WT Light Chain

```
DIQVTQSPSS LSASVGDRVT ITCITSTDID DDMNWYQQKP GKVPKLLISG GNTLRPGVPS     60
RFSGSGSGTD FTLTISSLQP EDVATYYCLQ SDSLPYTFGQ GTKVEIKRTV AAPSVFIFPP     120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT     180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC  (SEQ ID NO:1)                  214
```

WT Heavy Chain

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY     60
ADDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG     120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL     180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT  (SEQ ID NO:2)        223
```

FIG. 1A

VL Domain

```
WT           DIQVTQSPSSLSASVGDRVTITCITSTDIDDDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP
TM           DIQVTQSPSSLSASVGDRVTITCITSTDIESDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP
TM.D92E      DIQVTQSPSSLSASVGDRVTITCITSTDIESDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP
SIESD         DIQVTQSPSSLSASVGDRVTITCITSTSIESDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP
SIESD.N103S  DIQVTQSPSSLSASVGDRVTITCITSTSIESDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP

WT           EDVATYYCLQSDSLPYTFGQGTKVEIK      (SEQ ID NO:3)
TM           EDVATYYCLQSDSLPYTFGQGTKVEIK      (SEQ ID NO:16)
TM.D92E      EDVATYYCLQSESLPYTFGQGTKVEIK      (SEQ ID NO:18)
SIESD        EDVATYYCLQSDSLPYTFGQGTKVEIK      (SEQ ID NO:19)
SIESD.N103S  EDVATYYCLQSDSLPYTFGQGTKVEIK      (SEQ ID NO:19)
```

VH Domain

```
WT           EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYADDFKGRFVFSLDTSVSTAY
TM           EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYAEDFKGRFVFSLDTSVSTAY
TM.D92E      EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYAEDFKGRFVFSLDTSVSTAY
SIESD        EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYAEDFKGRFVFSLDTSVSTAY
SIESD.N103S  EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYAEDFKGRFVFSLDTSVSTAY

WT           LQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS      (SEQ ID NO:4)
TM           LQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS      (SEQ ID NO:17)
TM.D92E      LQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS      (SEQ ID NO:17)
SIESD        LQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS      (SEQ ID NO:17)
SIESD.N103S  LQISSLKAEDTAVYYCEREGGVSNWGQGTLVTVSS      (SEQ ID NO:20)
```

FIG. 1B

SIESD (AFD.v8) Light Chain

```
DIQVTQSPSS LSASVGDRVT ITCITSTSIE SDMNWYQQKP GKVPKLLISG GNTLRPGVPS      60
RFSGSGSGTD FTLTISSLQP EDVATYYCLQ SDSLPYTFGQ GTKVEIKRTV AAPSVFIFPP     120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT     180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC  (SEQ ID NO:26)                 214
```

SIESD (AFD.v8) Heavy Chain

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY      60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG     120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL     180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT   (SEQ ID NO:27)      223
```

Cys-Modified SIESD (AFD.v8) Heavy Chain

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY      60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG     120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL     180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTC   (SEQ ID NO:30)     223
```

Cys-Pro-Pro-Cys-Modified SIESD (AFD.v8) Heavy Chain

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY      60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG     120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL     180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPC (SEQ ID NO:31)    223
```

FIG. 1C

SIESD.N103S (AFD.v14) Light Chain

DIQVTQSPSS LSASVGDRVT ITCITSTSIE SDMNWYQQKP GKVPKLLISG GNTLRPGVPS 60
RFSGSGSGTD FTLTISSLQP EDVATYYCLQ SDSLPYTFGQ GTKVEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC (SEQ ID NO:28) 214

SIESD.N103S (AFD.v14) Heavy Chain

EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY 60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVSNWGQGTL VTVSSASTKG 120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL 180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT (SEQ ID NO:29) 223

Cys-Modified SIESD.N103S (AFD.v14) Heavy Chain

EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY 60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVSNWGQGTL VTVSSASTKG 120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL 180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTC (SEQ ID NO:32) 223

Cys-Modified SIESD.N103S (AFD.v14) Heavy Chain

EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY 60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVSNWGQGTL VTVSSASTKG 120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL 180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPC (SEQ ID NO:33) 223

FIG. 1D

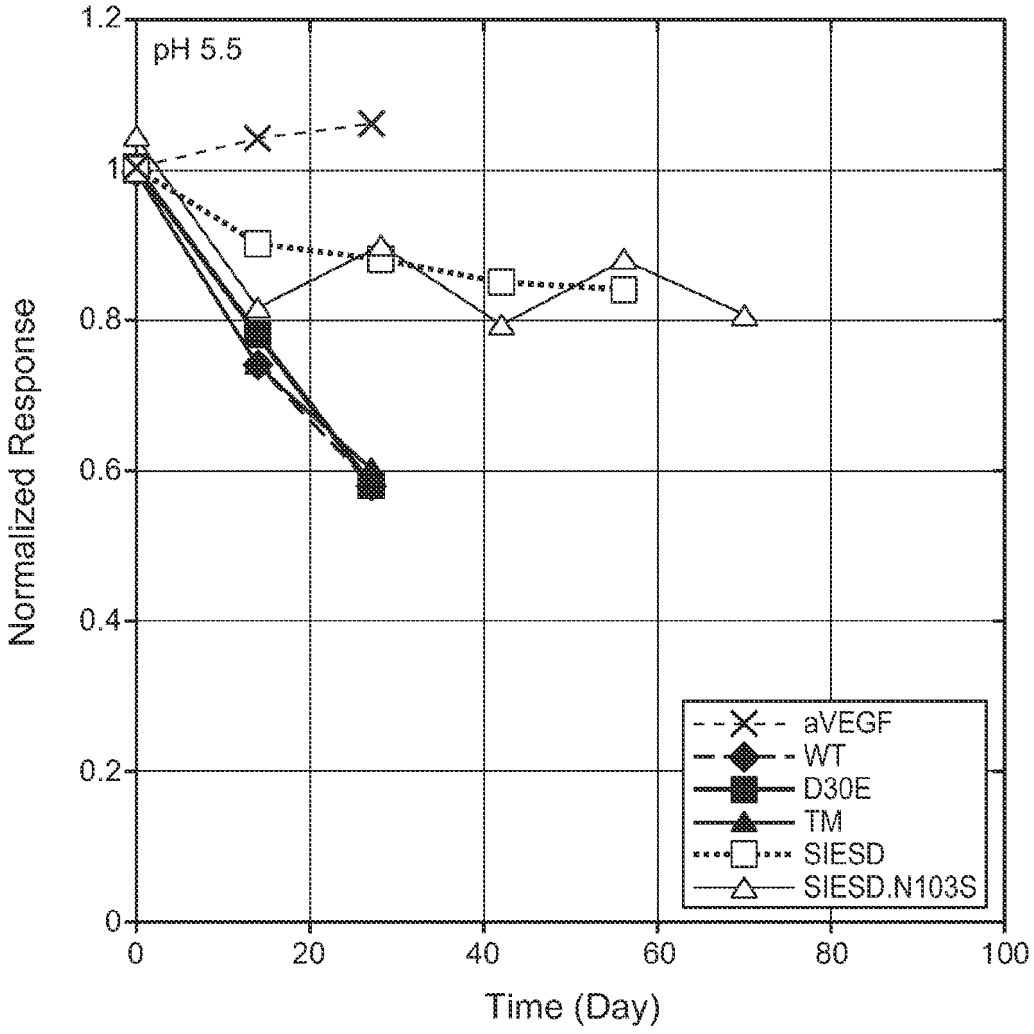

pH 5.5
Normalized Response
Time (Day)
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
aVEGF
WT
D30E
TM
SIESD
SIESD.N103S

FIG. 2A

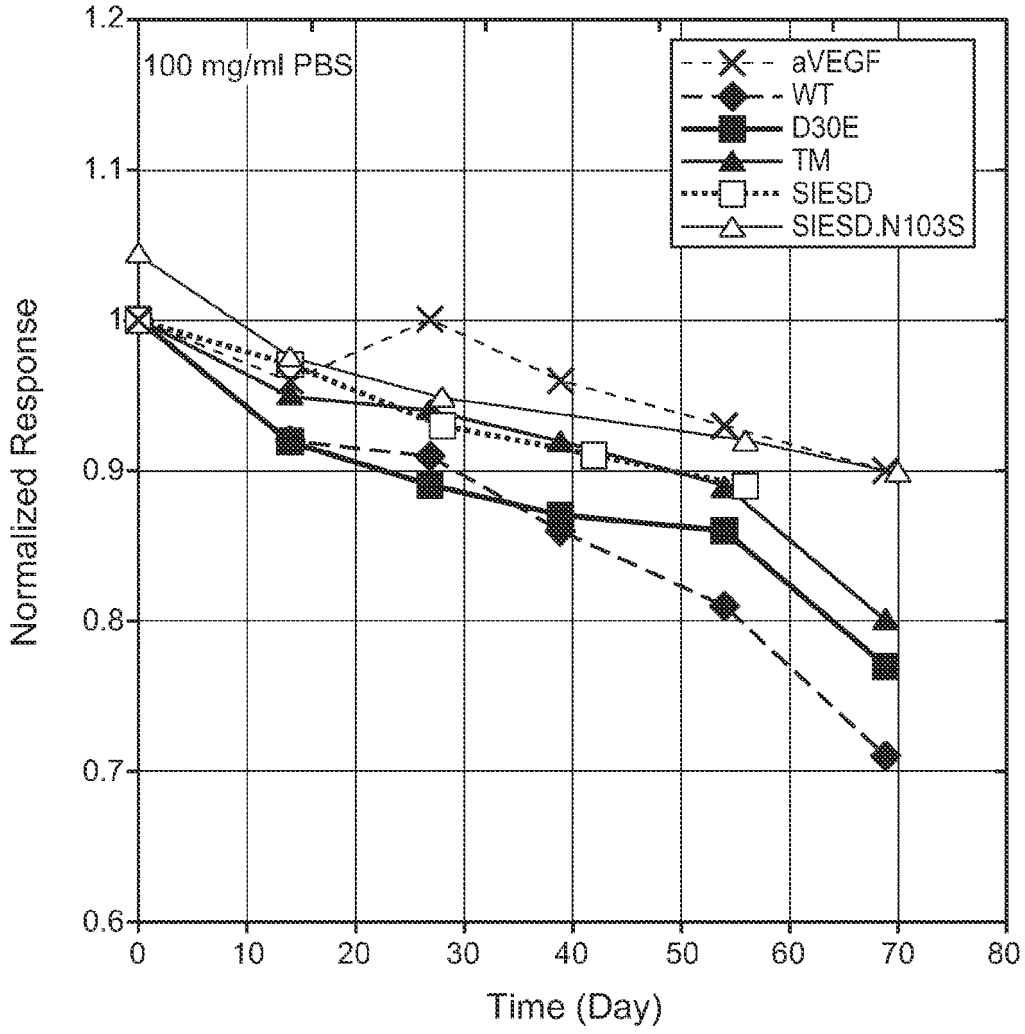

100 mg/ml PBS
aVEGF
WT
D30E
TM
SIESD
SIESD.N103S
Normalized Response
Time (Day)
1.2
1.1
1
0.9
0.8
0.7
0.6
0
10
20
30
40
50
60
70
80

FIG. 2B

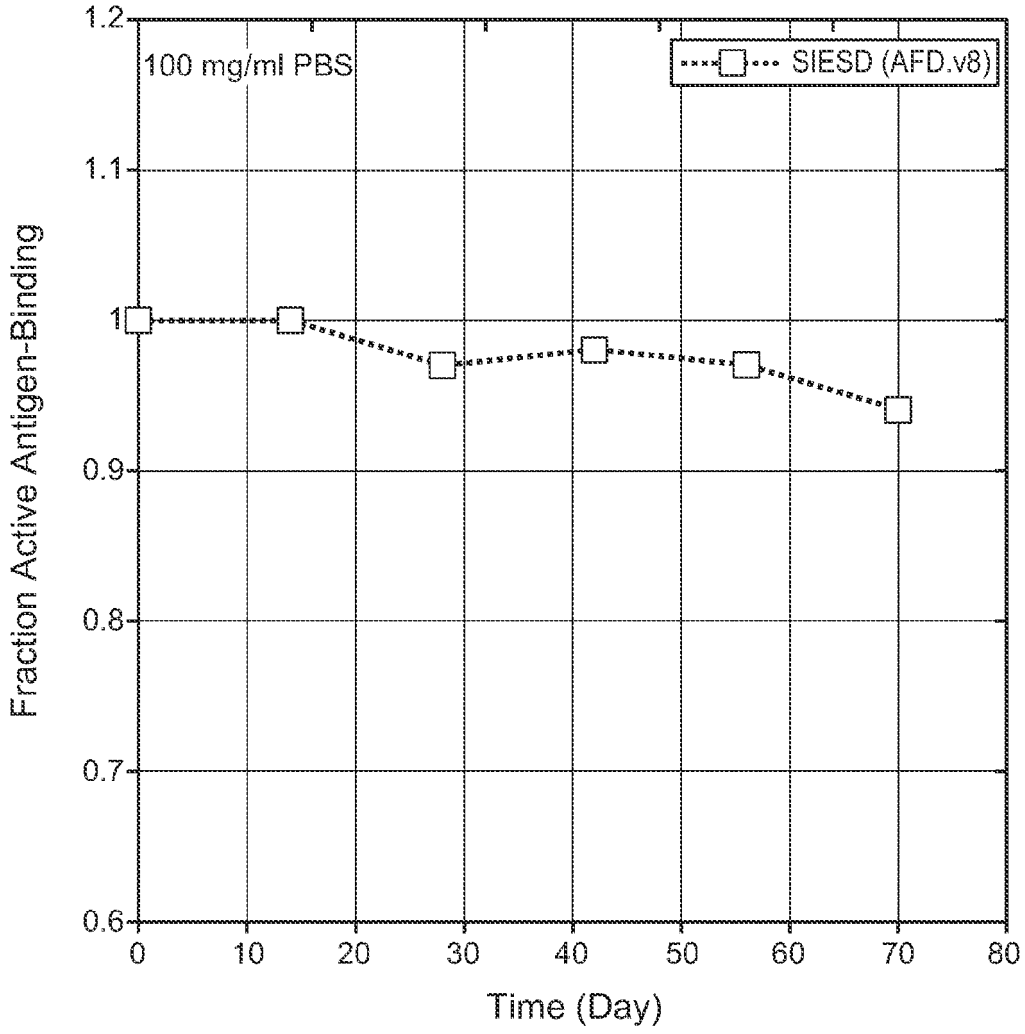

100 mg/ml PBS
SIESD (AFD.v8)
Fraction Active Antigen-Binding
1.2
1.1
1
0.9
0.8
0.7
0.6
0
10
20
30
40
50
60
70
80
Time (Day)

FIG. 2C

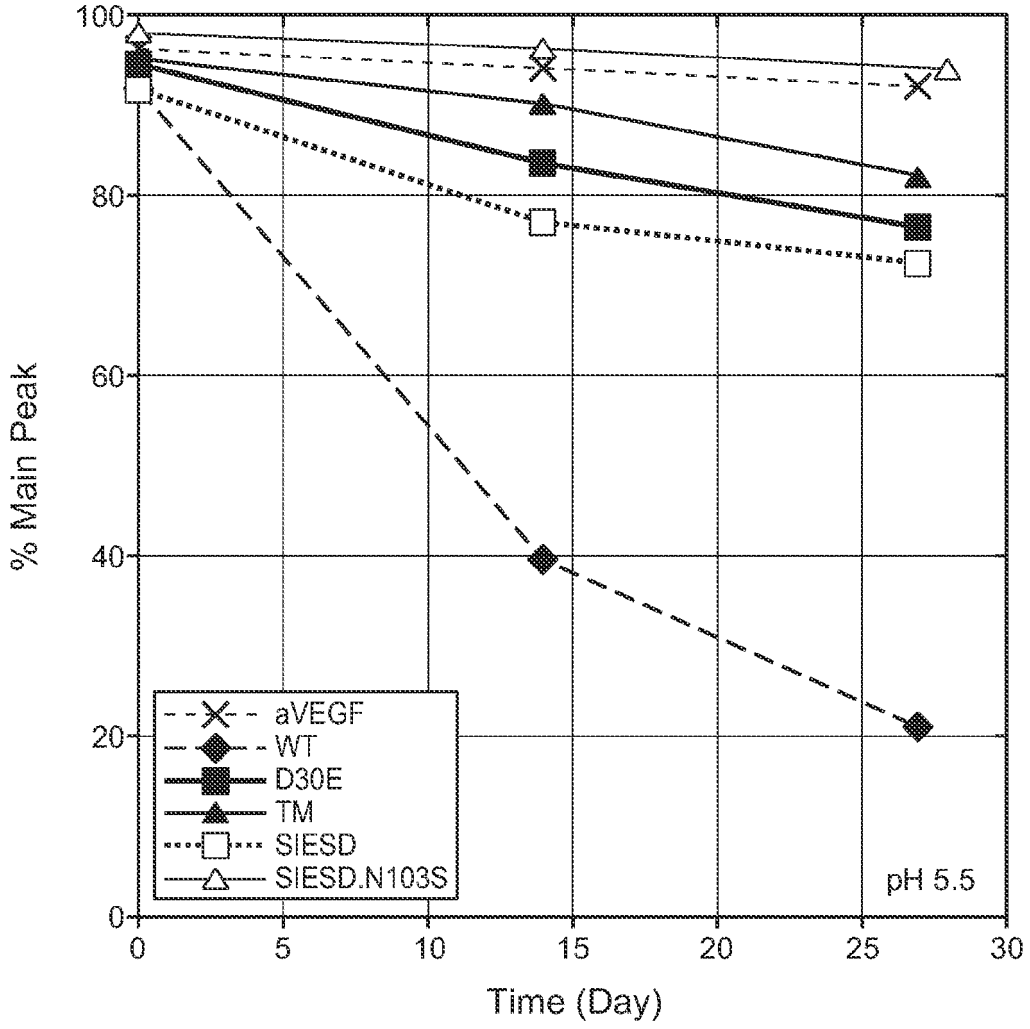

100
80
60
40
20
0
% Main Peak
0
5
10
15
20
25
30
Time (Day)
aVEGF
WT
D30E
TM
SIESD
SIESD.N103S
pH 5.5

FIG. 3A

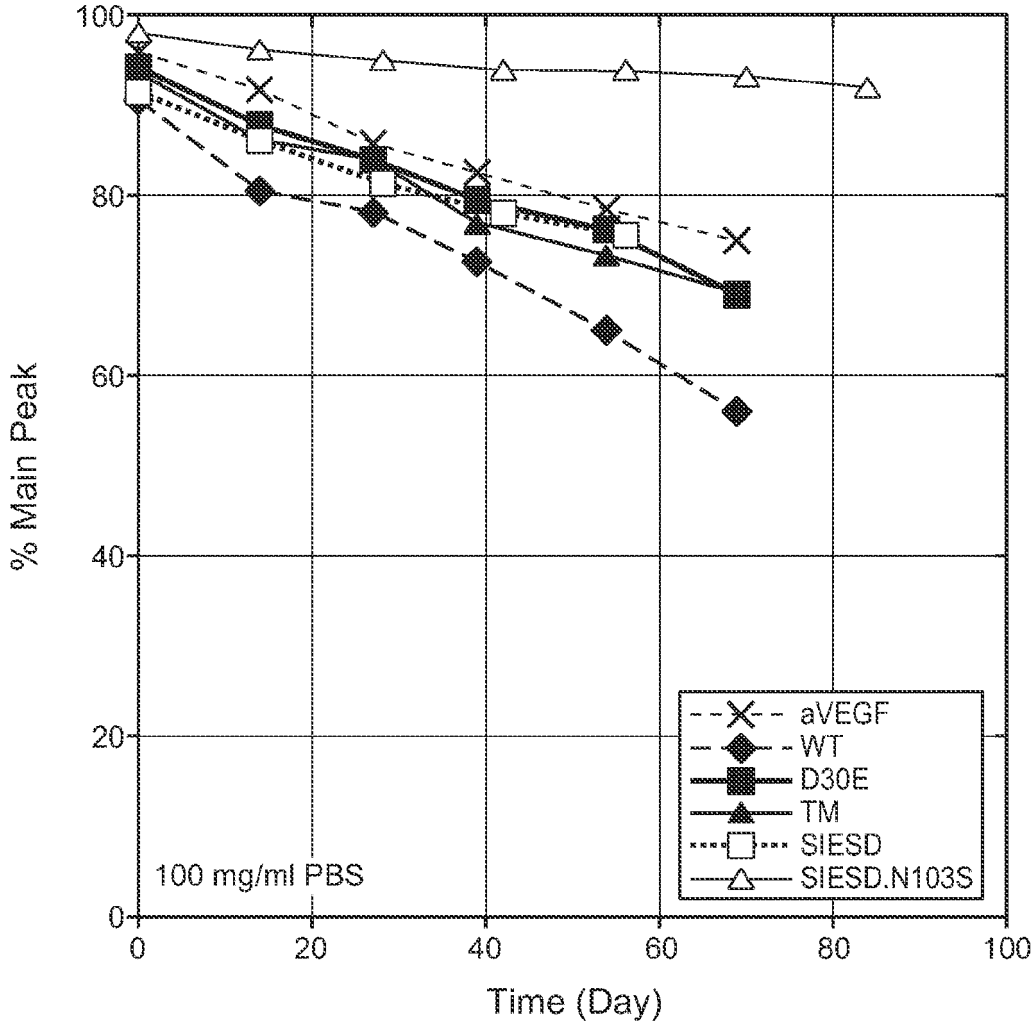

100
80
60
40
20
0
% Main Peak
0
20
40
60
80
100
Time (Day)
100 mg/ml PBS
aVEGF
WT
D30E
TM
SIESD
SIESD.N103S

FIG. 3B

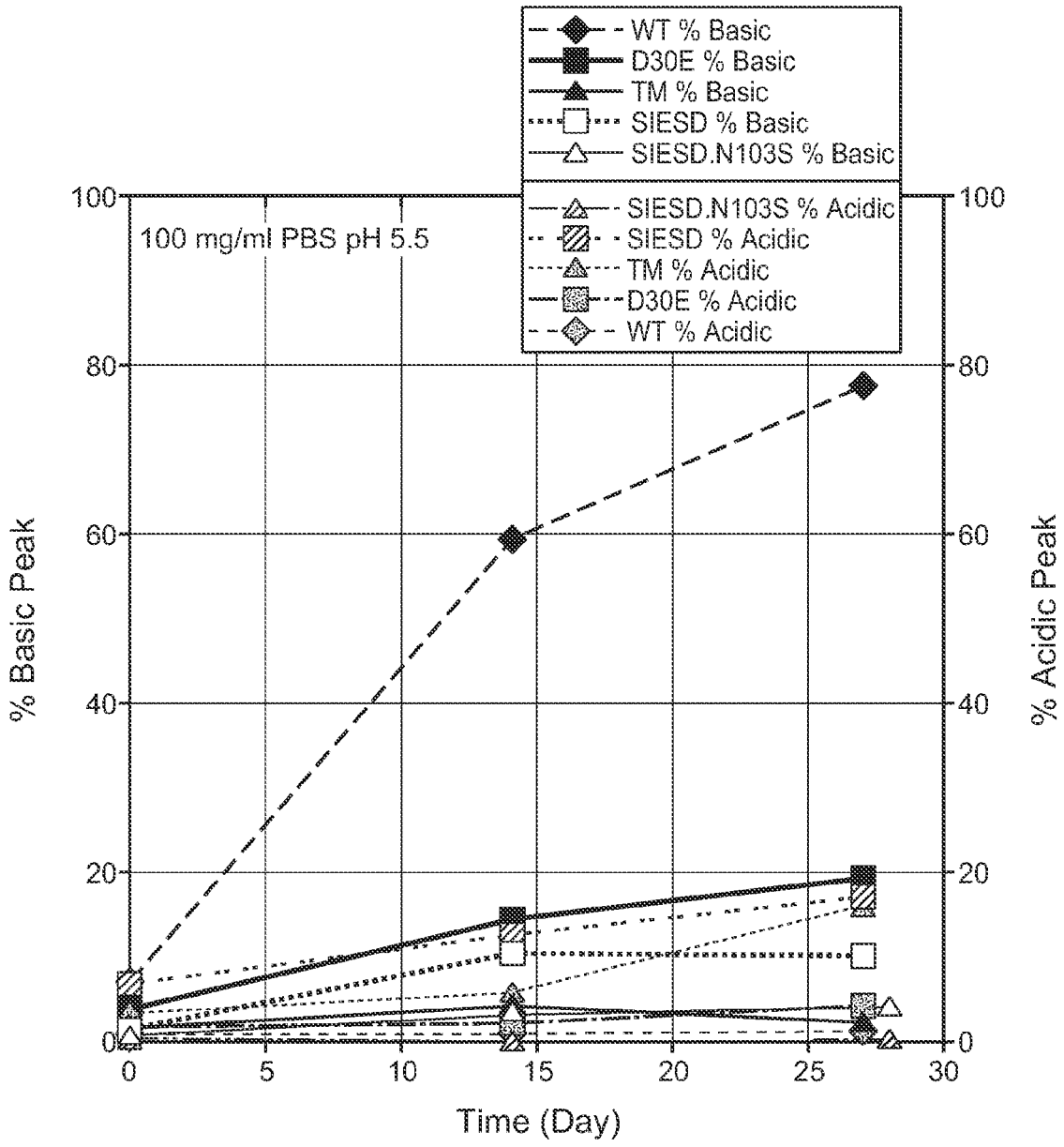

WT % Basic
D30E % Basic
TM % Basic
SIESD % Basic
SIESD.N103S % Basic
SIESD.N103S % Acidic
SIESD % Acidic
TM % Acidic
D30E % Acidic
WT % Acidic
100 mg/ml PBS pH 5.5
% Basic Peak
% Acidic Peak
Time (Day)
0
20
40
60
80
100
0
5
10
15
20
25
30

FIG. 4A

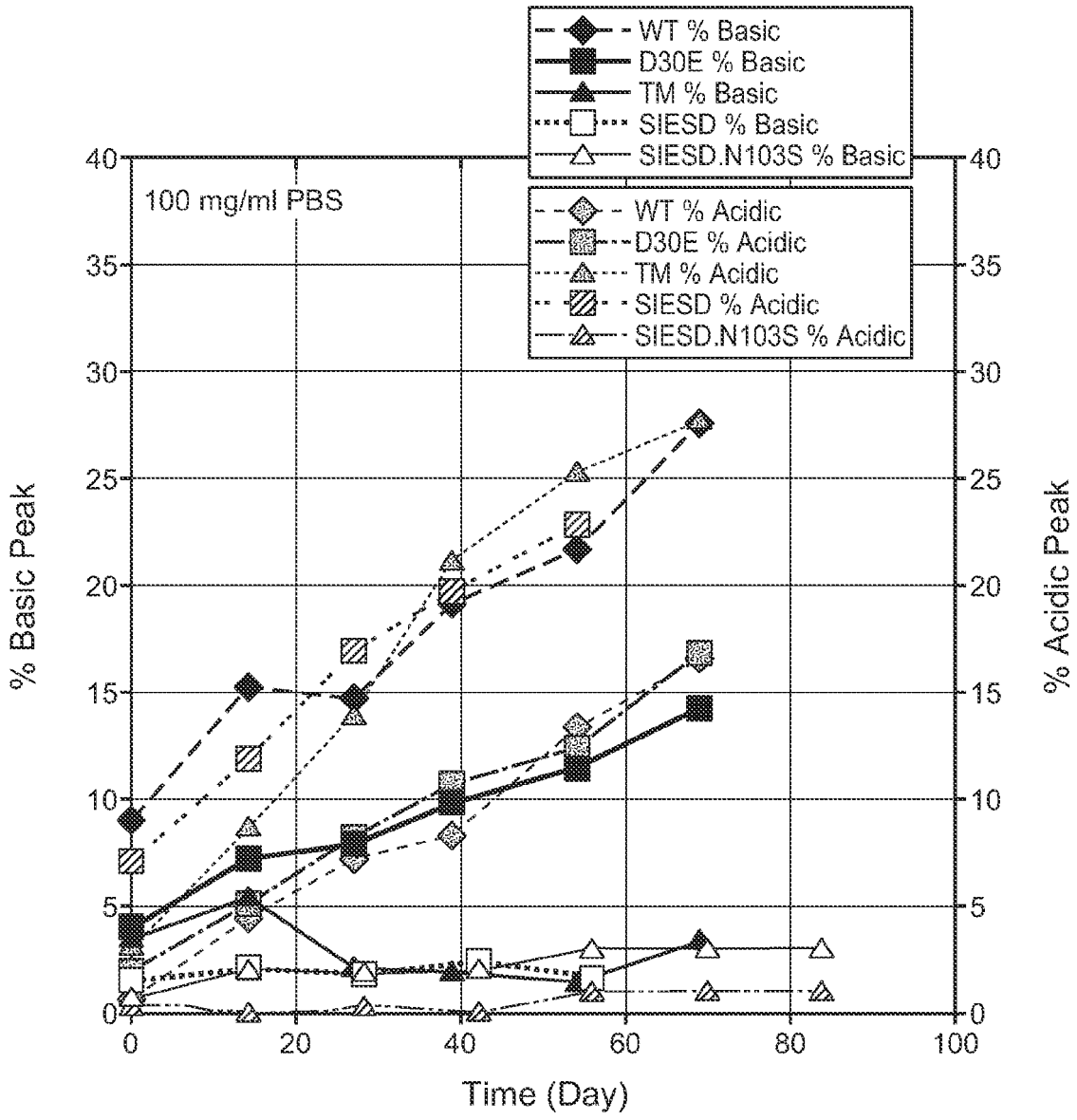

WT % Basic
D30E % Basic
TM % Basic
SIESD % Basic
SIESD.N103S % Basic
WT % Acidic
D30E % Acidic
TM % Acidic
SIESD % Acidic
SIESD.N103S % Acidic
100 mg/ml PBS
% Basic Peak
% Acidic Peak
Time (Day)
0
5
10
15
20
25
30
35
40
0
20
40
60
80
100

FIG. 4B

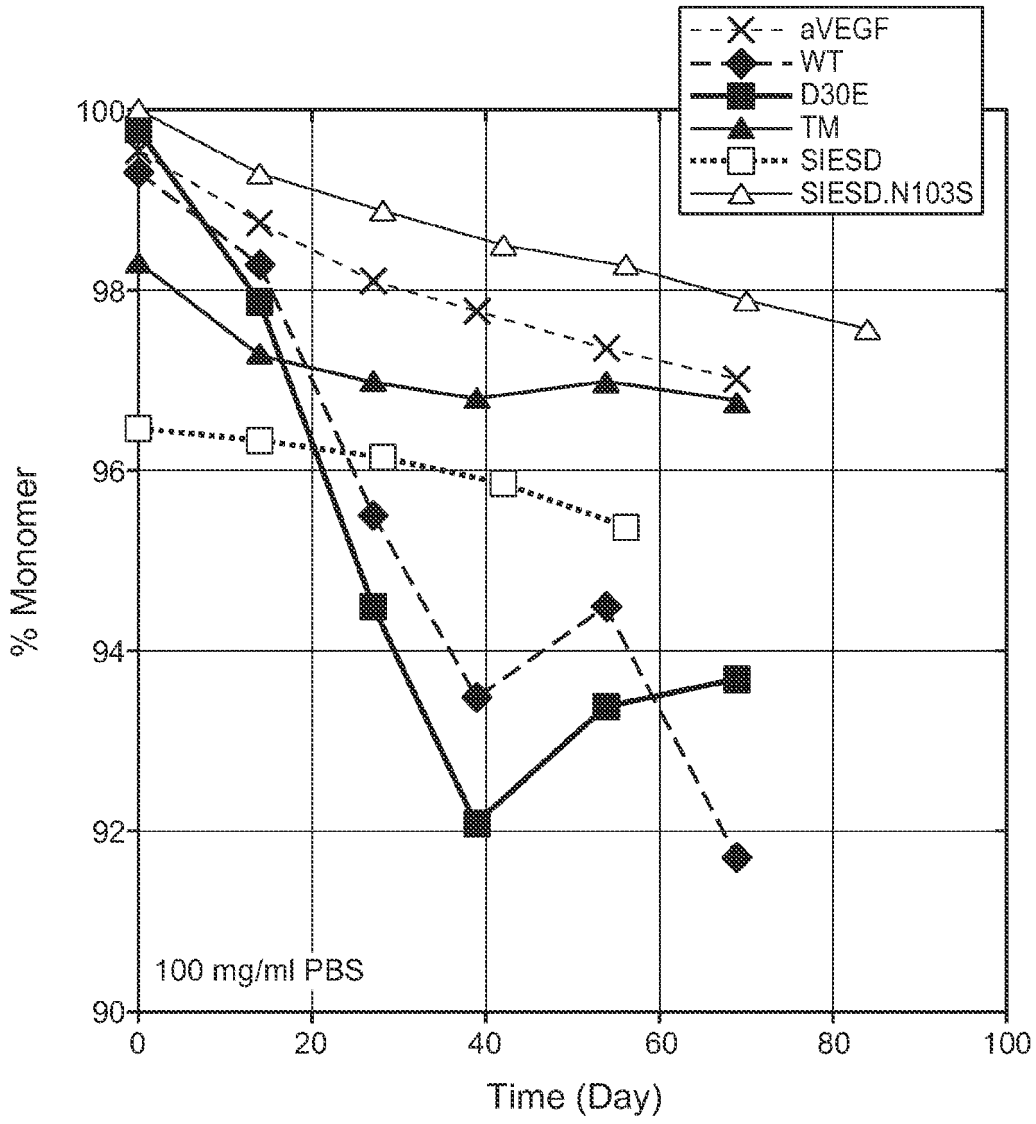

aVEGF
WT
D30E
TM
SIESD
SIESD.N103S
100
98
96
94
92
90
% Monomer
100 mg/ml PBS
0
20
40
60
80
100
Time (Day)

FIG. 5

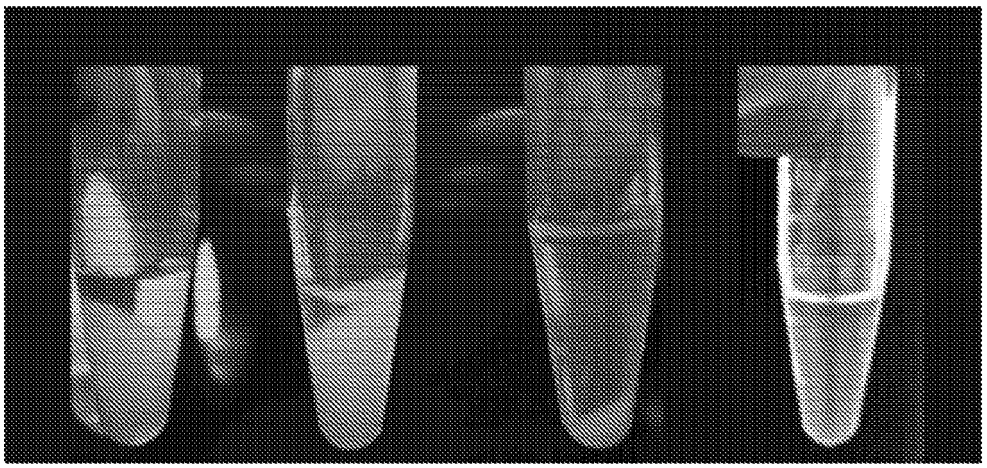

aFD WT
D30E
(AFD.v2)
TM
(AFD.v6)
SIESD
(AFD.v8)

FIG. 6

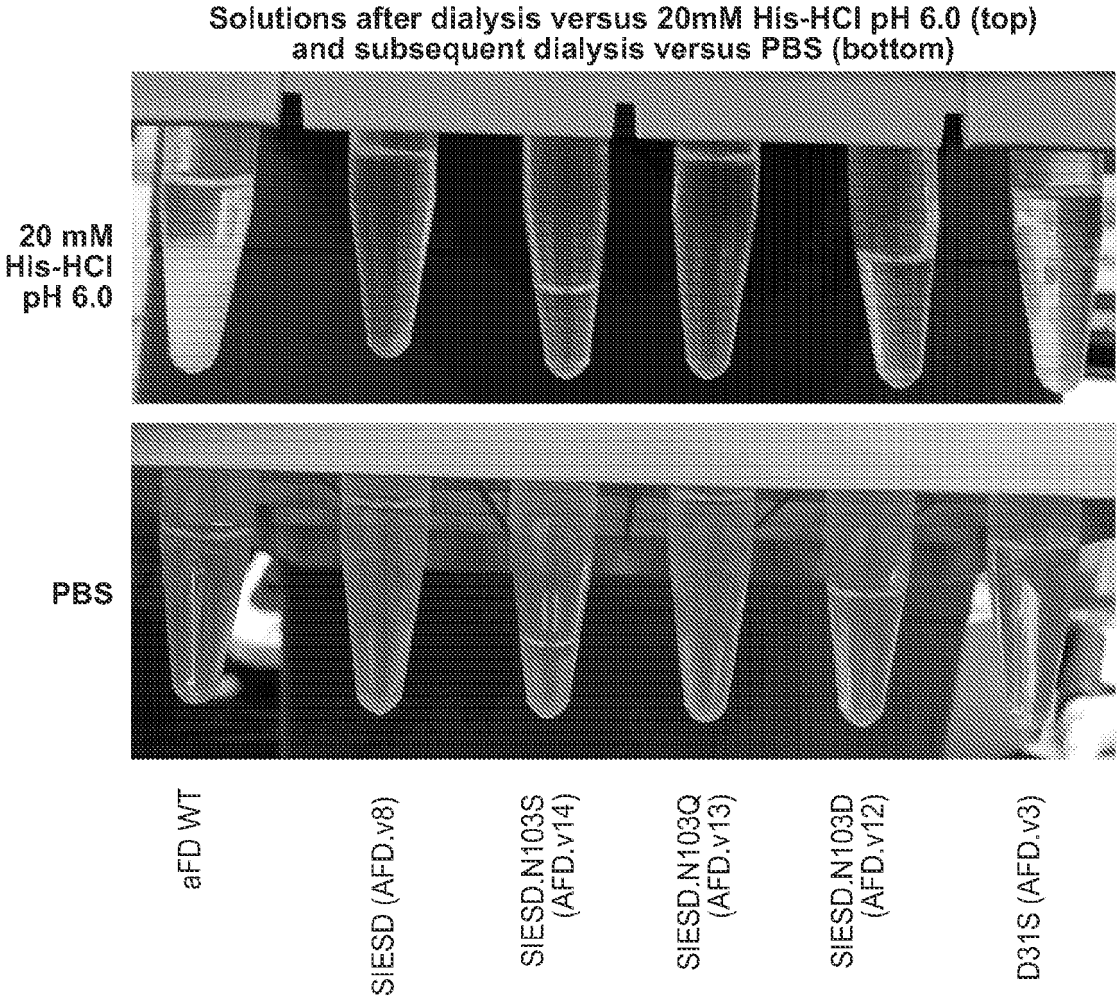

Solutions after dialysis versus 20mM His-HCl pH 6.0 (top)
and subsequent dialysis versus PBS (bottom)
20 mM
His-HCl
pH 6.0
PBS
aFD WT
SIESD (AFD.v8)
SIESD.N103S
(AFD.v14)
SIESD.N103Q
(AFD.v13)
SIESD.N103D
(AFD.v12)
D31S (AFD.v3)

FIG. 7

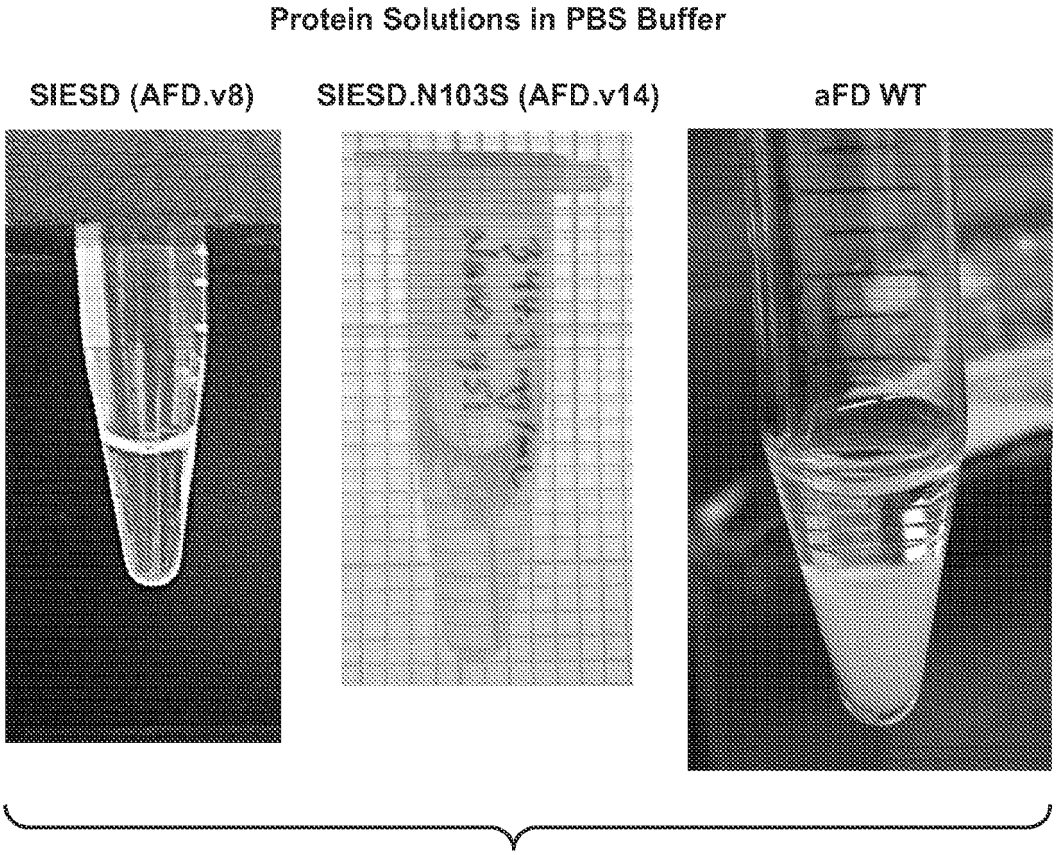
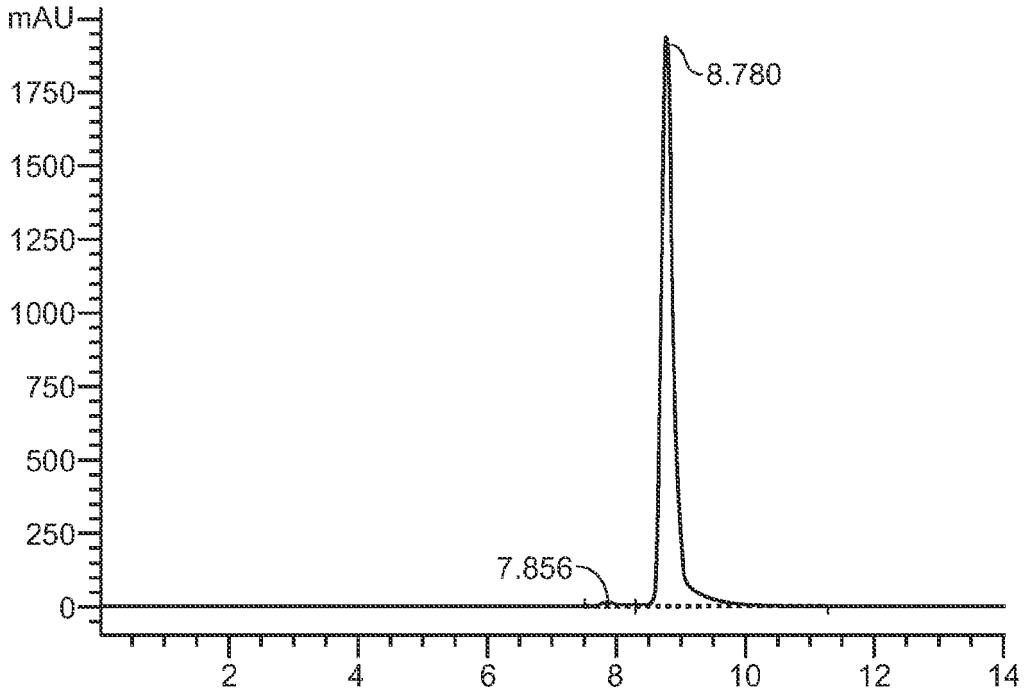

Protein Solutions in PBS Buffer
SIESD (AFD.v8)
SIESD.N103S (AFD.v14)
aFD WT

FIG. 8

SEC Profile for 372 mg/mL SIESD.N103S (AFD.v14) in PBS

DAD1 A, Sig=280, 2 Ref=off



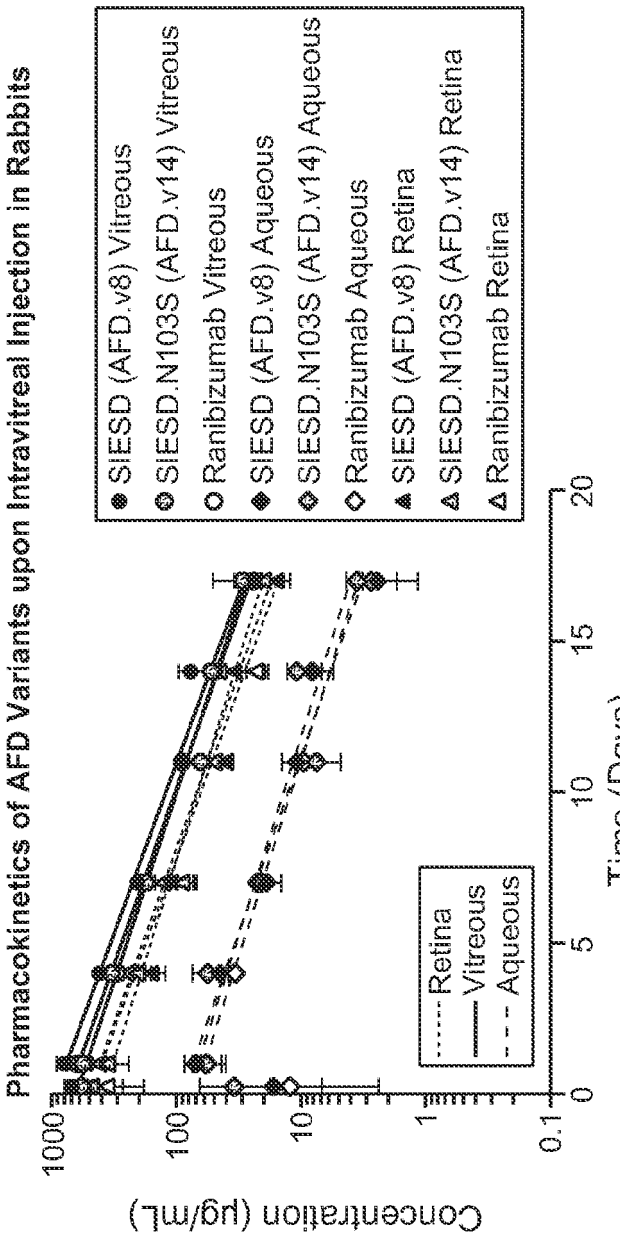
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=280, 2 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.856	VV	0.2537	241.13916	12.69158	0.94195
2	8.780	VB	0.1986	2.53587e4	1939.25708	99.05805
Totals:				2.55999e4	1951.94866	

FIG. 9



	Vitreous (Liquid)				Aqueous		Retina	
Molecule*	Half-life (Days)	AUC_{all} (Day*µg/mL)	CL (mL/Day)	V_{ss} (mL)	Half-life (Days)	AUC_{all} (Day*µg/mL)	Half-life (Days)	AUC_{all} (Day*µg/mL)
SIESD (AFD.v8)	3.4	4460	0.22	1.0	3.5	411	3.4	2489
SIESD.N103S (AFD.v14)	3.7	3450	0.28	1.4	4.0	432	4.0	2199
Ranibizumab	3.5	3600	0.27	1.3	3.9	353	3.5	2453

*1 mg/eye dose, study 14-0995

FIG. 10

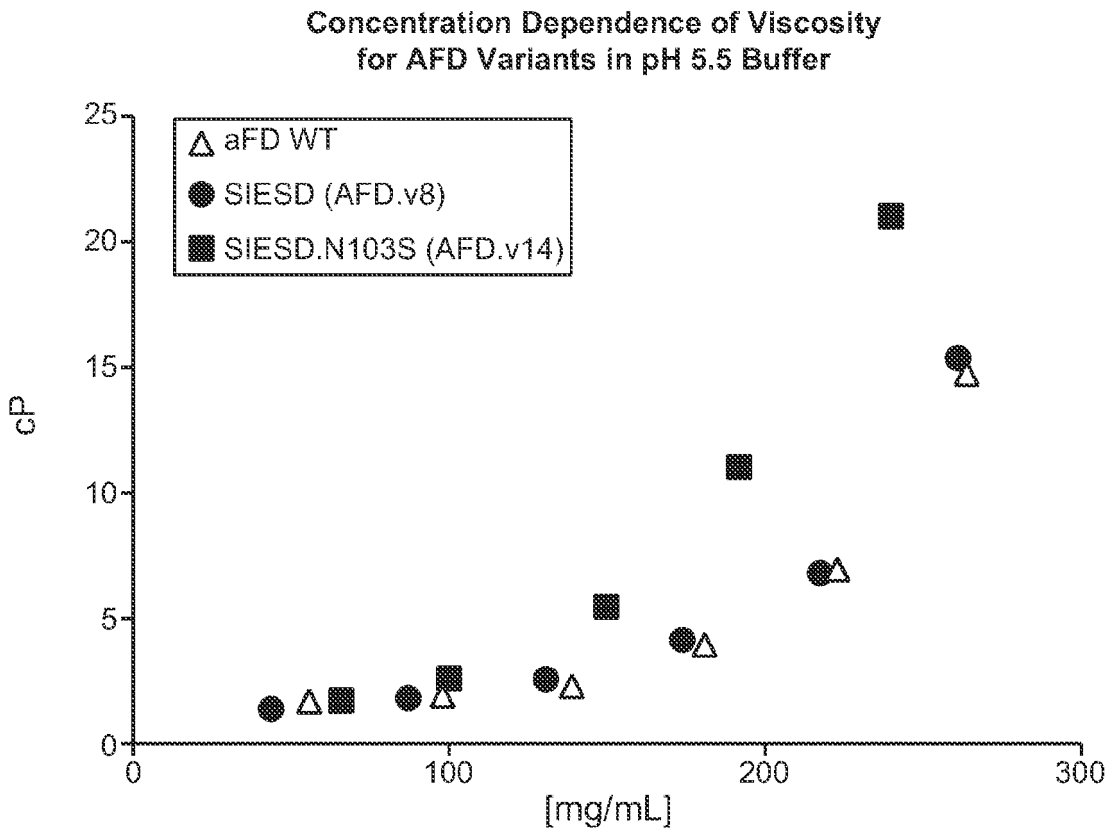

Concentration Dependence of Viscosity
for AFD Variants in pH 5.5 Buffer
aFD WT
SIESD (AFD.v8)
SIESD.N103S (AFD.v14)
cP
25
20
15
10
5
0
0
100
200
300
[mg/mL]

FIG. 11