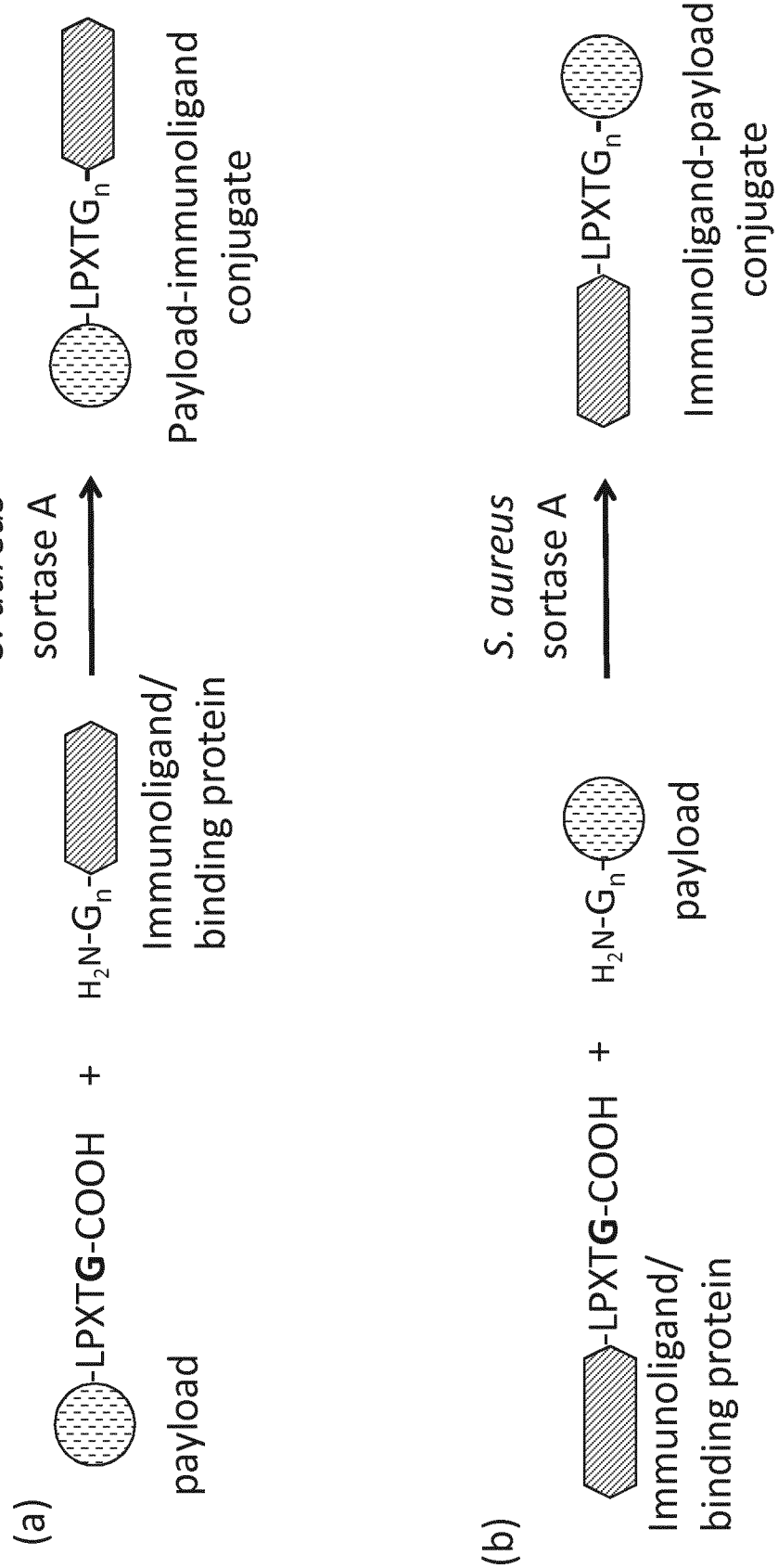

(a)
-LPXTG-COOH
payload
+
H2N-Gn-
Immunoligand/
binding protein
S. aureus
sortase A
-LPXTGn-
Payload-immunoligand
conjugate
(b)
-LPXTG-COOH
Immunoligand/
binding protein
+
H2N-Gn-
payload
S. aureus
sortase A
-LPXTGn-
Immunoligand-payload
conjugate

Fig. 1

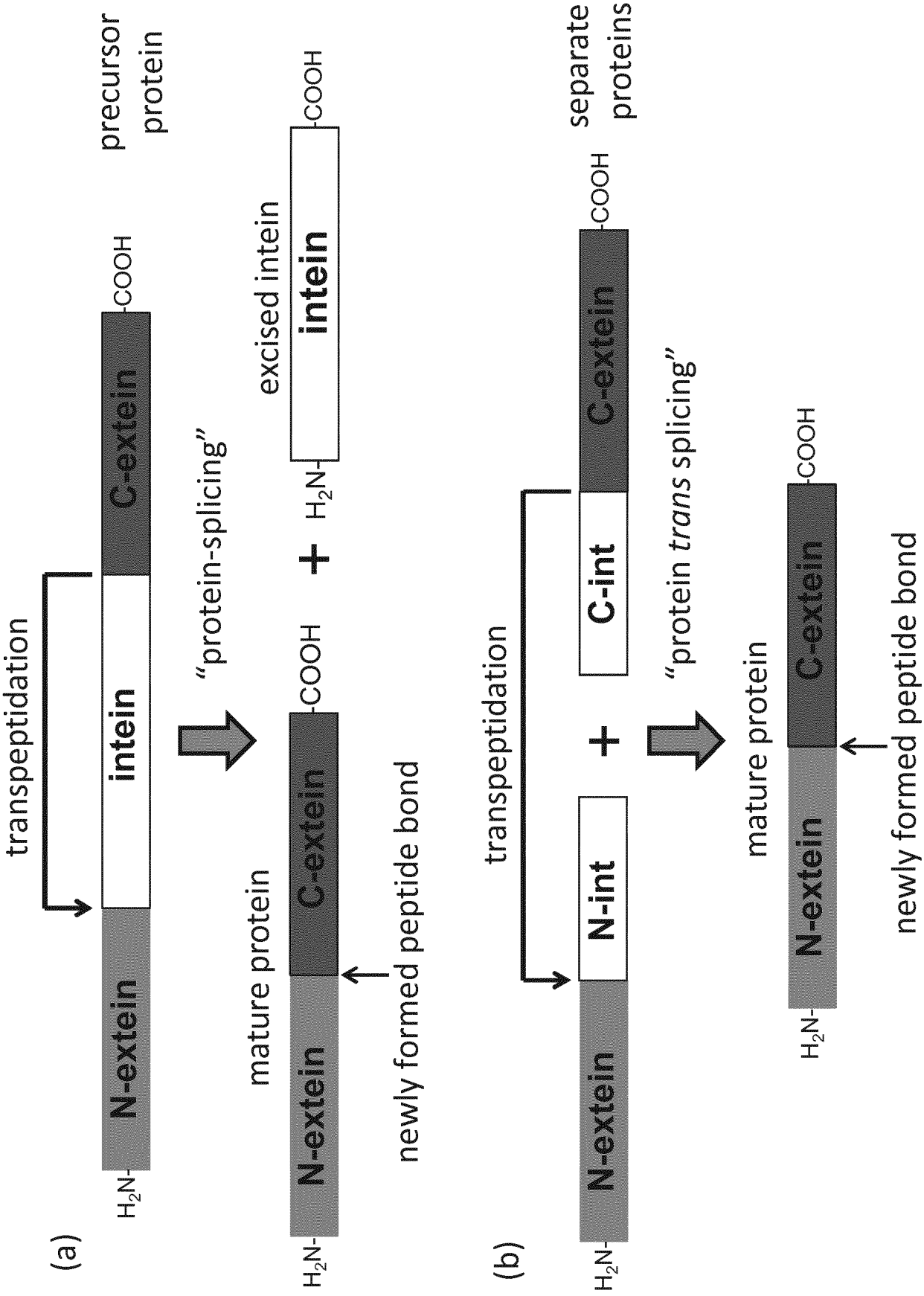

(a)
transpeptidation
precursor protein
intein
N-extein
C-extein
COOH
H2N-
"protein-splicing"
excised intein
intein
COOH
H2N-
+
mature protein
C-extein
N-extein
COOH
H2N-
newly formed peptide bond
(b)
transpeptidation
N-int
N-extein
H2N-
+
C-int
C-extein
COOH
"protein trans splicing"
separate proteins
mature protein
C-extein
N-extein
COOH
H2N-
newly formed peptide bond

Fig. 2

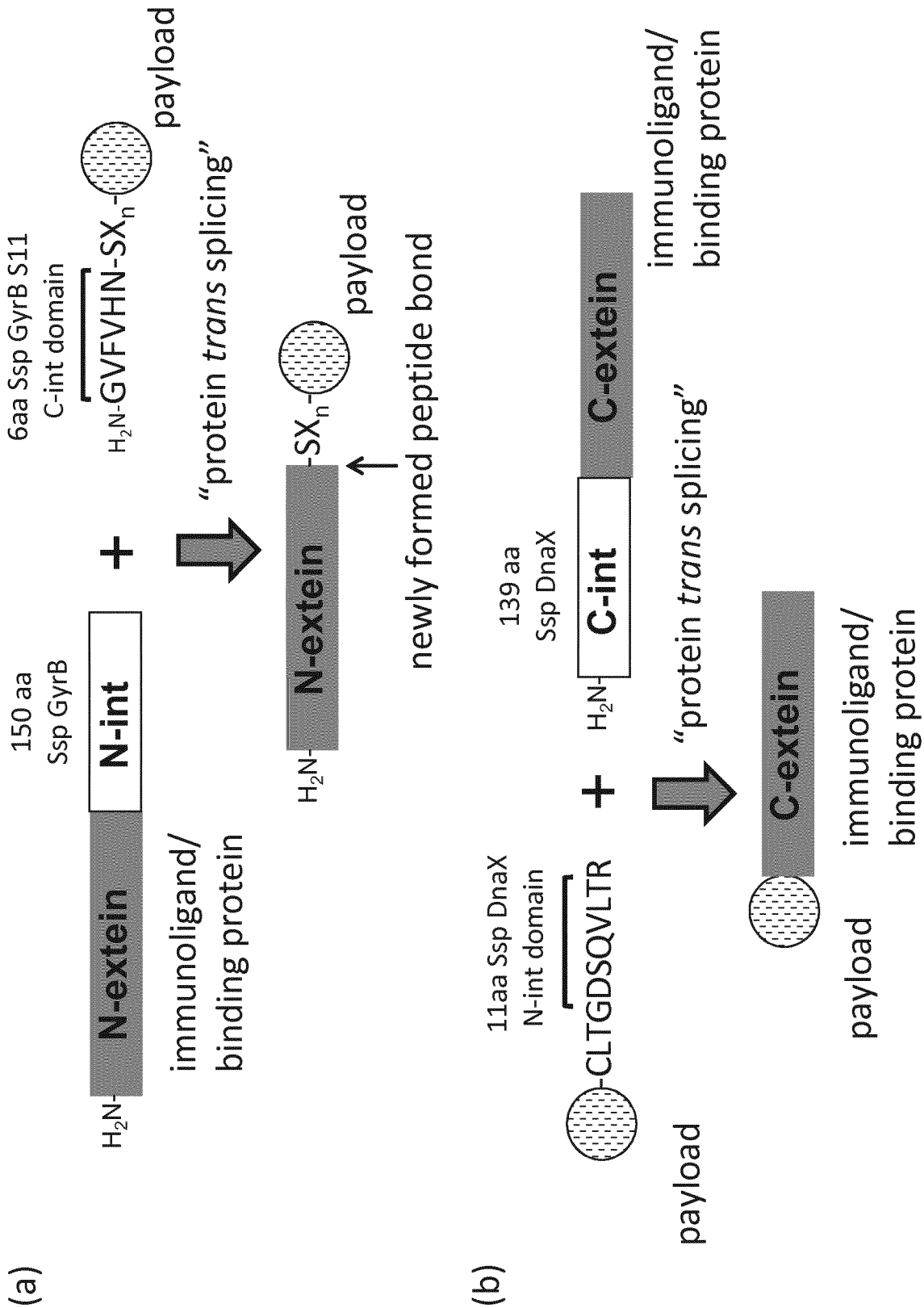

(a)
150 aa
Ssp GyrB
N-int
N-extein
H2N-
immunoligand/
binding protein
+
6aa Ssp GyrB S11
C-int domain
H2N-GVFVHN-SXn-
payload
"protein trans splicing"
N-extein
H2N-
-SXn-
payload
newly formed peptide bond
(b)
11aa Ssp DnaX
N-int domain
-CLTGDSQVLTR
payload
+
139 aa
Ssp DnaX
H2N-
C-int
C-extein
"protein trans splicing"
C-extein
payload
immunoligand/
binding protein
immunoligand/
binding protein

Fig. 3

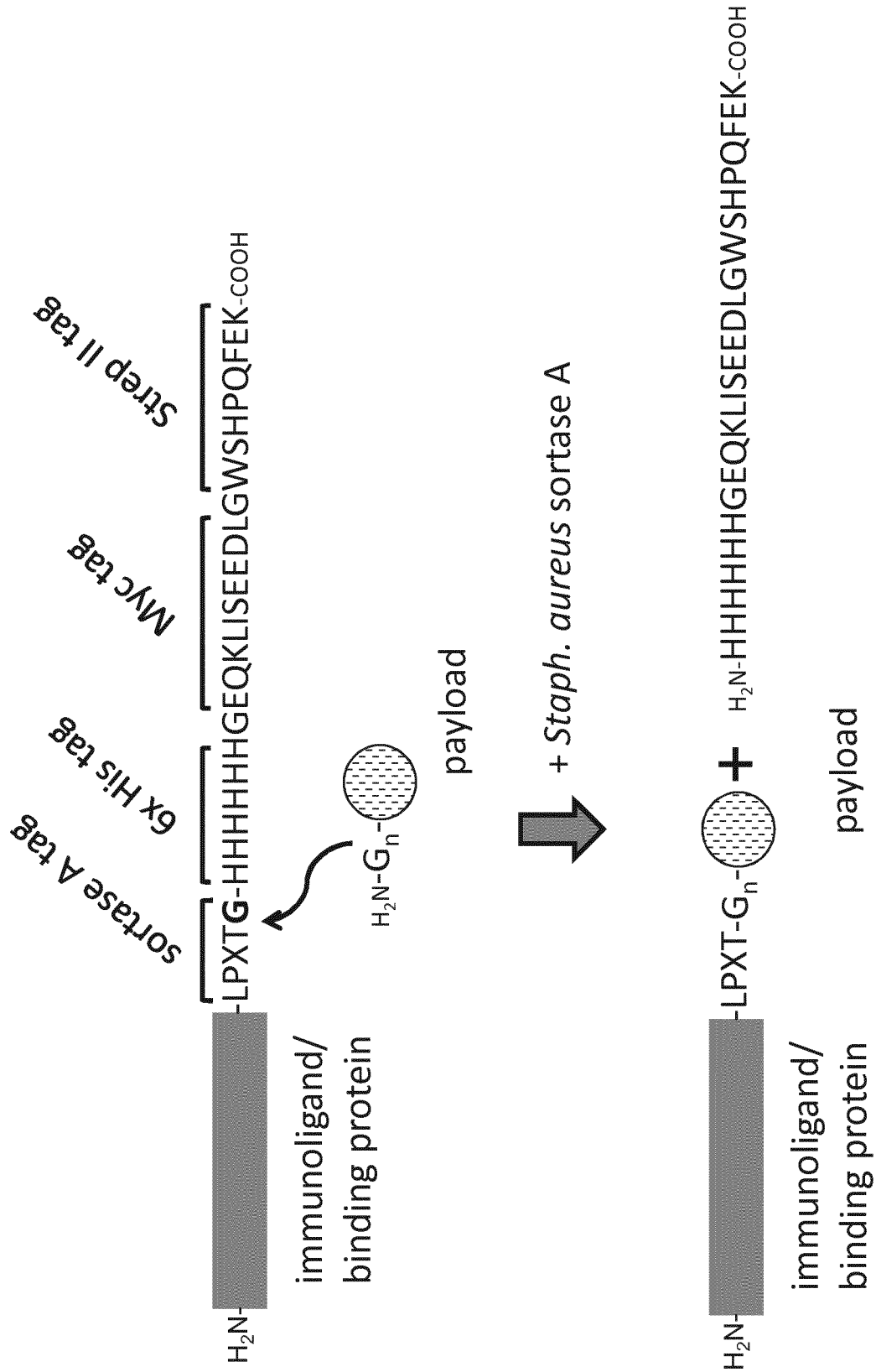

H2N-
immunoligand/
binding protein
-LPXTG-HHHHHHGEQKLISEEDLGWSHPQFEK-COOH
sortase A tag
6x His tag
Myc tag
Strep II tag
H2N-Gn-
payload
+ Staph. aureus sortase A
H2N-
immunoligand/
binding protein
-LPXT-Gn-
+
H2N-HHHHHHGEQKLISEEDLGWSHPQFEK-COOH
payload

Fig. 4 (a)

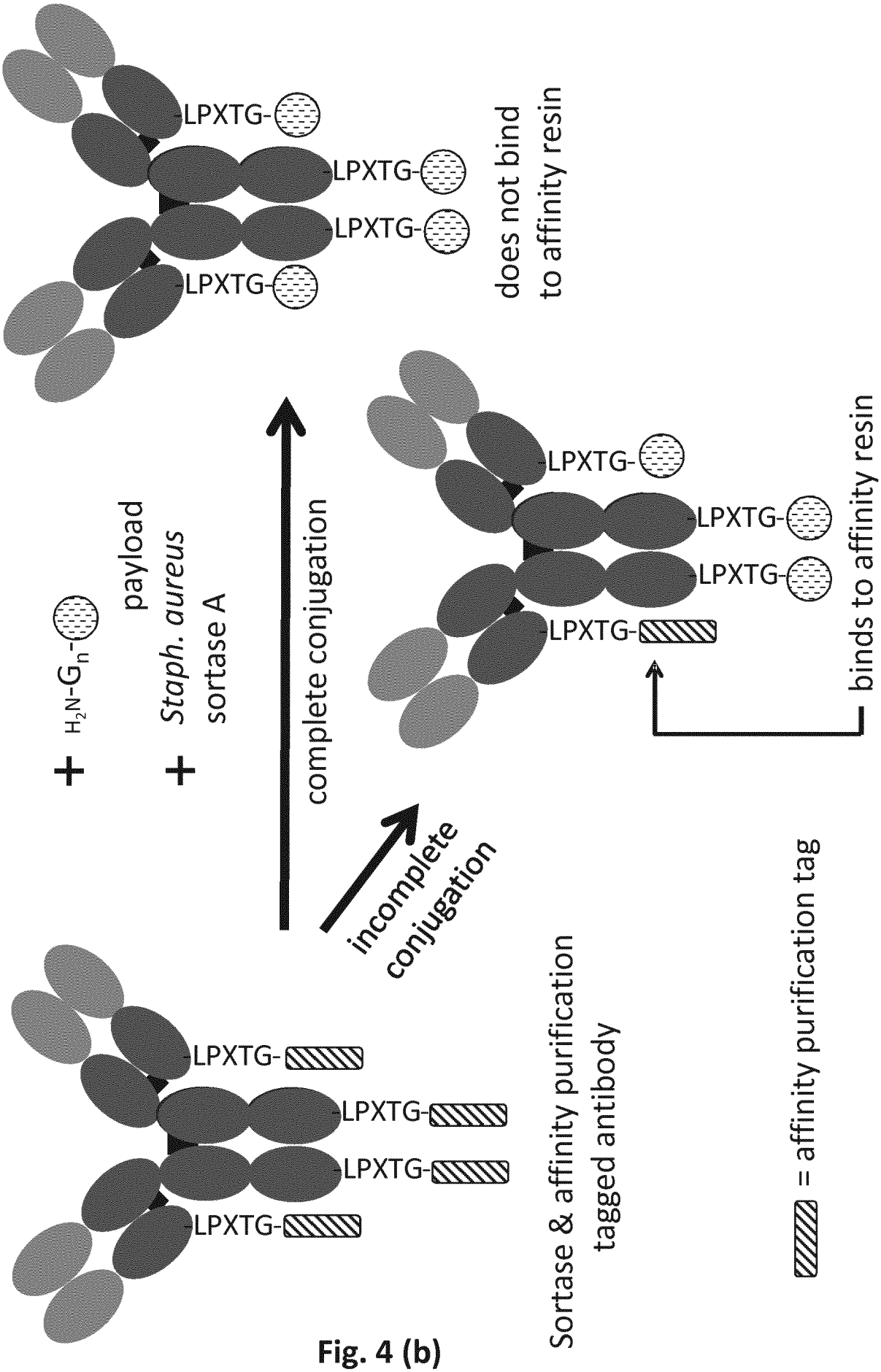

LPXTG-
LPXTG-
LPXTG-
LPXTG-
does not bind
to affinity resin
+ H_2N-G_n-payload
+ *Staph. aureus*
sortase A
complete conjugation
incomplete
conjugation
LPXTG-
LPXTG-
LPXTG-
LPXTG-
LPXTG-
LPXTG-
binds to affinity resin
Sortase & affinity purification
tagged antibody
= affinity purification tag

Fig. 4 (b)

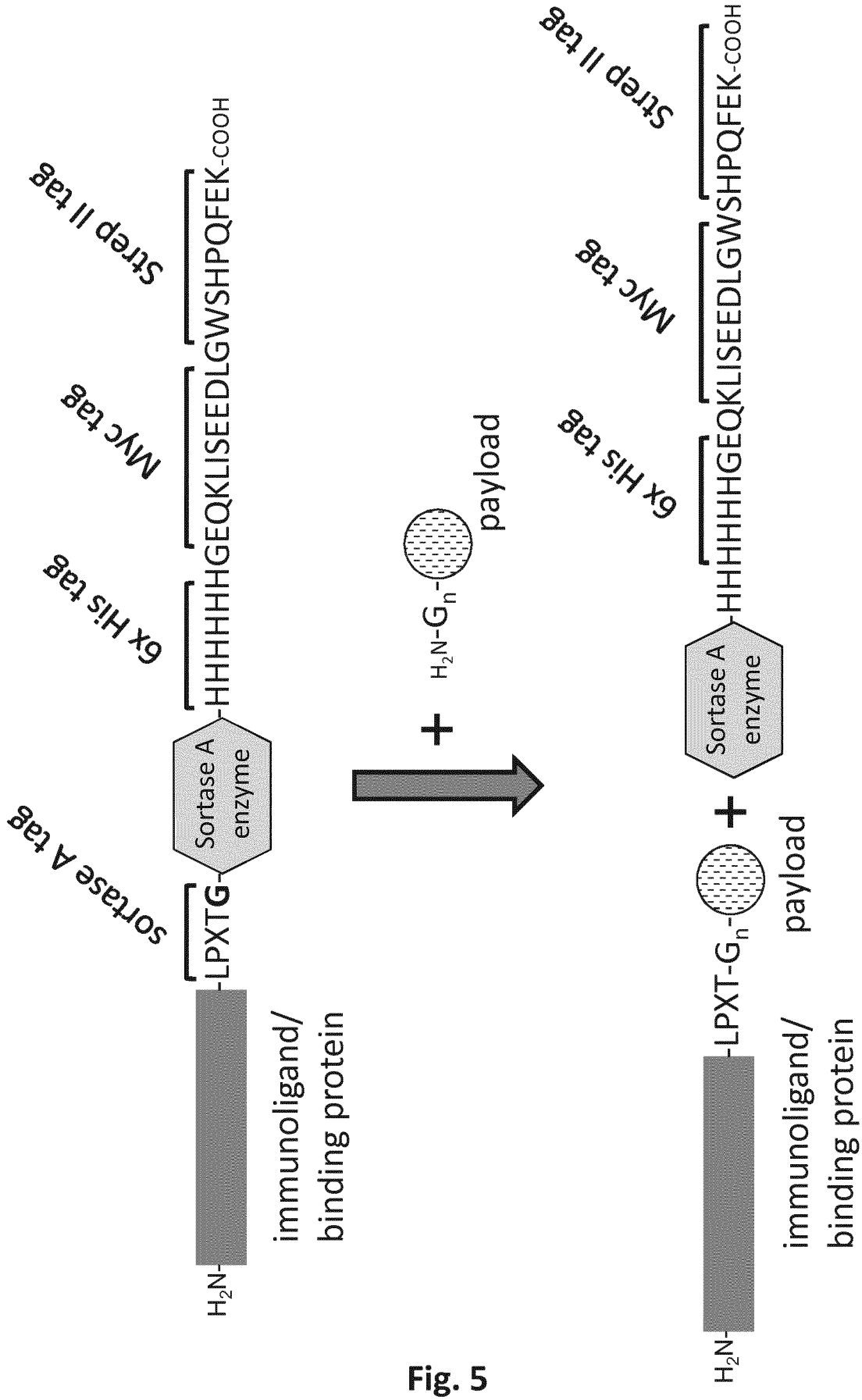

H2N-
immunoligand/
binding protein
-LPXTG-
Sortase A enzyme
-HHHHHHGEQKLISEEDLGWSHPQFEK-COOH
Sortase A tag
6x His tag
Myc tag
Strep II tag
+
H2N-Gn-
payload
Sortase A enzyme
-LPXT-Gn-
payload
-HHHHHHGEQKLISEEDLGWSHPQFEK-COOH

Fig. 5

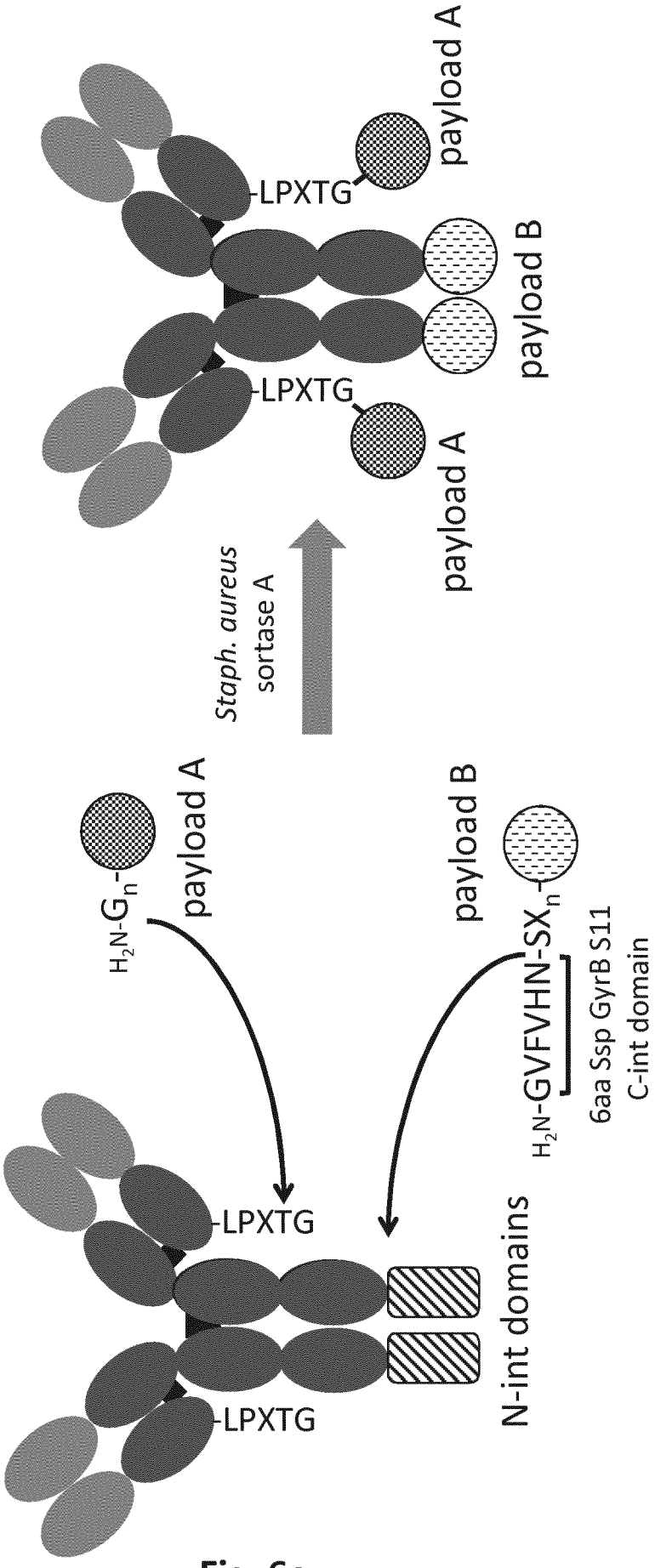

payload A
LPXTG
payload B
LPXTG
payload A
Staph. aureus
sortase A
H2N-Gn-
payload A
payload B
H2N-GVFVHN-SXn-
6aa Ssp GyrB S11
C-int domain
LPXTG
N-int domains
LPXTG

Fig. 6a

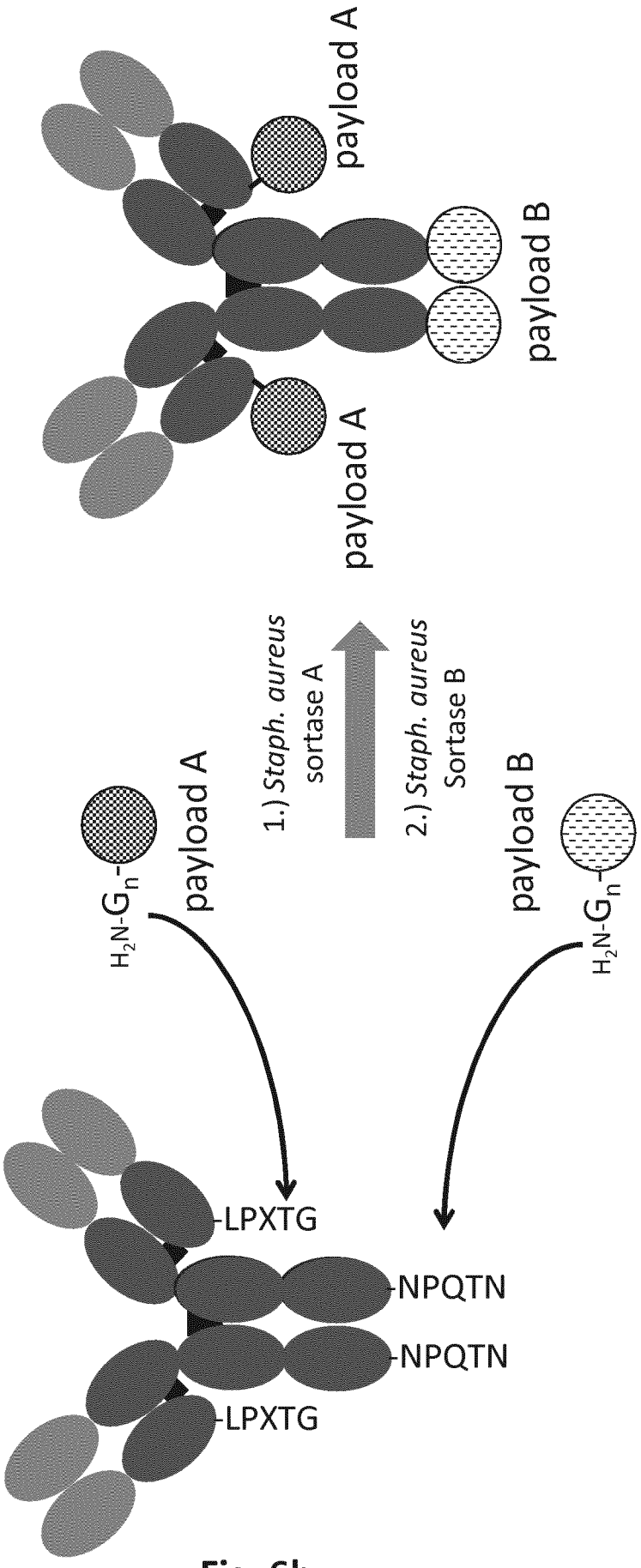

payload A
payload B
payload A
1.) Staph. aureus sortase A
2.) Staph. aureus Sortase B
payload A
payload B
H2N-Gn-
H2N-Gn-
LPXTG
NPQTN
NPQTN
LPXTG

Fig. 6b

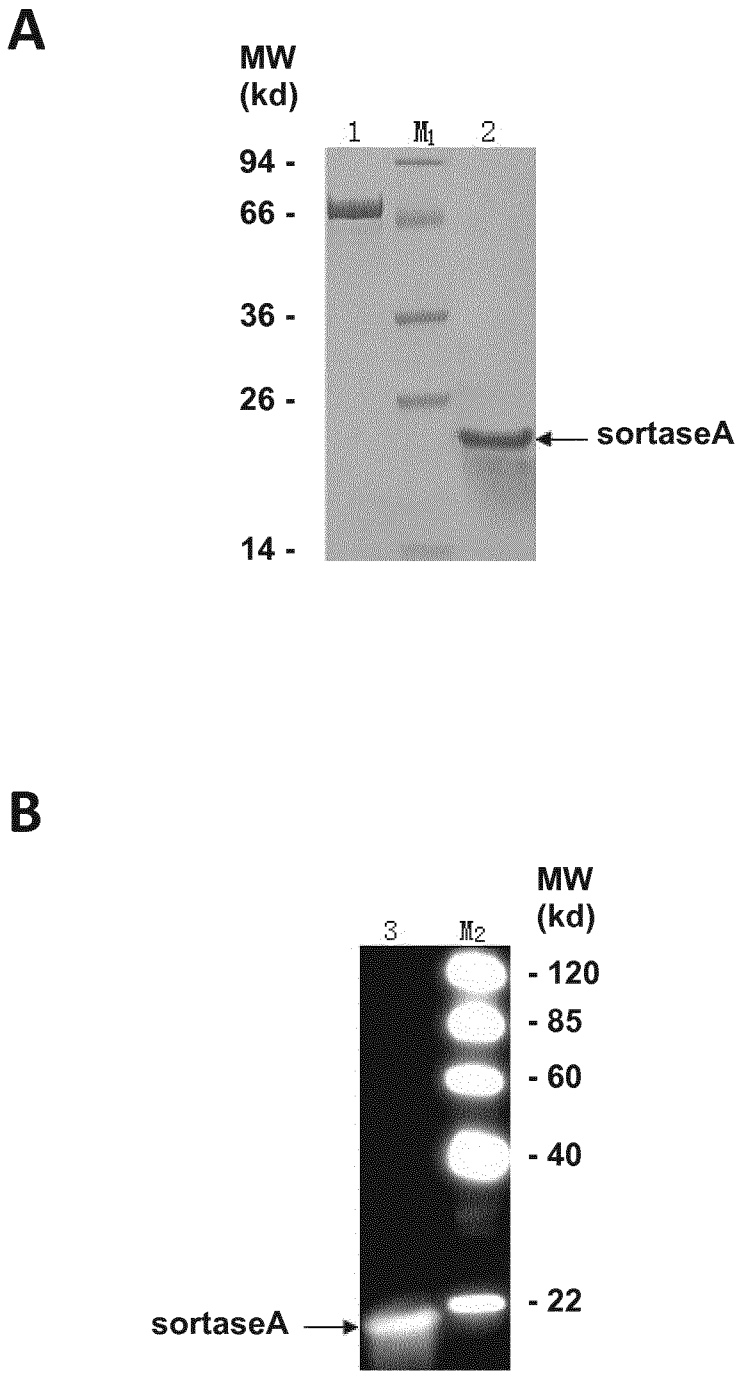
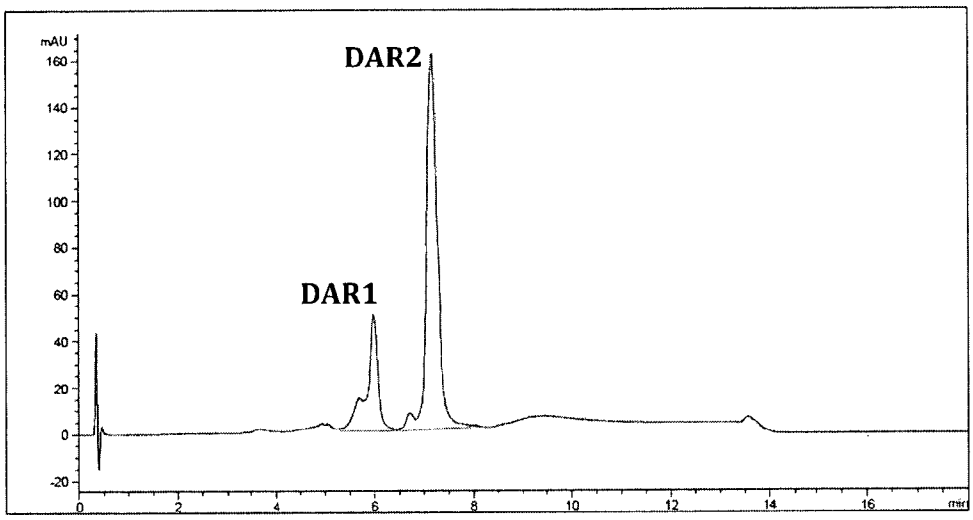

A
MW
(kd)
1
M1
2
94 -
66 -
36 -
26 -
14 -
sortaseA
B
MW
(kd)
3
M2
- 120
- 85
- 60
- 40
- 22
sortaseA

Fig. 7

A


mAU
160
140
120
100
80
60
40
20
0
-20
DAR2
DAR1
0
2
4
6
8
10
12
14
16
min

B

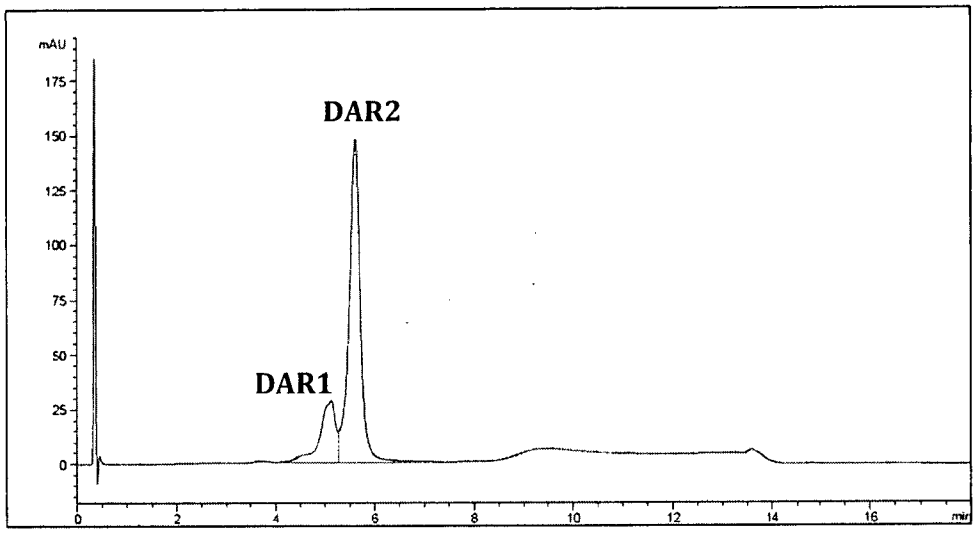

mAU
175
150
125
100
75
50
25
0
DAR2
DAR1
0
2
4
6
8
10
12
14
16
min

Fig. 8

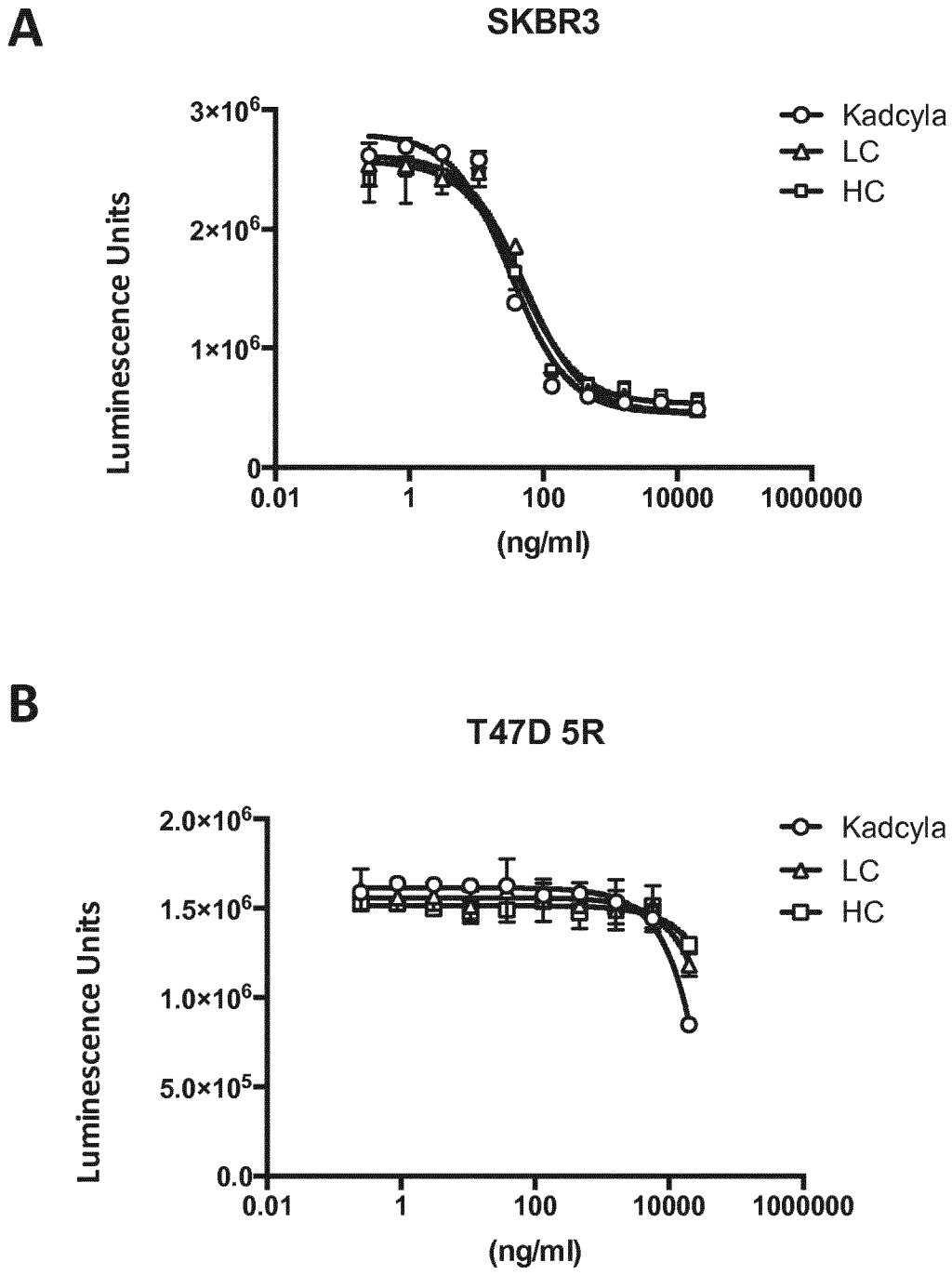

A
SKBR3
Luminescence Units
3×10^6
2×10^6
1×10^6
0
0.01
1
100
10000
1000000
(ng/ml)
Kadcyla
LC
HC
B
T47D 5R
Luminescence Units
2.0×10^6
1.5×10^6
1.0×10^6
5.0×10^5
0.0
0.01
1
100
10000
1000000
(ng/ml)
Kadcyla
LC
HC

Fig. 9

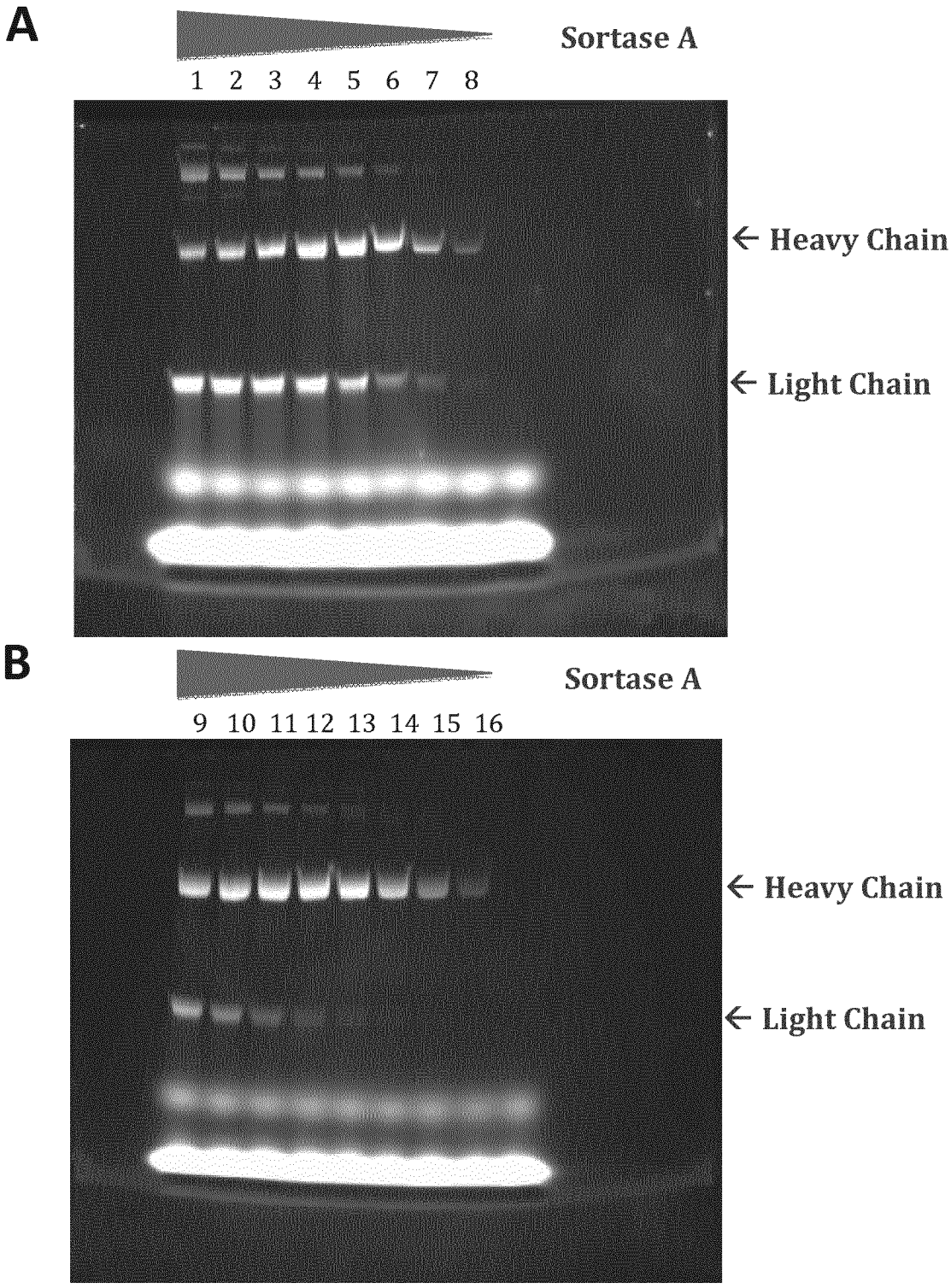

A
Sortase A
1 2 3 4 5 6 7 8
← Heavy Chain
← Light Chain
B
Sortase A
9 10 11 12 13 14 15 16
← Heavy Chain
← Light Chain

Fig. 10

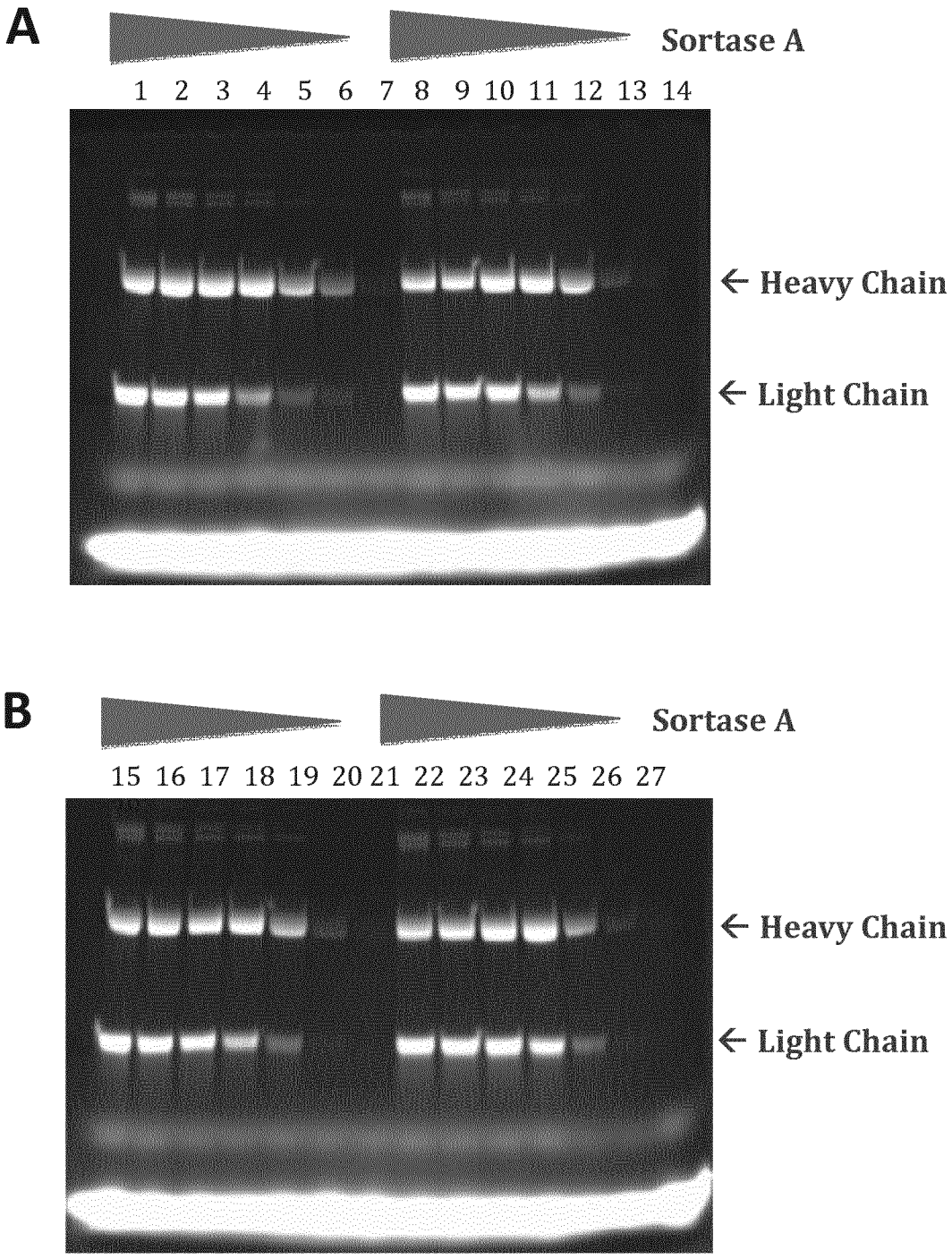
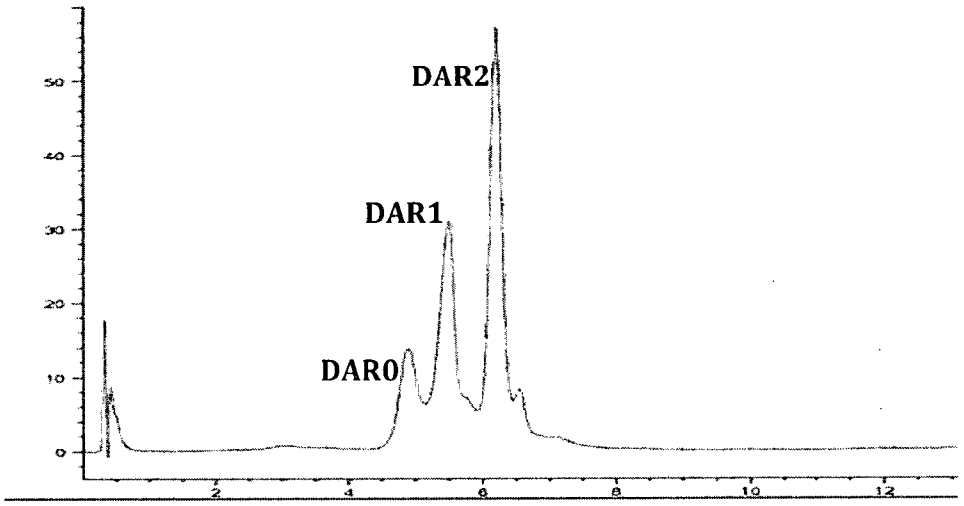

A
Sortase A
1 2 3 4 5 6 7 8 9 10 11 12 13 14
← Heavy Chain
← Light Chain
B
Sortase A
15 16 17 18 19 20 21 22 23 24 25 26 27
← Heavy Chain
← Light Chain

Fig. 11

A


DAR2
DAR1
DAR0
50
40
30
20
10
0
2
4
6
8
10
12

B

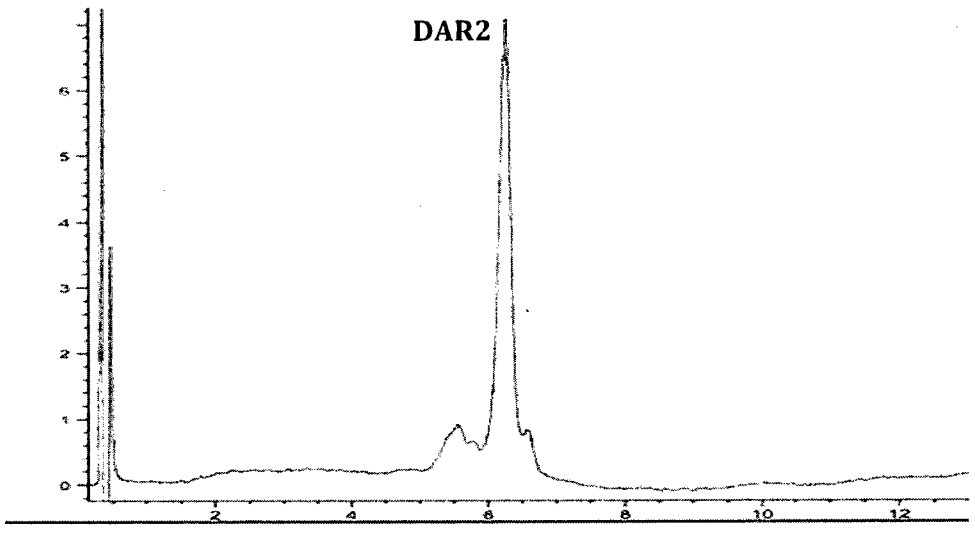
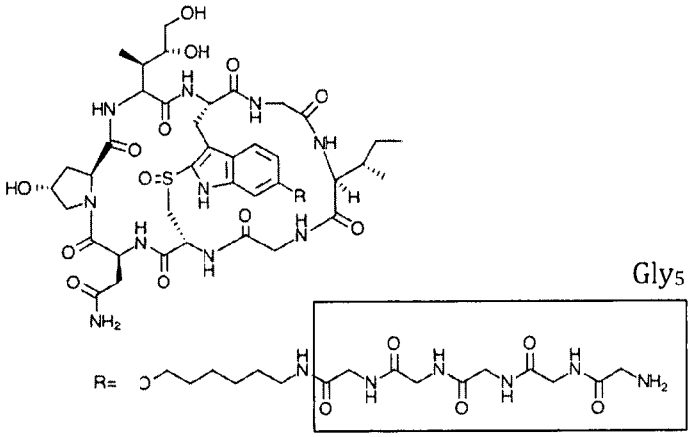
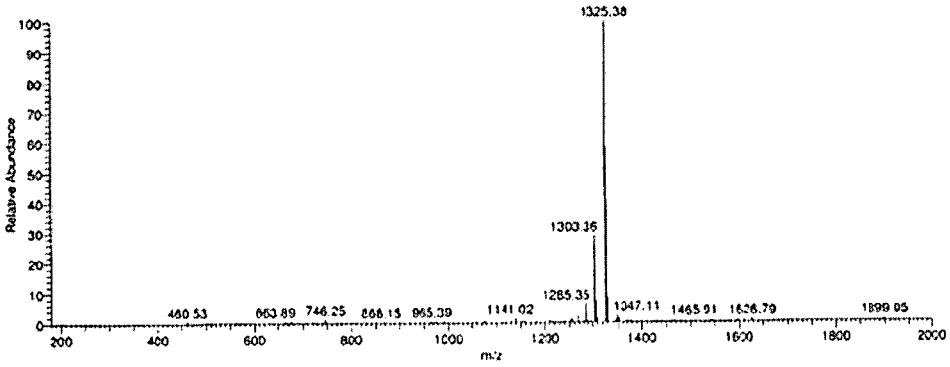

DAR2
6
5
4
3
2
1
0
2
4
6
8
10
12

Fig. 12



Gly_5-modified alpha-amanitin

Mass Spectral Analysis of Gly_5-modified alpha-amanitin:



RP-HPLC Analysis of Gly_5-modified alpha-amanitin:

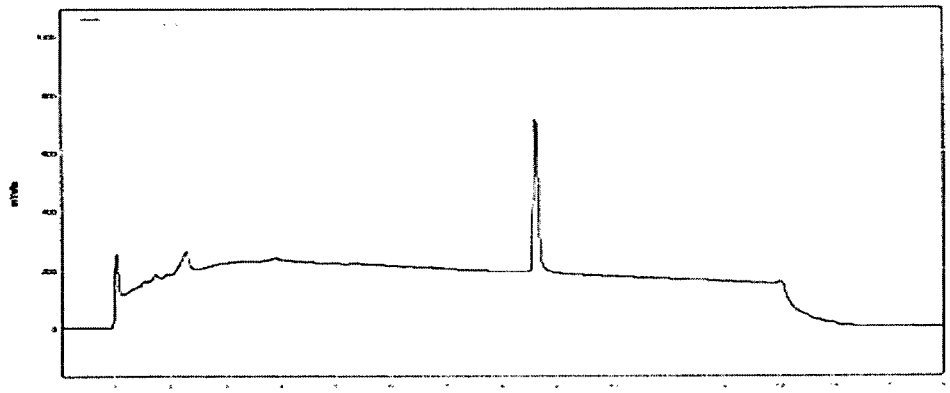
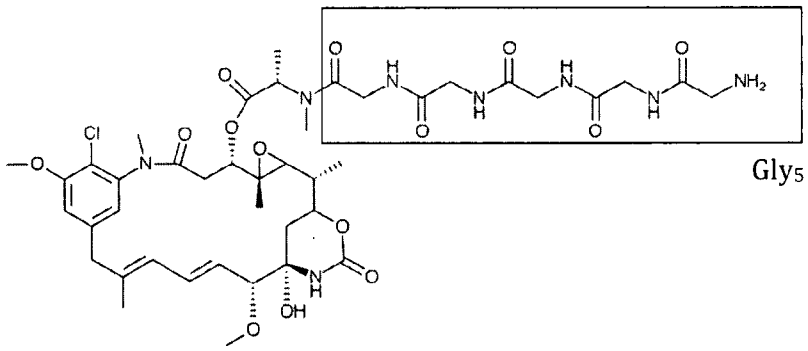
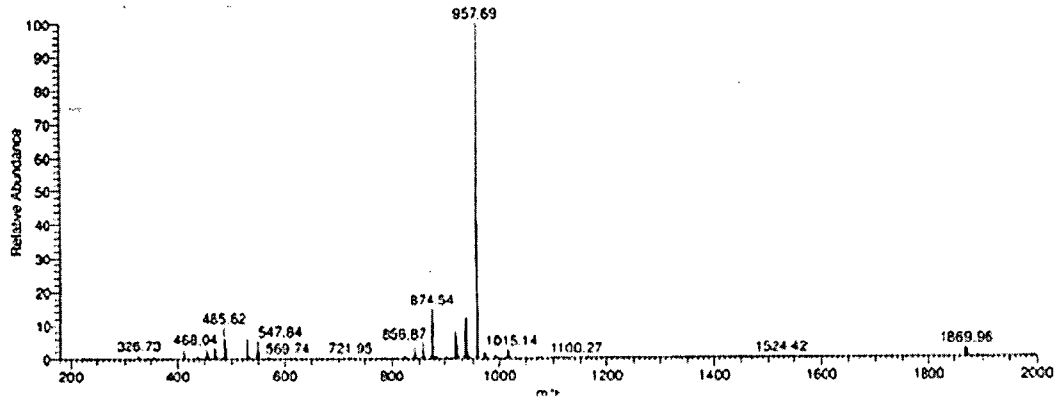


Fig. 13 A



Gly_5-modified maytansine

Mass Spectral Analysis of Gly_5-modified maytansine:



RP-HPLC Analysis of Gly_5-modified maytansine:

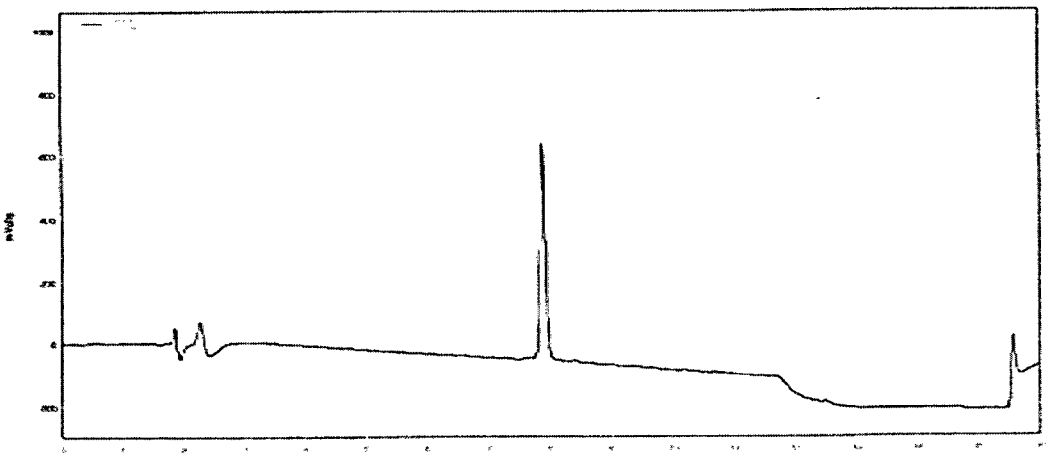
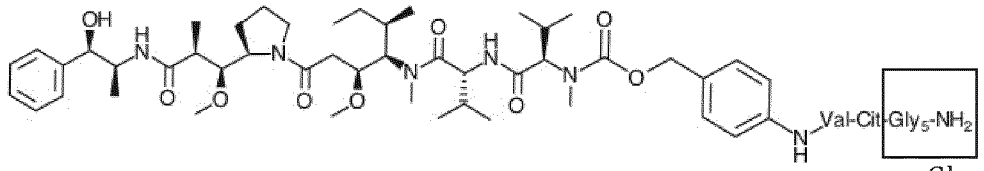


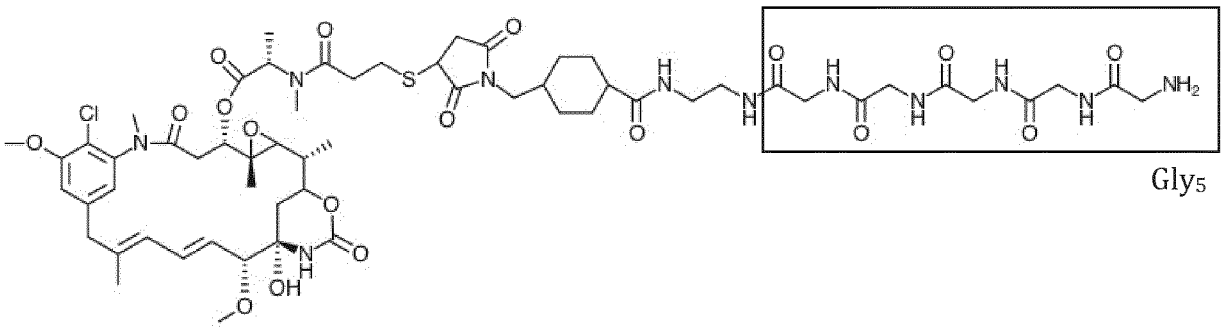
Fig. 13 B

structure 1:



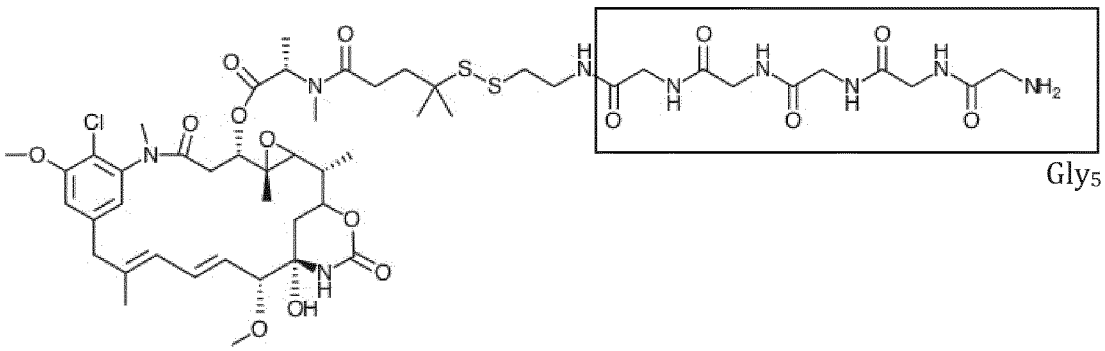
Gly_5-vc-PAB-MMAE

structure 2:



Gly_5-DM1

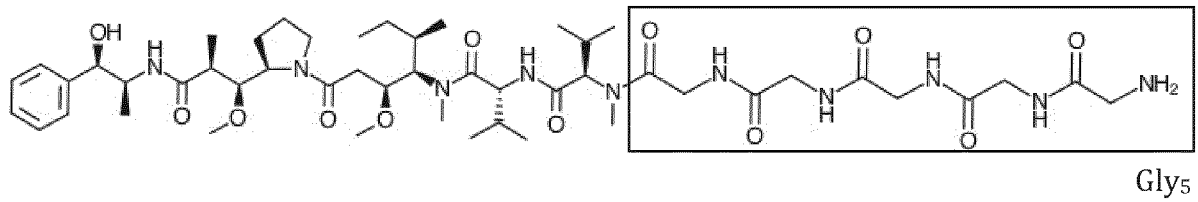
structure 3:



Gly_5-DM4

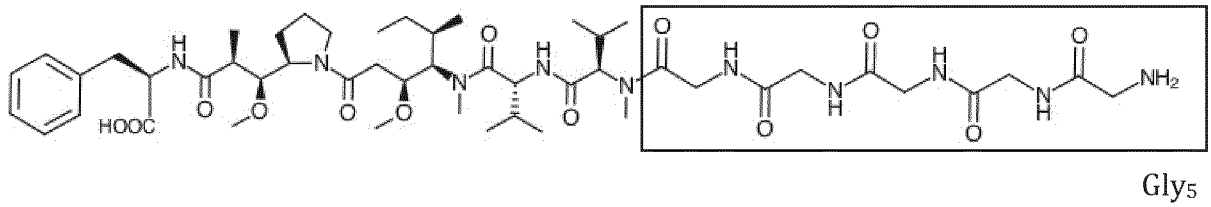
Fig. 14 A

structure 4:



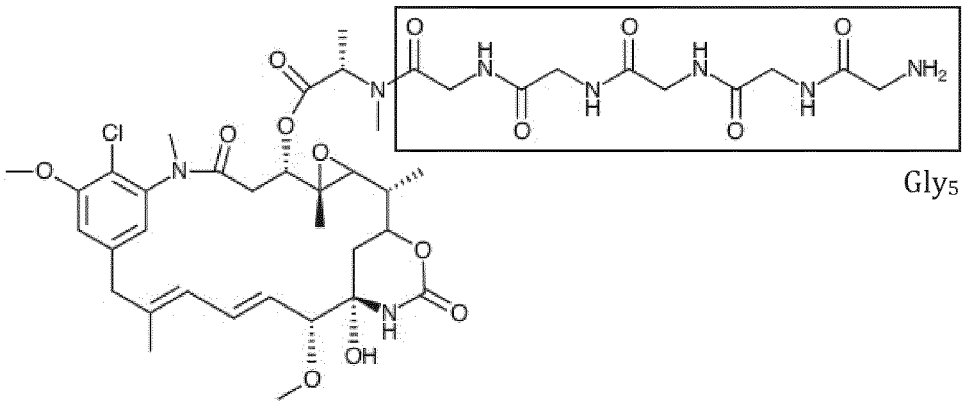
Gly_5-MMAE

structure 5:



Gly_5-MMAF

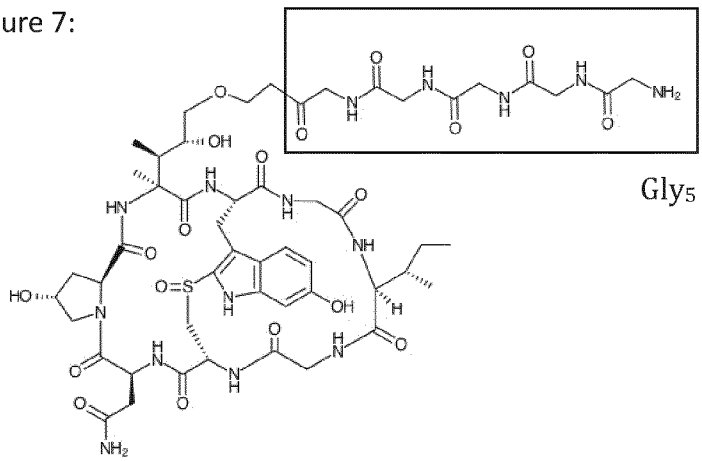
structure 6:



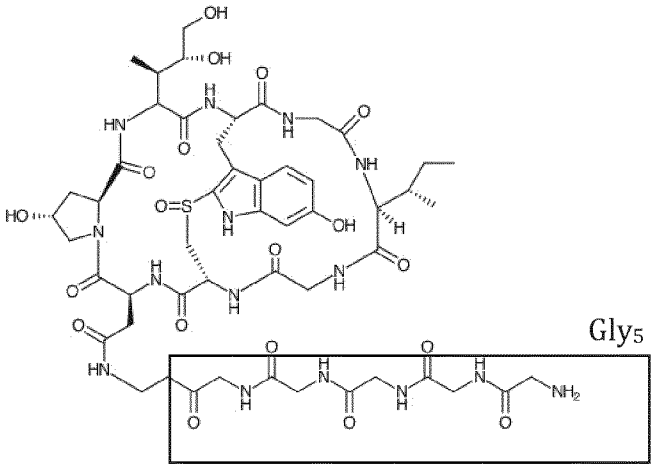
Gly_5-Maytansine

Fig. 14 B

structure 7:

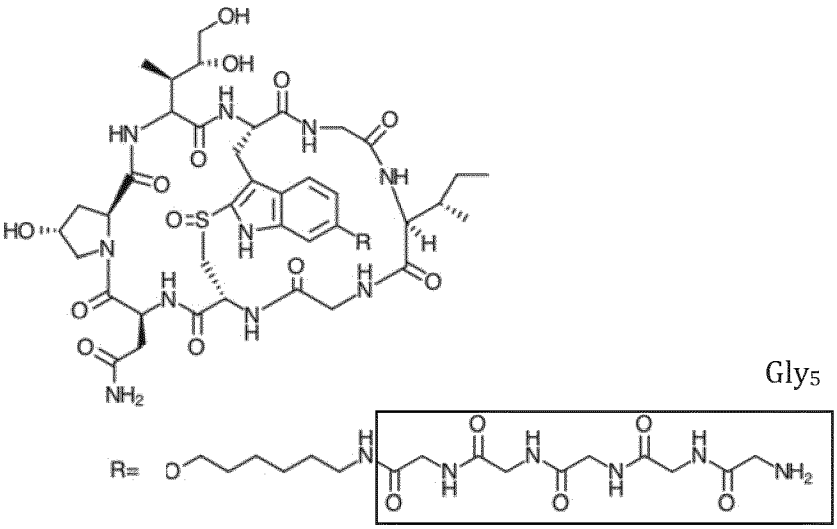


structure 8:



Gly5-alpha-amanitin (II)

structure 9:



Gly5-alpha-amanitin (III)

Fig. 14 C

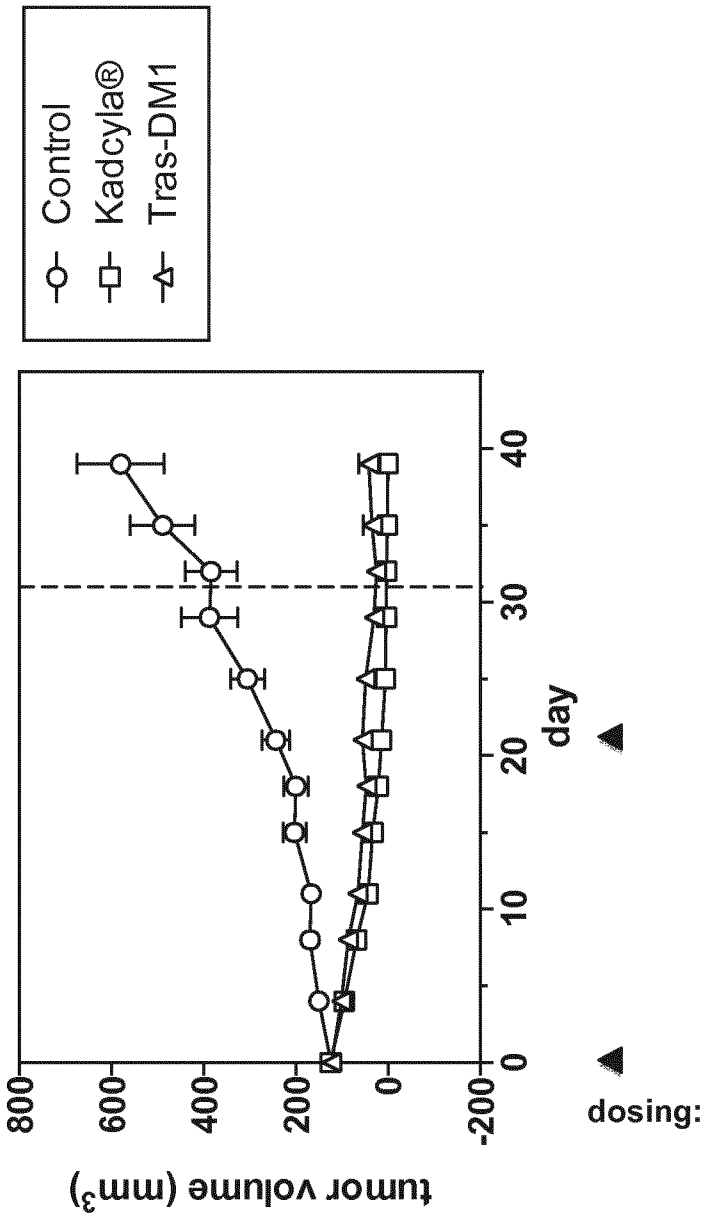

Control
Kadcyla®
Tras-DM1
tumor volume (mm³)
800
600
400
200
0
-200
0
10
20
30
40
day
dosing:

Fig. 15

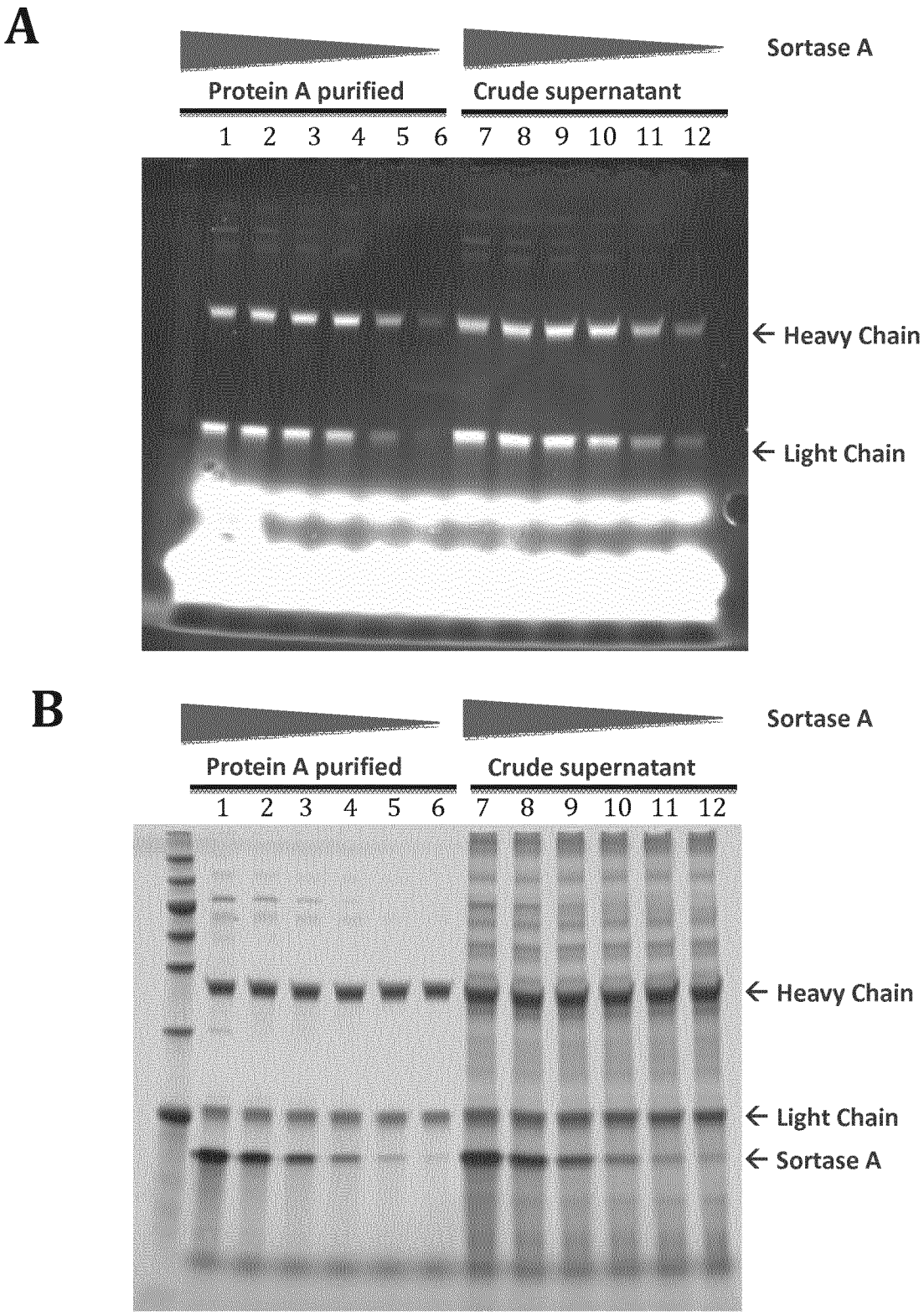

A
Sortase A
Protein A purified
Crude supernatant
1 2 3 4 5 6 7 8 9 10 11 12
← Heavy Chain
← Light Chain
B
Sortase A
Protein A purified
Crude supernatant
1 2 3 4 5 6 7 8 9 10 11 12
← Heavy Chain
← Light Chain
← Sortase A

Fig. 16