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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY,DOCKET,NO	TOT CLAIMS	IND CLAIMS
62/435,409	12/16/2016		130	129319.00437.BWH24258.01		

CONFIRMATION NO. 1669

FILING RECEIPT



0000000089222949

26710
QUARLES & BRADY LLP
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SUITE 2350
MILWAUKEE, WI 53202-4426

Date Mailed: 02/15/2017

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The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 62/435,409**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

** SMALL ENTITY **

Title

SYSTEM AND METHOD FOR PROTEIN CORONA SENSOR ARRAY FOR EARLY DETECTION
OF DISEASES

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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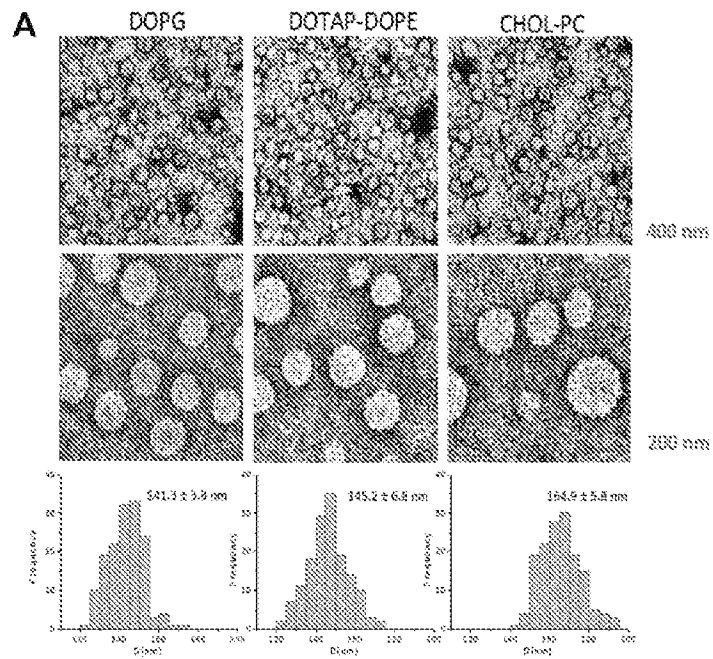


FIG. 1A

B	Bare Liposomes							
		TEM diameter (nm)	SD	Size intensity (nm)	SD	PdI	SD	Zeta-potential (mV)
	NOVA	143.2	6.8	133.30	11.40	0.17	0.04	46.30
	NOVAP	141.3	3.8	146.6	3.25	0.113	0.076	59.1
	CNC	164.9	5.8	172.9	7.8	0.05	0.001	-3.5
	Corona Coated Liposomes							
	NOVA							
		Size Intensity (nm)	SD	PdI	SD	Zeta-potential (mV)	SD	Protein Assay (ug)
	Healthy	178.70	2.82	0.406	0.052	-42.70	1.31	7.49
	Pancreas	205.23	4.27	0.262	0.066	-35.60	0.72	6.66
	Lung	192.27	4.87	0.280	0.028	-34.43	0.75	6.87
	Glioblastoma	203.50	6.70	0.475	0.047	-38.50	0.32	6.46
	Meningioma	198.60	4.30	0.353	0.031	-27.83	3.00	6.25
	Myeloma	169.50	4.80	0.265	0.040	-22.60	3.28	8.57
	NOVAP							
	Healthy	232.97	3.36	0.33	0.09	-38.13	1.09	5.86
	Pancreas	240.37	4.20	0.35	0.02	-29.90	1.32	6.86
	Lung	200.47	8.52	0.36	0.03	-23.27	1.03	6.03
	Glioblastoma	235.30	7.87	0.38	0.05	-30.70	1.44	5.81
	Meningioma	233.77	4.22	0.34	0.06	-27.37	0.45	6.49
	Myeloma	242.43	6.50	0.43	0.09	-9.39	0.37	6.03
	CNC							
	Healthy	251.40	7.60	0.379	0.039	-28.4	2.34	3.36
	Pancreas	262.50	3.07	0.270	0.068	-21.3	2.09	4.18
	Lung	228.69	2.15	0.276	0.074	-36.8	1.26	4.38
	Glioblastoma	229.47	1.87	0.297	0.040	-36.6	0.96	3.07
	Meningioma	228.97	1.63	0.413	0.074	-32.5	3.03	2.99
	Myeloma	261.27	1.82	0.327	0.024	-6.6	0.41	5.56

FIG. 1B

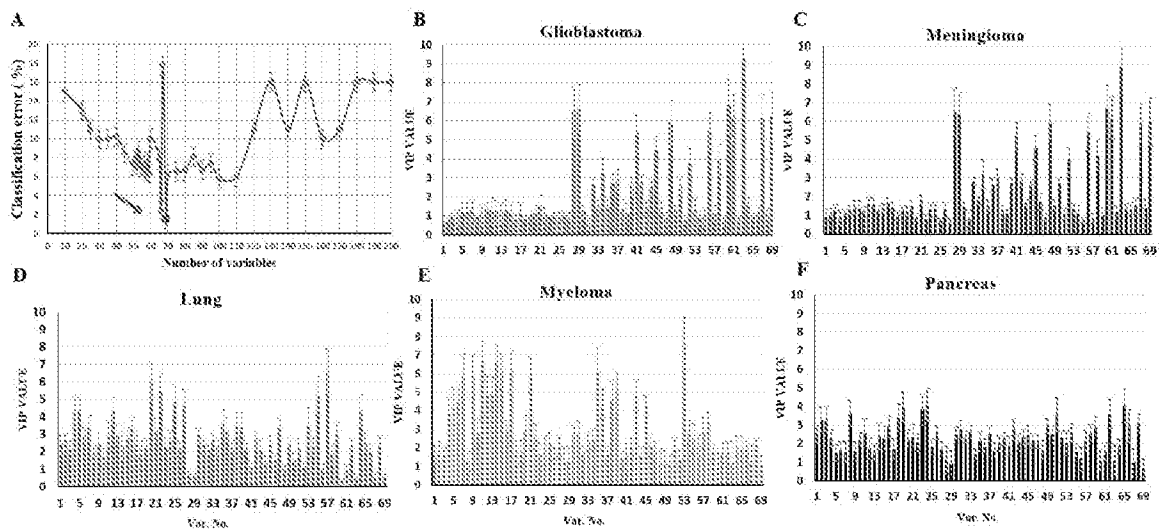


FIG. 2A-F

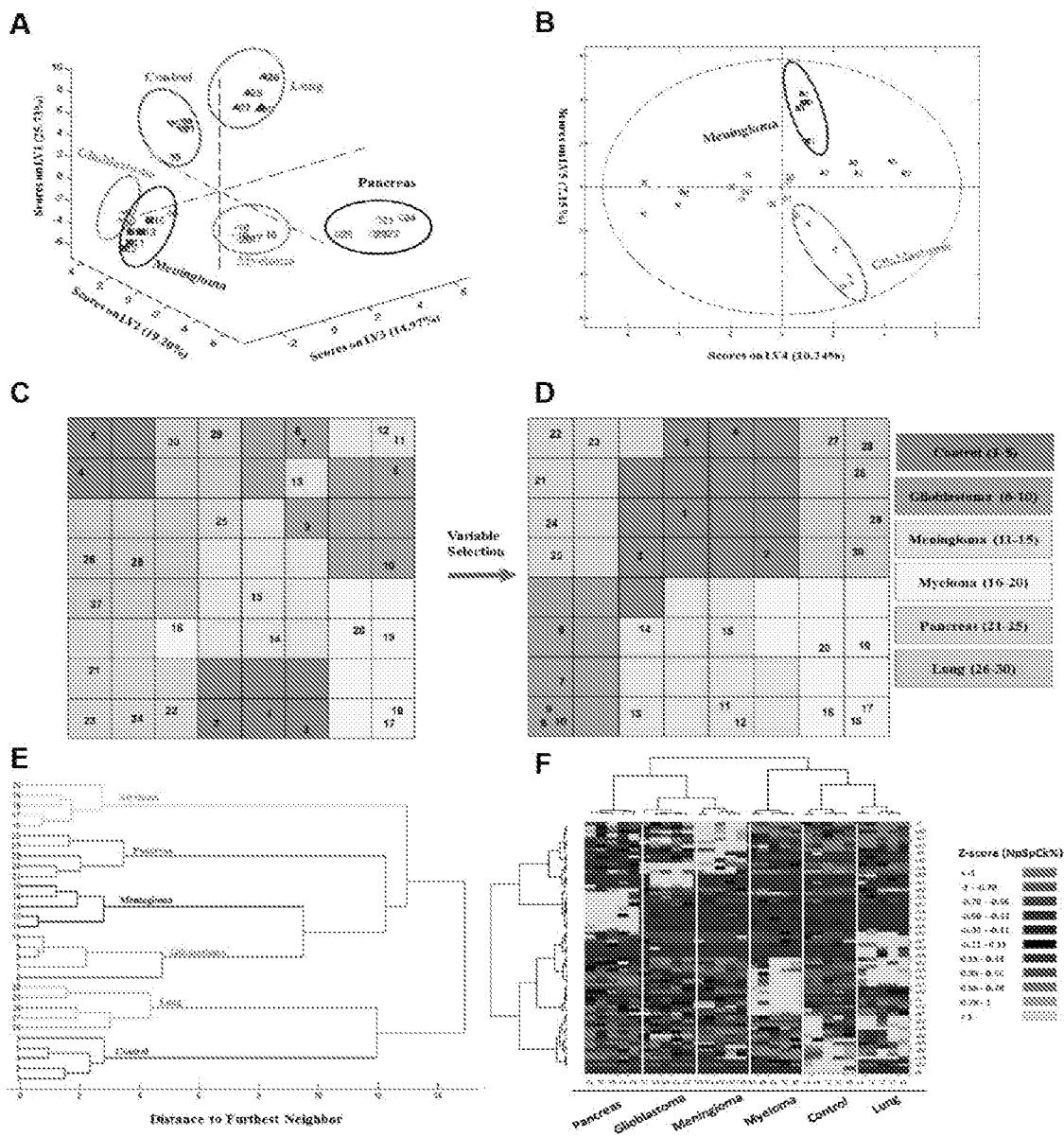


FIG. 3A-F.

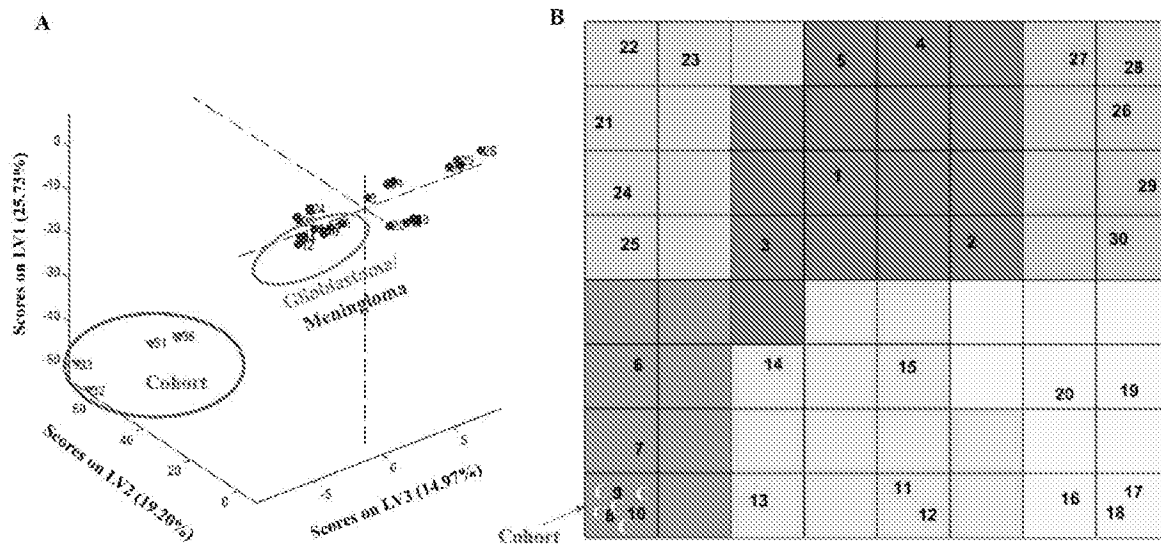


FIG. 4A-B

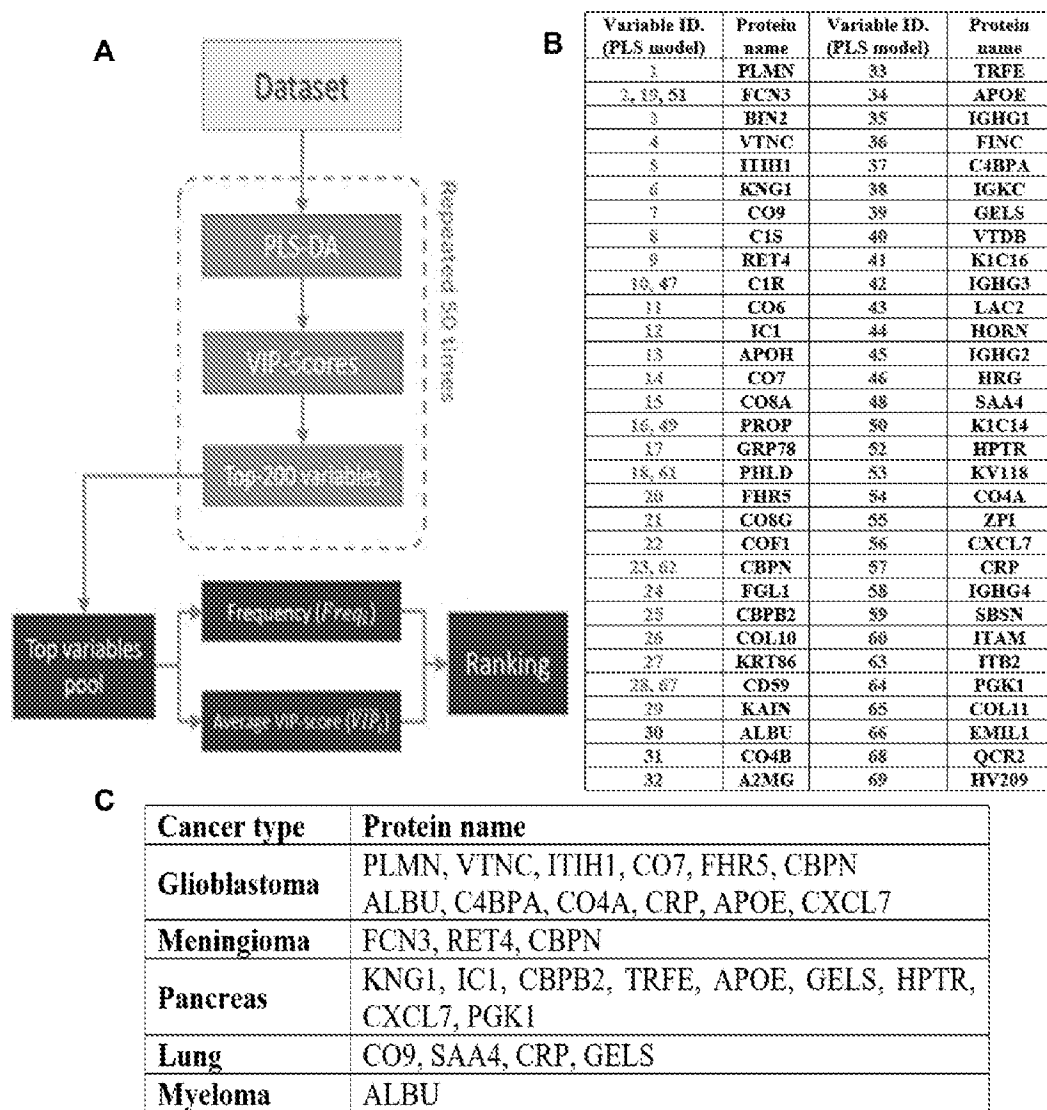
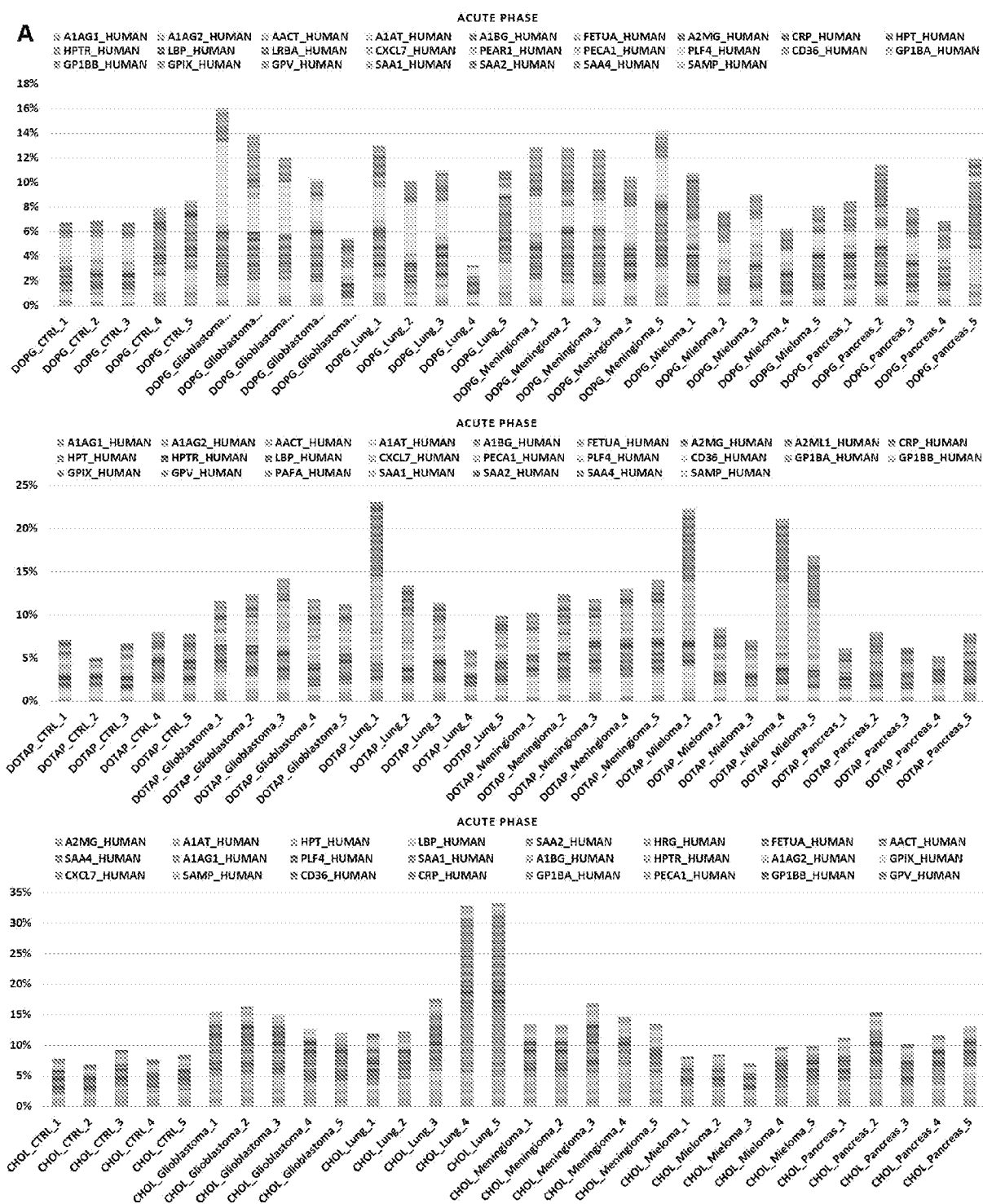


FIG. 5A-C.



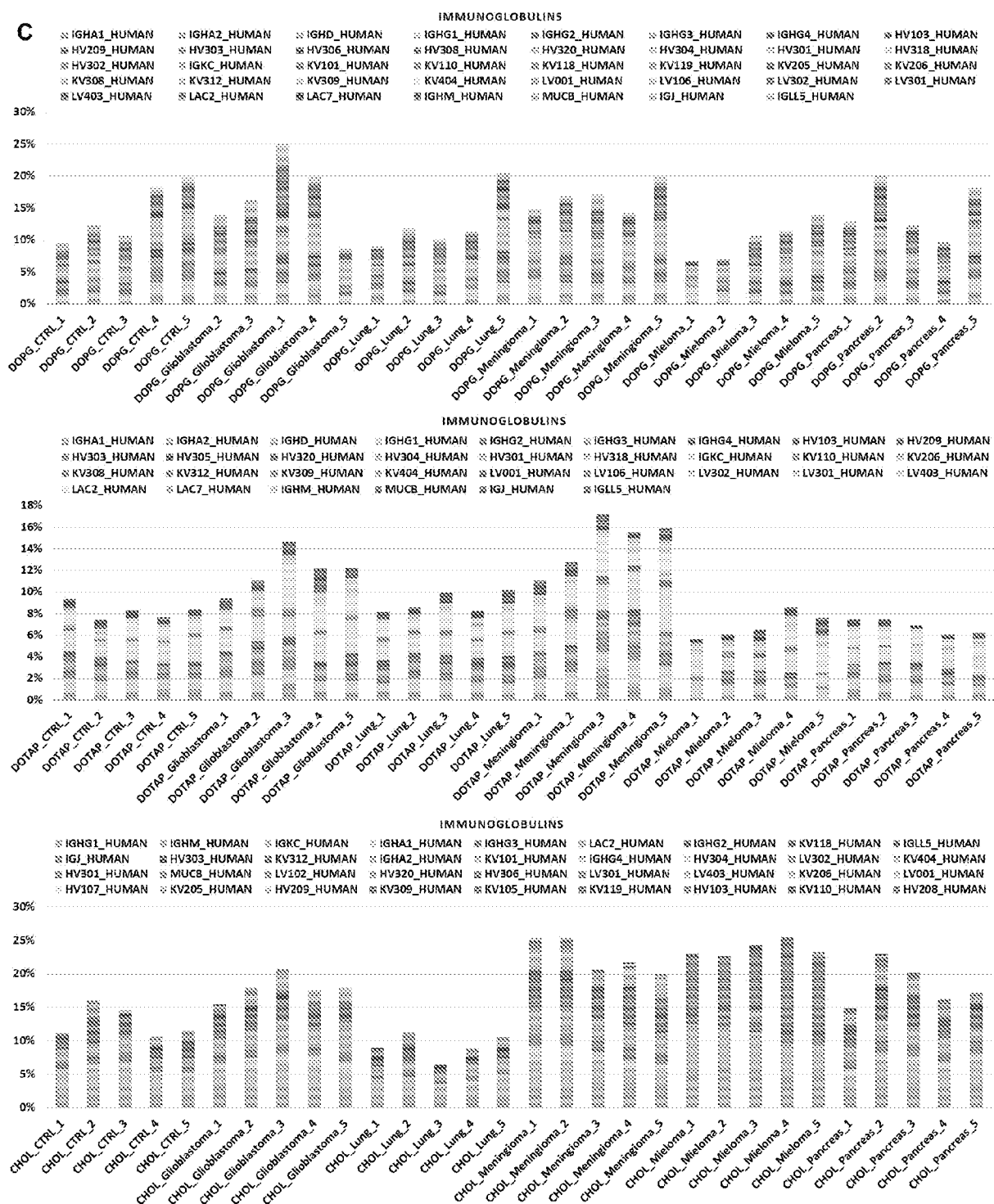


FIG. 6C

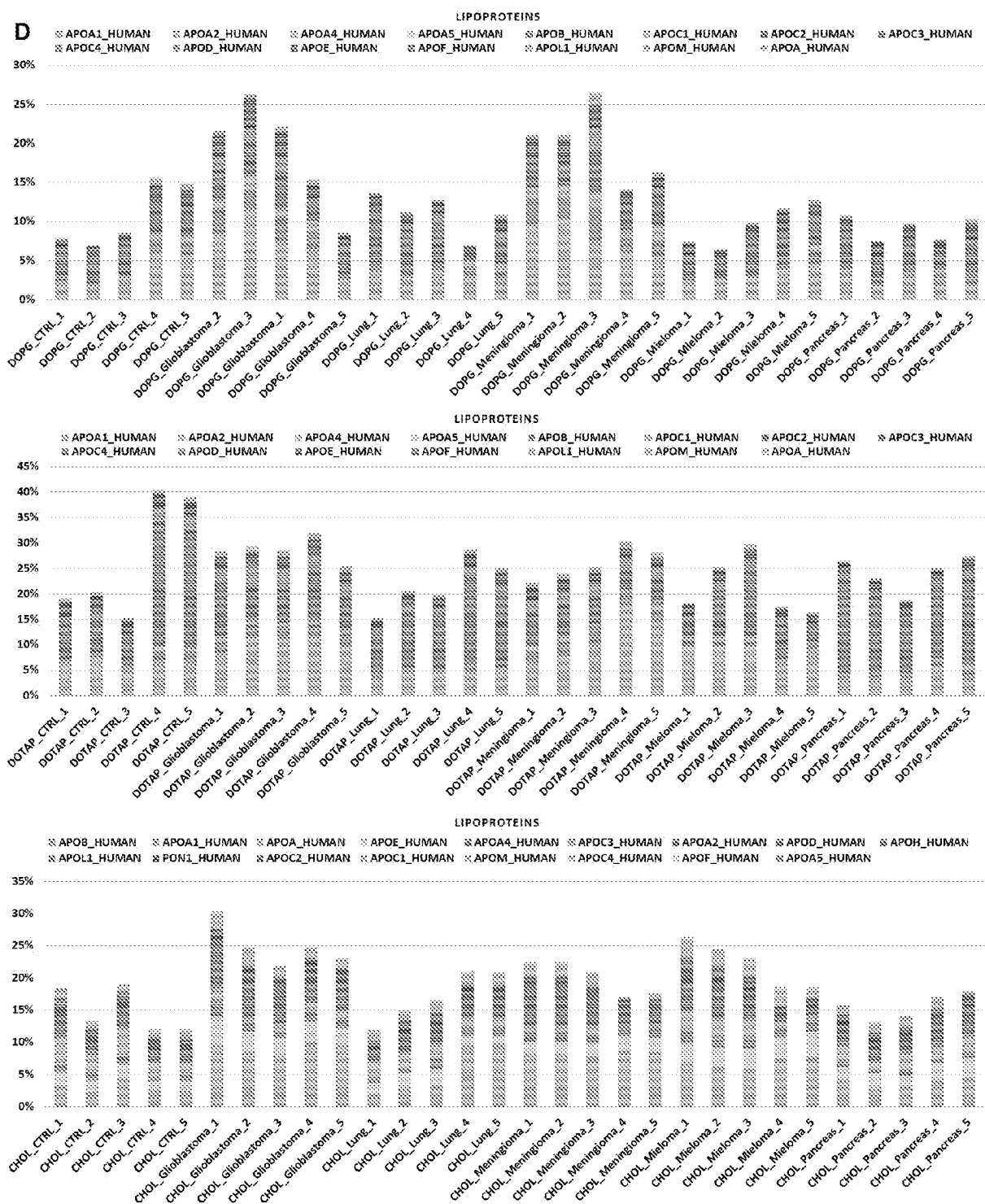
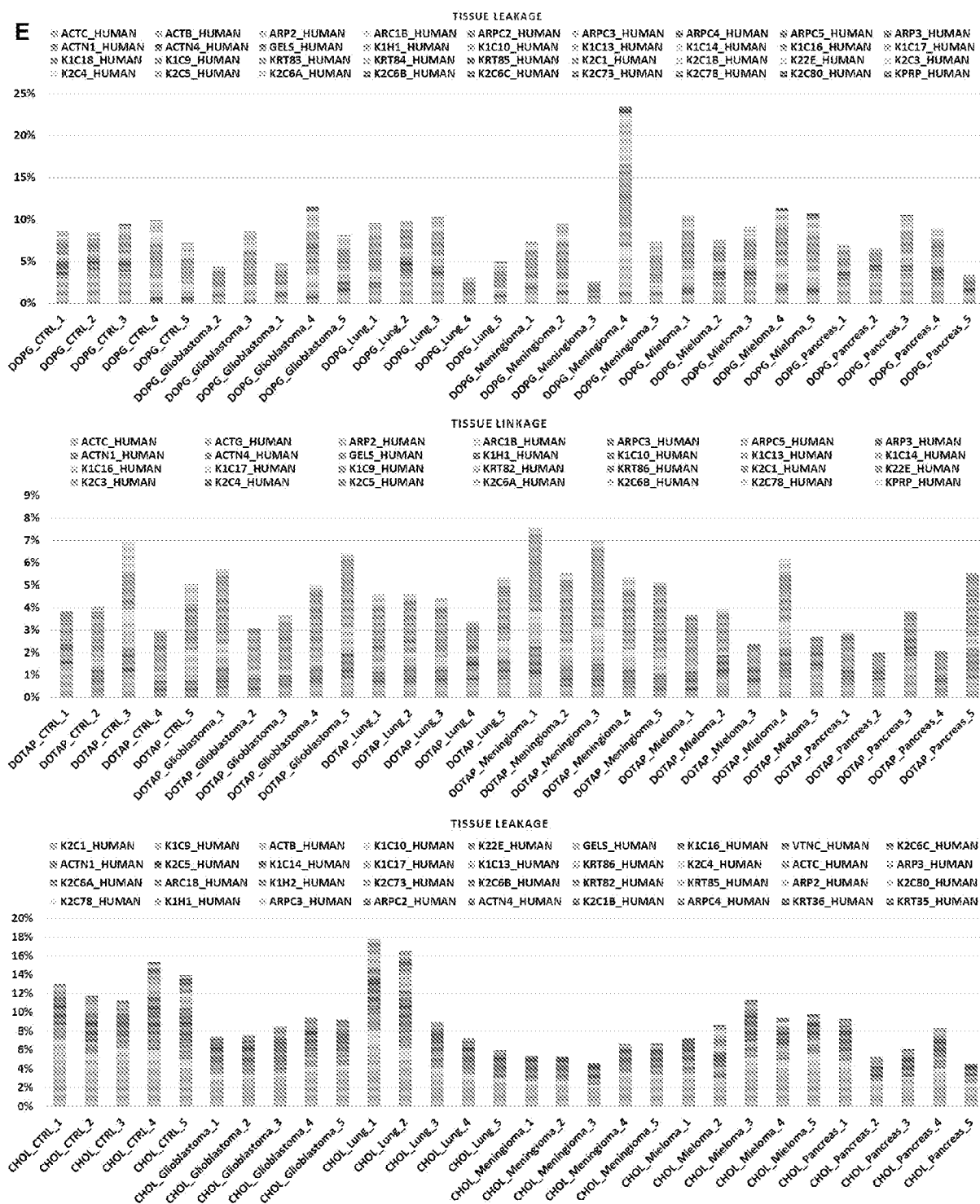
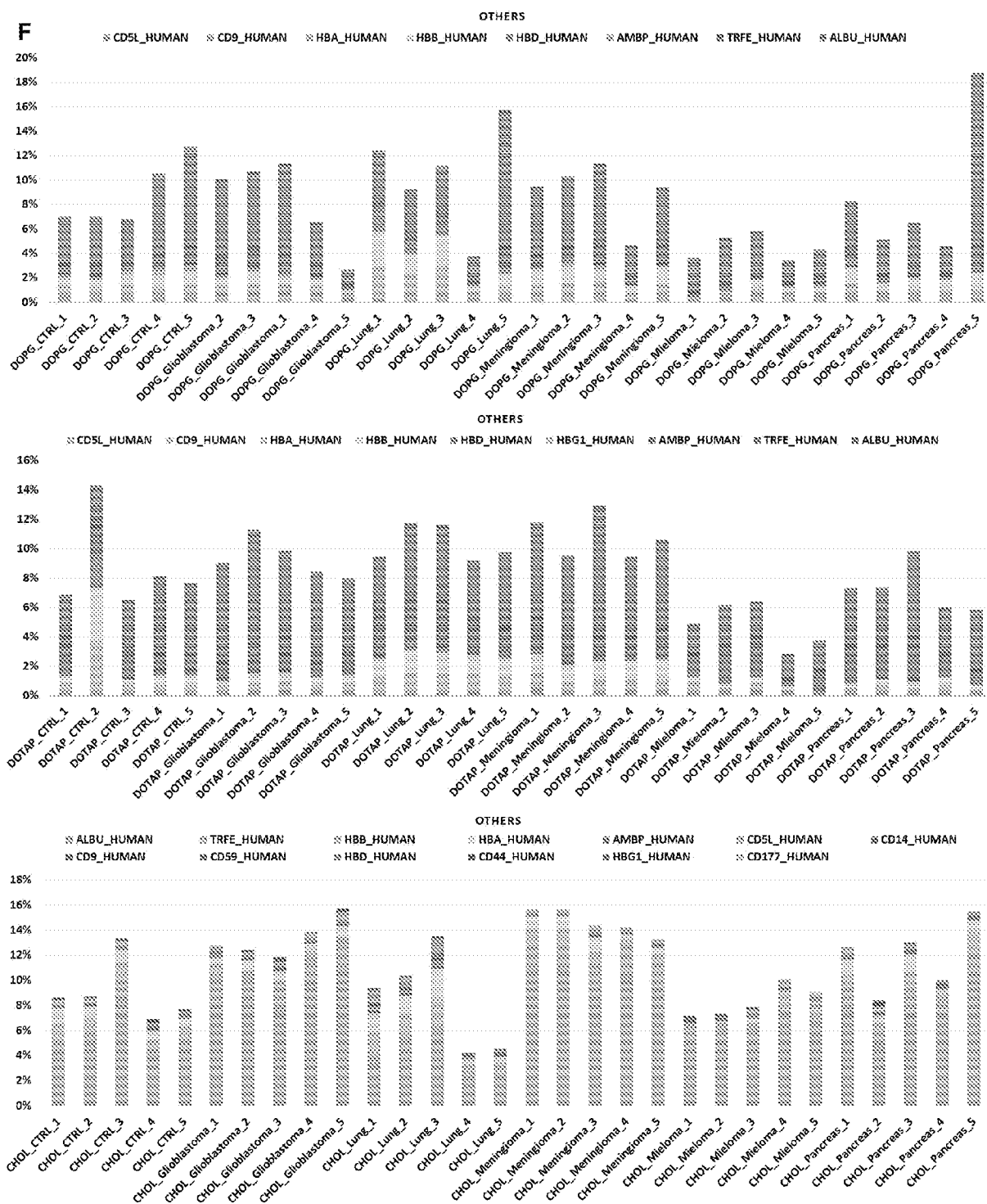


FIG. 6D





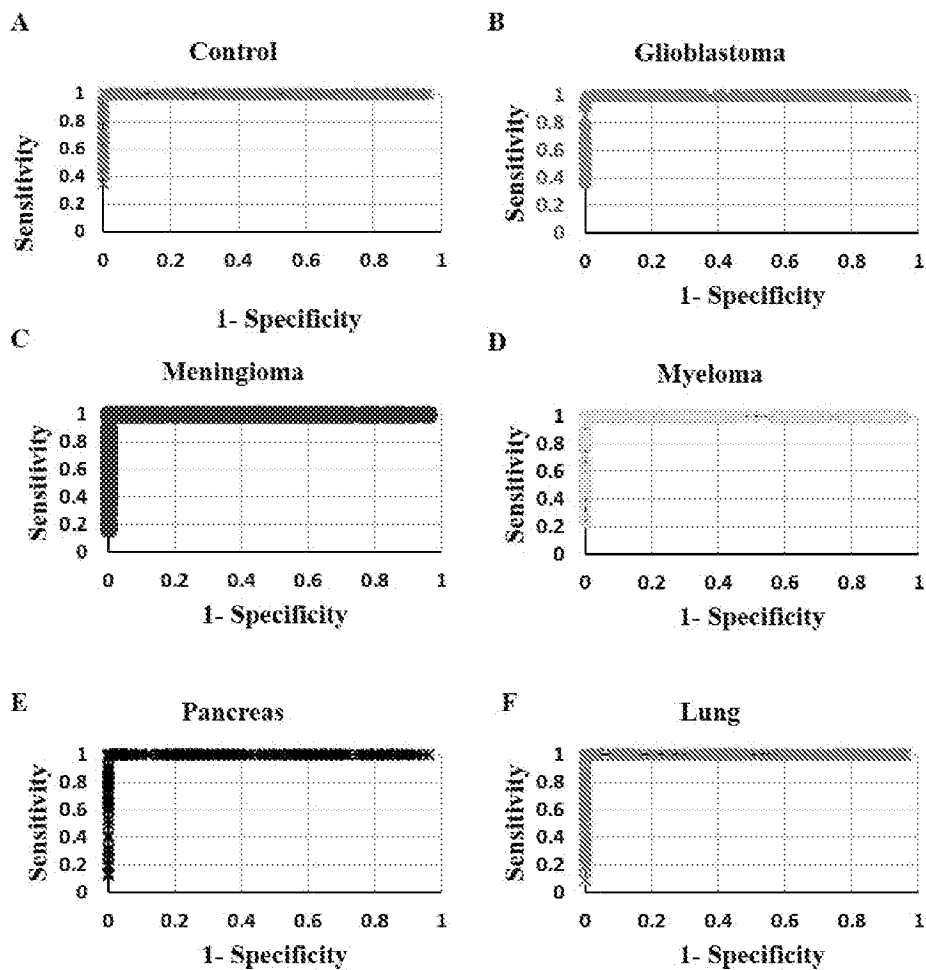


FIG. 7A-F

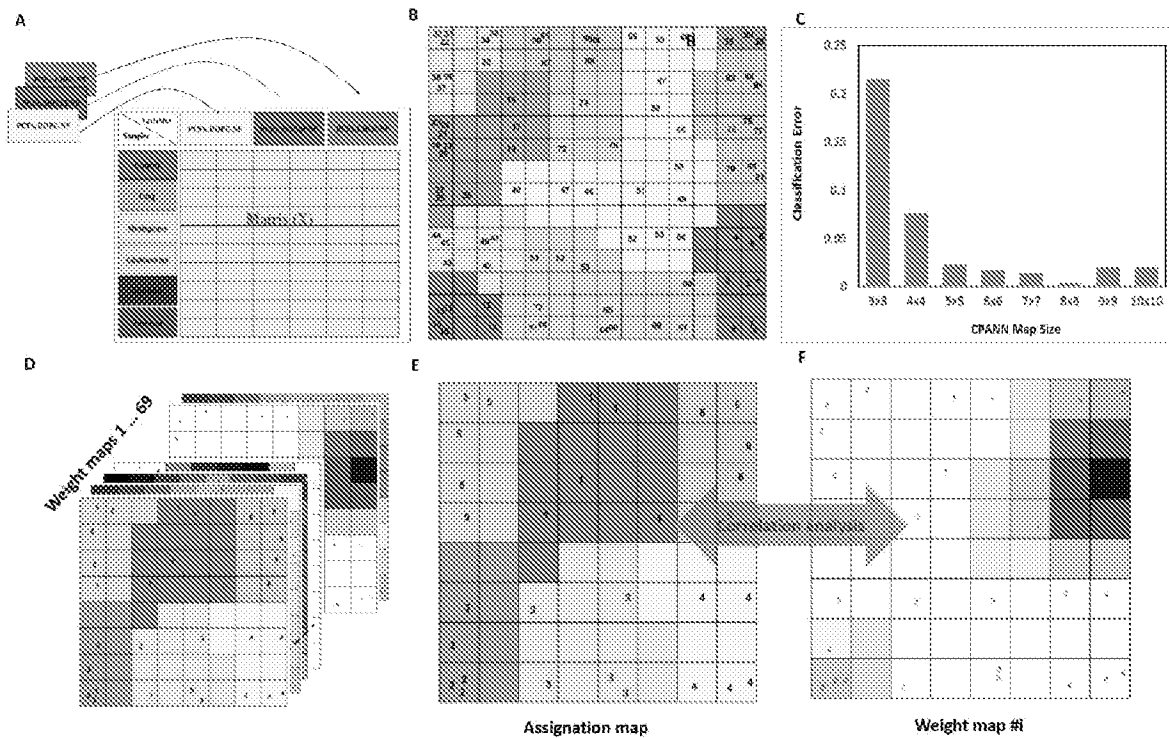


FIG. 8A-F

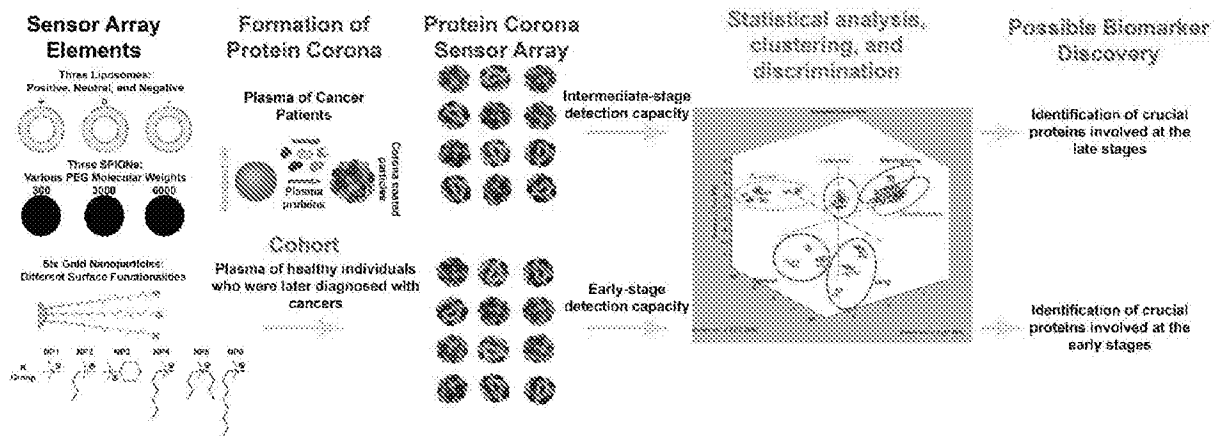
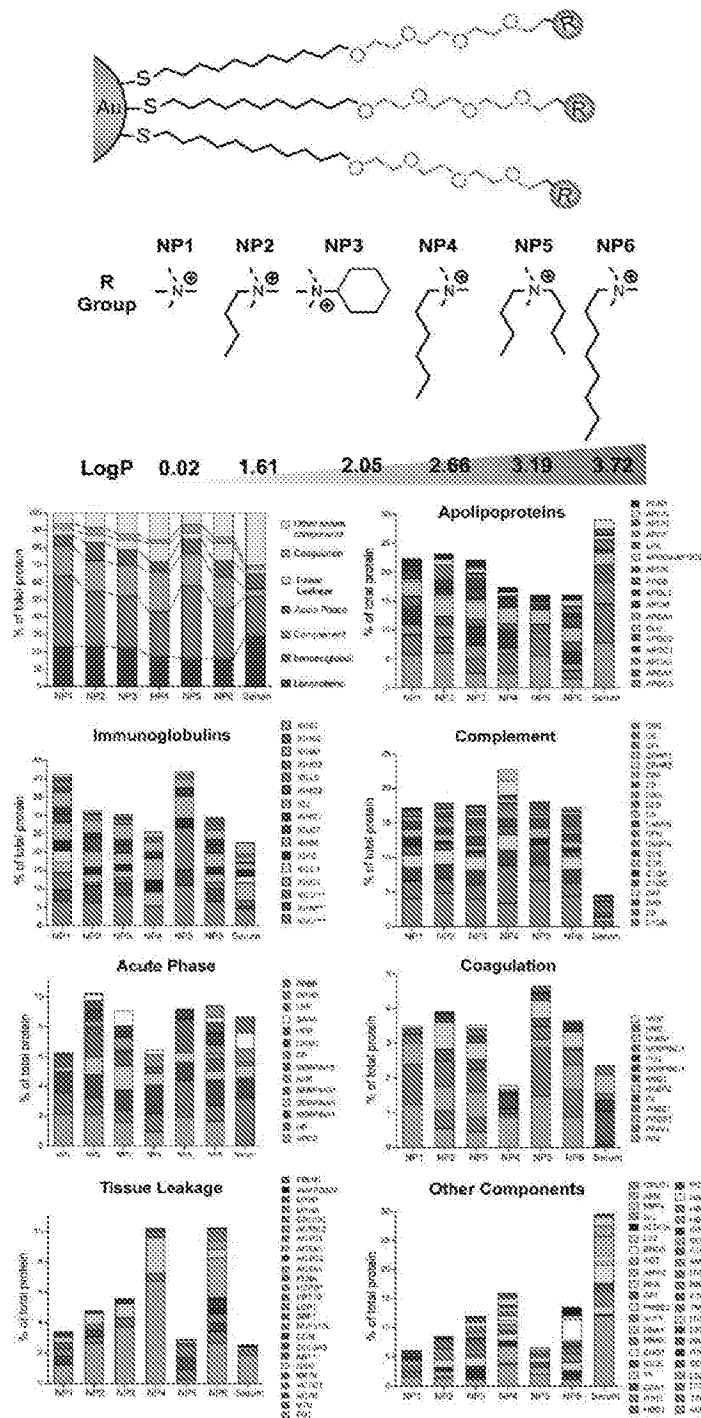


FIG. 9



From Saha et al. "Regulation of Macrophage Recognition through the Interplay of Nanoparticle Surface Functionality and Protein Corona" ACS Nano 2016 10 (4), 4421-4430

FIG. 10

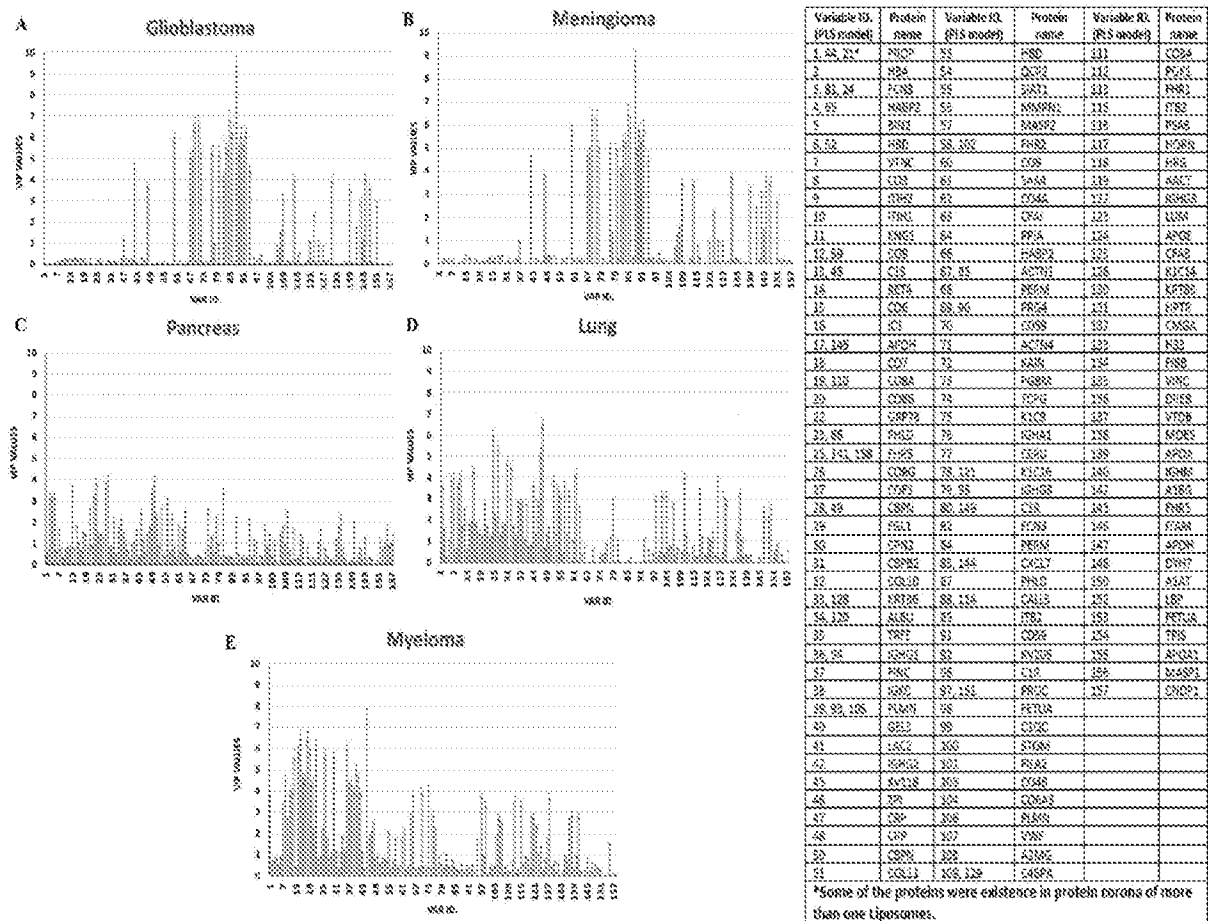


FIG. 12

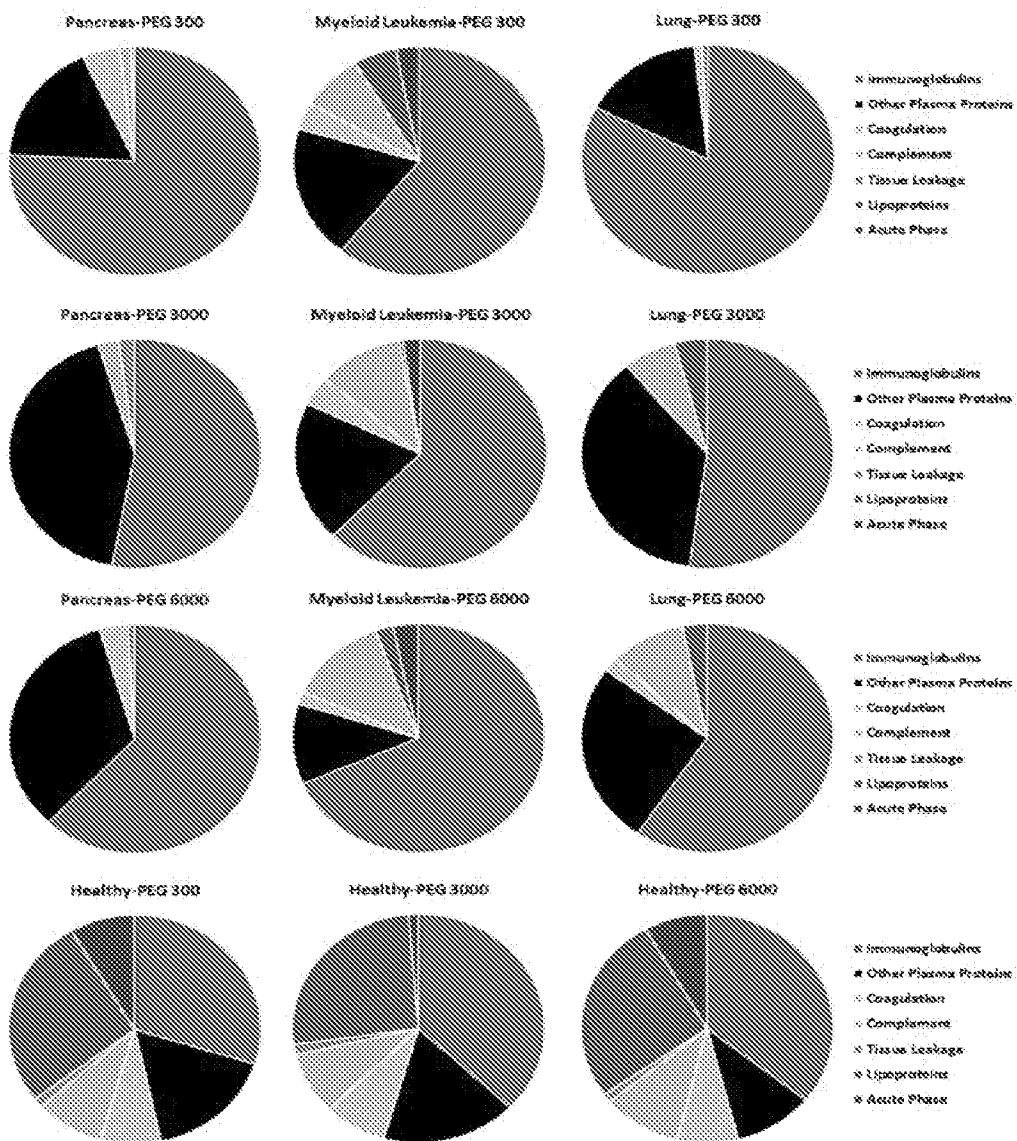


FIG. 13

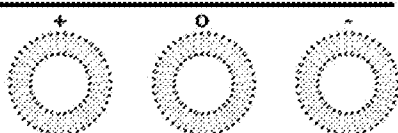
Sensor Array Elements

Various types of NPs (organic, inorganic, and or polymeric) with different physicochemical properties

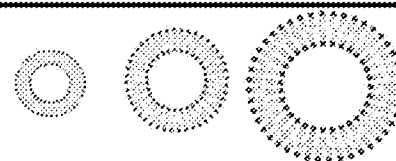
Examples

Liposomes

Different Surface Charges/Functionalities



Different Sizes



Different Lipid Compositions and conjugates

e.g., cholesterol



Superparamagnetic iron oxide nanoparticles

Different Surface Charges/Functionalities



Different Sizes



Different Shapes



Gold Nanoparticles

Different Surface Charges/Functionalities



Different Sizes



Different Shapes

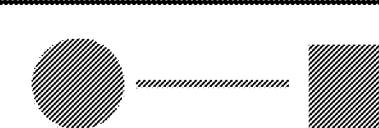


FIG. 14

Formation of protein corona

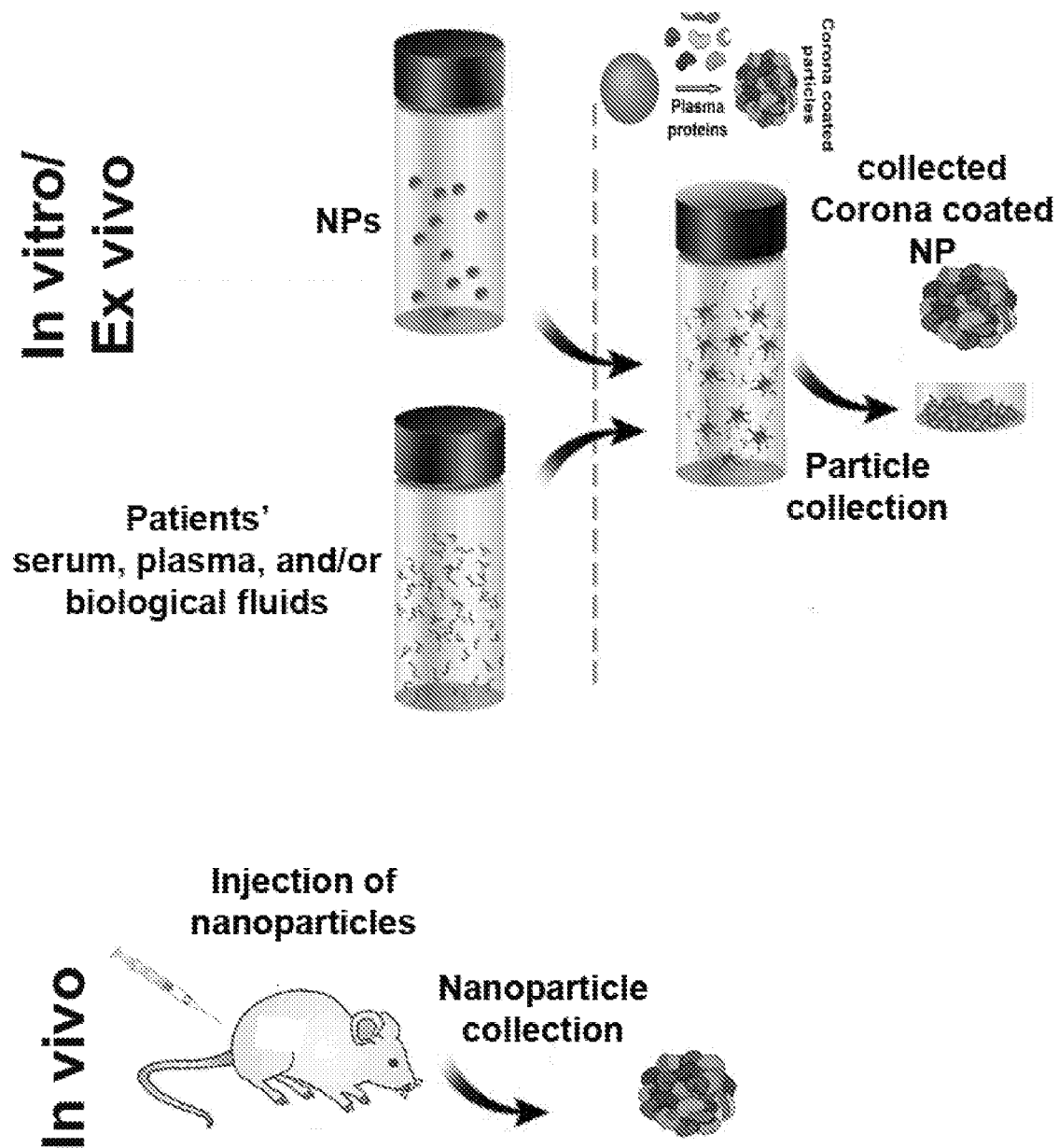


FIG. 15

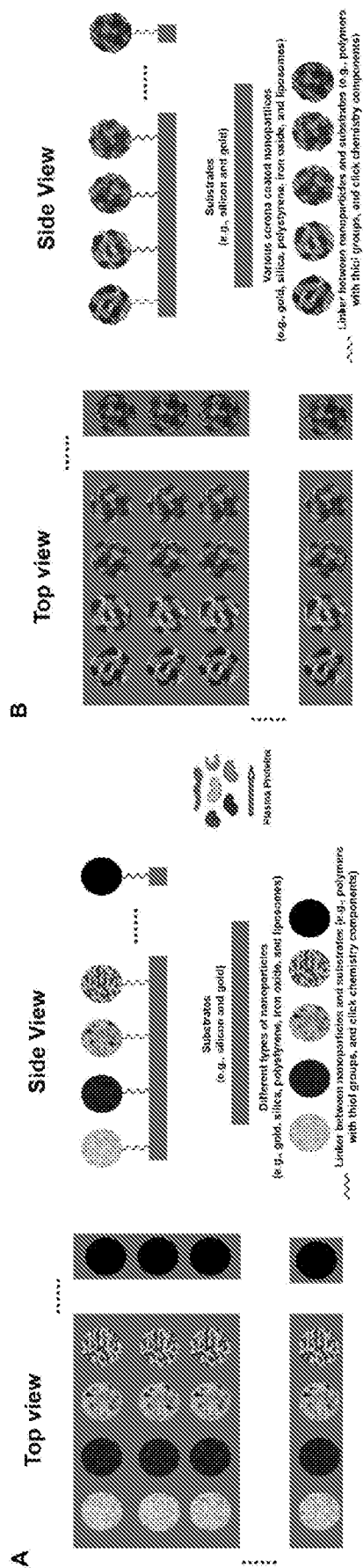


FIG. 16

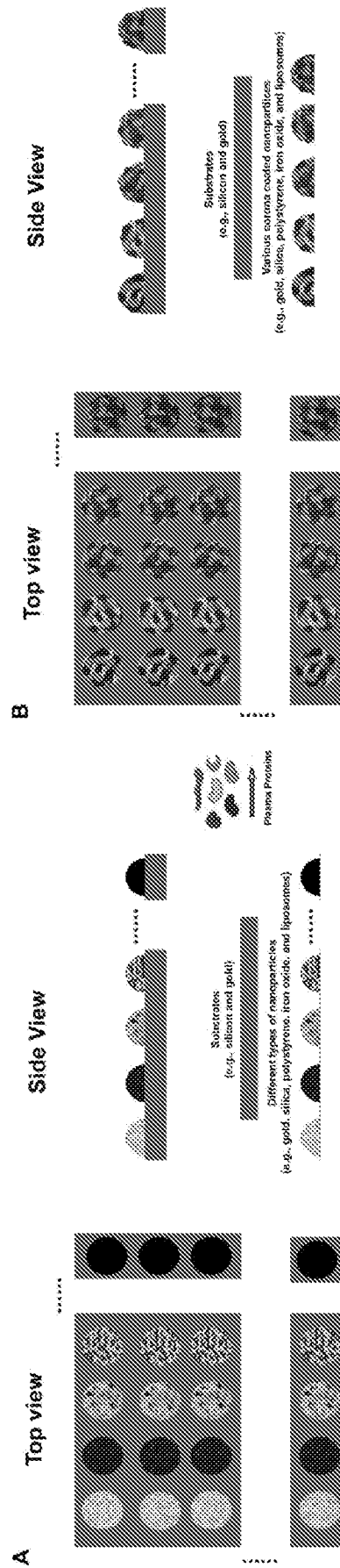


FIG. 17

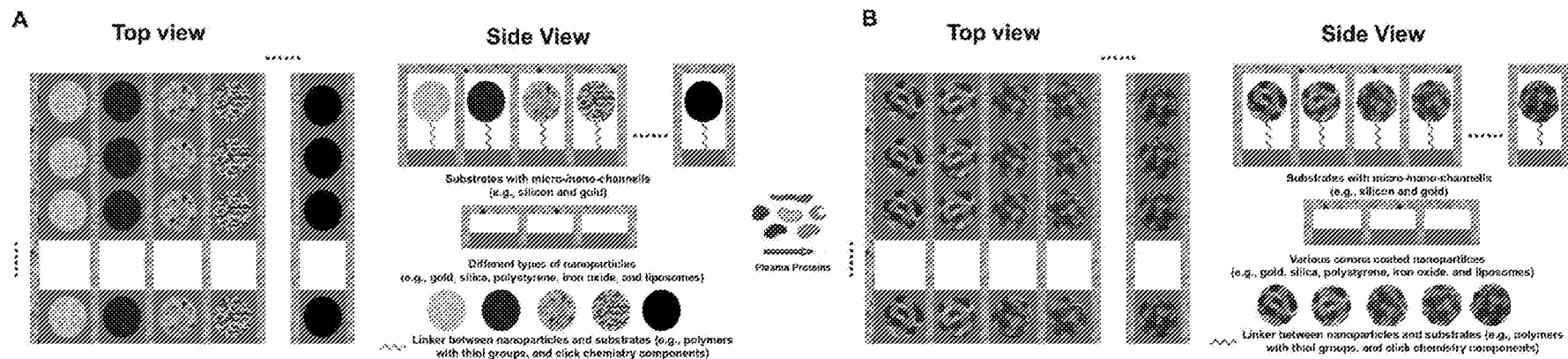


FIG. 18

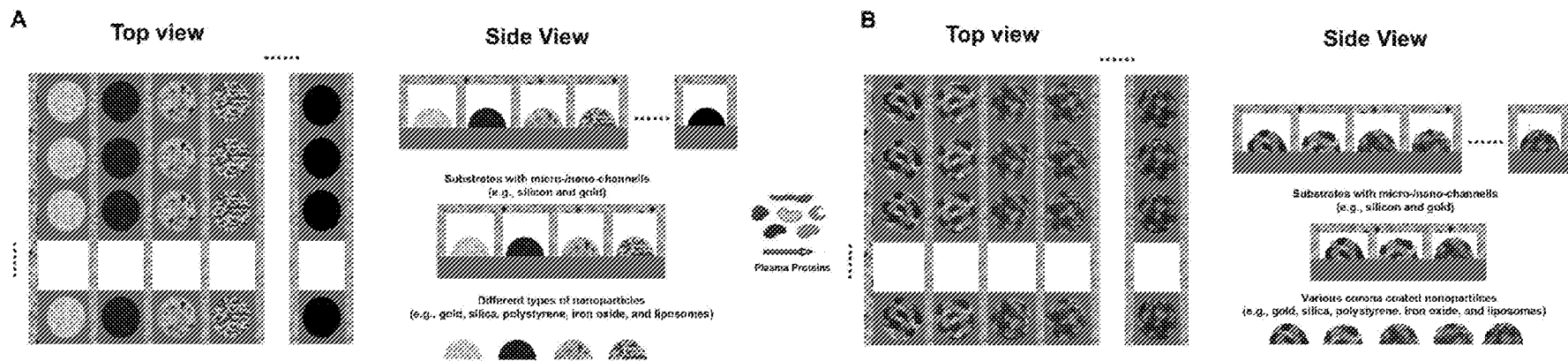


FIG. 19

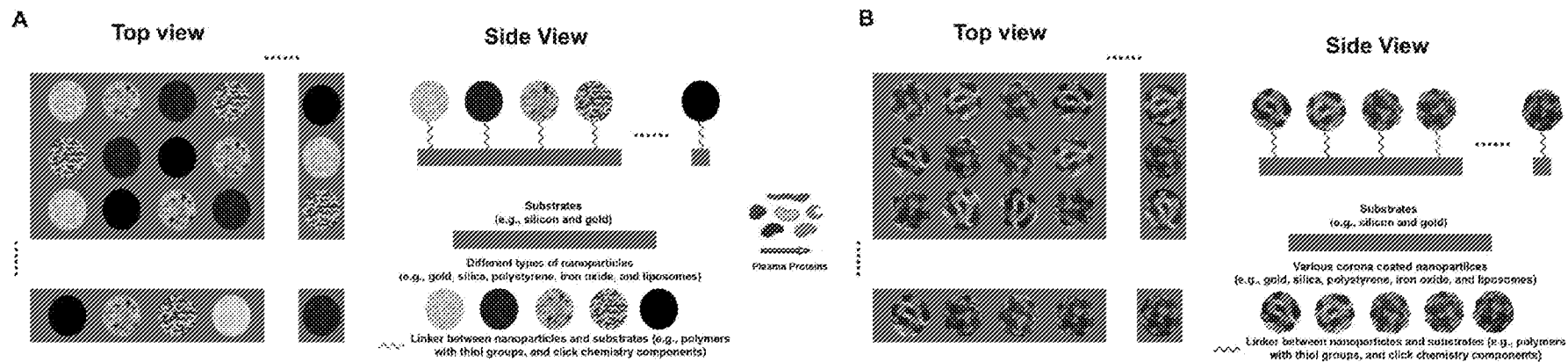


FIG. 20

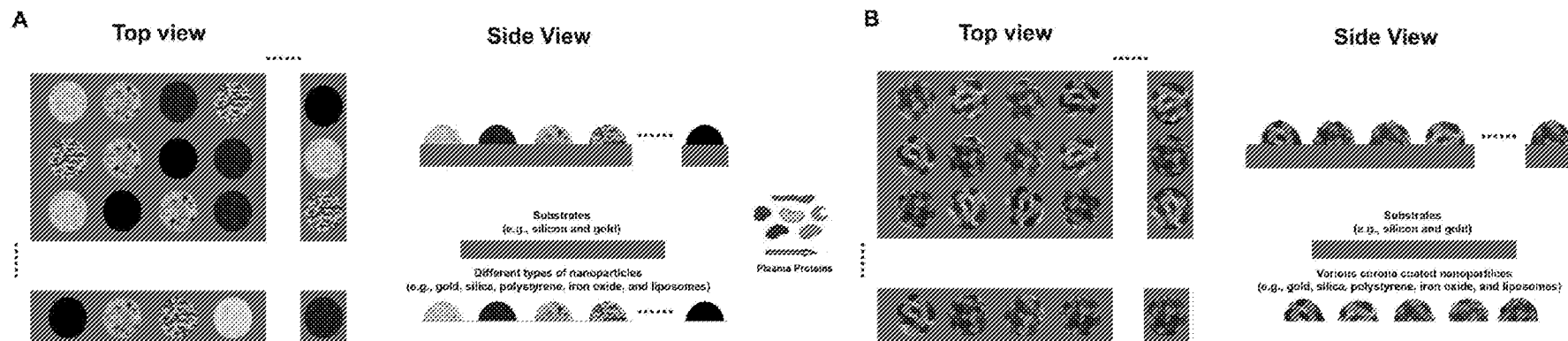


FIG. 21

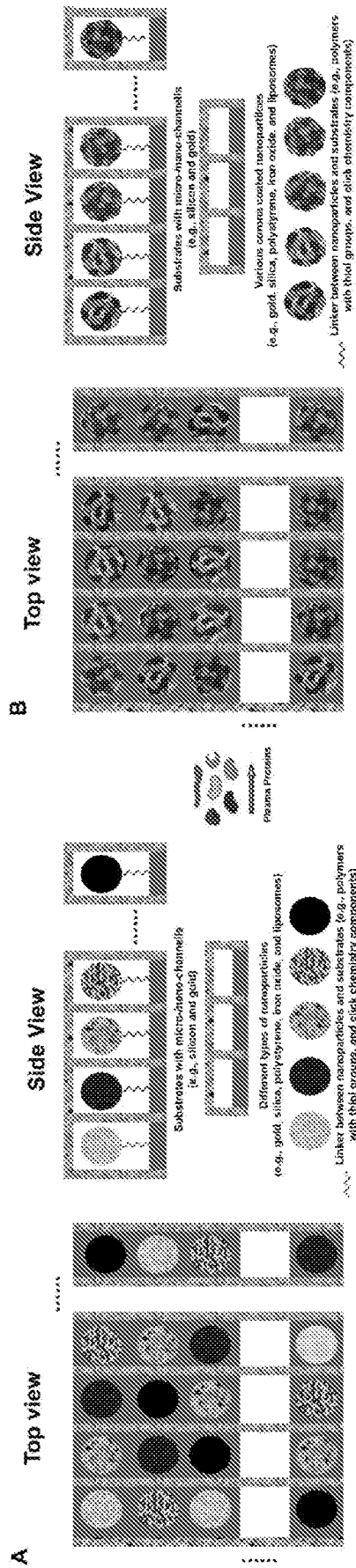


FIG. 22

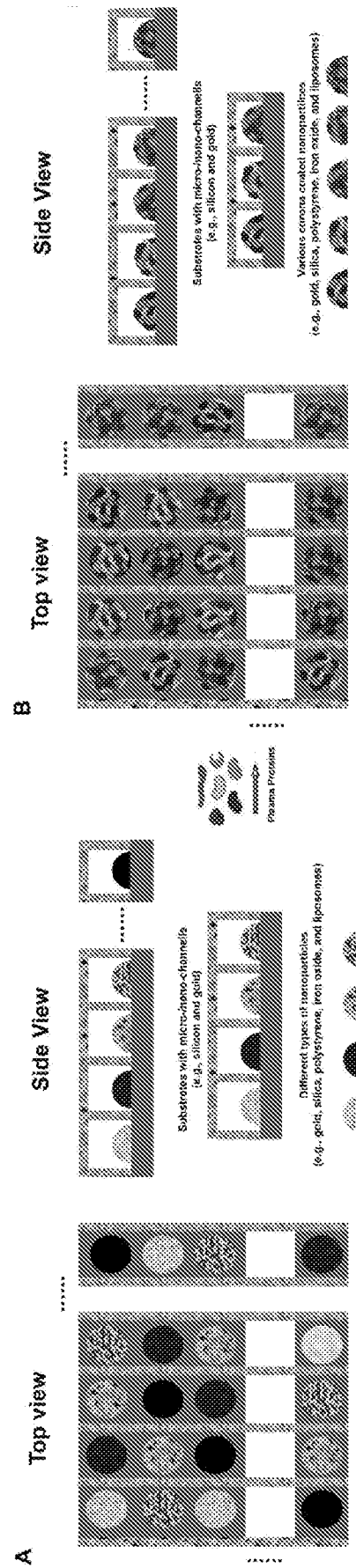


FIG. 23

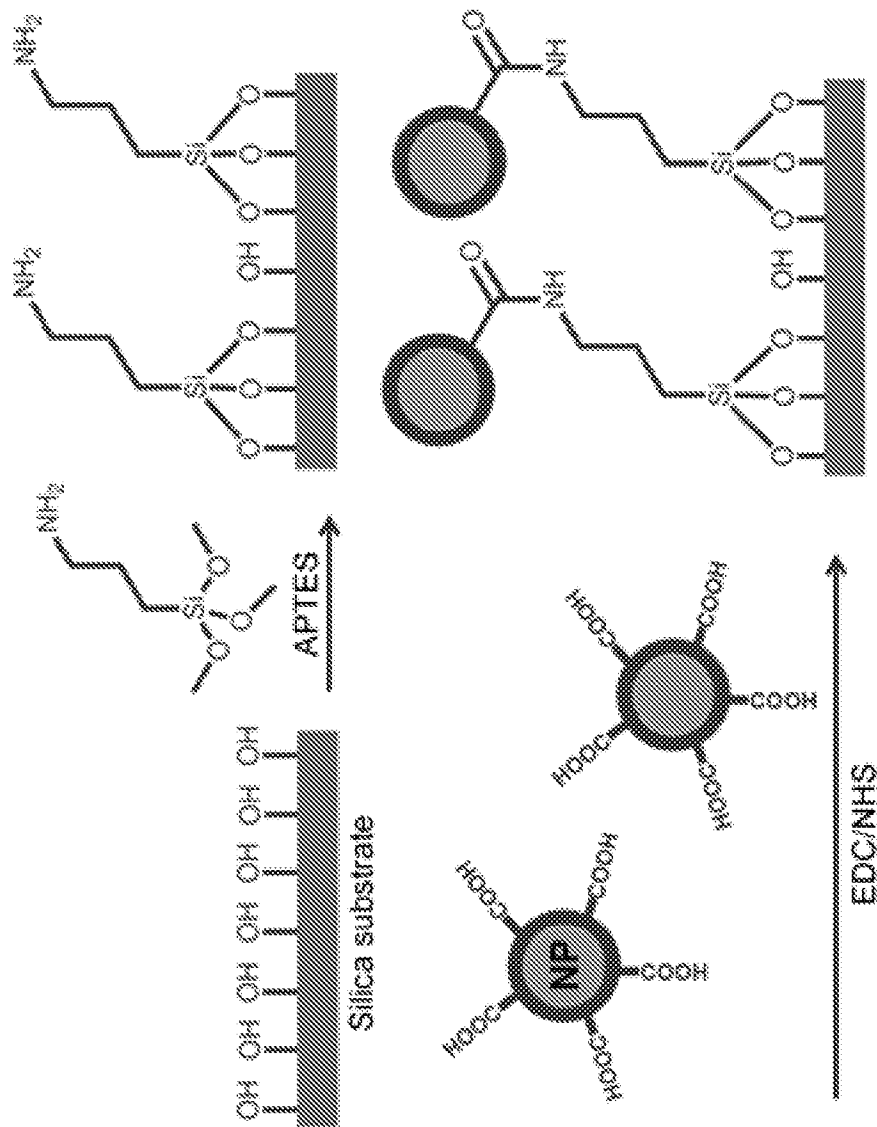


FIG. 24

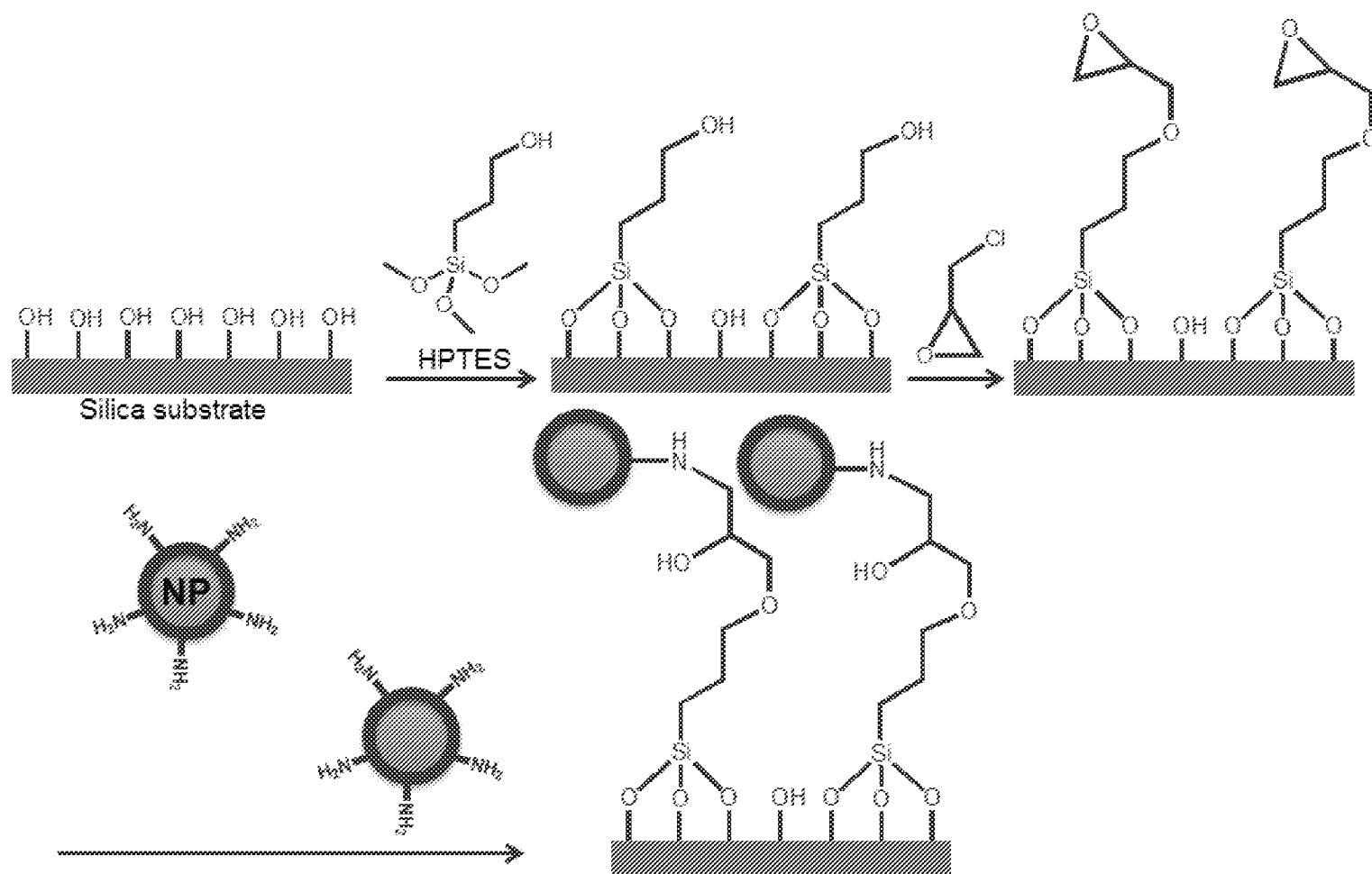


FIG. 25

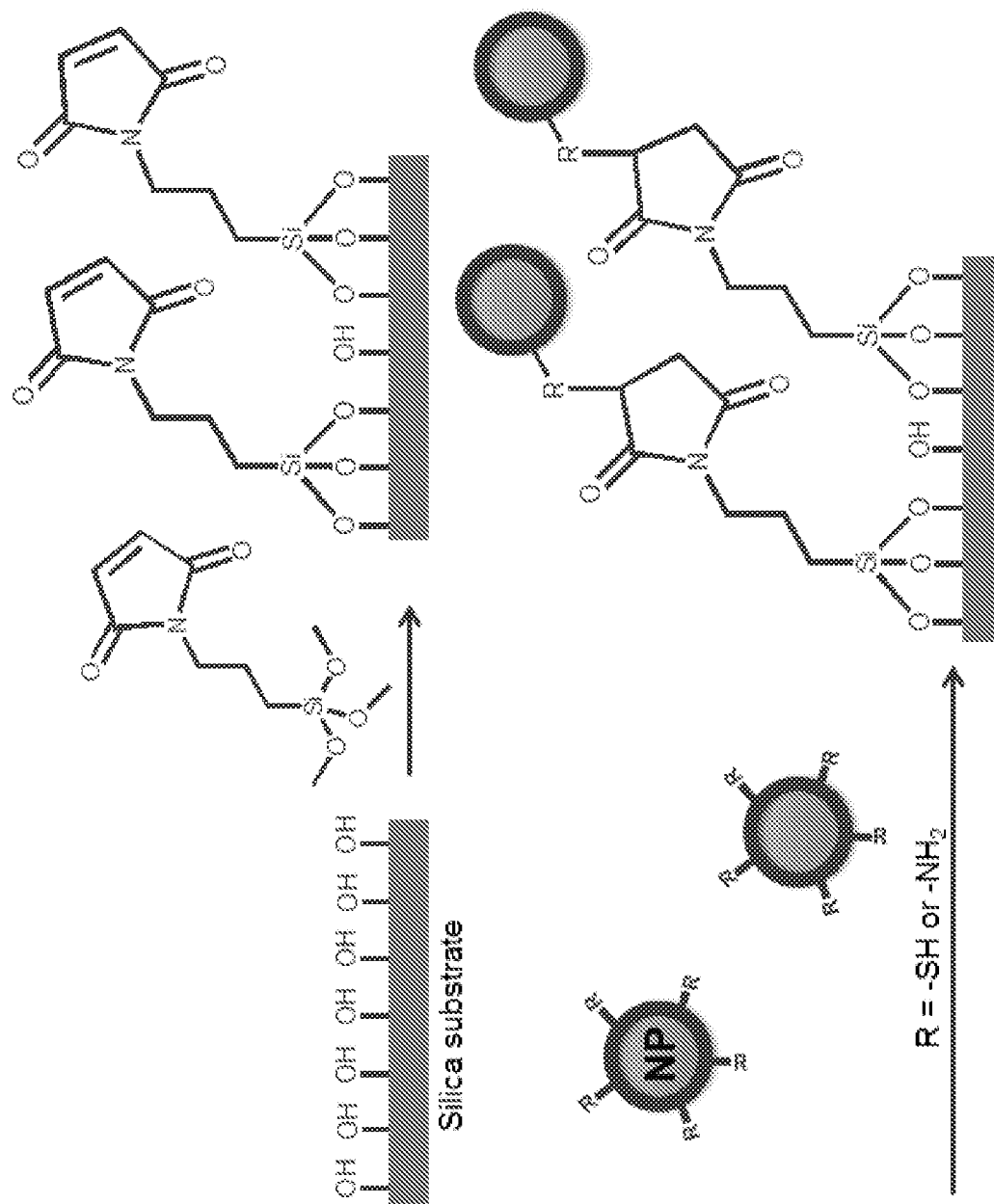


FIG. 26

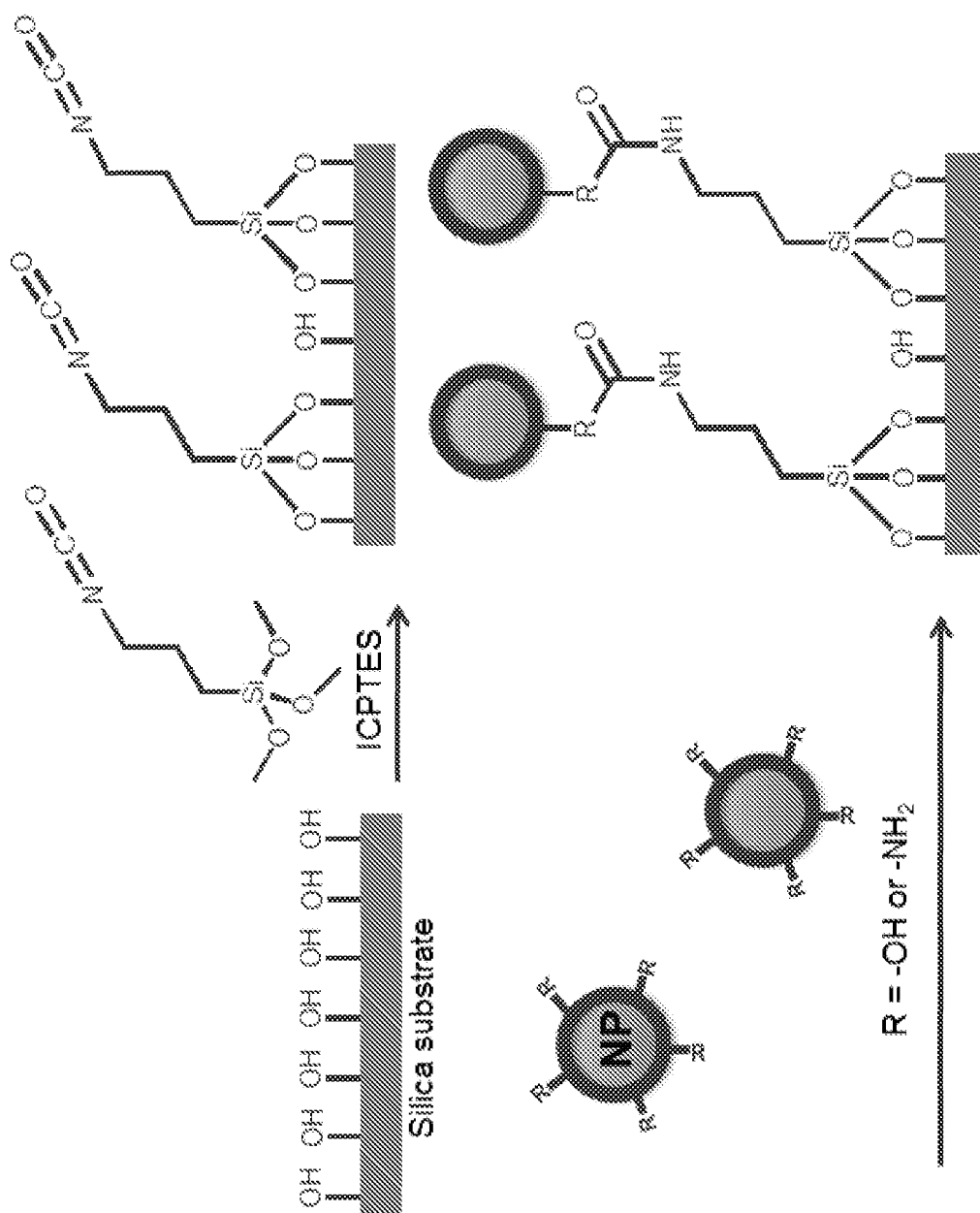


FIG. 27

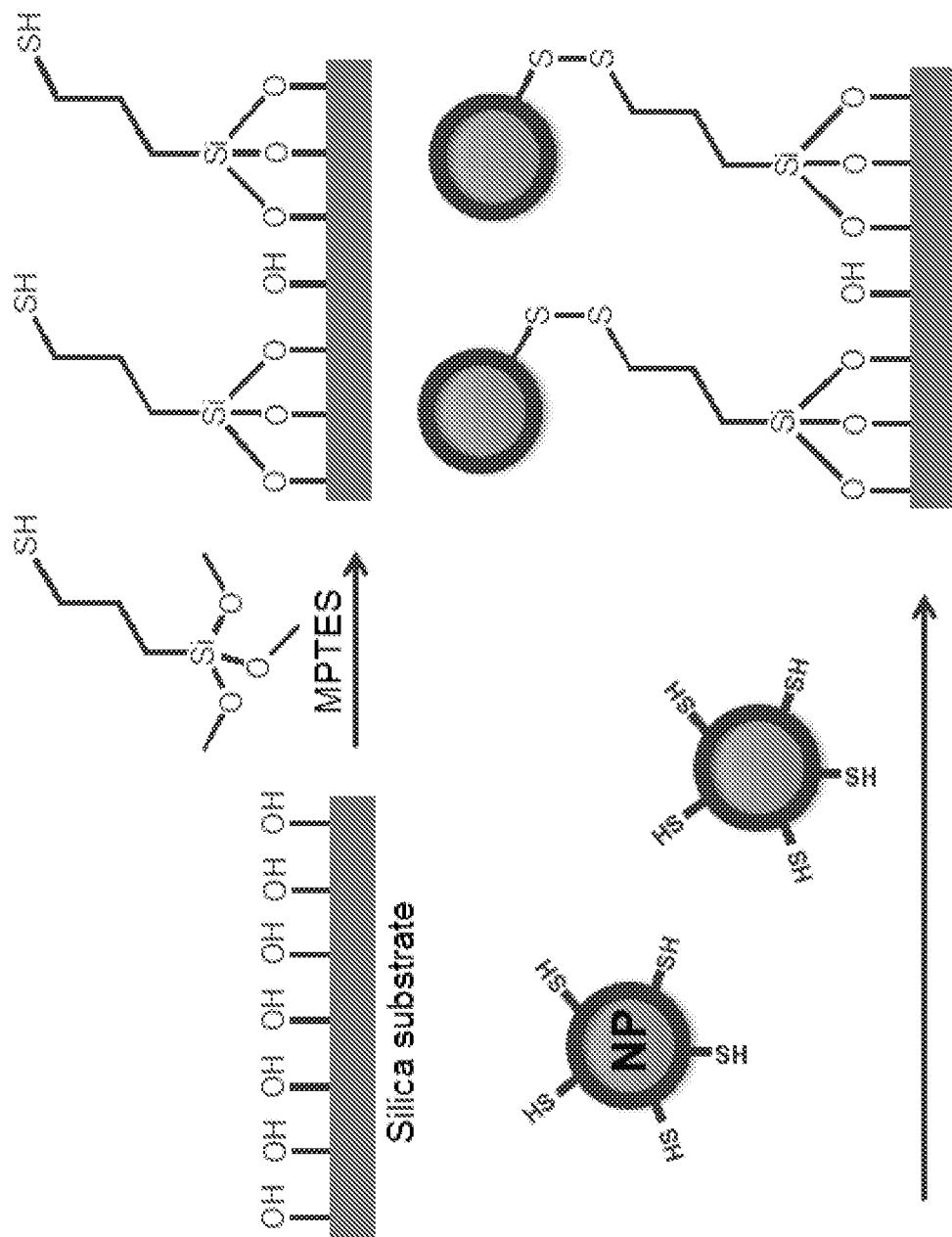


FIG. 28

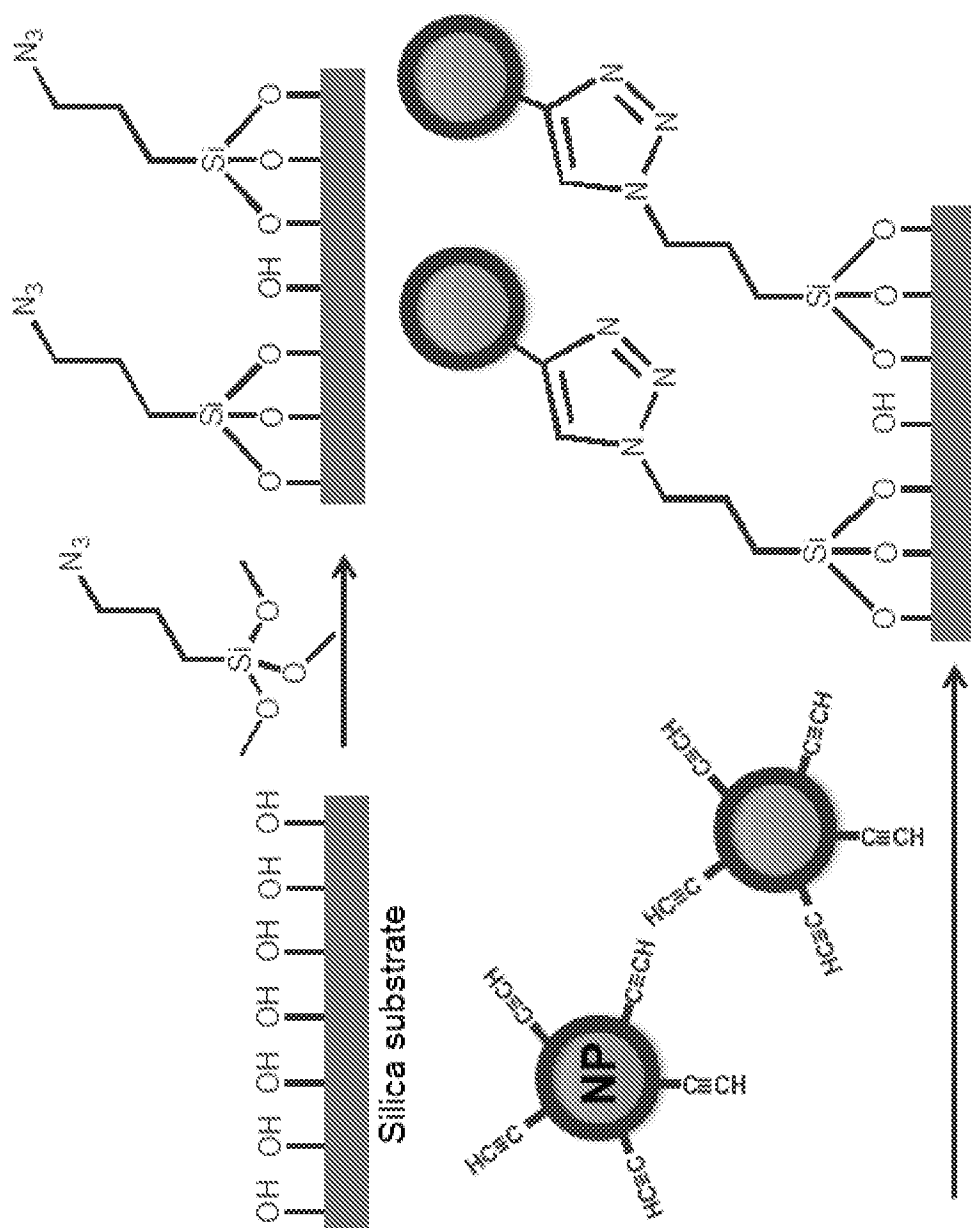


FIG. 29

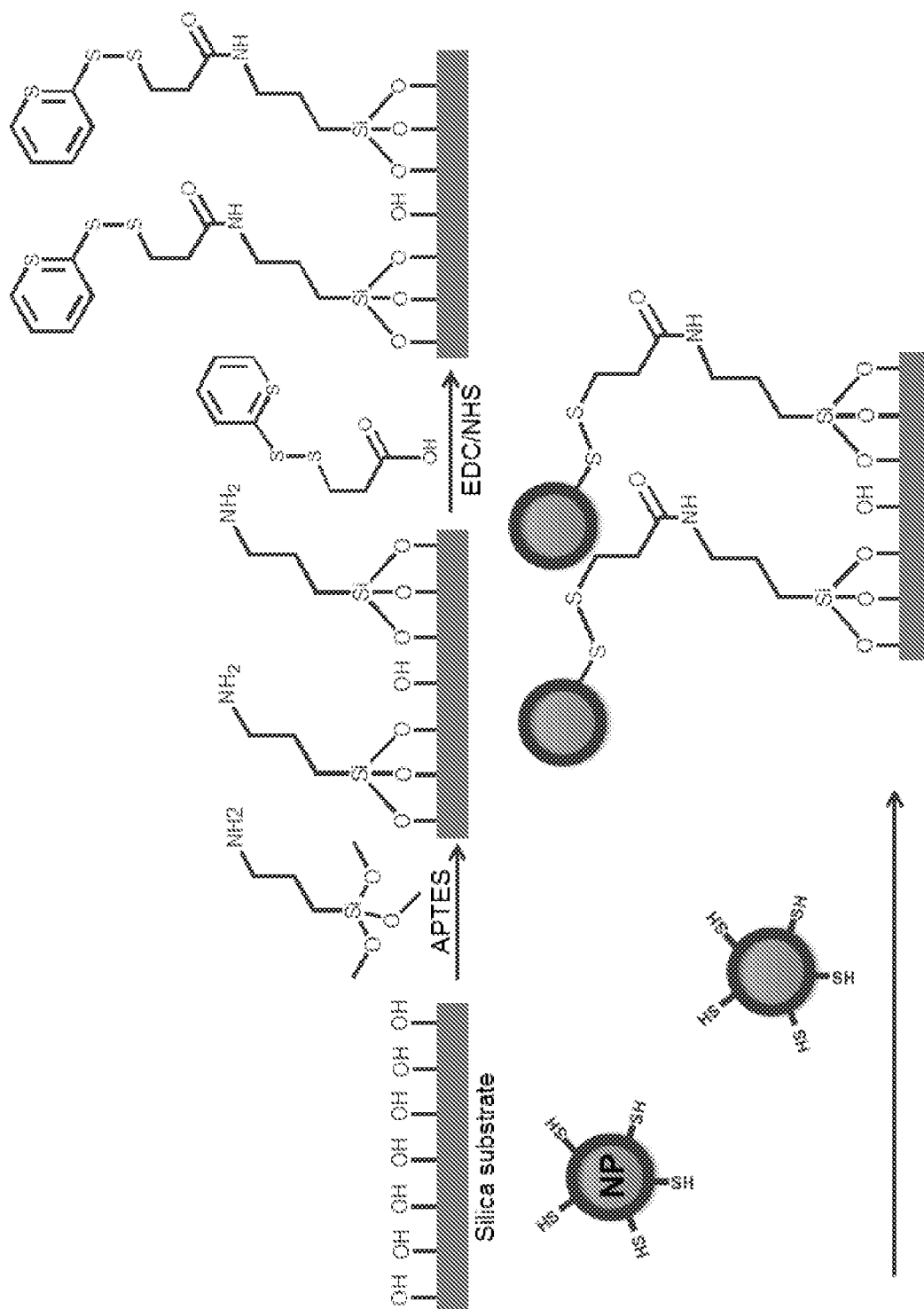


FIG. 30

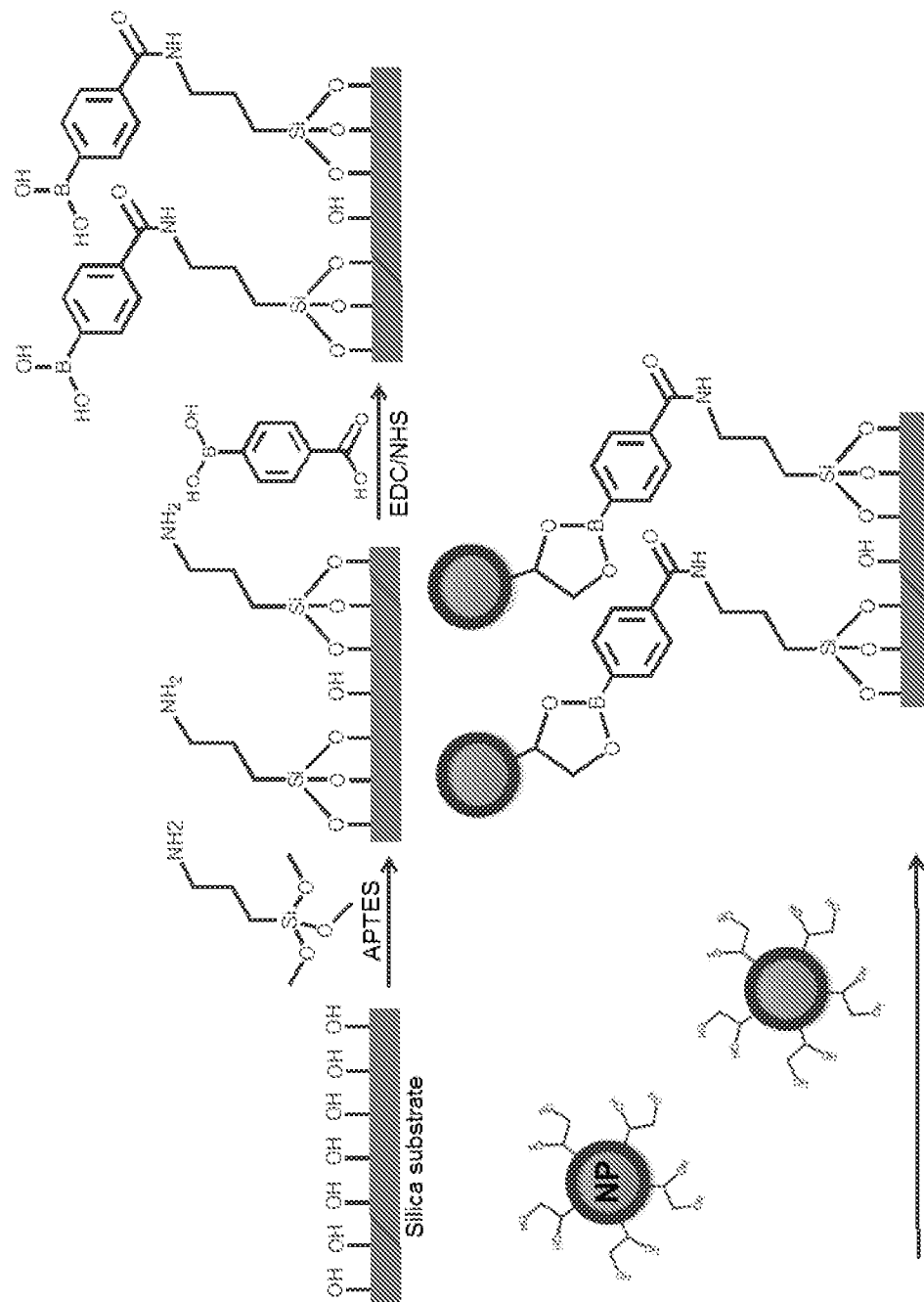


FIG. 31

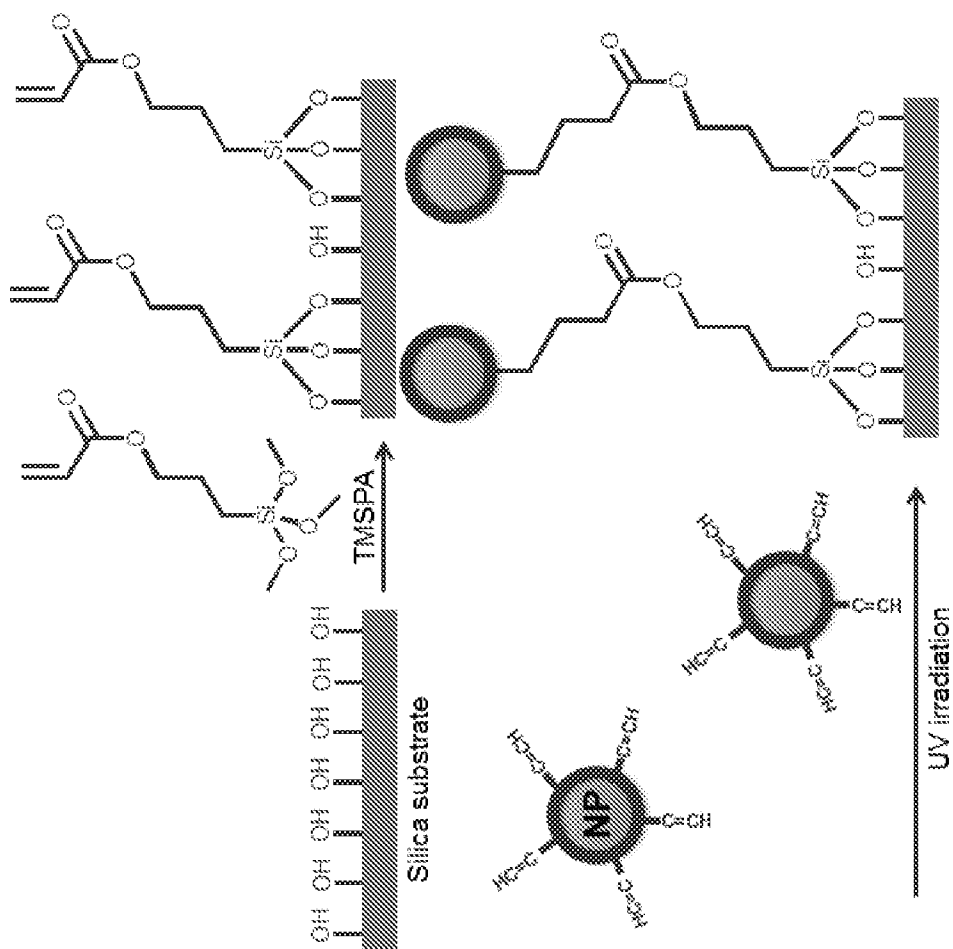


FIG. 32

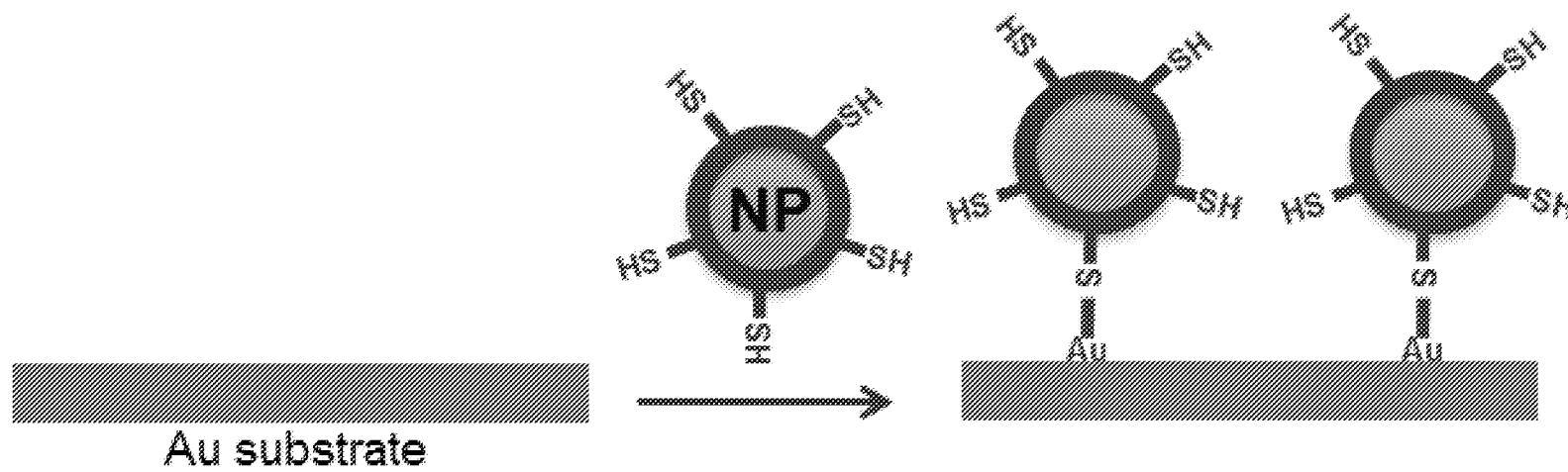


FIG. 33

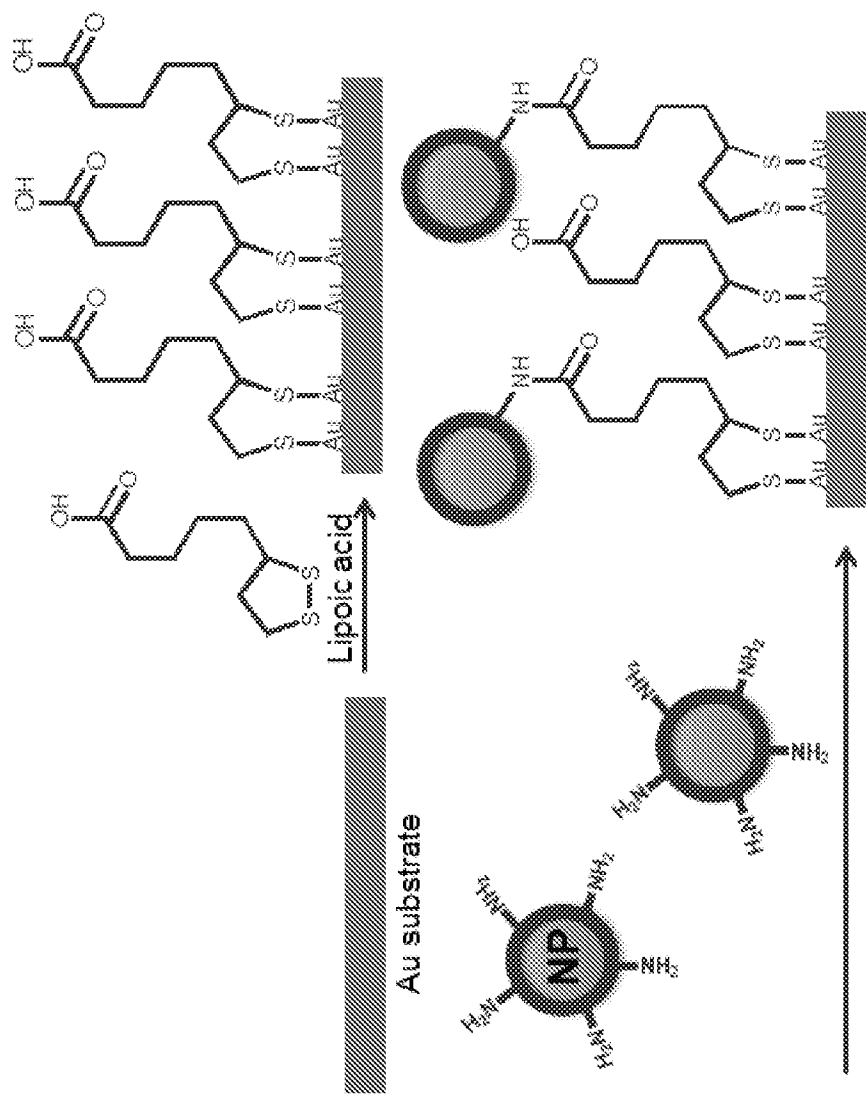


FIG. 34

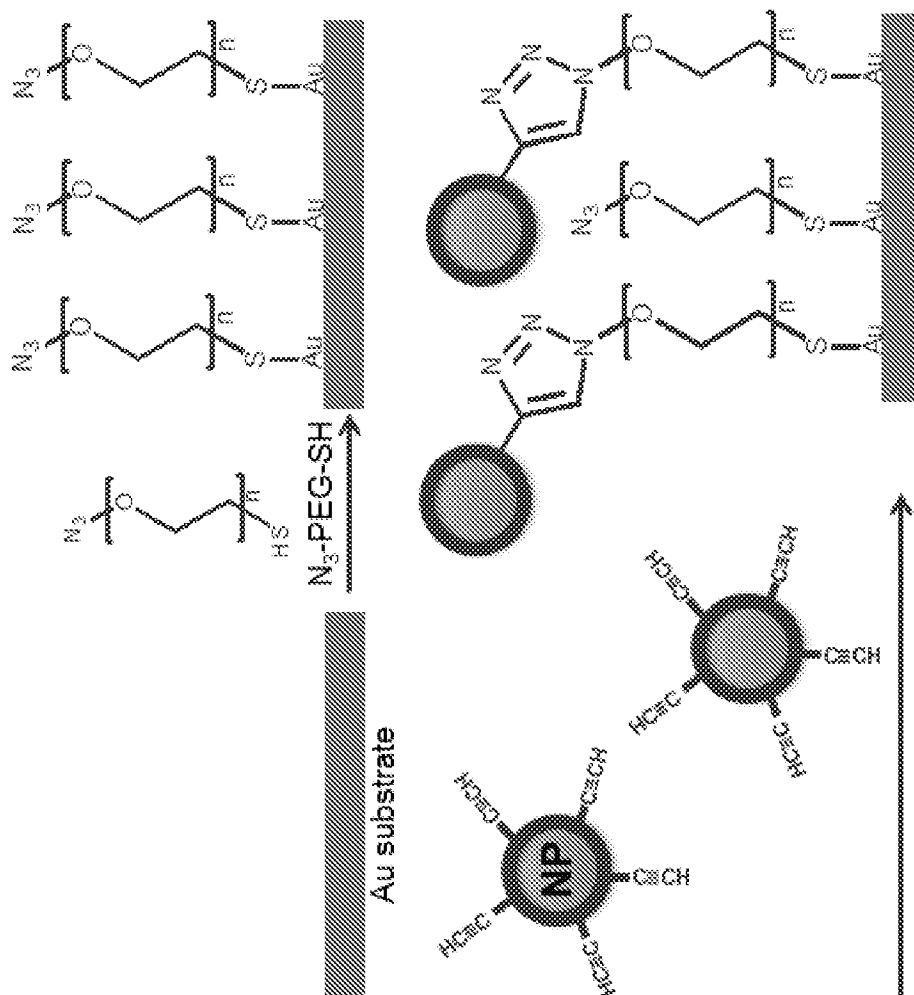


FIG. 35

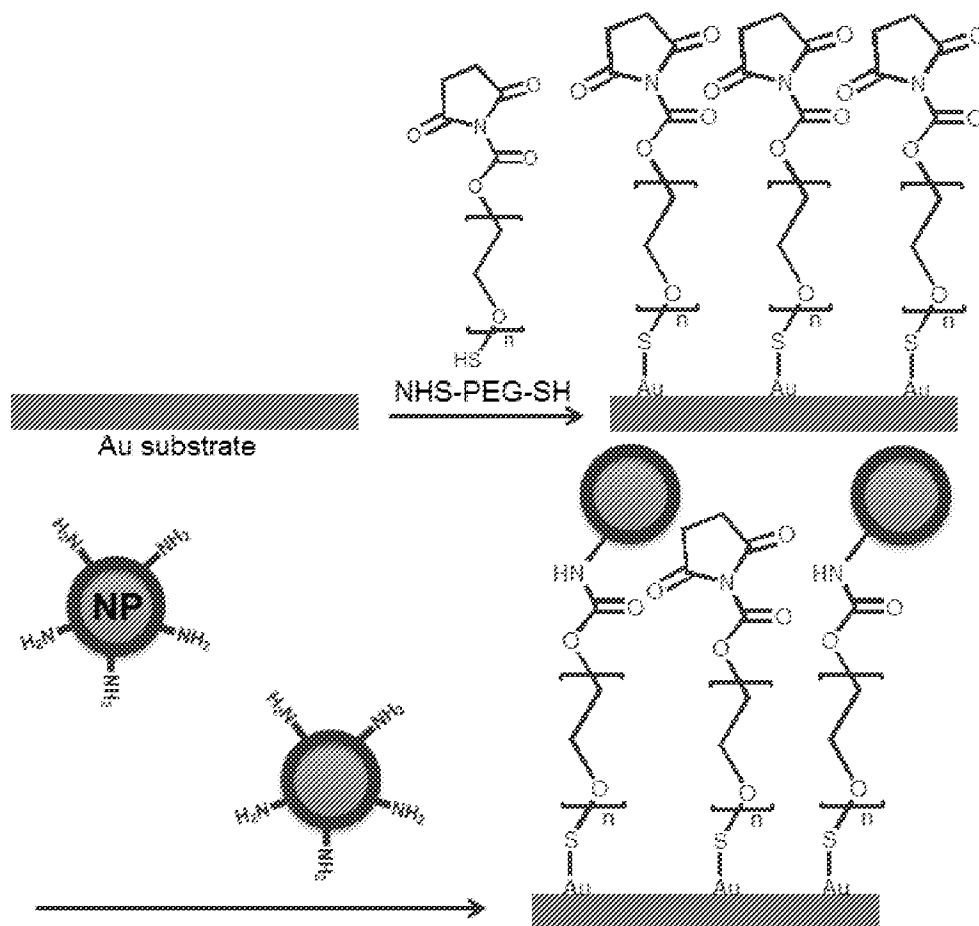


FIG. 36

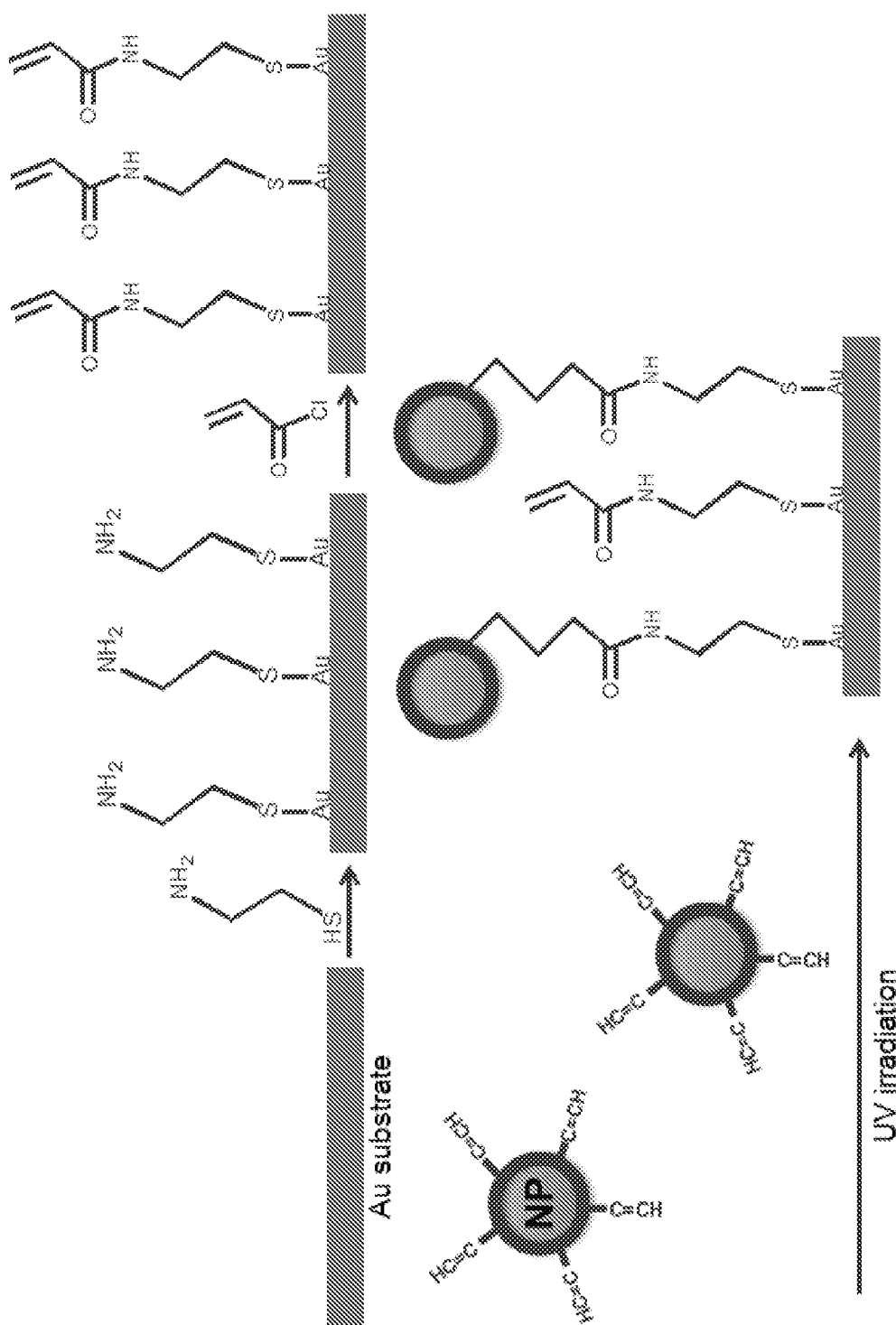
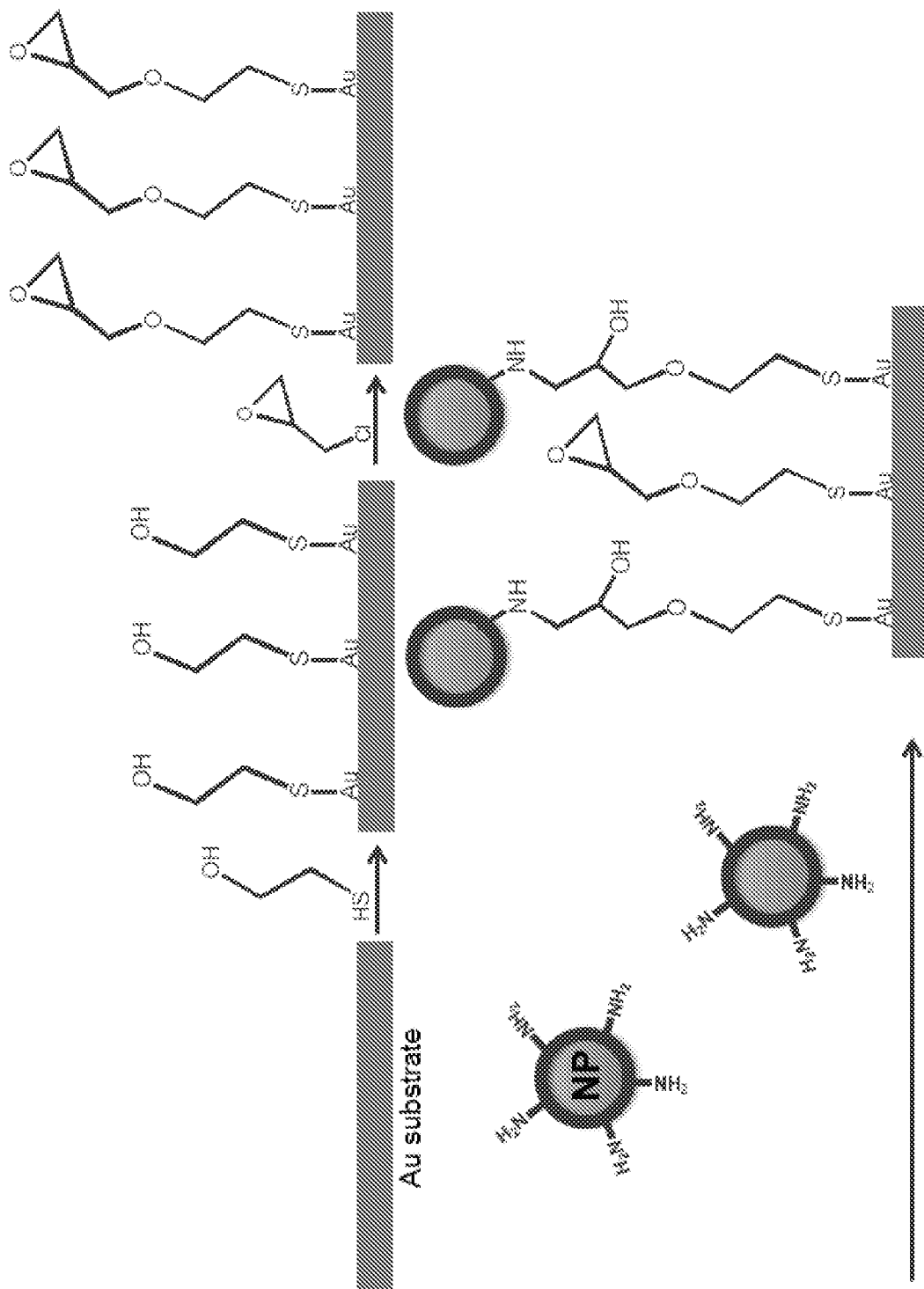


FIG. 37



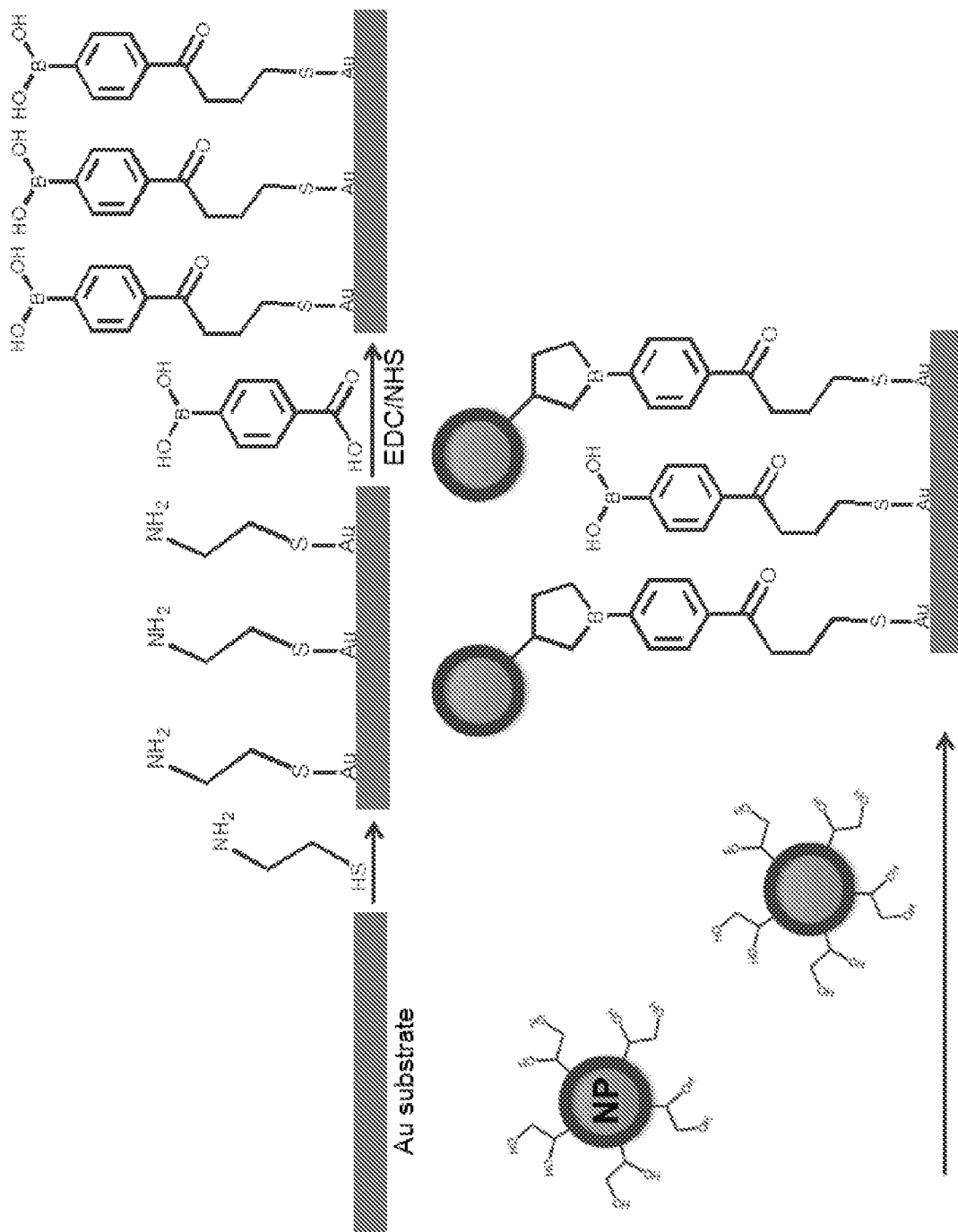


FIG. 39

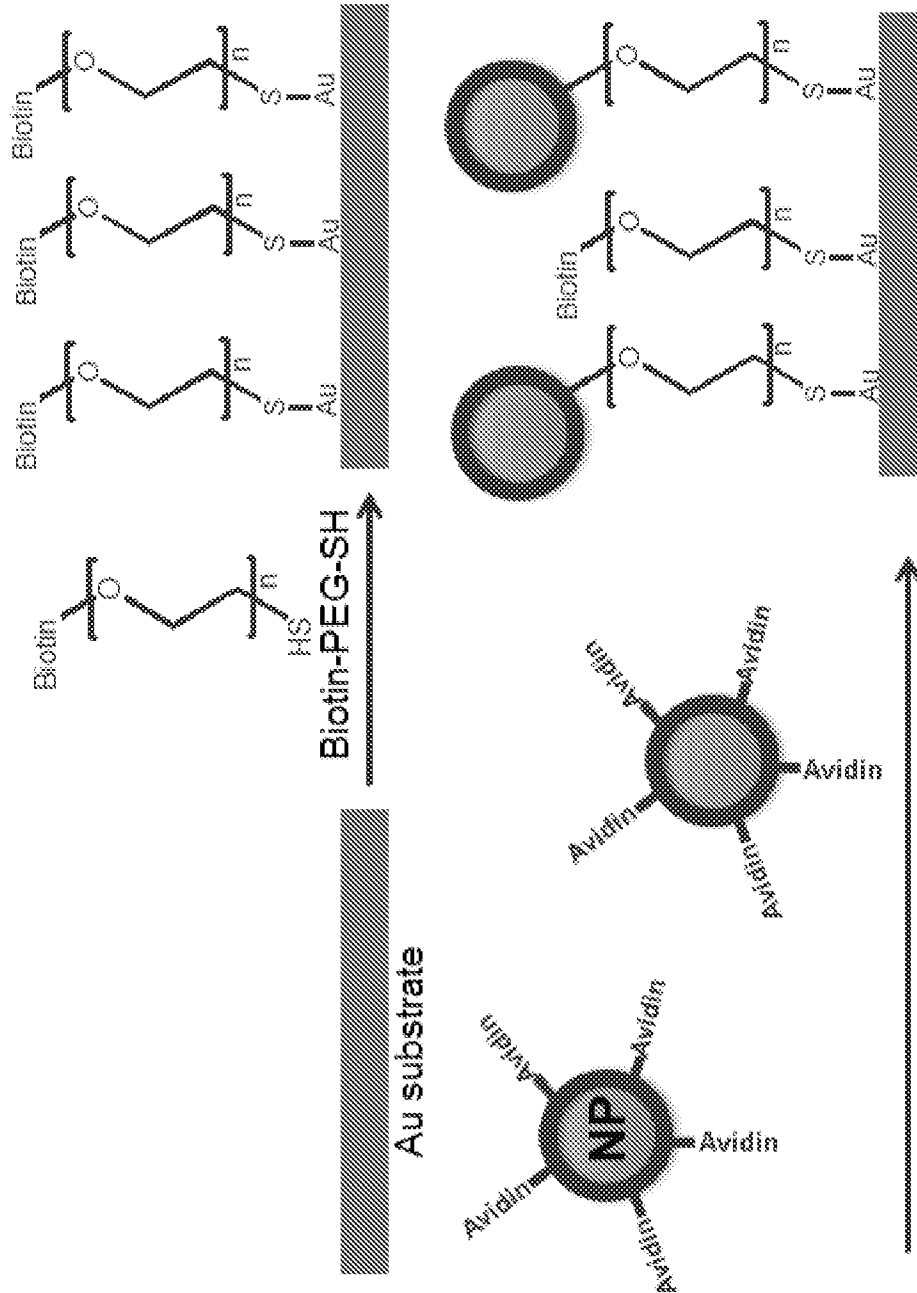


FIG. 40

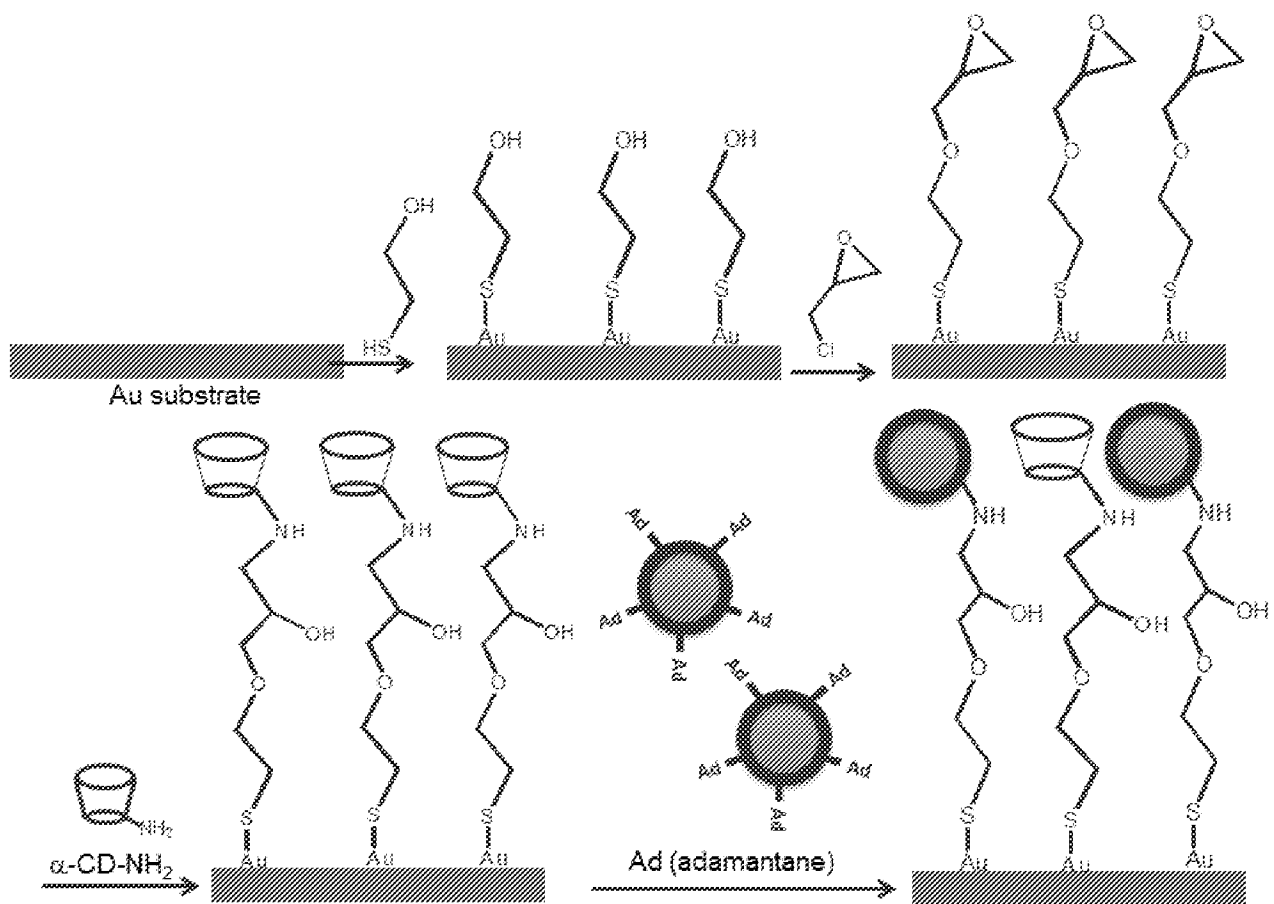


FIG. 41

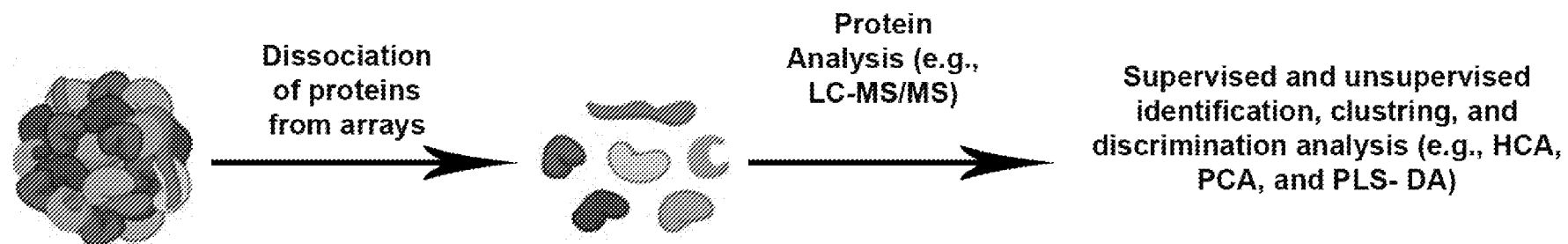


FIG. 42

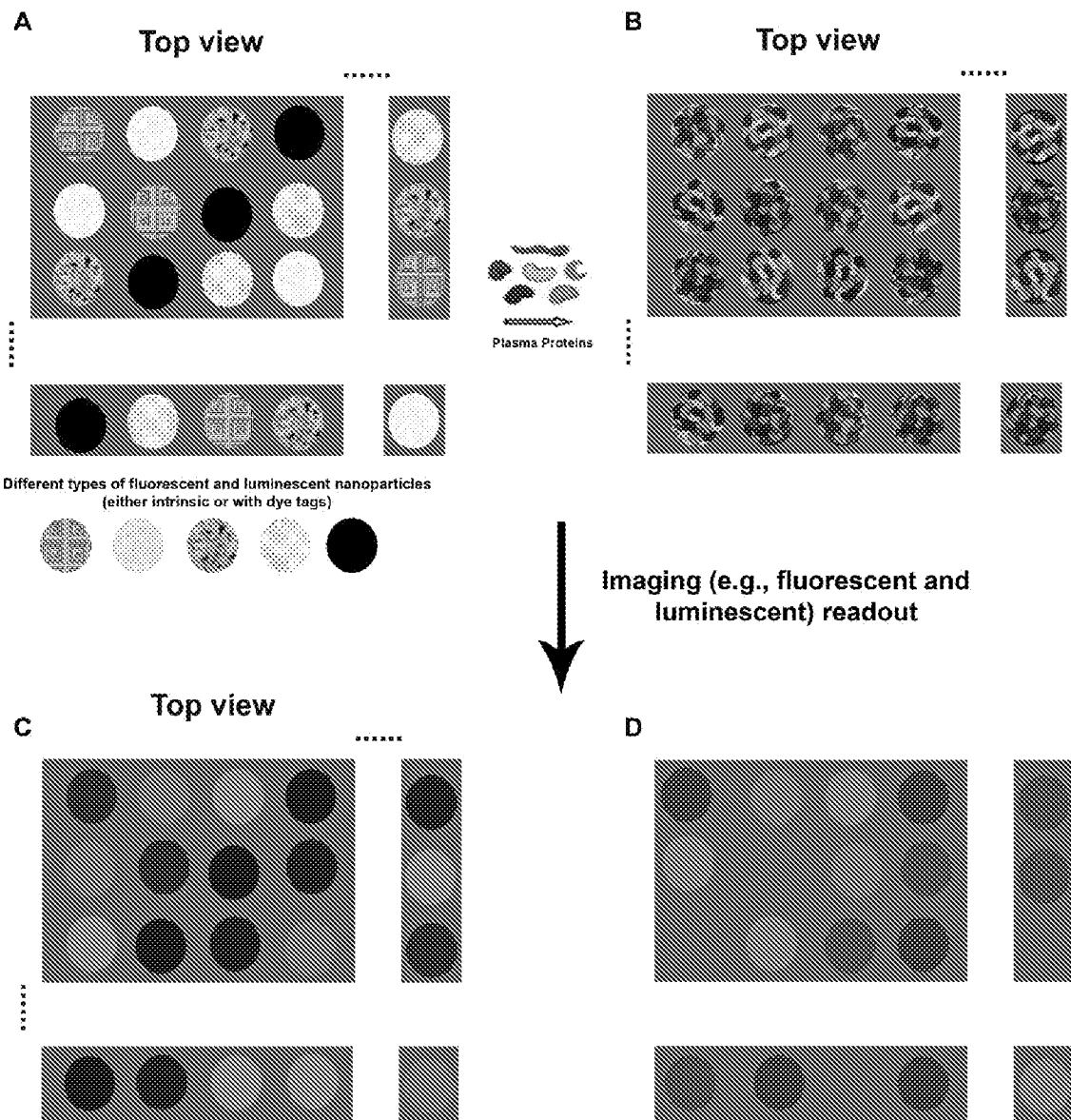


FIG. 43

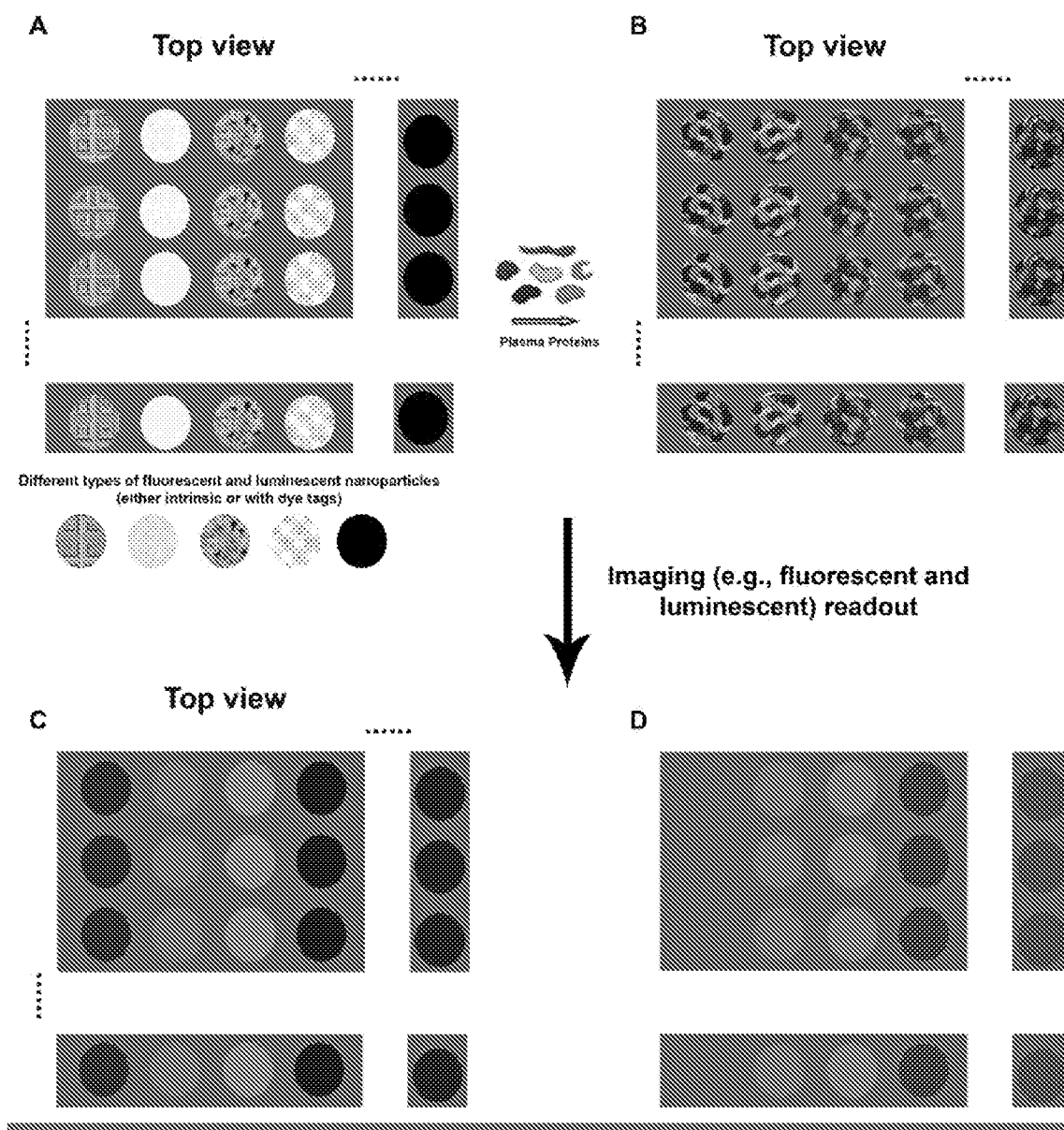


FIG. 44

SYSTEM AND METHOD FOR PROTEIN CORONA SENSOR ARRAY FOR EARLY DETECTION OF DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] N/A

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] N/A

BACKGROUND OF THE INVENTION

[0003] The field of the invention is related to sensor arrays for the detection and diagnosis of different disease states, particularly, the invention relates to the ability to diagnose or prognose diseases or disorders.

[0004] 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 you treat a disease early, you may be able 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.

[0005] Early diagnosis of cancer is crucial, as many types of cancers can be successfully treated in their early stages.¹⁻⁴ 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.^{1, 4, 5} 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.³⁹⁻⁴¹ 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.⁴² 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 cancers⁴²⁻⁴⁵; although huge numbers of risk factors and biomarkers have been introduced⁴⁶⁻⁴⁹, a broadly relevant platform for early detection of a wide range of cancers remains elusive^{38, 50, 51}.

[0006] 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 at very early stages.⁶

SUMMARY OF THE INVENTION

[0007] The present invention provides a sensitive versatile sensor array for detection of a wide range of diseases and disorders and determination of disease states in a subject. The uniqueness of the present invention is the combination of this recognition of a biomolecular fingerprint from a sample from a subject and the ability to determine a disease state for that subject on a continuum of health.

[0008] In some aspect, the invention provides a sensor array comprising a plurality of nanoscale sensor elements, wherein the plurality of nanoscale sensor elements differ from each other in at least one physicochemical property and the plurality of sensor elements comprises at least two sensor elements. In some aspects, each sensor element is able to bind a plurality of biomolecules in a sample to produce a biomolecule corona signature, wherein each nanoscale sensor elements has a distinct biomolecule corona signature from the other.

[0009] In some aspects, the plurality of sensor elements produces a plurality of biomolecule corona signatures when contacted by the sample, wherein the combination of the plurality of biomolecule corona signatures produces a biomolecule fingerprint for the sample.

[0010] In some aspects, the nanoscale sensor elements are linked to the substrate.

[0011] In some aspects, the invention provides methods of detecting a disease

state in a subject comprising: obtaining a sample from the subject; contacting the sample with a sensor array, wherein the sensor array comprises a plurality of nanoscale sensor elements, wherein the plurality of nanoscale sensor elements differ from each other in at least one physicochemical property and the plurality of sensor elements comprises at least two sensor elements, and determining a biomolecule fingerprint associated with the sample, wherein the biomolecule fingerprint may differentiate the disease state of the subject. In some aspects, the method further comprises comparing the biomolecule fingerprint of the sample to a panel of biomolecule fingerprints associated with a plurality of disease states to determine which disease state is associated with the sample.

[0012] In another aspect, the invention provides a method of determining a biomolecule fingerprint associated with at least disease state or at least one disease or a disorder, the method comprising the steps of: (a) obtaining a samples from at least two subjects diagnosed with the disease state or the at least one disease or disorder; (b) contacting each sample with a sensor array described herein, and (c) determining a biomolecule fingerprint for the sensor array that is associated with the disease state or at least one disease or disorder. In some aspects, step (c) further comprises detecting the composition of the biomolecule corona of each sensor element, wherein the combination of the composition of each biomolecule corona between the different sensor elements produce the biomolecule fingerprint associated with the sample. In other aspects, step (c) comprises dissociating the biomolecule corona from each nanoscale sensor element and assaying the plurality of biomolecules of each biomolecule corona, wherein the combination of biomolecules assayed produced the biomolecule fingerprint.

[0013] In yet another aspect, the invention provides a method of diagnosing or prognosing a disease or disorder in a subject, comprising obtaining a sample from a subject; contacting the sample with a sensor array of any one of claims 1-21 to produce a biomolecule fingerprint, comparing the biomolecule fingerprint to a panel of biomolecule fingerprints associated with a plurality of diseases or disorders; and diagnosing or prognosing the disease or disorder.

[0014] In yet another aspect, the invention provides a method of identifying a pattern of biomarkers associated with a disease or disorder, the method comprising: (a) obtaining a samples from at least two subjects diagnosed with the disease or disorder

and at least two control subjects; (b) contacting each sample with a sensor array to produce a plurality of biomolecule corona for each subject, and (c) comparing the composition of the plurality of biomolecule corona of the subjects with the disease or disorder to the composition of the plurality of biomolecule corona of the control subjects to determine a pattern of biomarkers associated with the disease or disorder.

[0015] In some aspects, the disease or disorder is cancer, endocrine disorder, cardiovascular disease, inflammatory disease or a neurological disease.

[0016] In some aspects, the invention provides a kit for diagnosing or prognosing a disease or disorder, the kit comprising: a sensor array comprising a plurality of nanoscale sensor elements, wherein the plurality of nanoscale sensor elements differ from each other in at least one physicochemical property and the plurality of sensor elements comprises at least two sensor elements.

[0017] In other aspects, the invention provides kit for determining and/or detecting at least one biomarker associated with a disease or disorder, comprising at least one sensor array comprising a plurality of nanoscale sensor elements, wherein the plurality of nanoscale sensor elements differ from each other in at least one physicochemical property and the plurality of sensor elements comprises at least two sensor elements.

[0018] The foregoing and other aspects and advantages of the invention will appear from the following description. In the description, reference is made to the accompanying drawings which form a part hereof, and in which there is shown by way of illustration a preferred embodiment of the invention. Such embodiment does not necessarily represent the full scope of the invention, however, and reference is made therefore to the claims and herein for interpreting the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0020] **FIG. 1A.** TEM images of liposomes with their size distribution profiles.

[0021] **FIG. 1B.** Physicochemical properties of different liposomes before and after interactions with human plasma from patients with different diseases. DLS and Zeta-Potential data on various liposomes before interactions with human plasma and

corona complexes free from excess plasma, obtained following incubation with plasma from healthy and cancer patients (Pdi: Polydispersity index from cumulant fitting).

[0022] FIG. 1C. Classification of identified corona on sensor array elements according to their physiological functions in human plasma of healthy and patients having different types of cancers. (complement proteins are shown here as an example; other protein categories, including coagulation, tissue leakage, lipoproteins, acute phase, immunoglobulins, and other plasma proteins, are shown in FIG. 6A-F).

[0023] FIG. 2A. Predictor discovery and contribution from each individual predictor to separation of each class by PLS discrimination analysis. Predictor exploration by weighted VIP was performed by adding the ranked variables to the PLS-DA model one by one and calculating the classification error for internal cross-validation (10-fold). Decreasing the classification error led to the discovery of a minimal set of 69 predictors with the highest possible importance for separating each class from the others.

[0024] FIG. 2B. The contribution of each individual marker to separation of each class based on the PLS discrimination analysis. VIP plot ranking markers of 69 selected variables for their contribution to separation of each class from PLS discrimination analysis. VIP score >1 indicates important protein leading to good prediction of class membership, whereas variables with VIP scores <1 indicate unimportant proteins for each class. FIG. 2B depicts the results for the 69 variables for glioblastoma.

[0025] FIG. 2C. Depicts the VIP values for the 69 variables for meningioma.

[0026] FIG. 2D. Depicts the VIP values for the 69 variables for lung cancer.

[0027] FIG. 2E. Depicts the VIP values for the 69 variables for myeloma.

[0028] FIG. 2F. Depicts the VIP Values for the 69 variables for pancreatic cancer.

[0029] FIG. 3A. PLS-DA plot showing the separation of different cancerous samples from each other and from controls. PLS score-plot obtained using PLS-toolbox, projecting the objects into the subspace created by the 1st, 2nd, and 3rd latent variables of the model.

[0030] FIG. 3B. PLS-DA plot showing the separation of different cancerous samples from each other and from controls. Objects displayed where the 4th and 5th latent variables of the model are shown. As can be seen, meningioma and glioblastoma cases were not separated in three dimensions appropriately, but they can be separated in the fourth and fifth dimensions of the PLS model.

[0031] FIG. 3C. Assignment map obtained by CPANN with all variables and selected variables. (C) The assignment map obtained by training of a CPANN network (8×8 neurons) using whole data set (1823 variables). The mapping quality is not good and there are conflicts of different types of cancer in term of mapping.

[0032] FIG. 3D. Assignment map attained by training of a CPANN network (8×8 neurons) using 69 variables. High dimensional input vectors (samples) are mapped on a two-dimensional network of neurons, preserving similarity and topology. Colors indicate the similarity of a neuron to a specific type of input vector (class type). This map also demonstrates the importance of the predictor selection step and the effect of deletion of non-informative and irrelevant predictors on the model quality.

[0033] FIG. 3E. Dendrogram depicting the 51 proteins identified as capable of distinguishing among the six groups of cancer.

[0034] FIG. 3F. The 51 proteins identified as capable of distinguishing among the six groups are presented in a 'Heat Map' generated with an unsupervised cluster algorithm (Agglomerative HCA with furthest neighbor linkage). Visual inspection of both the dendrogram (FIG. 3E) and heat map (FIG. 3F) demonstrates cancer-specific protein corona signature and clear clustering of six groups of samples (five groups of cancerous samples plus normal samples) and also expected similarities among five patients from each group. The heat map also indicates substantial differences in the patterns of variables (markers) of different cancers (each column represents a patient, and each row represents a protein). Higher and lower protein levels are indicated in red and green, respectively.

[0035] FIG. 4A. Prediction of cohort samples using the proposed linear and non-linear classification models. PLS-DA plot indicates that the models can predict the class membership of cohort samples. The position and orientation of cohort samples are illustrated by the PLS-DA score plot. The PLS-DA score plot obtained by replacing absent values with zero. Model-generated predictions for five cohort samples indicate that all cohort samples belong to class 2, Glioblastoma, consistent with clinical diagnosis after 10 years.

[0036] FIG. 4B. CPANN map indicate that the models can predict the class membership of cohort samples. Assignment map obtained by CPANN (8×8) trained using only the top 69 variables. The cohort samples are also mapped and indicated by

label 'C'. Sample numbers are indicated on each neuron. The neuron color (assigned label) is decided based on the similarity between the class label (a 6x1 binary vector) and the weight vector in the output layer of the corresponding neuron. The pattern of regions for each class shows that employing all biomarkers for discrimination of different cancer types is highly recommended and that the built model has a minimal risk of classification error.

[0037] FIG. 5A. Schematic representation of study outline. Informative variable selection and classification model building.

[0038] FIG. 5B. The protein name and ID of 69 selected variables are listed. Some of the proteins were present in the protein corona of more than one liposome (DOPG, DOTAP and CHOL are denoted by color: green, red, and blue, respectively).

[0039] FIG. 5C. The disease-specific biomarkers covered as significant variables by the proposed models.

[0040] FIG. 6A. Classification of identified coronas by sensor array elements according to their physiological functions including acute phase, in human plasma of healthy subjects and patients having different types of cancers.

[0041] FIG. 6B. Classification of identified coronas by sensor array elements according to their physiological functions, including coagulation in human plasma of healthy subjects and patients having different types of cancers.

[0042] FIG. 6C. Classification of identified coronas by sensor array elements according to their physiological functions, including immunoglobulins in human plasma of healthy subjects and patients having different types of cancers.

[0043] FIG. 6D. Classification of identified coronas by sensor array elements according to their physiological functions, including lipoproteins in human plasma of healthy subjects and patients having different types of cancers.

[0044] FIG. 6E. Classification of identified coronas by sensor array elements according to their physiological functions, including tissue leakage in human plasma of healthy subjects and patients having different types of cancers.

[0045] FIG. 6F. Classification of identified coronas by sensor array elements according to their physiological functions, including other plasma proteins in human plasma of healthy subjects and patients having different types of cancers.

[0046] FIG. 7A-F. Receiver operating characteristic (ROC) plot derived from PLS-DA based on the top 69 ranked variables for five cancer and control classes. ROC

plot of sensitivity (True Positive Rate, Y-axis) versus 1 – specificity (False Positive Rate, X-axis) based on a PLS-DA built upon the 69 markers with the highest contribution for six classes.

[0047] FIG. 8A. Schematic representation of unfolding a three-way data matrix into a two-way matrix.

[0048] FIG. 8B. Assignment map obtained by CPANN (14×14) trained using 90 samples (replicates) with all 1823 variables. Sample numbers are indicated on each neuron. The neuron color (assigned label) is decided based on the similarity between the class label (a 6x1 binary vector) and the weight vector in the output layer of the corresponding neuron. Despite using all biomarkers, there are some distinct similarities between samples of the same cancer class. Replicated samples are also mapped on adjacent or the same neurons

[0049] FIG. 8C. Classification error of CPANN map was calculated at different map size using 10-fold cross validation.

[0050] FIG. 8D. The CPANN network has 69 weight layer, which is equal to the number of variables used to train the model. The i^{th} weight layer reflects the effect of the i^{th} variable (biomarker) on the pattern of the assignment map.

[0051] FIG. 8E The correlation of the assignment map and 69 weight layers (weight maps) can be calculated and could help identify the biomarkers related to each cancer class. It can also be visually decided; similarity can be monitored by absolute value of Correlation Coefficient of two maps.

[0052] FIG. 8F. The correlation of the assignment map and 69 weight layers (weight maps) can be calculated and could help identify the biomarkers related to each cancer class. It can also be visually decided; similarity can be monitored by absolute value of Correlation Coefficient of two maps.

[0053]

[0054] FIG. 9. Schematic presentation of the corona sensor array for early detection of cancers and discovery of new cross-reactive biomarkers.

[0055] FIG. 10. Depiction of gold nanoparticles used in an embodiment of the sensor array of the present invention with increasing hydrophobicity. Log P denotes relative hydrophobicity of the functional head groups. Classification of identified corona on NP1-NP6 according to their physiological functions in healthy human serum. See Saha et al. "Regulation of Macrophage Recognition through the Interplay of

Nanoparticle Surface Functionality and Protein Corona" ACS Nano 2016 10 (4), 4421-4430, incorporated by reference in its entirety.

[0056] FIG. 11. Contribution of immunoglobulins to the identified corona of three different liposomes according to their physiological functions in cancerous and healthy human plasmas.

[0057] FIG. 12A-E. Contribution from each individual marker to case-control separation from PLS discrimination analysis. Variable Importance of Projection (VIP) plot ranking markers of 158 selected variables for their contribution to case-control separation from PLS discrimination analysis. A) Glioblastoma vs. control B) Meningioma vs. control C) Pancreas vs. control D) Lung vs. control E) Myeloma vs. control. The table shows the protein names.

[0058] FIG. 13. Classification of identified protein corona on iron oxide nanoparticles (with PEG molecular weights of 300, 3000, and 6000) after interaction with cohort plasma of healthy people who later (after 8 years) ended up with myeloid leukemia, lung, or pancreas cancers and also plasma of people who remained healthy.

[0059] FIG. 14. Examples of the types of nanoscale sensor elements that can be used for the sensor arrays. Different types of nanoparticles (e.g., organic, inorganic, and polymeric nanoparticles) with various physicochemical properties (e.g., different, surface properties, sizes, and shapes) can be used as sensor elements. Sensor array can be created by minimum two elements to an unlimited number of elements.

[0060] FIG. 15. Example of one method of collection of corona coated particles in in vitro, ex vivo, and in vivo conditions. The particles get incubated with biological fluids (e.g., plasma of patients with different type of disease) and the corona coated particles get collected and stored for analysis.

[0061] FIG. 16A. Examples of conjugation of nano-object materials (with different physicochemical properties) to substrates (with different physicochemical properties) to make a protein corona sensor array chip (A) before interactions with protein source (e.g., human plasma of various disease). The specific protein corona will form on the surface of nano-objects, with different physicochemical properties. The substrates may also get coated by several types of proteins, which have negligible effects on the detection efficacy of the chip.

[0062] FIG. 16B. Examples of conjugation of nano-object materials (with different physicochemical properties) to substrates (with different physicochemical

properties) to make a protein corona sensor array chip after interactions with protein source (e.g., human plasma of various disease).

[0063] FIG. 17A. Examples of protein corona sensor array chip with nano-curvatures (produced by wide variety of available approaches like lithography and mold casting), with different physicochemical properties, before interactions with protein source (e.g., human plasma of various disease). The specific protein corona will form on the surface of nano-objects, with different physicochemical properties. The substrates may also get coated by several types of proteins, which have negligible effects on the detection efficacy of the chip.

[0064] FIG. 17B. Examples of protein corona sensor array chip with nano-curvatures (produced by wide variety of available approaches like lithography and mold casting), with different physicochemical properties after interactions with protein source (e.g., human plasma of various disease). The specific protein corona will form on the surface of nano-objects, with different physicochemical properties. The substrates may also get coated by several types of proteins, which have negligible effects on the detection efficacy of the chip.

[0065] FIG. 18A. Examples of conjugation of nano-object materials (with different physicochemical properties) to substrates (with different physicochemical properties) to make a protein corona sensor array micro/nano fluidic chip before and (b) after interactions with protein source (e.g., human plasma of various disease). The specific protein corona will form on the surface of nano-objects, with different physicochemical properties. The substrates may also get coated by several types of proteins, which have negligible effects on the detection efficacy of the chip.

[0066] FIG. 18B. Examples of conjugation of nano-object materials to substrates to make a protein corona sensor array micro/nano fluidic chip after interactions with protein source (e.g., human plasma of various disease). The specific protein corona will form on the surface of nano-objects, with different physicochemical properties.

[0067] FIG. 19A. Examples of protein corona sensor array micro/nano fluidic chip with nano-curvatures (produced by wide variety of available approaches like lithography and mold casting), with different physicochemical properties, before interactions with protein source (e.g., human plasma of various disease).

[0068] FIG. 19B. Examples of protein corona sensor array micro/nano fluidic chip with nano-curvatures (produced by wide variety of available approaches like

lithography and mold casting), with different physicochemical properties, after interactions with protein source (e.g., human plasma of various disease).

[0069] FIG. 20A. Examples of conjugation of random-ordered nano-object materials (with different physicochemical properties) to substrates (with different physicochemical properties) to make a protein corona sensor array chip (A) before interactions with protein source (e.g., human plasma of various disease).

[0070] FIG. 20B. Examples of conjugation of random-ordered nano-object materials (with different physicochemical properties) to substrates (with different physicochemical properties) to make a protein corona sensor array chip (b) after interactions with protein source (e.g., human plasma of various disease).

[0071] FIG. 21A. Examples of protein corona sensor array chip with random-ordered nano-curvatures (produced by wide variety of available approaches like lithography and mold casting), with different physicochemical properties, (A) before interactions with protein source (e.g., human plasma of various disease).

[0072] FIG. 21B. Examples of protein corona sensor array chip with random-ordered nano-curvatures with different physicochemical properties (b) after interactions with protein source (e.g., human plasma of various disease).

[0073] FIG. 22A. Examples of conjugation of random-ordered nano-object materials (with different physicochemical properties) to substrates (with different physicochemical properties) to make a protein corona sensor array micro/nano fluidic chip (A) before interactions with protein source (e.g., human plasma of various disease).

[0074] FIG. 22B. Examples of conjugation of random-ordered nano-object materials to substrates to make a protein corona sensor array micro/nano fluidic chip (b) after interactions with protein source (e.g., human plasma of various disease).

[0075] FIG. 23A. Examples of protein corona sensor array micro/nano fluidic chip with random-ordered nano-curvatures (produced by wide variety of available approaches like lithography and mold casting), with different physicochemical properties, (A) before interactions with protein source (e.g., human plasma of various disease).

[0076] FIG. 23B. Examples of protein corona sensor array micro/nano fluidic chip with random-ordered nano-curvatures with different physicochemical properties after interactions with protein source (e.g., human plasma of various disease).

[0077] FIG. 24. Example of conjugation of nanoparticles (NPs) to the silica

substrate surface (as representative substrate) via the amidation reaction between the amino groups on silica substrate surface and carboxylic acid groups on NP surface.

[0078] FIG. 25. Example of conjugation of NPs to the silica substrate surface (as representative substrate) via the ring-opening reaction between the epoxy groups on silica substrate surface and amino groups on NP surface.

[0079] FIG. 26. Example of conjugation of NPs to the silica substrate surface (as representative substrate) via the Michael Addition reaction between the maleimide groups on silica substrate surface and thiol or amino groups on NP surface.

[0080] FIG. 27. Example of conjugation of NPs to the silica substrate surface (as representative substrate) via the urethane reaction between the isocyanate groups on silica substrate surface and hydroxyl or amino groups on NP surface.

[0081] FIG. 28. Example of conjugation of NPs to the silica substrate surface (as representative substrate) via the oxidation reaction between the thiol groups on silica substrate surface and the ones on NP surface.

[0082] FIG. 29. Example of conjugation of NPs to the silica substrate surface (as representative substrate) via the “Click” chemistry between azide groups on silica substrate surface and alkyne groups on NP surface.

[0083] FIG. 30. Example of conjugation of NPs to the silica substrate surface (as representative substrate) via the thiol exchange reaction between 2-pyridyldithiol groups on silica substrate surface and thiol groups on NP surface.

[0084] FIG. 31. Example of conjugation of NPs to the silica substrate surface (as representative substrate) via the coordination reaction between boronic acid groups on silica substrate surface and diol groups on NP surface.

[0085] FIG. 32. Example of conjugation of NPs to the silica substrate surface (as representative substrate) via the UV light-irradiated addition reaction between C=C bonds on silica substrate surface and C=C bonds on NP surface.

[0086] FIG. 33. Example of conjugation of NPs to the gold substrate surface (as representative substrate) via Au-thiol bonds.

[0087] FIG. 34. Example of conjugation of NPs to the gold substrate surface (as representative substrate) via the amidation reaction between the carboxylic acid groups on gold substrate surface and the amino groups on NP surface.

[0088] FIG. 35. Example of conjugation of NPs to the gold substrate surface (as representative substrate) via “Click” chemistry between the azide groups on gold

substrate surface and the alkyne groups on NP surface.

[0089] FIG. 36. Example of conjugation of NPs to the gold substrate surface (as representative substrate) via urethane reaction between the NHS groups on gold substrate surface and the amino groups on NP surface.

[0090] FIG. 37. Example of conjugation of NPs to the silica substrate surface (as representative substrate) via the ring-opening reaction between the epoxy groups on gold substrate surface and amino groups on NP surface.

[0091] FIG. 38. Example of conjugation of NPs to the gold substrate surface (as representative substrate) via the coordination reaction between boronic acid groups on silica substrate surface and diol groups on NP surface.

[0092] FIG. 39. Example of conjugation of NPs to the gold substrate surface (as representative substrate) via the UV light-irradiated addition reaction between C=C bonds on gold substrate surface and C=C bonds on NP surface.

[0093] FIG. 40. Example of conjugation of NPs to the gold substrate surface (as representative substrate) via the "Ligand-Receptor" interaction between biotin on gold substrate surface and avidin on NP surface.

[0094] FIG. 41. Example of conjugation of NPs to the gold substrate surface (as representative substrate) via the "Host-Guest" interaction between α -cyclodextrin (α -CD) on gold substrate surface and adamantine (Ad) on NP surface.

[0095] FIG. 42. Dissociation of proteins from the surface of nanoparticles and their corona composition analysis. Analysis of the protein corona sensor array data with supervised and unsupervised approaches to identify and discriminate diseases.

[0096] FIG. 43 Example of sensor array with random order nanoscale sensor elements for fluorescence or luminescence readout.

[0097] FIG. 44. Example of sensor array with order nanoscale sensor elements for fluorescence or luminescence readout.

DETAILED DESCRIPTION OF THE INVENTION

[0098] The present invention has been described in terms of one or more preferred embodiments, and it should be appreciated that many equivalents, alternatives, variations, and modifications, aside from those expressly stated, are possible and within the scope of the invention.

[0099] The present invention provides sensor arrays and methods of use for the

prognosis, diagnosis and detection of a disease state in a subject. The sensor array of the present invention differ from known sensor arrays that involve individual sensors that detect specific biomolecules. In the present sensor array, the biomolecules do not have to be known, as the system does not rely on the presence or absence of a specific biomolecules or amounts of specific disease markers. This new sensor array is able to detect changes in the compositions of the biomolecule corona associated with the different sensor elements. This ability to detect relative changes or patterns (either the actual biomolecules associated with the different sensor elements or in the amounts and/or conformations of each biomolecule associated with each sensor element) allows for determining a unique biomolecule fingerprint for each array. This biomolecule fingerprint can stratify different health and disease states of subjects. In some embodiments, the biomolecular fingerprint is not only able to differentiate between healthy subjects and subjects in various different stages of a disease or disorder but also to determine a pre-disease state in a subject where the subject will develop the disease or disorder at a later time. This is significantly different and novel over systems in the art that measure or detect specific biomarkers associated with a disease or disorder to provide a predisposition (e.g. a chance or likelihood) of developing the disease. The present sensor and methods is able to detect a disease before any signs or symptoms, in other words, can pre-diagnose the disease before any specific signs or symptoms appear.

[00100] The uniqueness of the present invention is the combination of this recognition of a biomolecular fingerprint from a sample from a subject and the ability to determine a disease state for that subject on a continuum of health.

[00101] The present invention is based on work by the inventors that have shown that the surface of sensor elements, e.g. nanoparticles, is rapidly covered with a layer of different biomolecules, including proteins, to form a "biomolecule corona" when contacted with a biological sample. The type, amount, and categories of the biomolecules that make up these biomolecule corona 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.

[00102] 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. Embodiments of the invention will be discussed in more detail below.

[00103] The present invention provides sensor arrays comprising, consisting essentially of or consists of a plurality of nanoscale sensor elements wherein the plurality of nanoscale sensor elements differ from each other in at least one physiocochemical property. In some embodiments, each nanoscale sensor element is able to bind a plurality of biomolecules in a sample to produce a biomolecule corona signature. In some embodiments, each nanoscale sensor elements has a distinct biomolecule corona signature.

[00104] The plurality of nanoscale 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 nanoscale sensor elements.

[00105] As used herein, the term "nanoscale sensor element" and "sensor element" may be used interchangeably and refer to elements that are able to bind to a plurality of biomolecules when in contact with a sample.

[00106] The term "plurality of nanoscale sensor elements" refers to more than one, for example, at least two nanoscale sensor elements. In some embodiments, the plurality of nanoscale elements includes at least two nanoscale elements to at least 1000 nanoscale elements, preferably about two nanoscale elements to about 100 nanoscale elements. In suitable embodiments, the array comprises at least two to at least 100 nanoscale elements, alternatively at least two to at least 50 nanoscale elements, alternatively at least 2 to 30 nanoscale elements, alternatively at least 2 to 20 nanoscale elements, alternatively at least 2 to 10 nanoscale elements, alternatively at least 3 to at least 50 nanoscale elements, alternatively at least 3 to at least 30 nanoscale elements, alternatively at least 3 to at least 20 nanoscale elements, alternatively at least 3 to at least 10 nanoscale elements, alternatively at least 4 to at least 50 nanoscale elements, alternatively at least 4 to at least 30 nanoscale elements, alternatively at least

4 to at least 20 nanoscale elements, alternatively at least 4 to at least 10 nanoscale elements, and including any number 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, etc.).

[00107] As used herein, the term "biomolecule corona" refers to the plurality of different biomolecules that are able to bind to a sensor element. The term "biomolecule corona" encompasses "protein corona" which is a term used in the art to refer to the proteins, lipids and other plasma components that bind 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 the art, see, e.g., 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, Jan 2016 pp. 295-304, Mahmoudi et al. "Emerging understanding of the protein corona at the nano-bio interfaces" *Nanotoday* 11(6) Dec. 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 in the art, adsorption curve shows 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) exhibits dynamic exchange. Proteins that adsorb with high affinity form what is known as the "hard" corona, consisting of tightly bound proteins that do not readily desorb, and proteins that adsorb with low affinity form the "soft" corona, consisting of loosely bound proteins. Soft and hard corona can also be defined based on their exchange times. Hard corona usually shows 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.

[00108] The term "biomolecule corona signature" refers to the composition, signature or pattern of different biomolecules that are bound to each separate sensor element. The signature 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 sensor element. 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, 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, but also the nature of the sample and the duration of exposure.

[00109] In some embodiments, the biomolecule corona signature comprises the biomolecules found in a soft corona and a hard corona

[00110] 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 corona signatures of each 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 confirmation of the biomolecule that binds to each sensor element.

[00111] In some embodiments, the sensor array comprises, consists essentially of or consists of a first nanoscale sensor element that produces a first biomolecule corona signature and at least one second nanoscale sensor element that produces at least one second biomolecule corona signature when the sensor array is contacted with a biological sample. A biomolecule fingerprint is the combination of the first biomolecule signature and the at least one second biomolecule signature. It is contemplated that the biomolecule signature can be made from at least two biomolecule corona signatures to as many different biomolecule signatures are assayed, e.g. at least 100 different biomolecule corona signatures. The biomolecule corona may be assayed separately for each sensor element and combined to determine the biomolecule fingerprint or the y can be assayed at the same time to develop the biomolecule signature at once.

[00112] The term "sample" refers to a biological sample obtained from a subject. Suitable biological samples include, but are not limited to, biological fluids, including, but not limited to, systemic blood, plasma, serum, lung lavage, cell lysates, menstrual blood, urine, processed tissue samples, amniotic fluid, cerebrospinal fluid, tears, saliva,

semen and the like. In a preferred embodiment, the sample is a blood or serum sample. Blood plasma contains several thousands of different proteins with twelve orders of magnitude differences in the concentrations of these proteins. The present sensor array is able to detect changes within these blood samples over time or over disease states of the subject.

[00113] In some embodiments, the biological fluid are prepared by methods and kits known in the art. For example, some biological samples (e.g. menstrual blood, blood samples, semen, etc.) may first be centrifuged at low speed to remove cell debris, blood clots and other cellular components that may interfere with the array. In other embodiments, for example, tissue specimens may be processed, e.g. tissue samples may be minced or homogenized, treated with enzymes to break up the tissue and/or centrifuged to remove cellular debris allowing for the assaying and extraction of the biomolecules within the tissue samples. Suitable methods of isolating and/or properly preparing and storing blood samples are known in the art, and may include, but are not limited, the addition of an anti-coagulant agent.

[00114] Suitable nanoscale sensor elements include, but are not limited to, for example, particles, such as organic particles, non-organic particles or combinations thereof. In some embodiments the particles are, for example, nanoparticles, microparticles, micelles, liposomes, iron oxide, graphene, silica, protein-based particles, polystyrene, silver, and gold particles, quantum dots, palladium, platinum, titanium, and combinations thereof. One skilled in the art would be able to select and prepare suitable particles. Suitable nanoscale sensor elements are less than 1 micron in at least one direction. In some aspects, the nanoscale sensor elements are less than about 100 nm in at least one direction.

[00115] In some embodiments, the plurality of nanoscale sensor elements comprises, consists essentially of, or consists of a plurality of particles, wherein each particle is differentiated for each other by at least one physicochemical property such that each nanoscale sensor element has a unique biomolecule corona signature when placed in contact with the same sample.

[00116] The physicochemical property of the nanoscale sensor element found in an array refer to, for example, the composition, size, surface charge, hydrophobicity, hydrophilicity, surface functionality (surface functional groups), surface topography, surface curvature and shape. The term composition encompasses the use of different

types of materials and differences in the chemical and/or physical properties of materials, for example, conductivity of the material chosen between the nanoscale sensor elements.

[00117] Surface curvature is generally determined by the nanoparticle size. Thus, at a nanometer scale, as the size of the nanoparticles changes, the surface curvature of the particle changes, and this change of the surface curvature affects the binding selectivity of the surface. For example, at certain curvature, the surface of the particle may have a binding affinity for a specific type of biomolecule where a different curvature will have a different binding affinity and/or a binding affinity for a different biomolecule. The curvature can be adjusted to create a plurality of sensor elements with altered affinity for different biomolecules.

[00118] Surface morphology may also be modified by methods such as patterning the surface to provide different affinities, engineering surface curvatures on multiple length scales and the like. Patterning the surface is provided by, for example, forming the sensor elements by block polymerization in which the at least two blocks have different chemistries, forming the nanoparticles using mixtures of at least two different polymers and phase separating the polymers during polymerization, and/or cross-linking the separate polymers following phase separation. Engineered surface curvature on multiple length scales is provided, for example, by employing Pickering emulsions (Sacanna et al. 2007) stabilized by finely divided particles for the synthesis of nanoparticles. In some embodiments, finely divided particles are selected from, for example, silicates, aluminates, titanates, metal oxides such as aluminum, silicon, titanium, nickel, cobalt, iron, manganese, chromium, or vanadium oxides, carbon blacks, and nitrides or carbides, e.g., boron nitride, boron carbide, silicon nitride, or silicon carbide, among others.

[00119] For example, the nanoscale sensor elements may each be functionalized to have different physicochemical properties. Suitable methods of functionalizing the nanoscale sensor elements are known in the art and depend on composition of the nanoscale sensor element (e.g. gold, iron oxide, silica, silver, etc.), and include, but not limited to, for example aminopropyl functionalized, amine functionalized, boronic acid functionalized, carboxylic acid functionalized, methyl functionalized, *N*-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.

[00120] In other embodiments, 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 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 nanoparticles, 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.

[00121] In some embodiments, the particles are liposomes. Suitable liposomes are known in the art and include, but are not limited to, for example, DOPG (1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol), DOTAP(1,2-Dioleoyl-3-trimethylammonium-propane)- DOPE (dioleoylphosphatidylethanolamine), CHOL (DOPC-Cholesterol), and combinations thereof.

[00122] Suitable liposomes may be solid lipid nanoparticles (SLN) which can be made of solid lipid, emulsifier and/or water/solvent. SLN may include, but are not limited to, a combination of the following ingredients: triglycerides (tri-stearin), partial glycerides (Imwitor), fatty acids (stearic acid, palmitic acid), and steroids (cholesterol) and waxes (cetyl palmitate). Various emulsifiers and their combination (Pluronic F 68, F 127) have been used to stabilize the lipid dispersion. Suitable ingredients for the use in preparing SNL sensor elements include, but are not limited to, e.g., phospholipids, glycerol, poloxamer 188, soy phosphatidyl choline, Compritol, cetyl palmitate, PEG 2000, PEG 4500, Tween 85, ethyl oleate, Na alginate, ethanol/butanol, tristearin glyceride, PEG 400, isopropyl myristate, Pluronic F68, Tween 80, trimyristin, tristearin, trilaurin, stearic acid, glyceryl caprate as Capmul®MCM C10, theobroma oil, triglyceride coconut oil, 1-octadecanol, glycerol behenate as Compritol® 888 ATO, glycerol palmitostearate as Precirol® ATO 5, and cetyl palmitate wax and the like.

[00123] In some embodiments, the plurality of sensor elements comprise, consist essentially of, or consist of a plurality of half particles of different geometric shapes which can be made by molding technology, 3D printing or 4D printing. Suitable half particles are known in the art, and include, but are not limited to half and partial particles in any geometric shape, for example, spheres, rods, cubes and combinations

thereof. Suitably, in one embodiment, the plurality of half particles have different physicochemical properties made by 3-D printing.

[00124] In some embodiments, the nanoscale sensor elements are made by 3D or 4 D printing. Suitable methods of 3D and 4D printing of nanoscale sensor elements are known in the art. Suitable material for 3D and 4D printing include, but is not limited to, e.g., plastics and synthetic polymers (e.g., poly-ethylene glycol-diacrylate (PEG-DA), poly(e-caprolactone) (PCL), poly(propylene oxide (PPO), poly(ethylene oxide) (PEO) etc.), metals, powders, glass, ceramics, and hydrogels. Suitable shapes made by 3D or 4D printing include, but are not limited to, for example, full or partial spheres (e.g. 3/4 or half spheres), rods, cubes, or other geometrical or non-geometrical shapes.

[00125] 3D printing techniques include, but are not limited to, microextrusion printing, inkjet bioprinting, laser-assisted bioprinting, stereolithography, omnidirectional printing, and stamp printing.

[00126] In some embodiments, the nanoscale sensor elements are nanoparticles. Suitable nanoparticles are known in the art and include, but are not limited to, for example, natural or synthetic polymers, copolymers, terpolymers (with the cores being composed of metals or inorganic oxides, including magnetic cores). Suitable polymeric nanoparticles include, but are not limited to, e.g., polystyrene; poly(lysine), chitosan, dextran, poly(acrylamide) and its derivatives such as N-isopropylacrylamide, N-tertbutylacrylamide, N,N-dimethylacrylamide, polyethylene glycol, poly(vinyl alcohol), gelatin, starch, degradable (bio)polymers, silica and the like.

[00127] In various embodiments, 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. In these methods, the first or second functional groups can include an amine group, a maleimide group, a hydroxyl group, a carboxyl group, a pyridylthiol group, or an azide group.

[00128] In certain embodiments, the nanoparticles can comprise polymers that include, for example, a sodium polystyrene sulfonate (PSS), polyethylene oxide (PEO), polyoxyethylene glycol, polyethylene glycol (PEG), polyethylene imine (PEI), polylactic acid, polycaprolactone, polyglycolic acid, poly(lactide-co-glycolide polymer (PLGA), cellulose ether polymer, polyvinylpyrrolidone, vinyl acetate, polyvinylpyrrolidone-vinyl acetate copolymer, polyvinyl alcohol (PVA), acrylate, polyacrylic acid (PAA), vinyl acetate, crotonic acid copolymers, polyacrylamide, polyethylene phosphonate, polybutene phosphonate, polystyrene, polyvinylphosphonate, polyalkylene, carboxy vinyl polymer, sodium alginate, carrageenan, xanthan gum, gum acacia, Arabic gum, guar gum, pullulan, agar, chitin, chitosan, pectin, karaya gum, locust bean gum, maltodextrin, amylose, corn starch, potato starch, rice starch, tapioca starch, pea starch, sweet potato starch, barley starch, wheat starch, hydroxypropylated high amylose starch, dextrin, levan, elsinan, gluten, collagen, whey protein isolate, casein, milk protein, soy protein, keratin, or a gelatin, or a copolymer, derivative, or mixture thereof.

[00129] In other embodiments, the polymer can be or include a polyethylene, polycarbonate, polyanhydride, polyhydroxyacid, polypropylfumerate, polycaprolactone, polyamide, polyacetal, polyether, polyester, poly(orthoester), polycyanoacrylate, polyvinyl alcohol, polyurethane, polyphosphazene, polyacrylate, polymethacrylate, polycyanoacrylate, polyurea, polystyrene, or a polyamine, or a copolymer, derivative, or mixture thereof.

[00130] In some embodiments, the sensor array comprises a substrate. Regardless of the identity of the nanoscale sensor element, this invention can be embodied by a matrix of nanoscale sensor elements immobilized on, connected with and/or coupled to a solid substrate. The substrate may comprise, consist essentially of or consist of polydimethylsiloxane (PDMS), silica, gold or gold coated substrate, silver or silver coated substrate, platinum or platinum coated substrate, zinc or zinc coated substrate, carbon coated substrate and the like. One skilled in the art would be able to select an appropriate substrate for the sensor array. In some embodiments, the sensor elements and the substrate are made of the same element, for example, gold. In some embodiments, the substrate and nanoparticles form a chip.

[00131] In some embodiments, the nanoscale sensor elements are non-covalently attached to the substrate. Suitable methods of non-covalent attachment are known in the art and include, but are not limited to, for example, metal coordination, charge

interaction, hydrophobic-hydrophobic interaction, chelation and the like. In other embodiments, the nanoscale sensor elements are covalently attached to the substrate. Suitable methods of covalently linking the sensor elements and the substrates include, but are not limited to, for example, click chemistry, irradiation, and the like.

[00132] For purposes of illustration only, methods of attaching the nanoscale sensor elements to substrates is demonstrated in Figures 24-41. For example, sensor elements may be conjugated to a substrate (e.g. silica substrate) via the amidation reaction between the amino groups on silica substrate surface and carboxylic acid groups on NP surface (FIG. 24), via the ring-opening reaction between the epoxy groups on silica substrate surface and amino groups on NP surface (FIG. 25), via the Michael Addition reaction between the maleimide groups on silica substrate surface and thiol or amino groups on NP surface (FIG. 26), via the urethane reaction between the isocyanate groups on silica substrate surface and hydroxyl or amino groups on NP surface (FIG. 27), via the oxidation reaction between the thiol groups on silica substrate surface and the ones on NP surface (FIG. 28), via the “Click” chemistry between azide groups on silica substrate surface and alkyne groups on NP surface (FIG. 29), via the thiol exchange reaction between 2-pyridyldithiol groups on silica substrate surface and thiol groups on NP surface (FIG. 30), via the coordination reaction between boronic acid groups on silica substrate surface and diol groups on NP surface (FIG. 31), via the UV light-irradiated addition reaction between C=C bonds on substrate surface and C=C bonds on NP surface (FIG. 32) and the like. Suitable methods of conjugating sensor elements to gold substrate are known in the art and include, for example, conjugation via Au-thiol bonds (FIG. 33), via the amidation reaction between the carboxylic acid groups on gold substrate surface and the amino groups on NP surface (FIG. 34), via “Click” chemistry between the azide groups on gold substrate surface and the alkyne groups on NP surface (FIG. 35), via urethane reaction between the NHS groups on gold substrate surface and the amino groups on NP surface (FIG. 36), via the ring-opening reaction between the epoxy groups on gold substrate surface and amino groups on NP surface (FIG. 37), via the coordination reaction between boronic acid groups on silica substrate surface and diol groups on NP surface (FIG. 38), via the UV light-irradiated addition reaction between C=C bonds on gold substrate surface and C=C bonds on NP surface (FIG. 39), via the “Ligand-Receptor” interaction between biotin on gold substrate surface and avidin on NP surface (FIG. 40), via the “Host-Guest” interaction

between α -cyclodextrin (α -CD) on gold substrate surface and adamantine (Ad) on NP surface (FIG. 41), and the like.

[00133] In another example, so-called “click chemistry” can be used to attach the functional surface groups to the core structures of the nanoparticles (see, e.g., the Sigma Aldrich catalog and U.S. Pat. No. 7,375,234, which are both incorporated herein by reference in their entireties). Of the reactions comprising the click chemistry field, one example is the Huisgen 1,3-dipolar cycloaddition of alkynes to azides to form 1,4-disubstituted-1,2,3-triazoles. The copper (I)-catalyzed reaction is mild and very efficient, requiring no protecting groups, and requiring no purification in many cases. The azide and alkyne functional groups are generally inert to biological molecules and aqueous environments. The triazole has similarities to the ubiquitous amide moiety found in nature, but unlike amides, is not susceptible to cleavage. Additionally, they are nearly impossible to oxidize or reduce.

[00134] The plurality of nanoscale sensor elements may be attached to the substrate randomly or in a distinct pattern. The nanoscale sensor elements may be substantially uniformly positioned. The pattern of the arranged sensor elements may vary according to the pattern in which the sensor elements are attached to the substrate. Each nanoscale sensor element is separated by a distance. The distance between the nanoparticles arranged on the substrate may vary depending on the length of the linker used to attach or other fabrication conditions. According to various embodiments, the plurality of sensor elements on the array can be fabricated having a desired inter-element distance and pattern. Suitable distinct patterns are known in the art, including, but not limited to, parallel lines, squares, circles, triangles and the like. Further, the nanoscale sensor elements may be arranged in rows, or columns. In some embodiments, the substrate is a flat substrate, in other embodiments, the substrate is in the form of microchannels or nanochannels. For illustrative purposes only, suitable embodiments are described in FIG. 16-23. The nanoscale sensor elements may be contained within microchannels or nanochannels that restrict or control the flow of the sample through the sensor array. Suitable microchannels can range from 10 μm to about 100 μm in size.

[00135] In some embodiments, a channel is formed by lithography, etching, embossing, or molding of a polymeric surface. In general, the fabrication process may involve one or more of any of these processes, and different parts of the array may be

fabricated using different methods and assembled or bonded together.

[00136] Lithography involves use of light or other form of energy such as electron beam to change a material. Typically, a polymeric material or precursor (e.g. photoresist, a light-resistant material) is coated on a substrate and is selectively exposed to light or other form of energy. Depending on the photoresist, exposed regions of the photoresist either remain or are dissolved in subsequent processing steps known generally as "developing." This process results in a pattern of the photoresist on the substrate. In some embodiments, the photoresist is used as a master in a molding process. In some embodiments, a polymeric precursor is poured on the substrate with photoresist, polymerized (i.e. cured) and peeled off.

[00137] In some embodiments, the photoresist is used as a mask for an etching process. For example, after patterning photoresist on a silicon substrate, channels can be etched into the substrate using a deep reactive ion etch (DRIE) process or other chemical etching process known in the art (e.g. plasma etch, KOH etch, HF etch, etc.). The photoresist is removed, and the substrate is bonded to another substrate using one of any bonding procedures known in the art (e.g. anodic bonding, adhesive bonding, direct bonding, eutectic bonding, etc.). Multiple lithographic and etching steps and machining steps such as drilling may be included as required.

[00138] In some embodiments, a polymeric substrate may be heated and pressed against a master mold for an embossing process. The master mold may be formed by a variety of processes, including lithography and machining. The polymeric substrate is then bonded with another substrate to form channels and/or a mixing apparatus. Machining processes may be included if necessary.

[00139] In some embodiments, a molten polymer or metal or alloy is injected into a suitable mold and allowed to cool and solidify for an injection molding process. The mold typically consists of two parts that allow the molded component to be removed. Parts thus manufactured may be bonded to result in the substrate.

[00140] In some embodiments, sacrificial etch may be used to form channels. Lithographic techniques may be used to pattern a material on a substrate. This material is covered by another material of different chemical nature. This material may undergo lithography and etch processes, or other machining process. The substrate is then exposed to a chemical agent that selectively removes the first material. Channels are formed in the second material, leaving voids where the first material was present before

the etch process.

[00141] In some embodiments, microchannels are directly machined into a substrate by laser machining or CNC machining. Several layers thus machined may be bonded together to obtain the final substrate.

[00142] In some embodiments, the width or height of each channel ranges from approximately 1 μm to approximately 1000 μm . In some embodiments, the width or height of each channel ranges from approximately 5 μm to approximately 500 μm . In some embodiments, the width or height of each channel ranges from approximately 10 μm to approximately 100 μm . In some embodiments, the width or height of each channel a ranges from approximately 25 μm to approximately 100 μm . In some embodiments, the width or height of each channel ranges from approximately 50 μm to approximately 100 μm . In some embodiments, the width or height of each channel ranges from approximately 75 μm to approximately 100 μm . In some embodiments, the width or height of each channel ranges from approximately 10 μm to approximately 75 μm . In some embodiments, the width or height of each channel ranges from approximately 10 μm to approximately 50 μm . In some embodiments, the width or height of each channel ranges from approximately 10 μm to approximately 25 μm .

[00143] In some embodiments, the maximum width or height of a channel is approximately 1 μm , approximately 5 μm , approximately 10 μm , approximately 20 μm , approximately 30 μm , approximately 40 μm , approximately 50 μm , approximately 60 μm , approximately 70 μm , approximately 80 μm , approximately 90 μm , approximately 100 μm , approximately 250 μm , approximately 500 μm , or approximately 1000 μm .

[00144] In some embodiments, the width of each channel ranges from approximately 5 μm to approximately 100 μm . In some embodiments, the width of a channel is approximately 5 μm , approximately 10 μm , approximately 15 μm , approximately 20 μm , approximately 25 μm , approximately 30 μm , approximately 35 μm , approximately 40 μm , approximately 45 μm , approximately 50 μm , approximately 60 μm , approximately 70 μm , approximately 80 μm , approximately 90 μm , or approximately 100 μm . I

[00145] In some embodiments, the height of each channel ranges from approximately 10 μm to approximately 1000 μm . In some embodiments, the height of a channel is approximately 10 μm , approximately 100 μm , approximately 250 μm , approximately 400 μm , approximately 500 μm , approximately 600 μm , approximately

750 μm , or approximately 1000 μm . In specific embodiments, the height of the channel(s) through which the sample flows is approximately 500 μm . In specific embodiments, the height of the channel(s) through which the sample flows is approximately 500 μm .

[00146] In some embodiments, the length of each channel ranges from approximately 100 μm to approximately 10 cm. In some embodiments, the length of a channel is approximately 100 μm , approximately 1.0 mm, approximately 10 mm, approximately 100 mm, approximately 500 mm, approximately 600 mm, approximately 700 mm, approximately 800 mm, approximately 900 mm, approximately 1.0 cm, approximately 1.1 cm, approximately 1.2 cm, approximately 1.3 cm, approximately 1.4 cm, approximately 1.5 cm, approximately 5 cm, or approximately 10 cm. In specific embodiments, the length of the channel(s) through which the sample flows is approximately 1.0 cm. In specific embodiments, the length of the channel(s) through which the sample flows is approximately 1.0 cm.

[00147] In some embodiments, the biomolecule fingerprint is associated with the development of a disease or disorder and/or is able associated with a disease state of the subject.

[00148] In some embodiments, the biomolecule fingerprint is able to determine a disease state for a subject. The term "disease state" for a subject as used herein refers to the ability of sensor array of the present technology to be able to differentiate between the different states of a disease within a subject. This term encompasses a pre-disease state or precursor state of a disease or disorder (a state in which the subject may not have any outward signs or symptoms of the disease or disorder but will develop the disease or disorder in the future) and a disease state in which the subject has a stage of the disease or disorder (e.g., an early, intermediate or late stage of the disease or disorder). In other words, the disease state is a spectrum that encompasses a continuum regarding the health of a subject with respect to a disease or disorder. The array of the present invention is able to distinguish different diseases states for a subject by determining a biomolecule fingerprint which can be compared to a differing biomolecule fingerprints that are associated with different disease states on the spectrum and to healthy subjects. In another example, the biomolecule fingerprint may be associated with a pre-disease state or precursor disease state, in which the subject appears healthy at the time with no outward signs or symptoms of a disease, but will

develop the disease in the future. In another example, the biomolecule fingerprint may indicate that the subject has the disease, and is able to distinguish if the disease is in the early, intermediate or late stages by the unique biomolecule fingerprint associated with each stage.

[00149] As discussed above, the disease state also includes a precursor state of a disease or disorder. This precursor state is a state in which the subject does not have any outward signs or symptoms of the disease or disorder (although there may be submacro changes within the biomolecules of the subject found in their blood or other biological fluids) but will develop the disease or disorder in the future. This precursor state can also be described as a state in which the first pathological changes of a disease are seen, e.g. changes in the biomolecule fingerprint of a biological sample from the patient.

[00150] The array can also determine a specific biomolecule fingerprint associated with a healthy, non-disease state, where the subject will not develop the disease in the future. In this case, the subject has no evidence at the time of the test that they either have the disease or will develop the disease in the future.

[00151] The innovation in this sensor arrays and associated methods differ from the current methods of detecting or measuring the presence or absence or levels of certain biomarkers to predict if a subject may have a pre-disposition or likelihood of developing a disease or disorder at very early stages of the disease, before any signs or symptoms can be monitored can be assayed. Suitably, the array is able to differentiate the health of the subject from no disease or disorder, having a precursor of a disease or disorder, and having the disease or disorder. However, the invention is not limited to those embodiments and covers the spectrum of other disease states that may occur within the continuum of health and disease of a subject.

[00152] Further, the innovation of the present invention sensor arrays can be demonstrated by the following example. The sensor array of the present invention are very sensitive and able to detect not only changes in small amounts of biomolecules within a sample, but rely on the interactions between the biomolecules. For example, not to be bound by any theory but in order to illustrate the uniqueness of the present sensory array, a theoretical example is described. If, for example, biological samples are collected from a subject over time (e.g., before any signs and symptoms of the disease, pre-disease state and during early and intermediate stages of a disease). In

these samples, by just measuring a level of a biomolecule X in the sample (e.g. quantitation of amount) it may be found that the concentration does not alter over the different disease states. Thus, measuring biomolecule X would not be a useful marker for the disease. However, using the present sensor array, although the level of biomarker X may be the same, the interaction of biomolecule X with other biomolecules, Y and Z, may change the composition of the protein corona signature associated with the sample over time and samples. For example, biomarker X may change its association from associating with biomolecule Y to biomolecule Z, or over time interaction of X with Y and Z leads to a conformational change in X. This type of changes, that does not change the overall concentration but will change the unique biomolecule fingerprint associated with the disease state (that includes biomolecule X) would allow for the distinction and association with other disease states. This would not be discovered if you use methods of the art of just measuring the quantitation of biomolecule X within the sample, as this level stayed the same throughout the entire disease. This is an unexpectedly highly specific and useful assay not seen before. With this assay, biomarkers of a disease that would not previously been characterized as biomarkers can be determined including, patterns of biomarkers, as these marker may not change in absolute amounts within the samples, but will have a consistent and measurable change with regard to the interaction with the nanoscale sensor elements in the sensor array.

[00153] The present sensor array may be used in a variety of methods described herein. The ability to determine a unique biomolecule fingerprint for samples provides a novel and innovative means to measure a specific disease state in a subject. These biomolecule fingerprints can be used to determine the disease state of a subject, diagnosing or prognosing a disease in a subject or identifying 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 discussed above, 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. Therefore, the sensor array of the present invention provides a unique opportunity to be able to develop biomolecule fingerprints associated with the pre-stages or precursor states of the disease.

[00154] It is understood that even before a disease has progressed into showing any measurable signs or symptoms, that at the macroscopic level, there are changes taking place within the body and biological systems of a subject. Being able to recognize these early pre-disease signs contemplated by use of the sensor array of the present invention. The inventors have found that by comparing the biomolecule fingerprint of a subject during different times of a disease state (e.g. before a disease has shown symptoms and developed, after early signs or symptoms of the disease, and/or at late stages of the disease) provides a unique biomolecule fingerprint linked to the different disease states linked to the disease progression. In other words, a biomolecule fingerprint can be determined that would allow one to be able to identify subjects that are going to develop a disease at a later time. This would allow for early monitoring and early treatment, greatly improving the outcome of the subject diagnosed with the disease.

[00155] 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.

[00156] The step of determining a biomolecule fingerprint associated with the sample may comprise detecting the biomolecule corona signature for at least two nanoscale 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 nanoscale 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 nanoscale elements are assayed at the same time or in the same sample.

[00157] In some embodiments, the method of determining the biomolecule fingerprint comprises detecting and determining the biomolecular corona signatures of

the at least two nanoscale sensor elements. In some embodiments, 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. Depending on the design of the array, in some instances the composition of each biomolecule corona signature of each nanoscale sensor element is assayed independently, and the results are combined to produce the biomolecule fingerprint (e.g. each nanoscale 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). In another embodiment, the at least two nanoscale sensor elements are on the same array 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. This later method would be the method of choice if using a chip array technology.

[00158] Methods of assaying the plurality of biomolecules that make up the biomolecule corona signature or the biomolecule fingerprint are known in the art and 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 a preferred embodiment, the assay is by liquid chromatography, mass spectrometry or a combination thereof.

[00159] In a preferred embodiment, the sensor assay is a non-label array.

[00160] In some embodiments, it is contemplated that labelled arrays may be used, wherein a corona signature is able to be determined as a change in signal (e.g. fluorescence, luminescence, charge, colorimetric dyes). Suitable example is shown in FIG. 42 and 43. For example, an array may include chemically responsive colorants in a printable formulation for detection and identification of biomolecules based on the equilibrium interactions with the biomolecules and responsive dyes.

[00161] In some embodiments, the nanoscale sensor element comprises 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 the biomolecule corona, it will effect 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 nanoscale sensor element can be measured and compared to the background fluorescence to produce the biomolecule fingerprint.

[00162] The 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.

[00163] The term "cancer" and "tumor" as used herein interchangeably and are meant to encompass any cancer, neoplastic and preneoplastic disease that is characterized by abnormal growth of cells. Cancer may, for example, be 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, cystadenocarcinoma, 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, craniopharyngiomas, 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 a preferred embodiment, 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, and neuroendocrine cancer.

[00164] 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.

[00165] 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.

[00166] 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.

[00167] For illustrative purposes, in one embodiment the sensor arrays may distinguish the different stages of atherosclerosis, including, but not limited to, the different degrees of stenosis in a subject.

[00168] 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.

[00169] 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.

[00170] 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.

[00171] The sensor arrays and methods of the present invention in some embodiments are able to detect the early stages of a disease or disorder. Early stages of the disease refers to when the first signs or symptoms of a disease may manifest within a subject. Usually diseases able to be caught in either pre-disease development or in the early states are easier to treat and provide a more positive outcome for the patient. For example, for cancer, the early stages of a disease may include stage 0 and stage 1 cancer. Stage 0 cancer describes the cancer in situ, which means "in place" signifying that the cancer is still located in the place it started and have not spread to nearby tissues. This stage of cancer is often highly curable, usually by removing the entire tumor with surgery. Stage 1 cancer is usually 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.

[00172] In some embodiments, the sensor arrays and methods are able to detect intermediate stages of the disease. Intermediate states of the disease describes 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.

[00173] Further, the 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.

[00174] In some embodiments, the methods of the present technology include comparing the protein fingerprint of the sample to a panel of protein 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 invention allows for the ability to characterize and detect the changes in biomolecule fingerprints over time in the subject by comparing 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. In some embodiments, samples can 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).

[00175] For illustrative purposes only, the examples have shown that the methods and sensor arrays of the present invention are able to distinguish not only between different types of cancers, but also between the different stages of the cancer (e.g. early stages of cancer).

[00176] Methods of determining a biomolecule fingerprint associated with at least one disease or disorder and/or a disease state are contemplated. The methods comprise the steps of obtaining a sample from at least two subjects diagnosed with the at least one disease or disorder or having the same disease state; contacting each sample with a sensor array described herein to determining a biomolecule fingerprint for each sensor array, and analyzing the fingerprint of the at least two samples to determine a biomolecule fingerprint associated with the at least one disease or disorder and/or disease state.

[00177] The method of determining the biomolecule fingerprint associated with the disease or disorder and/or disease state include the analysis of the biomolecule fingerprints of the at least two samples. This determination or analysis is done by methods known in the art, including, for example, a wide variety of supervised and unsupervised data analysis and clustering approaches such as hierarchical cluster analysis (HCA), principal component analysis (PCA), and Partial least squares Discriminant Analysis (PLS-DA). In other words, the biomolecule fingerprint of each sample are compared/analyzed with each other to determine with statistical significance what patterns are common between the individual fingerprints to determine a biomolecule fingerprint that is associated with the disease or disorder or disease state.

[00178] In some embodiments, the method includes obtaining samples from control subjects which are contacted with the sensor array to produce a control biomolecule fingerprint. These control biomolecule fingerprints can then be used to compare to the biomolecule fingerprints of the subjects with a disease or disorder and/or specific disease state to determine a biomolecule fingerprint specific to that disease or disorder and/or specific disease state.

[00179] The method may include, for example, obtaining control sample from at least one control subject, contacting the control sample with the sensor array to produce a plurality of control biomolecule corona, and assaying the plurality of biomolecules of each control biomolecule corona. The method may further comprise comparing the plurality of biomolecules of the plurality of control biomolecule corona with the plurality of biomolecules of the plurality of biomolecule corona from the subject with the disease or disorder to determine a biomolecule fingerprint associated with the at least one disease or disorder.

[00180] Methods of diagnosing or prognosing a disease or disorder are also contemplated. The methods comprise obtaining a sample from a subject; contacting the sample with a sensor array to produce a biomolecule fingerprint, and comparing the biomolecule fingerprint to a panel of biomolecule fingerprints associated with a plurality of diseases or disorders; and diagnosing or prognosing the disease or disorder.

[00181] In some embodiments, methods of identifying patterns of biomarkers or specific biomarkers associated with a disease or disorder are contemplated. Suitable methods, include, for example, performing the methods described above (e.g. obtaining a samples from at least two subjects diagnosed with the disease or disorder and at least two control subjects; contacting each sample with the nanoscale sensor array to produce a biomolecule fingerprint, and comparing the biomolecule fingerprint of the subjects with the disease or disorder to the biomolecule fingerprint of the control subjects to determine at least one pattern and/or biomarker associated with the disease or disorder. Suitable, the method may comprise at least 2 disease subjects and at least two control subjects, alternatively at least 5 disease subjects and at least 5 control subjects, alternatively at least 10 disease subjects and at least 10 control subjects, alternatively at least 15 disease subjects and at least 15 control subjects, alternatively at least 20 disease subjects and at least 20 control subjects, and includes any variations in between (e.g. disease subjects from at least 2-100, and control subjects from at least 2-100).

[00182] In some embodiments, the arrays and methods allow for the determination of a pattern of biomarkers associated with the disease state or disease or disorder or, in some embodiments, specific biomarkers that are associated with the disease or disorder. Not only will biomarkers that may be associated with a disease state be able to be identified, for example, biomarkers listed herein, but new biomarkers

or patterns of biomarkers that may be associated with a disease state or a disease or disorder may be determined. As discussed above, some biomarkers or patterns of biomarkers for a specific disease or disorder may be a change in a biomolecule associated with the sensor array of the present invention and differ from what is usually referred to as biomarkers in the art, e.g., and increase expression of a specific biomolecule associated with a disease. As discussed above, it may be the interaction of a biomolecule, e.g. biomolecule X, with other biomolecules, e.g. biomolecule Y and Z, that results in the ability to associate with a specific disease state and may not correlate with any change in the absolute concentration of biomarker X in the sample over time or disease state. Thus, a molecule that would not in the conventional sense be considered a biomarker since it does not change in absolute concentration in a sample from the pre-disease to disease state, may in view of the present disclosure be considered a biomolecule as its relative changes that are measured by the array of the present invention are associated with a disease state. In other words, it may be an increase or decrease in the interaction of biomolecule X (due to the interactions of X with the nanoscale sensor elements and other biomolecules in the sample) with the array that provides a signal that a biomarker is associated with a disease state.

[00183] Suitable cancer biomarkers include, but are not limited to, for example, AHSG (α 2-HS-Glycoprotein), AKR7A2 (Aflatoxin B1 aldehyde reductase), AKT3 (PKB γ), ASGR1 (ASGPR1), BDNF, BMP1 (BMP-1), BMPER, C9, CA6 (Carbonic anhydrase VI), CAPG (CapG), CDH1 (Cadherin-1), CHRDL1 (Chordin-Like 1), CKB-CKM- (CK-MB), CLIC1 (chloride intracellular channel 1), CMA1 (Chymase), CNTN1 (Contactin-1), COL18A1 (Endostatin), CRP, CTSL2 (Cathepsin V), DDC (dopa decarboxylase), EGFR (ERBB1), FGA-FGB-FGG (D-dimer), FN1 (Fibronectin FN1.4), GHR (Growth hormone receptor), GPI (glucose phosphate isomerase), HMGB1 (HMG-1), HNRNPAB (hnRNP A/B), HP (Haptoglobin, Mixed Type), HSP90AA1 (HSP 90 α), HSPA1A (HSP 70), IGFBP2 (IGFBP-2), IGFBP4 (IGFBP-4), IL12B-IL23A (IL-23), ITIH4 (Inter- α -trypsin inhibitor heavy chain H4), KIT (SCF sR), KLK3-SERPINA3 (PSA-ACT), L1CAM (NCAM-L1), LRIG3, MMP12 (MMP-12), MMP7 (MMP-7), NME2 (NDP kinase B), PA2G4 (ErbB3 binding protein Ebp1), PLA2G7 (LpPLA2/PAFAH), PLAUR (suPAR), PRKACA (PRKA C- α), PRKCB (PKC- β -II), PROK1 (EG-VEGF), PRSS2 (Trypsin-2), PTN (Pleiotrophin), SERPINA1 (α 1-Antitrypsin), STC1 (Stanniocalcin-1), STX1A (Syntaxin 1A), TACSTD2 (GA733-1 protein), TFF3 (Trefoil factor 3), TGFBI (β IGH3), TPI1 (Triosephosphate isomerase),

TPT1 (Fortilin), YWHAG (14-3-3 protein γ), YWHAH (14-3-3 protein ϵ), prostate cancer biomarkers, for example, PSA, Pro-PSA, PHI, PCA3, TMPRSS3:ERG, PCMT, MTEN, breast cancer markers, for example, epidermal growth factor receptor 2 (HER2) oncogene, melanoma biomarker BRAF, lung cancer biomarker EML4-ALK, A2ML1, BAX, C10orf47, Clorf62, CSDA, EIFC3, ETFB, GABARAPL2, GUKL, GZMH, HIST1H3B, HLA-A, HSP90AA1, NRG1, PRDX5, PTMA, RABAC1, RABAGAP1L, RPL22, SAP 18, SEPW1, SOX1, EGFR, EGFRvIII, apolipoprotein AI, apolipoprotein CIII, myoglobin, tenascin C, MSH6, claudin-3, claudin-4, caveolin-1, coagulation factor III, CD9, CD36, CD37, CD53, CD63, CD81, CD136, CD147, Hsp70, Hsp90, Rab13, Desmocollin-1, EMP-2, CK7, CK20, GCDF15, CD82, Rab-5b, Annexin V, MFG-E8, HLA-DR, a miR200 microRNA, MDC, NME-2, KGF, PIGF, Flt-3L, HGF, MCP1, SAT-1, MIP-1-b, GCLM, OPG, TNF RII, VEGF-D, ITAC, MMP-10, GPI, PPP2R4, AKR1B1, Amy1A, MIP-1b, P-Cadherin, EPO and the like. For example, biomarkers for breast cancer include, but are not limited to, ER/PR, HER-2/neu, and the like. Biomarkers for colorectal cancer include, but are not limited to, for example, EGFR, KRAS, UGT1A1, and the like. Biomarkers associated with leukemia/lymphoma include, but are not limited to, e.g., CD20 antigen, CD30, FIP1L1-PDGFRalpha, PDGFR, Philadelphia Chromosome (BCR/ABL), PML/RAR alpha, TPMT, UGT1A1, and the like. Biomarker associated with lung cancer include but are not limited to, e.g., ALK, EGFR, KRAS and the like. Biomarkers are known in the art, and can be found in, for example, Bigbee W, Herberman RB. Tumor markers and immunodiagnosis. In: Bast RC Jr., Kufe DW, Pollock RE, et al., editors. Cancer Medicine. 6th ed. Hamilton, Ontario, Canada: BC Decker Inc., 2003; Andriole G, Crawford E, Grubb R, et al. Mortality results from a randomized prostate-cancer screening trial. New England Journal of Medicine 2009; 360(13):1310–1319; Schröder FH, Hugosson J, Roobol MJ, et al. Screening and prostate-cancer mortality in a randomized European study. New England Journal of Medicine 2009; 360(13):1320–1328; Buys SS, Partridge E, Black A, et al. Effect of screening on ovarian cancer mortality: the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Randomized Controlled Trial. JAMA 2011; 305(22):2295–2303; Cramer DW, Bast RC Jr, Berg CD, et al. Ovarian cancer biomarker performance in prostate, lung, colorectal, and ovarian cancer screening trial specimens. Cancer Prevention Research 2011; 4(3):365–374; Sparano JA, Gray RJ, Makower DF, et al. Prospective validation of a 21-gene expression assay in breast cancer. New England Journal of Medicine 2015; First published online September 28, 2015. doi: 10.1056/NEJMoa1510764, incorporated by

reference in their entirety.

[00184] Suitable, this methods can be used to determine biomarkers associated with cancer. For example, in one embodiment the cancer is glioblastoma, and wherein the biomarker is selected from the group consisting of HABP1, VTNC, CO3, ITIH2, ITIH1, CO7, FHR5, CBPN, ALBU, PLMN, CO4A, PRDX2, VWF, C4BPA, APOB, HBB, CNDP1, CRP, SAA4, APOE, CSCL7 and combinations thereof. In another embodiment, the cancer is meningioma, and wherein the biomarker is selected from the group consisting of FCN3, RET4, HABP2, CBPN and combinations thereof. In another embodiment, the cancer is pancreatic cancer and wherein the biomarker is selected from the group consisting of KNG1, IC1, CBPB2, TRFE, GELS, CXCL7, HPTR, PGK1, AACT, LUM, APOE, FIBB, APOA2, A1BG, A1AT, LBP, APOA1, H4, FIBG and combinations thereof. In another embodiment, the cancer is lung cancer and the biomarker is selected from the group consisting of COO, CRP, SAA4, APOA1, A1AT, GELS and combinations thereof. In another embodiment, the cancer is myeloma and the biomarker is ALBU.

[00185] Biomarkers may also be associated with the cardiovascular disease which are known in the art and include, but are not limited to, lipid profile, glucose, and hormone level and physiological biomarkers based on measurement of levels of important biomolecules such as serum ferritin, triglyceride to HDLp (high density lipoproteins) ratio, lipophorin-cholesterol ratio, lipid-lipophorin ratio, LDL cholesterol level, HDLp and apolipoprotein levels, lipophorins and LTPs ratio, sphingolipids, Omega-3 Index, and ST2 level, among others. Suitable biomarkers for cardiovascular disease can be found in the art, for example, but not limited to, in van Holten et al. "Ciculating Biomarkers for Predicting Cardiovascular Disease Risk; a Systemic Review and Comprehensive Overview of Meta-Analyses" PLoS One, 2013 8(4): e62080, incorporated by reference in its entirety.

[00186] Biomarkers may also be associated with a neurological disease. Suitable biomarkers are known in the art and include, but are not limited to, e.g., A β 1-42, t-tau and p-tau 181, α -synuclein, among others. *See, e.g.,* Chintamaneni and Bhaskar "Biomarkers in Alzheimer's Disease: A Review" ISRN Pharmacol. 2012. 2012: 984786. Published online 2012 Jun 28, incorporated by reference in its entirety.

[00187] Biomarkers for inflammatory diseases are known in the art and include, but are not limited to, e.g., cytokines/chemokines, immune-related effectors, acute-phase proteins [C-reactive protein (CRP) and serum amyloid A (SAA)], reactive oxygen

species (ROS) and reactive nitrogen species (RNS), prostaglandins and cyclooxygenase (COX)-related factors, and mediators such as transcription factors and growth factors, which can include, for example, C-reactive protein (CRP), S100, LIF, CXCL1, CXCL2, CXCL4, CXCL5, CXCL8, CXCL9, CXCL10, CCL2, CCL23, IL-1 β , IL-1Ra, TNF, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IFN γ , CXCR1, CXCR4, CXCR5, GM-CSF, GM-CSFR, G-CSF, G-CSFR, EGF, VEGFA, LEP, SAA1, VCAM1, CRP, MMP1, MMP3, TNFRSF1A, RETN, CHI3L1, antinuclear antibodies (ANA), rheumatoid factor (RF), antibodies against cyclic citrullinated peptide (anti-CCP)] and for chronic IBD (fecal calprotectin), among others. Suitable biomarkers for inflammatory bowel disease, for example, include CRP, ESR, pANCA, ASCA, and fecal calprotectin. *See, e.g.,* Yi Fengming and Wu Jianbing, "Biomarkers of Inflammatory Bowel Disease," *Disease Markers*, vol. 2014, Article ID 710915, 11 pages, 2014. doi:10.1155/2014/710915, incorporated by reference in its entirety.

[00188] The terms "individual," "subject," and "patient" are used interchangeably herein irrespective of whether the subject has or is currently undergoing any form of treatment. As used herein, the term "subject" generally refers to any vertebrate, including, but not limited to a mammal. Examples of mammals including primates, including simians and humans, equines (e.g., horses), canines (e.g., dogs), felines, various domesticated livestock (e.g., ungulates, such as swine, pigs, goats, sheep, and the like), as well as domesticated pets (e.g., cats, hamsters, mice, and guinea pigs). Preferably, the subject is a human.

[00189] The arrays and methods described herein can be used under a number of different conditions to provide the desired biomolecule footprint. For example, the size of the nanoscale sensor elements, the rate of flow of the sample through the sensor, the time of incubating the sensor array with the sample and the temperature at which the sensor array is incubated can all be changed to provide a reproducible biomolecule footprint. Suitable sizes of the nanoscale sensor element include sensor elements that have less than one micron in at least one direction.

[00190] Suitable time for incubating the array or plurality of nanoscale sensor elements include, at least a few seconds, e.g. at least 10 seconds to about 24 hours, for example 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, and include any time and increment in between (e.g. 10 seconds, 11, 12, 13, 14, 15, 15, 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, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60 seconds, etc.; 1 minute, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 15, 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, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, etc.; 1 hour, 2 hours, 3, hours, 4, hours, 5 hours, 6 hours, 7 hours, 8 hours etc.)

[00191] Further, the temperature at which the assay is performed can be determined by one skilled in the art, and includes temperatures between about 4 °C to about 40 °C, alternatively from about 4 °C to about 20 °C, alternatively from about 10°C to about 15 °C, alternatively from about 10 °C to about 40 °C, for example, at about 4 °C, about 5 °C, about 6°C, about 7 °C, about 8 °C, about 9°C, about 10°C, about 11 °C, about 12 °C, about 13°C, about 14 °C, about 15°C, about 16°C, about 17 °C, about 18°C, about 19°C, about 20°C, about 21 °C, about 22 °C, about 25°C, about 30°C, about 35 °C, about 37°C, etc. Suitable, the assay may be performed at room temperature (e.g. around about 37°C, for example from about 35 °C to about 40°C).

[00192] The methods of the present invention may comprise contacting the sample with the array. The contacting of the sample with the array may be using any suitable flow rate in which the sample can flow over the sensor array. In some aspects, the flow velocity of the streams, the Reynolds number, or the relative cross sectional areas of the flow streams can be altered to provide adequate contact between the sample and the array.

[00193] For example, in embodiments using a nanochannel or microchannel the cross sectional area of the first stream can be more than 1% of the cross sectional area

of the channel. In another example, the cross sectional area of the first stream can be less than 90% of the cross sectional area of the channel. The cross sectional area ratio can be 10:1 to 1:10, 1:5 to 5:1, 1:3 to 3:1, 1:2 to 2:1 or 1:1.

[00194] In certain other circumstances, the flow of the sample over the array has a Reynolds number at the location of the introduction of the sample of between 300 and 1,000,000. In some instances, the location of the introduction of the sample is a nanochannel or microchannel.

[00195] Kits

[00196] Aspects of the present disclosure that are described with respect to methods can be utilized in the context of the sensor array or kits discussed in this disclosure. Similarly, aspects of the present disclosure that are described with respect to the sensor array and methods can be utilized in the context of the kits, and aspects of the present disclosure that are described with respect to kits can be utilized in the context of the methods and sensor array.

[00197] This disclosure provides kits. The kits can be suitable for use in the methods described herein. Suitable kits include a kit for determining a biomolecule fingerprint for a sample comprising a sensor array as described herein. In one aspect, the kit provides a sensor array comprising at least two nanoscale sensor elements which have differing physicochemical properties from each other. In some aspects, the kits provides a comparative panel of biomolecule fingerprint in order to use the biomolecule fingerprint to determine a disease state for the subject. In some aspects, instructions on how to determine the biomolecule fingerprint are included. In some suitable embodiments, the sensor arrays are provided as chip arrays in the kit.

[00198] In other aspects, kits for determining a disease state of a subject or diagnosing or prognosing a disease in a subject are provided. Suitable kits include a sensor array comprising at least two nanoscale sensor elements which have differing physicochemical properties from each other to determining a biomolecule fingerprint. Further, the kit may further include a comparative panel of biomolecule fingerprint in of different disease state or different diseases or disorders. Instructions on determining the biomolecule fingerprint and analysis are provided.

[00199] The following non-limiting examples are included for purposes of illustration only, and are not intended to limit the scope of the range of techniques and protocols in which the compositions and methods of the present invention may find

utility, as will be appreciated by one of skill in the art and can be readily implemented.

Examples

[00200] Example 1: Label-free sensor array for early detection of cancer.

[00201] The present Example provides a label-free sensor array for early detection of various cancers. The sensor array consists of three different cross-reactive liposomes with various surface charges (i.e., cationic (DOPG (1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol))), anionic (DOTAP(1,2-Dioleoyl-3-trimethylammonium-propane)-DOPE(dioleoylphosphatidylethanolamine)), and neutral (CHOL (DOPC-Cholesterol))), whose protein corona composition changes in response to their interactions with the plasma of patients who have different types of various cancers, i.e., lung, pancreas, myeloma, meningioma, and glioblastoma. Although no single protein corona composition is specific for any one cancer type, the changes in the corona composition pattern provide a unique "fingerprint" for each type of cancer.

[00202] Hard corona profiles of the sensor array elements using plasma from patients with cancers at early, intermediate, and advanced stages. The composition of the protein corona that forms on the surface of sensor array elements (nanoparticles) is strongly dependent on the physicochemical properties of those nanoparticles⁴² and, at the same time, can be strongly affected by the type of disease present in the donor of the human plasma used for incubation^{40,41}. The size and charge of the corona-coated nanoparticles, after incubation with plasma from patients with five different types of cancers (i.e., glioblastoma multiforme, lung cancer, meningioma, multiple myeloma, and pancreatic cancer) and healthy individuals (see Table 1), were probed using dynamic light scattering (DLS/Nanosight) and transmission electron microscopy (TEM), and the results demonstrated that the physicochemical properties of the corona-coated nanoparticles depended substantially on the type of cancer (Fig. 1A,B).

[00203] Table 1. General information on patients (and their cancer types) whose plasma was used in this study.

	AGE	GENDER	CANCER STAGE	CANCER STAGE category
PANCREAS 1	81	M	TNM: cT4 N+M+	Advanced
PANCREAS 2	76	M	TNM: cT2 N+	Moderate

PANCREAS 3	60	F	TNM: cT4 N+	Advanced
PANCREAS 4	75	M	TNM: cT3 N+M+	Advanced
PANCREAS 5	61	F	TNM: cT3 N+M+	Advanced
LUNG 1	45	F	TNM: T1A N0 M0	Early
LUNG 2	24	M	TNM: T1A N0 M0	Early
LUNG 3	44	F	TNM: T1A N0 M0	Early
LUNG 4	48	F	TNM: T1A N0 M0	Early
LUNG 5	40	M	TNM: T1A N0 M0	Early
MYELOMA 1	41	F	Onset II (52% Plasmacells in Bone Marrow; Monoclonal IgG-k)	Moderate
MYELOMA 2	60	F	Onset I (28% Plasmacells in Bone Marrow; Monoclonal IgG-k)	Early
MYELOMA 3	57	F	Onset I (8% Plasmacells in Bone Marrow; Monoclonal IgG-k)	Early
MYELOMA 4	63	M	Onset I (9% Plasmacells in Bone Marrow; Monoclonal IgG-k)	Early
MYELOMA 5	75	M	Onset II (42% Plasmacells in Bone Marrow; Monoclonal IgG-k)	Moderate
GLIOBLASTOMA 1	58	M	WHO 4	Advanced
GLIOBLASTOMA 2	75	M	WHO 4	Advanced
GLIOBLASTOMA 3	76	M	WHO 4	Advanced
GLIOBLASTOMA 4	73	M	WHO 4	Advanced
GLIOBLASTOMA 5	76	F	WHO 4	Advanced
MENINGIOMA 1	82	F	WHO 2	Moderate
MENINGIOMA 2	50	F	WHO 2	Moderate
MENINGIOMA 3	80	M	WHO 2	Moderate
MENINGIOMA 4	67	M	WHO 2	Moderate
MENINGIOMA 5	64	M	WHO 2	Moderate

[00204] Quantitative evaluation of the total protein adsorbed onto the nanoparticles was performed *via* the BCA or NanoOrange assay, and the results showed significant differences in the amounts of adsorbed proteins after incubation in plasma from patients with various types of cancers (**Fig. 1B**). The quantitative evaluation of the total protein adsorbed on the surface of liposomes showed strong dependency of protein amount on cancer type (**Fig. 1B**). The protein corona composition at the surface of three liposomes was evaluated by liquid chromatography-mass spectrometry (LC-MS/MS) in which the abundance of 1,800 known proteins was defined. The contribution

of individual proteins and their categories (*i.e.*, complement, coagulation, tissue leakage, lipoproteins, acute phase, immunoglobulins, and other plasma proteins) to the corona composition were defined (**Fig. 1C; Fig. 6A-F**). These results demonstrated significant associations between the protein composition and not only the cancer type but also the type of sensor element (*i.e.*, type of nanoparticle).

[00205] According to an extensive body of literature, there are strong relationships between cancer development and variations in complement⁴³⁻⁴⁶, coagulation⁴⁷⁻⁵⁰, tissue leakage^{51,52}, lipoproteins⁵³⁻⁵⁷, acute phase^{58,59}, and immunoglobulins⁶⁰⁻⁶². Therefore, the cross-reactive interactions of these protein categories with nanoparticles may provide unique “fingerprints” for each type of cancer, which may facilitate cancer identification and discrimination. Consequently, one would expect the protein corona sensor array to cross-reactively adsorb a wide range of proteins involved in cancer induction and development that could be used for cancer identification and discrimination.

[00206] Develop supervised classification analysis to identify and discriminate among cancers using the protein corona sensor array outcomes. In order to investigate whether protein corona fingerprints (PCFs) of various sensor elements could be utilized as a biosensors and form unique patterns for different diseases, we have applied focused classification approaches to proteomic data on three liposomes' protein corona composition (cationic, anionic, and neutral). Details of the methods are described in the Methods section. A weighted-variable importance in the projection (VIP) score is introduced and applied for ranking of variables based on partial least squares discriminant analysis (PLS-DA) as a linear projection method. Selection of the most relevant variables (proteins) in building the classification model can be guided by a set of obtained ranked variables. In this regard, top ranked variables were added to the model one by one, and the classification error of the PLS-DA model was monitored. We observed that the classification model has the minimum error by using only the top 69 features (Fig. 2A). The new 69-dimensional feature space was successfully used to discriminate 30 samples belonging to six classes by PLS-DA with a high classification accuracy (0.97) using leave-one-out and 10-fold cross-validation (Fig. 2A,B). The classification parameters are given in Table 2. The contribution of each single selected protein to the separation of each cancer group (VIP) is plotted on the y- and x- axis, respectively, to provide a visual representation of the relative specificity of the findings

(Fig. 2B-F). The proteins with higher VIP scores could be considered the best informative or diagnostic set to discriminate each disease from controls and from among all cancer categories.

[00207] Table 2. Classification results obtained from two developed and non-linear models for six groups of samples.

Models/ classification parameters		PLS-DA, 10 fold CV, LV=5 20 iteration	CPANN (8*8) 10 fold CV
Specificity (CV)	1	1.00	1.00
	2	0.96	0.96
	3	1.00	1.00
	4	1.00	1.00
	5	1.00	1.00
	6	1.00	1.00
Sensitivity (CV)	1	1.00	1.00
	2	1.00	1.00
	3	1.00	0.80
	4	1.00	1.00
	5	1.00	1.00
	6	1.00	1.00
Class error (CV)	1	0.00	0.00
	2	0.03	0.02
	3	0.00	0.00
	4	0.00	0.00
	5	0.00	0.00
	6	0.00	0.00
1) Control 2) Glioblastoma 3) Meningioma 4) Myeloma 5) Pancreas 6) Lung cancers			

[00208] PLS-DA and counter propagation artificial neural network (CPANN) were then applied to the selected variables, and whole samples as supervised classification, linear, and non-linear approaches, respectively. It should be noted that the primary data set with all variables before variable selection, has poor discrimination and could not be separated into six groups.

[00209] Next, to further verify and analyze the data, we decided to take advantage of a non-linear classification method. Visualizing the feature space can help us understand the hidden structures and topological relationships among the patterns. To reduce the dimensionality of the feature space while preserving the topological relation in the data structure, the CPANN (a supervised variant of self-organizing maps/SOM) was used to learn and predict the class membership of the patterns, simultaneously producing a two-dimensional map of neurons and provide valuable information (from a non-linear approach) about the data structure. Details of the CPANN are provided in the Methods section. Different sizes for the CPANN map were checked using 10-fold cross-validation; a map including 64 (8×8) neurons was chosen due to the minimum classification error (**Fig. 7C**). Moreover, the topological structure of data in the high-dimensional space is reflected in the assignment map produced by CPANN (**Fig. 4B**). Considering the similarity of the neurons to the input vectors, the map can be partitioned into six distinct zones related to different type of cancers and control samples. Samples with the same class label are mapped onto nearby or the same neurons, which means that the selected variables provide information valuable in discriminating the samples in the feature space. The relative position and orientation of six zones on the map can contribute qualitative information on the similarities between types of cancers. To represent the effect of variable selection on the quality of mapping, another CPANN was trained with all 1823 variables, and the resulting map shows that the selected biomarkers (variables) play an important role in discriminating among cancer types and classifying them properly (**Fig. 4A**).

[00210] On the basis of the obtained results, both linear and non-linear models showed high accuracy, deduced from their acceptable specificity, sensitivity, and class error values. Consistent with these findings, unsupervised clustering (HCA) based on 69 markers was able to strongly separate, various type of cancerous and control samples (**Fig. 3E-F**). As can be seen in **Fig. 3**, there is close similarity between the glioblastoma and meningioma groups of samples, implying difficulty in discrimination, most probably

related to similar plasma proteomics patterns in these two brain cancers. These results reflect the fact that the plasma concentrations of many proteins in the corona differ considerably, not only among subjects with different types of cancers, but among healthy individuals as well.

[00211] To illustrate the biosensors' capability for pattern recognition, a set of analyses was performed on the data obtained from individual nanoparticles. As expected, the pattern of cancer-specific fingerprints could not be extracted solely from each nanoparticle's PCF. We found that pattern-recognition techniques applied to protein abundance in the protein corona formed on three different liposomes (cationic, anionic, and neutral) correctly distinguished not only cancerous from control samples, but also each type of cancer under consideration from the others.

[00212] Identification of proteins with crucial roles in cancer detection and discrimination as promising biomarkers for specific types of cancers. The use of biomarkers both before cancer diagnosis (in risk assessment and screening/early detection) and after diagnosis (in monitoring therapy, selecting additional therapy, and detecting recurrence) would yield substantial therapeutic and health-economic benefits.[1] To understand the potential biological relevance of the 69 selected proteins that discriminate cancerous samples, we manually searched through previously published reports in PubMed on protein biomarkers of specific types of cancers that are upregulated or downregulated according to different disease stages. The resulting data were compared with the selected proteins in the model to identify matched markers and determine the biological relevance of the proposed model. Interestingly, we noted significant numbers of biomarkers specific to five investigated groups of cancers among the selected predictors that had been reported as specific cancer biomarkers (Fig. 5B).[2-5] [6, 7] [8-23] [24-27] [28]

[00213] The high specificity of the selected markers for discriminating among the five groups of cancers, which derives from the introduced protein corona sensor array approach, demonstrates a significant correlation with the work now under way in the complex cancer proteomics space; therefore this strategy not only provides a basis for cancer prediction but also translates that promise into reality. It is noteworthy that the discrimination between different groups occurs as a result of several predictors (and not individual biomarkers) that change simultaneously in a systematic manner, forming patterns unique to each specific type of cancer. On the basis of this evidence, the most

informative predictors selected by the proposed model that have not already been reported as cancer-specific biomarkers may have great potential as new diagnosis biomarker candidates. To define their role in cancer development, the variation and functionality of these promising candidates in cancer patients should be carefully monitored. By focusing on the unique patterns derived from huge numbers of subjects via a set of informative predictors, researchers should be able to predict cancers at different stages more accurately than is possible using current methods.

[00214] Cohort data analysis. To probe the capacity of this innovative sensor array for very early detection of cancers, we used cohort plasma from healthy people who were diagnosed with one of the five types of cancers several years after plasma collection. Using the cohort samples, we evaluated whether our proposed models, both linear and non-linear, with 69 selected predictors could be utilized for cancer prediction.

[00215] To this end, the values related to 69 variables were put into the model, and the class membership of each cohort object was predicted given the fixed optimum parameters values. It is noteworthy that 19 variables (proteins), out of the 69 variables, were absent in the proteomics profile of protein corona sensor array of cohort samples; therefore their amount in the validation data matrix was zero. Interestingly, both linear and non-linear models provided good predictions for all five samples. The observed distance between training and cohort samples (**Fig. 4A**), most probably influenced by zero values that were added to data matrix for those 19 absent variables. As shown in **Fig. 4B**, the cohort samples were placed in the correct neuron related to Glioblastoma in the CPANN map.

[00216] METHODS

[00217] Liposomes. Cholesterol (Chol) was purchased from Sigma Aldrich (St. Louis, MO, USA). DOPC (dioleoylphosphatidylcholine), DOPE (dioleoylphosphatidylethanolamine), DOPG (1,2-dioleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol)), and DOTAP (1,2-Dioleoyl-3-trimethylammonium-propane) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). DOPG, DOTAP-DOPE (1:1 molar ratio) and DOPC-Chol (1:1 molar ratio) liposomes were prepared by dissolving appropriate amounts of lipids 9:1 (v/v) in chloroform:methanol. The chloroform:methanol mixture was evaporated by rotary-evaporation. Lipid films were kept under vacuum overnight and hydrated with phosphate saline buffer (PBS) 10 mmol/l (pH 7.4) to a final lipid

concentration of 1 mg/ml. The liposome suspensions obtained were sized by extrusion through a 50-nm polycarbonate carbonate filter by the Avanti Mini-Extruder (Avanti Polar Lipids, Alabaster, AL).

[00218]

[00219] Human plasma collection, preparation, and storage. Human plasma (HP) was collected from healthy and cancer patients diagnosed with glioblastoma multiforme, lung cancer, meningioma, multiple myeloma, or pancreatic cancer. The present study was approved by the Ethical Committees of the Sapienza University of Rome (glioblastoma multiforme, meningioma, multiple myeloma), the University of Napoli Federico II (lung cancer), and the University Campus Bio-Medico di Roma (pancreatic cancer). In brief, blood was collected by venipuncture of healthy subjects and cancer patients by means of a BD P100 Blood Collection System (Franklin Lakes, NJ, USA) with push-button technology that reduces blood waste while minimizing the risk of contamination. After clot formation, samples were centrifuged at 1000 x g for 5 min to pellet the blood cells, and the supernatant was removed. After confirming the absence of hemolysis, plasma collected from each donor (1 ml) was split into 200-microliter aliquots and stored at -80° C in labeled Protein LoBind tubes until use. For analysis, the aliquots were thawed at 4°C and then allowed to warm at room temperature (RT).

[00220] Cohort plasma samples. We used human plasma from healthy people diagnosed with brain, lung, and pancreatic cancers within eight years after plasma collection. The plasma samples were collected through the NIH-funded Golestan Cohort Study, performed by the National Cancer Institute (NCI) in the USA, the International Agency for Research on Cancer (IARC) in France, and the Tehran University of Medical Sciences (TUMS) in Iran. This study involved the collection and storage of plasma from 50,000 healthy subjects, over 1,000 of whom went on to develop various types of cancers in subsequent years. Samples from five individuals per cancer were used in this study. These important plasma samples provide us the unique opportunity to probe the capacity of our innovative protein corona sensor array for early detection of cancers.

[00221] Size and zeta-potential. Bare liposomes were incubated with HP (1:1 v/v) for 1 hour at 37°C. Subsequently samples were centrifuged at 14000 rpm for 15 minutes at 4°C to pellet liposome-HP complexes. The resulting pellet was washed three times with phosphate-buffered saline (PBS) and resuspended in ultrapure water. For

size and zeta-potential measurements, 10 μL of each sample were diluted with 990 μL of distilled water. All size and zeta-potential measurements were performed at RT using a Zetasizer Nano ZS90 (Malvern, U.K.) equipped with a 5-mW HeNe laser (wavelength $\lambda = 632.8 \text{ nm}$) and a digital logarithmic correlator. The normalized intensity autocorrelation functions were analyzed by the CONTIN method to obtain the distribution of the diffusion coefficient D of the particles. D is converted into an effective hydro- dynamic radius R_H by the Stokes–Einstein equation ($R_H = K_B T / 6\pi\eta D$), where $K_B T$ is the thermal energy and η is the solvent viscosity. Electrophoretic mobility of samples, u , was measured by laser Doppler electrophoresis. Zeta-potential was calculated by the Smoluchowski relation (zeta potential = $u\eta/\epsilon$) where η and ϵ are the viscosity and the permittivity of the solvent phase, respectively). Size and zeta-potential of liposome-HP complexes are given as mean $\pm$ standard deviation (S.D.) of five independent measurements.

[00222] Protein assay. Liposome formulations were incubated with HP (1:1 v/v) for 1 hour at 37°C. Afterwards, liposome-HP complexes were pelleted at 15000 x g for 15 minutes at 4°C and washed three times with PBS. Washed pellet was resuspended in urea 8 mol/l, NH_4CO_3 50 mmol/l. 10 microliters of each sample were added to five wells of a 96-well plate. Protein quantification was made adding 150 microliters/well of Protein Assay reagent (Pierce, Thermo Scientific, Waltham, MA, USA). The multiwell was shaken and incubated at room temperature for 5 minutes. Absorbance was measured with GloMax Discover System (Promega, Madison, WI, USA) at 660 nm. Background effects were properly corrected and the protein concentration was calculated using the standard curve. Results are given as mean $\pm$ S.D. of five independent replicates.

[00223] Protein identification and quantification. The incubation procedure was performed as described elsewhere (Label-free quantitative analysis for studying the interactions between nanoparticles and plasma proteins. Analytical and Bioanalytical Chemistry, 2013, 405, 2-3, 635-645, incorporated by reference in its entirety). Liposome formulations were incubated with HP (1:1 v/v) for 1 hour at 37°C. Samples were centrifuged at 15000 x g for 15 min to pellet liposome-HP complexes. The pellet was washed three times with 10 mmol/l Tris HCl (pH 7.4), 150 mmol/l NaCl and 1 mmol/l EDTA. After washing, the pellet was air dried and resuspended in the digestion buffer. The digestion and peptide desalting were carried out as previously described (Shotgun proteomic analytical approach for studying proteins adsorbed onto liposome surface.

Analytical and Bioanalytical Chemistry, 2013, 401, 4, 1195-1202, incorporated by reference in its entirety). In brief, pellet was resuspended in 40 microliters of urea 8 mol/l, NH_4CO_3 50 mmol/l and digested by adding 2 micrograms of trypsin. The digested peptides were desalted using SPE C18 column, reconstituted with a suitable volume of a 0.1% formic acid solution, and stored at -80°C until analysis. Digested peptides were analyzed by nano-high-performance liquid chromatography (HPLC) coupled to tandem mass spectrometry (MS/MS). NanoHPLC MS/MS analysis was carried out using a Dionex Ultimate 3000 (Dionex Corporation Sunnyvale, CA, U.S.A.) directly connected to a hybrid linear ion trap-Orbitrap mass spectrometer (Orbitrap LTQ-XL, Thermo Scientific, Bremen, Germany) by a nanoelectrospray ion source. Peptide mixtures were enriched on a $300\text{ }\mu\text{m ID} \times 5\text{ mm}$ Acclaim PepMap 100 C18 precolumn (Dionex Corporation Sunnyvale, CA, U.S.A.), employing a premixed mobile phase made up of ddH₂O/ACN, 98/2 (v/v) containing 0.1% (v/v) HCOOH, at a flow-rate of 10 microliters/min. Peptide mixtures were then separated by reversed-phase (RP) chromatography. The largest set of peptides was detected using a 3-hour optimized LC gradient composed of a mobile phase A of ddH₂O/HCOOH (99.9/0.1, v/v) and a mobile phase B of ACN/HCOOH (99.9/:0.1, v/v). MS spectra of eluting peptides were collected over an m/z range of 350-1700 using a resolution setting of 60,000 (full width at half-maximum at m/z 400), operating in the data-dependent mode. MS/MS spectra were collected for the five most abundant ions in each MS scan. Further details can be found elsewhere (Shotgun proteomic analytical approach for studying proteins adsorbed onto liposome surface. Analytical and Bioanalytical Chemistry, 2013, 401, 4, 1195-1202). For each experimental condition three independent samples (biological replicates) were prepared, each of which was measured in triplicate (technical replicates), yielding nine measurements for each experimental condition. RAW data files were submitted to Mascot (v2.3, Matrix Science, London, UK) using Thermo-Finishingan LCQ/DECA RAW file data import filter to perform database searches against the non-redundant Swiss-Prot database (09-2014, 546000 sequences, Homo Sapiens taxonomy restriction). For the database search, trypsin was specified as the proteolytic enzyme with a maximum of two missed cleavages. Carbamidomethylation was set as fixed modification of cysteine, whereas oxidation of methionine was chosen as variable modification. The monoisotopic mass tolerance for precursor ions and fragmentation ions were set to 10 ppm and 0.8 Da, respectively. Charge state of +2 or +3 were selected as precursor ions.

Proteome output files were submitted to the commercial software Scaffold (v3.6, Proteome Software, Portland, Oregon, USA). Peptide identifications were validated if they surpassed a 95% probability threshold set by the PeptideProphet algorithm. Protein identifications were accepted if they could be established at greater than 99.0% probability and contained at least two unique peptides. Proteins that contained shared peptides and could not be differentiated on the basis of MS/MS analysis alone were grouped to satisfy the principles of parsimony. Unweighted spectrum counts (USC) were used to assess the consistency of biological replicates in quantitative analysis, and normalized spectrum counts (NCS) was used to retrieve protein abundance.

[00224] Statistical Analysis. All statistical analyses were performed using PLS, Kohonen, and CPANN toolboxes, and graphs were created using Microsoft Excel, XLSTAT, and MATLAB.

[00225] Data matrix. Each row of the predictor matrix (X) relating to each individual is derived from all proteins' abundance obtained from the three-protein corona sensor array (**Fig. 7A**). In the preprocessing step, the normalized data in matrix X, relative protein abundance (RPA), were auto-scaled.

[00226] Classification and Clustering.

[00227] Partial least squares discriminant analysis (PLS-DA). Partial least squares discriminant analysis is a well-known multivariate approach regarded as a linear classification and dimension reduction method consisting of two main parts a structural part, which searches for latent variables as linear combinations of original independent variables (*i.e.*, data matrix X), which have the maximum covariation with the corresponding dependent-variables (*i.e.*, class membership, Y). The measured components include the latent variables as scores and loadings, which show how the latent variables and their original ones are related. Based on the ability of PLSDA to reduce the dimensionality of the data, it allows a linear mapping and graphical visualization of the different data patterns. PLS-DA is particularly well suited to deal with highly collinear and noisy patterns. The main problems associated with the large dataset in proteomics are the large number of monitored variables (*i.e.*, proteins) and relatively small number of samples. Hence, there may be a high redundancy among variables, which render many of them uninformative and irrelevant to the classification. In this way, eliminating uninformative variables or finding new uncorrelated ones may improve the predictive performance of classification. Since in biomedical applications

such as the present work, we must not only make decisions about whether a sample belongs to one of a number of known groups, but also determine which variables are most relevant for the best discrimination between classes, a method like PLS-DA is a good candidate approach for finding uncorrelated new latent variables while preserving the variation of the data [31]. The impotence power of the original variables to produce latent projections can also be calculated by the variable importance in the projection (VIP) analysis and can play a significant role in to decide about variables [32].

[00228] Identifying the most relevant variables based on weighted VIP. The partial least squares discriminant analysis (PLS- DA) was used to explore the VIP values associated with variables. VIP is a combined measure of how much a variable contributes to a description of the two sets of data: the dependent (Y) and the independent variables (X). The weights in a PLS model reflect the covariance between the independent and dependent variables, and the inclusion of the weights allows VIP to reflect not only how well the dependent variable is described but also how important that information is for the model of the independent variables [33].

[00229] An approach based on VIP score was developed to identify the best subset of variables. VIP scores can be calculated by performing PLS-DA on the dataset. In that approach, VIP scores of variables are calculated 50 times, each time using a random permutation of training and validation sets (random training sets were selected iteratively by considering 80-percent coverage of each class of objects). Considering the most important variables, the large VIP-score values (>2), the top 200 variables can be selected at each repetition and added to the top-variables pool. Afterward, a frequency of occurrence ($Freq_i$) and an average VIP-score ($\overline{VIP}_i$) for each variable can be obtained according to the top-variables pool. Thus, the selection of variable i (which has a high $\overline{VIP}_i$ value and low $\overline{VIP}_i$ value) is less recommended because of dependency on the training and validation sets. Therefore, the $\overline{VIP}_i$ value of each variable can be weighted by $Freq_i$, and ranking of the most relevant variables can be done using the weighted $\overline{VIP}_i$. Fig_appr is a schematic diagram of the proposed approach. Selection of the most relevant variables to build the classification model can be guided by the obtained ranking as follows: The highly ranked variables were added one by one to the dataset, and the classification error of PLS-DA was calculated to find the minimum number of relevant predictors (FIG. 2A).

[00230] Counter-Propagation Artificial Neural Network (CPANN). The

counter-propagation artificial neural network (CPANN) is a supervised variant of self-organizing map that consists of two layers of neurons arranged on a predefined $N \times N$ grid. CPANN can be used to map data from a high-dimensional feature space to a low-dimensional (typically 2) discrete space of neurons as well as to predict the class membership of the unknown samples. The input vectors (sample feature vector) and corresponding class membership vectors (a binary vector) are presented to the input and output layer of CPANN, respectively. The weight correction of the neurons in both layers performs based on competitive learning and cooperation of the neurons (See Table 3) [34, 35]. Hence, similar input vectors can be mapped on the same or adjacent neurons and vice versa. The final assignation map properly reveals the structure of the data in feature space and preserves the distance of patterns in the low-dimensional grid of neurons. **Fig. 8C** shows a high-quality assignation map of CPANN using top-ranked biomarkers. According to the distinct regions for each class, the risk of classification error is minimized. The proper size of the map can be decided by performing 10-fold cross-validation at different map sizes. The trained CPANN can be used to assign a class membership to an unlabeled sample [29]. Presence of redundant and uninformative variables in training data will affect the quality of the map and increase the risk of an error of classification (**Fig. 3C**). The process is a nonlinear mapping, which helps visualize a high-dimensional input object on a two-dimensional neuron grid. It is a self-organized procedure and solves the issue of classification in a transparent way. More details about the CPANN method can be found in the following references.

[00231] Hierarchical clustering analysis (HCA). Hierarchical clustering analysis is an unsupervised method widely used to explore and visualize whole heterogeneous large data sets like those often used in proteomics into distinct and homogeneous clusters. Strategies for hierarchical clustering can be divided into two categories: agglomerative and divisive methods. The agglomerative procedure first separates each object into its own individual cluster and then combines the clusters sequentially; similar objects or clusters are merged until every object belongs to only one cluster. The divisive procedure, in contrast, starts with all of the objects in one large cluster and gradually partitions them into smaller clusters until each object is in an individual cluster. Finally, objects are organized into a dendrogram whose branches are the defined clusters. In cluster analysis, to identify homogeneous sub-groups, the two important concepts, similarity (determining a numerical value for the similarity

between objects and constructing a similarity matrix) and linkage (connection of an object to a group or not) should be defined [36, 37]. Herein, we applied agglomerative hierarchical clustering with furthest-neighbor linkage algorithm for unsupervised analysis based on the selected variables.

[00232] Cohort sample prediction. The predictive ability of both linear and non-linear variables, with 69 selected predictors, were assessed by cohort samples analysis. To this end, the values related to 69 variables were put into the model, and the class membership of each cohort object was predicted at the fixed optimum parameters values. The 19 variables (proteins) in the proteomics profile of our protein corona sensor array of cohort samples were not detected, and zero were considered for these variables in validation data matrix. Interestingly, both linear and non-linear models provide good predictions for all five samples. The distance between training and cohort samples shown in **Fig. 4A** is most probably related to zero values that were added to the data matrix for those 19 absent variables. We examined the effect of replaced zero values for absent variables by deleting these 19 proteins from both data matrices of the training and prediction sets; then the PLS-DA model was built and cohort samples were predicted. As expected, no large distance between cohort and training samples was observed.

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[00233] Table 3. Correlation coefficient of CPANN weight map for each variable in the six classes.

Biomarker ID	CC(1) Control	CC(2) Glioblastoma	CC(3) Meningioma	CC(4) Myeloma	CC(5) Pancreas	CC(6) Lung
1323	-0.16	0.04	0.80	-0.41	0.16	-0.51
1282	-0.47	-0.04	0.11	0.90	-0.03	-0.37
1518	-0.22	0.76	-0.12	-0.12	-0.15	0.05
742	0.38	-0.06	-0.21	-0.72	-0.03	0.58
1462	-0.14	-0.25	-0.17	-0.20	-0.12	0.89
1373	-0.30	-0.13	-0.10	0.98	-0.21	-0.16
1466	-0.37	0.03	0.84	-0.31	0.14	-0.37
1313	-0.28	0.03	0.59	-0.05	0.37	-0.68
1630	0.97	-0.23	-0.31	-0.26	-0.19	-0.19
815	-0.33	-0.15	-0.24	0.65	-0.45	0.61
882	-0.16	-0.16	-0.25	-0.13	-0.16	0.91
1284	-0.47	-0.01	0.42	0.74	-0.34	-0.28
891	-0.37	-0.05	-0.33	0.55	-0.34	0.68
954	-0.19	-0.10	-0.25	-0.20	0.99	-0.20
782	-0.37	-0.32	-0.39	0.66	0.02	0.50
820	-0.34	-0.24	-0.43	0.36	0.02	0.72
1556	-0.18	-0.09	-0.25	-0.19	0.98	-0.20
1286	0.41	-0.35	0.18	-0.31	-0.51	0.40
868	0.15	-0.28	-0.44	0.16	-0.32	0.72
1308	0.42	0.26	-0.38	-0.45	-0.31	0.46
881	-0.12	-0.09	-0.30	-0.18	0.98	-0.24
848	-0.47	-0.04	-0.07	0.72	-0.49	0.47
1267	0.56	-0.01	-0.01	-0.61	-0.44	0.39
950	-0.18	-0.13	-0.26	-0.14	0.96	-0.20
1561	0.95	-0.24	-0.35	-0.28	-0.10	-0.17
856	-0.33	-0.18	-0.21	0.64	-0.43	0.60
895	-0.03	-0.16	-0.32	-0.20	-0.13	0.87
1270	-0.60	0.65	0.57	-0.08	-0.02	-0.37
1476	0.42	-0.11	-0.39	-0.32	-0.32	0.65
1416	0.59	-0.05	-0.10	-0.61	-0.36	0.40
786	-0.15	-0.19	-0.32	0.02	0.94	-0.25
751	-0.38	-0.23	-0.36	0.33	0.05	0.70
1316	0.81	-0.04	-0.43	-0.35	-0.22	0.11
1351	0.87	-0.35	-0.21	-0.05	-0.36	-0.12
992	0.90	-0.24	-0.29	-0.21	-0.17	-0.18
1272	-0.45	0.37	0.74	-0.17	0.07	-0.51
1363	-0.63	0.12	0.81	-0.12	0.05	-0.19
1486	-0.33	0.66	0.07	-0.18	-0.26	0.22
1496	-0.18	-0.08	-0.24	-0.18	0.93	-0.19
96	-0.23	-0.13	-0.22	-0.18	0.92	-0.11
1294	-0.23	-0.08	0.01	0.78	-0.08	-0.37
1027	-0.18	0.69	-0.02	-0.26	-0.05	-0.02
1007	-0.29	-0.11	0.86	-0.08	-0.23	-0.23
1362	-0.21	-0.11	-0.05	-0.23	0.87	-0.25

1302	0.29	-0.32	-0.37	0.75	-0.20	-0.19
802	-0.51	-0.22	-0.15	0.55	0.57	-0.13
1265	-0.37	0.33	0.67	-0.17	0.16	-0.59
1489	-0.45	0.10	0.78	-0.20	0.03	-0.26
878	-0.09	-0.26	-0.32	0.85	-0.06	-0.08
1338	-0.45	0.82	0.12	-0.07	-0.05	-0.16
826	0.18	0.40	0.38	-0.55	0.02	-0.47
1424	-0.19	-0.21	-0.32	-0.09	0.09	0.77
335	-0.20	-0.10	-0.27	-0.15	0.93	-0.16
879	-0.20	-0.03	-0.19	-0.21	0.89	-0.20
1305	-0.57	0.14	0.78	0.14	-0.03	-0.43
978	-0.12	-0.15	-0.25	-0.09	-0.17	0.82
769	-0.01	-0.36	-0.58	0.42	0.01	0.56
1567	-0.16	0.58	-0.15	-0.16	0.18	-0.14
35	0.60	-0.31	-0.39	-0.37	-0.13	0.46
1632	-0.15	-0.12	0.64	-0.10	-0.16	-0.19
1431	0.53	-0.35	0.42	-0.44	-0.24	-0.16
788	-0.45	0.11	-0.13	0.87	-0.22	-0.03
1333	-0.40	-0.10	-0.32	0.03	0.69	0.22
1357	0.47	0.01	-0.56	-0.14	-0.30	0.50
997	-0.16	-0.13	-0.22	-0.12	-0.16	0.83
1325	0.68	-0.19	0.04	-0.41	-0.44	0.13
1275	-0.45	0.50	-0.08	0.52	0.05	-0.34
1315	-0.15	0.34	0.66	-0.62	0.08	-0.33
1545	-0.12	-0.05	-0.26	-0.15	-0.21	0.85

For each variable, the correlation coefficient of corresponding weight map with the assignment map pattern can be calculated.

CC = 0: indicates no correlation between biomarker and the related class;

1 > CC(i) > 0: accordance between the biomarker intensity and cancer related class.

0 < CC < -1: an inverse correlation between biomarker value and cancer-related class.

The CC values >0.5 or <-0.5 are colored. For example, the weight map of biomarker 1282 is highly correlated with the cancer class 4 pattern on the assignment map, and it may be an important biomarker for the samples from patients with myeloma.

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Example 2: Additional sensor array construction for detection of diseases

[00325] A sensor array consisting of 12 different cross-reactive nanoparticles including three liposomes, three superparamagnetic iron oxide nanoparticles, and six gold nanoparticles are made as detailed in Figure 9. These types of liposomes (DOPG

(1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)), DOTAP(1,2-Dioleoyl-3-trimethylammonium-propane)-DOPE(dioleoylphosphatidylethanolamine), and CHOL (DOPC-Cholesterol)) with negative, neutral, and positive surface charges are synthesized according to our previous reports^{27, 29} as depicted in Figure 9. Ultra-uniform PEG-coated superparamagnetic iron oxide nanoparticles 20 nm in size and of various PEG molecular weights (*i.e.*, 300, 3000, and 6000) are obtained from Micromod®. Gold nanoparticles, with core size of ~ 2 nm, and different surface functionalities, as shown in Figure 10, are synthesized according to our previous report.²⁸

[00326] All of the nanoparticles are the same size but have different surface properties, which are expected to form significantly different protein corona compositions in response to the plasma of patients developing various types of cancers. The sensor array probes the capability of the protein corona sensor array to identify and discriminate among cancer types via the patient's plasma proteins, as described in Example 1.

[00327] Samples from a wide range of cancers including lung, pancreas, myeloma, meningioma, glioblastoma, esophageal squamous cell carcinoma, and gastric adenocarcinoma are probed. Protein corona arrays based on different nanoparticles (liposomes, iron oxide, and gold) are designed to probe the variation in biomolecule fingerprint among various cancer types. The sensor assay will also allow for the identification of proteins that are markers for specific types of cancers, including new and unknown biomarkers for different cancer cell types. The pattern recognition that can be determined based on the different types of cancer or the stage of cancer.

[00328] A wide range of human plasmas from healthy people that were later diagnosed with various types of cancers several years after plasma collection will be analyzed. The plasma samples were collected through a NIH-funded cohort study, named the Golestan Cohort Study, performed by the National Cancer Institute (NCI) in the USA, the International Agency for Research on Cancer (IARC) in France, and the Tehran University of Medical Sciences (TUMS) in Iran. This study involved the collection and storage of plasma from 50,000 healthy subjects. Over 1,000 of these subjects went on to develop various types of cancers in subsequent years. The samples are stored at IARC and being used by our team for analysis. These

important plasma samples provide us a unique opportunity to probe the capacity of our innovative protein corona sensor array for early detection of cancers. In addition, we will also assess and identify proteins useful for the identification and discrimination of these cancers.

[00329] We believe that the outcomes from applying this innovative sensor array to cohort plasma samples will not only be instrumental in the detection and screening of cancers at early stages but also help identify novel protein markers involved in cancer development. Our sensor array component choices have more specific capability compared to other developed methods to provide fingerprints for a wide variety of proteins in a non-specific, cross-reactive manner for identification and discrimination of cancers.

The hard corona profiles of the sensor array elements are probed using plasma from patients with cancers at intermediate and advanced stages:

[00330] The composition of the protein corona that forms on the surface of sensor array elements is strongly dependent on the physicochemical properties of those nanoparticles⁹⁷ and, at the same time, can be strongly affected by the type of disease present in the donor of the human plasma used for incubation^{14, 15}. To prepare the corona-coated nanoparticles, the 12 nanoparticles prepared are incubated with human plasma (separately) of nine types of cancers (lung, pancreas, myeloma, myeloid leukemia, meningioma, glioblastoma, breast, esophageal squamous cell carcinoma, and gastric adenocarcinoma) and isolated from free proteins *via* a well-defined centrifugation approach^{33, 60, 61}. Centrifugation is usually performed at 13000g for 30 min at 15°C. The supernatant will be removed and the collected particles would be re-dispersed in 500 microlitter of PBS. The procedure will be repeated to get the loosely attached proteins removed. In order to remove the loosely attached proteins from the surface of nanoparticles, the collected nanoparticles will be redispersed in cold PBS (15°C) and collected via centrifugation. The size and charge of the corona-coated nanoparticles will then be determined using DLS/Nanosight and compared to their initial values obtained in buffer. Quantitative evaluation of the total protein adsorbed onto the nanoparticles will be performed *via* the BCA or NanoOrange assay, while qualitative shotgun proteomics analysis identify the proteins adsorbed onto the surface of the 12 nanoparticles. Briefly, after separation

of proteins from the surface of nanoparticles (according to the protocol (Saha, K.; Rahimi, M.; Yazdani, M.; Kim, S. T.; Moyano, D. F.; Hou, S.; Das, R.; Mout, R.; Rezaee, F.; Mahmoudi, M. ACS nano 2016, 10, (4), 4421-4430, incorporated by reference), proteins are injected into a liquid chromatography-mass spectrometry (LC-MS/MS) apparatus. The proteins are identified from the resulting data through screening of relevant databases. To obtain the total number of LC-MS/MS spectra for all peptides attributed to a matched protein, semi-^aPolydispersity index from cumulant fitting quantitative assessment of the protein amount will be conducted through spectral counting (SpC). The normalized SpC (NpSpC) amounts of each protein identified in the LC-MS/MS spectra will be calculated using the following equation:⁹⁸

$$\text{[00331]} \quad NpSpCk = \left(\frac{\frac{SpC}{(M_w)_k}}{\sum_{t=1}^n \left(\frac{SpC}{(M_w)_t} \right)} \right) \times 100$$

where NpSpCk is the normalized percentage of spectral count (i.e., raw counts of ions) for protein k, SpC is the spectral count, and Mw is the molecular weight (in kDa) of the protein k.

Develop supervised and unsupervised clustering analysis to identify and discriminate among cancers using the sensor array outcomes:

[00332] In order to investigate whether protein corona fingerprints (PCFs) of various sensor elements could be utilized as a biosensors and form unique patterns for different diseases (biomolecule corona signature), we have applied focused classification approaches to proteomic data from three liposomes' protein corona composition (cationic, anionic, and neutral) as described in Example 1.

[00333] Example 3: Conjugation of nanoparticles to the substrate to make sensor arrays.

[00334] Different types of particles may be used as nanoscale sensor elements in the practice of this invention. Further, different methods of conjugation of the nanoscale sensor elements to the substrate are provided. Additionally, the nanoscale sensor elements may be attached to substrate in different patterns.

[00335] Specific examples of different configurations of nanoscale sensor elements on different substrates are exemplified in Fig. 16-43.

CLAIMS

1. A sensor array comprising a plurality of nanoscale sensor elements,
wherein the plurality of nanoscale sensor elements differ from each other in at least one physicochemical property,
wherein the plurality of sensor elements comprises at least two sensor elements;
and
wherein each sensor element is able to bind a plurality of biomolecules in a sample to produce a biomolecule corona signature, wherein each nanoscale sensor elements has a distinct biomolecule corona signature from the other.
2. The sensor array of claim 1, wherein the plurality of sensor elements produces a plurality of biomolecule corona signatures when contacted by the sample, wherein the combination of the plurality of biomolecule corona signatures produces a biomolecule fingerprint for the sample.
3. The sensor array of any one of the preceding claims, wherein the biomolecule corona signature comprises at least one protein, at least one polypeptide, at least one lipid, at least one metabolome, at least one oligonucleotide or combination thereof.
4. The sensor array of any one of the preceding claims, wherein the first nanoscale sensor element produces a first biomolecule corona and the second nanoscale sensor element produces a second biomolecule corona when the sensor array is contacted with the sample.
5. The sensor array of any one of the preceding claims, wherein the at least one physicochemical property is selected from the group consisting of composition, size, surface charge, hydrophobicity, hydrophilicity, surface functionality, surface topography, and shape.
6. The sensor array of any one of the preceding claims, wherein the sensor array further comprises a substrate.

7. The sensor array of claim 6, wherein the nanoscale sensor elements are covalently linked to the substrate.
8. The sensor array of claim 6, wherein the nanoscale sensor elements are non-covalently linked to the substrate.
9. The sensor array of any one of claims 6-8, wherein the substrate binds to the plurality of nanoscale sensory elements in a distinct pattern.
10. The sensor array of any one of the preceding claims, wherein the plurality of sensor elements are a plurality of particles.
11. The sensor array of any one of the preceding claims, wherein the plurality of sensor elements are a plurality of particles immobilized on a substrate.
12. The sensor array of any one of the preceding claims, wherein the sensor elements are organic particles, non-organic particles or combinations thereof.
13. The sensor array of any one of the preceding claims, wherein the sensor elements are particles and the particles are selected from the group consisting of nanoparticles, microparticles, micelles, liposomes, iron oxide, graphene, silica, protein-based particles, polystyrene, silver, and gold particles, quantum dots, and combination therefore.
14. The sensor array of claim 13, wherein the particles are liposomes selected from the group consisting of DOPG (1,2-dioleoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol), DOTAP(1,2-Dioleoyl-3 trimethylammonium-propane)- DOPE (dioleoylphosphatidylethanolamine), CHOL (DOPC-Cholesterol), and combinations thereof.

15. The sensor array of any one of claims 1-12, wherein the plurality of sensor elements comprise a plurality of half particles of different shapes made by molding technology.
16. The sensor array of claim 15, wherein the plurality of half particles are in a shape selected from the group consisting of spheres, rods, cubes and combinations thereof.
17. The sensor array of any one of claims 1-12, wherein the plurality of sensor elements comprise a plurality of half particles of different physicochemical properties (e.g., spheres, rods, and cubes) made by 3-D printing.
18. The sensor array of any one of the preceding claims, wherein the sensor array comprises at least 2 to at least 100 nanoscale sensor elements.
19. The sensor array of any one of claims 2-18, wherein the biomolecule fingerprint is associated with a disease or disorder.
20. The sensor array of claim 19, wherein the disease or disorder is cancer, cardiovascular disease, endocrine disease, inflammatory disease, or a neurological disease.
21. The sensor array of claim 20, wherein the disease is cancer selected from the group consisting of lung cancer, pancreas cancer, myeloma, myeloid leukemia, meningioma, glioblastoma, breast cancer, esophageal squamous cell carcinoma, gastric adenocarcinoma, prostate, bladder, ovarian, thyroid, and neuroendocrine cancer.
22. A method of detecting a disease state in a subject comprising:
 - (a) obtaining a sample from the subject;
 - (b) contacting the sample with a sensor array of any one of claims 1-21,
 - (c) determining a biomolecule fingerprint associated with the sample,wherein the biomolecule fingerprint differentiates the disease state of the subject.

23. The method of claim 22, wherein the disease state is selected from no disease or disorder, having a precursor of a disease or disorder, and having disease or disorder.
24. The method of claim 22 or 23, wherein step (c) comprises determining the combined composition of the plurality of biomolecule corona associated with the plurality of nanoscale sensor elements to produce the biomolecule fingerprint of the sample.
25. The method of claim 22 or 23, wherein step (c) comprises
assaying a biomolecule corona signature associated with each sensor element,
and
combining the plurality of biomolecule corona signatures to produce the biomolecule fingerprint associated with the sample.
26. The method of any one of claims 22-25, wherein step (c) comprises
separating the plurality of biomolecules of each biomolecule corona from each sensor elements and
assaying the plurality of biomolecules to determine the biomolecule composition of the biomolecule corona to determine the biomolecule fingerprint.
27. The method of any one of claims 22-26, wherein the assaying of the plurality of biomolecule is selected from the group consisting of gel-electrophoresis, liquid chromatography, mass spectrometry, NMR, FTIR, Circular Dichroism, Raman spectrometry, and a combination thereof.
28. The method of any one of claims 22-27, wherein the biomolecule fingerprint is associated with a disease state that is associated with a stage of a disease or disorder.
29. The method of any one of claims 22-28, wherein the disease or disorder is selected from the group consisting of cancer, cardiovascular disease, endocrine disease, inflammatory disease, and neurological disease.

30. The method of any one of claims 22-29 wherein the disease state is associated with cancer.
31. The method of claims 30, wherein the cancer selected from the group consisting of lung cancer, pancreas cancer, myeloma, myeloid leukemia, meningioma, glioblastoma, breast cancer, esophageal squamous cell carcinoma, gastric adenocarcinoma, prostate, bladder, ovarian, thyroid, and neuroendocrine.
32. The method of any one of claims 22-31, wherein the method detects the disease state that is associated with an early stage of a disease or disorder.
33. The method of any one of claims 22-32, wherein the method further comprises:
comparing the biomolecule fingerprint of the sample to a panel of biomolecule fingerprints associated with a plurality of disease states to determine which disease state is associated with the sample.
34. A method of determining a biomolecule fingerprint associated with at least disease state or at least one disease or a disorder, the method comprising the steps of:
(a) obtaining a samples from at least two subjects diagnosed with the disease state or the at least one disease or disorder;
(b) contacting each sample with the sensor array of any one of claims 1-21, and
(c) determining a biomolecule fingerprint for the sensor array that is associated with the disease state or at least one disease or disorder.
35. The method of claim 34, wherein step (c) further comprises detecting the composition of the biomolecule corona of each sensor element, wherein the combination of the composition of each biomolecule corona between the different sensor elements produce the biomolecule fingerprint associated with the sample.
36. The method of claim 34 or 35, wherein step (c) further comprises dissociating the biomolecule corona from each nanoscale sensor element to produce a plurality of biomolecules and detecting the plurality of biomolecules to produce the biomolecule fingerprint.

37. The method of any one of claims 34-36, wherein the method further comprises obtaining control sample from at least one control subject, contacting the control sample with the sensor array of any one of claims 1-21 to produce a biomolecule fingerprint for the control sample, and comparing the biomolecule fingerprint of the control sample to the biomolecule fingerprint from the subject with the disease state or at least one disease or disorder to determine a biomolecule fingerprint associated with the disease state or at least one disease or disorder.
38. The method of any one of claims 34-37, wherein the detecting of the plurality of biomolecules is selected from gel-electrophoresis, liquid chromatography, mass spectrometry, NMR, FTIR, Circular Dichroism, Raman spectrometry, and a combination thereof.
39. A method of diagnosing or prognosing a disease or disorder in a subject, obtaining a sample from a subject; contacting the sample with a sensor array of any one of claims 1-21 to produce a biomolecule fingerprint, comparing the biomolecule fingerprint to a panel of biomolecule fingerprints associated with a plurality of diseases or disorders; and diagnosing or prognosing the disease or disorder.
40. The method of claim 39, wherein the disease or disorder is cancer, cardiovascular disease, endocrine disease, inflammatory disease, or a neurological disease.
41. The method of claim 40, wherein the disease is cancer selected from the group consisting of lung cancer, pancreas cancer, myeloma, myeloid leukemia, meningioma, glioblastoma, breast cancer, esophageal squamous cell carcinoma, and gastric adenocarcinoma.

42. The method of any one of claims 39-41, wherein the method is able to diagnose early stage of a cancer.

43. A method of identifying a pattern of biomarkers associated with a disease or disorder, the method comprising:

(a) obtaining a samples from at least two subjects diagnosed with the disease or disorder and at least two control subjects;

(b) contacting each sample with the nanoscale sensor array of any one of claims 1-21 to produce a plurality of biomolecule corona for a plurality of nanoscale sensor elements for each subject, and

(c) comparing the composition of the plurality of biomolecule corona of the subjects with the disease or disorder to the composition of the plurality of biomolecule cornona of the control subjects to determine a pattern of biomarkers associated with the disease or disorder.

44. The method of claim 43, wherein step (a) comprises obtaining samples from at least 5 subjects diagnosed with the disease or disorder and at least 5 control subjects.

45. The method of claim 43 or 44, wherein the composition of the plurality of biomolecule corona is determined by gel-electrophoresis, liquid chromatography, mass spectrometry, NMR, FTIR, Circular Dichroism, Raman spectroscopy, and a combination thereof.

46. The method of any one of claims 43- 45, wherein the disease or disorder is cancer, cardiovascular disease, or a neurological disease.

47. The method of claim 46, wherein the disease is cancer selected from the group consisting of lung cancer, pancreas cancer, myeloma, myeloid leukemia, meningioma, glioblastoma, breast cancer, esophageal squamous cell carcinoma, and gastric adenocarcinoma.

48. The method of claim 43 or 44, wherein the cancer is glioblastoma, and wherein the biomarker is selected from the group consisting of HABP1, VTNC, C03, ITIH2, ITIH1,

CO7, FHR5, CBPN, ALBU, PLMN, CO4A, PRDX2, VWF, C4BPA, APOB, HBB, CNDP1, CRP, SAA4, APOE, CSCL7 and combinations thereof.

49. The method of claim 43 or 44, wherein the cancer is meningioma, and wherein the biomarker is selected from the group consisting of FCN3, RET4, HABP2, CBPN and combinations thereof.

50. The method of claim 43 or 44, wherein the cancer is pancreatic cancer and wherein the biomarker is selected from the group consisting of KNG1, IC1, CBPB2, TRFE, GELS, CXCL7, HPTR, PGK1, AACT, LUM, APOE, FIBB, APOA2, A1BG, A1AT, LBP, APOA1, H4, FIBG and combinations thereof.

51. The method of claim 43 or 44, wherein the cancer is lung cancer and the biomarker is selected from the group consisting of COO, CRP, SAA4, APOA1, A1AT, GELS and combinations thereof.

52. The method of claim 43 or 44, wherein the cancer is myeloma and the biomarker is ALBU.

53. A kit for diagnosing or prognosing a disease or disorder, the kit comprising:
a sensor array of any one of claims 1-21.

54. A kit for determining and/or detecting at least one biomarker associated with a disease or disorder, comprising at least one sensor array of any one of claims 1-21.

TITLE

ABSTRACT OF THE DISCLOSURE

The present disclosure provides sensor arrays for detecting biomolecules and methods of use. In some embodiments, the sensor arrays are capable of determining a disease state in a subject.

Provisional Application for Patent Cover Sheet

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

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Title of Invention

SYSTEM AND METHOD FOR PROTEIN CORONA SENSOR ARRAY FOR
EARLY DETECTION OF DISEASES

Attorney Docket Number (if applicable)

129319.00437.BWH24258.01

Correspondence Address

Direct all correspondence to (select one):

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☐ Firm or Individual Name

Customer Number

26710

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

Yes, the invention was made by an agency of the United States Government. The U.S. Government agency name is:

Yes, the invention was under a contract with an agency of the United States Government. The name of the U.S. Government agency and Government contract number are:

Entity Status

Applicant asserts small entity status under 37 CFR 1.27 or applicant certifies micro entity status under 37 CFR 1.29

- ☒ Applicant asserts small entity status under 37 CFR 1.27
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Signature	Angeline R. Babel/			Date (YYYY-MM-DD)	2016-12-16
First Name	Angeline	Last Name	Babel	Registration Number (If appropriate)	60886

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **This form can only be used when in conjunction with EFS-Web. If this form is mailed to the USPTO, it may cause delays in handling the provisional application.**

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	129319.00437.BWH24258.01
		Application Number	
Title of Invention	SYSTEM AND METHOD FOR PROTEIN CORONA SENSOR ARRAY FOR EARLY DETECTION OF DISEASES		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

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Inventor	1				Remove
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PREOMICS EXHIBIT 1017

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	129319.00437.BWH24258.01
		Application Number	
Title of Invention	SYSTEM AND METHOD FOR PROTEIN CORONA SENSOR ARRAY FOR EARLY DETECTION OF DISEASES		

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Application Information:

Title of the Invention	SYSTEM AND METHOD FOR PROTEIN CORONA SENSOR ARRAY FOR EARLY DETECTION OF DISEASES		
Attorney Docket Number	129319.00437.BWH24258.01	Small Entity Status Claimed	☒
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	47	Suggested Figure for Publication (if any)	

Filing By Reference:

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For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	129319.00437.BWH24258.01
		Application Number	
Title of Invention	SYSTEM AND METHOD FOR PROTEIN CORONA SENSOR ARRAY FOR EARLY DETECTION OF DISEASES		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status		Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
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Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013. NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	129319.00437.BWH24258.01
	Application Number	
Title of Invention	SYSTEM AND METHOD FOR PROTEIN CORONA SENSOR ARRAY FOR EARLY DETECTION OF DISEASES	

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When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

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NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	129319.00437.BWH24258.01
		Application Number	
Title of Invention	SYSTEM AND METHOD FOR PROTEIN CORONA SENSOR ARRAY FOR EARLY DETECTION OF DISEASES		

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant	1	Remove
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section. Clear		
☒ Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
Person to whom the inventor is obligated to assign.		Person who shows sufficient proprietary interest
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:		
Name of the Deceased or Legally Incapacitated Inventor:		
If the Applicant is an Organization check here. ☒		
Organization Name	BRIGHAM AND WOMEN'S HOSPITAL, INC.	
Mailing Address Information For Applicant:		
Address 1	75 Francis Street	
Address 2		
City	Boston	State/Province MA
Country	US	Postal Code 02115
Phone Number		Fax Number
Email Address		
Additional Applicant Data may be generated within this form by selecting the Add button. Add		

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	129319.00437.BWH24258.01
		Application Number	
Title of Invention	SYSTEM AND METHOD FOR PROTEIN CORONA SENSOR ARRAY FOR EARLY DETECTION OF DISEASES		

Assignee	1			
Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.				
Remove				
If the Assignee or Non-Applicant Assignee is an Organization check here. ☐				
Prefix	Given Name	Middle Name	Family Name	Suffix
Mailing Address Information For Assignee including Non-Applicant Assignee:				
Address 1				
Address 2				
City		State/Province		
Country ⁱ		Postal Code		
Phone Number		Fax Number		
Email Address				
Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add				

Signature:[Remove](#)

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Angeline R. Babel/		Date (YYYY-MM-DD)	2016-12-16
First Name	Angeline	Last Name	Babel	Registration Number
				60886
Additional Signature may be generated within this form by selecting the Add button. Add				

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	129319.00437.BWH24258.01
		Application Number	
Title of Invention	SYSTEM AND METHOD FOR PROTEIN CORONA SENSOR ARRAY FOR EARLY DETECTION OF DISEASES		

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal				
Application Number:				
Filing Date:				
Title of Invention:		SYSTEM AND METHOD FOR PROTEIN CORONA SENSOR ARRAY FOR EARLY DETECTION OF DISEASES		
First Named Inventor/Applicant Name:		Morteza Mahmoudi		
Filer:		Angeline R. Babel/Angela Schulz		
Attorney Docket Number:		129319.00437.BWH24258.01		
Filed as Small Entity				
Filing Fees for Provisional				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
PROVISIONAL APPLICATION FILING FEE	2005	1	130	130
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				130

Electronic Acknowledgement Receipt

EFS ID:	27817594
Application Number:	62435409
International Application Number:	
Confirmation Number:	1669
Title of Invention:	SYSTEM AND METHOD FOR PROTEIN CORONA SENSOR ARRAY FOR EARLY DETECTION OF DISEASES
First Named Inventor/Applicant Name:	Morteza Mahmoudi
Customer Number:	26710
Filer:	Angeline R. Babel/Angela Schulz
Filer Authorized By:	Angeline R. Babel
Attorney Docket Number:	129319.00437.BWH24258.01
Receipt Date:	16-DEC-2016
Filing Date:	
Time Stamp:	16:05:32
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$ 130
RAM confirmation Number	121916INTEFSW00001602170055
Deposit Account	
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File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Drawings-other than black and white line drawings	129319_00437_drawings.pdf	18331023	no	47
			a10e48fae02c7f290d9060e58296c083aca1e0d0		
Warnings:					
Information:					
2		129319_00437_application.pdf	597174	yes	80
			17bc4dd3ee22650f9c8eb4555e914d7086f67107		
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Specification		1	71	
	Claims		72	79	
	Abstract		80	80	
Warnings:					
Information:					
3	Provisional Cover Sheet (SB16)	129319_00437_cover_sheet.pdf	1477246	no	3
			035abb07e281803abf8074fee98df2f3f0c852f6		
Warnings:					
Information:					
4	Application Data Sheet	129319_00437_ADS.pdf	1822883	no	8
			9e197e70321388f23e1e29ed123a5726a3792eaf		
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	30270	no	2
			2b462d26e4030f103159cbfb6cfe754db0aa3ac8		

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Warnings:	
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Total Files Size (in bytes):	22258596
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Application Number: 62435409

Document Date: 12/16/2016

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