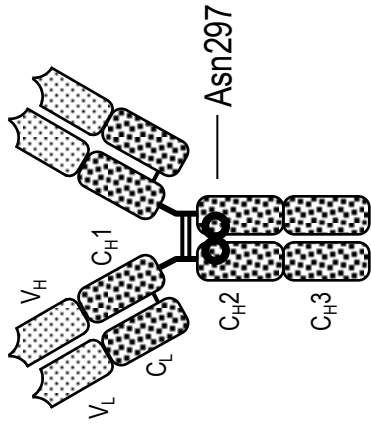
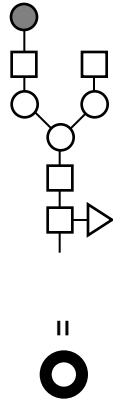


Existing Carbohydrates

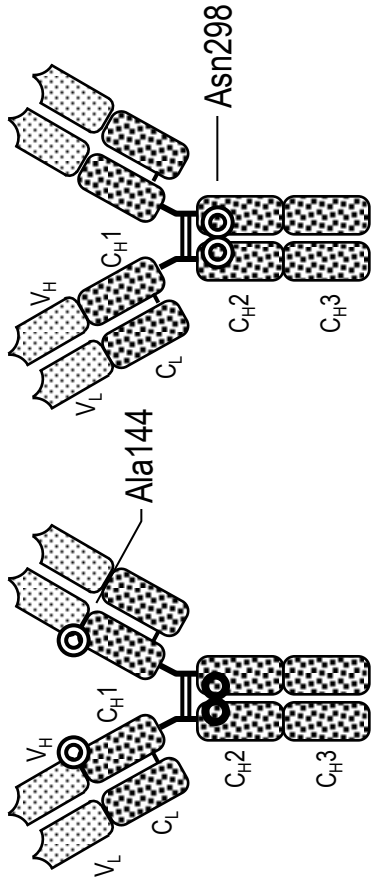
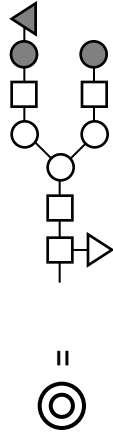
A.



B.



Engineered Glycosylation Sites



C.

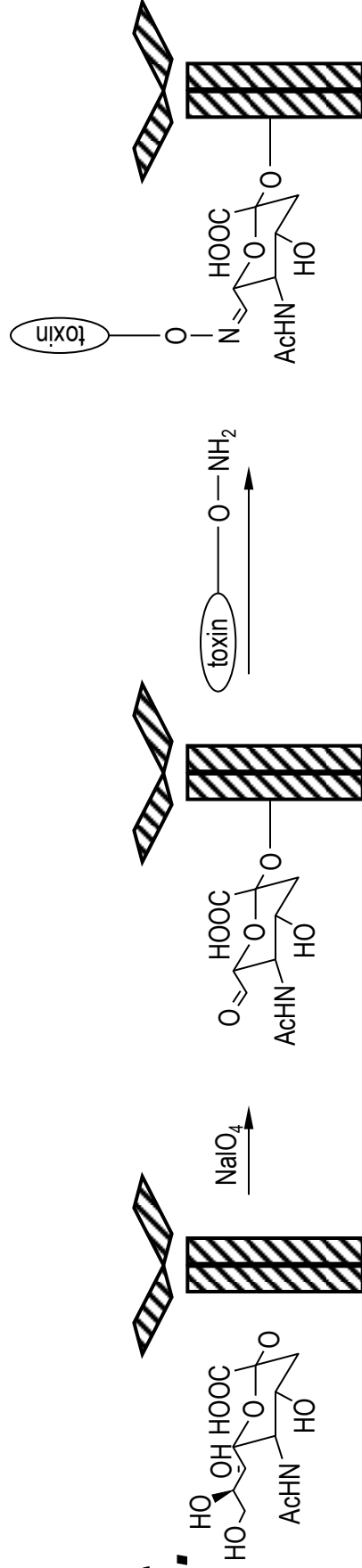


Fig. 1

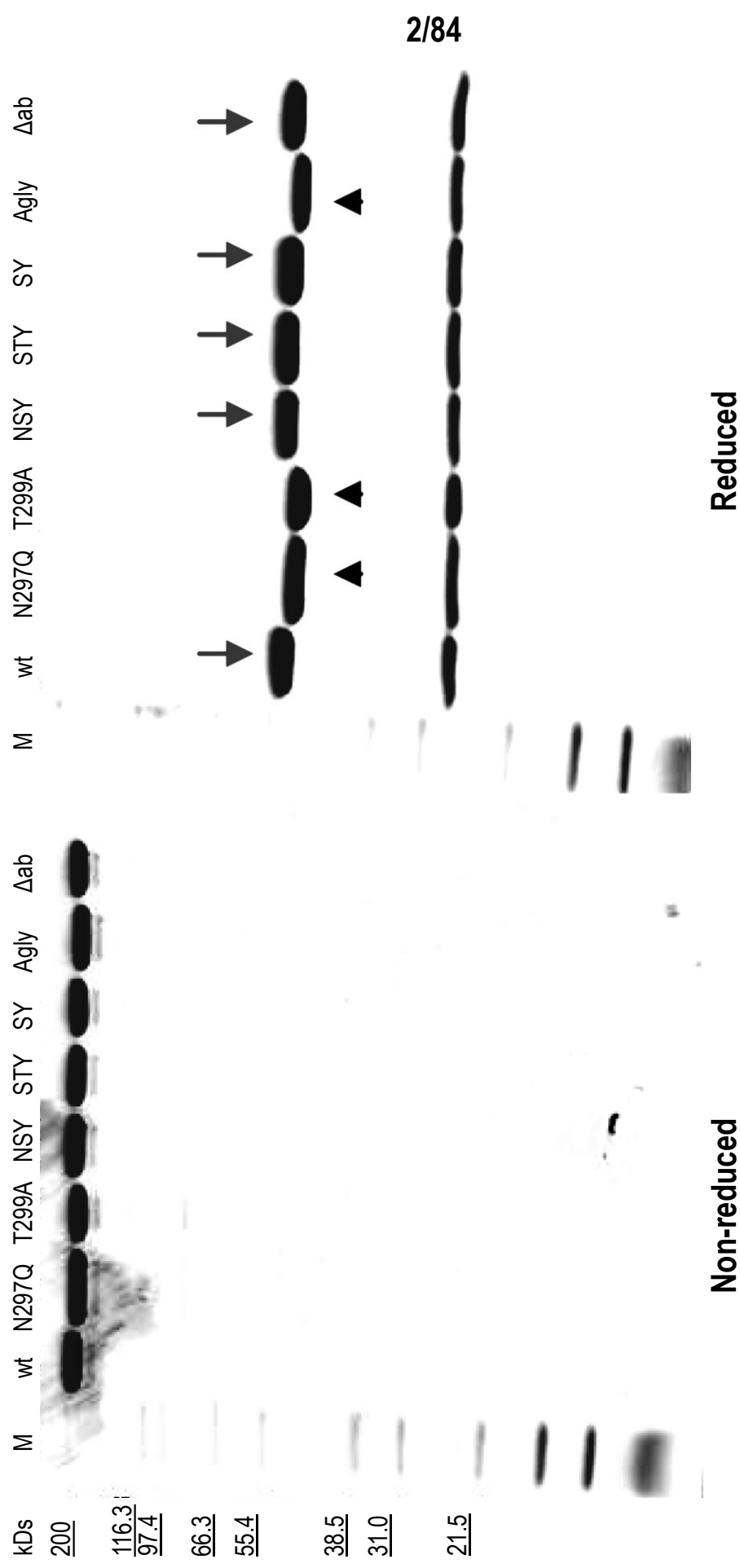
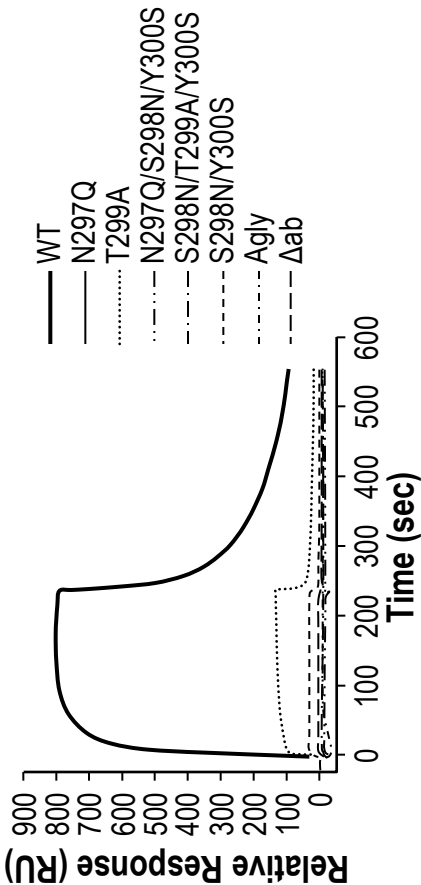


Fig. 2

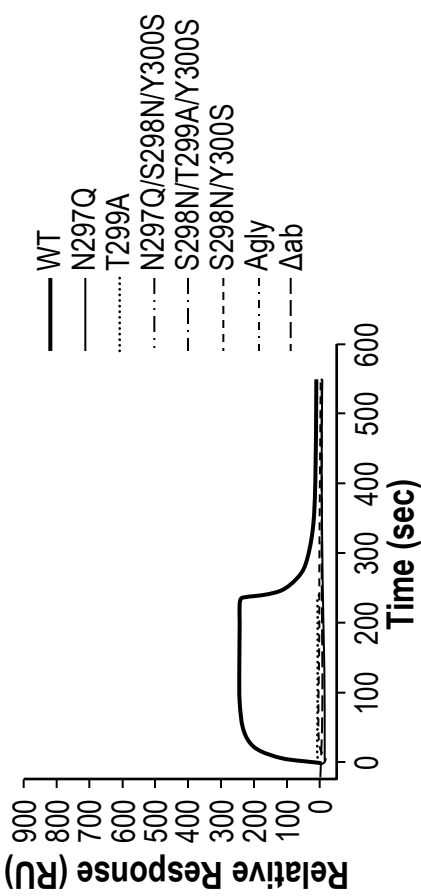
Fullscale: CD16a-Val158

Human FcγRIIIa-Val158 Binding of αβTCR HEBE1 IgG mutants



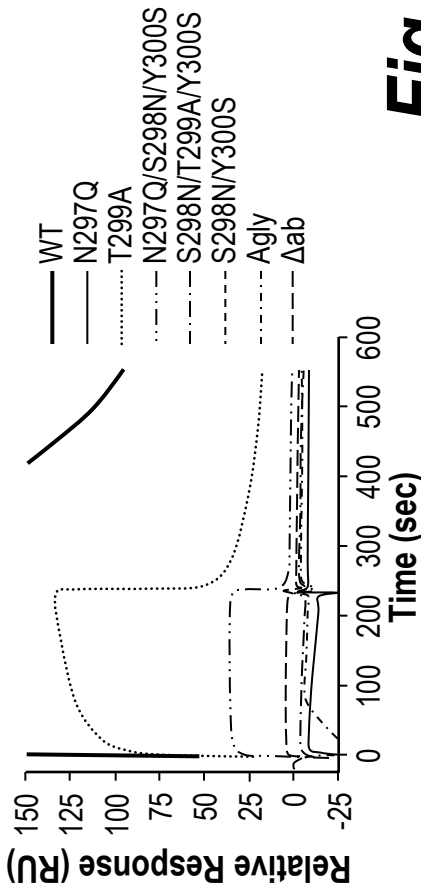
Fullscale: CD16a-Phe158

Human FcγRIIIa-Phe158 Binding of αβTCR HEBE1 IgG mutants



zoomed:

Human FcγRIIIa-Val158 Binding of αβTCR HEBE1 IgG mutants



zoomed:

Human FcγRIIIa-Phe158 Binding of αβTCR HEBE1 IgG mutants

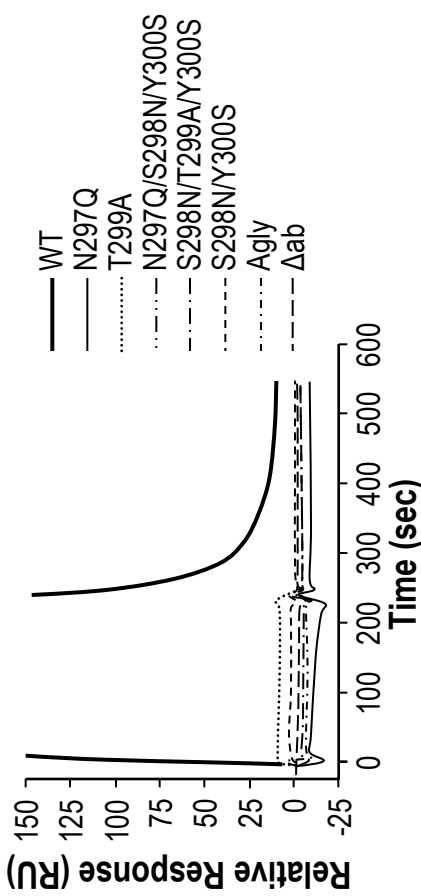


Fig. 3

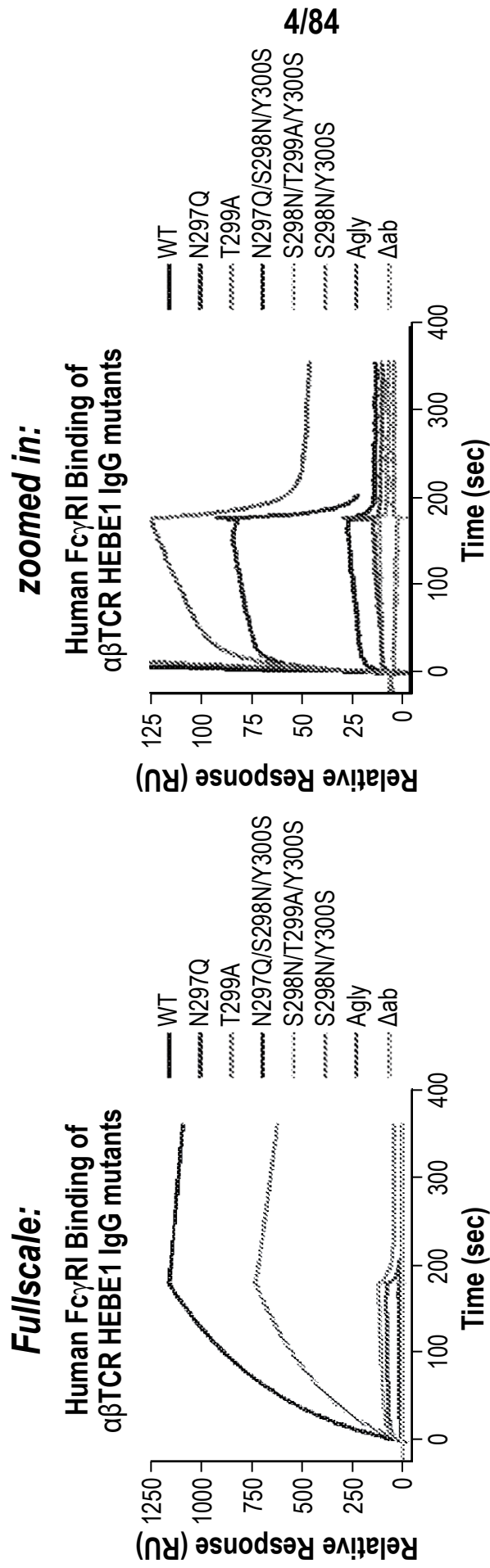


Fig. 4

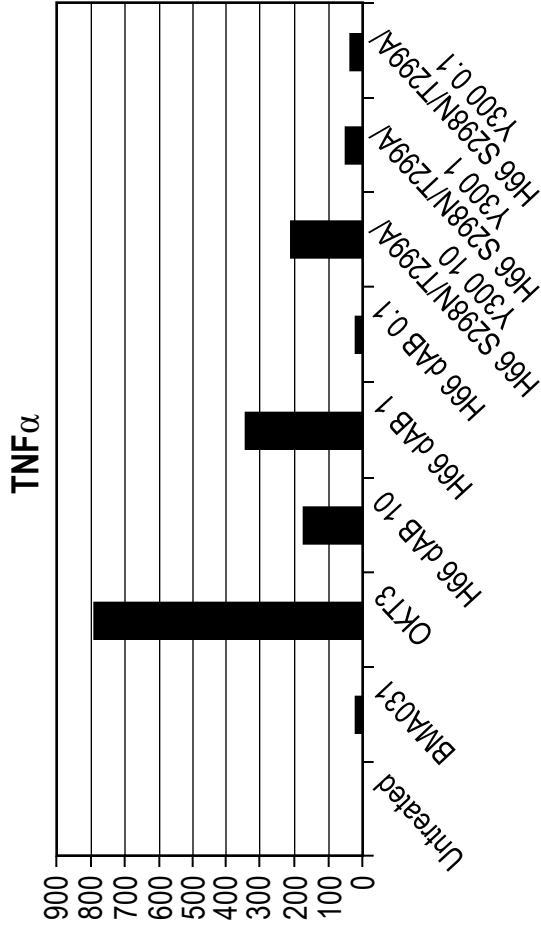
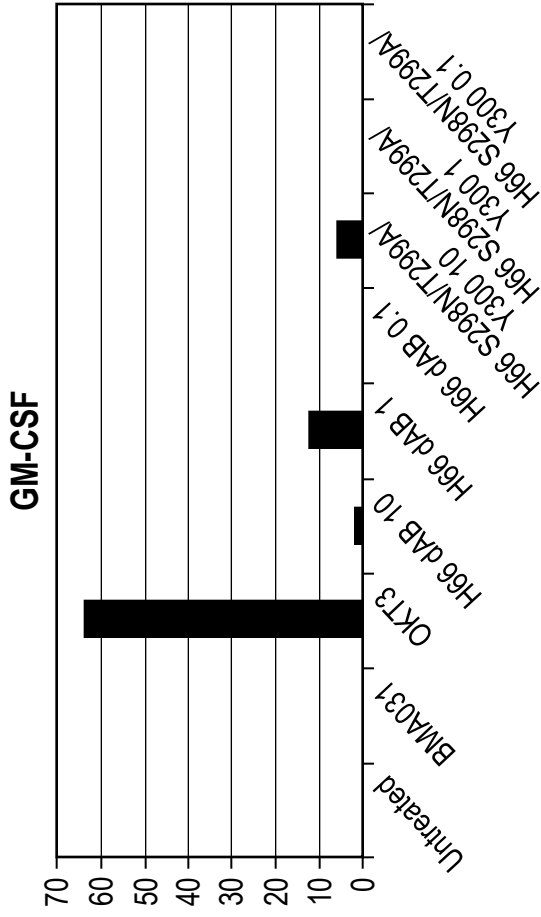
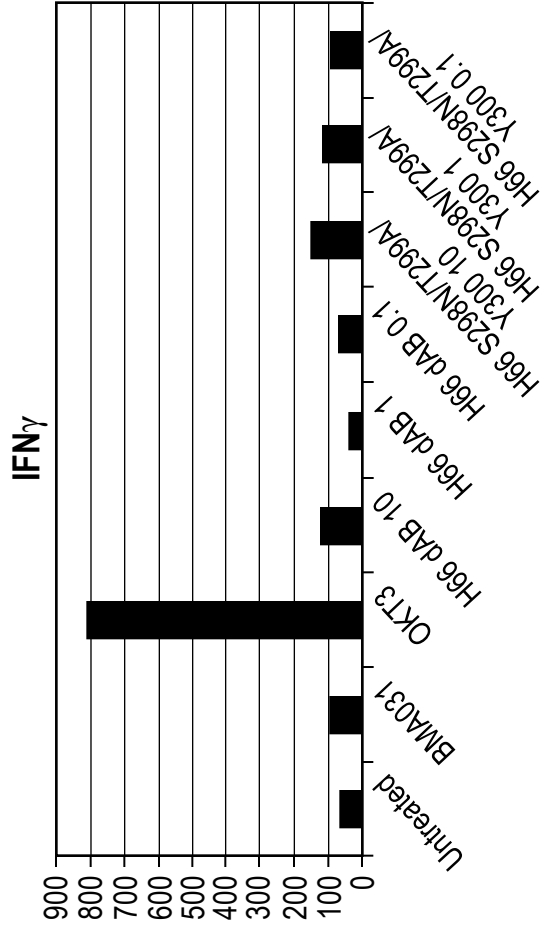
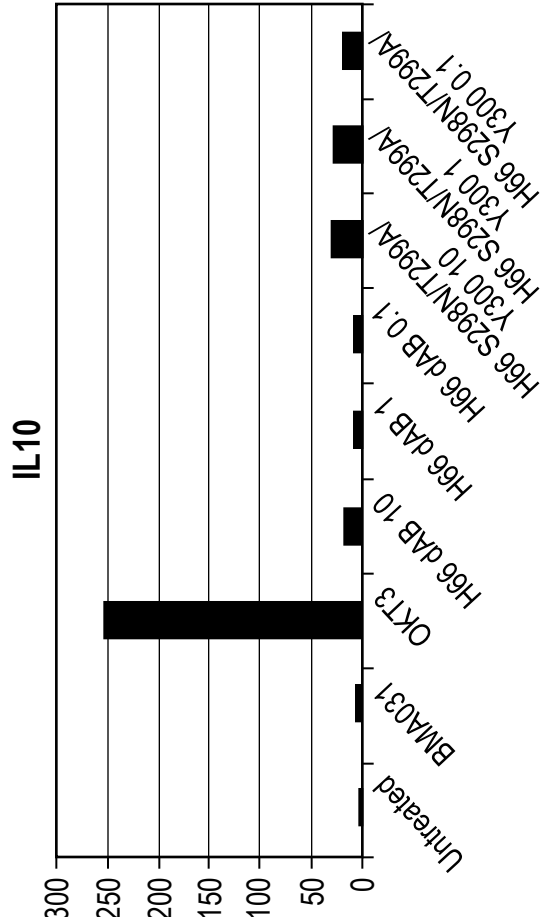


Fig. 5

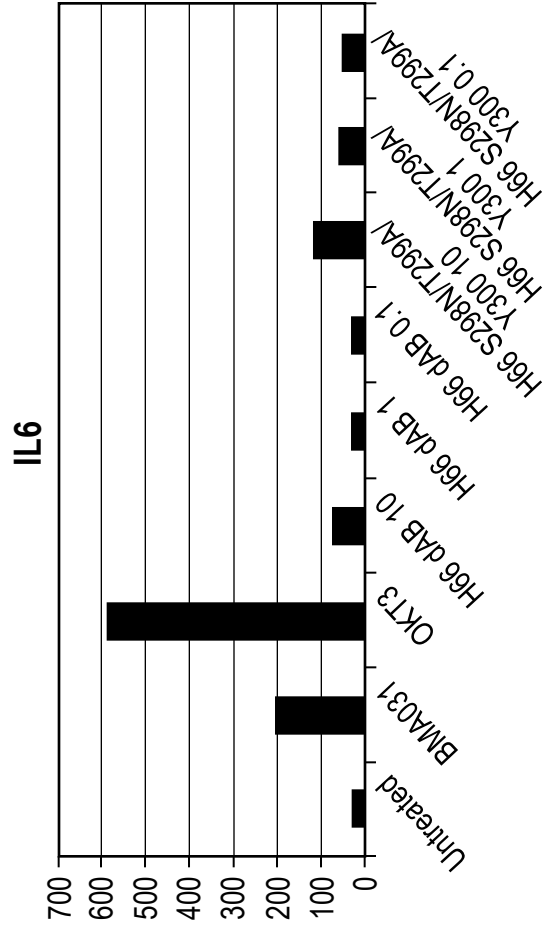
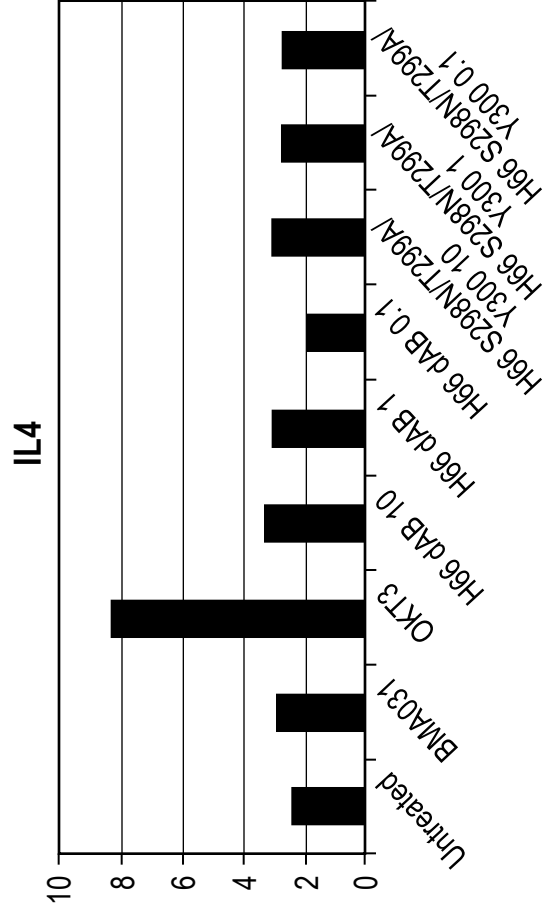
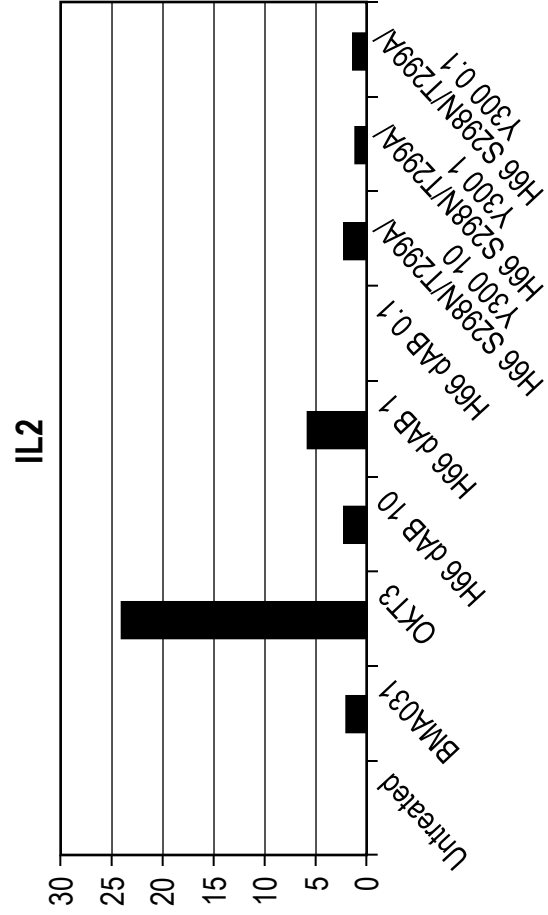


Fig. 6

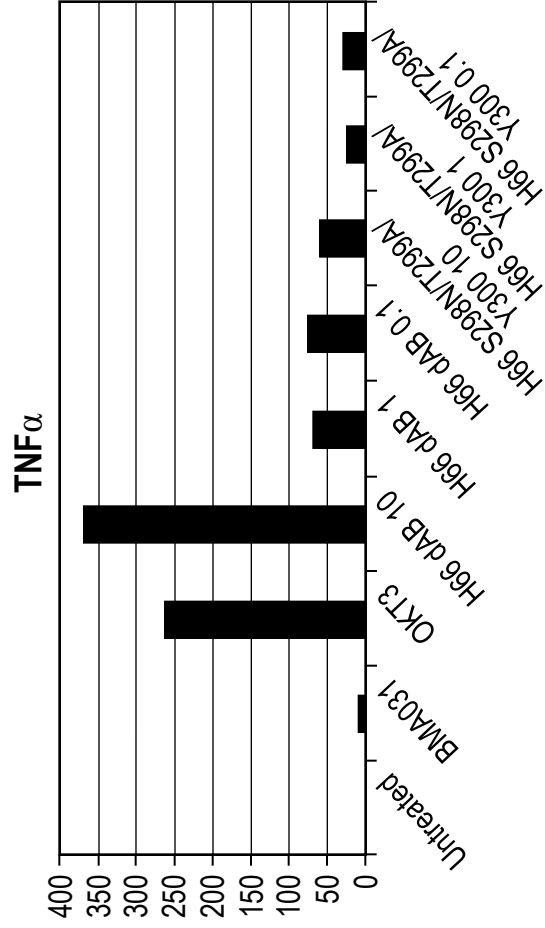
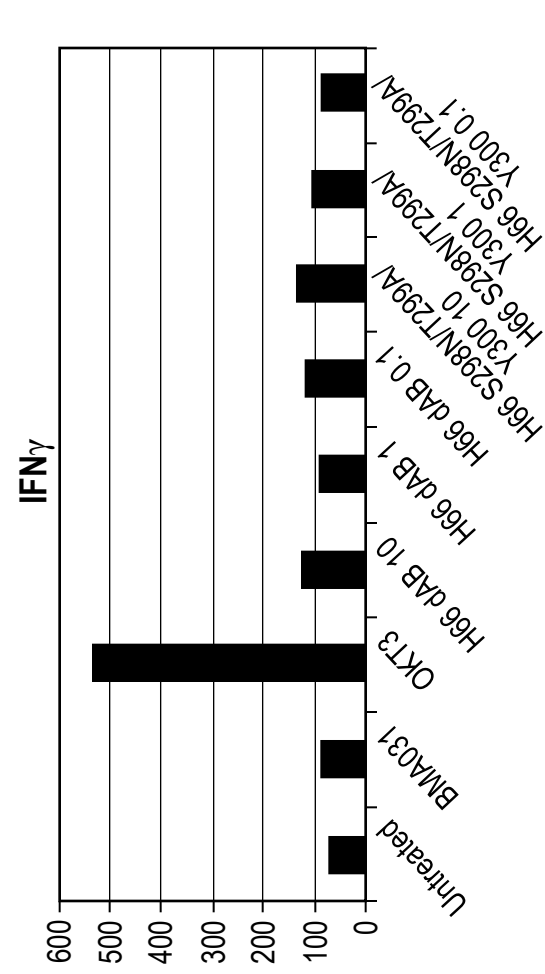
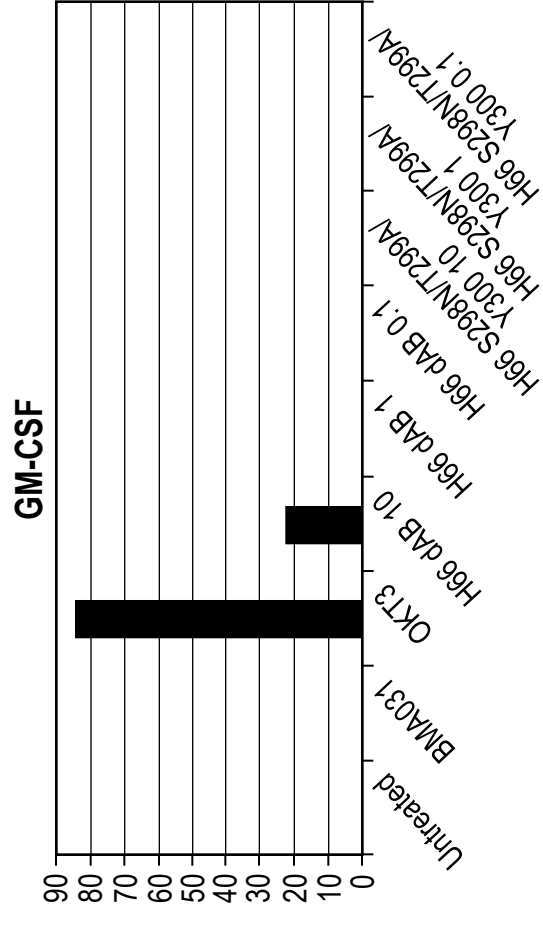
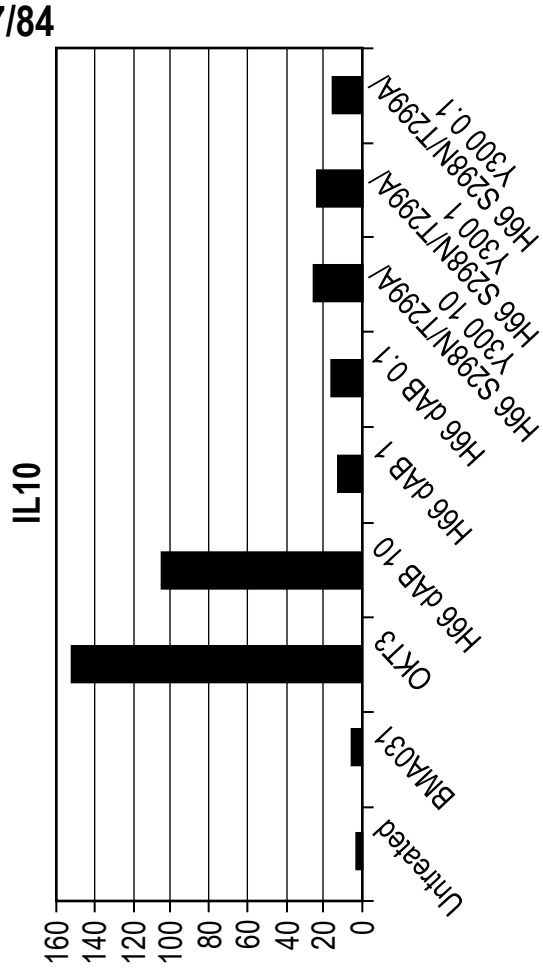


Fig. 7

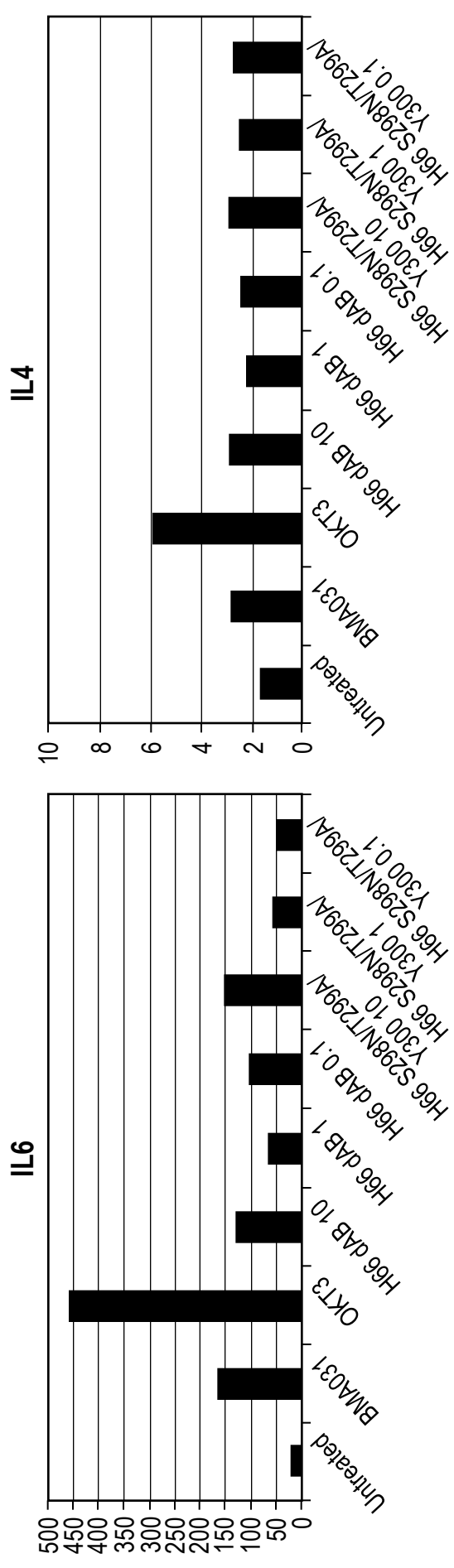
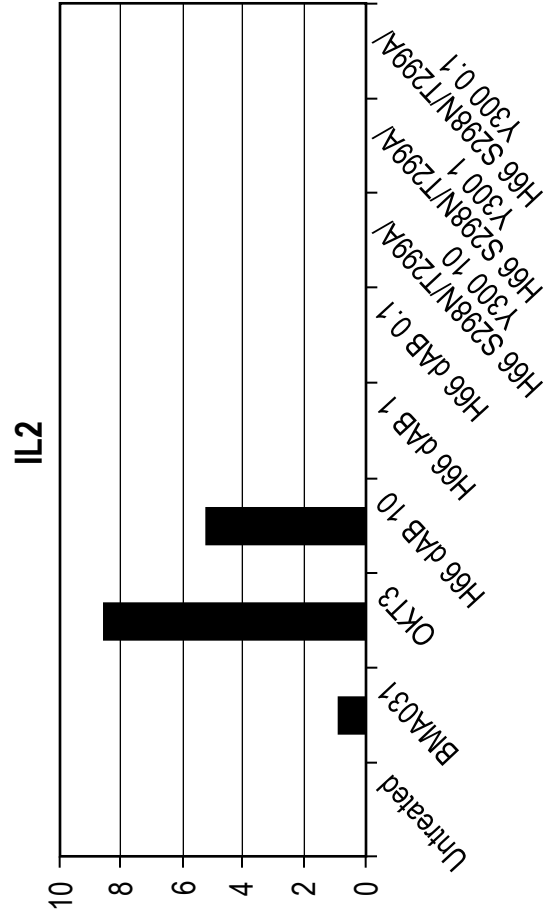
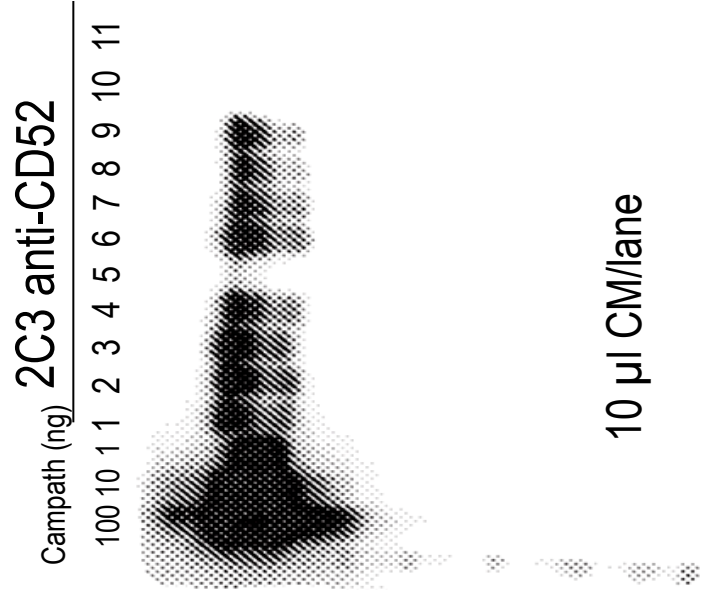


Fig. 8

A.

Western Blot



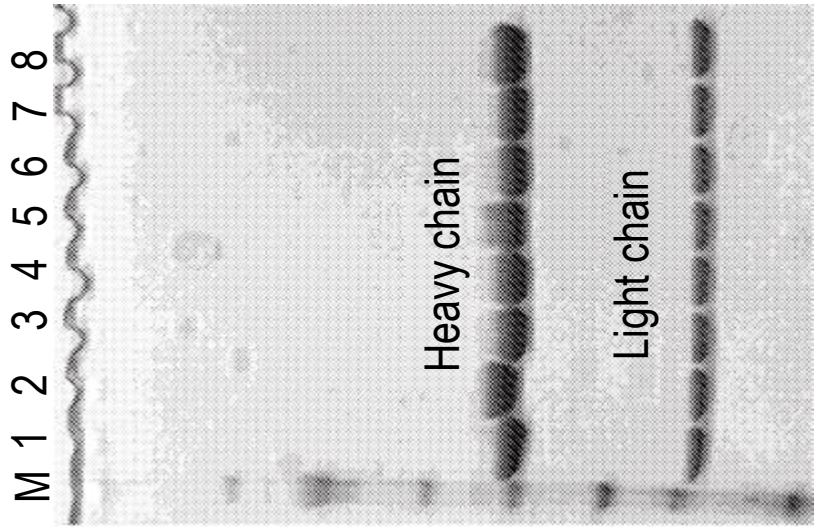
- 1 – WT
- 2 – WT
- 3 – A114N
- 4 – Y436S
- 5 – Y436T
- 6 – S440N
- 7 – S442N
- 8 – NGT
- 9 – 298N/Y300S
- 10 – Mock
- 11 – Media

B.

Biacore

Unknown	Conc (µg/mL)	Relative Response (RU)	Calc Conc (M)	Calc Conc (µg/mL)
2C3 in media	3.00	855.75	2.45E-08	3.669
2C3 in media	3.00	860.96	2.47E-08	3.710
2C3 in media	3.00	866.89	2.51E-08	3.758
A114N		146.5	1.60E-09	0.240
Mock		87.84	8.62E-10	0.129
NGT		124.52	1.30E-09	0.196
S298N/Y300S		112.14	1.15E-09	0.172
S440N		146.36	1.60E-09	0.240
S442N		121.21	1.26E-09	0.189
WT (Katya)		148.41	1.63E-09	0.244
WT (Tim)		148.08	1.62E-09	0.244
Y436S		158.72	1.78E-09	0.267
Y436T		84.27	8.23E-10	0.123
Incubated media		87.38	8.57E-10	0.129

Fig. 9



Lane	Mutant	mg/ml
1	wt	2.66
2	A114N	2.99
3	Y436S	1.61
4	S298N/Y300S	0.99
5	Y436T	0.41
6	S440N	1.21
7	S442N	1.62
8	NGT	2.21

Fig. 11

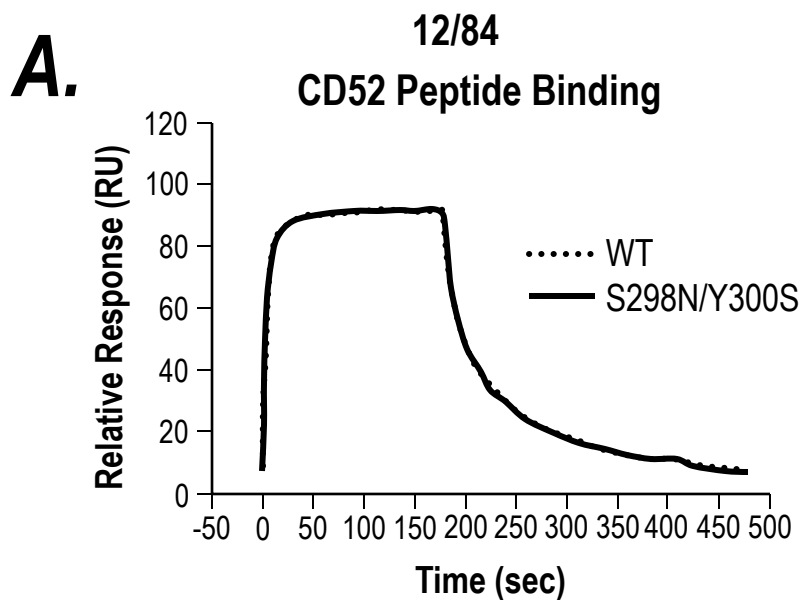
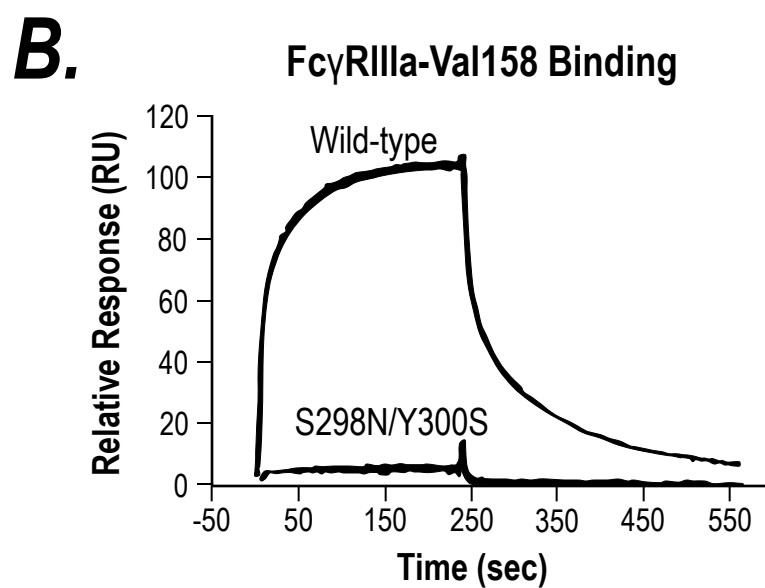
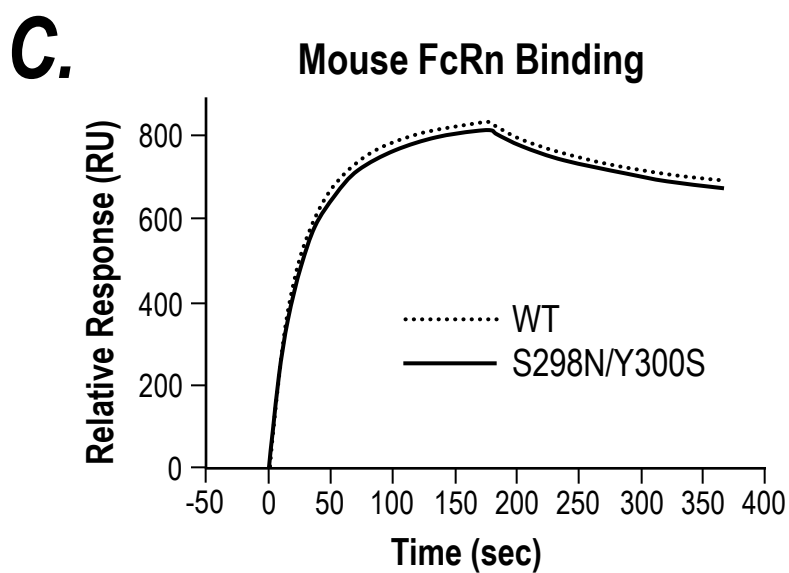


Fig. 12

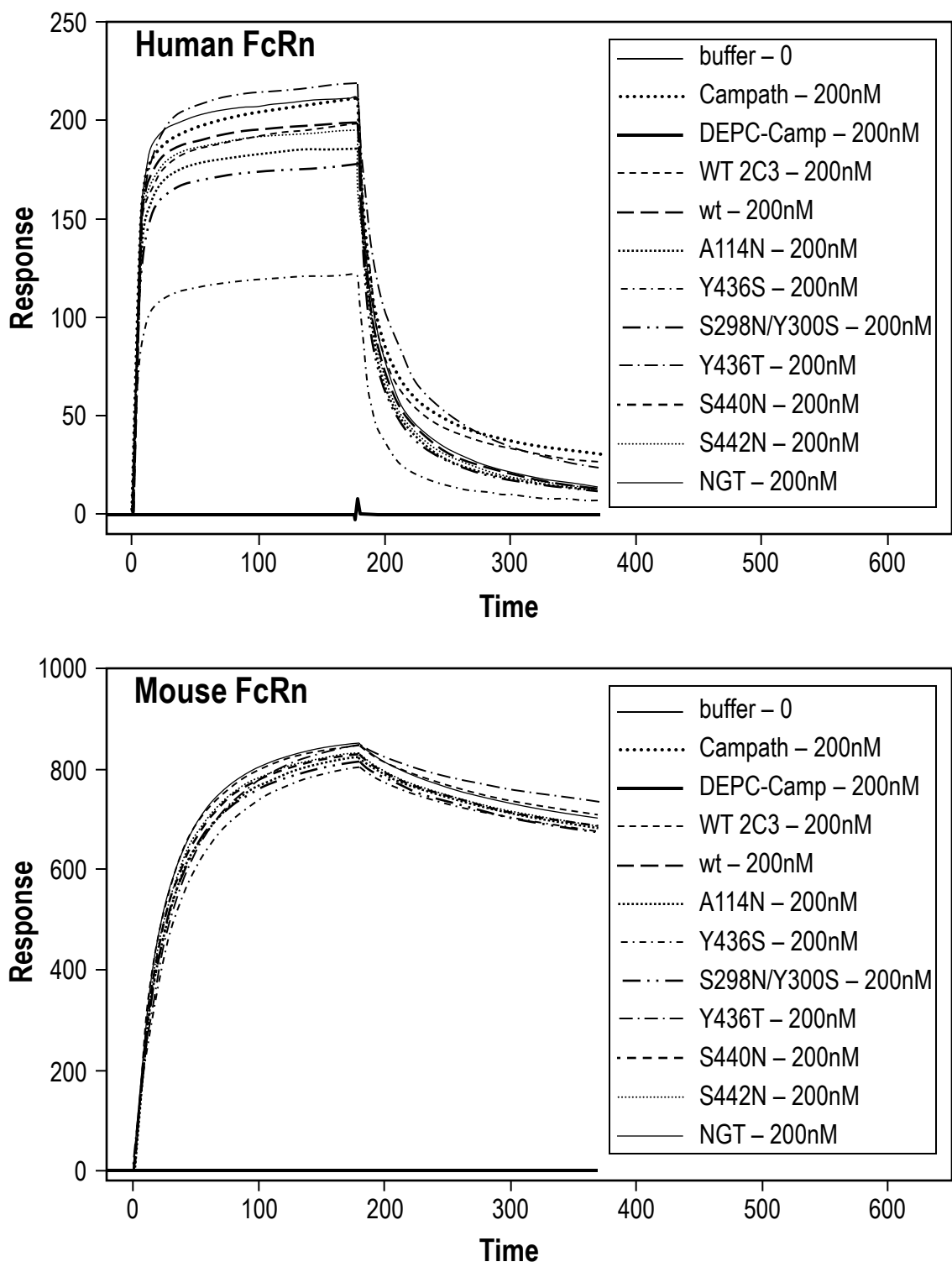
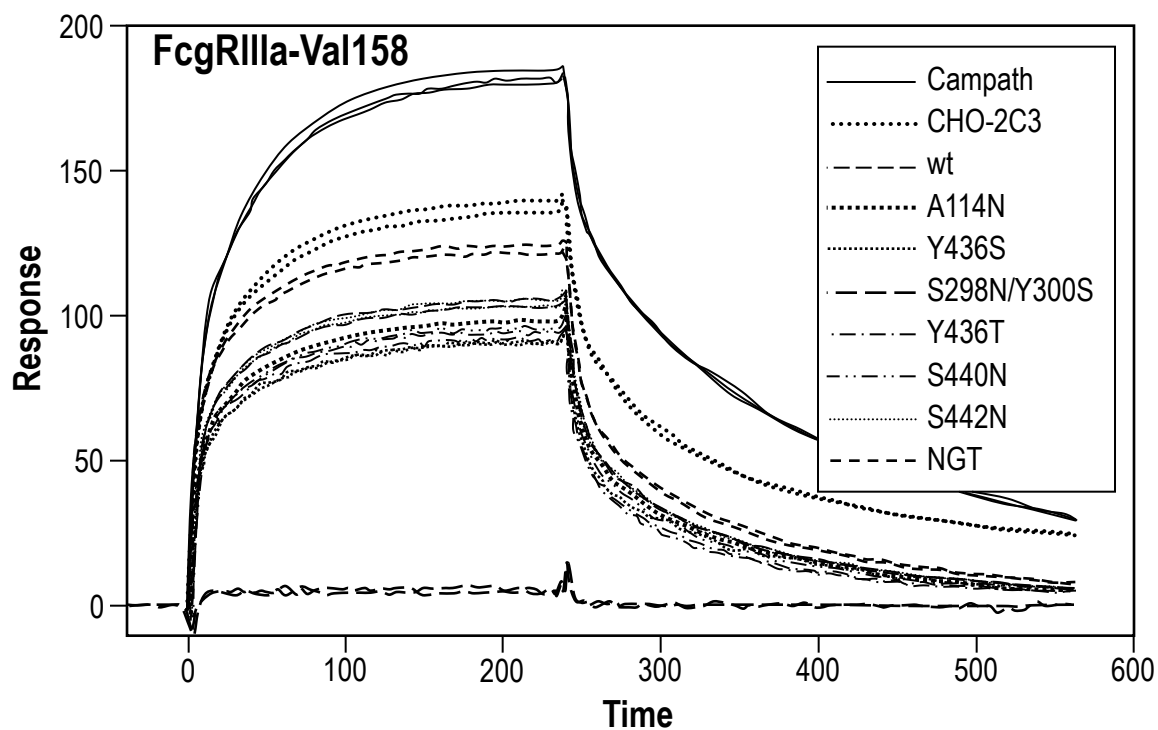
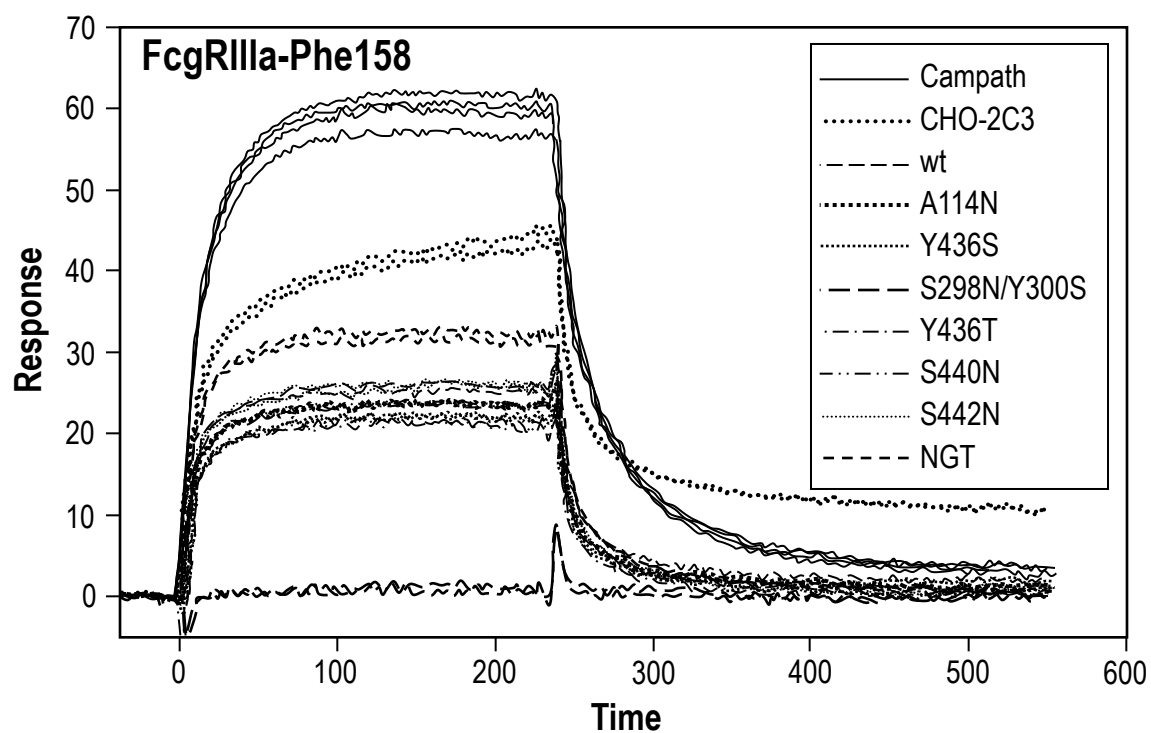
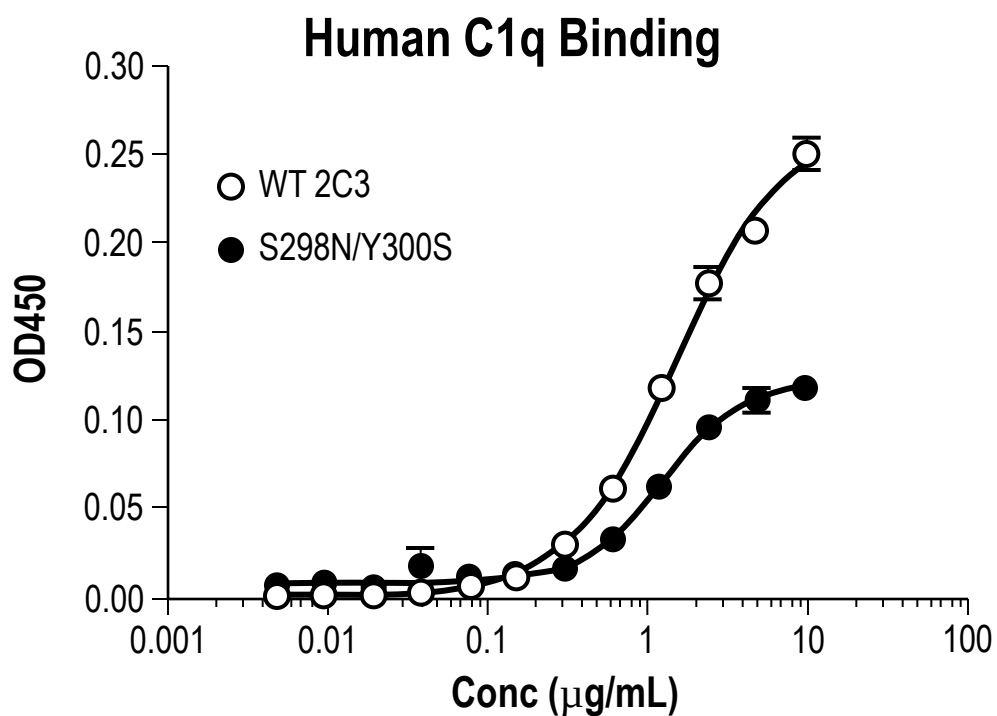
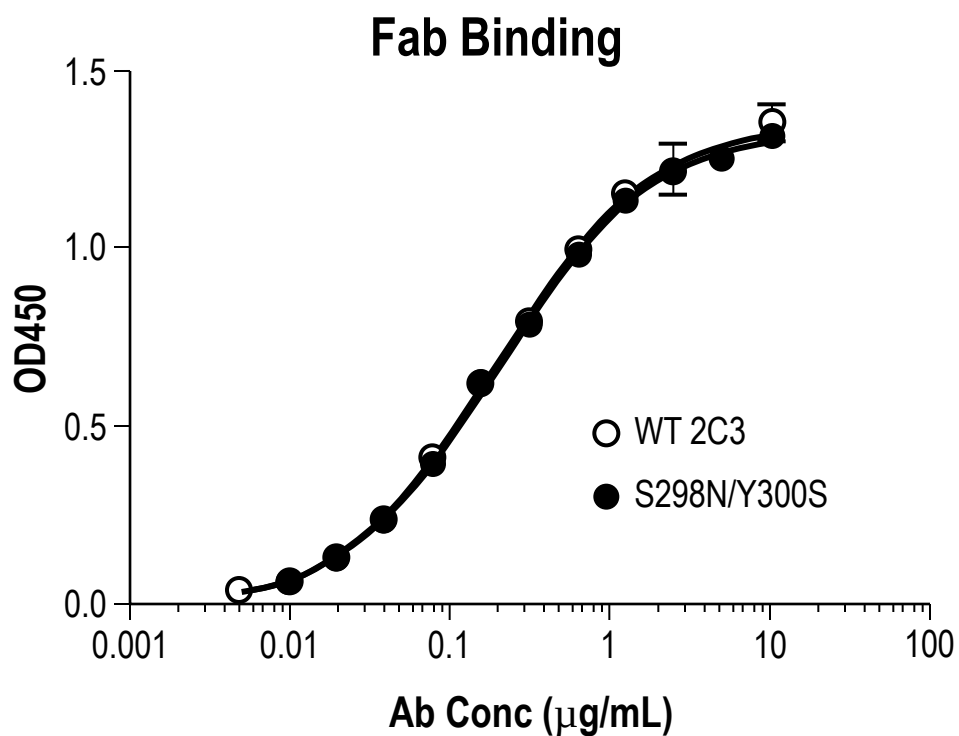


Fig. 13

A.**B.****Fig. 14**

A.**B.****Fig. 15**

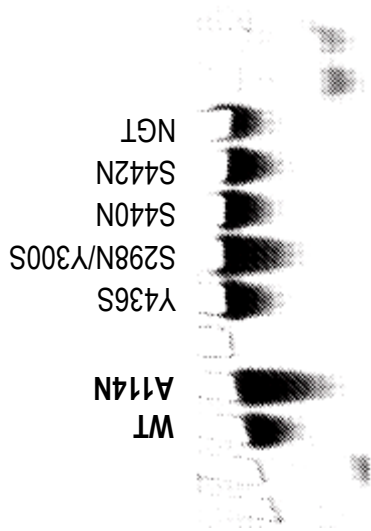
Sample	k_a ($\times 10^6/\text{Ms}$)	k_d ($\times 10^{-2}/\text{s}$)	R_{max} (RU)	K_D (nM)
GLD52	7.0	1.7	67.0	2.44
WT 2C3	6.0	1.1	64.2	1.75
A114N	4.7	1.1	59.5	2.45
Y436S	5.9	1.0	66.9	1.73
S298N/Y300S	5.7	1.0	63.3	1.80
Y436T	4.8	0.9	65.7	1.95
S440N	5.8	1.1	66.8	1.84
S442N	5.7	1.1	66.2	1.85
NGT	7.9	1.1	70.2	1.35

Fig. 16

Sample	K_{on} ($\times 10^6 M^{-1} s^{-1}$)	K_{off} ($\times 10^{-2} s^{-1}$)	KD (nM)
WT 2C3	5.2	1.1	2.1
A114N	5.3	1.3	2.4

Fig. 17

A.



B.

Mass reconstruction of Deconvolution of +TOF MS: 34.114 to 45.998 min from aCD52_147.wiff

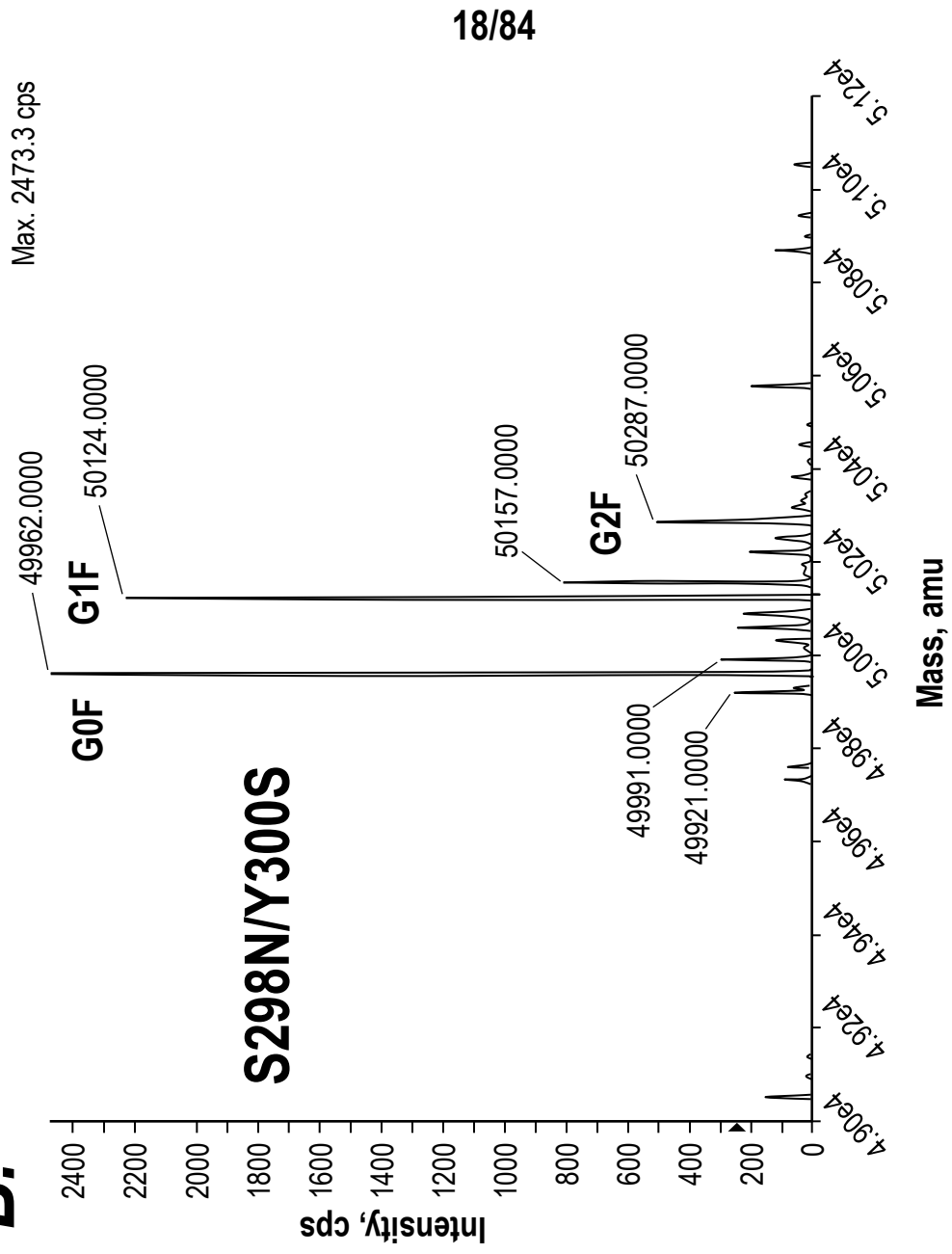


Fig. 18A and Fig. 18B

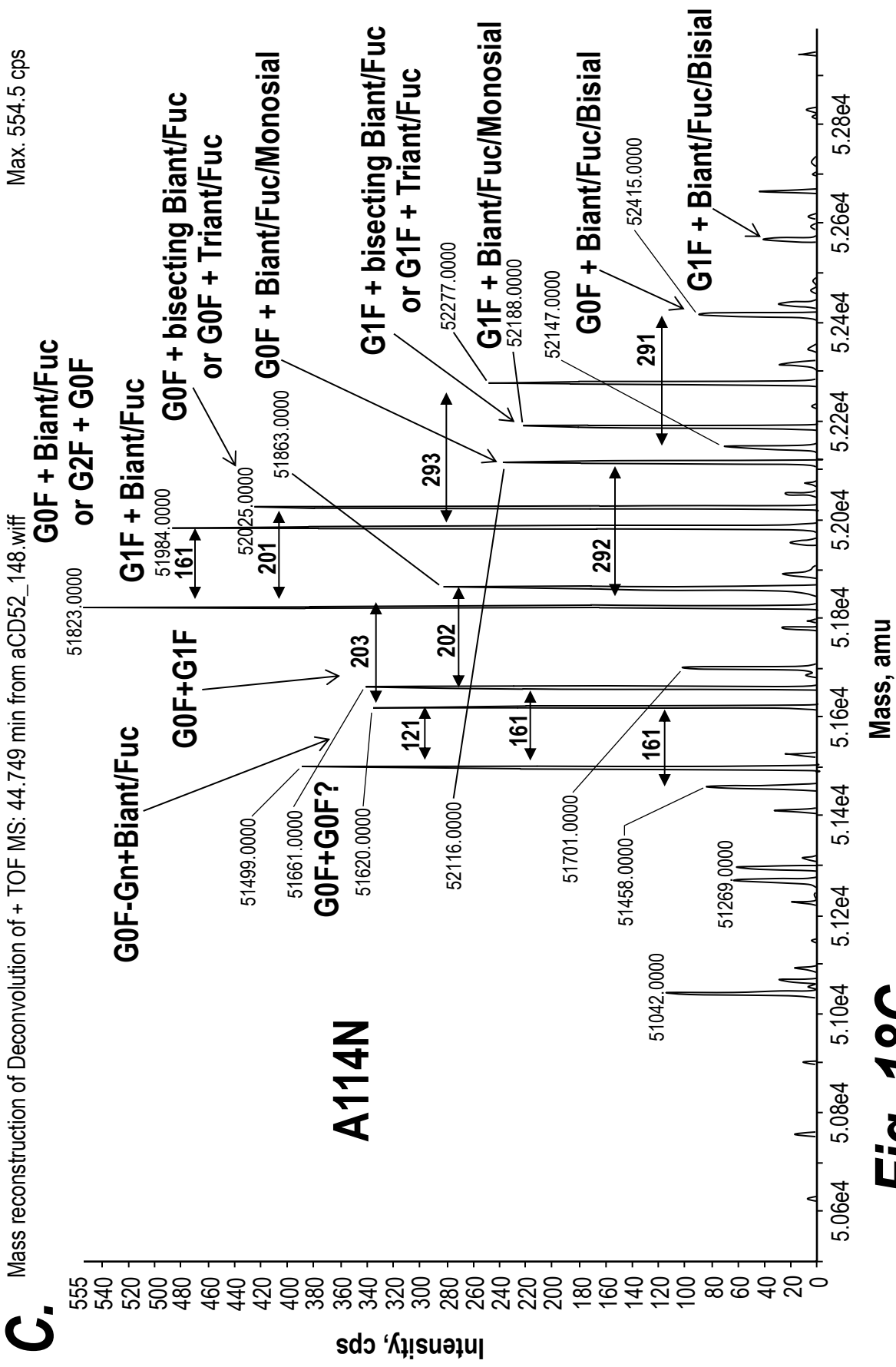


Fig. 18C

D.

Mass reconstruction of Deconvolution of + TOF MS: 34.181 to 45.998 min from azCD52_145.wiff Max. 5519.9 cps

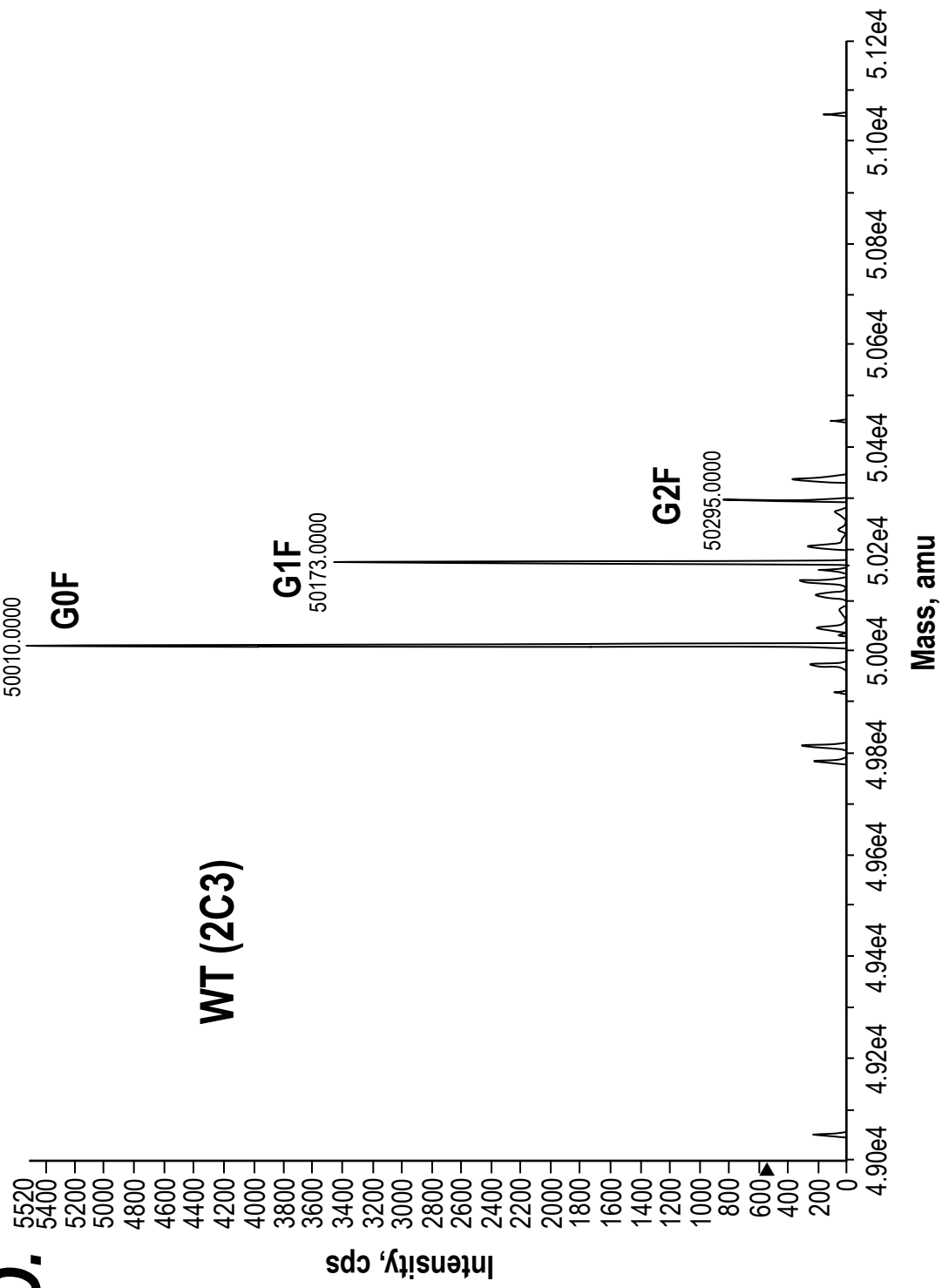


Fig. 18D

A.

Sample	lot #	Octet Conc (µg/mL)
Mock media	11/23/2009	too low
wt 2C3	11/23/2009	2.54
A114N	11/23/2009	2.83
S298N/Y300S	11/23/2009	1.36
S440N	11/23/2009	1.32
S442N	11/23/2009	1.21
Y436S	11/23/2009	1.92
Y436T	11/23/2009	0.34
NGT	11/23/2009	1.90

B.

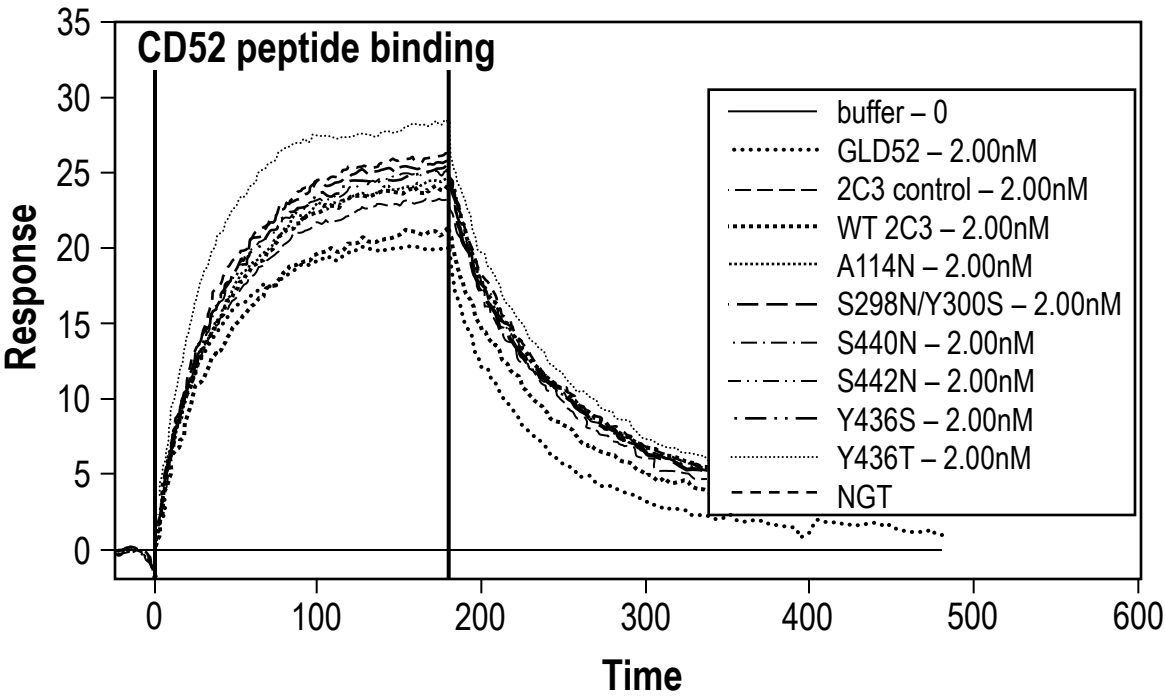


Fig. 19

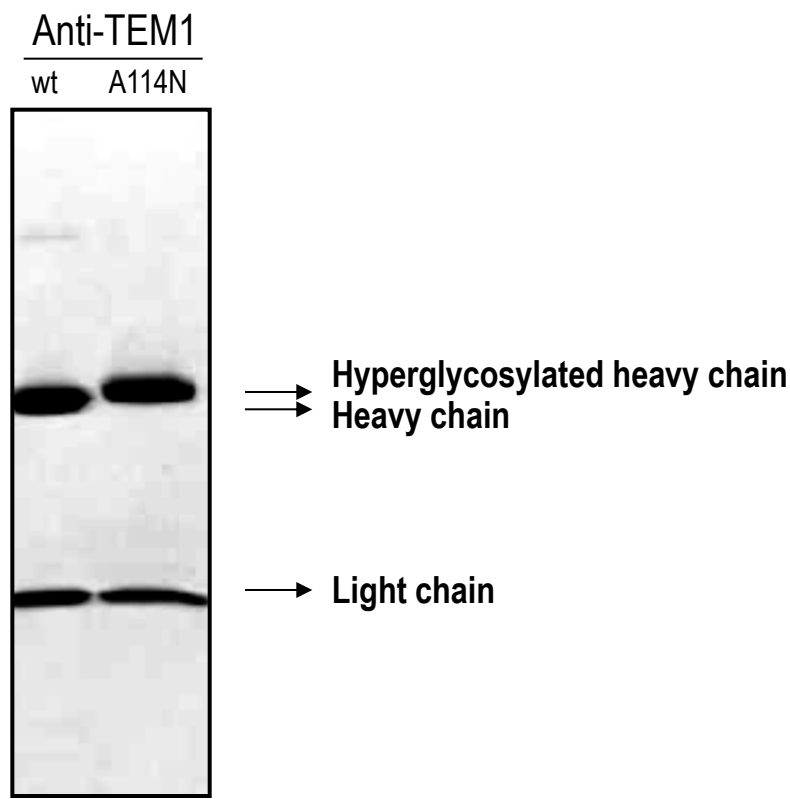
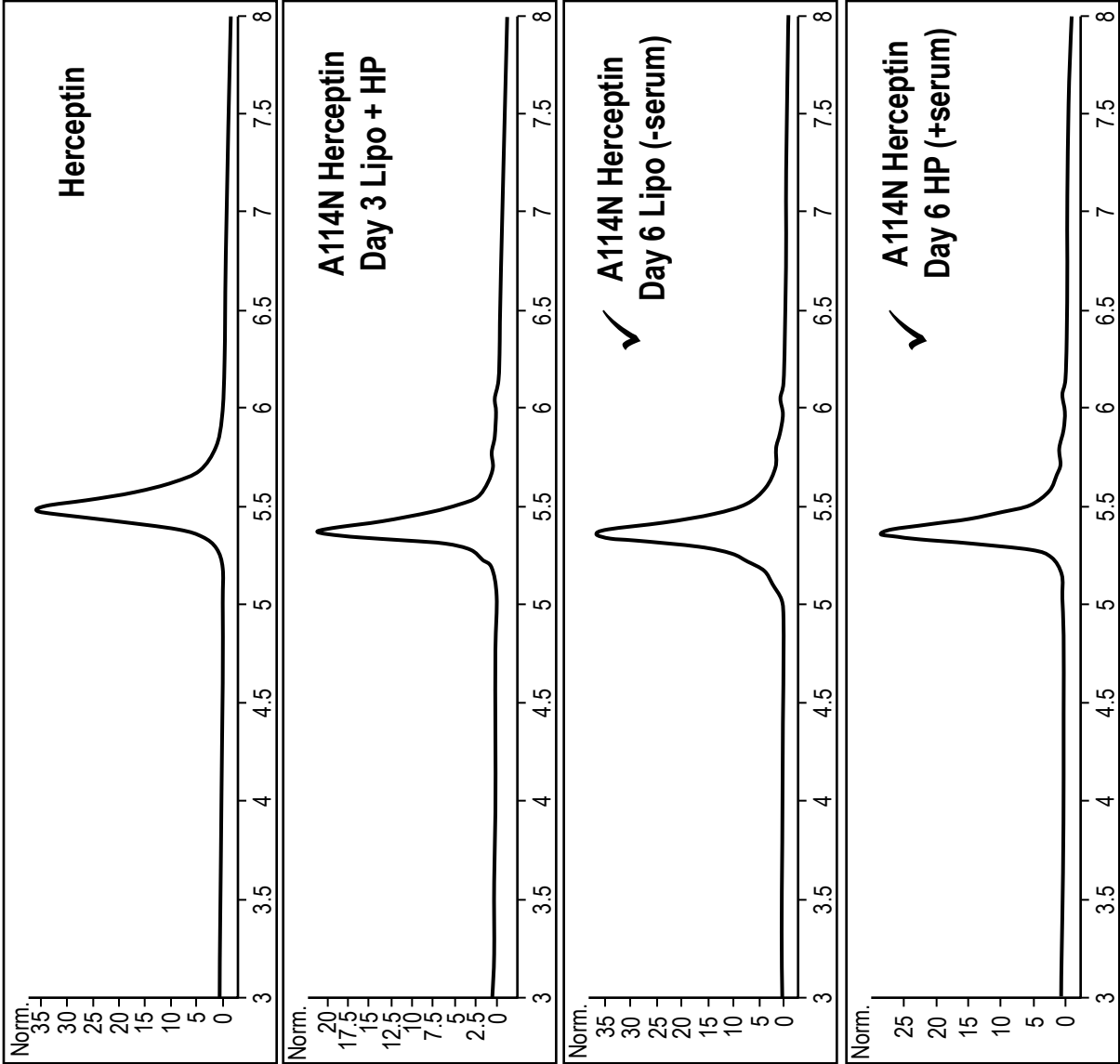


Fig. 20

HIC Analysis of Purified A114N Herceptin



10% Tris-glycine SDS-PAGE

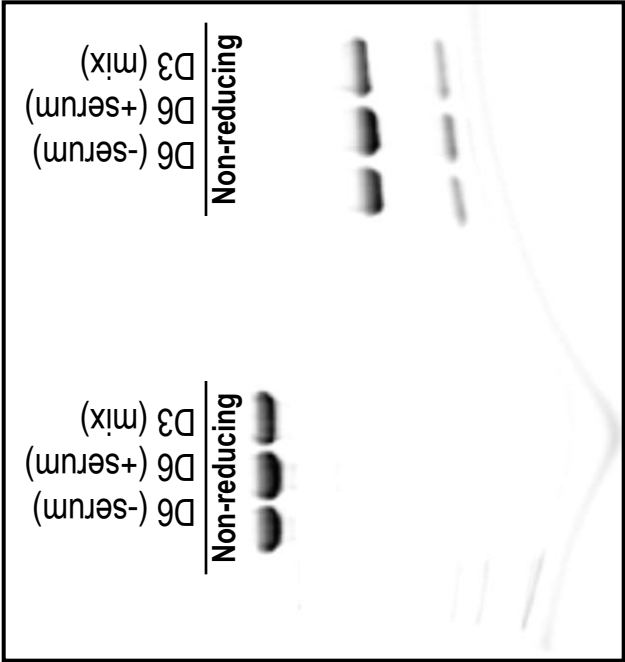


Fig. 21

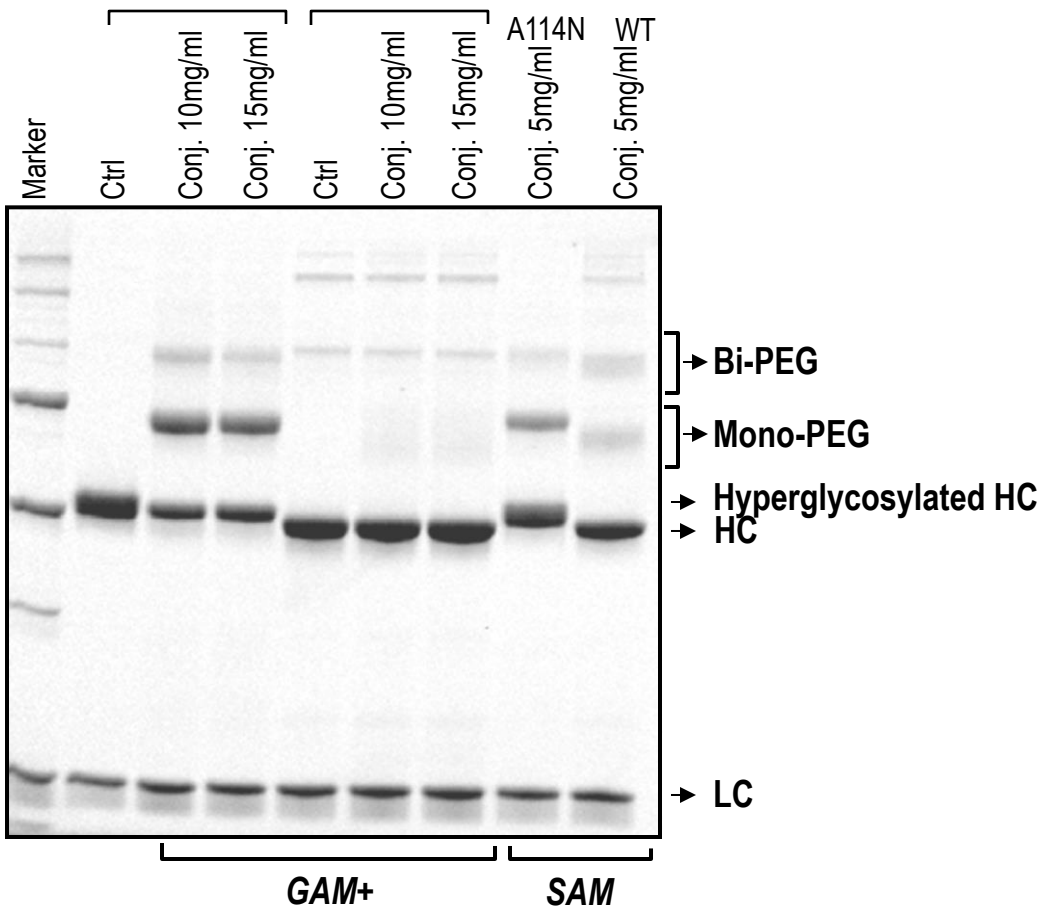


Fig. 22

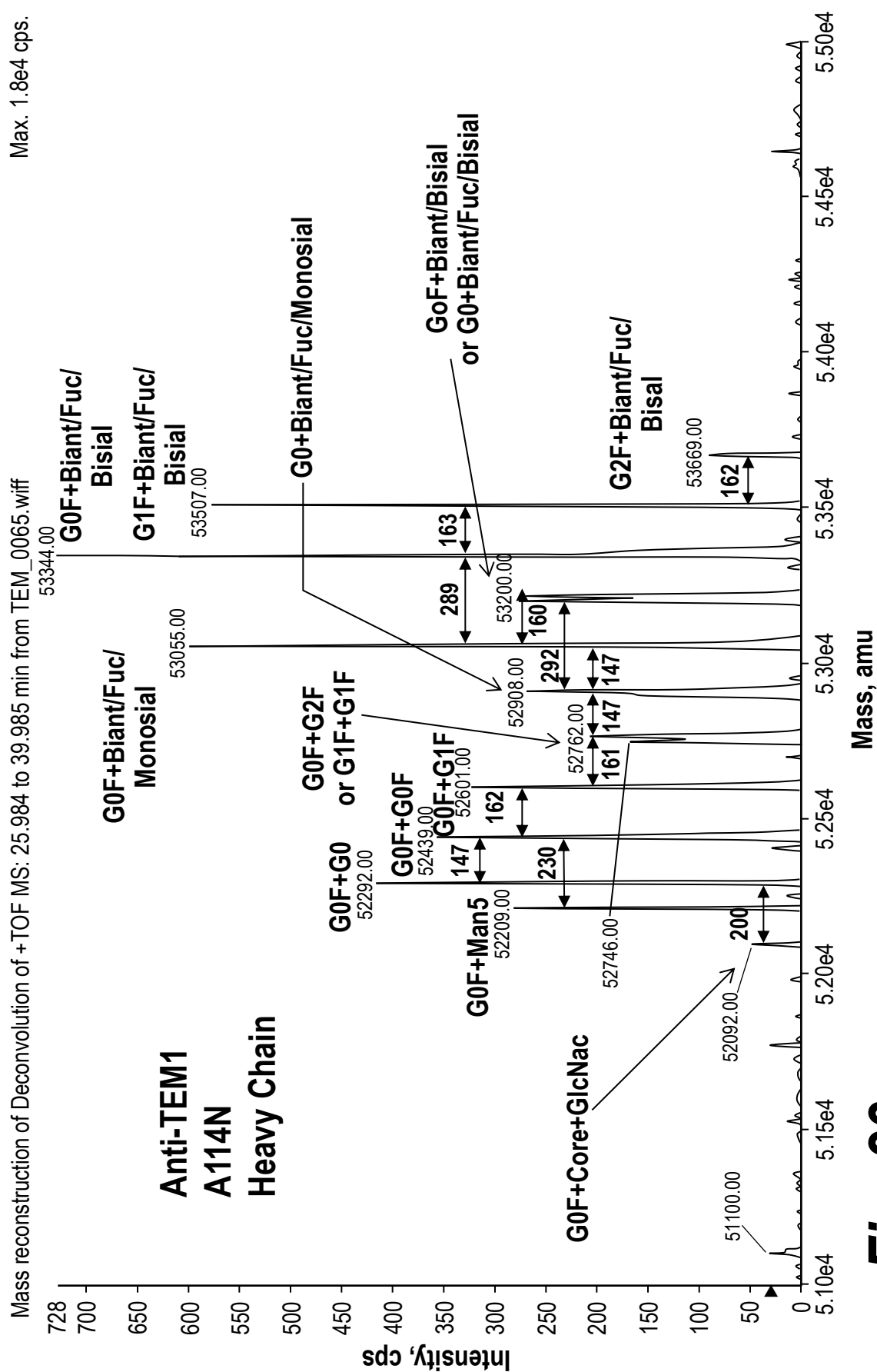
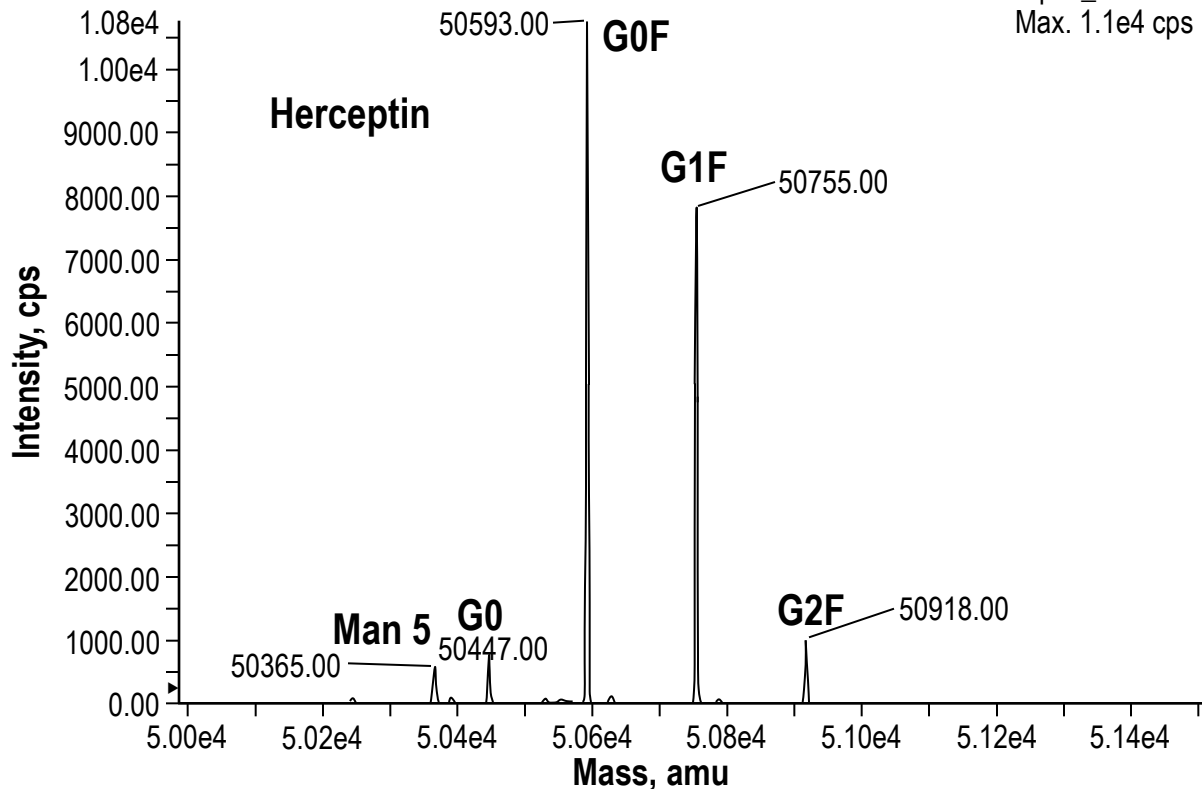


Fig. 23

26/84

Mass reconstruction of Deconvolution of +TOF MS: 26.217 to 37.001 min from Herceptin_001.wiff
Max. 1.1e4 cps



Mass reconstruction of Deconvolution of +TOF MS: 26.401 to 37.051 min from Herceptin_002.wiff
Max. 7231.3 cps

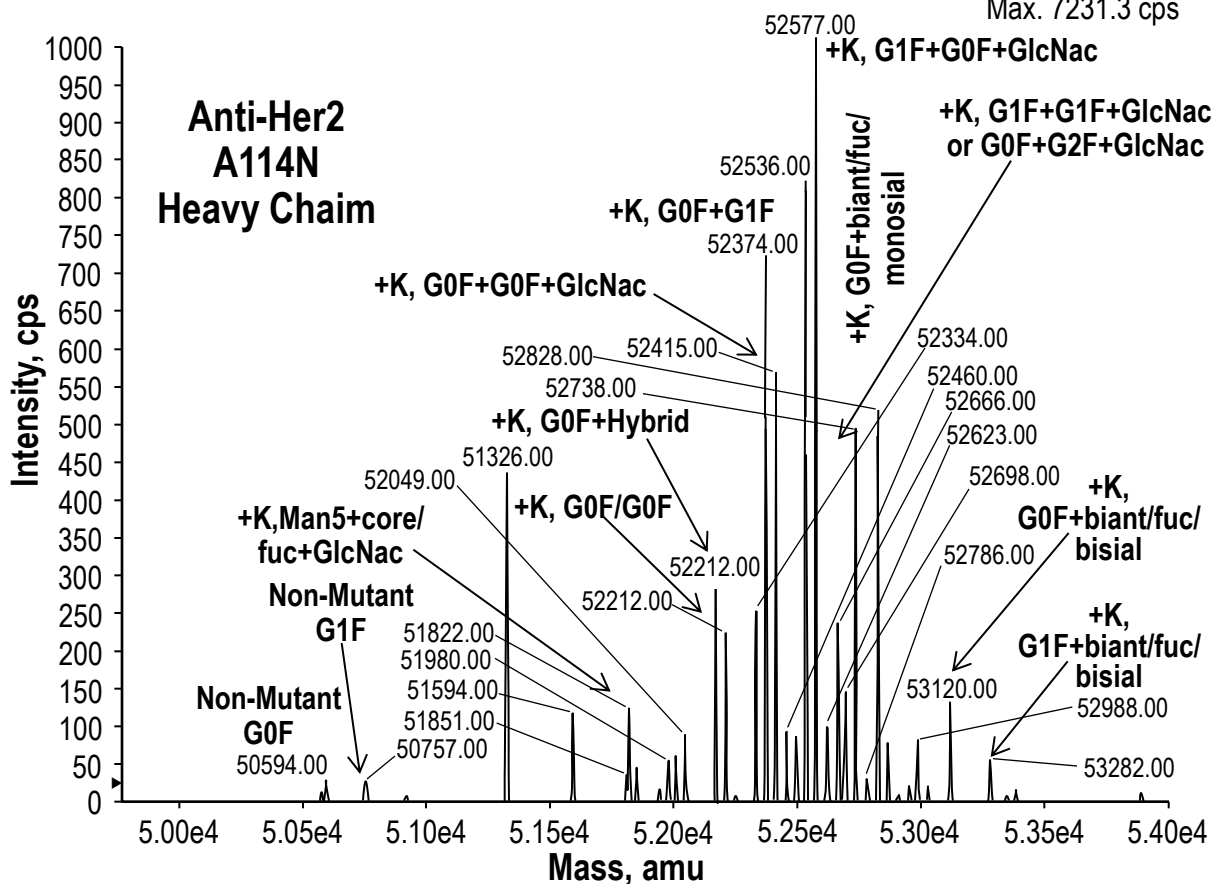


Fig. 24

A.

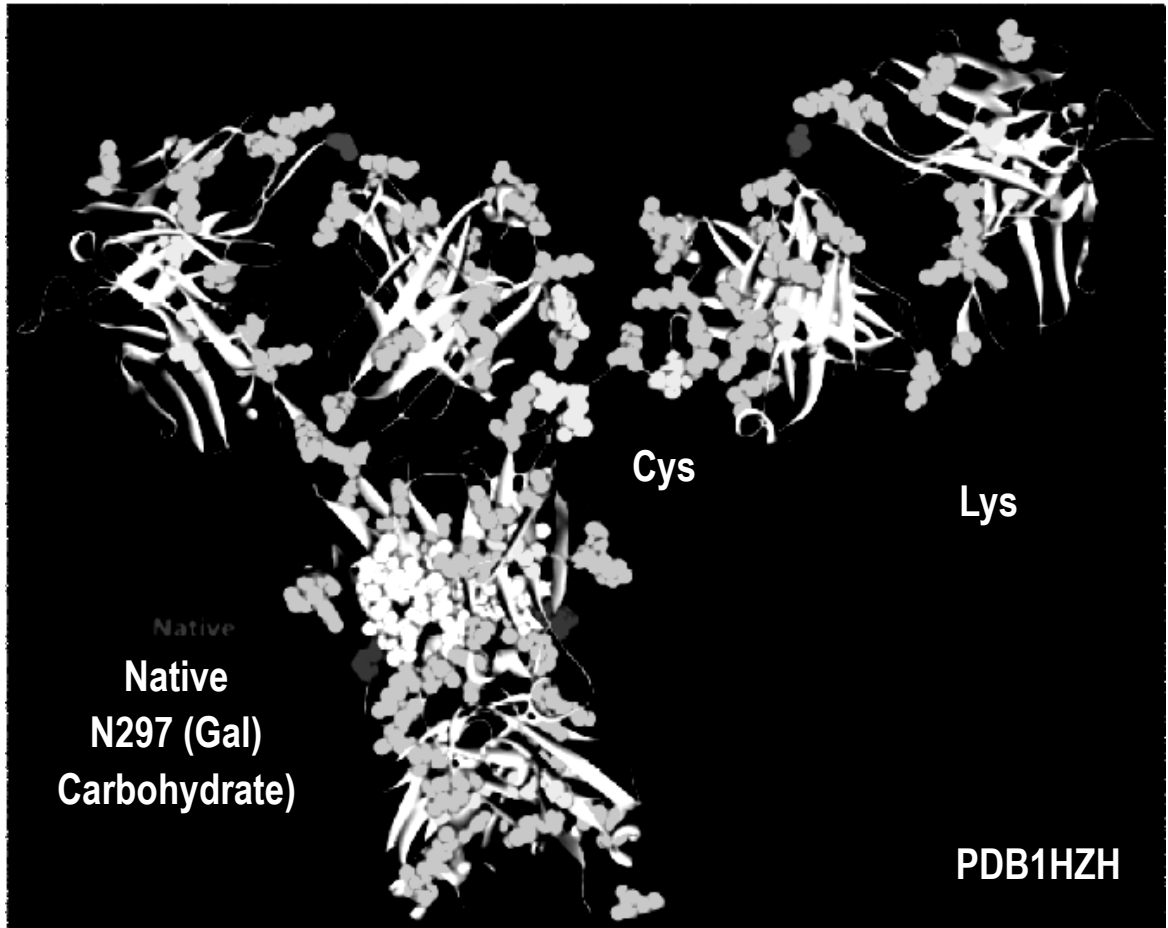


Fig. 25A

C.

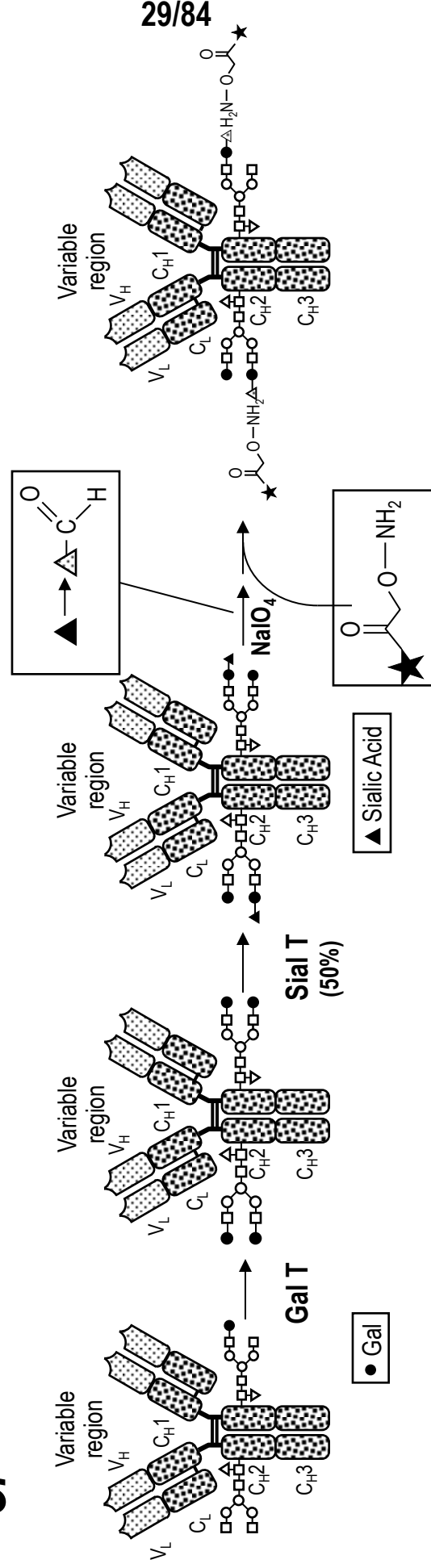


Fig. 25C

Synthesis of aminoxy-Cys-MC-VC-PABC-MMAE and aminoxy-Cys-MC-VC-PABC-PEG8-Do10

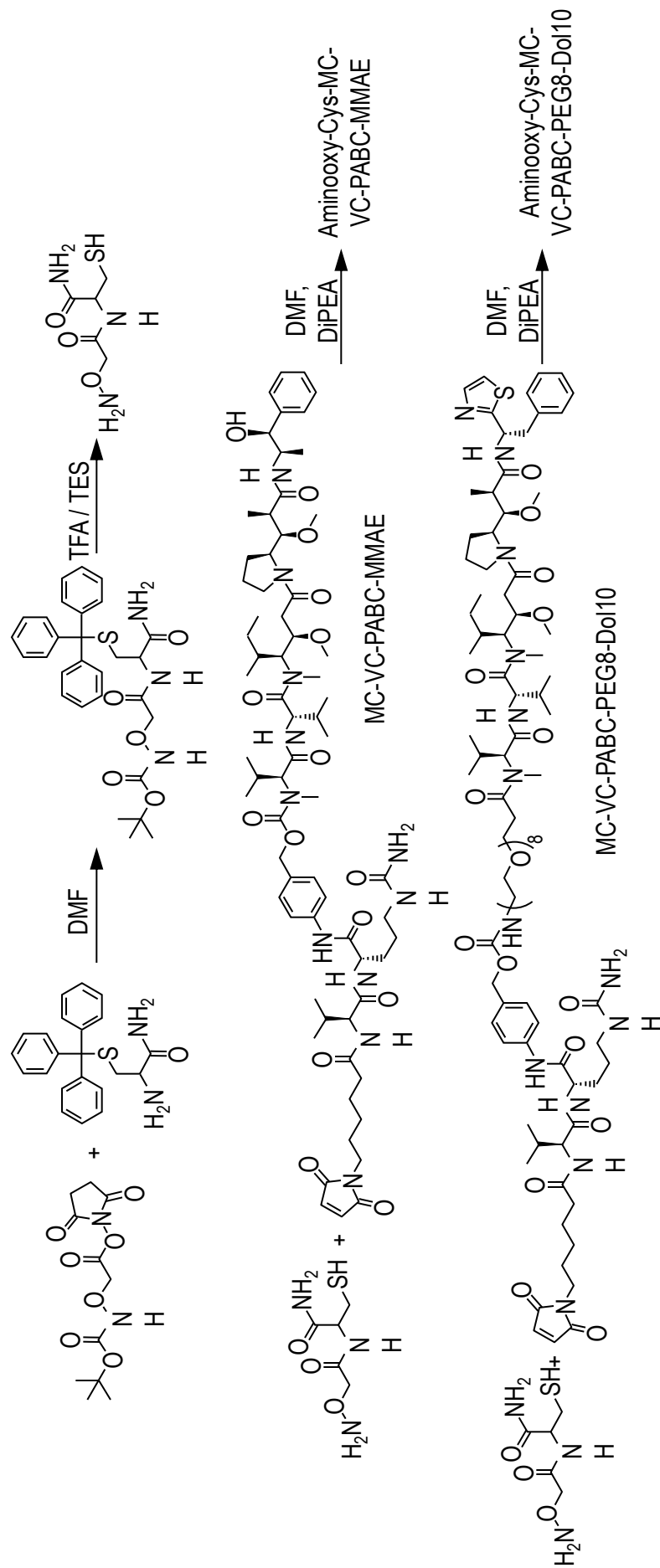
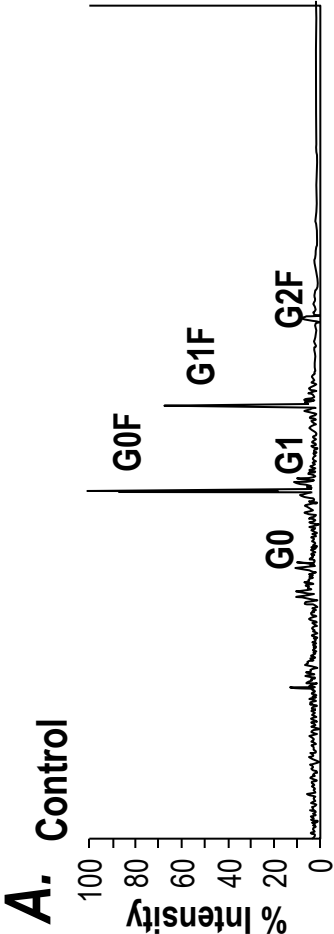
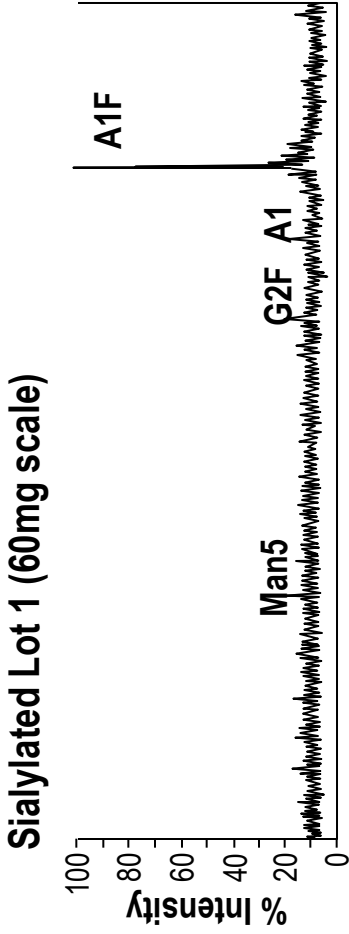


Fig. 26



B.

	Sialic acid, mol/mol
Control	0
Sialylated Lot 1	2.2 ± 0
Sialylated Lot 2	2.3 ± 0.1

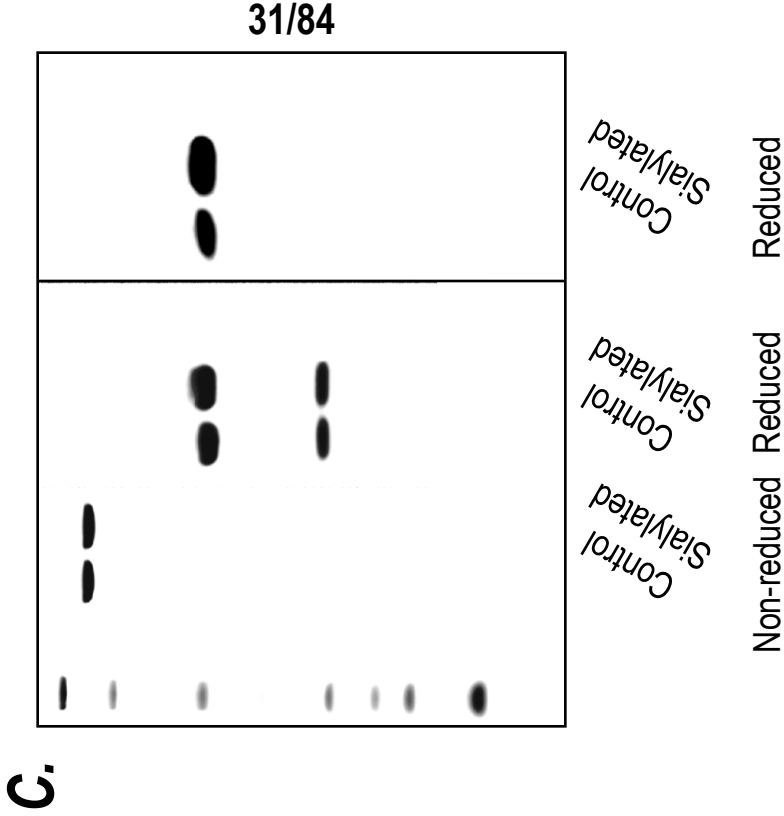


Fig. 27

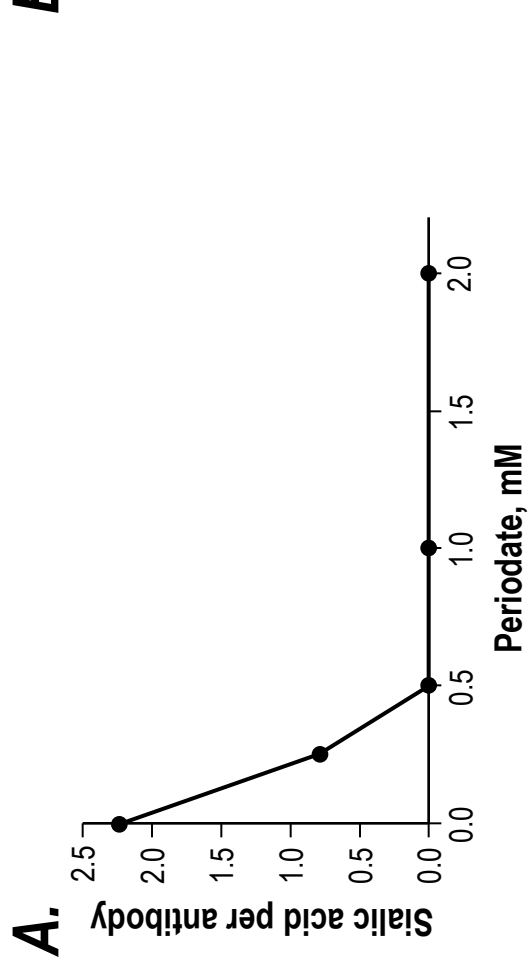
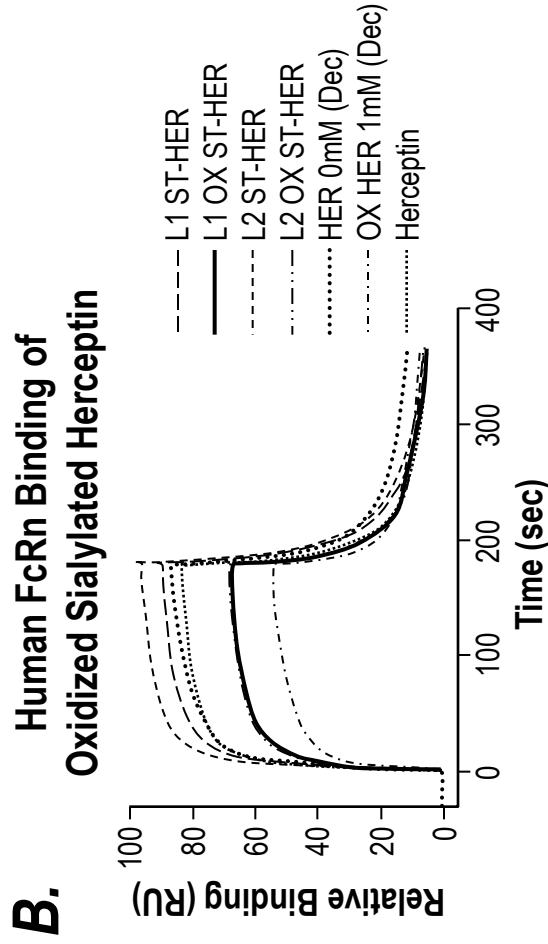
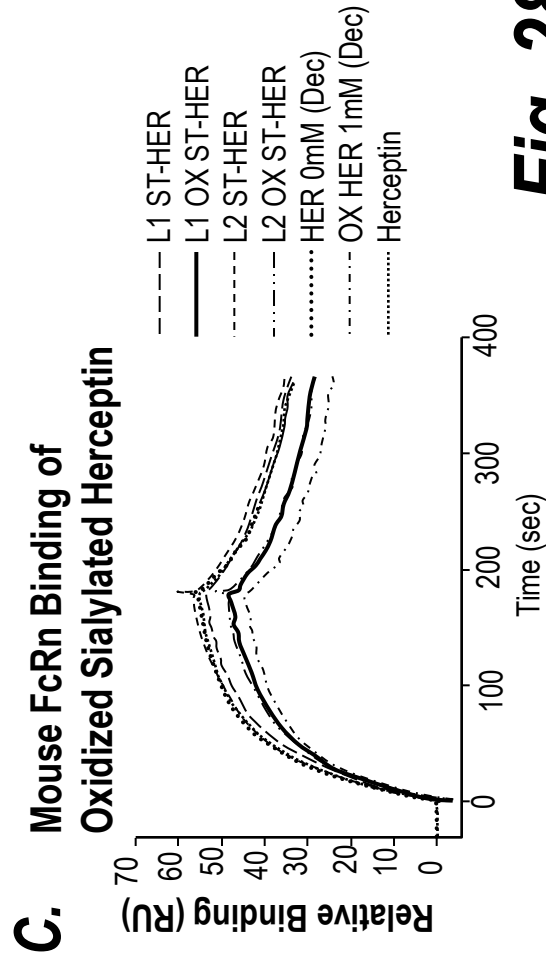
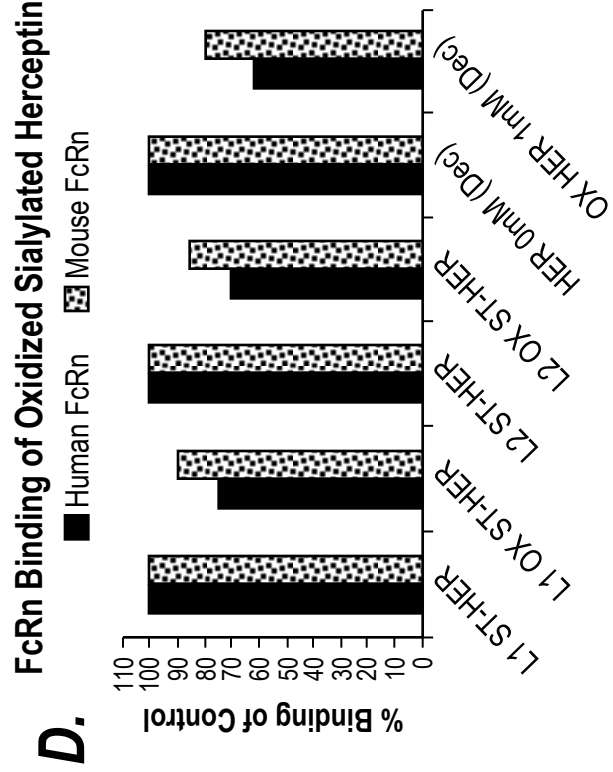
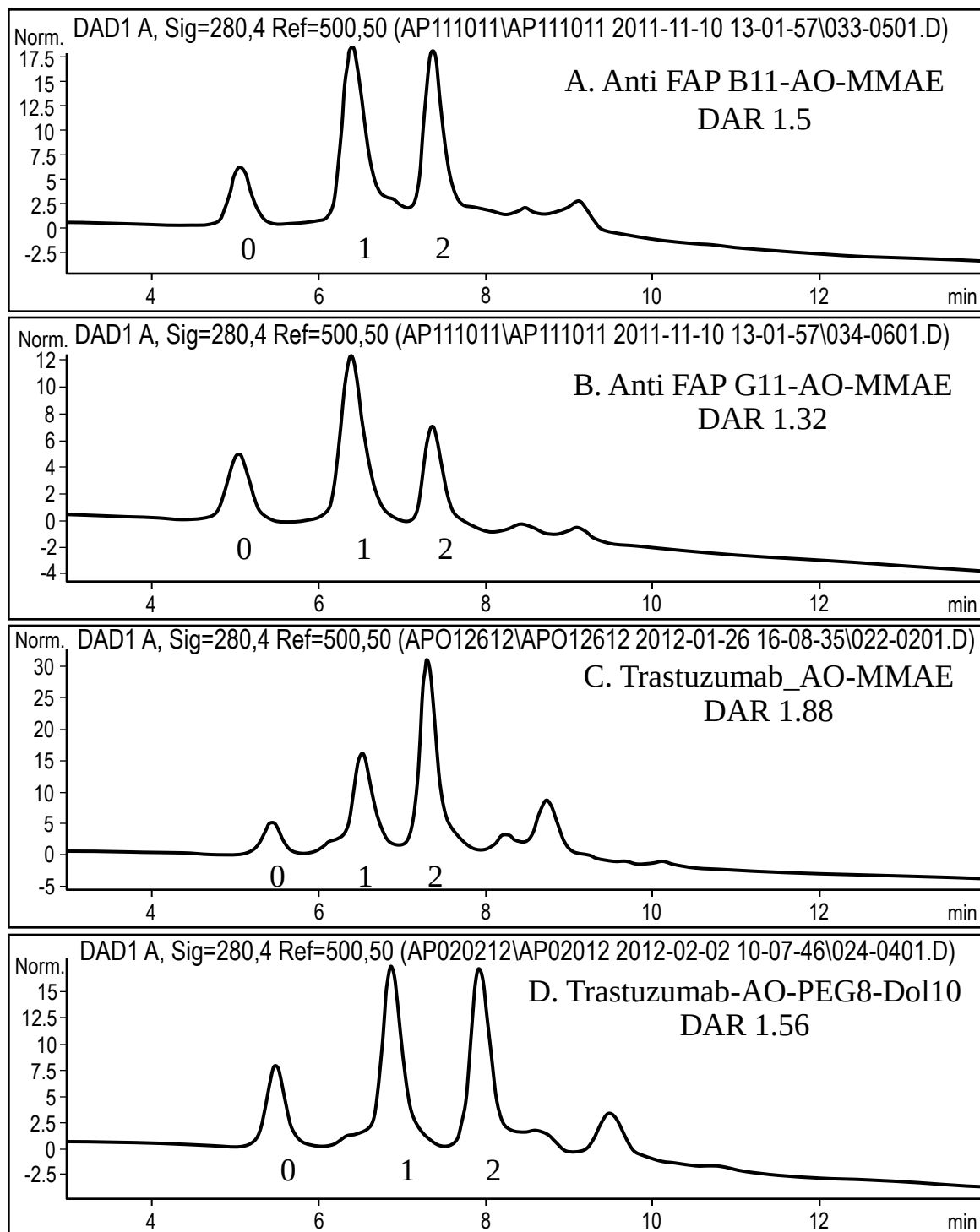
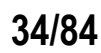


Fig. 28

**Fig. 29**

MC-VC-PABC-MMAE



WT + Toxin



Fig. 30

Fig. 30

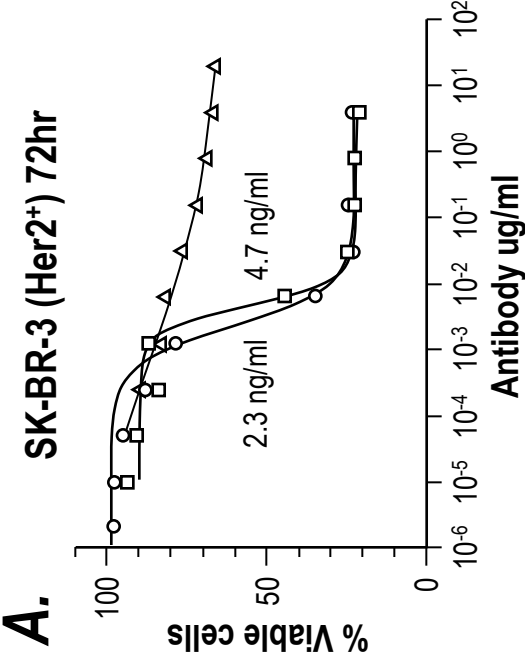
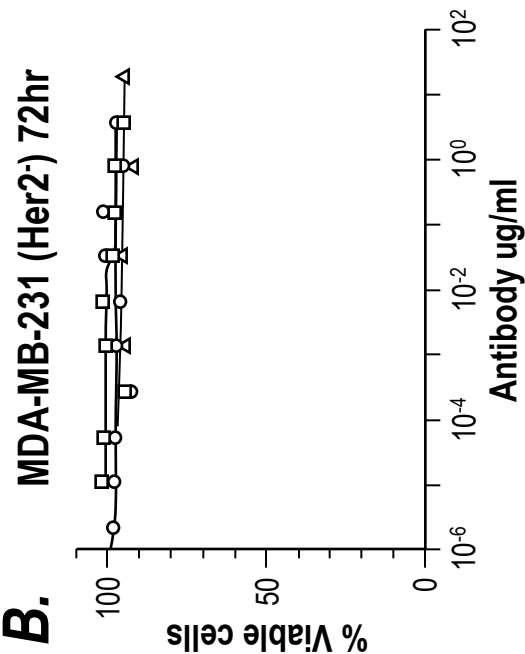
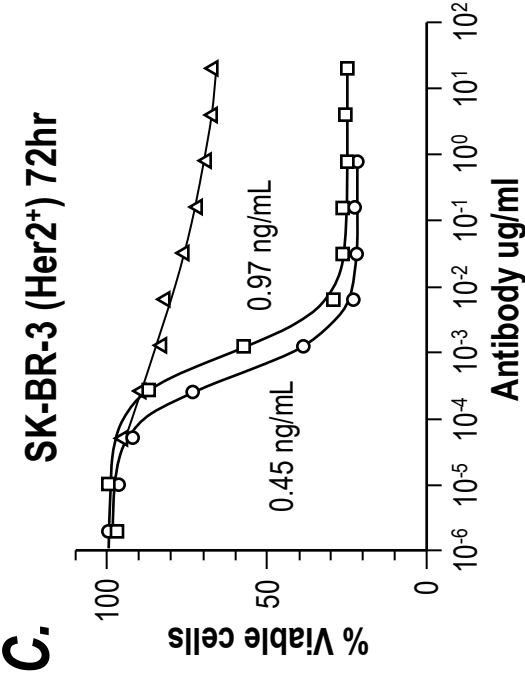
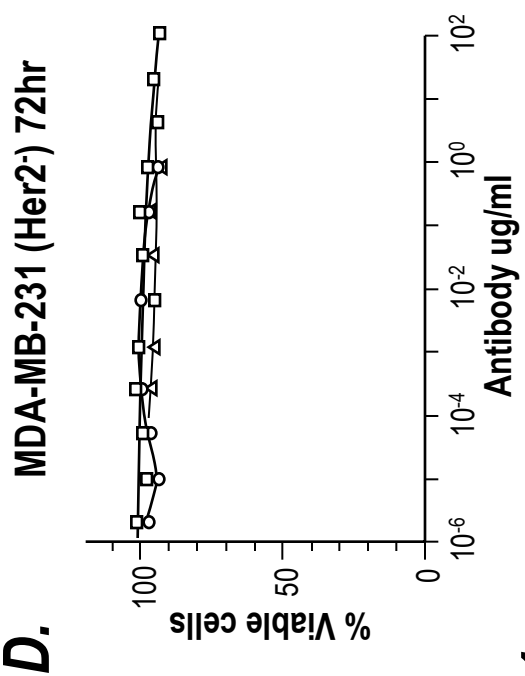


Figure 31: please align the legend so that symbols and words line up

Fig. 31

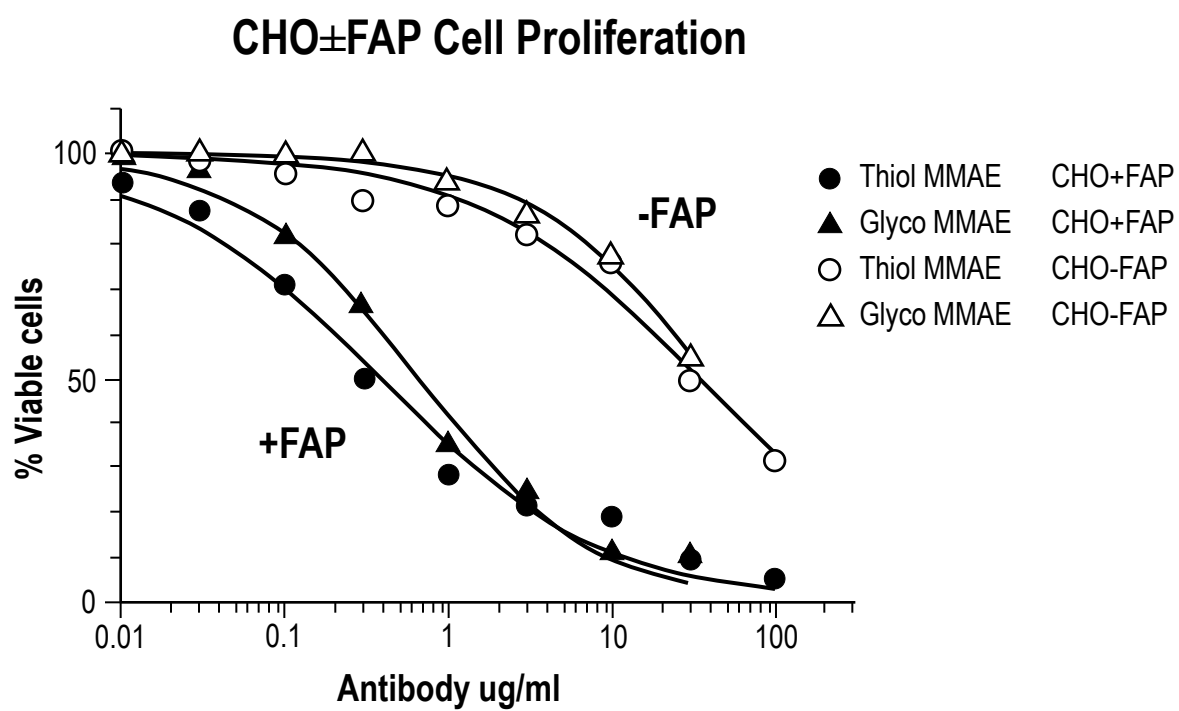


Fig. 32

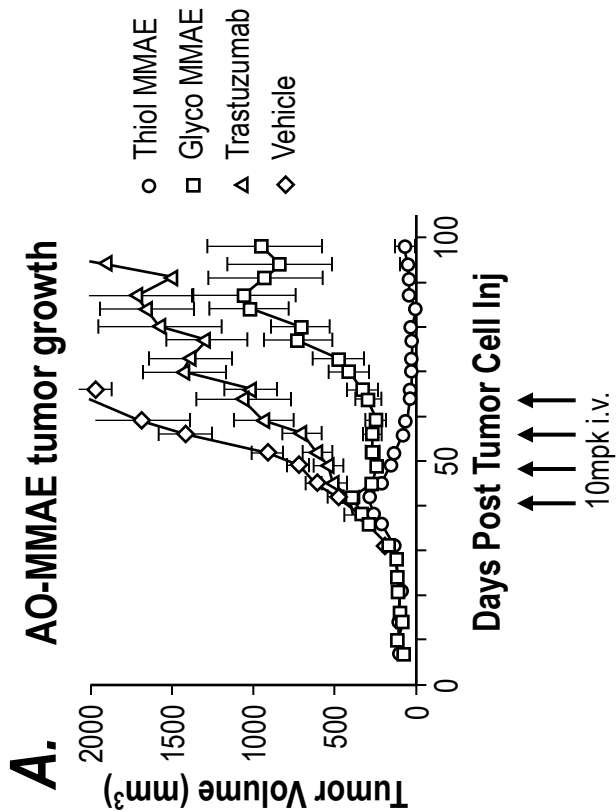
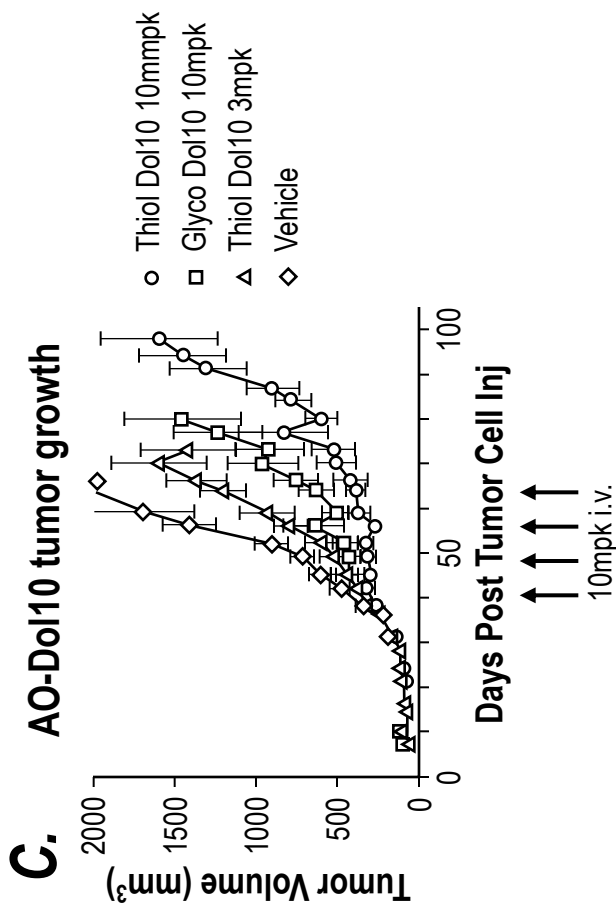
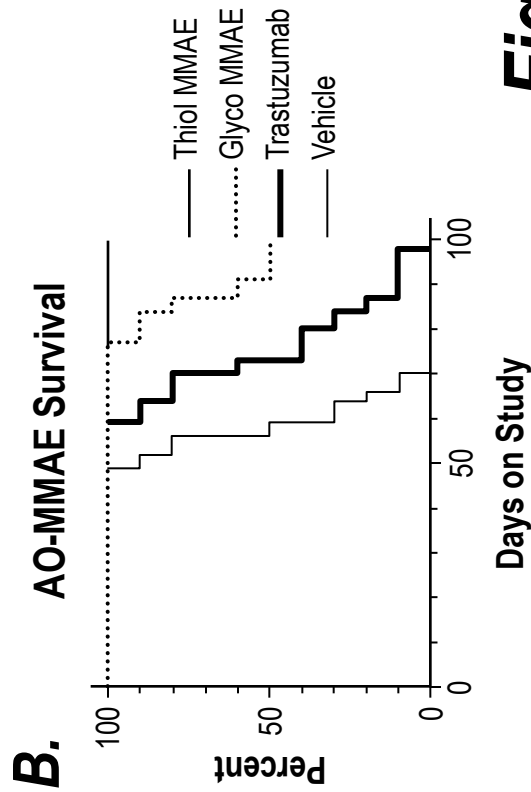
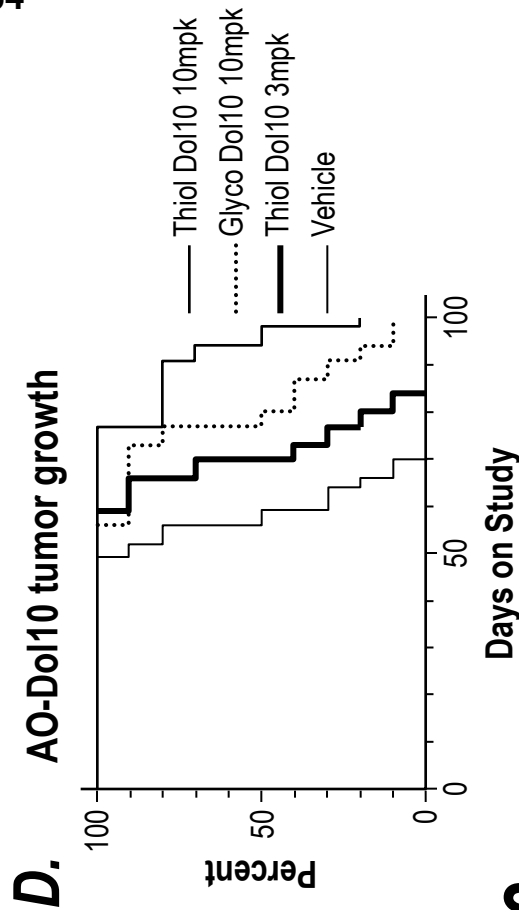


Fig. 33

LC/MS of S298N/T299A/Y300S (NNAS) heavy chain

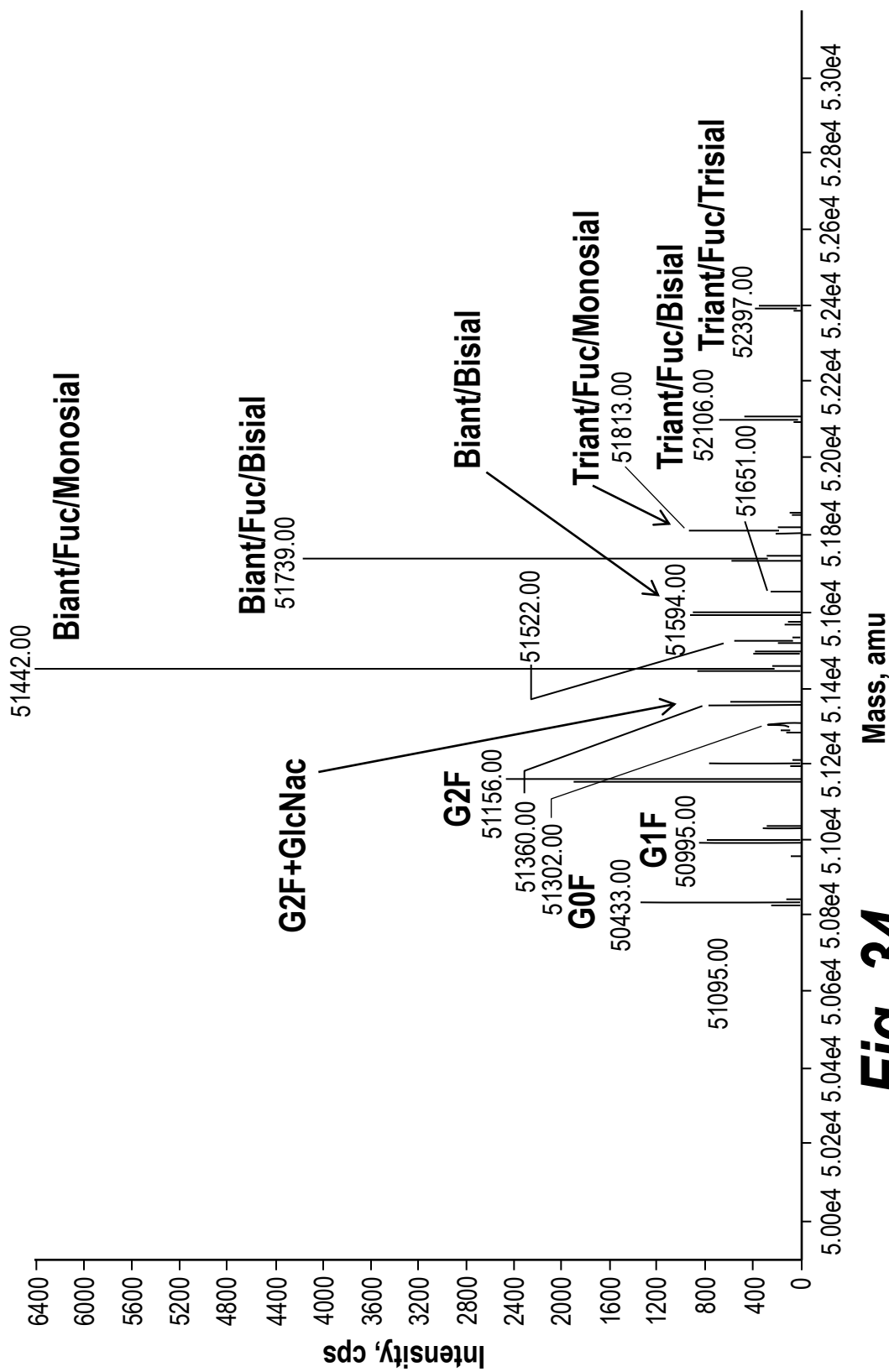


Fig. 34

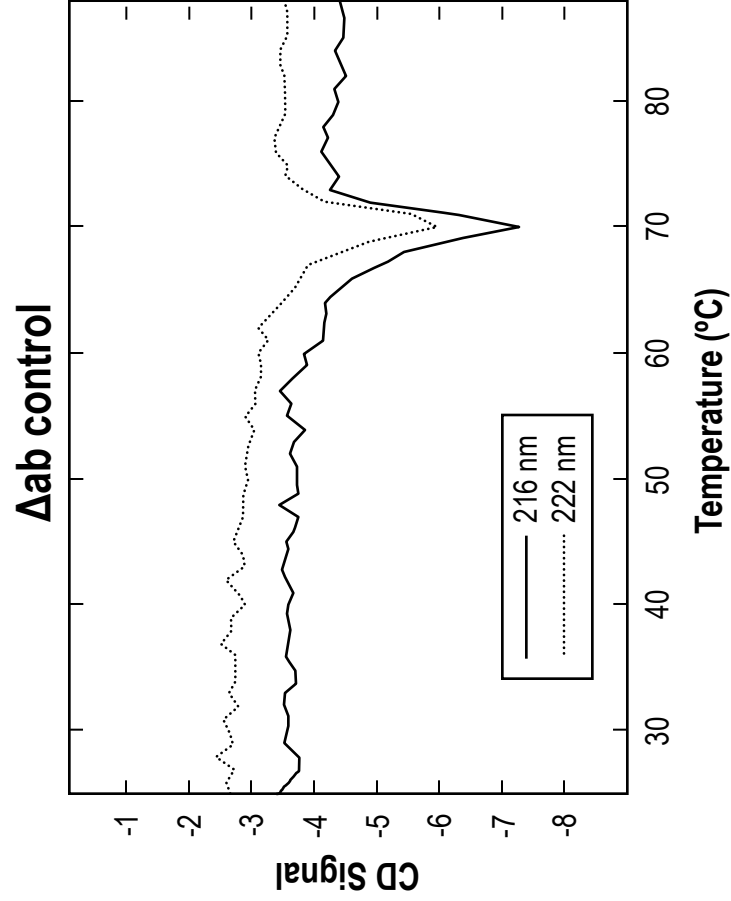
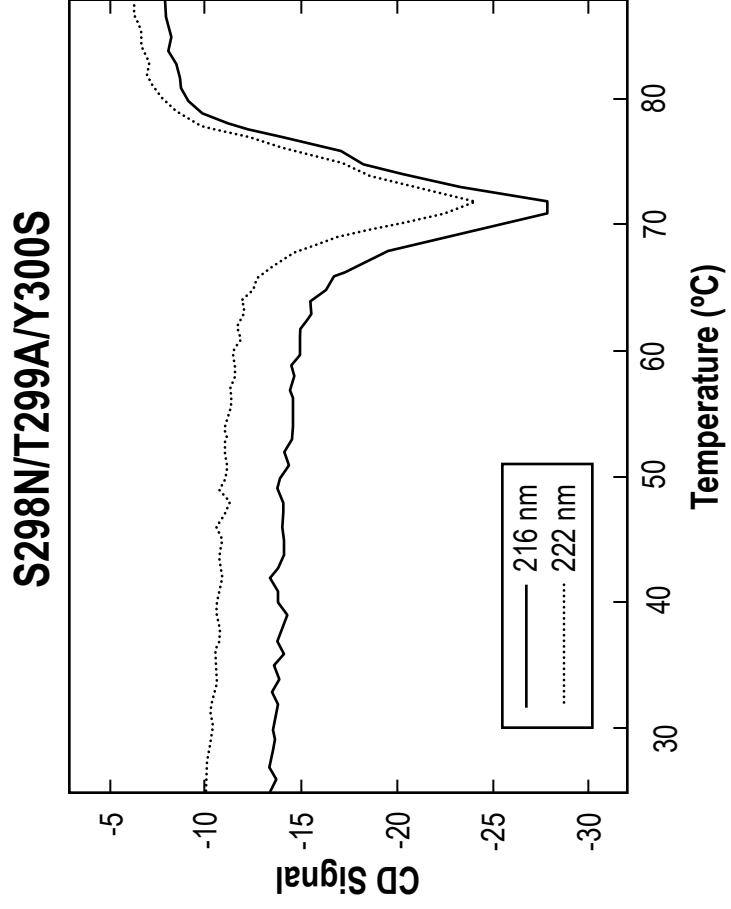


Fig. 35

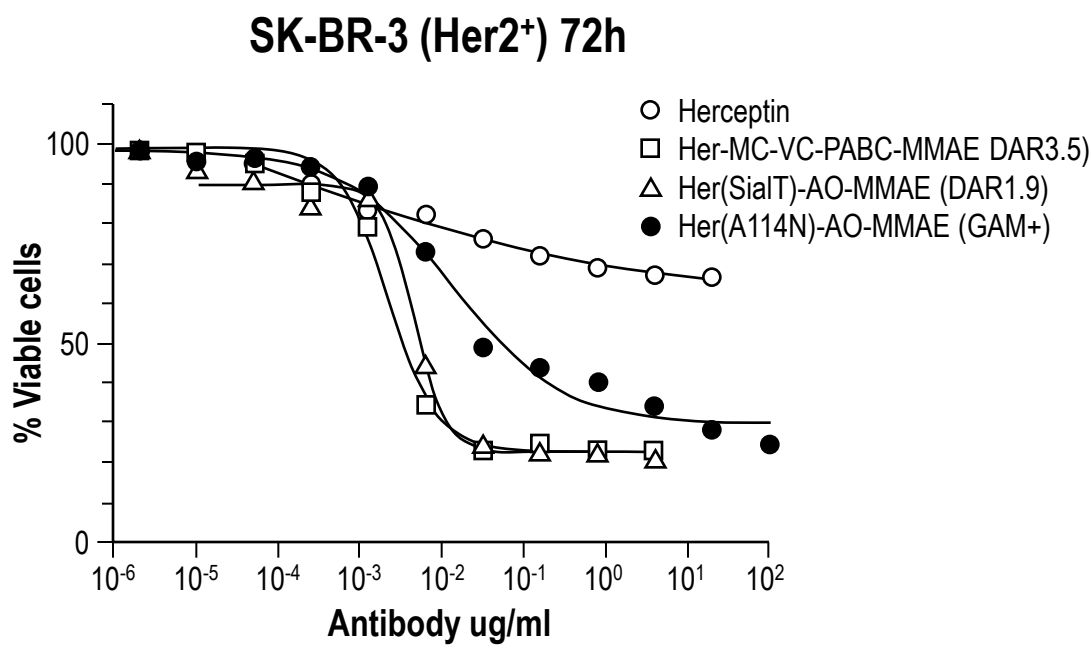
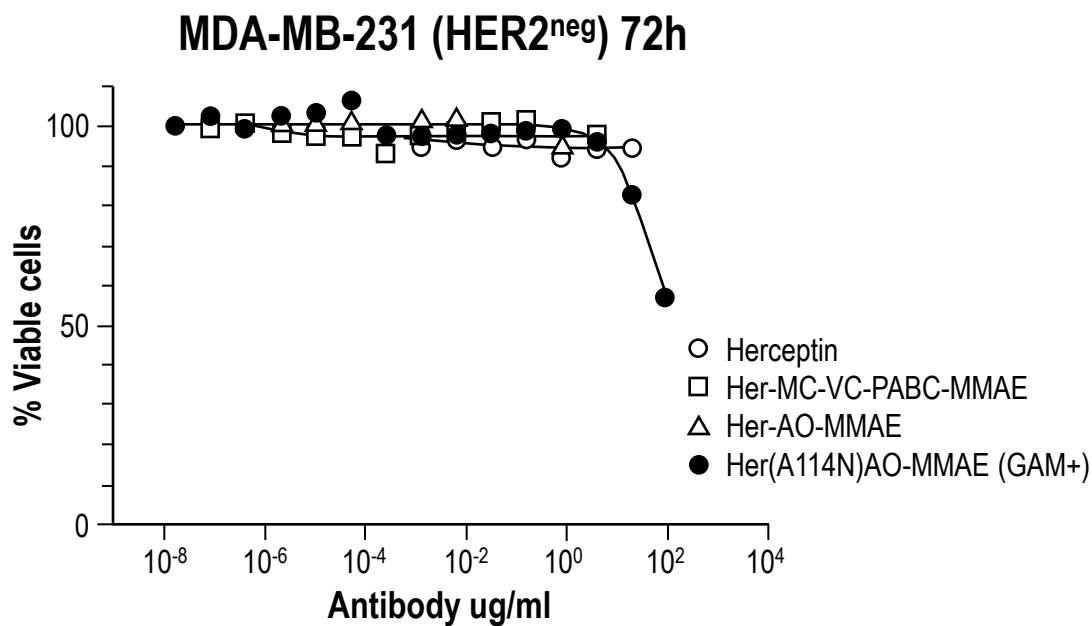


Fig. 36

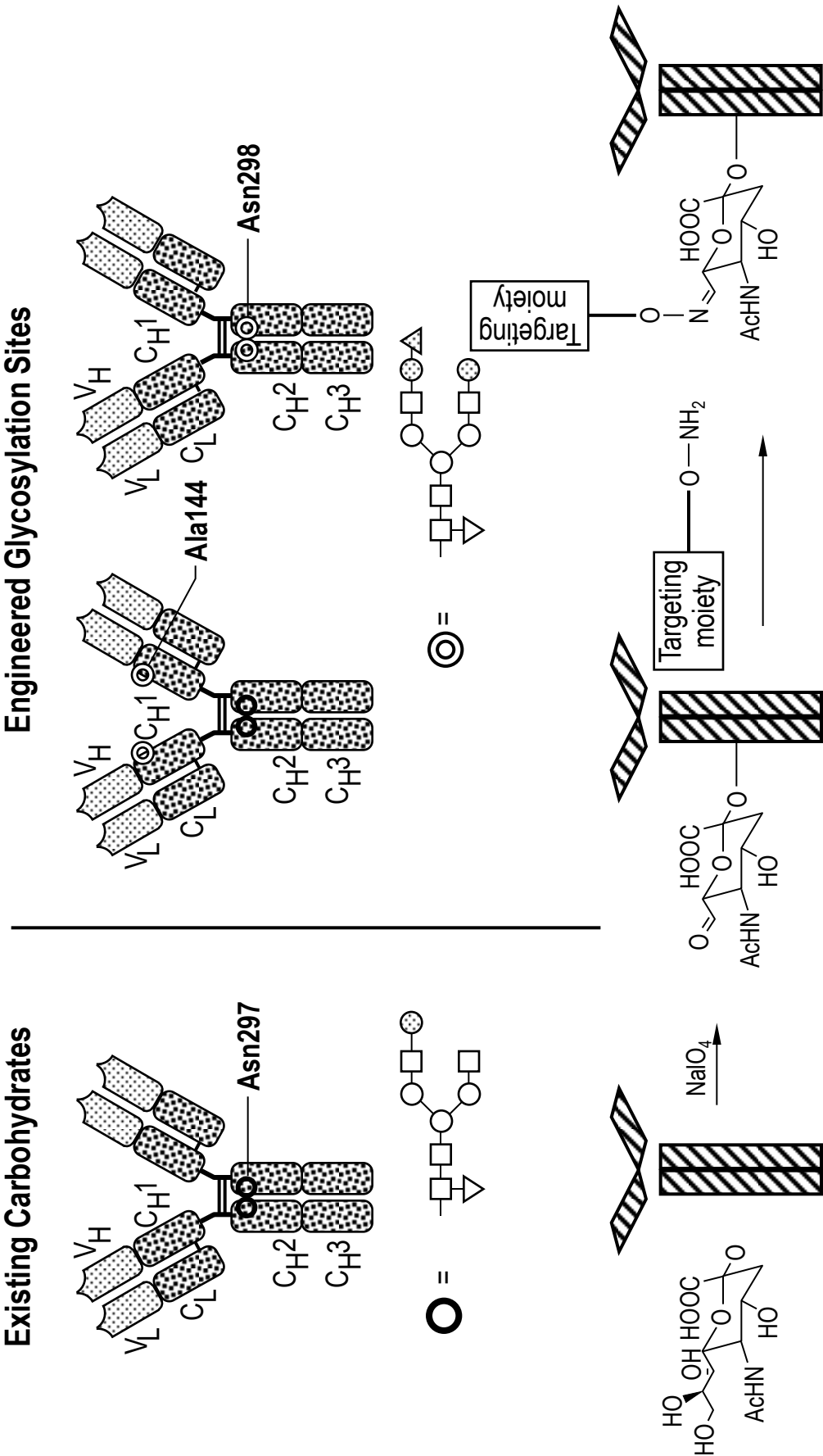


Fig. 37

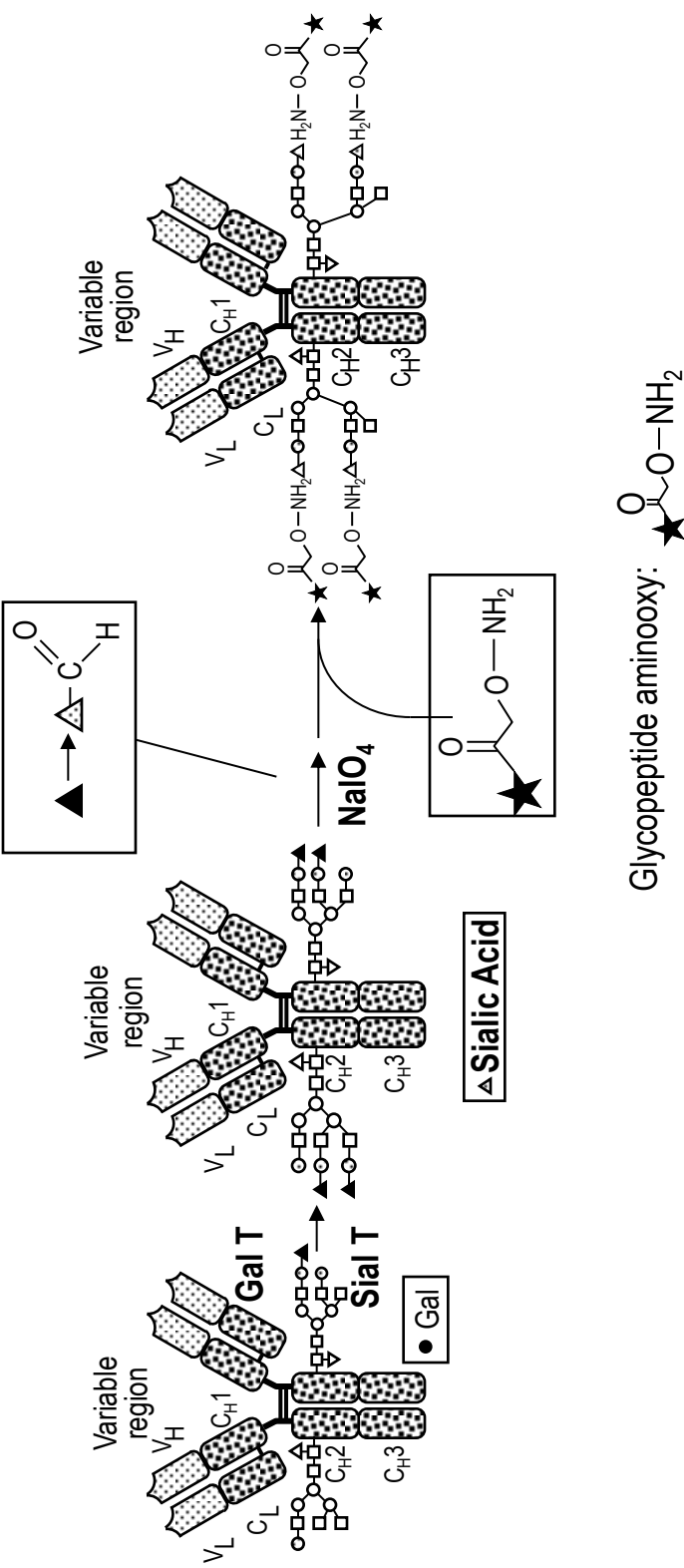


Fig. 38

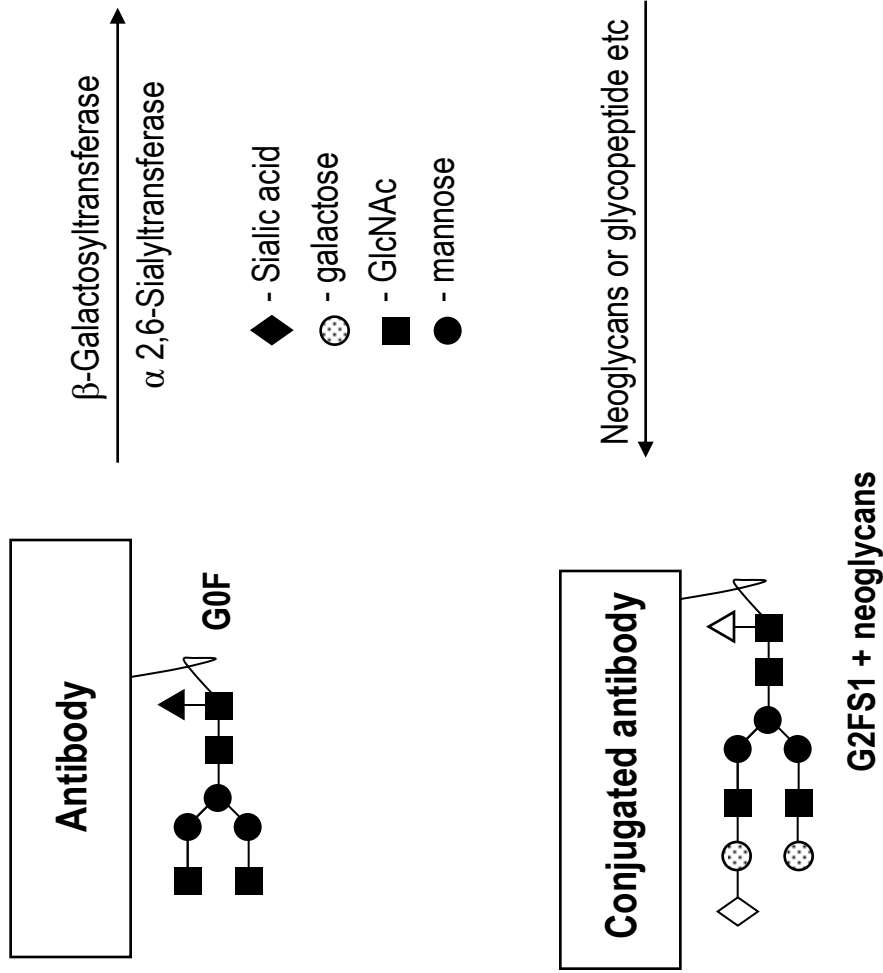
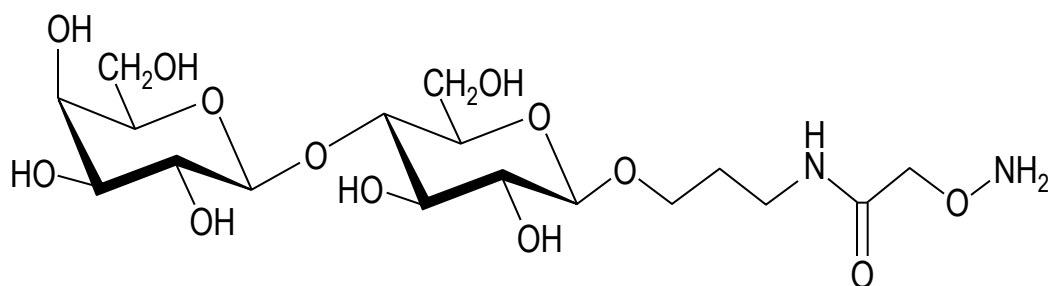


Fig. 39

Lactose aminooxy for native glycans (SAM or GAM):



Exact Mass: 472.19
Molecular Weight: 472.44

bisM6P hexamannose aminooxy (SAM or GAM):

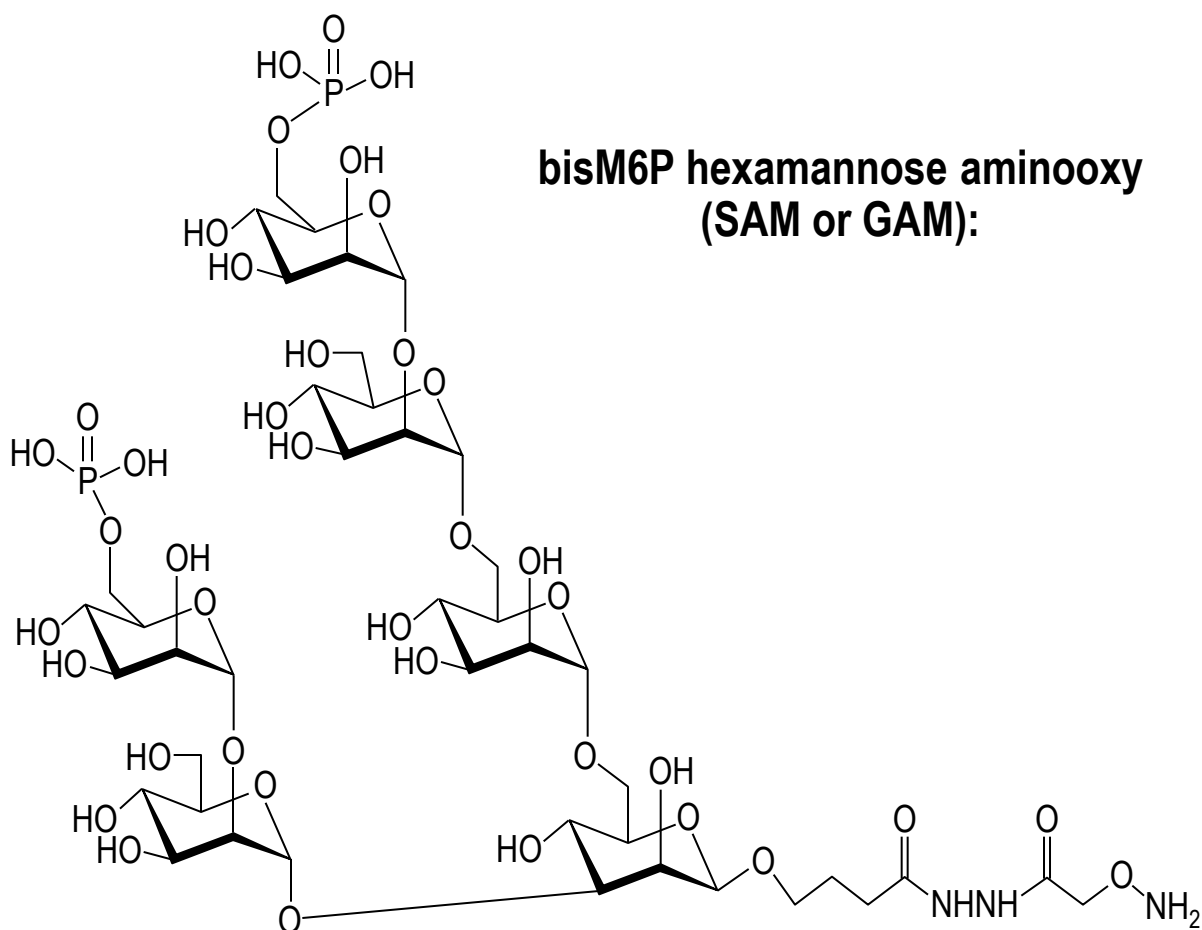
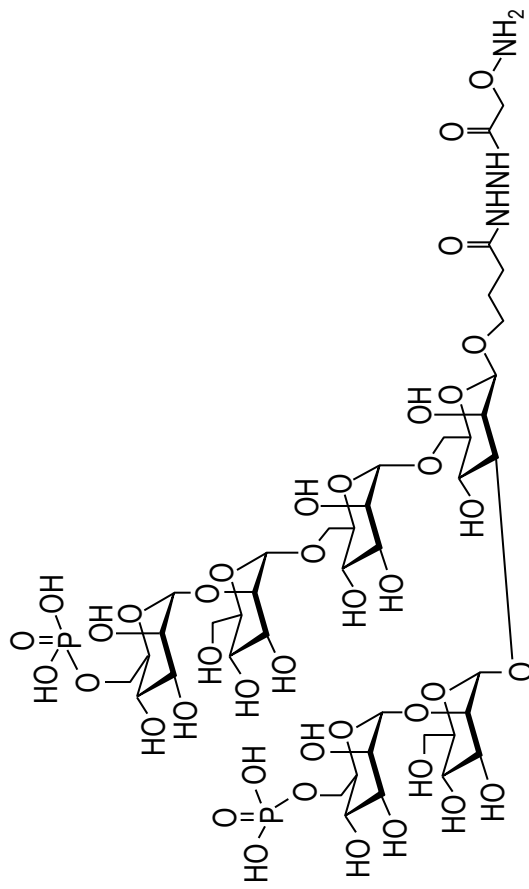
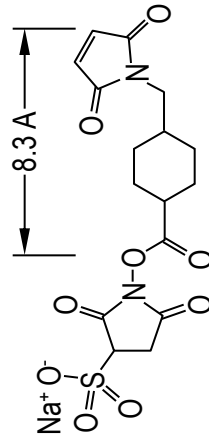


Fig. 40

Fig. 41

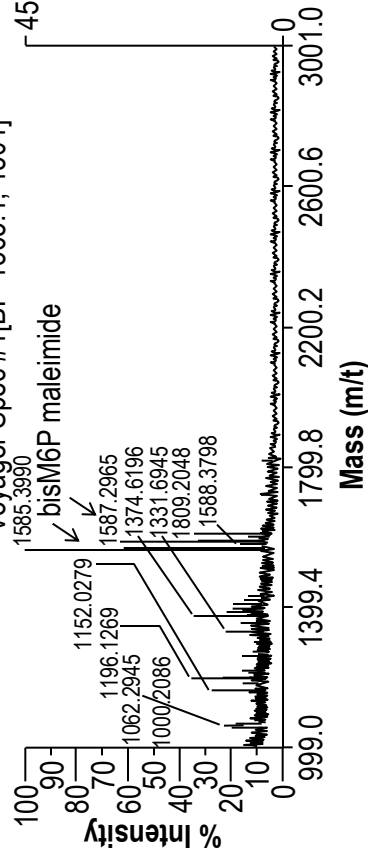
bisM6P hexamannose aminoxy
(SAM on Fc glycans):



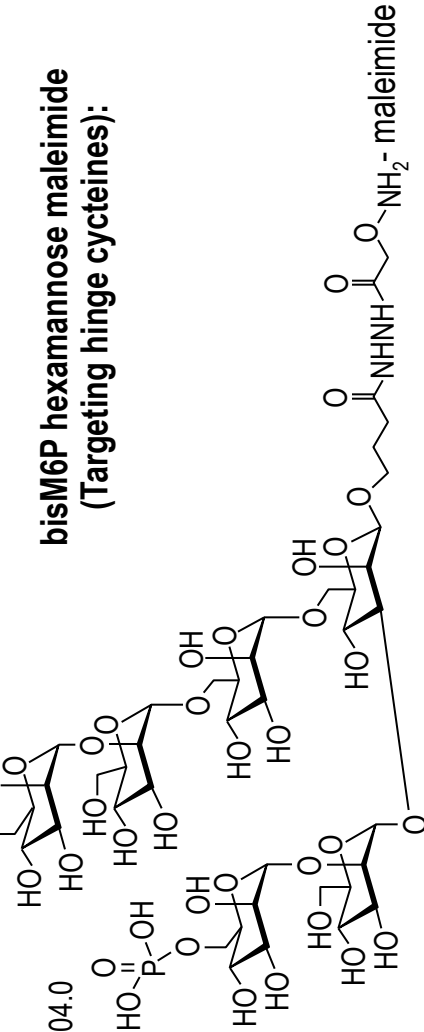
Sulfo SMCC

Voyager Spec #1 [BP-1565.4, 4504]

bisM6P maleimide



bisM6P hexamannose maleimide
(Targeting hinge cycteines):



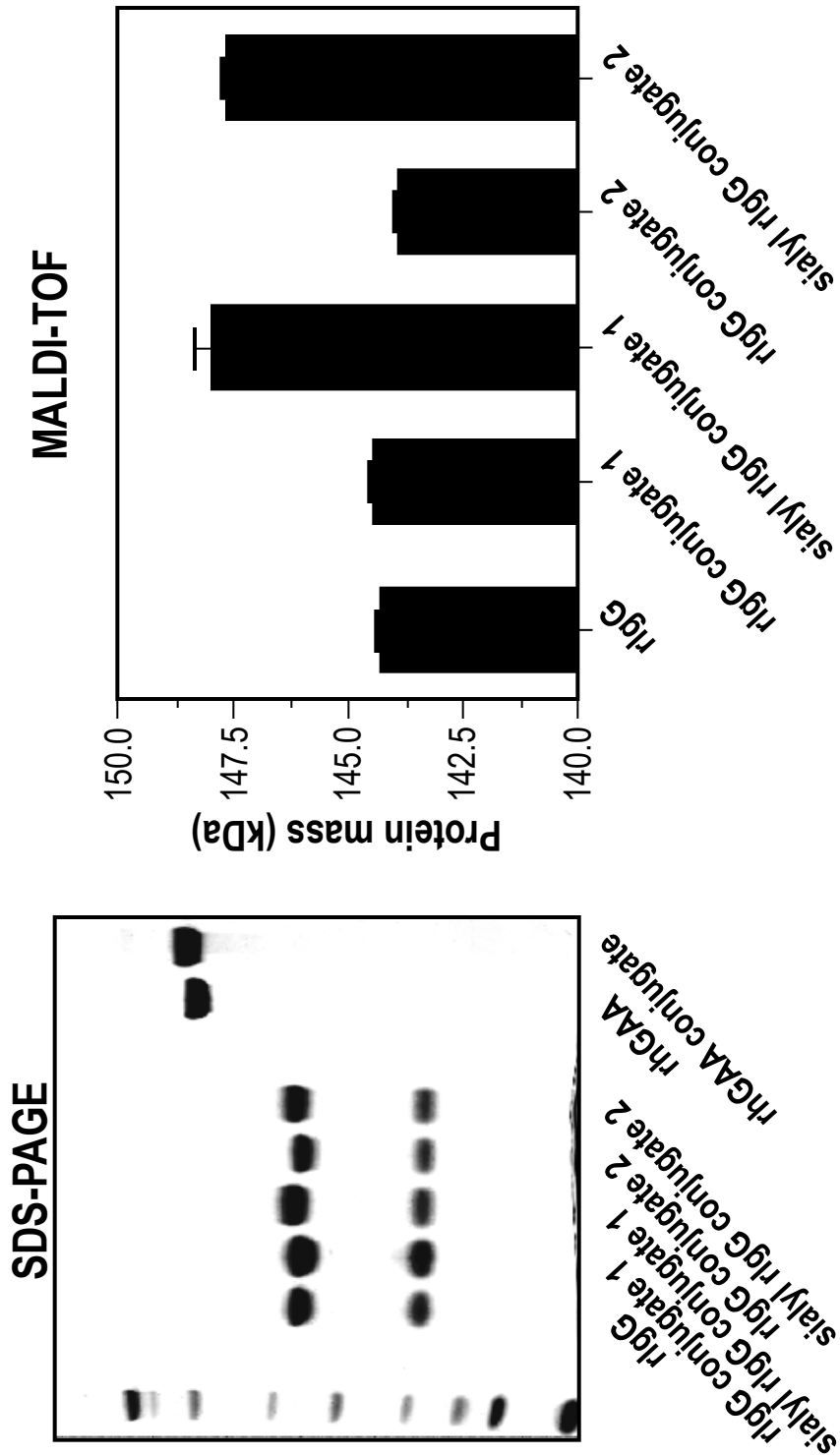


Fig. 42

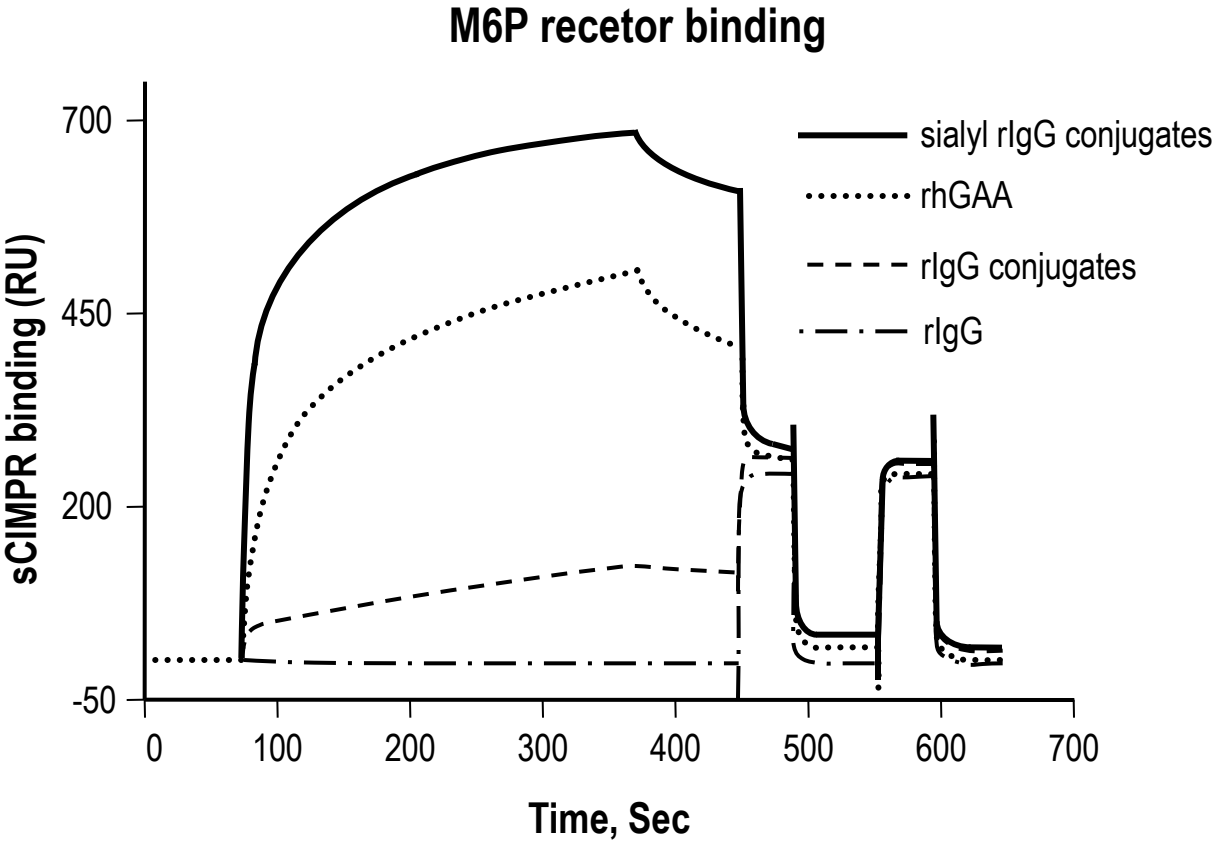
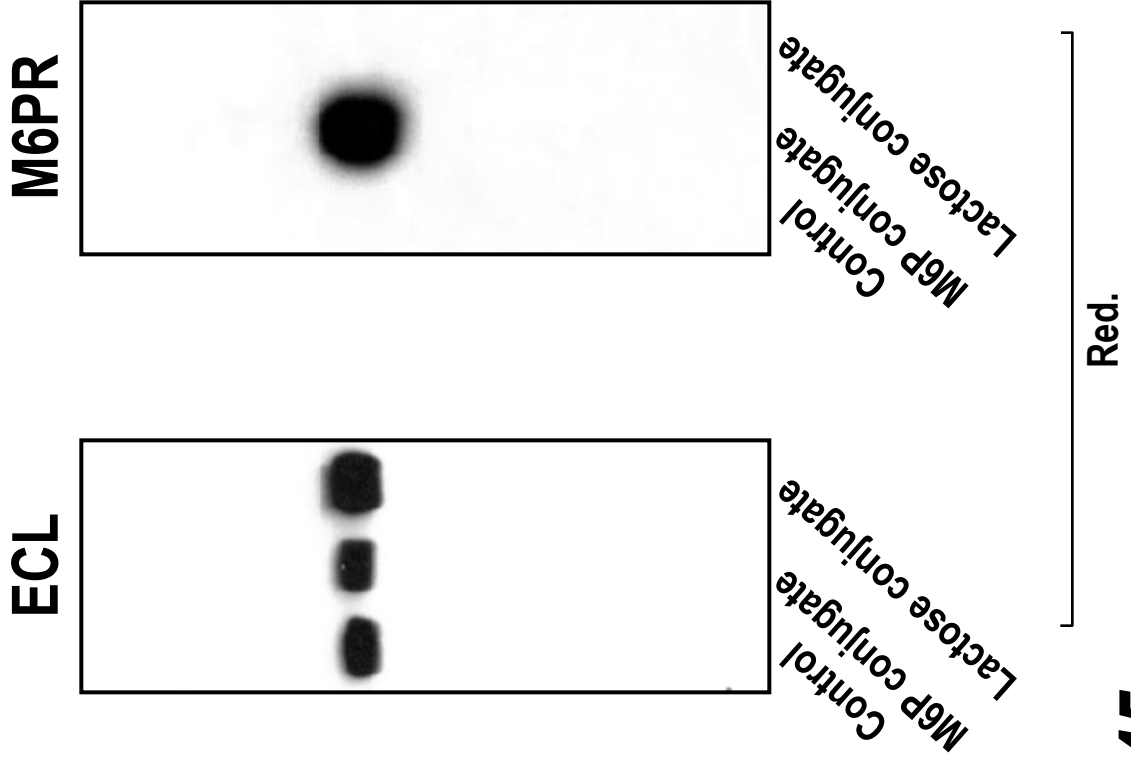
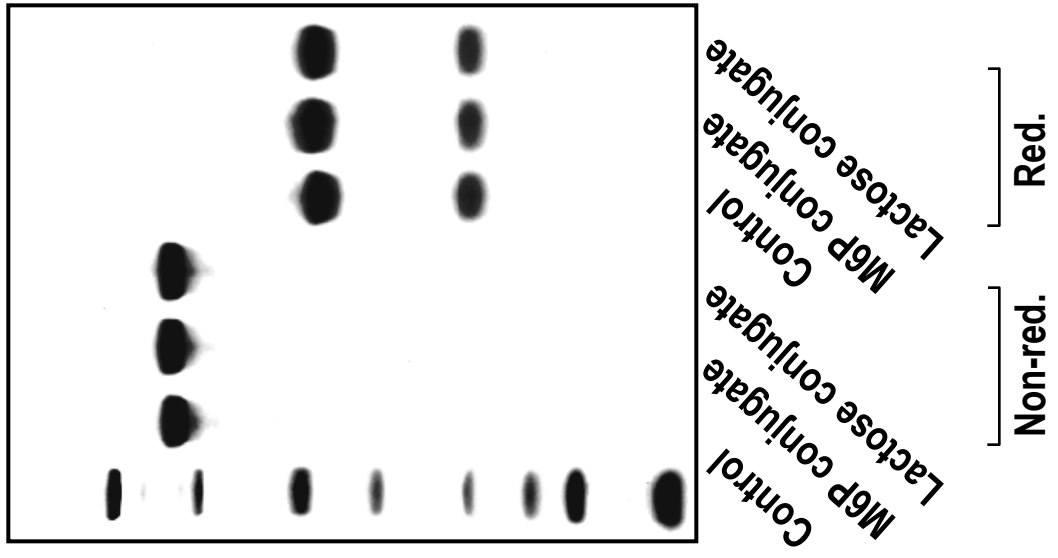


Fig. 43



Samples	MW (kDa)	# Glycan
Control	145.42 $\pm$ 0.24	0.00
M6P conjugate	147.95 $\pm$ 0.09	1.92
Lactose conjugate	146.35 $\pm$ 0.36	1.96

Fig. 46

Galactose quantitation:

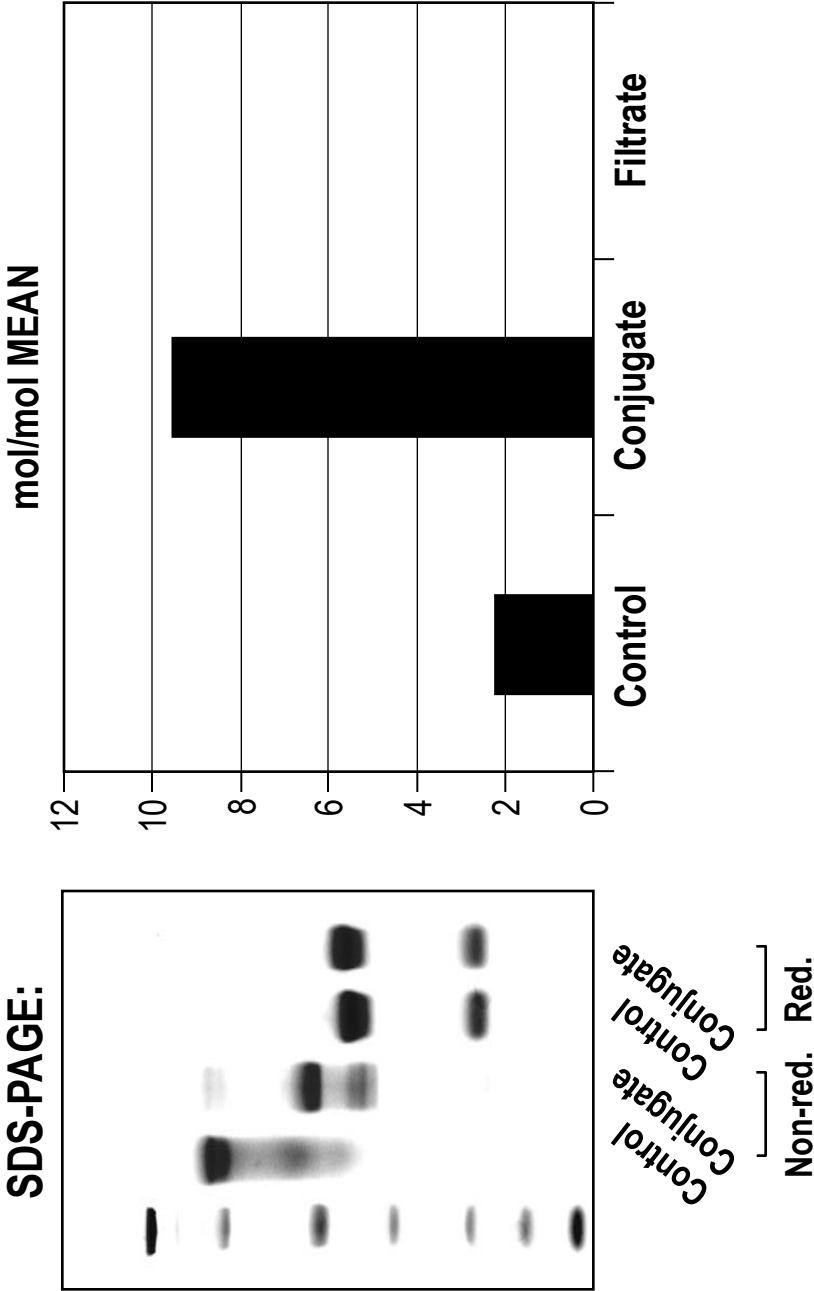


Fig. 48

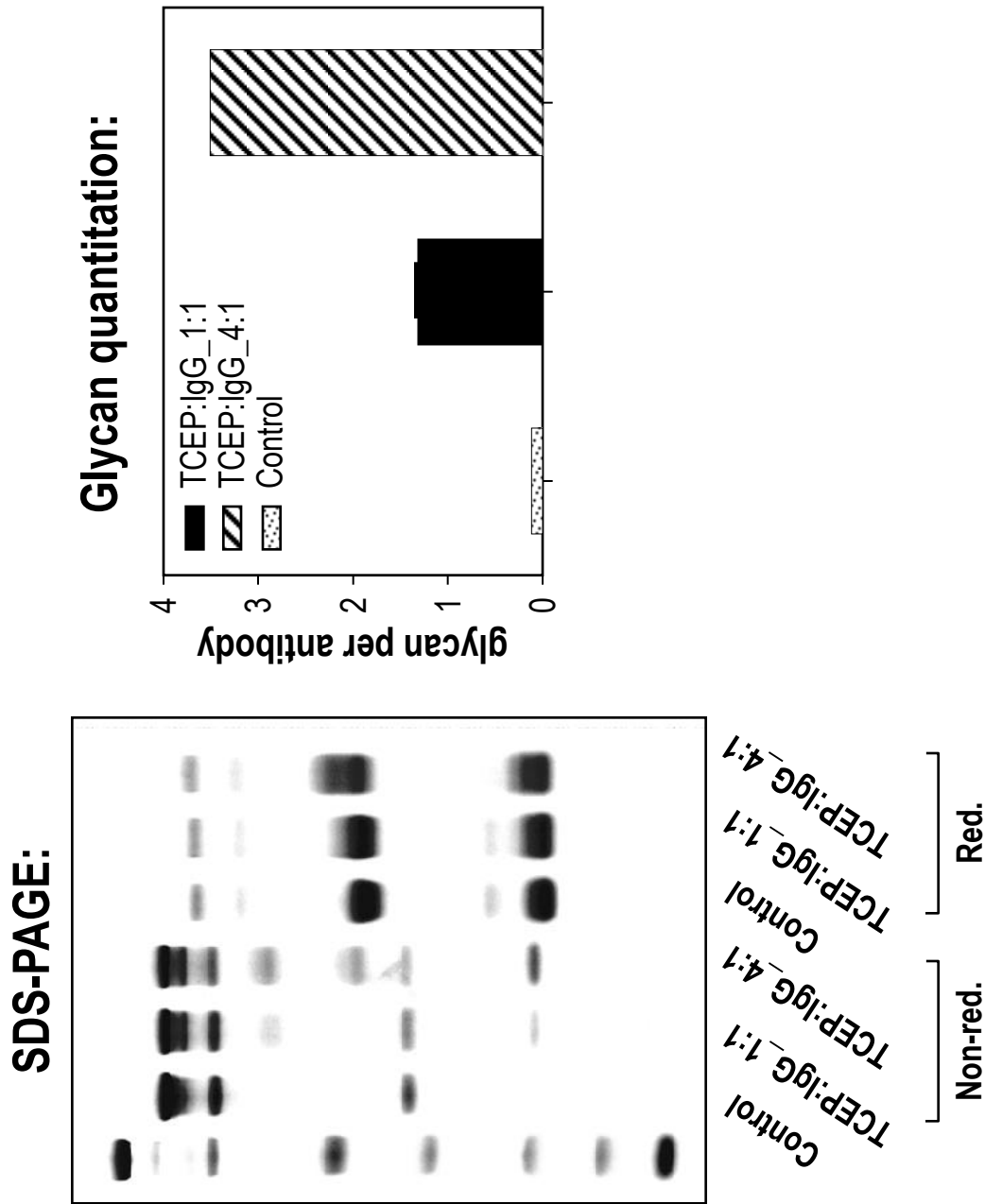
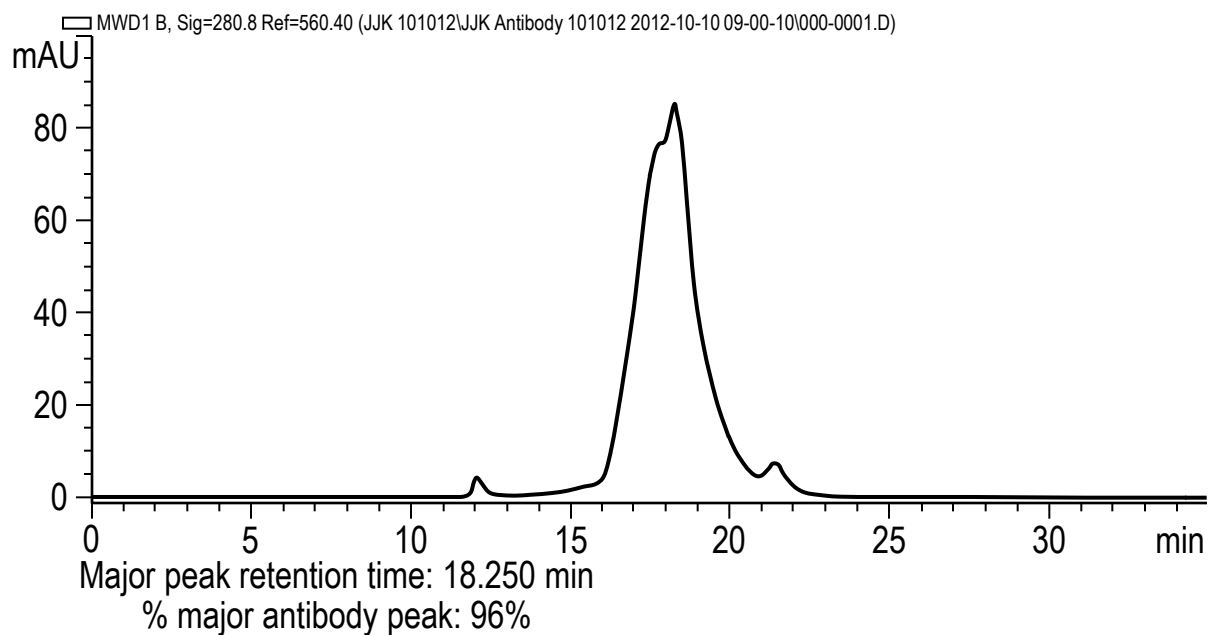


Fig. 49

Control:



Lactose maleimide conjugate:

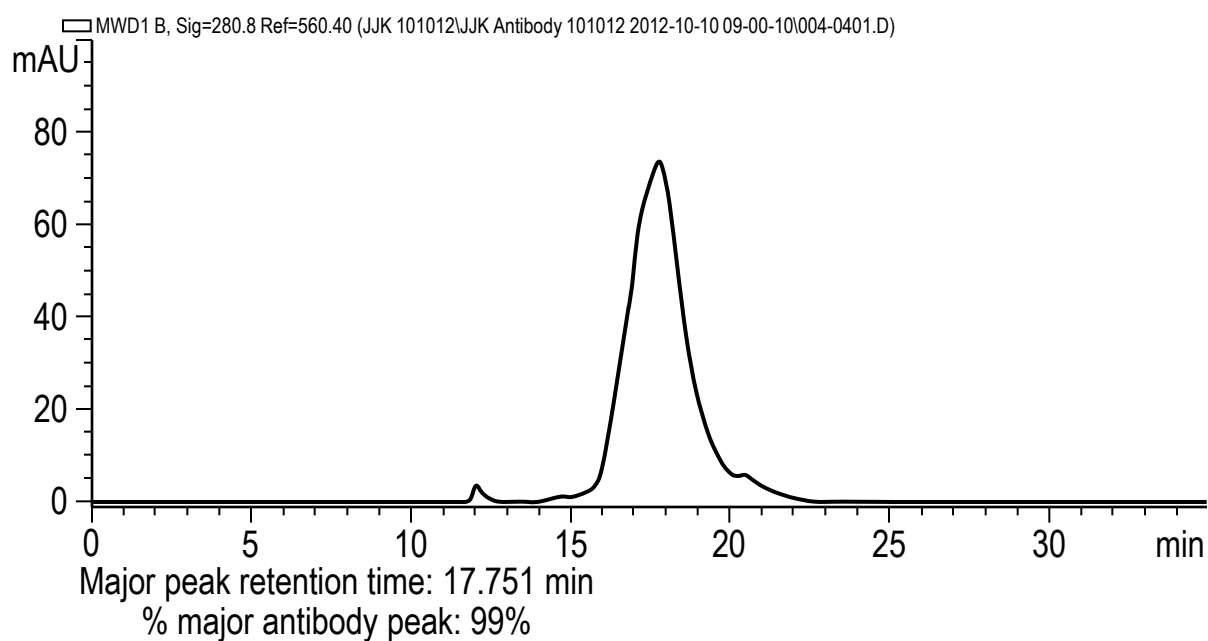
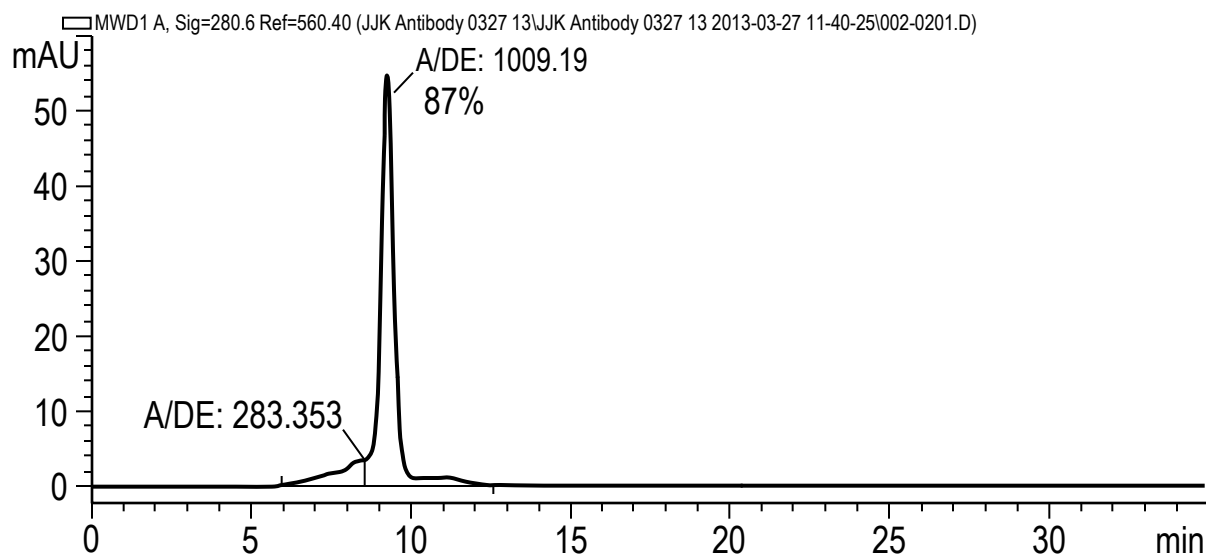


Fig. 50

Control:



M6P conjugate:

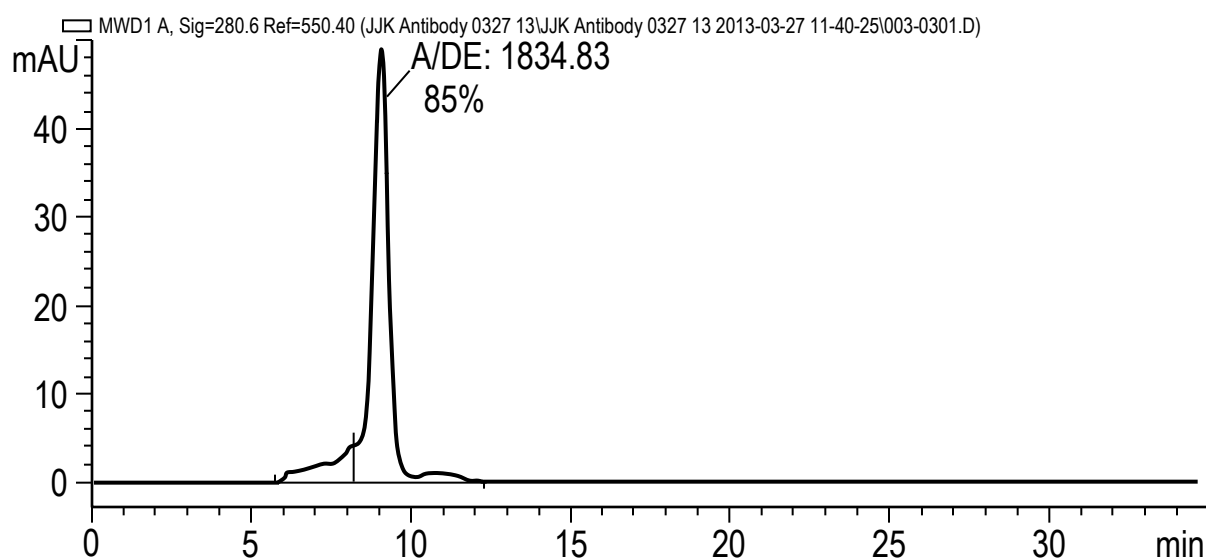
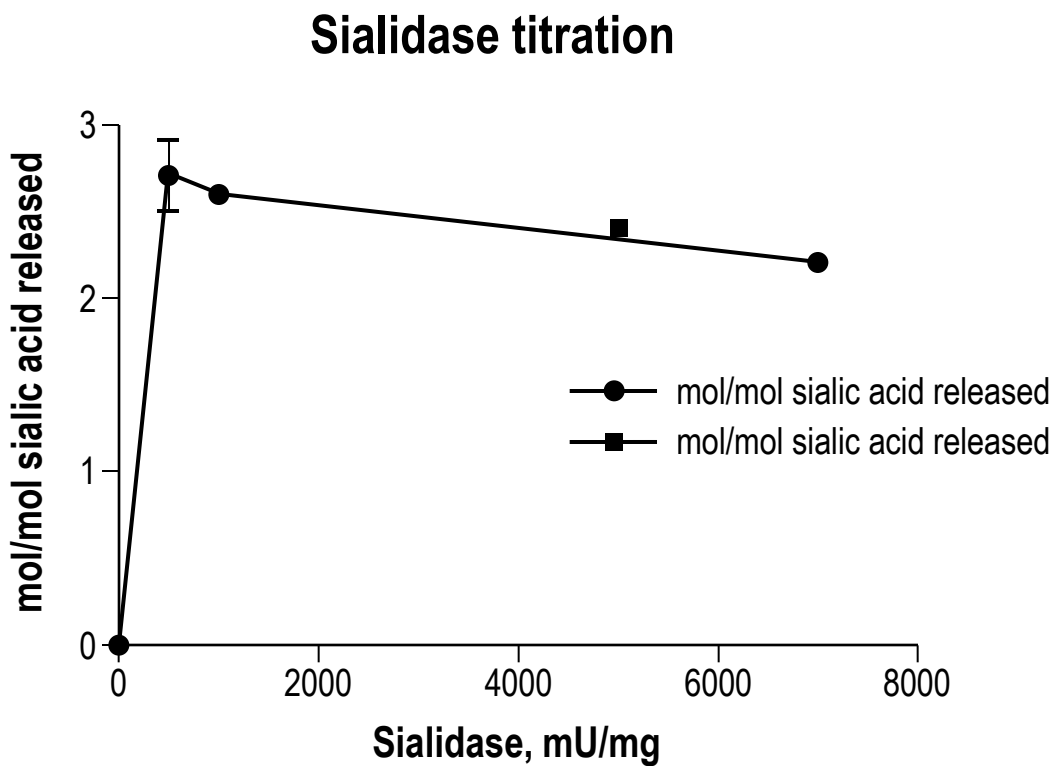


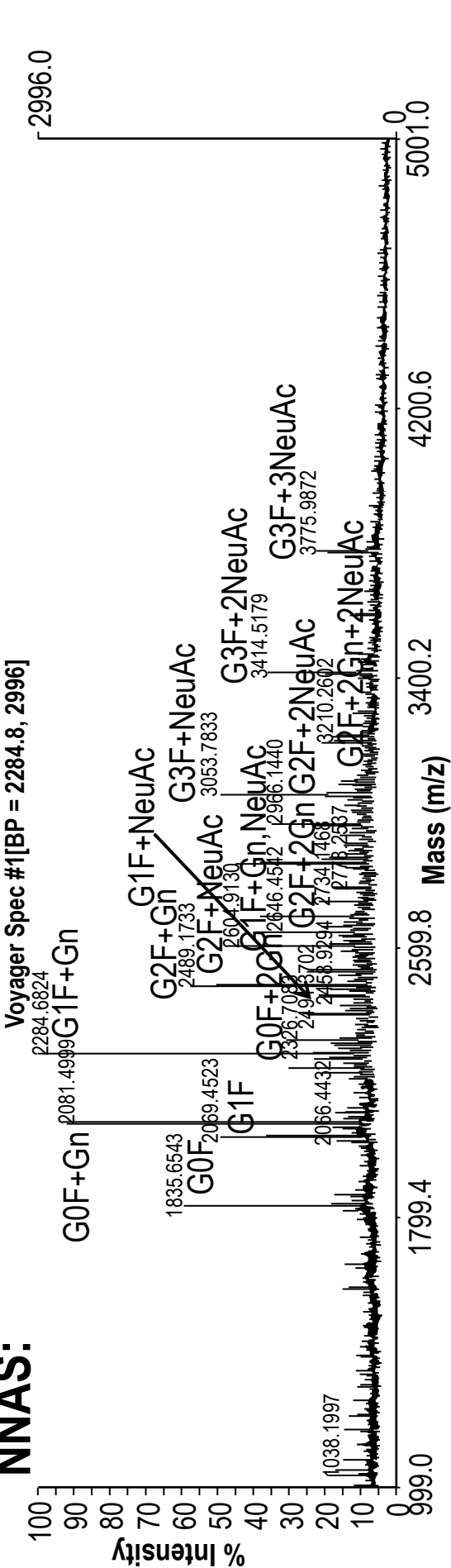
Fig. 51



	mol per mol sialic acid
NNAS	2.4 ± 0
Sialylated NNAS	3.7 ± 0.2
Desialylated and galactosylated NNAS	0 ± 0

Fig. 52

NNAS:



Desialylation and galactosylation

NNAS desialylated and galactosylated:

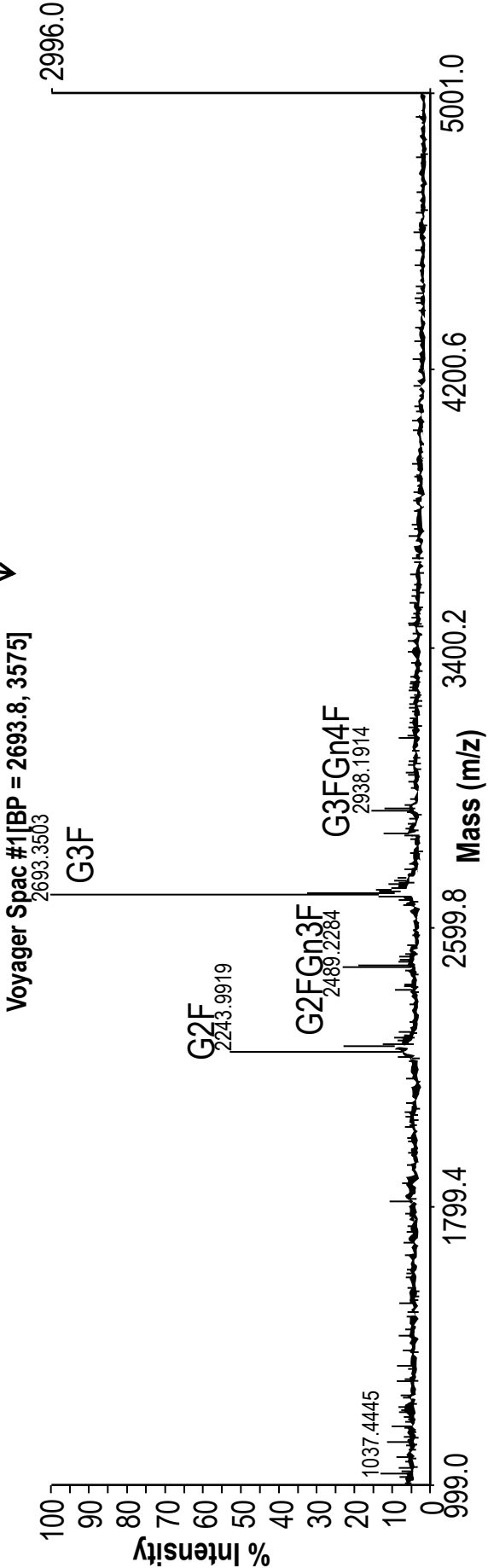


Fig. 53

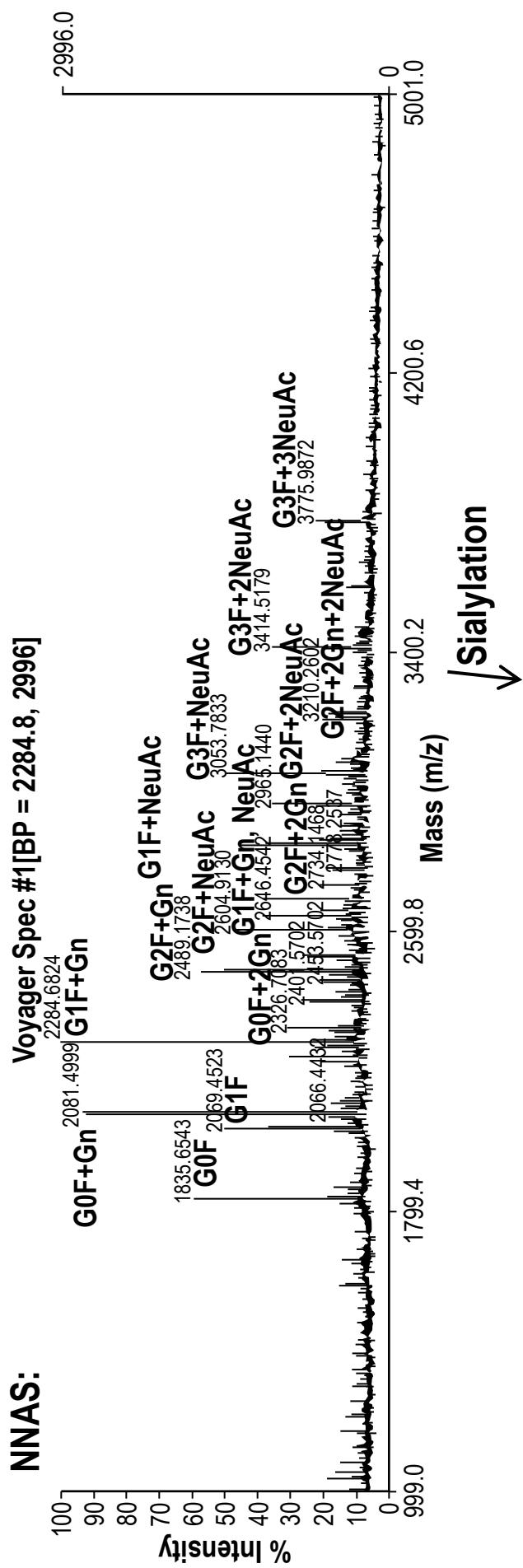
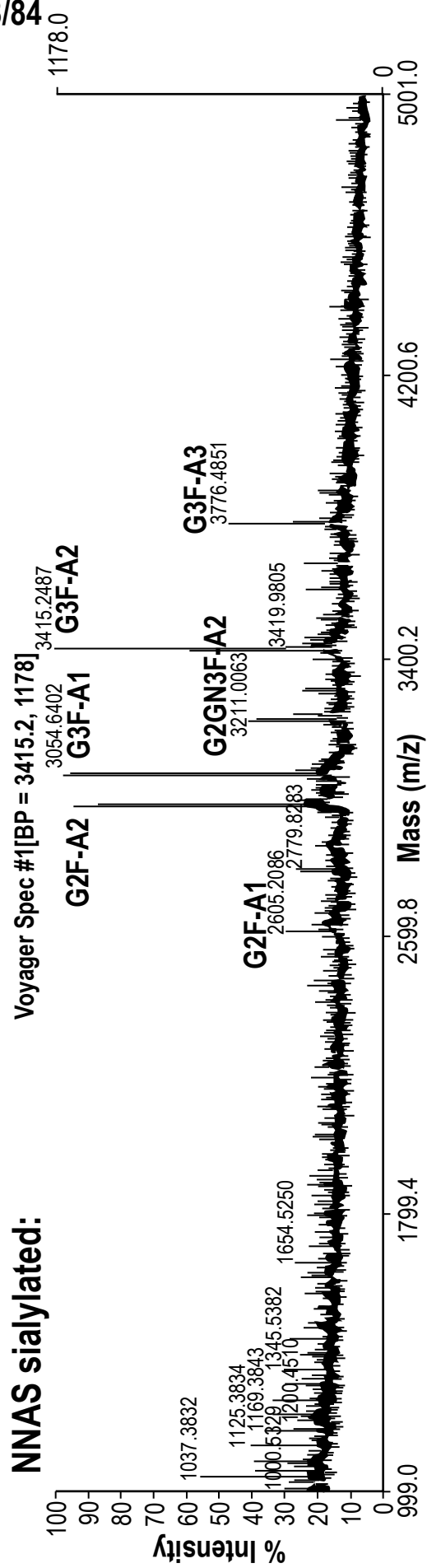
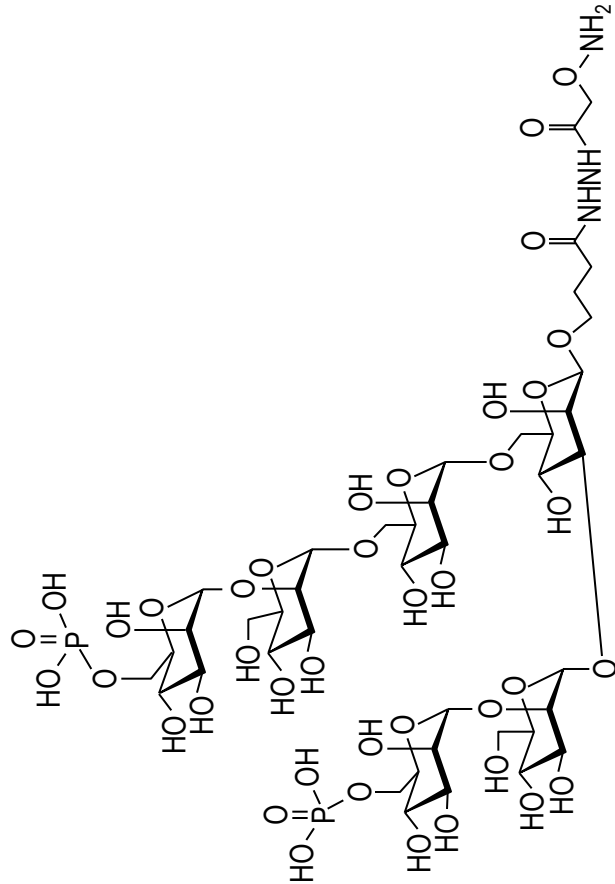
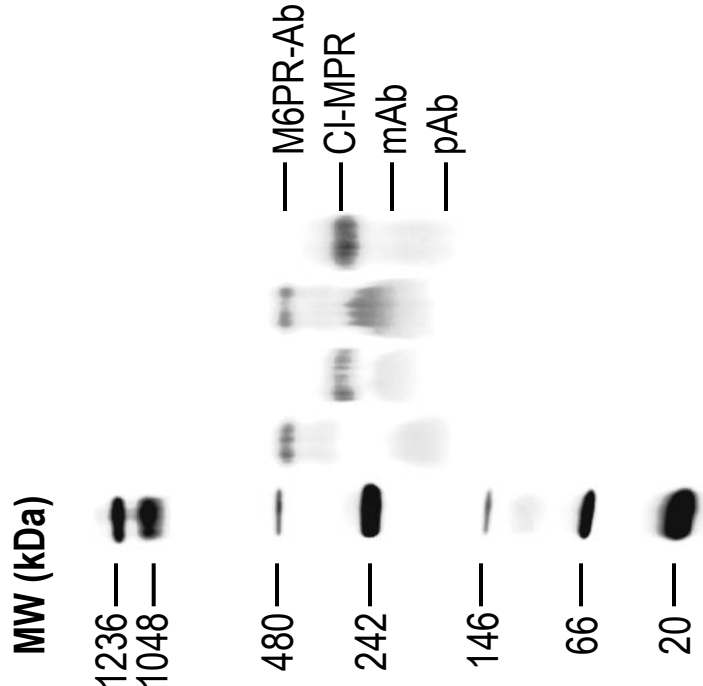


Fig. 54

bisM6P hexasaccharide aminoxy
(SAM):



M6P conjugate at Fc (pAb, 3 glycans)	*		
mAb 3F11		*	
M6P conjugate at Fc (mAb, 1.5 glycans)			*
pAb			*

Fig. 55

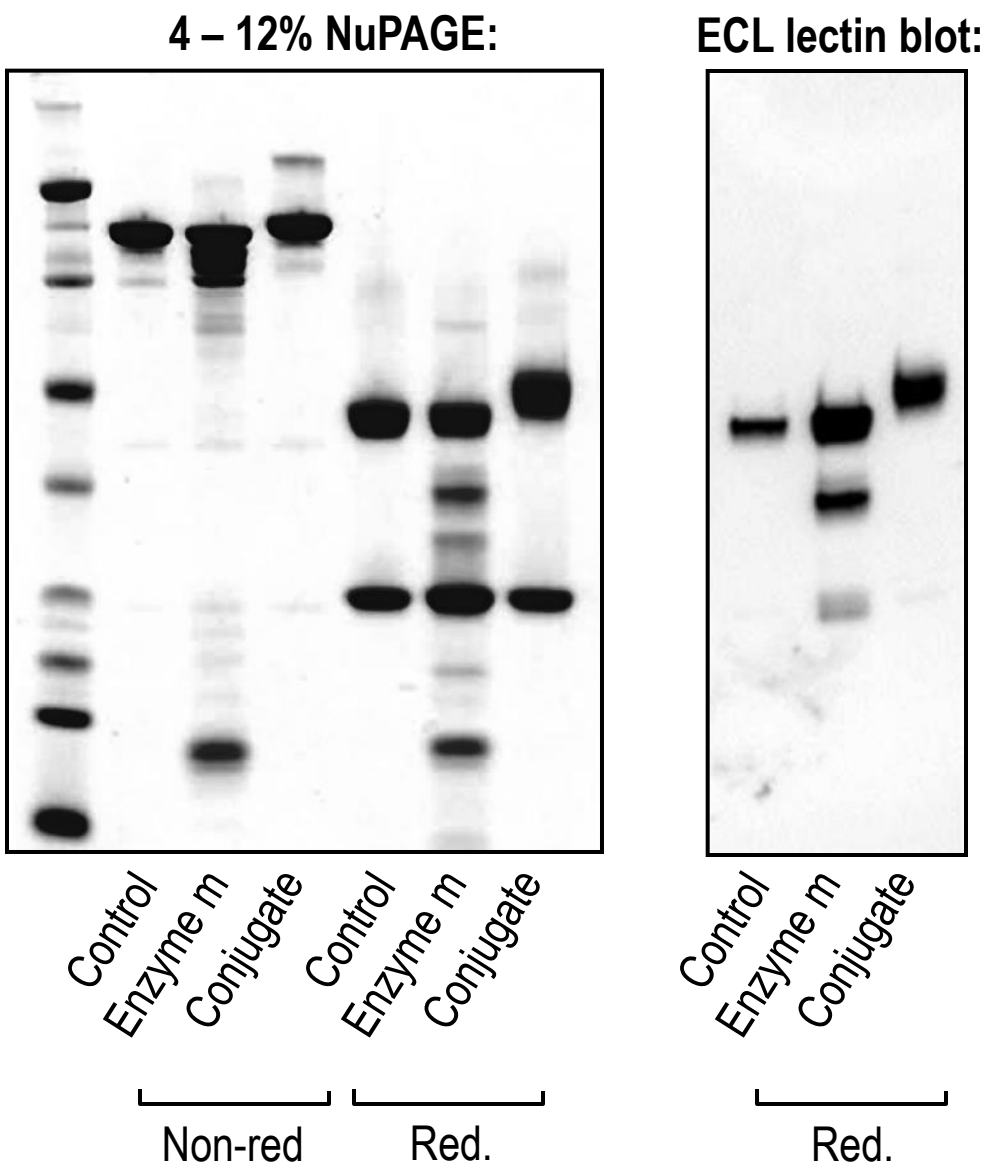


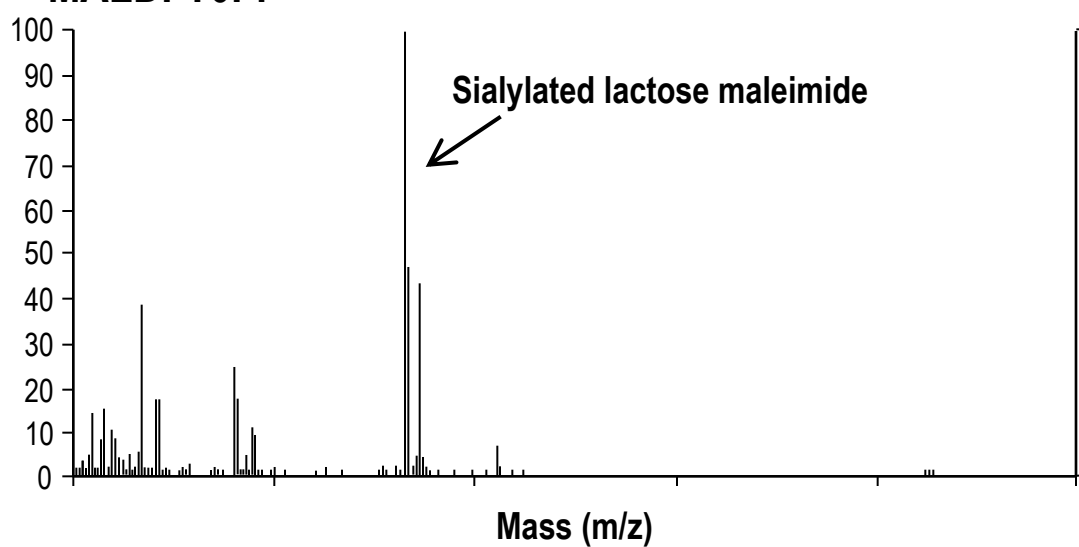
Fig. 56

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	mol galactose per mol AB	mol glycopeptide per mol Ab
NNAS	1.4 + 0	0
NNAS desialylated/galactosylated	7.4 + 0.3	0
NNAS conjugate	9.4 + 0	3.1

Fig. 57

MALDI-TOF:



Dionex HPLC:

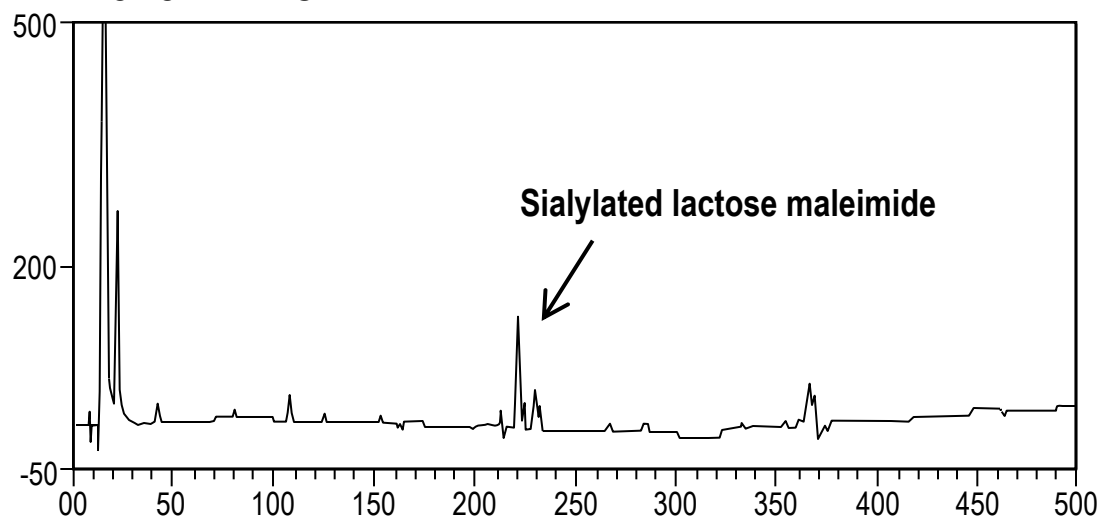
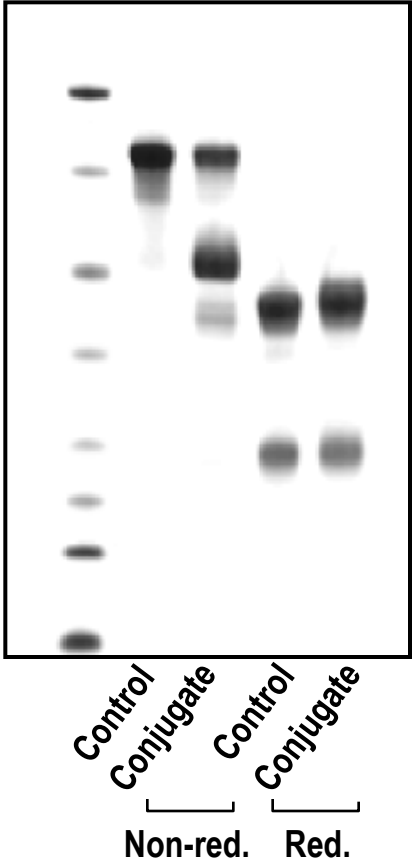


Fig. 58

A

4-12% NuPAGE:



B

Sialic acid analysis:

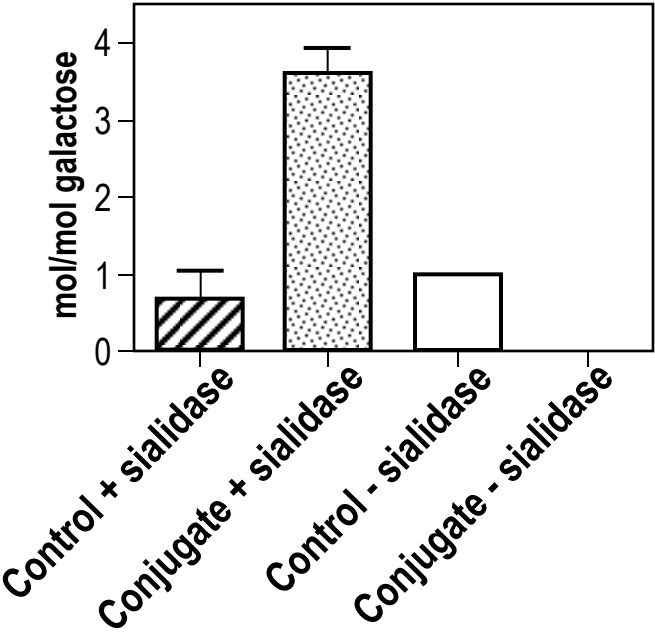


Fig. 59

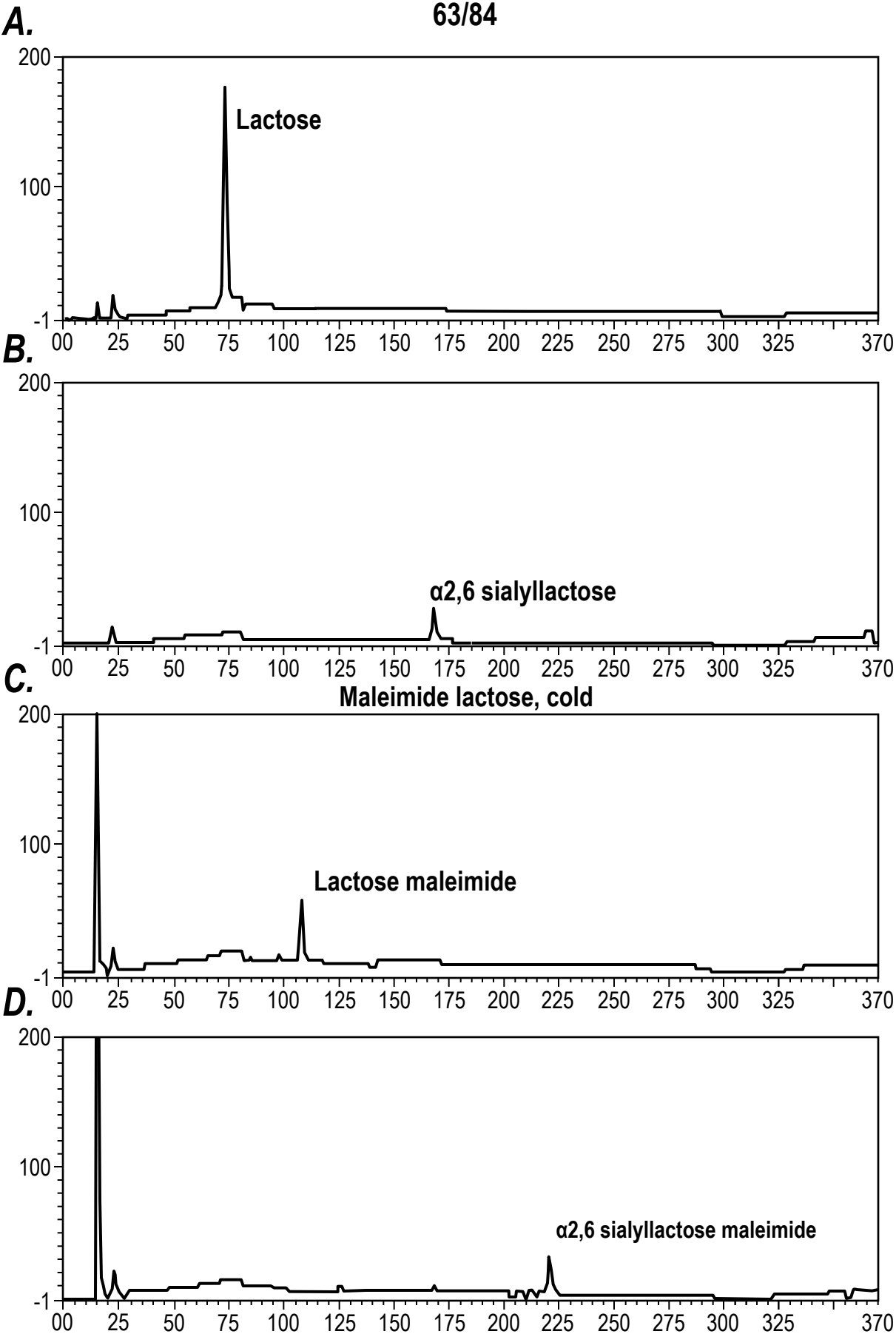


Fig. 60

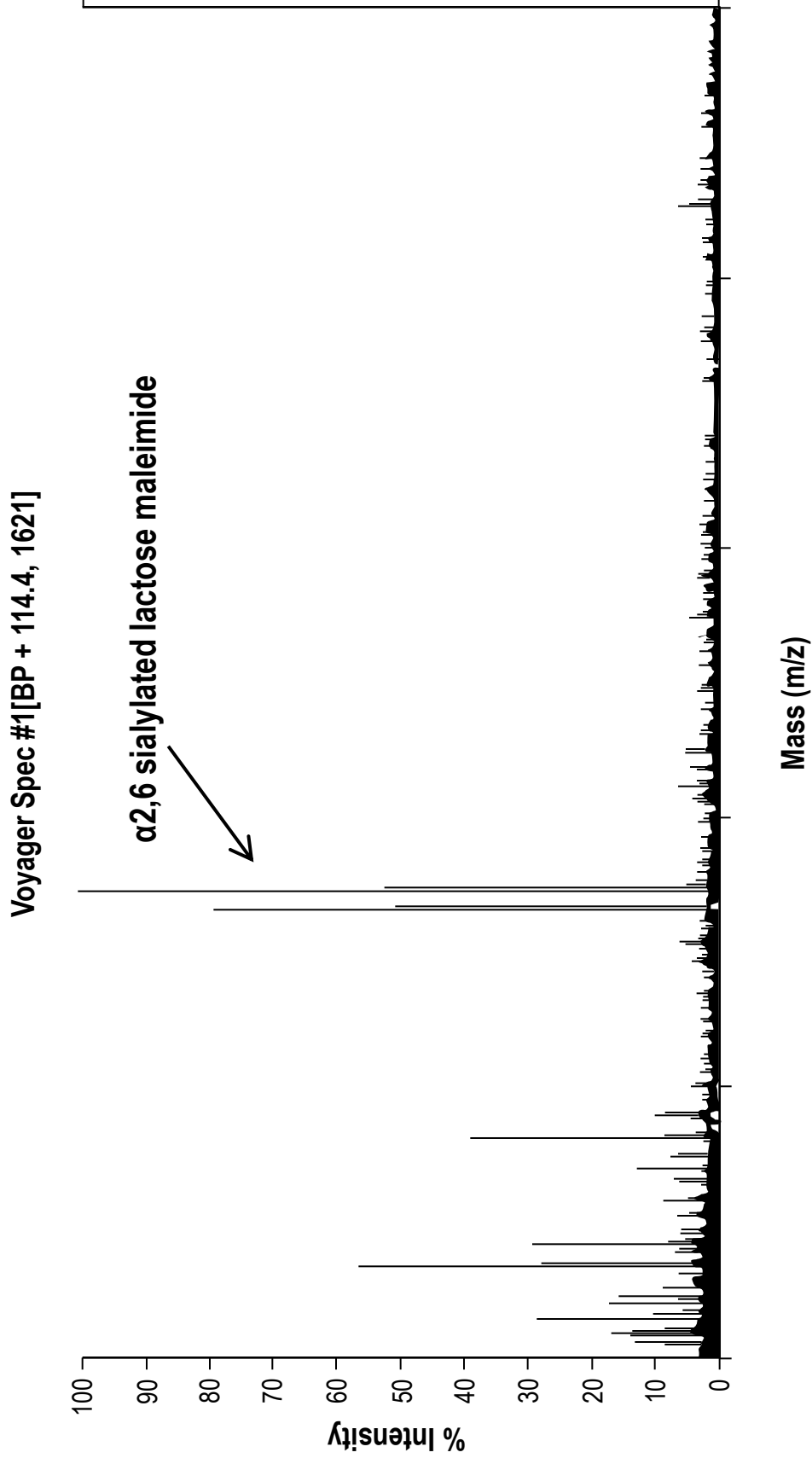
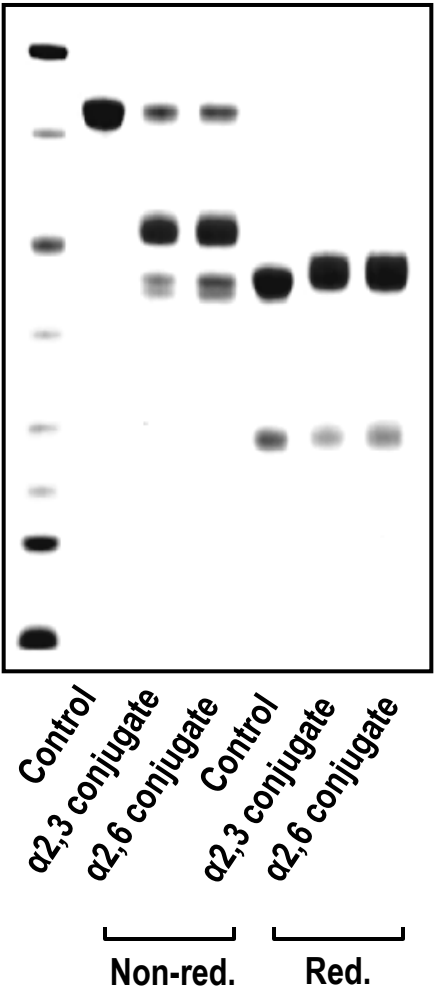


Fig. 61

A.

4-12% NuPAGE:



B.

Sialic acid analysis:

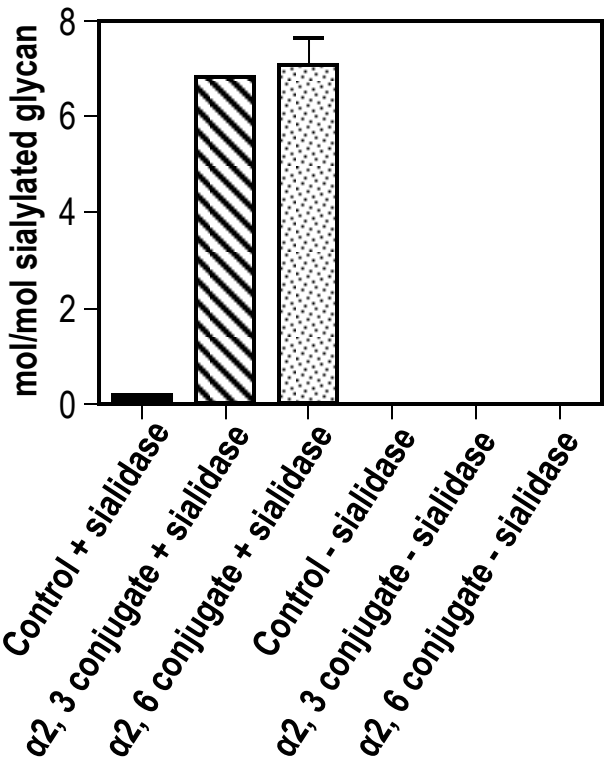


Fig. 62

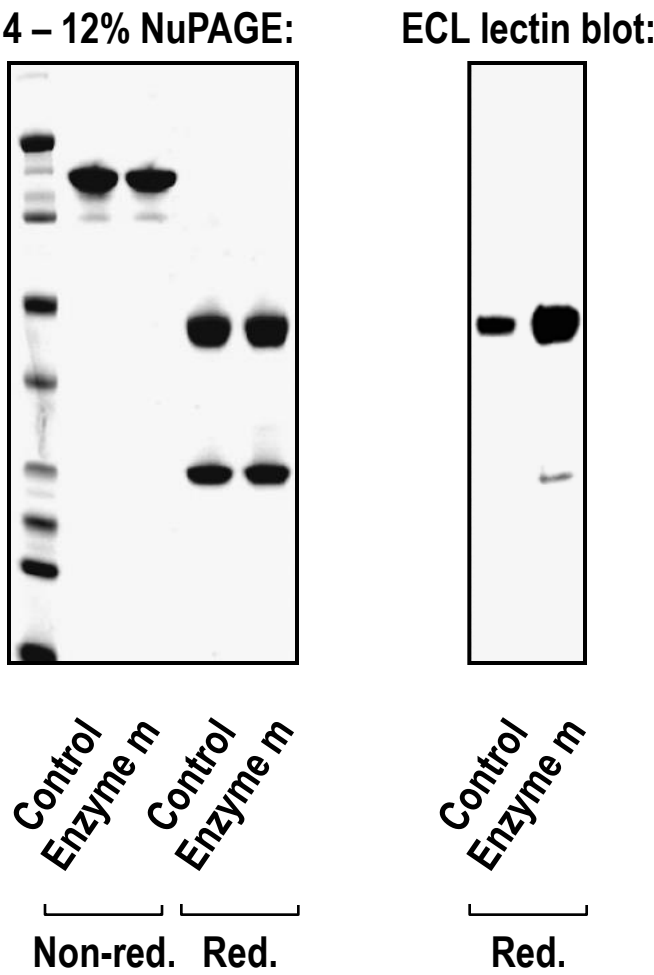
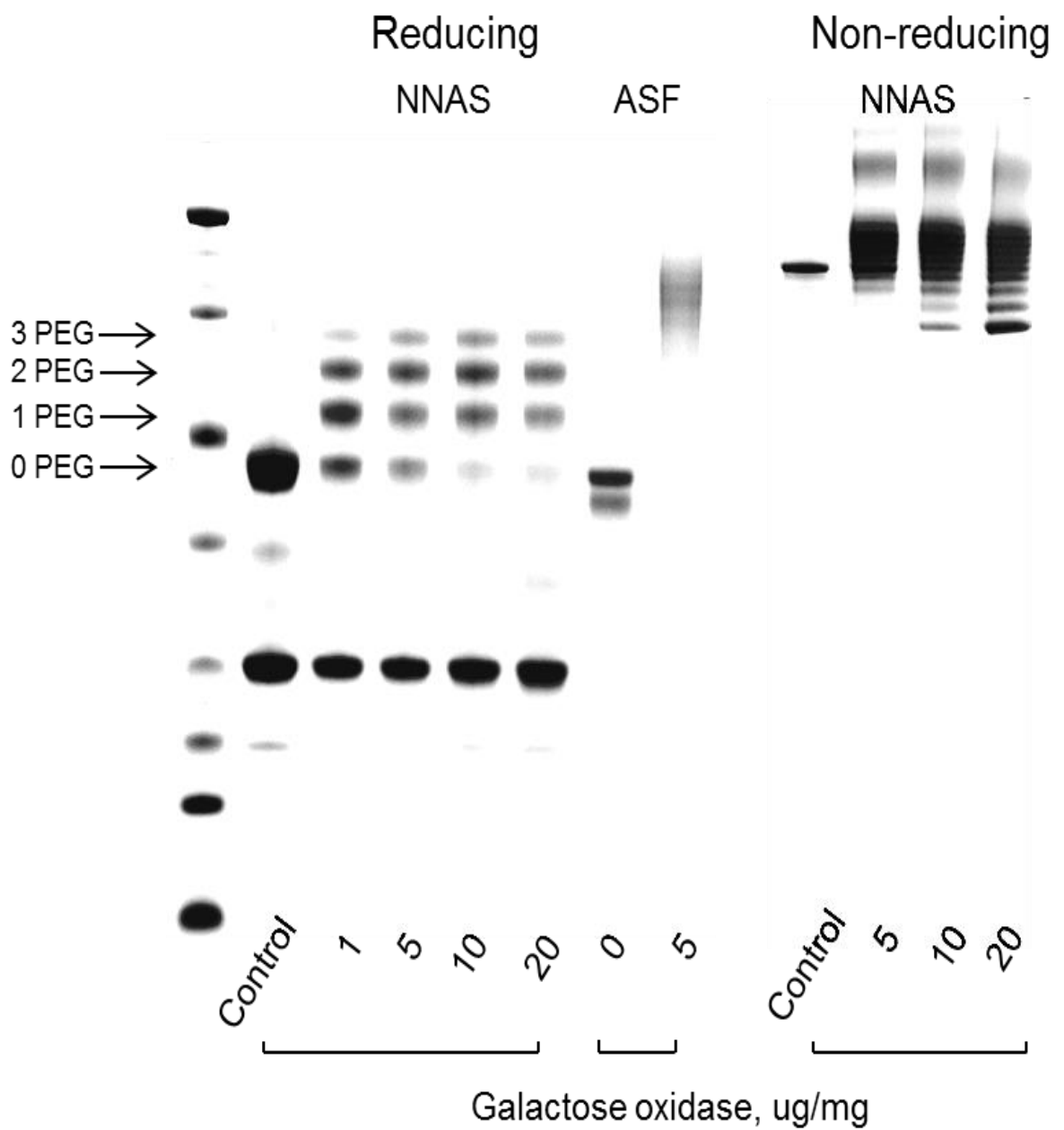


Fig. 63

**Fig. 64**

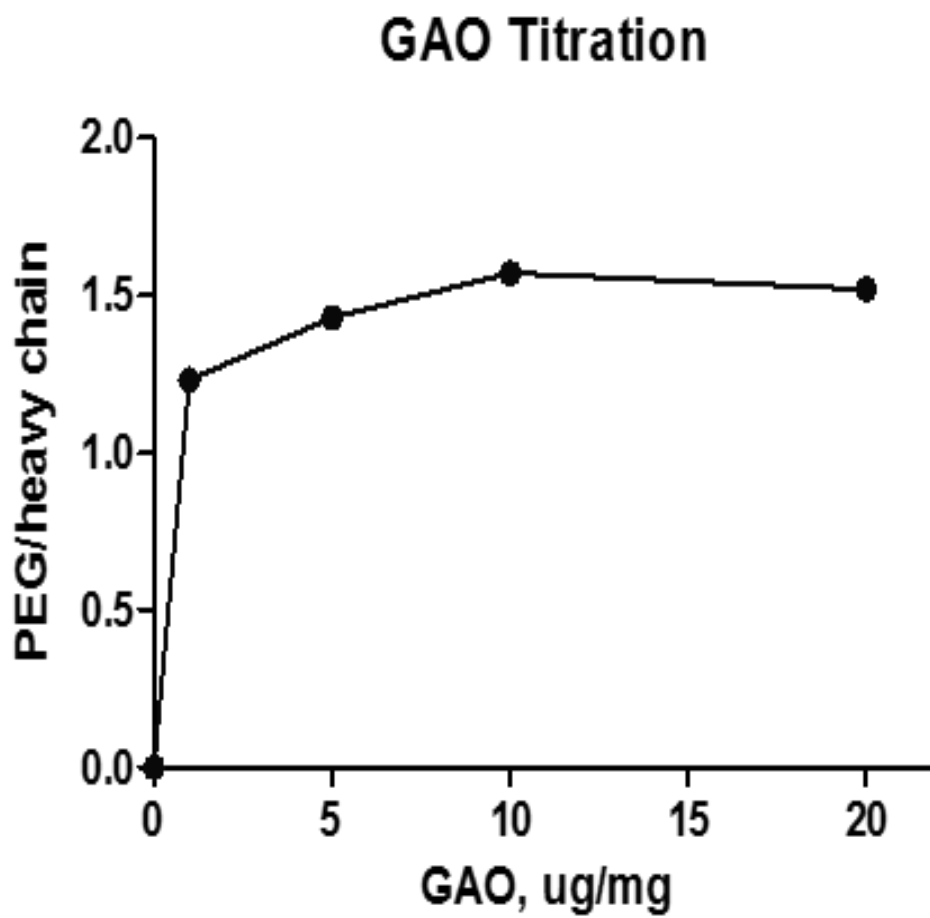
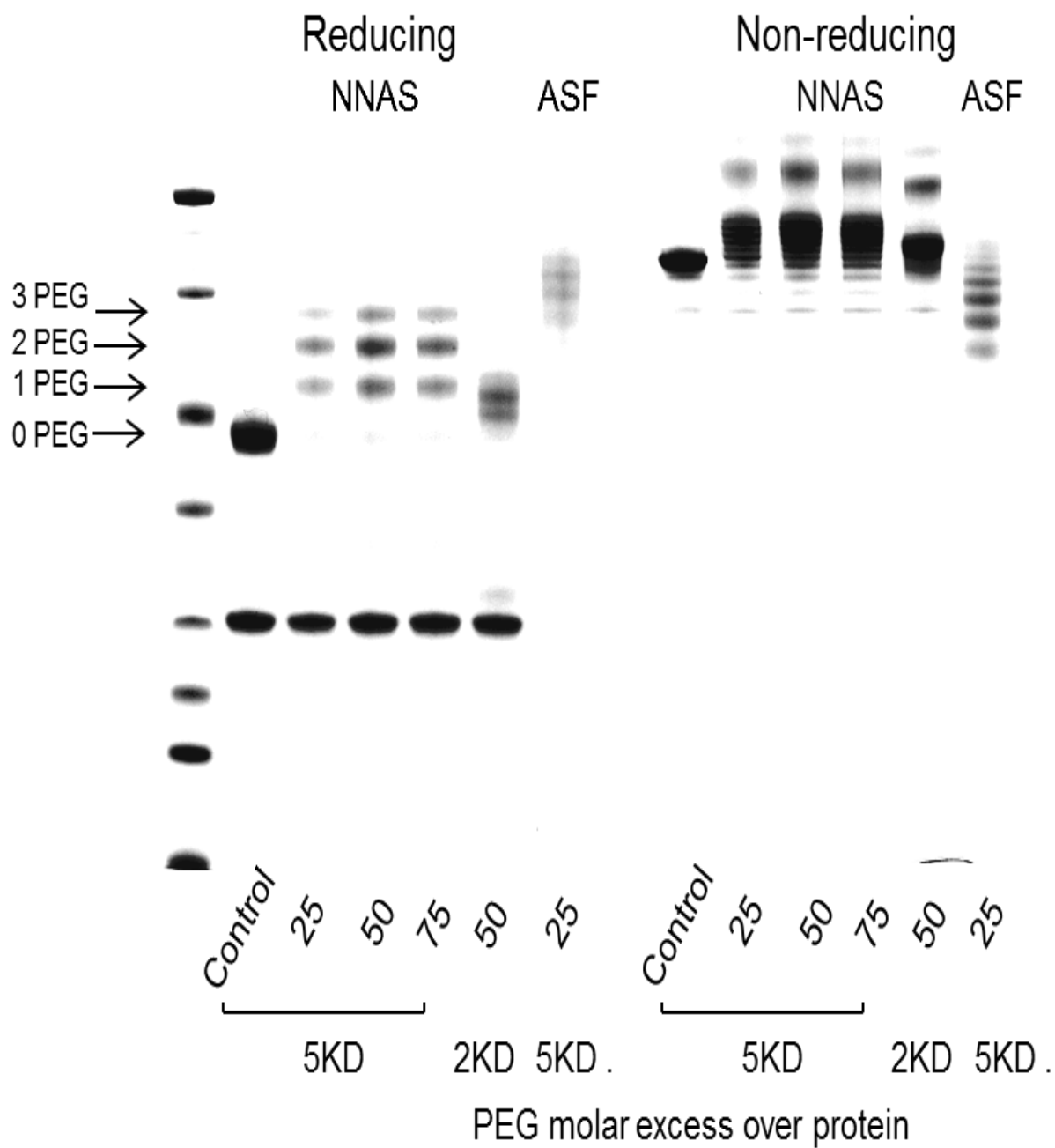


Fig. 65

**Fig. 66**

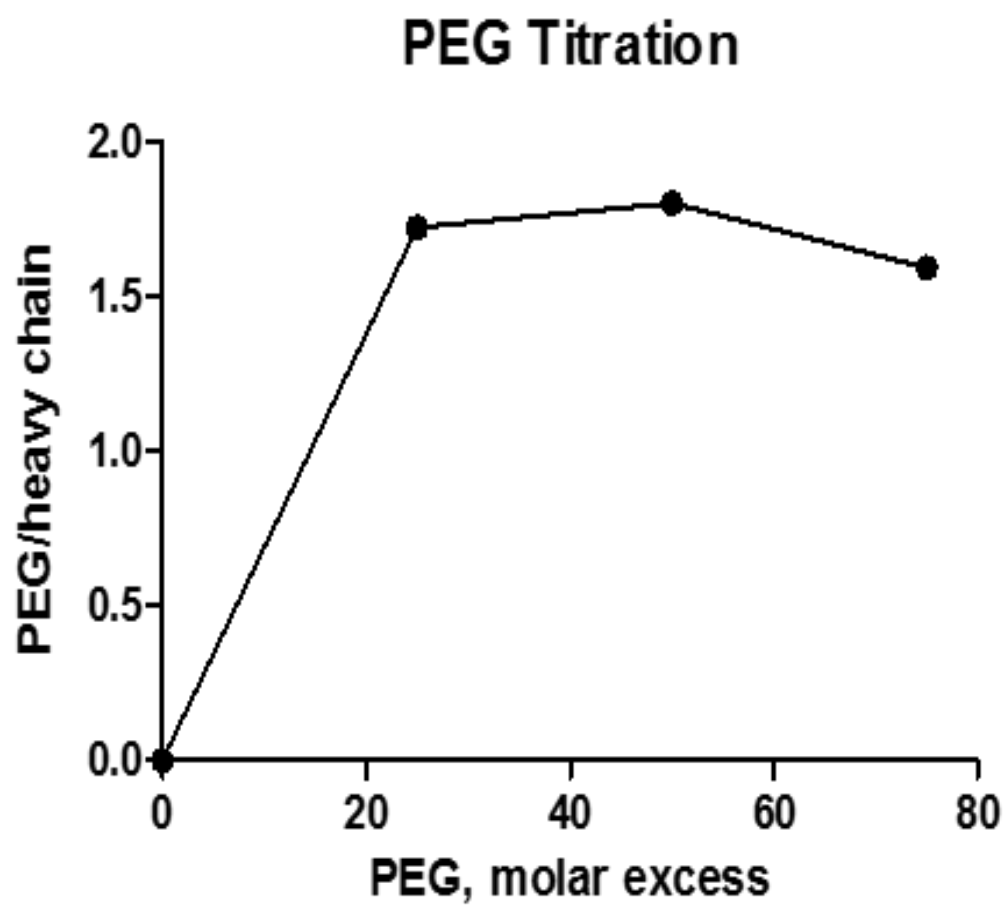
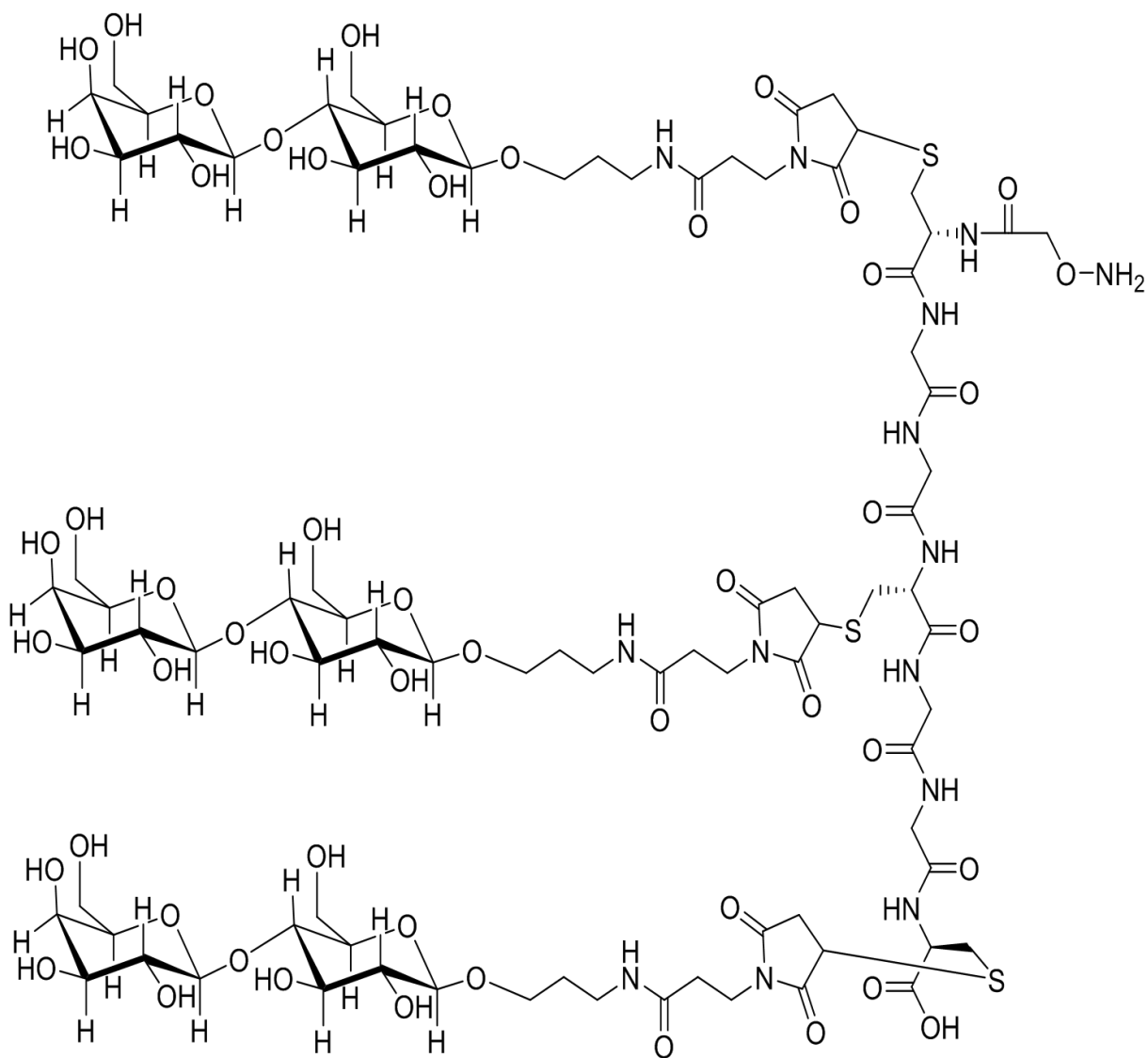


Fig. 67



Glycopeptide aminoxy: lactose₃-Cys₃-Gly₄

Fig. 68

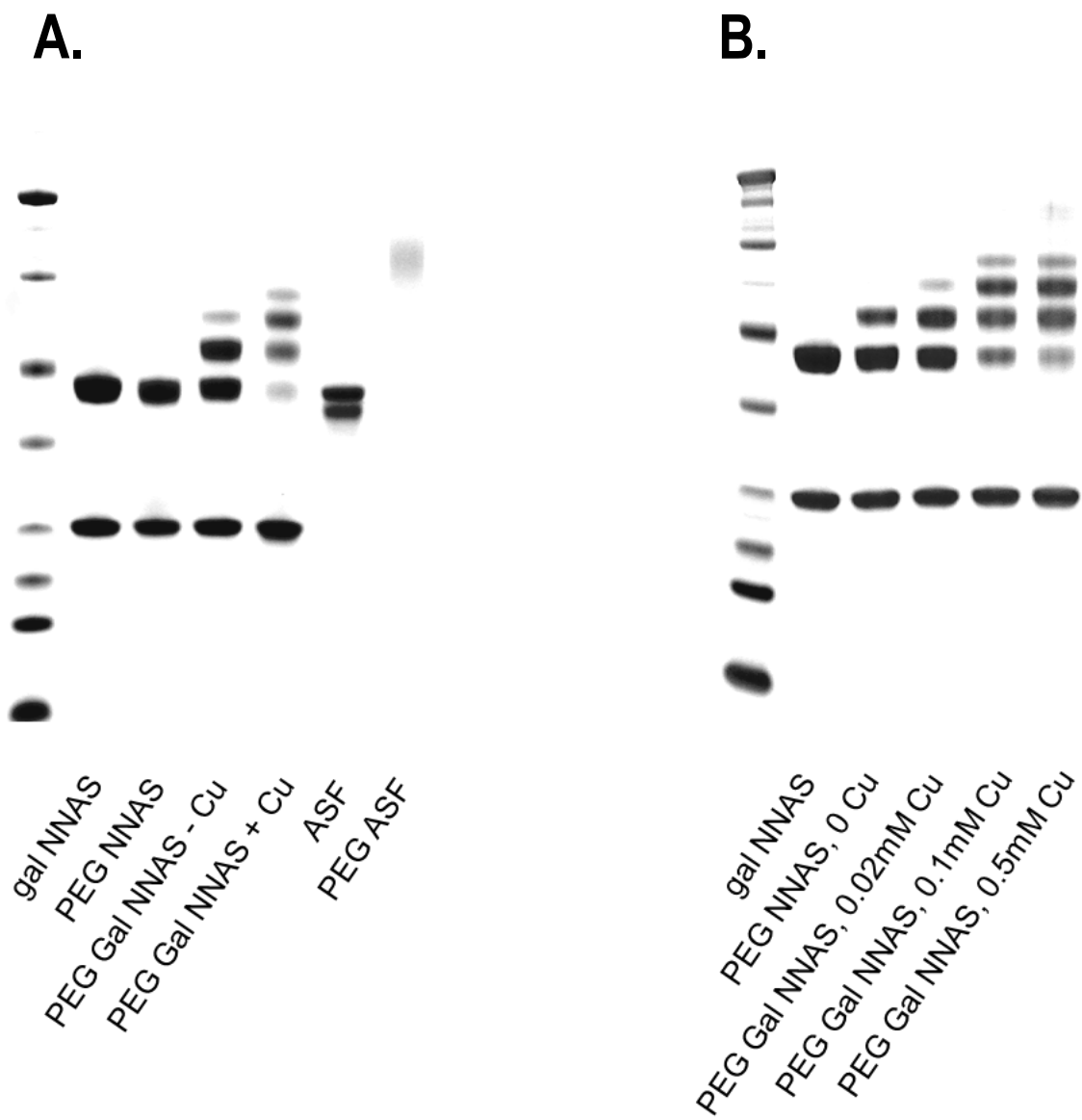


Fig. 69

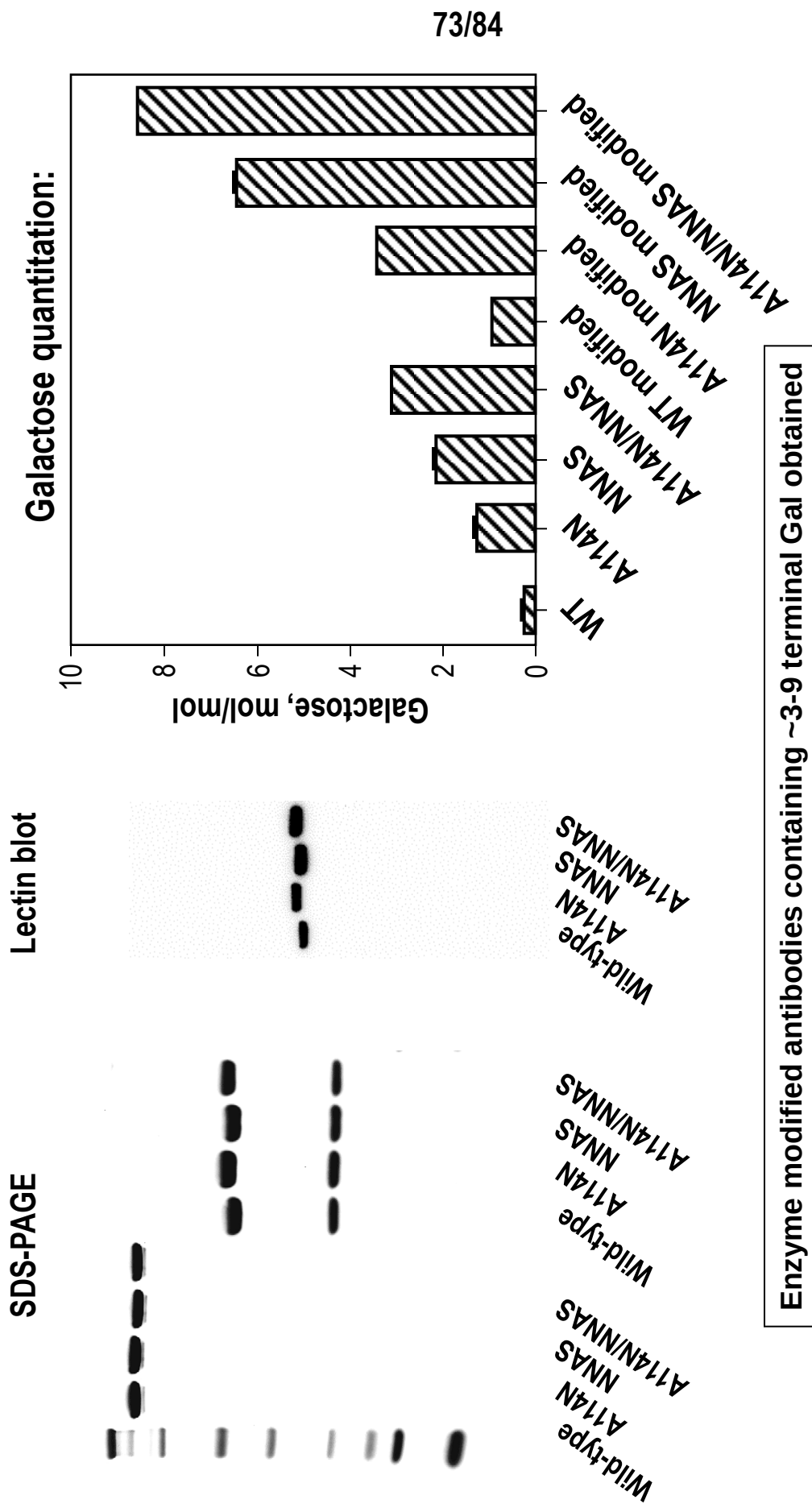


Fig. 70

	Mol per mol, Mean $\pm$ SD
Wild-type	0.1 $\pm$ 0
A114N	0.5 $\pm$ 0
NNAS	2.3 $\pm$ 0
A114N/NNAS	2.7 $\pm$ 0

Fig. 71

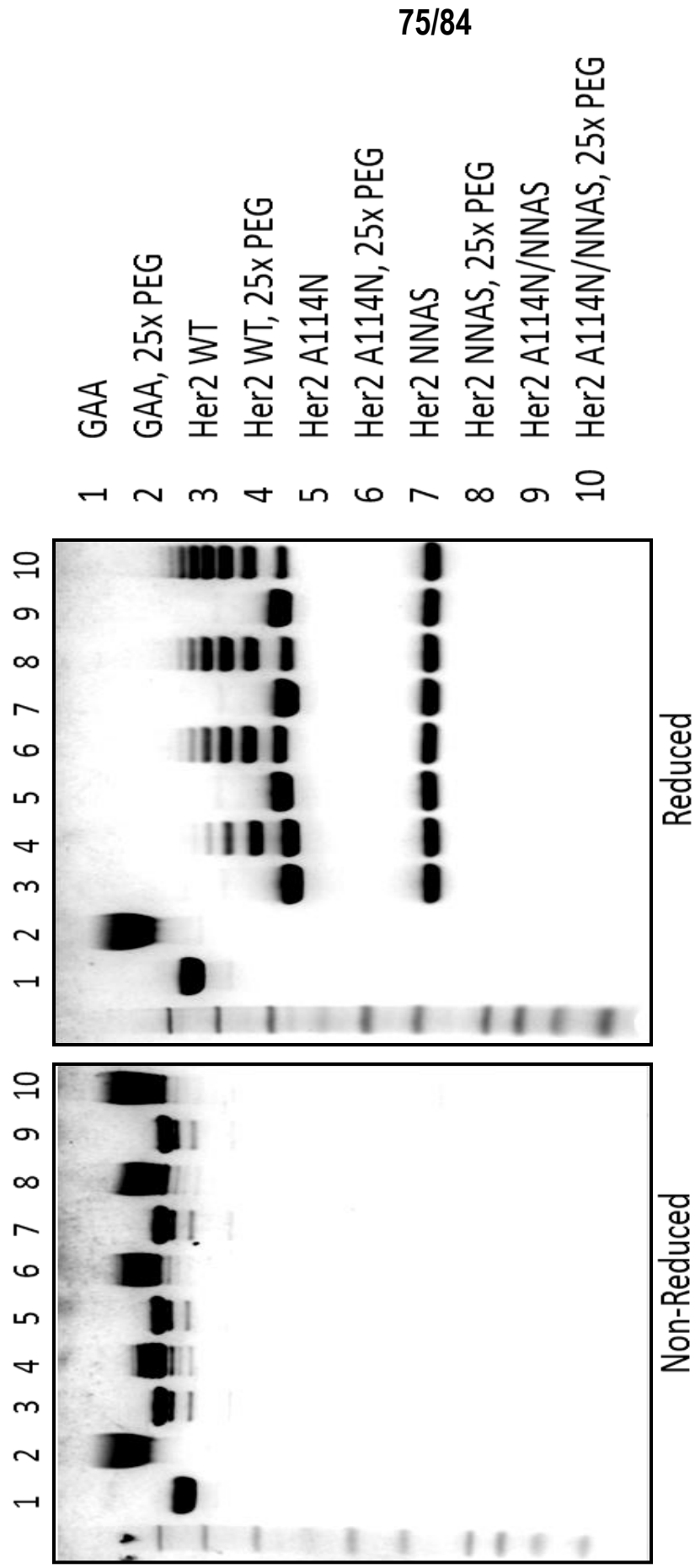
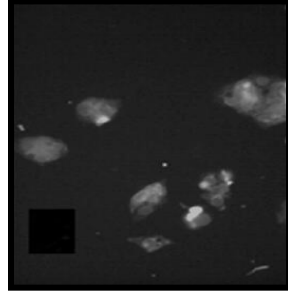


Fig. 72

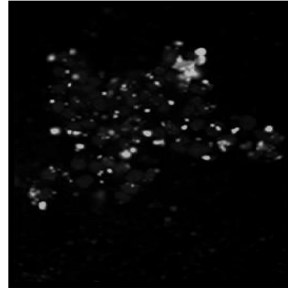
	Mol per mol, Mean + SD
Wild-type	1.4
A114N	2.7
NNAS	3.4
A114N/NNAS	4.6

Fig. 73

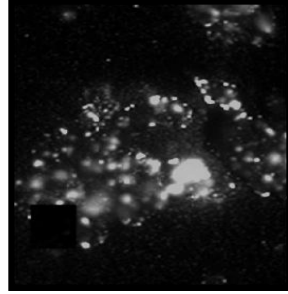
Polyclonal antibody uptake into HepG2 cells



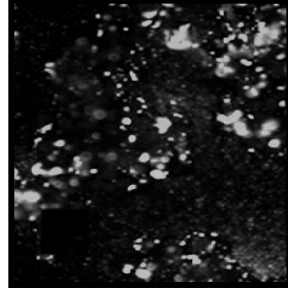
Parent Ab
1 galactose/Ab



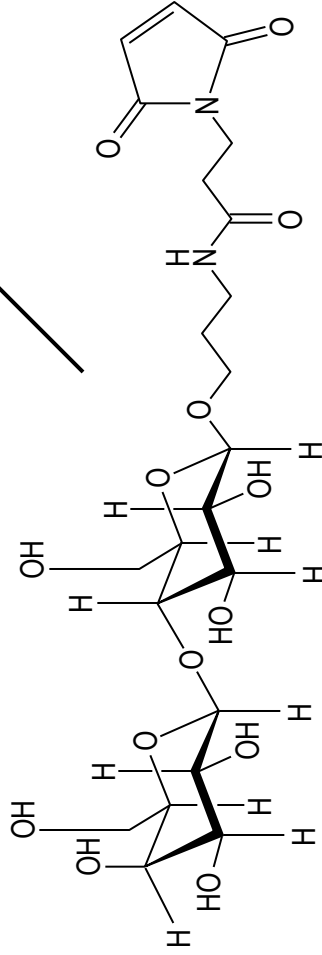
Gal Transferrase
4 galactose / Ab



Gal-glc to Hinge
7 galactose / Ab

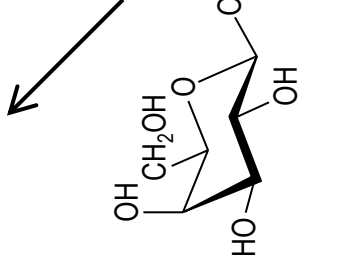


Gal-glc to 297
2 galactose / Ab



Exact Mass: 550.20
Molecular Weight: 550.51

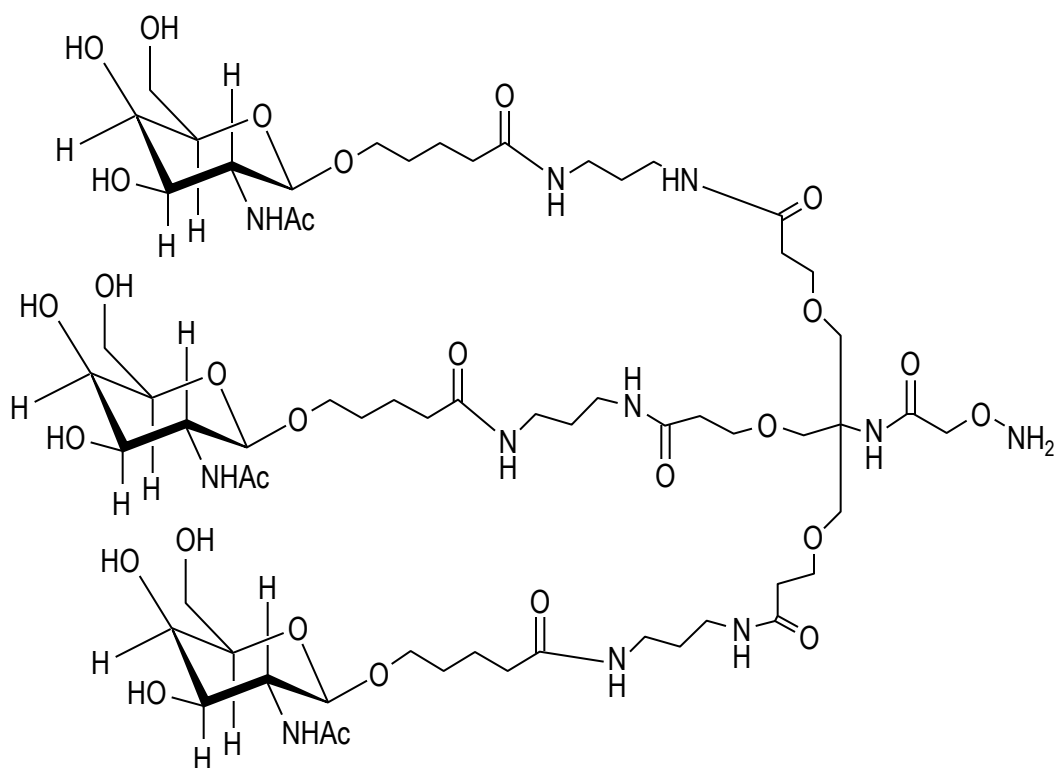
Lactose maleimide



Exact Mass: 472.19
Molecular Weight: 472.44

Lactose aminoxy

Fig. 74

***Fig. 75***

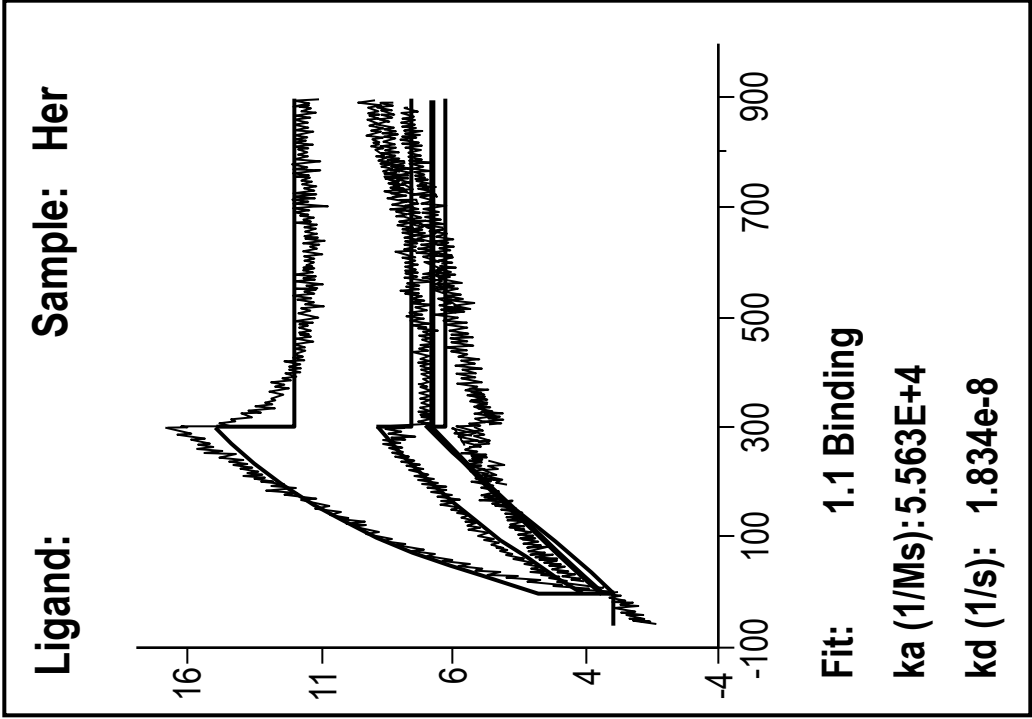
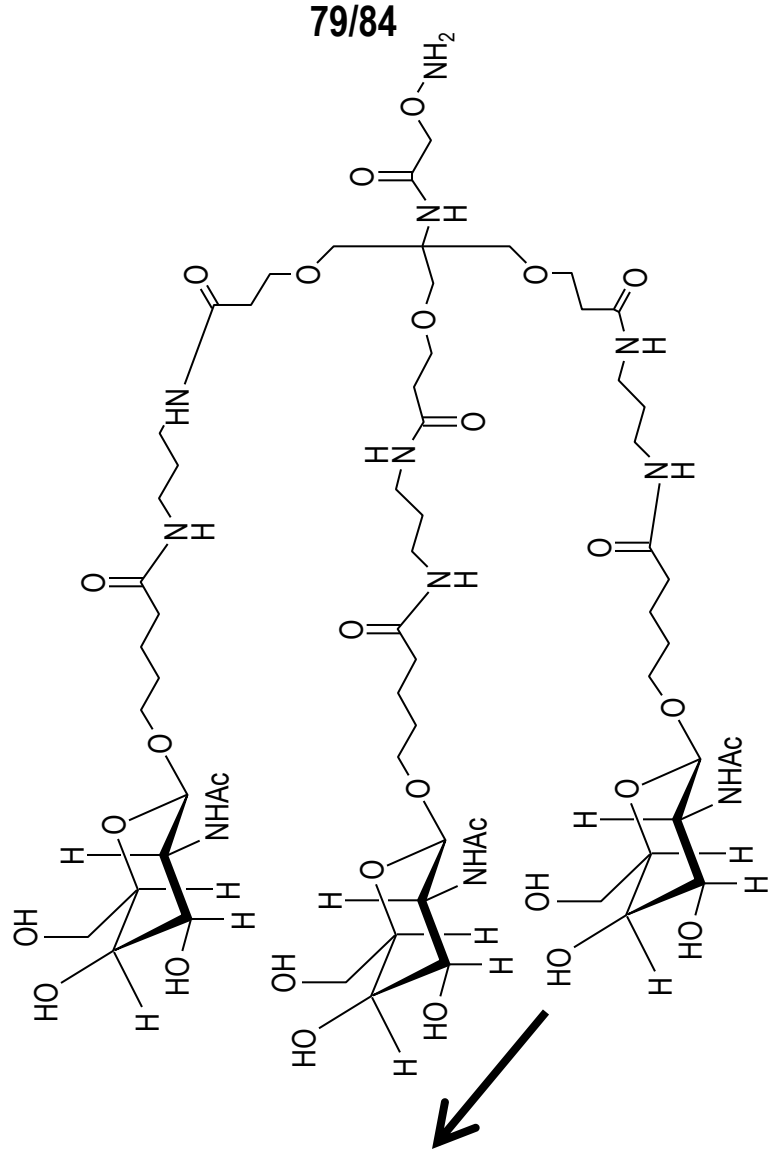
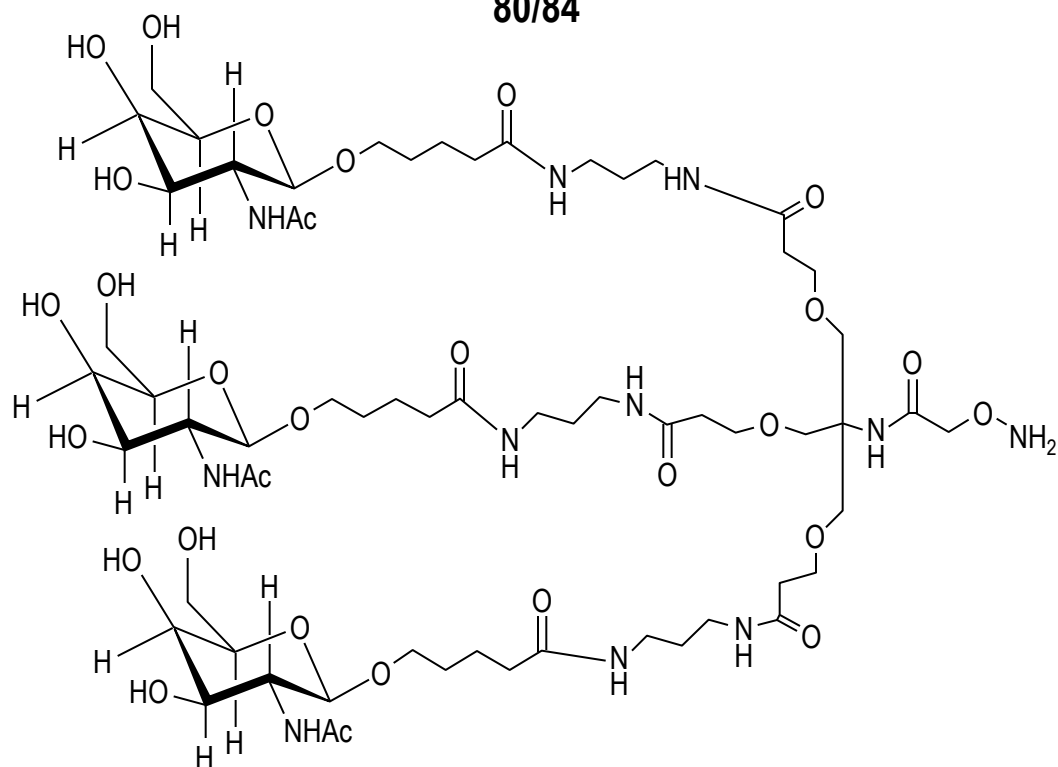
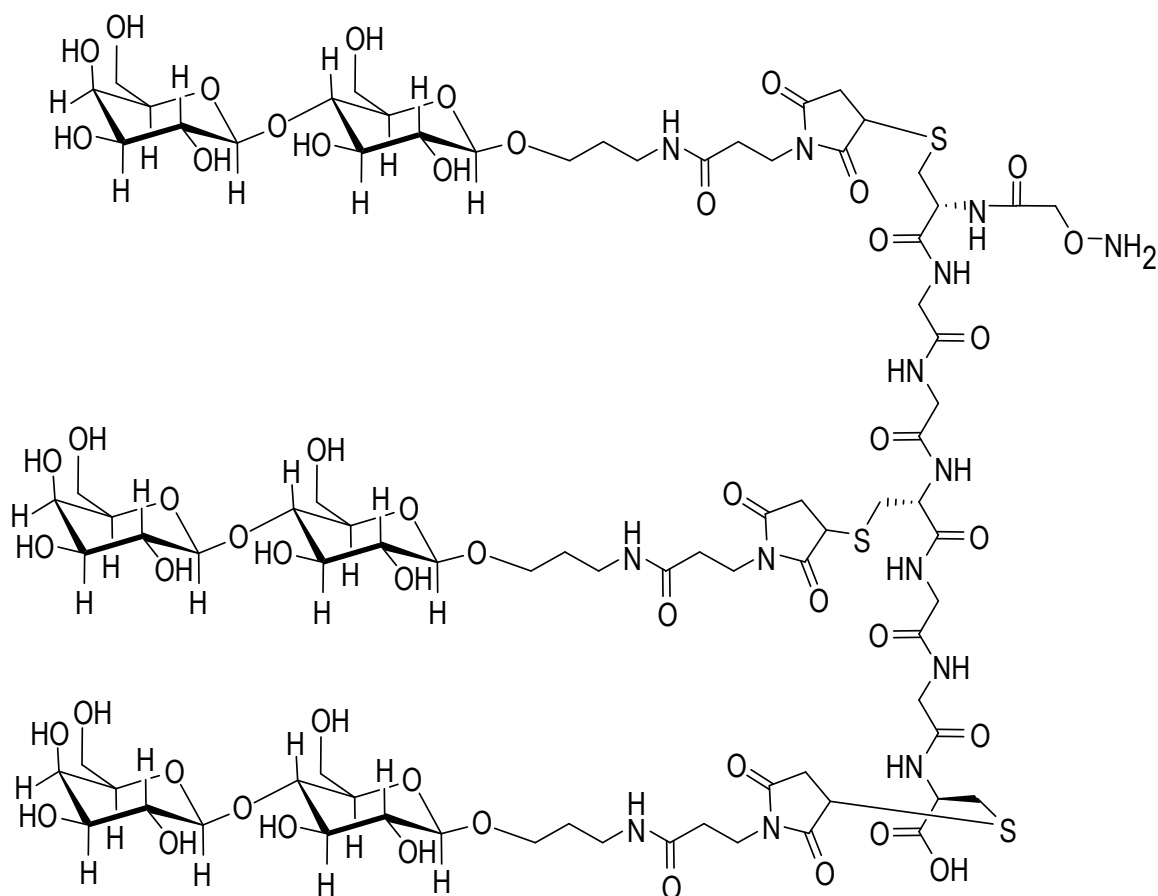


Fig. 76

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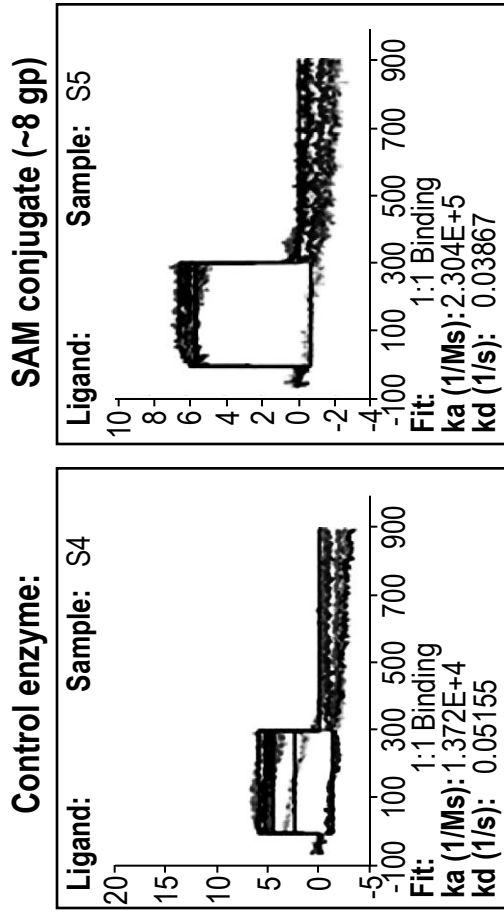
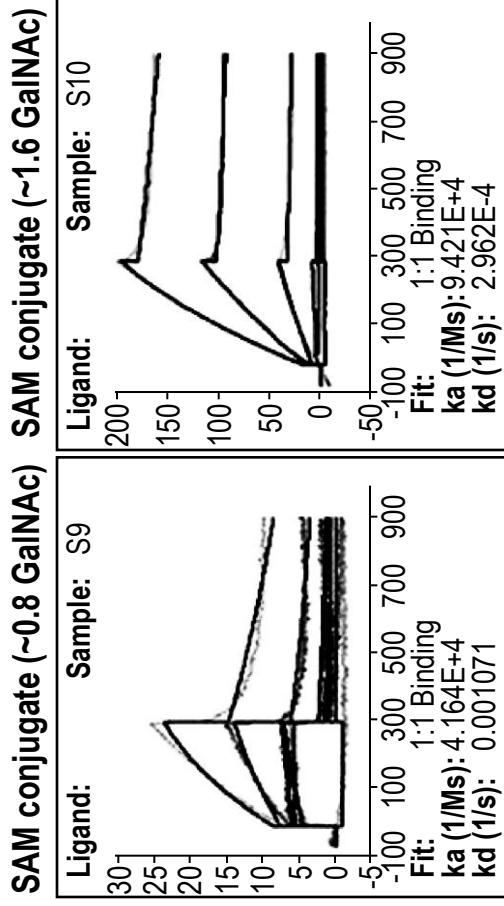
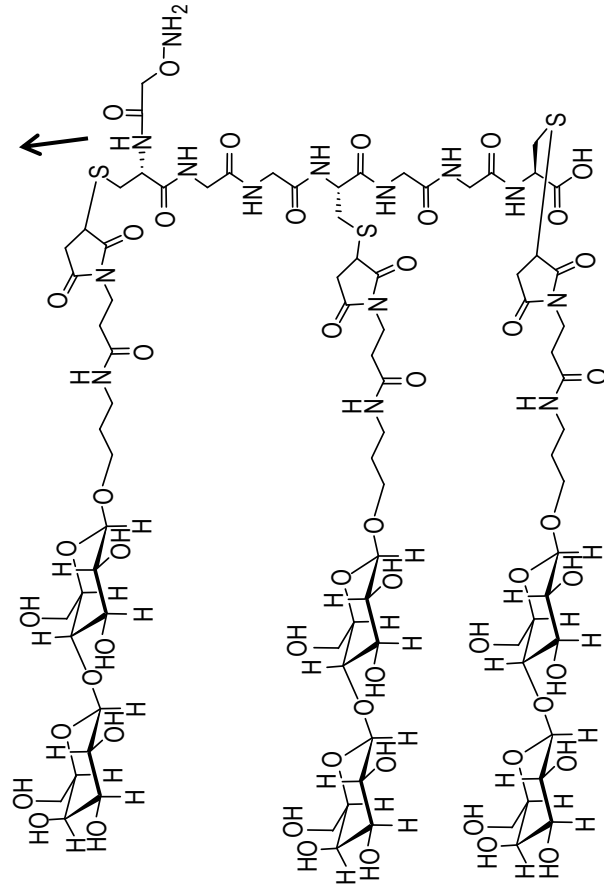


Trivalent GalNAc containing glycan

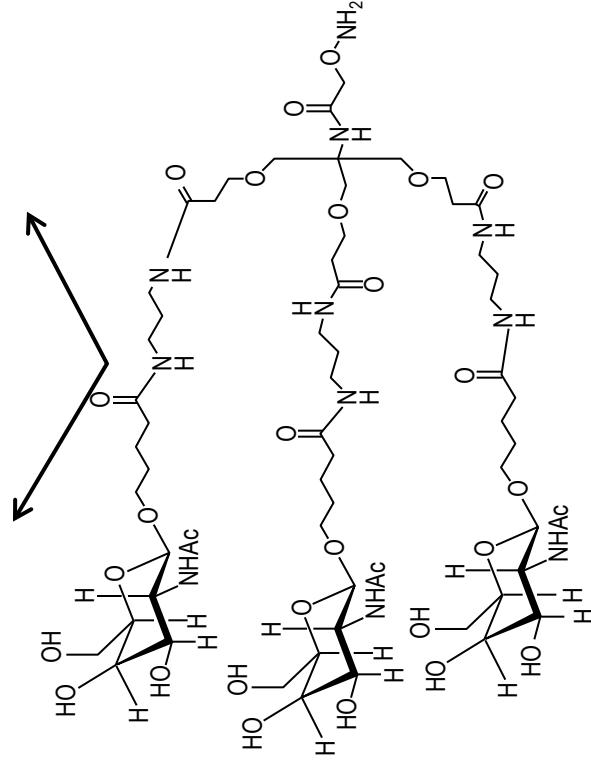


Trivalent Galactose containing glycopeptide

Fig. 77

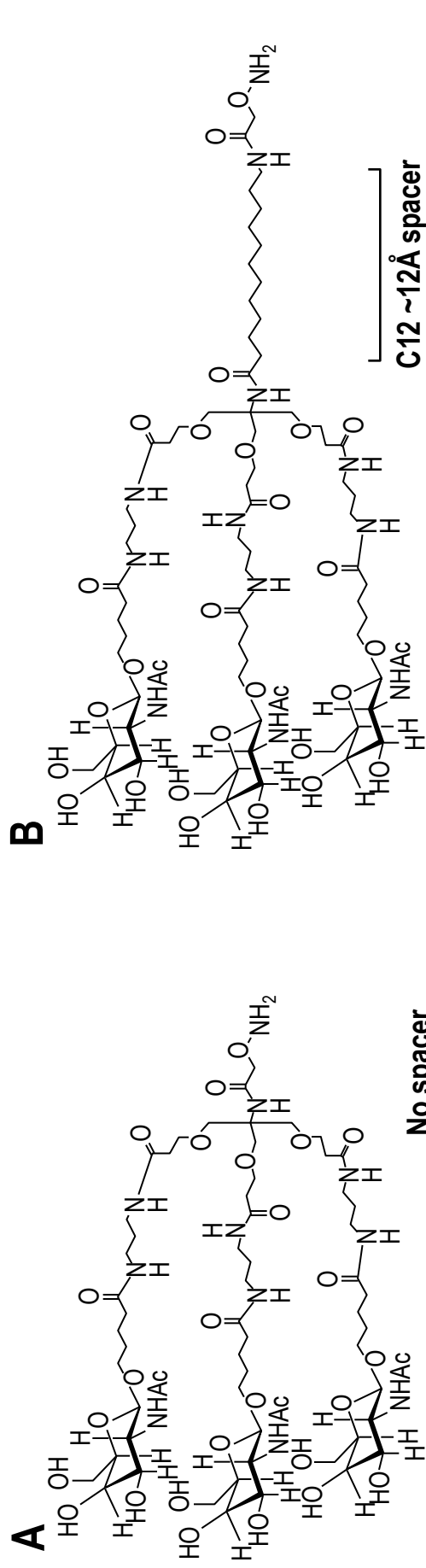


Trivalent Galactose containing glycopeptide



Trivalent GalNAc containing glycan

Fig. 78



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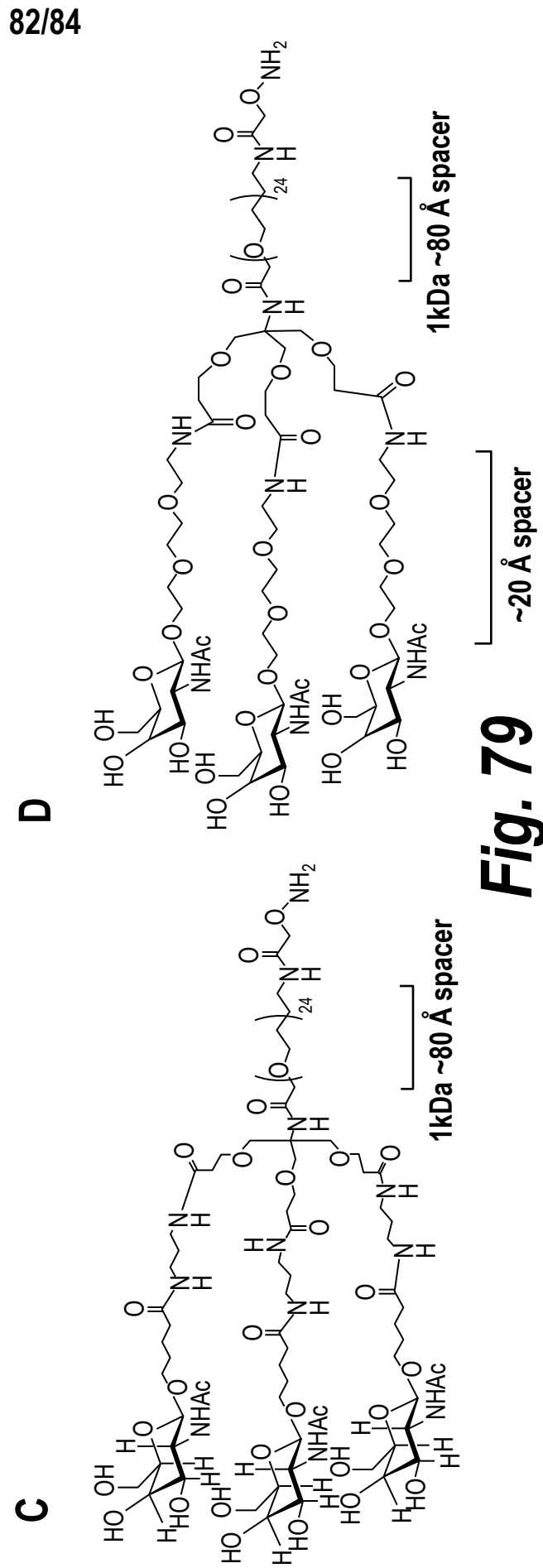
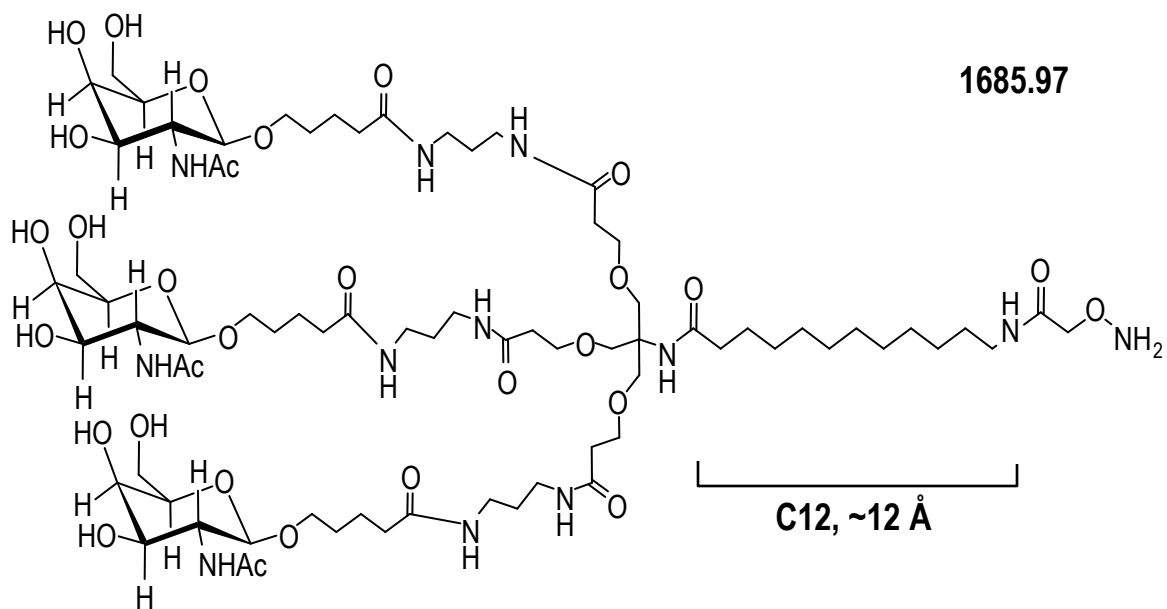


Fig. 79

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Trivalent GalNAc glycan C12

Intact protein MALDI analysis

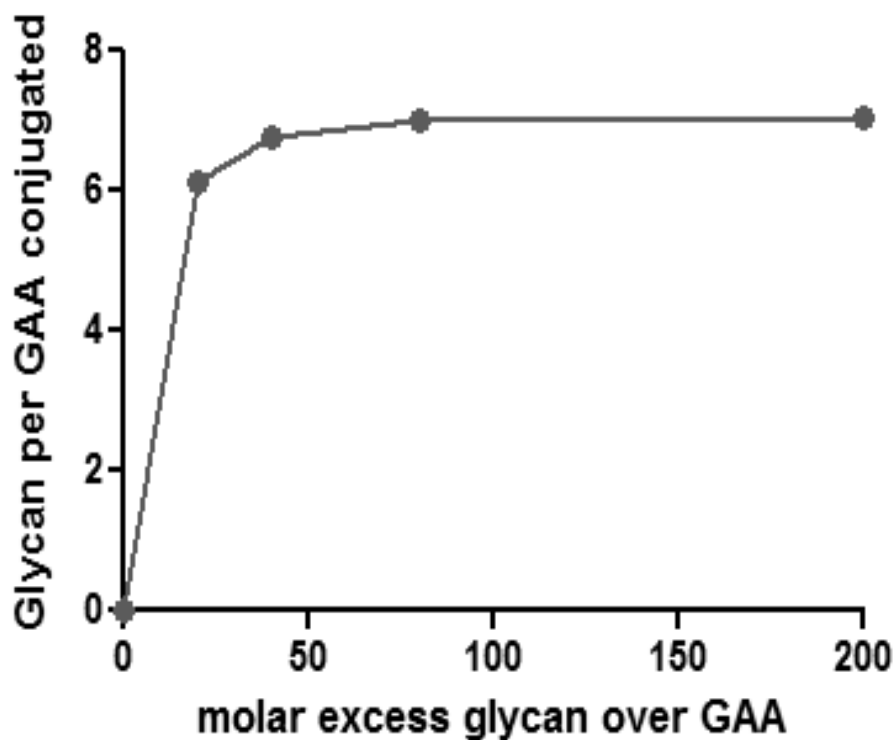


Fig. 80

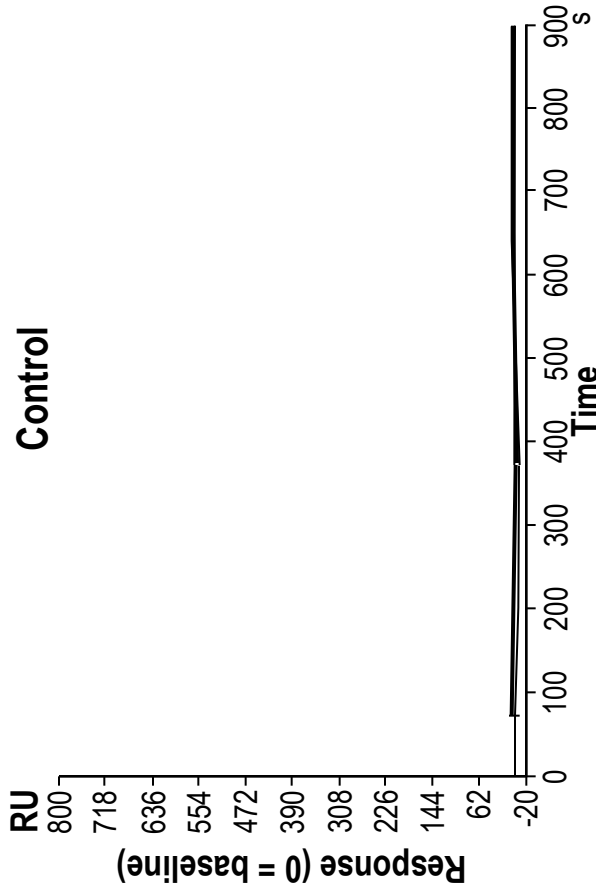
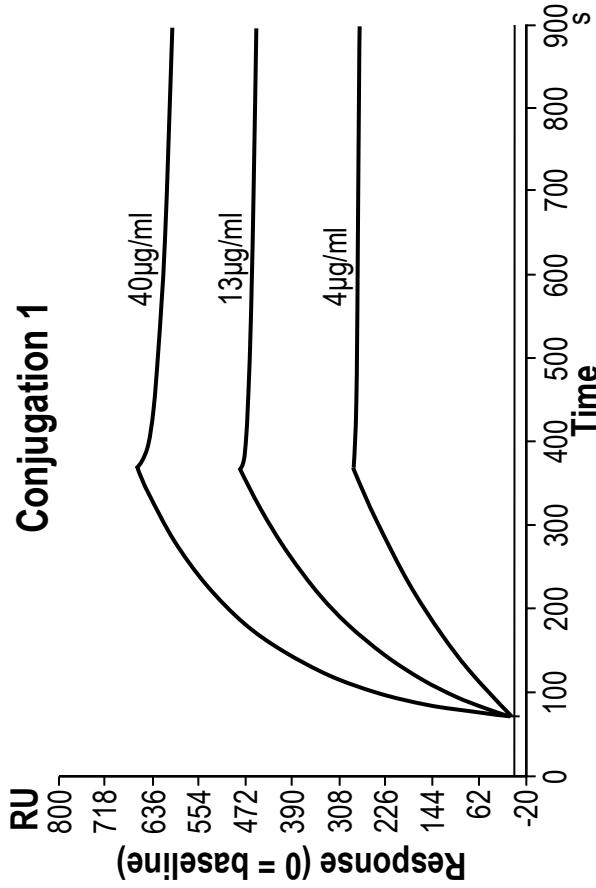
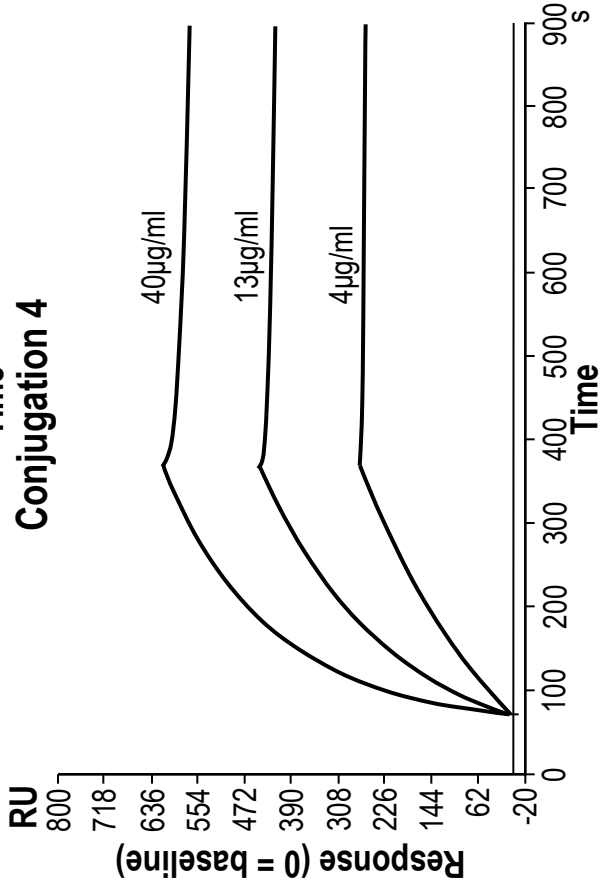


Fig. 81