Figure 1

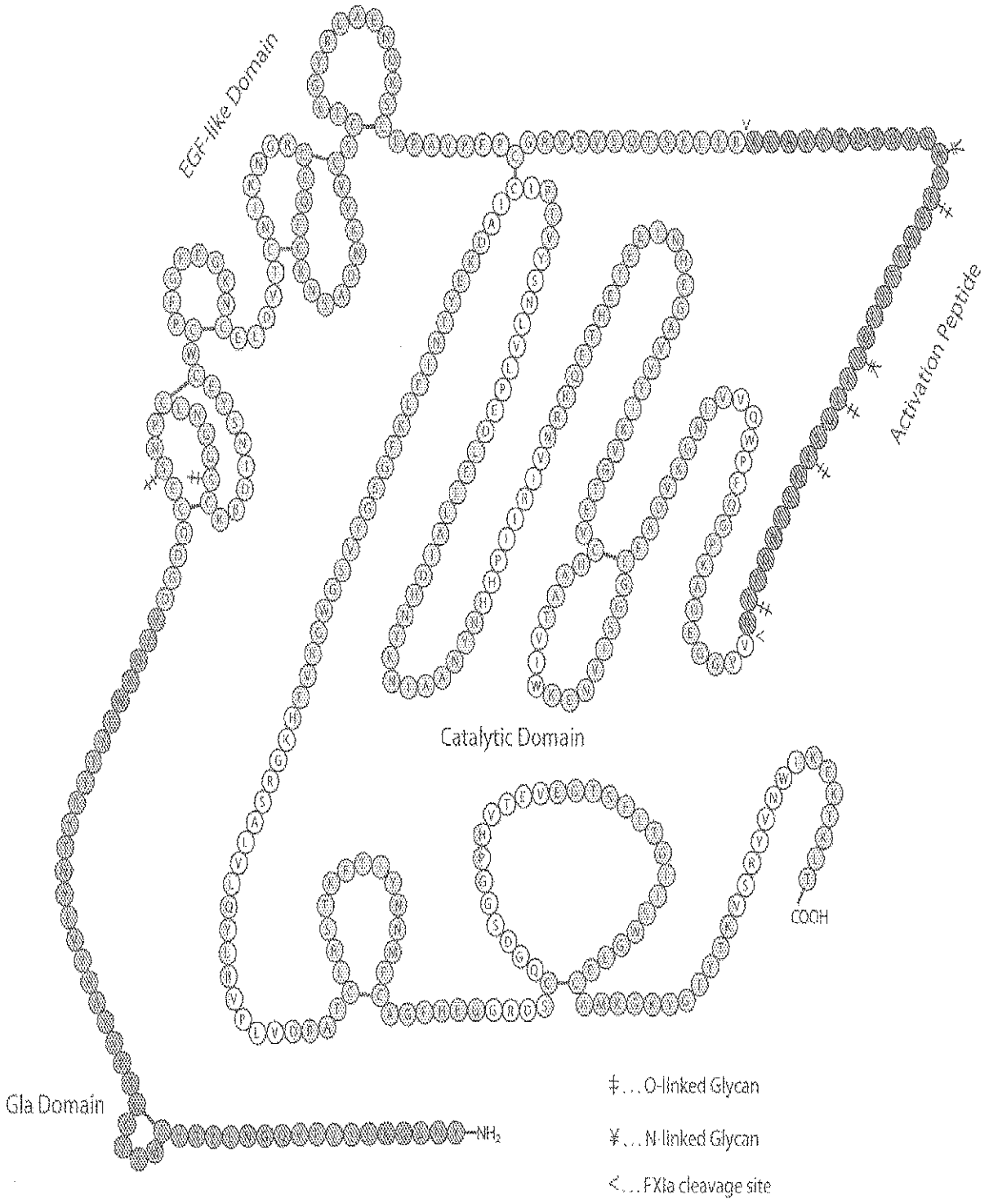

EGF-like Domain
Activation Peptide
Catalytic Domain
COOH
Gla Domain
NH2
‡ . . . O-linked Glycan
¥ . . . N-linked Glycan
< . . . FXIa cleavage site

Figure 2

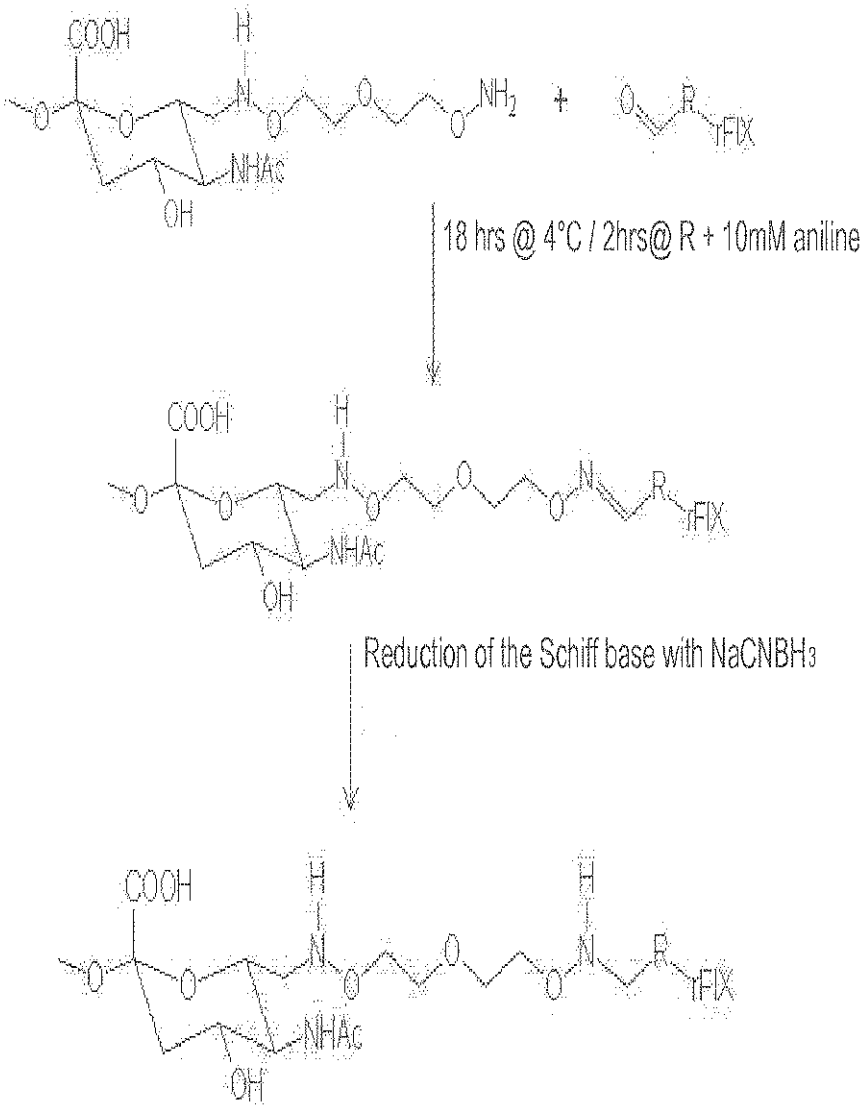

COOH
H
N
O
NHAc
OH
NH2
+
R
FIX
18 hrs @ 4°C / 2hrs@ R + 10mM aniline
Reduction of the Schiff base with NaCNBH3

Figure 3

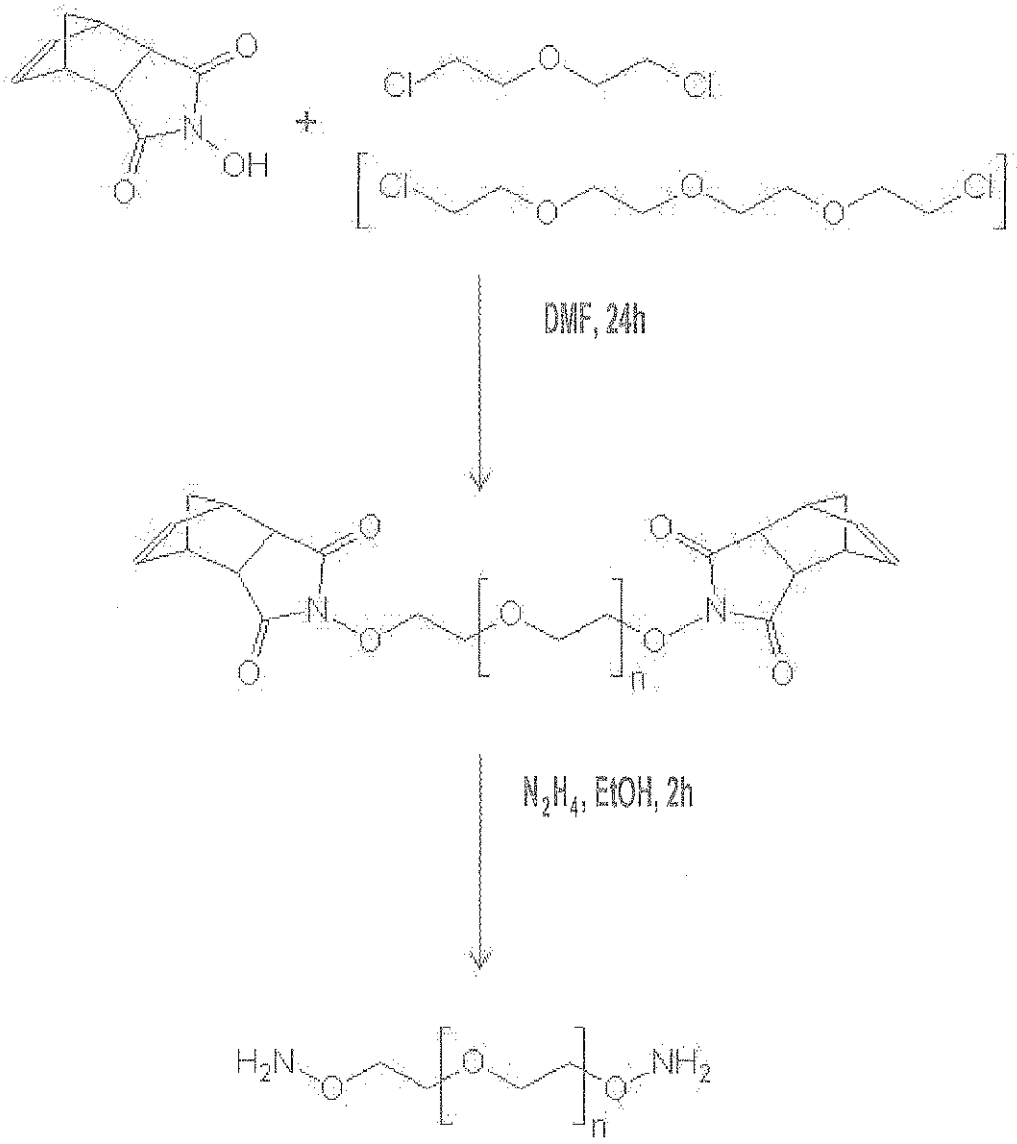
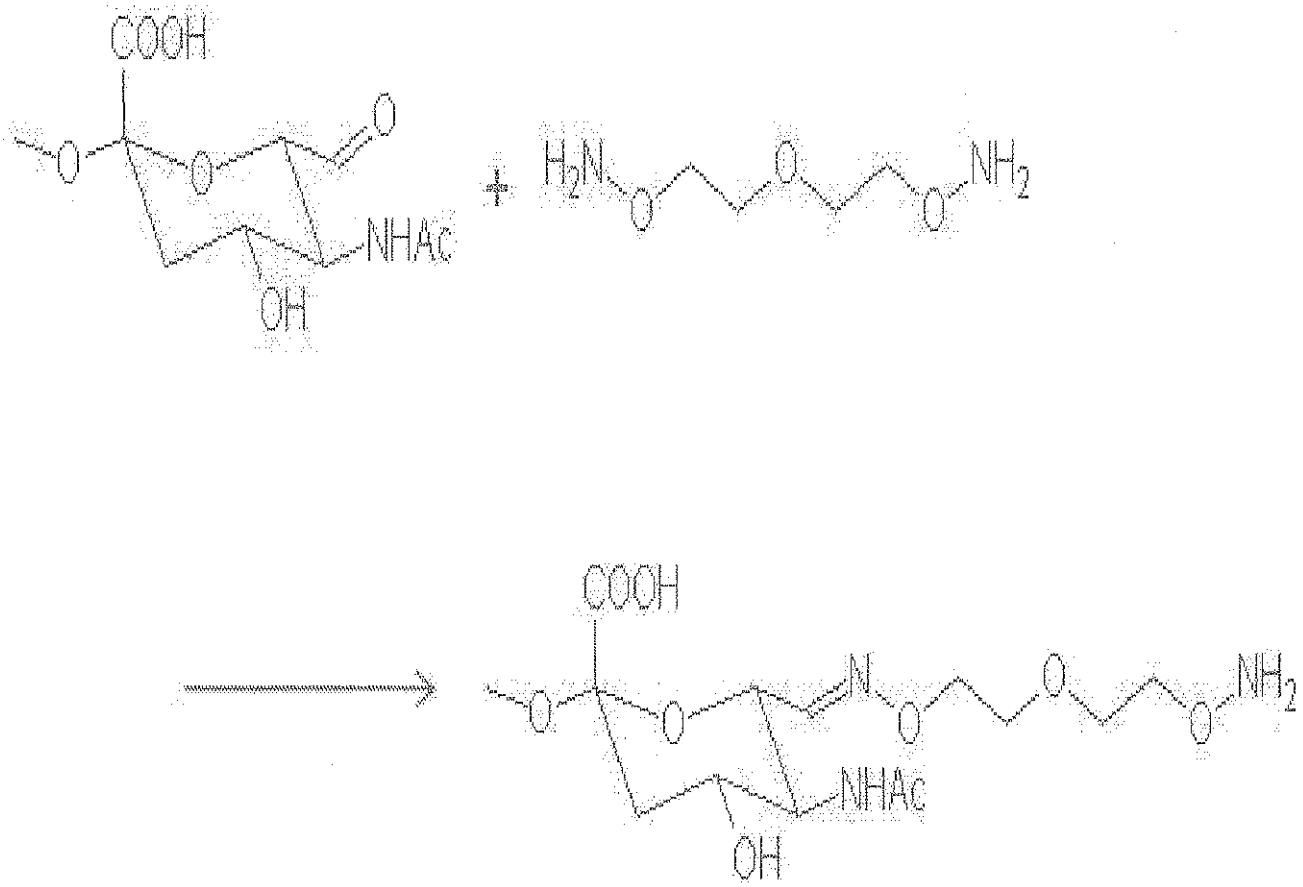


Figure 4



Reduction step to stabilize Schiff base:

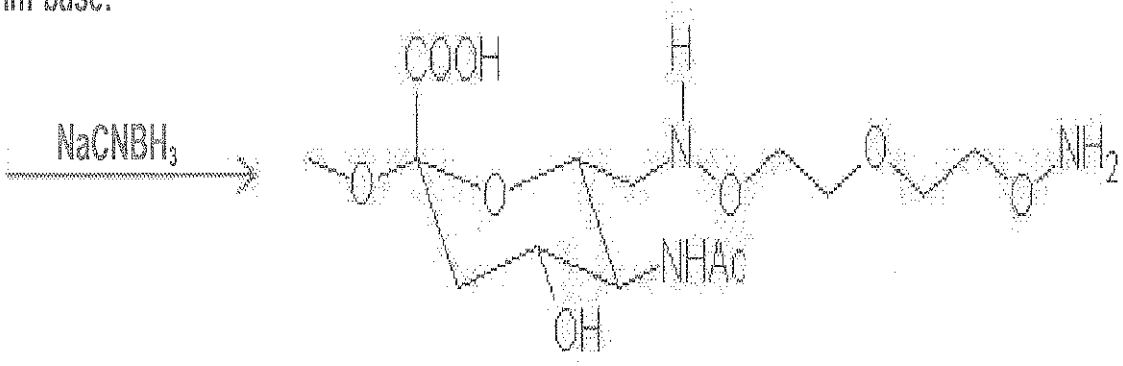
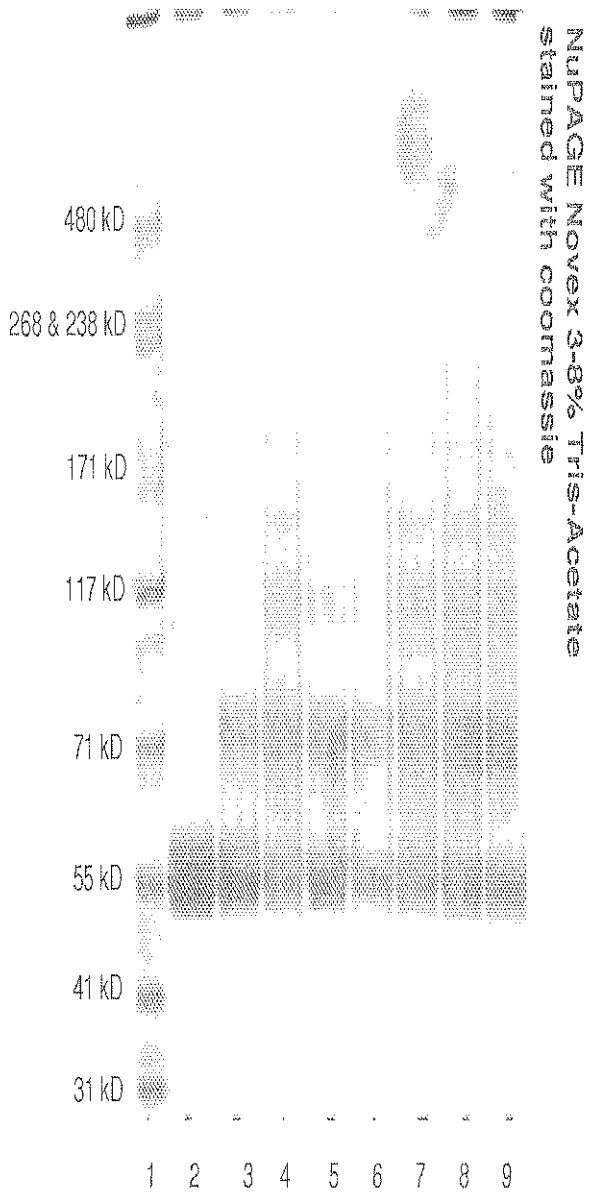


Figure 5A



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

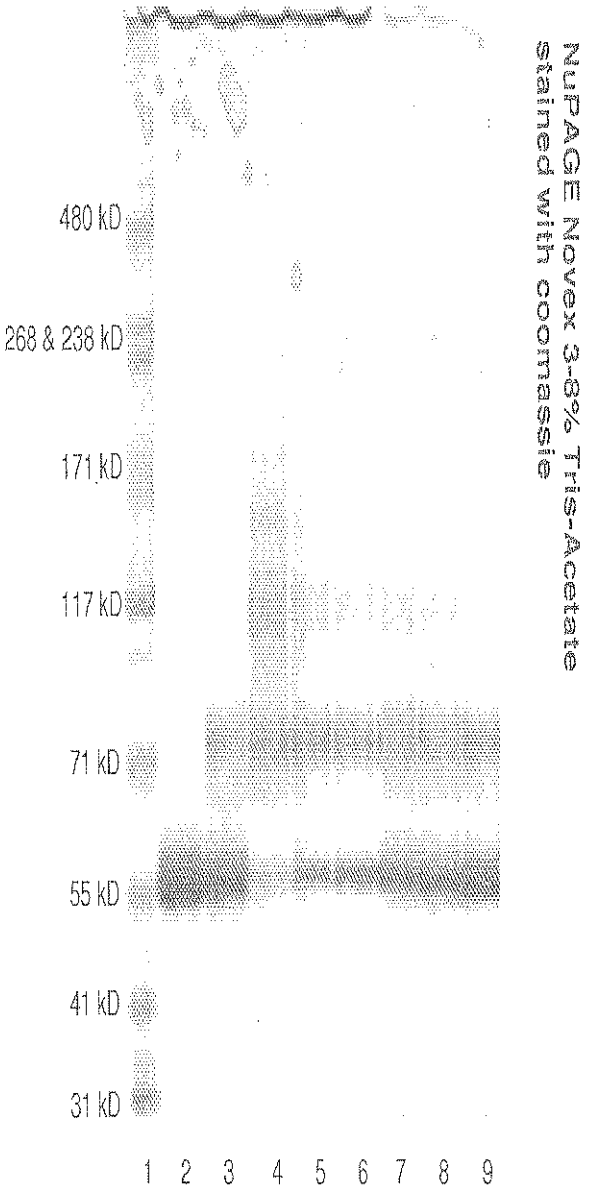
Lane 6: rFIX + 100μM NaIO4, 10mM aniline, 5X PSA

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 $NaIO_4$, 0mM catalyst, 5X PSA

Lane 4: rFIX + 100μM $NaIO_4$, 10mM aniline, 5X PSA

Lane 5: rFIX + 100μM $NaIO_4$, 10mM o-aminobenzoic acid, 5X PSA

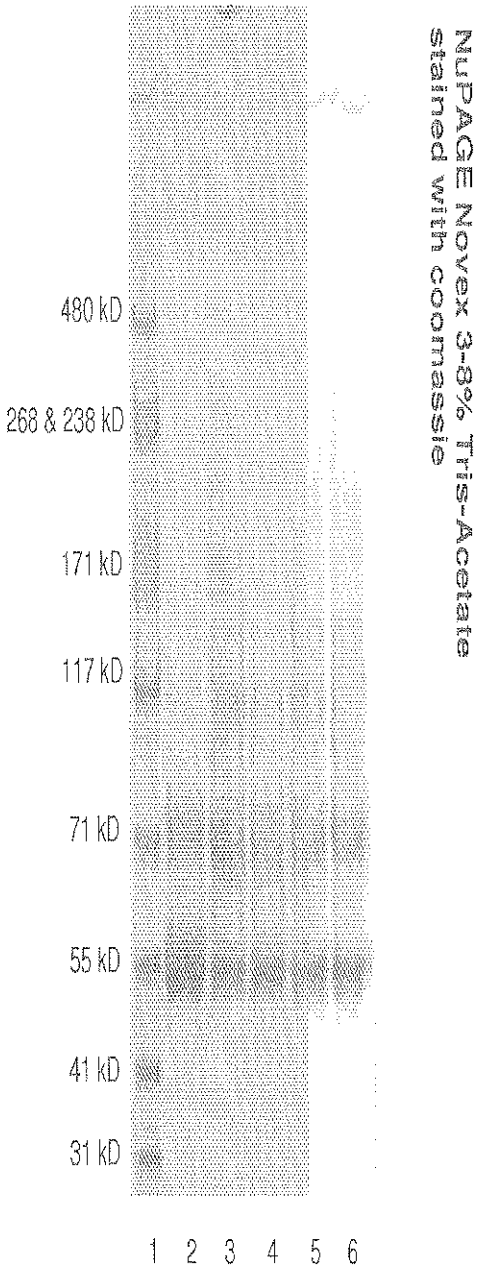
Lane 6: rFIX + 100μM $NaIO_4$, 10mM m-aminobenzoic acid, 5X PSA

Lane 7: rFIX + 100μM $NaIO_4$, 10mM p-aminobenzoic acid, 5X PSA

Lane 8: rFIX + 100μM $NaIO_4$, 10mM p-aminobenzamide, 5X PSA

Lane 9: rFIX + 100μM $NaIO_4$, 10mM psulfanilic acid, 5X PSA

Figure 5C



Lane 1: HiMark MW Standard

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

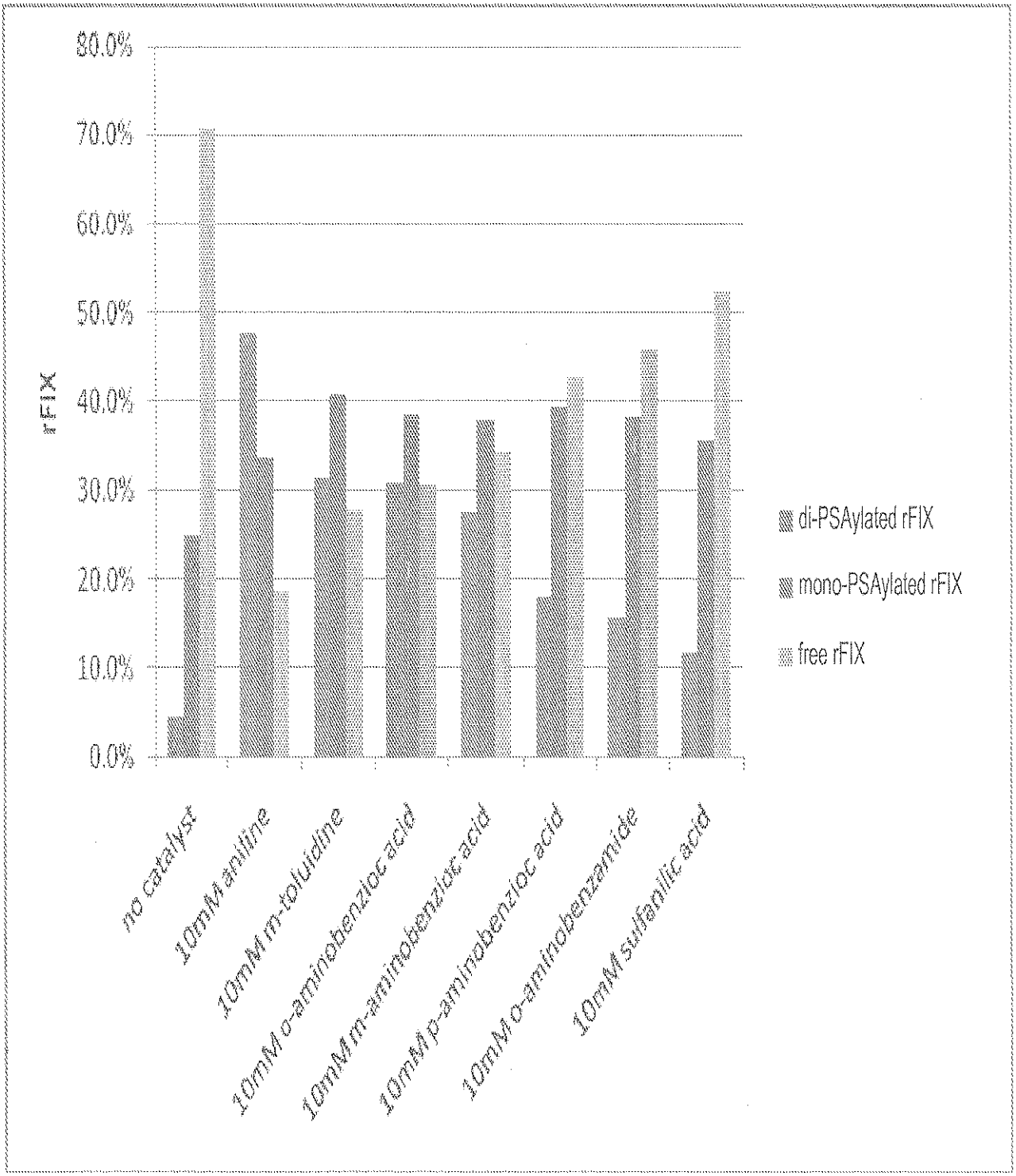
Lane 3: rFIX + 100μM NaIO4, 10mM aniline, 5X PSA

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
di-PSAylated rFIX
mono-PSAylated rFIX
free rFIX
no catalyst
10mM anitine
10mM m-toluidine
10mM o-aminobenzoic acid
10mM m-aminobenzoic acid
10mM p-aminobenzoic acid
10mM o-aminobenzamide
10mM sulfanilic acid