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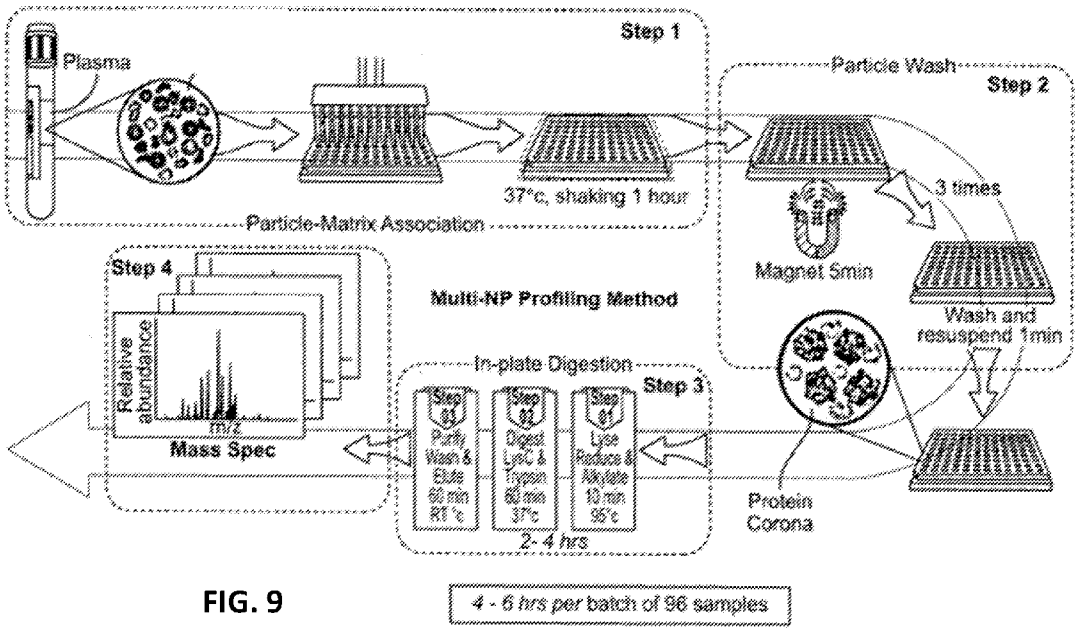

FIG. 9

(57) Abstract: Systems and methods for automated sample preparation and processing of protein corona are described herein, as well as its application in the discovery of advanced diagnostic tools as well as therapeutic agents. In some aspects, the present disclosure provides an automated apparatus for generating a subset of biomolecules from a complex biological sample, the automated apparatus comprising: (i) a substrate comprising a plurality of partitions, wherein the plurality of partitions comprises a plurality of particles; (II) a sample storage unit comprising the complex biological sample; and (iii) a loading unit that is movable at least across the substrate.

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SYSTEMS AND METHODS FOR SAMPLE PREPARATION, DATA GENERATION, AND PROTEIN CORONA ANALYSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to and benefit from U.S. Provisional Application No. 62/883,107 filed August 5, 2019, the entire contents of which is herein incorporated by reference.

BACKGROUND

[0002] Broad scale implementation of proteomic information in science and medicine has lagged behind genomics in large part because of complexities inherent in protein molecules themselves, necessitating complex workflows that limit the scalability of such analyses. Disclosed herein are systems, methods and kits for rapid and automated sample preparation, processing of proteomic data and the identification of key biomarkers associated with diseased states.

SUMMARY

[0003] The present disclosure provides automated systems, methods and kits for protein corona preparation and analysis. In some aspects, the present disclosure provides an automated apparatus for generating a subset of biomolecules from a complex biological sample, the automated apparatus comprising: (i) a substrate comprising a plurality of partitions, wherein the plurality of partitions comprises a plurality of particles; (ii) a sample storage unit comprising the complex biological sample; and (iii) a loading unit that is movable at least across the substrate, wherein the loading unit transfers one or more volumes of the complex biological sample in the sample storage unit to the plurality of partitions on the substrate, thereby contacting the plurality of particles in the plurality of partitions with biomolecules of the complex biological sample to form biomolecule coronas, thereby generating the subset of biomolecules of the complex biological sample, and wherein a dynamic range of the subset of biomolecules is compressed relative to a dynamic range of biomolecules present in the complex biological sample. In some embodiments, the substrate is a multi-well plate. In some embodiments, the subset of biomolecules comprises at least 20% to at least 60% of the types of biomolecules from the complex biological sample within a 6 order of magnitude concentration range. In some embodiments, the subset of biomolecules comprises at least 20% to at least 60% of the types of proteins from the complex biological sample within a 6 order of magnitude concentration range. In some embodiments, the automated apparatus generates the subset of biomolecules from a complex biological sample in less than 7 hours.

[0004] In some embodiments, the automated apparatus comprises an incubation element that agitates or heats volumes of the plurality of particles within volumes of the complex biological sample in the plurality of partitions. In some embodiments, the incubation element is configured to shake, mix, stir, spin, vibrate, be static, or any combination thereof. In some embodiments, the wherein the incubation element is configured to heat and/or incubate the substrate to a temperature between about 20 °C and about 100 °C.

[0005] In some embodiments, the plurality of partitions is at least partially covered or sealed. In some embodiments, a partition from among the plurality of partitions is covered or sealed. In some embodiments, the automated apparatus comprises the ability to add or remove a lid on the substrate, wherein the lid covers at least one of the partitions from among the plurality of partitions.

[0006] In some embodiments, the automated apparatus comprises a unit comprising a resuspension solution. In some embodiments, the resuspension solution comprises Tris EDTA 150mM KCl 0.05% CHAPS buffer. In some embodiments, the resuspension solution comprises 10 mM Tris HCl pH 7.4, 1 mM EDTA.

[0007] In some embodiments, the apparatus comprises a unit comprising a denaturing solution. In some embodiments, the denaturing solution comprises a protease. In some embodiments, the denaturing solution comprises a reductant, a methylating agent, guanidine, urea, sodium deoxycholate, acetonitrile, or any combination thereof. In some embodiments, the denaturing solution generates an average peptide fragment with a mass of less than 4600 Daltons.

[0008] In some embodiments, the loading unit comprises a plurality of pipettes. In some embodiments, the loading unit is configured to dispense 10 uL to 400 uL of a solution into one or more partitions of the plurality of partitions. In some embodiments, the loading unit is configured to dispense 5 uL to 150 uL of a solution into one or more partitions of the plurality of partitions. In some embodiments, the loading unit is configured to dispense 35 uL to 80 uL of a solution into one or more partitions of the plurality of partitions. In some embodiments, the solution is selected from the group consisting of a wash solution, the resuspension solution, the denaturing solution, a buffer and a reagent. In some embodiments, the loading unit is configured to dispense 10 uL to 400 uL of the complex biological sample into one or more partitions of the plurality of partitions. In some embodiments, the loading unit is configured to dispense 5 uL to 150 uL of the complex biological sample into one or more partitions of the plurality of partitions. In some embodiments, the loading unit is configured to dispense 35 uL to 80 uL of the complex biological sample into one or more partitions of the plurality of partitions.

[0009] In some embodiments, the complex biological sample comprises a biofluid from a subject. In some embodiments, the complex biological sample comprises plasma, serum, urine, cerebrospinal fluid, synovial fluid, tears, saliva, whole blood, milk, nipple aspirate, ductal lavage, vaginal fluid, nasal fluid, ear fluid, gastric fluid, pancreatic fluid, trabecular fluid, lung lavage, sweat, crevicular fluid, semen, prostatic fluid, sputum, fecal matter, bronchial lavage, fluid from swabbings, bronchial aspirants, fluidized solids, fine needle aspiration samples, tissue homogenates, lymphatic fluid. cell culture samples, or any combination thereof.

[0010] In some embodiments, the automated apparatus further comprises a magnet. In some embodiments, one or more particles of the plurality of particles is a magnetic particle, and the substrate and the magnet are in proximity such that the one or more magnetic particles are immobilized on the substrate

[0011] In some embodiments, the automated apparatus further comprises a housing, the substrate and the loading unit are located in the housing, and the housing is at least partially enclosed.

[0012] In some embodiments, the compressed dynamic range comprises an increase in the number of types of biomolecules whose concentrations are within 6 orders of magnitude of the most abundant biomolecule in the sample. In some embodiments, the compressed dynamic range comprises an increase in the number of types of biomolecules whose concentrations are within 5 orders of magnitude of the most abundant biomolecule in the sample. In some embodiments, the compressed dynamic range comprises an increase in the number of types of biomolecules whose concentrations are within 4 orders of magnitude of the most abundant biomolecule in the sample. In some embodiments, the compressed dynamic range comprises an increase in the number of types of proteins whose concentrations are within 6 orders of magnitude of the most abundant protein in the sample. In some embodiments, the increase in the number of types of biomolecules whose concentrations are within 6 orders of magnitude of the most concentrated biomolecule in the sample is at least 25%, 50%, 100%, 200%, 300%, 500%, or 1000%. In some embodiments, the compressed dynamic range comprises an increase in the number of types of proteins whose concentrations are within 6 orders of magnitude of the most abundant protein in the sample. In some embodiments, the increase in the number of types of proteins whose concentrations are within 6 orders of magnitude of the most abundant protein in the sample is at least 25%, 50%, 100%, 200%, 300%, 500%, or 1000%.

[0013] In some embodiments, the dynamic range of the biomolecules of the biomolecule coronas is a first ratio of a top decile of biomolecules to a bottom decile of biomolecules in the plurality of biomolecule coronas. In some embodiments, the dynamic range of the biomolecules of the

biomolecule coronas is a first ratio comprising a span of the interquartile range of biomolecules in the plurality biomolecule coronas.

[0014] In some embodiments, the generating enriches low abundance biomolecules from the complex biological sample. In some embodiments, the low abundance biomolecules are biomolecules at concentrations of 10ng/mL or less in the complex biological sample. In some embodiments, the subset of biomolecules from the complex biological sample comprises proteins.

[0015] In some embodiments, changes of at most 10 mg/mL in the lipid concentration of the complex biological sample result in changes of less than 10%, 5%, 2%, or 1% in the composition of the proteins in the subset of biomolecules generated from the complex biological sample.

[0016] In some embodiments, at least two particles from among the plurality of particles differ in at least one physicochemical property. In some embodiments, the at least one physicochemical property is selected from the group consisting of: composition, size, surface charge, hydrophobicity, hydrophilicity, surface functionality, surface topography, surface curvature, porosity, core material, shell material, shape, and any combination thereof. In some embodiments, the surface functionality comprises aminopropyl functionalization, amine functionalization, boronic acid functionalization, carboxylic acid functionalization, methyl functionalization, N-succinimidyl ester functionalization, PEG functionalization, streptavidin functionalization, methyl ether functionalization, triethoxylpropylaminosilane functionalization, thiol functionalization, PCP functionalization, citrate functionalization, lipoic acid functionalization, BPEI functionalization. In some embodiments, a particle from among the plurality of particles is selected from the group consisting of: micelles, liposomes, iron oxide particles, silver particles, gold particles, palladium particles, quantum dots, platinum particles, titanium particles, silica particles, metal or inorganic oxide particles, synthetic polymer particles, copolymer particles, terpolymer particles, polymeric particles with metal cores, polymeric particles with metal oxide cores, polystyrene sulfonate particles, polyethylene oxide particles, polyoxyethylene glycol particles, polyethylene imine particles, polylactic acid particles, polycaprolactone particles, polyglycolic acid particles, poly(lactide-co-glycolide polymer particles, cellulose ether polymer particles, polyvinylpyrrolidone particles, polyvinyl acetate particles, polyvinylpyrrolidone-vinyl acetate copolymer particles, polyvinyl alcohol particles, acrylate particles, polyacrylic acid particles, crotonic acid copolymer particles, polyethlene phosphonate particles, polyalkylene particles, carboxy vinyl polymer particles, sodium alginate particles, carrageenan particles, xanthan gum particles, gum acacia particles, Arabic gum particles, guar gum particles, pullulan particles, agar particles, chitin particles, chitosan particles,

pectin particles, karaya tum particles, locust bean gum particles, maltodextrin particles, amylose particles, corn starch particles, potato starch particles, rice starch particles, tapioca starch particles, pea starch particles, sweet potato starch particles, barley starch particles, wheat starch particles, hydroxypropylated high amylose starch particles, dextrin particles, levan particles, elsinan particles, gluten particles, collagen particles, whey protein isolate particles, casein particles, milk protein particles, soy protein particles, keratin particles, polyethylene particles, polycarbonate particles, polyanhydride particles, polyhydroxyacid particles, polypropylfumerate particles, polycaprolactone particles, polyamine particles, polyacetal particles, polyether particles, polyester particles, poly(orthoester) particles, polycyanoacrylate particles, , polyurethane particles, polyphosphazene particles, polyacrylate particles, polymethacrylate particles, polycyanoacrylate particles, polyurea particles, polyamine particles, polystyrene particles, poly(lysine) particles, chitosan particles, dextran particles, poly(acrylamide) particles, derivatized poly(acrylamide) particles, gelatin particles, starch particles, chitosan particles, dextran particles, gelatin particles, starch particles, poly-β-amino-ester particles, poly(amido amine) particles, poly lactic-co-glycolic acid particles, polyanhydride particles, bioreducible polymer particles, and 2-(3-aminopropylamino)ethanol particles, and any combination thereof. In some embodiments, one or more particles of the plurality of particles adsorbs at least 100 types of proteins upon contacting the complex biological sample. In some embodiments, the plurality of particles comprises at least 2 distinct particle types, at least 3 distinct particle types, at least 4 distinct particle types, at least 5 distinct particle types, at least 6 distinct particle types, at least 7 distinct particle types, at least 8 distinct particle types, at least 9 distinct particle types, at least 10 distinct particle types, at least 11 distinct particle types, at least 12 distinct particle types, at least 13 distinct particle types, at least 14 distinct particle types, at least 15 distinct particle types, at least 20 distinct particle types, at least 25 particle types, or at least 30 distinct particle types.

[0017] In some embodiments, biomolecules of the biomolecule coronas comprise a number of protein groups. In some embodiments, the number of protein groups comprises from 1 to 20,000 protein groups. In some embodiments, the number of protein groups comprises from 100 to 10,000 protein groups. In some embodiments, the number of protein groups comprises from 100 to 5000 protein groups. In some embodiments, the number of proteins groups comprises from 300 to 2,200 protein groups. In some embodiments, the number of proteins groups comprises from 1,200 to 2,200 protein groups.

[0018] In some embodiments, at least two partitions of the plurality of partitions comprise different buffers. In some embodiments, the different buffers differ in pH, salinity, osmolarity,

viscosity, dielectric constant, or any combination thereof. In some embodiments, at least two partitions of the plurality of partitions comprise different ratios of buffer and the complex biological sample. In some embodiments, one or more partitions of the plurality of partitions comprises 1 pM to 100 nM nanoparticles. In some embodiments, at least two partitions of the plurality of partitions comprise different concentrations of nanoparticles.

[0019] In some embodiments, the automated apparatus further comprises a purification unit. In some embodiments, the purification unit comprises a solid phase extraction (SPE) plate.

[0020] Various aspects of the present disclosure provide an automated system comprising: (i) an automated apparatus configured to isolate the subset of biomolecules from the biological sample; (ii) a mass spectrometer configured to receive the subset of biomolecules and to generate data comprising mass spectrometric or tandem mass spectrometric signals; and (iii) a computer comprising one or more computer processors and a computer readable medium comprising machine-executable code that, upon execution by the one or more computer processors, implements a method comprising: generating a biomolecule fingerprint and assigning a biological state based on the biomolecule fingerprint.

[0021] In some embodiments, the biomolecule fingerprint comprises a plurality of distinct biomolecule corona signatures. In some embodiments, the biomolecule fingerprint comprises at least 5, 10, 20, 40, or 80, 150 or 200 distinct biomolecule corona signatures. In some embodiments, the computer is configured to process the data comprising the intensity, APEX, spectral count or number of peptides, or Ion mobility behavior of the mass spectrometric or tandem mass spectrometric signal between a plurality of the distinct biomolecule corona signatures. In some embodiments, the computer is configured to process data from between 100 and 2000 mass spectrometric or tandem mass spectrometric signals between a plurality of the distinct biomolecule corona signatures. In some embodiments, the computer is configured to process the data comprising the intensities of between 10,000 and 5,000,000 mass spectrometric or tandem mass spectrometric signals between a plurality of the distinct biomolecule corona signatures. In some embodiments, the biomolecule fingerprint is generated from data from a single mass spectrometric or tandem mass spectrometric run. In some embodiments, the single mass spectrometric or tandem mass spectrometric run is performed in less than one hour. In some embodiments, the computer is configured to identify a biomolecule or characterize an unidentified molecular feature based on a mass spectrometric or tandem mass spectrometric signal and or ion mobility and chromatographic behavior, and wherein the computer provides a certainty threshold of at least 95% to identify a feature or characterize and unidentified feature. In some embodiments, the automated system is configured to generate the biomolecule

fingerprint from the complex biological sample in less than about 10 hours. In some embodiments, the determining comprises comparing the abundance of two biomolecules whose concentrations span at least 7 to at least 12 orders of magnitude in the complex biological sample.

[0022] In some embodiments, the computer is capable of distinguishing between two or more biological states associated with biomolecule fingerprints that differ by less than 10%, 5%, 2%, or 1%. In some embodiments, the biological state is a disease, disorder, or tissue abnormality. In some embodiments, the disease is an early phase or intermediate phase disease state. In some embodiments, the disease is cancer. In some embodiments, the cancer is a stage 0 cancer or a stage 1 cancer. In some embodiments, the cancer is selected from the group consisting of: lung cancer, pancreas cancer, myeloma, myeloid leukemia, meningioma, glioblastoma, breast cancer, esophageal squamous cell carcinoma, gastric adenocarcinoma, prostate cancer, bladder cancer, ovarian cancer, thyroid cancer, neuroendocrine cancer, colon carcinoma, ovarian cancer, head and neck cancer, Hodgkin's Disease, non-Hodgkin's lymphomas, rectum cancer, urinary cancers, uterine cancers, oral cancers, skin cancers, stomach cancer, brain tumors, liver cancer, laryngeal cancer, esophageal cancer, mammary tumors, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, Ewing's sarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystandeocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, endometrial cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioblastomas, neuronomas, craniopharingiomas, schwannomas, glioma, astrocytoma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemias and lymphomas, acute lymphocytic leukemia and acute myelocytic polycythemia vera, multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease, acute nonlymphocytic leukemias, chronic lymphocytic leukemia, chronic myelogenous leukemia, childhood-null acute lymphoid leukemia (ALL), thymic ALL, B-cell ALL, acute megakaryocytic leukemia, Burkitt's lymphoma, and T cell leukemia, small and large non-small cell lung carcinoma, acute granulocytic leukemia, germ cell tumors, endometrial cancer, gastric cancer, hairy cell leukemia, thyroid cancer and other cancers known in the art. In some embodiments, the biological state is a pre-disease state.

[0023] Various aspects of the present disclosure provide a method for distinguishing a biological state of a complex biological sample, the method comprising: providing the complex biological

sample to an automated apparatus to generate a subset of biomolecules; assaying the subset of biomolecules to generate a biomolecule fingerprint; and distinguishing a biological state of the complex biological sample with the biomolecule fingerprint.

[0024] In some embodiments, the biomolecule fingerprint comprises proteins. In some embodiments, the subset of biomolecules from the complex biological sample comprises a lower ratio of albumin to non-albumin peptides than the complex biological sample. In some embodiments, the subset of biomolecules comprises biomolecules that span at least 6 to at least 12 orders of magnitude in concentration range in the complex biological sample. In some embodiments, the subset of biomolecules comprises proteins that span at least 6 to at least 12 orders of magnitude in concentration range in the complex biological sample. In some embodiments, the biomolecule fingerprint comprises from 1 to 74,000 protein groups.

[0025] In some embodiments, the assaying comprises desorbing a plurality of biomolecules from a biomolecule corona from among the plurality of biomolecule coronas. In some embodiments, the assaying comprises chemically modifying a biomolecule from among the plurality of desorbed biomolecules. In some embodiments, the assaying comprises fragmenting a biomolecule from among the plurality of desorbed biomolecules. In some embodiments, the fragmenting comprises protease digestion. In some embodiments, the fragmenting comprises chemical peptide cleavage.

[0026] In some embodiments, the assaying comprises collecting the plurality of desorbed biomolecules. In some embodiments, the assaying comprises purifying the collected plurality of desorbed biomolecules. In some embodiments, the purifying comprises solid-phase extraction. In some embodiments, the purifying depletes non-protein biomolecules from the collected plurality of desorbed biomolecules. In some embodiments, the assaying comprises discarding the plurality of desorbed biomolecules. In some embodiments, the assaying comprises desorbing a first subset of biomolecules and a second set of biomolecules from a biomolecule corona from among the plurality of biomolecule coronas, analyzing a biomolecule from among the first subset of biomolecules, and analyzing a biomolecule from among the second subset of biomolecules.

[0027] In some embodiments, the assaying comprises analyzing a biomolecule corona from among the plurality of biomolecule coronas with mass spectrometry, tandem mass spectrometry, mass cytometry, mass cytometry, potentiometry, fluorimetry, absorbance spectroscopy, Raman spectroscopy, chromatography, electrophoresis, immunohistochemistry, PCR, next generation sequencing (NGS), or any combination thereof. In some embodiments, the assaying comprises mass spectrometry or tandem mass spectrometry. In some embodiments, the assaying comprises identifying the conformational state of a protein from among the subset of biomolecules. In some

embodiments, the assaying comprises identifying a post-translational modification on a protein from among the subset of biomolecules. In some embodiments, the distinguishing comprises comparing the relative abundances of at least 200 to at least 1000 biomolecules from the subset of biomolecules. In some embodiments, the assaying identifies biomolecules at concentrations of less than 10 ng/mL in the complex biological sample.

[0028] Various aspects of the present disclosure provide an automated apparatus for generating a subset of biomolecules from a complex biological sample, the automated apparatus comprising: a plurality of particles and the complex biological sample, wherein the automated apparatus is configured to generate the subset of biomolecules by contacting the plurality of particles with the complex biological sample to form a plurality of biomolecule coronas comprising the subset of biomolecules, and wherein a dynamic range of the subset of biomolecules is compressed relative to a dynamic range of biomolecules present in the complex biological sample. In some embodiments, the automated apparatus comprises a substrate. In some embodiments, the substrate comprises a multi-well plate. In some embodiments, the substrate is a multi-well plate. In some embodiments, the automated apparatus generates the subset of biomolecules from a complex biological sample in less than 7 hours.

[0029] In some embodiments, the automated apparatus comprises an incubation element. In some embodiments, the incubation element is configured to heat and/or incubate the plurality of particles and the complex biological sample to a temperature between 4 °C and 40 °C.

[0030] In some embodiments, the automated apparatus comprises at least one solution selected from the group consisting of a wash solution, a resuspension solution, a denaturing solution, a buffer and a reagent. In some embodiments, the resuspension solution comprises a Tris EDTA buffer, a phosphate buffer, and/or water. In some embodiments, the denaturing solution comprises a protease. In some embodiments, the denaturing solution comprises a small molecule capable of performing peptide cleavage.

[0031] In some embodiments, the automated apparatus comprises a loading unit comprising a plurality of pipettes. In some embodiments, each pipette of the plurality of pipettes is configured to dispense about 5 uL – 150 uL of the solution, the complex biological sample, and/or the plurality of particles. In some embodiments, the complex biological sample comprises plasma, serum, urine, cerebrospinal fluid, synovial fluid, tears, saliva, whole blood, milk, nipple aspirate, ductal lavage, vaginal fluid, nasal fluid, ear fluid, gastric fluid, pancreatic fluid, trabecular fluid, lung lavage, sweat, crevicular fluid, semen, prostatic fluid, sputum, fecal matter, bronchial lavage, fluid from swabbings, bronchial aspirants, fluidized solids, fine needle aspiration samples, tissue homogenates, lymphatic fluid, cell culture samples, or any combination thereof.

In some embodiments, the automated apparatus comprises a magnet. In some embodiments, the automated apparatus comprises a filter.

[0032] In some embodiments, the compressed dynamic range comprises an increase in the number of types of biomolecules whose concentrations are within 4 to 6 orders of magnitude of the most abundant biomolecule in the sample. In some embodiments, the types of biomolecules comprises protein. In some embodiments, the dynamic range of the biomolecules of the biomolecule coronas is a first ratio of a top decile of biomolecules to a bottom decile of biomolecules in the plurality of biomolecule coronas. In some embodiments, the generating enriches low abundance biomolecules from the complex biological sample. In some embodiments, the low abundance biomolecules are biomolecules at concentrations of 10ng/mL or less in the complex biological sample.

[0033] In some embodiments, at least two particles from among the plurality of particles differ in at least one physicochemical property. In some embodiments, the at least one physicochemical property is selected from the group consisting of: composition, size, surface charge, hydrophobicity, hydrophilicity, surface functionality, surface topography, surface curvature, porosity, core material, shell material, shape, and any combination thereof. In some embodiments, a particle from among the plurality of particles is selected from the group consisting of: micelles, liposomes, iron oxide particles, silver particles, gold particles, palladium particles, quantum dots, platinum particles, titanium particles, silica particles, metal or inorganic oxide particles, synthetic polymer particles, copolymer particles, terpolymer particles, polymeric particles with metal cores, polymeric particles with metal oxide cores, polystyrene sulfonate particles, polyethylene oxide particles, polyoxyethylene glycol particles, polyethylene imine particles, polylactic acid particles, polycaprolactone particles, polyglycolic acid particles, poly(lactide-co-glycolide polymer particles, cellulose ether polymer particles, polyvinylpyrrolidone particles, polyvinyl acetate particles, polyvinylpyrrolidone-vinyl acetate copolymer particles, polyvinyl alcohol particles, acrylate particles, polyacrylic acid particles, crotonic acid copolymer particles, polyethlene phosphonate particles, polyalkylene particles, carboxy vinyl polymer particles, sodium alginate particles, carrageenan particles, xanthan gum particles, gum acacia particles, Arabic gum particles, guar gum particles, pullulan particles, agar particles, chitin particles, chitosan particles, pectin particles, karaya tum particles, locust bean gum particles, maltodextrin particles, amylose particles, corn starch particles, potato starch particles, rice starch particles, tapioca starch particles, pea starch particles, sweet potato starch particles, barley starch particles, wheat starch particles, hydroxypropylated high amylose starch particles, dextrin particles, levan particles, elsinan particles, gluten particles, collagen particles,

whey protein isolate particles, casein particles, milk protein particles, soy protein particles, keratin particles, polyethylene particles, polycarbonate particles, polyanhydride particles, polyhydroxyacid particles, polypropylfumerate particles, polycaprolactone particles, polyamine particles, polyacetal particles, polyether particles, polyester particles, poly(orthoester) particles, polycyanoacrylate particles, , polyurethane particles, polyphosphazene particles, polyacrylate particles, polymethacrylate particles, polycyanoacrylate particles, polyurea particles, polyamine particles, polystyrene particles, poly(lysine) particles, chitosan particles, dextran particles, poly(acrylamide) particles, derivatized poly(acrylamide) particles, gelatin particles, starch particles, chitosan particles, dextran particles, gelatin particles, starch particles, poly-β-amino-ester particles, poly(amido amine) particles, poly lactic-co-glycolic acid particles, polyanhydride particles, bioreducible polymer particles, and 2-(3-aminopropylamino)ethanol particles, and any combination thereof.

[0034] In some embodiments, biomolecules of the biomolecule coronas comprise a number of protein groups. In some embodiments, the number of protein groups comprises from 1 to 20,000 protein groups. In some embodiments, the number of protein groups comprises from 100 to 10,000 protein groups. In some embodiments, the number of protein groups comprises from 100 to 5000 protein groups. In some embodiments, the number of proteins groups comprises from 300 to 2,200 protein groups. In some embodiments, the number of proteins groups comprises from 1,200 to 2,200 protein groups.

[0035] In some embodiments, the automated apparatus comprises a purification unit. In some embodiments, the purification unit comprises a solid phase extraction (SPE) plate.

[0036] Various aspects of the present disclosure provide a method for generating a subset of biomolecules from a complex biological sample, the method comprising: providing the complex biological sample to an automated apparatus, wherein the automated apparatus contacts the complex biological sample with a plurality of particles to generate biomolecule coronas, wherein the automated apparatus processes the biomolecule coronas to generate the subset of biomolecules, and wherein a dynamic range of the subset of biomolecules is compressed relative to a dynamic range of biomolecules present in the complex biological sample.

[0037] In some embodiments, the method comprises assaying the subset of biomolecules to generate a biomolecule fingerprint. In some embodiments, the assaying identifies biomolecules at concentrations of less than 10 ng/mL in the complex biological sample. In some embodiments, the assaying comprises analyzing biomolecule coronas with mass spectrometry, tandem mass spectrometry, mass cytometry, mass cytometry, potentiometry, fluorimetry, absorbance spectroscopy, Raman spectroscopy, chromatography, electrophoresis, immunohistochemistry, or

any combination thereof. In some embodiments, the assaying comprises mass spectrometry or tandem mass spectrometry.

[0038] In some embodiments, the method comprises distinguishing a biological state of the complex biological sample with the biomolecule fingerprint. In some embodiments, the biomolecule fingerprint comprises a plurality of distinct biomolecule corona signatures. In some embodiments, the biomolecule fingerprint comprises at least 5, 10, 20, 40, or 80, 150 or 200 distinct biomolecule corona signatures. In some embodiments, the biological state is a disease, disorder, or tissue abnormality. In some embodiments, the disease is an early phase or intermediate phase disease state. In some embodiments, the disease is cancer. In some embodiments, the cancer is a stage 0 cancer or a stage 1 cancer. In some embodiments, the cancer is selected from the group consisting of: lung cancer, pancreas cancer, myeloma, myeloid leukemia, meningioma, glioblastoma, breast cancer, esophageal squamous cell carcinoma, gastric adenocarcinoma, prostate cancer, bladder cancer, ovarian cancer, thyroid cancer, neuroendocrine cancer, colon carcinoma, ovarian cancer, head and neck cancer, Hodgkin's Disease, non-Hodgkin's lymphomas, rectum cancer, urinary cancers, uterine cancers, oral cancers, skin cancers, stomach cancer, brain tumors, liver cancer, laryngeal cancer, esophageal cancer, mammary tumors, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, Ewing's sarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystandeocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, endometrial cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioblastomas, neuronomas, craniopharingiomas, schwannomas, glioma, astrocytoma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemias and lymphomas, acute lymphocytic leukemia and acute myelocytic polycythemia vera, multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease, acute nonlymphocytic leukemias, chronic lymphocytic leukemia, chronic myelogenous leukemia, childhood-null acute lymphoid leukemia (ALL), thymic ALL, B-cell ALL, acute megakaryocytic leukemia, Burkitt's lymphoma, and T cell leukemia, small and large non-small cell lung carcinoma, acute granulocytic leukemia, germ cell tumors, endometrial cancer, gastric cancer, hairy cell leukemia, or thyroid cancer. In some embodiments, the biological state is a pre-disease state.

[0039] Various aspects of the present disclosure provide an automated apparatus to identify proteins in a biological sample, the automated apparatus comprising: a sample preparation unit; a

substrate comprising a plurality of channels; a plurality of pipettes; a plurality of solutions, a plurality of nanoparticles, and wherein the automated apparatus is configured to form a protein corona and digest the protein corona.

[0040] Various aspects of the present disclosure provide an automated apparatus to identify proteins in a biological sample, the automated apparatus comprising: a sample preparation unit; a substrate comprising a plurality of channels; a plurality of pipettes; a plurality of solutions, a plurality of nanoparticles, wherein the automated apparatus is configured to form a protein corona and digest the protein corona, and wherein at least one of the solutions is TE 150mM KCl 0.05% CHAPS buffer.

[0041] In some aspects, the sample preparation unit is configured to add the plurality of nanoparticles to the substrate with the plurality of pipettes. In some aspects, the sample preparation unit is configured to add the biological sample to the substrate with the plurality of pipettes. In some aspects, the sample preparation unit is configured to incubate the plurality of nanoparticles and the biological sample to form the protein corona. In some aspects, the sample preparation unit is configured to separate the protein corona from the supernatant to form a protein corona pellet. In some aspects, the sample preparation unit is configured to reconstitute the protein corona pellet with TE 150mM KCl 0.05% CHAPS buffer.

[0042] In some aspects, the automated apparatus comprises a magnetic source. In some aspects, the automated apparatus is configured for BCA, gel, or trypsin digestion of the protein corona. In some aspects, the automated apparatus is enclosed. In some aspects, the automated apparatus is sterilized before use. In some aspects, the automated apparatus is configured to a mass spectrometry. In some aspects, the automated apparatus is temperature controlled.

[0043] Various aspects of the present disclosure provide a method of identifying proteins in a biological sample, the method comprising: adding the biological sample to an automated apparatus; generating proteomic data from the automated apparatus; and quantifying the proteomic data. In some embodiments, the method further comprises incubating a plurality of nanoparticles with the biological sample in the automated apparatus to form a protein corona. In some embodiments, the method further comprises separating the protein corona from the supernatant in the automated apparatus. In some embodiments, the method further comprises digesting the protein corona to form the digested sample in the automated apparatus. In some embodiments, the method further comprises washing the digested sample in the automated apparatus. In some embodiments, quantifying the proteomic data comprises providing the proteomic data to a mass spectrometry. In some embodiments, the biological sample is a biofluid. In some embodiments, the biofluid is serum or plasma.

[0044] In some aspects, the present disclosure provides an automated system comprising a network of units with differentiated functions in distinguishing states of a complex biological sample using a plurality of particles having surfaces with different physicochemical properties wherein: a first unit comprises a multichannel fluid transfer instrument for transferring fluids between units within the system; a second unit comprises a support for storing a plurality of biological samples; a third unit comprises a support for a sensor array plate possessing partitions that comprise the plurality of particles having surfaces with different physicochemical properties for binding a population of analytes within the complex biological sample; a fourth unit comprises supports for storing a plurality of reagents; a fifth unit comprises supports for storing a reagent to be disposed of; a sixth unit comprises supports for storing consumables used by the multichannel fluid transfer instrument; and wherein the system is programed to perform a series of steps comprising: contacting the complex biological sample with a specified partition of the sensor array; incubating the complex biological sample with the plurality of particles contained within the partition of the sensor array plate; removing all components from a partition except the plurality of particles and a population of analytes interacting with a particle; and preparing a sample for mass spectrometry.

[0045] In some embodiments, the first unit comprises a degree of mobility that enables access to all other units within the system. In some embodiments, the first unit comprises a capacity to perform pipetting functions.

[0046] In some embodiments, the support of the second and/or third unit comprises support for a single plate, a 6 well plate, a 12 well plate, a 96 well plate, or a rack of microtubes. In some embodiments, the second and/or unit comprises a thermal unit capable of modulating the temperature of said support and a sample. In some embodiments, the second and/or third unit comprises a rotational unit capable of physically agitating and/or mixing a sample.

[0047] In some embodiments, the plurality of particles having surfaces with different physicochemical properties for binding a population of analytes within the complex biological sample are immobilized to a surface within a partition of the sensory array. In some embodiments, the plurality of particles comprises a plurality of magnetic nanoparticles with different physicochemical properties for binding a population of analytes within the complex biological sample. In some embodiments, the system comprises a step wherein the sensor array plate is transferred to an additional seventh unit that comprises a magnetized support and a thermal unit capable of modulating the temperature of said support and a sample and incubated for an additional amount of time.

[0048] In some embodiments, the fourth unit comprises a set of reagents for: generating the sensor array plate; washing an unbound sample; and/or preparing a sample for mass spectrometry. In some embodiments, contacting the biological sample with a specified partition of the sensor array comprises pipetting a specified volume of the biological sample into the specific partition of the sensor array. In some embodiments, contacting the biological sample with a specified partition of the sensor array comprises pipetting a volume corresponding to a 1:1, 1:2: 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:15, or 1:20 ratio of a plurality of particles in a solution to the biological sample.

[0049] In some embodiments, contacting the biological sample with a specified partition of the sensor array comprises pipetting a volume of at least 10 microliters, at least 50 microliters, at least 100 microliters, at least 250 microliters, at least 500 microliters, or at least 1000 microliters the biological sample into the specific partition of the sensor array.

[0050] In some embodiments, incubating the biological sample with the plurality of particles contained within the partition of the sensor array plate comprises an incubation time of at least about 10 seconds, at least about 15 seconds, at least about 20 seconds, at least about 25 seconds, at least about 30 seconds, at least about 40 seconds, at least about 50 seconds, at least about 60 seconds, at least about 90 seconds, at least about 2 minutes, at least about 3 minutes, at least about 4 minutes, at least about 5 minutes, at least about 6 minutes, at least about 7 minutes, at least about 8 minutes, at least about 9 minutes, at least about 10 minutes, at least about 15 minutes, at least about 20 minutes, at least about 25 minutes, at least about 30 minutes, at least about 45 minutes, at least about 50 minutes, at least about 60 minutes, at least about 90 minutes, at least about 2 hours, at least about 3 hours, at least about 4 hours, at least about 5 hours, at least about 6 hours, at least about 7 hours, at least about 8 hours, at least about 9 hours, at least about 10 hours, at least about 12 hours, at least about 14 hours, at least about 15 hours, at least about 16 hours, at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, or at least about 24 hours.

[0051] In some embodiments, incubating the biological sample with the plurality of particles contained within the partition of the substrate comprises an incubation temperature between about 4° C to about 40° C. Incubating the biological sample with the plurality of particles contained within the partition of the substrate may comprise an incubation temperature between about 4° C to about 37° C. Incubating the biological sample with the plurality of particles contained within the partition of the substrate may comprise an incubation temperature between about 4° C to about 100° C.

[0052] In some embodiments, removing all components from a partition except the plurality of particles and a population of analytes interacting with a particle comprises a series of wash steps.

[0053] In some embodiments, the second unit can facilitate a transfer of the sample for mass spectrometry to a mass spectrometry unit.

[0054] In some aspects, the present disclosure provides an automated apparatus to identify proteins in a biological sample, the automated apparatus comprising: a sample preparation unit; a substrate comprising a plurality of channels; a plurality of pipettes; a plurality of solutions, a plurality of nanoparticles, and wherein the automated apparatus is configured to form a protein corona and digest the protein corona.

[0055] In some aspects, the present disclosure provides an automated apparatus to identify proteins in a biological sample, the automated apparatus comprising: a sample preparation unit; a substrate comprising a plurality of channels; a plurality of pipettes; a plurality of solutions, a plurality of nanoparticles, wherein the automated apparatus is configured to form a protein corona and digest the protein corona, and wherein at least one of the solutions is TE 150mM KCl 0.05% CHAPS buffer.

[0056] In some embodiments, the sample preparation unit is configured to add the plurality of nanoparticles to the substrate with the plurality of pipettes. In some embodiments, wherein the sample preparation unit is configured to add the biological sample to the substrate with the plurality of pipettes. In some embodiments, the sample preparation unit is configured to incubate the plurality of nanoparticles and the biological sample to form the protein corona.

[0057] In some embodiments, the sample preparation unit is configured to separate the protein corona from the supernatant to form a protein corona pellet. In some embodiments, the sample preparation unit is configured to reconstitute the protein corona pellet with TE 150mM KCl 0.05% CHAPS buffer.

[0058] In some embodiments, the automated apparatus further comprises a magnetic source. In some embodiments, the automated apparatus is configured for BCA, gel, or trypsin digestion of the protein corona.

[0059] In some embodiments, the automated apparatus is enclosed. In some embodiments, the automated apparatus is sterilized before use. In some embodiments, the automated apparatus is configured to a mass spectrometry. In some embodiments, the automated apparatus is temperature controlled.

[0060] In some aspects, the present disclosure provides a method of identify proteins in a biological sample, the method comprising: adding the biological sample to the automated

apparatus disclosed herein; generating proteomic data from the automated apparatus; and quantifying the proteomic data.

[0061] In some embodiments, the method further comprises incubating a plurality of nanoparticles with the biological sample in the automated apparatus to form a protein corona. In some embodiments, the method further comprises separating the protein corona from the supernatant in the automated apparatus. In some embodiments, the method further comprises digesting the protein corona to form the digested sample in the automated apparatus.

[0062] In some embodiments, the method further comprises washing the digested sample in the automated apparatus. In some embodiments, quantifying the proteomic data comprises providing the proteomic data to a mass spectrometry.

[0063] In some embodiments, the biological sample is a biofluid. In some embodiments, the biofluid is serum or plasma.

[0064] Another aspect of the present disclosure provides a non-transitory computer readable medium comprising machine executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[0065] Another aspect of the present disclosure provides a system comprising one or more computer processors and computer memory coupled thereto. The computer memory comprises machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0066] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0067] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0068] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained

by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also "Figure" and "FIG." herein), of which:

[0069] FIG. 1 shows a schematic illustration of the steps for generating data using nanoparticle or protein corona methods.

[0070] FIG. 2 shows an example illustration of the steps for generating data using nanoparticle or protein corona methods and units of the automated system in which they can take place.

[0071] FIG. 3 shows an example layout of the system and coupling to a continuous MS for high throughput applications.

[0072] FIG. 4 shows an example illustration of sensor array analyte capture methods.

[0073] FIG. 5 shows a step-wise illustration of automated sample processing for magnetic sensor array particles.

[0074] FIG. 6 shows a step-wise illustration of automated sample processing for immobilized sensor array particles.

[0075] FIG. 7 shows surface chemistries for magnetic nanoparticle sensor arrays.

[0076] FIG 8 shows an example of protein corona-based methods for detecting disease biomarkers in a cancer patient (referring to US20180172694A1, incorporated by reference in its entirety herein).

[0077] FIG. 9 shows a process for proteomic analysis. The process is tailored for high-throughput and automation that can be run in hours and across multiple samples in parallel. The process includes particle-matrix association, particle wash (x3), formation of the protein corona, in-plate digestion, and mass spectrometry. Using the process, it may take only 4 to 6 hours per batch of 96 samples. One nanoparticle, or more, at a time may be incubated with a sample.

[0078] FIG. 10 shows the protein counts (number of proteins identified from corona analysis) collected on pluralities of particles comprising from 1 particle type to 12 particle types. Each particle from among a plurality of particles may be comprise unique materials, surface functionalization, and/or physical property (e.g., size or shape). Pooled plasma from a group of healthy subjects was used. Counts are the numbers of unique proteins collected from a plurality of particles and observed in about 2 hour mass spectrometry (MS) runs. 1318 proteins were identified from the sample contacted with a plurality of particles comprising 12 particle types.

[0079] FIG. 11 shows the distribution of the presence-filtered, cluster quality-filtered, median-normalized MS feature intensities for the 56-sample NSCLC comparative study. Each line represents the density of the $\log_2$ feature intensity for either a diseased sample or a control sample. Density is plotted from 0.00 to 0.15 on the y-axis, and $\log_2$ feature intensity is plotted

from 15 to 35 on the x-axis. At the highest peak located near a $\log_2$ feature intensity of about 28, with densities ranging from about 0.13 to about 0.17, the two highest traces correspond to control samples, while the lowest trace corresponds to a diseased sample. The remaining control and diseased traces are distributed between the highest and lowest traces. At the two shoulder peaks, occurring at about 20 $\log_2$ feature intensity and about 23 $\log_2$ feature intensity, the highest two traces are control traces and the lowest two traces are control traces at the 20 $\log_2$ feature intensity peak, and the highest traces is a diseased trace at the 23 $\log_2$ feature intensity.

[0080] FIG. 12 shows changed features in a non-small cell lung cancer (NSCLC) pilot study using poly(N-(3-(dimethylamino)propyl) methacrylamide) (PDMAPMA)-coated SPION particles. Seven MS features were identified as statistically, significantly different between 28 subjects with Stage IV NSCLC (with associated co-morbidities and treatment effects) and 28 age- and gender-matched, apparently healthy subjects. The table at bottom is a list of the seven proteins that were significantly different. This includes 5 known proteins and 2 unknown proteins. If a peptide-spectrum match was made for MS2 data associated with the feature, that peptide sequence (and charge) as well as the potential parent protein are indicated; if an MS2 match was not associated with the feature, both the peptide and the protein are marked as "Unknown".

[0081] FIG. 13 shows correlation of the maximum intensities of particle corona proteins and plasma proteins to the published concentration of the same proteins. The blue plotted lines are linear regression models to the data and the shaded regions represent the standard error of the model fit. The dynamic range of the samples assayed with particles ("S-003," "S-007," and "S-011", detailed in TABLE 1) exhibited a compressed dynamic range as compared to the plasma sample not assayed with particles ("Plasma"), as shown by the decrease in slopes of the linear fits. The slopes of each plot are 0.47, 0.19, 0.22, and 0.18 for, plasma without particles, plasma with S-003 particles, plasma with S-007 particles, and plasma with S-011 particles, respectively.

[0082] FIG. 14 shows the dynamic range compression of a protein corona analysis assay with mass spectrometry as compared to mass spectrometry without particle corona formation. Protein intensities of common proteins identified in particle corona in the plasma samples assayed in **FIG. 13** ("Nanoparticle MS ln Intensity") are plotted against the protein intensity identified by mass spectrometry of plasma without particles ("Plasma MS ln Intensity"). The lightest dotted line shows a slope of 1, indicating the dynamic range of mass spectrometry without particles. The slopes of the linear fits to the protein intensity was 0.12, 0.36, and 0.093 for S-003, S-007, and S-011 particles, respectively. The grayed area indicates the standard error region of the regression fit.

DETAILED DESCRIPTION

[0083] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

[0084] Whenever the term "at least," "greater than," or "greater than or equal to" precedes the first numerical value in a series of two or more numerical values, the term "at least," "greater than" or "greater than or equal to" applies to each of the numerical values in that series of numerical values. For example, greater than or equal to 1, 2, or 3 is equivalent to greater than or equal to 1, greater than or equal to 2, or greater than or equal to 3.

[0085] Whenever the term "no more than," "less than," or "less than or equal to" precedes the first numerical value in a series of two or more numerical values, the term "no more than," "less than," or "less than or equal to" applies to each of the numerical values in that series of numerical values. For example, less than or equal to 3, 2, or 1 is equivalent to less than or equal to 3, less than or equal to 2, or less than or equal to 1.

[0086] As used herein, a "feature" identified by mass spectrometry includes a signal at a specific combination of retention time and m/z (mass-to-charge ratio), where each feature has an associated intensity. Some features are further fragmented in a second mass spectrometry analysis (MS2) for identification.

[0087] As used herein, the term "sensor element" refers to elements that are able to bind to a plurality of biomolecules when in contact with a sample and encompasses the term "nanoscale sensor element". A sensor element may be a particle, such as a nanoparticle, or microparticle. A sensor element may be a surface or a portion of a surface. A sensor element may comprise a particle or plurality of particles. A sensor element may comprise a plurality of surfaces capable of adsorbing or binding biomolecules. A sensor element may comprise a porous material, such as a material into which biomolecules can intercalate.

[0088] As used herein, a "sensor array" may comprise a plurality of sensor elements wherein the plurality of sensor elements (e.g., particles) comprises multiple types of sensor elements. The sensor elements may be different types that differ from each other in at least one physicochemical property. A sensor array may be a substrate with a plurality of partitions containing a plurality of sensor elements (e.g., particles). For example, a sensor array may comprise a multi-well plate with a plurality of particles distributed between the plurality of wells. A sensor array may be a substrate comprising a plurality of partitions, wherein the plurality of

partitions comprises a plurality of particles. In some embodiments, each sensor element or particle is able to bind a plurality of biomolecules in a sample to produce a biomolecule corona signature. In some embodiments, each sensor element (e.g., particle type) has a distinct biomolecule corona signature.

[0089] As used herein, the term "biomolecule corona" refers to the plurality of different biomolecules that bind to a sensor element. The term "biomolecule corona" may refer to the proteins, lipids and other plasma components that bind to particles (e.g., nanoparticles) when they come into contact with biological samples or biological system. For use herein, the term "biomolecule corona" also encompasses both the soft and hard protein corona as referred to in Milani et al. "Reversible versus Irreversible Binding of Transferring to Polystyrene Nanoparticles: Soft and Hard Corona" ACS NANO, 2012, 6(3), pp. 2532-2541; Mirshafiee et al. "Impact of protein pre-coating on the protein corona composition and nanoparticle cellular uptake" Biomaterials vol. 75, January 2016 pp. 295-304, Mahmoudi et al. "Emerging understanding of the protein corona at the nano-bio interfaces" Nanotoday 11(6) December 2016, pp. 817-832, and Mahmoudi et al. "Protein-Nanoparticle Interactions: Opportunities and Challenges" Chem. Rev., 2011, 111(9), pp. 5610-5637, the contents of which are incorporated by reference in their entireties. As described therein, an adsorption curve may show the build-up of a strongly bound monolayer up to the point of monolayer saturation (at a geometrically defined protein-to-NP ratio), beyond which a secondary, weakly bound layer is formed. While the first layer is irreversibly bound (hard corona), the secondary layer (soft corona) may exhibit dynamic exchange. Proteins that adsorb with high affinity may form the "hard" corona, comprising tightly bound proteins that do not readily desorb, and proteins that adsorb with low affinity may form the "soft" corona, comprising loosely bound proteins. Soft and hard corona can also be characterized based on their exchange times. Hard corona may show much larger exchange times in the order of several hours. See, e.g., M. Rahman et al. Protein-Nanoparticle Interactions, Spring Series in Biophysics 15, 2013, incorporated by reference in its entirety.

[0090] The term "biomolecule" refers to biological components that may be involved in corona formation, including, but not limited to, for example, proteins, polypeptides, polysaccharides, a sugar, a lipid, a lipoprotein, a metabolite, an oligonucleotide, metabolome or combination thereof. It is contemplated that the biomolecule coronas of distinct particles may contain some of the same biomolecules, may contain distinct biomolecules with regard to the other sensor elements, and/or may differ in level or quantity, type or conformation of the biomolecule that binds to each sensor element. In one embodiment, the biomolecule is selected from the group of proteins, nucleic acids, lipids, and metabolomes.

[0091] The term “biomolecule corona signature” refers to the composition, signature or pattern of different biomolecules that are bound to each type of particle or separate sensor element. The signature may not only refers to the different biomolecules but also the differences in the amount, level or quantity of the biomolecule bound to the sensor element, or differences in the conformational state of the biomolecule that is bound to the particle or sensor element. It is contemplated that the biomolecule corona signatures of each distinct type of sensor elements may contain some of the same biomolecules, may contain distinct biomolecules with regard to the other sensor elements, and/or may differ in level or quantity, type or conformation of various biomolecules. The biomolecule corona signature may depend on not only the physicochemical properties of the sensor element (e.g., particle), but also the nature of the sample and the duration of exposure to the biological sample.

[0092] Disclosed herein are compositions and methods for multi-omic analysis.“Multi-omic(s)” or“multiomic(s)” can refer to an analytical approach for analyzing biomolecules at a large scale, wherein the data sets are multiple omes, such as proteome, genome, transcriptome, lipidome, and metabolome. Non-limiting examples of multi-omic data include proteomic data, genomic data, lipidomic data, glycomic data, transcriptomic data, or metabolomics data.

[0093] “Biomolecule” in “biomolecule corona” can refer to any molecule or biological component that can be produced by, or is present in, a biological organism. Non-limiting examples of biomolecules include proteins (protein corona), polypeptides, oligopeptides, polyketides, polysaccharides, a sugar, a lipid, a lipoprotein, a metabolite, an oligonucleotide, a nucleic acid (DNA, RNA, micro RNA, plasmid, single stranded nucleic acid, double stranded nucleic acid), metabolome, as well as small molecules such as primary metabolites, secondary metabolites, and other natural products, or any combination thereof. In some embodiments, the biomolecule is selected from the group of proteins, nucleic acids, lipids, and metabolomes.

[0094] Currently, there are a small number of protein-based biomarkers in use today for clinical diagnosis, and in spite of extensive efforts to analyze the plasma proteome for the expansion of markers, relatively few new candidates have been accepted as clinically useful indicators. The plasma proteome contains >10,000 proteins and potentially an order of magnitude more protein isoforms with a concentration range spanning over 10 orders of magnitude (from mg/mL to pg/mL). These attributes, combined with a lack of convenient molecular tools for proteome analysis, make comprehensive studies of the plasma proteome exceptionally challenging. Approaches to overcome the broad dynamic range of proteins in biological samples must be capable of identifying and quantifying against a background of thousands of unique proteins and even more protein variants. However, there are no existing technologies that are capable of

simultaneous measurement of proteins across the entire plasma concentration range in a format with a sufficient throughput and with a practical cost profile to allow for appropriately-sized studies with robust prospects for validation and replication. These challenges not only limit the discovery of novel disease biomarkers, but have been a bottleneck against the adoption of proteogenomics and protein annotation of genomic variants. Advances in mass spectrometry (MS) along with development of improved data analytics have offered tools for deep and broad proteomic analysis. Several attempts have been made to substantially improve the detection of low abundance proteins, such as depletion of highly abundant proteins, plasma fractionation, and peptide fractionation. It is now possible to identify over 4,500 proteins in plasma. However, current approaches are fairly complex and time-consuming (days to weeks), and thus require a tradeoff between depth of protein coverage and sample throughput. Consequently, a simple and robust strategy for comprehensive and rapid analysis of the available body of information in the proteome remains an unmet need.

[0095] Additionally, the earlier a disease is diagnosed, the more likely that the disease can be cured or successfully managed leading to a better prognosis for the patient. When a disease is treated early, it may be possible to prevent or delay problems from the disease and may improve the outcomes for the patient, including extending the patient's life and/or quality of life.

[0096] Early diagnosis of cancer is crucial, as many types of cancers can be successfully treated in their early stages. For example, five-year survival after early diagnosis and treatment of breast, ovarian, and lung cancers is 90%, 90%, and 70%, respectively, compared to 15%, 5%, and 10% for patients diagnosed at the most advanced stage of disease. Once cancer cells leave their tissue of origin, successful treatment using available established therapeutics becomes very unlikely. Although recognizing the warning signs of cancers and taking prompt action may lead to early diagnosis, the majority of cancers (e.g., lung) show symptoms only after cancer cells have already invaded the surrounding tissues and metastasized throughout the body. For example, more than 60% of patients with breast, lung, colon, and ovarian cancer have concealed or even metastatic colonies by the time their cancers are detected. Therefore, there is an urgent need for development of an effective approach for early detection of cancer. Such an approach should have the sensitivity to identify a cancer at various stages and the specificity to give a negative result when the person being tested is free of the cancer. There have been extensive efforts to develop methods for early detection of cancers; although huge numbers of risk factors and biomarkers have been introduced, a broadly relevant platform for early detection of a wide range of cancers remains elusive.

[0097] As various types of cancers can change the composition of blood plasma—even in their early stages—one promising approach for early detection is molecular blood analysis for biomarkers. Although this strategy has already worked for a few cancers (like PSA for prostate cancer), there are not yet specific biomarkers for early detection of the majority of cancers. For such cancers (e.g., lung), none of the defined candidate circulating biomarkers has been clinically validated, and very few have reached late-stage clinical development. Therefore, there is an urgent need for novel approaches to improve our ability to detect cancer, as well as other diseases, at very early stages.

Automated Sample Preparation

[0098] The present disclosure provides systems and methods for automated sample preparation, data generation, protein corona analysis. As is depicted in **FIG. 1**, the systems and methods can comprise (1) contacting a sample to particles (e.g., in a particle mixture) on a sensor array, substrate, plate, or within partitions on any of the foregoing, (2) allowing biomolecules in the sample to bind to the particles, (3) removing unbound sample from the particles, and (4) preparing a sample for analysis (e.g., using mass spectrometry ("MS")). For example, in (1), a method of the present disclosure can comprise contacting a biological sample to a plurality of particles. In (2), the sample may be incubated with the plurality of particles so as to promote biomolecule adsorption to the particles. In (3), unbound sample may be removed while retaining the particles and the biomolecules adsorbed to the particles. In (4) the adsorbed biomolecules may be desorbed from the particles and preparing them for mass spectrometric analysis by which example data may be generated.

[0099] The present disclosure provides automated systems, methods and kits for biomolecule corona preparation and analysis. The automated apparatus may perform at least the aforementioned data generating steps outlined in **FIG. 1** using various units illustrated in **FIG. 2**. The automated apparatus may contain a substrate with a plurality of partitions containing sensor elements 205 and a biological sample 210. The loading unit 215 on the apparatus may transfer a portion of the biological sample 210 into a partition on the substrate 205, leading to adsorption of biomolecules from the biological sample onto a sensor element in the partition on the substrate. The automated apparatus may then remove unbound biomolecules from the partition, optionally transferring the unbound sample into a waste receptacle 220. The remaining biomolecules (e.g., biomolecules adsorbed to the sensor element) may be desorbed, collected, and prepared for mass spectrometric analysis. The reagents **225** may comprise a buffer, such as a resuspension buffer capable of desorbing biomolecules from a biomolecule corona or a denaturation buffer capable

of denaturing or fragmenting a biomolecule. Reagents (e.g., a buffer or protease) 225 may also be loaded using the loading unit 215 to facilitate any of the foregoing.

[0100] In some aspects, the present disclosure provides an automated system comprising a network of units with differentiated functions in distinguishing states of a complex biological sample using a plurality of particles having surfaces with different physicochemical properties wherein: a first unit comprises a multichannel fluid transfer instrument for transferring fluids between units within the system; a second unit comprises a support for storing a plurality of biological samples; a third unit comprises a support for a sensor array plate (e.g., a substrate comprising a plurality of partitions comprising sensor elements, such as a 96 well plate containing nanoparticles) possessing partitions that comprise the plurality of particles having surfaces with different physicochemical properties for detecting a binding interaction between a population of analytes within the complex biological sample and the plurality of particles; a fourth unit comprises supports for storing a plurality of reagents; a fifth unit comprises supports for storing a reagent to be disposed of; a sixth unit comprises supports for storing consumables used by the multichannel fluid transfer instrument; and wherein the system is programed to perform a series of steps comprising: contacting the biological sample with a specified partition of the sensor array; incubating the biological sample with the plurality of particles contained within the partition of the sensor array plate; removing all components from a partition except the plurality of particles and a population of analytes interacting with a particle; and preparing a sample for mass spectrometry.

[0101] An example of such an apparatus is provided in **FIG. 3**. The apparatus comprises an automated pipette that is able to transfer volumes between a biological sample storage unit, a substrate comprising a plurality of partitions comprising a plurality of sensor elements, a waste collection unit, a unit comprising a denaturation solution, and a unit comprising a resuspension solution. The automated apparatus can perform a biomolecule corona assay which comprises transferring a portion of the biological sample into a partition within the substrate comprising a sensor element, incubating the portion of the sample with the sensor element to allow biomolecules from the biological sample to bind to the sensor element, removing contents from the partition comprising biomolecules that are not bound to the sensor elements, and then preparing the biomolecules that remained within the partition for mass spectrometric (MS) analysis (e.g., LC-MS).

[0102] The loading may comprise a degree of mobility that enables access to all other unit within the system. The loading may comprise a capacity to perform pipetting functions.

[0103] The system or apparatus of the present disclosure may comprise support for a single plate, a 6 well plate, a 12 well plate, a 96 well plate, a 192 well plate, a 384 well plate, or a rack of microtubes. In some embodiments, the system or apparatus of the present disclosure may comprise a thermal unit capable of modulating the temperature of said support and a sample. In some embodiments, the system or apparatus of the present disclosure may comprise a rotational unit capable of physically agitating and/or mixing a sample.

[0104] In some embodiments, the plurality of particles comprises surfaces with different physicochemical properties for detecting a binding interaction between a population of analytes within the complex biological sample and the plurality of particles are immobilized to a surface with a partition of the sensory array. In some embodiments, the plurality of particles comprises a plurality magnetic nanoparticles in a solution with different physicochemical properties for detecting a binding interaction between a population of analytes within the complex biological sample and the plurality of particles. In some embodiments, the system comprises a step wherein the sensor array plate is transferred to an additional seventh unit that comprises a magnetized support and a thermal unit capable of modulating the temperature of said support and a sample and incubated for an additional amount of time.

[0105] In some embodiments, the fourth unit comprises a set of reagents for: generating the sensor array plate; washing an unbound sample; and/or preparing a sample for mass spectrometry. In some embodiments, contacting the biological sample with a specified partition of the sensor array comprises pipetting a specified volume of the biological sample into the specific partition of the sensor array. In some embodiments, contacting the biological sample with a specified partition of the sensor array comprises pipetting a volume corresponding to a 1:1, 1:2: 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:15, or 1:20 ratio of a plurality of particles in a solution to the biological sample.

[0106] In some embodiments, contacting the biological sample with a specified partition of the sensor array comprises pipetting a volume of at least 10 microliters, at least 50 microliters, at least 100 microliters, at least 250 microliters, at least 500 microliters, or at least 1000 microliters the biological sample into the specific partition of the sensor array.

Automated Apparatus

[0107] In some aspects, the present disclosure provides an automated apparatus for generating a subset of biomolecules from a biological sample, comprising: a substrate comprising a plurality of partitions, a first unit comprising the biological sample, and a loading unit that is movable across the substrate and is capable of transferring a volume (e.g., a volume of buffer) between different units of the apparatus. In some cases, the substrate is a multi-well plate.

[0108] The plurality of partitions may comprise a plurality of sensor elements. The plurality of sensor elements may comprise particles. The plurality of sensor elements may be particles (e.g., nanoparticles or microparticles).

[0109] A partition from among the plurality of partitions may comprise 1 to 100 types of sensor elements (e.g., distinct particle types). A partition from among the plurality of partitions may comprise 2 to 50 types of sensor elements. A partition from among the plurality of partitions may comprise 2 to 5 types of sensor elements. A partition from among the plurality of partitions may comprise 3 to 8 types of sensor elements. A partition from among the plurality of partitions may comprise 4 to 10 types of sensor elements. A partition from among the plurality of partitions may comprise 5 to 12 types of sensor elements. A partition from among the plurality of partitions may comprise 6 to 15 types of sensor elements. A partition from among the plurality of partitions may comprise 8 to 20 types of sensor elements.

[0110] Two or more partitions from among the plurality of partitions may comprise different quantities of sensor elements. two or more partitions from among the plurality of partitions may comprise different types of sensor elements. A partition amongst a plurality of partitions may comprise a combination of types and/or quantities of sensor element(s) that differs from other partitions in the plurality. A subset of partitions in a plurality of partitions may each contain a combination of distinct sensor elements that is distinct from other partitions in the plurality.

[0111] Sensor elements may be stored in dry form inside of or within the partitions. Dry sensor elements may be reconstituted or rehydrated prior to use. Sensor elements may also be stored within solutions. For example, a substrate partition may comprise a solution comprising a high concentration of particles.

[0112] Partitions from among the plurality of partitions comprise different concentrations or amounts (e.g., by mass/molar amount per unit volume of sample) of sensor elements. A partition from among the plurality of partitions may comprise from 1 pM to 100 nM of sensor elements. A partition from among the plurality of partitions comprise may from 1 pM to 500 pM of sensor elements. A partition from among the plurality of partitions may comprise from 10 pM to 1 nM of sensor elements. A partition from among the plurality of partitions may comprise from 100 pM to 10 nM of sensor elements.A partition from among the plurality of partitions may comprise from 500 pM to 100 nM of sensor elements. A partition from among the plurality of partitions may comprise from 50 μg/ml to 300 μg/ml of sensor elements. A partition from among the plurality of partitions may comprise from 100 μg/ml to 500 μg/ml of sensor elements. A partition from among the plurality of partitions may comprise from 250 μg/ml to 750 μg/ml of sensor elements. A partition from among the plurality of partitions may comprise from 400

μg/ml to 1 mg/ml of sensor elements. A partition from among the plurality of partitions may comprise from 600 μg/ml to 1.5 mg/ml of sensor elements. A partition from among the plurality of partitions may comprise from 800 μg/ml to 2 mg/ml of sensor elements. A partition from among the plurality of partitions may comprise from 1 mg/ml to 3 mg/ml of sensor elements. A partition from among the plurality of partitions may comprise from 2 mg/ml to 5 mg/ml of sensor elements. A partition from among the plurality of partitions may comprise more than 5 mg/ml of sensor elements.

[0113] The loading unit may be configured to move between and transfer volumes (e.g., a volume of a solution or a powder) between any units, compartments, or partitions within the apparatus. The loading unit may be configured to move precise volumes (e.g., within 0.1%, 0.01%, 0.001% of the specified volume). The loading unit may be configured to collect a volume from the substrate or a compartment or partition within the substrate, and dispense the volume back into the substrate or compartment or partition within the substrate, or to dispense the volume or a portion of the volume into a different unit, compartment, or partition. The loading unit may be configured to move multiple volumes simultaneously, such as 2 to 400 separate volumes. The loading unit may comprise a plurality of pipette tips.

[0114] The loading unit may be configured to move a volume of a liquid. The volume may be about 0.1 μl, 0.2 μl, 0.3 μl, 0.4 μl, 0.5 μl, 0.6 μl, 0.7 μl, 0.8 μl, 0.9 μl, 1 μl, 2 μl, 3 μl, 4 μl, 5 μl, 6 μl, 7 μl, 8 μl, 9 μl, 10 μl, 12 μl, 15 μl, 20 μl, 25 μl, 30 μl, 40 μl, 50 μl, 60 μl, 70 μl, 80 μl, 90 μl, 100 μl, 120 μl, 150 μl, 180 μl, 200 μl, 250 μl, 300 μl, 400 μl, 500 μl, 600 μl, 800 μl, 1 ml, or more than 1 ml. The liquid may be a biological sample or a solution.

[0115] In some cases, the solution comprises a wash solution, a resuspension solution, a denaturing solution, a buffer, a reagent (e.g., a reducing reagent), or any combination thereof. In some cases, the solution comprises a biological sample.

[0116] In part owing to these functionalities, the loading unit can be capable of partitioning a sample. In some embodiments, this comprises dividing a sample into a number of partitions. A sample can be divided into at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 200, 250, 300, 350, 400, 500, or more partitions. A sample can be divided into 96, 192, or 384 partitions. The automated apparatus can comprise multiple substrates comprising partitions. The automated apparatus may comprise 1, 2, 3, 4, 5, or more substrates comprising partitions. In some cases, the loading unit loads different volumes of the biological sample into different partitions. In some cases, the loading unit loads identical volumes into two or more partitions. The volume of biological sample loaded into a partition may be about 0.1 μl, 0.2 μl, 0.3 μl, 0.4 μl, 0.5 μl, 0.6 μl, 0.7 μl, 0.8 μl, 0.9 μl, 1 μl, 2 μl, 3 μl, 4

μl, 5 μl, 6 μl, 7 μl, 8 μl, 9 μl, 10 μl, 12 μl, 15 μl, 20 μl, 25 μl, 30 μl, 40 μl, 50 μl, 60 μl, 70 μl, 80 μl, 90 μl, 100 μl, 120 μl, 150 μl, 180 μl, 200 μl, 250 μl, 300 μl, 400 μl, 500 μl, 600 μl, 800 μl, 1 ml, or more than 1 ml. The volume of biological sample loaded into a partition may be about 10 μl to 400 μl. The volume of biological sample loaded into a partition may be about 5 μl to 150 μl. The volume of biological sample loaded into a partition may be about 35 μl to 80 μl. In some cases, the loading unit may partition two or more biological samples. For example, a sample storage unit may comprise two biological samples that the system partitions into one well plate. In some embodiments, the loading unit can facilitate a transfer of the sample for mass spectrometry to a mass spectrometry unit.

[0117] The system may be configured to perform a dilution on a sample or a sample partition. A sample or sample partition may be diluted with buffer, water (e.g., purified water), a non-aqueous solvent, or any combination thereof. The diluent may be stored in the automated apparatus prior to dispensation into a substrate partition. The automated apparatus may store a plurality of diluents differing in pH, salinity, osmolarity, viscosity, dielectric constant, or any combination thereof. The diluents may be used to adjust the chemical properties of a sample or sample partition. The automated apparatus may dilute a sample or sample partition by 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 8-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 75-fold, 100-fold, 150-fold, 200-fold, 300-fold, 400-fold, 500-fold or greater. The automated apparatus may perform different dilutions on two samples or sample partitions. The system may perform different dilutions on each partition from among a plurality of partitions. For example, the system may perform different dilutions on each of the 96 sample partitions in a 96 well plate. In some cases, the different dilutions comprise different degrees of dilution (e.g., 2-fold vs. 4-fold). In some cases, the different dilutions comprise dilution with different solutions (e.g., different buffers). In some cases, two sample partitions may be made to differ in one or more chemical properties, such as pH, salinity, or viscosity.

[0118] In some cases, the system may modify the chemical composition of a sample or sample partition. The system may modify or adjust the pH, salinity, osmolarity, dielectric constant, viscosity, buffer types, salt types, sugar types, detergent types, or any combination thereof for a sample or sample partition. Such modification or adjustments may comprise mixing a reagent from the fourth unit with a sample or sample partition. The system may differently modify the chemical composition of two samples or sample partitions.

[0119] A system or automated apparatus of the present disclosure may also comprise an incubation element. The incubation element may contact, support, or hold another component of the automated apparatus (e.g., the substrate or a unit). The incubation unit may contact, support,

or hold multiple components of the automated apparatus. The incubation element may contact the substrate to facilitate heat transfer between the incubation element and the substrate. The incubation unit may be configured to control the temperature of the one or more components of the automated apparatus, such as by heating or cooling. The incubation element may be capable of cooling a component of the apparatus to from 20 °C to 1 °C. The incubation element may be capable of heating a component of the apparatus to from 25 °C to 100 °C. The incubation element may be capable of setting the temperature a component of the apparatus to from 4 °C to 37 °C. The incubation element may be configured to heat or cool different portions of a component of the automated apparatus to different temperatures. For example, the incubation element may hold a first partition in the substrate at 30 °C and a second partition in the substrate at 35 °C. The incubation element may control the temperature of a sample or partition. The incubation element may comprise a temperature sensor (e.g., a thermocouple) for detecting the temperature within a partition or container. The incubation element may calibrate its heating or cooling to the readout from the temperature sensor.

[0120] The incubation element may be configured to physically agitate a component of the automated apparatus. The agitation may be in the form of shaking or spinning, vibrating, rocking, sonicating, or any combination thereof. The incubation element may be capable of providing multiple agitation intensities and/or frequencies. For example, the incubation element may comprise multiple settings for shaking at different frequencies and amplitudes. The incubation element may also be capable of stirring and or mixing a volume (e.g., a portion of the biological sample).

[0121] The automated apparatus may comprise a unit comprising a resuspension solution. The loading unit may be capable of transferring a volume of the resuspension solution to a partition from among the plurality of partitions of the substrate. In some cases, this results in the dilution of a sample present within the partition and can further result in the desorption of a plurality of biomolecules from a biomolecule corona disposed on a sensor element within the partition. The quantity of biomolecules desorbed from a biomolecule corona can depend on the volume of the resuspension solution added to the partition, the temperature of the partition, the composition of the resuspension solution (e.g., the salinity, osmolarity, viscosity, dielectric constant, or pH), the volume of the biological sample within the partition, and the sensor element type and the composition of biomolecules in the biomolecule corona. The transfer of a volume of the resuspension solution into a partition may result in the desorption of less than 5% of the biomolecules from a biomolecule corona. The transfer of a volume of the resuspension solution into a partition may result in the desorption of 10% to 20% of the biomolecules from a

biomolecule corona. The transfer of a volume of the resuspension solution into a partition may result in the desorption of 20% to 30% of the biomolecules from a biomolecule corona. The transfer of a volume of the resuspension solution into a partition may result in the desorption of 30% to 40% of the biomolecules from a biomolecule corona. The transfer of a volume of the resuspension solution into a partition may result in the desorption of 40% to 50% of the biomolecules from a biomolecule corona. The transfer of a volume of the resuspension solution into a partition may result in the desorption of 50% to 60% of the biomolecules from a biomolecule corona. The transfer of a volume of the resuspension solution into a partition may result in the desorption of 60% to 70% of the biomolecules from a biomolecule corona. The transfer of a volume of the resuspension solution into a partition may result in the desorption of 70% to 80% of the biomolecules from a biomolecule corona. The transfer of a volume of the resuspension solution into a partition may result in the desorption of 80% to 90% of the biomolecules from a biomolecule corona. The transfer of a volume of the resuspension solution into a partition may result in the desorption of more than 90% of the biomolecules from a biomolecule corona.

[0122] In some cases, multiple rounds of desorption are performed. In each round, the supernatant comprising the desorbed biomolecules may be collected, analyzed, or discarded. The types and abundances of biomolecules in the supernatant may differ between desorption rounds. The automated apparatus may perform one or more desorption and discard cycles (i.e., washes), followed by one or more desorption cycles comprising sample collection and/or analysis.

[0123] The resuspension solution may be tailored to optimize enrichment of particular biomarkers. The resuspension solution may comprise a buffer, such as Tris-EDTA (TE), CHAPS, PBS, citrate, HEPES, MES, CHES, or another bio buffer. The resuspension solution may comprise Tris EDTA (TE) 150mM KCl 0.05% CHAPS buffer. The resuspension solution may comprise 10 mM TrisHCl pH 7.4, 1 mM EDTA. The resuspension solution may also contain or be highly purified water (e.g., distilled or deionized water). Biomolecule desorption may be augmented by heating or agitation by an incubation element. The supernatant may be transferred to a new partition following desorption. A resuspension solution may be used to dilute a sample.

[0124] The automated apparatus may comprise a unit comprising a denaturing solution. The denaturing solution may comprise a protease. The denaturing solution may comprise a chemical capable of performing peptide cleavage (e.g., cyanogen bromide, formic acid, or hydroxylamine, 2-nitro-5-thiocyanatobenzoic acid). The denaturing solution may comprise a chemical denaturant such as guanidine, urea, sodium deoxycholate, acetonitrile, trichloroacetic acid, acetic acid,

sulfosalicylic acid, sodium bicarbonate, ethanol, perchlorate, dodecyl sulfate, or any combination thereof. The denaturing solution may comprise a reductant, such as 2-mercaptoethanol, dithiothreitol, or tris(2-carboxyethyl)phosphine. The protease may be trypsin. The denaturing solution may be added to a partition following desorption. The denaturing solution may be added to a partition comprising biomolecule coronas.

[0125] The automated apparatus may comprise a magnet or array of magnets. The automated apparatus may capable of moving the substrate onto and off of the magnet or array of magnets. The array of magnets may be structured so that a plurality of magnets from the array of magnets can rest directly underneath underneath a plurality of partitions from the substrate. The magnet may be capable of immobilizing magnetic sensor elements (e.g., magnetic particles such as coated or uncoated super paramagnetic iron oxide nanoparticles) within a partition on the substrate. For example, the magnet may prevent magnetic nanoparticles from being removed from a partition during a wash step. The magnet may also create a pellet from a collection of magnetic particles. The magnet may create a particle pellet in less than 10 minutes. The magnet may create a particle pellet in less than 5 minutes. The particle pellet may comprise a particle with a biomolecule corona.

[0126] The automated apparatus may comprise a purification unit. The purification unit may comprise a plurality of partitions comprising an adsorbent or resin. The purification unit may comprise a solid-phase extraction array or plate. The solid-phase extraction array or plate may comprise a polar stationary phase material. The solid-phase extraction array or plate may comprise a non-polar stationary phase material. The solid-phase extraction array or plate may comprise a C18 stationary phase material (e.g., octadecyl group silica gel). The automated apparatus may comprises a unit with a conditioning solution for the purification unit (e.g., a conditioning solution for a solid-phase extraction material). The automated apparatus may comprise a unit with an elution solution for removing biomolecules from the purification unit.

[0127] In some embodiments, components are removed from a partition, except for the plurality of sensor elements and a population of analytes interacting with the plurality of sensor elements (i.e., a wash step). In some instances, the automated apparatus may perform a series of wash steps. A wash step may remove biomolecules that are not bound to the sensor elements within the partition. A wash step may desorb a subset of biomolecules bound to sensor elements within a partition. For example, a wash step may result in the desorption and removal of a subset of soft corona analytes, while leaving the majority of hard corona analytes bound to the sensor element.

[0128] In some aspects, the present disclosure provides an automated apparatus to identify proteins in a biological sample, the automated apparatus comprising: a sample preparation unit; a

substrate comprising a plurality of channels; a plurality of pipettes; a plurality of solutions, a plurality of particles, and wherein the automated apparatus is configured to form a protein corona and digest the protein corona.

[0129] In some aspects, the present disclosure provides an automated apparatus to identify proteins in a biological sample, the automated apparatus comprising: a sample preparation unit; a substrate comprising a plurality of channels; a plurality of pipettes; a plurality of solutions, a plurality of nanoparticles, wherein the automated apparatus is configured to form a protein corona and digest the protein corona, and wherein at least one of the solutions is TE 150mM KCl 0.05% CHAPS buffer.

[0130] In some embodiments, the sample preparation unit is configured to add the plurality of nanoparticles to the substrate with the plurality of pipettes. In some embodiments, wherein the sample preparation unit is configured to add the biological sample to the substrate with the plurality of pipettes. In some embodiments, the sample preparation unit is configured to incubate the plurality of nanoparticles and the biological sample to form the protein corona.

[0131] In some embodiments, the sample preparation unit is configured to separate the protein corona from the supernatant to form a protein corona pellet. In some embodiments, the sample preparation unit is configured to reconstitute the protein corona pellet with TE 150mM KCl 0.05% CHAPS buffer.

[0132] In some embodiments, the automated apparatus further comprises a magnetic source. In some embodiments, the automated apparatus is configured for BCA, gel, or trypsin digestion of the protein corona.

[0133] In some embodiments, the automated apparatus is enclosed. In some embodiments, the automated apparatus is sterilized before use. In some embodiments, the automated apparatus is configured to a mass spectrometry. In some embodiments, the automated apparatus is temperature controlled.

Assaying Methods

[0134] In some aspects, the present disclosure provides a method of identify proteins in a biological sample. In some cases the method comprises: adding the biological sample to the automated apparatus disclosed herein; generating proteomic data from the automated apparatus; and quantifying the proteomic data.

[0135] In some embodiments, the method comprises incubating a plurality of biomolecules with the biological sample in the automated apparatus to form a biomolecule corona. In some embodiments, incubating the biological sample with the plurality of sensor elements (e.g., particles) contained within the partition of the substrate comprises an incubation time of at least

about 10 seconds, at least about 15 seconds, at least about 20 seconds, at least about 25 seconds, at least about 30 seconds, at least about 40 seconds, at least about 50 seconds, at least about 60 seconds, at least about 90 seconds, at least about 2 minutes, at least about 3 minutes, at least about 4 minutes, at least about 5 minutes, at least about 6 minutes, at least about 7 minutes, at least about 8 minutes, at least about 9 minutes, at least about 10 minutes, at least about 15 minutes, at least about 20 minutes, at least about 25 minutes, at least about 30 minutes, at least about 45 minutes, at least about 50 minutes, at least about 60 minutes, at least about 90 minutes, at least about 2 hours, at least about 3 hours, at least about 4 hours, at least about 5 hours, at least about 6 hours, at least about 7 hours, at least about 8 hours, at least about 9 hours, at least about 10 hours, at least about 12 hours, at least about 14 hours, at least about 15 hours, at least about 16 hours, at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, or at least about 24 hours. In some cases, two wells will have two different incubation times. In some embodiments, incubating the biological sample with the plurality of particles contained within the partition of the substrate comprises an incubation temperature between about 4° C to about 37° C. In some embodiments, incubating the biological sample with the plurality of particles contained within the partition of the substrate comprises an incubation temperature between about 4° C to about 100° C.

[0136] The method, systems, and apparatus of the present disclosure may comprise covering or sealing a partition on the substrate. This may comprise covering a surface of the apparatus with a lid or a seal. The lid or seal may prevent solutions or species from leaving a partition (e.g., evaporating from a partition). The automated apparatus may be configured to place and/or remove the lid or seal. The lid or seal may be pierceable (e.g., may comprise a septum), thereby allowing a syringe or needle to enter a substrate partition without removal of the lid or seal.

[0137] In some cases, the system, apparatus and method of the present disclosure further comprise preparing analytes from the biomolecule corona for analysis (e.g., mass spectrometric analysis). This can comprise separating the biomolecule corona from the supernatant in the automated apparatus. The biomolecule corona may be separated from the supernatant by removing the supernatant and then desorbing a plurality of proteins from the biomolecule corona into a desorbate solution (e.g., a resuspension solution). In some cases, a first portion of biomolecules from a biomolecule corona are desorbed from the biomolecule corona and discarded, and a second portion of biomolecules from a biomolecule corona are desorbed from the biomolecule corona and collected (e.g., for analysis). Multiple portions of biomolecules from a biomolecule corona may be separately, desorbed, collected, and analyzed.

[0138] In some cases, biomolecules within a biomolecule corona are denatured, fragmented, chemically modified, or any combination thereof. These treatments may be performed on desorbed biomolecules or on biomolecule coronas. The plurality of biomolecules desorbed from a biomolecule corona may comprise 1%, 2%, 3%, 4%, 5%, 6%, 8%, 10%, 12%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or greater than 99% of the biomolecules from the biomolecule corona. The desorption may performed for different lengths of time, including 5 seconds, 15 seconds, 30 seconds, 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 8 minutes, 10 minutes, 12 minutes, 15 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 1.5 hours, 2 hours, 3 hours , 4 hours, 5 hours, 6 hours, 8 hours, 12 hours, or longer. In some cases, the desorption comprises physical agitation, such as shaking or sonication. The percent of proteins desorbed from a particle corona may depend on the desorption time, the chemical composition of the desorbate solution into which proteins are desorbed (e.g., pH or buffer-type), the desorption temperature, the form and intensity of physical agitation applied, or any combination thereof. Additionally, the types of proteins desorbed from a protein corona can be responsive to desorption conditions and methods. The types of proteins desorbed from a protein corona may differ by 1%, 2%, 3%, 4%, 5%, 6%, 8%, 10%, 12%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, or more between two desorption conditions or methods.

[0139] In some cases, preparing analytes from a biomolecule corona for analysis comprises digesting the biomolecule corona, a subset of biomolecules within the protein corona, or biomolecules desorbed from the biomolecule corona to form a digested sample in the automated apparatus. Preparing analytes from the biomolecule corona for analysis may also comprise chemically modifying a biomolecule from the biomolecule corona, such as methylating or reducing the biomolecule.

[0140] Desorbed biomolecules may be collected for further analysis (e.g., mass spectrometric analysis). The automated apparatus may perform the collecting, for example by collecting a volume of sample from a substrate partition comprising biomolecules desorbed from biomolecule coronas. A method may involve placing partition or plurality of partitions (e.g., a well plate) may be placed directly within an instrument for performing said analysis.

[0141] A method may comprise multiple rounds of preparing analytes from a biomolecule corona for analysis. A method may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more rounds of preparation. In some cases, each round produces a separate sample for analysis (e.g., desorbed biomolecules may be collected after each round and subjected to mass spectrometric analysis). Two rounds may comprise desorbing different pluralities of proteins from a biomolecule corona. Two rounds may also comprise different desorption methods or conditions, such as different

desorbate solution volumes, different desorbate solution types (e.g., desorbate solutions comprising different buffers or osmolarities), different temperatures, or different types and degrees of physical agitation. Two or more successive rounds of preparation from a single biomolecule corona (e.g., desorption and collection of a first subset of biomolecules from a biomolecule corona followed by desorption and collection of a second subset of biomolecules from a biomolecule corona) may generate two sets of biomolecules. This may inform detection or analysis of biomolecule interactions within a protein corona. As such, multiple rounds of preparation from a single biomolecule corona may be used to generate a number biomolecule subsets which exceed the number of partitions or types of sensor. For example, a method utilizing a substrate with 96 partitions (e.g., a 96 well plate) may generate as many as 960 unique biomolecule subsets if each partition comprises a unique combination of particles and solution conditions, and 10 rounds of analyte preparation are performed on each partition.

[0142] A different number of rounds of analyte preparation may be performed in separate partitions. Partitions may also be subjected to different analyte preparation conditions. Performing more rounds of analyte preparation can increase the number of proteins or types of proteins collected for analysis (e.g., generate more proteins that fall within concentration ranges accessible for simultaneous mass spectrometric detection). The number proteins or types of proteins detected when multiple rounds of analyte preparation are performed may be 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, or more than 200% higher than if a single round of analyte preparation was performed.

[0143] In some cases, the method comprises immobilizing a sensor element within a partition. The immobilization may prevent the sensor element from being removed from the partition when a volume is removed from the partition (e.g., the loading unit removes 95% of the solution from the partition). Immobilization may be performed, for example, chemically (e.g., covalent or non-covalent binding to a substrate). Chemical immobilization may comprise reacting a sensor element with a surface of the partition. Chemical immobilization may also comprise non-covalently associating a sensor element with a surface of the partition. For example, a sensor element may comprise biotin moieties that bind to streptavidin bound to a surface of a partition. Immobilization may be achieved by applying a magnetic field to hold a magnetic sensor element within a partition. For example, a plurality of sensor elements may comprise a plurality of magnetic particles, and the substrate and the magnet may be in proximity such that the one or more magnetic particles are immobilized within a partition in the substrate. Immobilization may be achieved by providing a substrate with a sensor element formed or embedded within a

partition on the substrate. For example, a sensor element may be a half-particle formed on the surface of a partition within the substrate.

[0144] In some cases, sensor element immobilization allows a biomolecule corona to be separated from the sensor element. This may comprise desorbing a plurality of biomolecules from a biomolecule corona associated with a sensor element, immobilizing the sensor element within the partition, and then collecting the solution with the plurality of biomolecules from the biomolecule corona, thereby separating at least a portion of a biomolecule corona from a sensor element.

[0145] **FIG. 4** illustrates examples of methods comprising immobilization sensor elements, which can be performed by the automated apparatus of the present disclosure. These methods utilize particles **402** and **411** to capture a subset of biomolecules **403** and **404** in a sample.

[0146] Panel **400** shows a partition **401** containing particles **402** and biomolecules. The particles are suspended within the partition, and have adsorbed biomolecules **403** from the sample, thereby forming biomolecule coronas. A number of biomolecules **404** may not adsorb to the particles, and will instead be suspended within the partition. Panel **410** shows an alternative method, comprising particles **411** that are formed on the surface of the partition.

[0147] Panels **420** and **430** show two methods for immobilizing the particles. In panel **420**, the particles are collected onto the bottom of the partition by a magnet **421**. In panel **430**, the particles are crosslinked to the partition via linkers **431**. Both methods result in the particles becoming immobilized to the partition. Throughout the immobilization process, particle-adsorbed biomolecules **403** remain adsorbed to the particles, while the unbound biomolecules **404** remain unbound from the particles.

[0148] Panel **440** shows the results of wash steps on the partitions from panels **410**, **420**, and **430**. In all three cases, the wash removes unbound biomolecules from the partition, while leaving the immobilized particles and the biomolecules adsorbed to them. Panel **450** then shows desorption of the biomolecule coronas, wherein a first plurality of biomolecules **451** elute from the particles, and a second plurality of biomolecules **403** remain adsorbed to the particles. The ratio of eluted to adsorbed biomolecules and the types of biomolecules eluted from the particles depends on the elution conditions (e.g., temperature, degree and type of physical agitation, solution conditions such as pH). The eluted biomolecules can be collected (e.g., by the loading unit) for further processing (e.g., fragmentation) or direct analysis.

[0149] **FIG. 5** shows an example of a sample preparation method that can be performed by the automated apparatus of the present disclosure. This method utilizes sensor elements **512** to generate a subset of biomolecules from a biological sample **502**. The biological sample (shown

in panel **500**), which is stored in a sample container **501**, comprises a number of biomolecules. A volume of the sample can optionally be processed **504** (e.g., cells within the sample can be lysed, nucleic acids and proteins can be fragmented, the sample can be filtered to remove large biomolecules, etc.), and then added to a partition **511** comprising sensor elements **512**. As is depicted in panel **520** a portion of the biomolecules **521** can bind to the sensor elements, separating them from a portion of biomolecules **522** that does not bind to the sensor elements. As is shown in panel **530**, the sensor elements can then be immobilized within the partition by bringing the partition in contact with a magnet **531**. The partition can then undergo a wash cycle (e.g., addition of buffer to the partition followed by removal of sample from the partition), resulting in the removal of the portion of biomolecules **522** that did not bind to the sensor elements (shown in panel **540**). The bound biomolecules **521** can be eluted from the sensor elements and collected for further processing or analysis.

[0150] FIG. 6 illustrates a sample preparation method that can be performed by the automated apparatus of the present disclosure. This method utilizes sensor elements **512** that are formed on the surface of a substrate partition **511** to collect biomolecules **503** from a sample **502**. The biological sample is transferred **504** from a sample holding unit **501** to the substrate partition **511**. As is shown in panel **520**, the sensor elements will adsorb a first portion of the biomolecules from the sample **521**, while a second portion **522** will remain unbound. Panel **530** depicts the removal of the unbound biomolecules, which leaves the sensor elements **512** and sensor element-bound biomolecules **521** within the partition. These biomolecules can subsequently be desorbed from the sensor elements and collected (e.g., by the loading unit) for further processing or analysis.

[0151] The methods disclosed herein may comprise filtering a sensor element from a solution. For example, the method may comprise desorbing a plurality of biomolecules from a biomolecule corona associated with a sensor element, and filtering the solution such that the sensor element is collected on the filter and the plurality of biomolecules remain in solution. The filtering may be performed after denaturation (e.g., digestion). The filtering may also remove a plurality of biomolecules or biological species such as intact proteins (e.g., undigested proteins from the biological sample or proteases).

[0152] In some cases, the method comprises a purification step. The purification step can precede or follow preparation of analytes from a biomolecule corona. A purification step may comprise transferring a biological sample (e.g., biomolecules eluted and collected from a biomolecule corona) to a purification unit (e.g., a chromatography column) or partition within a purification unit. Purification may involve transferring a plurality of sample partitions from the

substrate into separate partitions in the purification unit. The purification unit may comprise a solid-phase extraction plate. The purification step may remove reagents (e.g., chemicals and enzymes) from the denaturation solution. Following purification, the biological sample may be recollected for further enrichment or chemical treatment within the substrate or purification unit, or may be collected for direct analysis (e.g., mass spectrometric analysis).

[0153] Collectively, the methods of the present disclosure enable a high degree of profiling depth for biological samples. The subset of biomolecules collected in the methods of the present disclosure may enable, without further manipulation or modification of said subset of biomolecules, mass spectrometric detection of at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 12%, at least 15 %, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, or more than 60% of the types of biomolecules in the biological sample from which the subset of biomolecules were collected. The subset of biomolecules may enable, without further manipulation or modification of said subset of biomolecules, mass spectrometric detection of at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 12%, at least 15 %, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, or more than 50% of the types of proteins in a sample. The subset of biomolecules collected on a sensor element or prepared for analysis may enable, without further manipulation or modification of said subset of biomolecules, simultaneous mass spectrometric detection of two biomolecules (e.g., proteins) spanning 6, 7, 8, 9, 10, 11, 12 or more orders of magnitude in a sample. For example, the two biomolecules may be desorbed and collected at concentrations within 6 orders of magnitude within a single sample, fragmented, and then submitted for mass spectrometric analysis.

[0154] In some cases, a type of sensor element (e.g., all sensor elements of a given type that are within contact of a single sample) adsorbs at least 100 to at least 300 types of proteins upon contacting a biological sample. A type of sensor element may adsorb at least 200 to at least 500 types of proteins upon contacting a biological sample. A type of sensor element may adsorb at least 300 to at least 800 types of proteins upon contacting a biological sample. A type of sensor element may adsorb at least 400 to at least 1000 types of proteins upon contacting a biological sample. A type of sensor element may adsorb at least 500 to at least 1200 types of proteins upon contacting a biological sample.

[0155] In some cases, the proteins collected from a plurality of sensor elements will be identified on the level of protein groups. The plurality of protein groups collected on sensor elements in a partition may comprise from 1 to 20,000 protein groups. The plurality of protein groups collected on sensor elements in a partition may comprise from 100 to 10,000 protein groups. The

plurality of protein groups collected on sensor elements in a partition may comprise from 100 to 5,000 protein groups. The plurality of protein groups collected on sensor elements in a partition may comprise from 300 to 2,200 protein groups. The plurality of protein groups collected on sensor elements in a partition may comprise from 1,200 to 2,200 protein groups. The plurality of protein groups collected on sensor elements in a partition may comprise from 20,000 to 25,000 protein groups. The plurality of protein groups collected on sensor elements in a partition may comprise from 25,000 to 30,000 protein groups. The plurality of protein groups collected on sensor elements in a partition may comprise from 30,000 to 50,000 protein groups.

[0156] The methods of the present disclosure can result in the enrichment of low abundance biomolecules (e.g., proteins) from a biological sample. A low abundance biomolecule may be a biomolecule at a concentration of 10ng/mL or less in a biological sample.

[0157] The methods of the present disclosure can result in the enrichment of biomolecules (e.g., proteins) present at a concentration that is at least 6 orders of magnitude lower than the concentration of the most abundant biomolecule of the same type in the same sample (e.g., a low abundance protein may be a protein whose concentration is at least 6 orders of magnitude lower than the most abundant protein in the sample). Databases, such as the Carr database (Keshishian et al., Mol. Cell Proteomics 14, 2375–2393 (2015), Plasma Proteome Database (plasmaproteomedatabase.org)) characterizing the plasma proteome, may provide a basis of comparison such that one can ascertain whether a protein or biomolecule detected is enriched relative to other biomolecule(s) present in a plasma sample. Similar databases may be used for other types of biological samples.

[0158] In particular cases, the biological sample comprises blood, plasma, or serum, and a biomolecule corona comprises a lower proportion of albumin to non-albumin proteins than the biological sample. The ratio of albumin to non-albumin proteins may be 20%, 30%, 40%, 50%, 60%, or 70% lower in a biomolecule corona than in the sample from which proteins were adsorbed.

[0159] The concentration range of a plurality of biomolecules may be compressed upon formation of a biomolecule corona. For example, the automated apparatus may increase the number of types of biomolecules whose concentrations are within 6 orders of magnitude of the most concentrated biomolecule in the sample by at least 25%, 50%, 100%, 200%, 300%, 500%, or 1000%. Analogously, the compressed dynamic range may comprise an increase in the number of types of proteins whose concentrations are within 6 orders of magnitude of the most abundant biomolecule in the sample. The automated apparatus may increase the number of types of proteins whose concentrations are within 6 orders of magnitude of the most concentrated protein

in the sample by at least 25%, 50%, 100%, 200%, 300%, 500%, or 1000%. The automated apparatus may enrich a subset of biomolecules from a biological sample, and the subset of biomolecules may comprise at least 10% of the types of biomolecules from the biological sample within a 6 order of magnitude concentration range. The automated apparatus may enrich a subset of biomolecules from a biological sample, and the subset of biomolecules may comprise at least 20% of the types of biomolecules from the biological sample within a 6 order of magnitude concentration range. The automated apparatus may enrich a subset of biomolecules from a biological sample, and the subset of biomolecules may comprise at least 30% of the types of biomolecules from the biological sample within a 6 order of magnitude concentration range. The automated apparatus may enrich a subset of biomolecules from a biological sample, and the subset of biomolecules may comprise at least 40% of the types of biomolecules from the biological sample within a 6 order of magnitude concentration range. The automated apparatus may enrich a subset of biomolecules from a biological sample, and the subset of biomolecules may comprise at least 50% of the types of biomolecules from the biological sample within a 6 order of magnitude concentration range. The automated apparatus may enrich a subset of biomolecules from a biological sample, and the subset of biomolecules may comprise at least 60% of the types of biomolecules from the biological sample within a 6 order of magnitude concentration range. The automated apparatus may enrich a subset of biomolecules from a biological sample, and the subset of biomolecules may comprise at least 70% of the types of biomolecules from the biological sample within a 6 order of magnitude concentration range. The automated apparatus may enrich a subset of biomolecules from a biological sample, and the subset of biomolecules may comprise at least 10% of the types of proteins from the biological sample within a 6 order of magnitude concentration range. The automated apparatus may enrich a subset of biomolecules from a biological sample, and the subset of biomolecules may comprise at least 20% of the types of proteins from the biological sample within a 6 order of magnitude concentration range. The automated apparatus may enrich a subset of biomolecules from a biological sample, and the subset of biomolecules may comprise at least 30% of the types of proteins from the biological sample within a 6 order of magnitude concentration range. The automated apparatus may enrich a subset of biomolecules from a biological sample, and the subset of biomolecules may comprise at least 40% of the types of proteins from the biological sample within a 6 order of magnitude concentration range. The automated apparatus may enrich a subset of biomolecules from a biological sample, and the subset of biomolecules may comprise at least 50% of the types of proteins from the biological sample within a 6 order of magnitude concentration range. The automated apparatus may enrich a subset of biomolecules from a

biological sample, and the subset of biomolecules may comprise at least 60% of the types of proteins from the biological sample within a 6 order of magnitude concentration range. The automated apparatus may enrich a subset of biomolecules from a biological sample, and the subset of biomolecules may comprise at least 70% of the types of proteins from the biological sample within a 6 order of magnitude concentration range.

[0160] The methods and sensor elements of the present disclosure may be tailored so that biomolecule corona composition is invariant with respect to sample lipid concentration. Changes of at most 10% in the lipid concentration in a biological sample may result in changes of less than 5%, 2%, 1%, or 0.1% in the composition of the proteins in a biomolecule corona. Changes of at most 10% in the lipid concentration in a biological sample may result in changes of less than 5%, 2%, 1%, or 0.1% in the number of types of proteins in a biomolecule corona. Changes of at most 10% in the lipid concentration in a biological sample may result in changes of less than 5%, 2%, 1%, or 0.1% in the total number of proteins in a biomolecule corona.

[0161] In some embodiments, the method further comprises washing the digested sample in the automated apparatus. In some embodiments, quantifying the proteomic data comprises providing the proteomic data to a mass spectrometer. In some embodiments, the biological sample is a biofluid. In some embodiments, the biofluid is serum or plasma.

[0162] In some cases, the entire assay time from a single sample, such as a pooled plasma sample, including sample preparation and LC-MS, can be about 8 hours. The entire assay time from a single sample, such as a pooled plasma sample, including sample preparation and LC-MS, can be about at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 9 hours, at least 10 hours, under 20 hours, under 19 hours, under 18 hours, under 17 hours, under 16 hours, under 15 hours, under 14 hours, under 13 hours, under 12 hours, under 11 hours, under 10 hours, under 9 hours, under 8 hours, under 7 hours, under 6 hours, under 5 hours, under 4 hours, under 3 hours, under 2 hours, under 1 hour, at least 5 min to 10 min, at least 10 min to 20 min, at least 20 min to 30 min, at least 30 min to 40 min, at least 40 min to 50 min, at least 50 min to 60 min, at least 1 hour to 1.5 hours, at least 1.5 hour to 2 hours, at least 2 hour to 2.5 hours, at least 2.5 hour to 3 hours, at least 3 hour to 3.5 hours, at least 3.5 hour to 4 hours, at least 4 hour to 4.5 hours, at least 4.5 hour to 5 hours, at least 5 hour to 5.5 hours, at least 5.5 hour to 6 hours, at least 6 hour to 6.5 hours, at least 6.5 hour to 7 hours, at least 7 hour to 7.5 hours, at least 7.5 hour to 8 hours, at least 8 hour to 8.5 hours, at least 8.5 hour to 9 hours, at least 9 hour to 9.5 hours, or at least 9.5 hour to 10 hours.

Dynamic Range

[0163] The biomolecule corona analysis methods described herein may comprise assaying biomolecules in a sample of the present disclosure across a wide dynamic range. The dynamic range of biomolecules assayed in a sample may be a range of measured signals of biomolecule abundances as measured by an assay method (e.g., mass spectrometry, chromatography, gel electrophoresis, spectroscopy, or immunoassays) for the biomolecules contained within a sample. For example, an assay capable of detecting proteins across a wide dynamic range may be capable of detecting proteins of very low abundance to proteins of very high abundance. The dynamic range of an assay may be directly related to the slope of assay signal intensity as a function of biomolecule abundance. For example, an assay with a low dynamic range may have a low (but positive) slope of the assay signal intensity as a function of biomolecule abundance, e.g., the ratio of the signal detected for a high abundance biomolecule to the ratio of the signal detected for a low abundance biomolecule may be lower for an assay with a low dynamic range than an assay with a high dynamic range. In specific cases, dynamic range may refer to the dynamic range of proteins within a sample or assaying method.

[0164] The biomolecule corona analysis methods described herein may compress the dynamic range of an assay. The dynamic range of an assay may be compressed relative to another assay if the slope of the assay signal intensity as a function of biomolecule abundance is lower than that of the other assay. For example, a plasma sample assayed using protein corona analysis with mass spectrometry may have a compressed dynamic range compared to a plasma sample assayed using mass spectrometry alone, directly on the sample or compared to provided abundance values for plasma proteins in databases (e.g., the database provided in Keshishian et al., Mol. Cell Proteomics 14, 2375–2393 (2015), also referred to herein as the “Carr database”), as shown in **FIG. 13** and **FIG. 14**. The compressed dynamic range may enable the detection of more low abundance biomolecules in a biological sample using biomolecule corona analysis with mass spectrometry than using mass spectrometry alone.

[0165] In some embodiments, the dynamic range of a proteomic analysis assay may be the ratio of the signal produced by highest abundance proteins (e.g., the highest 10% of proteins by abundance) to the signal produced by the lowest abundance proteins (e.g., the lowest 10% of proteins by abundance). Compressing the dynamic range of a proteomic analysis may comprise decreasing the ratio of the signal produced by the highest abundance proteins to the signal produced by the lowest abundance proteins for a first proteomic analysis assay relative to that of a second proteomic analysis assay. The protein corona analysis assays disclosed herein may

compress the dynamic range relative to the dynamic range of a total protein analysis method (e.g., mass spectrometry, gel electrophoresis, or liquid chromatography).

[0166] Provided herein are several methods for compressing the dynamic range of a biomolecular analysis assay to facilitate the detection of low abundance biomolecules relative to high abundance biomolecules. For example, a particle type of the present disclosure can be used to serially interrogate a sample. Upon incubation of the particle type in the sample, a biomolecule corona comprising forms on the surface of the particle type. If biomolecules are directly detected in the sample without the use of said particle types, for example by direct mass spectrometric analysis of the sample, the dynamic range may span a wider range of concentrations, or more orders of magnitude, than if the biomolecules are directed on the surface of the particle type. Thus, using the particle types disclosed herein may be used to compress the dynamic range of biomolecules in a sample. Without being limited by theory, this effect may be observed due to more capture of higher affinity, lower abundance biomolecules in the biomolecule corona of the particle type and less capture of lower affinity, higher abundance biomolecules in the biomolecule corona of the particle type.

[0167] A dynamic range of a proteomic analysis assay may be the slope of a plot of a protein signal measured by the proteomic analysis assay as a function of total abundance of the protein in the sample. Compressing the dynamic range may comprise decreasing the slope of the plot of a protein signal measured by a proteomic analysis assay as a function of total abundance of the protein in the sample relative to the slope of the plot of a protein signal measured by a second proteomic analysis assay as a function of total abundance of the protein in the sample. The protein corona analysis assays disclosed herein may compress the dynamic range relative to the dynamic range of a total protein analysis method (e.g., mass spectrometry, gel electrophoresis, or liquid chromatography).

Automated Systems

[0168] Various aspects of the present disclosure provide an automated system comprising an automated apparatus configured to isolate a subset of biomolecules from a biological sample, a mass spectrometer configured to receive the subset of biomolecules and to generate data comprising mass spectrometric or tandem mass spectrometric signals, and a computer comprising one or more computer processors and a computer readable medium comprising machine-executable code that, upon execution of the code, generates a biological fingerprint and assigns a biological state based on the biological fingerprint.

[0169] In many cases, the automated apparatus comprises a sensor element or plurality of sensor elements which adsorb biomolecules from biological solutions, thereby forming biomolecule

coronas. They type, amount, and categories of the biomolecules that make up these biomolecule coronas are strongly related to the physicochemical properties of the sensor elements themselves and the complex interactions between the different biomolecules themselves and the sensor elements. These interactions lead to the production of a unique biomolecule corona signature for each sensor element. In other words, depending on which biomolecules interact with the sensor element not only influences the makeup of the biomolecule corona but also can alter which other different biomolecules can also interact with that specific sensor element.

[0170] Different sensor elements each with their own biomolecule corona signature can be contacted with a sample to produce a unique biomolecule fingerprint for that sample. This fingerprint can then be used to determine a disease state of a subject. A plurality of sensor elements may be able to bind a plurality of biomolecules in a sample to produce a biomolecule corona signature. A plurality of sensor elements may have distinct biomolecule corona signatures. In particular cases, each type of sensor element has a distinct biomolecule corona signature. For example, a plurality of particles comprising 5 pM of each of 5 types of particles could have one biomolecule corona signature for each particle type.

[0171] The plurality of sensor elements when contacted with a sample produces a plurality of biomolecule corona signatures which together form a biomolecule fingerprint. The "biomolecule fingerprint" is the combined composition or pattern of biomolecules of at least two biomolecule corona signatures for the plurality of sensor elements. The biomolecule fingerprint may comprise at least 5, 10, 20, 40, 80, 150, or 200 distinct biomolecule corona signatures.

[0172] In some cases, the automated system is configured so that the biomolecule corona may be assayed separately for each sensor element, allowing the biomolecule corona signature to be determined for each element. More broadly, the automated system may be configured so that each sample partition (e.g., each well in the substrate) can be assayed separately, so that the combined set of biomolecule corona signatures may be determined for each partition.

[0173] Analogously, the computer may be configured to compare data from multiple biomolecule corona signatures, partitions, or separate subsets of biomolecules collected from an individual partition (e.g., through multiple rounds of desorption). This can provide a profiling sensitivity that is not possible with conventional methods. Many biological states (such as many pre-disease states) create minute variance in a patient's biological sample (e.g., blood, urine, etc.) that are often not discernible from biomarker analysis alone. The power of the present apparatuses, systems, methods, and sensor elements in part, stems from the interdependence of sensor element characteristics and biological sample composition on biomolecule corona composition, so that a small change in the populations, chemical states (e.g., post-translational

modification status), or even conformations of sparsely populated biomolecules can have a major impact on the biomolecule corona signature for a particular sensor element. Furthermore, a biological state which may not be evident from a single set of data may be clearly elucidated by the correlation between disparate biomolecule abundances across multiple biomolecule corona signatures or sample partition measurements. Thus, a combination of nearly identical biomolecule corona signatures can distinguish healthy subjects from cancer-ridden subjects with a high degree of accuracy.

[0174] In some cases, the computer is configured to process the data comprising the intensity, APEX, spectral count or number of peptides, Ion mobility behavior of the mass spectrometric or tandem mass spectrometric signal between a plurality of the distinct biomolecule corona signatures. The computer may be configured to process between 5,000 and 5,000,000 signals between a plurality of the distinct biomolecule corona signatures or sample partitions. The computer may be configured to process between 10,000 and 5,000,000 signals between a plurality of the distinct biomolecule corona signatures or sample partitions. The computer may be configured to compare between 20,000 and 200,000 signals between a plurality of the distinct biomolecule corona signatures or sample partitions. The computer may be configured to compare between 400,000 and 1,000,000 signals between a plurality of the distinct biomolecule corona signatures or sample partitions. The computer may be configured to compare between 600,000 and 2,000,000 signals between a plurality of the distinct biomolecule corona signatures or sample partitions. The computer may be configured to compare between 1,000,000 and 5,000,000 signals between a plurality of the distinct biomolecule corona signatures or sample partitions. In some cases, the signals comprise mass spectrometric or tandem mass spectrometric signals.

[0175] An aspect of the present disclosure provides methods for generating a biomolecule fingerprint from one or more sets of mass spectrometric data, tandem mass spectrometric data, chromatographic data, ion mobility data, or any combination thereof. In some cases, mass spectrometric data, tandem mass spectrometric data, chromatographic data, or ion mobility data may be used to determine the concentration of a biomolecule from a biological sample. A plurality of sample partitions may be subjected to a separate mass spectrometric or tandem mass spectrometric runs. A plurality of sample partitions may also be pooled and collectively analyzed in a single mass spectrometric or tandem mass spectrometric run. Multiple mass spectrometric runs may be coupled with multiple different chromatographic methods (e.g., different columns, buffers, or gradients). A single mass spectrometric or tandem mass spectrometric run is performed in less than two hours, less than one hour, or less than half an hour.

[0176] Aspects of the present disclosure provide methods for identifying biological states and biomolecules with high degrees of certainty and accuracy. The computer may be configured to identify a biomolecule or characterize an unidentified molecular feature based on a mass spectrometric or tandem mass spectrometric signal and or ion mobility and chromatographic behavior with a probability or certainty threshold of at least 95%. The computer may associate a biomolecule fingerprint with a biological state with at least 70% accuracy, at least 75% accuracy, at least 80% accuracy, at least 85% accuracy, at least 90% accuracy, at least 92% accuracy, at least 95% accuracy, at least 96% accuracy, at least 97% accuracy, at least 98% accuracy, at least 99% accuracy, or 100 % accuracy. The computer may associate a biomolecule fingerprint with a biological state with at least 70% sensitivity, at least 75% sensitivity, at least 80% sensitivity, at least 85% sensitivity, at least 90% sensitivity, at least 92% sensitivity, at least 95% sensitivity, at least 96% sensitivity, at least 97% sensitivity, at least 98% sensitivity, at least 99% sensitivity, or 100 % sensitivity. The computer may be capable of distinguishing between two biological states associated with biological fingerprints that differ by less than 20%, 15%, 10%, or 8%, 5%, 3%, 2%, or 1%. In some aspects, a biomolecule identification is validated if a threshold level of diagnostic signals are detected. For example, if a threshold number of three uniquely assignable peptide fragment signals is provided for protein group identification in a mass spectrometric assay, then two peptide fragment signals corresponding to a particular protein group will not be counted.

Sensor Elements

[0177] As used herein, the term “sensor element” refers to elements that are able to bind to a plurality of biomolecules when in contact with a sample and encompasses the term “particle”. The sensor element may be an element from about 5 nanometers (nm) to about 50000 nm in at least one direction. Suitable sensor elements include, for example, but not limited to a sensor element from about 5 nm to about 50,000 nm in at least one direction, including, about 5 nm to about 40000 nm, alternatively about 5 nm to about 30000 nm, alternatively about 5 nm to about 20,000 nm, alternatively about 5 nm to about 10,000 nm, alternatively about 5 nm to about 5000 nm, alternatively about 5 nm to about 1000 nm, alternatively about 5 nm to about 500 nm, alternatively about 5 nm to 50 nm, alternatively about 10 nm to 100 nm, alternatively about 20 nm to 200 nm, alternatively about 30 nm to 300 nm, alternatively about 40 nm to 400 nm, alternatively about 50 nm to 500 nm, alternatively about 60 nm to 600 nm, alternatively about 70 nm to 700 nm, alternatively about 80 nm to 800 nm, alternatively about 90 nm to 900 nm, alternatively about 100 nm to 1000 nm, alternatively about 1000 nm to 10000 nm, alternatively about 10000 nm to 50000 nm and any combination or amount in between (e.g. 5 nm, 10 nm, 15

nm, 20 nm, 25 nm, 30 nm, 35 nm, 40 nm, 45 nm, S0 nm, 55 nm, 60 nm, 65 nm, 70 nm, 80 nm, 90 nm, 100 nm, 125 nm, 150 nm, 175 nm, 200 nm, 225 nm, 250 nm, 275 nm, 300 nm, 350 nm, 400 nm, 450 nm, 500 nm, 550 nm, 600 nm, 650 nm, 700 nm, 750 nm, 800 nm, 850 nm, 900 nm, 1000 nm, 1200 nm, 1300 nm, 1400 nm, 1500 nm, 1600 nm, 1700 nm, 1800 nm, 1900 nm, 2000 nm, 2500 nm, 3000 nm, 3500 nm, 4000 nm, 4500 nm, 5000 nm, 5500 nm, 6000 nm, 6500 nm, 7000 nm, 7500 nm, 8000 nm, 8500 nm, 9000 nm, 10000 nm, 11000 nm, 12000 nm, 13000 nm, 14000 nm, 15000 nm, 16000 nm, 17000 nm, 18000 nm, 19000 nm, 20000 nm, 25000 nm, 30000 nm, 35000 nm, 40000 nm, 45000 nm, 50000 nm and any number in between). A nanoscale sensor element refers to a sensor element that is less than 1 micron in at least one direction. Suitable examples of ranges of nanoscale sensor elements include, but are not limited to, for example, elements from about 5 nm to about 1000 nm in one direction, including, from example, about 5 nm to about 500 nm, alternatively about 5 nm to about 400 nm, alternatively about 5 nm to about 300 nm, alternatively about 5 nm to about 200 nm, alternatively about 5 nm to about 100 nm, alternatively about 5 nm to about 50 nm, alternatively about 10 nm to about 1000 nm, alternatively about 10 nm to about 750 nm, alternatively about 10 nm to about 500 nm, alternatively about 10 nm to about 250 nm, alternatively about 10 nm to about 200 nm, alternatively about 10 nm to about 100 nm, alternatively about S0 nm to about 1000 nm, alternatively about 50 nm to about 500 nm, alternatively about 50 nm to about 250 nm, alternatively about 50 nm to about 200 nm, alternatively about 50 nm to about 100 nm, and any combinations, ranges or amount in-between (e.g. 5 nm, 10 nm, 15 nm, 20 nm, 25 nm, 30 nm, 35 nm, 40 nm, 45 nm, S0 nm, 55 nm, 60 nm, 65 nm, 70 nm, 80 nm, 90 nm, 100 nm, 125 nm, 150 nm, 175 nm, 200 nm, 225 nm, 250 nm, 275 nm, 300 nm, 350 nm, 400 nm, 450 nm, 500 nm, 550 nm, 600 nm, 650 nm, 700 nm, 750 nm, 800 nm, 850 nm, 900 nm, 1000 nm, etc.). In reference to the sensor arrays described herein, the use of the term sensor element includes the use of a nanoscale sensor element for the sensor element and associated methods.

[0178] The term “plurality of sensor elements” refers to more than one, for example, at least two sensor elements. In some embodiments, the plurality of sensor elements includes at least two sensor elements to at least 10^{15} sensor elements. In some embodiments, the plurality of sensor elements includes 10^{6}-10^{7}, 10^{6}-10^{8}, 10^{6}-10^{9}, 10^{6}-10^{10}, 10^{6}-10^{11}, 10^{6}-10^{12}, 10^{6}-10^{13}, 10^{6}-10^{14}, 10^{6}-10^{15}, 10^{7}-10^{8}, 10^{7}-10^{9}, 10^{7}-10^{10}, 10^{7}-10^{11}, 10^{7}-10^{12}, 10^{7}-10^{13}, 10^{7}-10^{14}, 10^{7}-10^{15}, 10^{8}-10^{9}, 10^{8}-10^{10}, 10^{8}-10^{11}, 10^{8}-10^{12}, 10^{8}-10^{13}, 10^{8}-10^{14}, 10^{8}-10^{15}, 10^{9}-10^{10}, 10^{9}-10^{11}, 10^{9}-10^{12}, 10^{9}-10^{13}, 10^{9}-10^{14}, 10^{9}-10^{15}, 10^{10}-10^{11}, 10^{10}-10^{12}, 10^{10}-10^{13}, 10^{10}-10^{14}, 10^{10}-10^{15}, 10^{11}-10^{12}, 10^{11}-10^{13}, 10^{11}-10^{14}, 10^{11}-10^{15}, 10^{12}-10^{13}, 10^{12}-10^{14}, 10^{12}-10^{15}, 10^{13}-10^{14}, 10^{13}-10^{15}, or 10^{14}-10^{15} different sensor elements.

[0179] In some embodiments, a plurality of sensor elements comprises a plurality of types of sensor elements. A plurality of sensor elements may comprise at least two to at least 1000 types of sensor elements, alternatively at least two to at least 50 types of sensor elements, alternatively at least 2 to 30 types of sensor elements, alternatively at least 2 to 20 types of sensor elements, alternatively at least 2 to 10 types of sensor elements, alternatively at least 3 to at least 50 types of sensor elements, alternatively at least 3 to at least 30 types of sensor elements, alternatively at least 3 to at least 20 types of sensor elements, alternatively at least 3 to at least 10 types of sensor elements, alternatively at least 4 to at least 50 types of sensor elements, alternatively at least 4 to at least 30 types of sensor elements, alternatively at least 4 to at least 20 types of sensor elements, alternatively at least 4 to at least 10 types of sensor elements, and including any number of types of sensor elements contemplated in between (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, etc.). The plurality of sensor elements may comprise at least 6 types of sensor elements to at least 20 types of sensor elements, or alternatively at least 6 types of sensor elements to at least 10 types of sensor elements.

[0180] In some cases, increasing the number of sensor elements can be a method for increasing the number of biomolecules (e.g., proteins) that can be identified in a given sample. An example of how increasing panel size may increase the number of identified proteins is shown in FIG. 10. This figure shows the number of proteins identified from corona analysis in assays utilizing panels with 1 to 12 particle types. In these assays, distinct proteins, as opposed to protein groups, were identified through mass spectrometric analysis. The number of types of proteins identified increased with increasing number of particle types, spanning from 419 unique identified proteins when one particle type was used to collect proteins, to 1318 unique identified proteins for when 12 types of particles were used to collect proteins.

[0181] The sensor elements may be functionalized to have a wide range of physicochemical properties. Suitable methods of functionalizing the sensor elements are known in the art and depend on composition of the sensor element (e.g. gold, iron oxide, silica, silver, etc.), and include, but are not limited to, for example aminopropyl functionalized, amine functionalized, boronic acid functionalized, carboxylic acid functionalized, methyl functionalized, succinimidyl ester functionalized, PEG functionalized, streptavidin functionalized, methyl ether functionalized, triethoxylpropylaminosilane functionalized, thiol functionalized, PCP functionalized, citrate functionalized, lipoic acid functionalized, BPEI functionalized, carboxyl

functionalized, hydroxyl functionalized, and the like. In one embodiment, the sensor elements may be functionalized with an amine group (-NH_2 or a carboxyl group (COOH). In some embodiments, the nanoscale sensor elements are functionalized with a polar functional group. Non-limiting examples of the polar functional group comprise carboxyl group, a hydroxyl group, a thiol group, a cyano group, a nitro group, an ammonium group, an imidazolium group, a sulfonium group, a pyridinium group, a pyrrolidinium group, a phosphonium group or any combination thereof. In some embodiments, the functional group is an acidic functional group (e.g., sulfonic acid group, carboxyl group, and the like), a basic functional group (e.g., amino group, cyclic secondary amino group (such as pyrrolidyl group and piperidyl group), pyridyl group, imidazole group, guanidine group, etc.), a carbamoyl group, a hydroxyl group, an aldehyde group and the like. In some embodiments, the polar functional group is an ionic functional group. Non-limiting examples of the ionic function group comprise an ammonium group, an imidazolium group, a sulfonium group, a pyridinium group, a pyrrolidinium group, a phosphonium group. In some embodiments, the sensor elements are functionalized with a polymerizable functional group. Non-limiting examples of the polymerizable functional group include a vinyl group and a (meth)acrylic group. In some embodiments, the functional group is pyrrolidyl acrylate, acrylic acid, methacrylic acid, acrylamide, 2-(dimethylamino)ethyl methacrylate, hydroxyethyl methacrylate and the like.

[0182] The physicochemical properties of the sensor elements may be modified by modification of the surface charge. For example, the surface can be modified to provide a net neutral charge, a net positive surface charge, a net negative surface charge, or a zwitterionic charge. The charge of the surface can be controlled either during synthesis of the element or by post-synthesis modification of the charge through surface functionalization. For polymeric sensor elements (e.g., polymeric particles), differences in charge can be obtained during synthesis by using different synthesis procedures, different charged comonomers, and in inorganic substances by having mixed oxidation states.

[0183] Non-limiting examples of the plurality of sensor elements include, but are not limited to, (a) a plurality of sensor elements made of the same material but differing in physiochemical properties, (b) a plurality of sensor elements where one or more sensor element is made of a different material with the same or differing physiochemical properties, (c) a plurality of sensor elements made of the same material differing in size, (d) a plurality of sensor elements made of different material with relatively the same size; (e) a plurality of sensor elements made of different material and made of different sizes, (f) a plurality of sensor elements in which each element is made of a different material, (g) a plurality of sensor elements having different

charges, among others. The plurality of sensor elements can be in any suitable combination of two or more sensor elements in which each sensor element provides a unique biomolecule corona signature. For example, the plurality of sensor elements may include one or more liposomes and one or more particles described herein. In one embodiment, the plurality of sensor elements can be a plurality of liposomes with varying lipid content and/or varying charges (cationic/anionic/neutral). In another embodiment, the plurality of sensors may contain one or more nanoparticle made of the same material but of varying sizes and physiochemical properties. In another embodiment, the plurality of sensors may contain one or more particle made of differing materials (e.g. silica and polystyrene) with similar or varying sizes and/or physiochemical properties (e.g. modifications, for example, $-NH_2$, -COOH functionalization). These combinations are purely provided as examples and are non- limiting to the scope of the disclosure.

[0184] A sensor element may comprise a particle, such as a nanoparticle or a microparticle. A sensor element may be a particle, such as a nanoparticle or a microparticle. A sensor element may comprise a surface or a portion of a surface of a material. A sensor element may comprise a porous material (e.g., a polymer matrix) into which biomolecules can intercalate. A sensor element may comprise a material with projections, such as polymers, oligomers, or metal dendrites. A sensor element may comprise an aggregate of particles, such as a nanoworm.

Particle Materials

[0185] A plurality of particles disclosed herein can be made of a variety of different materials. A plurality of particles can comprise specific types of nanoparticles to identify a broad range of proteins in the sample, or to selectively assay for a particular protein or set of proteins of interest.

[0186] A plurality of particles may comprise at least 1 particle distinct type, at least 2 distinct particle types, at least 3 distinct particle types, at least 4 distinct particle types, at least 5 distinct particle types, at least 6 distinct particle types, at least 7 distinct particle types, at least 8 distinct particle types, at least 9 distinct particle types, at least 10 distinct particle types, at least 1 1 distinct particle types, at least 12 distinct particle types, at least 13 distinct particle types, at least 14 distinct particle types, at least 15 distinct particle types, at least 16 distinct particle types, at least 17 distinct particle types, at least 18 distinct particle types, at least 19 distinct particle types, at least 20 distinct particle types, at least 25 distinct particle types, at least 30 distinct particle types, at least 35 distinct particle types, at least 40 distinct particle types, at least 45 distinct particle types, at least 50 distinct particle types, at least 55 distinct particle types, at least 60 distinct particle types, at least 65 distinct particle types, at least 70 distinct particle types, at least 75 distinct particle types, at least 80 distinct particle types, at least 85 distinct particle types, at

least 90 distinct particle types, at least 95 distinct particle types, at least 100 distinct particle types, from 1 to 5 distinct particle types, from 5 to 10 distinct particle types, from 10 to 15 distinct particle types, from 15 to 20 distinct particle types, from 20 to 25 distinct particle types, from 25 to 30 distinct particle types, from 30 to 35 distinct particle types, from 35 to 40 distinct particle types, from 40 to 45 distinct particle types, from 45 to 50 distinct particle types, from 50 to 55 distinct particle types, from 55 to 60 distinct particle types, from 60 to 65 distinct particle types, from 65 to 70 distinct particle types, from 70 to 75 distinct particle types, from 75 to 80 distinct particle types, from 80 to 85 distinct particle types, from 85 to 90 distinct particle types, from 90 to 95 distinct particle types, from 95 to 100 distinct particle types, from 1 to 100 distinct particle types, from 20 to 40 distinct particle types, from 5 to 10 distinct particle types, from 3 to 7 distinct particle types, from 2 to 10 distinct particle types, from 6 to 15 distinct particle types, or from 10 to 20 distinct particle types. A plurality of particles may comprise from 3 to 10 particle types. A plurality of particles may comprise from 4 to 11 distinct particle types. A plurality of particles may comprise from 5 to 15 distinct particle types. A plurality of particles may comprise from 5 to 15 distinct particle types. A plurality of particles may comprise from 8 to 12 distinct particle types. A plurality of particles may comprise from 9 to 13 distinct particle types. A plurality of particles may comprise 10 distinct particle types. The particle types may include nanoparticles.

[0187] For example, the present disclosure a plurality of particles having at least 2 distinct particle types, at least 3 different surface chemistries, at least 4 different surface chemistries, at least 5 different surface chemistries, at least 6 different surface chemistries, at least 7 different surface chemistries, at least 8 different surface chemistries, at least 9 different surface chemistries, at least 10 different surface chemistries, at least 11 different surface chemistries, at least 12 different surface chemistries, at least 13 different surface chemistries, at least 14 different surface chemistries, at least 15 different surface chemistries, at least 20 different surface chemistries, at least 25 different surface chemistries, at least 30 different surface chemistries, at least 35 different surface chemistries, at least 40 different surface chemistries, at least 45 different surface chemistries, at least 50 different surface chemistries, at least 100 different surface chemistries, at least 150 different surface chemistries, at least 200 different surface chemistries, at least 250 different surface chemistries, at least 300 different surface chemistries, at least 350 different surface chemistries, at least 400 different surface chemistries, at least 450 different surface chemistries, at least 500 different surface chemistries, from 2 to 500 different surface chemistries, from 2 to 5 different surface chemistries, from 5 to 10 different surface chemistries, from 10 to 15 different surface chemistries, from 15 to 20 different surface

chemistries, from 20 to 40 different surface chemistries, from 40 to 60 different surface chemistries, from 60 to 80 different surface chemistries, from 80 to 100 different surface chemistries, from 100 to 500 different surface chemistries, from 4 to 15 different surface chemistries, or from 2 to 20 different surface chemistries.

[0188] The present disclosure provides a plurality of particles having at least 2 different physical properties, at least 3 different physical properties, at least 4 different physical properties, at least 5 different physical properties, at least 6 different physical properties, at least 7 different physical properties, at least 8 different physical properties, at least 9 different physical properties, at least 10 different physical properties, at least 1 1 different physical properties, at least 12 different physical properties, at least 13 different physical properties, at least 14 different physical properties, at least 15 different physical properties, at least 20 different physical properties, at least 25 different physical properties, at least 30 different physical properties, at least 35 different physical properties, at least 40 different physical properties, at least 45 different physical properties, at least 50 different physical properties, at least 100 different physical properties, at least 150 different physical properties, at least 200 different physical properties, at least 250 different physical properties, at least 300 different physical properties, at least 350 different physical properties, at least 400 different physical properties, at least 450 different physical‘ properties, at least 500 different physical properties, from 2 to 500 different physical properties, from 2 to 5 different physical properties, from 5 to 10 different physical properties, from 10 to 15 different physical properties, from 15 to 20 different physical properties, from 20 to 40 different physical properties, from 40 to 60 different physical properties, from 60 to 80 different physical properties, from 80 to 100 different physical properties, from 100 to 500 different physical properties, from 4 to 15 different physical properties, or from 2 to 20 different physical properties.

[0189] Particles can be made from various materials. For example, nanoparticle materials consistent with the present disclosure include metals, polymers, magnetic materials, and lipids. Magnetic nanoparticles may be iron oxide nanoparticles. Examples of metal materials include any one of or any combination of gold, silver, copper, nickel, cobalt, palladium, platinum, iridium, osmium, rhodium, ruthenium, rhenium, vanadium, chromium, manganese, niobium, molybdenum, tungsten, tantalum, iron and cadmium, or any other material described in US7749299.

[0190] Examples of polymers include any one of or any combination of polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl

alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, or polyamines, a polyalkylene glycol (e.g., polyethylene glycol (PEG)), a polyester (e.g., poly(lactide-co-glycolide) (PLGA), polylactic acid, or polycaprolactone), or a copolymer of two or more polymers, such as a copolymer of a polyalkylene glycol (e.g., PEG) and a polyester (e.g., PLGA). In some embodiments, the polymer is a lipid-terminated polyalkylene glycol and a polyester, or any other material disclosed in US9549901. A polymer may also be a liposome.

[0191] Examples of lipids that can be used to form the nanoparticles of the present disclosure include cationic, anionic, and neutrally charged lipids. For example, nanoparticles can be made of any one of or any combination of dioleoylphosphatidylglycerol (DOPG), diacylphosphatidylcholine, diacylphosphatidylethanolamine, ceramide, sphingomyelin, cephalin, cholesterol, cerebrosides and diacylglycerols, dioleoylphosphatidylcholine (DOPC), dimyristoylphosphatidylcholine (DMPC), and dioleoylphosphatidylserine (DOPS), phosphatidylglycerol, cardiolipin, diacylphosphatidylserine, diacylphosphatidic acid, N-dodecanoyl phosphatidylethanolamines, N-succinyl phosphatidylethanolamines, N-glutarylphosphatidylethanolamines, lysylphosphatidylglycerols, palmitoyloleyolphosphatidylglycerol (POPG), lecithin, lysolecithin, phosphatidylethanolamine, lysophosphatidylethanolamine, dioleoylphosphatidylethanolamine (DOPE), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), palmitoyloleoyl-phosphatidylethanolamine (POPE) palmitoyloleoylphosphatidylcholine (POPC), egg phosphatidylcholine (EPC), distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), palmitoyloleyolphosphatidylglycerol (POPG), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, palmitoyloleoyl-phosphatidylethanolamine (POPE), 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), phosphatidylserine, phosphatidylinositol, sphingomyelin, cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicetylphosphate, and cholesterol, or any other material listed in US9445994.

[0192] In various cases, the core of the nanoparticles can include an organic particle, an inorganic particle, or a particle including both organic and inorganic materials. For example, the particles can have a core structure that is or includes a metal particle, a quantum dot particle, a metal oxide particle, or a core-shell particle. For example, the core structure can be or include a polymeric particle or a lipid-based particle, and the linkers can include a lipid, a surfactant, a polymer, a hydrocarbon chain, or an amphiphilic polymer. For example, the linkers can include

polyethylene glycol or polyalkylene glycol, e.g., the first ends of the linkers can include a lipid bound to polyethelene glycol (PEG) and the second ends can include functional groups bound to the PEG. A particle may have a core-shell structure. In some cases, a particle has a core comprising a first material or composite and a plurality of shells comprising different materials or composites. In some cases, a particle has a magnetic core surrounded by a non-magnetic or plurality of non-magnetic shells. For example, a particle may comprise a magnetic iron oxide core surrounded by a non-magnetic polymer shell. In some cases, magnetic core has a 10 nm to 500 nm diameter, and the shell has a 5 nm to 100 nm thickness.

[0193] Examples of particle types consistent with the present disclosure are shown in **TABLE 1** below. Additional examples of particles, such as magnetic core nanoparticles (MNP) and corresponding surface chemistries are illustrated in **FIG. 7**.

TABLE 1: Particle Types

P#	Description	Vendor
HX-13 or S-001	Carboxylate (Citrate)	Seer
HX-19 or S-002	Phenol-formaldehyde coated	Seer
HX-31 or S-004	Polystyrene coated	Seer
HX-38 or S-005	Polystyrene/carboxylate coated	Seer
HX-42 or S-006	Silica coated, amine	Seer
HX-57 or S-008	Benzoic acid	Seer
HX-58 or S-009	PVBTMAC coated (Vinylbenzyltrimethylammonium chloride)	Seer
HX-59 or S-010	Carboxylate, PAA coated	Seer
P-033	Carboxylate	Polysciences
P-039	Polystyrene Carboxyl	Micro Particles
P-041	Carboxylic acid	OceanNanoTech
P-047	Silica	OceanNanoTech
P-048	Carboxylic acid	OceanNanoTech

P#	Description	Vendor
P-053	Amino	Spherotech
P-056	Silica Amino	Spherotech
P-063	Jeffamine	Spherotech
P-064	Polystyrene	Spherotech
P-065	Silica	Spherotech
P-069	Original coating	OceanNanoTech
P-073	Dextran based	Kisker Biotech
P-074	Silica Silanol	Kisker Biotech
HX-20 or S-003	Silica-coated superparamagnetic iron oxide NPs (SPION)	Seer
HX-56 or S-007	poly(N-(3-(dimethylamino)propyl) methacrylamide) (PDMAPMA)-coated SPION	Seer
HX-86 or S-011	poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA)-coated SPION	Seer

Properties of Particles

[0194] Nanoparticles that are consistent with the present disclosure can be made and used in methods of forming protein coronas after incubation in a biofluid at a wide range of sizes. For example, the nanoparticles disclosed herein can be at least 10 nm, at least 100 nm, at least 200 nm, at least 300 nm, at least 400 nm, at least 500 nm, at least 600 nm, at least 700 nm, at least 800 nm, at least 900 nm, from 10 nm to 50 nm, from 50 nm to 100 nm, from 100 nm to 150 nm, from 150 nm to 200 nm, from 200 nm to 250 nm, from 250 nm to 300 nm, from 300 nm to 350 nm, from 350 nm to 400 nm, from 400 nm to 450 nm, from 450 nm to 500 nm, from 500 nm to 550 nm, from 550 nm to 600 nm, from 600 nm to 650 nm, from 650 nm to 700 nm, from 700 nm to 750 nm, from 750 nm to 800 nm, from 800 nm to 850 nm, from 850 nm to 900 nm, from 100 nm to 300 nm, from 150 nm to 350 nm, from 200 nm to 400 nm, from 250 nm to 450 nm, from 300 nm to 500 nm, from 350 nm to 550 nm, from 400 nm to 600 nm, from 450 nm to 650 nm, from 500 nm to 700 nm, from 550 nm to 750 nm, from 600 nm to 800 nm, from 650 nm to 850 nm, from 700 nm to 900 nm, or from 10 nm to 900 nm.

[0195] Additionally, particles can have a homogenous size distribution or a heterogeneous size distribution. Polydispersity index (PDI), which can be measured by techniques such as dynamic light scattering, is a measure of the size distribution. A low PDI indicates a more homogeneous size distribution and a higher PDI indicates a more heterogeneous size distribution. In some cases, a plurality of particles has a PDI of 0.01 to 0.1, 0.1 to 0.5, 0.5 to 1, 1 to 5, 5 to 20, or greater than 20.

[0196] Particles disclosed herein can have a range of different surface charges. Particles can be negatively charged, positively charged, or neutral in charge. In some embodiments, particles have a surface charge of -500 mV to -450 mV, -450 mV to -400 mV, -400 mV to -350 mV, -350 mV to -300 mV, -300 mV to -250 mV, -250 mV to -200 mV, -200 mV to -150 mV, -150 mV to -100 mV, -100 mV to -90 mV, -90 mV to -80 mV, -80 mV to -70 mV, -70 mV to -60 mV, -60 mV to -50 mV, -50 mV to -40 mV, -40 mV to -30 mV, -30 mV to -20 mV, -20 mV to -10 mV, -10 mV to 0 mV, 0 mV to 10 mV, 10 mV to 20 mV, 20 mV to 30 mV, 30 mV to 40 mV, 40 mV to 50 mV, 50 mV to 60 mV, 60 mV to 70 mV, 70 mV to 80 mV, 80 mV to 90 mV, 90 mV to 100 mV, 100 mV to 110 mV, 110 mV to 120 mV, 120 mV to 130 mV, 130 mV to 140 mV, 140 mV to 150 mV, 150 mV to 200 mV, 200 mV to 250 mV, 250 mV to 300 mV, 300 mV to 350 mV, 350 mV to 400 mV, 400 mV to 450 mV, 450 mV to 500 mV, -500 mv to -400 mV, -400 mv to -300 mV, -300 mv to -200 mV, -200 mv to -100 mV, -100 mv to 0 mV, 0 mv to 100 mV, 100 mv to 200 mV, 200 mv to 300 mV, 300 mv to 400 mV, or 400 mv to 500 mV.

[0197] Various particle morphologies are consistent with the particle types in panels of the present disclosure. For example, particles may be spherical, colloidal, square shaped, rods, wires, cones, pyramids, or oblong.

Biomolecule Coronas

[0198] Provided herein are automated apparatuses, systems, methods, and sensor elements capable of generating biomolecule coronas comprising, consisting essentially of or consisting of a plurality of sensor elements wherein the plurality of sensor elements differ from each other in at least one physicochemical property. The plurality of sensor elements may comprise a plurality of particles (e.g., nanoparticles). The plurality of sensor elements may be a plurality of particles. A plurality of sensor elements may be able to bind a plurality of biomolecules in a complex biological sample to produce a biomolecule corona signature. A plurality of sensor elements may comprise a plurality of distinct biomolecule corona signatures.

[0199] A biomolecule of interest (e.g., a low abundance protein) may be enriched in a biomolecule corona relative to the untreated sample (e.g., a sample that is not assayed using particles). The biomolecule of interest may be a protein. The biomolecule corona may be a protein corona. A level of enrichment may be the percent increase or fold increase in relative abundance of the biomolecule of interest (e.g., number of copies of the biomolecule of interest versus the total number of biomolecules) in the biomolecule corona as compared to the biological sample from which the biomolecule corona was collected. A biomolecule of interest may be enriched in a biomolecule corona by increasing the abundance of the biomolecule of interest in the biomolecule corona as compared to the sample that has not been contacted to the

sensor element. A biomolecule of interest may be enriched by decreasing the abundance of a biomolecule that is in high abundance biological sample.

[0200] A biomolecule corona analysis assay may be used to rapidly identify low abundance biomolecules in a biological sample (e.g., a biofluid). Biomolecule corona analysis may be used to identify at least about 500 low abundance biomolecules in a biological sample in no more than about 8 hours from first contacting the biological sample with a sensor element (e.g., a particle). Biomolecule corona analysis may identify at least about 1000 low abundance biomolecules in a biological sample in no more than about 8 hours from first contacting the biological sample with a sensor element. Biomolecule corona analysis may identify at least about 500 low abundance biomolecules in a biological sample in no more than about 4 hours from first contacting the biological sample with a sensor element. Biomolecule corona analysis may identify at least about 1000 low abundance biomolecules in a biological sample in no more than about 4 hours from first contacting the biological sample with a sensor element.

[0201] A biomolecule corona signature may comprise a protein, a peptide, a polysaccharide, an oligosaccharide, a monosaccharide, a metabolite, a lipid, a nucleic acid, or any combination thereof. The biomolecule corona signature may be a protein corona signature. The biomolecule corona signature may be a polysaccharide corona signature. The biomolecule corona signature may be a metabolite corona signature. The biomolecule corona signature may be a lipidomic corona signature. The biomolecule corona signature may comprise the biomolecules found in a soft corona and a hard corona. The soft corona may be a soft protein corona. The hard corona may be a hard protein corona.

[0202] The biomolecule corona signature refers to the composition, signature or pattern of different biomolecules that are bound to each separate sensor element or each nanoparticle. In some cases, the biomolecule corona signature is a protein corona signature. In another case, the biomolecule corona signature is a polysaccharide corona signature. In yet another case, the biomolecule corona signature is a metabolite corona signature. In some cases, the biomolecule corona signature is a lipidomic corona signature. The signature can refer to the different biomolecules. It can also refer to the differences in the amount, level or quantity of the biomolecule bound to the sensor element or the nanoparticle, or differences in the conformational state of the biomolecule that is bound to the sensor element or the particle. It is contemplated that the biomolecule corona signatures of each sensor elements may contain some of the same biomolecules, may contain distinct biomolecules with regard to the other sensor elements or nanoparticles, and/or may differ in level or quantity, type or confirmation of the biomolecule. The biomolecule corona signature may depend on not only the physicochemical

properties of the sensor element or the particle, but also the nature of the sample and the duration of exposure. In some embodiments, the biomolecule corona signature comprises the biomolecules found in a soft corona and a hard corona.

[0203] In some embodiments, a plurality of sensor elements includes a first sensor element that produces a first biomolecule corona signature and at least one second sensor element (e.g., at least one nanoparticle) that produces at least one second biomolecule corona signature when the sensor array is contacted with a complex biological sample. In some cases, each type of sensor element from among a plurality of sensor elements produces a different biomolecule corona signature.

[0204] The plurality of sensor elements when contacted with a sample produces a plurality of biomolecule corona signatures which together can form a biomolecule fingerprint. The "biomolecule fingerprint" refers to the combined composition or pattern of biomolecules of at least two biomolecule corona signatures for the plurality of sensor elements. It is contemplated that the biomolecule fingerprint can be made from at least two biomolecule corona signatures to as many different biomolecule signatures are assayed, e.g. at least 1000 different biomolecule corona signatures. The biomolecule corona can be assayed separately for each sensor element to determine the biomolecule corona signature for each sensor element (e.g., each nanoparticle or each liposome) and combined to form the biomolecule fingerprint. In some cases, the biomolecule fingerprint can be developed by assaying the two or more biomolecule coronas simultaneously.

Identified Proteins

[0205] The automated apparatuses, systems, methods, and sensor elements (e.g., particles) disclosed herein can be used to identify a number of biomolecules, proteins, peptides, or protein groups. Feature intensities, as disclosed herein, refers to the intensity of a signal from an analytical measurement, for example the intensity of a mass to charge ratio from a mass spectrometry run of a sample. Using the data analysis methods described herein, feature intensities of peptides and peptide fragments can be sorted into protein groups. Protein groups refer to two or more proteins that are identified by a shared peptide sequence. Alternatively, a protein group can refer to one protein that is identified using a unique identifying sequence. For example, if in a sample, a peptide sequence is assayed that is shared between two proteins (Protein 1: XYZZX and Protein 2: XYZYZ), a protein group could be the "XYZ protein group" having two members (protein 1 and protein 2). Alternatively, if the peptide sequence is unique to a single protein (Protein 1), a protein group could be the "ZZX" protein group having one member (Protein 1). Each protein group can be supported by more than one peptide sequence.

Protein detected or identified according to the instant disclosure can refer to a distinct protein detected in the sample (e.g., distinct relative other proteins detected using mass spectrometry). Thus, analysis of proteins present in distinct coronas corresponding to the distinct sensor element types yields a high number of feature intensities. This number decreases as feature intensities are processed into distinct peptides, further decreases as distinct peptides are processed into distinct proteins, and further decreases as peptides are grouped into protein groups (two or more proteins that share a distinct peptide sequence).

[0206] The automated apparatuses, systems, methods, and sensor elements (e.g., particles) disclosed herein can be used to identify at least at least 100 protein groups, at least 200 protein groups, at least 300 protein groups, at least 400 protein groups, at least 500 protein groups, at least 600 protein groups, at least 700 protein groups, at least 800 protein groups, at least 900 protein groups, at least 1000 protein groups, at least 1100 protein groups, at least 1200 protein groups, at least 1300 protein groups, at least 1400 protein groups, at least 1500 protein groups, at least 1600 protein groups, at least 1700 protein groups, at least 1800 protein groups, at least 1900 protein groups, at least 2000 protein groups, at least 2100 protein groups, at least 2200 protein groups, at least 2300 protein groups, at least 2400 protein groups, at least 2500 protein groups, at least 2600 protein groups, at least 2700 protein groups, at least 2800 protein groups, at least 2900 protein groups, at least 3000 protein groups, at least 3100 protein groups, at least 3200 protein groups, at least 3300 protein groups, at least 3400 protein groups, at least 3500 protein groups, at least 3600 protein groups, at least 3700 protein groups, at least 3800 protein groups, at least 3900 protein groups, at least 4000 protein groups, at least 4100 protein groups, at least 4200 protein groups, at least 4300 protein groups, at least 4400 protein groups, at least 4500 protein groups, at least 4600 protein groups, at least 4700 protein groups, at least 4800 protein groups, at least 4900 protein groups, at least 5000 protein groups, at least 10000 protein groups, at least 20000 protein groups, at least 100000 protein groups, from 100 to 5000 protein groups, from 200 to 4700 protein groups, from 300 to 4400 protein groups, from 400 to 4100 protein groups, from 500 to 3800 protein groups, from 600 to 3500 protein groups, from 700 to 3200 protein groups, from 800 to 2900 protein groups, from 900 to 2600 protein groups, from 1000 to 2300 protein groups, from 1000 to 3000 protein groups, from 3000 to 4000 protein groups, from 4000 to 5000 protein groups, from 5000 to 6000 protein groups, from 6000 to 7000 protein groups, from 7000 to 8000 protein groups, from 8000 to 9000 protein groups, from 9000 to 10000 protein groups, from 10000 to 11000 protein groups, from 11000 to 12000 protein groups, from 12000 to 13000 protein groups, from 13000 to 14000 protein groups, from 14000 to 15000 protein groups, from 15000 to 16000 protein groups, from 16000 to 17000 protein groups, from 17000 to 18000

protein groups, from 18000 to 19000 protein groups, from 19000 to 20000 protein groups, from 20000 to 25000 protein groups, from 25000 to 30000 protein groups, from 10000 to 20000 protein groups, from 10000 to 50000 protein groups, from 20000 to 100000 protein groups, from 2000 to 20000 protein groups, from 1800 to 20000 protein groups, or from 10000 to 100000 protein groups.

[0207] The automated apparatuses, systems, methods, and sensor elements (e.g., particles) disclosed herein can be used to identify at least at least 100 proteins, at least 200 proteins, at least 300 proteins, at least 400 proteins, at least 500 proteins, at least 600 proteins, at least 700 proteins, at least 800 proteins, at least 900 proteins, at least 1000 proteins, at least 1100 proteins, at least 1200 proteins, at least 1300 proteins, at least 1400 proteins, at least 1500 proteins, at least 1600 proteins, at least 1700 proteins, at least 1800 proteins, at least 1900 proteins, at least 2000 proteins, at least 2100 proteins, at least 2200 proteins, at least 2300 proteins, at least 2400 proteins, at least 2500 proteins, at least 2600 proteins, at least 2700 proteins, at least 2800 proteins, at least 2900 proteins, at least 3000 proteins, at least 3100 proteins, at least 3200 proteins, at least 3300 proteins, at least 3400 proteins, at least 3500 proteins, at least 3600 proteins, at least 3700 proteins, at least 3800 proteins, at least 3900 proteins, at least 4000 proteins, at least 4100 proteins, at least 4200 proteins, at least 4300 proteins, at least 4400 proteins, at least 4500 proteins, at least 4600 proteins, at least 4700 proteins, at least 4800 proteins, at least 4900 proteins, at least 5000 proteins, from 100 to 5000 proteins, from 200 to 4700 proteins, from 300 to 4400 proteins, from 400 to 4100 proteins, from 500 to 3800 proteins, from 600 to 3500 proteins, from 700 to 3200 proteins, from 800 to 2900 proteins, from 900 to 2600 proteins, from 1000 to 2300 proteins, from 1000 to 3000 proteins, from 3000 to 4000 proteins, from 4000 to 5000 proteins, from 5000 to 6000 proteins, from 6000 to 7000 proteins, from 7000 to 8000 proteins, from 8000 to 9000 proteins, from 9000 to 10000 proteins, from 10000 to 11000 proteins, from 11000 to 12000 proteins, from 12000 to 13000 proteins, from 13000 to 14000 proteins, from 14000 to 15000 proteins, from 15000 to 16000 proteins, from 16000 to 17000 proteins, from 17000 to 18000 proteins, from 18000 to 19000 proteins, from 19000 to 20000 proteins, from 20000 to 25000 proteins, from 25000 to 30000 proteins, or from 10000 to 20000 proteins.

[0208] The sensor elements disclosed herein can be used to identify the number of distinct proteins disclosed herein, and/or any of the specific proteins disclosed herein, over a wide dynamic range. For example, a plurality of particles disclosed herein comprising distinct particle types, can enrich for proteins in a sample, which can be identified using the methods of the present disclosure, over the entire dynamic range at which proteins are present in a sample (e.g.,

a plasma sample). A particle panel may include any number of distinct particle types disclosed herein, and may enrich and identify biomolecules over a concentration range of at least 2 to at least 12 orders of magnitude in a sample.

Disease Detection

[0209] The systems and methods described herein can be used for detection of markers in a sample from a subject, which are consistent with a particular biological (e.g., disease) state. The biological state may be a disease, disorder, or tissue abnormality. The disease state may be an early phase or intermediate phase disease state.

[0210] The systems and methods of the present disclosure can be used to detect a wide range of disease states in a given sample. For example, the systems and methods of the present disclosure can be used to detect a cancer. The cancer may be brain cancer, lung cancer, pancreatic cancer, glioblastoma, meningioma, myeloma, or pancreatic cancer.

[0211] In some cases, a biomolecule fingerprint can be used to determine the disease state of a subject, diagnose or prognose a disease in a subject or identify unique patterns of biomarkers that are associated with a disease state or a disease or disorder. For example, the changes in the biomolecule fingerprint in a subject over time (days, months, years) allows for the ability to track a disease or disorder in a subject (e.g. disease state) which may be broadly applicable to determination of a biomolecule fingerprint that can be associated with the early stage of a disease or any other disease state. As disclosed herein, the ability to detect a disease early on, for example cancer, even before it fully develops or metastasizes allows for a significant increase in positive outcomes for those patients and the ability to increase life expectancy and lower mortality associated with that disease.

[0212] The automated apparatuses, systems, methods, and sensor elements (e.g., particles) disclosed herein can provide a unique opportunity to be able to develop biomolecule fingerprints associated with the pre-stages or precursor states of the disease in a high-throughput fashion. The present disclosure provides for large scale, fast processing of samples to generate biomolecule fingerprints in a high throughput manner, thereby allowing for large scale determination of disease state of a subject, diagnosis or prognosis a disease in a subject or identification of unique patterns of biomarkers that are associated with a disease state or a disease or disorder, across many subjects.

[0213] In some embodiments, a method of detecting a disease or disorder in a subject are provided. The method comprises the steps of (a) obtaining a sample from the subject; (b) contacting the sample with a sensor array as described herein, and (c) determining a biomolecule fingerprint associated with the sample, wherein the biomolecule fingerprint differentiates the

health of subject in a disease state, for example, from no disease or disorder, having a precursor of a disease or disorder, and having disease or disorder.

[0214] Determining whether a biomolecule fingerprint associated with the sample may comprise detecting the biomolecule corona signature for at least two sensor elements, wherein the combination of the at least two biomolecule corona signatures produces the biomolecule fingerprint. In some embodiments, the biomolecule corona signatures of the at least two sensor elements are assayed separately, and the results combined to determine the biomolecule fingerprint. In some embodiments the biomolecule corona signatures of the at least two elements are assayed at the same time or in the same sample.

[0215] The automated apparatuses, systems, sensor arrays, and methods described herein can be used to determine a disease state, and/or prognose or diagnose a disease or disorder. The diseases or disorders contemplated include, but are not limited to, for example, cancer, cardiovascular disease, endocrine disease, inflammatory disease, a neurological disease and the like.

[0216] In one embodiment, the disease or disorder is cancer. The term “cancer” is meant to encompass any cancer, neoplastic and preneoplastic disease that is characterized by abnormal growth of cells, including tumors and benign growths. Cancer may, for example, be lung cancer, pancreatic cancer, or skin cancer. In suitable embodiments, the automated apparatuses, systems, sensor arrays, and methods described herein are not only able to diagnose cancer (e.g. determine if a subject (a) does not have cancer, (b) is in a pre-cancer development stage, (c) is in early stage of cancer, (d) is in a late stage of cancer) but in some embodiments is able to determine the type of cancer. As demonstrated in the examples below, a sensor array comprising six sensor elements was able to accurately determine the disease state of the presence or absence of cancer. Additionally, the Examples demonstrate that a sensor array comprising six sensor elements was able to distinguish between different cancer types (e.g. lung cancer, glioblastoma, meningioma, myeloma and pancreatic cancer).

[0217] The automated apparatuses, systems, sensor arrays, and methods of the present disclosure can additionally be used to detect other cancers, such as acute lymphoblastic leukemia (ALL); acute myeloid leukemia (AML); cancer in adolescents; adrenocortical carcinoma; childhood adrenocortical carcinoma; unusual cancers of childhood; AIDS-related cancers; kaposi sarcoma (soft tissue sarcoma); AIDS-related lymphoma (lymphoma); primary cns lymphoma (lymphoma); anal cancer; appendix cancer - see gastrointestinal carcinoid tumors; astrocytomas, childhood (brain cancer); atypical teratoid/rhabdoid tumor, childhood, central nervous system (brain cancer); basal cell carcinoma of the skin - see skin cancer; bile duct cancer; bladder cancer; childhood bladder cancer ; bone cancer (includes ewing sarcoma and osteosarcoma and

malignant fibrous histiocytoma); brain tumors; breast cancer; childhood breast cancer; bronchial tumors, childhood; burkitt lymphoma - see non-hodgkin lymphoma; carcinoid tumor (gastrointestinal); childhood carcinoid tumors; carcinoma of unknown primary; childhood carcinoma of unknown primary; cardiac (heart) tumors, childhood; central nervous system; atypical teratoid/rhabdoid tumor, childhood (brain cancer); embryonal tumors, childhood (brain cancer); germ cell tumor, childhood (brain cancer); primary cns lymphoma; cervical cancer; childhood cervical cancer; childhood cancers; cancers of childhood, unusual; cholangiocarcinoma - see bile duct cancer; chordoma, childhood; chronic lymphocytic leukemia (CLL); chronic myelogenous leukemia (CML); chronic myeloproliferative neoplasms; colorectal cancer; childhood colorectal cancer; craniopharyngioma, childhood (brain cancer); cutaneous t-cell lymphoma - see lymphoma (mycosis fungoides and sézary syndrome); ductal carcinoma in situ (DCIS) - see breast cancer; embryonal tumors, central nervous system, childhood (brain cancer); endometrial cancer (uterine cancer); ependymoma, childhood (brain cancer); esophageal cancer; childhood esophageal cancer; esthesioneuroblastoma (head and neck cancer); ewing sarcoma (bone cancer); extracranial germ cell tumor, childhood; extragonadal germ cell tumor; eye cancer; childhood intraocular melanoma; intraocular melanoma; retinoblastoma; fallopian tube cancer; fibrous histiocytoma of bone, malignant, and osteosarcoma; gallbladder cancer; gastric (stomach) cancer; childhood gastric (stomach) cancer; gastrointestinal carcinoid tumor; gastrointestinal stromal tumors (GIST) (soft tissue sarcoma); childhood gastrointestinal stromal tumors; germ cell tumors; childhood central nervous system germ cell tumors (brain cancer); childhood extracranial germ cell tumors; extragonadal germ cell tumors; ovarian germ cell tumors; testicular cancer; gestational trophoblastic disease; hairy cell leukemia; head and neck cancer; heart tumors, childhood; hepatocellular (liver) cancer; histiocytosis, langerhans cell; hodgkin lymphoma; hypopharyngeal cancer (head and neck cancer); intraocular melanoma; childhood intraocular melanoma; islet cell tumors, pancreatic neuroendocrine tumors; kaposi sarcoma (soft tissue sarcoma); kidney (renal cell) cancer; langerhans cell histiocytosis; laryngeal cancer (head and neck cancer); leukemia; lip and oral cavity cancer (head and neck cancer); liver cancer; lung cancer (non-small cell and small cell); childhood lung cancer; lymphoma; male breast cancer; malignant fibrous histiocytoma of bone and osteosarcoma; melanoma; childhood melanoma; melanoma, intraocular (eye); childhood intraocular melanoma; merkel cell carcinoma (skin cancer); mesothelioma, malignant; childhood mesothelioma; metastatic cancer; metastatic squamous neck cancer with occult primary (head and neck cancer); midline tract carcinoma with nut gene changes; mouth cancer (head and neck cancer); multiple endocrine neoplasia syndromes; multiple myeloma/plasma cell neoplasms; mycosis fungoides (lymphoma);

myelodysplastic syndromes, myelodysplastic/myeloproliferative neoplasms; myelogenous leukemia, chronic (cml); myeloid leukemia, acute (aml); myeloproliferative neoplasms, chronic; nasal cavity and paranasal sinus cancer (head and neck cancer); nasopharyngeal cancer (head and neck cancer); neuroblastoma; non-hodgkin lymphoma; non-small cell lung cancer; oral cancer, lip and oral cavity cancer and oropharyngeal cancer (head and neck cancer); osteosarcoma and malignant fibrous histiocytoma of bone; ovarian cancer; childhood ovarian cancer; pancreatic cancer; childhood pancreatic cancer; pancreatic neuroendocrine tumors (islet cell tumors); papillomatosis (childhood laryngeal); paraganglioma; childhood paraganglioma; paranasal sinus and nasal cavity cancer (head and neck cancer); parathyroid cancer; penile cancer; pharyngeal cancer (head and neck cancer); pheochromocytoma; childhood pheochromocytoma; pituitary tumor; plasma cell neoplasm/multiple myeloma; pleuropulmonary blastoma; pregnancy and breast cancer; primary central nervous system (CNS) lymphoma; primary peritoneal cancer; prostate cancer; rectal cancer; recurrent cancer; renal cell (kidney) cancer; retinoblastoma; rhabdomyosarcoma, childhood (soft tissue sarcoma); salivary gland cancer (head and neck cancer); sarcoma; childhood rhabdomyosarcoma (soft tissue sarcoma); childhood vascular tumors (soft tissue sarcoma); ewing sarcoma (bone cancer); kaposi sarcoma (soft tissue sarcoma); osteosarcoma (bone cancer); soft tissue sarcoma; uterine sarcoma; sézary syndrome (lymphoma); skin cancer; childhood skin cancer; small cell lung cancer; small intestine cancer; soft tissue sarcoma; squamous cell carcinoma of the skin - see skin cancer; squamous neck cancer with occult primary, metastatic (head and neck cancer); stomach (gastric) cancer; childhood stomach (gastric) cancer; t-cell lymphoma, cutaneous - see lymphoma (mycosis fungoides and sèzary syndrome); testicular cancer; childhood testicular cancer; throat cancer (head and neck cancer); nasopharyngeal cancer; oropharyngeal cancer; hypopharyngeal cancer; thymoma and thymic carcinoma; thyroid cancer; transitional cell cancer of the renal pelvis and ureter (kidney (renal cell) cancer); carcinoma of unknown primary; childhood cancer of unknown primary; unusual cancers of childhood; ureter and renal pelvis, transitional cell cancer (kidney (renal cell) cancer; urethral cancer; uterine cancer, endometrial; uterine sarcoma; vaginal cancer; childhood vaginal cancer; vascular tumors (soft tissue sarcoma); vulvar cancer; wilms tumor and other childhood kidney tumors; or cancer in young adults.

[0218] In some cases, the disease or disorder is cardiovascular disease. As used herein, the terms “cardiovascular disease” (CVD) or “cardiovascular disorder” are used to classify numerous conditions affecting the heart, heart valves, and vasculature (e.g., veins and arteries) of the body and encompasses diseases and conditions including, but not limited to atherosclerosis, myocardial infarction, acute coronary syndrome, angina, congestive heart failure, aortic

aneurysm, aortic dissection, iliac or femoral aneurysm, pulmonary embolism, atrial fibrillation, stroke, transient ischemic attack, systolic dysfunction, diastolic dysfunction, myocarditis, atrial tachycardia, ventricular fibrillation, endocarditis, peripheral vascular disease, and coronary artery disease (CAD). Further, the term cardiovascular disease refers to subjects that ultimately have a cardiovascular event or cardiovascular complication, referring to the manifestation of an adverse condition in a subject brought on by cardiovascular disease, such as sudden cardiac death or acute coronary syndrome, including, but not limited to, myocardial infarction, unstable angina, aneurysm, stroke, heart failure, non-fatal myocardial infarction, stroke, angina pectoris, transient ischemic attacks, aortic aneurysm, aortic dissection, cardiomyopathy, abnormal cardiac catheterization, abnormal cardiac imaging, stent or graft revascularization, risk of experiencing an abnormal stress test, risk of experiencing abnormal myocardial perfusion, and death.

[0219] As used herein, the ability to detect, diagnose or prognose cardiovascular disease, for example, atherosclerosis, can include determining if the patient is in a pre-stage of cardiovascular disease, has developed early, moderate or severe forms of cardiovascular disease, or has suffered one or more cardiovascular event or complication associated with cardiovascular disease.

[0220] Atherosclerosis (also known as arteriosclerotic vascular disease or ASVD) is a cardiovascular disease in which an artery-wall thickens as a result of invasion and accumulation and deposition of arterial plaques containing white blood cells on the innermost layer of the walls of arteries resulting in the narrowing and hardening of the arteries. The arterial plaque is an accumulation of macrophage cells or debris, and contains lipids (cholesterol and fatty acids), calcium and a variable amount of fibrous connective tissue. Diseases associated with atherosclerosis include, but are not limited to, atherothrombosis, coronary heart disease, deep venous thrombosis, carotid artery disease, angina pectoris, peripheral arterial disease, chronic kidney disease, acute coronary syndrome, vascular stenosis, myocardial infarction, aneurysm or stroke. In one embodiment the automated apparatuses, compositions, and methods of the present disclosure may distinguish the different stages of atherosclerosis, including, but not limited to, the different degrees of stenosis in a subject.

[0221] In some cases, the disease or disorder is an endocrine disease. The term “endocrine disease” is used to refer to a disorder associated with dysregulation of endocrine system of a subject. Endocrine diseases may result from a gland producing too much or too little of an endocrine hormone causing a hormonal imbalance, or due to the development of lesions (such as nodules or tumors) in the endocrine system, which may or may not affect hormone levels. Suitable endocrine diseases able to be treated include, but are not limited to, e.g., Acromegaly,

Addison's Disease, Adrenal Cancer, Adrenal Disorders, Anaplastic Thyroid Cancer, Cushing's Syndrome, De Quervain's Thyroiditis, Diabetes, Follicular Thyroid Cancer, Gestational Diabetes, Goiters, Graves' Disease, Growth Disorders, Growth Hormone Deficiency, Hashimoto's Thyroiditis, Hurthle Cell Thyroid Cancer, Hyperglycemia, Hyperparathyroidism, Hyperthyroidism, Hypoglycemia, Hypoparathyroidism, Hypothyroidism, Low Testosterone, Medullary Thyroid Cancer, MEN 1, MEN 2A, MEN 2B, Menopause, Metabolic Syndrome, Obesity, Osteoporosis, Papillary Thyroid Cancer, Parathyroid Diseases, Pheochromocytoma, Pituitary Disorders, Pituitary Tumors, Polycystic Ovary Syndrome, Prediabetes, Silent, Thyroiditis, Thyroid Cancer, Thyroid Diseases, Thyroid Nodules, Thyroiditis, Turner Syndrome, Type 1 Diabetes, Type 2 Diabetes, and the like.

[0222] In some cases, the disease or disorder is an inflammatory disease. As referred to herein, inflammatory disease refers to a disease caused by uncontrolled inflammation in the body of a subject. Inflammation is a biological response of the subject to a harmful stimulus which may be external or internal such as pathogens, necrosed cells and tissues, irritants etc. However, when the inflammatory response becomes abnormal, it results in self-tissue injury and may lead to various diseases and disorders. Inflammatory diseases can include, but are not limited to, asthma, glomerulonephritis, inflammatory bowel disease, rheumatoid arthritis, hypersensitivities, pelvic inflammatory disease, autoimmune diseases, arthritis; necrotizing enterocolitis (NEC), gastroenteritis, pelvic inflammatory disease (PID), emphysema, pleurisy, pyelitis, pharyngitis, angina, acne vulgaris, urinary tract infection, appendicitis, bursitis, colitis, cystitis, dermatitis, phlebitis, rhinitis, tendonitis, tonsillitis, vasculitis, autoimmune diseases; celiac disease; chronic prostatitis, hypersensitivities, reperfusion injury; sarcoidosis, transplant rejection, vasculitis, interstitial cystitis, hay fever, periodontitis, atherosclerosis, psoriasis, ankylosing spondylitis, juvenile idiopathic arthritis, Behcet's disease, spondyloarthritis, uveitis, systemic lupus erythematosus, and cancer. For example, the arthritis includes rheumatoid arthritis, psoriatic arthritis, osteoarthritis or juvenile idiopathic arthritis, and the like.

[0223] The disease or disorder may be a neurological disease. Neurological disorders or neurological diseases are used interchangeably and refer to diseases of the brain, spine and the nerves that connect them. Neurological diseases include, but are not limited to, brain tumors, epilepsy, Parkinson's disease, Alzheimer's disease, ALS, arteriovenous malformation, cerebrovascular disease, brain aneurysms, epilepsy, multiple sclerosis, Peripheral Neuropathy, Post-Herpetic Neuralgia, stroke, frontotemporal dementia, demyelinating disease (including but are not limited to, multiple sclerosis, Devic's disease (i.e. neuromyelitis optica), central pontine myelinolysis, progressive multifocal leukoencephalopathy, leukodystrophies, Guillain-Barre

syndrome, progressing inflammatory neuropathy, Charcot-Marie-Tooth disease, chronic inflammatory demyelinating polyneuropathy, and anti-MAG peripheral neuropathy) and the like. Neurological disorders also include immune-mediated neurological disorders (IMNDs), which include diseases with at least one component of the immune system reacts against host proteins present in the central or peripheral nervous system and contributes to disease pathology. IMNDs may include, but are not limited to, demyelinating disease, paraneoplastic neurological syndromes, immune-mediated encephalomyelitis, immune-mediated autonomic neuropathy, myasthenia gravis, autoantibody-associated encephalopathy, and acute disseminated encephalomyelitis.

[0224] Methods, systems, and/or apparatuses of the present disclosure may be able to accurately distinguish between patients with or without Alzheimer's disease. These may also be able to detect patients who are pre-symptomatic and may develop Alzheimer's disease several years after the screening. This provides advantages of being able to treat a disease at a very early stage, even before development of the disease.

[0225] The systems, methods, and apparatuses of the present disclosure can detect a pre-disease stage of a disease or disorder. A pre-disease stage is a stage at which the patient has not developed any signs or symptoms of the disease. A pre-cancerous stage would be a stage in which cancer or tumor or cancerous cells have not be identified within the subject. A pre-neurological disease stage would be a stage in which a person has not developed one or more symptom of the neurological disease. The ability to diagnose a disease before one or more sign or symptom of the disease is present allows for close monitoring of the subject and the ability to treat the disease at a very early stage, increasing the prospect of being able to halt progression or reduce the severity of the disease.

[0226] The automated apparatuses, systems, sensor arrays, and methods of the present disclosure in some embodiments are able to detect the early stages of a disease or disorder. Early stages of the disease can refer to when the first signs or symptoms of a disease may manifest within a subject. The early stage of a disease may be a stage at which there are no outward signs or symptoms. For example, in Alzheimer's disease an early stage may be a pre-Alzheimer's stage in which no symptoms are detected yet the patient will develop Alzheimer's months or years later.

[0227] Identifying a disease in either pre-disease development or in the early states may often lead to a higher likelihood for a positive outcome for the patient. For example, diagnosing cancer at an early stage (stage 0 or stage 1) can increase the likelihood of survival by over 80%. Stage 0 cancer can describe a cancer before it has begun to spread to nearby tissues. This stage of cancer is often highly curable, usually by removing the entire tumor with surgery. Stage 1 cancer may

usually be a small cancer or tumor that has not grown deeply into nearby tissue and has not spread to lymph nodes or other parts of the body.

[0228] FIG. 8 presents a schematic overview of a cancer detection method that can be performed using the automated apparatus of the present disclosure. Whole blood samples can be collected from a range of patients, including healthy patients and patients with different types and stages of cancer. The whole blood can be fractionated into plasma samples, and then contacted with a plurality of types of particles, including positively charged, negatively charged, and neutral particles. Each particle type collects different types of proteins from the plasma samples, leading to each patient having a unique biomolecule fingerprint. The biomolecule fingerprint not only comprise the relative abundances of proteins on each particle type, but also the relative abundances of proteins across particle types. For example, an increase in the abundance of fibronectin on a first particle type may be a relevant indicator only when the abundance of complement component 4 is low on a second particle type. The biomolecule fingerprints can not only be used to determine which patients have cancer, but also to determine the stages and types of the cancers.

[0229] In some embodiments, the automated apparatuses, systems, sensor arrays, and methods are able to detect intermediate stages of the disease. Intermediate states of the disease describe stages of the disease that have passed the first signs and symptoms and the patient is experiencing one or more symptom of the disease. For example, for cancer, stage II or III cancers are considered intermediate stages, indicating larger cancers or tumors that have grown more deeply into nearby tissue. In some instances, stage II or III cancers may have also spread to lymph nodes but not to other parts of the body.

[0230] Further, the automated apparatuses, systems, sensor arrays, and methods are able to detect late or advanced stages of the disease. Late or advanced stages of the disease may also be called “severe” or “advanced” and usually indicates that the subject is suffering from multiple symptoms and effects of the disease. For example, severe stage cancer includes stage IV, where the cancer has spread to other organs or parts of the body and is sometimes referred to as advanced or metastatic cancer.

[0231] The methods of the present disclosure can include processing the biomolecule fingerprint of the sample against a collection of biomolecule fingerprints associated with a plurality of diseases and/or a plurality of disease states to determine if the sample indicates a disease and/or disease state. For example, samples can be collected from a population of subjects over time. Once the subjects develop a disease or disorder, the present disclosure allows for the ability to characterize and detect the changes in biomolecule fingerprints over time in the subject by

computationally analyzing the biomolecule fingerprint of the sample from the same subject before they have developed a disease to the biomolecule fingerprint of the subject after they have developed the disease. Samples can also be taken from cohorts of patients who all develop the same disease, allowing for analysis and characterization of the biomolecule fingerprints that are associated with the different stages of the disease for these patients (e.g. from pre-disease to disease states).

[0232] In some cases, the apparatuses, systems, compositions, and methods of the present disclosure are able to distinguish not only between different types of diseases, but also between the different stages of the disease (e.g. early stages of cancer). This can comprise distinguishing healthy subjects from pre-disease state subjects. The pre-disease state may be stage 0 or stage 1 cancer, a neurodegenerative disease, dementia, a coronary disease, a kidney disease, a cardiovascular disease (e.g., coronary artery disease), diabetes, or a liver disease. Distinguishing between different stages of the disease can comprise distinguishing between two stages of a cancer (e.g., stage 0 vs stage 1 or stage 1 vs stage 3).

Sample

[0233] The panels of the present disclosure can be used to generate proteomic data from protein coronas and subsequently associated with any of the biological states described herein. Samples consistent with the present disclosure include biological samples from a subject. The subject may be a human or a non-human animal. Biological samples may be a biofluid. For example, the biofluid may be plasma, serum, CSF, urine, tear, or saliva. Said biological samples can contain a plurality of proteins or proteomic data, which may be analyzed after adsorption of proteins to the surface of the various sensor element (e.g., particle) types in a panel and subsequent digestion of protein coronas. Proteomic data can comprise nucleic acids, peptides, or proteins.

[0234] A wide range of biological samples are compatible for use within the automated apparatuses of the present disclosure. The biological sample may comprise plasma, serum, urine, cerebrospinal fluid, synovial fluid, tears, saliva, whole blood, milk, nipple aspirate, ductal lavage, vaginal fluid, nasal fluid, ear fluid, gastric fluid, pancreatic fluid, trabecular fluid, lung lavage, sweat, crevicular fluid, semen, prostatic fluid, sputum, fecal matter, bronchial lavage, fluid from swabbings, bronchial aspirants, fluidized solids, fine needle aspiration samples, tissue homogenates, lymphatic fluid, cell culture samples, or any combination thereof. The biological sample may comprise multiple biological samples (e.g., pooled plasma from multiple subjects, or multiple tissue samples from a single subject). The biological sample may comprise a single type of biofluid or biomaterial from a single source.

[0235] The biological sample may be diluted or pre-treated. The biological sample may undergo depletion (e.g., the biological sample comprises serum) prior to use within the automated apparatus. The biological sample may also undergo physical (e.g., homogenization or sonication) or chemical treatment prior to use within the automated apparatus. The biological sample may be diluted prior to use within the automated apparatus. The dilution medium may comprise buffer or salts, or be purified water (e.g., distilled water). Different partitions of a biological sample may undergo different degrees of dilution. A biological sample or sample partition may undergo a 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 8-fold, 10-fold, 12-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 75-fold, 100-fold, 200-fold, 500-fold, or 1000-fold dilution.

[0236] In some embodiments, the panels of the present disclosure provide identification and measurement of particular proteins in the biological samples by processing of the proteomic data via digestion of coronas formed on sensor elements. Examples of proteins that can be identified and measured include highly abundant proteins, proteins of medium abundance, and low-abundance proteins. Examples of proteins that are highly abundant proteins include albumin and IgG.

[0237] In some embodiments, examples of proteins that can be measured and identified include albumin, immunoglobulin G (IgG), lysozyme, carcino embryonic antigen (CEA), receptor tyrosine-protein kinase erbB-2 (HER-2/neu), bladder tumor antigen, thyroglobulin, alpha-fetoprotein, prostate specific antigen (PSA), mucin 16 (CA125),carbohydrate antigen 19-9 (CA19.9), carcinoma antigen 15-3 (CA15.3), leptin, prolactin, osteopontin, insulin-like growth factor 2 (IGF-II), 4F2 cell-surface antigen heavy chain (CD98), fascin, sPigR, 14-3-3 eta, troponin I, B-type natriuretic peptide, breast cancer type 1 susceptibility protein (BRCA1), c-Myc proto-oncogene protein (c-Myc), interleukin-6 (IL-6), fibrinogen. Epidermal growth factor receptor (EGFR), gastrin, PH, Granulocyte colony-stimulating factor (G CSF), desmin, enolase 1 (NSE), folice-stimulating hormone (FSH), vascular endothelial growth factor (VEGF), P21, Proliferating cell nuclear antigen (PCNA), calcitonin, pathogenesis-related proteins (PR), luteinizing hormone (LH), somatostatin. S100, insulin. alpha-prolactin, Adrenocorticotropic hormone (ACTH), B-cell lymphoma 2 (Bcl 2), estrogen receptor alpha (ER alpha), antigen k (Ki-67), tumor protein (p53), cathepsin D, beta catenin, von Willebrand factor (VWF), CD15, k-ras, caspase 3, ENTH domain-containing protein (EPN), CD10, FAS, breast cancer type 2 susceptibility protein (BRCA2), CD30L, CD30, CGA, CRP, prothrombin, CD44, APEX, transferrin, GM-CSF, E-cadherin, interleukin-2 (IL-2), Bax, IFN-gamma, beta-2-MG, tumor necrosis factor alpha (TNF alpha), cluster of differentiation 340, trypsin, cyclin D1, MG B, XBP-

1, HG-1, YKL-40, S-gamma, NESP-55, netrin-1, geminin, GADD45A, CDK-6, CCL21, breast cancer metastasis suppressor 1 (BrMS1), 17betaHDI, platelet-derived growth factor receptor A (PDGRFA), P300/CBP-associated factor (Pcaf), chemokine ligand 5 (CCL5), matrix metalloproteinase-3 (MMP3), claudin-4, and claudin-3.

Methods of Analysis

[0238] The proteomic data of the sample can be identified, measured, and quantified using a number of different analytical techniques. For example, proteomic data can be analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) or any gel-based separation technique. Peptides and proteins can also be identified, measured, and quantified using an immunoassay, such as an enzyme-linked immunosorbent assay (ELISA). Alternatively, proteomic data can be identified, measured, and quantified using mass spectrometry, high performance liquid chromatography, LC-MS/MS, and other protein separation techniques.

[0239] In some cases, the method of determining the biomolecule fingerprint comprises detecting and determining the biomolecular corona signatures of the at least two sensor elements. This step can be done by separating the plurality of biomolecules attached to each sensor element (e.g. separating the biomolecule corona from the sensor element) and assaying the plurality of biomolecules to determine the composition of the plurality of biomolecule coronas to determine a biomolecule fingerprint. In some cases, the composition of each biomolecule corona signature of each sensor element is assayed independently, and the results are combined to produce the biomolecule fingerprint (e.g. each sensor element is in a separate channel or compartment wherein the specific composition of the biomolecule corona for that specific sensor element can be separately analyzed (e.g. either by detaching the biomolecules and assaying by mass spectrometry and/or chromatography or by detecting the plurality of biomolecules still attached to the sensor element by fluorescence, luminescence or other means). The at least two sensor elements may also be in the same partition and the composition of the biomolecule corona for the at least two sensor elements is assayed at the same time by dissociating the biomolecule corona from both sensor elements into one solution and assaying that solution to determining a biomolecule signature.

[0240] Methods of assaying the plurality of biomolecules that make up the biomolecule corona signature or the biomolecule fingerprint may include, but are not limited to, for example, gel-electrophoresis, liquid chromatography, mass spectrometry, nuclear magnetic resonance spectroscopy (NMR), Fourier transform infrared spectroscopy (FTIR), circular dichroism, Raman spectrometry, and a combination thereof. In some cases, the assaying comprises an analyte specific identification technique, such as ELISA, immunostaining, or nucleic acid

capture by hybridization. In a preferred embodiment, the assaying comprises liquid chromatography, mass spectrometry or a combination thereof.

[0241] As disclosed herein, nucleic acids may be processed by standard molecular biology techniques for downstream applications. Embodiments of the methods and compositions disclosed herein relate to nucleic acid (polynucleotide) sequencing. In some methods and compositions described herein, the nucleotide sequence of a portion of a target nucleic acid or fragment thereof may be determined using a variety of methods and devices. Examples of sequencing methods include electrophoretic, sequencing by synthesis, sequencing by ligation, sequencing by hybridization, single-molecule sequencing, and real time sequencing methods. In some embodiments, the process to determine the nucleotide sequence of a target nucleic acid or fragment thereof may be an automated process. In some embodiments, capture probes may function as primers permitting the priming of a nucleotide synthesis reaction using a polynucleotide from the nucleic acid sample as a template. In this way, information regarding the sequence of the polynucleotides supplied to the array may be obtained. In some embodiments, polynucleotides hybridized to capture probes on the array may serve as sequencing templates if primers that hybridize to the polynucleotides bound to the capture probes and sequencing reagents are further supplied to the array. Methods of sequencing using arrays have been described previously in the art.

[0242] In some embodiments involving sequencing on a substrate such as an array, paired end reads may be obtained on nucleic acid clusters. Methods for obtaining paired end reads are described in WO/07010252 and WO/07091077, each of which is incorporated herein by reference in its entirety. Paired end sequencing facilitates reading both the forward and reverse template strands of each cluster during one paired-end read. Generally, template clusters may be amplified on the surface of a substrate (e.g. a flow-cell) by bridge amplification and sequenced by paired primers sequentially. Upon amplification of the template strands, a bridged double stranded structure may be produced. This may be treated to release a portion of one of the strands of each duplex from the surface. The single stranded nucleic acid may be available for sequencing, primer hybridization and cycles of primer extension. After the first sequencing run, the ends of the first single stranded template may be hybridized to the immobilized primers remaining from the initial cluster amplification procedure. The immobilized primers may be extended using the hybridized first single strand as a template to resynthesize the original double stranded structure. The double stranded structure may be treated to remove at least a portion of the first template strand to leave the resynthesized strand immobilized in single stranded form. The resynthesized strand may be sequenced to determine a second read, whose location

originates from the opposite end of the original template fragment obtained from the fragmentation process.

[0243] Nucleic acid sequencing may be single-molecule sequencing or sequencing by synthesis. Sequencing may be massively parallel array sequencing (e.g., Illumina™ sequencing), which may be performed using template nucleic acid molecules immobilized on a support, such as a flow cell. For example, sequencing may comprise a first-generation sequencing method, such as Maxam-Gilbert or Sanger sequencing, or a high-throughput sequencing (e.g., next-generation sequencing or NGS) method. A high-throughput sequencing method may sequence simultaneously (or substantially simultaneously) at least about 10,000, 100,000, 1 million, 10 million, 100 million, 1 billion, or more polynucleotide molecules. Sequencing methods may include, but are not limited to: pyrosequencing, sequencing-by synthesis, single-molecule sequencing, nanopore sequencing, semiconductor sequencing, sequencing-by-ligation, sequencing-by-hybridization, Digital Gene Expression (Helicos), massively parallel sequencing, e.g., Helicos, Clonal Single Molecule Array (Solexa/Illumina), sequencing using PacBio, SOLiD, Ion Torrent, or Nanopore platforms.

[0244] A sensor element may comprise a complex with a first component and a polymer fluorophore or other quencher component chemically complementary to the first component where such a complex having an initial background or reference fluorescence. Once the first component comes into contact with a biomolecule (e.g., upon formation of a biomolecule corona), it can affect the quenching of the fluorophore and this change in fluorescence can be measured. After the sensor is irradiated and/or excited with a laser, the effect and/or change in fluorescence for each sensor element can be measured and compared to or processed against the background fluorescence to produce the biomolecule fingerprint.

Computer systems

[0245] The present disclosure provides computer control systems that are programmed to implement methods of the disclosure. This determination, analysis or statistical classification is done by methods known in the art, including, but not limited to, for example, a wide variety of supervised and unsupervised data analysis and clustering approaches such as hierarchical cluster analysis (HCA), principal component analysis (PCA), Partial least squares Discriminant Analysis (PLSDA), machine learning (also known as random forest), logistic regression, decision trees, support vector machine (SVM), k-nearest neighbors, naive bayes, linear regression, polynomial regression, SVM for regression, K-means clustering, and hidden Markov models, among others. The computer system can perform various aspects of analyzing the

protein sets or protein corona of the present disclosure, such as, for example, comparing/analyzing the biomolecule corona of several samples to determine with statistical significance what patterns are common between the individual biomolecule coronas to determine a protein set that is associated with the biological state. The computer system can be used to develop classifiers to detect and discriminate different protein sets or protein corona (e.g., characteristic of the composition of a protein corona). Data collected from the presently disclosed sensor array can be used to train a machine learning algorithm, specifically an algorithm that receives array measurements from a patient and outputs specific biomolecule corona compositions from each patient. Before training the algorithm, raw data from the array can be first denoised to reduce variability in individual variables.

[0246] Machine learning can be generalized as the ability of a learning machine to perform accurately on new, unseen examples/tasks after having experienced a learning data set. Machine learning may include the following concepts and methods. Supervised learning concepts may. include AODE; Artificial neural network, such as Backpropagation, Autoencoders, Hopfield networks, Boltzmann machines, Restricted Boltzmann Machines, and Spiking neural networks; Bayesian statistics, such as Bayesian network and Bayesian knowledge base; Case-based reasoning; Gaussian process regression; Gene expression programming; Group method of data handling (GMDH); Inductive logic programming; Instance-based learning; Lazy learning;

[0247] Learning Automata; Learning Vector Quantization; Logistic Model Tree; Minimum message length (decision trees, decision graphs, etc.), such as Nearest Neighbor Algorithm and Analogical modeling; Probably approximately correct learning (PAC) learning; Ripple down rules, a knowledge acquisition methodology; Symbolic machine learning algorithms; Support vector machines; Random Forests; Ensembles of classifiers, such as Bootstrap aggregating (bagging) and Boosting (meta-algorithm); Ordinal classification; Information fuzzy networks (IFN); Conditional Random Field; ANOVA; Linear classifiers, such as Fisher's linear discriminant, Linear regression, Logistic regression, Multinomial logistic regression, Naive Bayes classifier, Perceptron, Support vector machines; Quadratic classifiers; k-nearest neighbor; Boosting; Decision trees, such as C4.5, Random forests, ID3, CART, SLIQ SPRINT; Bayesian networks, such as Naive Bayes; and Hidden Markov models. Unsupervised learning concepts may include; Expectation-maximization algorithm; Vector Quantization; Generative topographic map; Information bottleneck method; Artificial neural network, such as Self-organizing map; Association rule learning, such as, Apriori algorithm, Eclat algorithm, and FPgrowth algorithm; Hierarchical clustering, such as Singlelinkage clustering and Conceptual clustering; Cluster analysis, such as, K-means algorithm, Fuzzy clustering, DBSCAN, and OPTICS algorithm; and

Outlier Detection, such as Local Outlier Factor. Semi-supervised learning concepts may include; Generative models; Low-density separation; Graph-based methods; and Co-training.

[0248] Reinforcement learning concepts may include; Temporal difference learning; Q-leaming; Learning Automata; and SARSA. Deep learning concepts may include; Deep belief networks; Deep Boltzmann machines; Deep Convolutional neural networks; Deep Recurrent neural networks; and Hierarchical temporal memory. A computer system may be adapted to implement a method described herein. The system includes a central computer server that is programmed to implement the methods described herein. The server includes a central processing unit (CPU, also "processor") which can be a single core processor, a multi core processor, or plurality of processors for parallel processing. The server also includes memory (e.g., random access memory, read-only memory, flash memory); electronic storage unit (e.g. hard disk); communications interface (e.g., network adaptor) for communicating with one or more other systems; and peripheral devices which may include cache, other memory, data storage, and/or electronic display adaptors. The memory, storage unit, interface, and peripheral devices are in communication with the processor through a communications bus (solid lines), such as a motherboard. The storage unit can be a data storage unit for storing data. The server is operatively coupled to a computer network ("network") with the aid of the communications interface. The network can be the Internet, an intranet and/or an extranet, an intranet and/or extranet that is in communication with the Internet, a telecommunication or data network. The network in some cases, with the aid of the server, can implement a peer-to-peer network, which' may enable devices coupled to the server to behave as a client or a server.

[0249] The storage unit can store files, such as subject reports, and/or communications with the data about individuals, or any aspect of data associated with the present disclosure.

[0250] The computer server can communicate with one or more remote computer systems through the network. The one or more remote computer systems may be, for example, personal computers, laptops, tablets, telephones, Smart phones, or personal digital assistants.

[0251] In some applications the computer system includes a single server. In other situations, the system includes multiple servers in communication with one another through an intranet, extranet and/or the internet.

[0252] The server can be adapted to store measurement data or a database as provided herein, patient information from the subject, such as, for example, medical history, family history, demographic data and/or other clinical or personal information of potential relevance to a particular application. Such information can be stored on the storage unit or the server and such data can be transmitted through a network.

[0253] Methods as described herein can be implemented by way of machine (or computer processor) executable code (or software) stored on an electronic storage location of the server, such as, for example, on the memory, or electronic storage unit. During use, the code can be executed by the processor. In some cases, the code can be retrieved from the storage unit and stored on the memory for ready access by the processor. In some situations, the electronic storage unit can be precluded, and machine-executable instructions are stored on memory.

[0254] Alternatively, the code can be executed on a second computer system.

[0255] Aspects of the systems and methods provided herein, such as the server, can be embodied in programming. Various aspects of the technology may be thought of as "products" or "articles of manufacture" typically in the form of machine (or processor) executable code and/or associated data that is carried on or embodied in a type of machine readable medium. Machine-executable code can be stored on an electronic storage unit, such memory (e.g., read-only memory, random-access memory, flash memory) or a hard disk. "Storage" type media can include any or all of the tangible memory of the computers, processors or the like, or associated modules thereof, such as various semiconductor memories, tape drives, disk drives and the like, which may provide non-transitory storage at any time for the software programming. All or portions of the software may at times be communicated through the Internet or various other telecommunication networks. Such communications, for example, may enable loading of the software from one computer or processor into another, for example, from a management server or host computer into the computer platform of an application server. Thus, another type of media that may bear the software elements includes optical, electrical, and electromagnetic waves, such as used across physical interfaces between local devices, through wired and optical landline networks and over various air-links. The physical elements that carry such waves, such as wired or wireless likes, optical links, or the like, also may be considered as media bearing the software. As used herein, unless restricted to non-transitory, tangible "storage" media, terms such as computer or machine "readable medium" can refer to any medium that participates in providing instructions to a processor for execution.

[0256] The computer systems described herein may comprise computer-executable code for performing any of the algorithms or algorithms-based methods described herein. In some applications the algorithms described herein will make use of a memory unit that is comprised of at least one database.

[0257] Data relating to the present disclosure can be transmitted over a network or connections for reception and/or review by a receiver. The receiver can be but is not limited to the subject to whom the report pertains; or to a caregiver thereof, e.g., a health care provider, manager, other

health care professional, or other caretaker; a person or entity that performed and/or ordered the analysis. The receiver can also be a local or remote system for storing such reports (e.g. servers or other systems of a “cloud computing” architecture). In one embodiment, a computer-readable medium includes a medium suitable for transmission of a result of an analysis of a biological sample using the methods described herein.

[0258] Aspects of the systems and methods provided herein can be embodied in programming. Various aspects of the technology may be thought of as "products" or "articles of manufacture" typically in the form of machine (or processor) executable code and/or associated data that is carried on or embodied in a type of machine readable medium. Machine executable code can be stored on an electronic storage unit, such as memory (e.g., read-only memory, random-access memory, flash memory) or a hard disk. "Storage" type media can include any or all of the tangible memory of the computers, processors or the like, or associated modules thereof, such as various semiconductor memories, tape drives, disk drives and the like, which may provide nontransitory storage at any time for the software programming. All or portions of the software may at times be communicated through the Internet or various other telecommunication networks. Such communications, for example, may enable loading of the software from one computer or processor into another, for example, from a management server or host computer into the computer platform of an application server. Thus, another type of media that may bear the software elements includes optical, electrical and electromagnetic waves, such as used across physical interfaces between local devices, through wired and optical landline networks and over various air-links. The physical elements that carry such waves, such as wired or wireless links, optical links or the like, also may be considered as media bearing the software. As used herein, unless restricted to non-transitory, tangible "storage" media, terms such as computer or machine "readable medium" refer to any medium that participates in providing instructions to a processor for execution.

[0259] Hence, a machine readable medium, such as computer- executable code, may take many forms, including but not limited to, a tangible storage medium, a carrier wave medium or physical transmission medium. Non-volatile storage media include, for example, optical or magnetic disks, such as any of the storage devices in any computer(s) or the like, such as may be used to implement the databases, etc. shown in the drawings. Volatile storage media include dynamic memory, such as main memory of such a computer platform. Tangible transmission media include coaxial cables; copper wire and fiber optics, including the wires that comprise a bus within a computer system. Carrier-wave transmission media may take the form of electric or electromagnetic signals, or acoustic or light waves such as those generated during radio

frequency (RF) and infrared (IR) data communications. Common forms of computer-readable media therefore include for example: a floppy disk, a flexible disk, hard disk, magnetic tape, any other magnetic medium, a CD-ROM, DVD or DVD-ROM, any other optical medium, punch cards paper tape, any other physical storage medium with patterns of holes, a RAM, a ROM, a PROM and EPROM, a FLASH-EPROM, any other memory chip or cartridge, a carrier wave transporting data or instructions, cables or links transporting such a carrier wave, or any other medium from which a computer may read programming code and/or data. Many of these forms of computer readable media may be involved in carrying one or more sequences of one or more instructions to a processor for execution.

EXAMPLES

[0260] The following examples are included to further describe some aspects of the present disclosure and should not be used to limit the scope of the disclosure.

Example 1: Formation of protein coronas with magnetic nanoparticles and biofluid with full resuspension

[0261] This exemplary procedure applies to creating protein coronas manually in biofluid samples using a panel of magnetic nanoparticles with full resuspension of nanoparticles. The systems and methods of the present disclosure may apply the procedures described herein.

[0262] ***Materials***:

[0263] The materials used in creating protein coronas is shown in **TABLE 2**.

TABLE 2: Equipment and reagents used in creating protein coronas

Equipment and Reagent	Supplier	Part number or Model Number
Reagent Grade Water	TEKNOVA	W1210 or equivalent
Reagent Grade Water	Corning	46-002-LF or equivalent
Microplate F-Bottom	Greiner	655901
Aluminum Adhesive Plate Sealers	VWR	29445-080 or equivalent
Microplate Shaker	VWR	12620-926 or equivalent
Vortexer	VWR	33570 or equivalent
Analytical Balance	Mettler Toledo	XP205
Single-channel pipettes (100-1000 μL)	Rainin	L-1000 or equivalent

Equipment and Reagent	Supplier	Part number or Model Number
Single-channel pipettes(20-200µL)	Rainin	L-200 or equivalent
Multi-channel pipette (100-1200µL)	Rainin	E12-1200 or equivalent
Pipette tips (1000µL)	Rainin	GPS-L1000 or equivalent
Pipette tips (20-200µL)	Rainin	GPS-L250 or equivalent
50 mL Reagent Reservoirs	VWR	82026-355 or equivalent
1x TE pH7.4	Quality Biological	351-010-131
CHAPS	Fisher	BP571-5
Potassium Chloride (KCl)	J.T. Baker	4001-01
Corning 1L bottle	Corning	430518
Nalgene Rapid Flow 1000mL 0.1µm or 0.2µm filter set	Nalgene	567-0010 or 567-0020

[0264] ***Storage and handling***:

[0265] The following reagents were stored at room temperature, as shown in **TABLE 3**:

TABLE 3: Reagents stored at room temperature

Reagent	Supplier	Part Number
1x TE pH7.4	Quality Biological	351-010-131
CHAPS	Fisher	BP571-5
Potassium Chloride (KCl)	J.T. Baker	4001-01
Reagent Grade Water	TEKNOVA	W1210
Reagent Grade Water	Corning	46-002-LF
TE 150mM KCl 0.05% CHAPS	Seer Inc.	SOP003

[0266] The following reagents were stored at about 2-8°C, as shown in **TABLE 4**:

TABLE 4: Reagents stored at 2-8°C

Reagent	Supplier	Part number
TE 150mM KCl 0.05% CHAPS	Seer Inc.	SOP003

[0267] ***Preparation***:

[0268] Biofluid samples were removed from the freezer and thawed thoroughly. The nanoparticles were sonicated and vortexed about 10 minutes before use. The TE 150mM KCl 0.05% CHAPS buffer was prepared before beginning the assay.

[0269] *TE 150mM KCl 0.05% CHAPS Buffer Preparation.* 11.18g potassium chloride and 500mg CHAPS were added to a Corning 1L bottle. 998.3g of 1X TE pH7.4 buffer was added. Using a house vacuum, the buffer was filtered with a 0.1µm or 0.2µm 1000mL filter set. The buffer can be stored at room temperature (for about 1 month) or at 2-8°C (for longer than 1 month). The buffer was mixed well before use.

[0270] *Nanoparticles Preparation.* The nanoparticles (aqueous) were diluted in Reagent Grade water to appropriate designated concentration. For dry powder nanoparticles, the dry powder nanoparticles were measured out on a scale before adding the appropriate volume of water to create needed concentration.

[0271] *Samples Preparation.* The samples were removed from the freezer. The samples were thawed thoroughly, and the samples were centrifuged at 16,000G for about 2 minutes.
The samples were either diluted with TE 150mM KCl 0.05% CHAPS Buffer (1:5) or kept as neat.

[0272] **FIG. 9** illustrates a sample preparation method consistent with the present disclosure. This method comprises 4 steps that generate a subset of biomolecules from a biological sample and then use the subset of biomolecules to generate a biomolecule fingerprint. The first step comprises transferring a plasma sample into a plurality of partitions (e.g., wells within a well plate) which comprises a plurality of sensor elements (e.g., magnetic nanoparticles). The sample is incubated within the partitions for 1 hour at 37 °C with shaking, thereby generating biomolecule coronas on the sensor elements. The plurality of partitions is then subjected to a magnetic field that is sufficiently strong to immobilize the sensor elements within the plurality of partitions. The plurality of partitions are then subjected to three washes (e.g., sequential addition and removal of a resuspension buffer) to remove biomolecules that did not adsorb to the sensor elements. After the 3rd wash, the particles are resuspended in buffer, resulting in the desorption of a subset of biomolecules from the biomolecule coronas. The subset of biomolecules is then

subjected to a set of denaturation and chemical treatment steps, including heating to 95 ºC, reduction and alkylation, protease digestion, and further washes. The subset of biomolecules isthen submitted for mass spectrometric analysis, which generates a biomolecule fingerprint for the sample.

[0273] ***Procedure***:

[0274] The reagents and equipment were prepared as described in the previous section (see "Preparation"). 100µL of diluted nanoparticles were loaded into each well using a multichannel pipette. 100µL of diluted samples per nanoparticle well were added using a pipette. The wells were mixed by aspiration with a pipette about 10 times. The plate was covered with an adhesive plate sealer and incubated for about 1 hours at 37 ºC on a plate shaker set to 300 rpm. After the about 1-hour incubation, the adhesive plate sealer was removed, and the plate was placed on a magnet for about 5 minutes to form a nanoparticle corona pellet at the well bottom. For washing, the supernatant was removed with a multichannel pipette. About 200µL of TE 150mM KCl 0.05% CHAPS Buffer was added using a pipette and fully resuspended the nanoparticles. The solution was placed back on the magnet for about 5 minutes. The washing step was repeated 3 times. The nanoparticle pellet was resuspended in an appropriate reagent for BCA, gel or trypsin digestion.

Example 2: Trypsin gold digest

[0275] ***Materials***:

[0276] The materials used in the trypsin gold digest is shown in **TABLE 5**.

TABLE 5: Reagents used in the trypsin gold digest

Reagent	Supplier	Part Number
Seppro Ammonium Bicarbonate	Sigma	52454-200mL
Urea	Fisher	BP169-500
DL-Dithiothreitol (DTT)	Sigma	4381-5G
Iodoacetamide (IAA)	GBiosciences	786-078
Trypsin Gold	Promega	V5280
Acetic Acid	VWR	BDH3096-2.5LPC

[0277] ***Preparation***:

[0278] *50 mM ABC (Ammonium Bicarbonate)*. 0.25 mL of 2M ABC was added to 9.75 mL of water to yield 10 mL of 50 mM ABC. The solution was vortexed and stored at 4ºC for up to a week.

[0279] *8M Urea.* 4.8 g of urea was weighed and 50 mM of ABC was added until close to about the 10 mL mark. The solution was vortexed and optionally swirled in 37°C incubator to help dissolve. 50 mM ABC was added to the 10 mL mark and vortexed.

[0280] *200mM DTT.* 0.031 g of DTT was weighed and 1 mL 50mM ABC was added. The solution was vortexed and stored away from light at 4°C.

[0281] *200mM IAA.* 400 uL 50mM ABC was added to 0.015g of premeasured IAA. The solution was vortexed and stored at 4°C. The solution was made right before use.

[0282] *Trypsin Gold Reconstitution.* The solution was prepared as per manufacture PI instructions. 100uL of 50mM Acetic Acid was added to 100ug of trypsin, and vortexed. The final concentration was 1ug/uL trypsin.

[0283] Sample/Trypsin Preparation

[0284] 40uL of 8M of urea was added to each sample. The solution was vortexed and sonicated for about 1 min. 2 uL of 200 mM DTT was added to each sample, and vortexed. The solution was incubated at room temperature for about 30min in the dark. 8 uL of 200mM IAA was added to each sample, and vortexed. The solution was incubated at room temperature for about 30min in the dark. 8 uL of 200 mM DTT was added to each sample, and vortexed. The solution was incubated at room temperature for about 30min in the dark. 50mM ABC was added so that the added urea was less than 2M. 110uL of 50mM ABC was added into 58uL of sample. The appropriate amount of trypsin was added to the samples. 3uL of reconstituted trypsin was added to each tube. The ratio of protein:trypsin = ~30ug protein:1ug trypsin. The solution was incubated at 37°C overnight. 17uL of 10%FA was added to stop digestion.

<u>Example 3</u>: Proteomic analysis of NSCLC samples and healthy controls

[0285] This example describes proteomic analysis of NSCLC samples and healthy controls. To demonstrate the utility of the corona analysis platform, the platform's ability was evaluated using a single particle type, poly(N-(3-(dimethylamino)propyl) methacrylamide) (PDMAPMA)-coated SPIONs, and serum samples from 56 subjects (28 with Stage IV NSCLC and 28 age- and gender-matched controls) to observe differences between the groups. The selected subject samples represented a reasonably balanced study to identify potential MS features that are different between the groups. Full data on subject annotation including disease status and co-morbidities are compiled in TABLE 5.

TABLE 5: Gender and age information for the patients from whom the serum samples were obtained.

Class	Gender	Age Mean (Standard Deviation)	Number
Healthy (Control)	Female	71.1(7.7)	19
	Male	72.4(11.1)	9
Non Small Cell Lung Cancer (Diseased)	Female	70.7(7.5)	19
	Male	75.6(13.6)	9

[0286] After collection and filtering of the MS1 features followed by log2 transformation of their intensity, the datasets were median scaled without respect to class. **FIG. 11** shows the normalized intensity distributions for all 56 subject datasets. All 56 sample MS raw data files from the NSCLC versus control study were processed by OpenMS pipeline scripts to extract MS1 features and their intensities and cluster them into feature groups based on overlapping mz and RT values within specified tolerances. Only those feature groups were retained that 1) had at least 50% presence of a feature in the group from at least one of the arms of the comparison and 2) had a feature group cluster quality above the 25th percentile. The retained features were median normalized without respect to class and used for subsequent univariate analytical comparison. There were no outliers by inspection of the distributions and all datasets were retained for the univariate analysis.

[0287] There did not appear to be any outlier datasets by inspection. Univariate comparison of feature group intensities between the classes was performed with a non-parametric, Wilcoxon Test (two-sided). The resulting p-value for the comparison was corrected for multiple testing using the method of Benjamini-Hochberg. Using an adjusted p-value cut-off of 0.05, a total of seven feature groups demonstrated statistical significance, as summarized in **FIG. 12**.

[0288] All five of the proteins identified as differentially abundant between the NSCLC-diseased and control groups have previously been implicated in cancer if not actually NSCLC itself. PON1, or paraoxanase-l, has a complicated pattern in lung cancer including the involvement of a relatively common minor allele variant (Q192R) as a risk factor. At the protein level, PON1 is modestly decreased in lung adenocarcinoma. SAA1 is an acute phase protein that has been shown to be overexpressed in NSCLC in MS-related studies, and the identified peptide was found to be increased 5.4-fold in diseased subjects. The matrisome factor tenascin C (TENA) has been shown to be increased in primary lung tumors and associated lymph node metastases compared with normal tissue, and the associated MS feature was found to be increased by 2-fold in this study. Neural cell adhesion molecule 1 (NCAMl) serves as a marker for diagnosing lung neuroendocrine tumors. FIBA peptides were identified by MS analysis with increased levels

correlating with advancing progression of lung cancer. Of special note are the two unknown features, Group2 and Group7, which show differences between control and diseased subjects. Group2 was found in 54 out of 56 subjects and had a modest 33% decrease in diseased subjects. In contrast, Group7 was found only in diseased subjects (14 out of 28 members of the class). These results demonstrated the potential utility for the particle corona to aid in identifying known and unknown markers for different disease states.

Example 4: Dynamic Range Compression of Plasma Using Protein Corona Analysis

[0289] This example describes dynamic range compression using particles to collect proteins from a plasma sample.

[0290] In order to evaluate the ability of particles to compress the measured dynamic range, measured and identified protein feature intensities were compared to the published values for the concentration of the same protein. First, the resulting peptide features for each protein was selected with the maximum MS-determined intensity of all possible features for a protein (using the OpenMS MS data processing tools to extract monoisotopic peak values), and then the intensities were modeled against the published abundance levels for those same proteins. **FIG. 13** shows correlation of the maximum intensities of particle corona proteins and plasma proteins to the published concentration of the same proteins. The blue plotted lines are linear regression models to the data and the shaded regions represent the standard error of the model fit. The dynamic range of the samples assayed with particles ("S-003," "S-007," and "S-011", detailed in TABLE 1) exhibited a compressed dynamic range as compared to the plasma sample not assayed with particles ("Plasma"), as shown by the decrease in slopes of the linear fits. The slopes of each plot are 0.47, 0.19, 0.22, and 0.18 for, plasma without particles, plasma with S-003 particles, plasma with S-007 particles, and plasma with S-011 particles, respectively. **FIG. 14** shows the dynamic range compression of a protein corona analysis assay with mass spectrometry as compared to mass spectrometry without particle corona formation. Protein intensities of common proteins identified in particle coronas in the plasma samples assayed in **FIG. 13** ("Nanoparticle MS ln Intensity") are plotted against the protein intensity identified by mass spectrometry of plasma without particles ("Plasma MS ln Intensity"). The lightest dotted line shows a slope of 1, indicating the dynamic range of mass spectrometry without particles. The slopes of the linear fits to the protein intensity was 0.12, 0.36, and 0.093 for S-003, S-007, and S-011 particles, respectively. The grayed area indicates the standard error region of the regression fit.

[0291] By comparing the regression model slopes and the intensity span of the measured data, the biomolecule coronas contain more proteins at lower abundances (measured or reported) than

does plasma. The dynamic range of those measured values was compressed (the slope of the regression model is reduced) for particle measurements as compared to plasma measurements. This was consistent with previous observations that particle can effectively compress the measured dynamic range for abundances in the resulting corona as compared to the original dynamic range in plasma, which could be attributable to the combination of absolute concentration of a protein, its binding affinity to particles, and its interactions with neighboring proteins. The results indicated that the biomolecule corona strategy facilitated the identification of a broad spectrum of plasma proteins, particularly those in the low abundance that are challenging for rapid detection by conventional proteomic techniques.

[0292] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. An automated apparatus for generating a subset of biomolecules from a complex biological sample, the automated apparatus comprising:
   (a) a substrate comprising a plurality of partitions, wherein the plurality of partitions comprises a plurality of particles;
   (b) a sample storage unit comprising the complex biological sample; and
   (c) a loading unit that is movable at least across the substrate,

   wherein the loading unit transfers one or more volumes of the complex biological sample in the sample storage unit to the plurality of partitions on the substrate, thereby contacting the plurality of particles in the plurality of partitions with biomolecules of the complex biological sample to form biomolecule coronas, thereby generating the subset of biomolecules of the complex biological sample, and

   wherein a dynamic range of the subset of biomolecules is compressed relative to a dynamic range of biomolecules present in the complex biological sample.
2. The automated apparatus of claim 1, further comprising an incubation element that agitates or heats volumes of the plurality of particles within volumes of the complex biological sample in the plurality of partitions.
3. The automated apparatus of claim 2, wherein the incubation element is configured to shake, mix, stir, spin, vibrate, be static, or any combination thereof.
4. The automated apparatus of claim 2 or 3, wherein the incubation element is configured to heat and/or incubate the substrate to a temperature between about 20 °C and about 100 °C.
5. The automated apparatus of any one of claims 1-4, wherein the plurality of partitions is at least partially covered or sealed.
6. The automated apparatus of claim 5, wherein the automated apparatus comprises the ability to add or remove a lid on the substrate, wherein the lid covers at least one of the partitions from among the plurality of partitions.
7. The automated apparatus of any one of claims 1-6, further comprising a unit comprising a resuspension solution.
8. The automated apparatus of claim 7, wherein the resuspension solution comprises Tris EDTA 150mM KCl 0.05% CHAPS buffer.
9. The automated apparatus of claim 7, wherein the resuspension solution comprises 10 mM Tris HCl pH 7.4, 1 mM EDTA.

10. The automated apparatus of any one of claims 1-9, further comprising a unit comprising a denaturing solution.
11. The automated apparatus of claim 10, wherein the denaturing solution comprises a protease.
12. The automated apparatus of claims 10 or 11, wherein the denaturing solution comprises a reductant, a methylating agent, guanidine, urea, sodium deoxycholate, acetonitrile, or any combination thereof.
13. The automated apparatus of any one of claims 10-12, wherein the denaturing solution generates an average peptide fragment with a mass of less than 4600 Daltons.
14. The automated apparatus of any one of claims 1-13, wherein the substrate is a multi-well plate.
15. The automated apparatus of any one of claims 1-14, wherein the loading unit comprises a plurality of pipettes.
16. The automated apparatus of any one of claims 1-15, wherein the loading unit is configured to dispense 10 uL to 400 uL of a solution into one or more partitions of the plurality of partitions.
17. The automated apparatus of claim 16, wherein the loading unit is configured to dispense 5 uL to 150 uL of a solution into one or more partitions of the plurality of partitions.
18. The automated apparatus of claim 16, wherein the loading unit is configured to dispense 35 uL to 80 uL of a solution into one or more partitions of the plurality of partitions.
19. The automated apparatus of any one of claims 16-18 , wherein the solution is selected from the group consisting of a wash solution, the resuspension solution, the denaturing solution, a buffer and a reagent.
20. The automated apparatus of any one of claims 1-19, wherein the loading unit is configured to dispense 10 uL to 400 uL of the complex biological sample into one or more partitions of the plurality of partitions.
21. The automated apparatus of claim 20, wherein the loading unit is configured to dispense 5 uL to 150 uL of the complex biological sample into one or more partitions of the plurality of partitions.
22. The automated apparatus of claim 20, wherein the loading unit is configured to dispense 35 uL to 80 uL of the complex biological sample into one or more partitions of the plurality of partitions.
23. The automated apparatus of any one of claims 1-22, wherein the complex biological sample comprises a biofluid from a subject.

24. The automated apparatus of claim 23, wherein the complex biological sample comprises plasma, serum, urine, cerebrospinal fluid, synovial fluid, tears, saliva, whole blood, milk, nipple aspirate, ductal lavage, vaginal fluid, nasal fluid, ear fluid, gastric fluid, pancreatic fluid, trabecular fluid, lung lavage, sweat, crevicular fluid, semen, prostatic fluid, sputum, fecal matter, bronchial lavage, fluid from swabbings, bronchial aspirants, fluidized solids, fine needle aspiration samples, tissue homogenates, lymphatic fluid. cell culture samples, or any combination thereof.
25. The automated apparatus of any one of claims 1-18, further comprising a magnet.
26. The automated apparatus of claim 25, wherein one or more particles of the plurality of particles is a magnetic particle, and wherein the substrate and the magnet are in proximity such that the one or more magnetic particles are immobilized on the substrate.
27. The automated apparatus of any one of claims 1-26, further comprising a housing, wherein the substrate and the loading unit are located in the housing, and wherein the housing is at least partially enclosed.
28. The automated apparatus of any one of claims 1-27, wherein the compressed dynamic range comprises an increase in the number of types of biomolecules whose concentrations are within 6 orders of magnitude of the most abundant biomolecule in the sample.
29. The automated apparatus of claim 28, wherein the compressed dynamic range comprises an increase in the number of types of biomolecules whose concentrations are within 5 orders of magnitude of the most abundant biomolecule in the sample.
30. The automated apparatus of claim 29, wherein the compressed dynamic range comprises an increase in the number of types of biomolecules whose concentrations are within 4 orders of magnitude of the most abundant biomolecule in the sample.
31. The automated apparatus of claims 30, wherein the compressed dynamic range comprises an increase in the number of types of proteins whose concentrations are within 6 orders of magnitude of the most abundant protein in the sample.
32. The automated apparatus of claim 31, wherein the increase in the number of types of biomolecules whose concentrations are within 6 orders of magnitude of the most concentrated biomolecule in the sample is at least 25%, 50%, 100%, 200%, 300%, 500%, or 1000%.
33. The automated apparatus of any one of claims 1-32, wherein the compressed dynamic range comprises an increase in the number of types of proteins whose concentrations are within 6 orders of magnitude of the most abundant protein in the sample.

34. The automated apparatus of claim 33, wherein the increase in the number of types of proteins whose concentrations are within 6 orders of magnitude of the most abundant protein in the sample is at least 25%, 50%, 100%, 200%, 300%, 500%, or 1000%.
35. The automated apparatus of any one of claims 1-34, wherein the subset of biomolecules comprises at least 20% to at least 60% of the types of biomolecules from the complex biological sample within a 6 order of magnitude concentration range.
36. The automated apparatus of claim 35, wherein the subset of biomolecules comprises at least 20% to at least 60% of the types of proteins from the complex biological sample within a 6 order of magnitude concentration range.
37. The automated apparatus of any one of claims 1-36, wherein the dynamic range of the biomolecules of the biomolecule coronas is a first ratio of a top decile of biomolecules to a bottom decile of biomolecules in the plurality of biomolecule coronas.
38. The automated apparatus of any one of claims 1-36, wherein the dynamic range of the biomolecules of the biomolecule coronas is a first ratio comprising a span of the interquartile range of biomolecules in the plurality biomolecule coronas.
39. The automated apparatus of any one of claims 1-38, wherein the generating enriches low abundance biomolecules from the complex biological sample.
40. The automated apparatus of claim 33, wherein the low abundance biomolecules are biomolecules at concentrations of 10ng/mL or less in the complex biological sample.
41. The automated apparatus of any one of claims 1-34, wherein the subset of biomolecules from the complex biological sample comprises proteins.
42. The automated apparatus of claim 41, wherein changes of at most 10 mg/mL in the lipid concentration of the complex biological sample result in changes of less than 10%, 5%, 2%, or 1% in the composition of the proteins in the subset of biomolecules generated from the complex biological sample.
43. The automated apparatus of any one of claims 1-42, wherein at least two particles from among the plurality of particles differ in at least one physicochemical property.
44. The automated apparatus of claim 43, wherein the at least one physicochemical property is selected from the group consisting of: composition, size, surface charge, hydrophobicity, hydrophilicity, surface functionality, surface topography, surface curvature, porosity, core material, shell material, shape, and any combination thereof.
45. The automated apparatus of claim 44, wherein the surface functionality comprises aminopropyl functionalization, amine functionalization, boronic acid functionalization, carboxylic acid functionalization, methyl functionalization, N-succinimidyl ester

functionalization, PEG functionalization, streptavidin functionalization, methyl ether functionalization, triethoxylpropylaminosilane functionalization, thiol functionalization, PCP functionalization, citrate functionalization, lipoic acid functionalization, BPEI functionalization.

46. The automated apparatus of any one of claims 1-45, wherein a particle from among the plurality of particles is selected from the group consisting of: micelles, liposomes, iron oxide particles, silver particles, gold particles, palladium particles, quantum dots, platinum particles, titanium particles, silica particles, metal or inorganic oxide particles, synthetic polymer particles, copolymer particles, terpolymer particles, polymeric particles with metal cores, polymeric particles with metal oxide cores, polystyrene sulfonate particles, polyethylene oxide particles, polyoxyethylene glycol particles, polyethylene imine particles, polylactic acid particles, polycaprolactone particles, polyglycolic acid particles, poly(lactide-co-glycolide polymer particles, cellulose ether polymer particles, polyvinylpyrrolidone particles, polyvinyl acetate particles, polyvinylpyrrolidone-vinyl acetate copolymer particles, polyvinyl alcohol particles, acrylate particles, polyacrylic acid particles, crotonic acid copolymer particles, polyethlene phosphonate particles, polyalkylene particles, carboxy vinyl polymer particles, sodium alginate particles, carrageenan particles, xanthan gum particles, gum acacia particles, Arabic gum particles, guar gum particles, pullulan particles, agar particles, chitin particles, chitosan particles, pectin particles, karaya tum particles, locust bean gum particles, maltodextrin particles, amylose particles, corn starch particles, potato starch particles, rice starch particles, tapioca starch particles, pea starch particles, sweet potato starch particles, barley starch particles, wheat starch particles, hydroxypropylated high amylose starch particles, dextrin particles, levan particles, elsinan particles, gluten particles, collagen particles, whey protein isolate particles, casein particles, milk protein particles, soy protein particles, keratin particles, polyethylene particles, polycarbonate particles, polyanhydride particles, polyhydroxyacid particles, polypropylfumerate particles, polycaprolactone particles, polyamine particles, polyacetal particles, polyether particles, polyester particles, poly(orthoester) particles, polycyanoacrylate particles, , polyurethane particles, polyphosphazene particles, polyacrylate particles, polymethacrylate particles, polycyanoacrylate particles, polyurea particles, polyamine particles, polystyrene particles, poly(lysine) particles, chitosan particles, dextran particles, poly(acrylamide) particles, derivatized poly(acrylamide) particles, gelatin particles, starch particles, chitosan particles, dextran particles, gelatin particles, starch particles, poly-β-amino-ester particles, poly(amido amine) particles, poly lactic-co-glycolic acid particles, polyanhydride particles, bioreducible

polymer particles, and 2-(3-aminopropylamino)ethanol particles, and any combination thereof.

47. The automated apparatus of any one of claims 1-46, wherein one or more particles of the plurality of particles adsorbs at least 100 types of proteins upon contacting the complex biological sample.
48. The automated apparatus of any one of claims 1-47, wherein the plurality of particles comprises at least 2 distinct particle types, at least 3 distinct particle types, at least 4 distinct particle types, at least 5 distinct particle types, at least 6 distinct particle types, at least 7 distinct particle types, at least 8 distinct particle types, at least 9 distinct particle types, at least 10 distinct particle types, at least 11 distinct particle types, at least 12 distinct particle types, at least 13 distinct particle types, at least 14 distinct particle types, at least 15 distinct particle types, at least 20 distinct particle types, at least 25 particle types, or at least 30 distinct particle types.
49. The automated apparatus of any one of claims 1-48, wherein biomolecules of the biomolecule coronas comprise a number of protein groups.
50. The automated apparatus of claim 49, wherein the number of protein groups comprises from 1 to 20,000 protein groups.
51. The automated apparatus of claim 50, wherein the number of protein groups comprises from 100 to 10,000 protein groups.
52. The automated apparatus of claim 51, wherein the number of protein groups comprises from 100 to 5000 protein groups.
53. The automated apparatus of claim 52, wherein the number of proteins groups comprises from 300 to 2,200 protein groups.
54. The automated apparatus of claim 53, wherein the number of proteins groups comprises from 1,200 to 2,200 protein groups.
55. The automated apparatus of any one of claims 1-54, wherein at least two partitions of the plurality of partitions comprise different buffers.
56. The automated apparatus of claim 55, wherein the different buffers differ in pH, salinity, osmolarity, viscosity, dielectric constant, or any combination thereof.
57. The automated apparatus of any one of claims 1-56, wherein at least two partitions of the plurality of partitions comprise different ratios of buffer and the complex biological sample.
58. The automated apparatus of any one of claims 1-57, wherein one or more partitions of the plurality of partitions comprises 1 pM to 100 nM nanoparticles.

59. The automated apparatus of any one of claims 1-58, wherein at least two partitions of the plurality of partitions comprise different concentrations of nanoparticles.
60. The automated apparatus of any one of claims 1-59, further comprising a purification unit.
61. The automated apparatus of claim 60, wherein the purification unit comprises a solid phase extraction (SPE) plate.
62. The automated apparatus of any one of claims 1-61, wherein the automated apparatus generates the subset of biomolecules from a complex biological sample in less than 7 hours.
63. An automated system comprising:
    (a) an automated apparatus of any one of claims 1-56 configured to isolate the subset of biomolecules from the biological sample;
    (b) a mass spectrometer configured to receive the subset of biomolecules and to generate data comprising mass spectrometric or tandem mass spectrometric signals; and
    (c) a computer comprising one or more computer processors and a computer readable medium comprising machine-executable code that, upon execution by the one or more computer processors, implements a method comprising:
        i. generating a biomolecule fingerprint, and
        ii. assigning a biological state based on the biomolecule fingerprint.
64. The automated system of claim 63, wherein the biomolecule fingerprint comprises a plurality of distinct biomolecule corona signatures.
65. The automated system of claim 64, wherein the biomolecule fingerprint comprises at least 5, 10, 20, 40, or 80, 150 or 200 distinct biomolecule corona signatures.
66. The automated system of any one of claims 63-65, wherein the computer is configured to process the data comprising the intensity, APEX, spectral count or number of peptides, or Ion mobility behavior of the mass spectrometric or tandem mass spectrometric signal between a plurality of the distinct biomolecule corona signatures.
67. The automated system of claim 66, wherein the computer is configured to process data from between 100 and 2000 mass spectrometric or tandem mass spectrometric signals between a plurality of the distinct biomolecule corona signatures.
68. The automated system of claim 67, wherein the computer is configured to process the data comprising the intensities of between 10,000 and 5,000,000 mass spectrometric or tandem mass spectrometric signals between a plurality of the distinct biomolecule corona signatures.
69. The automated system of any one of claims 63-68, wherein the biomolecule fingerprint is generated from data from a single mass spectrometric or tandem mass spectrometric run.

70. The automated system of claim 69, wherein the single mass spectrometric or tandem mass spectrometric run is performed in less than one hour.
71. The automated system of any one of claims 63-70, wherein the computer is configured to identify a biomolecule or characterize an unidentified molecular feature based on a mass spectrometric or tandem mass spectrometric signal and or ion mobility and chromatographic behavior, and wherein the computer provides a certainty threshold of at least 95% to identify a feature or characterize and unidentified feature.
72. The automated system of any one of claims 63-71, wherein the automated system is configured to generate the biomolecule fingerprint from the complex biological sample in less than about 10 hours.
73. The automated system of any one of claims 63-72, wherein the computer is capable of distinguishing between two or more biological states associated with biomolecule fingerprints that differ by less than 10%, 5%, 2%, or 1%.
74. The automated system of any one of claims 63-73, wherein the biological state is a disease, disorder, or tissue abnormality.
75. The automated system of claim 75, wherein the disease is an early phase or intermediate phase disease state.
76. The automated system of claims 74 or 75, wherein the disease is cancer.
77. The automated system of claim 76, wherein the cancer is a stage 0 cancer or a stage 1 cancer.
78. The automated system of claims 76 or 77, wherein the cancer is selected from the group consisting of: lung cancer, pancreas cancer, myeloma, myeloid leukemia, meningioma, glioblastoma, breast cancer, esophageal squamous cell carcinoma, gastric adenocarcinoma, prostate cancer, bladder cancer, ovarian cancer, thyroid cancer, neuroendocrine cancer, colon carcinoma, ovarian cancer, head and neck cancer, Hodgkin's Disease, non-Hodgkin's lymphomas, rectum cancer, urinary cancers, uterine cancers, oral cancers, skin cancers, stomach cancer, brain tumors, liver cancer, laryngeal cancer, esophageal cancer, mammary tumors, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, Ewing's sarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystandeocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, endometrial cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioblastomas, neuronomas, craniopharingiomas, schwannomas,

glioma, astrocytoma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemias and lymphomas, acute lymphocytic leukemia and acute myelocytic polycythemia vera, multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease, acute nonlymphocytic leukemias, chronic lymphocytic leukemia, chronic myelogenous leukemia, childhood-null acute lymphoid leukemia (ALL), thymic ALL, B-cell ALL, acute megakaryocytic leukemia, Burkitt's lymphoma, and T cell leukemia, small and large non-small cell lung carcinoma, acute granulocytic leukemia, germ cell tumors, endometrial cancer, gastric cancer, hairy cell leukemia, or thyroid cancer.

79. The automated system of any one of claims 74-78, wherein the biological state is a pre-disease state.

80. The automated system of any one of claims 63-79, wherein the determining comprises comparing the abundance of two biomolecules whose concentrations span at least 7 to at least 12 orders of magnitude in the complex biological sample.

81. A method for distinguishing a biological state of a complex biological sample, the method comprising:

(a) providing the complex biological sample to the automated apparatus of any one of claims 1-56 to generate a subset of biomolecules;
(b) assaying the subset of biomolecules to generate a biomolecule fingerprint; and
(c) distinguishing a biological state of the complex biological sample with the biomolecule fingerprint.

82. The method of claim 81, wherein the biomolecule fingerprint comprises proteins.

83. The method of claim 81 or 82, wherein the subset of biomolecules from the complex biological sample comprises a lower ratio of albumin to non-albumin peptides than the complex biological sample.

84. The method of any one of claims 81-83, wherein the subset of biomolecules comprises biomolecules that span at least 6 to at least 12 orders of magnitude in concentration range in the complex biological sample.

85. The method of any one of claims 81-84, wherein the subset of biomolecules comprises proteins that span at least 6 to at least 12 orders of magnitude in concentration range in the complex biological sample.

86. The method of any one of claims 81-85, wherein the biomolecule fingerprint comprises from 1 to 74,000 protein groups.

87. The method of any one of claims 81-86, wherein the assaying comprises desorbing a plurality of biomolecules from a biomolecule corona from among the plurality of biomolecule coronas.
88. The method of claim 87, wherein the assaying comprises chemically modifying a biomolecule from among the plurality of desorbed biomolecules.
89. The method of claim 87, wherein the assaying comprises fragmenting a biomolecule from among the plurality of desorbed biomolecules.
90. The method of claim 89, wherein the fragmenting comprises protease digestion.
91. The method of claim 89, wherein the fragmenting comprises chemical peptide cleavage.
92. The method of any one of claims claim 87-91, wherein the assaying comprises collecting the plurality of desorbed biomolecules.
93. The method of claim 92, wherein the assaying comprises purifying the collected plurality of desorbed biomolecules.
94. The method of claim 93, wherein the purifying comprises solid-phase extraction.
95. The method of claims 93 or 94, wherein the purifying depletes non-protein biomolecules from the collected plurality of desorbed biomolecules.
96. The method of claim 87, wherein the assaying comprises discarding the plurality of desorbed biomolecules.
97. The method of any one of claims 87-96, where the assaying comprises desorbing a first subset of biomolecules and a second set of biomolecules from a biomolecule corona from among the plurality of biomolecule coronas, analyzing a biomolecule from among the first subset of biomolecules, and analyzing a biomolecule from among the second subset of biomolecules.
98. The method of any one of claims 81-97, wherein the assaying comprises analyzing a biomolecule corona from among the plurality of biomolecule coronas with mass spectrometry, tandem mass spectrometry, mass cytometry, mass cytometry, potentiometry, fluorimetry, absorbance spectroscopy, Raman spectroscopy, chromatography, electrophoresis, immunohistochemistry, PCR, next generation sequencing (NGS), or any combination thereof.
99. The method of claim 98, wherein the assaying comprises mass spectrometry or tandem mass spectrometry.
100. The method of any one of claims 81-99, wherein the assaying comprises identifying the conformational state of a protein from among the subset of biomolecules.

101. The method of any one of claims 81-100, wherein the assaying comprises identifying a post-translational modification on a protein from among the subset of biomolecules.

102. The method of any one of claims 81-101, wherein the distinguishing comprises comparing the relative abundances of at least 200 to at least 1000 biomolecules from the subset of biomolecules.

103. The method of any one of claims 81-102, wherein the assaying identifies biomolecules at concentrations of less than 10 ng/mL in the complex biological sample.

104. An automated apparatus for generating a subset of biomolecules from a complex biological sample, the automated apparatus comprising:

a plurality of particles; and

the complex biological sample,

wherein the automated apparatus is configured to generate the subset of biomolecules by contacting the plurality of particles with the complex biological sample to form a plurality of biomolecule coronas comprising the subset of biomolecules, and

wherein a dynamic range of the subset of biomolecules is compressed relative to a dynamic range of biomolecules present in the complex biological sample.

105. The automated apparatus of claim 104, further comprising a substrate, wherein the substrate is a multi-well plate.

106. The automated apparatus of claim 104 or 105, further comprising an incubation element.

107. The automated apparatus of claim 106, wherein the incubation element is configured to heat and/or incubate the plurality of particles and the complex biological sample to a temperature between 4 °C and 40 °C.

108. The automated apparatus of any one of claims 104-107, further comprising at least one solution selected from the group consisting of a wash solution, a resuspension solution, a denaturing solution, a buffer and a reagent.

109. The automated apparatus of claim 108, wherein the resuspension solution comprises a Tris EDTA buffer, a phosphate buffer, and/or water.

110. The automated apparatus of claim 108, wherein the denaturing solution comprises a protease.

111. The automated apparatus of claim 108, wherein the denaturing solution comprises a small molecule capable of performing peptide cleavage.

112. The automated apparatus of any one of claims 104-111, further comprising a loading unit, wherein the loading unit comprises a plurality of pipettes.

113. The automated apparatus of claim 112, wherein each pipette of the plurality of pipettes is configured to dispense about 5 uL – 150 uL of the solution, the complex biological sample, and/or the plurality of particles.

114. The automated apparatus of any one of claim 104-113, wherein the complex biological sample comprises plasma, serum, urine, cerebrospinal fluid, synovial fluid, tears, saliva, whole blood, milk, nipple aspirate, ductal lavage, vaginal fluid, nasal fluid, ear fluid, gastric fluid, pancreatic fluid, trabecular fluid, lung lavage, sweat, crevicular fluid, semen, prostatic fluid, sputum, fecal matter, bronchial lavage, fluid from swabbings, bronchial aspirants, fluidized solids, fine needle aspiration samples, tissue homogenates, lymphatic fluid. cell culture samples, or any combination thereof.

115. The automated apparatus of any one of claims 104-114, further comprising a magnet.

116. The automated apparatus of any one of claims 104-115, further comprising a filter.

117. The automated apparatus of any one of claims 104-116, wherein the compressed dynamic range comprises an increase in the number of types of biomolecules whose concentrations are within 4 to 6 orders of magnitude of the most abundant biomolecule in the sample.

118. The automated apparatus of claim 117, wherein the types of biomolecules comprises protein.

119. The automated apparatus of any one of claims 104-118, wherein the dynamic range of the biomolecules of the biomolecule coronas is a first ratio of a top decile of biomolecules to a bottom decile of biomolecules in the plurality of biomolecule coronas.

120. The automated apparatus of any one of claims 104-119, wherein the generating enriches low abundance biomolecules from the complex biological sample.

121. The automated apparatus of claim 120, wherein the low abundance biomolecules are biomolecules at concentrations of 10ng/mL or less in the complex biological sample.

122. The automated apparatus of any one of claims 104-121, wherein at least two particles from among the plurality of particles differ in at least one physicochemical property.

123. The automated apparatus of claim 114, wherein the at least one physicochemical property is selected from the group consisting of: composition, size, surface charge, hydrophobicity, hydrophilicity, surface functionality, surface topography, surface curvature, porosity, core material, shell material, shape, and any combination thereof.

124. The automated apparatus of any one of claims 104-123, wherein a particle from among the plurality of particles is selected from the group consisting of: micelles, liposomes, iron oxide particles, silver particles, gold particles, palladium particles, quantum dots, platinum particles, titanium particles, silica particles, metal or inorganic oxide particles, synthetic

polymer particles, copolymer particles, terpolymer particles, polymeric particles with metal cores, polymeric particles with metal oxide cores, polystyrene sulfonate particles, polyethylene oxide particles, polyoxyethylene glycol particles, polyethylene imine particles, polylactic acid particles, polycaprolactone particles, polyglycolic acid particles, poly(lactide-co-glycolide polymer particles, cellulose ether polymer particles, polyvinylpyrrolidone particles, polyvinyl acetate particles, polyvinylpyrrolidone-vinyl acetate copolymer particles, polyvinyl alcohol particles, acrylate particles, polyacrylic acid particles, crotonic acid copolymer particles, polyethlene phosphonate particles, polyalkylene particles, carboxy vinyl polymer particles, sodium alginate particles, carrageenan particles, xanthan gum particles, gum acacia particles, Arabic gum particles, guar gum particles, pullulan particles, agar particles, chitin particles, chitosan particles, pectin particles, karaya tum particles, locust bean gum particles, maltodextrin particles, amylose particles, corn starch particles, potato starch particles, rice starch particles, tapioca starch particles, pea starch particles, sweet potato starch particles, barley starch particles, wheat starch particles, hydroxypropylated high amylose starch particles, dextrin particles, levan particles, elsinan particles, gluten particles, collagen particles, whey protein isolate particles, casein particles, milk protein particles, soy protein particles, keratin particles, polyethylene particles, polycarbonate particles, polyanhydride particles, polyhydroxyacid particles, polypropylfumerate particles, polycaprolactone particles, polyamine particles, polyacetal particles, polyether particles, polyester particles, poly(orthoester) particles, polycyanoacrylate particles, , polyurethane particles, polyphosphazene particles, polyacrylate particles, polymethacrylate particles, polycyanoacrylate particles, polyurea particles, polyamine particles, polystyrene particles, poly(lysine) particles, chitosan particles, dextran particles, poly(acrylamide) particles, derivatized poly(acrylamide) particles, gelatin particles, starch particles, chitosan particles, dextran particles, gelatin particles, starch particles, poly-β-amino-ester particles, poly(amido amine) particles, poly lactic-co-glycolic acid particles, polyanhydride particles, bioreducible polymer particles, and 2-(3-aminopropylamino)ethanol particles, and any combination thereof.

125. The automated apparatus of any one of claims 104-124, wherein biomolecules of the biomolecule coronas comprise a number of protein groups.

126. The automated apparatus of claim 125, wherein the number of protein groups comprises from 1 to 20,000 protein groups.

127. The automated apparatus of claim 125, wherein the number of protein groups comprises from 100 to 10,000 protein groups.

128. The automated apparatus of claim 125, wherein the number of protein groups comprises from 100 to 5000 protein groups.

129. The automated apparatus of claim 125, wherein the number of proteins groups comprises from 300 to 2,200 protein groups.

130. The automated apparatus of claim 125, wherein the number of proteins groups comprises from 1,200 to 2,200 protein groups.

131. The automated apparatus of any one of claims 104-130, further comprising a purification unit.

132. The automated apparatus of claim 131, wherein the purification unit comprises a solid phase extraction (SPE) plate.

133. The automated apparatus of any one of claims 104-132, wherein the automated apparatus generates the subset of biomolecules from a complex biological sample in less than 7 hours.

134. A method for generating a subset of biomolecules from a complex biological sample, the method comprising:

providing the complex biological sample to an automated apparatus,

wherein the automated apparatus contacts the complex biological sample with a plurality of particles to generate biomolecule coronas,

wherein the automated apparatus processes the biomolecule coronas to generate the subset of biomolecules, and

wherein a dynamic range of the subset of biomolecules is compressed relative to a dynamic range of biomolecules present in the complex biological sample.

135. The method of claim 134, wherein the automated apparatus is the automated apparatus of any one of claims 104-133.

136. The method of claim 134 or 135, further comprising assaying the subset of biomolecules to generate a biomolecule fingerprint.

137. The method of claim 136, wherein the assaying identifies biomolecules at concentrations of less than 10 ng/mL in the complex biological sample.

138. The method of any one of claims 136 or 137, wherein the assaying comprises analyzing biomolecule coronas with mass spectrometry, tandem mass spectrometry, mass cytometry, mass cytometry, potentiometry, fluorimetry, absorbance spectroscopy, Raman spectroscopy, chromatography, electrophoresis, immunohistochemistry, or any combination thereof.

139. The method of claim 138, wherein the assaying comprises mass spectrometry or tandem mass spectrometry

140. The method of any one of claims 134-139, further comprising distinguishing a biological state of the complex biological sample with the biomolecule fingerprint.

141. The method of claim 140, wherein the biomolecule fingerprint comprises a plurality of distinct biomolecule corona signatures.

142. The method of claim 140 or 141, wherein the biomolecule fingerprint comprises at least 5, 10, 20, 40, or 80, 150 or 200 distinct biomolecule corona signatures.

143. The method of any one of claims 140-142, wherein the biological state is a disease, disorder, or tissue abnormality.

144. The method of claim 143, wherein the disease is an early phase or intermediate phase disease state.

145. The method of claim 143 or 144, wherein the disease is cancer.

146. The method of claim 145, wherein the cancer is a stage 0 cancer or a stage 1 cancer.

147. The method of claims 145 or 146, wherein the cancer is selected from the group consisting of: lung cancer, pancreas cancer, myeloma, myeloid leukemia, meningioma, glioblastoma, breast cancer, esophageal squamous cell carcinoma, gastric adenocarcinoma, prostate cancer, bladder cancer, ovarian cancer, thyroid cancer, neuroendocrine cancer, colon carcinoma, ovarian cancer, head and neck cancer, Hodgkin's Disease, non-Hodgkin's lymphomas, rectum cancer, urinary cancers, uterine cancers, oral cancers, skin cancers, stomach cancer, brain tumors, liver cancer, laryngeal cancer, esophageal cancer, mammary tumors, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, Ewing's sarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystandeocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, endometrial cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioblastomas, neuronomas, craniopharingiomas, schwannomas, glioma, astrocytoma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemias and lymphomas, acute lymphocytic leukemia and acute myelocytic polycythemia vera, multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease, acute nonlymphocytic leukemias, chronic lymphocytic leukemia, chronic myelogenous leukemia, childhood-null acute lymphoid leukemia (ALL), thymic ALL, B-cell ALL, acute megakaryocytic leukemia, Burkitt's lymphoma, and T cell leukemia, small and large non-

small cell lung carcinoma, acute granulocytic leukemia, germ cell tumors, endometrial cancer, gastric cancer, hairy cell leukemia, or thyroid cancer.

148. The method of claim 143, wherein the biological state is a pre-disease state.

149. An automated system comprising a network of units with differentiated functions in distinguishing states of a complex biological sample using a plurality of particles having surfaces with different physiochemical properties wherein:

(a) a first unit comprises a multichannel fluid transfer instrument for transferring fluids between units within the system;

(b) a second unit comprises a support for storing a plurality of biological samples;

(c) a third unit comprises a support for a sensor array plate possessing partitions that comprise the plurality of particles having surfaces with different physiochemical properties for detecting a binding interaction between a population of analytes within the complex biological sample and the plurality of particles;

(d) a fourth unit comprises supports for storing a plurality of reagents;

(e) a fifth unit comprises supports for storing a reagent to be disposed of;

(f) a sixth unit comprises supports for storing consumables used by the multichannel fluid transfer instrument; and

wherein the system is programed to perform a series of steps comprising:

i. contacting the biological sample with a specified partition of the sensor array;

ii. incubating the biological sample with the plurality of particles contained within the partition of the sensor array plate;

iii. removing all components from a partition except the plurality of particles and a population of analytes interacting with a particle; and

iv. preparing a sample for mass spectrometry.

150. The automated system of claim 149, wherein the first unit comprises a degree of mobility that enables access to all other unit within the system.

151. The automated system of either of claims 149 or 150, wherein the first unit comprises a capacity to perform pipetting functions.

152. The automated system of any one of claims 149-151, wherein the support of the second and/or third unit comprises support for a single plate, a 6 well plate, a 12 well plate, a 96 well plate, or a rack of microtubes.

153. The automated system of any one of claims 149-152, wherein the second and/or unit comprises a thermal unit capable of modulating the temperature of said support and a sample.

154. The automated system of any one of claims 149-153, wherein the second and/or third unit comprises a rotational unit capable of physically agitating and/or mixing a sample.

155. The automated system of any one of claims 149-154, wherein the plurality of particles having surfaces with different physiochemical properties for detecting a binding interaction between a population of analytes within the complex biological sample and the plurality of particles are immobilized to a surface with a partition of the sensory array.

156. The automated system of any one of claims 149-155, wherein the plurality of particles having surfaces comprise a plurality magnetic nanoparticles in a solution with different physiochemical properties for detecting a binding interaction between a population of analytes within the complex biological sample and the plurality of particles.

157. The automated system of claim 156, comprising a step wherein the sensor array plate is transferred to an additional seventh unit that comprises a magnetized support and a thermal unit capable of modulating the temperature of said support and a sample and incubated for an additional amount of time.

158. The automated system of any one of claims 149-157, wherein the fourth unit comprises a set of reagents for:

(a) generating the sensor array plate;

(b) washing an unbound sample; and/or

(c) preparing a sample for mass spectrometry.

159. The automated system of any one of claims 149-158, wherein (i) contacting the biological sample with a specified partition of the sensor array comprises pipetting a specified volume of the biological sample into the specific partition of the sensor array.

160. The automated system of any one of claims 149-159, wherein (i) contacting the biological sample with a specified partition of the sensor array comprises pipetting a volume corresponding to a 1:1, 1:2: 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:15, or 1:20 ratio of a plurality of particles in a solution to the biological sample.

161. The automated system of any one of claims 149-160, wherein (i) contacting the biological sample with a specified partition of the sensor array comprises pipetting a volume of at least 10 microliters, at least 50 microliters, at least 100 microliters, at least 250 microliters, at least 500 microliters, or at least 1000 microliters the biological sample into the specific partition of the sensor array.

162. The automated system of any one of claims 149-161, wherein (ii) incubating the biological sample with the plurality of particles contained within the partition of the sensor array plate comprises an incubation time of at least about 10 seconds, at least about 15 seconds, at least about 20 seconds, at least about 25 seconds, at least about 30 seconds, at least about 40 seconds, at least about 50 seconds, at least about 60 seconds, at least about 90 seconds, at least about 2 minutes, at least about 3 minutes, at least about 4 minutes, at least about 5 minutes, at least about 6 minutes, at least about 7 minutes, at least about 8 minutes, at least about 9 minutes, at least about 10 minutes, at least about 15 minutes, at least about 20 minutes, at least about 25 minutes, at least about 30 minutes, at least about 45 minutes, at least about 50 minutes, at least about 60 minutes, at least about 90 minutes, at least about 2 hours, at least about 3 hours, at least about 4 hours, at least about 5 hours, at least about 6 hours, at least about 7 hours, at least about 8 hours, at least about 9 hours, at least about 10 hours, at least about 12 hours, at least about 14 hours, at least about 15 hours, at least about 16 hours, at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, or at least about 24 hours.

163. The automated system of any one of claims 149-162, wherein (ii) incubating the biological sample with the plurality of particles contained within the partition of the sensor array plate comprises an incubation temperature between about 4° C to about 40° C.

164. The automated system of any one of claims 149-163, wherein removing all components from a partition except the plurality of particles and a population of analytes interacting with a particle comprises a series of wash steps.

165. The automated system of any one of claims 149-164, wherein the second unit can facilitate a transfer of the sample for mass spectrometry to a mass spectrometry unit.

166. An automated apparatus to identify proteins in a biological sample, the automated apparatus comprising:

a sample preparation unit;

a substrate comprising a plurality of channels;

a plurality of pipettes;

a plurality of solutions,

a plurality of nanoparticles, and

wherein the automated apparatus is configured to form a protein corona and digest the protein corona.

167. An automated apparatus to identify proteins in a biological sample, the automated apparatus comprising:
a sample preparation unit;
a substrate comprising a plurality of channels;
a plurality of pipettes;
a plurality of solutions,
a plurality of nanoparticles,
wherein the automated apparatus is configured to form a protein corona and digest the protein corona, and
wherein at least one of the solutions is TE 150mM KCl 0.05% CHAPS buffer.

168. The automated apparatus of claims 166 or 167, wherein the sample preparation unit is configured to add the plurality of nanoparticles to the substrate with the plurality of pipettes.

169. The automated apparatus of any one of claims 166-168, wherein the sample preparation unit is configured to add the biological sample to the substrate with the plurality of pipettes.

170. The automated apparatus of any one of claims 166-169, wherein the sample preparation unit is configured to incubate the plurality of nanoparticles and the biological sample to form the protein corona.

171. The automated apparatus of any one of claims 166-170, wherein the sample preparation unit is configured to separate the protein corona from the supernatant to form a protein corona pellet.

172. The automated apparatus of any one of claims 166-171, wherein the sample preparation unit is configured to reconstitute the protein corona pellet with TE 150mM KCl 0.05% CHAPS buffer.

173. The automated apparatus of any one of claims 166-172, further comprising a magnetic source.

174. The automated apparatus of any one of claims 166-173, wherein the automated apparatus is configured for BCA, gel, or trypsin digestion of the protein corona.

175. The automated apparatus of any one of claims 166-174, wherein the automated apparatus is enclosed.

176. The automated apparatus of any one of claims 166-175, wherein the automated apparatus is sterilized before use.

177. The automated apparatus of any one of claims 166-176, wherein the automated apparatus is configured to a mass spectrometry.

178. The automated apparatus of any one of claims 166-177, wherein the automated apparatus is temperature controlled.

179. A method of identifying proteins in a biological sample, the method comprising:
adding the biological sample to the automated apparatus of any one of claims 166-178;
generating proteomic data from the automated apparatus; and
quantifying the proteomic data.

180. The method of claim 179, further comprising incubating a plurality of nanoparticles with the biological sample in the automated apparatus to form a protein corona.

181. The method of claim 179 or 180, further comprising separating the protein corona from the supernatant in the automated apparatus.

182. The method of any one of claims 179-181, further comprising digesting the protein corona to form the digested sample in the automated apparatus.

183. The method of any one of claims 179-182, further comprising washing the digested sample in the automated apparatus.

184. The method of any one of claims 179-183, wherein quantifying the proteomic data comprises providing the proteomic data to a mass spectrometry.

185. The method of any one of claims 179-184, wherein the biological sample is a biofluid.

186. The method of any one of claims 179-185, wherein the biofluid is serum or plasma.

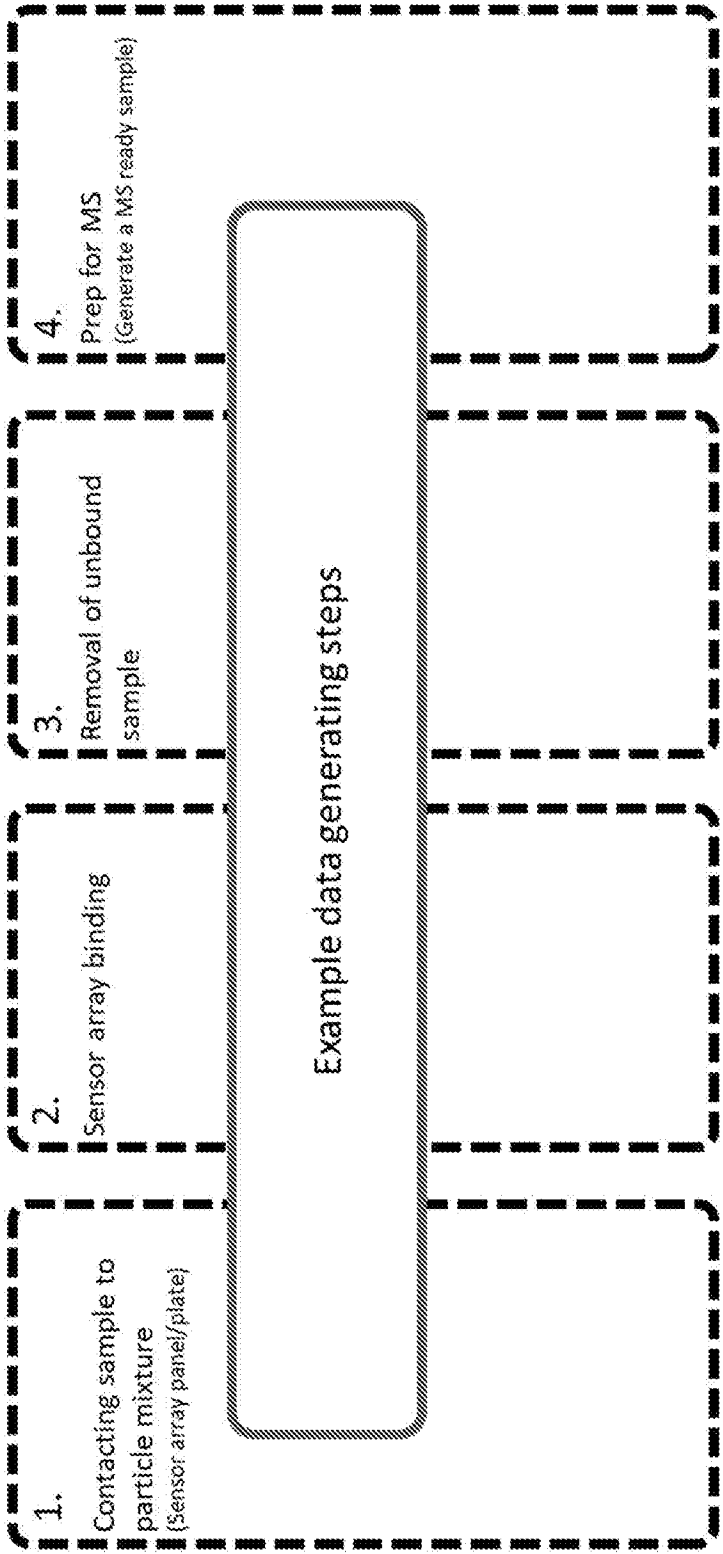

1.
Contacting sample to particle mixture
(Sensor array panel/plate)
2.
Sensor array binding
3.
Removal of unbound sample
4.
Prep for MS
(Generate a MS ready sample)
Example data generating steps

FIG. 1

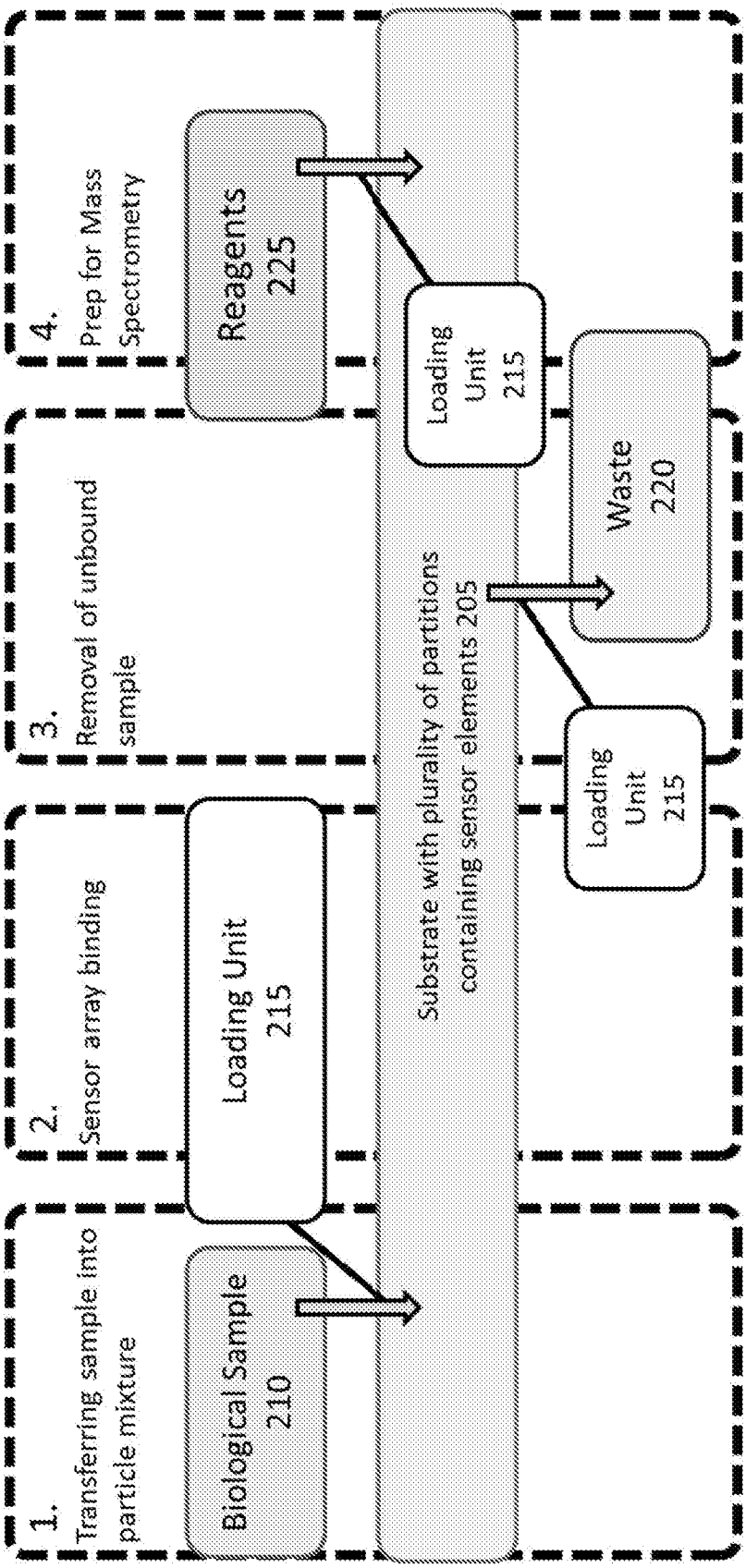

1.
Transferring sample into particle mixture
2.
Sensor array binding
3.
Removal of unbound sample
4.
Prep for Mass Spectrometry
Biological Sample 210
Loading Unit 215
Reagents 225
Substrate with plurality of partitions containing sensor elements 205
Loading Unit 215
Waste 220
Loading Unit 215

FIG. 2

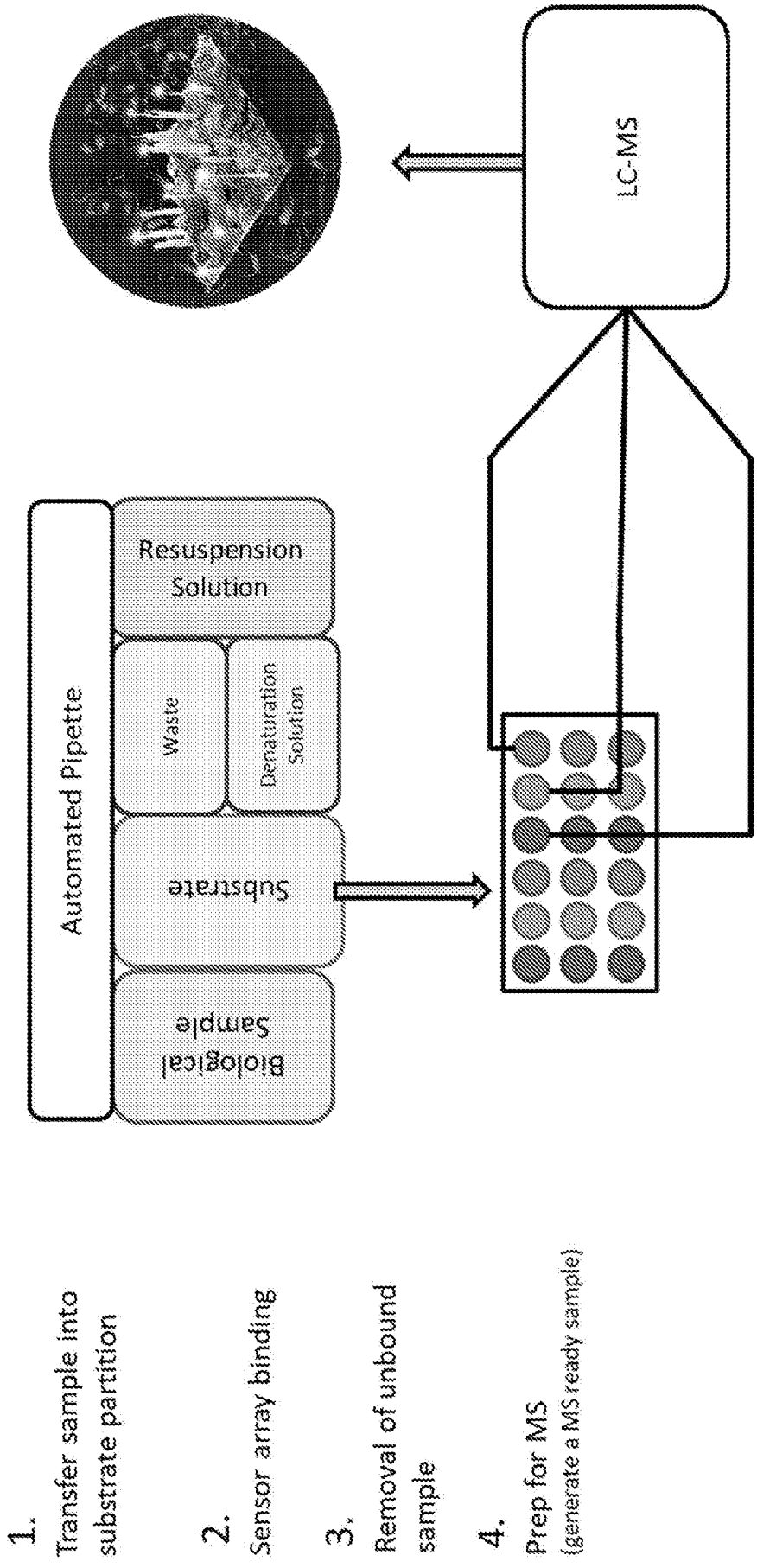
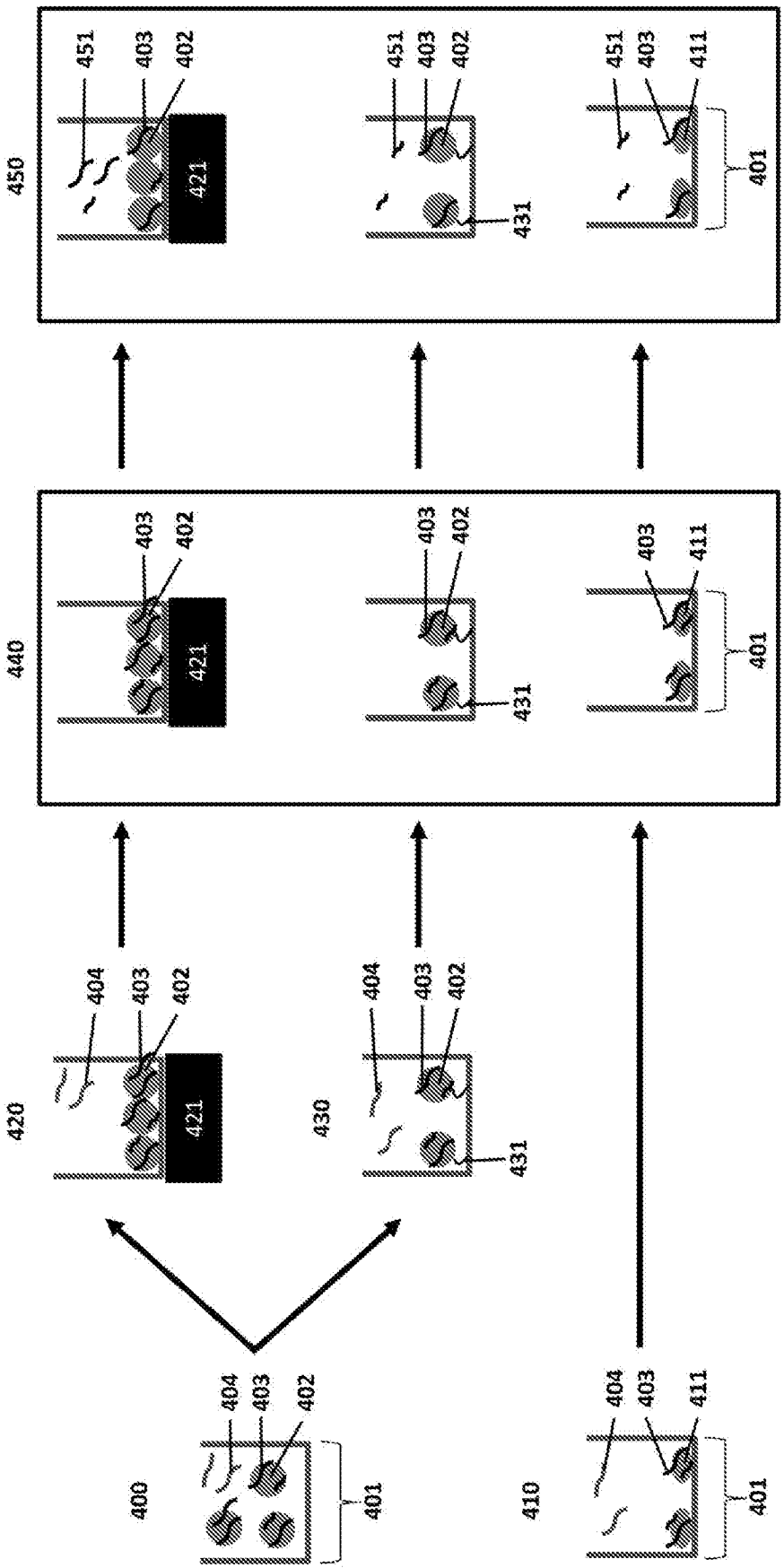

1.
Transfer sample into substrate partition
2.
Sensor array binding
3.
Removal of unbound sample
4.
Prep for MS
(generate a MS ready sample)
Automated Pipette
Biological Sample
Substrate
Waste
Denaturation Solution
Resuspension Solution
LC-MS

FIG. 3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440
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450
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411
403
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402
403
451
421
402
403
451

FIG. 4

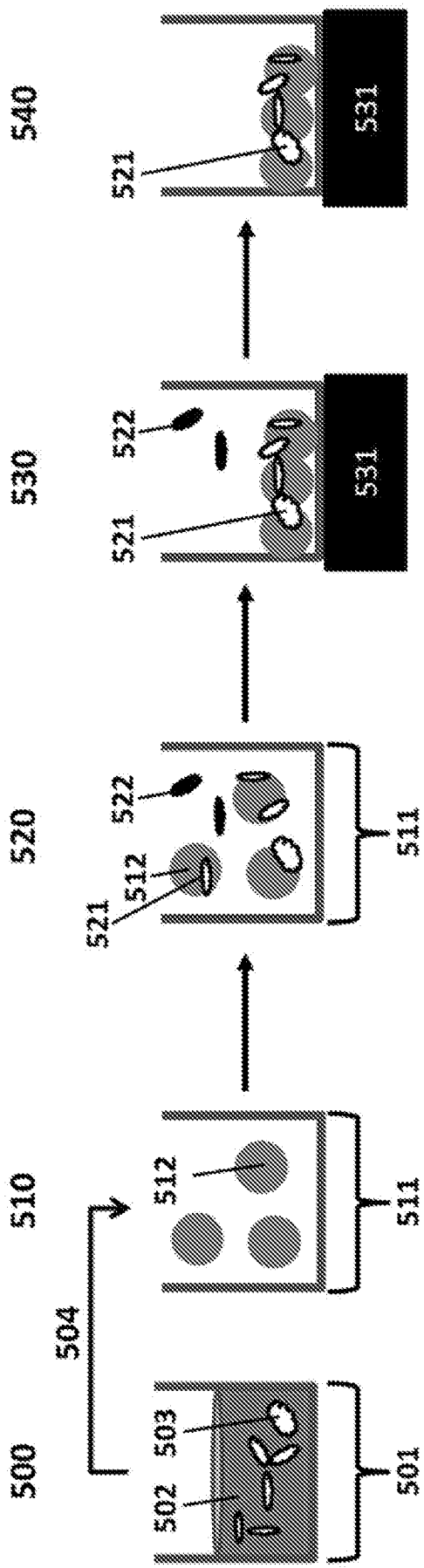


FIG. 5

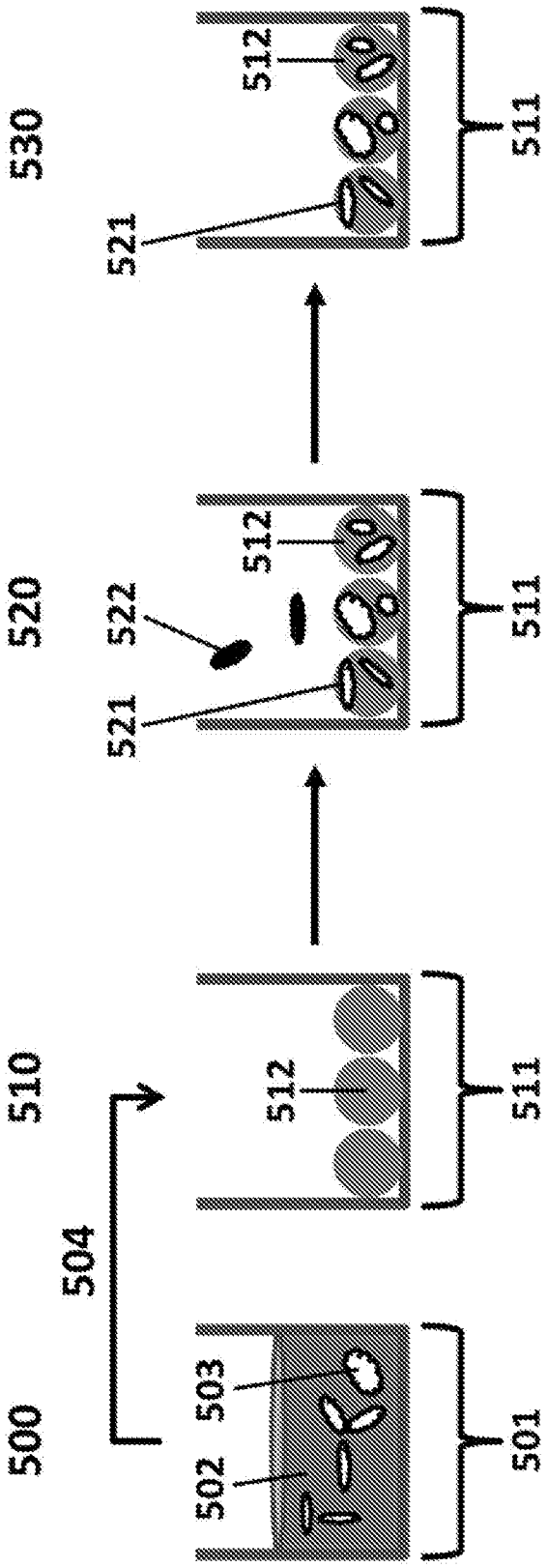


FIG.6

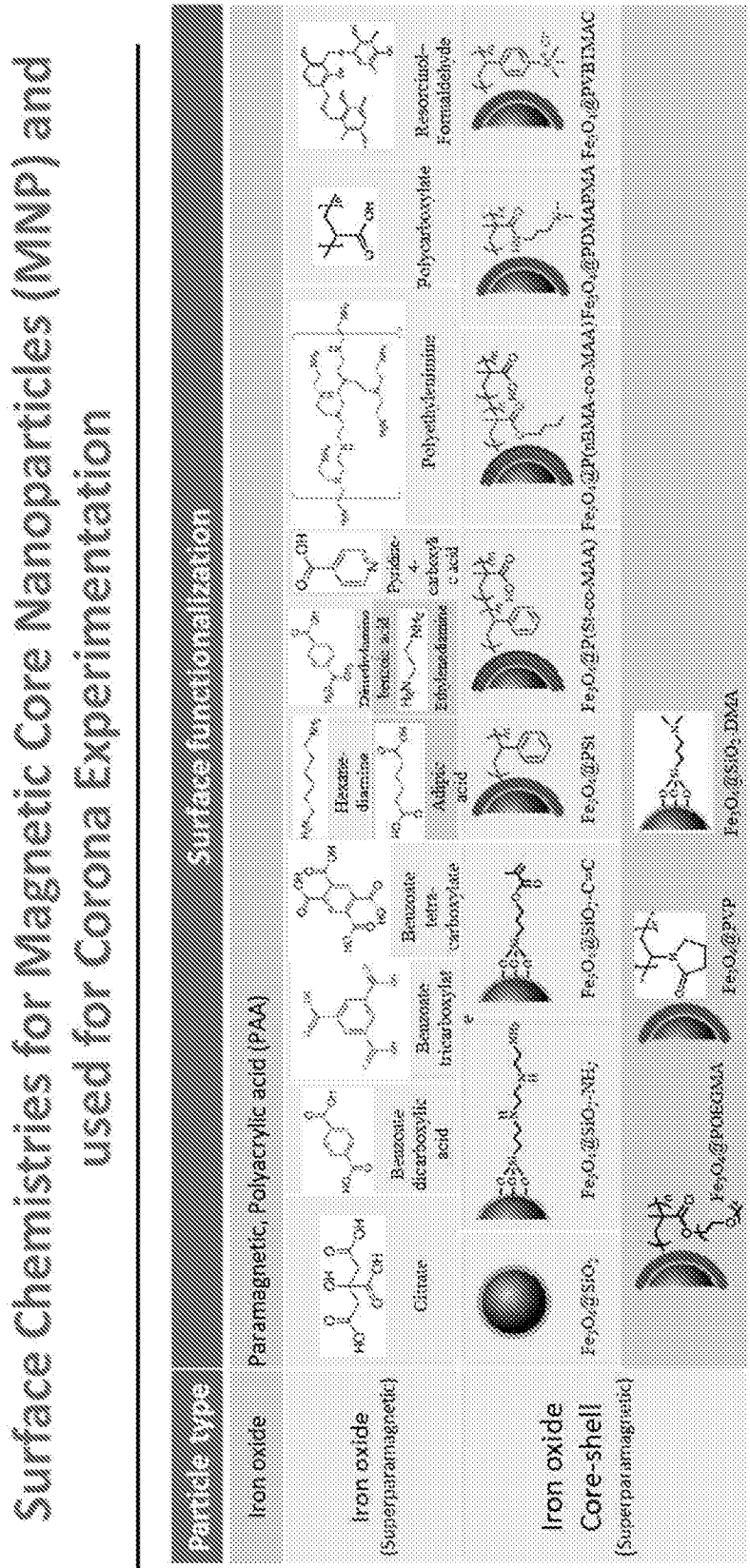

Surface Chemistries for Magnetic Core Nanoparticles (MNP) and used for Corona Experimentation
Particle Type
Surface Functionalization
Iron oxide
Paramagnetic, Polyacrylic acid (PAA)
Iron oxide
(Superparamagnetic)
Citrate
Benzoate dicarboxylic acid
Benzoate tricarboxylate
Benzoate tetra-carboxylate
Hexane-diamine
Dimethylamino benzoic acid
Pyridine-4-carboxylic acid
Adipic acid
Ethylene-diamine
Polyethylenimine
Polycarboxylate
Resorcinol-Formaldehyde
Iron oxide
Core-shell
(Superparamagnetic)
Fe3O4@SiO2
Fe3O4@SiO2-NH2
Fe3O4@SiO2-C=C
Fe3O4@PS
Fe3O4@P(St-co-MAA)
Fe3O4@P(nBMA-co-MAA)
Fe3O4@PDMAPMA
Fe3O4@PVBTMAC
Fe3O4@PGMRMA
Fe3O4@PVP
Fe3O4@SiO2-DMA

FIG. 7

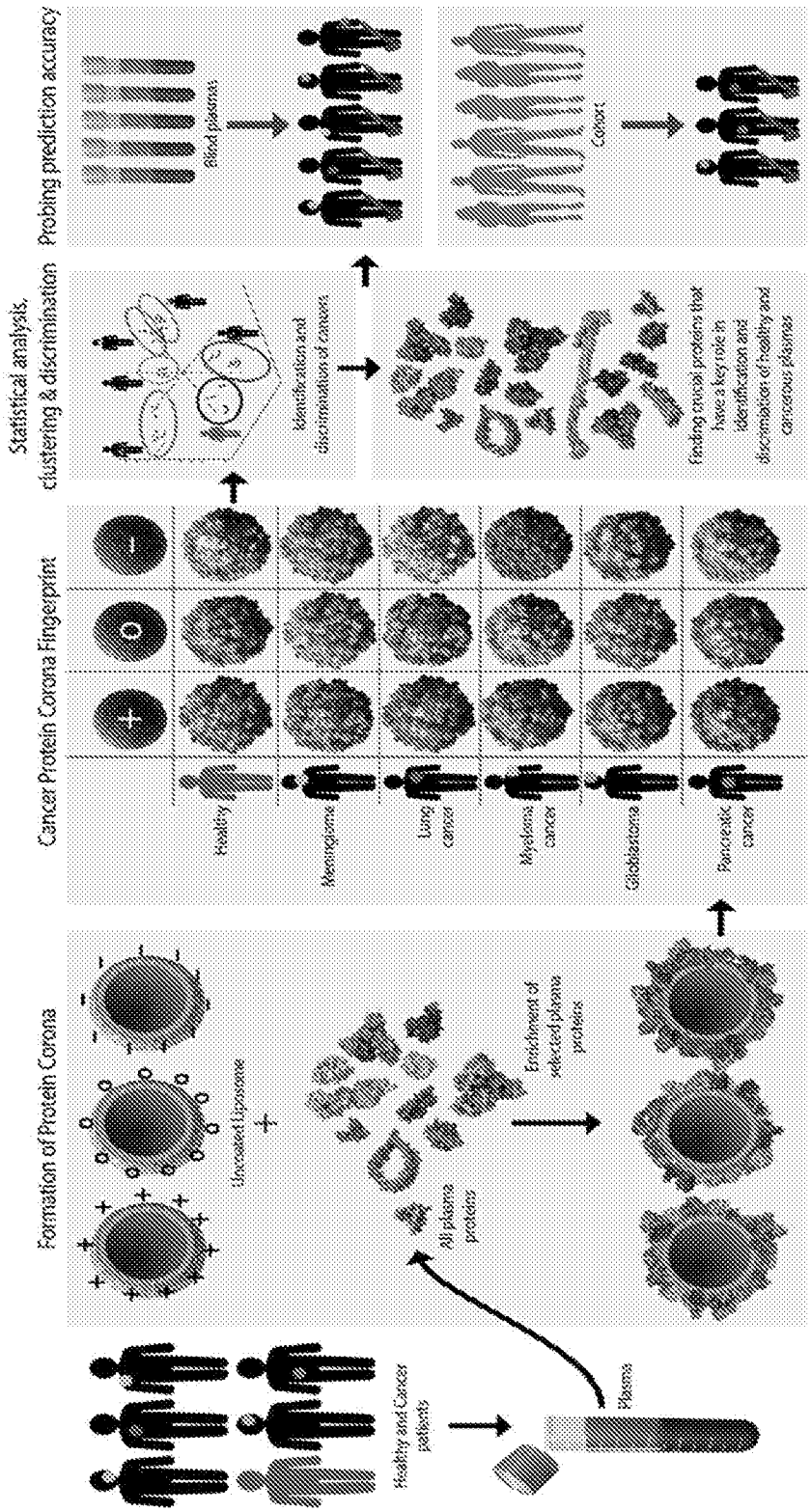

Formation of Protein Corona
Uncoated Liposome
All plasma proteins
Enrichment of selected plasma proteins
Healthy and Cancer patients
Plasma
Cancer Protein Corona Fingerprint
Healthy
Meningioma
Lung cancer
Myeloma cancer
Glioblastoma
Pancreatic cancer
Statistical analysis, clustering & discrimination
Identification and discrimination of cancers
Finding crucial proteins that have a key role in identification and discrimination of healthy and cancerous plasmas
Probing prediction accuracy
Blind plasmas
Cohort

FIG. 8

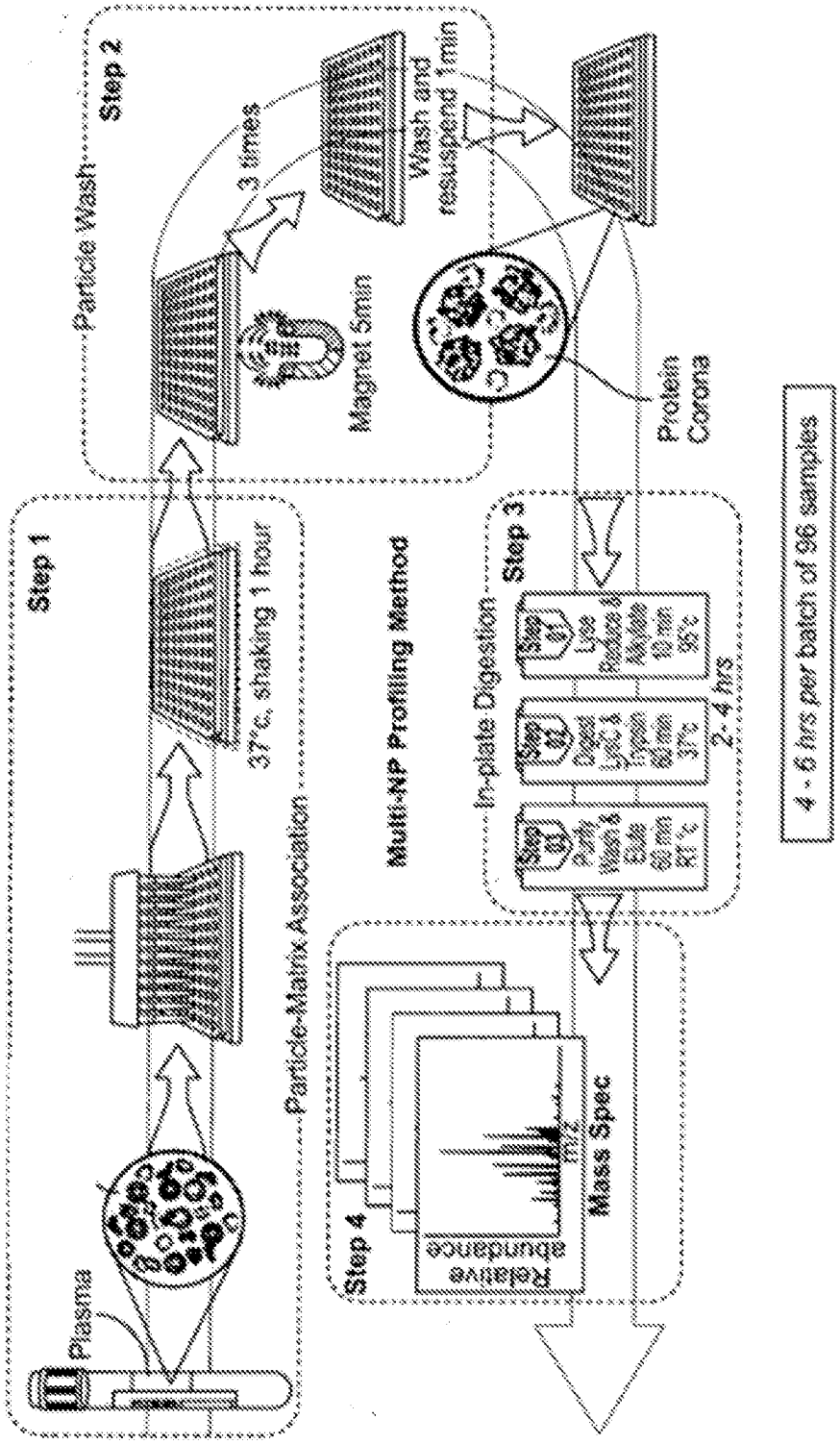

Step 1
Plasma
Particle-Matrix Association
37°c, shaking 1 hour
Step 2
Particle Wash
3 times
Wash and resuspend 1min
Magnet 5min
Protein Corona
Multi-NP Profiling Method
Step 3
In-plate Digestion
2- 4 hrs
4 - 6 hrs per batch of 96 samples
Step 4
Relative abundance
m/z
Mass Spec

FIG. 9

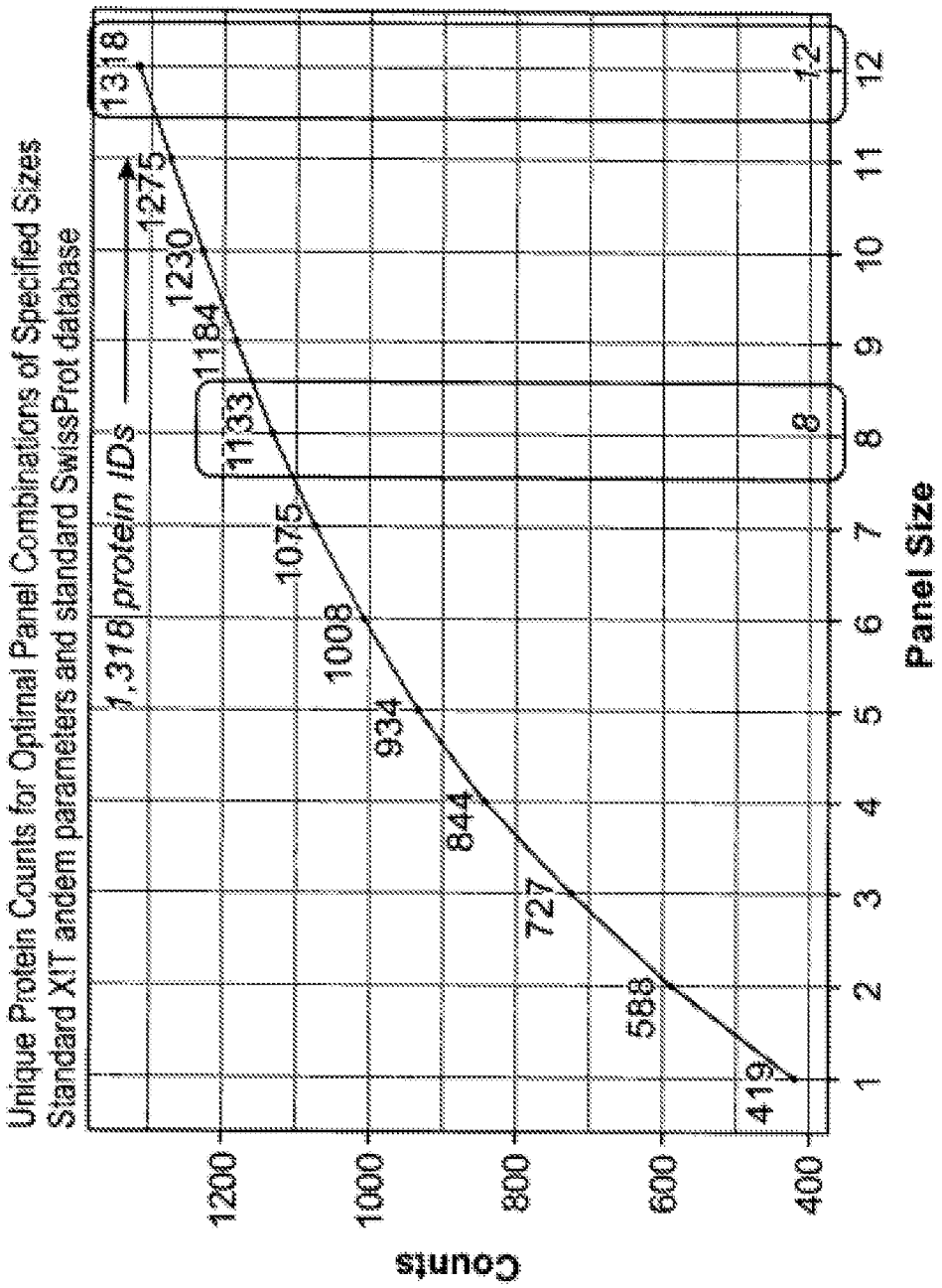

Unique Protein Counts for Optimal Panel Combinations of Specified Sizes
Standard XIT andem parameters and standard SwissProt database
1,318 protein IDs
1318
1275
1230
1184
1133
1075
1008
934
844
727
588
419
8
12
Counts
1200
1000
800
600
400
1
2
3
4
5
6
7
8
9
10
11
12
Panel Size

FIG. 10

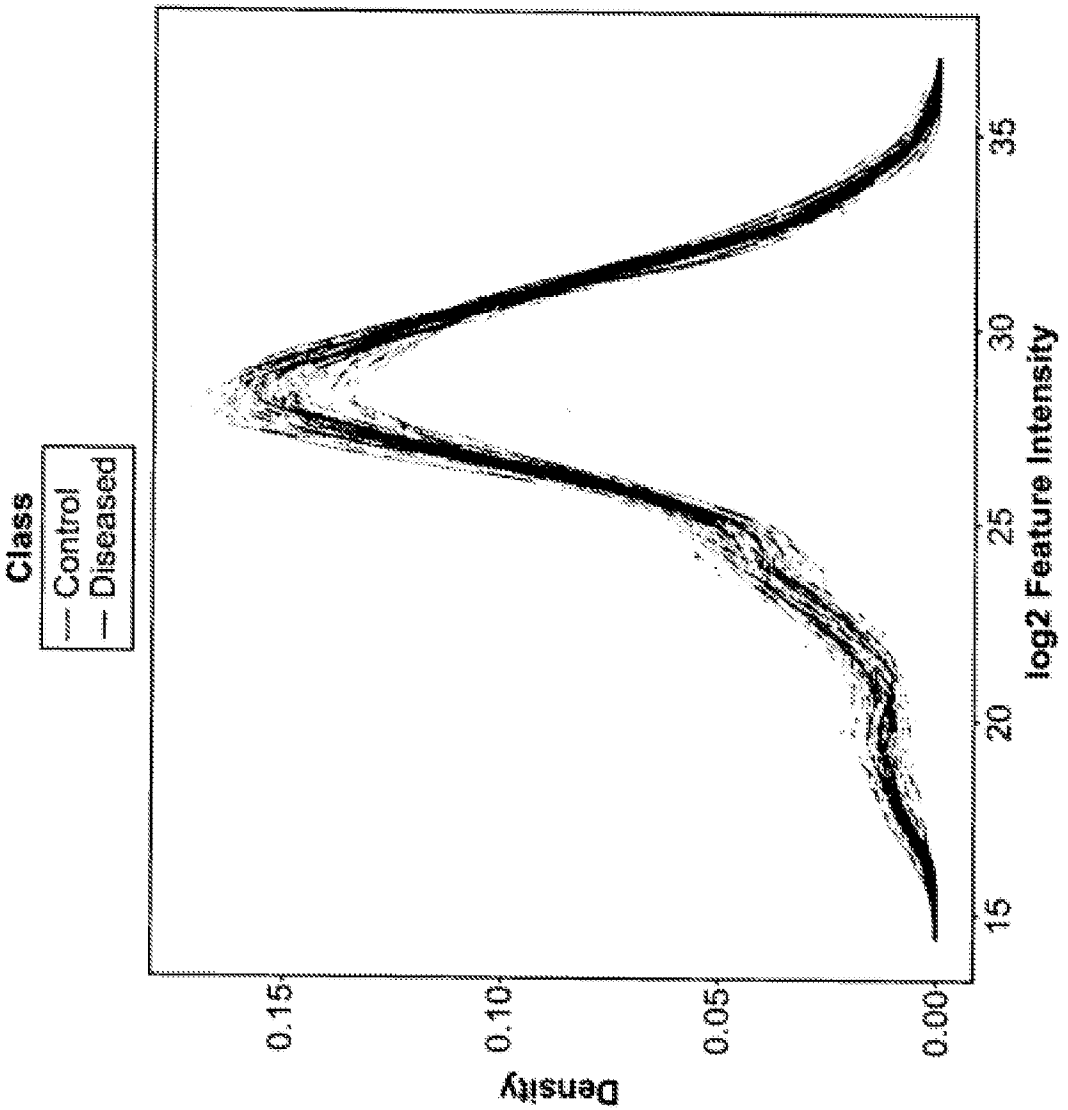
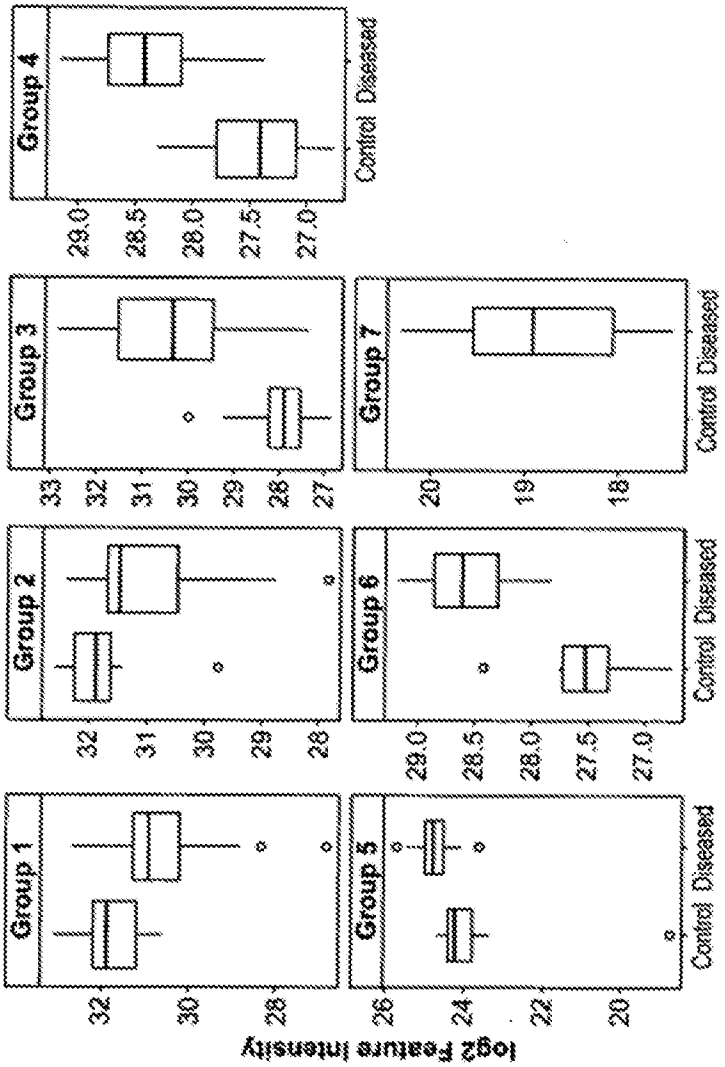

Class
Control
Diseased
Density
0.15
0.10
0.05
0.00
15
20
25
30
35
log2 Feature Intensity

FIG. 11



Feature Group	Protein	Peptide_Charge	NumControl	NumDiseased	log2 Difference	Adj. P-value
Group 1	PON1	YVYIAELLAHK_3	28	28	0.95	0.0398
Group 2	Unknown	Unknown	26	28	0.41	0.398
Group 3	SAA1	FFGHGAEDSLADQAANEWGR_3	22	24	-2.44	0.0019
Group 4	TENA	AATHYTTTR_3	20	17	-1.03	0.0097
Group 5	NCAM1	AGEQDATHLK_3	17	18	-0.54	0.0398
Group 6	FIBA	GSESGIFTNTK_2	12	19	-1.08	0.0107
Group 7	Unknown	Unknown	0	14	NA	NA

FIG. 12

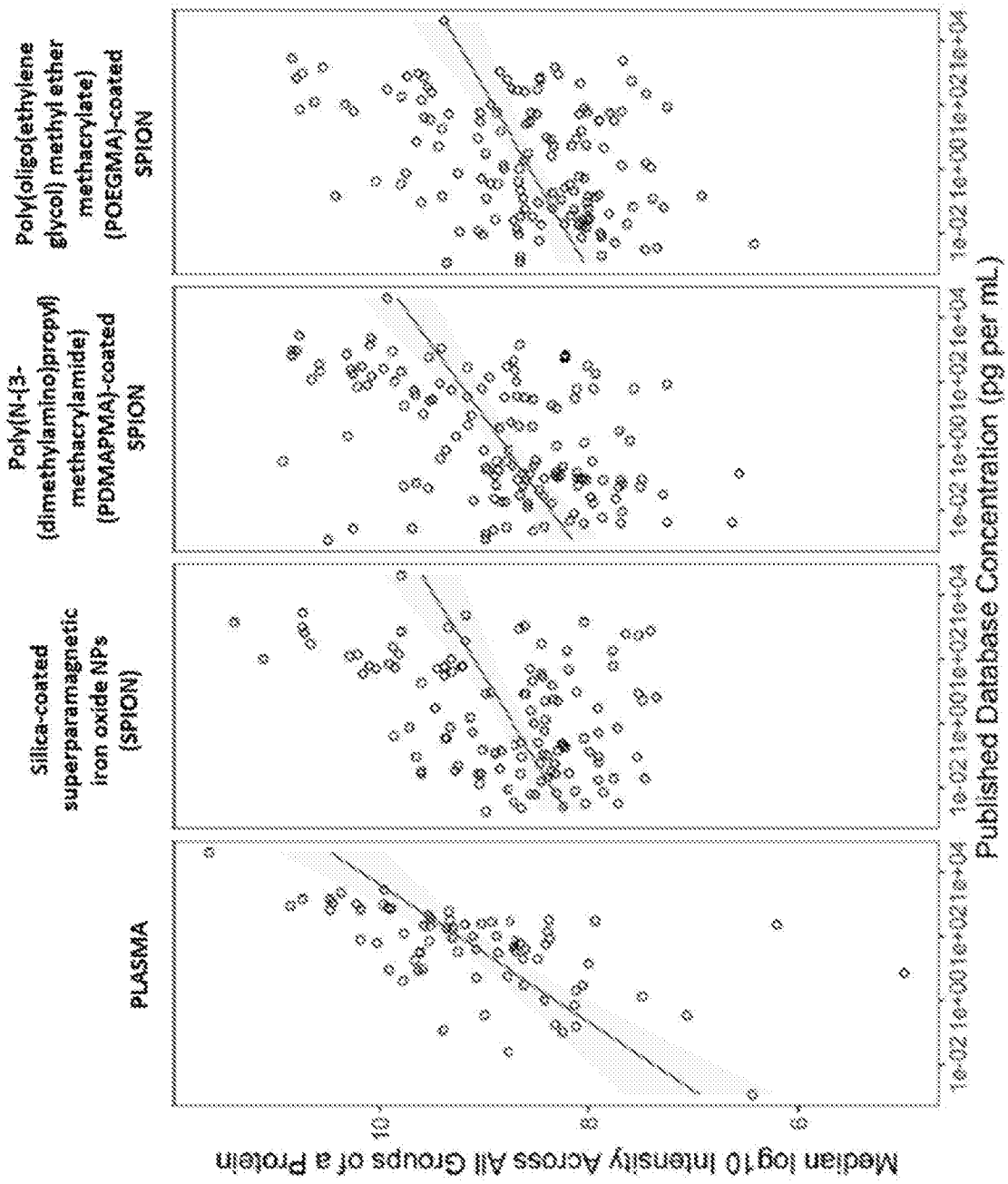

PLASMA
Silica-coated superparamagnetic iron oxide NPs (SPION)
Poly(N-[3-(dimethylamino)propyl] methacrylamide) (PDMAPMA)-coated SPION
Poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA)-coated SPION
Median log10 Intensity Across All Groups of a Protein
10
8
6
1e-02 1e+00 1e+02 1e+04
Published Database Concentration (pg per mL)

FIG. 13

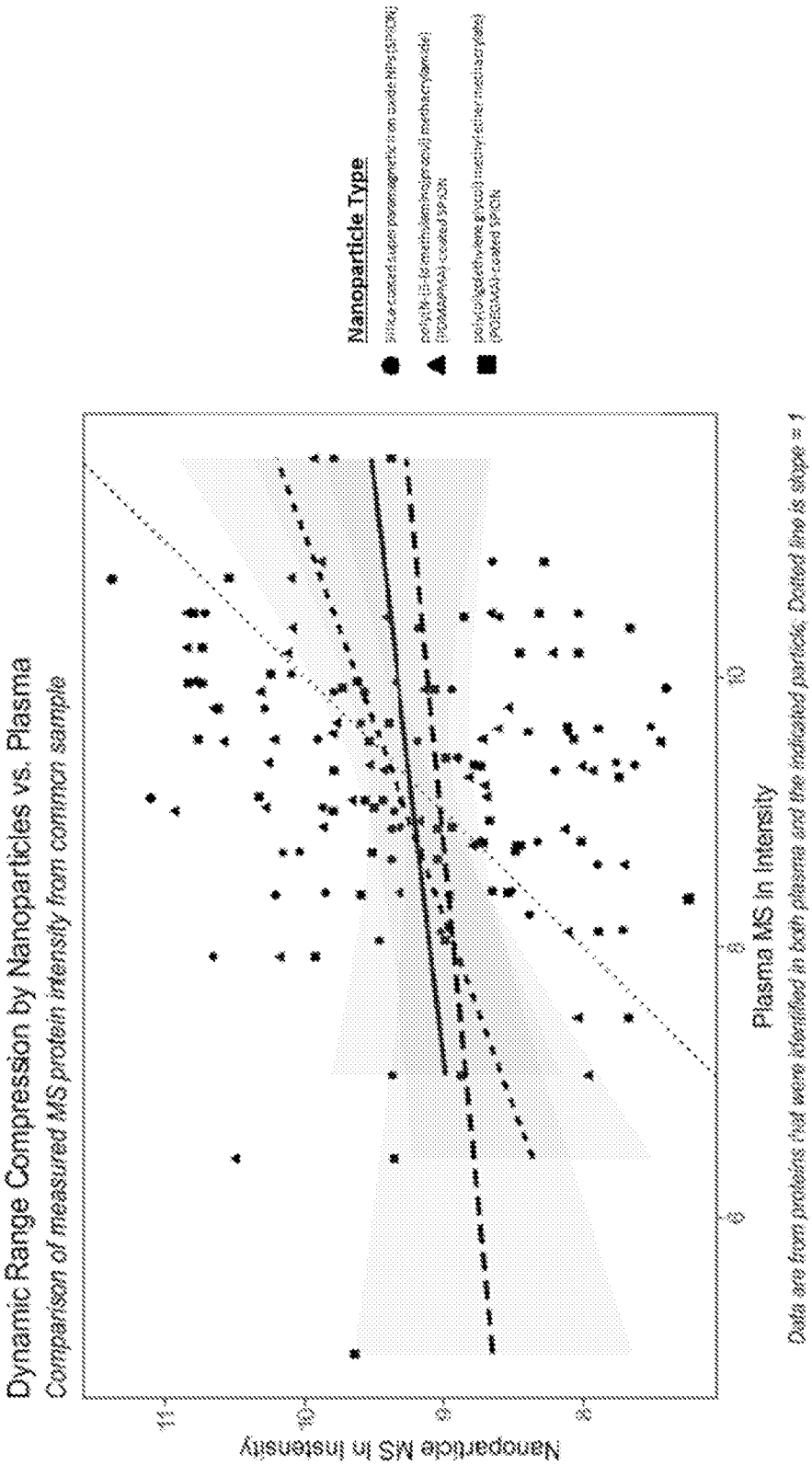

Dynamic Range Compression by Nanoparticles vs. Plasma
Comparison of measured MS protein intensity from common sample
Nanoparticle Type
Nanoparticle MS In Intensity
Plasma MS In Intensity
11
10
9
8
8
9
10
Data are from proteins that were identified in both plasma and the indicated particle. Dotted line is slope = 1

FIG. 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/044908

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 14/47; C12P 21/02; C12Q 1/37 (2020.01)
CPC - G01N 33/54326; G01N 33/54346; G01N 33/6848 (2020.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2018/0172694 A1 (THE BRIGHAM AND WOMEN'S HOSPITAL, INC.) 21 June 2018 (21.06.2018) entire document	104 ---------- 1-4, 105-107, 134, 166, 168
Y	US 6,649,419 B1 (ANDERSON) 18 November 2003 (18.11.2003) entire document	1-4, 105-107, 166, 168
Y	SALEHI-REYHANI, A. "Evaluating single molecule detection methods for microarrays with high dynamic range for quantitative single cell analysis," Scientific Reports, 20 December 2017 (20.12.2017), Vol. 7, No. 17957, Pgs. 1-12. entire document	1-4, 134
Y	US 2016/0327554 A1 (SIWARD CRYSTAL TECHNOLOGY CO., LTD.) 10 November 2016 (10.11.2016) entire document	168
A	US 2013/0084561 A1 (COULL et al) 04 April 2013 (04.04.2013) entire document	1-4, 104-107, 134, 149-151, 166-168
T	WO 2020/198209 A1 (SEER, INC.) 01 October 2020 (01.10.2020) entire document	1-4, 104-107, 134, 149-151, 166-168

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 October 2020	12 JAN 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/044908

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-103, 108-133, 135-148, 152-165, 169-186
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.