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Critical Considerations for Analytical Methods Used in Biosimilar Development

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CASSS CMC Strategy Forum Europe 2015

Biosimilar Development and Registration: Lessons Learned

Outlines

- Points for consideration for biosimilar development
- Points for consideration for analytical methods should be sensitive and capable of differentiating meaningful differences
- Overview of selected fingerprint methods and applications

Biosimilar product development begins with establishing target quality product profiles

Define critical quality attributes for the reference product

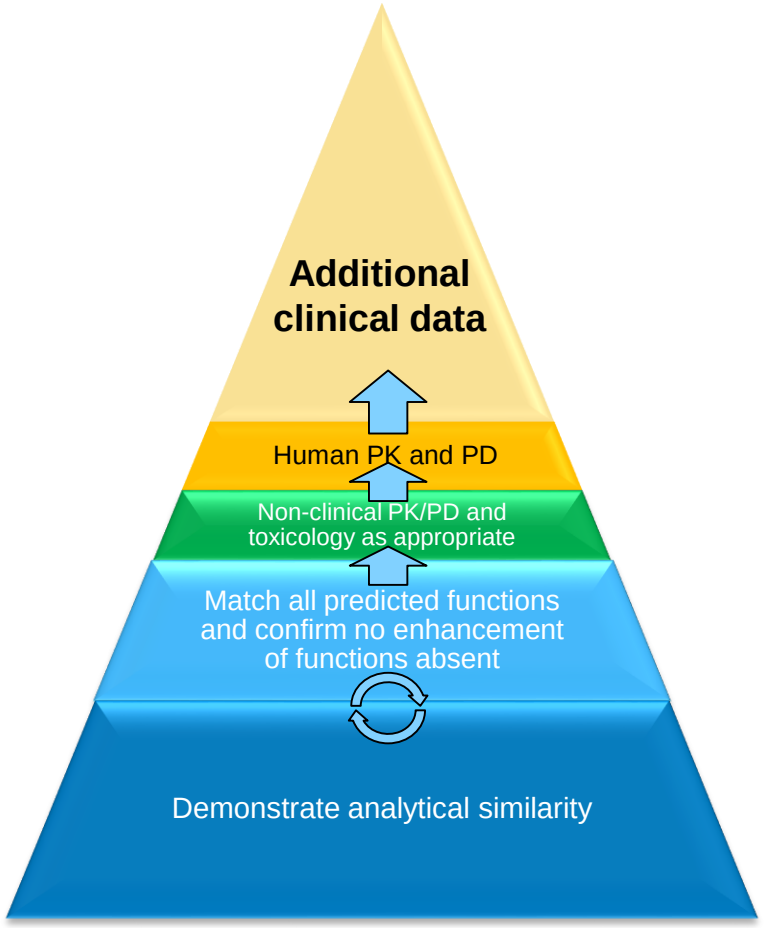
- Known mechanism of actions, biological functions, safety, and immunogenicity profiles

Establish product quality profiles based on the target reference product

- Characterize reference product to establish targets and ranges for critical product quality attributes

Develop biosimilar products to match the target reference product

- Match reference product profiles with greater emphasis on matching all biological functions



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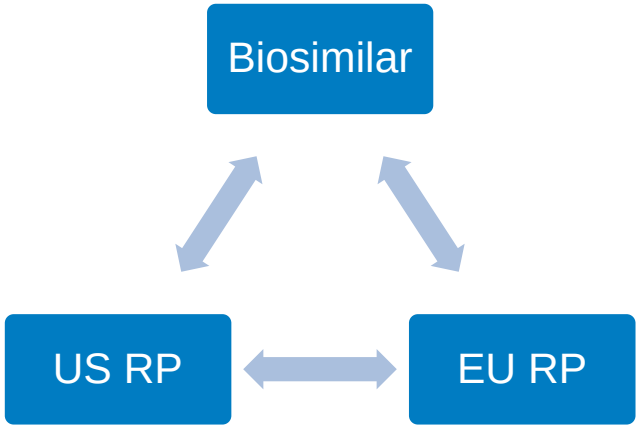
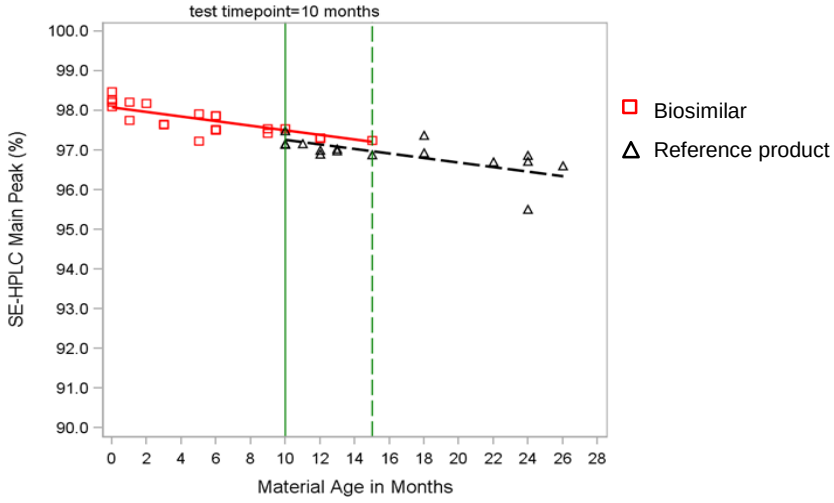
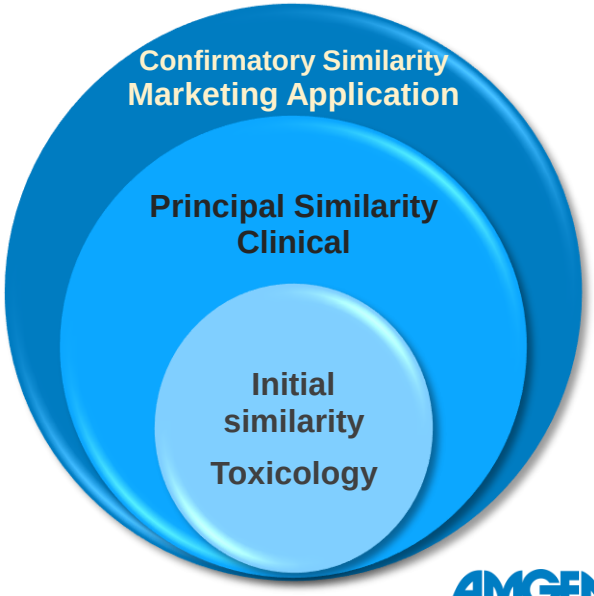
Approaches for analytical similarity assessment and data management

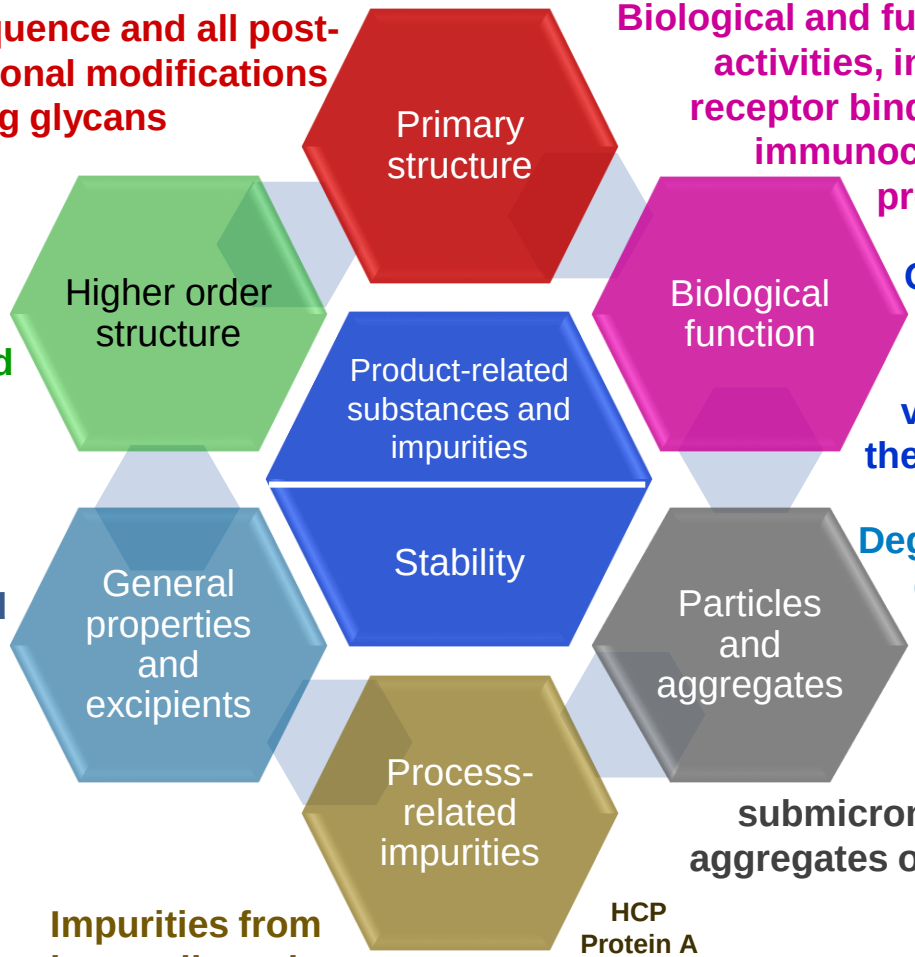
- Cumulative knowledge of reference products on the market helps to understand range and variability of the innovator manufacturing process
- Similarity is a series of pair-wise comparisons
- Sample age at the time of testing should be factored in when comparing stability-indicating attributes







Comprehensive analytical similarity assessment reduces the degree of uncertainty



Whole protein MS
HC and LC MS
peptide map
Disulfide structure
Glycan map
Isoelectric point
Extinction coefficient
Immuno-based
Identity

Attributes related to the amino acid sequence and all post-translational modifications including glycans

Biological and functional activities, including receptor binding and immunochemical properties

Potency assay
Antigen binding
Effector functions (ADCC/CDC)
Fc receptor family (FcγR) binding activities
Neonatal Fc receptor (FcRn)
Other MoA

Integrity of the secondary, tertiary, and quaternary structure

FTIR
near UV CD
DSC

Quantitative levels of product variants and their identities

SE-HPLC
CEX-HPLC
reduced CE-SDS
non-reduced CE-SDS

Properties of the finished drug product including strength and formulation

Protein concentration
Volume
Osmolality
pH
Appearance
Color
Clarity
polysorbate 20

Degradation profiles denoting stability

Forced degradation
Accelerated stability
Stressed stability

Subvisible and submicron particles, and aggregates of various sizes

HIAC
MFI
DLS
FFF
AUC-SV
SE-HPLC-LS

Impurities from host cells and downstream process

HCP
Protein A
DNA
2D SDS-PAGE
HCP LC/MS/MS

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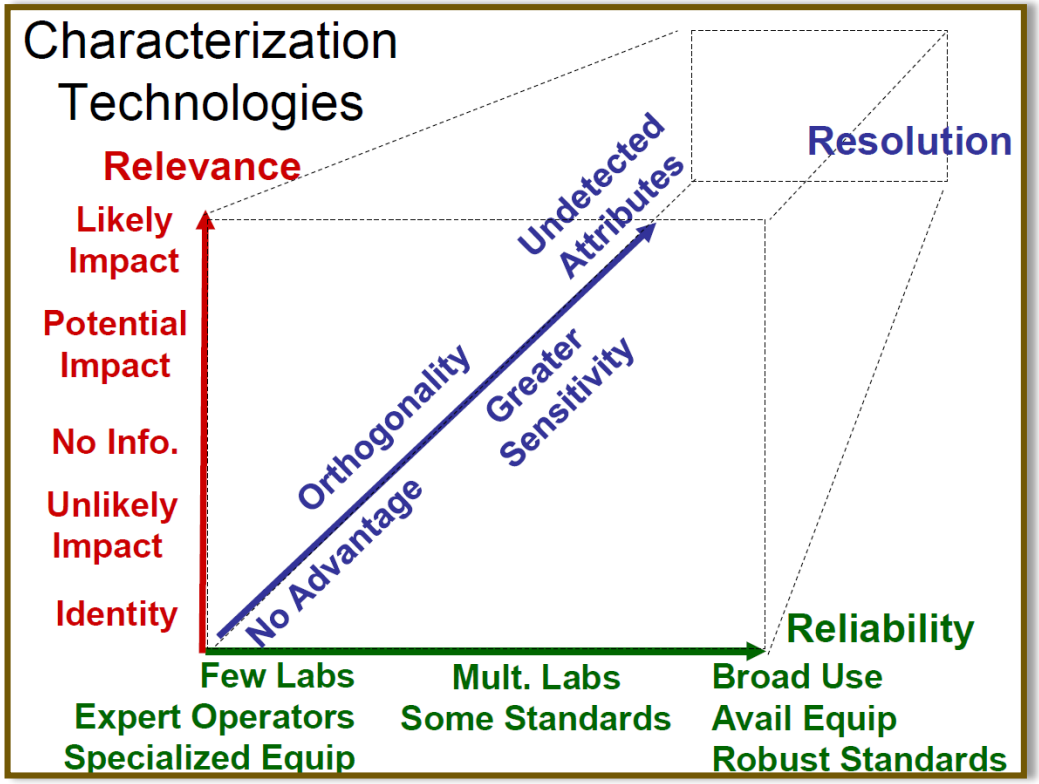
Choice and performance of analytical tools matter

Methods provide meaningful information and may predict clinical performance



Slide from Kozlowski, S (CDER) presentation at 2014 Biomanufacturing Technology Summit, Rockville, MD, June 13, 2014

Methods should are sensitive and capable of resolving differences which are critical attributes

Methods should be qualified and fit-for-use for intended purposes

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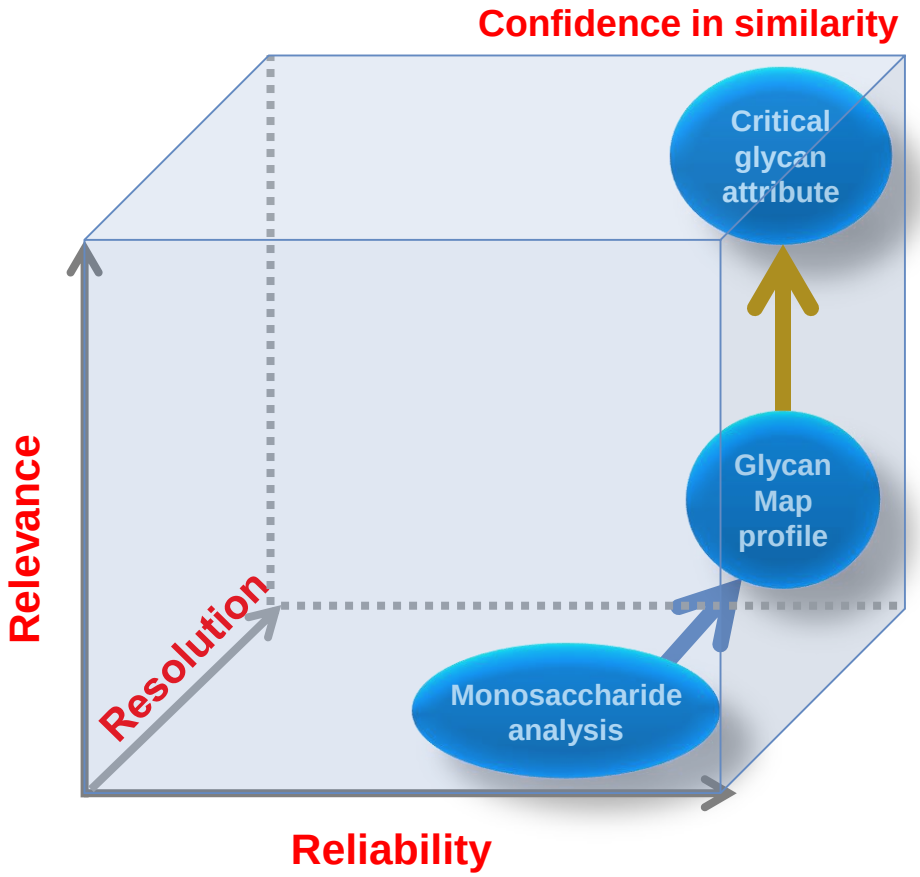
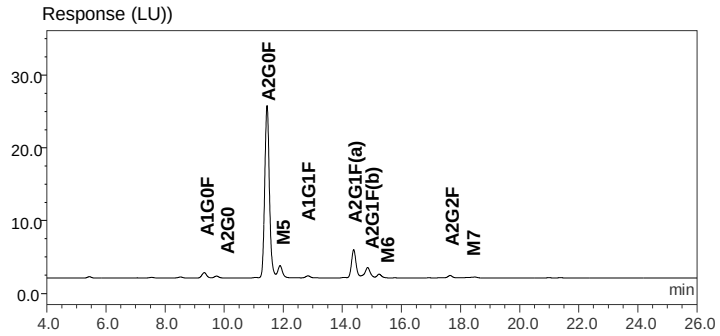
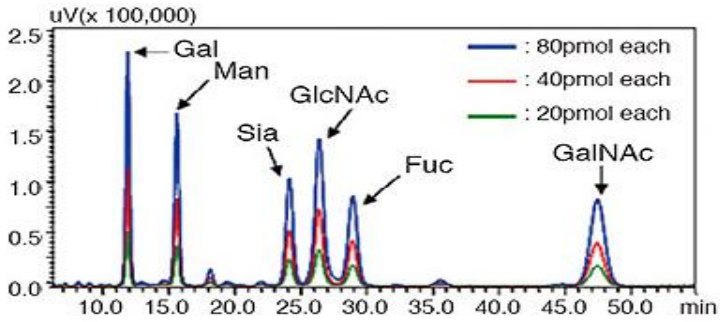
Understand the biological relevance of measured quality attributes are important

Measuring the quality attributes by retaining the important structural characteristics of the attributes

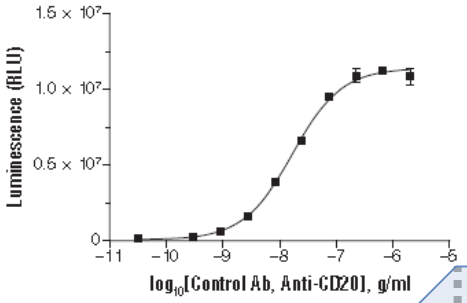
Increase understanding for the biological relevance of different glycan attributes and ensure the critical attributes are controlled properly in order to match biological functions



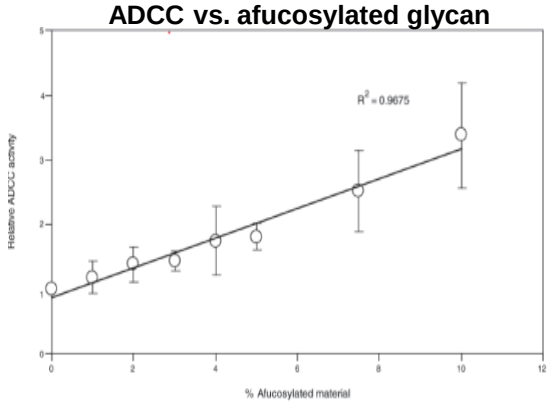




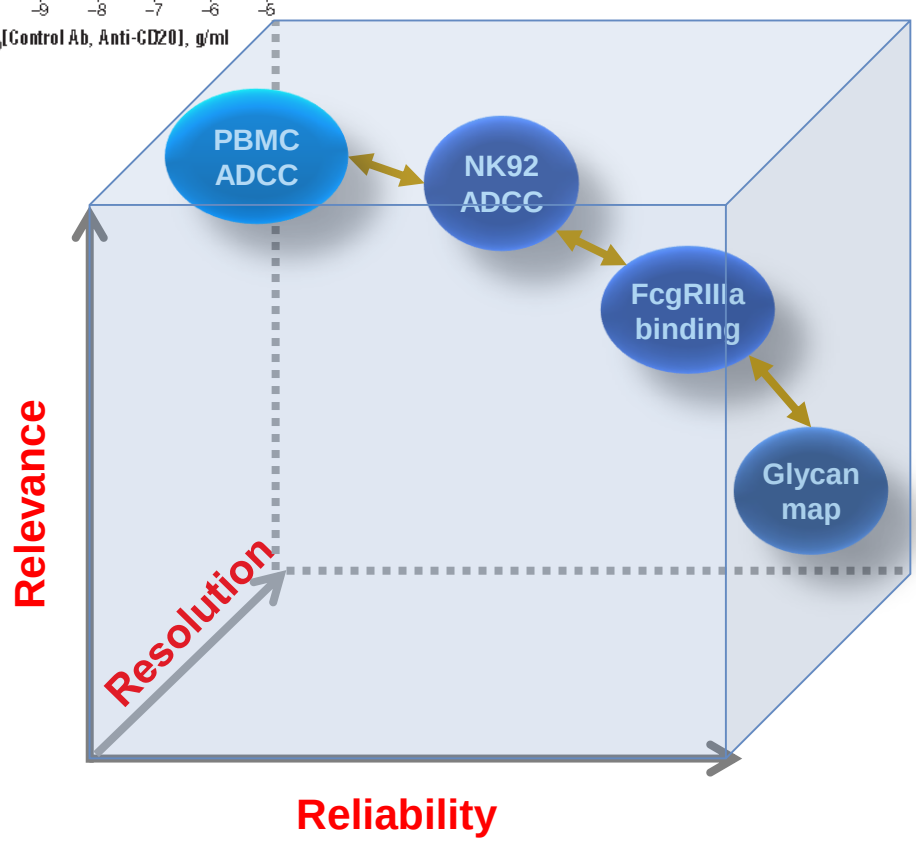
Knowing the correlation of orthogonal methods help predict potential biological impact



Include orthogonal methods with increasing biological relevance to discern clinically meaningful differences and confirm functional similarity

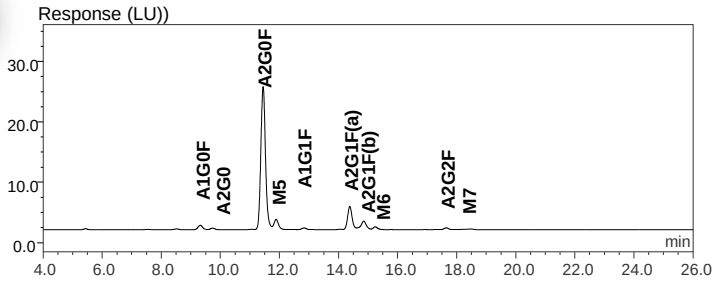


Chung, mAbs, 326-340, May 2012



Use high resolution and reliable methods to guide the process and product development.

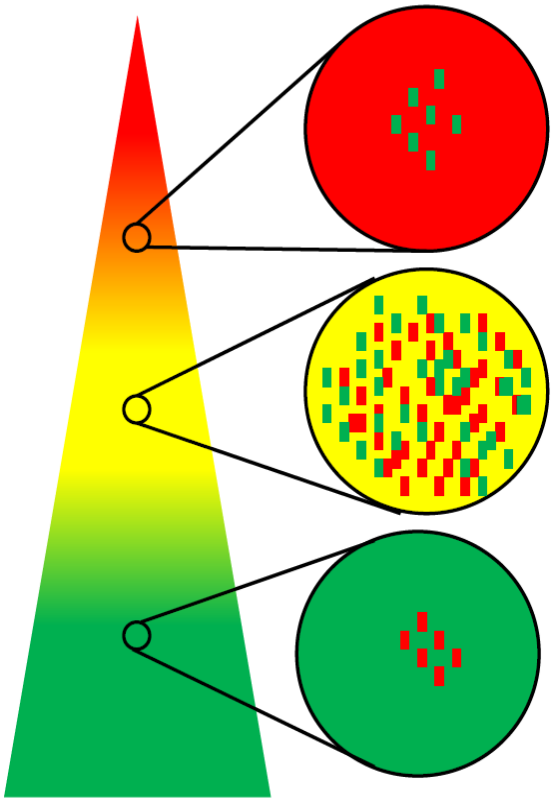
Better suited for process control purpose

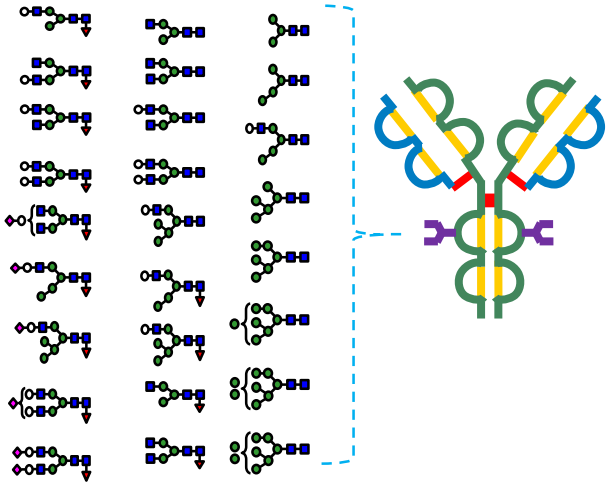


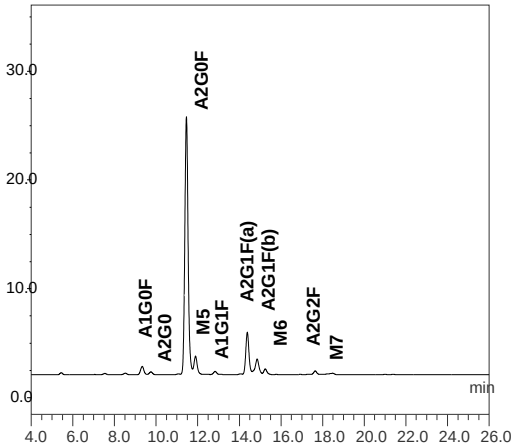
Analytical methods should have sufficient resolution and capable of measuring critical quality attributes

The biological relevance and importance may not be in proportion to its relative abundance.

The devil is in the detail







Glycan Type	Impact to function	Sensitivity to difference
No glycan	No ADCC	Low to medium
Bisecting GN	Increase ADCC	Low to medium
High mannose	Clearance and ADCC	Medium
Terminal Gal	Increase CDC	Low
Sialic acid	Anti-inflammatory and ADCC	Low
Afucosylated	Increase ADCC	High

Adapted from Kozlowski, S (CDER) presentation at CASSS WCBP, Washington DC, January 31, 2013

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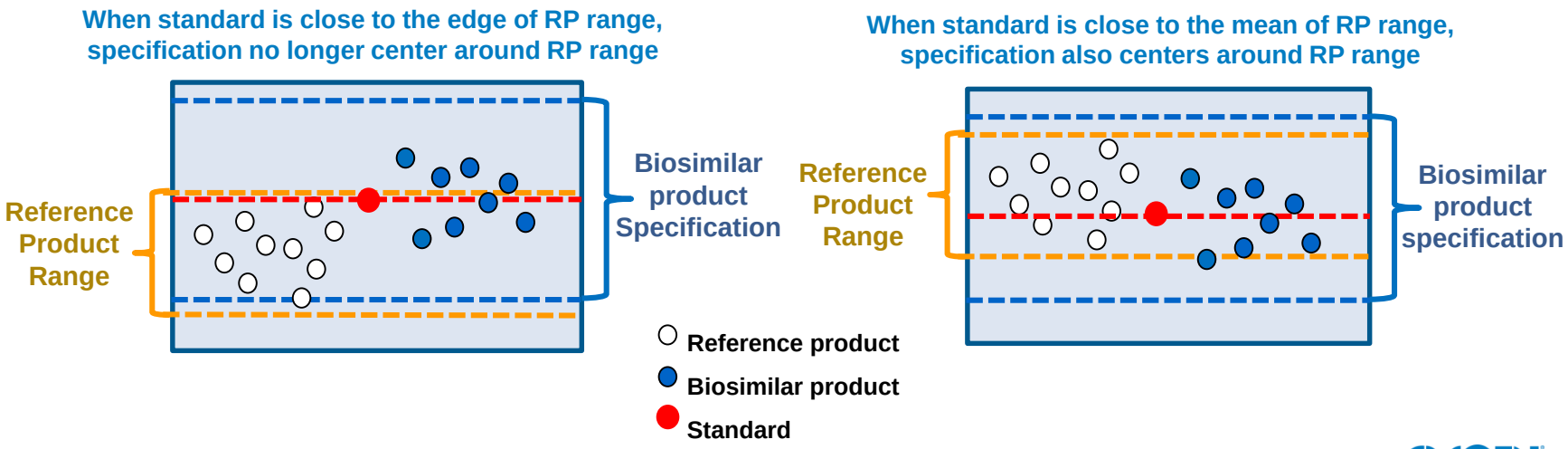
Management of standards used in biological assays is important to ensure reliability

- Selection of standards for biological assays for reporting relative activities should represent reference product
- Ideally the same standard should be used across orthogonal biological assays
- Bridging of different standards to allow pooling of results
- Proper system suitability and assay controls should be well described in procedures, including criteria for passing and failing results
- Methods should be qualified and shown to be fit for purpose

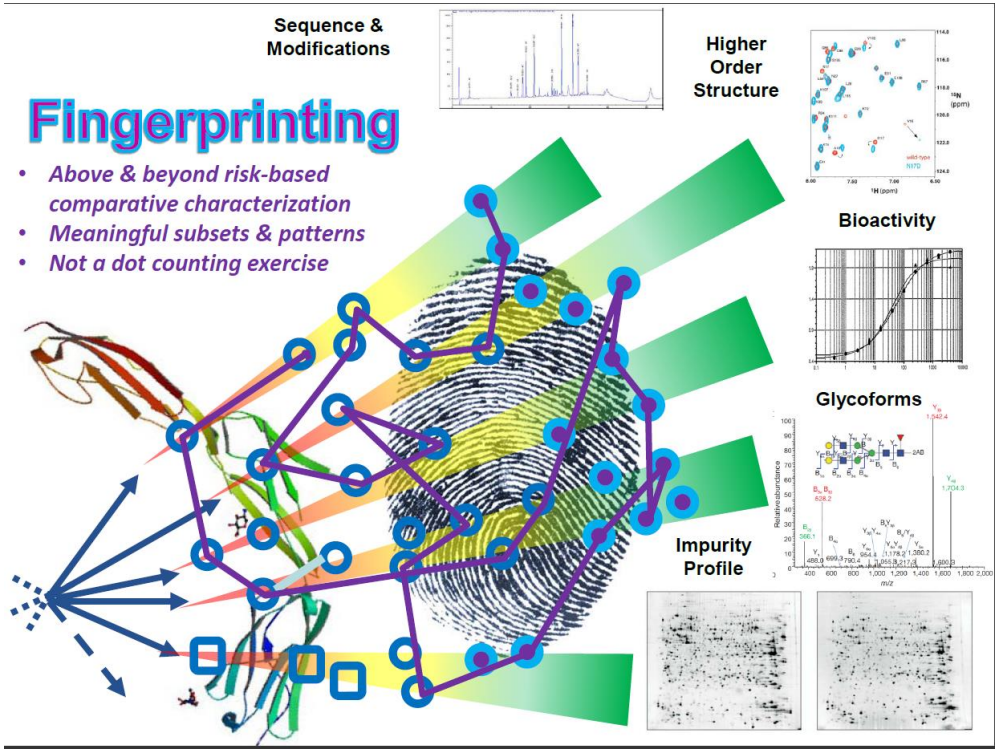


Fingerprint-like similarity versus Fingerprint-like methods

FDA Definition of Fingerprint-like:

a term to describe integrated, multi-parameter approaches that are extremely sensitive in identifying analytical differences.

FDA DRAFT GUIDANCE
"Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product"



Koslowski, presentation at CASSS WCBP Conference, Washington DC, Jan, 31 2013

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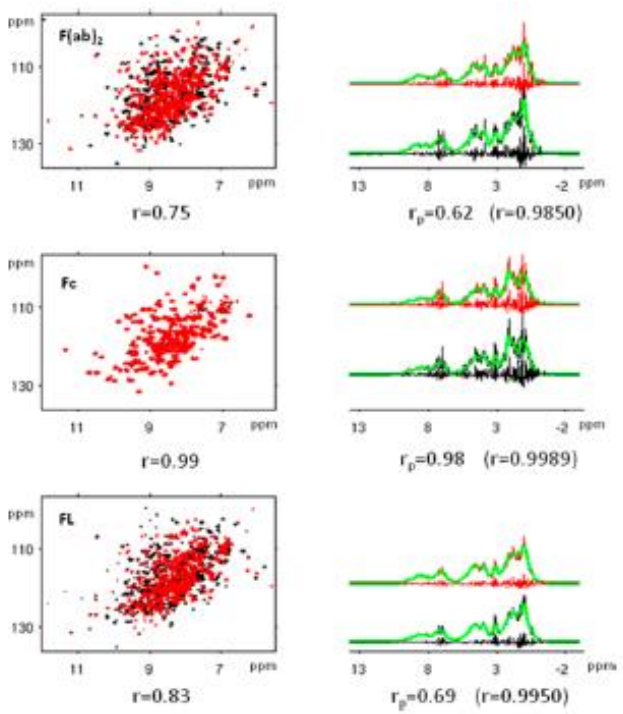
Analytical methods which may provide fingerprint-like profiles

- Methods investigate the overall conformational integrity
  - 1-D NMR
  - 2-D NMR
  - Crystallography
  - Antibody conformation array
  - H/D exchange
- Methods investigate the heterogeneous characteristics
  - Peptide map
  - Glycan map

Can anyone of these methods demonstrate fingerprint-like similarity in its own right?

^{1}H NMR provides fingerprint pattern

- Applications for monoclonal antibodies and less complex proteins have been shown
- Visual profile comparison could be subjective. An objective comparison for spectrum similarity would require complex mathematical algorithm



L. Poppe, Anal. Chem. 2013, 85, 9623-9629

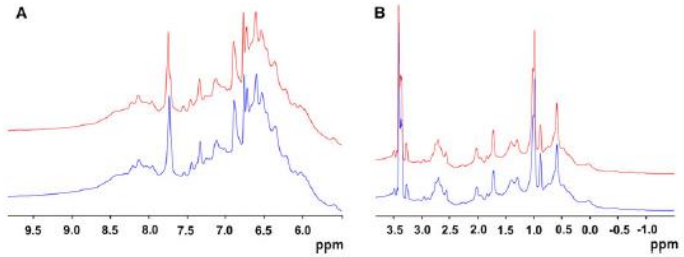
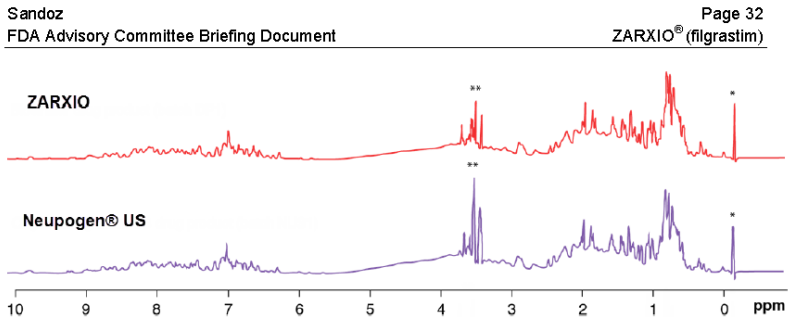


Fig. 2 Comparison of 1D ^{1}H NMR spectra of GP2013 and originator rituximab. *Blue line* GP2013; *red line* originator rituximab. **a** Zoom of the amide region of the NMR spectra. **b** Zoom of the aliphatic region of the NMR spectra

V. Visser et. al, BioDrugs, 07 May 2013



1D-{1H}-nuclear magnetic resonance (NMR) spectra of ZARXIO, Neupogen US and Neupogen EU. * indicates d4-TSP signal; ** signals between 3.4 and 3.9 ppm correspond to formulation components, i.e. are not protein related. d4-TSP = 2,2,3,3-d4 sodium 3-(trimethylsilyl)propionate.

Similarity is assessed based on pattern similarity and little information can be discerned on the differences and their impact to purity, safety, and efficacy

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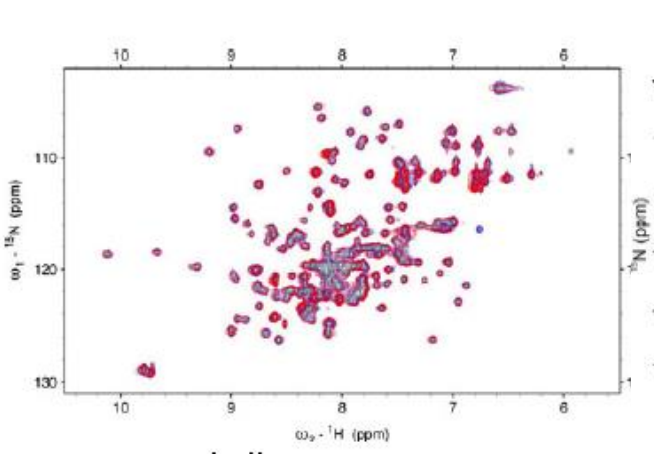
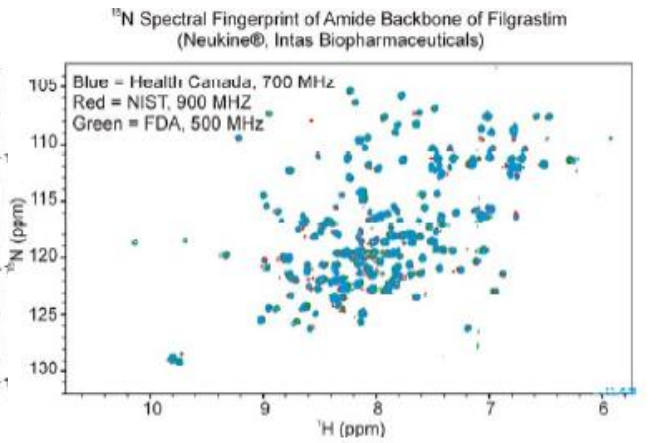
2D-NMR has been applied for smaller non-glycosylated protein

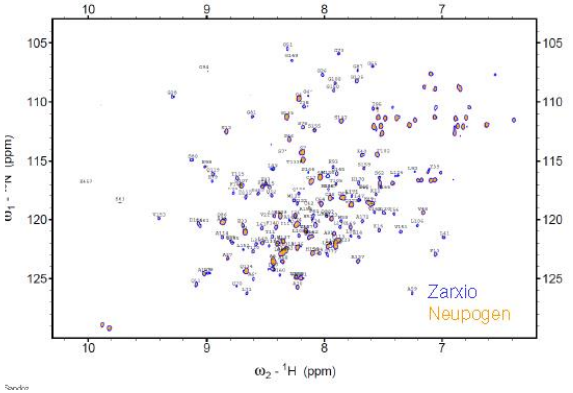
- Several publications on filgrastim (small non-glycosylated protein of ~ 19,000 Dalton) 1H-15N 2D-NMR spectral (pattern) similarity
- Provides higher resolution in structural information compared to 1D-NMR down to the single amino acid level



M. Levy, Anal. Bioanal. Chem. 22 Nov 2013



R. Brinson, AAPS, 2013
D. Hodgson, WCBP, 2012



Sandoz presentation to ODAC, 07 Jan. 2015

May not be suitable for glycoprotein and monoclonal antibodies which is structurally more complex than filgrastim

Crystallography has been applied to investigate sub-domains of large protein

- Typically require changing formulation to allow crystallization of protein of sub-domains of mAb
- Crystallization process generally serves as a purification step selecting the most “homogeneous” population, and could miss low abundant sub-populations

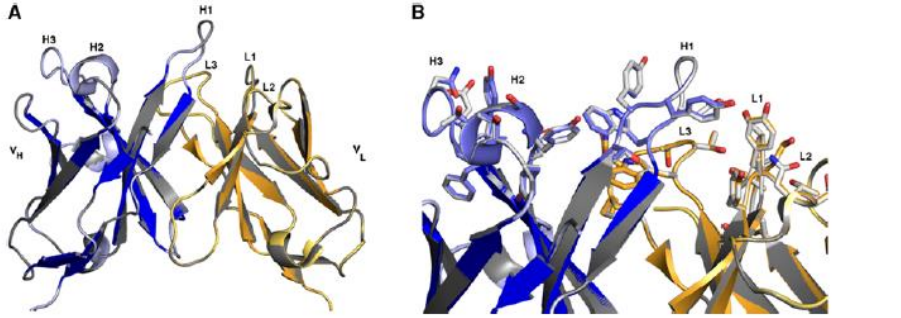


Fig. 3 Super positioned view on the complementarity determining regions (CDRs) of GP2013 and originator rituximab. **a** Ribbon representation of the CDRs. The heavy chain and light chain structures of Fab GP2013 are colored in *blue* and *orange*, respectively. The structure of Fab originator rituximab is colored in *gray*. **b** Ribbon representation of the CDRs with the side chains shown as a stick model, colored according to the chemical atom type (C GP2013 in *blue* and *orange*, C MabThera® in *gray*, O in *red*, and N in *blue*). CDR loops are labeled

V. Visser et. al, BioDrugs, 07 May 2013

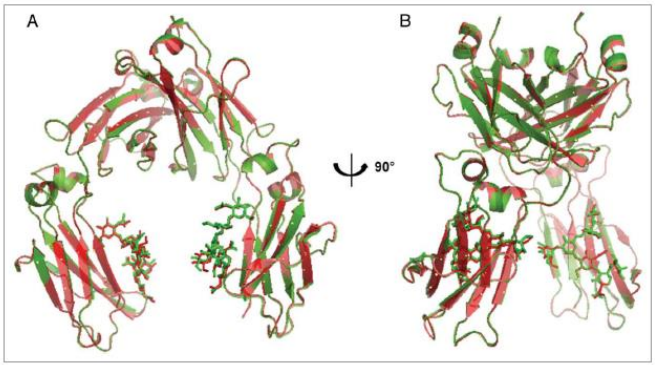


Figure 5. Superimposition of CT-P13 Fc (green) and RMP Fc (red) crystal structures: (**A**) front view; (**B**) side view.

S. K. Jung, mAbs 6:5, 1163--1177; September/October 2014

Does not investigate heterogeneous populations or discern sub-populations which may have different biological activities

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H/D Exchange provides site-specific information on structural differences

- H/D exchange require long experimental time with multiple testing time points.
- Investigate differences in the exchange rate comparing two products under the same condition. Results could be informative in regards to detailed structural differences at specific locations

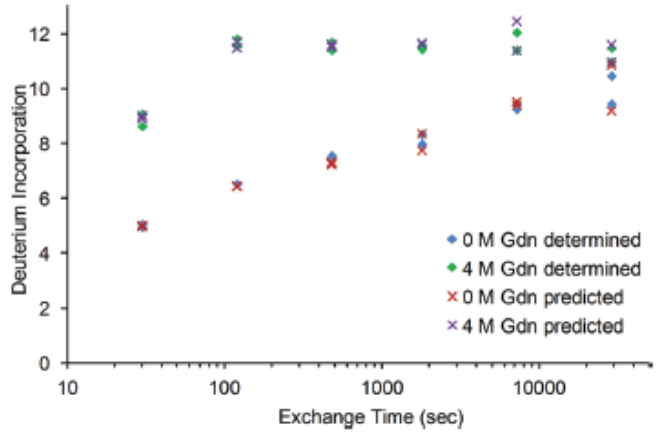
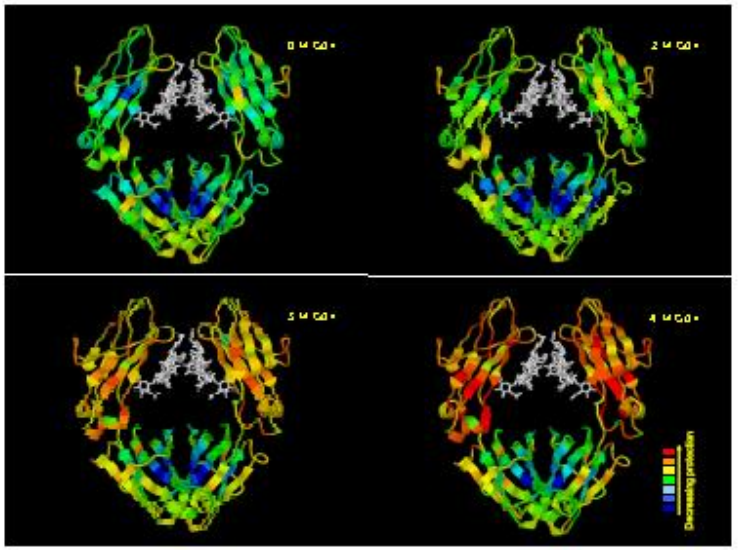


Figure 4. Comparison of experimentally determined deuterium levels and levels predicted by the model for the heavy-chain peptide 1-22 at 0 and 4 M of guanidine.

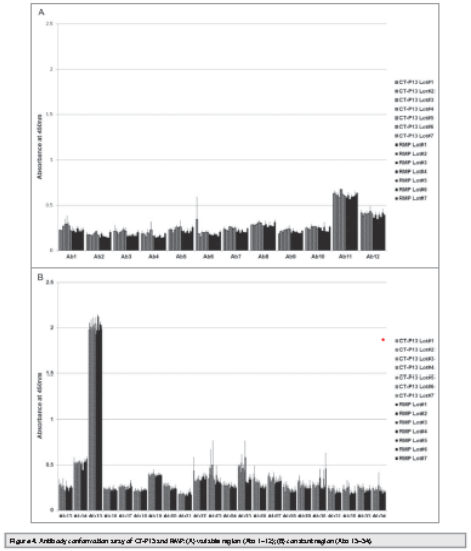


Z. Zhang **Anal. Chem. 2012, 84, 4942−4949**

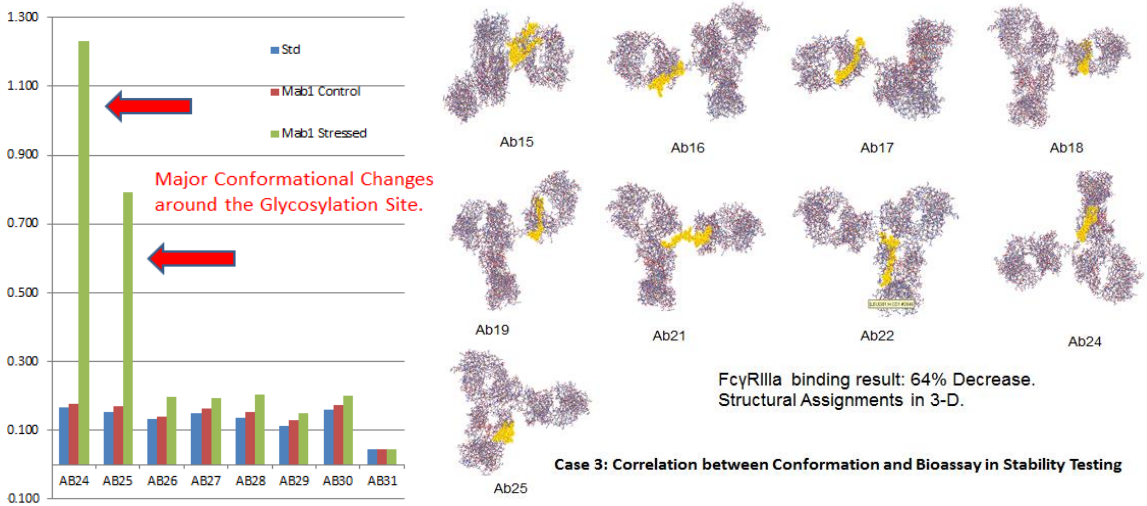
Interpretation of complex results requires experienced subject matter experts

Antibody conformation array could identify new epitopes due to changes in conformation

- Evidences of regional conformational change may predict impact to biological activities mediated through specific antigen or receptor recognition
- Reagents maybe high cost and experiment is relatively low throughput



S. K. Jung, mAbs 6:5, 1163--1177; September/October 2014



X. Wang, CHI's 6th Annual Biotherapeutics Analytical Summit Comparability for Biologics & Biosimilars March 13, 2015

Orthogonal to biological assays in investigating structure/functional differences

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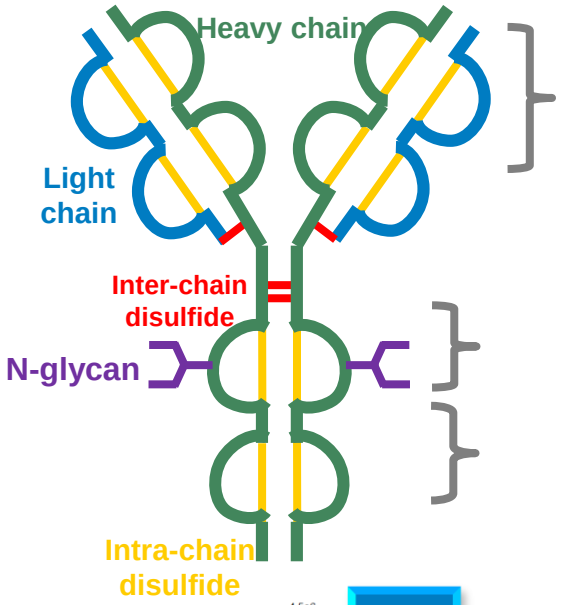
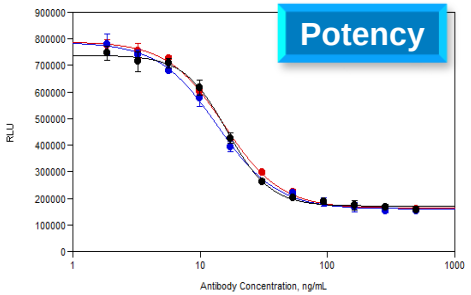
Capabilities and gaps of the fingerprinting methods

Technique	Capabilities	Gaps
1D-[1H]-NMR	Sensitive to detect differences for both small and large proteins including monoclonal antibodies	Difficult to interpret results and primarily rely on pattern similarity. May not determine specific site and levels of differences
2D-[1H-15N] HSQC NMR	Sensitive to detecting differences down to amino acid levels for small proteins	May not be suitable for complex biologics, such as glycoprotein and monoclonal antibodies
X-ray crystallography	Primary application for smaller protein or fragments of monoclonal antibody. Assess sub-populations which are homogenous in nature	Limited resolution to structural differences for large protein. May omit minor components with unknown safety or efficacy impact.
H/D exchange	Provide detailed structural differences at specific locations	Long experimental time. Interpretation of complex results requires experienced subject matter experts
Ab conformational array	Data analysis is less complex compared to the other fingerprinting methods. Provides regional structural information	Require product-specific reagents which could be costly or may not be available

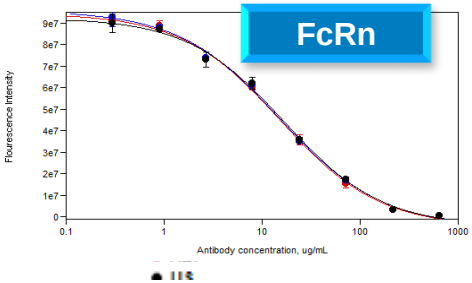
Biological assays can be more sensitive to structural and conformational changes



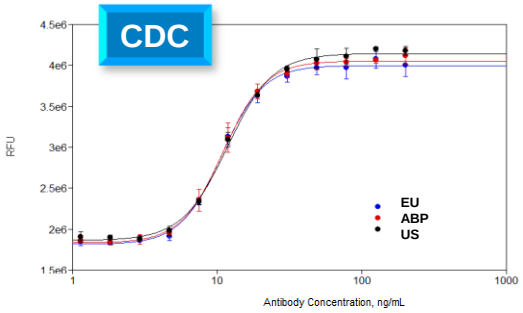
Potency Reflective of Target Binding and Mechanism of Action

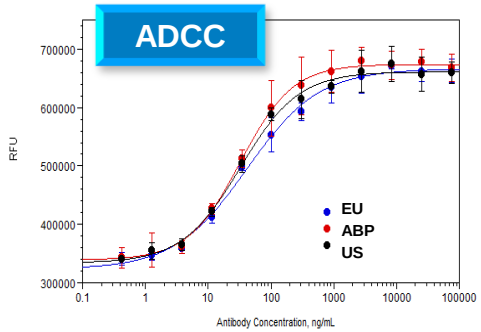


Binding Region for FcRn (PK)



Binding Region for FcγR, Effector Function (CDC, ADCC)





Biological and functional assays are used to investigate structure/functional differences which are clinically meaningful for biosimilarity

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Summary

- Analytical tools used in biosimilar development are critical and should be fit-for-purpose
- FDA definition of “fingerprint-like” is a term to describe integrated, multi-parameter approaches that are extremely sensitive in identifying analytical differences
- There is no one method alone capable of providing “fingerprint-like” structure.
- Biological assays are essential to confirm structural/functional integrity

Acknowledgements

Leszek Poppe

Pavel Bondarenko

Zhongqi Zhang

John Gabrielson

Brad Jordan

Izydor Apostol

Linda Narhi