CSL Limited Presentation

Protein Characterisation of monoclonal Antibody CSL 360

Outline

- Bioanalytical Sciences Roles, Instrumentation and Capabilities
- Monoclonal Antibody CSL360
- CSL360 manufactured for phase I trials at CMO
- CSL360 manufactured for phase II trials at CSL
- Characterisation of CSL360 Protein Isoforms

Bioanalytical Sciences – Instrumentation and Capabilities

- LC-MS Mass Spectrometry:
- N-terminal Protein Sequencer
- Biacore 3000
- HPLCs

- Peptide mapping
- N-linked glycan release and profiling
- In-gel digestion → peptide extraction → Peptide identification by MS/MS

Bioanalytical Sciences - Roles

- Release tests for biopharmaceuticals
  Tests for: purity impurities, identity, content/potency

- Characterisation assays
  - Protein chemical characterisation of clinical lots and reference lots for regulatory requirements
  - Lot-to-lot comparability characterisation to support process changes (e.g. Expression system, Seed strain, Manufacturing cell bank, Upstream / downstream process)

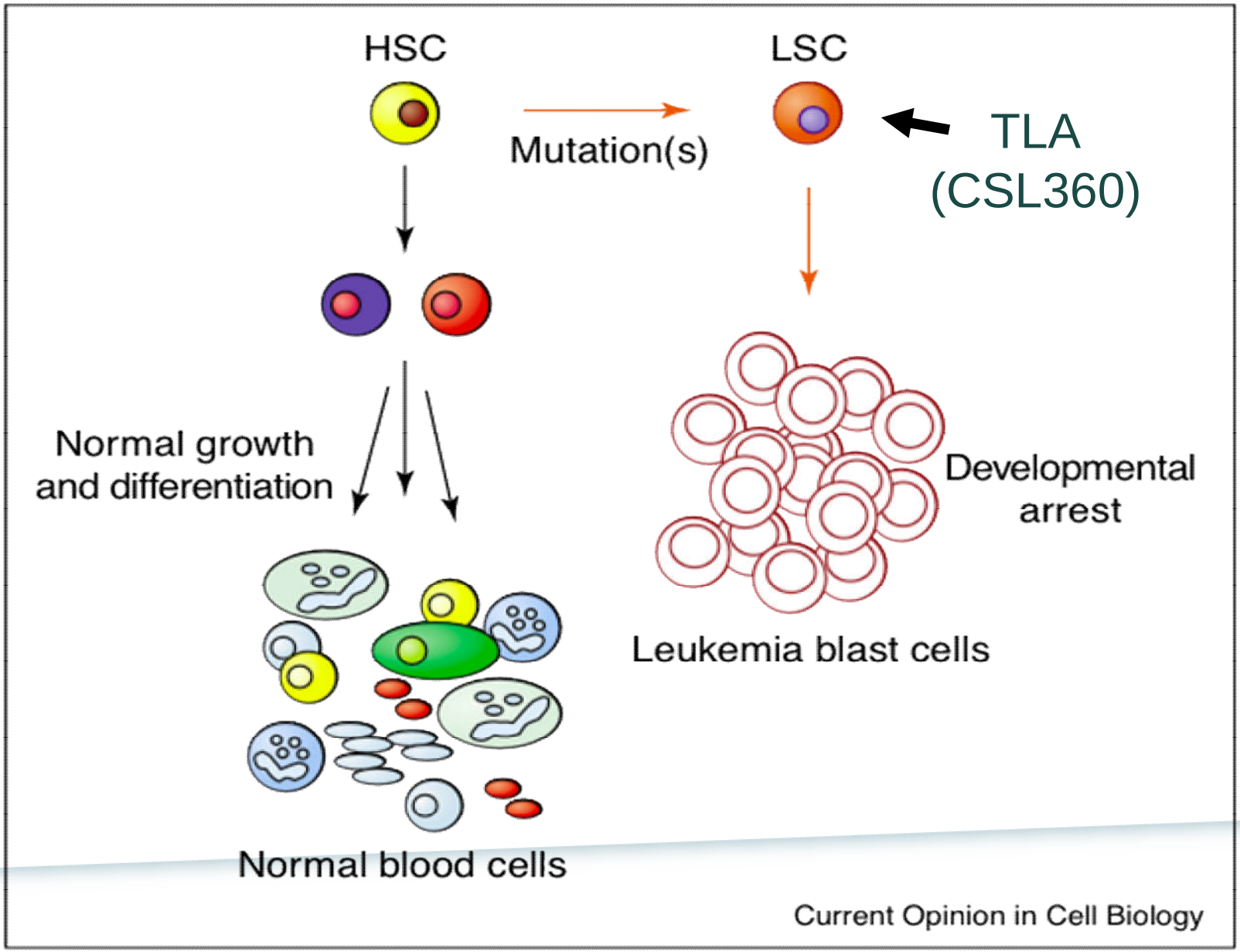
- Validation of release tests and use during phase I to III clinical trials
- Transfer of release tests to and from contract testing laboratories

CSL 360 Target Product Proposition

- A chimeric monoclonal antibody ($IgG_1\kappa$) targeting CD123 (IL-3R α-chain) positive human leukaemic stem cells to be used as an intravenous treatment of acute myeloid leukaemia (AML)

- Based on a unique IL-3R α-chain neutralising mouse monoclonal antibody (murine mAb 7G3)

Therapeutic Concept



Acute Myeloid Leukemia

- US Incidence 10,500 pa
- 18% 5 year survival, often months
- First line therapy = chemo +/- BMT
- 80% relapse/ refractory
- Mylotarg (anti CD33-calicheamycin)
  - Approved by FDA for relapsed AML if > 60
  - poor efficacy, liver toxicity

Manufacture of CSL360 at CMO for Phase I

Protein Characterisation Approach to demonstrate Lot Comparability

Aims:

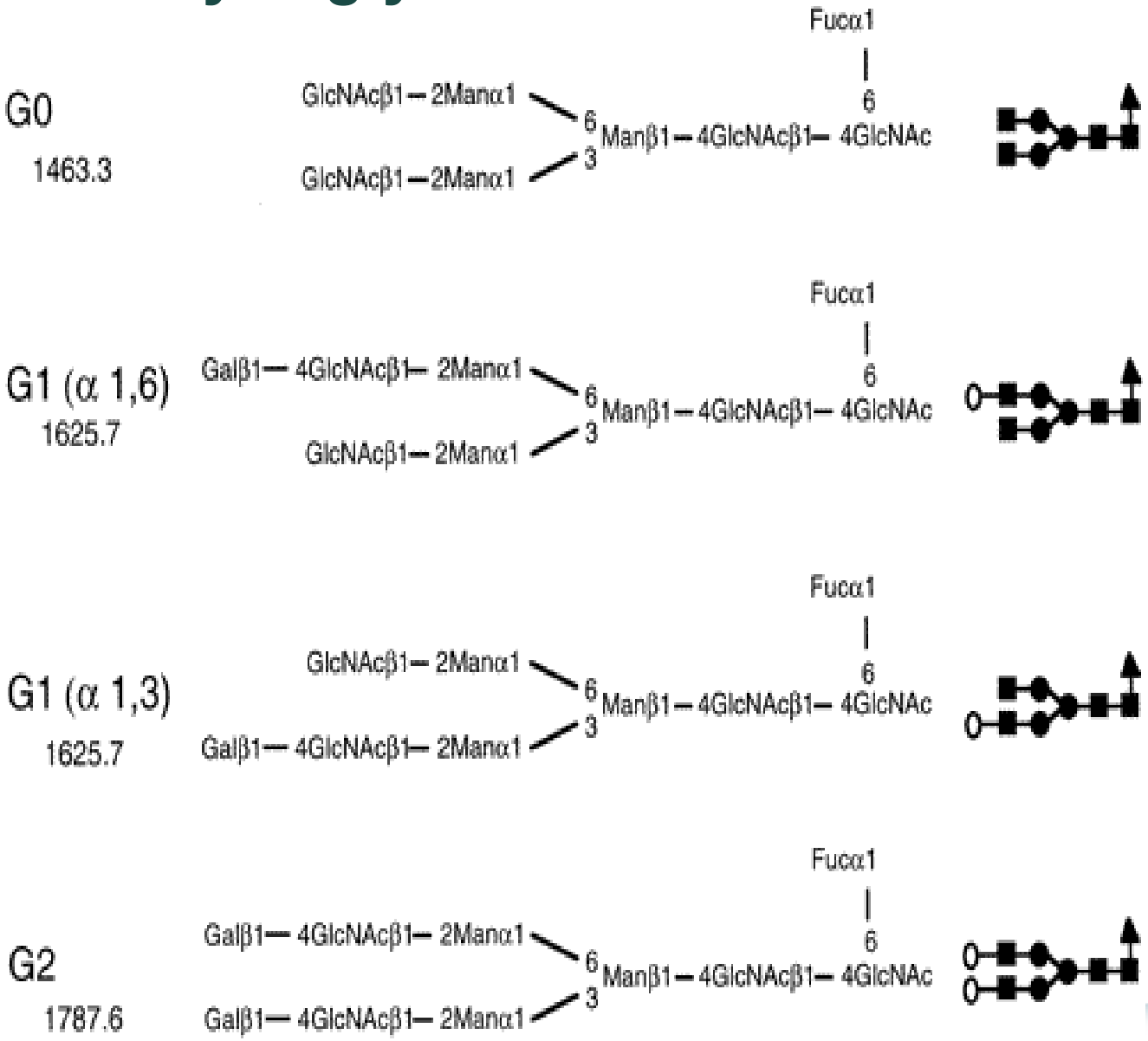
- Comparability CSL360 lots from Contract Manufacturer
- Define the Protein Structure of early CSL360 Material

  - Isoelectric Focusing
  - Cation Exchange Chromatography
  - Molecular Weight of Heavy and Light chains
  - Peptide fingerprint profile, LC-MS peptide mapping
  - Biacore binding analysis
  - Characterisation of N-glycans

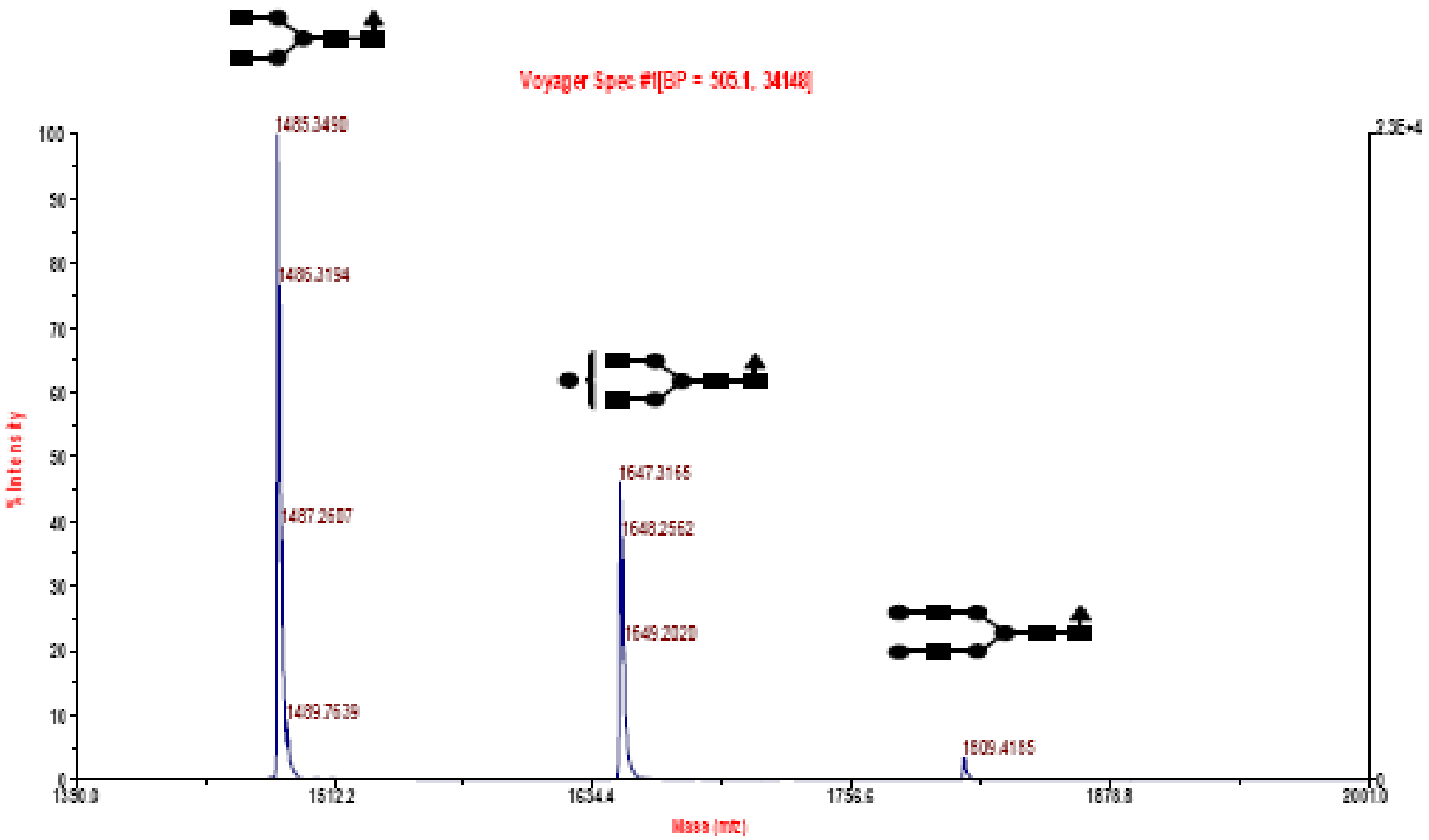
Characterisation of N-glycans on CSL360

- Release N-linked glycans by PNGase, partially purify
- Molecular ion mass by MALDI MS
- Fluorescently label glycans
- HPLC profile for quantitation

Diantennary N-glycan Structures



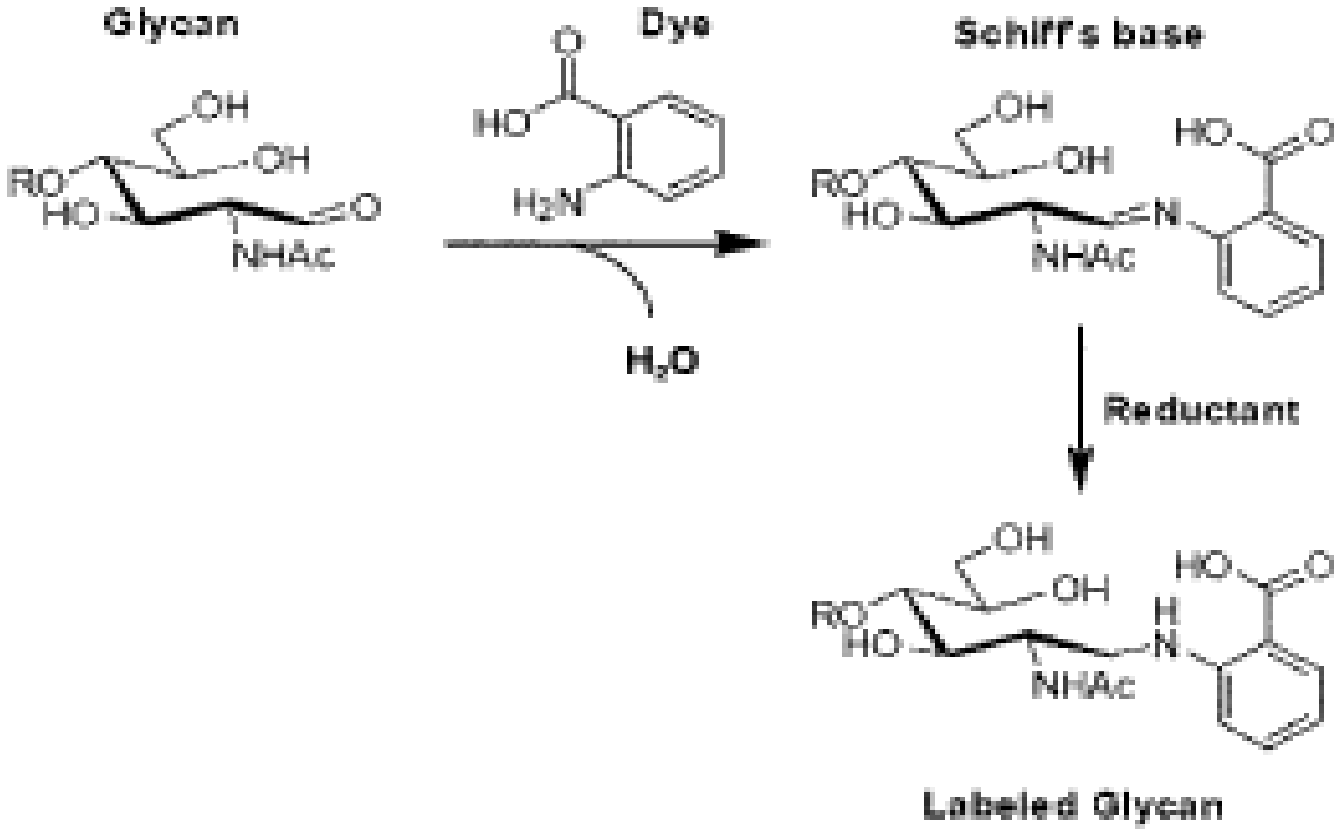
MALDI-TOF MS of released CSL360 N-glycans



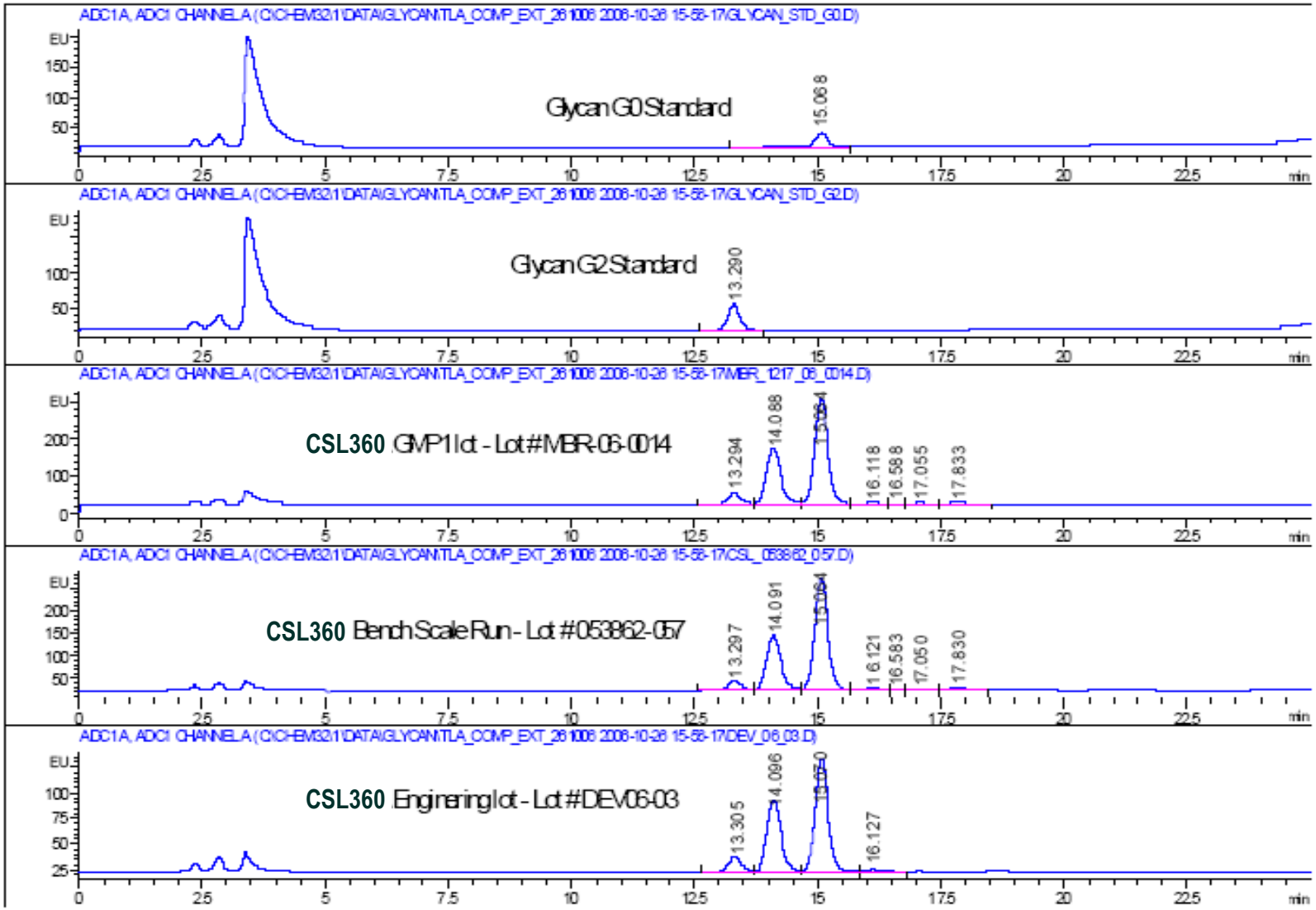
Observed and Theoretical Masses of released CSL360 N-glycans by MALDI-TOF

	Experimentally determined Mass (m/z)				Theoretical Mass (Da)	
	BSR2 Lot	Engineering Lot	GMP1 Lot	GMP2 Lot	Glycan Mass	Glycan Adduct Mass + Na^+
G2	1810.82	1810.01	1809.42	1810.45	1787.63	1810.62
G1	1647.97	1648.17	1647.32	1648.62	1625.49	1648.48
G0	1485.93	1485.55	1485.35	1486.67	1463.35	1486.34

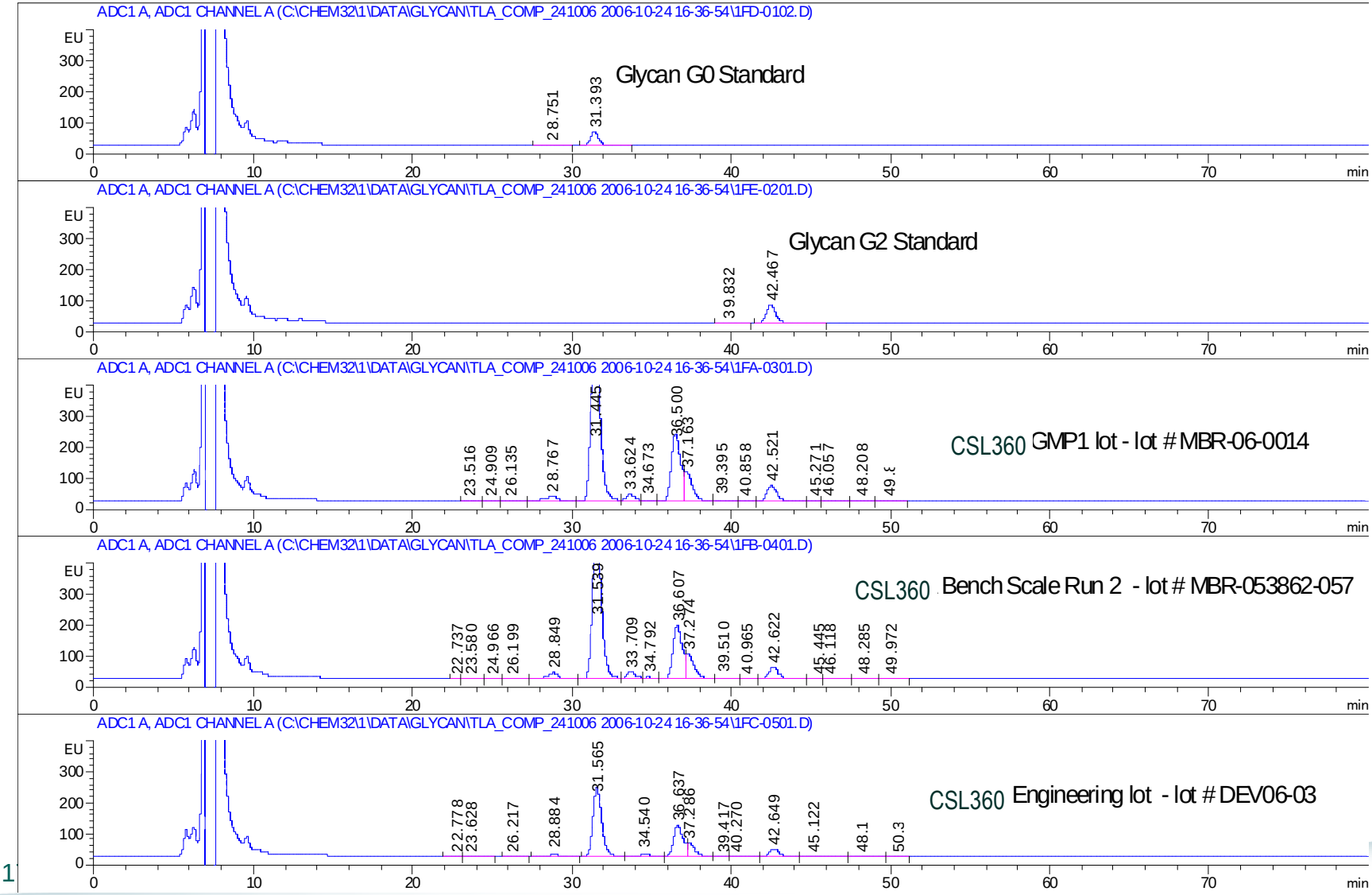
Fluorescent labelling of released N-glycans



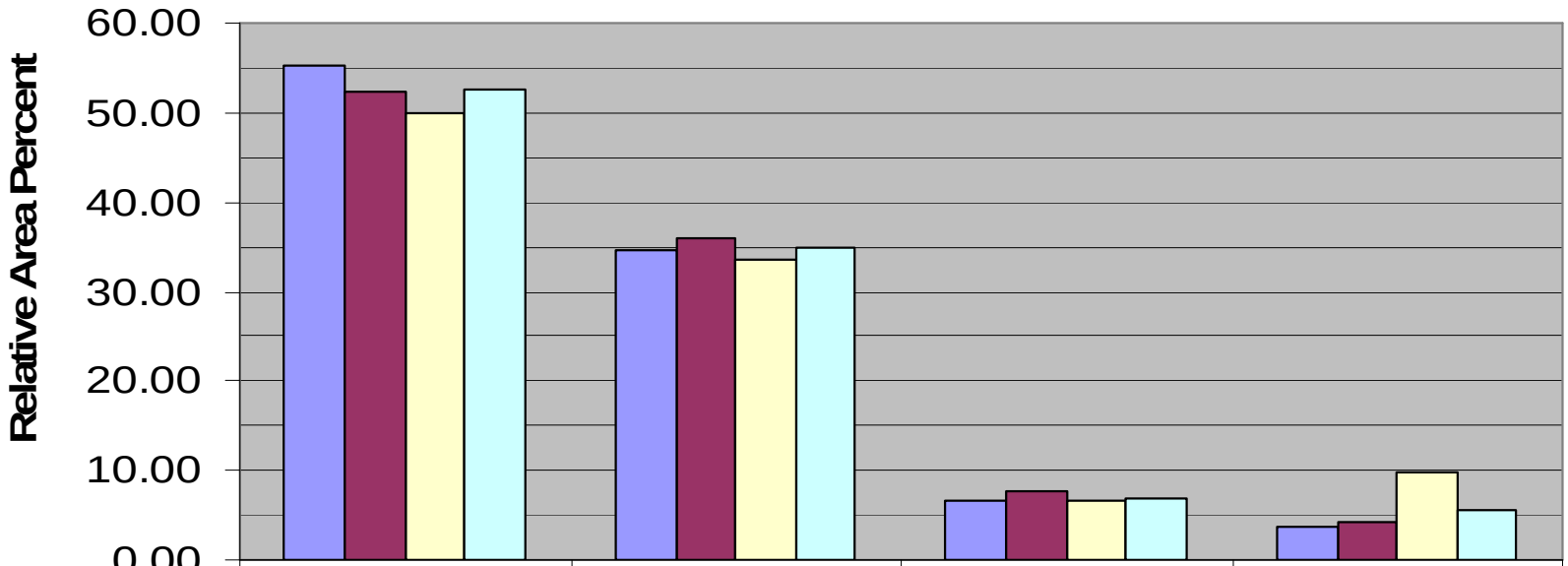
Ion Exchange Chromatogram of labelled CSL360 N-glycans



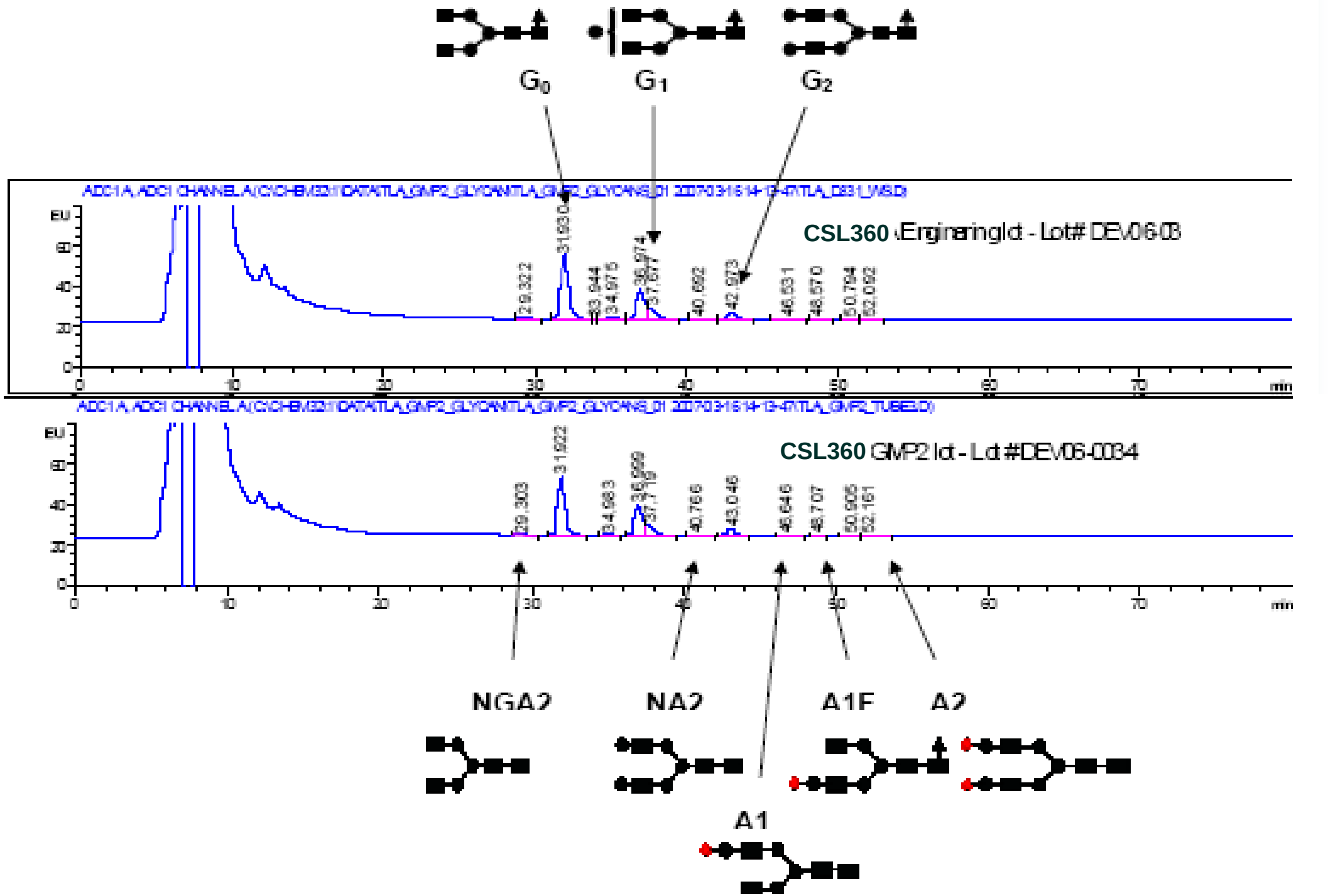
Normal Phase Chromatogram of labelled CSL360 N-glycans



Inter - Laboratory Study of CSL360 N-glycans



	Go	G1	G2	Other Peaks
CMO-IEX	55.20	34.50	6.60	3.70
CSL-IEX	52.26	35.83	7.61	4.30
CSL-NP (Exp -1)	50.00	33.49	6.68	9.83
CSL-NP (Exp -2)	52.66	34.91	6.75	5.66


G0
G1
G2
CSL360 Engineering Id - Lot# DEV06-03
29.322
31.930
33.944
34.975
36.974
37.677
40.692
42.973
46.631
48.570
50.794
52.092
min
CSL360 GMP2 Id - Lot #DEV06-0034
29.303
31.922
34.983
36.999
37.719
40.766
43.045
46.646
48.707
50.905
52.161
min
NGA2
NA2
A1F
A2
A1

Quantitation of CSL360 N-glycan HPLC profile (% peak areas)

		Bench Scale Run 2	Engineering Lot	GMP 1 Lot	GMP 2 Lot	
		053862-057	DEV06-03	MBR-06-0014	MBR-06-0034	TLA
	NGA2	2.7	1.9	2.2	1.0	
G0	**(NGA2F)**	**57.3**	**52.7**	**56.1**	**49.4**	
G1	**(NA1F)**	**29.4**	**34.9**	**31.2**	**38.0**	
	NA2	0.2	0.4	0.2	0.5	
G2	**(NA2F)**	**5.1**	**6.8**	**6.0**	**8.5**	
	A1	0.2	0.3	0.2	0.3	
	A1F	0.0	0.1	0.1	0.1	
	A2	0.1	0.2	0.1	0.2	
	A2F	ND	ND	ND	ND	
	A3	ND	ND	ND	ND	
Not identified peak areas		4.9	2.8	4.0	2.0	
		100.0	100.0	100.0	100.0	
Total of non-fucosylated		3.2	2.8	2.7	2.0	
Total of sialylated		0.32	0.58	0.32	0.64	

Summary:
Fluorescence HPLC profiling of N-glycans from CMO

- Three major glycans account for >90% of N-linked glycans on CSL360
- Four CSL360 lots manufactured at CMO have an essentially similar glycan profile
- NP-HPLC allows for quantitation of minor additional species of glycans
- Some 10 additional forms were detected, the most abundant individual species was 2.7%

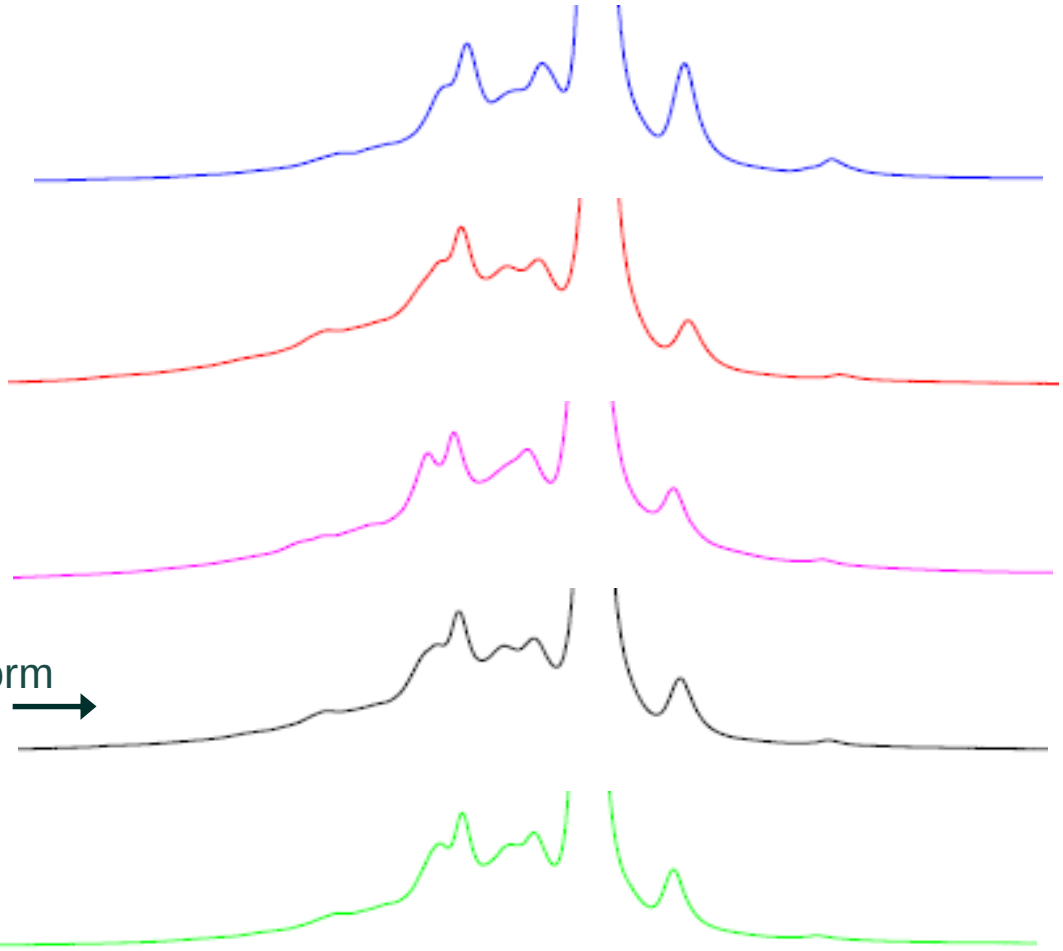
Development Progress for CSL360

- CMO material in clinical safety trial
- Tech Transfer from CMO to CSL
- Process improvements:
  - Yield ↑ 5 x
  - Improved manufacturability
  - Improved quality, e.g. viral clearance ↑ 10 logs

Protein Characterisation of CLS360 lots manufactured in 2008:

- Isoelectric focusing
- Cation Exchange Chromatography
- Molecular weight of Heavy and Light chains
- Peptide fingerprint profile/peptide map
- Biacore binding analysis, kinetic study
- N-glycan analysis

Cation Exchange Chromatography Profiles of CSL360 Lots manufactured at CSL in 2008



CMO Ref Lot DEV03-06

CSL GMP 1 Lot 526901T55

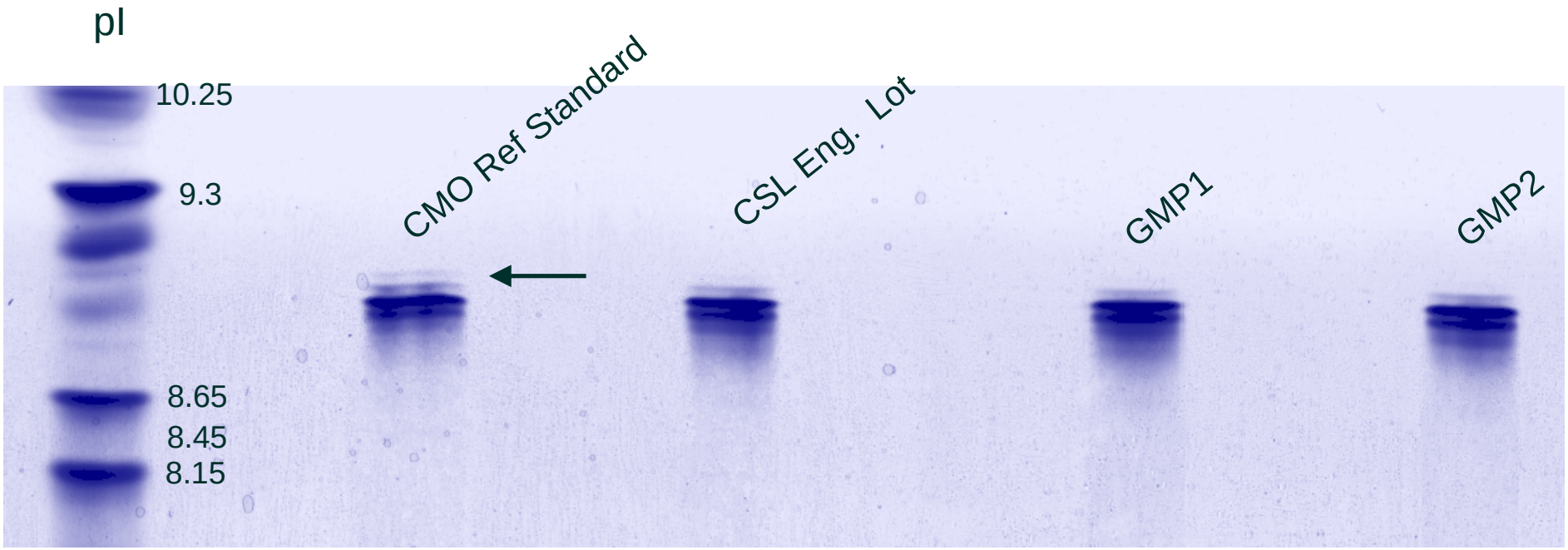
CSL Eng Lot 8269000177

Study
aterial for isoform
analysis

CSL GMP 2 Lot 526901T59

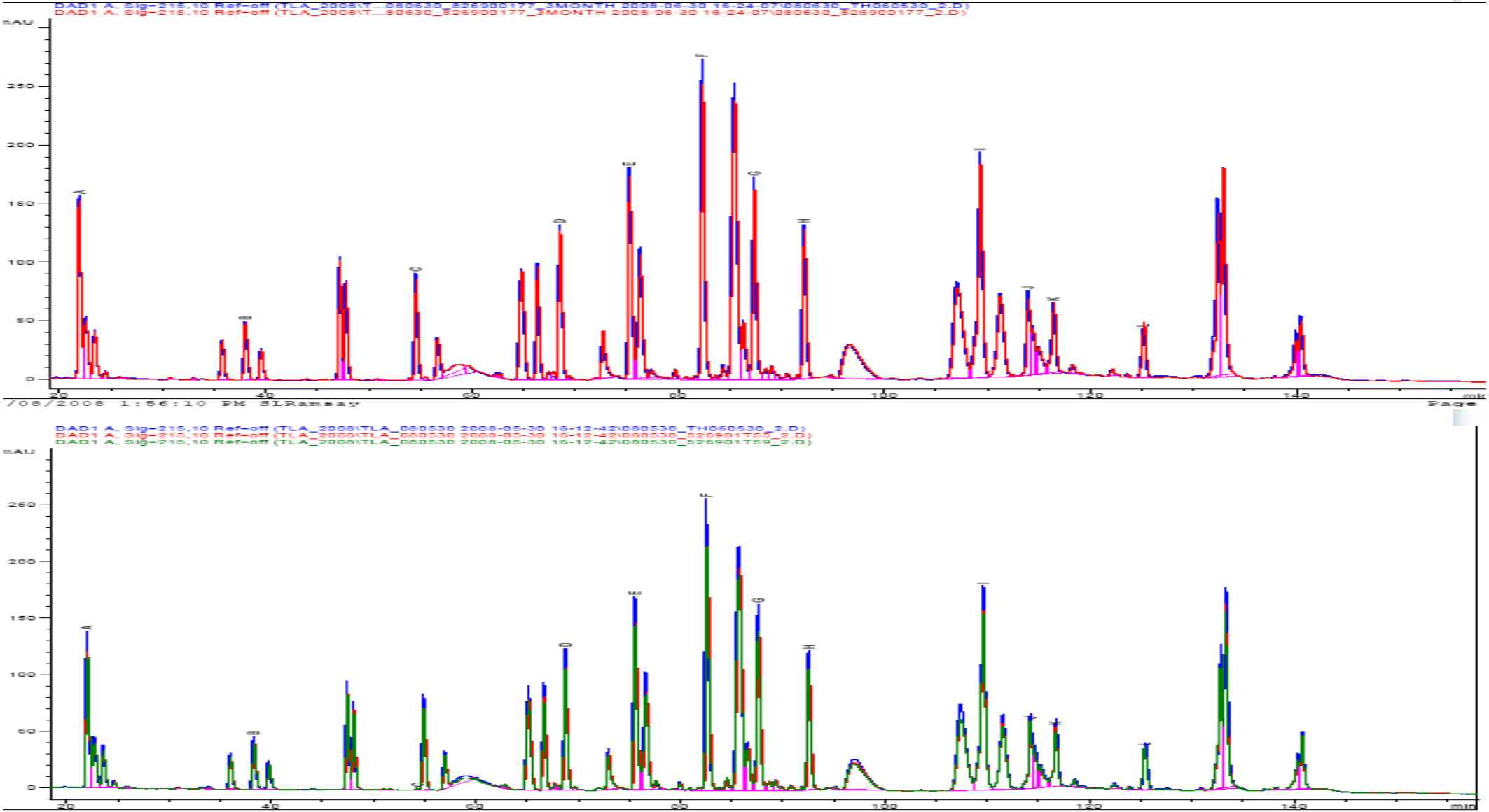
CSL GMP3 Lot 526901T85

IEF of CSL360 lots manufactured at CSL in 2008



- Major banding pattern is similar between CMO and CSL
- Most basic species is absent in new CSL360 lots

Peptide Fingerprint Profile of CSL360 lots mfg in 2008



Masses of CSL360 Heavy and Light chains by LC-MS

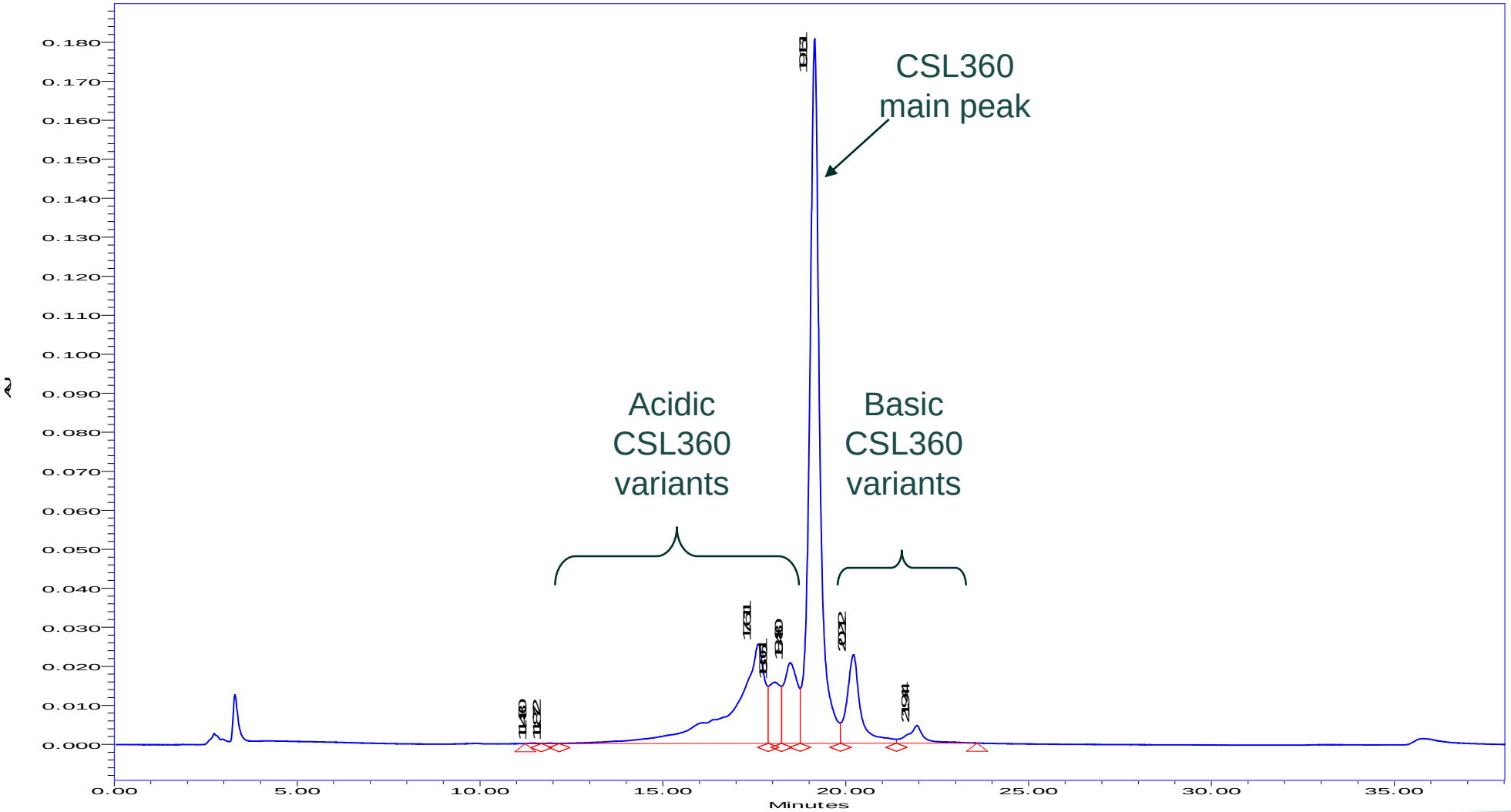
Heavy

Sample	Measured Mass (M_r)(Da)	Theoretical Mass (Da)	Mass Range of CMO Ref Std ± 3 SD (Da)
CMO Reference standard	51,413.5	51,410.8	51,407.2 – 51,419.8
CSL Engineering Lot	51,411.5		
GMP lot 1	51,410.8		
GMP lot 2	51,410.1		
GMP lot 3	51,412.2		

Light

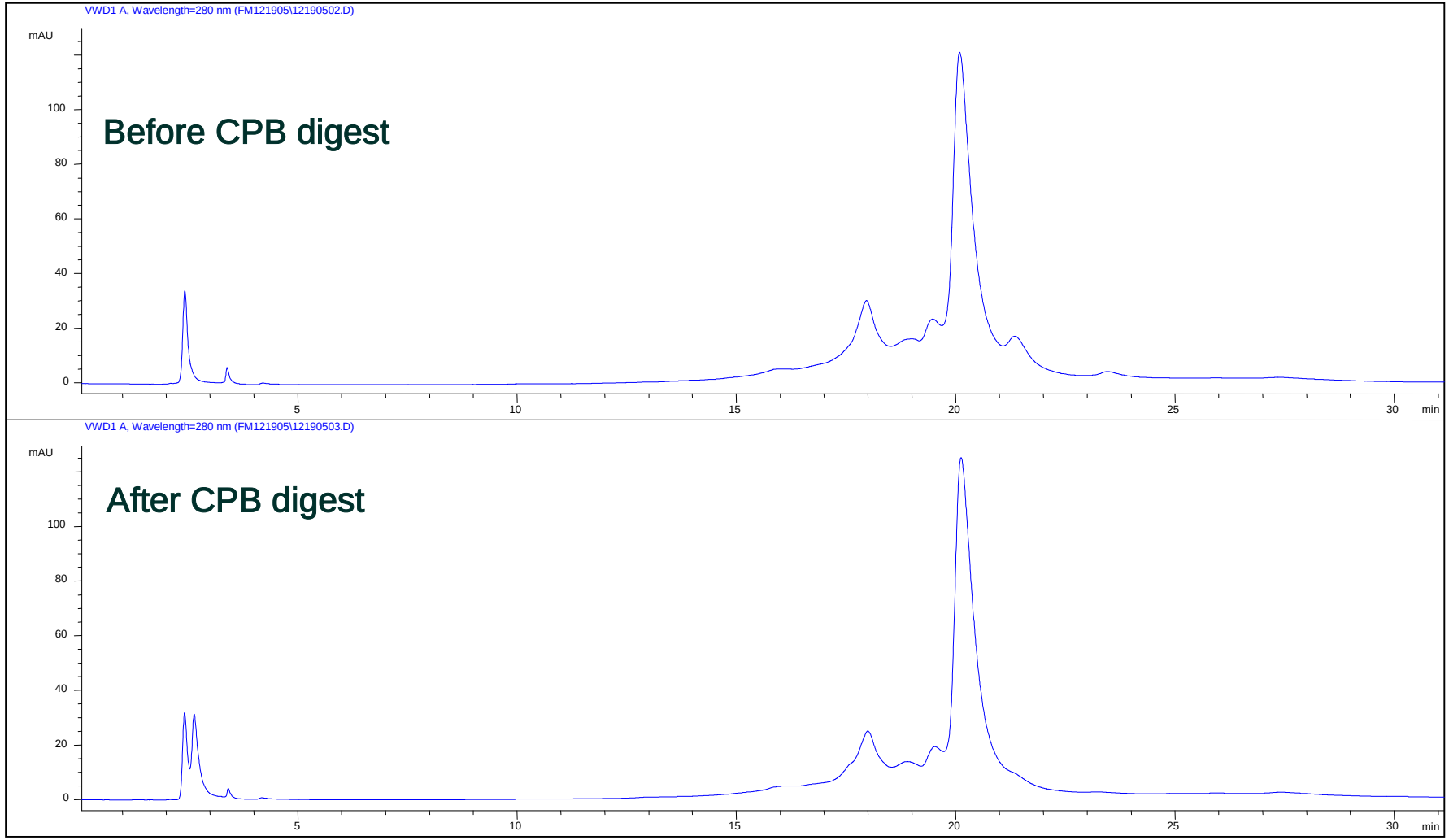
Sample	Measured Mass (Mr) (Da)	Theoretical Mass (Da)	Mass Range of CMO Ref Std ± 3 SD (Da)
CMO reference standard	24,463.4	24,463.2	24,462.2 24,464.6
CSL Engineering Lot	24,463.4		
GMP lot 1	24,462.5		
GMP lot 2	24,463.0		
GMP lot 3	24,463.3		

Cation Exchange Chromatography Profile

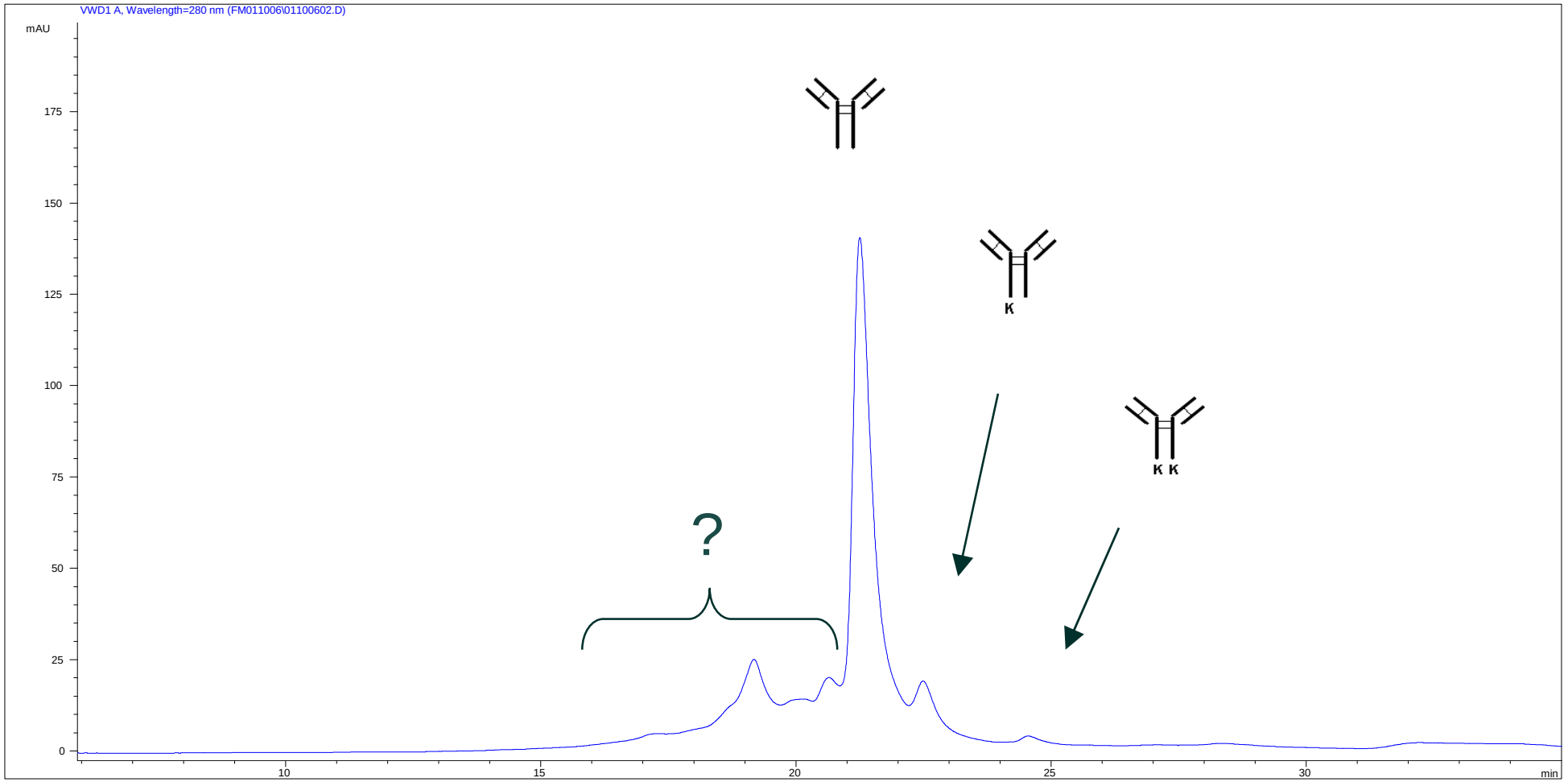


SampleName TLA_D831_WS_TH060530A - 40ug; Injection 1; Injection Volume 40.00; Date Acquired 5/09/2006 2:17:31 PM; Sample Set Name IXC_IgG_Qual_Repeatability; Acq Method Set IXC_ICOS_IgG_01

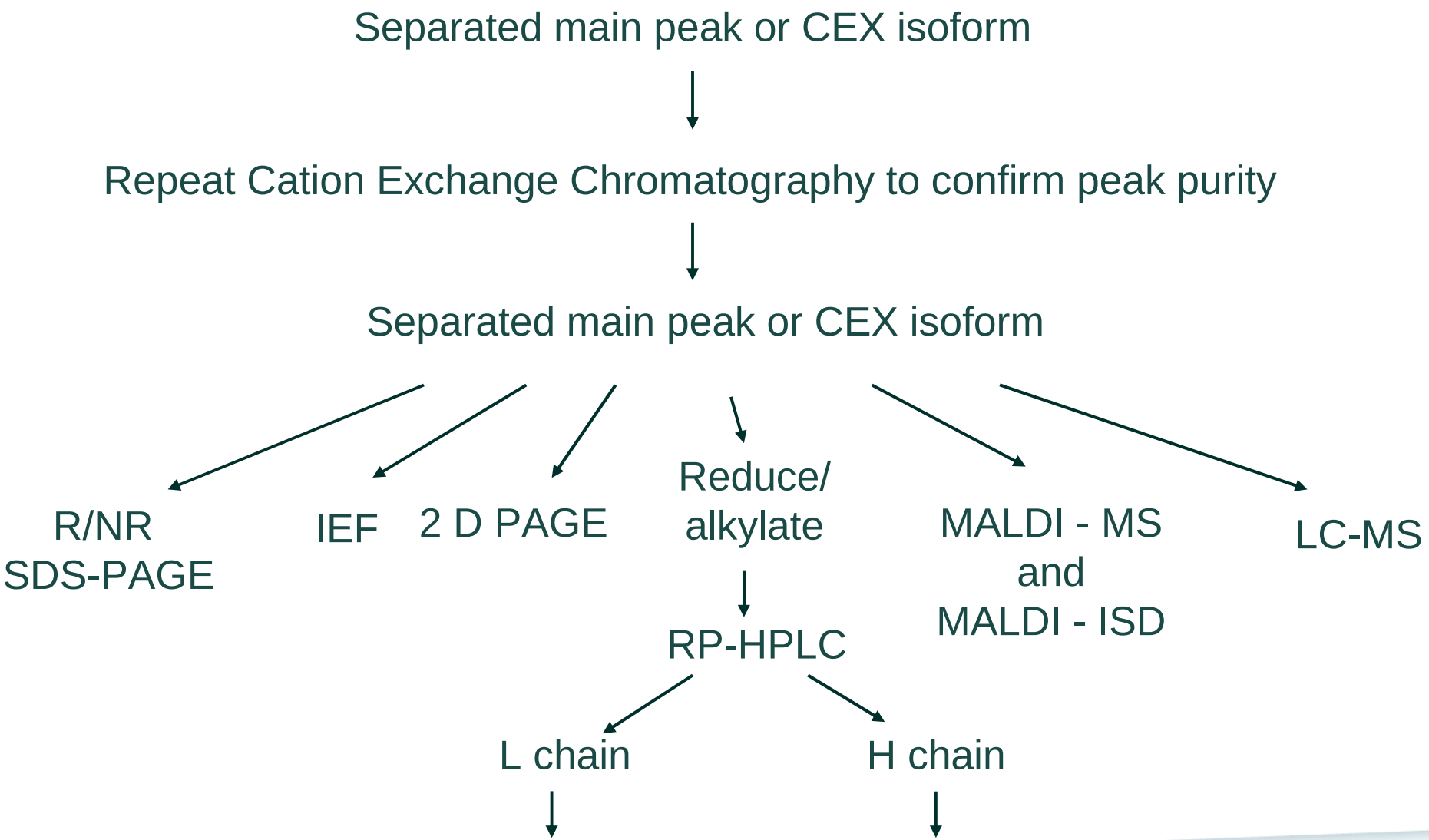
CEX Method - Carboxypeptidase B



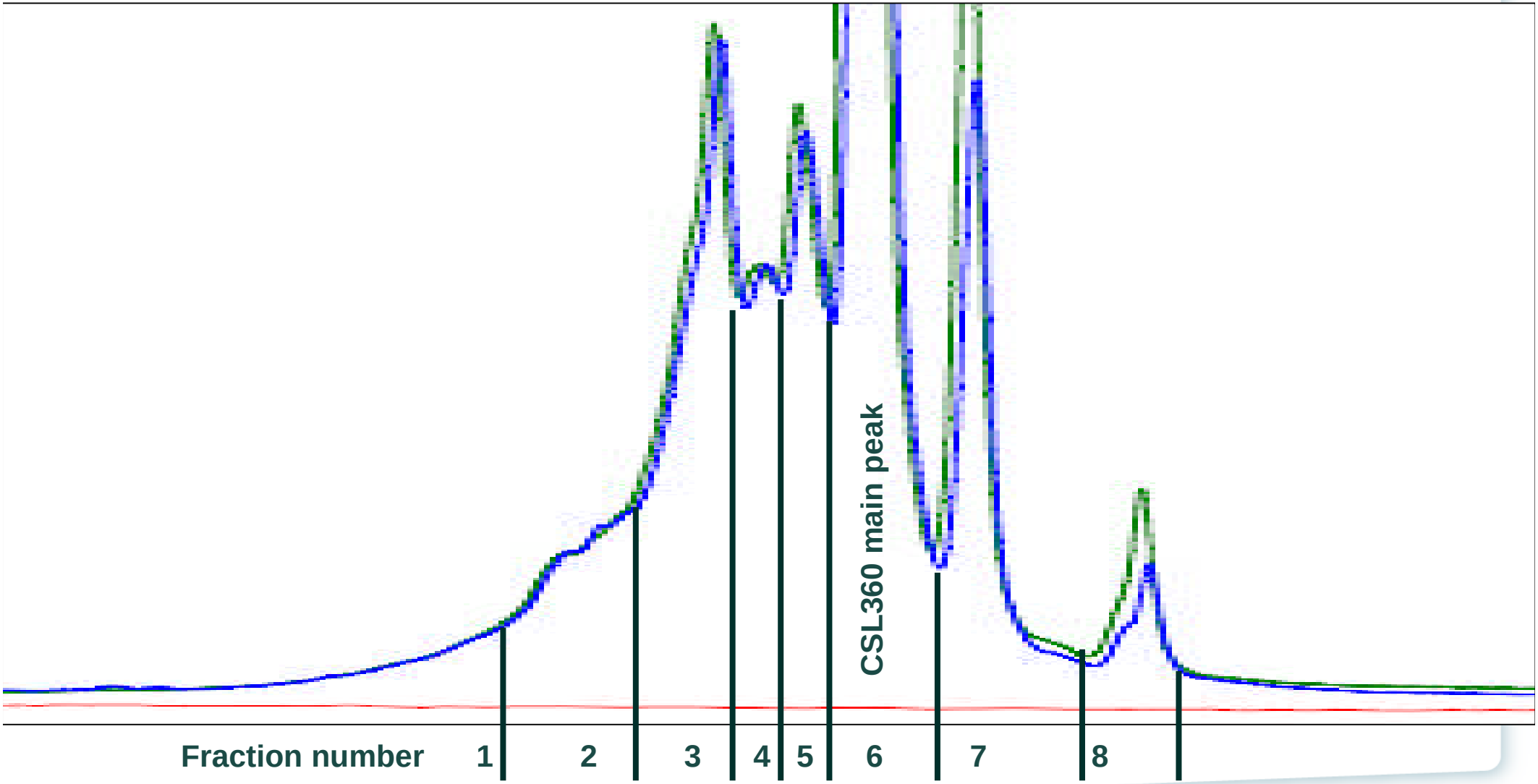
CEX Method - Hypothetical Structures



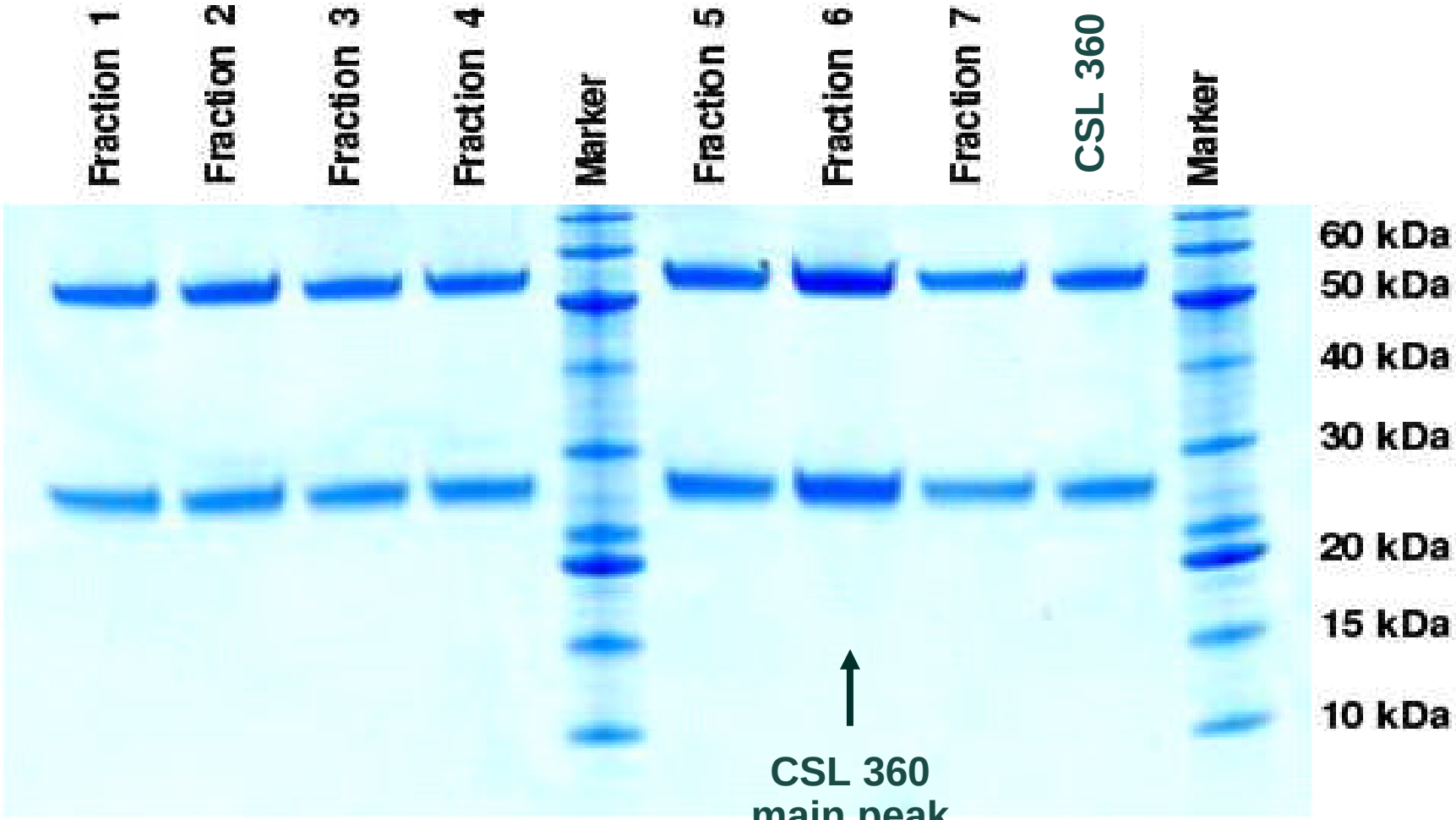
Characterisation of CSL360 Isoforms



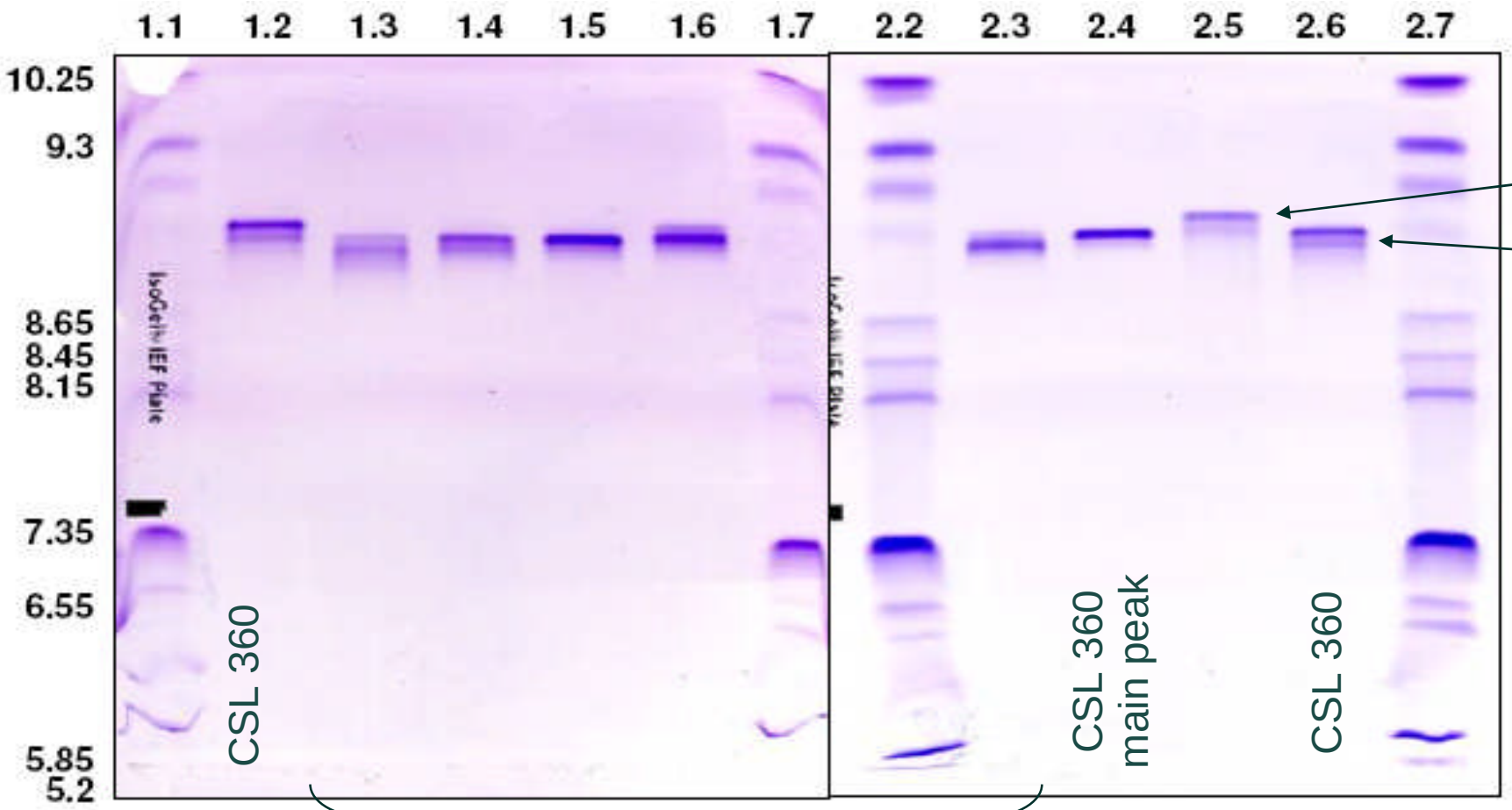
Fractionation of CSL360 CEX Peaks



Reducing SDS-PAGE of CEX Fractions



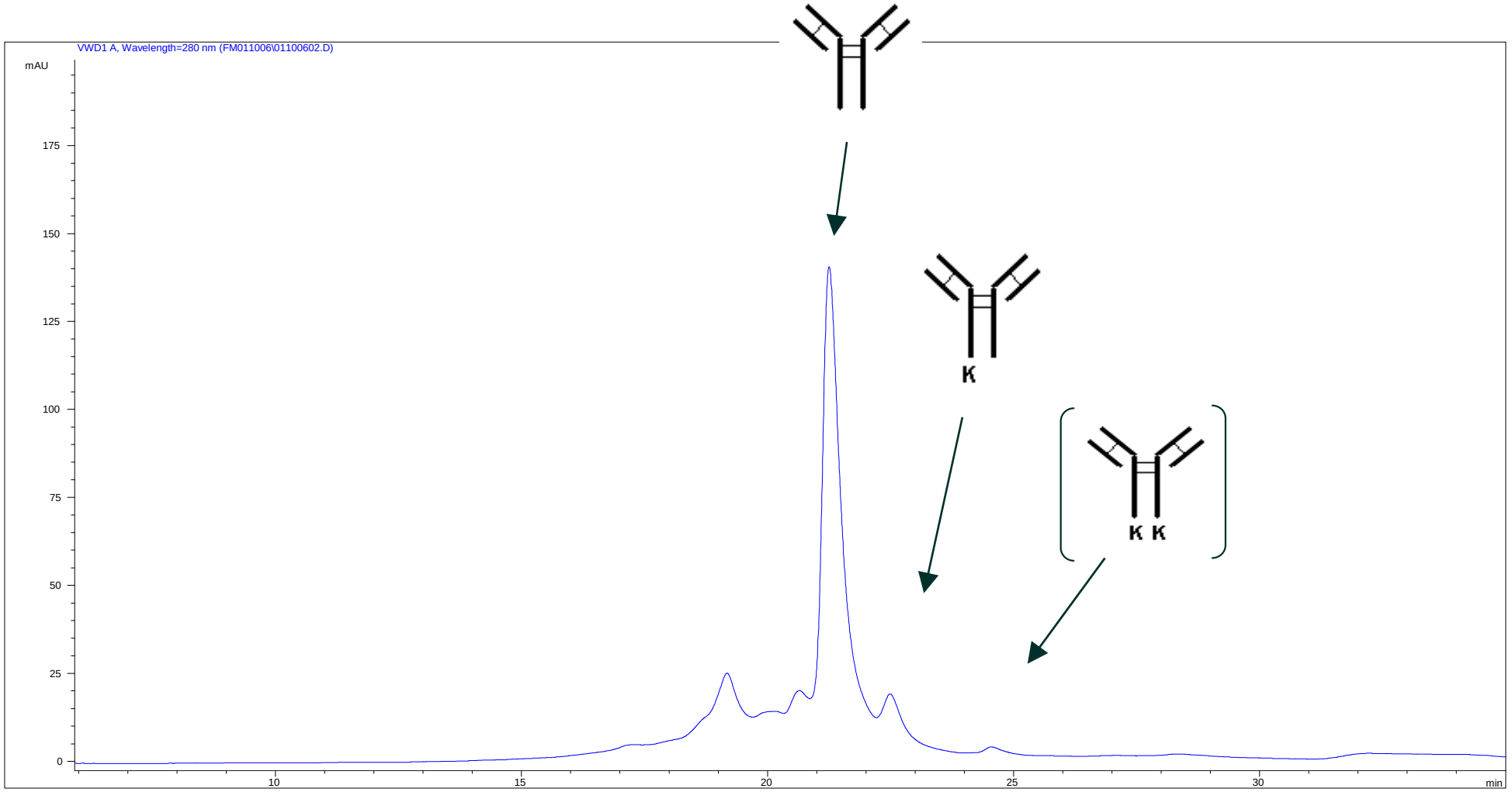
IEF of Cation Exchange Chromatography Fractions



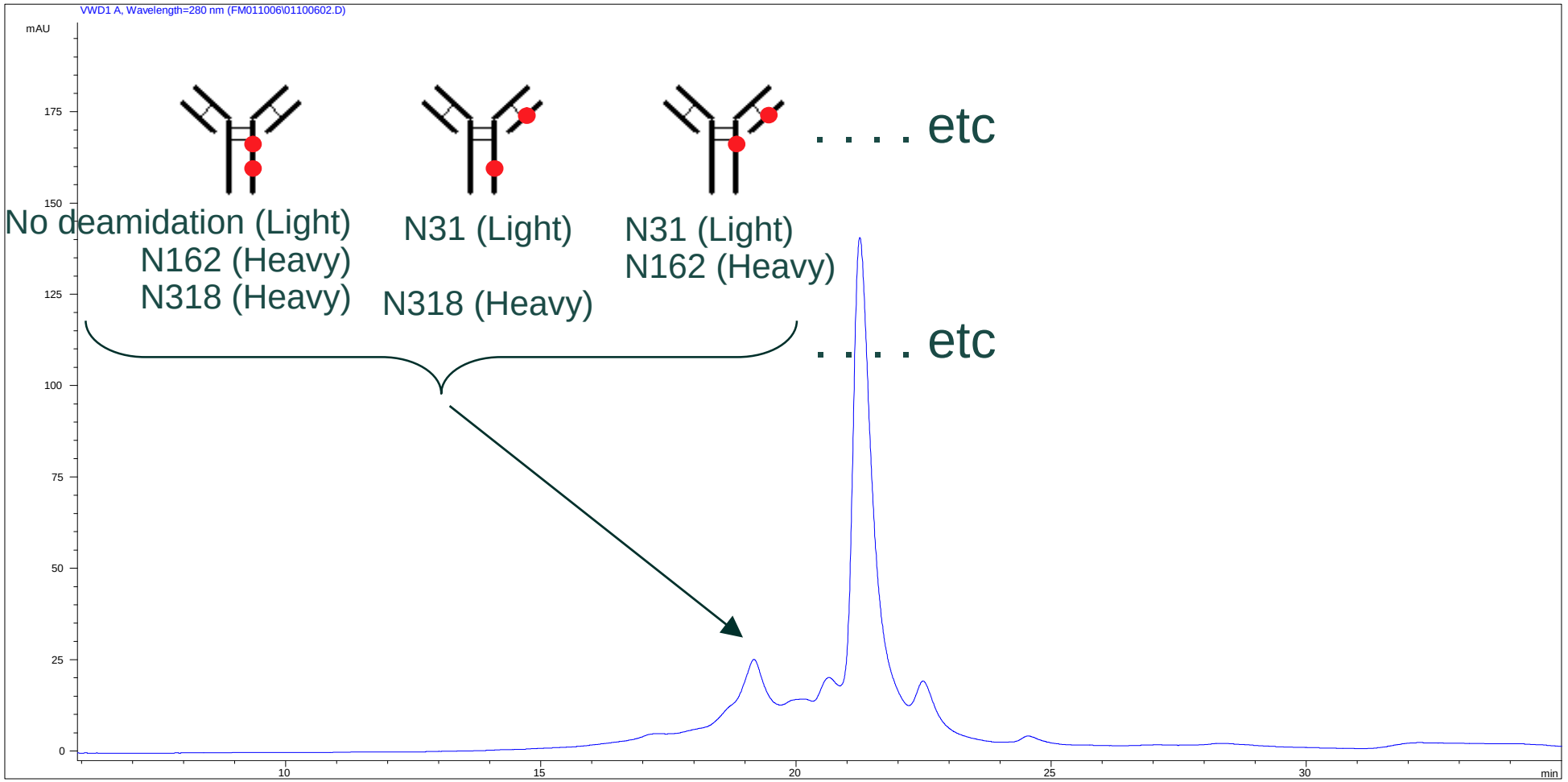
Structures of CSL360 Cation Exchange Isoforms

- IEF “ladder” confirms charge states of CEX Fractions
- Structure of CSL360 Carboxy terminal Lys variants were confirmed using several methods:
  - Carboxy B digestion
  - MALDI sequence
  - LC-MS peptide mapping
- Acidic and main species have no major structural differences, i.e. deletions, truncations, large mol.wt. PTMs
- Acidic species likely to consist of subtle modifications & combinations

Confirmed Structures in CSL360 main peak and basic peaks



Potential Structures in CEX Peak 2



Summary

- Protein Characterisation is an essential tool to support Process Development for recombinant Biopharmaceuticals
- Analytical methods are sensitive:
  Protein structure differences are likely to be observed on process changes
- Recognise the need for an “envelope” of product variability
- Know your molecule - define structure of molecule that goes into Tox and clinical studies
- Hi tech analytics is fun but classical methods (electrophoresis) sensitive & informative