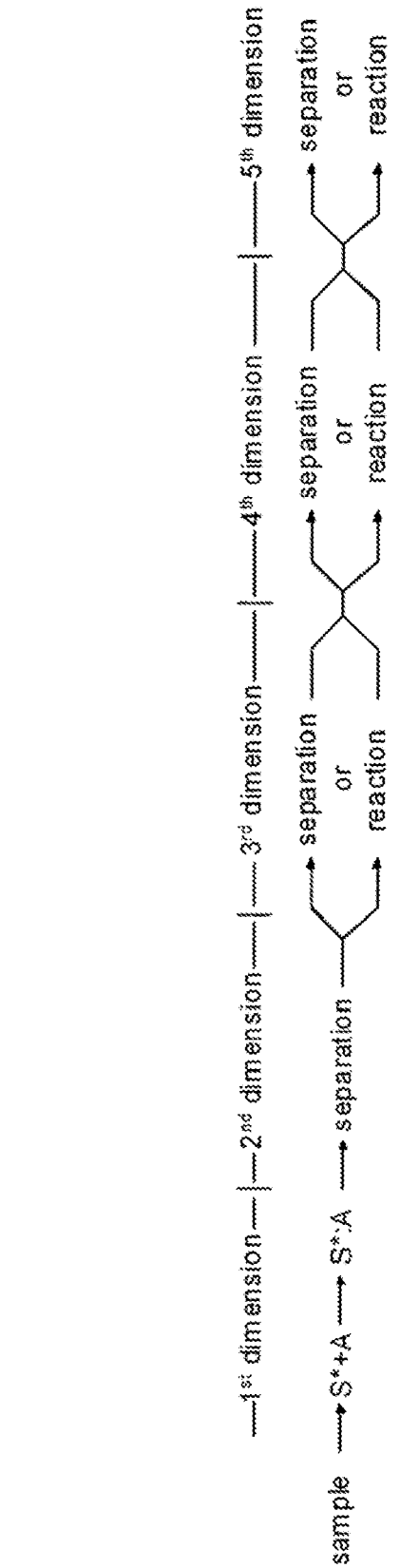

1st dimension
2nd dimension
3rd dimension
4th dimension
5th dimension
sample
S*+A
S*:A
separation
or
reaction
separation
or
reaction
separation
or
reaction

FIG. 1

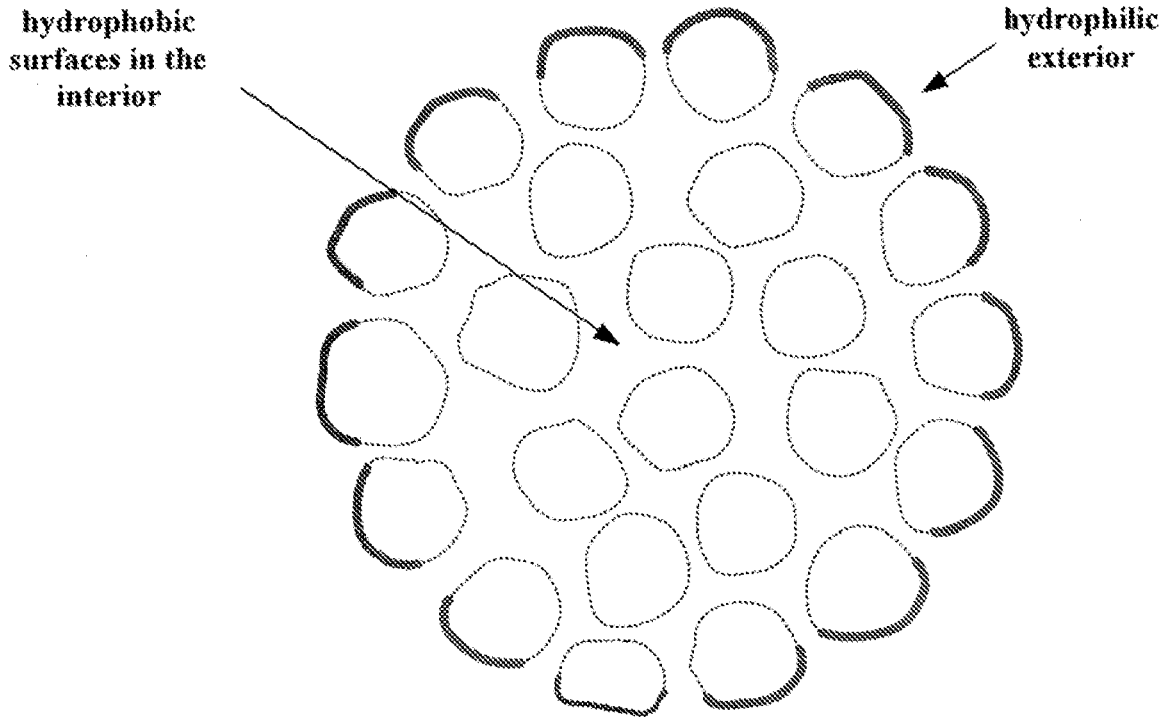

hydrophobic surfaces in the interior
hydrophilic exterior

FIG. 2

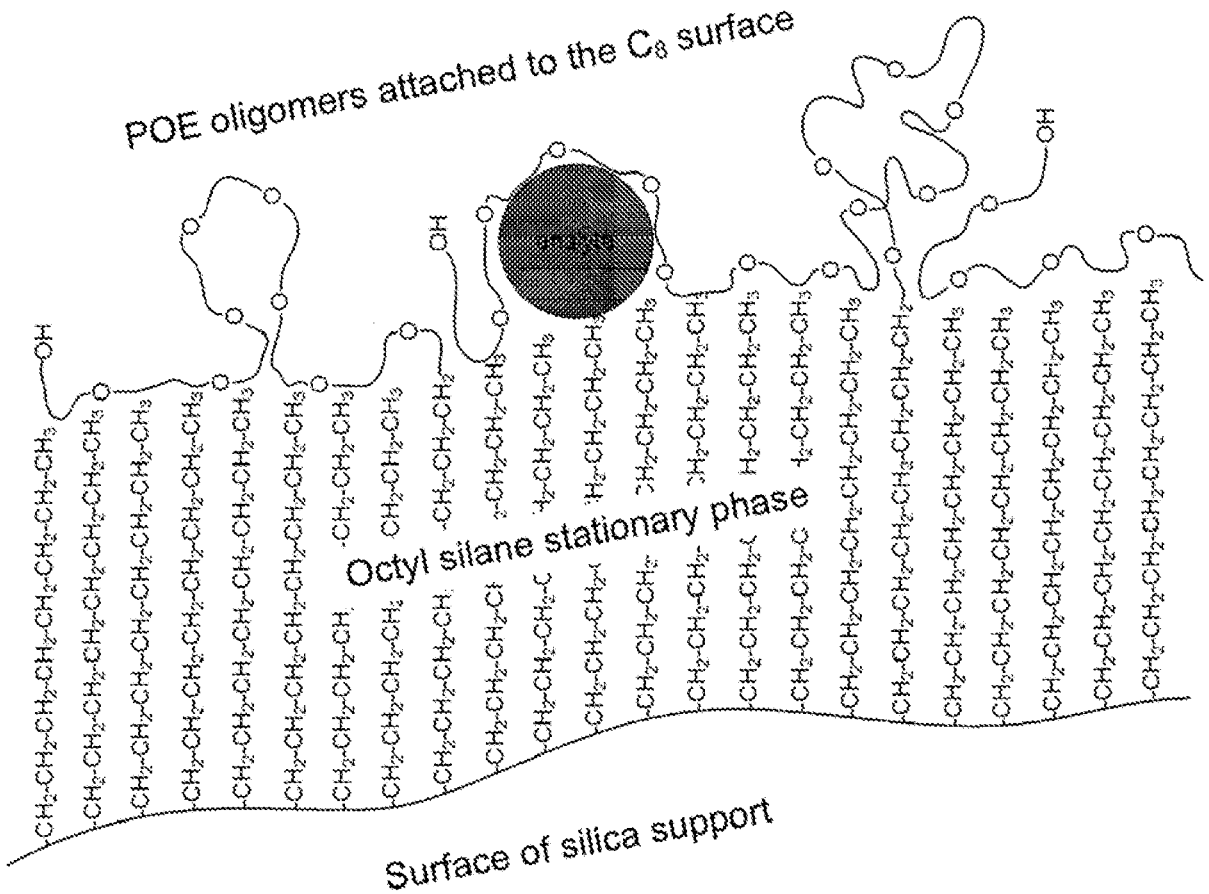

POE oligomers attached to the C8 surface
Octyl silane stationary phase
Surface of silica support

FIG. 3

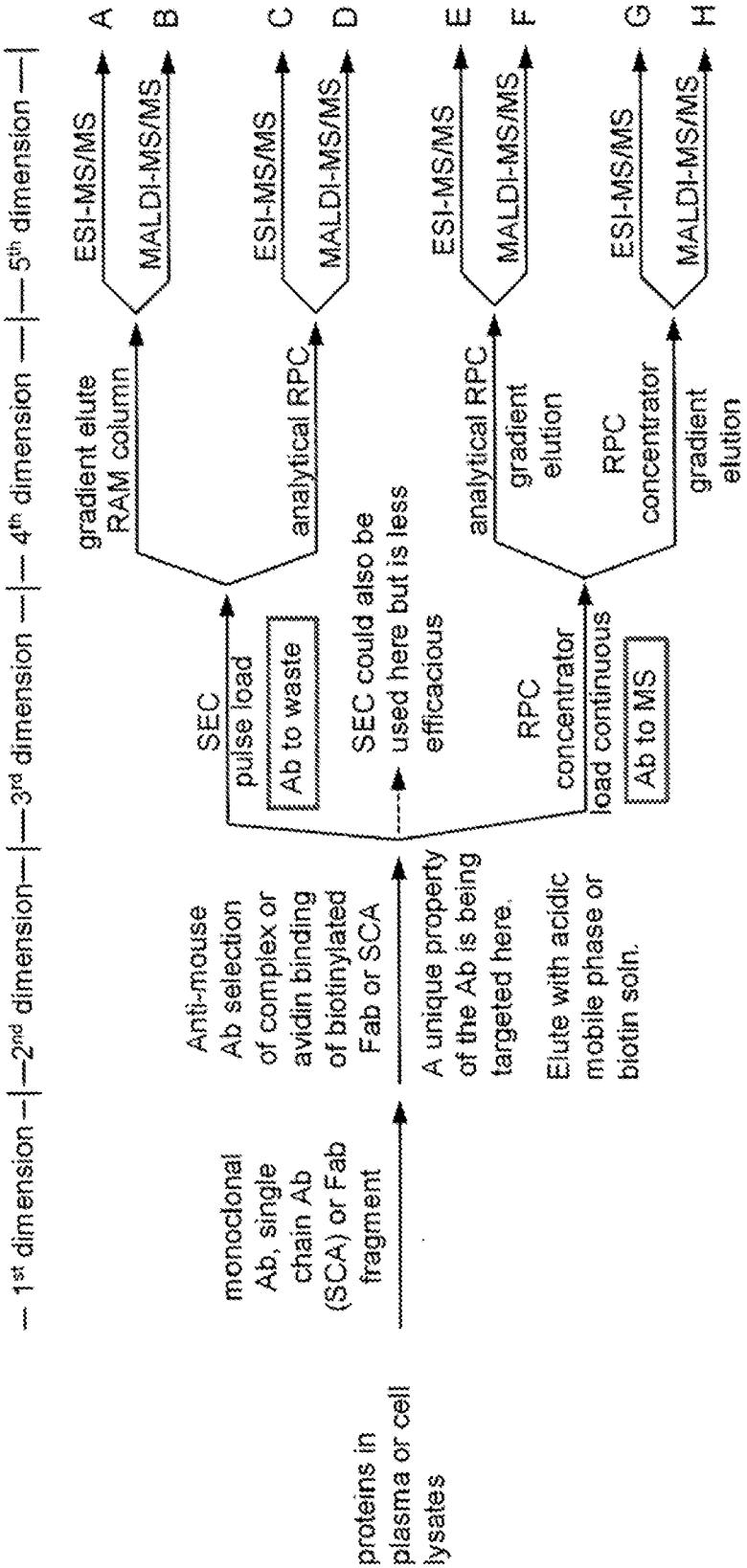

1st dimension
2nd dimension
3rd dimension
4th dimension
5th dimension
proteins in plasma or cell lysates
monoclonal Ab, single chain Ab (SCA) or Fab fragment
Anti-mouse Ab selection of complex or avidin binding of biotinylated Fab or SCA
A unique property of the Ab is being targeted here.
Elute with acidic mobile phase or biotin soln.
SEC
pulse load
Ab to waste
SEC could also be used here but is less efficacious
RPC concentrator
load continuous
Ab to MS
gradient elute RAM column
analytical RPC
analytical RPC gradient elution
RPC concentrator gradient elution
ESI-MS/MS
MALDI-MS/MS
ESI-MS/MS
MALDI-MS/MS
ESI-MS/MS
MALDI-MS/MS
A
B
C
D
E
F
G
H

FIG. 4

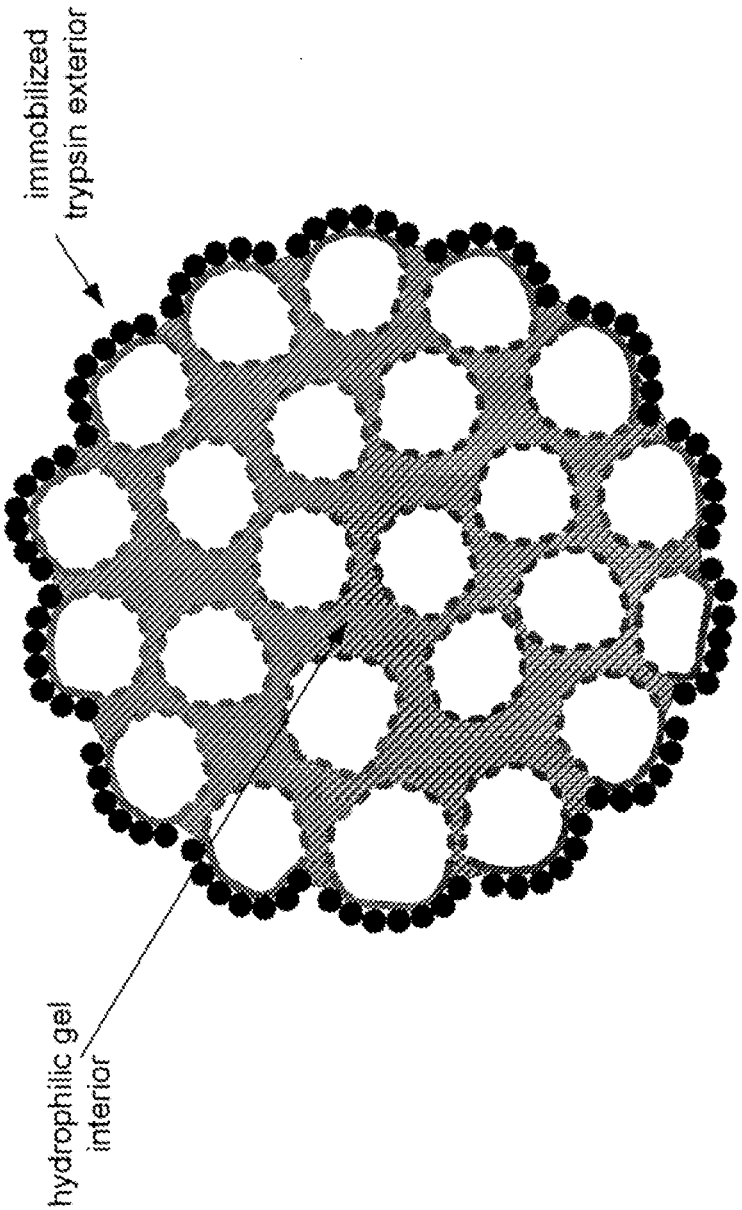


FIG. 5

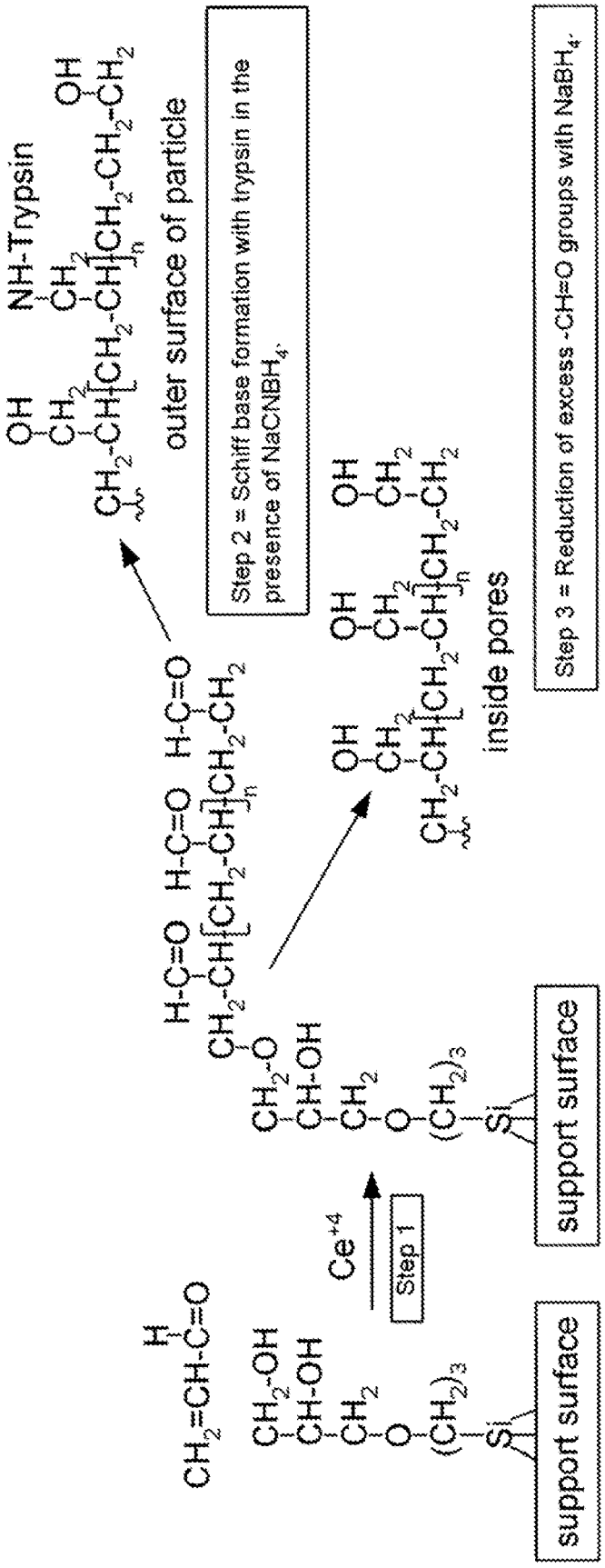

support surface
support surface
Step 1
Ce+4
inside pores
outer surface of particle
NH-Trypsin
Step 2 = Schiff base formation with trypsin in the presence of NaCNBH4.
Step 3 = Reduction of excess -CH=O groups with NaBH4.

FIG. 6

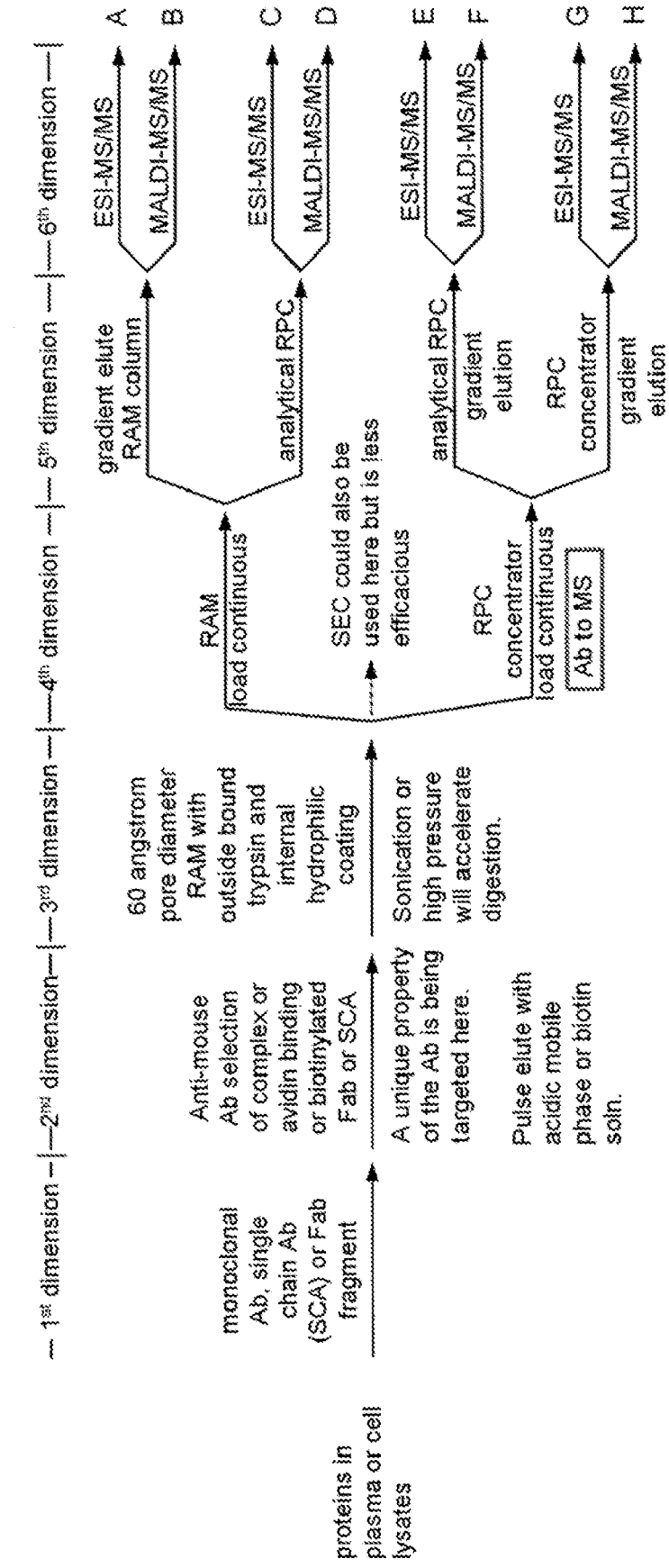

1st dimension
2nd dimension
3rd dimension
4th dimension
5th dimension
6th dimension
proteins in plasma or cell lysates
monoclonal Ab, single chain Ab (SCA) or Fab fragment
Anti-mouse Ab selection of complex or avidin binding or biotinylated Fab or SCA
60 angstrom pore diameter RAM with outside bound trypsin and internal hydrophilic coating
A unique property of the Ab is being targeted here.
Sonication or high pressure will accelerate digestion.
Pulse elute with acidic mobile phase or biotin soln.
RAM load continuous
SEC could also be used here but is less efficacious
RPC concentrator load continuous
Ab to MS
analytical RPC gradient elution
RPC concentrator gradient elution
gradient elute RAM column
analytical RPC
ESI-MS/MS
MALDI-MS/MS
A
B
C
D
E
F
G
H

FIG. 7

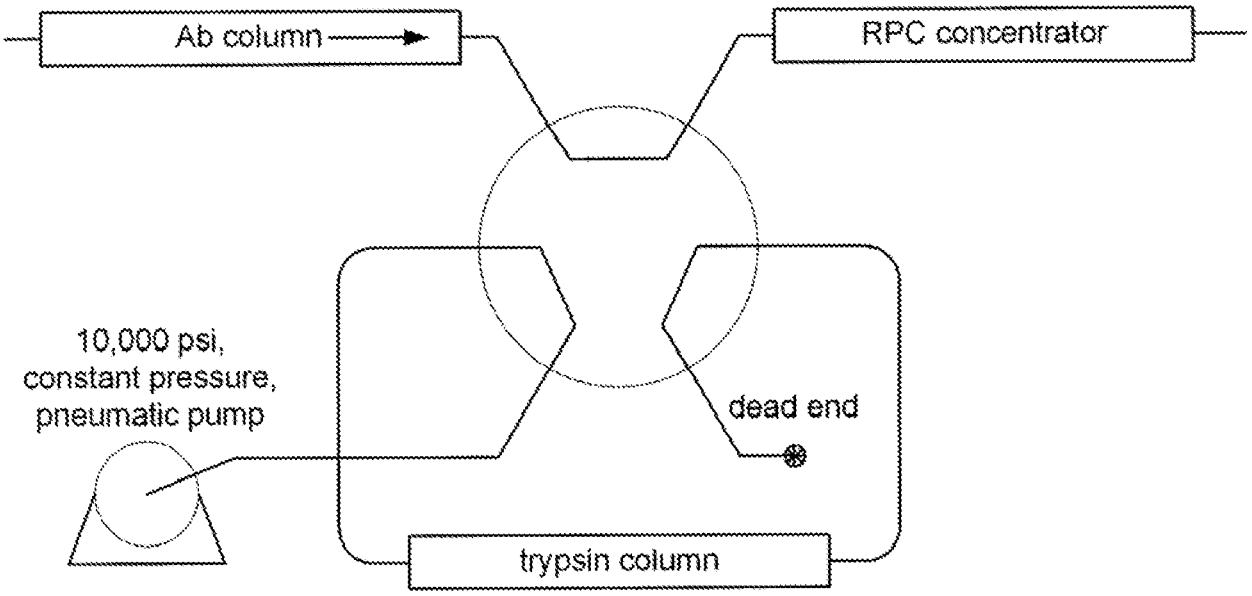

Ab column
RPC concentrator
10,000 psi,
constant pressure,
pneumatic pump
dead end
trypsin column

FIG. 8

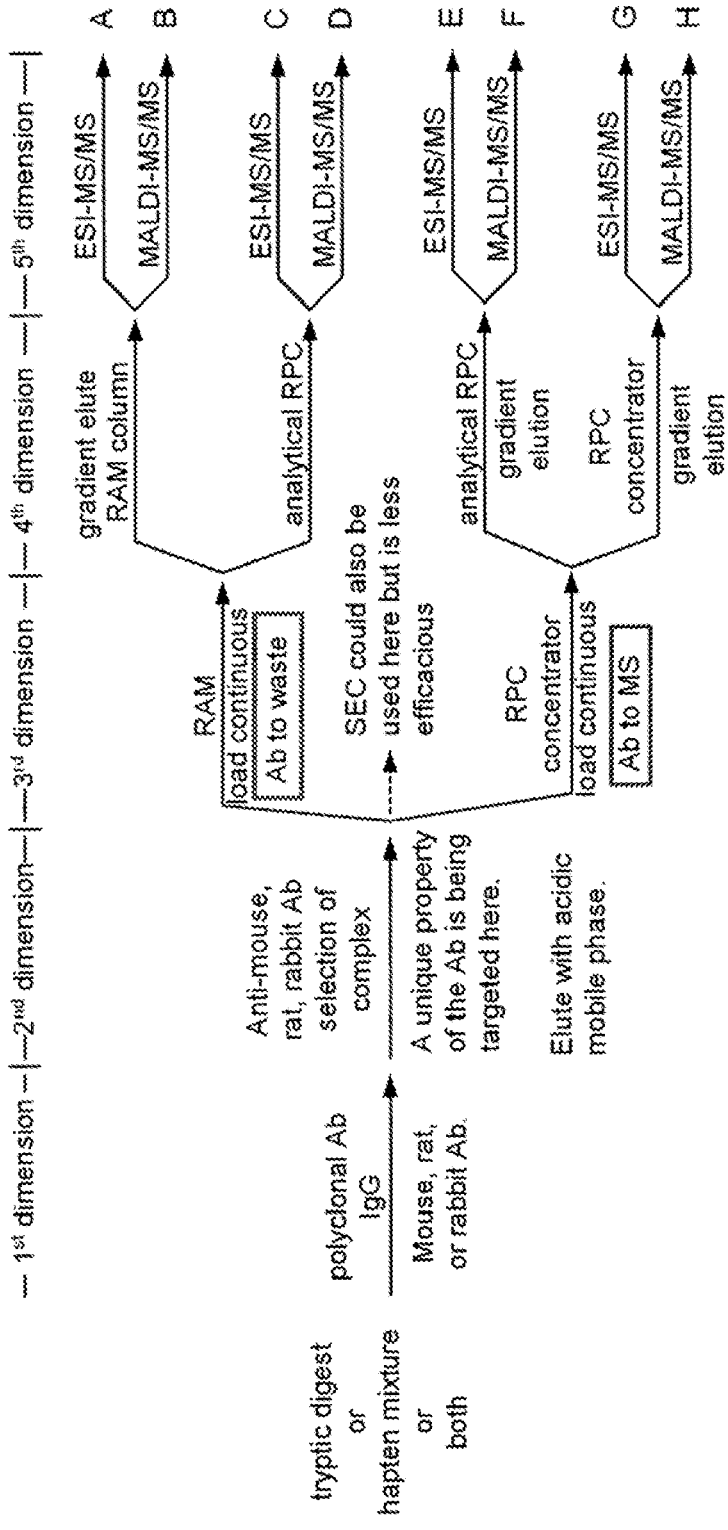

1st dimension
2nd dimension
3rd dimension
4th dimension
5th dimension
tryptic digest or hapten mixture or both
polyclonal Ab IgG
Mouse, rat, or rabbit Ab.
Anti-mouse, rat, rabbit Ab selection of complex
A unique property of the Ab is being targeted here.
RAM
load continuous
Ab to waste
SEC could also be used here but is less efficacious
RPC concentrator
load continuous
Ab to MS
Elute with acidic mobile phase.
gradient elute RAM column
analytical RPC
analytical RPC
gradient elution
RPC concentrator
gradient elution
ESI-MS/MS
MALDI-MS/MS
ESI-MS/MS
MALDI-MS/MS
ESI-MS/MS
MALDI-MS/MS
ESI-MS/MS
MALDI-MS/MS
A
B
C
D
E
F
G
H

FIG. 9

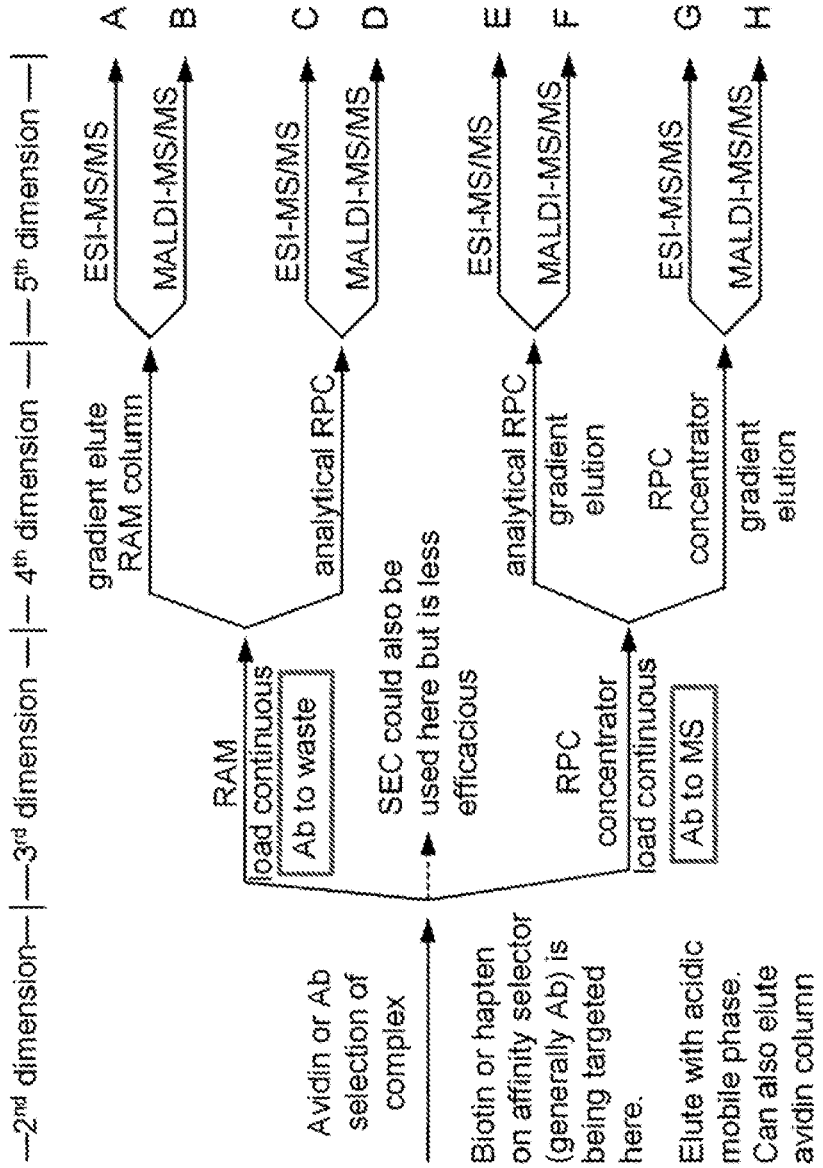

1st dimension
2nd dimension
3rd dimension
4th dimension
5th dimension
tryptic digest or hapten mixture or both
polyclonal Ab IgG
Ab is tagged with biotin or hapten
Avidin or Ab selection of complex
Biotin or hapten on affinity selector (generally Ab) is being targeted here.
Elute with acidic mobile phase. Can also elute avidin column with biotin.
RAM load continuous
Ab to waste
SEC could also be used here but is less efficacious
RPC concentrator load continuous
Ab to MS
gradient elute RAM column
analytical RPC
analytical RPC gradient elution
RPC concentrator gradient elution
ESI-MS/MS
MALDI-MS/MS
ESI-MS/MS
MALDI-MS/MS
ESI-MS/MS
MALDI-MS/MS
ESI-MS/MS
MALDI-MS/MS
A
B
C
D
E
F
G
H

FIG. 10

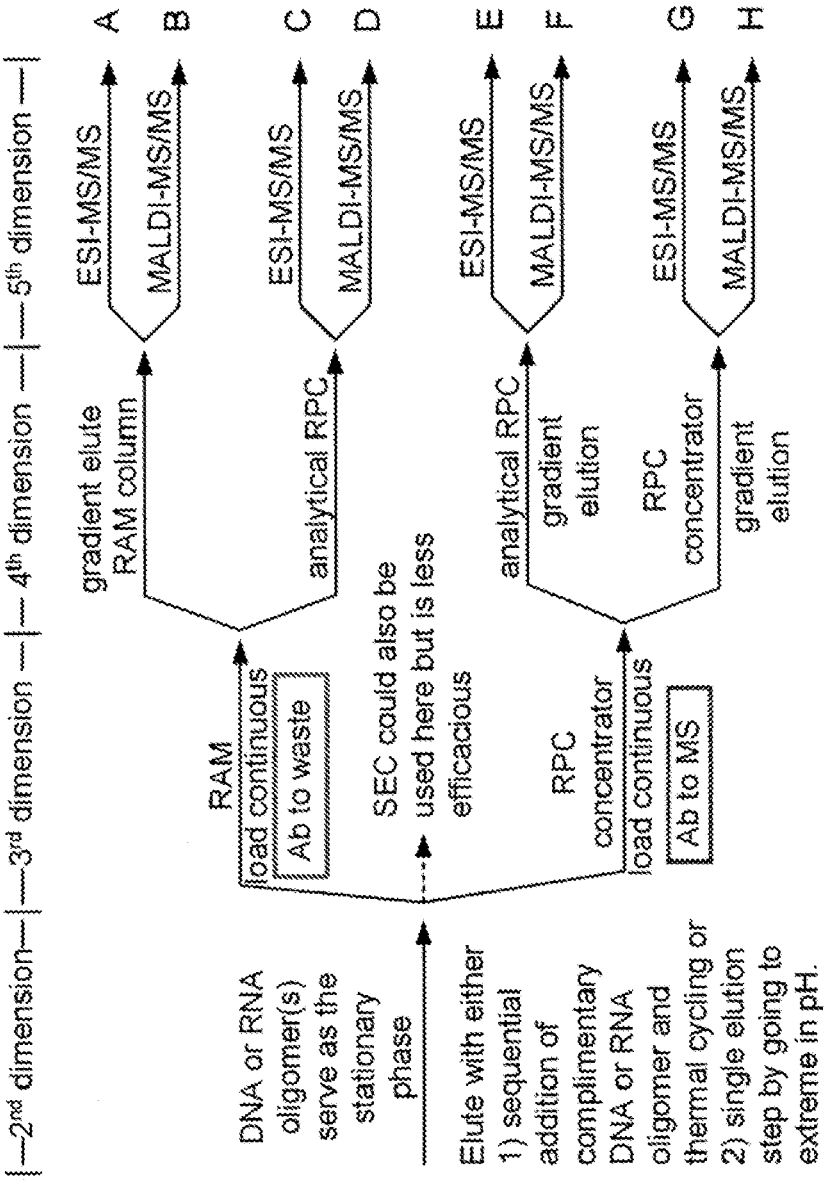

1st dimension
2nd dimension
3rd dimension
4th dimension
5th dimension
tryptic digest or hapten mixture or both
polyclonal Ab IgG
Antibodies are tagged with DNA or RNA oligomers
DNA or RNA oligomer(s) serve as the stationary phase
Elute with either 1) sequential addition of complimentary DNA or RNA oligomer and thermal cycling or 2) single elution step by going to extreme in pH.
RAM
load continuous
Ab to waste
SEC could also be used here but is less efficacious
RPC concentrator
load continuous
Ab to MS
gradient elute RAM column
analytical RPC
analytical RPC
gradient elution
RPC concentrator
gradient elution
ESI-MS/MS
MALDI-MS/MS
ESI-MS/MS
MALDI-MS/MS
ESI-MS/MS
MALDI-MS/MS
A
B
C
D
E
F
G
H

FIG. 11

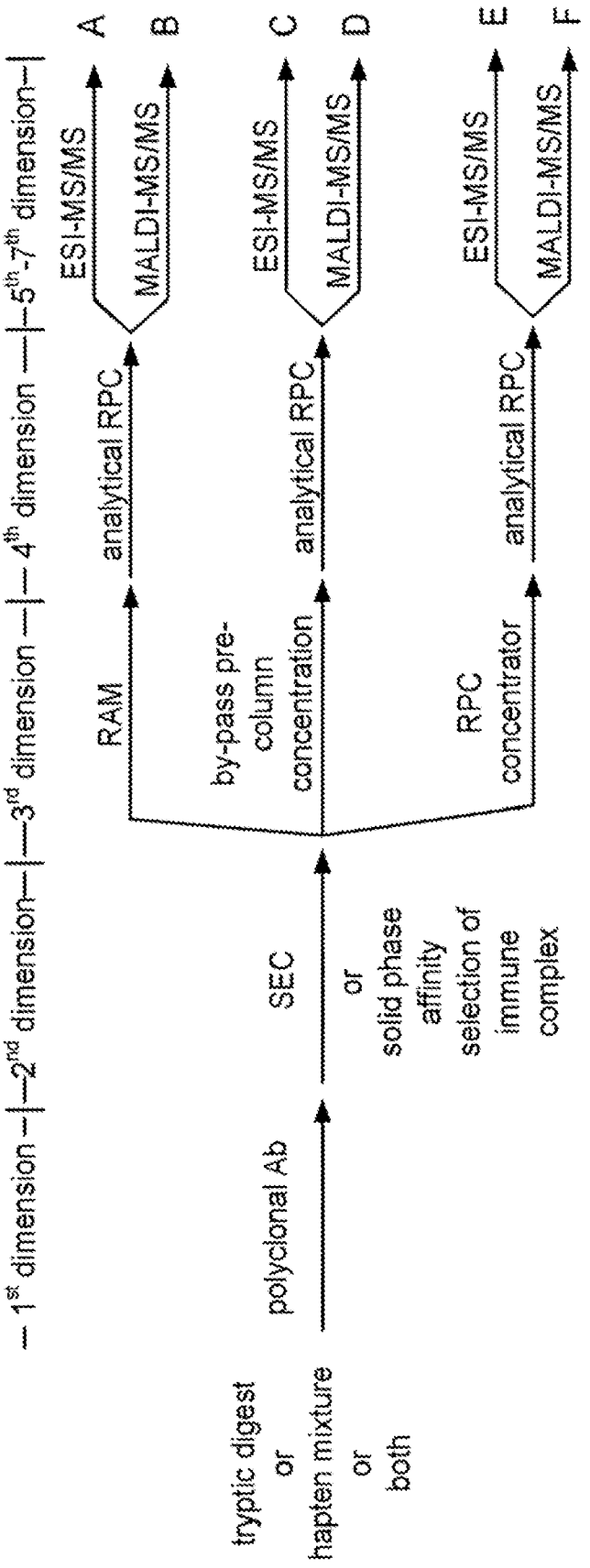

1st dimension
2nd dimension
3rd dimension
4th dimension
5th-7th dimension
tryptic digest or hapten mixture or both
polyclonal Ab
SEC
or solid phase affinity selection of immune complex
RAM
by-pass pre-column concentration
RPC concentrator
analytical RPC
analytical RPC
analytical RPC
ESI-MS/MS
MALDI-MS/MS
ESI-MS/MS
MALDI-MS/MS
ESI-MS/MS
MALDI-MS/MS
A
B
C
D
E
F

FIG. 12

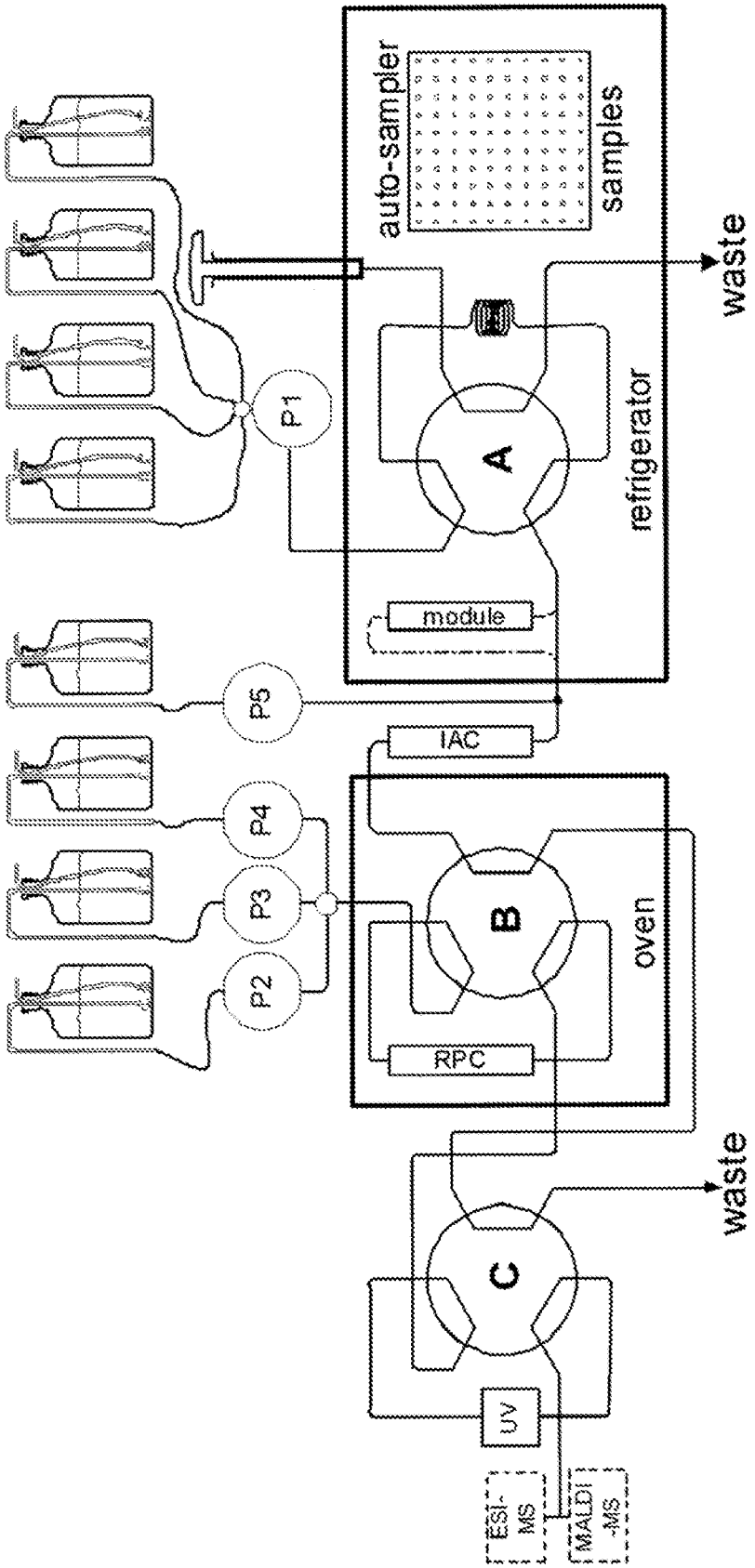

auto-sampler
samples
waste
refrigerator
A
P1
module
P5
IAC
P4
P3
P2
B
oven
RPC
waste
C
UV
ESI-MS
MALDI-MS

FIG. 13