Using Structure/Function Relationships to Identify CQAs and Develop Analytical Specifications

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Specifications

ICH Q6B: "A specification is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described."

Options for setting Acceptance Criteria (AC):

- Published guidelines (sterility, endotoxins, etc)
- Manufacturing capability / clinical experience
- Link AC to product safety and efficacy

Use structure / function data to set "clinically relevant" AC based on impact to biological functions

Analytical Control Strategies for PTM CQAs

Key Topics of the Presentation

Multi-point, forced degradation studies with structural modeling and full analytical characterization are the foundation for PTM control strategy. Use these data to:

- Clearly identify PTM CQAs (deamidation & oxidation)
- Set “clinically relevant” AC based on S/F correlations rather than statistical analysis of mfg batches

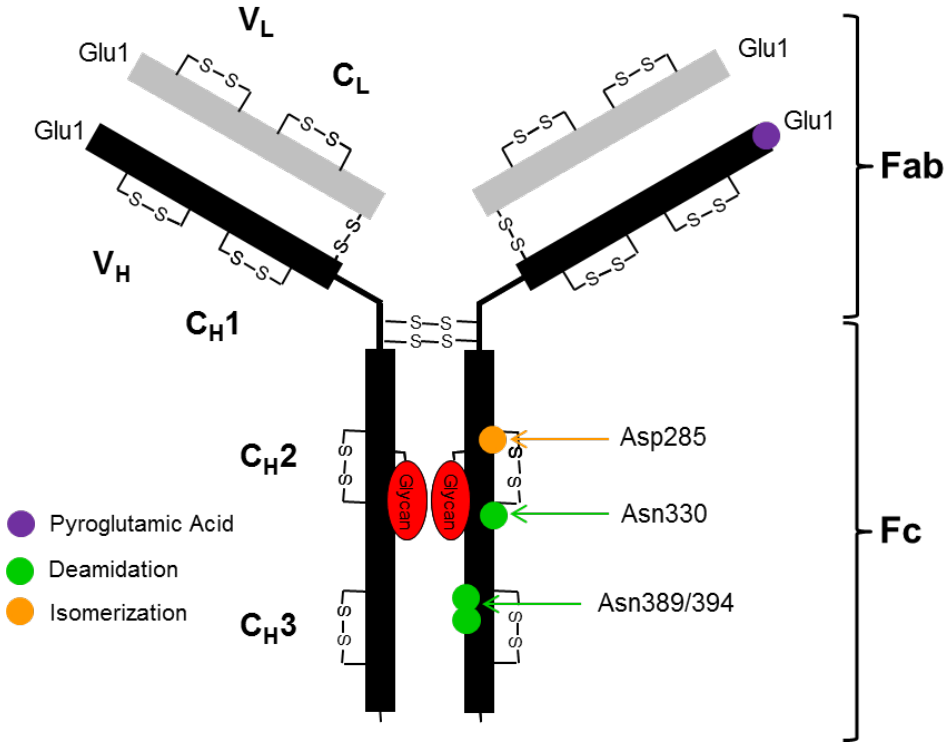
Forced Deg Workflow

- Evaluate heat, peroxides, light, pH and glucose stress
- Prep 4-6 samples under each stress condition
- Fully characterize all samples using the assays below to identify CQAs and establish S/F correlations

Quality Attributes	Analytical Methods
Primary Structure	Peptide Map
Glycosylation and Glycation	Oligo Map and Intact Mass
Charge Heterogeneity	cIEF
Size Heterogeneity	SE-HPLC and cSDS
Higher Order Structure	CD, AUC and DSC
Biological Functions (depending on MOA)	Bioassay and Ag Binding
	CDC, ADCC, FcγR and FcRn Binding

Heat Stress Impacts Biological Function

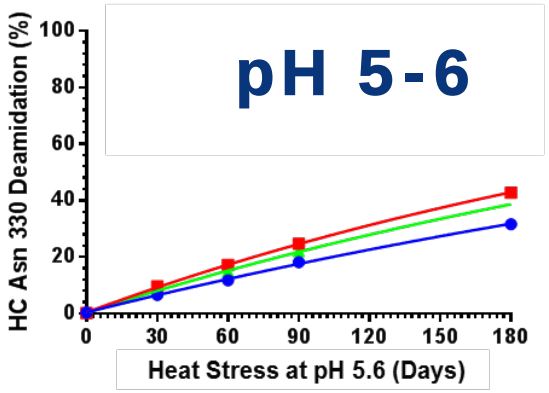
6M Heat Stress Study in pH 5.5 Formulation Buffer at 37°C

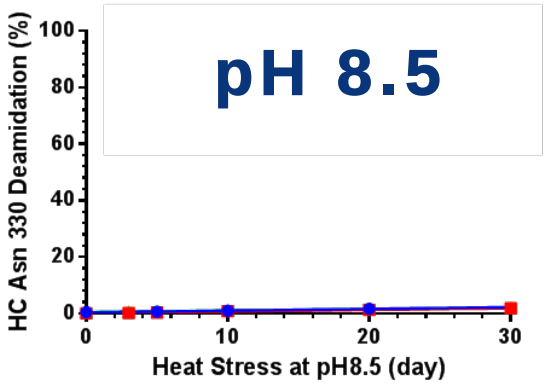


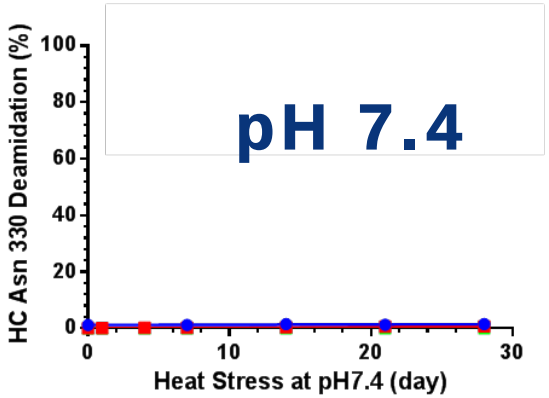
- Large decreases in CDC, ADCC and FcγRIIIa binding (>50%)
- Small increases in N-terminal cyclization, Asp isomerization and HC Asn389/394 deamidation (<5%)
- Large increases in HC Asn330 deamidation & acidic peaks by cIEF

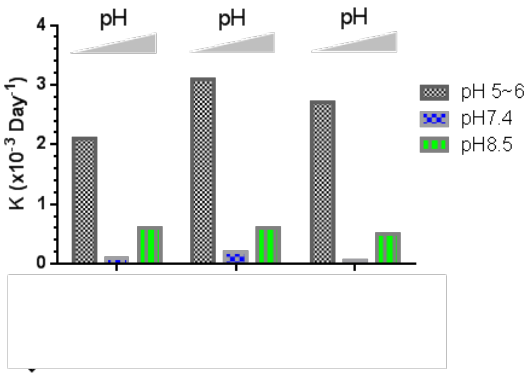
Unique pH Profile for HC Asn330 Deamidation

Comparison of 3 IgG1 mAbs at 37ºC





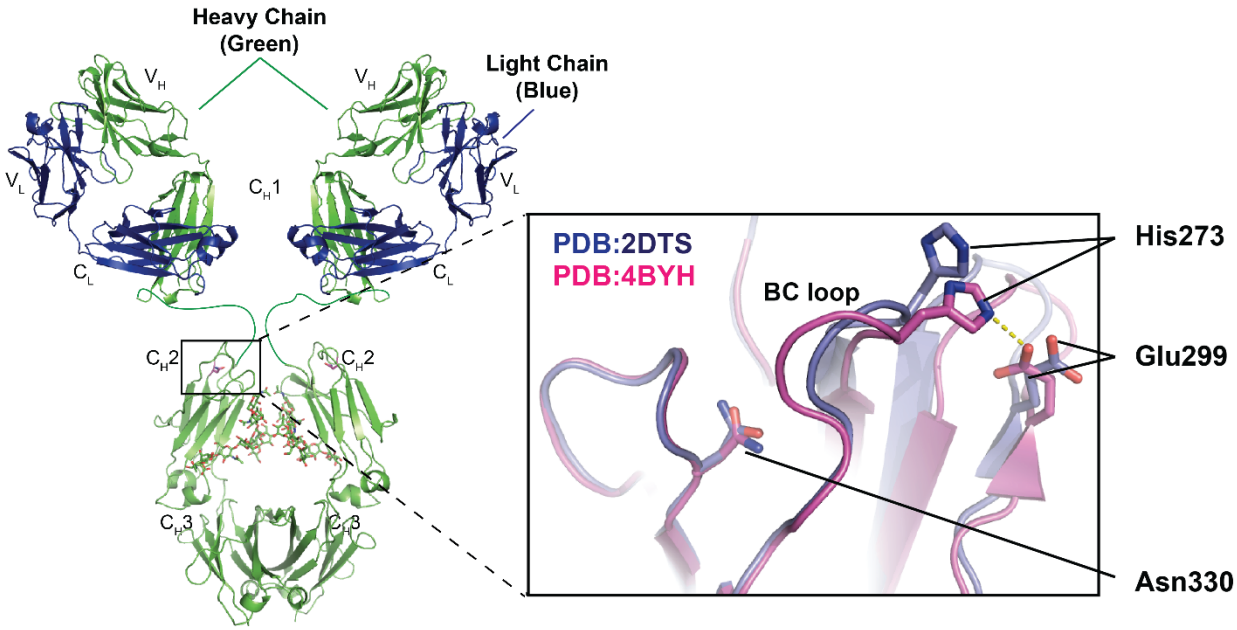




- Asn330 deamidation occurs very slowly in mild acidic buffer (6%/mon at 37ºC)
- Asn330 deamidation **does not** occur at neutral or higher pH. Manufacturing process variation has little impact on Asn330 deamidation.

Unique pH Profile for HC Asn330 Deamidation

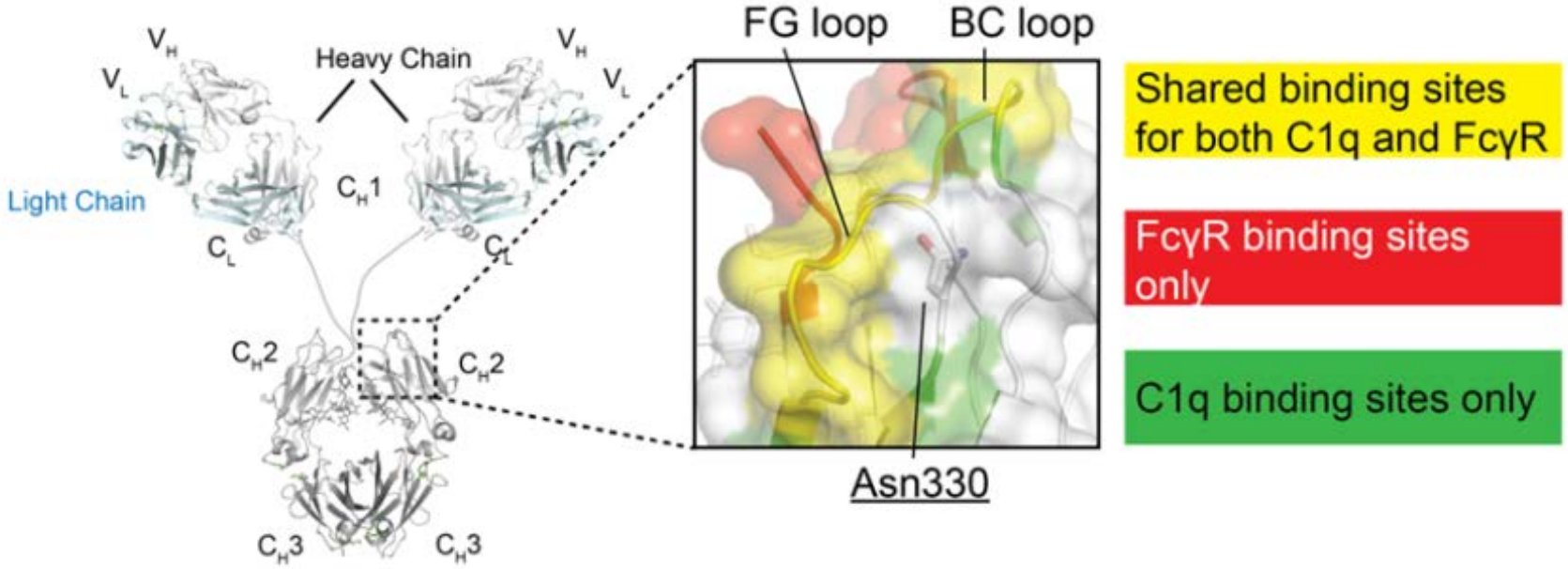
Molecular Mechanism



- His sidechain imidazole ring has a pKa around 6.0.
- Below pH 6, protonation of His273 helps form salt bridge with Glu299, which increases the solvent accessibility of Asn330.
- Above pH 6, deprotonation of His273 breaks the salt bridge with Glu299 and reduces the solvent accessibility of Asn330.
- Below pH 3, deamidation occurs by acid hydrolysis at very slow rate.

HC Asn330 Deamidation is a CQA Based on Structural Modeling and Bioassay Data

HC Asn330 sits near regions involved in FcγRIIIa binding needed for ADCC activity and C1q binding needed for CDC activity

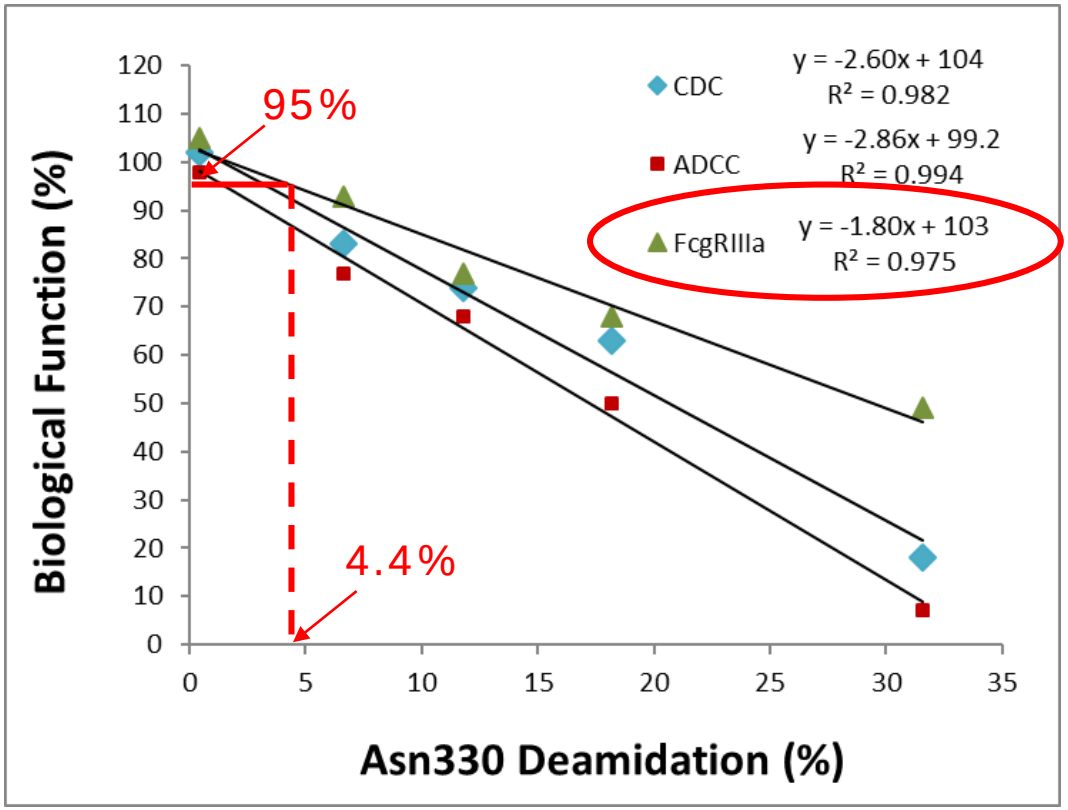


Asked to have analytical control even though deamidation rate on stability was very slow (0.4% / year at 5ºC)

Set “Clinically Relevant” AC Based on S/F Correlations

4.4% Asn330 Deamidation has 95% FcγRIIIa Binding

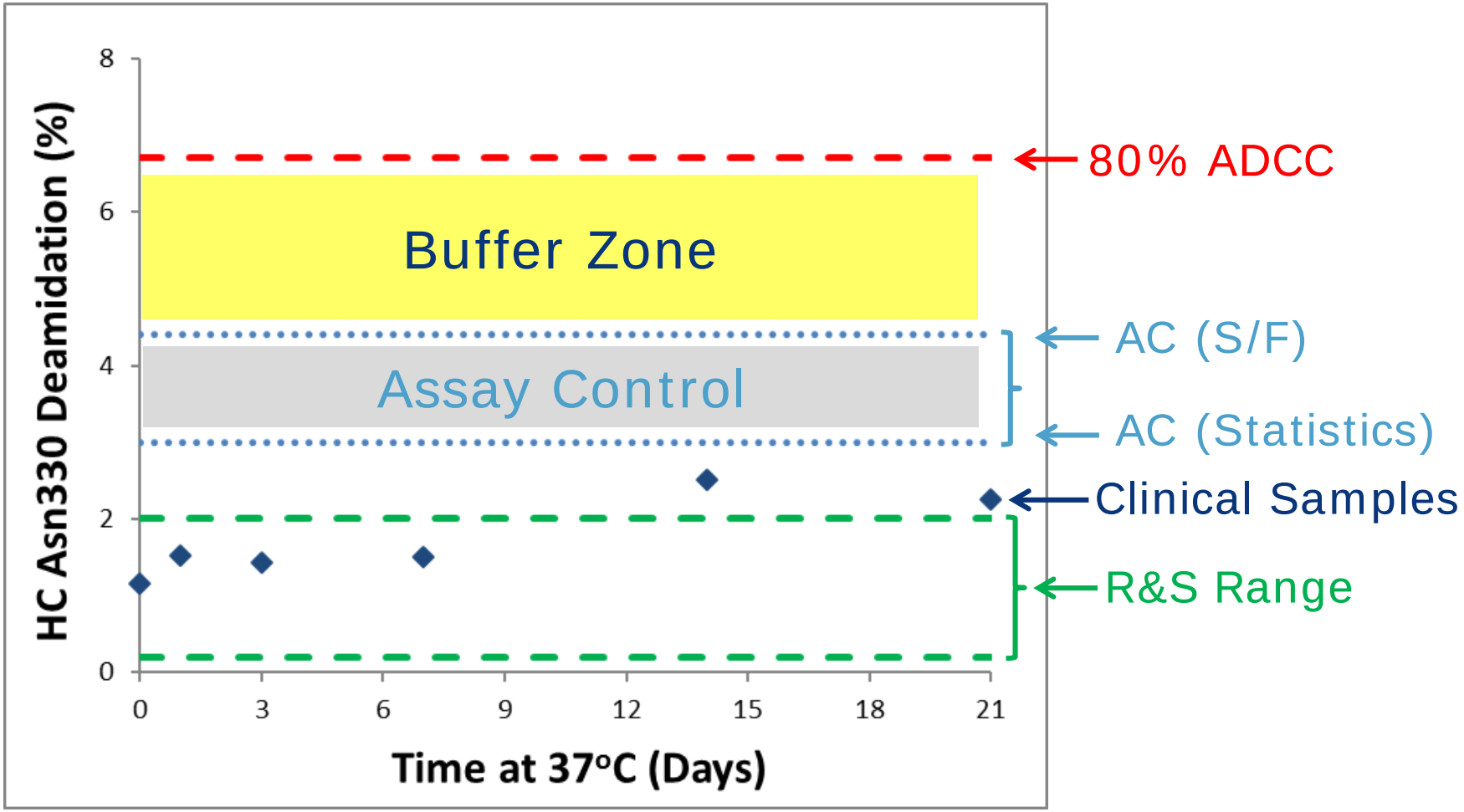
Asn330 deamidation correlates with loss of CDC, ADCC & FcγRIIIa binding



Limit: 95% FcγRIIIa binding correlates with 4.4% Asn330 deamidation
Compared to ≤3.0% from statistical analysis of mfg batches

HC Asn330 Deamidation is Still Well Controlled

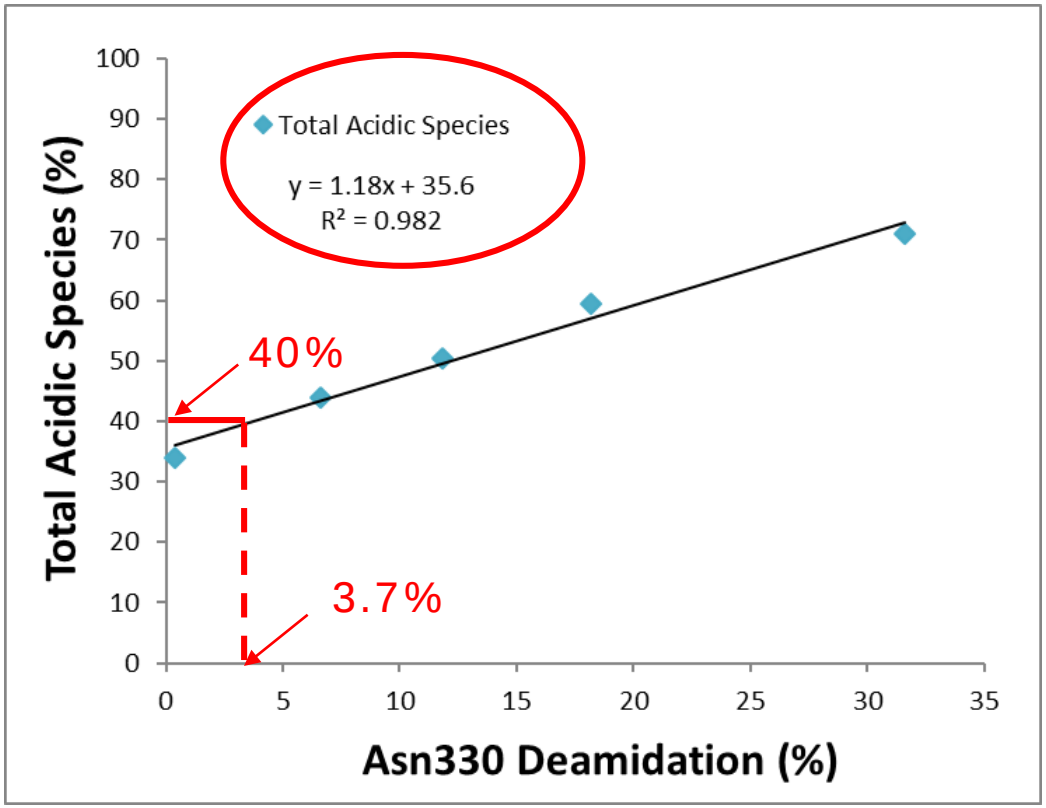
Based on Data from Heat Stress Study at pH 5.5



Control of Asn330 Deamidation by cIEF

Data from Heat Stress Study at pH 5.5

Asn330 Deamidation correlates with increase in acidic peaks by cIEF



Release / Stability Acceptance Criteria: 23-40% acidic peaks

Limit: 40% Acidic Peaks correlates with 3.7% Asn330 deamidation

Increase in Acidic Peaks Due Primarily to Asn330 Deamidation & Unique to Heat Stress at pH 5.5

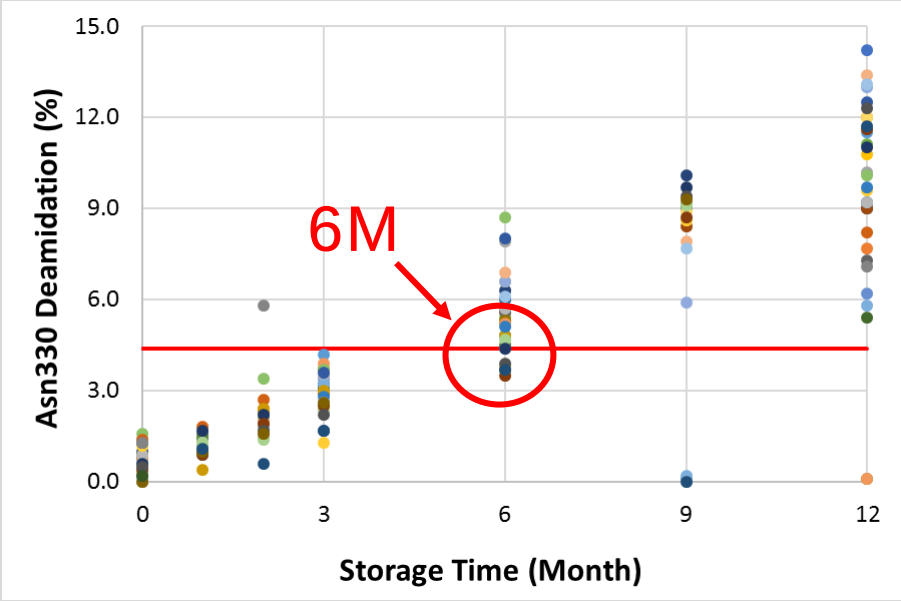
Attribute	T0	1M at 37C	2M at 37C	3M at 37C	6M at 37C
Peptide Map					
HC Glu 1 Cyclization	1.1	1.7	2.4	3.2	5.2
HC Asp285 Isomerization	0.4	1.3	2.0	3.0	5.1
HC Asn320 Deamidation	6.9	6.9	7.2	6.9	7.4
HC Asn330 Deamidation	0.4	6.6	11.8	18.2	31.6
HC Asn389/394 Deamidation	8.8	9.8	10.4	11.0	12.7
HC Asn439 Deamidation	1.0	1.0	1.0	1.1	1.1
cIEF					
Main Peak	62.1	48.2	40.6	32.1	21.3
Acidic peaks	33.9	44.0	50.4	59.4	71.0
Basic Peaks	3.9	7.8	9.0	8.5	7.7

Forced deg conditions	Glucose Stress	Peroxide Stress	Photo Stress	Heat Stress (pH 5.5) 6M at 37C	High pH Stress (8.5)	Low pH Stress (3.2)
Asn330 Deamidation	0.7	0.5	0.8	31.6	1.7	1.3

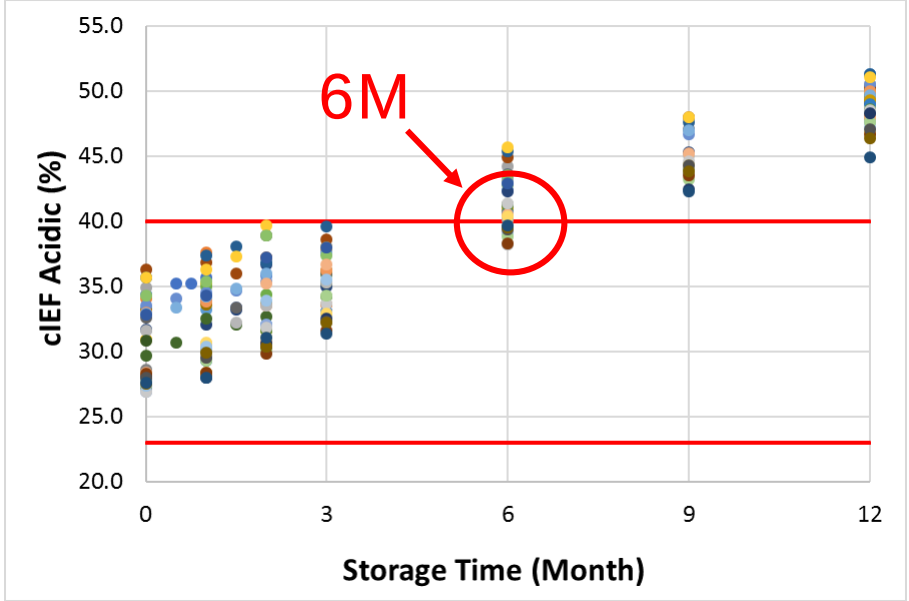
Control of HC Asn330 Deamidation

AC for cIEF and Peptide Map are Equally Effective

Peptide Map
Asn330 Deamidation



cIEF
Total Acidic Peaks

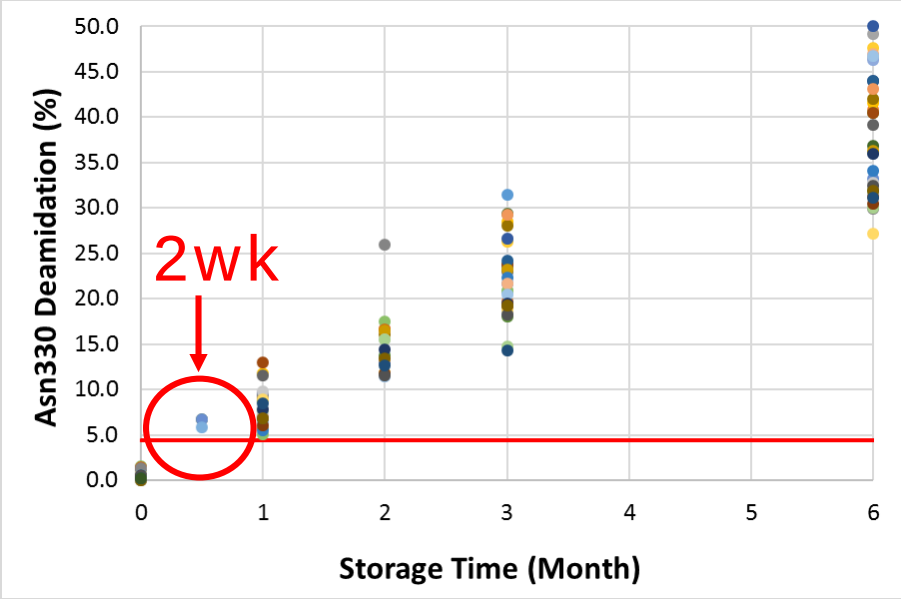


- 25°C Stability data for 36 DP batches (color coded)
- AC indicated by the red bars in the graphs
- AC for cIEF based on statistical analysis of batch results

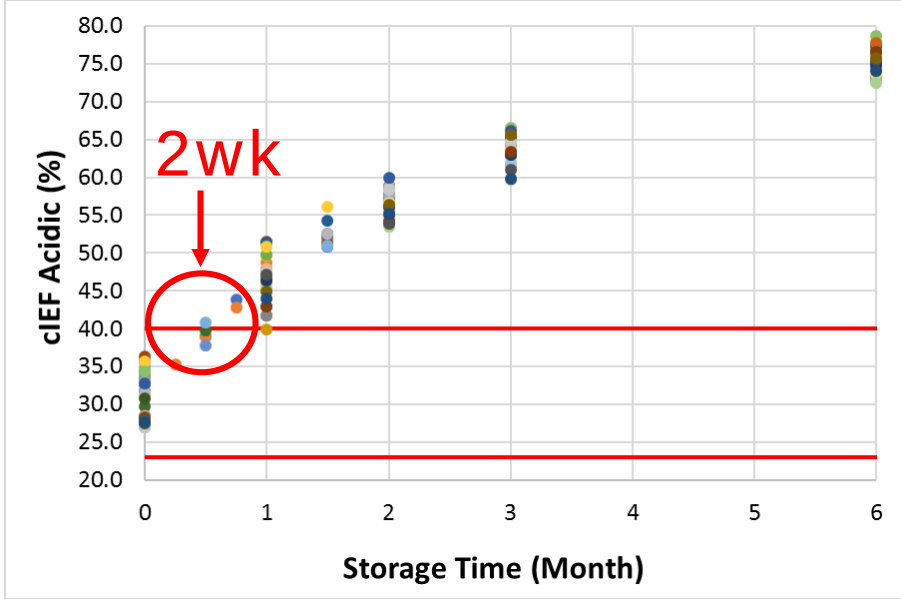
Control of HC Asn330 Deamidation

AC for cIEF and Peptide Map are Equally Effective

Peptide Map
Asn330 Deamidation



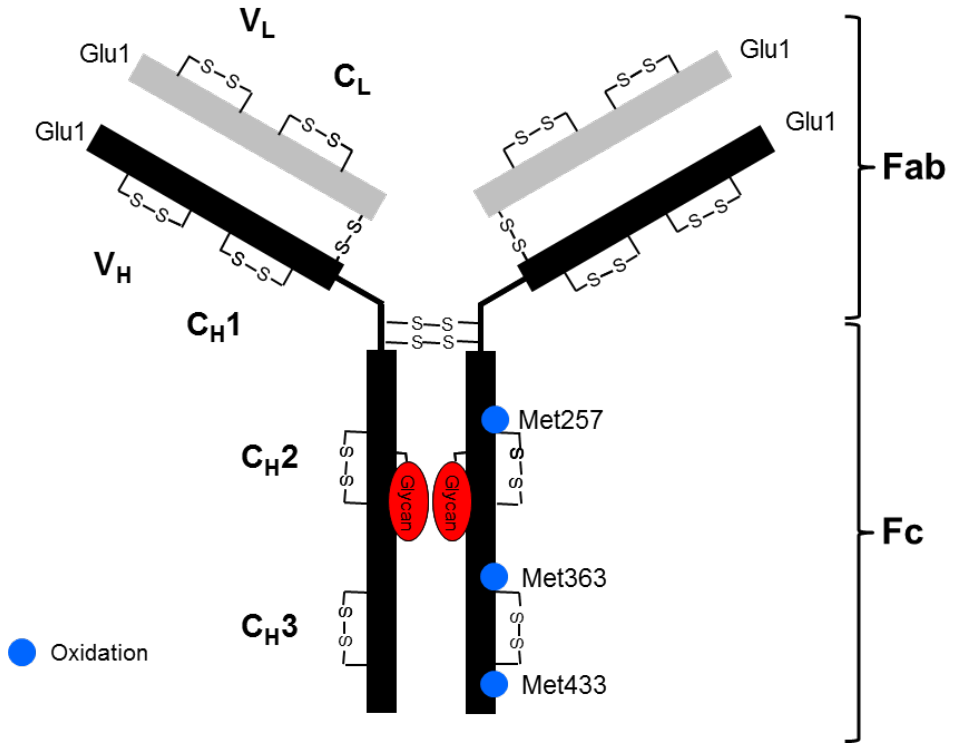
cIEF
Total Acidic Peaks



- 40°C Stability data for 36 DP batches (color coded)
- AC indicated by the red bars in the graphs
- AC for cIEF based on statistical analysis of batch results

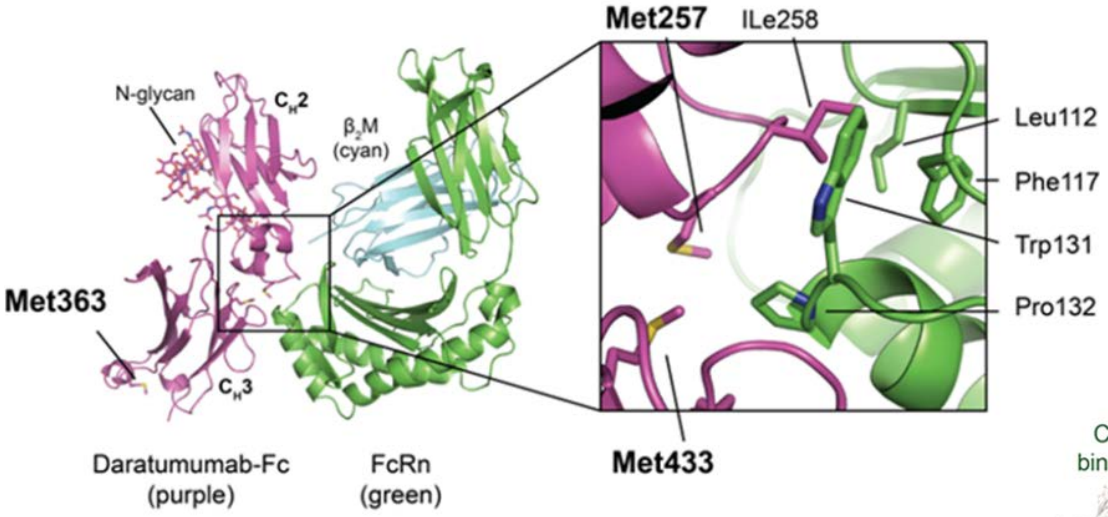
Methionine Oxidation is a Critical Deg Pathway

Hydrogen Peroxide Stress Study



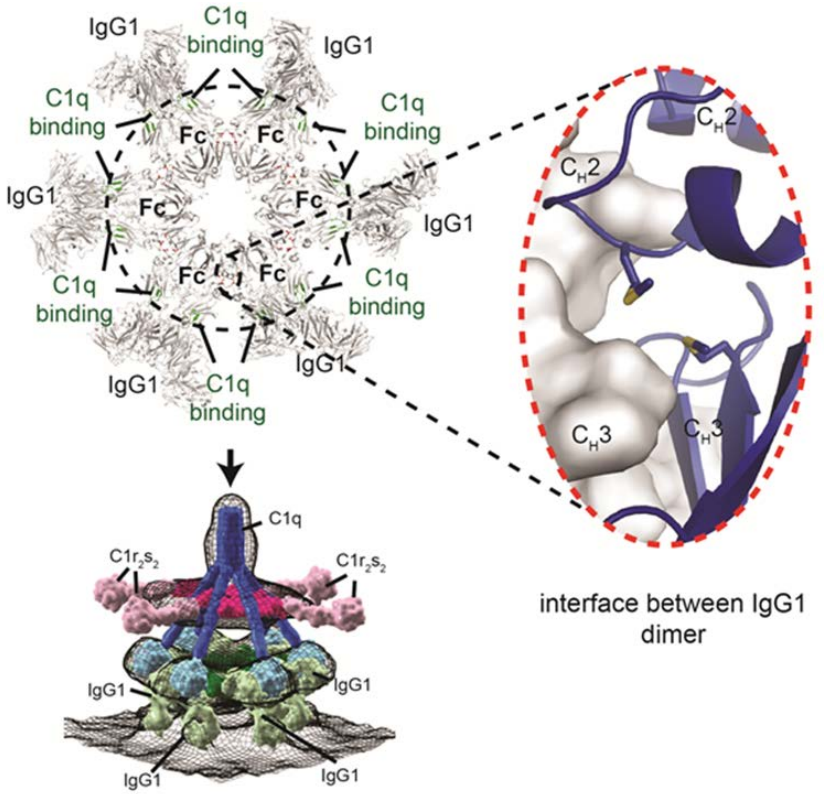
- Large decreases in CDC and FcRn binding (>50%)
- Large increases in Met oxidation (>80%), but no impact on Trp oxidation or other structural attributes

Only HC Met257 and Met 433 Oxidation are CQAs Based on Structural Modeling and Bioassay Data



FcRn Binding

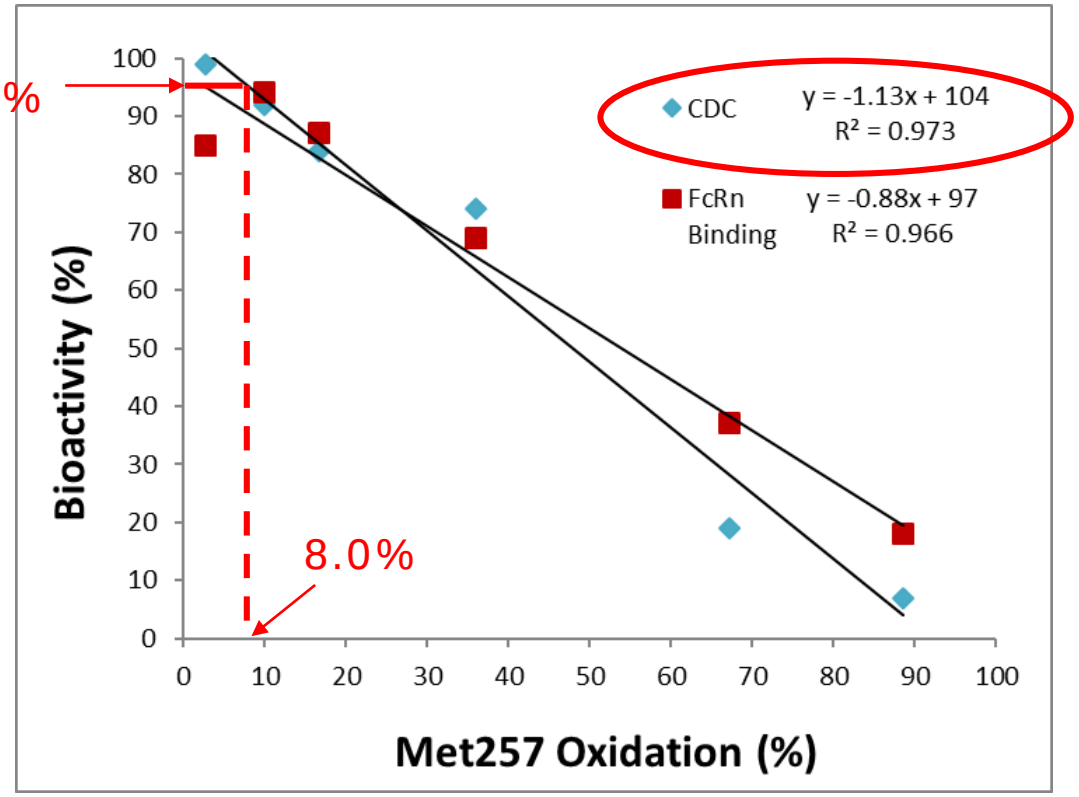
CDC Activity



Set “Clinically Relevant” AC Based on S/F Correlations

8.0% HC Met257 Oxidation has 95% CDC Activity

Met257 oxidation impacts FcRn binding and CDC activity



Limit: 95% CDC correlates with 8.0% Met257 oxidation

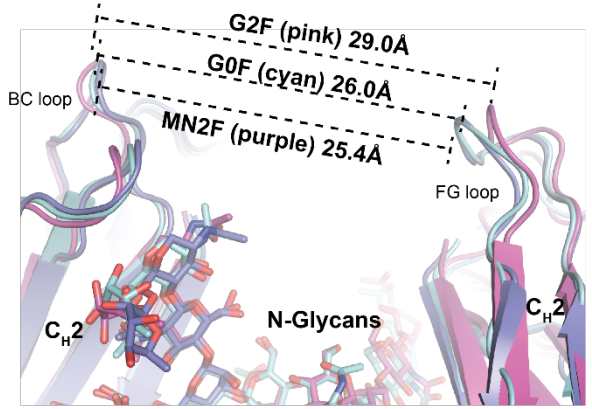
Compared to ≤5.6% from statistical analysis of mfg batches

Glycosylation is a CQA

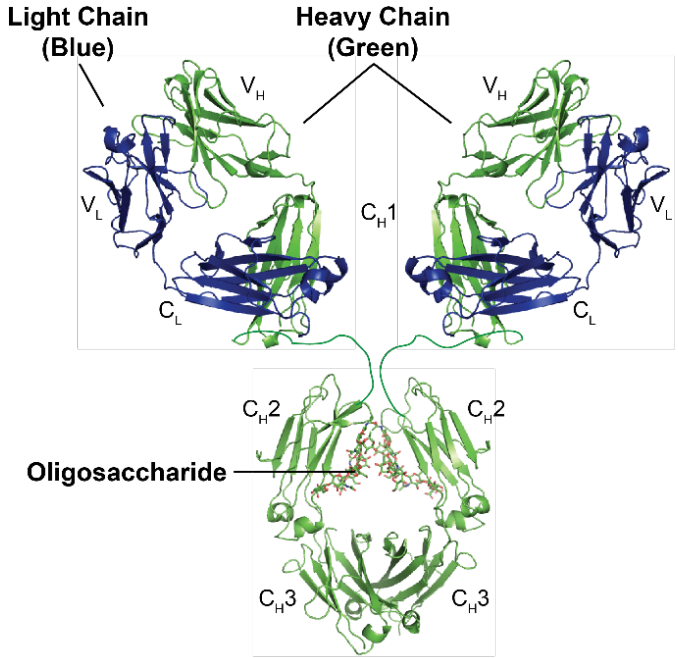
Single Point, Glycoform Enrichment Studies

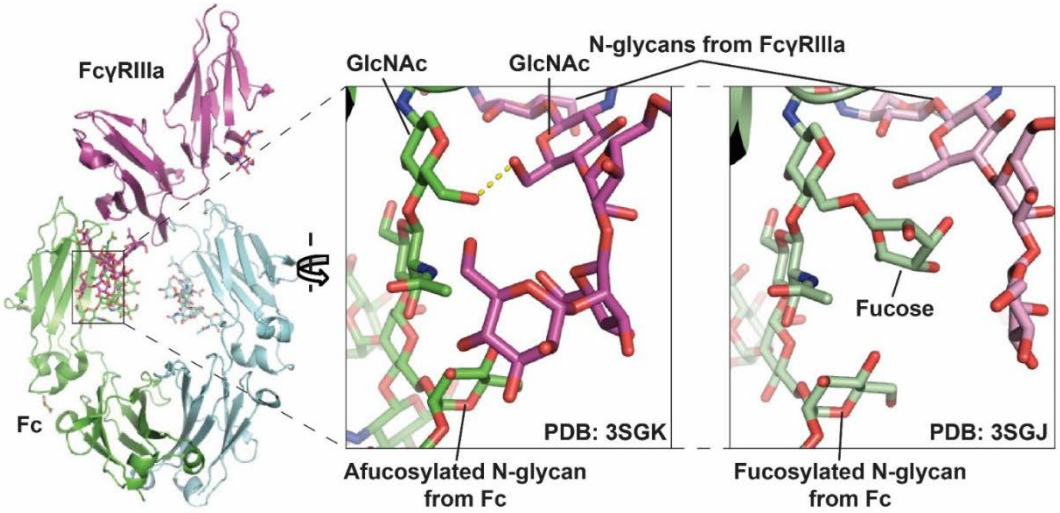
Terminal galactose and core fucose impacted CDC, ADCC and FcγRIIIa binding

Krapp, S et al (2003) JMB 325:979-989



G2F (pink) PDB: 1H3V
G0F (cyan) PDB: 1H3X
MN2F (purple) PDB: 1H3T

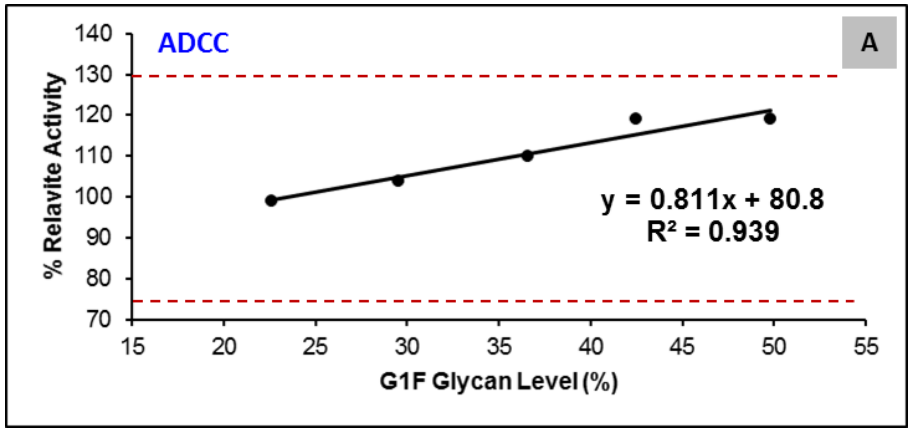


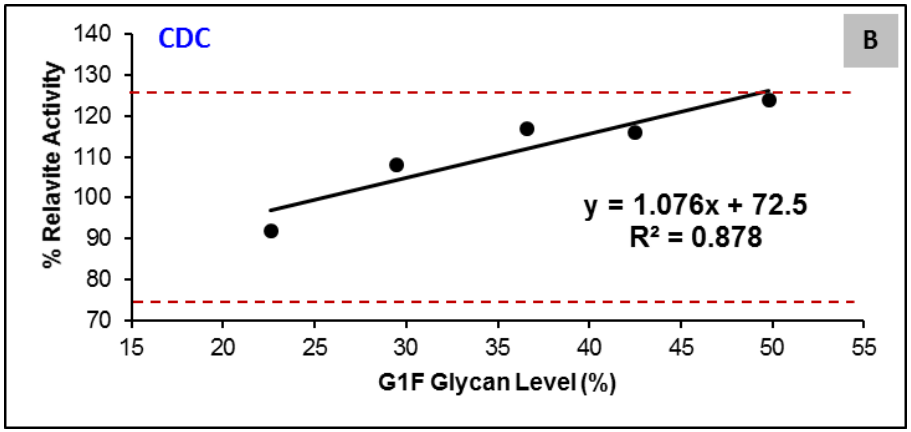


Ferrara C et al (2011) PNAS 108:12669-12674

Set "Clinically Relevant" AC Based on S/F Correlations

Results of G1F Spiking Study





- Current AC for G1F is 15-27% based on statistical analysis of clinical batches
- mAb with up to 50% G1F met the AC for ADCC and CDC activity

	Activity (%)	
Acceptance Criteria	ADCC	CDC
15-27%	93-103	89-102
15-32%	93-107	89-107

5% increase in G1F
=4% increase in ADCC
=5% increase in CDC

Summary
Analytical Control Strategy for PTM CQAs

- Multi-point, forced degradation or glycoform enrichment studies provide a wealth of information
- Can clearly identify PTM CQAs (deamidation, oxidation and terminal galactose)
- Can set “clinically relevant” AC based on impact to function rather than statistical analysis of mfg batches
- Can develop robust analytical control strategies using acceptance criteria from multiple methods

THANKS!!

Analytical Development

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