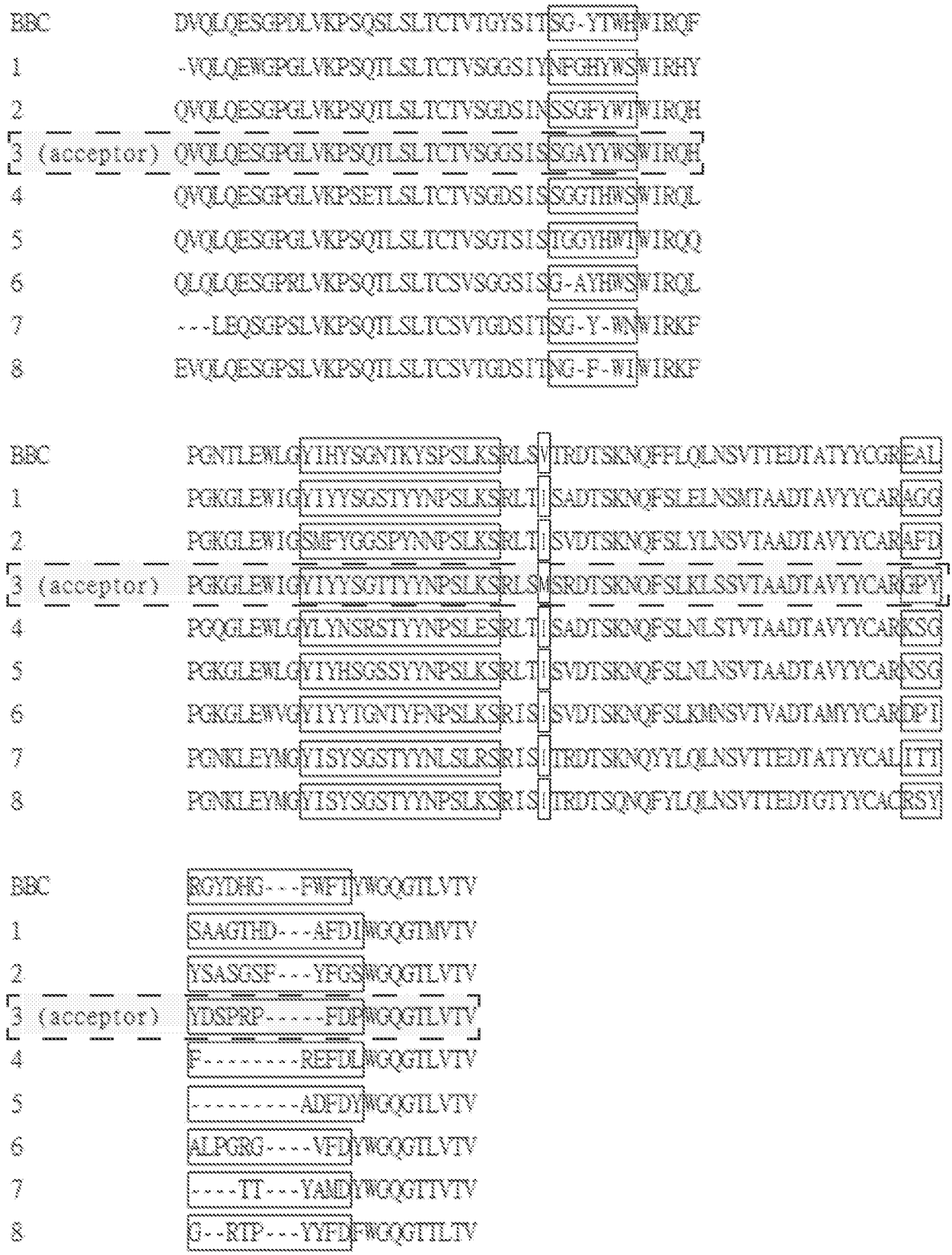

BBC DVQLQESGPDLVKPSQSLSLTCTVTGYSITSG-YTWHWIRQF
1 -VQLQEWGPGLVKPSQTLSLTCTVSGGSITYNFGHYWSWIRHY
2 QVQLQESGPGLVKPSQTLSLTCTVSGDSINSSGFYWIWIRQH
3 (acceptor) QVQLQESGPGLVKPSQTLSLTCTVSGGSISSGAYYWSWIRQH
4 QVQLQESGPGLVKPSETLSLTCTVSGDSISSGGTHWSWIRQL
5 QVQLQESGPGLVKPSQTLSLTCTVSGTSISTGGYHWTWIRQQ
6 QLQLQESGPRLVKPSQTLSLTCSVSGGSISG-AYHWSWIRQL
7 ---LEQSGPSLVKPSQTLSLTCSVTGDSITSG-Y-WNWIRKF
8 EVQLQESGPSLVKPSQTLSLTCSVTGDSITNG-F-WIWIRKF
BBC PGNTLEWLGYIHYSGNTKYSPSLKSRLSVTRDTSKNQFFLQLNSVTTEDTATYYCGREAL
1 PGKGLEWIGYIYYSGSTYYNPSLKSRLTISADTSKNQFSLELNSMTAADTAVYYCARAGG
2 PGKGLEWIGSMFYGGSPYNNPSLKSRLTISVDTSKNQFSLYLNSVTAADTAVYYCARAFD
3 (acceptor) PGKGLEWIGYIYYSGITYYNPSLKSRLSMSRDTSKNQFSLKLSSVTAADTAVYYCARGPY
4 PGQGLEWLGYLYNSRSTYYNPSLESRLTISADTSKNQFSLNLSTVTAADTAVYYCARKSG
5 PGKGLEWLGYIYHSGSSYYNPSLKSRLTISVDTSKNQFSLNLNSVTAADTAVYYCARNSG
6 PGKGLEWVGYIYYTGNTYFNPSLKSRISISVDTSKNQFSLKMNSVTVADTAMYYCARDPI
7 PGNKLEYMGYISYSGSTYYNLSLRSRISITRDTSKNQYYLQLNSVTTEDTATYYCALITT
8 PGNKLEYMGYISYSGSTYYNPSLKSRISITRDTSQNQFYLQLNSVTTEDTGTYYCACRSY
BBC RGYDHG---FWFTIWGQGTLVTV
1 SAAGTHD---AFDIWGQGTMVTV
2 YSASGSF---YFGSWGQGTLVTV
3 (acceptor) YDSPRP-----FDPWGQGTLVTV
4 F--------REFDLWGQGTLVTV
5 ---------ADFDYWGQGTLVTV
6 ALPGRG----VFDIWGQGTLVTV
7 ----TT---YAMDIWGQGTTVTV
8 G--RTP---YYFDFWGQGTLLTV

FIG. 1

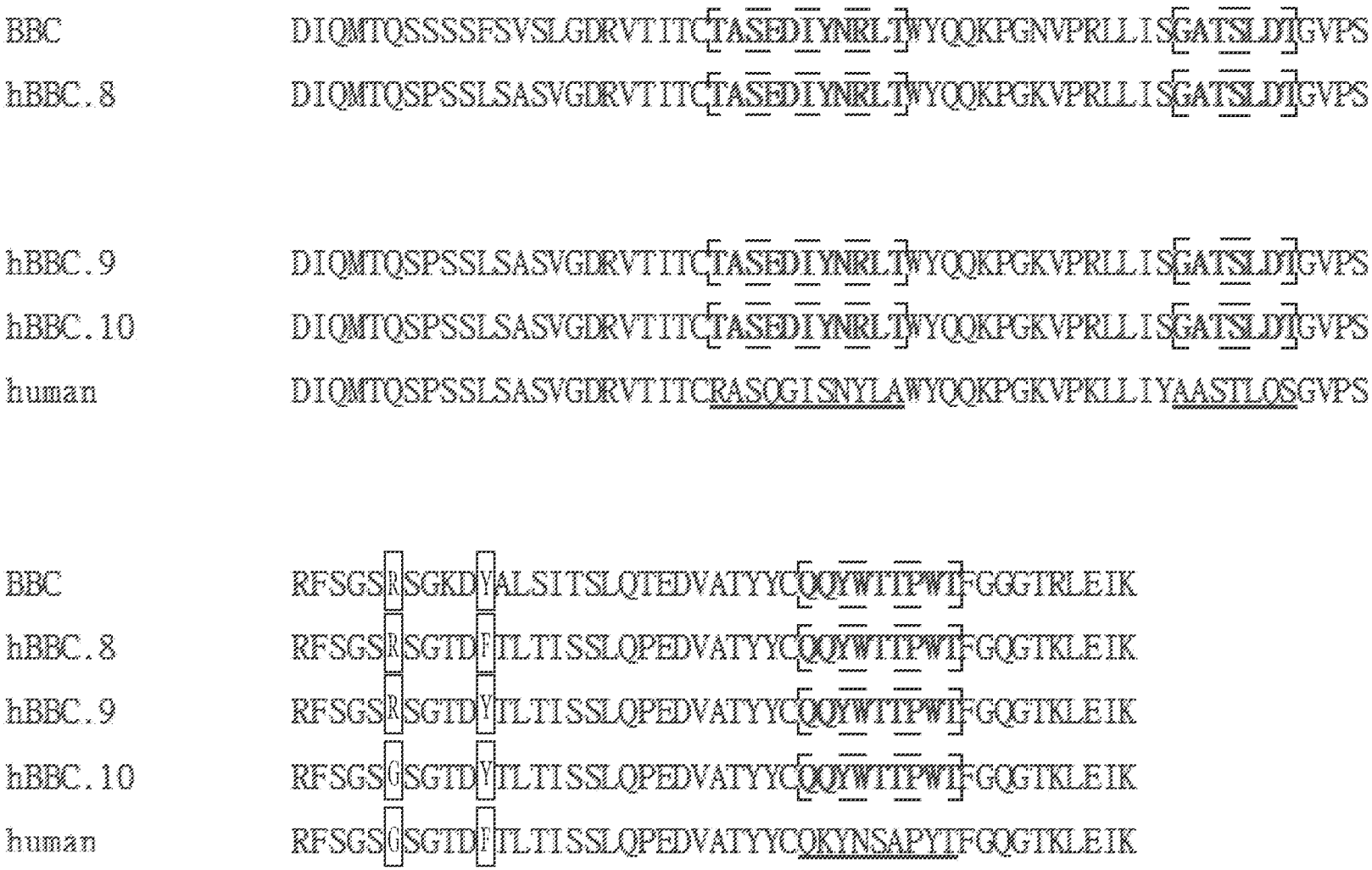

BBC DIQMTQSSSSFSVSLGDRVTITCTASEDIYNRLTWYQQKPGNVPRLLISGATSLDTGVPS
hBBC.8 DIQMTQSPSSLSASVGDRVTITCTASEDIYNRLTWYQQKPGKVPRLLISGATSLDTGVPS
hBBC.9 DIQMTQSPSSLSASVGDRVTITCTASEDIYNRLTWYQQKPGKVPRLLISGATSLDTGVPS
hBBC.10 DIQMTQSPSSLSASVGDRVTITCTASEDIYNRLTWYQQKPGKVPRLLISGATSLDTGVPS
human DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPKLLIYAASTLQSGVPS
BBC RFSGSRSGKDYALSITSLQTEDVATYYCQQYWTTPWTFGQGTRLEIK
hBBC.8 RFSGSRSGTDFTLTISSLQPEDVATYYCQQYWTTPWTFGQGTKLEIK
hBBC.9 RFSGSRSGTDYTLTISSLQPEDVATYYCQQYWTTPWTFGQGTKLEIK
hBBC.10 RFSGSQSGTDYTLTISSLQPEDVATYYCQQYWTTPWTFGQGTKLEIK
human RFSGSQSGTDFTLTISSLQPEDVATYYCQKYNSAPYTFGQGTKLEIK

FIG. 2A

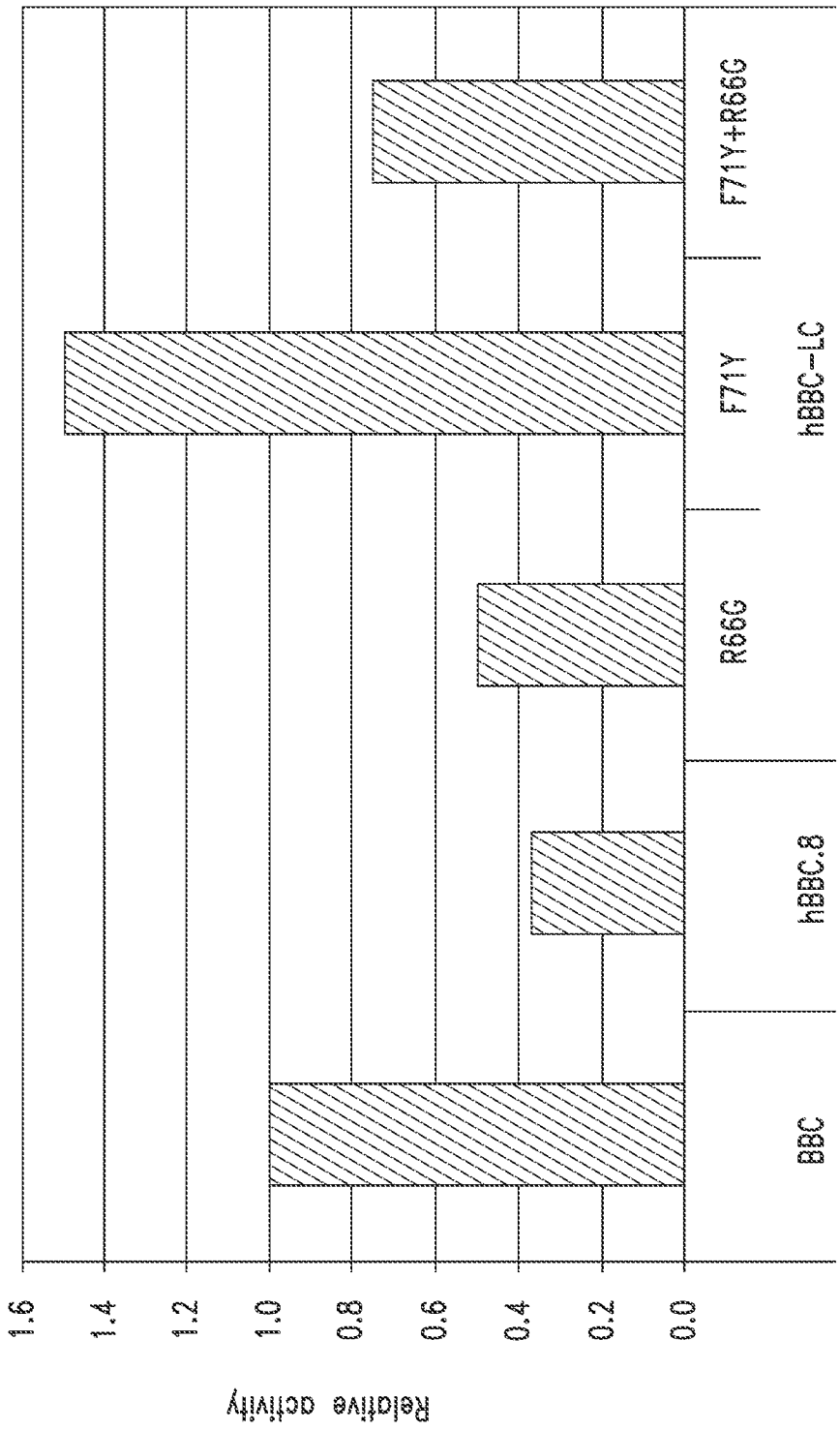

Relative activity
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
BBC
hBBC.8
R66G
F71Y
F71Y+R66G
hBBC-LC
AGS

FIG. 2B

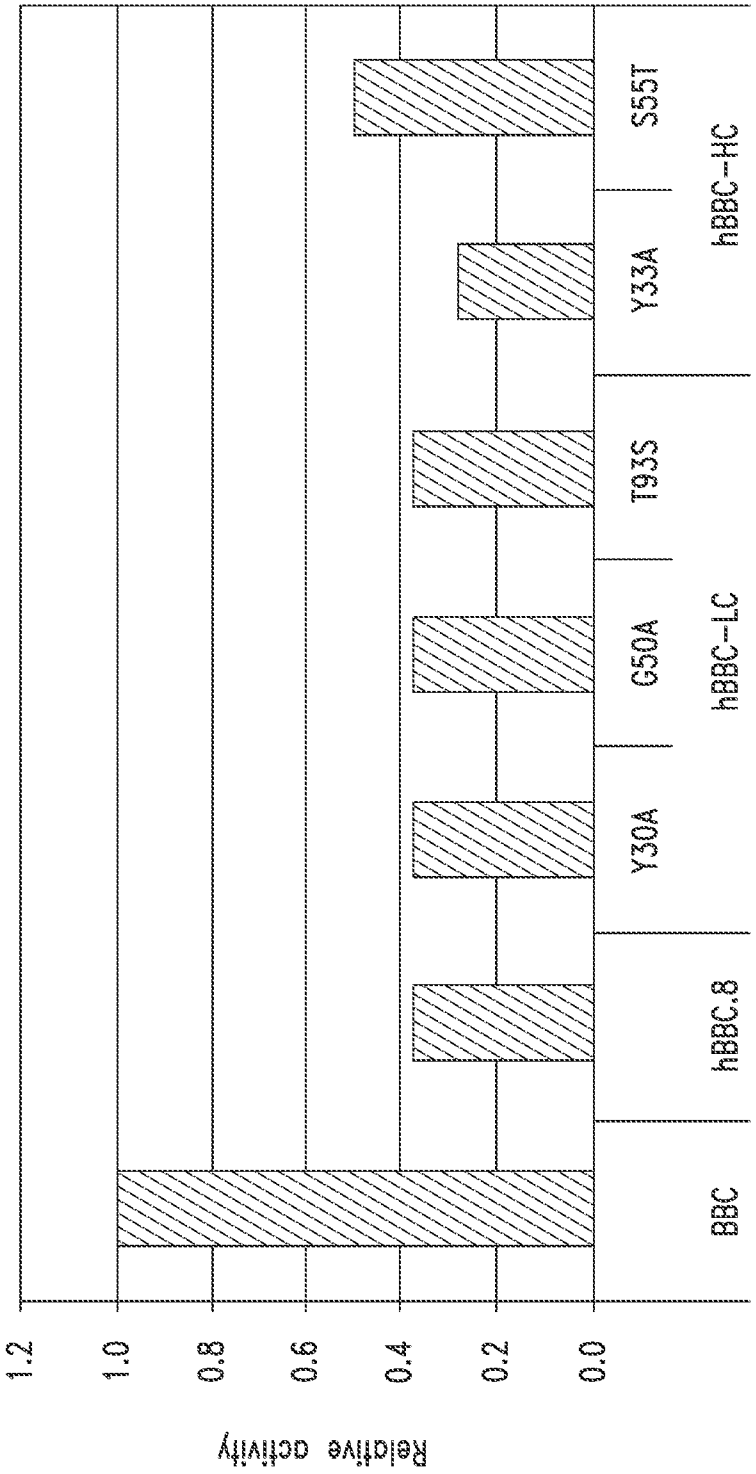

Relative activity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
BBC
hBBC.8
Y30A
G50A
T93S
Y33A
S55T
hBBC-LC
hBBC-HC
AGS

FIG. 2C

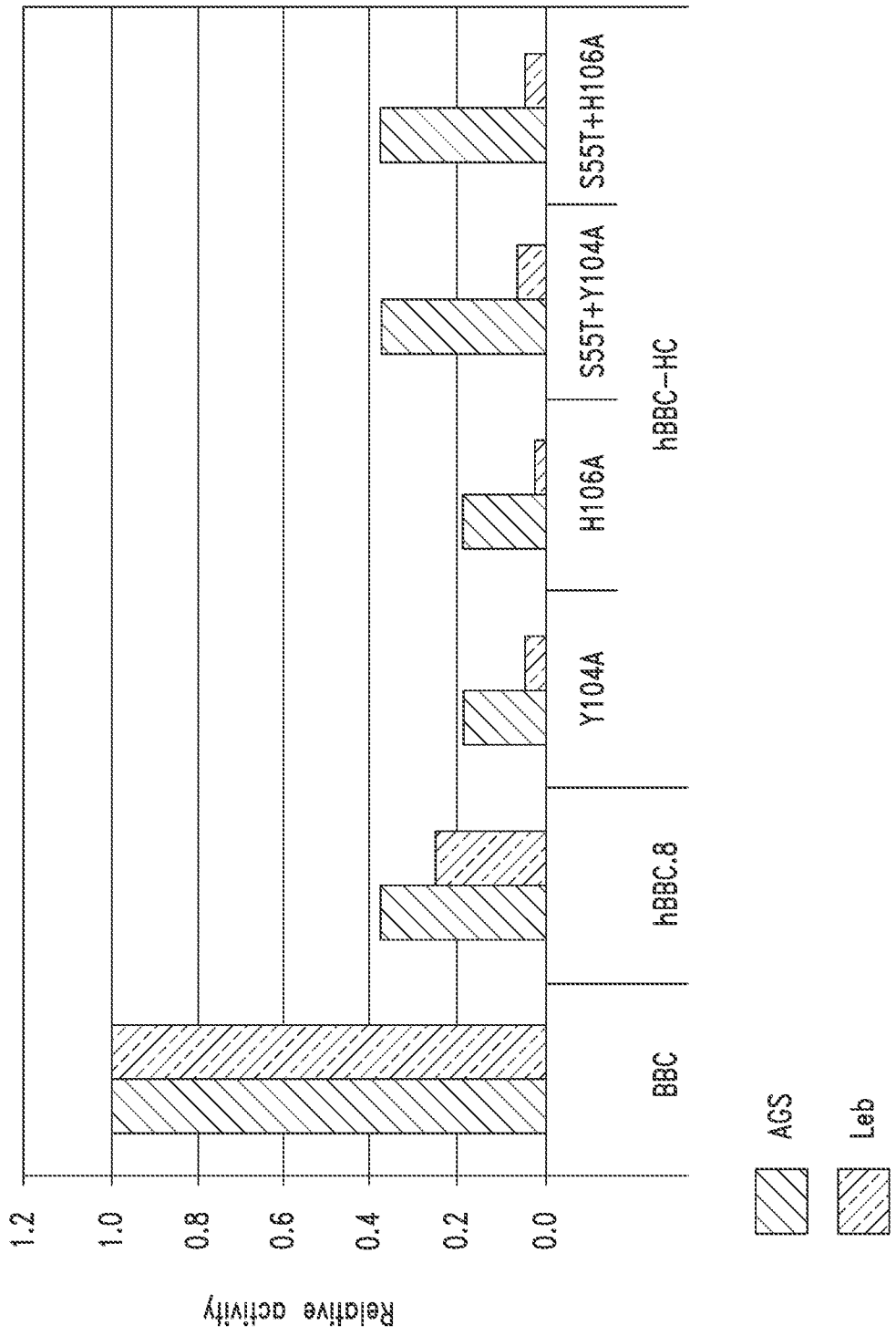

Relative activity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
BBC
hBBC.8
Y104A
H106A
S55T+Y104A
S55T+H106A
hBBC-HC
AGS
Leb

FIG. 2D

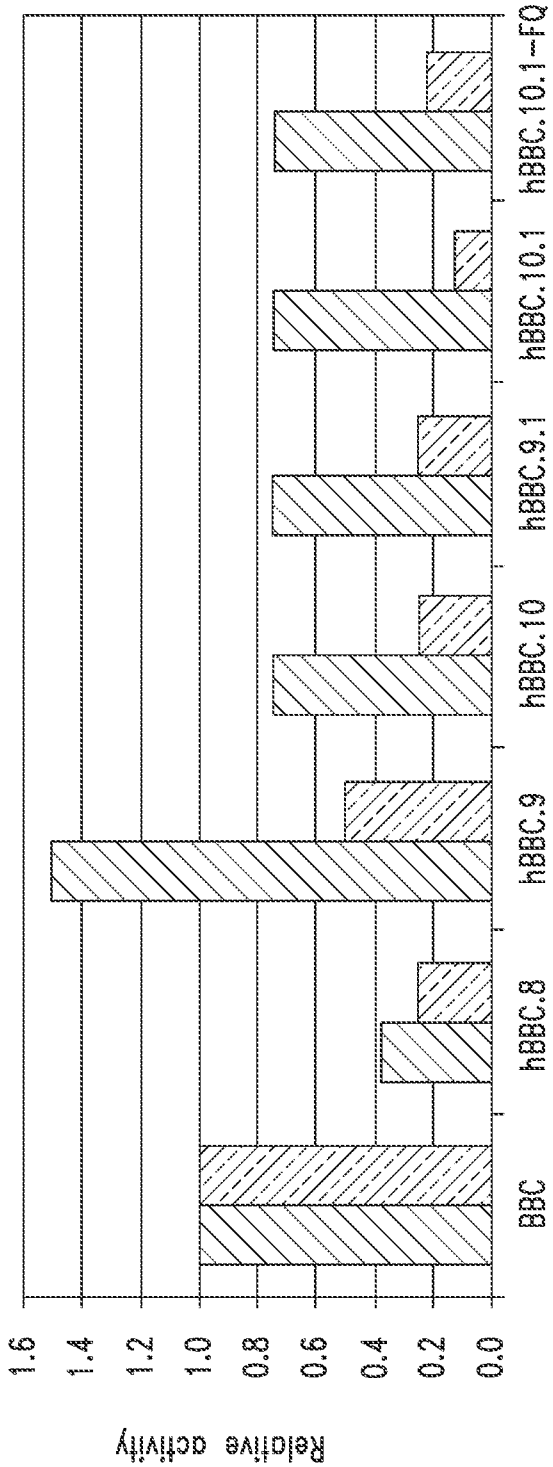

Relative activity
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
BBC
hBBC.8
hBBC.9
hBBC.10
hBBC.9.1
hBBC.10.1
hBBC.10.1-FQ
AGS
Leb

FIG. 2E

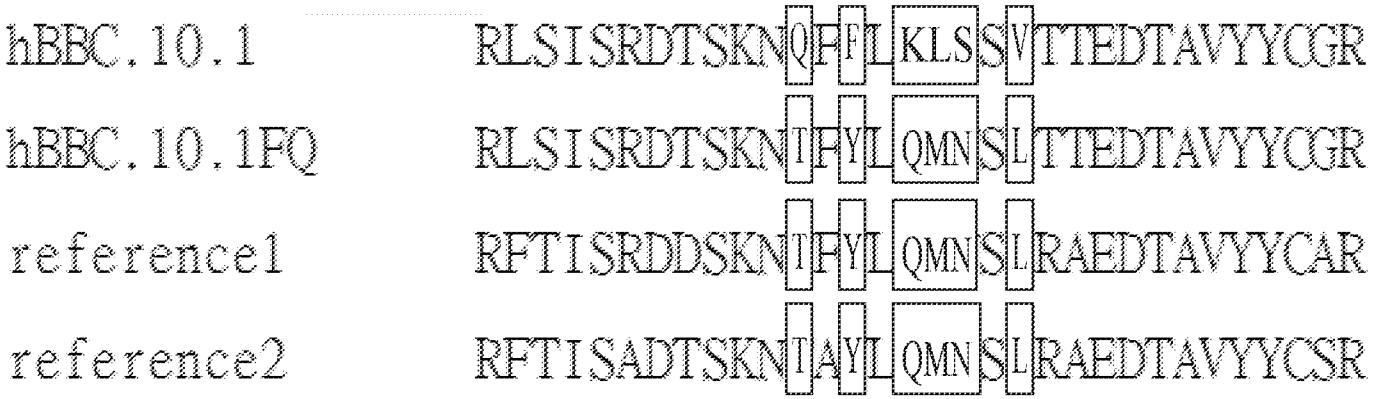

hBBC.10.1 RLSISRDTSKNQFFLKLSSVTTEDTAVYYCGR
hBBC.10.1FQ RLSISRDTSKNIFYLQMNSLTTEDTAVYYCGR
reference1 RFTISRDDSKNIFYLQMNSLRAEDTAVYYCAR
reference2 RFTISADTSKNIAYLQMNSLRAEDTAVYYCSR

FIG. 3

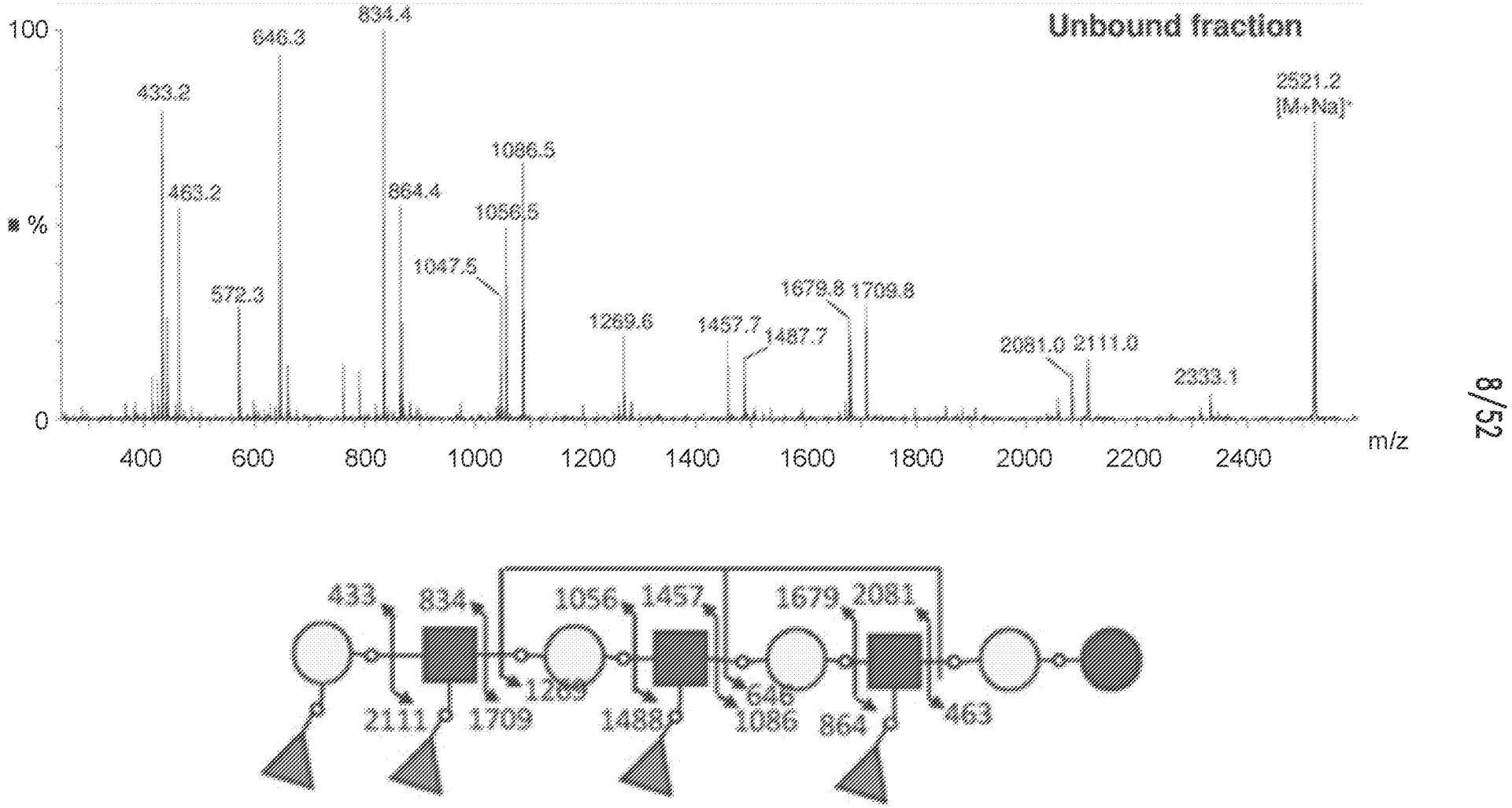
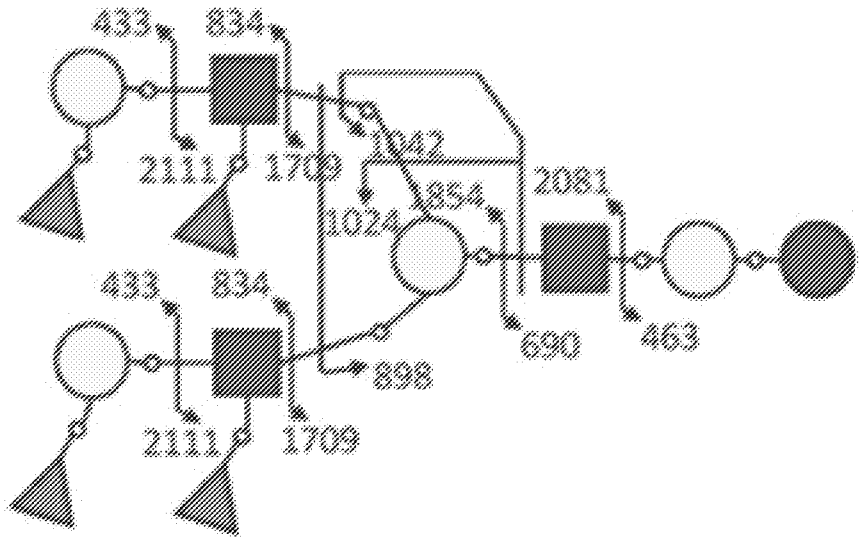

Unbound fraction
100
0
%
433.2
646.3
834.4
463.2
864.4
1086.5
1056.5
572.3
1047.5
1269.6
1457.7
1487.7
1679.8
1709.8
2081.0
2111.0
2333.1
2521.2
[M+Na]+
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
m/z
433
834
1056
1457
1679
2081
2111
1709
1269
1488
1086
648
864
463

FIG. 4A


433
834
2111
1709
1042
1854
2081
1024
433
834
690
463
898
2111
1709

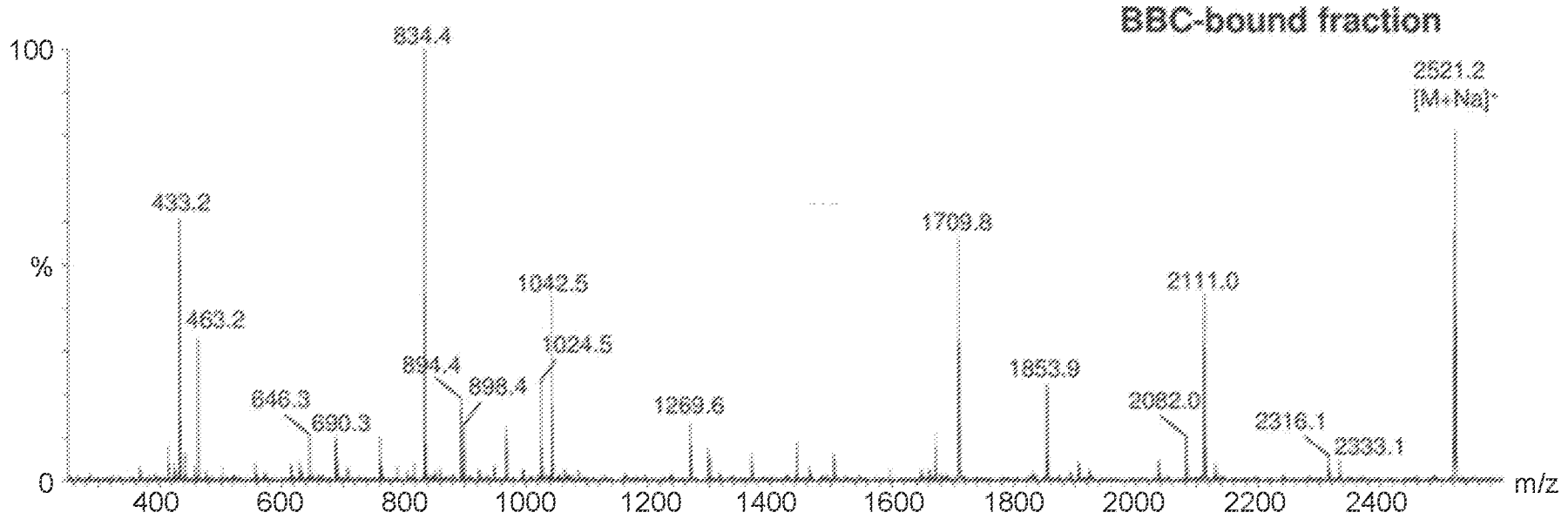

BBC-bound fraction
100
%
0
834.4
2521.2
[M+Na]+
433.2
1709.8
1042.5
2111.0
463.2
1024.5
894.4
898.4
1853.9
646.3
690.3
1269.6
2082.0
2316.1
2333.1
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
m/z

FIG. 4B

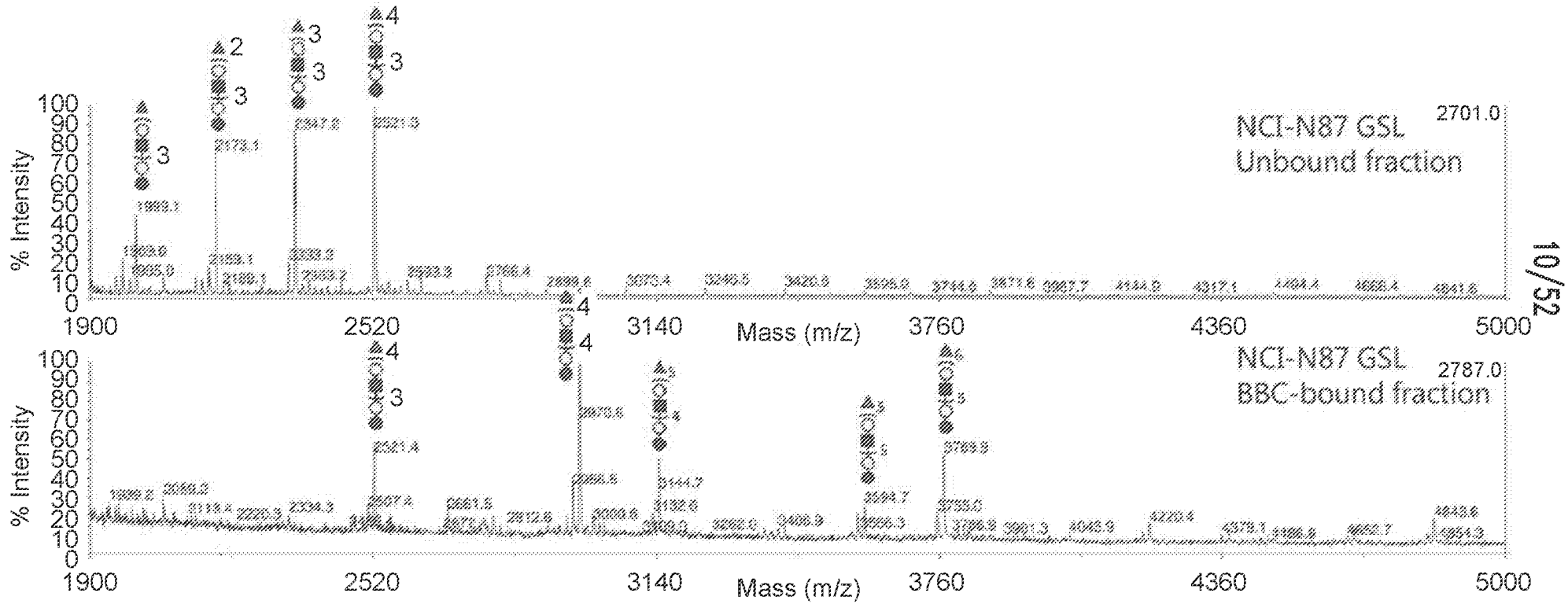

NCI-N87 GSL
Unbound fraction
2701.0
NCI-N87 GSL
BBC-bound fraction
2787.0
% Intensity
Mass (m/z)
1900
2520
3140
3760
4360
5000

FIG. 5A

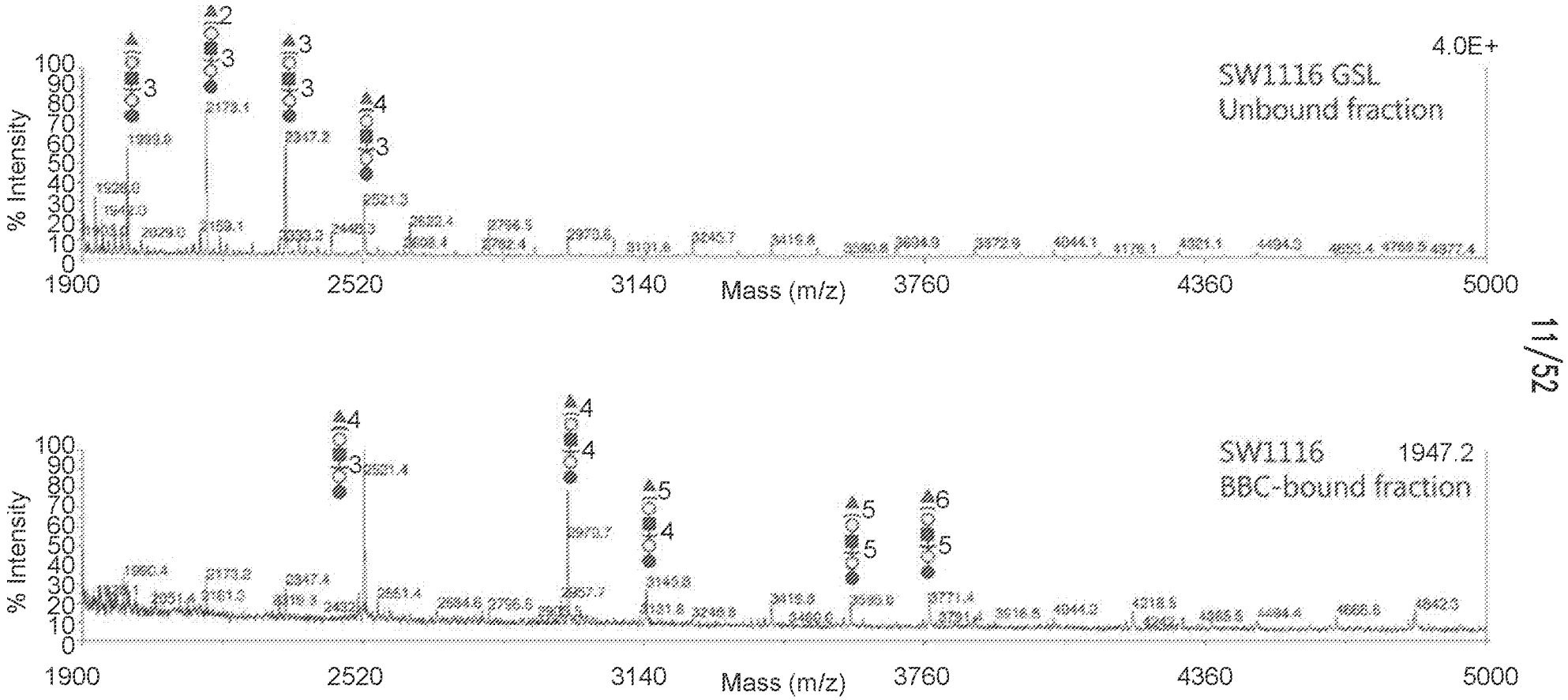

4.0E+
SW1116 GSL
Unbound fraction
% Intensity
100
90
80
70
60
50
40
30
20
10
0
1900
2520
3140
Mass (m/z)
3760
4360
5000
SW1116
1947.2
BBC-bound fraction
% Intensity
100
90
80
70
60
50
40
30
20
10
0
1900
2520
3140
Mass (m/z)
3760
4360
5000

FIG. 5B

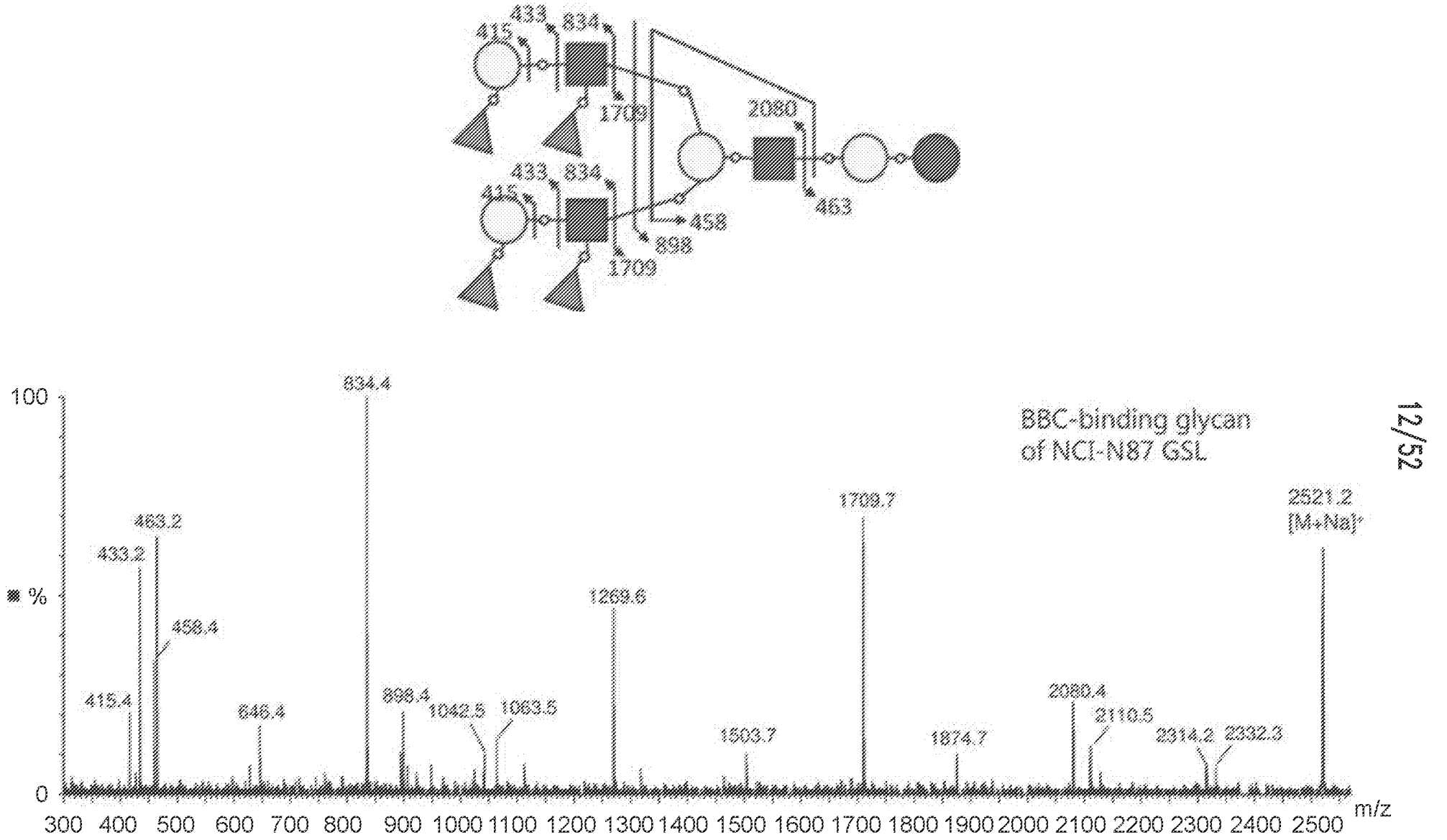
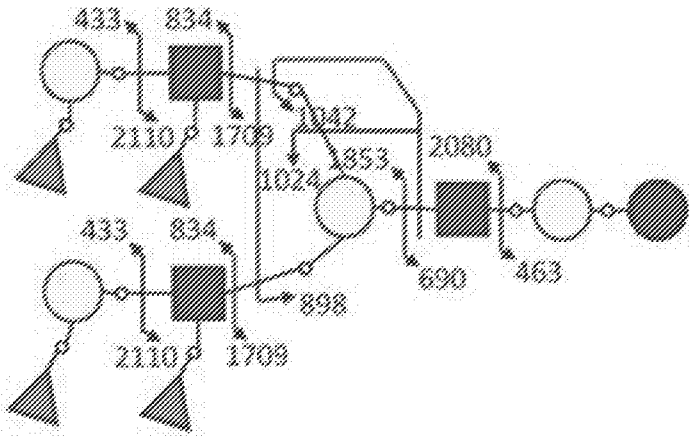

415
433
834
1709
2080
463
458
898
BBC-binding glycan
of NCI-N87 GSL
2521.2
[M+Na]+
1709.7
834.4
463.2
433.2
458.4
1269.6
415.4
646.4
898.4
1042.5
1063.5
1503.7
1874.7
2080.4
2110.5
2314.2
2332.3
100
%
0
m/z
300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000 2100 2200 2300 2400 2500

FIG. 6A


433
834
2110
1709
1042
1024
853
2080
690
463
433
834
898
2110
1709

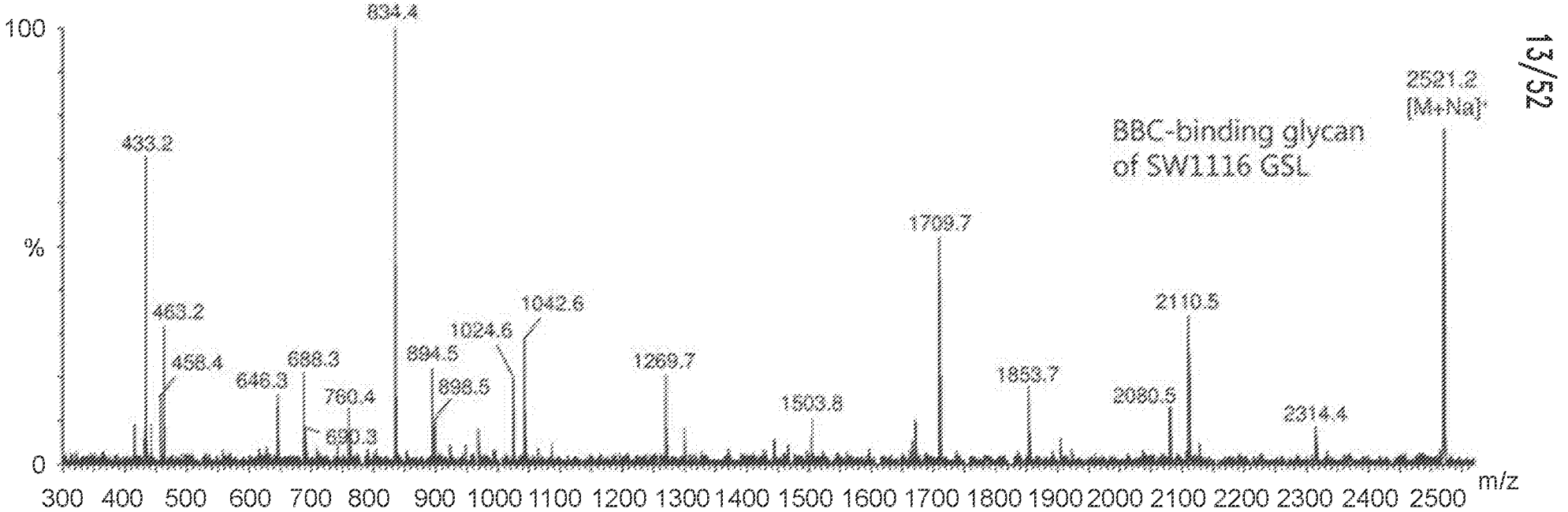
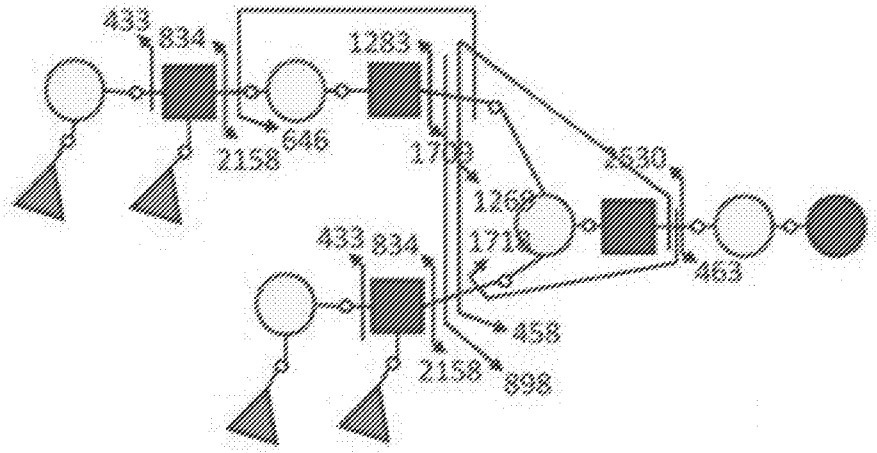

100
%
0
834.4
433.2
463.2
458.4
646.3
688.3
760.4
690.3
894.5
898.5
1024.6
1042.6
1269.7
1503.8
1709.7
1853.7
2080.5
2110.5
2314.4
2521.2
[M+Na]+
BBC-binding glycan
of SW1116 GSL
300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000 2100 2200 2300 2400 2500
m/z

FIG. 6B


433
834
1283
2158
646
1718
2530
1268
1718
433
834
463
458
2158
898

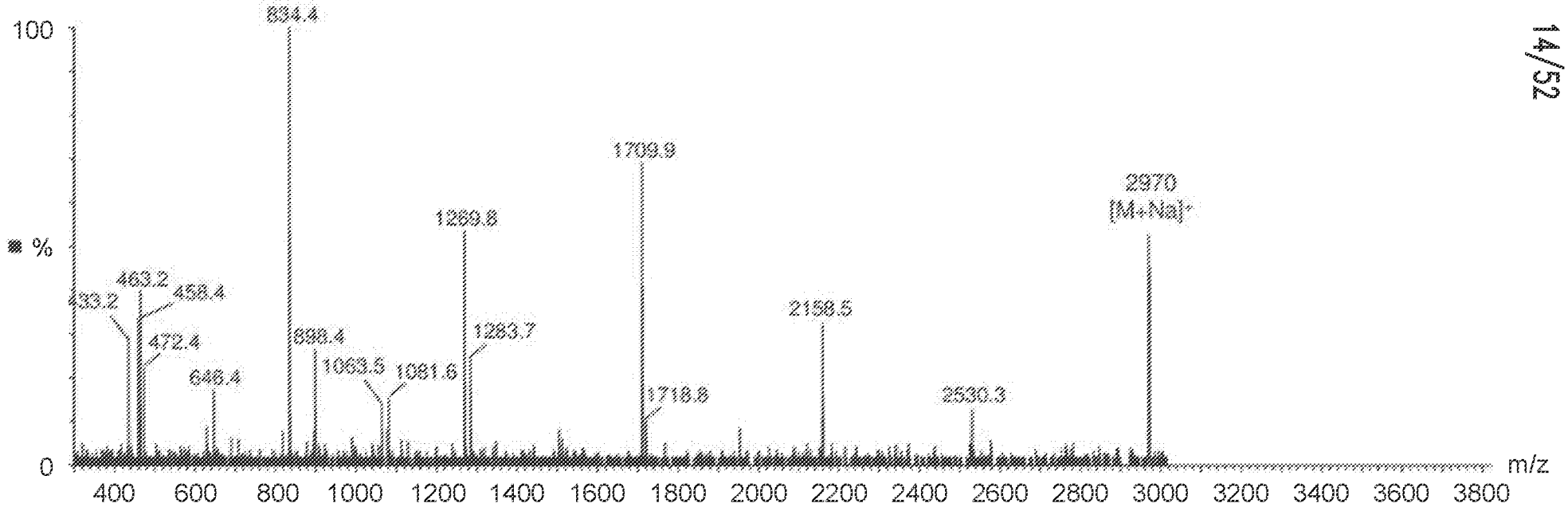

100
%
0
834.4
1709.9
1259.8
2970
[M+Na]+
463.2
458.4
433.2
472.4
646.4
898.4
1063.5
1081.6
1263.7
2158.5
1718.8
2530.3
m/z
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800

FIG. 7A

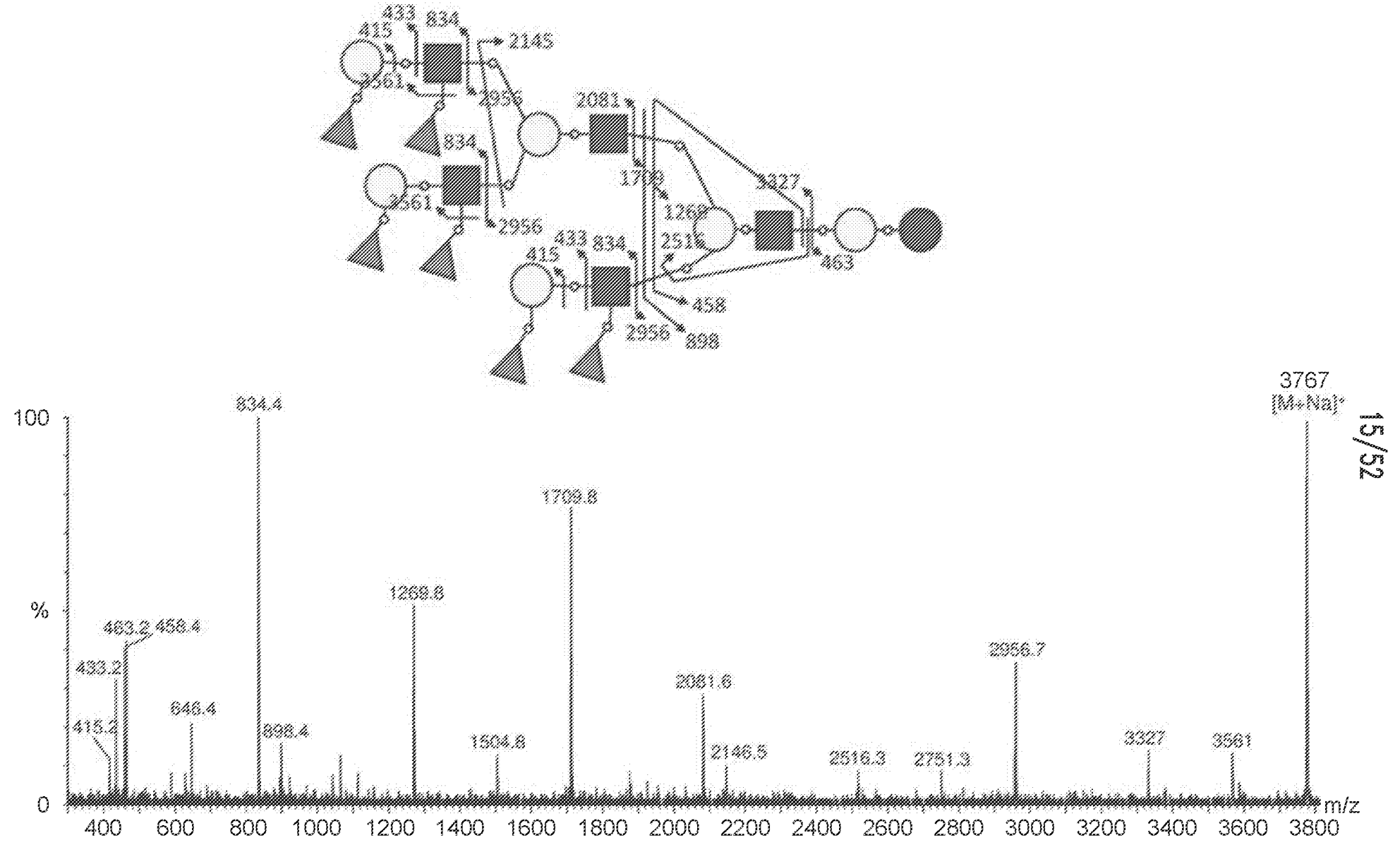

433
834
415
2145
561
2956
2081
834
1709
3327
561
1269
2956
2516
433
834
415
463
458
2956
898
3767
[M+Na]+
834.4
100
1709.8
1269.8
%
463.2
458.4
433.2
2956.7
2081.6
646.4
415.2
898.4
1504.8
2146.5
2516.3
2751.3
3327
3561
0
m/z
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800

FIG. 7B

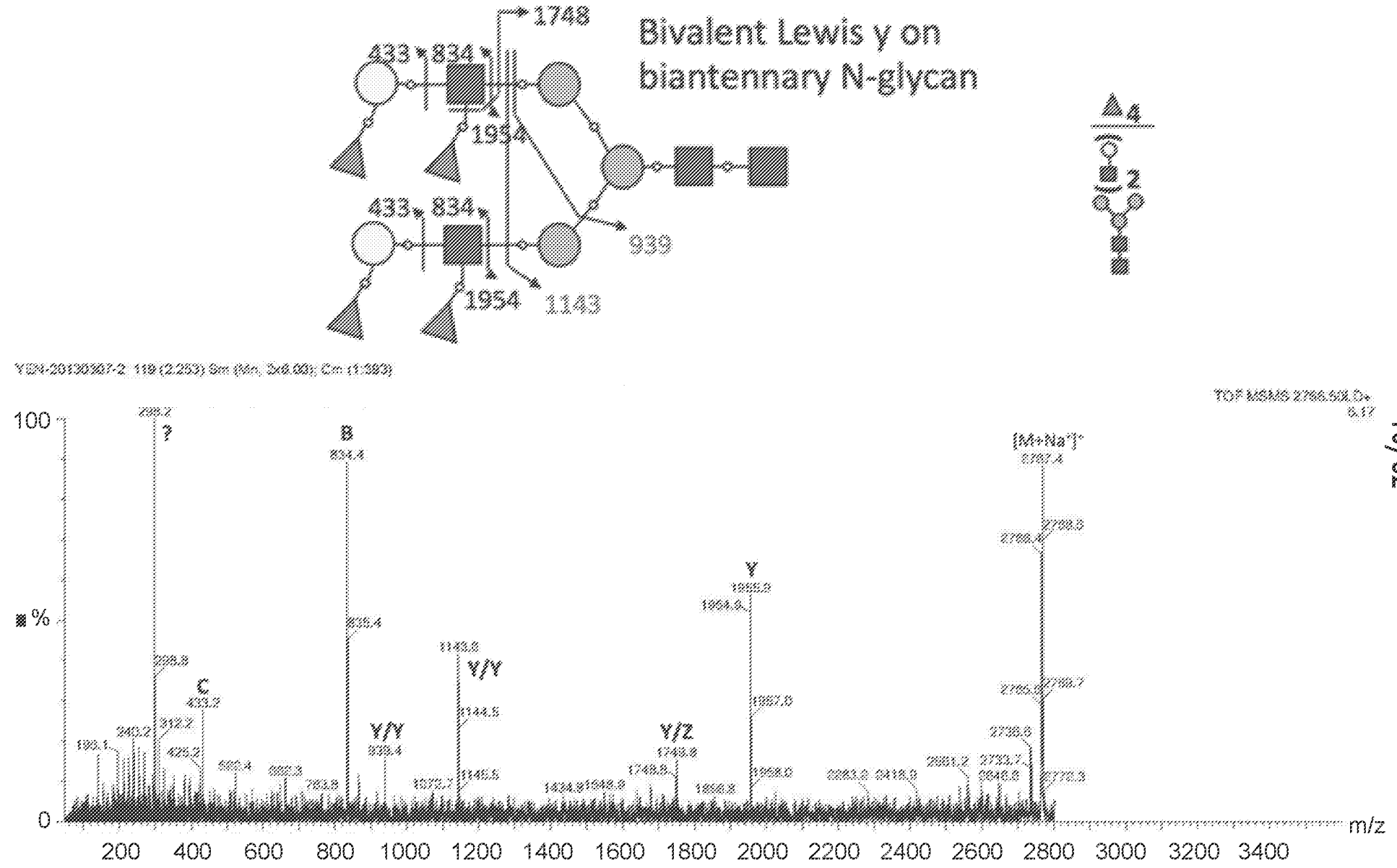

Bivalent Lewis y on
biantennary N-glycan
1748
433
834
1954
939
1143
4
2
100
%
0
?
B
C
Y/Y
Y/Z
Y
[M+Na]+
m/z
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200
3400

FIG. 8A

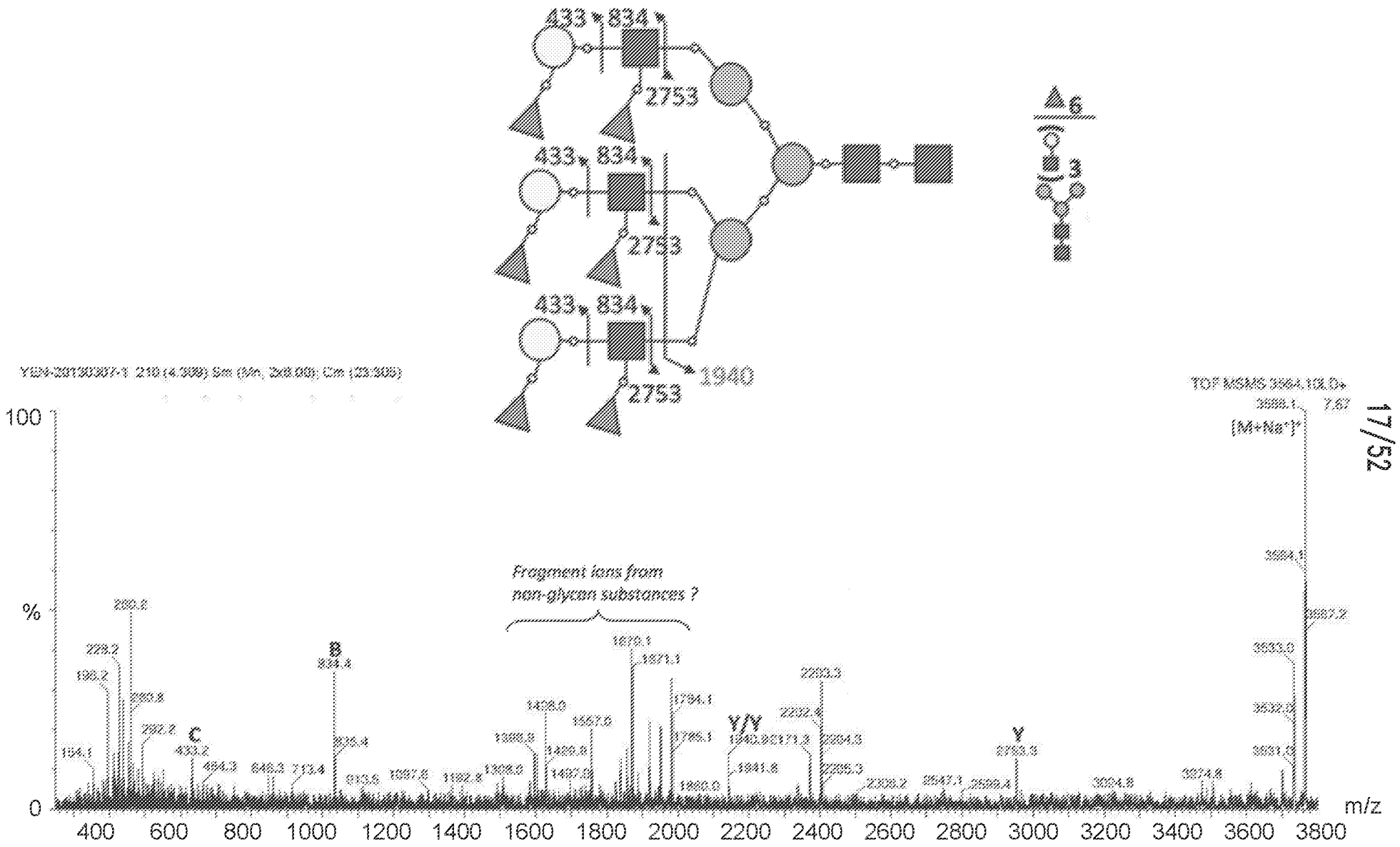

433
834
2753
1940
6
3
100
%
0
m/z
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800
Fragment ions from
non-glycan substances ?
B
C
Y/Y
Y
[M+Na+]+
7.67

FIG. 8B

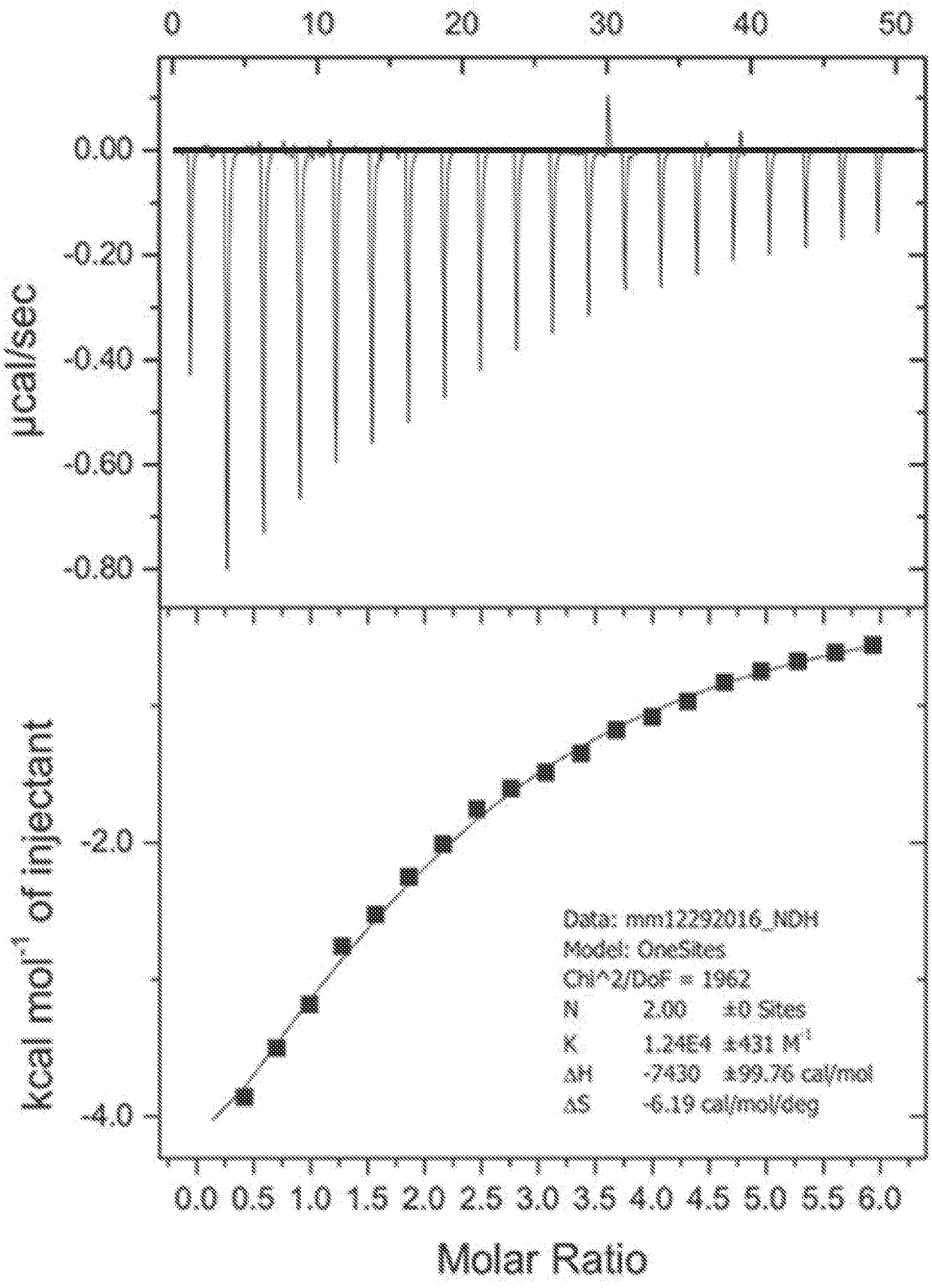

0
10
20
30
40
50
0.00
-0.20
-0.40
-0.60
-0.80
µcal/sec
kcal mol-1 of injectant
-2.0
-4.0
Data: mm12292016_NDH
Model: OneSites
Chi^2/DoF = 1962
N 2.00 ±0 Sites
K 1.24E4 ±431 M-1
ΔH -7430 ±99.76 cal/mol
ΔS -6.19 cal/mol/deg
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0
Molar Ratio

FIG. 9A

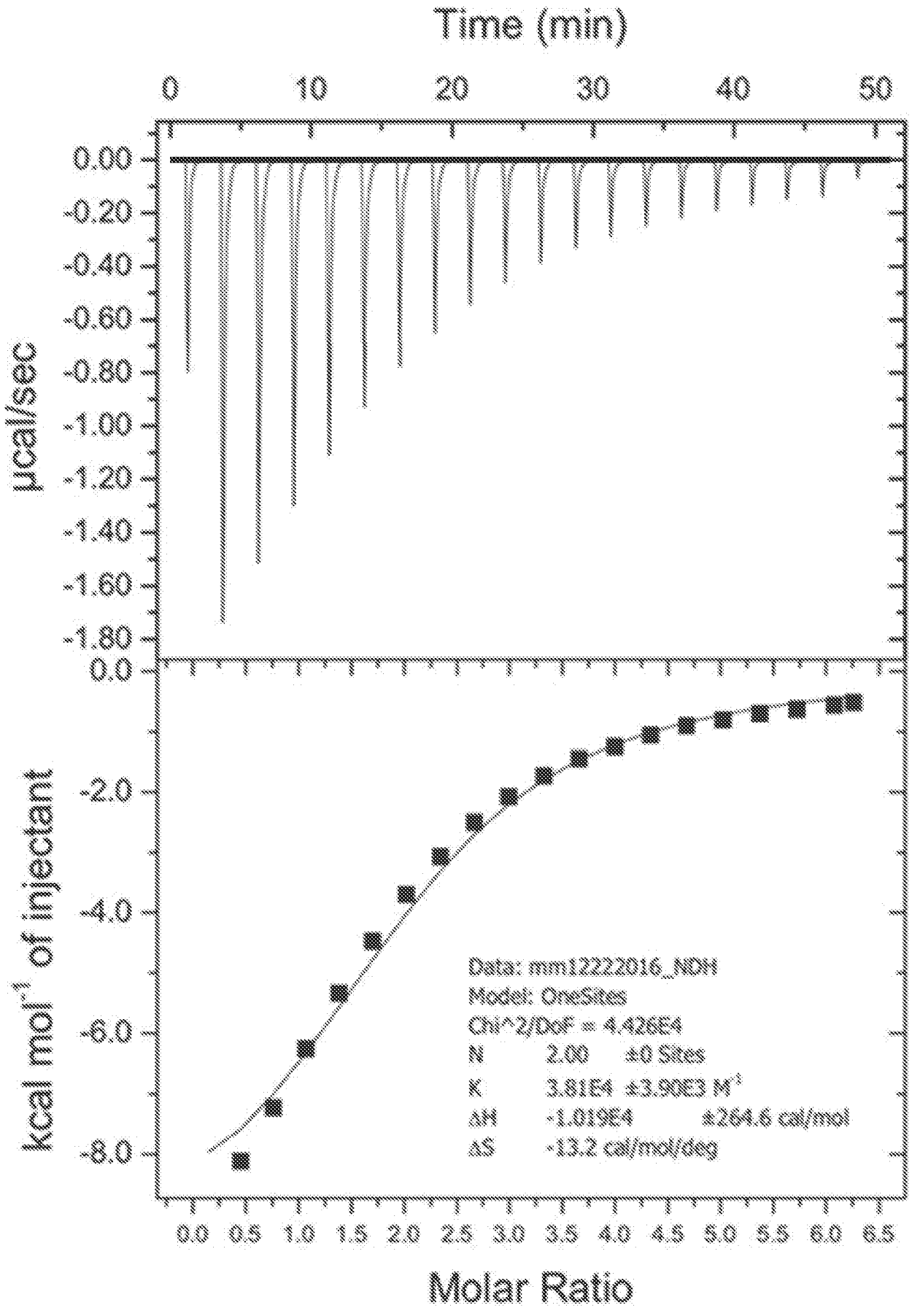

Time (min)
0
10
20
30
40
50
0.00
-0.20
-0.40
-0.60
-0.80
-1.00
-1.20
-1.40
-1.60
-1.80
µcal/sec
0.0
-2.0
-4.0
-6.0
-8.0
kcal mol-1 of injectant
Data: mm12222016_NDH
Model: OneSites
Chi^2/DoF = 4.426E4
N 2.00 ±0 Sites
K 3.81E4 ±3.90E3 M-1
ΔH -1.019E4 ±264.6 cal/mol
ΔS -13.2 cal/mol/deg
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
Molar Ratio

FIG. 9B

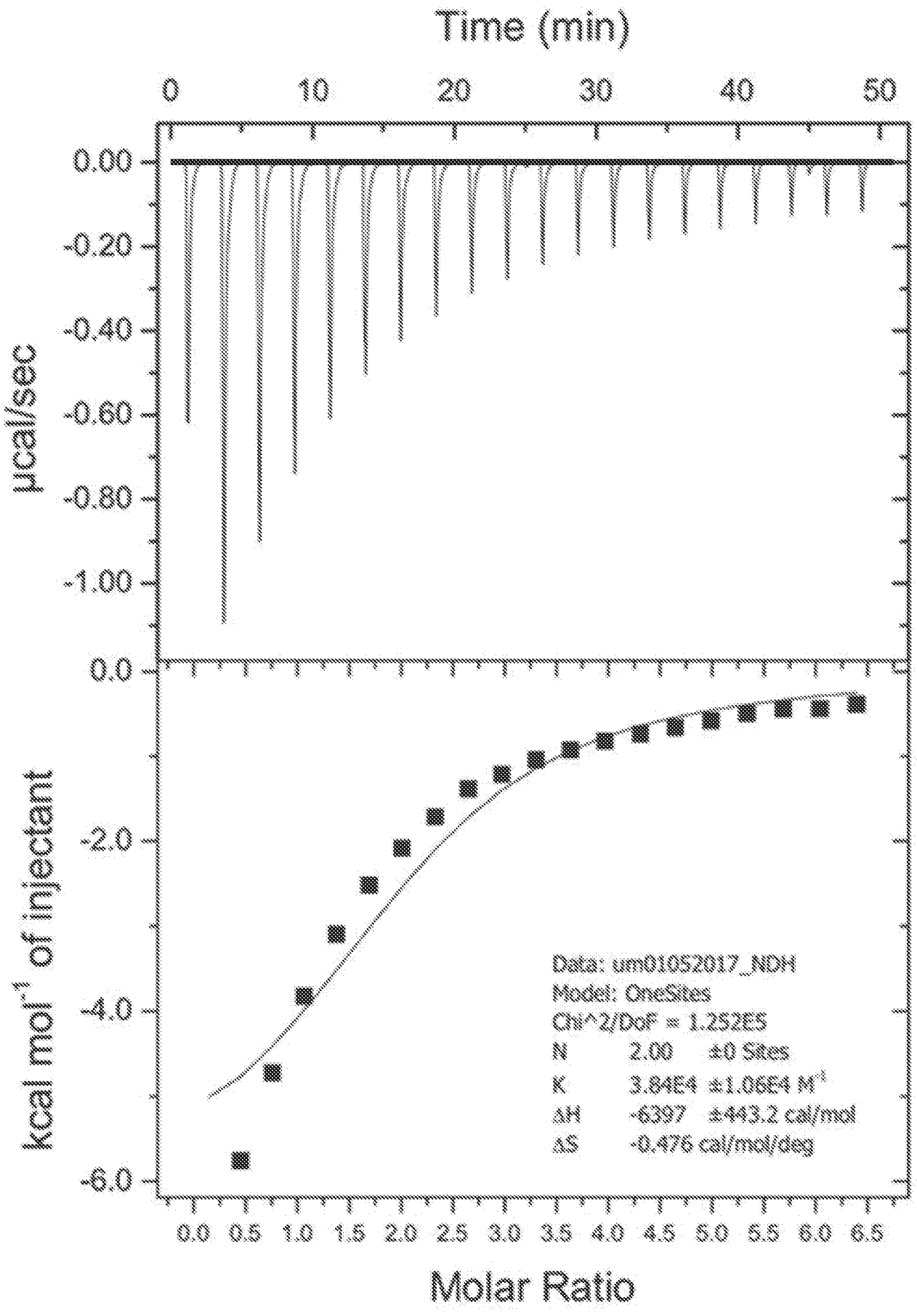

Time (min)
0
10
20
30
40
50
0.00
-0.20
-0.40
-0.60
-0.80
-1.00
0.0
µcal/sec
kcal mol-1 of injectant
-2.0
-4.0
-6.0
Data: um01052017_NDH
Model: OneSites
Chi^2/DoF = 1.252E5
N 2.00 ±0 Sites
K 3.84E4 ±1.06E4 M-1
ΔH -6397 ±443.2 cal/mol
ΔS -0.476 cal/mol/deg
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5
Molar Ratio

FIG. 10A

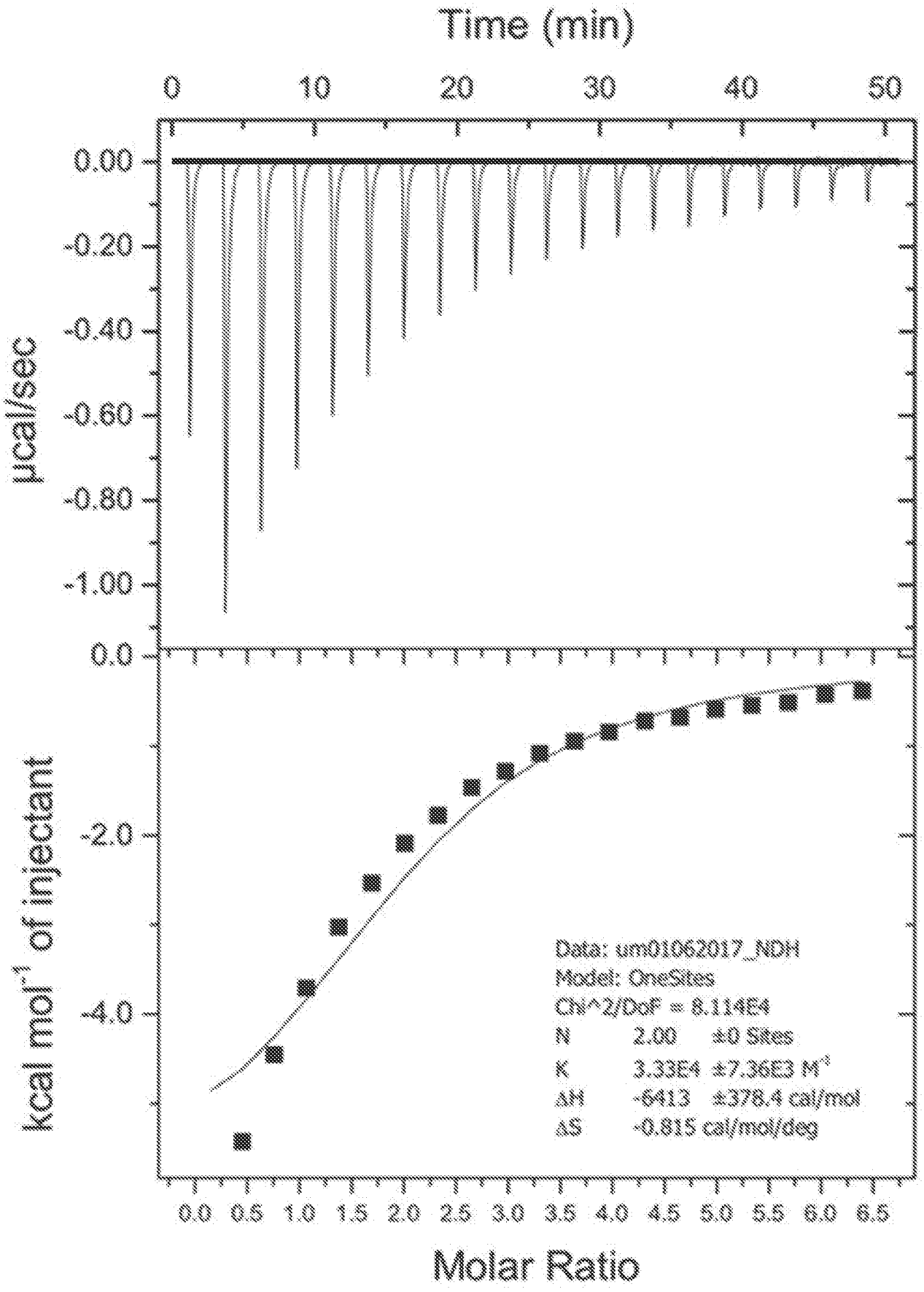

Time (min)
0
10
20
30
40
50
0.00
-0.20
-0.40
-0.60
-0.80
-1.00
µcal/sec
0.0
-2.0
-4.0
kcal mol-1 of injectant
Data: um01062017_NDH
Model: OneSites
Chi^2/DoF = 8.114E4
N 2.00 ±0 Sites
K 3.33E4 ±7.36E3 M-1
ΔH -6413 ±378.4 cal/mol
ΔS -0.815 cal/mol/deg
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
Molar Ratio

FIG. 10B

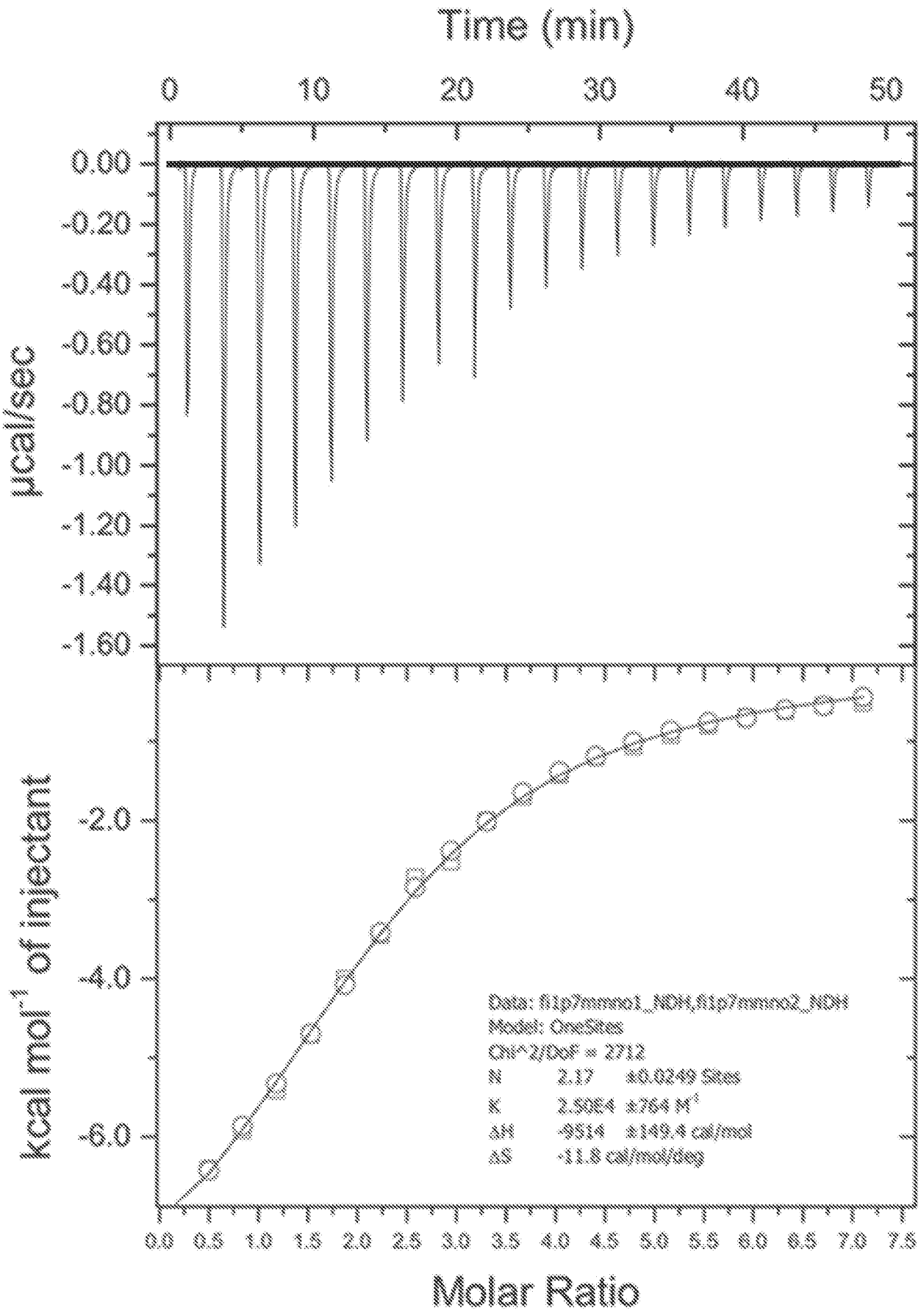

Time (min)
0
10
20
30
40
50
0.00
-0.20
-0.40
-0.60
-0.80
-1.00
-1.20
-1.40
-1.60
µcal/sec
-2.0
-4.0
-6.0
kcal mol-1 of injectant
Data: fi1p7mmno1_NDH,fi1p7mmno2_NDH
Model: OneSites
Chi^2/DoF = 2712
N 2.17 ±0.0249 Sites
K 2.50E4 ±764 M-1
ΔH -9514 ±149.4 cal/mol
ΔS -11.8 cal/mol/deg
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Molar Ratio

FIG. 10C

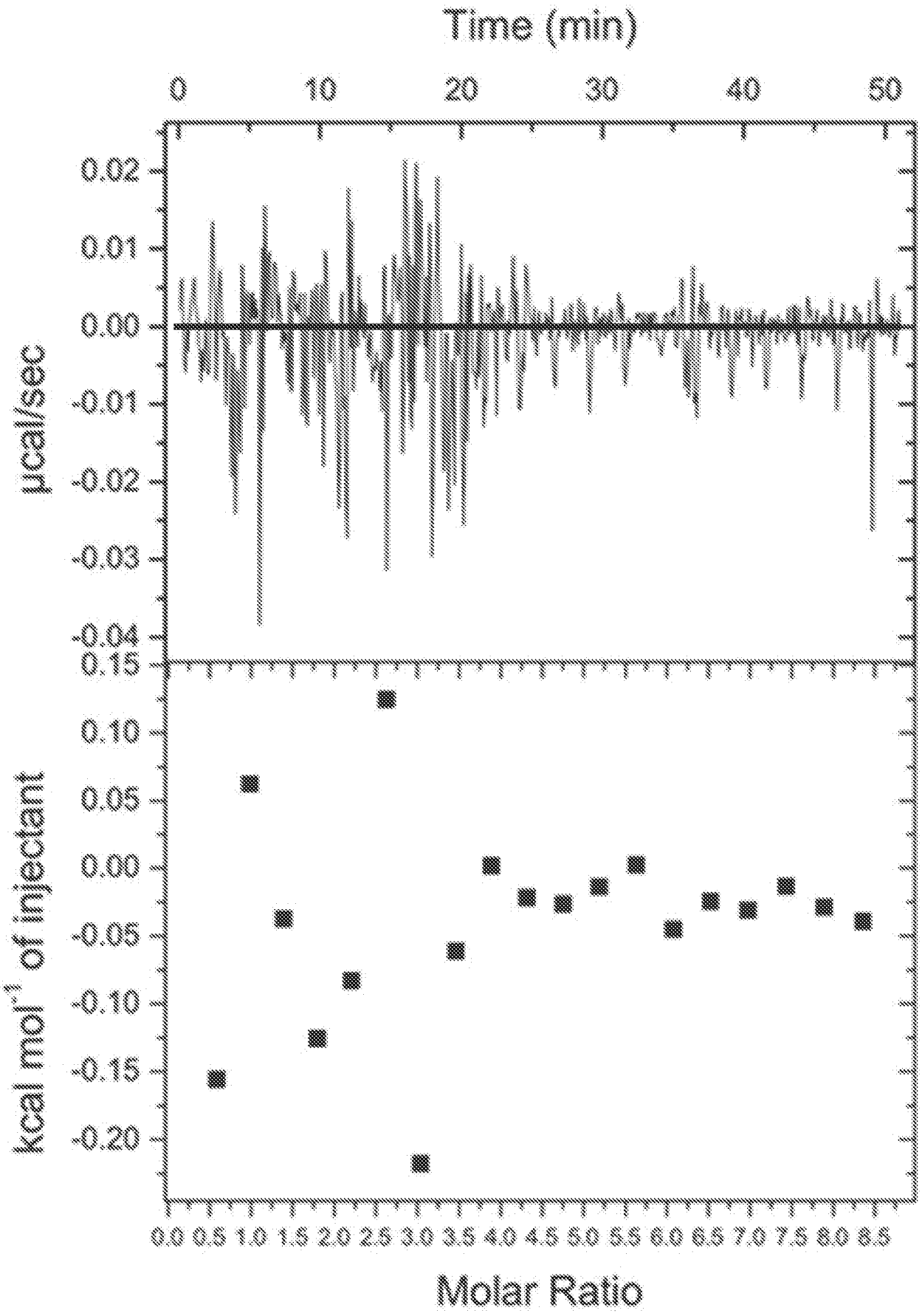

Time (min)
0
10
20
30
40
50
0.02
0.01
0.00
-0.01
-0.02
-0.03
-0.04
µcal/sec
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
kcal mol-1 of injectant
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5
Molar Ratio

FIG. 11A

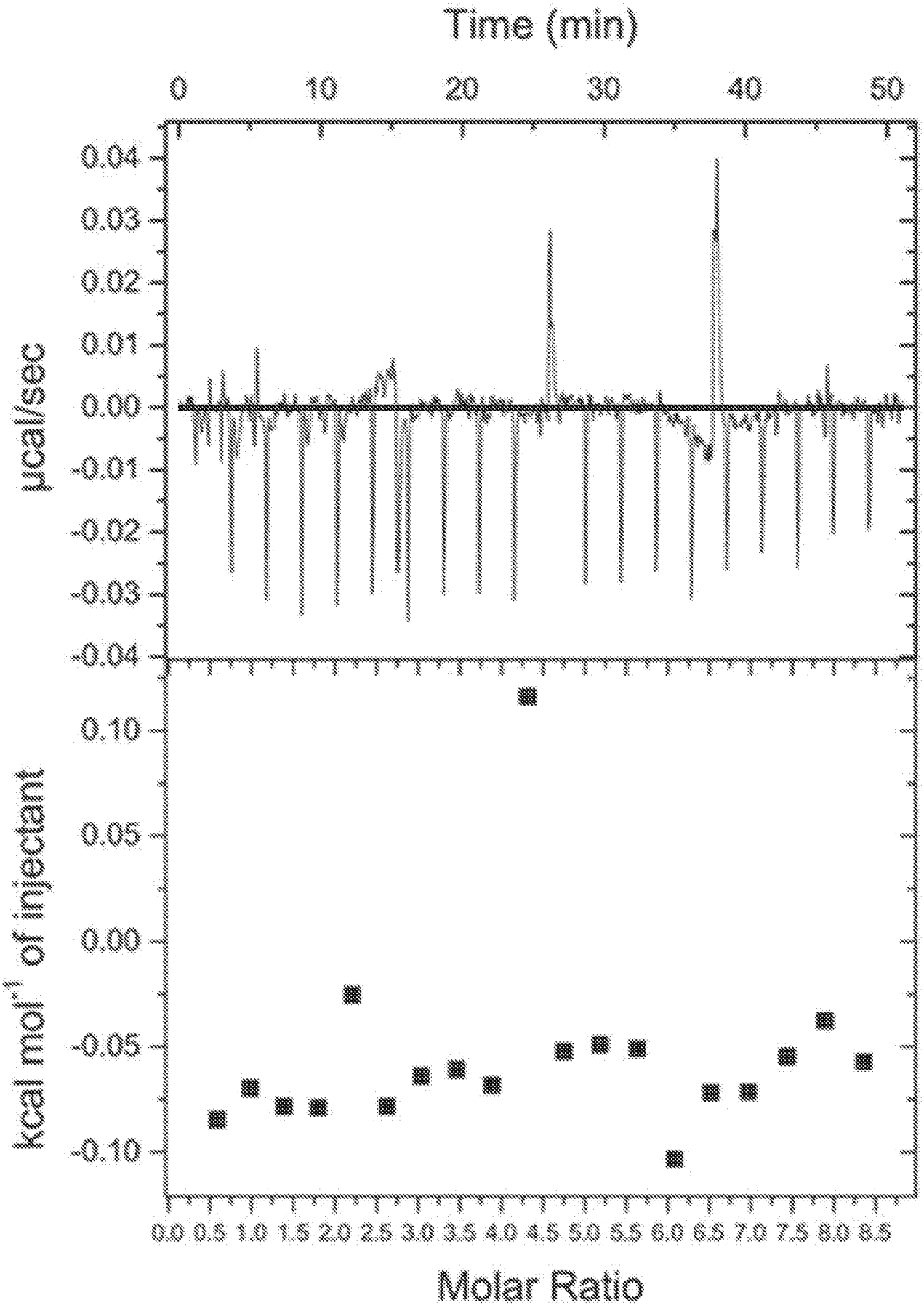

Time (min)
0
10
20
30
40
50
µcal/sec
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
-0.04
kcal mol-1 of injectant
0.10
0.05
0.00
-0.05
-0.10
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5
Molar Ratio

FIG. 11B

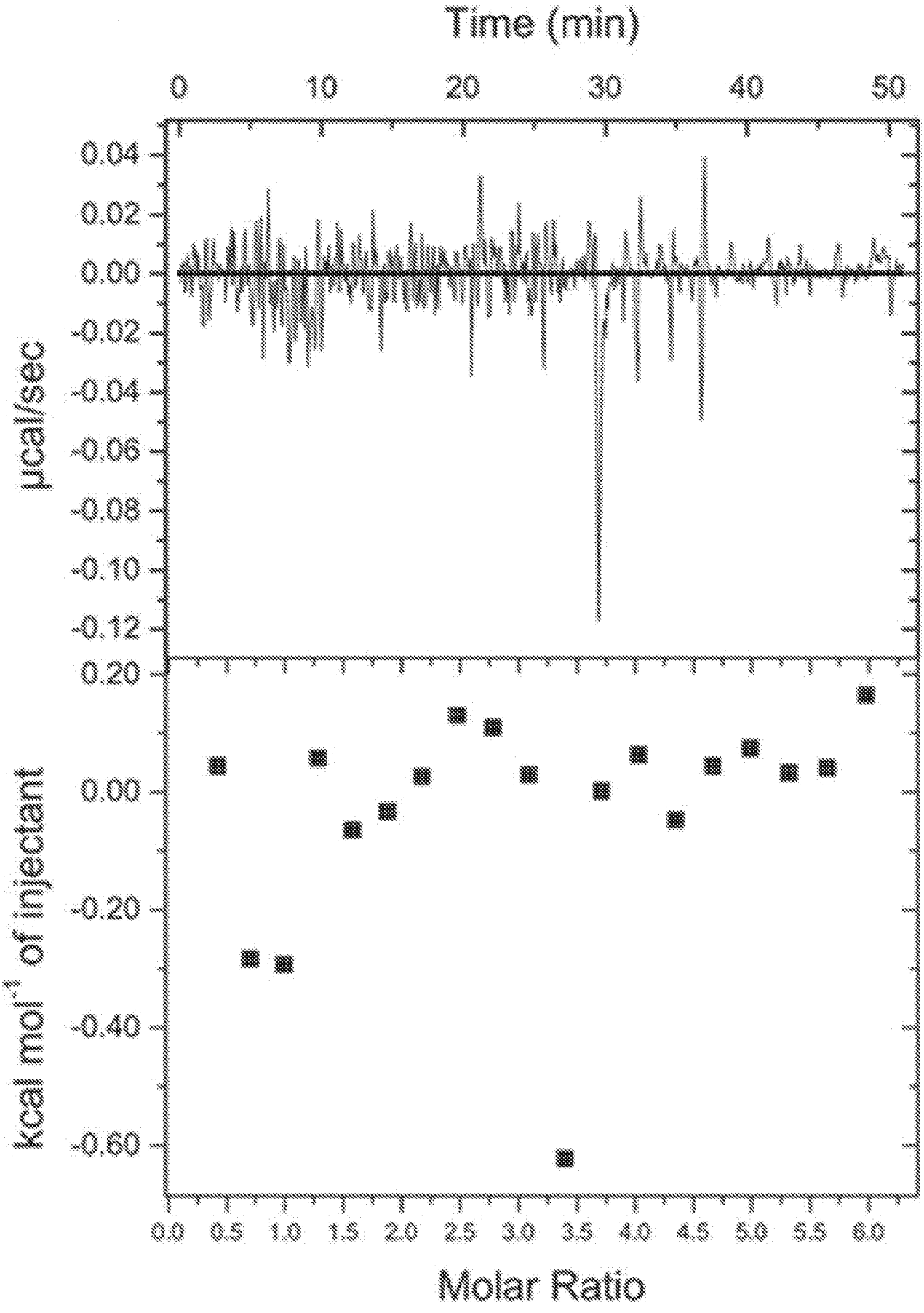

Time (min)
0
10
20
30
40
50
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
-0.12
µcal/sec
0.20
0.00
-0.20
-0.40
-0.60
kcal mol-1 of injectant
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
Molar Ratio

FIG. 11C

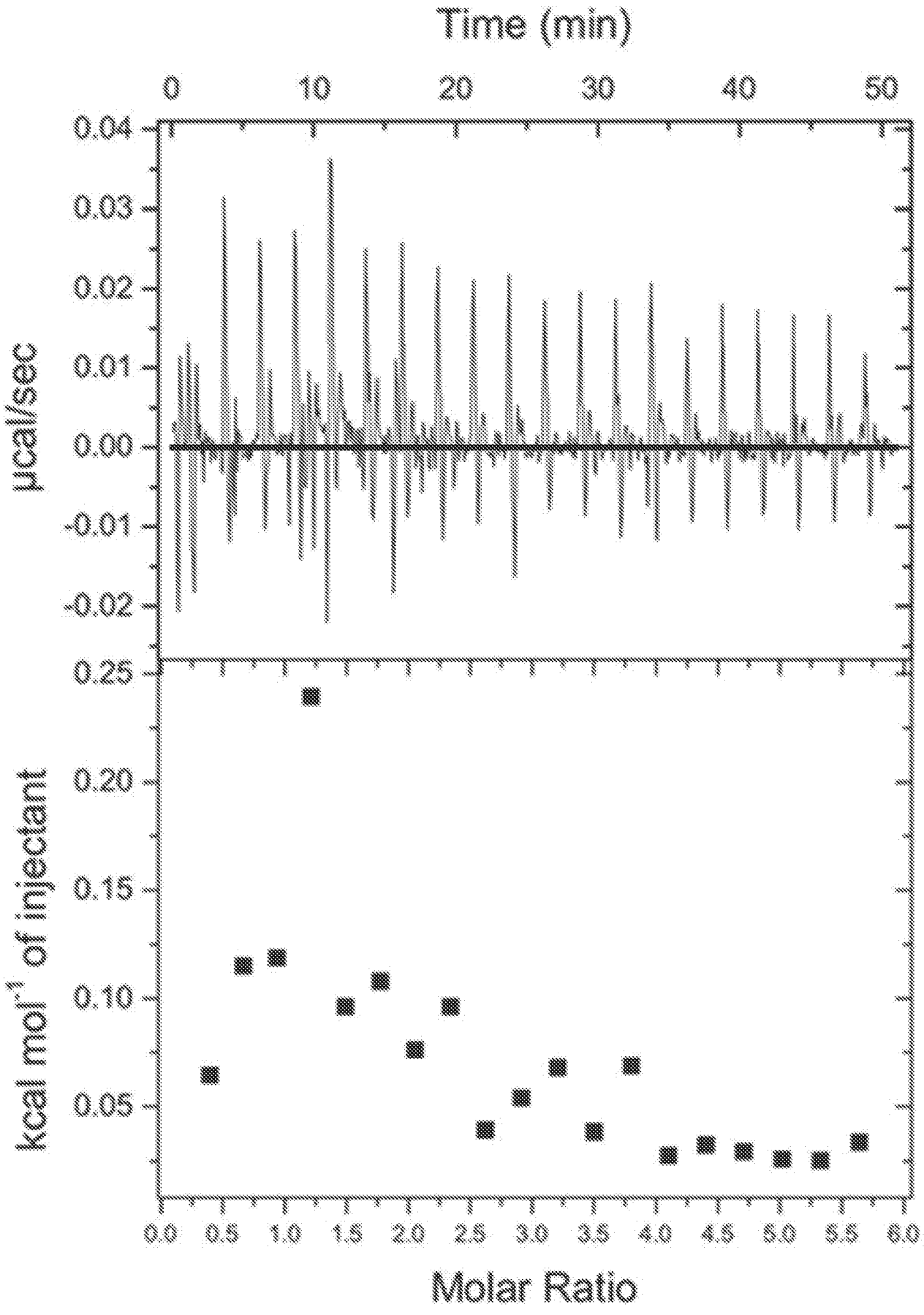

Time (min)
0
10
20
30
40
50
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
µcal/sec
0.25
0.20
0.15
0.10
0.05
kcal mol-1 of injectant
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
Molar Ratio

FIG. 11D

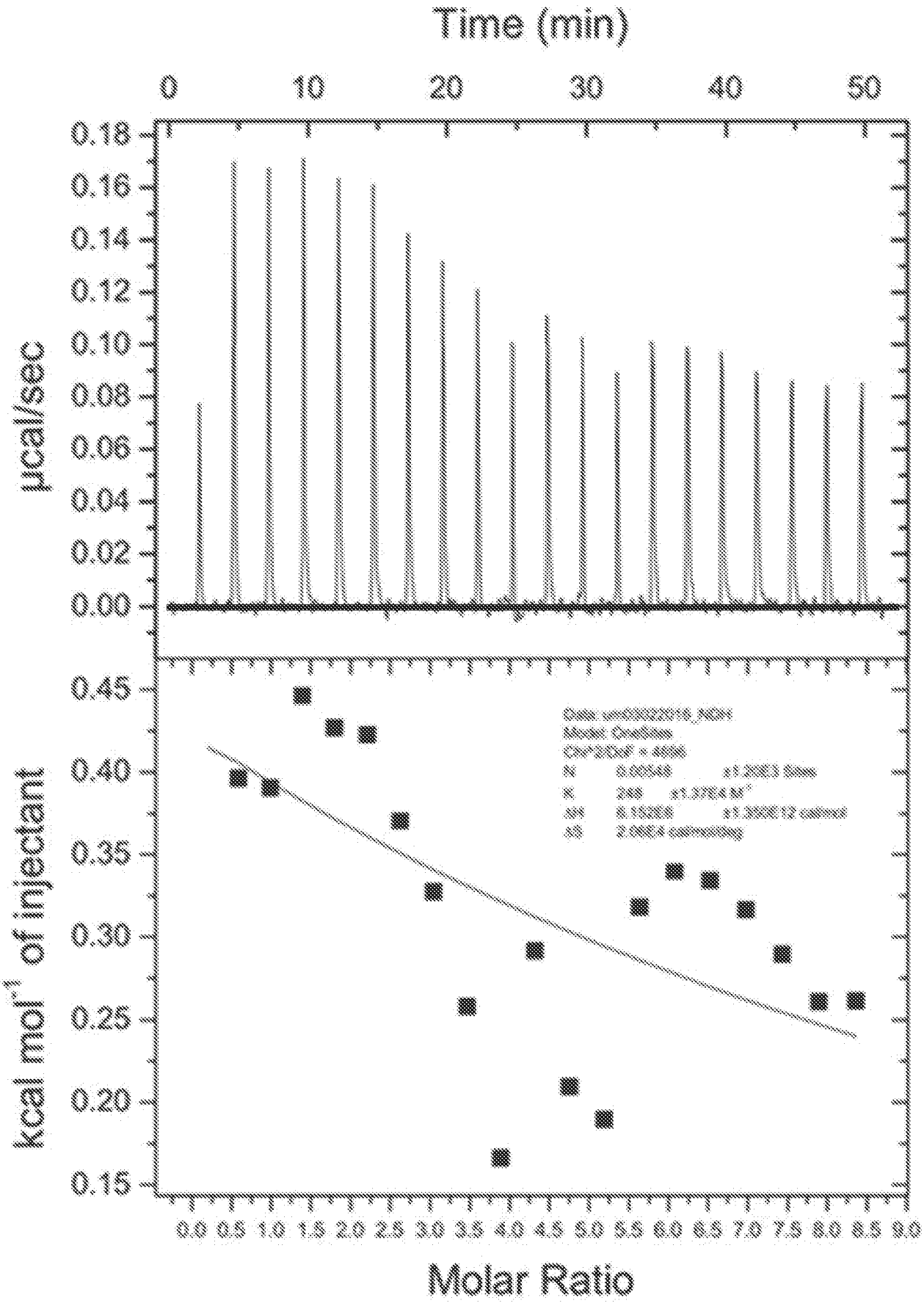

Time (min)
0
10
20
30
40
50
µcal/sec
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
kcal mol-1 of injectant
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0
Molar Ratio

FIG. 11E

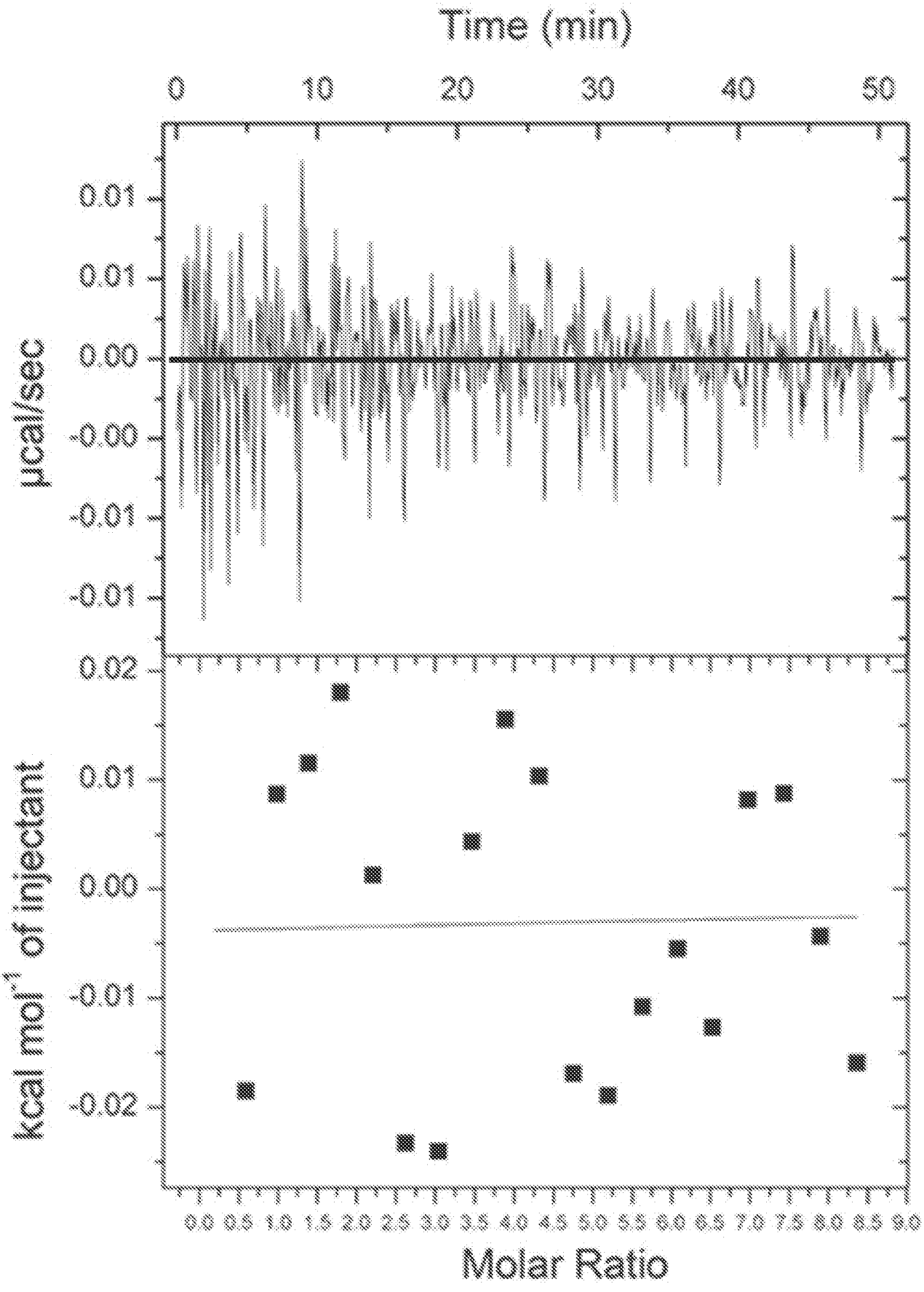

Time (min)
0
10
20
30
40
50
µcal/sec
0.01
0.01
0.00
-0.00
-0.01
-0.01
0.02
kcal mol-1 of injectant
0.01
0.00
-0.01
-0.02
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0
Molar Ratio

FIG. 11F

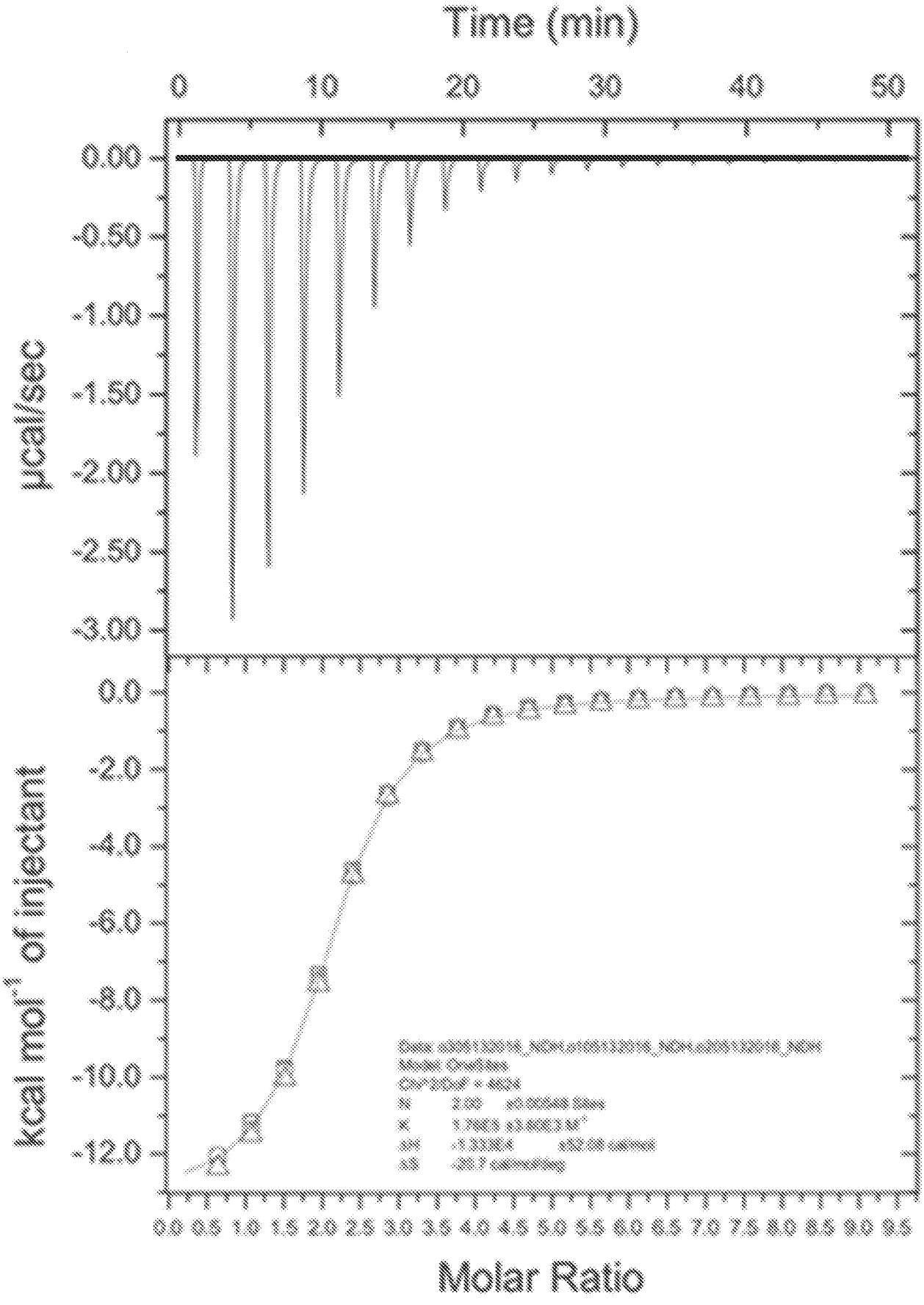

Time (min)
0
10
20
30
40
50
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
-3.00
µcal/sec
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
kcal mol-1 of injectant
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5
Molar Ratio

FIG. 12A

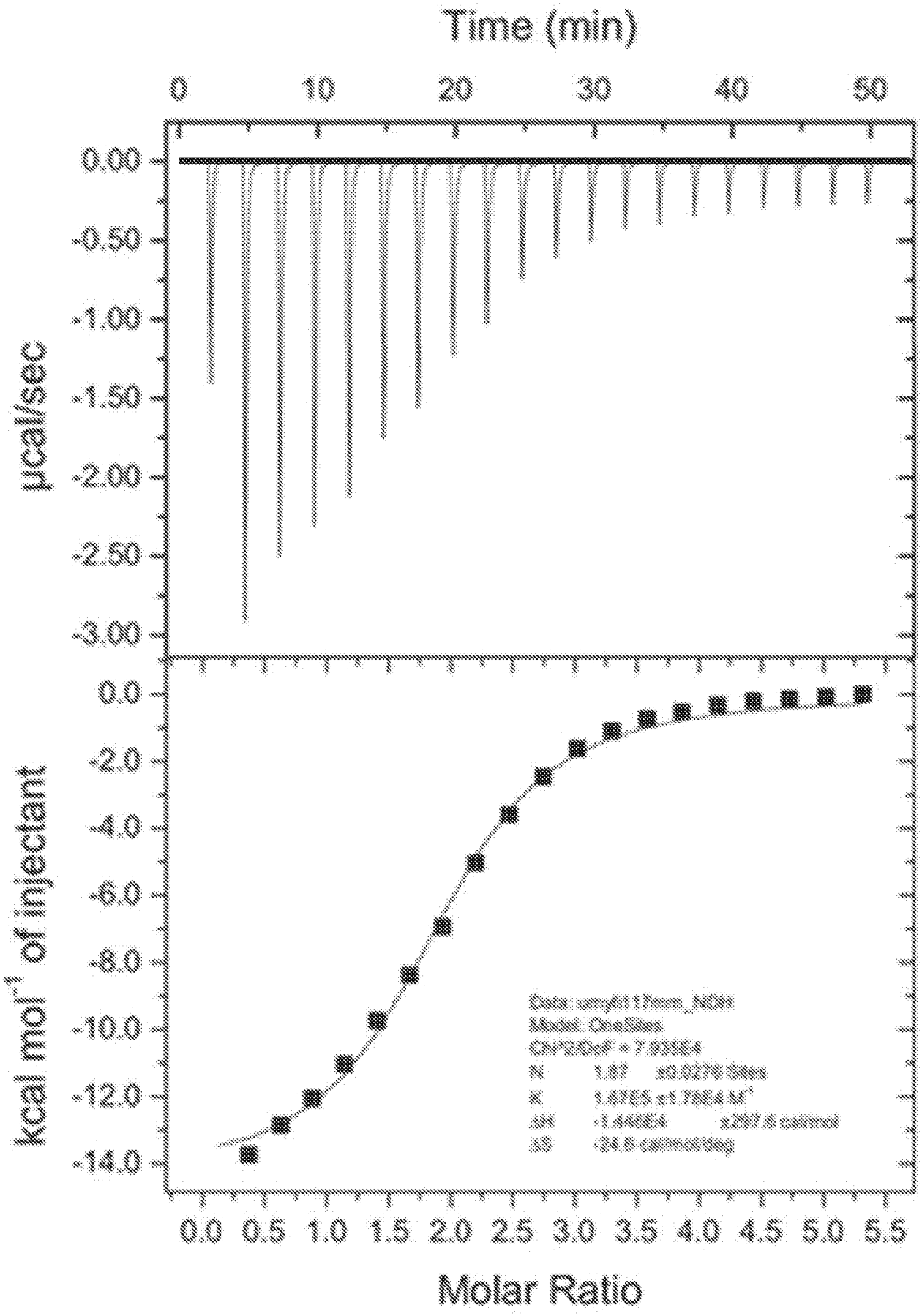

Time (min)
0
10
20
30
40
50
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
-3.00
µcal/sec
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-14.0
kcal mol-1 of injectant
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
Molar Ratio

FIG. 12B

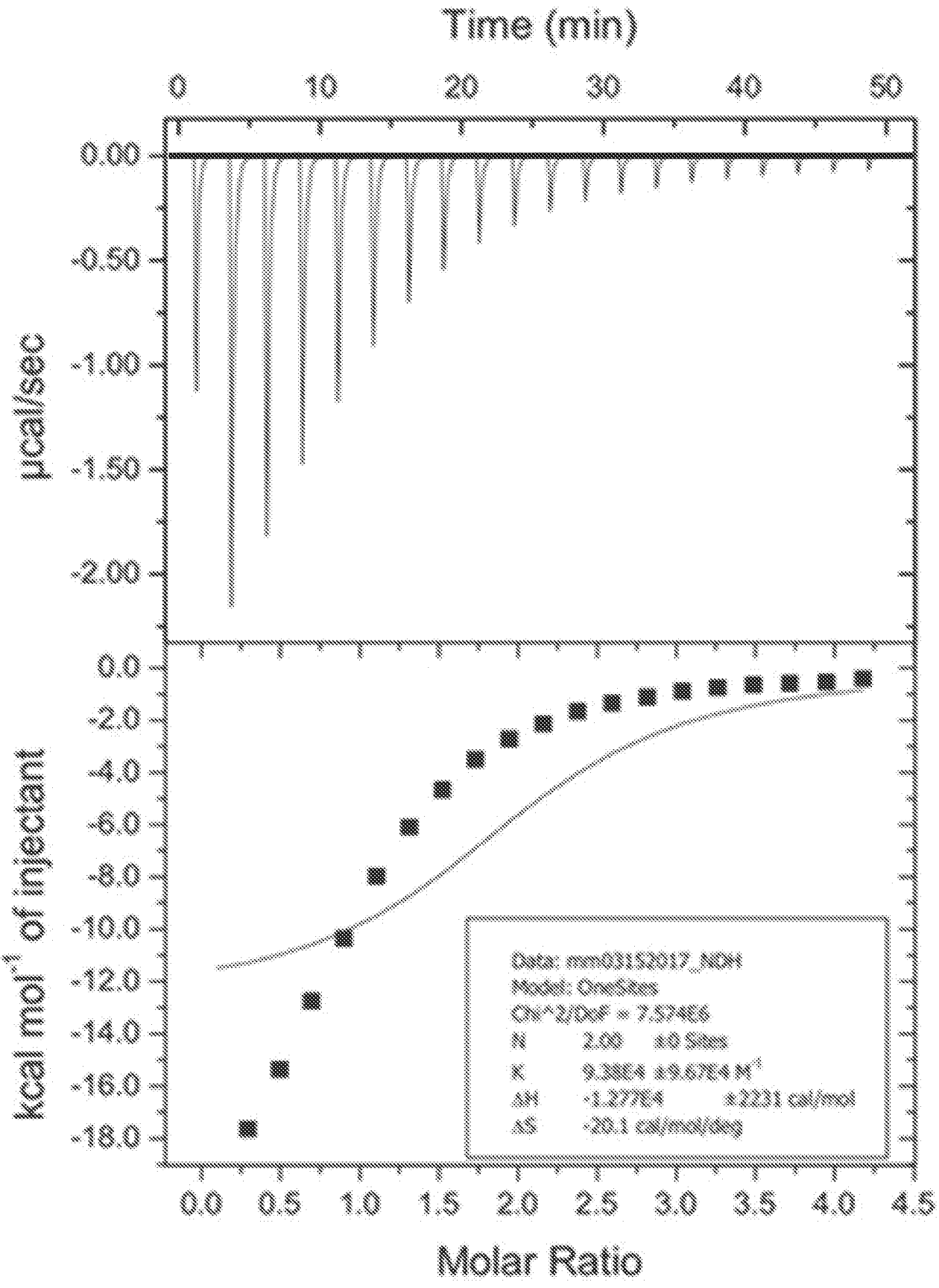

Time (min)
0
10
20
30
40
50
0.00
-0.50
-1.00
-1.50
-2.00
µcal/sec
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-14.0
-16.0
-18.0
kcal mol-1 of injectant
Data: mm03152017_NOH
Model: OneSites
N 2.00 ±0 Sites
ΔH -1.27E4 ±2231 cal/mol
ΔS -20.1 cal/mol/deg
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Molar Ratio

FIG. 12C

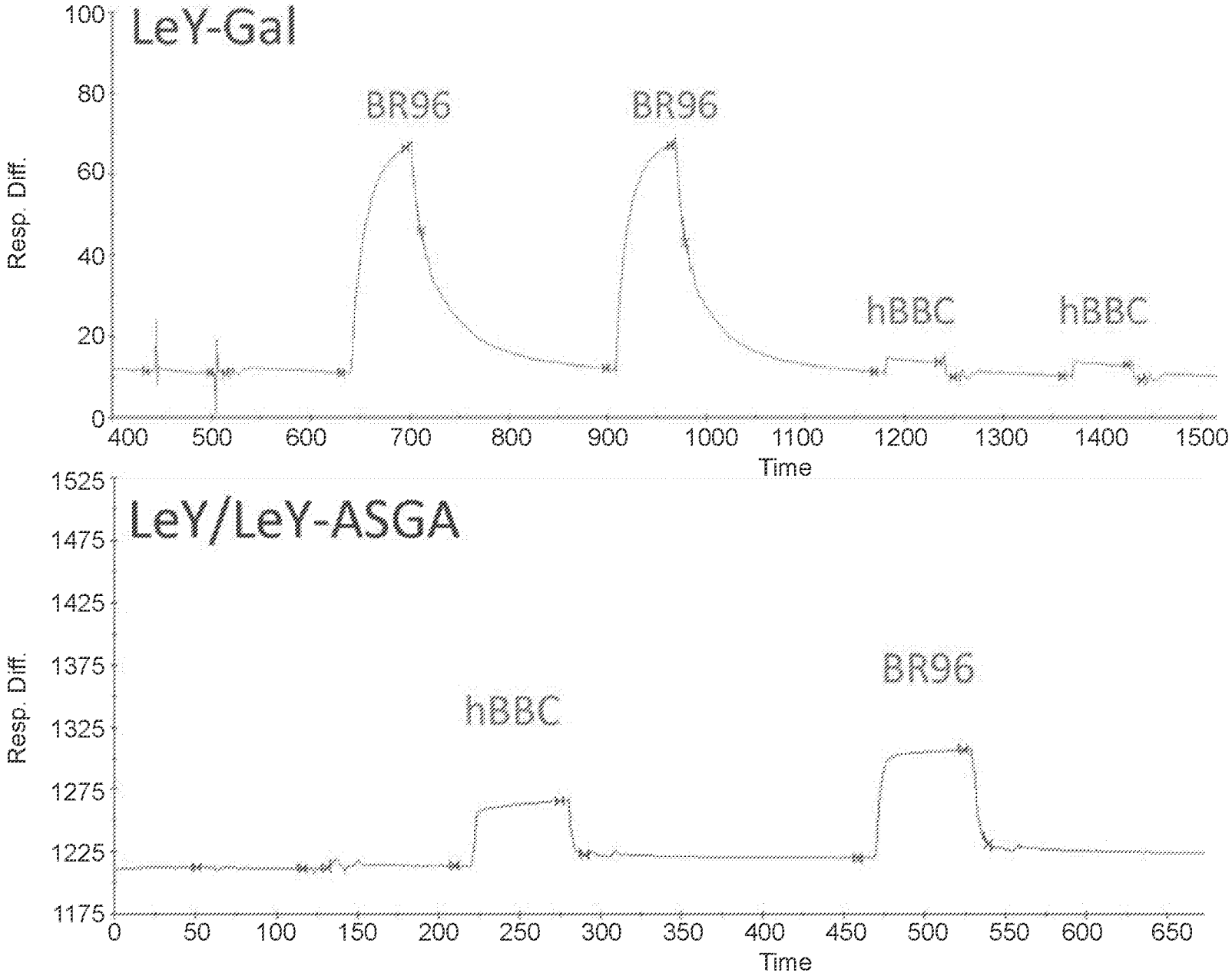

LeY-Gal
BR96
BR96
hBBC
hBBC
Resp. Diff.
Time
LeY/LeY-ASGA
hBBC
BR96
Resp. Diff.
Time

FIG. 13A

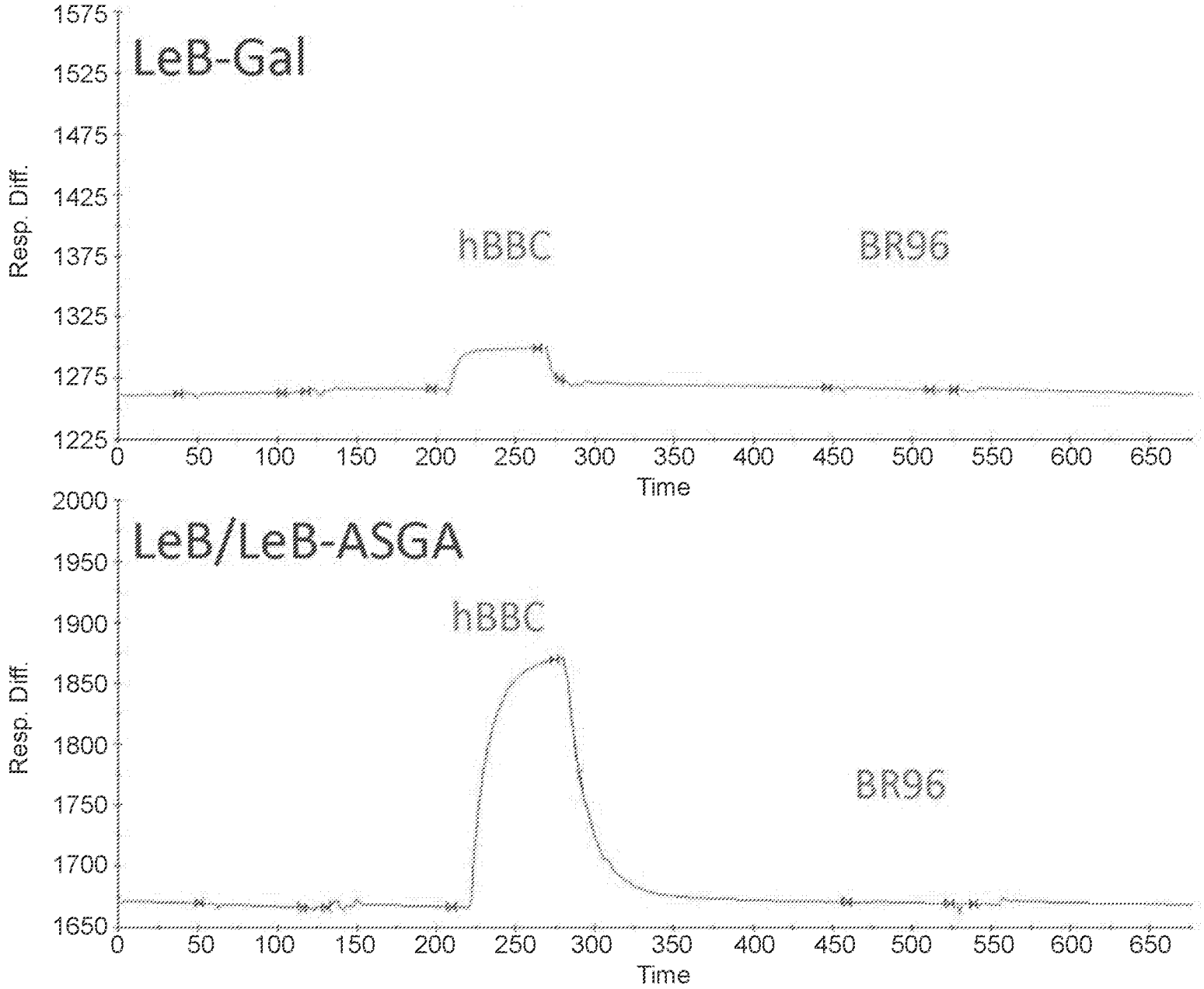

LeB-Gal
hBBC
BR96
Resp. Diff.
1575
1525
1475
1425
1375
1325
1275
1225
0
50
100
150
200
250
300
350
400
450
500
550
600
650
Time
LeB/LeB-ASGA
hBBC
BR96
Resp. Diff.
2000
1950
1900
1850
1800
1750
1700
1650
0
50
100
150
200
250
300
350
400
450
500
550
600
650
Time

FIG. 13B

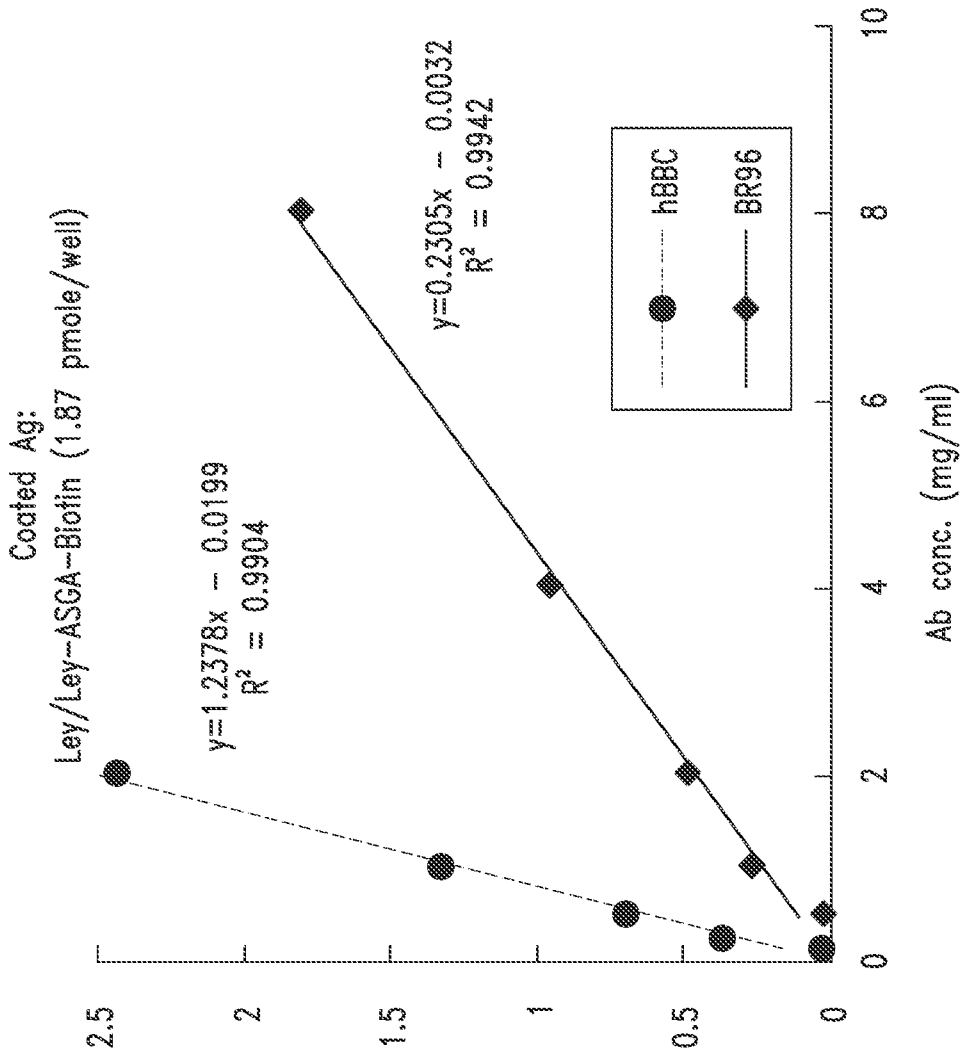

Coated Ag:
Ley/Ley-ASGA-Biotin (1.87 pmole/well)
y=1.2378x - 0.0199
R² = 0.9904
y=0.2305x - 0.0032
R² = 0.9942
hBBC
BR96
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
Ab conc. (mg/ml)

FIG. 14A

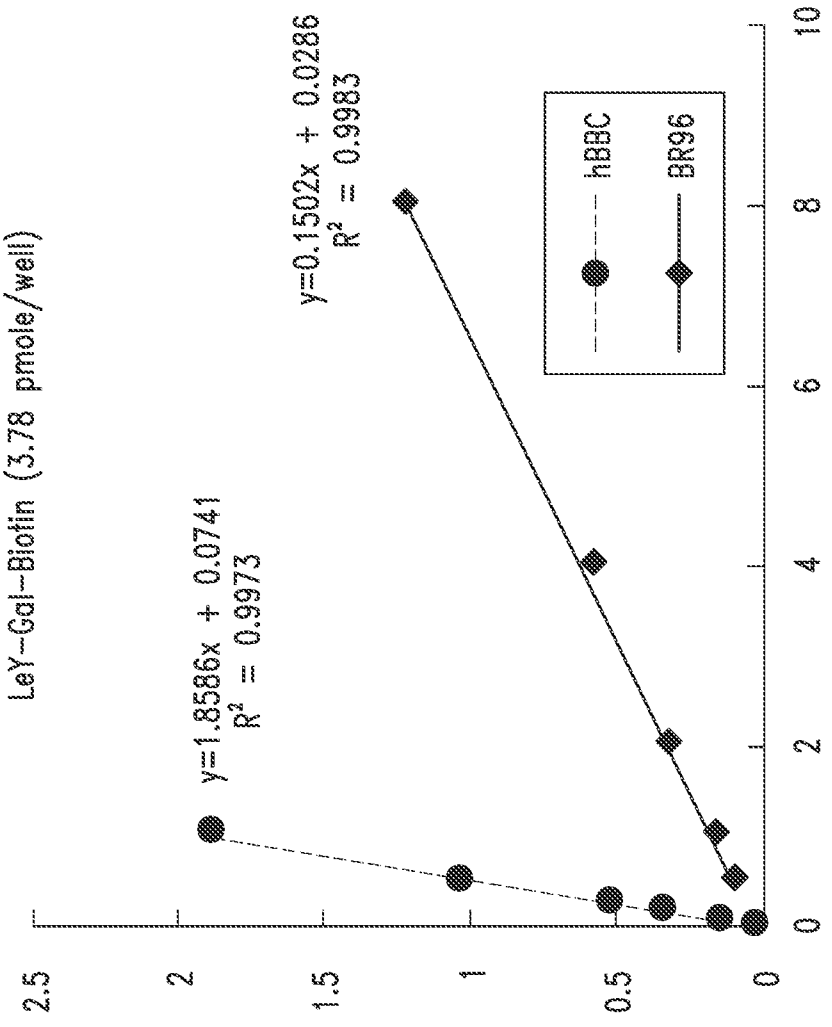

Coated Ag:
LeY-Gal-Biotin (3.78 pmole/well)
y=1.8586x + 0.0741
R² = 0.9973
y=0.1502x + 0.0286
R² = 0.9983
hBBC
BR96
Ab conc. (mg/ml)
0
0.5
1
1.5
2
2.5
0
2
4
6
8
10

FIG. 14B

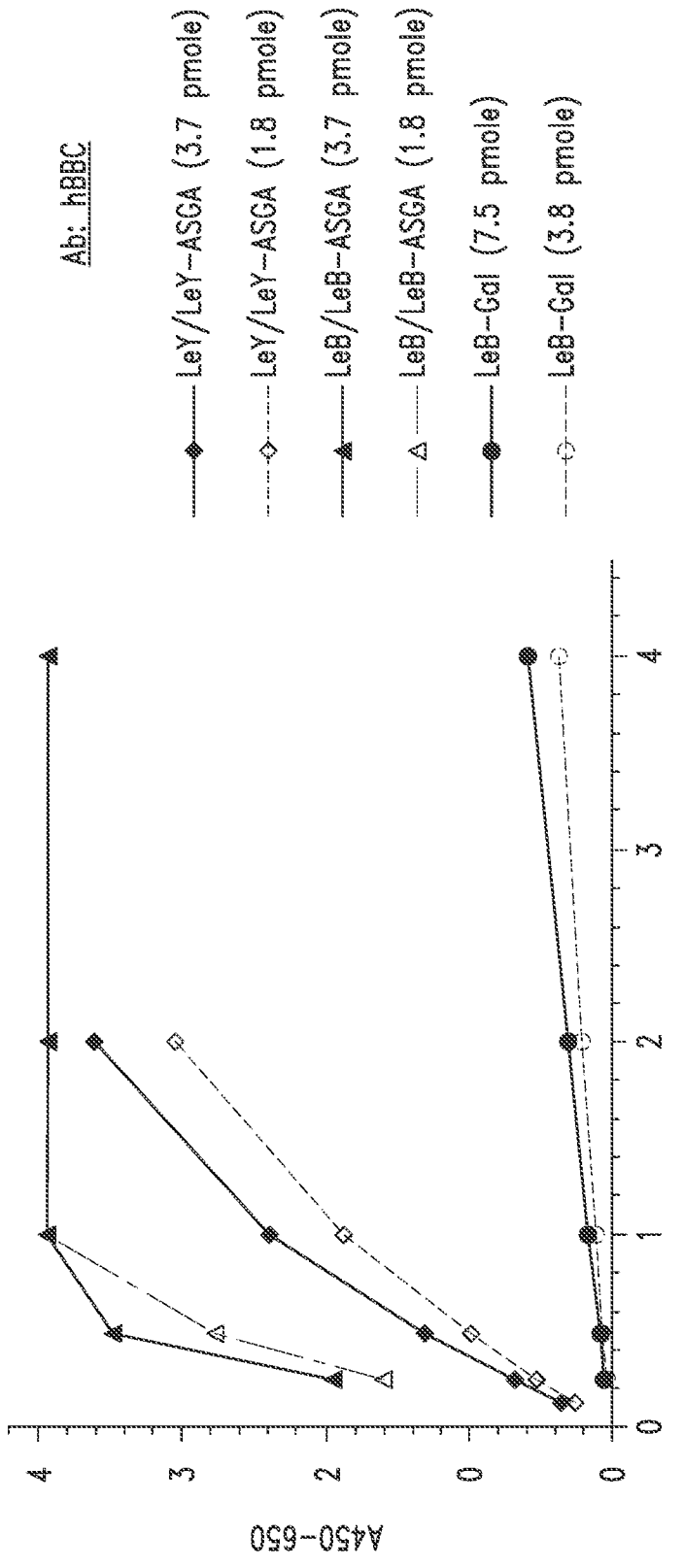

Ab: hBBC
LeY/LeY-ASGA (3.7 pmole)
LeY/LeY-ASGA (1.8 pmole)
LeB/LeB-ASGA (3.7 pmole)
LeB/LeB-ASGA (1.8 pmole)
LeB-Gal (7.5 pmole)
LeB-Gal (3.8 pmole)
A450-650
hBBC [C] µg/ml
0
2
3
4

FIG. 15

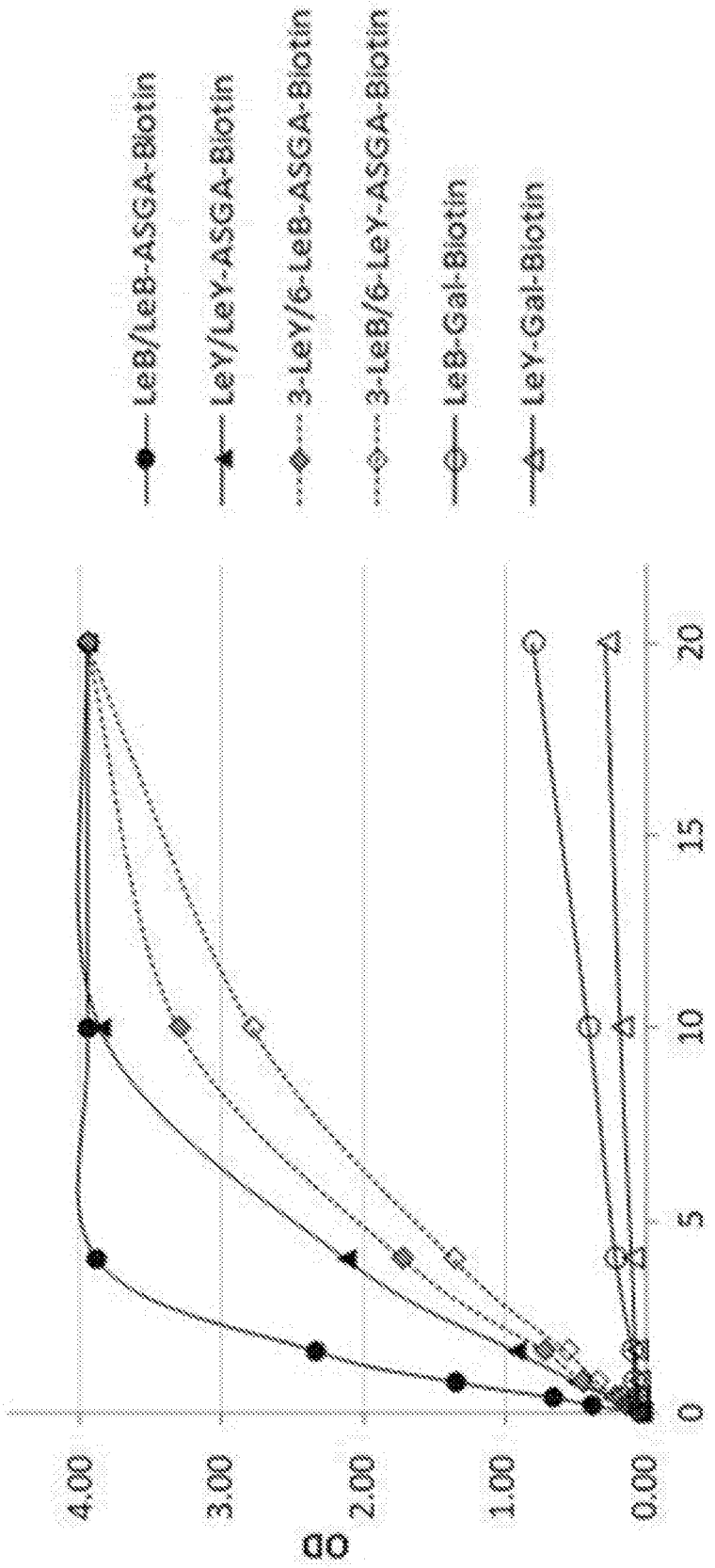

LeB/LeB-ASGA-Biotin
LeY/LeY-ASGA-Biotin
3-LeY/6-LeB-ASGA-Biotin
3-LeB/6-LeY-ASGA-Biotin
LeB-Gal-Biotin
LeY-Gal-Biotin
OD
4.00
3.00
2.00
1.00
0.00
0
5
10
15
20
hBBC (µg/ml)

FIG. 16A

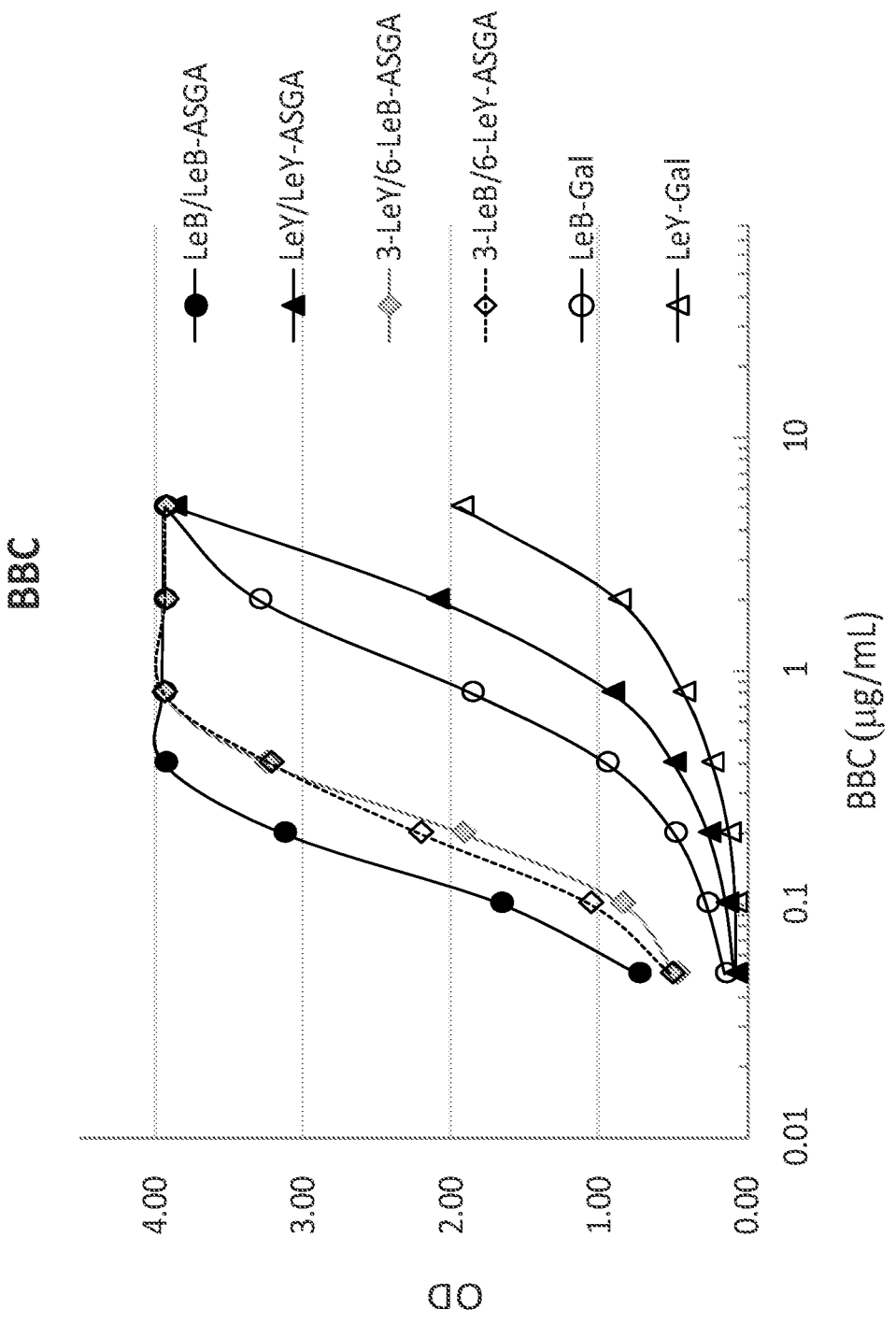

BBC
LeB/LeB-ASGA
LeY/LeY-ASGA
3-LeY/6-LeB-ASGA
3-LeB/6-LeY-ASGA
LeB-Gal
LeY-Gal
OD
4.00
3.00
2.00
1.00
0.00
0.01
0.1
1
10
BBC (µg/mL)

FIG. 16B

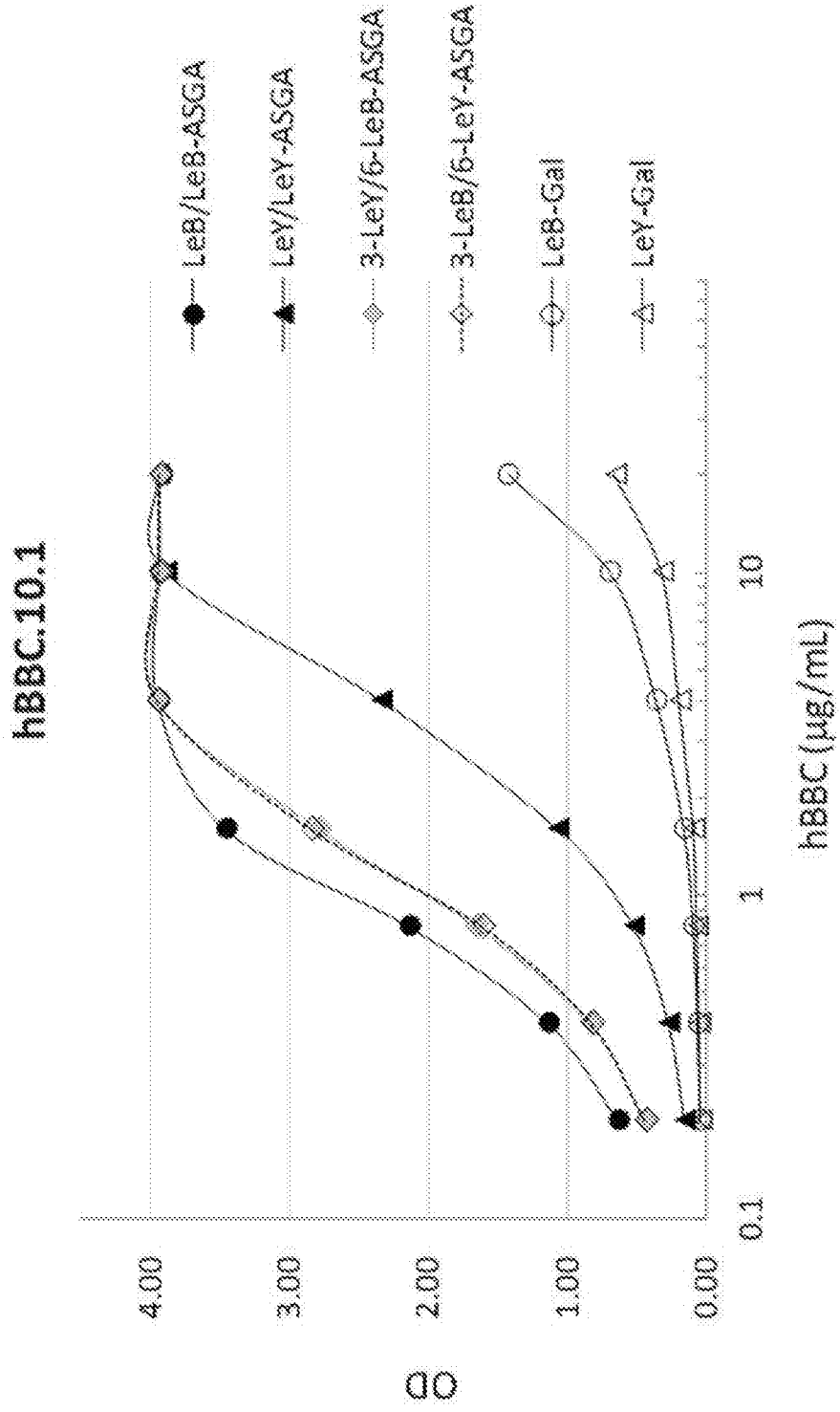

hBBC.10.1
LeB/LeB-ASGA
LeY/LeY-ASGA
3-LeY/6-LeB-ASGA
3-LeB/6-LeY-ASGA
LeB-Gal
LeY-Gal
4.00
3.00
2.00
1.00
0.00
OD
0.1
1
10
hBBC (µg/mL)

FIG. 16C

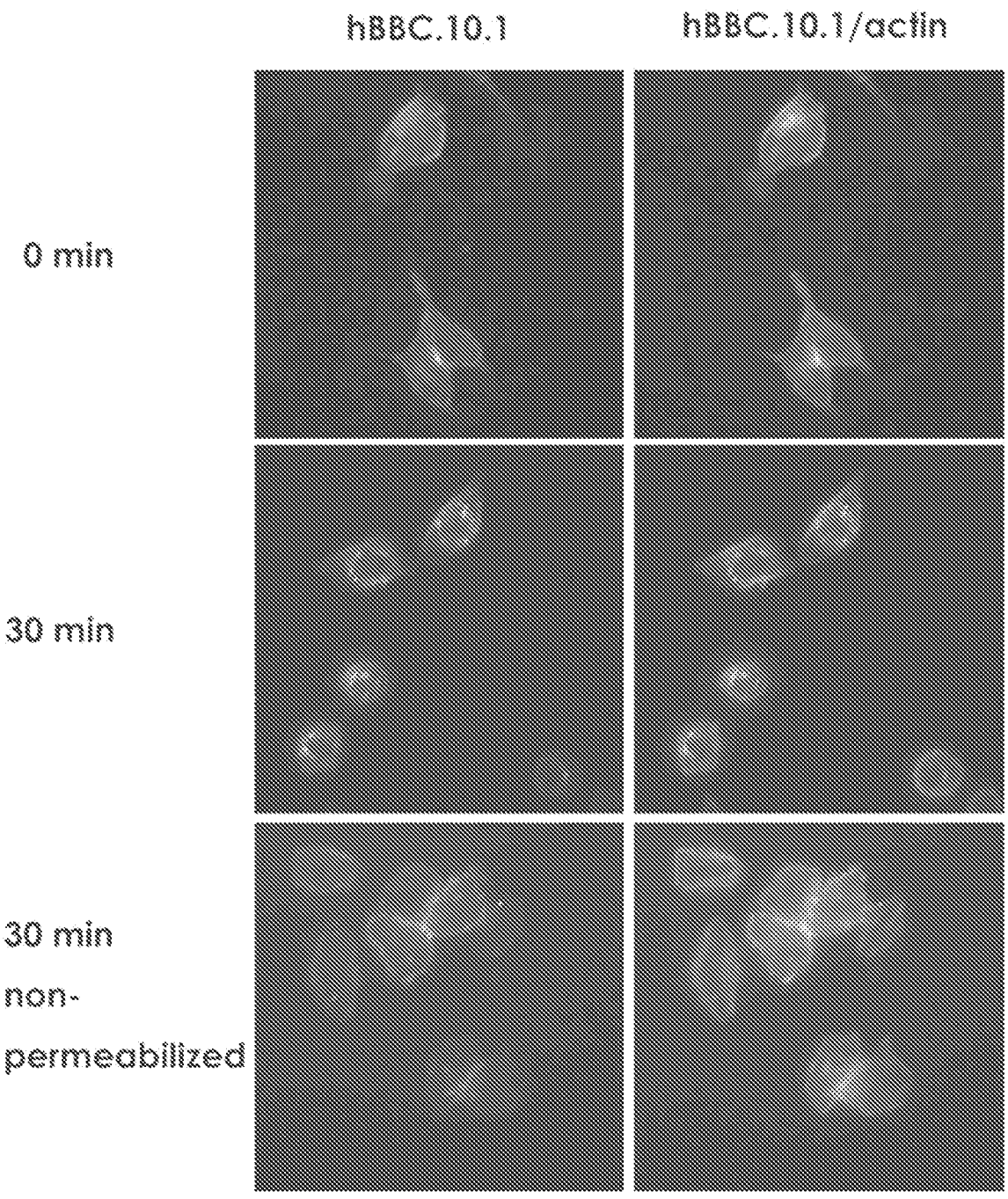


FIG. 17

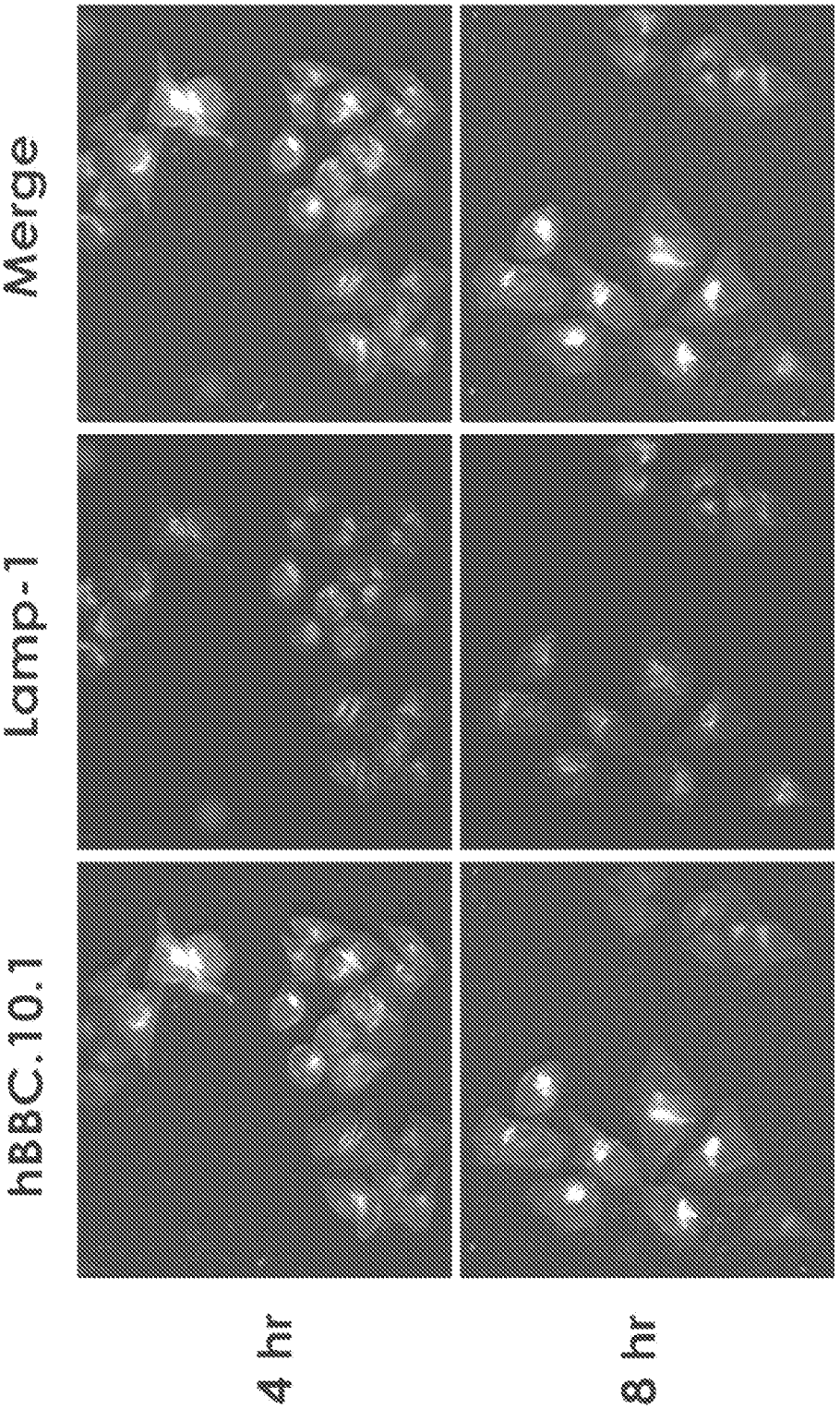


FIG. 18

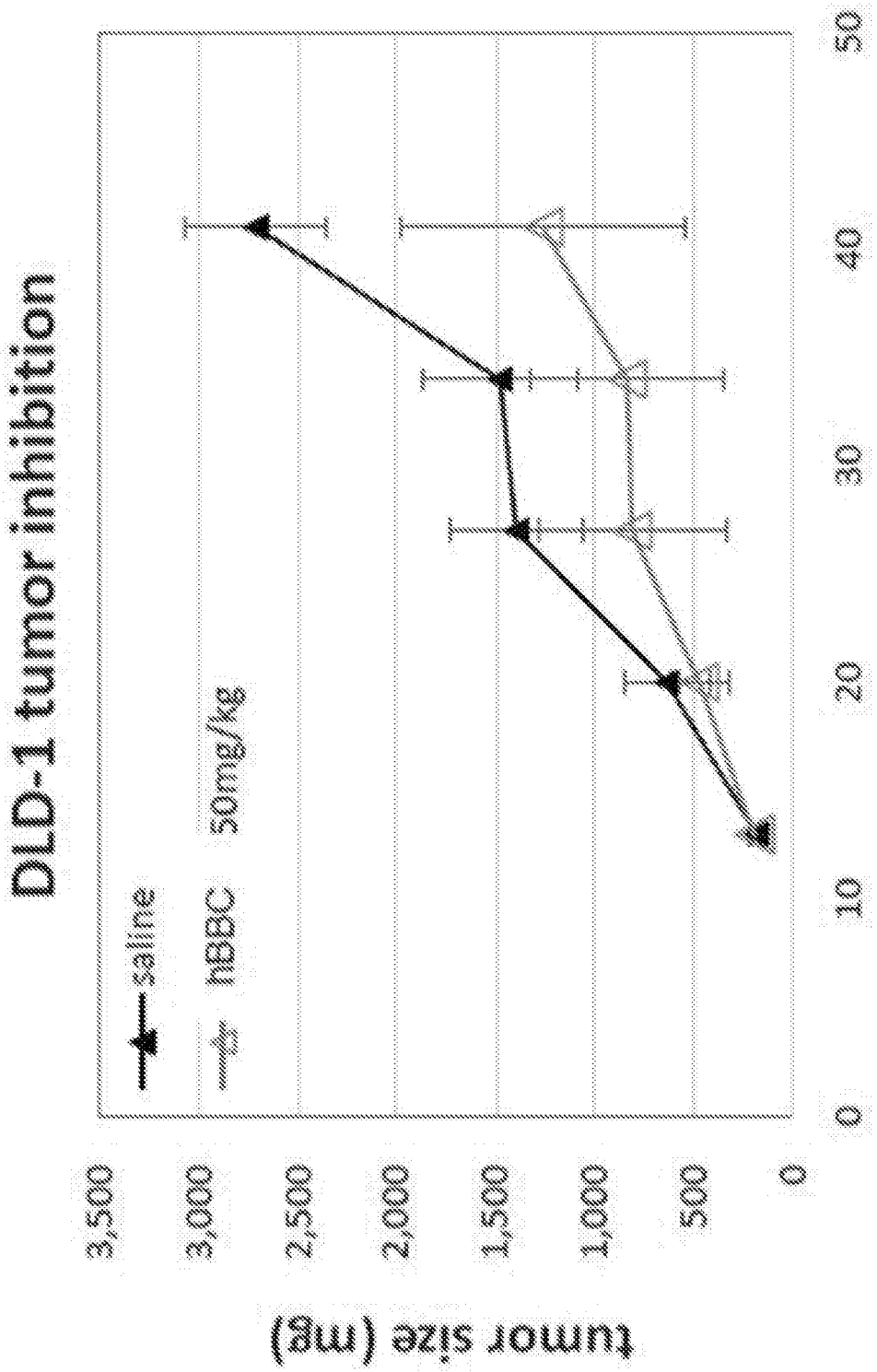

DLD-1 tumor inhibition
saline
hBBC 50mg/kg
tumor size (mg)
3,500
3,000
2,500
2,000
1,500
1,000
500
0
0
10
20
30
40
50
days after tumor inoculation

FIG. 19A

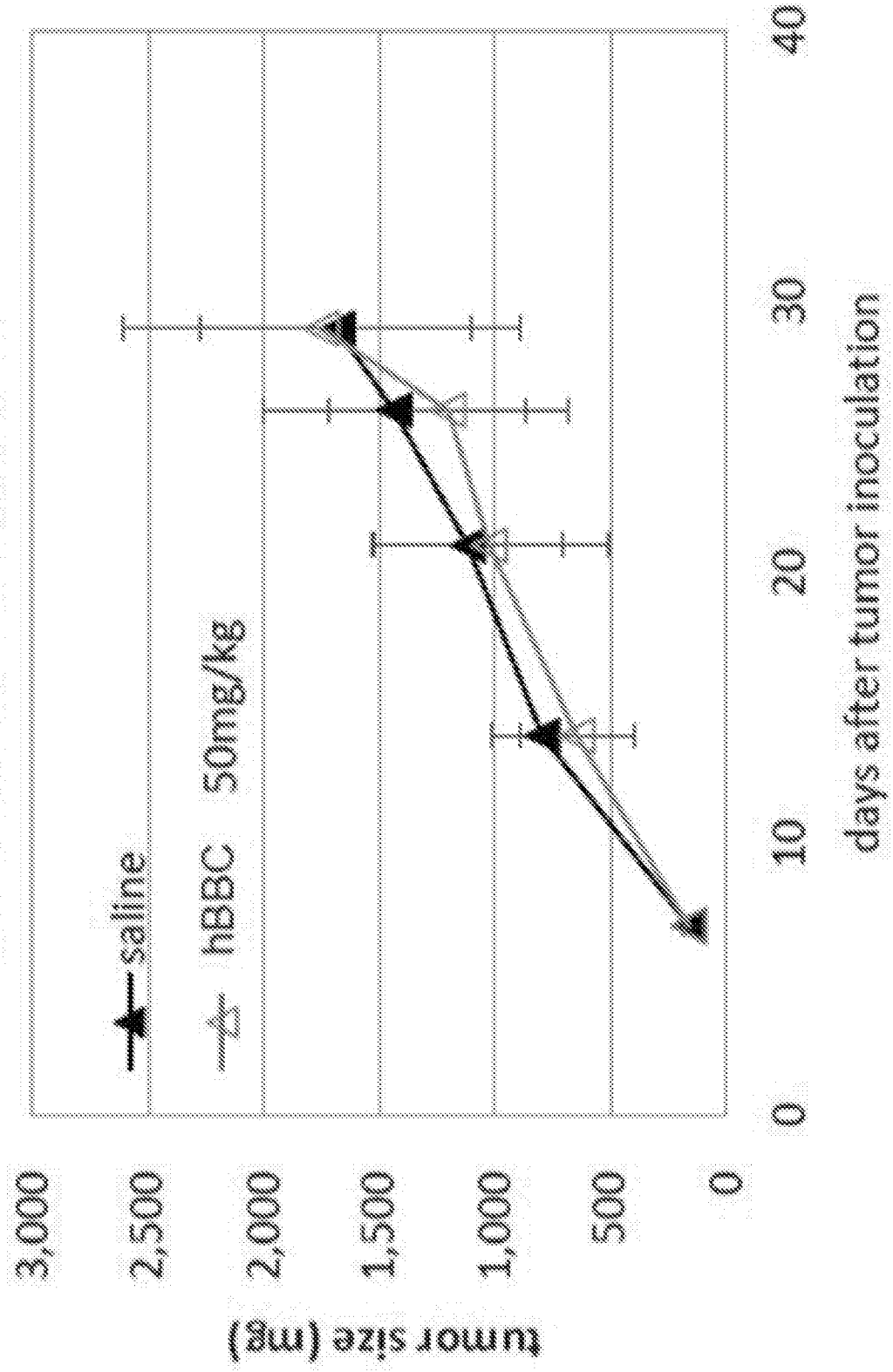

COLO 205 tumor inhibition
saline
hBBC 50mg/kg
tumor size (mg)
3,000
2,500
2,000
1,500
1,000
500
0
0
10
20
30
40
days after tumor inoculation

FIG. 19B

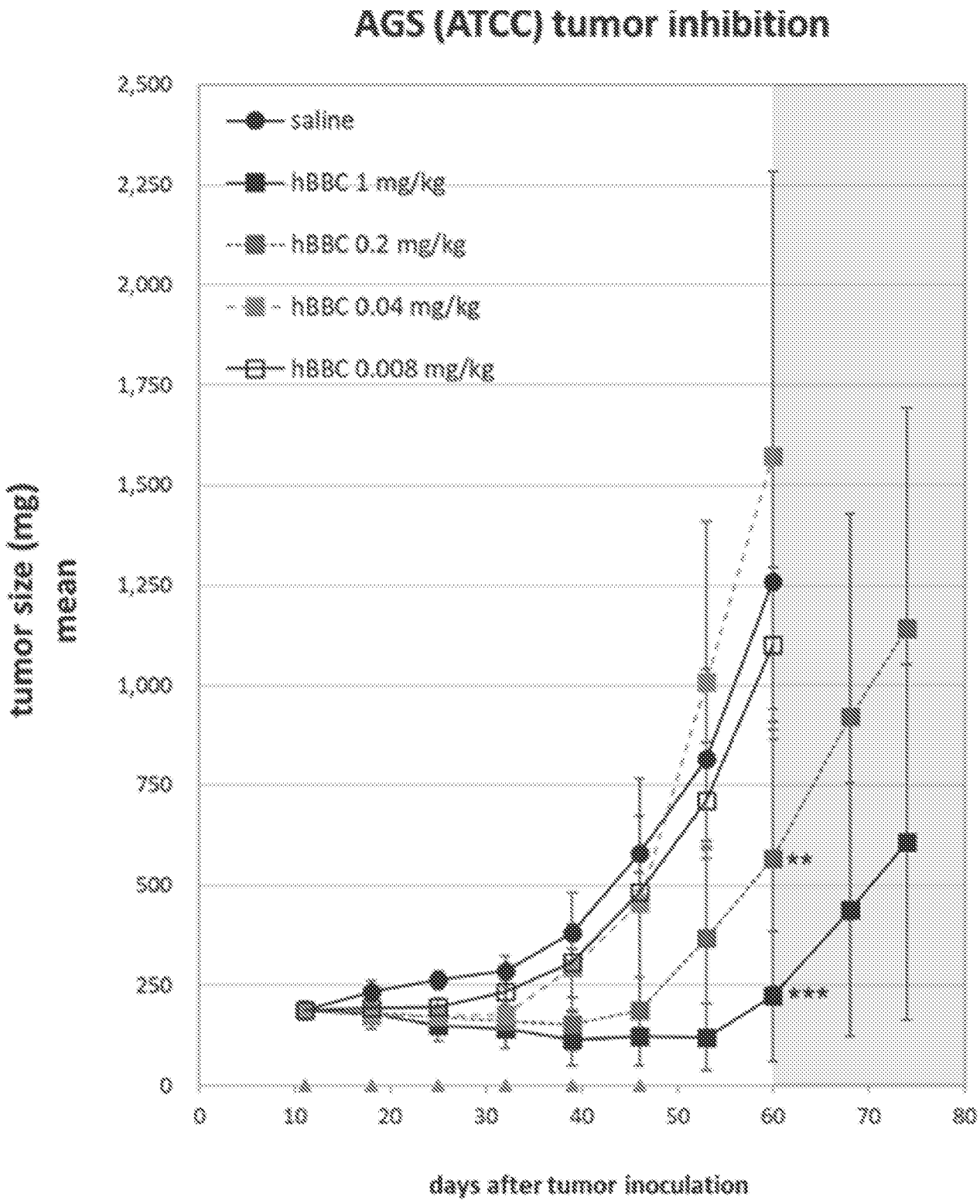

AGS (ATCC) tumor inhibition
saline
hBBC 1 mg/kg
hBBC 0.2 mg/kg
hBBC 0.04 mg/kg
hBBC 0.008 mg/kg
tumor size (mg)
mean
2,500
2,250
2,000
1,750
1,500
1,250
1,000
750
500
250
0
0
10
20
30
40
50
60
70
80
**
***
days after tumor inoculation

FIG. 20A

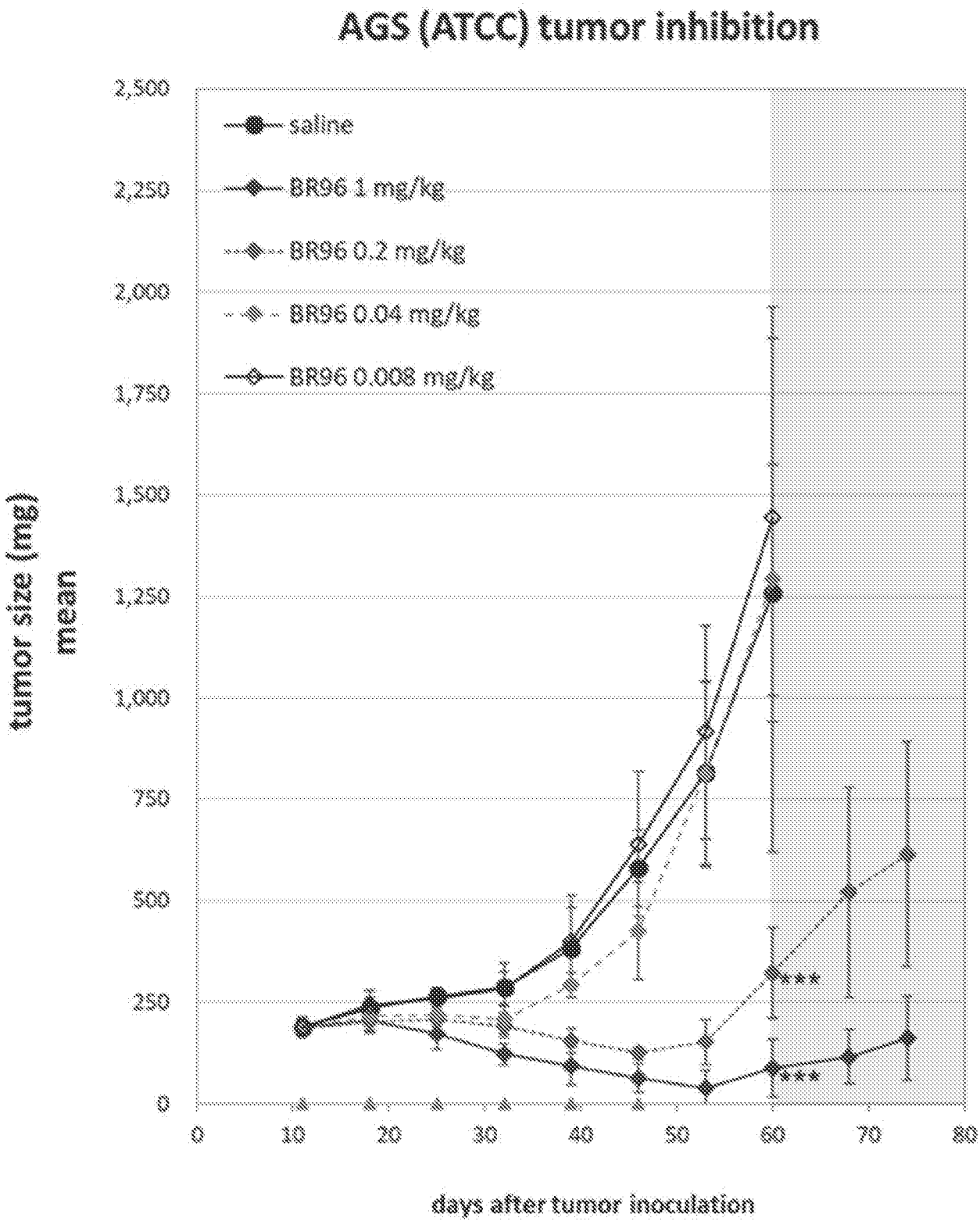

AGS (ATCC) tumor inhibition
saline
BR96 1 mg/kg
BR96 0.2 mg/kg
BR96 0.04 mg/kg
BR96 0.008 mg/kg
tumor size (mg)
mean
2,500
2,250
2,000
1,750
1,500
1,250
1,000
750
500
250
0
0
10
20
30
40
50
60
70
80
***
***
days after tumor inoculation

FIG. 20B

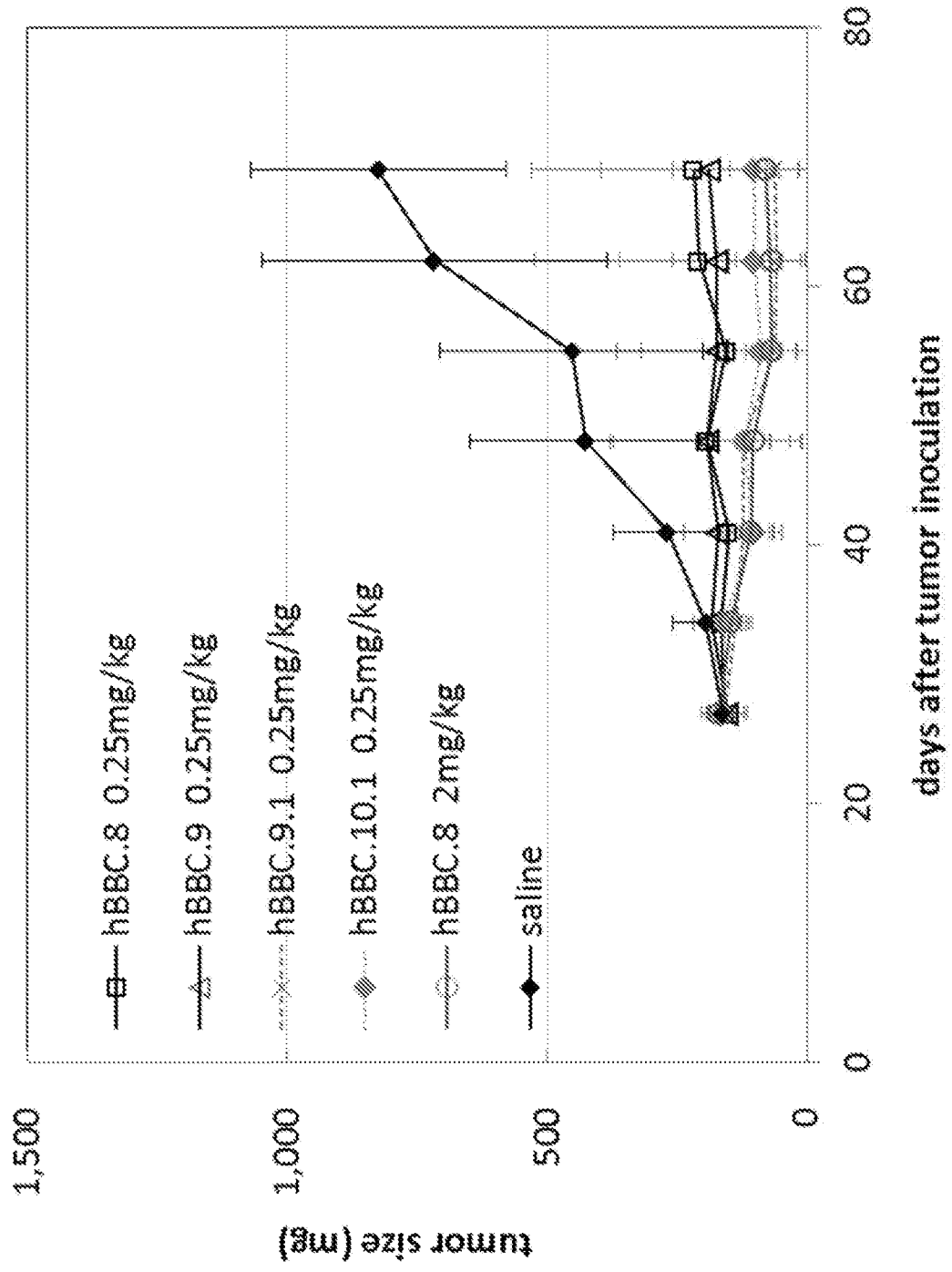

1,500
1,000
500
0
tumor size (mg)
0
20
40
60
80
days after tumor inoculation
hBBC.8 0.25mg/kg
hBBC.9 0.25mg/kg
hBBC.9.1 0.25mg/kg
hBBC.10.1 0.25mg/kg
hBBC.8 2mg/kg
saline

FIG. 20C

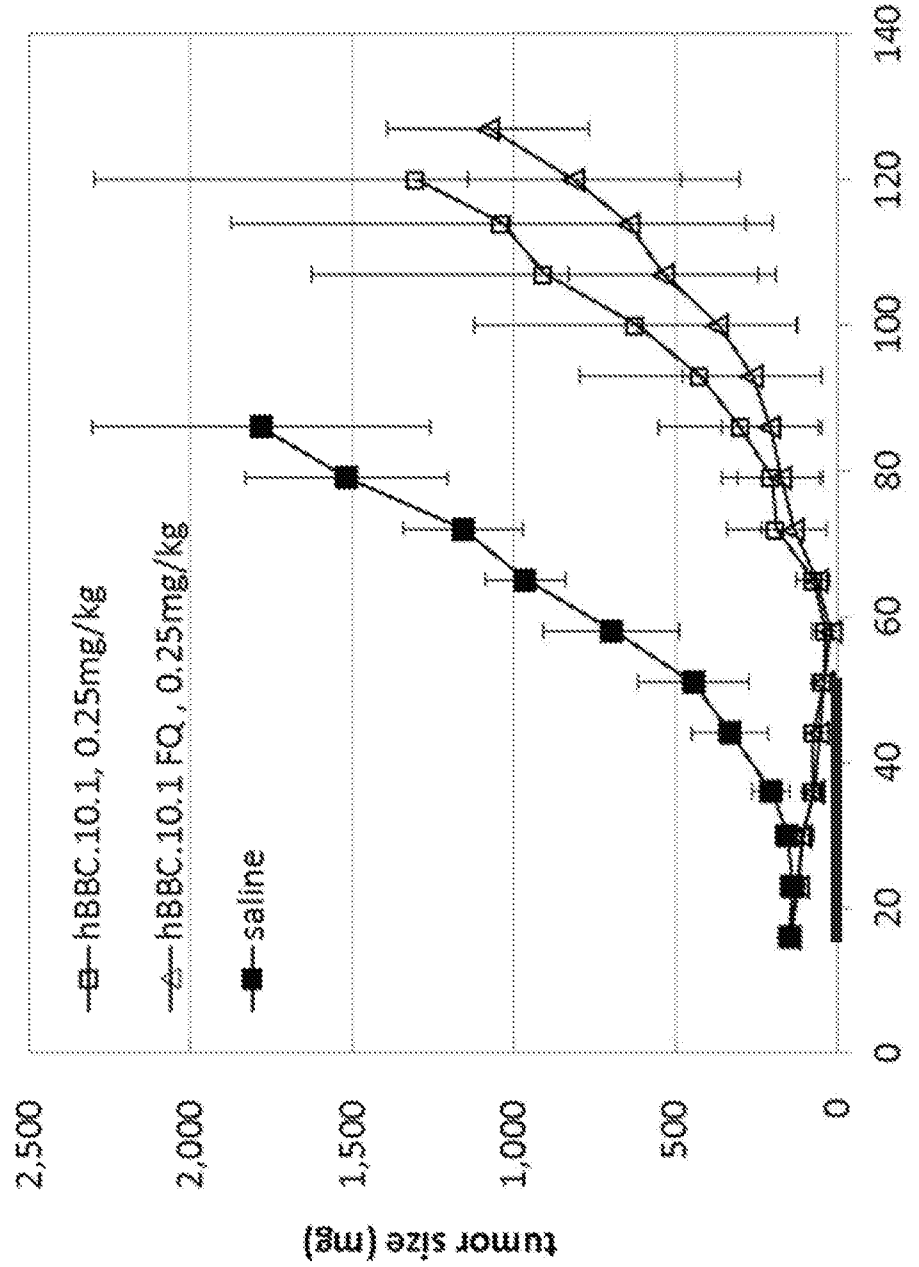

tumor size (mg)
2,500
2,000
1,500
1,000
500
0
0
20
40
60
80
100
120
140
days after tumor inoculation
hBBC.10.1, 0.25mg/kg
hBBC.10.1 FQ, 0.25mg/kg
saline

FIG. 20D

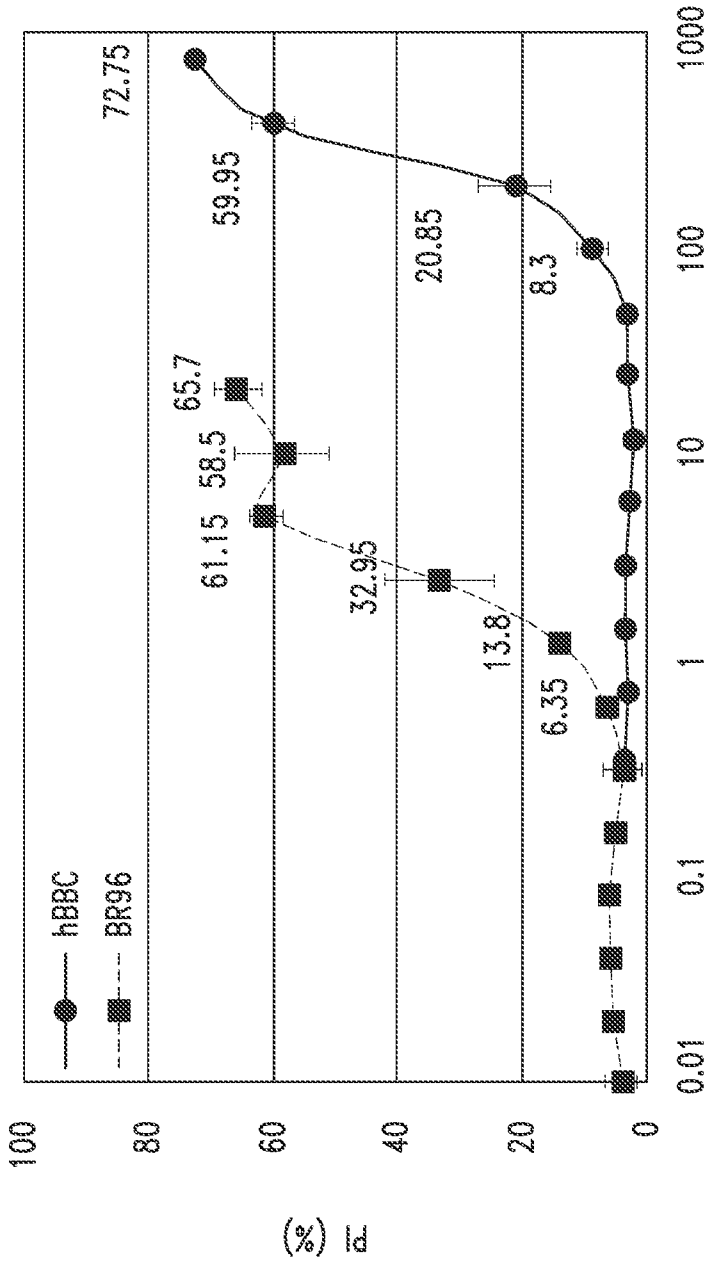

hBBC vs. BR96
hBBC
BR96
72.75
59.95
65.7
58.5
61.15
32.95
20.85
13.8
8.3
6.35
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
Antibody (con., μg/ml)
(%)

FIG. 21

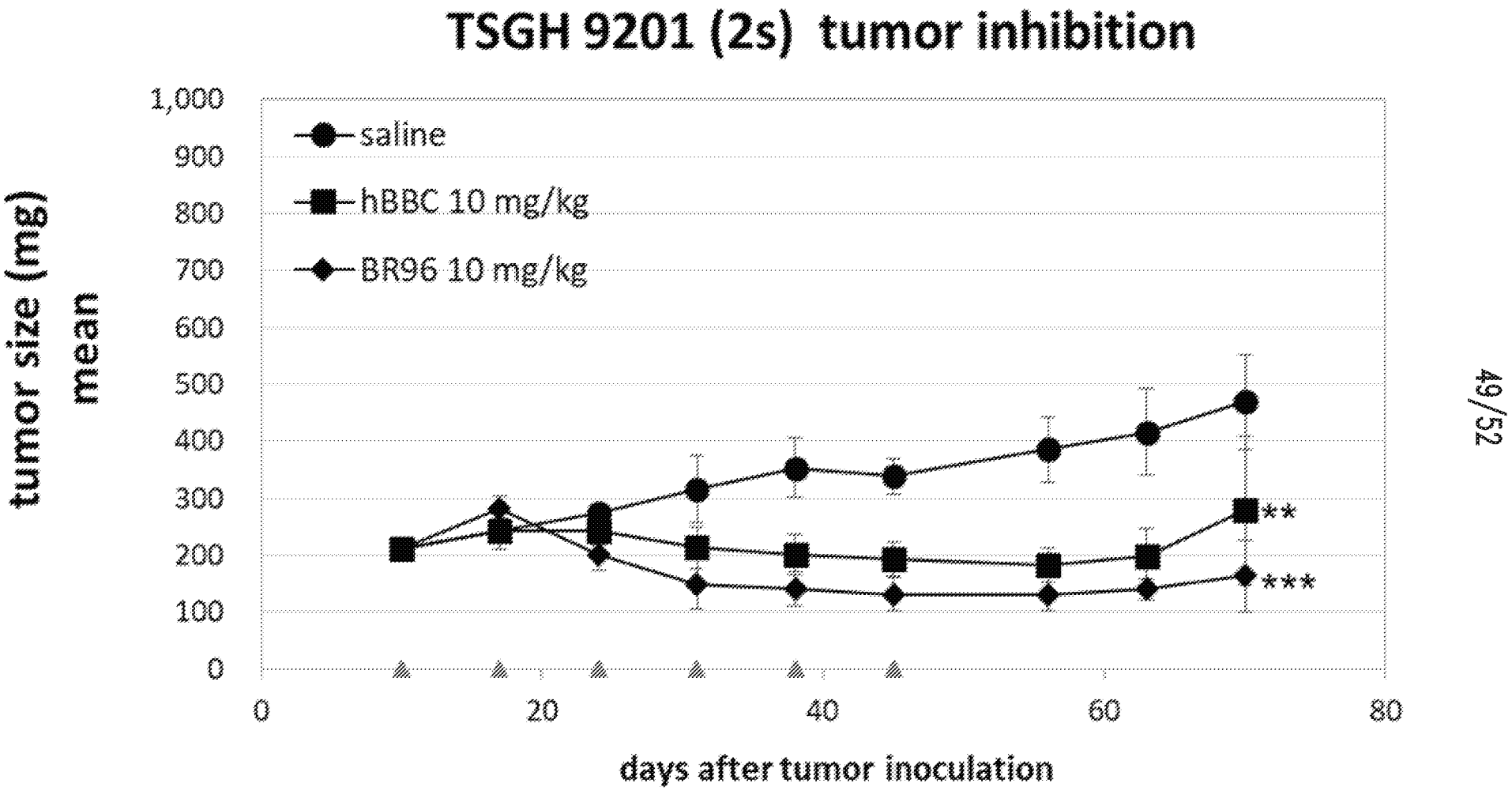

TSGH 9201 (2s) tumor inhibition
tumor size (mg)
mean
1,000
900
800
700
600
500
400
300
200
100
0
saline
hBBC 10 mg/kg
BR96 10 mg/kg
**
***
0
20
40
60
80
days after tumor inoculation

FIG. 22

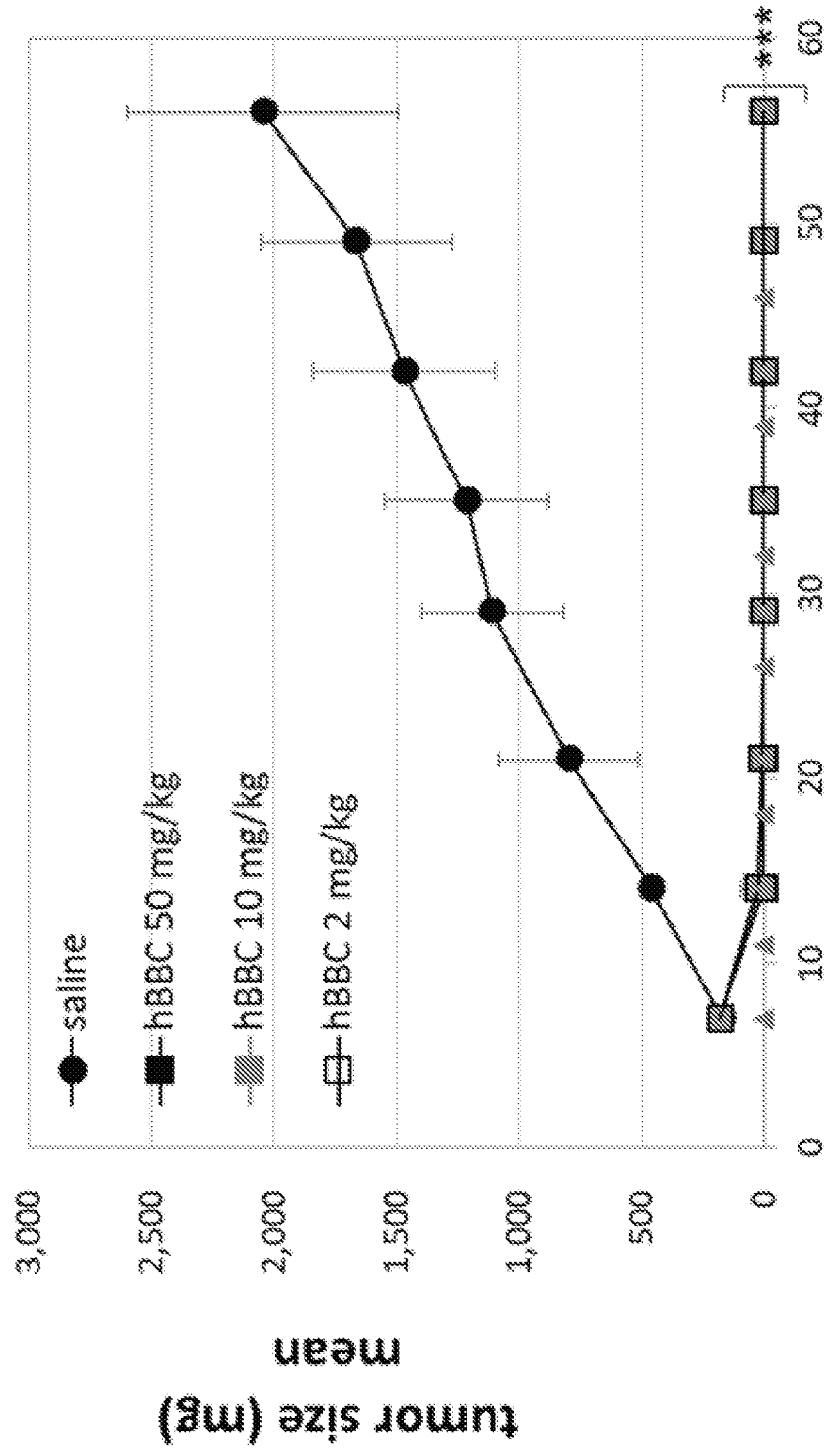

COLO 201 tumor inhibition
tumor size (mg)
mean
3,000
2,500
2,000
1,500
1,000
500
0
saline
hBBC 50 mg/kg
hBBC 10 mg/kg
hBBC 2 mg/kg
0
10
20
30
40
50
60
***
days after tumor inoculation

FIG. 23

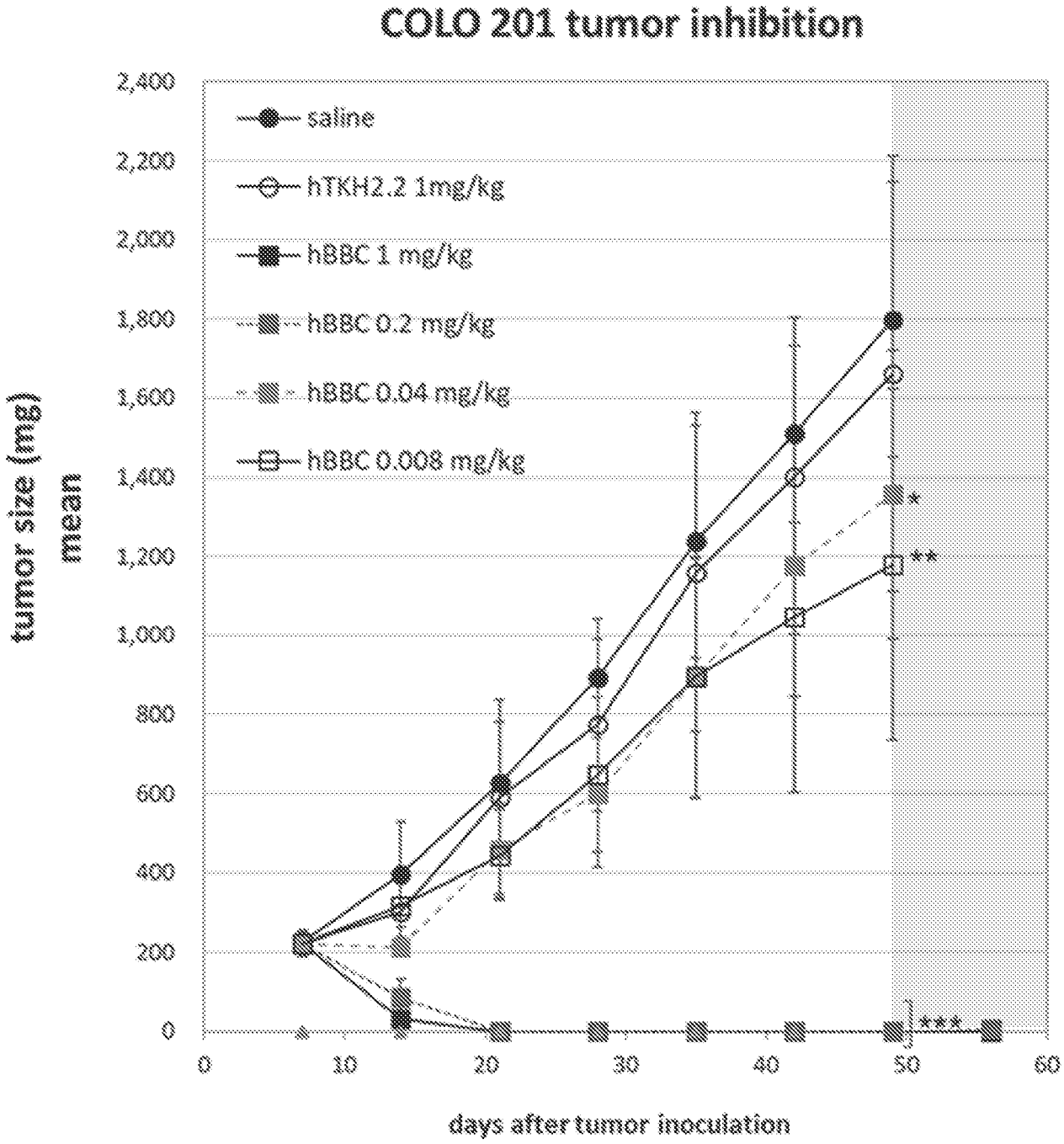

COLO 201 tumor inhibition
tumor size (mg)
mean
2,400
2,200
2,000
1,800
1,600
1,400
1,200
1,000
800
600
400
200
0
saline
hTKH2.2 1mg/kg
hBBC 1 mg/kg
hBBC 0.2 mg/kg
hBBC 0.04 mg/kg
hBBC 0.008 mg/kg
*
**
***
0
10
20
30
40
50
60
days after tumor inoculation

FIG. 24A

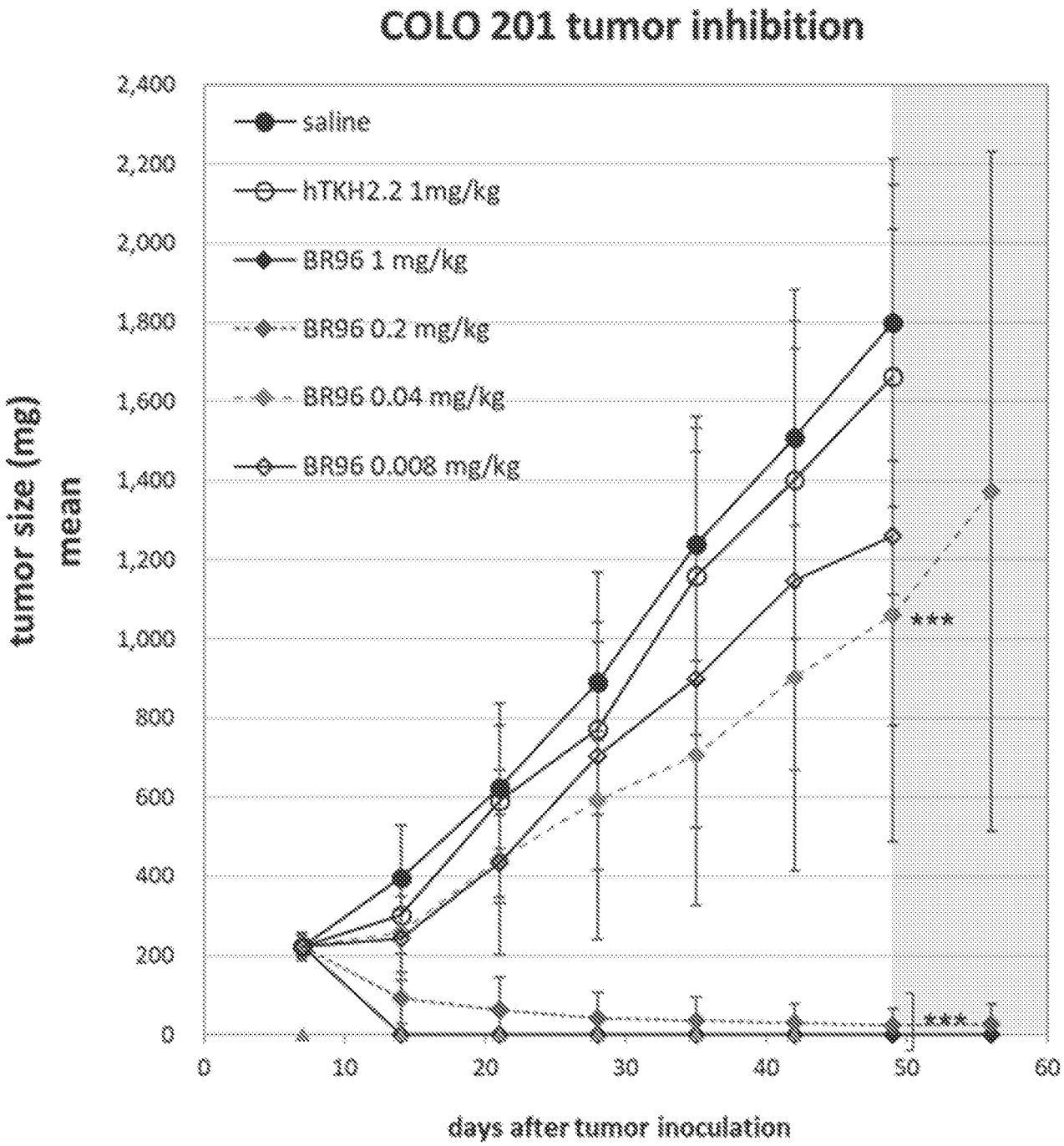

COLO 201 tumor inhibition
saline
hTKH2.2 1mg/kg
BR96 1 mg/kg
BR96 0.2 mg/kg
BR96 0.04 mg/kg
BR96 0.008 mg/kg
tumor size (mg)
mean
2,400
2,200
2,000
1,800
1,600
1,400
1,200
1,000
800
600
400
200
0
0
10
20
30
40
50
60
***
***
days after tumor inoculation

FIG. 24B