

CLAIMS

WHAT IS CLAIMED IS:

1. A method for assaying a biological sample using a substrate, the method comprising:
 - (a) contacting said biological sample with said substrate to form thereon a biomolecule corona which comprises biomolecules from said biological sample, wherein said substrate has a first surface area to mass ratio;
 - (b) assaying said biomolecule corona to identify said biomolecules, wherein the number of different biomolecules identified is higher than the number of different biomolecules identified when said biological sample is contacted with a substrate having a second surface area to mass ratio which is different from said first surface area to mass ratio.
2. The method of claim 1, wherein said second surface area to mass ratio is greater than said first surface area to mass ratio.
3. The method of claim 1 or 2, wherein said substrate having said first surface area to mass ratio has a greater surface area than said substrate having said second surface area to mass ratio.
4. The method of any one of claims 1-3, wherein said substrate having said first surface area to mass ratio has at least 50% greater surface area than said substrate having said second surface area to mass ratio.
5. The method of any one of claims 1-4, wherein said substrate having said first surface area to mass ratio has at least 100% greater surface area than said substrate having said second surface area to mass ratio.
6. The method of any one of claims 1-5, wherein said substrate having said first surface area to mass ratio has at least 200% greater surface area than said substrate having said second surface area to mass ratio.
7. The method of any one of claims 1-6, wherein said substrate having said first surface area to mass ratio has at least 500% greater surface area than said substrate having said second surface area to mass ratio.
8. The method of any one of claims 1-7, wherein said substrate having said first surface area to mass ratio and said substrate having said second surface area to mass ratio have densities differing from each other by at most 25%.
9. The method of any one of claims 1-8, wherein said substrate having said first surface area to mass ratio and said substrate having said second surface area to mass ratio have densities differing from each other by at most 10%.

10. The method of any one of claims 1-9, wherein said first substrate having said first surface area to mass ratio and said substrate having said second surface area to mass ratio have different shapes.
11. The method of any one of claims 1-10, wherein said substrate having said first surface area to mass ratio comprises a first nanoparticle and said substrate having said second surface area to mass ratio comprises a second nanoparticle.
12. The method of claim 11, wherein said first nanoparticle and said second nanoparticle are of a same particle type.
13. The method of claim 11 or 12, wherein said first nanoparticle or said second nanoparticle has a diameter of about 80 nm to about 500 nm.
14. The method of any one of claims 11-13, wherein first nanoparticle or said second nanoparticle has a diameter of about 120 nm to about 350 nm.
15. The method of any one of claims 11-13, wherein said first nanoparticle or said second nanoparticle has a diameter of at least 100 nm.
16. The method of claim 11, 12 or 15, wherein said first nanoparticle or said second nanoparticle has a diameter of at most 500 nm.
17. The method of any one of claims 11-16, wherein said first nanoparticle or said second nanoparticle has a polydispersity index of at most 1.
18. The method of any one of claims 11-17, wherein said first nanoparticle or said second nanoparticle has an oblong geometry.
19. The method of any one of claims 11-18, wherein said first nanoparticle or said second nanoparticle has a substantially spherical geometry.
20. The method of any one of claims 11-19, wherein said first nanoparticle or said second nanoparticle comprises a core material and a shell material.
21. The method of claim 20, wherein said core material comprises a metal, an oxide, a nitride, a ceramic, a carbon material, a silicon material, a polymer, or any combination thereof.
22. The method of claim 20 or 21, wherein said shell material comprises a polymer, a saccharide, a lipid, a peptide, a self-assembled monolayer, a sol-gel, a hydrogel, a glass, or any combination thereof.
23. The method of any one of claims 20-22, wherein said core material has a greater density than said shell material.
24. The method of any one of claims 20-23, wherein said shell material comprises at least two materials, and wherein said at least two materials are phase separated.

25. The method of any one of claims 1-24, wherein said substrate having said first surface area to mass ratio or said substrate having said second surface area to mass ratio comprises a surface functionalization.
26. The method of claim 25, wherein said surface functionalization comprises a polar functional group, an acidic functional group, a basic functional group, a charged functional group, a polymerizable functional group, or any combination thereof.
27. The method of either of claims 25 or 26, wherein said surface functionalization comprises an aminopropyl functionalization, an amine functionalization, a boronic acid functionalization, a carboxylic acid functionalization, a methyl functionalization, an N-succinimidyl ester functionalization, a PEG functionalization, a streptavidin functionalization, a methyl ether functionalization, a triethoxylpropylaminosilane functionalization, a thiol functionalization, a PCP functionalization, a citrate functionalization, a lipoic acid functionalization, a BPEI functionalization, carboxyl functionalization, a hydroxyl functionalization, or any combination thereof.
28. The method of any one of claims 25-27, wherein said surface functionalization comprises an average density of at least about 1 functional group per 20 nm^2 on a surface of said substrate having said first surface area to mass ratio or on a surface of said substrate having said second surface area to mass ratio.
29. The method of any one of claims 25-28, wherein said surface functionalization comprises an average density of about 1 functional group per 30 nm^2 to about 1 functional group per 60 nm^2 on a surface of said substrate having said first surface area to mass ratio or on a surface of said substrate having said second surface area to mass ratio.
30. The method of any one of claims 1-29, wherein said substrate having said first surface area to mass ratio or said second surface area to mass ratio comprises a microparticle.
31. The method of claim 30, wherein said microparticle has a diameter of about 1 micron to about 2 microns.
32. The method of claim 30 or 31, wherein said microparticle has a diameter of less than about 1.5 microns.
33. The method of any one of claims 30-32, wherein said substrate having said second surface area to mass ratio comprises a microparticle.
34. The method of any one of claims 30-32, wherein said substrate having said second surface area to mass ratio comprises a nanoparticle.

35. The method of any one of claims 1-29, wherein said substrate having said first surface area to mass ratio comprises a nanoparticle and said substrate having said second surface area to mass ratio comprises said microparticle.
36. The method of any one of claims 1-35, wherein said substrate having said first surface area to mass ratio and said substrate having said second surface area to mass ratio substrate are particles having diameters differing from each other by at most 10%.
37. The method of any one of claims 1-36, wherein said biomolecule corona comprises at most 0.1% of the biological mass of said biological sample.
38. The method of any one of claims 1-37, wherein said biomolecule corona comprises at most 0.01% of the biological mass of said biological sample.
39. The method of any one of claims 1-38, wherein said biomolecule corona comprises at most 0.001% of the biological mass of said biological sample.
40. The method of any one of claims 1-39, wherein said biomolecule corona comprises at most 0.0001% of the biological mass of said biological sample.
41. The method of any one of claims 1-40, wherein the number of different biomolecules identified is at least 5% higher than the number of different biomolecules identified when said biological sample is contacted with said substrate having said second surface area to mass ratio.
42. The method of any one of claims 1-41, wherein the number of different biomolecules identified is at least 10% higher than the number of different biomolecules identified when said biological sample is contacted with said substrate having said second surface area to mass ratio.
43. The method of any one of claims 1-42, wherein the number of different biomolecules identified is at least 25% higher than the number of different biomolecules identified when said biological sample is contacted with said substrate having said second surface area to mass ratio.
44. The method of any one of claims 1-43, wherein said substrate having said first surface area to mass ratio forms a colloid upon said contacting with said biological sample.
45. The method of any one of claims 1-44, wherein said substrate having said second surface area to mass ratio does not form a colloid upon being contacted with said biological sample.
46. The method of any one of claims 1-45, wherein said contacting of (a) is conducted for less than one hour.
47. The method of any one of claims 1-46, wherein said contacting of (a) is conducted for less than 30 minutes.
48. The method of any one of claims 1-47, wherein said assaying of (b) is performed prior to said biomolecule corona achieving equilibrium.

49. The method of any one of claims 1-48, wherein the composition of said biomolecule corona subjected to said assaying of (b) and the composition of said biomolecule corona subsequent to said biomolecule corona achieving said equilibrium share at most 85% of proteins in common.
50. The method of any one of claims 1-49, wherein said substrate comprises a particle selected from the group consisting of a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.
51. The method of any one of claims 1-50, wherein said substrate comprises at least two particles selected from the group consisting of a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.
52. The method of any one of claims 1-51, wherein said substrate comprises at least three particles selected from the group consisting of a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.
53. The method of any one of claims 1-52, wherein said substrate comprises at least four particles selected from the group consisting of a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.
54. The method of any one of claims 1-53, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.

55. The method of any one of claims 1-53, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a poly(N-(3-(dimethylamino)propyl) methacrylamide) (PDMAPMA) surface.
56. The method of any one of claims 1-55, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA) surface.
57. The method of any one of claims 1-56, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface.
58. The method of any one of claims 1-57, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface.
59. The method of any one of claims 1-58, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a dextran surface.
60. The method of any one of claims 1-59, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a surface with a mixed chemistry based on amine-epoxy chemistry.
61. The method of any one of claims 1-60, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a Polyzwitterion coated (Poly(N-[3-(Dimethylamino)propyl]methacrylamide-co-[2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide, P(DMAPMA-co-SBMA)) surface.
62. The method of any one of claims 1-61, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising styrene surface comprising an oleic acid functionalization.
63. The method of any one of claims 1-62, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a boronated styrene surface.
64. The method of any one of claims 1-63, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a carboxylated styrene surface.
65. The method of any one of claims 1-64, wherein said substrate comprises a superparamagnetic iron oxide microparticle (SPION) comprising a carboxylated styrene surface.
66. The method of any one of claims 1-65, wherein said substrate comprises a superparamagnetic iron oxide microparticle (SPION) comprising a strongly acidic silica surface.
67. The method of any one of claims 1-66, wherein said substrate comprises at least one particle from the group consisting of a silica-coated SPION, a

poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.

68. The method of any one of claims 1-67, wherein said substrate comprises at least two particles from the group consisting of a silica-coated SPION, a

poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.

69. The method of any one of claims 1-68, wherein said substrate comprises at least three particles from the group consisting of a silica-coated SPION, a

poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.

70. The method of any one of claims 1-69, wherein said substrate comprises at least four particle from the group consisting of a silica-coated SPION, a

poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.

71. The method of any one of claims 1-70, wherein said substrate comprises a silica-coated SPION, a poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-

Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.

72. A method of assaying a biological sample using a substrate, the method comprising:

- (a) contacting said biological sample with said substrate to form thereon a biomolecule corona which comprises biomolecules from said biological sample, wherein said substrate has a surface area to mass ratio of from 1 to 6000 cm²/mg; and
- (b) assaying said biomolecule corona to identify said biomolecules, wherein the number of different biomolecules identified is higher than the number of different biomolecules identified when said biological sample is assayed with an amount of said substrate that is 10% or more greater than said amount of said substrate used in (a).

73. The method of claim 72, wherein said biomolecule corona comprises at least 1 micrograms (μg) biomolecules per milligram (mg) substrate.

74. The method of claim 72 or 73, wherein said biomolecule corona comprises at most 1 micrograms (μg) biomolecules per milligram (mg) substrate.
75. The method of any one of claims 72-74, wherein said biomolecule corona comprises at least 1 μg biomolecules per 100 square centimeter (cm^2) substrate.
76. The method of any one of claims 72-75, wherein said biomolecule corona comprises at most 1 microgram (μg) biomolecules per 100 square centimeter (cm^2) substrate.
77. The method of any one of claims 72-76, wherein said substrate comprises a nanoparticle.
78. The method of claim 77, wherein said nanoparticle has a diameter of at least 50 nm.
79. The method of claim 77 or 78, wherein said nanoparticle has a diameter of at most 500 nm.
80. The method of any one of claims 77-79, wherein said nanoparticle has a diameter of about 80 nm to about 500 nm.
81. The method of any one of claims 77-80, wherein said nanoparticle comprises a diameter of about 120 nm to about 350 nm.
82. The method of any one of claims 77-81, wherein said nanoparticle has a polydispersity index of at most 1.
83. The method of any one of claims 77-82, wherein said nanoparticle has an oblong geometry.
84. The method of any one of claims 77-83-, wherein said nanoparticle has a substantially spherical geometry.
85. The method of any one of claims 77-84, wherein said nanoparticle is zwitterionic.
86. The method of claim 85, wherein said nanoparticle comprises an amine functionalization and a sulfuryl or organosulfur functionalization.
87. The method of either of claims 85 or 86, wherein said zwitterionic nanoparticle is zwitterionic over a pH range of at least 4.
88. The method of any one of claims 77-87, wherein said nanoparticle comprises a core material and a shell material.
89. The method of claim 88, wherein said core material comprises a metal, an oxide, a nitride, a ceramic, a carbon material, a silicon material, a polymer, or any combination thereof.
90. The method of either of claims 88 or 89, wherein said core material comprises silica.
91. The method of any one of claims 88-90, wherein said core material comprises a metal or a metal oxide.
92. The method of claim 91, wherein said core material comprises iron oxide.
93. The method of any one of claims 88-92, wherein said core material is magnetic.

94. The method of claim 93, wherein said core material is superparamagnetic.
95. The method of any one of claims 88-94, wherein said shell material has a thickness that is less than about 10 nm.
96. The method of any one of claims 88-95, wherein said shell material has a thickness that is greater than about 10 nm.
97. The method of any one of claims 88-96, wherein said shell material comprises a polymer, a saccharide, a lipid, a peptide, a self-assembled monolayer, a silicon material, a sol-gel, a hydrogel, a glass, or any combination thereof.
98. The method of claim 97, wherein said shell material comprises dextran.
99. The method of claim 97 or 98, wherein said shell material comprises polystyrene, N-(3-(Dimethylamino)propyl)methacrylamide (DMAPMA), or a combination thereof.
100. The method of claim 99, wherein said polystyrene or said DMAPMA is functionalized.
101. The method of any one of claims 88-100, wherein said shell material comprises two materials with a degree of phase separation.
102. The method of any one of claims 88-101, wherein said core material has a greater density than said shell material.
103. The method of any one of claims 72-102, wherein said substrate comprises a surface functionalization.
104. The method of claim 103, wherein said surface functionalization comprises a polar functional group, an acidic functional group, a basic functional group, a charged functional group, a polymerizable functional group, or any combination thereof.
105. The method of either of claims 103 or 104, wherein said surface functionalization comprises an aminopropyl functionalization, an amine functionalization, a boronic acid functionalization, a carboxylic acid functionalization, a methyl functionalization, an N-succinimidyl ester functionalization, a PEG functionalization, a streptavidin functionalization, a methyl ether functionalization, a triethoxylpropylaminosilane functionalization, a thiol functionalization, a PCP functionalization, a citrate functionalization, a lipoic acid functionalization, a BPEI functionalization, carboxyl functionalization, a hydroxyl functionalization, or any combination thereof.
106. The method of any one of claims 103-105, wherein said surface functionalization has an average density of at least about 20 functional groups /cm² on a surface of said substrate.
107. The method of any one of claims 103-106, wherein said surface functionalization has an average density of about 30 functional groups / cm² to about 60 functional groups / cm².

108. The method of any one of claims 72-107, wherein said substrate comprises a positively charged particle, a neutral particle, a negatively charged particle, or a combination thereof.
109. The method of any one of claims 72-108, wherein said substrate comprises a silica particle, an amine functionalized particle, a polyethylene glycol-functionalized particle, or a combination thereof.
110. The method of any one of claims 72-109, wherein said substrate comprises a carboxylate functionalized particle.
111. The method of any one of claims 72-110, wherein said substrate comprises a carboxylate functionalized styrene particle.
112. The method of any one of claims 72-111, wherein said substrate comprises a dextran-coated particle.
113. The method of any one of claims 72-112, wherein said substrate comprises a sulfuryl functionalized particle.
114. The method of claim 113, wherein said sulfuryl functionalized particle further comprises a positively charged surface functionalization.
115. The method of any one of claims 72-114, wherein said substrate comprises a microparticle.
116. The method of claim 115, wherein said microparticle has a diameter of less than about 5 microns.
117. The method of claim 115 or 116, wherein said microparticle has a diameter of less than about 2 microns.
118. The method of any one of claims 115-117, wherein said microparticle has a diameter of about 1 micron to about 2 microns.
119. The method of any one of claims 72-118, wherein said substrate comprises a plurality of particles with different densities.
120. The method of any one of claims 72-119, wherein said substrate has a density of at least about 0.01 gram per cubic centimeter (g/cm^3).
121. The method of any one of claims 72-120, wherein said substrate has a density of less than about 15 g/cm^3 .
122. The method of any one of claims 72-121, wherein said substrate has a density of between about 0.05 gram per cubic centimeter (g/cm^3) and about 10 g/cm^3 .
123. The method of any one of claim 72-122, wherein said substrate has a density of between about 0.8 gram per cubic centimeter (g/cm^3) and about 3 g/cm^3 .

124. The method of any one of claims 72-123, wherein said biomolecules identified in (b) span at least 1 order of magnitude greater in concentration than biomolecules identified when said biological sample is assayed with an amount of said substrate that is 10% or more greater than said amount of said substrate used in (a).
125. The method of any one of claims 72-124, wherein said biological sample comprises plasma, and wherein said identified biomolecules comprise a lower proportion of albumin and globulins than biomolecules identified when said biological sample is assayed with an amount of said substrate that is 10% or more greater than said amount of said substrate used in (a).
126. The method of any one of claims 72-125, wherein said assaying of (b) comprises digesting said biomolecules.
127. The method of claim 126, wherein said assaying of (b) further comprises desorbing said biomolecules from said substrate subsequent to said digesting.
128. The method of any one of claims 72-127, wherein said assaying of (b) comprises identifying a post-translational modification of said biomolecules.
129. The method of claim 128, wherein said post-translational modification comprises cleavage, N-terminal extension, glycosylation, iodination, acetylation, degradation, acylation, biotinylation, amidation, alkylation, methylation, terminal amino acid cyclization, adenylation, ADP-ribosylation, sulfonation, prenylation, hydroxylation, decarboxylation, glutamylation, glycosylation, isoprenylation, lipoylation, phosphorylation, sulfurylation, or any combination thereof.
130. The method of any one of claims 72-129, wherein said substrate comprises a particle selected from the group consisting of a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.
131. The method of any one of claims 72-130, wherein said substrate comprises at least two particles selected from the group consisting of a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.
132. The method of any one of claims 72-131, wherein said substrate comprises at least three particles selected from the group consisting of a superparamagnetic iron oxide particle (SPION)

comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.

133. The method of any one of claims 72-132, wherein said substrate comprises at least four particles selected from the group consisting of a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.

134. The method of any one of claims 72-133, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.

135. The method of any one of claims 72-134, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a poly(N-(3-(dimethylamino)propyl) methacrylamide) (PDMAEMA) surface.

136. The method of any one of claims 72-135, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA) surface.

137. The method of any one of claims 72-136, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface.

138. The method of any one of claims 72-137, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface.

139. The method of any one of claims 72-138, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a dextran surface.

140. The method of any one of claims 72-139, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a surface with a mixed chemistry based on amine-epoxy chemistry.

141. The method of any one of claims 72-140, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a Polyzwitterion coated (Poly(N-[3-

(Dimethylamino)propyl]methacrylamide-co-[2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide, P(DMAPMA-co-SBMA)) surface.

142. The method of any one of claims 72-141, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising styrene surface comprising an oleic acid functionalization.

143. The method of any one of claims 72-142, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a boronated styrene surface.

144. The method of any one of claims 72-143, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a carboxylated styrene surface.

145. The method of any one of claims 72-144, wherein said substrate comprises a superparamagnetic iron oxide microparticle (SPION) comprising a carboxylated styrene surface.

146. The method of any one of claims 72-145, wherein said substrate comprises a superparamagnetic iron oxide microparticle (SPION) comprising a strongly acidic silica surface.

147. The method of any one of claims 72-146, wherein said substrate comprises at least one particle from the group consisting of a silica-coated SPION, a poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.

148. The method of any one of claims 72-147, wherein said substrate comprises at least two particles from the group consisting of a silica-coated SPION, a poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.

149. The method of any one of claims 72-148, wherein said substrate comprises at least three particles from the group consisting of a silica-coated SPION, a poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.

150. The method of any one of claims 72-149, wherein said substrate comprises at least four particle from the group consisting of a silica-coated SPION, a poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.

151. The method of any one of claims 72-150, wherein said substrate comprises a silica-coated SPION, a poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.
152. A method of identifying biomolecules in a biological sample, comprising:
- (a) contacting a first portion of said biological sample with a first concentration of a particle, thereby generating a first biomolecule corona;
 - (b) contacting a second portion of said biological sample with a second concentration of said particle, thereby generating a second biomolecule corona, said second concentration being different than said first concentration; and
 - (c) assaying said first biomolecule corona and said second biomolecule corona to identify biomolecules or biomolecule groups comprised therein, wherein the number of biomolecules or biomolecule groups comprises in said first biomolecule corona differs from the number of biomolecules or biomolecule groups comprised in said second biomolecule corona by at least 10%.
153. The method of claim 152, wherein said contacting of (a) and said contacting of (b) comprise the same conditions.
154. The method of either of claims 152 or 153, wherein said first concentration of said particle and said second concentration of said particle differ by at most 1 order of magnitude.
155. The method of either of claims 152 or 153, wherein said first concentration of said particle and said second concentration of said particle differ by at least 1 order of magnitude.
156. The method of any one of claims 152-155, wherein said first concentration of said particle and said second concentration of said particle are between 100 nanogram/milliliter (ng/mL) and 100 milligram/milliliter (mg/mL).
157. The method of any one of claims 152-156, wherein said particle comprises a plurality of particles.
158. The method of claim 157, wherein particles of said plurality of particles differ from one another by at least 1 physicochemical property.
159. The method of any one of claims 152-158, wherein said assaying of (c) comprises identifying a thermodynamic parameter for binding of a biomolecule or biomolecule group from said first biomolecule corona or said second biomolecule corona.
160. The method of any one of claims 152-159, wherein said particle contacted to said first portion of said biological sample and said particle contacted to said second portion of said

biological sample comprise substantially similar zeta potentials following formation of said first and said second biomolecule coronas.

161. The method of any one of claims 152-160, wherein said first concentration is greater than said second concentration.

162. The method of any one of claims 152-161, wherein the ratio of albumin to non-albumin biomolecules in said first biomolecule corona and said second biomolecule corona differ by at least 20%.

163. The method of any one of claims 152-162, wherein the ratio of sub-microgram per milliliter biomolecules from said biological sample in the first biomolecule corona and said second biomolecule corona differs by at least 20%.

164. The method of any one of claims 152-163, wherein 100 or more biomolecules or biomolecule groups are identified in (c).

165. The method of any one of claims 152-164, wherein about 100 to about 1200 biomolecules or biomolecule groups are identified in (c).

166. The method of any one of claims 152-165, wherein about 300 to about 600 biomolecules or biomolecule groups are identified in (c).

167. The method of any one of claims 152-166, wherein at most about 100 biomolecules or biomolecule groups are identified in (c).

168. The method of any one of claims 152-167, wherein at most about 50 biomolecules or biomolecule groups are identified in (c).

169. The method of either of claims 167 or 168, wherein a median concentration of said at most about 100 biomolecules or biomolecule groups in said biological sample is at most 1 $\mu\text{g/mL}$.

170. The method of any one of claims 152-169, wherein said assaying of (c) generates a greater average number of signals per identified biomolecule than assaying either said first biomolecule corona or said second biomolecule corona alone.

171. The method of any one of claims 152-170, wherein said assaying of (c) comprises identifying a biomolecule or a biomolecule group which is not identifiable from assaying said first biomolecule corona or said second biomolecule corona alone.

172. The method of any one of claims 152-171, wherein a dynamic range of said biomolecules or biomolecule groups identified in (c) is at least 1 greater than dynamic ranges of the biomolecules or biomolecule groups in both said first biomolecule corona and said second biomolecule corona.

173. The method of any one of claims 152-172, wherein said substrate comprises a particle selected from the group consisting of a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.

174. The method of any one of claims 152-173, wherein said substrate comprises at least two particles selected from the group consisting of a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.

175. The method of any one of claims 152-174, wherein said substrate comprises at least three particles selected from the group consisting of a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.

176. The method of any one of claims 152-175, wherein said substrate comprises at least four particles selected from the group consisting of a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.

177. The method of any one of claims 152-176, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a silica surface, a SPION comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface, a SPION comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface, a SPION comprising a carboxyl functionalized polystyrene surface, and a SPION comprising a dextran coating.

178. The method of any one of claims 152-177, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a poly(N-(3-(dimethylamino)propyl) methacrylamide) (PDMAEMA) surface.

179. The method of any one of claims 152-178, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA) surface.
180. The method of any one of claims 152-179, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising an N-(3-Trimethoxysilylpropyl)diethylenetriamine surface.
181. The method of any one of claims 152-180, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a Poly(dimethyl aminopropyl methacrylamide) (Dimethylamine) surface.
182. The method of any one of claims 152-181, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a dextran surface.
183. The method of any one of claims 152-182, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a surface with a mixed chemistry based on amine-epoxy chemistry.
184. The method of any one of claims 152-183, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a Polyzwitterion coated (Poly(N-[3-(Dimethylamino)propyl]methacrylamide-co-[2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide, P(DMAPMA-co-SBMA)) surface.
185. The method of any one of claims 152-184, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising styrene surface comprising an oleic acid functionalization.
186. The method of any one of claims 152-185, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a boronated styrene surface.
187. The method of any one of claims 152-186, wherein said substrate comprises a superparamagnetic iron oxide particle (SPION) comprising a carboxylated styrene surface.
188. The method of any one of claims 152-187, wherein said substrate comprises a superparamagnetic iron oxide microparticle (SPION) comprising a carboxylated styrene surface.
189. The method of any one of claims 152-188, wherein said substrate comprises a superparamagnetic iron oxide microparticle (SPION) comprising a strongly acidic silica surface.
190. The method of any one of claims 152-189, wherein said substrate comprises at least one particle from the group consisting of a silica-coated SPION, a poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.

191. The method of any one of claims 152-190, wherein said substrate comprises at least two particles from the group consisting of a silica-coated SPION, a poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.
192. The method of any one of claims 152-191, wherein said substrate comprises at least three particles from the group consisting of a silica-coated SPION, a poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.
193. The method of any one of claims 152-192, wherein said substrate comprises at least four particle from the group consisting of a silica-coated SPION, a poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.
194. The method of any one of claims 152-193, wherein said substrate comprises a silica-coated SPION, a poly(dimethylaminopropylmethacrylamide)-coated SPION, an N-(3-Trimethoxysilylpropyl)diethylenetriamine-coated SPION, a 1,6-hexanediamine-coated SPION, and an N1-(3-(trimethoxysilyl)propyl)hexane-1,6-diamine functionalized, silica-coated SPION.
195. A system comprising:
- computer memory comprising data comprising information of biomolecules or biomolecule groups corresponding to a plurality of different biomolecule coronas, wherein said plurality of different biomolecule coronas comprises a first biomolecule corona which is formed upon contacting a first portion of a biological sample with a first concentration of a particle and a second biomolecule corona which is formed upon contacting a second portion of said biological sample with a second concentration of said particle, said second concentration being different than said first concentration; and
 - a computer in communication with said computer memory, wherein said computer comprises a computer processor and computer readable medium comprising machine-executable code that, upon execution by said computer processor, implements a method comprising:
 - (i) receiving said data from said computer memory; and

- (ii) identifying at least a subset of said biomolecules or biomolecule groups present in said first biomolecule corona and said second biomolecule corona, based on at least partially on said data.

196. The system of claim 195, wherein said data comprises mass spectrometric signals associated with said biomolecules or said biomolecule groups.

197. The system of claim 195 or 196, wherein (ii) comprises identifying a biomolecule or biomolecule group of said plurality of biomolecules or biomolecule groups present in said first biomolecule corona and not present in said second biomolecule corona.

198. The system of any one of claims 195-197, wherein (ii) comprises identifying a protein isoform of said plurality of biomolecules or biomolecule groups present in said first biomolecule corona and not present in said second biomolecule corona.

199. The system of any one of claims 195-198, wherein (ii) comprises identifying a post-translational protein of said plurality of biomolecules or biomolecule groups present in said first biomolecule corona and not present in said second biomolecule corona.

200. The system of any one of claims 195-199, wherein (ii) comprises identifying at least a 10% difference between said first biomolecule corona and said second biomolecule corona in terms of said biomolecules or biomolecule groups present in said first biomolecule corona and said second biomolecule corona.

201. The system of any one of claims 195-200, wherein said identifying comprises computationally modeling at least a portion of said data.

202. The system of claim 201, wherein said computational modeling comprises hierarchical cluster analysis (HCA), Partial least squares Discriminant Analysis (PLSDA), machine learning, logistic regression, decision tree modeling, k-nearest neighbors, naive Bayes, linear regression, polynomial regression, singular value decomposition, K-means clustering, hidden Markov modeling, or any combination thereof.

203. The system of any one of claims 195-202, wherein said identifying comprises comparing said data against reference data.

204. The system of any one of claims 195-203, wherein said data are transmitted to the computer memory over a communication network.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of assaying a biological sample using a substrate, the method comprising:
 - (a) contacting the biological sample with the substrate to form thereon a biomolecule corona which comprises biomolecules from the biological sample, wherein the substrate has a surface area to mass ratio of from 1 to 6000 cm²/mg, and wherein the biomolecule corona comprises at most 0.1% by mass of the biomolecules in the biological sample; and
 - (b) assaying the biomolecule corona to identify the biomolecules, wherein the number of different biomolecules identified is higher than the number of different biomolecules identified when the biological sample is assayed with an amount of the substrate that is 10% or more greater than the amount of the substrate used in (a).
2. The method of claim 1, wherein the biomolecule corona comprises at most 0.01% by mass of the biomolecules in the biological sample.
3. The method of claim 1 or 2, wherein the number of different biomolecules identified is higher by at least 5%.
4. The method of any one of claims 1-3, wherein the contacting is performed for at least 15 minutes.
5. The method of claim 4, wherein the contacting is performed for at most 2 hours.
6. The method of any one of claims 1-5, wherein the surface area to mass ratio provides an equilibrium time of at least 24 hours.
7. The method of any one of claims 1-6, wherein the substrate comprises a particle.

8. The method of claim 7, wherein the particle is magnetic.
9. The method of any one of claims 1-8, wherein the biological sample comprises human serum or plasma.
10. The method of any one of claims 1-9, wherein the biomolecule corona comprises at least 1 μg biomolecules per 100 square centimeter (cm^2) substrate.
11. The method of any one of claims 1-10, wherein the assaying comprises performing mass spectrometry.
12. The method of claim 11, wherein the mass spectrometry comprise liquid chromatography tandem mass spectrometry.
13. The method of any one of claims 1-12, wherein the number of different biomolecules identified is at least 100.
14. The method of any one of claims 1-13, wherein the biomolecules comprise proteins.
15. The method of any one of claims 1-14, wherein the biomolecules are identified over a dynamic range of at least 6 magnitudes.
16. The method of any one of claims 1-15, wherein the assaying is performed prior to the biomolecule corona achieving equilibrium.
17. The method of claim 16, wherein the biomolecule corona comprises at most 85% of proteins in common in composition with the biomolecule corona achieving equilibrium.

18. The method of any one of claims 1-17, wherein the biomolecule corona comprises a protein isoform not present in a biomolecule corona formed with a surface area to mass ratio that is different from the surface area to mass ratio in (a).
19. The method of any one of claims 1-18, wherein the biomolecule corona comprises a protein with a post-translational modification not present in a biomolecule corona formed with a surface area to mass ratio that is different from the surface area to mass ratio in (a).
20. The method of any one of claims 1-19, wherein the substrate comprises a carboxyl functionalization.