FIG. 1

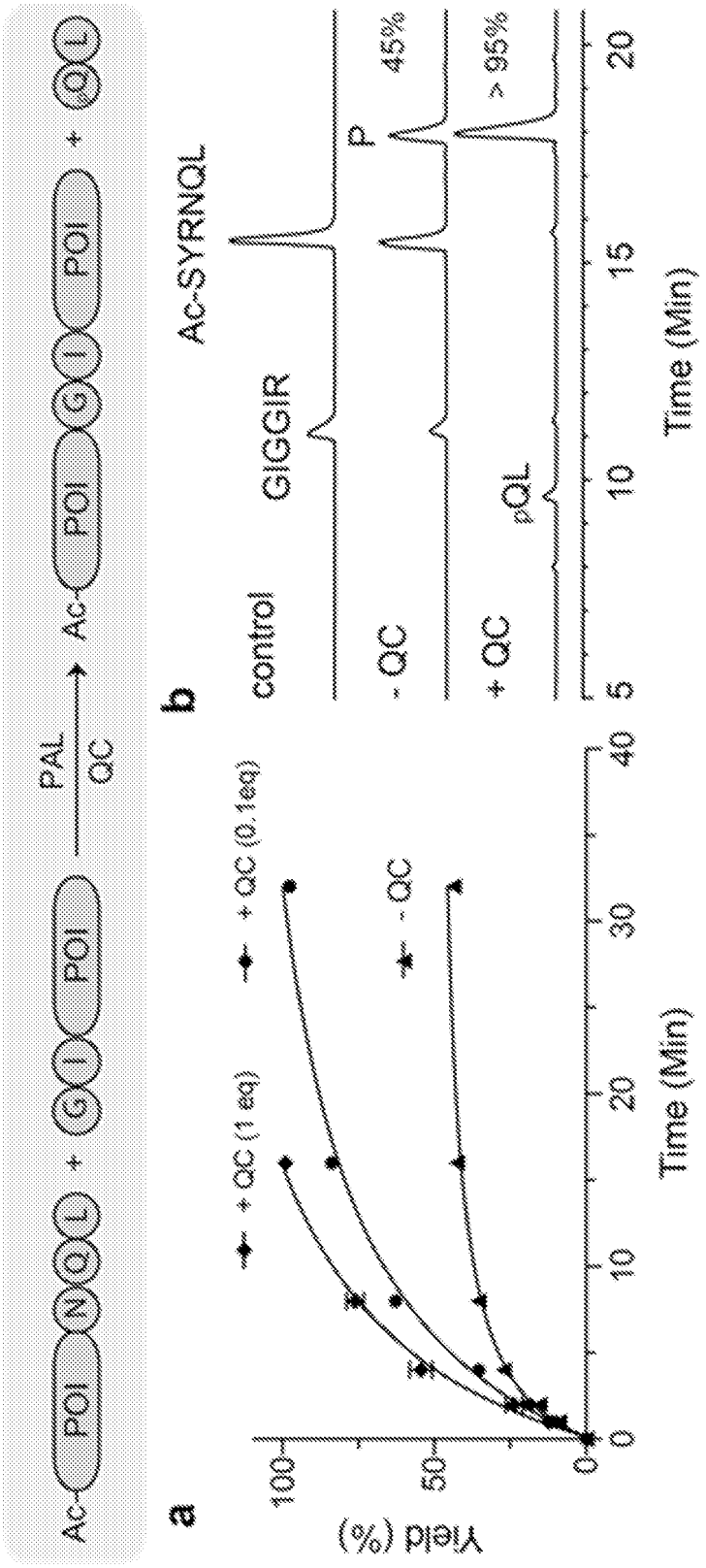

Ac-POI-N-Q-L + G-I-POI
PAL
QC
Ac-POI-G-I-POI + Q-L
a
Yield (%)
100
50
0
0
10
20
30
40
Time (Min)
+ QC (1 eq)
+ QC (0.1eq)
- QC
b
Ac-SYRNQL
control
GIGGIR
P
45%
- QC
+ QC
pQL
> 95%
5
10
15
20
Time (Min)

FIG. 2

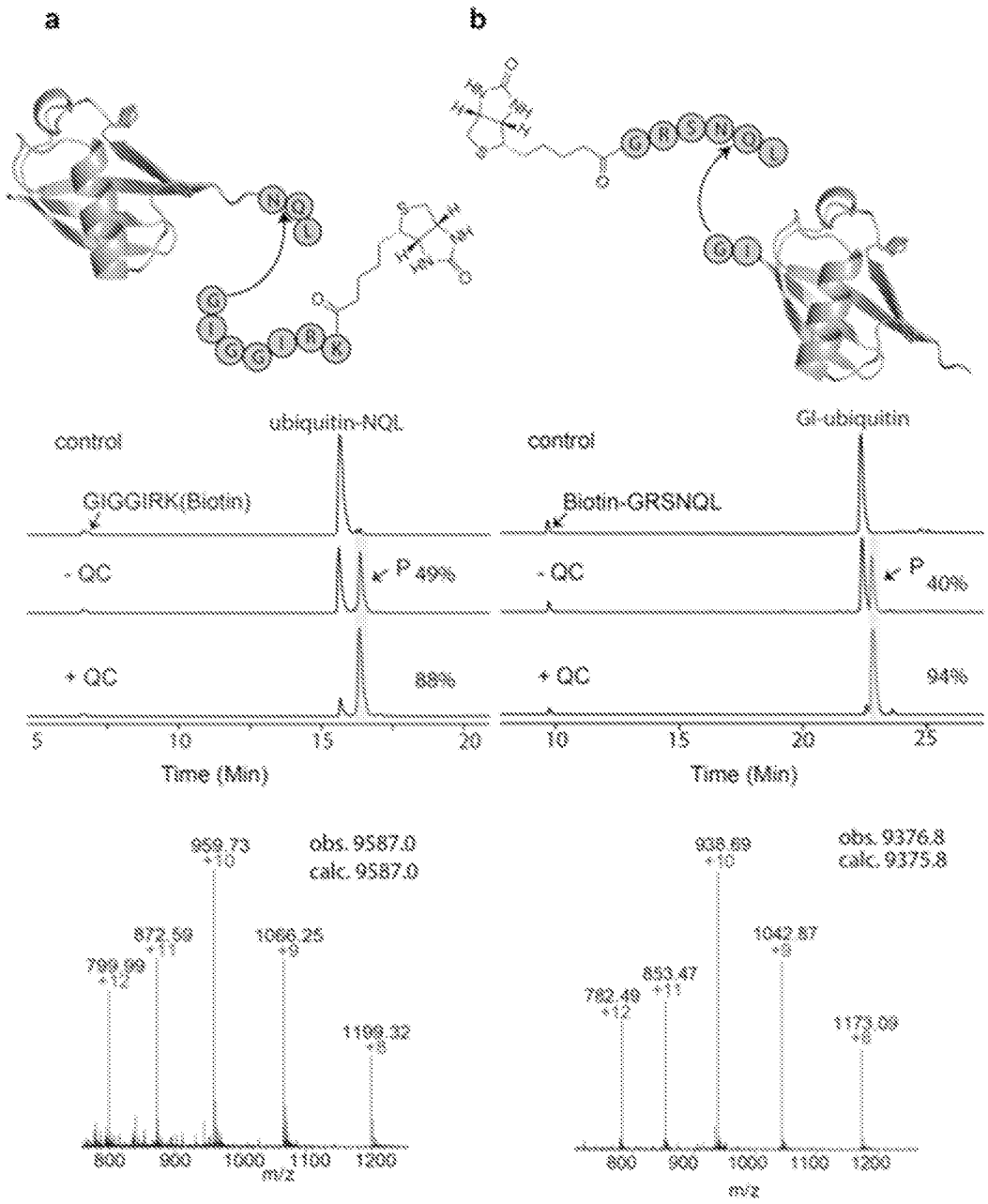

a
b
control
ubiquitin-NQL
GIGGIRK(Biotin)
- QC
+ QC
P 49%
88%
Time (Min)
control
GI-ubiquitin
Biotin-GRSNQL
- QC
+ QC
P 40%
94%
Time (Min)
obs. 9587.0
calc. 9587.0
959.73
872.59
798.99
1066.25
1199.32
m/z
obs. 9376.8
calc. 9375.8
938.89
853.47
782.49
1042.87
1173.09
m/z

FIG. 3

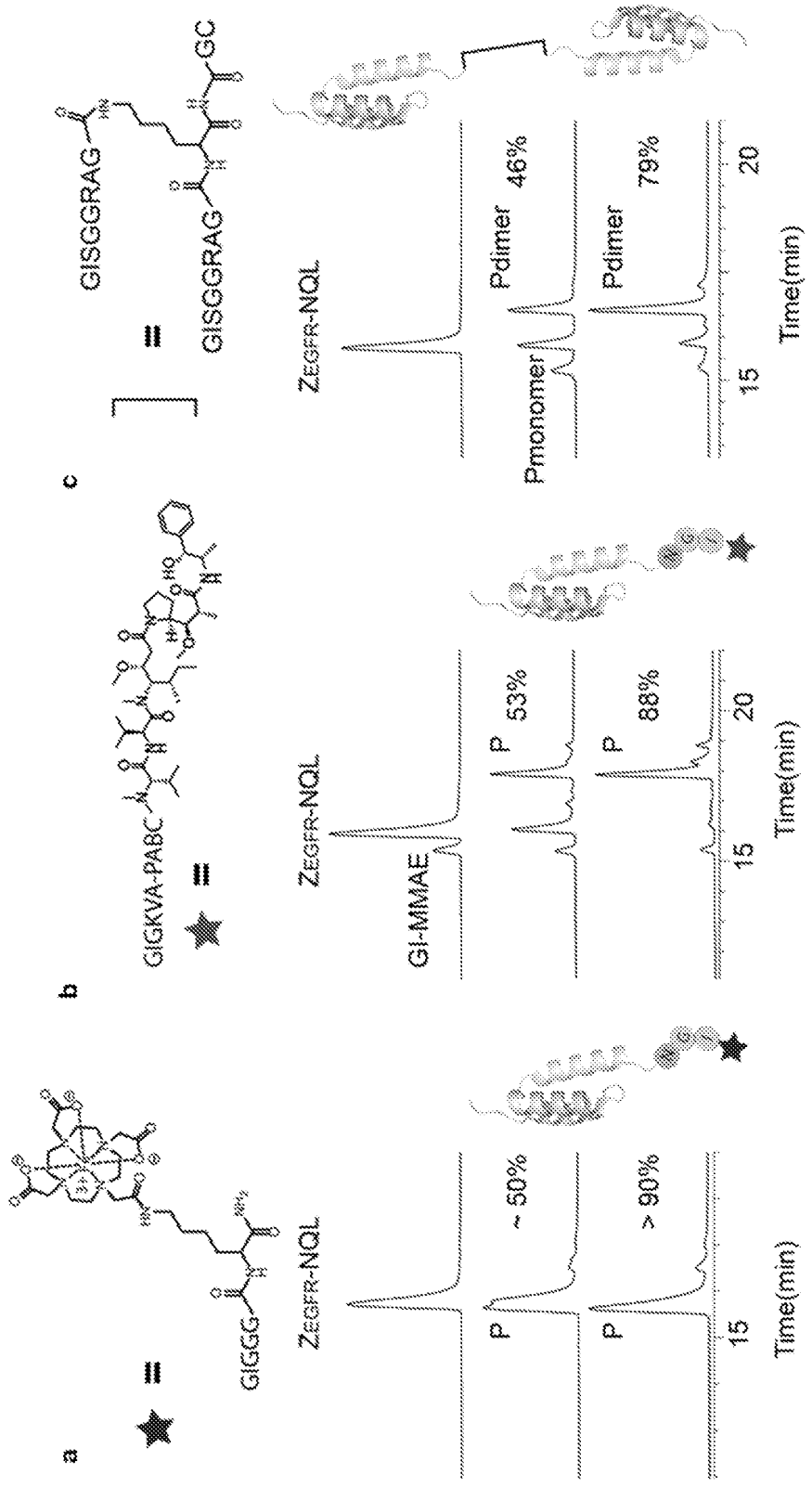

a
GIGGG
ZEGFR-NQL
P
~ 50%
P
> 90%
15
Time(min)
b
GIGKVA-PABC
GI-MMAE
ZEGFR-NQL
P
53%
P
88%
15
20
Time(min)
c
GISGGRAG
GISGGRAG
GC
ZEGFR-NQL
Pmonomer
Pdimer
46%
Pdimer
79%
15
20
Time(min)

FIG. 4

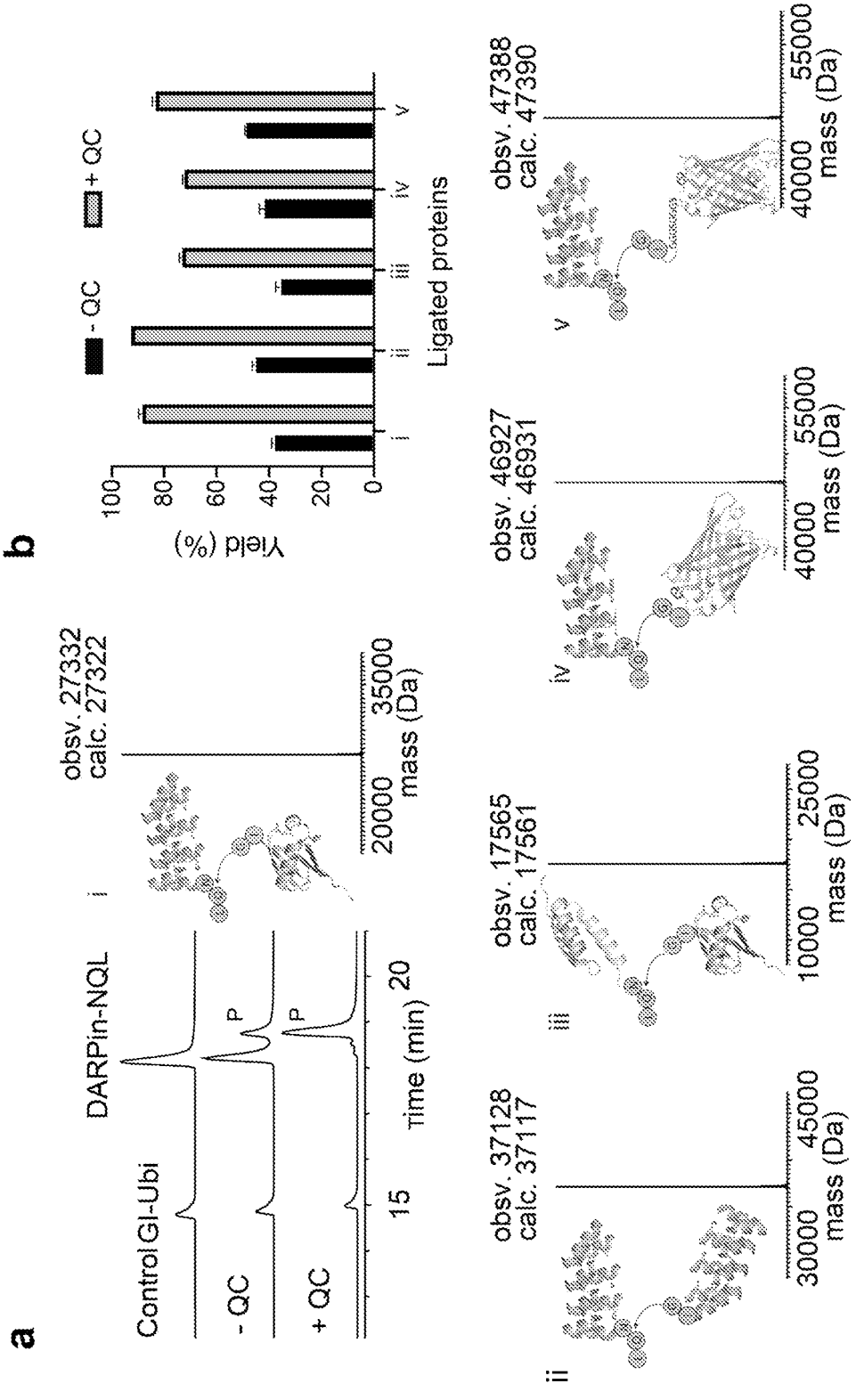

a
Control Gl-Ubi
DARPin-NQL
i
obsv. 27332
calc. 27322
- QC
+ QC
P
P
15
Time (min)
20
20000
35000
mass (Da)
b
Yield (%)
100
80
60
40
20
0
- QC
+ QC
i
ii
iii
iv
v
Ligated proteins
ii
obsv. 37128
calc. 37117
30000
45000
mass (Da)
iii
obsv. 17565
calc. 17561
10000
25000
mass (Da)
iv
obsv. 46927
calc. 46931
40000
55000
mass (Da)
v
obsv. 47388
calc. 47390
40000
55000
mass (Da)

FIG. 5

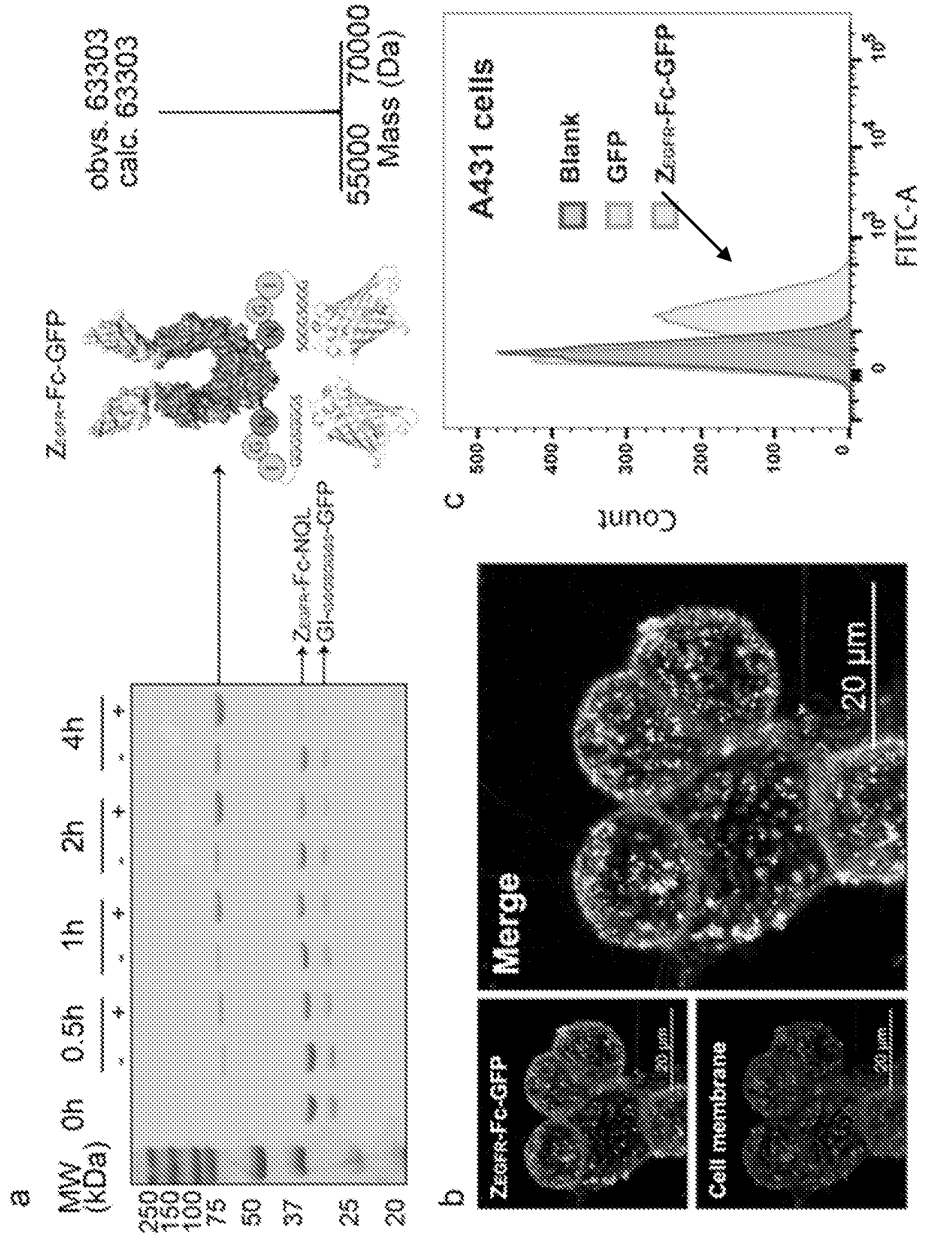

a
MW (kDa)
0h
0.5h
1h
2h
4h
250
150
100
75
50
37
25
20
obvs. 63303
calc. 63303
55000
70000
Mass (Da)
b
Merge
20 μm
Cell membrane
c
A431 cells
Blank
GFP
Count
FITC-A

FIG. 6

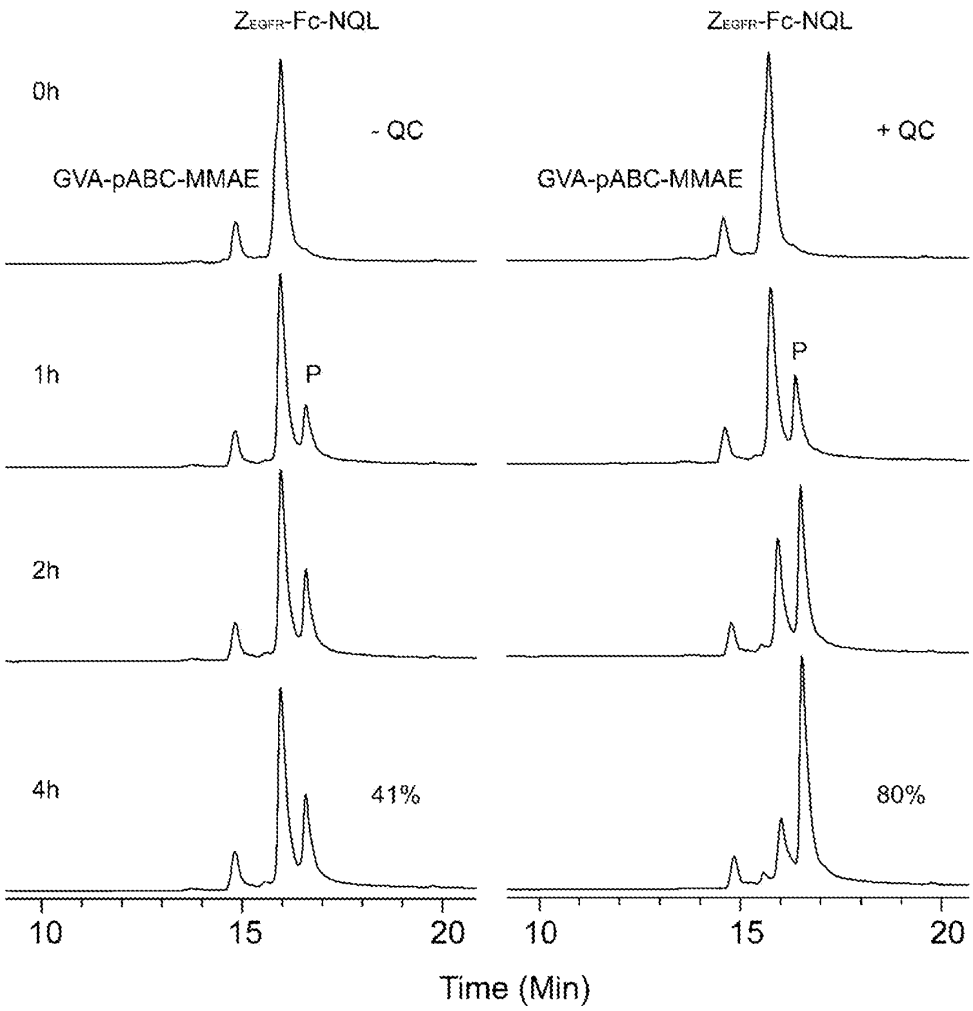

ZEGFR-Fc-NQL
ZEGFR-Fc-NQL
0h
- QC
+ QC
GVA-pABC-MMAE
GVA-pABC-MMAE
1h
P
P
2h
4h
41%
80%
10
15
20
10
15
20
Time (Min)

FIG. 7

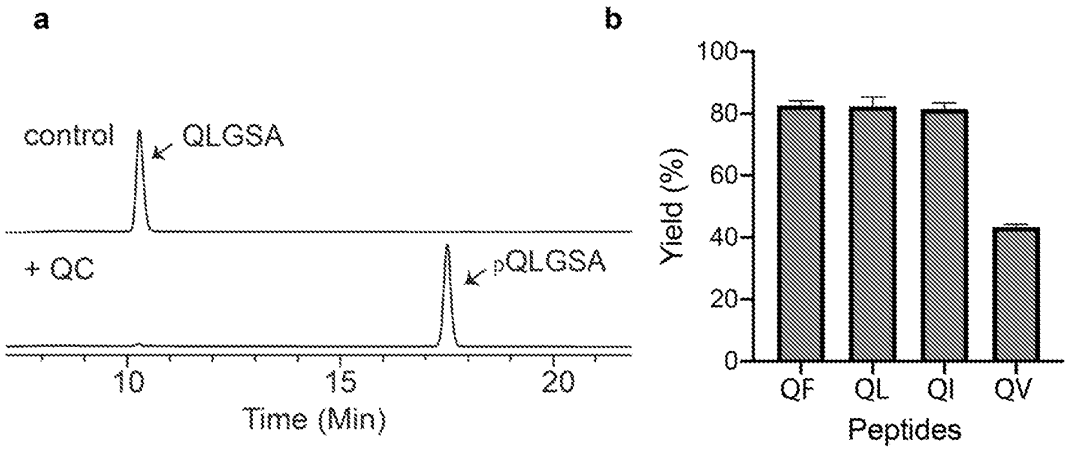

a
control
QLGSA
+ QC
pQLGSA
10
15
20
Time (Min)
b
100
80
60
40
20
0
Yield (%)
QF
QL
QI
QV
Peptides

FIG. 8

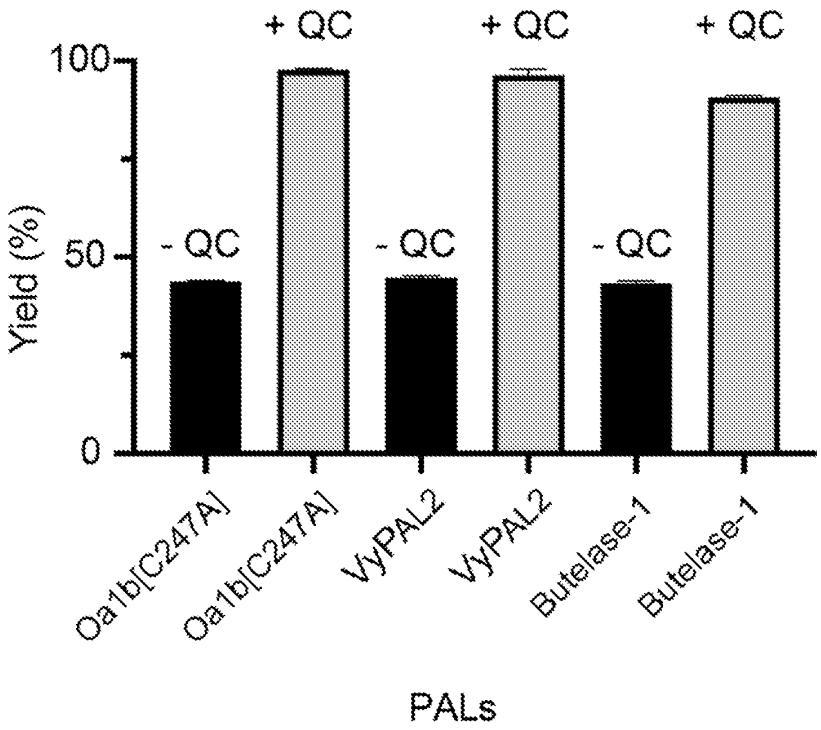

+ QC
+ QC
+ QC
100
50
0
Yield (%)
- QC
- QC
- QC
Oa1b[C247A]
Oa1b[C247A]
VyPAL2
VyPAL2
Butelase-1
Butelase-1
PALs

FIG. 9

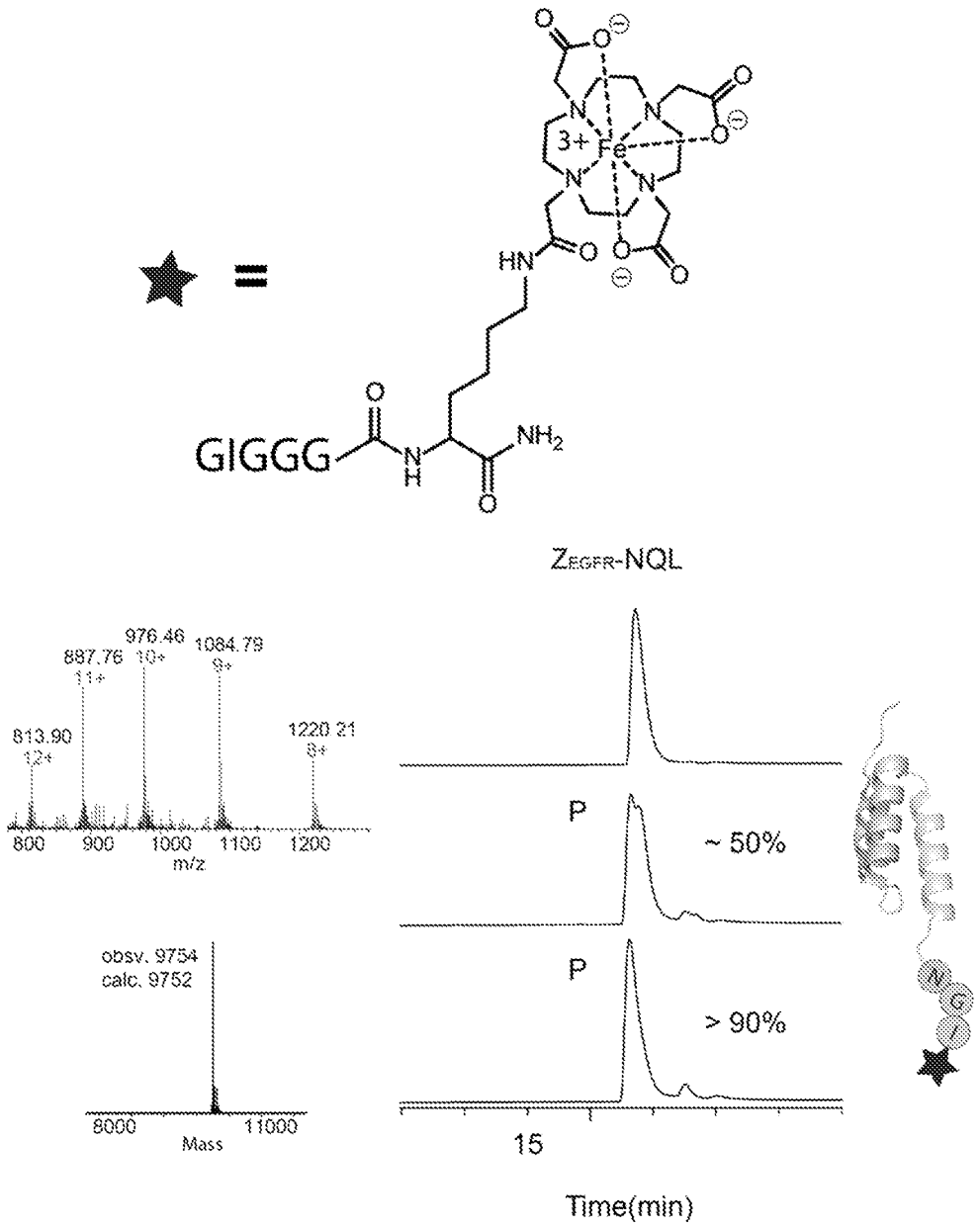

=
3+
Fe
HN
GIGGG
NH2
ZEGFR-NQL
887.76
11+
976.46
10+
1084.79
9+
813.90
12+
1220.21
8+
800
900
1000
1100
1200
m/z
obsv. 9754
calc. 9752
8000
11000
Mass
P
~ 50%
P
> 90%
15
Time(min)

FIG. 10

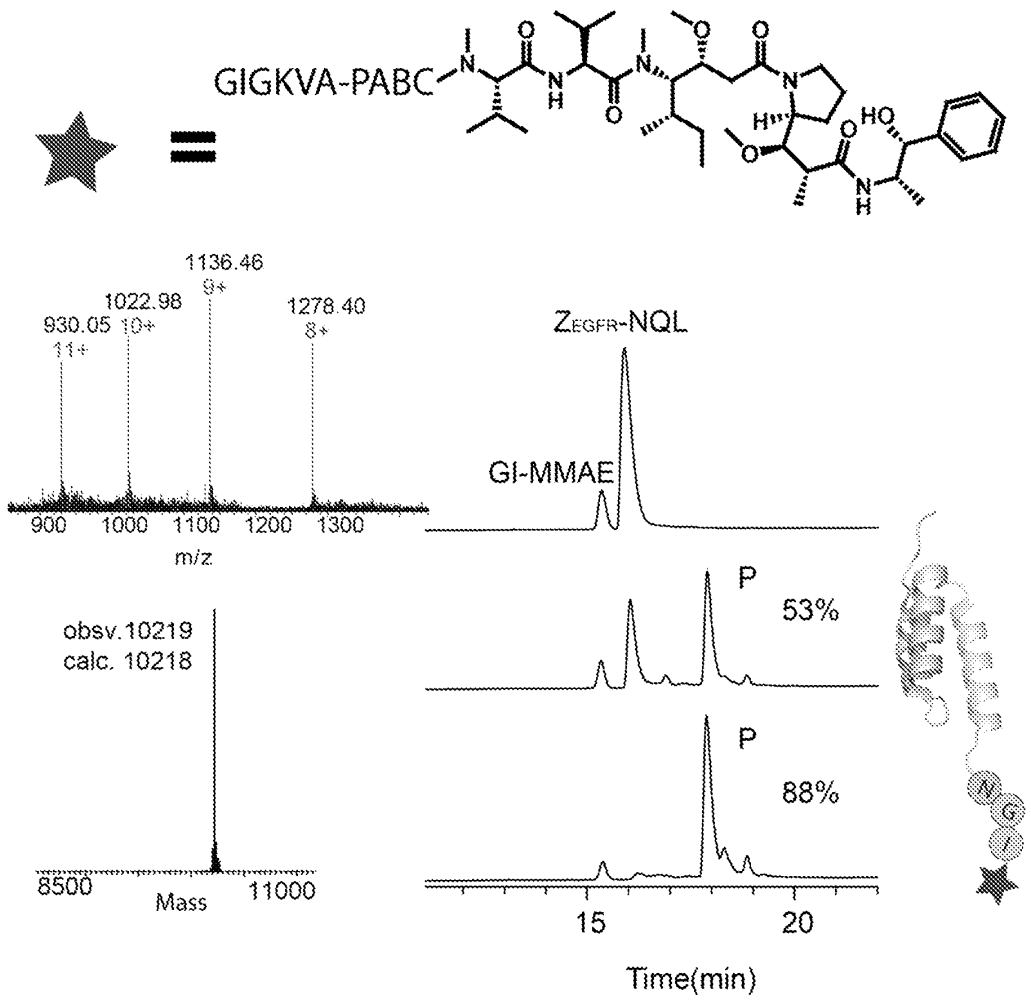

GIGKVA-PABC
1136.46
9+
1022.98
10+
930.05
11+
1278.40
8+
900
1000
1100
1200
1300
m/z
obsv.10219
calc. 10218
8500
Mass
11000
ZEGFR-NQL
GI-MMAE
P
53%
P
88%
15
20
Time(min)

FIG. 11

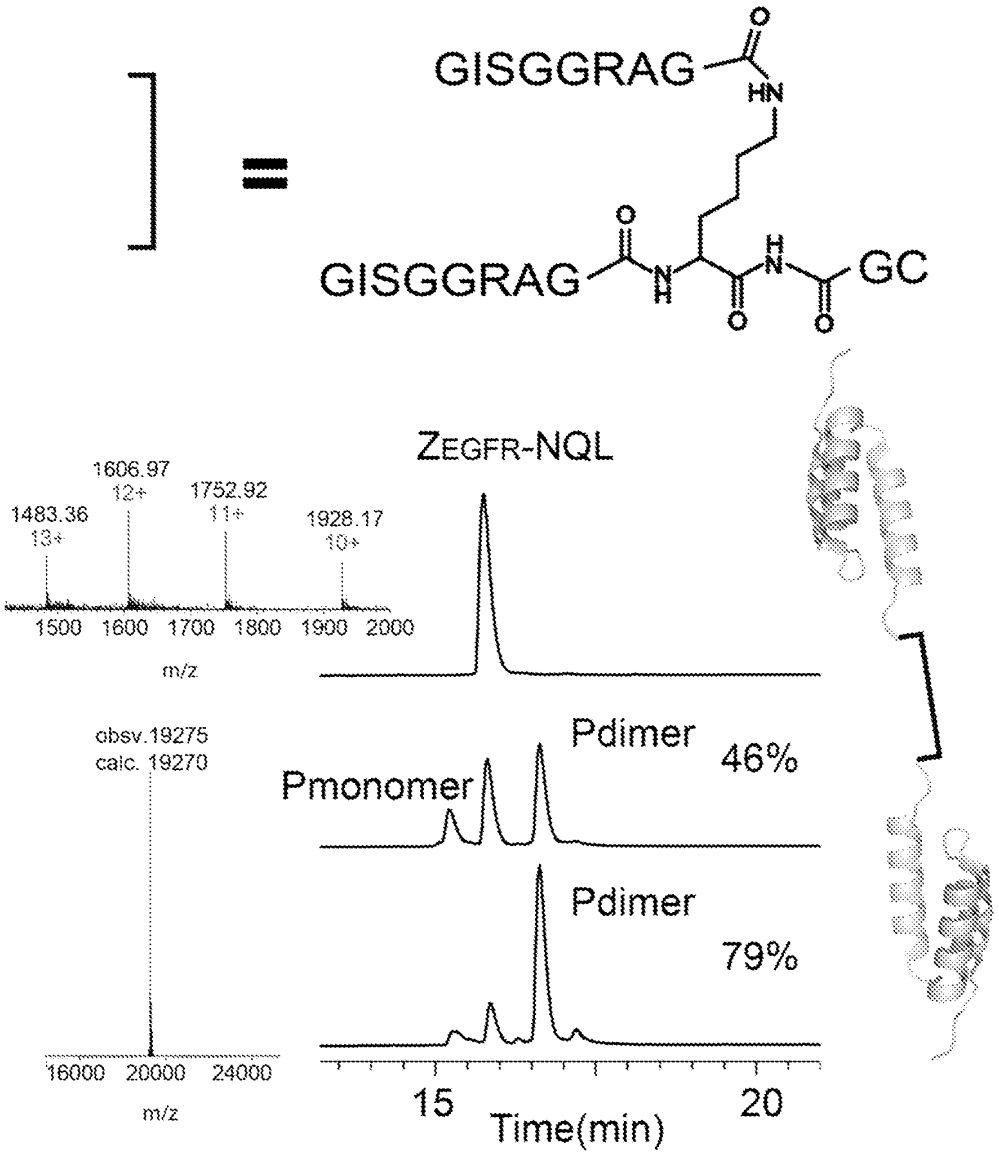
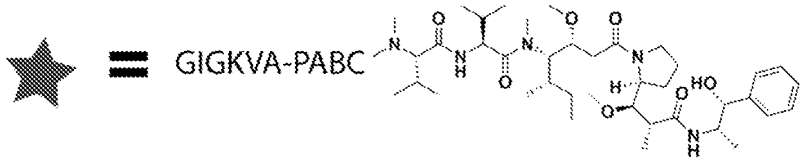

GISGGRAG
HN
GISGGRAG
N
H
H
N
GC
ZEGFR-NQL
1606.97
12+
1752.92
11+
1483.36
13+
1928.17
10+
1500 1600 1700 1800 1900 2000
m/z
obsv.19275
calc. 19270
16000 20000 24000
m/z
Pdimer
46%
Pmonomer
Pdimer
79%
15
20
Time(min)

FIG. 12


GIGKVA-PABC

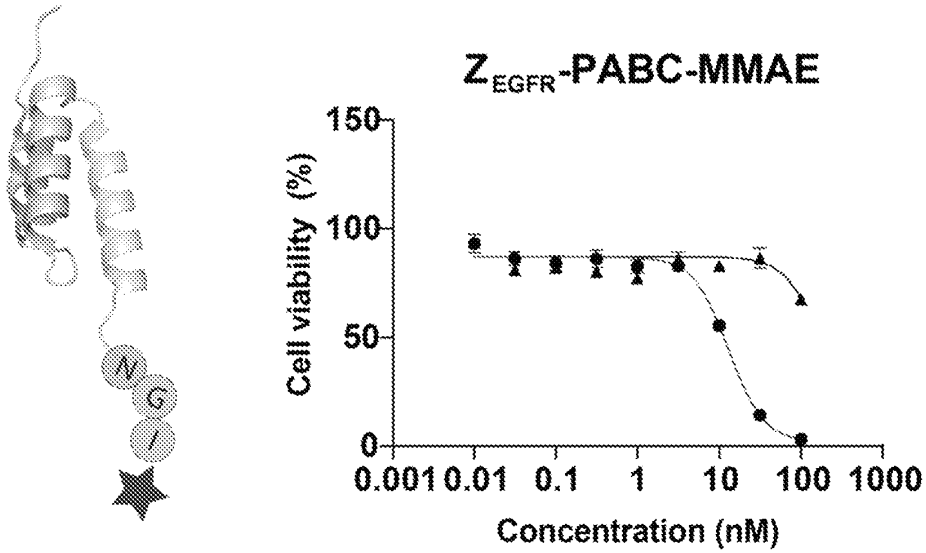

N
G
I
Z_{EGFR}-PABC-MMAE
Cell viability (%)
150
100
50
0
0.001 0.01 0.1 1 10 100 1000
Concentration (nM)

FIG. 13

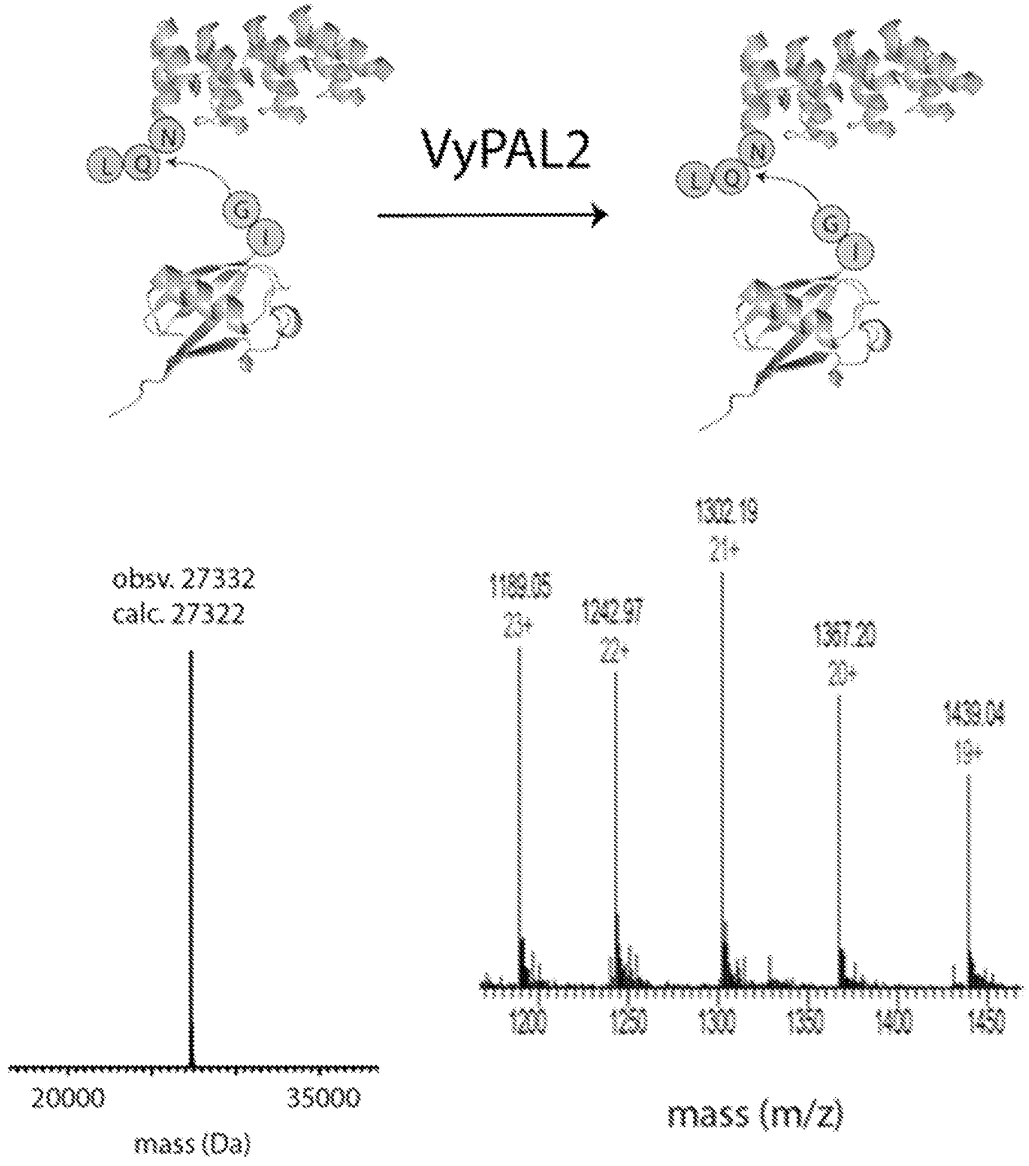

VyPAL2
obsv. 27332
calc. 27322
20000
35000
mass (Da)
1189.05
22+
1242.97
22+
1302.19
21+
1367.20
20+
1439.04
19+
1200
1250
1300
1350
1400
1450
mass (m/z)

FIG. 13 (cont'd)

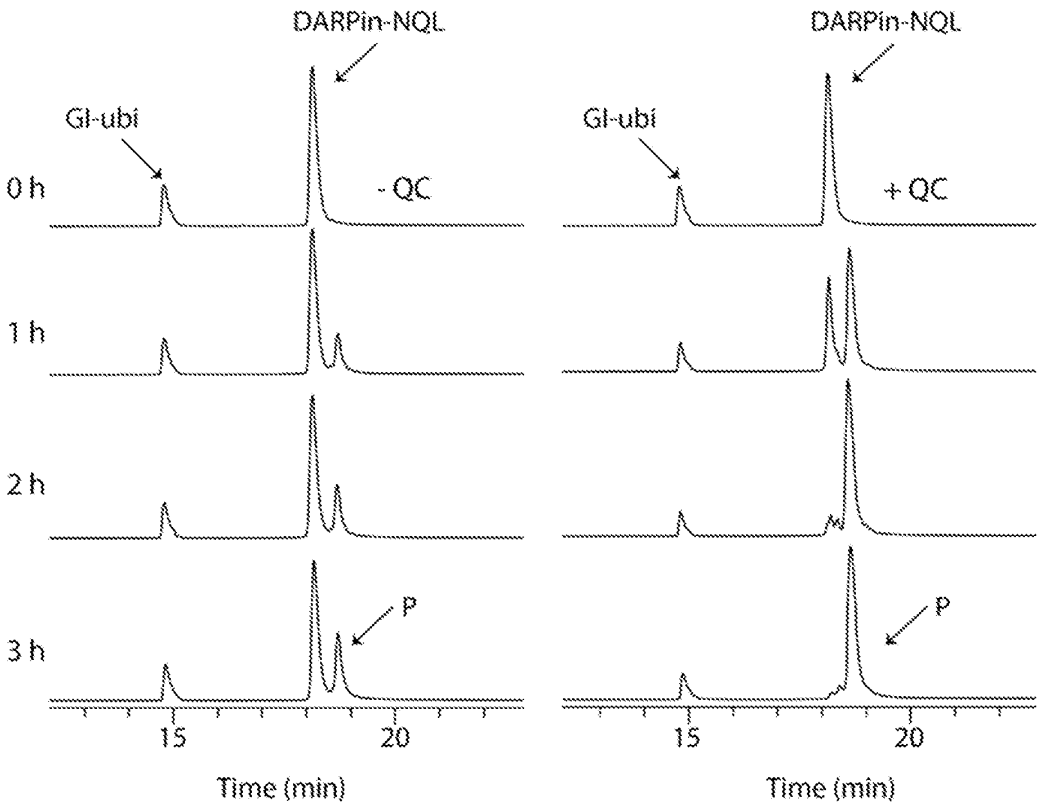

DARPin-NQL
Gl-ubi
0 h
- QC
1 h
2 h
3 h
P
15
20
Time (min)
DARPin-NQL
Gl-ubi
+ QC
P
15
20
Time (min)

FIG. 14

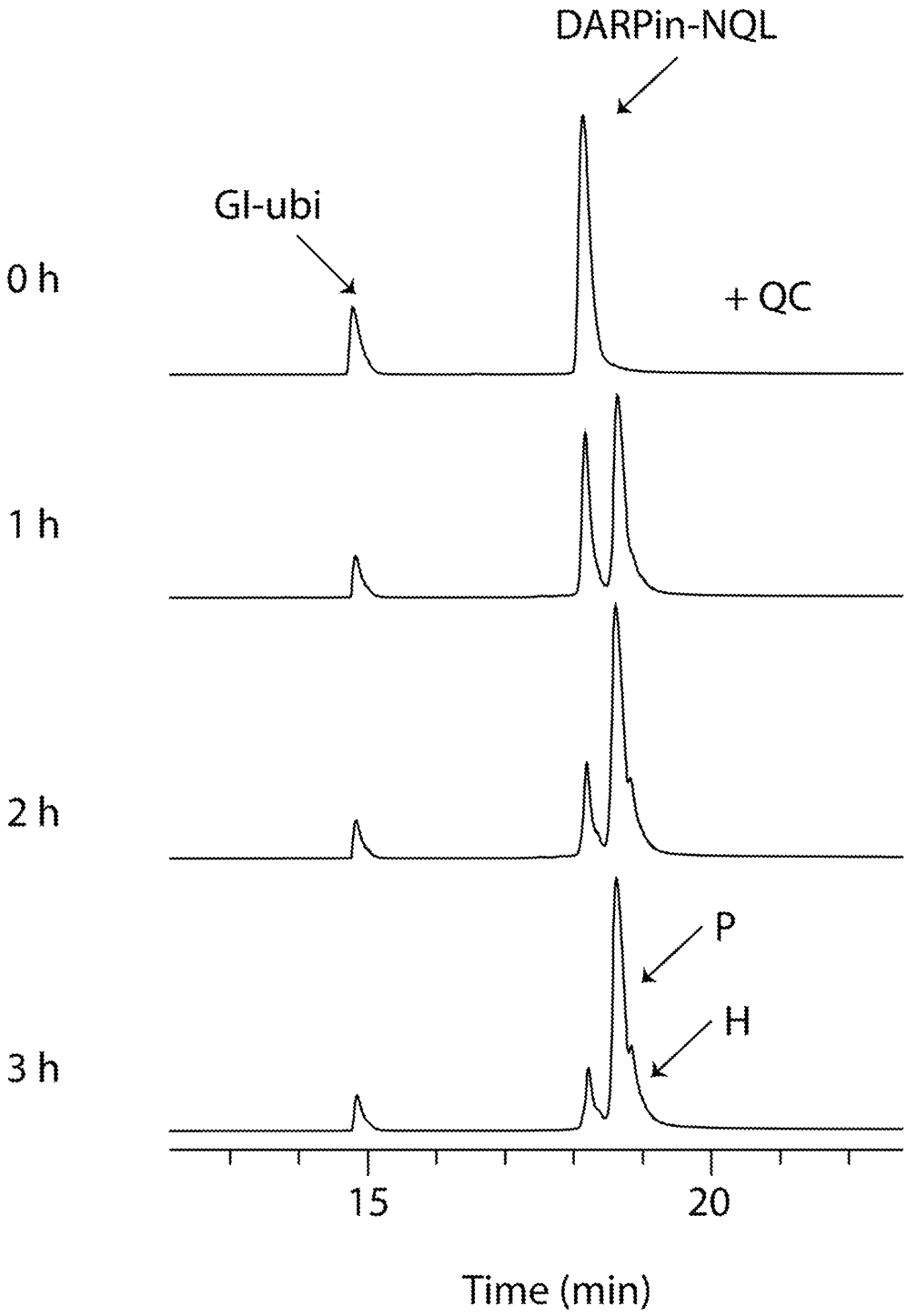


FIG. 15

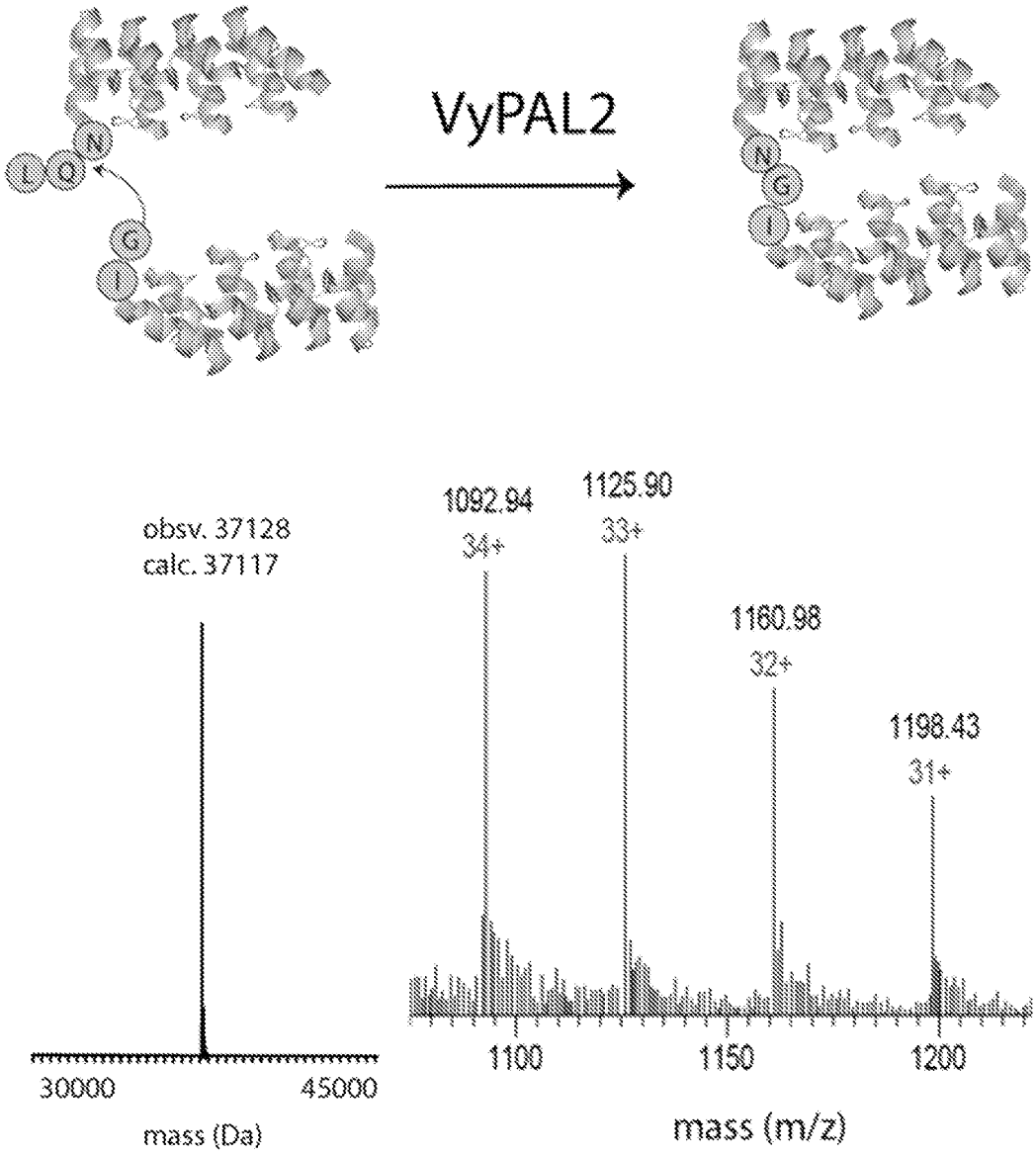

VyPAL2
obsv. 37128
calc. 37117
30000
45000
mass (Da)
1092.94
34+
1125.90
33+
1160.98
32+
1198.43
31+
1100
1150
1200
mass (m/z)

FIG. 15 (cont'd)

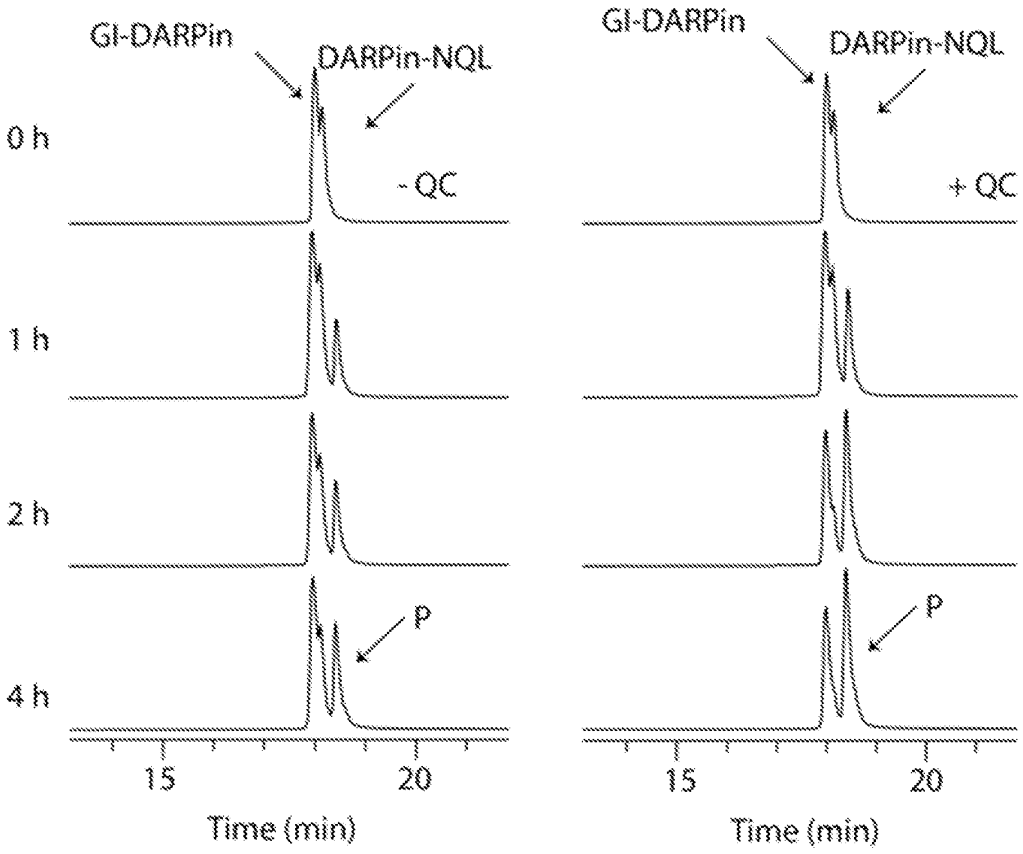


FIG. 16

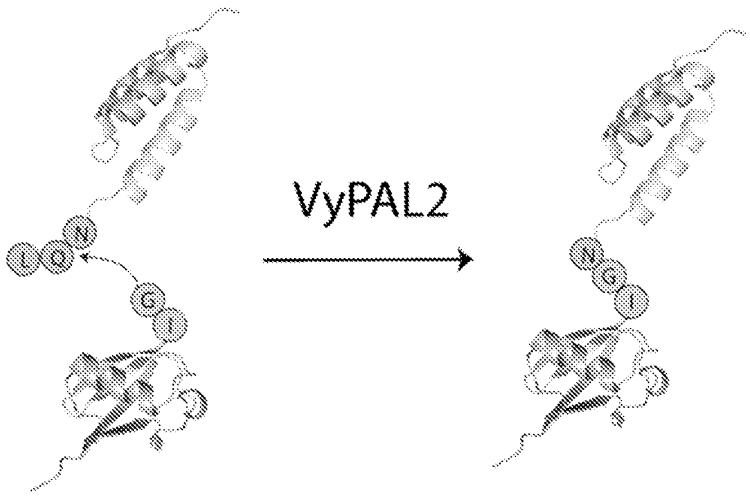


FIG. 16 (cont'd)

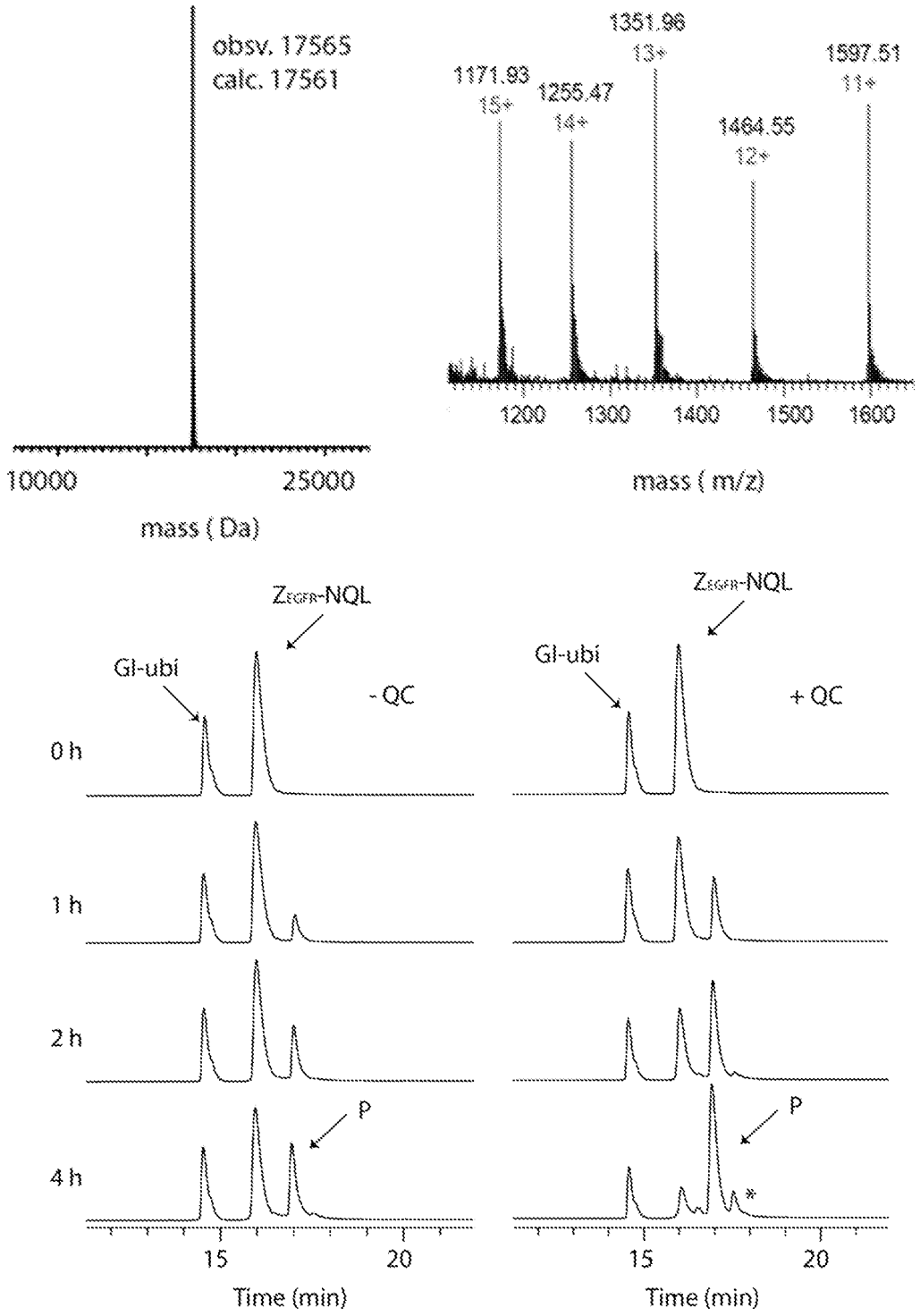

obsv. 17565
calc. 17561
10000
25000
mass (Da)
1171.83
15+
1255.47
14+
1351.96
13+
1464.55
12+
1597.51
11+
1200
1300
1400
1500
1600
mass (m/z)
ZEGFR-NQL
Gl-ubi
- QC
+ QC
0 h
1 h
2 h
4 h
P
*
15
20
Time (min)

FIG. 17

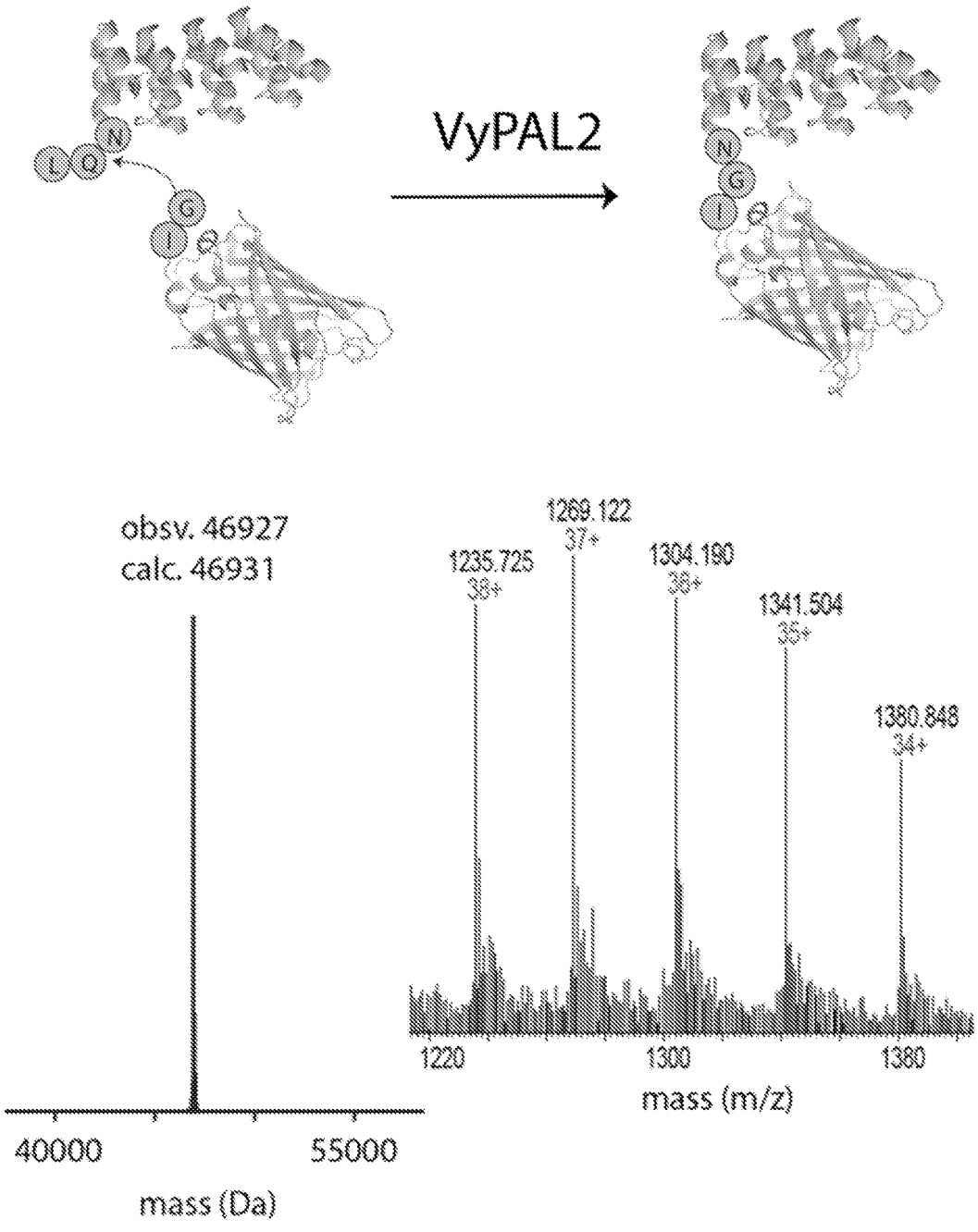

VyPAL2
obsv. 46927
calc. 46931
40000
55000
mass (Da)
1269.122
37+
1235.725
38+
1304.190
36+
1341.504
35+
1380.848
34+
1220
1300
1380
mass (m/z)

FIG. 17 (cont'd)

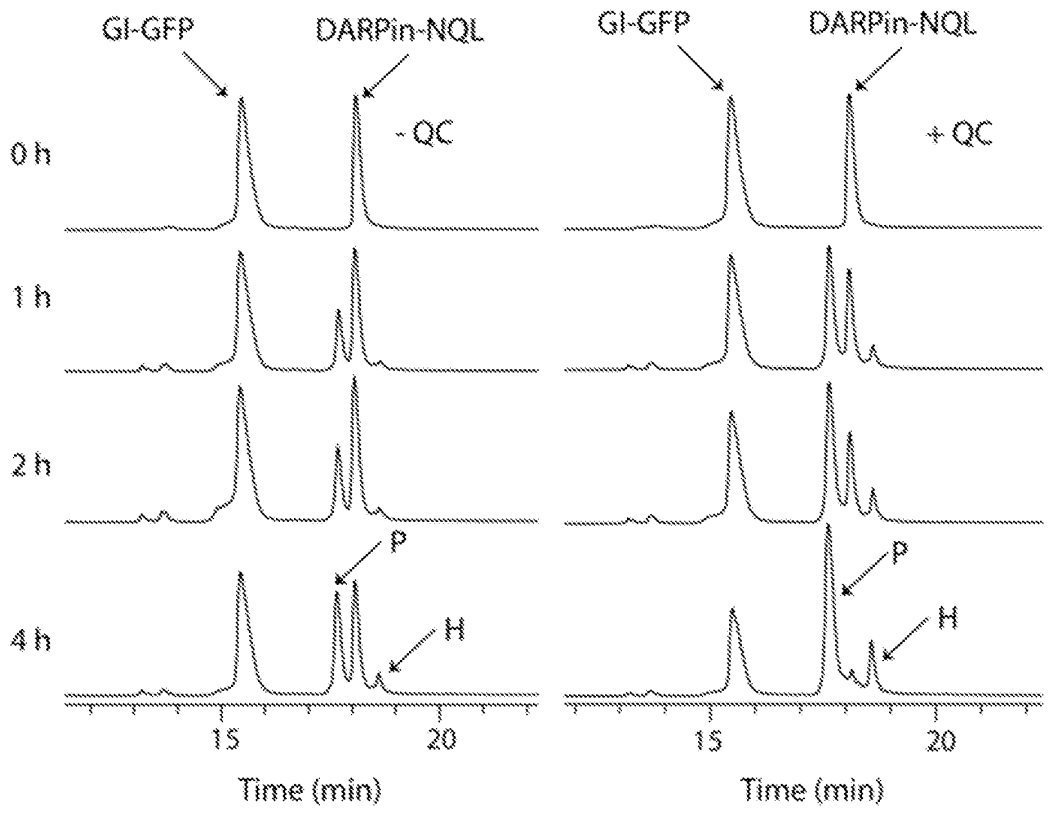


FIG. 18

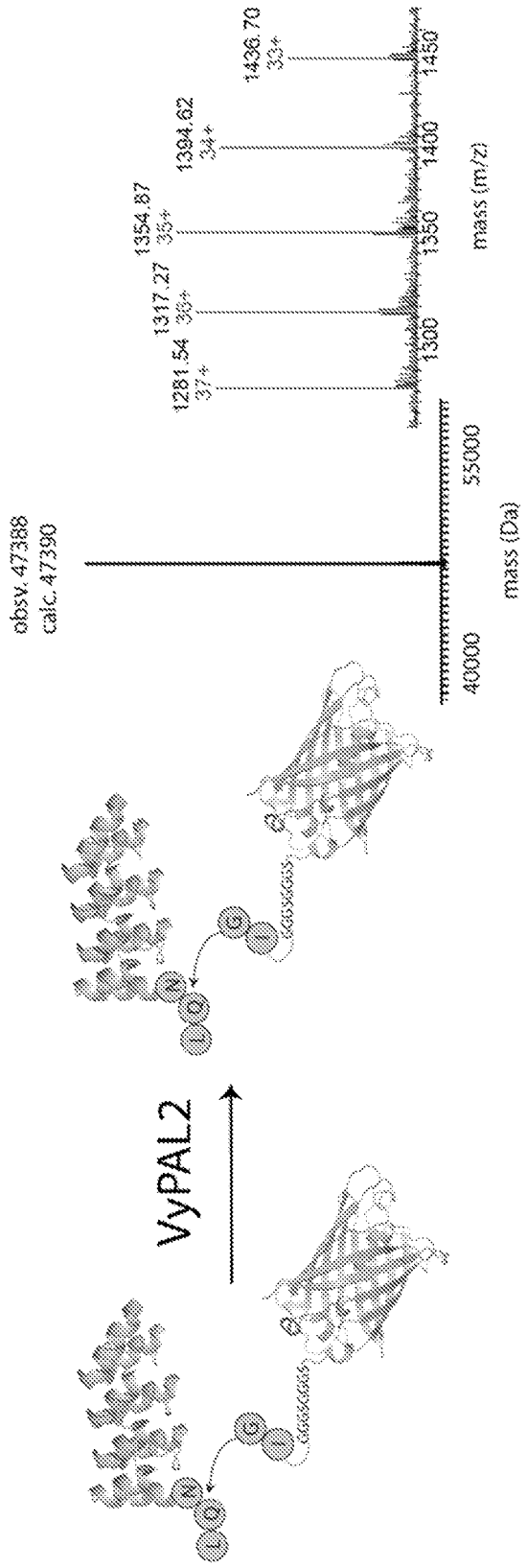

VyPAL2
obsv. 47388
calc. 47390
40000
55000
mass (Da)
1281.54
37+
1317.27
36+
1354.87
35+
1394.62
34+
1436.70
33+
1300
1350
1400
1450
mass (m/z)

FIG. 18 (cont'd)

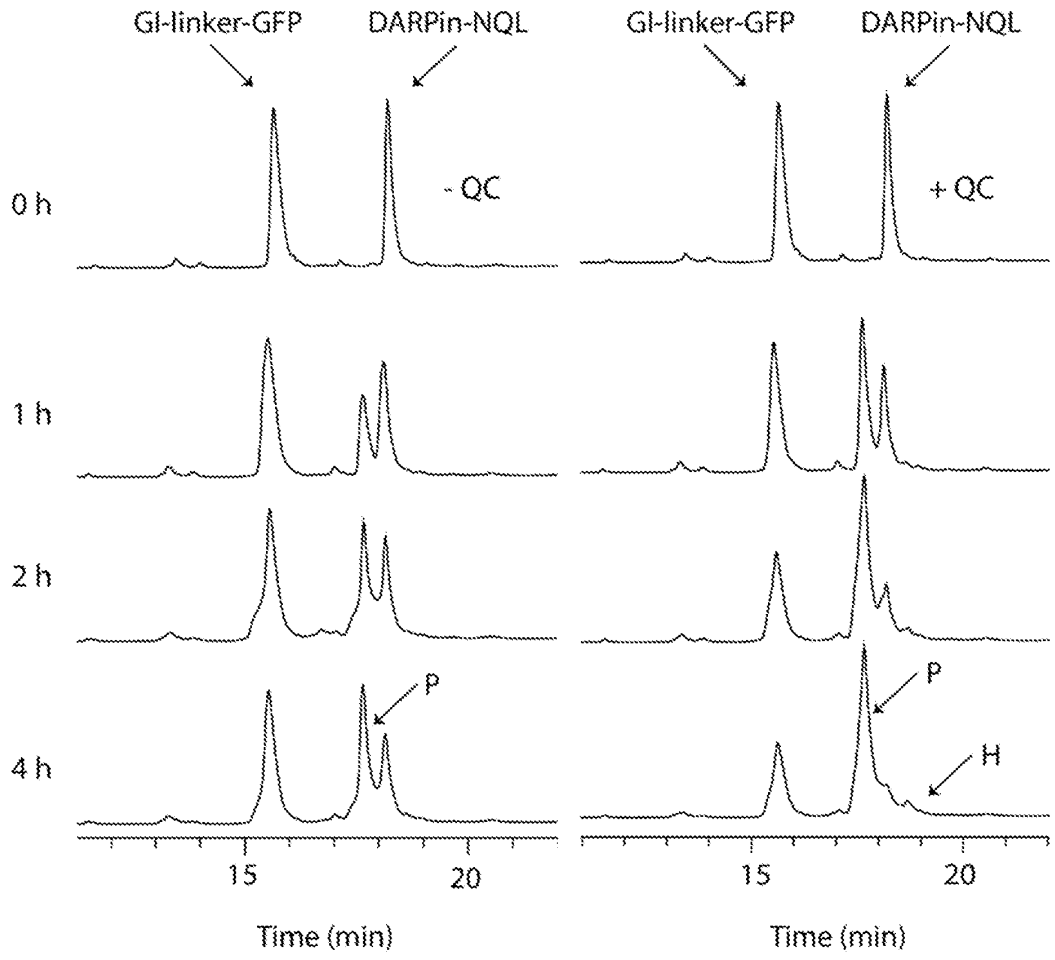

Gl-linker-GFP
DARPin-NQL
Gl-linker-GFP
DARPin-NQL
0 h
- QC
+ QC
1 h
2 h
4 h
P
P
H
15
20
15
20
Time (min)
Time (min)

FIG. 19

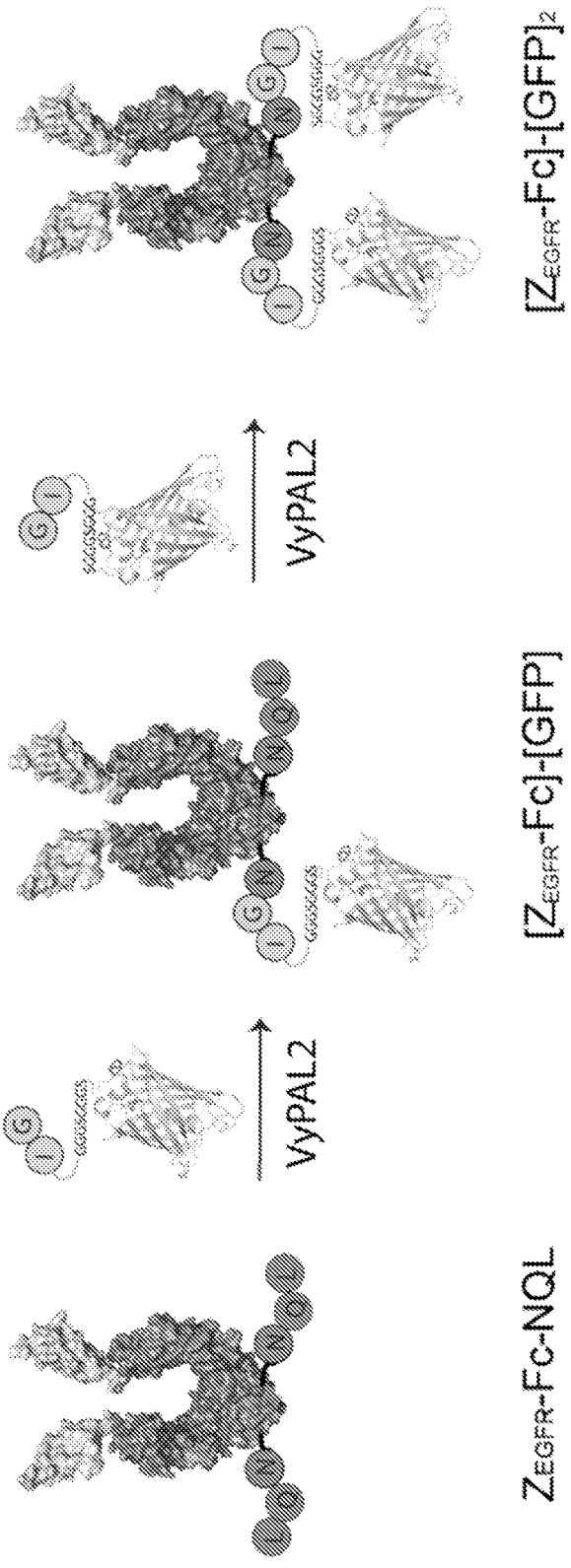


FIG. 19 (cont'd)

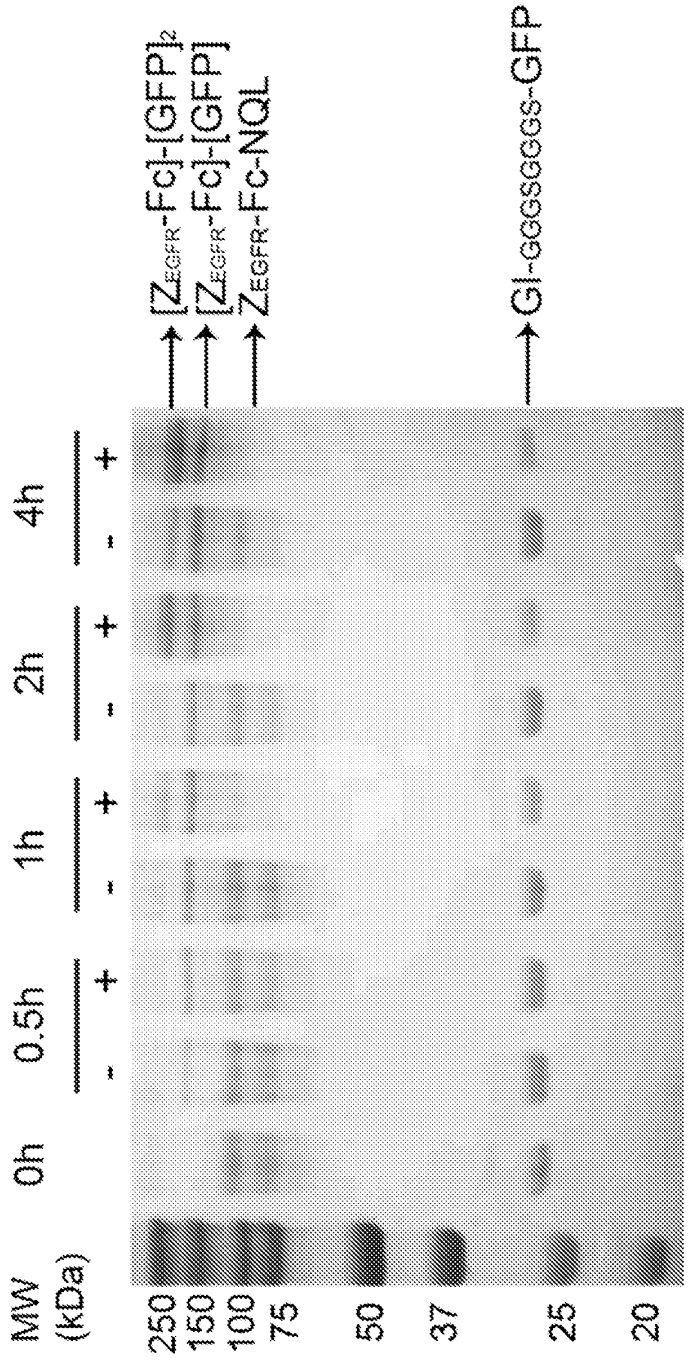

MW (kDa)
0h
0.5h
1h
2h
4h
-
+
-
+
-
+
-
+
-
+
250
150
100
75
50
37
25
20
[ZEGFR-Fc]-[GFP]2
[ZEGFR-Fc]-[GFP]
ZEGFR-Fc-NQL
GI-GGGSGGGS-GFP

FIG. 20

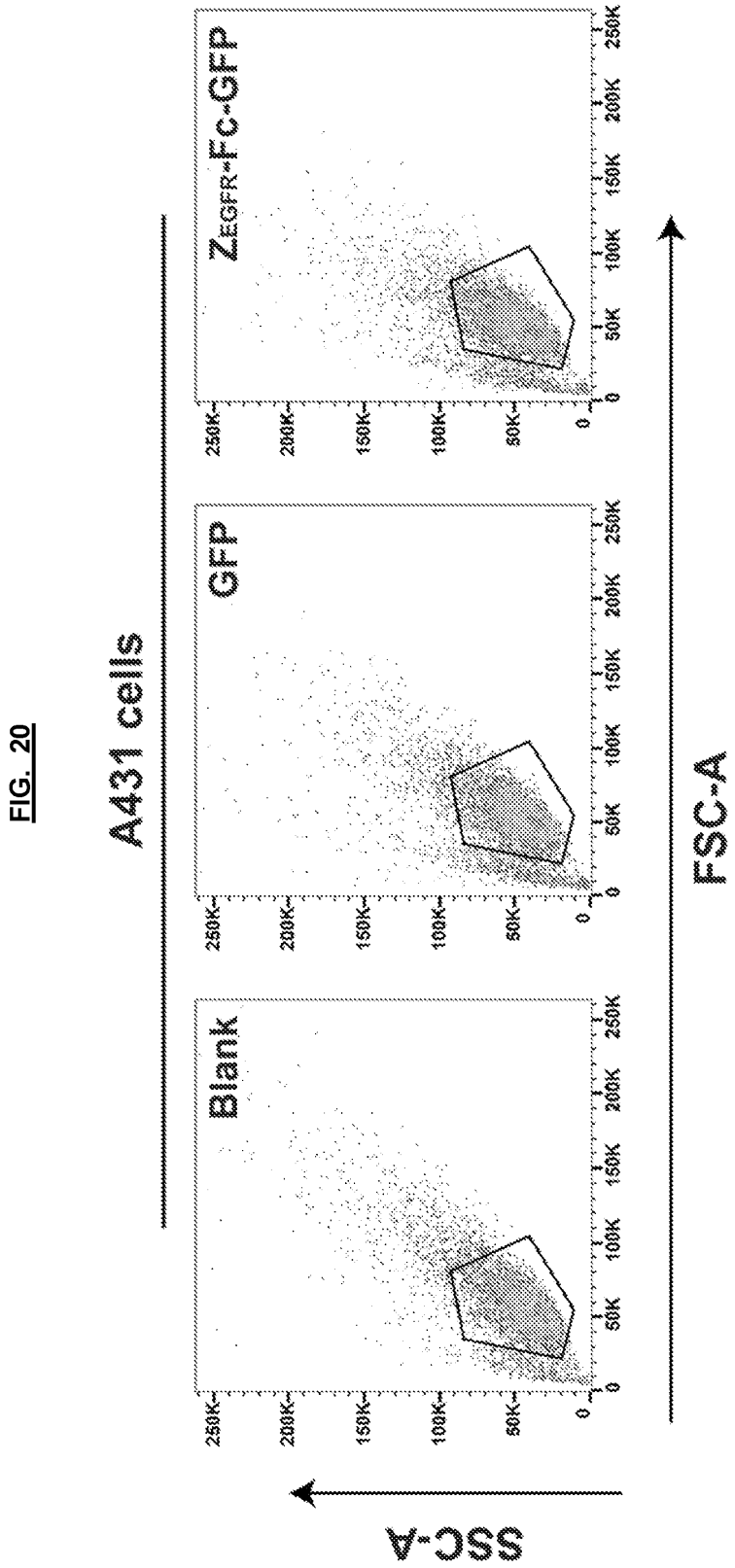

A431 cells
Blank
GFP
ZEGFR-Fc-GFP
SSC-A
FSC-A
250K
200K
150K
100K
50K
0

FIG. 20 (cont'd)

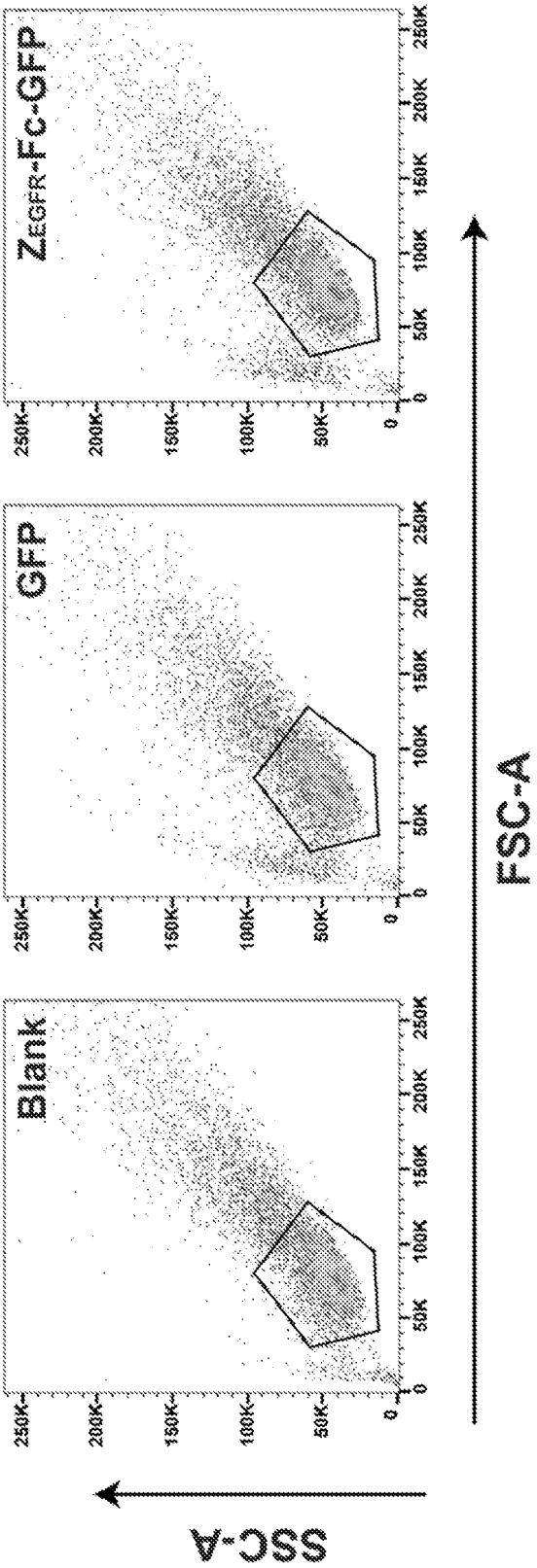

MCF7 cells
Blank
GFP
ZEGFR-Fc-GFP
SSC-A
FSC-A
250K
200K
150K
100K
50K
0

FIG. 20 (cont'd)

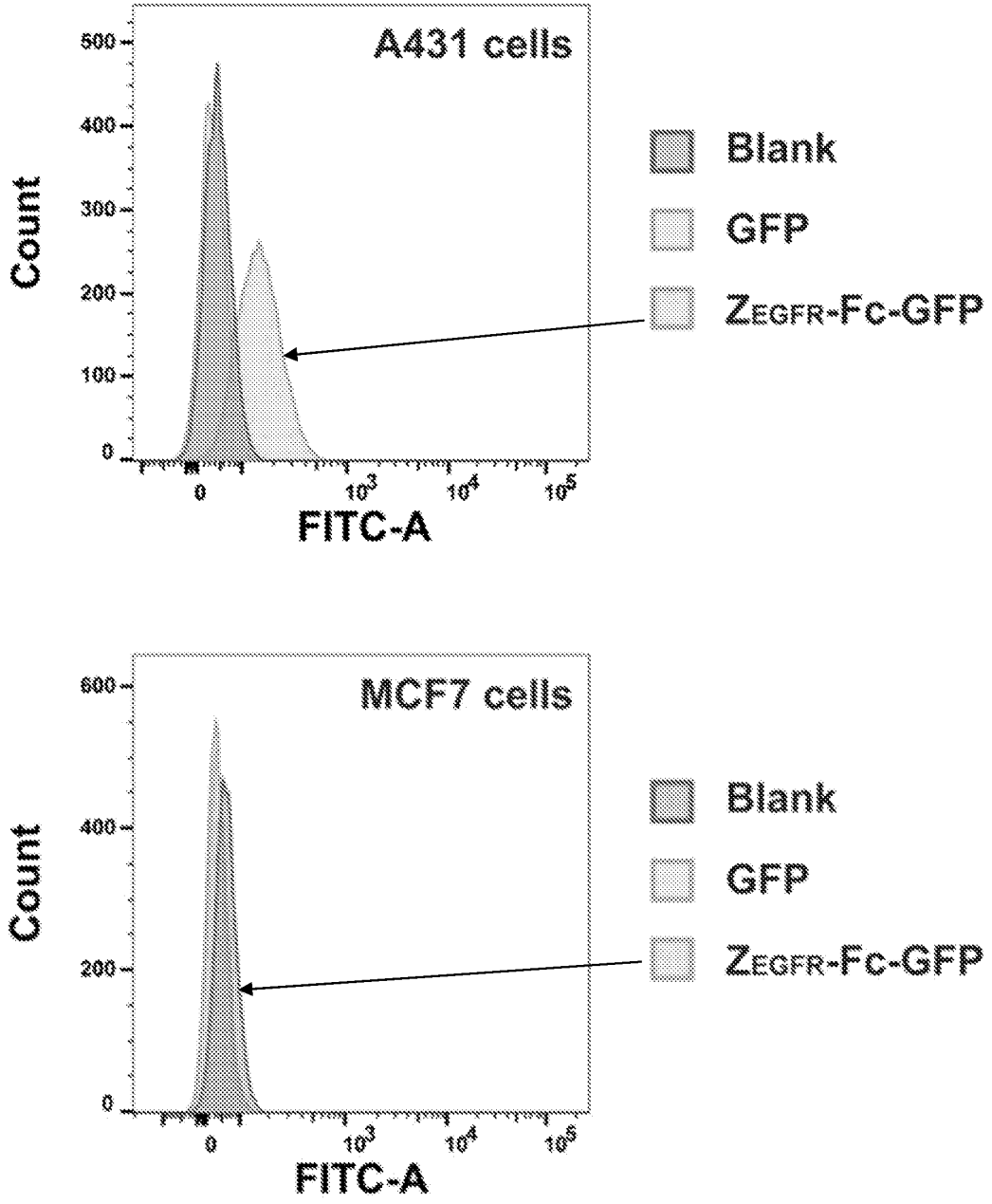

A431 cells
Count
FITC-A
Blank
GFP
ZEGFR-Fc-GFP
MCF7 cells
Count
FITC-A
Blank
GFP
ZEGFR-Fc-GFP

FIG. 21

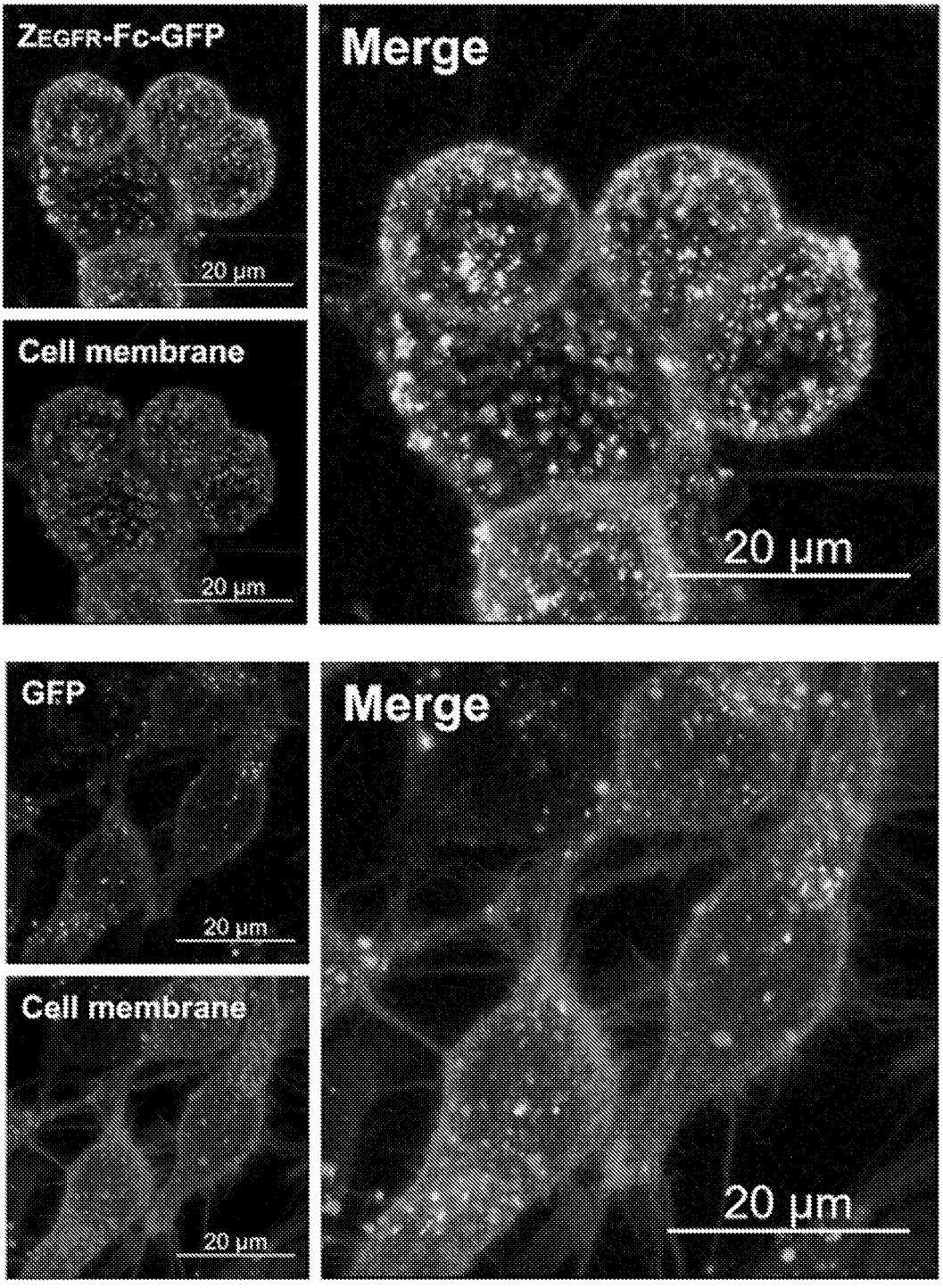

ZEGFR-Fc-GFP
20 μm
Merge
20 μm
Cell membrane
20 μm
GFP
20 μm
Merge
20 μm
Cell membrane
20 μm

FIG. 22

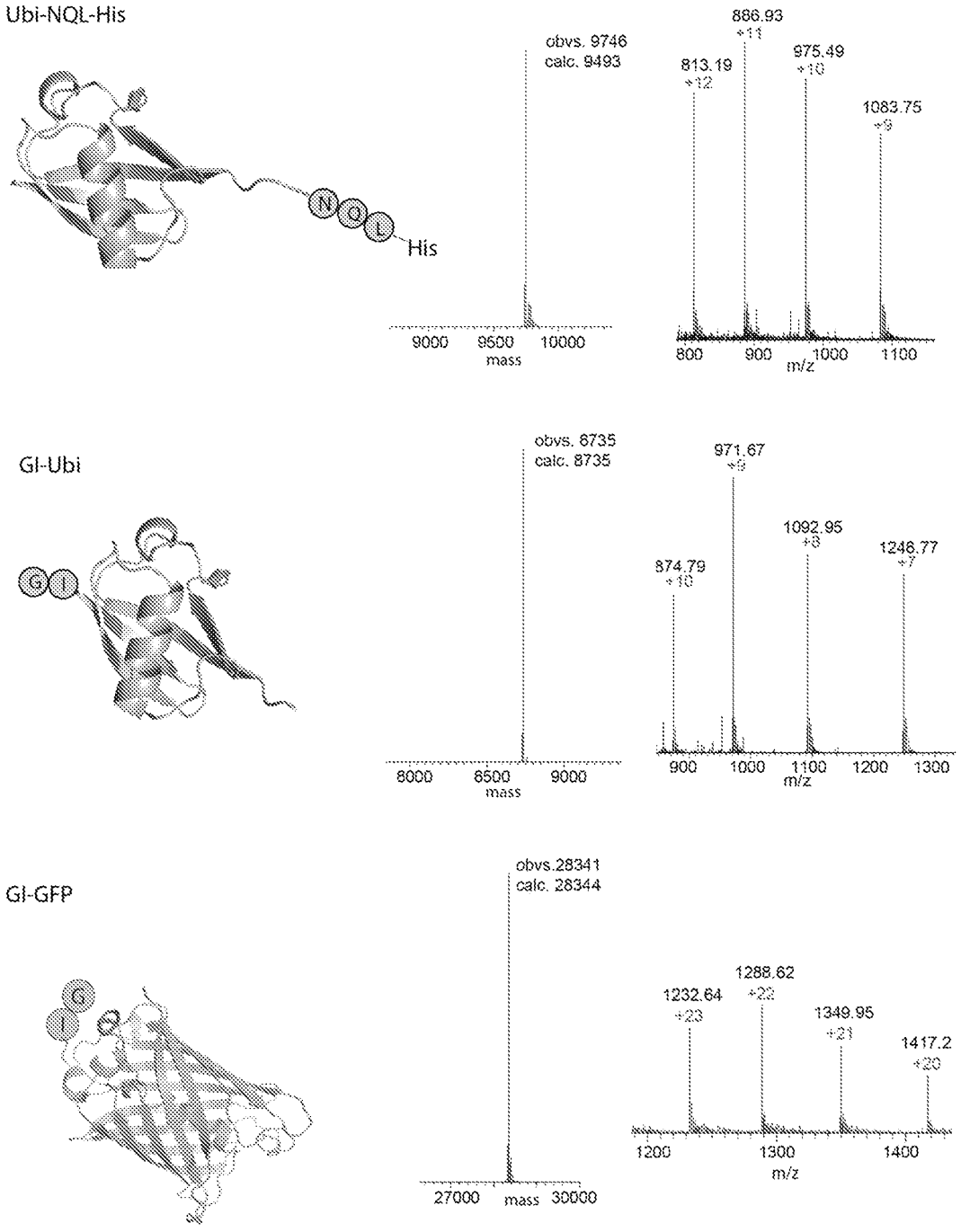
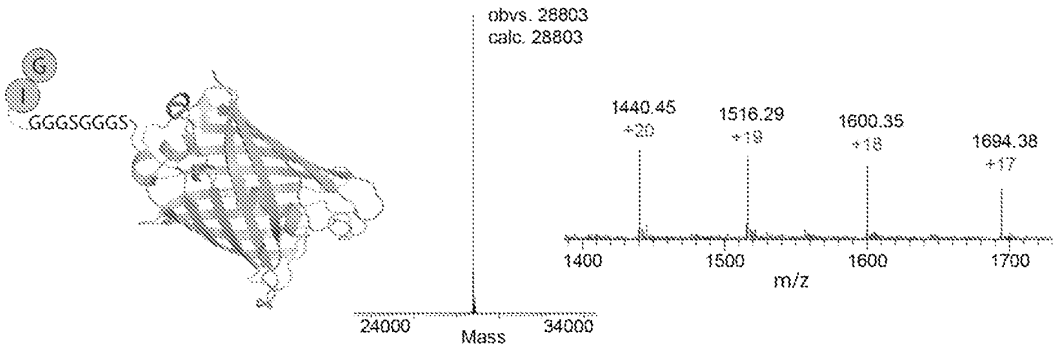
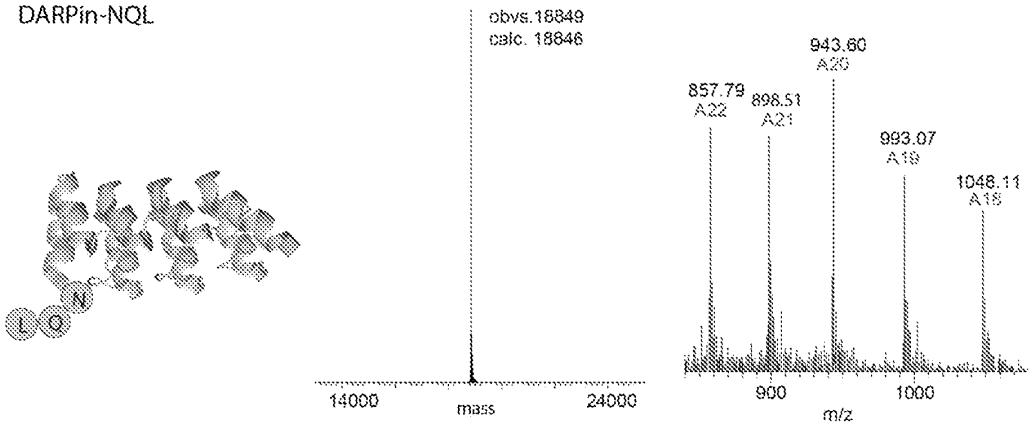

Ubi-NQL-His
N
Q
L
His
obvs. 9746
calc. 9493
9000
9500
10000
mass
886.93
+11
813.19
+12
975.49
+10
1083.75
+9
800
900
1000
1100
m/z
Gl-Ubi
G
I
obvs. 8735
calc. 8735
8000
8500
9000
mass
971.67
+9
1092.95
+8
874.79
+10
1246.77
+7
900
1000
1100
1200
1300
m/z
Gl-GFP
G
I
obvs.28341
calc. 28344
27000
mass
30000
1288.62
+22
1232.64
+23
1349.95
+21
1417.2
+20
1200
1300
1400
m/z

FIG. 22 (cont'd)

GI-GGGSGGGS-GFP



DARPin-NQL



GI-DARPin

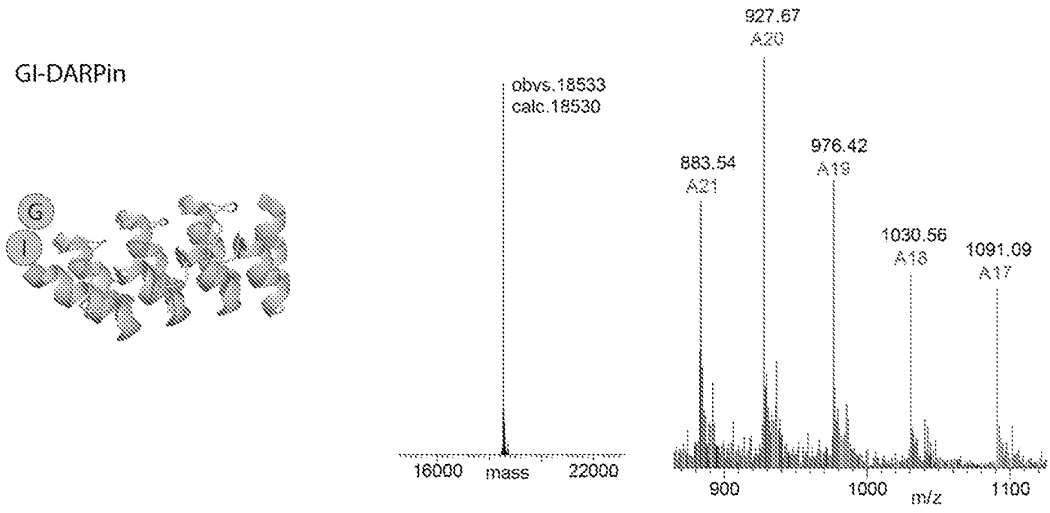
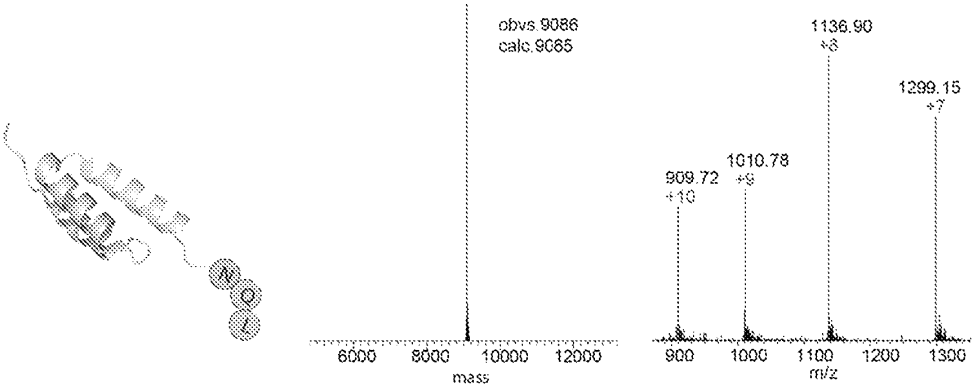


FIG. 22 (cont'd)

ZEGFR-NQL



ZEGFR-Fc-NQL monomer

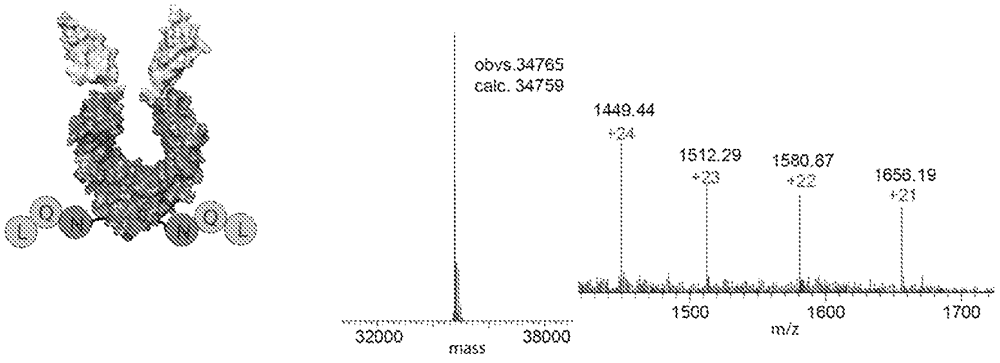
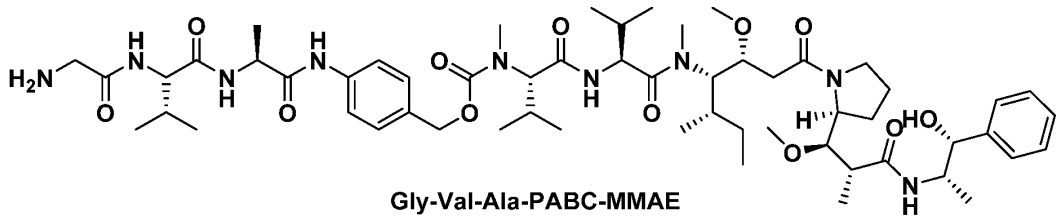


FIG. 23



Gly-Val-Ala-PABC-MMAE

FIG. 24

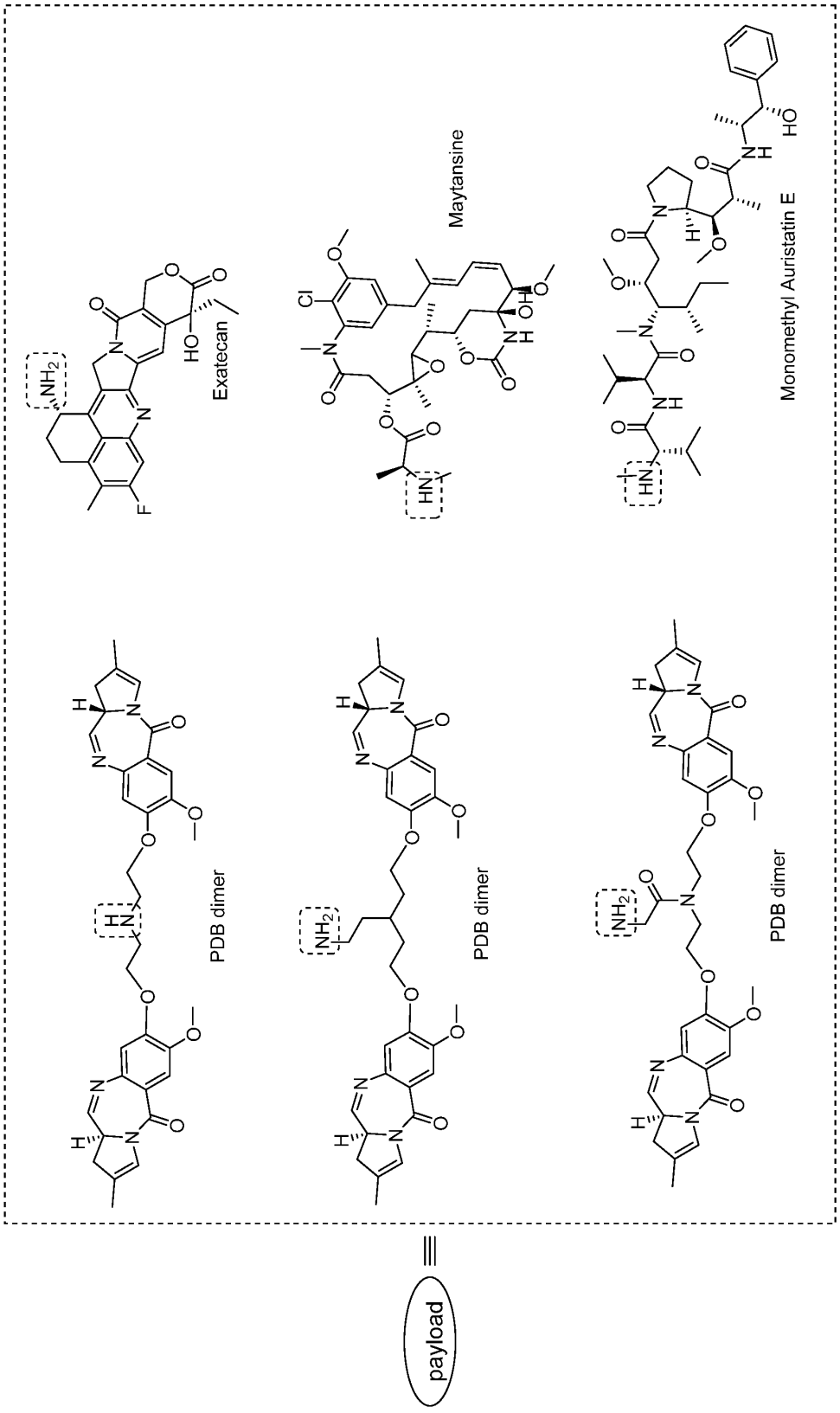

payload
PDB dimer
PDB dimer
PDB dimer
Exatecan
Maytansine
Monomethyl Auristatin E

FIG. 25

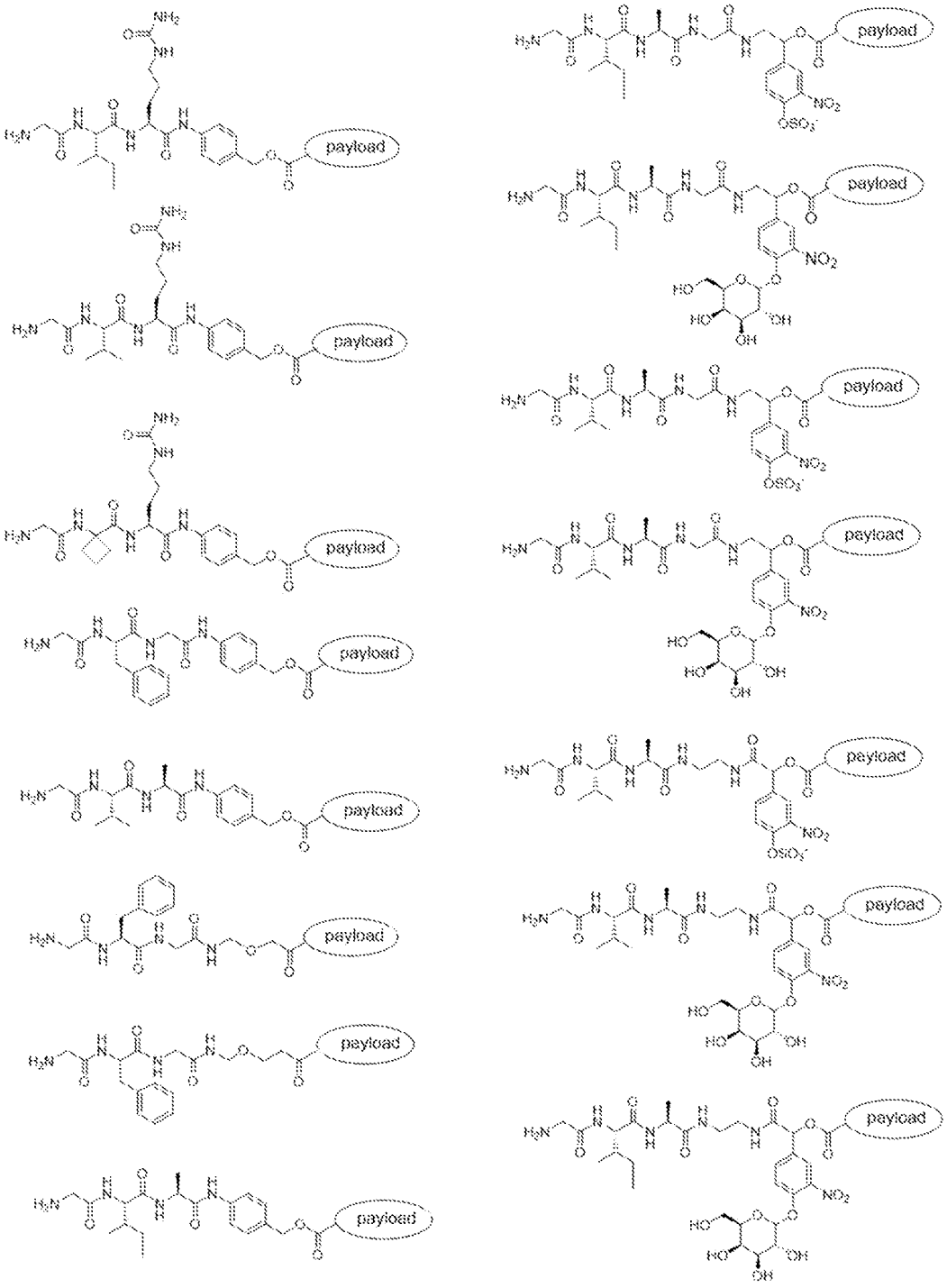


FIG. 26

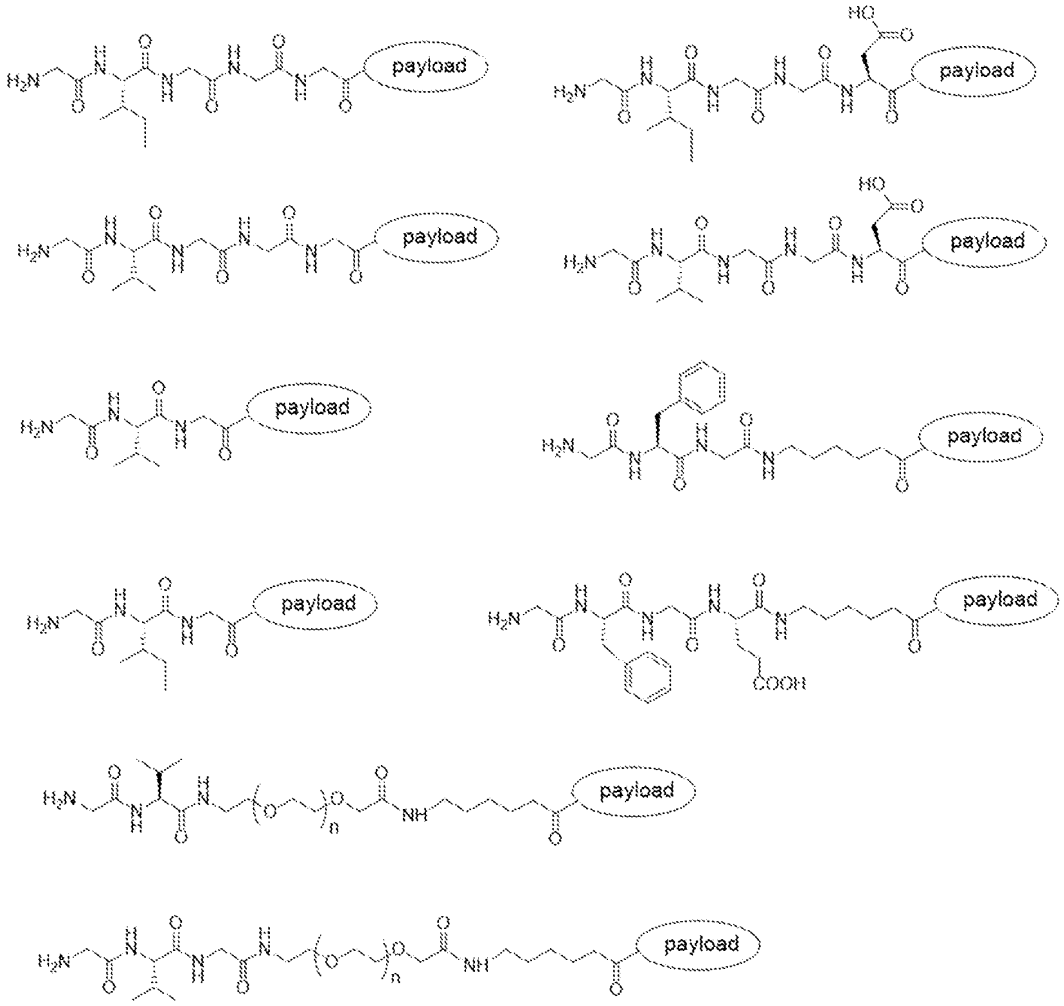

payload
payload
payload
payload
payload
payload
payload
payload
payload
payload
COOH
NH
NH

FIG. 27

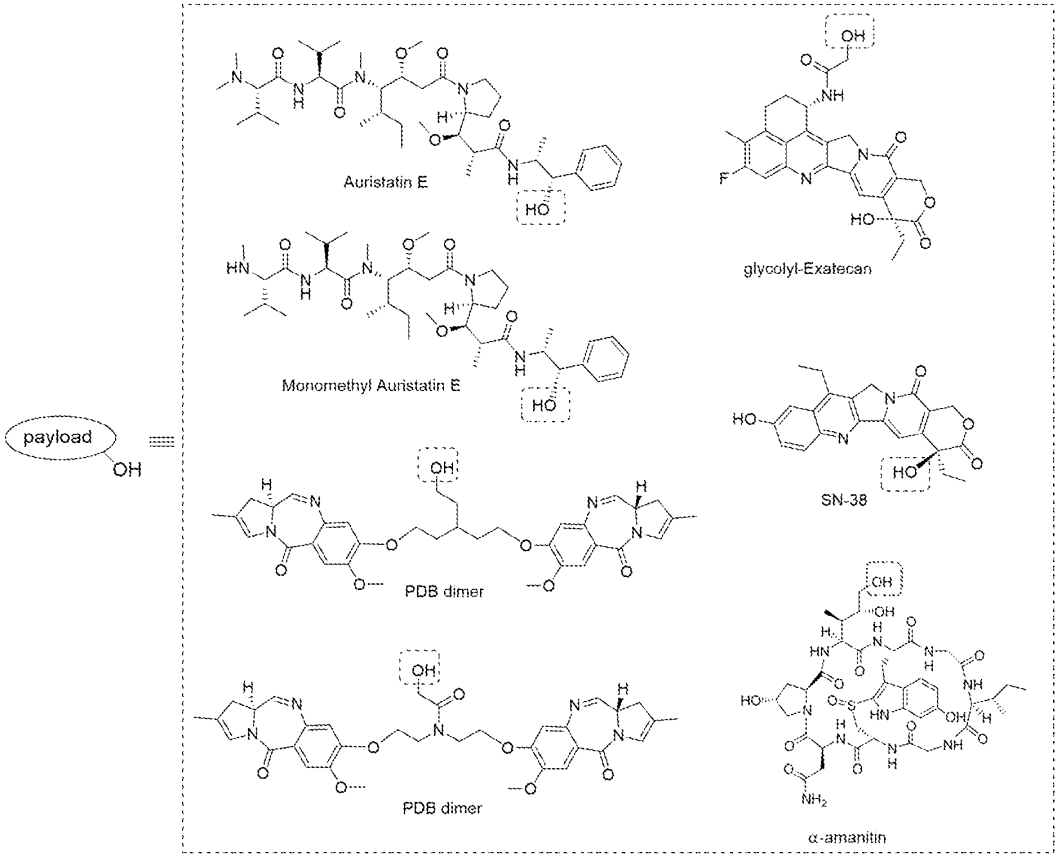

payload
OH
Auristatin E
Monomethyl Auristatin E
PDB dimer
PDB dimer
glycolyl-Exatecan
SN-38
α-amanitin

FIG. 28

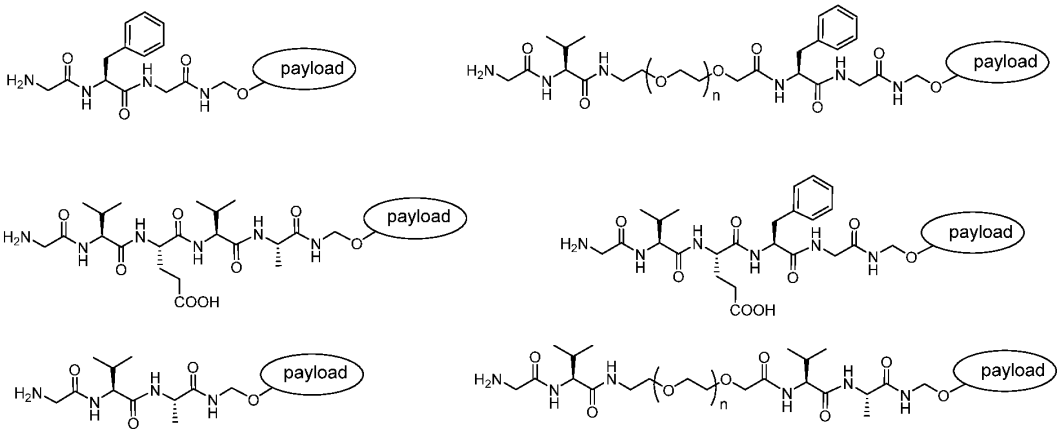

payload
payload
payload
payload
payload
payload
COOH
COOH

FIG. 29

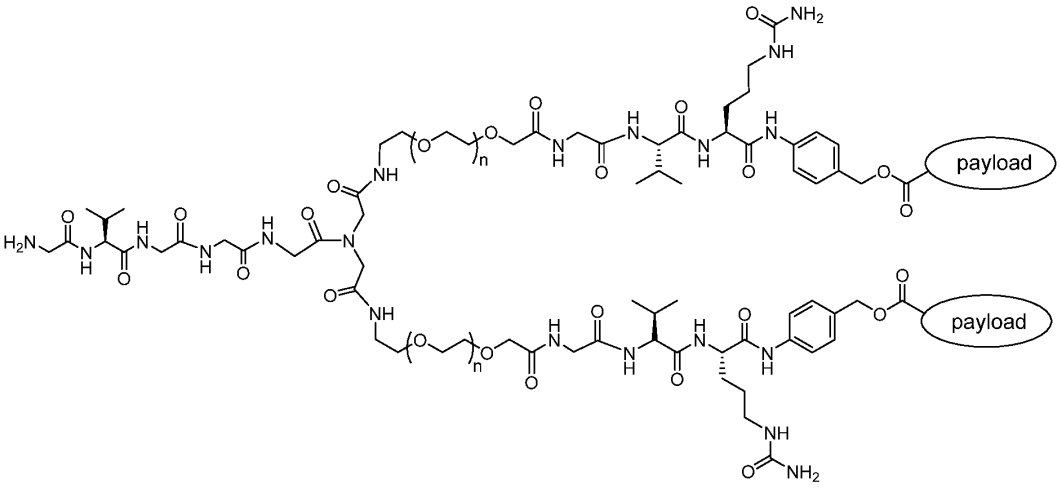


FIG. 30

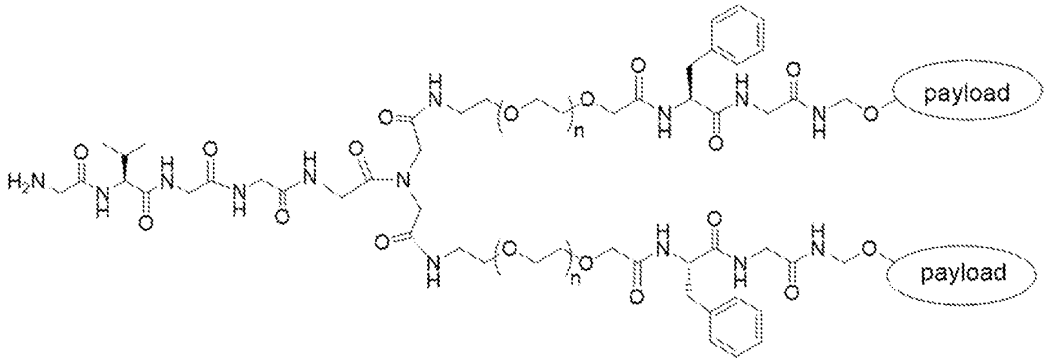


FIG. 31

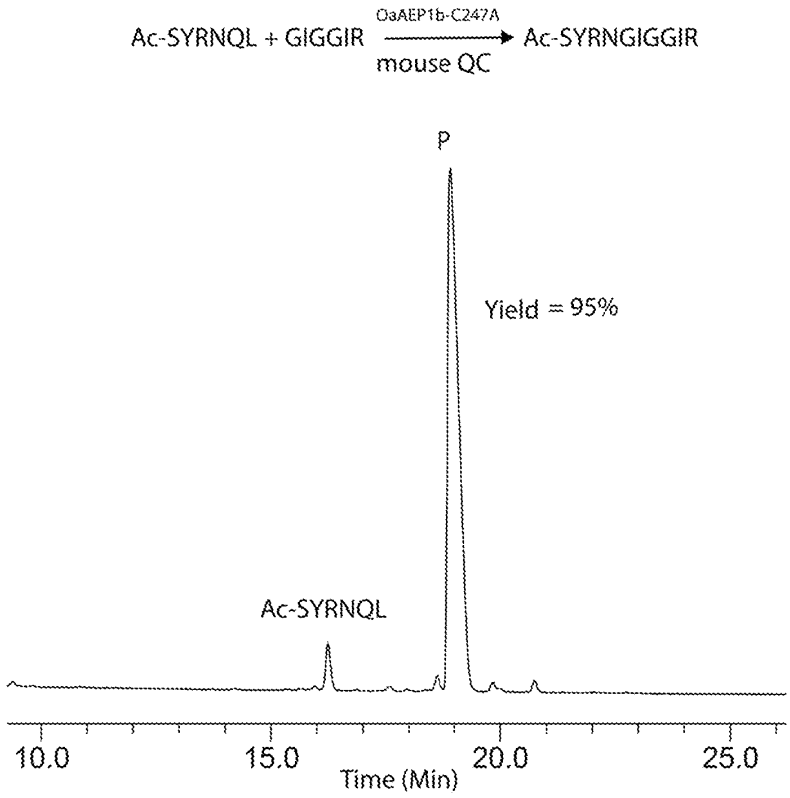
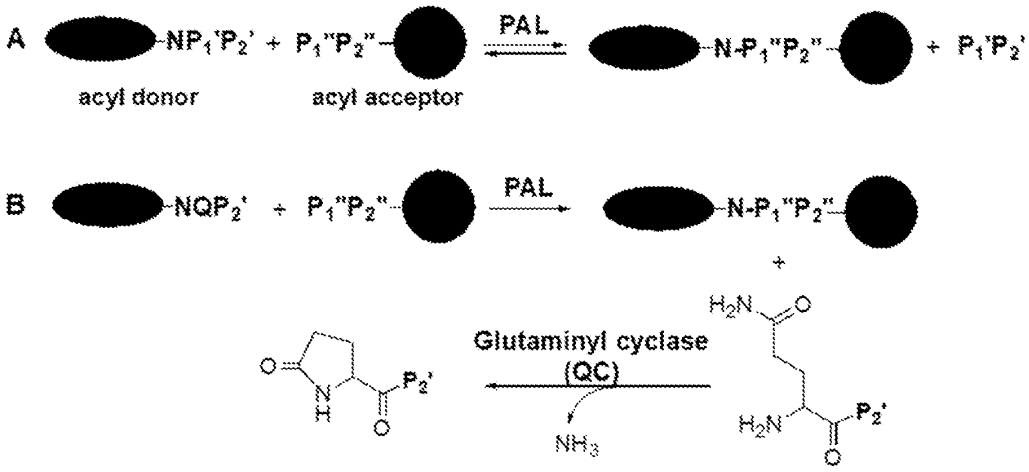

Ac-SYRNQL + GIGGIR
OaAEP1b-C247A
mouse QC
Ac-SYRNGIGGIR
P
Yield = 95%
Ac-SYRNQL
10.0
15.0
20.0
25.0
Time (Min)

FIG. 32


A
NP1'P2'
P1"P2"
PAL
N-P1"P2"
P1'P2'
acyl donor
acyl acceptor
B
NQP2'
P1"P2"
PAL
N-P1"P2"
Glutaminyl cyclase
(QC)
NH3