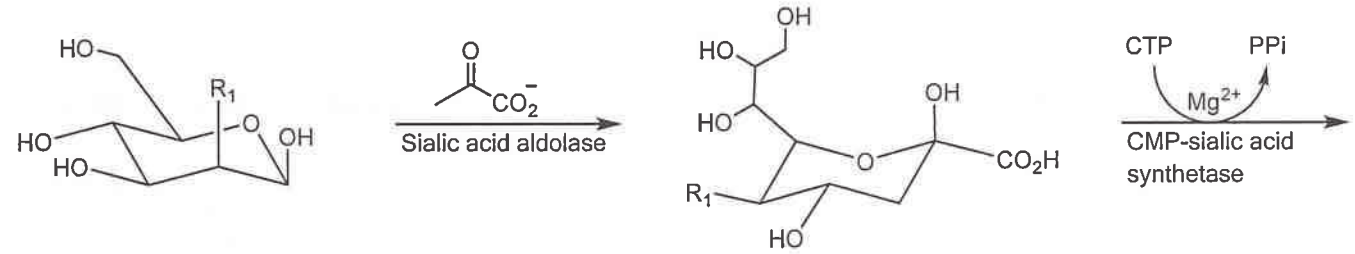
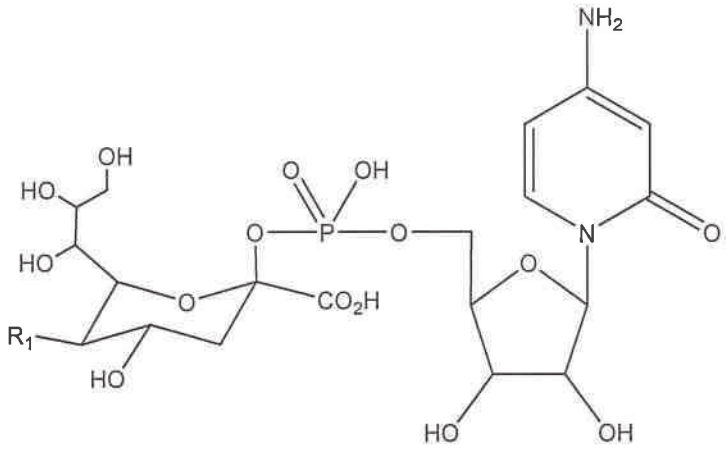
Figure 1



Sugar or sugar derivative

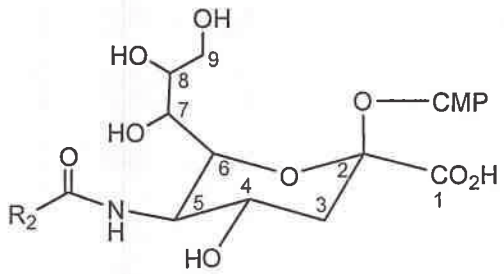
Sialic acid or sialic acid derivative



CMP-sialic acid derivative

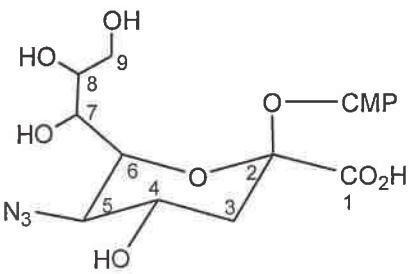
$R_1 = NH(C=O)CH_3$
$R_1 = NH(C=O)CH_2CH_2(C=O)CH_3$
$R_1 = NH(C=O)CH_2OH$
$R_1 = NH(C=O)CH_2N_3$
$R_1 = NH(C=O)SH$
$R_1 = OH$
$R_1 = N_3$

Figure 2

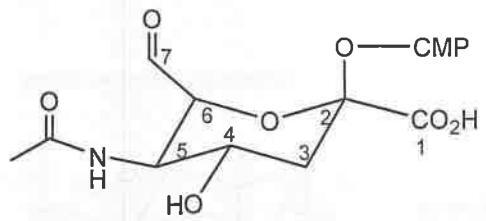


CMP-sialic acid C5 N-acetyl derivative

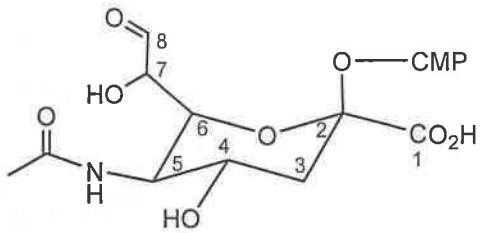
$R_2 = CH_3$
$R_2 = CH_2CH_2(C=O)CH_3$
$R_2 = CH_2OH$
$R_2 = CH_2N_3$
$R_2 = SH$



CMP-sialic acid C5 azide derivative



CMP-sialic acid C7 aldeyhyde derivative



CMP-sialic acid C8 aldehyde derivative

Figure 3

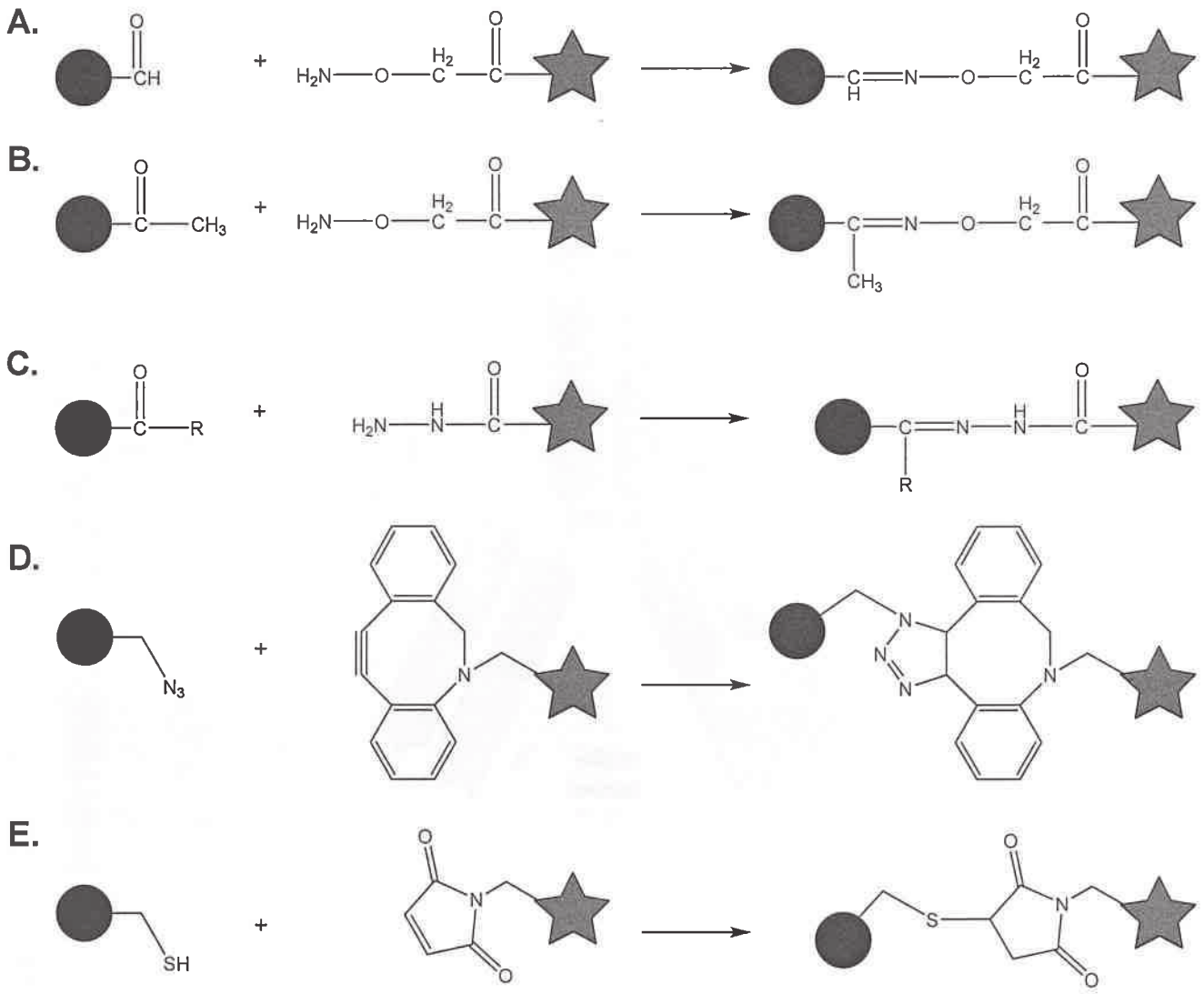


Figure 4

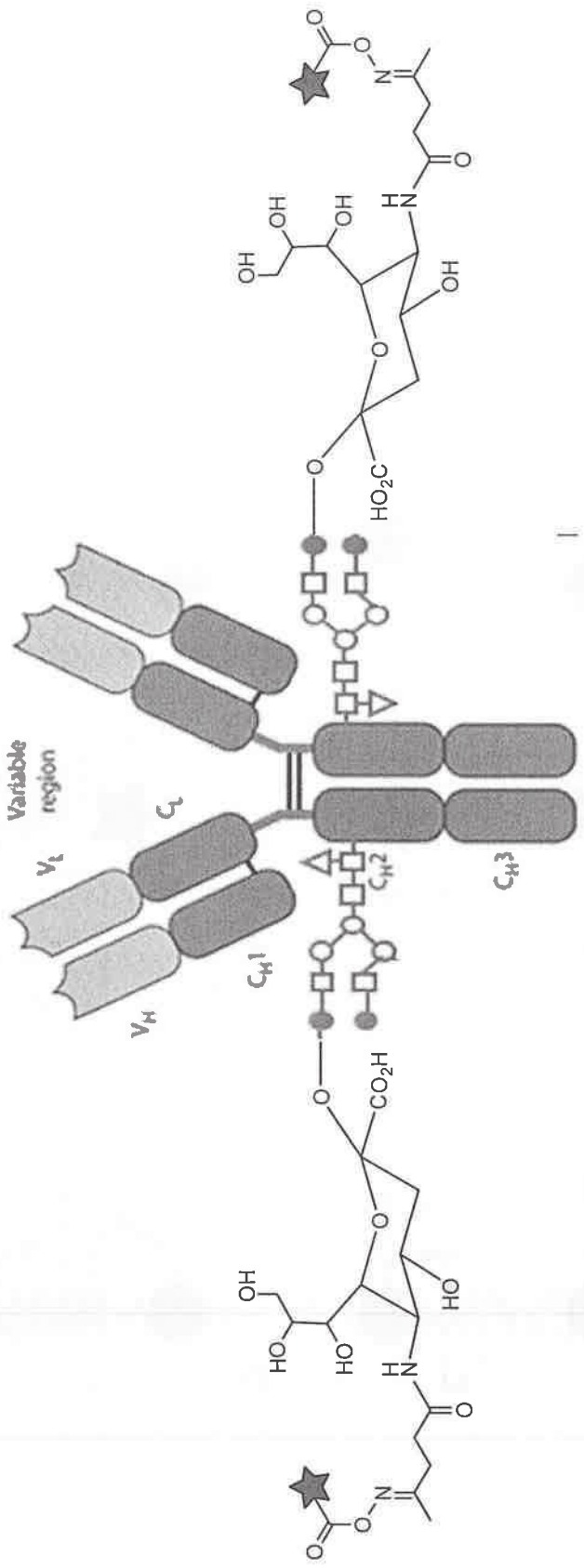


Figure 5

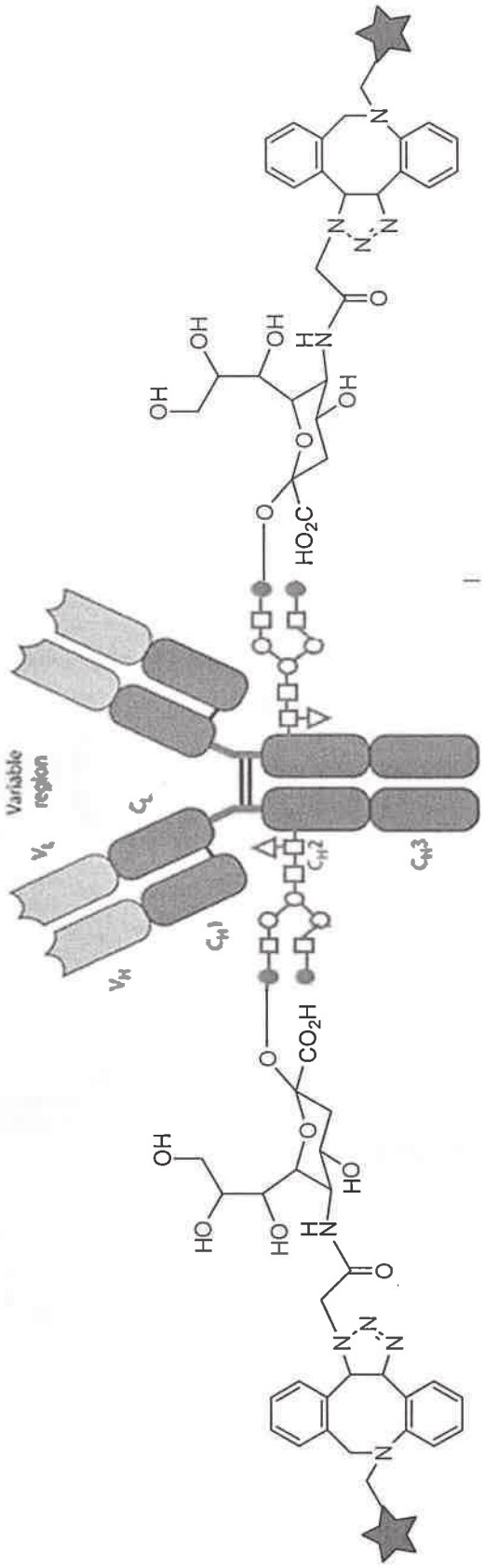


Figure 6

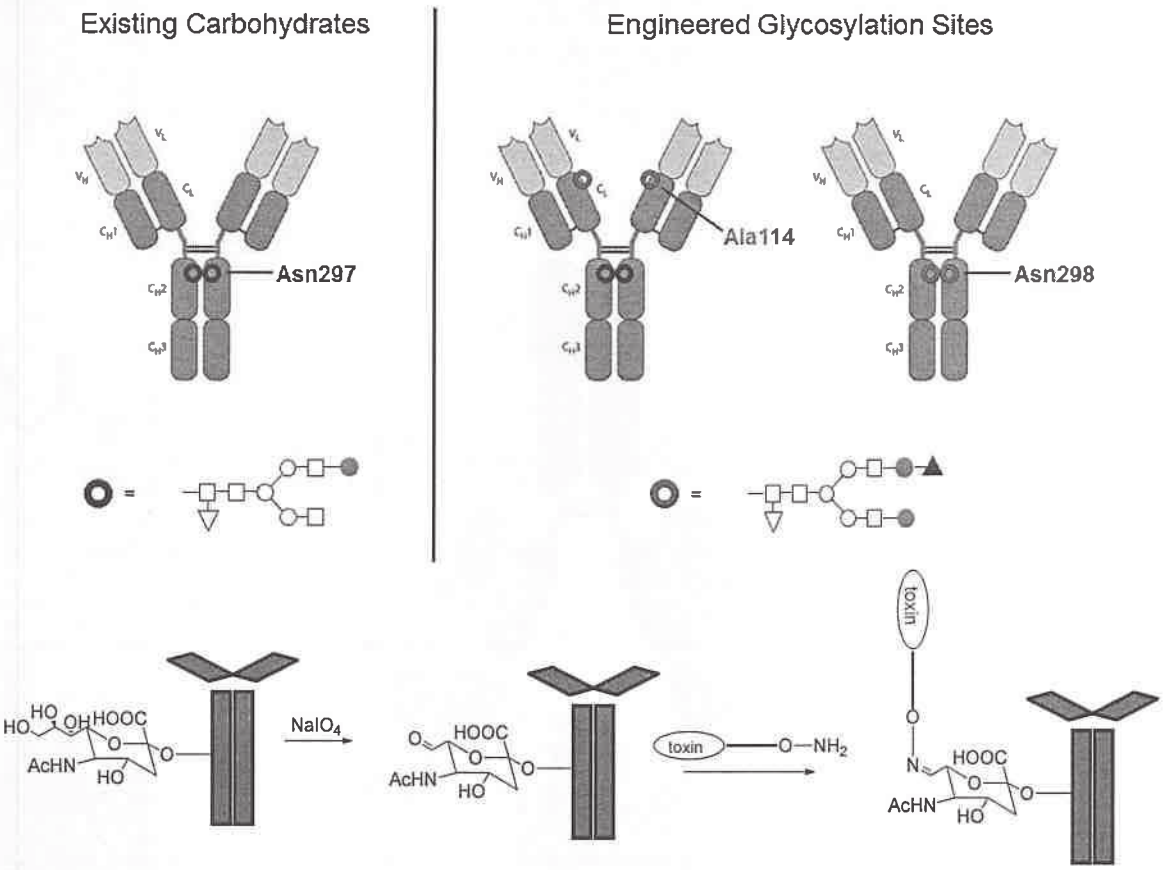

Existing Carbohydrates
Engineered Glycosylation Sites
Asn297
Ala114
Asn298
NaIO4
toxin
AcHN
HO
HOOC

Figure 7

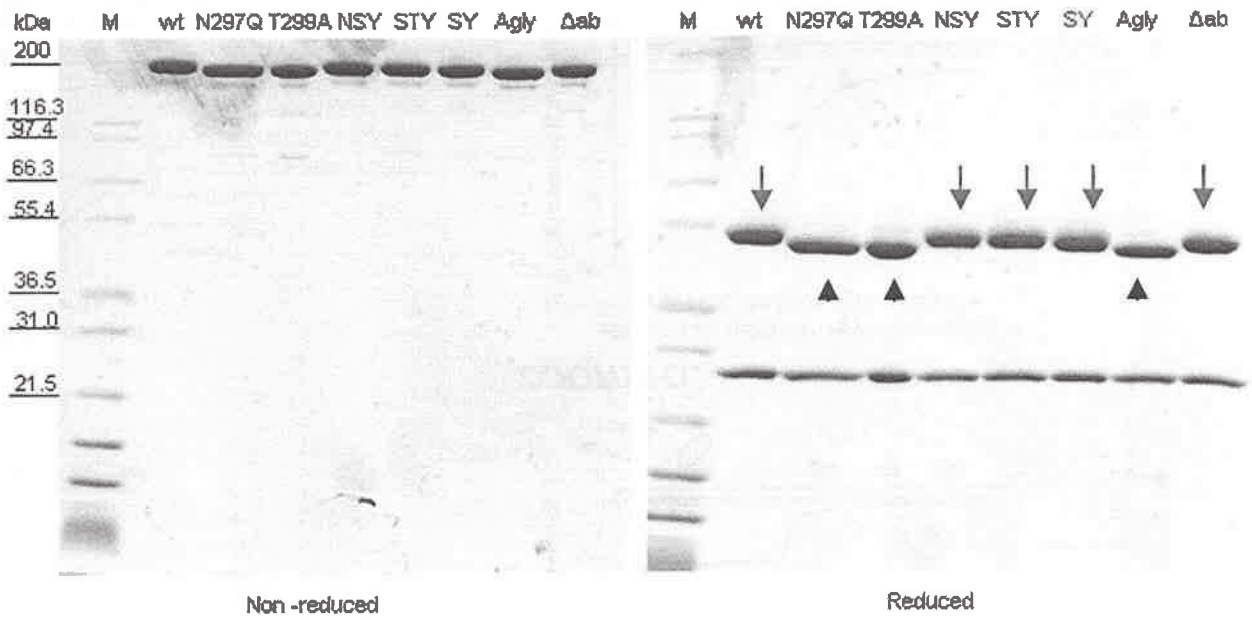
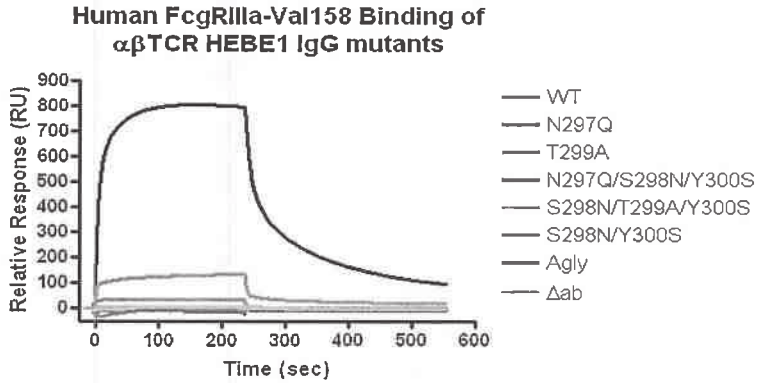
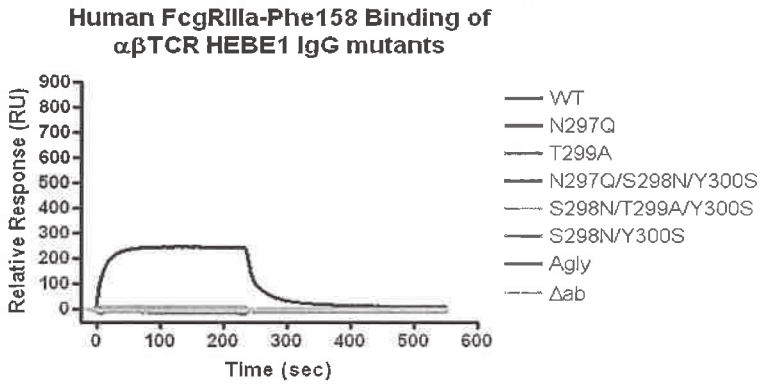

kDa
M
wt
N297Q
T299A
NSY
STY
SY
Agly
Δab
200
116.3
97.4
66.3
55.4
36.5
31.0
21.5
Non -reduced
Reduced

Figure 8

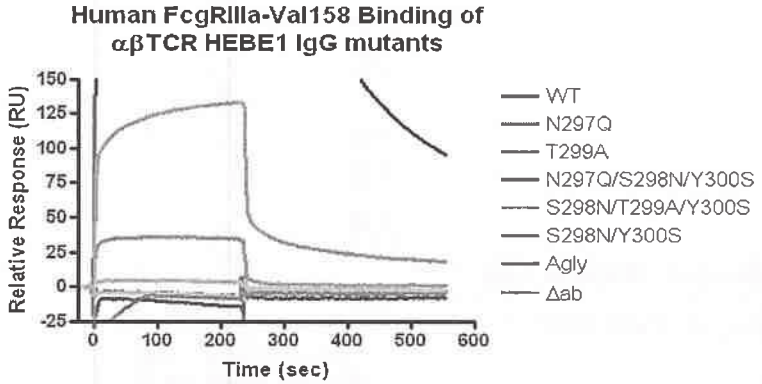
Fullscale: CD16a-Val158



Fullscale: CD16a-Phe158



zoomed:



zoomed:

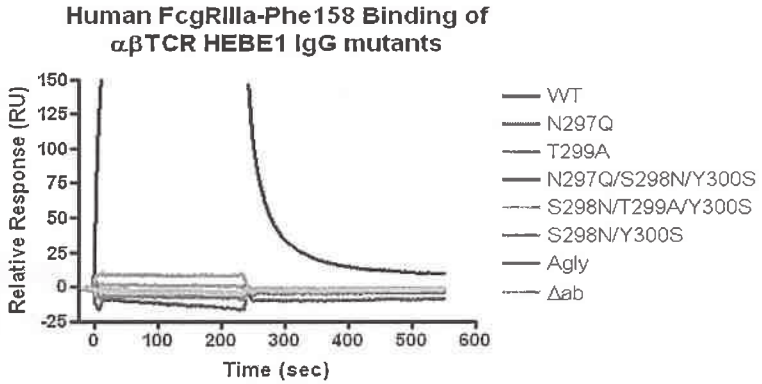
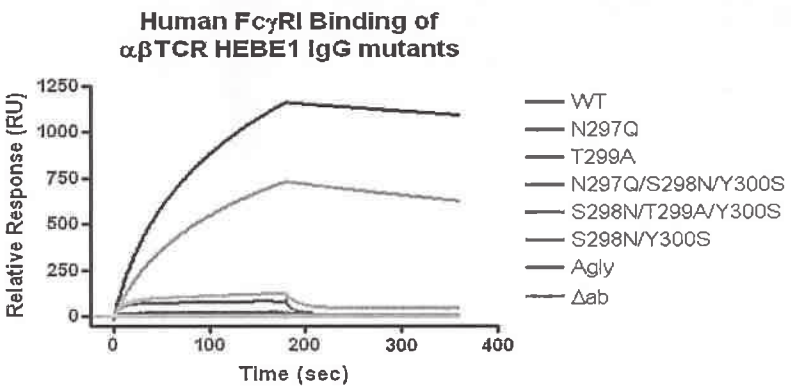


Figure 9

Fullscale:



zoomed in:

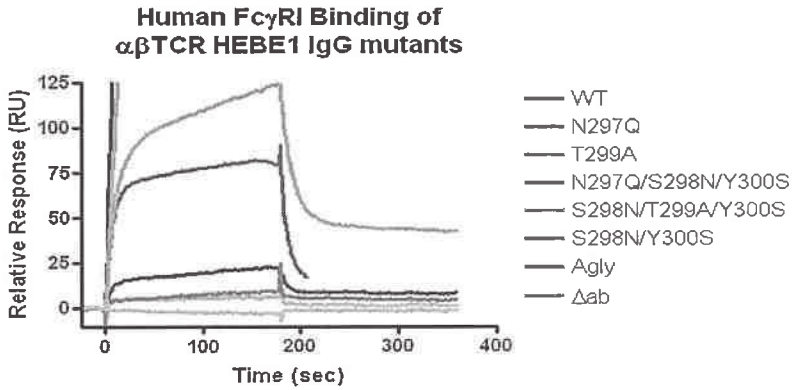


Figure 10

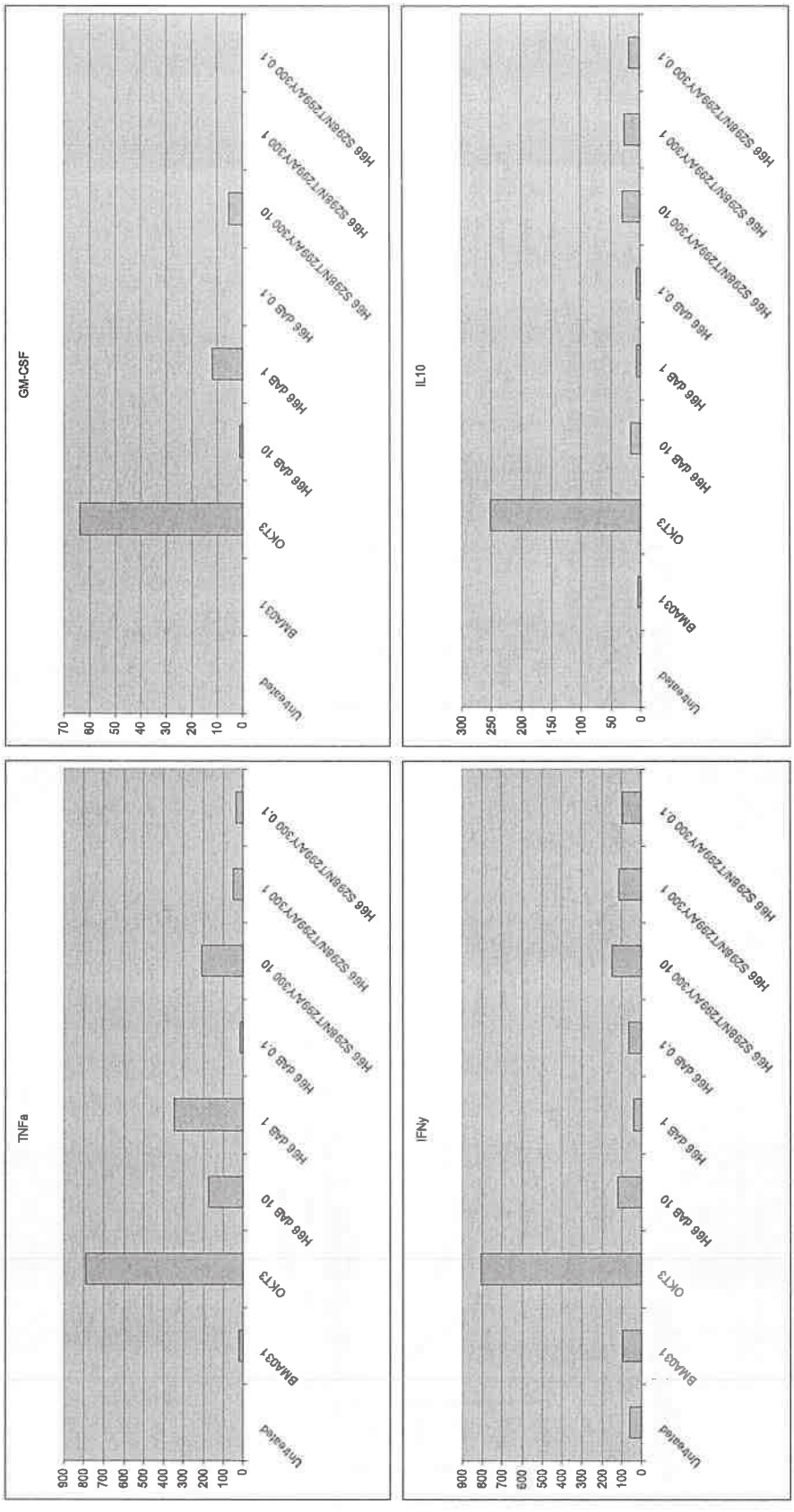

GM-CSF
TNFa
IL10
IFNγ
Untreated
BMA031
OKT3
H66 dAg 10
H66 dAg 1
H66 dAg 0.1
H66 SZ9&NTZ99A/Y300 10
H66 SZ9&NTZ99A/Y300 1
H66 SZ9&NTZ99A/Y300 0.1

Figure 11

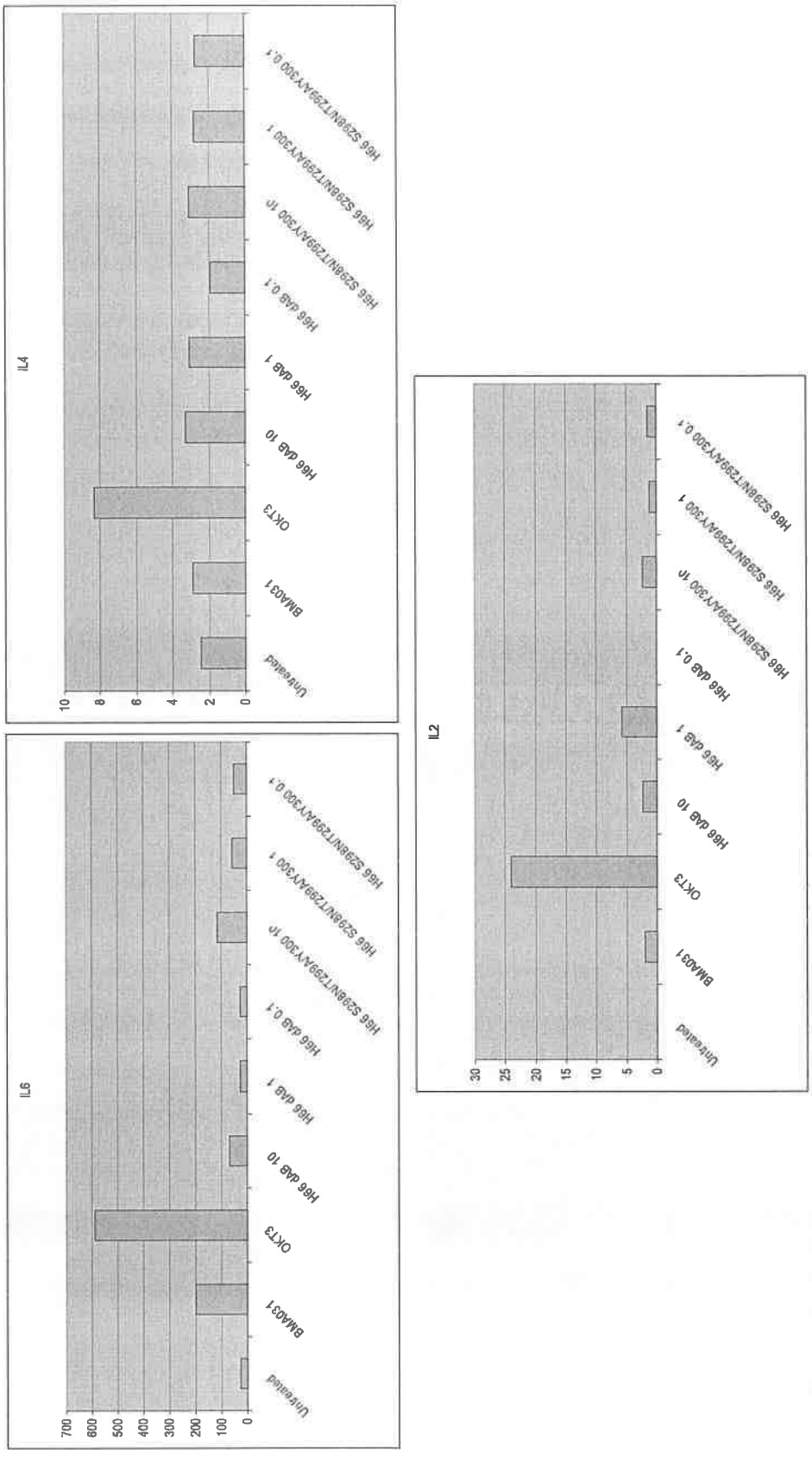

IL4
IL6
IL2
Unstained
BMA031
OKT3
H66 dAg 10
H66 dAg 1
H66 dAg 0,1
H66 S298N/T299A/Y300 1n
H66 S298N/T299A/Y300 1
H66 S298N/T299A/Y300 0,1

Figure 12

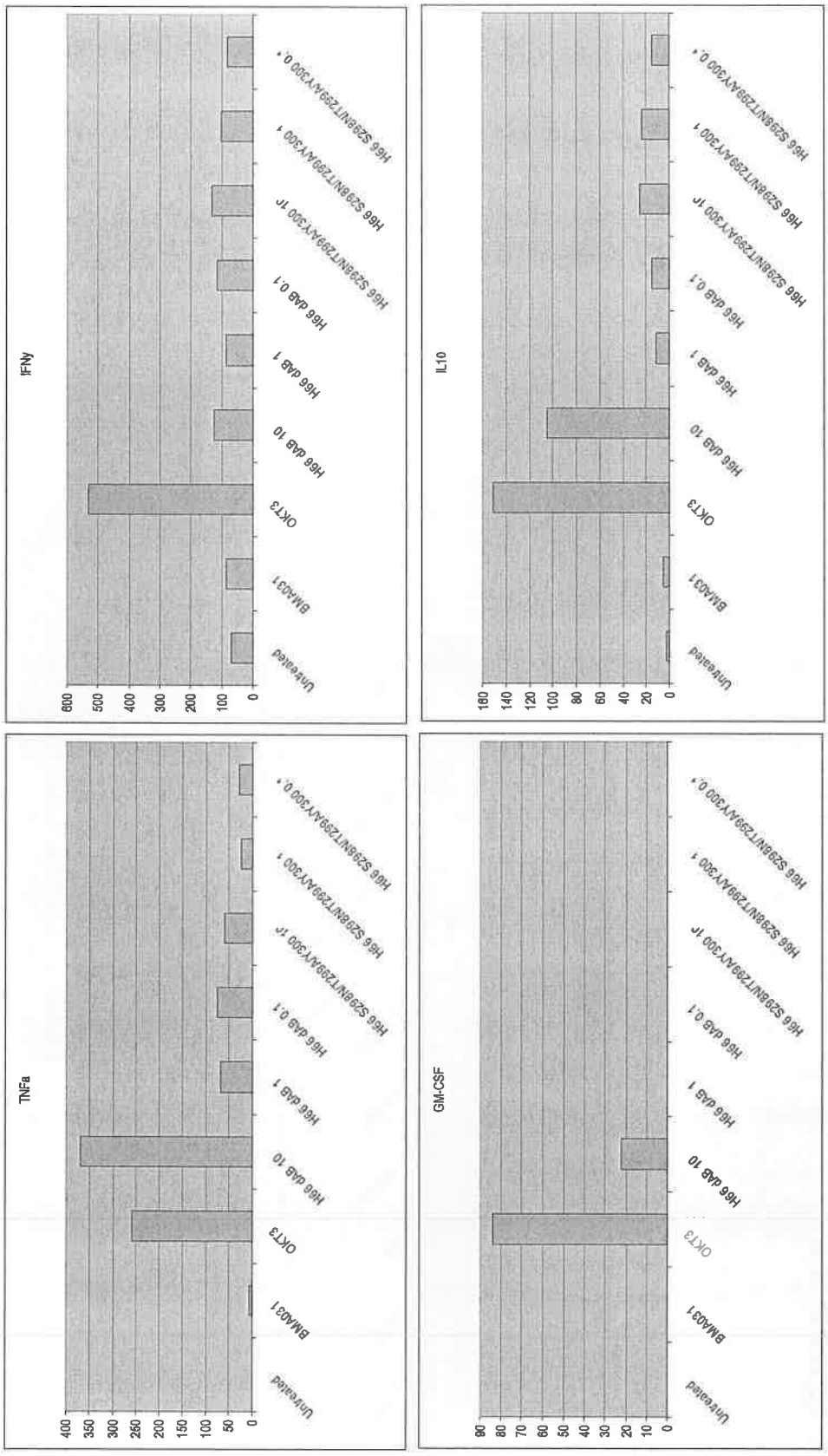

IFNγ
TNFa
IL10
GM-CSF
Untreated
BMA031
OKT3
H66 dAb 10
H66 dAb 1
H66 dAb 0.1
H66 S29BN/T29BA/V300 1F
H66 S29BN/T29BA/V300 1
H66 S29BN/T29BA/V300 0.1

Figure 13

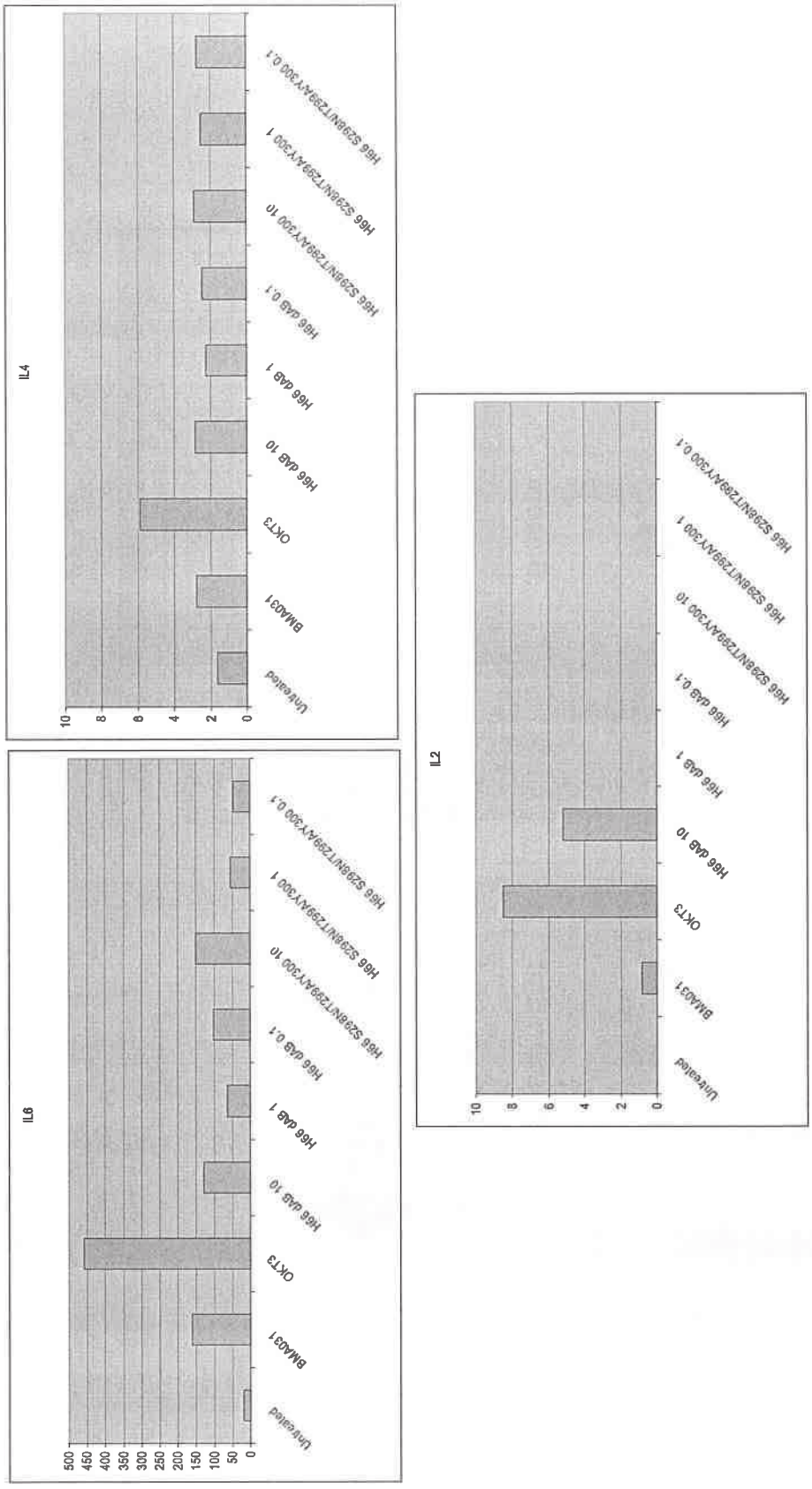
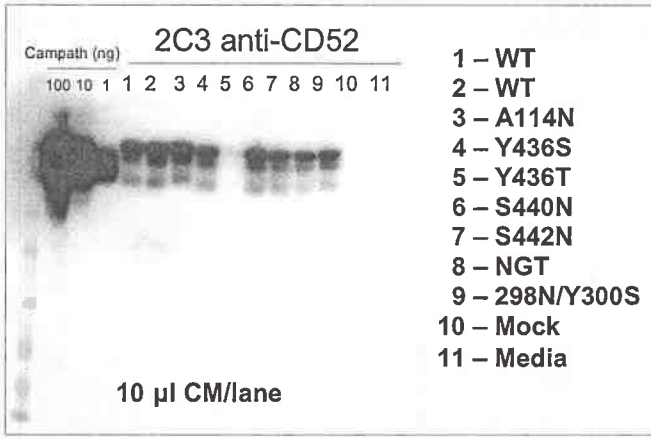

IL4
IL6
IL2
Untreated
BMA031
OKT3
H66 dAg 10
H66 dAg 1
H66 dAg 0,1
H66 S298N/T299A/Y300 10
H66 S298N/T299A/Y300 1
H66 S298N/T299A/Y300 0,1

Figure 14

A.

Western Blot



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**

Figure 15

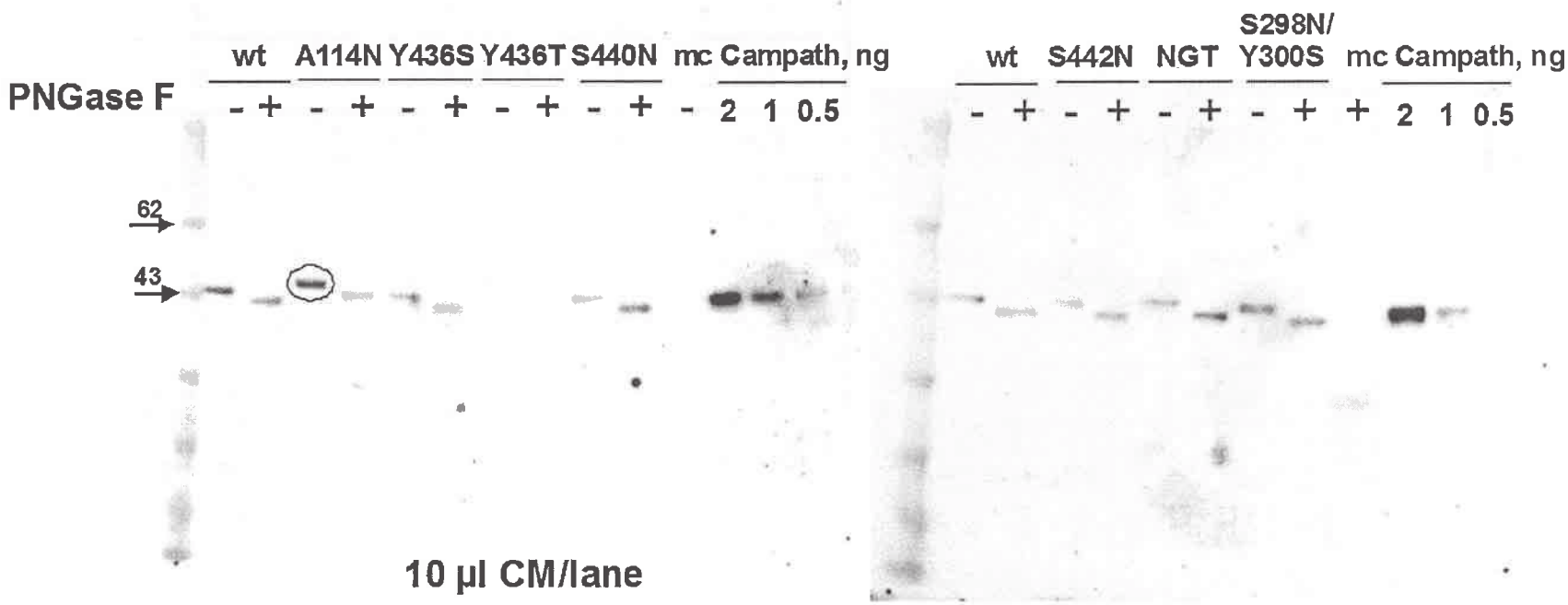

wt
A114N
Y436S
Y436T
S440N
mc
Campath, ng
wt
S442N
NGT
S298N/
Y300S
mc
Campath, ng
PNGase F
- + - + - + - + - + - 2 1 0.5
- + - + - + - + + 2 1 0.5
62
43
10 µl CM/lane

Figure 16

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

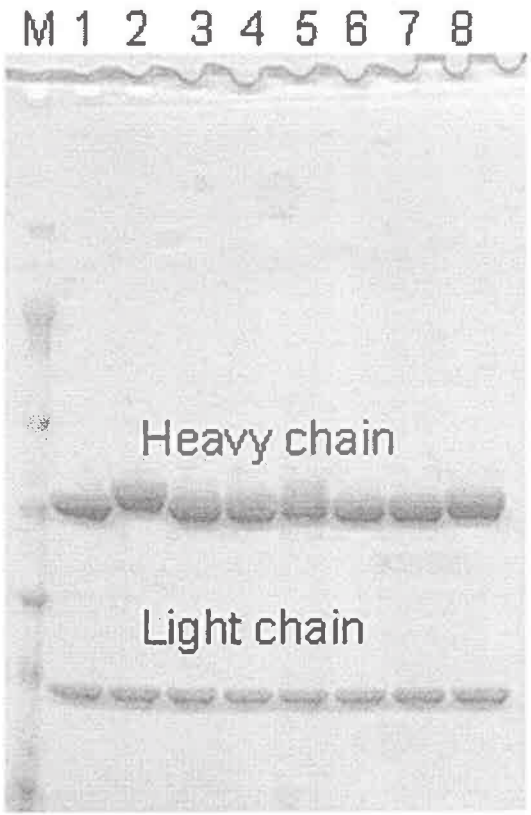
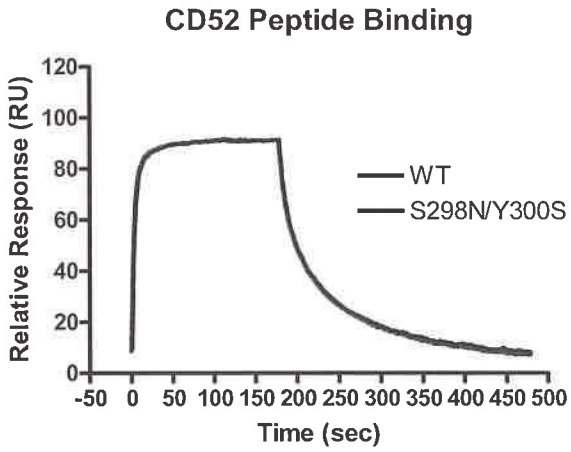
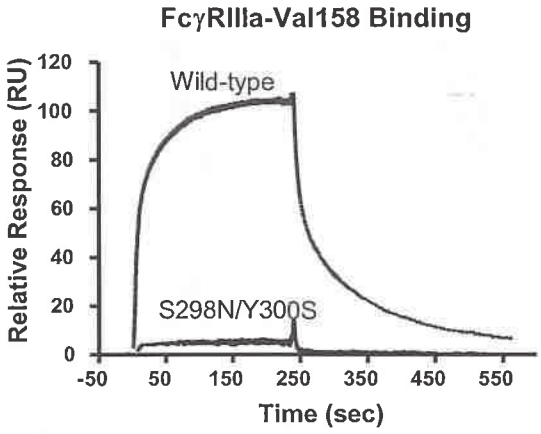


Figure 17

A.



B.



C.

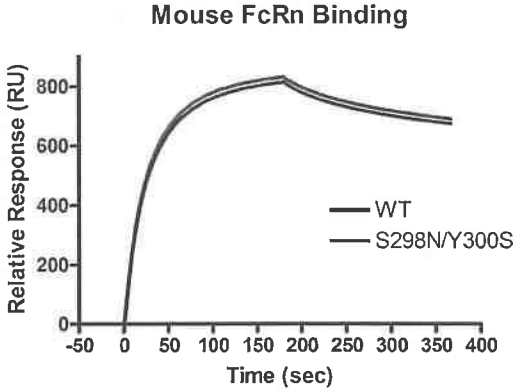


Figure 18

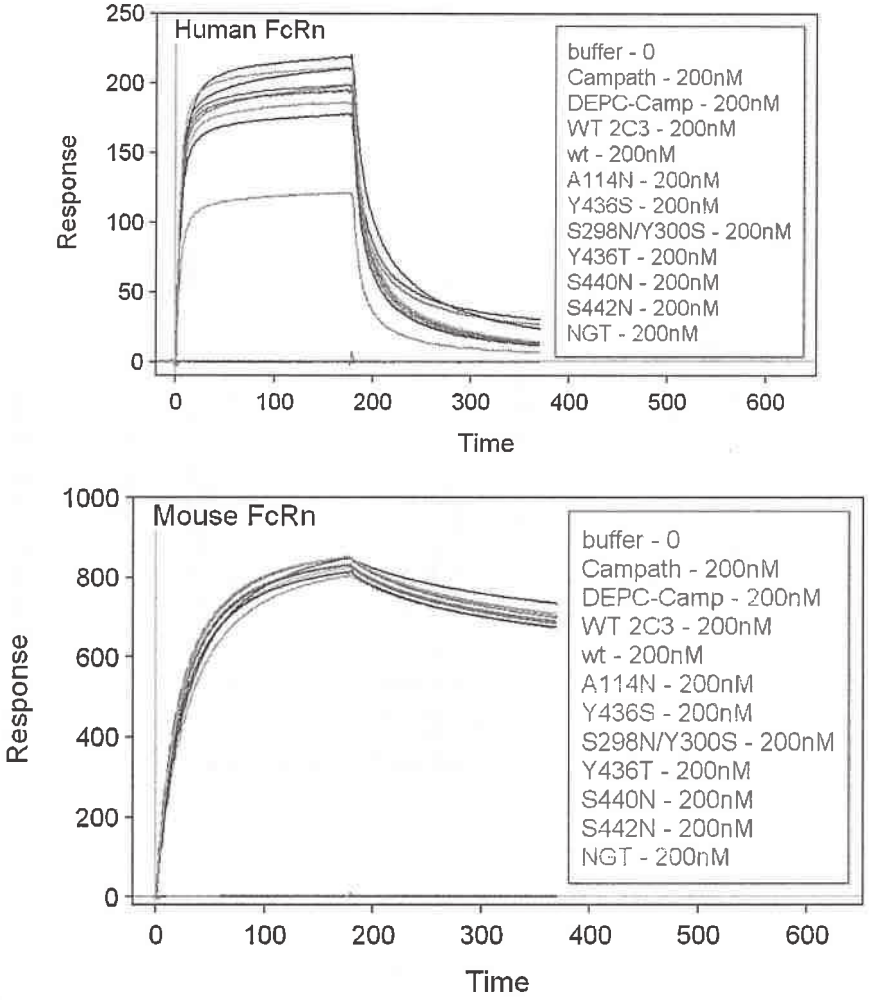
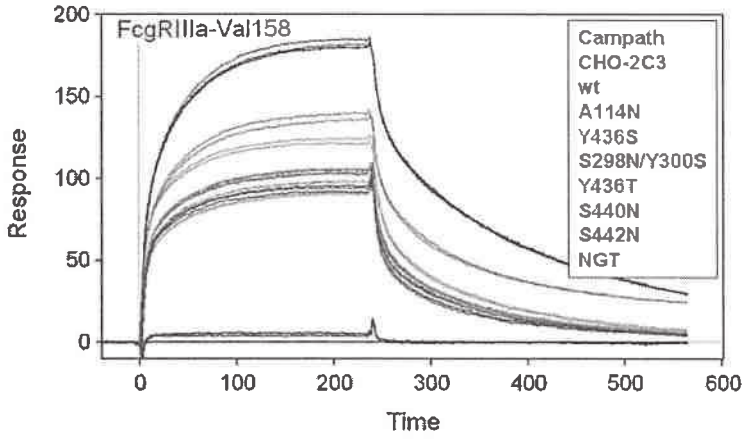

Human FcRn
Response
Time
buffer - 0
Campath - 200nM
DEPC-Camp - 200nM
WT 2C3 - 200nM
wt - 200nM
A114N - 200nM
Y436S - 200nM
S298N/Y300S - 200nM
Y436T - 200nM
S440N - 200nM
S442N - 200nM
NGT - 200nM
Mouse FcRn
Response
Time
buffer - 0
Campath - 200nM
DEPC-Camp - 200nM
WT 2C3 - 200nM
wt - 200nM
A114N - 200nM
Y436S - 200nM
S298N/Y300S - 200nM
Y436T - 200nM
S440N - 200nM
S442N - 200nM
NGT - 200nM

Figure 19

A.


FcgRIIIa-Val158
Campath
CHO-2C3
wt
A114N
Y436S
S298N/Y300S
Y436T
S440N
S442N
NGT
Response
Time
200
150
100
50
0
0
100
200
300
400
500
600

B.

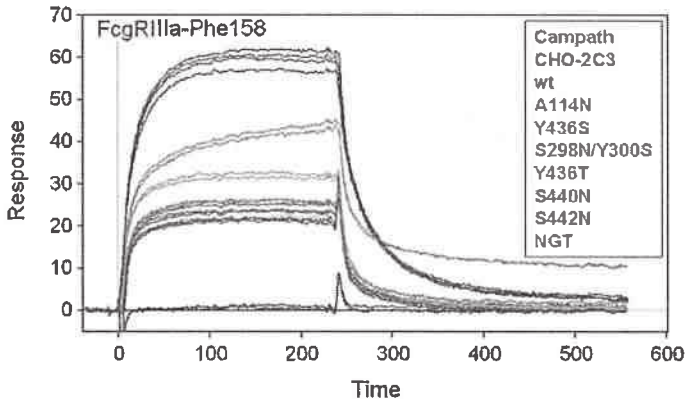
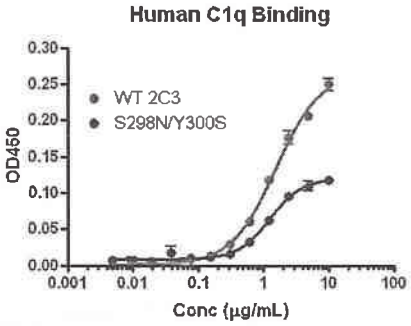

FcgRIIIa-Phe158
Campath
CHO-2C3
wt
A114N
Y436S
S298N/Y300S
Y436T
S440N
S442N
NGT
Response
Time
70
60
50
40
30
20
10
0
0
100
200
300
400
500
600

Figure 20

A.


Human C1q Binding
OD450
0.30
0.25
0.20
0.15
0.10
0.05
0.00
WT 2C3
S298N/Y300S
0.001
0.01
0.1
1
10
100
Conc (µg/mL)

B.

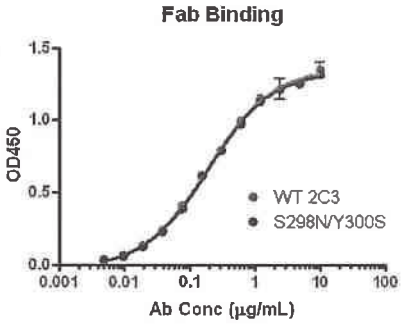

Fab Binding
OD450
1.5
1.0
0.5
0.0
WT 2C3
S298N/Y300S
0.001
0.01
0.1
1
10
100
Ab Conc (µg/mL)

Figure 21

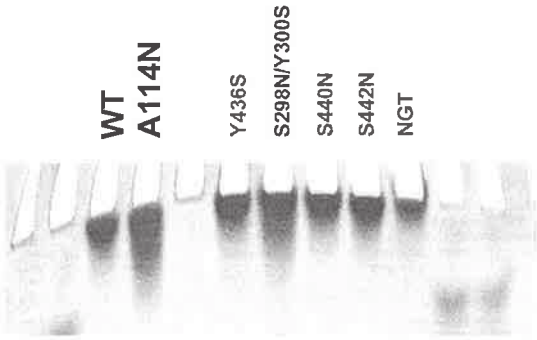
Sample	k_a ($x10^6$/Ms)	k_d ($x10^{-2}$/s)	R_{max} (RU)	K_D (nM)
GLD52	7.0	1.7	67.0	2.44
WT 2C3	6.0	1.1	64.2	1.75
A114N	4.7	1.1	59.5	2.45
Y436S	5.9	1.0	66.9	1.73
S298N/Y300S	5.7	1.0	63.3	1.80
Y436T	4.8	0.9	65.7	1.95
S440N	5.8	1.1	66.8	1.84
S442N	5.7	1.1	66.2	1.85
NGT	7.9	1.1	70.2	1.35

Figure 22

Sample	Kon ($x10^{6}M^{-1}s^{-1}$)	Koff ($x10^{-2}s^{-1}$)	KD (nM)
WT 2C3	5.2	1.1	2.1
A114N	5.3	1.3	2.4

Figure 23

A.


WT
A114N
Y436S
S298N/Y300S
S440N
S442N
NGT

B.

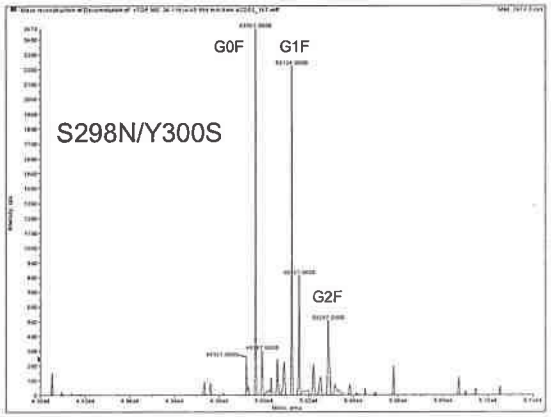
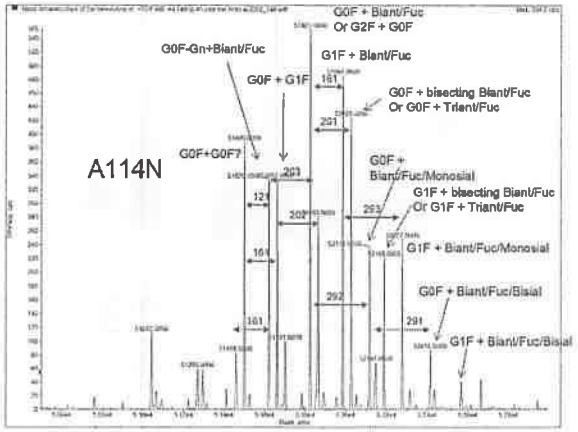

G0F
G1F
S298N/Y300S
G2F

Figure 23

C.


A114N
G0F + Biant/Fuc
Or G2F + G0F
G0F-Gn+Biant/Fuc
G1F + Biant/Fuc
G0F + G1F
G0F + bisecting Biant/Fuc
Or G0F + Triant/Fuc
G0F+G0F7
G0F +
Biant/Fuc/Monosial
G1F + bisecting Biant/Fuc
Or G1F + Triant/Fuc
G1F + Biant/Fuc/Monosial
G0F + Biant/Fuc/Bisial
G1F + Biant/Fuc/Bisial

D.

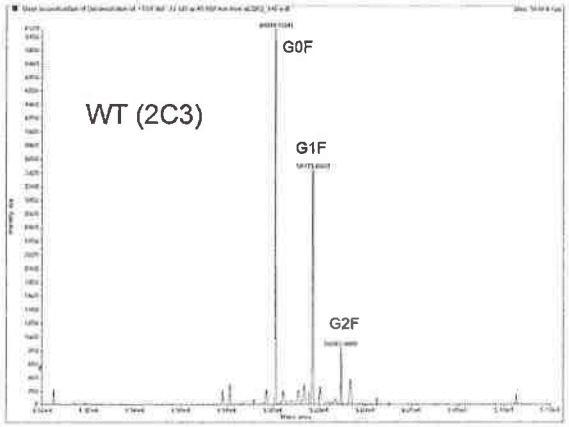

WT (2C3)
G0F
G1F
G2F

Figure 24

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.

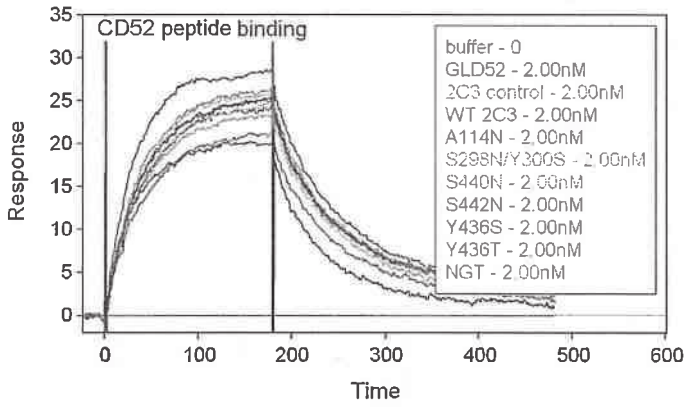


Figure 25

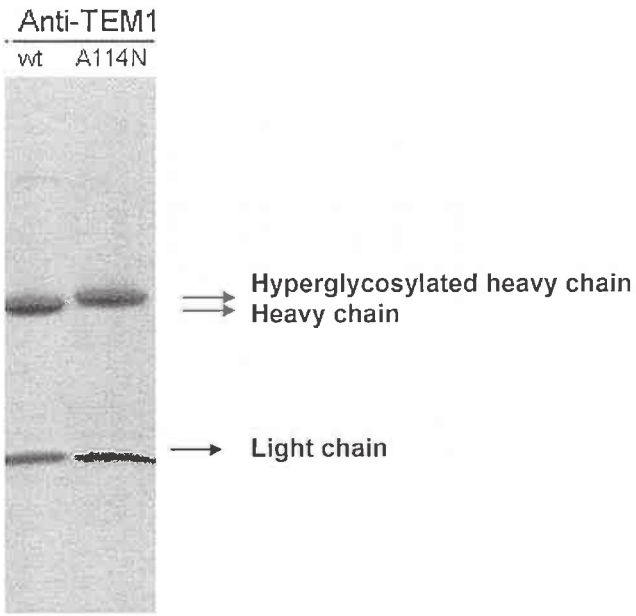
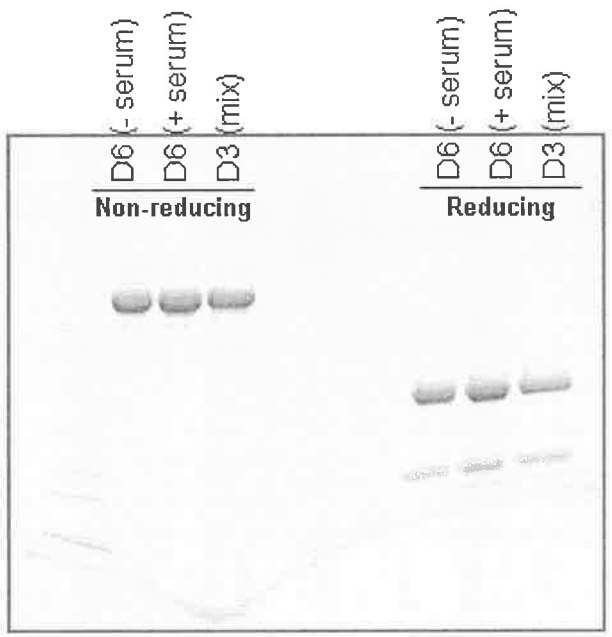

Anti-TEM1
wt
A114N
Hyperglycosylated heavy chain
Heavy chain
Light chain

Figure 26

10% Tris-glycine SDS-PAGE



HIC Analysis of Purified A114N Herceptin

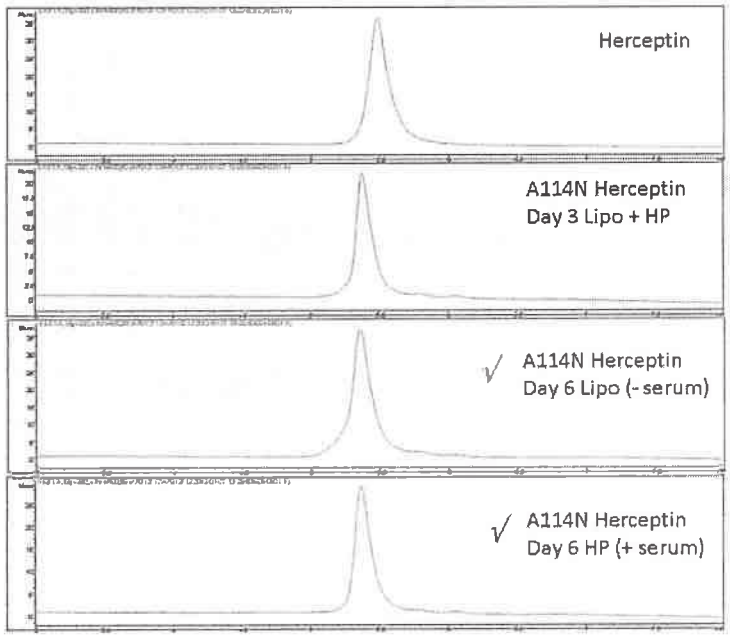


Figure 27

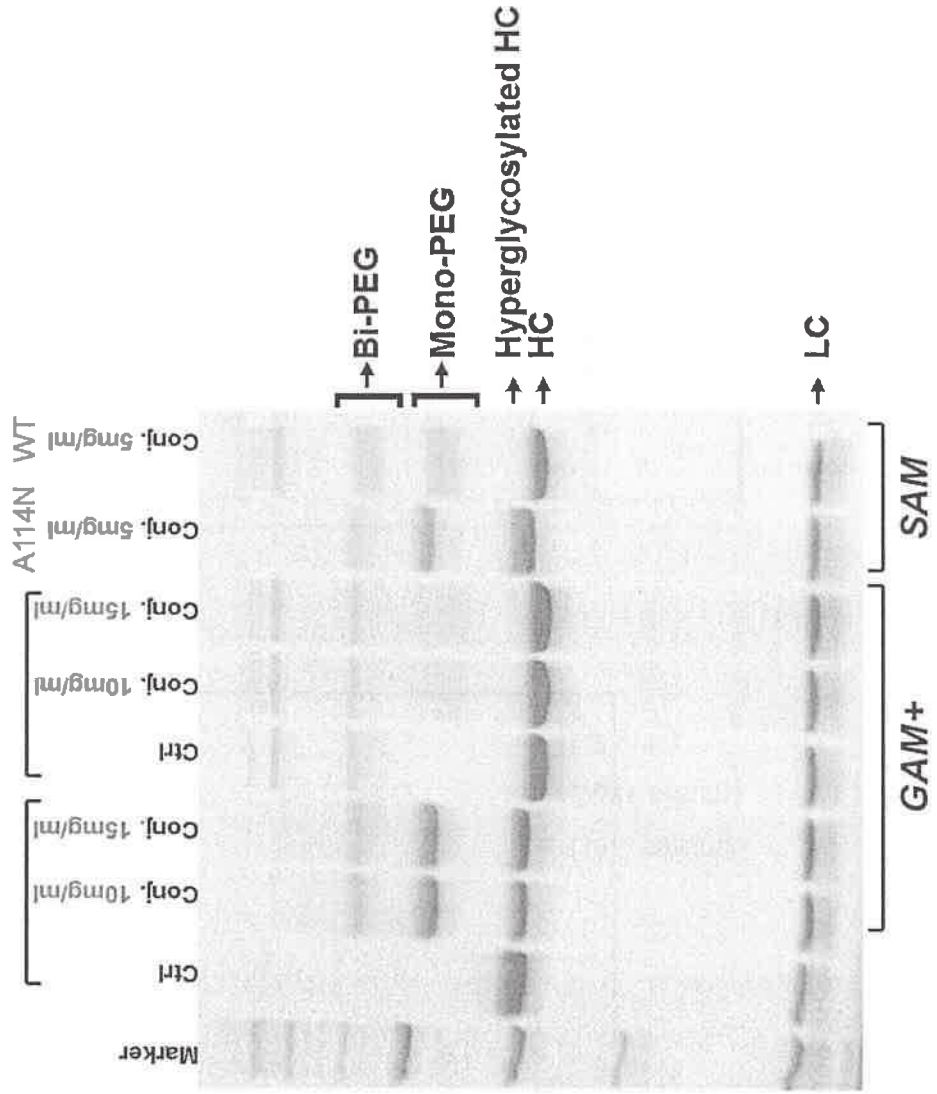

Marker
Ctrl
Conj. 10mg/ml
Conj. 15mg/ml
Ctrl
Conj. 10mg/ml
Conj. 15mg/ml
A114N
Conj. 5mg/ml
WT
Conj. 5mg/ml
Bi-PEG
Mono-PEG
Hyperglycosylated HC
HC
LC
GAM+
SAM

Figure 28

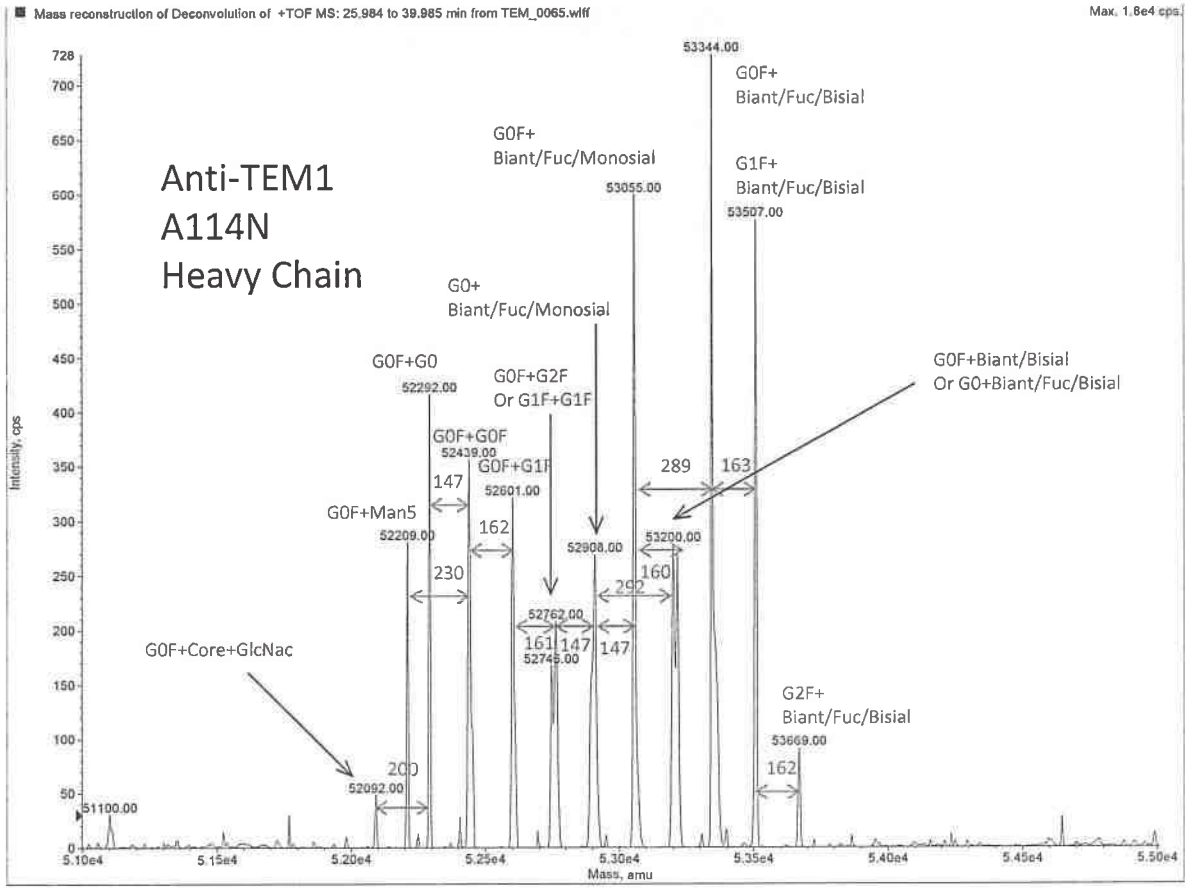
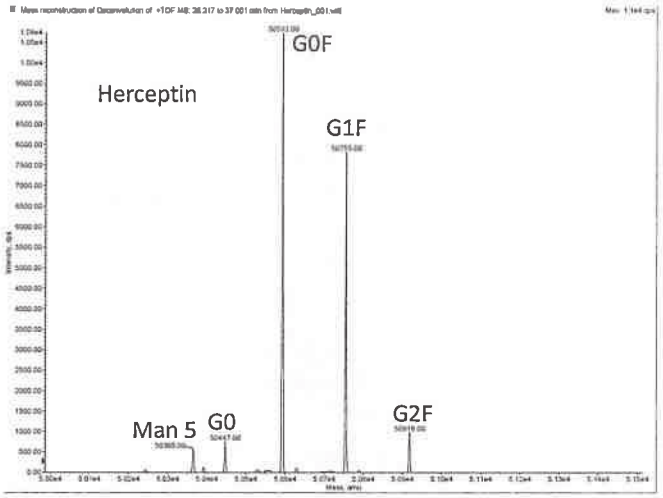

Mass reconstruction of Deconvolution of +TOF MS: 25.984 to 39.985 min from TEM_0065.wiff
Max. 1.8e4 cps.
Anti-TEM1
A114N
Heavy Chain
G0F+
Biant/Fuc/Bisial
53344.00
G0F+
Biant/Fuc/Monosial
53055.00
G1F+
Biant/Fuc/Bisial
53507.00
G0+
Biant/Fuc/Monosial
G0F+G0
52292.00
G0F+G2F
Or G1F+G1F
G0F+Biant/Bisial
Or G0+Biant/Fuc/Bisial
G0F+G0F
52439.00
G0F+G1F
52601.00
G0F+Man5
52209.00
52908.00
53200.00
147
162
230
289
163
160
262
52762.00
161
147
147
52745.00
G0F+Core+GlcNac
G2F+
Biant/Fuc/Bisial
53669.00
162
200
52092.00
51100.00
Intensity, cps
Mass, amu

Figure 29


Herceptin
G0F
G1F
Man 5
G0
G2F

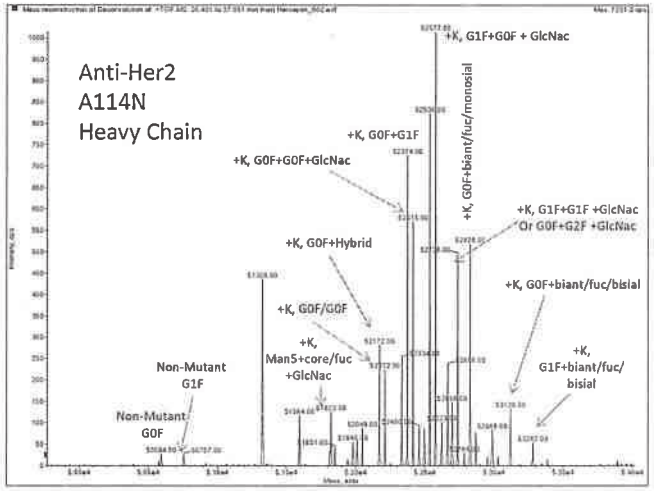
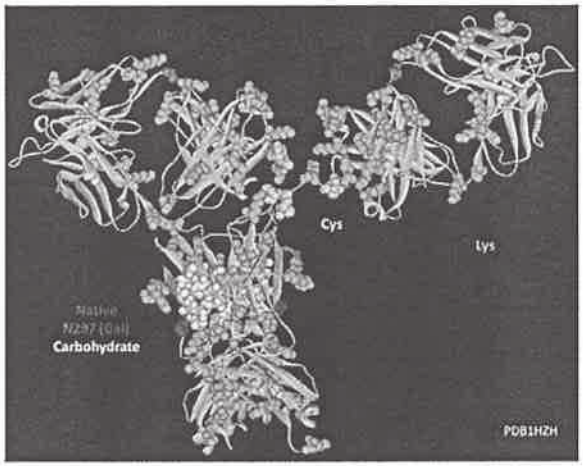
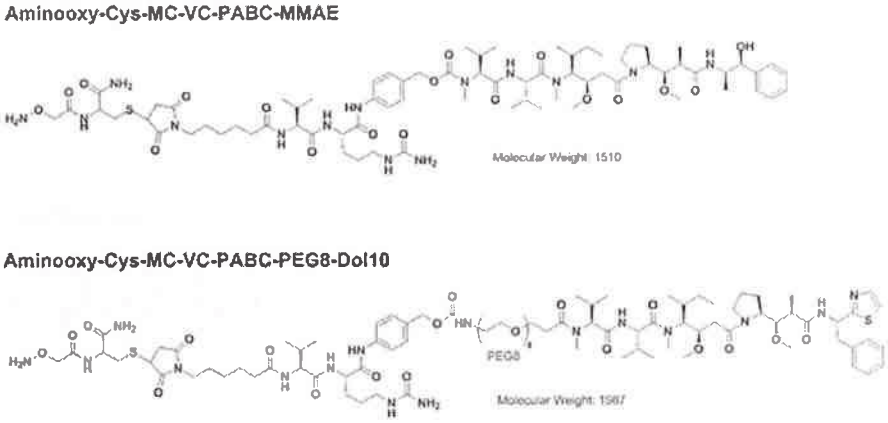

Anti-Her2
A114N
Heavy Chain
+K, G1F+G0F + GlcNac
+K, G0F+G1F
+K, G0F+G0F+GlcNac
+K, G0F+biant/fuc/monosial
+K, G1F+G1F +GlcNac
Or G0F+G2F +GlcNac
+K, G0F+Hybrid
+K, G0F+biant/fuc/bisial
+K, G0F/G0F
+K,
Man5+core/fuc
+GlcNac
+K,
G1F+biant/fuc/
bisial
Non-Mutant
G1F
Non-Mutant
G0F

Figure 30

A.



B.



C.

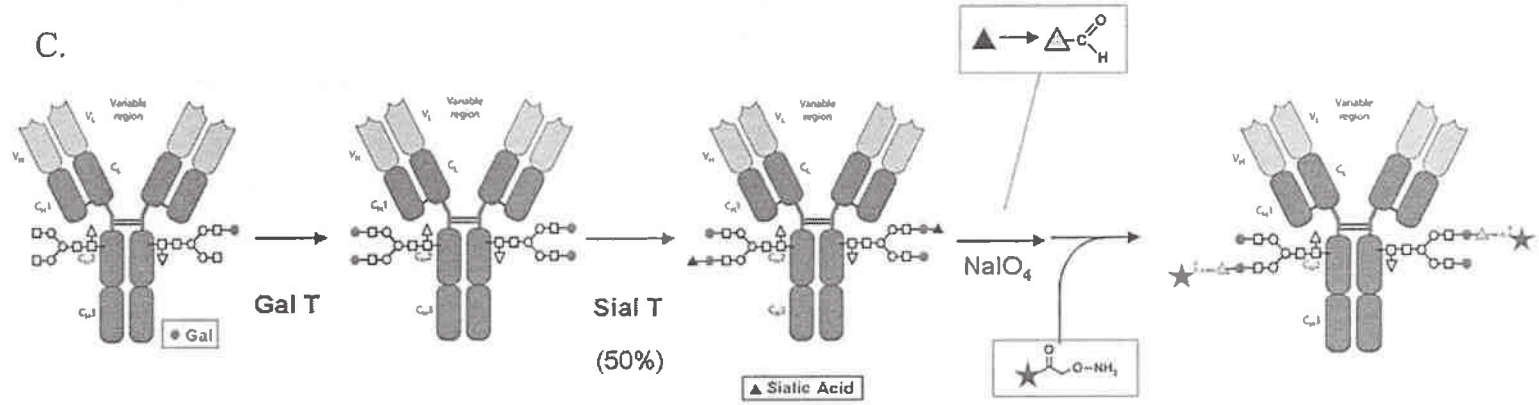


Figure 31

*Synth*esis of aminooxy-Cys-MC-VC-PABC-MMAE and aminooxy-Cys-MC-VC-PABC-PEG8-Dol10

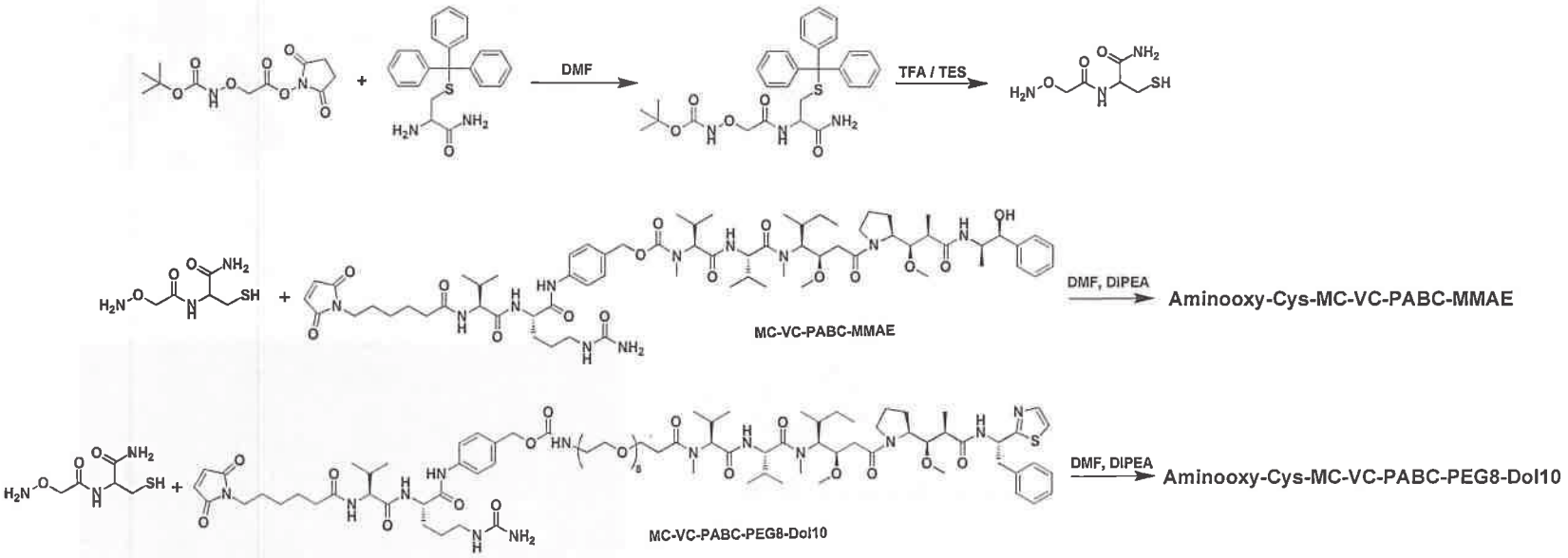
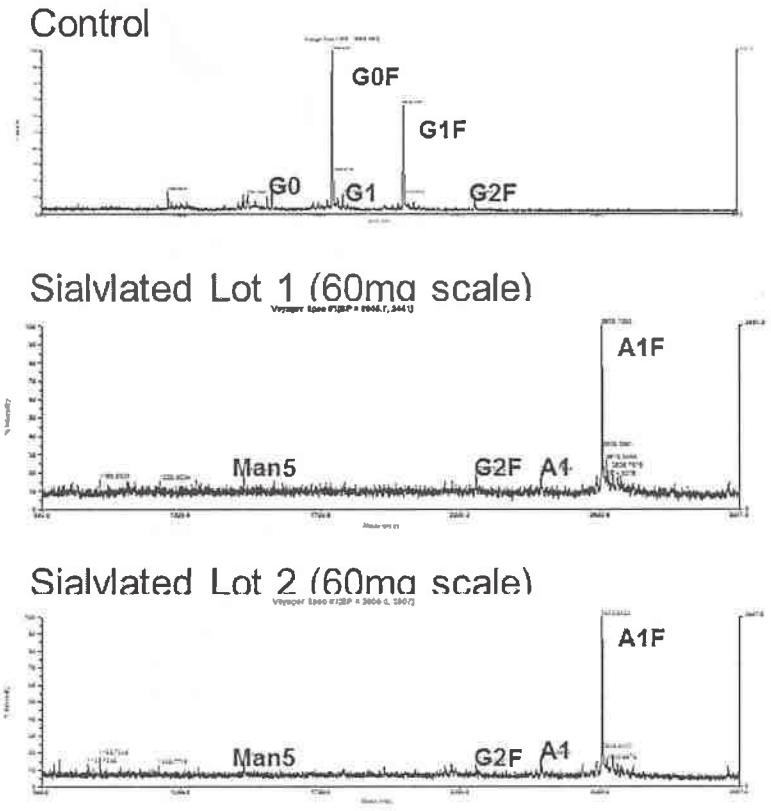


Figure 32

A.



B.

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

C.

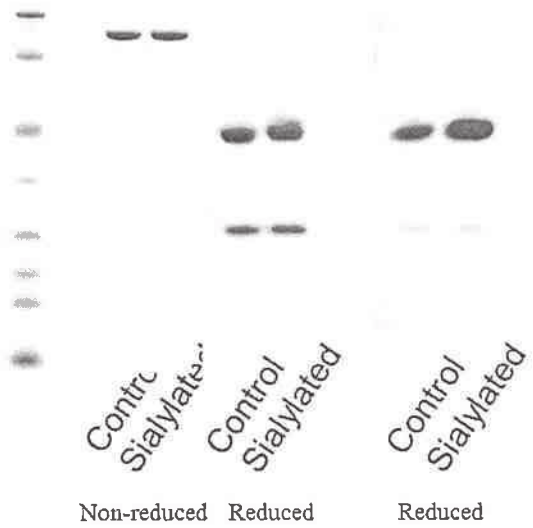


Figure 33

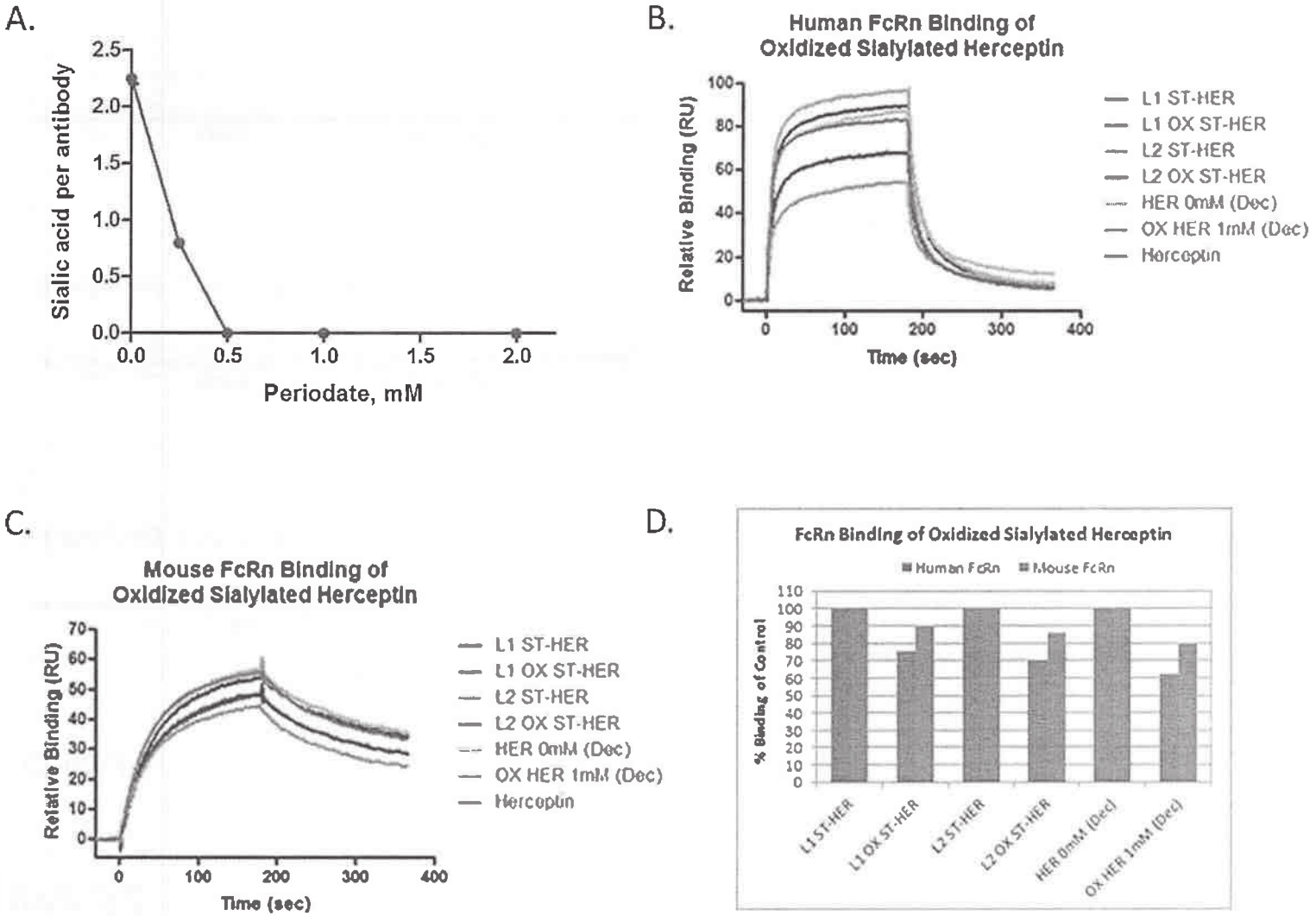

A.
Sialic acid per antibody
2.5
2.0
1.5
1.0
0.5
0.0
0.0
0.5
1.0
1.5
2.0
Periodate, mM
B.
Human FcRn Binding of Oxidized Sialylated Herceptin
Relative Binding (RU)
100
80
60
40
20
0
0
100
200
300
400
Time (sec)
L1 ST-HER
L1 OX ST-HER
L2 ST-HER
L2 OX ST-HER
HER 0mM (Dec)
OX HER 1mM (Dec)
Herceptin
C.
Mouse FcRn Binding of Oxidized Sialylated Herceptin
Relative Binding (RU)
70
60
50
40
30
20
10
0
0
100
200
300
400
Time (sec)
L1 ST-HER
L1 OX ST-HER
L2 ST-HER
L2 OX ST-HER
HER 0mM (Dec)
OX HER 1mM (Dec)
Herceptin
D.
FcRn Binding of Oxidized Sialylated Herceptin
Human FcRn
Mouse FcRn
% Binding of Control
110
100
90
80
70
60
50
40
30
20
10
0
L1 ST-HER
L1 OX ST-HER
L2 ST-HER
L2 OX ST-HER
HER 0mM (Dec)
OX HER 1mM (Dec)

Figure 34

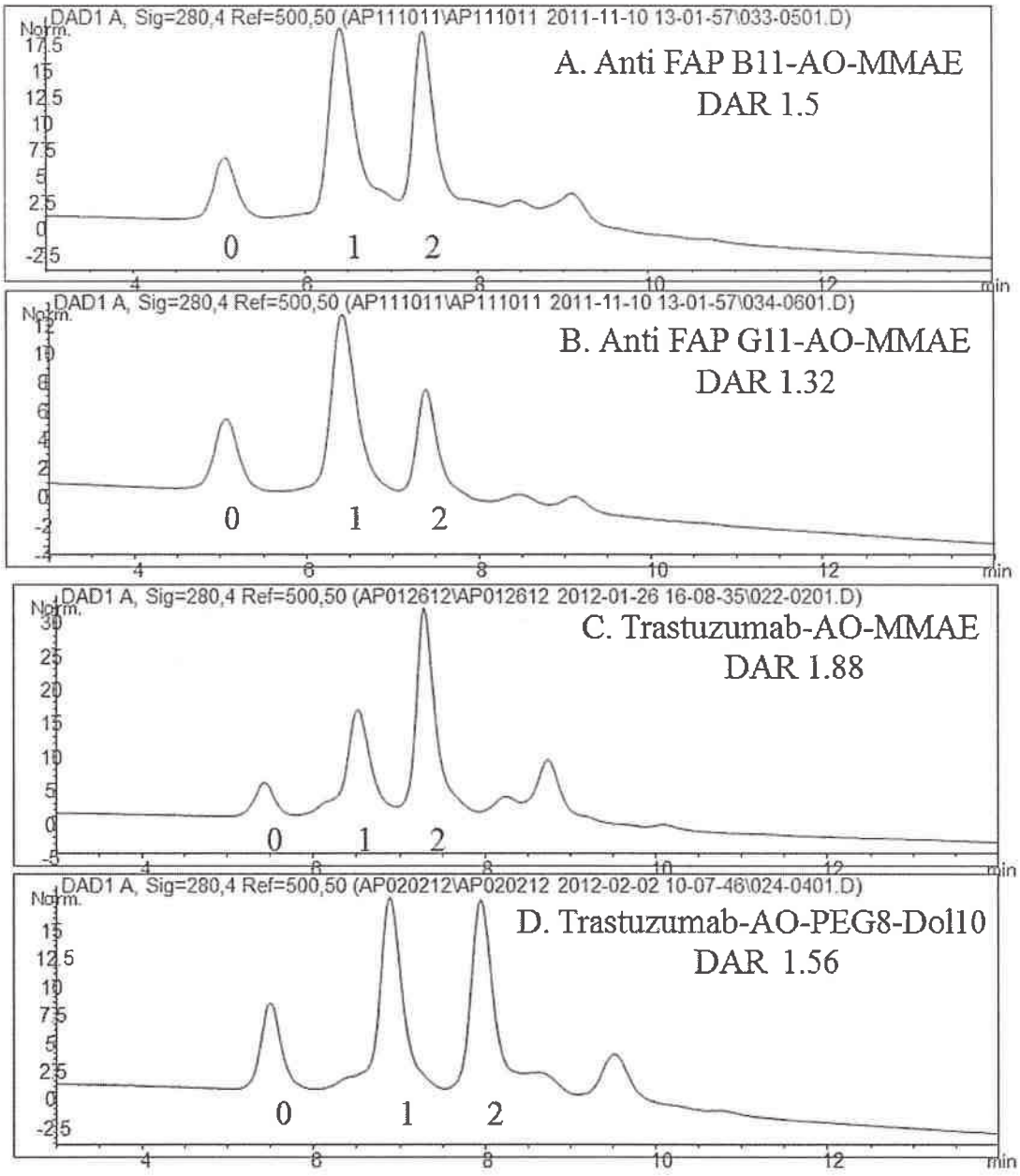
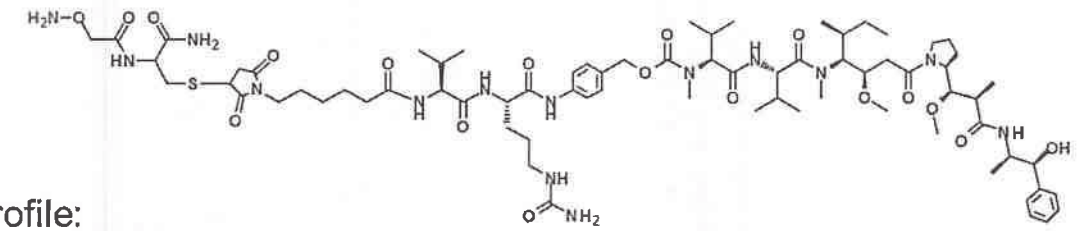

DAD1 A, Sig=280,4 Ref=500,50 (AP111011\AP111011 2011-11-10 13-01-57\033-0501.D)
Norm.
A. Anti FAP B11-AO-MMAE
DAR 1.5
0
1
2
min
DAD1 A, Sig=280,4 Ref=500,50 (AP111011\AP111011 2011-11-10 13-01-57\034-0601.D)
Norm.
B. Anti FAP G11-AO-MMAE
DAR 1.32
0
1
2
min
DAD1 A, Sig=280,4 Ref=500,50 (AP012612\AP012612 2012-01-26 16-08-35\022-0201.D)
Norm.
C. Trastuzumab-AO-MMAE
DAR 1.88
0
1
2
min
DAD1 A, Sig=280,4 Ref=500,50 (AP020212\AP020212 2012-02-02 10-07-46\024-0401.D)
Norm.
D. Trastuzumab-AO-PEG8-Dol10
DAR 1.56
0
1
2
min

Figure 35

Aminooxy

MC-VC-PABC-MMAE



HIC Profile:

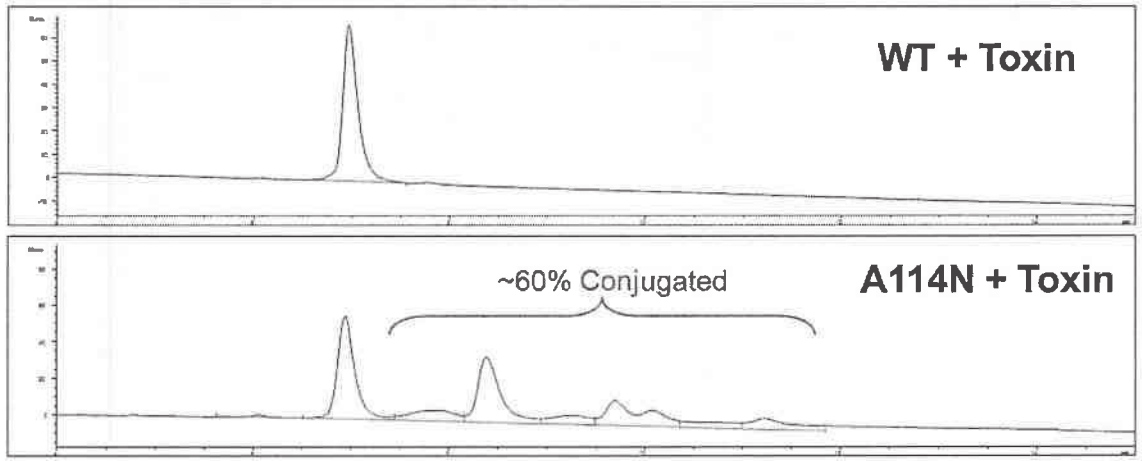


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Figure 36

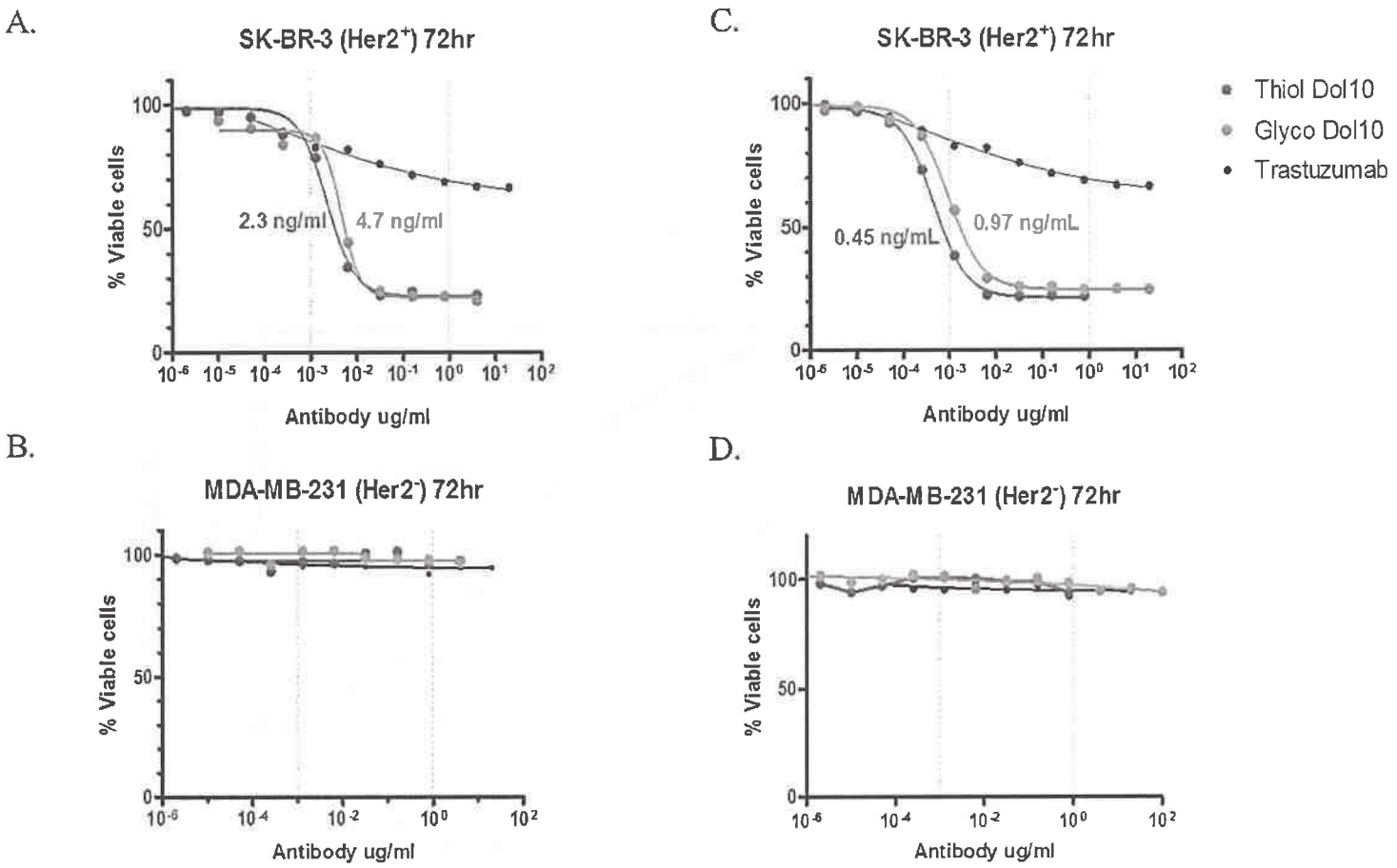

A.
SK-BR-3 (Her2+) 72hr
% Viable cells
Antibody ug/ml
2.3 ng/ml
4.7 ng/ml
B.
MDA-MB-231 (Her2-) 72hr
% Viable cells
Antibody ug/ml
C.
SK-BR-3 (Her2+) 72hr
% Viable cells
Antibody ug/ml
0.45 ng/mL
0.97 ng/mL
Thiol Dol10
Glyco Dol10
Trastuzumab
D.
MDA-MB-231 (Her2-) 72hr
% Viable cells
Antibody ug/ml

Figure 37

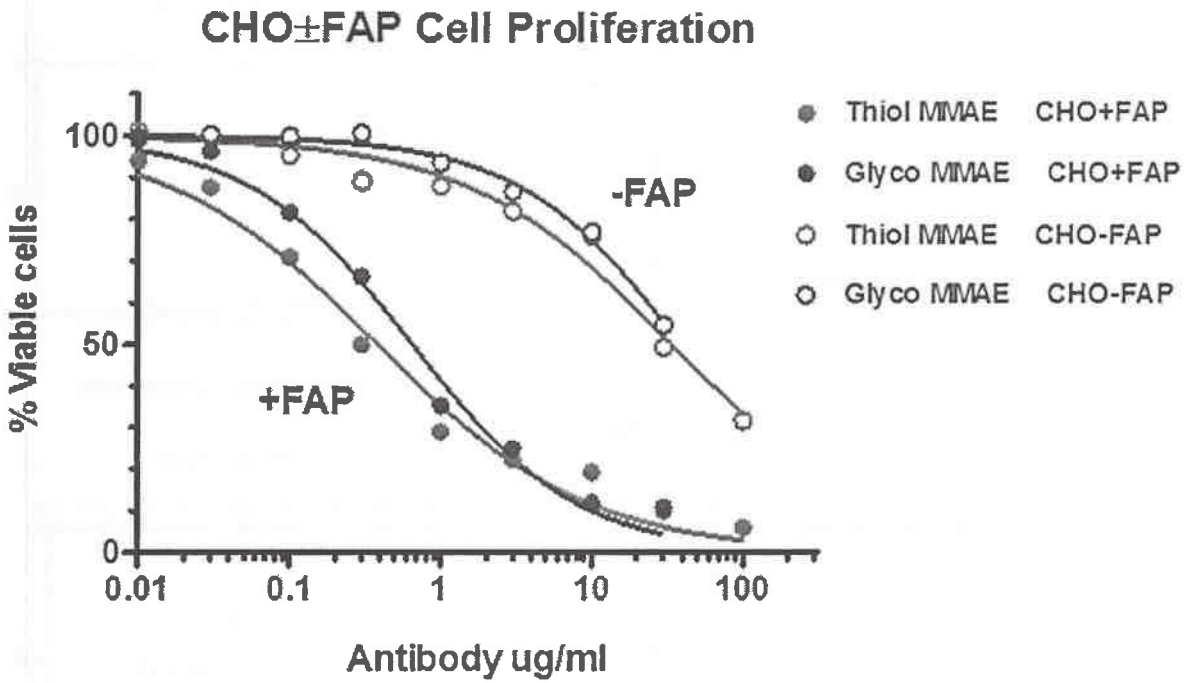
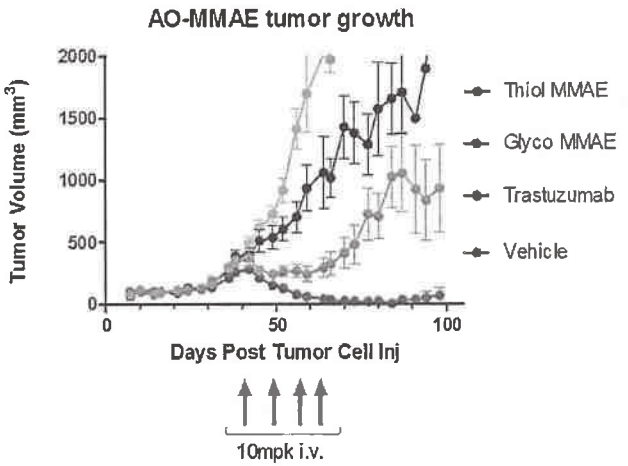
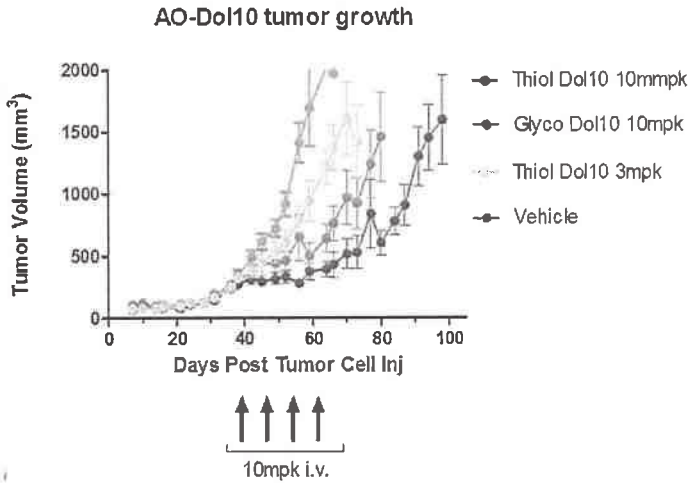

CHO±FAP Cell Proliferation
% Viable cells
100
50
0
0.01
0.1
1
10
100
Antibody ug/ml
-FAP
+FAP
Thiol MMAE CHO+FAP
Glyco MMAE CHO+FAP
Thiol MMAE CHO-FAP
Glyco MMAE CHO-FAP

Figure 38

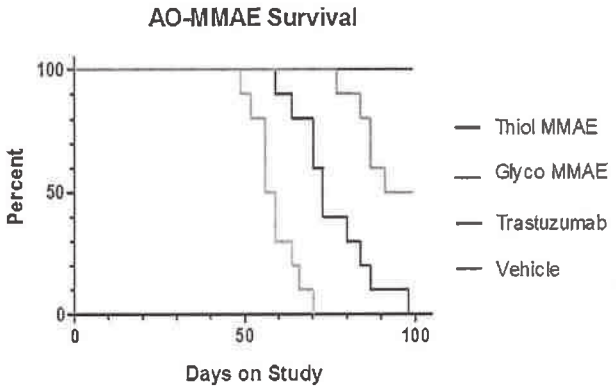
A.



C.



B.



D.

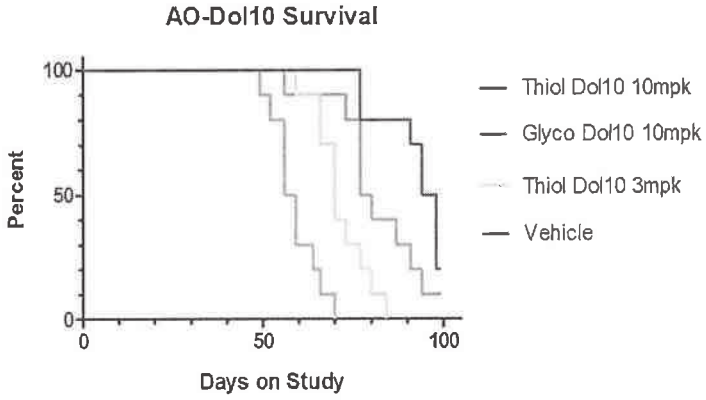


Figure 39

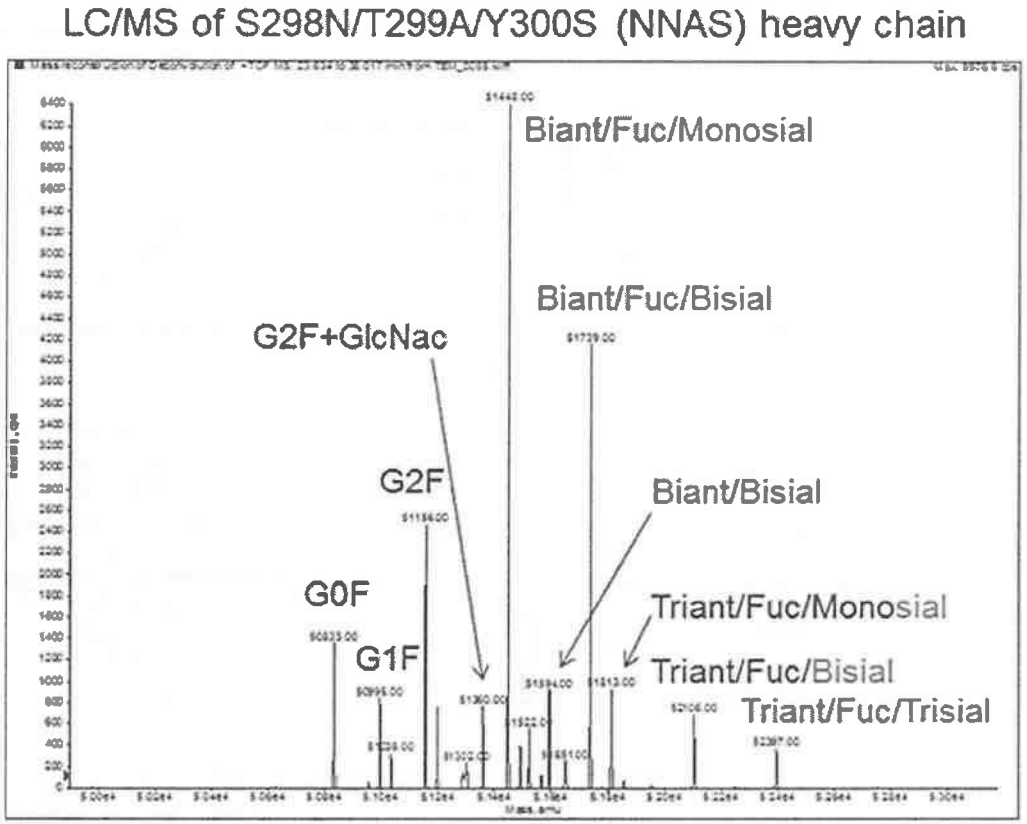

LC/MS of S298N/T299A/Y300S (NNAS) heavy chain
Biant/Fuc/Monosial
Biant/Fuc/Bisial
G2F+GlcNac
G2F
Biant/Bisial
G0F
Triant/Fuc/Monosial
G1F
Triant/Fuc/Bisial
Triant/Fuc/Trisial

Figure 40

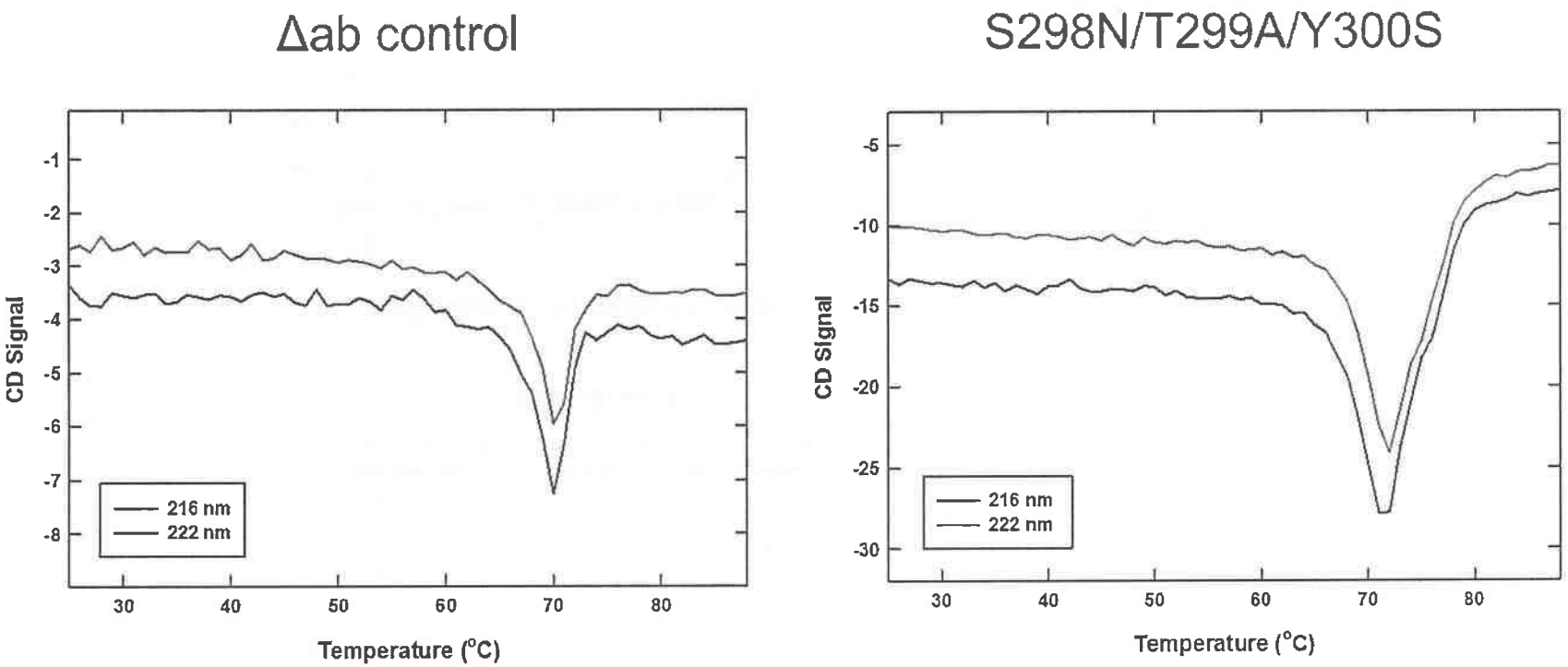

Δab control
S298N/T299A/Y300S
CD Signal
Temperature (°C)
216 nm
222 nm
-1
-2
-3
-4
-5
-6
-7
-8
-5
-10
-15
-20
-25
-30
30
40
50
60
70
80

Figure 41

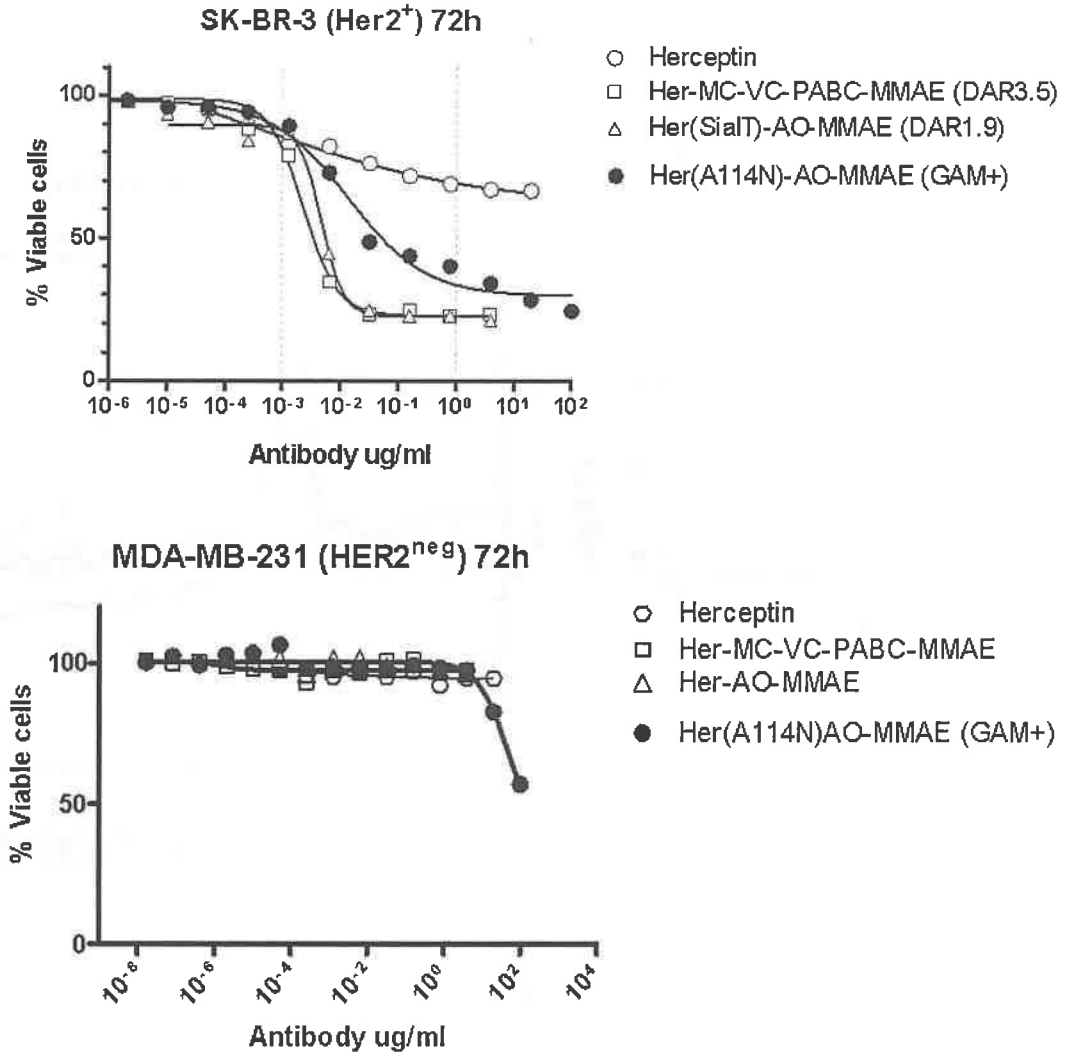

SK-BR-3 (Her2+) 72h
% Viable cells
100
50
0
10-6 10-5 10-4 10-3 10-2 10-1 100 101 102
Antibody ug/ml
Herceptin
Her-MC-VC-PABC-MMAE (DAR3.5)
Her(SialT)-AO-MMAE (DAR1.9)
Her(A114N)-AO-MMAE (GAM+)
MDA-MB-231 (HER2neg) 72h
% Viable cells
100
50
0
10-8 10-6 10-4 10-2 100 102 104
Antibody ug/ml
Herceptin
Her-MC-VC-PABC-MMAE
Her-AO-MMAE
Her(A114N)AO-MMAE (GAM+)

Figure 42

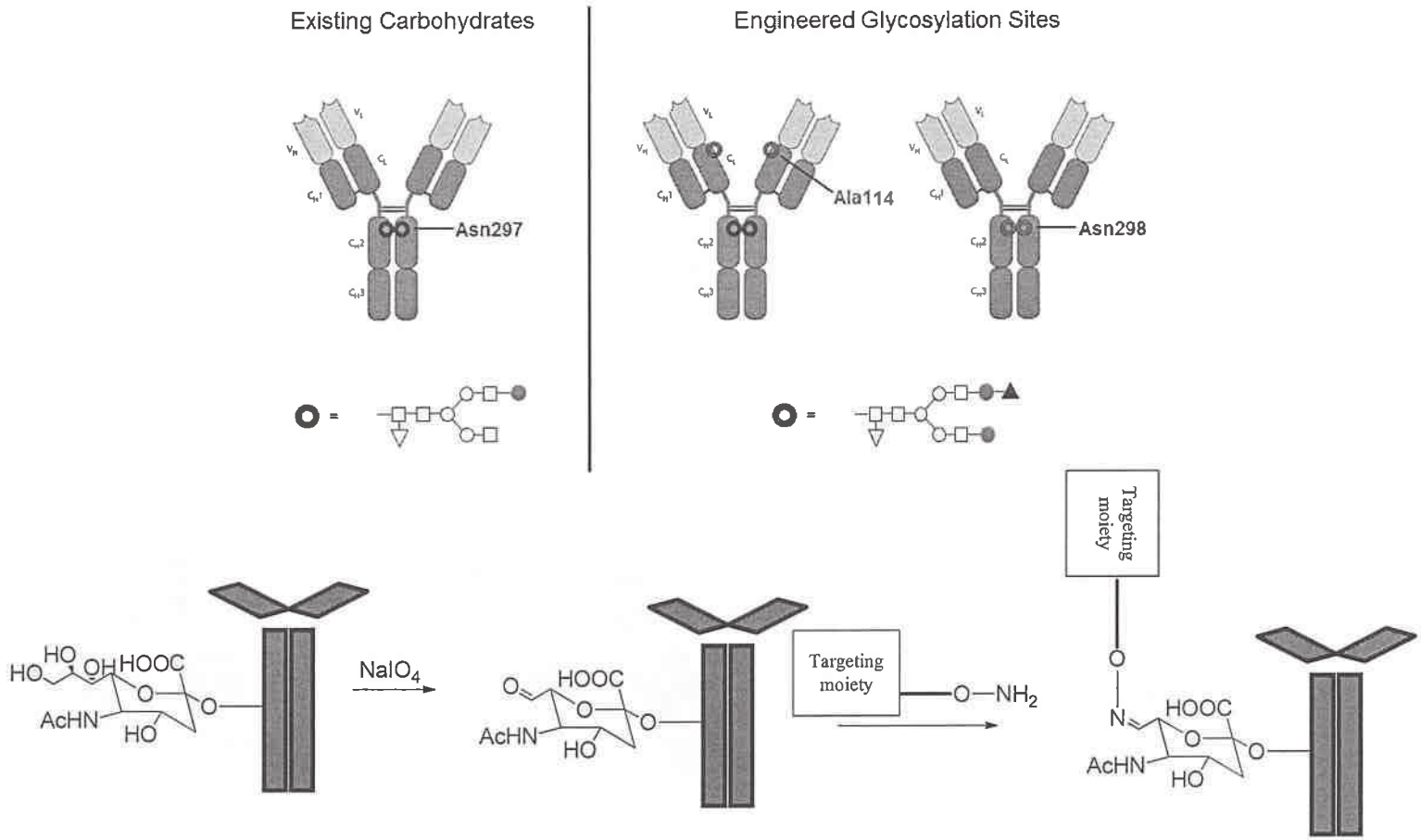
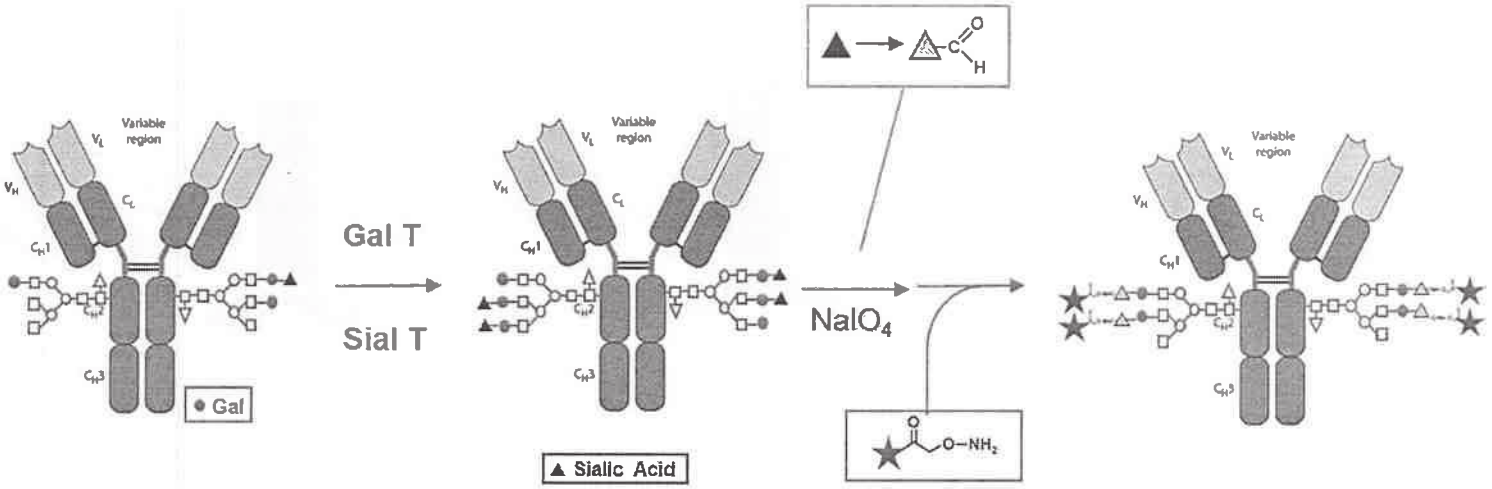

Existing Carbohydrates
Engineered Glycosylation Sites
Asn297
Ala114
Asn298
NaIO4
Targeting moiety
Targeting moiety
O—NH2
HOOC
AcHN
HO
OH

Figure 43



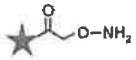
Glycopeptide aminooxy: 

Figure 44

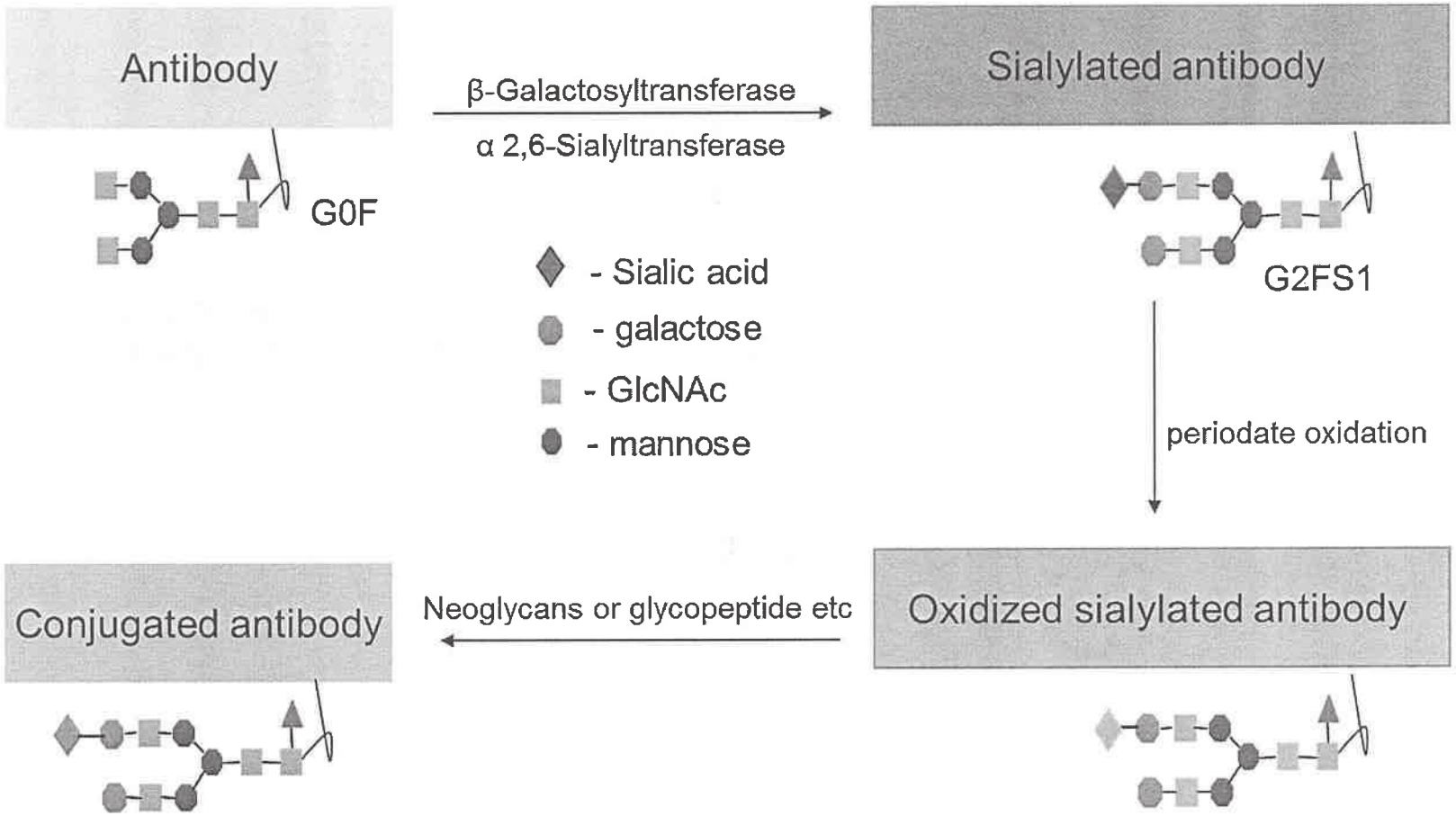
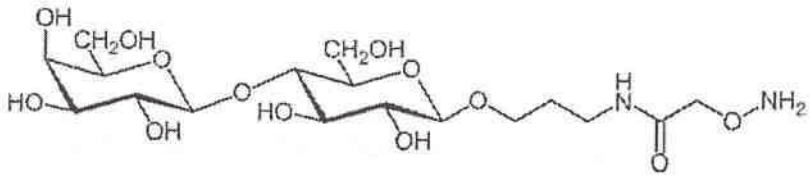

Antibody
G0F
β-Galactosyltransferase
α 2,6-Sialyltransferase
Sialylated antibody
G2FS1
- Sialic acid
- galactose
- GlcNAc
- mannose
periodate oxidation
Conjugated antibody
Neoglycans or glycopeptide etc
Oxidized sialylated antibody
G2FS1+ neoglycans
G2Foxidized S1

Figure 45

Lactose aminooxy for native glycans (SAM or GAM):



Exact Mass: 472.19
Molecular Weight: 472.44

bisM6P hexamannose aminooxy (SAM or GAM):

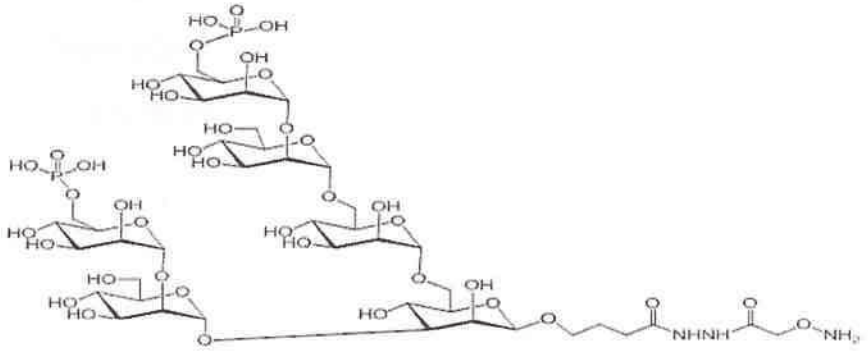
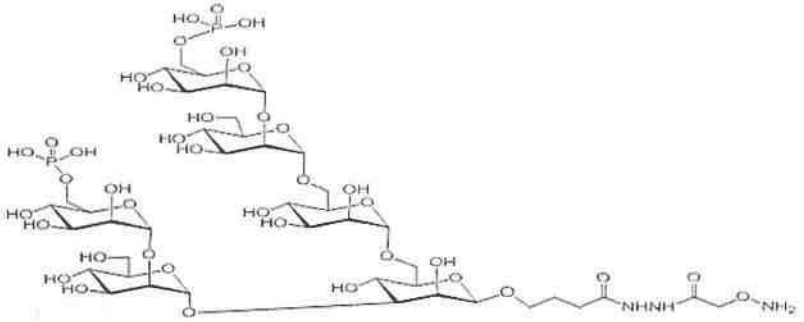
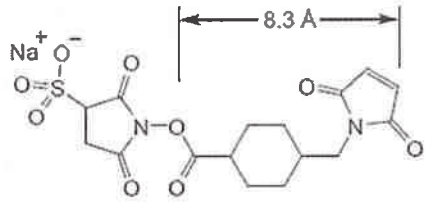


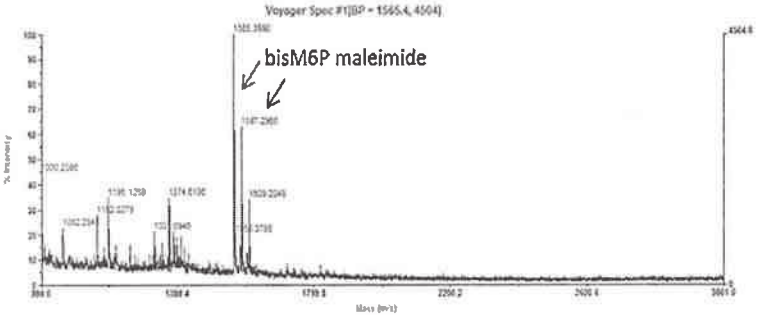
Figure 46

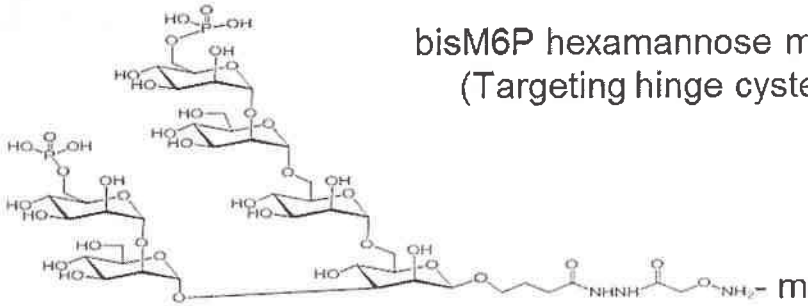
bisM6P hexamannose aminooxy
(SAM on Fc glycans):





Sulfo SMCC





bisM6P hexamannose maleimide
(Targeting hinge cysteines):

- maleimide

Figure 47

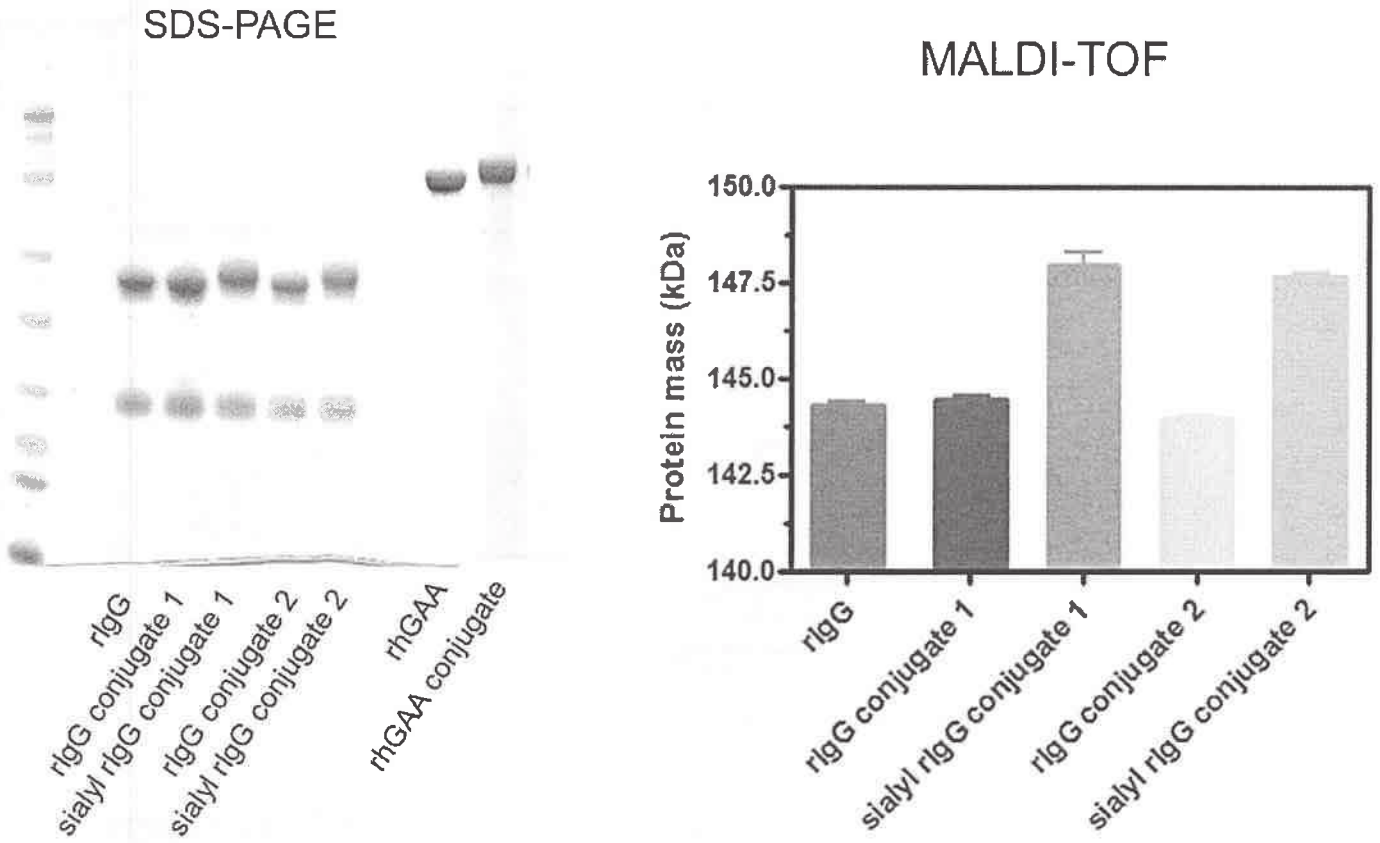

SDS-PAGE
rIgG
rIgG conjugate 1
sialyl rIgG conjugate 1
rIgG conjugate 2
sialyl rIgG conjugate 2
rhGAA
rhGAA conjugate
MALDI-TOF
Protein mass (kDa)
150.0
147.5
145.0
142.5
140.0
rIgG
rIgG conjugate 1
sialyl rIgG conjugate 1
rIgG conjugate 2
sialyl rIgG conjugate 2

Figure 48

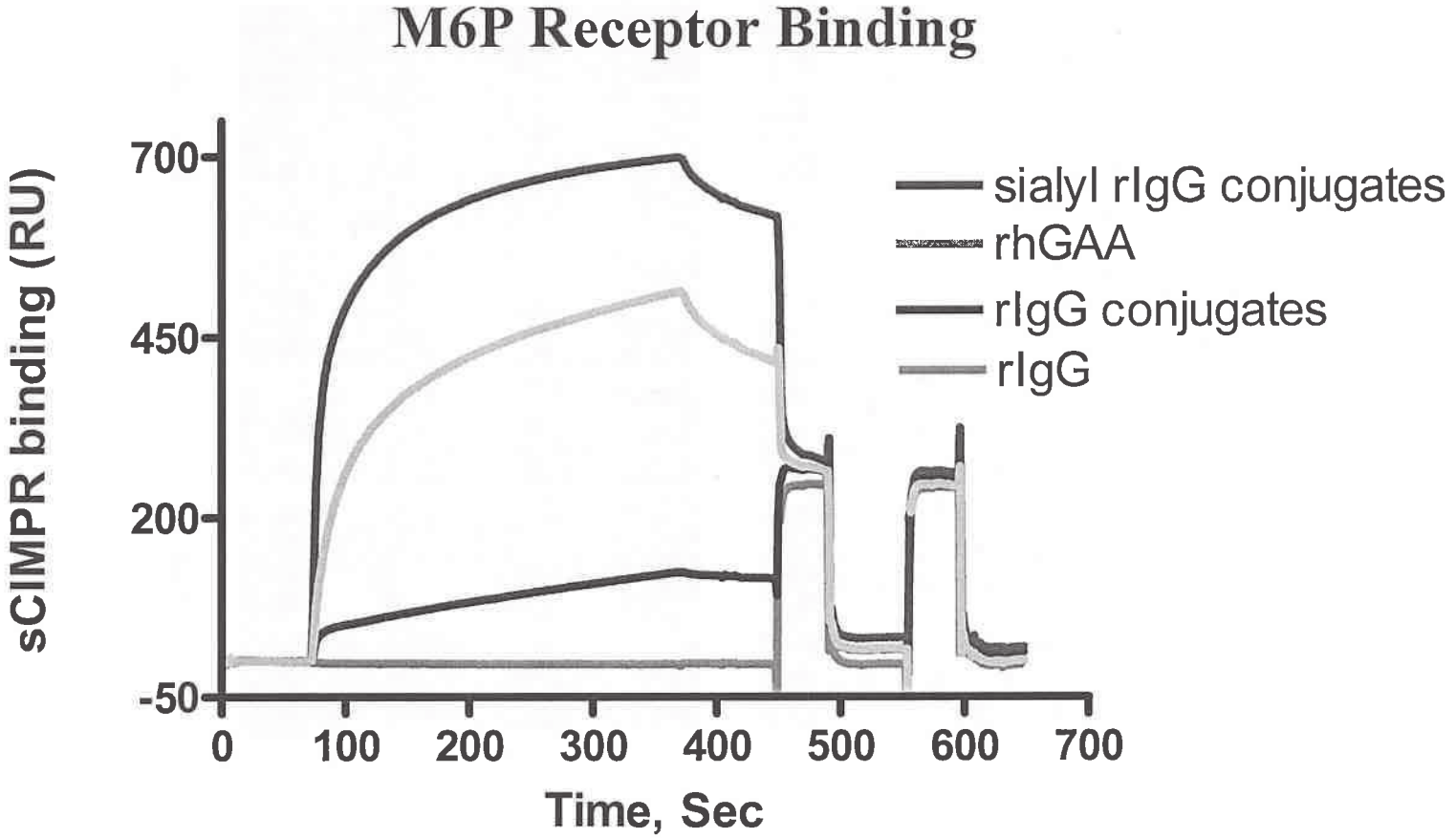

M6P Receptor Binding
sCIMPR binding (RU)
700
450
200
-50
0
100
200
300
400
500
600
700
Time, Sec
sialyl rIgG conjugates
rhGAA
rIgG conjugates
rIgG

Figure 49

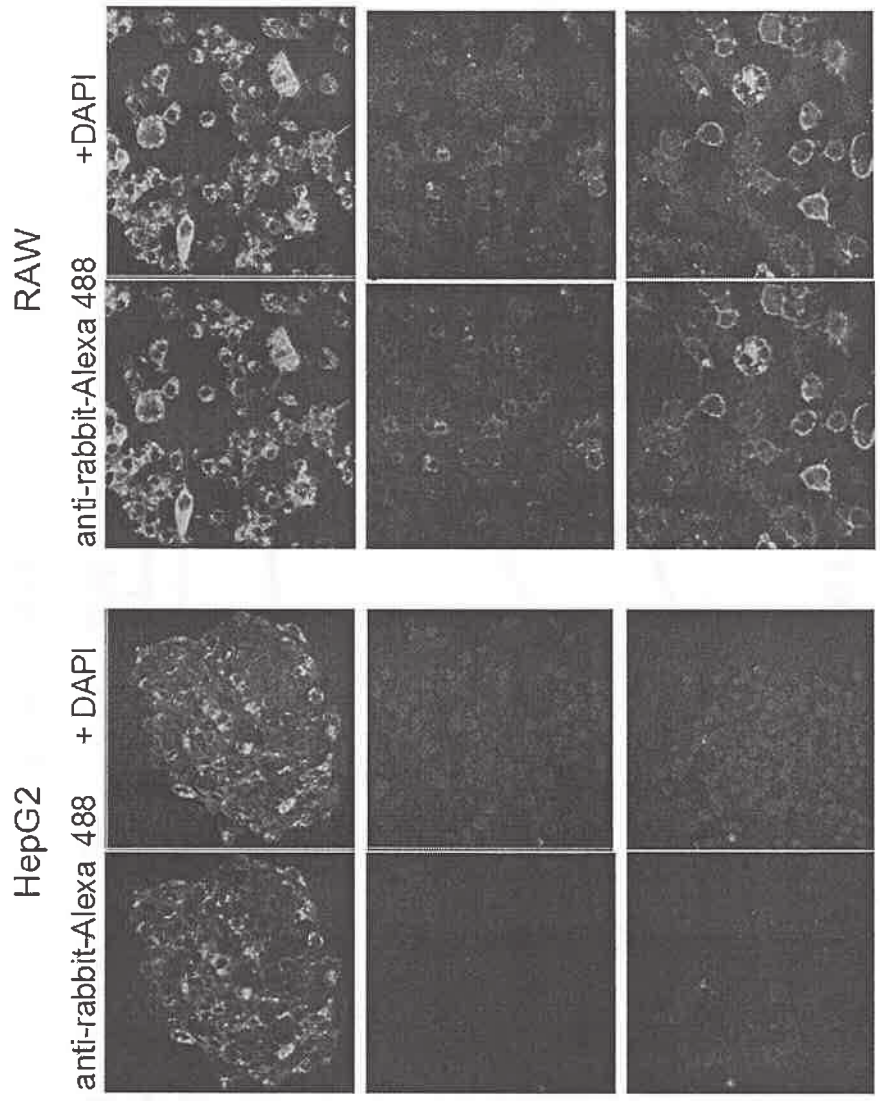

HepG2
anti-rabbit-Alexa 488
+ DAPI
RAW
anti-rabbit-Alexa 488
+DAPI
M6P-IgG
Conj Ctrl
Native IgG

Figure 50

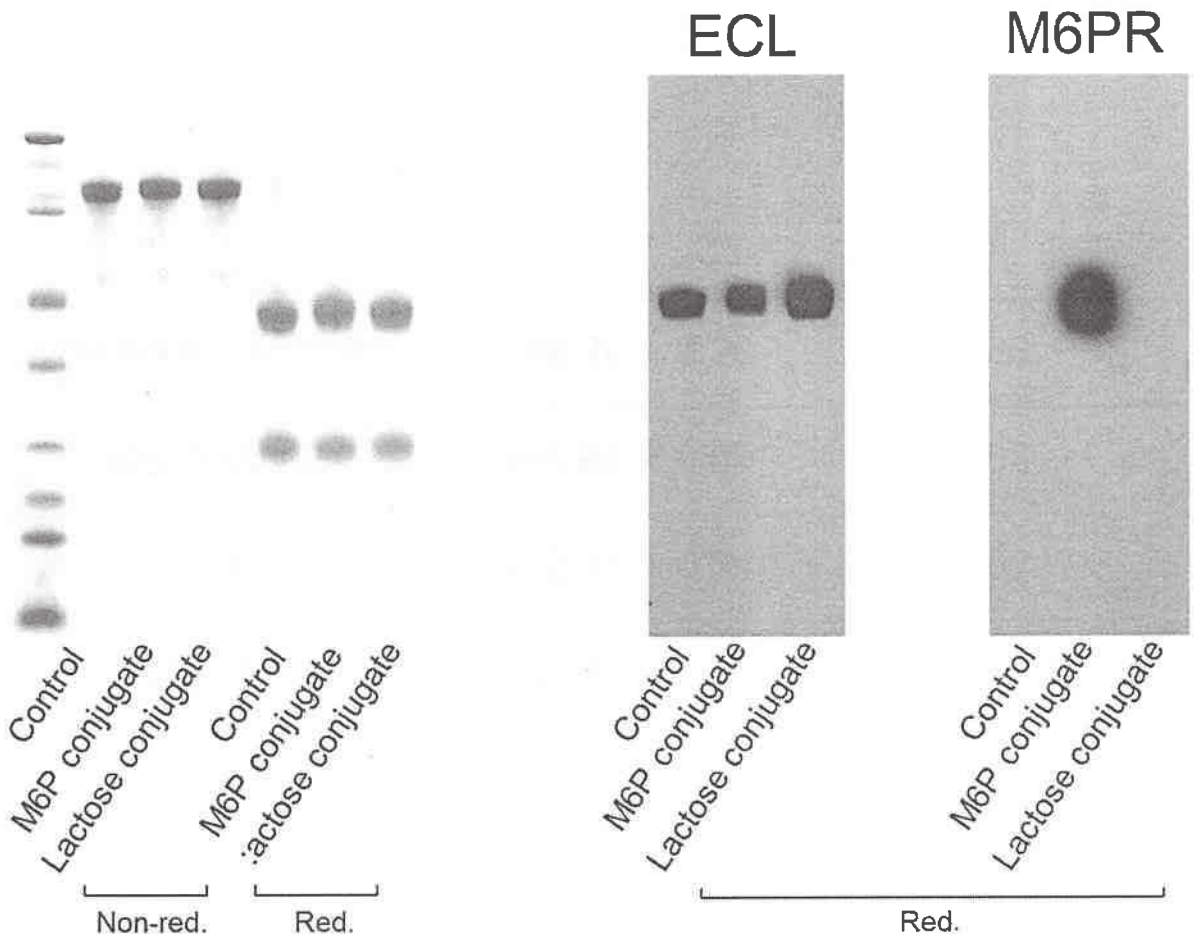

ECL
M6PR
Control
M6P conjugate
Lactose conjugate
Control
M6P conjugate
:actose conjugate
Non-red.
Red.
Control
M6P conjugate
Lactose conjugate
Control
M6P conjugate
Lactose conjugate
Red.

Figure 51

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

Figure 52

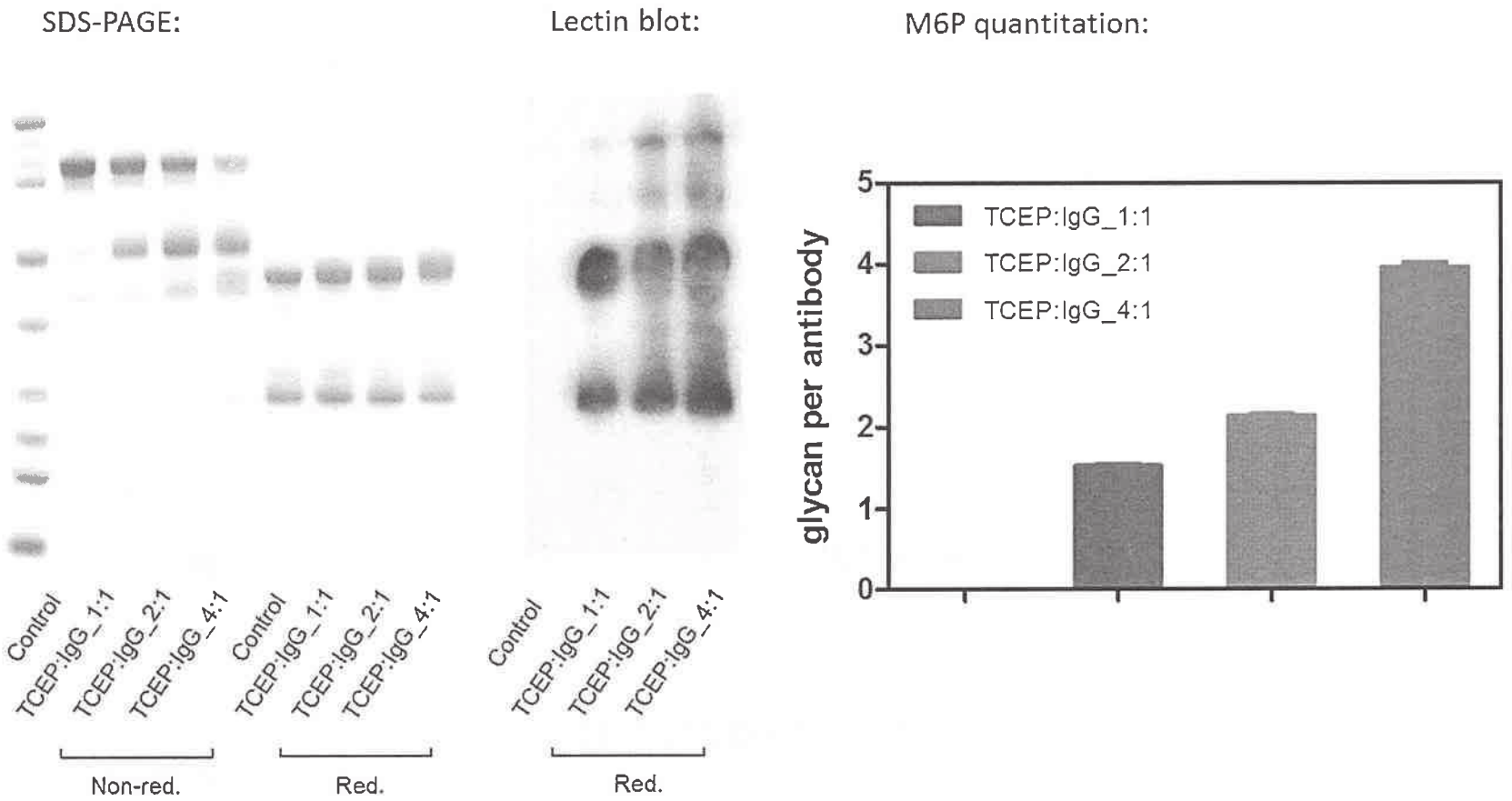

SDS-PAGE:
Lectin blot:
M6P quantitation:
Control
TCEP:IgG_1:1
TCEP:IgG_2:1
TCEP:IgG_4:1
Non-red.
Red.
Red.
glycan per antibody
0
1
2
3
4
5
TCEP:IgG_1:1
TCEP:IgG_2:1
TCEP:IgG_4:1

Figure 53

SDS-PAGE:

Galactose quantitation:

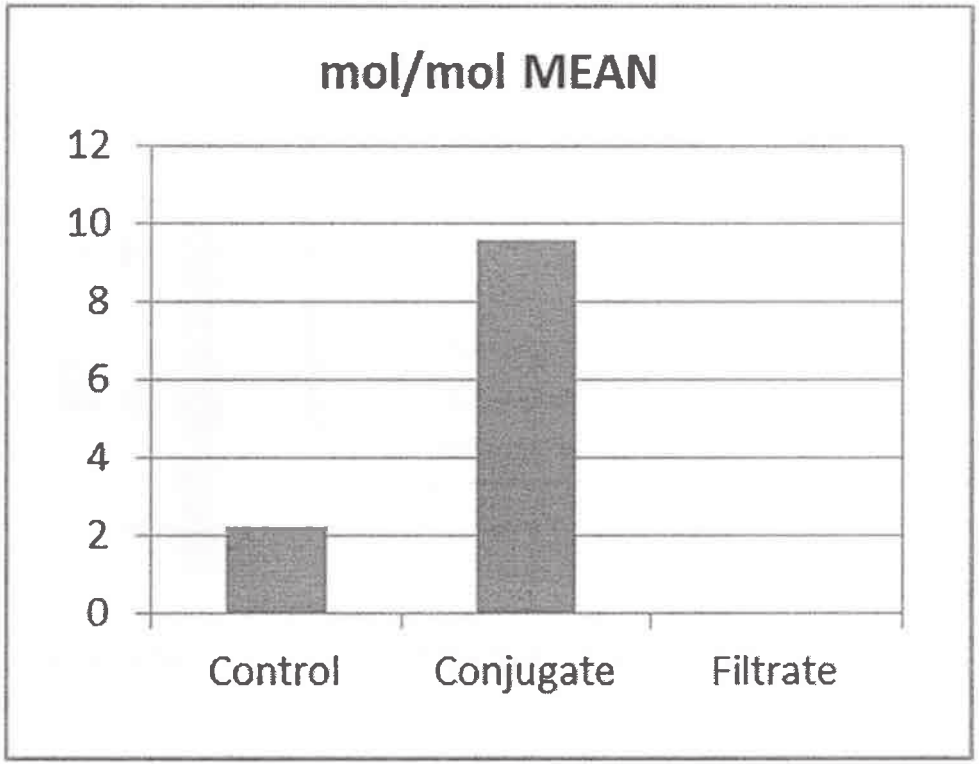
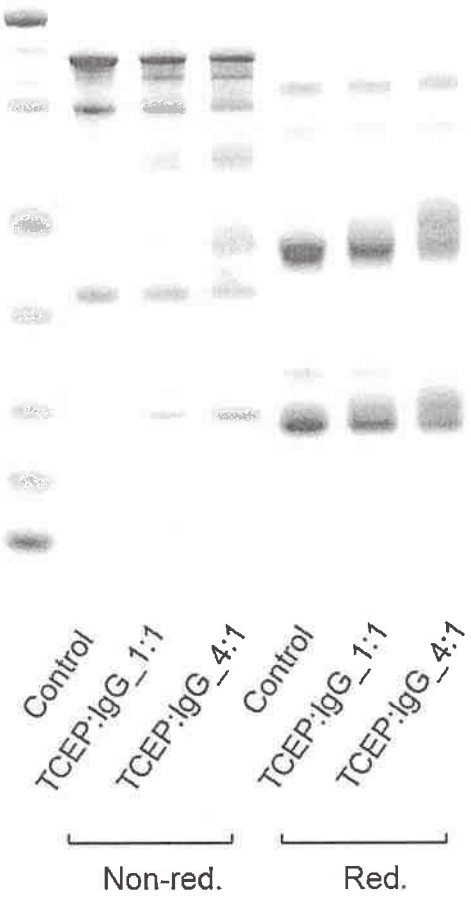


Figure 54

SDS-PAGE:



Glycan quantitation:

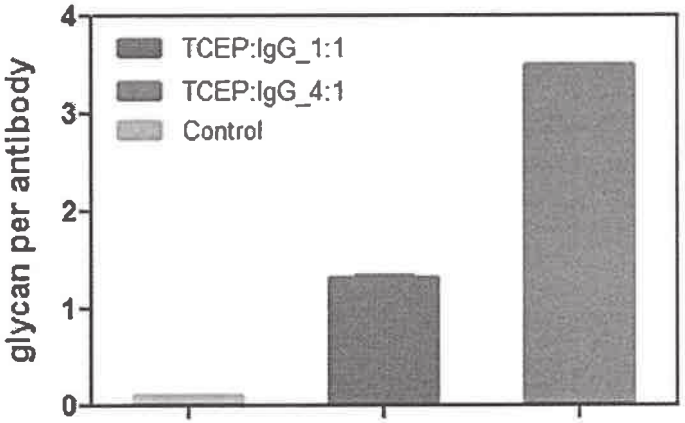
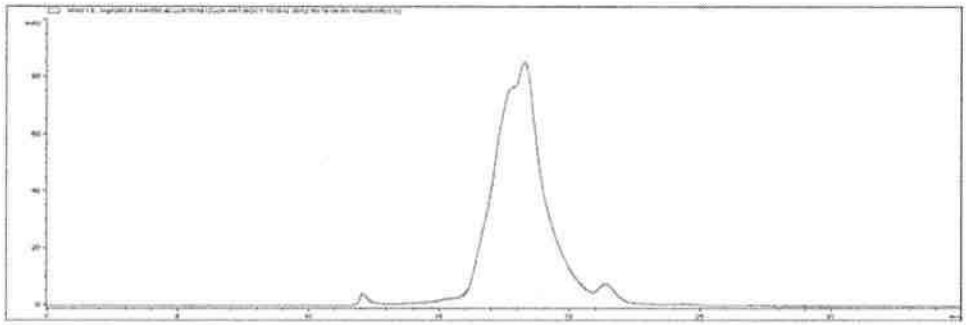


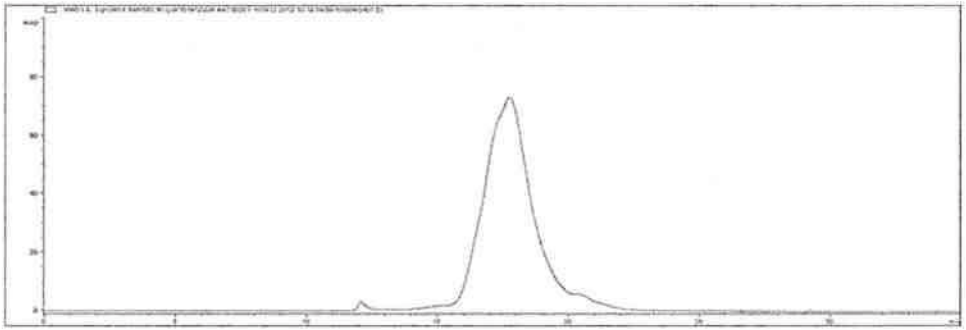
Figure 55

Control:



Major peak retention time: 18.250min
% major antibody peak: 96%

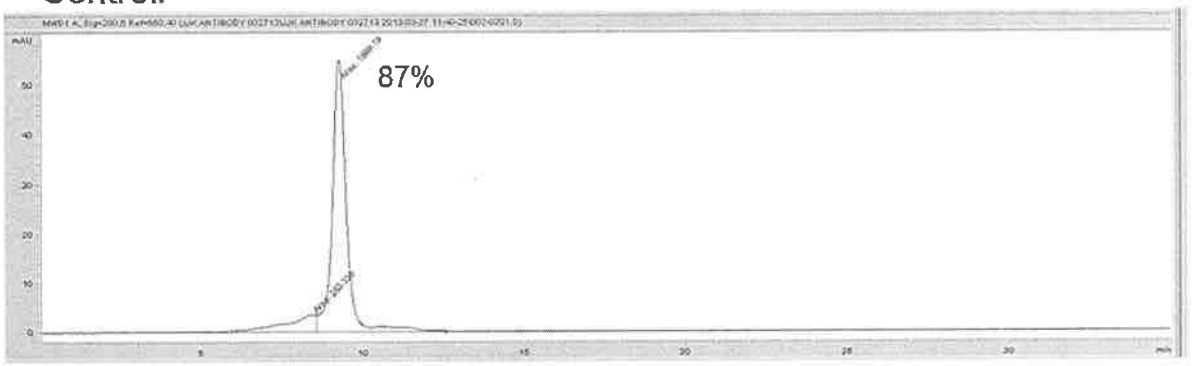
Lactose maleimide conjugate:



Major peak retention time: 17.751min
% major antibody peak: 99%

Figure 56

Control:



M6P conjugate:

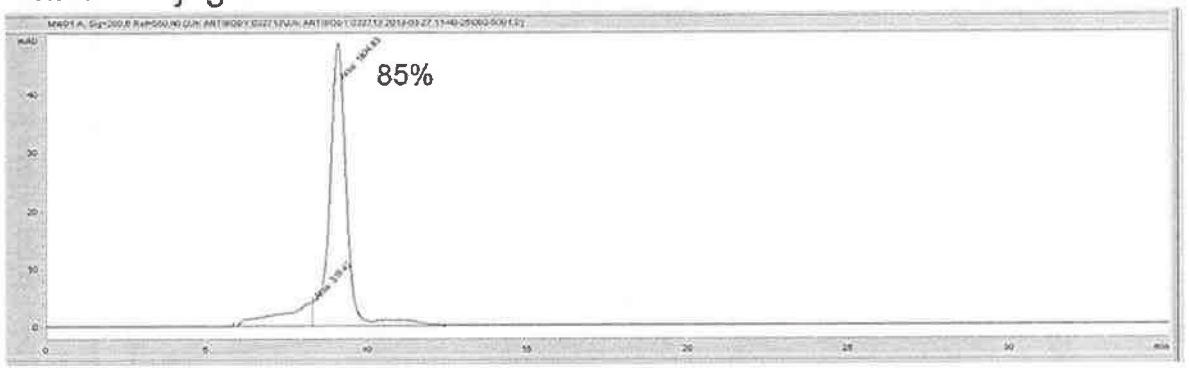
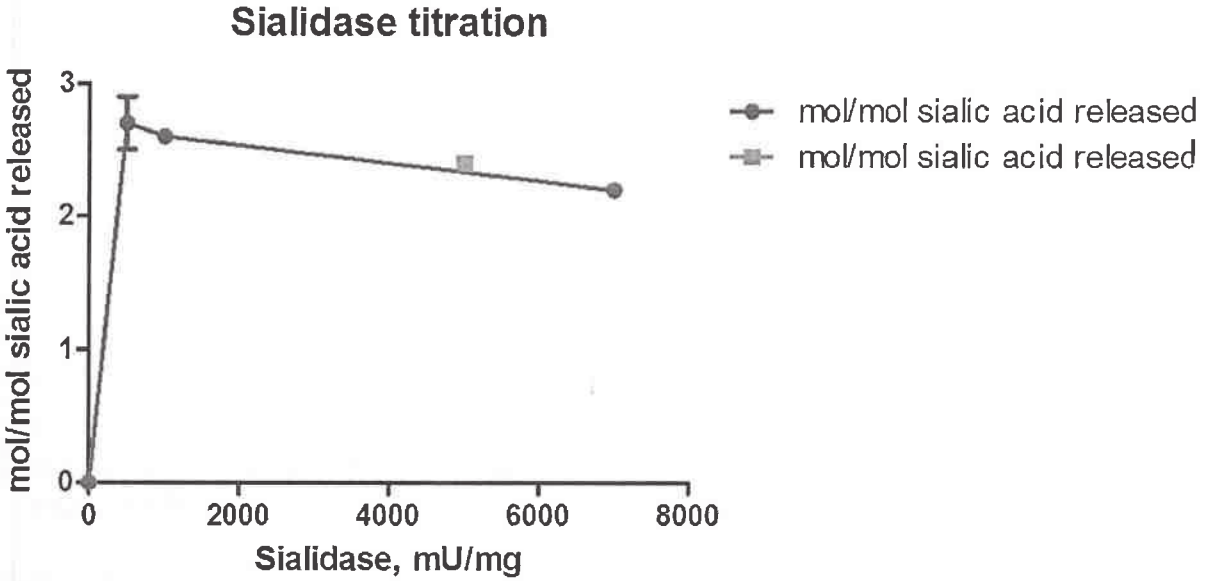


Figure 57



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

Figure 58

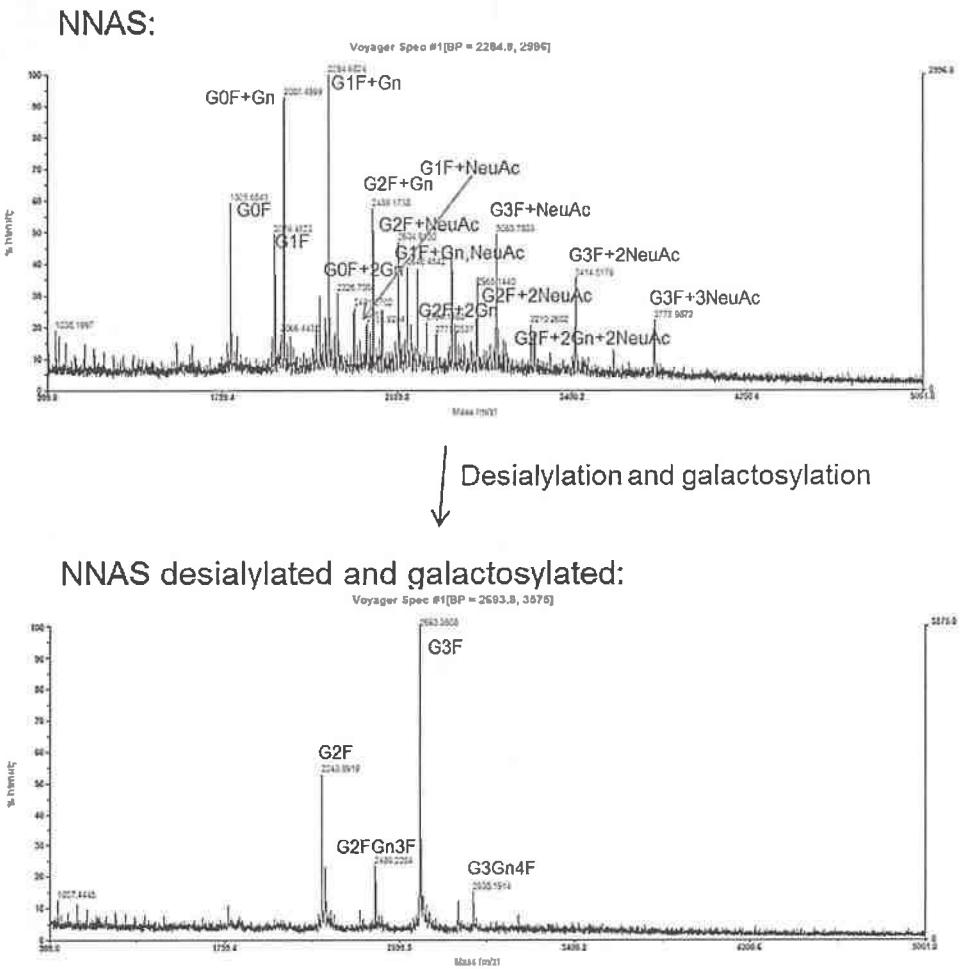

NNAS:
G0F+Gn
G1F+Gn
G1F+NeuAc
G2F+Gn
G0F
G2F+NeuAc
G3F+NeuAc
G1F
G1F+Gn,NeuAc
G3F+2NeuAc
G0F+2Gn
G2F+2NeuAc
G3F+3NeuAc
G2F+2Gn
G3F+2Gn+2NeuAc
Desialylation and galactosylation
NNAS desialylated and galactosylated:
G3F
G2F
G2FGn3F
G3Gn4F

Figure 59

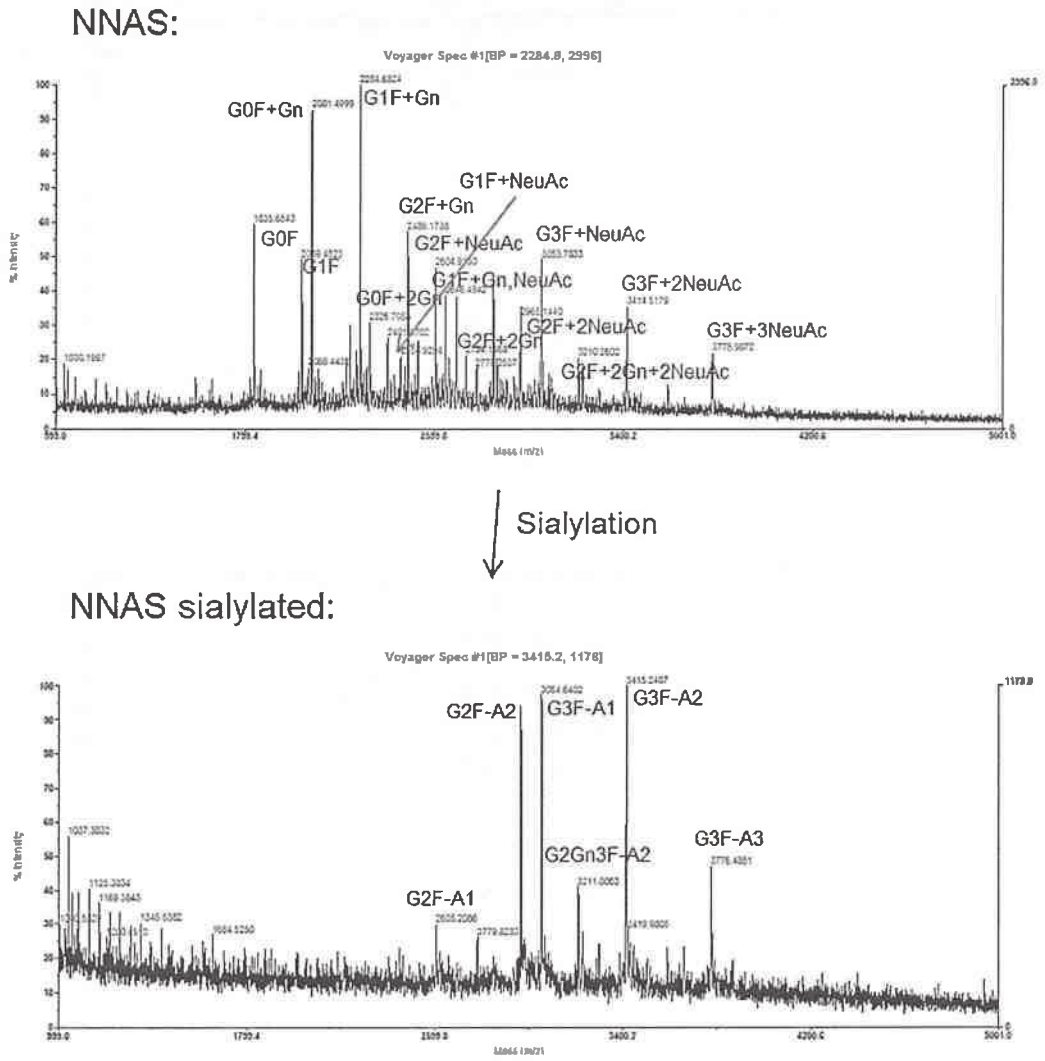
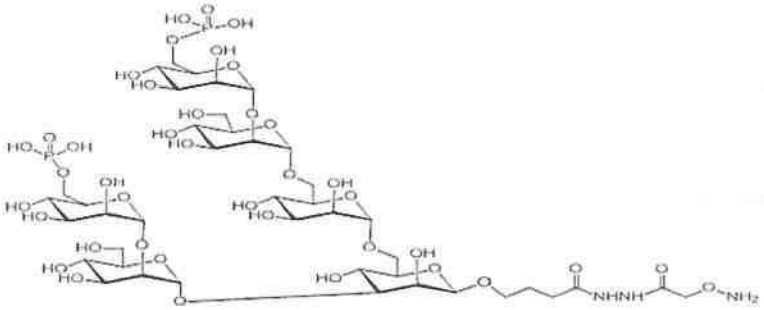
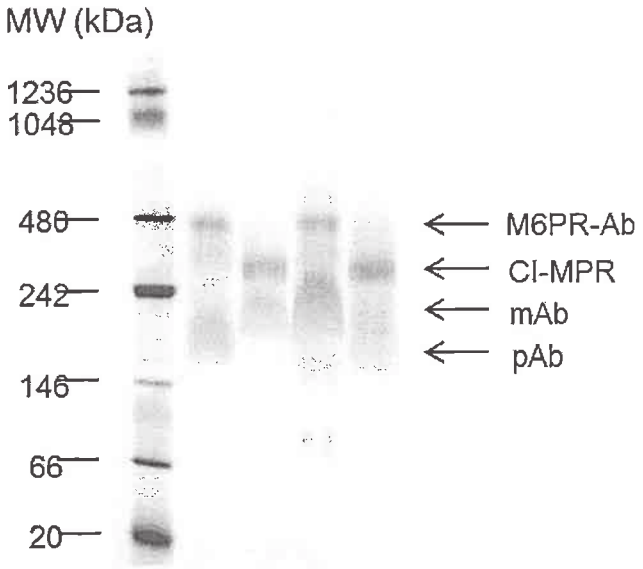

NNAS:
Voyager Spec #1[BP = 2284.8, 2996]
G0F+Gn
G1F+Gn
G1F+NeuAc
G2F+Gn
G0F
G2F+NeuAc
G3F+NeuAc
G1F
G1F+Gn,NeuAc
G3F+2NeuAc
G0F+2Gn
G2F+2NeuAc
G3F+3NeuAc
G2F+2Gn
G2F+2Gn+2NeuAc
Sialylation
NNAS sialylated:
Voyager Spec #1[BP = 3418.2, 1178]
G2F-A2
G3F-A1
G3F-A2
G3F-A3
G2Gn3F-A2
G2F-A1

Figure 60

bisM6P hexasaccharide aminooxy (SAM):





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

Figure 61

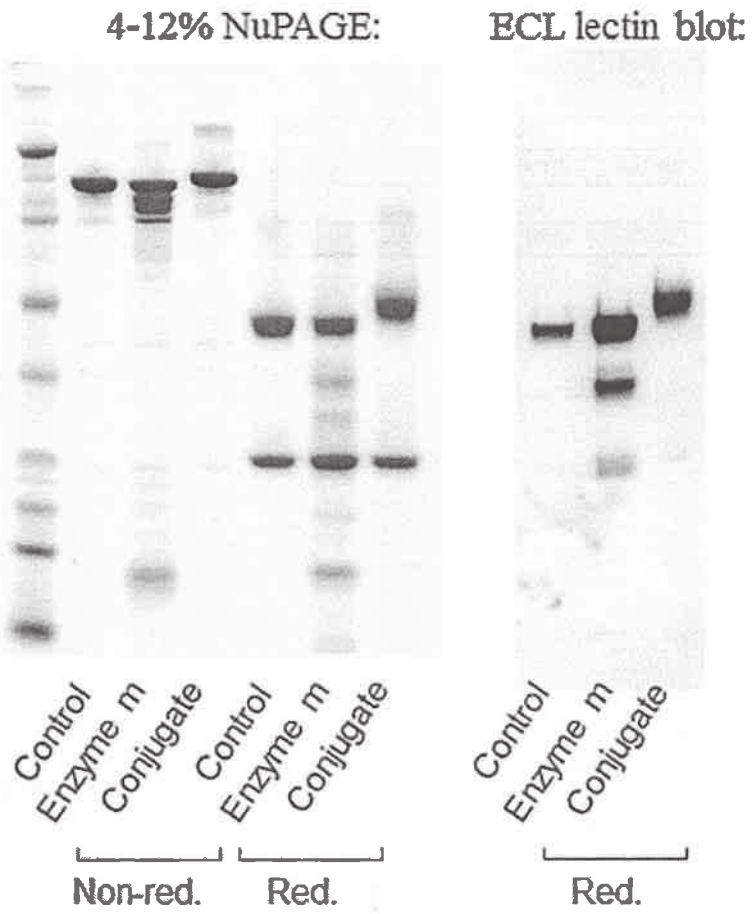
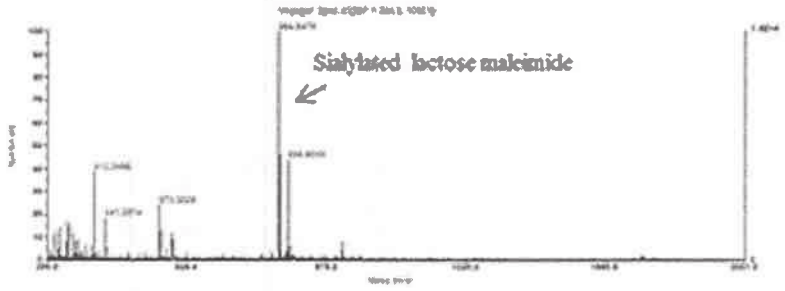

4-12% NuPAGE:
ECL lectin blot:
Control
Enzyme m
Conjugate
Control
Enzyme m
Conjugate
Control
Enzyme m
Conjugate
Non-red.
Red.
Red.

Figure 62

	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

Figure 63

MALDI-TOF:



Dionex HPLC:

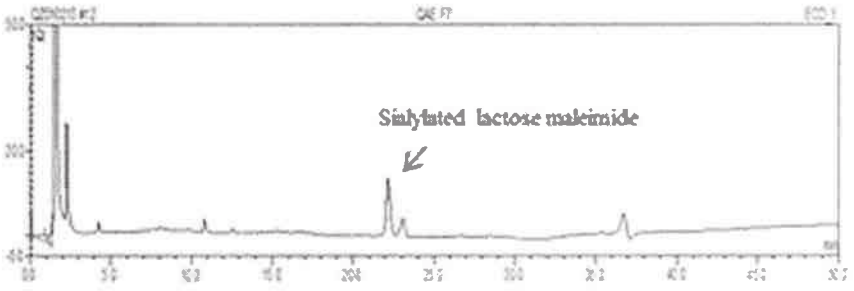


Figure 64

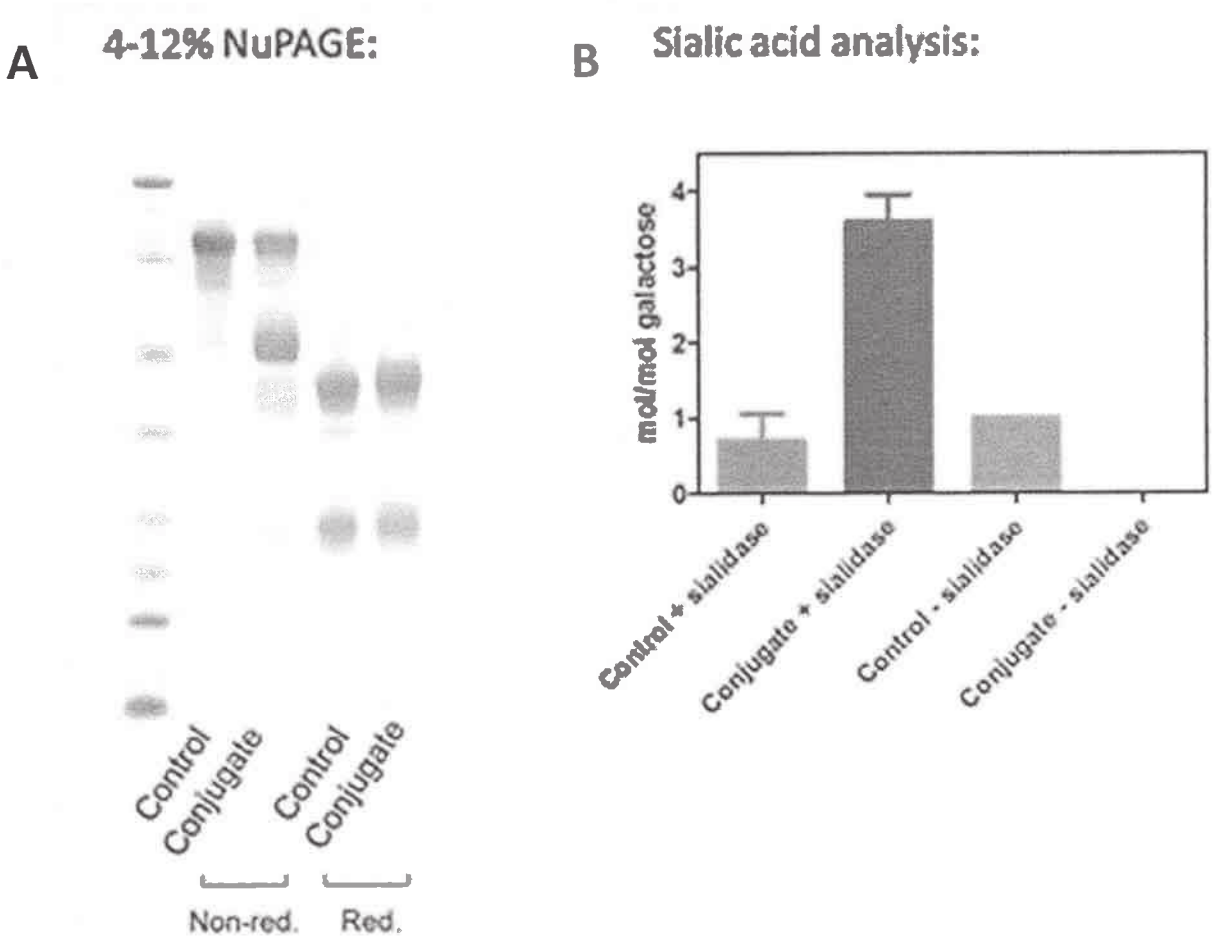

A
4-12% NuPAGE:
Control
Conjugate
Control
Conjugate
Non-red.
Red.
B
Sialic acid analysis:
mol/mol galactose
0
1
2
3
4
Control + sialidase
Conjugate + sialidase
Control - sialidase
Conjugate - sialidase

Figure 65

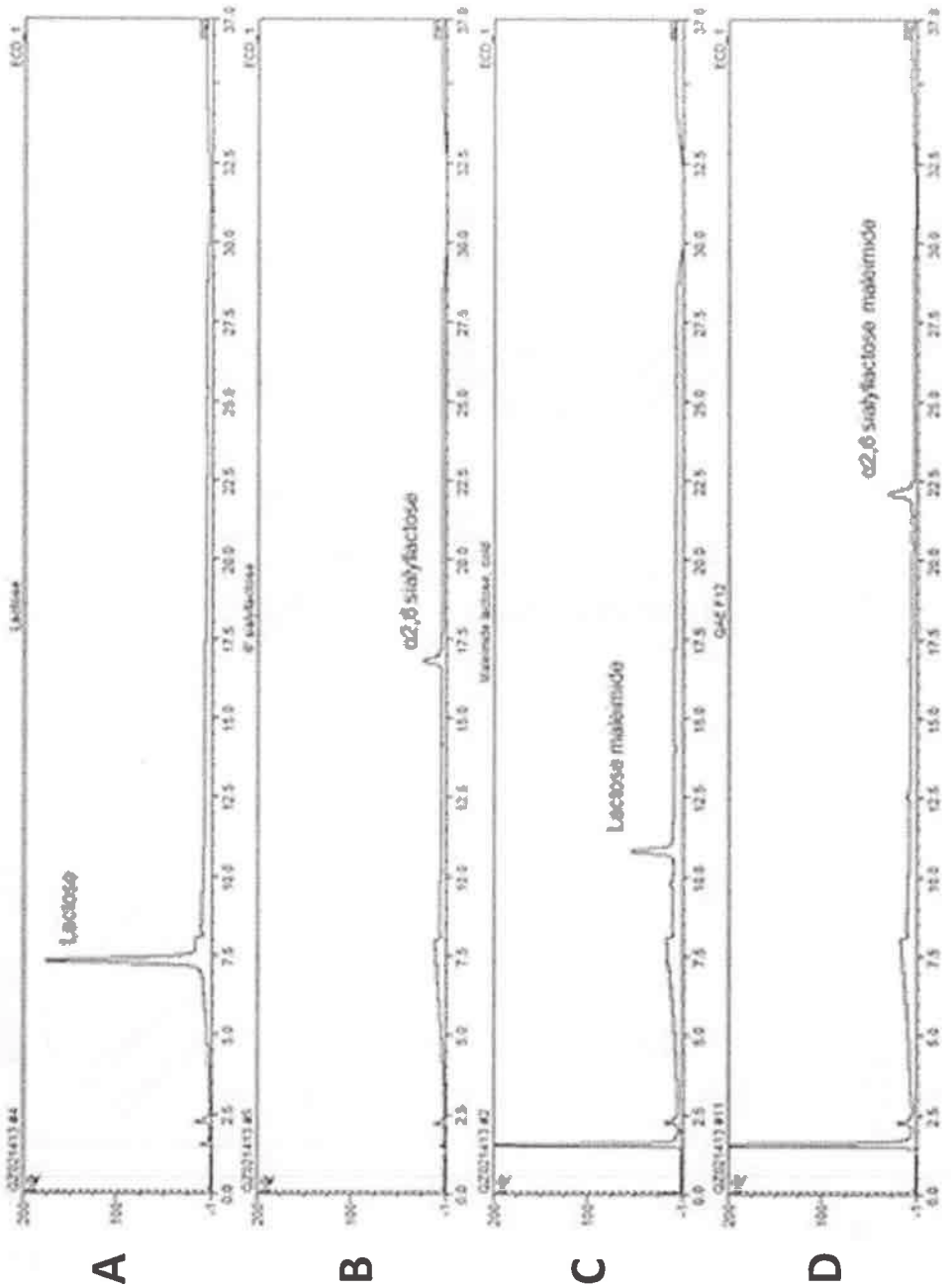

A
Lactose
B
α2,6 sialyllactose
C
Lactose maleimide
D
α2,6 sialyllactose maleimide

Figure 66

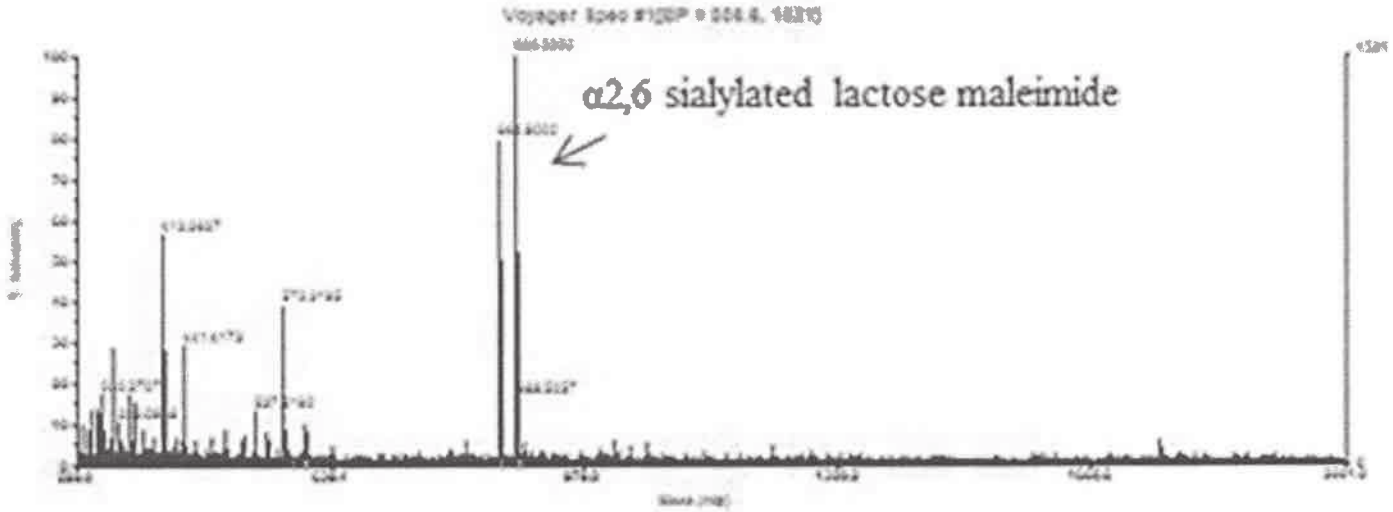


Figure 67

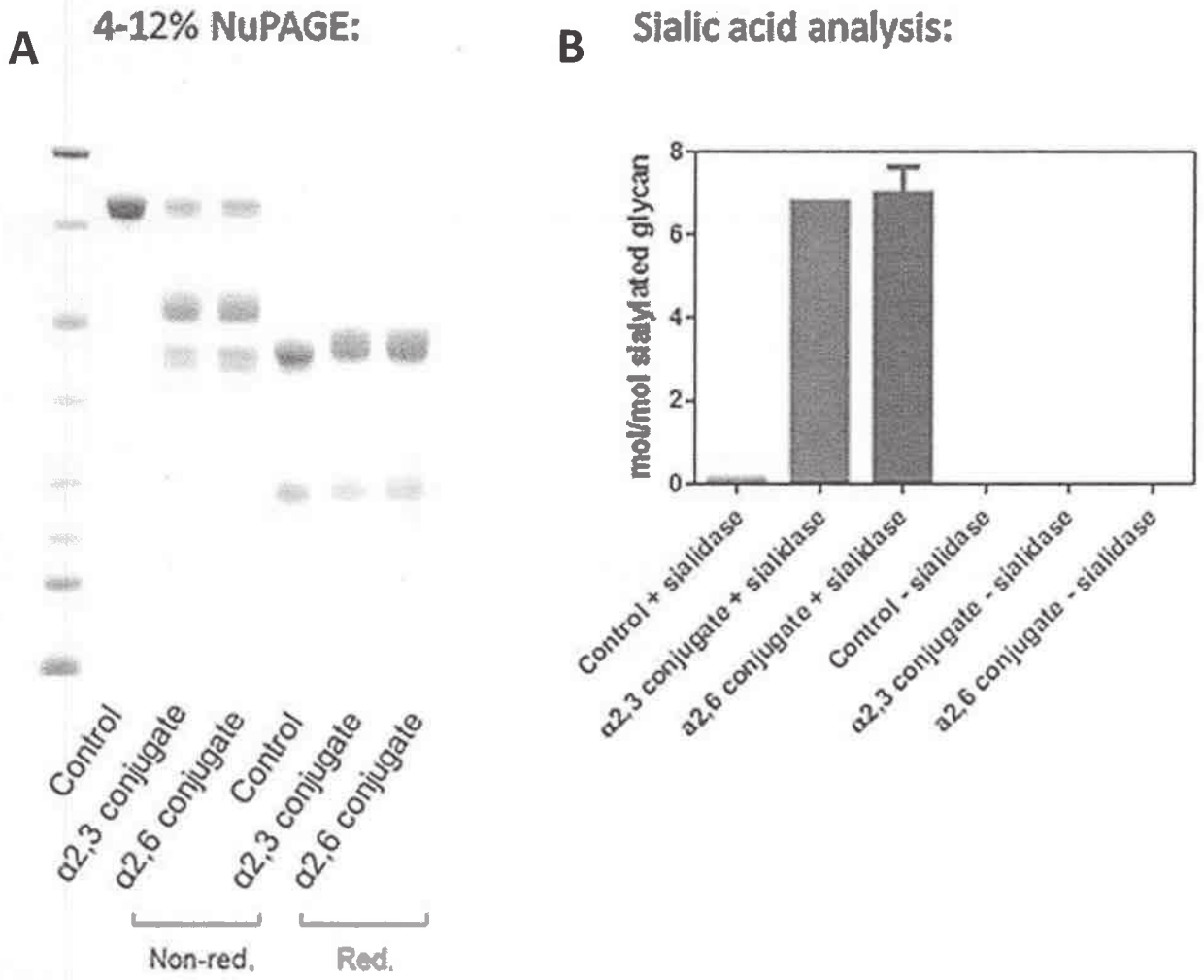

A
4-12% NuPAGE:
Control
α2,3 conjugate
α2,6 conjugate
Control
α2,3 conjugate
α2,6 conjugate
Non-red.
Red.
B
Sialic acid analysis:
mol/mol sialylated glycan
0
2
4
6
8
Control + sialidase
α2,3 conjugate + sialidase
α2,6 conjugate + sialidase
Control - sialidase
α2,3 conjugate - sialidase
α2,6 conjugate - sialidase

Figure 68

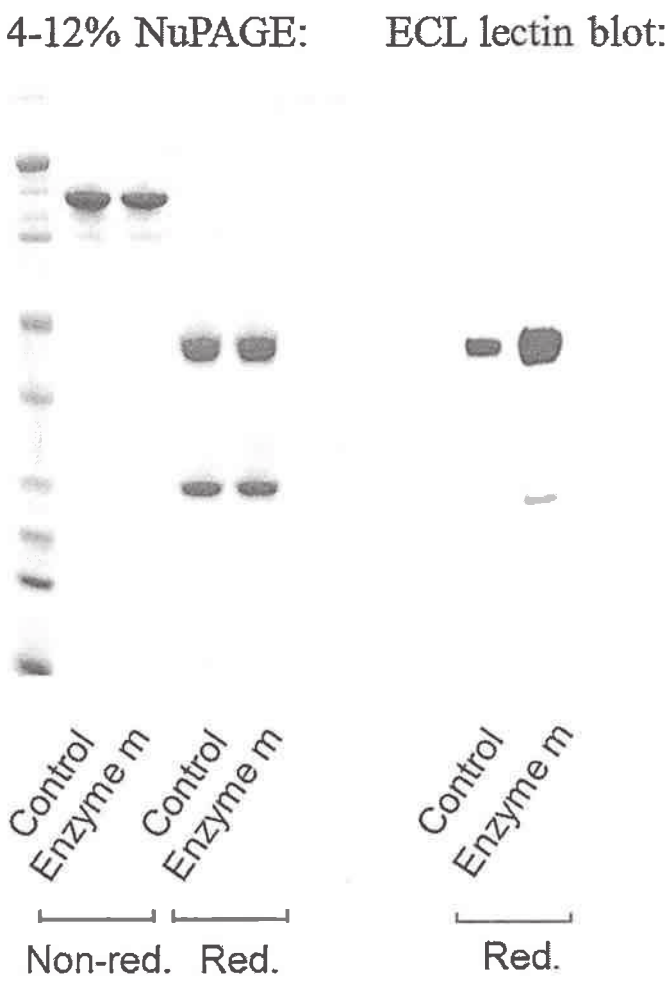

4-12% NuPAGE:
ECL lectin blot:
Control
Enzyme m
Control
Enzyme m
Control
Enzyme m
Non-red.
Red.
Red.

Figure 69

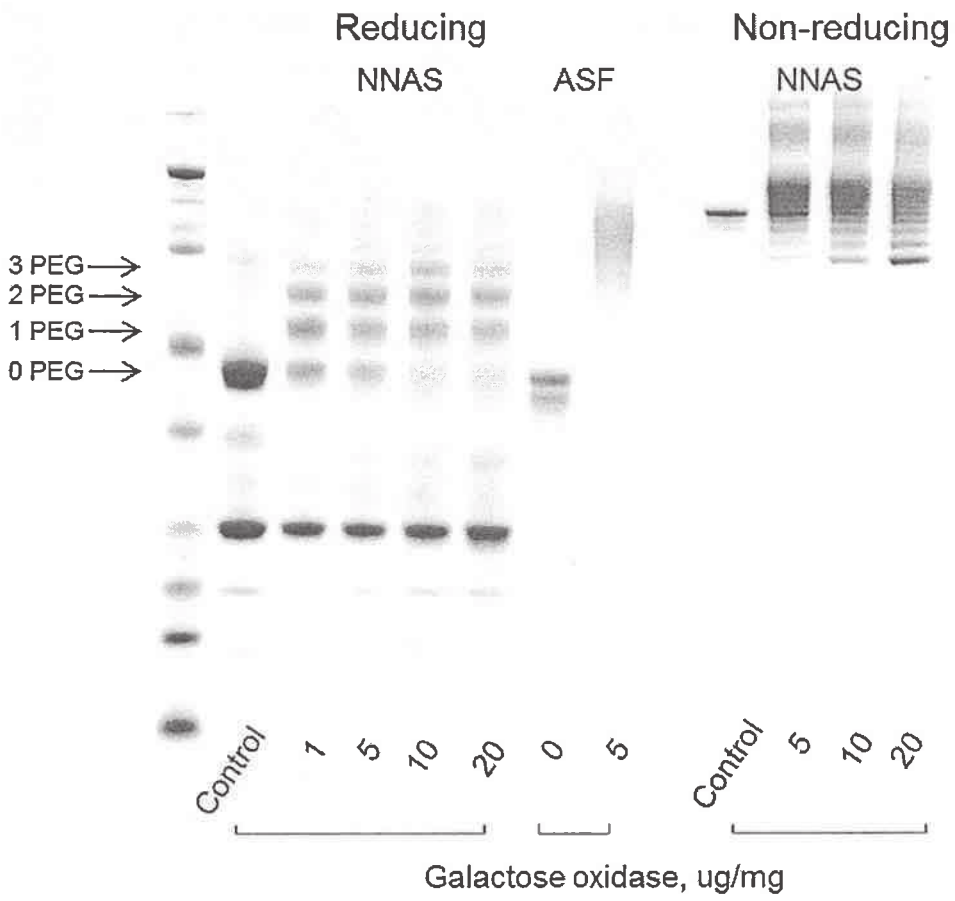

Reducing
Non-reducing
NNAS
ASF
NNAS
3 PEG
2 PEG
1 PEG
0 PEG
Control
1
5
10
20
0
5
Control
5
10
20
Galactose oxidase, ug/mg

Figure 70

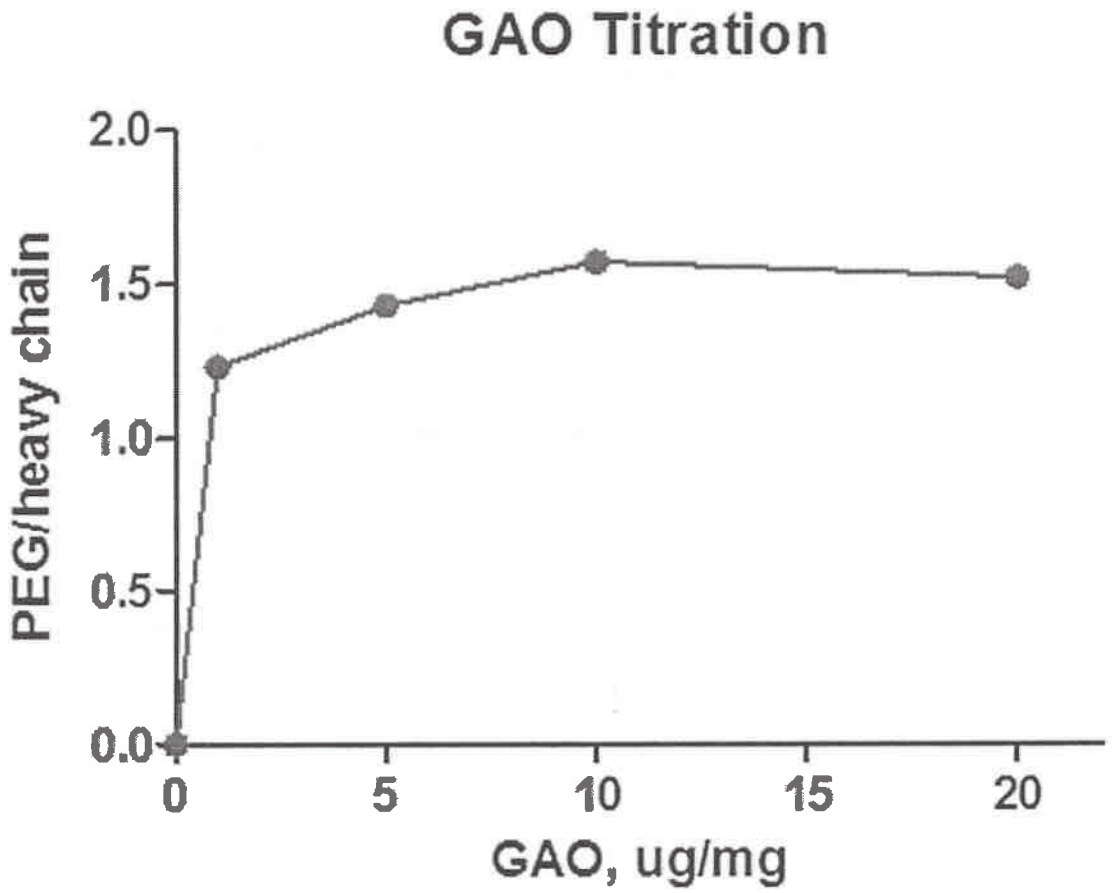

GAO Titration
PEG/heavy chain
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
GAO, ug/mg

Figure 71

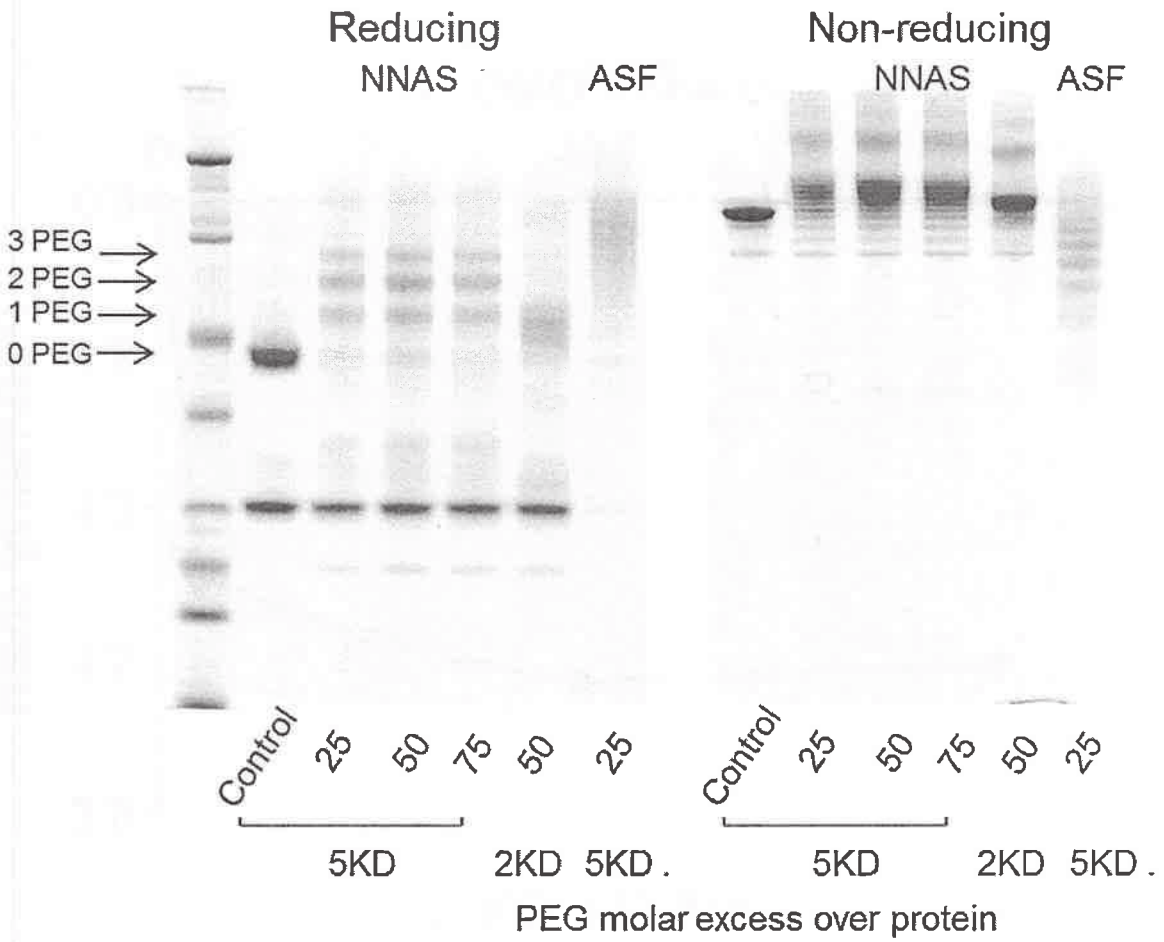

Reducing
NNAS
ASF
Non-reducing
NNAS
ASF
3 PEG
2 PEG
1 PEG
0 PEG
Control
25
50
75
50
25
Control
25
50
75
50
25
5KD
2KD
5KD .
5KD
2KD
5KD .
PEG molar excess over protein

Figure 72

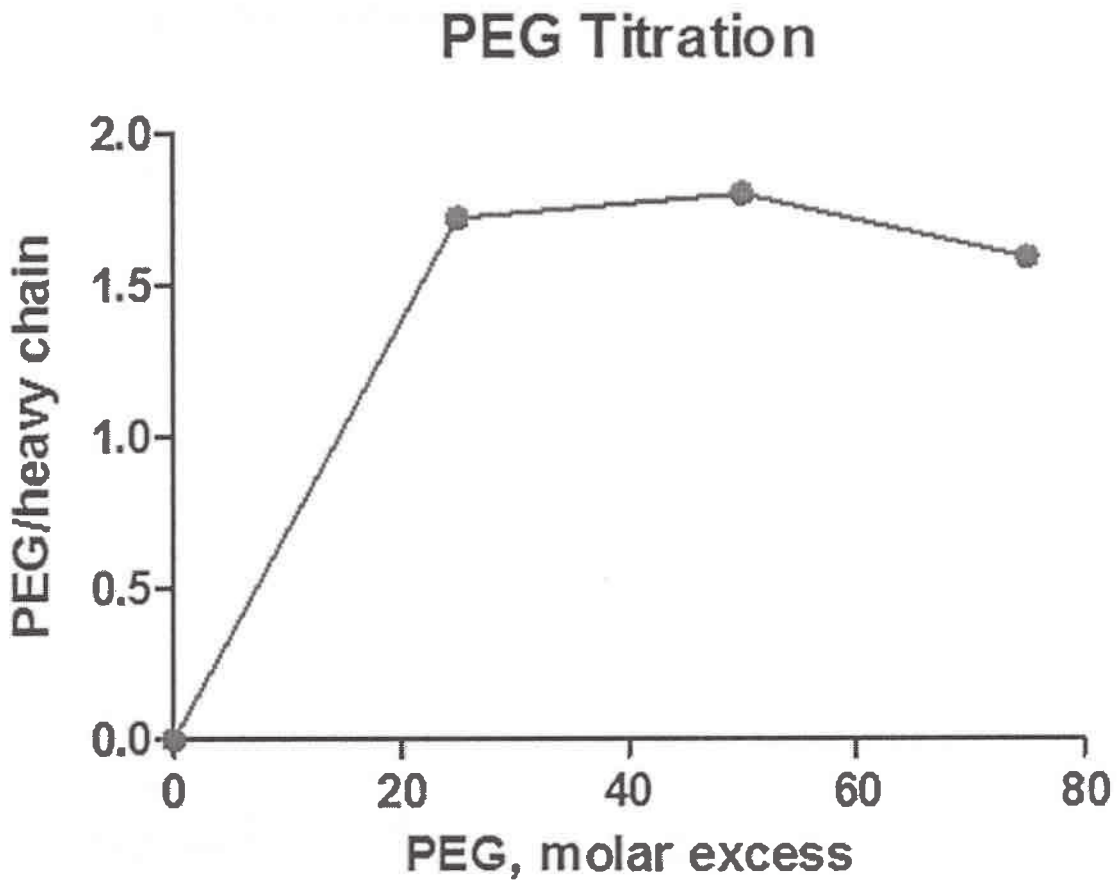
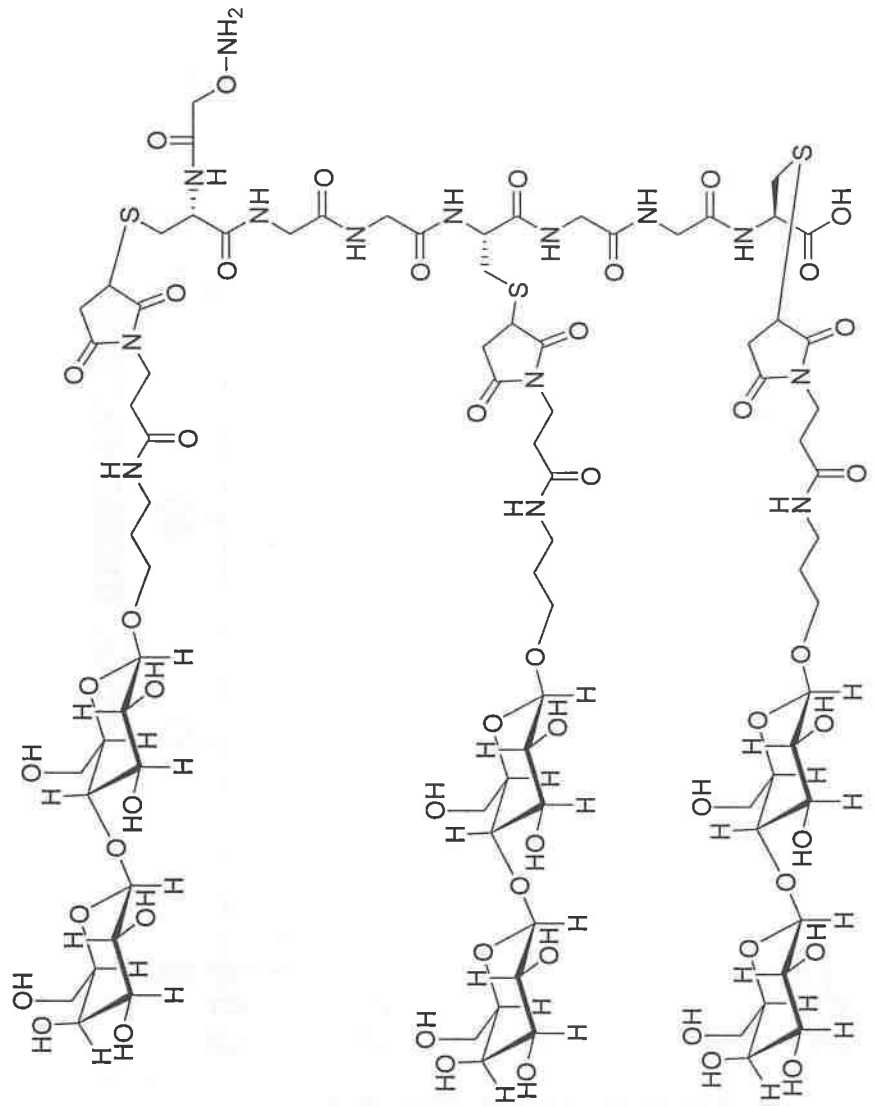

PEG Titration
PEG/heavy chain
2.0
1.5
1.0
0.5
0.0
0
20
40
60
80
PEG, molar excess

Figure 73

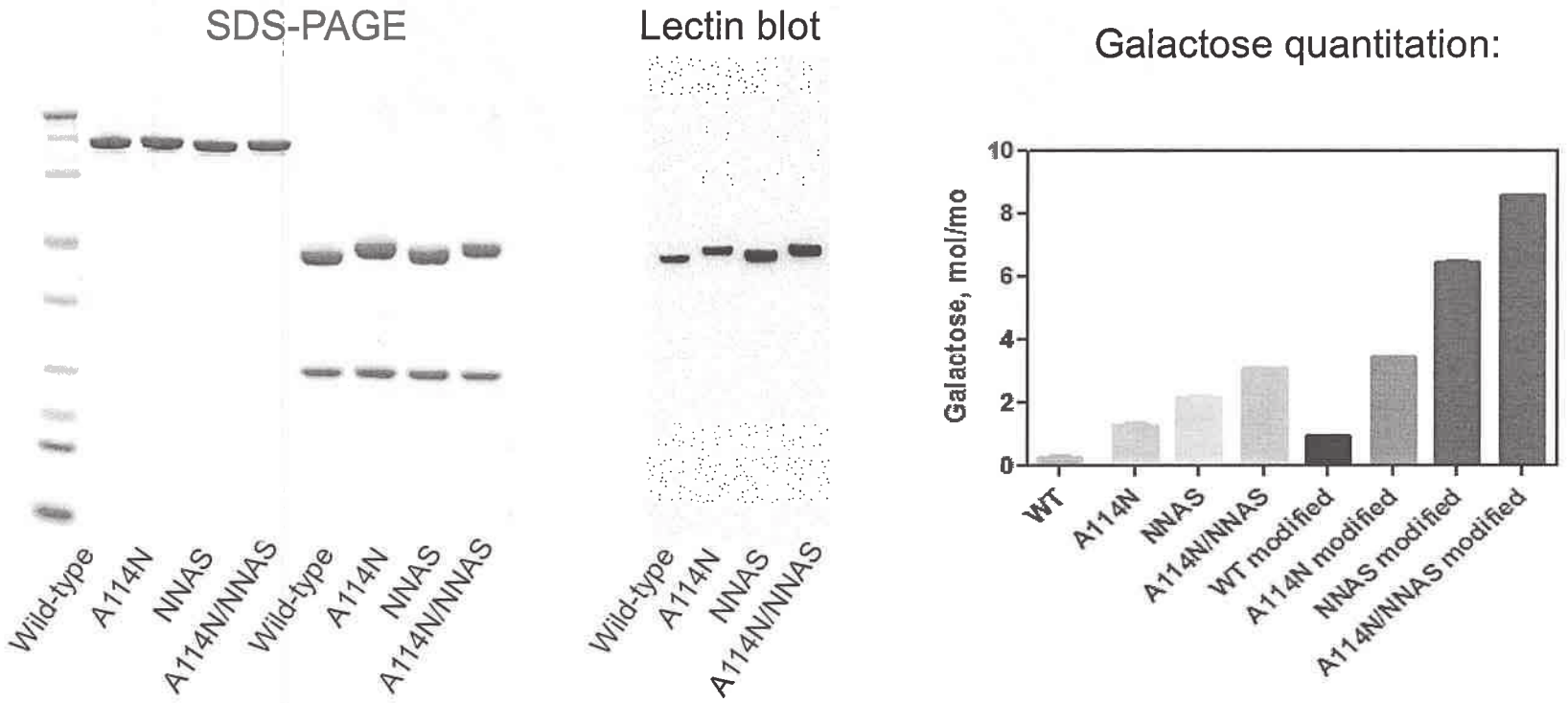


Glycopeptide aminooxy: lactose$_3$-Cys$_3$-Gly$_4$

Figure 74

A
gal
PEG
PEG Gal NNAS -
PEG Gal NNAS +
AS
PEG
B
Gal
PEG Gal NNAS. 0
PEG Gal NNAS. 0.02mM
PEG Gal NNAS. 0.1mM
PEG Gal NNAS. 0.5mM

Figure 75

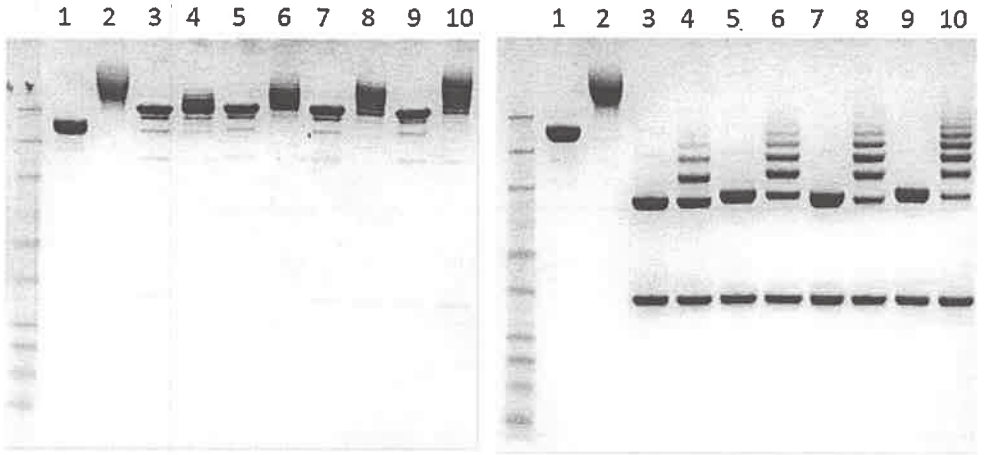


Enzyme modified antibodies containing ~3-9 terminal Gal obtained

Figure 76

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

Figure 77



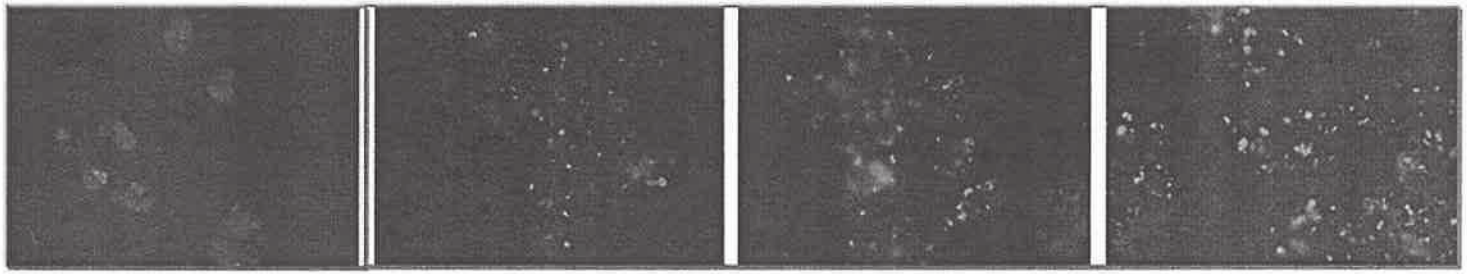
1 GAA
2 GAA, 25x PEG
3 Her2 WT
4 Her2 WT, 25x PEG
5 Her2 A114N
6 Her2 A114N, 25x PEG
7 Her2 NNAS
8 Her2 NNAS, 25x PEG
9 Her2 A114N/NNAS
10 Her2 A114N/NNAS, 25x PEG

Figure 78

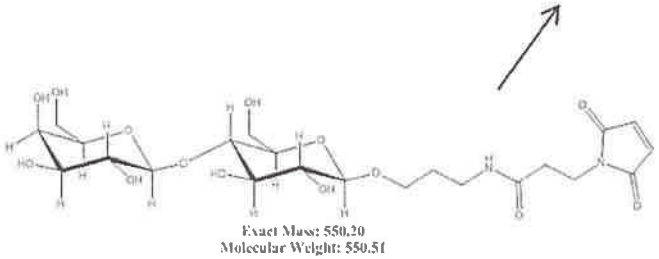
	Mol per mol, Mean + SD
Wild-type	1.4
A114N	2.7
NNAS	3.4
A114N/NNAS	4.6

Figure 79

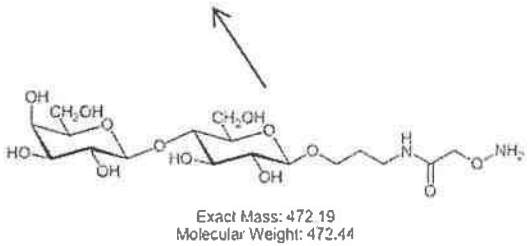
Polyclonal antibody uptake into HepG2 cells



Parent Ab	Gal Transferase	Gal-glc to Hinge	Gal-glc to 297
1 galactose/Ab	4 galactose / Ab	7 galactose / Ab	2 galactose / Ab



Lactose maleimide



Lactose aminooxy

Figure 80

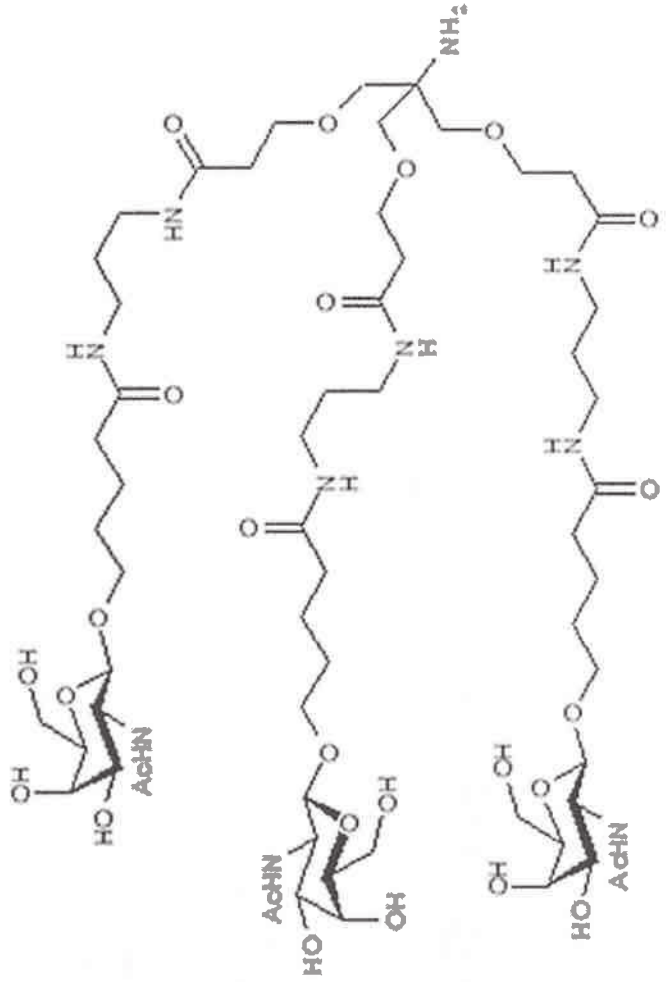
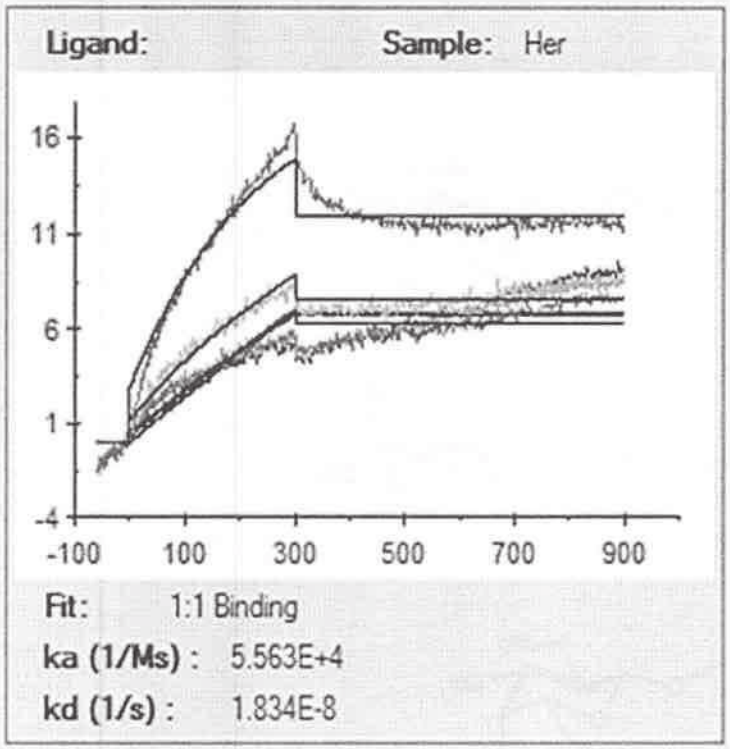


Figure 81


Ligand:
Sample: Her
16
11
6
1
-4
-100
100
300
500
700
900
Fit: 1:1 Binding
ka (1/Ms) : 5.563E+4
kd (1/s) : 1.834E-8

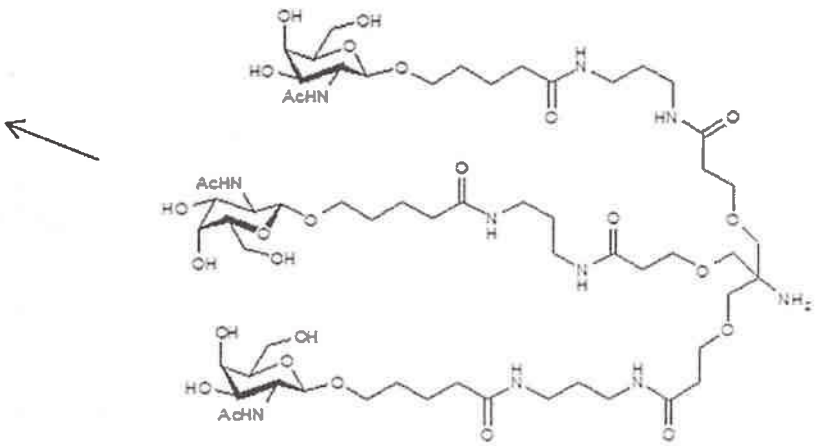
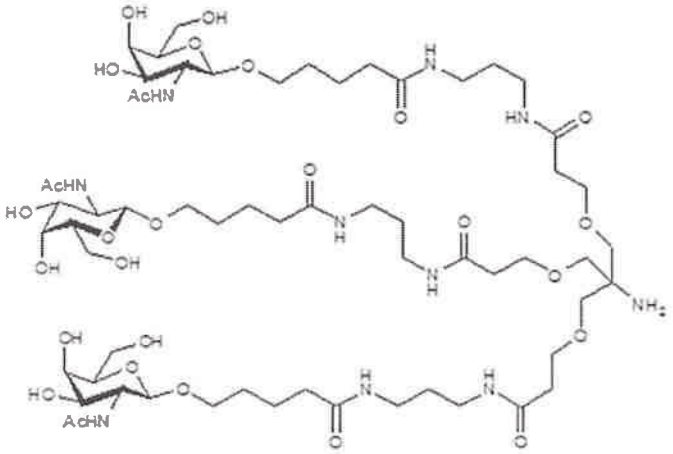
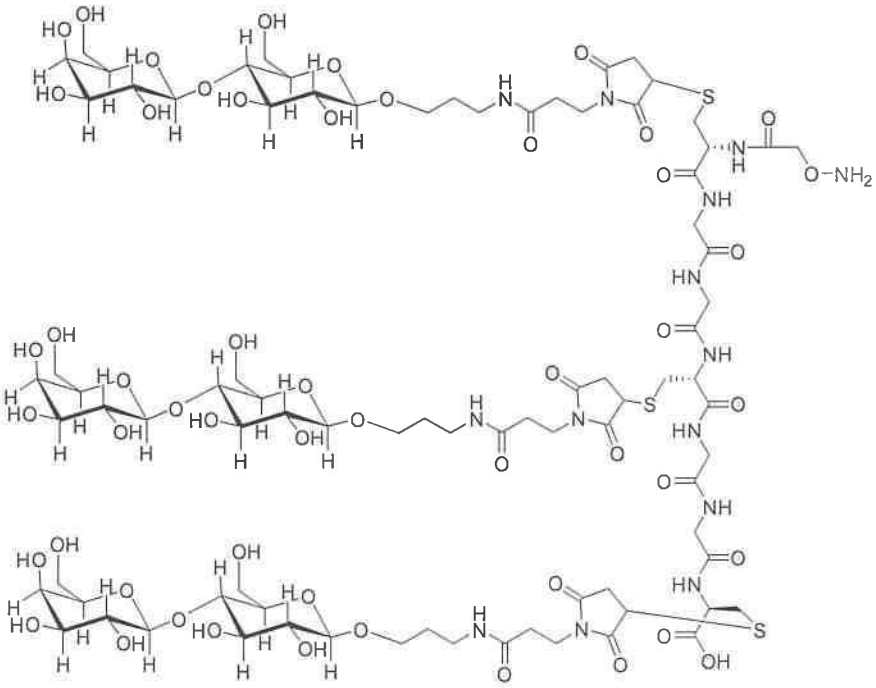

OH
OH
HO
AcHN
O
N
H
HN
O
AcHN
HO
OH
OH
O
N
H
O
N
H
O
O
NH2
OH
OH
HO
AcHN
O
N
H
N
H
O
O

Figure 82

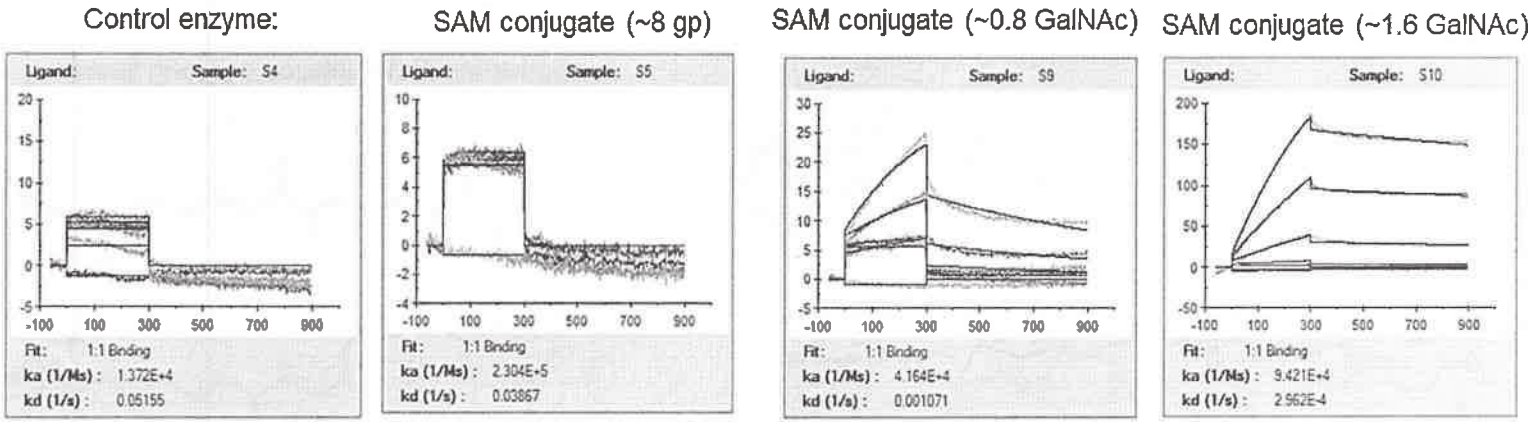


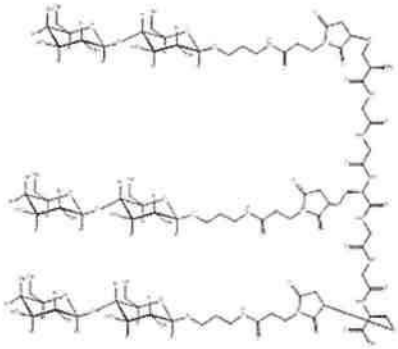
Trivalent GalNAc containing glycopeptide



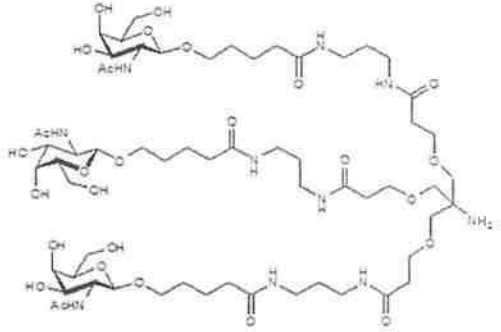
Trivalent Galactose containing glycopeptide

Figure 83





Trivalent Galactose containing glycopeptide



Trivalent GalNAc containing glycopeptide

Figure 84

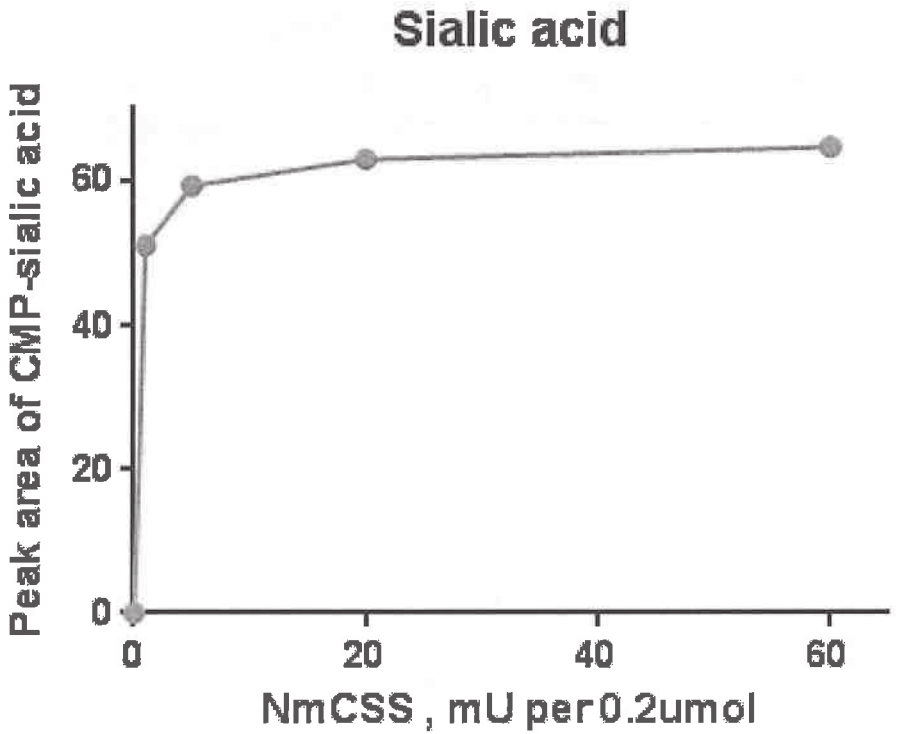

Sialic acid
Peak area of CMP-sialic acid
0
20
40
60
0
20
40
60
NmCSS , mU per 0.2umol

Figure 85

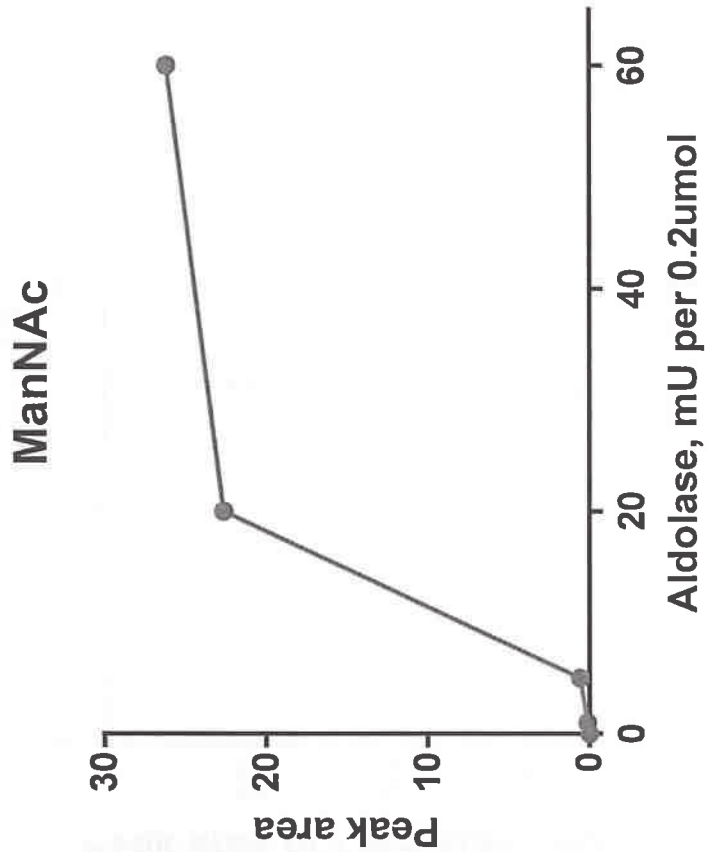


Figure 86

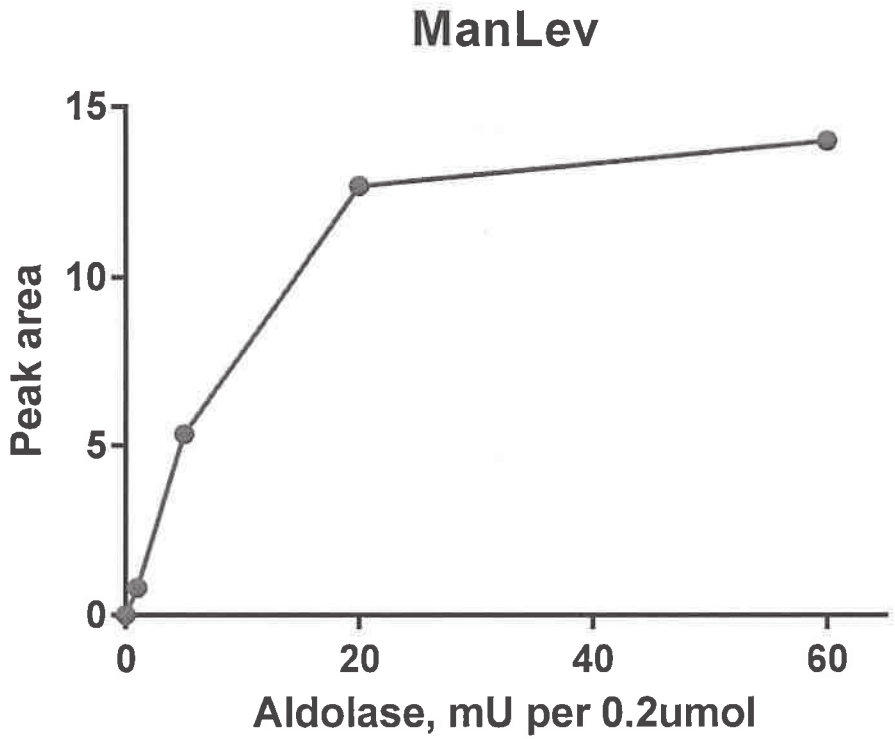


Figure 87

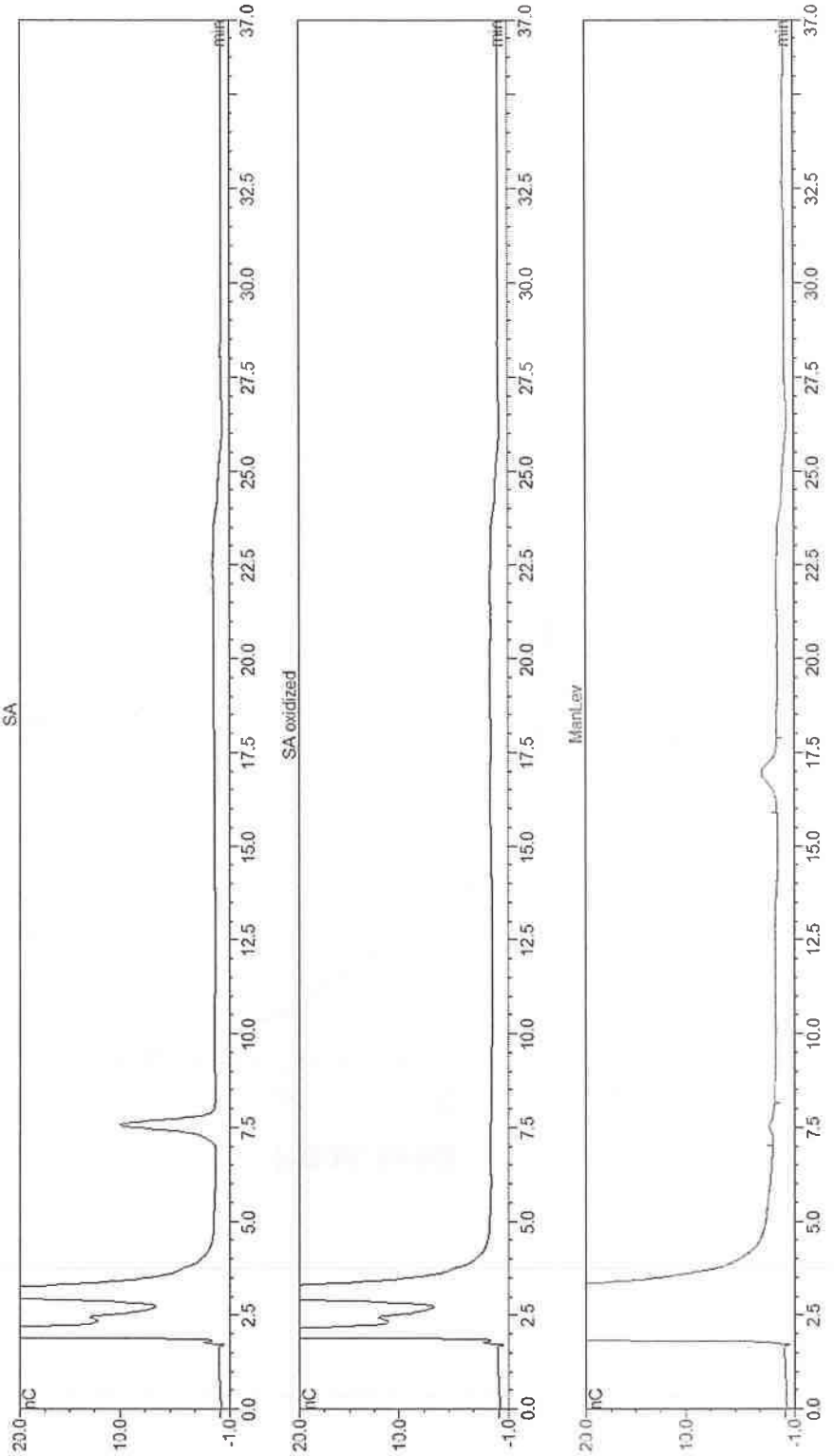

SA
SA oxidized
ManLev
nC
min
20.0
10.0
-1.0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
27.5
30.0
32.5
37.0

Figure 88

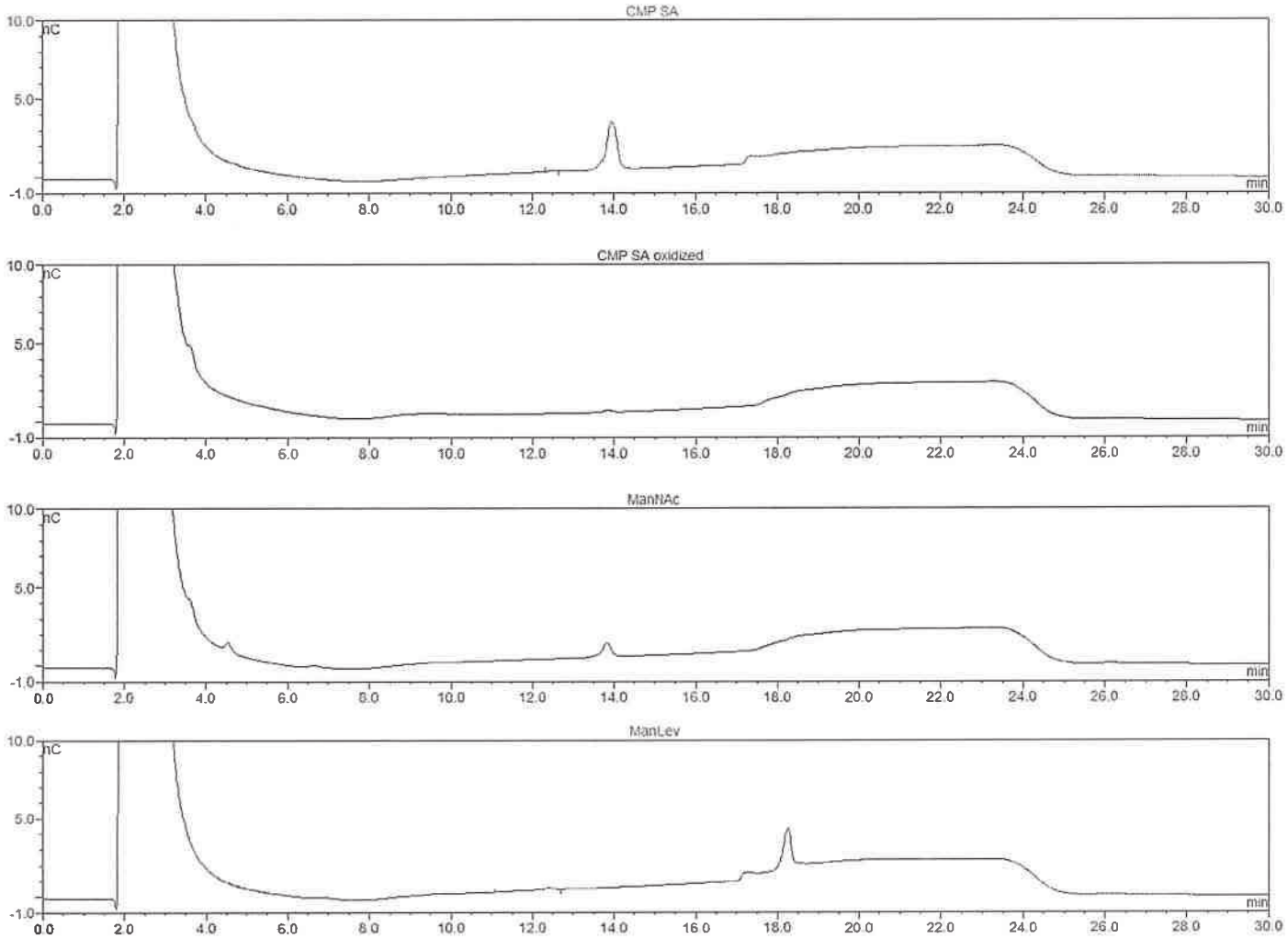


Figure 89

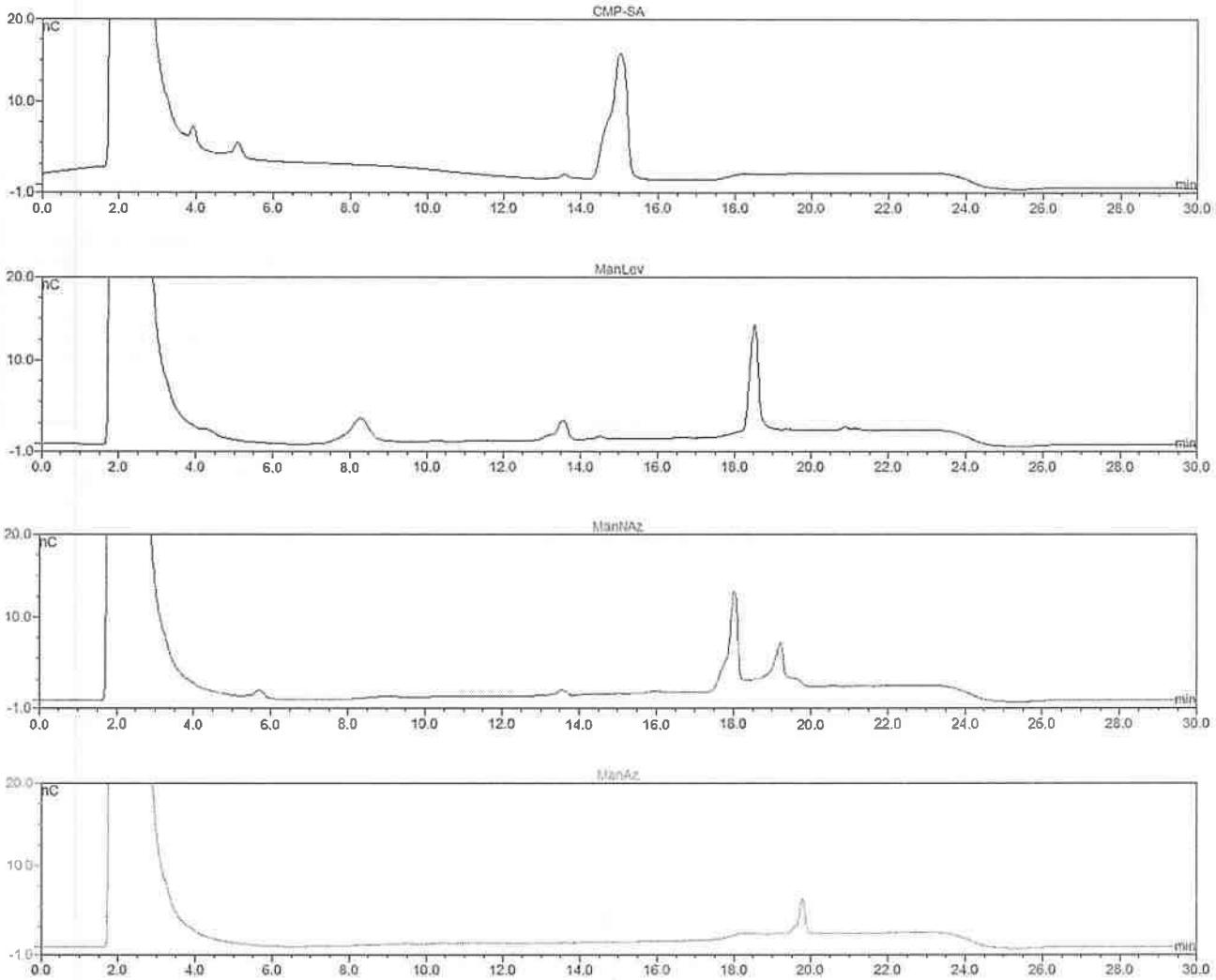


Figure 90

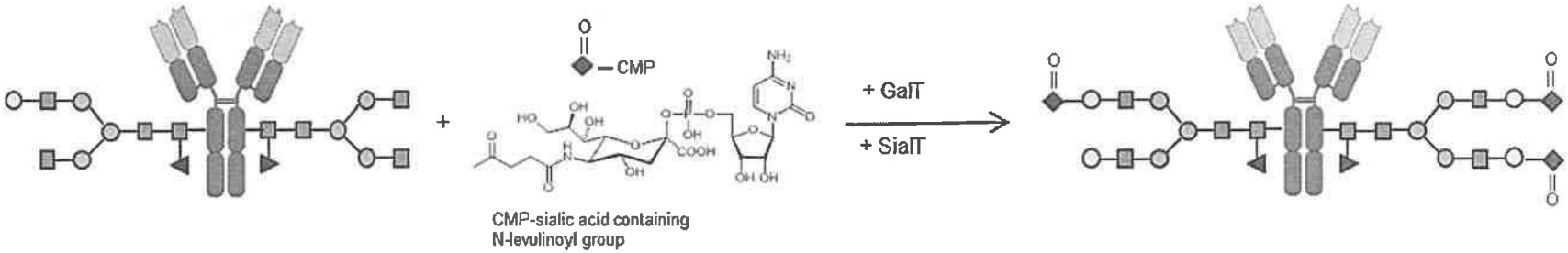

+
CMP
+ GalT
+ SialT
CMP-sialic acid containing
N-levulinoyl group

Figure 91

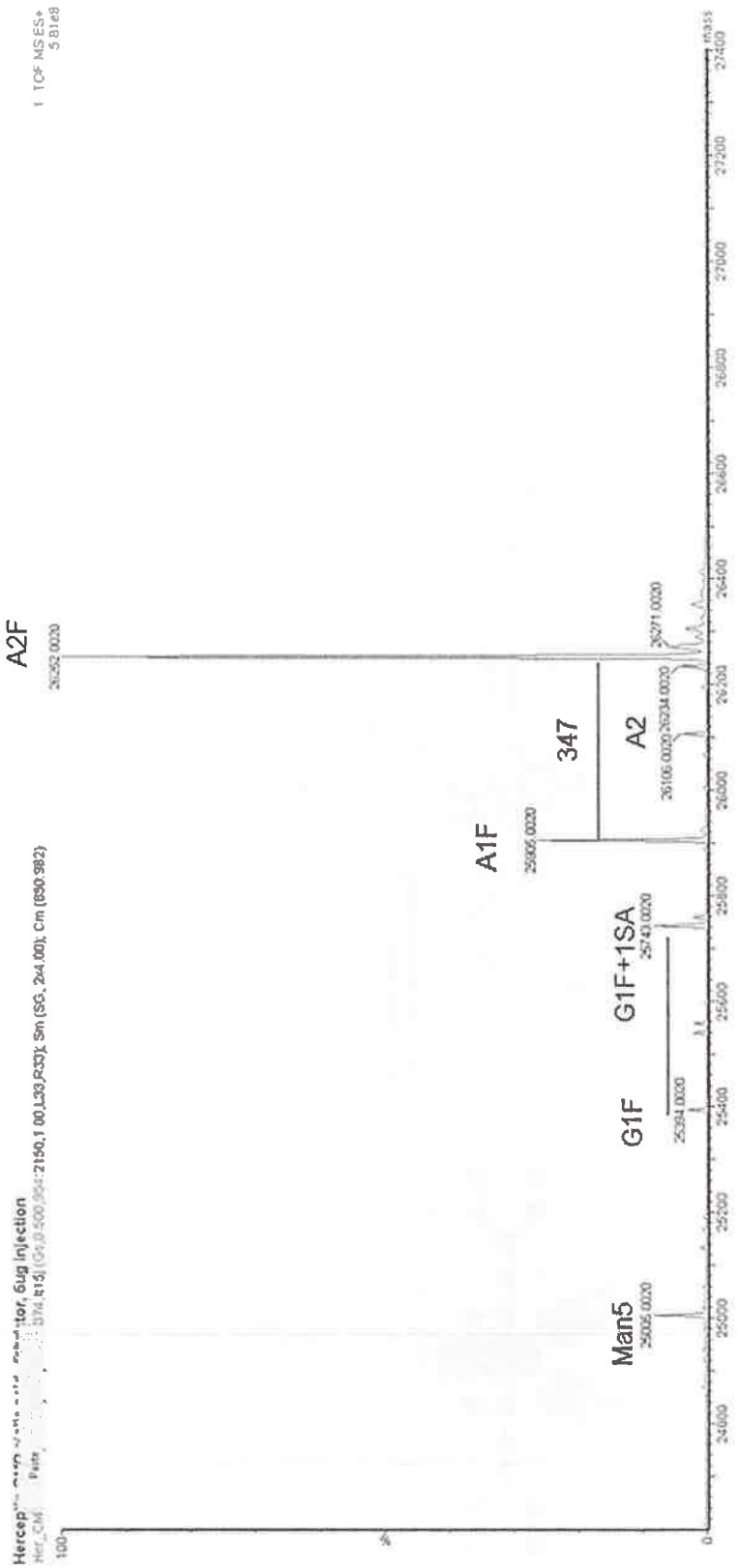


Figure 92

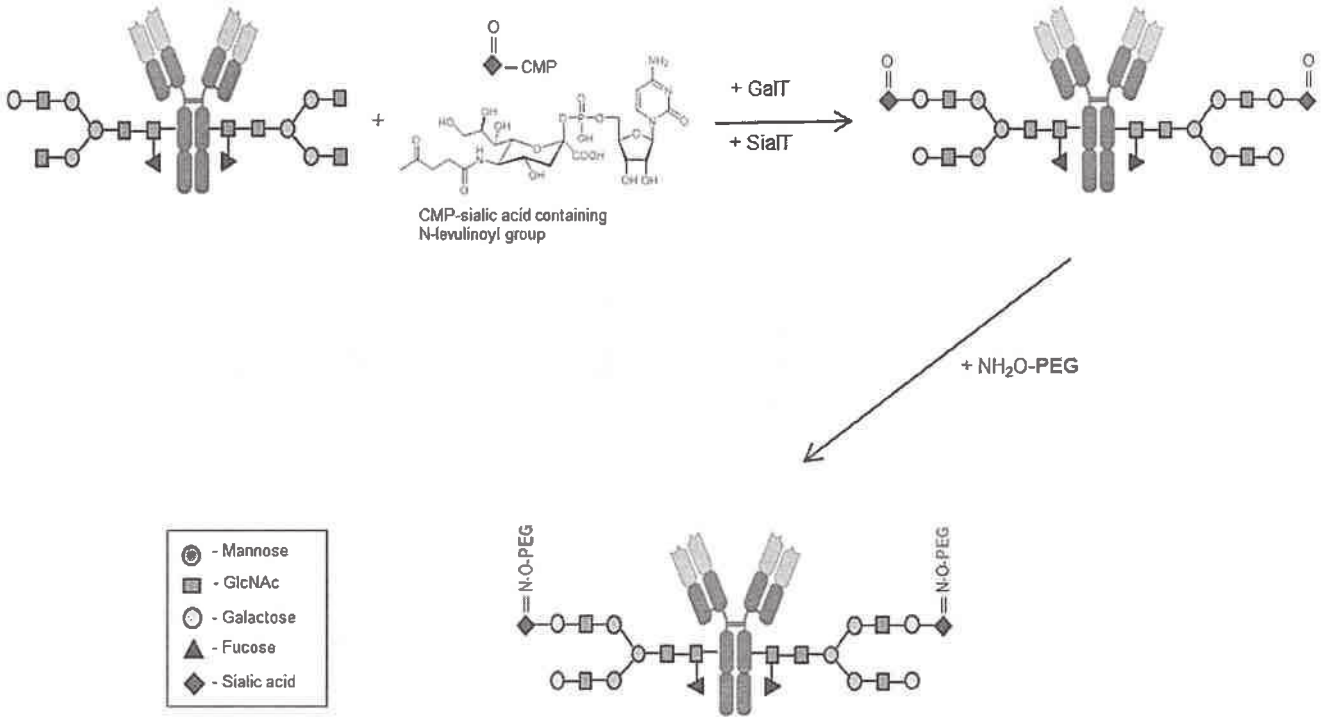

CMP
+ GalT
+ SialT
CMP-sialic acid containing
N-levulinoyl group
+ NH2O-PEG
- Mannose
- GlcNAc
- Galactose
- Fucose
- Sialic acid
N-O-PEG
N-O-PEG

Figure 93

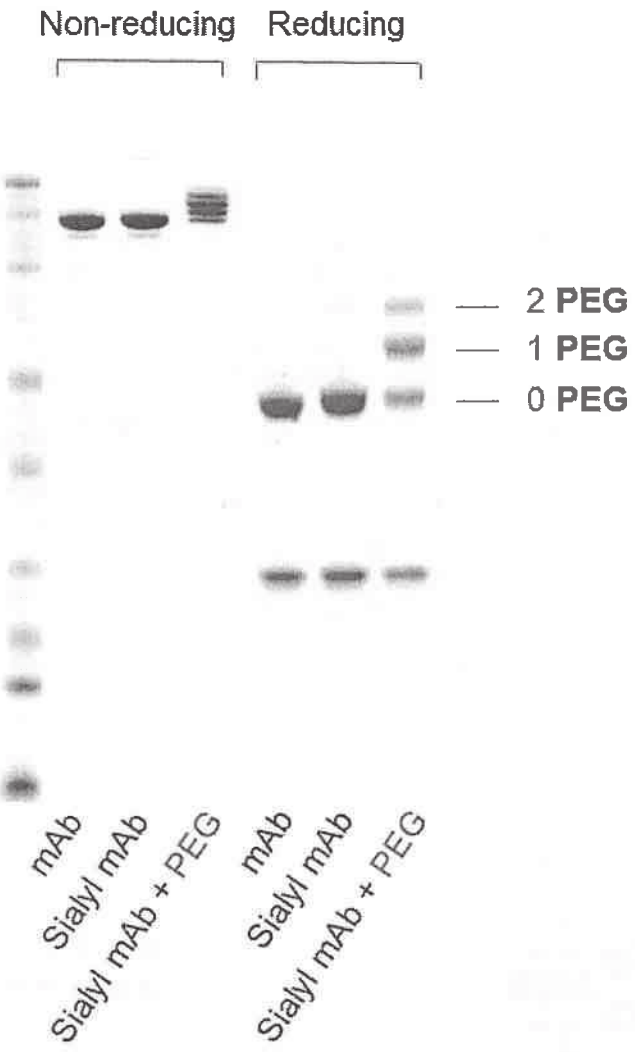

Non-reducing
Reducing
2 PEG
1 PEG
0 PEG
mAb
Sialyl mAb
Sialyl mAb + PEG
mAb
Sialyl mAb
Sialyl mAb + PEG

Figure 94

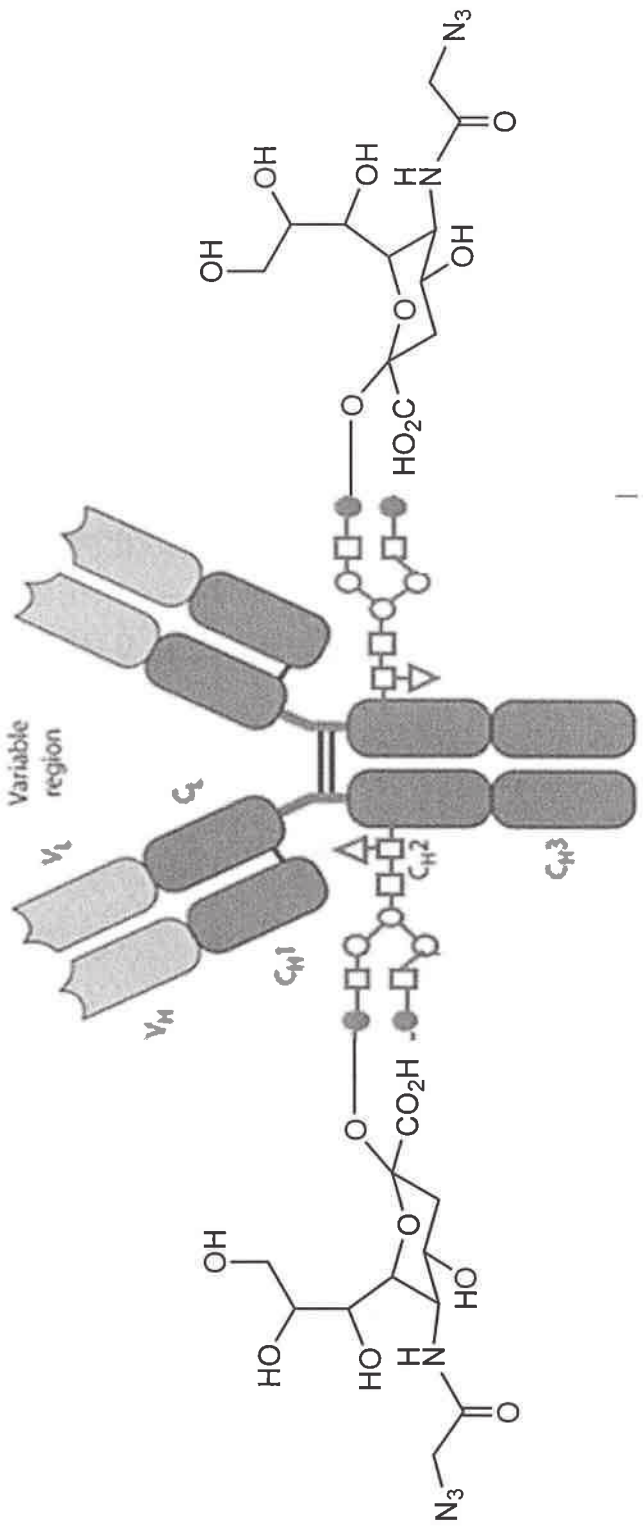
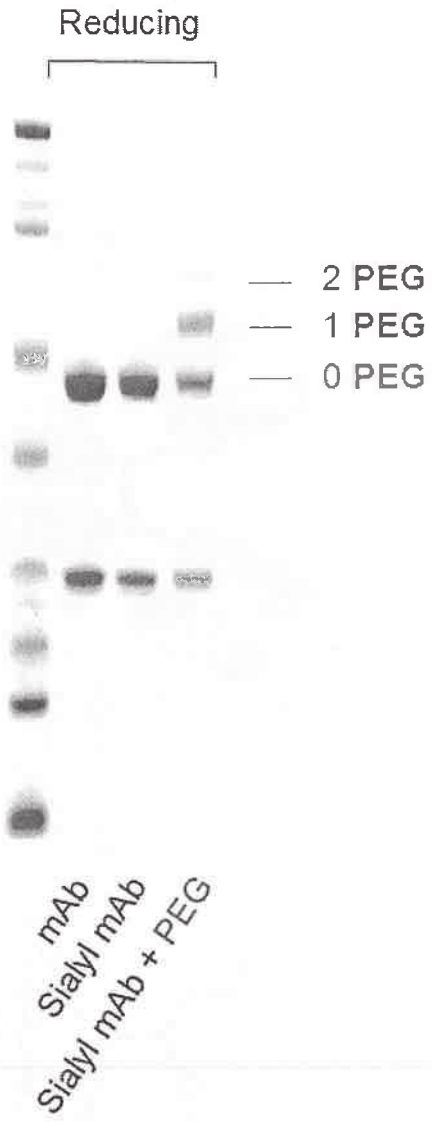



Figure 95