Figure 1

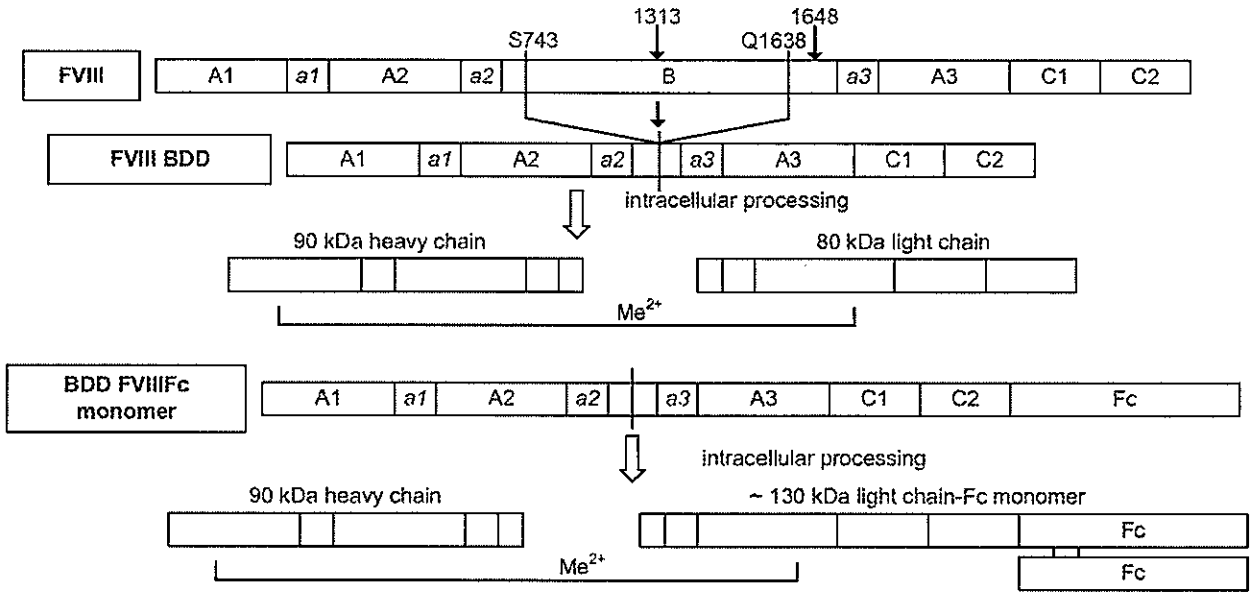
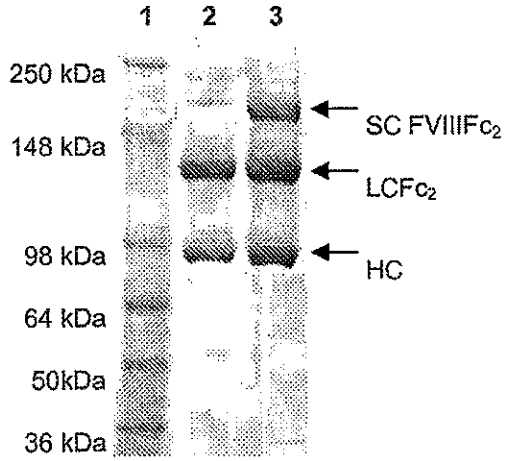
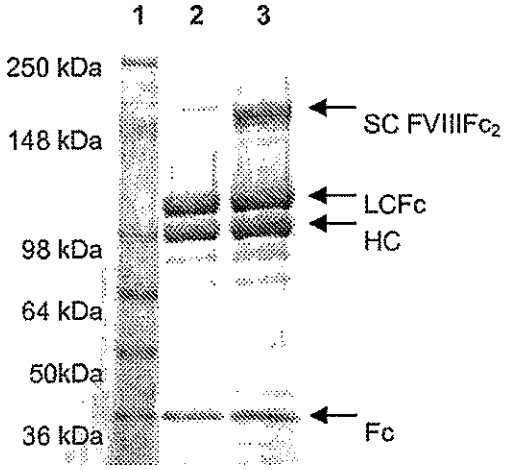

1313
1648
S743
Q1638
FVIII
A1
a1
A2
a2
B
a3
A3
C1
C2
FVIII BDD
intracellular processing
90 kDa heavy chain
80 kDa light chain
Me2+
BDD FVIIIFc monomer
Fc
intracellular processing
90 kDa heavy chain
~ 130 kDa light chain-Fc monomer
Fc
Fc
Me2+

Figure 2

A



B



1. SeeBlue MW Marker
2. rFVIIIFc/PC5
3. rFVIIIFc

Figure 2C

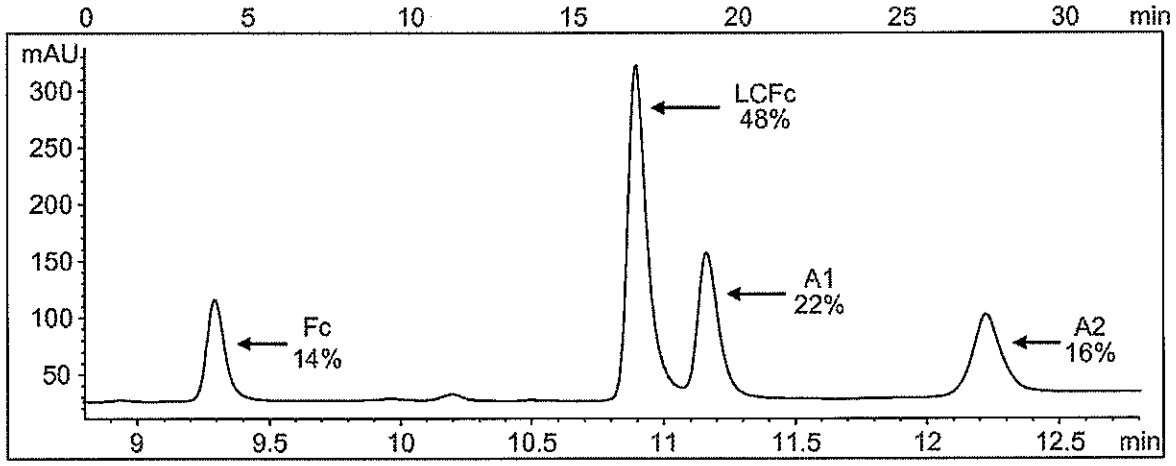


Fig. 2D

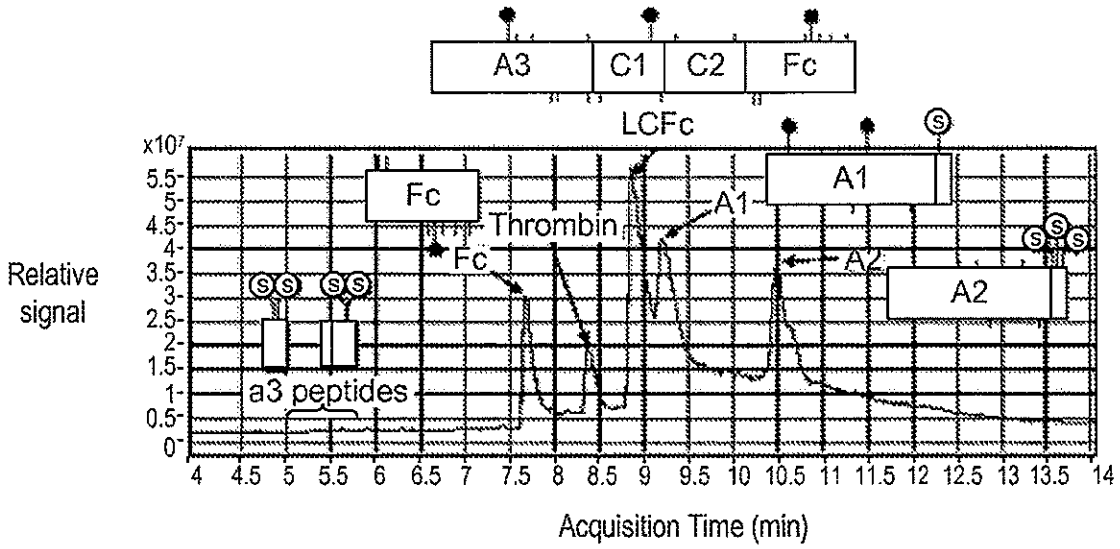
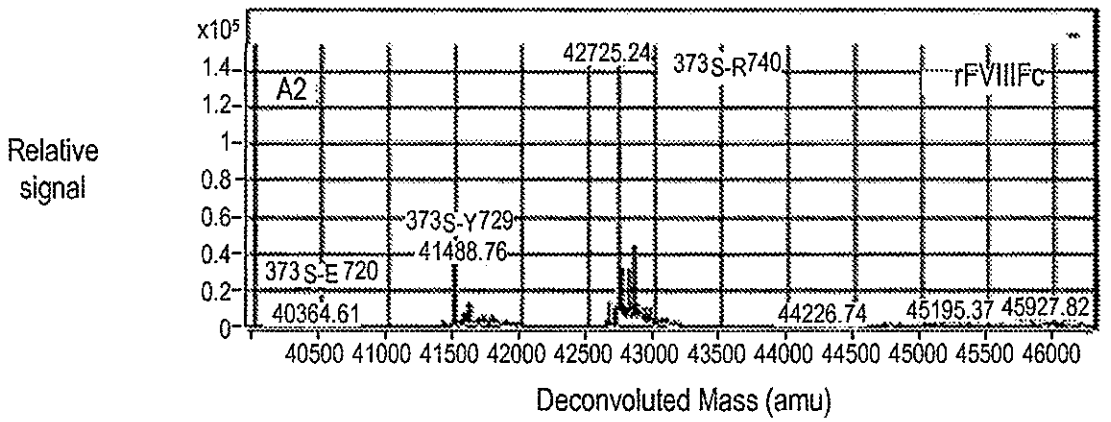

A3
C1
C2
Fc
LCFc
x10⁷
Relative signal
Fc
Thrombin
A1
A1
Fc
A2
A2
a3 peptides
Acquisition Time (min)

Fig. 2E


x10⁵
Relative signal
A2
42725.24
373S-R740
rFVIIIFc
373S-Y729
41488.76
373S-E720
40364.61
44226.74
45195.37
45927.82
Deconvoluted Mass (amu)

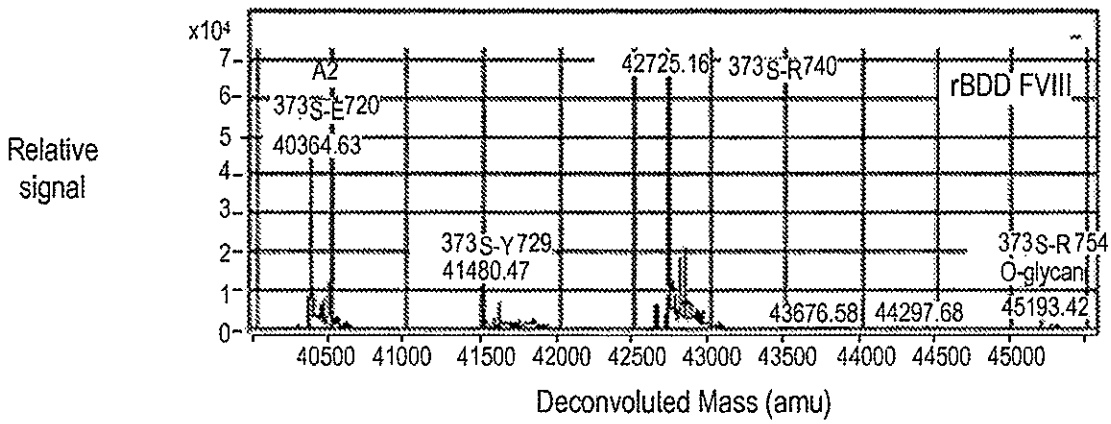
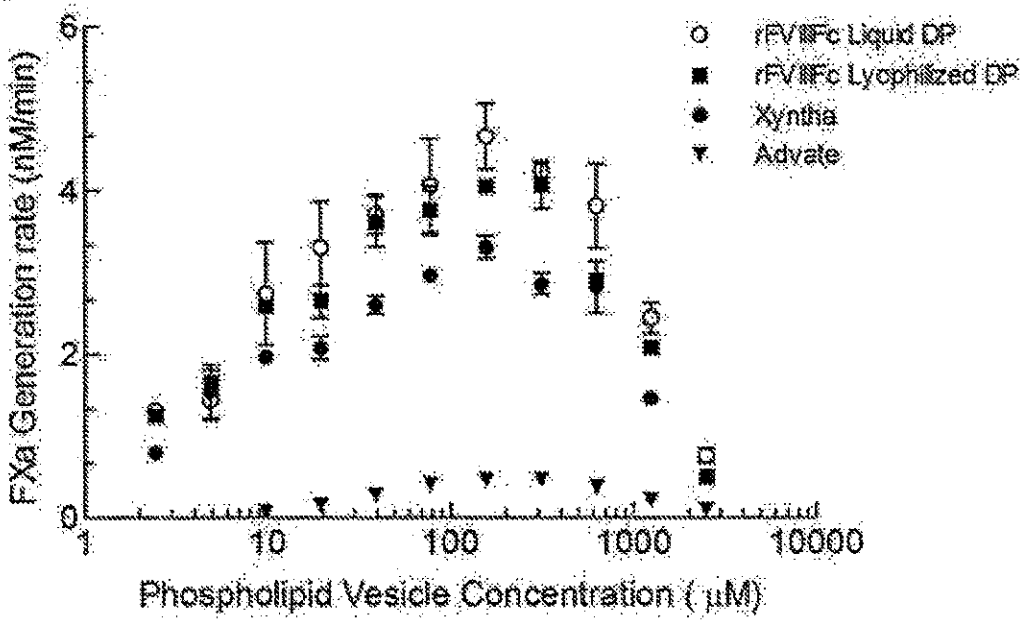
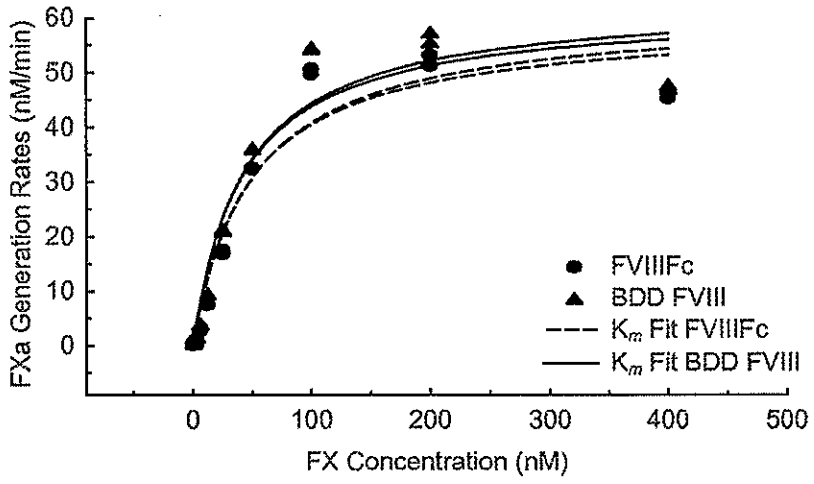

x10⁴
Relative signal
A2
373S-E720
40364.63
42725.16
373S-R740
rBDD FVIII
373S-Y729
41480.47
373S-R754
O-glycan
43676.58
44297.68
45193.42
Deconvoluted Mass (amu)

Figure 3

A.


rFVIIIFc Liquid DP
rFVIIIFc Lyophilized DP
Xynthia
Advate
FXa Generation rate (nM/min)
0
2
4
6
10
100
1000
10000
Phospholipid Vesicle Concentration (μM)

B.


FXa Generation Rates (nM/min)
0
10
20
30
40
50
60
0
100
200
300
400
500
FX Concentration (nM)
FVIIIFc
BDD FVIII
K_m Fit FVIIIFc
K_m Fit BDD FVIII

C.

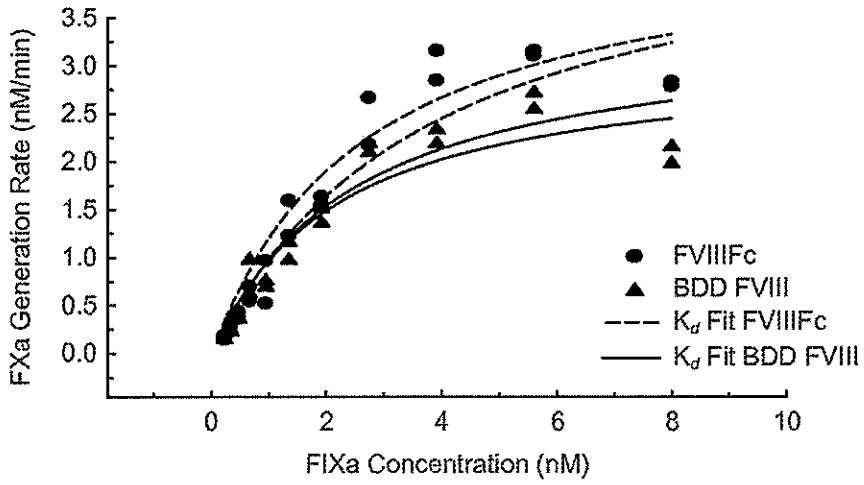

FXa Generation Rate (nM/min)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0
2
4
6
8
10
FIXa Concentration (nM)
FVIIIFc
BDD FVIII
K_d Fit FVIIIFc
K_d Fit BDD FVIII

Figure 4

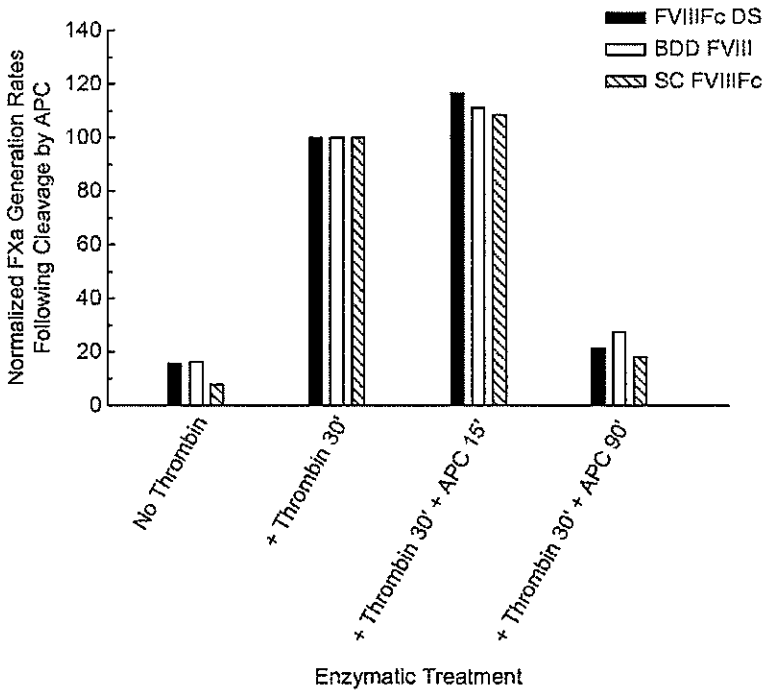

FVIIIFc DS
BDD FVIII
SC FVIIIFc
Normalized FXa Generation Rates Following Cleavage by APC
140
120
100
80
60
40
20
0
No Thrombin
+ Thrombin 30'
+ Thrombin 30' + APC 15'
+ Thrombin 30' + APC 90'
Enzymatic Treatment

Figure 5A

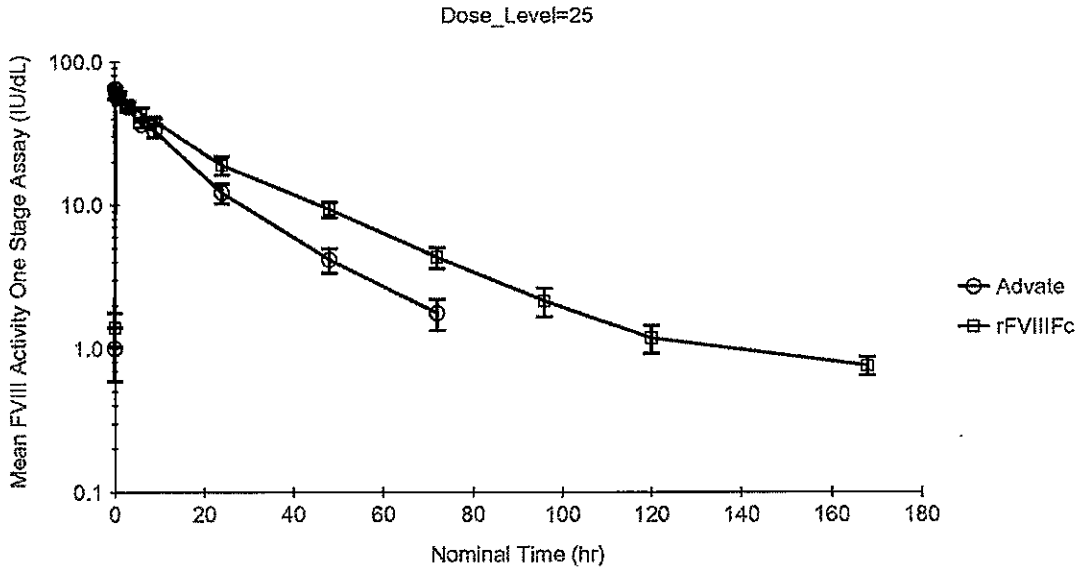

Dose_Level=25
Mean FVIII Activity One Stage Assay (IU/dL)
100.0
10.0
1.0
0.1
0
20
40
60
80
100
120
140
160
180
Nominal Time (hr)
Advate
rFVIIIFc

Figure 5B

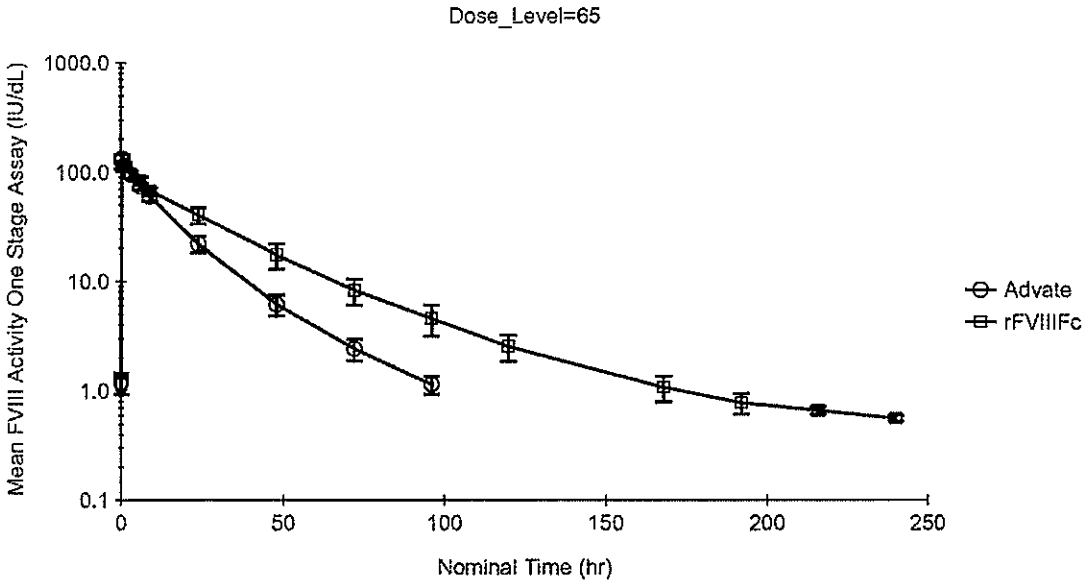

Dose_Level=65
Mean FVIII Activity One Stage Assay (IU/dL)
1000.0
100.0
10.0
1.0
0.1
0
50
100
150
200
250
Nominal Time (hr)
Advate
rFVIIIFc

Figure 5C

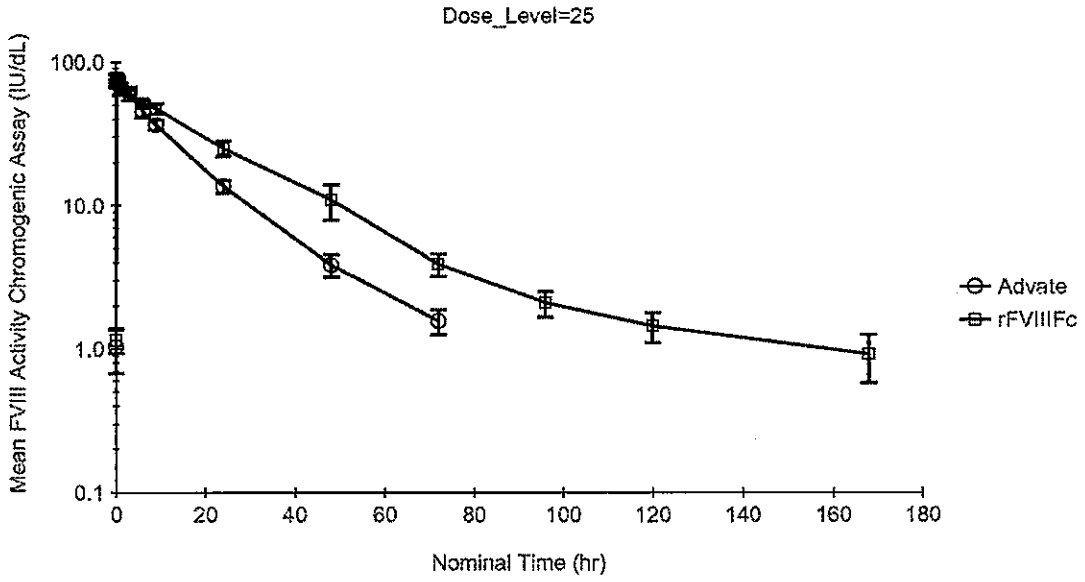

Dose_Level=25
Mean FVIII Activity Chromogenic Assay (IU/dL)
100.0
10.0
1.0
0.1
0
20
40
60
80
100
120
140
160
180
Nominal Time (hr)
Advate
rFVIIIFc

Figure 5D

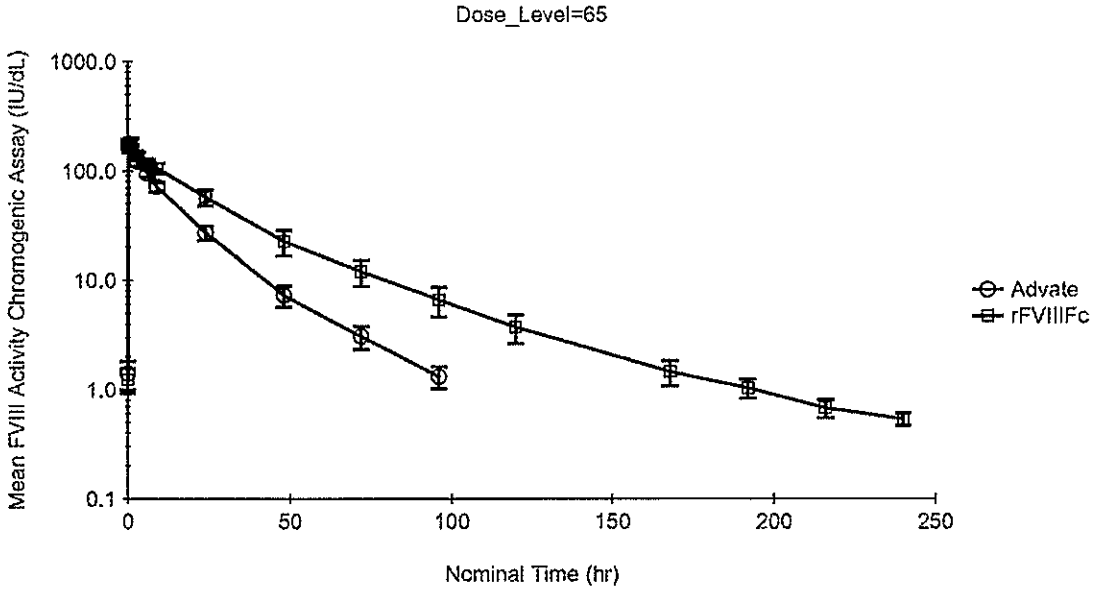

Dose_Level=65
Mean FVIII Activity Chromogenic Assay (IU/dL)
1000.0
100.0
10.0
1.0
0.1
0
50
100
150
200
250
Nominal Time (hr)
Advate
rFVIIIFc

Figure 6A

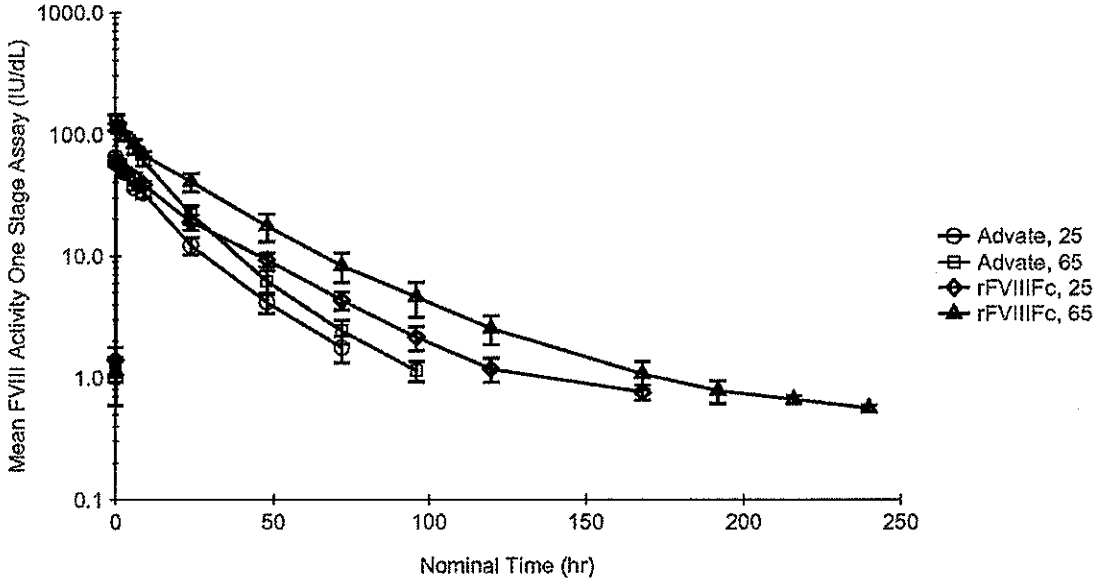

1000.0
100.0
10.0
1.0
0.1
Mean FVIII Activity One Stage Assay (IU/dL)
0
50
100
150
200
250
Nominal Time (hr)
Advate, 25
Advate, 65
rFVIIIFc, 25
rFVIIIFc, 65

Figure 6B

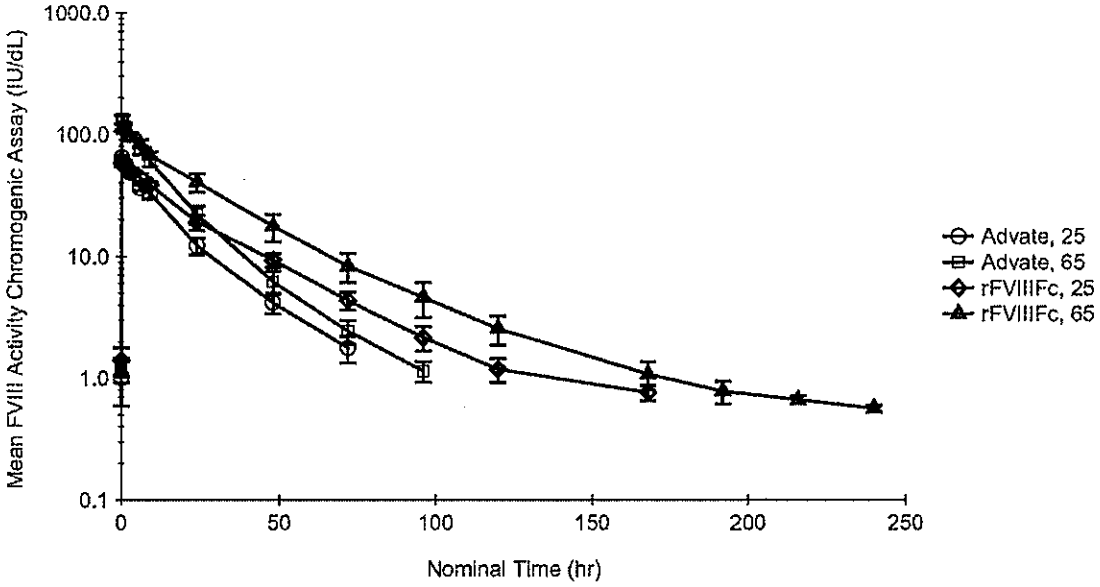

Mean FVIII Activity Chromogenic Assay (IU/dL)
1000.0
100.0
10.0
1.0
0.1
0
50
100
150
200
250
Nominal Time (hr)
Advate, 25
Advate, 65
rFVIIIFc, 25
rFVIIIFc, 65

Figure 7A

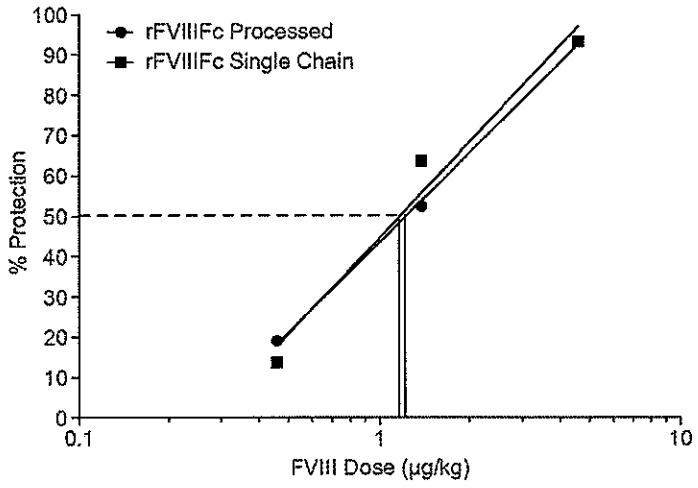

rFVIIIFc Processed
rFVIIIFc Single Chain
% Protection
100
90
80
70
60
50
40
30
20
10
0
0.1
1
10
FVIII Dose (µg/kg)

Figure 7B

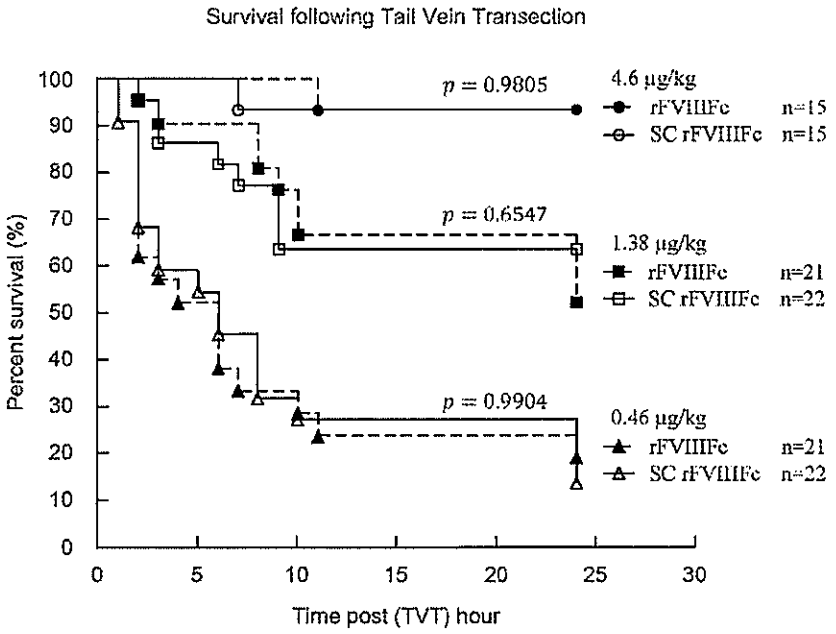

Survival following Tail Vein Transection
Percent survival (%)
Time post (TVT) hour
p = 0.9805
p = 0.6547
p = 0.9904
4.6 µg/kg
rFVIIIFc n=15
SC rFVIIIFc n=15
1.38 µg/kg
rFVIIIFc n=21
SC rFVIIIFc n=22
0.46 µg/kg
rFVIIIFc n=21
SC rFVIIIFc n=22

Figure 7C

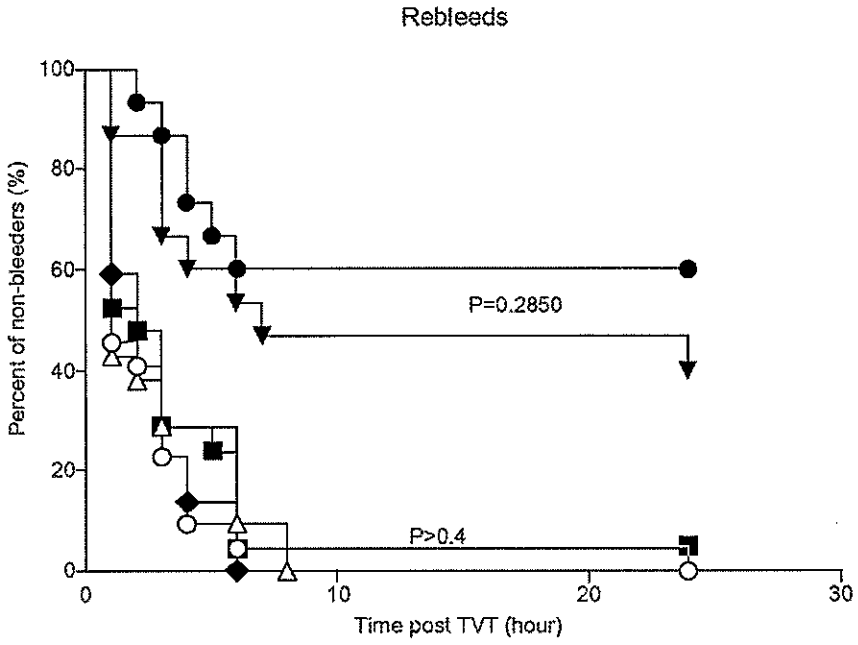

Rebleeds
Percent of non-bleeders (%)
100
80
60
40
20
0
P=0.2850
P>0.4
0
10
20
30
Time post TVT (hour)

Figure 8 Study Design

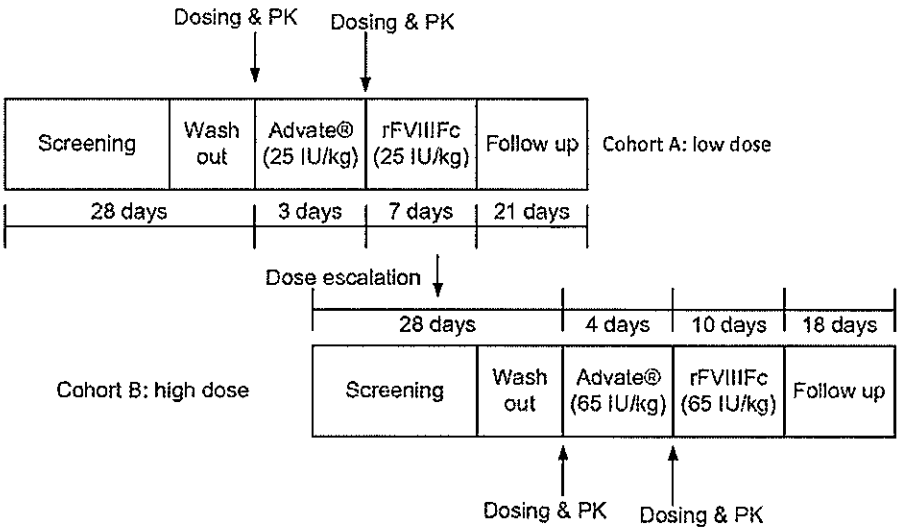


Figure 9

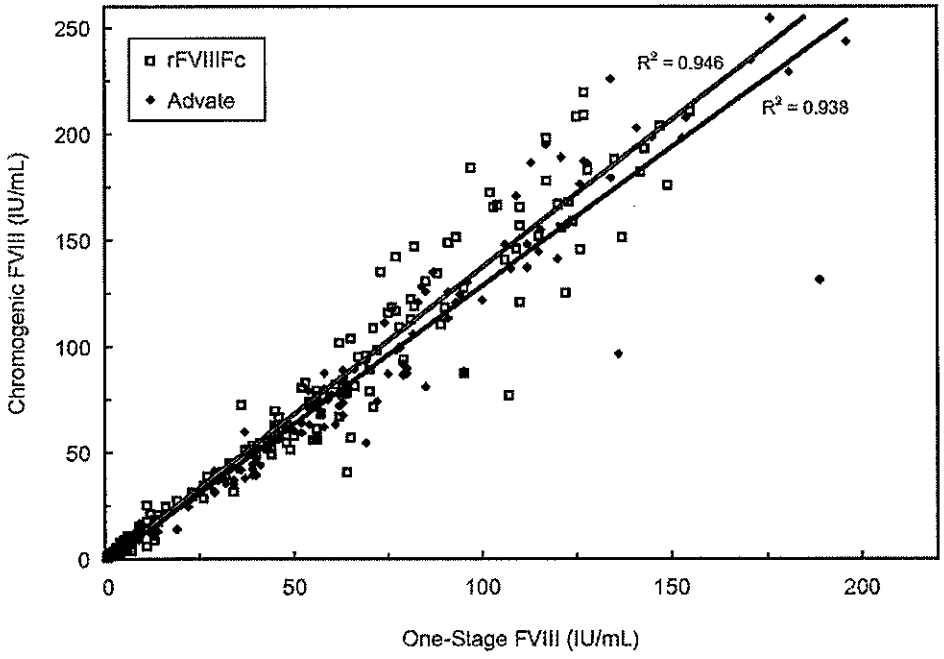
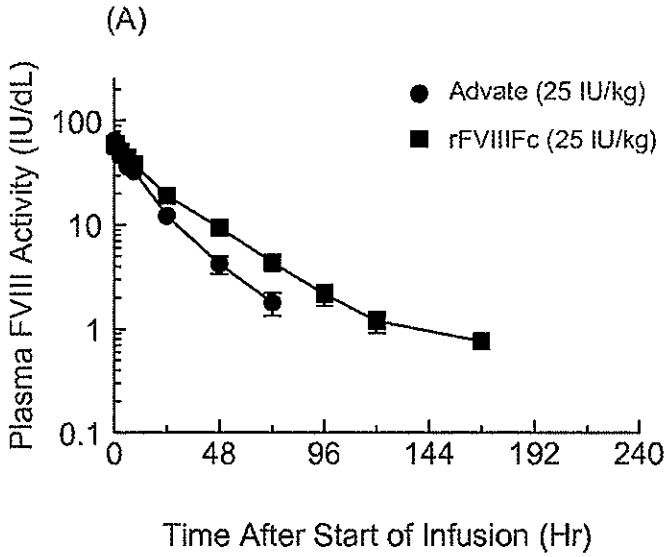

rFVIIIFc
Advate
R^2 = 0.946
R^2 = 0.938
Chromogenic FVIII (IU/mL)
One-Stage FVIII (IU/mL)
0
50
100
150
200
250

Figure 10


(A)
Plasma FVIII Activity (IU/dL)
100
10
1
0.1
0
48
96
144
192
240
Advate (25 IU/kg)
rFVIIIFc (25 IU/kg)
Time After Start of Infusion (Hr)

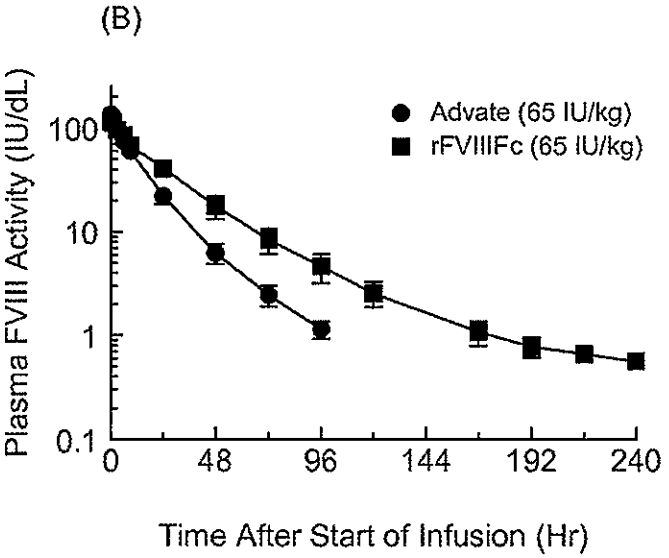

(B)
Plasma FVIII Activity (IU/dL)
100
10
1
0.1
0
48
96
144
192
240
Advate (65 IU/kg)
rFVIIIFc (65 IU/kg)
Time After Start of Infusion (Hr)

Figure 11

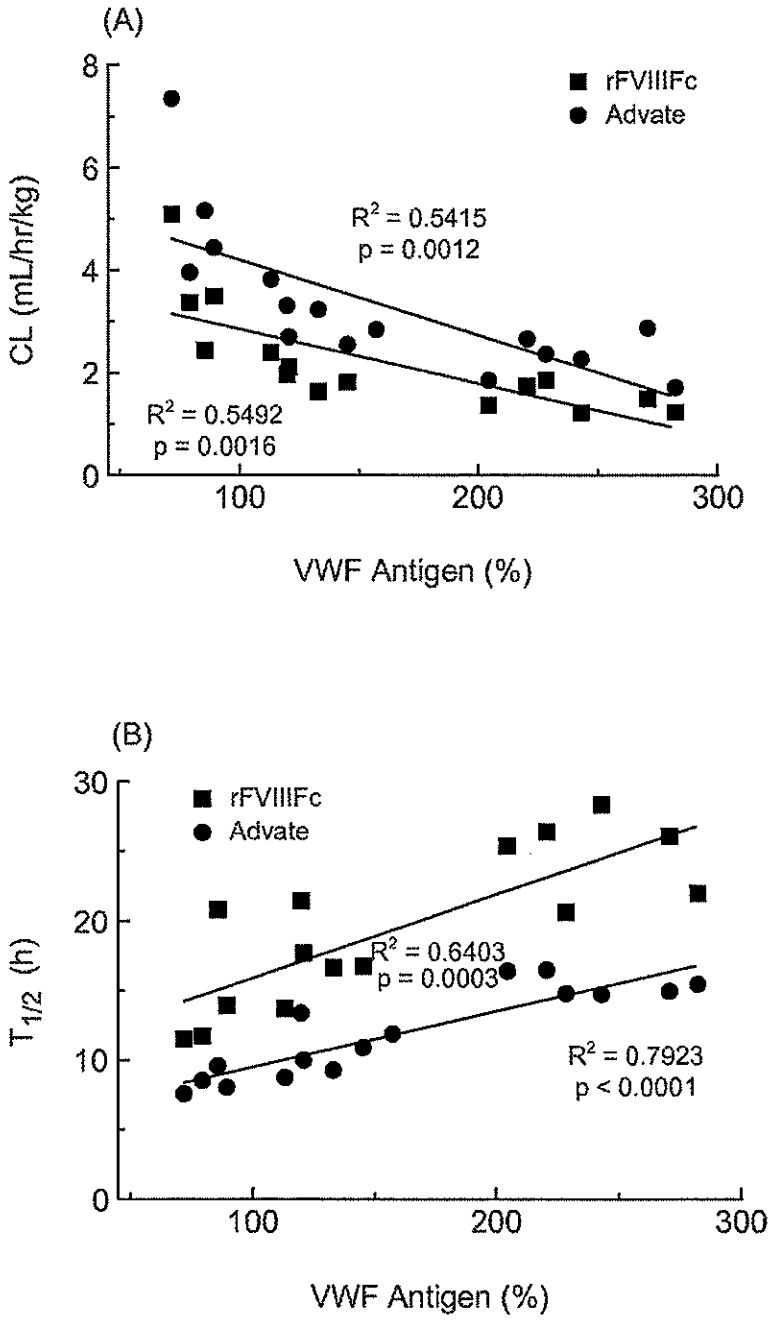

(A)
rFVIIIFc
Advate
CL (mL/hr/kg)
R^2 = 0.5415
p = 0.0012
R^2 = 0.5492
p = 0.0016
VWF Antigen (%)
(B)
rFVIIIFc
Advate
$T_{1/2}$ (h)
R^2 = 0.6403
p = 0.0003
R^2 = 0.7923
p < 0.0001
VWF Antigen (%)

Figure 12

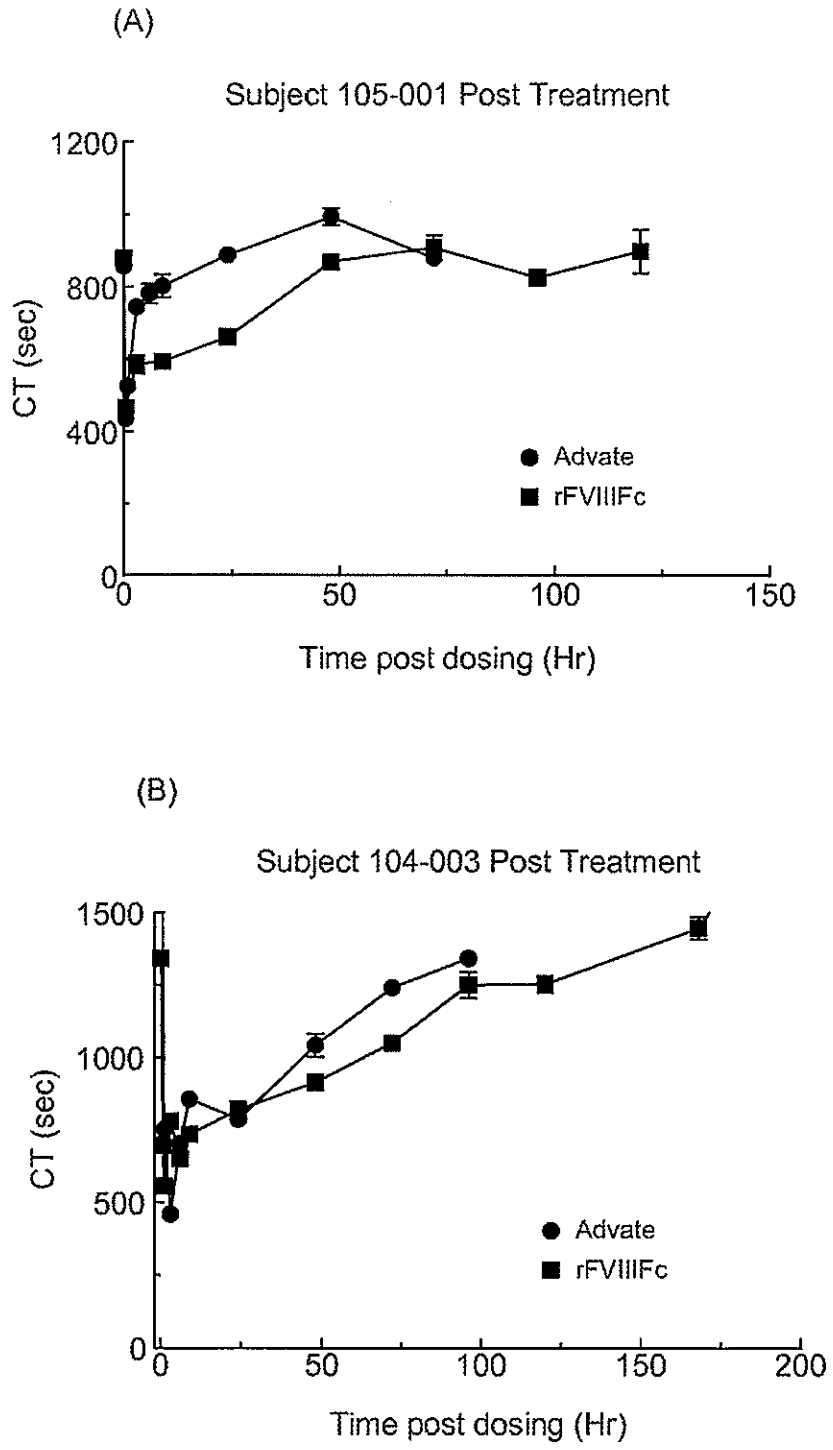

(A)
Subject 105-001 Post Treatment
CT (sec)
1200
800
400
0
0
50
100
150
Time post dosing (Hr)
Advate
rFVIIIFc
(B)
Subject 104-003 Post Treatment
CT (sec)
1500
1000
500
0
0
50
100
150
200
Time post dosing (Hr)
Advate
rFVIIIFc

Figure 13A

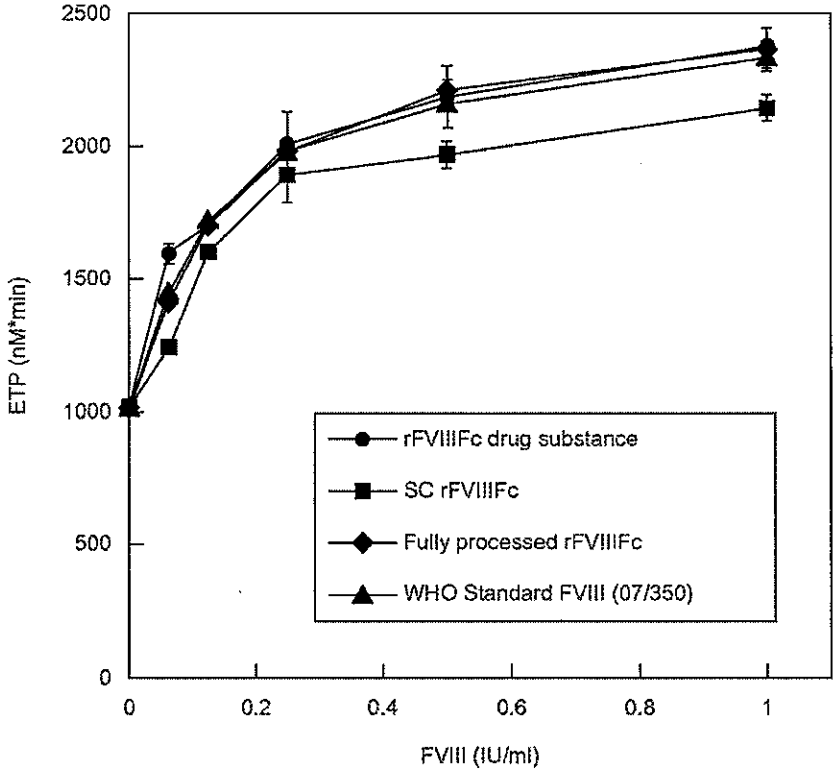

2500
2000
1500
1000
500
0
ETP (nM*min)
0
0.2
0.4
0.6
0.8
1
FVIII (IU/ml)
rFVIIIFc drug substance
SC rFVIIIFc
Fully processed rFVIIIFc
WHO Standard FVIII (07/350)

Figure 13B

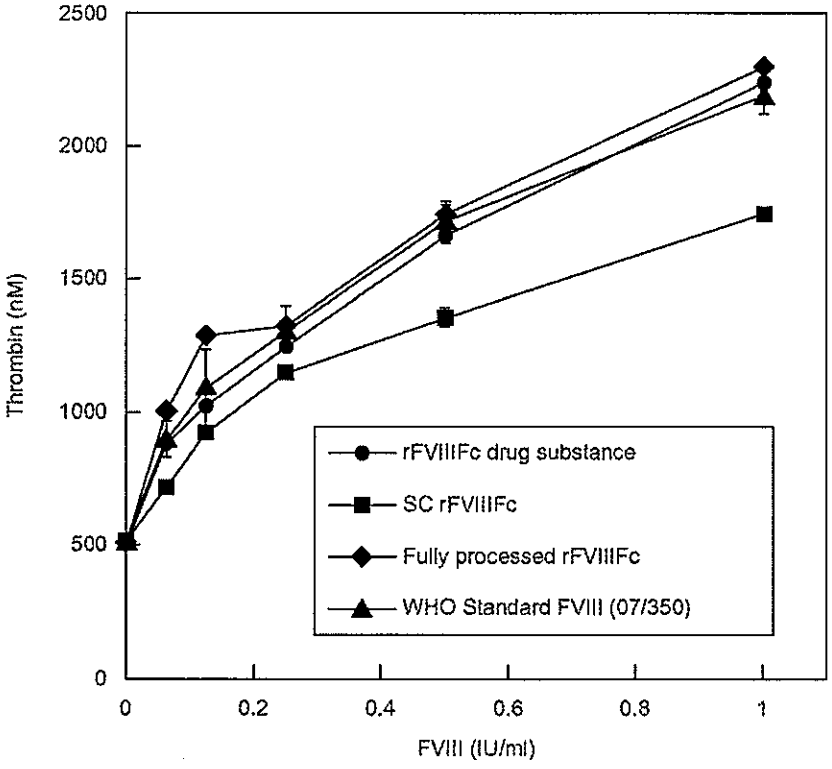
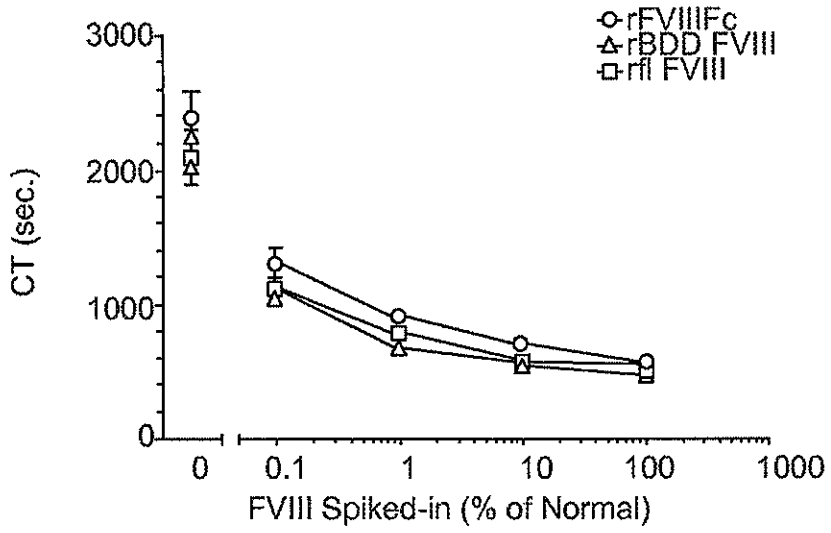
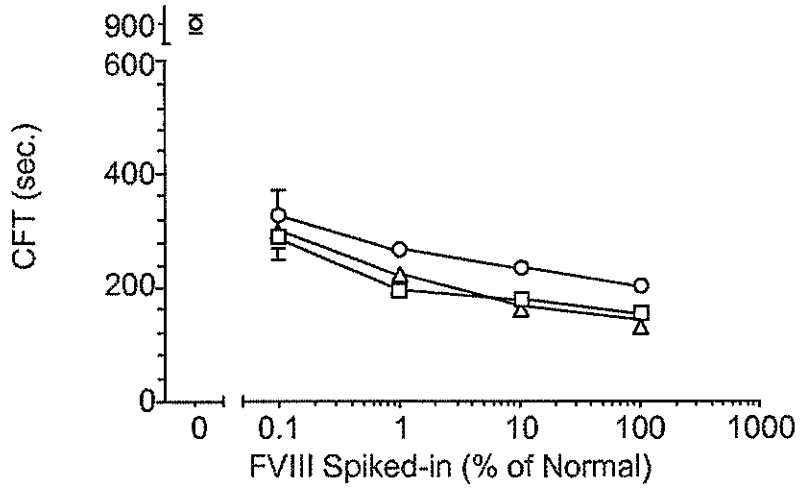

2500
2000
1500
1000
500
0
Thrombin (nM)
0
0.2
0.4
0.6
0.8
1
FVIII (IU/ml)
rFVIIIFc drug substance
SC rFVIIIFc
Fully processed rFVIIIFc
WHO Standard FVIII (07/350)

Figure 14

A.



B.



C.

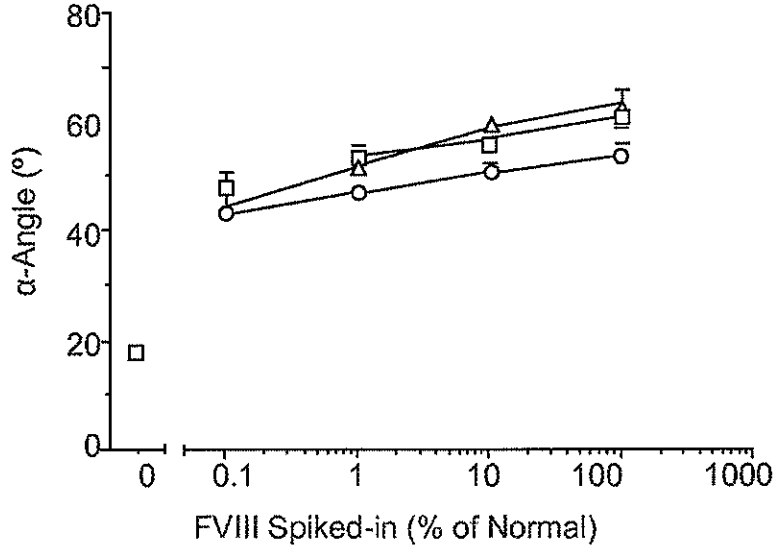
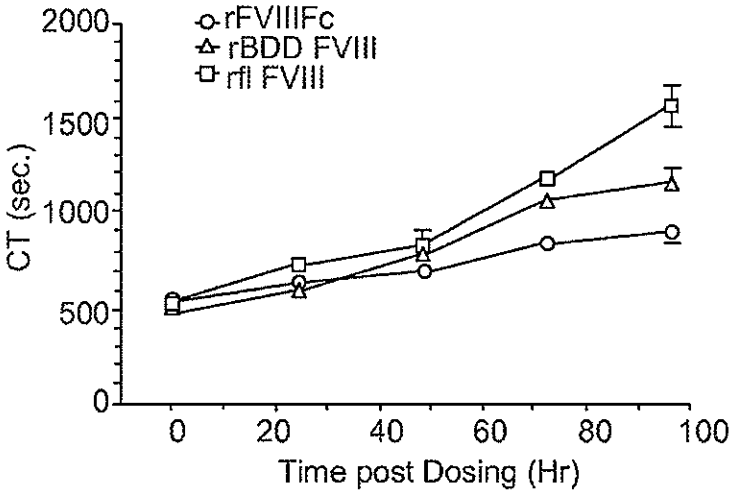
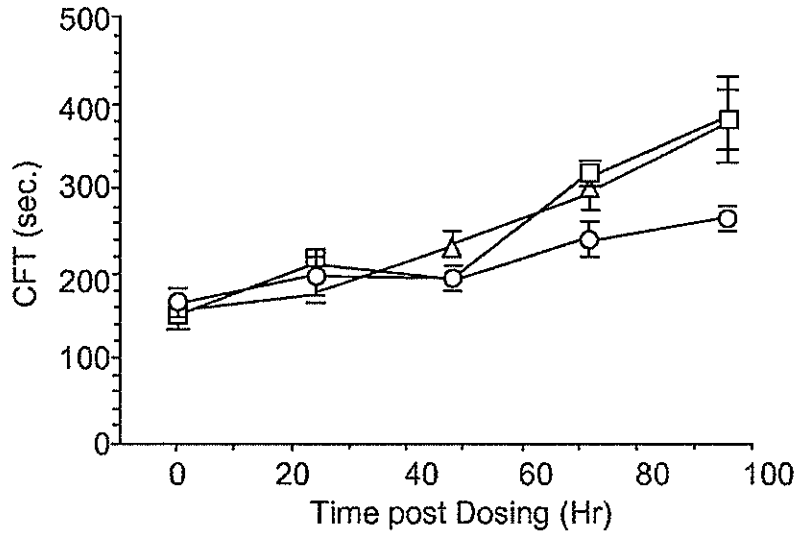


Figure 15

A.



B.



C.

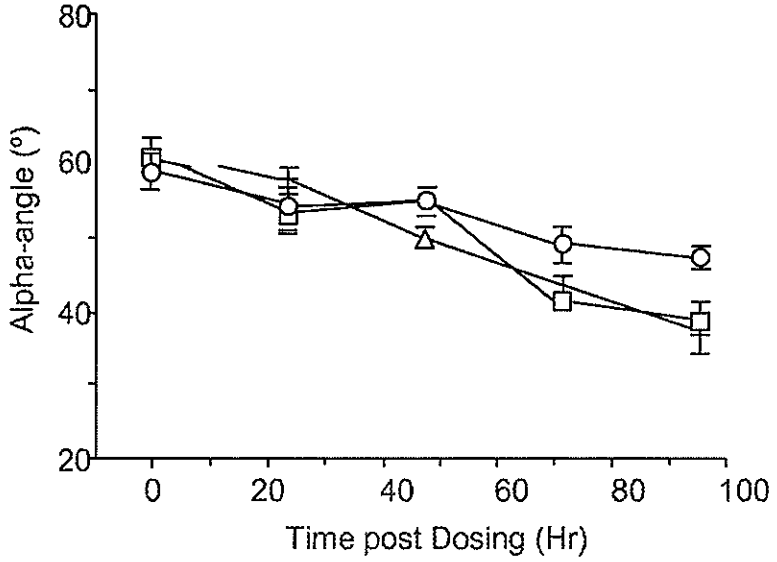
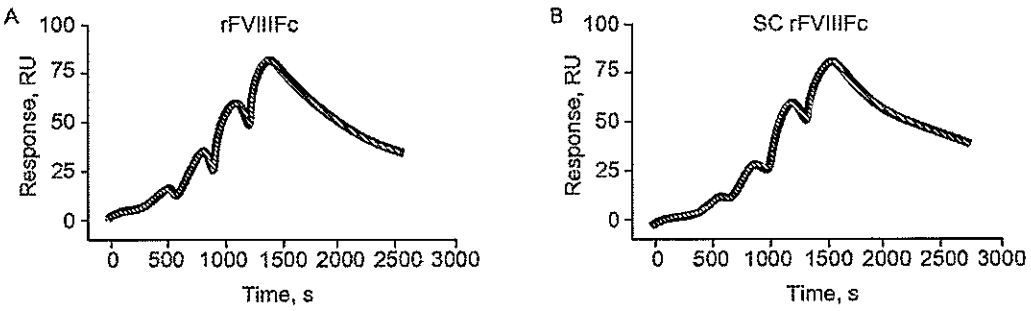


Figure 16A



Binding curve, black; 1:1 fit, red
RU, resonance units; SC, single chain

Figure 16B

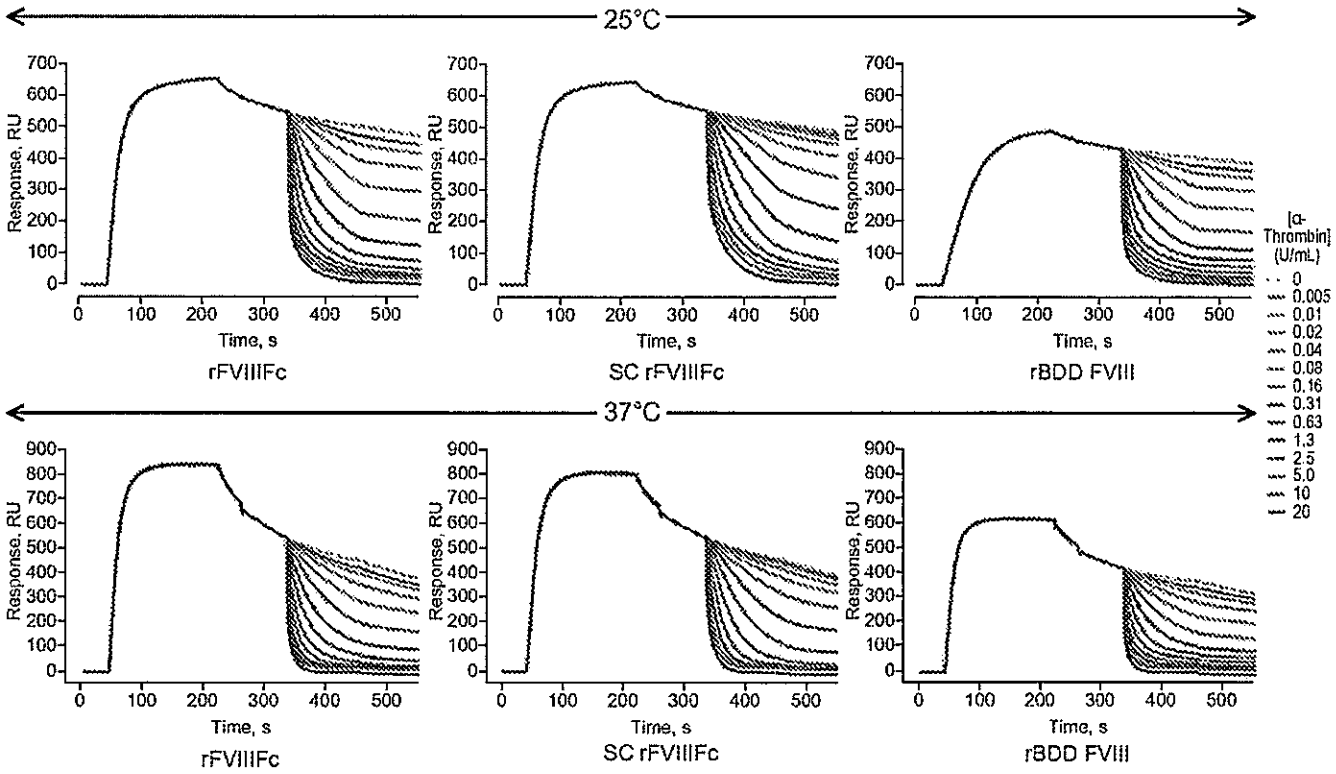

25°C
37°C
Response, RU
Time, s
rFVIIIFc
SC rFVIIIFc
rBDD FVIII
[α-Thrombin] (U/mL)
0
0.005
0.01
0.02
0.04
0.08
0.16
0.31
0.63
1.3
2.5
5.0
10
20

Figure 16C

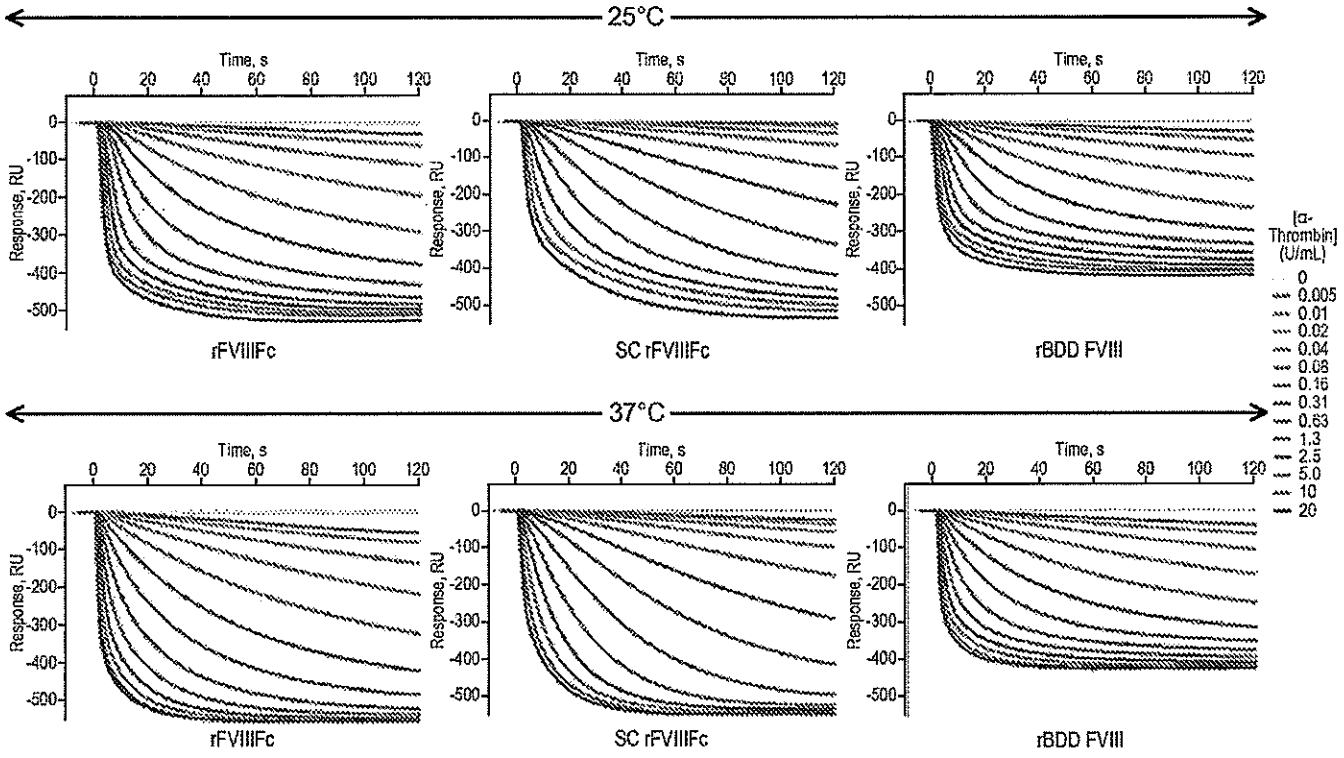

25°C
37°C
Time, s
Response, RU
rFVIIIFc
SC rFVIIIFc
rBDD FVIII
[α-Thrombin] (U/mL)
0
0.005
0.01
0.02
0.04
0.08
0.16
0.31
0.63
1.3
2.5
5.0
10
20

Figure 16D

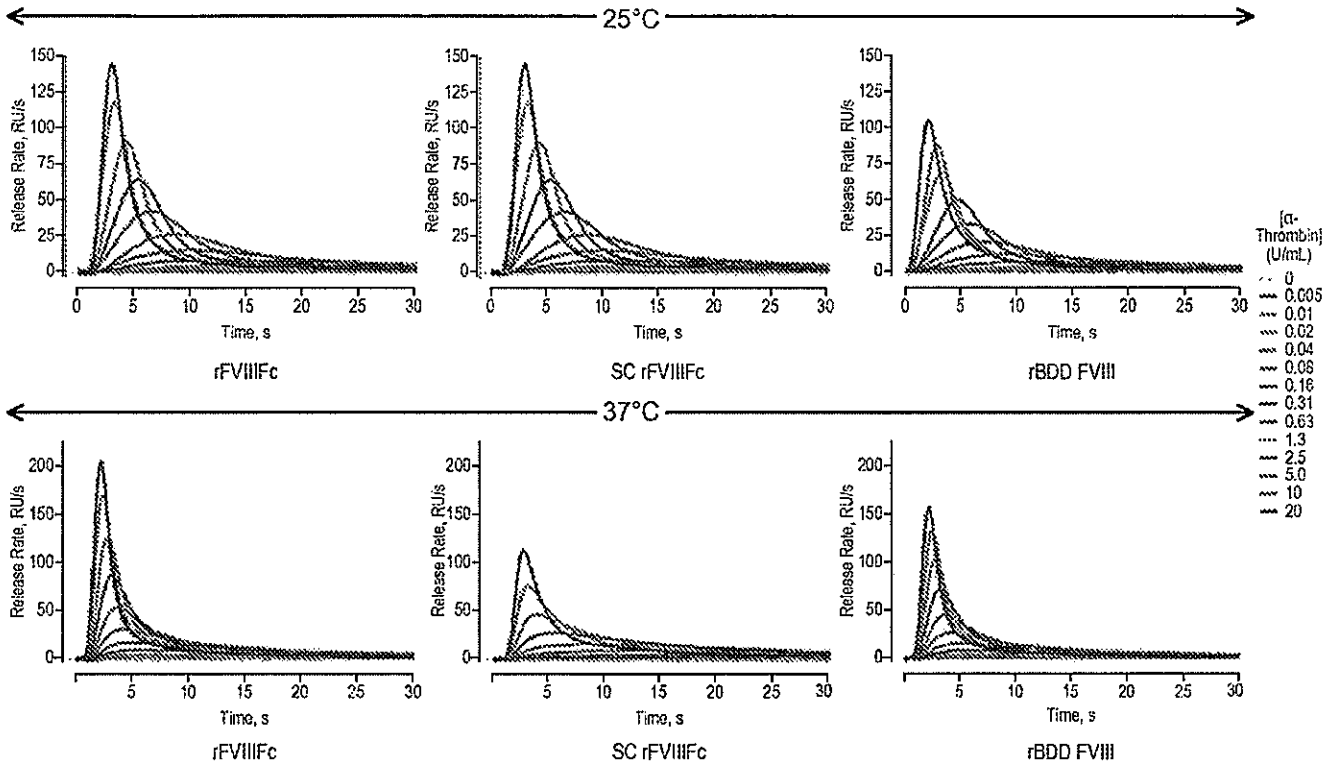
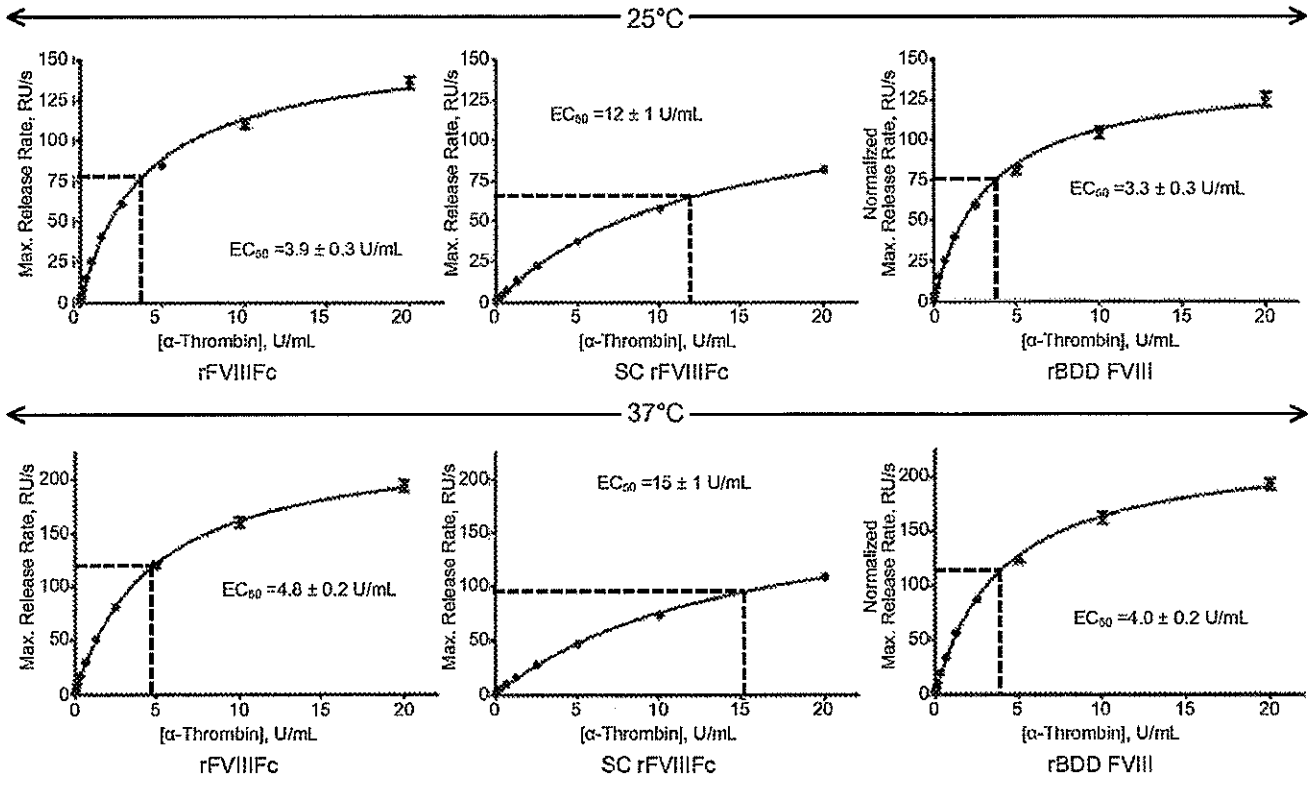

25°C
37°C
Release Rate, RU/s
Time, s
rFVIIIFc
SC rFVIIIFc
rBDD FVIII
[α-Thrombin] (U/mL)
0
0.005
0.01
0.02
0.04
0.08
0.16
0.31
0.63
1.3
2.5
5.0
10
20

Figure 16E


25°C
Max. Release Rate, RU/s
EC50 =3.9 ± 0.3 U/mL
[α-Thrombin], U/mL
rFVIIIFc
EC50 =12 ± 1 U/mL
SC rFVIIIFc
Normalized Max. Release Rate, RU/s
EC50 =3.3 ± 0.3 U/mL
rBDD FVIII
37°C
EC50 =4.8 ± 0.2 U/mL
EC50 =15 ± 1 U/mL
EC50 =4.0 ± 0.2 U/mL