

OVERVIEW

Purpose: To determine sites of post-translational modification (PTM) and scrambled disulfide bonds (sDSB) of the IgG1 monoclonal antibody adalimumab under stressed conditions using LC-MSMS

Methods: Samples were deglycosylated, reduced (or not), alkylated and digested to generate peptide fragments analyzable by reverse-phase high resolution mass spectrometry

Results: Several PTM were identified as either heat or stress induced; seven sDSB were measured; peptide coverage for HC and LC was > 95%; all predicted DSB measured

INTRODUCTION

Background: mAb adalimumab of IgG1 class; 451 amino acids (aa) in heavy chain (HC); 214 aa in light chain (LC); total MW ~ 150kDa; 16 DSB comprised of 8 HC intrachain, 4 LC intrachain, 2 HC interchain and 2 hinge

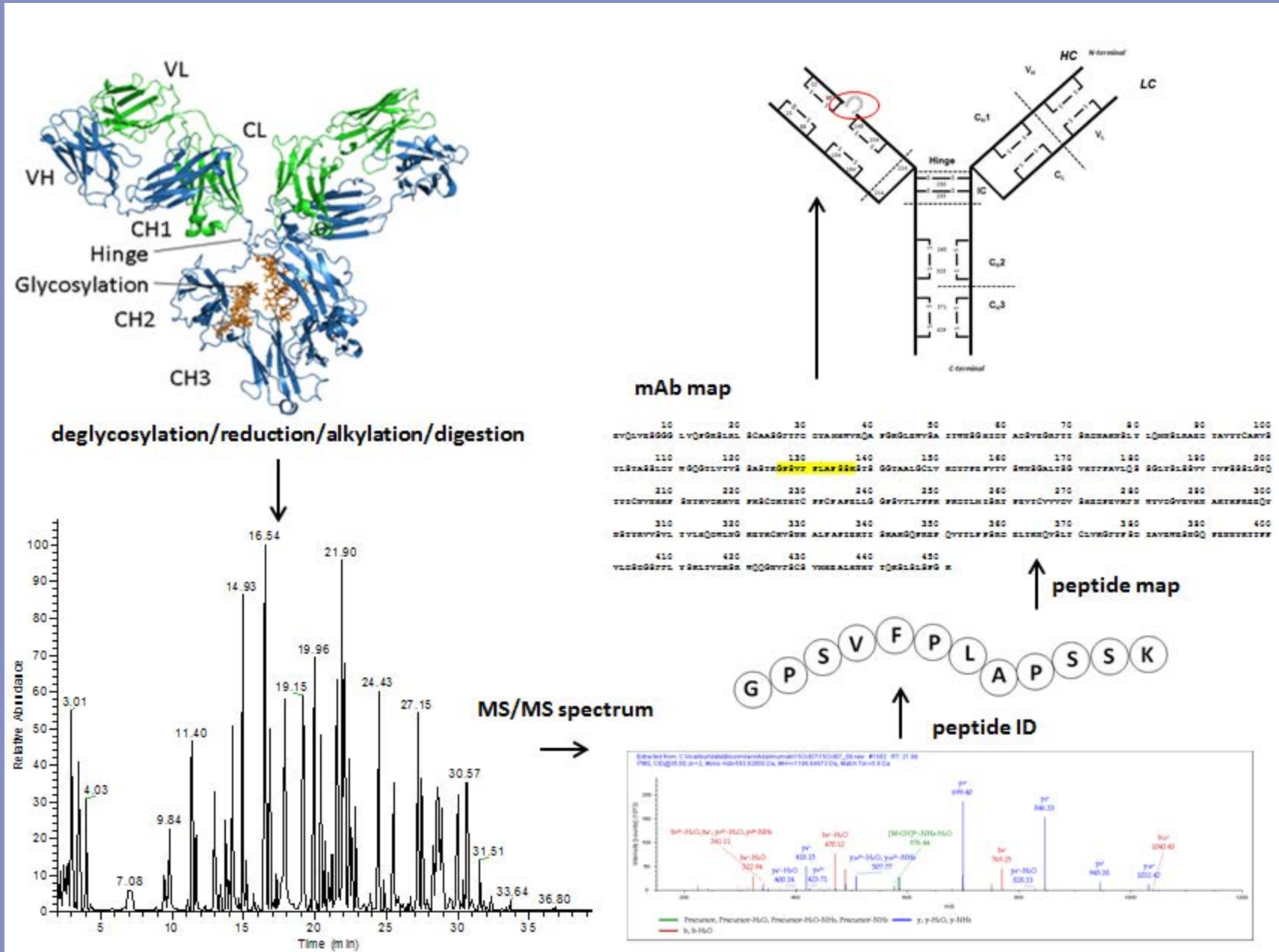
Sample: Pharmacy purchased Humera (40 mg/0.8 mL) used at 10 mg/mL

Stressed conditions:

- Temperature at 40 °C over 6 weeks
- White light (765 W/m²) exposure over 24 hr
- pH 9.0 and temperature at 40 °C for 48 hr
- Rapid heating to 75 °C

METHODS

Workflow schematic:

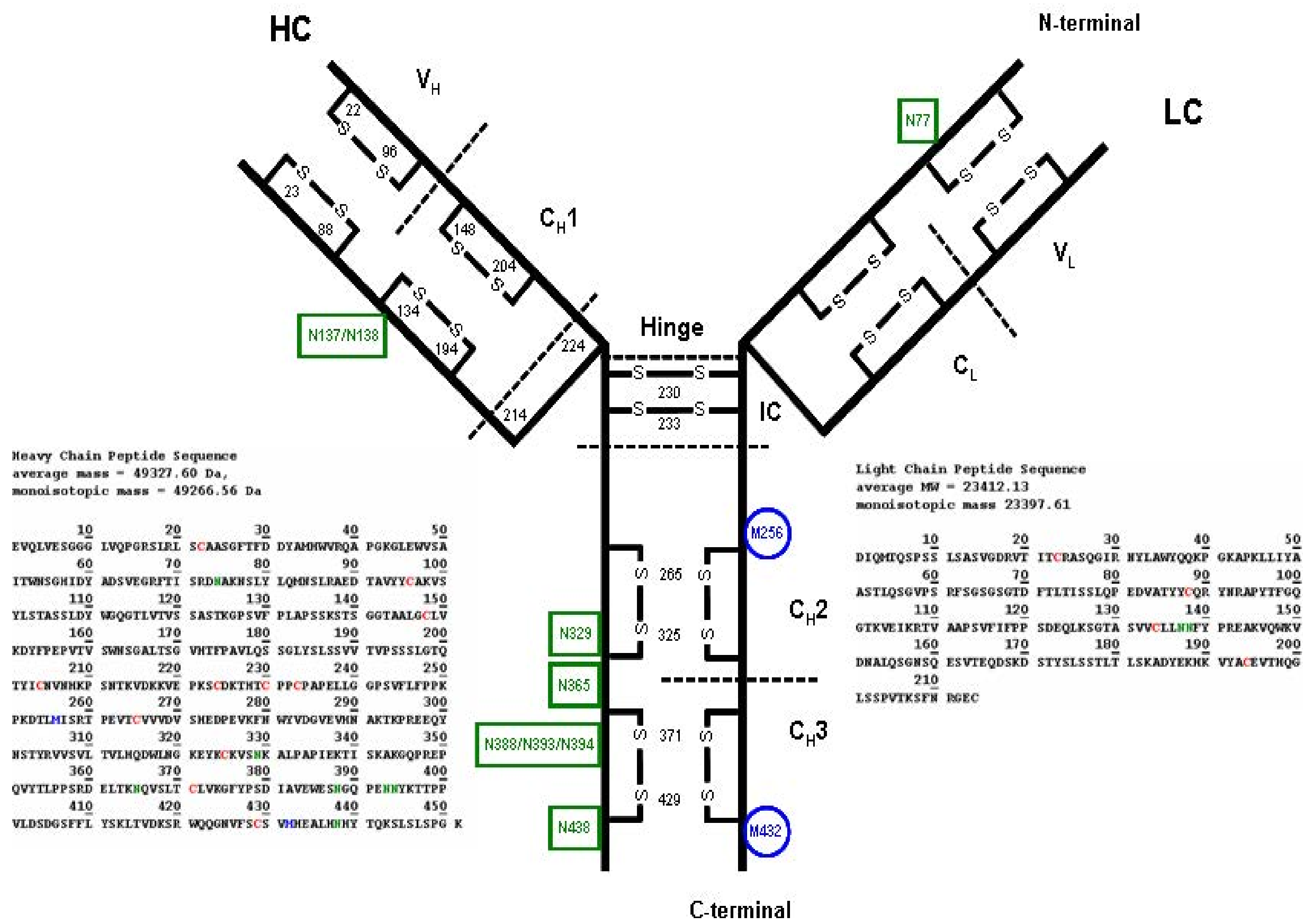


Sample preparation:

- Denatured (8M urea/100 mM tris, pH 8)
- Deglycosylated w/ PNGase-F (1:25, w/w)
- Reduced (25 mM DTT)
- Alkylated 50 mM IAA)
- Tandem digestion, Lys-C (1:25 w/w), trypsin (1:25 w/w)

RESULTS

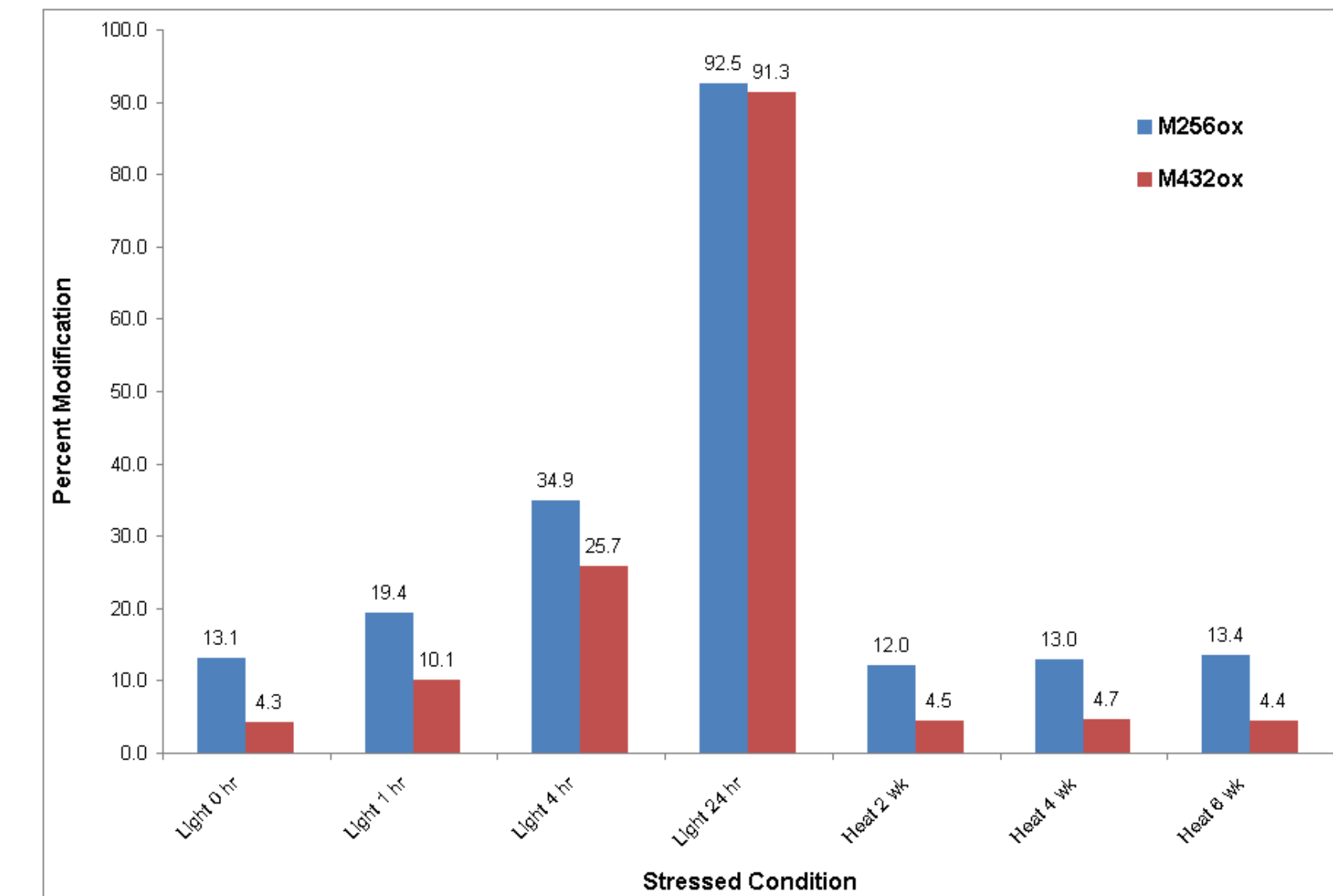
Post-Translational Modifications



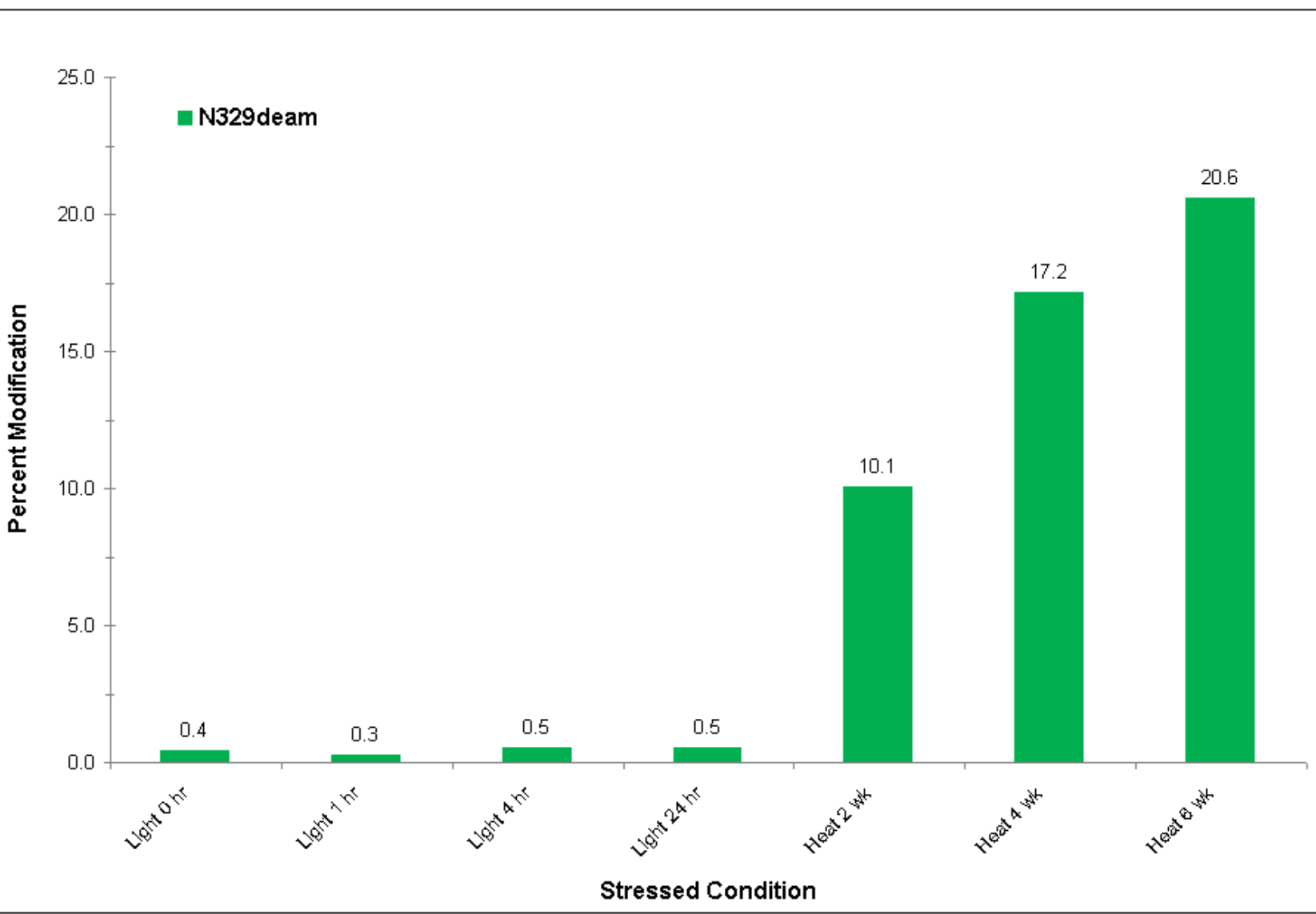
Localization of Post-Translational Modifications

Peptide ID	Modified Peptide	Site	Light 0 hr *	Light 1 hr	Light 4 hr	Light 24 hr	Heat 2 wk †	Heat 4 wk	Heat 6 wk
HCL-3	(77)NSLYQMNSLRAEDTAVYYCAK(98)	N77	1.29	1.41	1.30	1.42	1.38	1.39	1.31
HCL-15	(253)DTLMISRTPEVTCVVVDSHEDPEVK(278)	M256	13.06	19.35	34.93	92.46	12.03	12.99	13.41
HCL-21	(327)VSNIK(330)	N329	0.43	0.27	0.54	0.52	10.07	17.17	20.61
HCL-26	(365)NOVSLTCLVK(374)	N365	2.74	2.62	2.98	2.68	2.78	2.83	2.97
HCL-27	(375)GYFSPDIAVEWESNGOPENNYK(396)	N388/N393/N394	4.92	7.80	5.19	7.52	9.75	5.61	5.10
HCL-27	(375)GYFSPDIAVEWESNGOPENNYK(396)	N388/N393/N394	---	3.72	4.12	4.55	3.46	4.35	5.82
HCL-30	(419)SRWQGNVFCSSVMHEALHHYTK(443)	M432	4.30	10.09	25.72	91.25	4.51	4.67	4.36
HCL-30	(419)SRWQGNVFCSSVMHEALHHYTK(443)	N438	3.41	3.29	3.36	1.39	2.92	3.03	3.15
LCL-7	(127)SGTASVCLLNFFYPREAK(145)	N137/N138	1.91	1.74	1.67	1.75	1.63	1.85	1.89

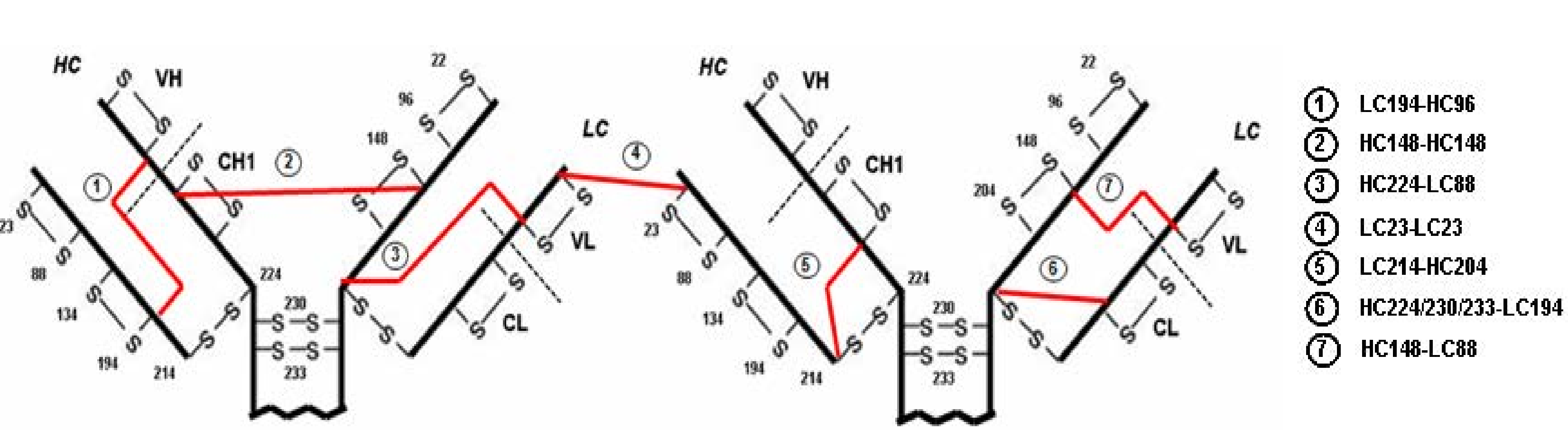
Methionine Oxidations



Asparagine Deamidation



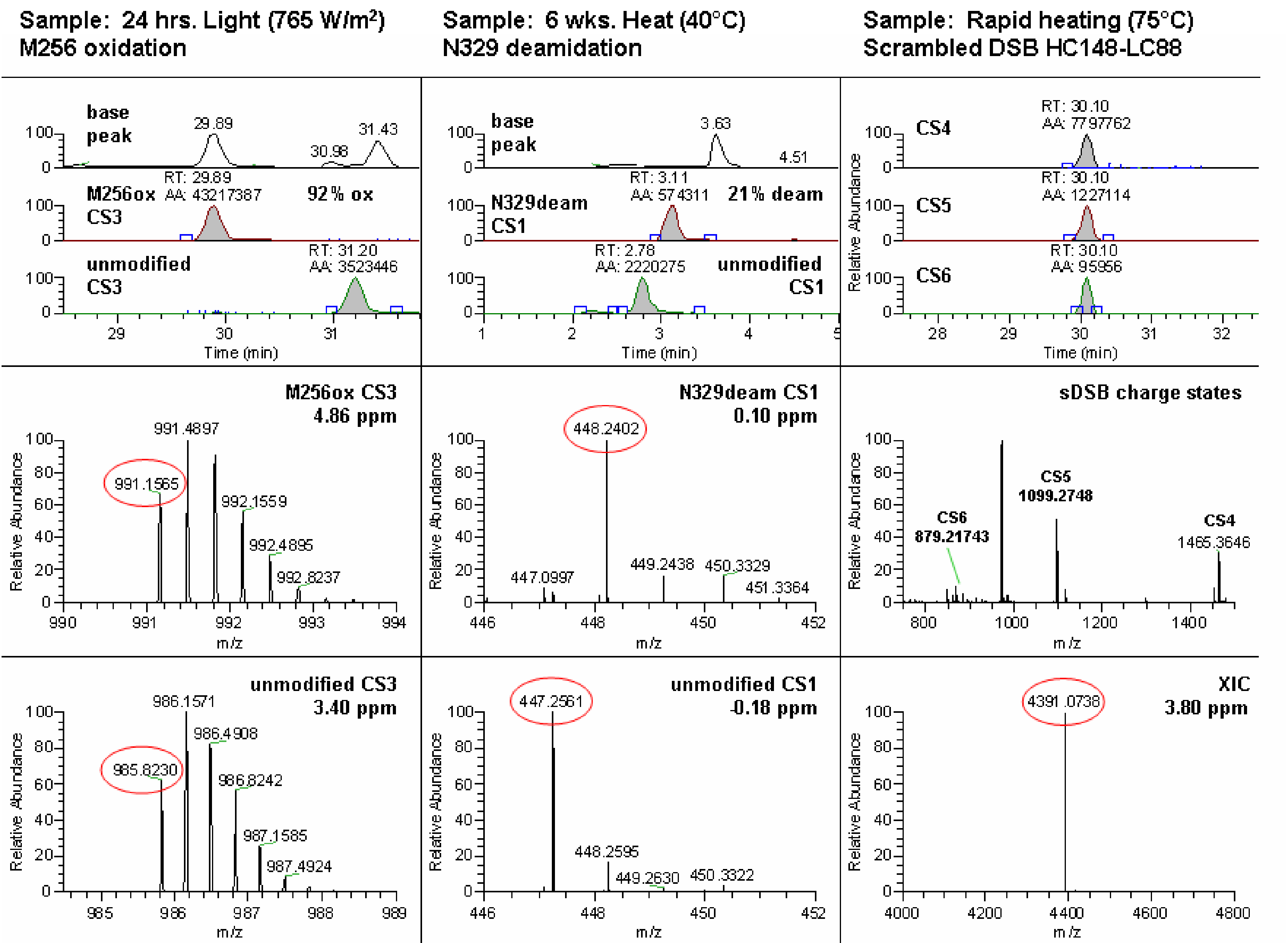
Scrambled Disulfide Bonds



Localization of Scrambled Disulfide Bonds

ID	Stressed Condition	Scrambled Linkage	Scrambled DSB	mass accuracy (ppm)	Peptide 1 :: Domain	Peptide 2 :: Domain
1	both	LC194-HC36	LC_V191-K207+HC_A88-K98	1.08	LC_VYACEVTHOGLSSPVTK :: C _L	HC_AEDTAVYYCAK :: V _H
2	both	HC148-HC148	HC_S138-K151+HC_S138-K151	2.23	HC_STSGGTAALGCLVK :: C _H 1	HC_STSGGTAALGCLVK :: C _H 1
3	heat †	HC224-LC88	HC_S223-K226+LC_F62-R80	2.37	HC_SCDK :: Hinge	LC_FSGSGSGTDFLTISLLOPEDVATYYCQR :: V _L
4	both	LC23-LC23	LC_V19-R24+LC_V19-R24	0.09	LC_VTITCR :: V _L	LC_VTITCR :: V _L
5	heat *	LC214-HC204	LC_G212-C214+HC_D152-K209	1.66	LC_GEC :: Hinge	HC_DYFPEPVTYSWNISGALTSVGHVTFPAVLSSGL YSLSSVITVPSSSLGTQTYICNVNHK :: C _H 1
6	light	HC224/230/233-LC194	HC_S223-K250+LC_H189-K207	4.60	HC_SCDKTHTCPPCPAPELLGGPSVFLFPFK :: IC	LC_HKVIYACEVTHOGLSSPVTK :: C _L
7	heat	HC148-LC88	HC_S138-K151+LC_F62-R80	3.80	HC_STSGGTAALGCLVK :: C _H 1	LC_FSGSGSGTDFLTISLLOPEDVATYYCQR :: V _L

Representative Spectra



Instrument Conditions:

Parameter	Specification		
Waters Acquity Ultra Performance Liquid Chromatograph			
Column	Waters X-Select Peptide CSH C18 XP Column 2.1 x 150 mm, 2.5 µm		
Mobile Phase	A: 0.05% (v/v) TFA in water B: 0.05% (v/v) TFA in acetonitrile		
Temperature	60°C		
Flow rate	0.200 mL/min		
Injection volume	10 µL		
Wavelength	214 nm		
Gradient	Time (min)	%A	%B
	Initial	97	3
	3.0	97	3
	50.0	60	40
	55.0	40	80
	56.0	3	97
	61.0	3	97
	70.0	97	3
Parameter	Specification		
Thermo Fisher Scientific LTQ-Orbitrap XL Mass Spectrometer			
Ion Polarity	Positive		
Duration (min)	61		
Tune Method	Updated Angiotensin Tune		
Lock Mass (Da)	391.2843		
Number of Scan Events	5		
Scan Event 1	FTMS, resolution 30000; Mass range: 200-2000 Da Scan Type: Full; Data Type: Profile		
Scan Events 2-5	ITMS/MS on top four most intense ions from scan event 1 Data Type: Centroid		
Activation Type	CID		
Isolation Width (m/z)	2		
Normalized Collision Energy (%)	35		
Default Charge State	4		
Activation Q	0.25		
Activation Time (ms)	30		
Divert Valve to Waste	Initial 2 minutes		
Dynamic Exclusion	Repeat Count: 1, Repeat Duration: 10 s Exclusion Duration: 4 s, Exclusion List Size: 150		

CONCLUSIONS

Possible sites of modification have been presented for adalimumab under stressed conditions.

- Methionine 256 and 432 showed an increase in oxidation over a 24 hour period when exposed to white light at 765 W/m².
- Asparagine 329 showed an increase in deamidation over a six week period when exposed to a elevated temperature of 40° C.
- Seven scrambled disulfide bonds were measured upon exposure to white light for 24 hours or rapid heating to 75° C. The rearranged configurations had peptides in close proximity suggesting that these occurred quickly.
- The stressed condition of pH 9.0 at 40 C for 48 hours did not show any changes.

The expanding development of the biosimilars market will require these types of investigations to further characterize these promising potential protein therapeutics.