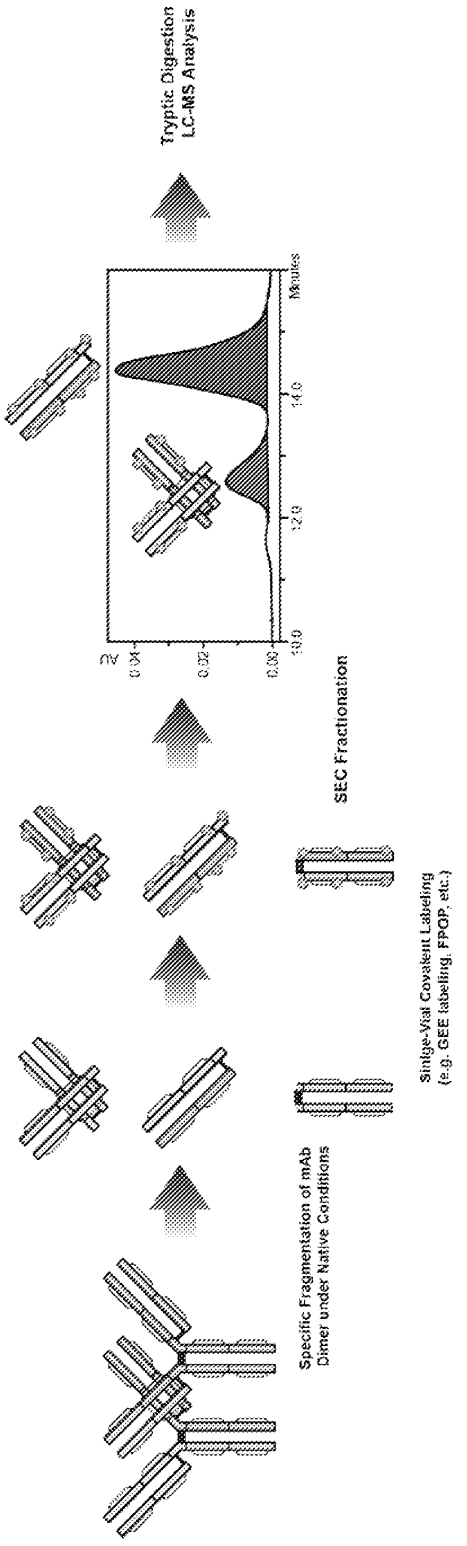

Specific Fragmentation of mAb
Dimer under Native Conditions
Single-Vial Covalent Labeling
(e.g. GEE labelling, FPOP, etc.)
SEC Fractionation
Tryptic Digestion
LC-MS Analysis
Minutes

Figure 1

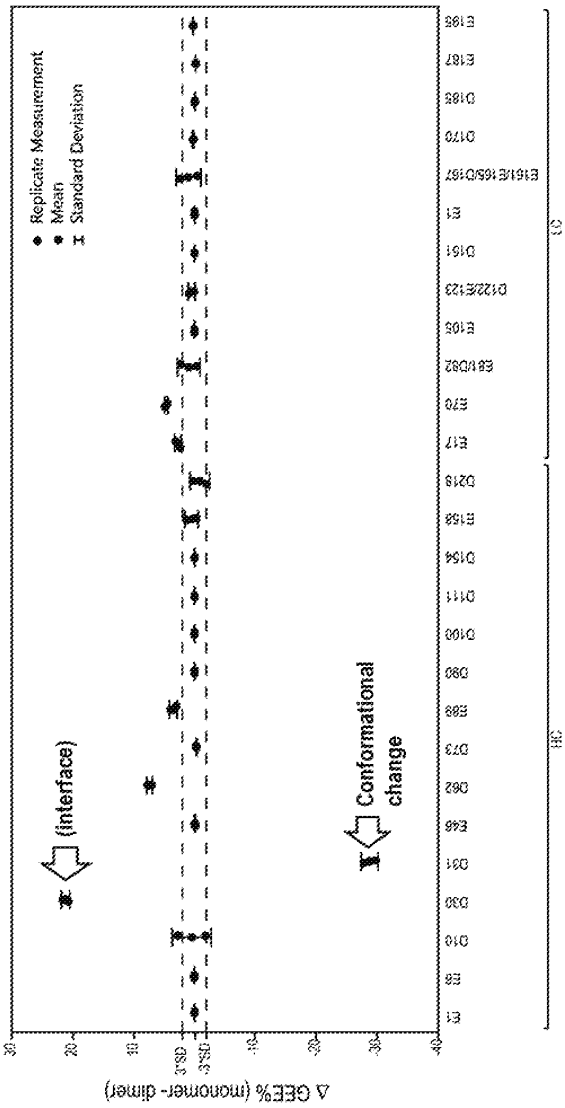

Replicate Measurement
Mean
Standard Deviation
(interface)
Conformational change
Δ GEE% (monomer- dimer)
Chain / residue
HC
LC

Figure 2

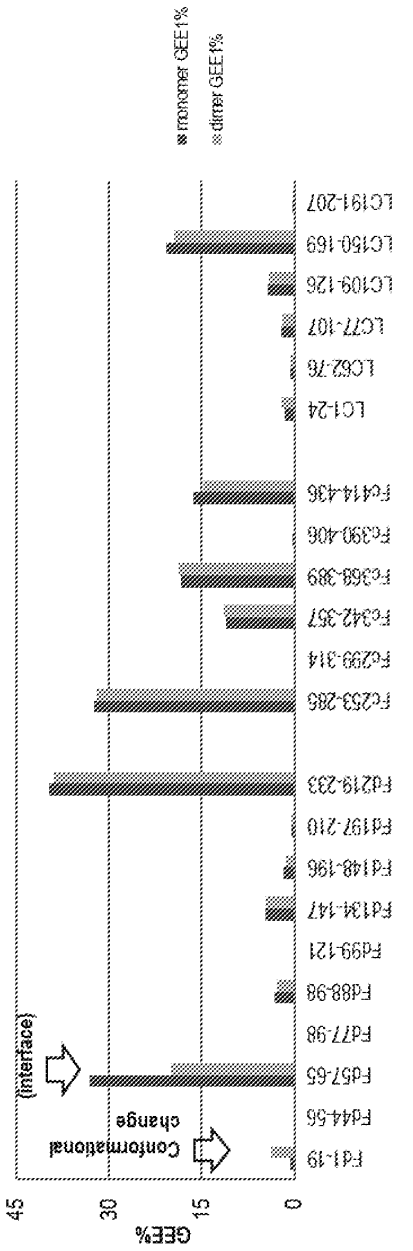


Figure 3A

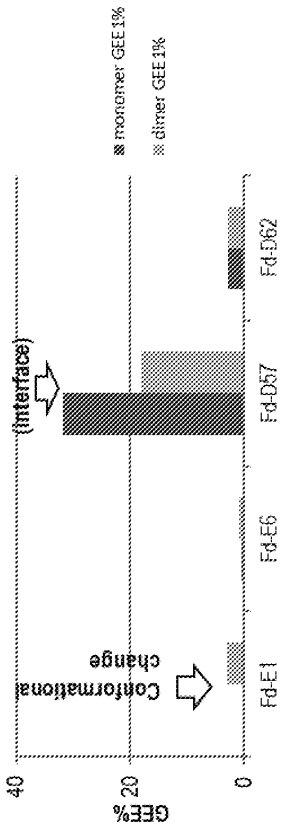


Figure 3B

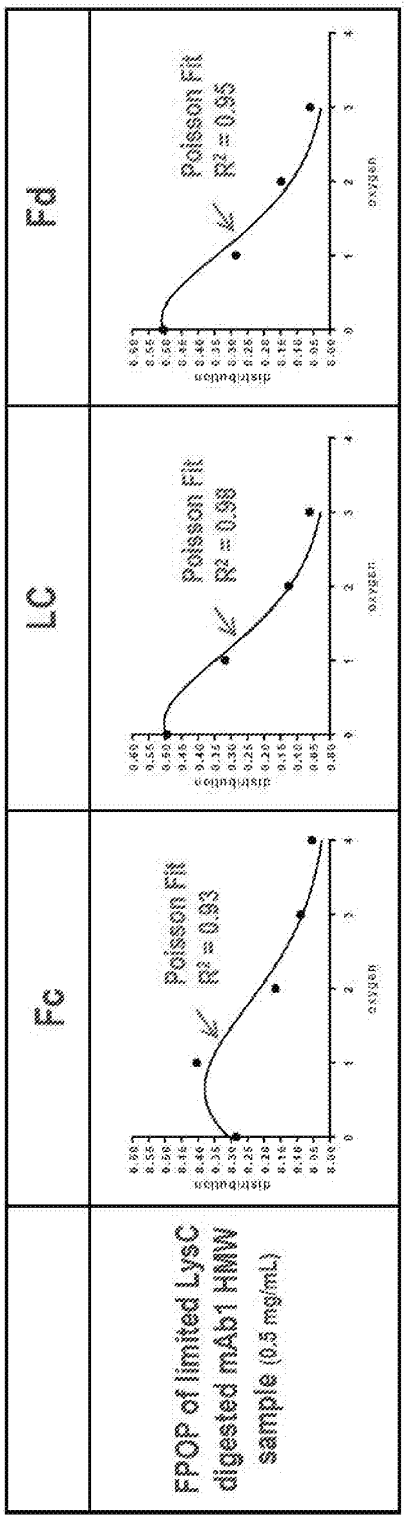


Figure 4A Figure 4B Figure 4C

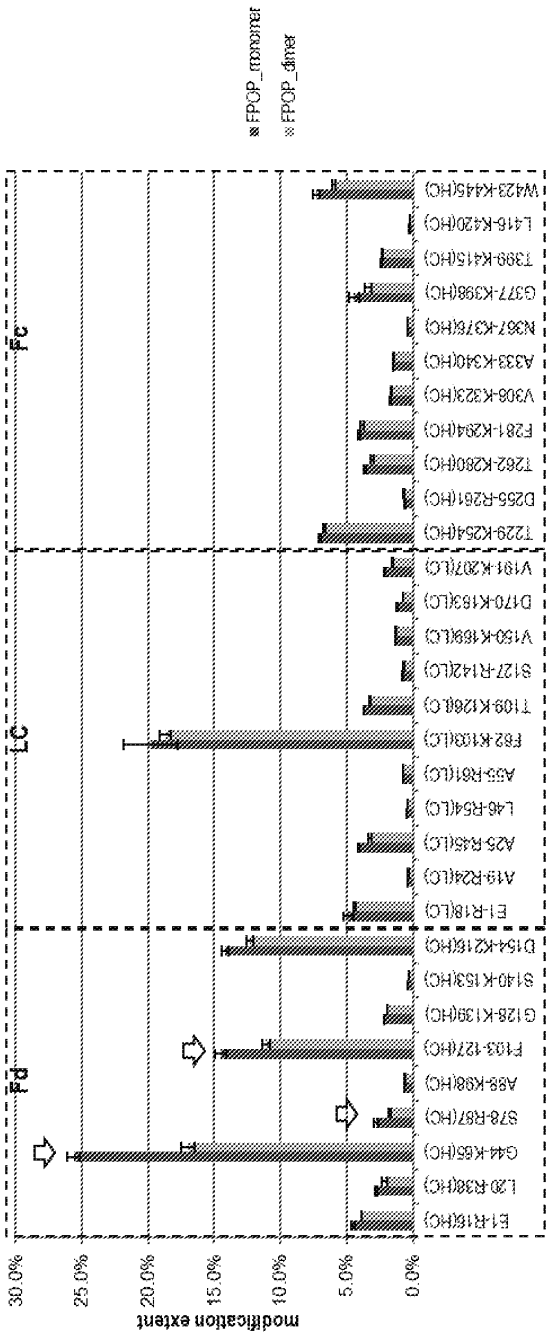

FPOP_monomer
FPOP_dimer
modification extent
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Fd
LC
Fc
E1-R18(HC)
L20-R38(HC)
G44-K65(HC)
S78-R87(HC)
A88-K98(HC)
F103-I27(HC)
G128-K139(HC)
S140-K153(HC)
D154-K216(HC)
E1-R18(LC)
A19-R24(LC)
A25-R45(LC)
L46-R54(LC)
A55-R61(LC)
F82-K103(LC)
T109-K126(LC)
S127-R142(LC)
V150-K169(LC)
D170-K183(LC)
V191-K207(LC)
T229-K254(HC)
D255-R261(HC)
T262-K280(HC)
F281-K294(HC)
V306-K323(HC)
A333-K340(HC)
N367-K376(HC)
G377-K398(HC)
T399-K415(HC)
L416-K420(HC)
W423-K445(HC)

Figure 5A

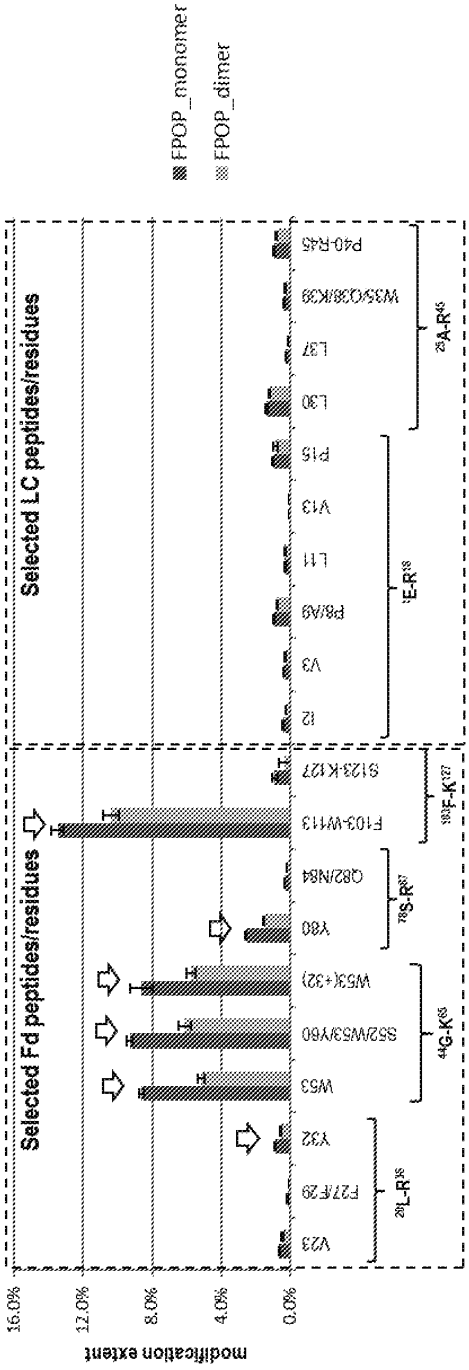

FPOP_monomer
FPOP_dimer
Selected LC peptides/residues
Selected Fd peptides/residues
modification extent
16.0%
12.0%
8.0%
4.0%
0.0%
I2
V3
P8/A9
L11
V13
P15
L30
L37
W35/Q38/K39
P40-R45
25A-R45
1E-R18
S123-K127
F103-W113
183F-K127
Q82/N84
Y80
78S-R87
W53(+32)
S52/W53/Y60
W53
44G-K65
Y32
F27/F29
V23
26L-R38

Figure 5B

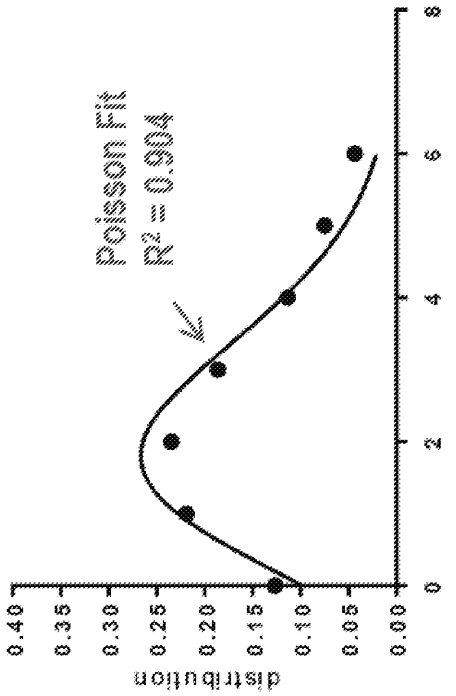


Figure 6B

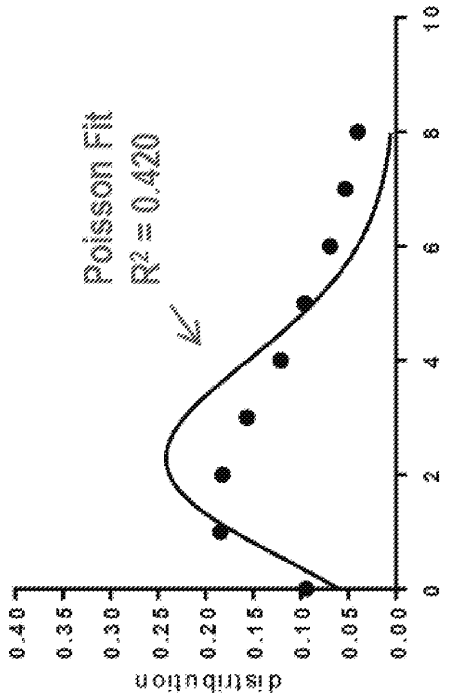
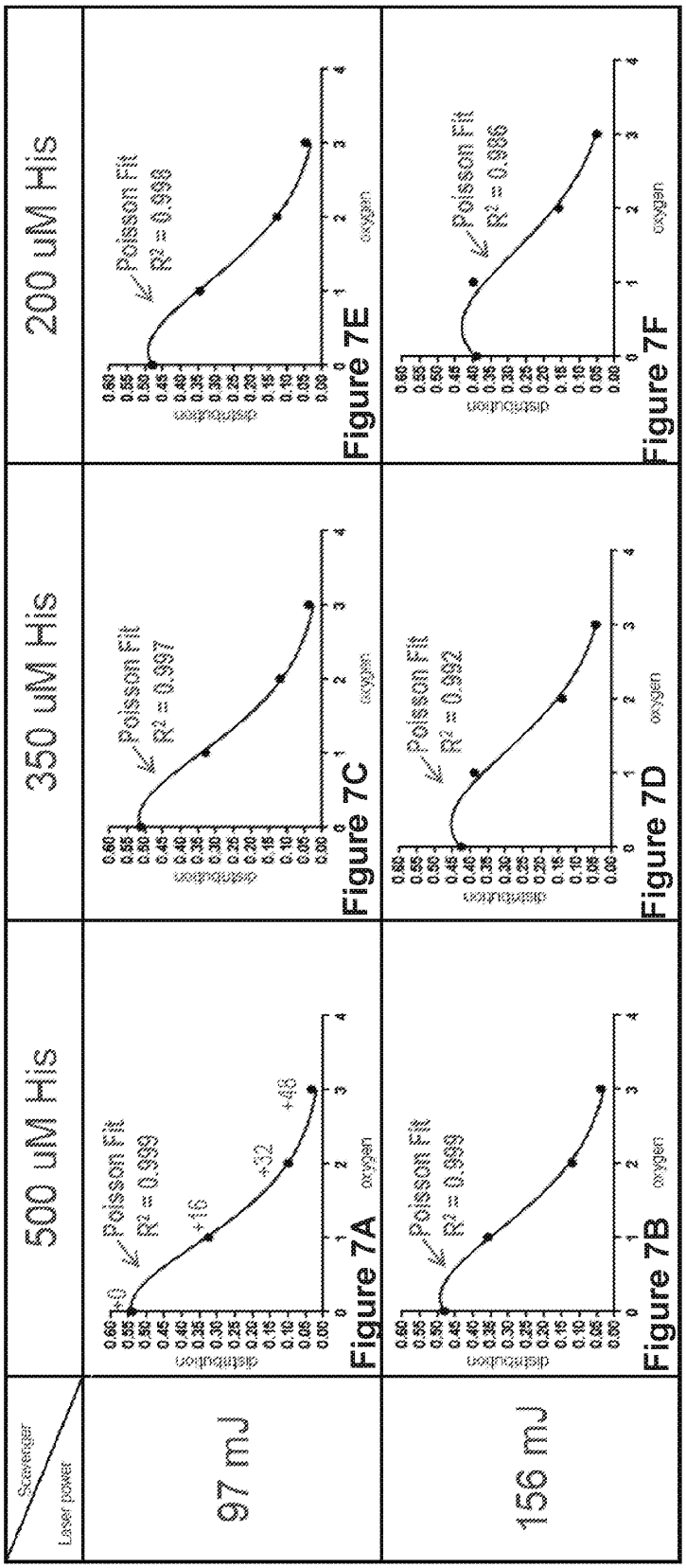
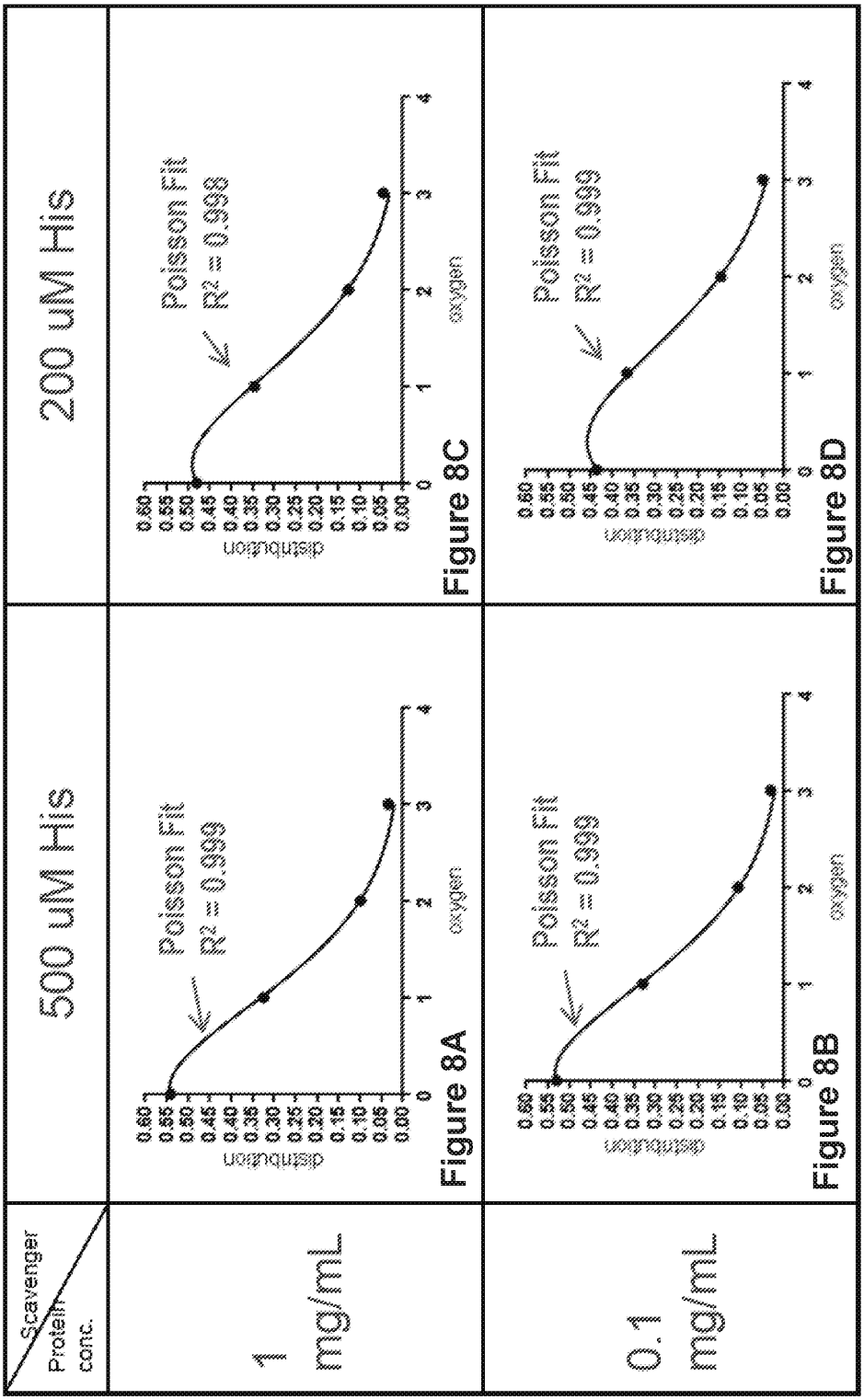
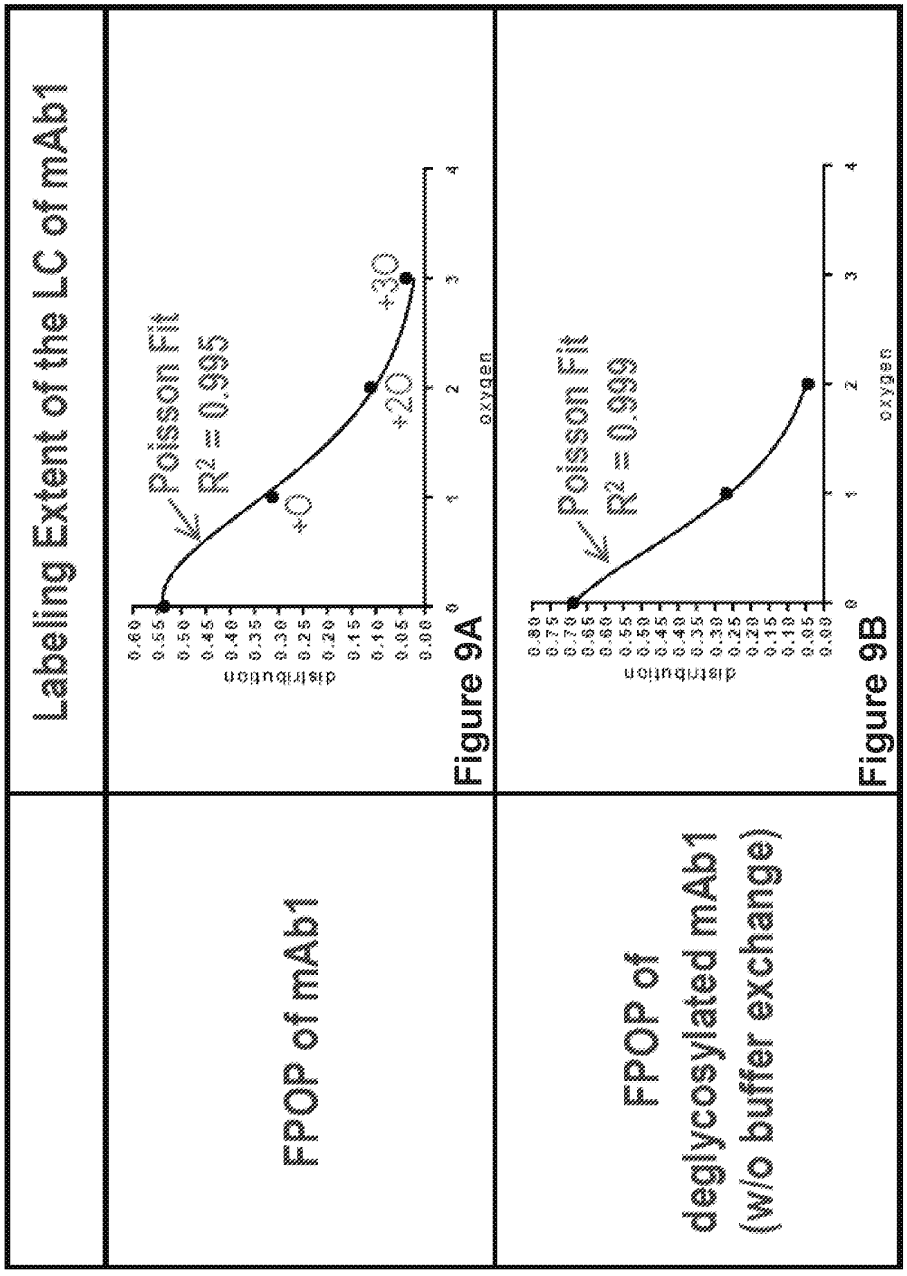


Figure 6A


Scavenger
Laser power
500 uM His
350 uM His
200 uM His
97 mJ
156 mJ
Poisson Fit
R² = 0.999
R² = 0.997
R² = 0.992
R² = 0.986
+0
+16
+32
+48
absorbance
oxygen
Figure 7A
Figure 7B
Figure 7C
Figure 7D
Figure 7E
Figure 7F


Scavenger
Protein
conc.
500 uM His
200 uM His
1 mg/mL
0.1 mg/mL
Poisson Fit
R² = 0.999
R² = 0.998
distribution
oxygen
Figure 8A
Figure 8B
Figure 8C
Figure 8D


Labeling Extent of the LC of mAb1
FPOP of mAb1
Poisson Fit
R² = 0.995
+0
+20
+30
distribution
oxygen
Figure 9A
FPOP of deglycosylated mAb1 (w/o buffer exchange)
Poisson Fit
R² = 0.999
distribution
oxygen
Figure 9B