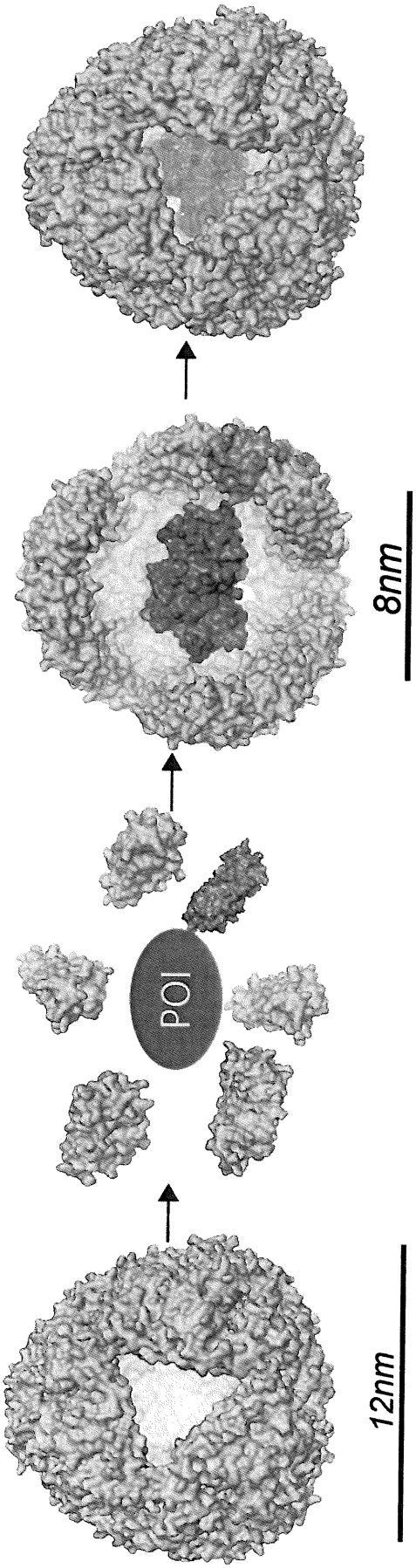
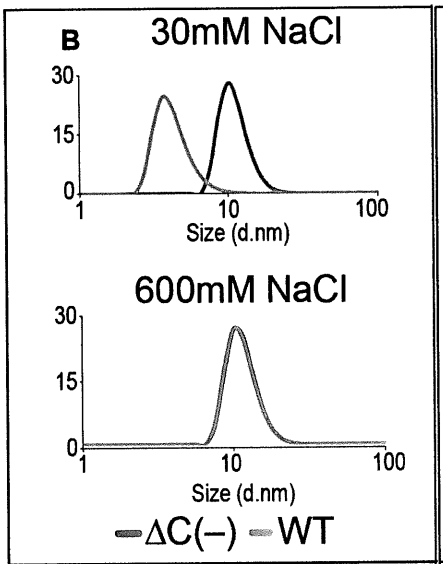


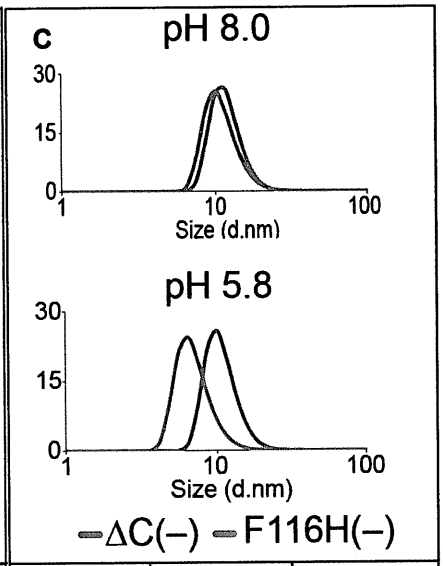
FIG. 1A

FIG. 1B



	30mM	600mM
WT	4.28 ± 1.3	11.12 ± 2.6
ΔC(+)	11.65 ± 2.8	11.51 ± 2.8
ΔC(–)	11.54 ± 2.8	11.56 ± 3.1
ΔC(+/–)	11.97 ± 2.9	11.11 ± 3.0

FIG. 1C



	pH 5.8	pH 8.0
ΔC(–)	11.42 ± 2.7	11.56 ± 3.1
F116H(+)	7.14 ± 2.0	11.29 ± 2.9
F116H(–)	7.16 ± 1.7	11.74 ± 3.1
F116H(+/–)	7.11 ± 0.9	11.35 ± 1.8

FIG. 2A

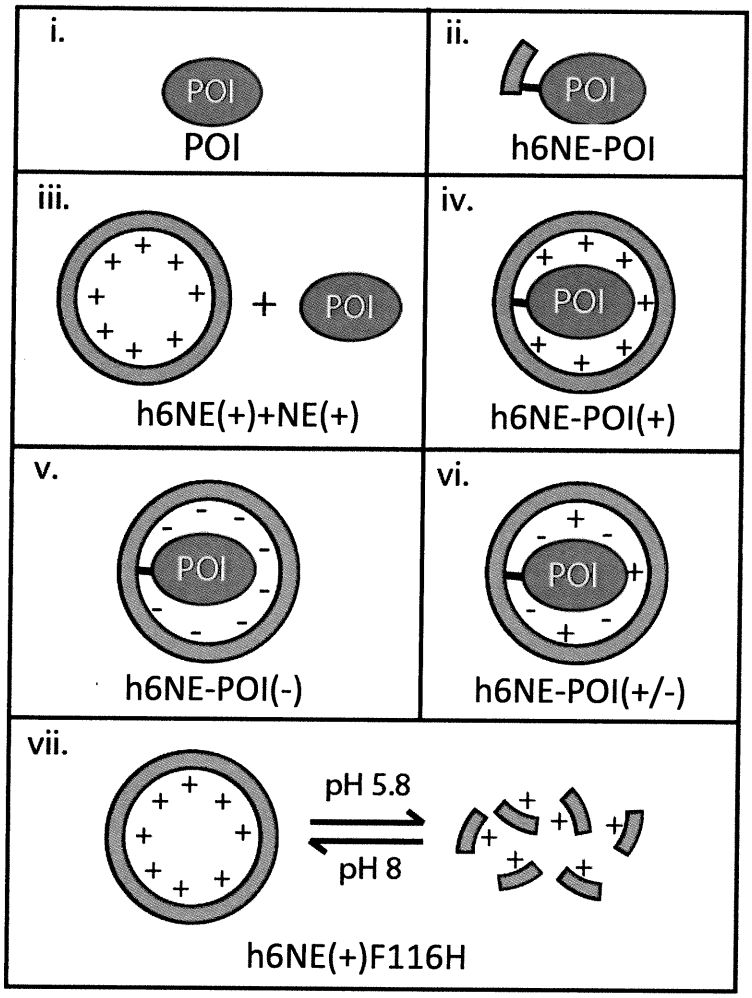

i.
POI
POI
ii.
POI
h6NE-POI
iii.
+
POI
h6NE(+)+NE(+)
iv.
POI
h6NE-POI(+)
v.
POI
h6NE-POI(-)
vi.
POI
h6NE-POI(+/-)
vii.
pH 5.8
pH 8
h6NE(+)F116H

FIG. 2B

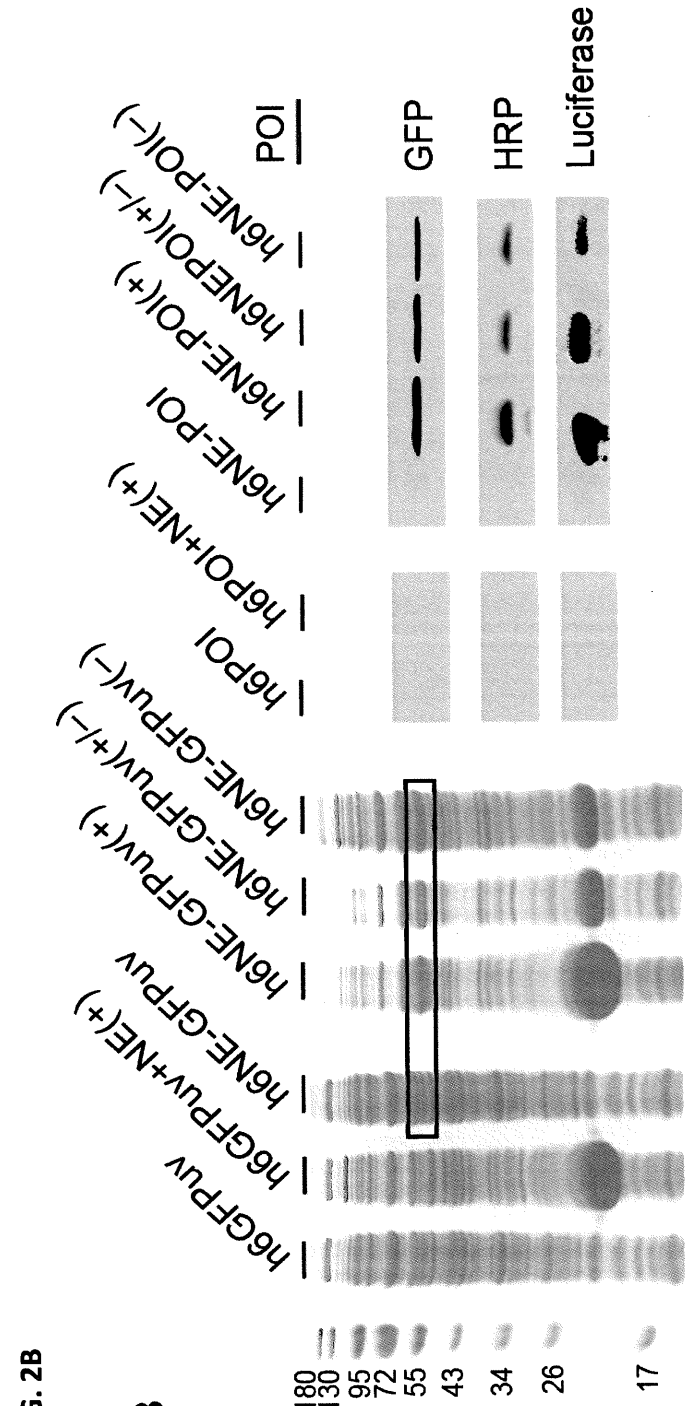

B
180
130
95
72
55
43
34
26
17
h6GFPuv
h6GFPuv+NE(+)
h6NE-GFPuv
h6NE-GFPuv(+)
h6NE-GFPuv(+/-)
h6NE-GFPuv(-)
h6POI
h6POI+NE(+)
h6NE-POI
h6NE-POI(+)
h6NEPOI(+/-)
h6NE-POI(-)
POI
GFP
HRP
Luciferase

FIG. 2C

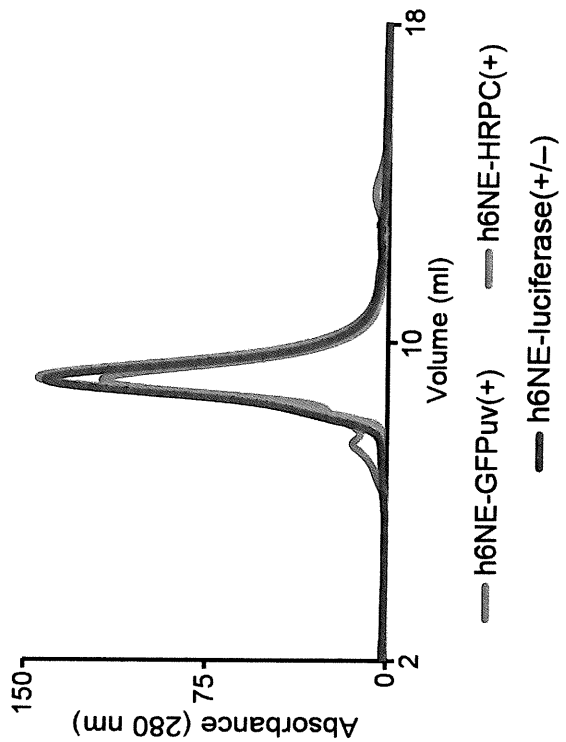

C
Absorbance (280 nm)
150
75
0
2
10
18
Volume (ml)
h6NE-GFPuv(+)
h6NE-HRPC(+)
h6NE-luciferase(+/–)

FIG. 2D

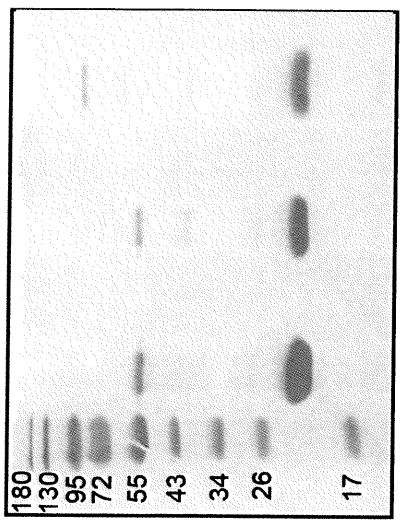

D
NE
180
130
95
72
55
43
34
26
17
h6NE-GFPuv(+)
h6NE-HRPC(+)
h6NE-luciferase(+/–)

FIG. 3A

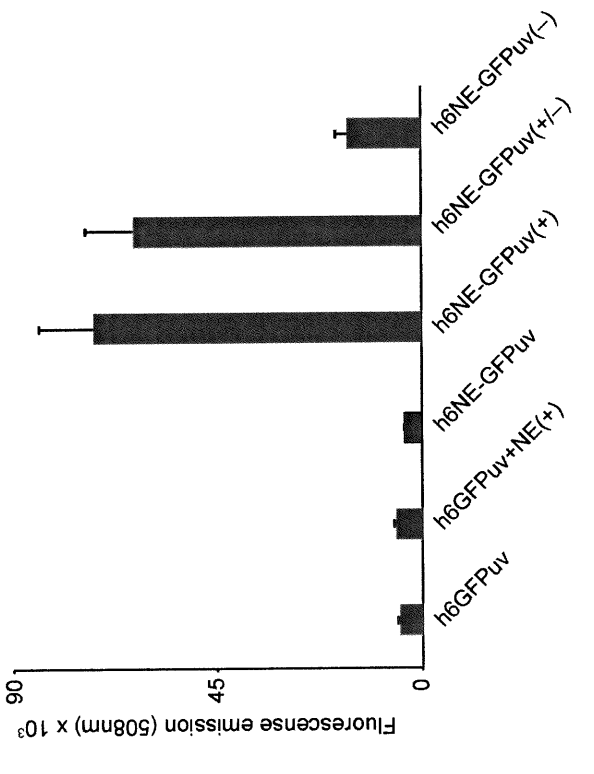

A
Fluorescense emission (508nm) x 10³
90
45
0
h6GFPuv
h6GFPuv+NE(+)
h6NE-GFPuv
h6NE-GFPuv(+)
h6NE-GFPuv(+/–)
h6NE-GFPuv(–)

FIG. 3B

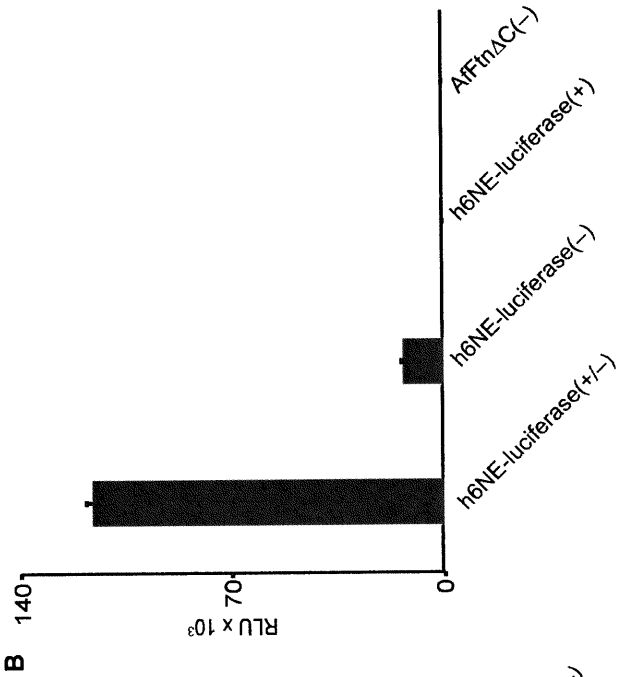

B
RLU x 10³
140
70
0
h6NE-luciferase(+/–)
h6NE-luciferase(–)
h6NE-luciferase(+)
AFFtnΔC(–)

FIG. 3C

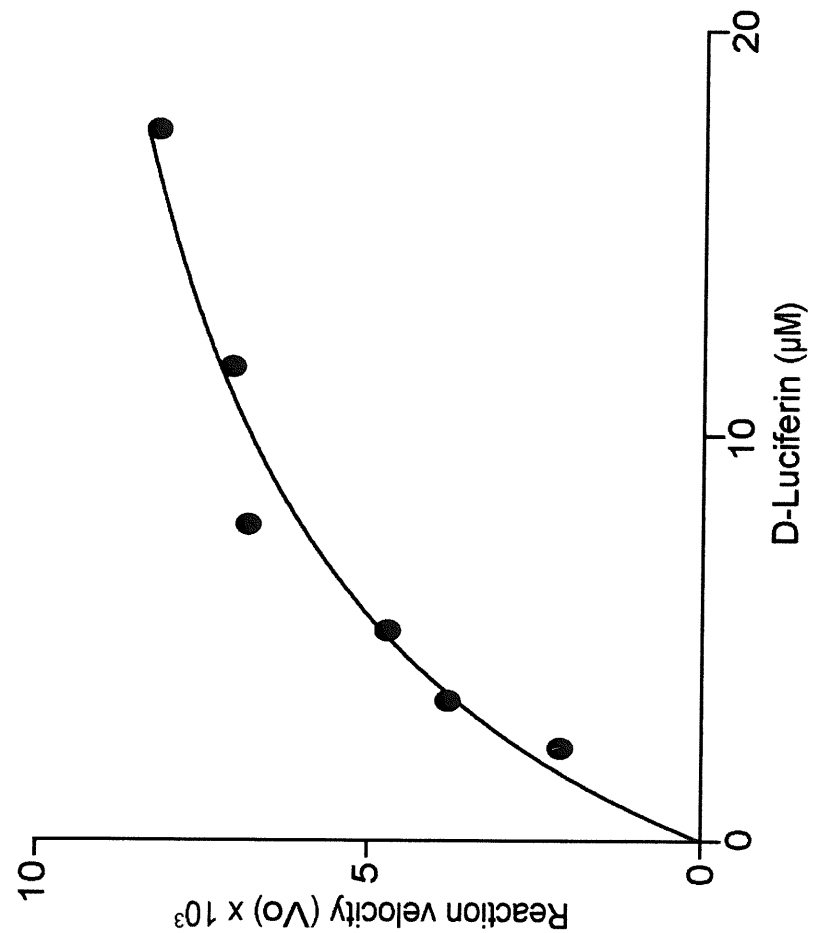


FIG. 3E

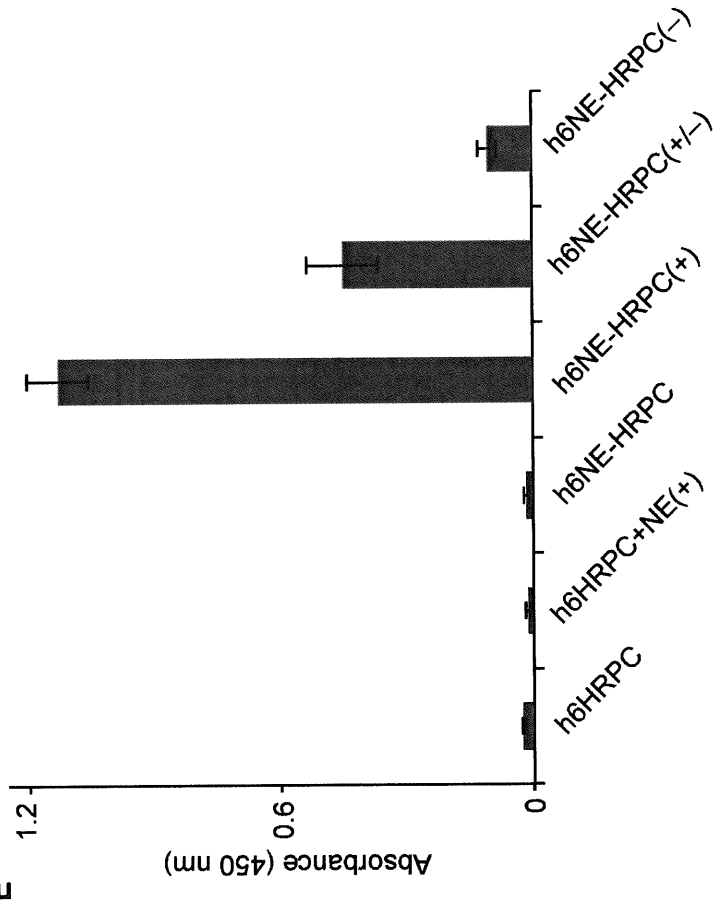
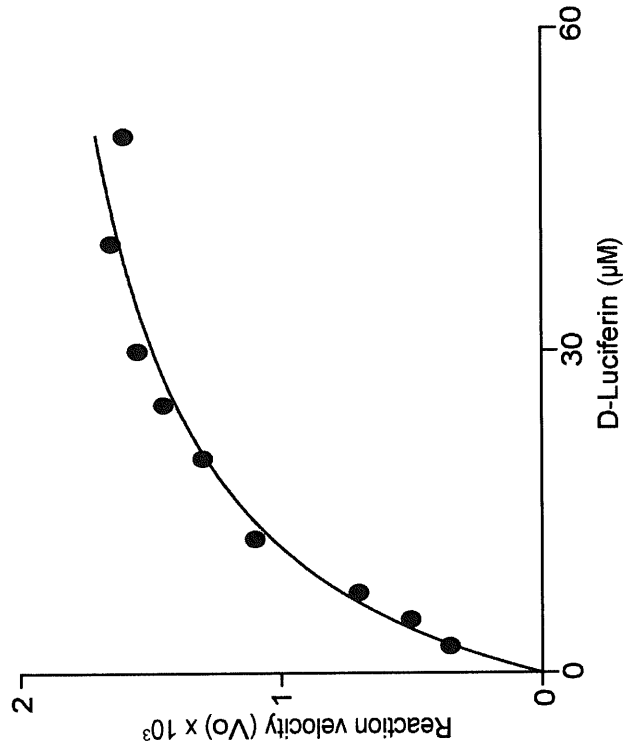

E
Absorbance (450 nm)
0
0.6
1.2
h6HRPC
h6HRPC+NE(+)
h6NE-HRPC
h6NE-HRPC(+)
h6NE-HRPC(+/–)
h6NE-HRPC(–)

FIG. 3D


D
Reaction velocity (Vo) x 10³
0
1
2
0
30
60
D-Luciferin (μM)

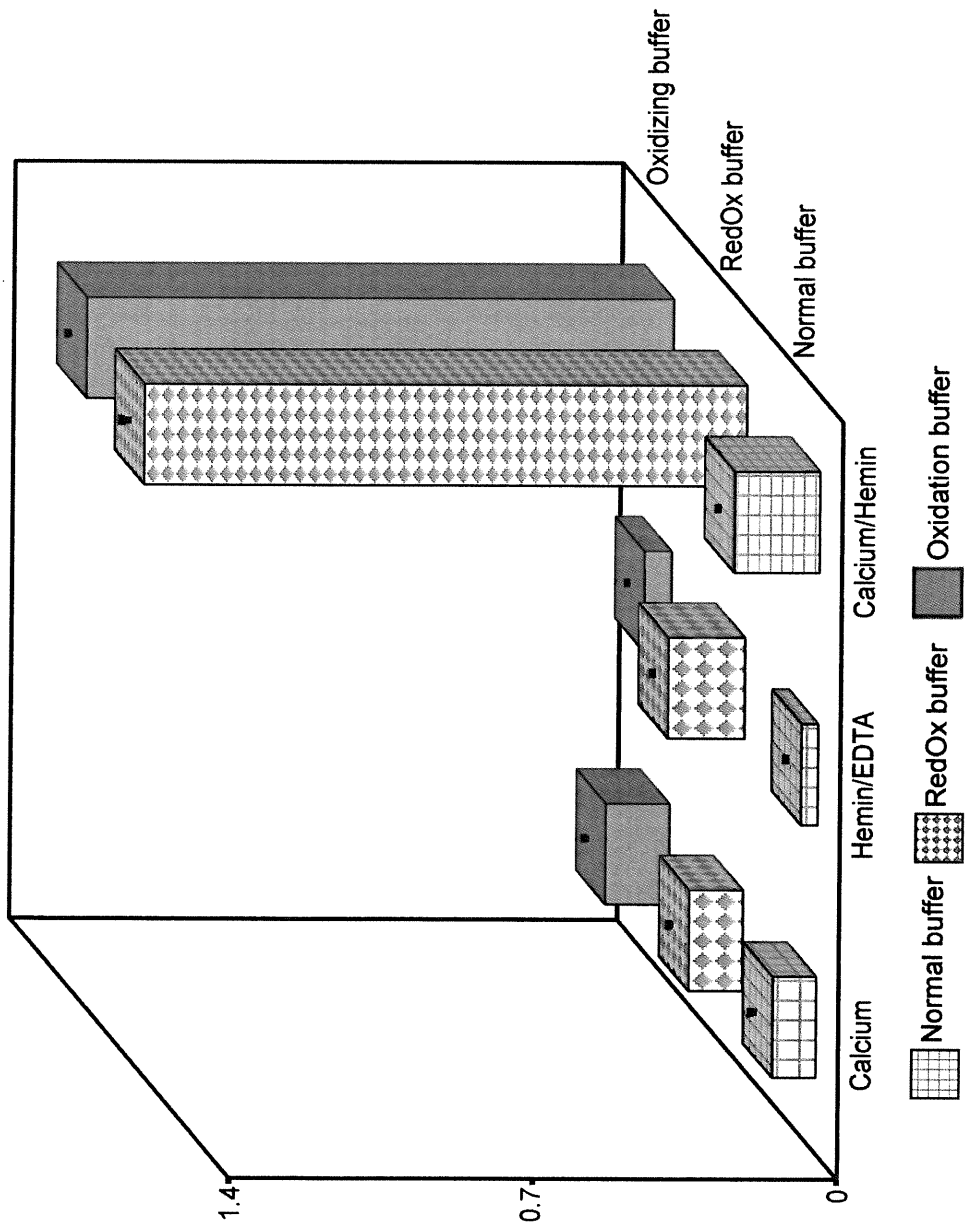

F
1.4
0.7
0
Calcium
Hemin/EDTA
Calcium/Hemin
Oxidizing buffer
RedOx buffer
Normal buffer
Normal buffer
RedOx buffer
Oxidation buffer

FIG. 3F

FIG. 4A

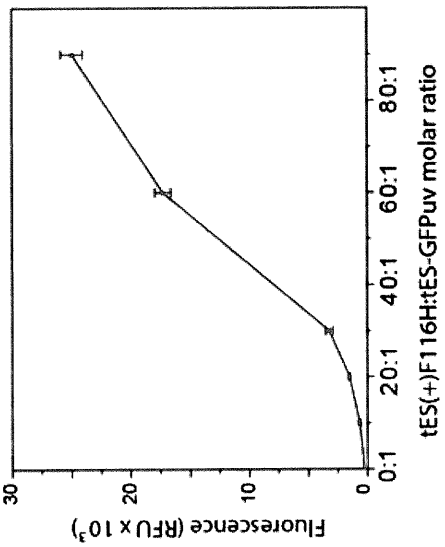


FIG. 4B

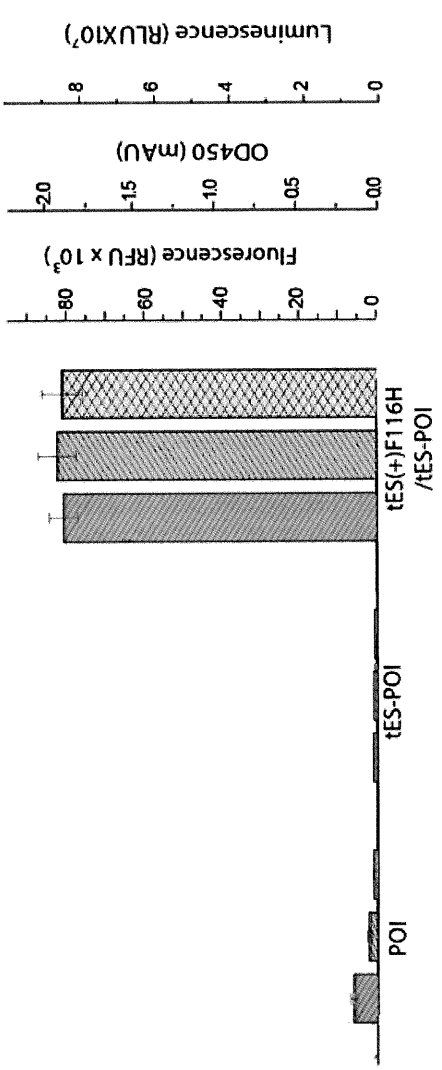


FIG. 4C

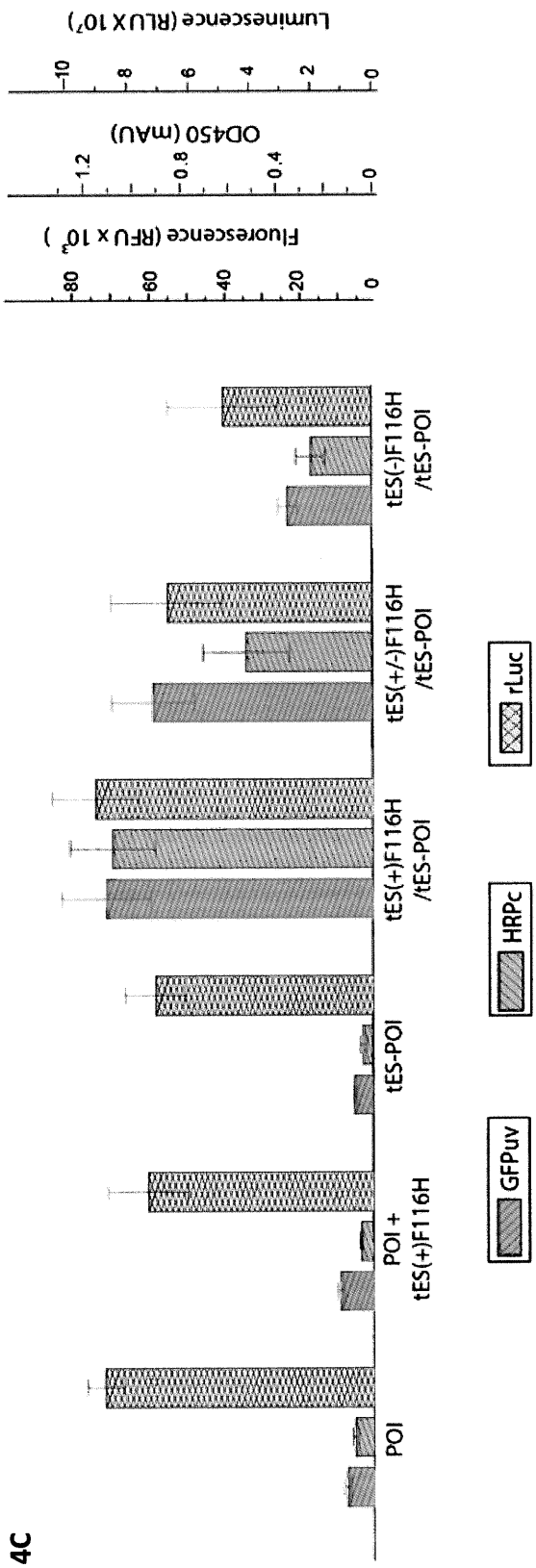


FIG. 5A

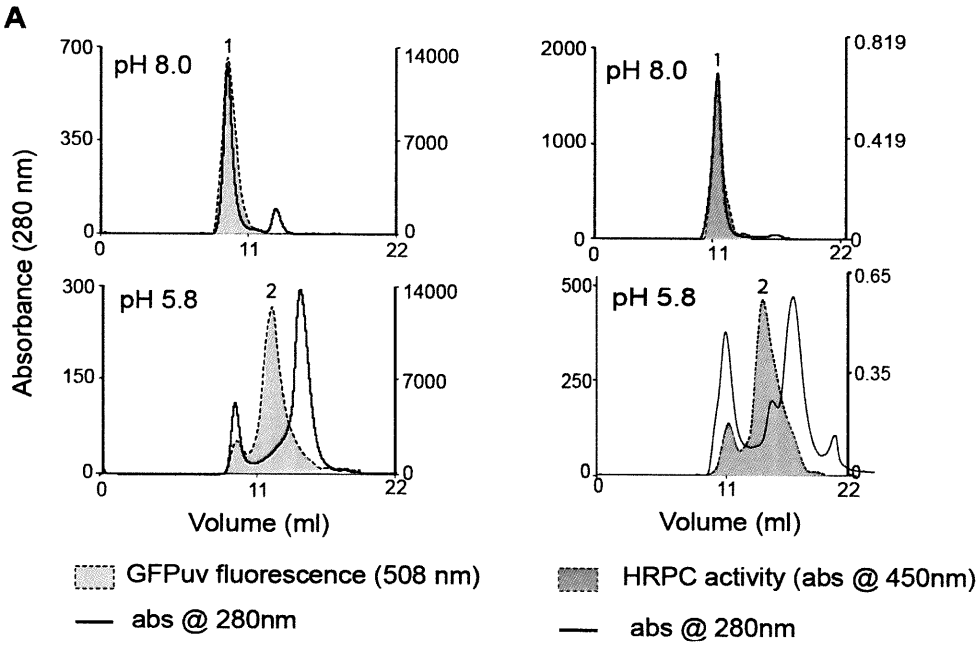

A
pH 8.0
pH 5.8
Absorbance (280 nm)
Volume (ml)
GFPuv fluorescence (508 nm)
abs @ 280nm
HRPC activity (abs @ 450nm)
abs @ 280nm

FIG. 5B

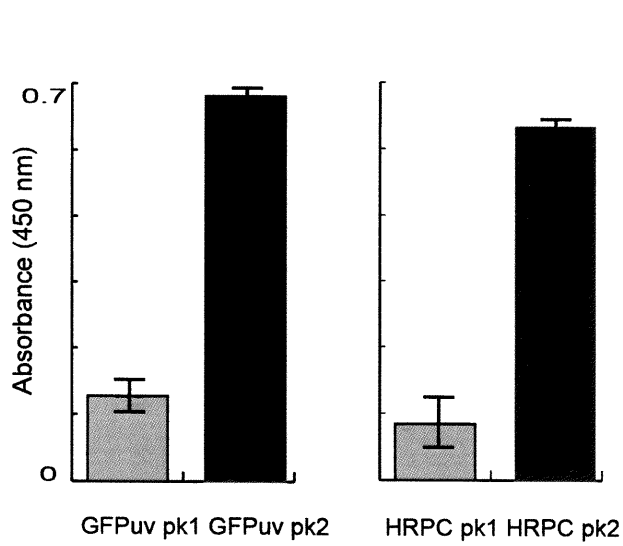

B
Absorbance (450 nm)
GFPuv pk1 GFPuv pk2
HRPC pk1 HRPC pk2

FIG. 5C

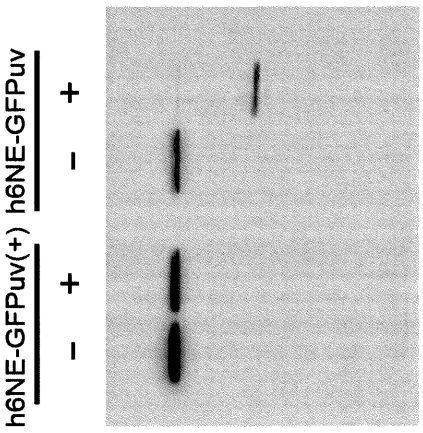

h6NE-GFPuv(+)
h6NE-GFPuv
– + – +
TEV
h6NE-GFPuv
GFPuv

FIG. 5D

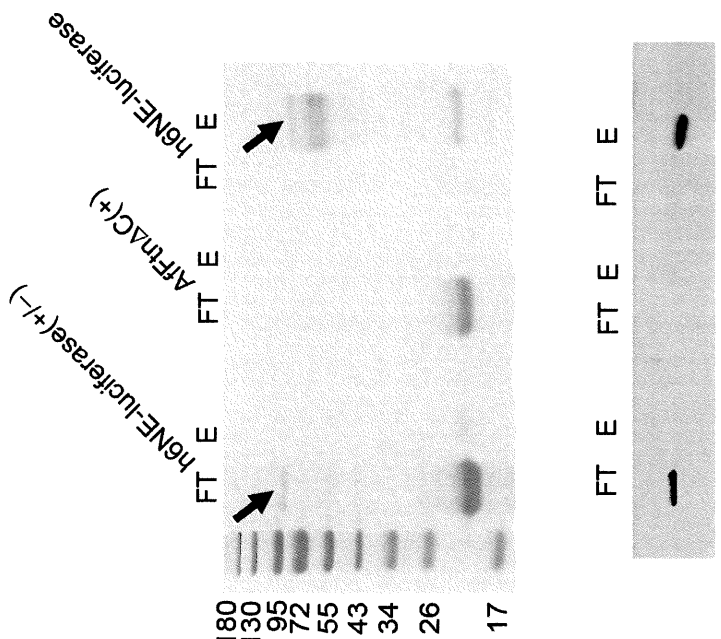

h6NE-luciferase
AFthiaC(+)
h6NE-luciferase(+/–)
FT E FT E FT E
180
130
95
72
55
43
34
26
17
FT E FT E FT E

FIG. 5E

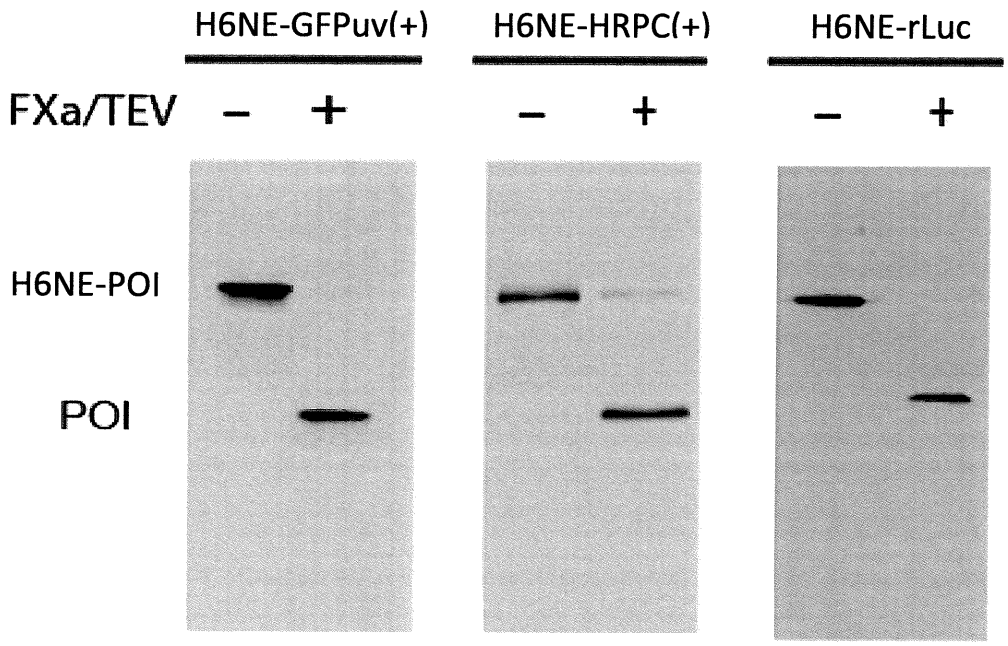
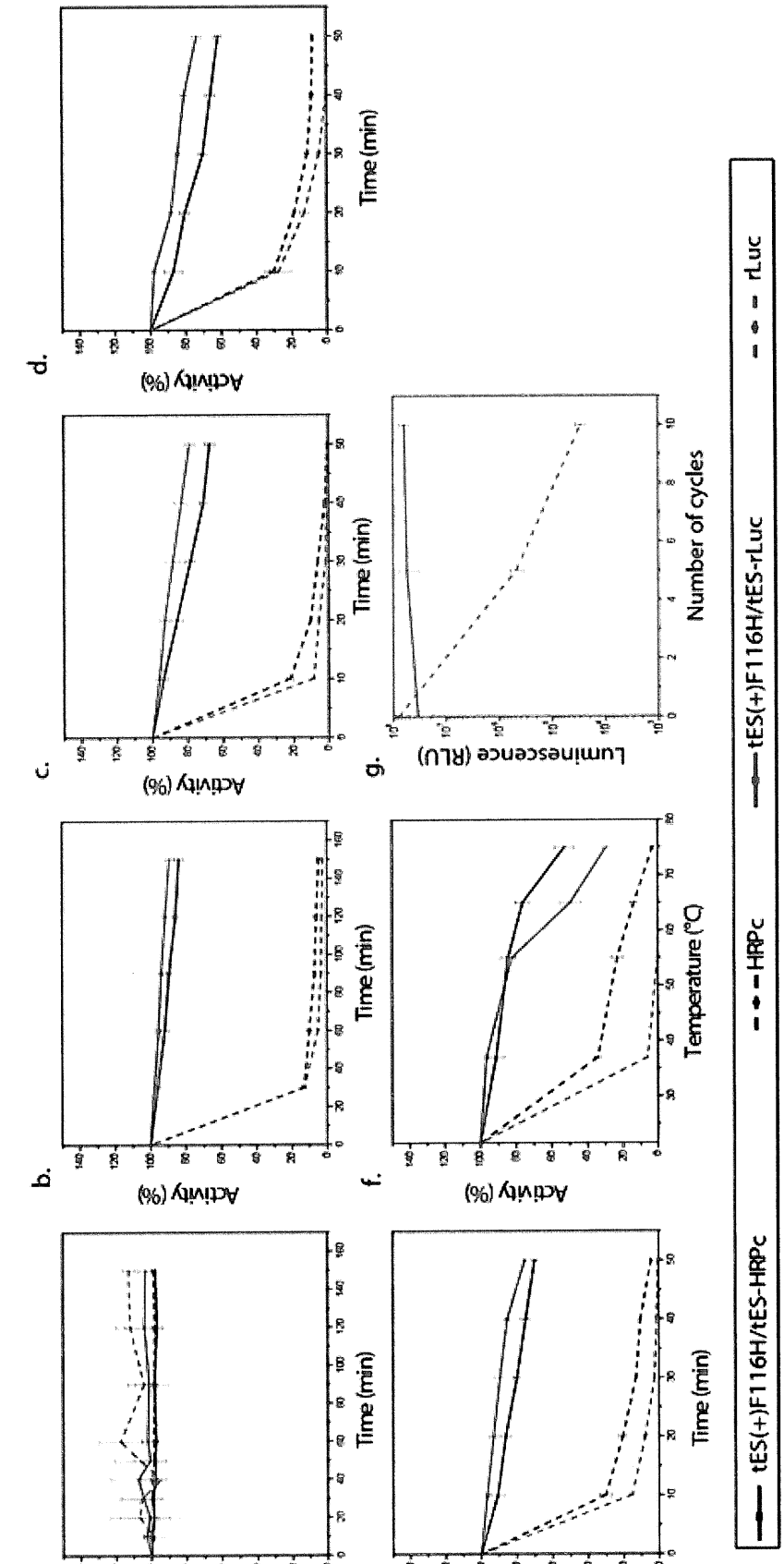


FIG. 6


a.
b.
c.
d.
e.
f.
g.
Activity (%)
Time (min)
Temperature (°C)
Luminescence (RLU)
Number of cycles
tES(+)F116H/tES-HRPc
HRPc
tES(+)F116H/tES-rLuc
rLuc

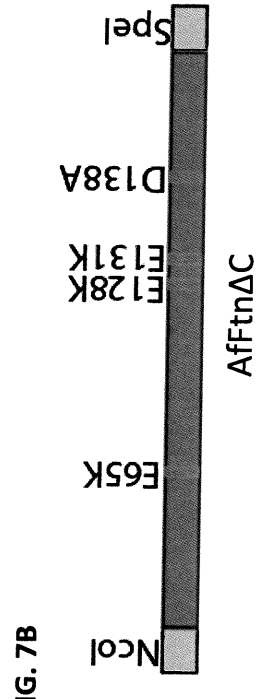


FIG. 7B

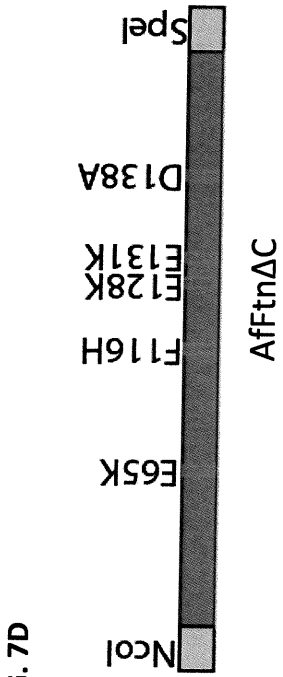


FIG. 7D

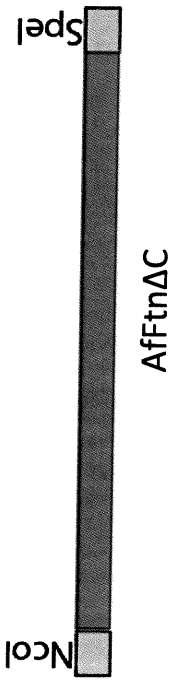


FIG. 7A

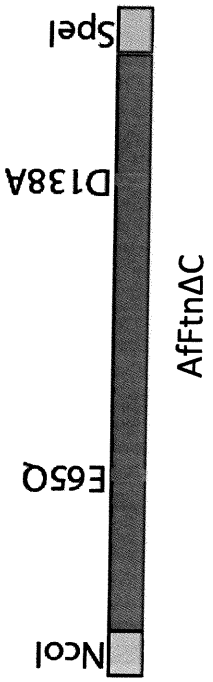


FIG. 7C

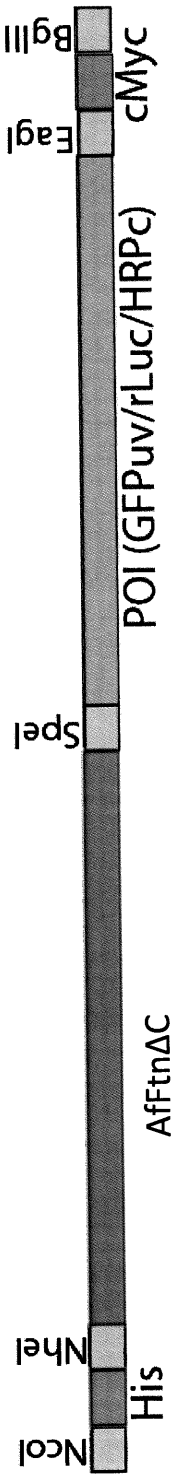

NcoI
His
NheI
AfFtnΔC
SpeI
POI (GFPuv/rLuc/HRPc)
EagI
cMyc
BglII

FIG. 7E

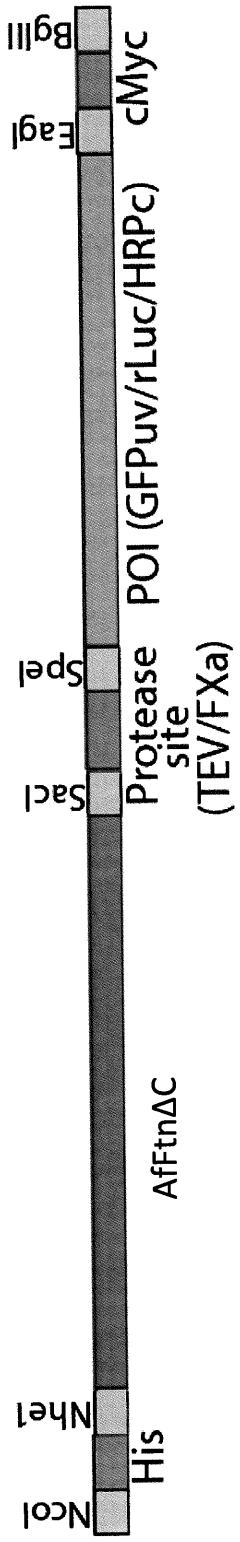

NcoI
His
NheI
AfFtnΔC
SacI
Protease site (TEV/FXa)
SpeI
POI (GFPuv/rLuc/HRPc)
EagI
cMyc
BglII

FIG. 7F

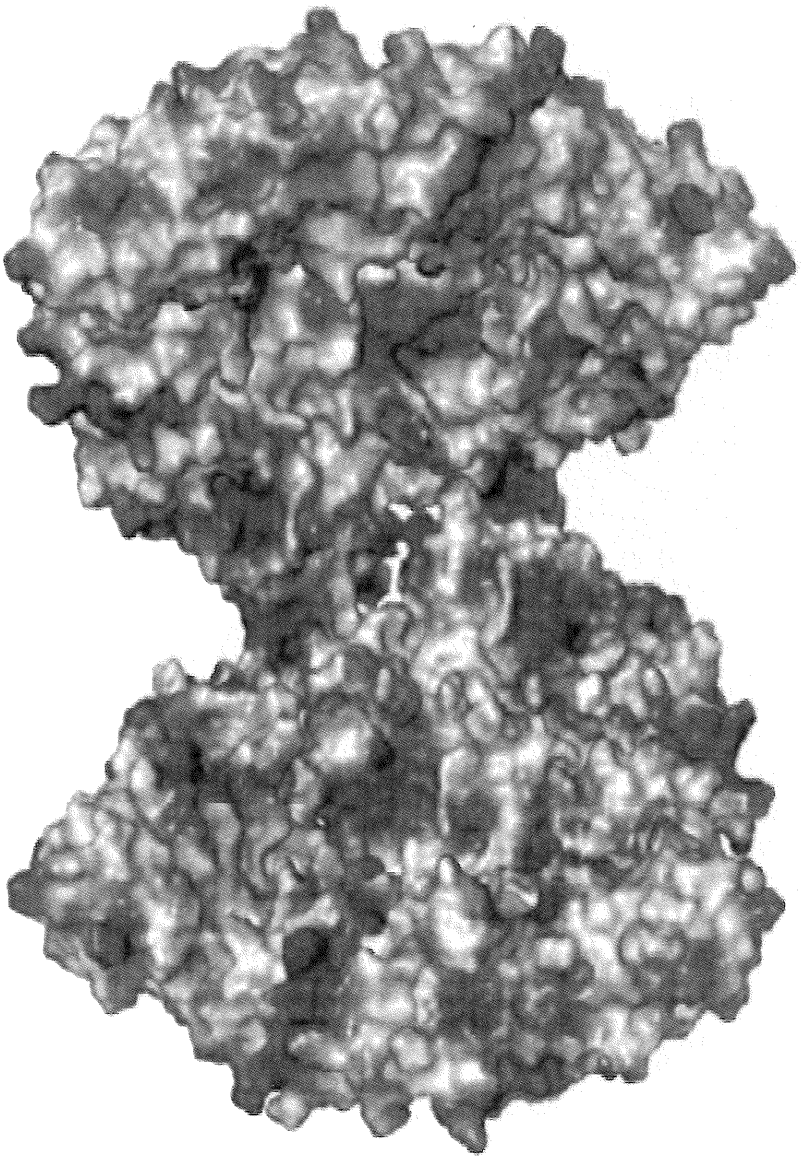


FIG. 8A

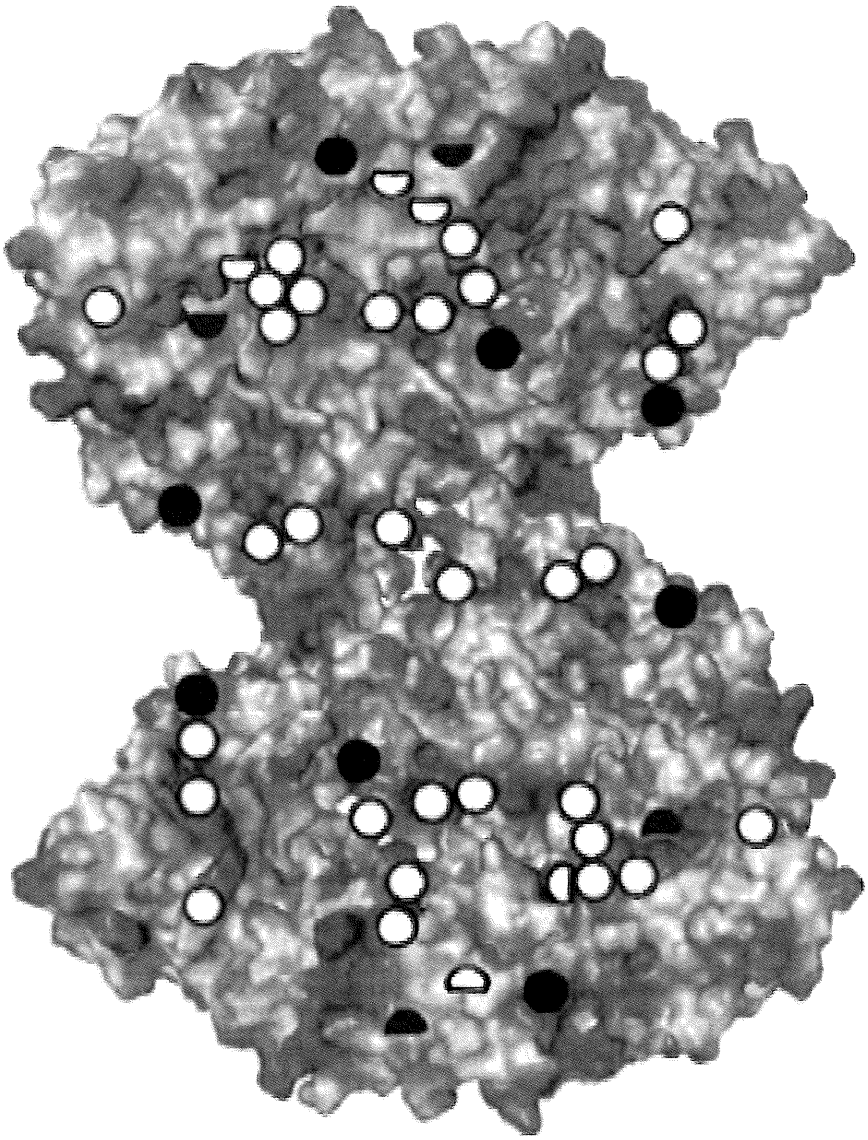


FIG. 8B

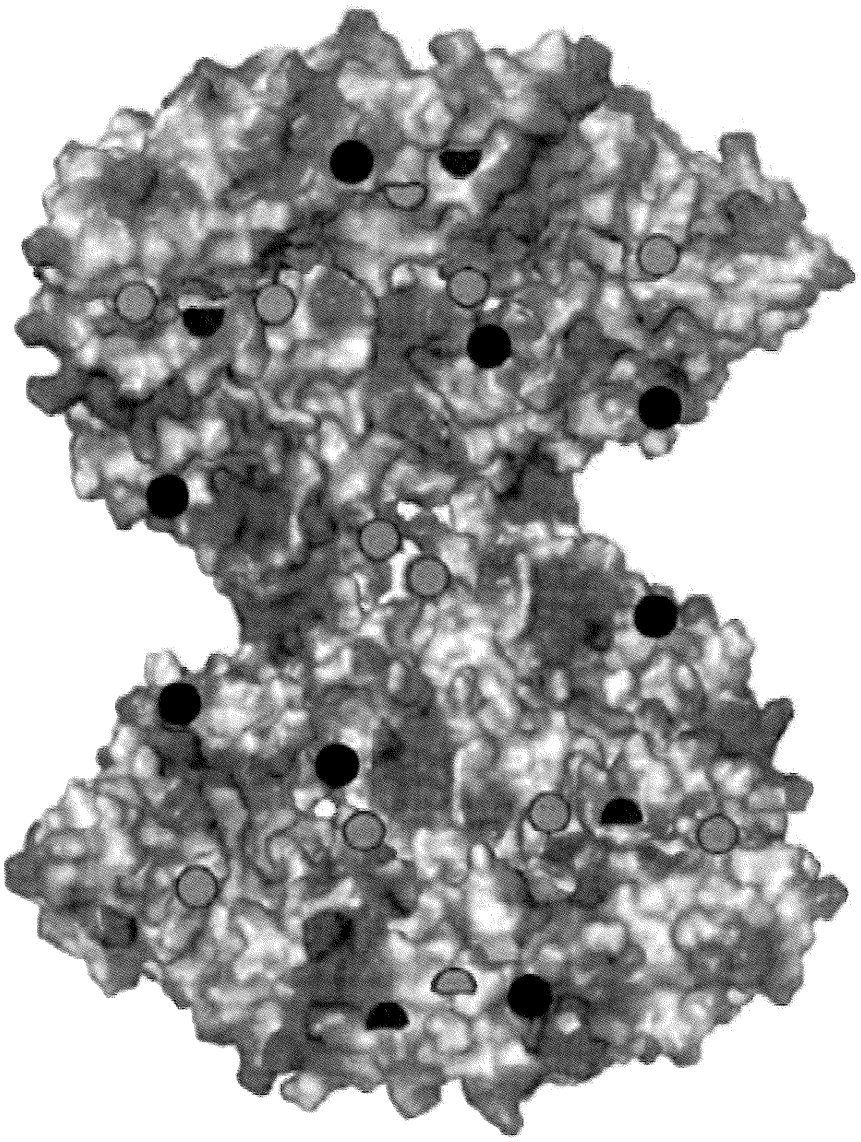


FIG. 8C

FIG. 9A

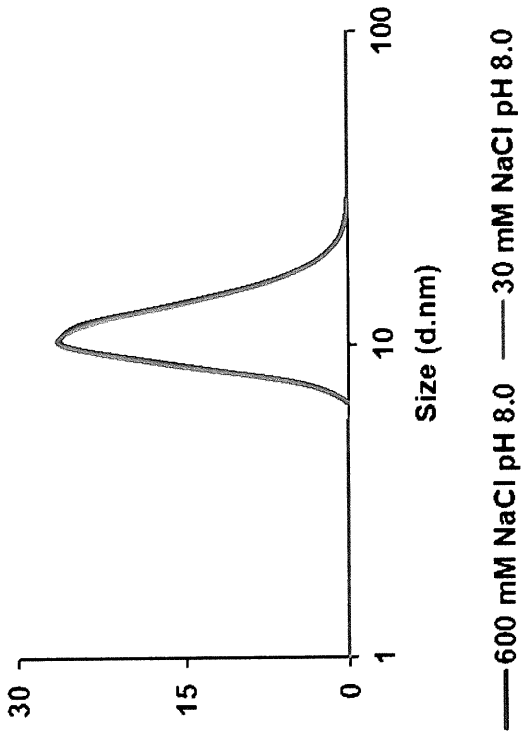

Number (percent)
30
15
0
1
10
100
Size (d.nm)
600 mM NaCl pH 8.0
30 mM NaCl pH 8.0

FIG. 9B

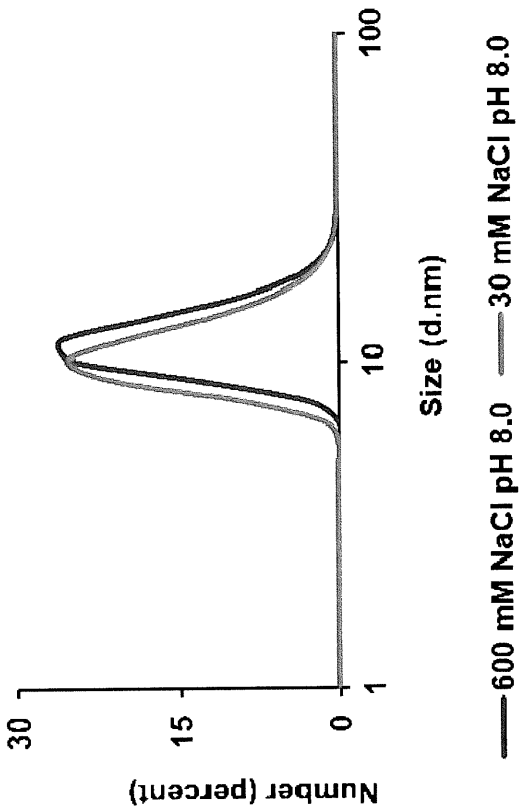

Number (percent)
30
15
0
1
10
100
Size (d.nm)
600 mM NaCl pH 8.0
30 mM NaCl pH 8.0

FIG. 9C

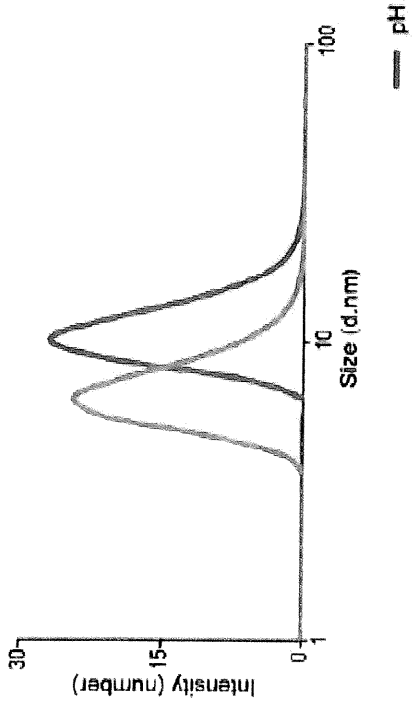


FIG. 9D

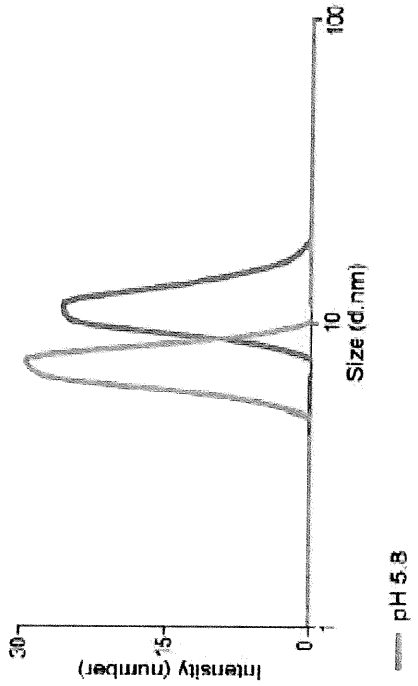


FIG. 9E

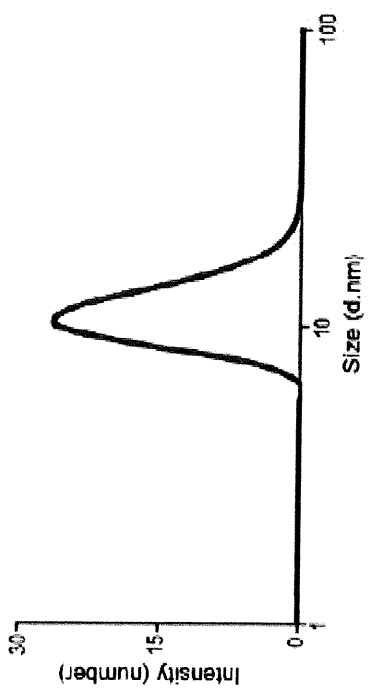


FIG. 9F

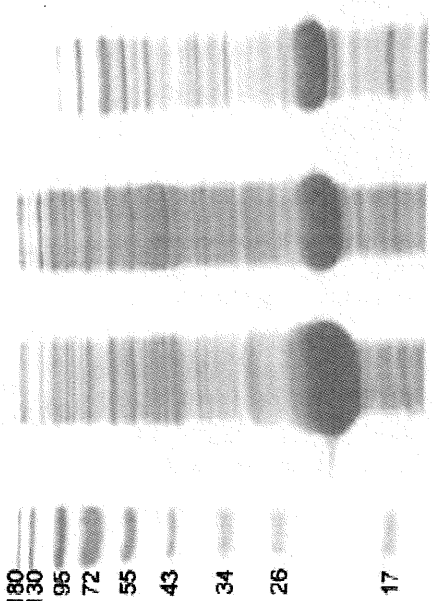
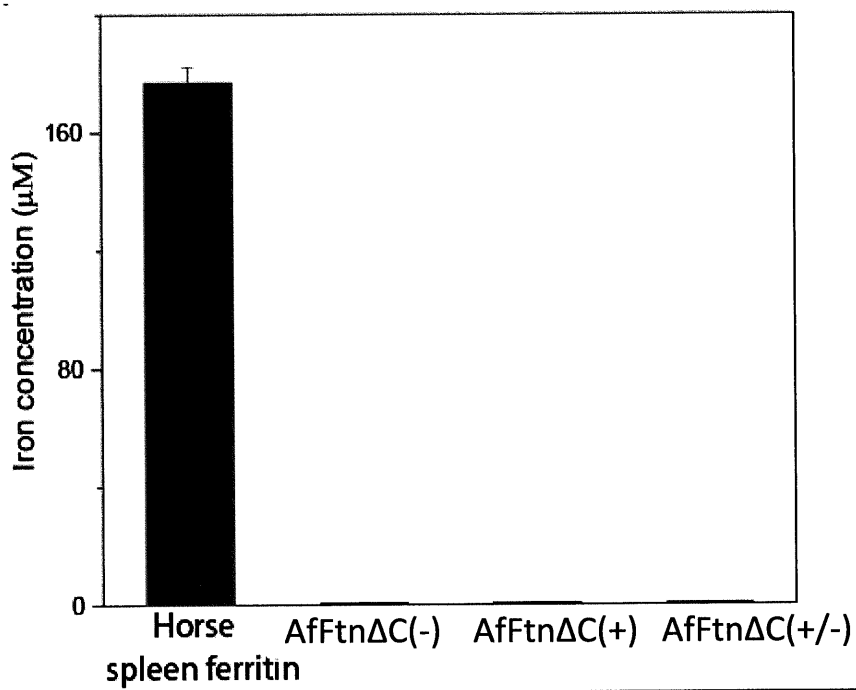
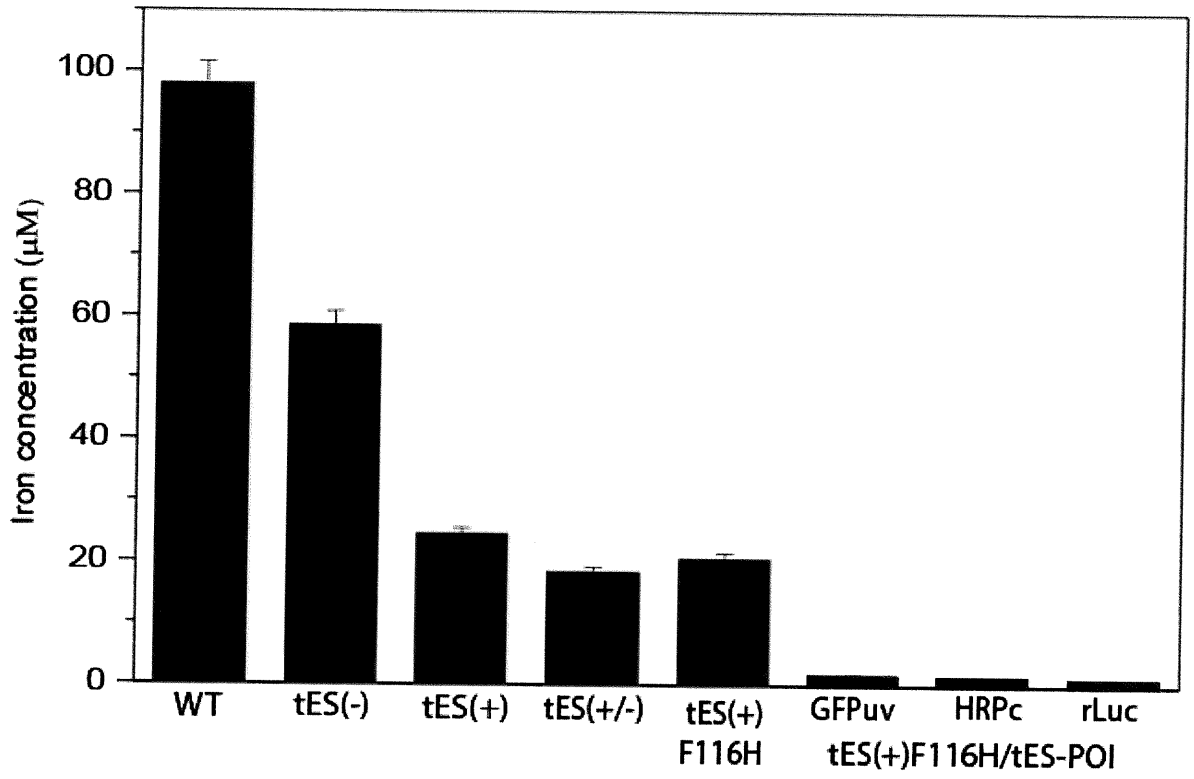


FIG. 10A



	Iron concentration (μM)
Horse spleen ferritin	176.98 ± 5.32
AfFtnΔC(-)	0.40 ± 0.019
AfFtnΔC(+)	0.39 ± 0.038
AfFtnΔC(+/-)	0.26 ± 0.034

FIG. 10B



	Iron concentration (μM)
WT	97.97 ± 3.51
tES(-)	58.61 ± 2.21
tES(+)	24.65 ± 0.90
tES(+/-)	18.45 ± 0.85
tES(+)F116H	20.66 ± 0.88
tES(+)F116H/tES-GFPuv	1.73 ± 0.09
tES(+)F116H/tES-HRPc	1.61 ± 0.02
tES(+)F116H/tES-rLuc	1.20 ± 0.03

FIG. 11A

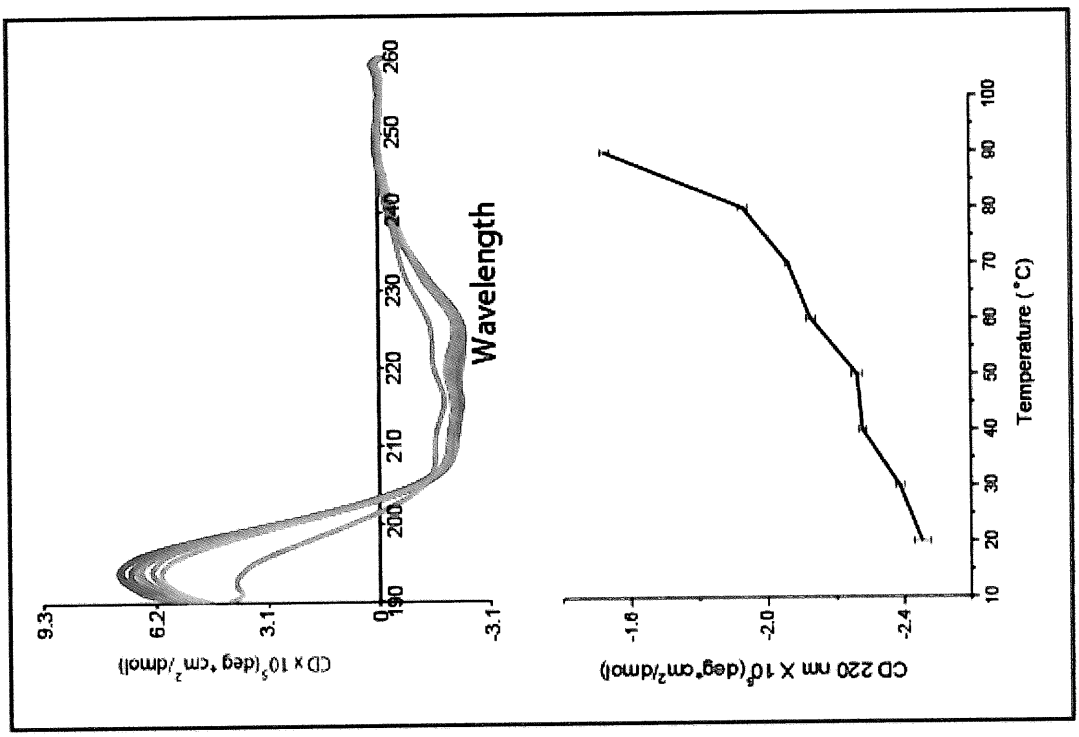


FIG. 11B

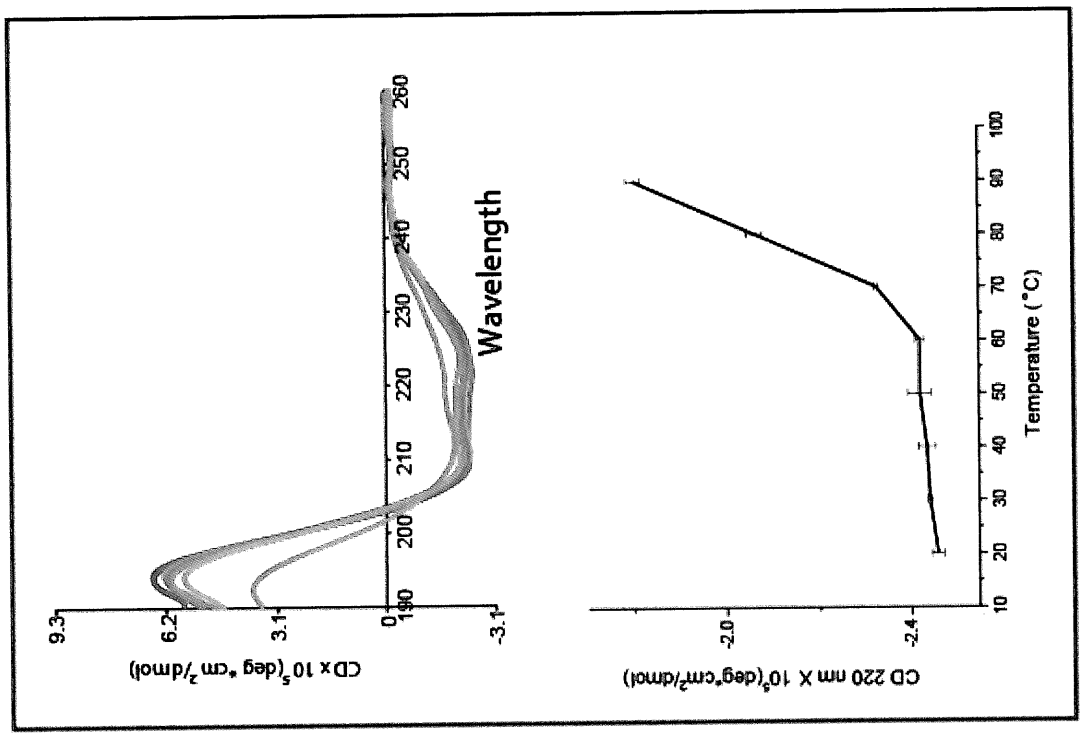
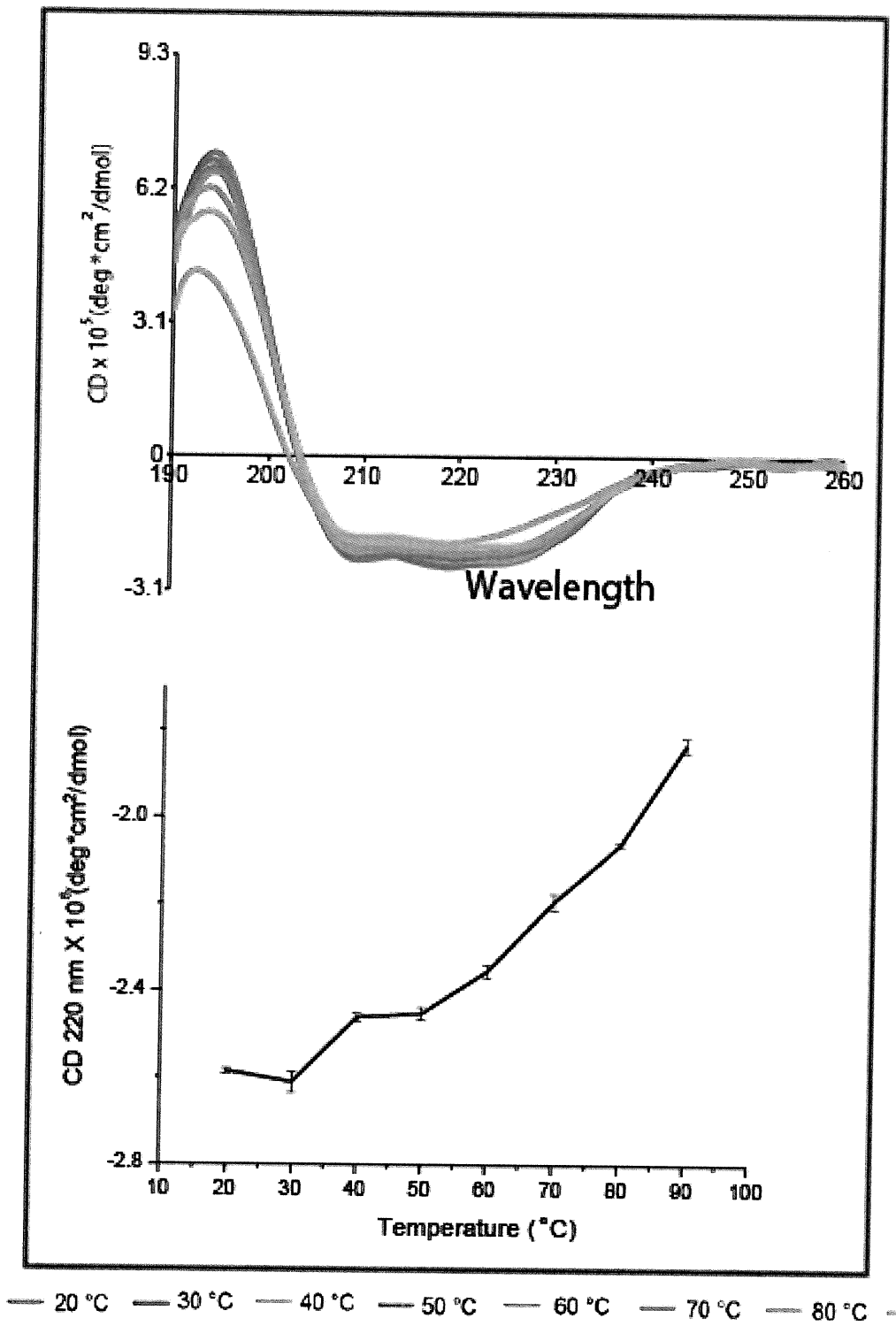


FIG. 11C


9.3
6.2
3.1
0
-3.1
CD x 10^5(deg*cm^2/dmol)
190
200
210
220
230
240
250
260
Wavelength
CD 220 nm X 10^5(deg*cm^2/dmol)
-2.0
-2.4
-2.8
10
20
30
40
50
60
70
80
90
100
Temperature (°C)
20 °C
30 °C
40 °C
50 °C
60 °C
70 °C
80 °C
90 °C

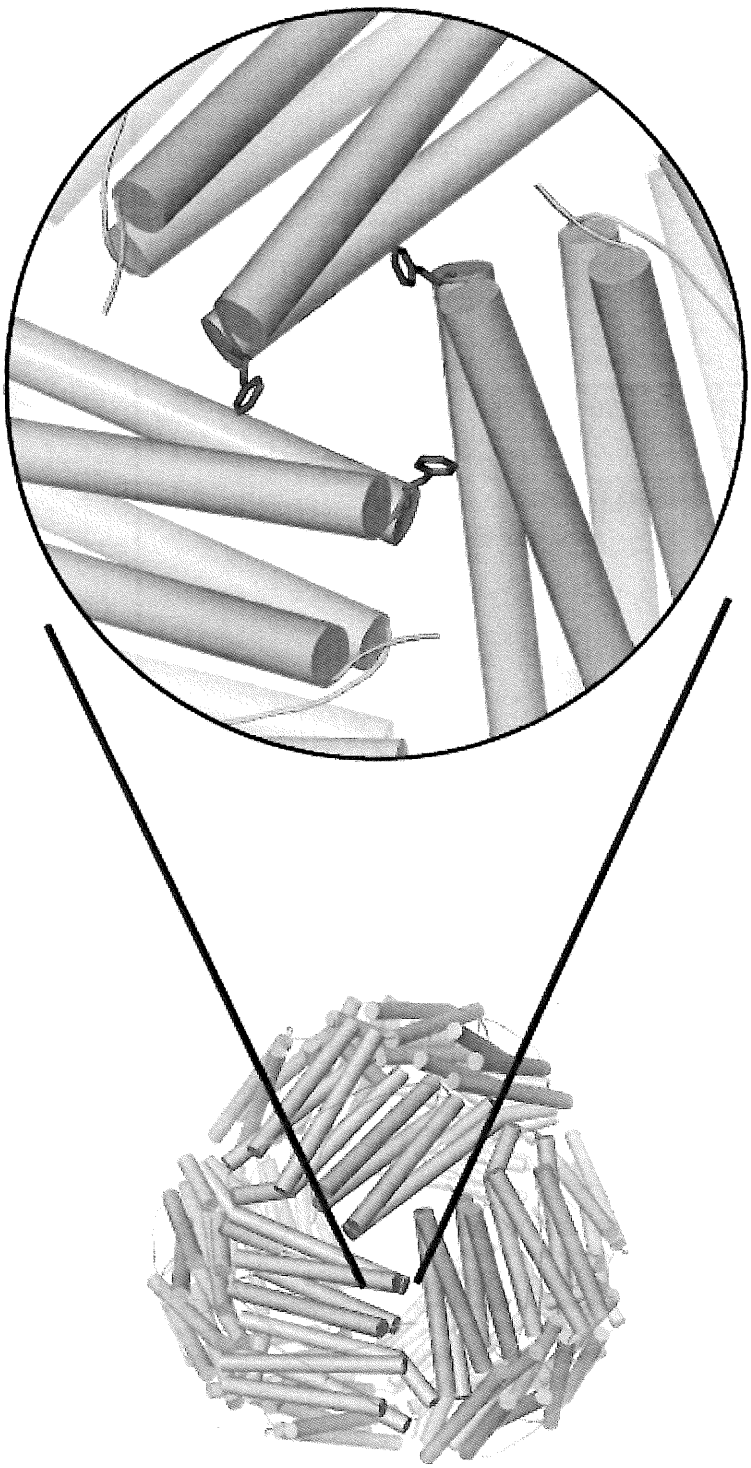


FIG. 12A

FIG. 12B

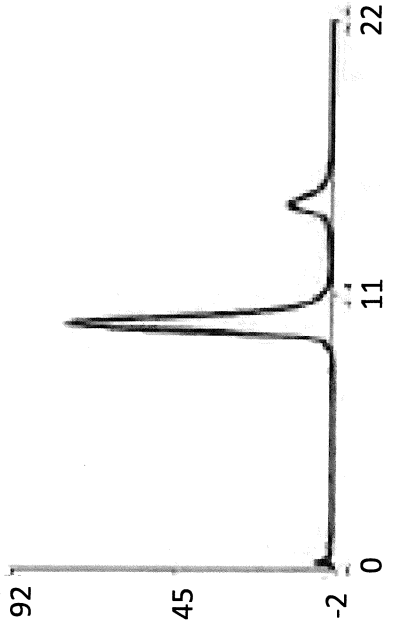


FIG. 12C

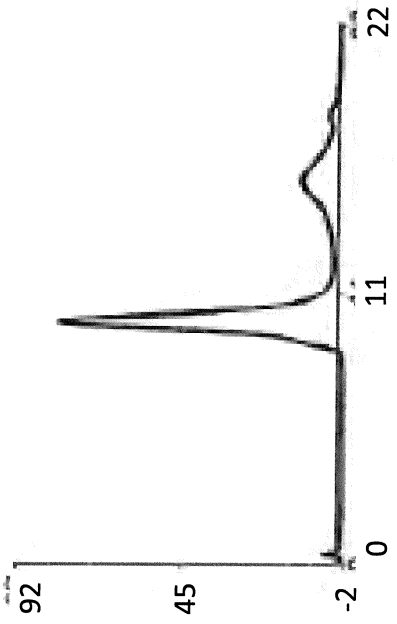


FIG. 12D

FIG. 12E

Absorbance (280 nm)

FIG. 12F

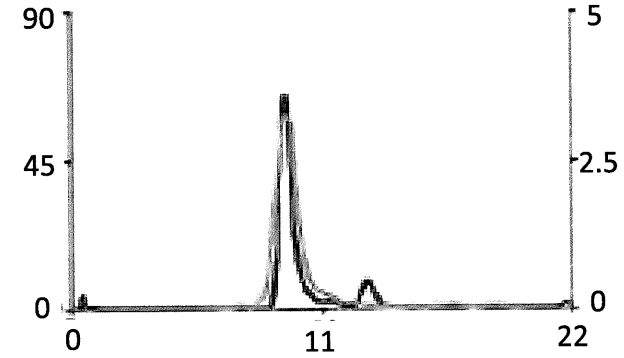


FIG. 12G

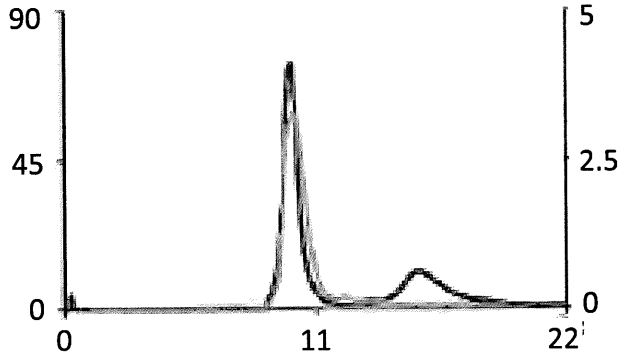


FIG. 12H

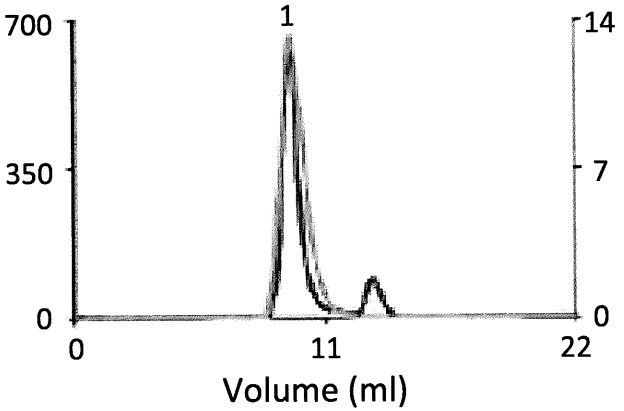
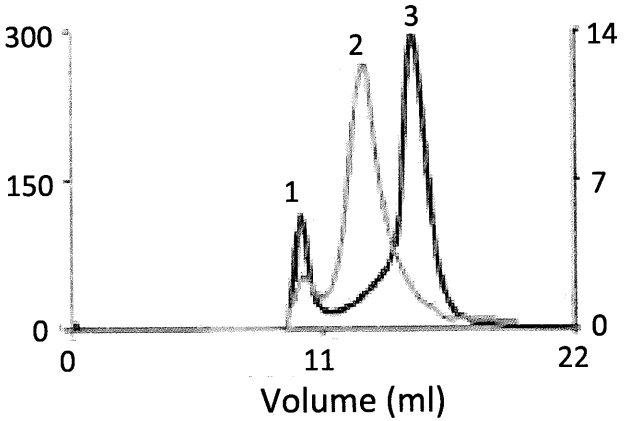


FIG. 12I



Absorbance (280 nm)

Fluorescence (509 nm) x 10^3

FIG. 13A

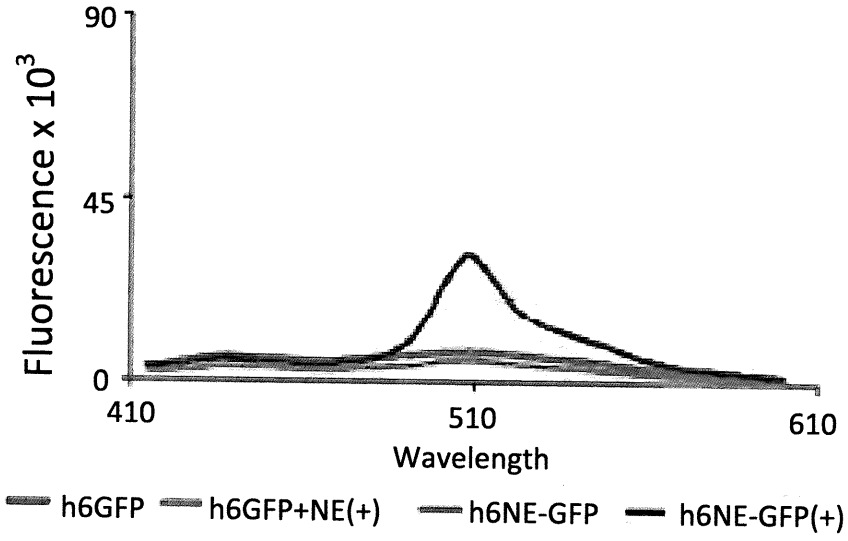


FIG. 13B

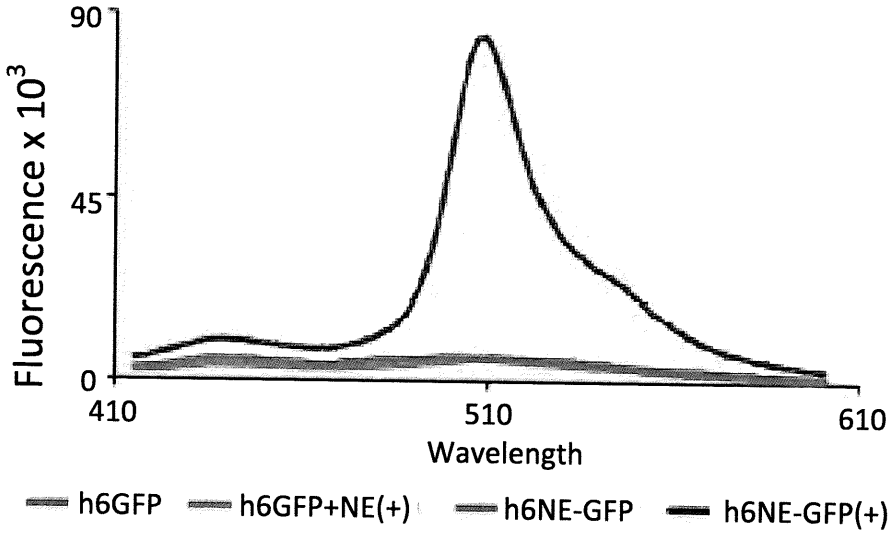


FIG. 13C

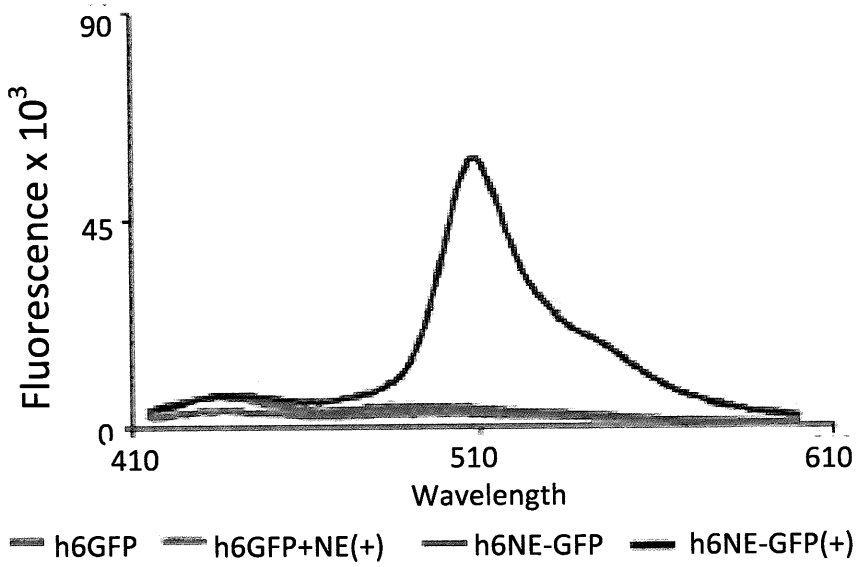


FIG. 13D

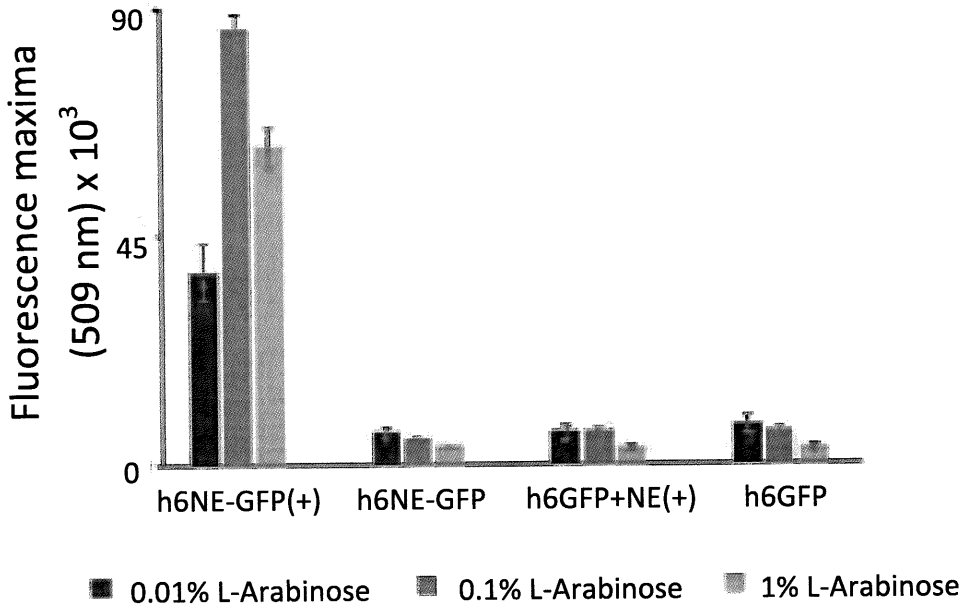


FIG. 14

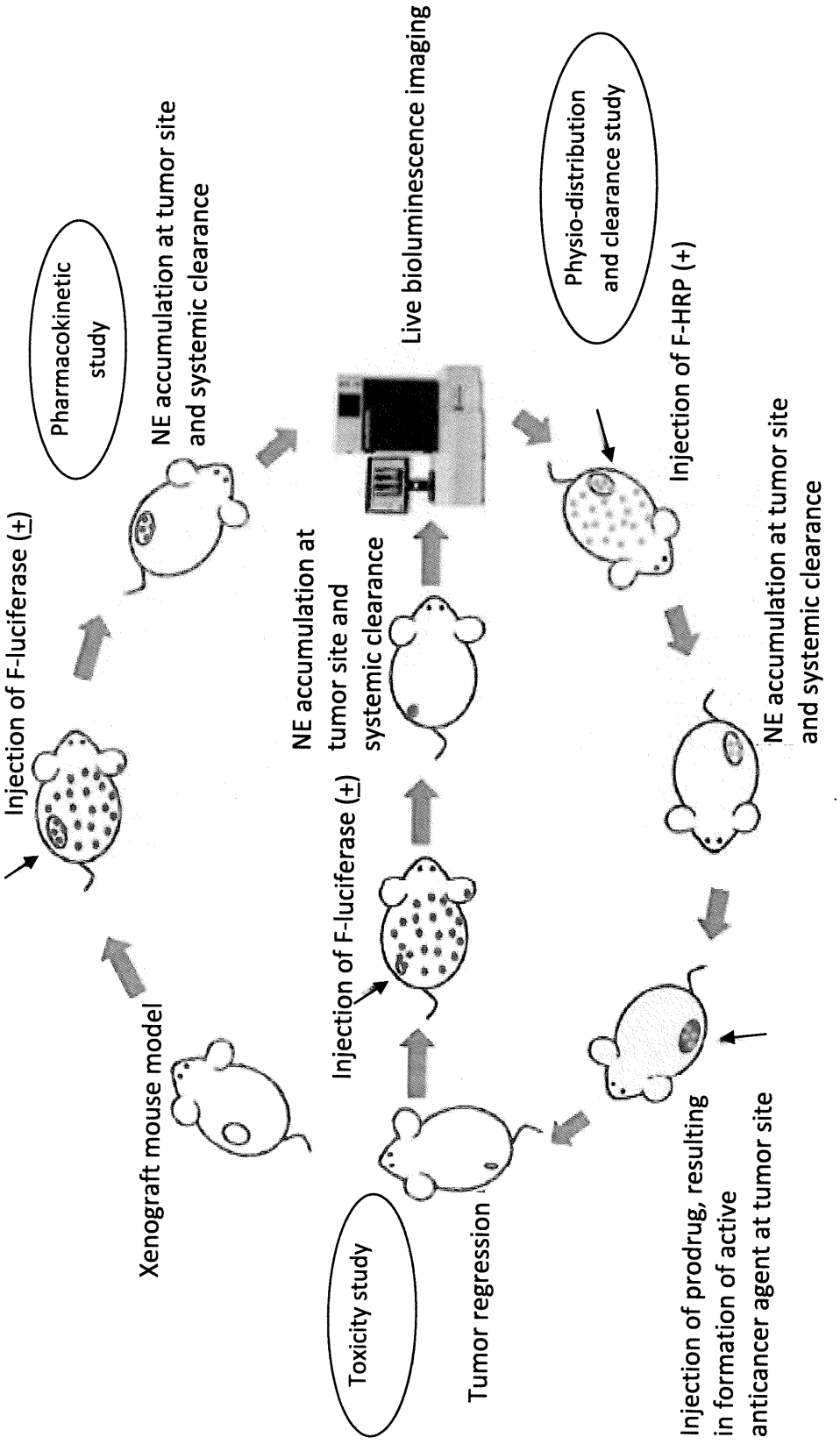

Xenograft mouse model
Injection of F-luciferase (±)
Pharmacokinetic study
NE accumulation at tumor site and systemic clearance
Injection of F-luciferase (±)
NE accumulation at tumor site and systemic clearance
Live bioluminescence imaging
Toxicity study
Tumor regression
Injection of prodrug, resulting in formation of active anticancer agent at tumor site
NE accumulation at tumor site and systemic clearance
Injection of F-HRP (+)
Physio-distribution and clearance study

FIG. 15A

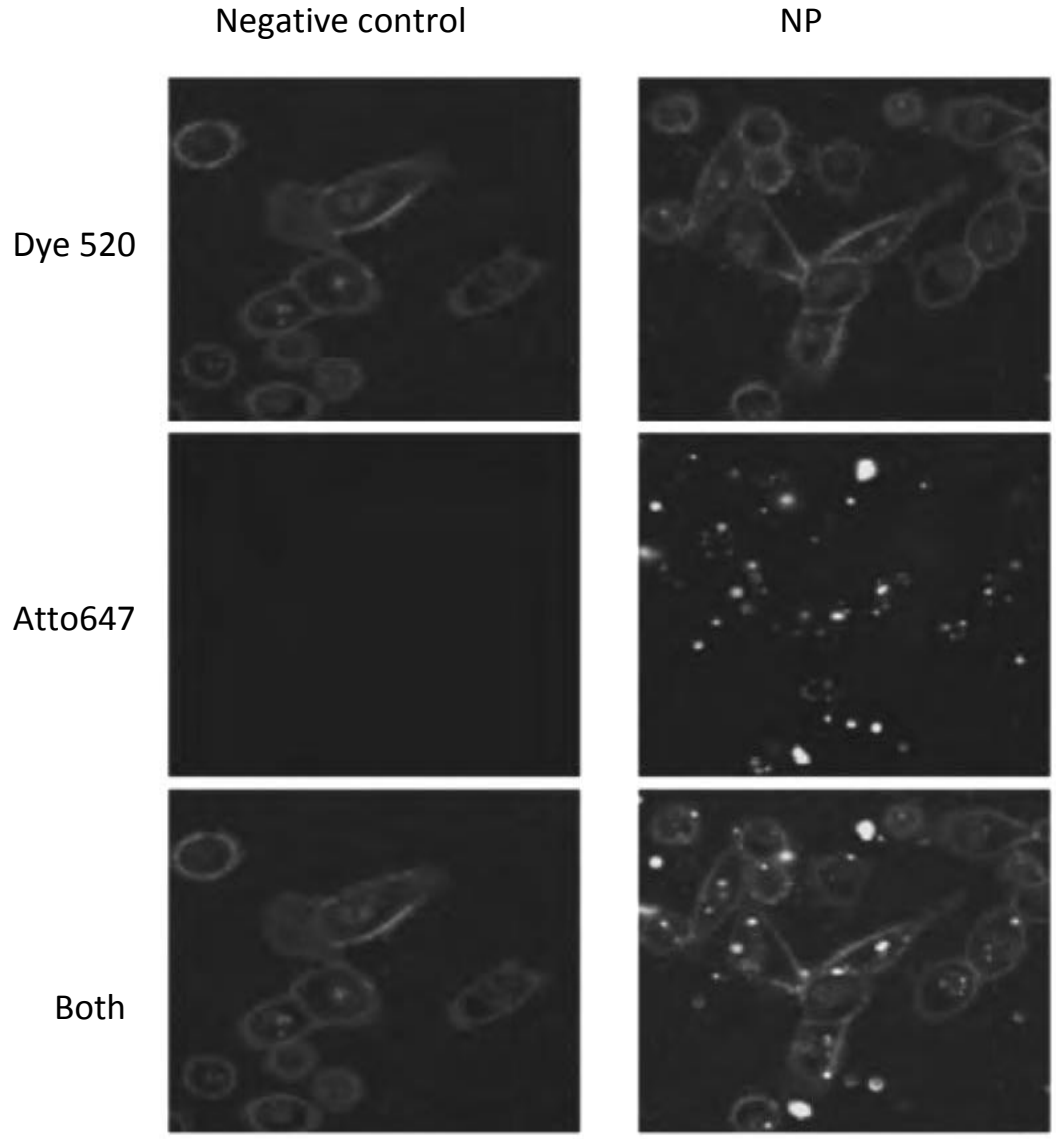


FIG. 15B

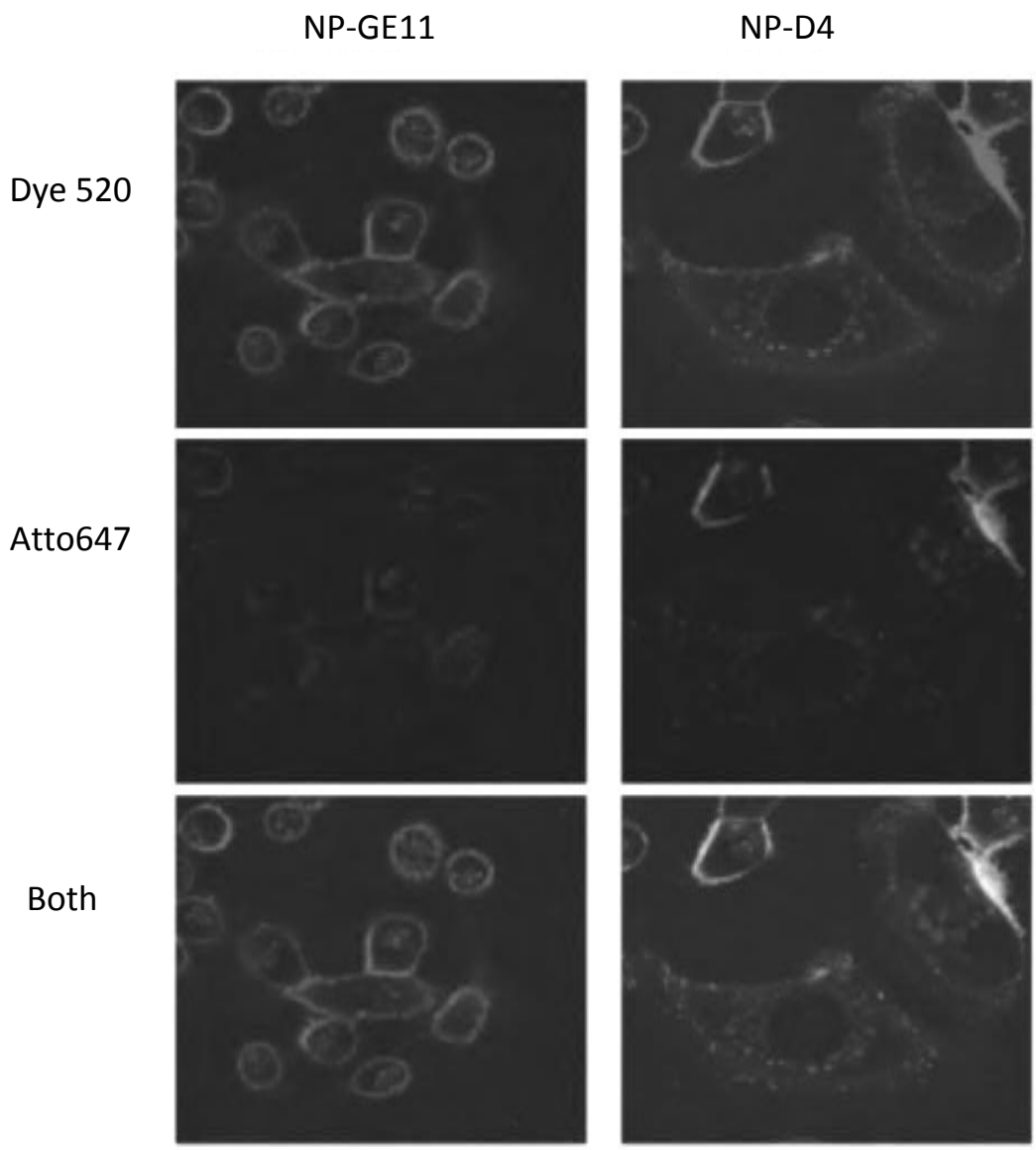


FIG. 16A

a.

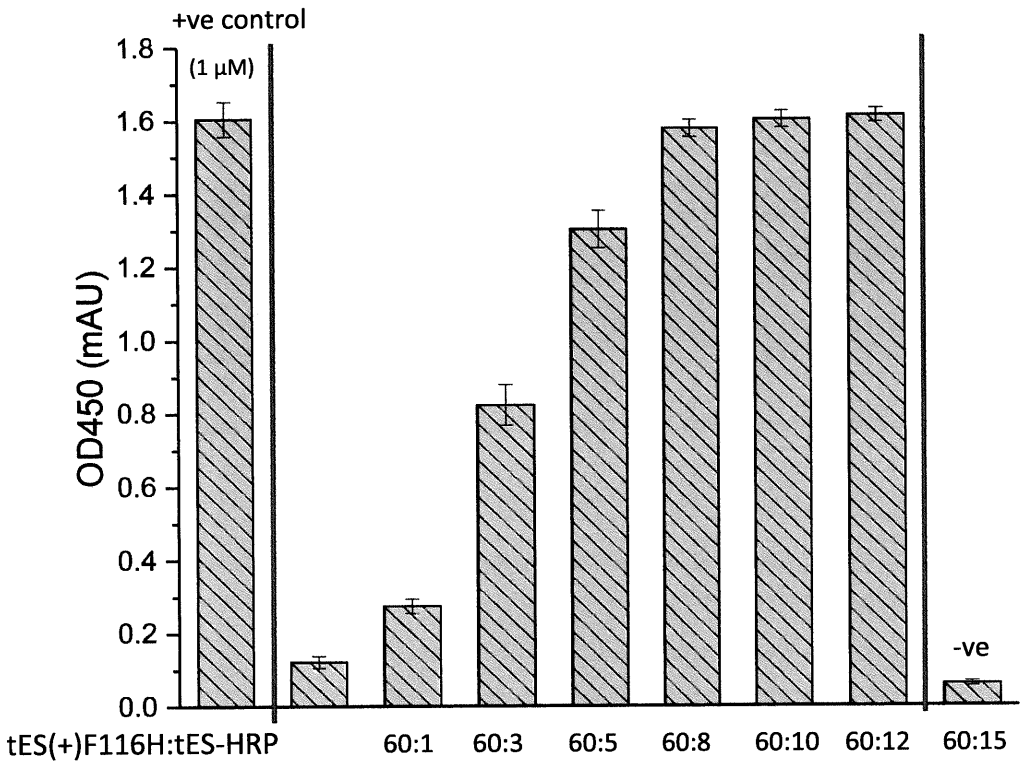


FIG. 16B

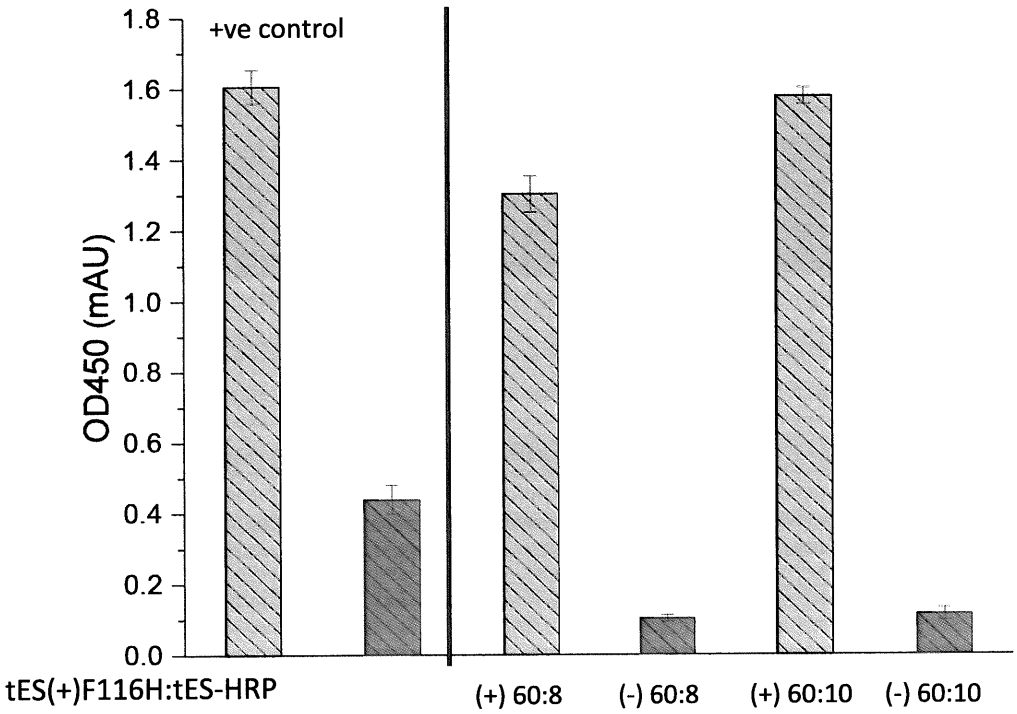


FIG. 17A

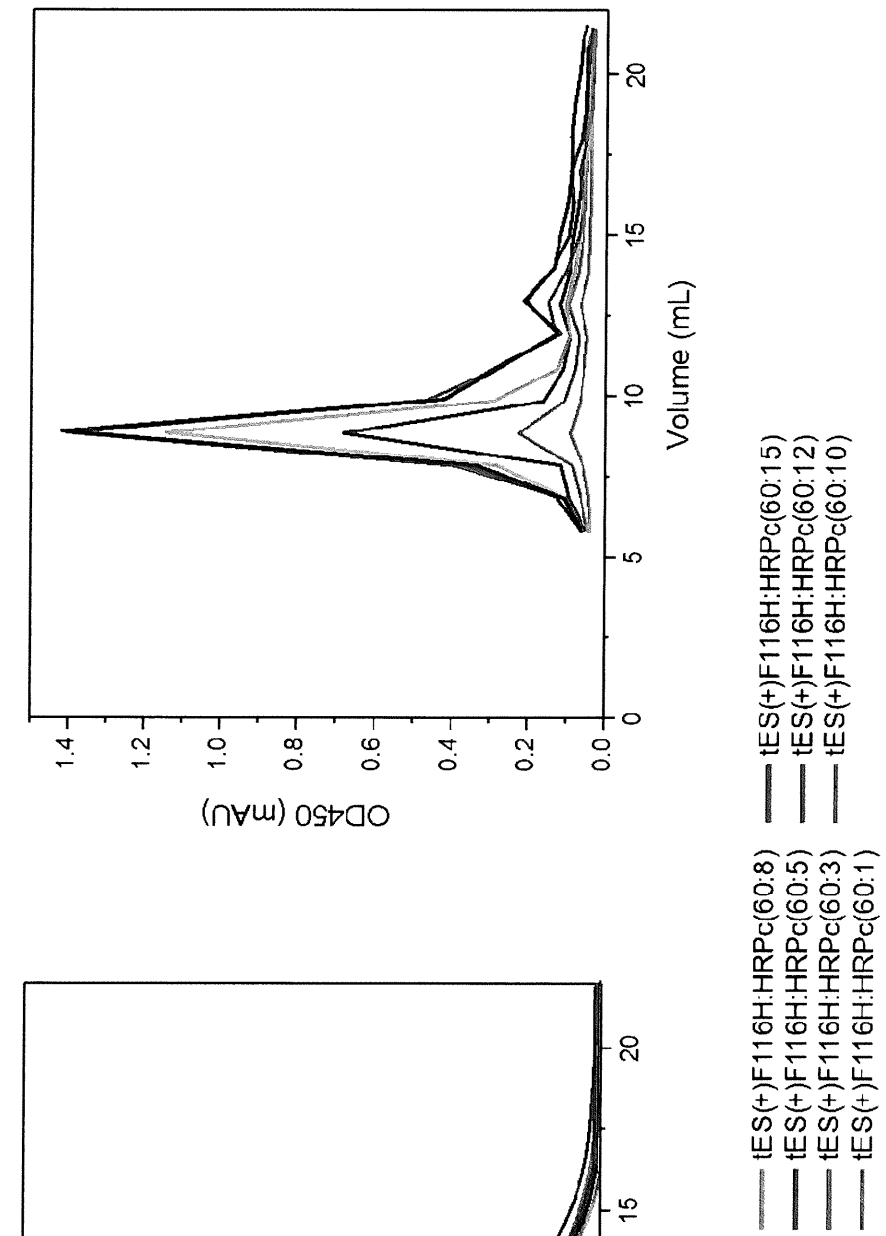

OD280 (mAU)
Volume (mL)
OD450 (mAU)
Volume (mL)
tES(+)F116H:HRPc(60:8)
tES(+)F116H:HRPc(60:5)
tES(+)F116H:HRPc(60:3)
tES(+)F116H:HRPc(60:1)
tES(+)F116H:HRPc(60:15)
tES(+)F116H:HRPc(60:12)
tES(+)F116H:HRPc(60:10)

FIG. 17B

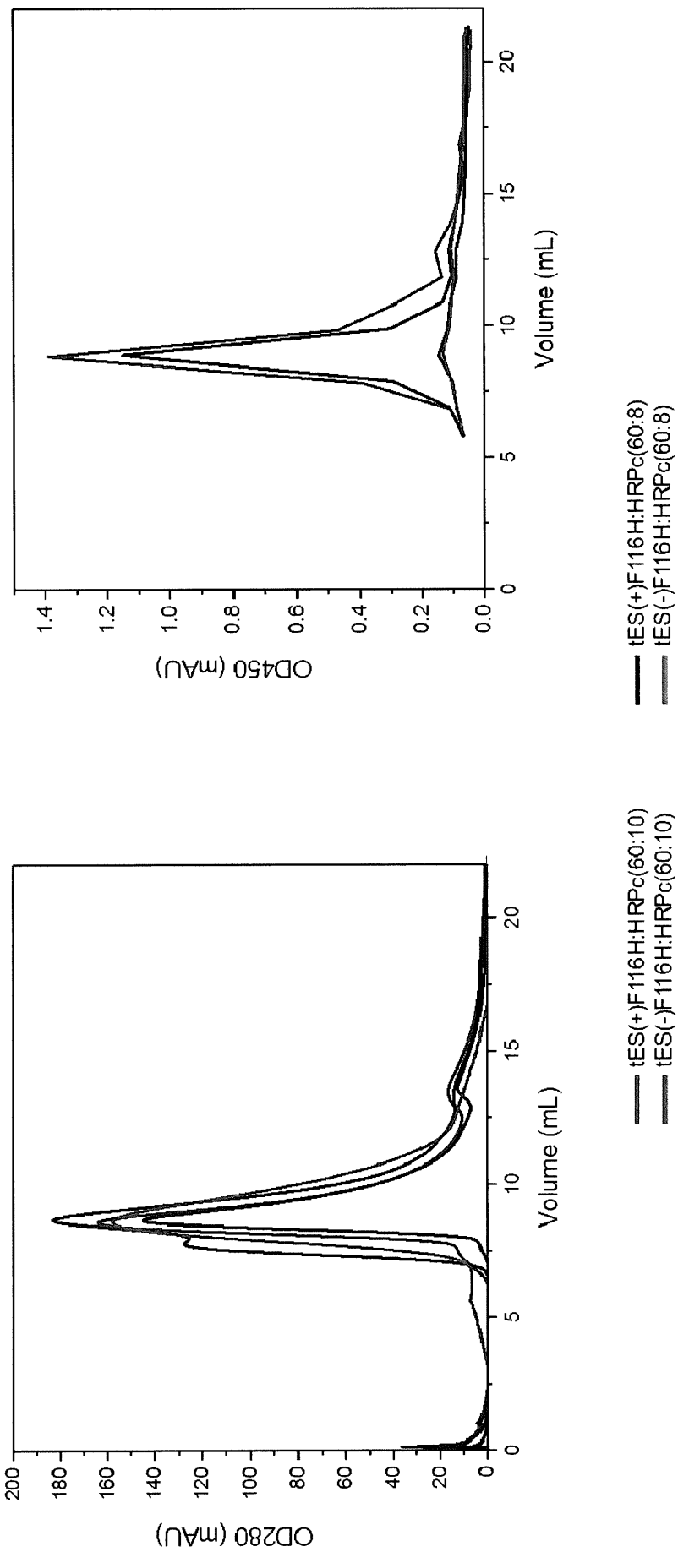

OD280 (mAU)
Volume (mL)
tES(+)F116H:HRPc(60:10)
tES(-)F116H:HRPc(60:10)
OD450 (mAU)
Volume (mL)
tES(+)F116H:HRPc(60:8)
tES(-)F116H:HRPc(60:8)

FIG. 17C

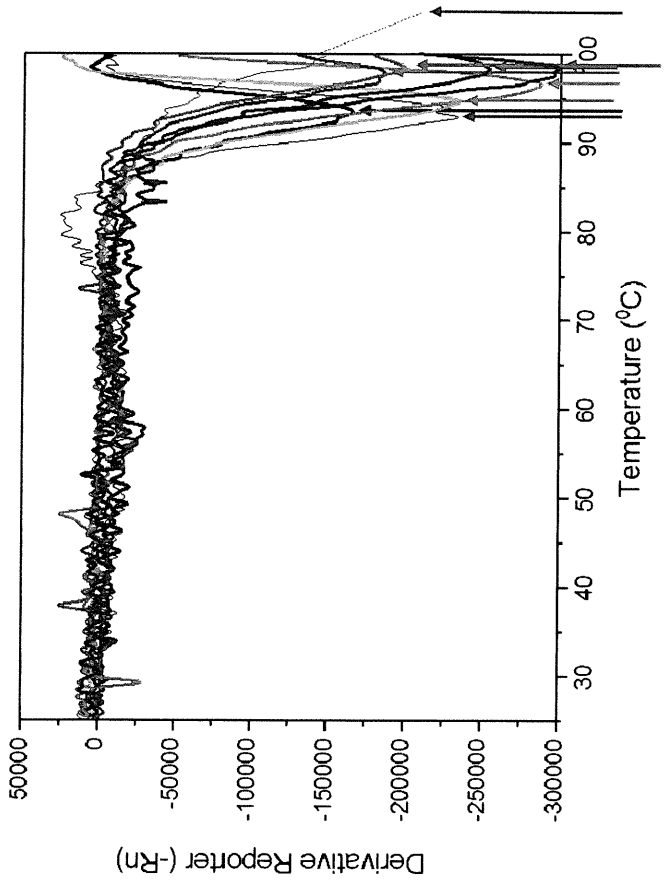


FIG. 17D

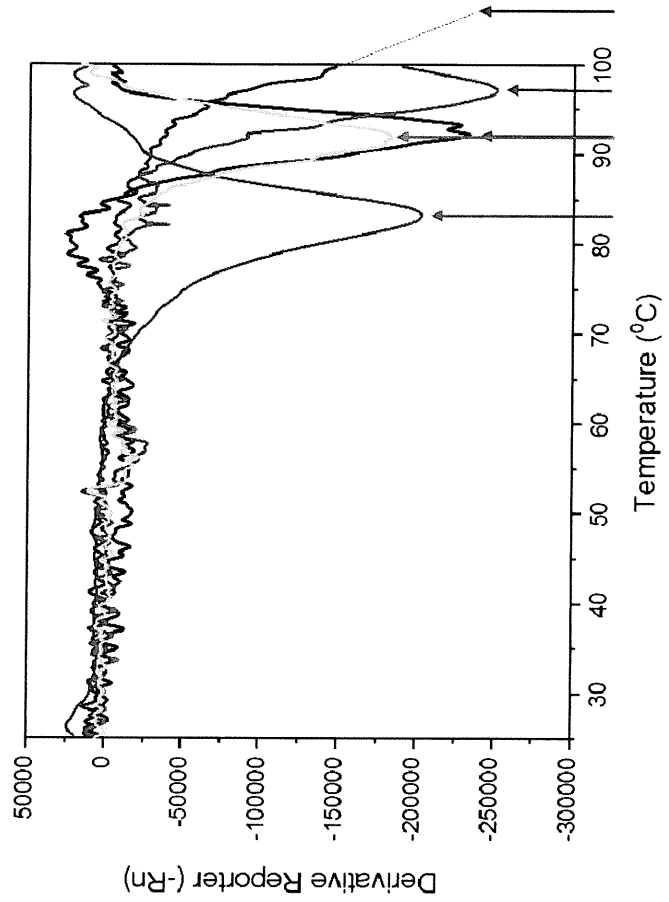


FIG. 18A

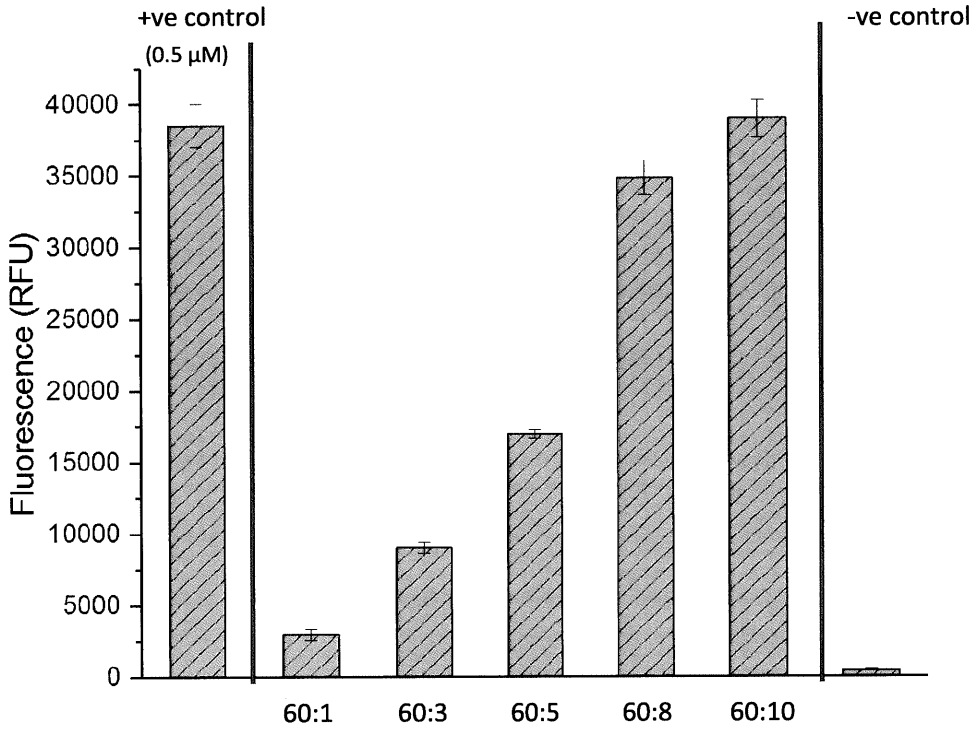
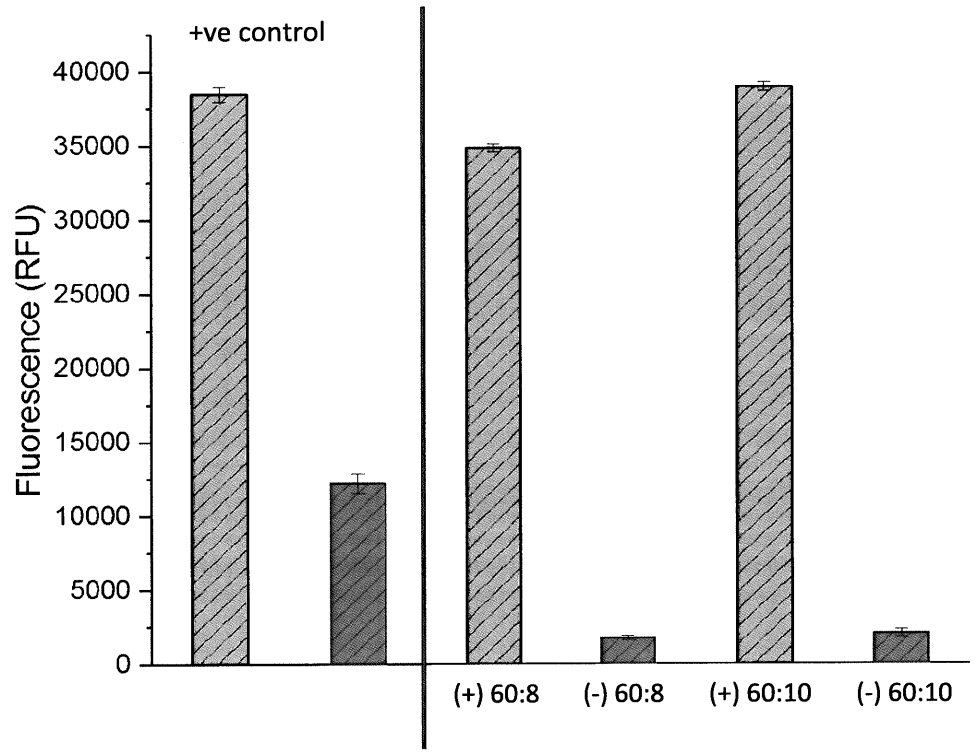


FIG. 18B



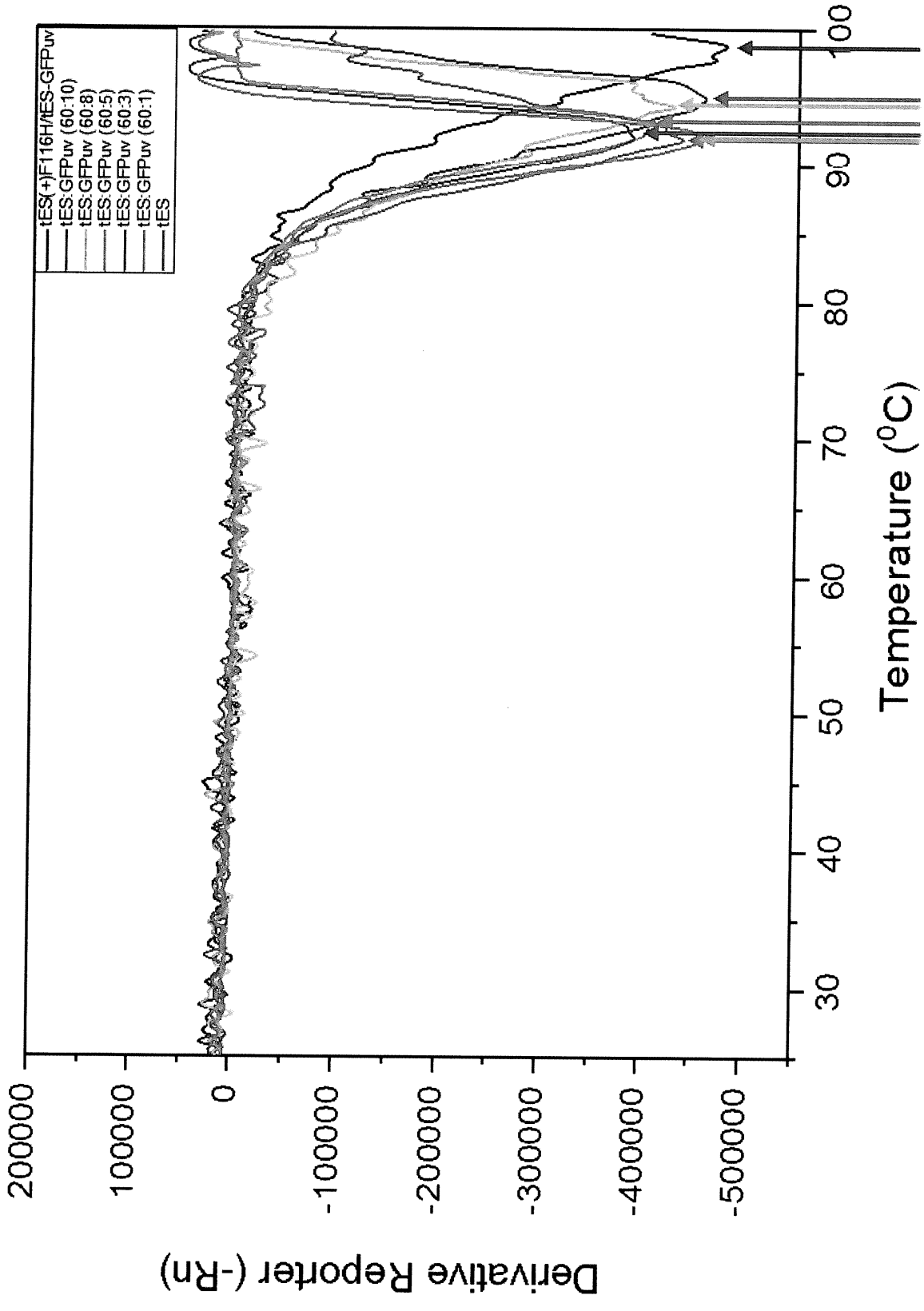

tES(+)F116H/tES-GFPuv
tES:GFPuv (60:10)
tES:GFPuv (60:8)
tES:GFPuv (60:5)
tES:GFPuv (60:3)
tES:GFPuv (60:1)
tES
200000
100000
0
-100000
-200000
-300000
-400000
-500000
Derivative Reporter (-Rn)
30
40
50
60
70
80
90
100
Temperature (°C)

FIG. 18C

FIG. 18D

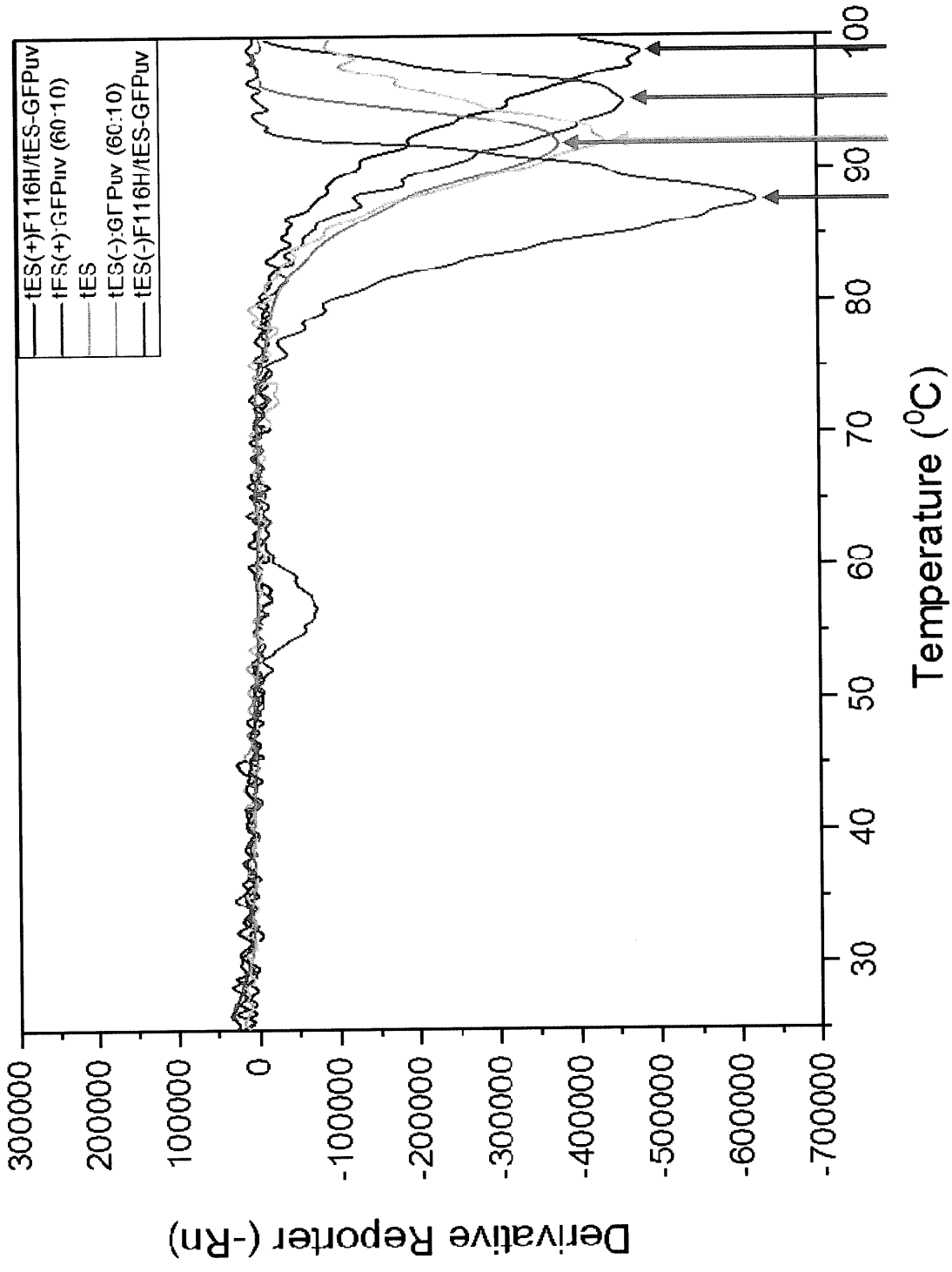

tES(+)F116H/tES-GFPuv
tES(+)GFPuv (60:10)
tES
tES(-)GFPuv (60:10)
tES(-)F116H/tES-GFPuv
300000
200000
100000
0
-100000
-200000
-300000
-400000
-500000
-600000
-700000
Derivative Reporter (-Rn)
30
40
50
60
70
80
90
100
Temperature (°C)

FIG. 19

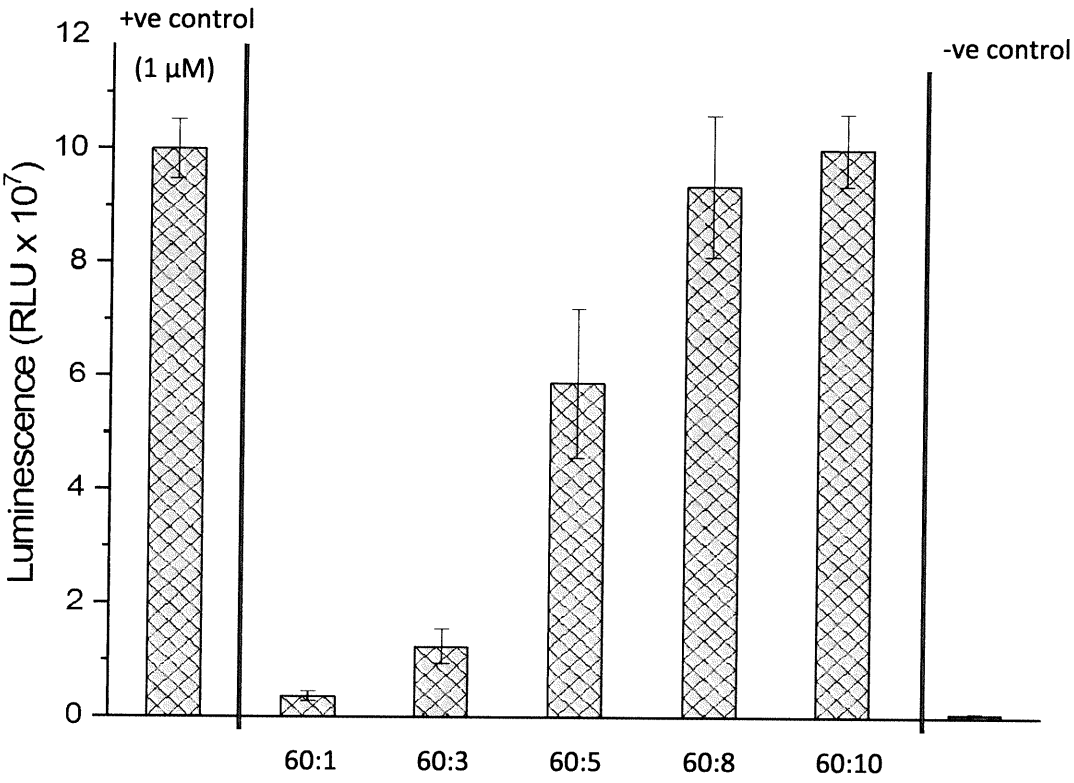

+ve control
(1 µM)
-ve control
Luminescence (RLU x 10^7)
12
10
8
6
4
2
0
60:1
60:3
60:5
60:8
60:10

FIG. 20

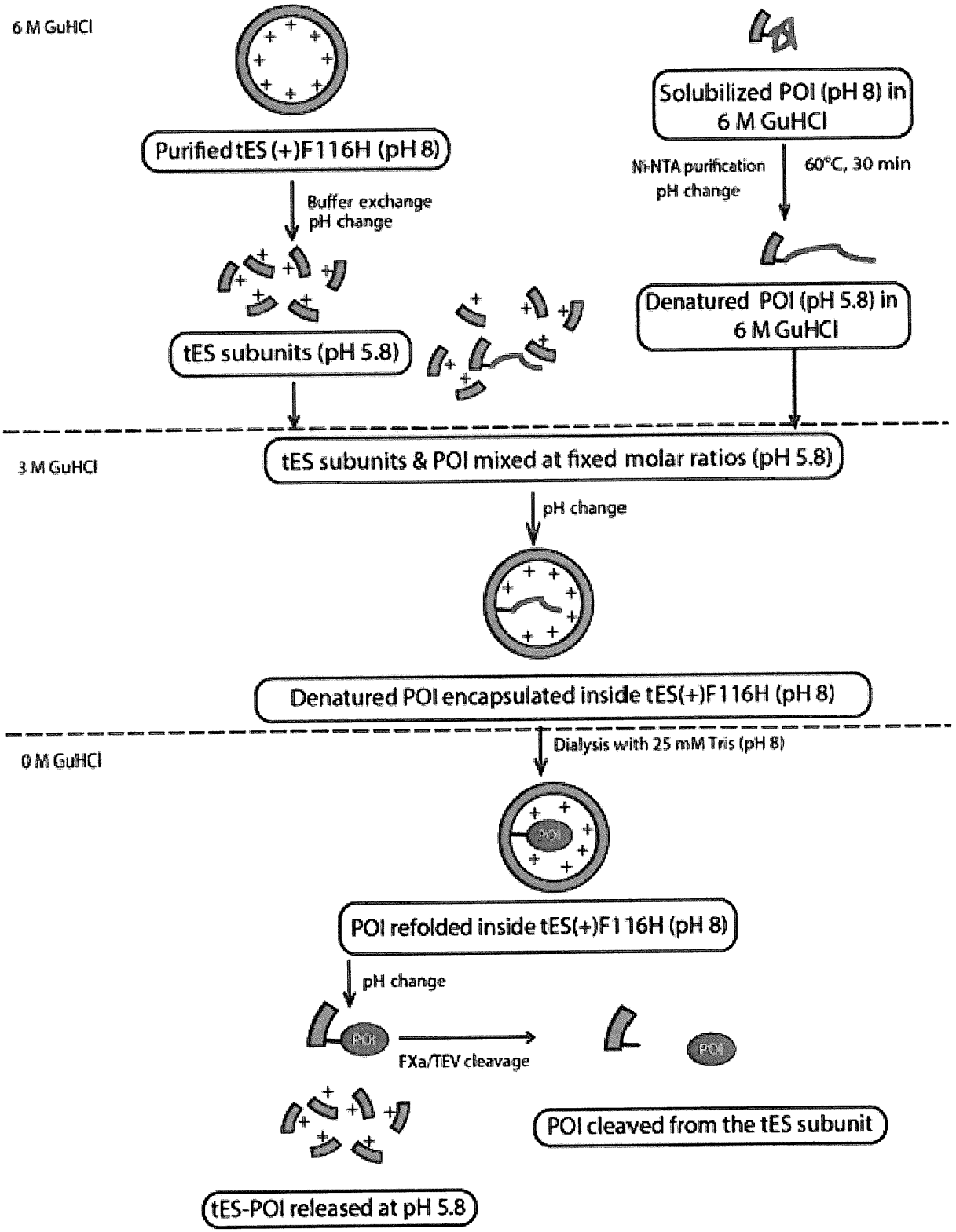

6 M GuHCl
Purified tES (+)F116H (pH 8)
Buffer exchange
pH change
tES subunits (pH 5.8)
Solubilized POI (pH 8) in
6 M GuHCl
Ni-NTA purification
pH change
60°C, 30 min
Denatured POI (pH 5.8) in
6 M GuHCl
3 M GuHCl
tES subunits & POI mixed at fixed molar ratios (pH 5.8)
pH change
Denatured POI encapsulated inside tES(+)F116H (pH 8)
0 M GuHCl
Dialysis with 25 mM Tris (pH 8)
POI
POI refolded inside tES(+)F116H (pH 8)
pH change
POI
FXa/TEV cleavage
POI
POI cleaved from the tES subunit
tES-POI released at pH 5.8

FIG. 21A

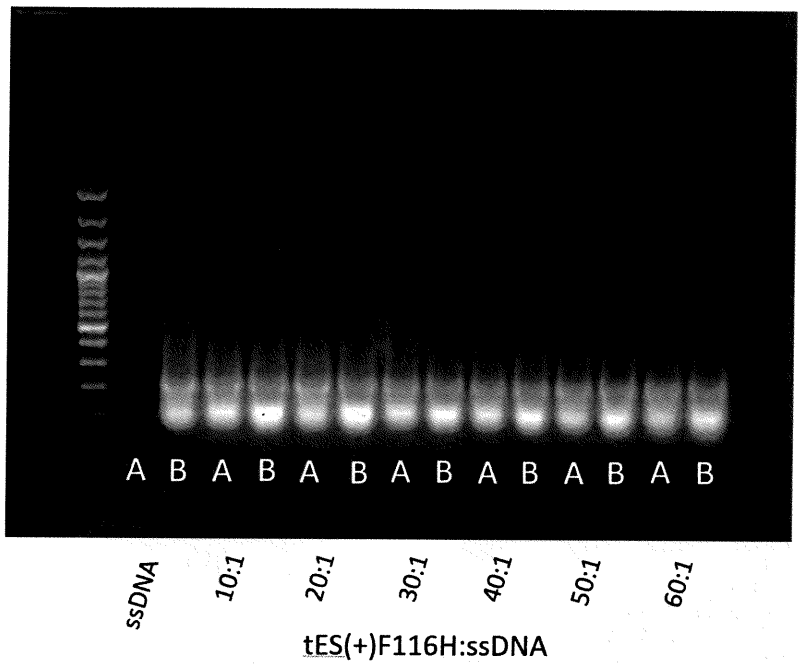


FIG. 21B

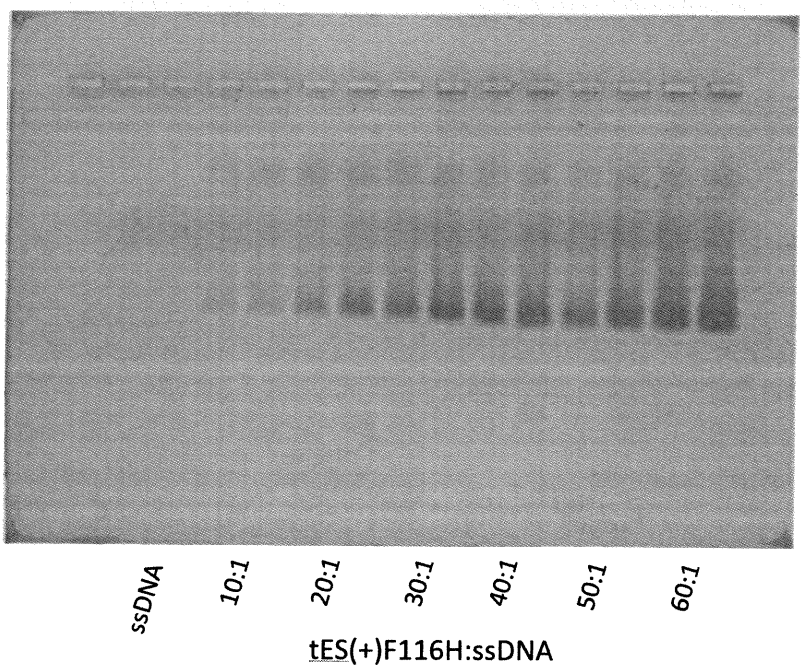


FIG. 22A

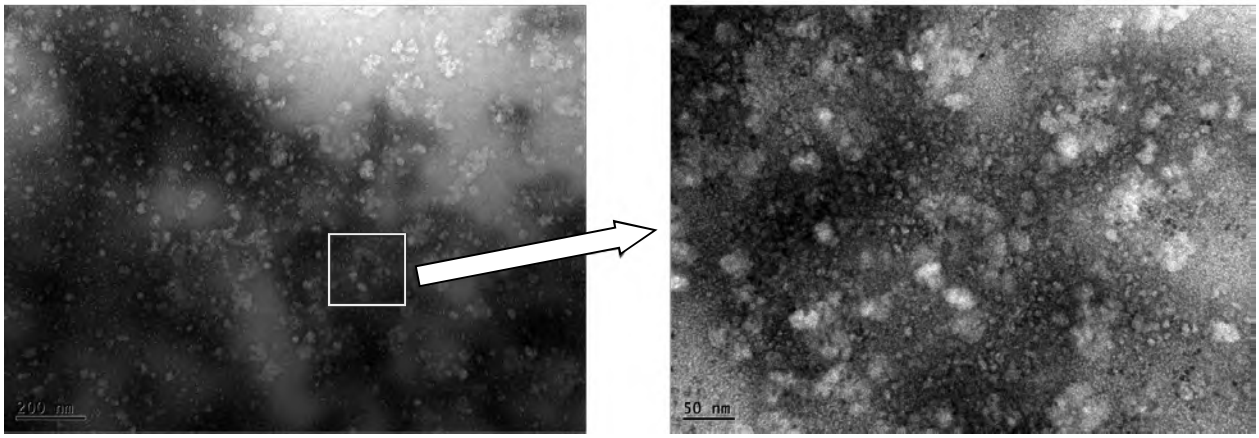


FIG. 22B

