Scalability and Manufacturing of Biosimilars

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Some of the common challenges for Biosimilar Developers

1. **Not having a lineally scalable equipments** (e.g. bioreactors of different configurations)

2. **Not having the right clones** (Biosimilarity not given proper emphasis during clone selection).

3. **Enzymatic and Process-induced product modifications** (Clips, Oxidation, Reduction).

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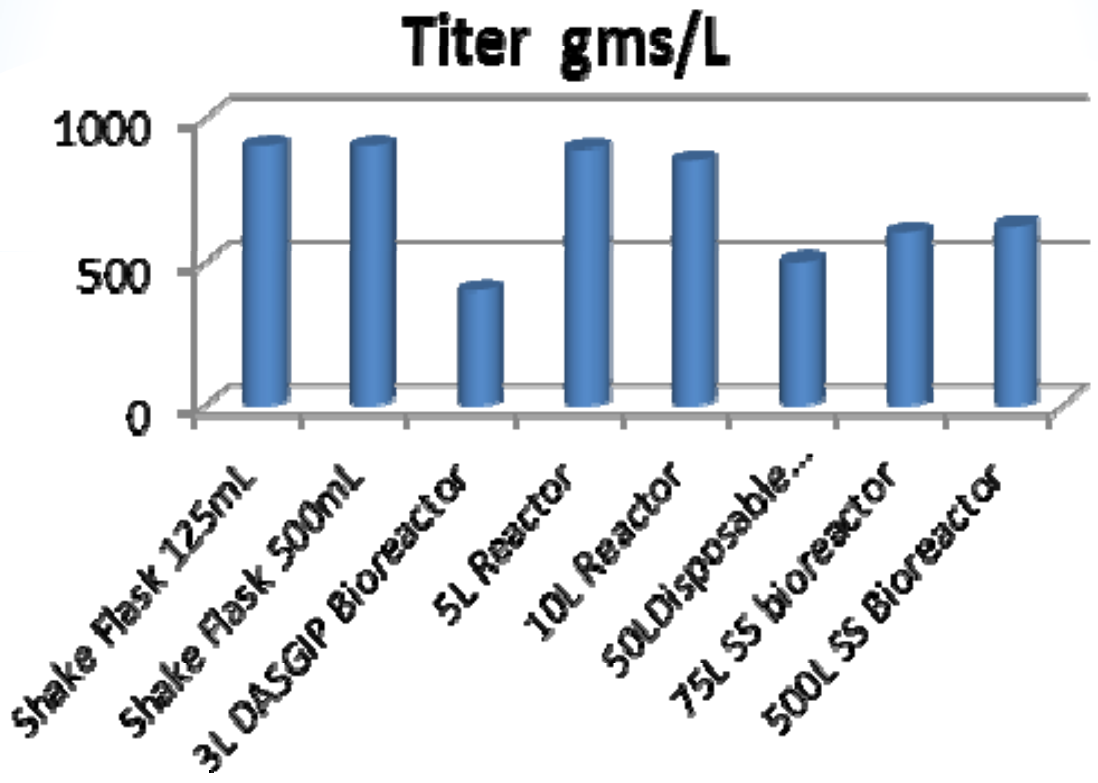
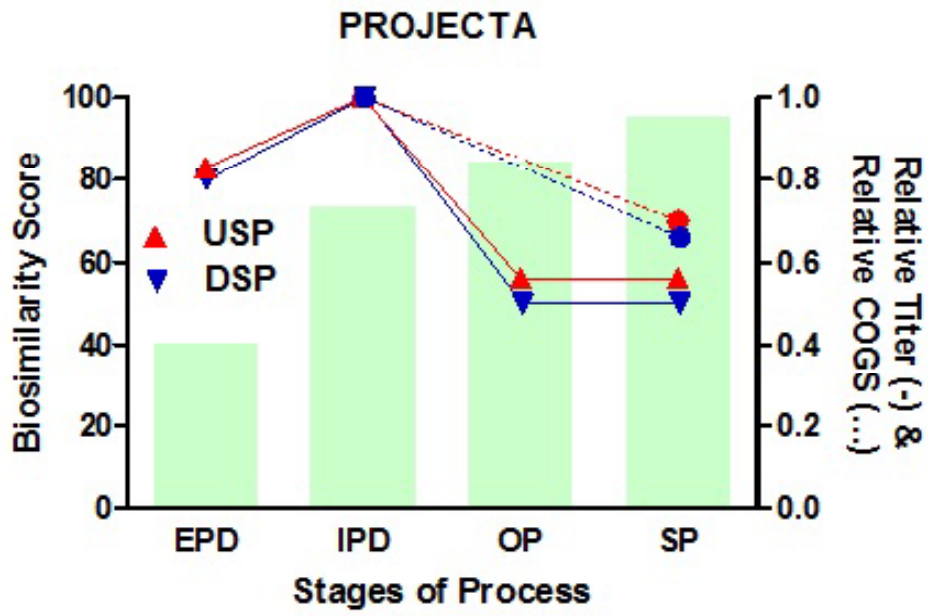
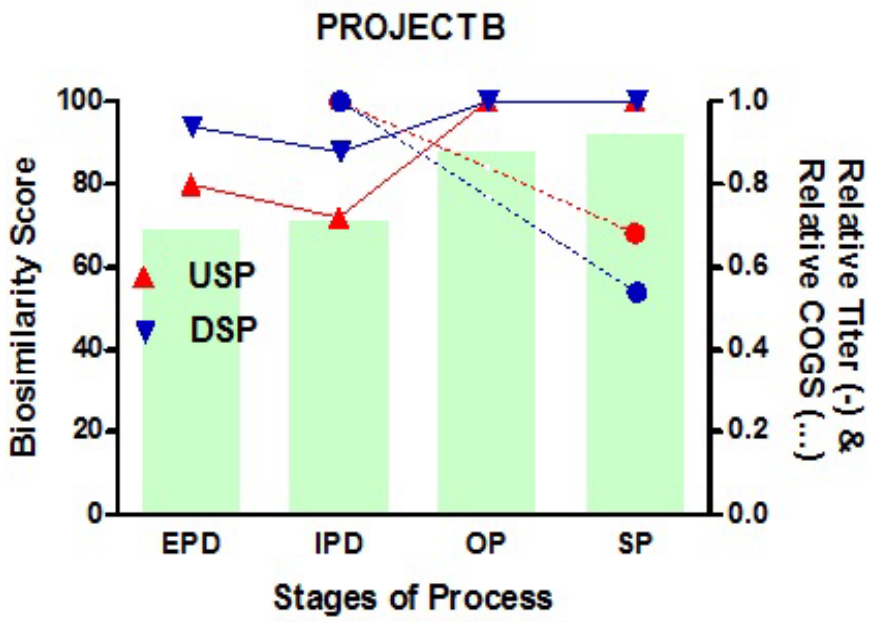
1. Not having linearly scalable equipments



2. Not having the right clone





EPD: Early Stage Process Development
IPD: Intermediate Stage Process Dev.
OP: Optimized Process
SP: Scaled-up Process

Shake Flask 125mL

Shake Flask 500mL

Glass Bioreactor 3L/5L/10L

Bioreactor 75L

SS Bioreactor 500L

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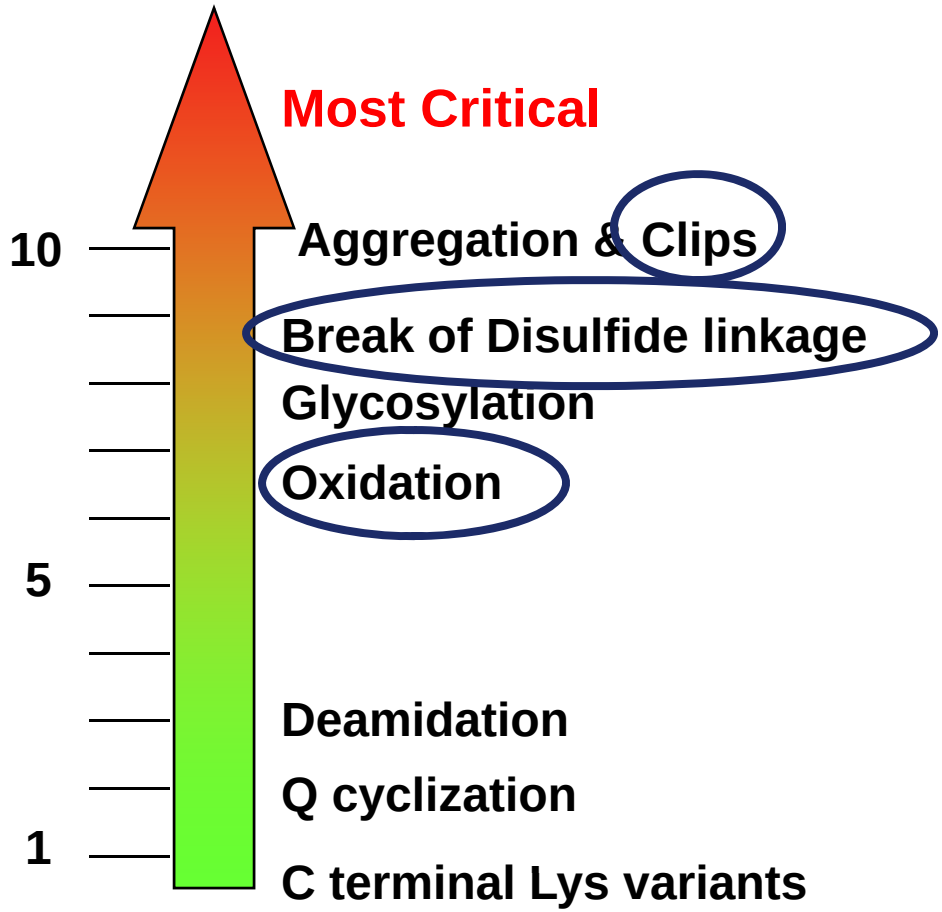
Control of Quality through process changes

Quality Attributes	% Change in levels through process development	Process Optimization in
Afucosylation	4.2 X	Upstream Process
Galactose index	2.7 X	
Man5	4.5 X	
Sialic acid	-	
Methionine Oxidation	10 X	Downstream Process
Clips/Truncations	20 X	
Aggregation	20 X	

Selecting the right clone is the key!!

Risk-based development strategy for monoclonal antibodies



3. Enzymatic and Process-induced product modifications (Clips, Oxidation, Reduction).

A. Proteolysis of target protein

B. Methionine Oxidation in mAbs

C. Reduction of disulfide bonds

A. Proteolysis of Target Protein

Process-born Enzymatic Modifications –

1. **Proteolysis**
2. **Deglycosylation**
3. **Desialyation**

- Proteolytic activity is detectable in CHO based products in the range of 1-5 ng Proteinase K equivalent/ml which can be demonstrated by use of protease inhibitors.
- Level of Protease activity varies with the method used for cell separation (centrifugation / filtration) and harvest conditions (pH, co-factors).

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Reports on Proteolysis of Biologics

Fragmentation of a Highly Purified Monoclonal Antibody Attributed to Residual CHO Cell Protease Activity

Sharon X. Gao,[1] Ying Zhang,[1] Kensey Stansberry-Perkins,[1] Alex Buko,[1] Shujun Bai, Vanessa Nguyen, Mark L. Brader Biotechnology and Bioengineering, Vol. 108, No. 4, April, 2011

[1]Department of Analytical Biochemistry, Biogen Idec, 5200 Research Place, San Diego, California; telephone: +1-858-401-8265; fax: 858-401-5031; e-mail: sharon.gao@biogenidec.com

Degradation of an Fc-Fusion Recombinant Protein by Host Cell Proteases: Identification of a CHO Cathepsin D Protease

Biotechnology and Bioengineering, Vol. 108, No. 4, April, 2011

Flavie Robert,[1] Horst Bierau,[2] Mara Rossi,[2] David Agugiaro,[2] Thomas Soranzo,[3] Hervé Broly,[1] Christine Mitchell-Logean[1]

[1]Biotech Process Sciences, Merck Serono Biotech Center,

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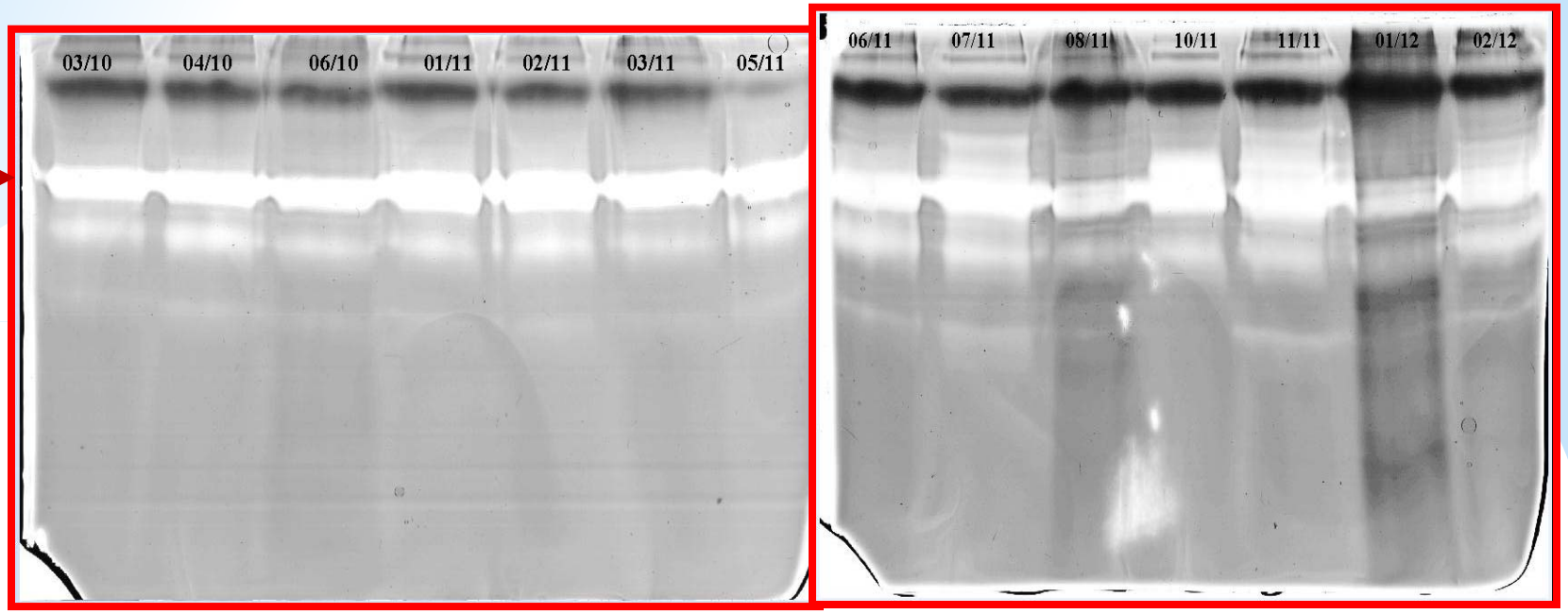
Metaloproteases are responsible!

1. Mapping of the proteolytic activities released by CHO K1 & DG44 cell lines show metalloproteinase
2. Proteinases were activated by Aminophenyl mercuric acetate - a MMP activator
3. SDS-PAGE and N terminal sequencing confirmed the presence of MMP3, MMP10 and MMP12 which showed significant homologies.
5. Mammalian MMPs are in the MW range 17-800 kDa.
6. Predominantly 92 kDa MMP9 was produced which acts on various substrates – gelatin & proteoglycans

Gelatin Zymograms of Harvests

92kDa
MMP9



Various Bioreactor conditions were tried such as different media, feed, tempearature, pH & osmolality, did not affect the expression of proteases

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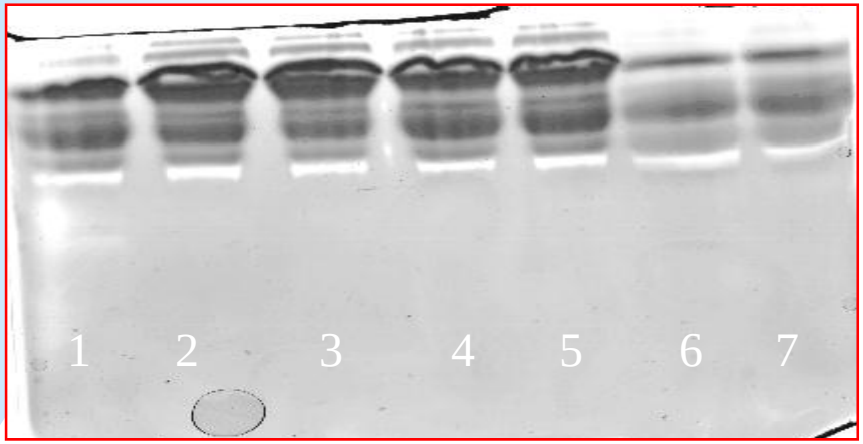
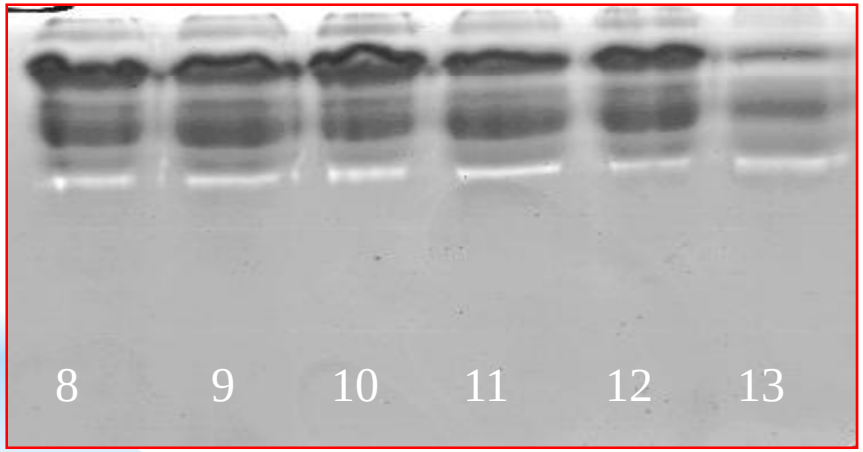
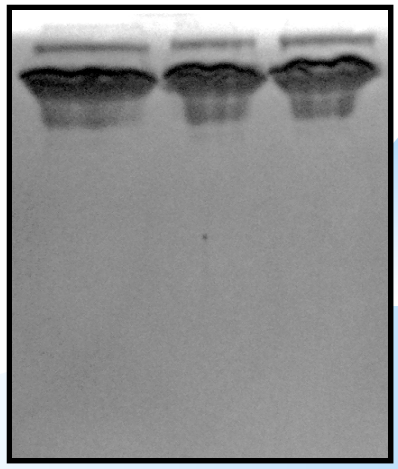
Gelatin Zymograms of Protein A eluates after column wash with different conditions





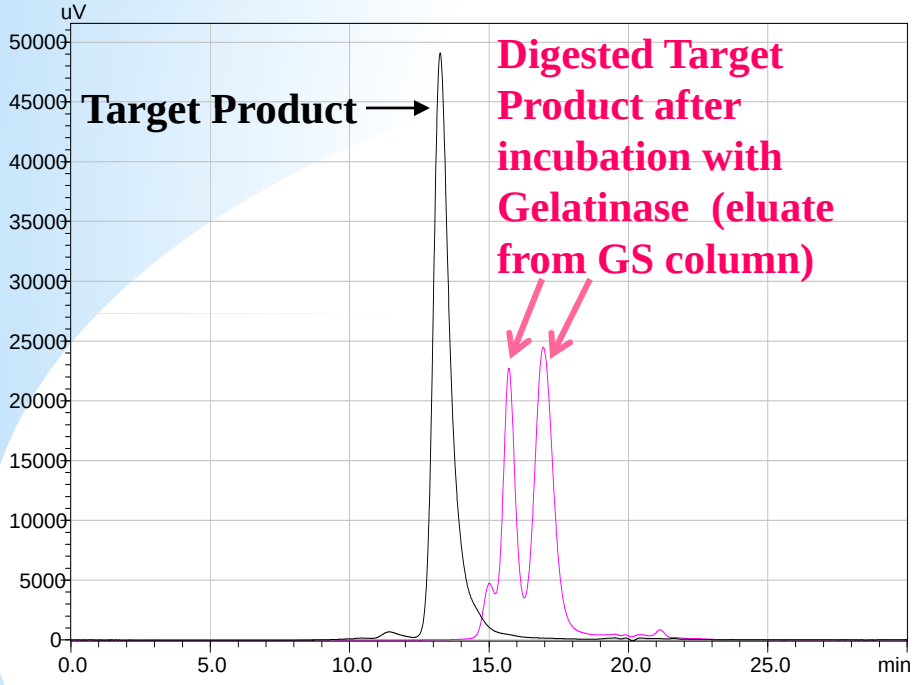
Lane 1:10% Propylene glycol wash
Lane 2: 0.5 M glycine
Lane 3: 0.5 M arginine HCl
Lane 4: 0.5 M GdnHCl
Lane 5: 1 M Calcium chloride
Lane 6: 0.5 M sodium sulfate
Lane 7: 1 M NaCl

Lane 8: 50 mM Sodium citatrate, 5% Ethanol
Lane 9: 50 mM Sodium citrate, pH 4.5
Lane 10: 1 M Magnesium sulfate
Lane 11: 1% Triton X-100
Lane 12: 1% Tween 20
Lane 13: 5% PEG 4000



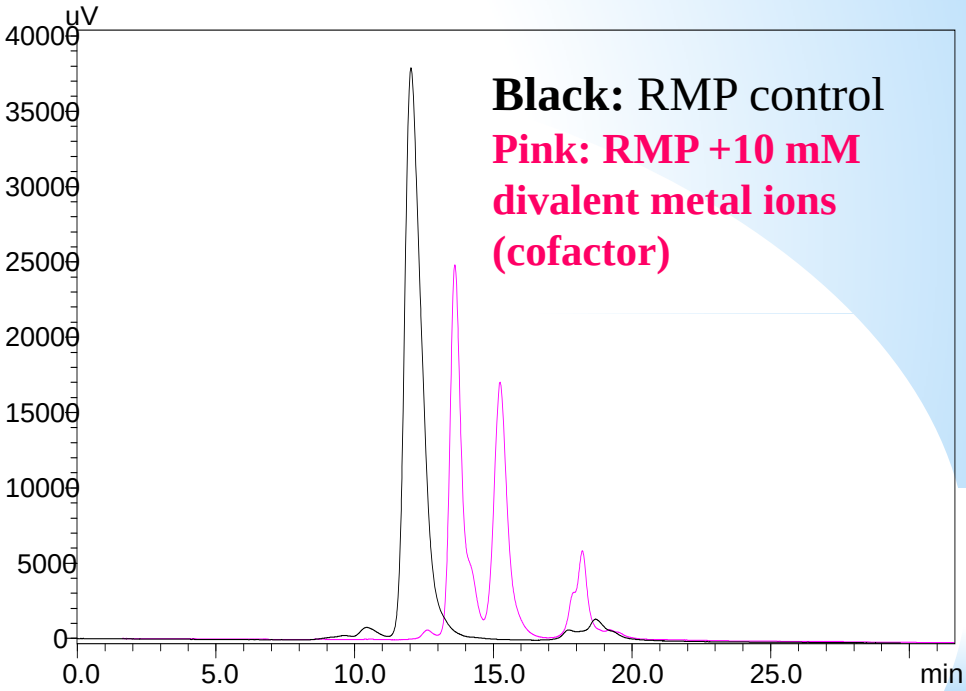
Protein A eluate with Solvent-Detergent-Salt wash
but it was not able to remove all the proteases →

Incubation of Target Product with Purified Gelatinase



SEC HPLC shows complete digestion of monoclonal antibodies at EPKD-G in the hinge region in the presence of Gelatinase

Incubation of RMP with protease cofactor



Complete degradation of RMP was observed when divalent metal ions (cofactor) were added implying that protease are present even in the RMP, but in the inactive form.

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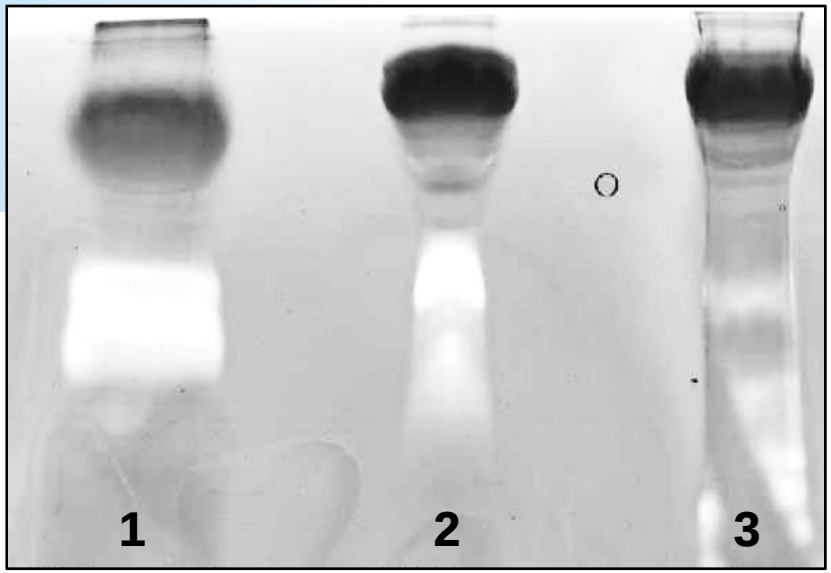
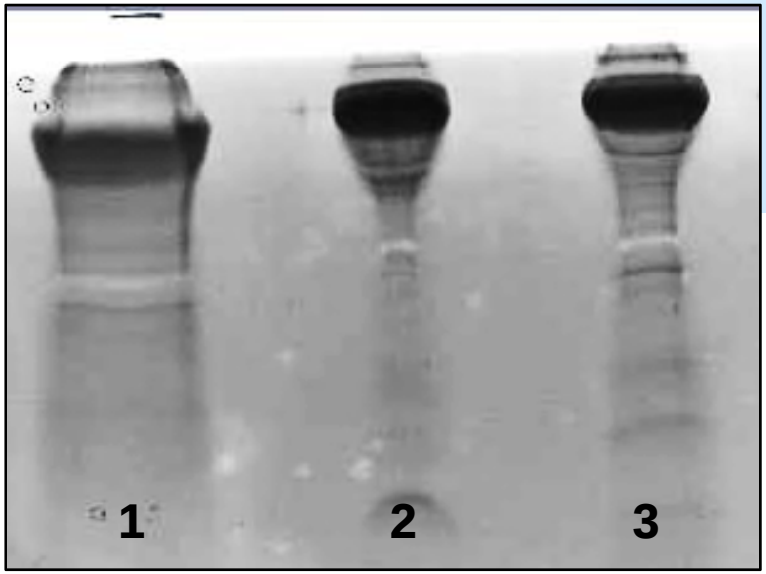
Protease co-expression a common phenomenon



Zymogram of 3 Target Proteins Harvests expressed in CHO K1



Zymogram of Protein A Eluates

Lane 1: Monoclonal antibody-1
Lane 2: Monoclonal antibody-2
Lane 3: Monoclonal antibody-3

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Stability Study

Sample	Temperature	% Purity	% LMW
Drug Substance without removal of proteases	4^0C	95.37	4.11
	25^0C	92.39	7.21
	40^0C	81.82	17.68
DS after protease & metal ion removal	4^0C	95.61	3.91
	25^0C	95.23	4.34
	40^0C	94.73	4.84

Approx. 30 different DSP strategies were tried with only two strategies showing complete removal.

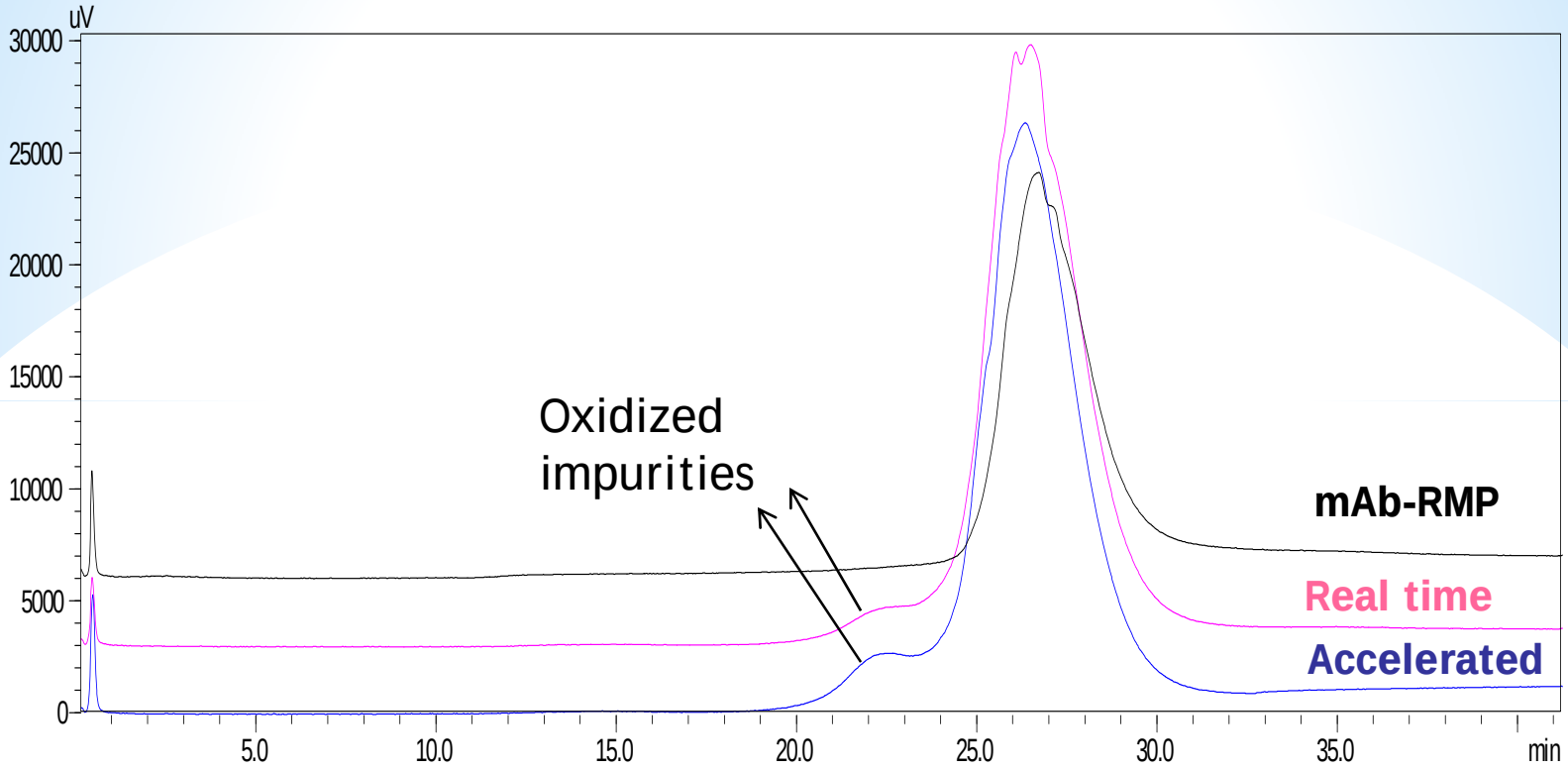
B. Oxidative damage in mAbs

Oxidation & its consequences

* Oxidation of methionine residues in mAbs affect its binding and can lead to changes in the Fc mediated activity and shelf-life.

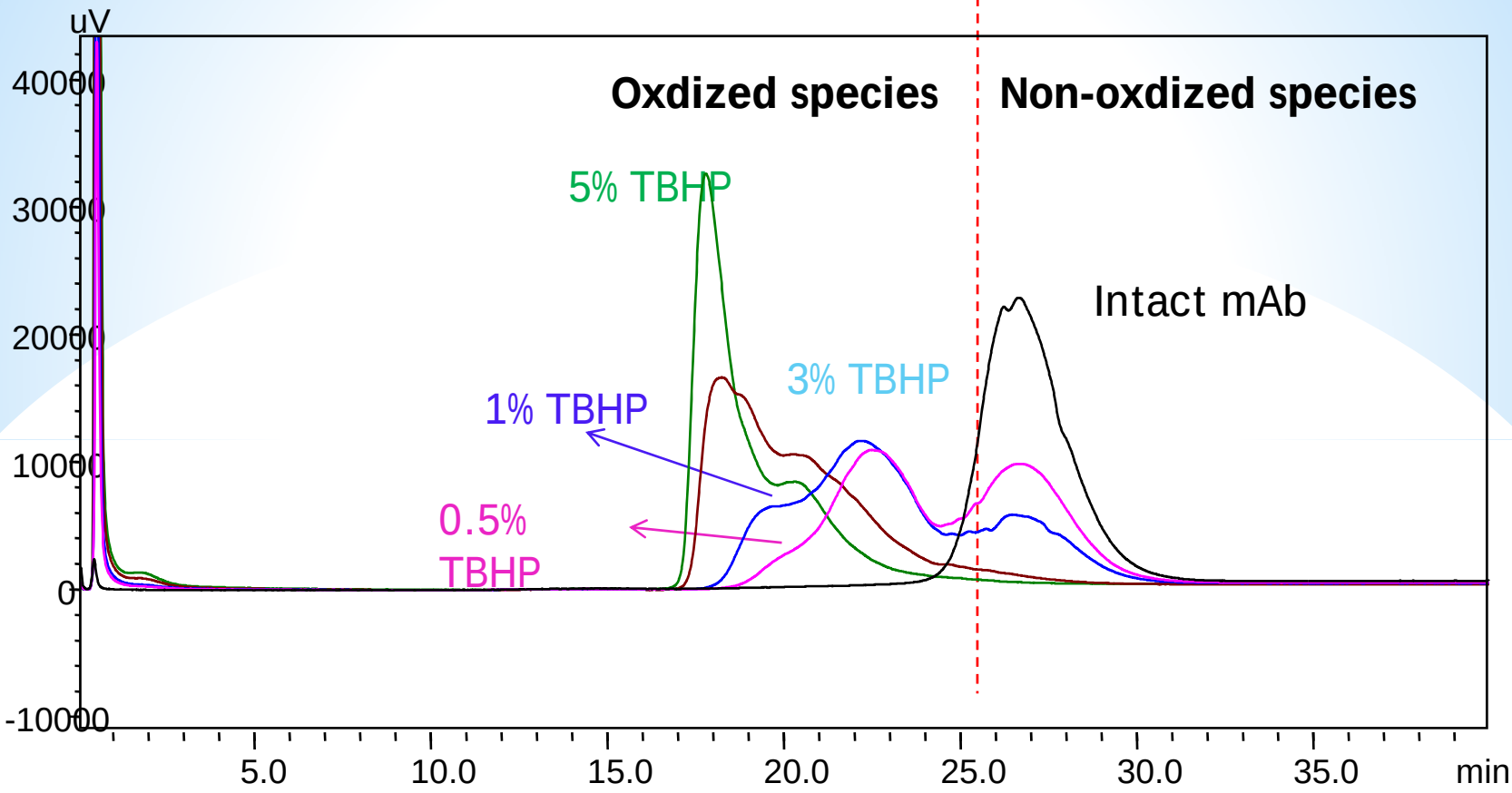
* Two Met residues, Met256 and Met432 are susceptible to oxidation.

Oxidation of mAb on storage



Samples	Oxidized impurities (%)	CDC activity
Zero Time	Nil	100%
Real Time sample (6M)	3.7	69%
Accelerated sample (6M)	6.1	62%

Oxidation states in mAbs



Induction of Oxidation: mAb incubated (37°C for 2 hrs) with different concentrations of TBHP (tert-butyl hydrogen peroxide)

Incubation of mAb with 5% TBHP resulted in complete oxidation of Met256 and Met432, while incubation with 1% TBHP resulted in mixed population of the antibody with different degree of Met oxidation.

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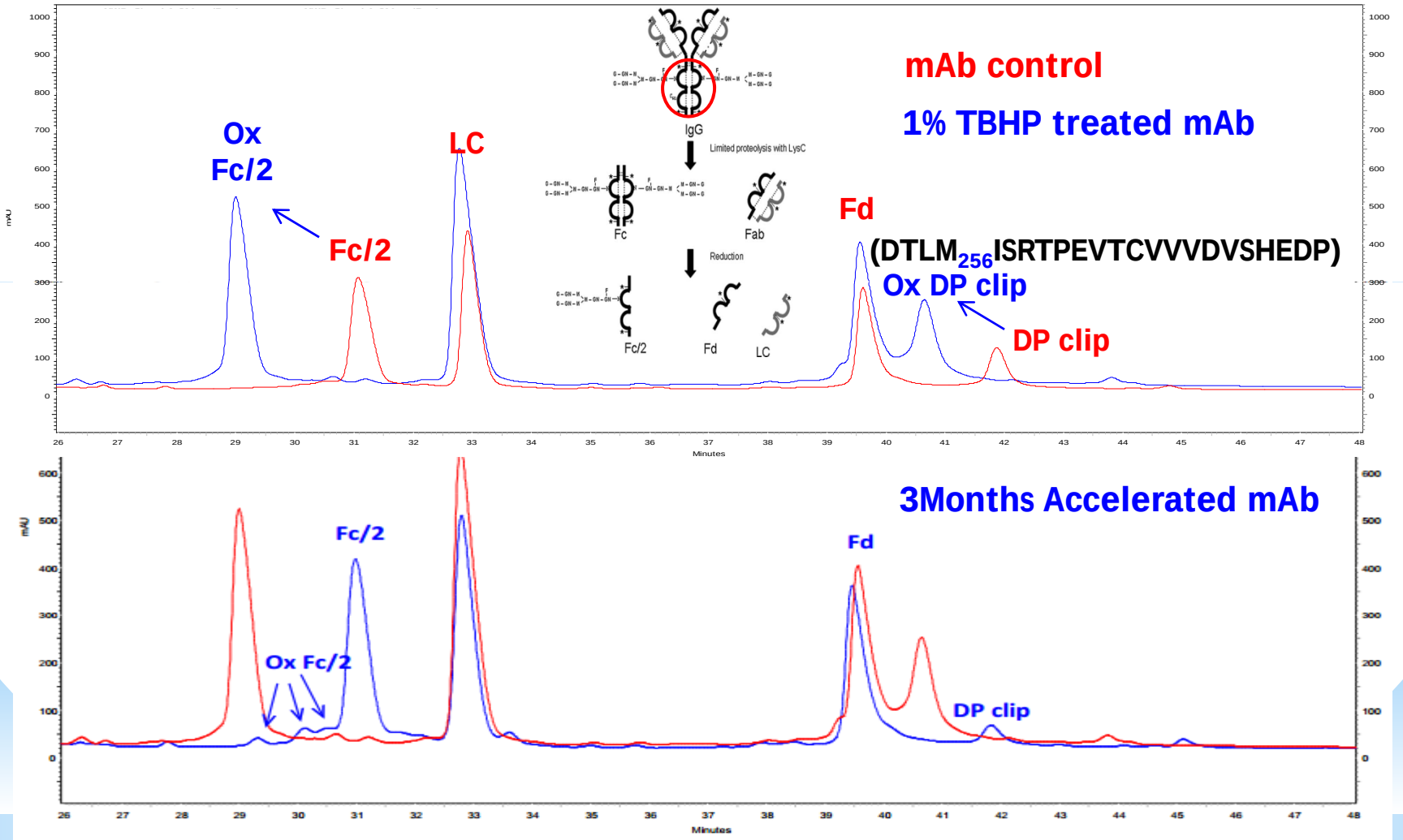
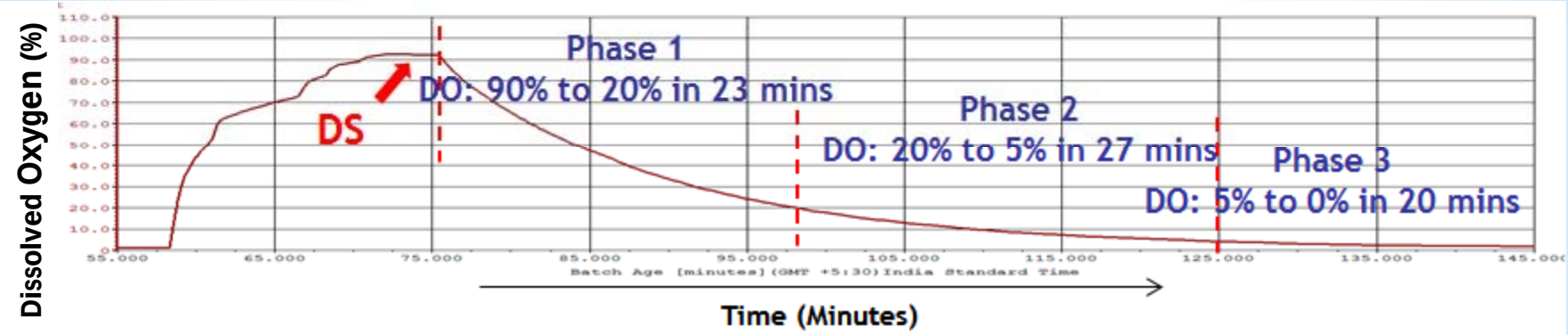
1% TBHP induced oxidation compared with 3M Acc mAb



11.4% Oxidiation of Fc/2 observed in 3M Accelerated sample

Dissolved oxygen induces oxidative damage



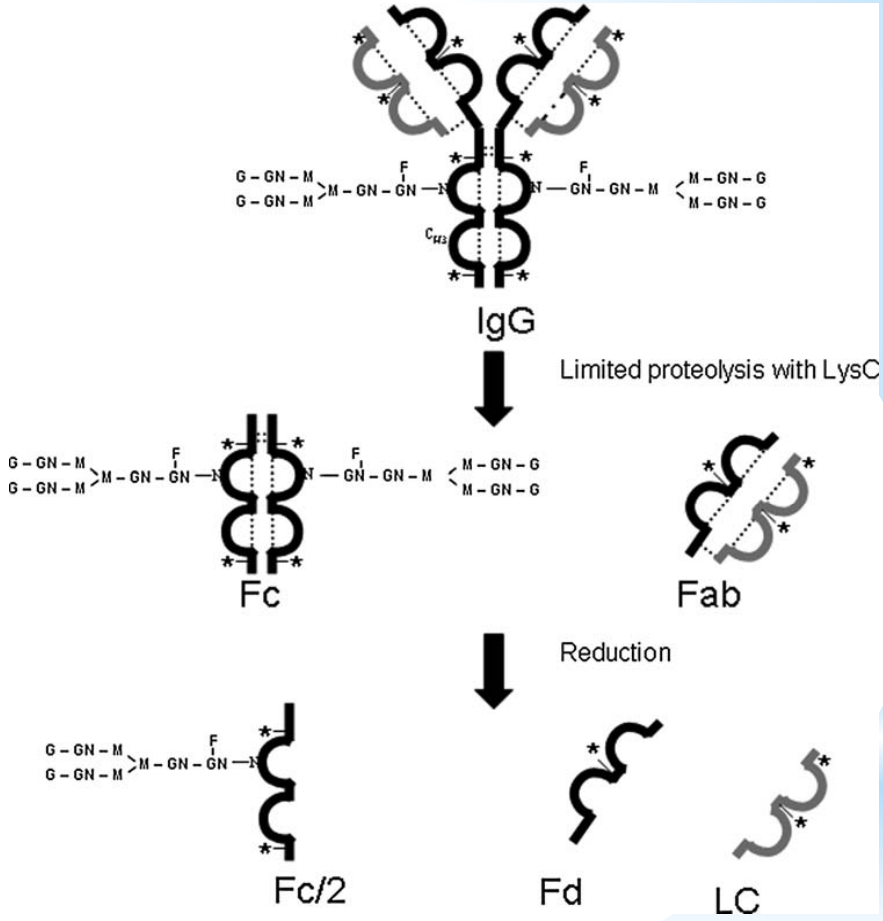
Nitrogen purging reduces dissolved oxygen

Measured Dissolved Oxygen (DO) in Drug Substance	Dissolved Oxygen after Nitrogen purging in DS	Conditions after purging with nitrogen	Re-dissolving of Oxygen (DO%) after 12 h
96%	**0%**	**Container kept open to atmosphere**	**25%**
97%	**0%**	**Closed container over head with Nitrogen**	**2%**

B. Reductive damage in mAbs

Reductive damage and its consequences

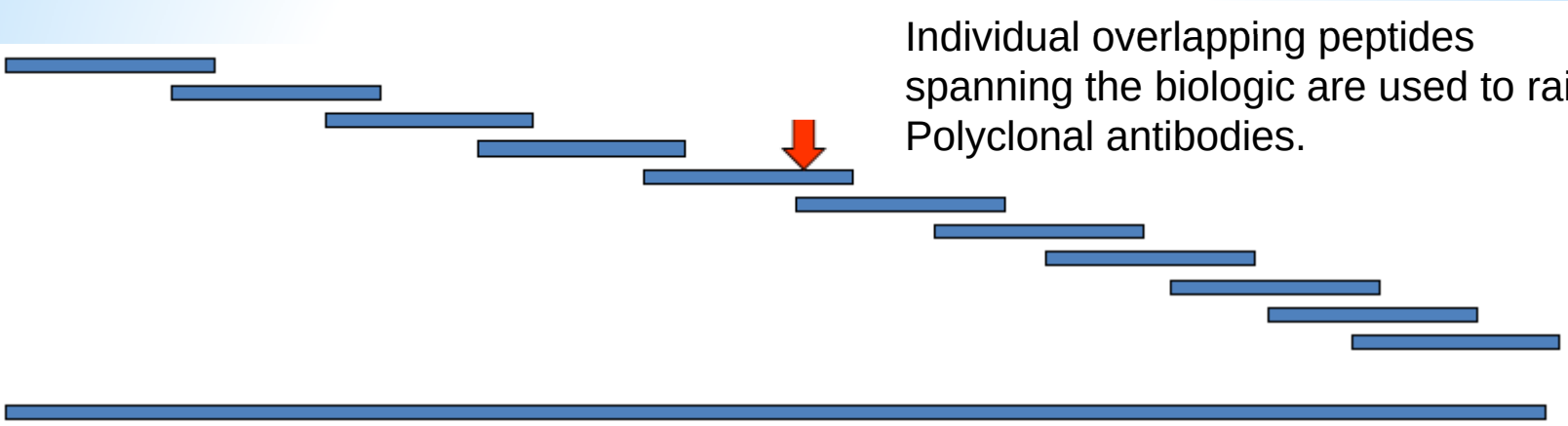
Reductive damage in mAbs can lead to disruption of one or more disulfide bonds which can lead to antibody fragments and / or affect functions such as target binding/ADCC/CDC.



Investigation of mAb stability

Stability Study	Potency (%)
At Zero time	96 %
After 6 months at real time-temperature	64

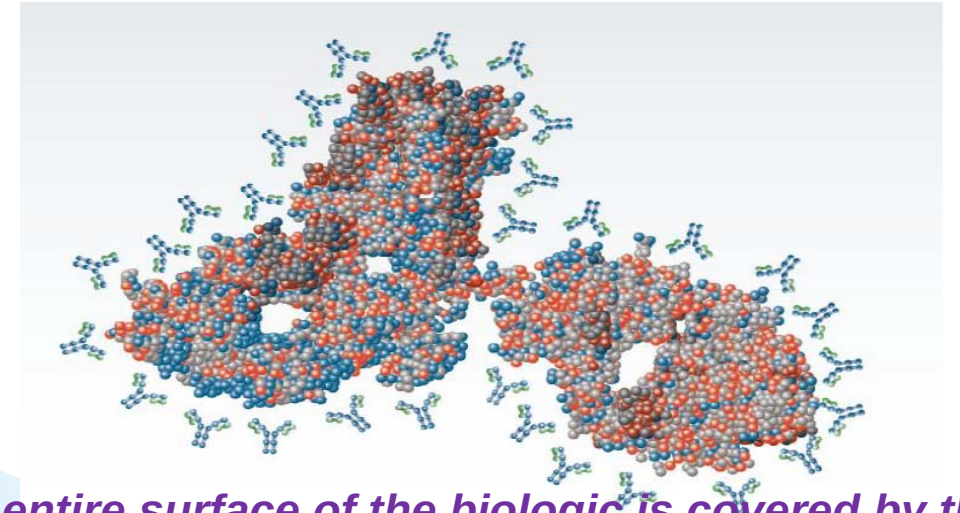
Array Bridge Analysis



Individual overlapping peptides spanning the biologic are used to raise Polyclonal antibodies.

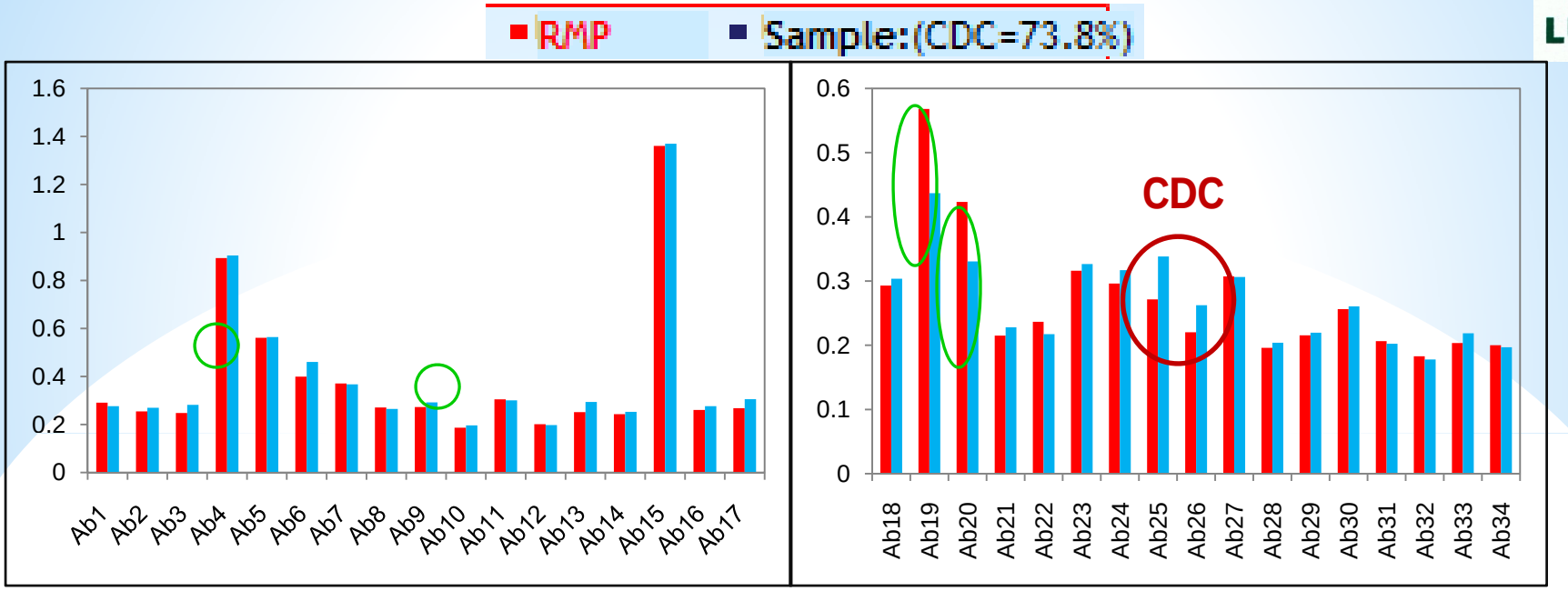
mAb Amino Acid Sequence

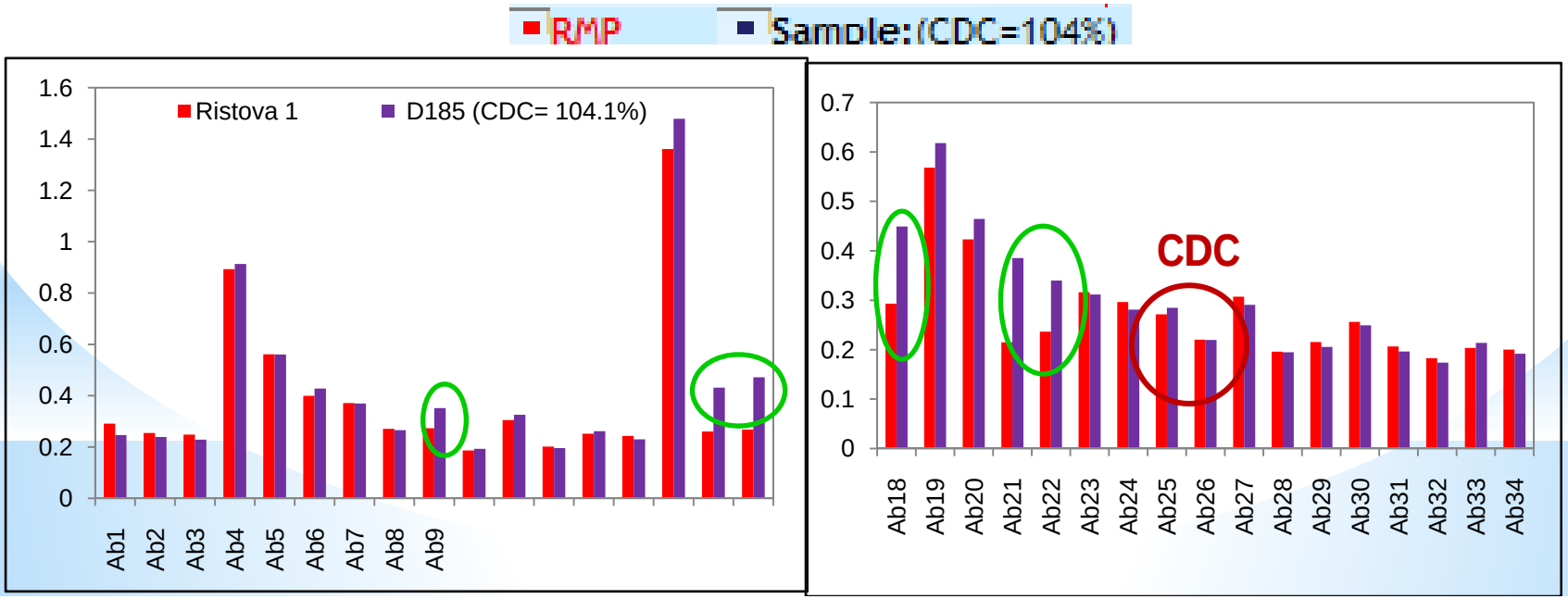
Antibody amino acid sequence is used to design the antibody array with overlapping regions to cover the whole mAb molecule



Potentially, the entire surface of the biologic is covered by the families of polyclonal antibodies, although in the native biologic the epitopes may be hidden

Array bridge results





What have we learned?

Metalloprotease activities are common in CHO K1 & DG44 cell lines and product that has target sites are at risk to clipping.

Process must be designed to remove proteases or their cofactors whenever clips are observed.

Oxidative damage is caused by dissolved oxygen In DS / DP and its removal gives better protection.

Reductive damage of disulfides during manufacture often leads to loss of one or more antibody functions.


Thank You !
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