Global Characterization of Oxidative Stresses of IgG1 mAb

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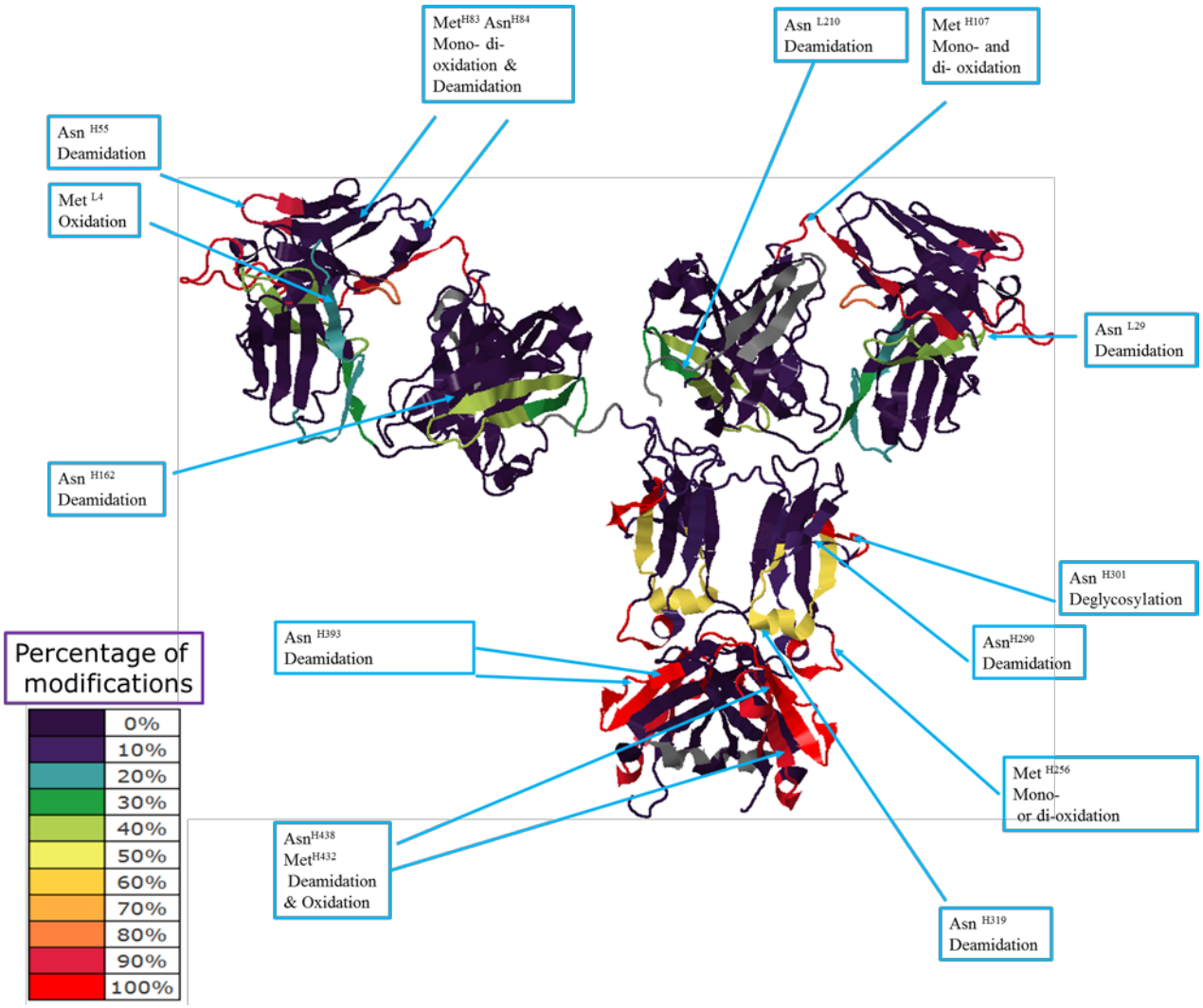
Why Global Characterization?

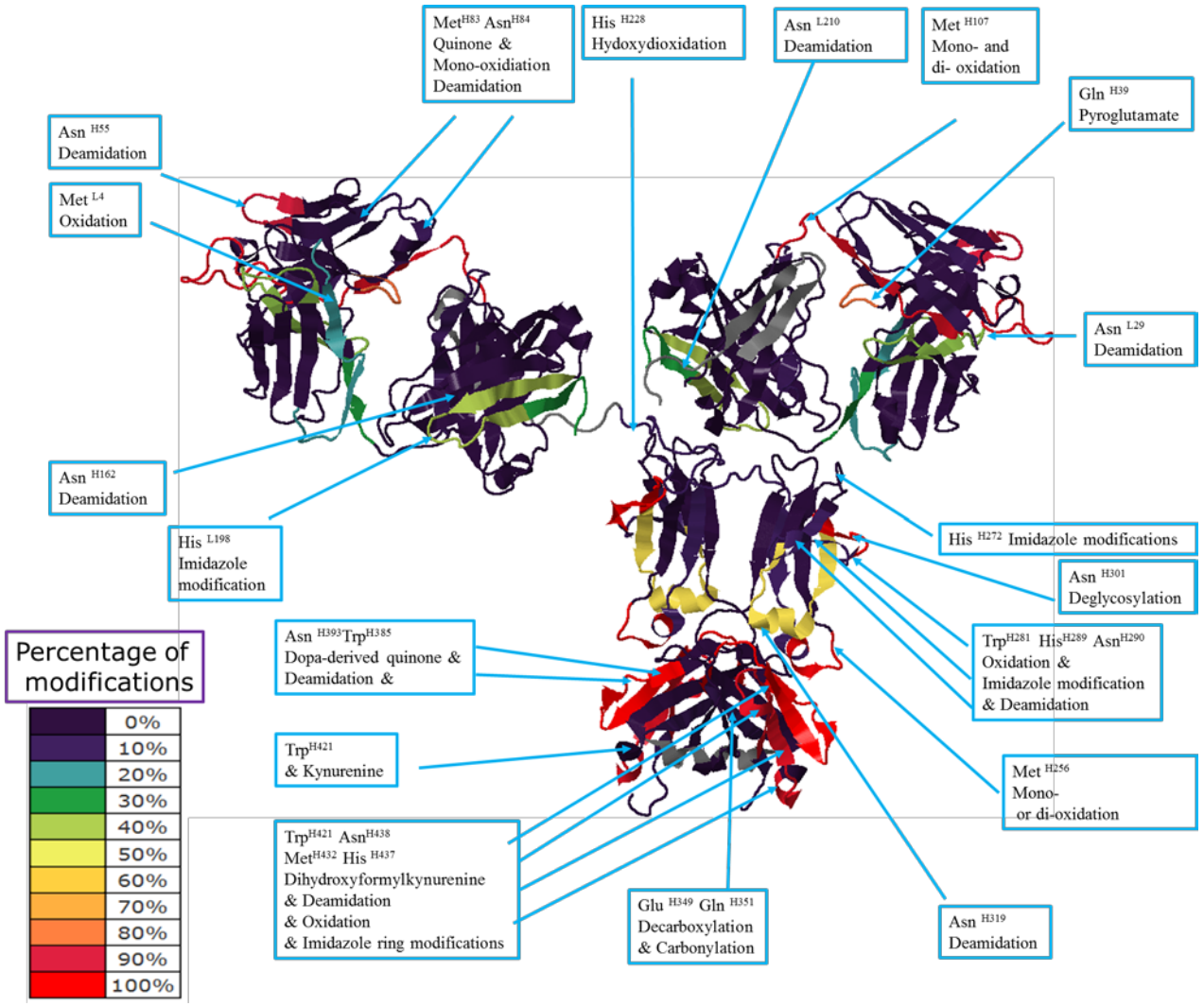
Majority of conventional protein stability characterization monitors Met and Asn



X-ray crystal structure source (PDB): 1HZH

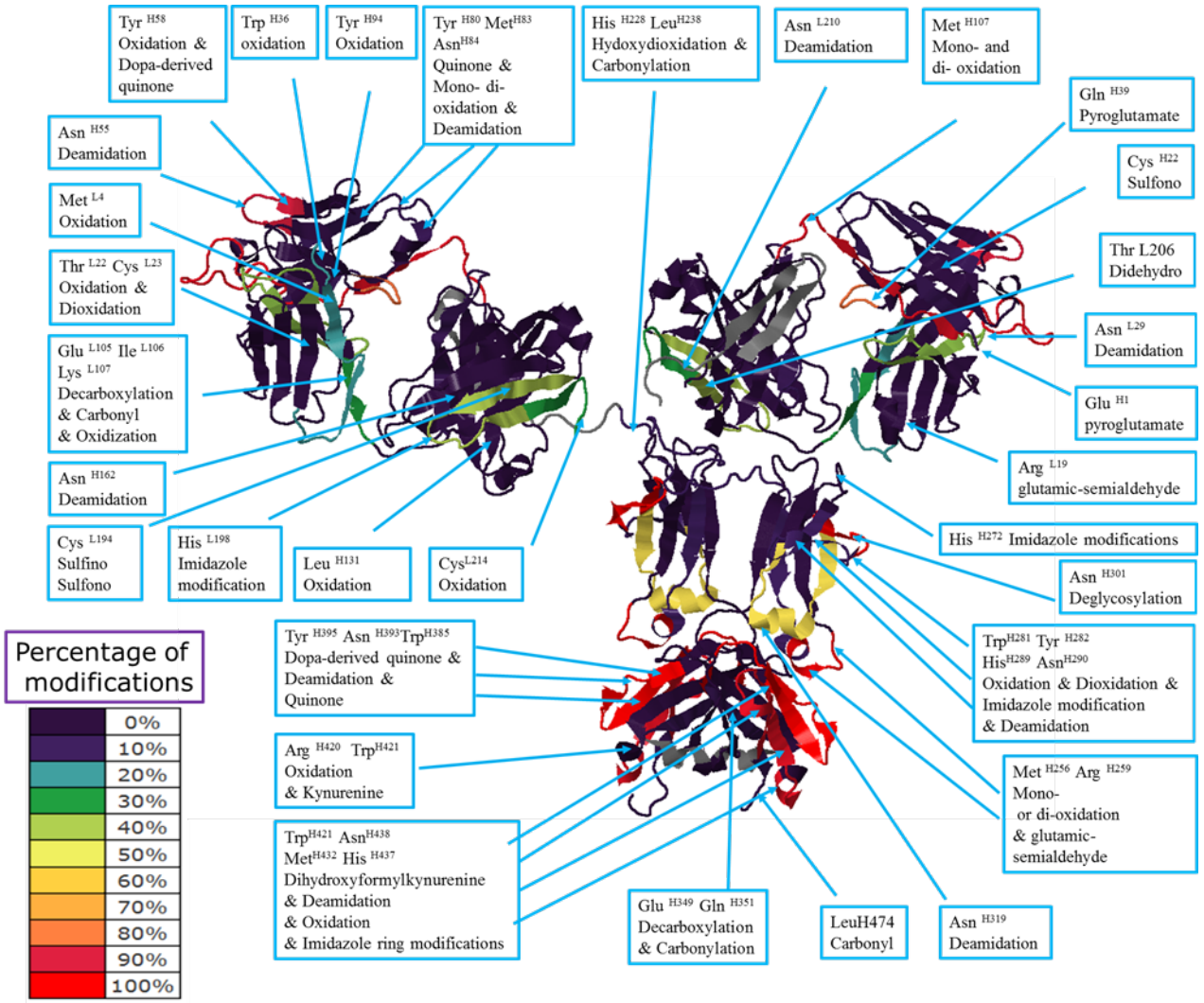
Why Global Characterization?

More advanced characterization would extend to Trp, His, or Gln



Global Characterization - Monitors 58 Modifications on IgG1 mAb

Many more modifications occur when protein is stressed



Why Global Characterization?

- His, Glu, Asp, Leu, Ile, Pro, Ser are commonly found at IgG1 and its ligand interfaces

IgG1 C1q complex

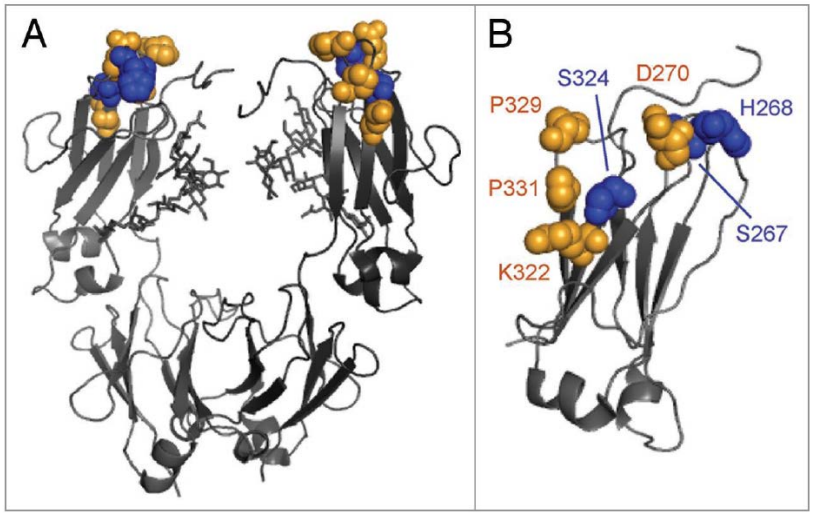
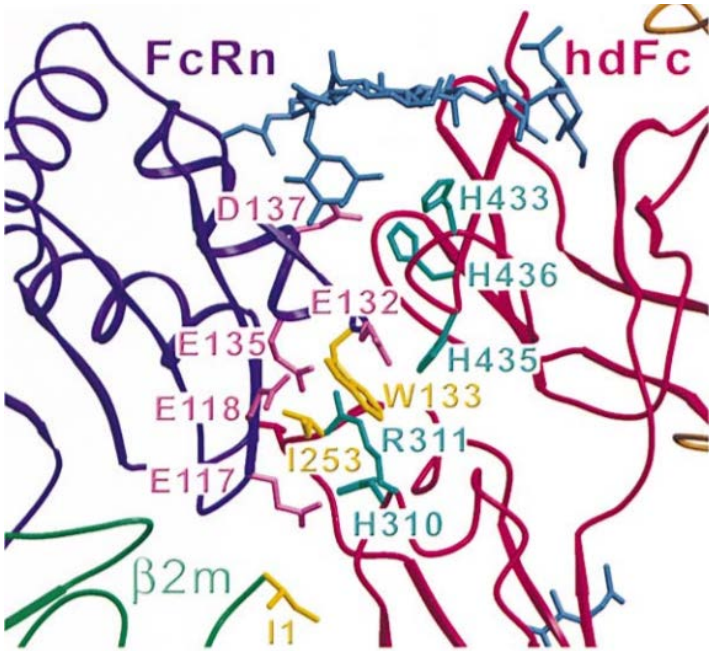


Figure 1. Cartoon representation of human IgG1 antibody Fc from Protein Data Bank record 1E4K[65] with positions at which substitution modulates C1q binding affinity highlighted as space-filling spheres. The putative C1q binding center (D270, K322, P329 and P331) is colored orange. Residues S267, H268 and S324 are indicated in blue. Oligosaccharides are represented as sticks. (A) Full Fc. (B) C_H2 domain only.

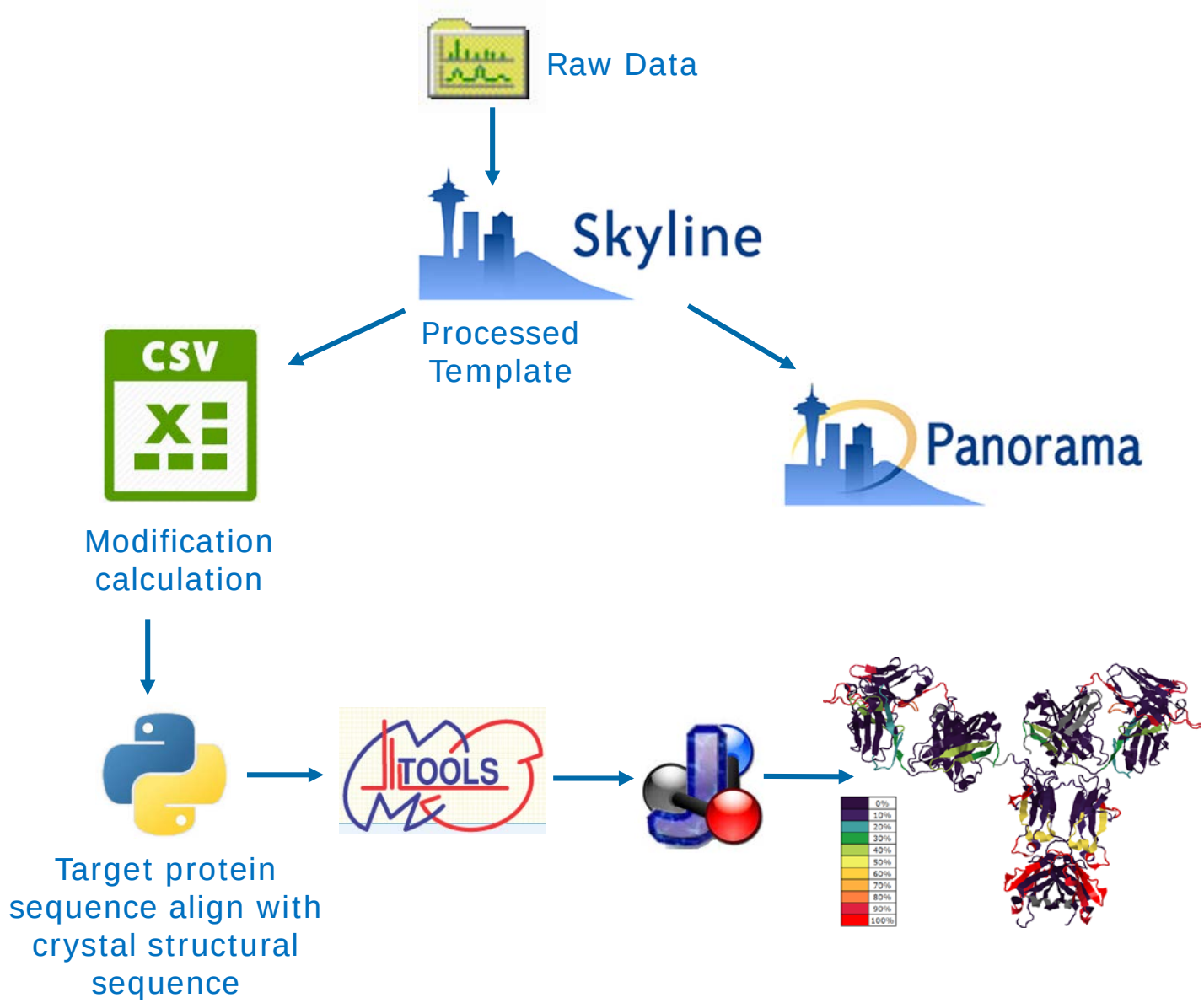
mAbs, 2010, 2:2, 181-189.

IgG1 FcRN complex



Molecular Cell, 2001, 7, 867–877.

Global Characterization For Protein Stability Analysis



Global Characterization For Protein Stability Analysis

Raw Data

Skyline

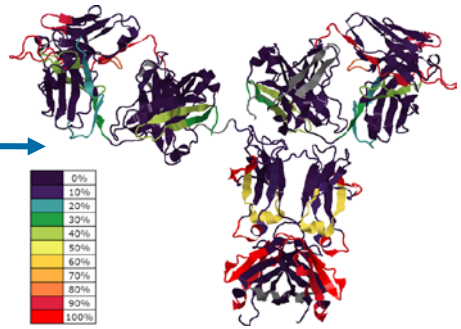
Processed Template

Final Goal:
From sample to Global Map within two days

Automated Modification calculation

Panorama

Target protein sequence align with crystal structural sequence

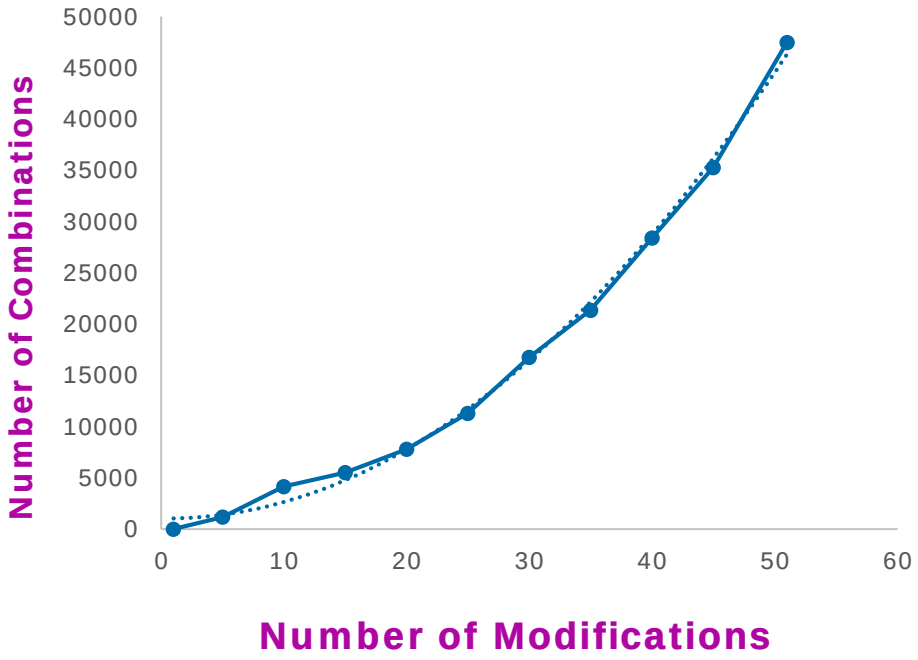


Challenge - Large Amount of Degradation Combinations

IgG1 [HC421-433]

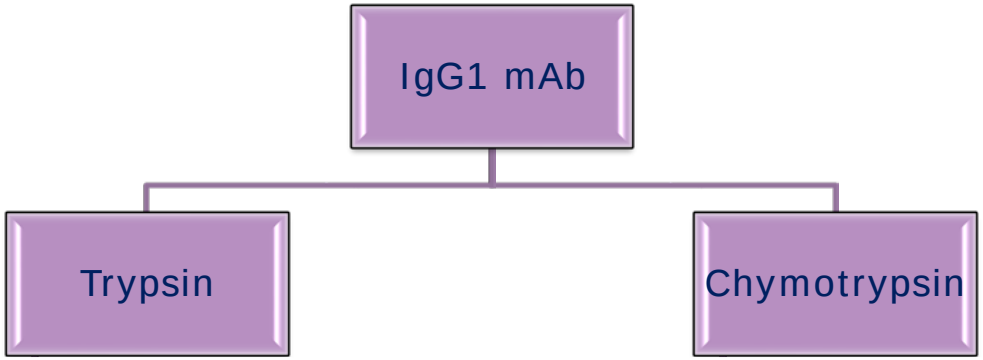
Trp-Gln-Gln-Gly-Asn-Val-Phe-Ser-Cys-Ser-Val-Met-His-Glu-Ala-Leu-His-Asn-His-Tyr-Thr-Gln-Lys

Assume 1, 2, or 3 modifications per peptide



Combination of IgG1 with all 58 modifications: Impossible to Process

Data Processing Strategy on Skyline



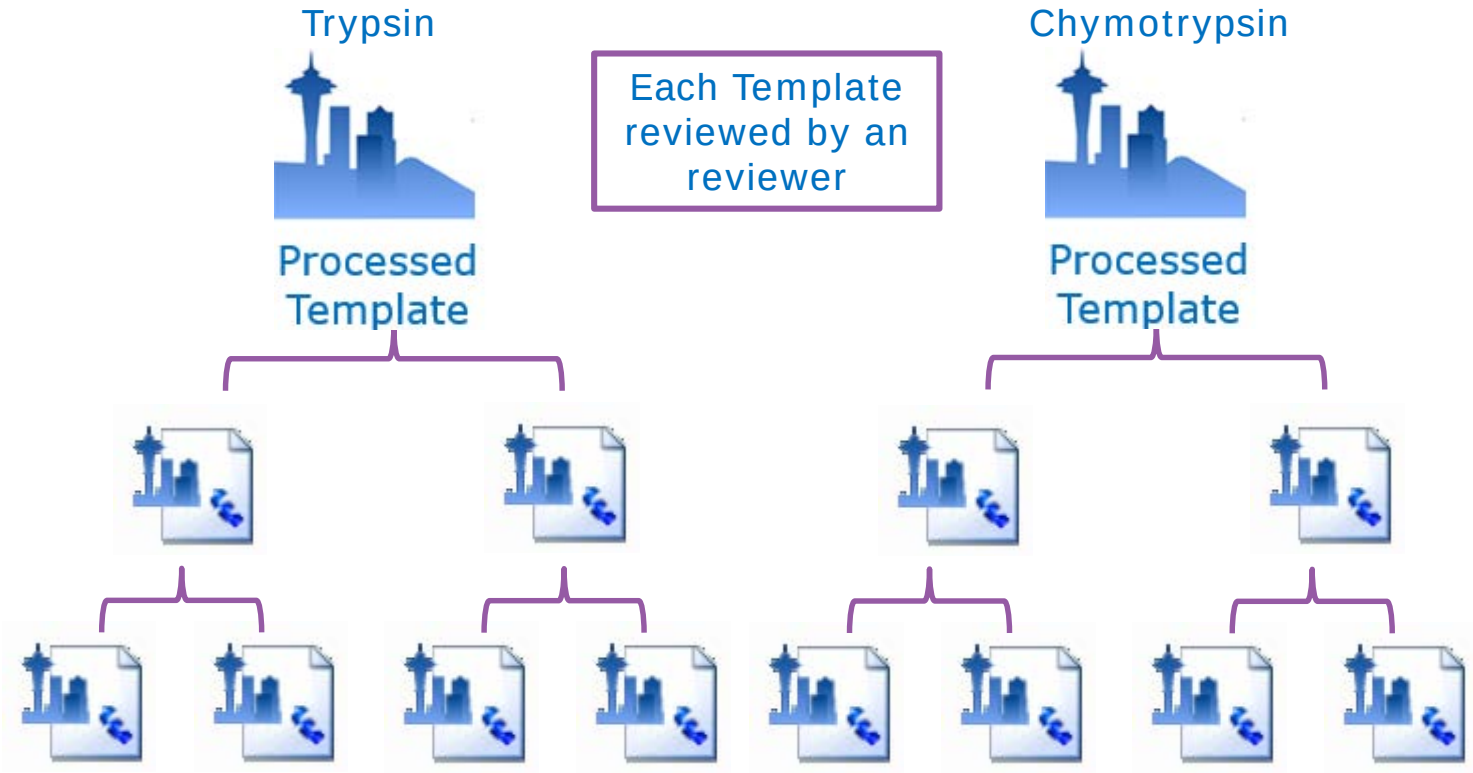
Process combinations of more reactive species

+

Process combinations of most reactive and less reactive species

- 3 modifications per peptide
- 1 and 2 miscleavage for trypsin and chymotrypsin
- 5 and 10 ppm for precursor and product ions
- 0.95 idotp filter
- Manually reviewed > 24,000 combinations
- 20-100 combinations left in each file

Data Processing Strategy on Skyline



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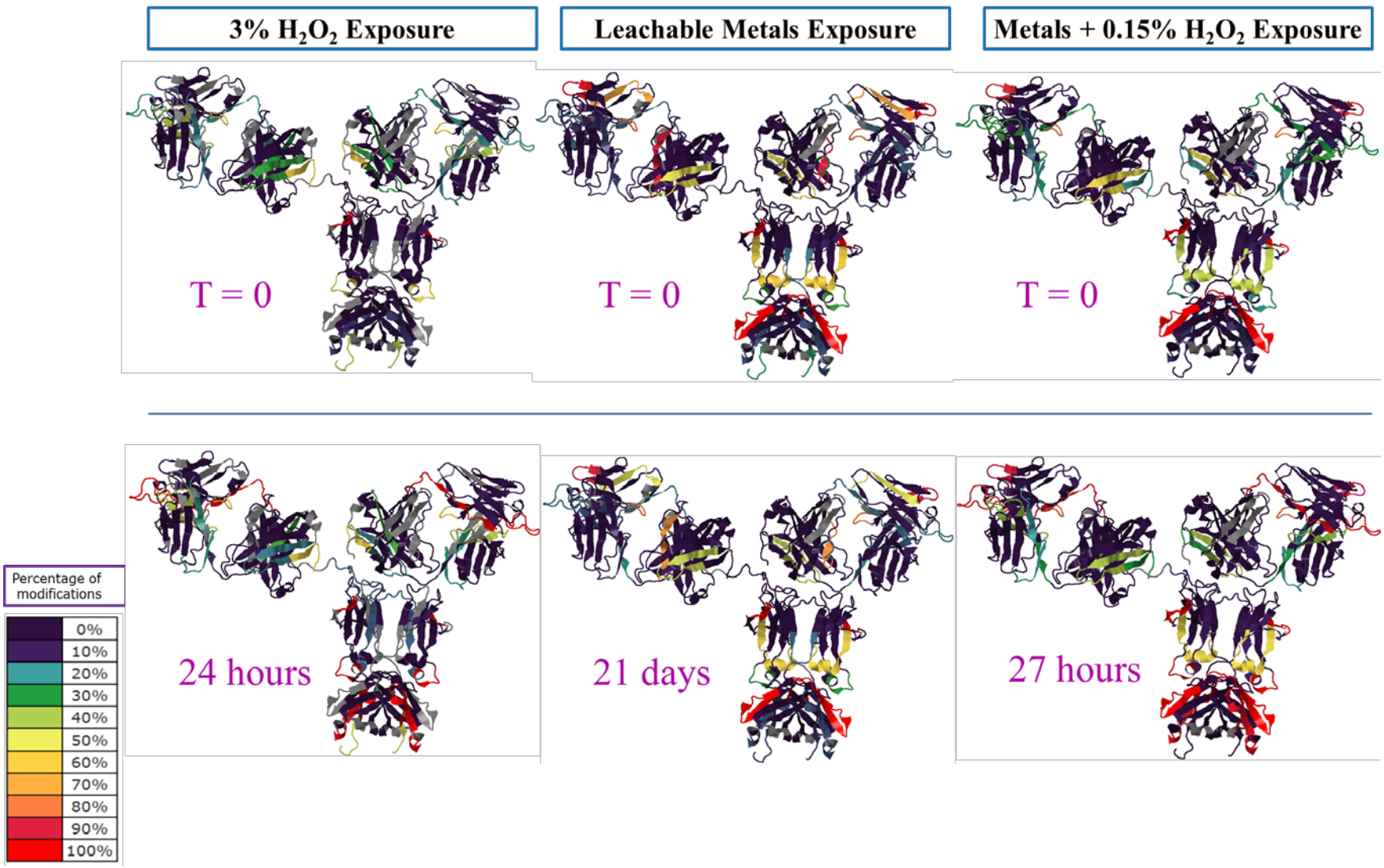
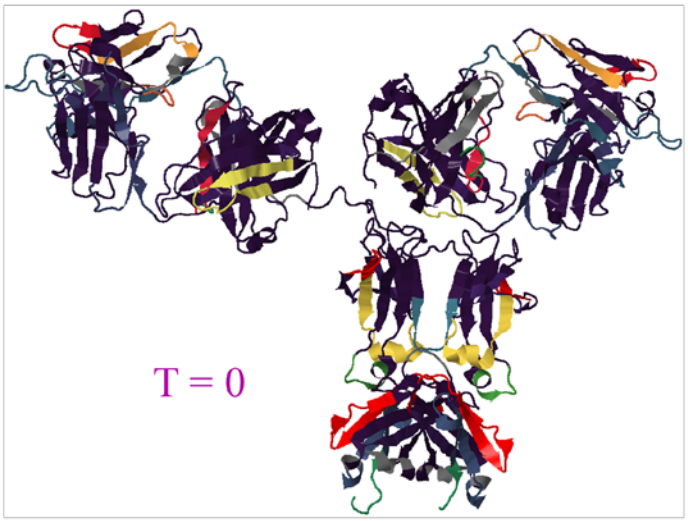
Stress Conditions For Template Generation

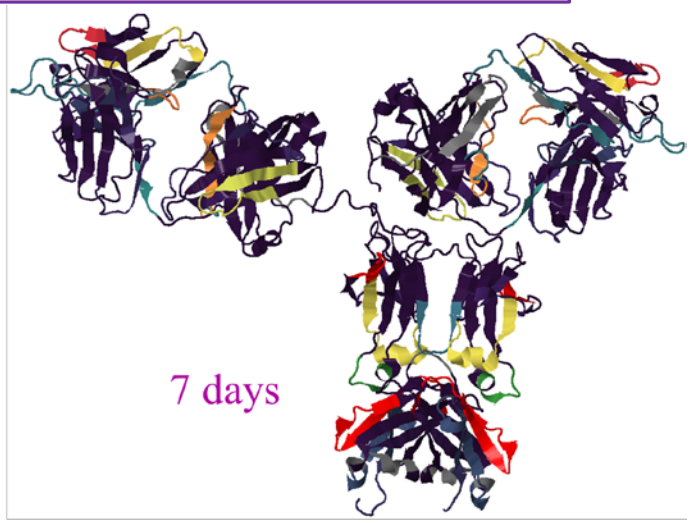


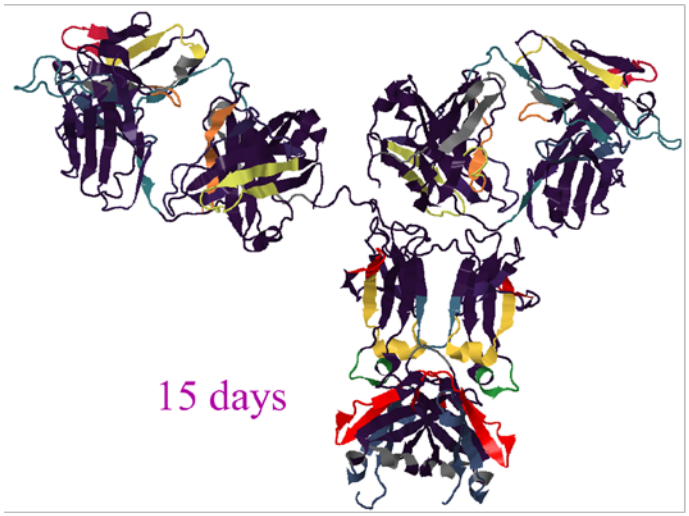
Global View - 3% H_2O_2 Treatment

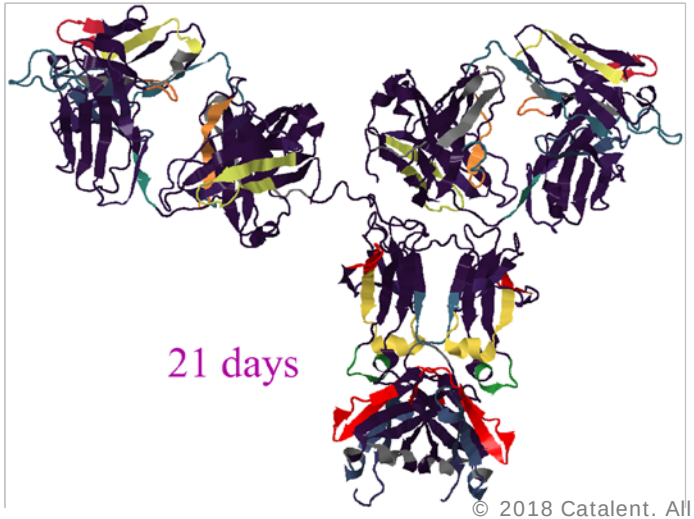
Global View - Leachable Metals Treatment

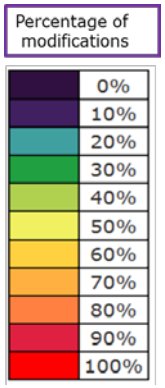
1 ppm Fe, 100 ppb Ni, 100 ppb Cr, at 25° C (±1° C)



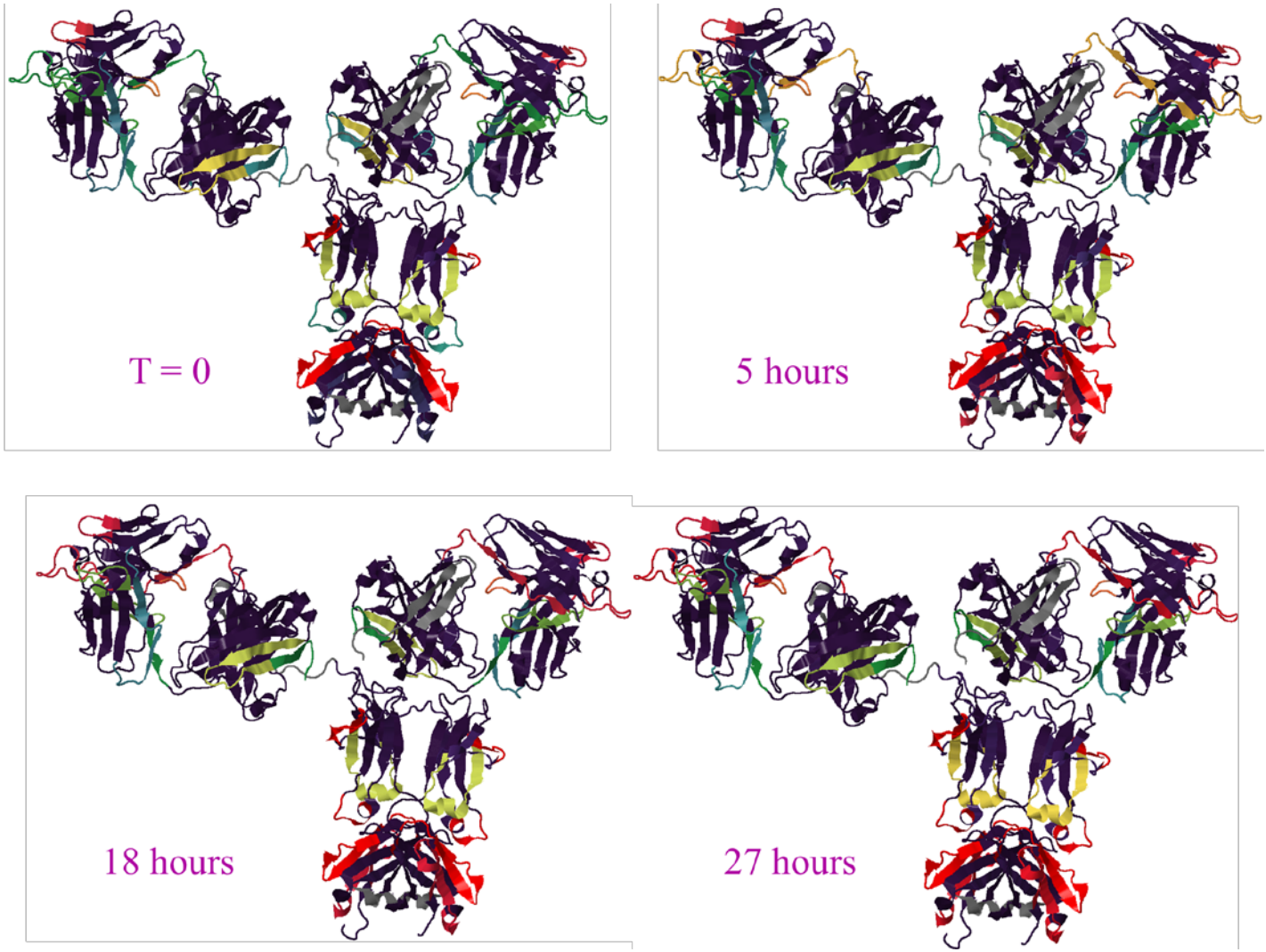








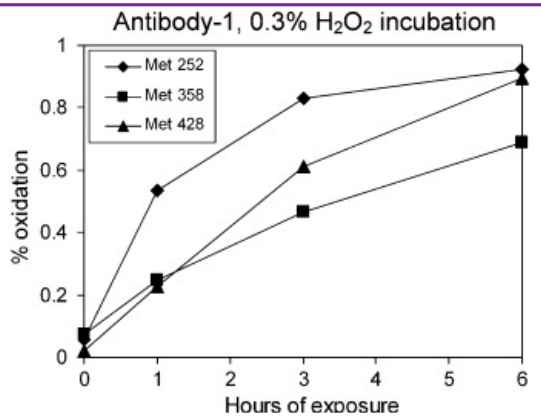
Global View - Leachable Metals + 0.15% H_2O_2

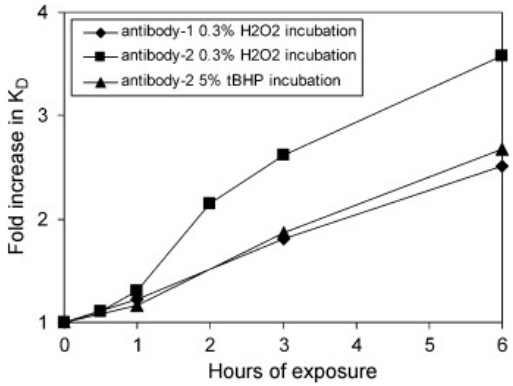


Global Characterization Reveals More Details

Protein[a]	Oxidative stress[b]	Chemical changes in primary structure[c]	Changes in higher-order structure	Biological consequences
IgG1 (humanized monoclonal)	H_2O_2	M252 (HC [d]) M428 (HC) M358 (HC)	Reduced melting temperature (*Tm*) of CH2 domain	Decreased binding to FcRn

1% of Met oxidations correspond to 4 fold increase of K_D



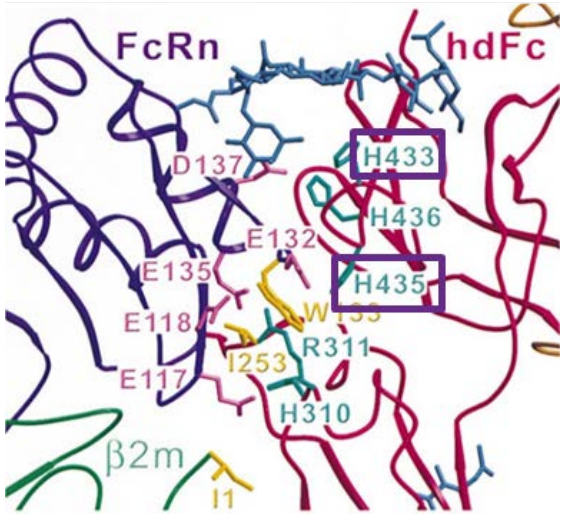


- His[433] Asn[434] His[435] on IgG1 were in direct contact with FcRn

Effect of Mutations in Human IgG1 Fc ON Binding to Human FcRn		
Mutation	Relative Binding	Predicted to Contact FcRn?
I253A	<0.10	Yes
S254A	<0.10	Yes
K288A	0.38	Yes
L309A	0.63	Yes
H433A	0.41	Yes
N434A	3.46	Yes
H435A	<0.10	Yes
Y436A	<0.10	Yes
T307A	1.81	Yes
Q311A	1.62	Yes

Table remade from Table 4 of Molecular Cell, 2001, 7, 867–877.

IgG1 FcRN complex



Pharm Res, 2014, 31:541–553
Molecular Immunology, 2009, 46: 1878–1882

Molecular Cell, 2001, 7, 867–877.

Scientific Advantage of Global Characterization

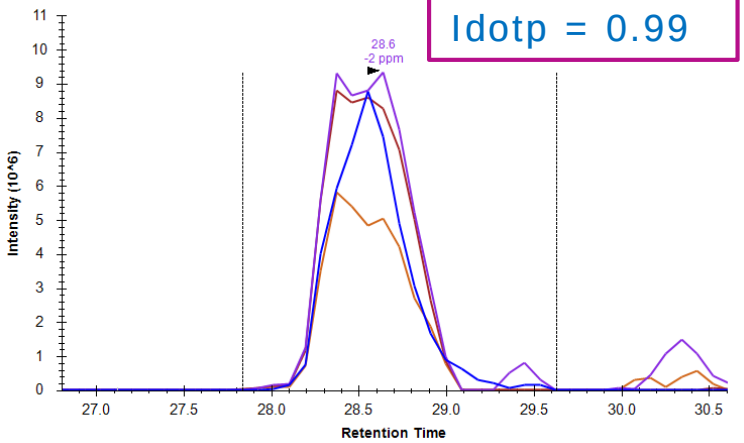
b 3 - 6 b7 b8 b9 b10-11 b13-16 b17 b18 B19-22

R.WQQGNVFSC[+57]SVM[+48]HEALH[-10]NHYTQK.S

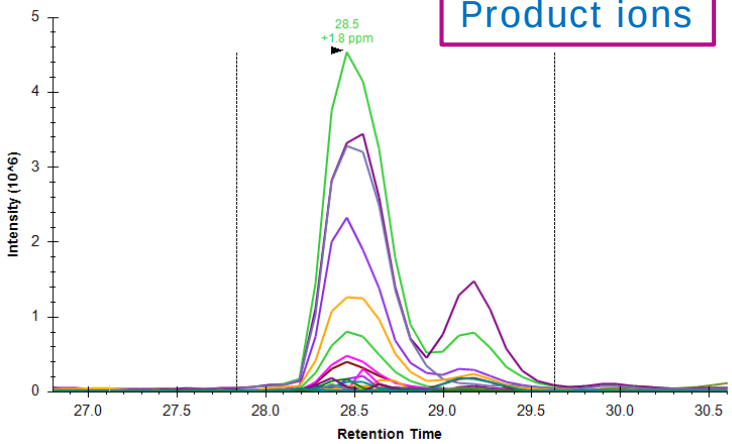
y17 y15 y13 y12 y11 y7 y6 y5 y4

[418, 439]

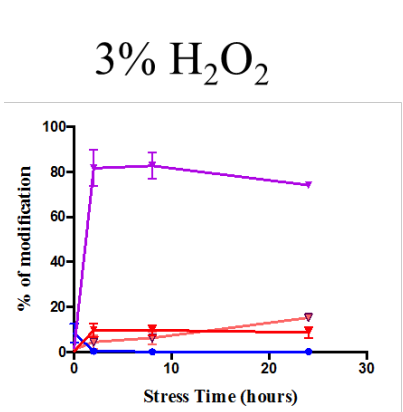
Precursor ions
Idotp = 0.99

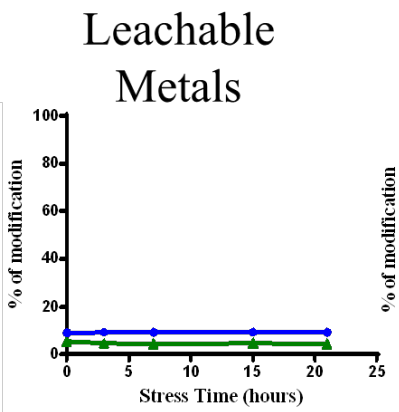


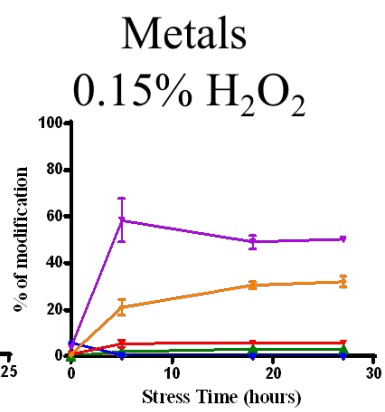
Product ions



WQQGNVFSC[+57]SVM[+16]HEALHNHYTQK
WQQGNVFSCSVM[+16]HEALHNHYTQK
WQQGNVFSC[+57]SVM[+16]HEALHN[+1]HYTQK
WQQGNVFSC[+57]SVMHEALHN[+1]HYTQK
W*QQGNVFSC*SVMHEALH*N*HYTQK
WQQGNVFSC[+57]SVM[+32]HEALHNHYTQK



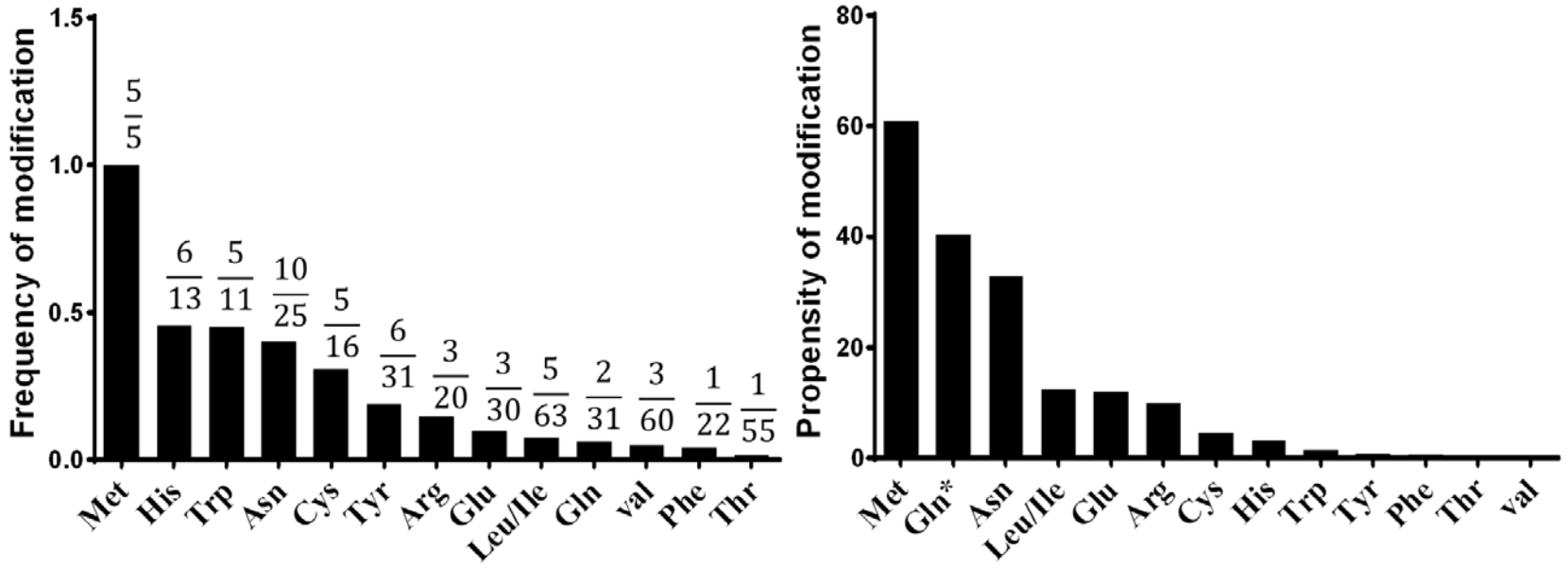




"W*QQGNVFSC*SVMHEALH*N*HYTQK" are sum of three combinations of modifications: "W[+64]QQGNVFSCSVMHEALH[-10]NHYTQK", "WQQGNVFSC[+57]SVM[+16]HEALH[-22]NHYTQK", and "W[+48]QQGNVFSC[+57]SVMHEALH[-10]NHYTQK".

H433 and N434 modification might also have an impact on Fc and FcRN binding

Frequencies of Modifications of metal + H_2O_2 Stressed IgG1



Conclusions and Future Directions

Conclusion:

- A global characterization template for IgG1 stability measurements was made by combining degradation profiles of IgG1 under three harsh stress conditions
- A semi-automated method was implemented to achieve global characterization of IgG1 and generate global characterization map

Future:

- Repeat and fully test the data processing template
- Achieve full data processing automation
- More processing templates for other molecules

Acknowledgements

Analytical Development:

- **Emma Doud Ph.D.**
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- **Lun (Luke) Xin**

Project Conducted Under:

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- **Victor Vinci Ph.D.**

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IgG 1 Exposed to leachable metals and 0.15% H_2O_2 for 27 hours

Questions?

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X-ray crystal structure source (PDB): 1HZH