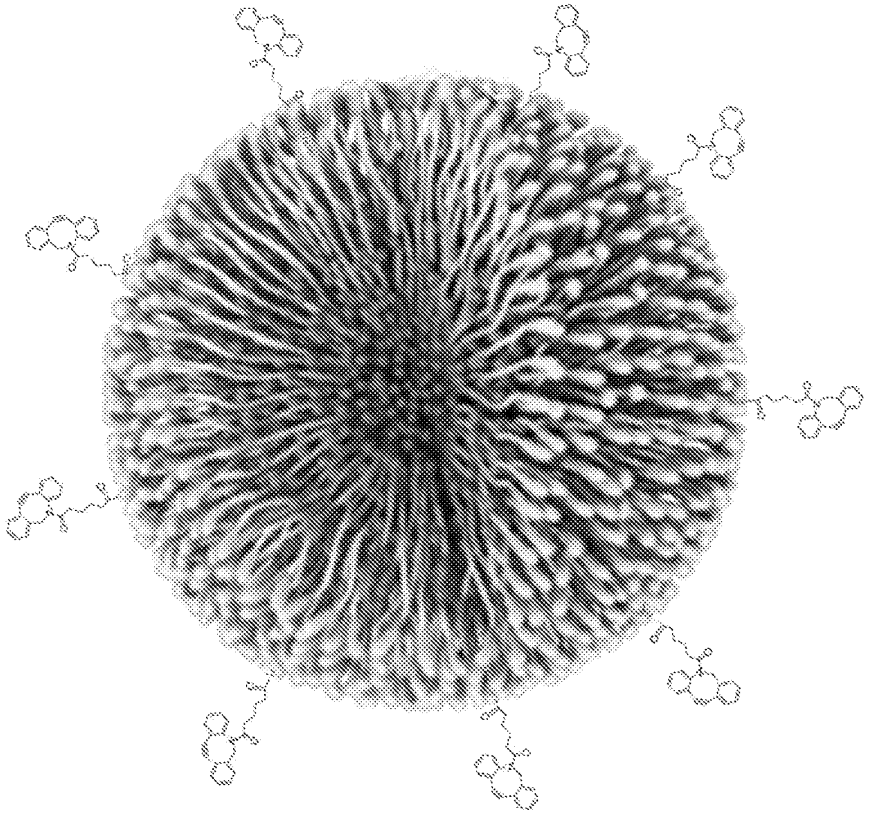
FIG. 1



Modular Nanoparticle with DBCO functional group for Cu-free Click Chemistry

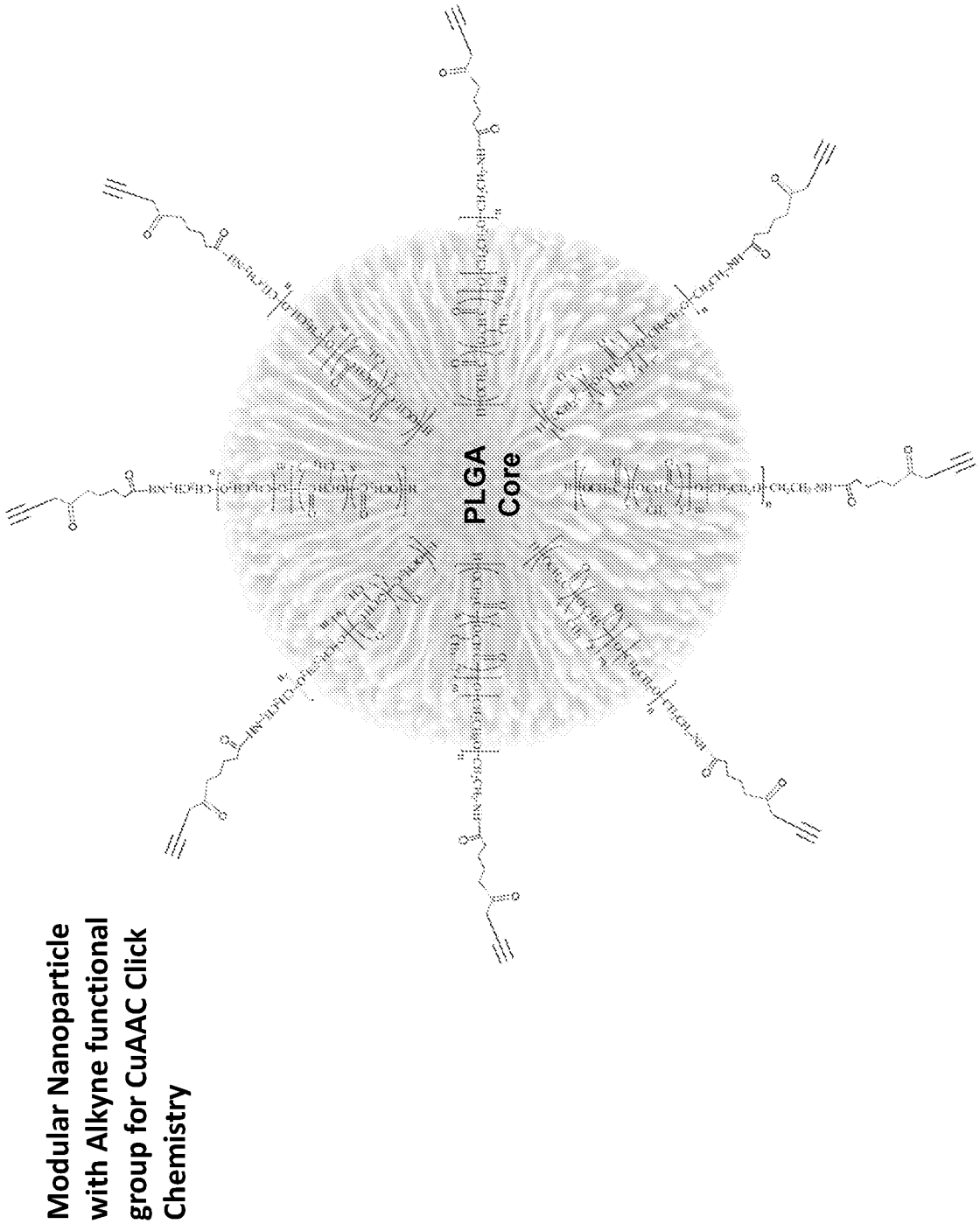

PLGA
Core
Modular Nanoparticle
with Alkyne functional
group for CuAAC Click
Chemistry

FIG. 2

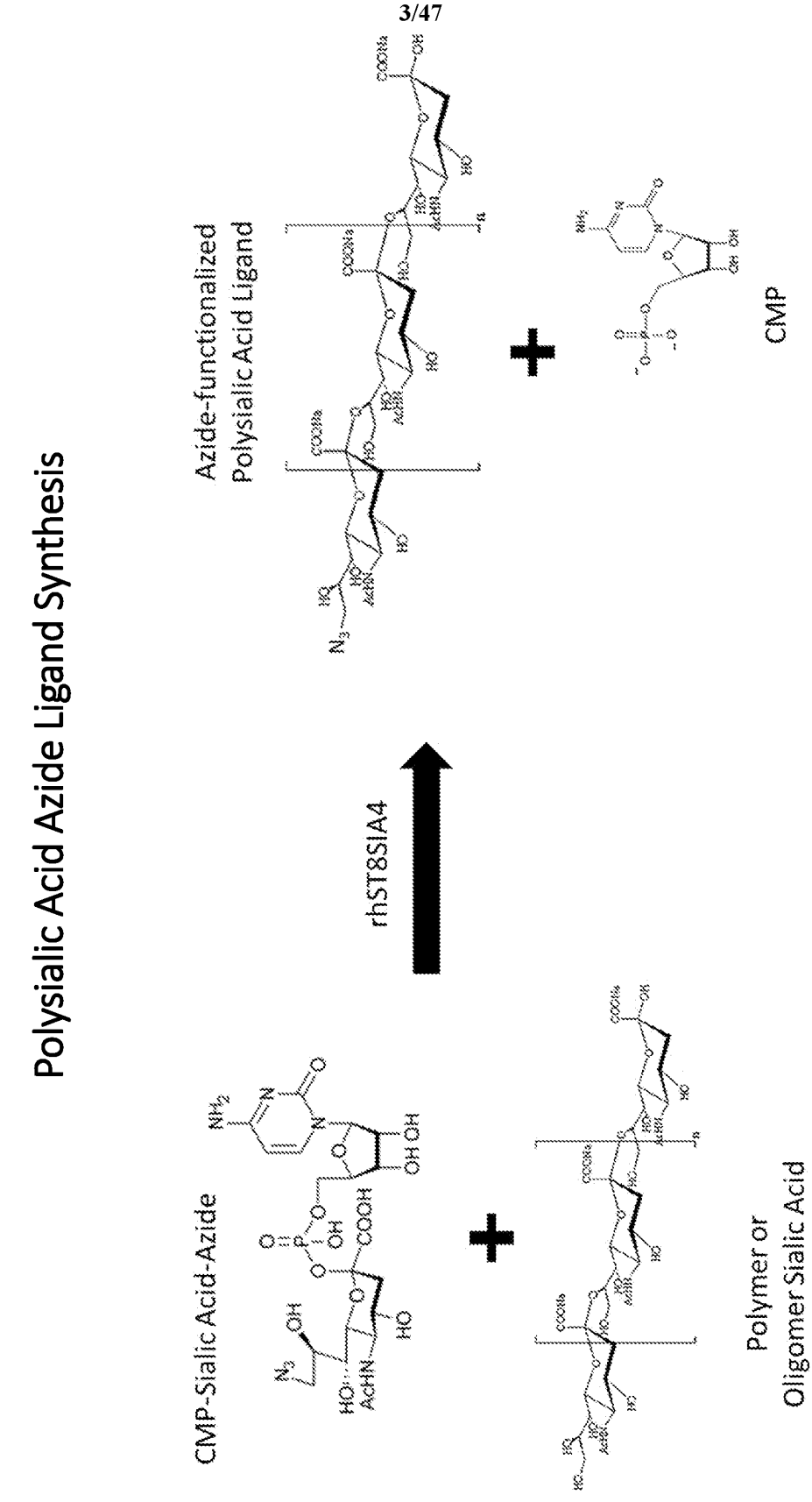

Polysialic Acid Azide Ligand Synthesis
CMP-Sialic Acid-Azide
rhST8SIA4
Azide-functionalized
Polysialic Acid Ligand
CMP
Polymer or
Oligomer Sialic Acid

FIG. 3

Modular Nanoparticle using DBCO for conjugation of sialyllactose-polysialic azide-azide ligand

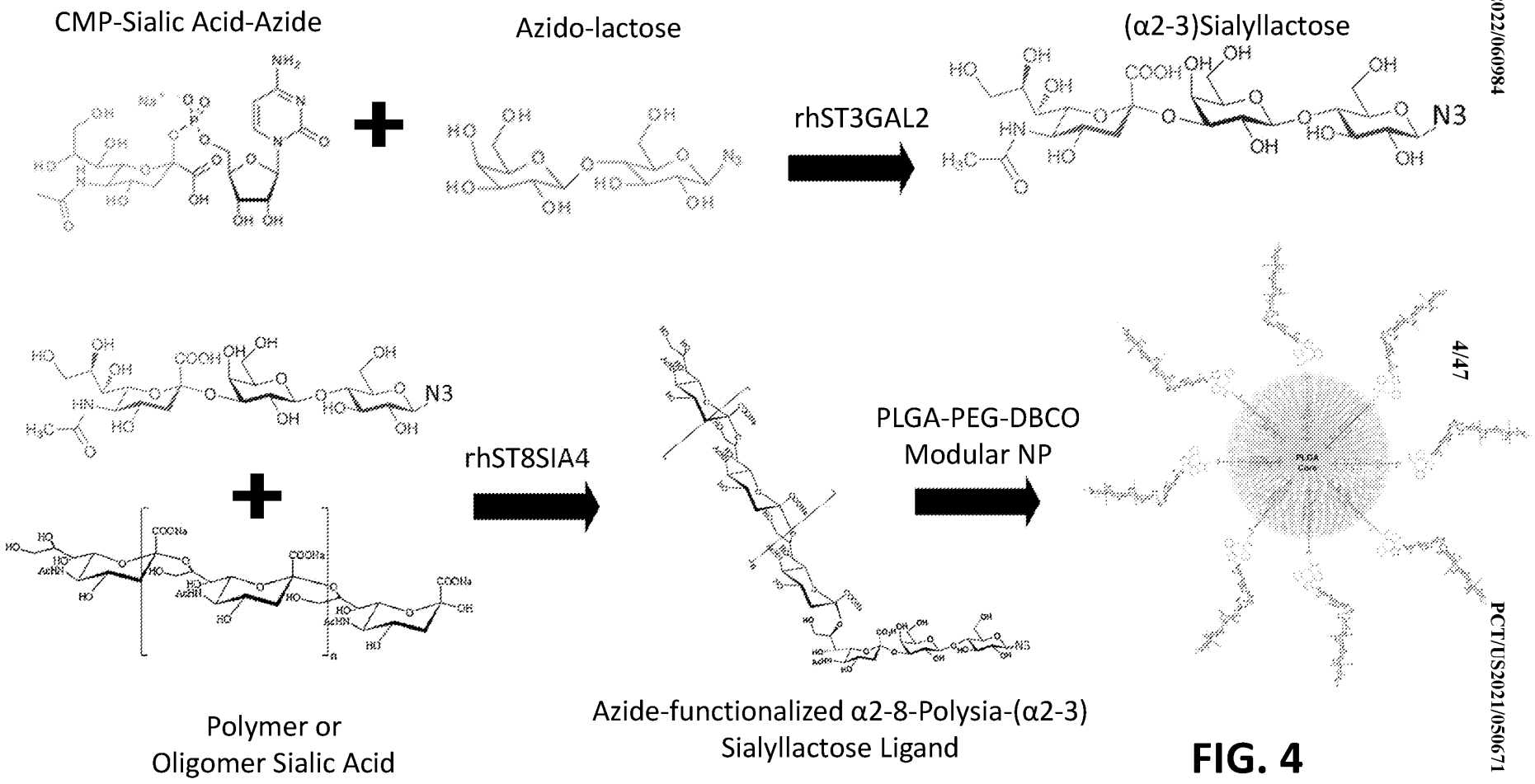


FIG. 4

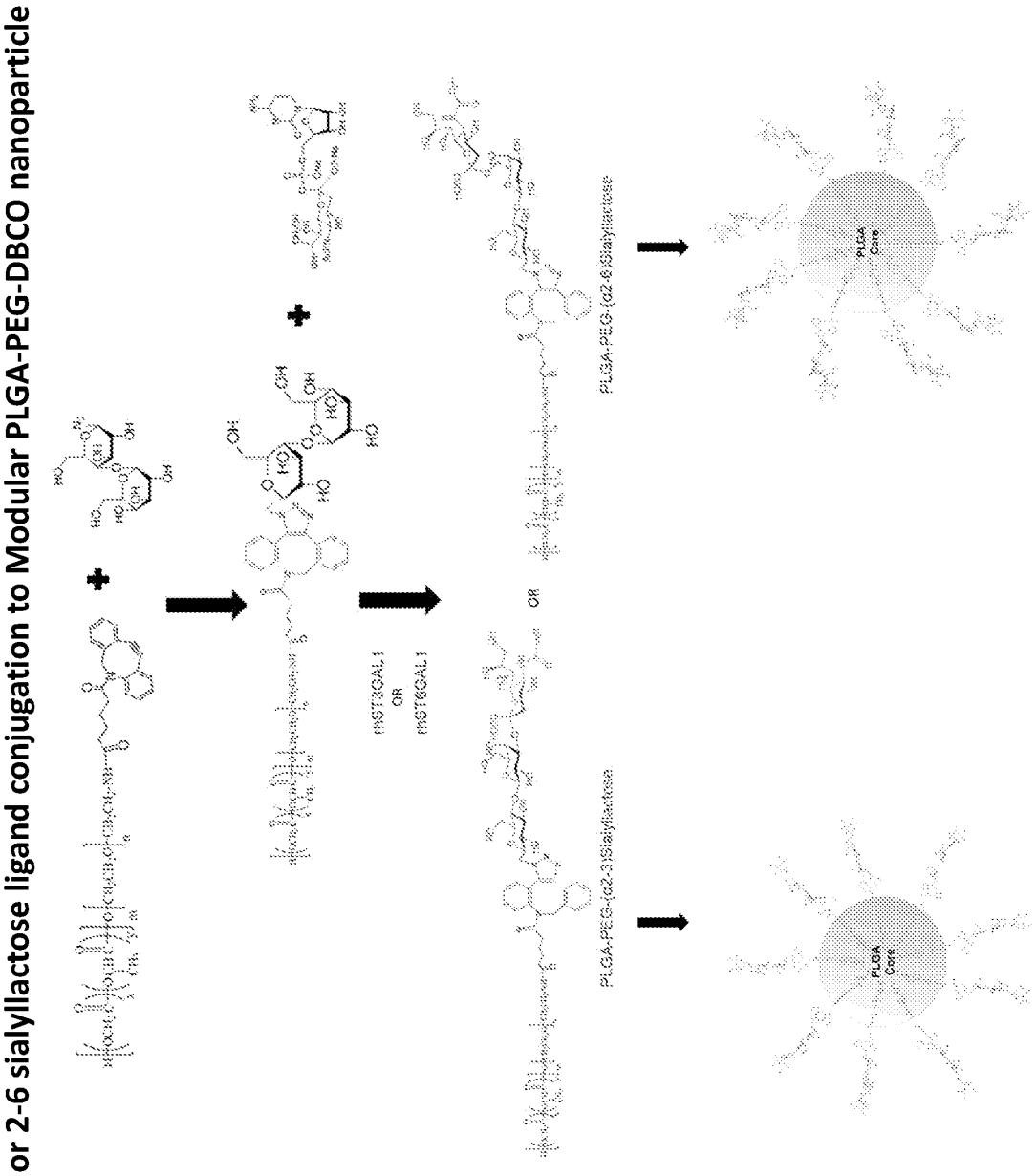
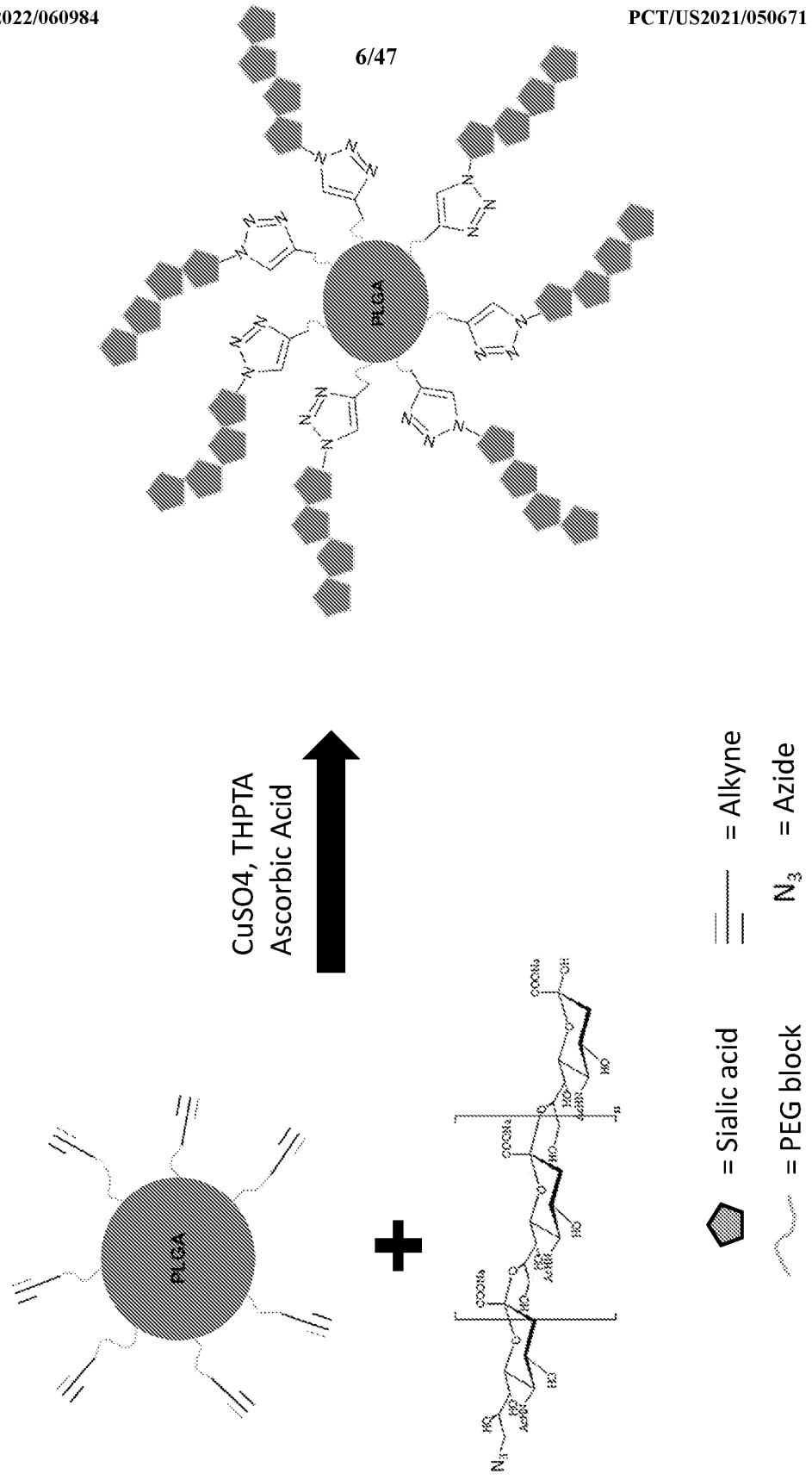

2-3 sialyllactose or 2-6 sialyllactose ligand conjugation to Modular PLGA-PEG-DBCO nanoparticle
mST3GAL1
OR
mST6GAL1
OR
PLGA-PEG-(α2-6)Sialyllactose
PLGA-PEG-(α2-3)Sialyllactose
PLGA Core
PLGA Core

FIG. 5

FIG. 6


PLGA
CuSO4, THPTA
Ascorbic Acid
PLGA
= Sialic acid
= Alkyne
= PEG block
N3 = Azide

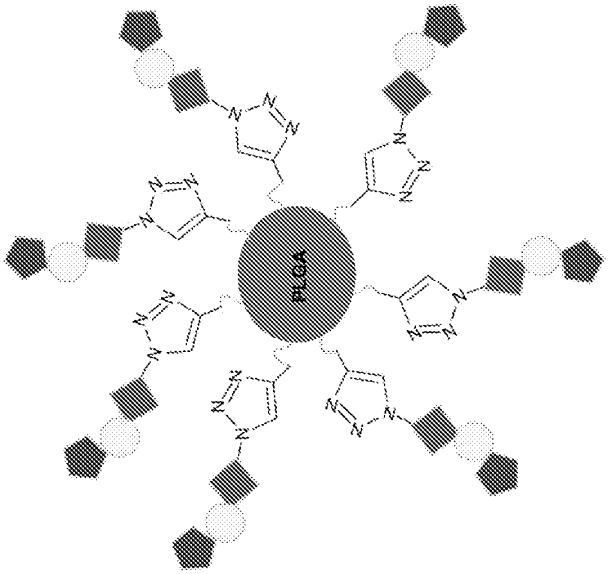


FIG. 7

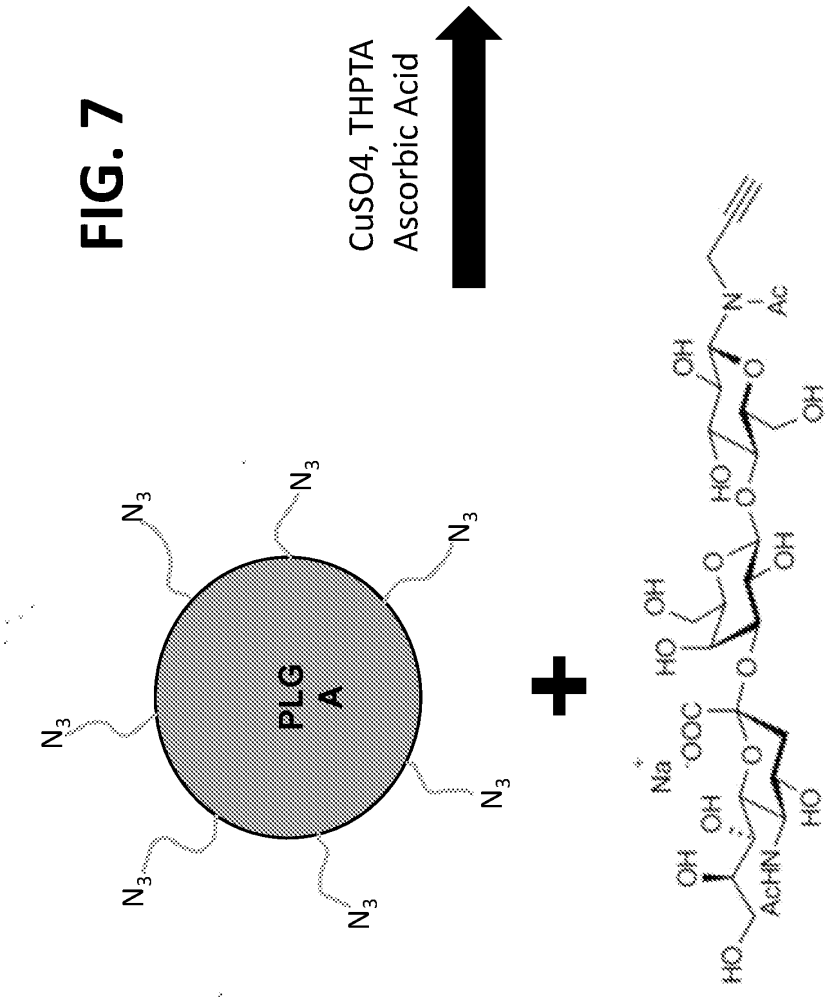


FIG. 8

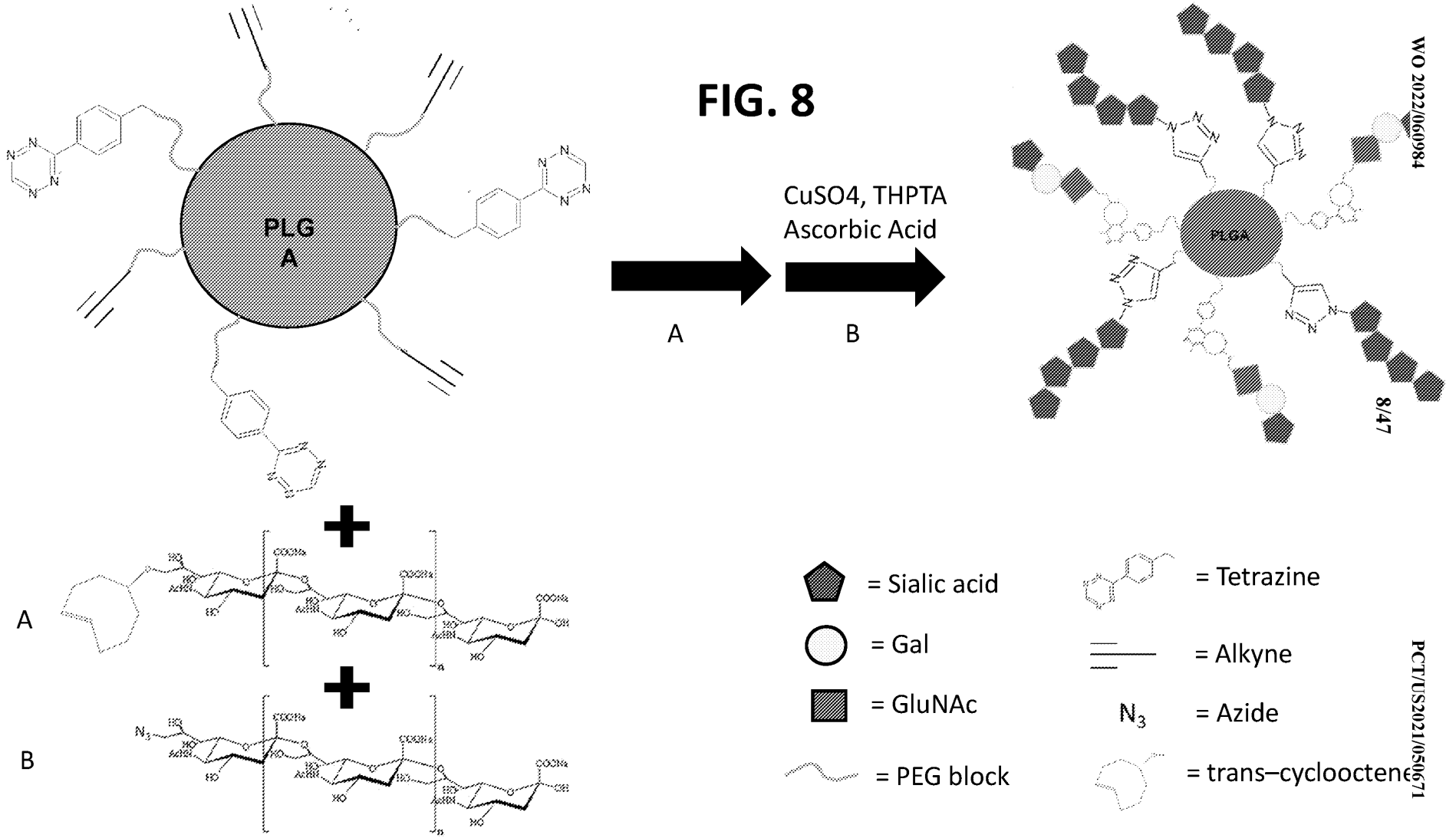

PLG
A
CuSO4, THPTA
Ascorbic Acid
A
B
PLGA
A
B
= Sialic acid
= Gal
= GluNAc
= PEG block
= Tetrazine
= Alkyne
N_3 = Azide
= trans–cyclooctene

FIG. 9

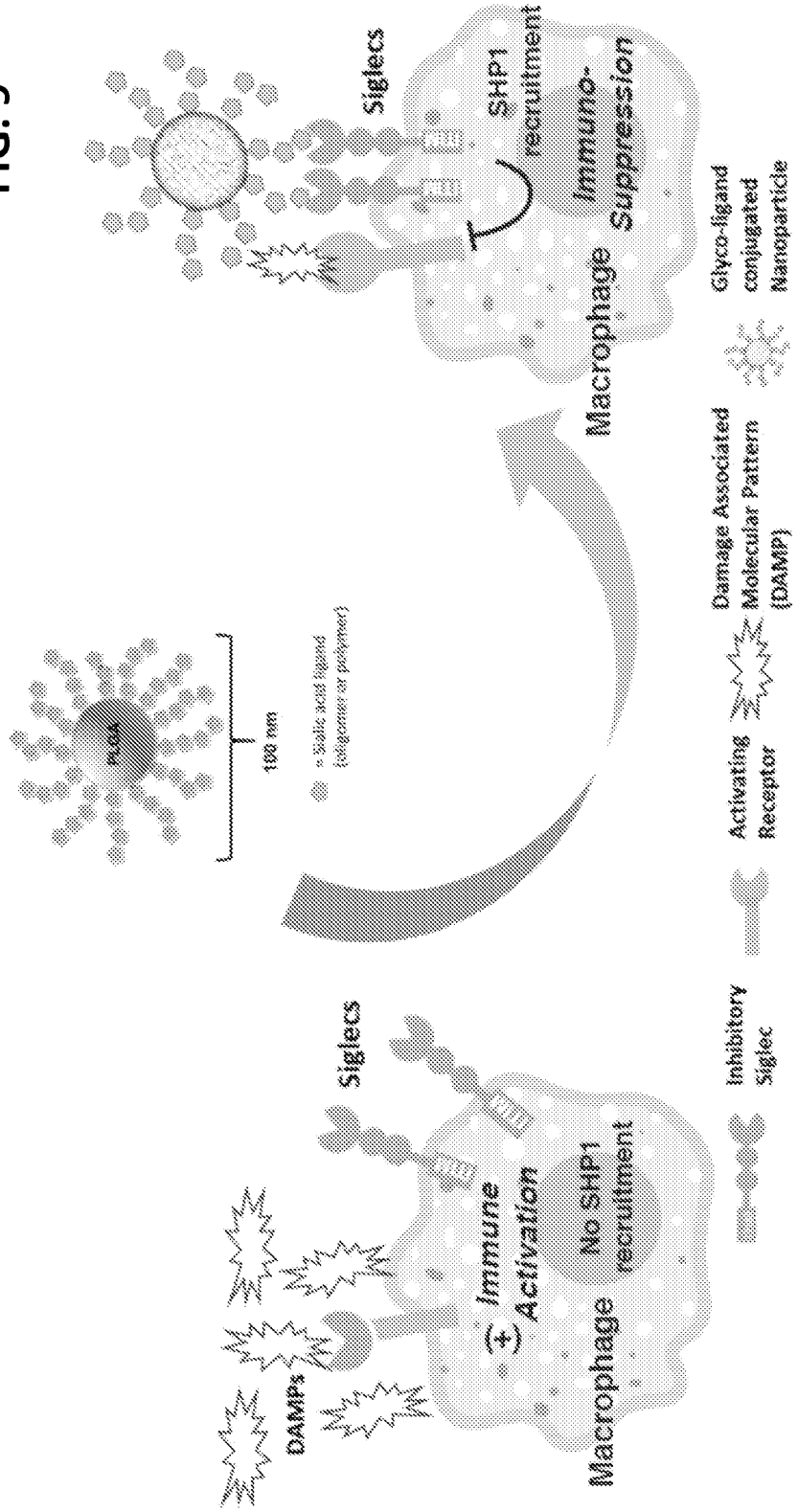

PLGA
100 nm
= Sialic acid ligand
(oligomer or polymer)
Siglecs
SHP1
recruitment
Immuno-
Suppression
Macrophage
Glyco-ligand
conjugated
Nanoparticle
Damage Associated
Molecular Pattern
(DAMP)
Activating
Receptor
Inhibitory
Siglec
DAMPs
Siglecs
Immune
Activation
No SHP1
recruitment
Macrophage

FIG. 10

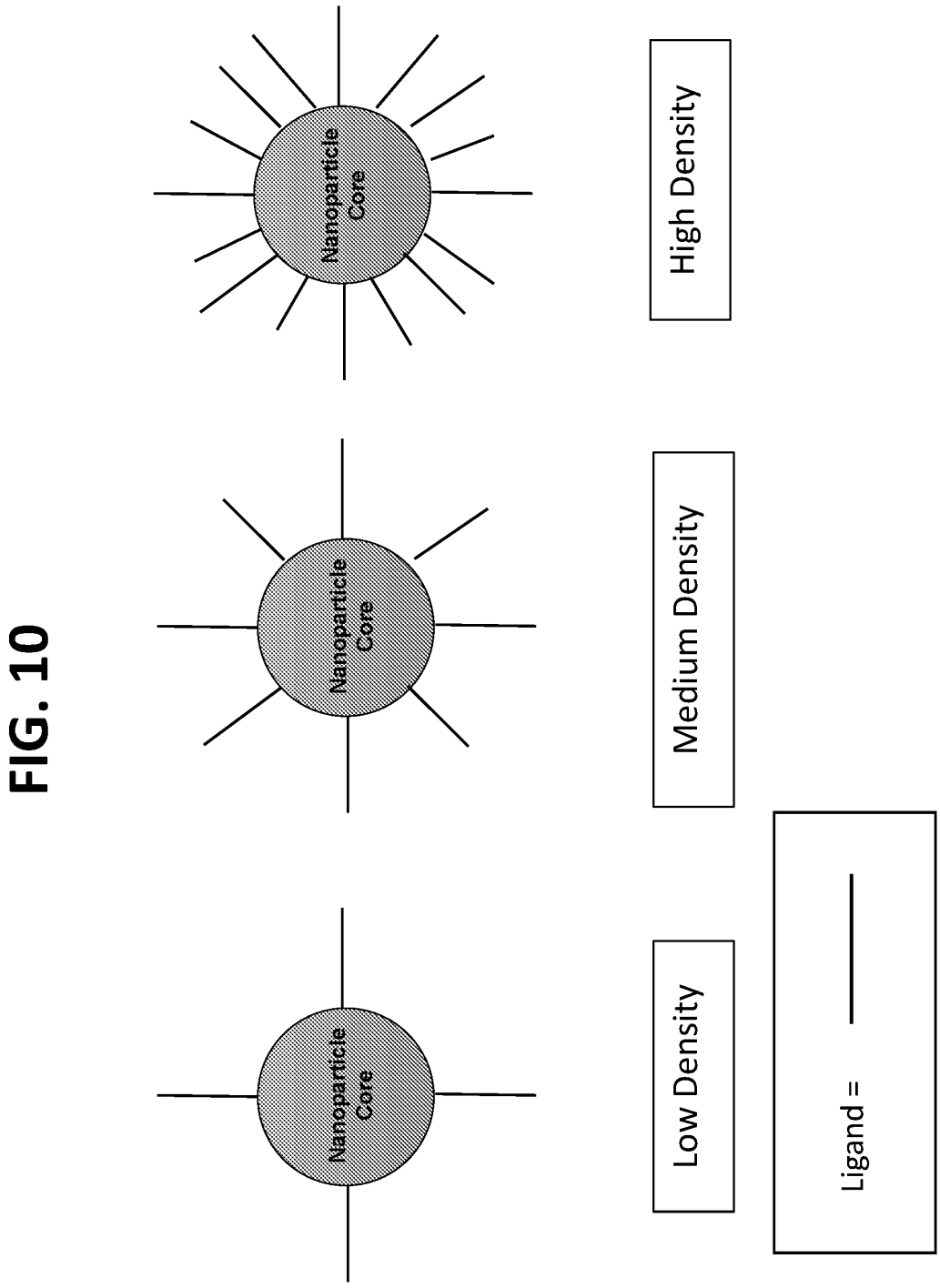
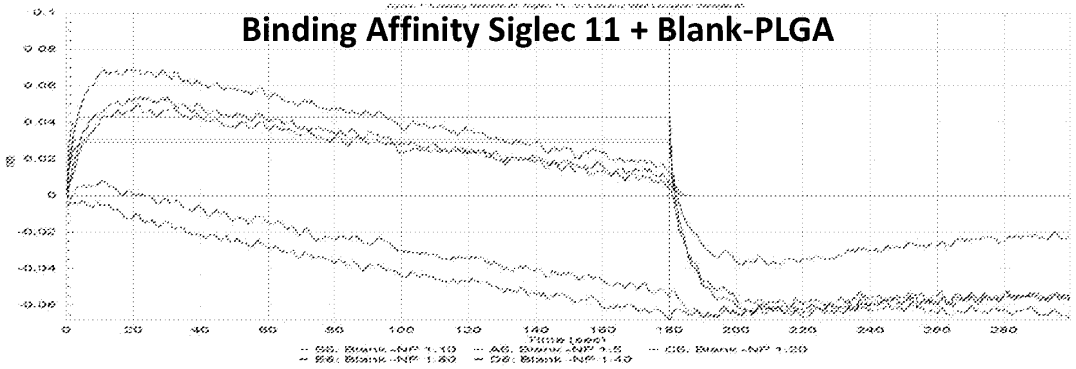
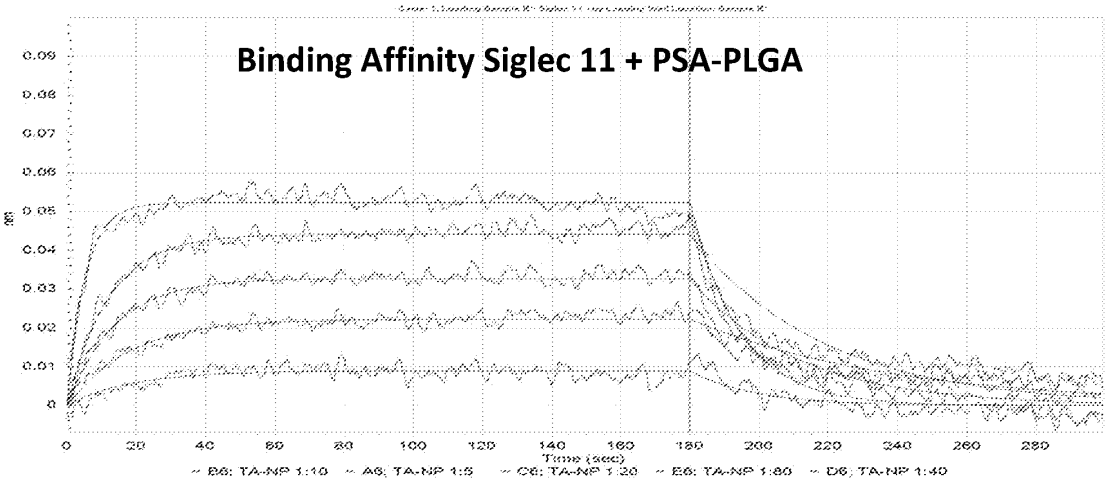

Nanoparticle Core
Nanoparticle Core
Nanoparticle Core
High Density
Medium Density
Low Density
Ligand =

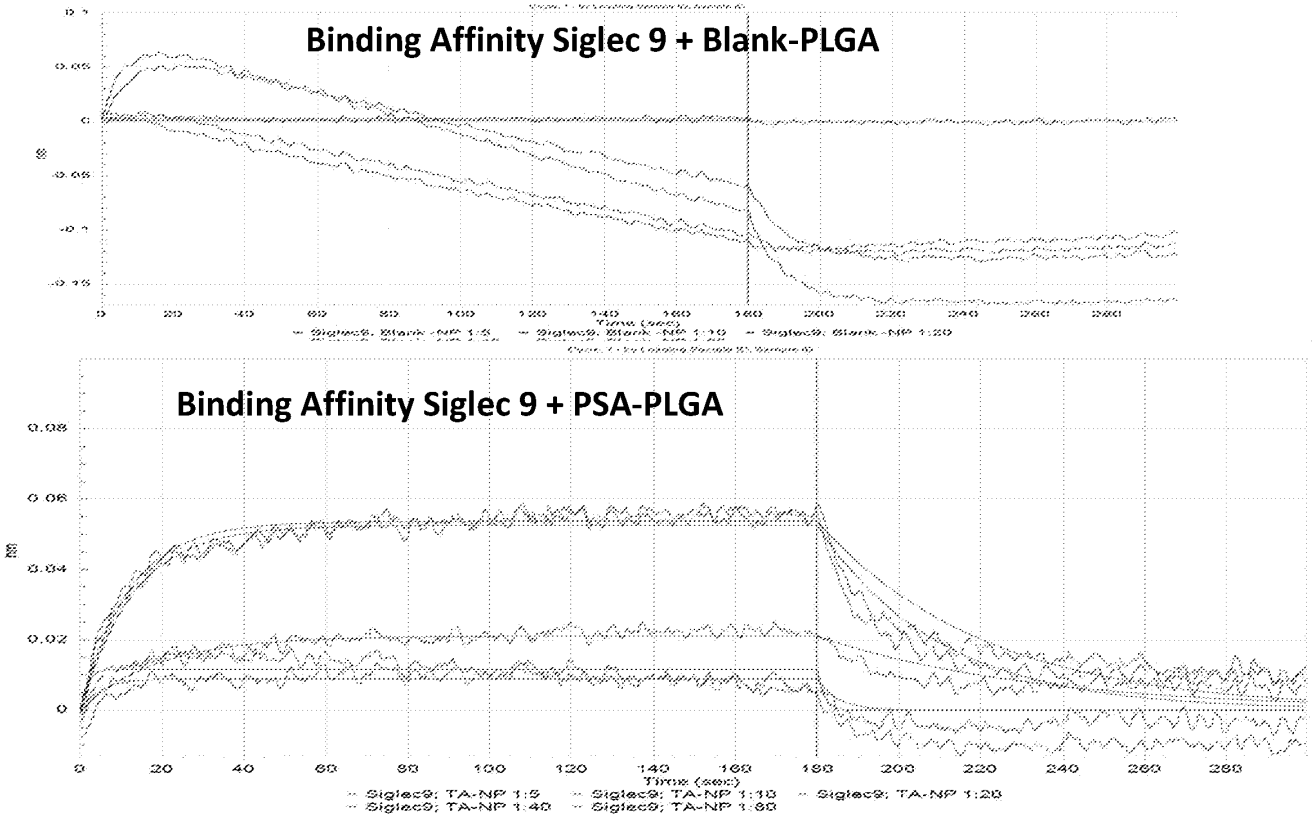
FIG. 11 (a) Nanoparticle binds with high affinity to Siglec 11 in a dose dependent fashion.





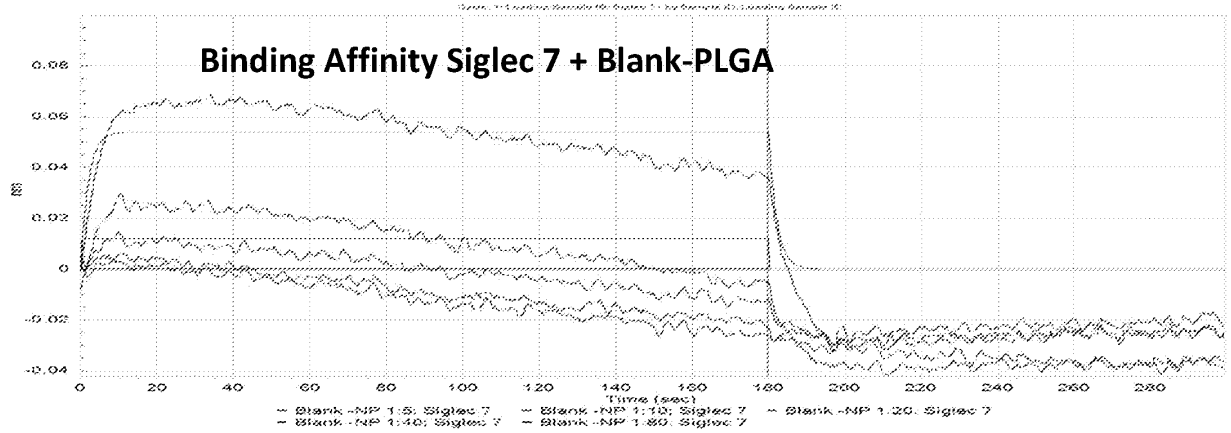
Sample ID on AHC (Anti-hIgG Fc Capture)	Response-Association	kdis(1/s) dissociation
Blank -NP 1:5	0.0185	NA
Blank -NP 1:10	0.0099	NA
Blank -NP 1:20	0.0141	NA
Blank -NP 1:40	-0.0501	NA
Blank -NP 1:80	-0.0629	NA
TA-NP 1:5	0.0488	6.98E-02
TA-NP 1:10	0.0444	2.62E-02
TA-NP 1:20	0.0336	3.24E-02
TA-NP 1:40	0.0235	1.70E-02
TA-NP 1:80	0.0068	5.38E-02

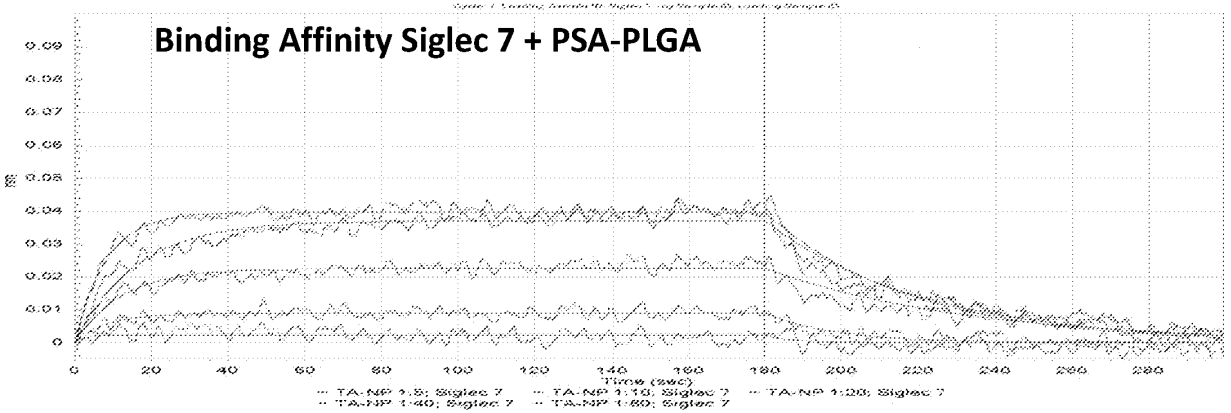
FIG. 11 (b) Nanoparticle binds with high affinity to Siglec 9 in a dose dependent fashion.



Sample ID on AHC (Anti-hIgG Fc Capture)	Response-Association	kdis(1/s) dissociation
Blank -NP 1:5	-0.077	NA
Blank -NP 1:10	-0.0545	NA
Blank -NP 1:20	-0.1001	NA
Blank -NP 1:40	-0.1067	NA
Blank -NP 1:80	0.0041	NA
TA-NP 1:5	0.0563	2.45E-02
TA-NP 1:10	0.0074	2.03E-01
TA-NP 1:20	0.0227	1.89E-02
TA-NP 1:40	0.0557	3.38E-02
TA-NP 1:80	0.0055	6.21E-01

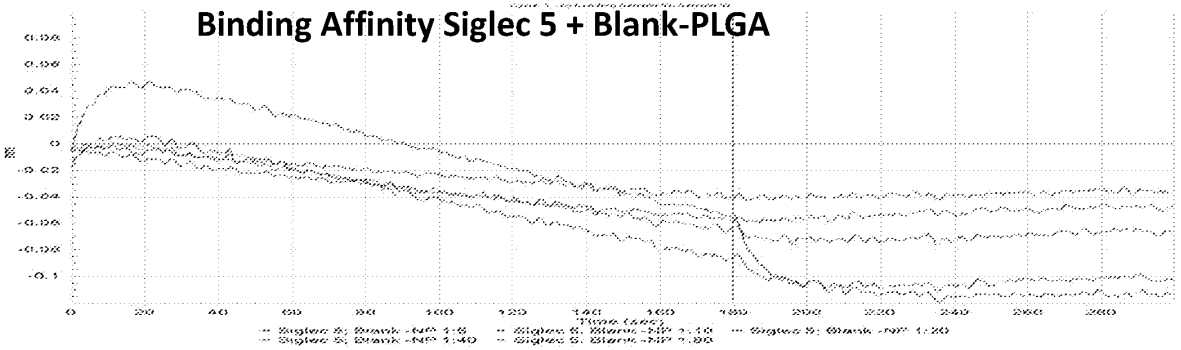
FIG. 11 (c) Nanoparticle binds with high affinity to Siglec 7 in a dose dependent fashion.

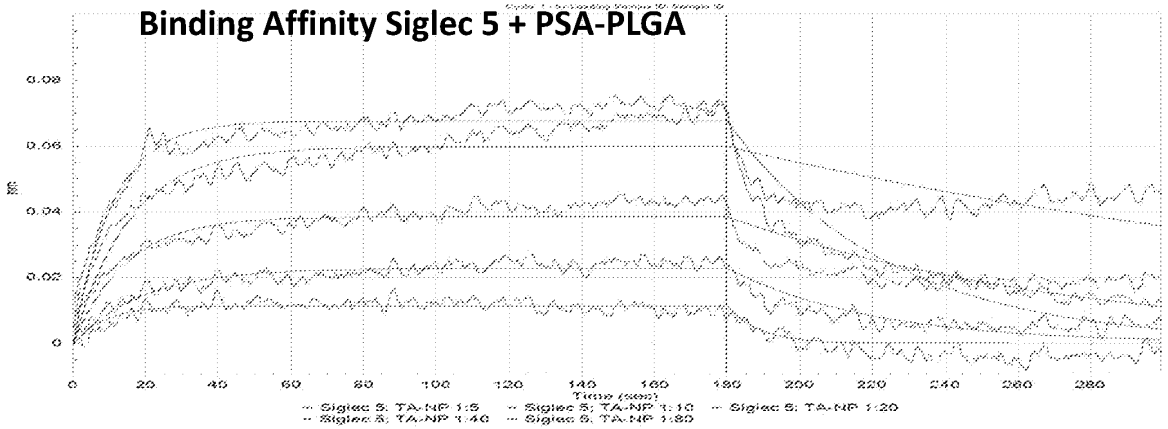




Sample ID on AHC (Anti-hIgG Fc Capture)	Response-Association	kdis(1/s) dissociation
Blank -NP 1:5	0.0377	NA
Blank -NP 1:10	-0.0056	NA
Blank -NP 1:20	-0.0118	NA
Blank -NP 1:40	-0.0253	NA
Blank -NP 1:80	-0.0199	NA
TA-NP 1:5	0.0398	2.69E-02
TA-NP 1:10	0.0385	2.43E-02
TA-NP 1:20	0.024	1.89E-02
TA-NP 1:40	0.0089	5.51E-02
TA-NP 1:80	0	1.52E-01

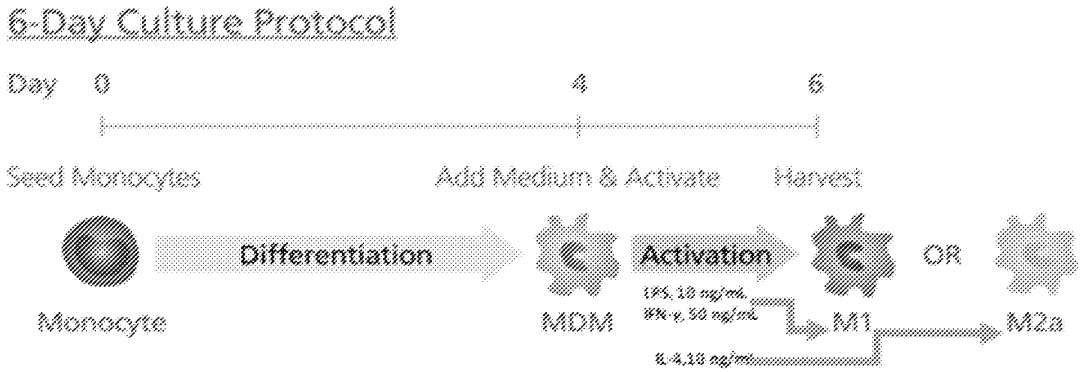
FIG. 11 (d) Nanoparticle binds with high affinity to Siglec 5 in a dose dependent fashion.





Sample ID AHC (Anti-hIgG Fc	Loading Sample ID	Response	kdis(1/s)
Blank -NP 1:5	Siglec 5	-0.0498	NA
Blank -NP 1:10	Siglec 5	-0.0843	NA
Blank -NP 1:20	Siglec 5	-0.063	NA
Blank -NP 1:40	Siglec 5	-0.0551	NA
Blank -NP 1:80	Siglec 5	-0.0389	NA
TA-NP 1:5	Siglec 5	0.0723	2.29E-02
TA-NP 1:10	Siglec 5	0.0694	4.31E-03
TA-NP 1:20	Siglec 5	0.0434	1.03E-02
TA-NP 1:40	Siglec 5	0.0242	2.51E-02
TA-NP 1:80	Siglec 5	0.0101	1.12E-01

FIG. 12 (a) Nanoparticle is non-toxic to peripheral blood monocytes as demonstrated by an MTT assay.



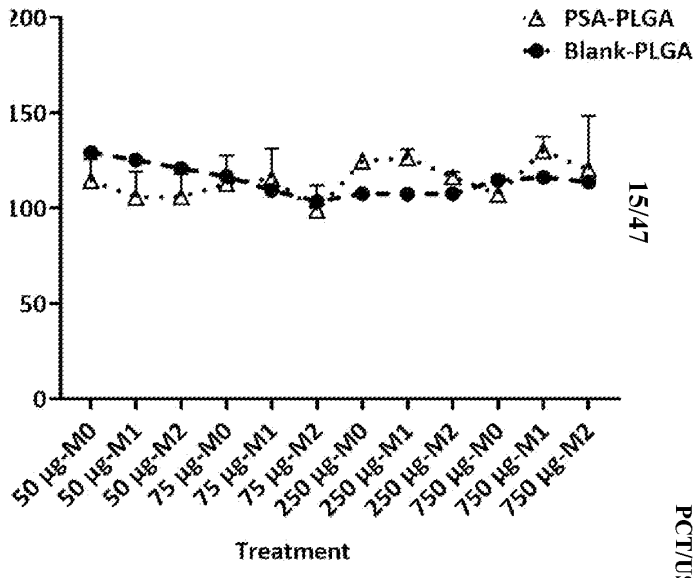
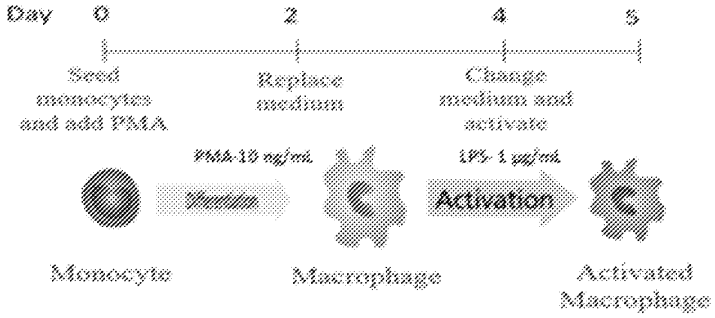


FIG. 12 (b) Nanoparticle is non-toxic to THP-1 monocyte derived macrophages as demonstrated by MTT assay



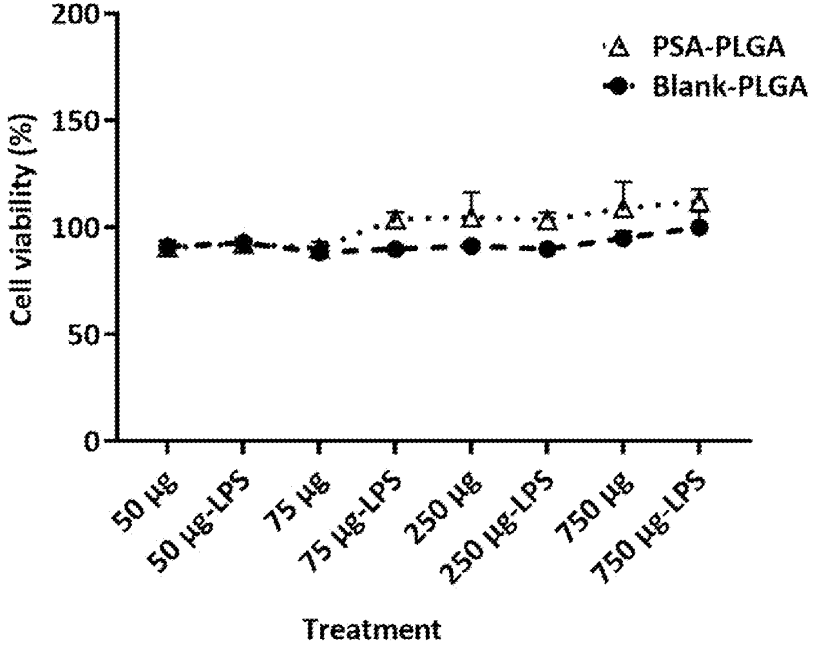


FIG. 13. Showing that exemplary nanoparticles increase IL-10 in activated macrophages .

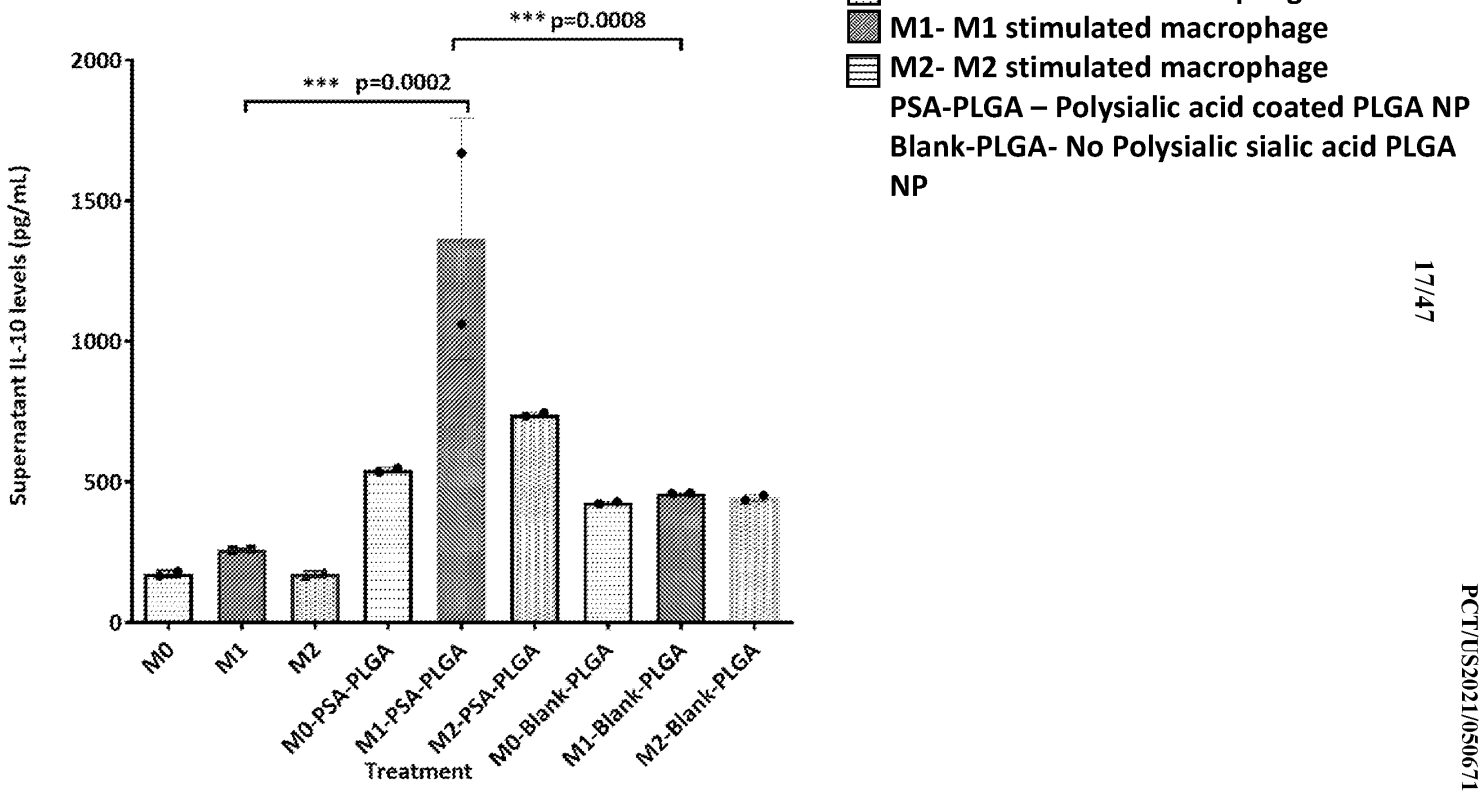


FIG. 14.(a) Nanoparticles increase CFH production

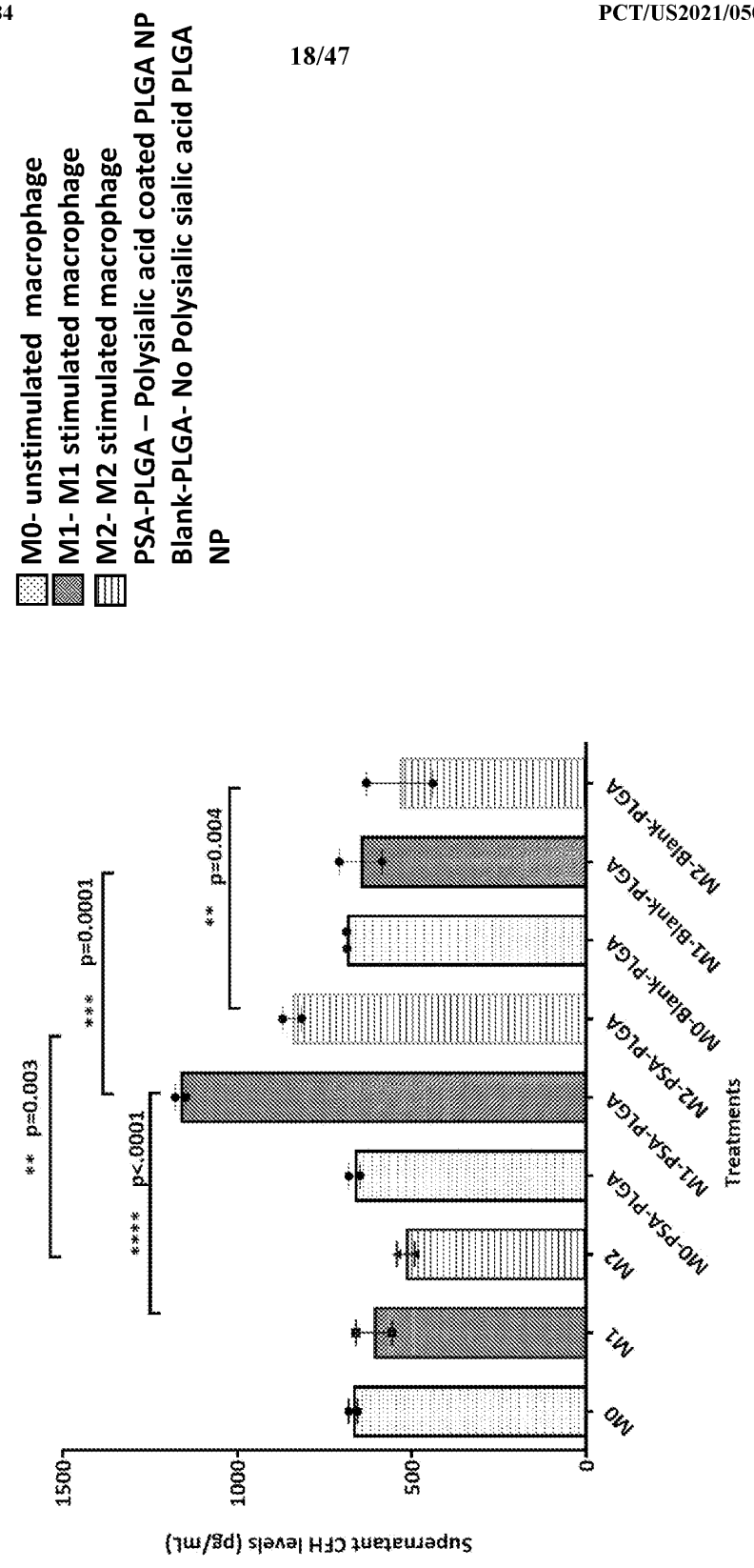


FIG. 14.(b) Nanoparticles decrease C3 production in macrophages

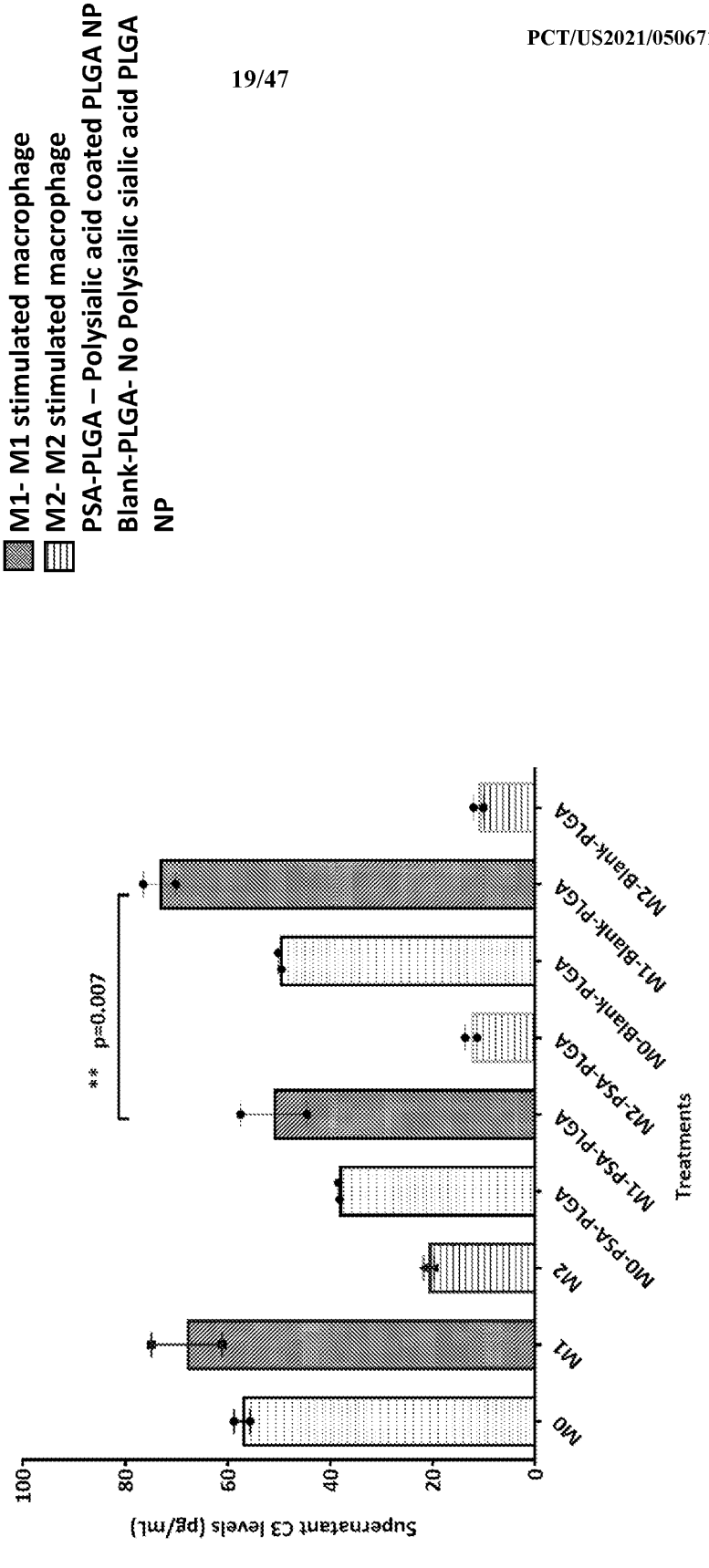


FIG. 15 Nanoparticles inhibit TNF-alpha in LPS-activated macrophages .

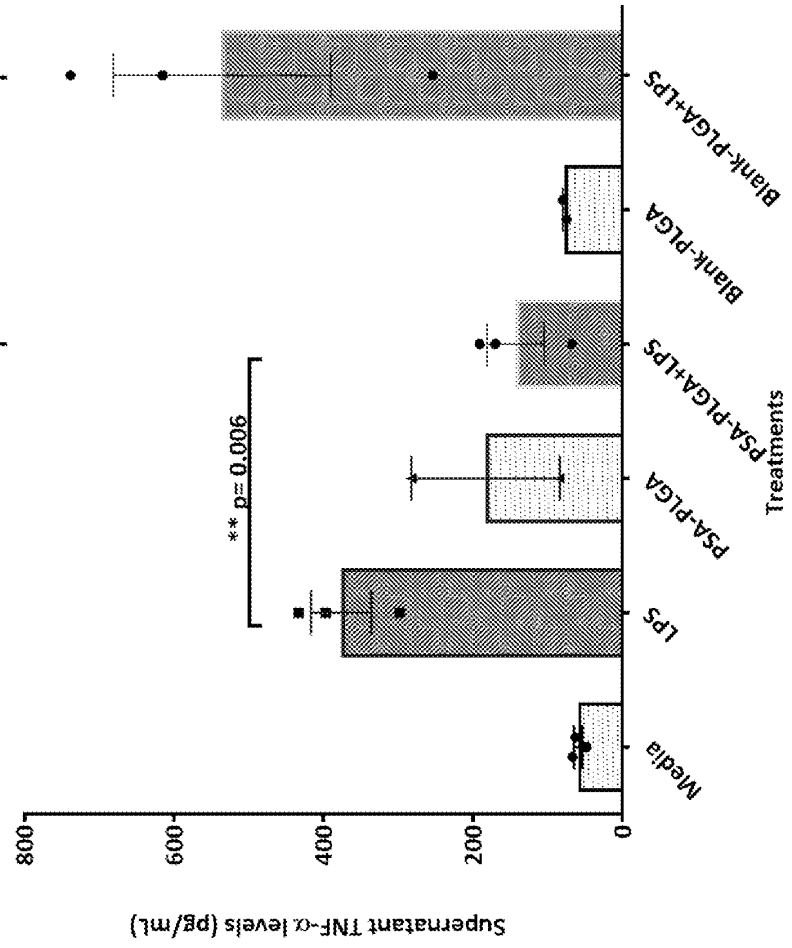
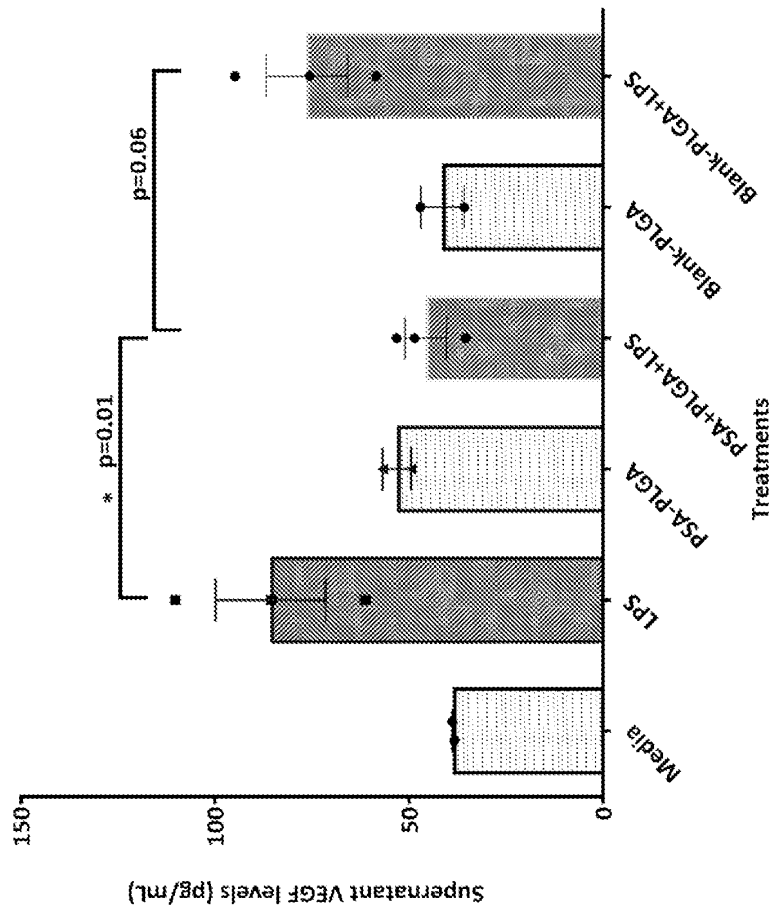


FIG. 16 Nanoparticles significantly reduce VEGF in LPS-activated macrophages.



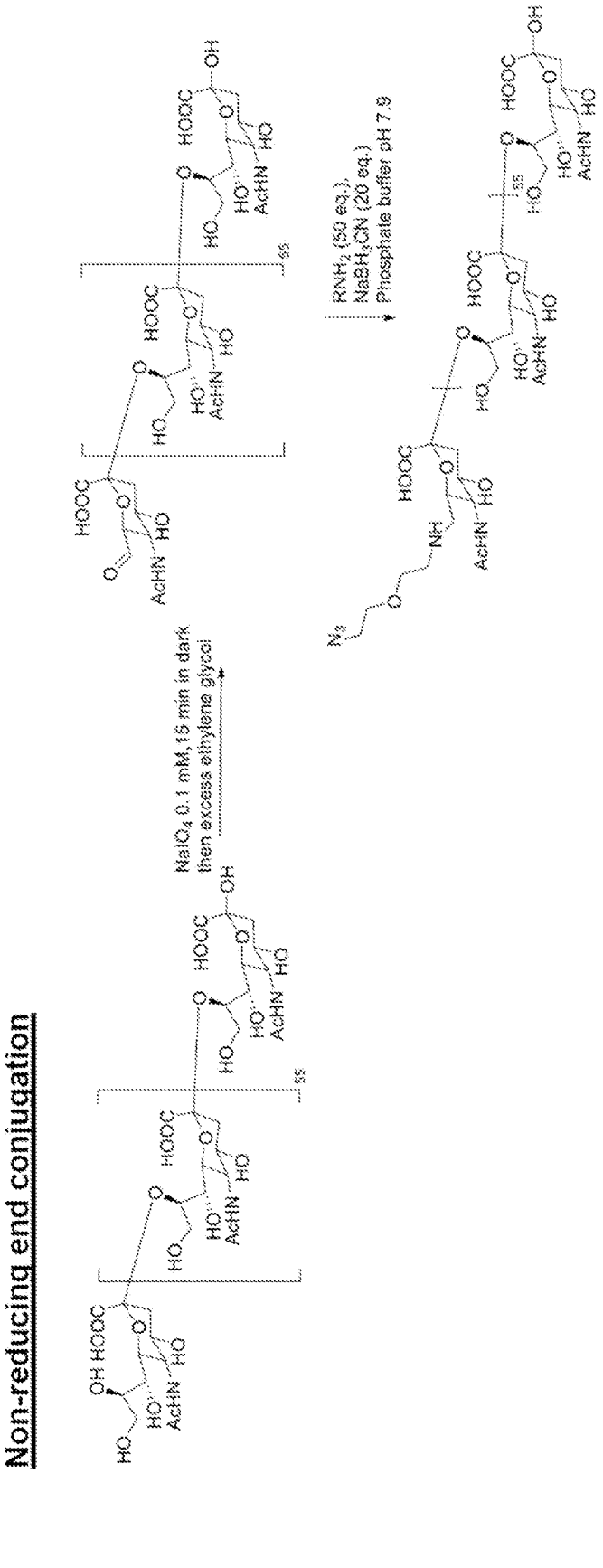

Non-reducing end conjugation
NaIO4 0.1 mM,15 min in dark
then excess ethylene glycol
RNH2 (50 eq.),
NaBH3CN (20 eq.)
Phosphate buffer pH 7.9

FIG. 17

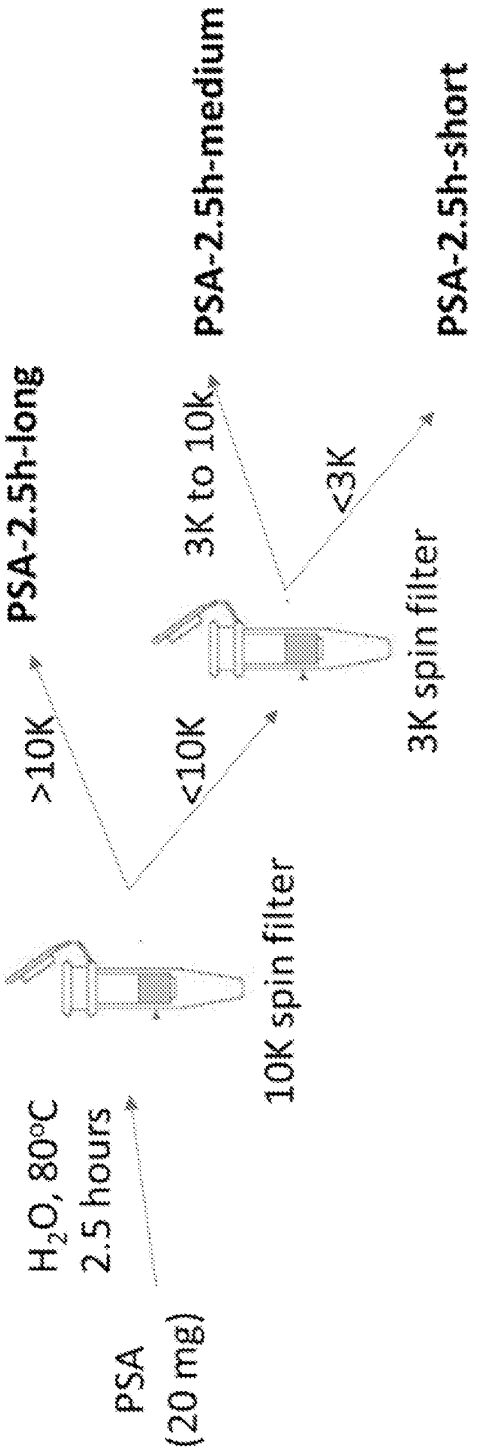

PSA
(20 mg)
H_2O, 80°C
2.5 hours
10K spin filter
>10K
<10K
PSA-2.5h-long
3K spin filter
3K to 10k
<3K
PSA-2.5h-medium
PSA-2.5h-short

FIG. 18

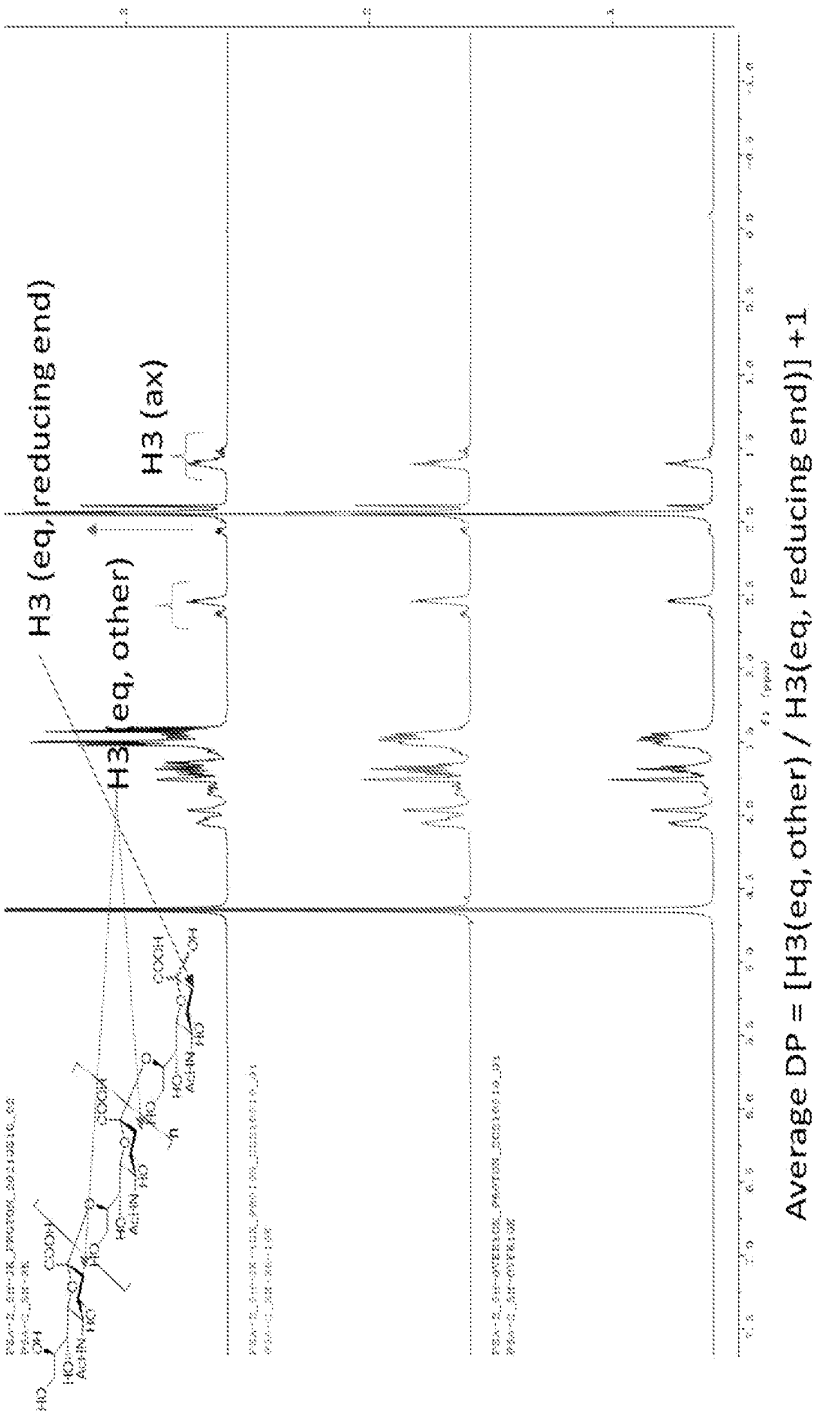

H3 (eq, reducing end)
H3 (eq, other)
H3
H3 (ax)
Average DP = [H3(eq, other) / H3(eq, reducing end)] +1

FIG. 19

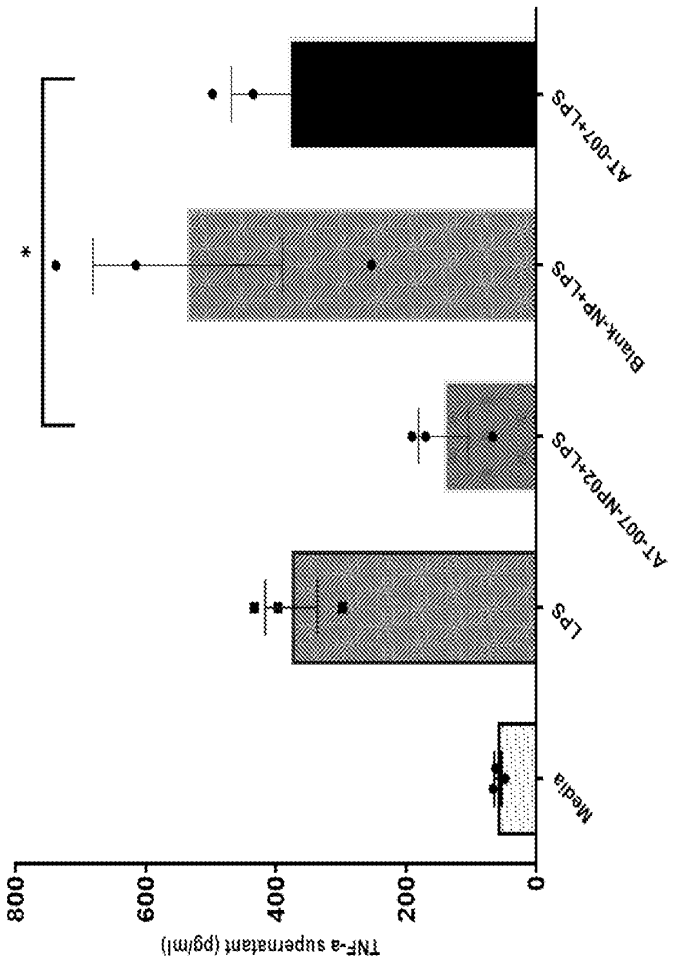

TNF-a supernatant (pg/ml)
800
600
400
200
0
Media
LPS
AT-007-NP02+LPS
Blank-NP+LPS
AT-007+LPS
*

FIG. 20

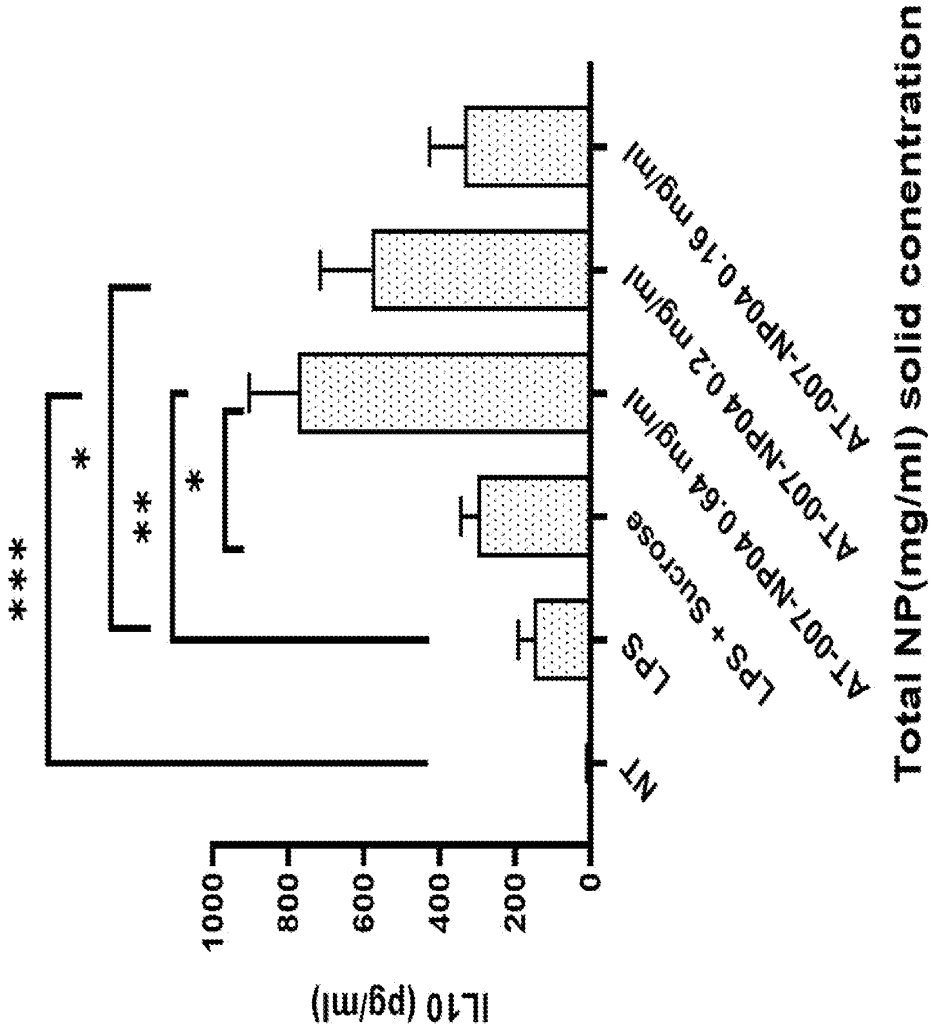

IL10 (pg/ml)
1000
800
600
400
200
0
NT
LPS
LPS + Sucrose
AT-007-NP04 0.64 mg/ml
AT-007-NP04 0.2 mg/ml
AT-007-NP04 0.16 mg/ml
Total NP(mg/ml) solid conentration
****
*
**
*

FIG. 21

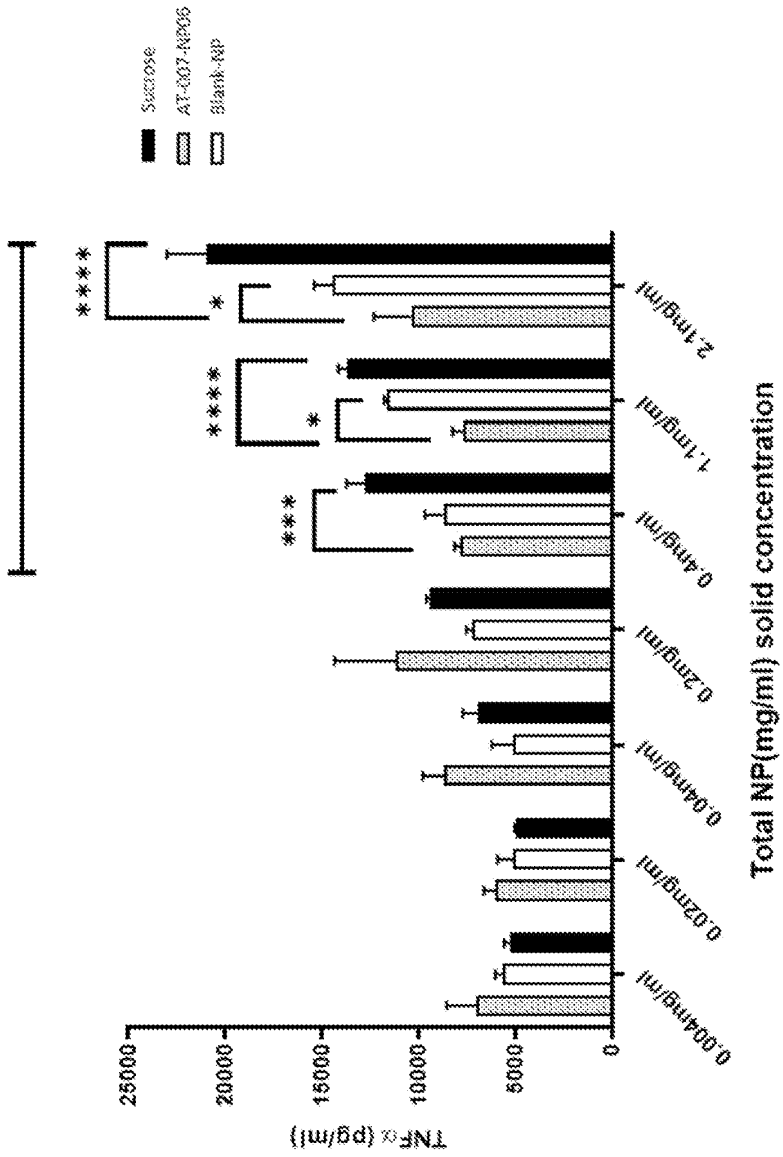

Sucrose
AT-007-NPs6
Blank-NP
TNFα (pg/ml)
25000
20000
15000
10000
5000
0
0.004mg/ml
0.02mg/ml
0.04mg/ml
0.2mg/ml
0.4mg/ml
1.1mg/ml
2.1mg/ml
Total NP(mg/ml) solid concentration
****
*
****
*
***

FIG. 22A

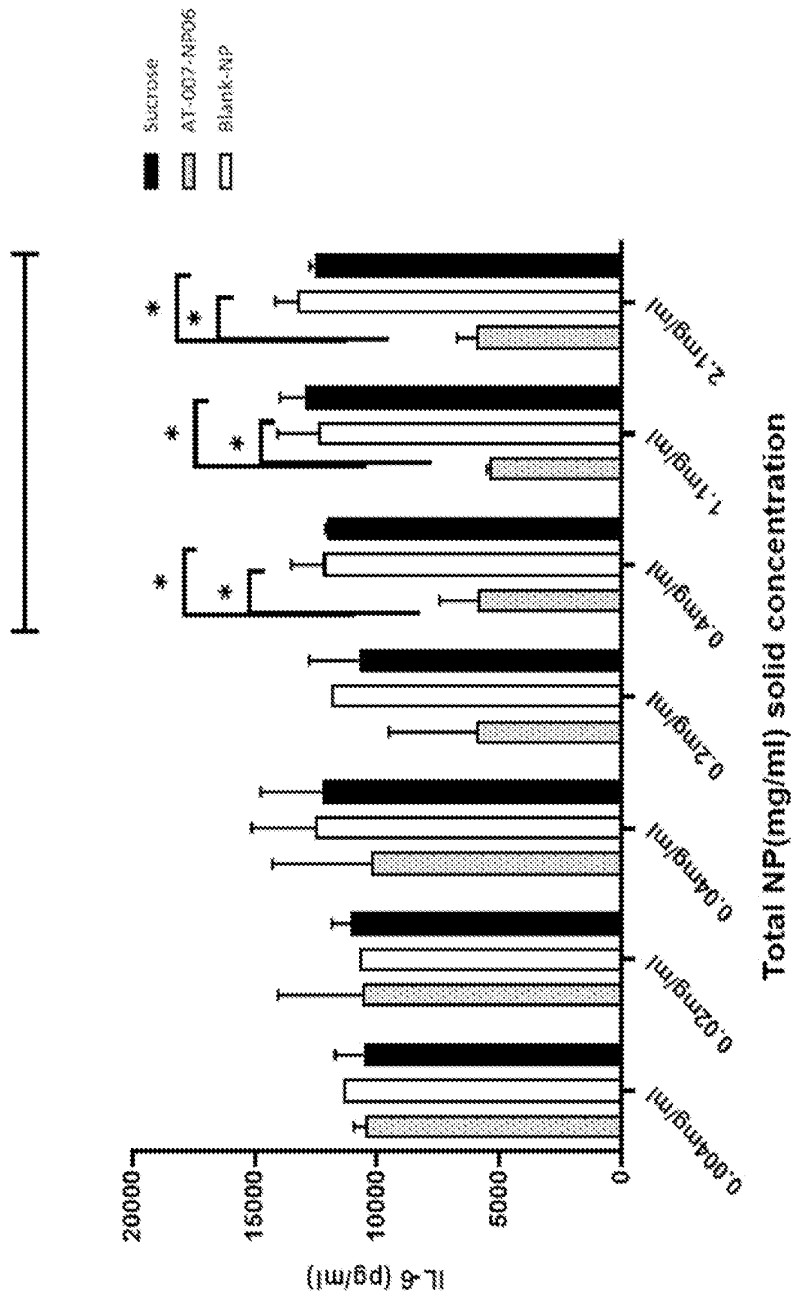

Sucrose
AT-007-NP06
Blank-NP
IL-6 (pg/ml)
20000
15000
10000
5000
0
0.004mg/ml
0.02mg/ml
0.04mg/ml
0.2mg/ml
0.4mg/ml
1.1mg/ml
2.1mg/ml
Total NP(mg/ml) solid concentration

FIG. 22B

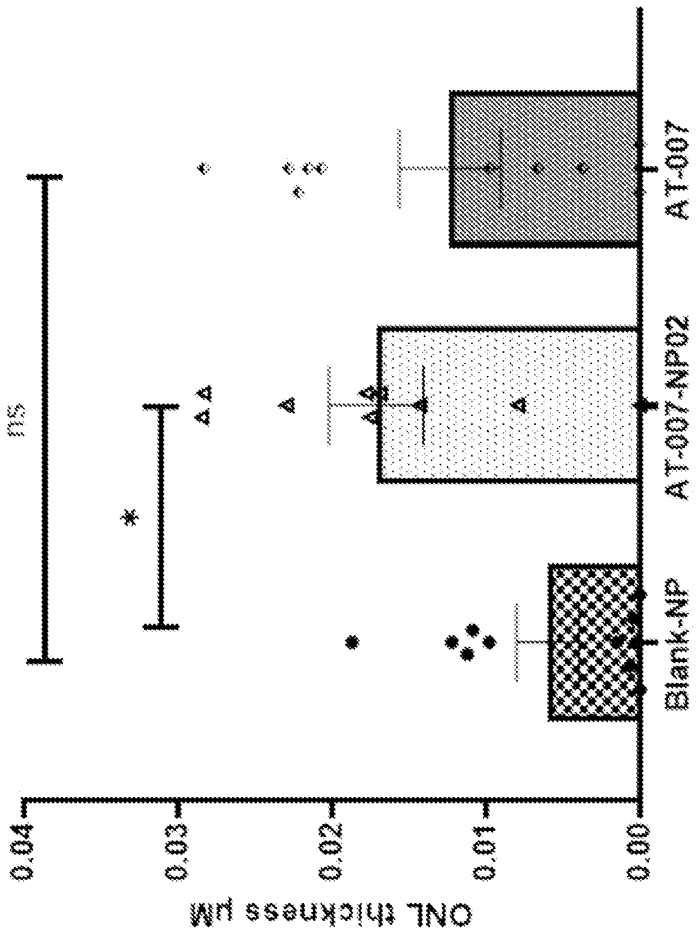

ONL thickness µM
0.04
0.03
0.02
0.01
0.00
ns
Blank-NP
AT-007-NP02
AT-007

FIG. 23

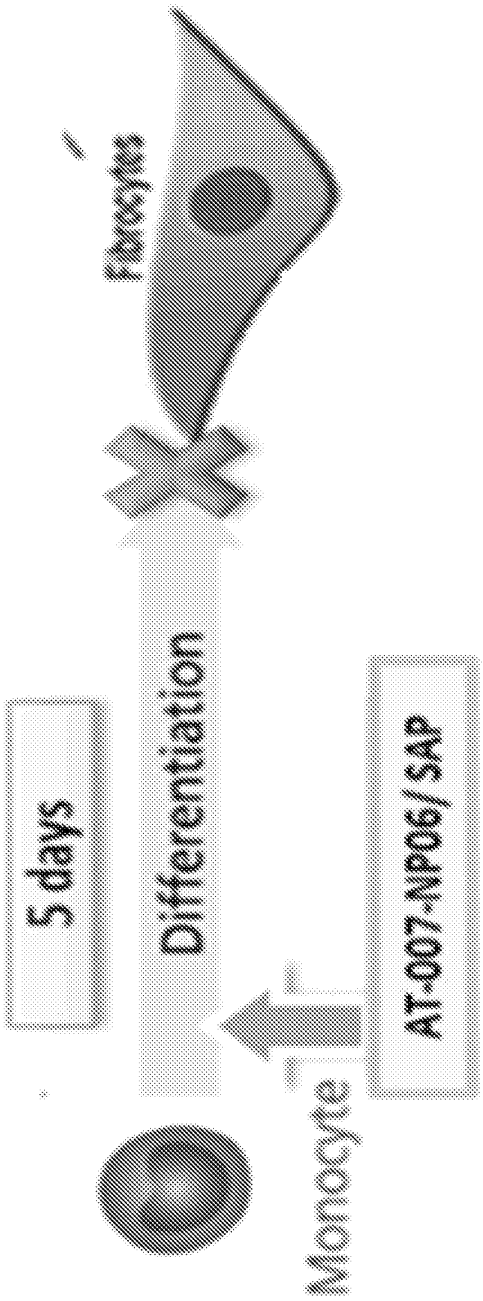

Fibrocytes
5 days
Differentiation
AT-007-NP06/ SAP
Monocyte

FIG. 24

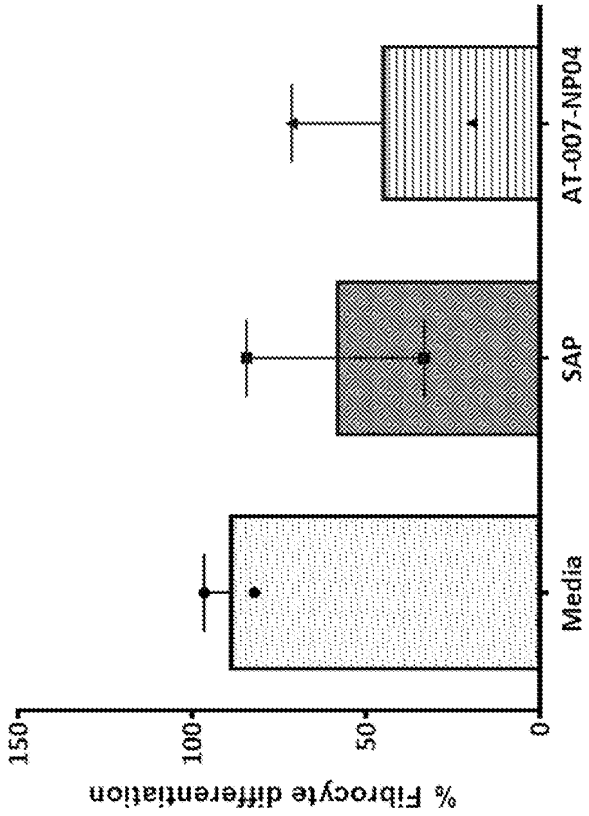

% Fibrocyte differentiation
150
100
50
0
Media
SAP
AT-007-NP04

FIG. 25

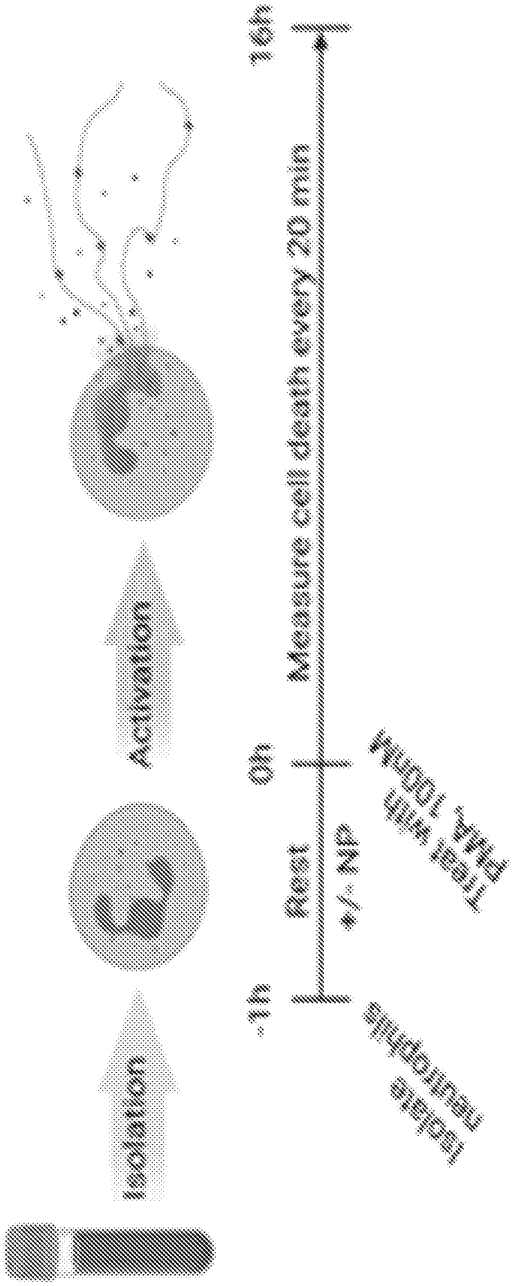

Isolation
Activation
-1h
Rest
0h
+/- NP
Treat with PMA-Ionm
Measure cell death every 20 min
16h
Isolate neutrophils

FIG. 26

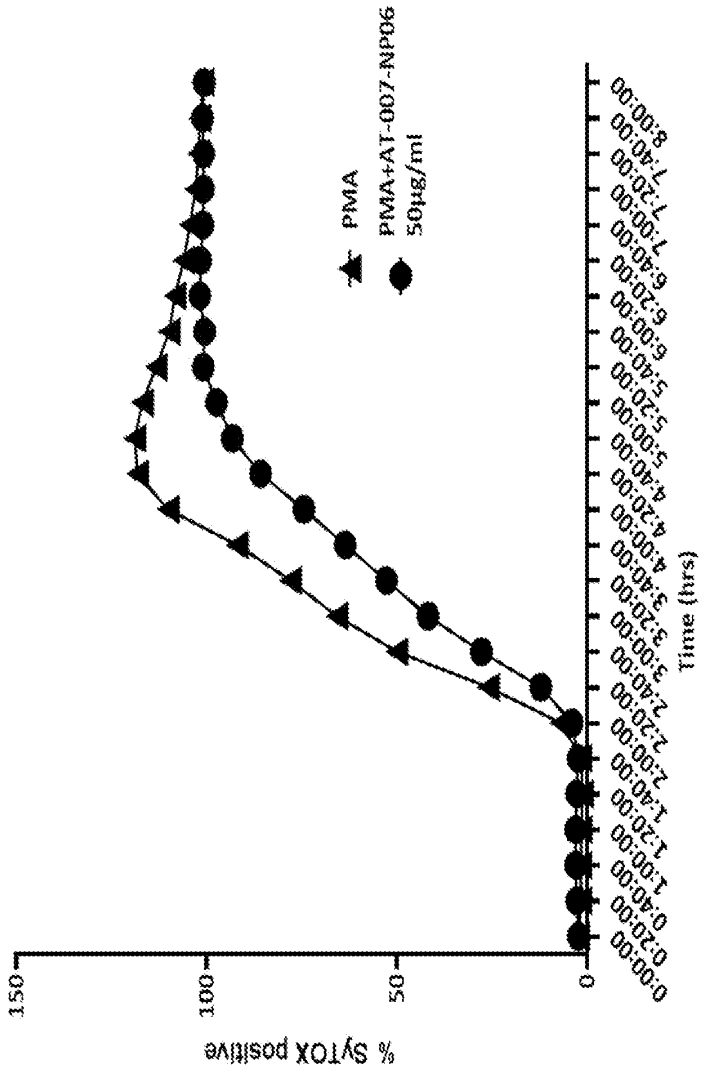

% SYTOX positive
150
100
50
0
Time (hrs)
0:00:00
0:20:00
0:40:00
1:00:00
1:20:00
1:40:00
2:00:00
2:20:00
2:40:00
3:00:00
3:20:00
3:40:00
4:00:00
4:20:00
4:40:00
5:00:00
5:20:00
5:40:00
6:00:00
6:20:00
6:40:00
7:00:00
7:20:00
7:40:00
8:00:00
PMA
PMA+AT-007-NP06
50µg/ml

FIG. 27

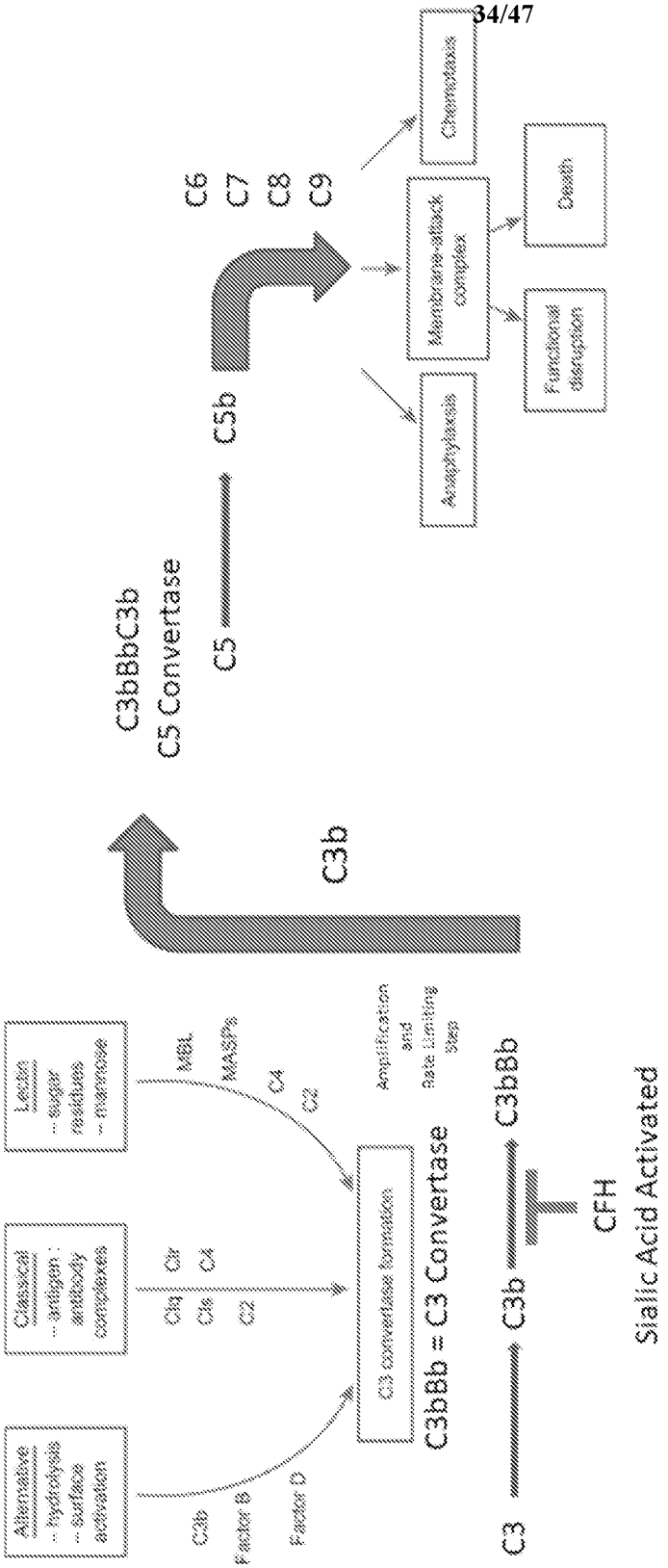

Alternative
– hydrolysis
– surface activation
Classical
– antigen : antibody complexes
Lectin
– sugar residues
– mannose
C3b
Factor B
Factor D
C1q
C1s
C1r
C4
C2
MBL
MASPs
C4
C2
C3 convertase formation
Amplification and Rate Limiting Step
C3bBb = C3 Convertase
C3
C3b
C3bBb
CFH
Sialic Acid Activated
C3b
C3bBbC3b
C5 Convertase
C5
C5b
C6
C7
C8
C9
Anaphylaxis
Membrane-attack complex
Functional disruption
Death
Chemotaxis

FIG. 28

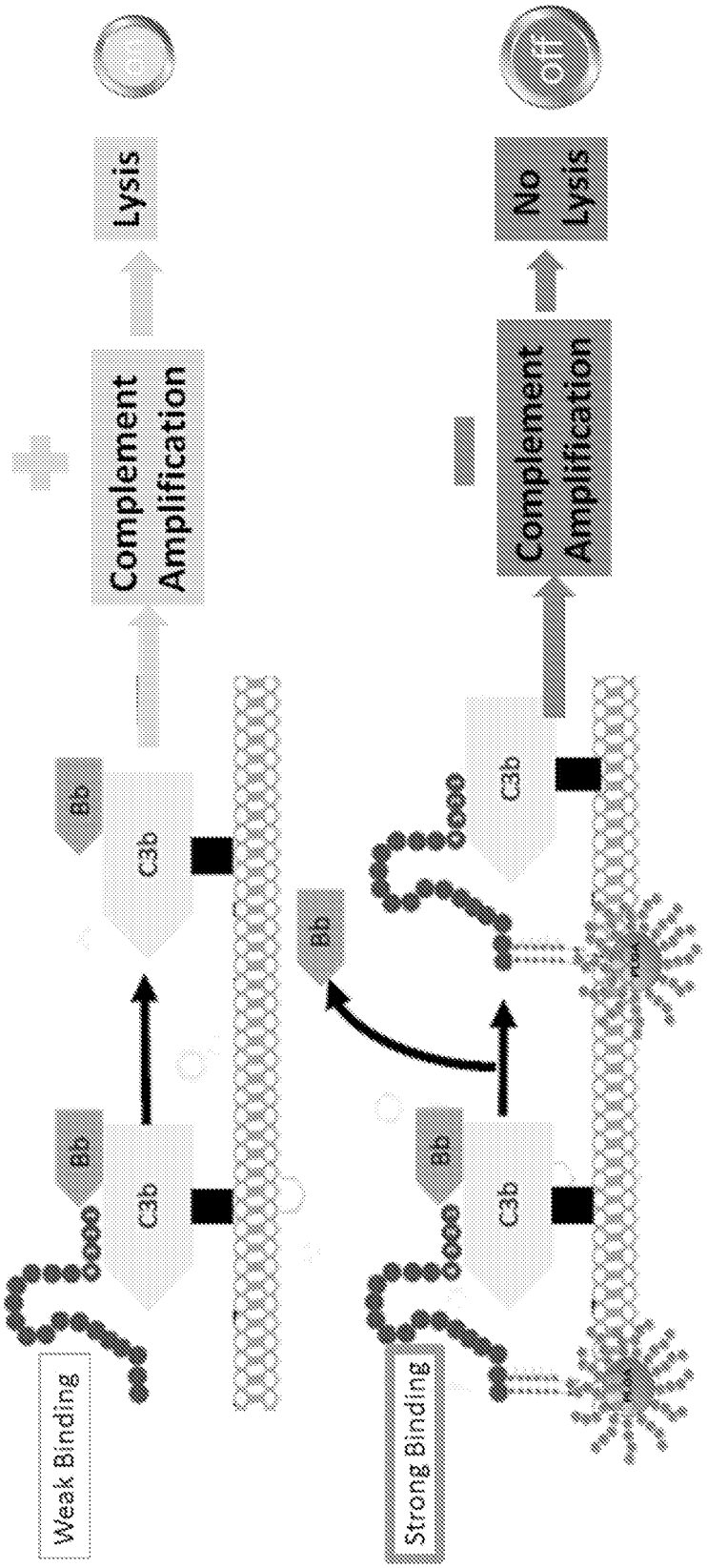

Weak Binding
Bb
C3b
Bb
C3b
Complement Amplification
Lysis
Strong Binding
Bb
C3b
Bb
C3b
Complement Amplification
No Lysis
Off

FIG. 29

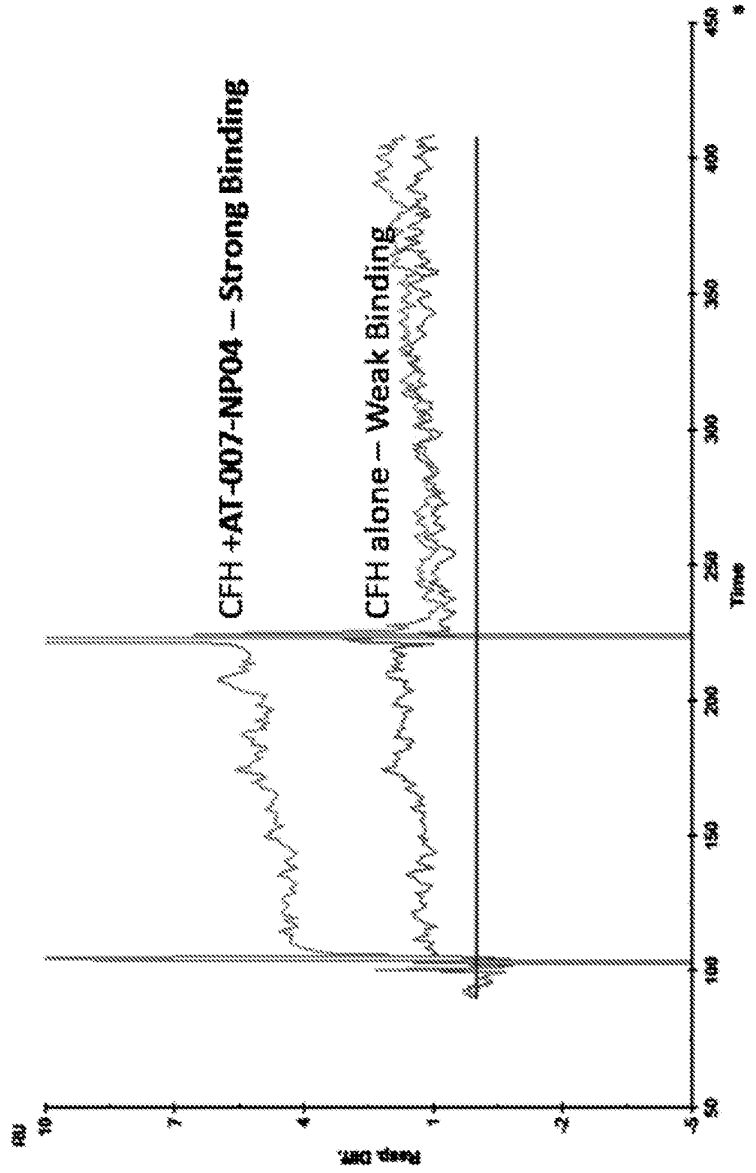

CFH +AT-007-NP04 – Strong Binding
CFH alone – Weak Binding
RU
Resp Diff.
Time
s
10
7
4
1
-2
-5
50
100
150
200
250
300
350
400
450

FIG. 30A

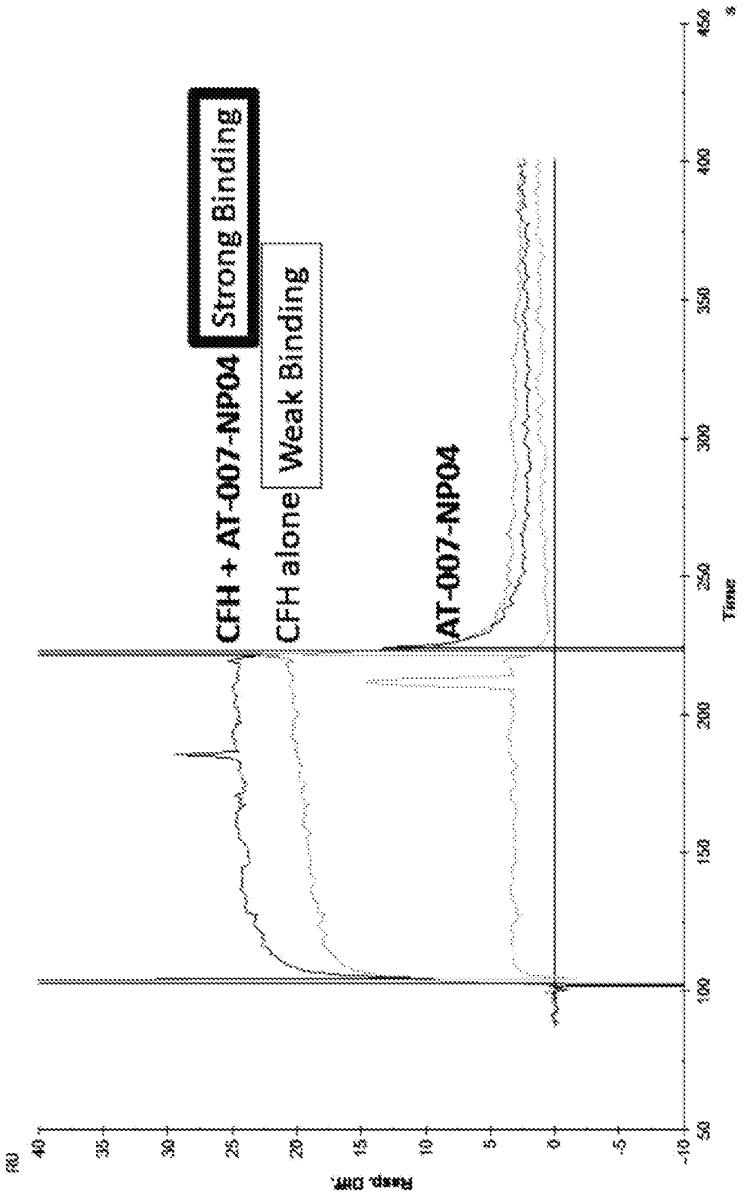

CFH + AT-007-NP04
Strong Binding
CFH alone
Weak Binding
AT-007-NP04
Resp. Diff.
Time
s
RU
40
35
30
25
20
15
10
5
0
-5
-10
50
100
150
200
250
300
350
400
450

FIG. 30B

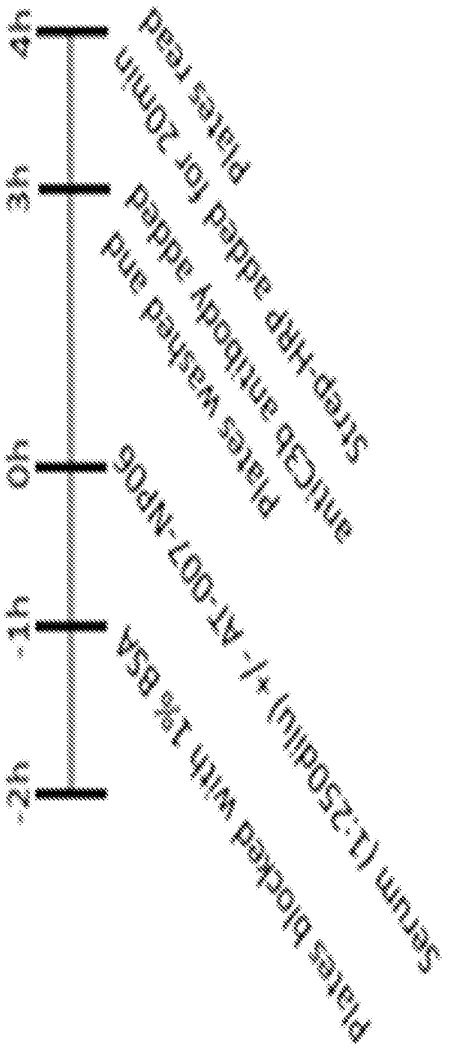

plates bloctked with 1% BSA
Serum (1:250dilu) +/- AT-007-NP06
plates washed and
anti-CD3 antibody added
Strep-HRP added for 20min
plates read
-1h
0h
1h
4h

FIG. 31A

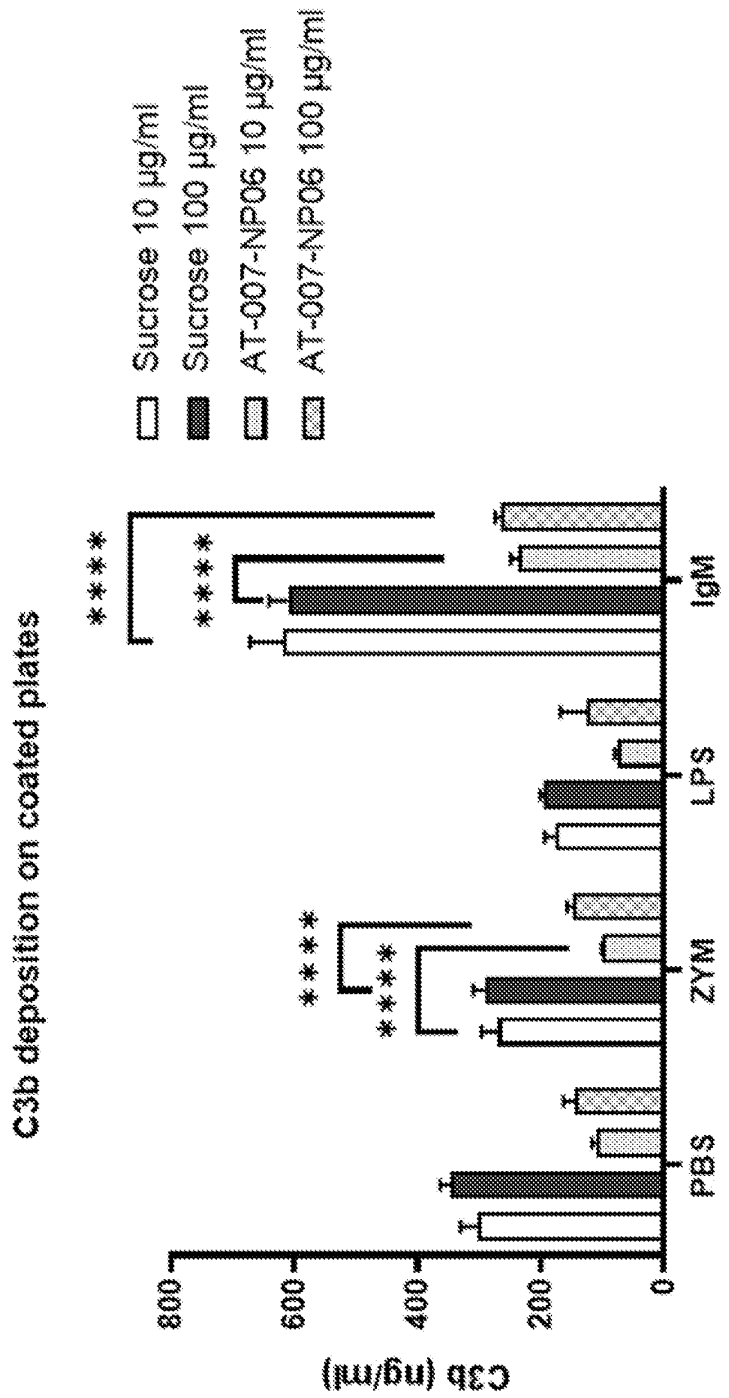

C3b deposition on coated plates
Sucrose 10 µg/ml
Sucrose 100 µg/ml
AT-007-NP06 10 µg/ml
AT-007-NP06 100 µg/ml
C3b (ng/ml)
800
600
400
200
0
PBS
ZYM
LPS
IgM
****
****
****
****

FIG. 31B

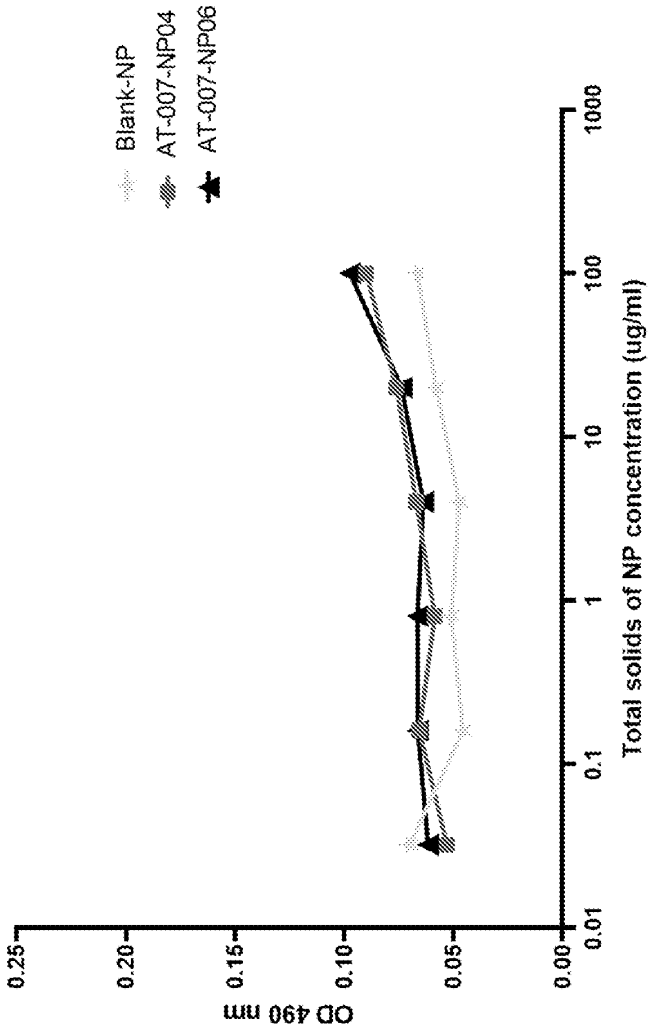

Blank-NP
AT-007-NP04
AT-007-NP06
OD 490 nm
0.25
0.20
0.15
0.10
0.05
0.00
0.01
0.1
1
10
100
1000
Total solids of NP concentration (ug/ml)

FIG. 32A

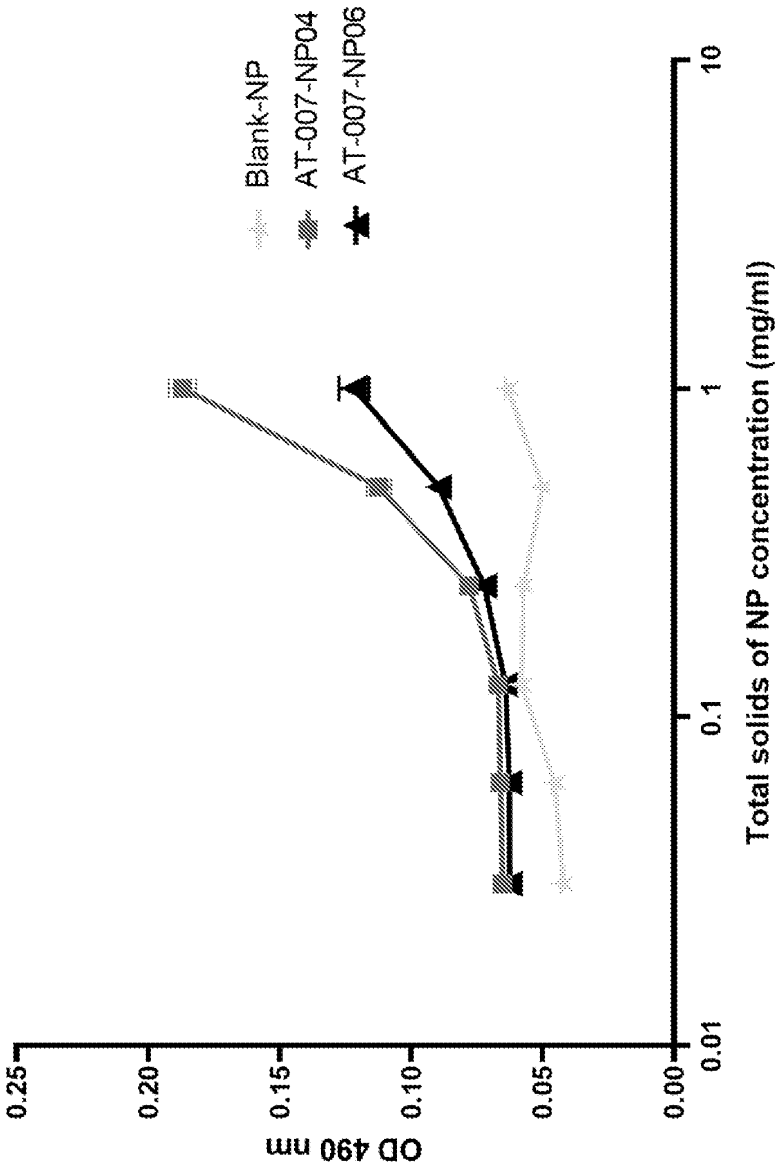

Blank-NP
AT-007-NP04
AT-007-NP06
OD 490 nm
0.25
0.20
0.15
0.10
0.05
0.00
0.01
0.1
1
10
Total solids of NP concentration (mg/ml)

FIG. 32B

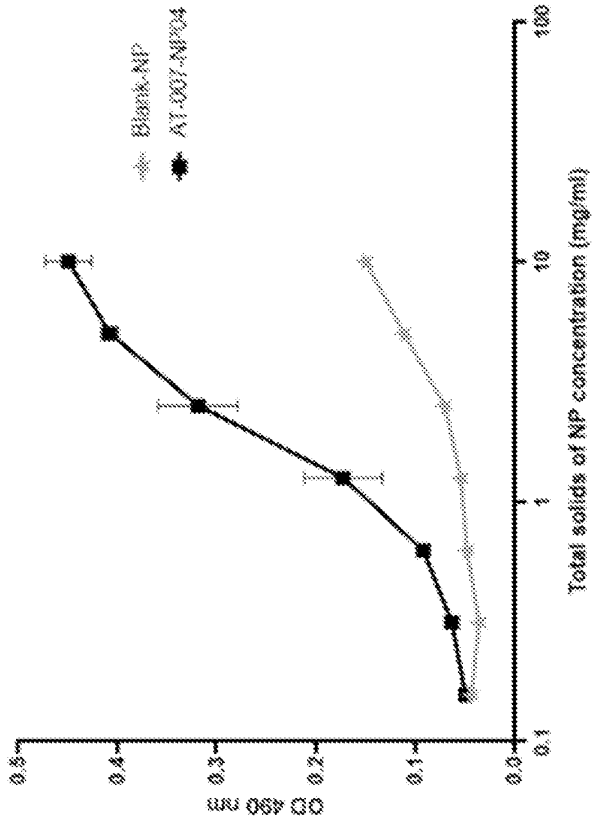

Blank NP
AT-007-NP24
OD 490 nm
0.5
0.4
0.3
0.2
0.1
0.0
0.1
1
10
100
Total solids of NP concentration (mg/ml)

FIG. 32C

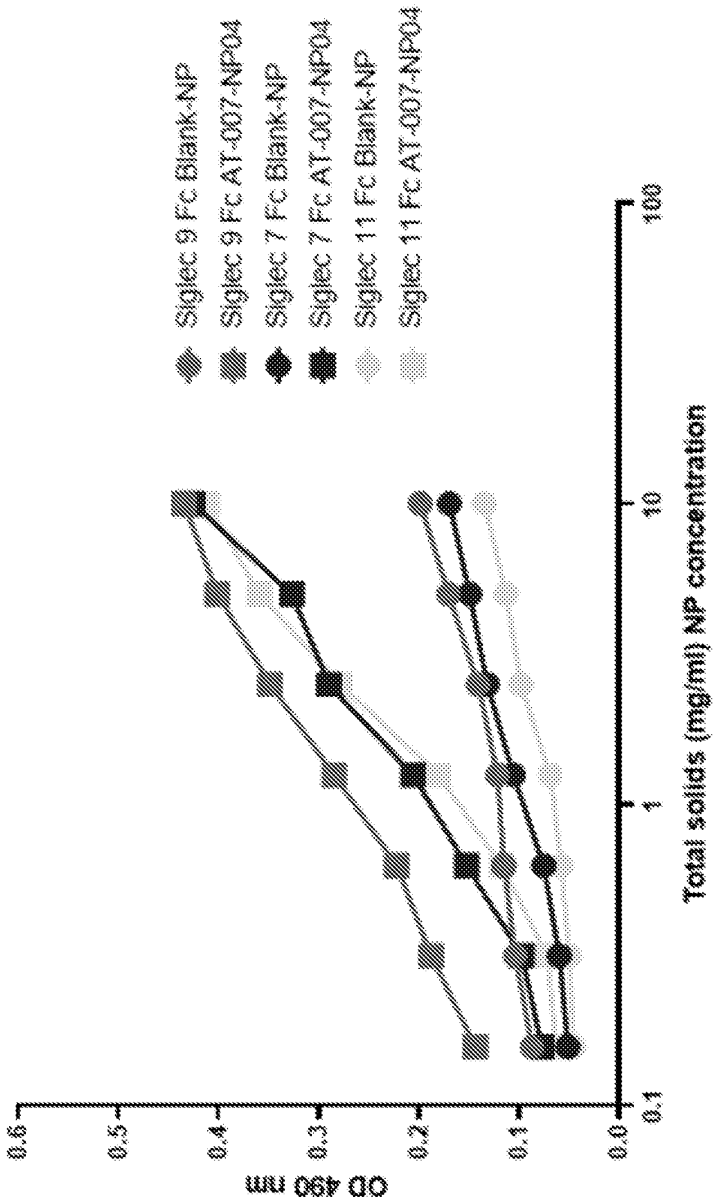

Siglec 9 Fc Blank-NP
Siglec 9 Fc AT-007-NP04
Siglec 7 Fc Blank-NP
Siglec 7 Fc AT-007-NP04
Siglec 11 Fc Blank-NP
Siglec 11 Fc AT-007-NP04
OD 490 nm
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.1
1
10
100
Total solids (mg/ml) NP concentration

FIG. 33

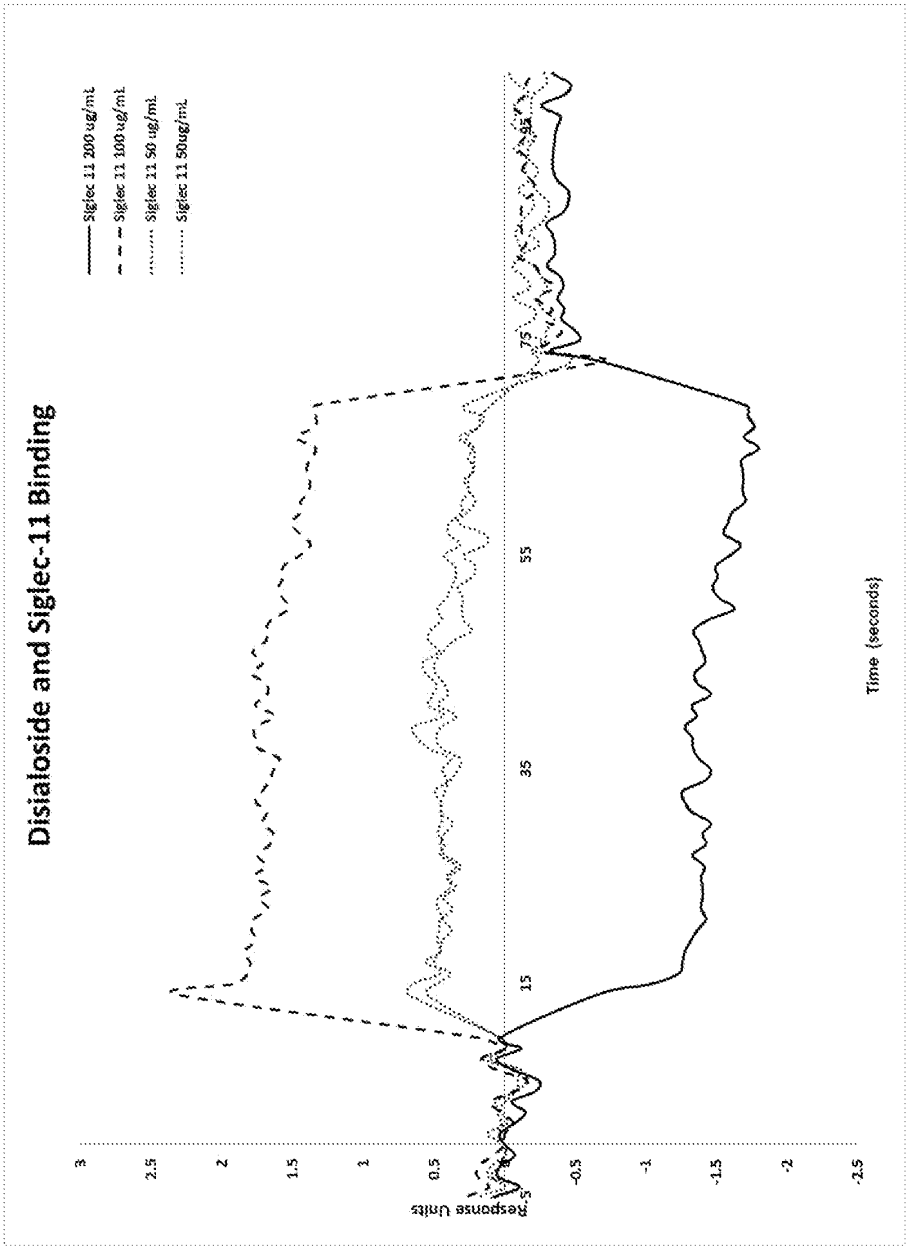

Disialoside and Siglec-11 Binding
Siglec 11 200 ug/mL
Siglec 11 100 ug/mL
Siglec 11 50 ug/mL
Siglec 11 50ug/mL
Response Units
3
2.5
2
1.5
1
0.5
-0.5
-1
-1.5
-2
-2.5
-5
15
35
55
75
Time (seconds)

FIG. 34A

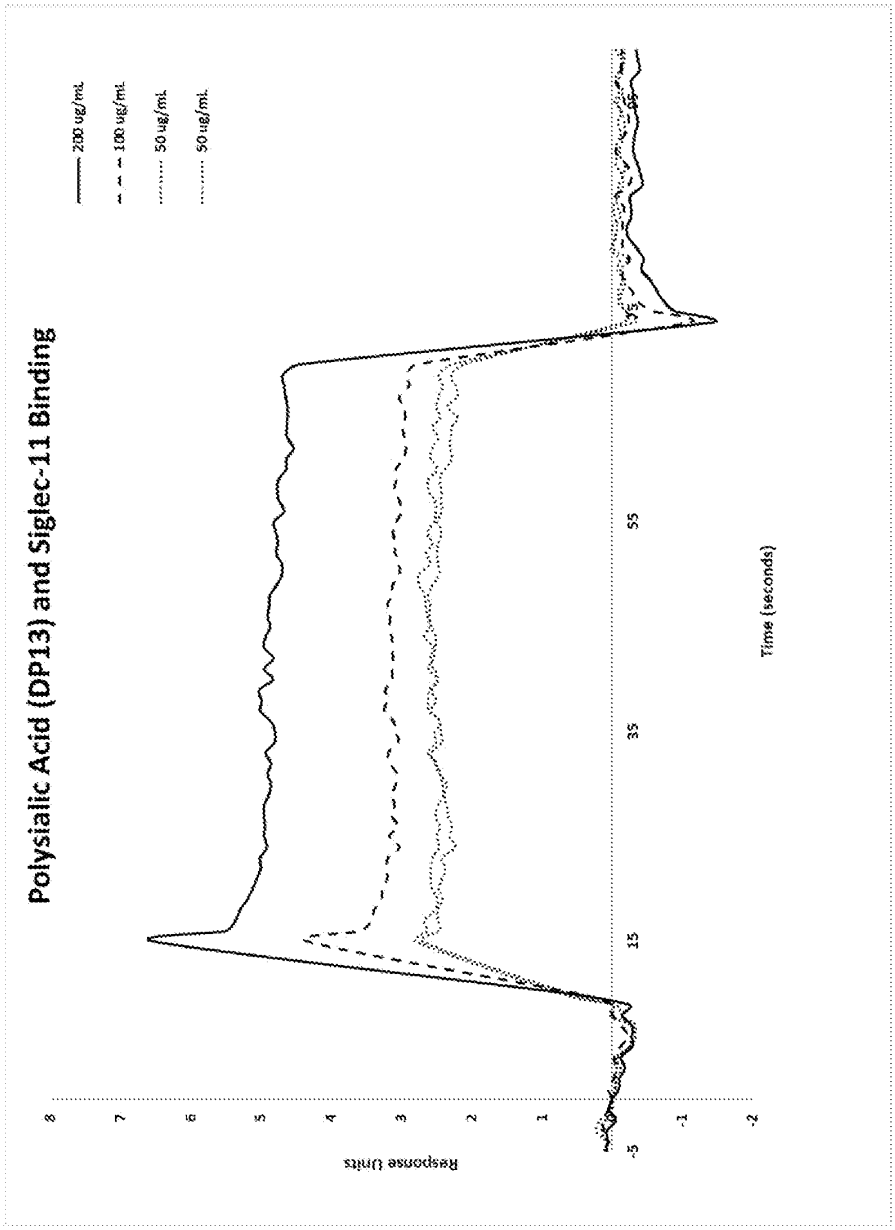

Polysialic Acid (DP13) and Siglec-11 Binding
200 ug/mL
100 ug/mL
50 ug/mL
50 ug/mL
Response Units
8
7
6
5
4
3
2
1
-1
-2
-5
15
35
55
Time (seconds)

FIG. 34B

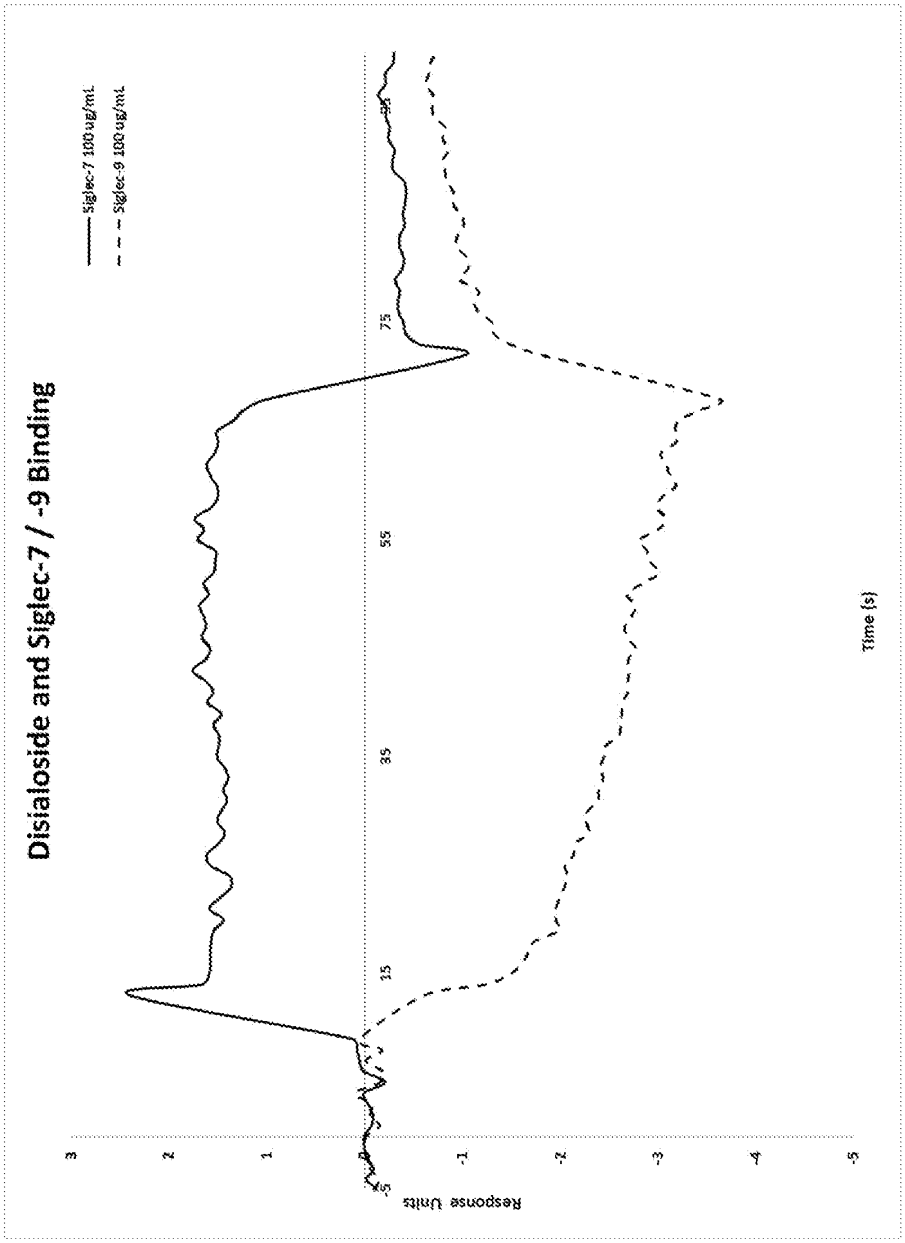

Disialoside and Siglec-7 / -9 Binding
Siglec-7 100 ug/mL
Siglec-9 100 ug/mL
Response Units
Time (s)
3
2
1
-1
-2
-3
-4
-5
15
35
55
75

FIG. 34C

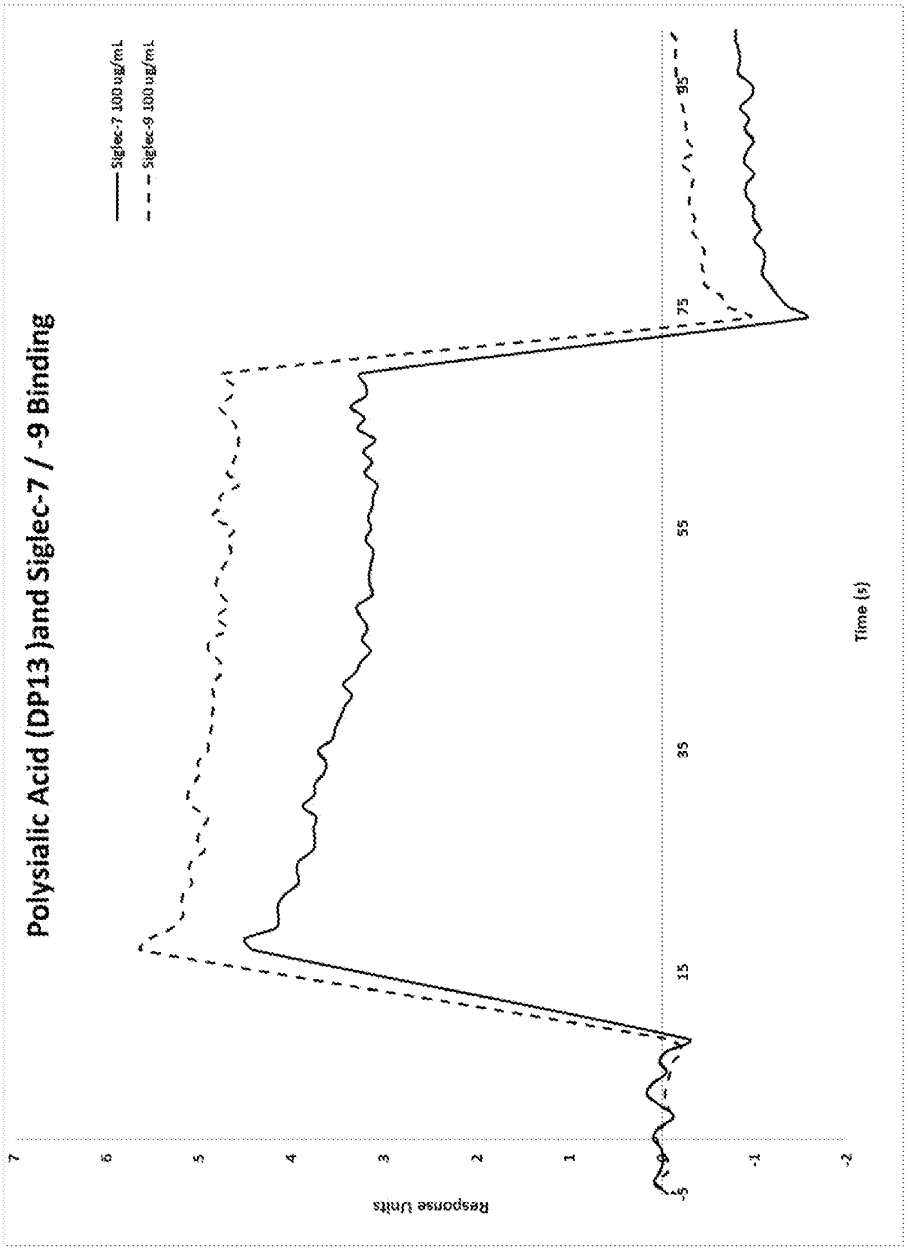

Polysialic Acid (DP13)and Siglec-7 / -9 Binding
Siglec-7 100 ug/mL
Siglec-9 100 ug/mL
Response Units
7
6
5
4
3
2
1
-1
-2
-5
15
35
55
75
95
Time (s)

FIG. 34D