Figure 1

**ASLDQTPRTA TRETGESLSI NCVLTDTSHI LFGTKWLWNN PGSTDWESIT IGGRYAESVN NQAKSFSLQI KDLTVEDSGT YYCKAQTIGR RKNLLPRPLV NGIAAMGYSS SDYDGAGTVL TVN**

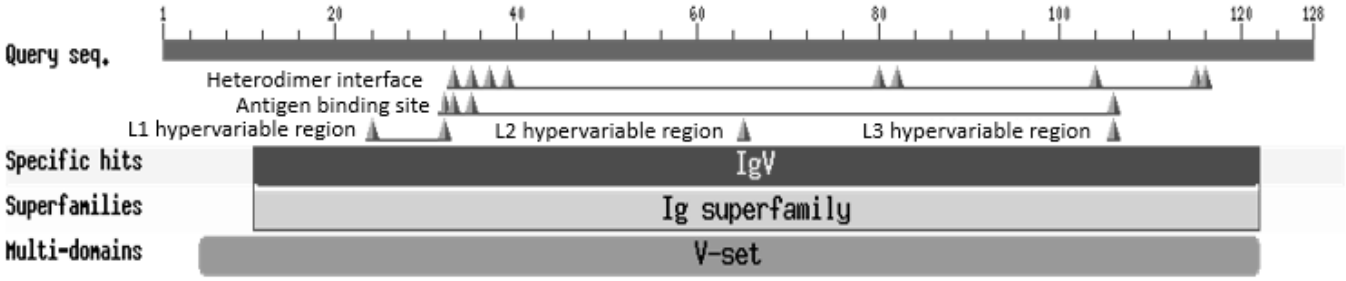


Figure 2

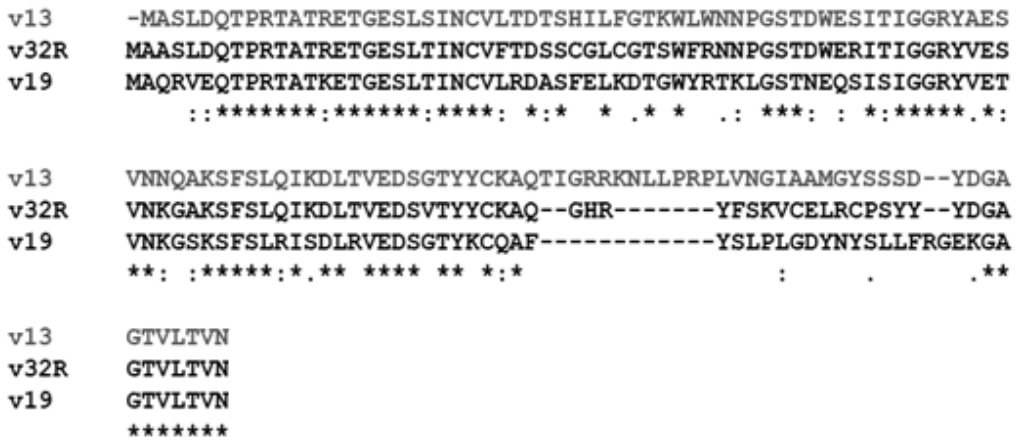
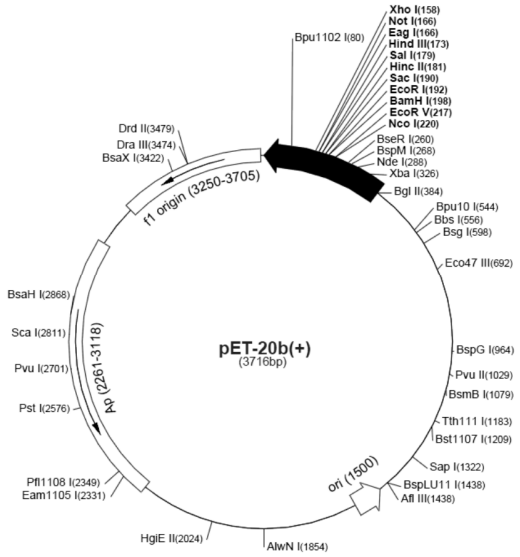

v13 -MASLDQTPRTATRETGESLSINCVLTDTSHILFGTKWLWNNPGSTDWESITIGGRYAES
v32R MAASLDQTPRTATRETGESLTINCVFTDSSCGLCGTSWFRNNPGSTDWERITIGGRYVES
v19 MAQRVEQTPRTATKETGESLTINCVLRDASFELKDTGWYRTKLGSTNEQSISIGGRYVET
::*******:******:****: *:* * .* * .: ***: : *:*****.*:
v13 VNNQAKSFSLQIKDLTVEDSGTYYCKAQTIGRRKNLLPRPLVNGIAAMGYSSSD--YDGA
v32R VNKGAKSFSLQIKDLTVEDSVTYYCKAQ--GHR-------YFSKVCELRCPSYY--YDGA
v19 VNKGSKSFSLRISDLRVEDSGTYKCQAF-----------YSLPLGDYNYSLLFRGEKGA
**: :*****:*.** **** ** *:* : . .**
v13 GTVLTVN
v32R GTVLTVN
v19 GTVLTVN
*******

Figure 3

**A**

| pET-20b(+) sequence landmarks | |
|---|---|
| T7 promoter | 353-369 |
| T7 transcription start | 352 |
| *pelB* coding sequence | 224-289 |
| Multiple cloning sites (*Nco* I - *Xho* I) | 158-225 |
| His•Tag coding sequence | 140-157 |
| T7 terminator | 26-72 |
| pBR322 origin | 1500 |
| *bla* coding sequence | 2261-3118 |
| f1 origin | 3250-3705 |




**B**

| pET-28a(+) sequence landmarks | |
|---|---|
| T7 promoter | 370-386 |
| T7 transcription start | 369 |
| His•Tag coding sequence | 270-287 |
| T7•Tag coding sequence | 207-239 |
| Multiple cloning sites (*Bam*H I - *Xho* I) | 158-203 |
| His•Tag coding sequence | 140-157 |
| T7 terminator | 26-72 |
| *lacI* coding sequence | 773-1852 |
| pBR322 origin | 3286 |
| Kan coding sequence | 3995-4807 |
| f1 origin | 4903-5358 |

The maps for pET-28b(+) and pET-28c(+) are the same as pET-28a(+) (shown) with the following exceptions: pET-28b(+) is a 5368bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-28c(+) is a 5367bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198.

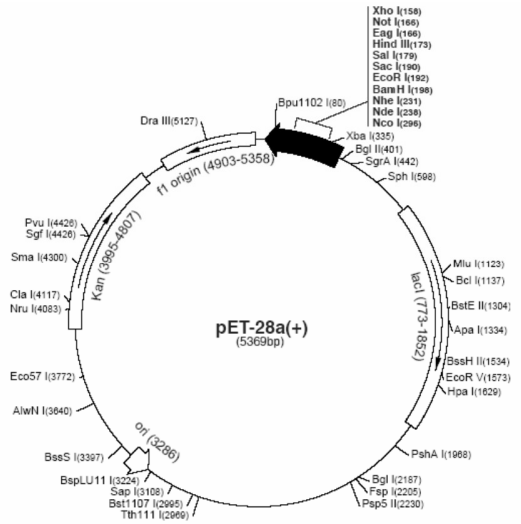


Figure 4

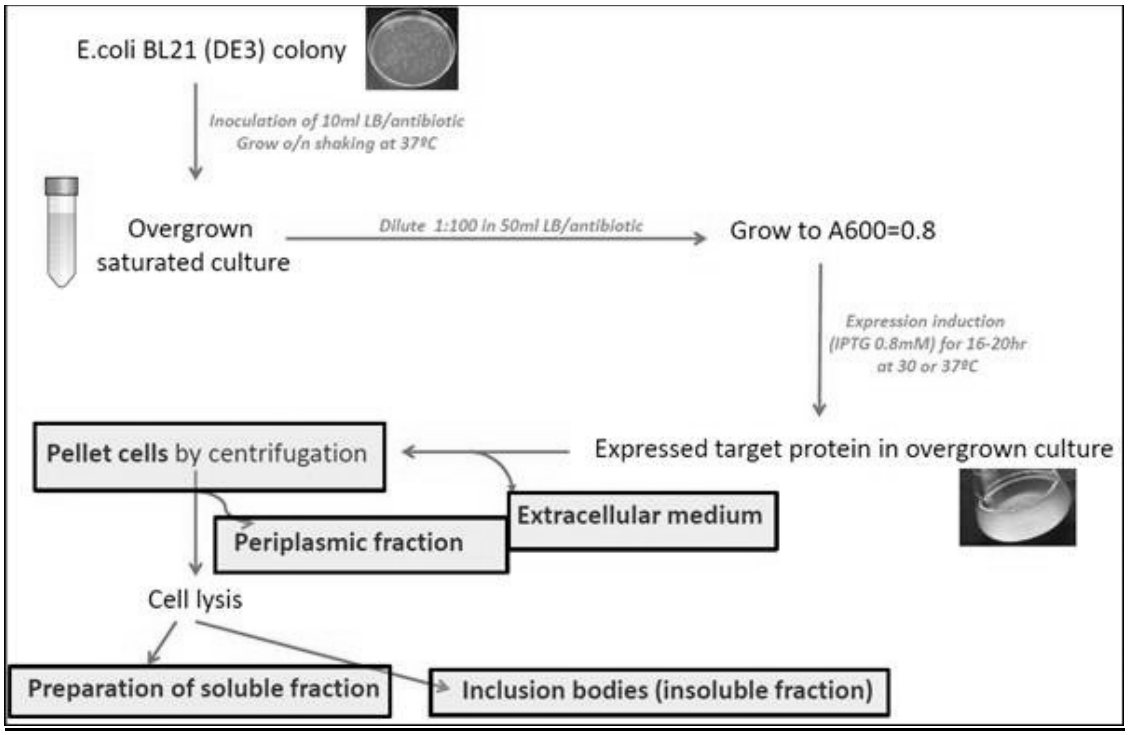

E.coli BL21 (DE3) colony
Inoculation of 10ml LB/antibiotic
Grow o/n shaking at 37ºC
Overgrown
saturated culture
Dilute 1:100 in 50ml LB/antibiotic
Grow to A600=0.8
Expression induction
(IPTG 0.8mM) for 16-20hr
at 30 or 37ºC
Expressed target protein in overgrown culture
Pellet cells by centrifugation
Extracellular medium
Periplasmic fraction
Cell lysis
Preparation of soluble fraction
Inclusion bodies (insoluble fraction)

Figure 5

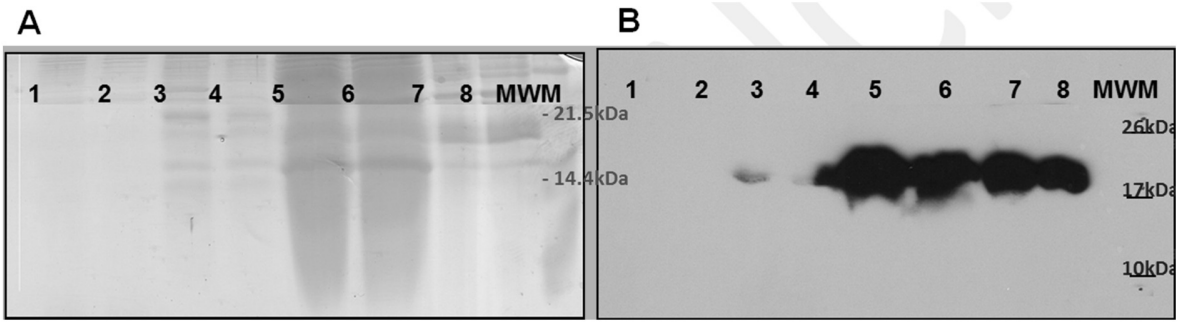



Figure 6

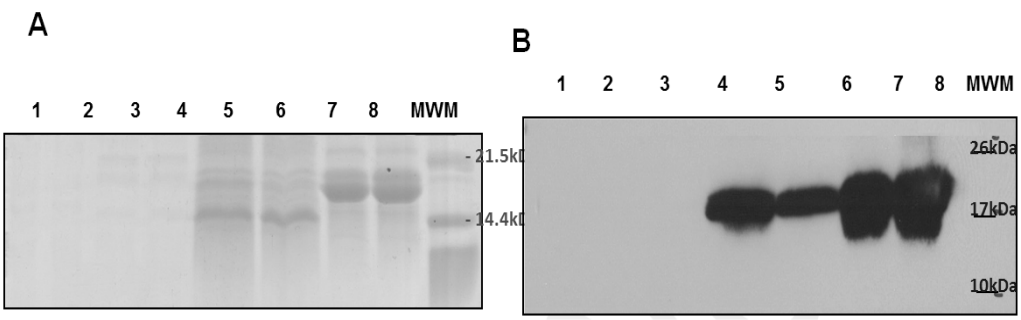


Figure 7

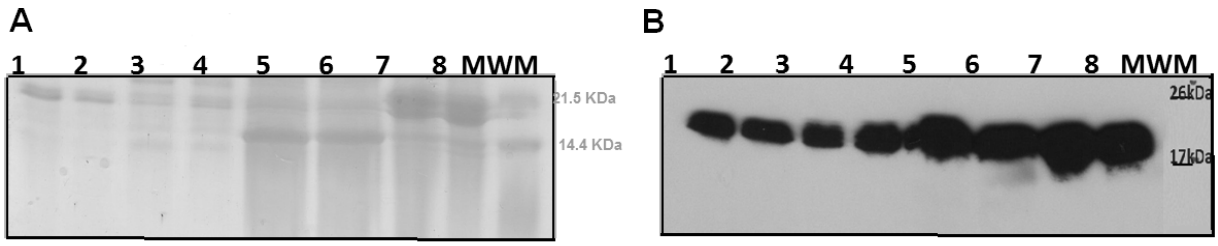



Figure 8

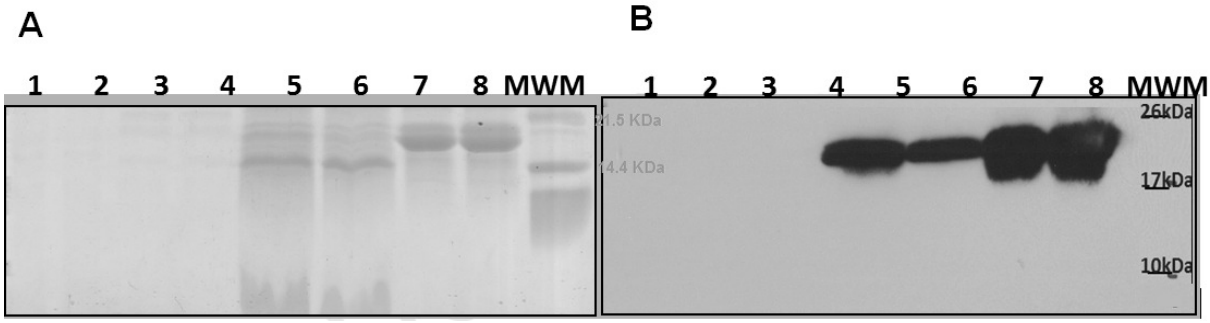


Figure 9

A

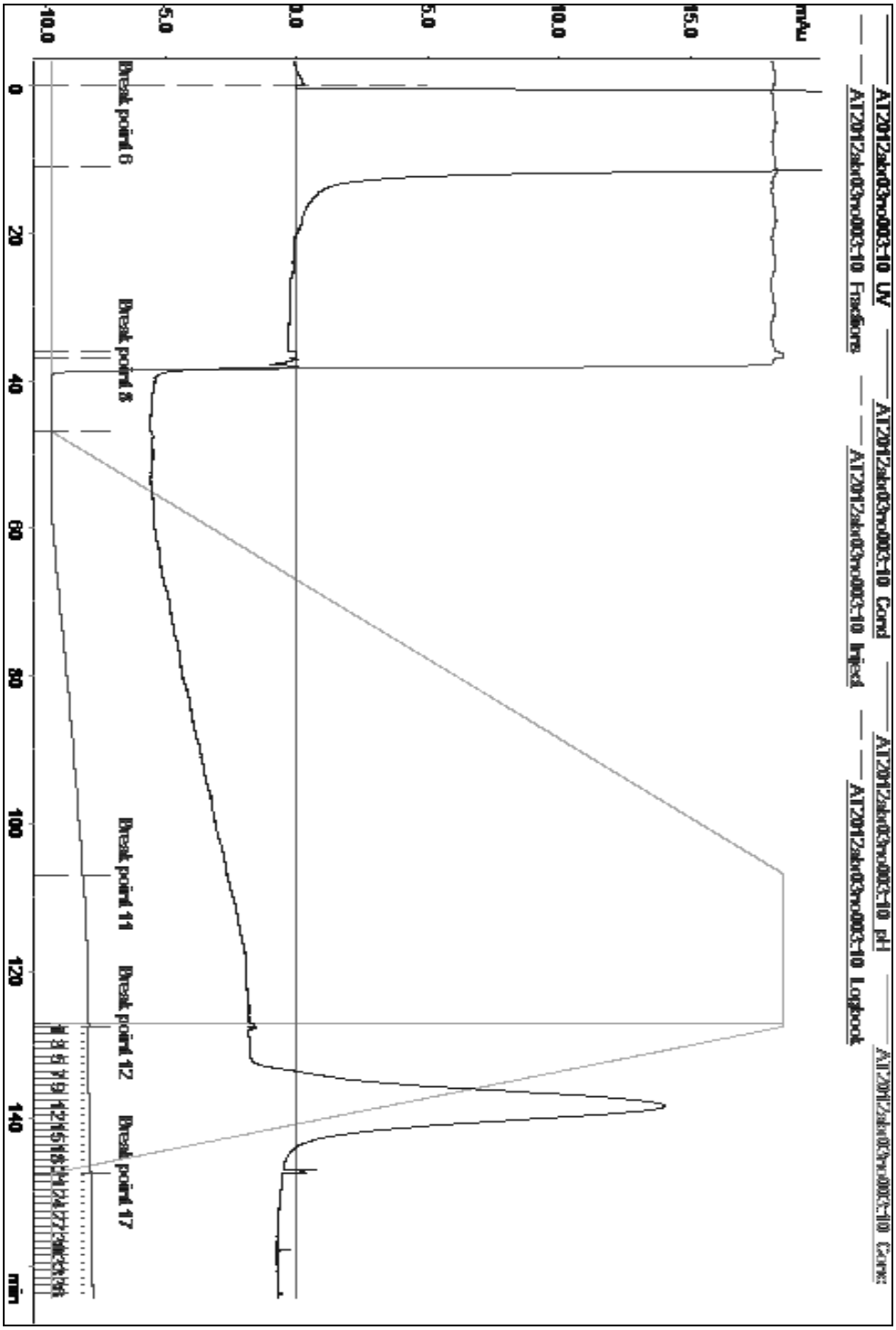

AT2012akv03nc0003-10 UV
AT2012akv03nc0003-10 Fractions
AT2012akv03nc0003-10 Cond
AT2012akv03nc0003-10 Injekt
AT2012akv03nc0003-10 pH
AT2012akv03nc0003-10 Logbook
mAu
15.0
10.0
5.0
0.0
-5.0
-10.0
Break point 6
Break point 8
Break point 11
Break point 12
Break point 17
0
20
40
60
80
100
120
140
min

Figure 9

B

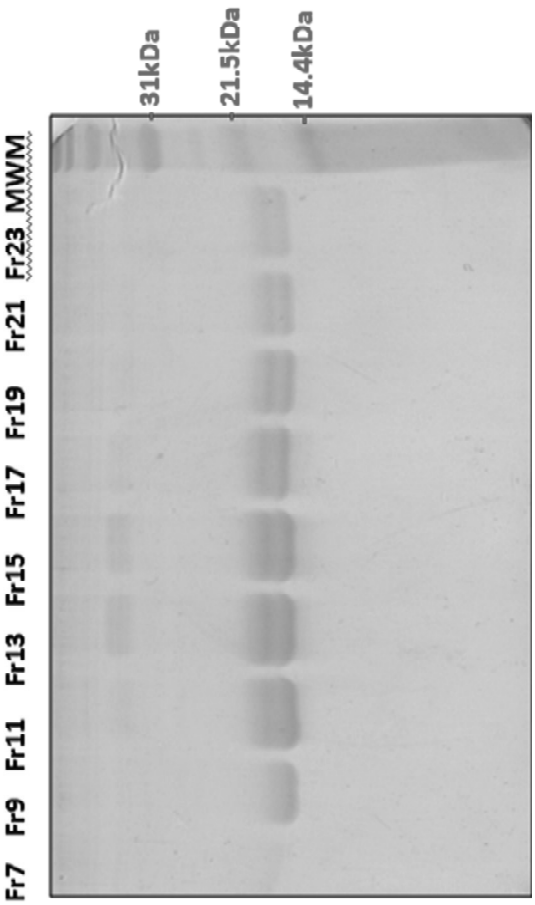

31kDa
21.5kDa
14.4kDa
Fr23
Fr21
Fr19
Fr17
Fr15
Fr13
Fr11
Fr9
Fr7
MWM

Figure 10

A

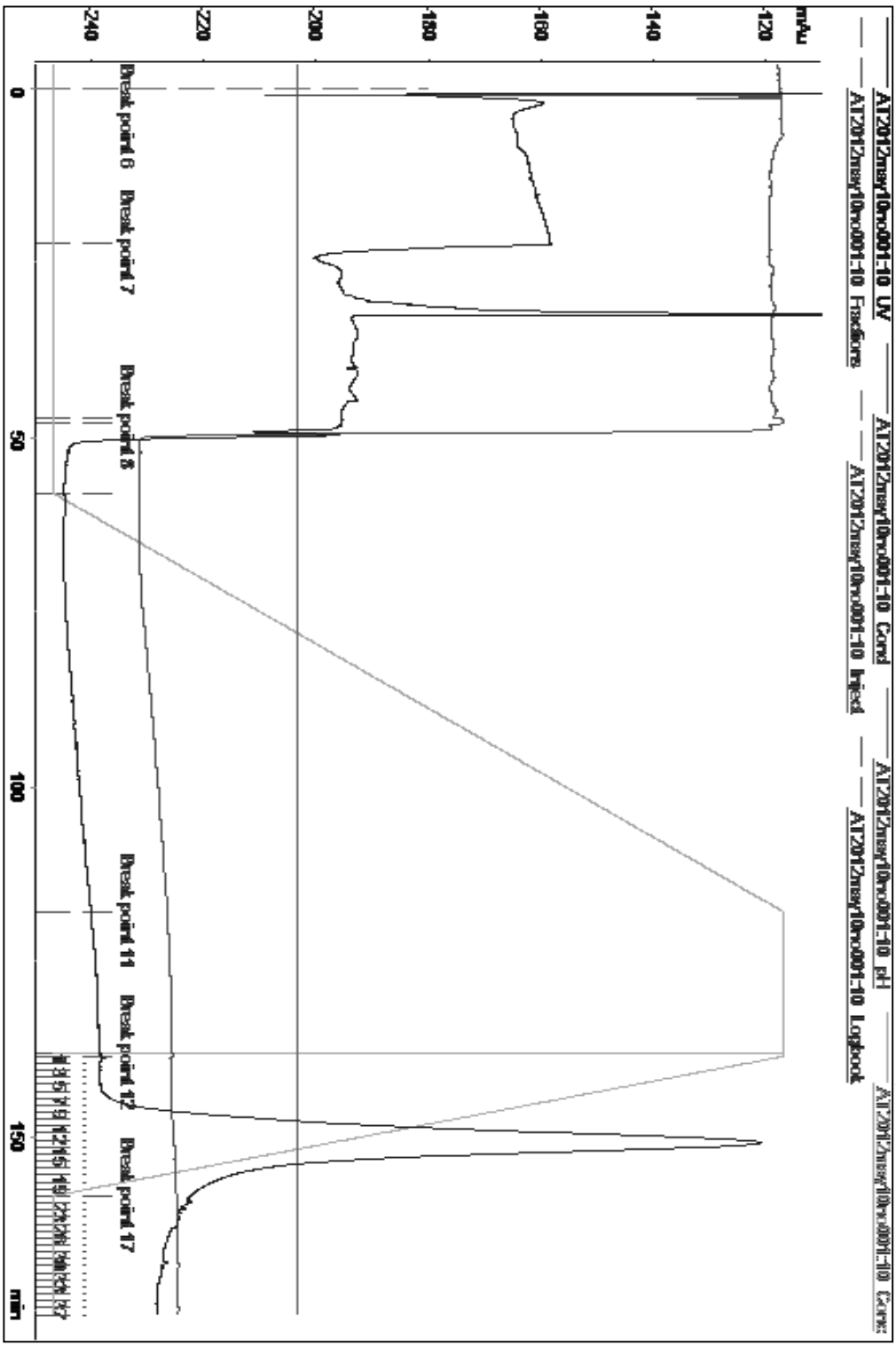

AT2012nasy10no001-10 UV
AT2012nasy10no001-10 Fractions
AT2012nasy10no001-10 Cond
AT2012nasy10no001-10 Inlet
AT2012nasy10no001-10 pH
AT2012nasy10no001-10 Logbook
mAu
120
140
160
180
200
220
240
Break point 6
Break point 7
Break point 8
Break point 11
Break point 12
Break point 17
0
50
100
150
min

Figure 10

**B**

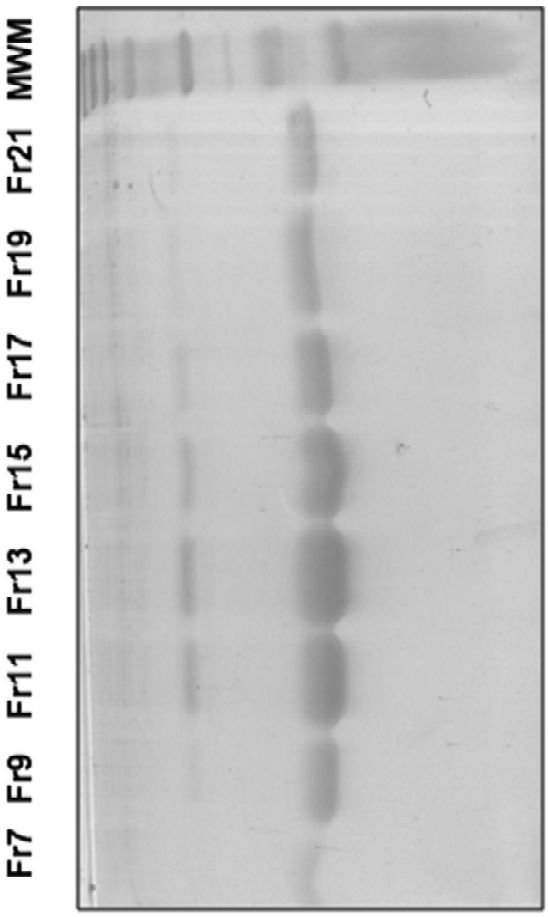


Figure 11

A

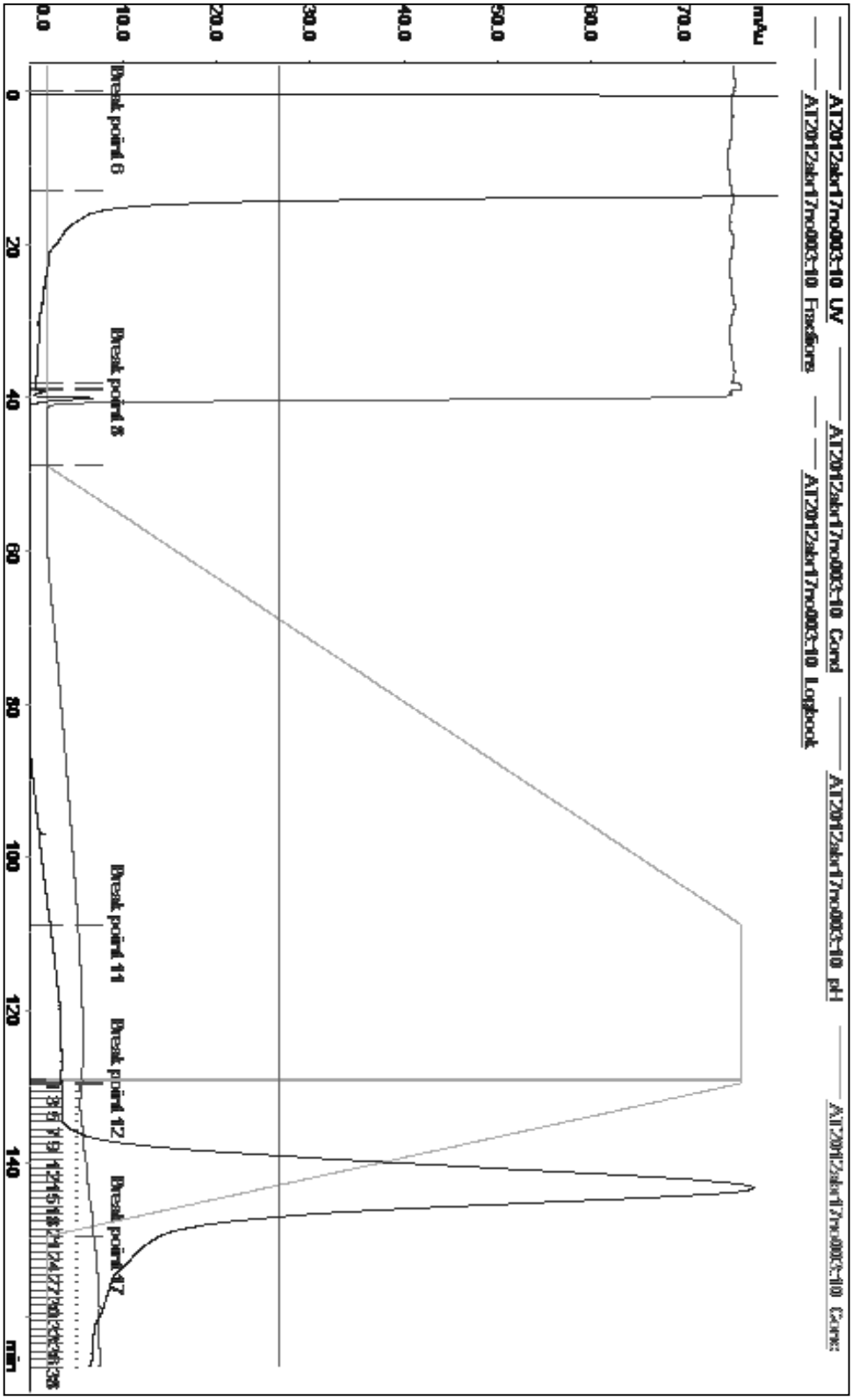

AT2012akr17no0003-10 UV
AT2012akr17no0003-10 Fractions
AT2012akr17no0003-10 Cond
AT2012akr17no0003-10 Logbook
AT2012akr17no0003-10 pH
mAu
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Break point 6
Break point 8
Break point 11
Break point 12
Break point 17
0
20
40
60
80
100
120
140
min

Figure 11

**B**

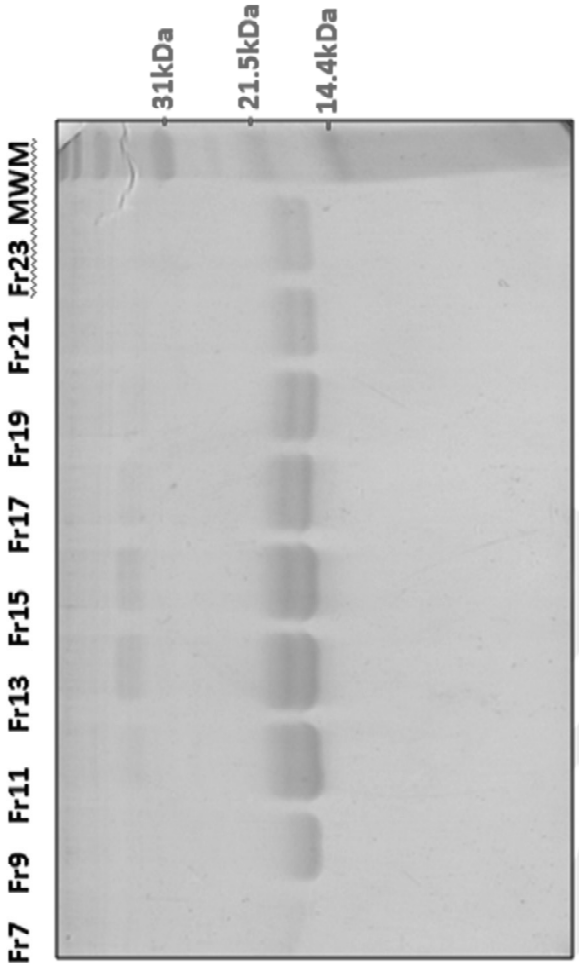

31kDa
21.5kDa
14.4kDa
MWM
Fr23
Fr21
Fr19
Fr17
Fr15
Fr13
Fr11
Fr9
Fr7

Figure 12

A

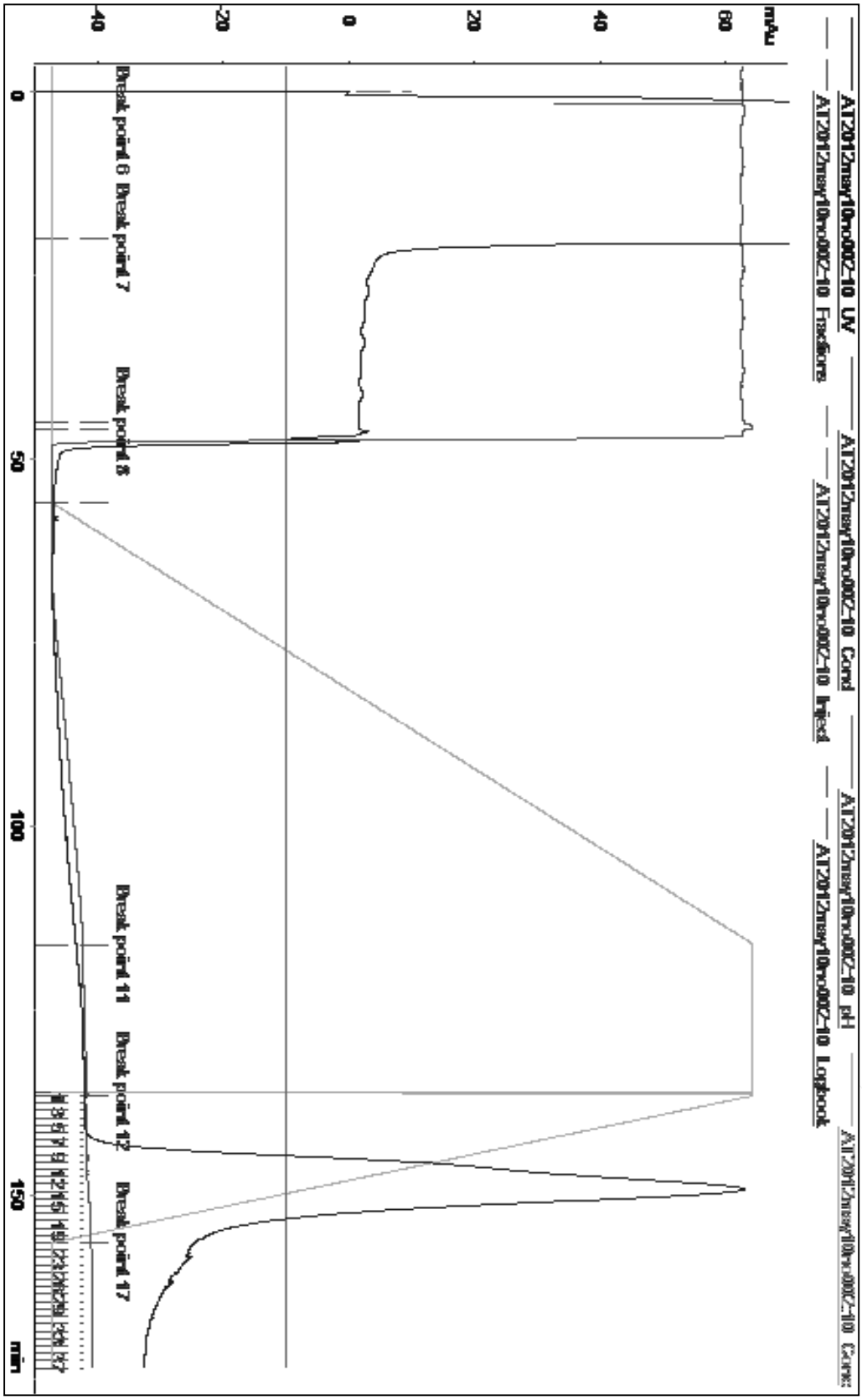

AT2012mary10no0002-10 UV
AT2012mary10no0002-10 Cond
AT2012mary10no0002-10 pH
AT2012mary10no0002-10 Conc
AT2012mary10no0002-10 Fractions
AT2012mary10no0002-10 Inject
AT2012mary10no0002-10 Logbook
mAu
80
60
40
20
0
ml
0
50
100
150
Break point 6
Break point 7
Break point 8
Break point 11
Break point 12
Break point 17

Figure 12

**B**

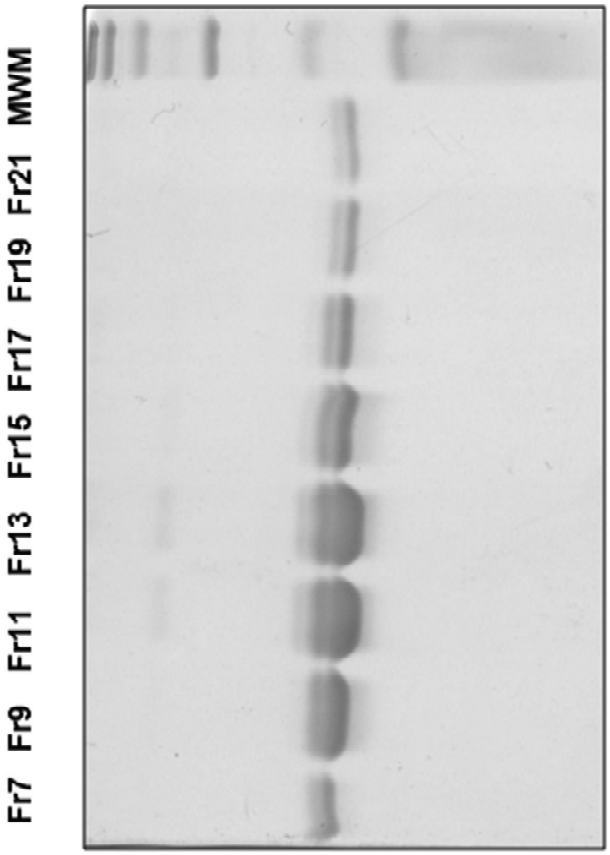


Figure 13

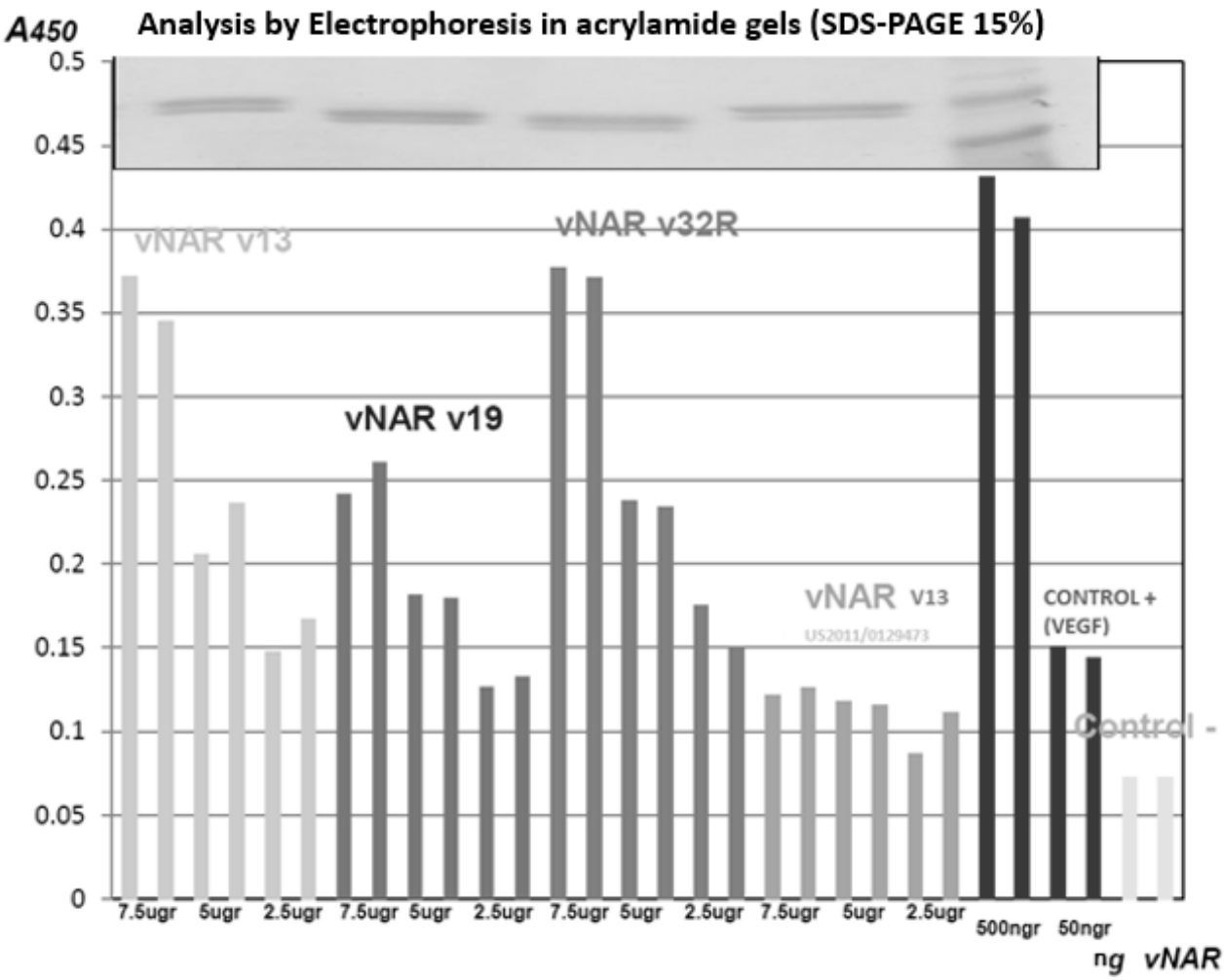

A450
Analysis by Electrophoresis in acrylamide gels (SDS-PAGE 15%)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
vNAR v13
vNAR v32R
vNAR v19
vNAR V13
US2011/0129473
CONTROL +
(VEGF)
Control -
7.5ugr
5ugr
2.5ugr
7.5ugr
5ugr
2.5ugr
7.5ugr
5ugr
2.5ugr
7.5ugr
5ugr
2.5ugr
500ngr
50ngr
ng vNAR

Figure 14

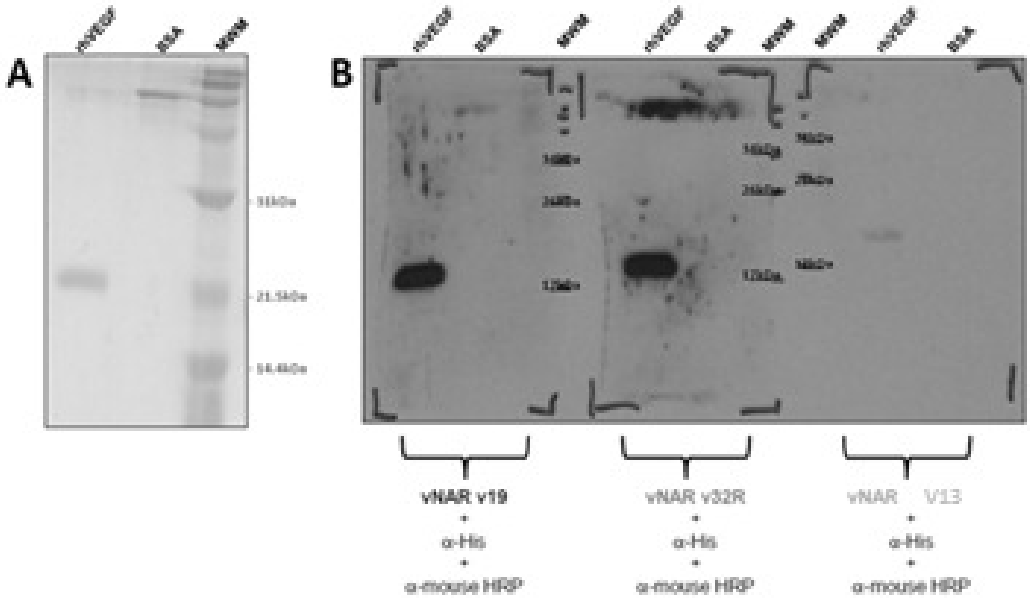



Figure 15

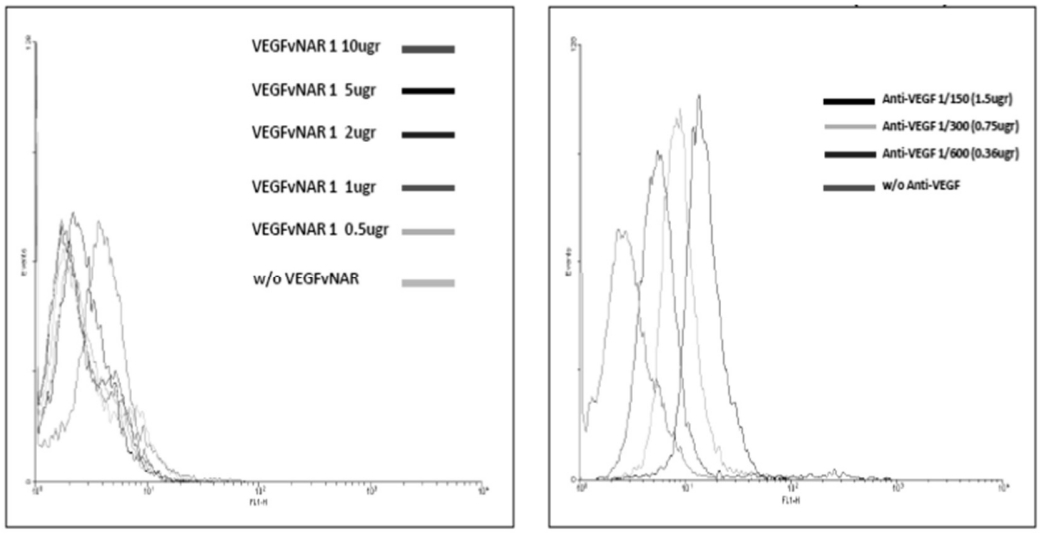


Figure 16

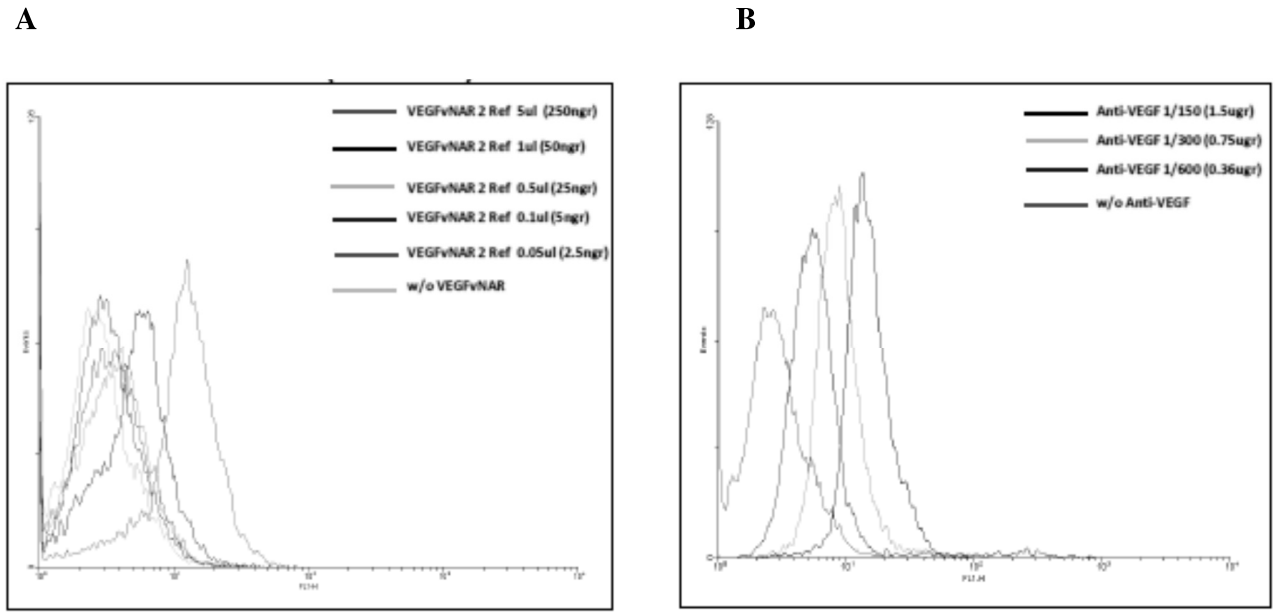



Figure 17

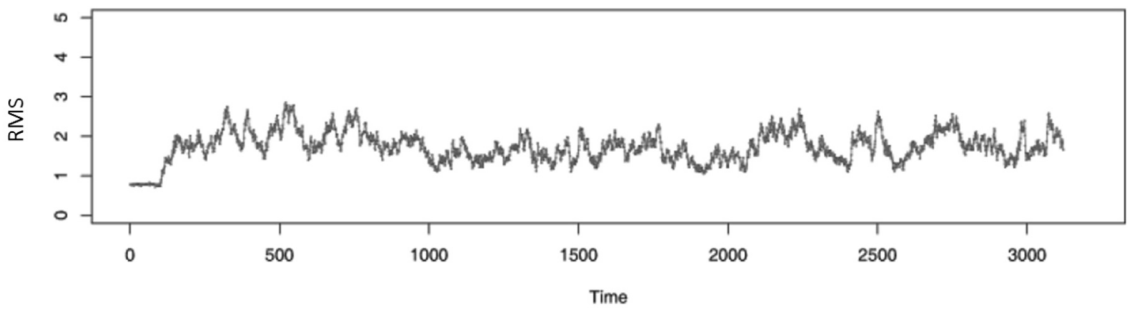


Figure 18

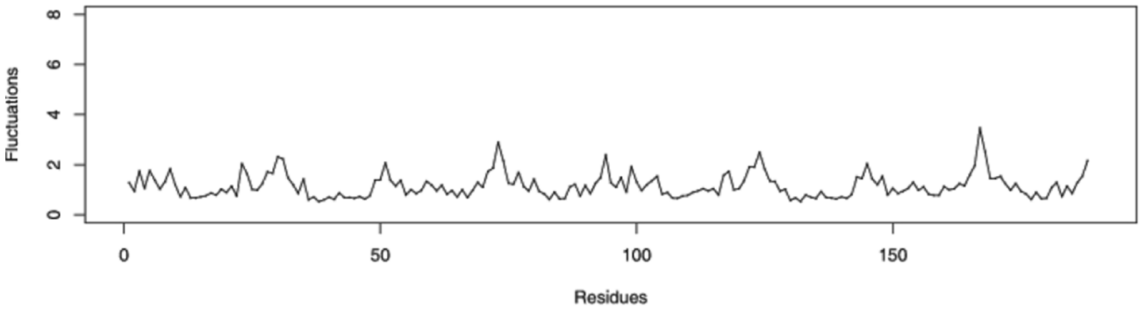


Figure 19

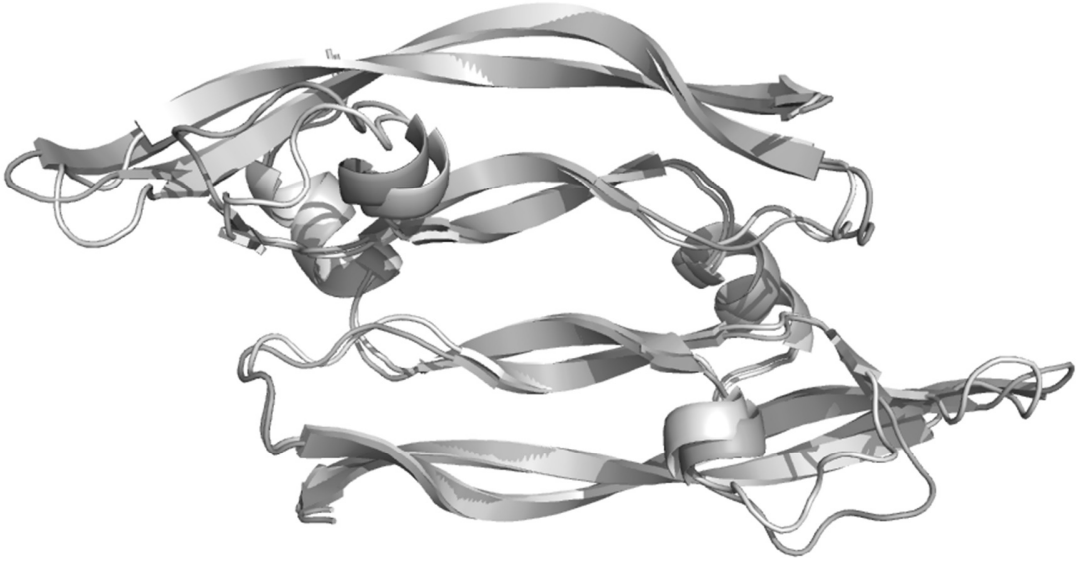


Figure 20

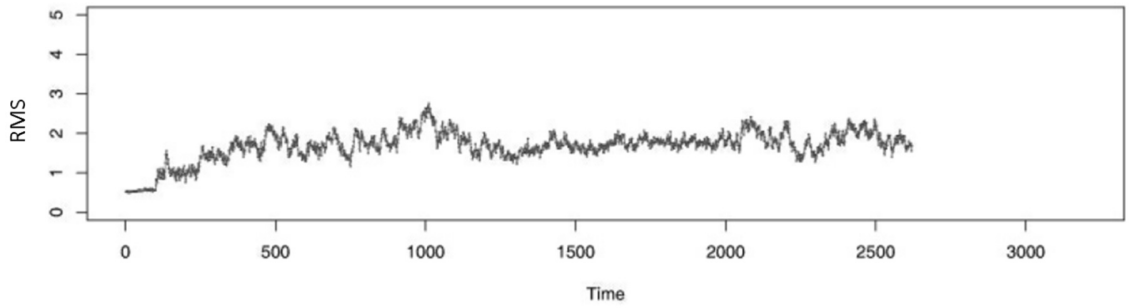



Figure 21

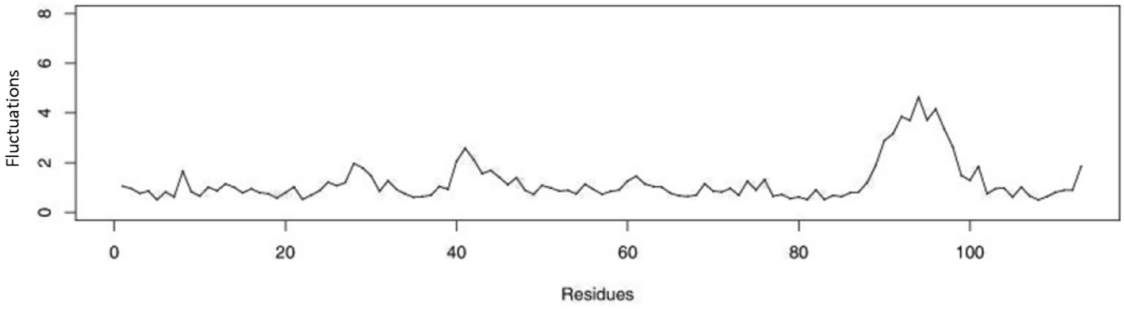


Figure 22

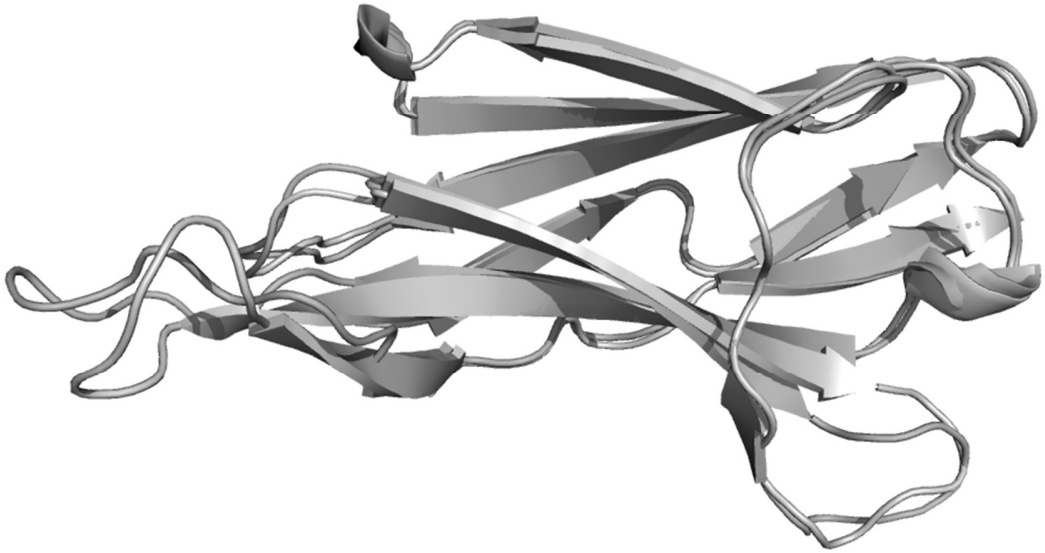


Figure 23

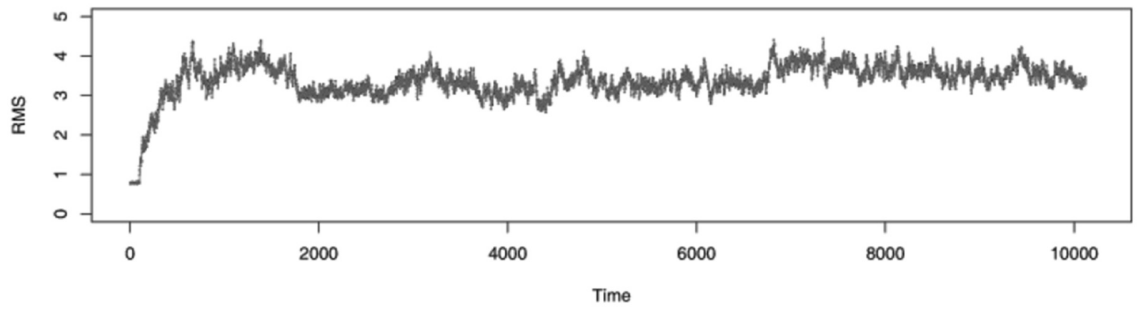


Figure 24

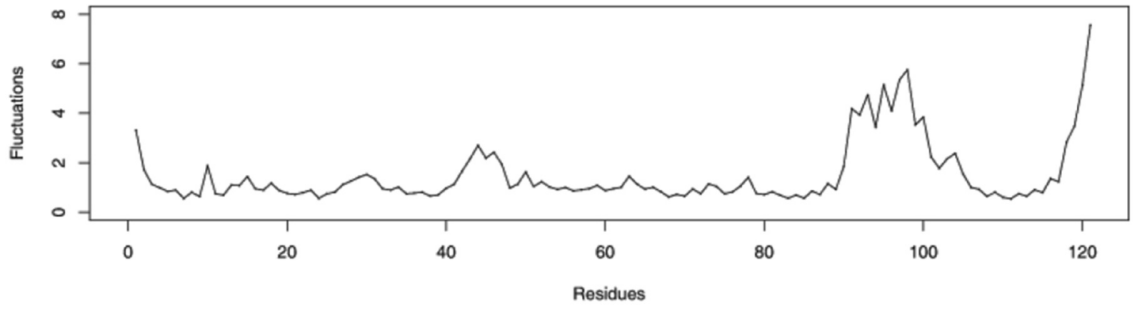



Figure 25



Figure 26

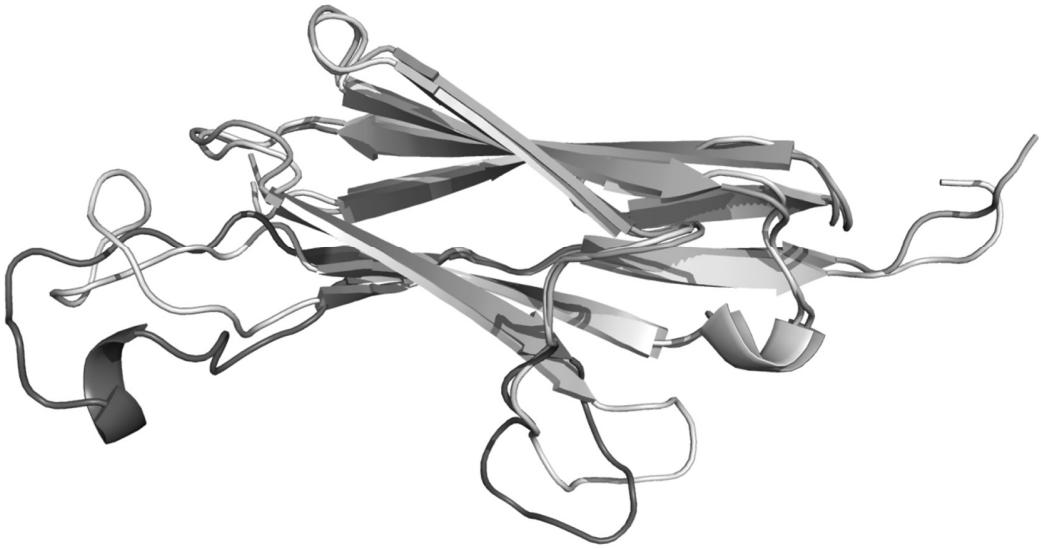


Figure 27

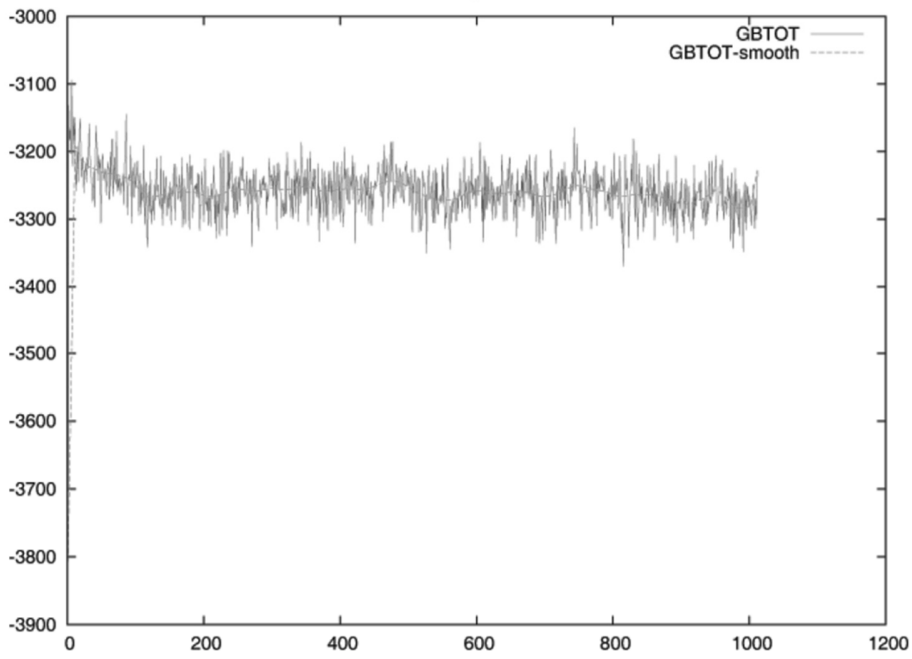


Figure 28

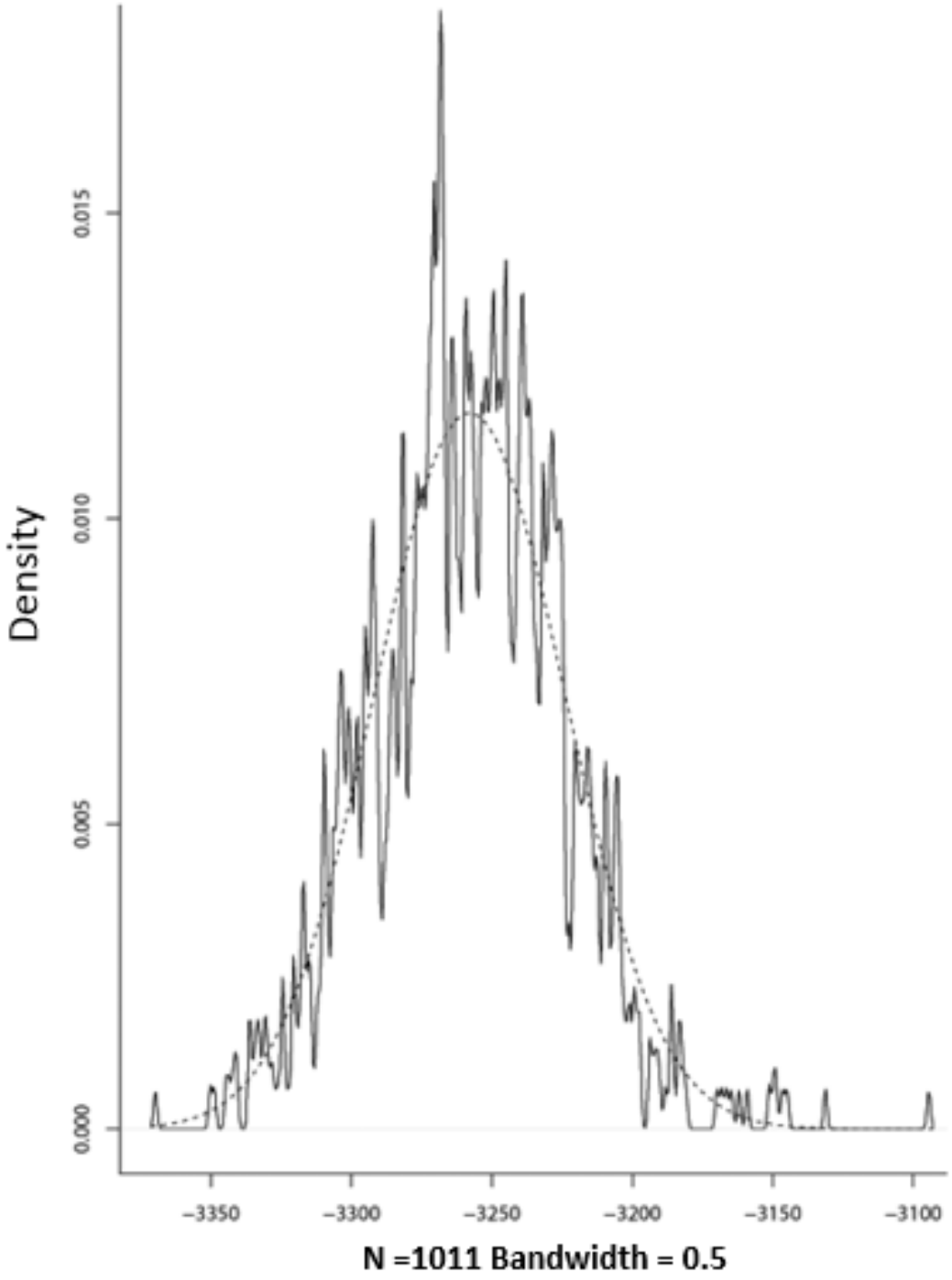

Density
0.000
0.005
0.010
0.015
−3350
−3300
−3250
−3200
−3150
−3100
N =1011 Bandwidth = 0.5

Figure 29



Figure 30

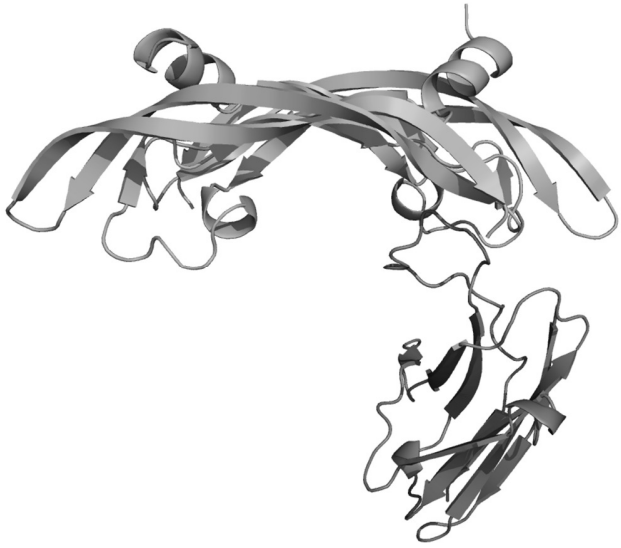


Figure 31

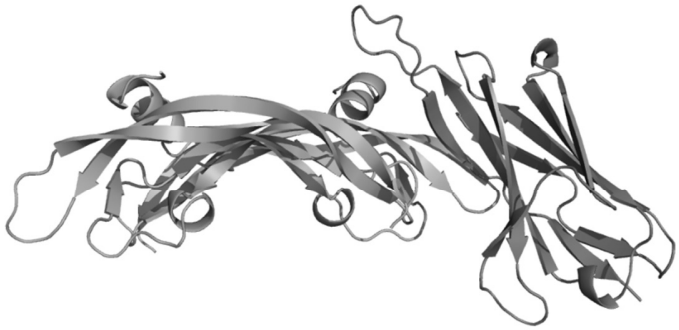


Figure 32



Figure 33

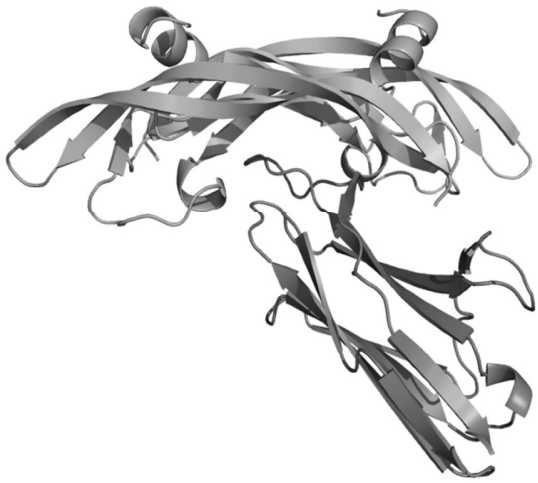


Figure 34

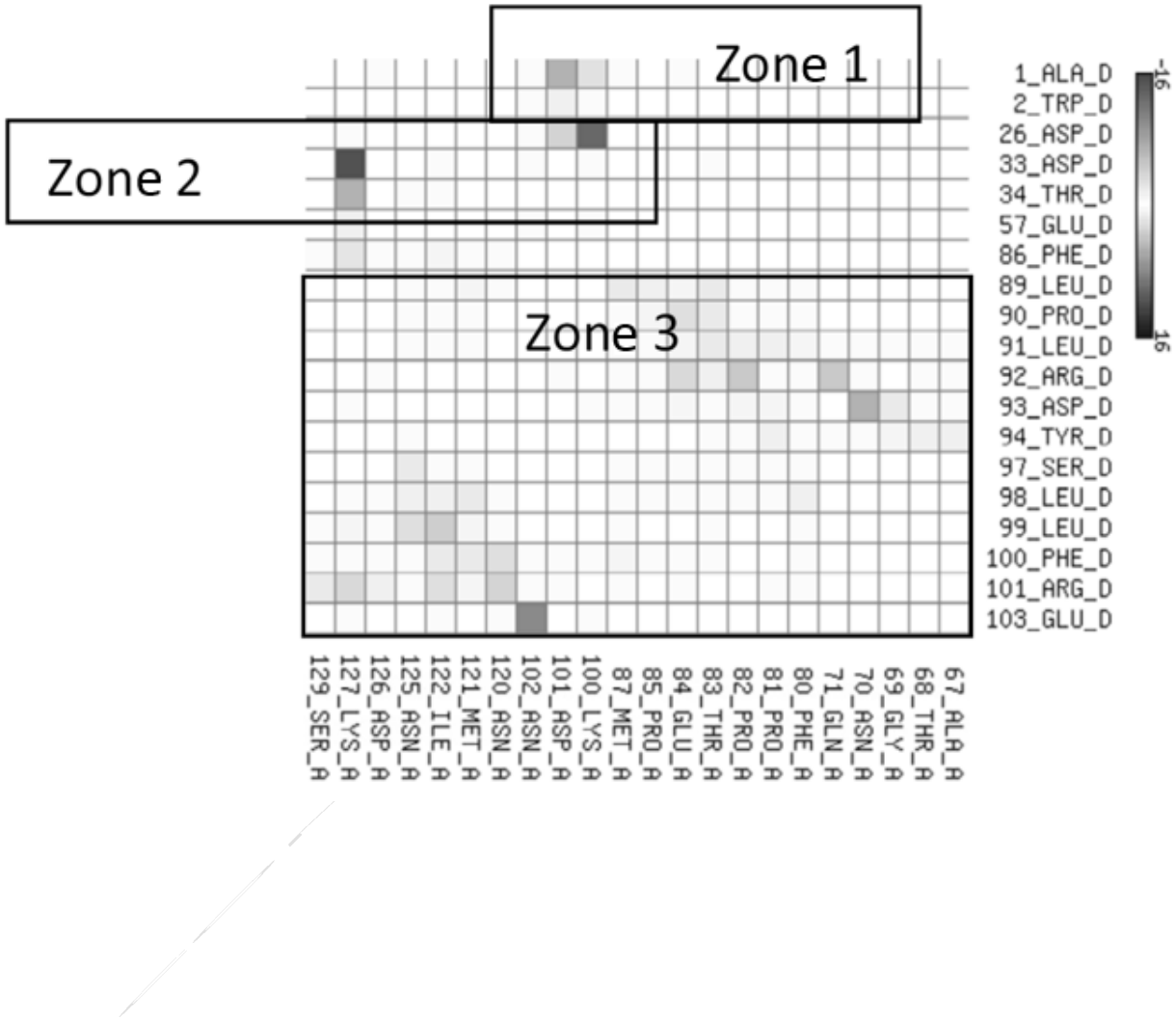

Zone 1
Zone 2
Zone 3
1_ALA_D
2_TRP_D
26_ASP_D
33_ASP_D
34_THR_D
57_GLU_D
86_PHE_D
89_LEU_D
90_PRO_D
91_LEU_D
92_ARG_D
93_ASP_D
94_TYR_D
97_SER_D
98_LEU_D
99_LEU_D
100_PHE_D
101_ARG_D
103_GLU_D
-16
16
129_SER_A
127_LYS_A
126_ASP_A
125_ASN_A
122_ILE_A
121_MET_A
120_ASN_A
102_ASN_A
101_ASP_A
100_LYS_A
87_MET_A
85_PRO_A
84_GLU_A
83_THR_A
82_PRO_A
81_PRO_A
80_PHE_A
71_GLN_A
70_ASN_A
69_GLY_A
68_THR_A
67_ALA_A

Figure 35a

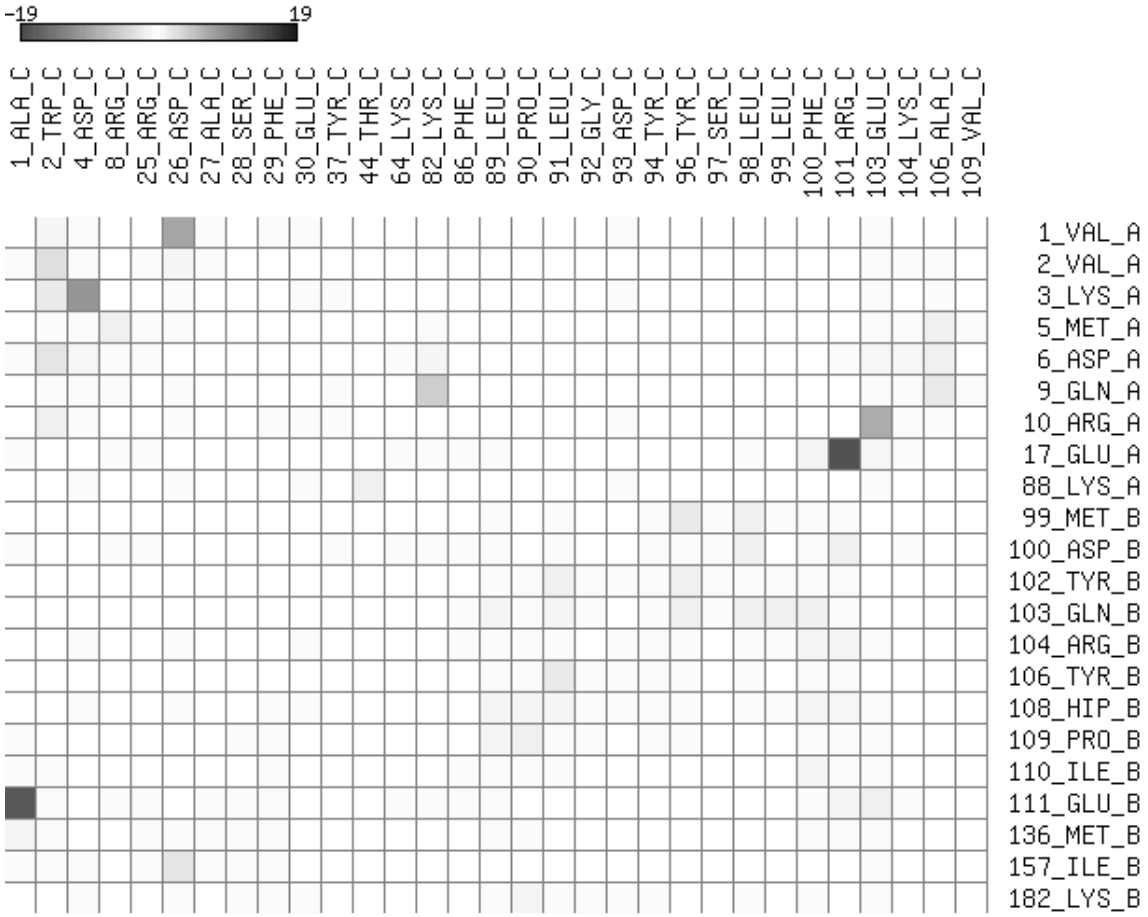

-19
19
1_ALA_C
2_TRP_C
4_ASP_C
8_ARG_C
25_ARG_C
26_ASP_C
27_ALA_C
28_SER_C
29_PHE_C
30_GLU_C
37_TYR_C
44_THR_C
64_LYS_C
82_LYS_C
86_PHE_C
89_LEU_C
90_PRO_C
91_LEU_C
92_GLY_C
93_ASP_C
94_TYR_C
96_TYR_C
97_SER_C
98_LEU_C
99_LEU_C
100_PHE_C
101_ARG_C
103_GLU_C
104_LYS_C
106_ALA_C
109_VAL_C
1_VAL_A
2_VAL_A
3_LYS_A
5_MET_A
6_ASP_A
9_GLN_A
10_ARG_A
17_GLU_A
88_LYS_A
99_MET_B
100_ASP_B
102_TYR_B
103_GLN_B
104_ARG_B
106_TYR_B
108_HIP_B
109_PRO_B
110_ILE_B
111_GLU_B
136_MET_B
157_ILE_B
182_LYS_B

Figure 35b

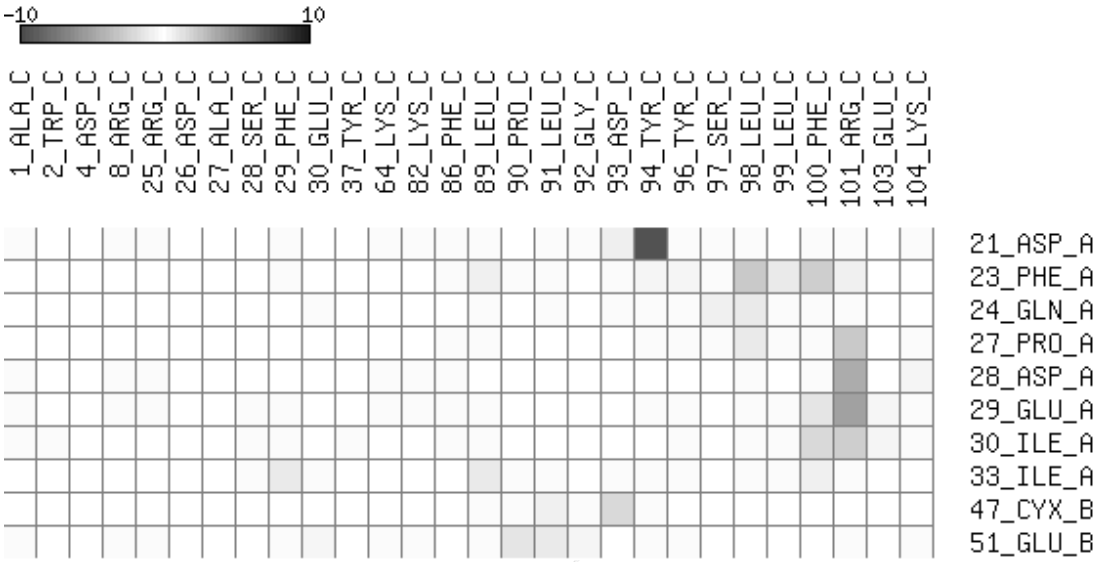



Figure 35c

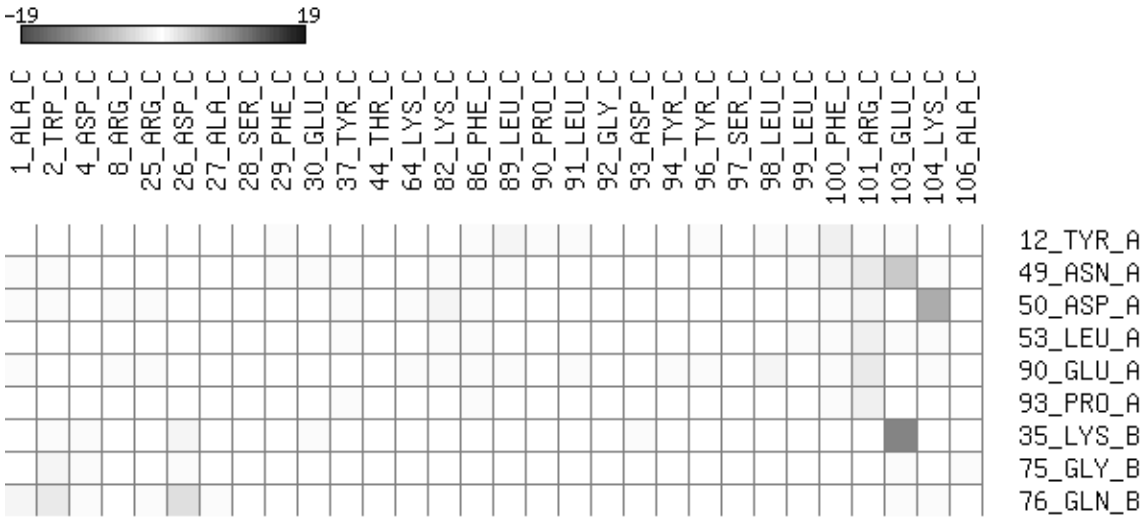


Figure 35d

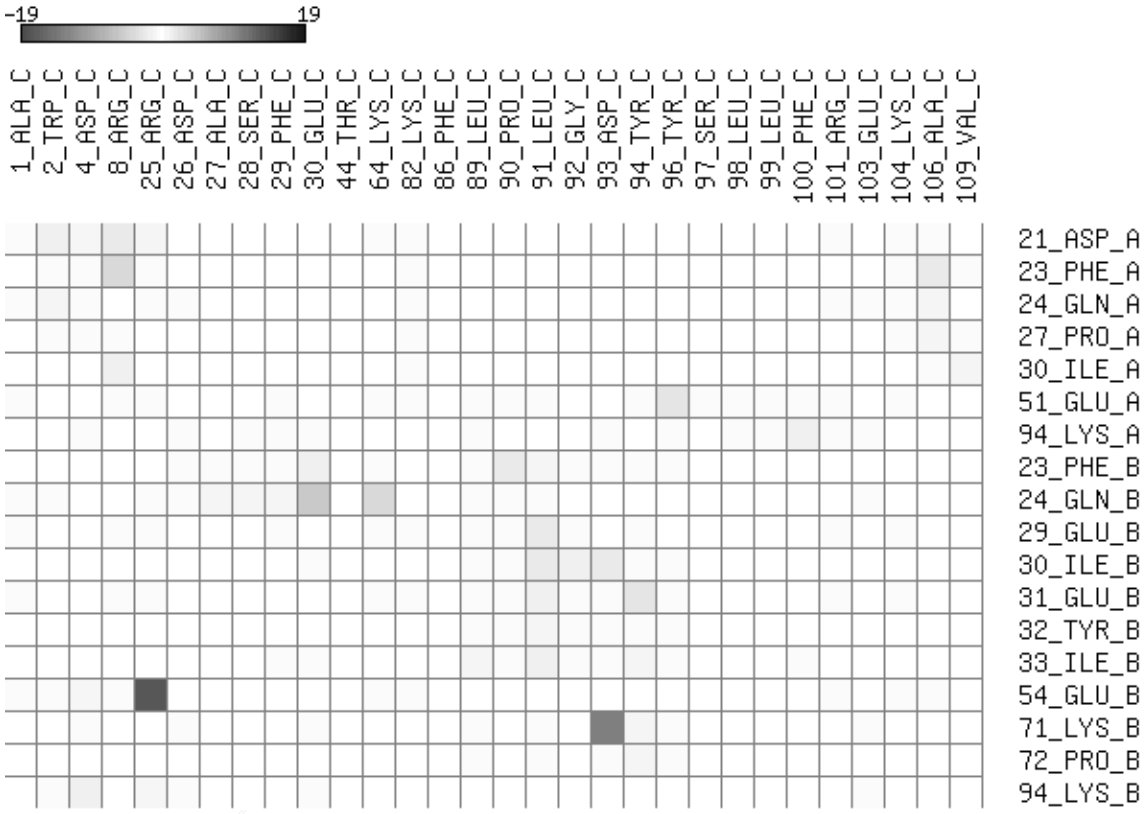

-19
19
1_ALA_C
2_TRP_C
4_ASP_C
8_ARG_C
25_ARG_C
26_ASP_C
27_ALA_C
28_SER_C
29_PHE_C
30_GLU_C
44_THR_C
64_LYS_C
82_LYS_C
86_PHE_C
89_LEU_C
90_PRO_C
91_LEU_C
92_GLY_C
93_ASP_C
94_TYR_C
96_TYR_C
97_SER_C
98_LEU_C
99_LEU_C
100_PHE_C
101_ARG_C
103_GLU_C
104_LYS_C
106_ALA_C
109_VAL_C
21_ASP_A
23_PHE_A
24_GLN_A
27_PRO_A
30_ILE_A
51_GLU_A
94_LYS_A
23_PHE_B
24_GLN_B
29_GLU_B
30_ILE_B
31_GLU_B
32_TYR_B
33_ILE_B
54_GLU_B
71_LYS_B
72_PRO_B
94_LYS_B

Figure 35e

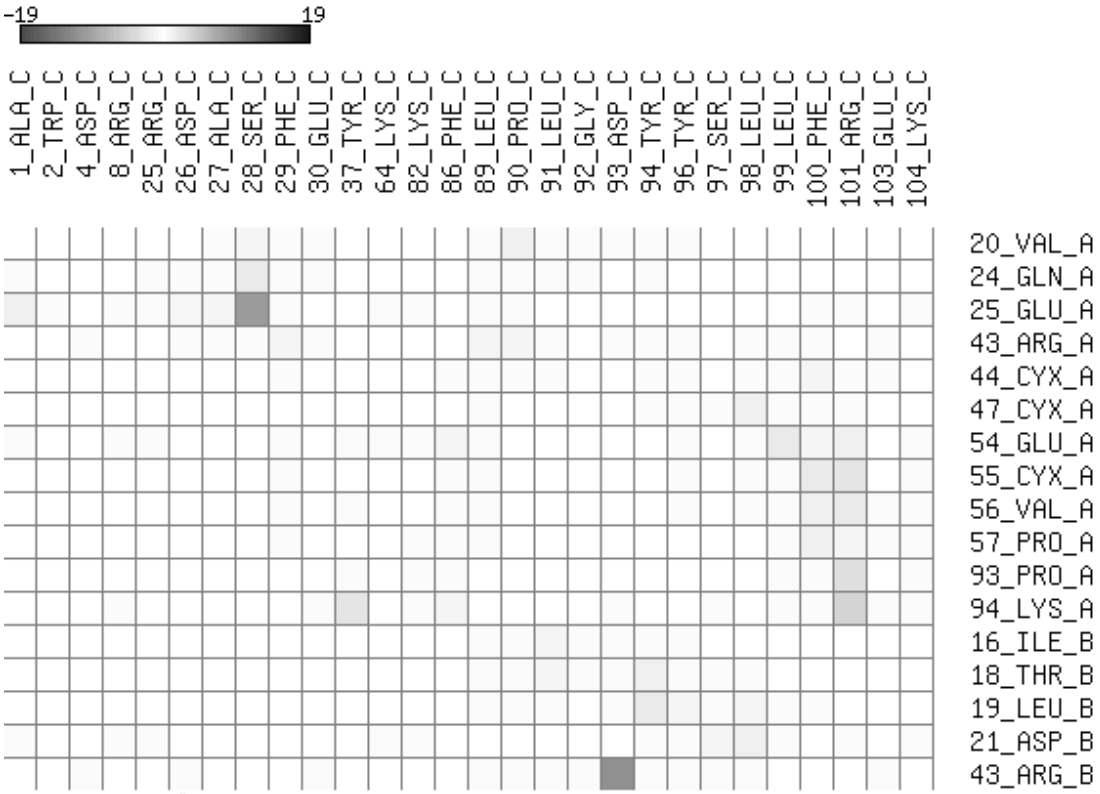

-19
19
1_ALA_C
2_TRP_C
4_ASP_C
8_ARG_C
25_ARG_C
26_ASP_C
27_ALA_C
28_SER_C
29_PHE_C
30_GLU_C
37_TYR_C
64_LYS_C
82_LYS_C
86_PHE_C
89_LEU_C
90_PRO_C
91_LEU_C
92_GLY_C
93_ASP_C
94_TYR_C
96_TYR_C
97_SER_C
98_LEU_C
99_LEU_C
100_PHE_C
101_ARG_C
103_GLU_C
104_LYS_C
20_VAL_A
24_GLN_A
25_GLU_A
43_ARG_A
44_CYX_A
47_CYX_A
54_GLU_A
55_CYX_A
56_VAL_A
57_PRO_A
93_PRO_A
94_LYS_A
16_ILE_B
18_THR_B
19_LEU_B
21_ASP_B
43_ARG_B

Figure 36

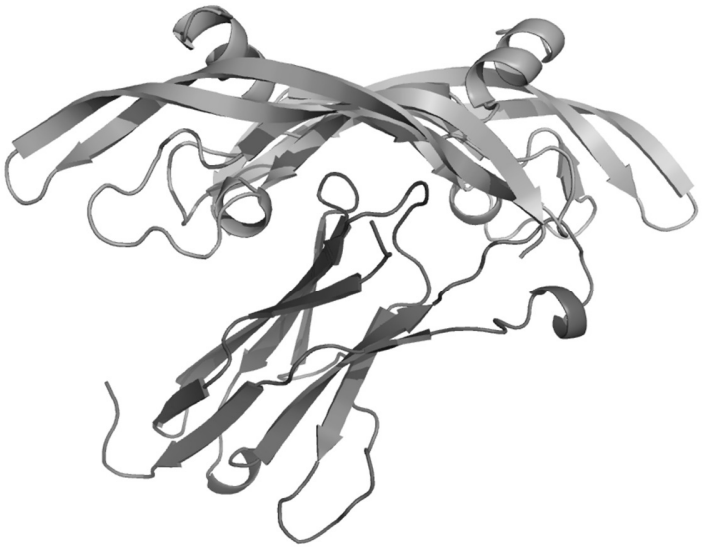


Figure 37

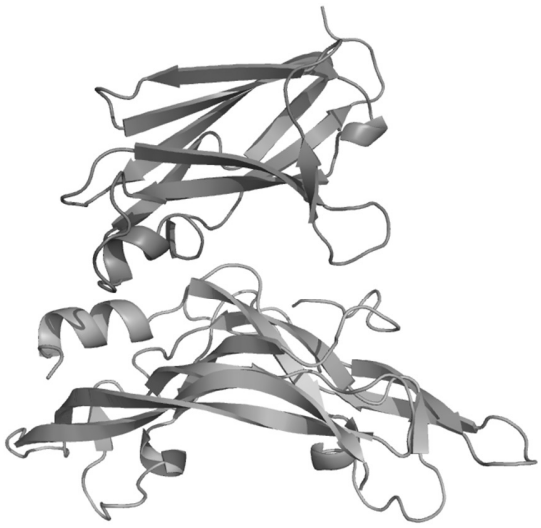


Figure 38



Figure 39



Figure 40a

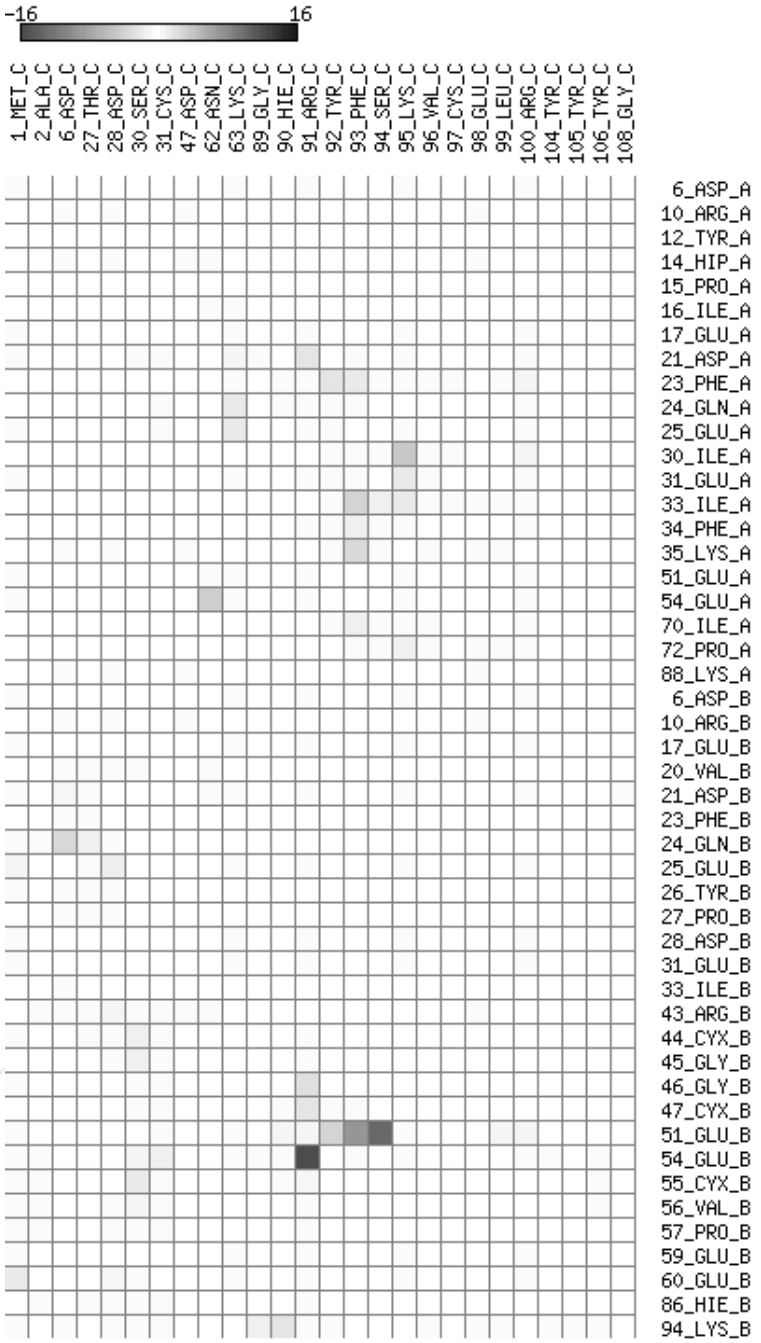

-16
16
1_MET_C
2_ALA_C
6_ASP_C
27_THR_C
28_ASP_C
30_SER_C
31_CYS_C
47_ASP_C
62_ASN_C
63_LYS_C
89_GLY_C
90_HIE_C
91_ARG_C
92_TYR_C
93_PHE_C
94_SER_C
95_LYS_C
96_VAL_C
97_CYS_C
98_GLU_C
99_LEU_C
100_ARG_C
104_TYR_C
105_TYR_C
106_TYR_C
108_GLY_C
6_ASP_A
10_ARG_A
12_TYR_A
14_HIP_A
15_PRO_A
16_ILE_A
17_GLU_A
21_ASP_A
23_PHE_A
24_GLN_A
25_GLU_A
30_ILE_A
31_GLU_A
33_ILE_A
34_PHE_A
35_LYS_A
51_GLU_A
54_GLU_A
70_ILE_A
72_PRO_A
88_LYS_A
6_ASP_B
10_ARG_B
17_GLU_B
20_VAL_B
21_ASP_B
23_PHE_B
24_GLN_B
25_GLU_B
26_TYR_B
27_PRO_B
28_ASP_B
31_GLU_B
33_ILE_B
43_ARG_B
44_CYX_B
45_GLY_B
46_GLY_B
47_CYX_B
51_GLU_B
54_GLU_B
55_CYX_B
56_VAL_B
57_PRO_B
59_GLU_B
60_GLU_B
86_HIE_B
94_LYS_B

Figure 40b

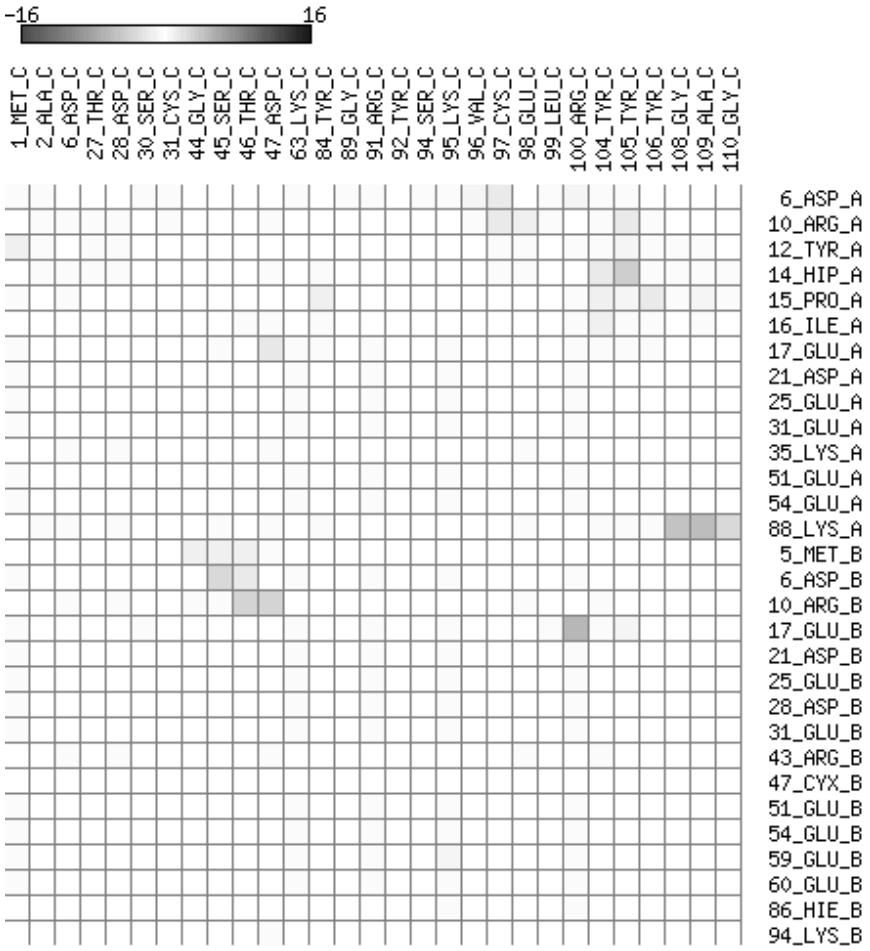

-16
16
1_MET_C
2_ALA_C
6_ASP_C
27_THR_C
28_ASP_C
30_SER_C
31_CYS_C
44_GLY_C
45_SER_C
46_THR_C
47_ASP_C
63_LYS_C
84_TYR_C
89_GLY_C
91_ARG_C
92_TYR_C
94_SER_C
95_LYS_C
96_VAL_C
97_CYS_C
98_GLU_C
99_LEU_C
100_ARG_C
104_TYR_C
105_TYR_C
106_TYR_C
108_GLY_C
109_ALA_C
110_GLY_C
6_ASP_A
10_ARG_A
12_TYR_A
14_HIP_A
15_PRO_A
16_ILE_A
17_GLU_A
21_ASP_A
25_GLU_A
31_GLU_A
35_LYS_A
51_GLU_A
54_GLU_A
88_LYS_A
5_MET_B
6_ASP_B
10_ARG_B
17_GLU_B
21_ASP_B
25_GLU_B
28_ASP_B
31_GLU_B
43_ARG_B
47_CYX_B
51_GLU_B
54_GLU_B
59_GLU_B
60_GLU_B
86_HIE_B
94_LYS_B

Figure 40c

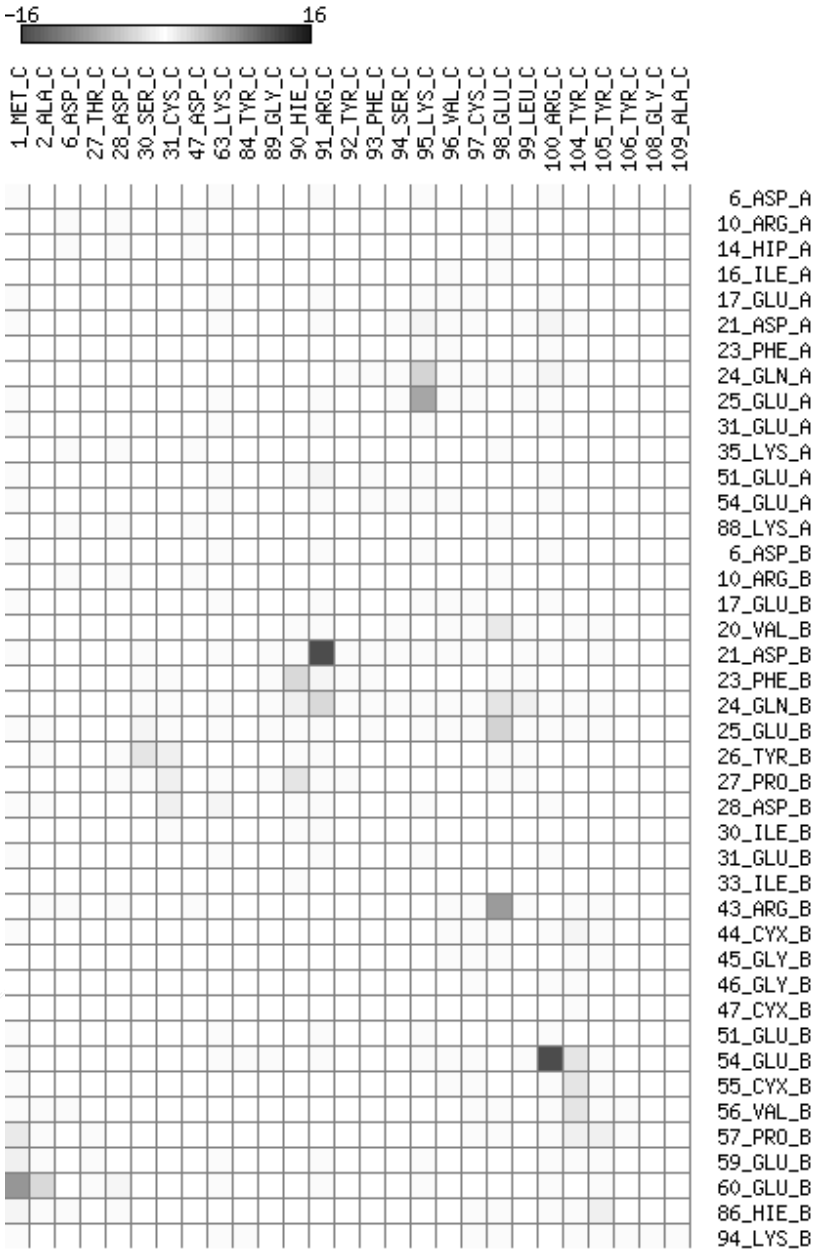

-16
16
1_MET_C
2_ALA_C
6_ASP_C
27_THR_C
28_ASP_C
30_SER_C
31_CYS_C
47_ASP_C
63_LYS_C
84_TYR_C
89_GLY_C
90_HIE_C
91_ARG_C
92_TYR_C
93_PHE_C
94_SER_C
95_LYS_C
96_VAL_C
97_CYS_C
98_GLU_C
99_LEU_C
100_ARG_C
104_TYR_C
105_TYR_C
106_TYR_C
108_GLY_C
109_ALA_C
6_ASP_A
10_ARG_A
14_HIP_A
16_ILE_A
17_GLU_A
21_ASP_A
23_PHE_A
24_GLN_A
25_GLU_A
31_GLU_A
35_LYS_A
51_GLU_A
54_GLU_A
88_LYS_A
6_ASP_B
10_ARG_B
17_GLU_B
20_VAL_B
21_ASP_B
23_PHE_B
24_GLN_B
25_GLU_B
26_TYR_B
27_PRO_B
28_ASP_B
30_ILE_B
31_GLU_B
33_ILE_B
43_ARG_B
44_CYX_B
45_GLY_B
46_GLY_B
47_CYX_B
51_GLU_B
54_GLU_B
55_CYX_B
56_VAL_B
57_PRO_B
59_GLU_B
60_GLU_B
86_HIE_B
94_LYS_B

Figure 40d

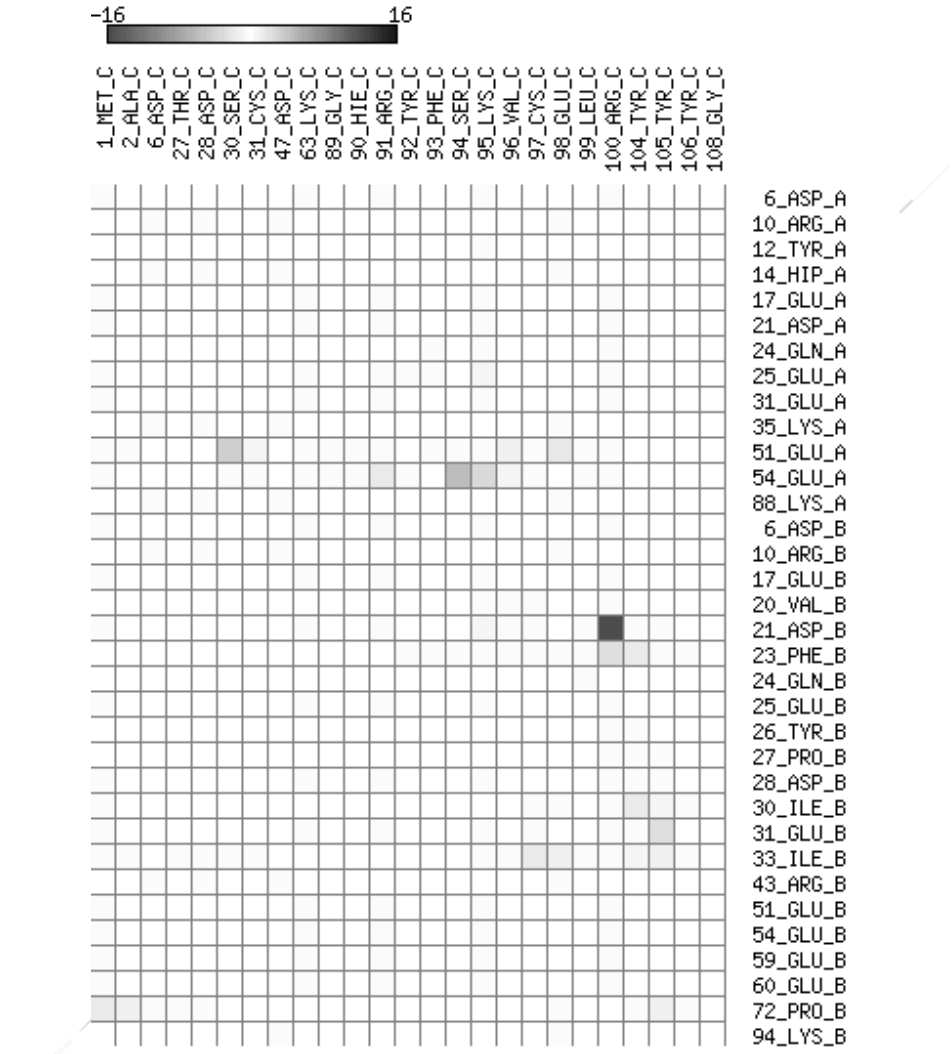

-16
16
1_MET_C
2_ALA_C
6_ASP_C
27_THR_C
28_ASP_C
30_SER_C
31_CYS_C
47_ASP_C
63_LYS_C
89_GLY_C
90_HIE_C
91_ARG_C
92_TYR_C
93_PHE_C
94_SER_C
95_LYS_C
96_VAL_C
97_CYS_C
98_GLU_C
99_LEU_C
100_ARG_C
104_TYR_C
105_TYR_C
106_TYR_C
108_GLY_C
6_ASP_A
10_ARG_A
12_TYR_A
14_HIP_A
17_GLU_A
21_ASP_A
24_GLN_A
25_GLU_A
31_GLU_A
35_LYS_A
51_GLU_A
54_GLU_A
88_LYS_A
6_ASP_B
10_ARG_B
17_GLU_B
20_VAL_B
21_ASP_B
23_PHE_B
24_GLN_B
25_GLU_B
26_TYR_B
27_PRO_B
28_ASP_B
30_ILE_B
31_GLU_B
33_ILE_B
43_ARG_B
51_GLU_B
54_GLU_B
59_GLU_B
60_GLU_B
72_PRO_B
94_LYS_B

Figure 41

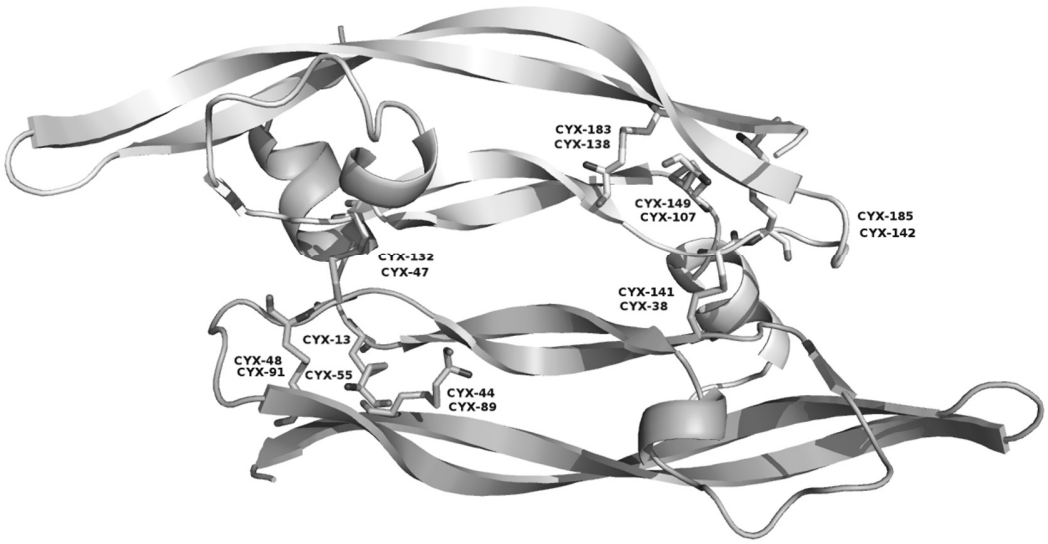



Figure 42

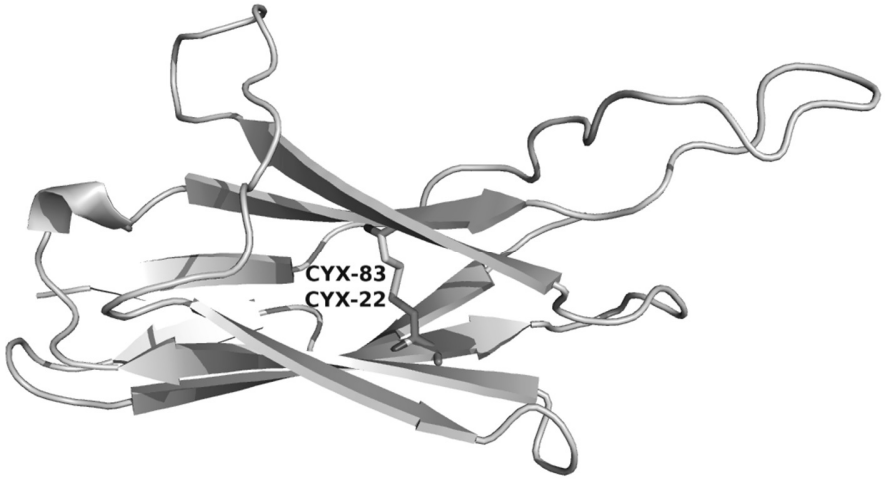


Figure 43

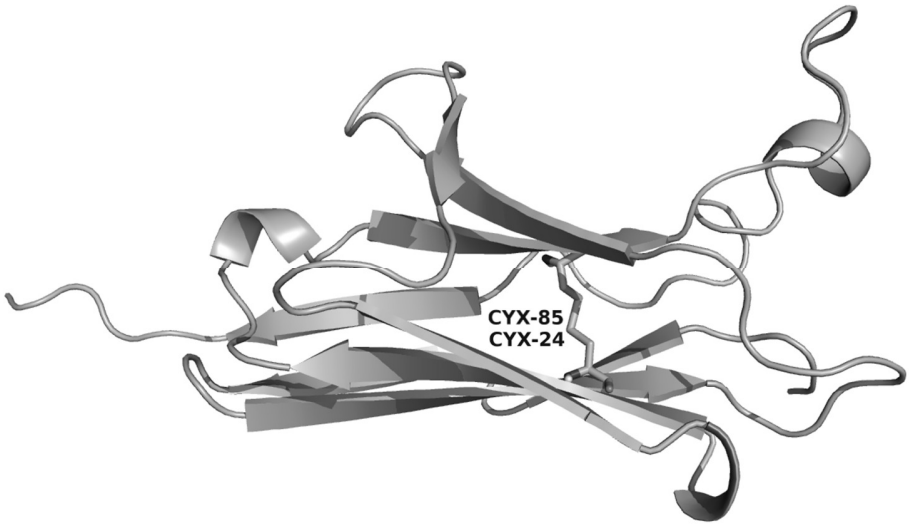


Figure 44

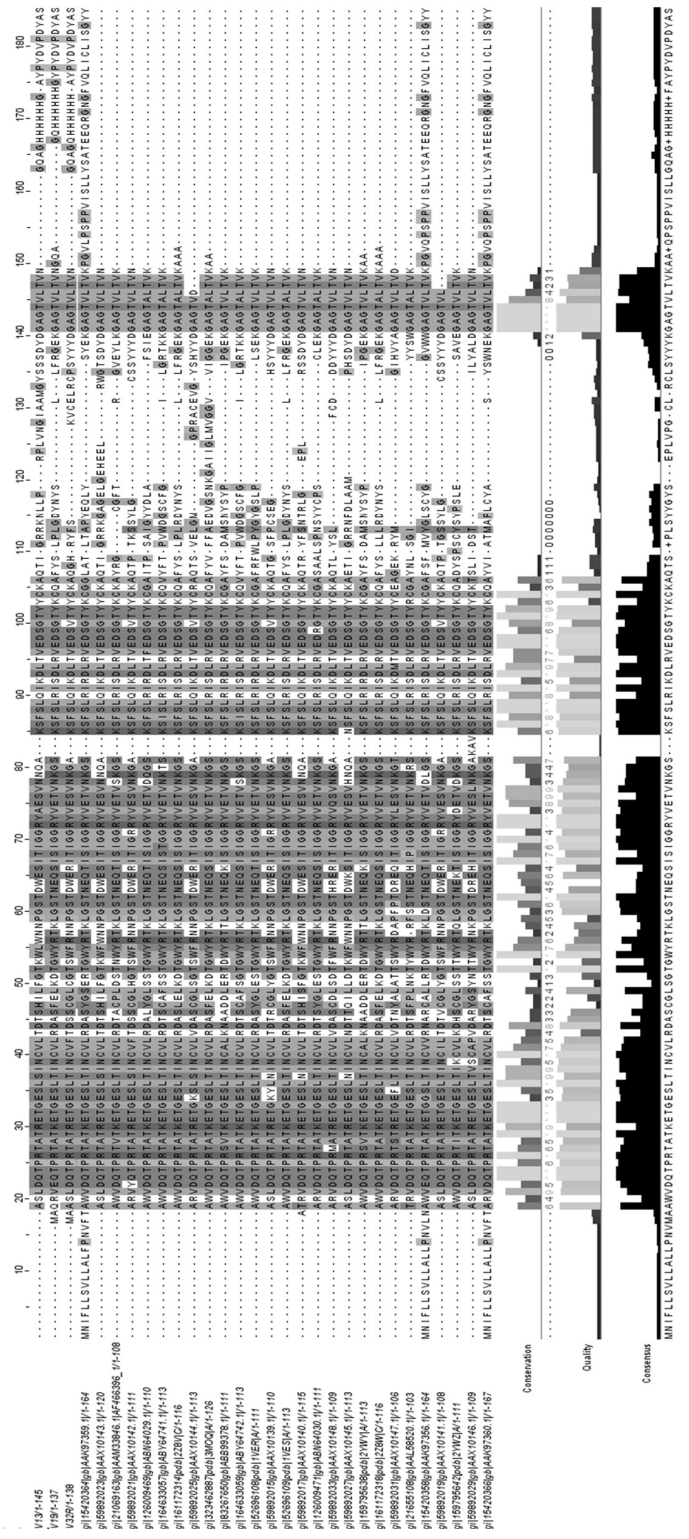


Figure 45



Figure 46a

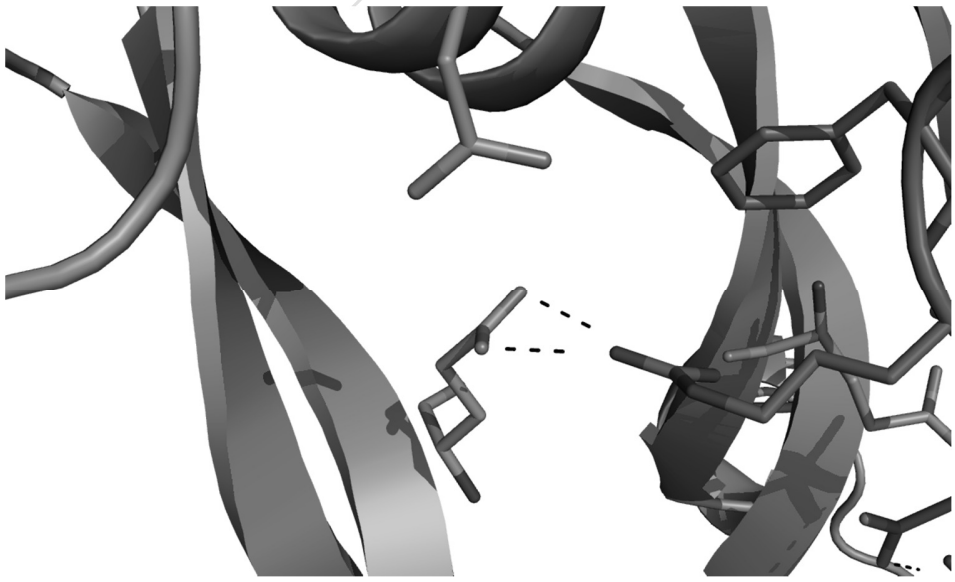


Figure 46b

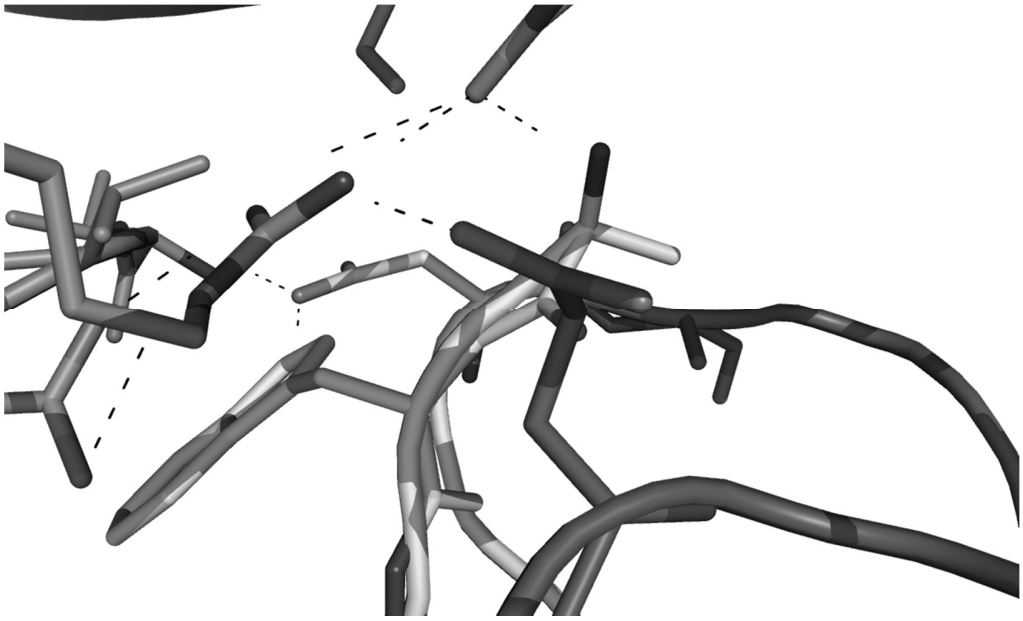


Figure 47



Figure 48a

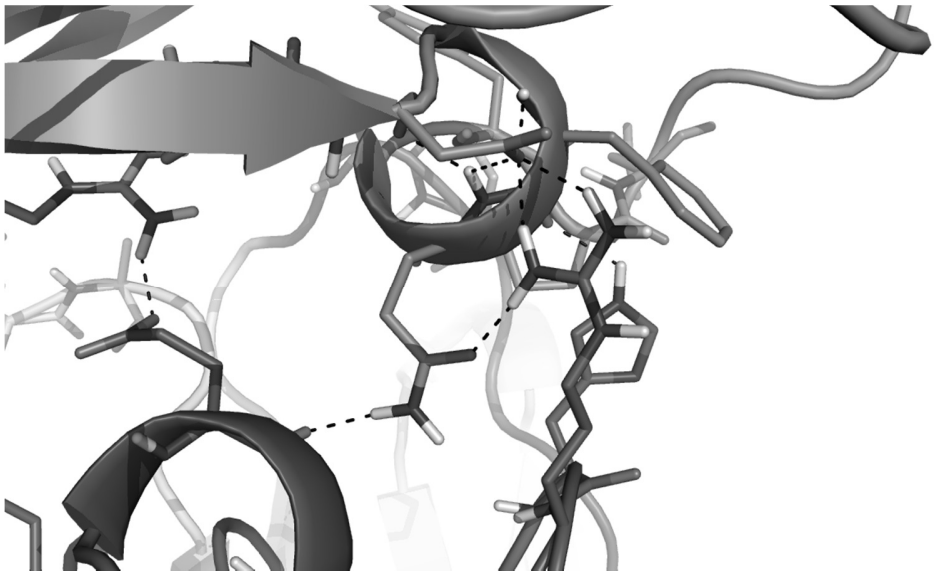


Figure 48b

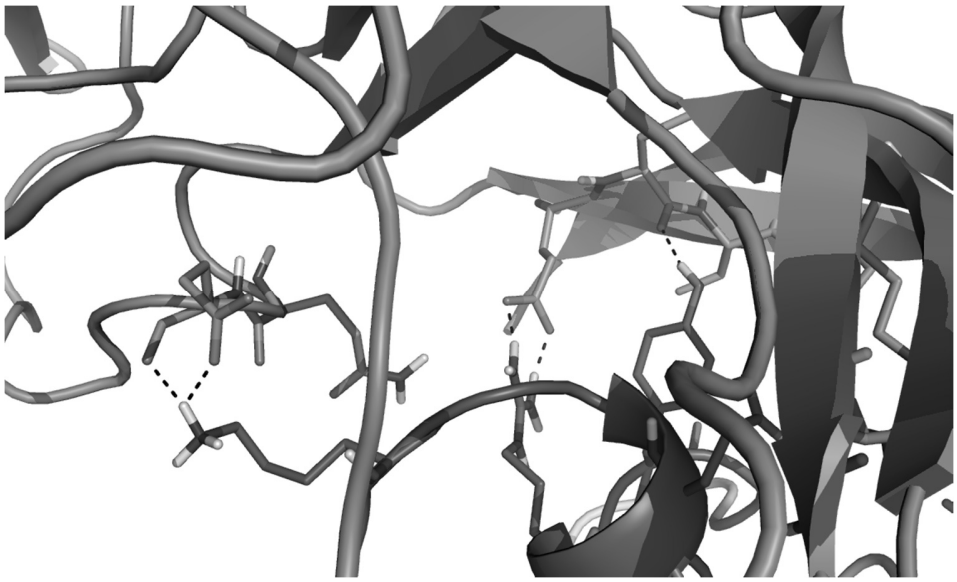


Figure 49

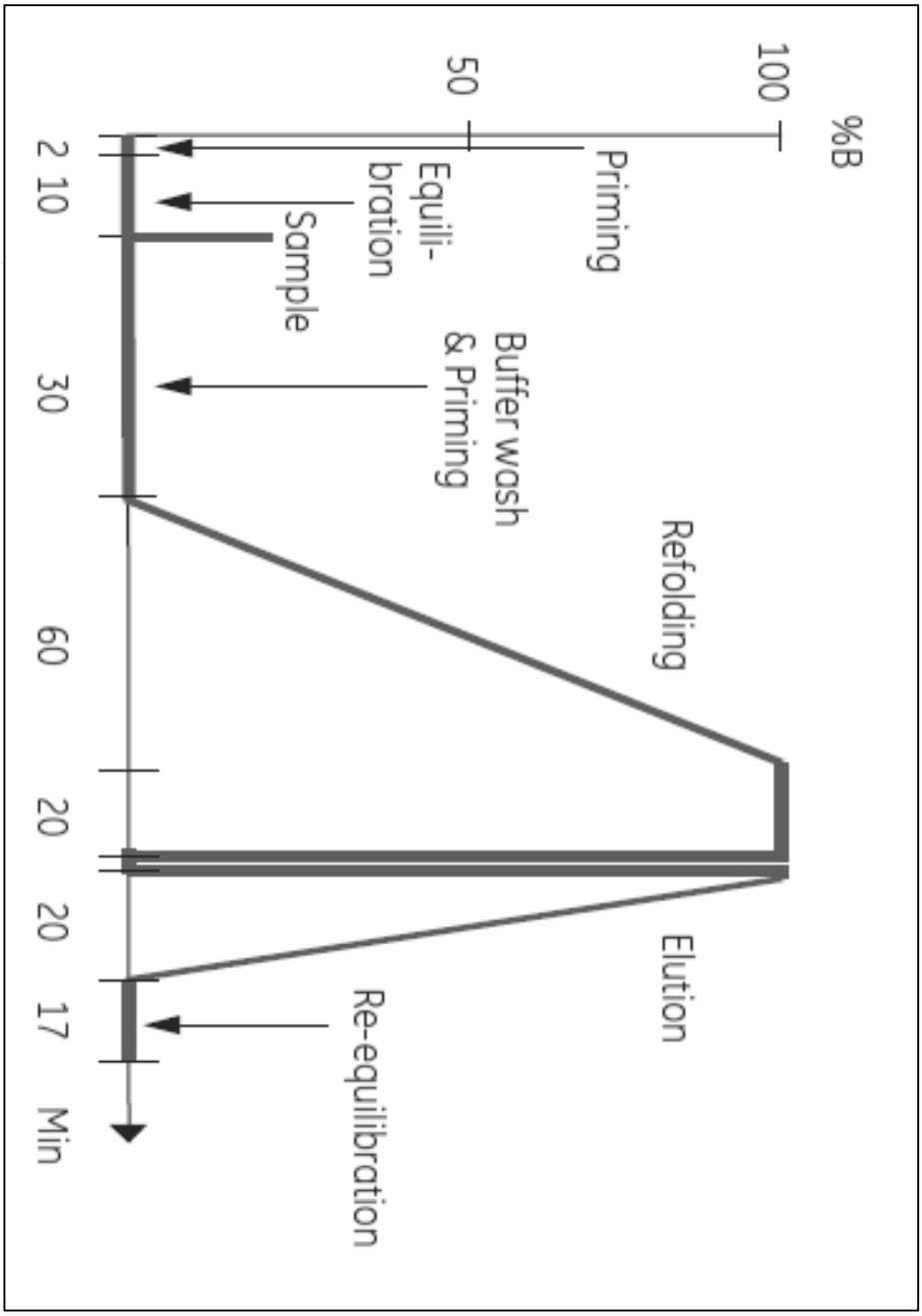

%B
100
50
2 10
30
60
20
20
17
Min
Priming
Equili-
bration
Sample
Buffer wash
& Priming
Refolding
Elution
Re-equilibration

Figure 50

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | VEGF | | VEGF | VEGF | VEGF | VEGF | VEGF | VEGF | VEGF | VEGF | VEGF | VEGF |
| B | 4 ng/mL | VEGF | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL |
| C | Suramine | 4 ng/mL | vNAR | vNAR | vNAR | vNAR | vNAR | vNAR | vNAR | vNAR | vNAR | vNAR |
| D | 100 µM | | 0.59 µg/mL | 1.17 µg/mL | 2.34 µg/mL | 4.69 µg/mL | 9.38 µg/mL | 18.75 µg/mL | 37.5 µg/mL | 75 µg/mL | 150 µg/mL | 300 µg/mL |
| E | | | VEGF | VEGF | VEGF | VEGF | VEGF | VEGF | VEGF | VEGF | VEGF | VEGF |
| F | Control | VEGF | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL | 4 ng/mL |
| G | without | 4 ng/mL | vNAR | vNAR | vNAR | vNAR | vNAR | vNAR | vNAR | vNAR | vNAR | vNAR |
| H | treatment | Buffer | 0.59 µg/mL | 1.17 µg/mL | 2.34 µg/mL | 4.69 µg/mL | 9.38 µg/mL | 18.75 µg/mL | 37.5 µg/mL | 75 µg/mL | 150 µg/mL | 300 µg/mL |

Figure 51

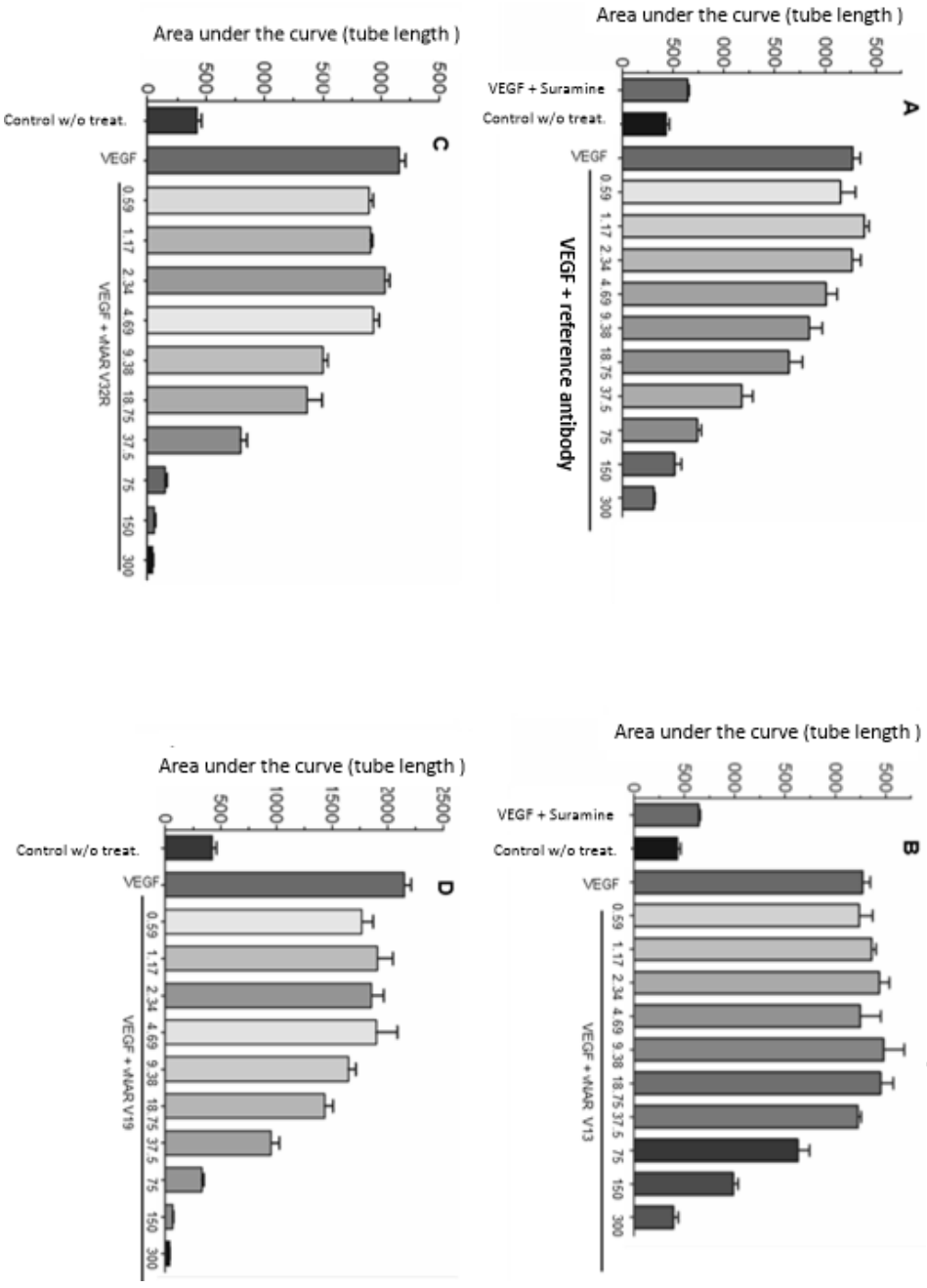

Area under the curve (tube length )
VEGF + Suramine
Control w/o treat.
VEGF
VEGF + reference antibody
A
Area under the curve (tube length )
Control w/o treat.
VEGF
VEGF + vNAR V32R
C
Area under the curve (tube length )
VEGF + Suramine
Control w/o treat.
VEGF
VEGF + vNAR V13
B
Area under the curve (tube length )
Control w/o treat.
VEGF
VEGF + vNAR V19
D

Figure 52

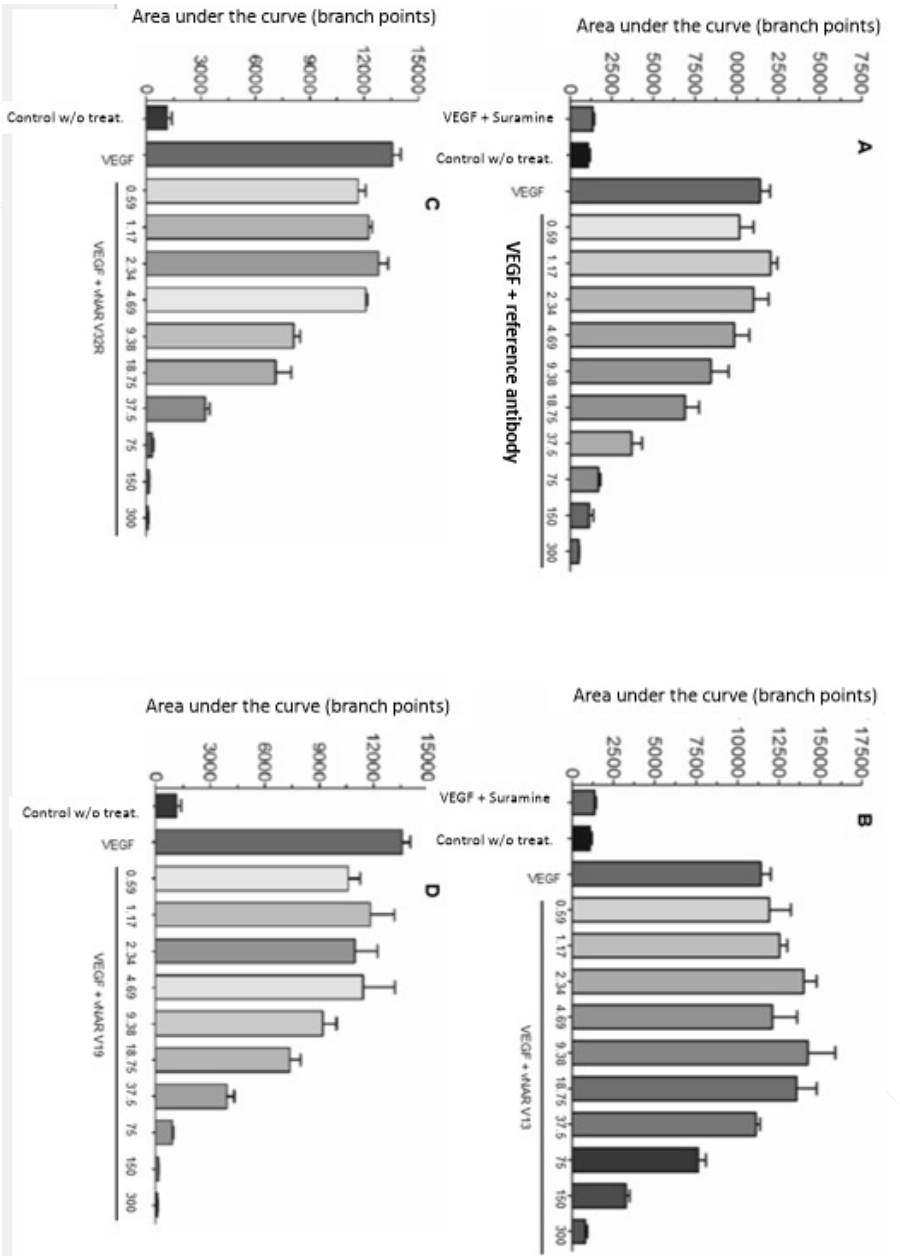

Area under the curve (branch points)
0
2500
5000
7500
10000
12500
15000
17500
VEGF + Suramine
Control w/o treat.
VEGF
VEGF + reference antibody
0.59
1.17
2.34
4.69
9.38
18.75
37.5
75
150
300
A
Area under the curve (branch points)
0
3000
6000
9000
12000
15000
Control w/o treat.
VEGF
VEGF + vNAR V32R
C
Area under the curve (branch points)
0
2500
5000
7500
10000
12500
15000
17500
VEGF + Suramine
Control w/o treat.
VEGF
VEGF + vNAR V13
B
Area under the curve (branch points)
0
3000
6000
9000
12000
15000
Control w/o treat.
VEGF
VEGF + vNAR V19
D

Figure 53

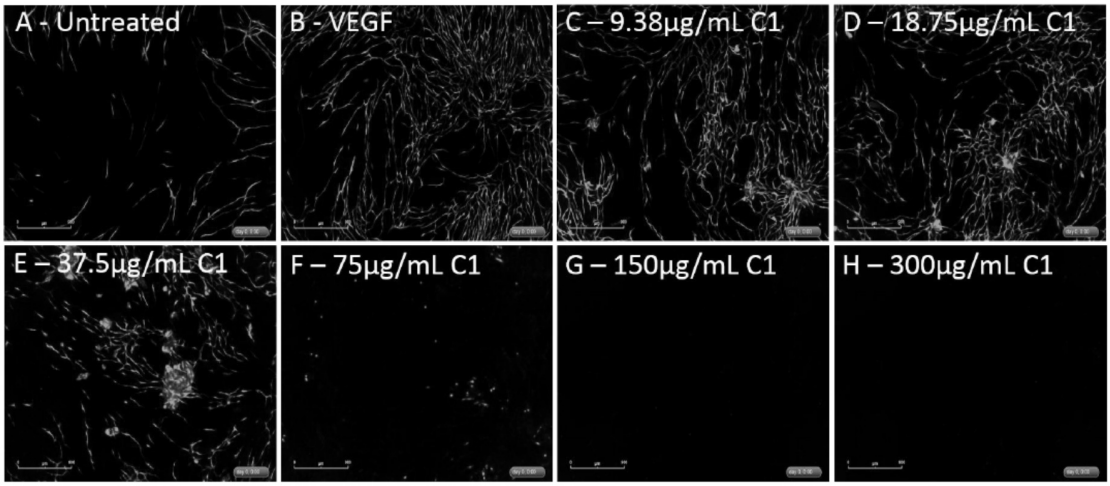



Figure 54

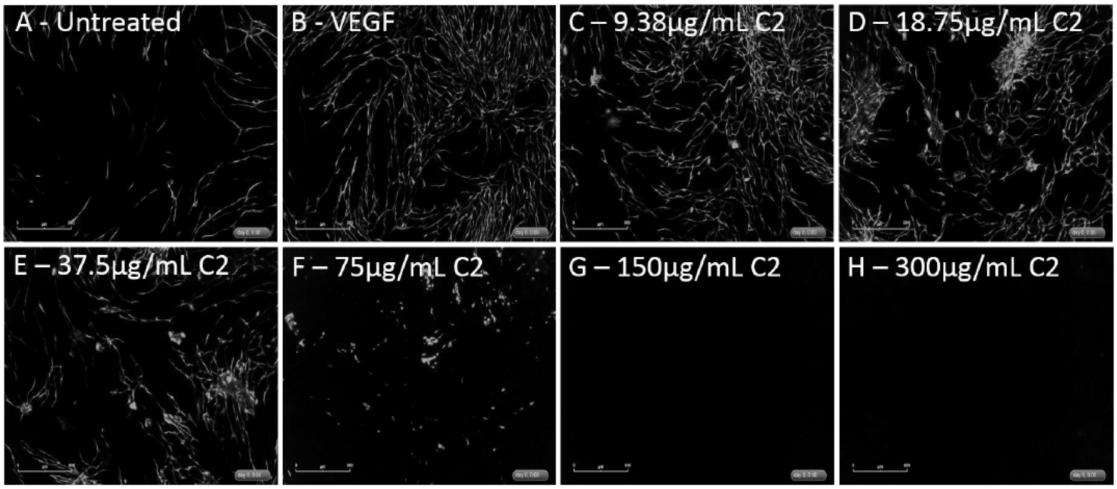


Figure 55.

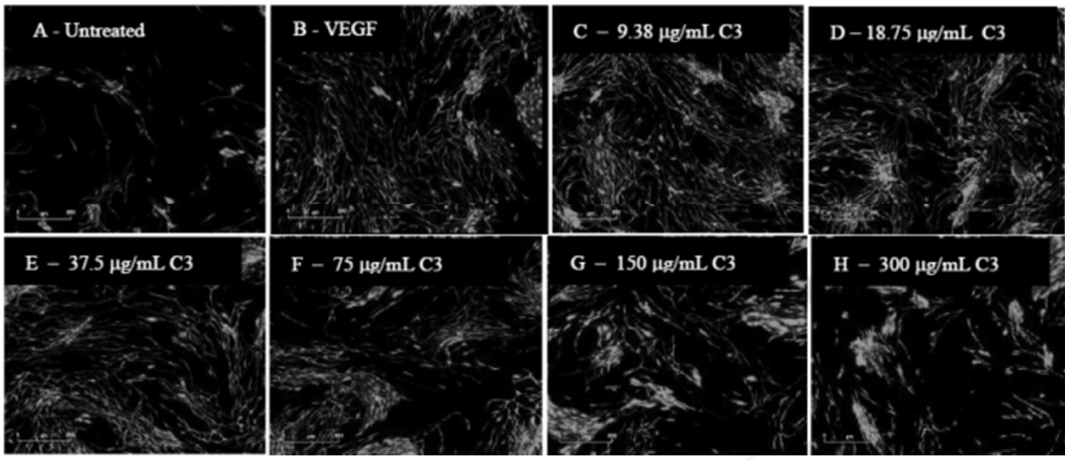



Figure 56.

