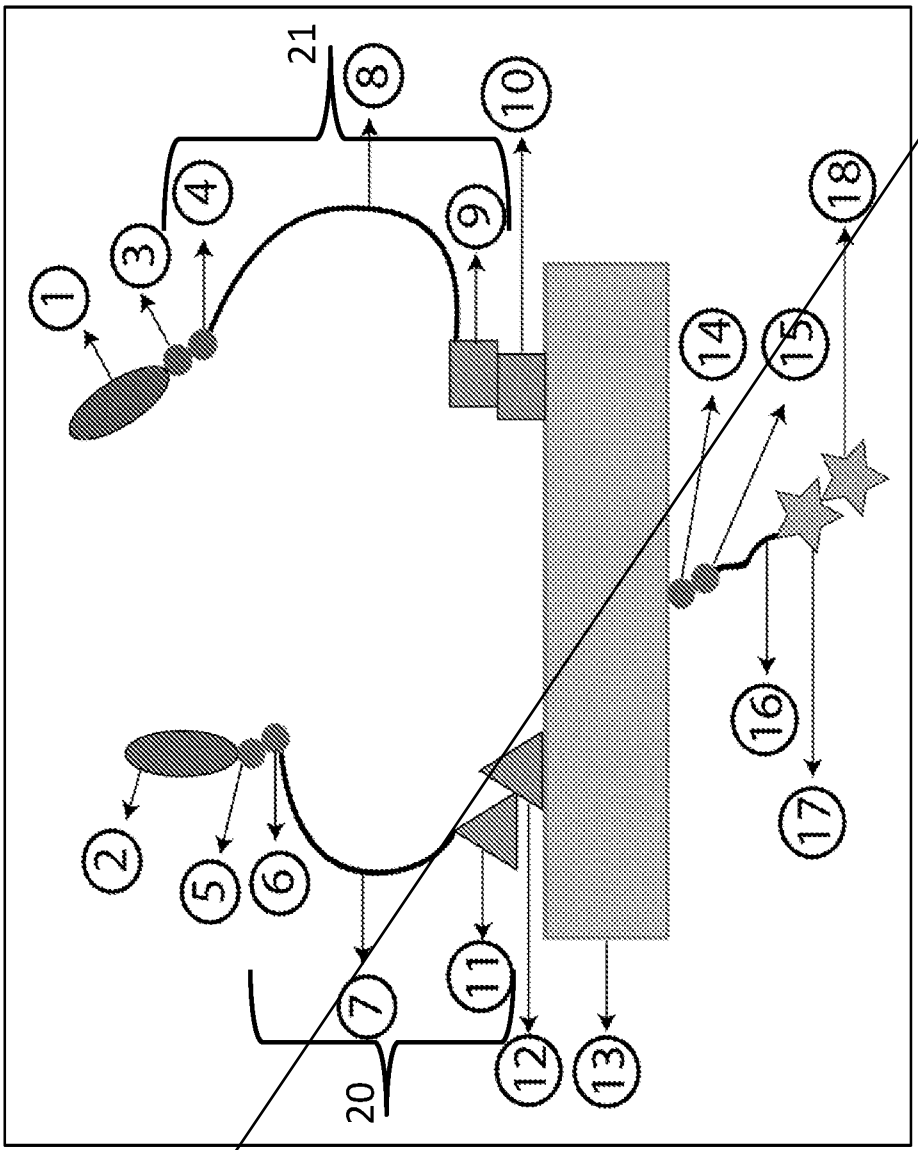


FIG. 1

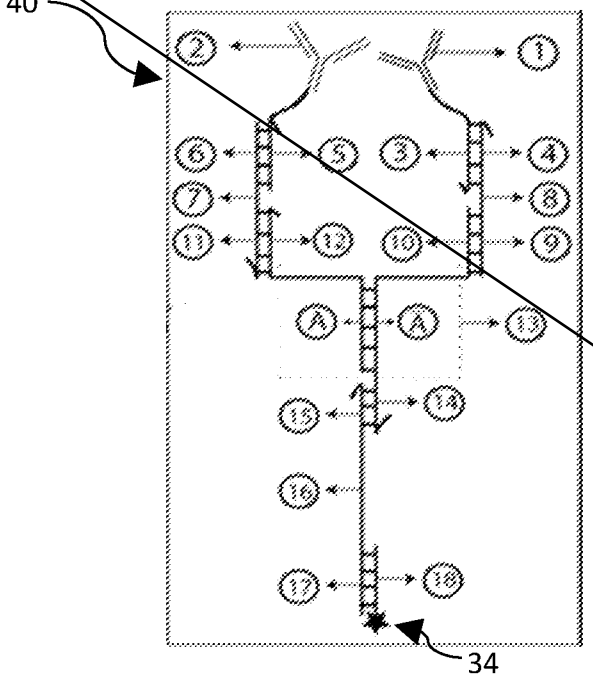


FIG. 2

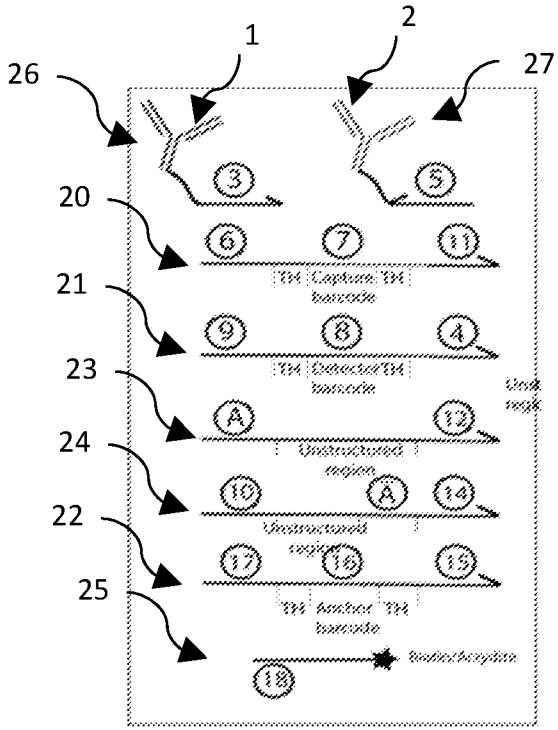


FIG. 3

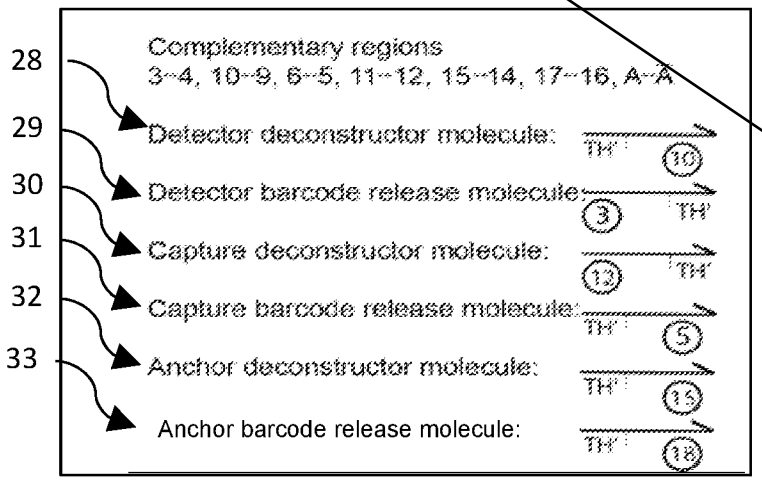


FIG. 4

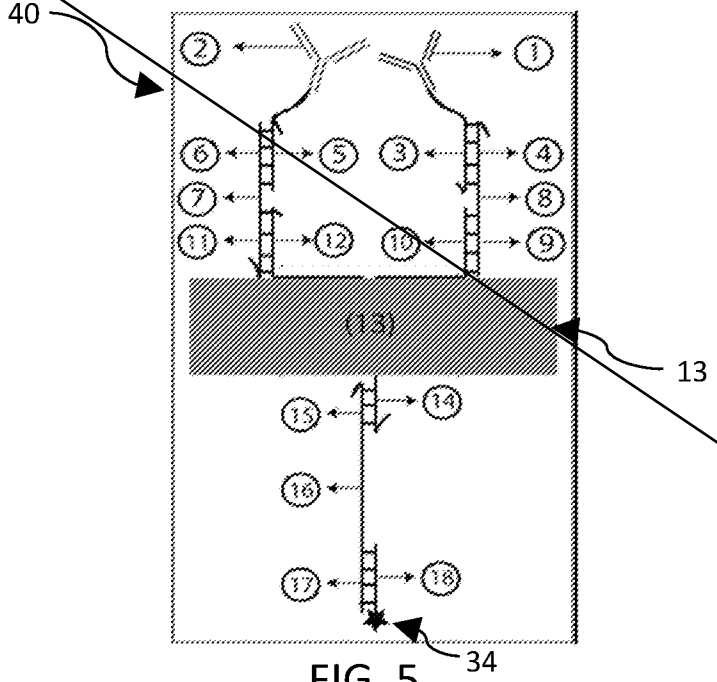


FIG. 5

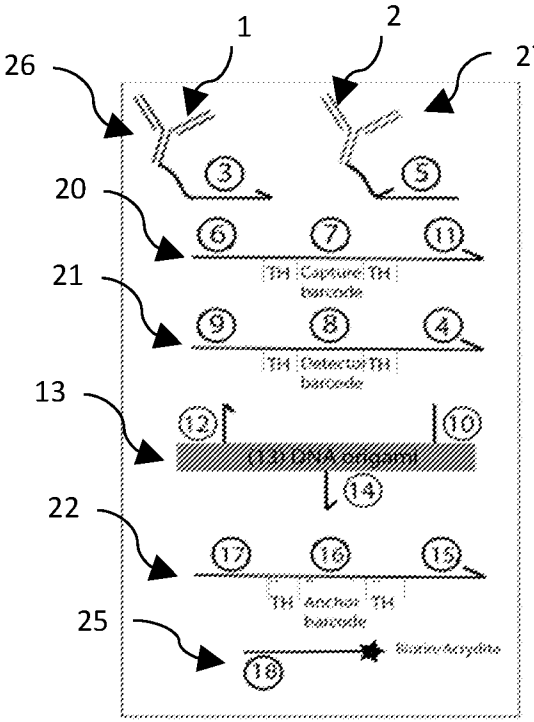


FIG. 6

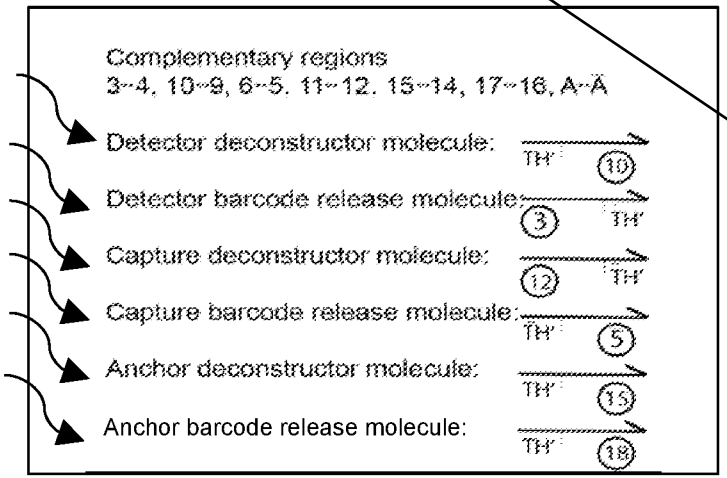


FIG. 7

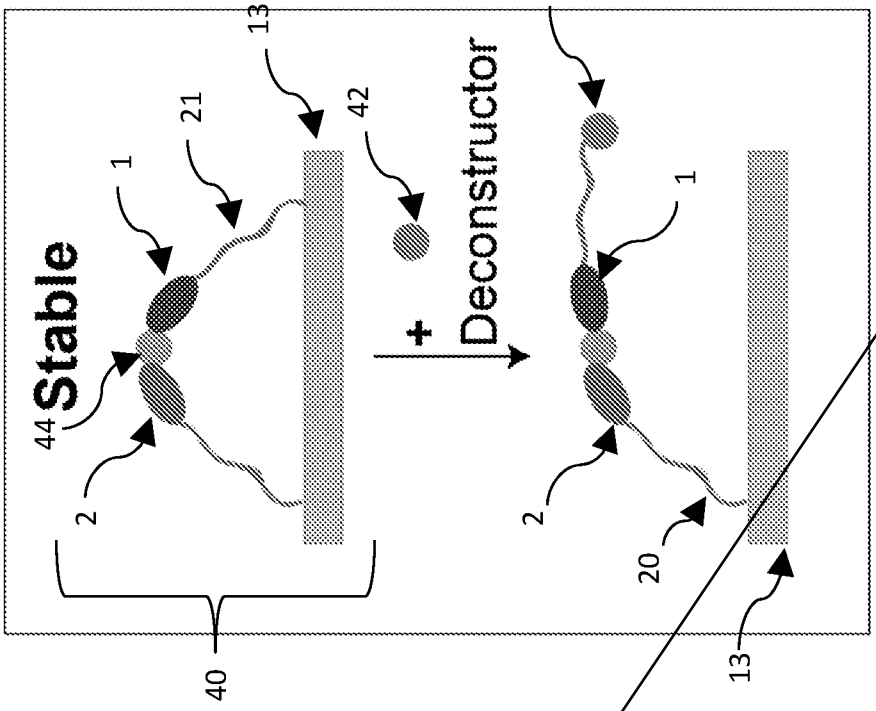

Stable
Deconstructor
1
2
13
21
42
44
40
20

FIG. 9

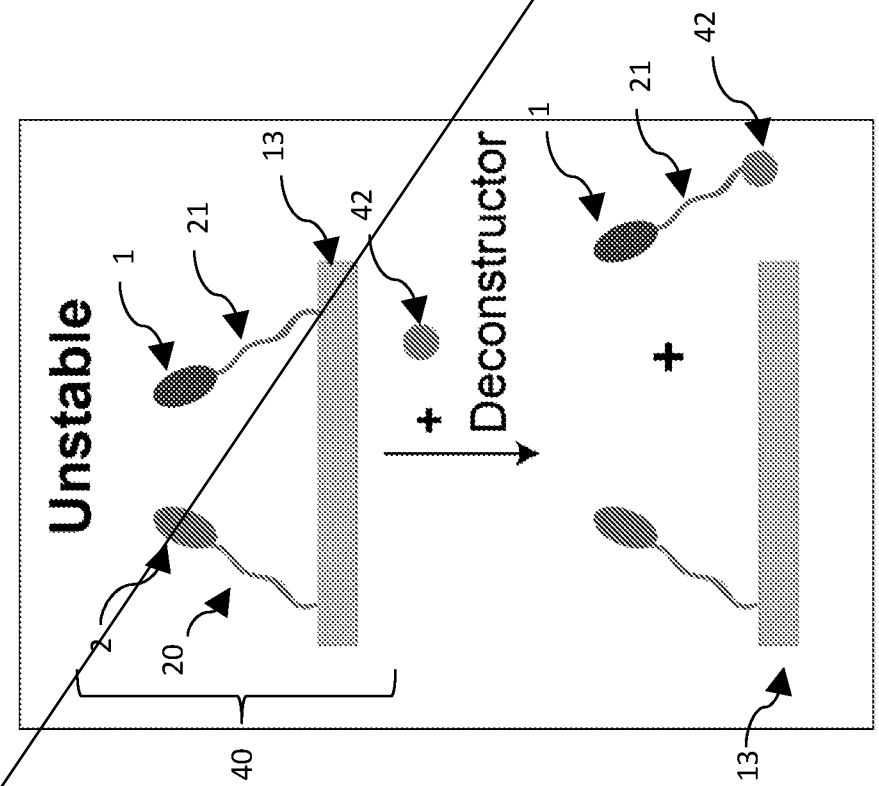

Unstable
Deconstructor
1
2
13
21
42
40
20

FIG. 8

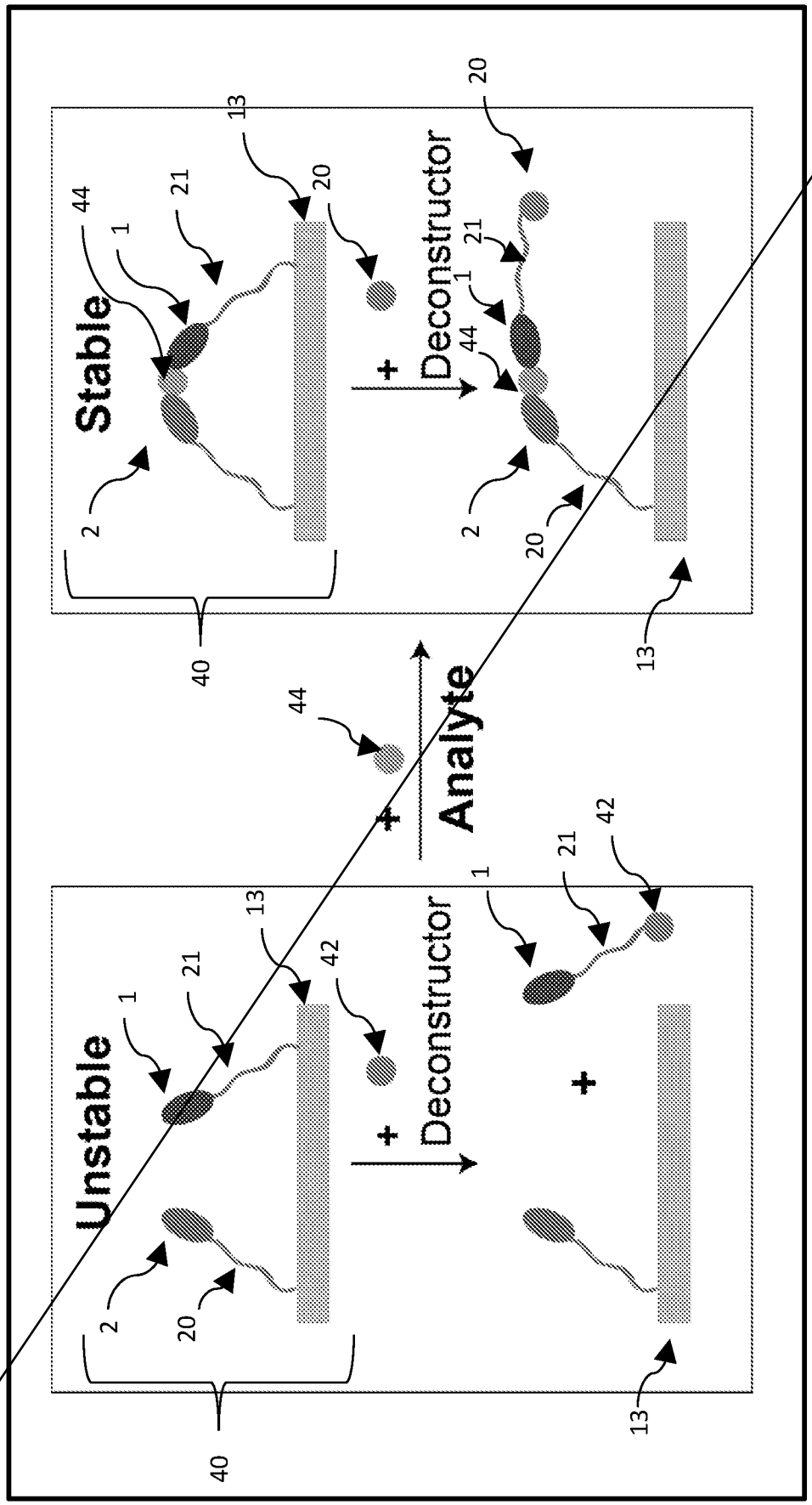

Stable
Deconstructor
Analyte
Unstable
Deconstructor
40
13
20
21
1
2
44
42

FIG. 10

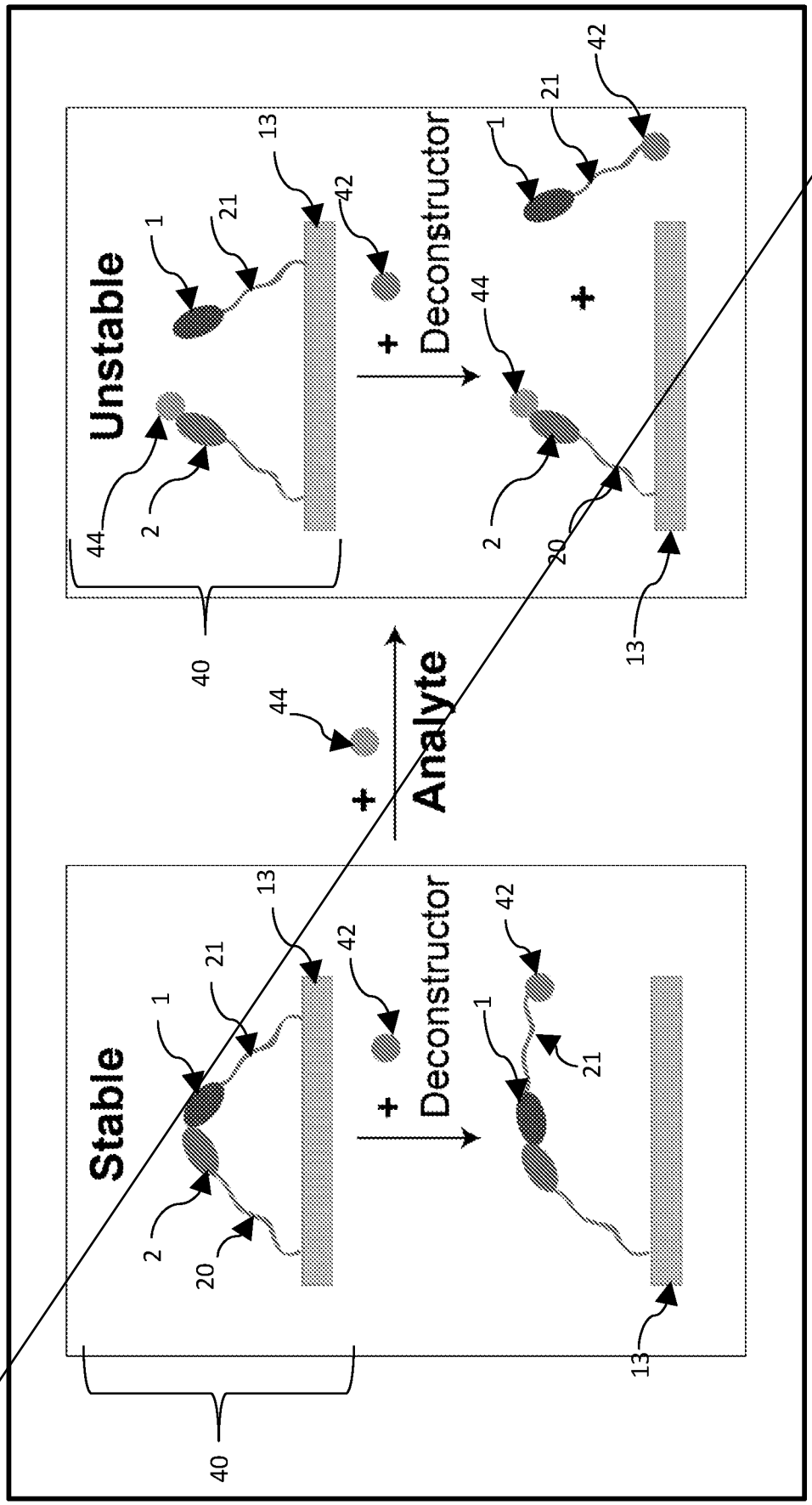

Unstable
Deconstructor
Analyte
Stable
Deconstructor
1
2
20
21
40
42
44
13

FIG. 11

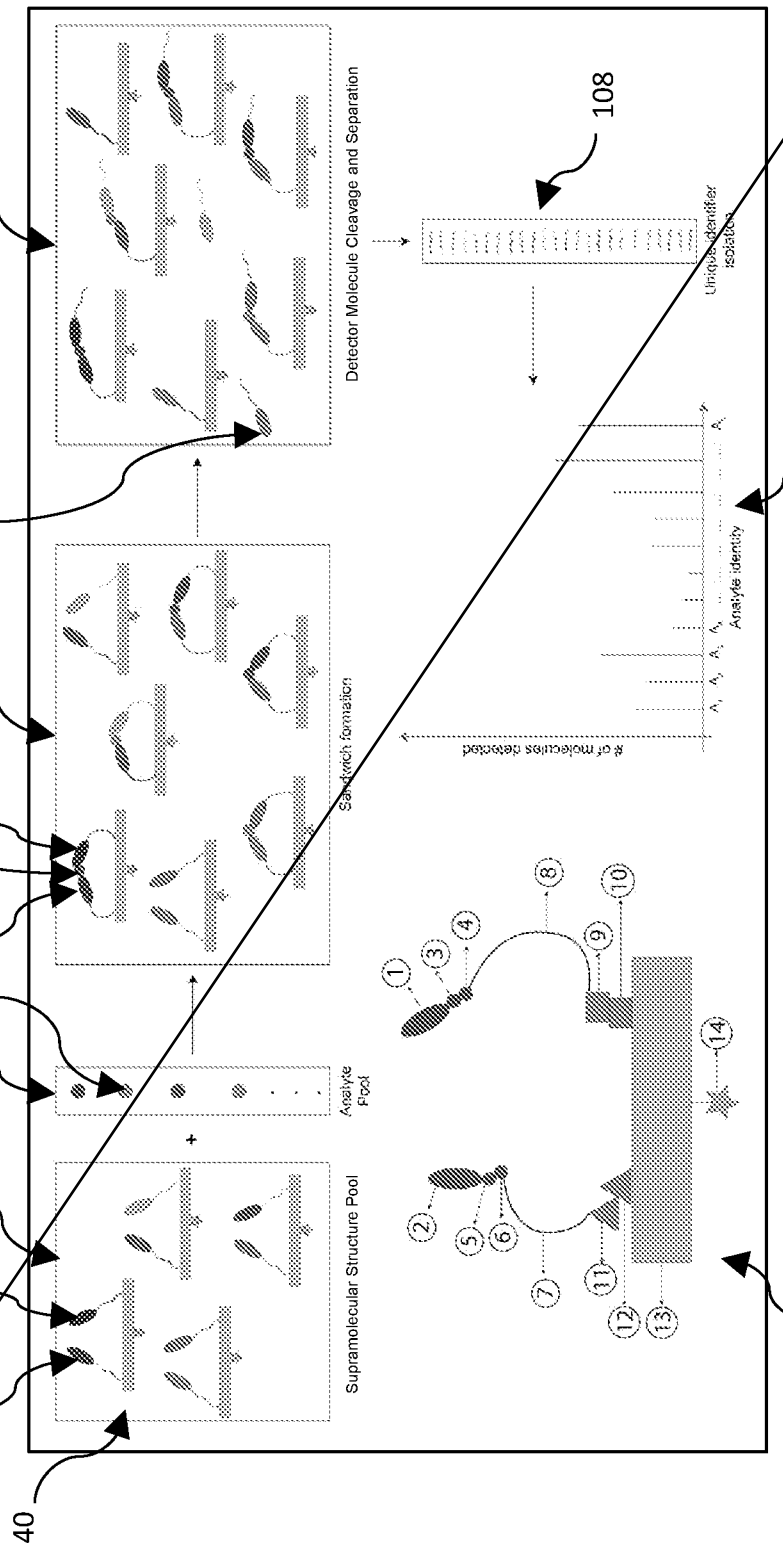

106
108
112
110
104
44
1
2
44
102
100
1
2
40
40
Detector Molecule Cleavage and Separation
Unique identifier isolation
Sandwich formation
Analyte identity
# of molecules detected
Analyte Pool
Supramolecular Structure Pool

FIG. 12

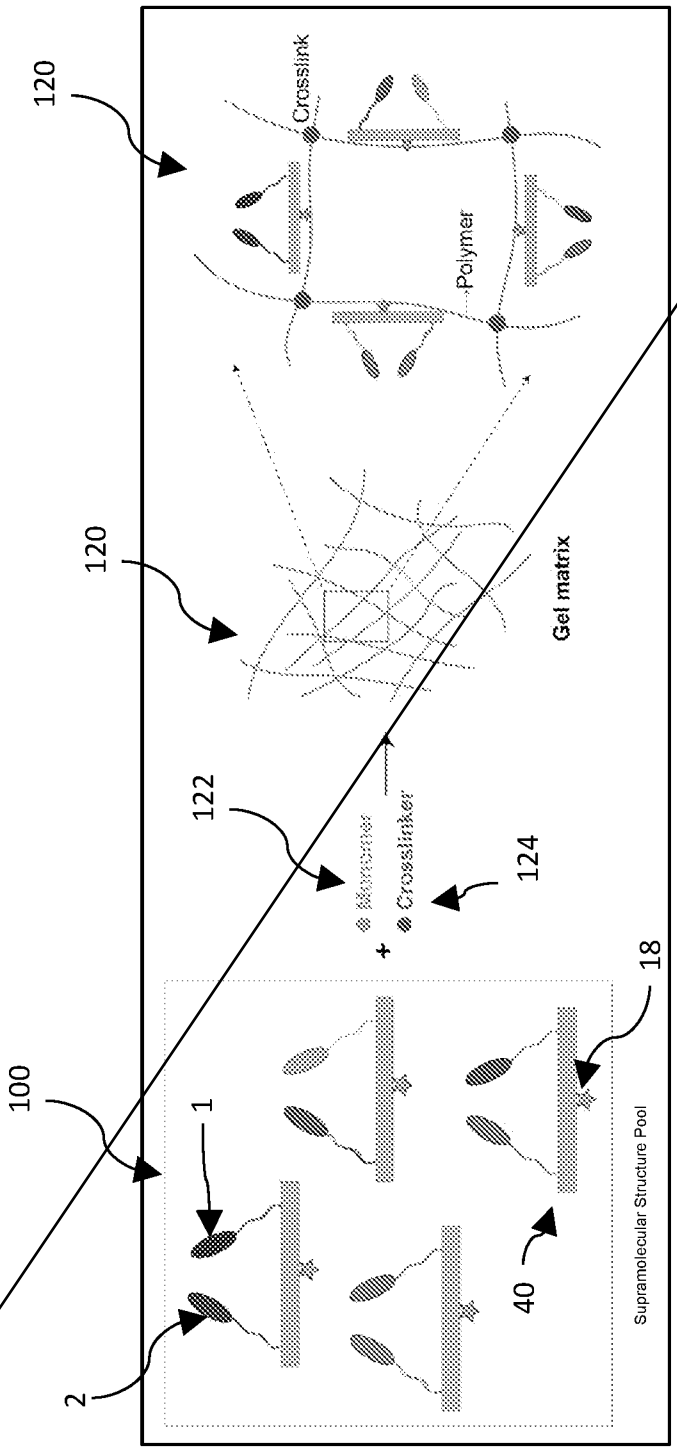

120
Crosslink
Polymer
120
Gel matrix
122
Monomer
Crosslinker
124
18
100
1
2
40
Supramolecular Structure Pool

FIG. 13

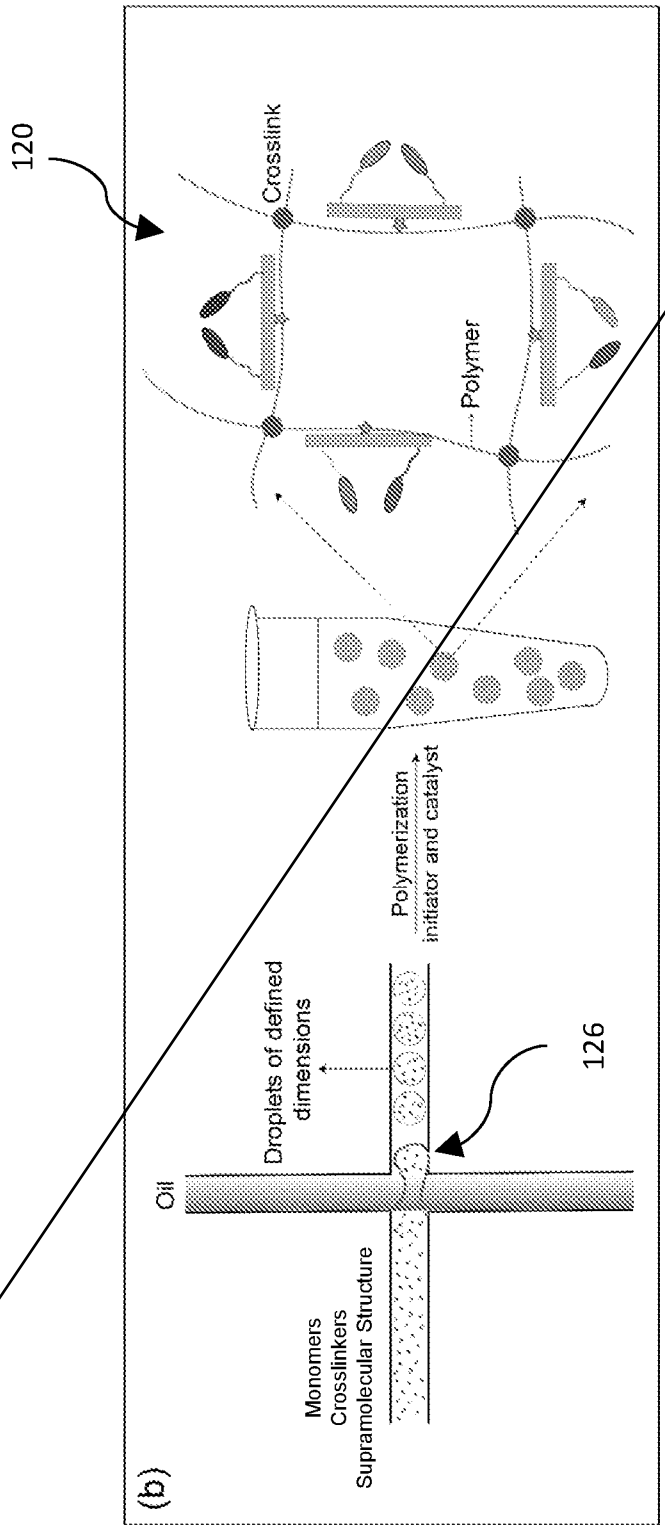

120
Crosslink
Polymer
Polymerization
initiator and catalyst
Droplets of defined
dimensions
126
Oil
Monomers
Crosslinkers
Supramolecular Structure
(b)

FIG. 14

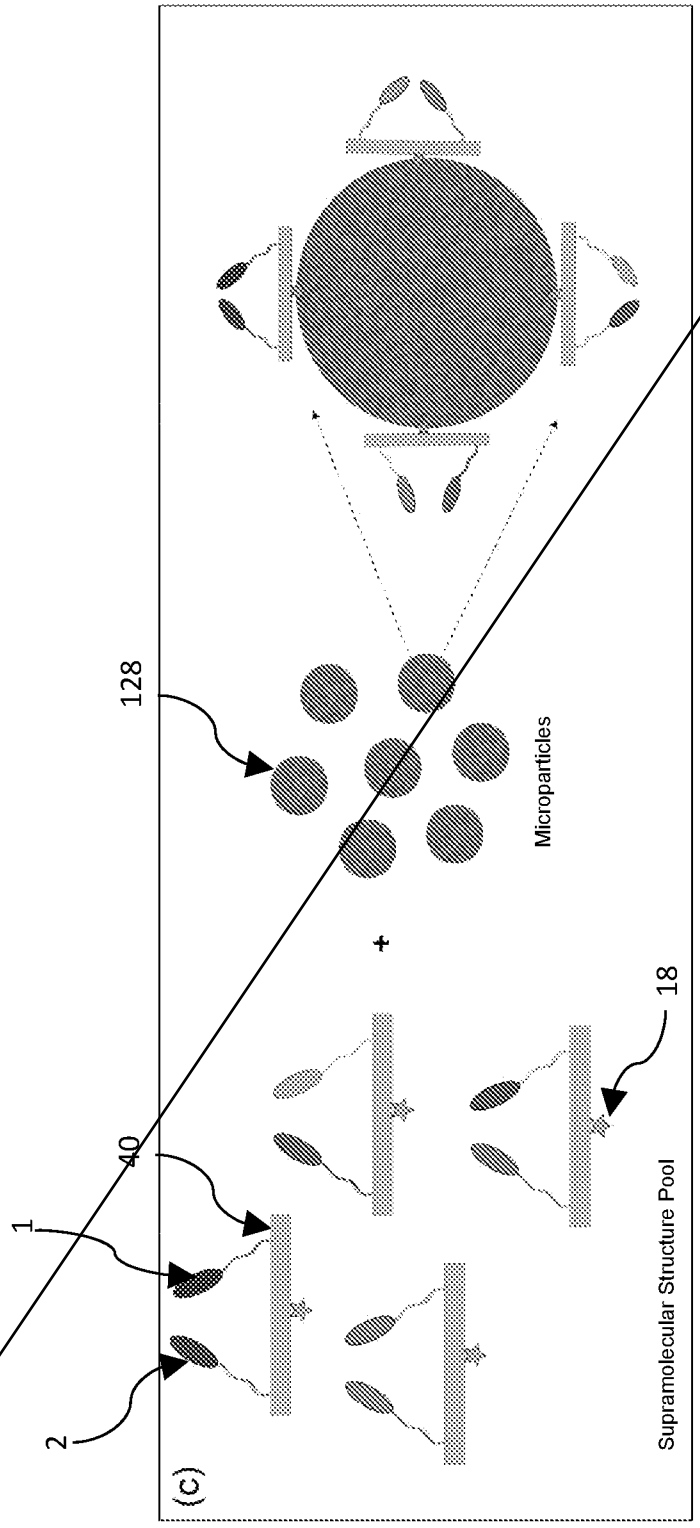

(c)
2
1
40
128
18
+
Microparticles
Supramolecular Structure Pool

FIG. 15

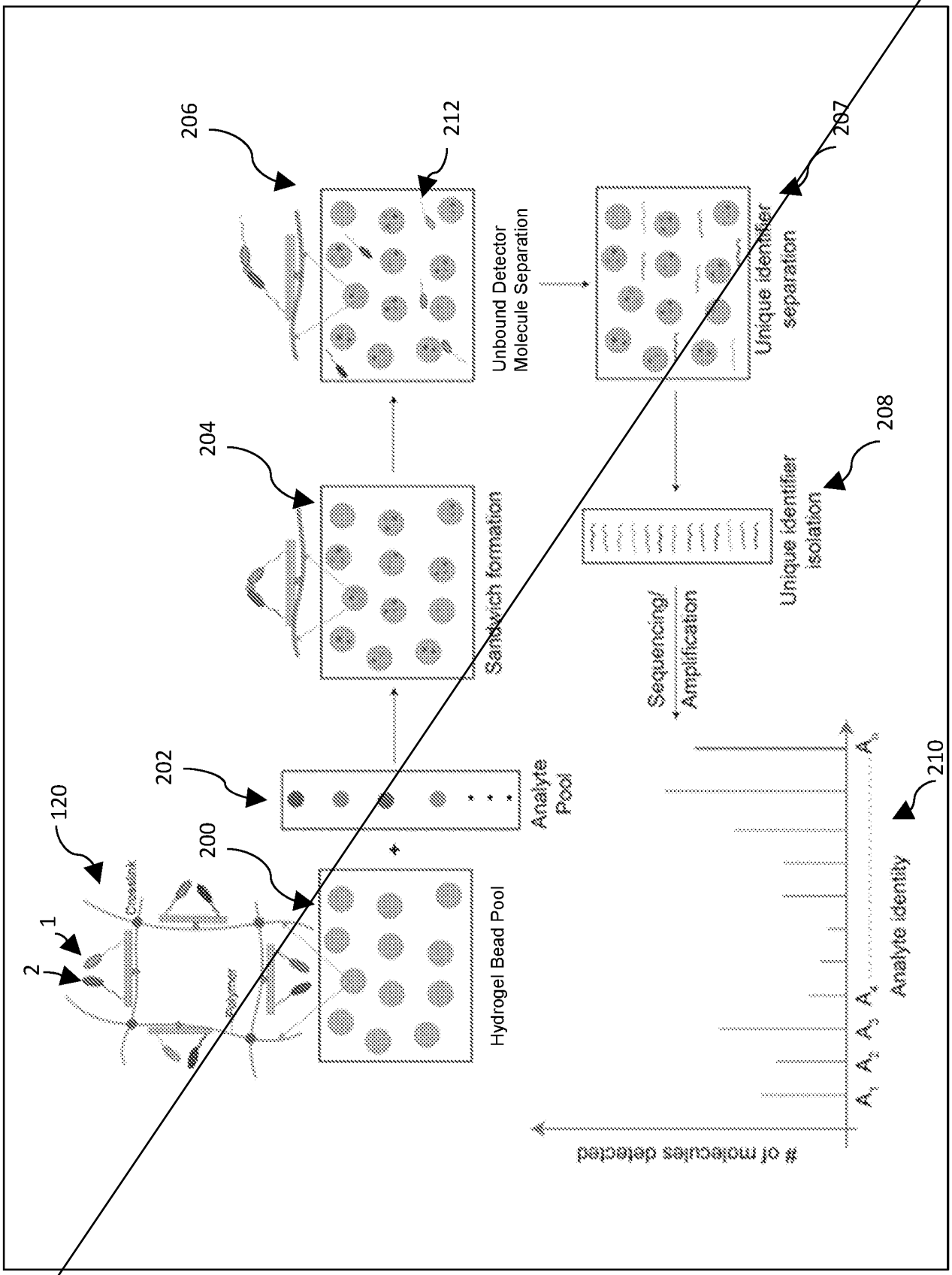

120
1
2
Capture
Enzyme
200
Hydrogel Bead Pool
202
Analyte Pool
204
Sandwich formation
206
212
Unbound Detector Molecule Separation
207
Unique identifier separation
208
Unique identifier isolation
Sequencing/ Amplification
210
Analyte identity
# of molecules detected
A1 A2 A3 AL

FIG. 16

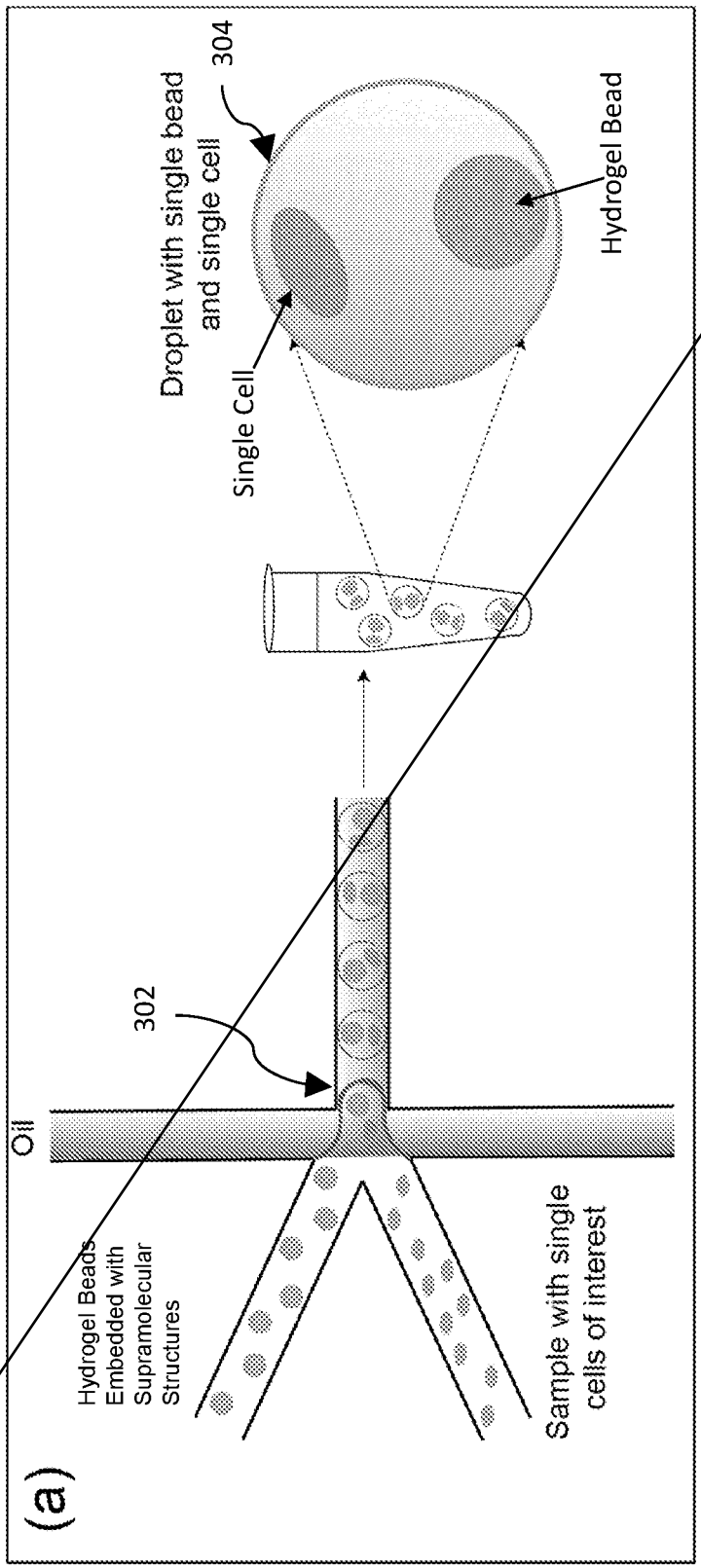

(a)
Hydrogel Beads Embedded with Supramolecular Structures
Oil
302
Sample with single cells of interest
Droplet with single bead and single cell
304
Single Cell
Hydrogel Bead

FIG. 17

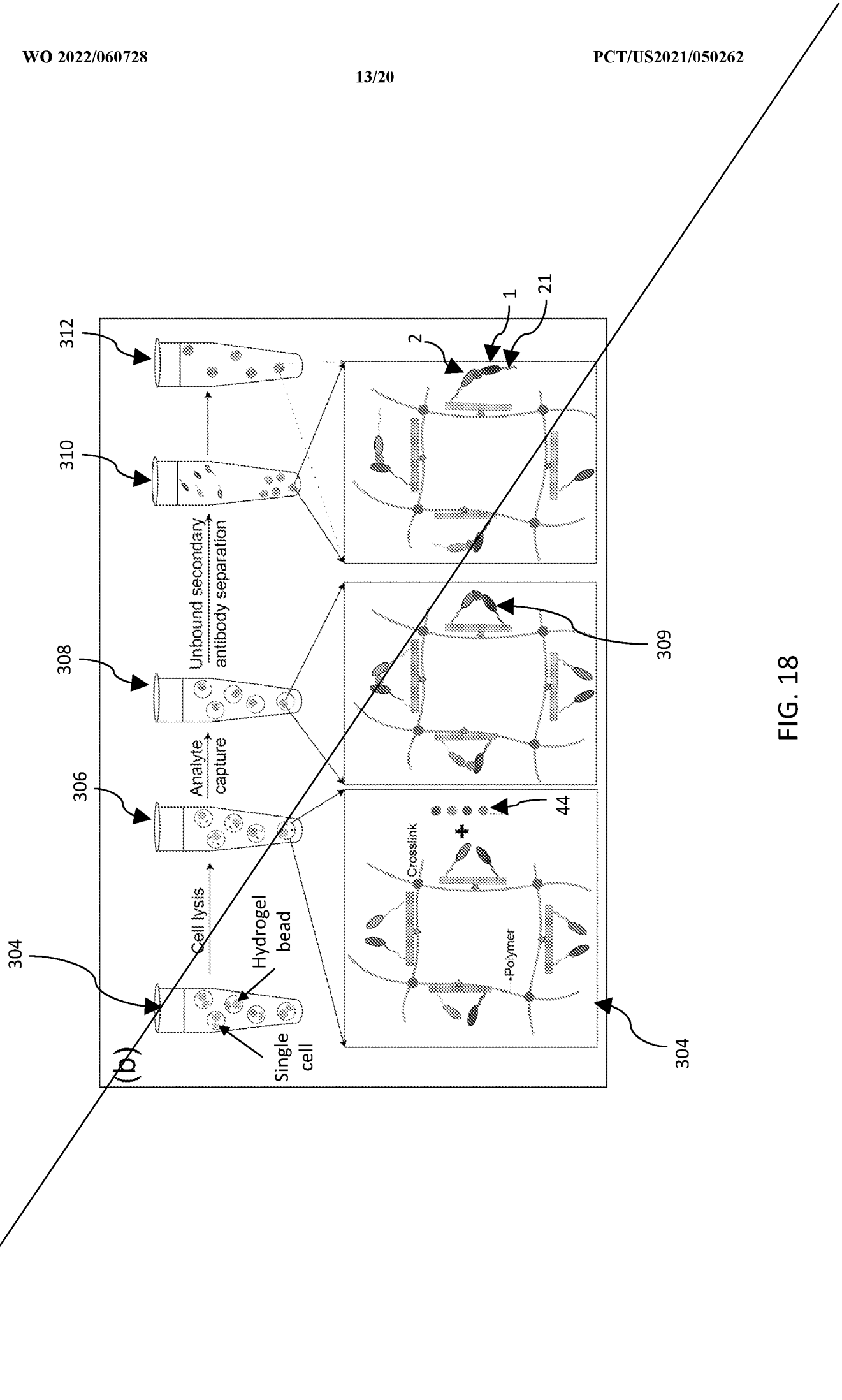

(b)
Single cell
Hydrogel bead
Cell lysis
Analyte capture
Unbound secondary antibody separation
Crosslink
Polymer
304
306
308
310
312
309
44
1
2
21

FIG. 18

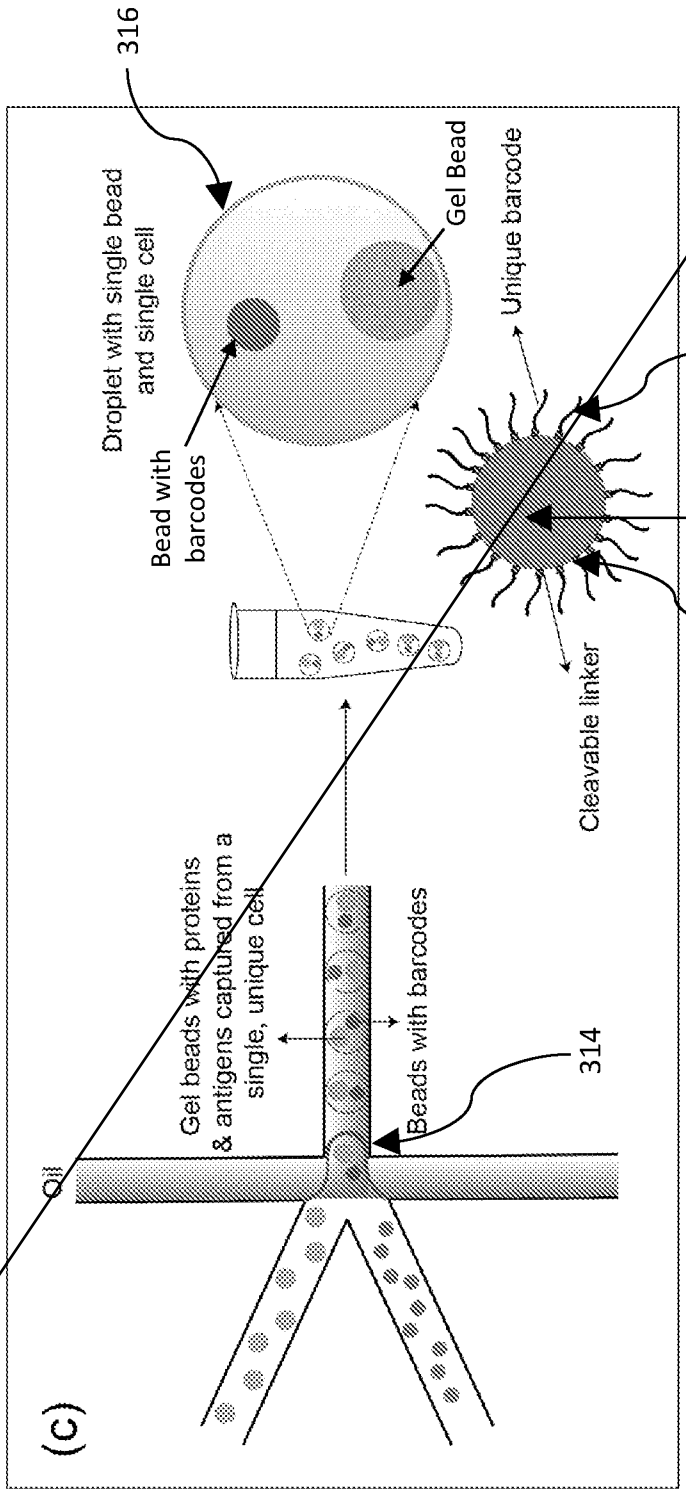

316
Droplet with single bead and single cell
Gel Bead
Unique barcode
320
Bead with barcodes
318
322
Cleavable linker
Gel beads with proteins & antigens captured from a single, unique cell
Beads with barcodes
314
Oil
(c)

FIG. 19

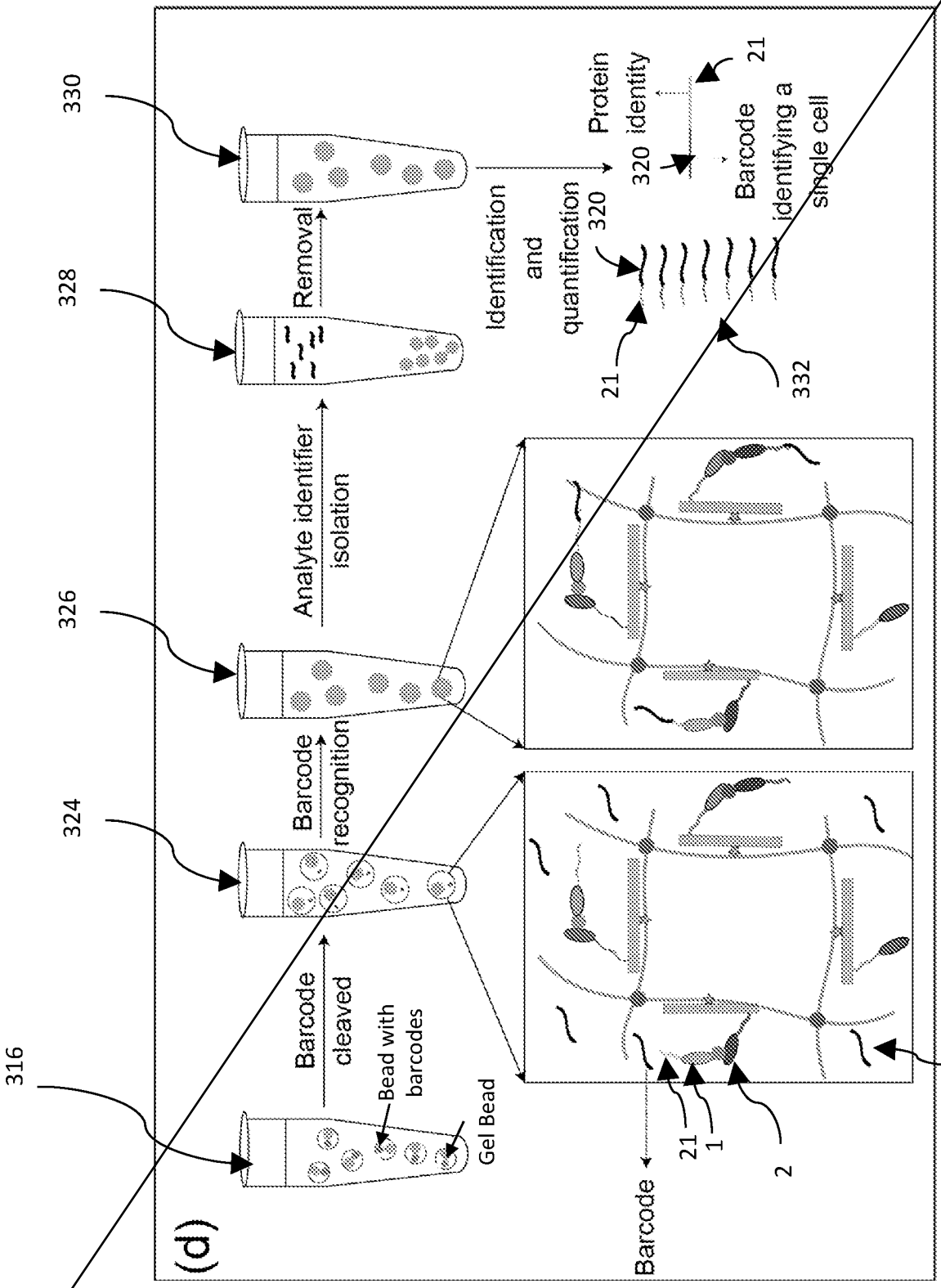

(d)
316
Gel Bead
Bead with barcodes
Barcode cleaved
324
Barcode recognition
326
Analyte identifier isolation
328
Removal
330
Identification and quantification
320
Protein identity
320
Barcode identifying a single cell
21
332
Barcode
21
1
2
332

FIG. 20

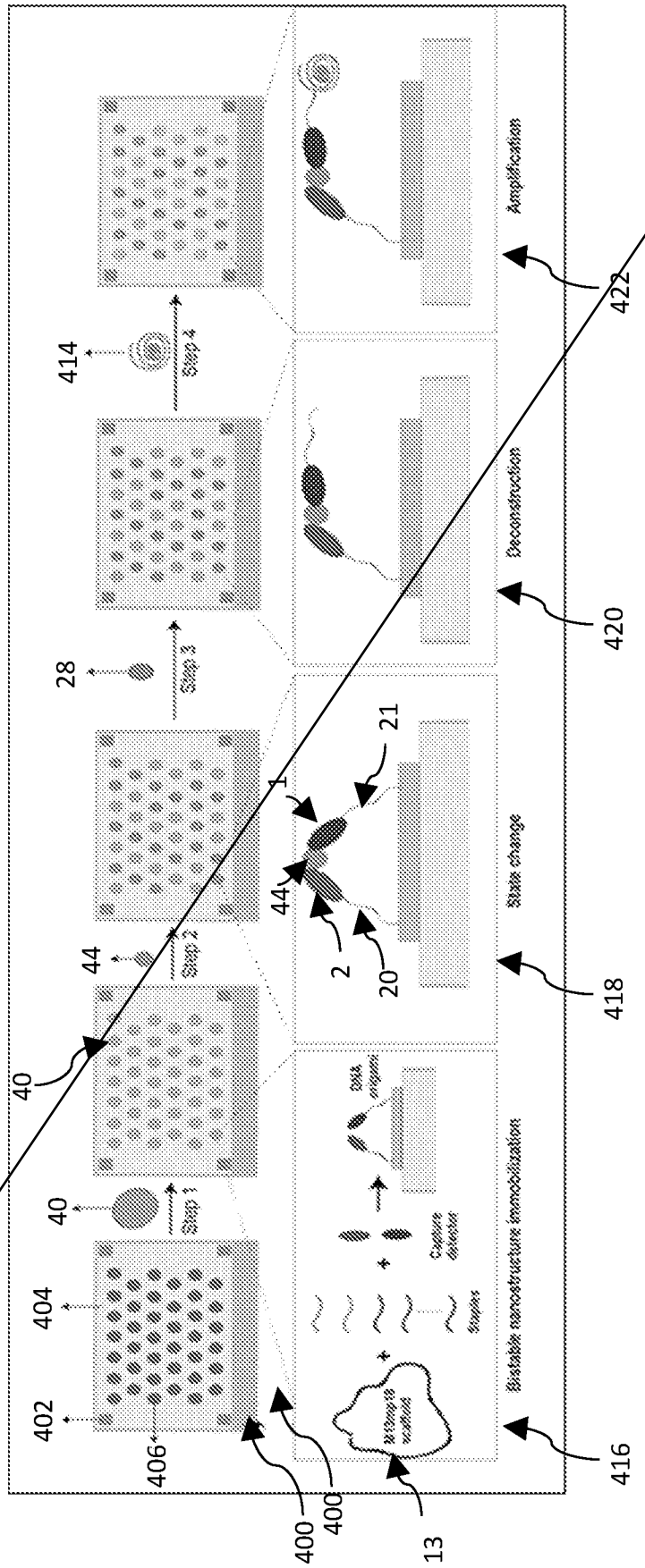

Step 1
Step 2
Step 3
Step 4
Amplification
Deconstruction
Base change
Base addition
Capture detection
Analyte
Biotinylated nanostructure immobilization
40
44
28
414
422
420
418
416
400
13
402
404
406
1
2
20
21

FIG. 21

S1
83 nt
S2
90 nt
Core
60 nt
Biotin
S1 + core
S2 + DC
3pt widget
Bridge
56 nt
widget
+ bridge
Deconstructor
Strand
(DC)
27 nt
Widget +
Bridge + DC
5pt widget
Release
30 nt
S1 + Core
+ Bridge
S2 + DC
+ Release
W1
W2
W3
W4
W5
W6
W7

FIG. 22

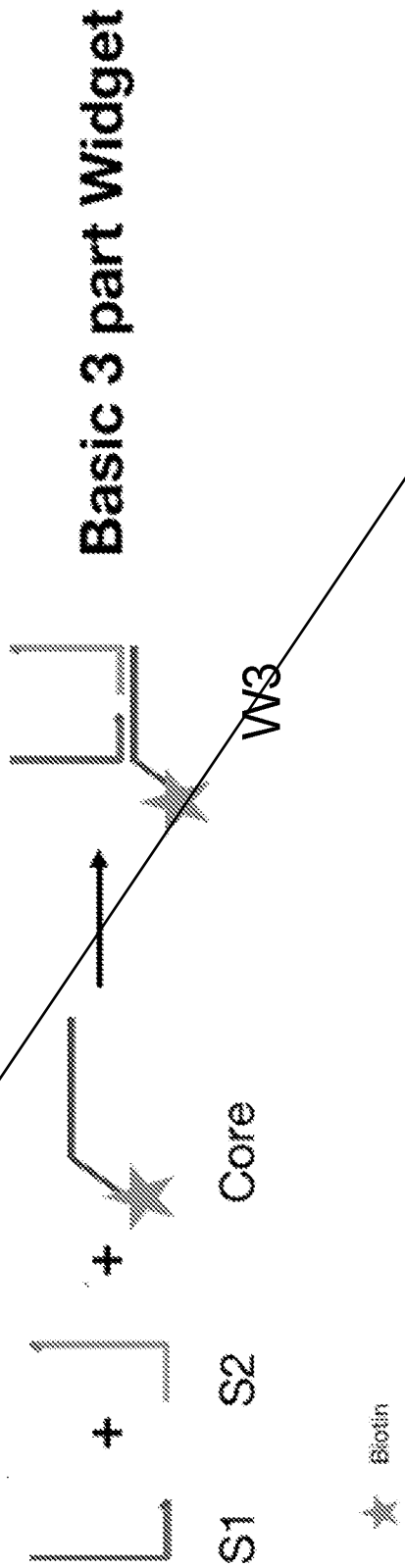


FIG. 23

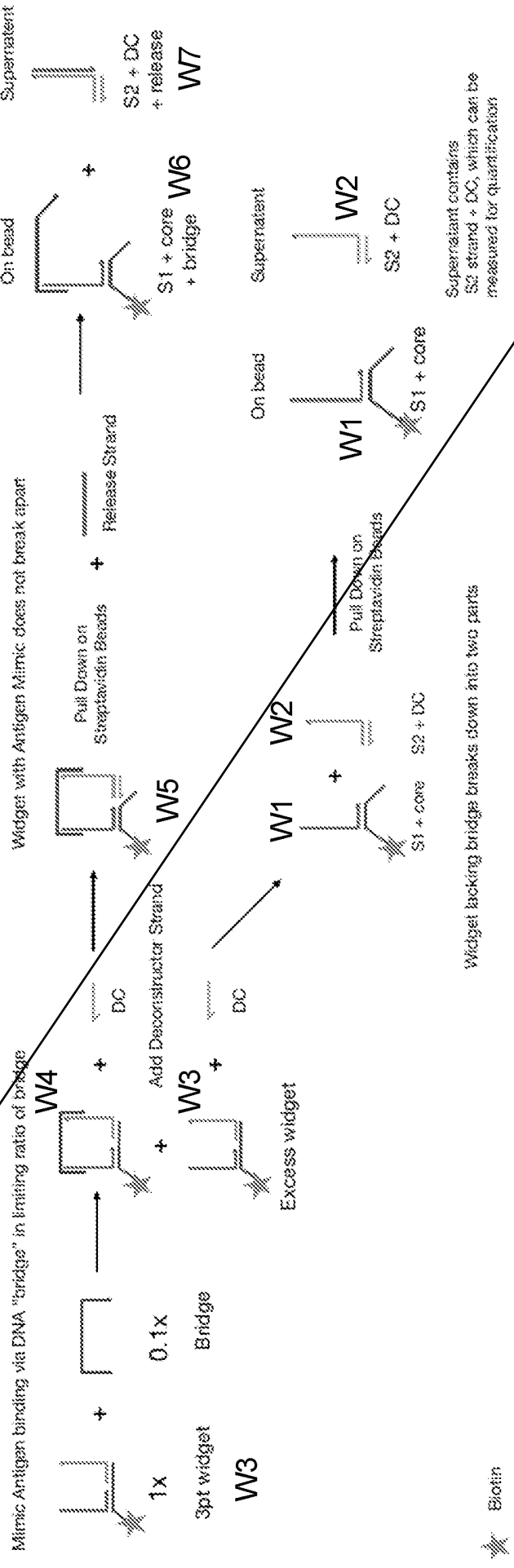

Mimic Antigen binding via DNA "bridge" in limiting ratio of bridge
1x
3pt widget
W3
+
0.1x
Bridge
W4
+
DC
Add Deconstructor Strand
+
W3
Excess widget
+
DC
Widget with Antigen Mimic does not break apart
W5
Pull Down on Streptavidin Beads
+
Release Strand
On bead
W6
S1 + core
+ bridge
+
Supernatant
W7
S2 + DC
+ release
Widget lacking bridge breaks down into two parts
W1
S1 + core
W2
S2 + DC
+
Pull Down on Streptavidin Beads
On bead
W1
S1 + core
Supernatant
W2
S2 + DC
Supernatant contains
S2 strand + DC, which can be
measured for quantification
Biotin

FIG. 24

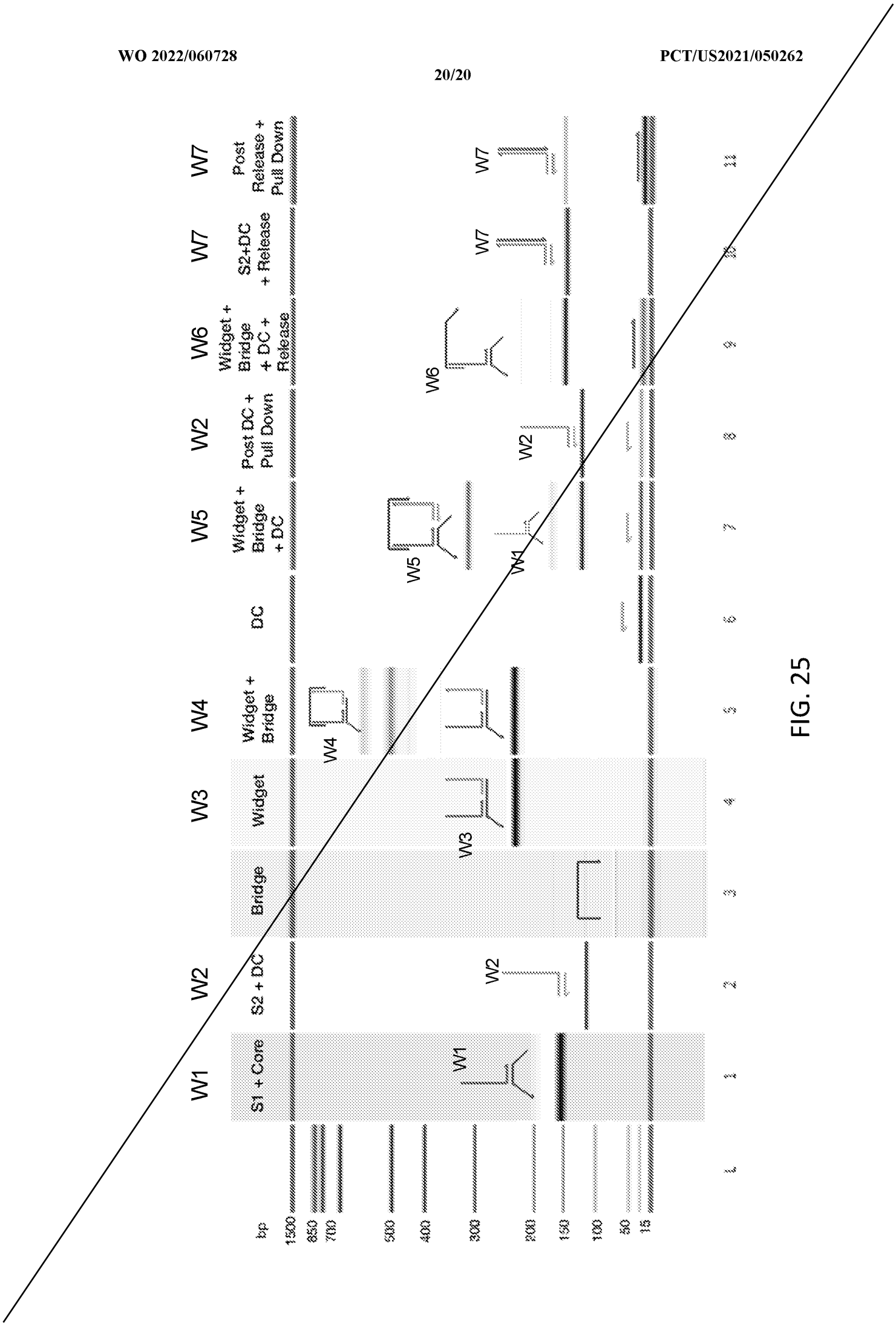

W1
W2
W3
W4
W5
W6
W7
S1 + Core
S2 + DC
Bridge
Widget
Widget + Bridge
DC
Widget + Bridge + DC
Post DC + Pull Down
Widget + Bridge + DC + Release
S2+DC + Release
Post Release + Pull Down
bp
1500
850
700
500
400
300
200
150
100
50
15
1
2
3
4
5
6
7
8
9
10
11

FIG. 25

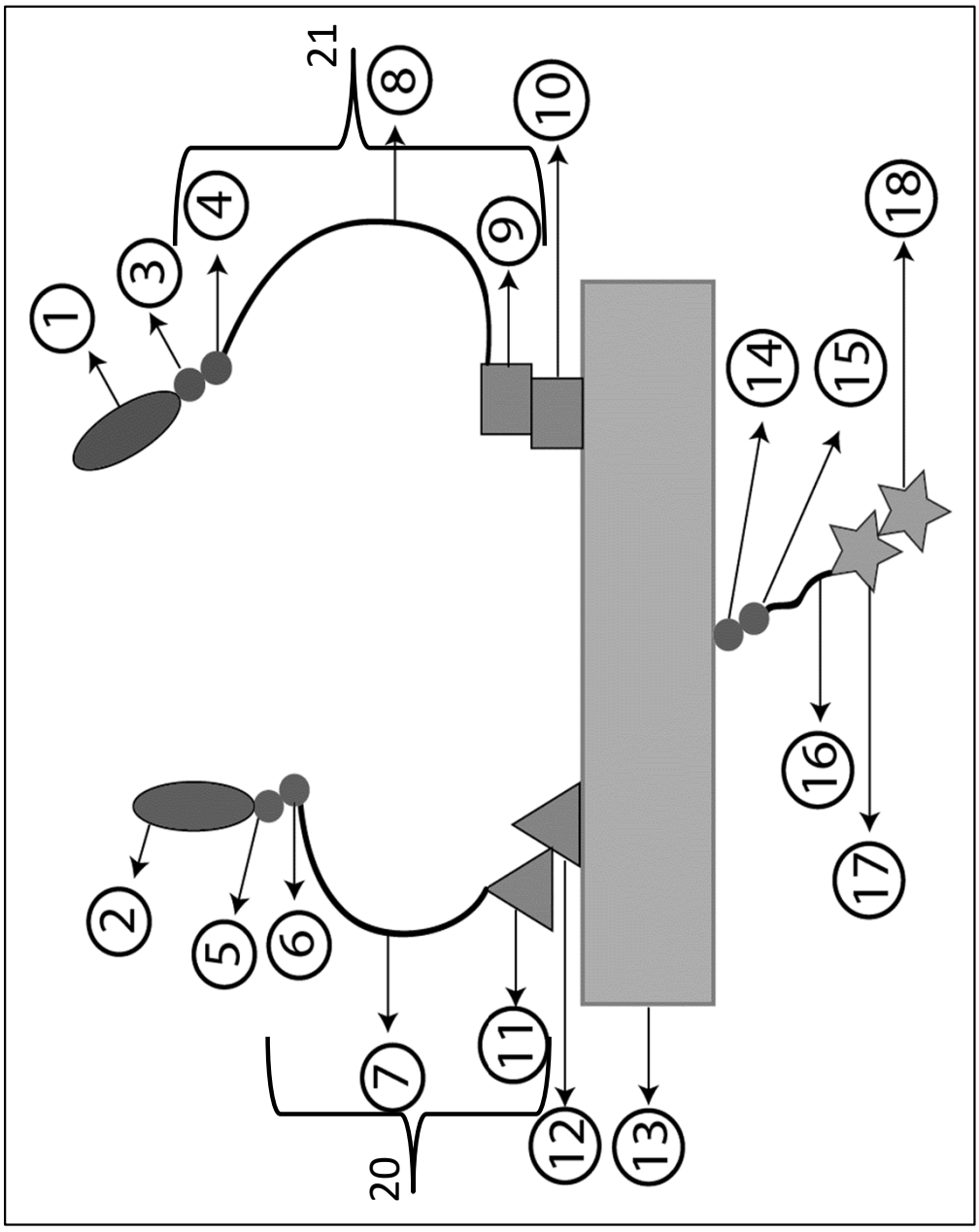


FIG. 1

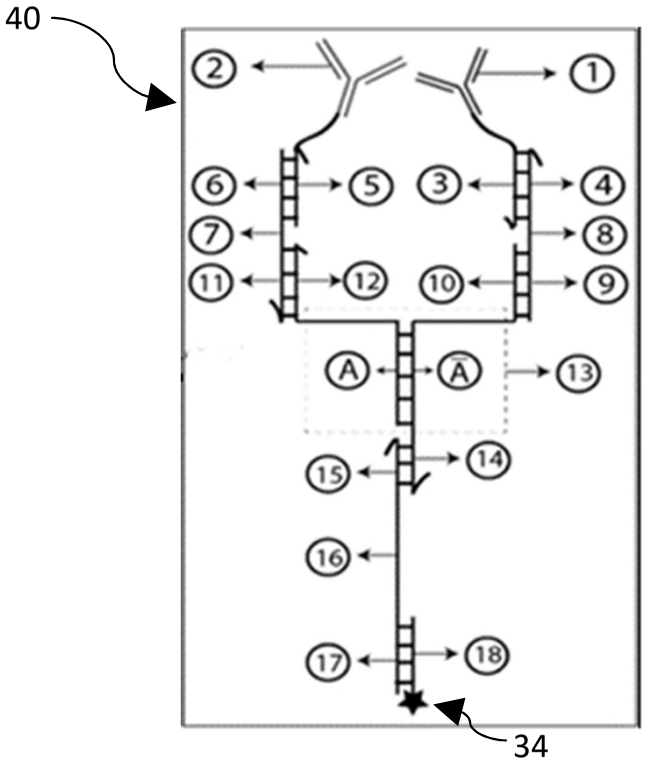


FIG. 2

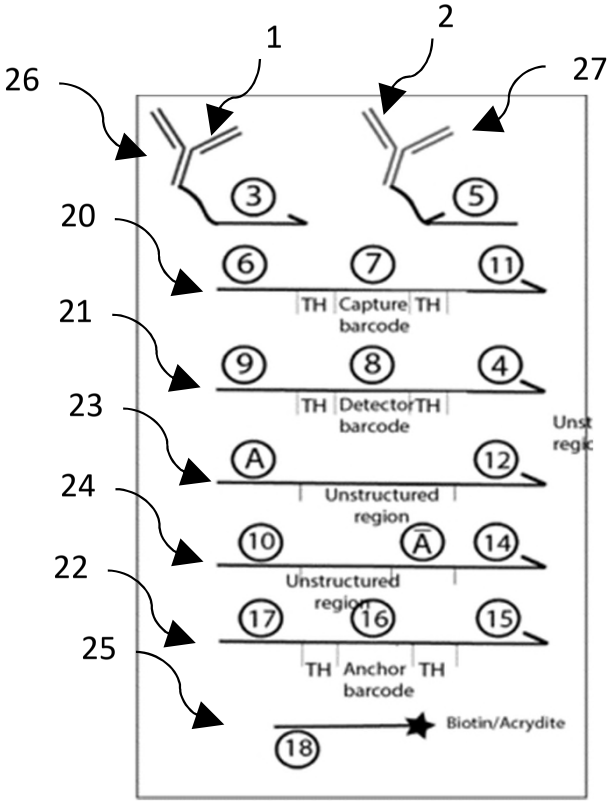


FIG. 3

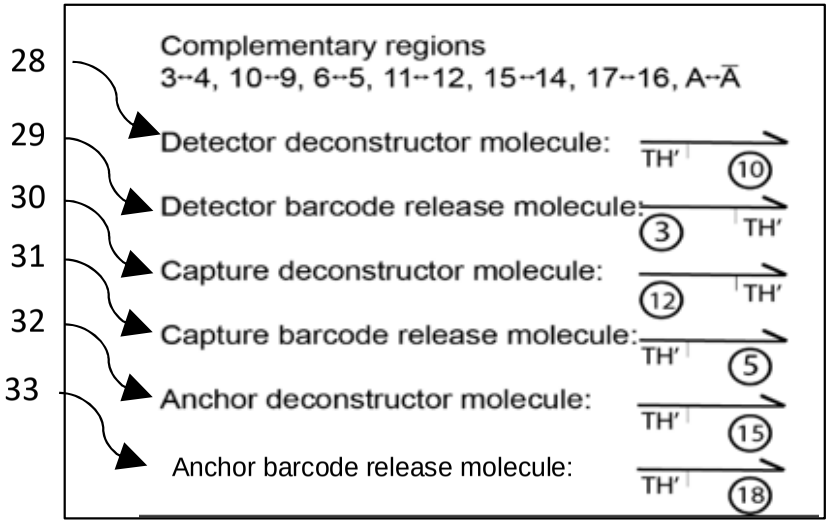


FIG. 4

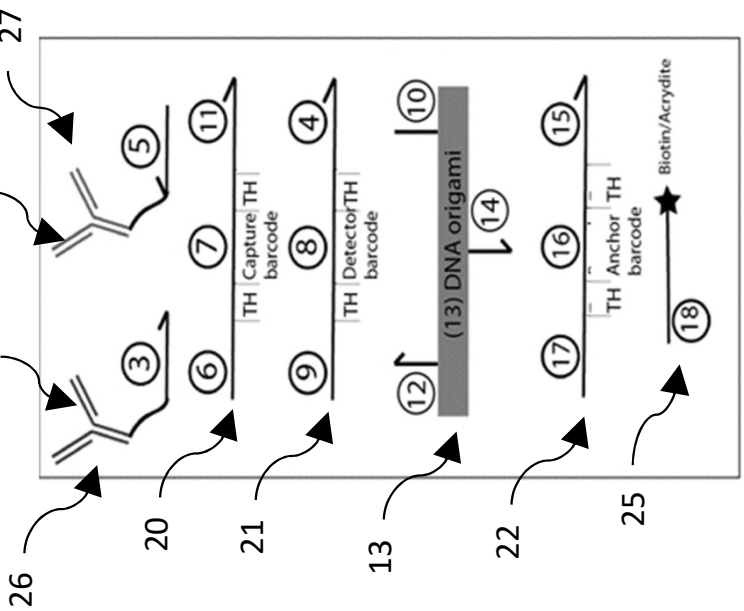


FIG. 6

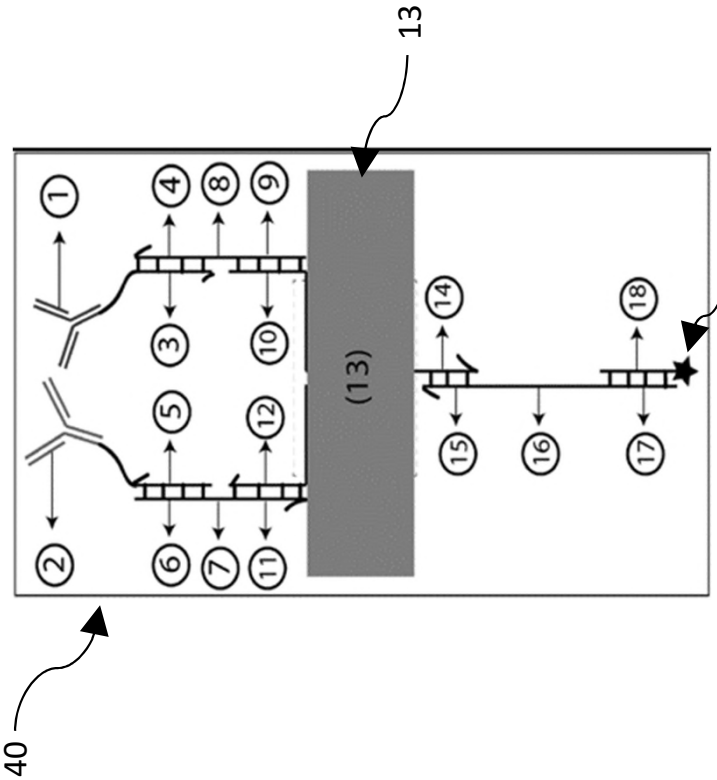


FIG. 5

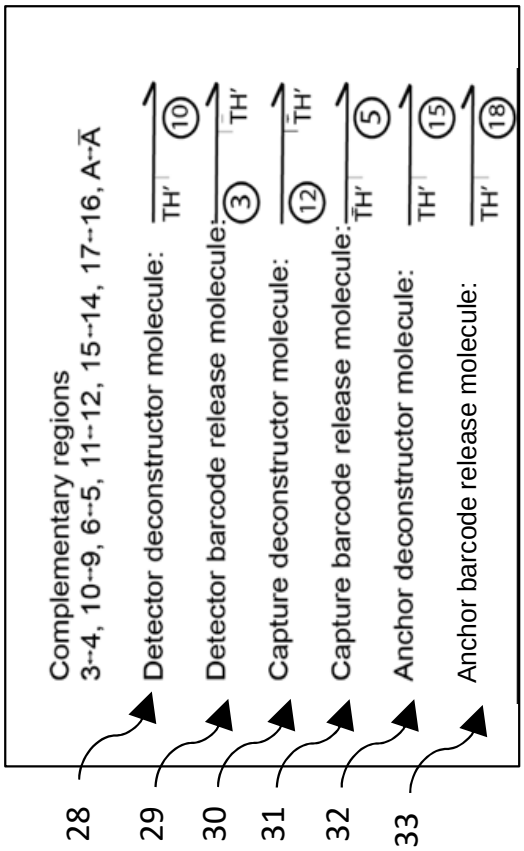


FIG. 7

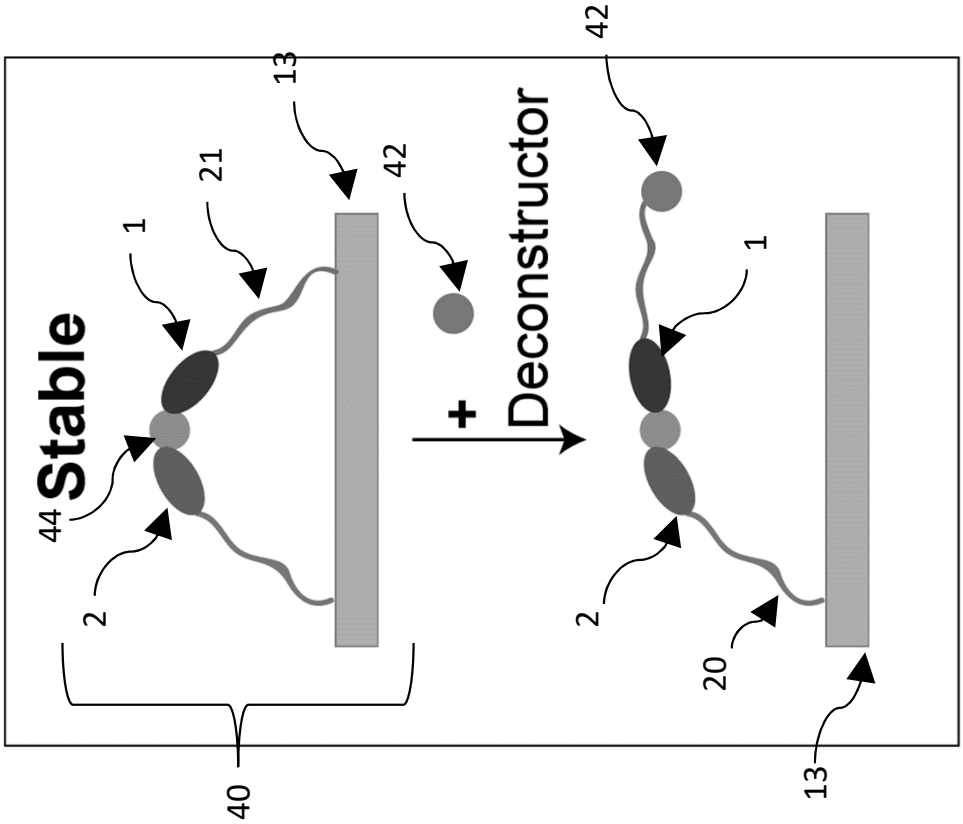

Stable
Deconstructor
44
1
21
13
42
2
40
20

FIG. 9

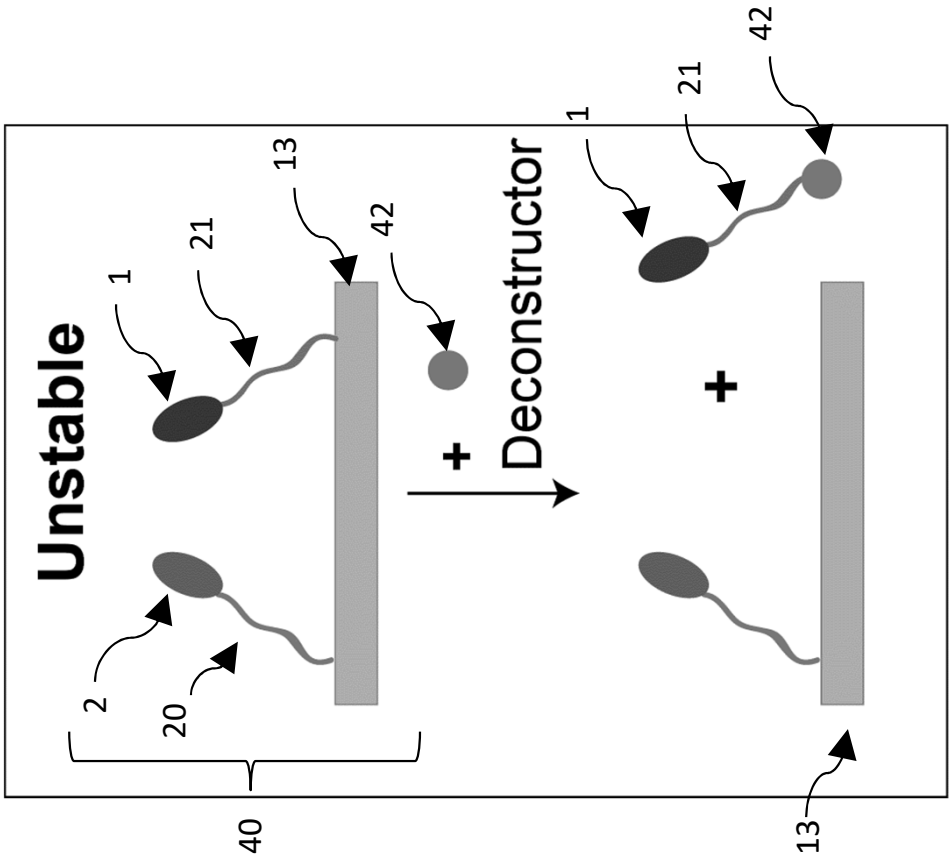

Unstable
Deconstructor
1
21
13
42
2
20
40

FIG. 8

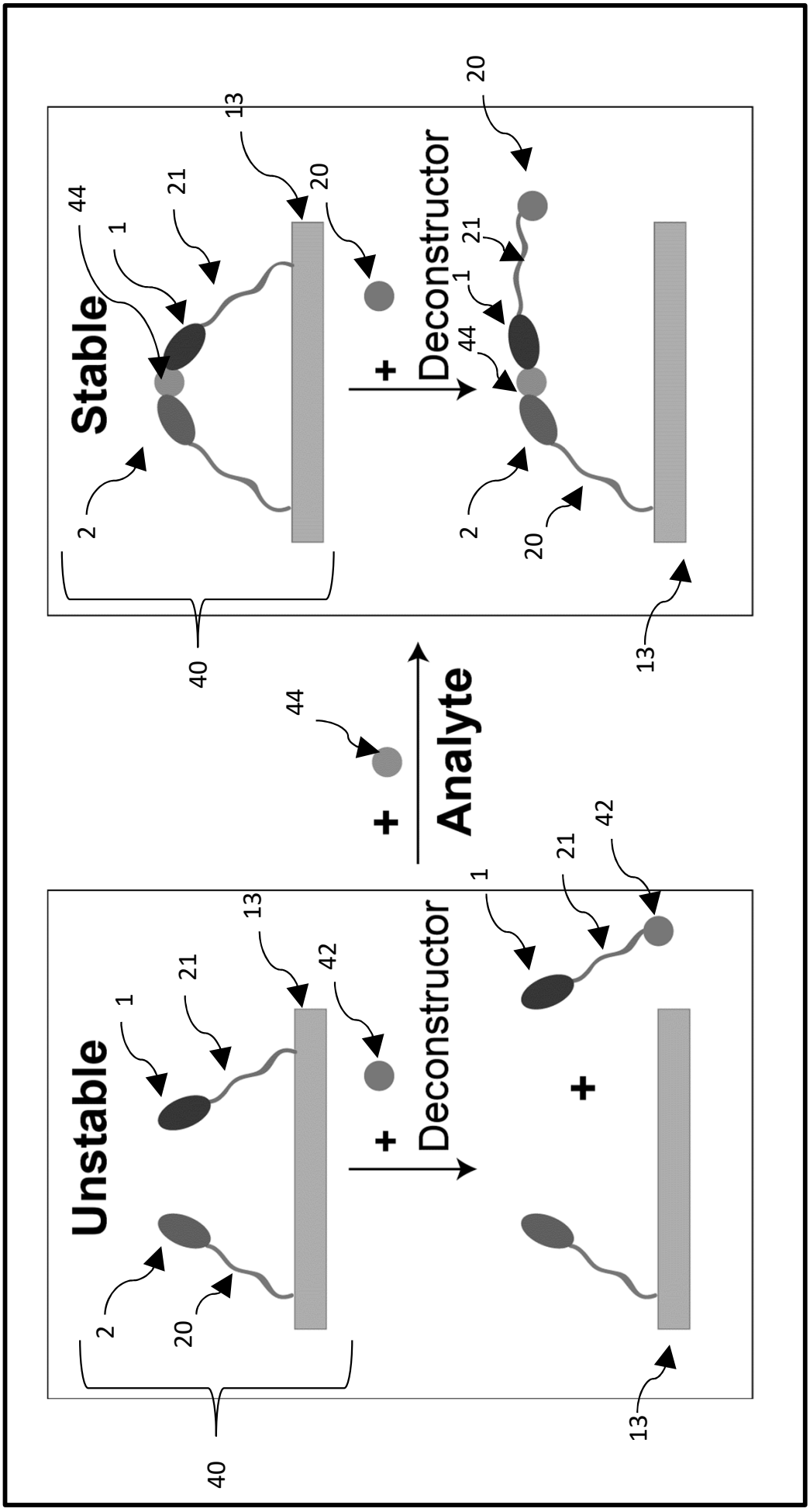

Unstable
Deconstructor
Analyte
Stable
Deconstructor
1
2
13
20
21
40
42
44

FIG. 10

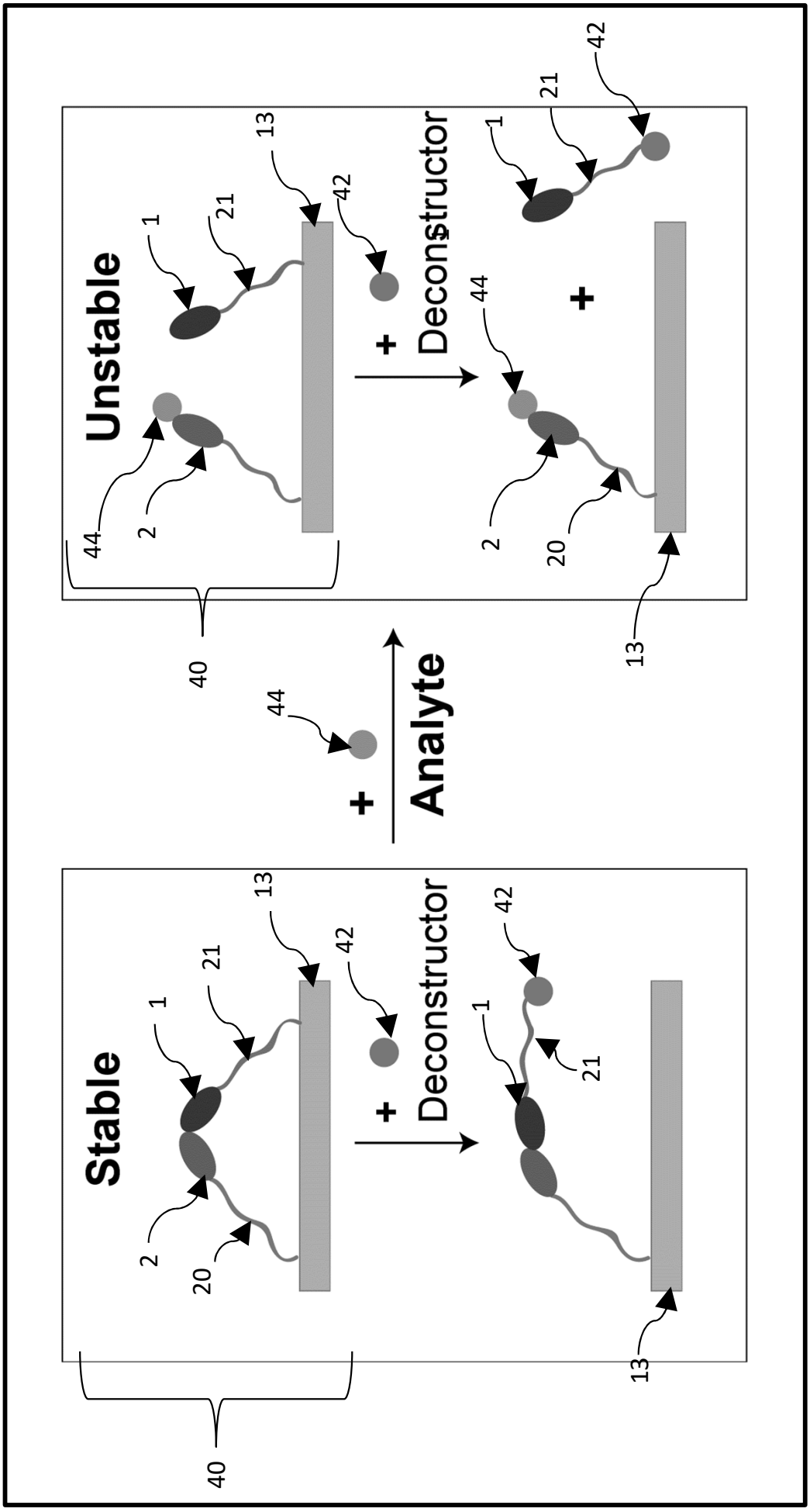

Stable
Deconstructor
Analyte
Unstable
Deconstructor
40
13
42
21
1
2
20
44

FIG. 11

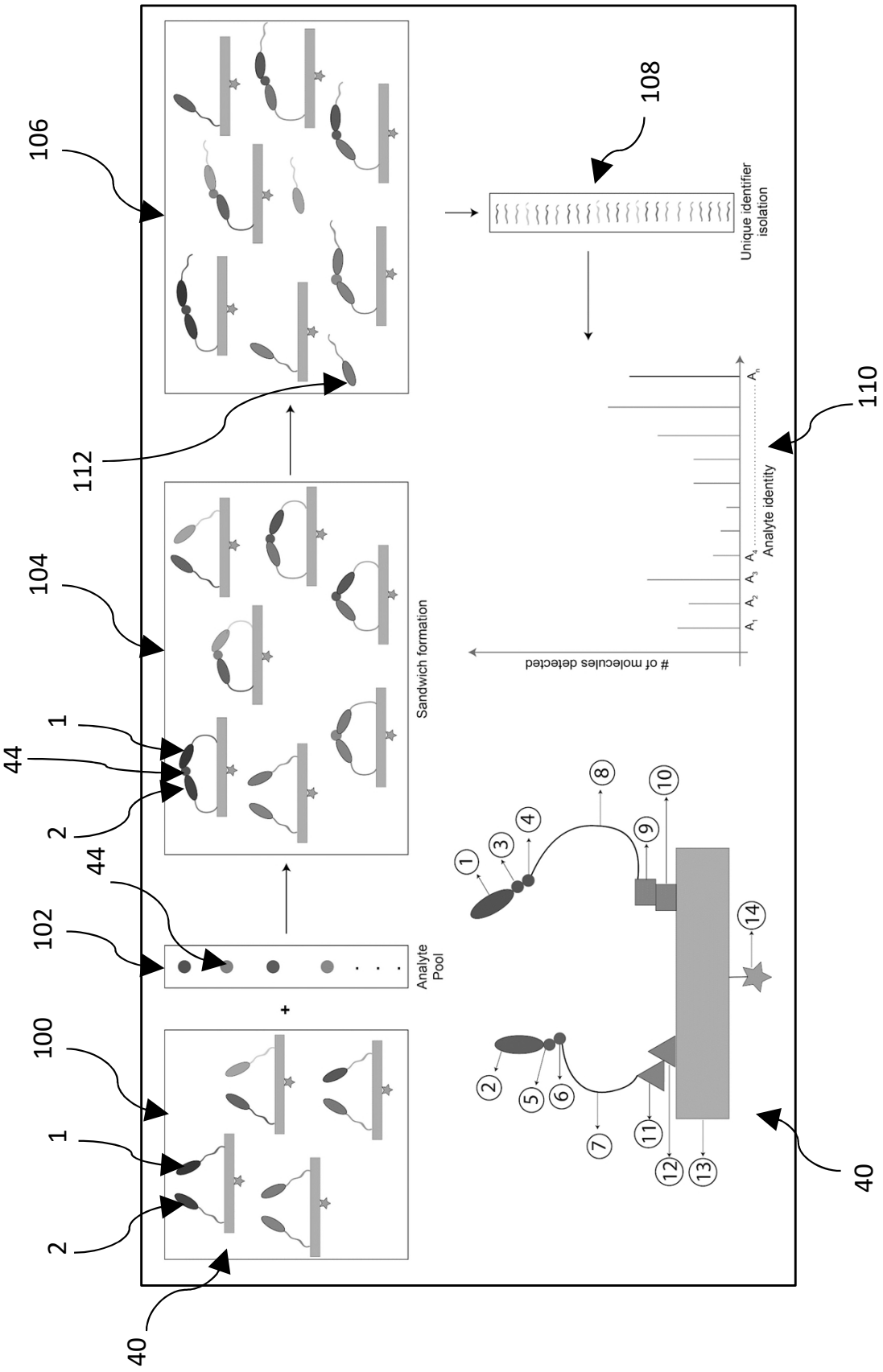

106
108
Unique identifier isolation
112
110
104
Sandwich formation
# of molecules detected
Analyte identity
1
44
2
44
102
Analyte Pool
100
1
2
40
40

FIG. 12

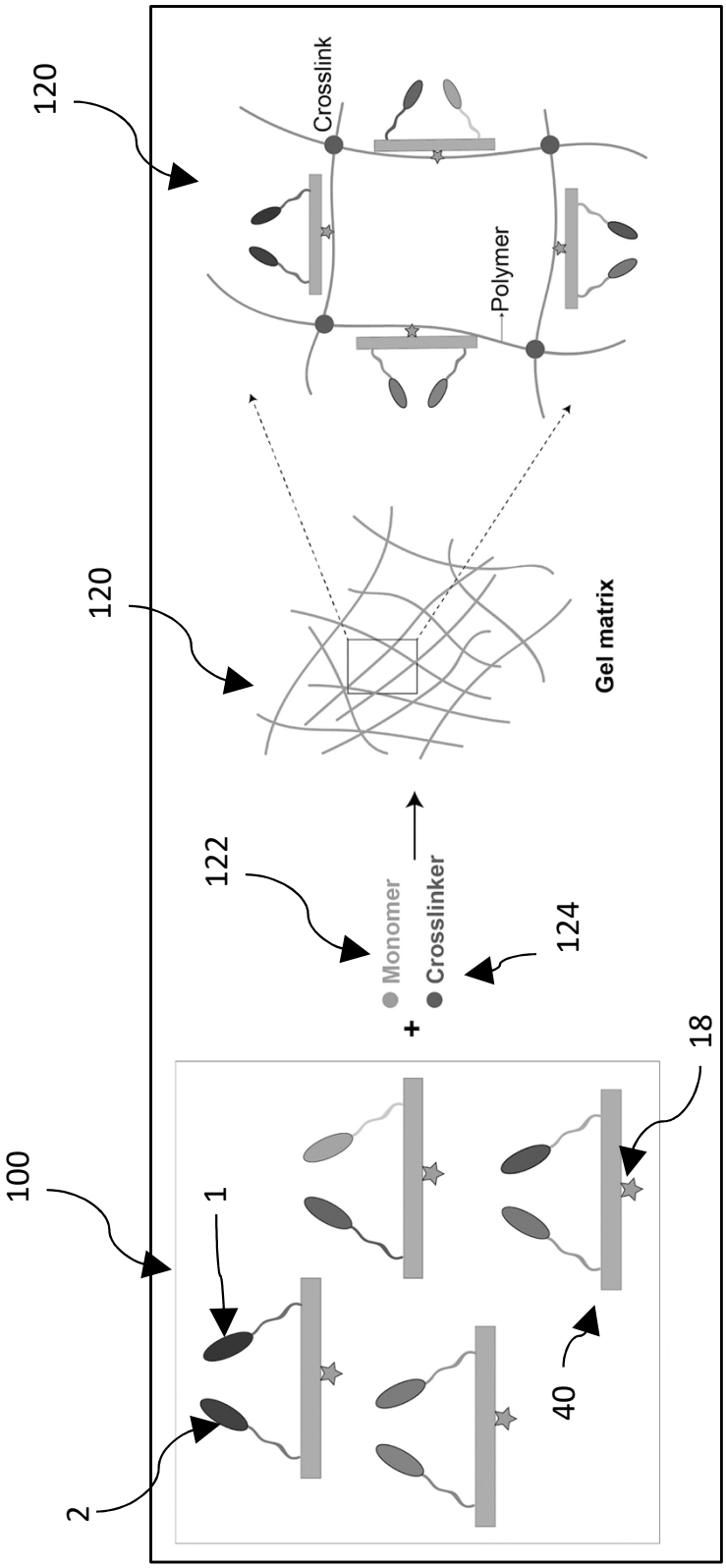

120
Crosslink
Polymer
120
Gel matrix
122
Monomer
Crosslinker
124
100
1
18
2
40

FIG. 13

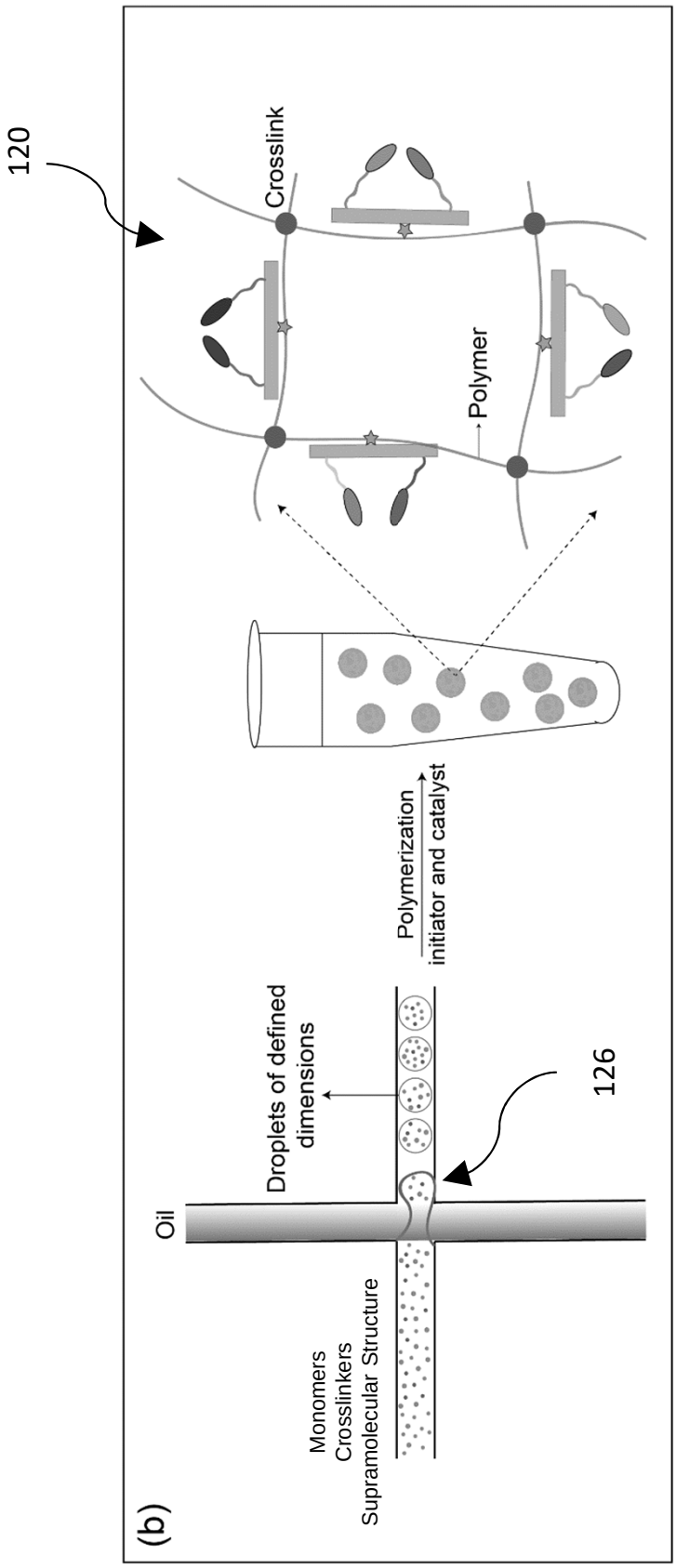

120
Crosslink
Polymer
Polymerization
initiator and catalyst
Droplets of defined
dimensions
126
Oil
Monomers
Crosslinkers
Supramolecular Structure
(b)

FIG. 14

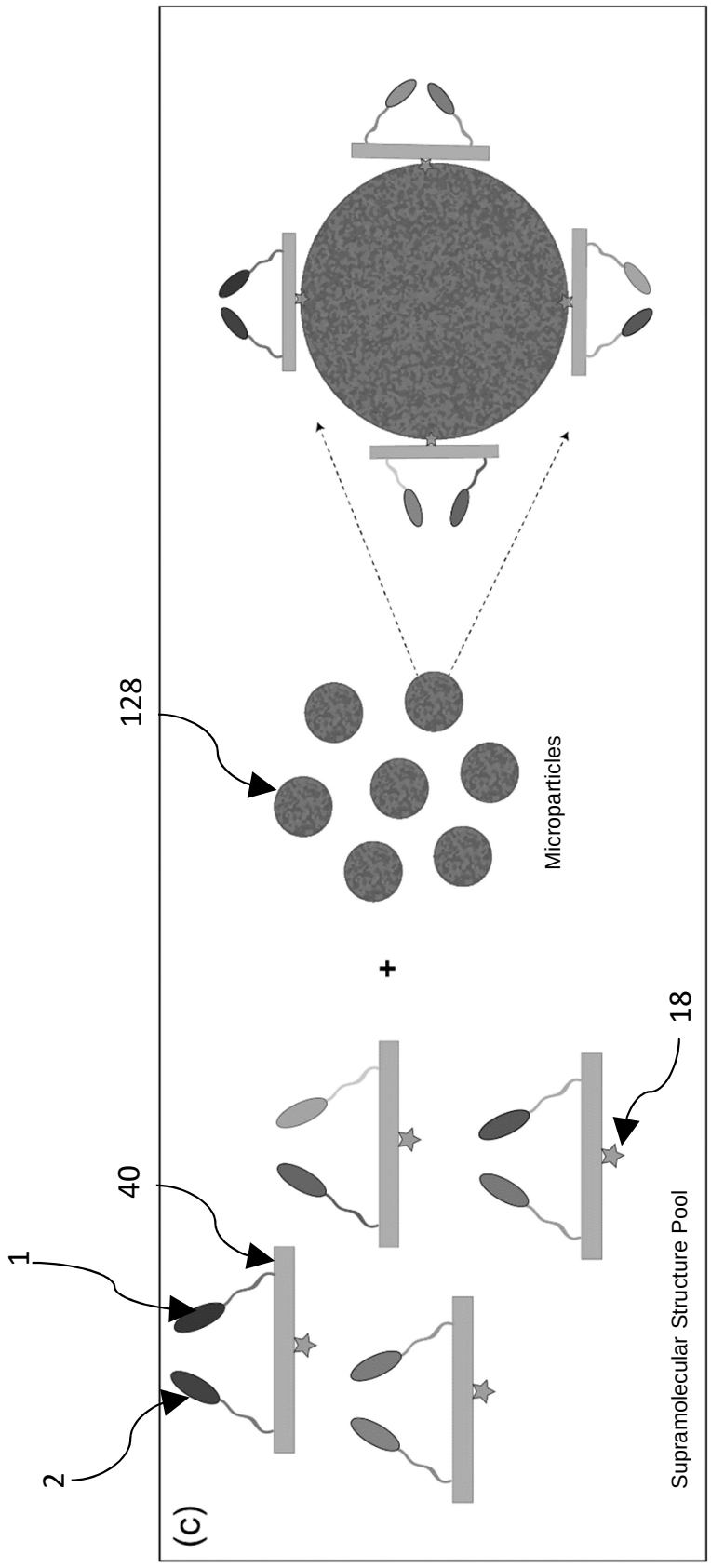

(c)
2
1
40
18
Supramolecular Structure Pool
+
128
Microparticles

FIG. 15

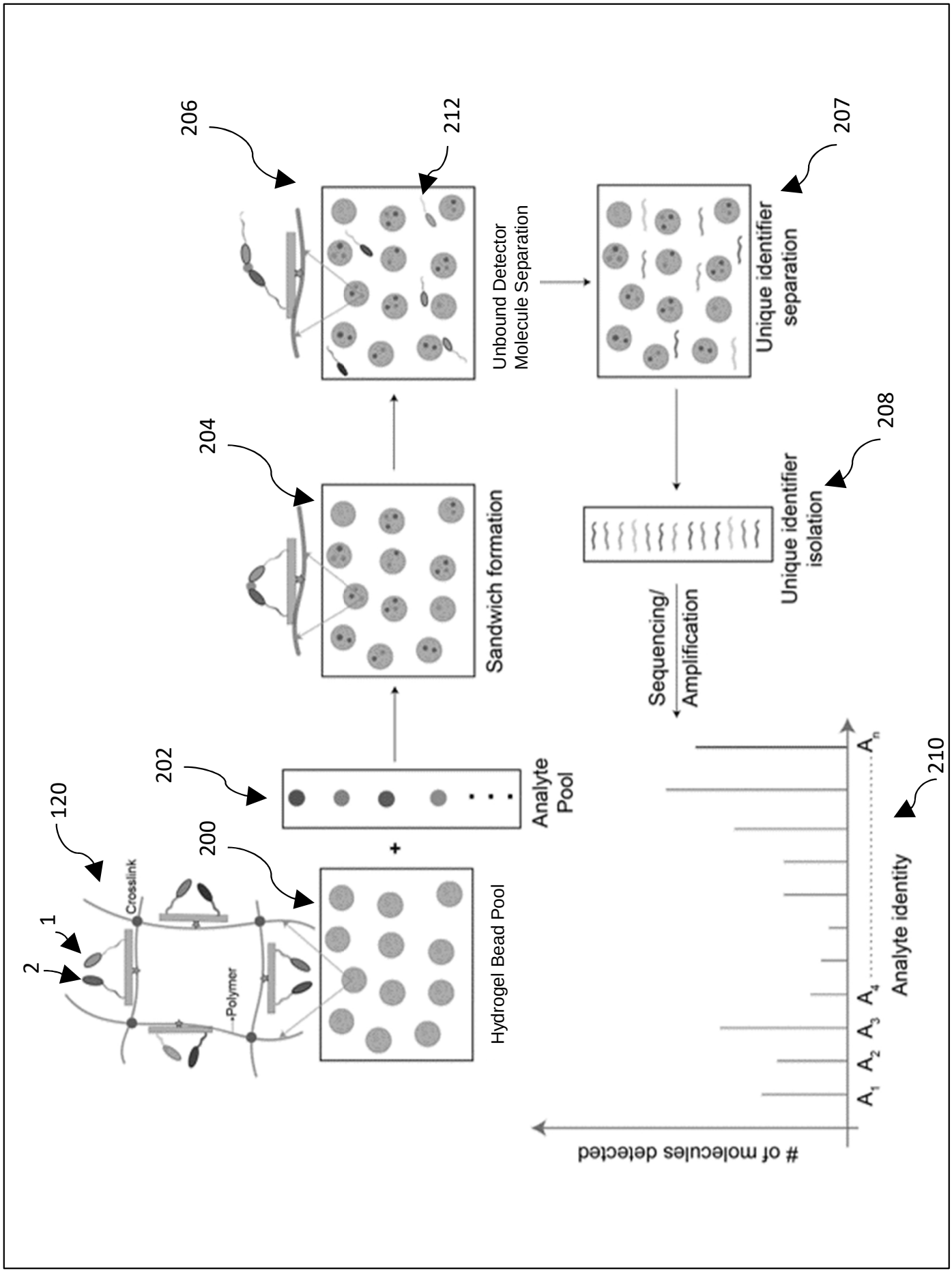

120
1
2
Crosslink
Polymer
200
Hydrogel Bead Pool
+
202
Analyte Pool
204
Sandwich formation
206
212
Unbound Detector Molecule Separation
207
Unique identifier separation
208
Unique identifier isolation
Sequencing/ Amplification
210
# of molecules detected
A1 A2 A3 A4 An
Analyte identity

FIG. 16

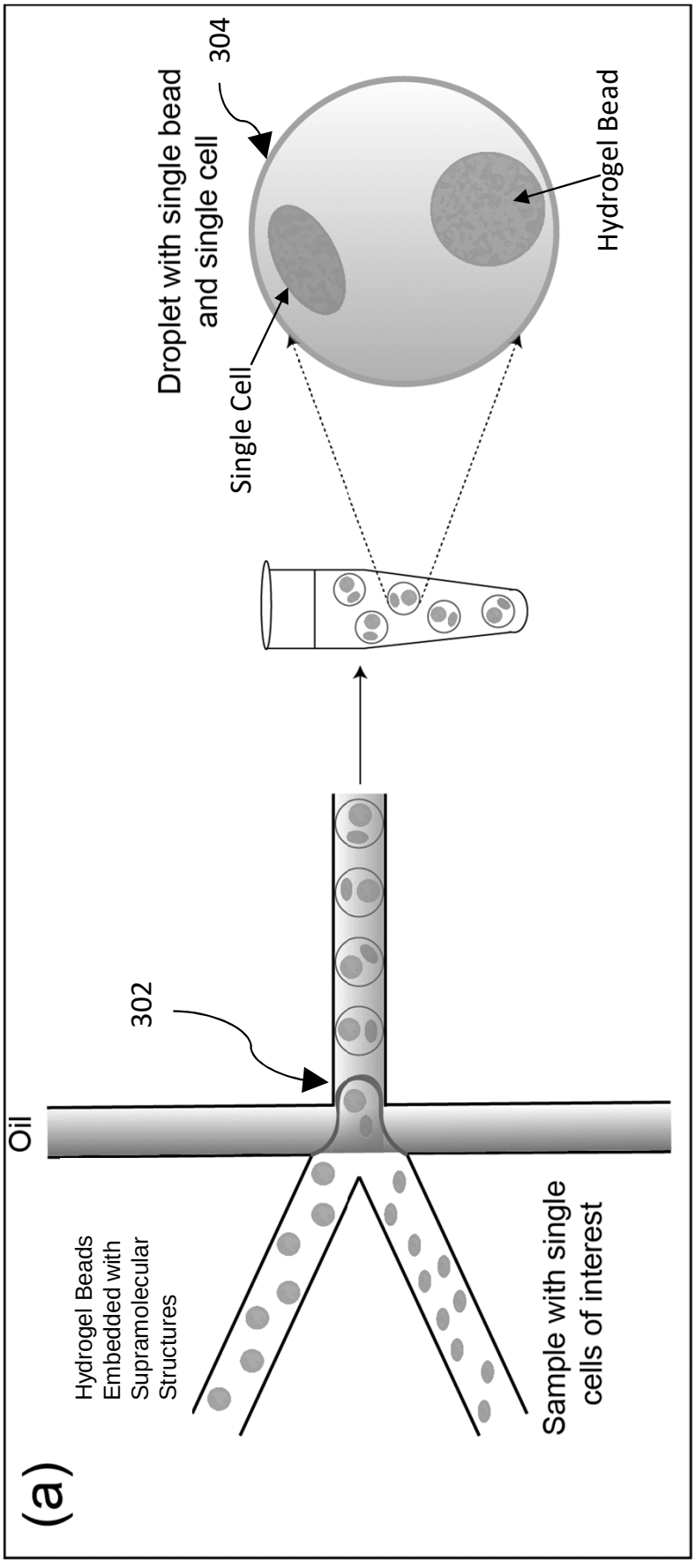

(a)
Hydrogel Beads Embedded with Supramolecular Structures
Oil
302
Sample with single cells of interest
Droplet with single bead and single cell
304
Single Cell
Hydrogel Bead

FIG. 17

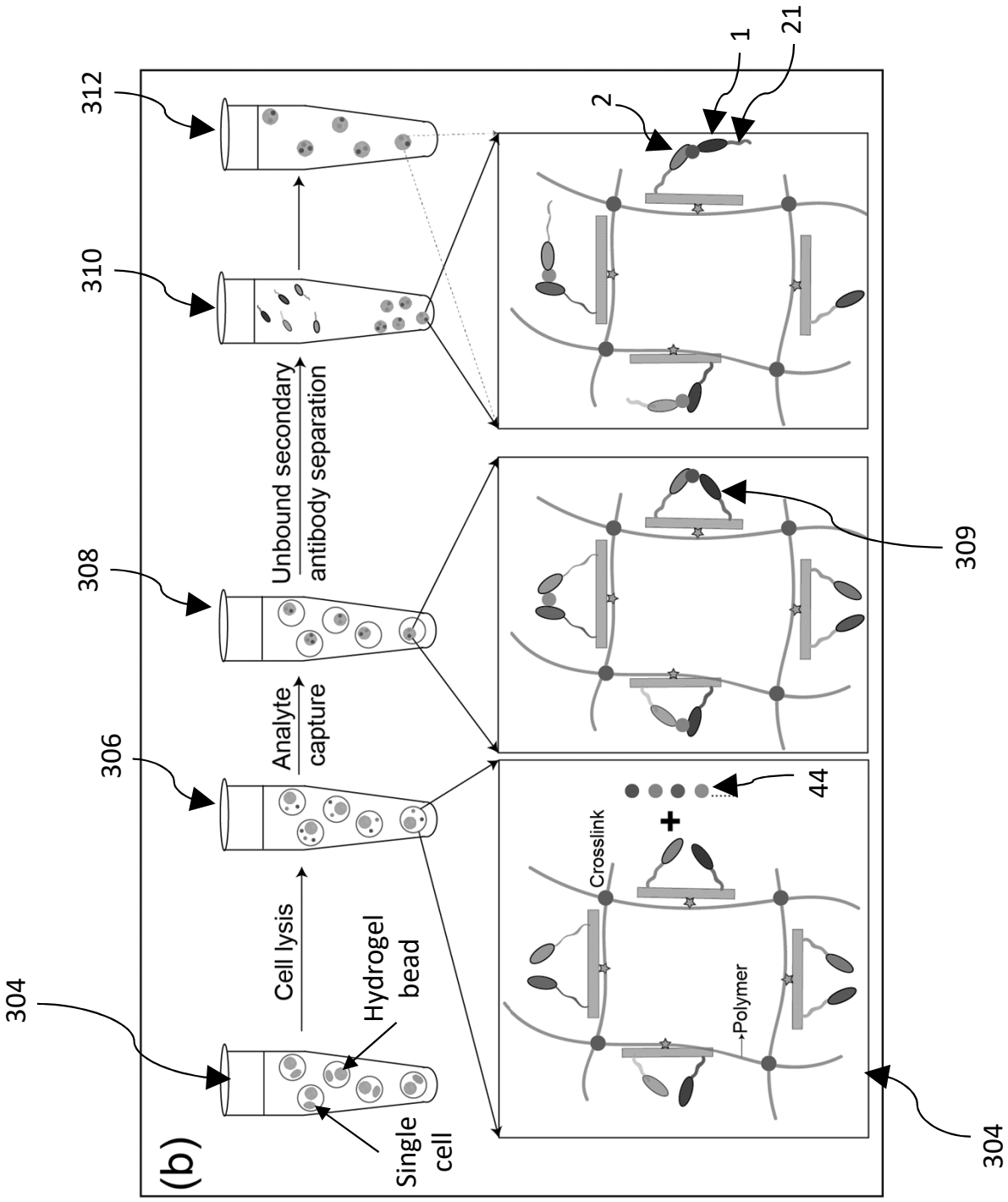

(b)
304
Single cell
Cell lysis
Hydrogel bead
306
Analyte capture
308
Unbound secondary antibody separation
310
312
Crosslink
Polymer
44
2
1
21
309
304

FIG. 18

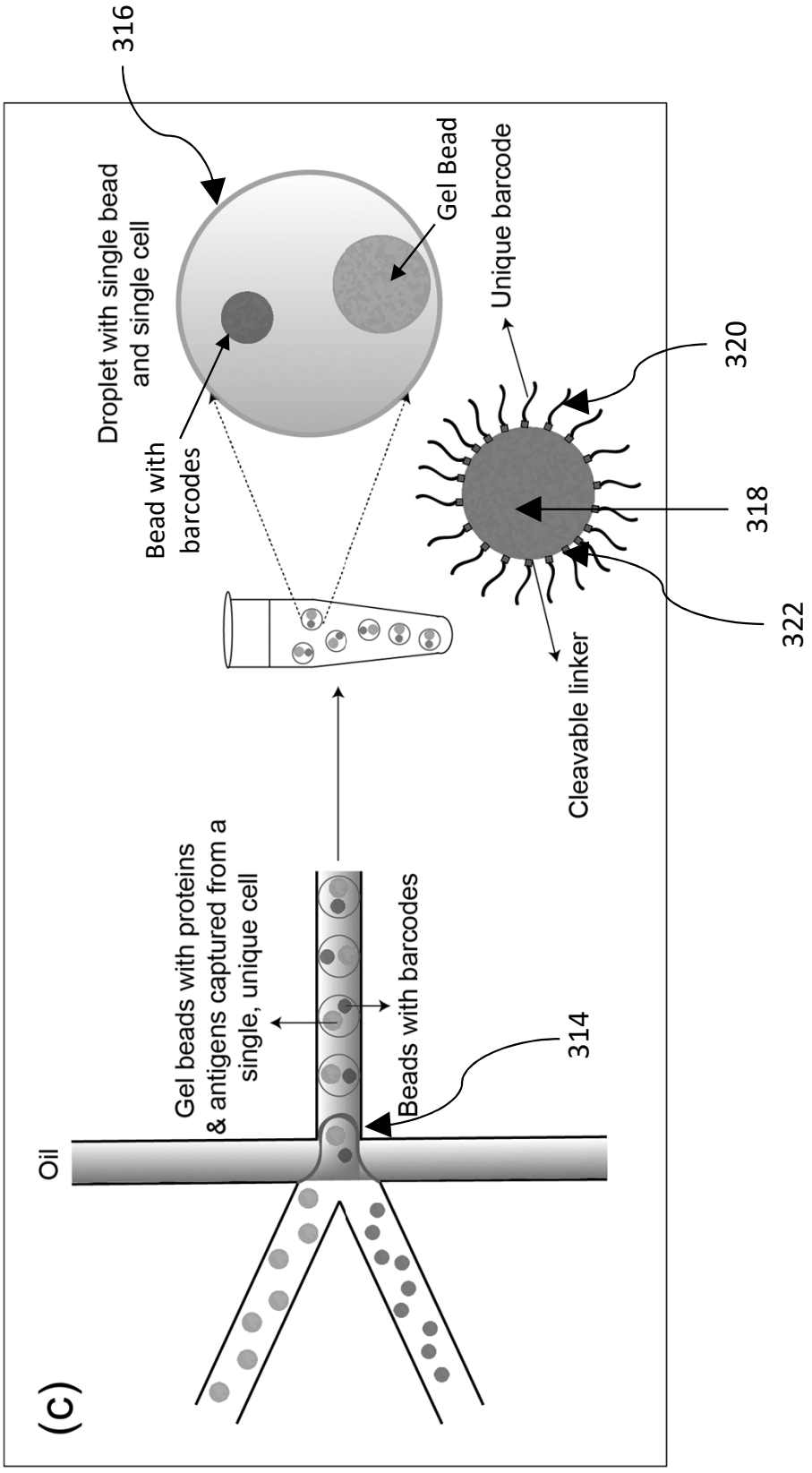

316
Droplet with single bead and single cell
Gel Bead
Unique barcode
Bead with barcodes
320
318
322
Cleavable linker
Gel beads with proteins & antigens captured from a single, unique cell
Beads with barcodes
314
Oil
(c)

FIG. 19

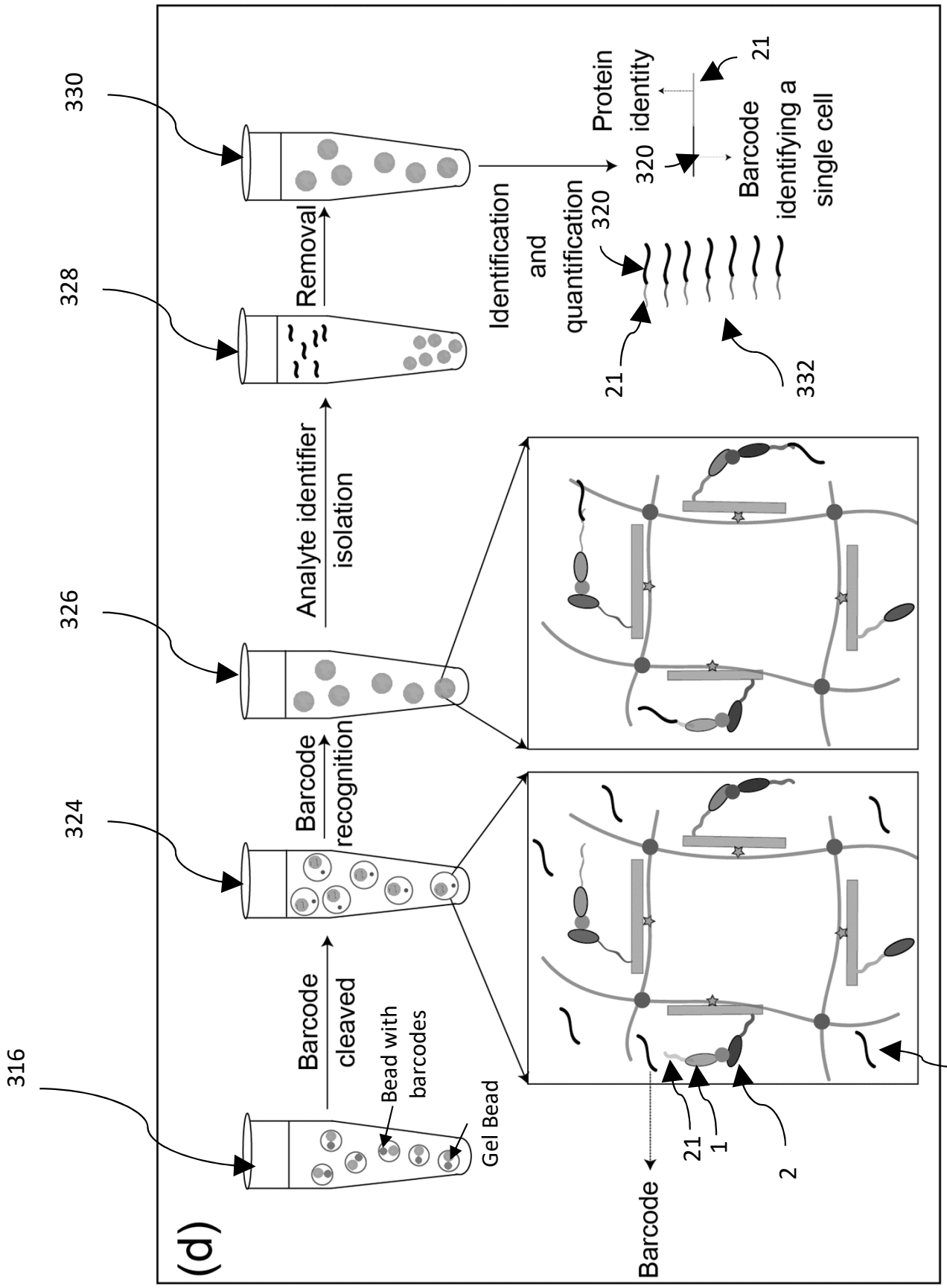

316
Gel Bead
Bead with barcodes
Barcode cleaved
324
Barcode recognition
326
Analyte identifier isolation
328
Removal
330
Identification and quantification
320
Protein identity
320
Barcode identifying a single cell
21
332
(d)
Barcode
21
1
2
332

FIG. 20

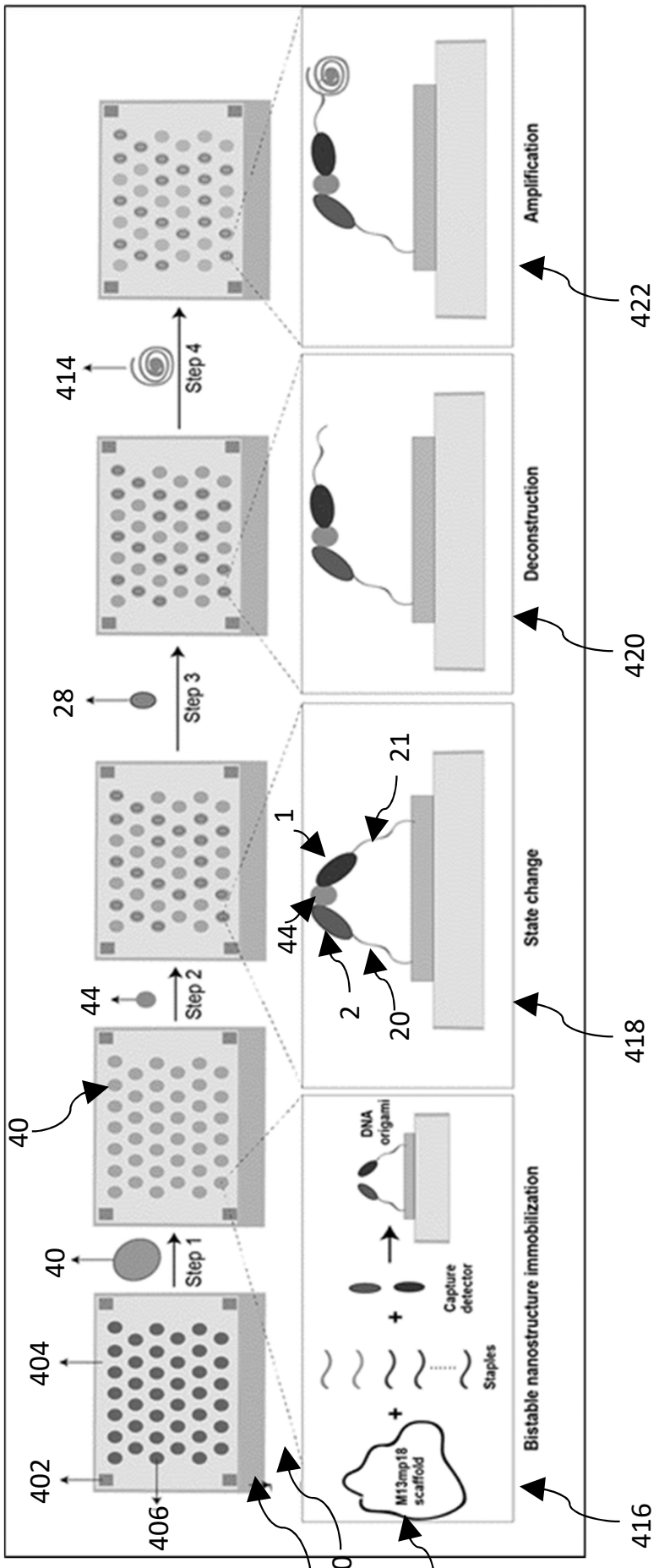

Bistable nanostructure immobilization
M13mp18 scaffold
Staples
Capture detector
DNA origami
State change
Deconstruction
Amplification
Step 1
Step 2
Step 3
Step 4
40
44
28
414
402
404
406
400
400
13
416
418
420
422
1
2
20
21
44

FIG. 21

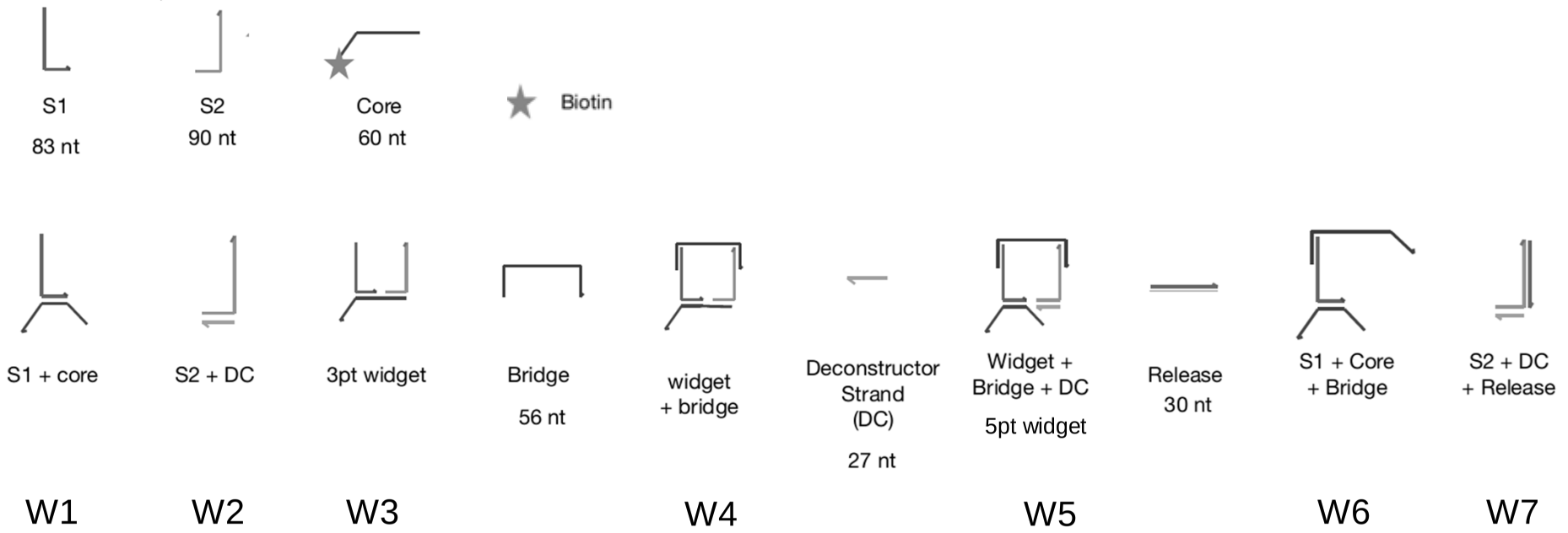

S1
83 nt
S2
90 nt
Core
60 nt
Biotin
S1 + core
S2 + DC
3pt widget
Bridge
56 nt
widget
+ bridge
Deconstructor
Strand
(DC)
27 nt
Widget +
Bridge + DC
5pt widget
Release
30 nt
S1 + Core
+ Bridge
S2 + DC
+ Release
W1
W2
W3
W4
W5
W6
W7

FIG. 22

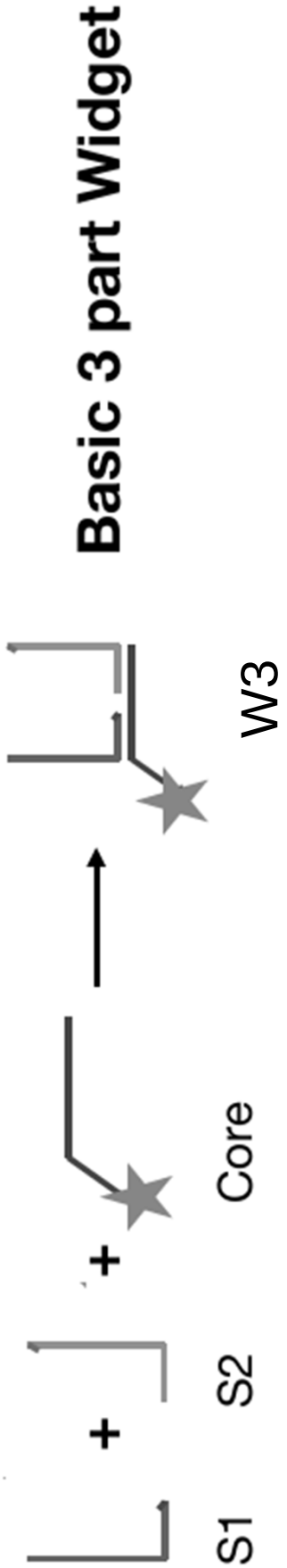


FIG. 23

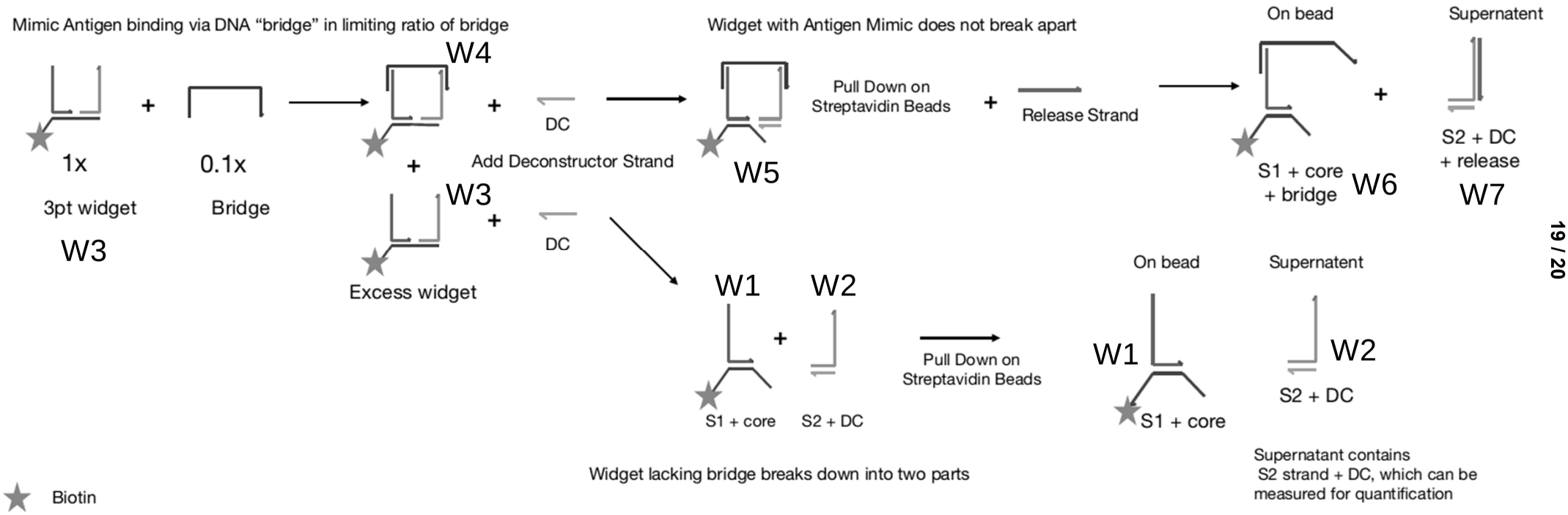

Mimic Antigen binding via DNA "bridge" in limiting ratio of bridge
1x
3pt widget
W3
0.1x
Bridge
W4
W3
Excess widget
DC
Add Deconstructor Strand
Widget with Antigen Mimic does not break apart
W5
Pull Down on Streptavidin Beads
Release Strand
On bead
S1 + core + bridge
W6
Supernatent
S2 + DC + release
W7
DC
W1
W2
S1 + core
S2 + DC
Pull Down on Streptavidin Beads
On bead
W1
S1 + core
Supernatent
W2
S2 + DC
Widget lacking bridge breaks down into two parts
Supernatant contains S2 strand + DC, which can be measured for quantification
Biotin

FIG. 24

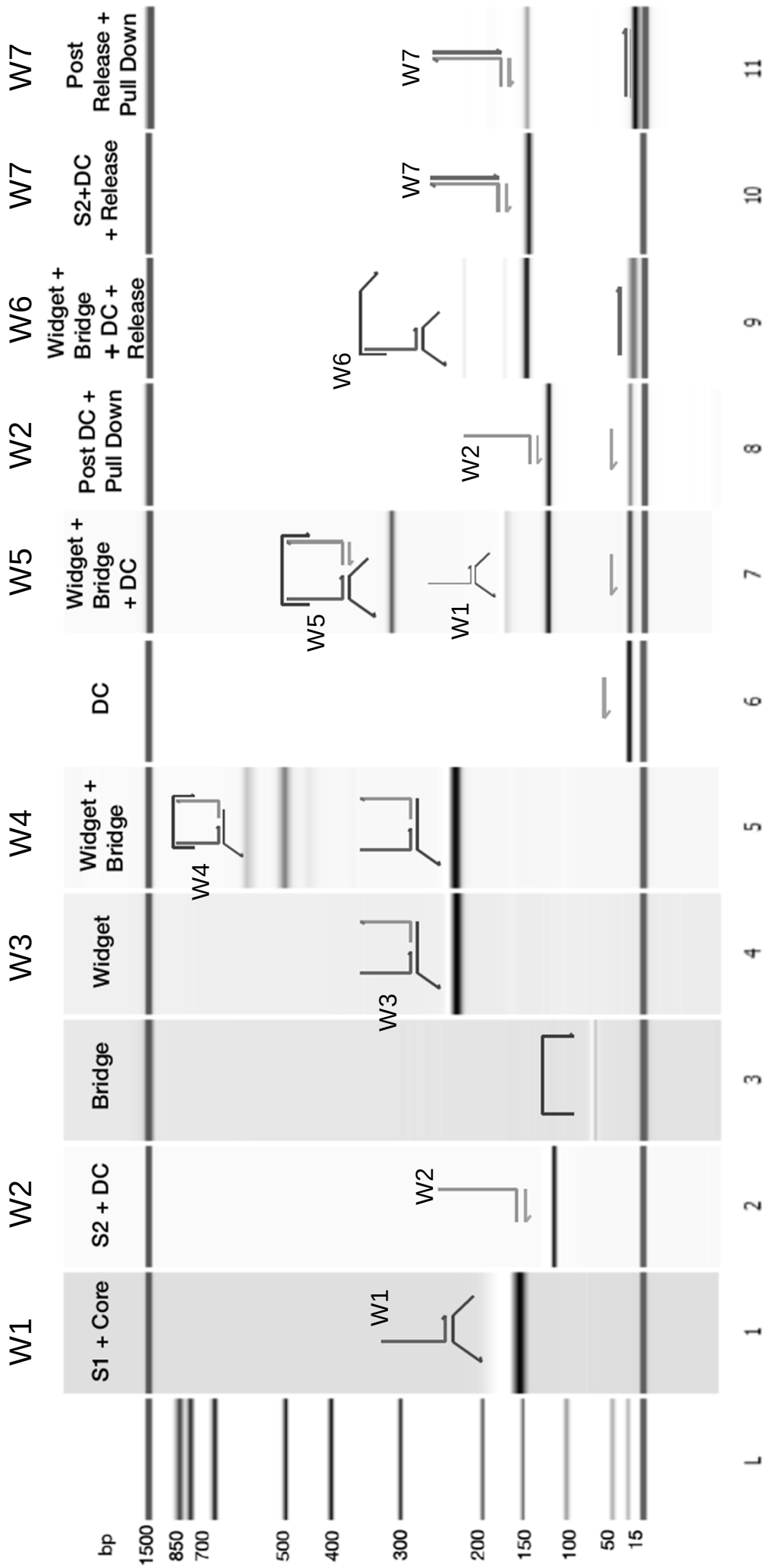

W1
W2
W3
W4
W5
W6
W7
S1 + Core
S2 + DC
Bridge
Widget
Widget + Bridge
DC
Widget + Bridge + DC
Post DC + Pull Down
Widget + Bridge + DC + Release
S2+DC + Release
Post Release + Pull Down
bp
1500
850
700
500
400
300
200
150
100
50
15
L
1
2
3
4
5
6
7
8
9
10
11

FIG. 25