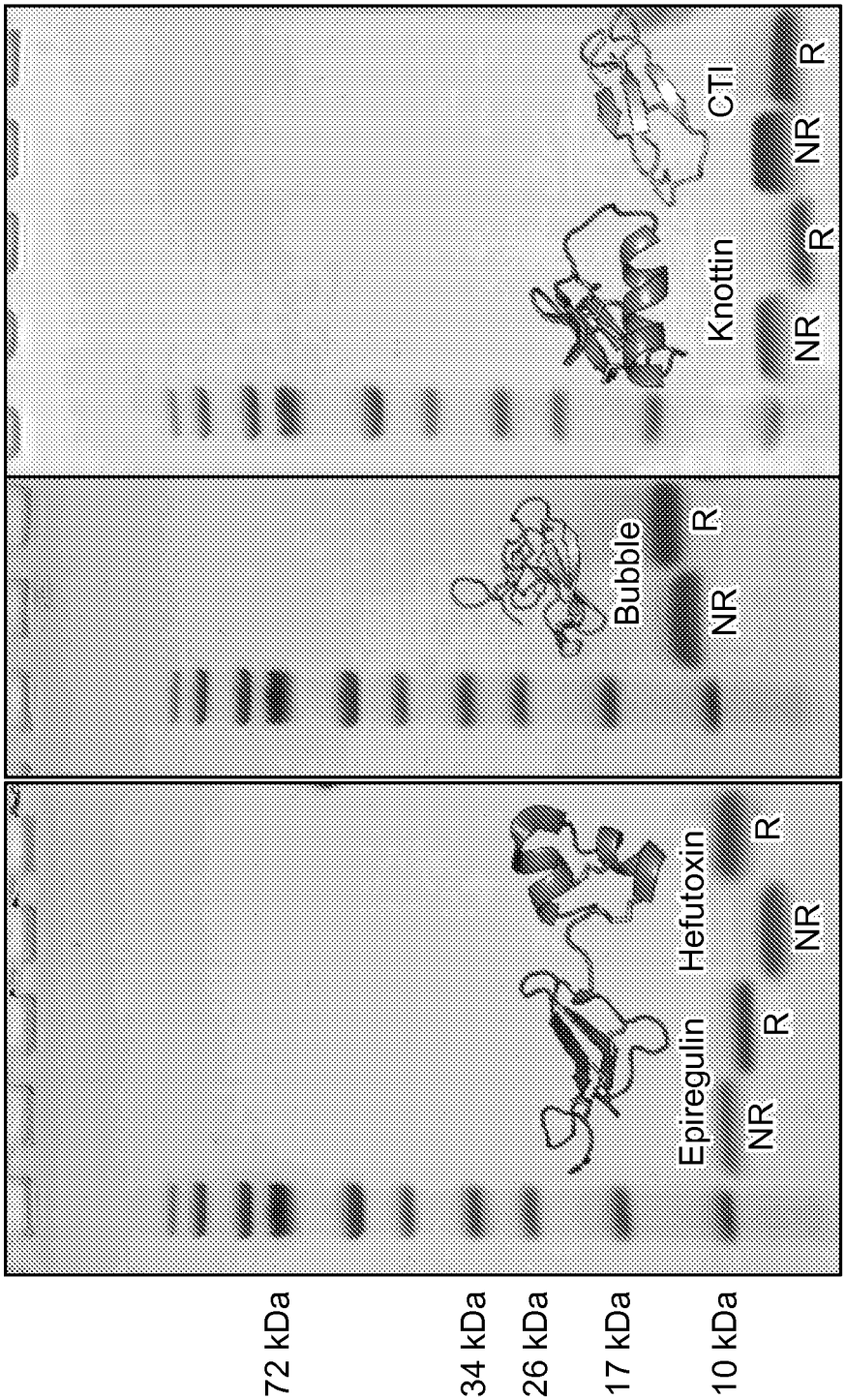

72 kDa
34 kDa
26 kDa
17 kDa
10 kDa
Epiregulin
NR
R
Hefutoxin
NR
R
Bubble
NR
R
Knottin
NR
R
CTI
NR
R

FIG. 1

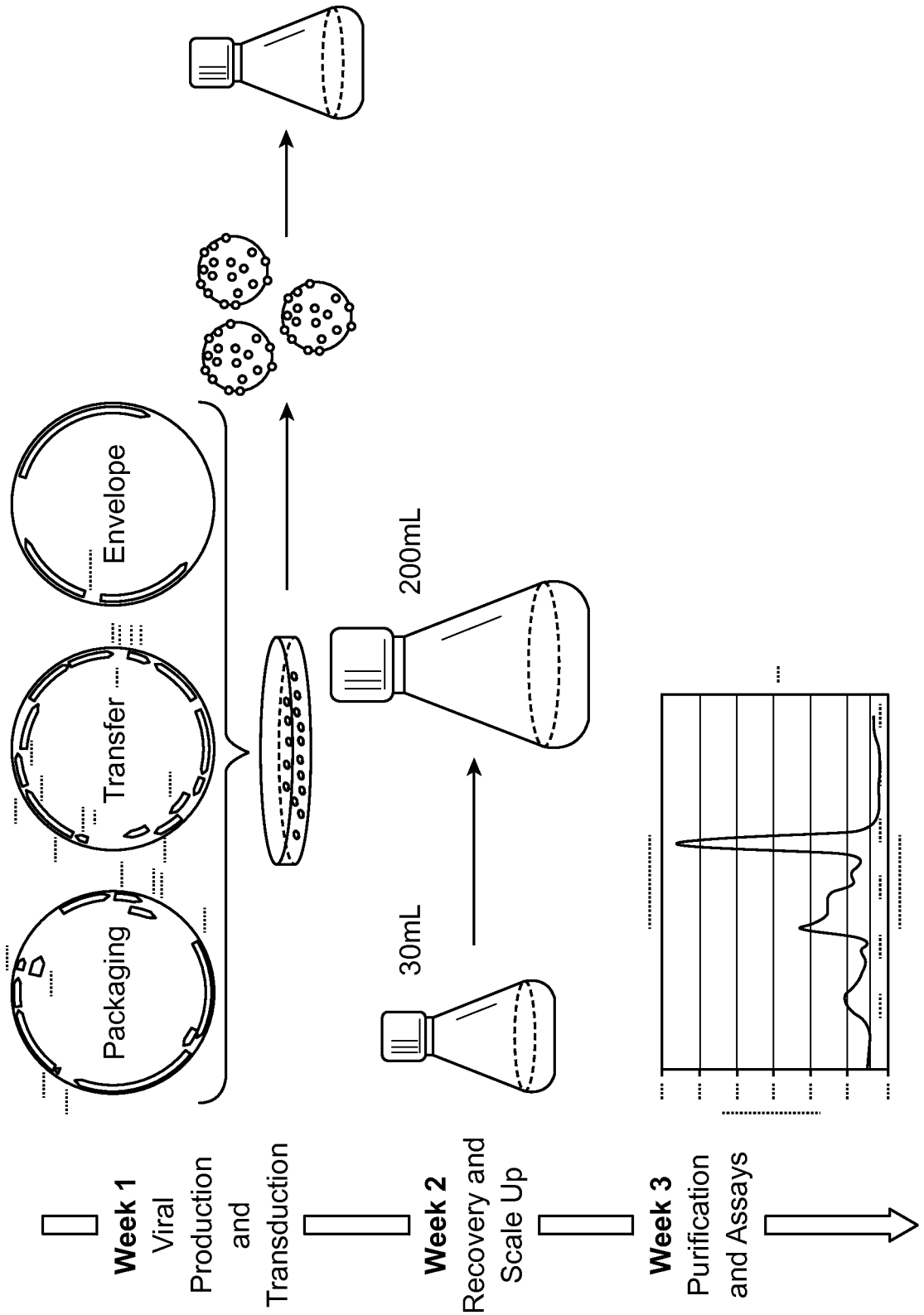

Envelope
Transfer
Packaging
200mL
30mL
Week 1
Viral
Production
and
Transduction
Week 2
Recovery and
Scale Up
Week 3
Purification
and Assays

FIG. 2

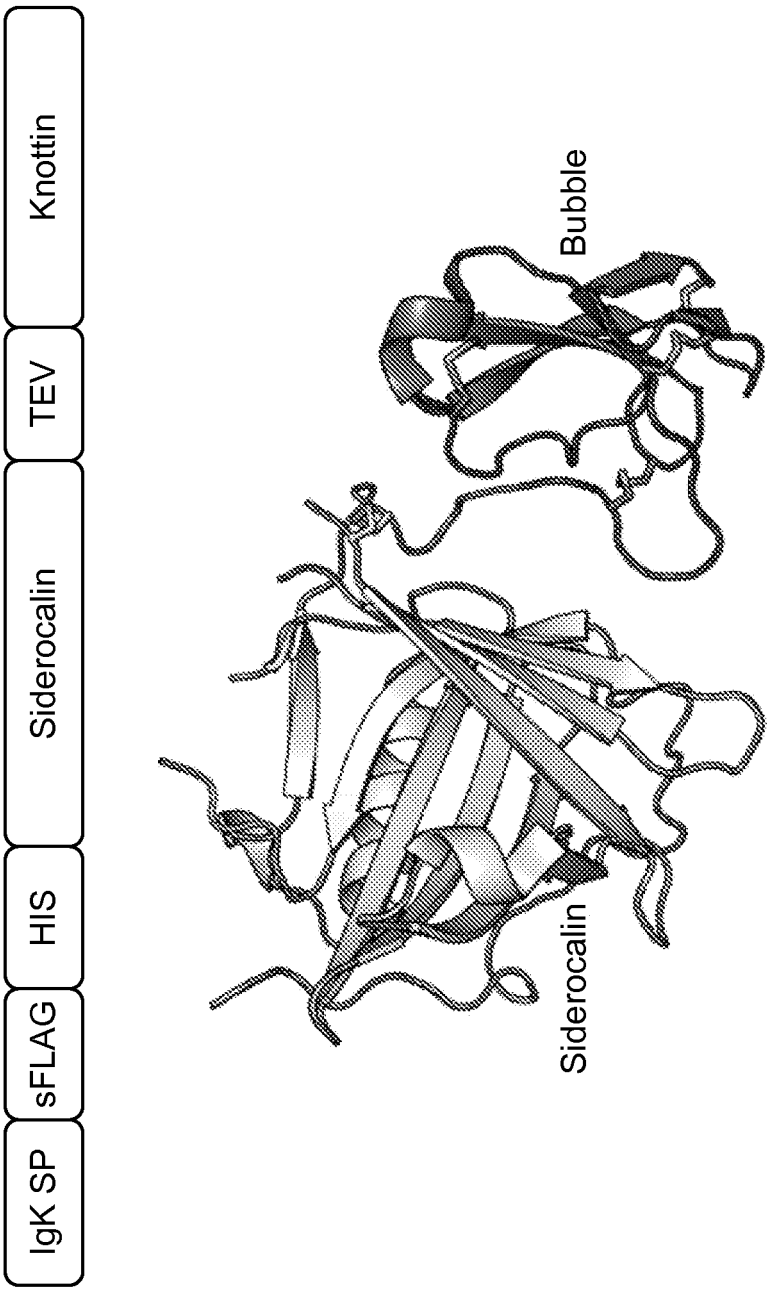

IgK SP
sFLAG
HIS
Siderocalin
TEV
Knottin
Bubble
Siderocalin

FIG. 3

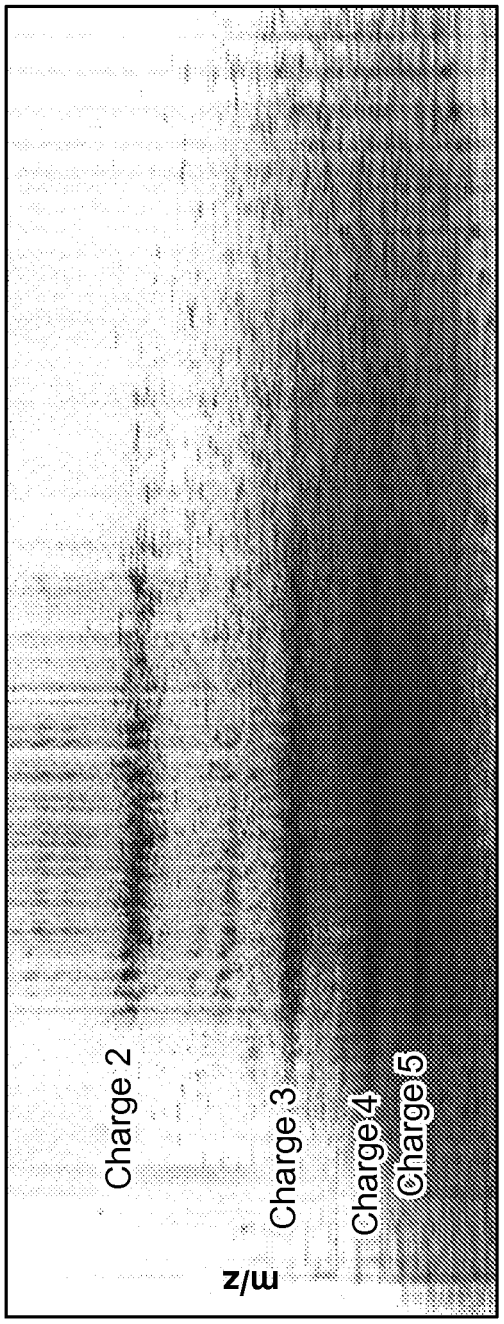


FIG. 4

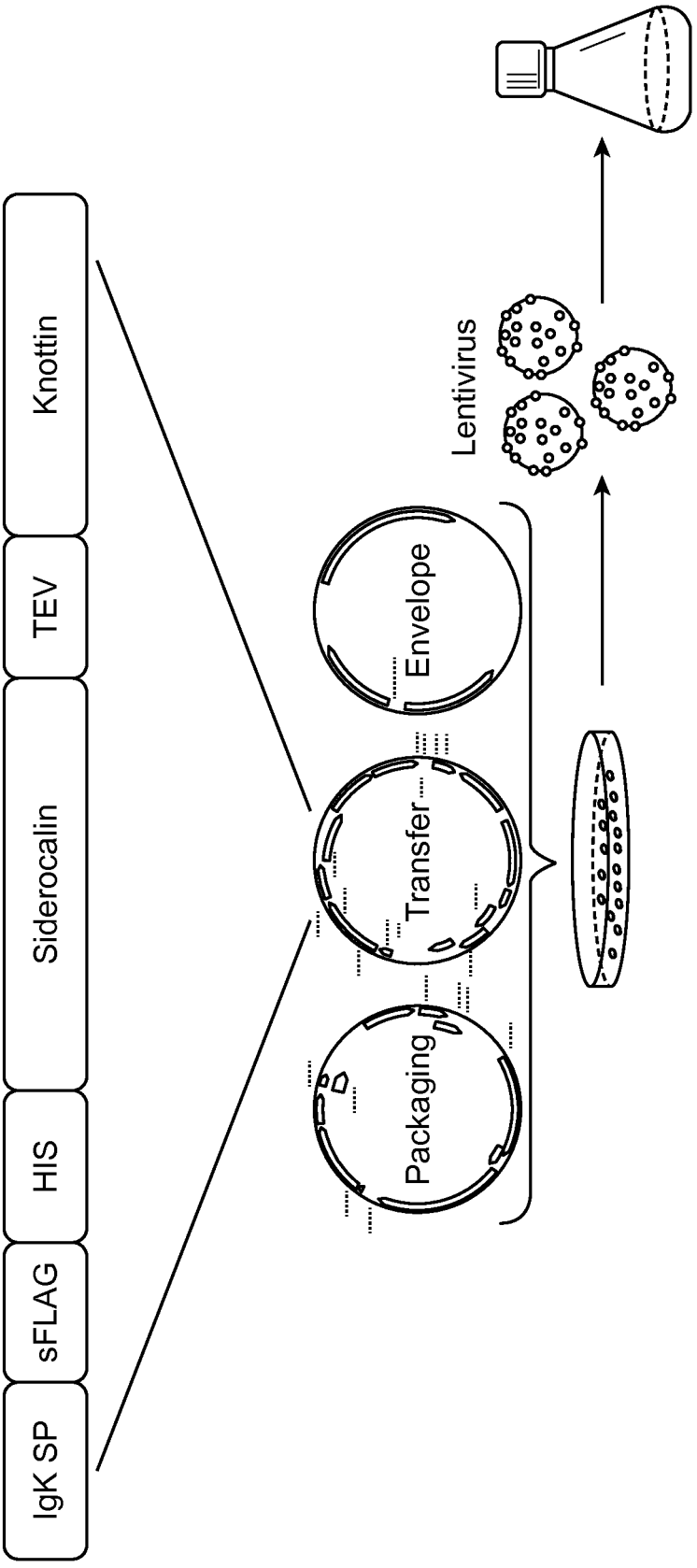

IgK SP
sFLAG
HIS
Siderocalin
TEV
Knottin
Packaging
Transfer
Envelope
Lentivirus

FIG. 5

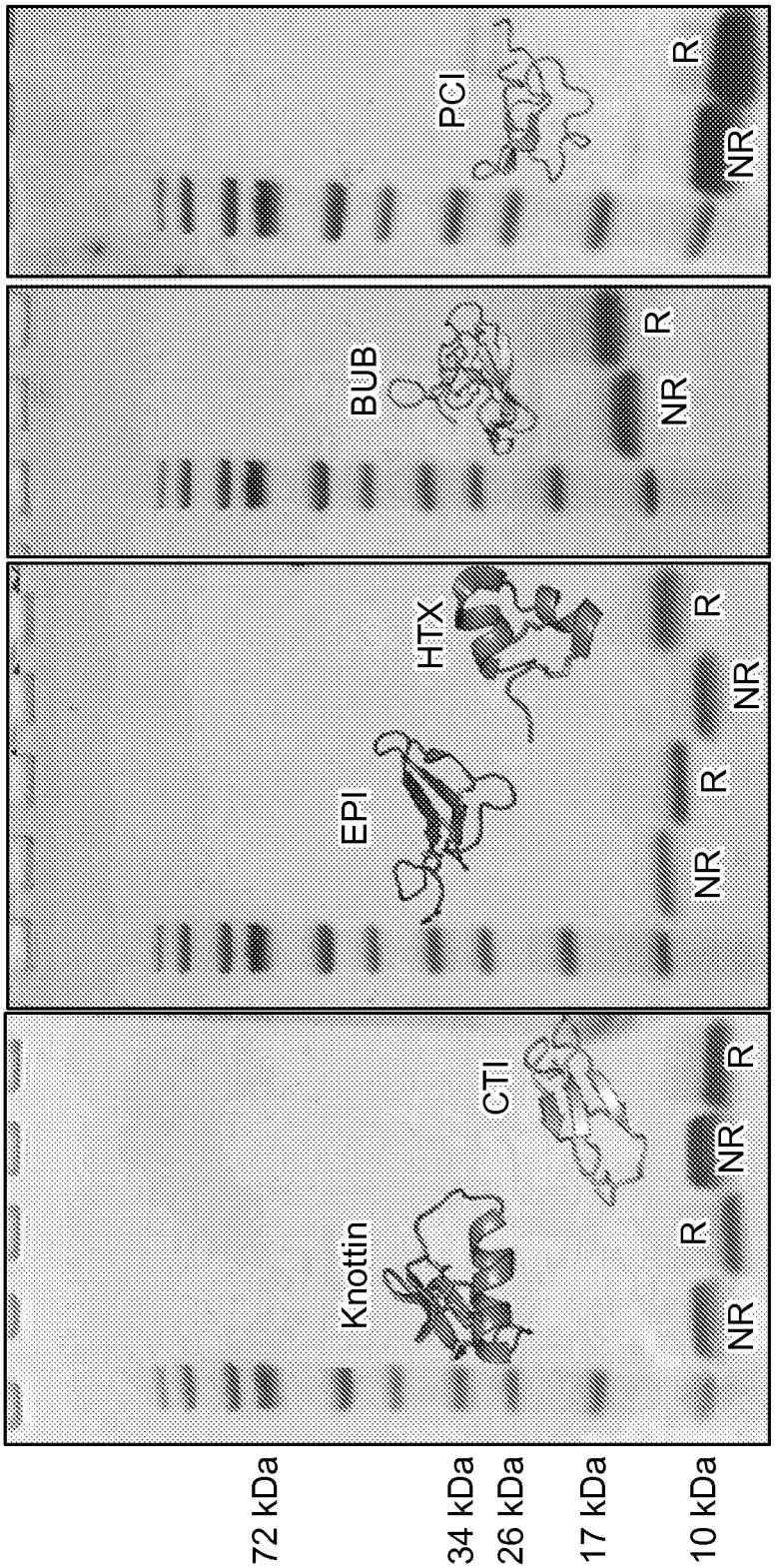

72 kDa
34 kDa
26 kDa
17 kDa
10 kDa
Knottin
CTI
EPI
HTX
BUB
PCI
R
NR

FIG. 6

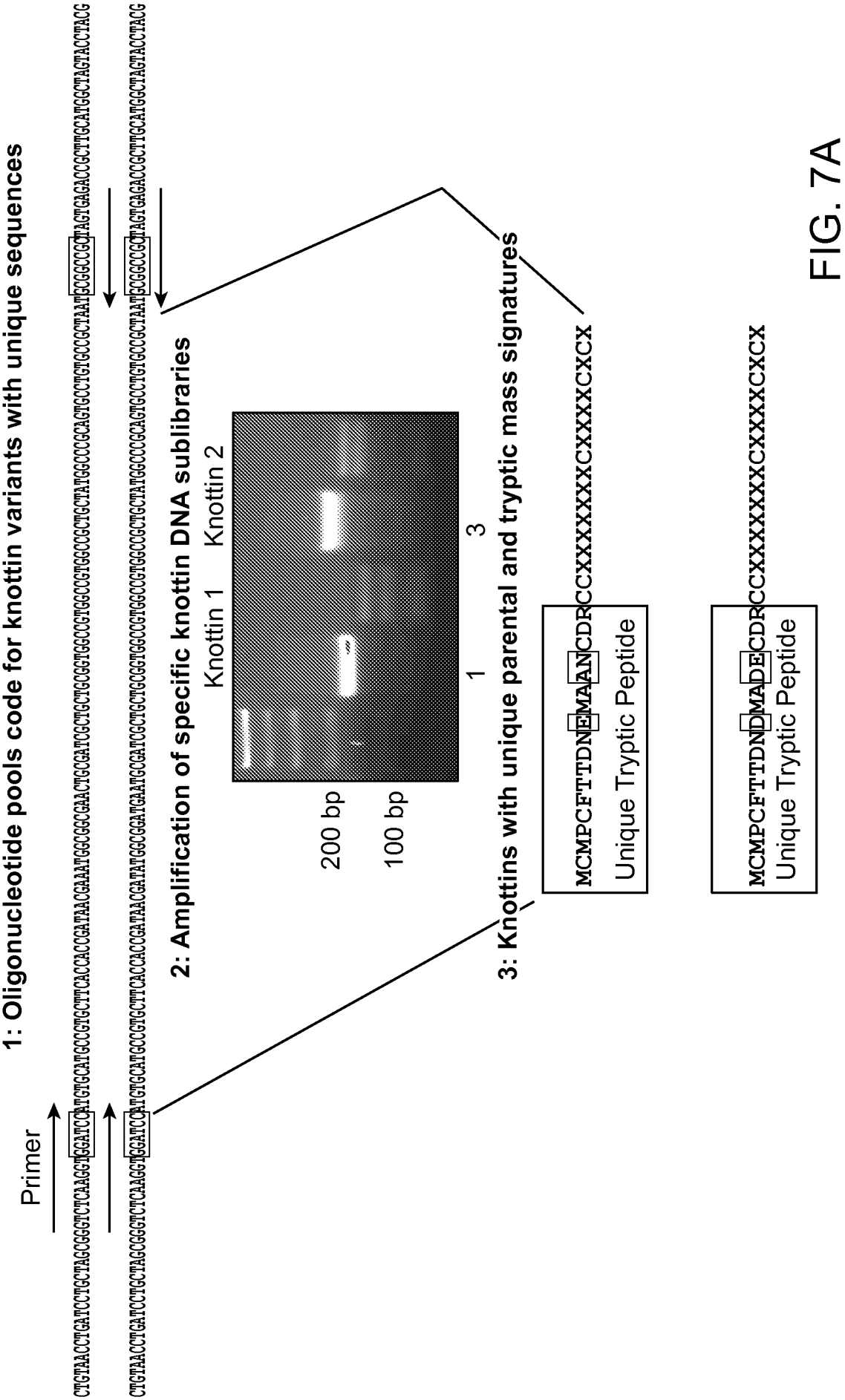

1: Oligonucleotide pools code for knottin variants with unique sequences
Primer
2: Amplification of specific knottin DNA sublibraries
Knottin 1
Knottin 2
200 bp
100 bp
1
3
3: Knottins with unique parental and tryptic mass signatures
MCMPCFTTDNEMAANCDRCCXXXXXXXXCXXXXCXCX
Unique Tryptic Peptide
MCMPCFTTDNDMADECDRCCXXXXXXXXCXXXXCXCX
Unique Tryptic Peptide

FIG. 7A

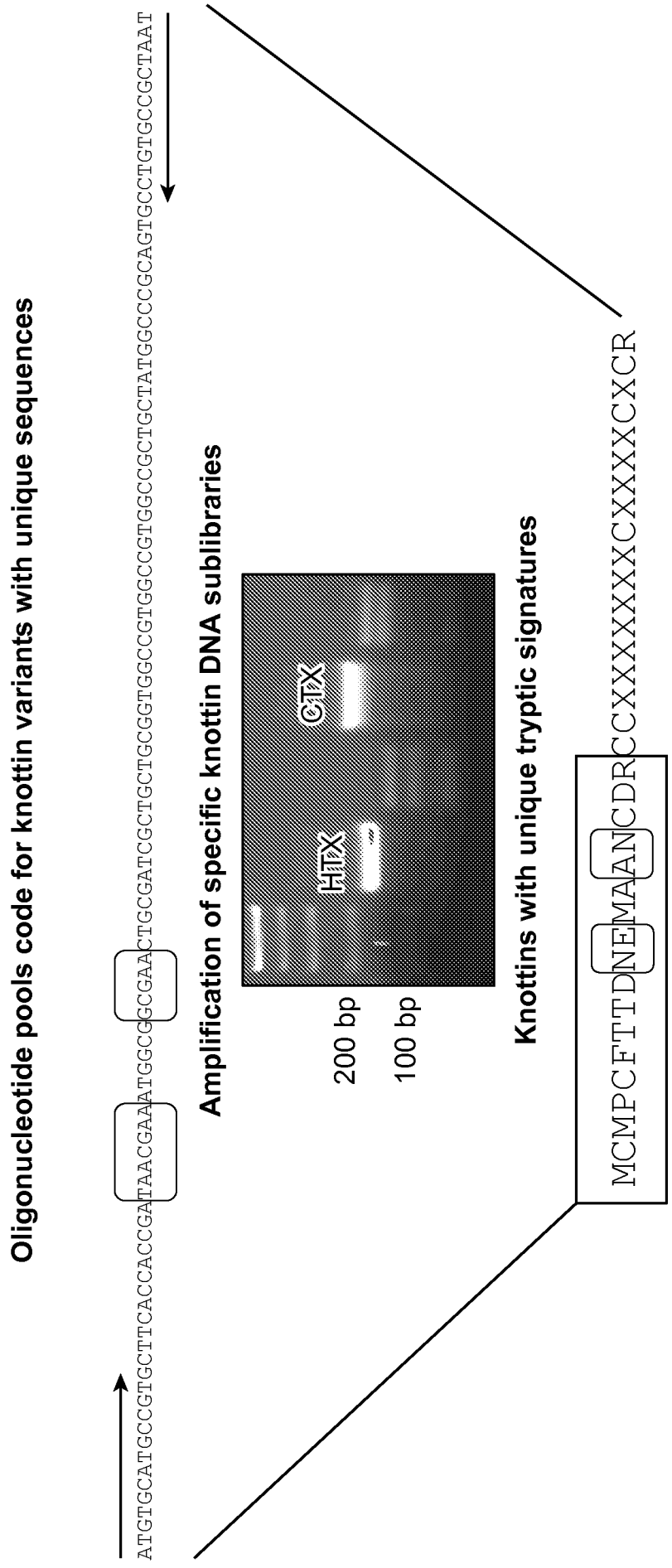

Oligonucleotide pools code for knottin variants with unique sequences
ATGTGCATGCCGTGCTTCACCACCGATAACGAAATGGCGGCGAACTGCGATCGCTGCTGCGGTGGCCGTGGCCGGCTGCTATGGCCCGCAGTGCCTGTGCCGCTAAT
Amplification of specific knottin DNA sublibraries
200 bp
100 bp
HTX
CTX
Knottins with unique tryptic signatures
MCMPCFTTDNEMAANCDRCCXXXXXXXXCXXXXCXCR

FIG. 7B

GSEISCEPGRTFRDRCNQCFCGFDGRRAACTIRACPNQ
GSEIHCQPGRTFRDRCNTCYCGWYGRSAACTLRACPNQ
GSEISCEPGGTFEDRCNVCRCGADGRSAGCTLRACPNQ
GSRIDCRPGRTFRDRCNTCMCGWTGHSAACTLRACPNQ
GSEISCEPGRTFRDRCNTCHCWADGRGAACTERACPNQ
GSESSCEPGATWRDRCNTCRCRADGRSAACTVRQCPNQ
GSEIFCIPGRTFRDRCNTCRCGRRGISAACTLRACPNQ
GSEISCEPGRTFRDRCNTCRCGADGRSAACTLFDCSLQ
GSEISCEPGRTSRDRCNTCRCGADGRSAACTLIDCPGQ
GSEISCEPGRTFRDRCNTCRCGADGRDAACTLGHCFFY
GSEISCEPGFTEQDRCNTCRCGAIGRSAACTLRACPNQ
GSEISCEPYSTFRDRCNTCSCGADGRGAACTMRACPNQ
GSEISCEPGRTFRDRCNTCRCGADGRSAACTLVACLIT
GSEIFCIPGVTFRFRCNTCRCGMDGRSAACTLRACPNQ
GSENDCHPGTTFRDRCNTCRCGGEGRSAACTLRACPNQ
GSQRSCEPGRTFRDRCNTCRCGADGRSAACTLFICPFQ
GSEISCEPGRTFRDRCNTCRCGADGRSAACTLFLCGFI
GSEISCEPDSTTRDRCNSCRCGNDGRSAACTLRACPNQ
GSEISCEPGRTFRDRCNTCRCGADGRSEACTLHNCREQ
GSDISCEPGSTHRDRCNTCRCSADGRSRACTLRACPNQ
GSEDSCEPGWTQRMRCNTCRCTADGRSAACTLRACPNQ
GSEISCEPGRTFRDRCNTCRCGADGRSAACTLHHCIYW
GSEVSCEPGRTFRSRCNDCVCGADGRHAACTIRACPNQ
GSEISCEPGRTFRDRCNTCRCGADGRSAACTLVFCHED
GSEISCEPWRTFRDRCNTCRCGADGRGAACTLVICGMQ
GSEISCEPGRTFRDRCNMCGCGADGRWARCTRRACPNQ
GSEISCEPGRTFRDRCNTCRCGADGRSAACTLFTCTYF
GSEISCEPGRTWRDRCNTCRCGADGRSAACTLNGCLLQ
GSEISCEPGRTFRDRCNTCRCGADGRSAACTLANCFFA

FIG. 8

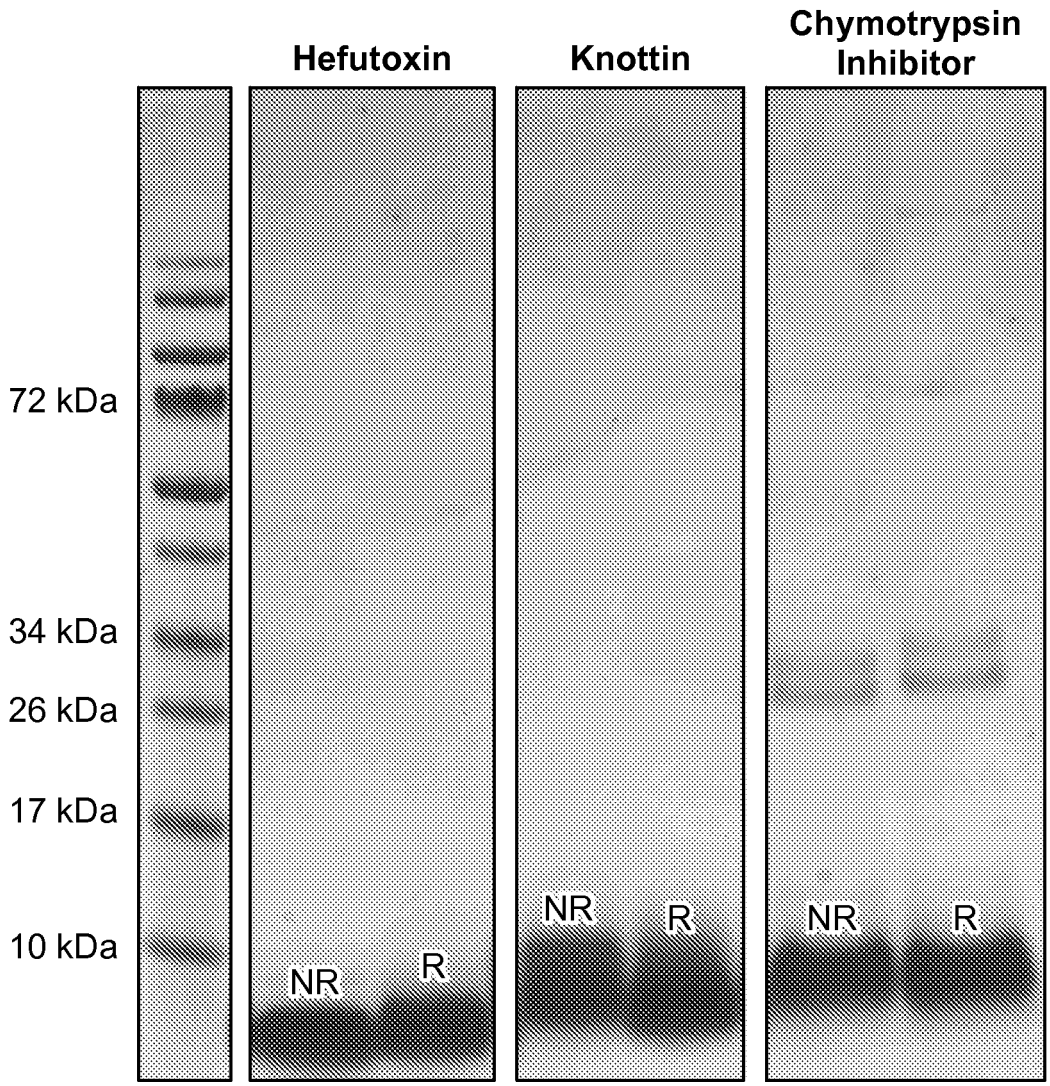

Hefutoxin
Knottin
Chymotrypsin Inhibitor
72 kDa
34 kDa
26 kDa
17 kDa
10 kDa
NR
R
NR
R
NR
R

FIG. 9

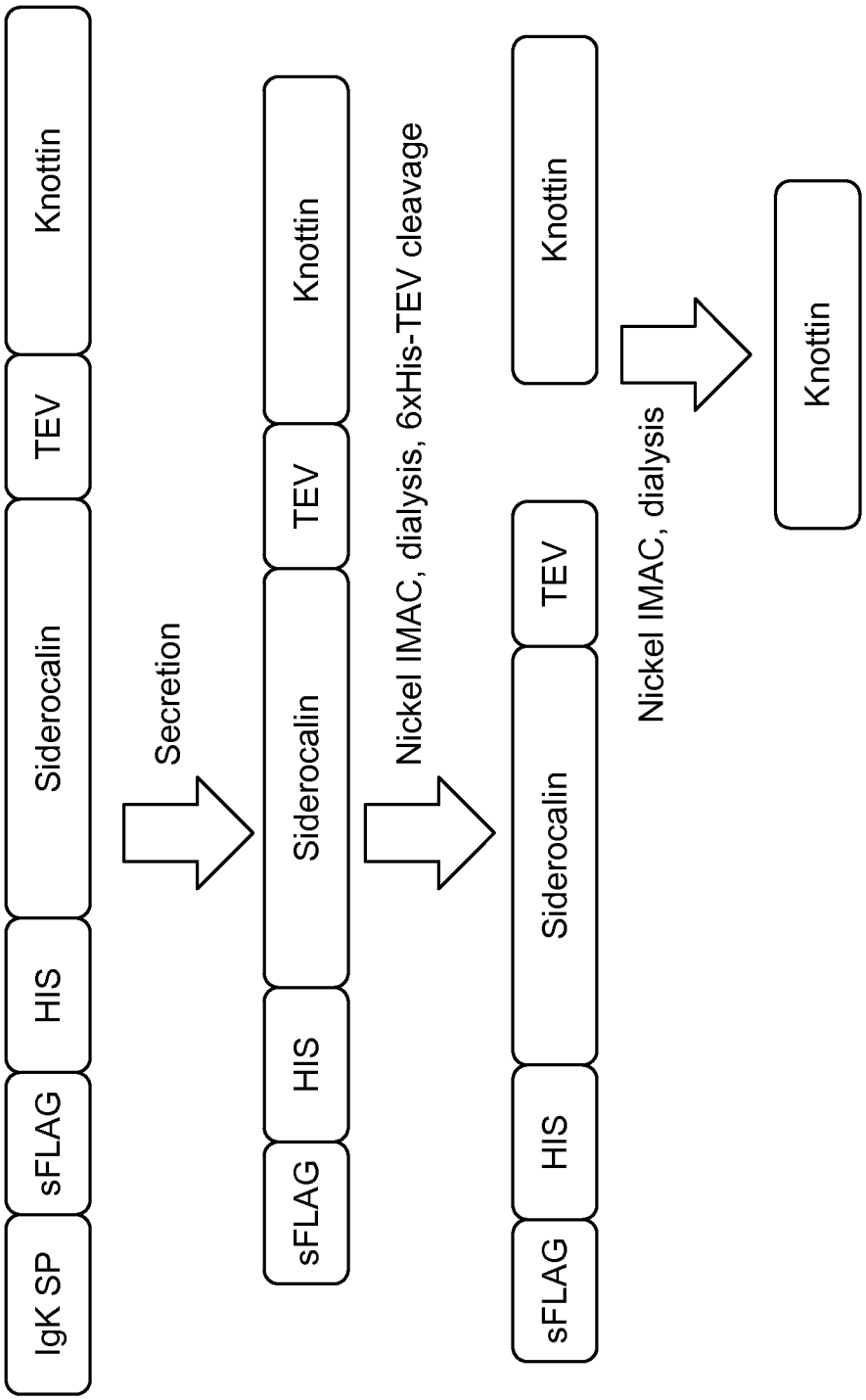

IgK SP
sFLAG
HIS
Siderocalin
TEV
Knottin
Secretion
sFLAG
HIS
Siderocalin
TEV
Knottin
Nickel IMAC, dialysis, 6xHis-TEV cleavage
sFLAG
HIS
Siderocalin
TEV
Knottin
Nickel IMAC, dialysis
Knottin

FIG. 10

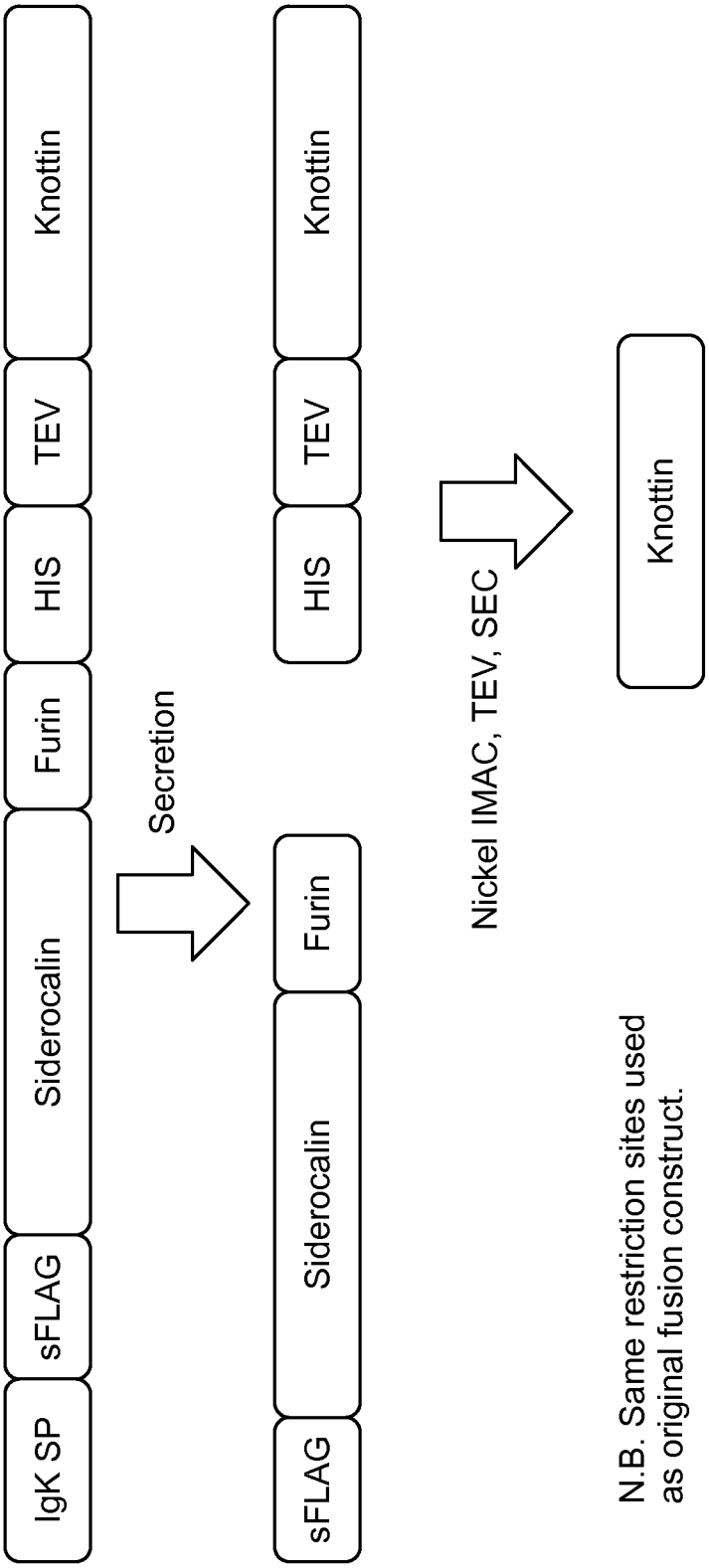

IgK SP
sFLAG
Siderocalin
Furin
HIS
TEV
Knottin
Secretion
sFLAG
Siderocalin
Furin
HIS
TEV
Knottin
Nickel IMAC, TEV, SEC
Knottin
N.B. Same restriction sites used as original fusion construct.

FIG. 11

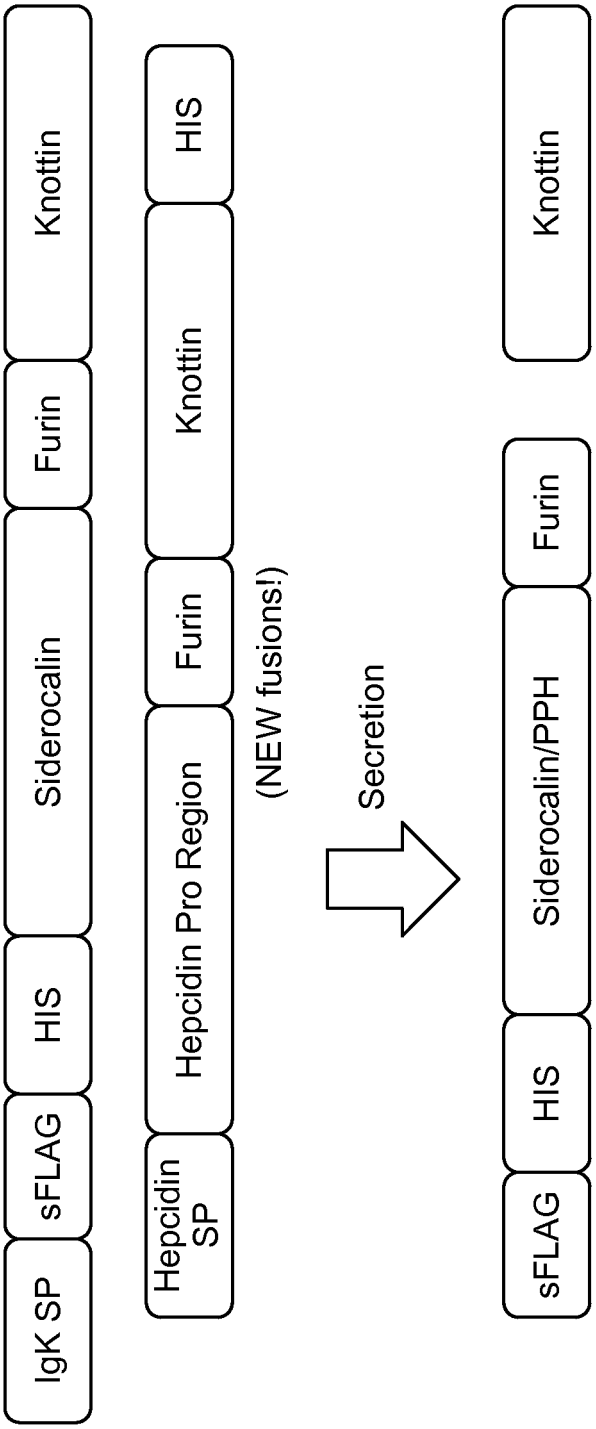

IgK SP
sFLAG
HIS
Siderocalin
Furin
Knottin
Hepcidin SP
Hepcidin Pro Region
Furin
Knottin
HIS
(NEW fusions!)
Secretion
sFLAG
HIS
Siderocalin/PPH
Furin
Knottin
N.B. Same restriction sites used as original fusion construct.

FIG. 12

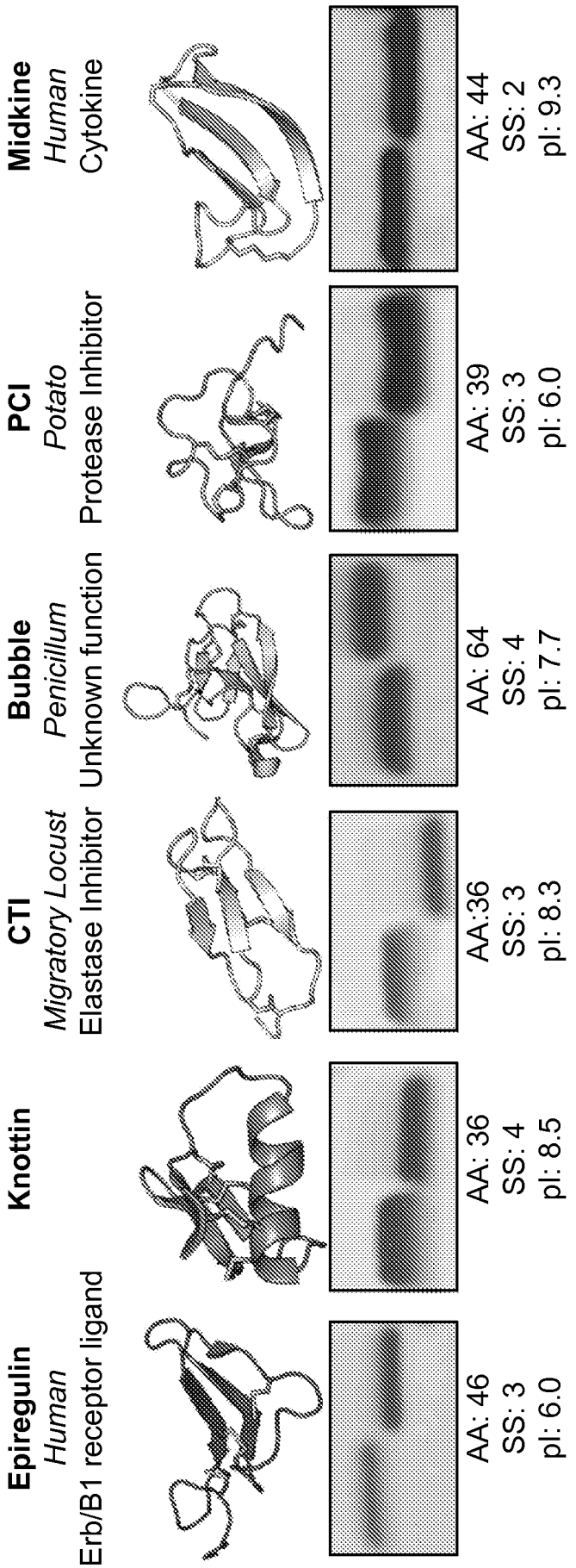

Epiregulin
Human
Erb/B1 receptor ligand
AA: 46
SS: 3
pI: 6.0
Knottin
AA: 36
SS: 4
pI: 8.5
CTI
Migratory Locust
Elastase Inhibitor
AA:36
SS: 3
pI: 8.3
Bubble
Penicillum
Unknown function
AA: 64
SS: 4
pI: 7.7
PCI
Potato
Protease Inhibitor
AA: 39
SS: 3
pI: 6.0
Midkine
Human
Cytokine
AA: 44
SS: 2
pI: 9.3

FIG. 13

```
                    1        10        20        30        40        50        60        70        80
H.sapiens           QDSTSDLIPAPPLSKVPLQQNFQDNQFQGKWYVVGLAGNAILREDKDPQKMYATIYELKEDKSYNVTSVLFRKK--KCDYWIRTFVPGCQP
P.troglodytes       QDSTSDLIPAPPLSKVPLQQNFQDNQFQGKWYVVGLAGNAILREDKDPQKMYATIYELKEDKSYNVTSVLFRKK--KCDYWIRTFVPGRQP
G.gorilla           QDSTSDLIPAPPLSKVPLQQNFQDNQFQGKWYVVGLAGNAILREDKDPQKMYATIYELKEDKSYNVTSVLFREKAQKCDYWIRTFVPGSQP
P.pygmaeus          QDSTSDLIPAPPLSKVPLQQNFQDNQFQGKWYVVGLAGNAIRREDKDSQKMYATIYELKEDKSYNVTSVLFRKK--KCDYWIRTFVPGSQP
M.mulatta           QDSSSDLIPAPPLSKVPLQQNFQDNQFQGKWYVVGLSGNAVGRKDEAPLKMYATIYELKEDKSYNVTSILFRKE--KCDYWIRTFVPGSQP
C.jacchus           QDSPSPLIPAPPLSKVPLQQNFQDNQFQGKWYVVGLAGNAIRREDQDSLKMYATIYELKEDKSYNVTSVLFRKAQ-KCDYWIRTFVPSSRP
M.musculus          QDSTQNLIPAPSLLTVPLQPDFRSDQFRGRWYVVGLAGNAVQKKTEGSFTMYSTIYELQENNSYNVTSILVRDQDQGCRYWIRTFVPSSRA
R.norvegicus        QDSTQNLIPAPPLISVPLQPGFWTERFQGRWFVVGLAANAVQKERQSRFTMYSTIYELQEDNSYNVTSILVRG--QGCRYWIRTFVPSSRP
O.princeps          QELTTDLIPVPSLRKIHVQKNFQSDQFQGKWYVVGLAGNNIHNSDQEHQQMYSTTYELKEDGSYNVTSTLLRQRNQQCDHWIRTFVPGSKL
O.cuniculus         QDPTPKLIPAPSLRRVPLQRNFQDEQFQGKWYVVGLAGNAVQKREEGQEPMYSTTYELNEDRSFNVTSTLLR--DQRCDHWIRTFVPTSRP
B.taurus            RSSSSRLLRAPPLSRIPLQPNFQADQFQGKWYTVGVAGNAIKKEEQDPLKMYSSNYELKEDGSYNVTSILLKDD--LCDYWIRTFVPSSQP
S.scrofa            QGTIPNWIPAPPLSKVPLQPNFQADQFQGKWYVVGLAGNAVKKEEQGRFKMYTTTYELKEDGSYNVISTLLRGQ--LCDNWIRTFVPSLQP
T.truncatus         QDSTPNLIPAPPLFRVPLQPNFQPDQFQGKWYIVGLAGNAFKKEKQGQFKMYATTYELKEDRSYNVTSALLRGKTQRCDHWIRTFVPSSRP
E.caballus          RDPAPKLIPAPPLDRVPLQPDFKDDQFQGKWYVVGVAGNAFKKEEQGQFTMYTTTYELKEDHSYNVTSILLR--DQNCDHWIRTFIPSSQP
M.murinus           QDSKEKLIPAPPLLRVPLQPDFQDDQFRETSWPRGSKMKETPAGSRDAGTGWATTYELK-DHSYNVTSTLLRQNG-KCDYWIRTFVLTSQP
L.africana          QTHSPTLIPAPPLLRVPLQPDFQDDKFQGKWYVIGLAGNAVEKKEQGQFKMYTTTYELKEDGSYNVTSTLLQEDG-KCSYWIRTFVPSFQP
P.capensis          QEPTPTLIPAPPLSSIPLKPNFHNDKFQGKWYVVGVAGNAITK-EKDPSLMYTTTYELRDDGSYNVTSTQFREKI-NCTHWTRTFVPTSQP
C.familiaris        QDSTPSLIPAPPPLKVPLQPDFQHDQFQGKWYVIGIAGNILKKEGHGQLKMYTTTYELKDDQSYNVTSTLLRNE--RCDYWNRDFVPSFQP
                                                  *                                                  * *
```

FIG. 14

```
                    90        100       110       120       130       140       150       160       170
H.sapiens           GEFTLGNIKSYPGLTSYLVRVVSTNYNQHAMVFFKKVSQN-REYFKITLYGRTKELTSELKENFIRFSKSLGLPENHIVFPVPIDQCIDG-
P.troglodytes       GEFTLGNIKSYPGLTSYLVRVVSTNYNQHAMVFFKKVSQN-REYFKITLYGRTKELTSELQENFIRFSKSLGLPENHIVFPVPIDQCIDG-
G.gorilla           GEFTLGNIKSYPGLTSYLVRVVSTNYNQHAMVFFKKVSQN-REYFKITLYGRTKELTSELKENFIRFSKSLGLPENHIVFPVPIDQCIDG-
P.pygmaeus          GEFTLGNTKGYPGLTSYLVRVVSTNYNQYAMVFFKKVSQN-REYFKITLYGRTKELTSELKENFIRFSKSLGLPENHIVFPAPIDQCIDG-
M.mulatta           GEFTLGNIQNHPGLTSYVVRVVSTNYKQYAMVFFKKVSQN-KEYFKITLYGRTKELTSELKENFIRFSKSLGLPENHIVFSVPIDQCING-
C.jacchus           GEFKLGNIESHPGLTSYIVRVVNTDYKQHAMVFFMKASHN-RKYFKVTLYGRTKELTSDLKENFTSFSKSLGLTENHIIFPVPIDQCIDG-
M.musculus          GQFTLGNMHRYPQVQSYNVQVATTDYNQFAMVFFRKTSEN-KQYFKITLYGRTKELSPELKERFTRFAKSLGLKDDNIIFSVPTDQCIDN-
R.norvegicus        GQFTLGNIHSYPQIQSYDVQVADTDYDQFAMVFFQKTSEN-KQYFKVTLYGRTKGLSDELKERFVSFAKSLGLKDNNIVFSVPTDQCIDN-
O.princeps          GHFNLGNIKSYPTLKSYLIRVVTTDYNQFAIVFFRKVYKNNKKFFKIVLYGRTKELSPELRGRFTSFAKTLGLTDNHIVFPAPIGQCIDD-
O.cuniculus         GQYNLGNIKSYPGVKNYIVRVVATDYSQYAMMFFRKGSRN-KQFFKTTLYGRTKELSPELRERFTRFAKSLGLPDDRIVFPTPIDQCIDD-
B.taurus            GQFTLGNIKSYRGIRSYTVRVVNTDYNQFAIVYFKKVQRK-KTYFKITLYGRTKELTPEVRENFINFAKSLGLTDDHIVFTVPIDRCIDDQ
S.scrofa            GQFKLGDIKKYSGLQSYVVRVVSTNYSQFAIVFFKKVSNN-QEYFKTTLYGRTKVLSPELKENFVRFAKSLGLSDDNIIFPVAIDQCIDGQ
T.truncatus         GQFTLGNIKGFPGVQSYTVRVATTNYNQFAIVYFKKVYKN-QEYFKTTLYGRTKELTPQLKENFIHFAKSLGLTDEYILFPVPIDKCIDDQ
E.caballus          GQFNLGDIKRYFGVQSYIVRVADTDYNQFAIVFFRKVYKN-QEYFKTTLYRRTKELTPELREKFISFAKSLGLTDDHIIFPVPIDQCIDEE
M.murinus           GQFALGNINRYPGIQSYTVRVVTTNYNQFAIVFFKKVSEN-KEYFKTTLYGRTKELPPELKENFIRFAKSLGLTEDHIIYPVPIDQCIDD-
L.africana          GQFNLGNIKNFPGLQSYTVRVTATNYNQFAIVFFKKVSKN-GEYFKTTLYGRTKELTPELKERFIRFAKSLGLS-DHIIFPVPIDRCIDD-
P.capensis          GQFSLGNIDKYPHLSSYTVRVTATNYNYFAIVYFKKVSKN-QEYFKTTLYKRIKKLTHGLKKHFIQFAKSLGLPDNHITFLVPTDRCIDDA
C.familiaris        GQFSLGDIQLYPGVQSYLVQVVATNYNQYALVYFRKVYKS-QEYFKITLYGRTKELPLELKKEFIRFAKSIGLTEDHIIFPVPIDQCIDE-
                                                  * *       *   *
```

FIG. 14 (Cont.)

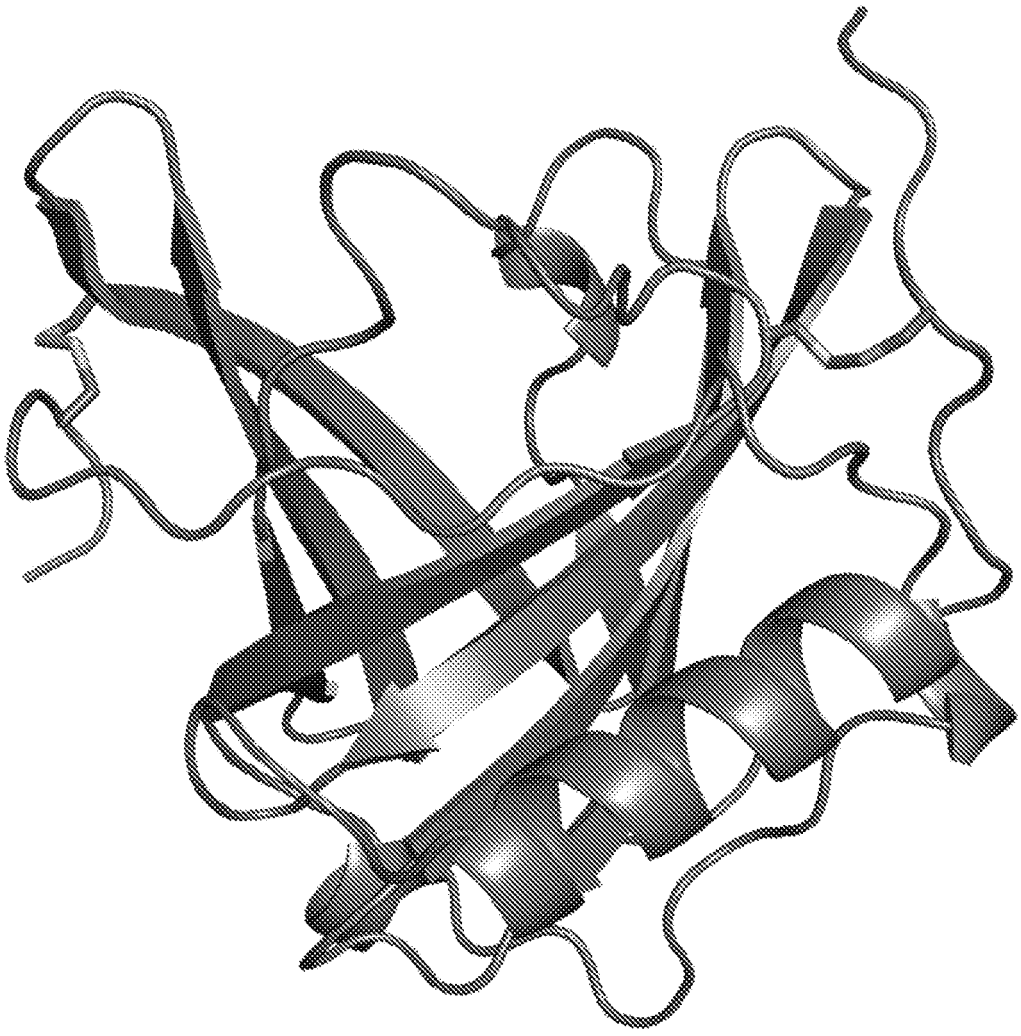


FIG. 15

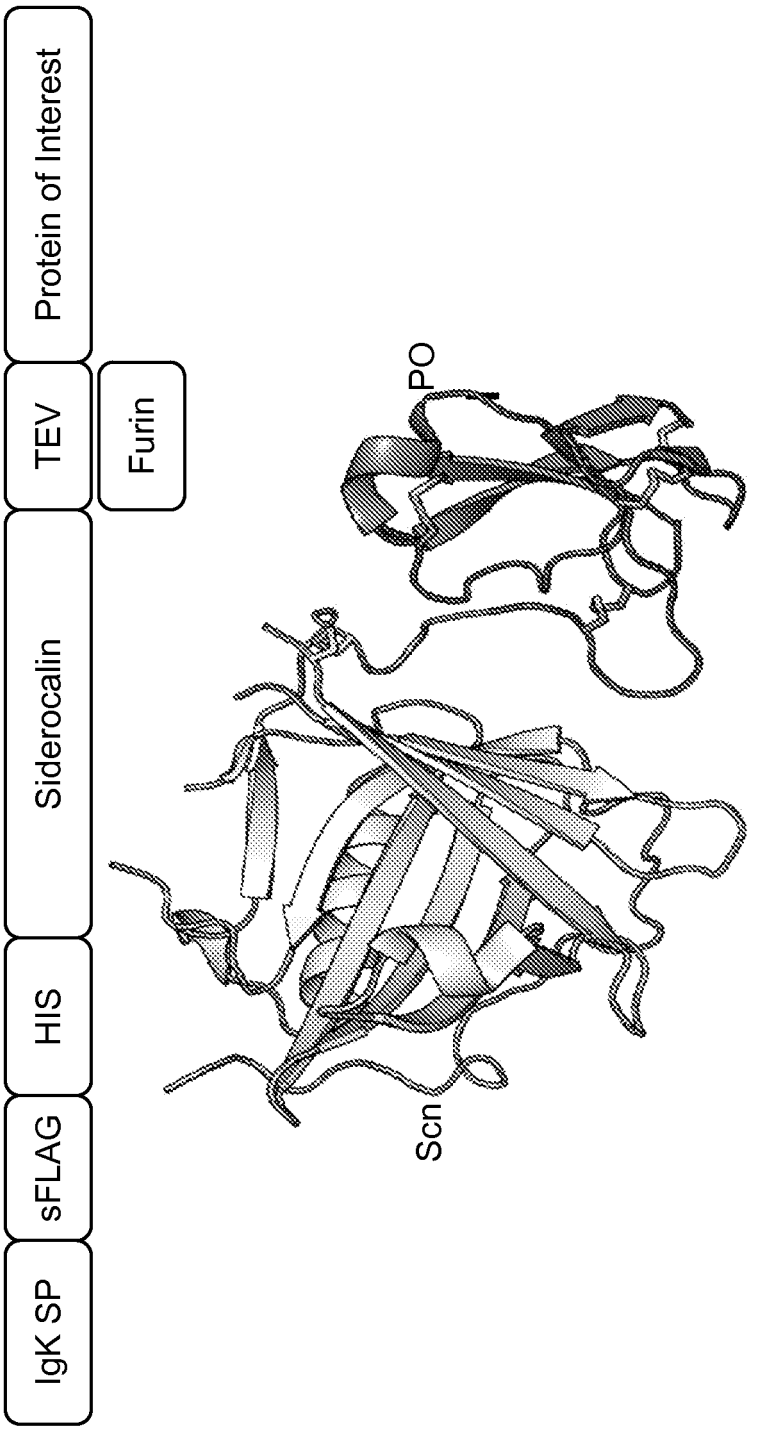

IgK SP
sFLAG
HIS
Siderocalin
TEV
Furin
Protein of Interest
PO
Scn

FIG. 16

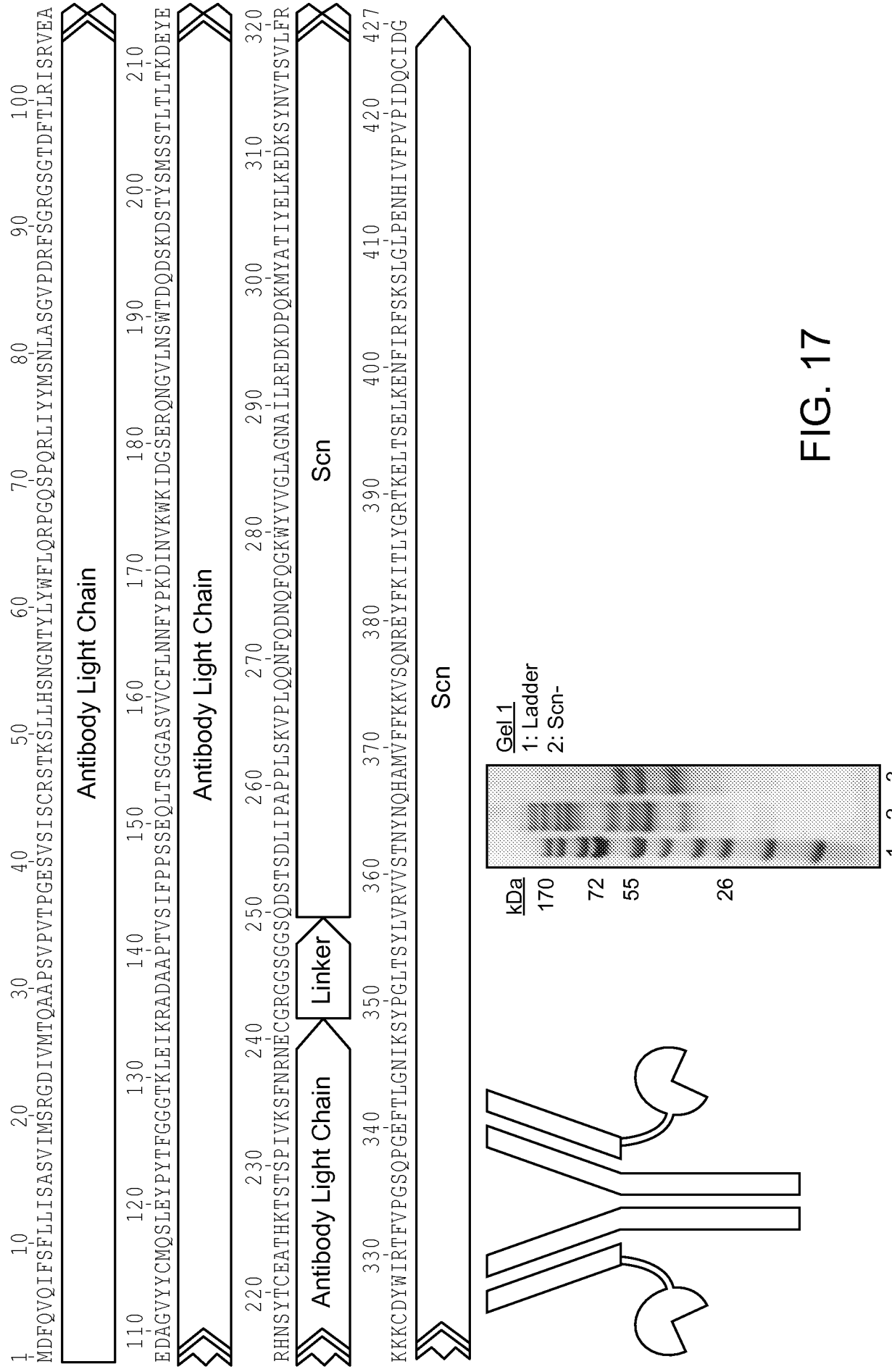
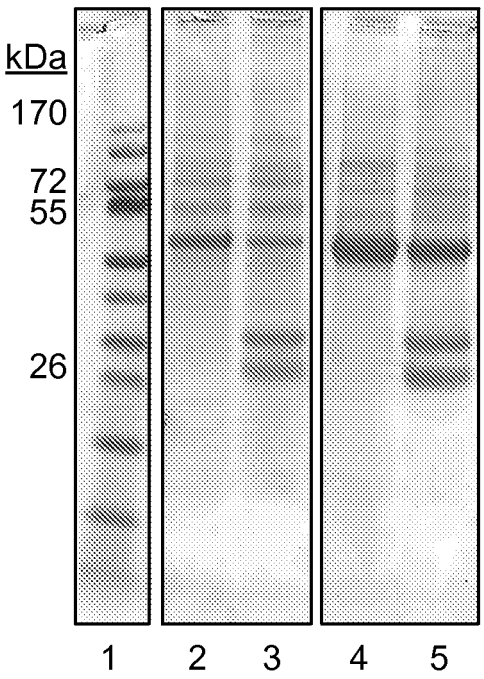

Antibody Light Chain
Antibody Light Chain
Antibody Light Chain
Linker
Scn
Scn
Gel 1
1: Ladder
2: Scn-
kDa
170
72
55
26
1 2 3

FIG. 17



Gel 1
1: Ladder
2: Scn-human HMOX1 fusion
3: Scn-human HMOX1 cleaved
4: Scn-murine HMOX1 fusion
5: Scn-murine HMOX1 cleaved

FIG. 18

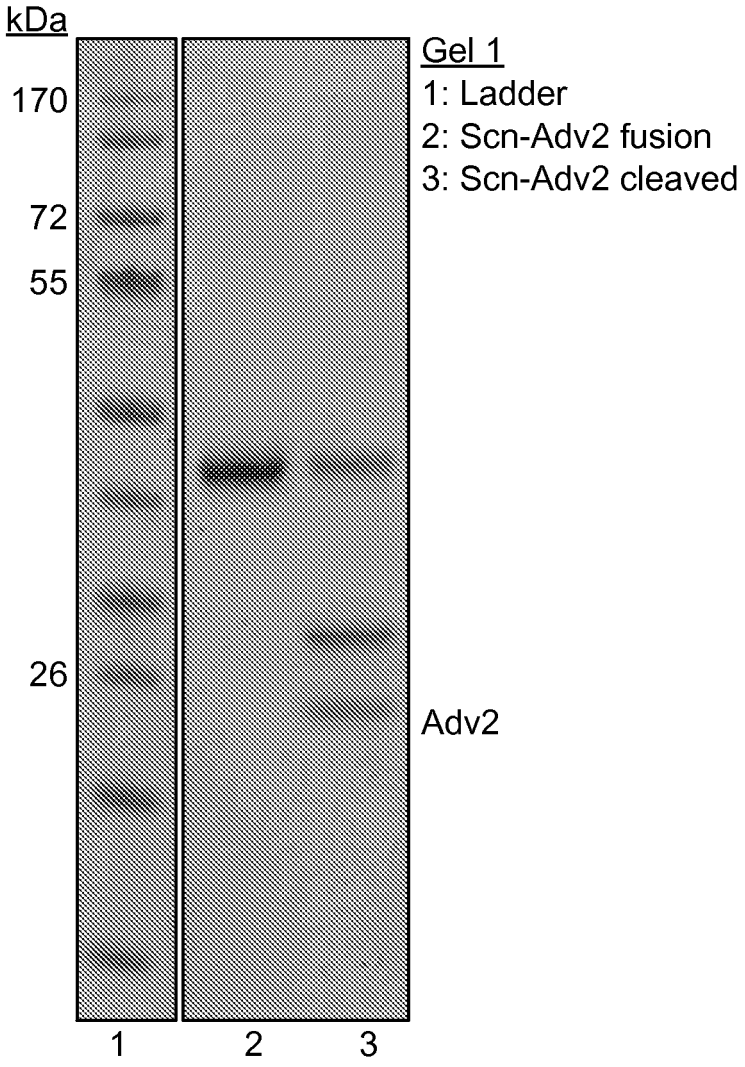

kDa
170
72
55
26
Gel 1
1: Ladder
2: Scn-Adv2 fusion
3: Scn-Adv2 cleaved
Adv2
1
2
3

FIG. 19

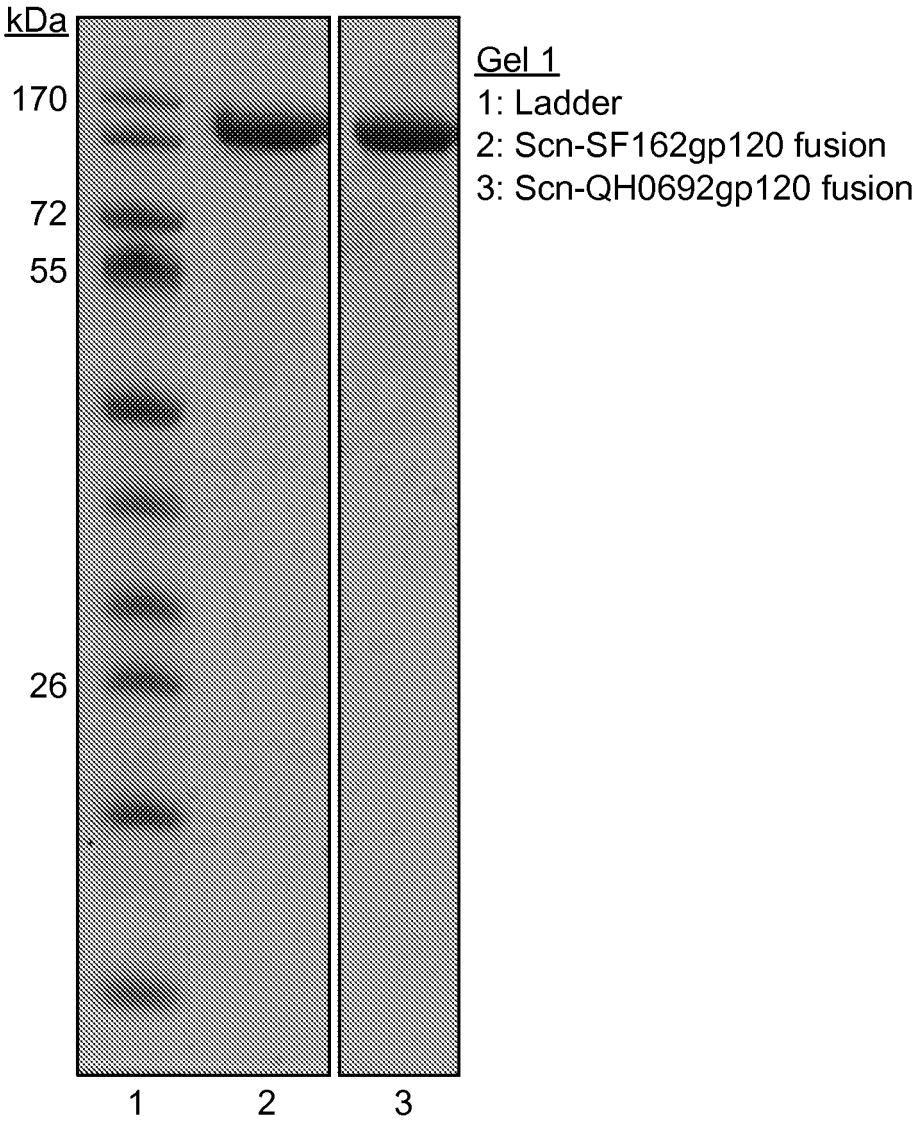

kDa
170
72
55
26
1
2
3
Gel 1
1: Ladder
2: Scn-SF162gp120 fusion
3: Scn-QH0692gp120 fusion

FIG. 20

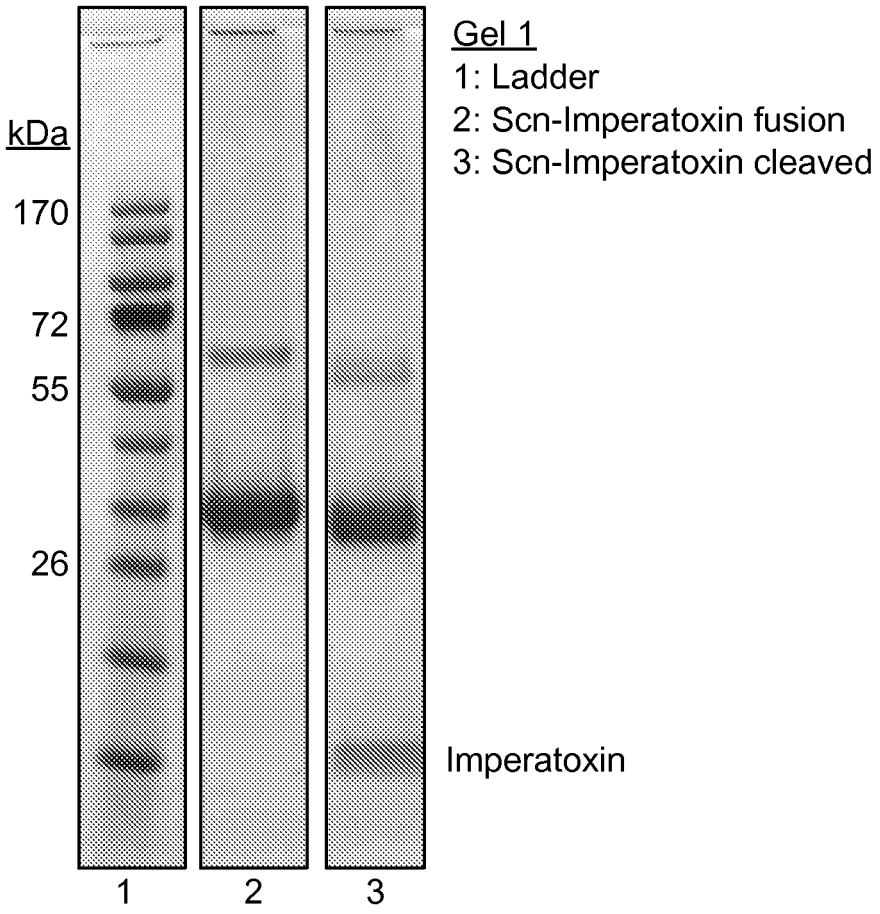

kDa
170
72
55
26
1
2
3
Gel 1
1: Ladder
2: Scn-Imperatoxin fusion
3: Scn-Imperatoxin cleaved
Imperatoxin

FIG. 21

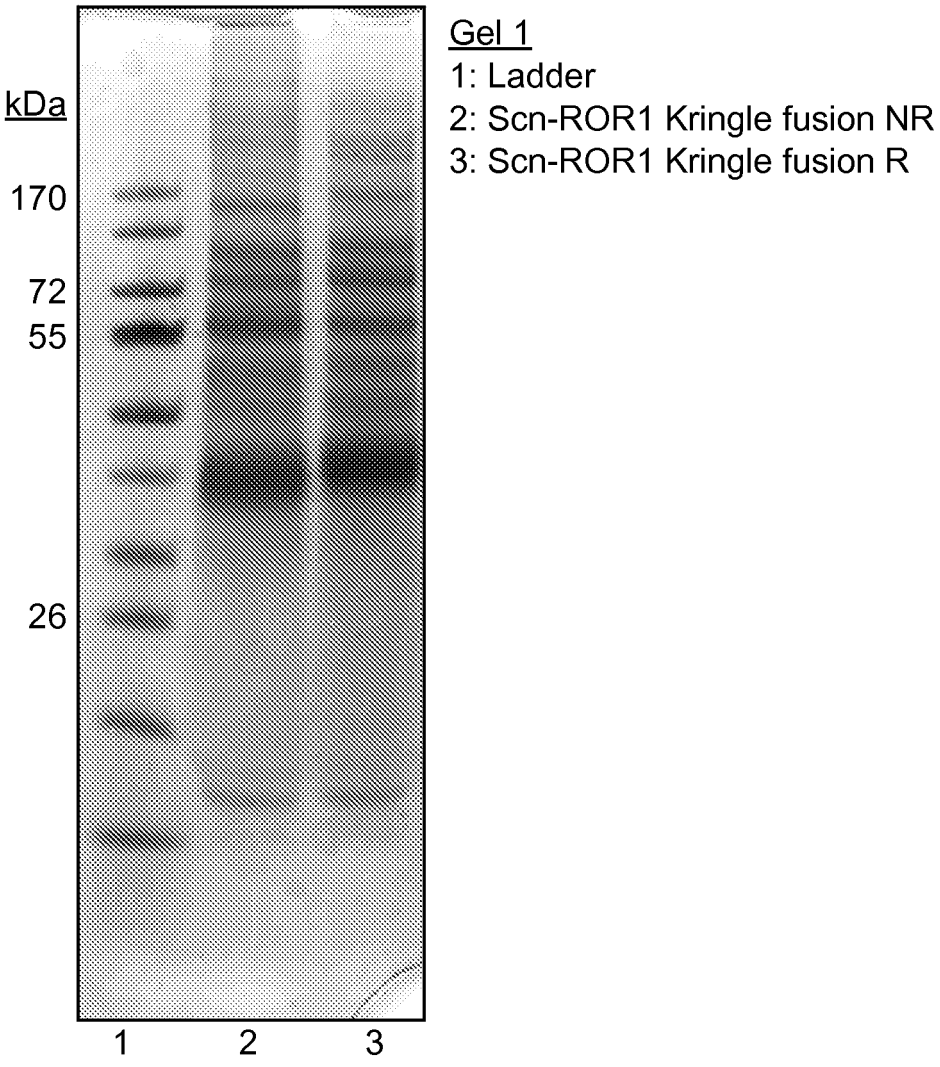

kDa
170
72
55
26
1
2
3
Gel 1
1: Ladder
2: Scn-ROR1 Kringle fusion NR
3: Scn-ROR1 Kringle fusion R

FIG. 22

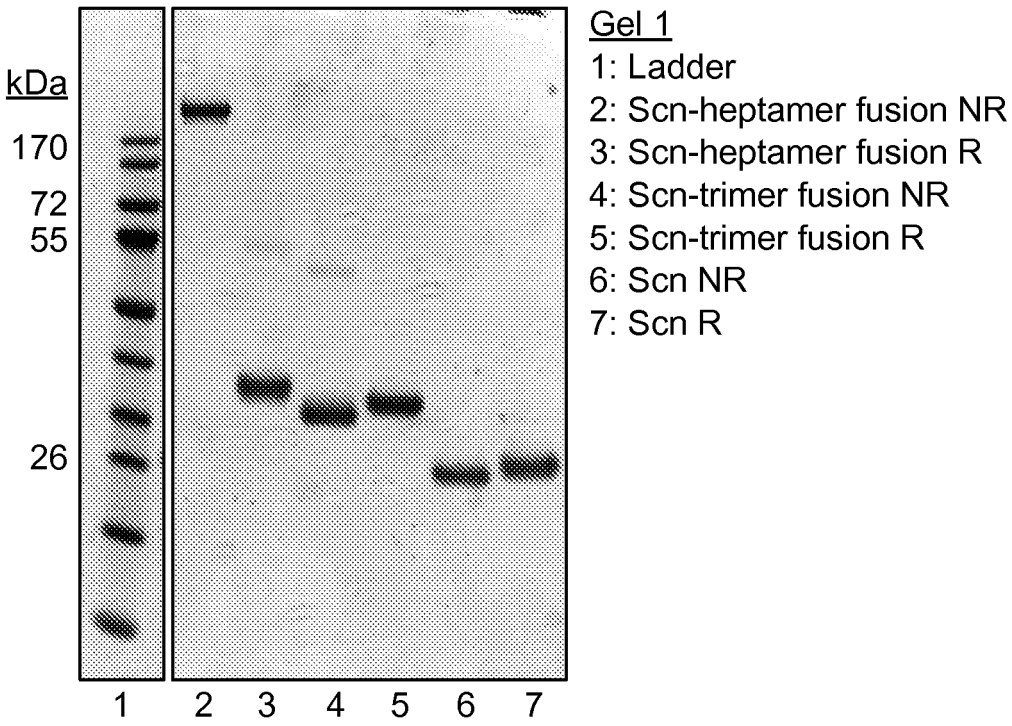

kDa
170
72
55
26
1
2
3
4
5
6
7
Gel 1
1: Ladder
2: Scn-heptamer fusion NR
3: Scn-heptamer fusion R
4: Scn-trimer fusion NR
5: Scn-trimer fusion R
6: Scn NR
7: Scn R

FIG. 23

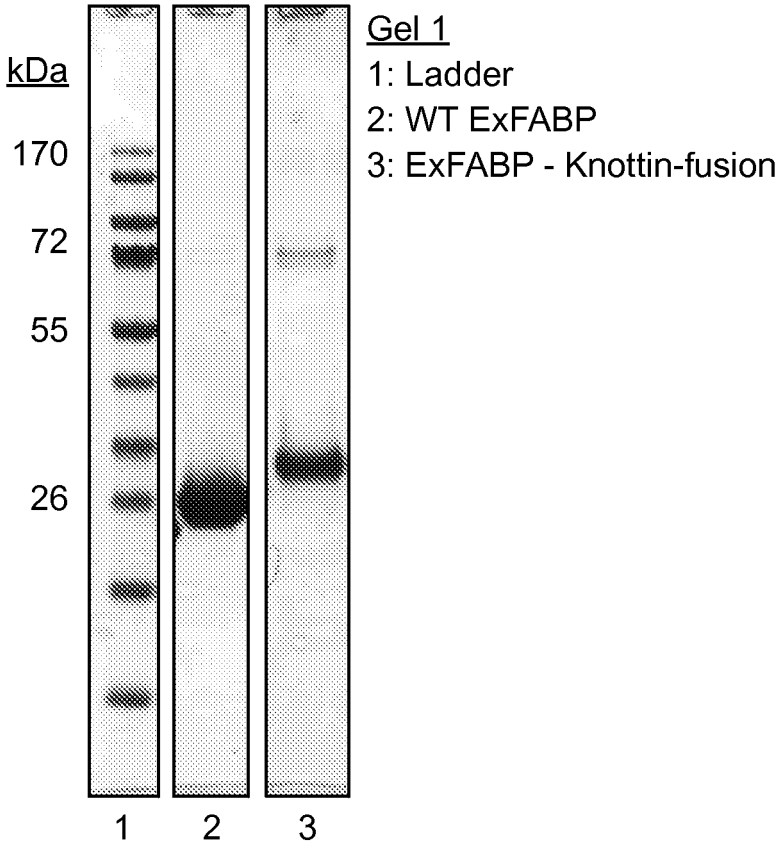

kDa
170
72
55
26
1
2
3
Gel 1
1: Ladder
2: WT ExFABP
3: ExFABP - Knottin-fusion

FIG. 24

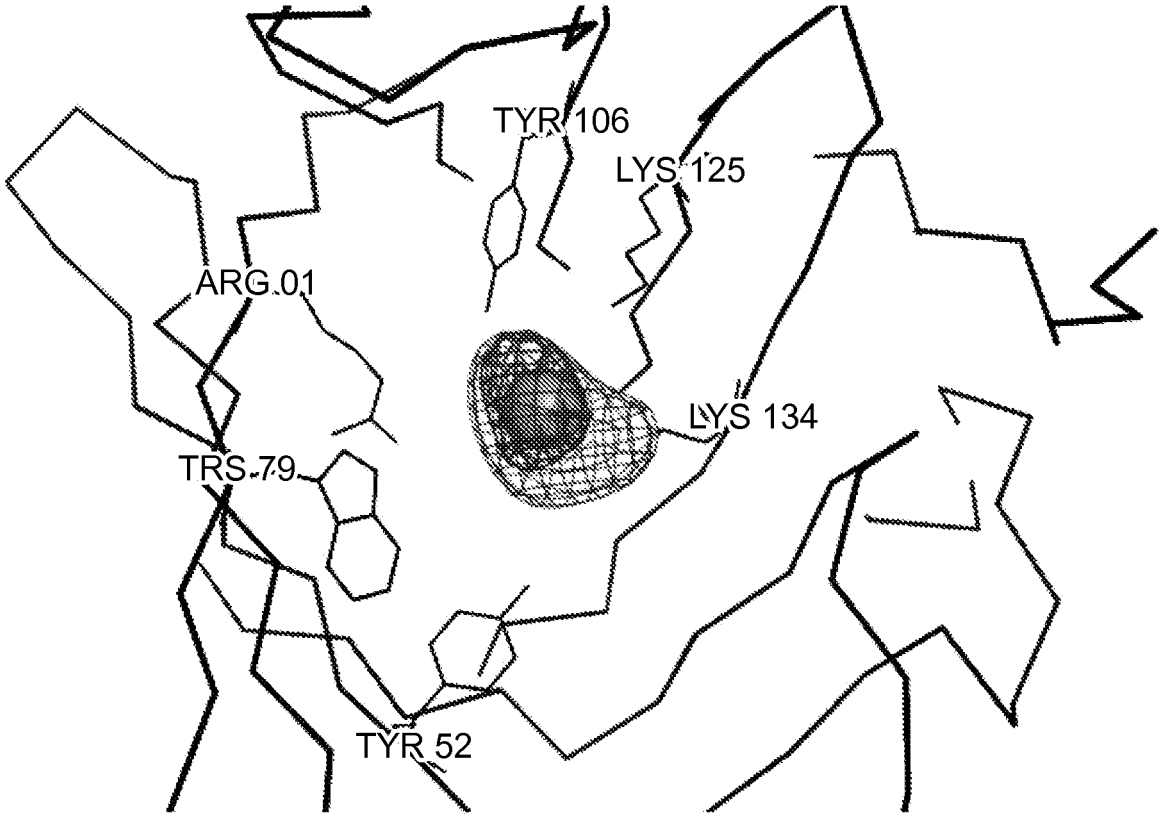


FIG. 25

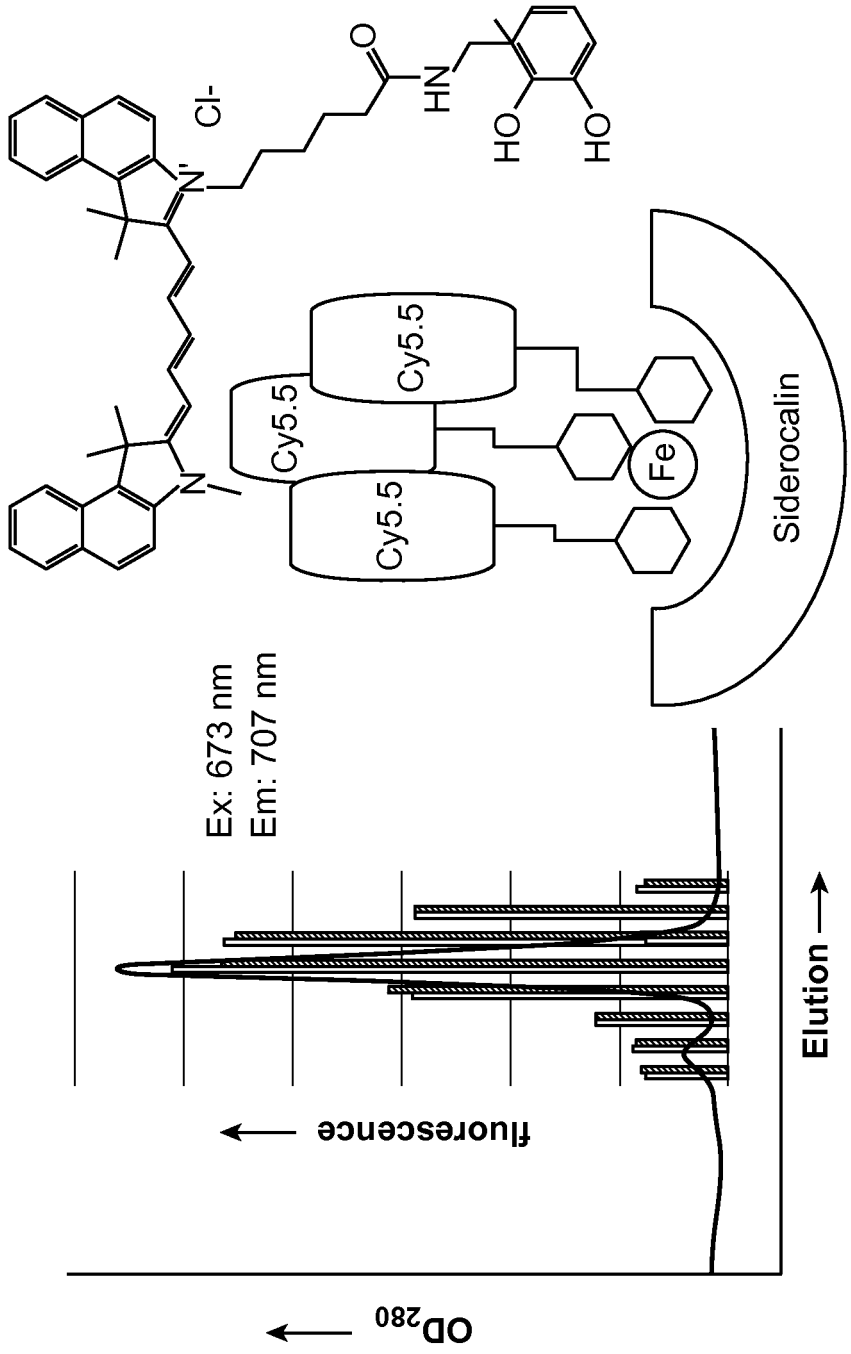

Cl-
N
N
O
HN
HO
HO
Cy5.5
Cy5.5
Cy5.5
Fe
Siderocalin
Ex: 673 nm
Em: 707 nm
fluorescence
Elution
OD280

FIG. 26