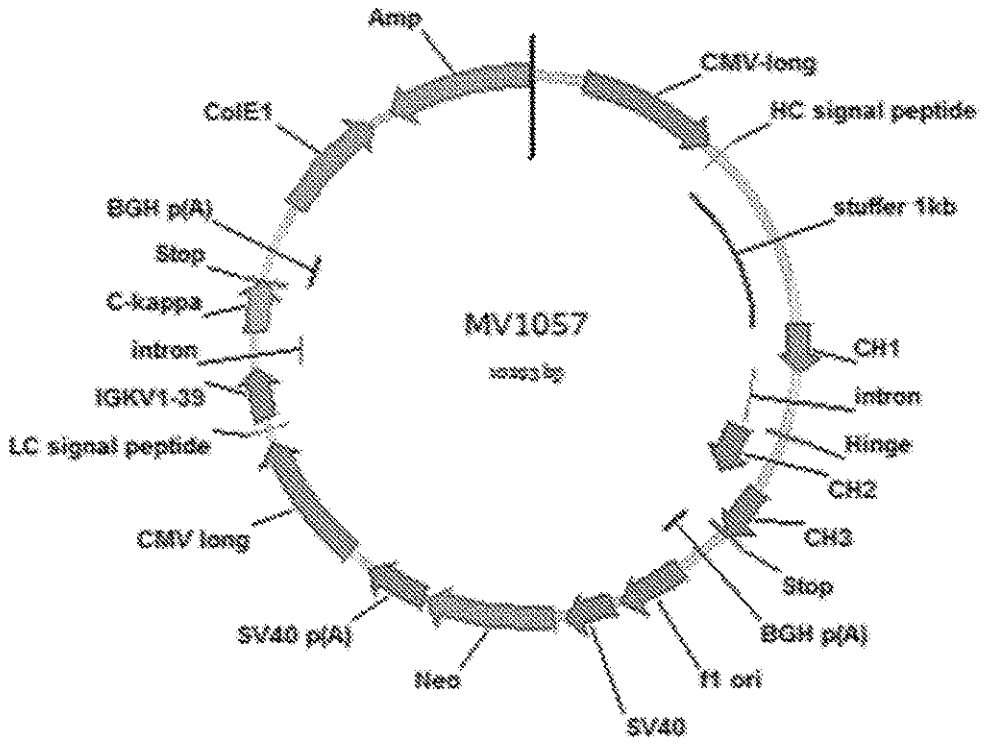
Figure 1:

A) schematic representation of construct vector MV1057



B) schematic representation of phage display vector MV1043

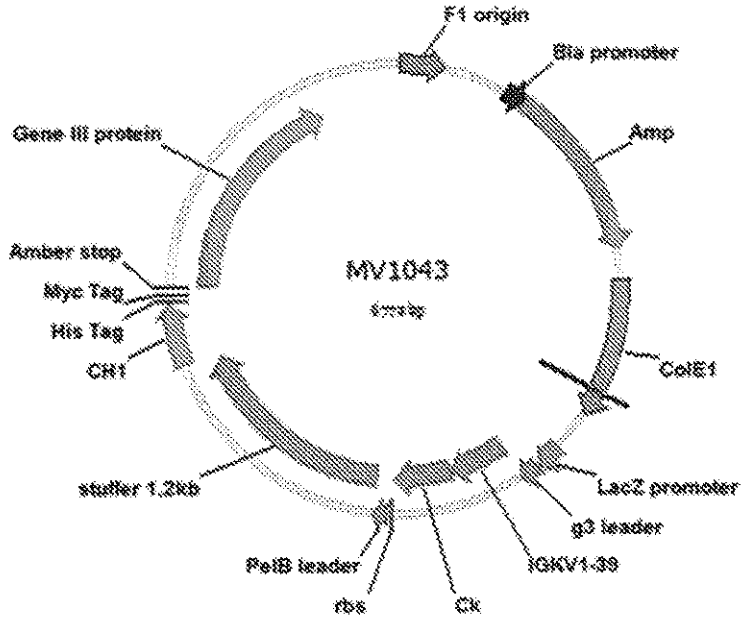


Figure 2: amino acid sequence of wildtype IgG1 Fc, as present in construct vector MV1057 (EU numbering scheme applied)

131 141 151 161 171 181

*SSK*STSGGTA 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

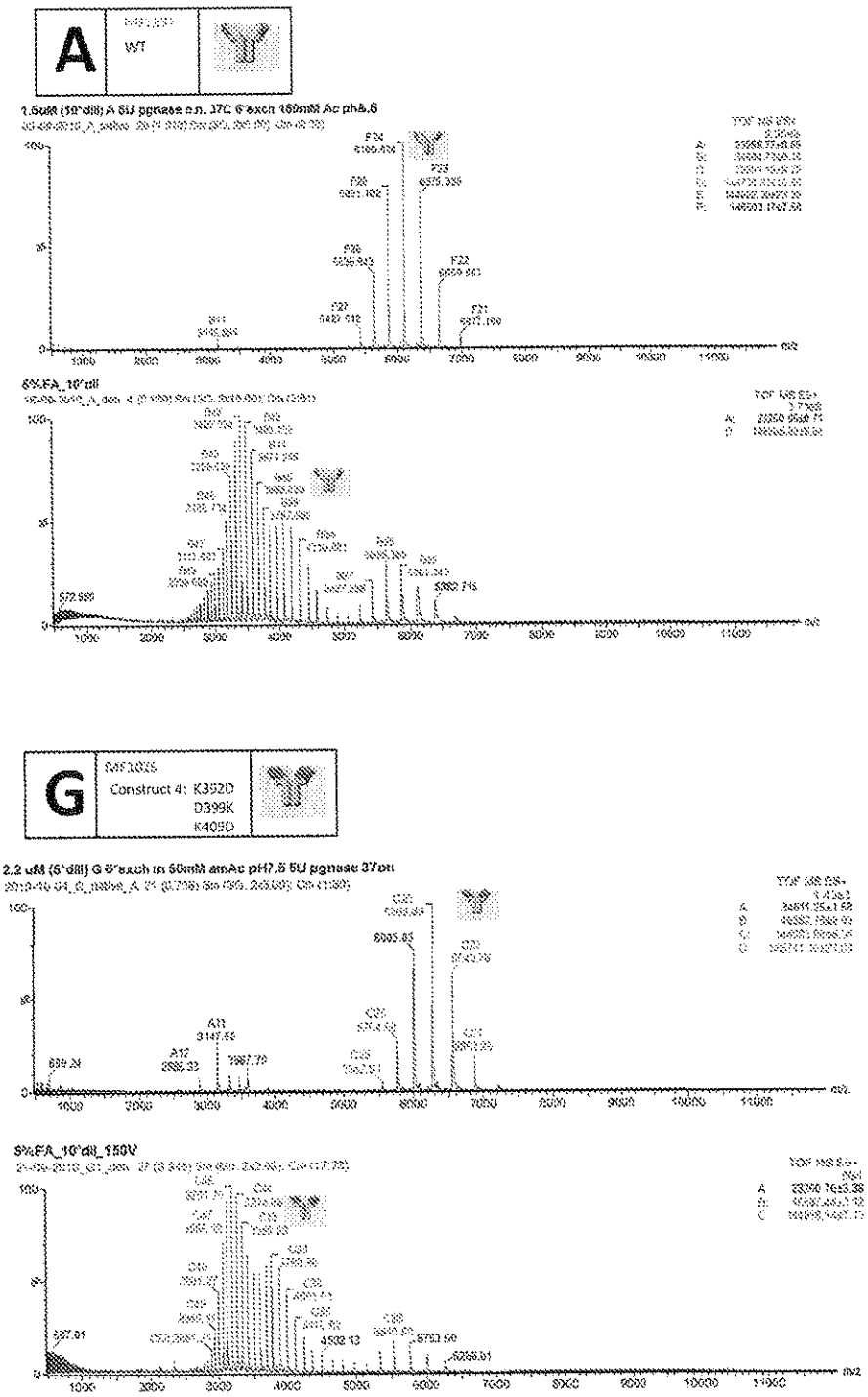
MF1122_VH
gaggtgcagctggtggagtctgggggaggcgtggtccagcctgggaggtccctgagactctcctgtgcag
cctctggattcaccttcagtagctatggcatgcactgggtccgccaggctccaggcaaggggctggagtg
ggtggcagttatatcatatgatggaagtaataaatactatgcagactccgtgaagggccgattcaccatc
tccagagacaattccaagaacacgctgtatctgcaaatgaacagcctgagagctgaggacacggccgtgt
attactgtgcaagagccctcttcacgaccatcgccatggactattggggccaaggtaccctggtcacc

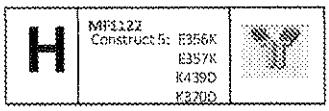
MF1122_VH
EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTI
SRDNSKNTLYLQMNSLRAEDTAVYYCARALFTTIAMDYWGQGTLVT

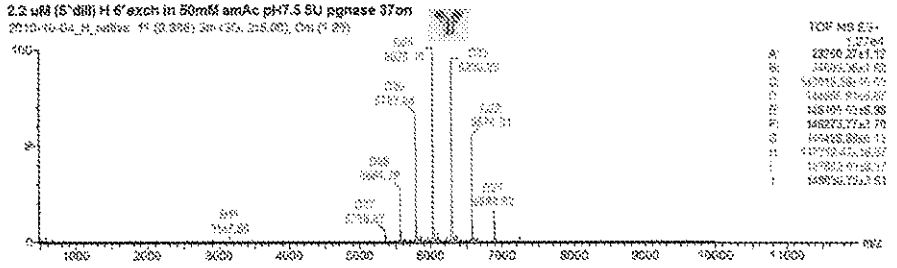
MF1337 VH
gaggtgcagctggtggagactggggctgaggtgaagaagccgggggcctcagtgaaggtctcctgcaaggcttct
gactacatcttcaccaaatatgacatcaactgggtgcgccaggcccctggacaagggcttgaatggatgggatgg
atgagcgctaacactggaaacacgggctatgcacagaagttccagggcagagtcaccatgaccagggacacgtcc
ataaacacagcctacatggagctgagcagcctgacatctggtgacacggccgtttatttctgtgcgaggagtagt
cttttcaagacagagacggcgccctactatcacttcgctctggacgtctggggccaagggaccacggtcacc

MF1337
VHEVQLVETGAEVKKPGASVKVSCKASDYIFTKYDINWVRQAPGQGLEWMGWMSANTGNTGYAQKFQGRVTMTRD
TSINTAYMELSSLTSGDTAVYFCARSSLFKTETAPYYHFALDVWGQGTTVT

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




H
M1912Z
Construct 5: E356K
E357K
K439D
K370D


2.3 ul8 (5`dil8) H 6'exch in 50mM amAc pH7.5 5U pgnase 37on
TOF MS ES+

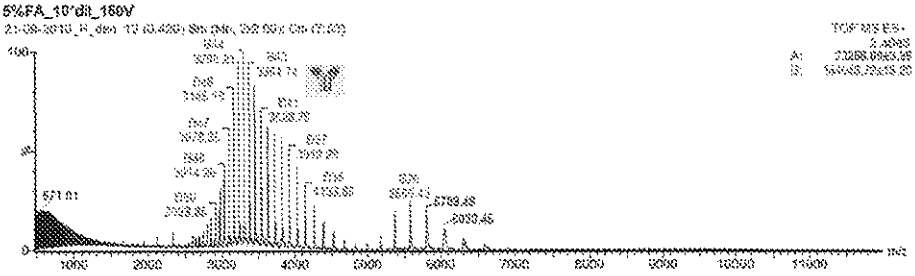

5%FA_10'dil_160V
TOF MS ES+
571.01
5789.48
6030.45

Figure 5

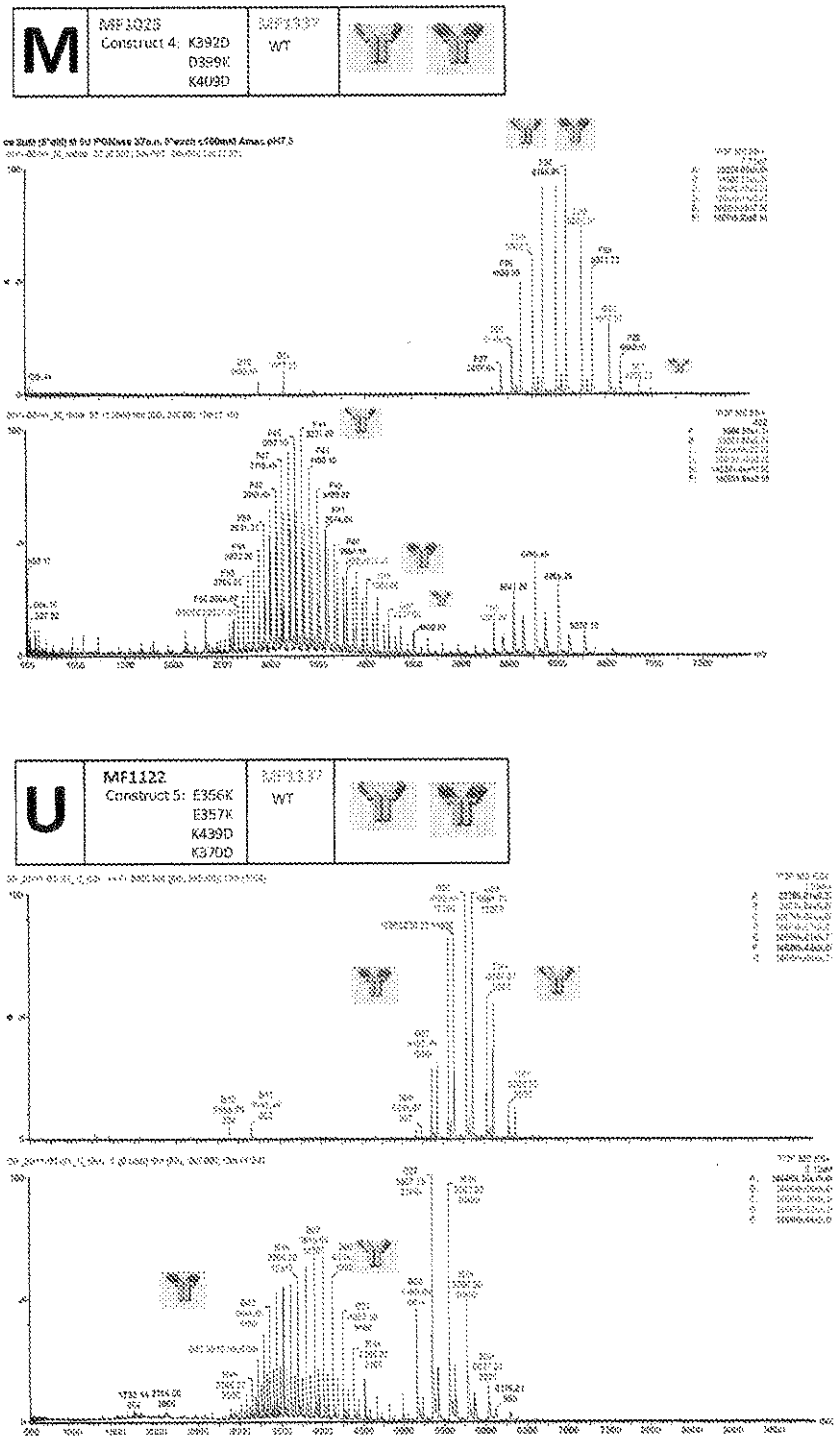

M
MF1023
Construct 4: K392D
D399K
K409D
WT
U
MF1122
Construct 5: E356K
E357K
K439D
K370D
WT

Figure 6:

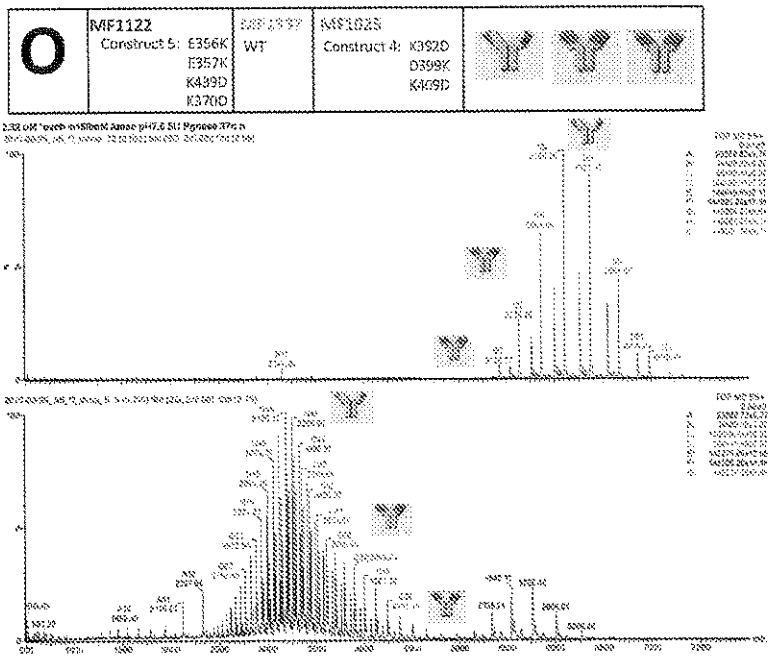
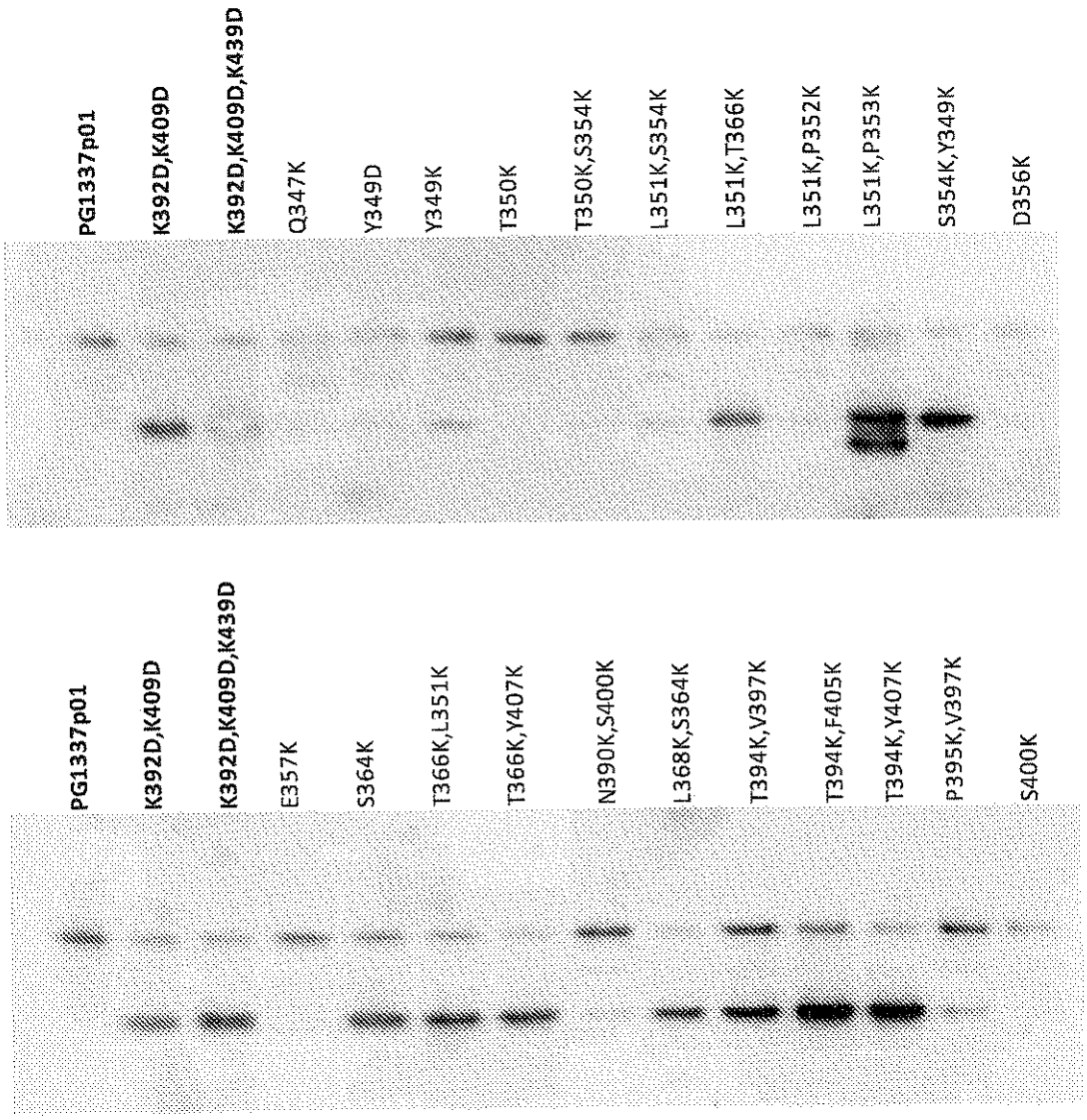
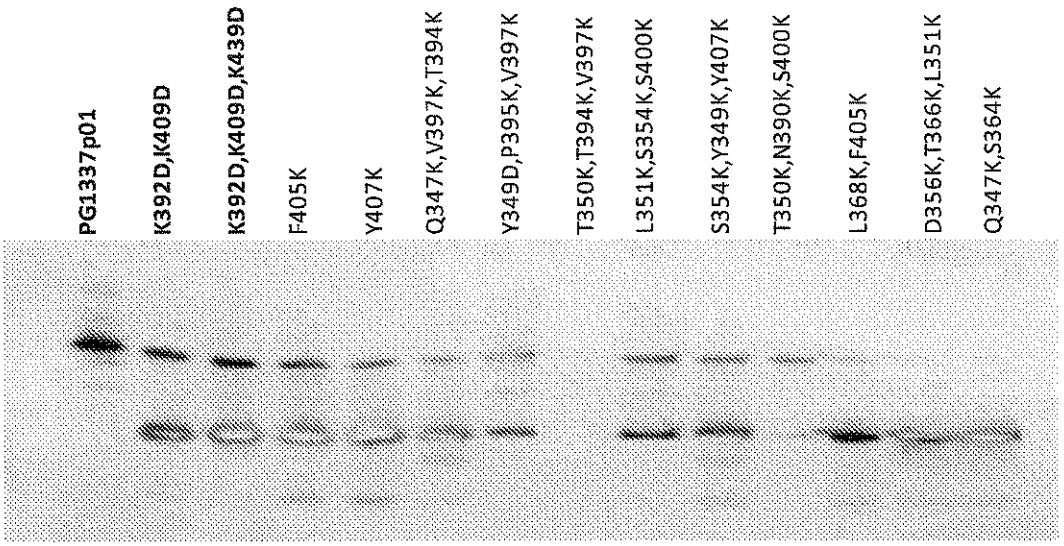

MF1122
Construct 5: E356K
E357K
K439D
K370D
WT
MF1025
Construct 4: K392D
D398K
K409D

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
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

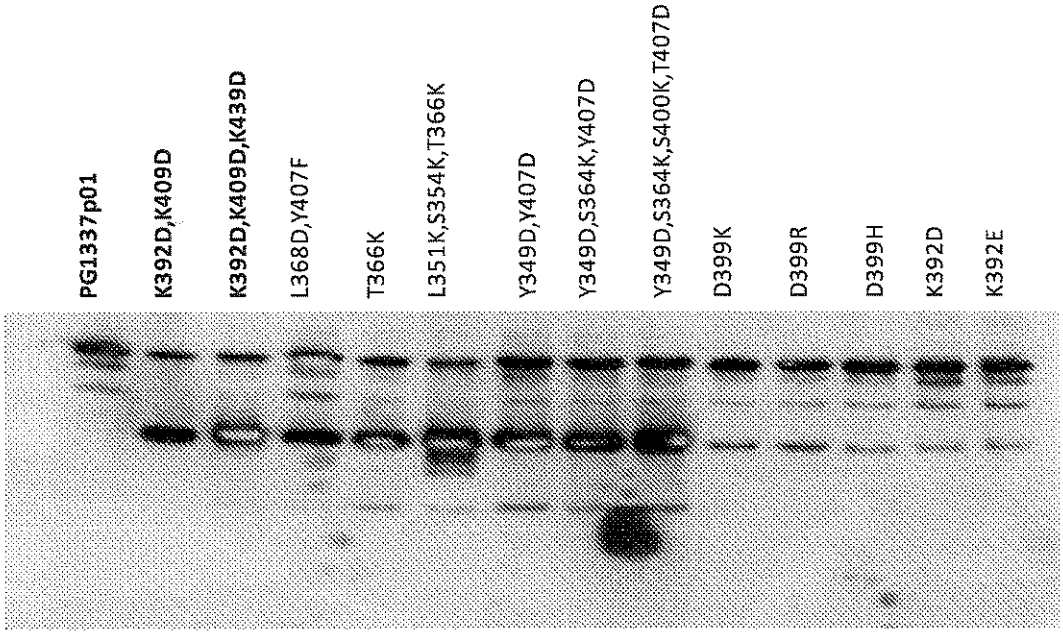
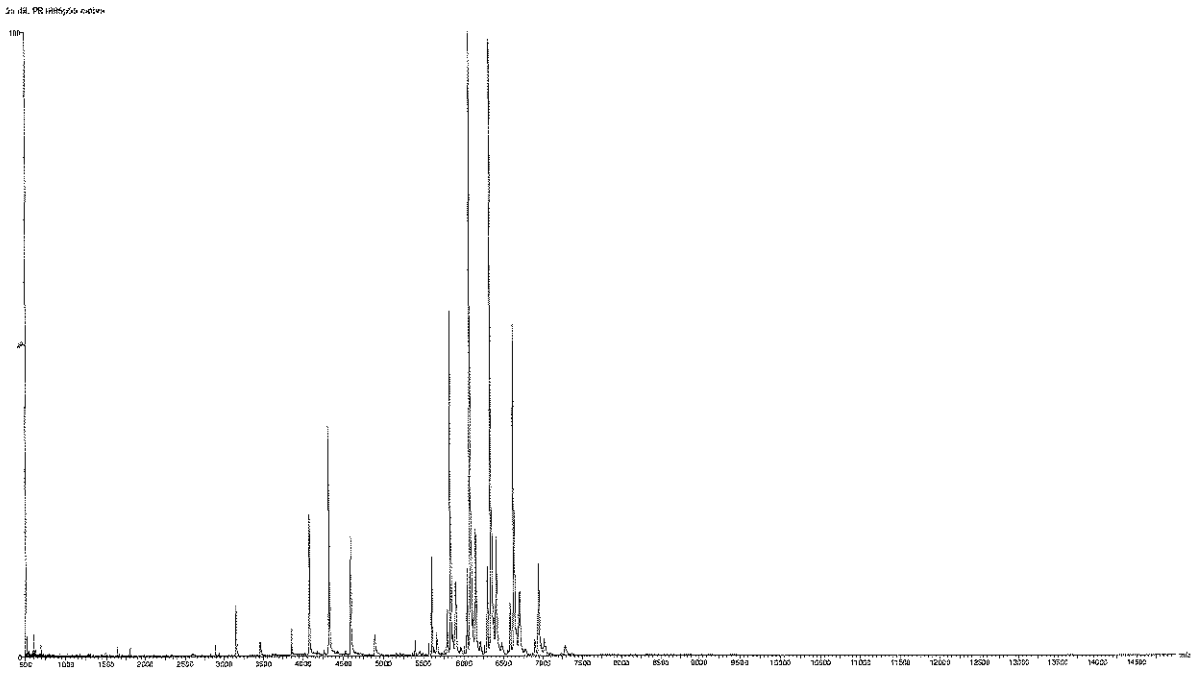
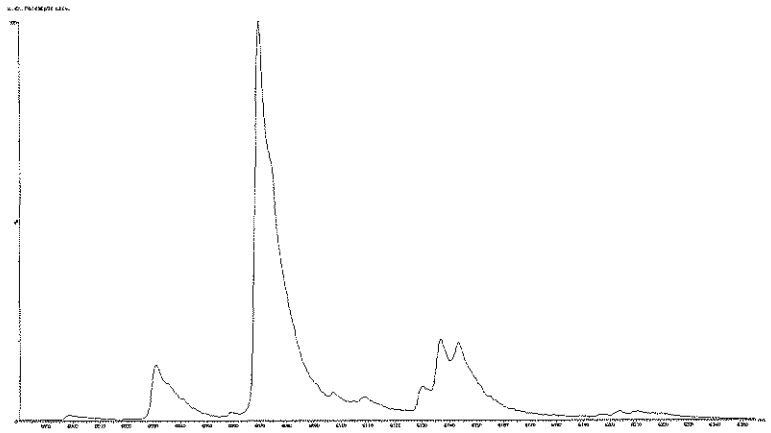

PG1337p01
K392D,K409D
K392D,K409D,K439D
L368D,Y407F
T366K
L351K,S354K,T366K
Y349D,Y407D
Y349D,S364K,Y407D
Y349D,S364K,S400K,T407D
D399K
D399R
D399H
K392D
K392E

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:

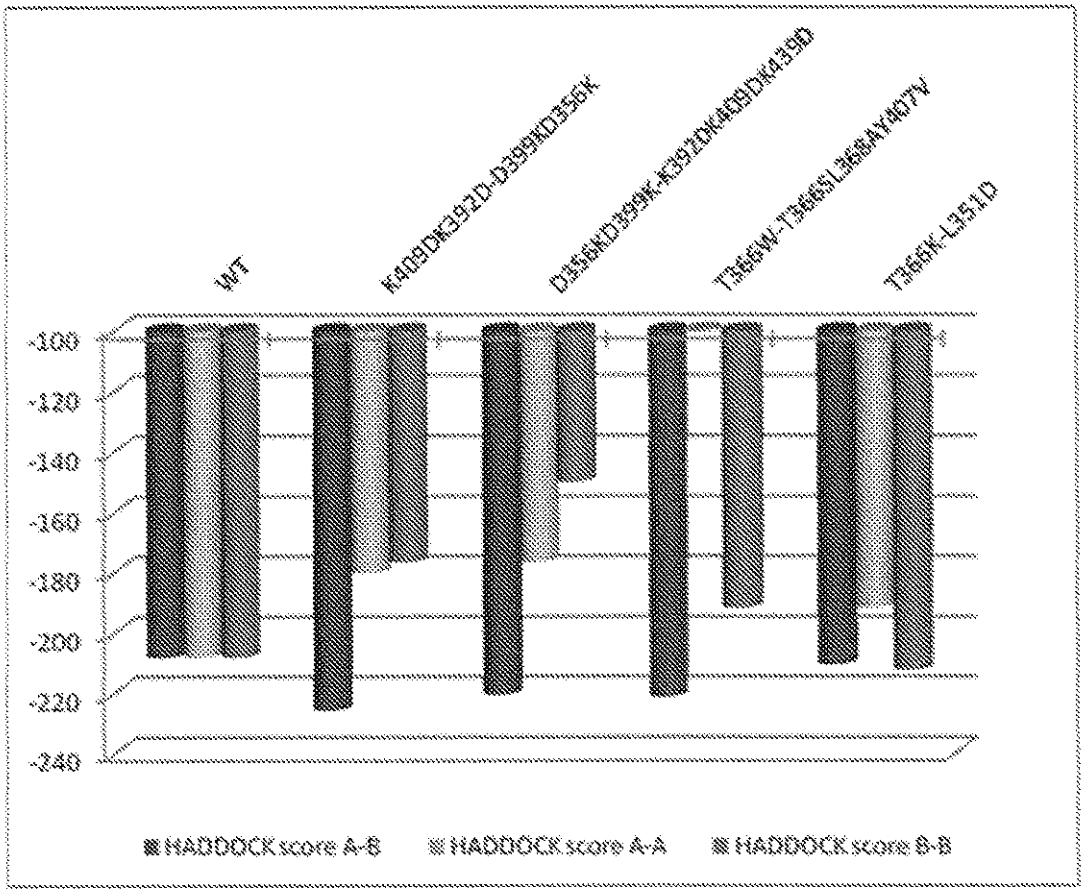
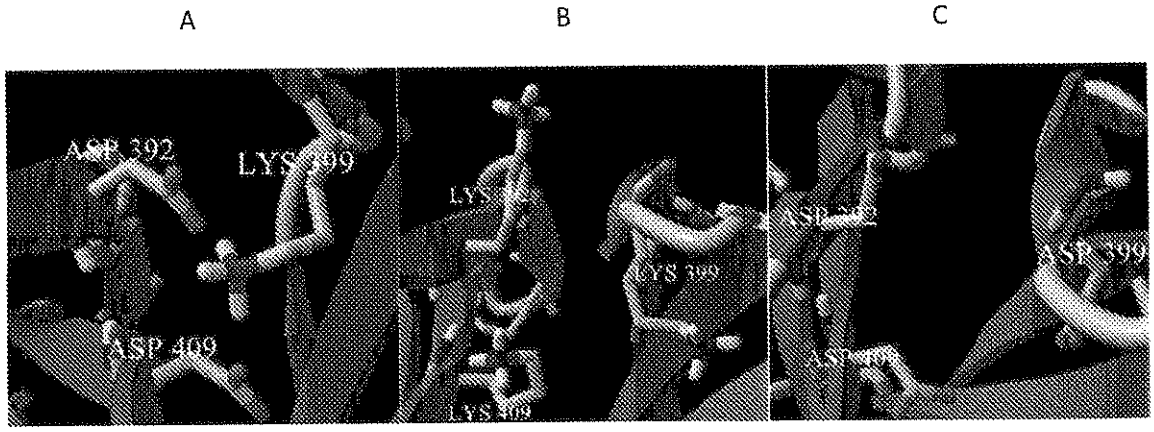

WT
K409DK392D-D399KD356K
D356KD399K-K392DK409DK439D
T366M-T366S L368AY407V
T366K-L351D
-100
-120
-140
-160
-180
-200
-220
-240
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

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:

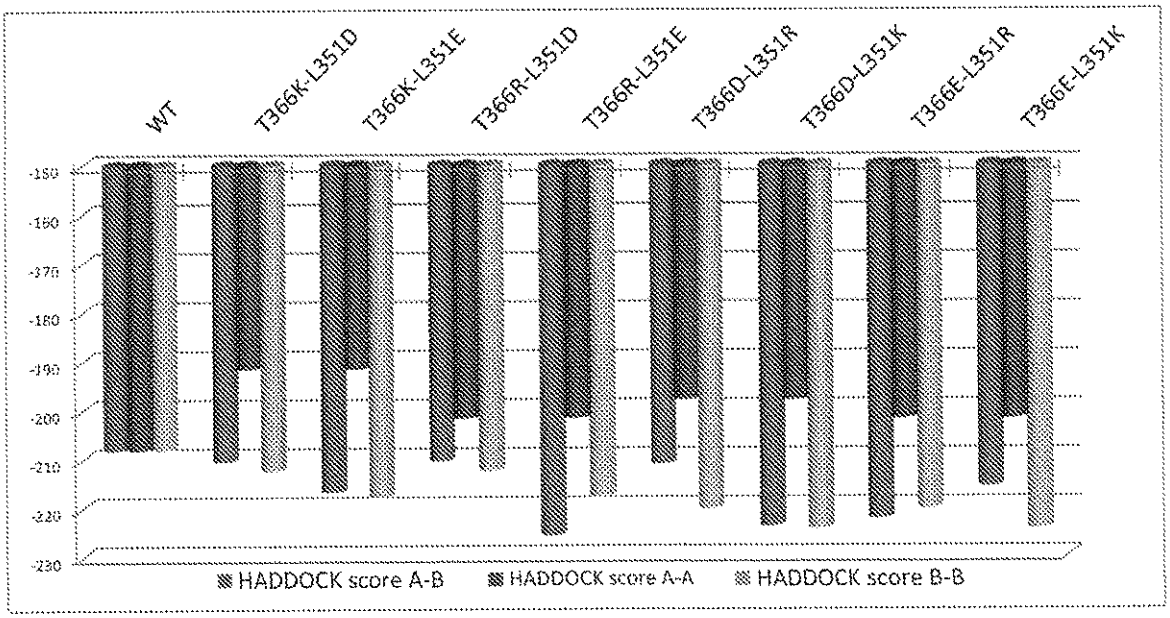
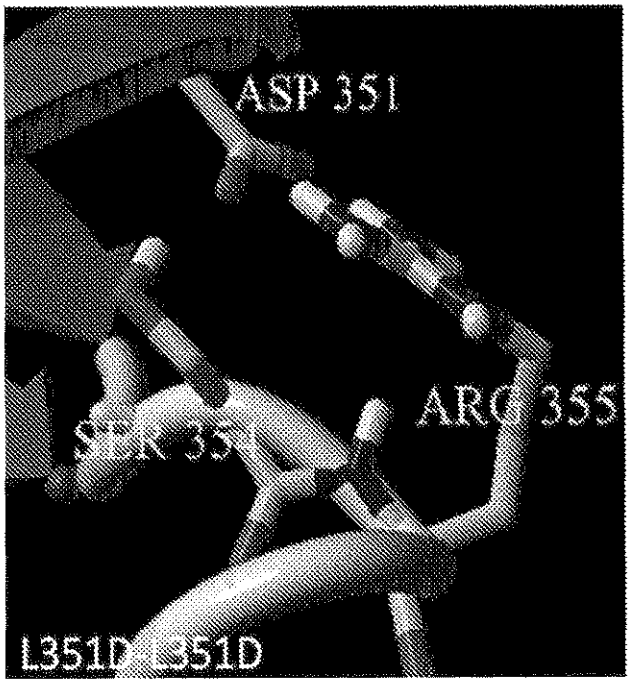

WT
T366K-L351D
T366K-L351E
T366R-L351D
T366R-L351E
T366D-L351R
T366D-L351K
T366E-L351R
T366E-L351K
-150
-160
-170
-180
-190
-200
-210
-220
-230
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Fig 12:

A


ASP 351
ARG 355
L351D L351D

B

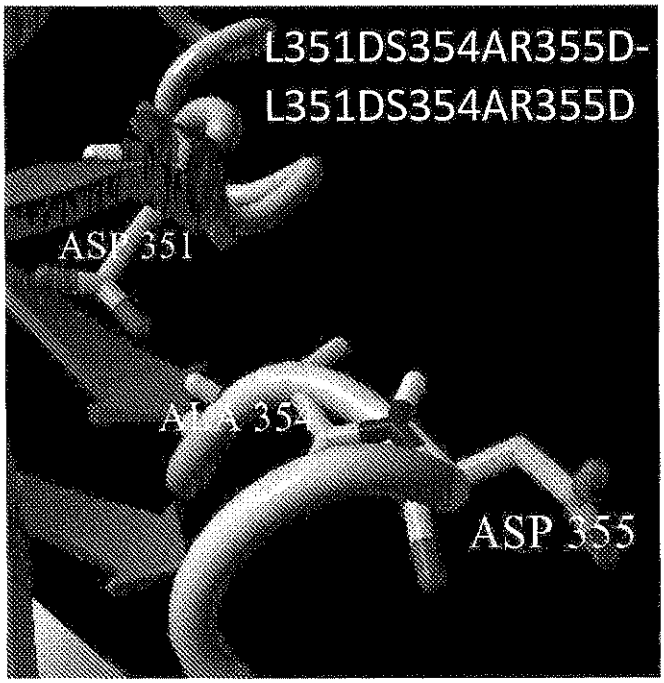

L351DS354AR355D-
L351DS354AR355D
ASP 351
ASP 355

Figure 13:

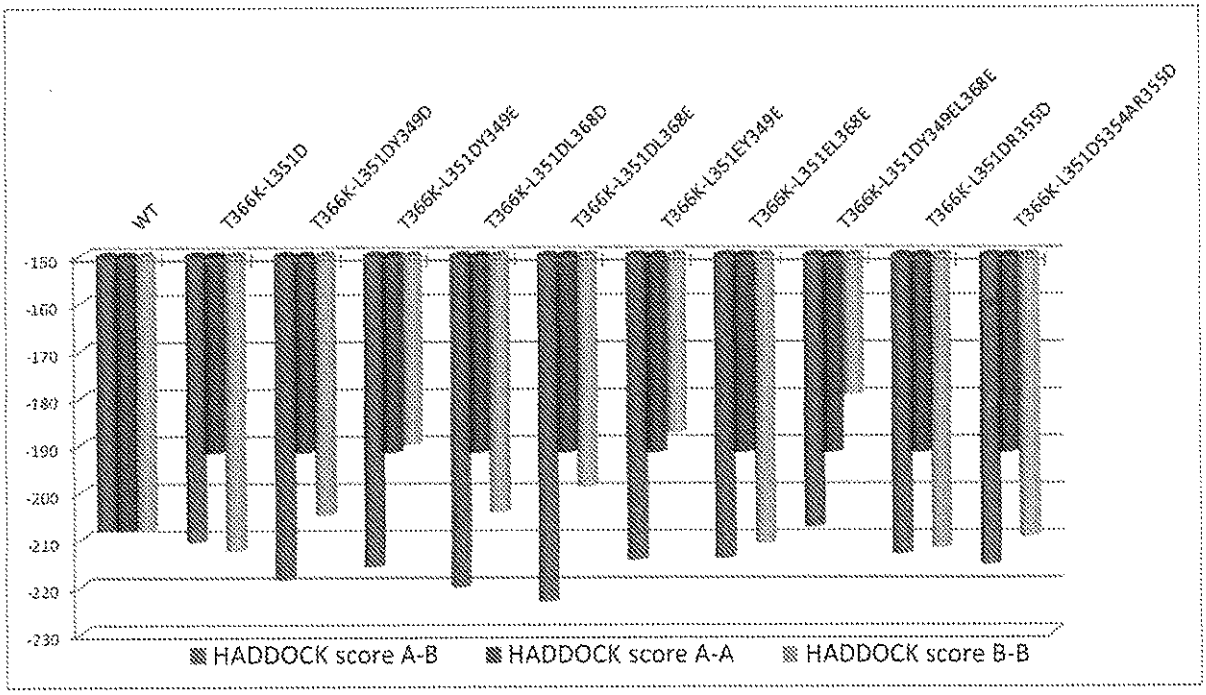

WT
T366K-L351D
T366K-L351DY349D
T366K-L351DY349E
T366K-L351DL368D
T366K-L351DL368E
T366K-L351EY349E
T366K-L351EL368E
T366K-L351DY349EL368E
T366K-L351DR355D
T366K-L351DS354AR355D
-150
-160
-170
-180
-190
-200
-210
-220
-230
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Figure 14:

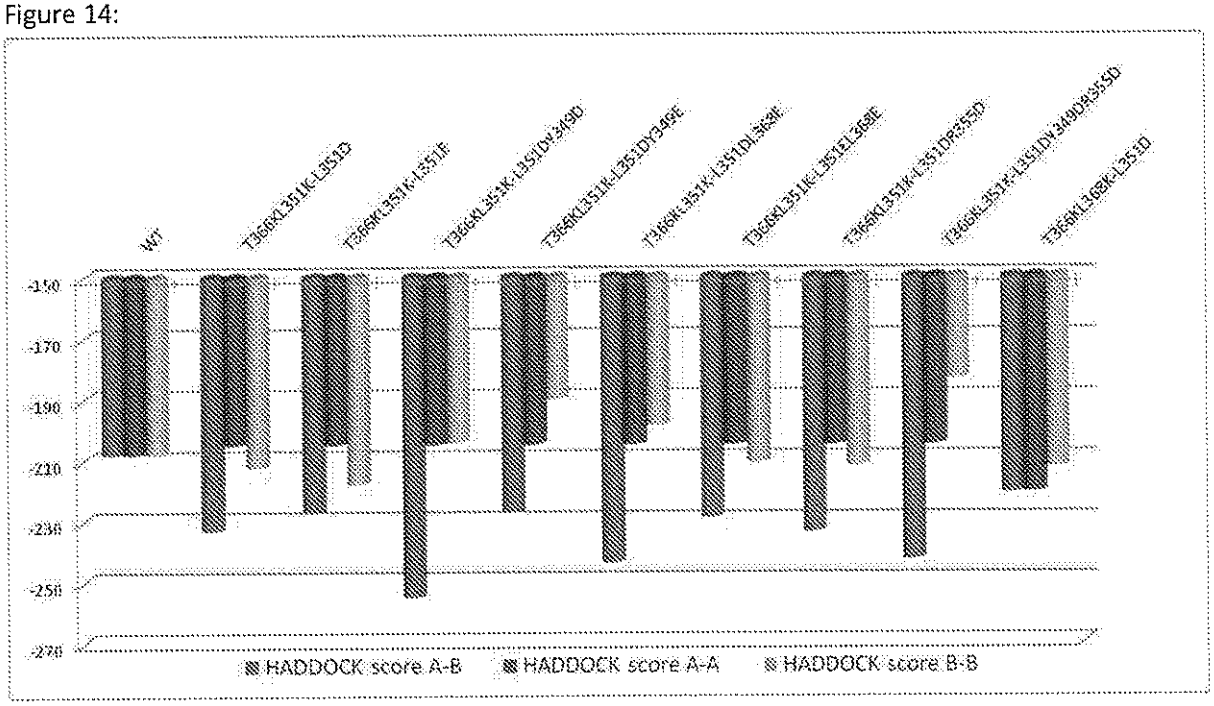

WT
T366K/L351K/L351D
T366K/L351K/L351E
T366K/L351K/L351D/Y349D
T366K/L351K/L351D/Y349E
T366K/L351K/L351D/L368E
T366K/L351K/L351E/L368E
T366K/L351K/L351D/R355D
T366K/L351K/L351D/Y349D/R355D
T366K/L368K/L351D
-150
-170
-190
-210
-230
-250
-270
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Figure 15:

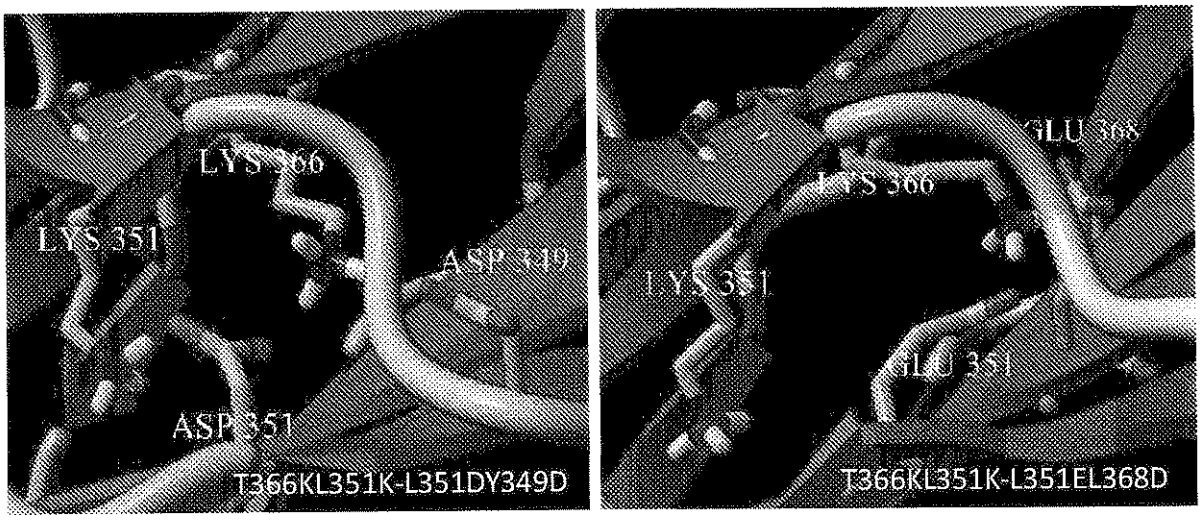

LYS 366
LYS 351
ASP 349
ASP 351
T366KL351K-L351DY349D
GLU 368
LYS 366
LYS 351
GLU 351
T366KL351K-L351EL368D

Figure 16:

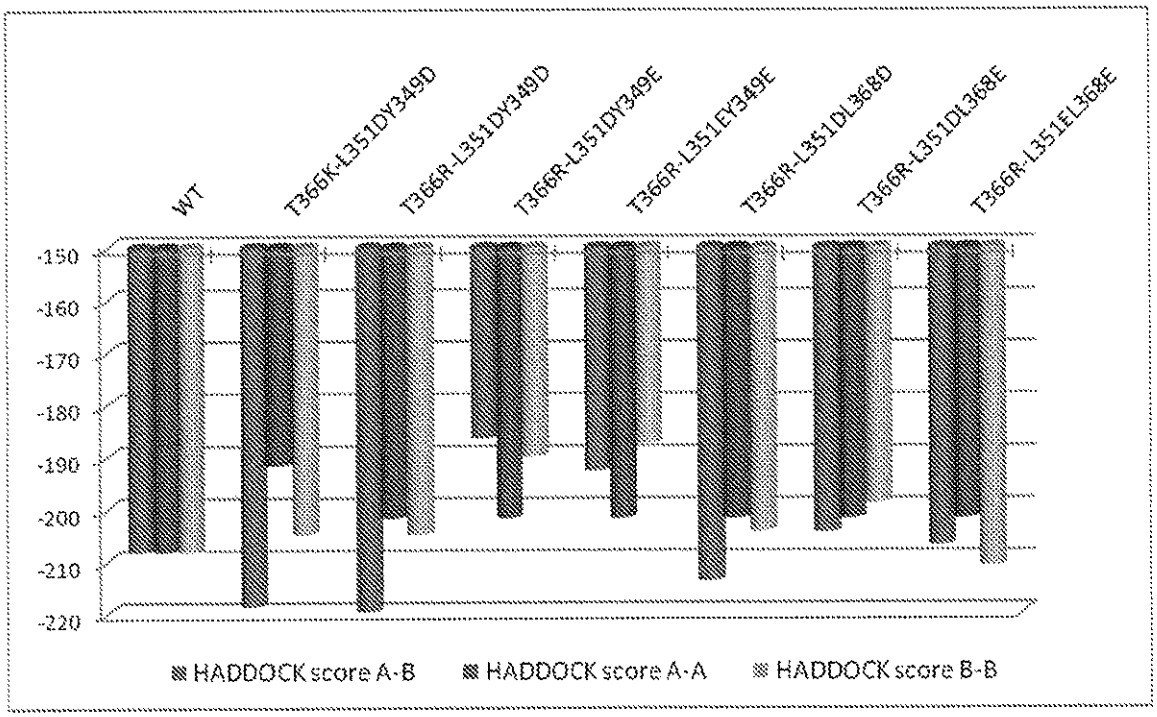

WT
T366K-L351DY349D
T366R-L351DY349D
T366R-L351DY349E
T366R-L351EY349E
T366R-L351DL368D
T366R-L351DL368E
T366R-L351EL368E
-150
-160
-170
-180
-190
-200
-210
-220
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Figure 17:

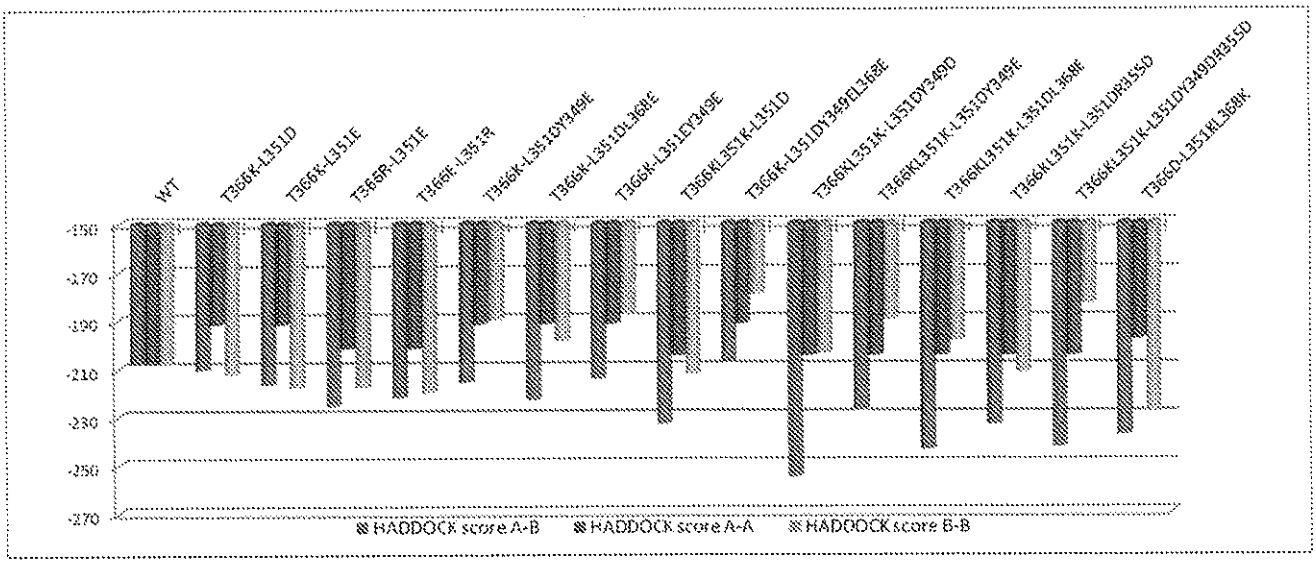

WT
T366K-L351D
T366K-L351E
T366R-L351E
T366E-L351R
T366K-L351DY349E
T366K-L351DL368E
T366K-L351EY349E
T366KL351K-L351D
T366K-L351DY349EL368E
T366KL351K-L351DY349D
T366KL351K-L351DY349E
T366KL351K-L351DL368E
T366KL351K-L351DR355D
T366KL351K-L351DY349DR355D
T366D-L351KL368K
-150
-170
-190
-210
-230
-250
-270
HADDOCK score A-B
HADDOCK score A-A
HADDOCK score B-B

Figure 18:

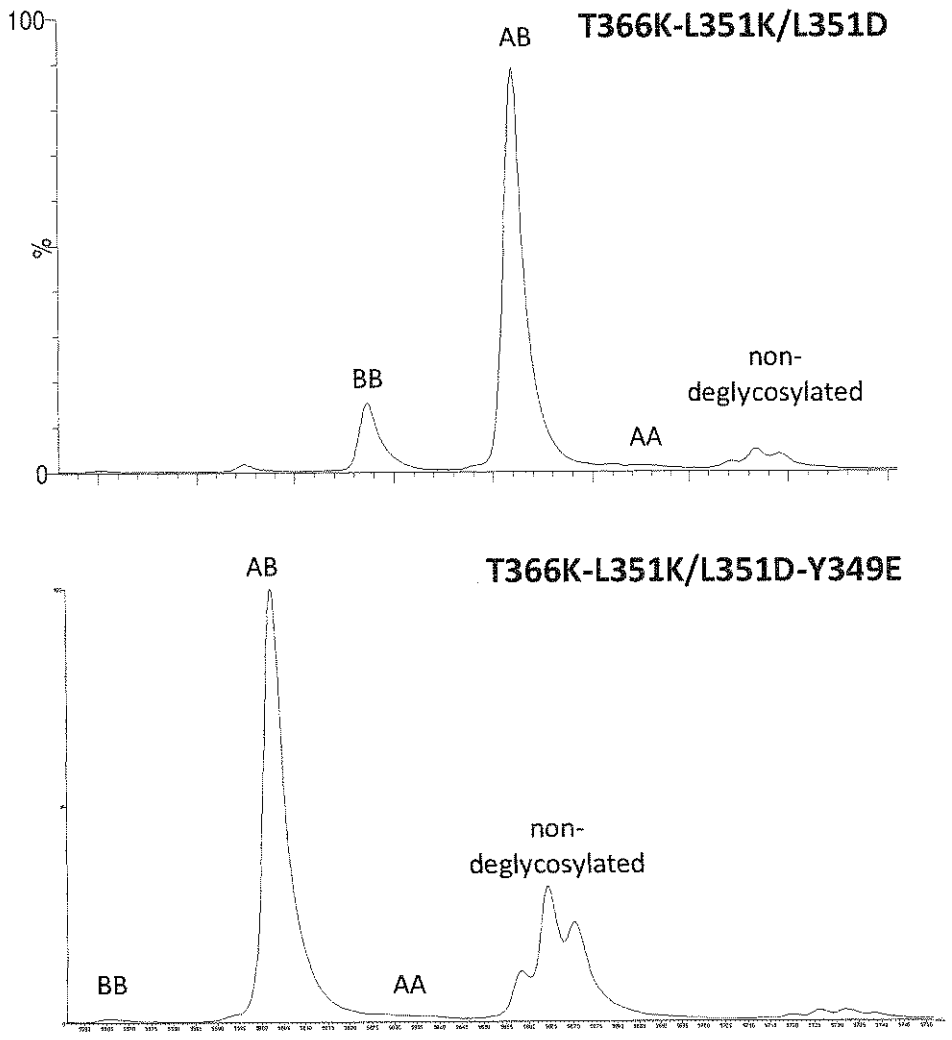

100
%
0
AB
T366K-L351K/L351D
BB
non-
deglycosylated
AA
AB
T366K-L351K/L351D-Y349E
non-
deglycosylated
BB
AA

Figure 19A:

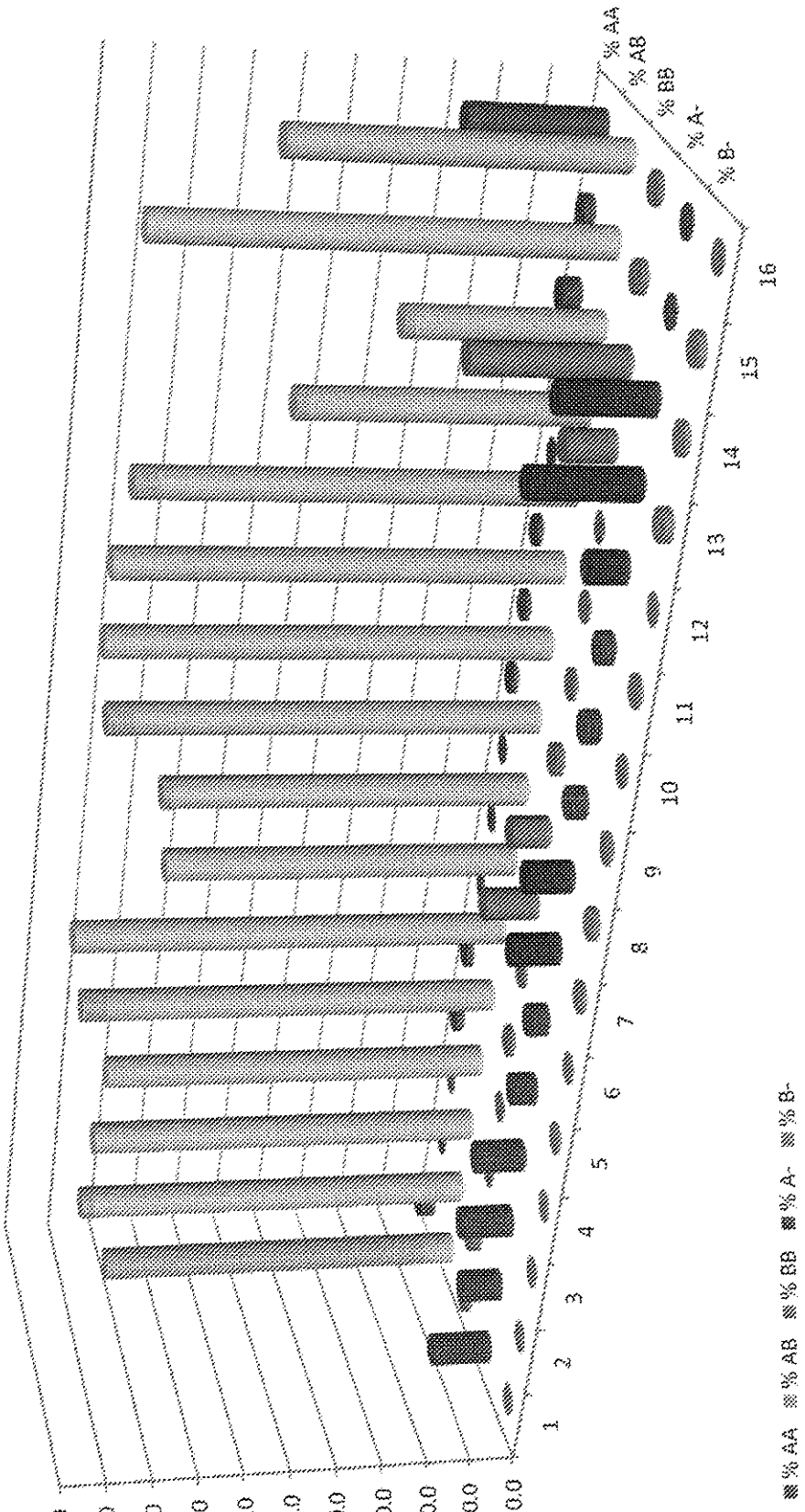

100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
% AA
% AB
% BB
% A-
% B-

Figure 19B:

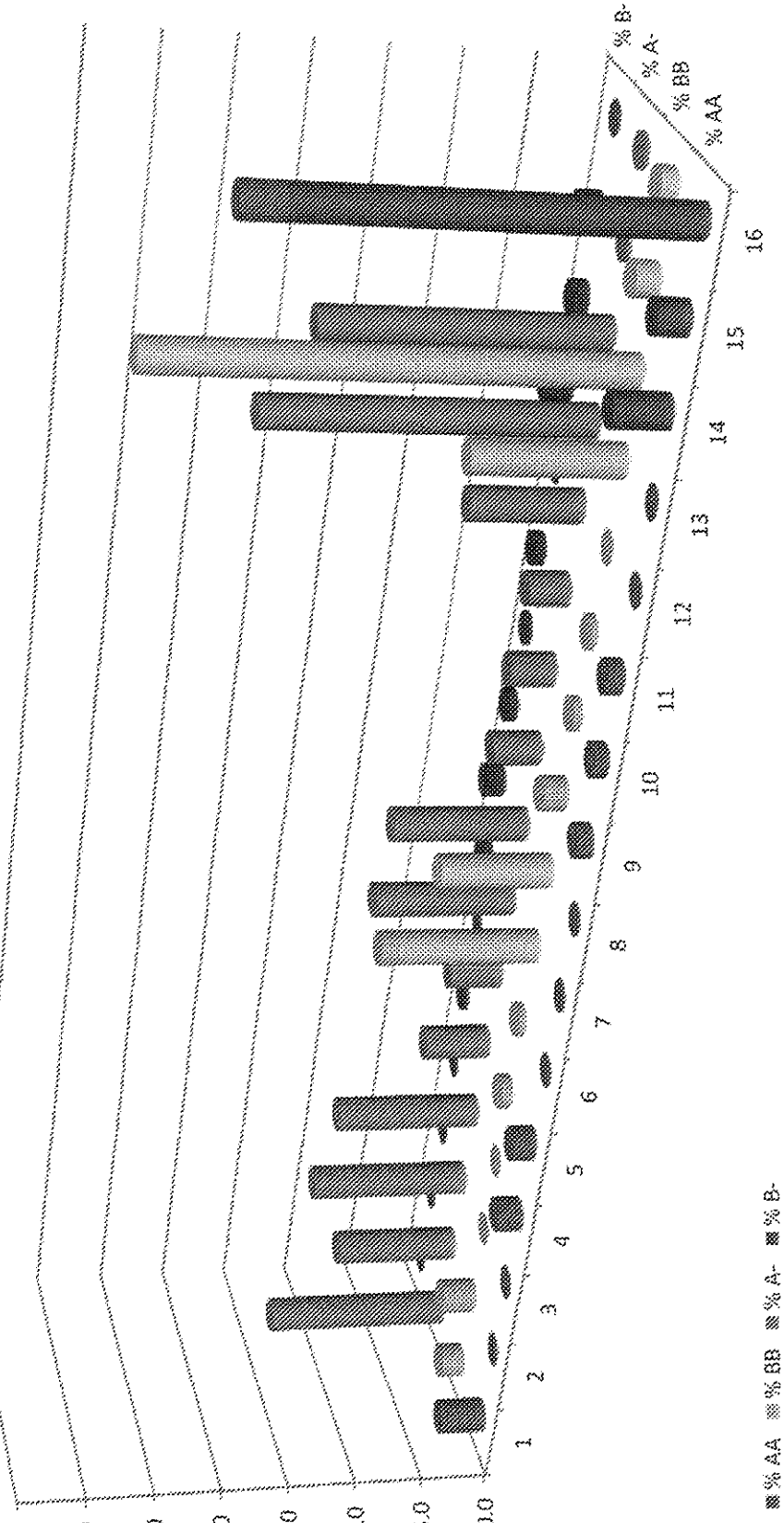

35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
% B-
% A-
% BB
% AA
% AA
% BB
% A-
% B-

Figure 20:

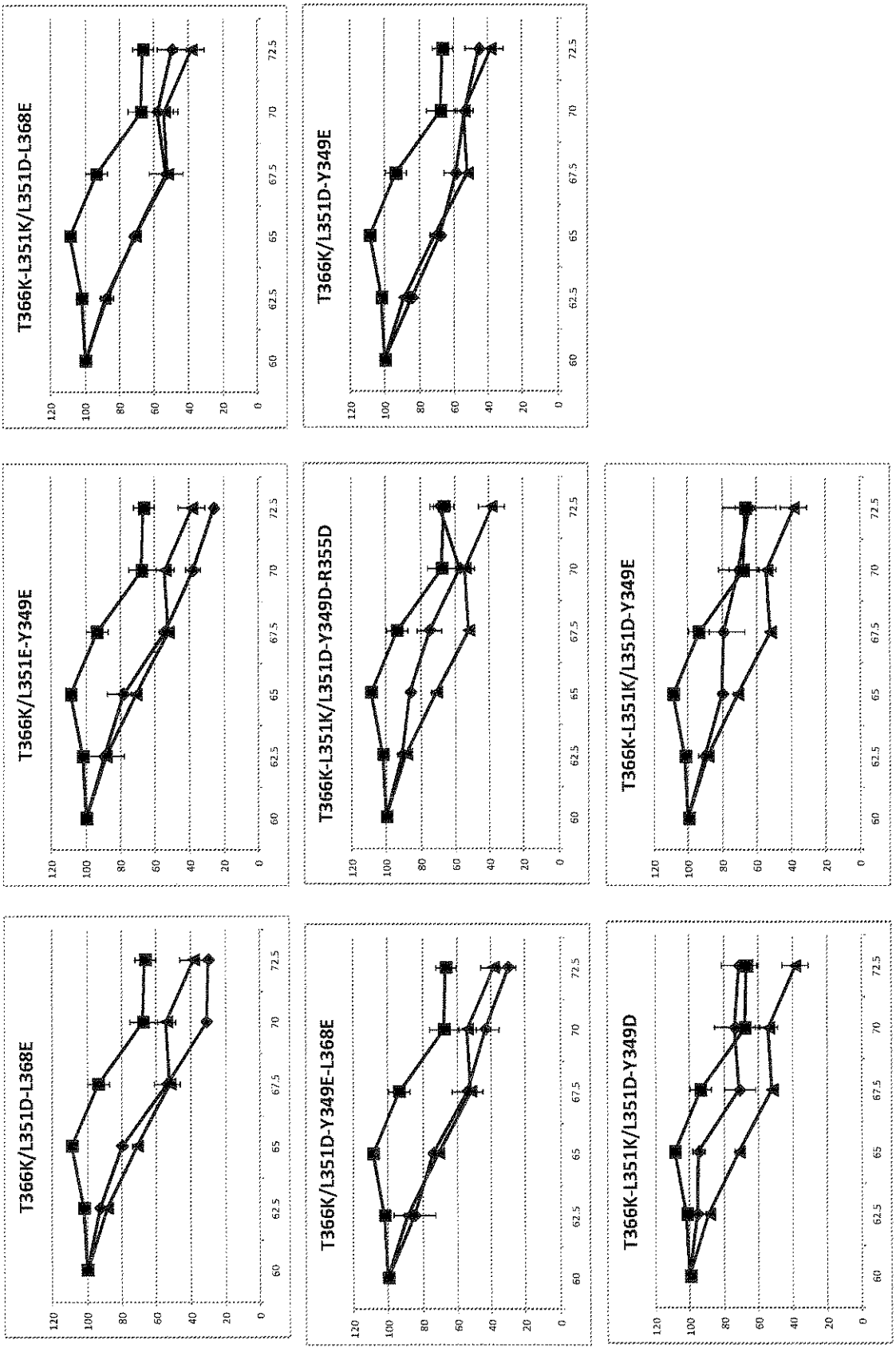

T366K-L351K/L351D-L368E
T366K/L351D-Y349E
T366K/L351E-Y349E
T366K-L351K/L351D-Y349D-R355D
T366K-L351K/L351D-Y349E
T366K/L351D-L368E
T366K/L351D-Y349E-L368E
T366K-L351K/L351D-Y349D

Figure 21:

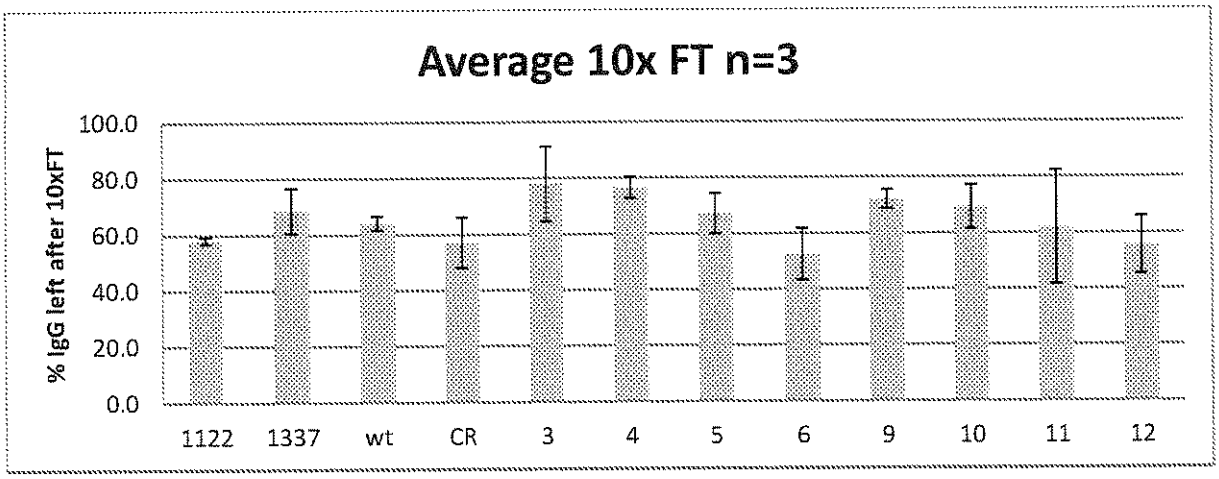
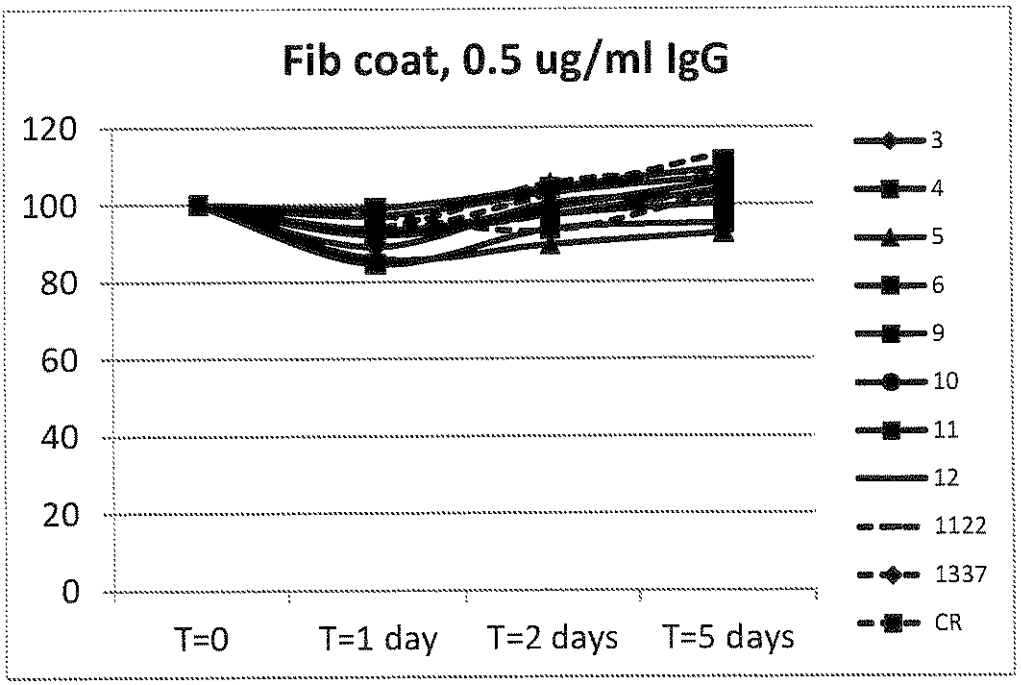

Average 10x FT n=3
% IgG left after 10xFT
100.0
80.0
60.0
40.0
20.0
0.0
1122
1337
wt
CR
3
4
5
6
9
10
11
12

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

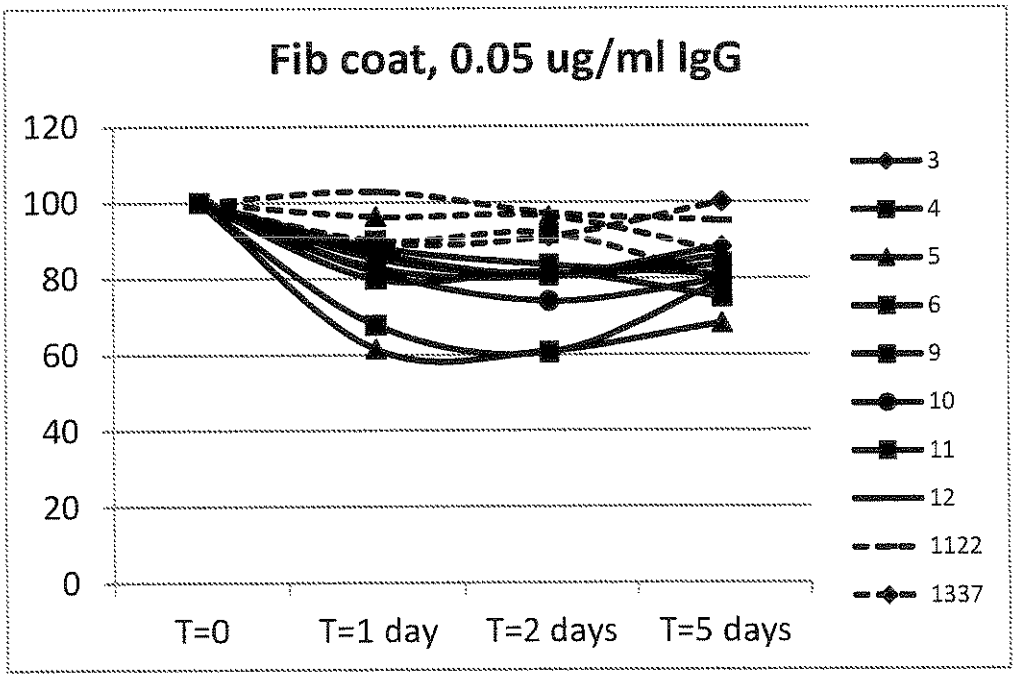
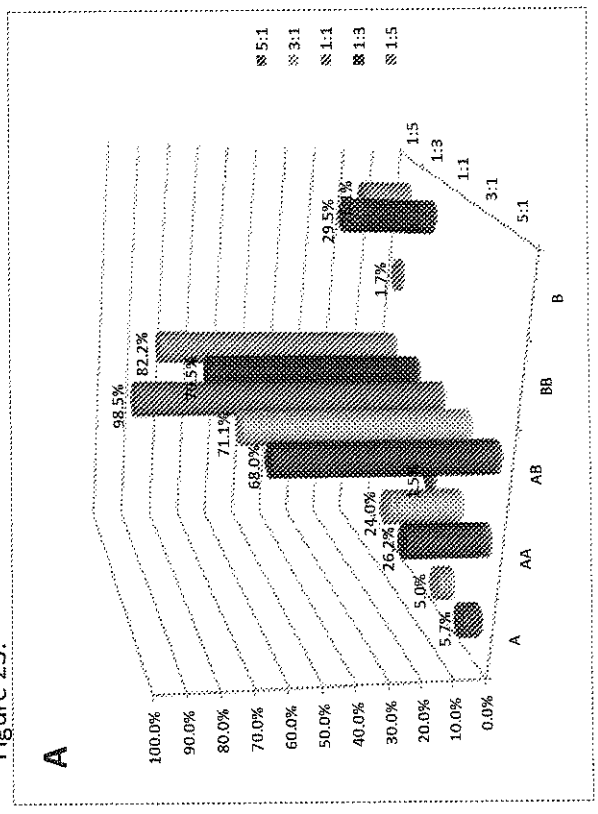
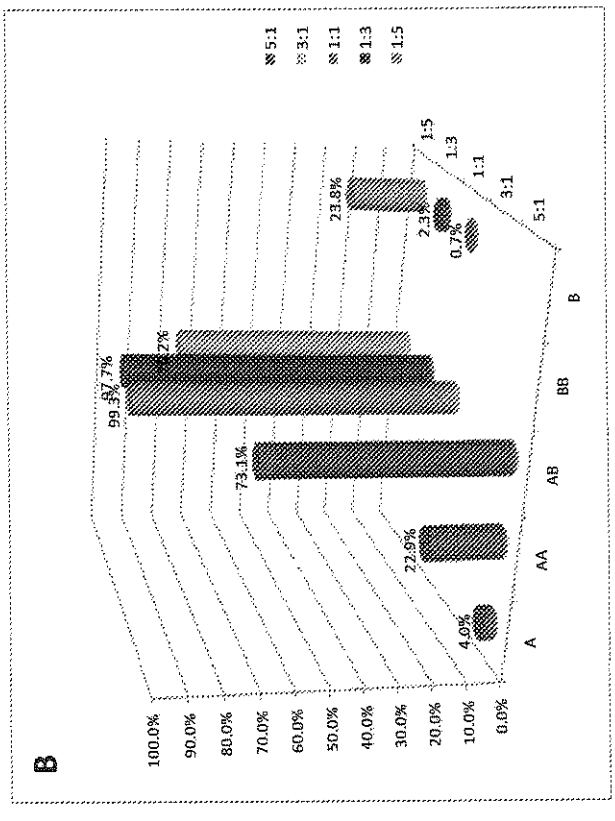
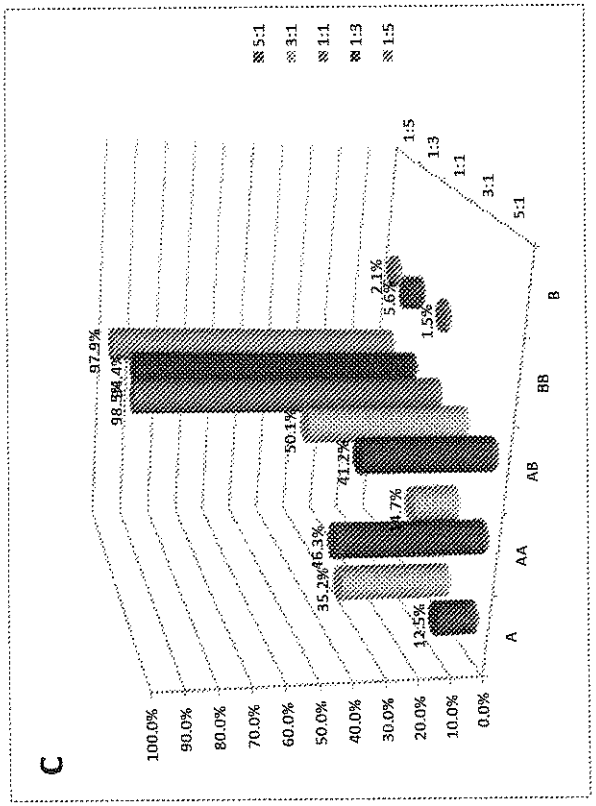

Fib coat, 0.05 ug/ml IgG
120
100
80
60
40
20
0
T=0
T=1 day
T=2 days
T=5 days
3
4
5
6
9
10
11
12
1122
1337

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				29.5%	16.1%
B					1.7%

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				2.3%	23.8%
B			0.7%		

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:

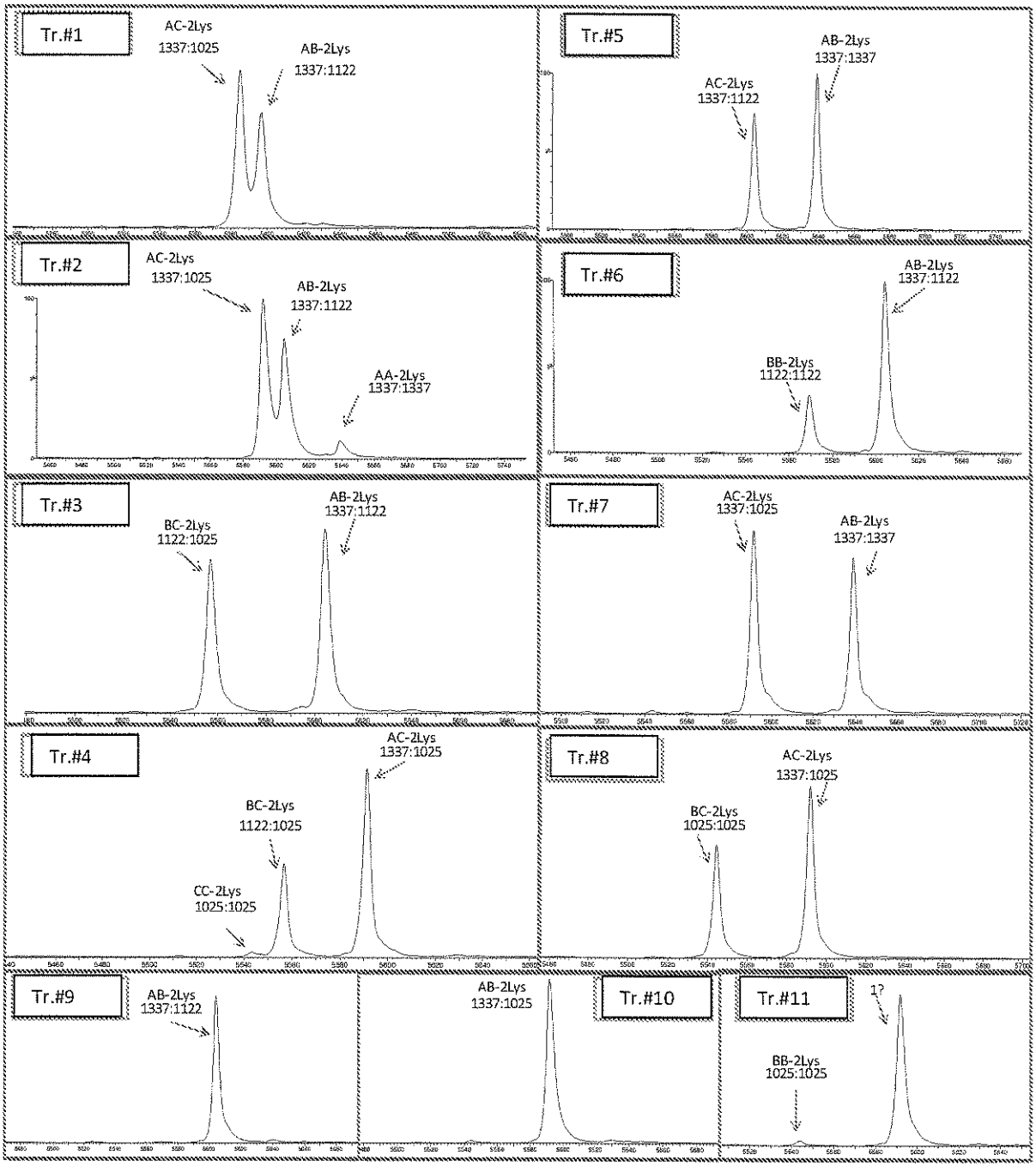

Tr.#1
AC-2Lys 1337:1025
AB-2Lys 1337:1122
Tr.#5
AB-2Lys 1337:1337
AC-2Lys 1337:1122
Tr.#2
AC-2Lys 1337:1025
AB-2Lys 1337:1122
AA-2Lys 1337:1337
Tr.#6
AB-2Lys 1337:1122
BB-2Lys 1122:1122
Tr.#3
BC-2Lys 1122:1025
AB-2Lys 1337:1122
Tr.#7
AC-2Lys 1337:1025
AB-2Lys 1337:1337
Tr.#4
AC-2Lys 1337:1025
BC-2Lys 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:

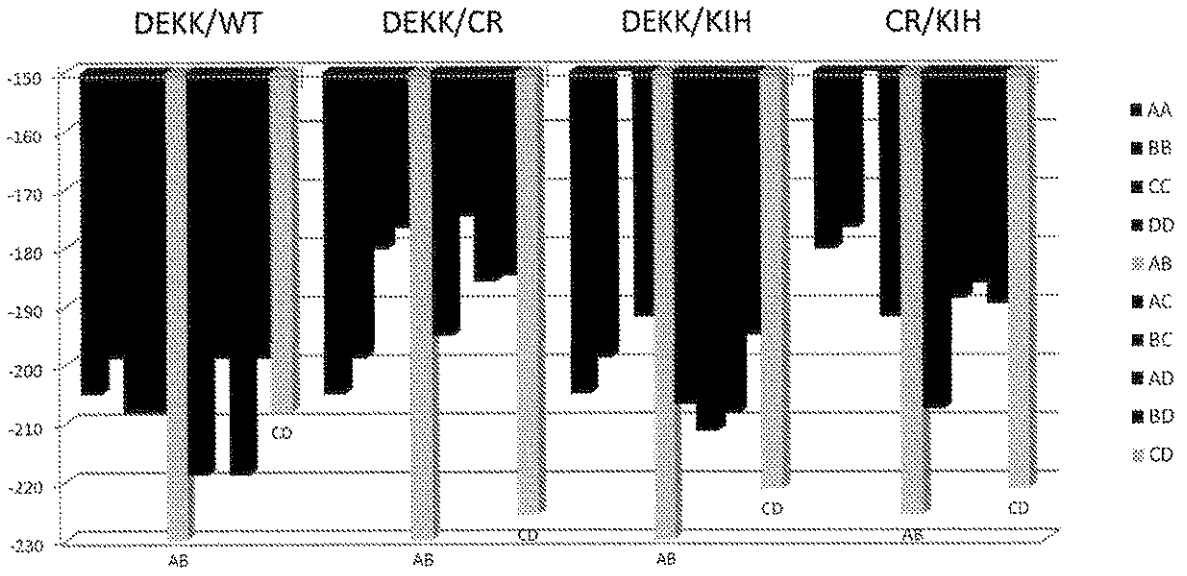

DEKK/WT
DEKK/CR
DEKK/KIH
CR/KIH
-150
-160
-170
-180
-190
-200
-210
-220
-230
AB
CD
AA
BB
CC
DD
AB
AC
BC
AD
BD
CD

Figure 26:

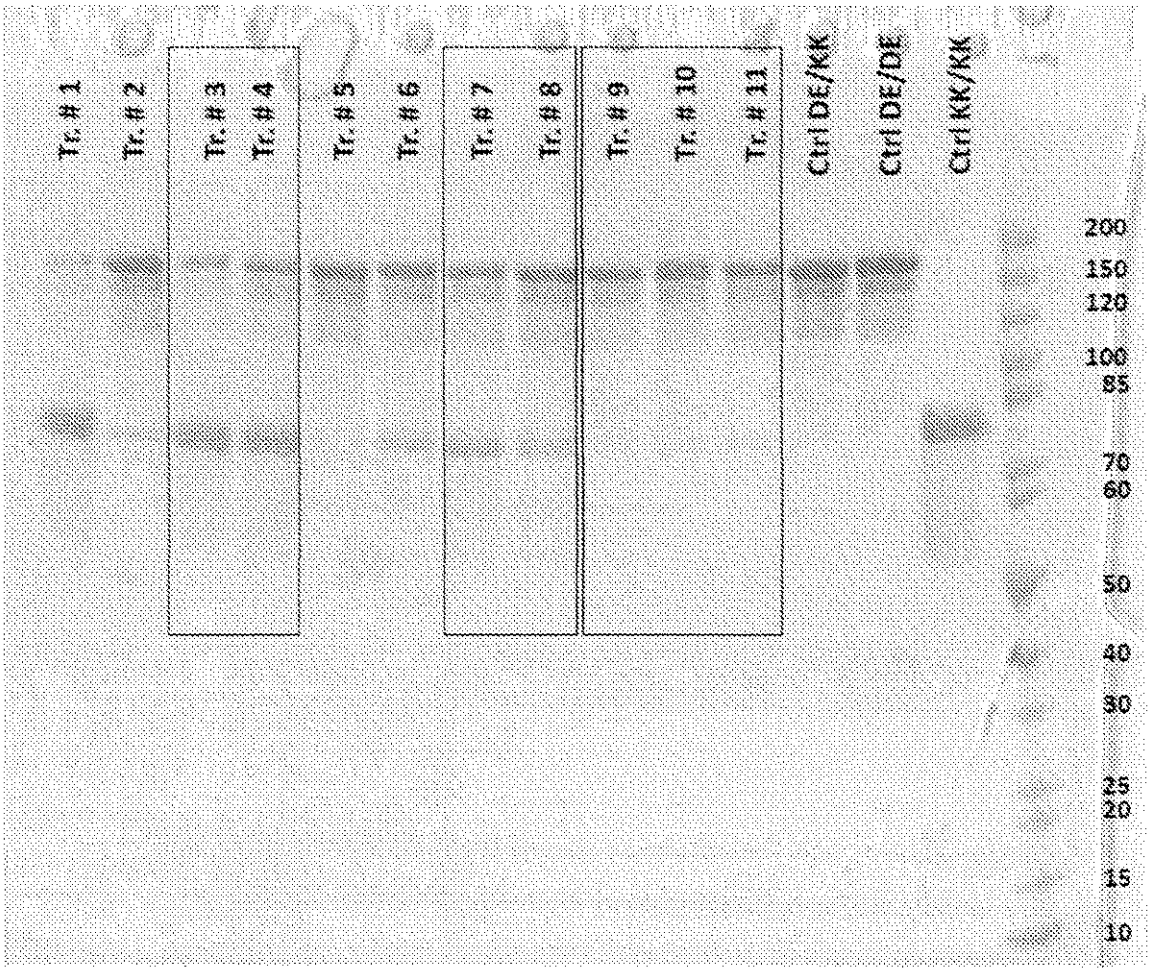

Tr. # 1
Tr. # 2
Tr. # 3
Tr. # 4
Tr. # 5
Tr. # 6
Tr. # 7
Tr. # 8
Tr. # 9
Tr. # 10
Tr. # 11
Ctrl DE/KK
Ctrl DE/DE
Ctrl KK/KK
200
150
120
100
85
70
60
50
40
30
25
20
15
10

Figure 27A:

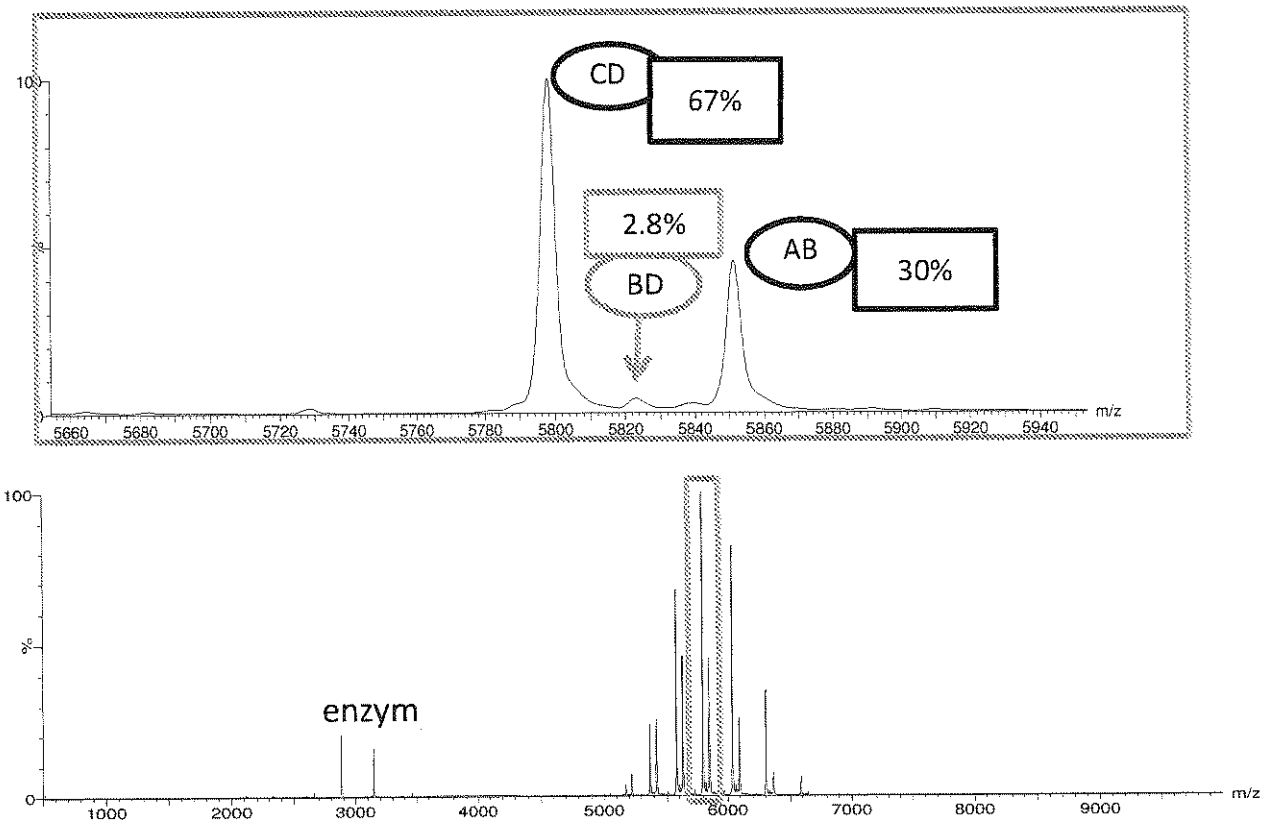
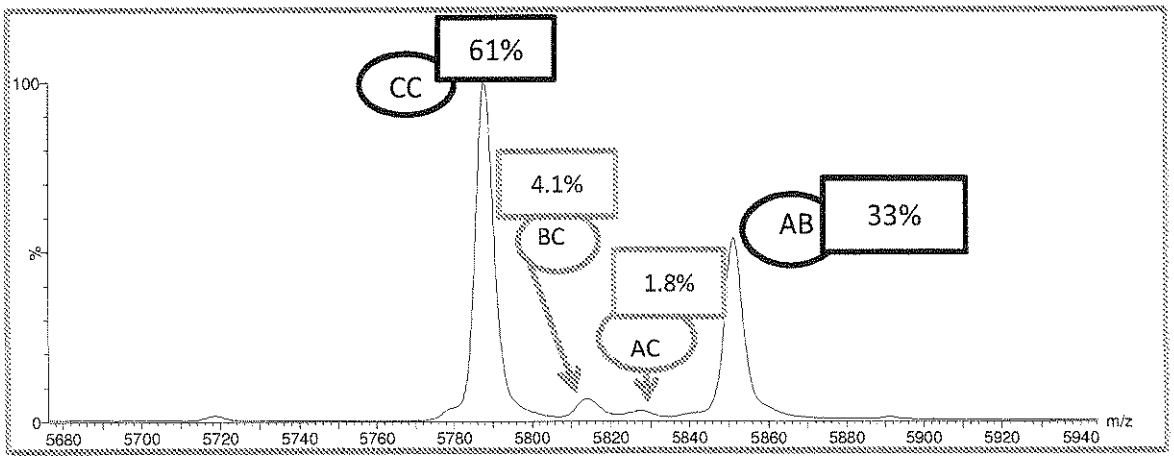

CD
67%
2.8%
BD
AB
30%
100
%
m/z
5660
5680
5700
5720
5740
5760
5780
5800
5820
5840
5860
5880
5900
5920
5940
100
%
0
enzym
1000
2000
3000
4000
5000
6000
7000
8000
9000
m/z

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

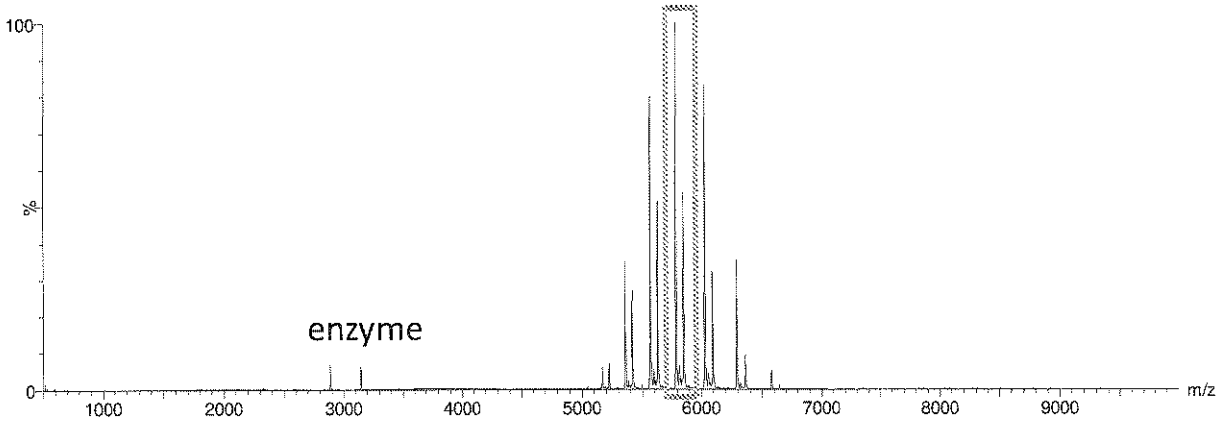

100
%
0
enzyme
1000
2000
3000
4000
5000
6000
7000
8000
9000
m/z

Figure 28:

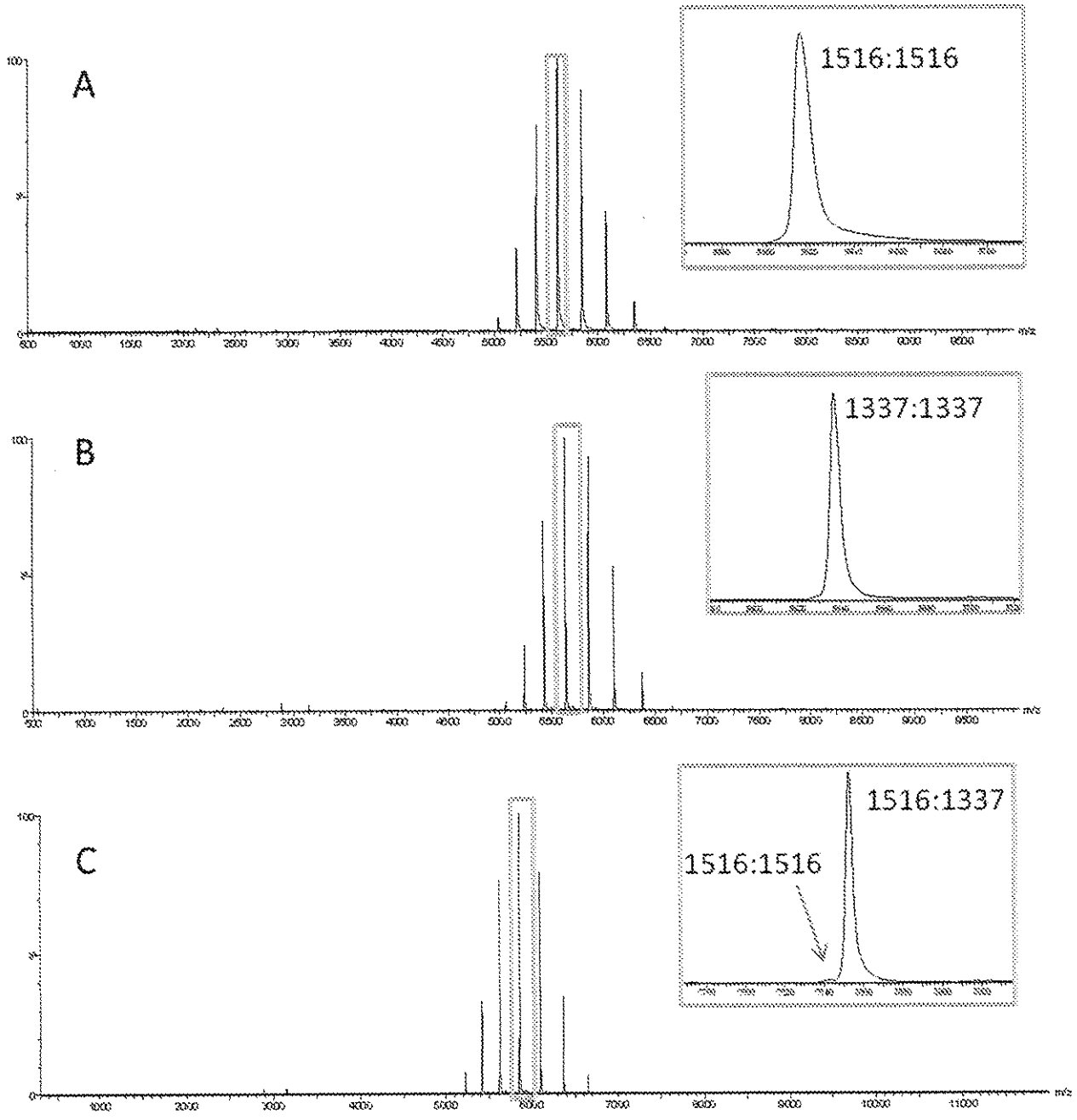
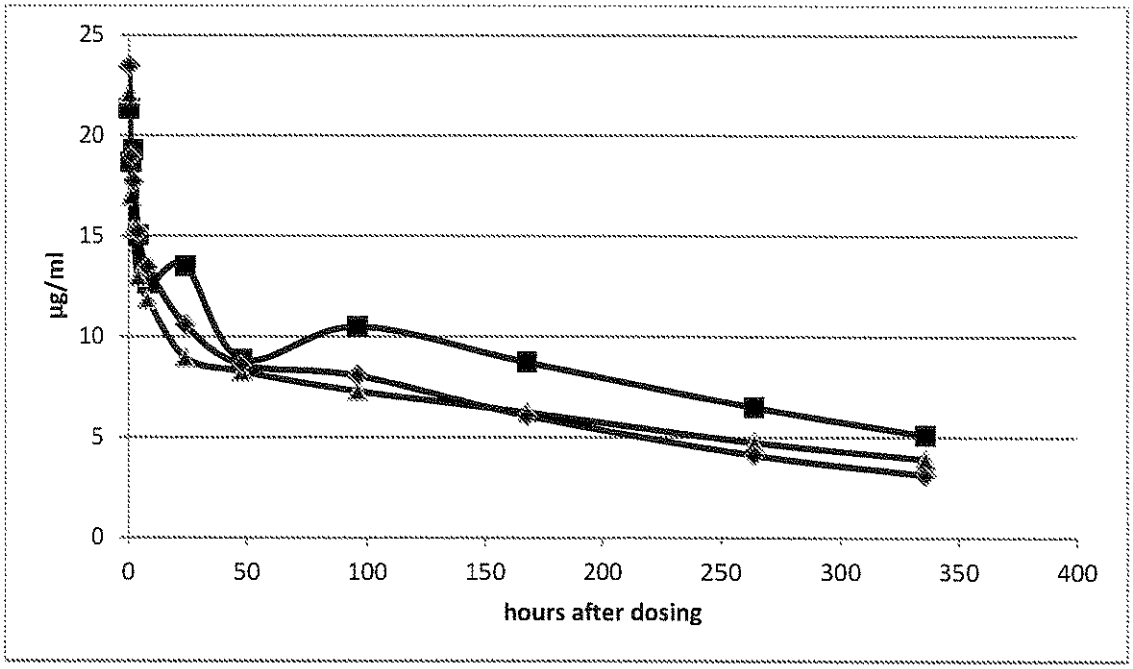


Figure 29:


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