Fig. 1

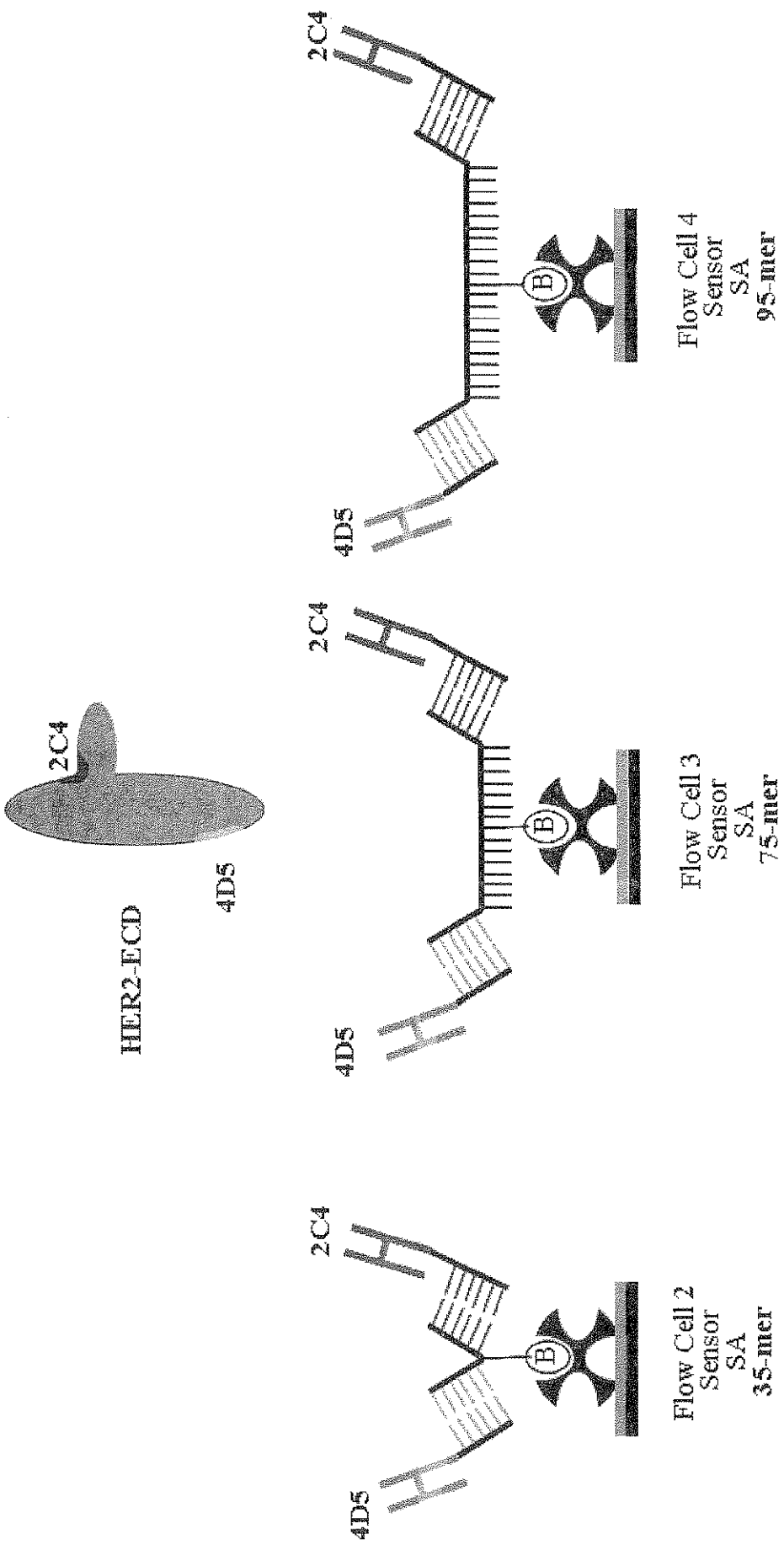

2C4
4D5
HER2-ECD
2C4
4D5
B
Flow Cell 4
Sensor
SA
95-mer
2C4
4D5
B
Flow Cell 3
Sensor
SA
75-mer
2C4
4D5
B
Flow Cell 2
Sensor
SA
35-mer

Fig. 2

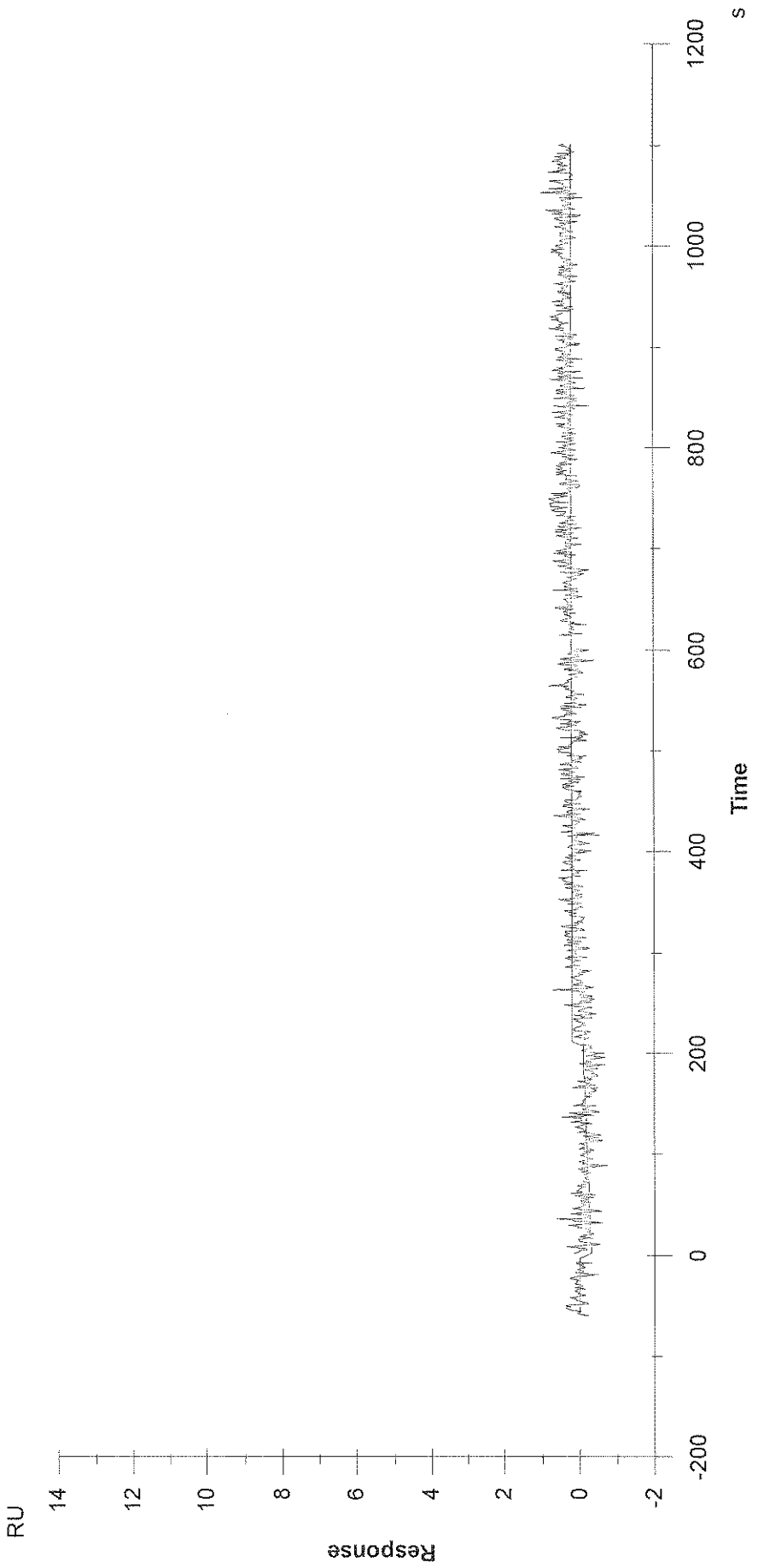

RU
14
12
10
8
6
4
2
0
-2
Response
-200
0
200
400
600
800
1000
1200
s
Time

Fig. 3

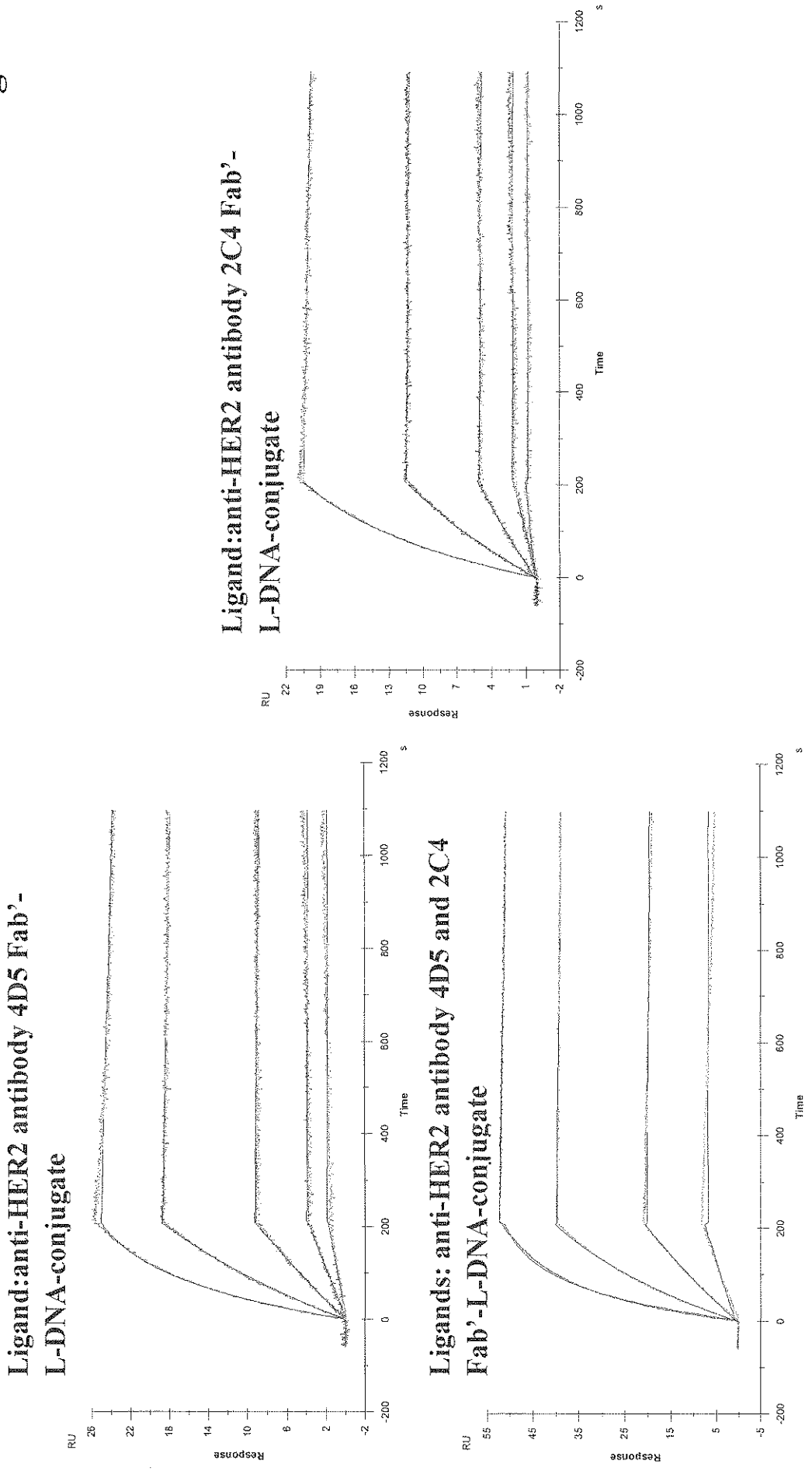

Ligand:anti-HER2 antibody 4D5 Fab'-L-DNA-conjugate
Ligand:anti-HER2 antibody 2C4 Fab'-L-DNA-conjugate
Ligands: anti-HER2 antibody 4D5 and 2C4 Fab'-L-DNA-conjugate
RU
Response
Time
s

Fig. 4

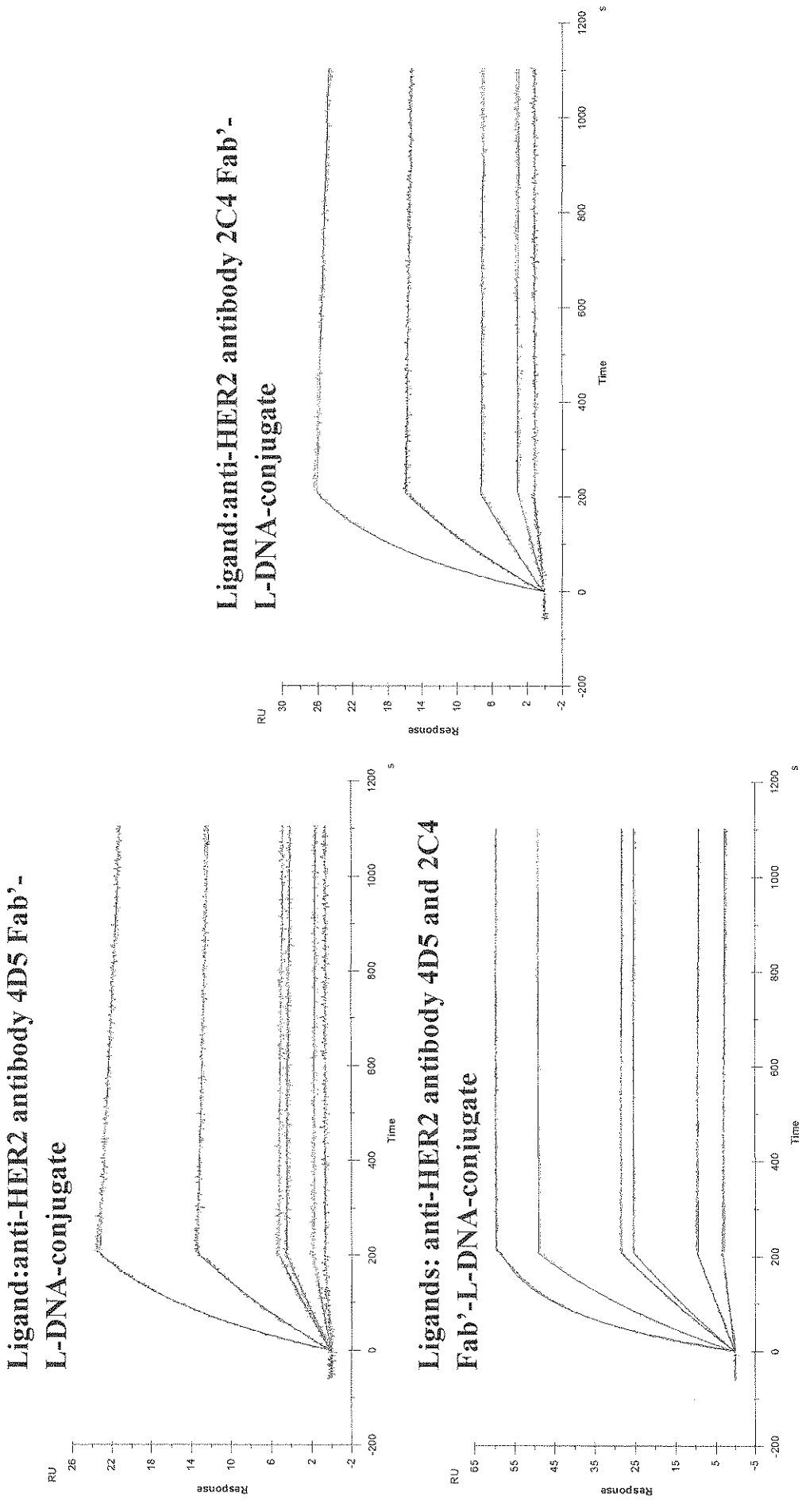
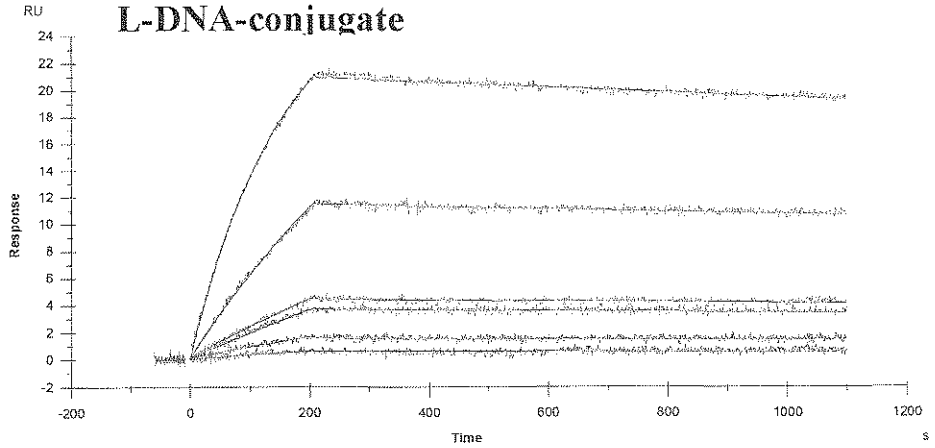
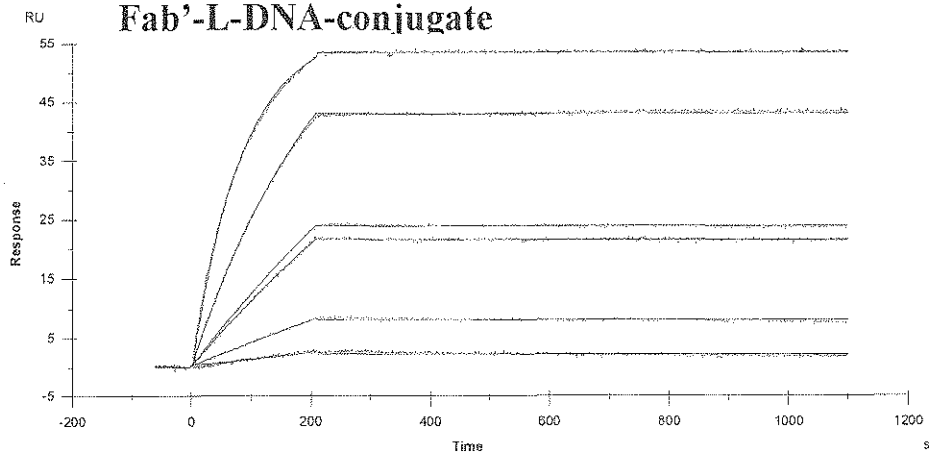

Ligand:anti-HER2 antibody 4D5 Fab'-L-DNA-conjugate
Ligand:anti-HER2 antibody 2C4 Fab'-L-DNA-conjugate
Ligands: anti-HER2 antibody 4D5 and 2C4 Fab'-L-DNA-conjugate
RU
Response
Time
s

Fig. 5

Ligand:anti-HER2 antibody 4D5 Fab'-L-DNA-conjugate



Ligands: anti-HER2 antibody 4D5 and 2C4 Fab'-L-DNA-conjugate



Ligand:anti-HER2 antibody 2C4 Fab'-L-DNA-conjugate

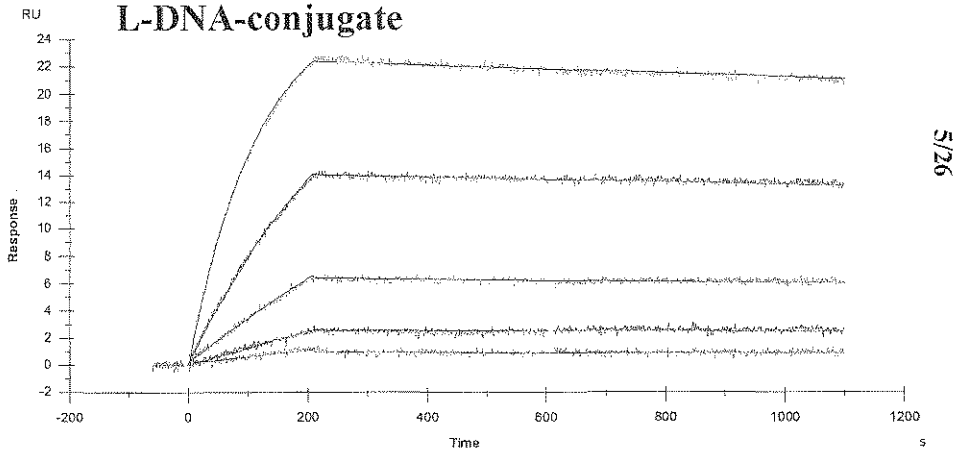


Fig. 6

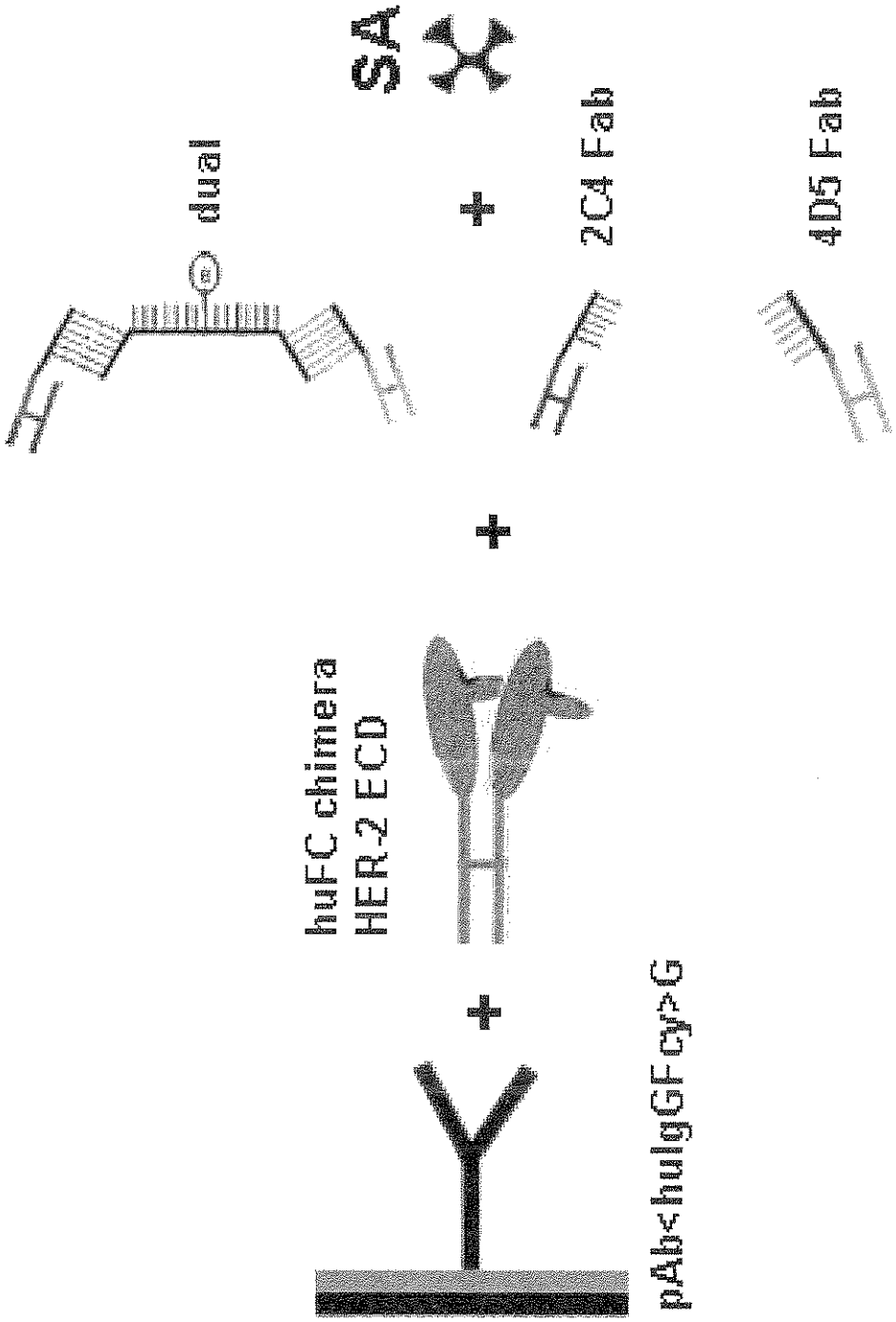


Fig. 7

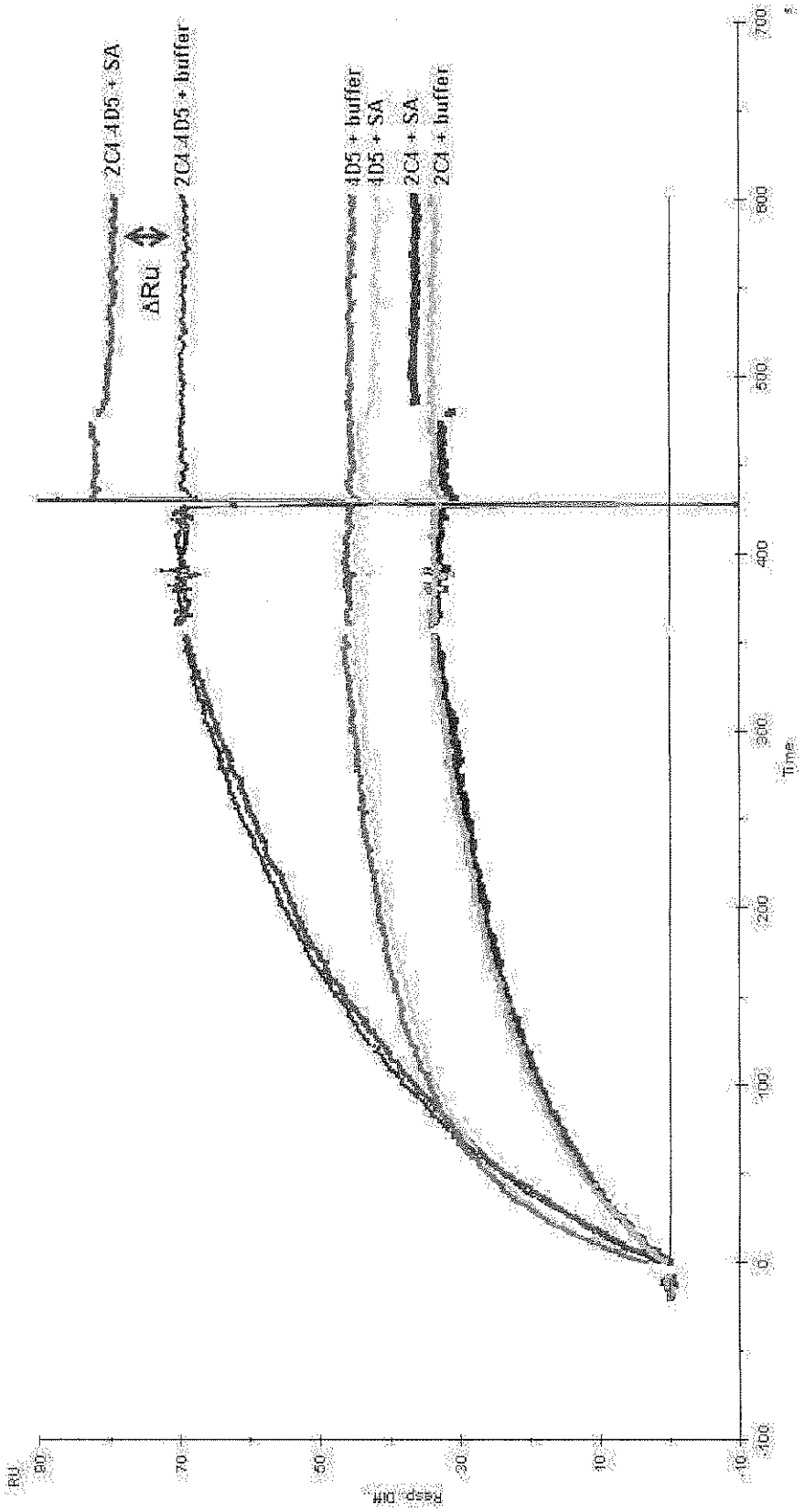

2C4:4D5 + SA
2C4:4D5 + buffer
ΔRu
4D5 + buffer
4D5 + SA
2C4 + SA
2C4 + buffer
RU
Resp. Diff
Time
s

Fig. 8

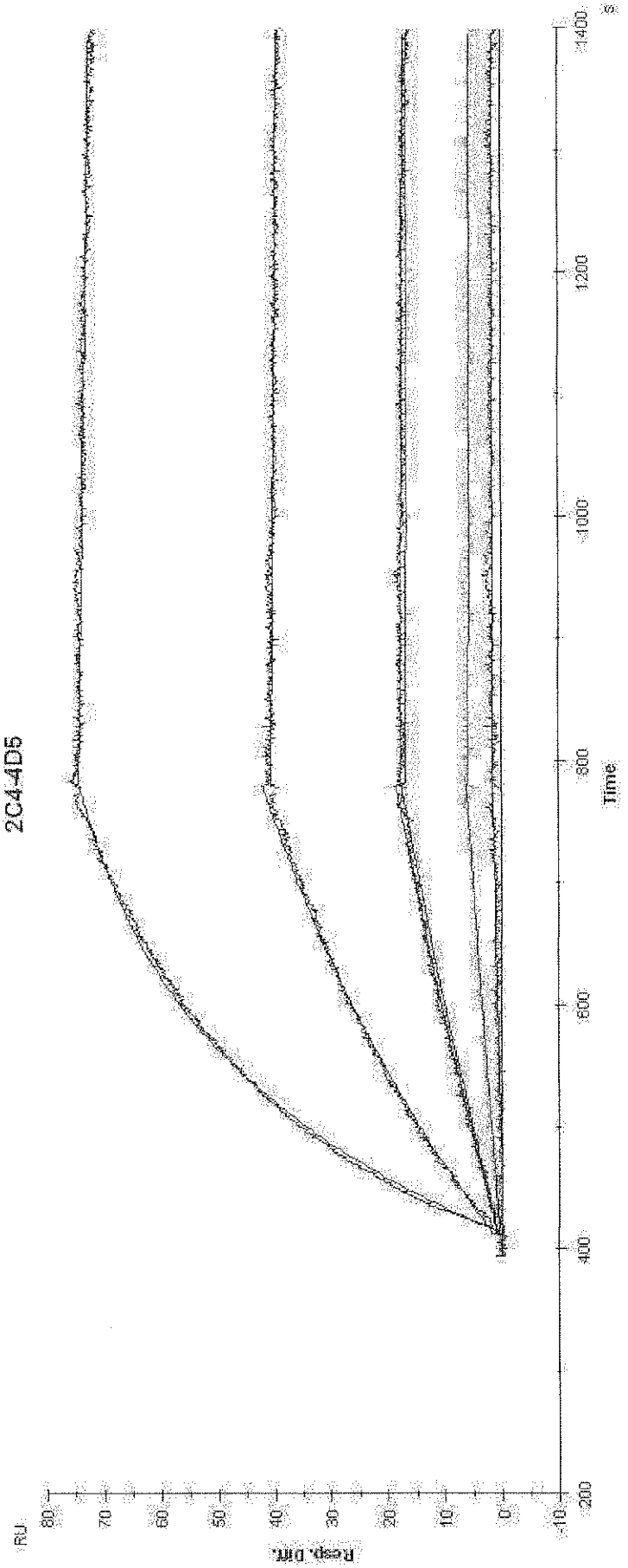

2C4-4D5
RU
Resp. Diff.
Time
s
80
70
60
50
40
30
20
10
0
-10
200
400
600
800
1000
1200
1400

Fig. 9

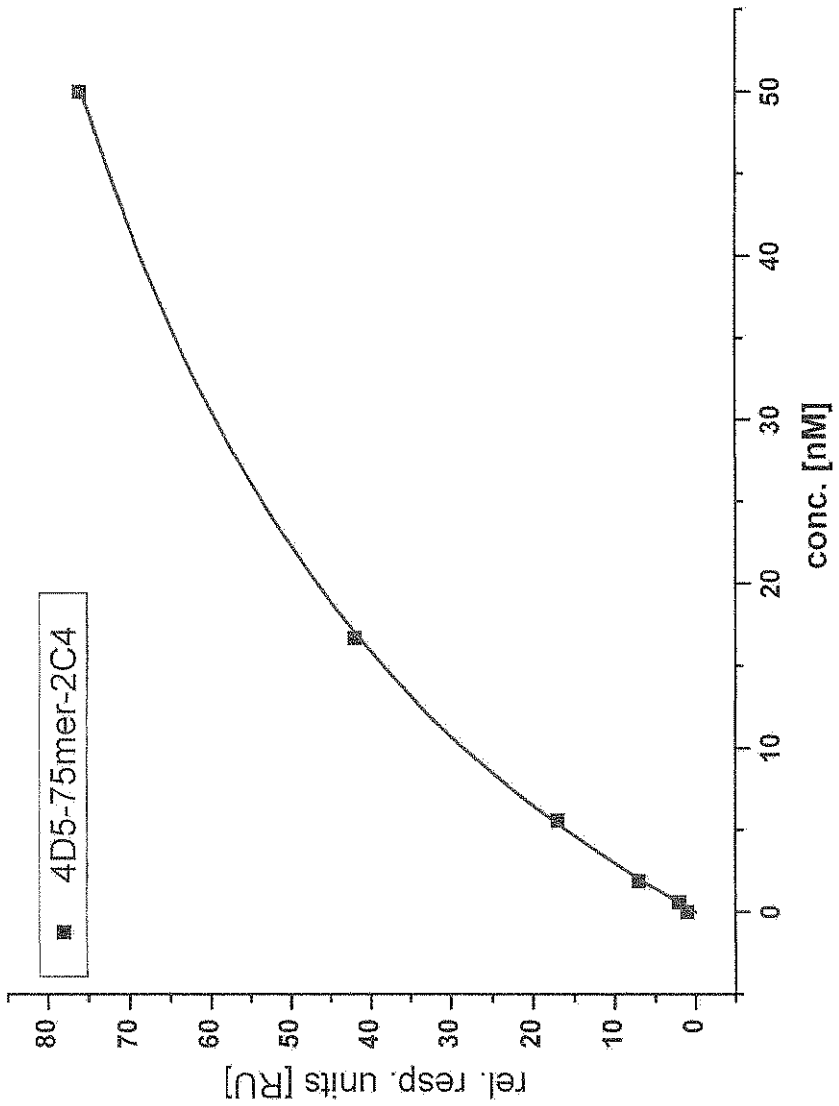

4D5-75mer-2C4
rel. resp. units [RU]
conc. [nM]
0
10
20
30
40
50
0
10
20
30
40
50
60
70
80

Fig. 10

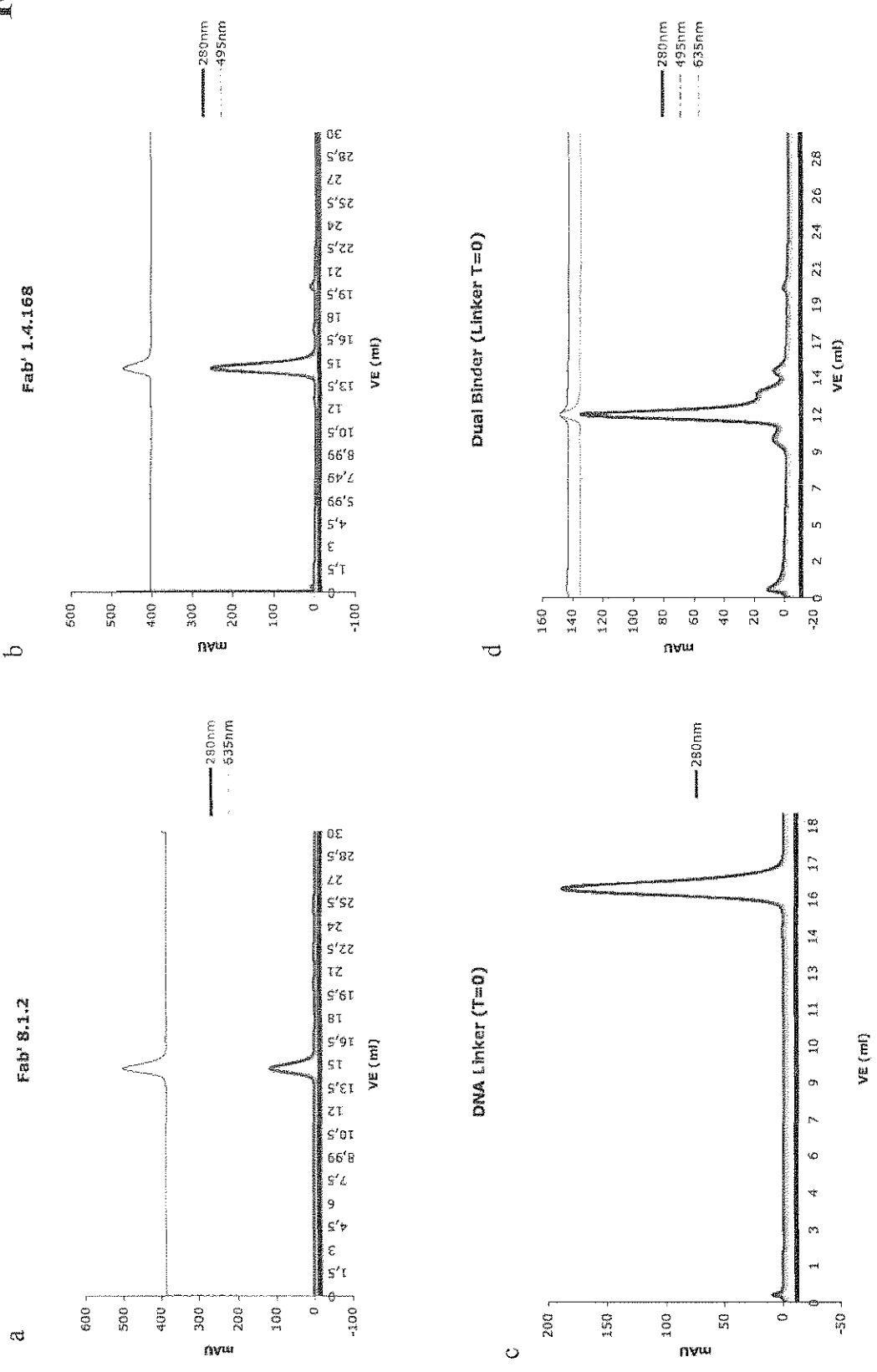

a
Fab' 8.1.2
280nm
635nm
mAU
VE (ml)
b
Fab' 1.4.168
280nm
495nm
mAU
VE (ml)
c
DNA Linker (T=0)
280nm
mAU
VE (ml)
d
Dual Binder (Linker T=0)
280nm
495nm
635nm
mAU
VE (ml)

Fig. 11

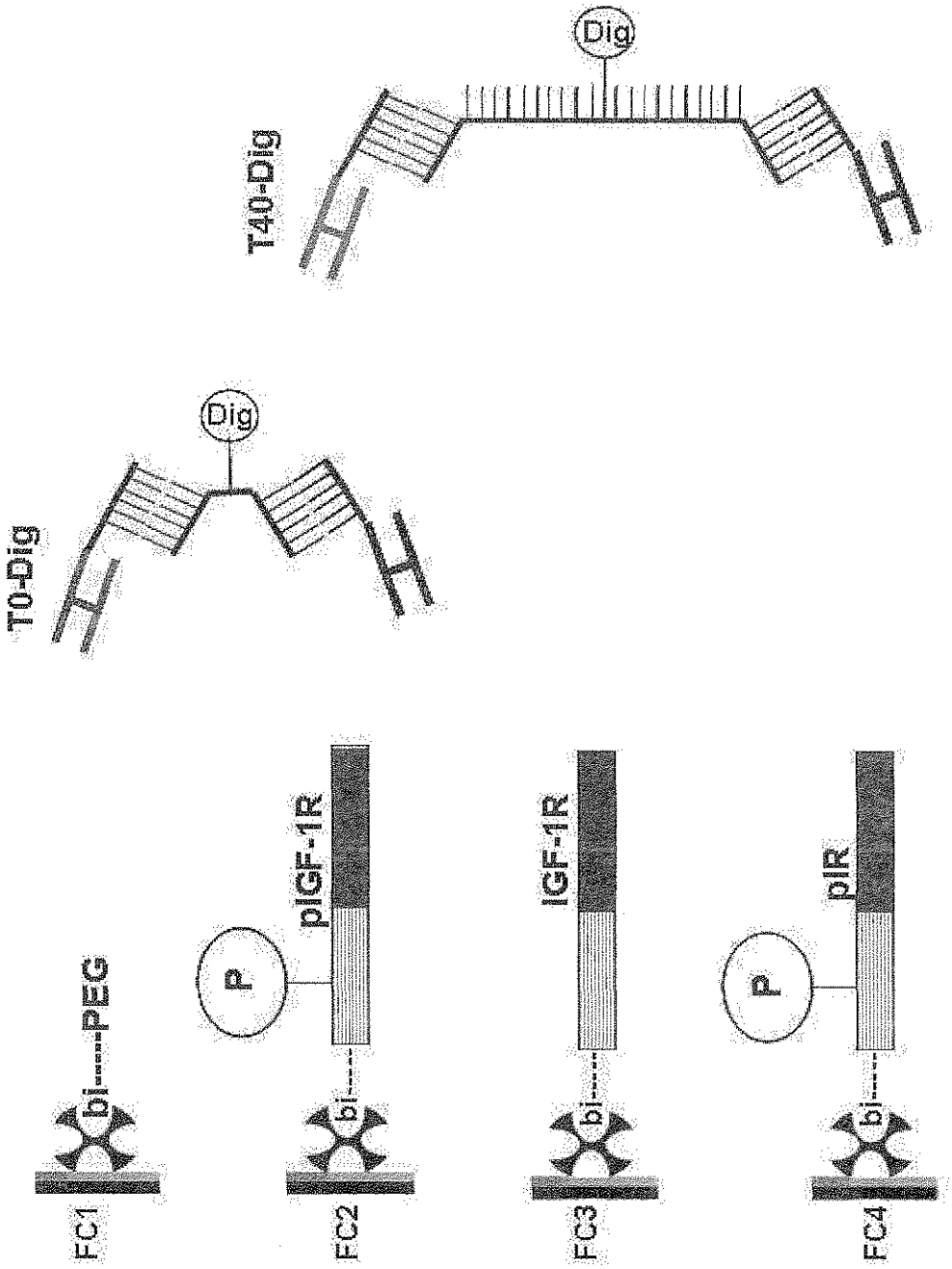

T40-Dig
Dig
T0-Dig
Dig
FC1
bi
PEG
FC2
P
pIGF-1R
bi
FC3
IGF-1R
bi
FC4
P
pIR
bi

Fig. 12

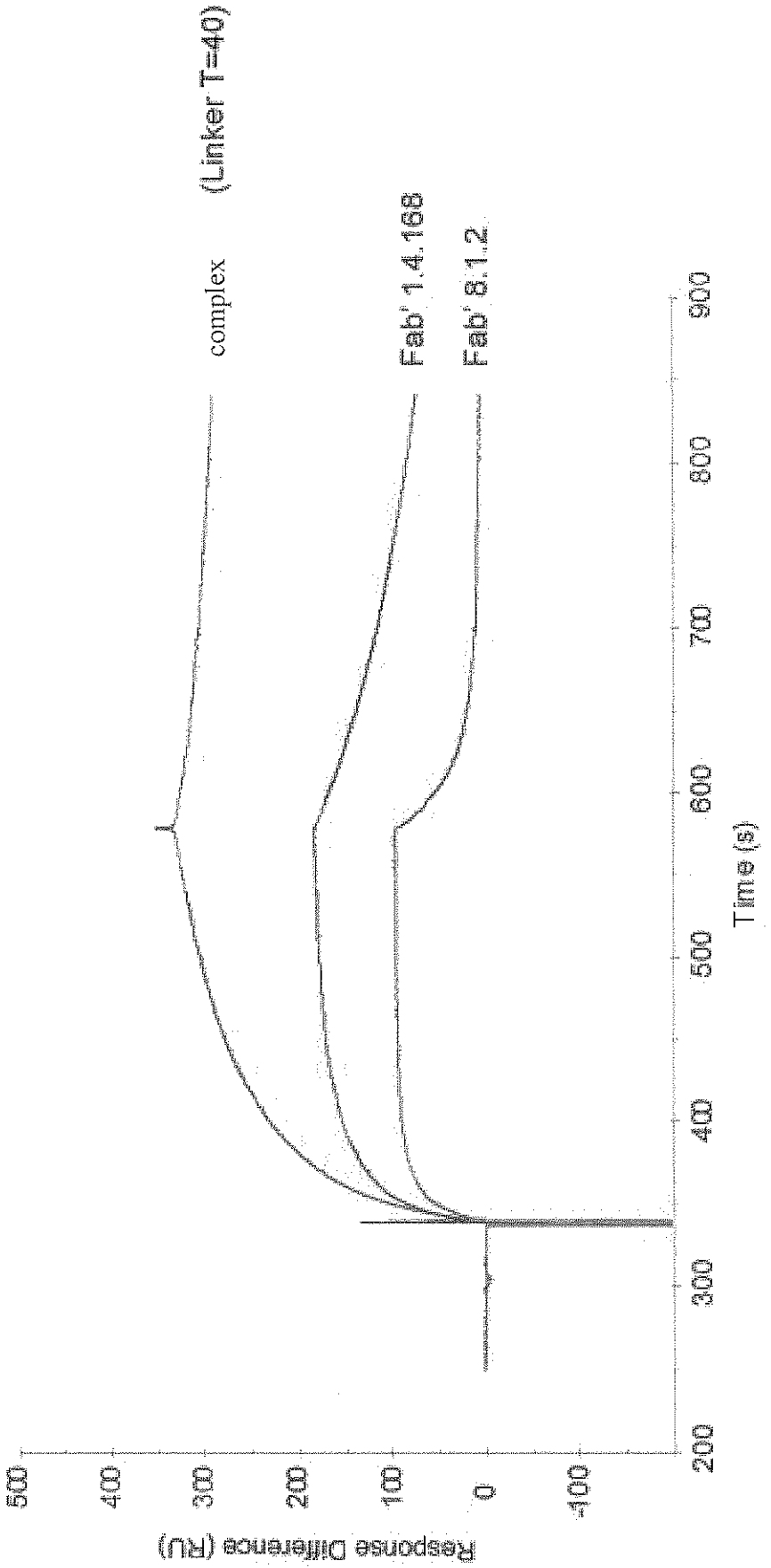

complex (Linker T=40)
Fab' 1.4.168
Fab' 8.1.2
Response Difference (RU)
Time (s)
500
400
300
200
100
0
-100
200
300
400
500
600
700
800
900

Fig. 13

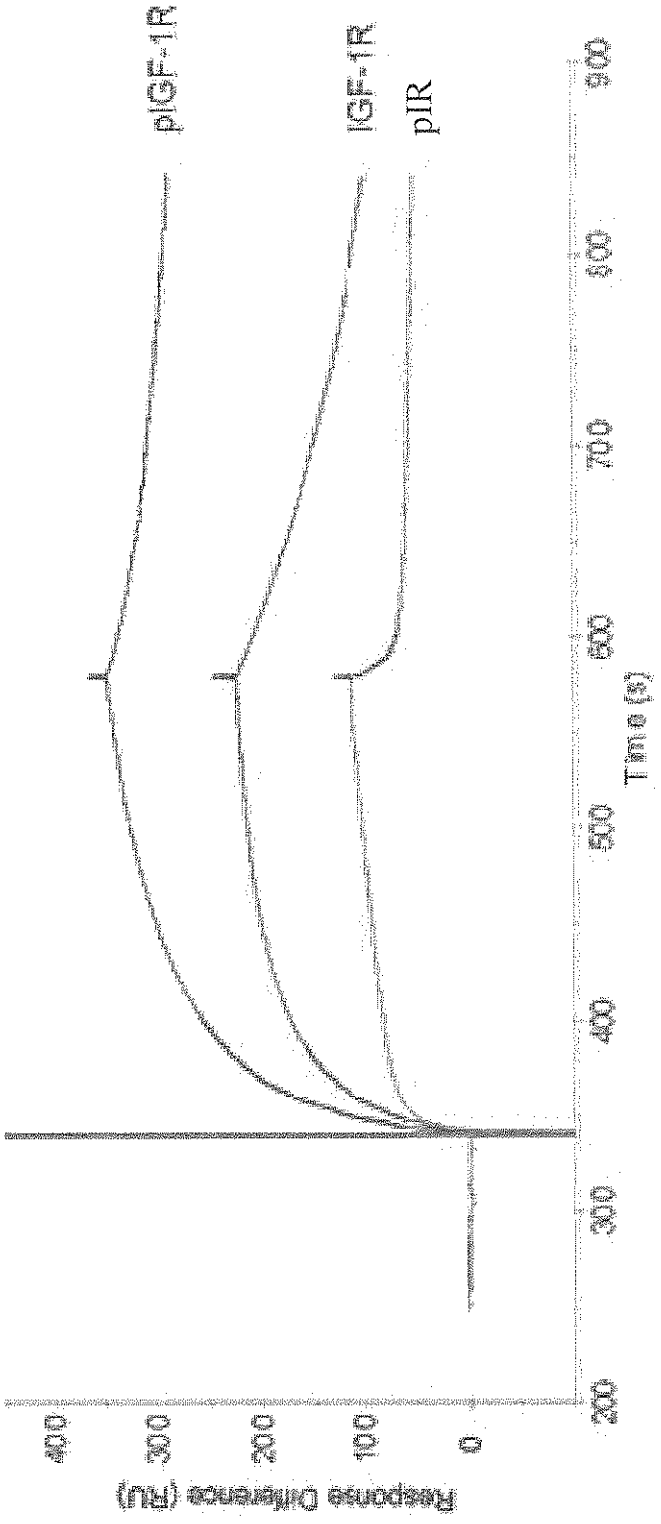

pIGF-1R
IGF-1R
pIR
Response Difference (RU)
0
0.01
0.02
0.03
0.04
200
300
400
500
600
700
800
900
Time (s)

Fig. 14

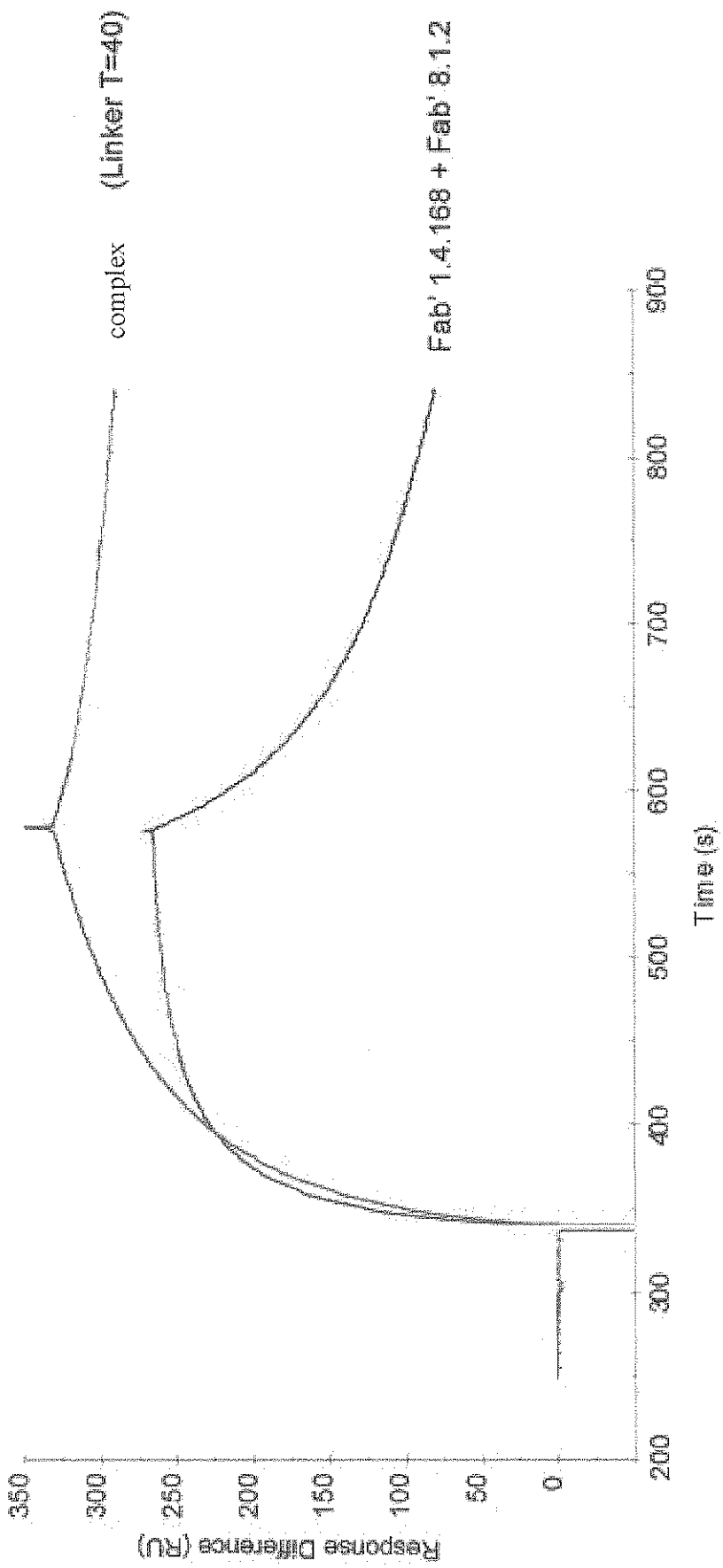

complex (Linker T=40)
Fab'1.4.168 + Fab'8.1.2
Response Difference (RU)
Time (s)
350
300
250
200
150
100
50
0
200
300
400
500
600
700
800
900

Fig. 15

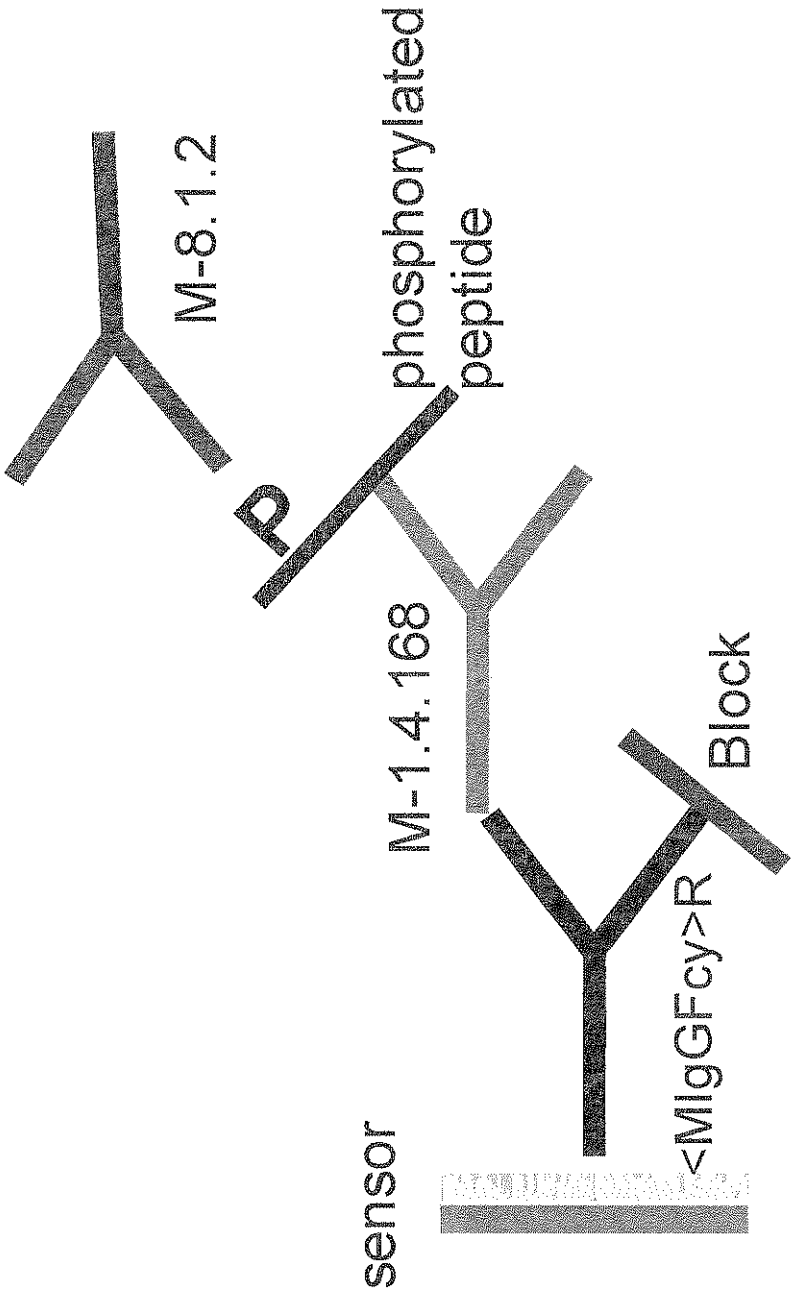

M-8.1.2
P
phosphorylated peptide
M-1.4.168
Block
<MIgGFcy>R
sensor

Fig. 16

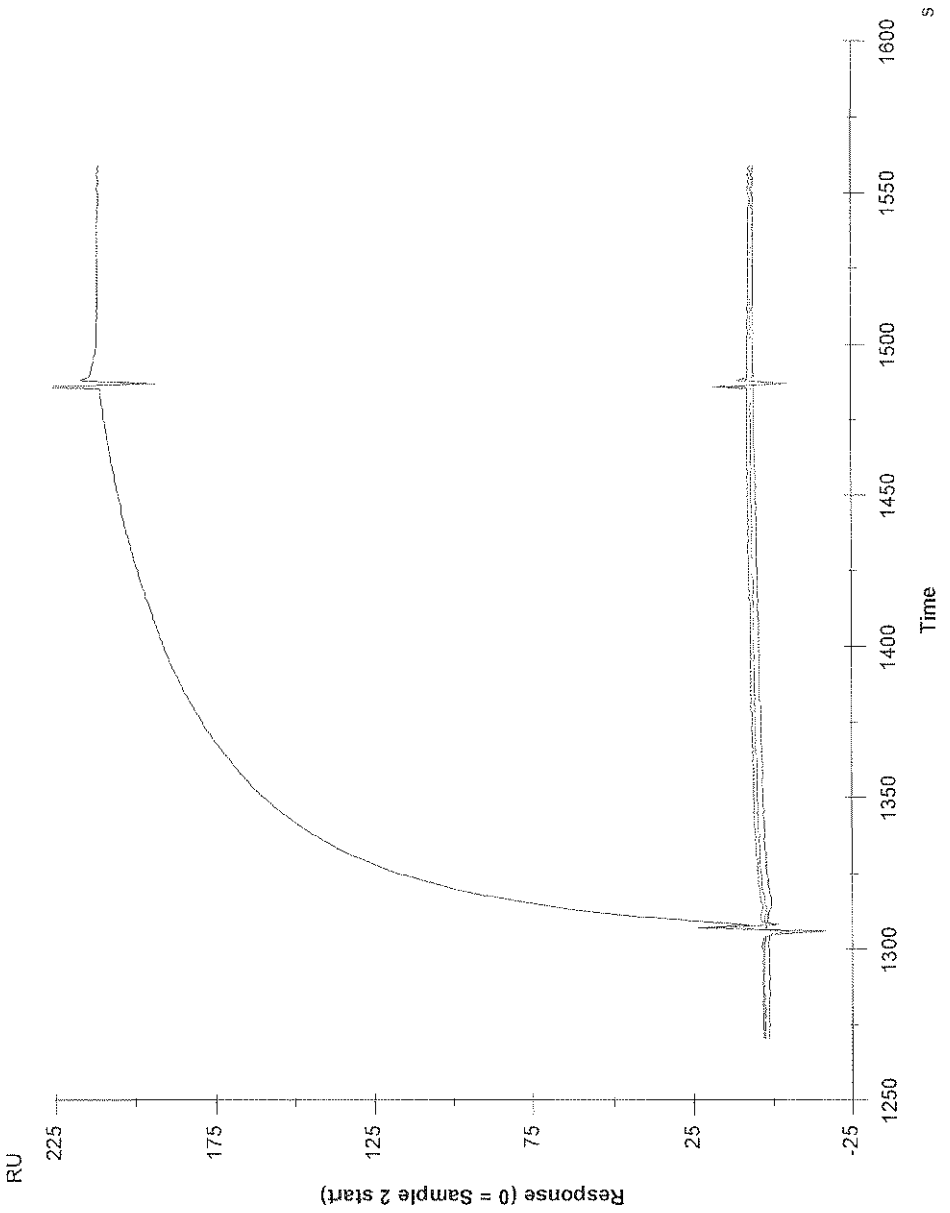

RU
225
175
125
75
25
-25
Response (0 = Sample 2 start)
1250
1300
1350
1400
1450
1500
1550
1600
Time
s

Fig. 17

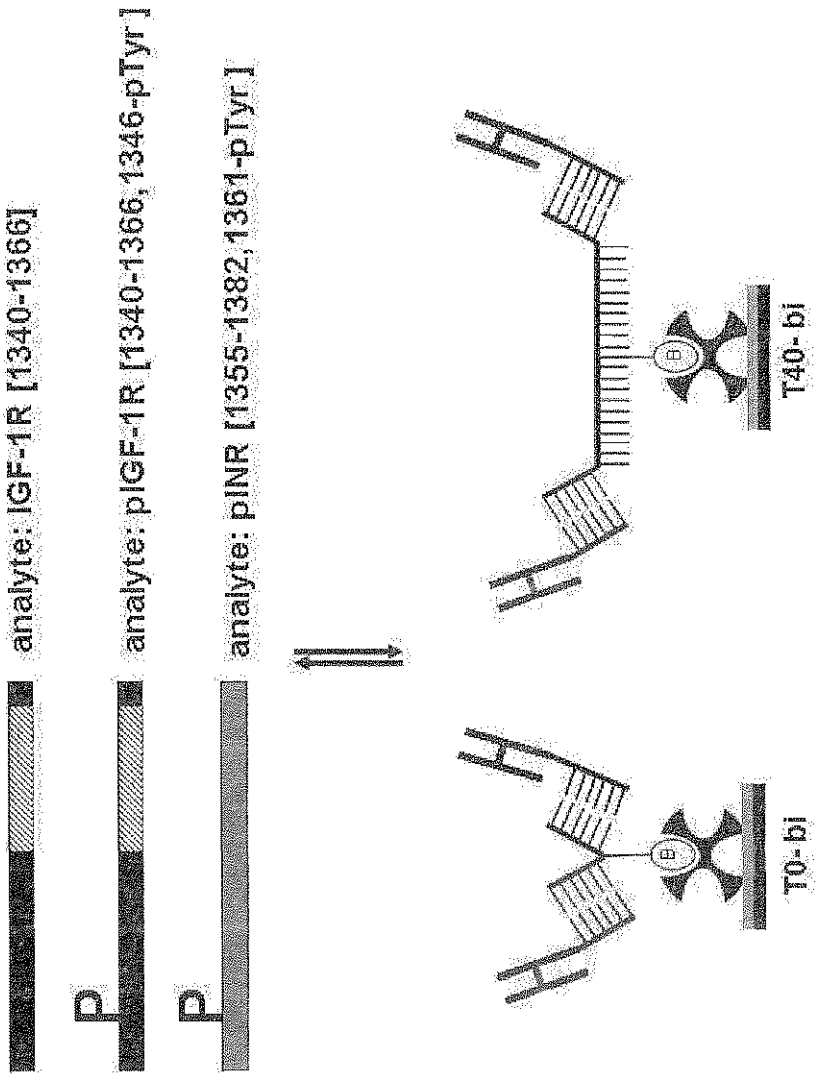

analyte: IGF-1R [1340-1366]
P
analyte: pIGF-1R [1340-1366;1346-pTyr]
P
analyte: pINR [1355-1382;1361-pTyr]
B
T0- bi
B
T40- bi

Fig. 18

Linker	Analyte	ssFab 1	ssFab 2	Ka (1/Ms)	kd (1/s)	t1/2 diss (min)	KD (M)	KD (nM)
T40	pIGF-1R	8.1.2	1.4.168	2,24E+06	2,79E-05	414	1,25E-11	0,01
		8.1.2	-	1,17E+06	2,21E-02	0,5	1,89E-08	19
		-	1.4.168	1,96E+06	4,19E-03	3	2,14E-09	2
	pINR	8.1.2	1.4.168	1,57E+06	3,70E-02	0,3	2,36E-08	24
		8.1.2	-	1,36E+06	4,45E-02	0,3	3,27E-08	33
		-	1.4.168	n.i.	n.i.	n.i.	n.i.	n.i.
	IGF-1R	8.1.2	1.4.168	2,73E+06	2,66E-03	4,3	9,73E-10	1
		8.1.2	-	n.i.	n.i.	n.i.	n.i.	n.i.
		-	1.4.168	3,30E+06	3,62E-03	3	1,10E-09	1
T0	pIGF-1R	8.1.2	1.4.168	1,75E+06	6,01E-05	192	3,44E-11	0,03
		8.1.2	-	1,03E+06	2,22E-02	1	2,15E-08	22
		-	1.4.168	1,12E+06	2,91E-03	4	2,59E-09	3
	pINR	8.1.2	1.4.168	1,70E+06	4,18E-02	0,3	2,46E-08	25
		8.1.2	-	1,09E+06	4,83E-02	0,2	4,41E-08	44
		-	1.4.168	n.d	n.d.	n.d	n.d.	n.d.
	IGF-1R	8.1.2	1.4.168	1,98E+06	2,38E-03	5	1,20E-09	1
		8.1.2	-	n.i.	n.i.	n.i.	n.i.	n.i.
		-	1.4.168	2,41E+06	3,26E-03	4	1,35E-09	1

Fig. 19

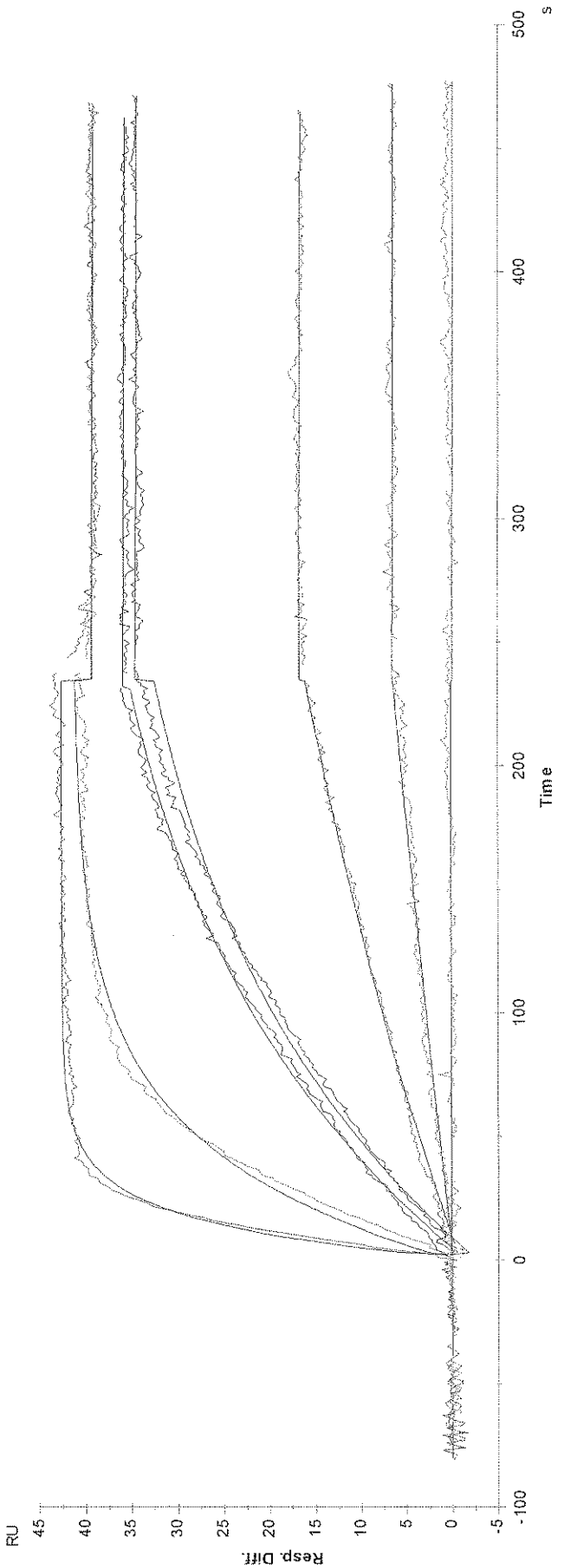

RU
45
40
35
30
25
20
15
10
5
0
-5
Resp. Diff.
-100
0
100
200
300
400
500
s
Time

Fig. 20

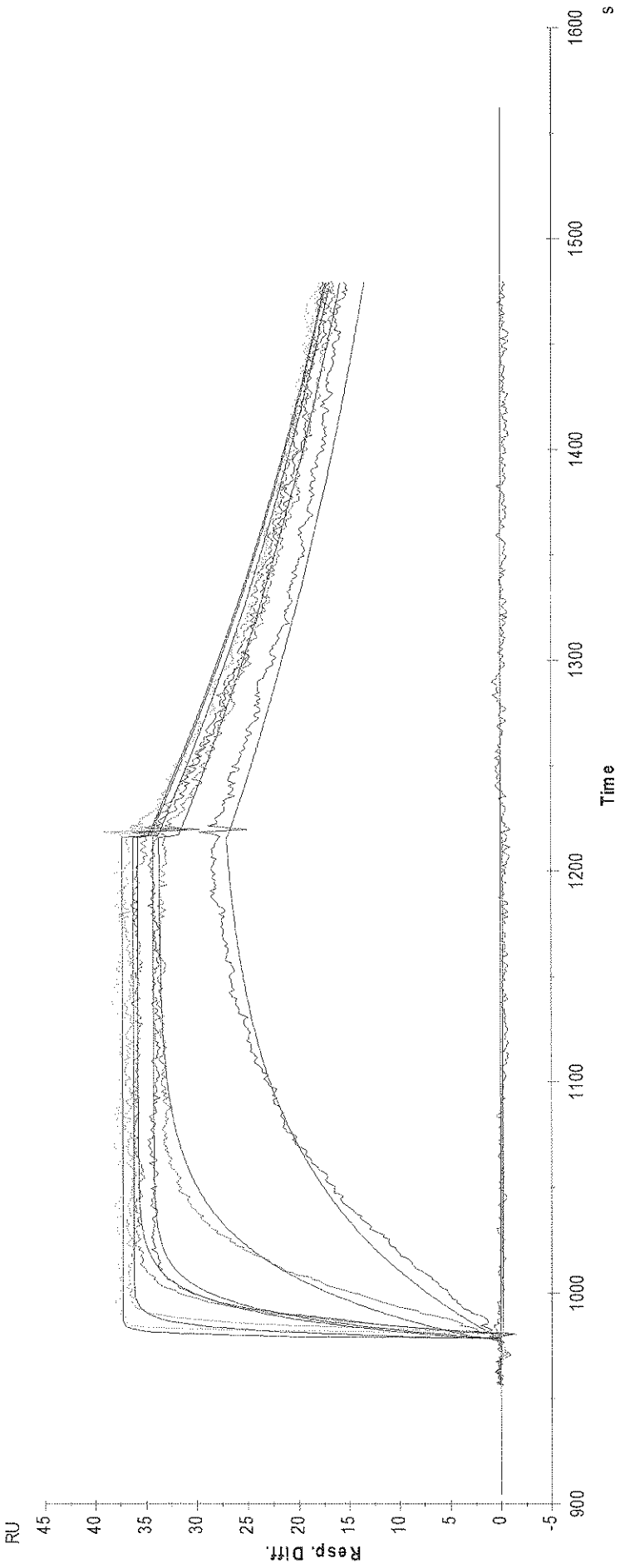

RU
45
40
35
30
25
20
15
10
5
0
-5
Resp. Diff.
900
1000
1100
1200
1300
1400
1500
1600
s
Time

Fig. 21

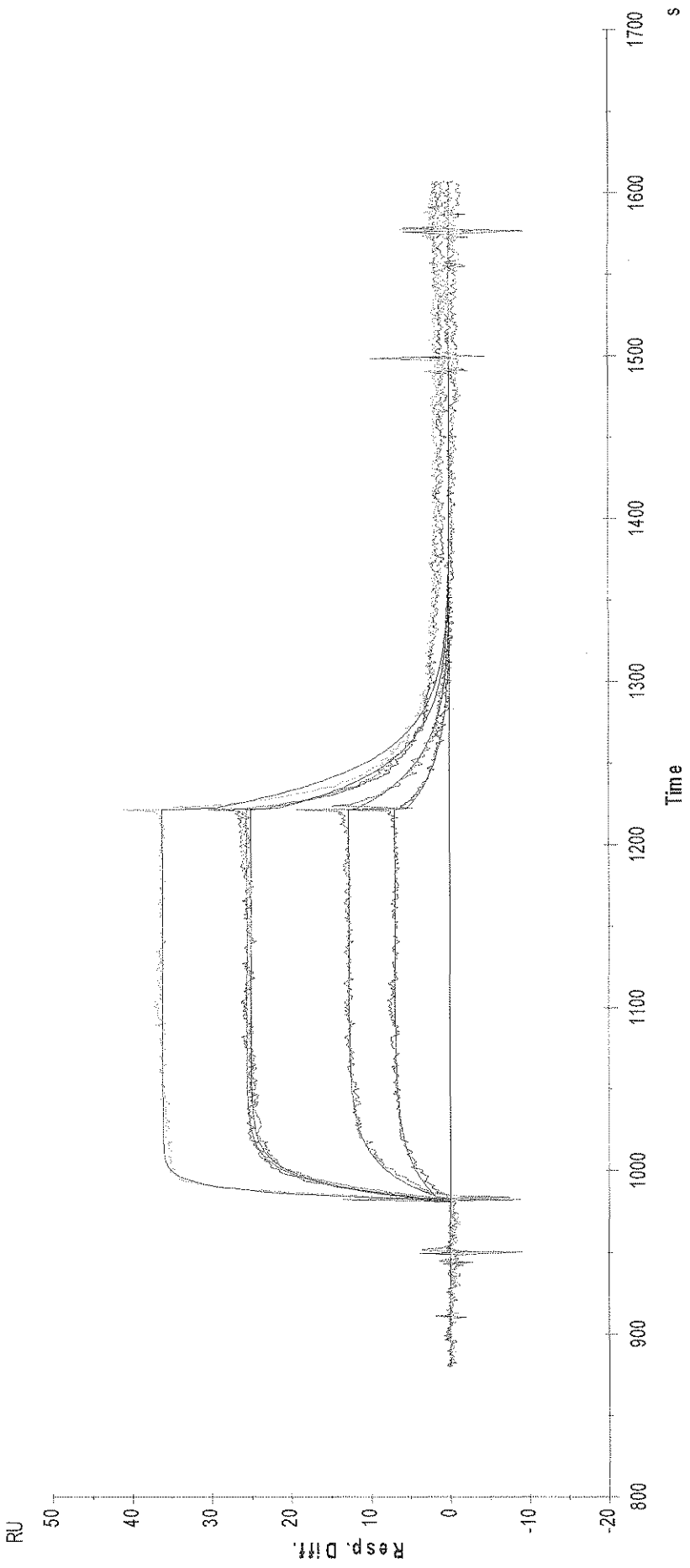

RU
50
40
30
20
10
0
-10
-20
Resp. Diff.
800
900
1000
1100
1200
1300
1400
1500
1600
1700
s
Time

Fig. 22

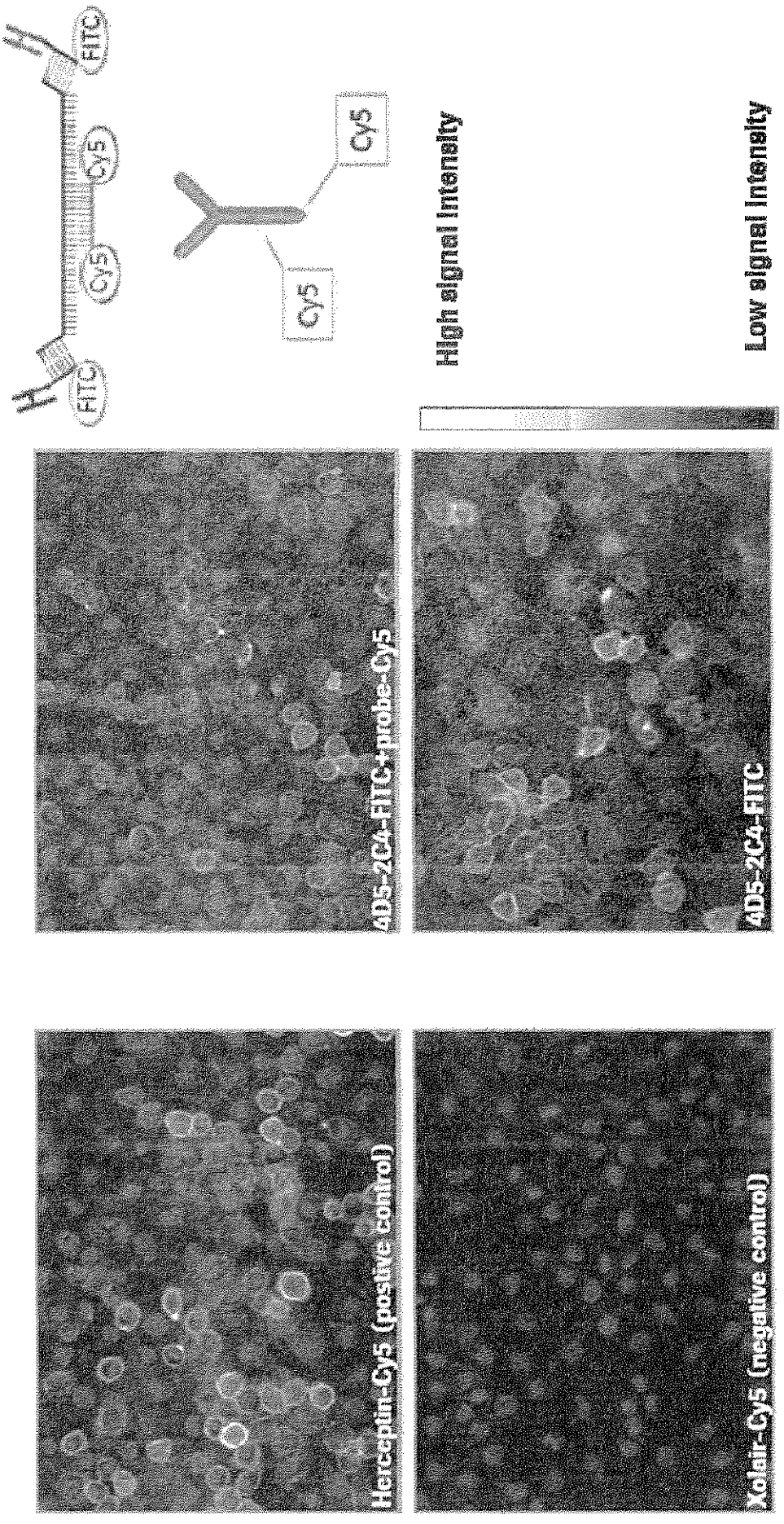

FITC
Cy5
Cy5
FITC
Cy5
Cy5
High signal Intensity
Low signal Intensity
4D5-2C4-FITC+probe-Cy5
4D5-2C4-FITC
Herceptin-Cy5 (positive control)
Xolair-Cy5 (negative control)

Fig. 23

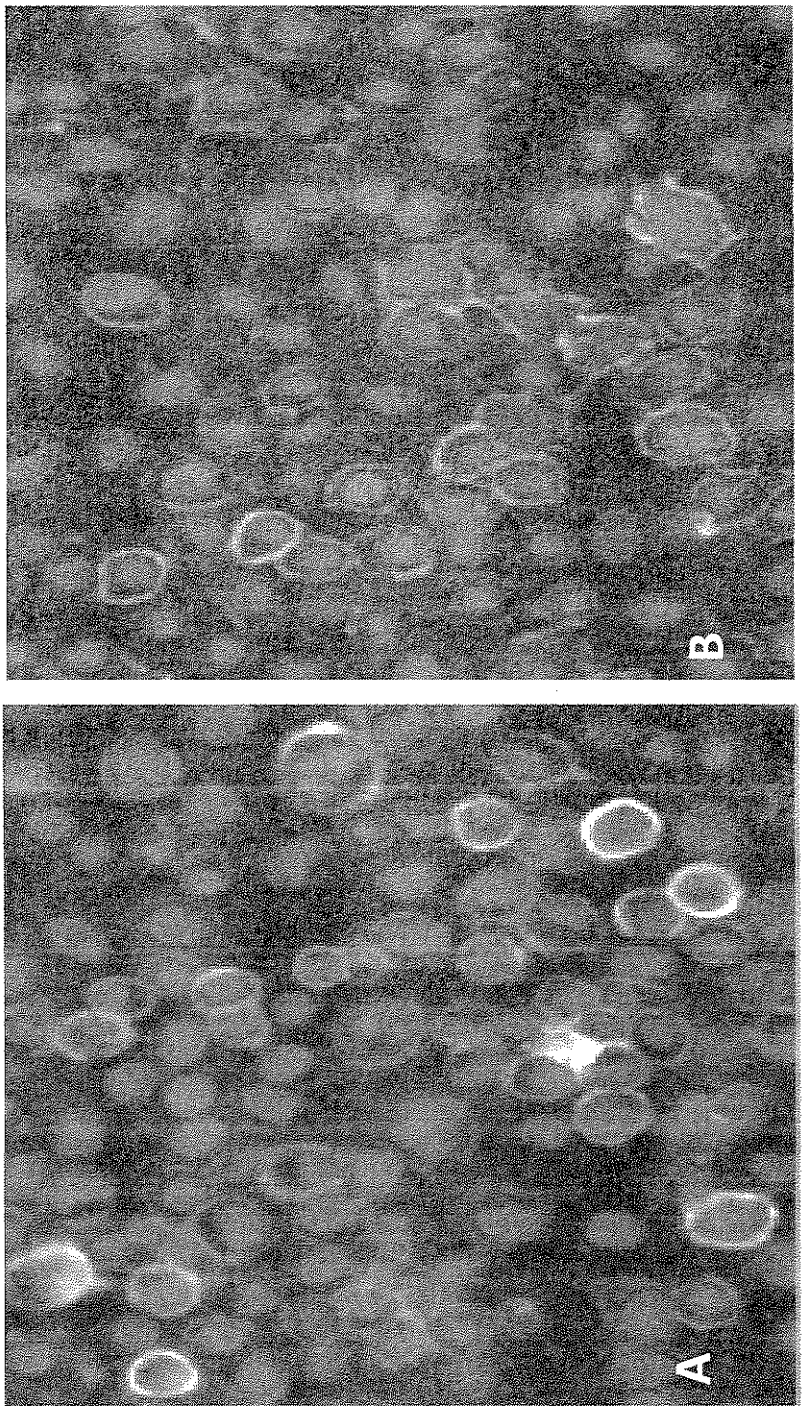
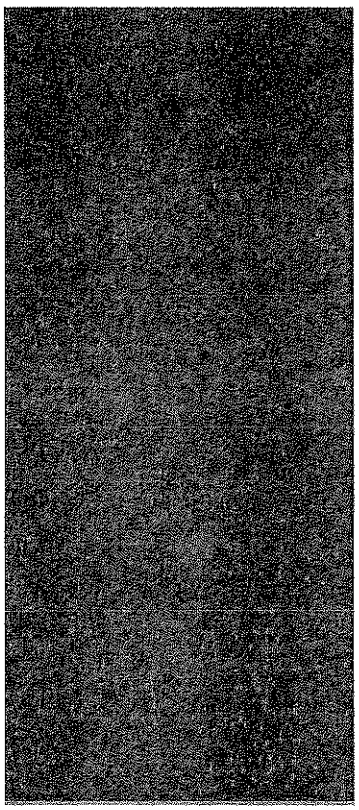


Fig. 24



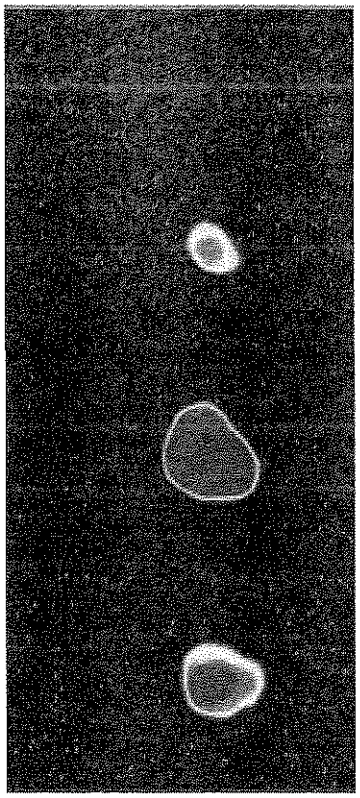


Fig. 25

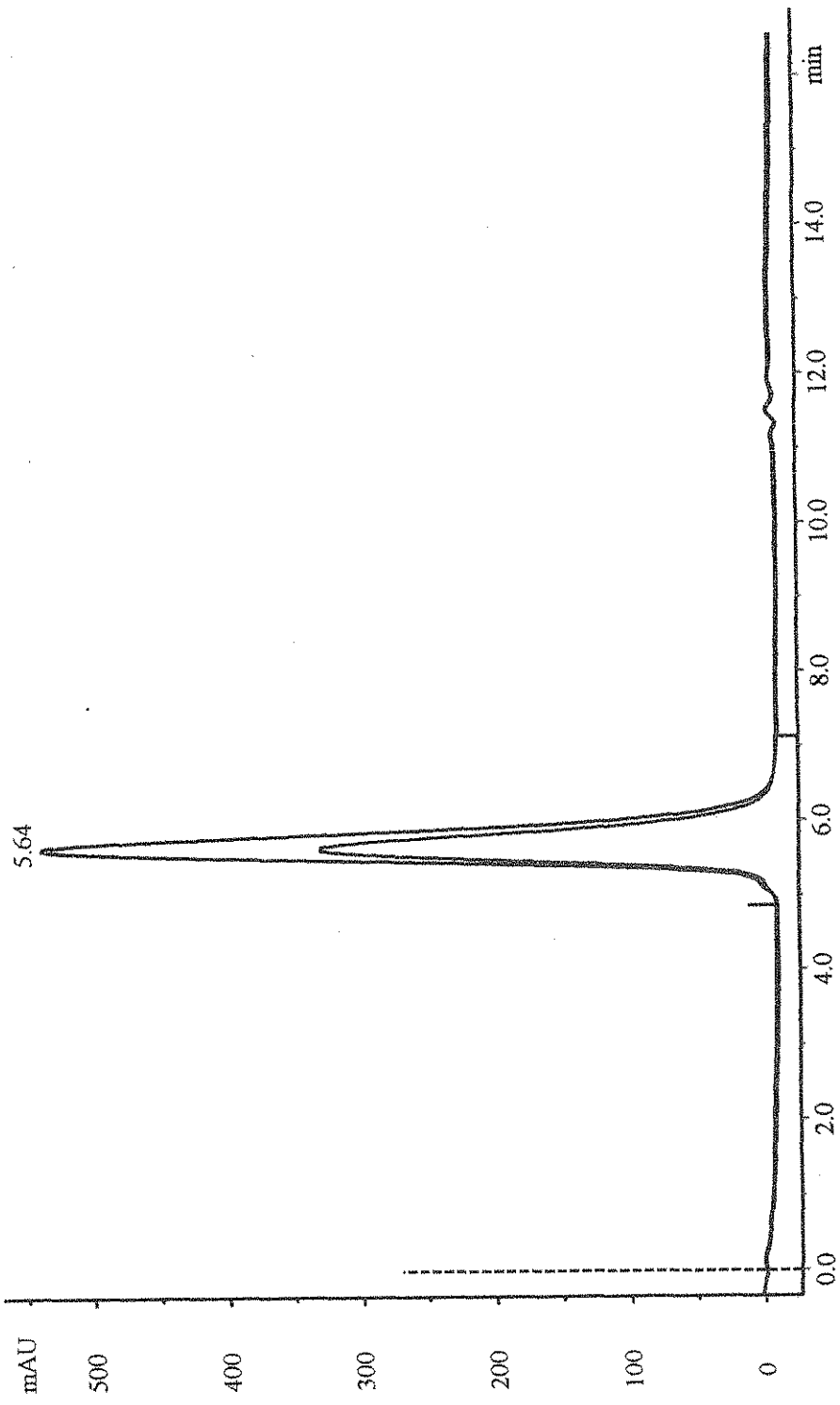
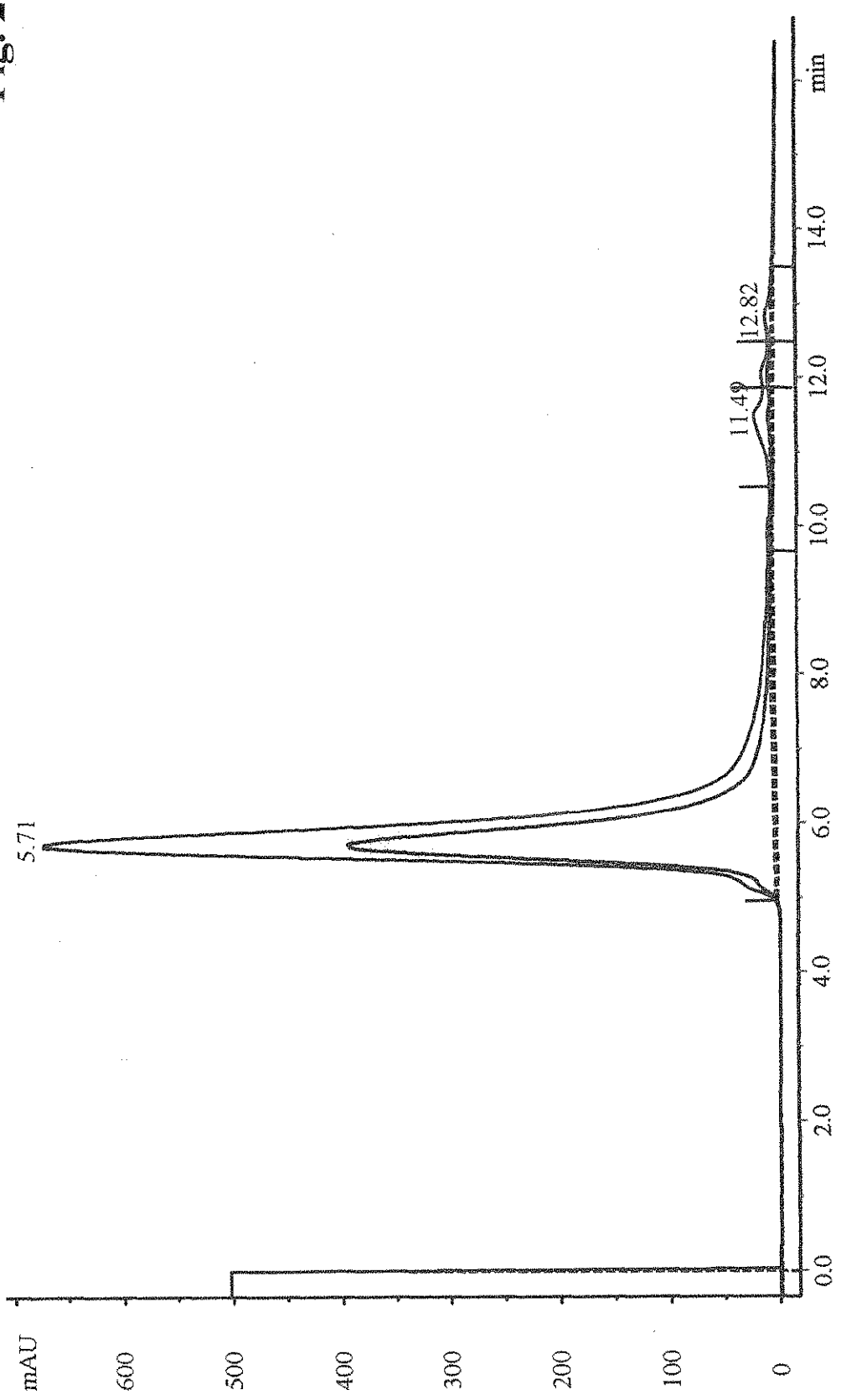


Fig. 26


mAU
600
500
400
300
200
100
0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
min
5.71
11.49
12.82