**Figure 1**

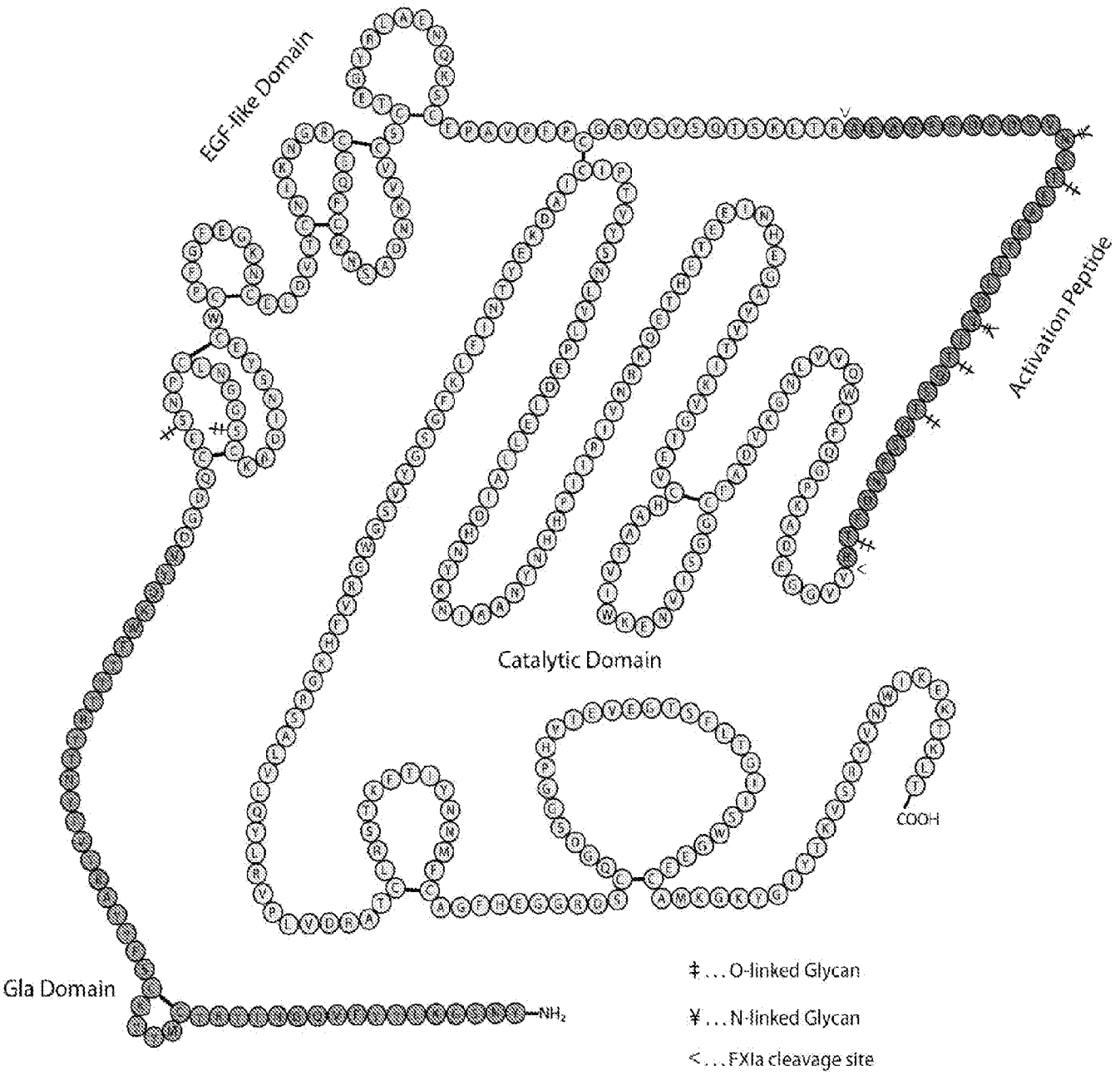

EGF-like Domain
Activation Peptide
Catalytic Domain
Gla Domain
COOH
NH2
‡ . . . O-linked Glycan
¥ . . . N-linked Glycan
< . . . FXIa cleavage site

**Figure 2**

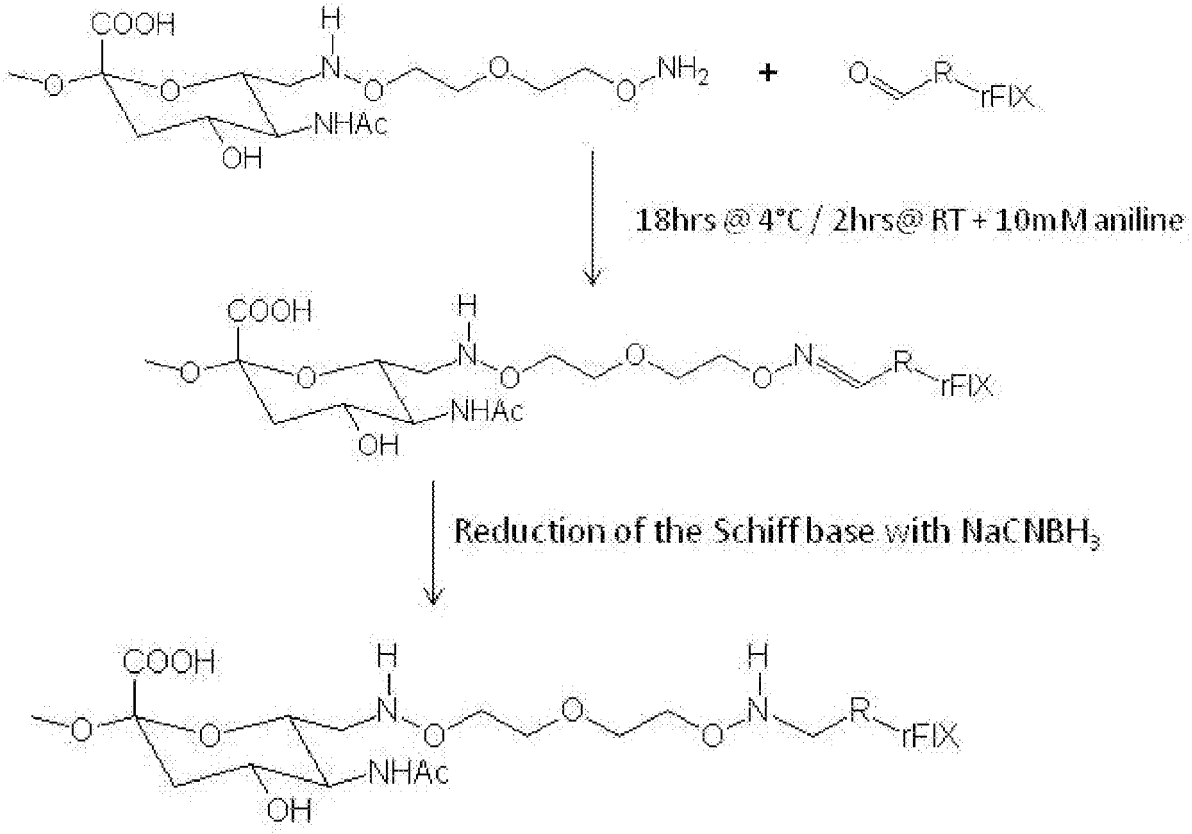

COOH
H
N
O
NHAc
OH
NH2
+
O
R
rFIX
18hrs @ 4°C / 2hrs@ RT + 10mM aniline
N
Reduction of the Schiff base with NaCNBH3

**Figure 3**

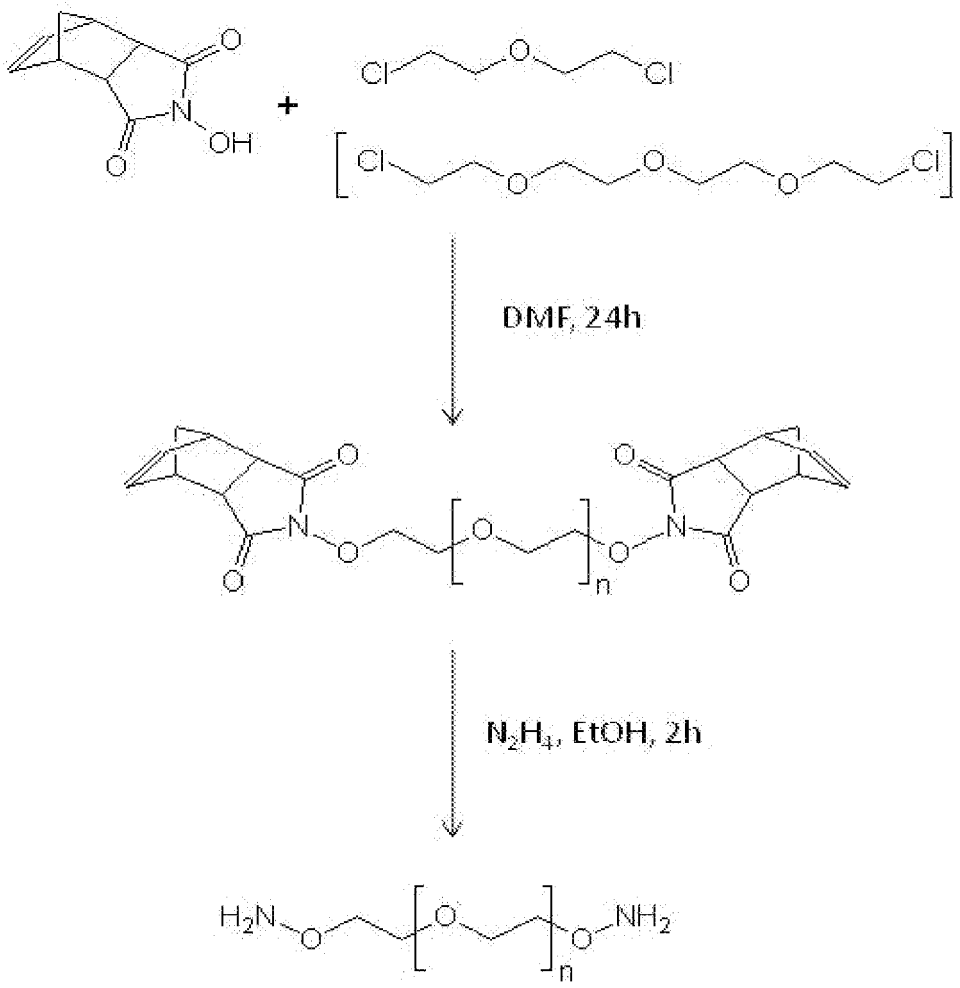


**Figure 4**

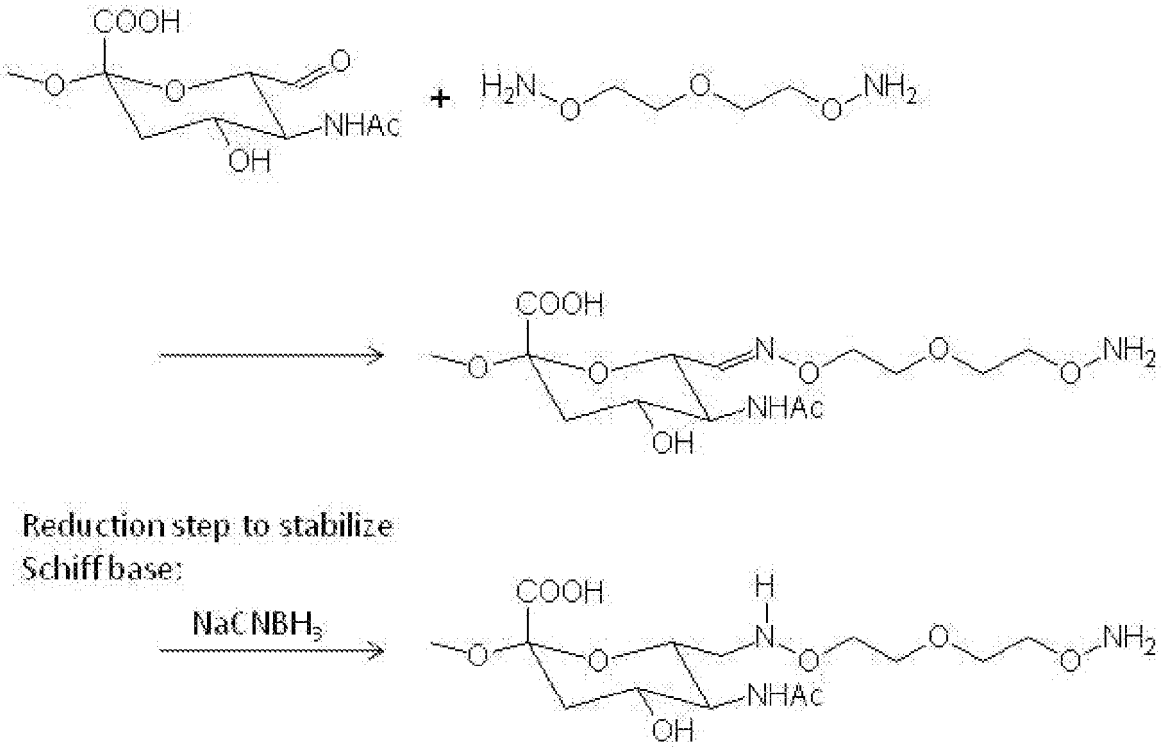

COOH
O
O
O
NHAc
OH
+
H2N
O
O
O
NH2
COOH
N
O
O
O
NHAc
OH
NH2
Reduction step to stabilize
Schiff base:
NaCNBH3
COOH
H
N
O
O
O
NHAc
OH
NH2

Figure 5

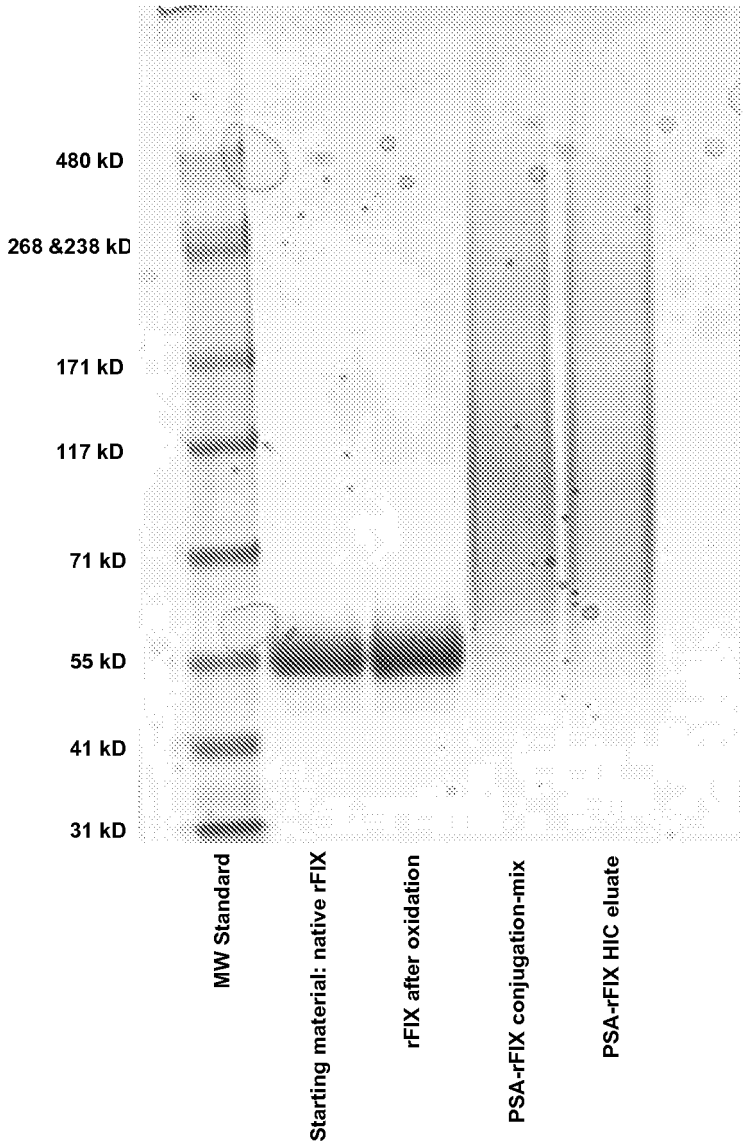

480 kD
268 &238 kD
171 kD
117 kD
71 kD
55 kD
41 kD
31 kD
MW Standard
Starting material: native rFIX
rFIX after oxidation
PSA-rFIX conjugation-mix
PSA-rFIX HIC eluate

Figure 6

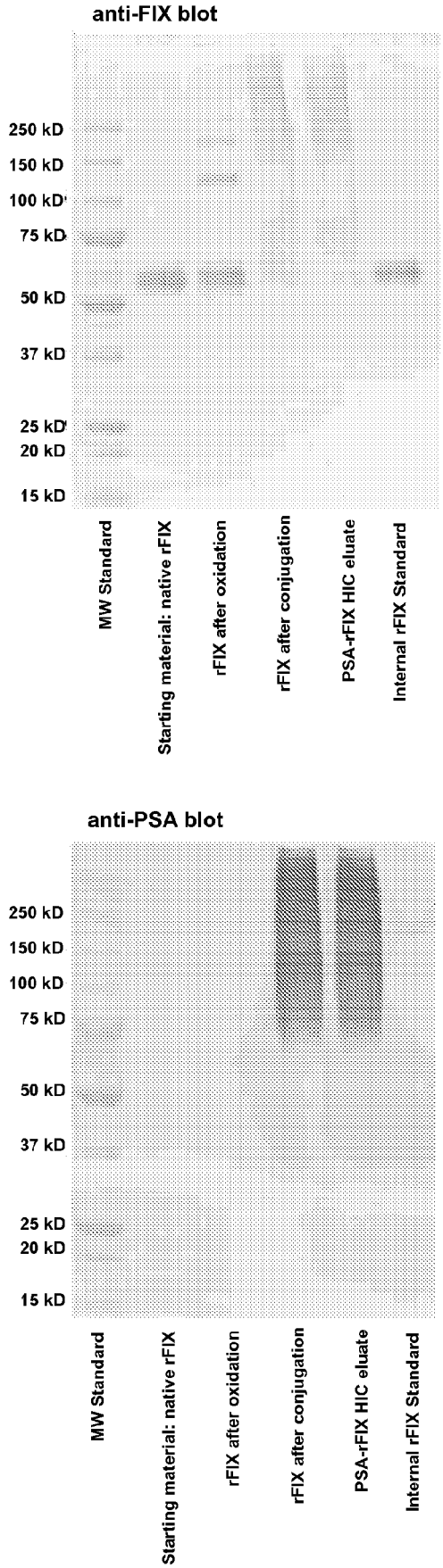

anti-FIX blot
250 kD
150 kD
100 kD
75 kD
50 kD
37 kD
25 kD
20 kD
15 kD
MW Standard
Starting material: native rFIX
rFIX after oxidation
rFIX after conjugation
PSA-rFIX HIC eluate
Internal rFIX Standard
anti-PSA blot
250 kD
150 kD
100 kD
75 kD
50 kD
37 kD
25 kD
20 kD
15 kD
MW Standard
Starting material: native rFIX
rFIX after oxidation
rFIX after conjugation
PSA-rFIX HIC eluate
Internal rFIX Standard

Figure 7

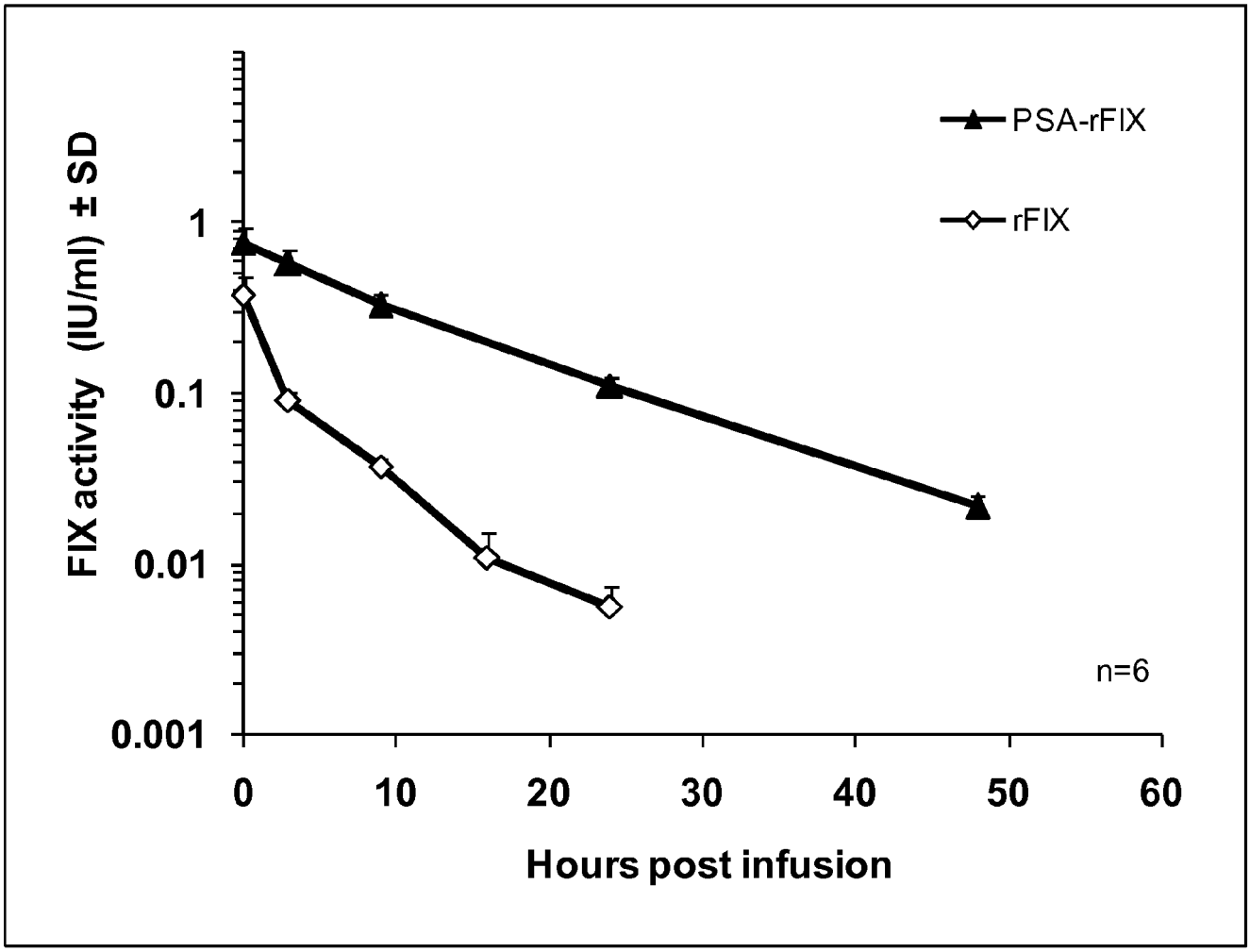
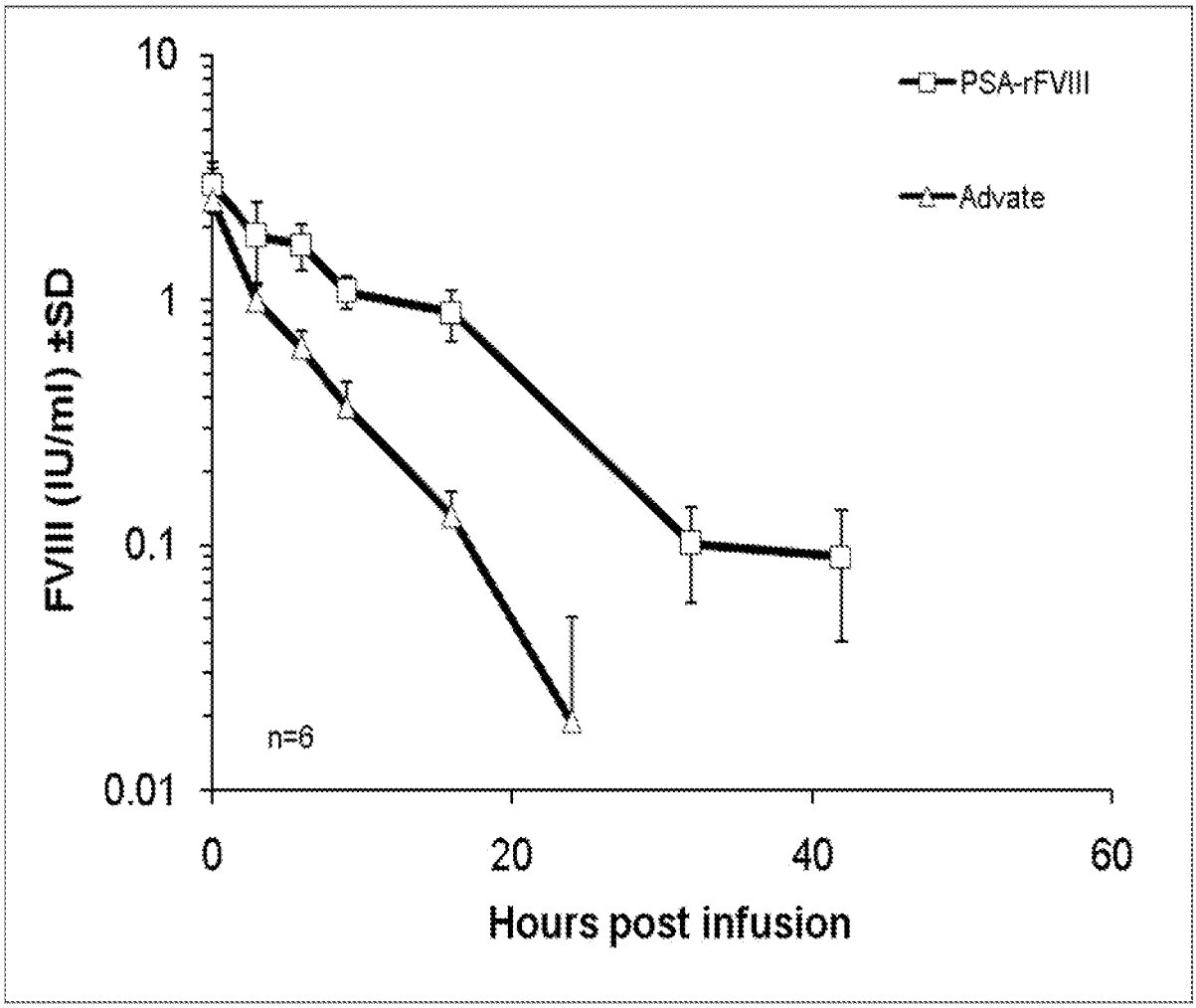

PSA-rFIX
rFIX
n=6
FIX activity (IU/ml) ± SD
1
0.1
0.01
0.001
0
10
20
30
40
50
60
Hours post infusion

Figure 8


10
1
0.1
0.01
FVIII (IU/ml) ±SD
n=6
0
20
40
60
Hours post infusion
PSA-rFVIII
Advate