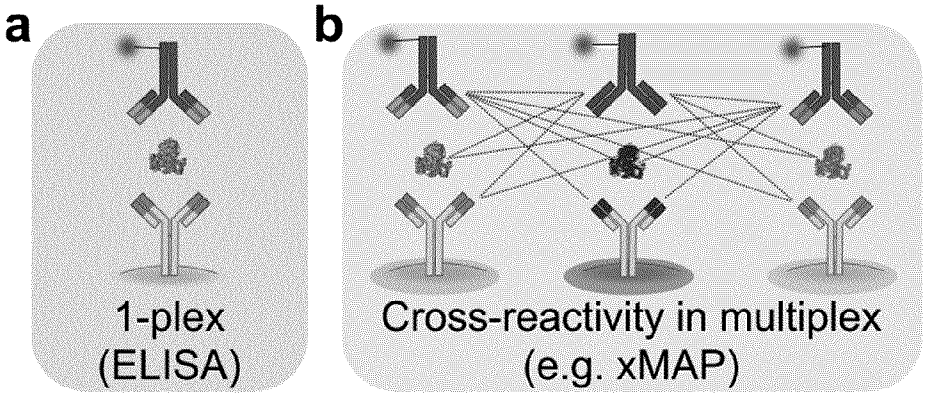
FIG. 1



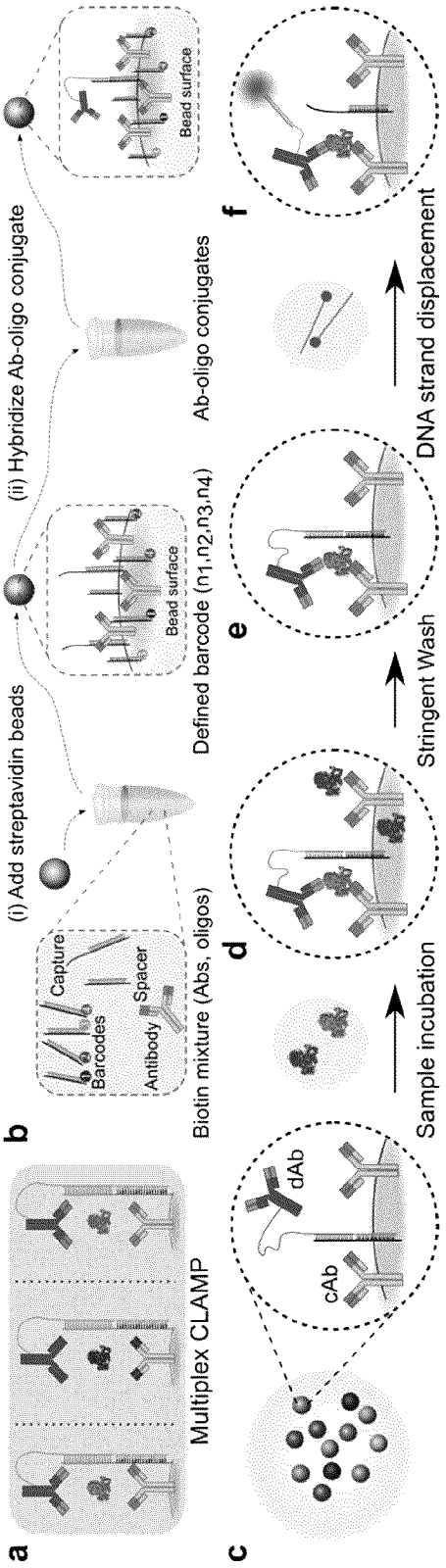

a
Multiplex CLAMP
b
Capture
Barcodes
Spacer
Antibody
Biotin mixture (Abs, oligos)
(i) Add streptavidin beads
Bead surface
Defined barcode (n1,n2,n3,n4)
(ii) Hybridize Ab-oligo conjugate
Bead surface
Ab-oligo conjugates
c
cAb
dAb
Sample incubation
d
Stringent Wash
e
DNA strand displacement
f

FIG. 2

FIG. 3

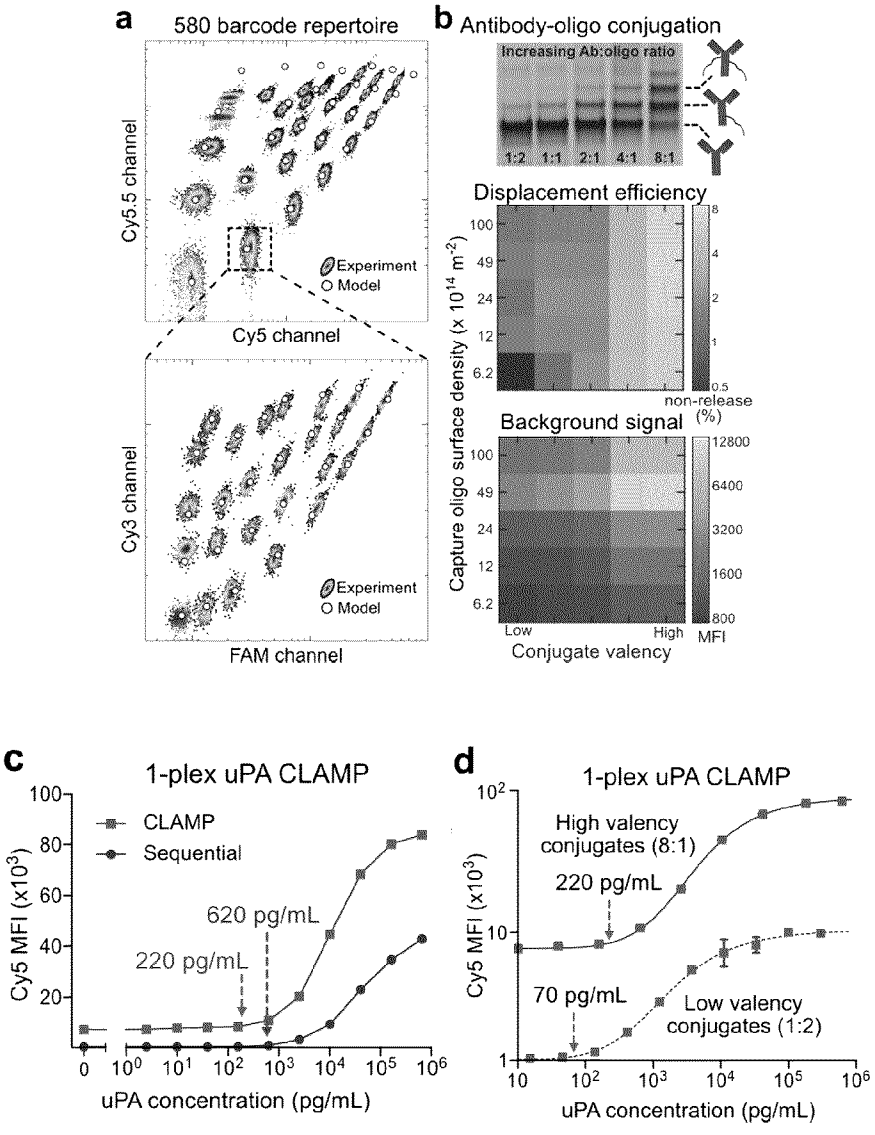

a
580 barcode repertoire
Cy5.5 channel
Experiment
Model
Cy5 channel
Cy3 channel
Experiment
Model
FAM channel
b
Antibody-oligo conjugation
Increasing Ab:oligo ratio
1:2 1:1 2:1 4:1 8:1
Displacement efficiency
Capture oligo surface density (x 10^14 m^-2)
100 49 24 12 6.2
8 4 2 1 0.5
non-release (%)
Background signal
12800 6400 3200 1600 800
MFI
Low High
Conjugate valency
c
1-plex uPA CLAMP
CLAMP
Sequential
620 pg/mL
220 pg/mL
Cy5 MFI (x10^3)
100 80 60 40 20
0 10^0 10^1 10^2 10^3 10^4 10^5 10^6
uPA concentration (pg/mL)
d
1-plex uPA CLAMP
High valency conjugates (8:1)
220 pg/mL
70 pg/mL
Low valency conjugates (1:2)
Cy5 MFI (x10^3)
10^2 10 1
10 10^2 10^3 10^4 10^5 10^6
uPA concentration (pg/mL)

FIG. 3 continued

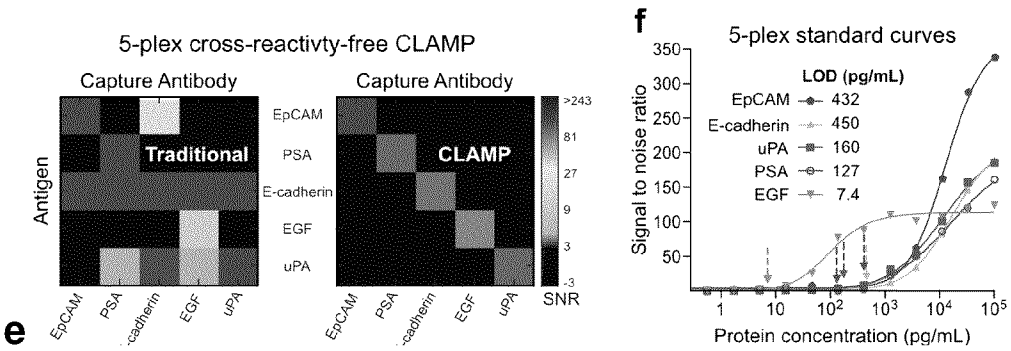


FIG. 4

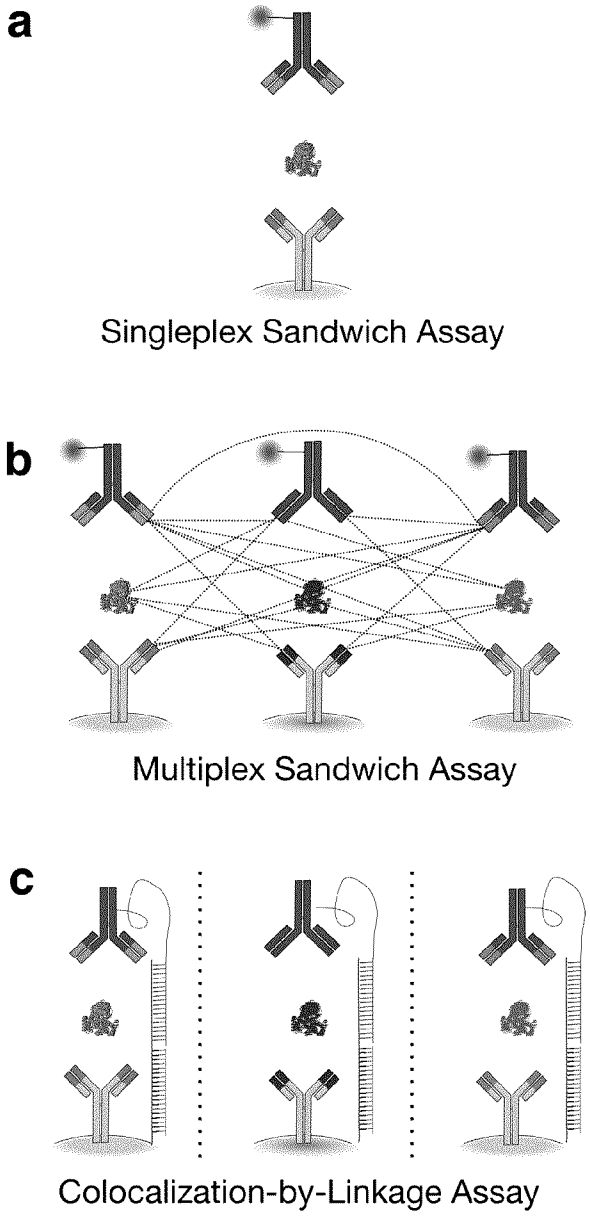

a
Singleplex Sandwich Assay
b
Multiplex Sandwich Assay
c
Colocalization-by-Linkage Assay

FIG 5.

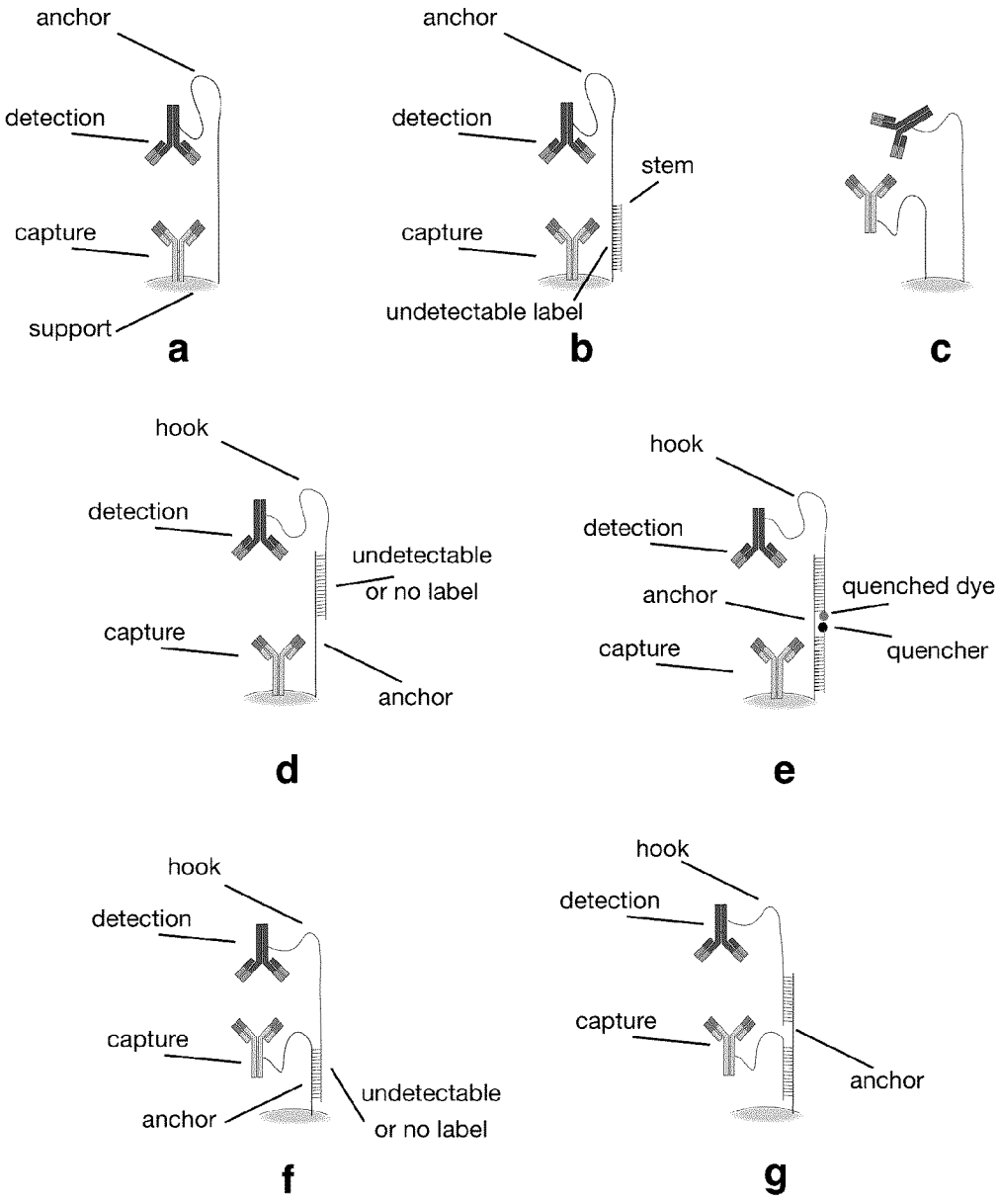

anchor
detection
capture
support
a
anchor
detection
stem
capture
undetectable label
b
c
hook
detection
undetectable
or no label
capture
anchor
d
hook
detection
anchor
quenched dye
capture
quencher
e
hook
detection
capture
anchor
undetectable
or no label
f
hook
detection
capture
anchor
g

FIG 6.

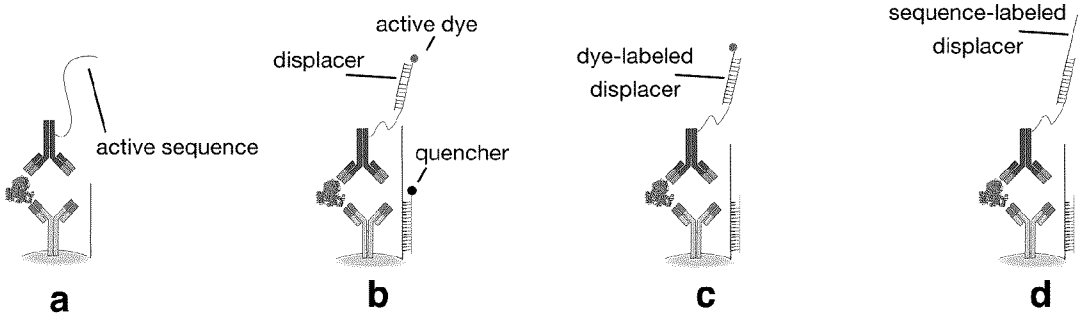
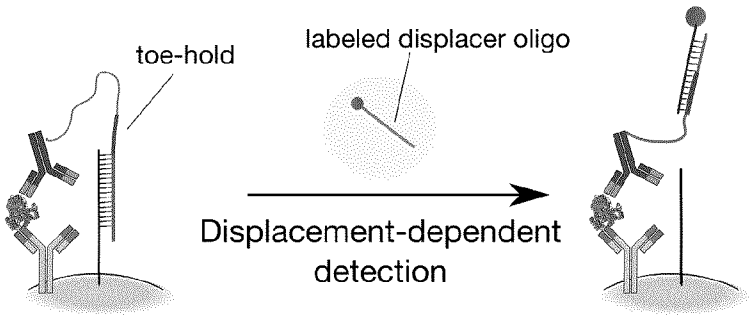

active sequence
active dye
displacer
quencher
dye-labeled
displacer
sequence-labeled
displacer
a
b
c
d

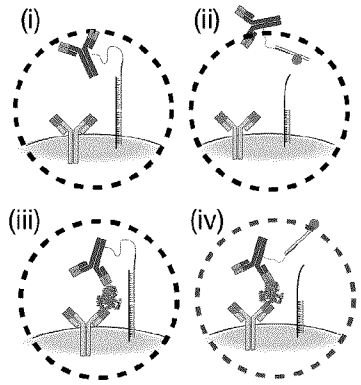
FIG. 7



a

AND gate

	analyte	displace	signal
(i)	0	0	0
(ii)	0	1	0
(iii)	1	0	0
(iv)	1	1	1



b

FIG. 8

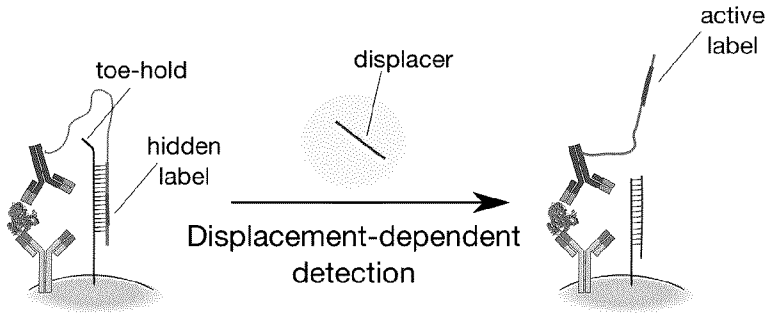


FIG. 9

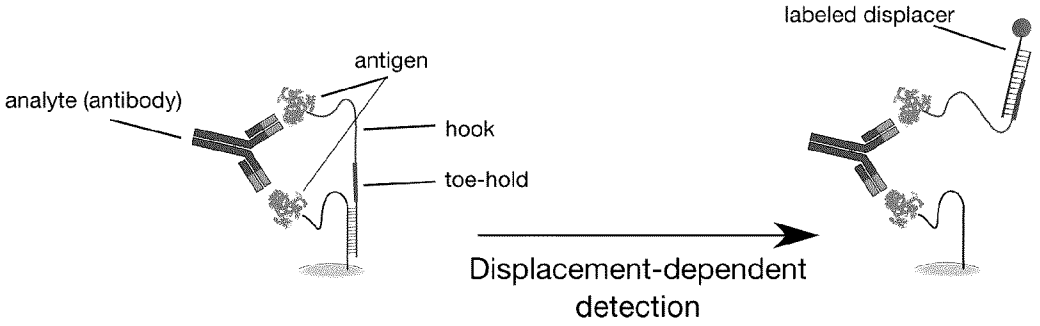


FIG. 10

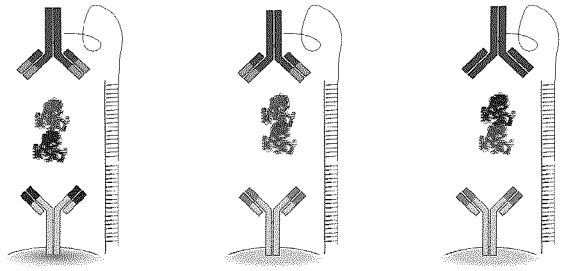


FIG. 11

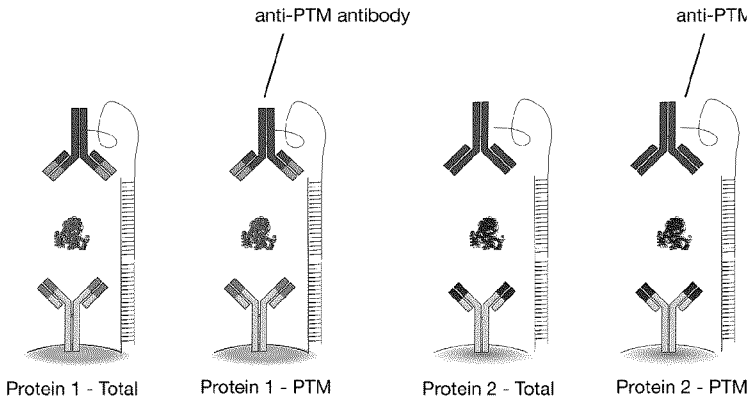

anti-PTM antibody
anti-PTM
Protein 1 - Total
Protein 1 - PTM
Protein 2 - Total
Protein 2 - PTM

FIG. 12

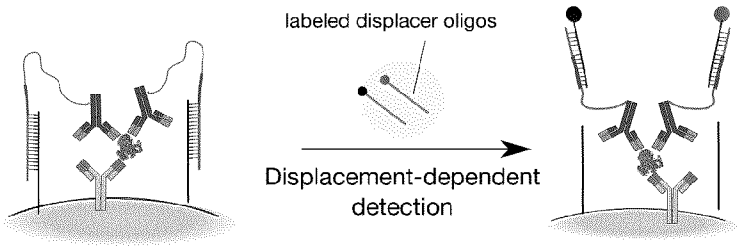

labeled displacer oligos
Displacement-dependent detection

FIG. 13

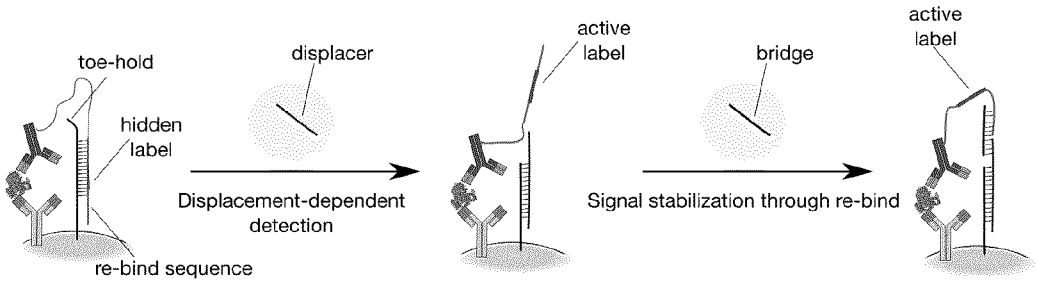

toe-hold
hidden label
re-bind sequence
displacer
Displacement-dependent detection
active label
bridge
Signal stabilization through re-bind
active label

FIG. 14

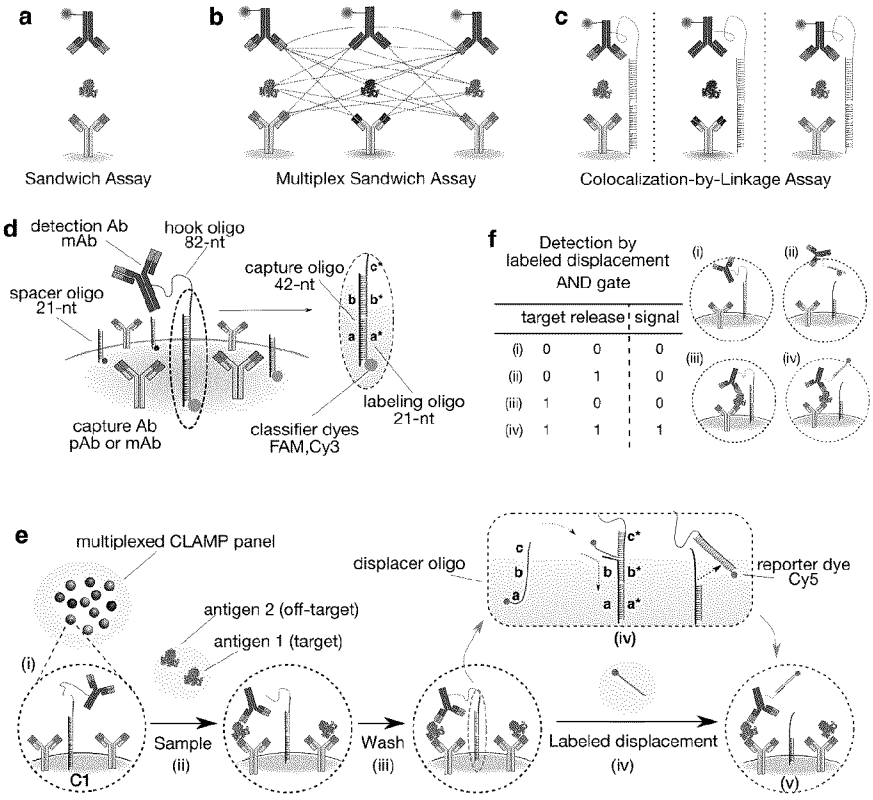

a
Sandwich Assay
b
Multiplex Sandwich Assay
c
Colocalization-by-Linkage Assay
d
detection Ab mAb
hook oligo 82-nt
spacer oligo 21-nt
capture oligo 42-nt
capture Ab pAb or mAb
classifier dyes FAM,Cy3
labeling oligo 21-nt
f
Detection by labeled displacement AND gate
target release
signal
(i) 0 0 0
(ii) 0 1 0
(iii) 1 0 0
(iv) 1 1 1
e
multiplexed CLAMP panel
antigen 2 (off-target)
antigen 1 (target)
displacer oligo
reporter dye Cy5
Sample (ii)
Wash (iii)
Labeled displacement (iv)
C1
(v)

FIG. 15

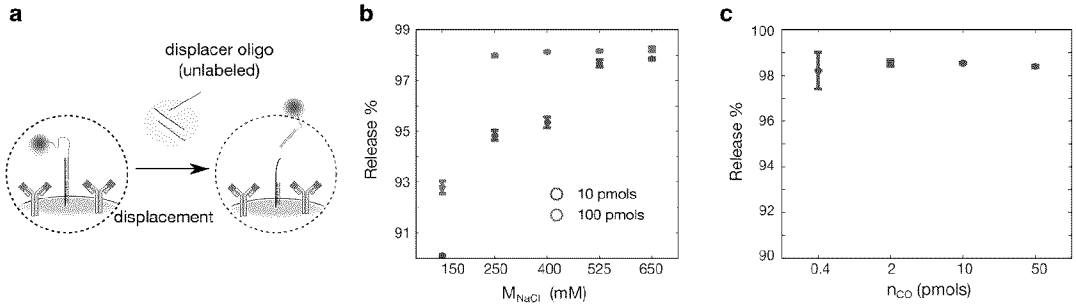


FIG. 16

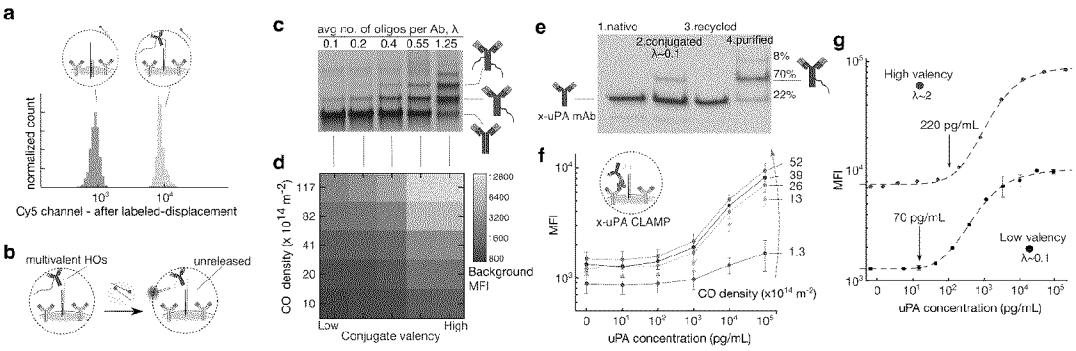


FIG. 17

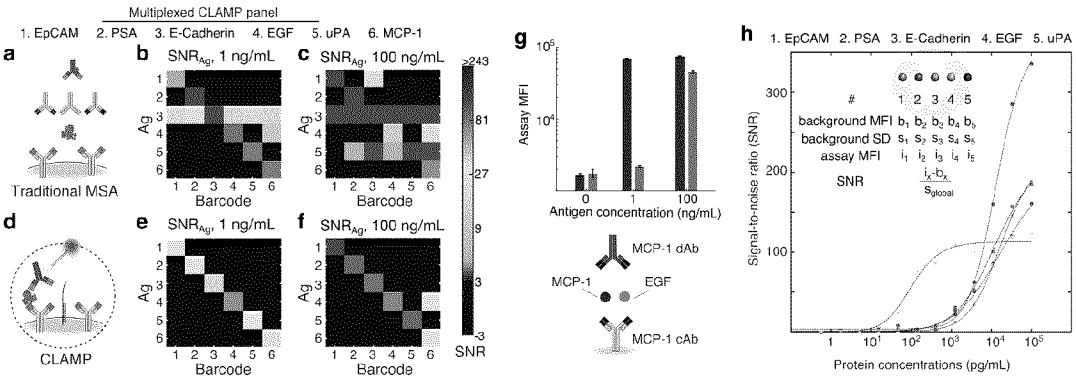


FIG. 18

MP	Strand	Modification	Sequence
CLAMP	Capture Oligo	Biotin	5'-Biotin-TTTTTTTTTTGTGGCGGCGGTGATTGGTTATTGAGAGTTTATG
	Spacer Oligo	Biotin	5'-Biotin- TTTTTTTTTGTGGCGGCGGTG
	Encoder Oligo		5'-CACCGCCGCCACAAAAAAAAA
	Encoder Oligo (FAM)	FAM	5'-CACCGCCGCCACAAAAAAAAA-FAM
	Encoder Oligo (Cy3)	Cy3	5'-CACCGCCGCCACAAAAAAAAA-Cy3
	Hook Oligo	Thiol	5'-Thiol-TTTTTTACTTTTCAACCACCACTCAACCATATTCAACTCATTCGCCATAAACTCATTCGCCATAAACTCTCAATAACCAAT
	Hook Oligo (Cy5)	Cy5	5'-Cy5-TTTTTTACTTTTCAACCACCACTCAACCATATTCAACTCATTCGCCATAAACTCATTCGCCATAAACTCTCAATAACCAAT
	Displacer Oligo (Cy5)		5'-ATTGGTTATTGAGAGTTTATGGCGAATGAG-Cy5
	Displacer Oligo		5'-ATTGGTTATTGAGAGTTTATGGCGAATGAG
	Labeler Oligo		5'-GTTGAGTGGTGGTTGATTGGTTGGGATTGA
HOP-MPs	Capture Oligo (2)	Biotin	5'-Biotin-TTTTTTTTTGTGGCGGCGGTGGTTGAGTGGTGGTTGGTGTTGATT
	Displacer Oligo (2)		5'-CTCGTCATAATGTAAACCGGGAATCAACACCAACCACCACTCAAC

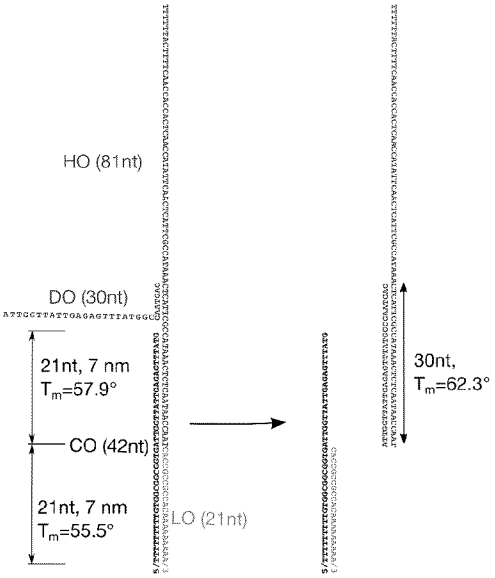


FIG. 19

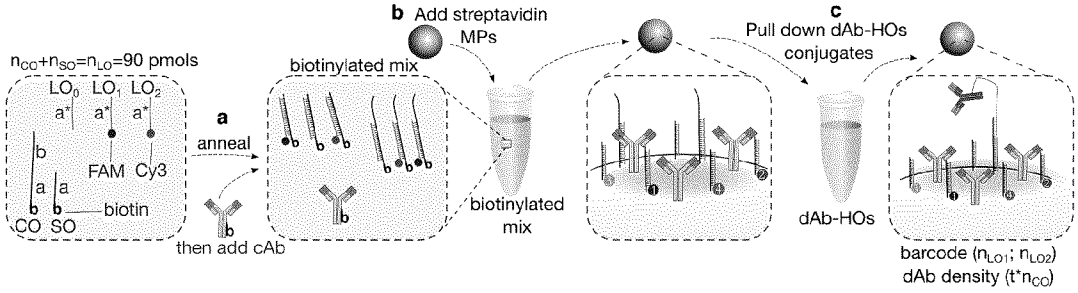

b Add streptavidin MPs
c Pull down dAb-HOs conjugates
$n_{CO}+n_{SO}=n_{LO}$=90 pmols
LO_0 LO_1 LO_2
a*
FAM Cy3
biotin
CO SO
a anneal
then add cAb
biotinylated mix
biotinylated mix
dAb-HOs
barcode (n_{LO1}; n_{LO2})
dAb density (t*n_{CO})

FIG. 20

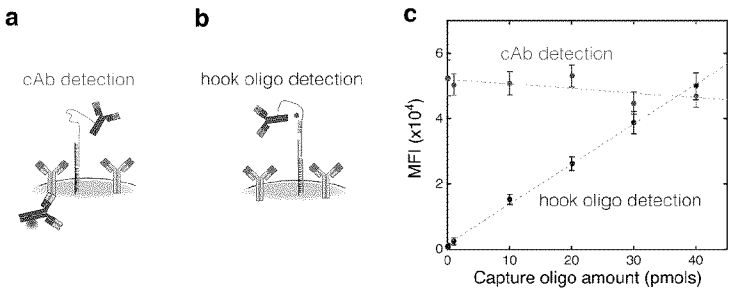

a
cAb detection
b
hook oligo detection
c
cAb detection
hook oligo detection
MFI (x10⁴)
0
2
4
6
0
10
20
30
40
Capture oligo amount (pmols)

FIG. 21

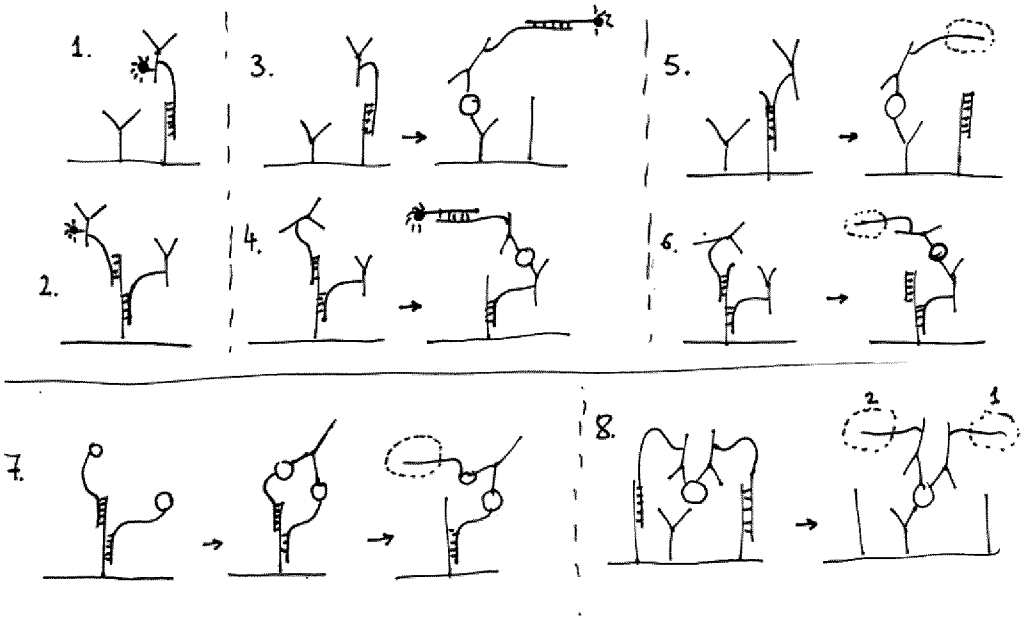


FIG. 22

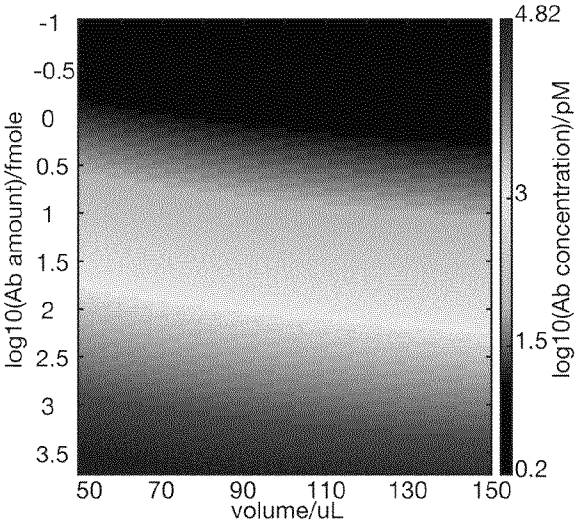

-1
-0.5
0
0.5
1
1.5
2
2.5
3
3.5
log10(Ab amount)/fmole
50
70
90
110
130
150
volume/uL
4.82
3
1.5
0.2
log10(Ab concentration)/pM

FIG. 23

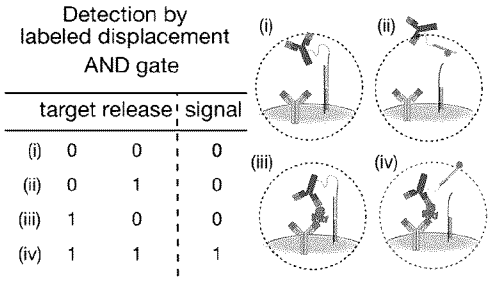

Detection by
labeled displacement
AND gate
target release
signal
(i) 0 0 0
(ii) 0 1 0
(iii) 1 0 0
(iv) 1 1 1
(i)
(ii)
(iii)
(iv)

FIG. 24

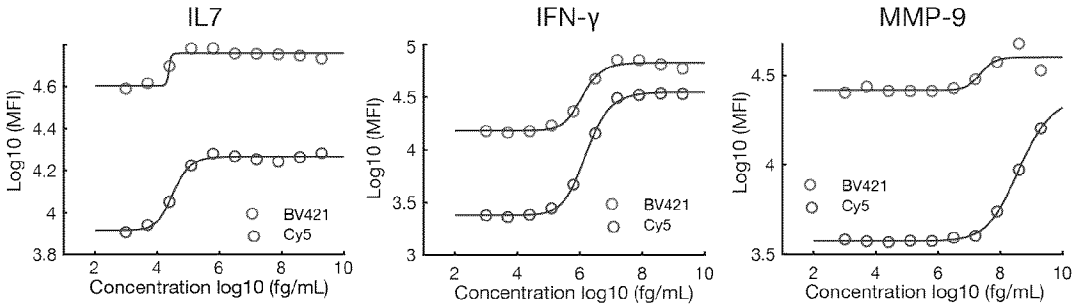

IL7
IFN-γ
MMP-9
Log10 (MFI)
Concentration log10 (fg/mL)
BV421
Cy5

FIG. 25

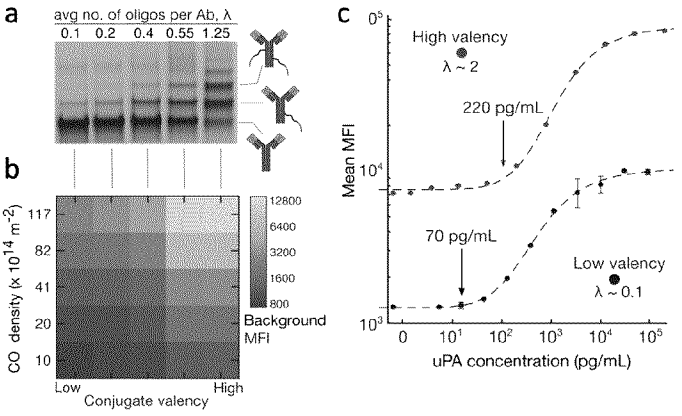
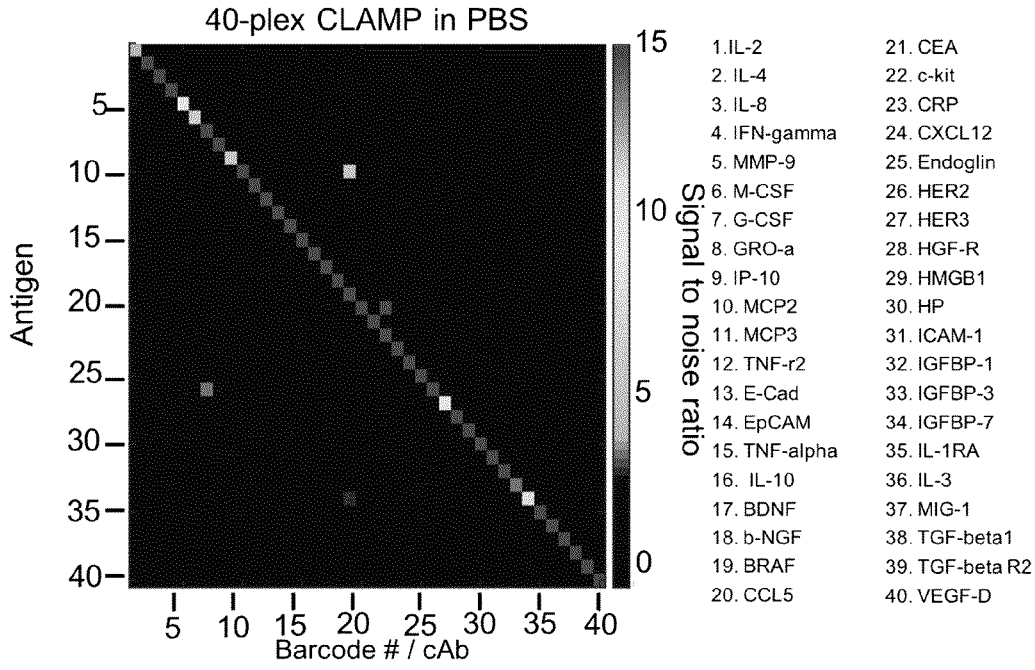

a
avg no. of oligos per Ab, λ
0.1 0.2 0.4 0.55 1.25
b
CO density (x 10^14 m^-2)
117
82
41
20
10
Low
High
Conjugate valency
12800
6400
3200
1600
800
Background MFI
c
Mean MFI
High valency
λ ~ 2
220 pg/mL
70 pg/mL
Low valency
λ ~ 0.1
uPA concentration (pg/mL)

FIG. 26


40-plex CLAMP in PBS
Antigen
Barcode # / cAb
Signal to noise ratio
1.IL-2
2. IL-4
3. IL-8
4. IFN-gamma
5. MMP-9
6. M-CSF
7. G-CSF
8. GRO-a
9. IP-10
10. MCP2
11. MCP3
12. TNF-r2
13. E-Cad
14. EpCAM
15. TNF-alpha
16. IL-10
17. BDNF
18. b-NGF
19. BRAF
20. CCL5
21. CEA
22. c-kit
23. CRP
24. CXCL12
25. Endoglin
26. HER2
27. HER3
28. HGF-R
29. HMGB1
30. HP
31. ICAM-1
32. IGFBP-1
33. IGFBP-3
34. IGFBP-7
35. IL-1RA
36. IL-3
37. MIG-1
38. TGF-beta1
39. TGF-beta R2
40. VEGF-D