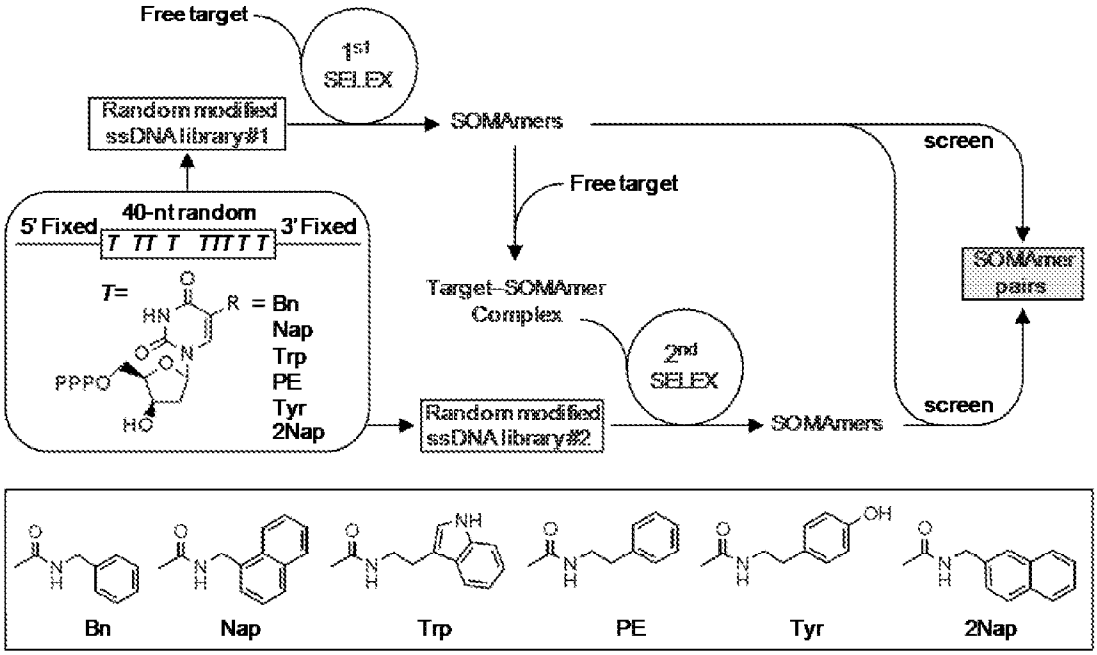

Free target
1st SELEX
Random modified ssDNA library#1
SOMAmers
screen
Free target
40-nt random
5' Fixed
T TT T TTT T T
3' Fixed
T=
R = Bn
Nap
Trp
PE
Tyr
2Nap
Target–SOMAmer Complex
2nd SELEX
SOMAmer pairs
Random modified ssDNA library#2
SOMAmers
screen
Bn
Nap
Trp
PE
Tyr
2Nap

FIG. 1A

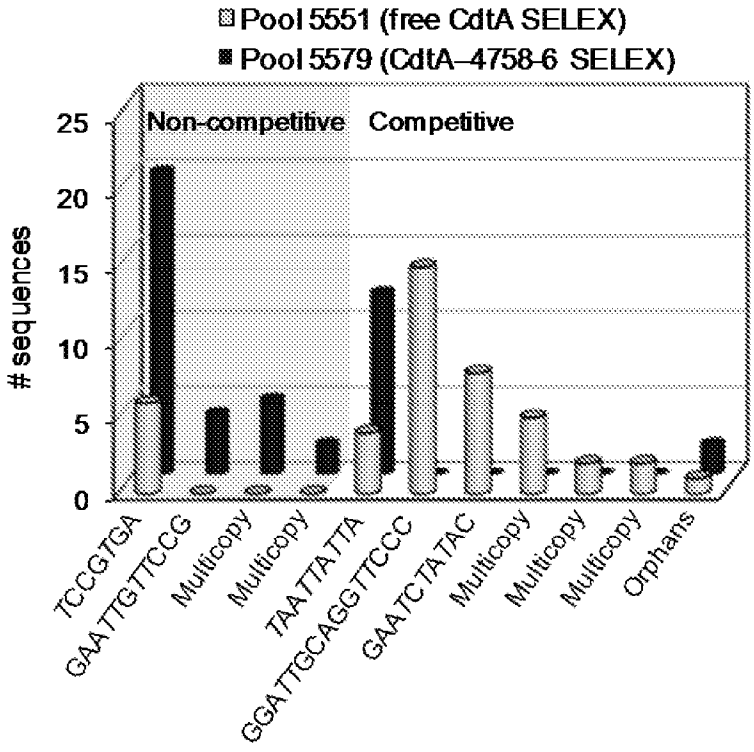

Pool 5551 (free CdtA SELEX)
Pool 5579 (CdtA–4758-6 SELEX)
Non-competitive
Competitive
sequences
25
20
15
10
5
0
TCCGTGA
GAATTGTTCCG
Multicopy
Multicopy
TAATTATTA
GGATTGCAGGTTCCC
GAATCTATAC
Multicopy
Multicopy
Multicopy
Orphans

FIG. 1B

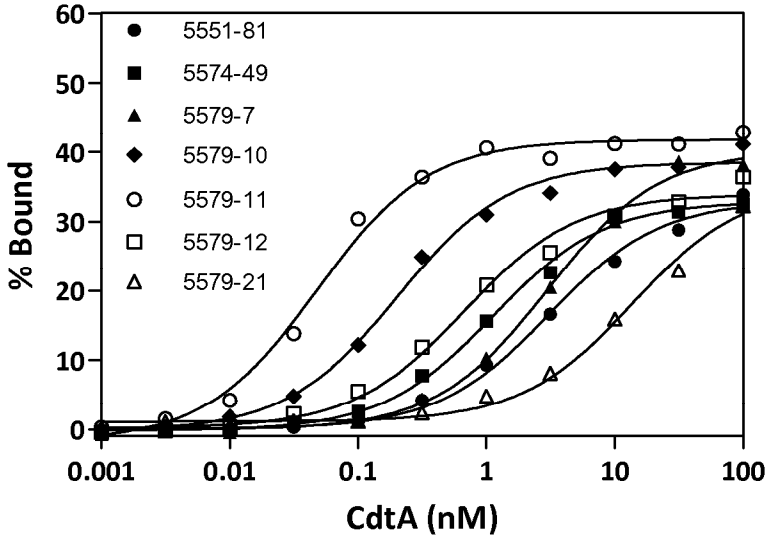


FIG. 1C

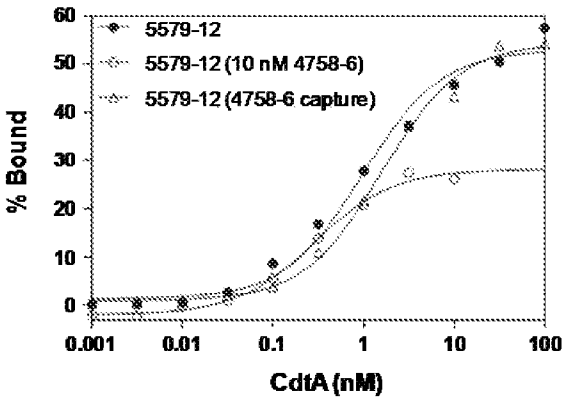


FIG. 1D

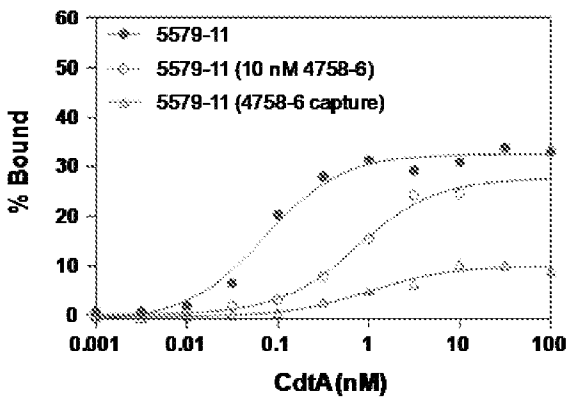


FIG. 1E

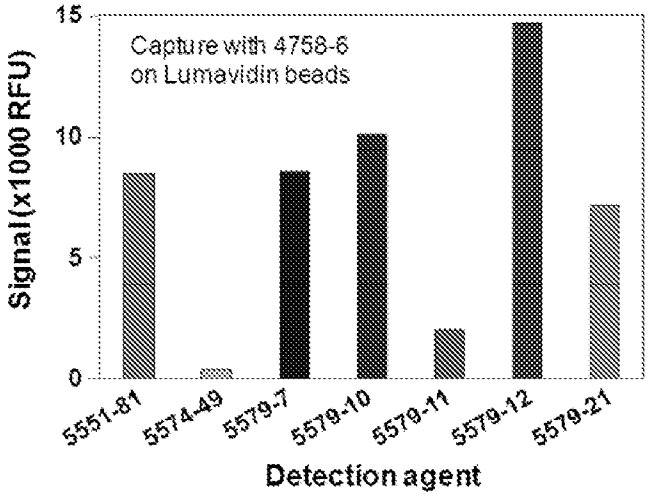

Capture with 4758-6
on Lumavidin beads
Signal (x1000 RFU)
15
10
5
0
5551-81
5574-49
5579-7
5579-10
5579-11
5579-12
5579-21
Detection agent

FIG. 1F

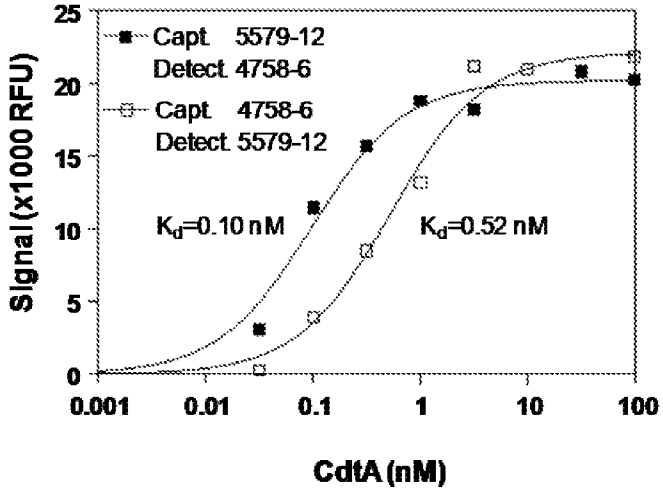

Capt. 5579-12
Detect. 4758-6
Capt. 4758-6
Detect. 5579-12
K_d=0.10 nM
K_d=0.52 nM
Signal (x1000 RFU)
25
20
15
10
5
0
0.001
0.01
0.1
1
10
100
CdtA (nM)

FIG. 1G

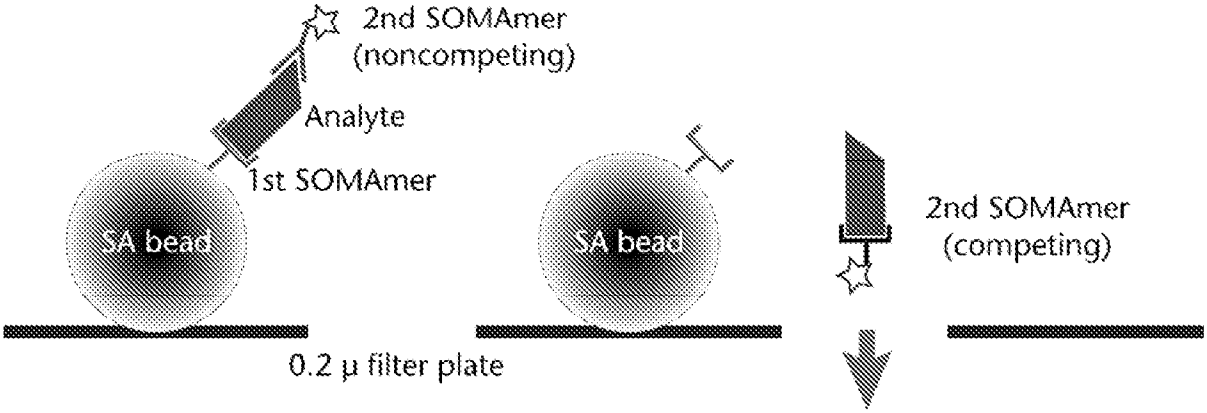

2nd SOMAmer
(noncompeting)
Analyte
1st SOMAmer
SA bead
SA bead
2nd SOMAmer
(competing)
0.2 µ filter plate

FIG. 2A

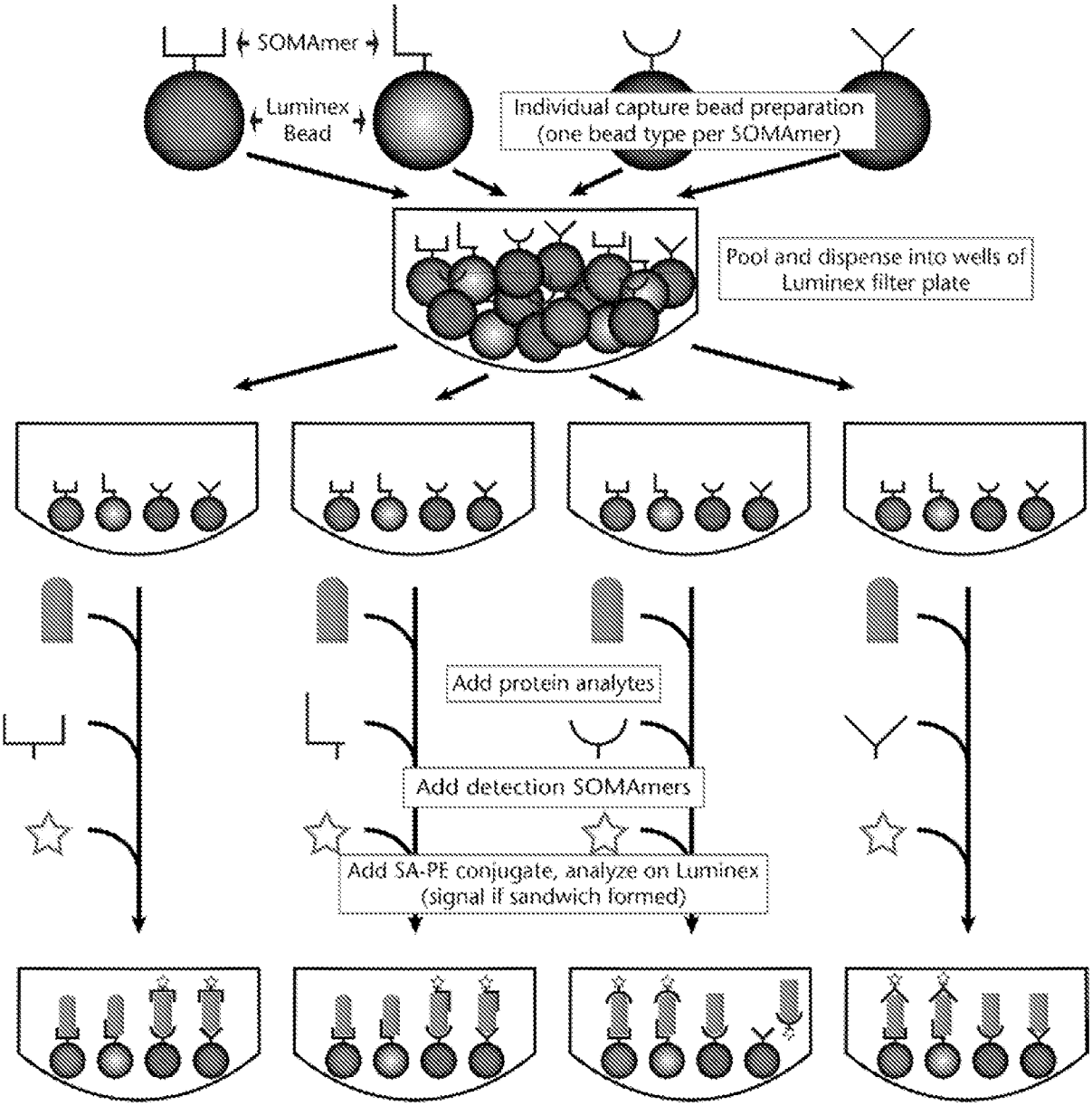

SOMAmer
Luminex
Bead
Individual capture bead preparation
(one bead type per SOMAmer)
Pool and dispense into wells of
Luminex filter plate
Add protein analytes
Add detection SOMAmers
Add SA-PE conjugate, analyze on Luminex
(signal if sandwich formed)

FIG. 2B

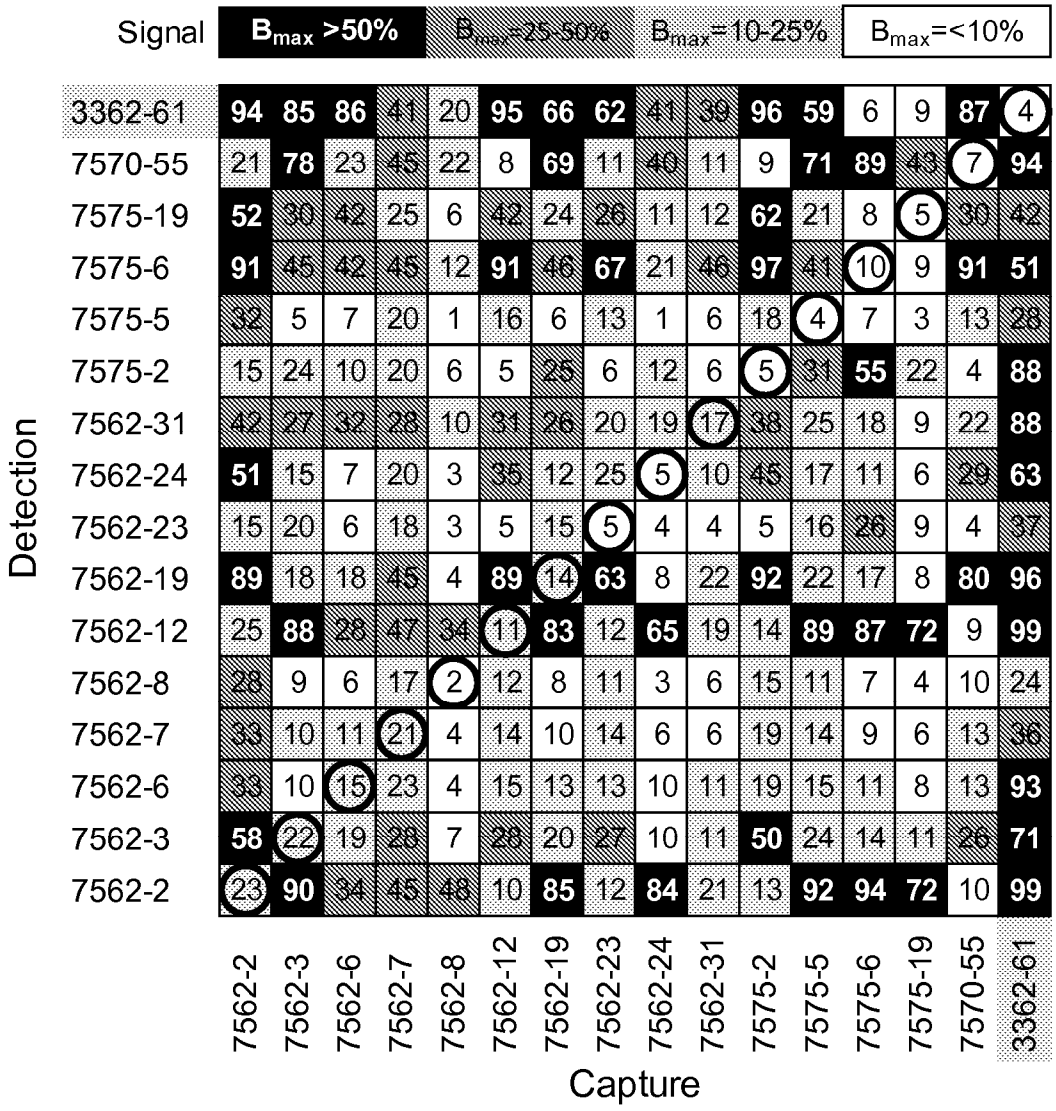
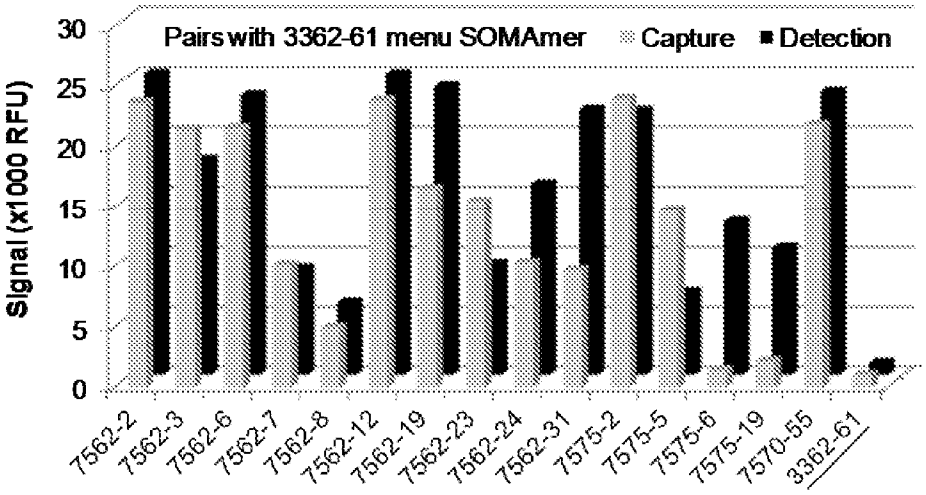

Signal
B_{max} >50%
B_{max}=25-50%
B_{max}=10-25%
B_{max}=<10%
Detection
3362-61 94 85 86 41 20 95 66 62 41 39 96 59 6 9 87 4
7570-55 21 78 23 45 22 8 69 11 40 11 9 71 89 43 7 94
7575-19 52 30 42 25 6 42 24 26 11 12 62 21 8 5 30 42
7575-6 91 45 42 45 12 91 46 67 21 46 97 41 10 9 91 51
7575-5 32 5 7 20 1 16 6 13 1 6 18 4 7 3 13 28
7575-2 15 24 10 20 6 5 25 6 12 6 5 31 55 22 4 88
7562-31 42 27 32 28 10 31 26 20 19 17 38 25 18 9 22 88
7562-24 51 15 7 20 3 35 12 25 5 10 45 17 11 6 29 63
7562-23 15 20 6 18 3 5 15 5 4 4 5 16 26 9 4 37
7562-19 89 18 18 45 4 89 14 63 8 22 92 22 17 8 80 96
7562-12 25 88 28 47 34 11 83 12 65 19 14 89 87 72 9 99
7562-8 28 9 6 17 2 12 8 11 3 6 15 11 7 4 10 24
7562-7 33 10 11 21 4 14 10 14 6 6 19 14 9 6 13 36
7562-6 33 10 15 23 4 15 13 13 10 11 19 15 11 8 13 93
7562-3 58 22 19 28 7 28 20 27 10 11 50 24 14 11 26 71
7562-2 23 90 34 45 48 10 85 12 84 21 13 92 94 72 10 99
7562-2
7562-3
7562-6
7562-7
7562-8
7562-12
7562-19
7562-23
7562-24
7562-31
7575-2
7575-5
7575-6
7575-19
7570-55
3362-61
Capture

FIG. 2C

FIG. 2D


Pairs with 3362-61 menu SOMAmer
Capture
Detection
Signal (x1000 RFU)
30
25
20
15
10
5
0
7562-2
7562-3
7562-6
7562-7
7562-8
7562-12
7562-19
7562-23
7562-24
7562-31
7575-2
7575-5
7575-6
7575-19
7570-55
3362-61

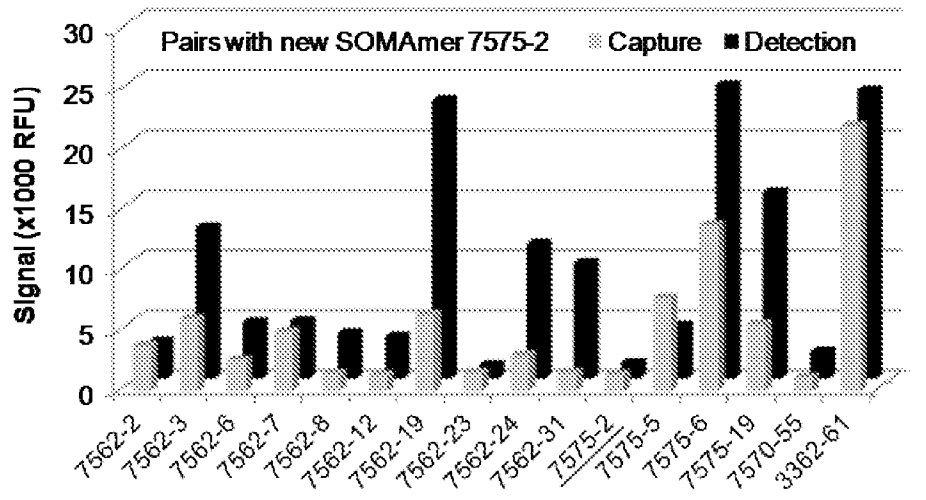
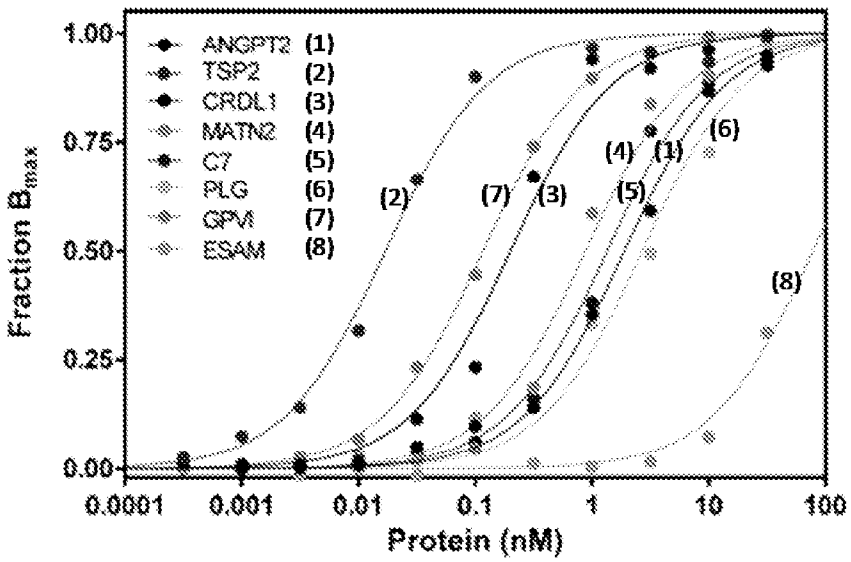

Pairs with new SOMAmer 7575-2
Capture
Detection
Signal (x1000 RFU)
30
25
20
15
10
5
0
7562-2
7562-3
7562-6
7562-7
7562-8
7562-12
7562-19
7562-23
7562-24
7562-31
7575-2
7575-5
7575-6
7575-19
7570-55
3362-61

FIG. 2E


1.00
0.75
0.50
0.25
0.00
Fraction B_{max}
0.0001
0.001
0.01
0.1
1
10
100
Protein (nM)
ANGPT2 (1)
TSP2 (2)
CRDL1 (3)
MATN2 (4)
C7 (5)
PLG (6)
GPVI (7)
ESAM (8)
(2)
(7)
(3)
(4)
(1)
(5)
(6)
(8)

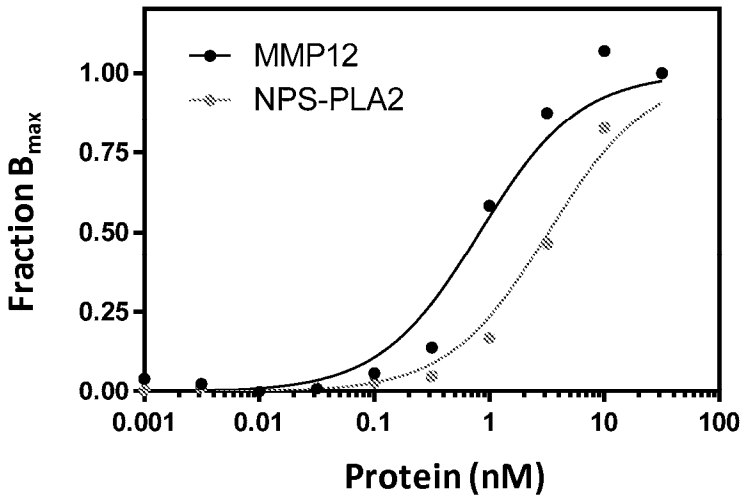

MMP12
NPS-PLA2
1.00
0.75
0.50
0.25
0.00
Fraction B_{max}
0.001
0.01
0.1
1
10
100
Protein (nM)

FIG. 2F

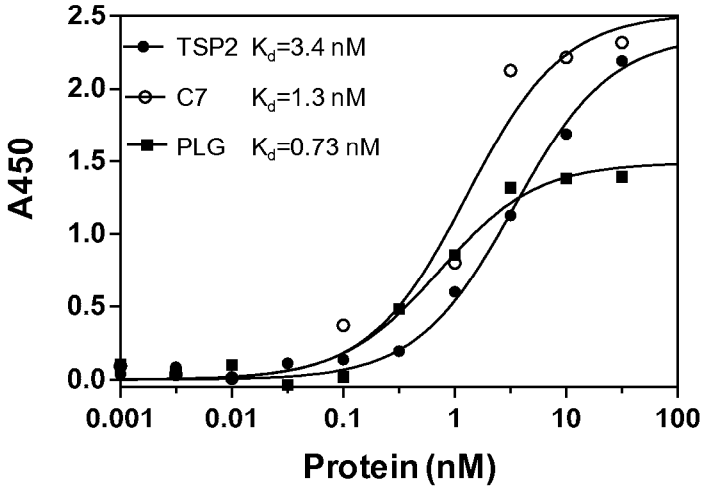

2.5
2.0
1.5
1.0
0.5
0.0
A450
TSP2 K_d=3.4 nM
C7 K_d=1.3 nM
PLG K_d=0.73 nM
0.001
0.01
0.1
1
10
100
Protein (nM)

FIG. 3