Figure 1

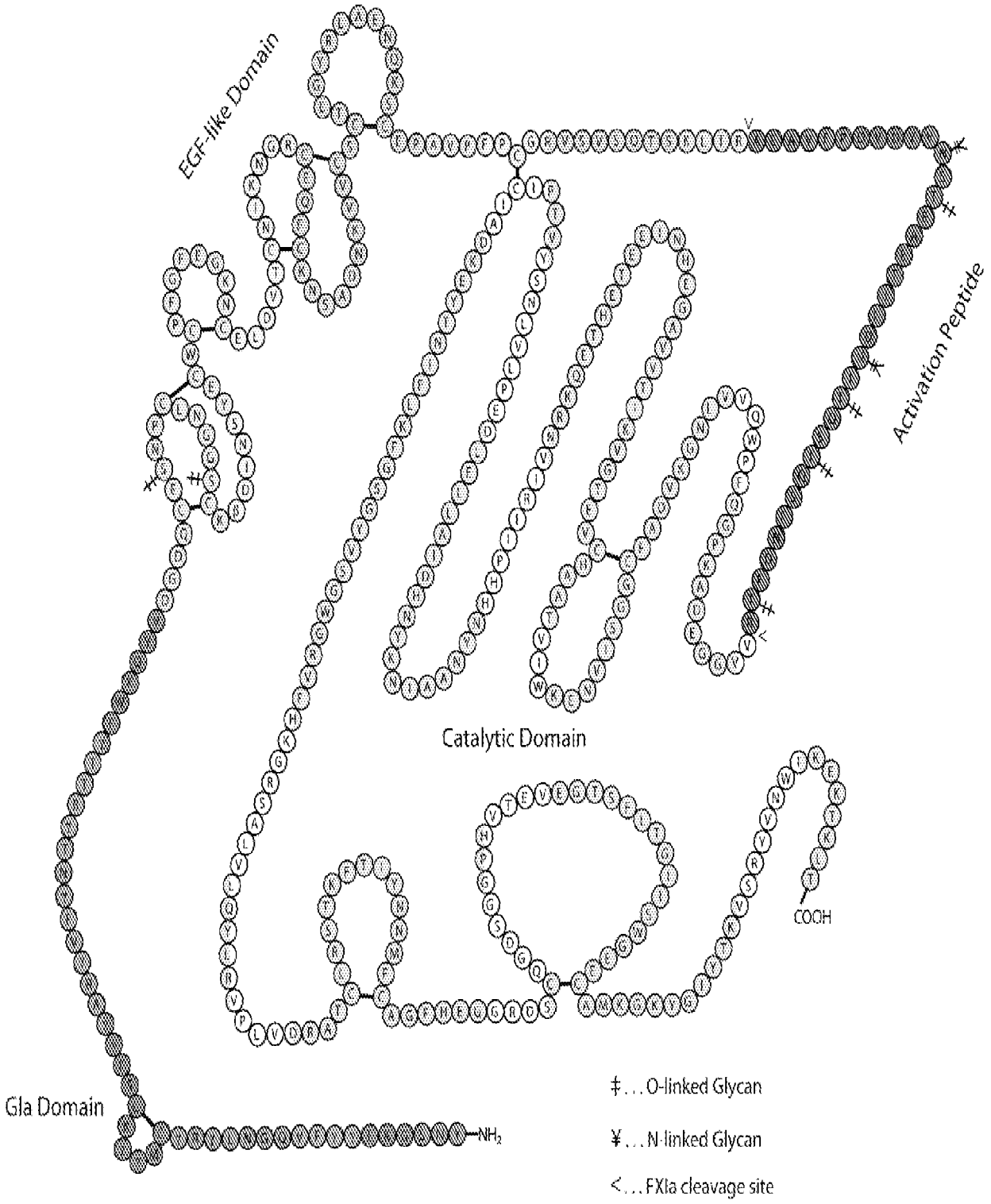

EGF-like Domain
Activation Peptide
Catalytic Domain
Gla Domain
COOH
NH2
‡...O-linked Glycan
¥...N-linked Glycan
<...FXIa cleavage site

Figure 2

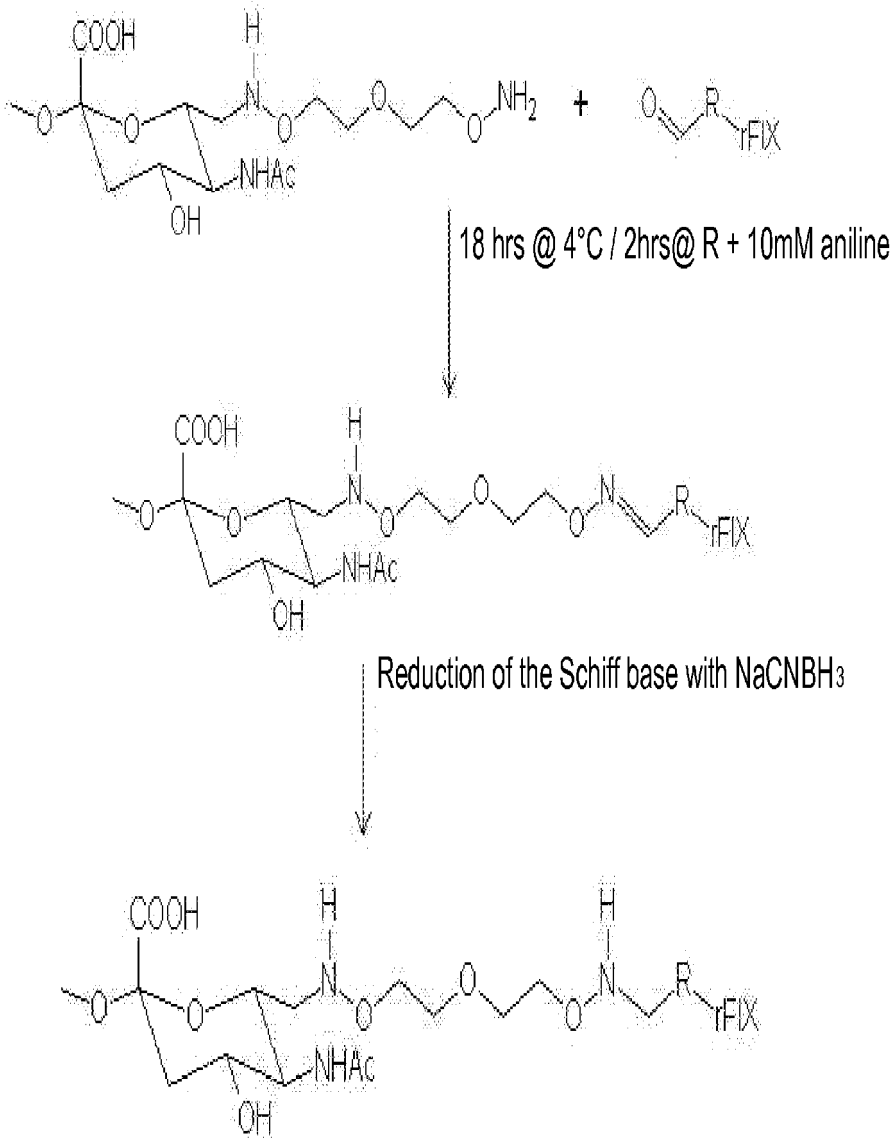

COOH
H
N
O
NH2
+
O
R
rFIX
NHAc
OH
18 hrs @ 4°C / 2hrs@ R + 10mM aniline
Reduction of the Schiff base with NaCNBH3

Figure 3

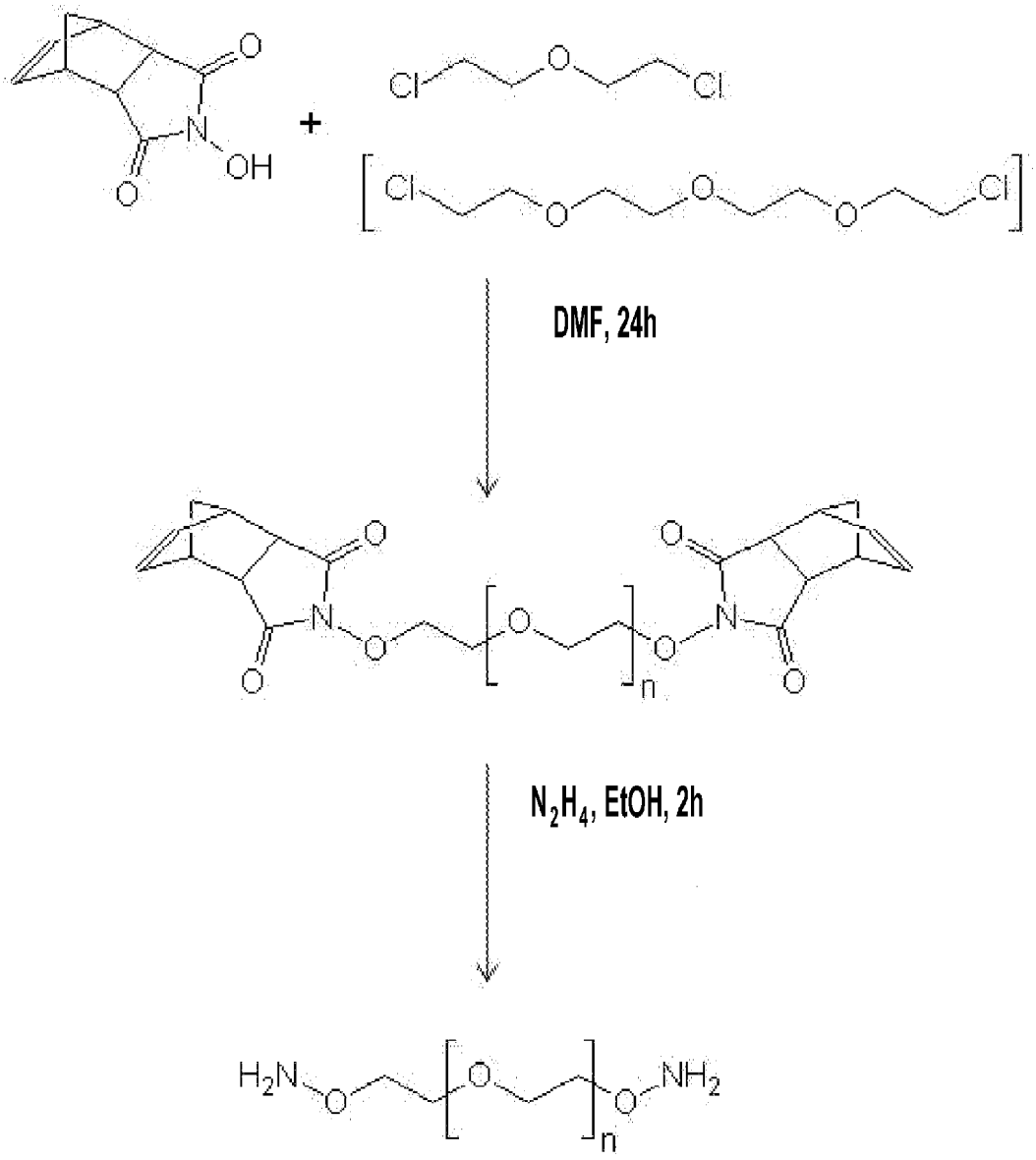
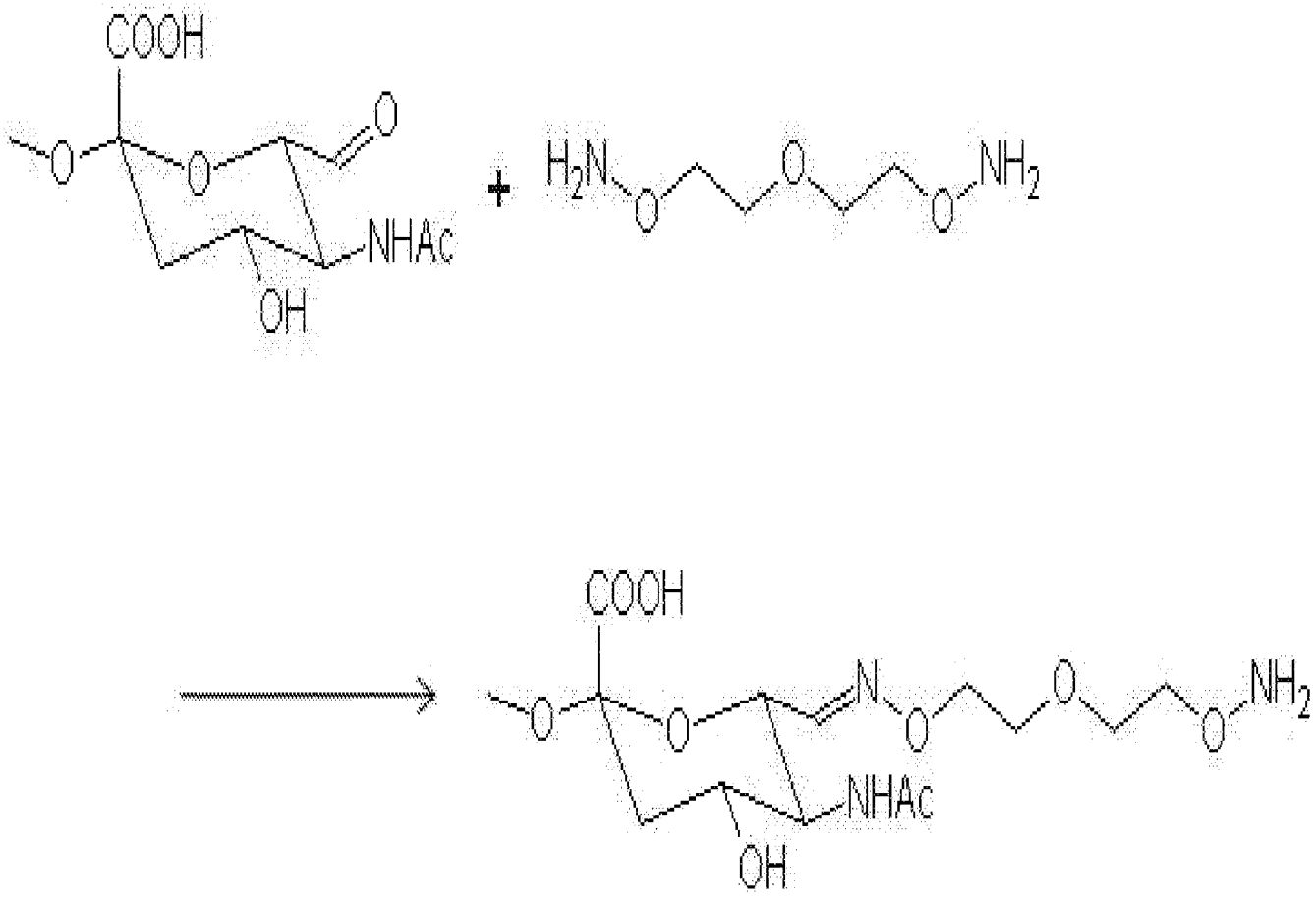

O
N
OH
O
+
Cl
O
Cl
Cl
O
O
O
Cl
DMF, 24h
O
N
O
O
O
n
O
N
O
N_2H_4, EtOH, 2h
H_2N
O
O
n
NH_2

Figure 4



Reduction step to stabilize Schiff base:

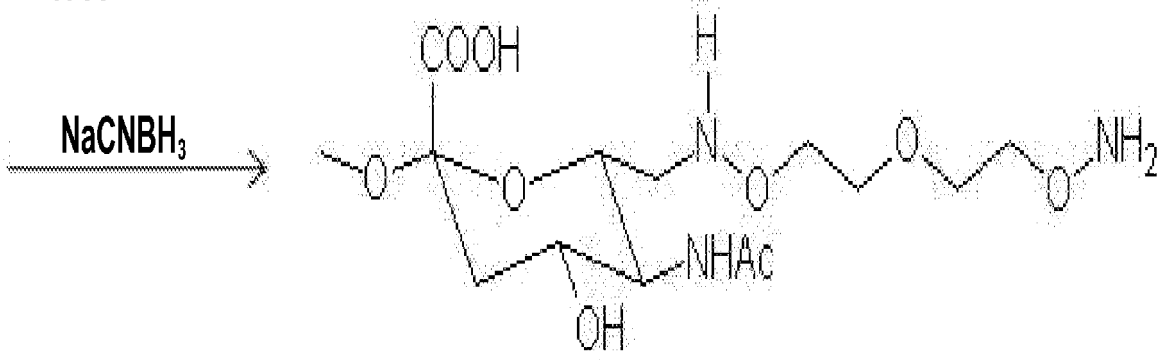
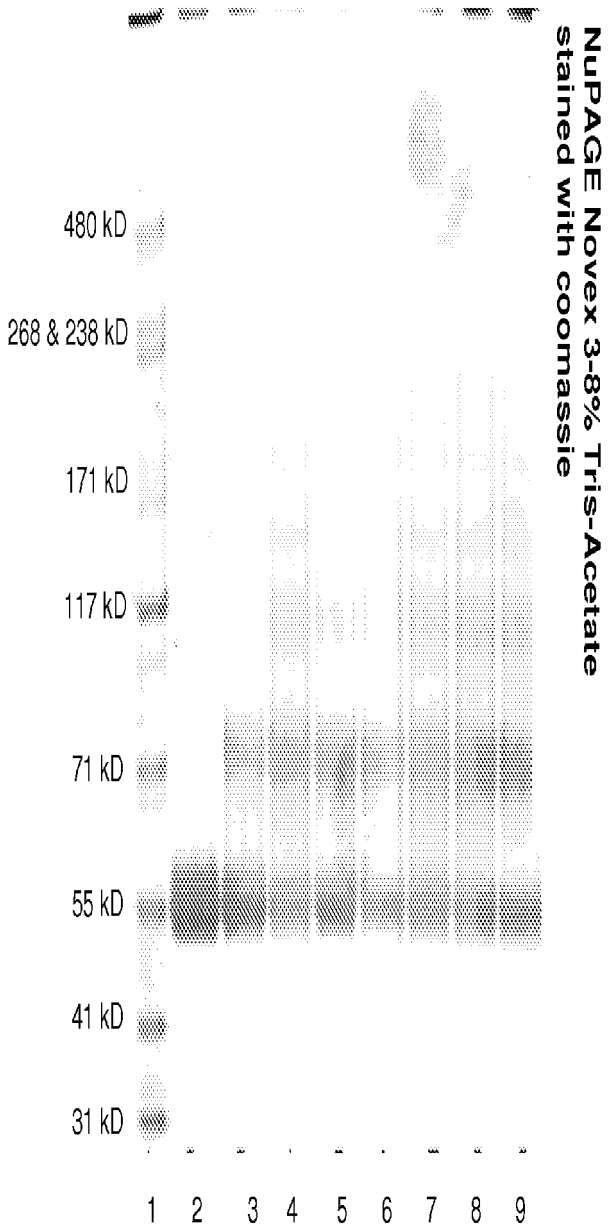


Figure 5A



Lane 1: HiMark MW Standard

Lane 2: rFIX Standard

Lane 3: rFIX + 100μM NaIO4, 0mM catalyst, 5X PSA

Lane 4: rFIX + 100μM NaIO4, 2.5mM aniline, 5X PSA

Lane 5: rFIX + 100μM NaIO4, 5mM aniline, 5X PSA

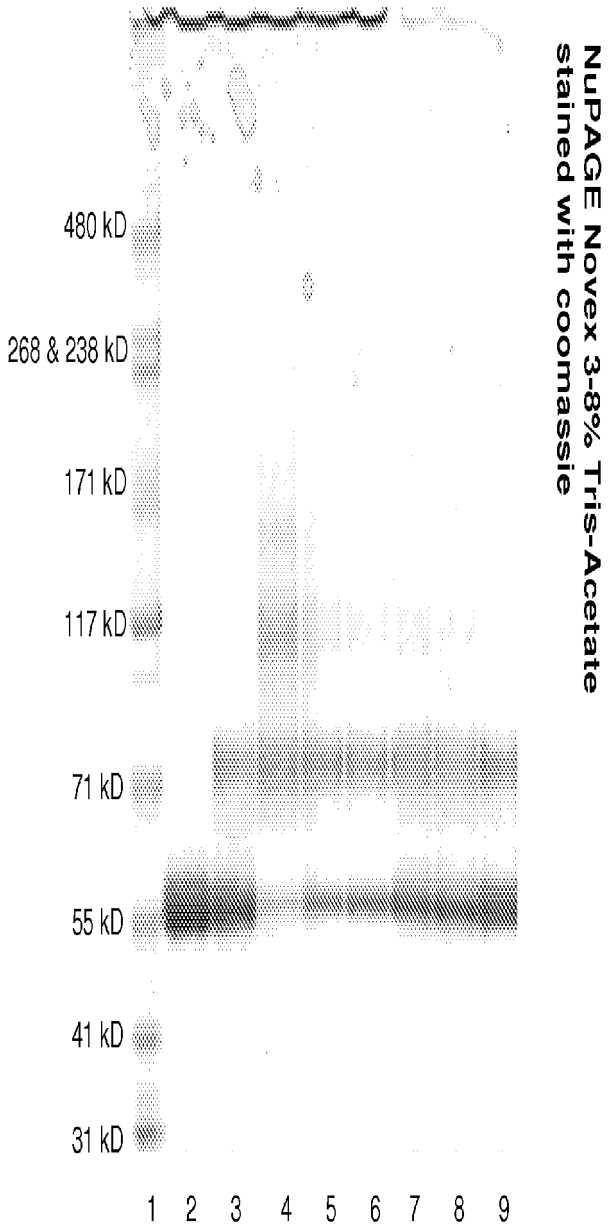
Lane 6: rFIX + 100μM NaIO4, 10mM aniline, 5X PSA

Lane 7: rFIX + 100μM NaIO4, 2.5mM m-toluidine, 5X PSA

Lane 8: rFIX + 100μM NaIO4, 5mM m-toluidine, 5X PSA

Lane 9: rFIX + 100μM NaIO4, 10mM m-toluidine, 5X PSA

Figure 5B



Lane 1: HiMark MW Standard

Lane 2: rFIX Standard

Lane 3: rFIX + 100μM NaIO4, 0mM catalyst, 5X PSA

Lane 4: rFIX + 100μM NaIO4, 10mM aniline, 5X PSA

Lane 5: rFIX + 100μM NaIO4, 10mM o-aminobenzoic acid, 5X PSA

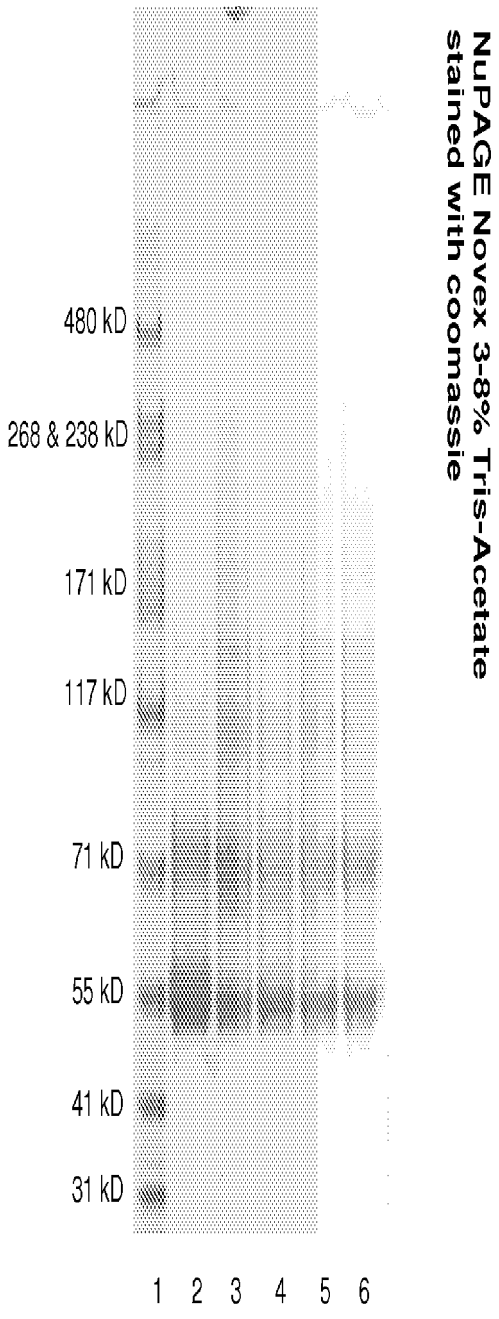
Lane 6: rFIX + 100μM NaIO4, 10mM m-aminobenzoic acid, 5X PSA

Lane 7: rFIX + 100μM NaIO4, 10mM p-aminobenzoic acid, 5X PSA

Lane 8: rFIX + 100μM NaIO4, 10mM p-aminobenzamide, 5X PSA

Lane 9: rFIX + 100μM NaIO4, 10mM psulfanilic acid, 5X PSA

Figure 5C



Lane 1: HiMark MW Standard

Lane 2: rFIX + 100μM NaIO4, 0mM catalyst, 5X PSA

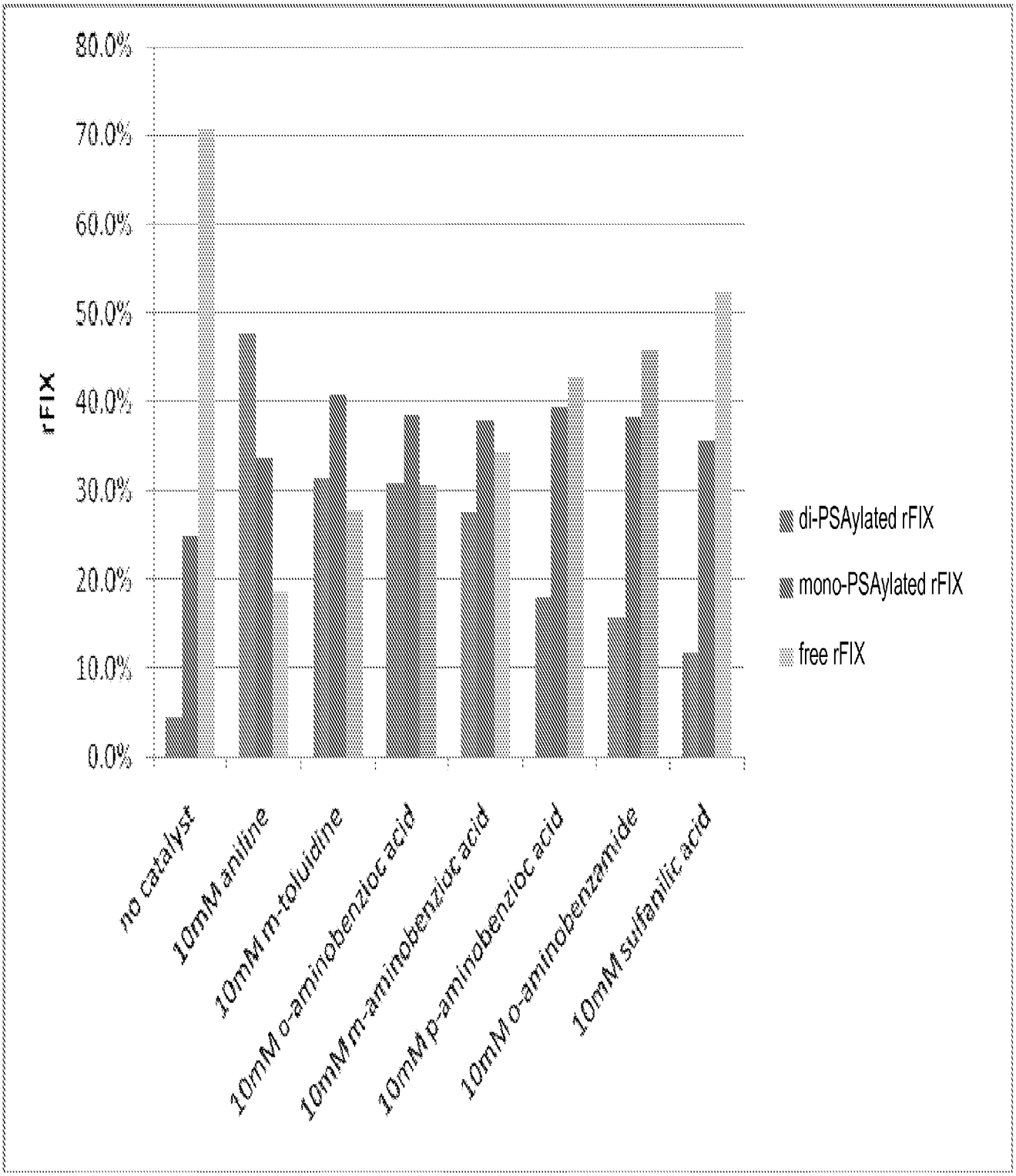
Lane 3: rFIX + 100μM NaIO4, 10mM aniline, 5X PSA

Lane 4: rFIX + 100μM NaIO4, 10mM o-anisidine, 5X PSA

Lane 5: rFIX + 100μM NaIO4, 10mM m-anisidine, 5X PSA

Lane 6: rFIX + 100μM NaIO4, 10mM m-toluidine, 5X PSA

Figure 6


80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
rFIX
no catalyst
10mM aniline
10mM m-toluidine
10mM o-aminobenzioc acid
10mM m-aminobenzioc acid
10mM p-aminobenzioc acid
10mM o-aminobenzamide
10mM sulfanilic acid
di-PSAylated rFIX
mono-PSAylated rFIX
free rFIX