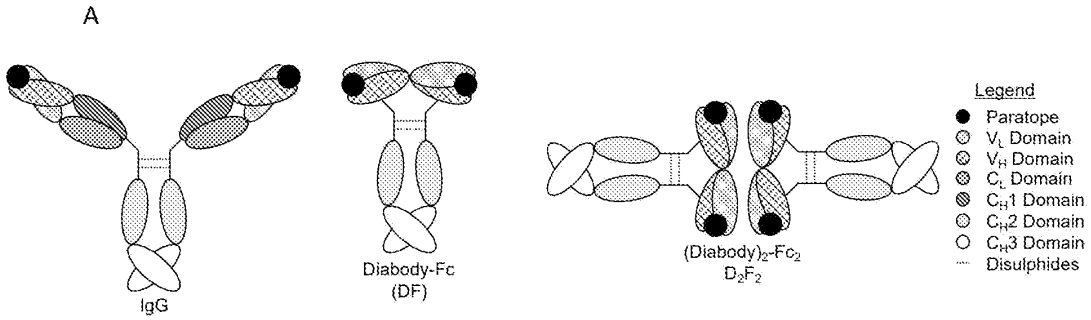
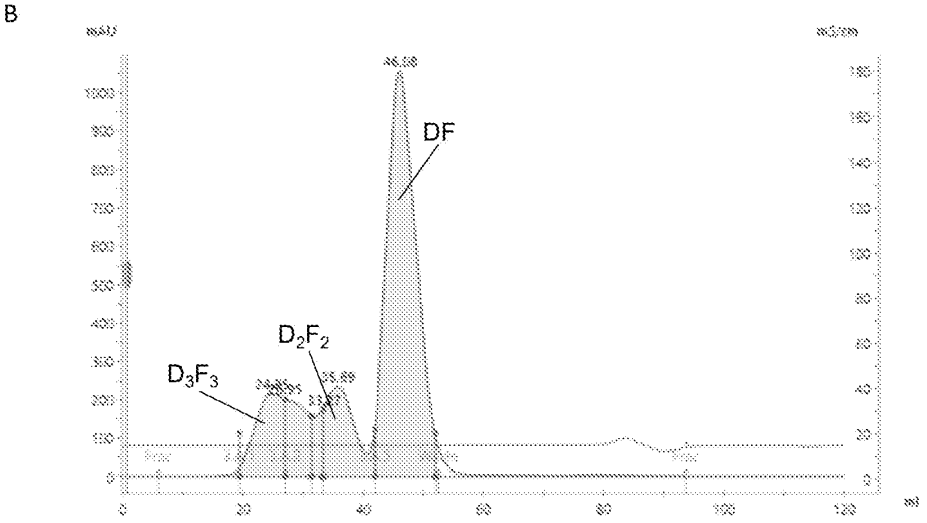

A
IgG
Diabody-Fc
(DF)
(Diabody)2-Fc2
D2F2
Legend
Paratope
VL Domain
VH Domain
CL Domain
CH1 Domain
CH2 Domain
CH3 Domain
Disulphides


B
DF
D2F2
D3F3

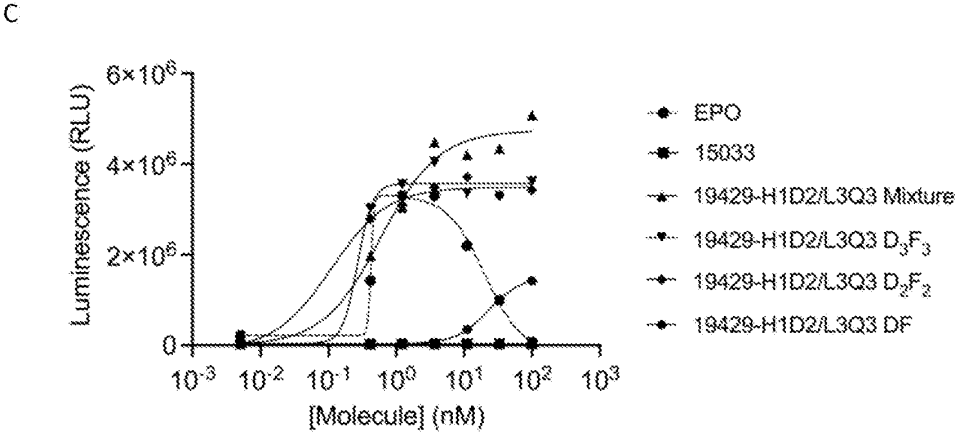

C
Luminescence (RLU)
6×10^6
4×10^6
2×10^6
0
10^-3
10^-2
10^-1
10^0
10^1
10^2
10^3
[Molecule] (nM)
EPO
15033
19429-H1D2/L3Q3 Mixture
19429-H1D2/L3Q3 D3F3
19429-H1D2/L3Q3 D2F2
19429-H1D2/L3Q3 DF

Fig. 1

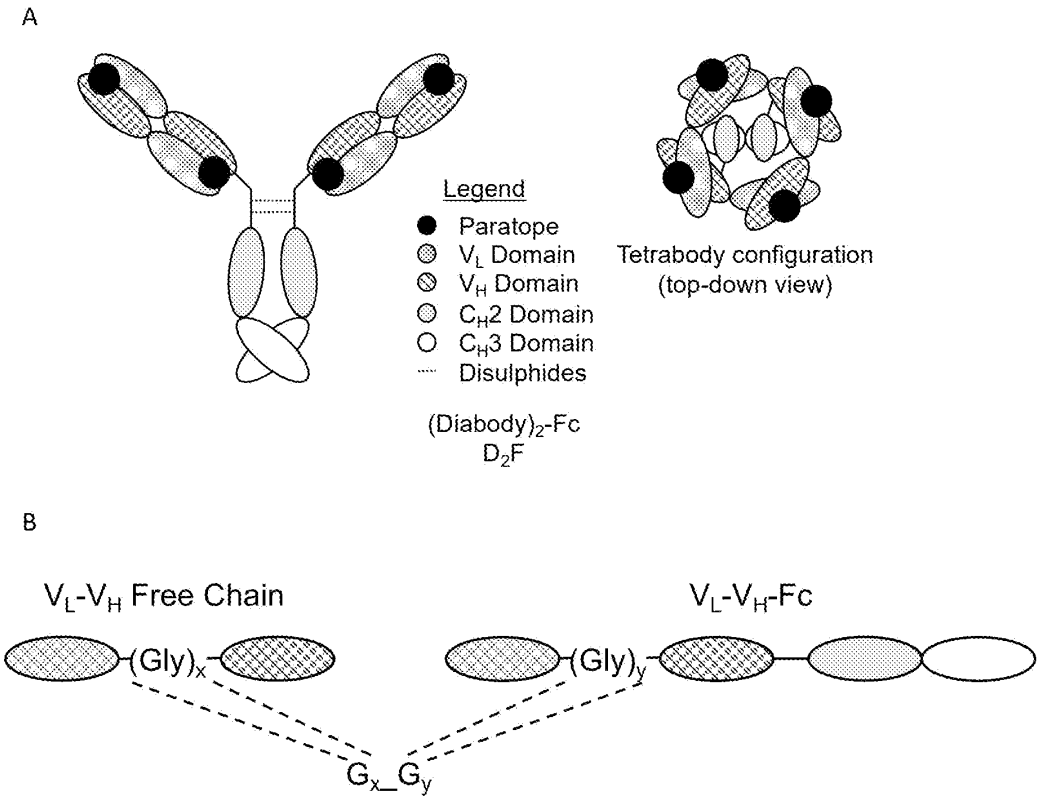
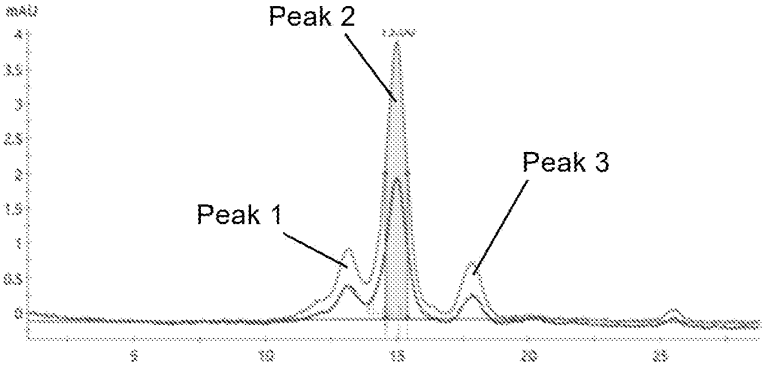
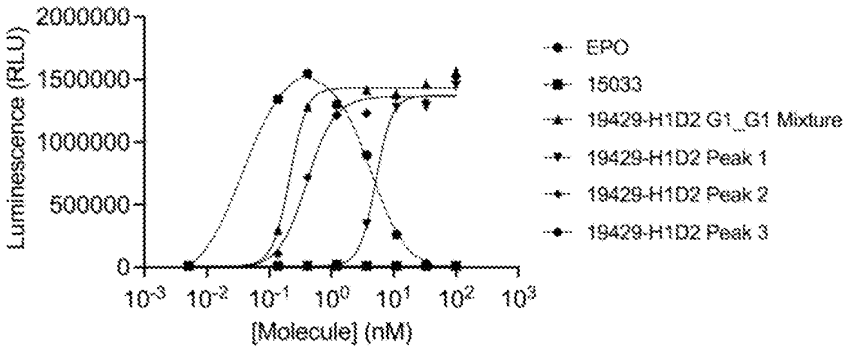

A
Legend
Paratope
V_L Domain
V_H Domain
C_H2 Domain
C_H3 Domain
Disulphides
$(Diabody)_2$-Fc
D_2F
Tetrabody configuration
(top-down view)
B
V_L-V_H Free Chain
V_L-V_H-Fc
$(Gly)_x$
$(Gly)_y$
G_x_G_y
C
Luminescence (RLU)
4000000
3000000
2000000
1000000
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
[Molecule] (nM)
EPO
15033
19429-H1D2 G0_G0
19429-H1D2 G0_G1
19429-H1D2 G0_G2
19429-H1D2 G1_G0
19429-H1D2 G1_G1
19429-H1D2 G1_G2

Fig. 2

D


Peak 2
Peak 3
Peak 1

E


Luminescence (RLU)
2000000
1500000
1000000
500000
0
10-3 10-2 10-1 100 101 102 103
[Molecule] (nM)
EPO
15033
19429-H1D2 G1_G1 Mixture
19429-H1D2 Peak 1
19429-H1D2 Peak 2
19429-H1D2 Peak 3

F

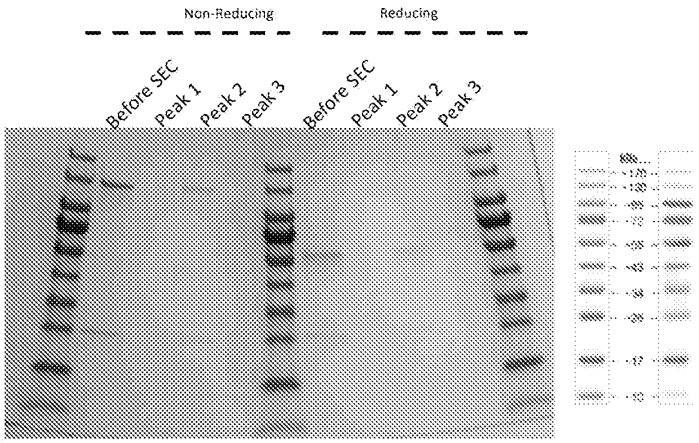
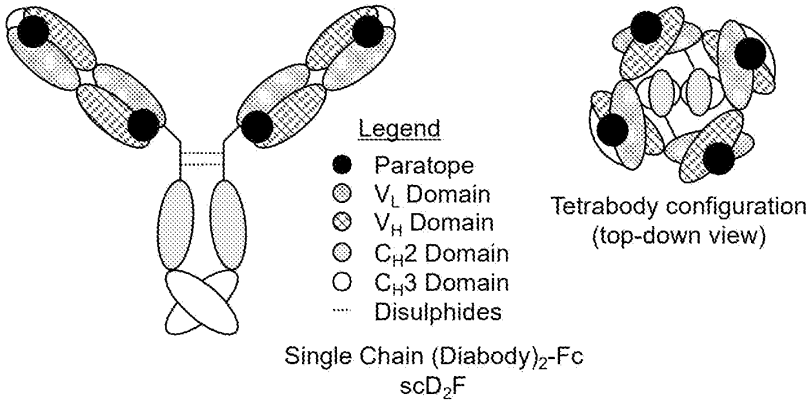
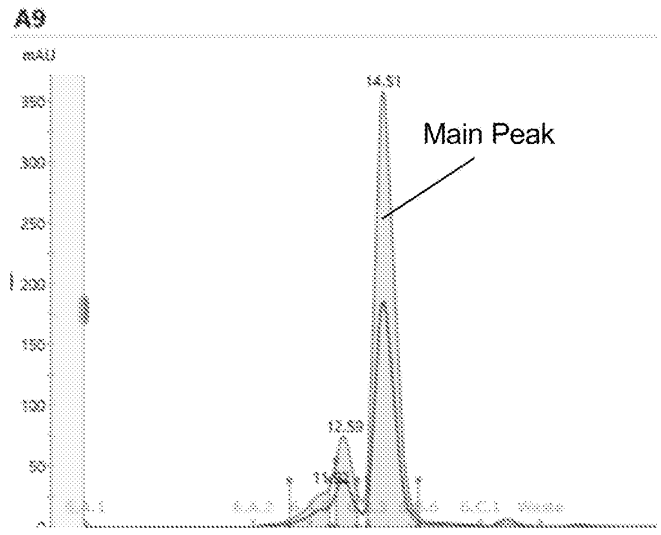

Non-Reducing
Reducing
Before SEC
Peak 1
Peak 2
Peak 3
Before SEC
Peak 1
Peak 2
Peak 3

Fig. 2

A


Legend
Paratope
V_L Domain
V_H Domain
C_H2 Domain
C_H3 Domain
Disulphides
Tetrabody configuration
(top-down view)
Single Chain (Diabody)$_2$-Fc
scD$_2$F

B


A9
mAU
Main Peak

C

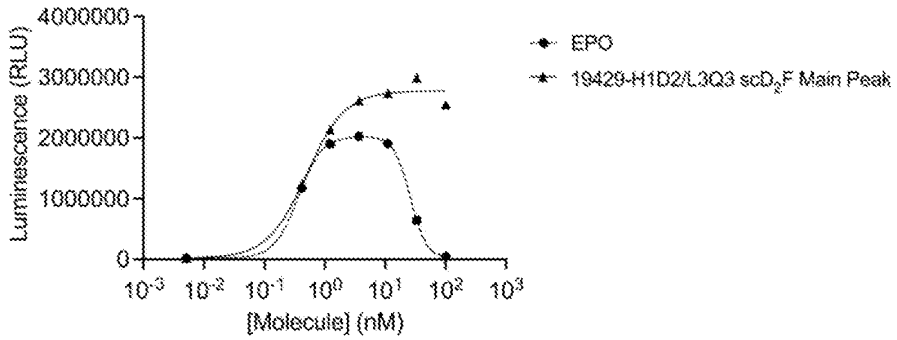
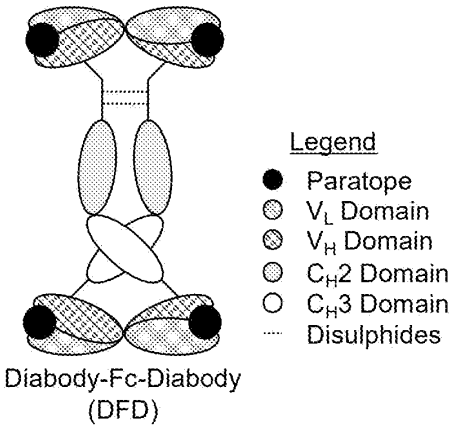
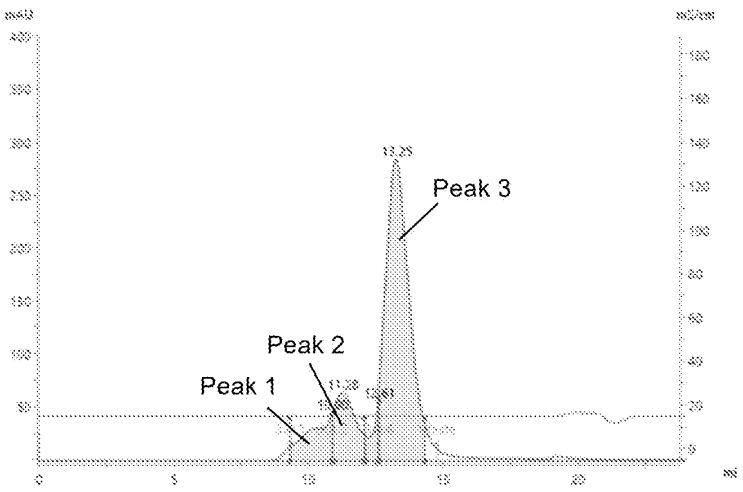

Luminescence (RLU)
4000000
3000000
2000000
1000000
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
[Molecule] (nM)
EPO
19429-H1D2/L3Q3 scD$_2$F Main Peak

Fig. 3

A


Legend
Paratope
V_L Domain
V_H Domain
C_H2 Domain
C_H3 Domain
Disulphides
Diabody-Fc-Diabody
(DFD)

B


Peak 3
Peak 2
Peak 1

C

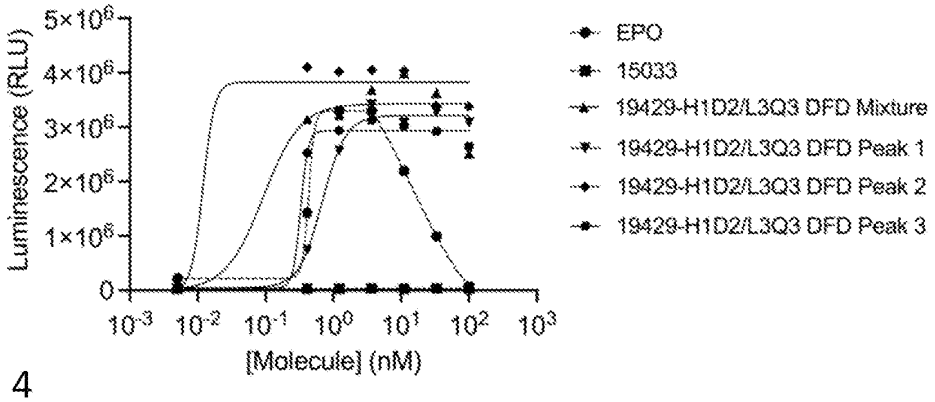
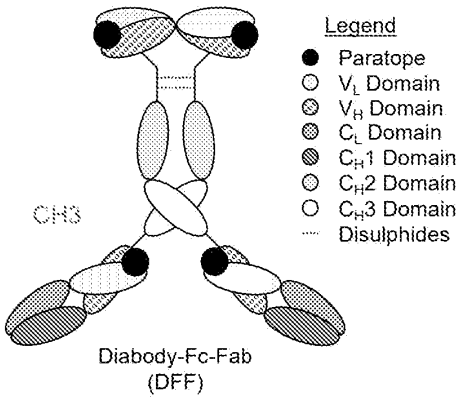
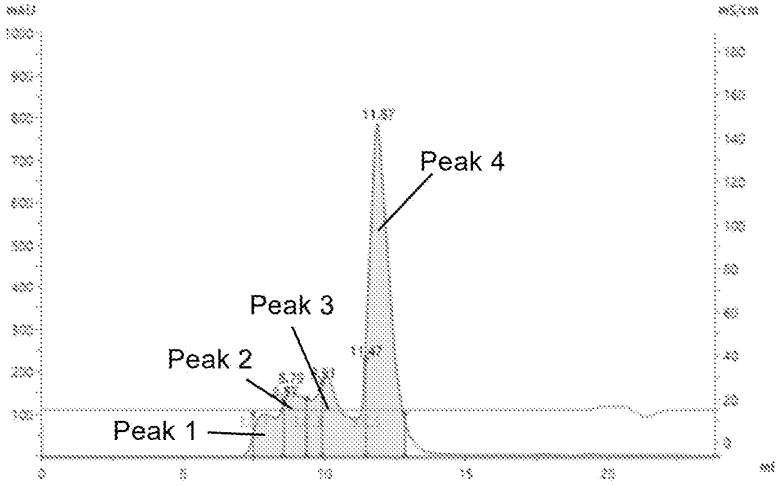

Luminescence (RLU)
5×10^6
4×10^6
3×10^6
2×10^6
1×10^6
0
10^-3 10^-2 10^-1 10^0 10^1 10^2 10^3
[Molecule] (nM)
EPO
15033
19429-H1D2/L3Q3 DFD Mixture
19429-H1D2/L3Q3 DFD Peak 1
19429-H1D2/L3Q3 DFD Peak 2
19429-H1D2/L3Q3 DFD Peak 3

Fig. 4

A


Legend
Paratope
V_L Domain
V_H Domain
C_L Domain
C_H1 Domain
C_H2 Domain
C_H3 Domain
Disulphides
CH3
Diabody-Fc-Fab
(DFF)

B


Peak 4
Peak 3
Peak 2
Peak 1

C

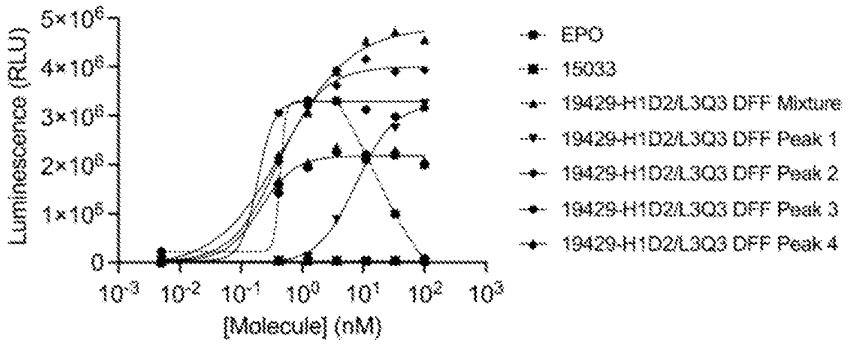
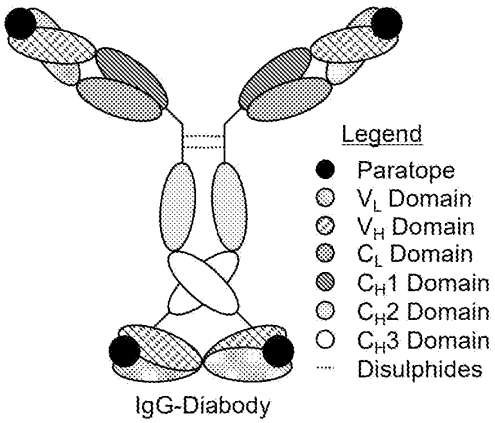
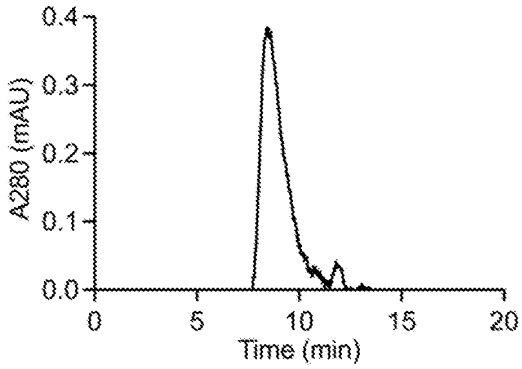

Luminescence (RLU)
5×10^6
4×10^6
3×10^6
2×10^6
1×10^6
0
10^{-3} 10^{-2} 10^{-1} 10^0 10^1 10^2 10^3
[Molecule] (nM)
EPO
15033
19429-H1D2/L3Q3 DFF Mixture
19429-H1D2/L3Q3 DFF Peak 1
19429-H1D2/L3Q3 DFF Peak 2
19429-H1D2/L3Q3 DFF Peak 3
19429-H1D2/L3Q3 DFF Peak 4

Fig. 5

A


Legend
Paratope
V_L Domain
V_H Domain
C_L Domain
C_H1 Domain
C_H2 Domain
C_H3 Domain
Disulphides
IgG-Diabody

B


A280 (mAU)
0.4
0.3
0.2
0.1
0.0
0
5
10
15
20
Time (min)

C

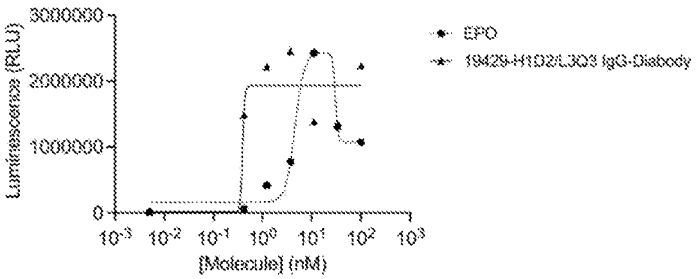
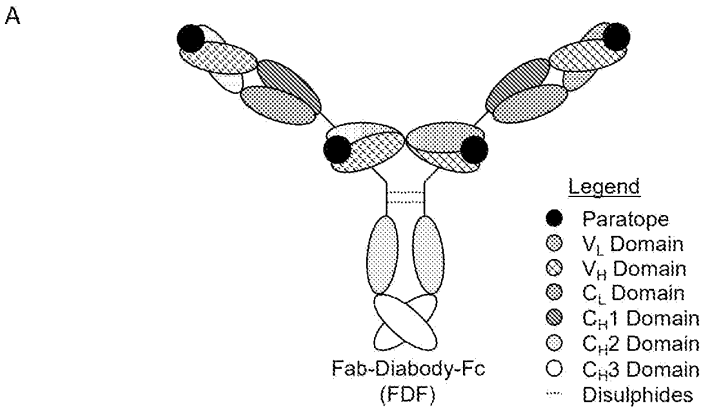
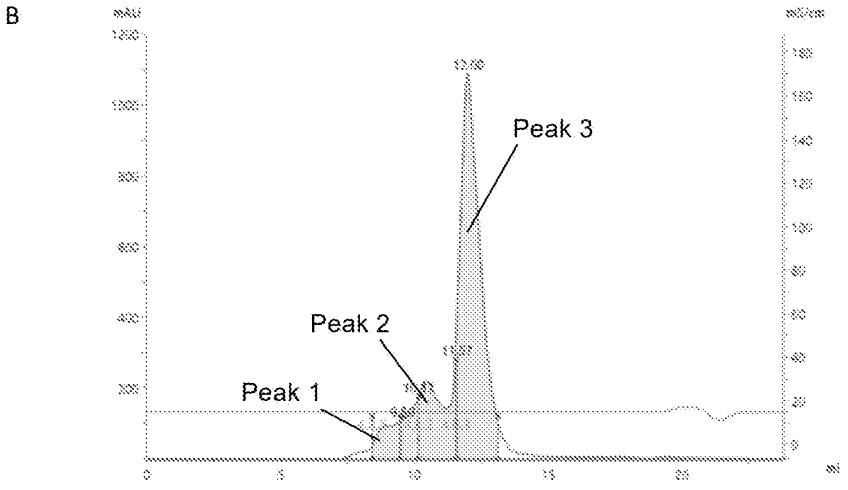

Luminescence (RLU)
3000000
2000000
1000000
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
[Molecule] (nM)
EPO
19429-H1D2/L3D3 IgG-Diabody

Fig. 6


A
Fab-Diabody-Fc
(FDF)
Legend
Paratope
V_L Domain
V_H Domain
C_L Domain
C_H1 Domain
C_H2 Domain
C_H3 Domain
Disulphides


B
Peak 3
Peak 2
Peak 1

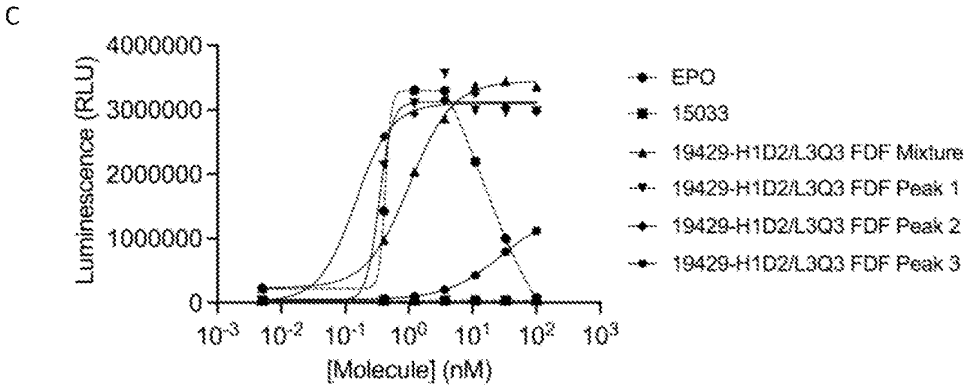
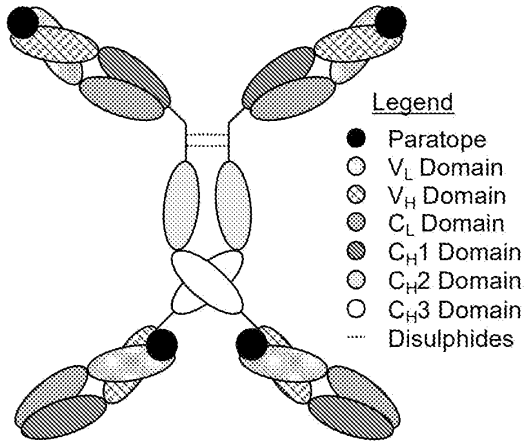
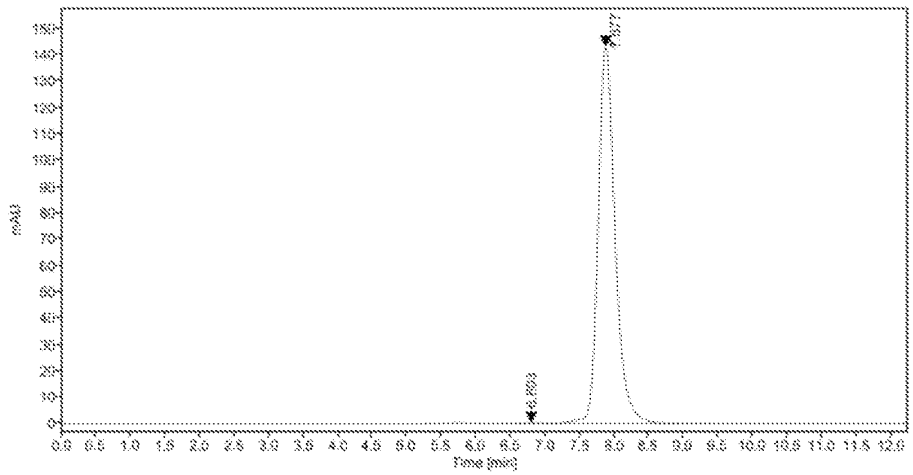

C
Luminescence (RLU)
4000000
3000000
2000000
1000000
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
[Molecule] (nM)
EPO
15033
19429-H1D2/L3Q3 FDF Mixture
19429-H1D2/L3Q3 FDF Peak 1
19429-H1D2/L3Q3 FDF Peak 2
19429-H1D2/L3Q3 FDF Peak 3

Fig. 7

A


Legend
Paratope
V_L Domain
V_H Domain
C_L Domain
C_H1 Domain
C_H2 Domain
C_H3 Domain
Disulphides
IgG-Fab

B



C

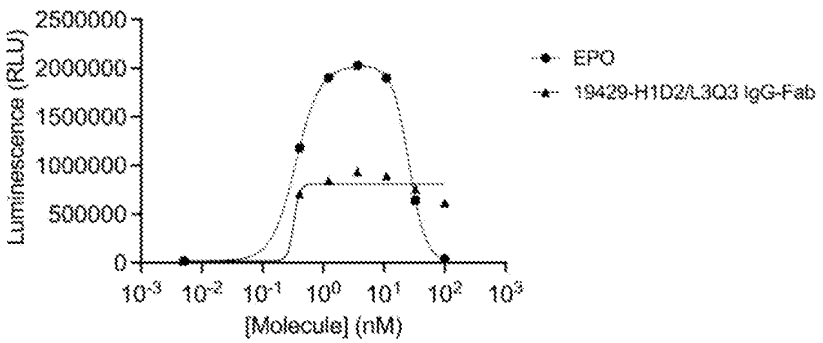
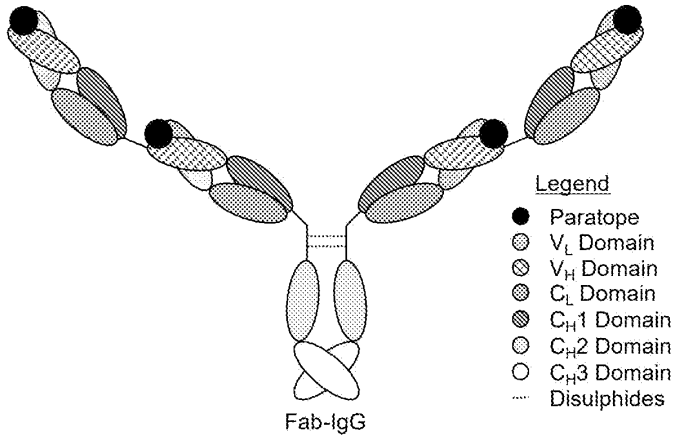
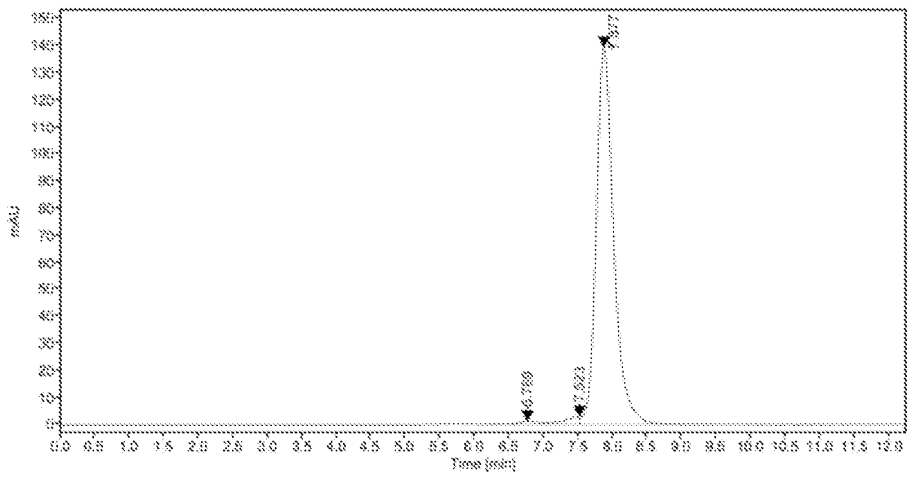

Luminescence (RLU)
2500000
2000000
1500000
1000000
500000
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
[Molecule] (nM)
EPO
19429-H1D2/L3Q3 IgG-Fab

Fig. 8

A


Legend
Paratope
V_L Domain
V_H Domain
C_L Domain
C_H1 Domain
C_H2 Domain
C_H3 Domain
Disulphides
Fab-IgG

B


mAU
Time [min]

C

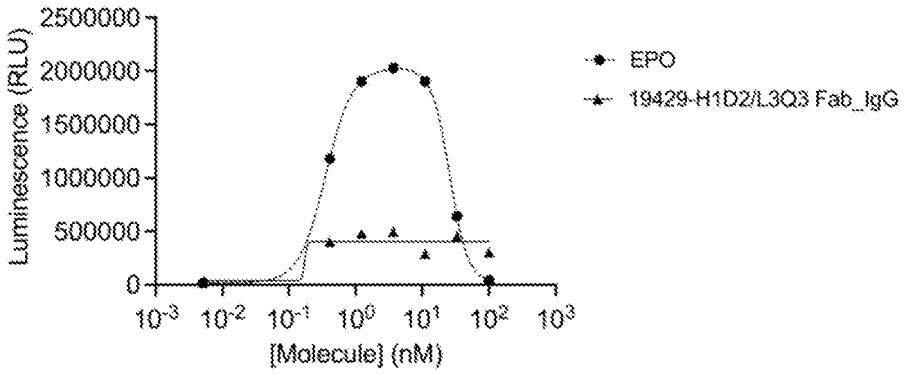
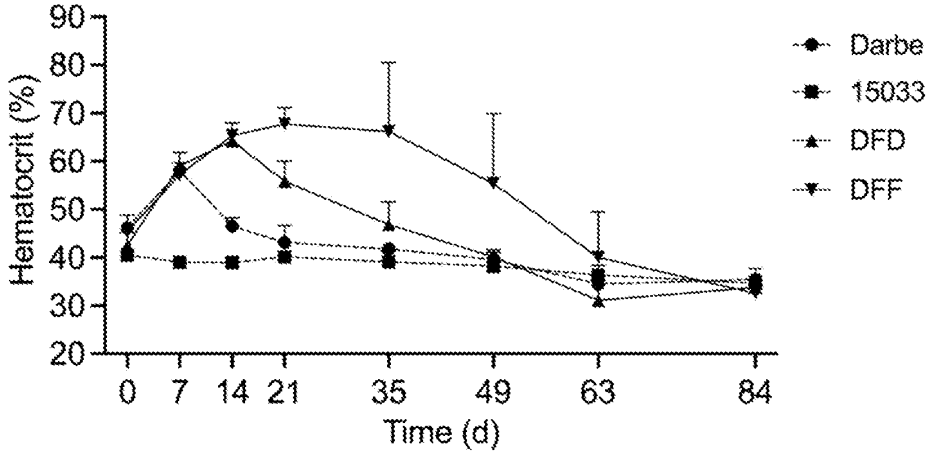

Luminescence (RLU)
2500000
2000000
1500000
1000000
500000
0
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
[Molecule] (nM)
EPO
19429-H1D2/L3Q3 Fab_IgG

Fig. 9

A


Hematocrit (%)
90
80
70
60
50
40
30
20
0 7 14 21 35 49 63 84
Time (d)
Darbe
15033
DFD
DFF

B

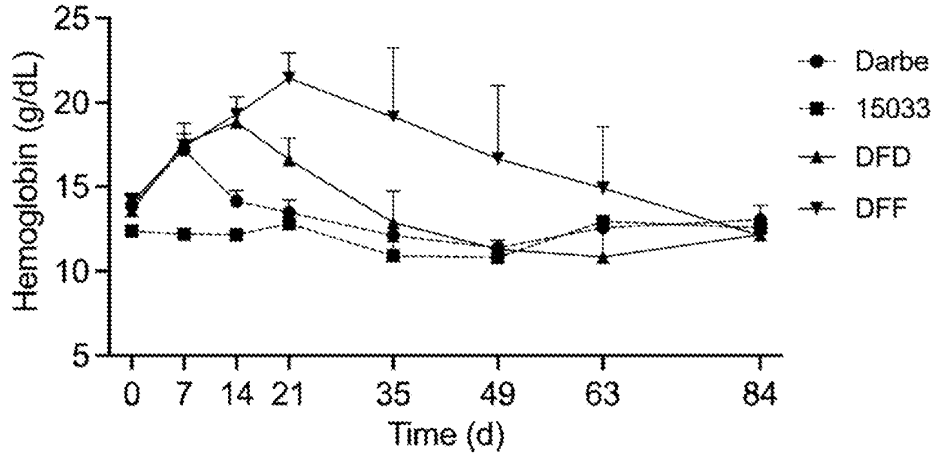

Hemoglobin (g/dL)
25
20
15
10
5
0 7 14 21 35 49 63 84
Time (d)
Darbe
15033
DFD
DFF

Fig. 10

C

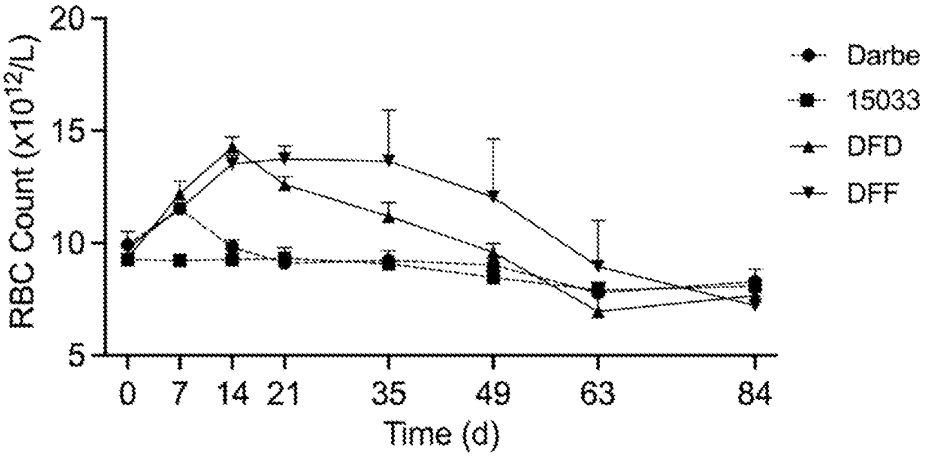
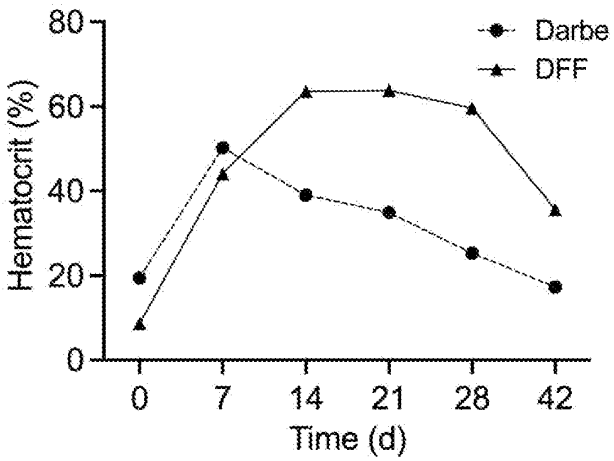

RBC Count (x10^12/L)
20
15
10
5
0
7
14
21
35
49
63
84
Time (d)
Darbe
15033
DFD
DFF

Fig. 10

A


Darbe
DFF
Hematocrit (%)
80
60
40
20
0
0
7
14
21
28
42
Time (d)

B

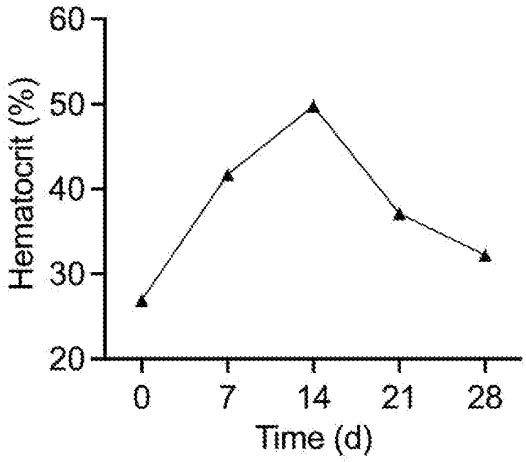

Hematocrit (%)
60
50
40
30
20
0
7
14
21
28
Time (d)

Fig. 11