

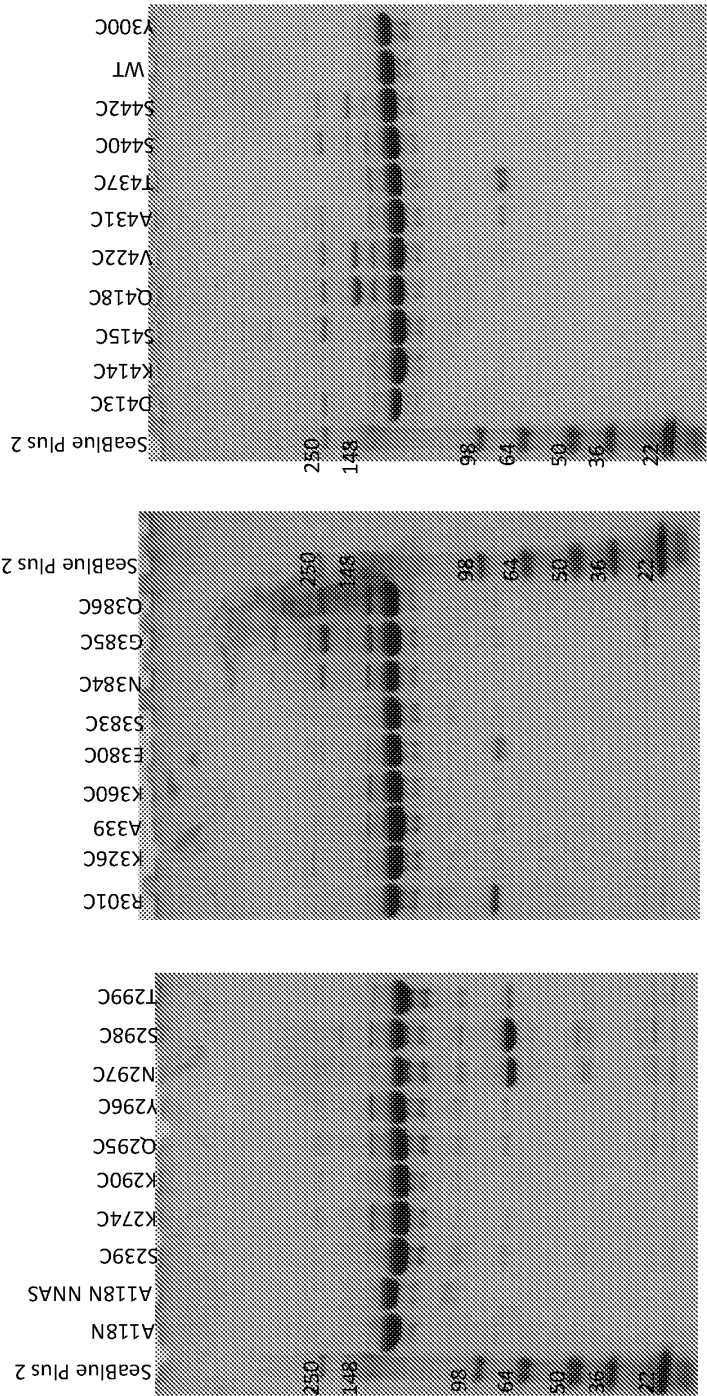


Fig. 1

Fig. 2

#	Mutant	Tm ₁	Tm ₂
18	S383C	68.8	81.9
19	E380C	63.3	81.8
20	S239C	69.8	81.8
21	K326C	66.5	81.8
22	K290C	72.2	82.0
23	Q295C	71.2	82.2
24	Y296C	70.6	81.9
25	N297C	57.6	81.6
26	S298C	72.0	82.6
27	T299C	61.2	82.4
28	R301C	62.3	82.5
29	A118N NNAS	60.0	81.0
30	WT	68.8	81.8

#	Mutant	Tm ₁	Tm ₂
1	Y300C	62.7	82.6
2	D413C	69.9	83.3
3	A339C	68.0	83.1
4	Q418C	70.2	83.4
5	A118N	70.4	83.3
6	A431C	67.0	83.2
7	K414C	71.7	84.0
8	Q386C	70.9	83.3
9	K274C	71.9	83.7
10	G385C	69.7	82.0
11	V422C	68.3	81.9
12	T437C	67.9	82.1
13	K360C	70.1	82.1
14	N384C	68.7	81.8
15	S440C	69.4	81.9
16	S442C	68.4	81.9
17	S415C	68.7	82.0

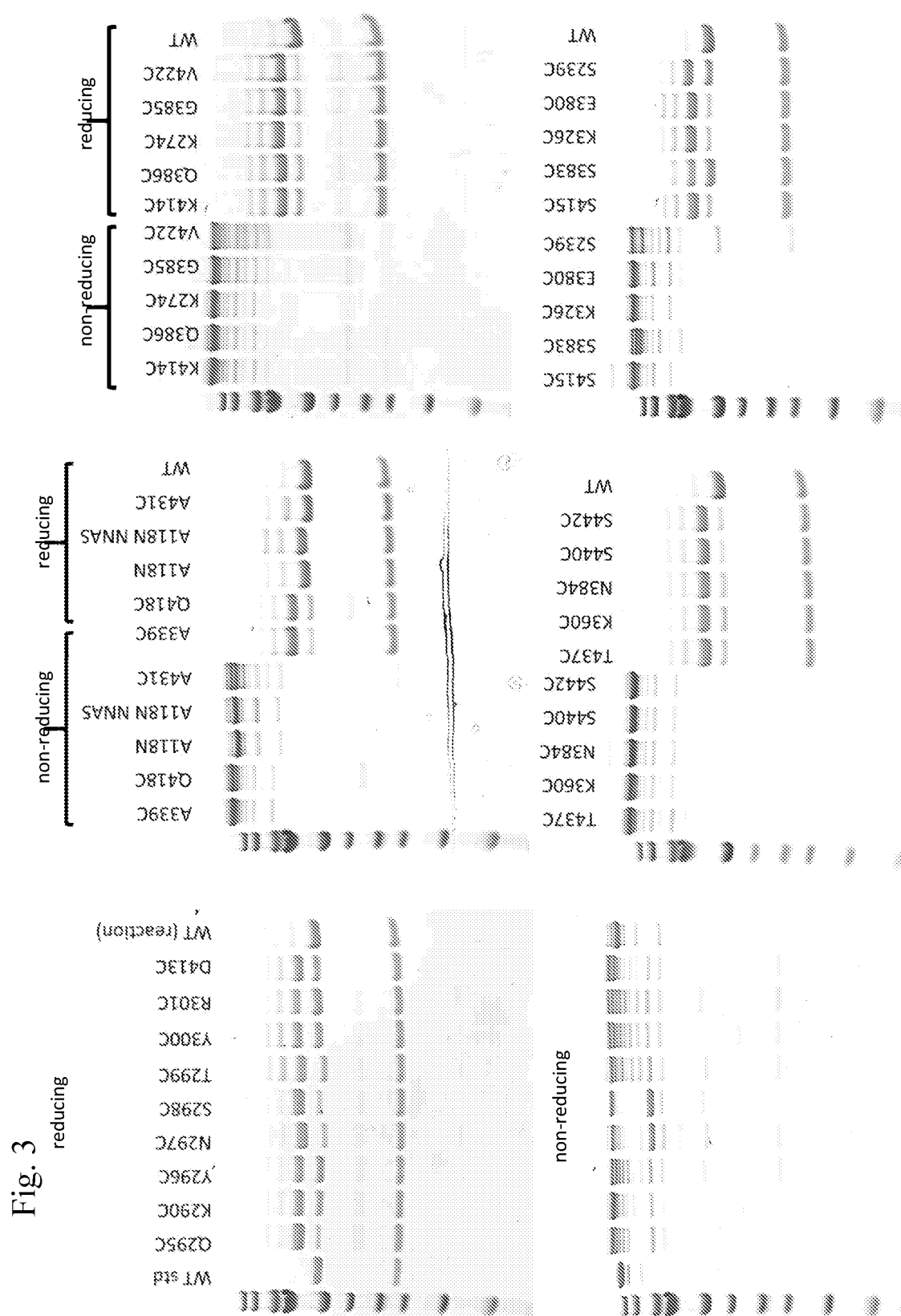


Fig. 4

Mutants	Mono-PEGylated	Multi-PEGylated	Un-PEGylated	PAR	Notes/issues mAb
A339C	77.6%	11.7%	10.7%	2.1	
A431C	21.4%	6.6%	72.0%	0.7	
D413C	53.2%	12.5%	34.3%	1.6	
E380C	60.2%	24.4%	15.4%	2.4	Some free HL
G385C	68.2%	18.6%	13.2%	2.2	Significant HMWS
K274C	71.4%	18.6%	9.0%	2.3	
K290C	72.6%	8.9%	18.5%	1.9	
K326C	64.6%	20.3%	15.1%	2.2	
K360C	65.0%	15.1%	19.9%	2.0	
K414C	59.7%	19.1%	21.2%	2.1	
N297C	73.1%	5.5%	21.4%	1.7	Significant free HL
N384C	68.2%	16.1%	15.7%	2.1	
Q295C	56.1%	9.2%	34.7%	1.5	
Q386C	63.7%	18.8%	17.5%	2.2	Some HMWS
Q418C	66.9%	12.2%	20.9%	1.9	Significant HMWS
R301C	36.0%	11.6%	52.5%	1.2	Some free HL
S239C	49.9%	13.9%	36.3%	1.6	
S298C	76.6%	5.9%	17.5%	1.8	Significant free HL
S383C	37.4%	14.9%	47.7%	1.4	
S415C	60.1%	27.7%	17.3%	2.3	
S440C	73.3%	17.3%	9.5%	2.3	
S442C	72.4%	16.2%	11.4%	2.2	
T299C	48.7%	23.0%	28.4%	2.0	
T437C	66.1%	17.8%	16.1%	2.2	Some free HL
V422C	71.9%	18.7%	9.4%	2.3	Some HMWS
Y296C	50.7%	4.8%	44.5%	1.0	
Y300C	43.9%	18.9%	37.2%	1.7	

Figure 1 consists of four bar charts arranged in a 2x2 grid, showing the effect of PEGylation on the stability of K274C and K290C antibodies. The x-axis for all charts lists 16 different antibody variants: K274C, V422C, S440C, S442C, Q386C, T437C, N384C, G385C, A339C, K290C, S440C, N384C, G385C, T437C, K290C, and Q386C.

Top Left Chart: % multi-PEGylated

Variant	% multi-PEGylated
K274C	~2.0%
V422C	~3.0%
S440C	~4.0%
S442C	~5.0%
Q386C	~6.0%
T437C	~7.0%
N384C	~8.0%
G385C	~9.0%
A339C	~10.0%
K290C	~11.0%
S440C	~12.0%
N384C	~13.0%
G385C	~14.0%
T437C	~15.0%
K290C	~16.0%
Q386C	~17.0%

Top Right Chart: % un-PEGylated

Variant	% un-PEGylated
K274C	~10.0%
V422C	~10.0%
S440C	~10.0%
S442C	~10.0%
Q386C	~10.0%
T437C	~10.0%
N384C	~10.0%
G385C	~10.0%
A339C	~10.0%
K290C	~10.0%
S440C	~10.0%
N384C	~10.0%
G385C	~10.0%
T437C	~10.0%
K290C	~10.0%
Q386C	~10.0%

Bottom Left Chart: % mono-PEGylated

Variant	% mono-PEGylated
K274C	~70.0%
V422C	~70.0%
S440C	~70.0%
S442C	~70.0%
Q386C	~70.0%
T437C	~70.0%
N384C	~70.0%
G385C	~70.0%
A339C	~70.0%
K290C	~70.0%
S440C	~70.0%
N384C	~70.0%
G385C	~70.0%
T437C	~70.0%
K290C	~70.0%
Q386C	~70.0%

Bottom Right Chart: PAR

Variant	PAR
K274C	~2.0
V422C	~2.0
S440C	~2.0
S442C	~2.0
Q386C	~2.0
T437C	~2.0
N384C	~2.0
G385C	~2.0
A339C	~2.0
K290C	~2.0
S440C	~2.0
N384C	~2.0
G385C	~2.0
T437C	~2.0
K290C	~2.0
Q386C	~2.0

Fig. 6

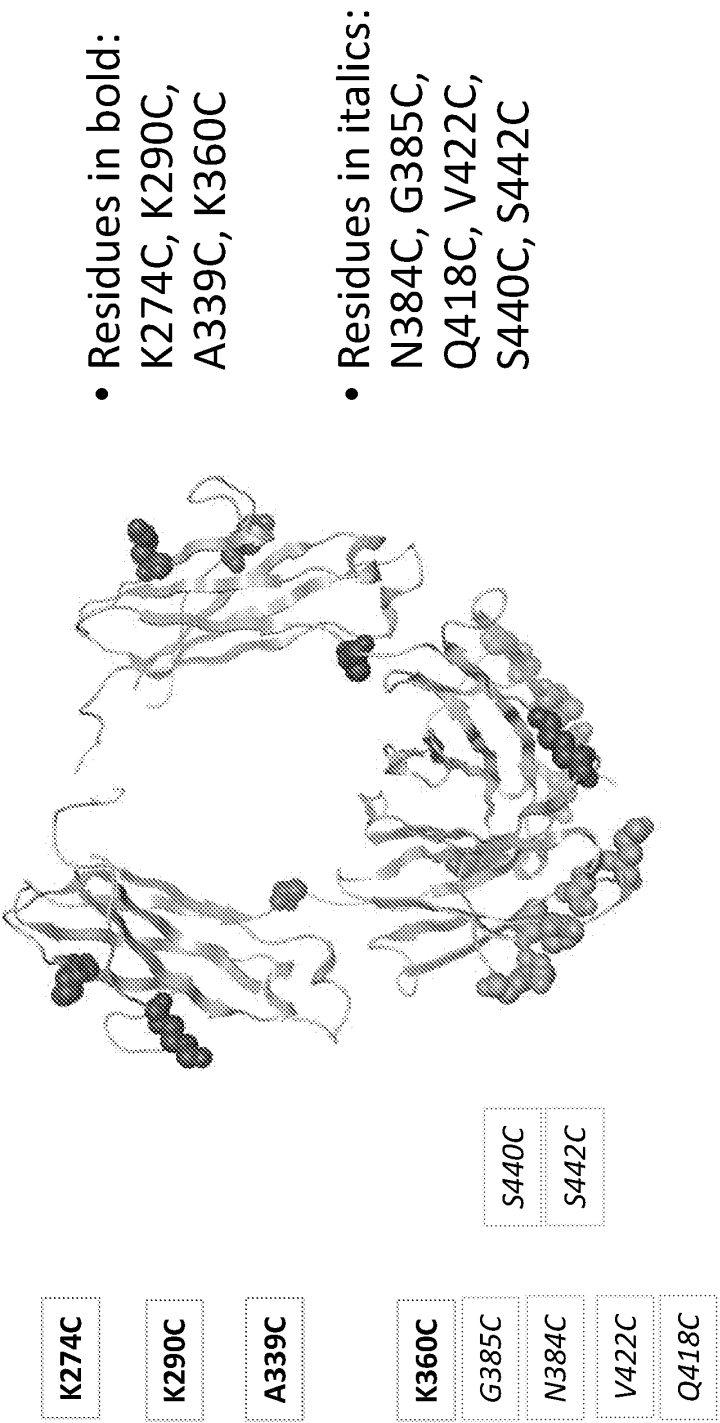


Fig. 7

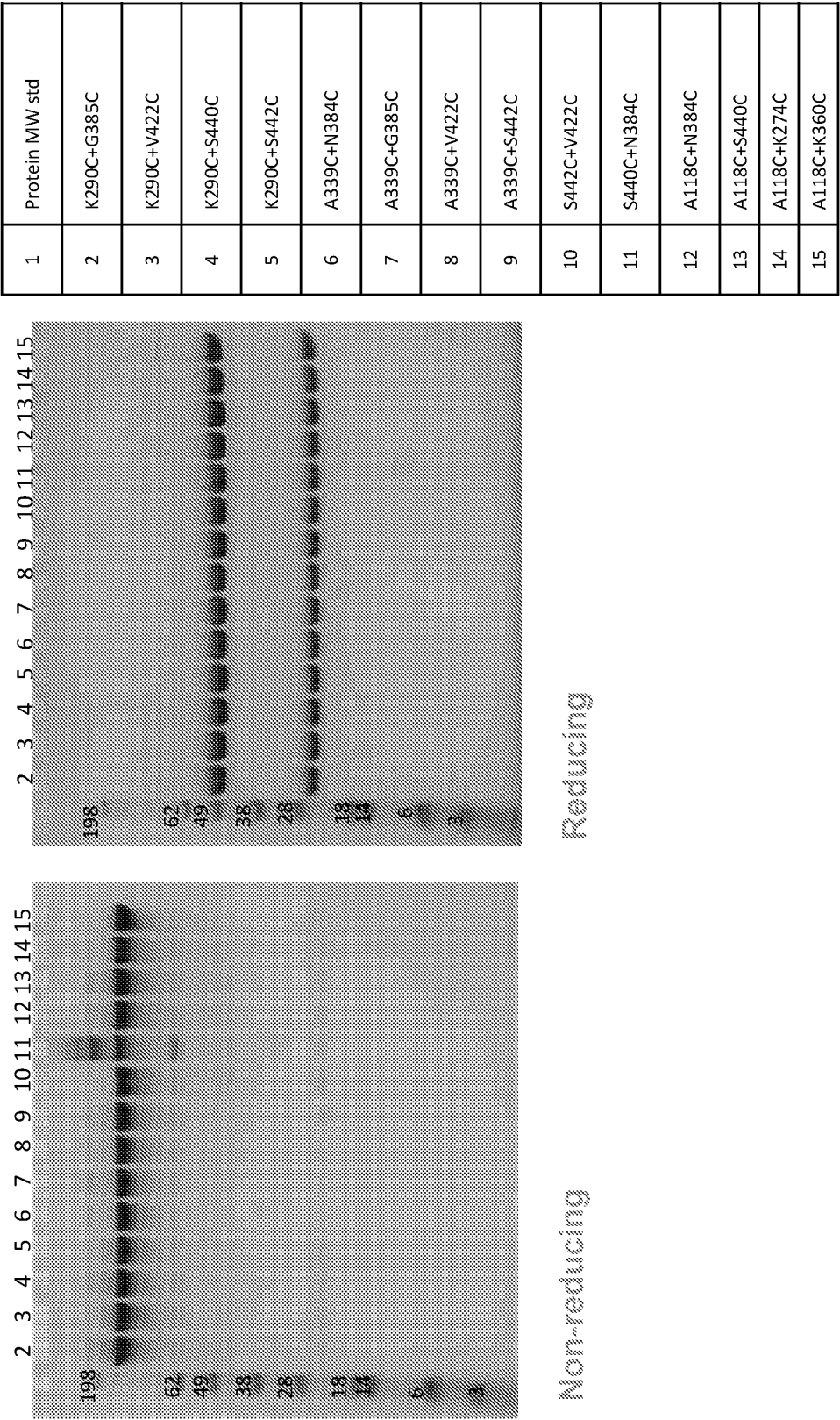


Fig. 8

Ab #	Antibody Mutations	nanoDSF Tm1 (°C)	nanoDSF Tm2 (°C)
1	WT parental antibody	68.5	81.6
<u>2</u>	<u>K290C + G385C</u>	<u>74.1</u>	<u>81.9</u>
<u>3</u>	<u>K290C + V422C</u>	<u>72.9</u>	<u>81.8</u>
<u>4</u>	<u>K290C + S440C</u>	<u>74.2</u>	<u>81.7</u>
<u>5</u>	<u>K290C + S442C</u>	<u>73.3</u>	<u>81.7</u>
<u>6</u>	<u>A339C + N384C</u>	<u>66.0</u>	<u>81.7</u>
<u>7</u>	<u>A339C + G385C</u>	<u>65.0</u>	<u>81.6</u>
<u>8</u>	<u>A339C + V422C</u>	<u>65.9</u>	<u>81.5</u>
<u>9</u>	<u>A339C + S442C</u>	<u>65.5</u>	<u>81.8</u>
10	S442C + V422C	69.5	82.2
11	S440C + N384C	70.2	82.0
12	A118C + N384C	67.7	82.6
13	A118C + G385C	68.2	82.7
14	A118C + V422C	67.9	82.5
15	A118C + S440C	69.8	82.5
16	A118C + S442C	68.3	82.6
17	A118C + K274C	69.3	82.4

Ab #	Antibody Mutations	nanoDSF Tm1 (°C)	nanoDSF Tm2 (°C)
<u>18</u>	<u>A118C + A339C</u>	<u>58.8</u>	<u>81.1</u>
19	A118C + K360C	68.3	82.9
20	A118C + Q418C	69.0	82.8
21	K274C + N384C	69.6	81.8
<u>22</u>	<u>K274C + G385C</u>	<u>70.8</u>	<u>81.7</u>
23	K274C + V422C	69.9	81.7
<u>24</u>	<u>K274C + S440C</u>	<u>71.7</u>	<u>81.7</u>
25	K274C + S442C	69.5	81.9
26	K274C + K360C	70.4	82.2
<u>27</u>	<u>K274C + A339C</u>	<u>66.4</u>	<u>81.8</u>
<u>28</u>	<u>K274C + K414C</u>	<u>72.3</u>	<u>82.2</u>
29	K360C + V422C	68.6	81.8
30	K360C + S440C	69.3	82.0
31	K360C + S442C	69.0	82.0
32	K360C + N384C	69.0	82.2
33	K360C + G385C	68.3	81.9
34	K360C + A339C	66.9	82.0
<u>35</u>	<u>K360C + K290C</u>	<u>72.5</u>	<u>82.5</u>

Fig. 9

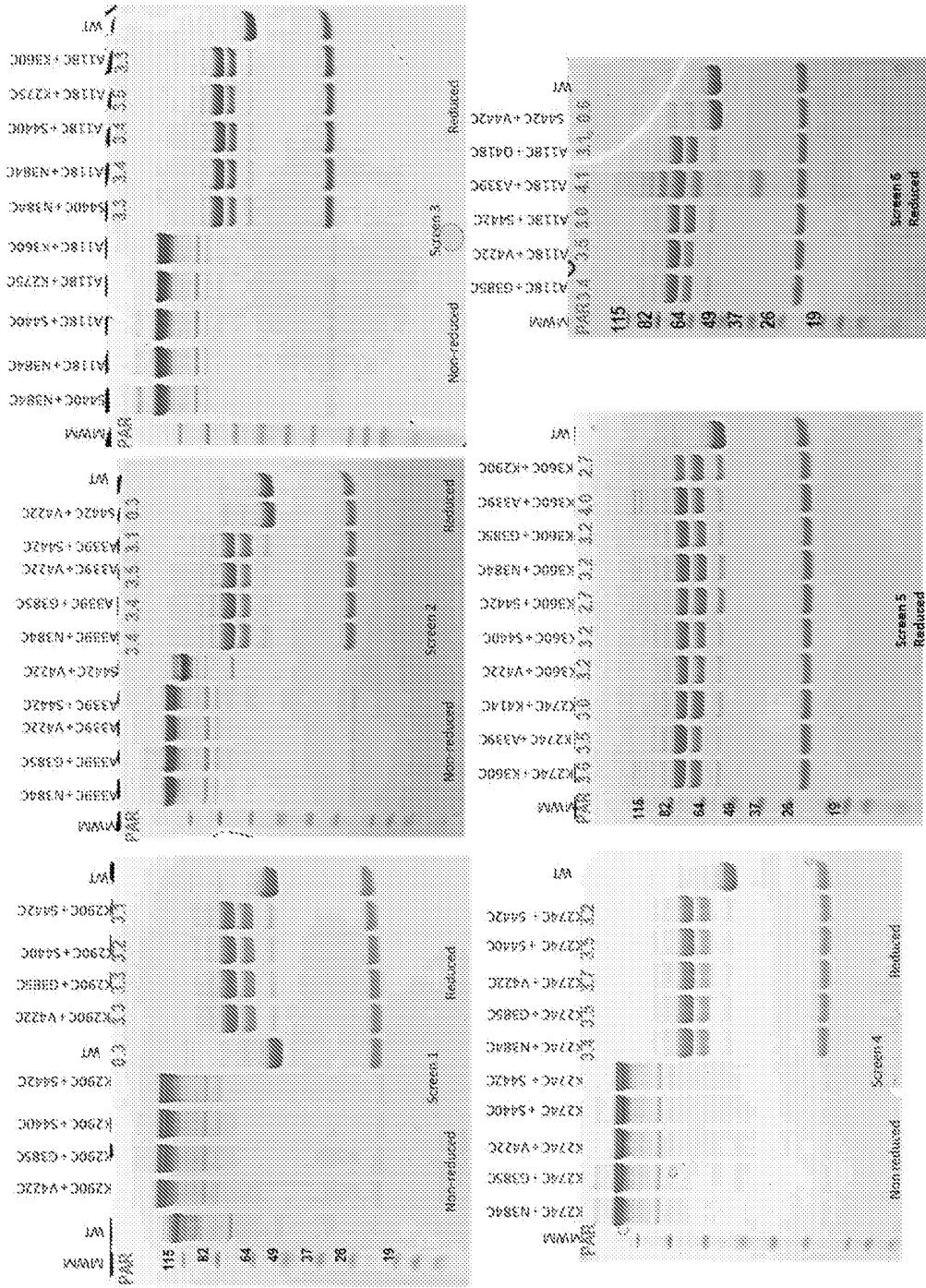


Fig. 10

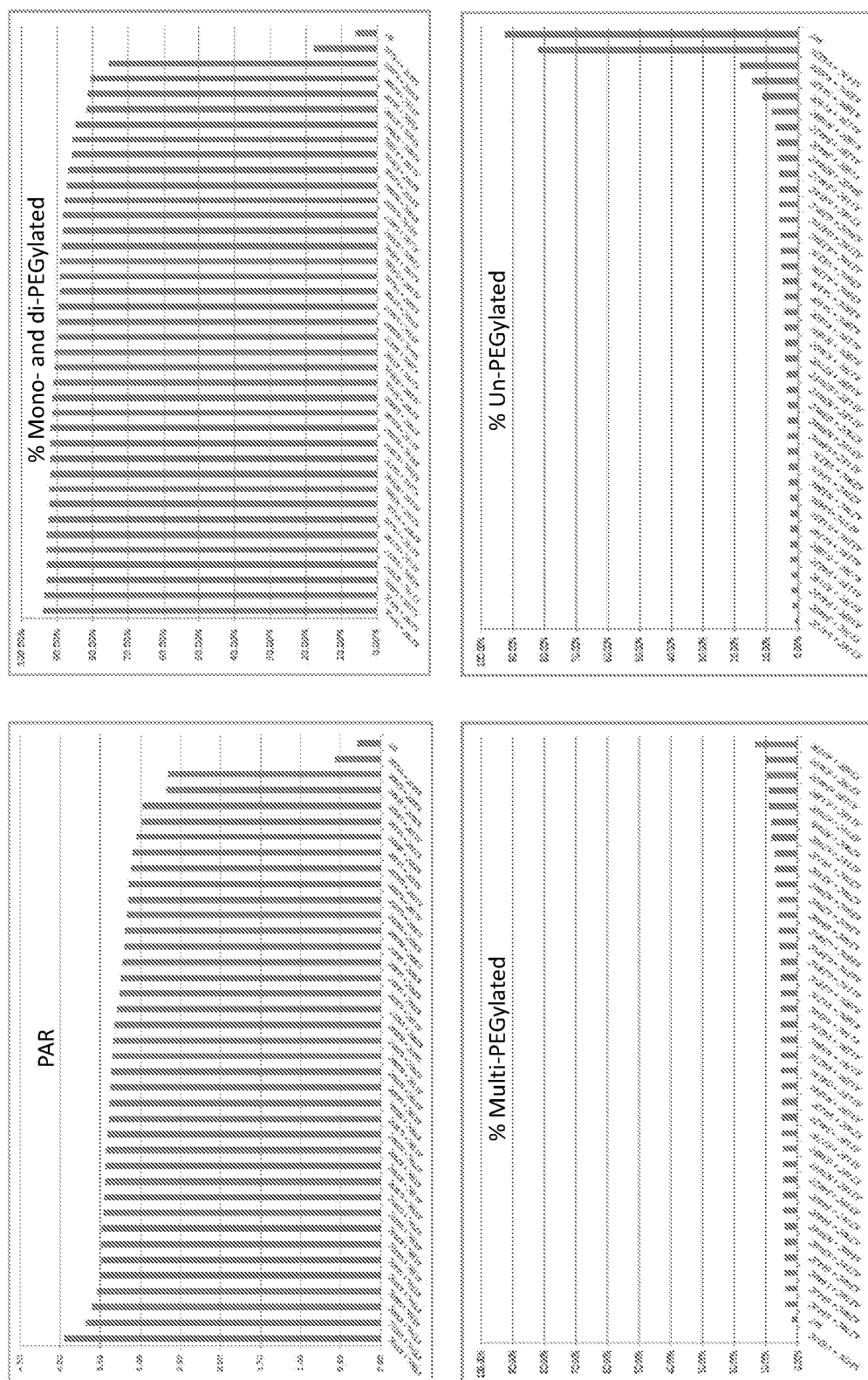


Fig. 11

Mutants	PAR	% Un-PEG	% Mono-&di-PEG	% Multi-PEG	SUM (% Mono-&di-PEG - % Un-PEGy - % Multi-PEG)
K360C + A339C	3.95	5.02%	81.63%	13.35%	0.633
K274C + V422C	3.68	0.82%	91.98%	7.21%	0.840
K274C + K360C	3.60	4.11%	85.73%	10.16%	0.715
A339C + S440C	3.54	2.60%	87.92%	9.48%	0.76
K274C + A339C	3.50	2.03%	90.80%	7.16%	0.816
K274C + S440C	3.49	1.47%	94.03%	4.50%	0.881
A118C + V422C	3.49	2.33%	92.35%	5.32%	0.847
A118C + K274C	3.48	2.47%	92.80%	4.73%	0.856
A339C + V422C	3.46	2.00%	92.88%	5.12%	0.858
K274C + G385C	3.45	2.36%	92.92%	4.60%	0.860
A339C + G385C	3.44	2.55%	91.98%	5.48%	0.840
A118C + A339C	3.43	5.44%	85.53%	9.02%	0.71
A339C + K290C	3.42	4.09%	87.02%	9.00%	0.74
K274C + N384C	3.41	2.76%	92.10%	5.14%	0.842
A118C + G385C	3.39	3.70%	90.86%	5.44%	0.817
K290C + N384C	3.38	4.39%	87.16%	8.46%	0.74
A118C + S440C	3.38	3.03%	92.98%	3.99%	0.860
A339C + N384C	3.37	3.22%	91.87%	4.91%	0.837
A118C + N384C	3.35	3.42%	91.99%	4.58%	0.840

Fig. 12

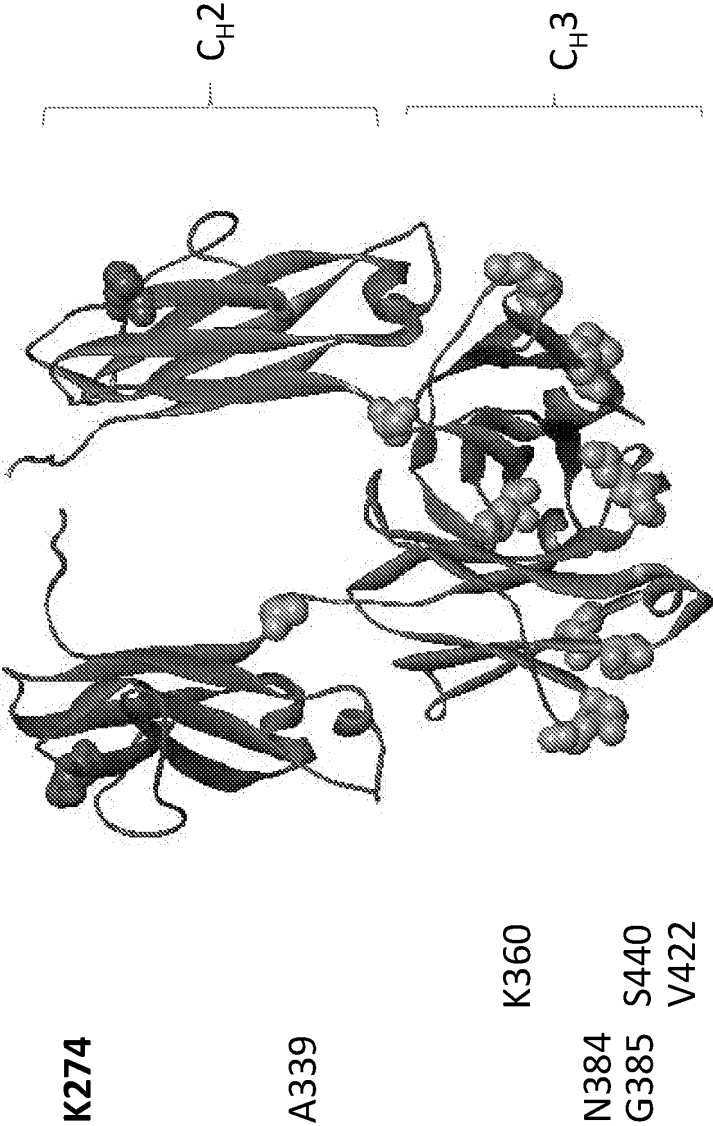


Fig. 13

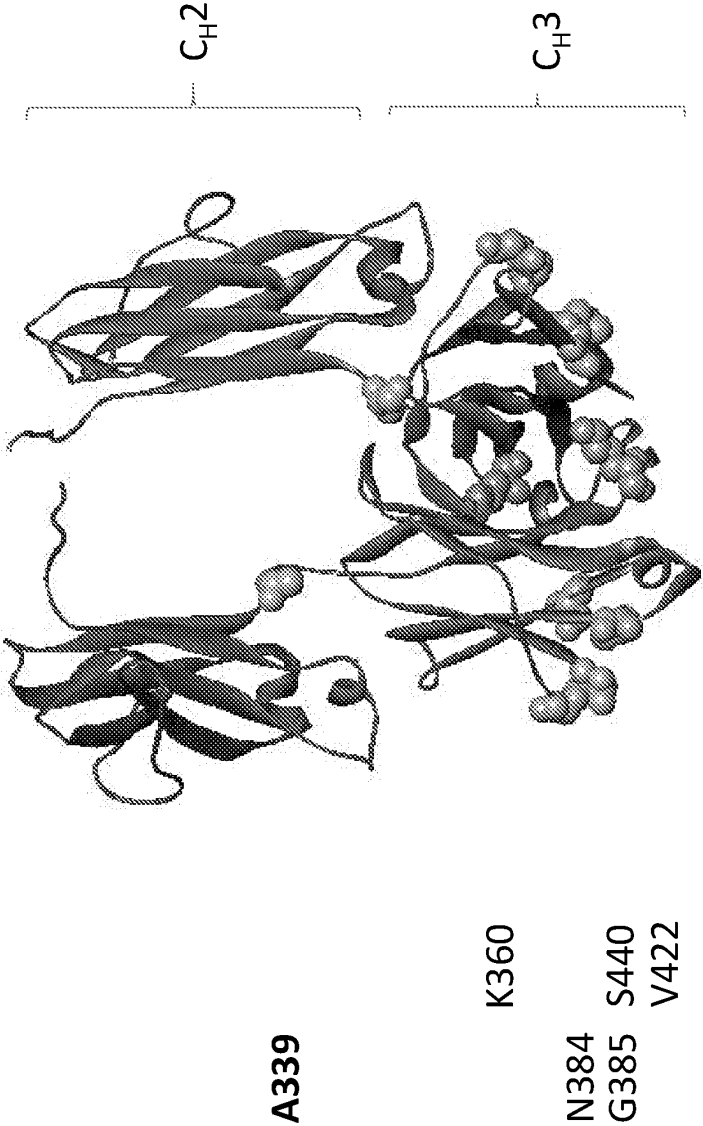


Fig. 14

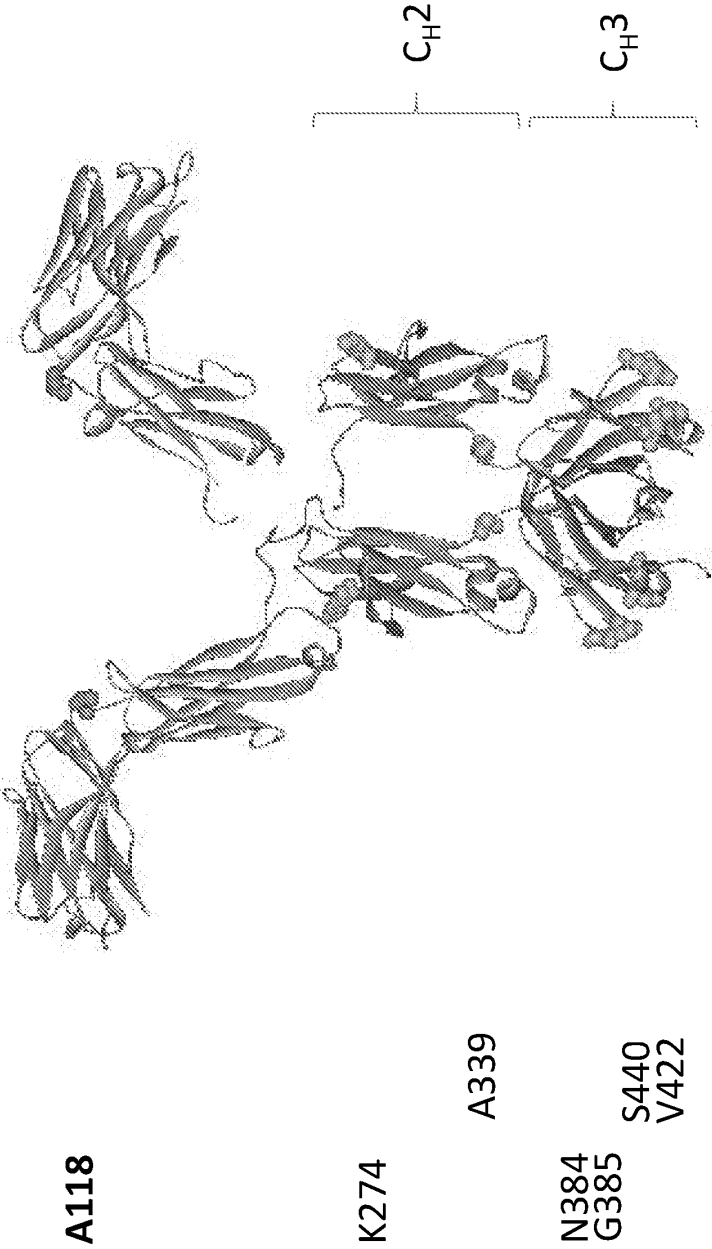


Fig. 15

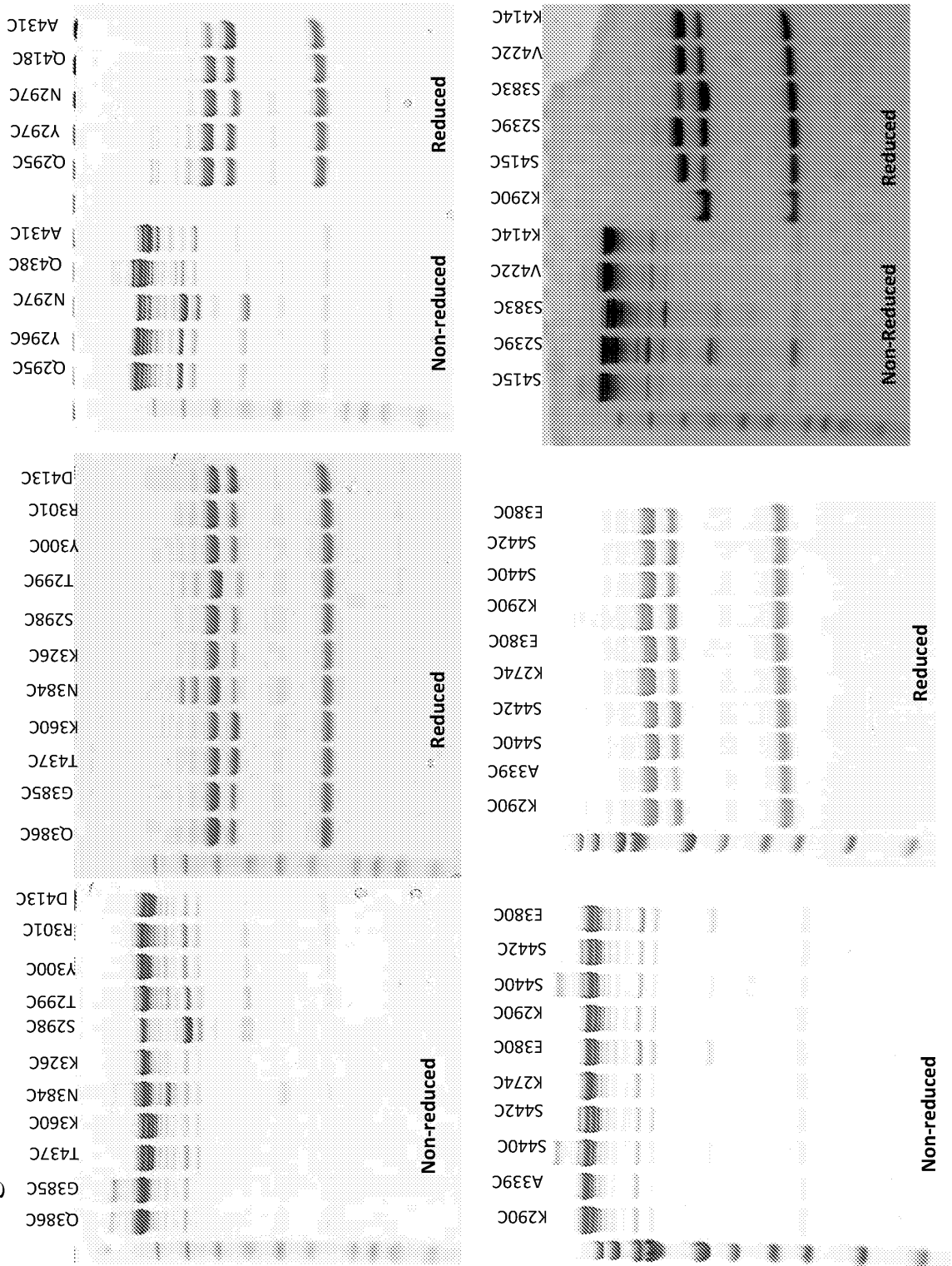


Fig. 16

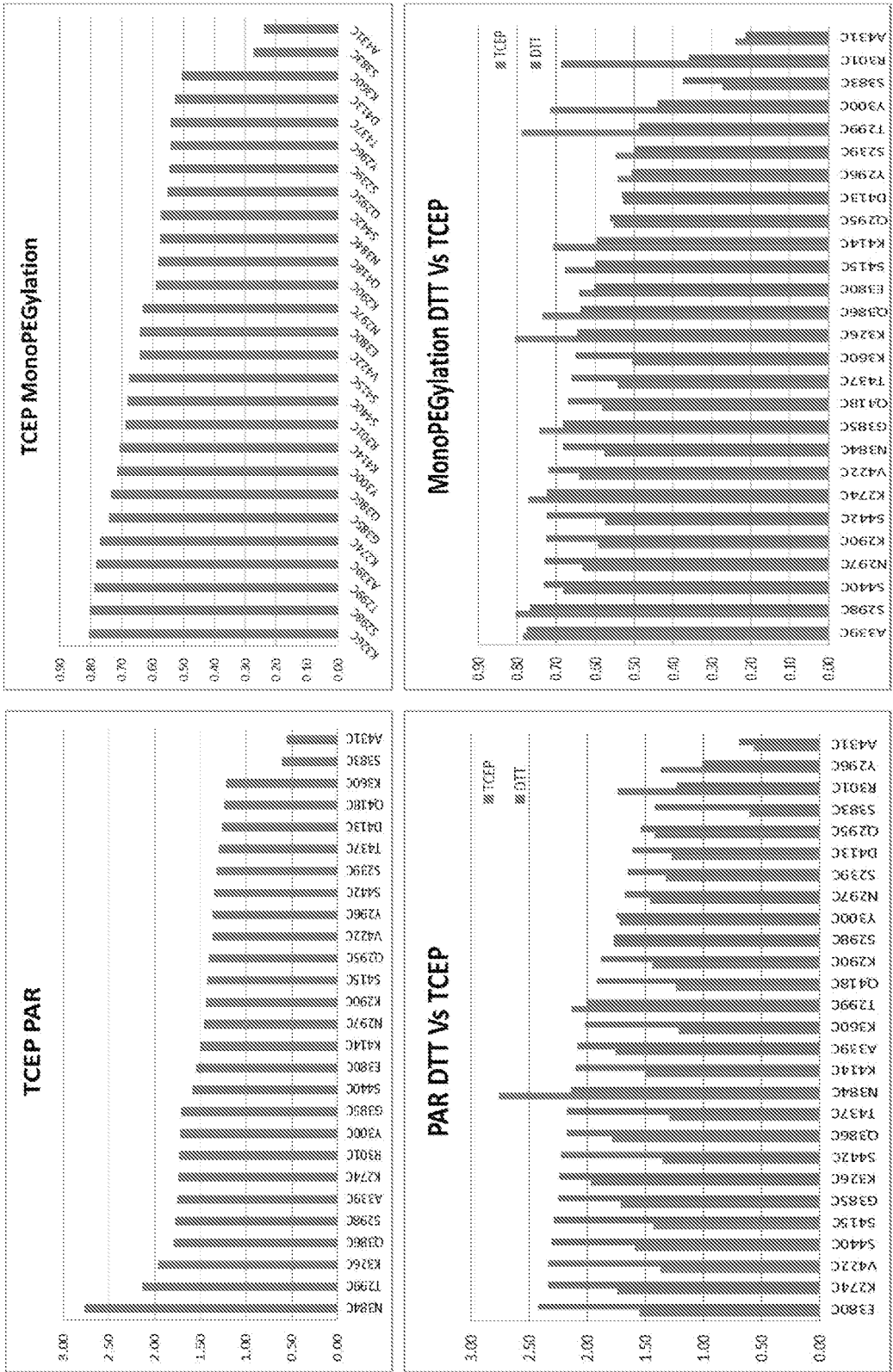


Fig. 16 (cont.)

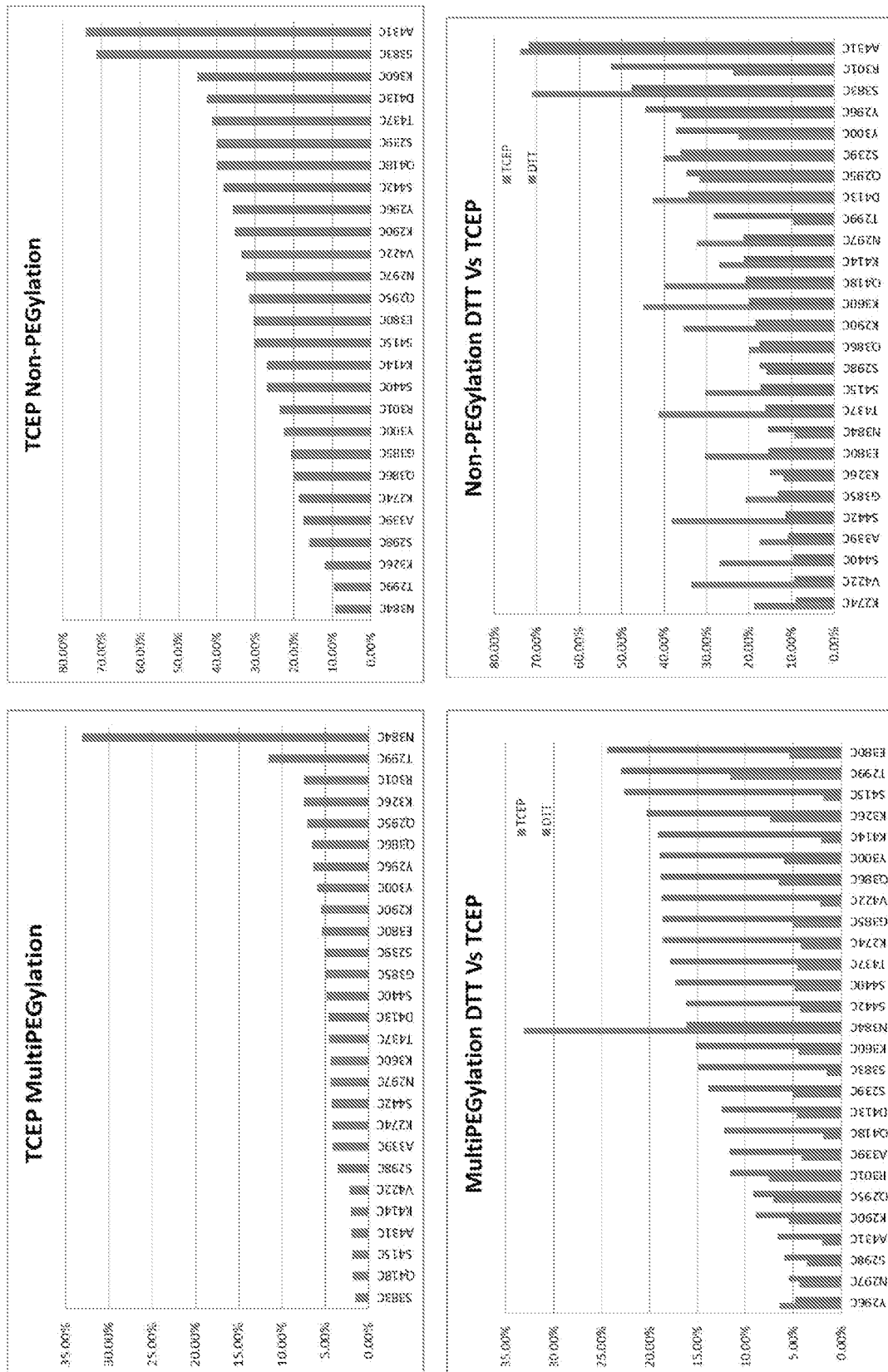
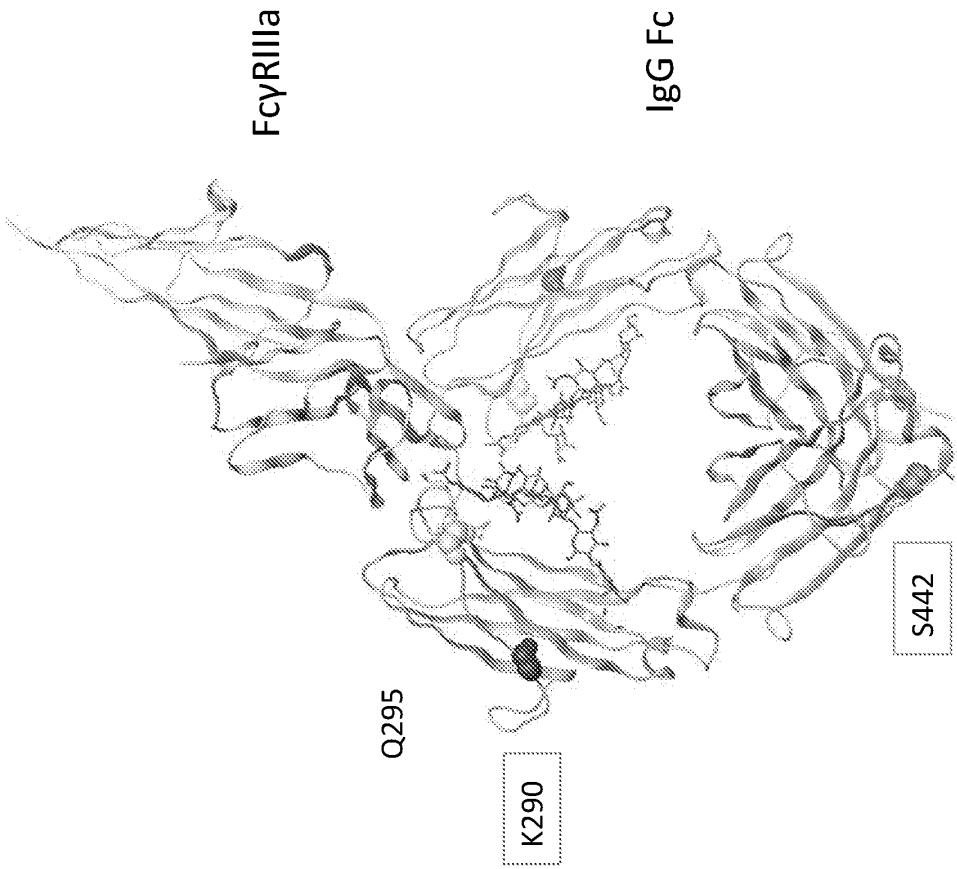


Fig. 17

Mutants	Mono-PEGylated	Multi-PEGylated	Un-PEGylated	PAR
A339C	78.34%	4.17%	17.50%	1.76
A431C	24.03%	2.00%	73.97%	0.56
D413C	52.81%	4.63%	42.56%	1.27
E380C	64.11%	5.44%	30.45%	1.54
G385C	74.23%	4.97%	20.80%	1.71
K274C	77.06%	4.20%	18.74%	1.74
K290C	59.05%	5.52%	35.44%	1.44
K326C	80.64%	7.44%	11.92%	1.97
K360C	50.57%	4.41%	45.01%	1.22
K414C	70.93%	2.04%	27.04%	1.50
N297C	63.21%	4.38%	32.40%	1.46
N384C	57.63%	33.07%	9.30%	2.77
Q295C	55.17%	7.07%	31.67%	1.42
Q386C	73.46%	6.52%	20.03%	1.79
Q418C	58.19%	1.85%	39.96%	1.24
R301C	68.76%	7.48%	23.76%	1.74
S239C	54.74%	5.10%	40.17%	1.33
S298C	80.44%	3.58%	15.98%	1.77
S383C	27.32%	1.51%	71.17%	0.61
S415C	67.77%	1.86%	30.37%	1.43
S440C	68.15%	4.86%	26.99%	1.59
S442C	57.40%	4.30%	38.30%	1.35
T299C	78.87%	11.54%	9.59%	2.14
T437C	54.15%	4.58%	41.27%	1.30
V422C	64.16%	2.22%	33.62%	1.37
Y296C	54.16%	6.37%	35.92%	1.37
Y300C	71.52%	5.97%	22.52%	1.72

Fig. 18



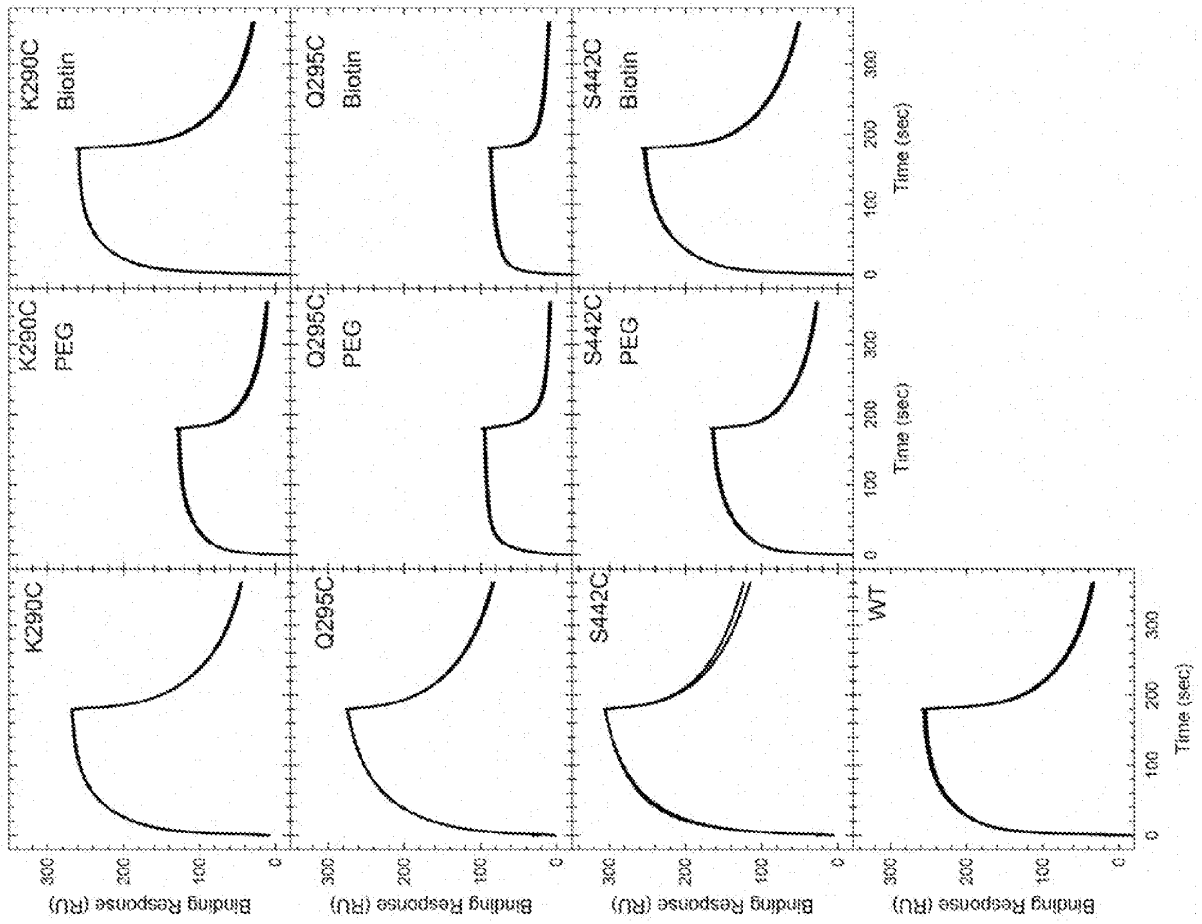


Fig. 19

Fig. 20

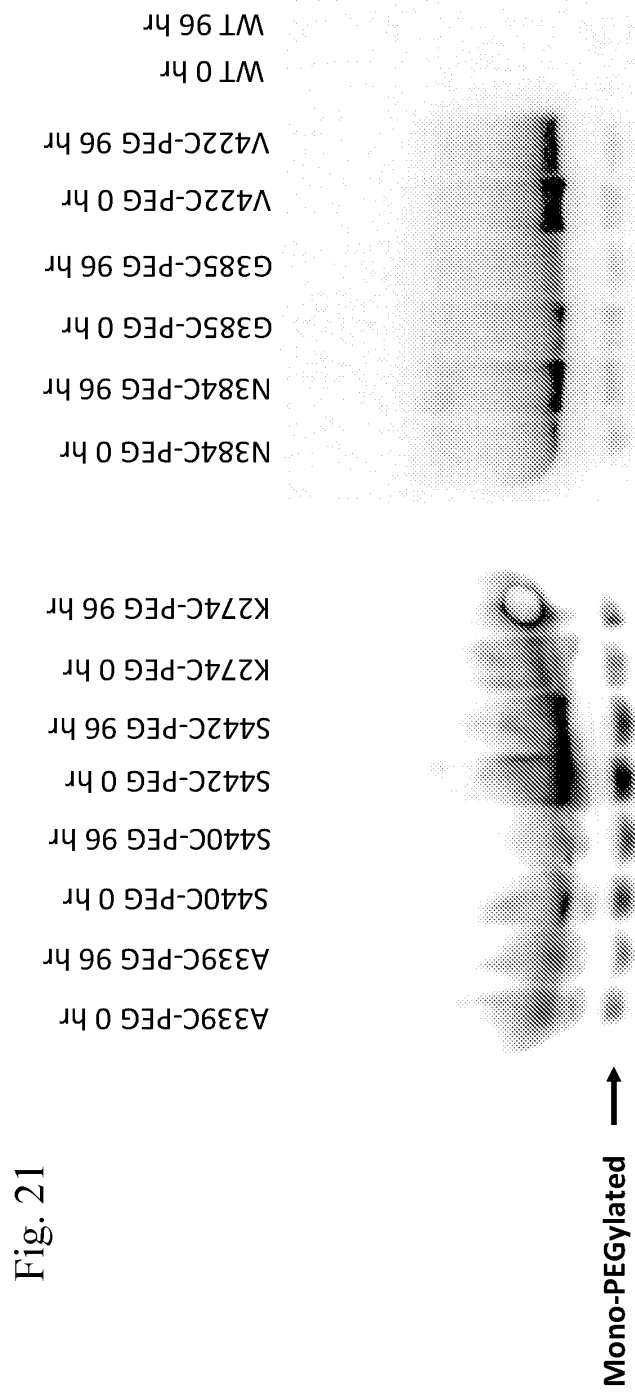


Fig. 21

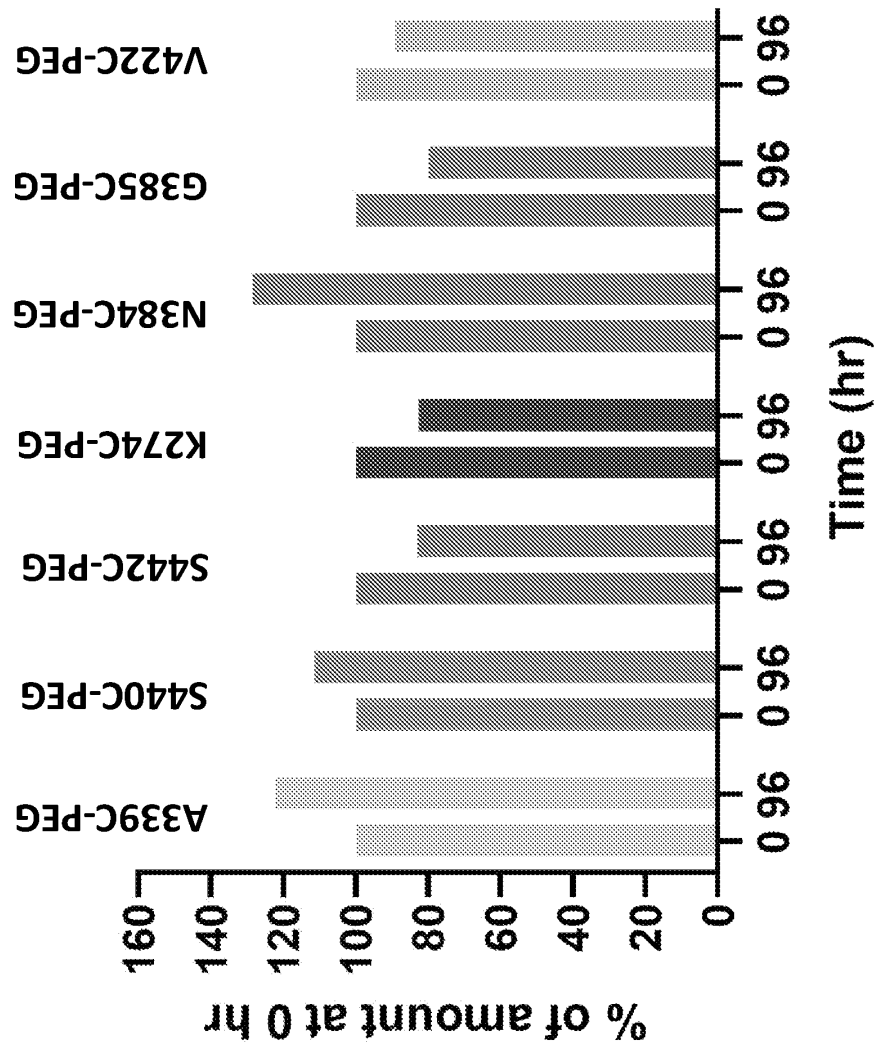


Fig. 22

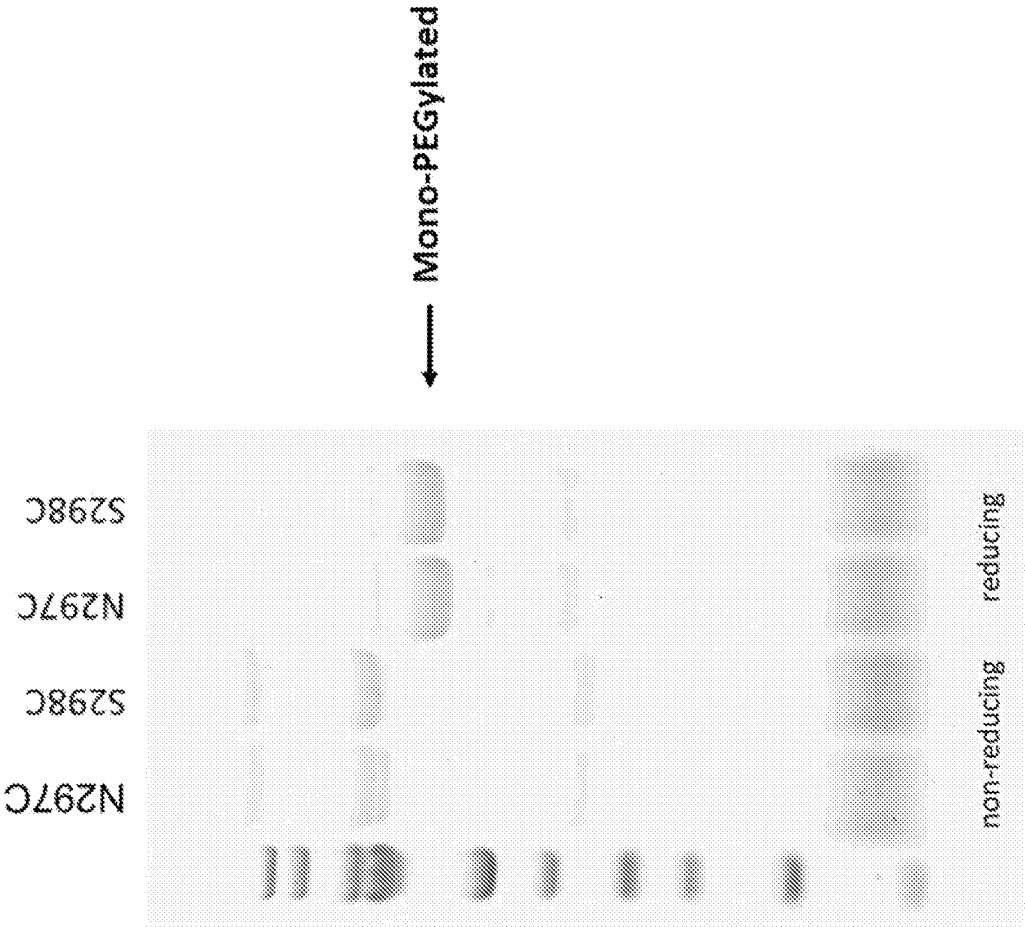


Fig. 23

Fig. 24

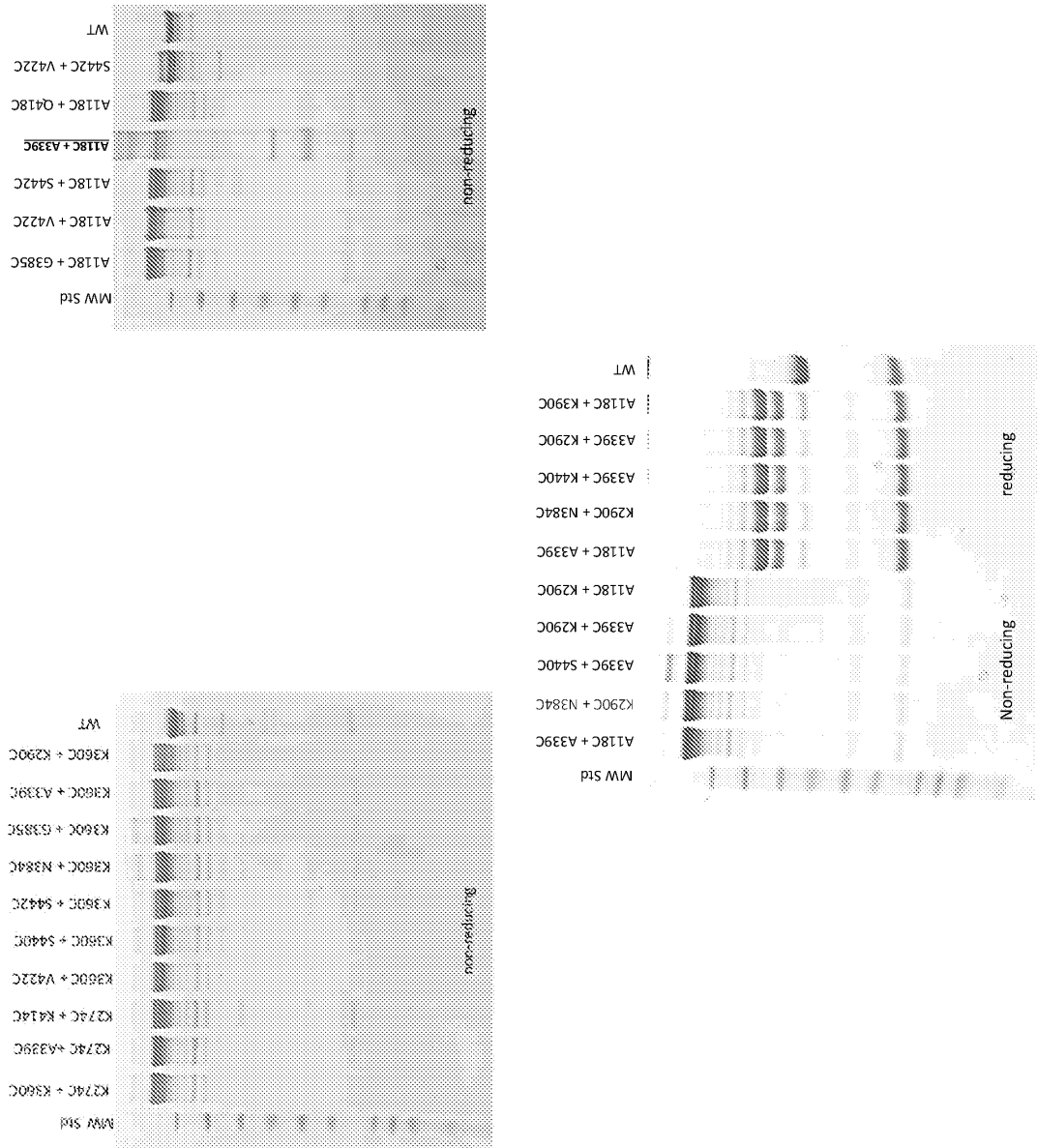


Fig. 25

Double cysteine mutants	PAR	% un-PEGylated	% mono- & di-PEGylated	% Multi-PEGylated	Selectivity (% mono- & di-PEG - % un-PEG - % Multi-PEG)
A114C + A39C	GREEN	YELLOW	GREEN	GREEN	YELLOW
A114C + G38C	GREEN	GREEN	GREEN	GREEN	GREEN
A114C + K274C	GREEN	GREEN	GREEN	GREEN	GREEN
A114C + K290C	YELLOW	RED	GREEN	GREEN	YELLOW
A114C + K360C	YELLOW	GREEN	GREEN	GREEN	GREEN
A114C + N384C	YELLOW	GREEN	GREEN	GREEN	GREEN
A114C + Q418C	YELLOW	YELLOW	GREEN	GREEN	GREEN
A114C + S40C	GREEN	GREEN	GREEN	GREEN	GREEN
A114C + S42C	YELLOW	YELLOW	GREEN	GREEN	GREEN
A114C + V422C	GREEN	GREEN	GREEN	GREEN	GREEN
A39C + G38C	GREEN	GREEN	GREEN	GREEN	GREEN
A39C + K290C	GREEN	GREEN	GREEN	GREEN	YELLOW
A39C + N384C	GREEN	GREEN	GREEN	GREEN	GREEN
A39C + S42C	YELLOW	YELLOW	GREEN	GREEN	GREEN
A39C + V422C	GREEN	GREEN	GREEN	GREEN	GREEN
A39C + S440C	GREEN	GREEN	GREEN	GREEN	GREEN
K274C + A39C	GREEN	GREEN	GREEN	GREEN	GREEN
K274C + G38C	GREEN	GREEN	GREEN	GREEN	GREEN
K274C + K360C	GREEN	GREEN	GREEN	YELLOW	YELLOW
K274C + K414C	YELLOW	YELLOW	GREEN	GREEN	GREEN
K274C + N384C	YELLOW	GREEN	GREEN	GREEN	GREEN
K274C + S440C	GREEN	GREEN	GREEN	GREEN	GREEN
K274C + S42C	YELLOW	GREEN	GREEN	GREEN	GREEN
K274C + V422C	GREEN	GREEN	GREEN	GREEN	GREEN
K290C + G38C	YELLOW	GREEN	GREEN	GREEN	YELLOW
K290C + N384C	GREEN	GREEN	GREEN	GREEN	GREEN
K290C + S440C	YELLOW	GREEN	GREEN	GREEN	GREEN
K290C + S42C	YELLOW	YELLOW	GREEN	GREEN	GREEN
K290C + V422C	YELLOW	GREEN	GREEN	GREEN	GREEN
K360C + A39C	GREEN	GREEN	GREEN	YELLOW	YELLOW
K360C + G38C	YELLOW	YELLOW	GREEN	GREEN	GREEN
K360C + K290C	YELLOW	RED	YELLOW	GREEN	RED
K360C + N384C	YELLOW	YELLOW	GREEN	GREEN	YELLOW
K360C + S440C	YELLOW	RED	GREEN	GREEN	GREEN
K360C + S42C	YELLOW	YELLOW	GREEN	GREEN	GREEN
K360C + V422C	YELLOW	YELLOW	GREEN	GREEN	GREEN
S440C + N384C	RED	RED	RED	RED	RED
S42C + V422C					

PAR: > 3.4 "Green" < 4; > 2.5
 "Yellow" < 3.3; > 0 "Red" <
 2.4

Mono-&di-PEGylated: > 85%
 "Green" < 100%; > 30%
 "Yellow" < 84.9%; > 5% "Red"
 < 29.9%

Multi-PEGylated: < 10%
 "Green" > 0%; > 10.1%
 "Yellow" < 20%; > 20.1%
 "Red" < 100%

Un-PEGylated: < 6% "Green"
 > 0%; > 6.1% "Yellow" < 10%;
 > 10.1% "Red" < 100%

Selectivity: > 0.7 "Green"
 < 1.0, > 0.51 "Yellow" < 0.69,
 > 0 "red" < 0.5