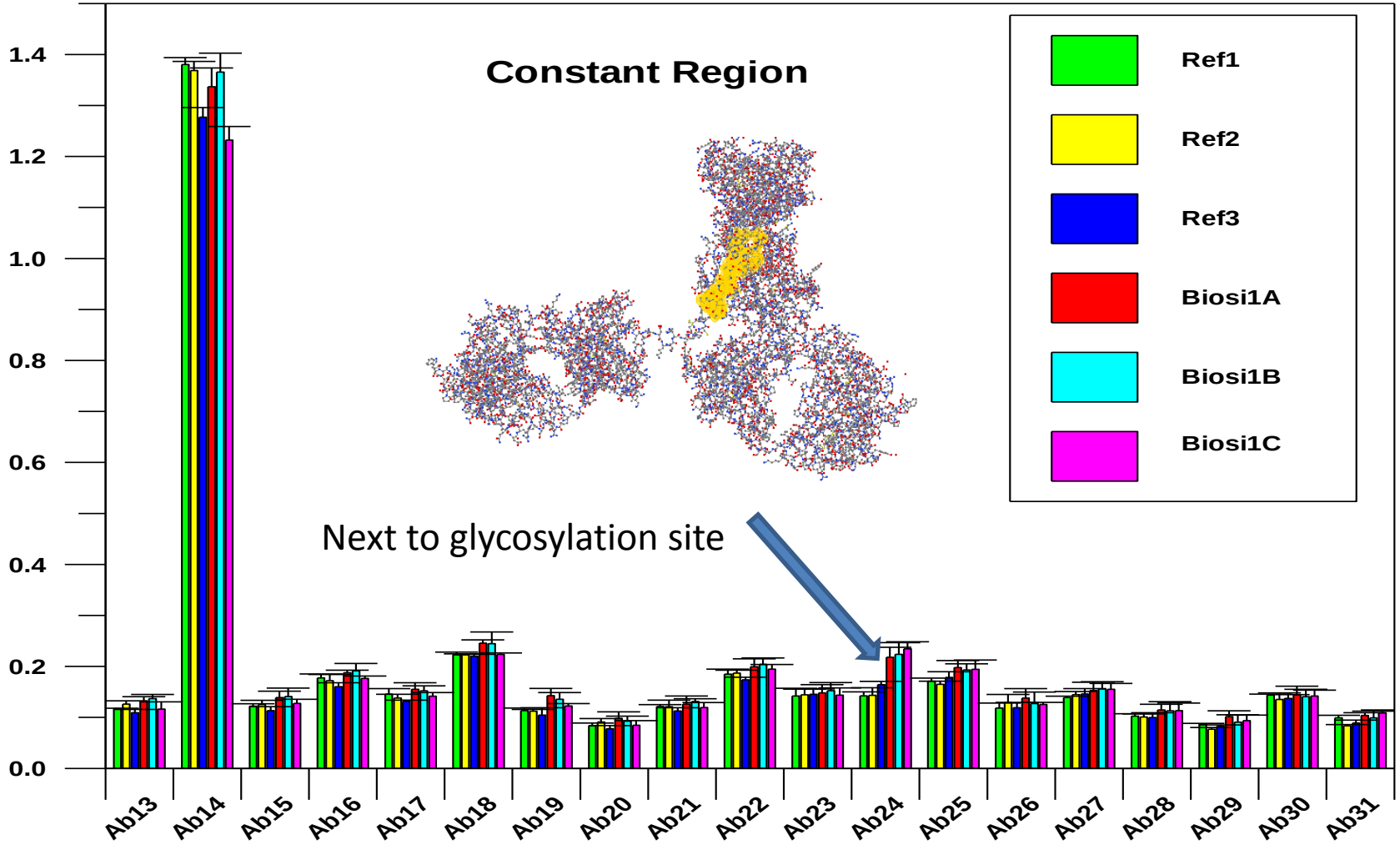
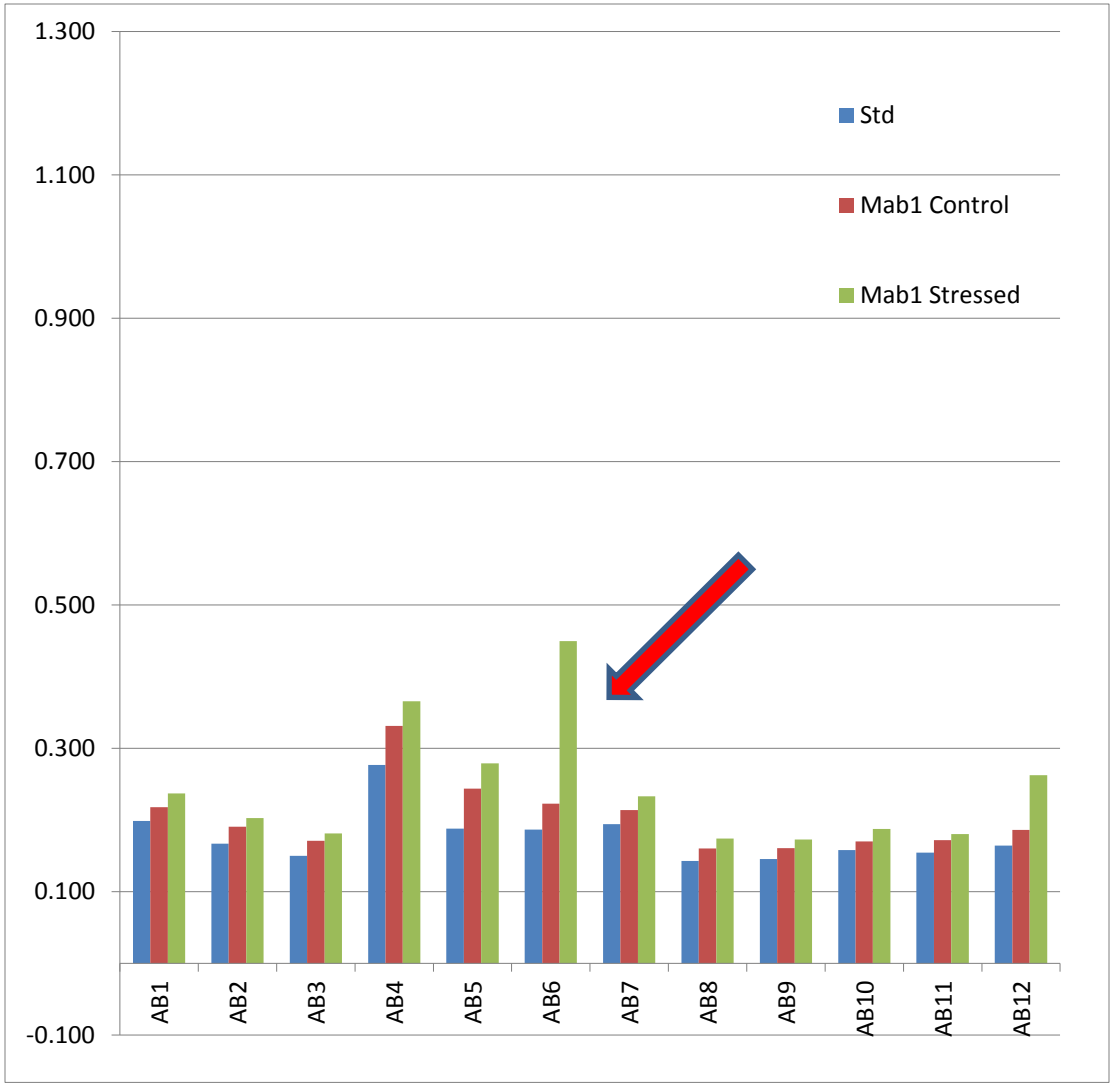
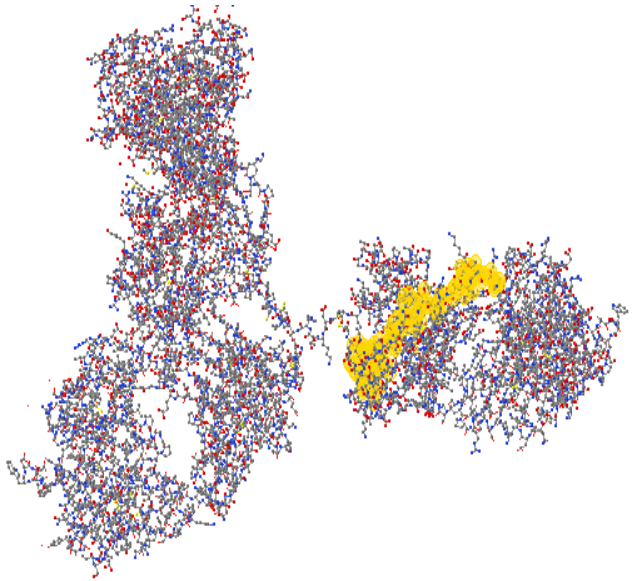


Bioassay and Clinical Testing Demonstrated Comparability, the biosimilar has been approved in both Europe and US

Case 2: Correlation Between Conformation and Bioassay in Stability Testing (Novel mAb)



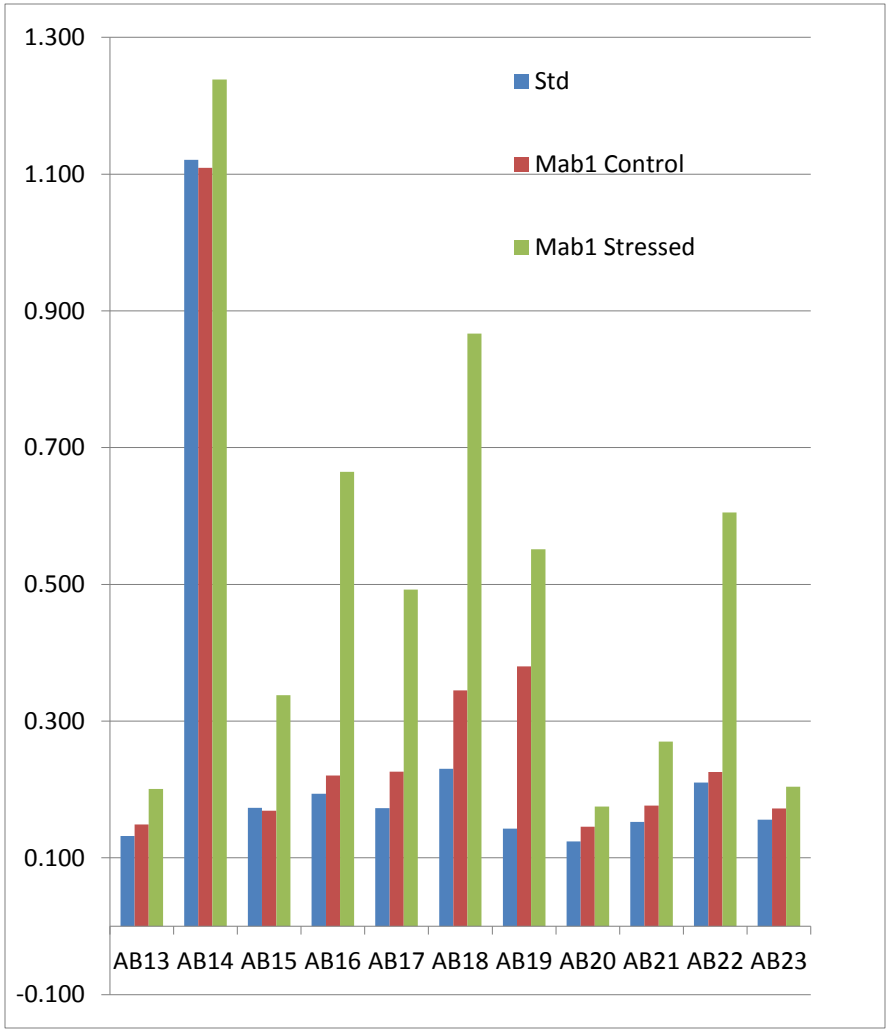


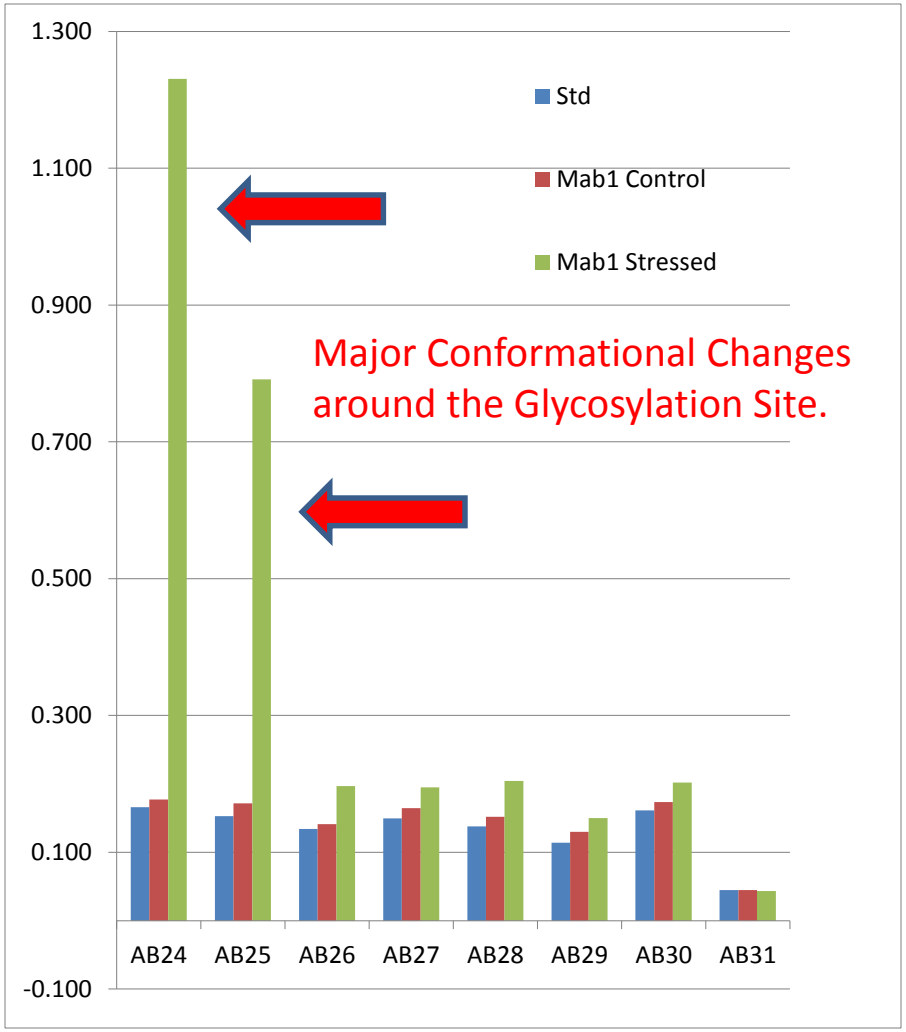
Ab6 is close to light chain CDR3
22% Bioassay Activity Decrease

The most significant difference in the variable region was seen at Ab6 suggesting a correlation between this site and the decrease in bioactivity (the more unfolding the higher the signal)

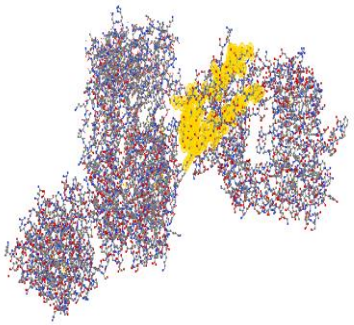
Case 2: Correlation between Conformation and Bioassay in Stability Testing (Novel mAb)

FcγRIIIa binding result: 64% Decrease

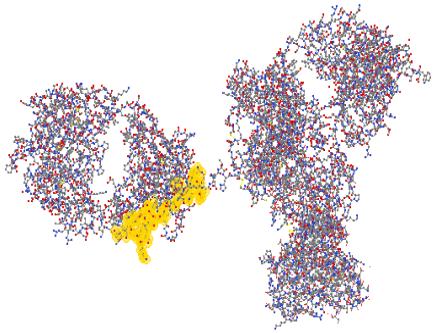




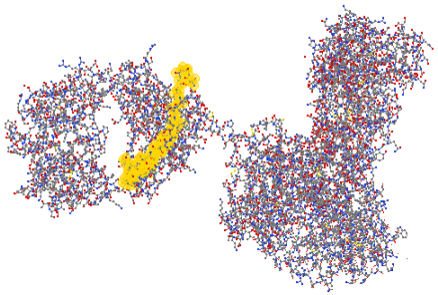
Ab15,16: LC Hinge Region; Ab17,18:HC, Fv-Fc domain
Ab24: HC Hinge Region.; Ab25: HC Glycosylation Site.



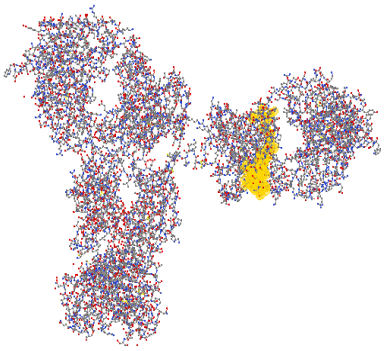
Ab15



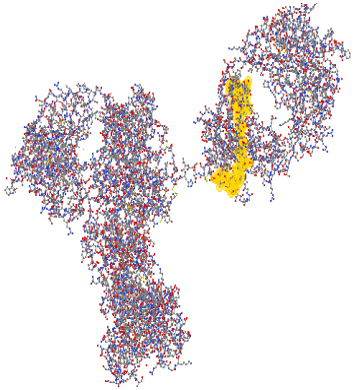
Ab16



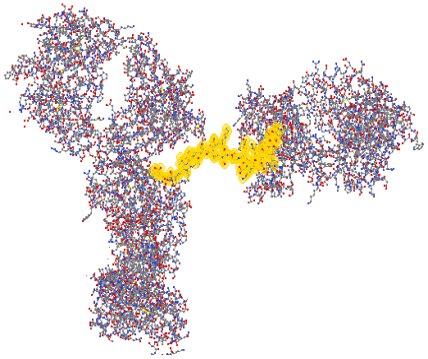
Ab17



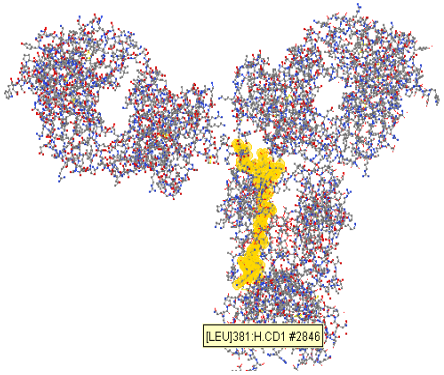
Ab18



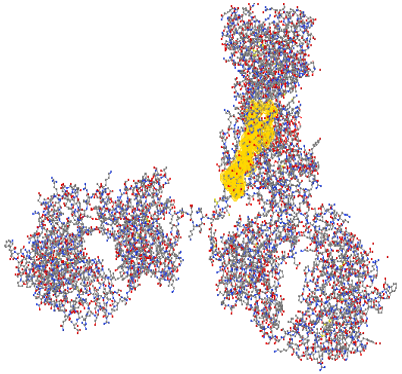
Ab19



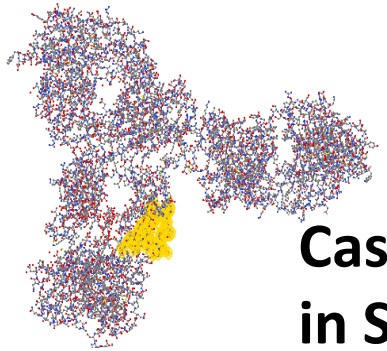
Ab21



Ab22



Ab24



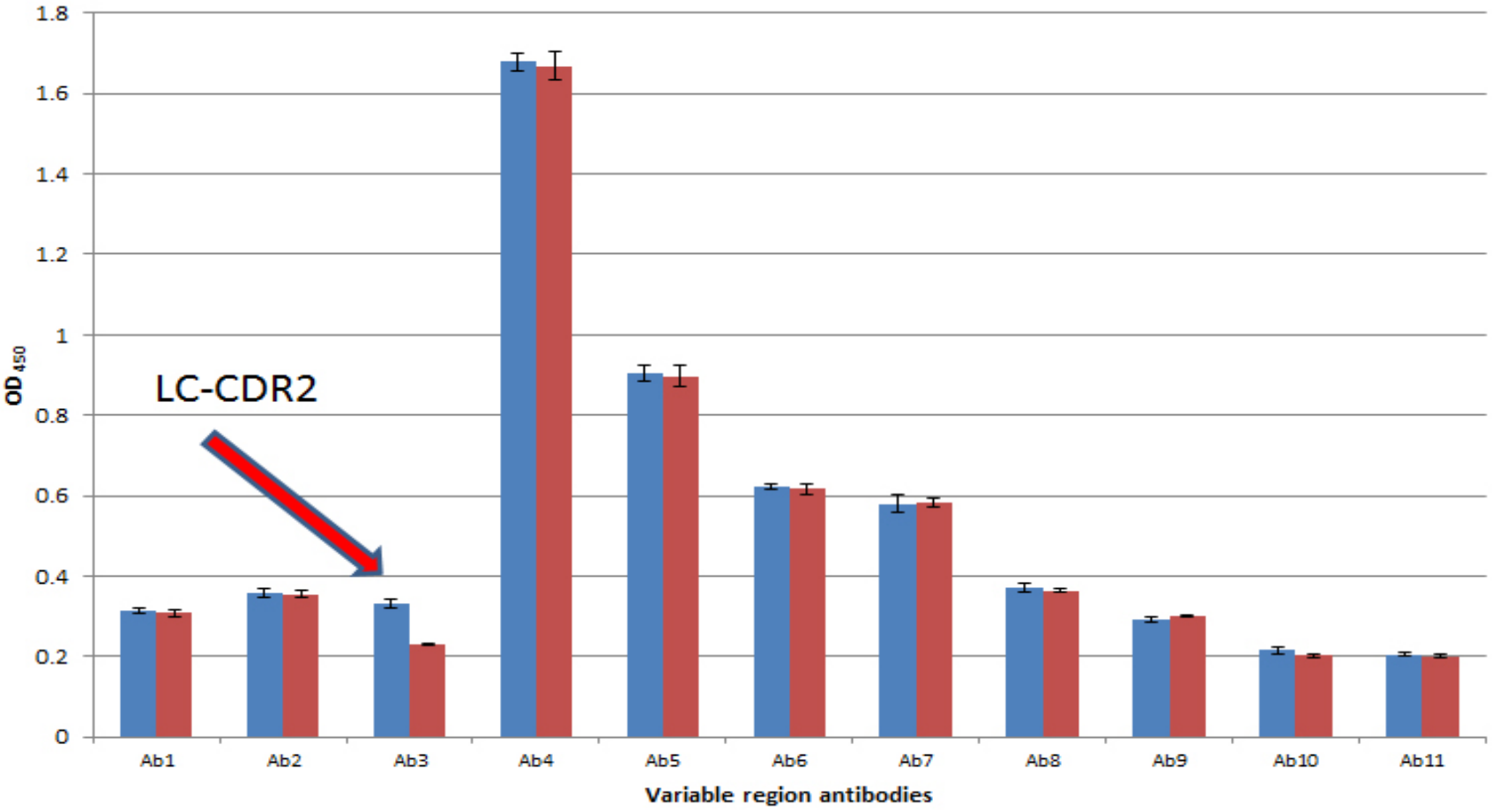
Ab25

FcγRIIIa binding result: 64% Decrease.
Structural Assignments in 3-D.

Case 2: Correlation between Conformation and Bioassay in Stability Testing

Case 3: Correlation Between Conformation and Bioassay in Biosimilar Stability Testing

ADCC Activity Decreased 40%, however there were no differences detected in glycosylation, oxidation, aggregation or any other physiochemical characteristics.



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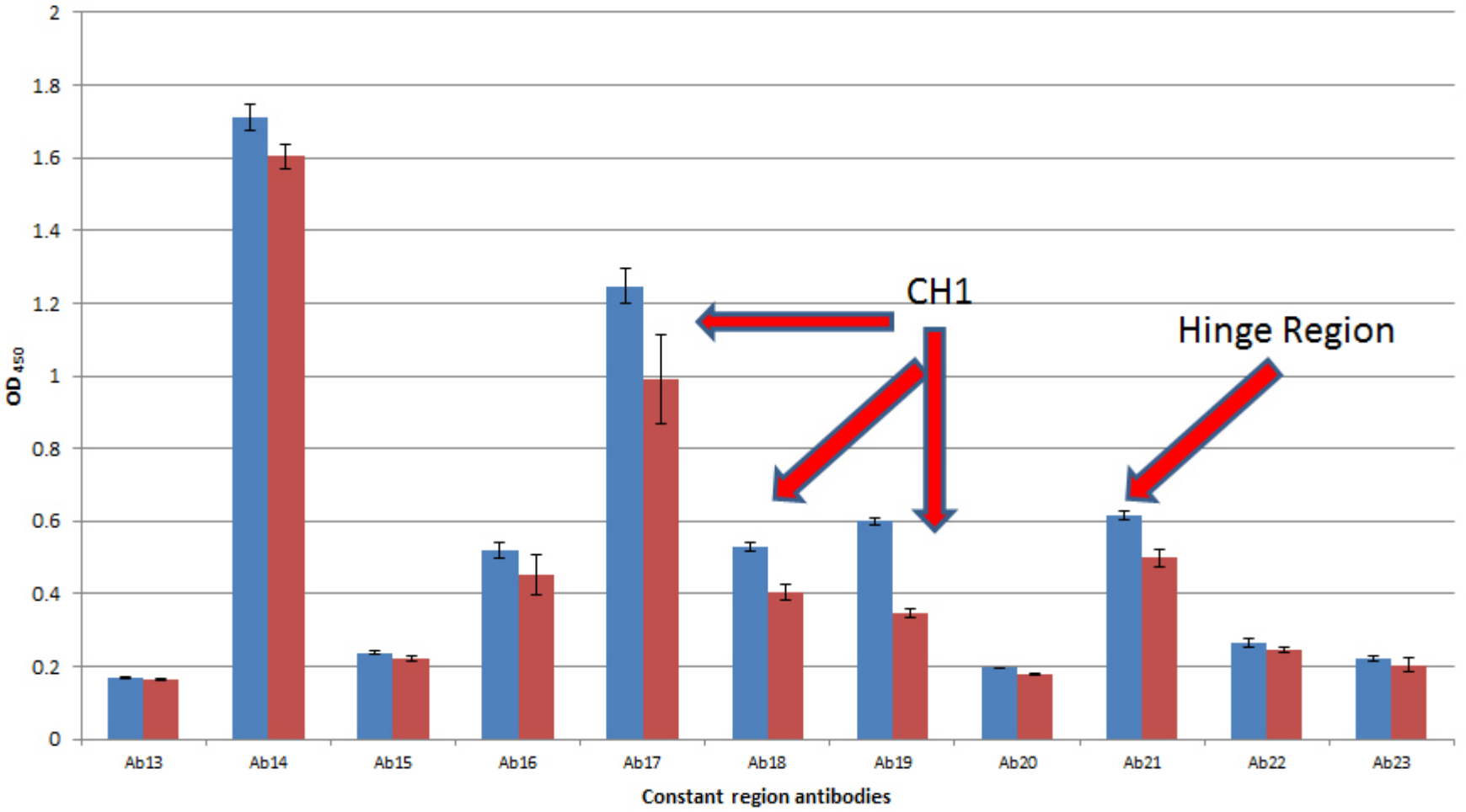

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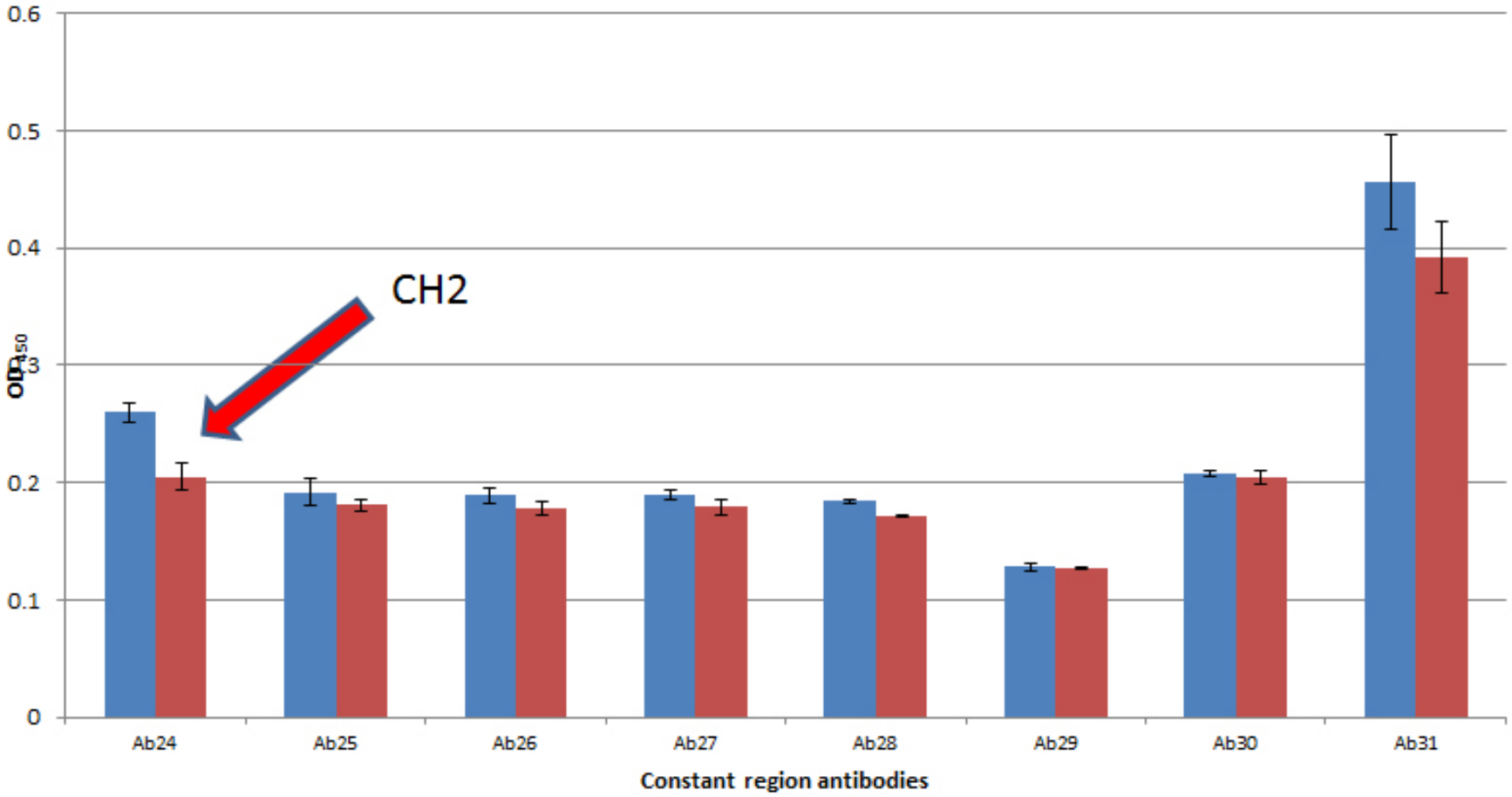
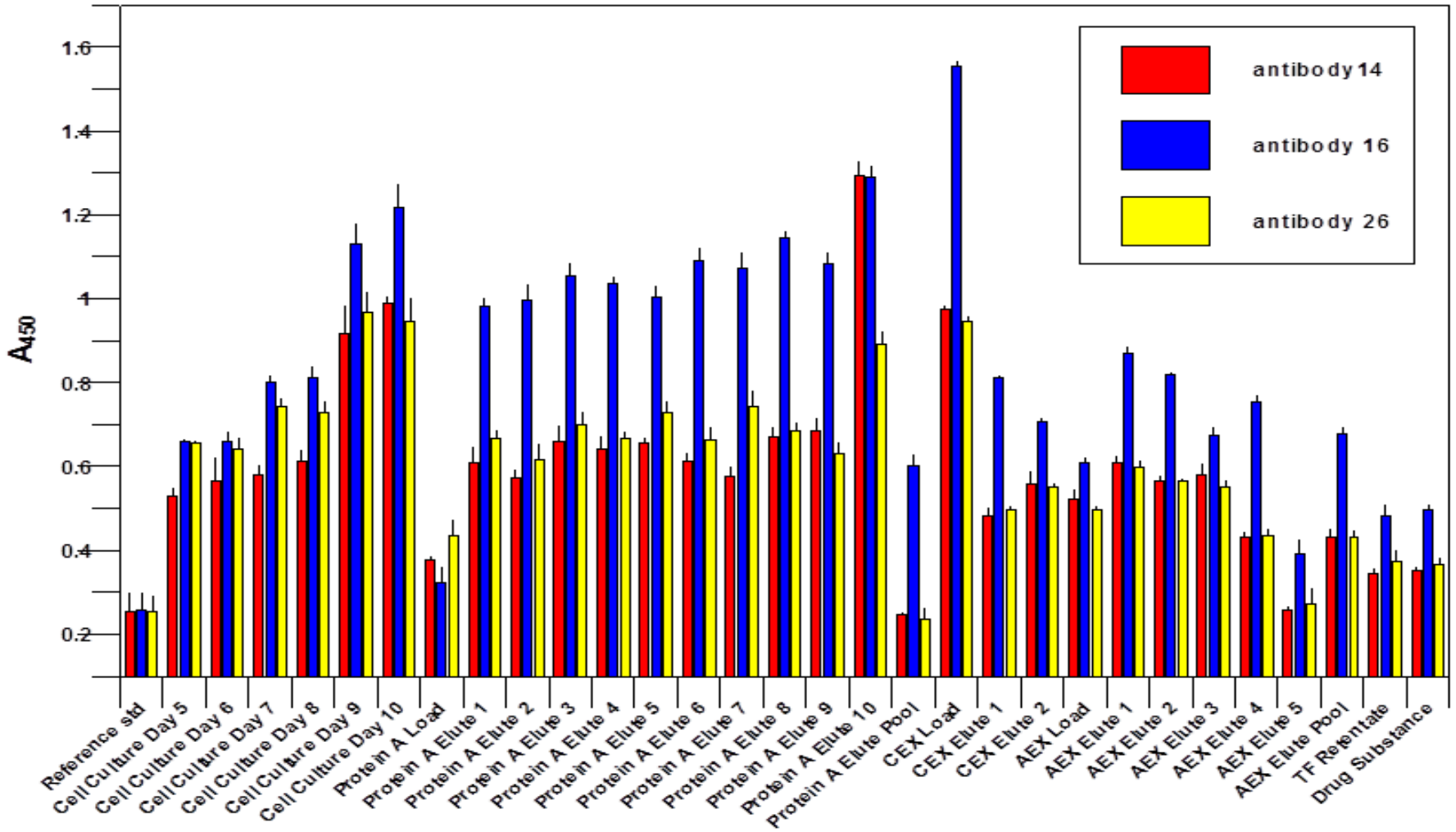


Case 4: Application of Conformational Array in Biosimilar In-process Development

The HOS impact of upstream and downstream process



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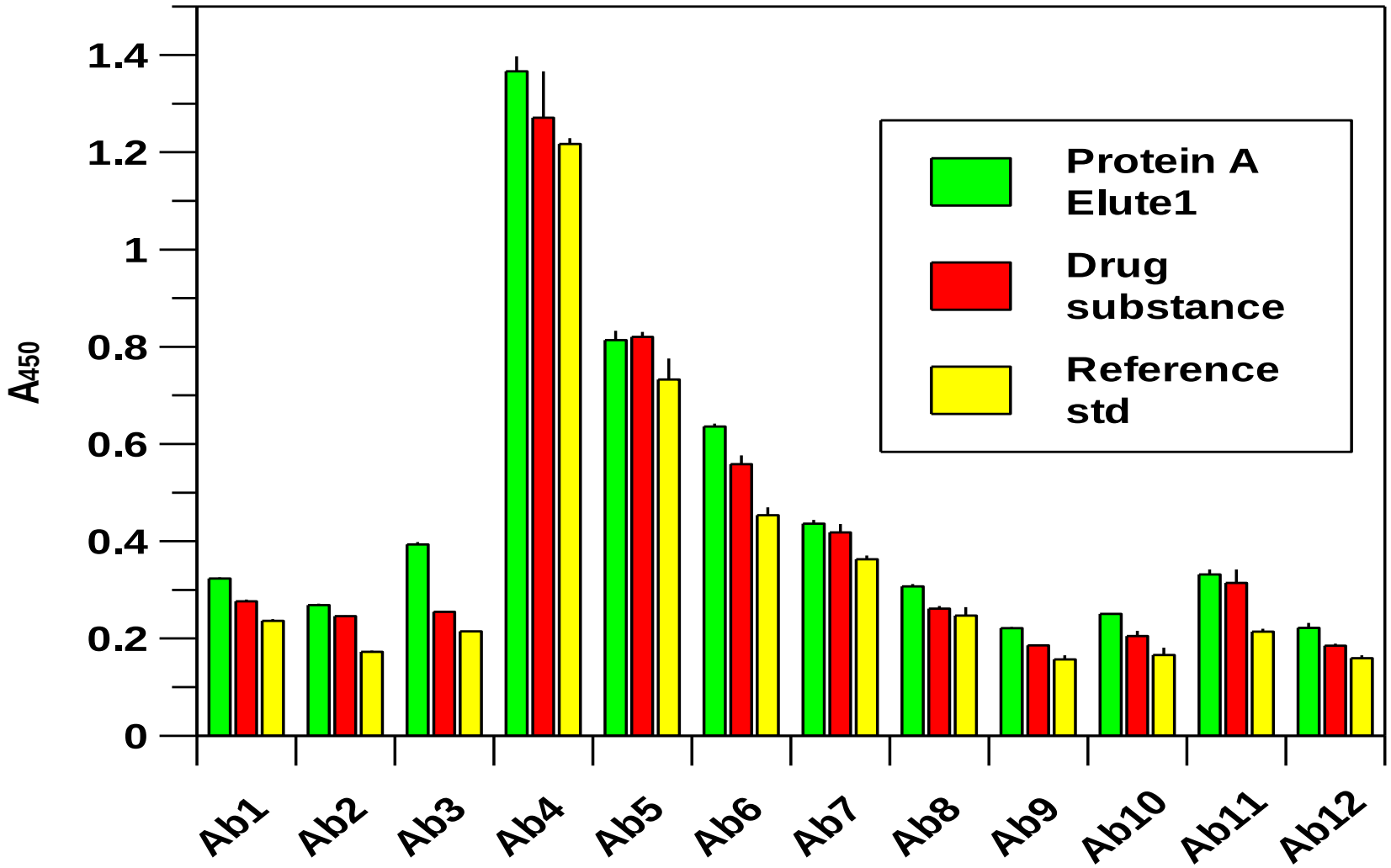

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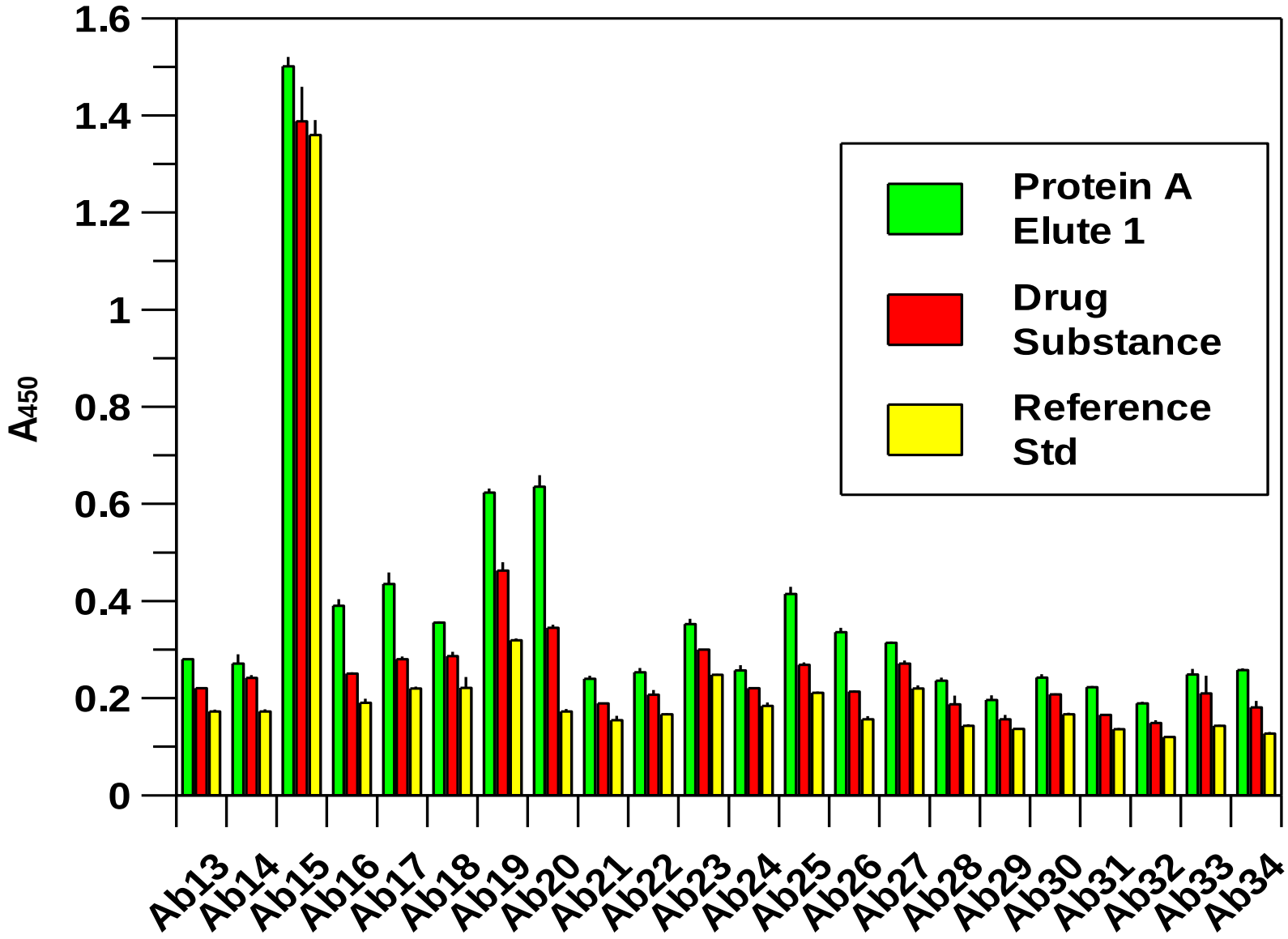
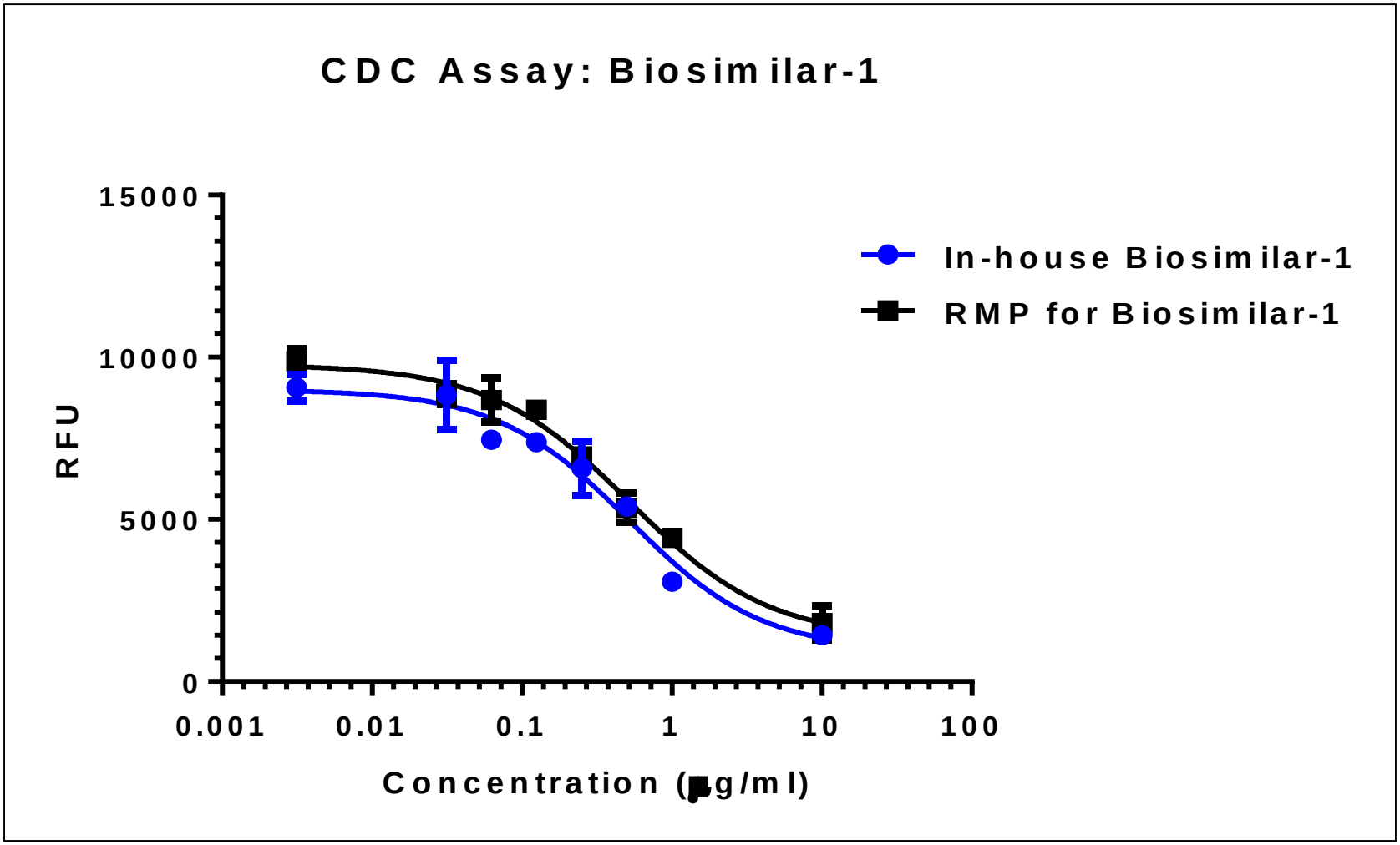


Case 4: Application of Conformational Array in Biosimilar In-process Development

Bioassay showed the biosimilar candidate and reference standard are comparable



4. Conclusions

- Protein Conformational Array was developed to characterize mAb HOS status.
- Each antibody array provides a unique signature for that mAb, reflecting its surface exposure and extent of exposure.
- The antibody array is sensitive, systematic and relatively high throughput.
- It correlates well with stability and bioassay data.
- It can detect changes that may not be detected with bioassays.
- It can be applied to many stages of biologics development, from cell line selection to product release.
- The technology can also be applied to novel mAb discovery and study antibody-drug conjugates (ADCs).