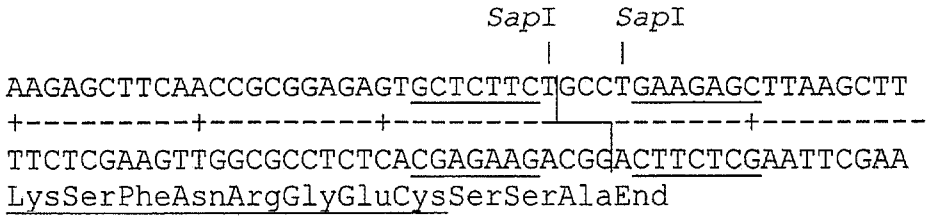
Figure 1

```
gccGCTCCAGCTGCACCTGCTCCAGCAGCACCTGCTGCACCAGCTCCGGCTGCTCCTGCT
   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   CGAGGTCGACGTGGACGAGGTCGTCGTGGACGACGTGGTCGAGGCCGACGAGGACGAcgg
AlaAlaProAlaAlaProAlaProAlaAlaProAlaAlaProAlaProAlaAlaProAla
```

Figure 2

A



B

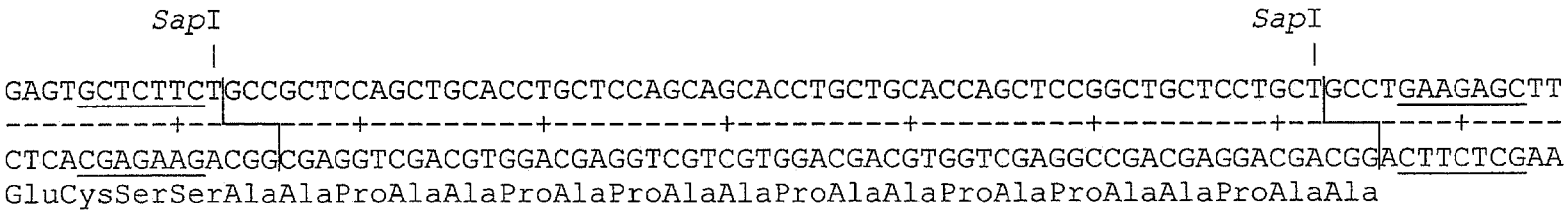


Figure 2 cont.
C

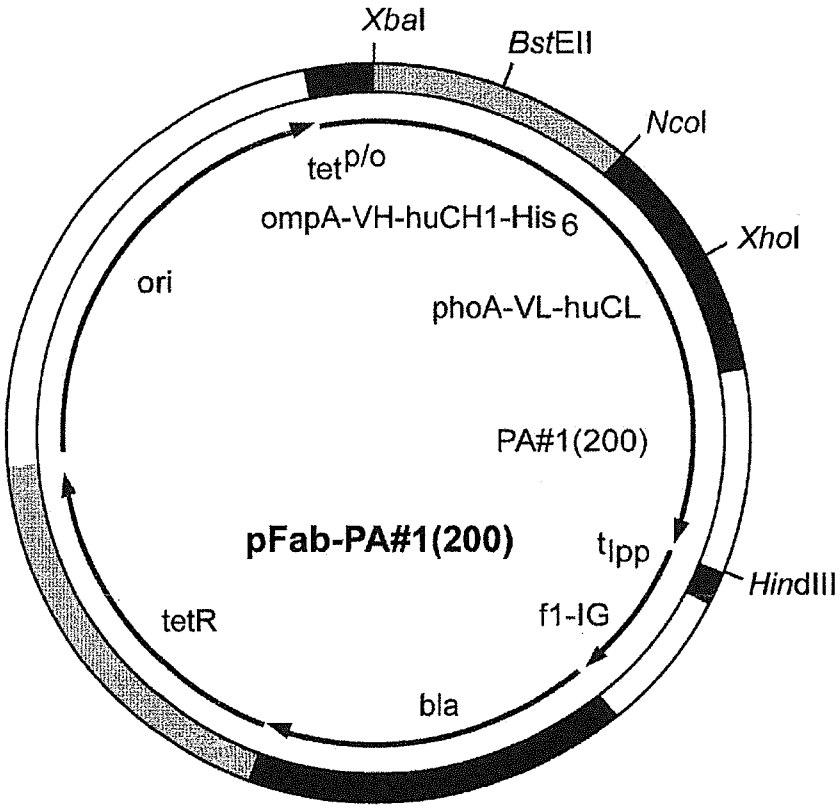


Figure 2 cont.

D

```
                SapI
                 |
GAAAAAGGCGCCAGCTCTTCTGCCTGTGATCTGCCTCAAACCCACAGCCTGGGTAGC...
----+----------+------+----------+----------+----------+--
CTTTTTCCGCGGTCGAGAAGACGGACACTAGACGGAGTTTGGGTGTCGGACCCATCG...
GluLysGlyAlaSerSerSerAlaCysAspLeuProGlnThrHisSerLeuGlySer...
                    +1
```

E

```
                SapI
                 |
GAAAAAGGCGCCAGCTCTTCTGCCGCTCCAGCTGCACCTGCTCCAGCAGCACCTGCTGCACCAGCTCCGGCTGCTCCTGCCTGTGAT...
----------+-------+-----+----------+----------+----------+----------+----------+----------+
CTTTTTCCGCGGTCGAGAAGACGGCGAGGTCGACGTGGACGAGGTCGTCGTGGACGACGTGGTCGAGGCCGACGAGGACGACGGACACTA...
GluLysGlyAlaSerSerSerAlaAlaProAlaProAlaAlaAlaProAlaAlaProAlaAlaAlaCysAsp...
                                                                        (1)
```

Figure 2 cont.
F

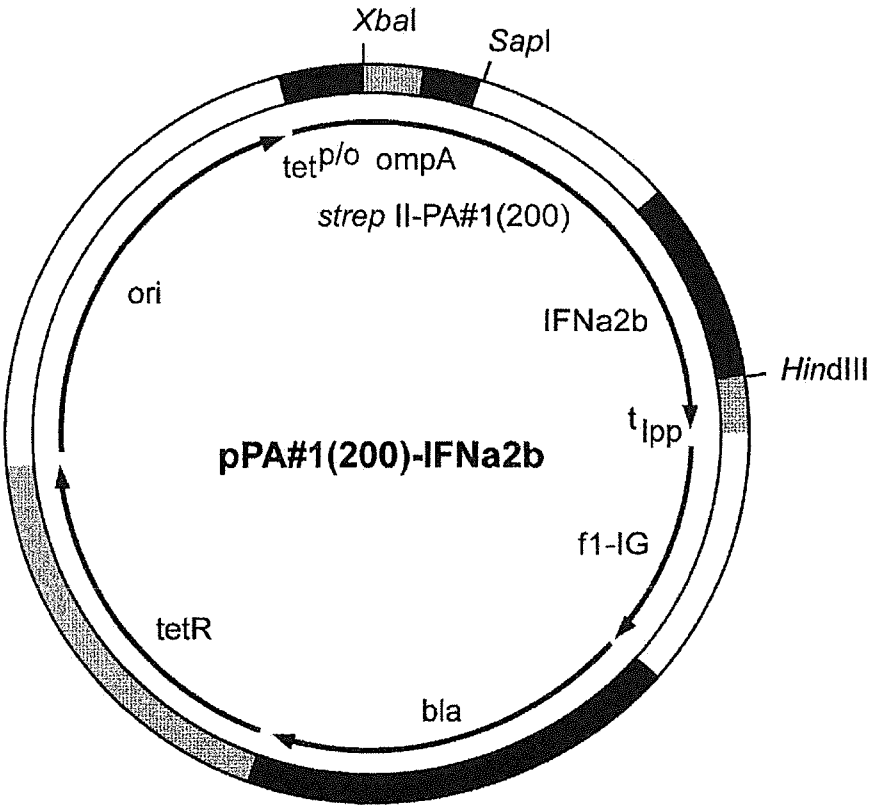
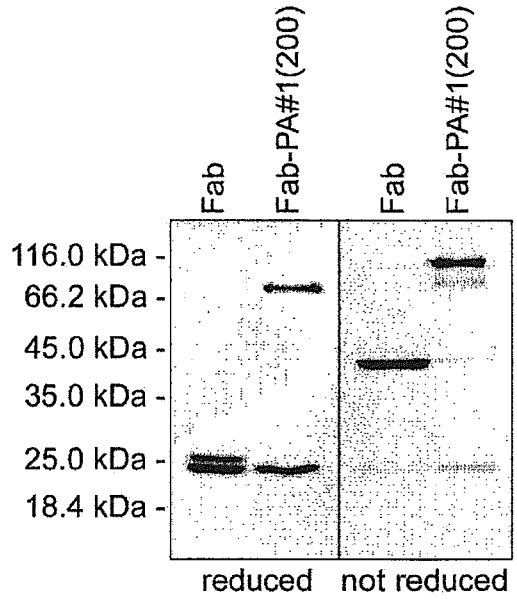


Figure 3

A


Fab
Fab-PA#1(200)
Fab
Fab-PA#1(200)
116.0 kDa -
66.2 kDa -
45.0 kDa -
35.0 kDa -
25.0 kDa -
18.4 kDa -
reduced
not reduced

B

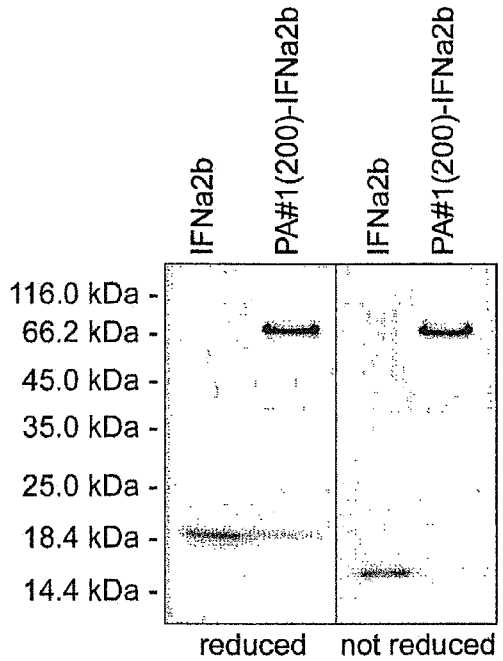
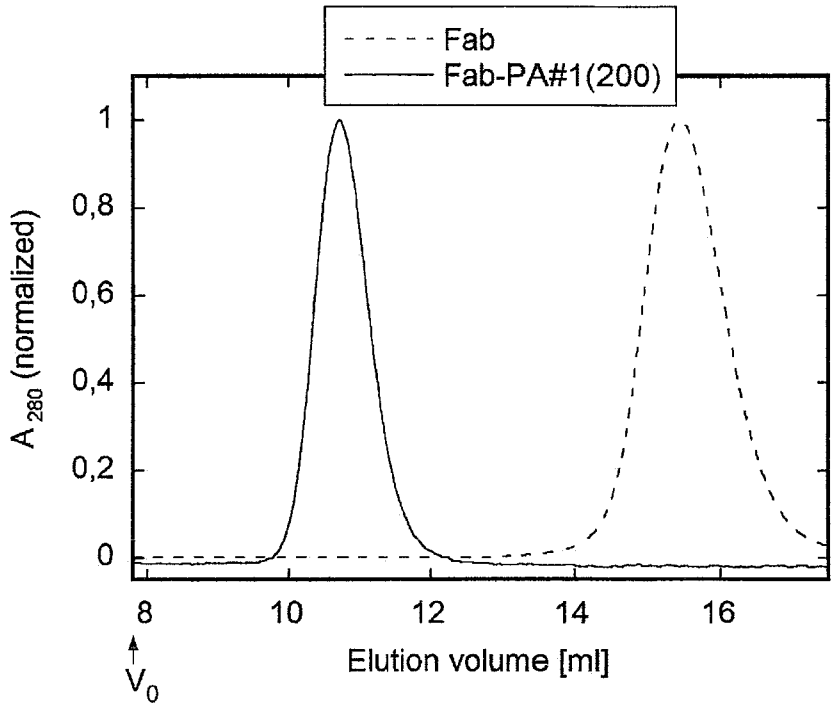

IFNa2b
PA#1(200)-IFNa2b
IFNa2b
PA#1(200)-IFNa2b
116.0 kDa -
66.2 kDa -
45.0 kDa -
35.0 kDa -
25.0 kDa -
18.4 kDa -
14.4 kDa -
reduced
not reduced

Figure 4

A


Fab
Fab-PA#1(200)
A280 (normalized)
1
0,8
0,6
0,4
0,2
0
8
10
12
14
16
V0
Elution volume [ml]

B

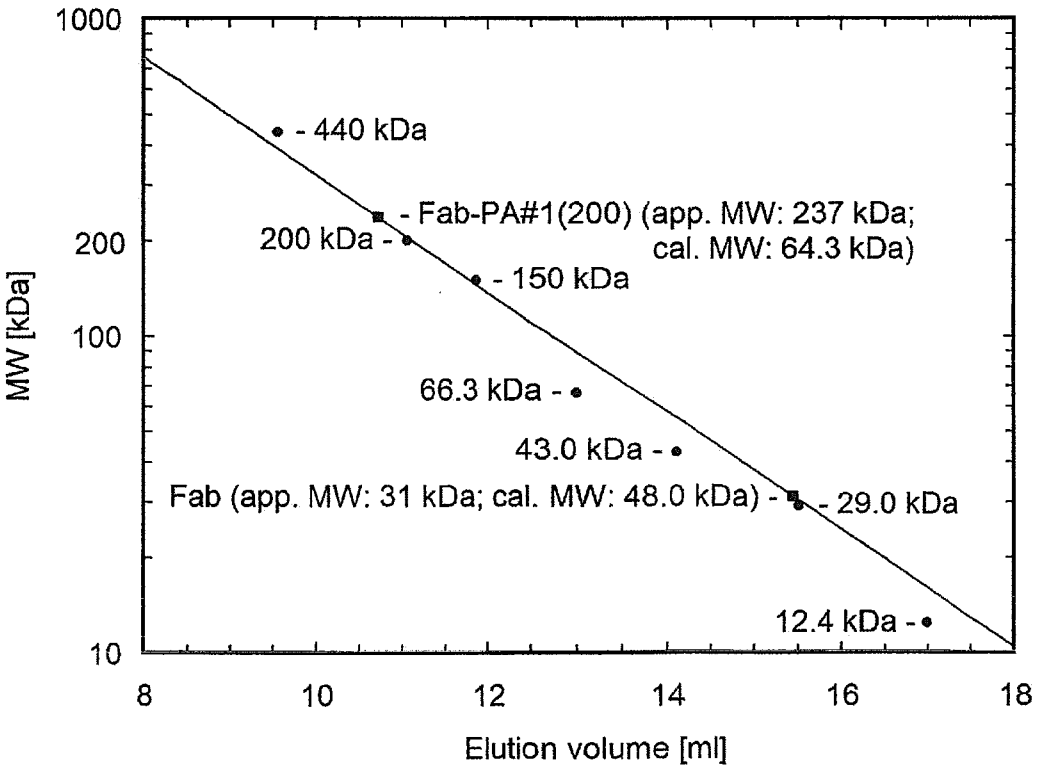
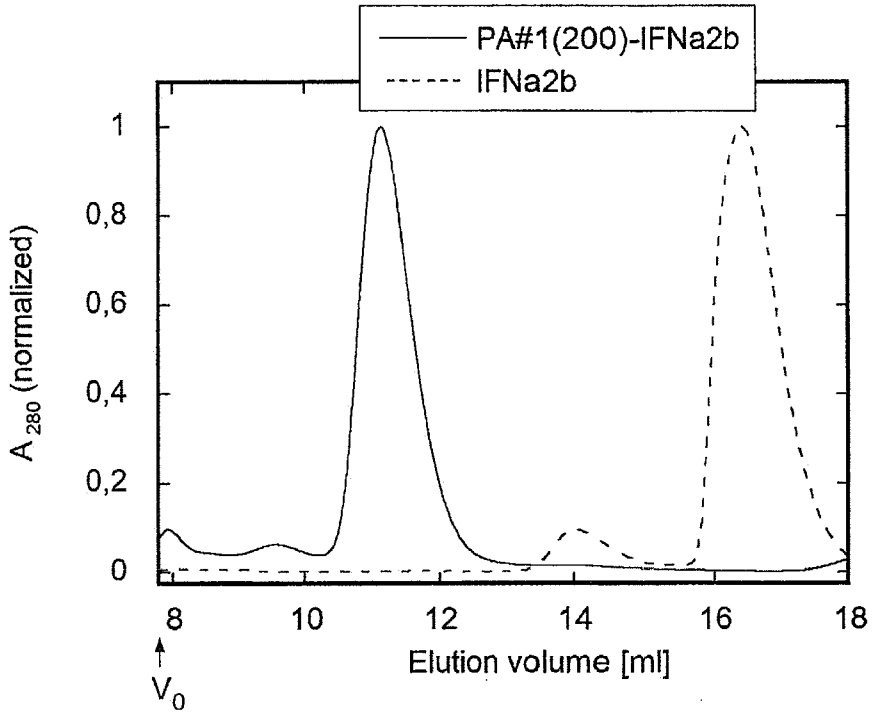

1000
200
100
10
MW [kDa]
- 440 kDa
- Fab-PA#1(200) (app. MW: 237 kDa;
cal. MW: 64.3 kDa)
200 kDa -
- 150 kDa
66.3 kDa -
43.0 kDa -
Fab (app. MW: 31 kDa; cal. MW: 48.0 kDa) -
- 29.0 kDa
12.4 kDa -
8
10
12
14
16
18
Elution volume [ml]

Figure 4 cont.

C



D

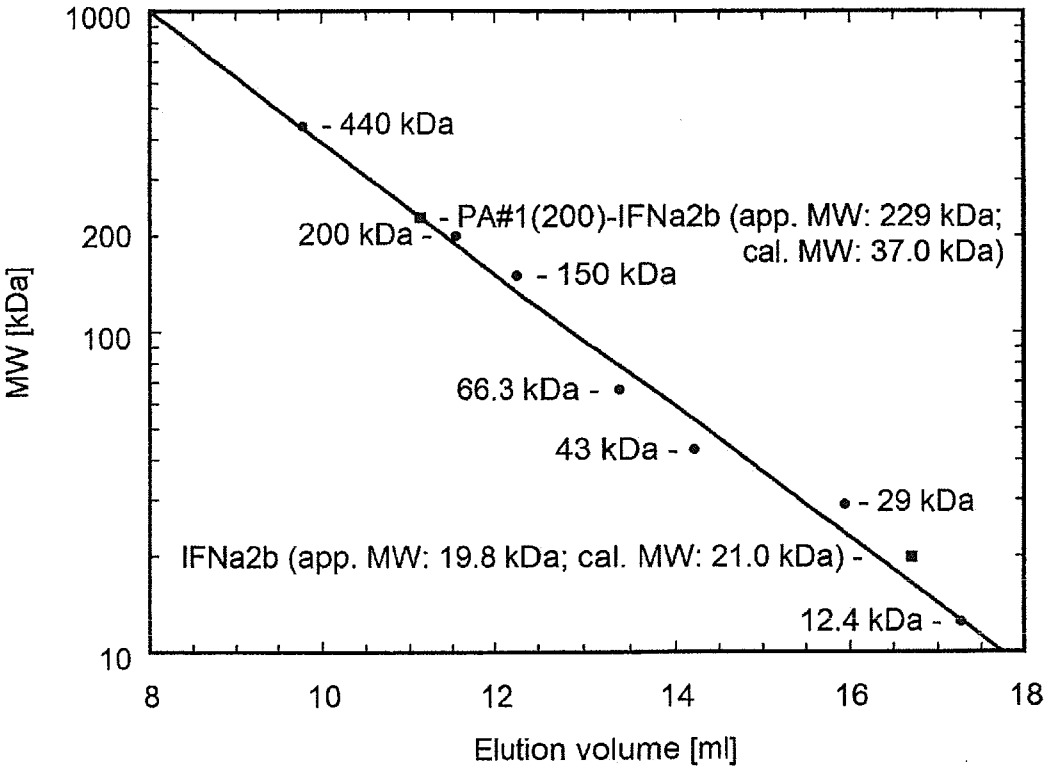
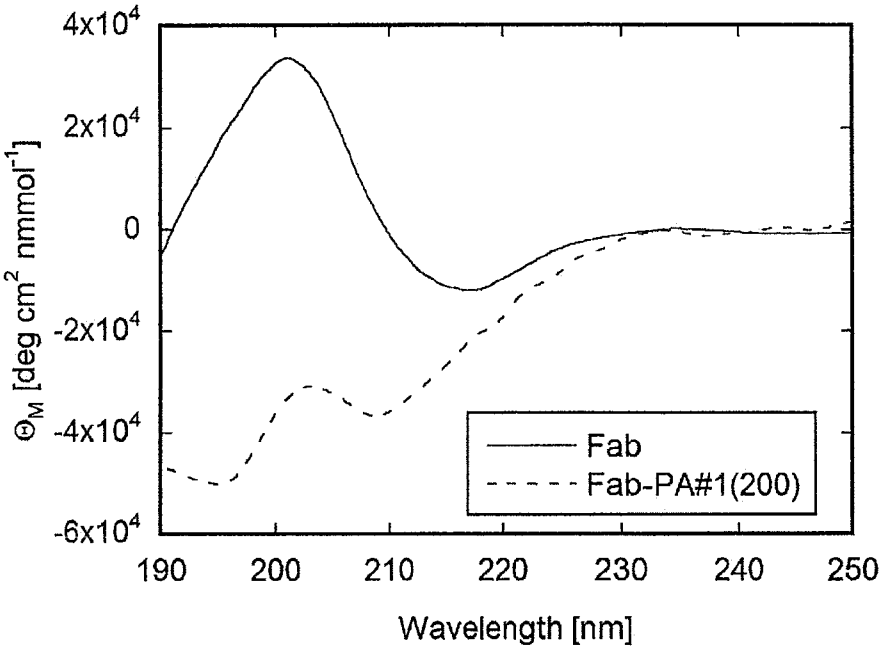


Figure 5

A


4x10^4
2x10^4
0
-2x10^4
-4x10^4
-6x10^4
Θ_M [deg cm^2 nmmol^-1]
190
200
210
220
230
240
250
Wavelength [nm]
Fab
Fab-PA#1(200)

B

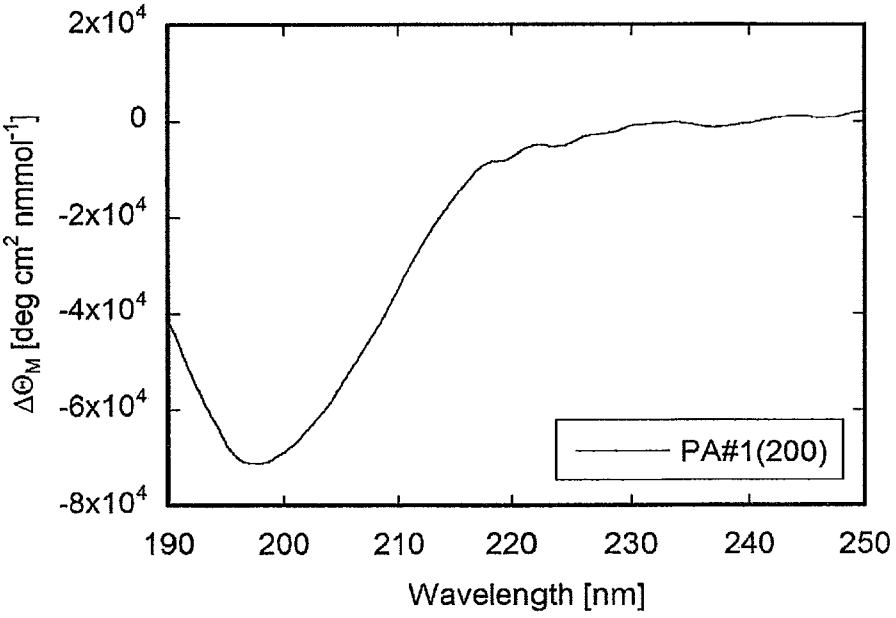
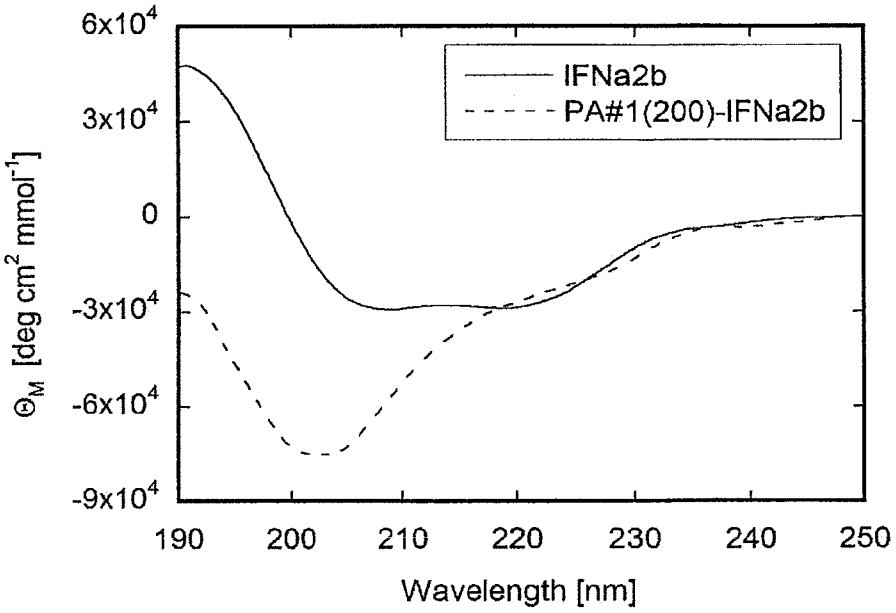

2x10^4
0
-2x10^4
-4x10^4
-6x10^4
-8x10^4
ΔΘ_M [deg cm^2 nmmol^-1]
190
200
210
220
230
240
250
Wavelength [nm]
PA#1(200)

Figure 5 cont.

C



D

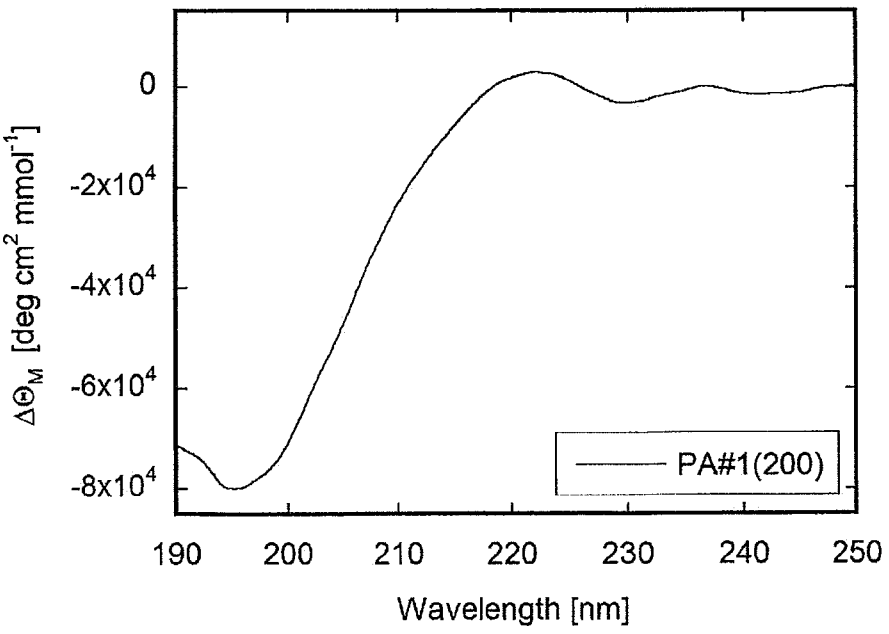


Figure 6

A

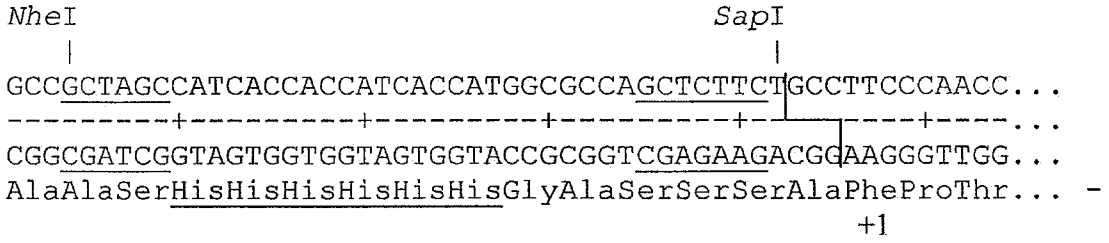

NheI
SapI
GCCGCTAGCCATCACCACCATCACCATGGCGCCAGCTCTTCTGCCTTCCCAACC...
CGGCGATCGGTAGTGGTGGTAGTGGTACCGCGGTCGAGAAGACGGAAGGGTTGG...
AlaAlaSerHisHisHisHisHisHisGlyAlaSerSerSerAlaPheProThr...
+1

Figure 6 cont.

B

```
NheI                                SapI
 |                                   |
GCCGCTAGCCATCACCACCATCACCATGGCGCCAGCTCTTCTGCCGCTCCAGCTGCACCTGCTCCAGCACCTGCTGCACCAGCTCCG
----------+----------+----------+----------+----------+----------+----------+----------+
CGGCGATCGGTAGTGGTGGTAGTGGTACCGCGGTCGAGAAGACGGCGAGGTCGACGTGGACGAGGTCGTCGTGGACGACGTGGTCGAGGC
AlaAlaSerHisHisHisHisHisGlyAlaSerSerAlaAlaProAlaProAlaAlaProAlaAlaProAlaPro

GCTGCTCCTGCTGCCTTCCCAACC...
---------+----------+----...
CGACGAGGACGACGGAAGGGTTGG...
AlaAlaProAlaAlaPheProThr...
        (1)
```

Figure 6 cont.

C

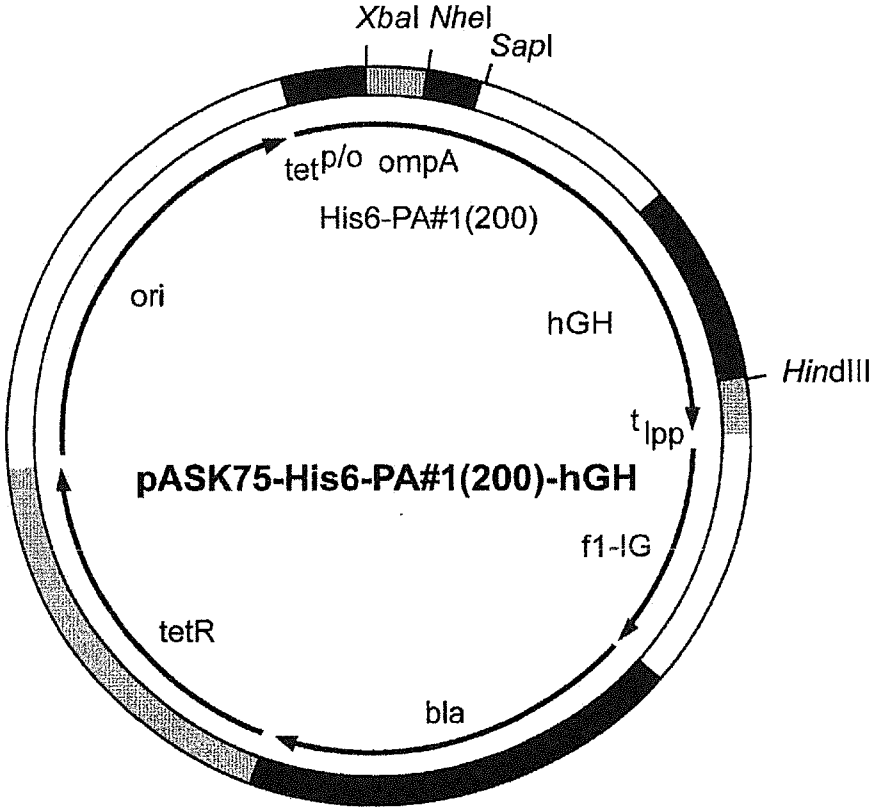


Figure 6 cont.

D

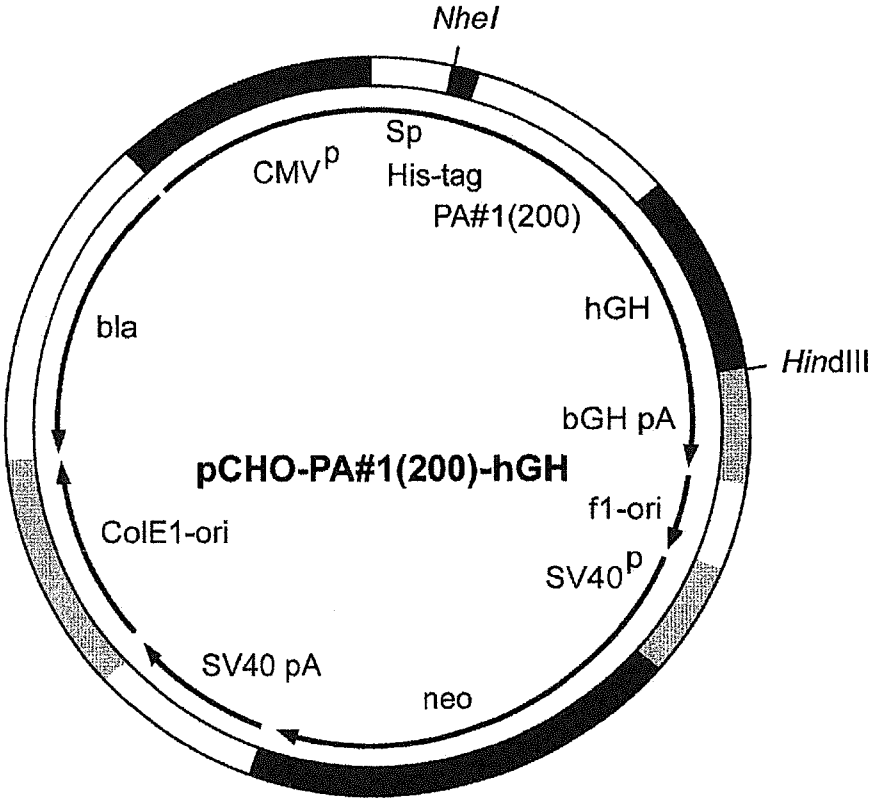


Figure 6 cont.

E

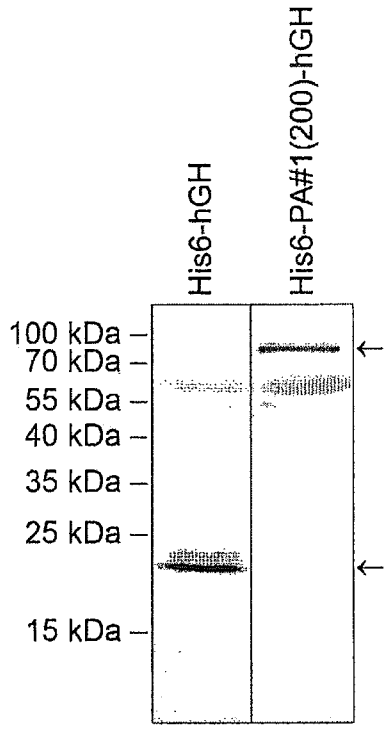


Figure 7

Chou-Fasman plot, 60 aa:



sheet

turns

Residue totals: H: 59 E: 0 T: 0

percent: H: 98.3 E: 0.0 T: 0.0

Figure 8:

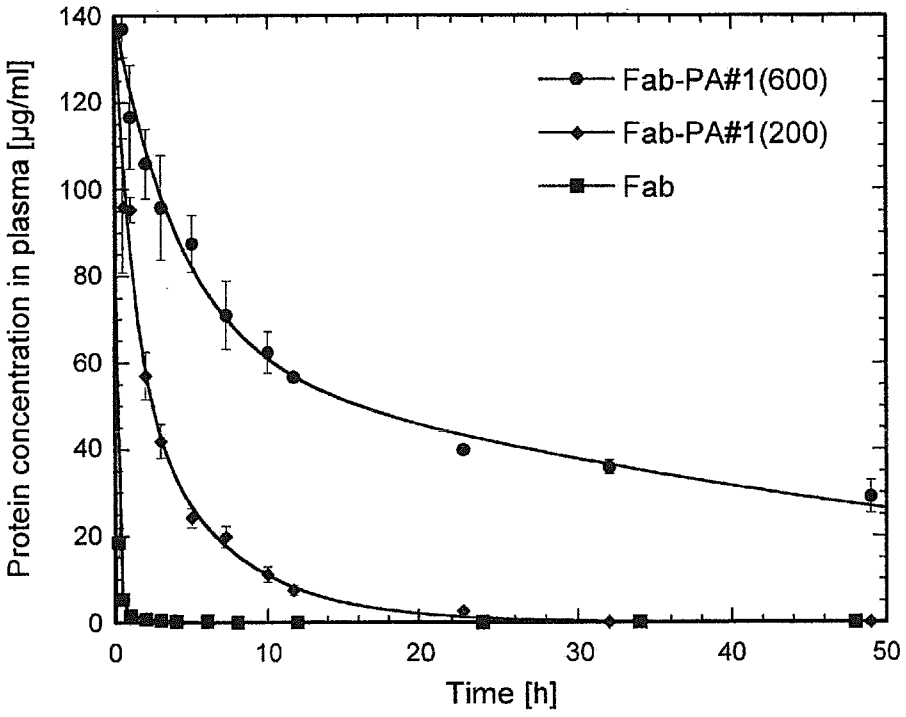

Protein concentration in plasma [µg/ml]
140
120
100
80
60
40
20
0
0
10
20
30
40
50
Time [h]
Fab-PA#1(600)
Fab-PA#1(200)
Fab

Fig. 9

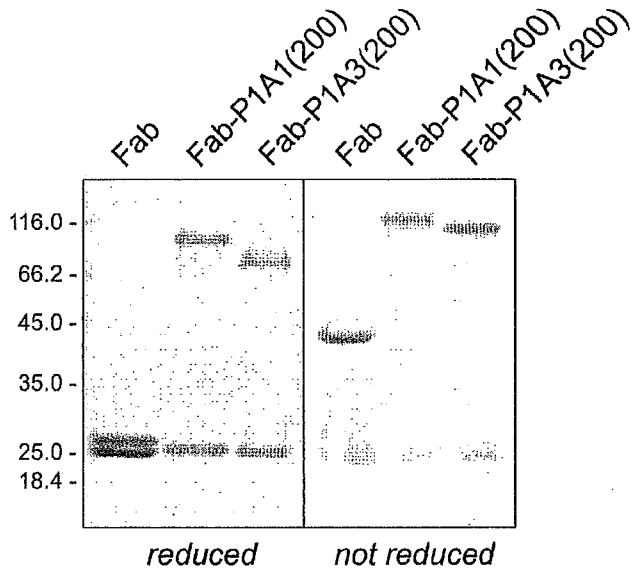

Fab
Fab-P1A1(200)
Fab-P1A3(200)
Fab
Fab-P1A1(200)
Fab-P1A3(200)
116.0
66.2
45.0
35.0
25.0
18.4
reduced
not reduced

Fig. 10

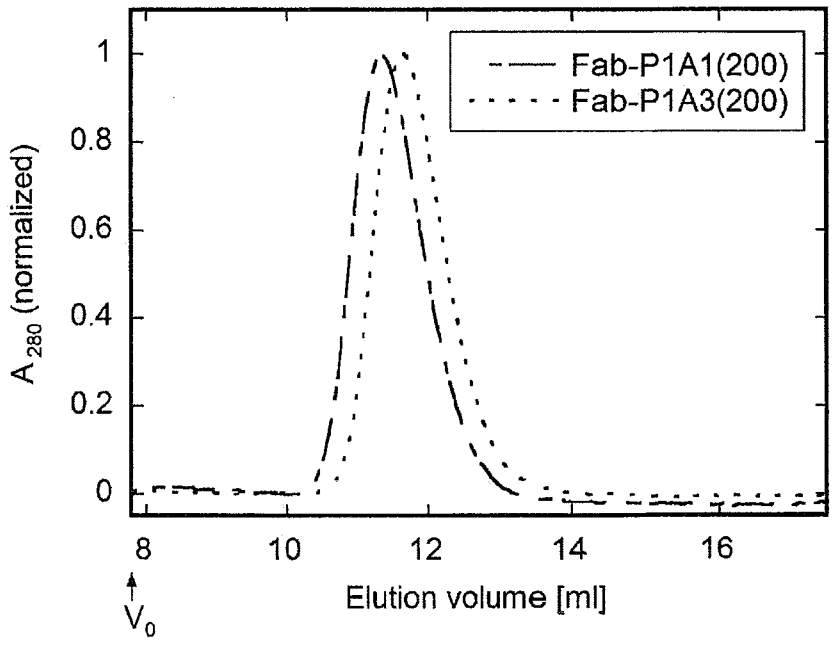
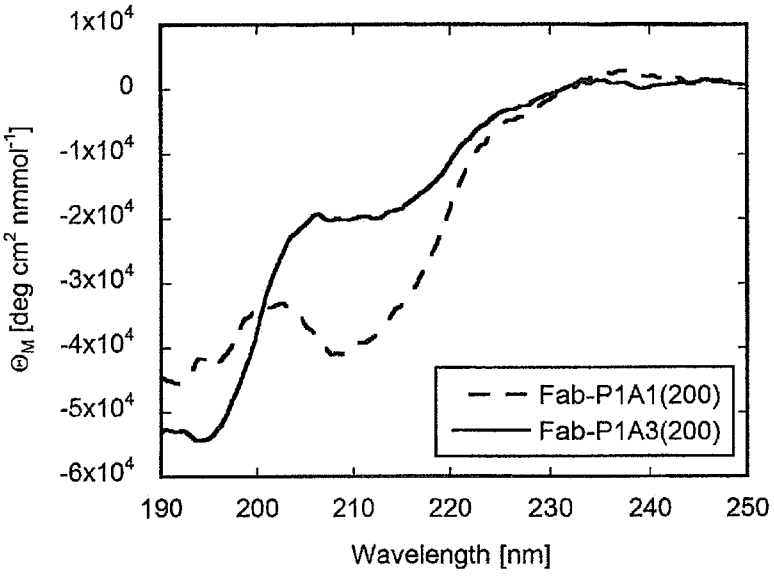

Fab-P1A1(200)
Fab-P1A3(200)
A280 (normalized)
1
0.8
0.6
0.4
0.2
0
8
10
12
14
16
V0
Elution volume [ml]

Fig. 11

A


1x10^4
0
-1x10^4
-2x10^4
-3x10^4
-4x10^4
-5x10^4
-6x10^4
Θ_M [deg cm^2 nmmol^-1]
190
200
210
220
230
240
250
Wavelength [nm]
Fab-P1A1(200)
Fab-P1A3(200)

B

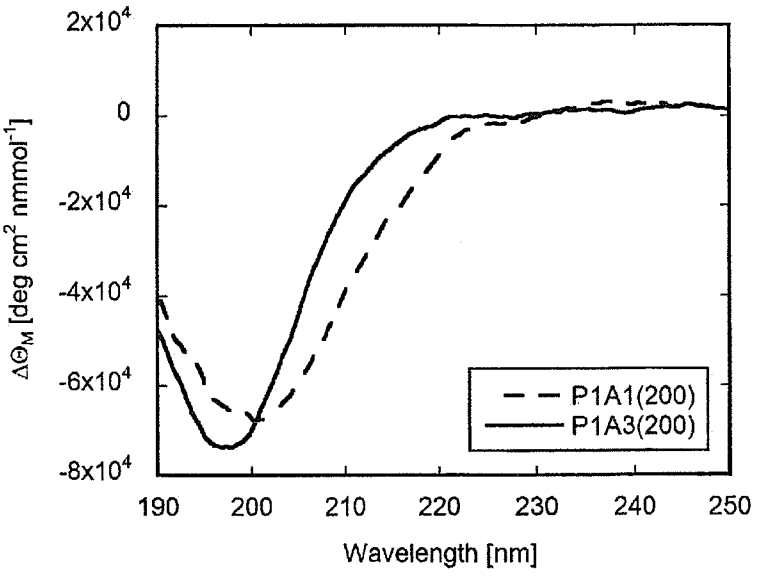
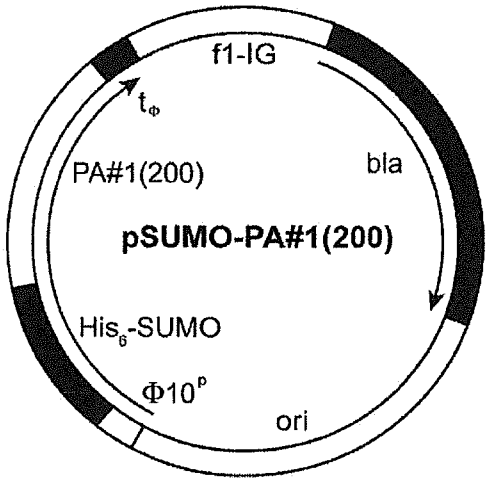
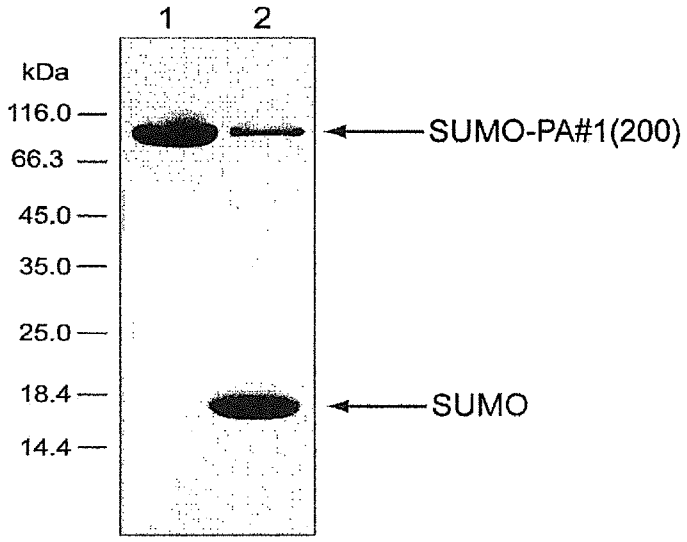

2x10^4
0
-2x10^4
-4x10^4
-6x10^4
-8x10^4
ΔΘ_M [deg cm^2 nmmol^-1]
190
200
210
220
230
240
250
Wavelength [nm]
P1A1(200)
P1A3(200)

Fig. 12

A



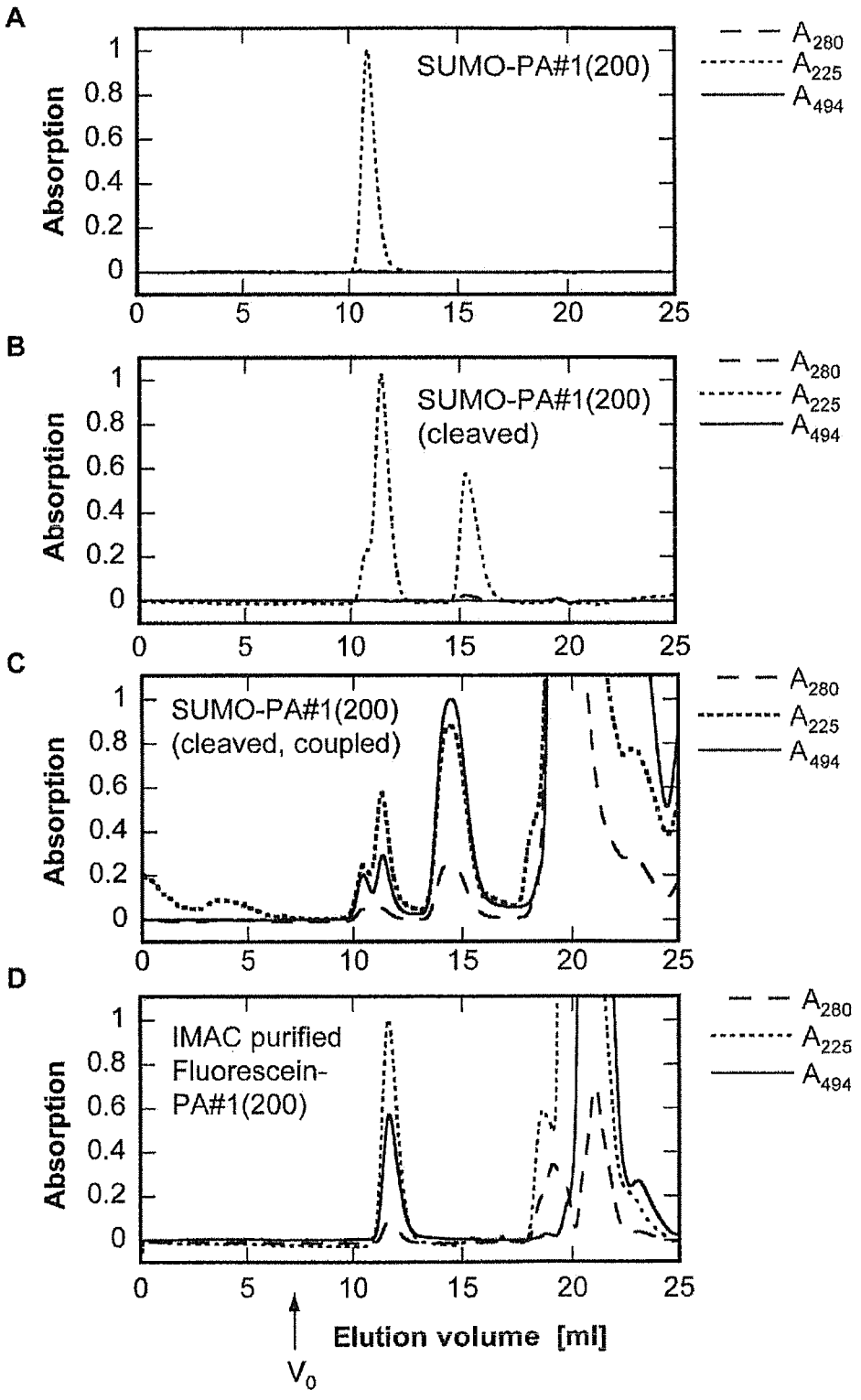
B

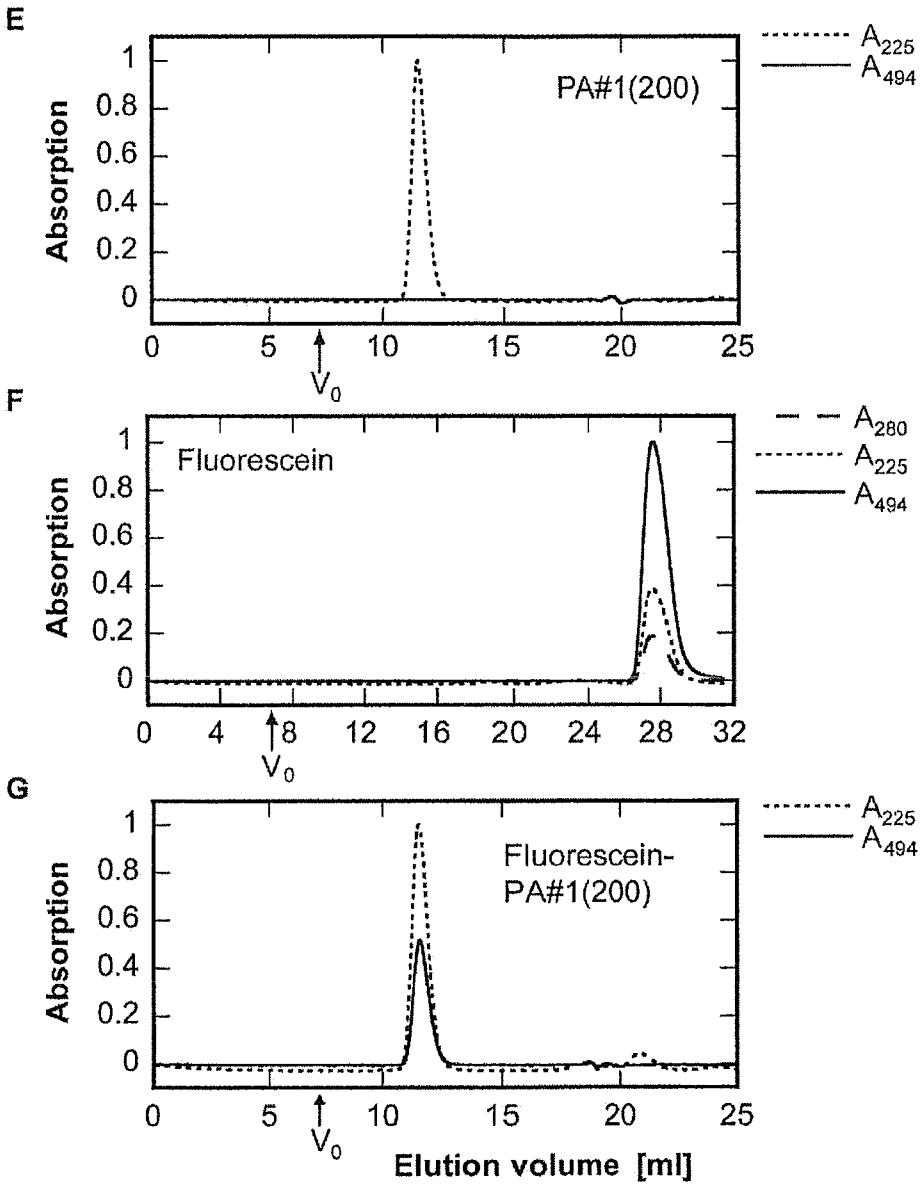


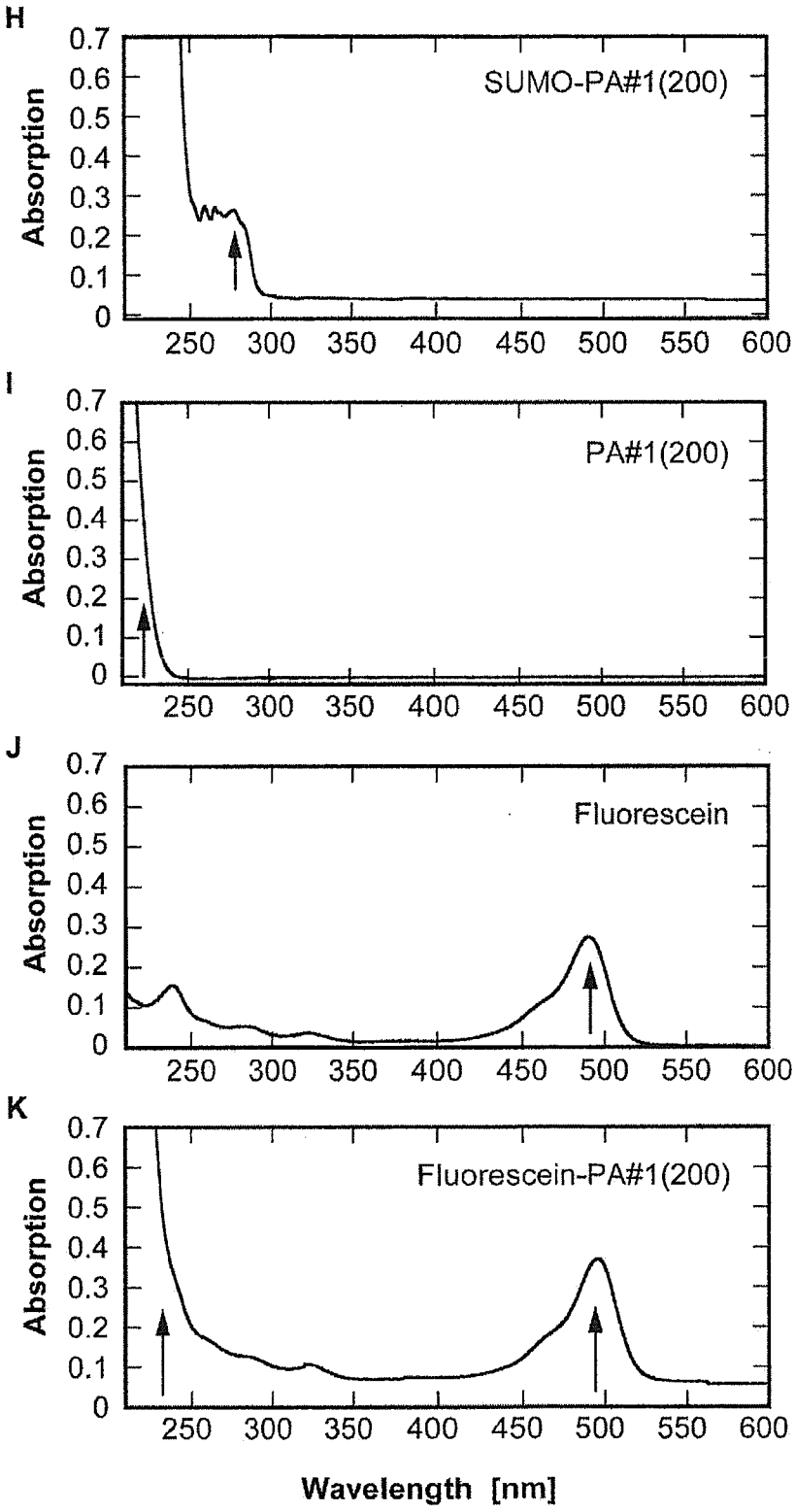
1: SUMO-PA#1(200) prior to cleavage

2: SUMO-PA#1(200) cleaved with SUMO protease

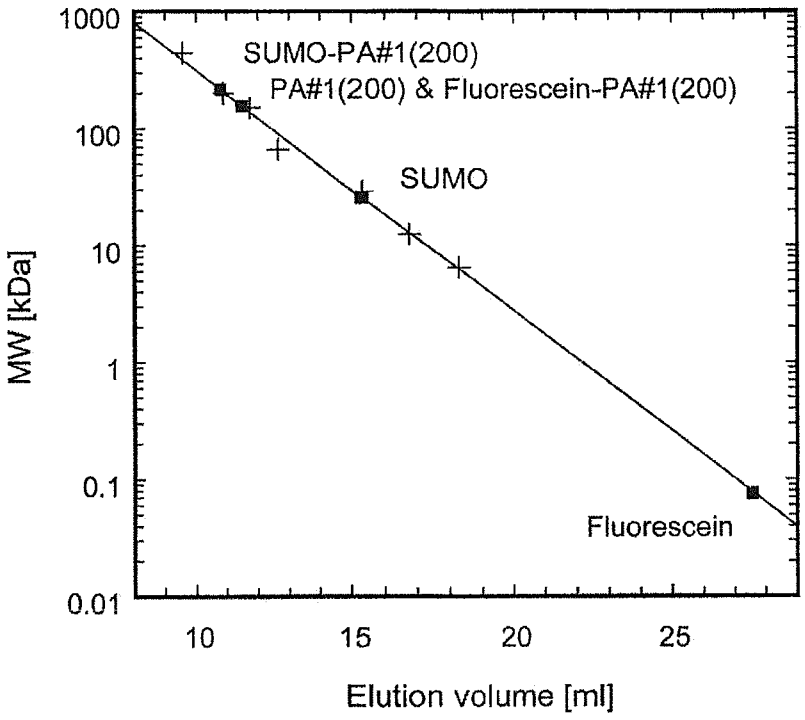
Fig. 13


A
SUMO-PA#1(200)
B
SUMO-PA#1(200)
(cleaved)
C
SUMO-PA#1(200)
(cleaved, coupled)
D
IMAC purified
Fluorescein-
PA#1(200)
Absorption
Elution volume [ml]
V_0
A_{280}
A_{225}
A_{494}


E
Absorption
PA#1(200)
A_{225}
A_{494}
V_0
F
Fluorescein
A_{280}
A_{225}
A_{494}
G
Fluorescein-
PA#1(200)
Elution volume [ml]


H
Absorption
SUMO-PA#1(200)
I
PA#1(200)
J
Fluorescein
K
Fluorescein-PA#1(200)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
250 300 350 400 450 500 550 600
Wavelength [nm]

L


1000
100
10
1
0.1
0.01
MW [kDa]
SUMO-PA#1(200)
PA#1(200) & Fluorescein-PA#1(200)
SUMO
Fluorescein
10
15
20
25
Elution volume [ml]

M

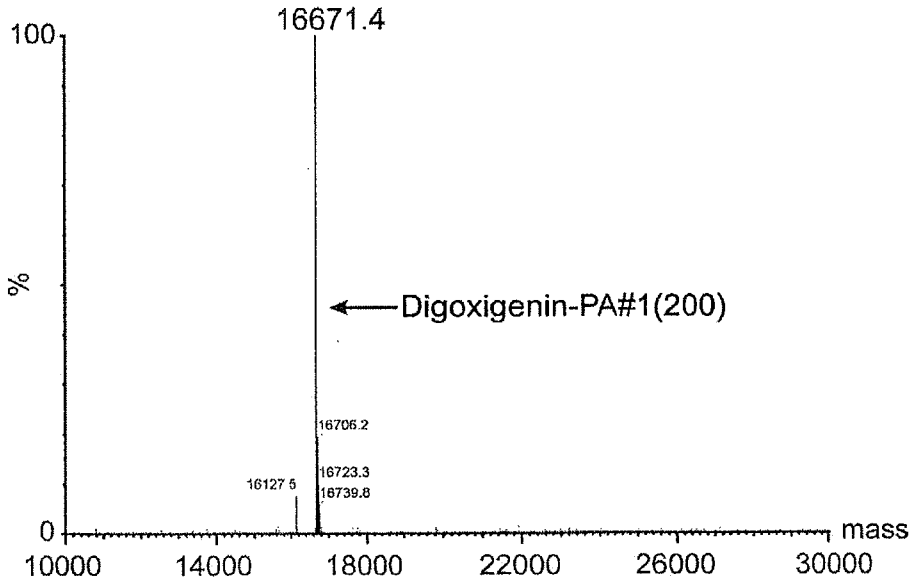
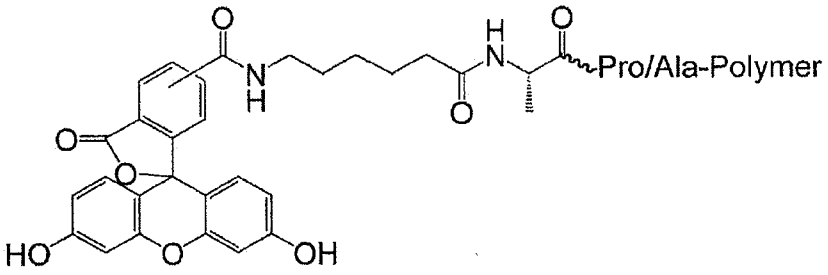
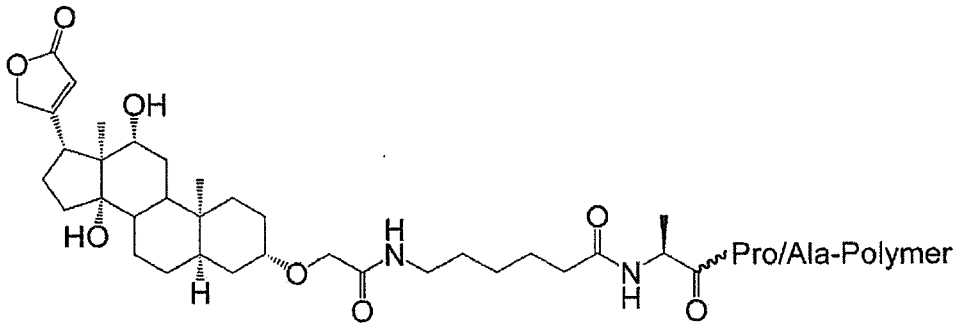

16671.4
100
%
Digoxigenin-PA#1(200)
16706.2
16723.3
16739.8
16127.5
0
mass
10000
14000
18000
22000
26000
30000

Fig. 14

A


O
N
H
H
N
O
O
Pro/Ala-Polymer
O
O
HO
O
OH

B


O
O
OH
HO
H
O
H
N
O
O
N
H
Pro/Ala-Polymer