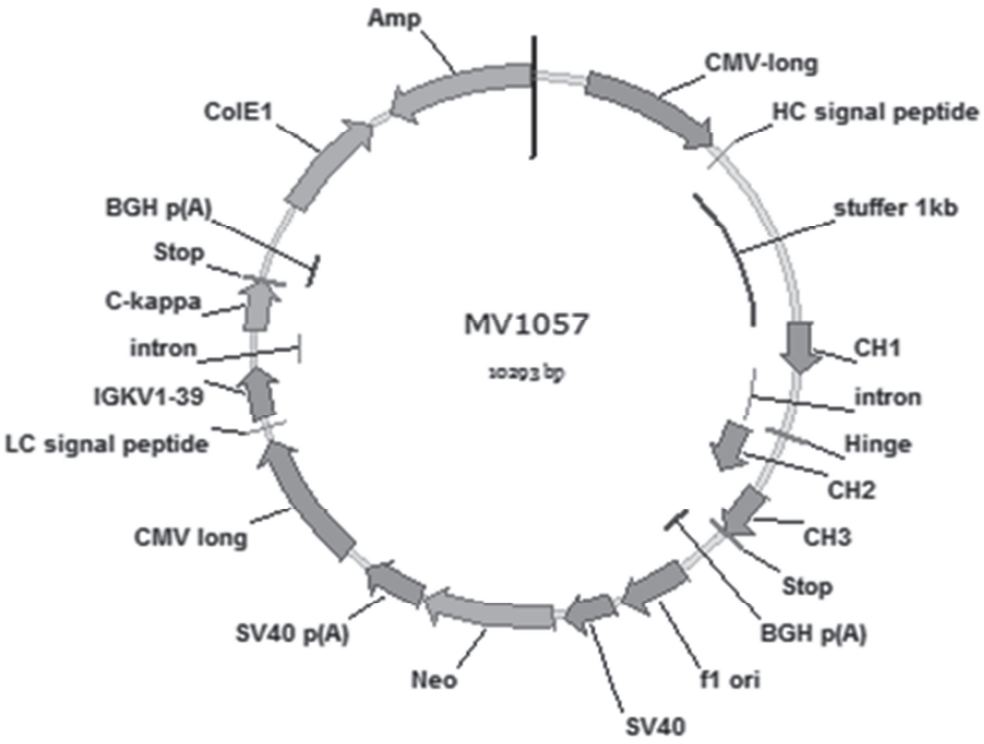
Figure 1:

A) schematic representation of construct vector MV1057




B) schematic representation of phage display vector MV1043

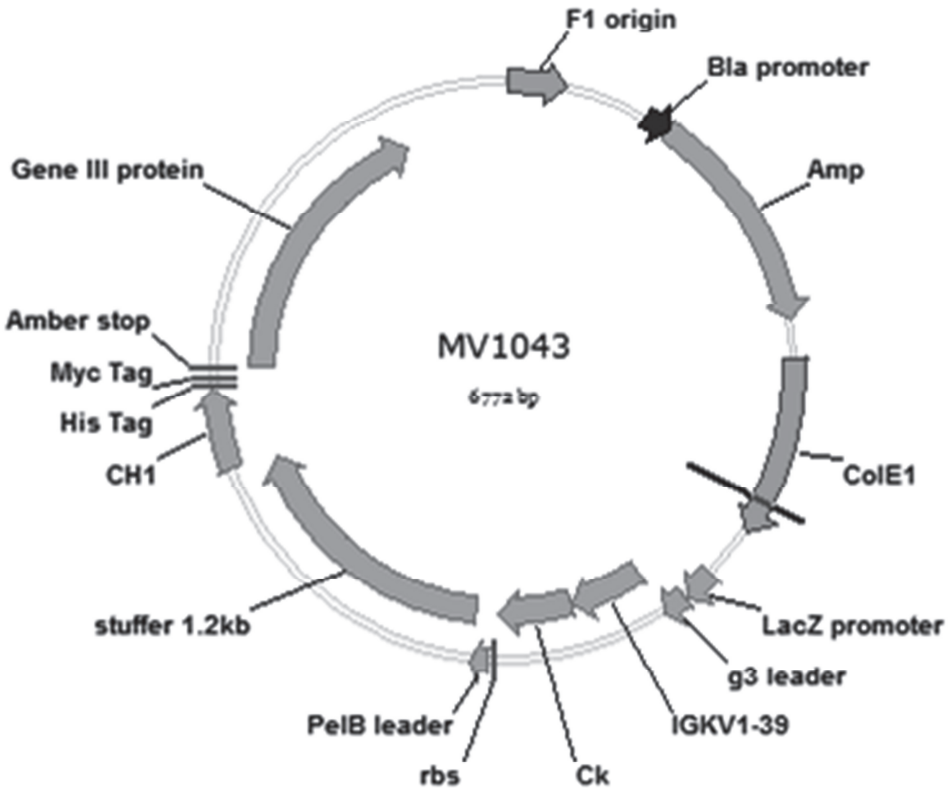


Figure 2: amino acid sequence of wildtype IgG1 Fc, as present in construct vector MV1057 (EU numbering scheme applied)

```
131         141         151         161         171         181
SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS
191         201         211         221         231         241
SSLGTQTYIC NVNHKPSNTK VDKRVEPKSC DKTHTCPPCP APELLGGPSV FLFPPKPKDT
251         261         271         281         291         301
LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH
311         321         331         341         351         361
QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK
371         381         391         401         411         421
GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
431         441
ALHNHYTQKS LSLSPGK
```

Figure 3: nucleotide and amino acid sequences of VH regions used

MF1025_VH
gaggtgcagctggtggagtctgggggaggcttggtacagcctgggggtccctgagactctcctgtgcag
cctctggattcacctttagcagctatgccatgagctgggtccgccaggctccagggaaggggctggagtg
ggtctcagctattagtggtagtggtggtagcacatactacgcagactccgtgaagggccggttcaccatc
tccagagacaattccaagaacacgctgtatctgcaaatgaacagcctgagagccgaggacacggccgtgt
attactgtgcaagggccgattggtgggcgacttttgactactggggccaaggtaccctggtcacc

MF1025_VH
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTI
SRDNSKNTLYLQMNSLRAEDTAVYYCARADWWATFDYWGQGTLVT

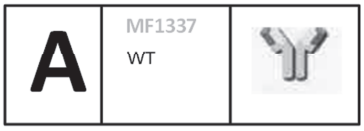
MF1122_VH
gaggtgcagctggtggagtctgggggaggcgtggtccagcctgggaggtccctgagactctcctgtgcag
cctctggattcaccttcagtagctatggcatgcactgggtccgccaggctccaggcaaggggctggagtg
ggtggcagttatatcatatgatggaagtaataaatactatgcagactccgtgaagggccgattcaccatc
tccagagacaattccaagaacacgctgtatctgcaaatgaacagcctgagagctgaggacacggccgtgt
attactgtgcaagagccctcttcacgaccatcgccatggactattggggccaaggtaccctggtcacc

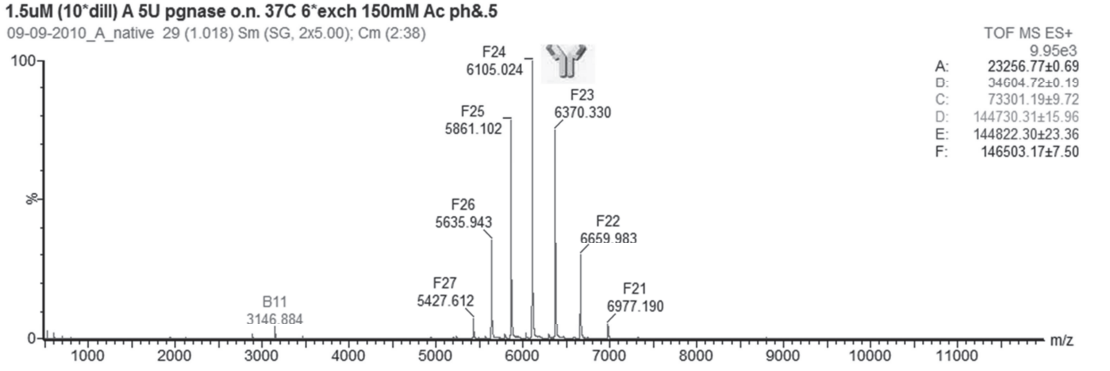
MF1122_VH
EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTI
SRDNSKNTLYLQMNSLRAEDTAVYYCARALFTTIAMDYWGQGTLVT

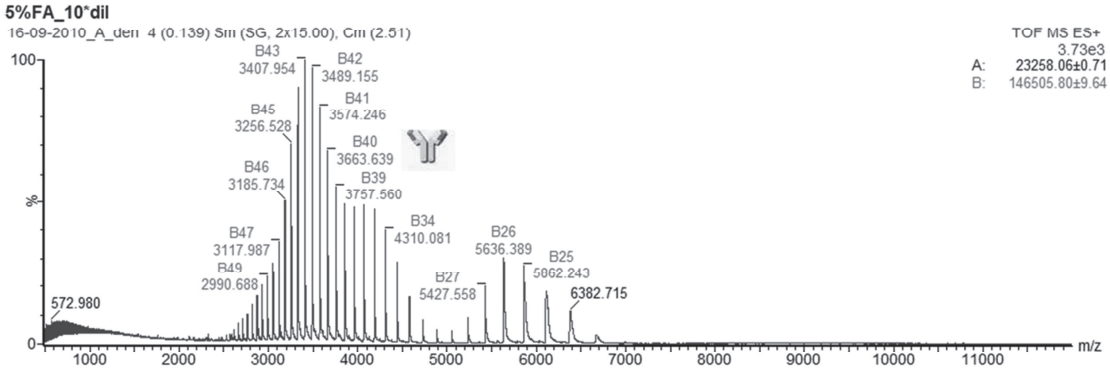
MF1337 VH
gaggtgcagctggtggagactggggctgaggtgaagaagccgggggcctcagtgaaggtctcctgcaaggcttct
gactacatcttcaccaaatatgacatcaactgggtgcgccaggcccctggacaagggcttgaatggatgggatgg
atgagcgctaacactggaaacacgggctatgcacagaagttccagggcagagtcaccatgaccagggacacgtcc
ataaacacagcctacatggagctgagcagcctgacatctggtgacacggccgtttatttctgtgcgaggagtagt
cttttcaagacagagacggcgccctactatcacttcgctctggacgtctggggccaagggaccacggtcacc

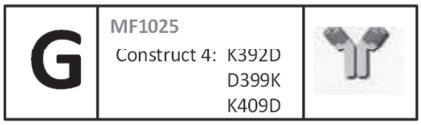
MF1337
VHEVQLVETGAEVKKPGASVKVSCKASDYIFTKYDINWVRQAPGQGLEWMGWMSANTGNTGYAQKFQGRVTMTRD
TSINTAYMELSSLTSGDTAVYFCARSSLFKTETAPYYHFALDVWGQGTTVT

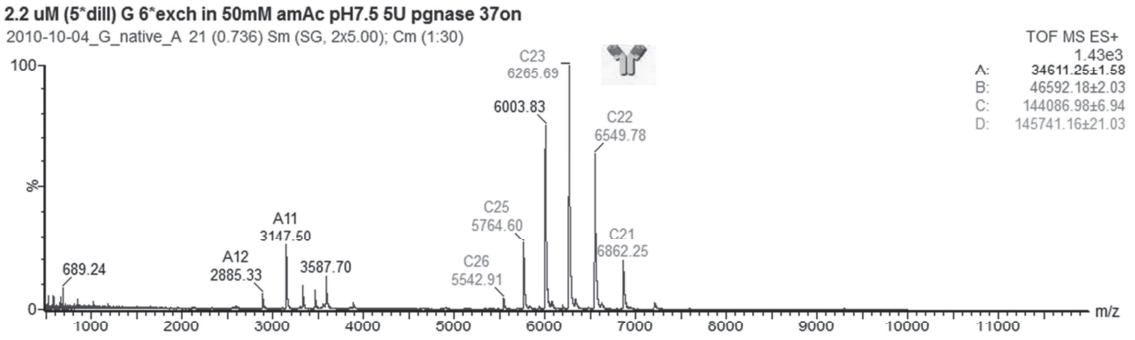
Figure 4: mass spec data of transfections A, G and H

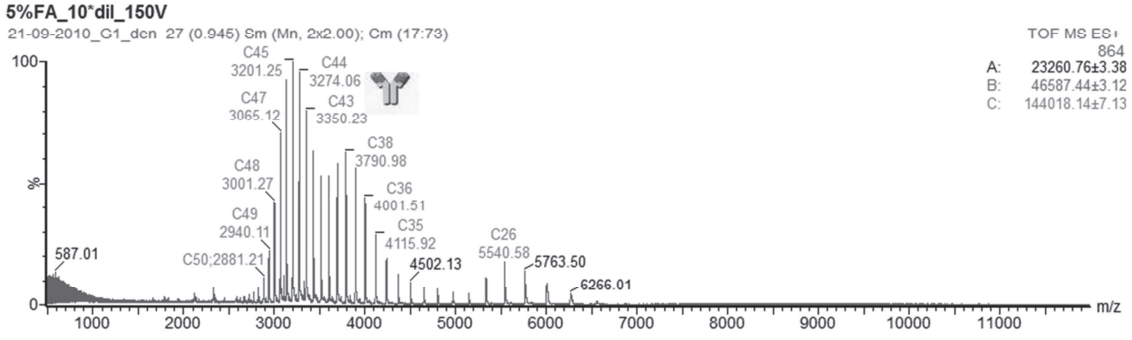


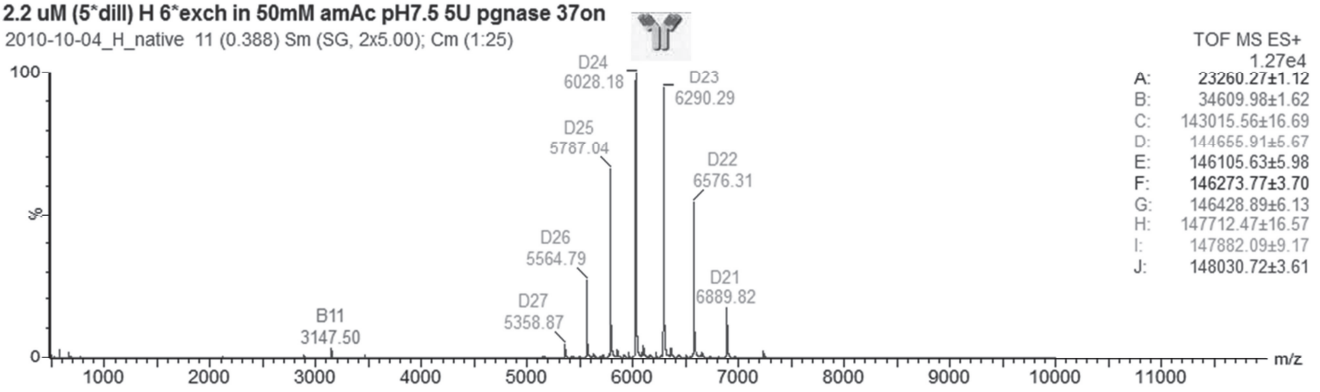



| H | MF1122<br>Construct 5: E356K<br>E357K<br>K439D<br>K370D |  |
|---|---|---|

**2.2 uM (5*dill) H 6*exch in 50mM amAc pH7.5 5U pgnase 37on**

2010-10-04_H_native 11 (0.388) Sm (SG, 2x5.00); Cm (1:25)




**5%FA_10*dil_150V**

21-09-2010_H_den 12 (0.420) Sm (Mn, 2x2.00); Cm (7:53)

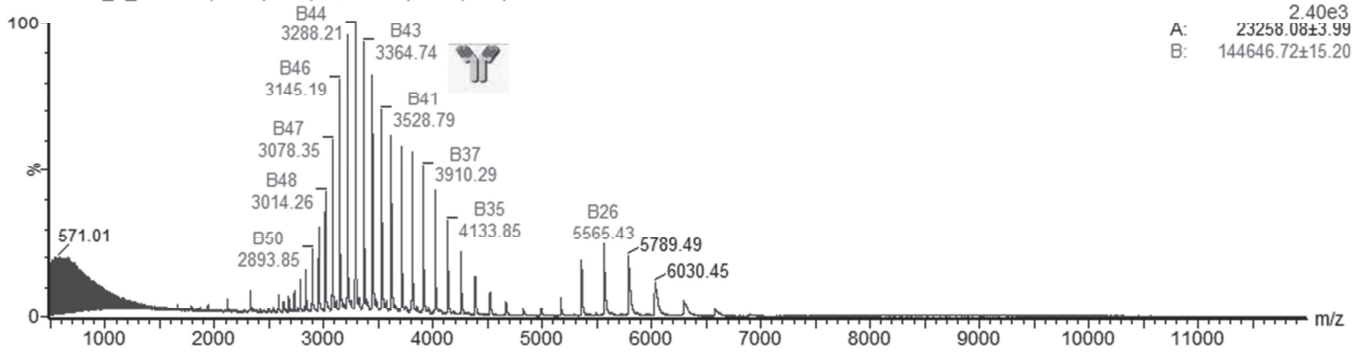
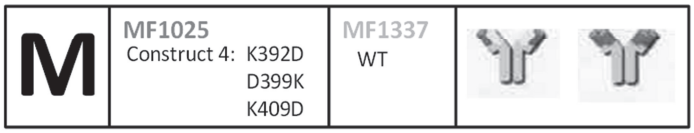
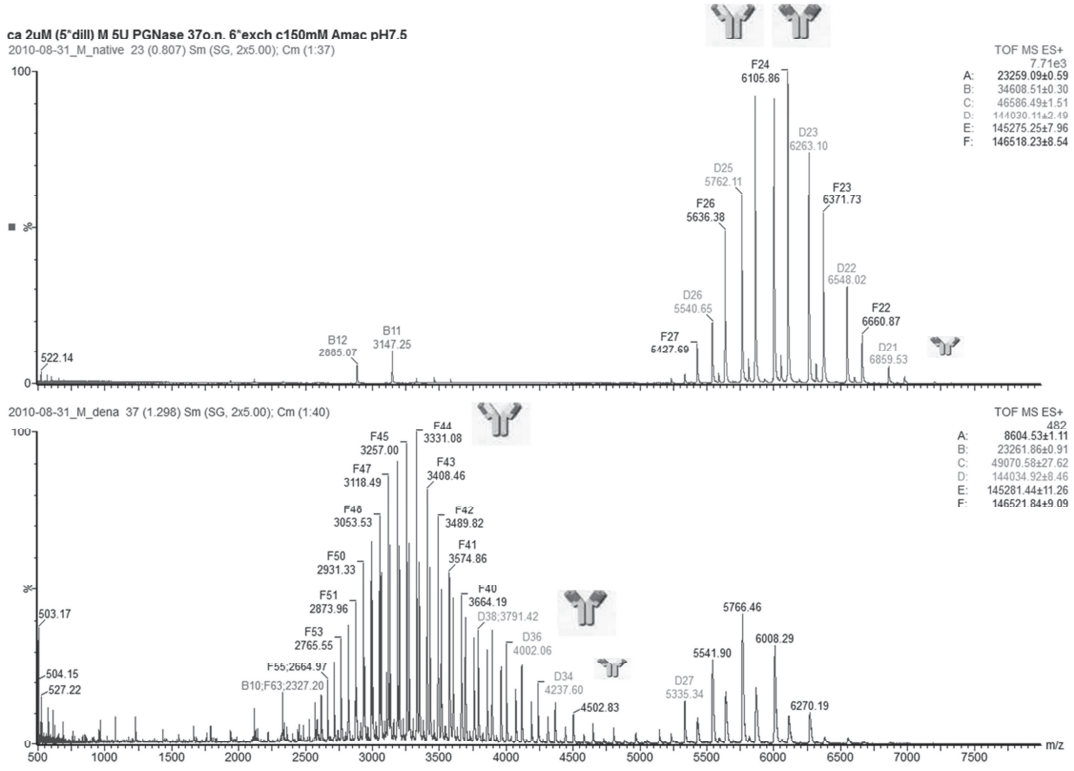
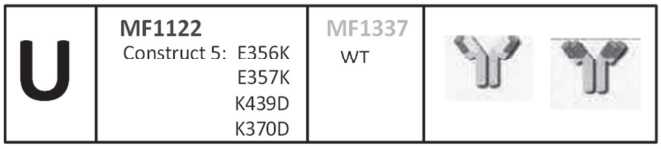


Figure 5


M
MF1025
Construct 4: K392D
D399K
K409D
MF1337
WT


ca 2uM (5*dill) M 5U PGNase 37o.n. 6*exch c150mM Amac pH7.5
2010-08-31_M_native 23 (0.807) Sm (SG, 2x5.00); Cm (1:37)
TOF MS ES+
7.71e3
A: 23259.09±0.59
B: 34608.51±0.30
C: 46586.49±1.51
D: 144030.11±2.49
E: 145275.25±7.96
F: 146518.23±8.54
F24 6105.86
D23 6263.10
D25 5762.11
F23 6371.73
F26 5636.38
D22 6548.02
D26 5540.65
F22 6660.87
F27 5427.69
D21 6859.53
B12 2885.07
B11 3147.25
522.14
2010-08-31_M_dena 37 (1.298) Sm (SG, 2x5.00); Cm (1:40)
TOF MS ES+
482
A: 8604.53±1.11
B: 23261.86±0.91
C: 49070.58±27.62
D: 144034.92±8.46
E: 145281.44±11.26
F: 146521.84±9.09
F44 3331.08
F45 3257.00
F47 3118.49
F43 3408.46
F46 3053.53
F42 3489.82
F50 2931.33
F41 3574.86
F51 2873.96
F40 3664.19
D38;3791.42
F53 2765.55
D36 4002.06
F55;2664.97
B10;F63;2327.20
D34 4237.60
D27 5335.34
4502.83
5766.46
6008.29
5541.90
6270.19
503.17
504.15
527.22
m/z


U
MF1122
Construct 5: E356K
E357K
K439D
K370D
MF1337
WT

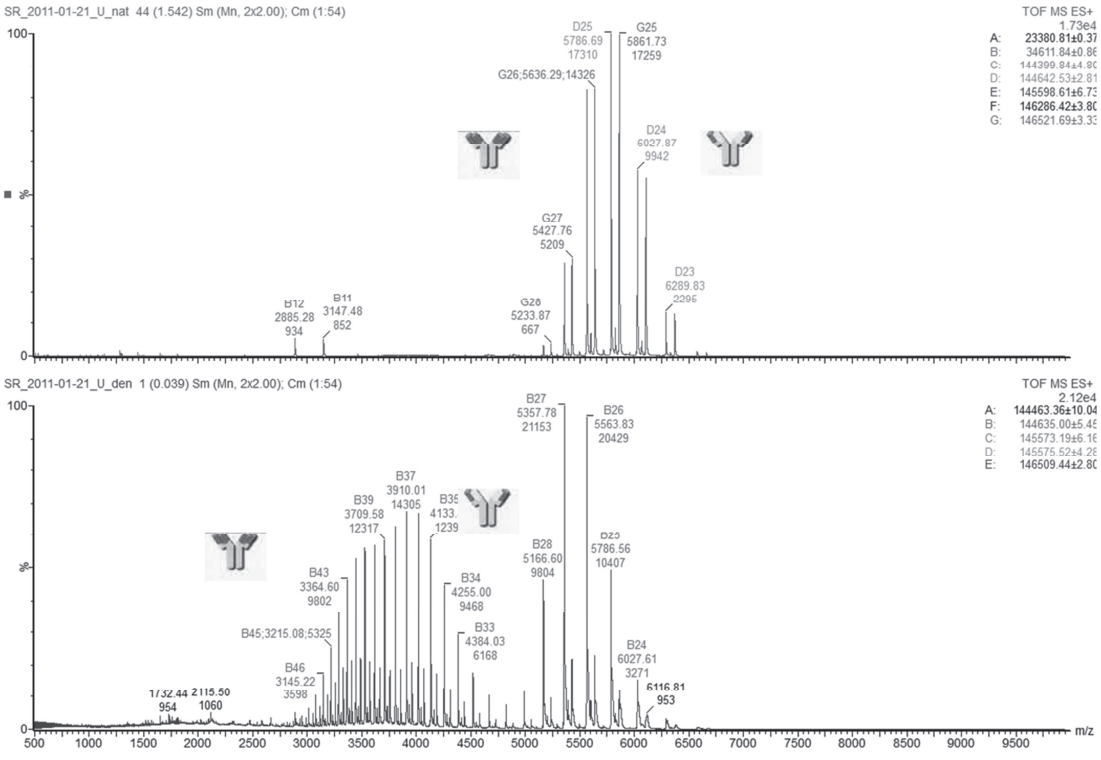

SR_2011-01-21_U_nat 44 (1.542) Sm (Mn, 2x2.00); Cm (1:54)
TOF MS ES+
1.73e4
A: 23380.81±0.37
B: 34611.84±0.86
C: 144399.84±4.80
D: 144642.53±2.81
E: 145598.61±6.73
F: 146286.42±3.80
G: 146521.69±3.33
D25 5786.69 17310
G25 5861.73 17259
G26;5636.29;14326
D24 6027.97 9942
G27 5427.76 5209
D23 6289.83
B11 3147.48 852
B12 2885.28 934
G28 5233.87 667
SR_2011-01-21_U_den 1 (0.039) Sm (Mn, 2x2.00); Cm (1:54)
TOF MS ES+
2.12e4
A: 144463.36±10.04
B: 144635.00±5.45
C: 145573.19±6.16
D: 145575.52±4.28
E: 146509.44±2.80
B27 5357.78 21153
B26 5563.83 20429
B37 3910.01 14305
B39 3709.58 12317
B35 4133. 1239
B28 5166.60 9804
D25 5786.56 10407
B43 3364.60 9802
B34 4255.00 9468
B45;3215.08;5325
B33 4384.03 6168
B46 3145.22 3598
B24 6027.61 3271
1732.44 2115.50 954 1060
6116.81 953
m/z

Figure 6:

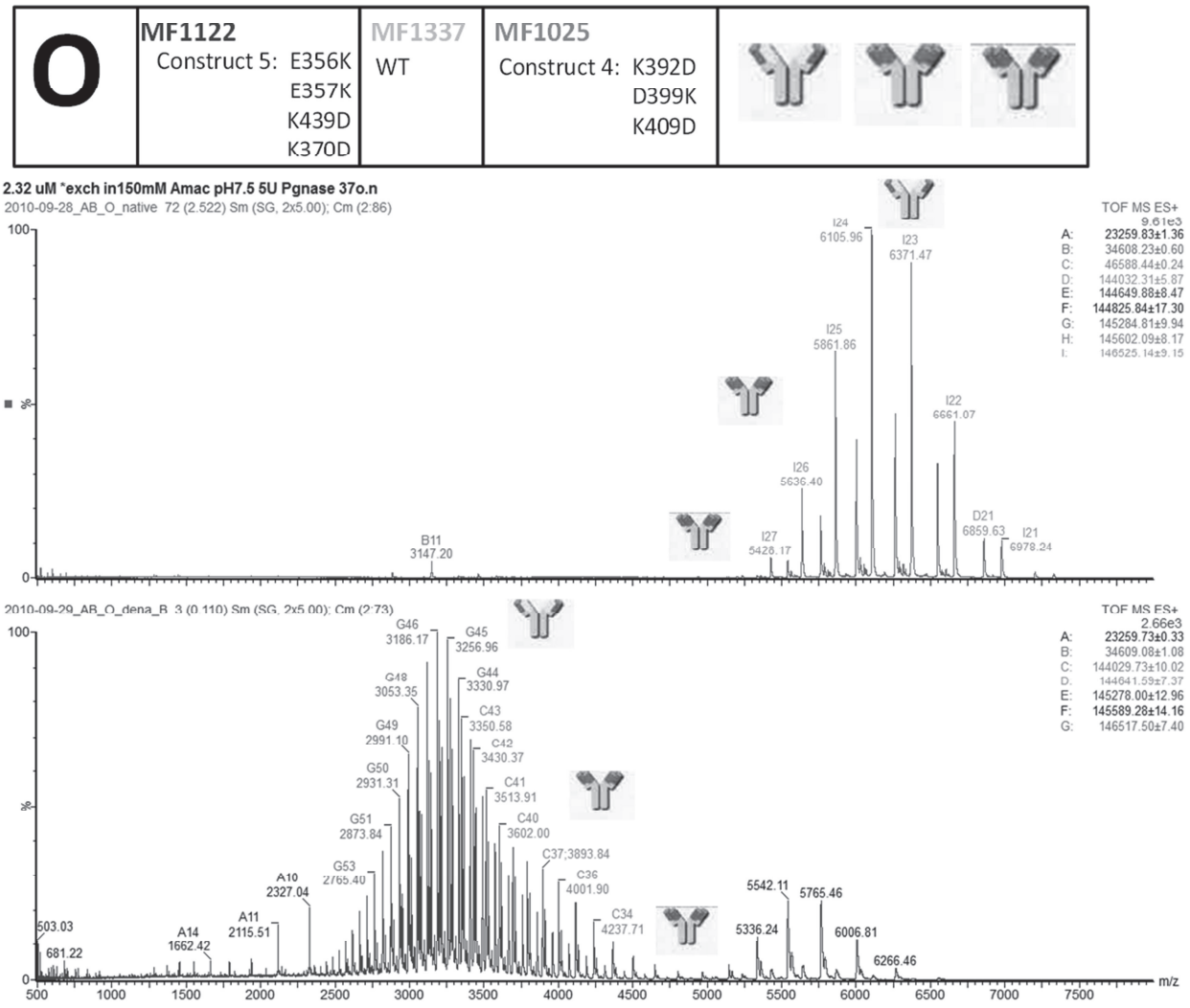
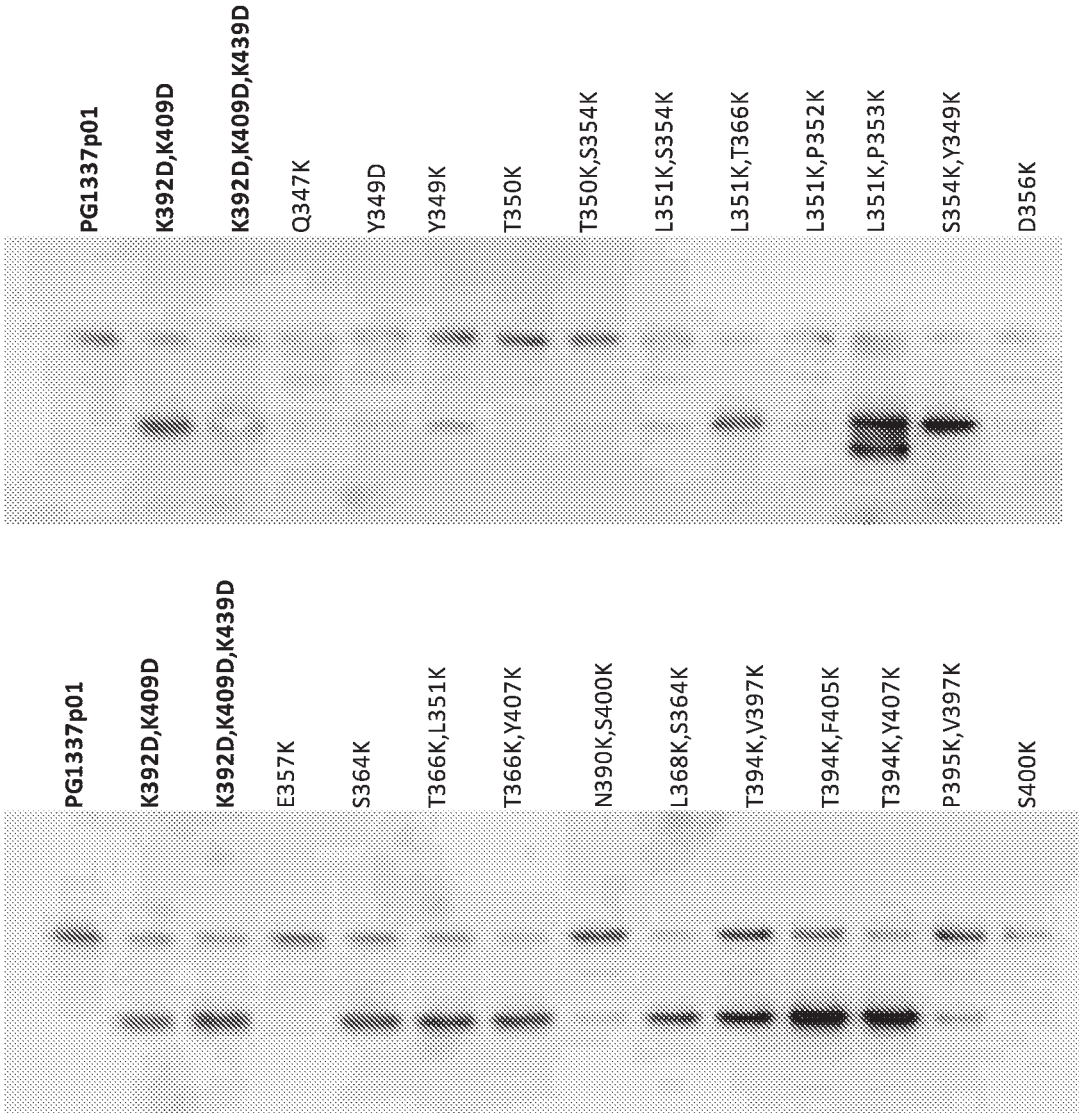

O
MF1122
Construct 5: E356K
E357K
K439D
K370D
MF1337
WT
MF1025
Construct 4: K392D
D399K
K409D
2.32 uM *exch in150mM Amac pH7.5 5U Pgnase 37o.n
2010-09-28_AB_O_native 72 (2.522) Sm (SG, 2x5.00); Cm (2:86)
TOF MS ES+
9.61e3
A: 23259.83±1.36
B: 34608.23±0.60
C: 46588.44±0.24
D: 144032.31±5.87
E: 144649.88±8.47
F: 144825.84±17.30
G: 145284.81±9.94
H: 145602.09±8.17
I: 146525.14±9.15
I24 6105.96
I23 6371.47
I25 5861.86
I22 6661.07
I26 5636.40
D21 6859.63
I21 6978.24
I27 5426.17
B11 3147.20
2010-09-29_AB_O_deng_B_3 (0.110) Sm (SG, 2x5.00); Cm (2:73)
TOF MS ES+
2.66e3
A: 23259.73±0.33
B: 34609.08±1.08
C: 144029.73±10.02
D: 144641.59±7.37
E: 145278.00±12.96
F: 145589.28±14.16
G: 146517.50±7.40
G46 3186.17
G45 3256.96
G48 3053.35
G44 3330.97
G49 2991.10
C43 3350.58
C42 3430.37
G50 2931.31
C41 3513.91
G51 2873.84
C40 3602.00
C37;3893.84
G53 2765.40
C36 4001.90
A10 2327.04
C34 4237.71
A11 2115.51
A14 1662.42
503.03
681.22
5542.11
5765.46
5336.24
6006.81
6266.46
%
m/z
100
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
6000
6500
7000
7500

Figure 7:


PG1337p01
K392D,K409D
K392D,K409D,K439D
Q347K
Y349D
Y349K
T350K
T350K,S354K
L351K,S354K
L351K,T366K
L351K,P352K
L351K,P353K
S354K,Y349K
D356K
PG1337p01
K392D,K409D
K392D,K409D,K439D
E357K
S364K
T366K,L351K
T366K,Y407K
N390K,S400K
L368K,S364K
T394K,V397K
T394K,F405K
T394K,Y407K
P395K,V397K
S400K

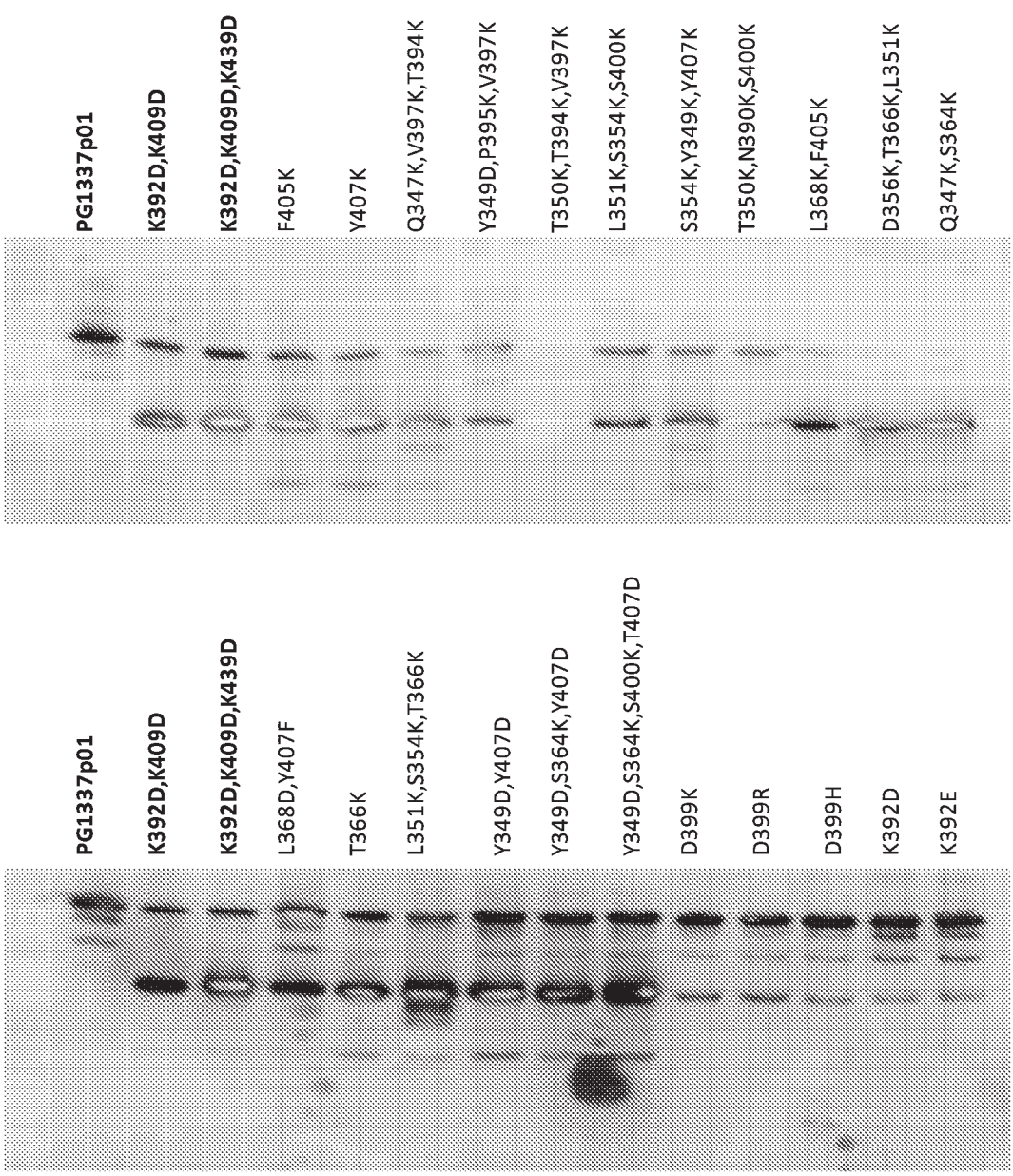
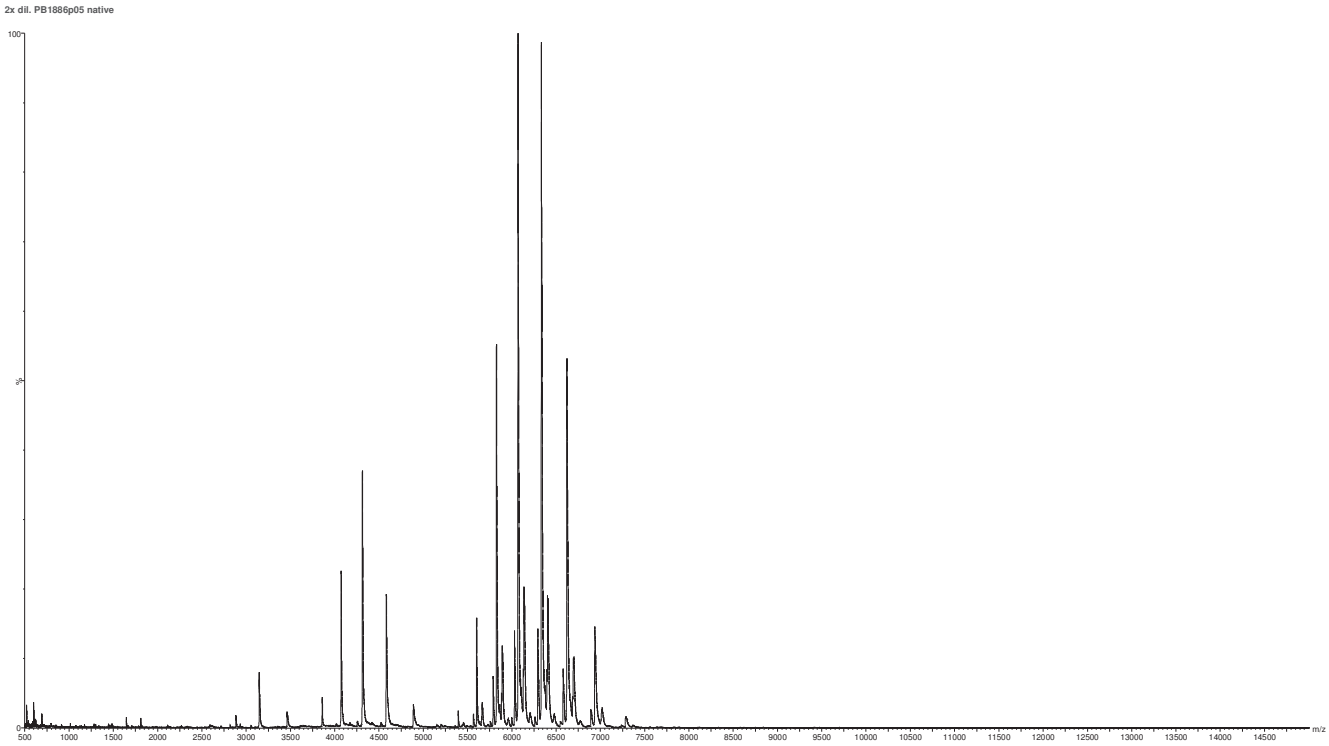
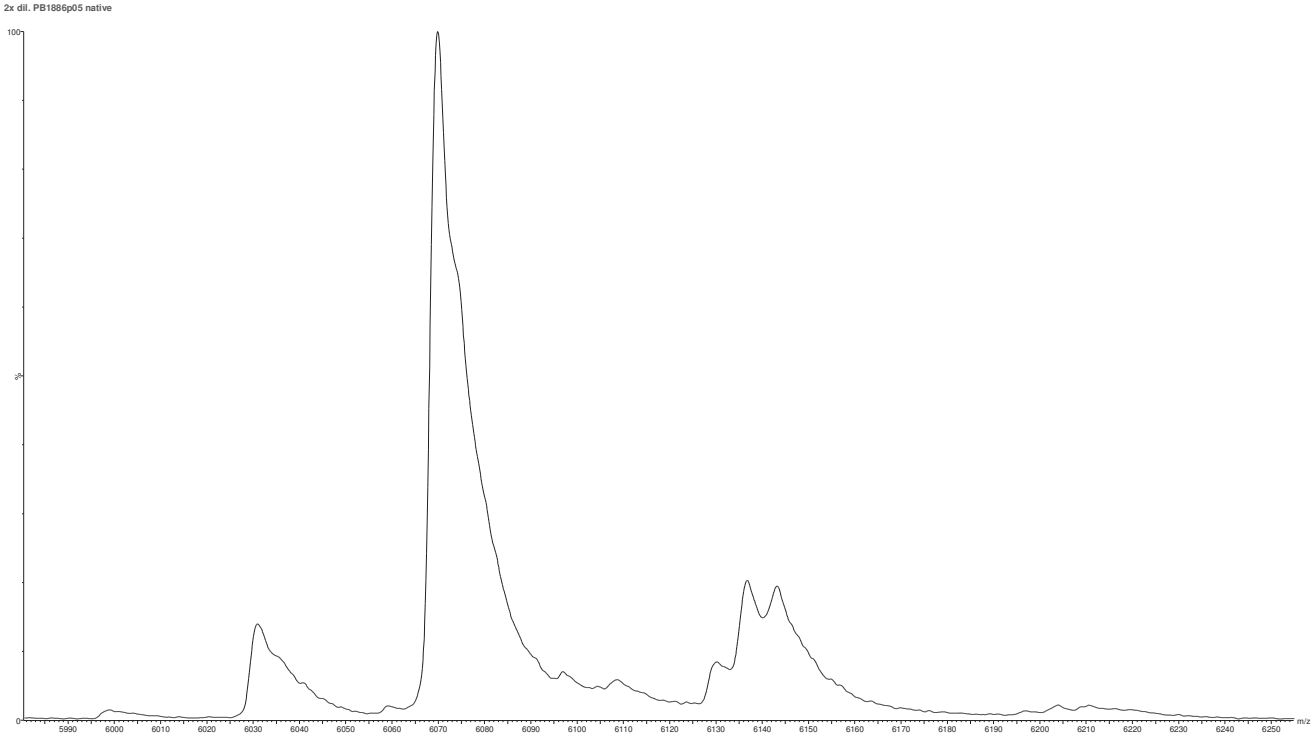

PG1337p01
K392D,K409D
K392D,K409D,K439D
L368D,Y407F
T366K
L351K,S354K,T366K
Y349D,Y407D
Y349D,S364K,Y407D
Y349D,S364K,S400K,T407D
D399K
D399R
D399H
K392D
K392E
PG1337p01
K392D,K409D
K392D,K409D,K439D
F405K
Y407K
Q347K,V397K,T394K
Y349D,P395K,V397K
T350K,T394K,V397K
L351K,S354K,S400K
S354K,Y349K,Y407K
T350K,N390K,S400K
L368K,F405K
D356K,T366K,L351K
Q347K,S364K

Figure 8:




A) Native MS spectrum of transfection sample ZO (T366K/L351'D)




B) Convoluted MS spectrum of transfection sample ZO (T366K/L351'D). The second/main peak represents the bispecific molecule.

Figure 9:

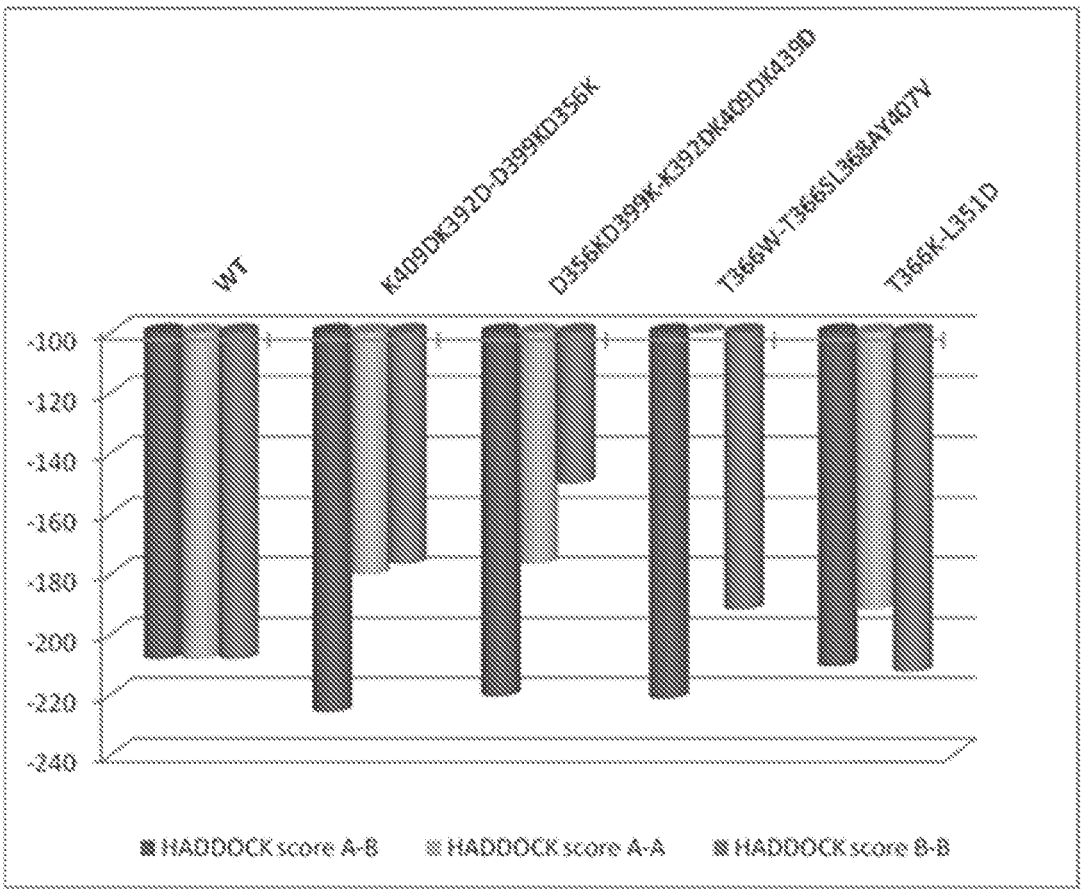
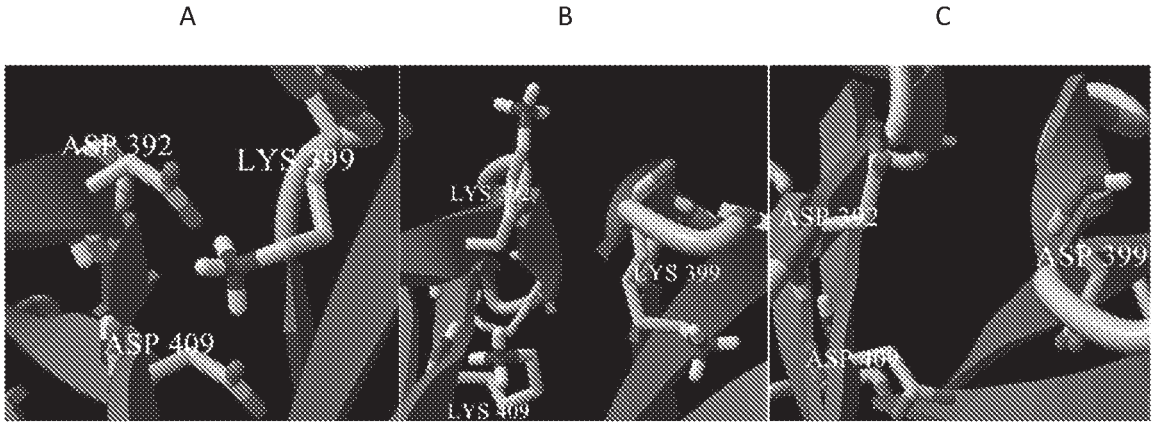

WT
K409DK392D-D399KD356K
D356KD399K-K392DK409DK439D
T366W-T366SL368AY407V
T366K-L351D
-100
-120
-140
-160
-180
-200
-220
-240
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Figure 10




A K409D:K392D/D399'K:E356'K

B D399K:E356K/D399'K:E356'K

C K409D:K392D/K409'D:K392'D

Figure 11:

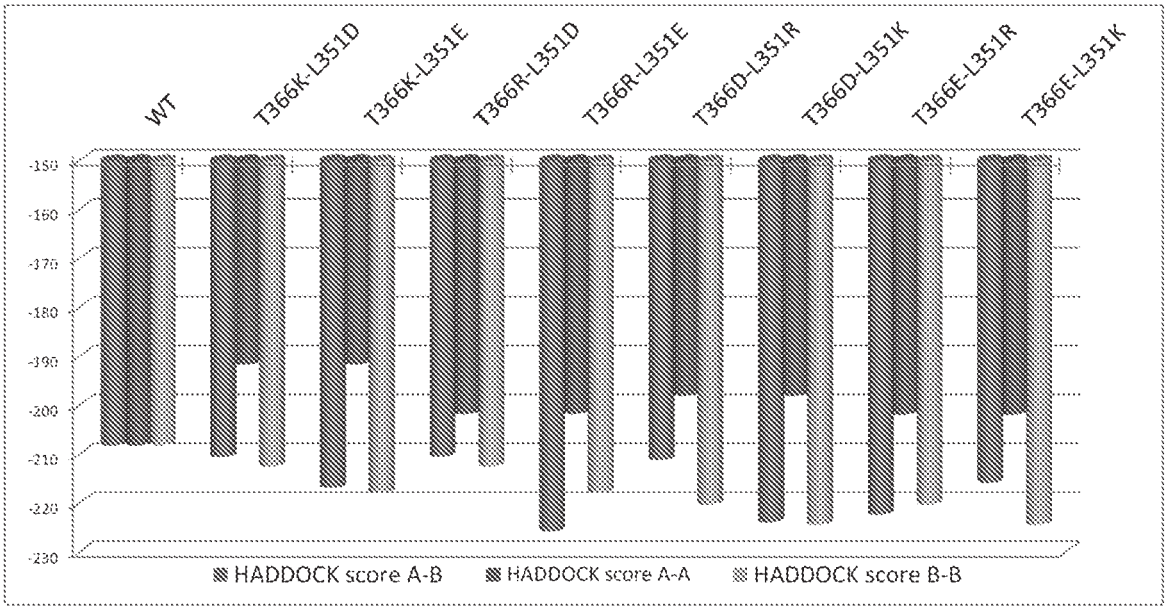
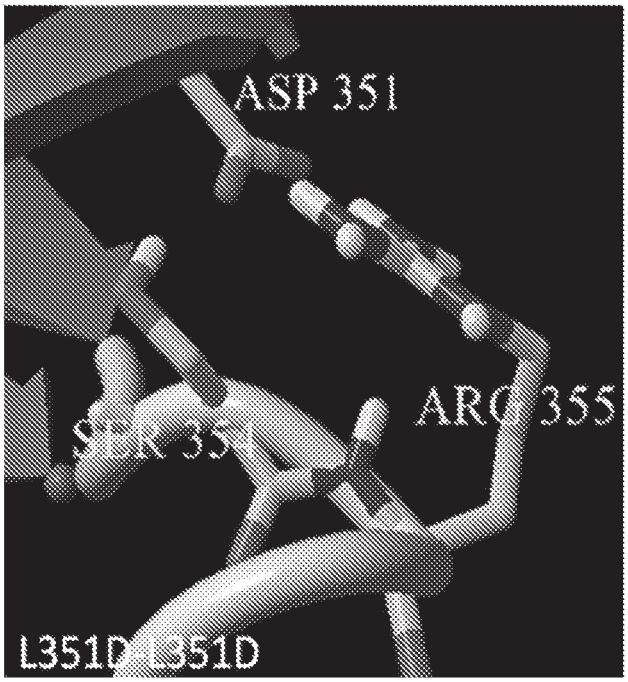

WT
T366K-L351D
T366K-L351E
T366R-L351D
T366R-L351E
T366D-L351R
T366D-L351K
T366E-L351R
T366E-L351K
-150
-160
-170
-180
-190
-200
-210
-220
-230
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Fig 12:

A


ASP 351
ARG 355
L351D L351D

B

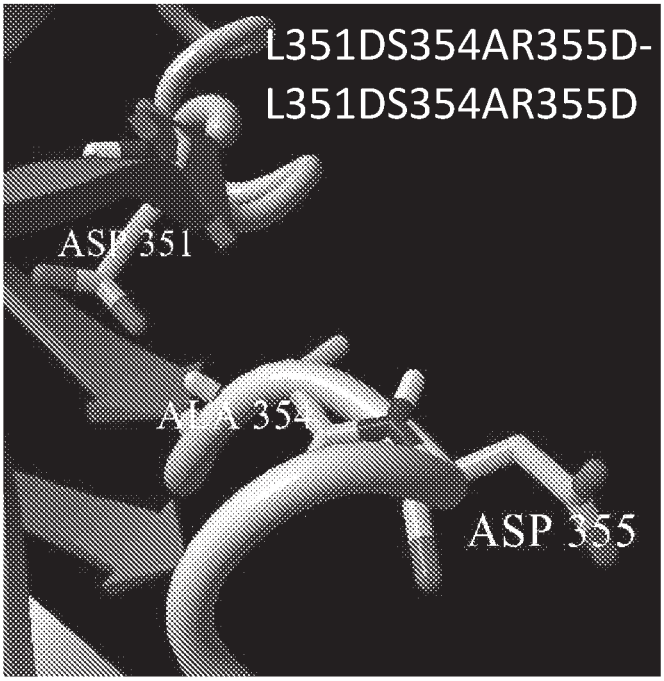

L351DS354AR355D-
L351DS354AR355D
ASP 351
ASP 355

Figure 13:

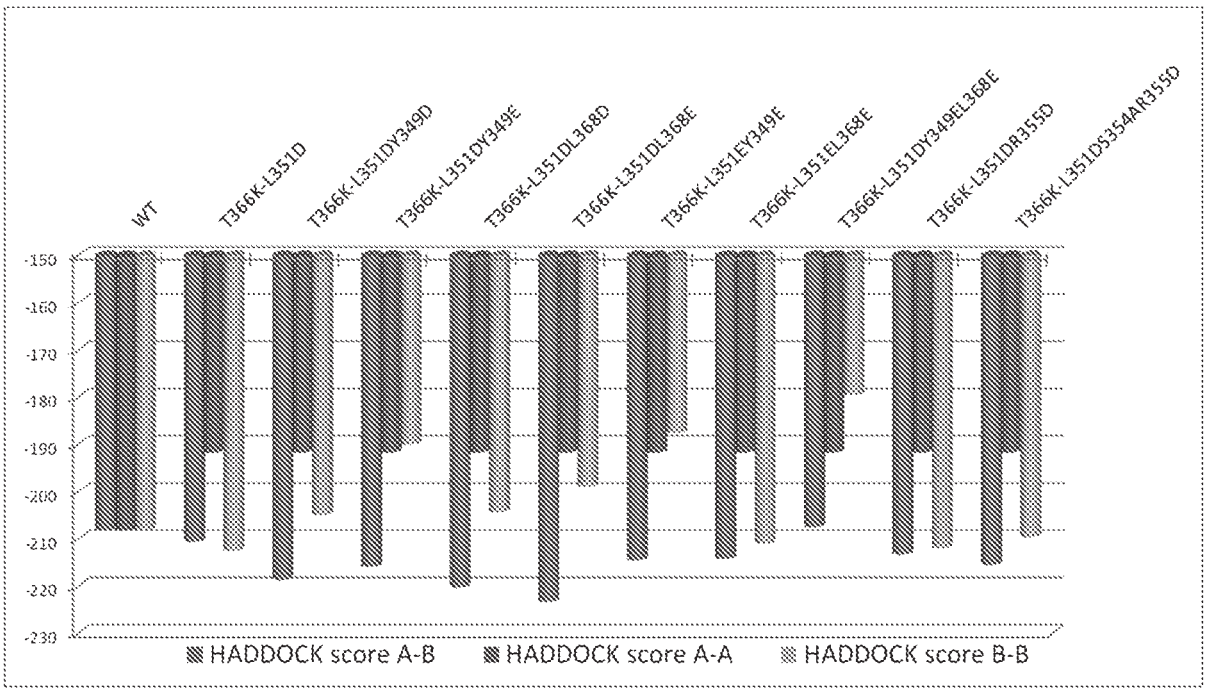

WT
T366K-L351D
T366K-L351DY349D
T366K-L351DY349E
T366K-L351DL368D
T366K-L351DL368E
T366K-L351EY349E
T366K-L351EL368E
T366K-L351DY349EL368E
T366K-L351DR355D
T366K-L351DS354AR355D
-150
-160
-170
-180
-190
-200
-210
-220
-230
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Figure 14:

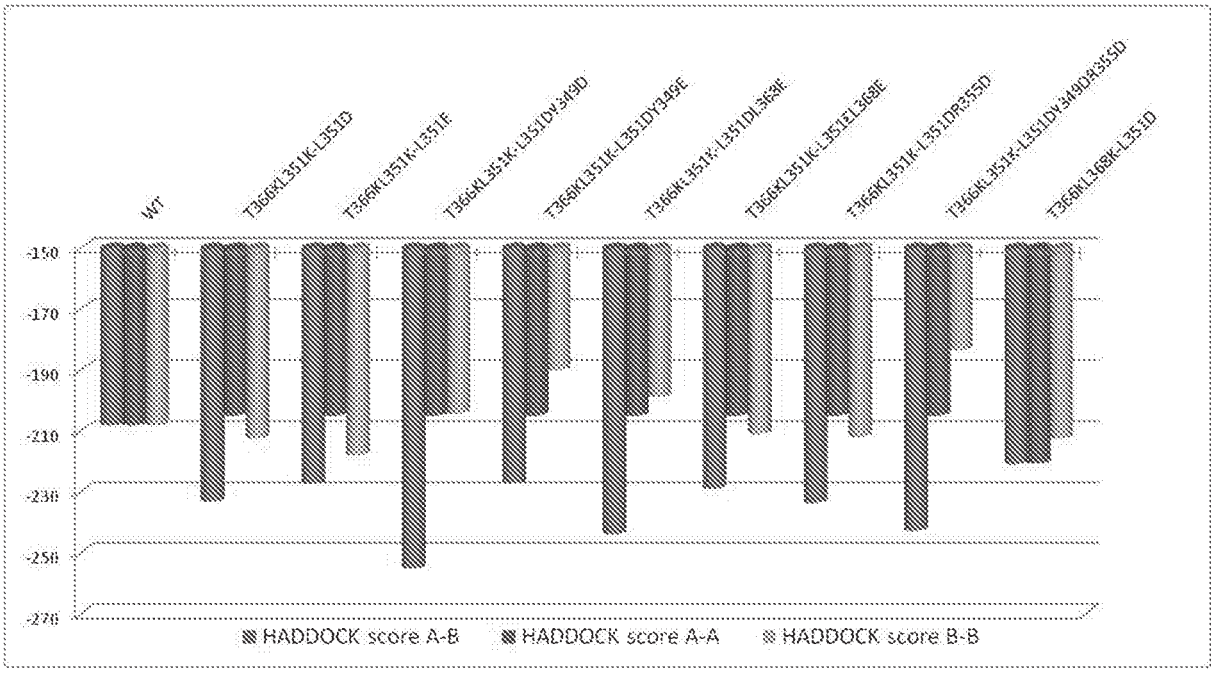

WT
T366KL351K-L351D
T366KL351K-L351E
T366KL351K-L351DY349D
T366KL351K-L351DY349E
T366KL351K-L351DL368E
T366KL351K-L351EL368E
T366KL351K-L351DR355D
T366KL351K-L351DY349DR355D
T366KL368K-L351D
-150
-170
-190
-210
-230
-250
-270
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Figure 15:

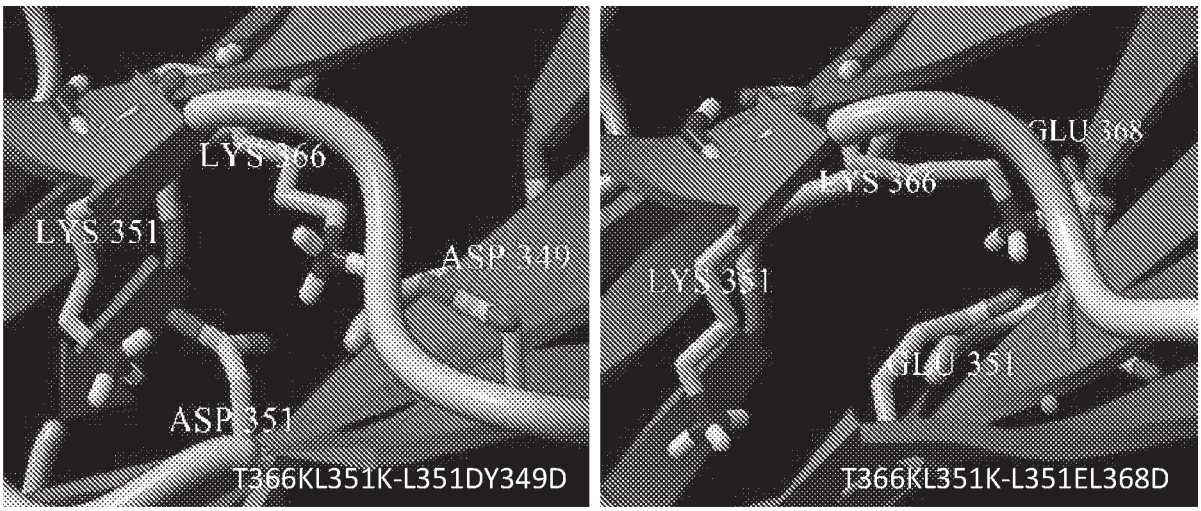

LYS 366
LYS 351
ASP 349
ASP 351
T366KL351K-L351DY349D
GLU 368
LYS 366
LYS 351
GLU 351
T366KL351K-L351EL368D

Figure 16:

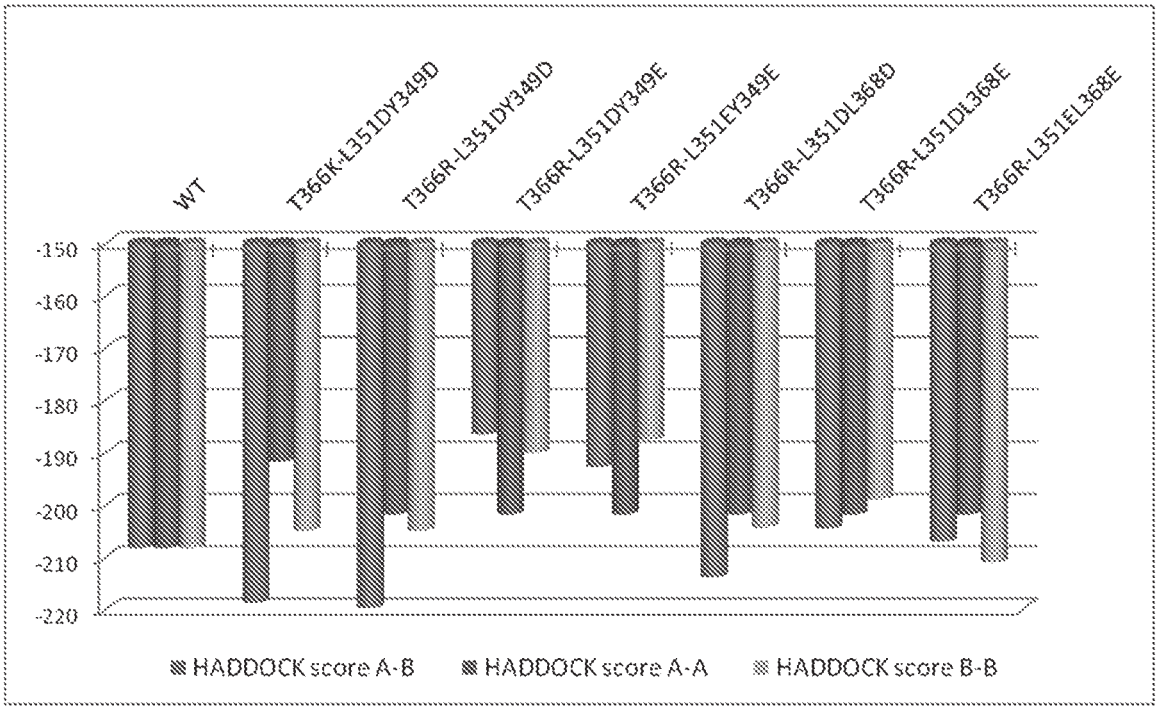

WT
T366K-L351DY349D
T366R-L351DY349D
T366R-L351DY349E
T366R-L351EY349E
T366R-L351DL368D
T366R-L351DL368E
T366R-L351EL368E
-150
-160
-170
-180
-190
-200
-210
-220
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Figure 17:

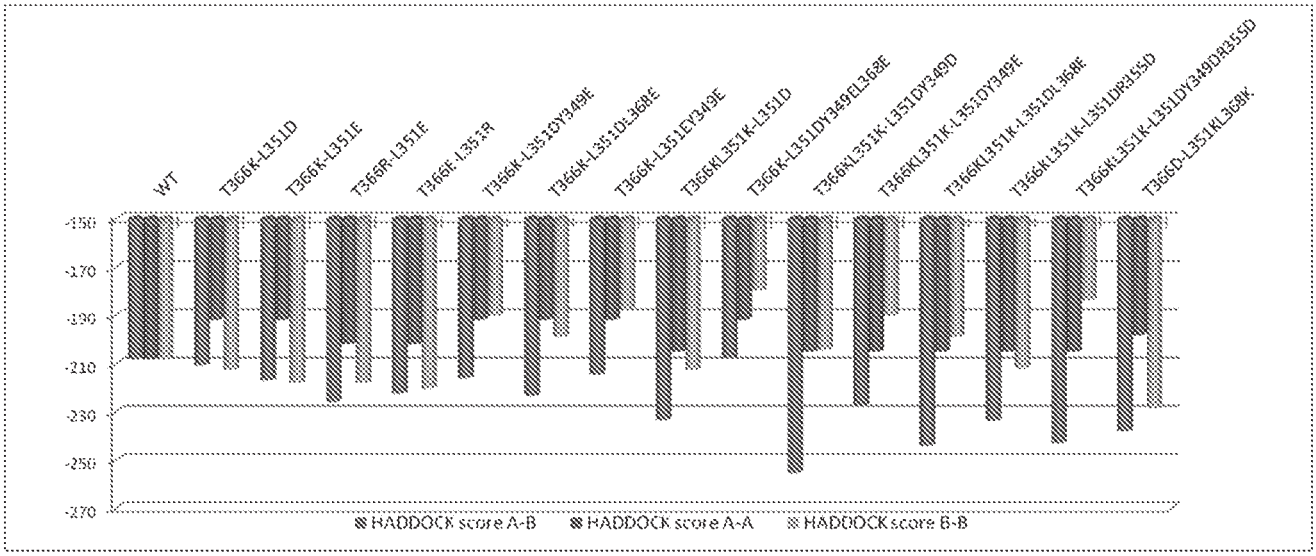

WT
T366K-L351D
T366K-L351E
T366R-L351E
T366E-L351R
T366K-L351DY349E
T366K-L351DE368E
T366K-L351EY349E
T366KL351K-L351D
T366K-L351DY349EL368E
T366KL351K-L351DY349D
T366KL351K-L351DY349E
T366KL351K-L351DE368E
T366KL351K-L351DR355D
T366KL351K-L351DY349DR355D
T366D-L351KL368K
-150
-170
-190
-210
-230
-250
-270
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Figure 18:

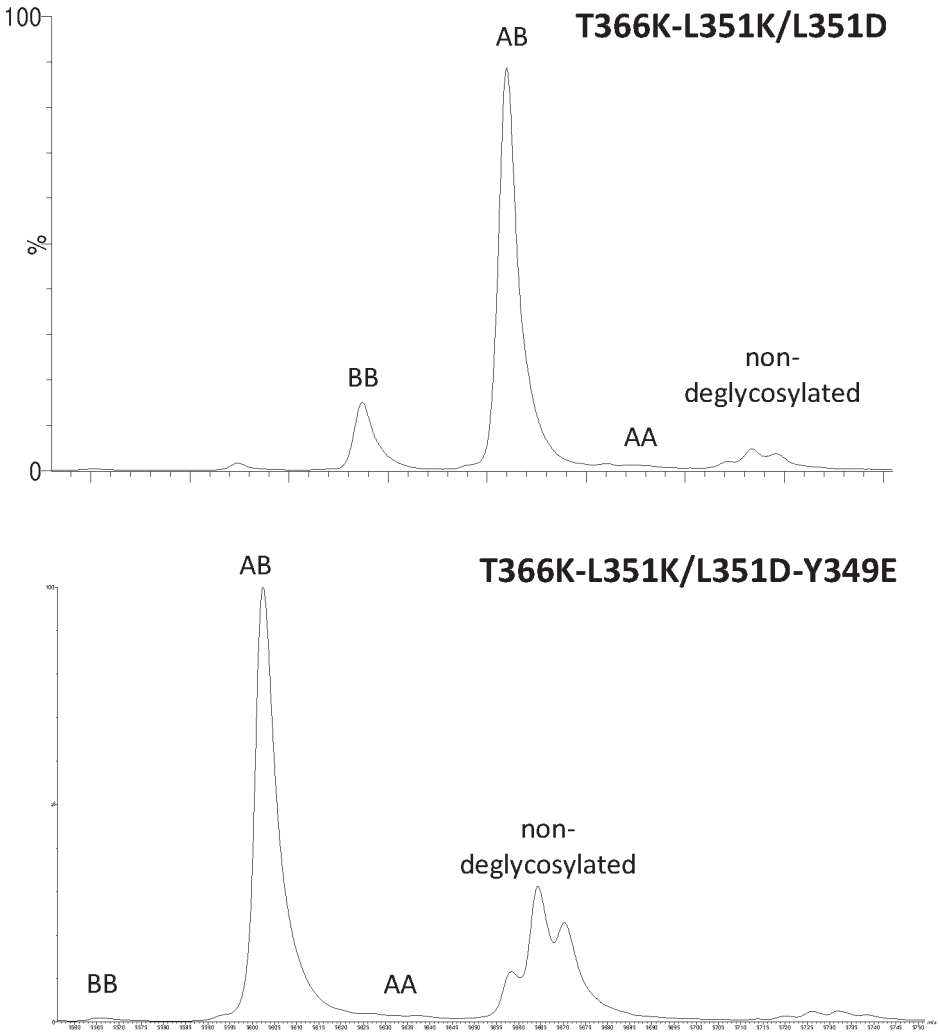

100
%
0
AB
T366K-L351K/L351D
BB
non-
deglycosylated
AA
AB
T366K-L351K/L351D-Y349E
non-
deglycosylated
BB
AA

Figure 19A:

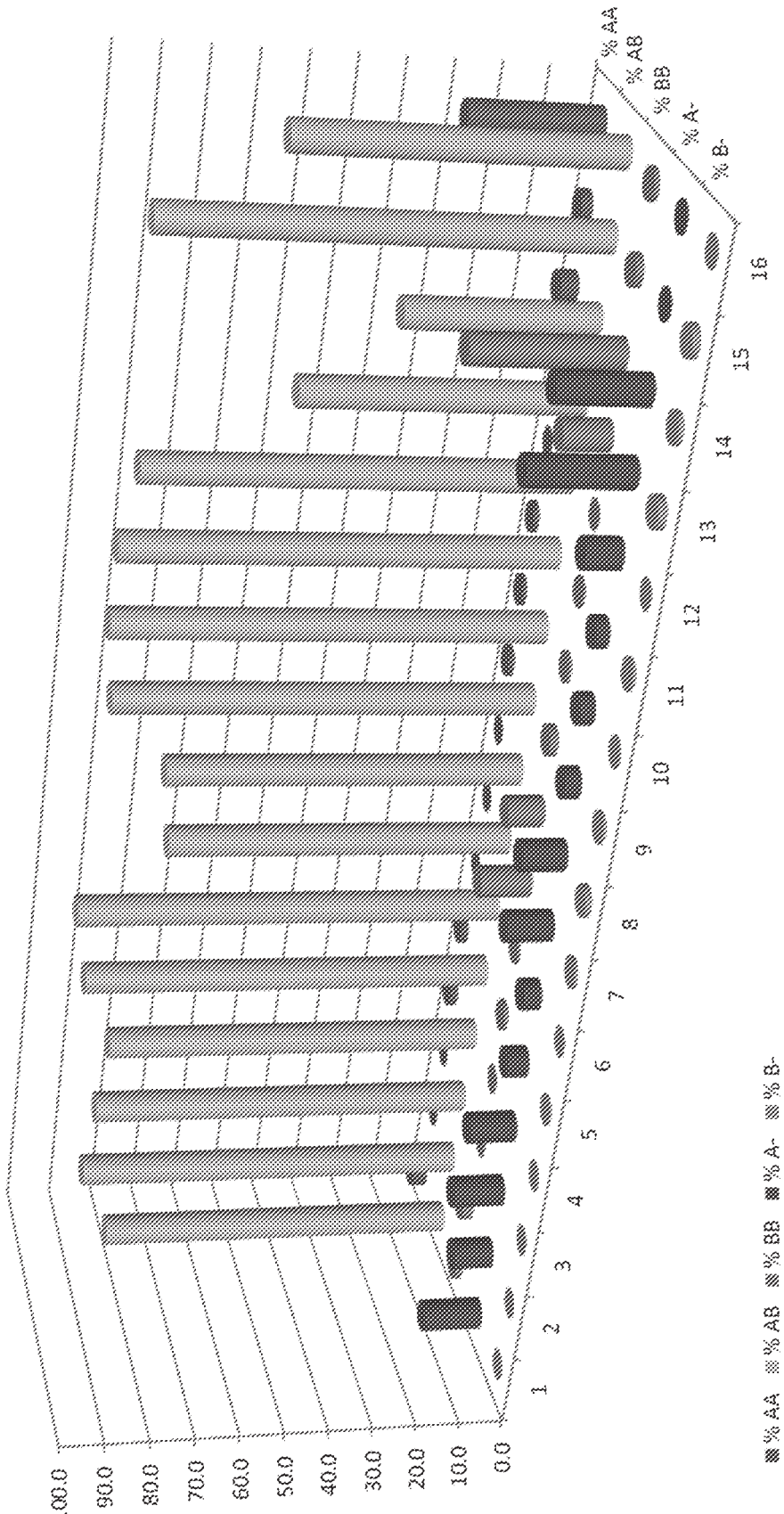

100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
% AA
% AB
% BB
% A-
% B-
% AA
% AB
% BB
% A-
% B-

Figure 19B:

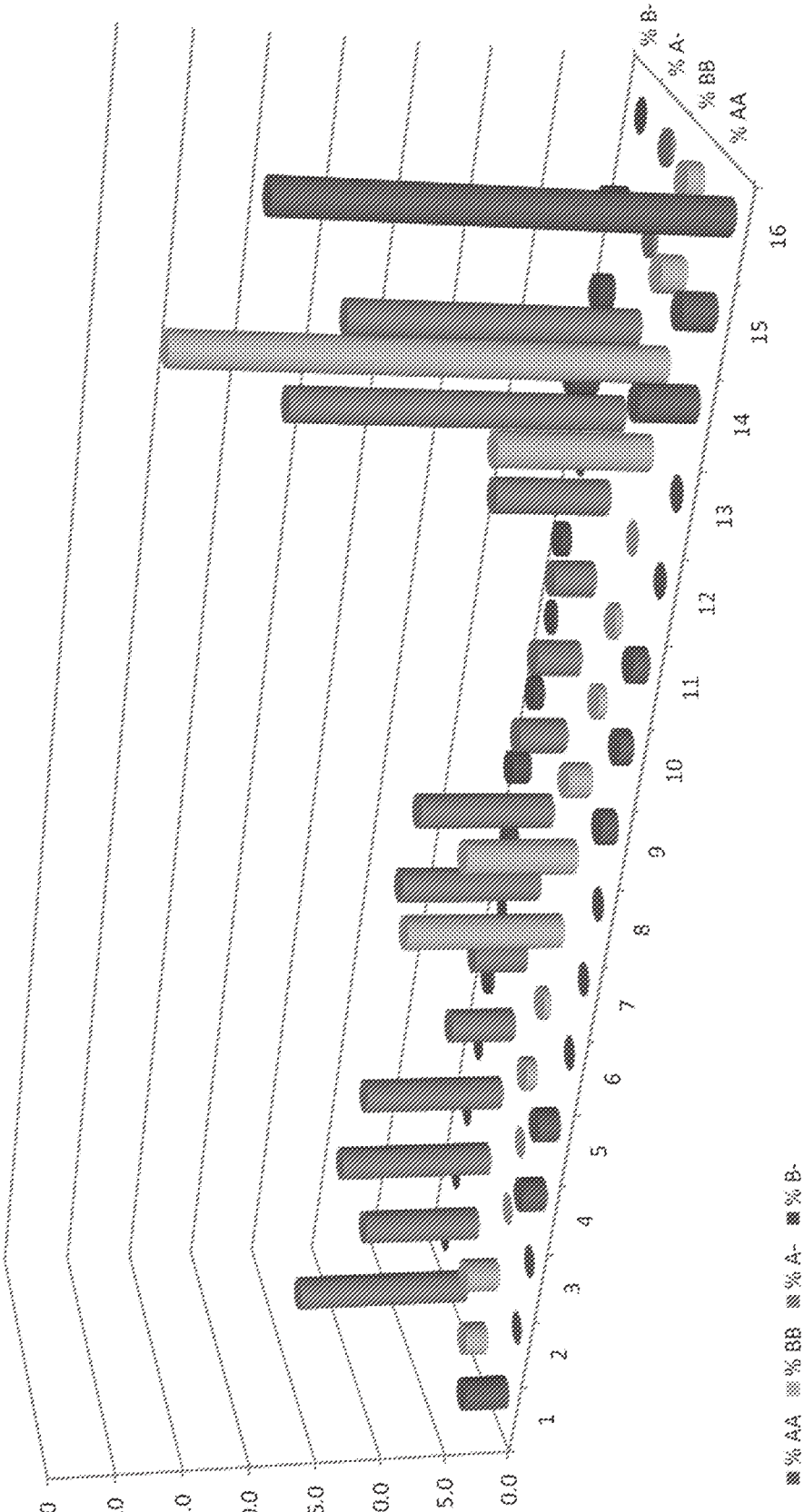

35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
% AA
% BB
% A-
% B-
% AA
% BB
% A-
% B-

Figure 20:

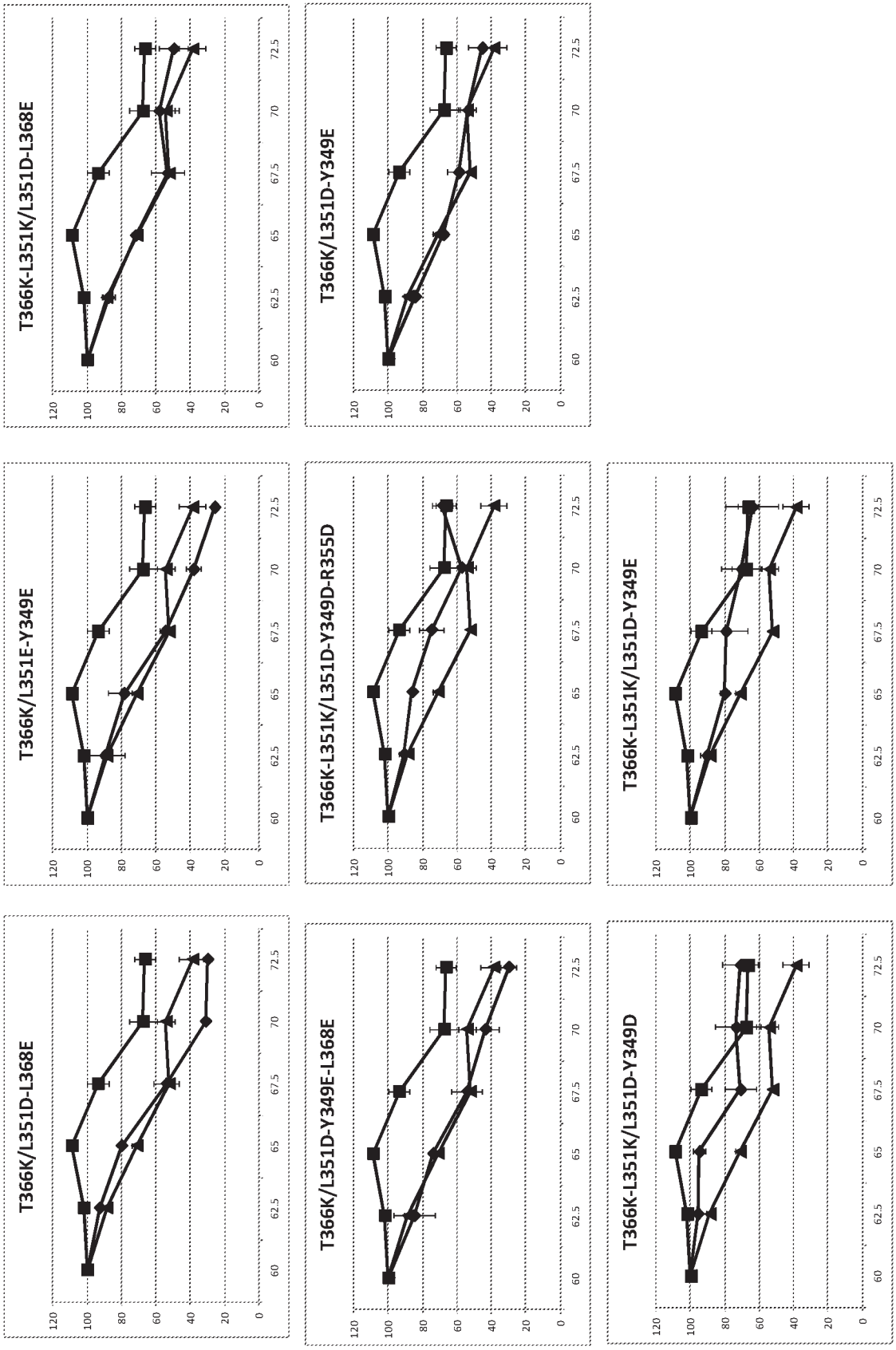

T366K-L351K/L351D-L368E
T366K/L351E-Y349E
T366K-L351K/L351D-Y349D-R355D
T366K/L351D-Y349E
T366K/L351D-L368E
T366K/L351D-Y349E-L368E
T366K-L351K/L351D-Y349E
T366K-L351K/L351D-Y349D
120
100
80
60
40
20
0
60
62.5
65
67.5
70
72.5

Figure 21:

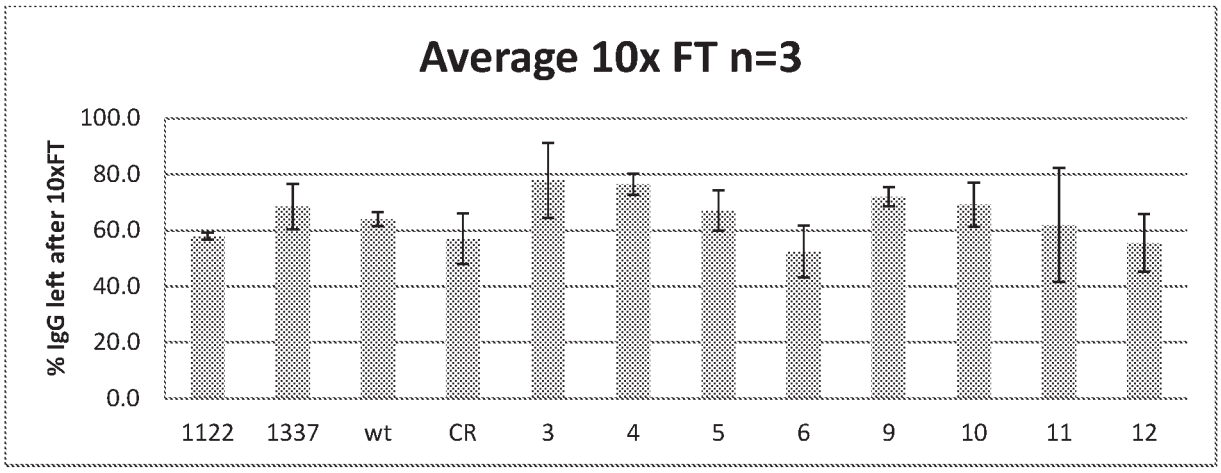
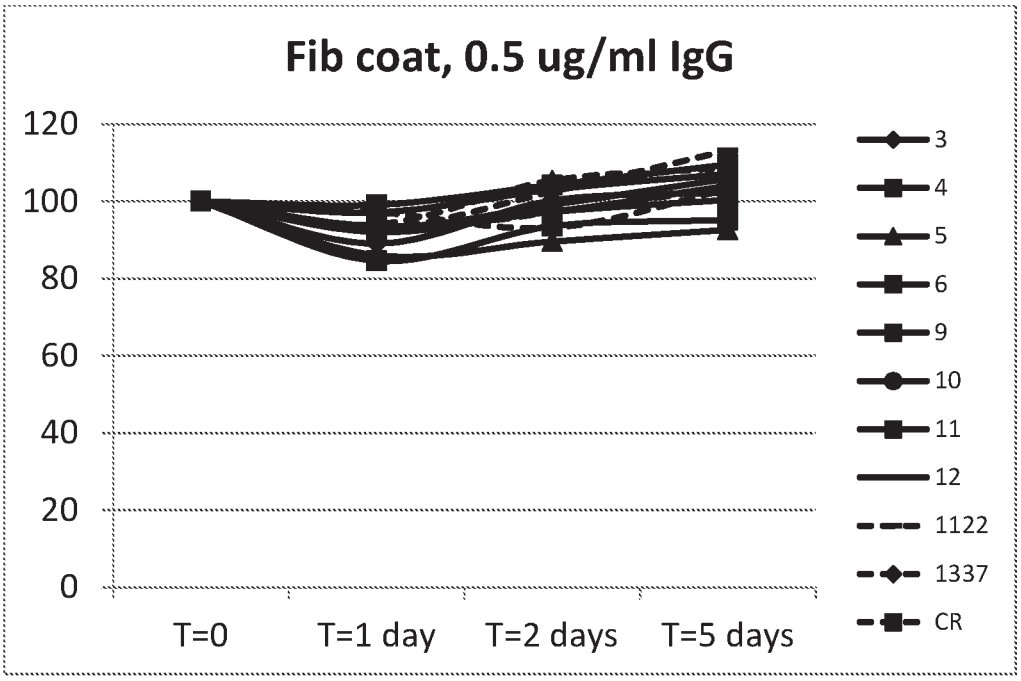

Average 10x FT n=3
% IgG left after 10xFT
100.0
80.0
60.0
40.0
20.0
0.0
1122
1337
wt
CR
3
4
5
6
9
10
11
12

Figure 22:

A


Fib coat, 0.5 ug/ml IgG
120
100
80
60
40
20
0
T=0
T=1 day
T=2 days
T=5 days
3
4
5
6
9
10
11
12
1122
1337
CR

B

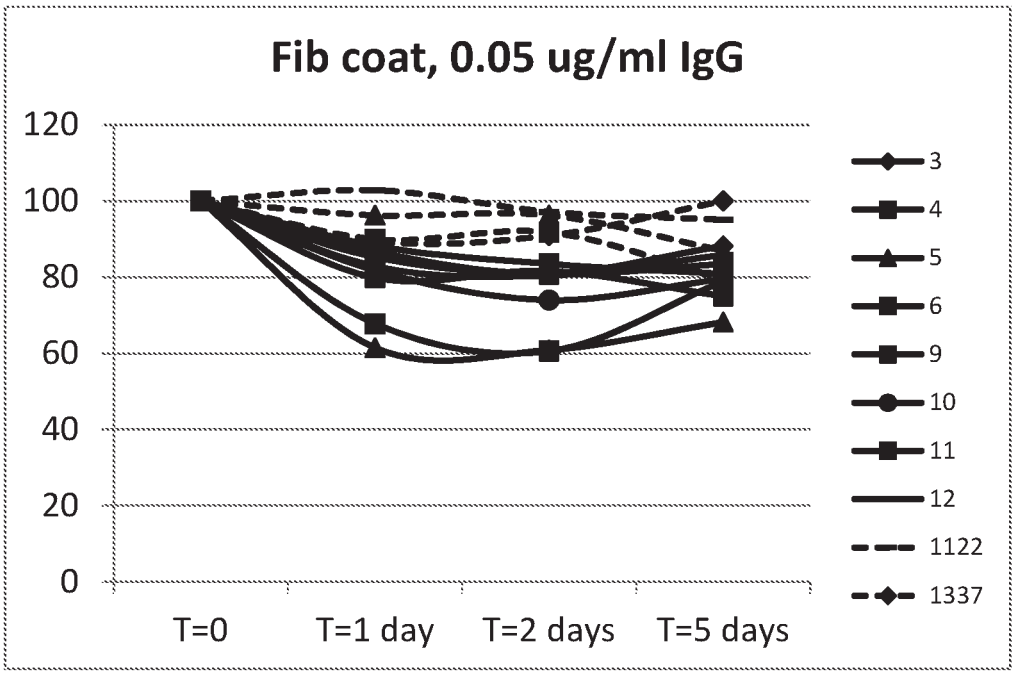
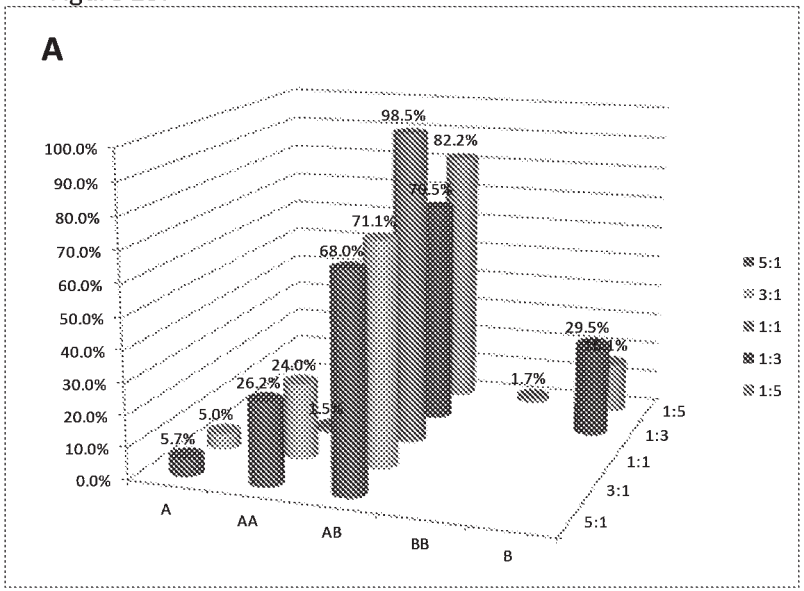
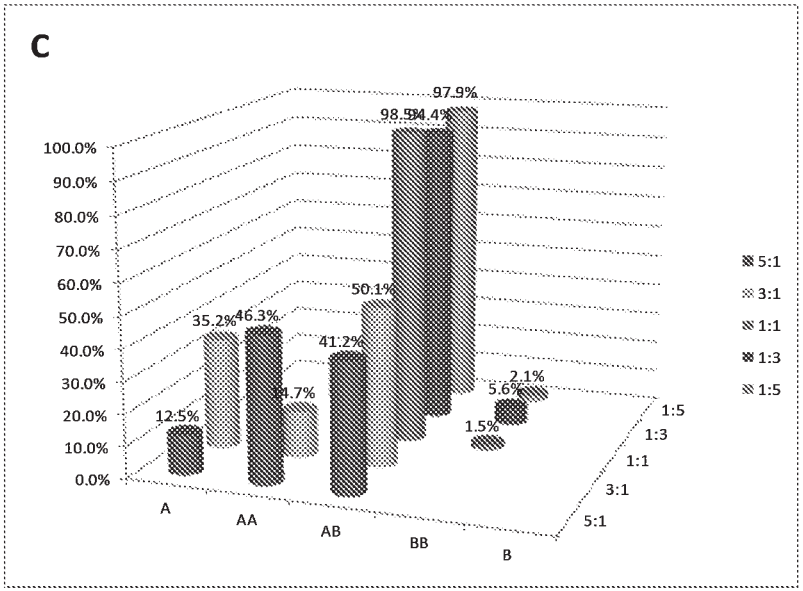
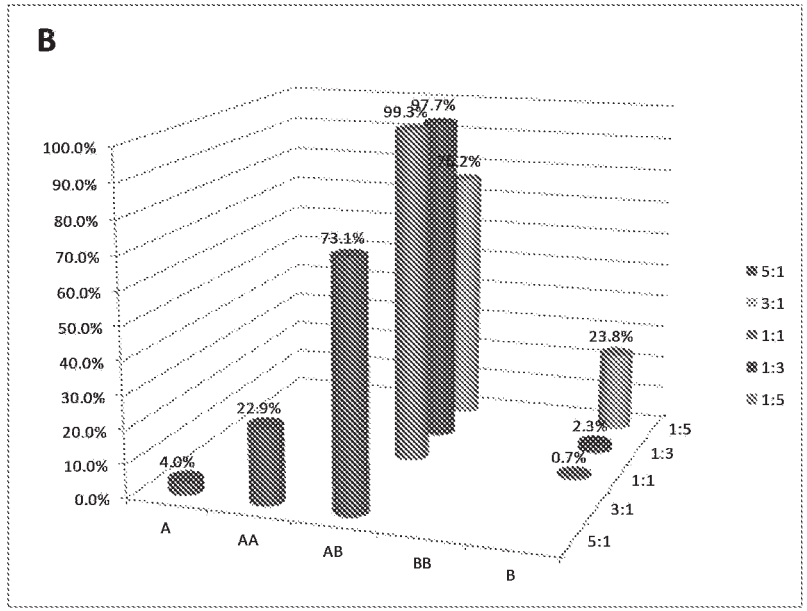

Fib coat, 0.05 ug/ml IgG
120
100
80
60
40
20
0
T=0
T=1 day
T=2 days
T=5 days
3
4
5
6
9
10
11
12
1122
1337

Figure 23:




A

| | 5:1 | 3:1 | 1:1 | 1:3 | 1:5 |
|---|---|---|---|---|---|
| A | 5.7% | 5.0% | | | |
| AA | 26.2% | 24.0% | 1.5% | | |
| AB | 68.0% | 71.1% | 98.5% | 70.5% | 82.2% |
| BB | | | | | 1.7% |
| B | | | | 29.5% | 16.1% |

B

| | 5:1 | 3:1 | 1:1 | 1:3 | 1:5 |
|---|---|---|---|---|---|
| A | 4.0% | | | | |
| AA | 22.9% | | | | |
| AB | 73.1% | | 99.3% | 97.7% | 76.2% |
| BB | | | | | |
| B | | | 0.7% | 2.3% | 23.8% |

C

| | 5:1 | 3:1 | 1:1 | 1:3 | 1:5 |
|---|---|---|---|---|---|
| A | 12.5% | 35.2% | | | |
| AA | 46.3% | 14.7% | | | |
| AB | 41.2% | 50.1% | 98.5% | 94.4% | 97.9% |
| BB | | | 1.5% | 5.6% | 2.1% |
| B | | | | | |

Figure 24:

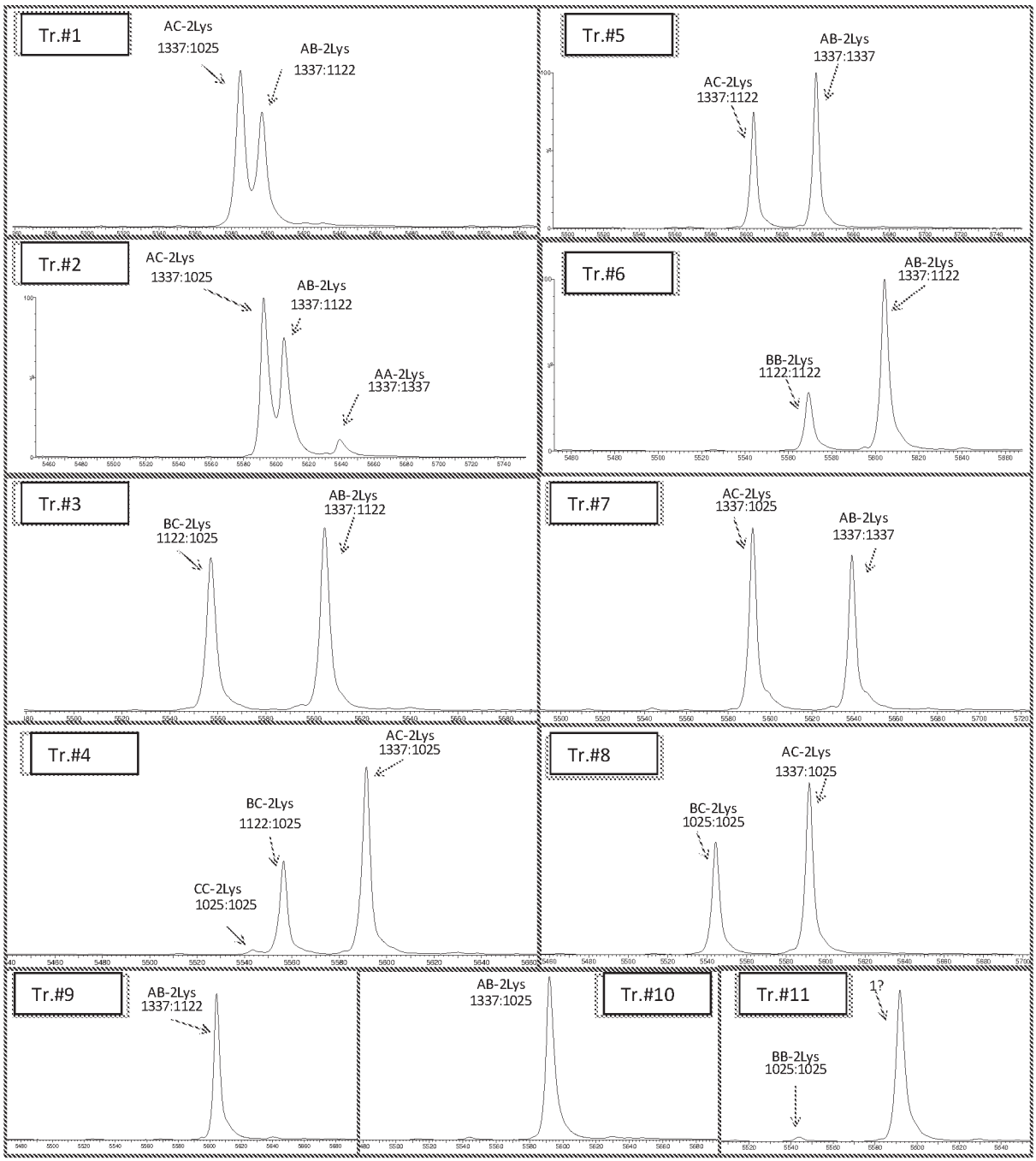

Tr.#1
AC-2Lys
1337:1025
AB-2Lys
1337:1122
Tr.#5
AB-2Lys
1337:1337
AC-2Lys
1337:1122
Tr.#2
AC-2Lys
1337:1025
AB-2Lys
1337:1122
AA-2Lys
1337:1337
Tr.#6
AB-2Lys
1337:1122
BB-2Lys
1122:1122
Tr.#3
AB-2Lys
1337:1122
BC-2Lys
1122:1025
Tr.#7
AC-2Lys
1337:1025
AB-2Lys
1337:1337
Tr.#4
AC-2Lys
1337:1025
BC-2Lys
1122:1025
CC-2Lys
1025:1025
Tr.#8
AC-2Lys
1337:1025
BC-2Lys
1025:1025
Tr.#9
AB-2Lys
1337:1122
AB-2Lys
1337:1025
Tr.#10
Tr.#11
1?
BB-2Lys
1025:1025

Figure 25:

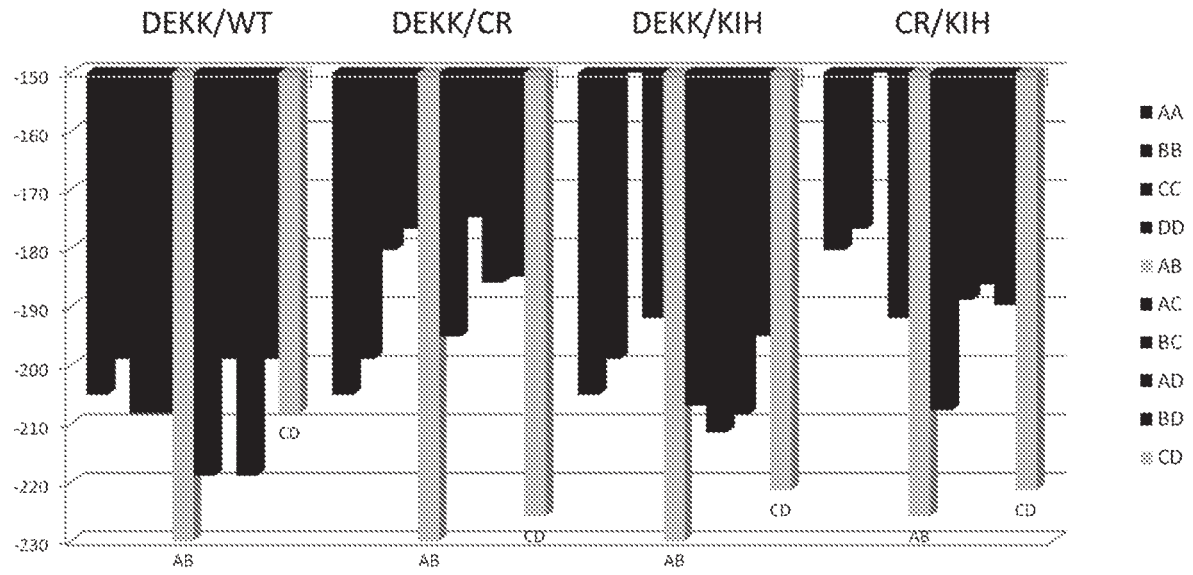

DEKK/WT
DEKK/CR
DEKK/KIH
CR/KIH
-150
-160
-170
-180
-190
-200
-210
-220
-230
AB
AB
AB
AB
CD
CD
CD
CD
AA
BB
CC
DD
AB
AC
BC
AD
BD
CD

Figure 26:

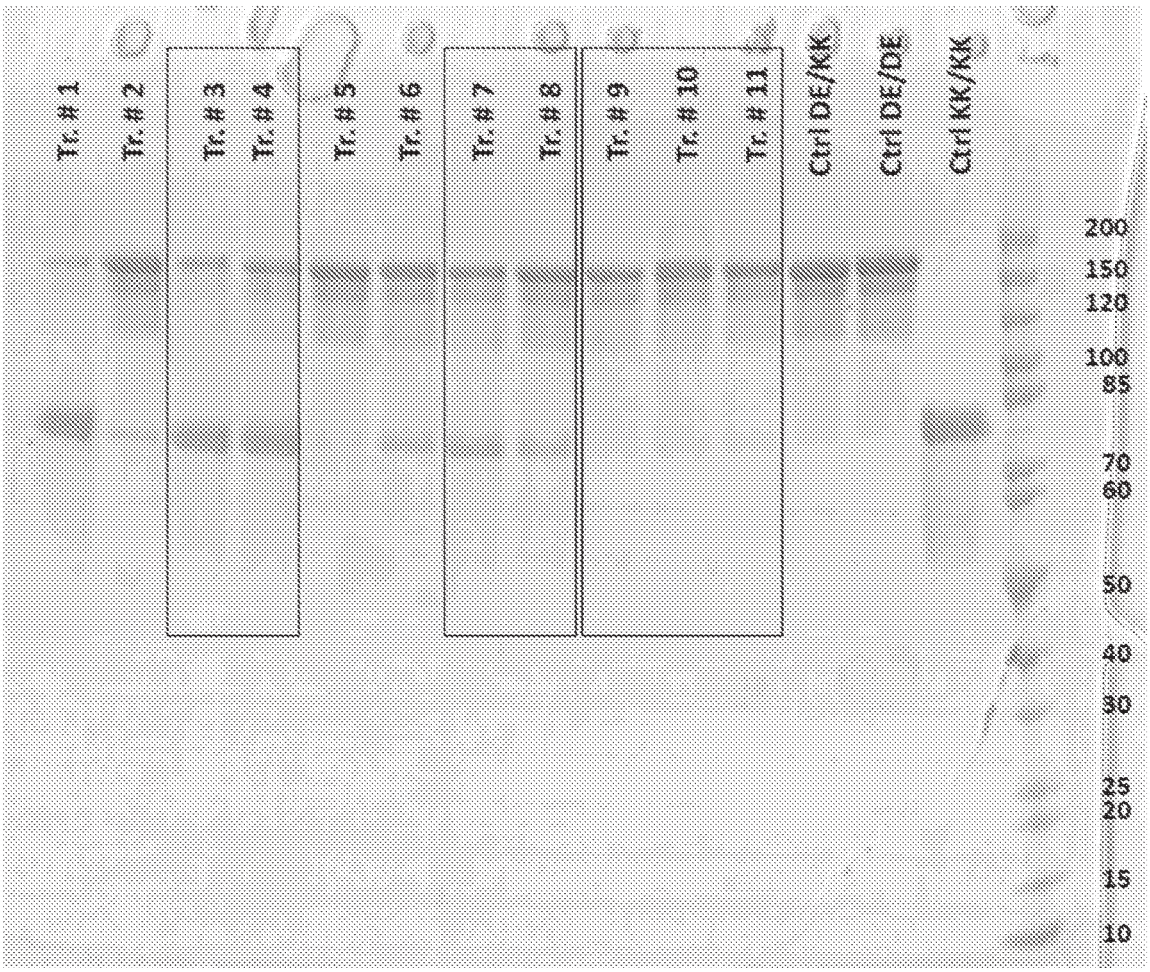

Tr. # 1
Tr. # 2
Tr. # 3
Tr. # 4
Tr. # 5
Tr. # 6
Tr. # 7
Tr. # 8
Tr. # 9
Tr. # 10
Tr. # 11
Ctrl DE/KK
Ctrl DE/DE
Ctrl KK/KK
200
150
120
100
85
70
60
50
40
30
25
20
15
10

Figure 27A:

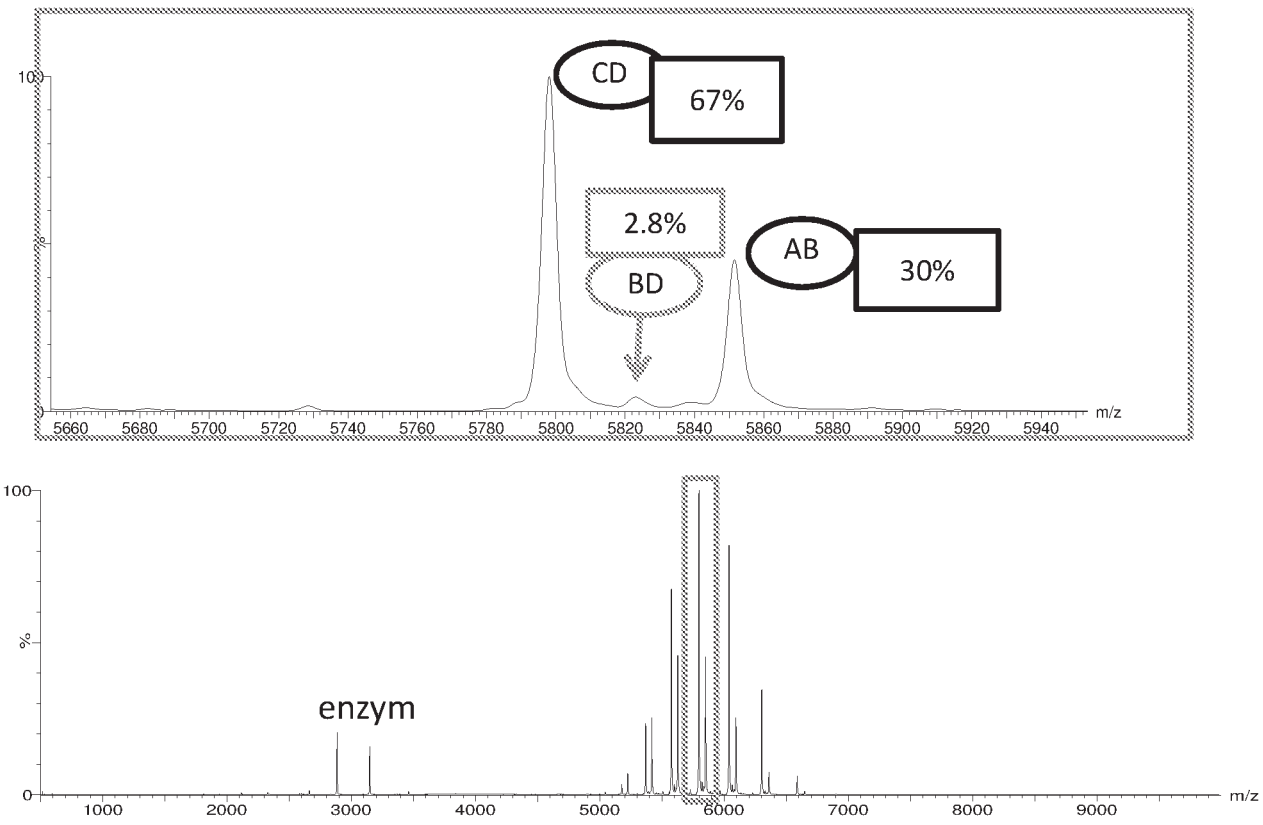
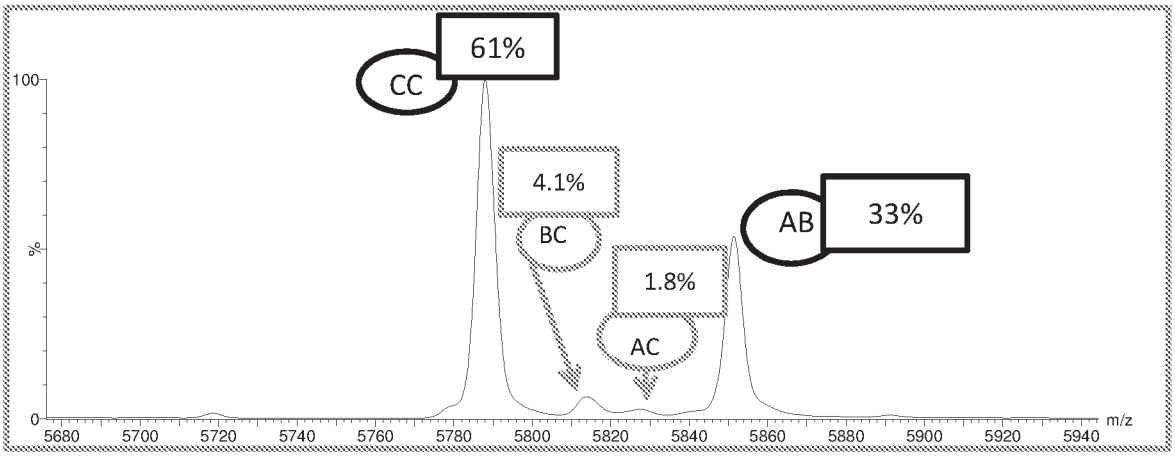

100
%
CD
67%
2.8%
BD
AB
30%
m/z
5660
5680
5700
5720
5740
5760
5780
5800
5820
5840
5860
5880
5900
5920
5940
100
%
enzym
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
m/z

Figure 27B:


61%
CC
4.1%
BC
1.8%
AC
AB
33%
100
%
0
5680
5700
5720
5740
5760
5780
5800
5820
5840
5860
5880
5900
5920
5940
m/z

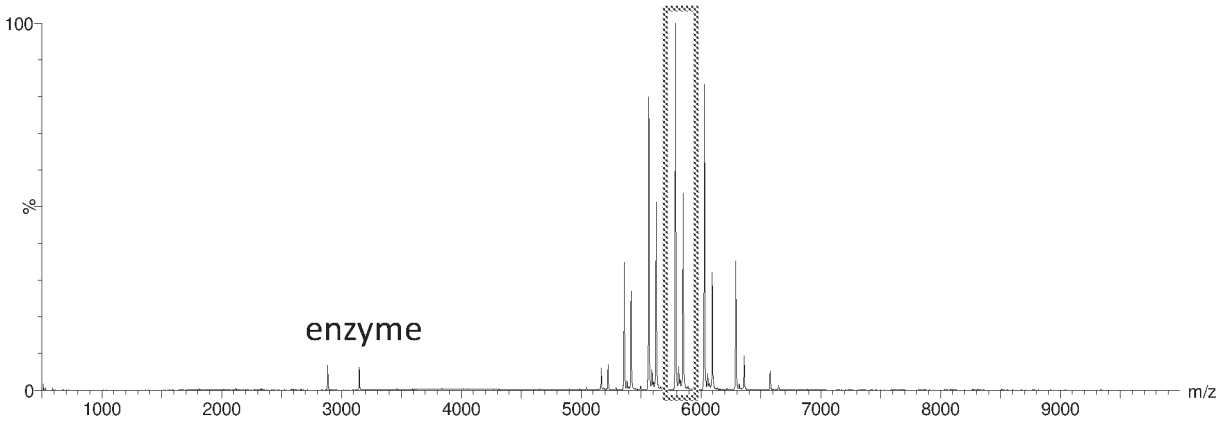

100
%
0
enzyme
1000
2000
3000
4000
5000
6000
7000
8000
9000
m/z

Figure 28:

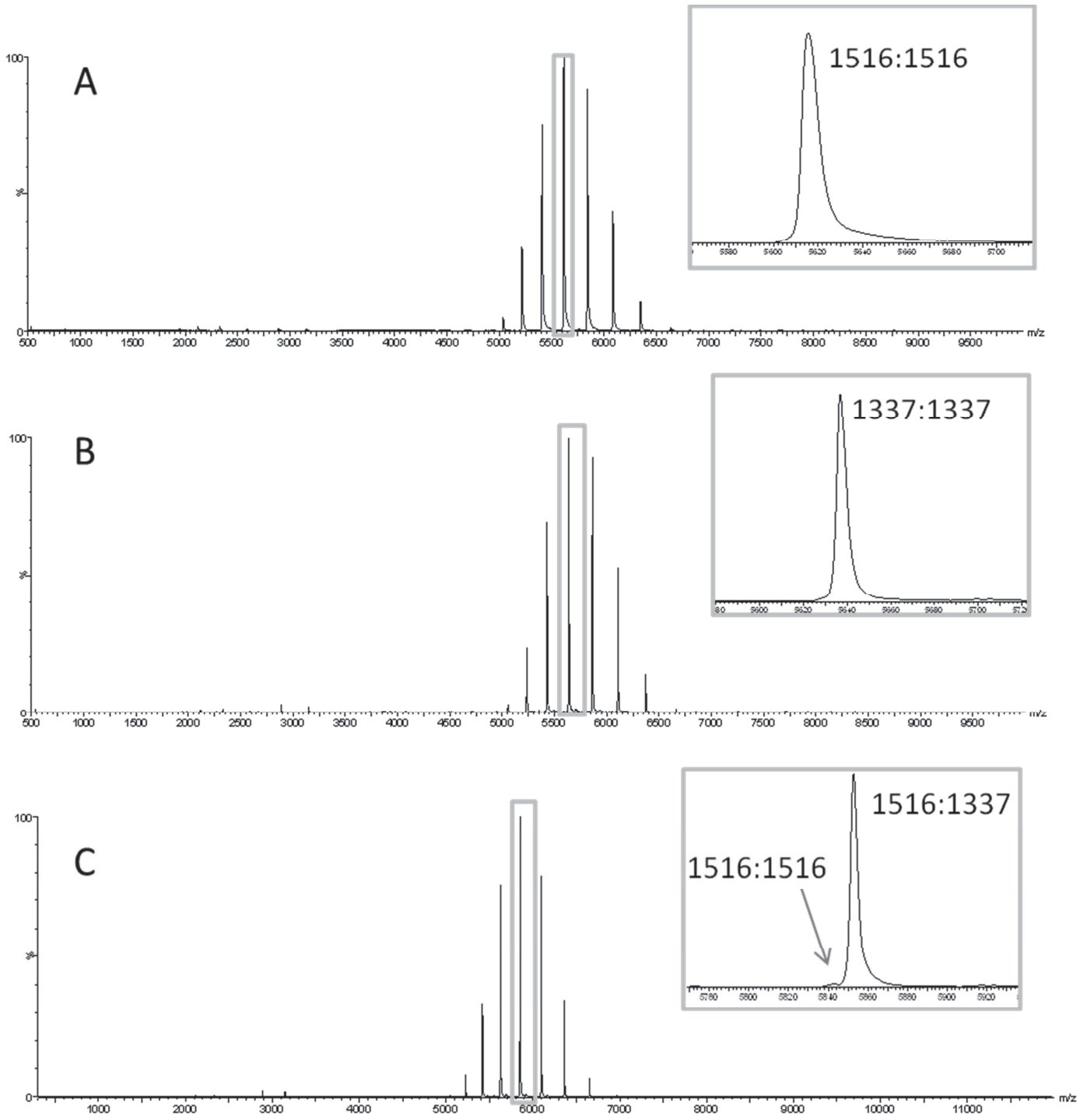
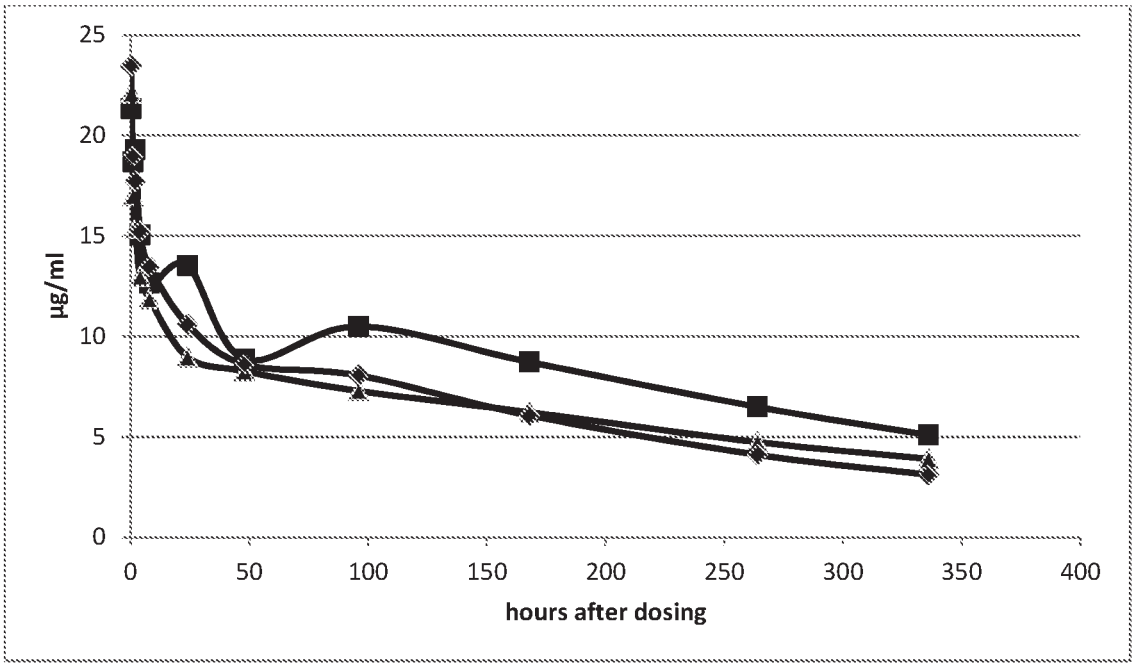


Figure 29:


25
20
15
10
5
0
µg/ml
0
50
100
150
200
250
300
350
400
hours after dosing