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(71) Applicant: **Ciphergen Biosystems, Inc.**
Fremont, CA 94555 (US)

(72) Inventor: **Boschetti, Egisto**
78290 Croissy sur Seine (FR)

(74) Representative: **Woods, Geoffrey Corlett**
J.A. Kemp & Co
14 South Square
Gray's Inn
London WC1R 5JJ (GB)

(54) **Methods for reducing the variance between analyte concentrations taken from complex sample mixtures**

(57) The present invention relates to the fields of molecular biology, combinatorial chemistry and biochemistry. Particularly, the present invention describes methods and kits for dynamically reducing the variance between analyte taken from complex mixtures.

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to the fields of molecular biology, combinatorial chemistry and biochemistry. Particularly, the present invention describes methods and kits for dynamically reducing the variance between analyte taken from complex mixtures.

BACKGROUND OF THE INVENTION

[0002] The full analysis of analytes in complex biological extracts is hindered by the large difference in concentration between individual analytes. In most biological mixtures some analytes are present at high concentration and others only present at trace-levels. As a result, the native concentration of analytes may not be adapted to the sensitivity of a given analytical method. For example, highly concentrated proteins may saturate the detection system and very low concentrations may be below the sensitivity of the analytical method, as occurs in human serum where the difference concentration between the most abundant protein (albumin) and the least abundant (e.g., some cellular factors) may reach levels as high as hundreds of millions.

[0003] Two ways are currently followed to reduce this gap: the first is to design more adapted instruments and the second is to eliminate the most abundant species so as to reduce the difference in the concentration of remaining analytes. This second method suffers from the fact that elimination of some analyte species also eliminates species that interact with them. Thus some species that may be of interest are eliminated. Moreover, highly abundant species are represented by several proteins (even several dozen in some situations) and therefore a number of specific methods would have to be designed to address each different abundant species. While eliminating proteins of high abundance may in some instance help, this approach does not allow concentrating very low abundance proteins that are still too dilute to be detected by current instruments.

SUMMARY OF THE INVENTION

[0004] A way to reduce the difference in concentrations between analytes rendering a complex mixture compatible with current instruments would be desirable, especially for protein profiling in view of identifying biomarkers of interest, particularly in those instances where high throughput analysis techniques are desirable.

[0005] Accordingly, the present invention provides methods for reducing the relative concentration differences between analytes in a sample. These methods have a number of steps including, in no particular order, a) providing a test sample comprising first different amounts of a plurality of different analytes, wherein the first different amounts have a first variance; b) contacting the test sample with amounts of each of a plurality of different binding moieties; c) capturing amounts of the different analytes from the test sample with the different binding moieties and removing unbound analytes; and d) isolating the captured analytes from the binding moieties to produce a second sample comprising second amounts of a plurality of different analytes, such that the second amounts have a second variance and the amounts of each of the plurality of different binding moieties are selected to capture amounts of the different analytes whereby the second variance is less than the first variance. In some aspects of the above methods the test sample is a biological fluid selected from the group consisting of blood, biopsy tissue amniotic fluid, urine, sweat, saliva, and a cell extract. In other aspects the sample is an environmental sample. Certain aspects of the described methods are for the analysis of at least 5, preferably at least 10, at least 100, at least 1000, at least 100,000, at least 1,000,000, at least 10,000,000 and at least 100,000,000 different analytes.

[0006] In some method aspects the binding moieties are contacted with the test sample by admixing. Alternative aspects include binding moieties that are in the form of a combinatorial library. Binding moieties of the methods may include bio-organic polymers, which may be selected from the group consisting of peptides, oligonucleotides and oligosaccharides. In some of these aspects the bio-organic polymer is a peptide at least four amino acids in length. In other aspects the peptides are antibodies. Particular aspects include peptide binding moieties that are surface molecules of a phage display library. Such peptide binding moieties include antibodies. In other aspects the bio-organic polymers acting as binding moieties are oligonucleotides of at least fifteen units in length. In particular aspects oligonucleotide binding moieties are aptamers. Binding moieties useful in the described methods also include oligosaccharides of at least five monosaccharide units in length. Other sources of binding moieties include molecular combinatorial libraries based on organic molecular scaffolds and composed of a large variety of diversomers.

[0007] The described methods may be performed with the analyte in a gaseous state, a liquid state, solution or in a solid suspension. Unbound analytes may be removed by techniques that include washing the captured analytes with a wash buffer.

[0008] Typically isolating the captured analytes using the described methods includes eluting the analytes from the binding moieties by contacting the captured analytes with an elution buffer and collecting the analytes in the elution buffer free of binding moieties. Elution buffers suitable for use with the methods of the present invention include a component selected from the group consisting of an acid, a base, a detergent, a chaotropic agent, a salt, an organic solvent, a competing ligand for the binding moiety or a mixture of two or more described groups.

[0009] Performance of the methods typically results in a second variance having a value at least 5%, more preferably 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 65%, 75%, 80%, 85%, advantageously 90%, 92%, 95% or 98% the value of the first variance. For example, if the first variance is 10 and the second variance is 9, the second variance has 90% the value of the first variance.

[0010] Typically, the methods involve adsorbing analytes to different binding moieties that are coupled to a solid support. In some aspects of the invention, the solid support comprises a hydrogel. in other aspects the solid support is a plurality of insoluble surfaces, each insoluble surface coupled to a single species of binding moiety. Preferred embodiments have solid supports that are insoluble surfaces, ideally in the form of spherical beads or irregular particles.

[0011] In certain aspects of the methods the binding moieties are coupled to the solid support through a linker. Linkers include molecules that covalently or non-covalently couple the binding moieties of the invention to a solid support, and include, but are not limited to, capture moieties. Exemplary capture moieties include FLAG tag, His tag and biotin.

[0012] Some embodiments of the described methods include detecting the isolated analytes. In certain aspects, detecting the analytes is performed using an assay selected from the group consisting of: colorimetric, spectrophotometric, magnetic resonance, ellipsometric, mass spectroscopic, electrophoretic (single and double dimension) and enzymatic. In some aspects where the detection assay is mass spectroscopic, the second variance of the isolated analytes allows quantifying of at least 30%, more preferably at least 35%, 40%, 45%, 50%, 60% or more of the captured analytes using a single detector setting.

[0013] The present invention also provides kits for detecting a plurality of analytes in a sample. The kit includes (a) a plurality of binding moieties, each binding moiety in an amount selected to capture a pre-determined amount of a different analyte; (b) a plurality of containers retaining components for sample preparation and analyte isolation; and (c) instructions for using the kit. In some aspects of the kits the binding moieties are coupled to the solid support. In other aspects the components for sample preparation comprise a wash solution sufficient for removing unbound material from a binding moiety specifically bound to an analyte, and an elution solution sufficient to release analyte specifically bound by a binding moiety.

[0014] Additional kit embodiments of the present invention include optional functional components that would allow one of ordinary skill in the art to perform any of the method variations described herein.

[0015] DEFINITIONSUnless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton *et al., Dictionary of Microbiology and Molecular Biology* (2nd ed. 1994); *The Cambridge Dictionary of Science and Technology* (Walker ed., 1988); *The Glossary of Genetics,* 5th Ed., R. Rieger *et al.* (eds.), Springer Verlag (1991); and Hale & Marham, *The Harper Collins Dictionary of Biology* (1991). As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

[0016] "Analyte" refers to any molecular moiety capable of binding to a binding moiety of the present invention in a manner that is not completely disrupted by contact with a wash solution as described herein. "Captured analyte" is any analyte bound by a binding moiety of the present invention after contact with a wash solution.

[0017] Analytes are found in "test samples." A test sample is any composition, preferably an aqueous solution, that may contain analytes of interest and is in a physical state which allows any analyte of interest present in the test sample to be contacted with a binding moiety of the present invention. Test samples may be taken from any source that potentially includes analytes of interest including environmental samples such as air, water, dirt, extracts and the like. Alternatively test samples may be taken from biological sources including biological fluids such as blood, biopsy tissue, amniotic fluid, milk, urine, sweat, saliva, cell expression supernatants and a cell extracts. The test sample may be an undiluted potion of the source material, or may be treated in a variety of ways including dilution, filtering, sifting, percolating, etc, that improves the properties of the source material making it more amenable for use in the methods of the present invention.

[0018] "Adsorbent" or "capture reagent" refers to any material capable of binding an analyte (e.g., a target polypeptide). "Chromatographic adsorbent" refers to a material typically used in chromatography. Chromatographic adsorbents include for example, ion exchange materials, metal chelators, hydrophobic interaction adsorbents, hydrophilic interaction adsorbents, dyes, and mixed mode adsorbents (e.g., hydrophobic attraction/electrostatic repulsion adsorbents). "Biospecific adsorbent" refers to an adsorbent comprising a biomolecule, e.g., a nucleotide, a nucleic acid molecule, an amino acid, a polypeptide, a simple sugar, a polysaccharide, a fatty acid, a lipid, a steroid or a conjugate of these (e.g., a glycoprotein, a lipoprotein, a glycolipid). In certain instances the biospecific adsorbent can be a macromolecular structure such as a multiprotein complex, a biological membrane or a virus. Examples of biospecific adsorbents are antibodies, receptor proteins and nucleic acids. Biospecific adsorbents typically have higher specificity for a target analyte than a chromatographic adsorbent. Further examples of adsorbents for use in SELDI can be found in U.S. Patent 6,225,047 (Hutchens and Yip, "Use of retentate chromatography to generate difference maps," May 1, 2001).

[0019] "Adsorb" refers to the detectable binding between an absorbent and an analyte either before or after washing with an eluant (selectivity threshold modifier).

[0020] "Capture moiety" refers to a composition that can specifically bind to certain types of adsorbent surfaces comprising a complementary binding partner for the particular capture moiety used. The chemistry involved in the binding reaction between a capture moiety and an adsorbent surface is dependent upon the nature of the capture moiety/adsorbent pair used. For example, hexahistidine sequences added to a polypeptide or protein chelate to adsorbents comprising nickel atoms, FLAG sequences are recognized and bound noncovalently by FLAG-specific antibodies, and adsorbent surfaces comprising receptors or enzymes can specifically bind capture moieties comprising their respective ligands and substrates, or homologues thereof. Typically, capture moieties are covalently attached to proteins, and serve as a means for anchoring the proteins to an adsorbent surface.

[0021] "Hydrogel" refers to a colloid in which particles are in the external or dispersion phase and water in the internal or dispersed phase.

[0022] "Solid support" refers to any insoluble surface including beads or plastic strips. The term also refers to a solid phase to which an adsorbent is attached or deposited.

[0023] "Binding moiety" refers to any first composition that recognizes and binds to a second composition in a manner that is determinative of the presence of the second composition in a heterogeneous population of molecules. Thus, under designated conditions, the first composition binds to the second composition at least two times the background and does not substantially bind in a significant amount to other molecules present in the sample.

[0024] In the case of binding moieties that are antibodies, specific binding may require selection of an antibody for its specificity. For example, polyclonal antibodies raised to Ras protein from specific species such as rat, mouse, or human can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with Ras protein and not with other proteins, except for polymorphic variants and alleles of Ras protein. This selection may be achieved by subtracting out antibodies that cross-react with Ras proteins from other species.

[0025] A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular analyte. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with an analyte (*see, e.g.,* Harlow & Lane, *Antibodies, A Laboratory Manual* (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically, a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

[0026] "Binding moiety" also refers to small molecules, particularly small biopolymers that are bound by analytes in solution. Binding moieties of the present invention include, but are not limited to, peptides, nucleic acids and oligosaccharides. Biopolymer binding moieties are typically constructed of at least 4 monomeric units, more preferably 6 monomeric units, desirably 10, 15, 20, 30 monomeric units or more.

[0027] Biopolymer binding moieties are particularly preferred in the present invention as they lend themselves to construction of "combinatorial libraries." A combinatorial library is a system using a random assortment of components at any positions in the linear arrangement of atoms, i.e., a combinatorial library of nucleic acid mutations could contain positions where all four bases have been randomly inserted at each position. Combinatorial libraries may be complete or incomplete. Complete combinatorial libraries of biopolymers are those libraries containing a representative of every possible permutation of monomers for a given polymer length. Incomplete libraries are those libraries lacking one or more possible permutation of monomers for a given polymer length.

[0028] Binding moieties may exist and interact with analytes detectable using the present invention in any physical state compatible with formation of molecular interactions, including gaseous, aqueous and organic suspensions and emulsions and, most preferably in a liquid state.

[0029] Binding moieties of the present invention may be in solution, suspension, or in any other situation allowing contact of the binding moiety with analyte including mounted on a solid support. One form of solid support is a "phage display library." A phage display library is formed from bacteriophage that has been recombinantly manipulated to express binding moiety as part of the phage protein coat. Using phage display, libraries of binding moieties may be easily constructed and screened for those members useful in binding analytes of interest.

[0030] "Variance" in the context of the present invention refers to the mathematical variance in the concentrations of analytes in a test sample. In simplest terms, the variance is the square of the standard deviation of all analyte concentrations in a test sample that are detected by at least one detection method. A preferred detection method is mass spectroscopy, where the amount of a detectable analyte is the area beneath the mass peak identified by the detector.

[0031] "Wash buffer" refers to a solution that may be used to wash and remove unbound material from an adsorbent surface. Wash buffers typically include salts that may or may not buffer pH within a specified range, detergents and optionally may include other ingredients useful in removing adventitiously associated material from a surface or complex.

[0032] "Elution buffer" refers to a solution capable of dissociating a binding moiety and an associated ana-

lyte. In some circumstances, an elution buffer is capable of disrupting the interaction between subunits if a complex. As with wash buffers, elution buffers may include detergents, salt, organic solvents and the like used separately or as mixtures. Typically, these latter reagents are present at higher concentrations in an elution buffer than in a wash buffer making the elution buffer more disruptive to molecular interactions. This ability to disrupt molecular interactions is termed "stringency," with elution buffers having greater stringency that wash buffers.

DETAILED DESCRIPTION

[0033] The present invention provides kits and methods that allow one of ordinary skill in the art to reduce the concentration variance of analytes of interest found in a complex mixture. This is accomplished by subjecting the complex mixture to selective adsorption using a large number of binding moieties, at least some of which recognize different analytes in the complex mixture. Using this technique, the amount analyte retained by the present invention for any analyte of high abundance in the complex mixture is limited by the amount of available binding moiety recognizing the high abundance analyte. Any high abundance analyte left over after saturation of the binding moiety is left in bulk solution and not collected by the present invention.

[0034] In contrast collection of low abundance analytes present in the complex mixture is not limited by the amount of binding moiety recognizing the respective low abundance analyte. Therefore for low abundance analytes, most if not all of the analyte is sequestered by the invention through adsorption to a complementary binding moiety. The invention thus has utility in analytical preparation of complex mixtures of molecules, such as biological samples. In blood serum for example, albumins are abundant, many hormones are present in intermediate concentrations, while paracrine factors and cellular markers may be present at minute concentrations. Using the present invention, the variance in analyte abundance observed in sera can be reduced, allowing all analytes of interest to be analyzed.

[0035] After adsorbing the analytes of interest, the analytes are optionally washed to remove excess analytes. Adsorbed analytes are then eluted from the binding moieties using, for example, an elution buffer. The resulting solution contains all analytes of interest free from binding moieties. However, unlike the original complex mixture, the variance in analyte concentration present in the resulting solution is relatively small as the concentration of high abundance analytes has been decreased and that of low abundance analytes increased relative to the original complex mixture. This modification in concentration variance between analytes allows for a large percentage of the analytes in the resulting solution to be detected without the recalibration of the detection device necessary for direct analysis of complex mixtures having components present at widely different concentrations.

[0036] Therefore, by providing a plurality of binding moieties, each recognizing a single analyte of interest present in a complex mixture, the present invention allows the composition of the complex mixture to be detected with minimal or no recalibration of the detection device, including detection of species that would otherwise not be detectable because either they were masked by high abundance analytes, or were present at too low a concentration to be detected by the method of analysis. This provides enormous benefits to high throughput analysis techniques that would otherwise be limited at the detection step by the need for multiple recalibrations, and/or multiple channels, and/or multiple detection steps or expensive and wasteful fractionation techniques necessitated by the large concentration range of the analytes present in many complex mixtures. Moreover, by concentrating low-abundance analytes, the invention allows detection of analytes that are only present in the sample in trace amounts. Using blood serum as an example certain analytes, such as some hormones, are present at only trace amounts in unconcentrated sera. Other analytes, such as albumin, are abundant, being present in amounts ranging from micromolar to hundreds of milimolar. The present invention concentrates the low abundance analytes relative to the high abundance analytes. Thus in preparation of the exemplary serum sample using the present invention, the concentration of hormones is increased relative to the concentration of albumin and other high abundance analytes. By bringing the concentrations of low and high abundance analytes from the sera closer together, the analyte composition can be determined both qualitatively and quantitatively using only one or a few sensitivity settings of the analytical instrumentation used to detect the analytes.

I. Reducing relative analyte concentrations in a sample

A. Suitable test samples

[0037] Test samples of the present invention may be in any form that allows analytes present in the test sample to be contacted with binding moieties of the present invention, as described herein. Suitable test samples include gases, powders, liquids, suspensions, emulsions permeable or pulverized solids, and the like. Preferably test solutions are liquids. Test samples may be taken directly from a source and used in the methods of the present invention without any preliminary manipulation. For example, a water sample may be taken directly from a aquifer and treated directly using the methods described herein.

[0038] Alternatively, the original sample may be manipulated in a variety of ways to enhance its suitability for testing. Such manipulations include depletion of certain analytes, concentrating, grinding, extracting, perco-

lating and the like. For example, solid samples may be pulverized to a powder, then extracted using an aqueous or organic solvent. The extract from the powder may then be subjected to the methods of the present invention. Gaseous samples may be bubbled or percolated through a solution to dissolve and/or concentrate components of the gas in a liquid prior to subjecting the liquid to methods of the present invention.

[0039] Test samples preferably contain at least four analytes of interest, more preferably at least 8, 15, 20, 50, 100, 1000, 100,000, 10,000,000 or more analytes of interest. In some circumstances, test samples suitable for manipulation using the methods of the present invention may include hundreds or thousands of analytes of interest. Preferably, the concentrations of analytes present in the test sample spans at least an order of magnitude, more preferably at least two, three, four or more orders of magnitude. Once subjected to the methods of the present invention, this concentration range for analytes detectable by at least one detection method will be decreased by at least a factor of two, more preferably a factor of 10, 20, 50, 100, 1000 or more.

[0040] Test samples may be collected using any suitable method. For example, environmental samples may be collected by dipping, picking, scooping, sucking, or trapping. Biological samples may be collected by swapping, scraping, withdrawing surgically or with a hypodermic needle, and the like. The collection method in each instance is highly dependent upon the sample source and the situation, with many alternative suitable techniques of collection well-known to those of skill in the art.

1. Biological test samples

[0041] A preferred test sample of the present is a biological sample, preferably a biological fluid. Biological samples that can be manipulated with the present invention include blood, biopsy tissue, lymphatic fluid, sputum, amniotic fluid, urine, sweat, saliva, milk, tissue samples, cell extracts, cell culture fluid, including supernatants of cells expressing an analyte of interest, and derivatives of these, such as serum or plasma. Analytes of interest in biological samples include proteins, lipids, nucleic acids and polysaccharides. More particularly, analytes of interest are cellular metabolites that are normally present in the animal, or are associated with a disease or infectious state such as a cancer, a viral infection, a parasitic infection, a bacterial infection and the like. Particularly interesting analytes are those that are markers for cellular stress. Analytes indicating that the animal is under stress is an early indicator of a number of disease states, including certain mental illnesses.

[0042] Analytes of interest also include those that are foreign to the animal, but found in tissue(s) of the animal. Particularly interesting analytes in this regard are toxins that may be produced by infecting organisms, or sequestered in an animal from the environment.

2. Environmental test samples

[0043] Environmental samples are another class of preferred test samples for use with the present invention. Preferred environmental samples include dirt, dust, dander, natural and synthetic fibers, water, plant materials, animal feces and the like. Preferred analytes in environmental samples include metal ions, natural and synthetic toxins, fertilizers, herbicides and insecticides, and markers for bacterial and viral agents such as structural proteins characteristic of the agent of interest. Particularly preferred analytes sought in environmental test samples are toxins, particularly toxins such as botulinum, ricin, anthrax toxins and the like. Disease-related analytes of interest present in environmental test samples include characteristic proteins and nucleic acids of botulinus, ebola, HIV, SARS, anthrax, plague, malaria, small pox, bovine spongiform, scrapie, etc.

B. Suitable binding moieties

[0044] Suitable binding moieties of the present invention include small organic molecules and biopolymers such as peptides, proteins, polynucleotides and oligosaccharides. Binding moieties of the present invention may be molecules having molecular weights of 100KDa or more, such as antibodies, but preferably are small molecules with a molecular weight in the range of 10KDa, more preferably around 1KDa, desirably less than 1KDa for example, less than 750, 500, 250 or 100Da. Particularly preferred binding moieties of the invention are bio-organic polymers, preferably bio-organic polymers of less than 15, more preferably less than 10, desirably less than 7, 6, 5, 4, or 3 monomeric units in length. Ideally, binding moieties of the present invention are coupled to an insoluble particulate material. Each insoluble particle preferably carries several copies of the same binding moiety, with each particle type coupling a different binding moiety.

[0045] Binding moieties may also be soluble combinatorial molecules. Soluble combinatorial molecules preferably comprise a capture moiety that allows the binding moiety to be coupled to a complementary solid support. Soluble binding moiety embodiments are typically contacted to the sample and allowed to bind analyte(s) of interest prior isolating the resulting complexes by coupling the binding moiety to a solid support.

[0046] Binding moieties of the present invention may be produced using any technique known to those of skill in the art. For example, binding moieties may be chemically synthesized, harvested from a natural source or, in the case of binding moieties that are bio-organic polymers, produced using recombinant techniques. For this latter reason, peptides having no more than 15, 10, 8, 6 or 4 amino acids are particularly advantageous, as they are easily produced using recombinant techniques. Moreover, peptide binding moieties may be produced in a manner that eases screening. For example, peptides

may be recombinantly produced as a phage display library where the peptide is presented as part of the phage coat. (See, e.g., Tang, Xiao-Bo, et al.; J. Biochem; 1997; pp. 686-690; vol. 122, No. 4). Presenting the peptide on the surface of the phage particle allows rapid throughput screening of combinatorial libraries of small peptides, a method that is also advantageous for screening combinatorial antibody libraries.

[0047] Nucleic acids are another preferred bio-organic polymer binding moiety. As with peptides, nucleic acids may be produced using synthetic or recombinant techniques well-known to those of skill in the art. Preferable nucleic acid binding moieties of the present invention are at least 4, more preferably 6, 8, 10, 15, or 20 nucleotides in length. Nucleic acid binding moieties include aptamers, i.e., double stranded DNA or single stranded RNA molecules that bind to specific molecular targets, such as a protein or metabolite.

[0048] Oligosaccharide binding moieties are also contemplated as part of the invention. Oligosaccharide binding moieties are preferably at least 5 monosaccharide units in length, more preferably 8, 10, 15, 20, 25 or more monosaccharide units in length.

[0049] Small organic molecules are also contemplated as binding moieties of the present invention. Typically, such molecules have properties that allow for ionic, hydrophobic or affinity interaction with the analyte. Small organic binding moieties include chemical groups traditionally used in chromatographic processes such as mono-, di- and tri-methyl amino ethyl groups, mono-, di- and tri-ethyl amino ethyl groups, sulphonyl, phosphoryl, phenyl, carboxymethyl groups and the like. This list is by no means exhaustive, as one of skill in the art will readily recognize thousands of chemical functional groups with ionic, hydrophobic or affinity properties compatible with use as binding moieties of the present invention. the production and use of combinatorial binding moiety libraries is discussed in more detail, below.

Capture Moieties

[0050] Binding moieties of the present invention optionally include capture moieties that allow targeted and/or reversible coupling of the binding moiety to a solid support. Exemplary capture moieties include epitope and his-tags, which are attached to the biomolecule to be captured to form a fusion protein. In these instances, a cleavable linker sequence, such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, Calif.) may be optionally included between the biomolecule and the capture moiety to facilitate isolation and/or separation of the components of the fusion molecule. Protein domains specifically recognized by designer ligands may also be used as capture moieties (See, e.g., Deisenhofer, J., Biochemistry 20 (1981) 2361-2370). Many other equivalent capture moieties are known in the art. See, e.g., Hochuli, *Chemische Industrie,* **12**: 69-70 (1989); Hochuli, *Genetic Engineering, Principle and Methods,* **12**:87-98 (1990), Plenum Press, N.Y.; and Crowe, *et al.* (1992) *OIAexpress: The High Level Expression & Protein Purification System,* QIAGEN, Inc. Chatsworth, Calif.; which are incorporated herein by reference. Antigenic determinants and other characteristic properties of the biomolecule to be adsorbed may also serve as capture moiety tags. Exemplary capture moieties include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field *et al.,* Mol. Cell. Biol., 8: 2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto (Evan *et al.,* Molecular and Cellular Biology, 5:3610-3616 (1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky *et al.,* Protein Engineering, 3(6):547-553 (1990)). Other tag polypeptides include the Flag-peptide (Hopp *et al.,* BioTechnology, 6: 1204-1210 (1988)); the KT3 epitope peptide (Martin *et al.,* Science, 255:192-194 (1992)); a α-tubulin epitope peptide (Skinner *et al.,* J. Biol. Chem., 266: 15163-15166 (1991)); and the T7 gene 10 protein peptide tag (Lutz-Freyermuth *et al.,* Proc. Natl. Acad. Sci. USA, 87: 6393-6397 (1990)).

[0051] A preferred capture moiety of the present invention is the "His-tag," a 6xHis tag that binds to Ni-NTA solid supports with high affinity allowing the binding moiety to be isolated in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., Chatsworth, Calif.). The his-tagged binding moiety can be released from the Ni-NTA support with nickel chelating agents. Imidazole is typically used for this purpose. Other chelating structures, such as IDA, CMA and TED can be used in analogous methods, known by those of ordinary skill in the art.

Combinatorial libraries

[0052] In one embodiment, binding moieties are identified by high throughput screening of combinatorial libraries containing a large number of potential binding moieties using assays known to those of skill in the art. In this manner the pool of binding moieties used in the invention is limited to those having a determined binding affinity and/or capacity. Alternatively, the technique can be used to identify only those binding moieties capable of recognizing analytes of interest.

[0053] A combinatorial chemical library is a collection of compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical "building blocks" such as reagents. For example, a complete linear combinatorial chemical library, such as a polypeptide library, is formed by combining a set of chemical building blocks (amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks. For biopolymer binding moieties of the present invention, lin-

ear length is preferably limited to less than 15, more preferably less than 10, desirably less than 7, 6, 5, 4, or 3 monomeric units. For peptide binding moieties, the length is preferably limited to 15, 10, 8, 6 or 4 amino acids. Nucleic acid binding moieties of the invention have preferred lengths of at least 4, more preferably 6, 8,10,15, or at least 20 nucleotides. Oligosaccharides are preferably at least 5 monosaccharide units in length, more preferably 8, 10, 15, 20, 25 or more monosaccharide units.

[0054] Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, nucleic acid, oligosaccharide and peptide libraries (*see, e.g.,* U.S. Patent 5,010,175, Furka, *Int. J. Pept. Prot. Res.* **37**:487-493 (1991) and Houghton *et al., Nature* **354**:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptides (e.g., PCT Publication No. WO 91/19735), encoded peptides (e.g., PCT Publication WO 93/20242), random bio-oligomers (e.g., PCT Publication No. WO 92/00091), benzodiazepines (e.g., U.S. Pat. No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs *et al., Proc. Nat. Acad. Sci. USA* **90**:6909-6913 (1993)), vinylogous polypeptides (Hagihara *et al., J. Amer. Chem. Soc.* **114**:6568 (1992)), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann *et al., J. Amer. Chem. Soc.* **114**: 9217-9218 (1992)), analogous organic syntheses of small compound libraries (Chen *et al., J. Amer. Chem. Soc.* **116**:2661 (1994)), oligocarbamates (Cho *et al., Science* **261**:1303 (1993)), and/or peptidyl phosphonates (Campbell *et al., J. Org. Chem.* **59**:658 (1994)), nucleic acid libraries (*see* Ausubel, Berger and Sambrook, all *supra*), peptide nucleic acid libraries (*see, e. g.,* U.S. Patent 5,539,083), antibody libraries (*see, e.g.,* Vaughn *et al., Nature Biotechnology,* **14(3)**:309-314 (1996) and PCT/US96/10287), carbohydrate libraries (*see, e.g.,* Liang *et al., Science,* **274**:1520-1522 (1996) and U.S. Patent 5,593,853), small organic molecule libraries (*see, e.g.,* benzodiazepines, Baum C&EN, Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337; benzodiazepines, 5,288,514, and the like).

[0055] In some peptide library embodiments, the peptides are expressed on the surface of recombinant bacteriophage to produce large, easily screened, libraries. Using the "phage method" (Scott and Smith, *Science* **249**:386-390, 1990; Cwirla, *et al, Proc. Natl. Acad. Sci.,* **87**:6378-6382, 1990; Devlin *et al., Science,* **49**:404-406, 1990), very large libraries can be constructed (10^6 -10^8 chemical entities). A second approach uses primarily chemical methods, of which the Geysen method (Geysen *et al., Molecular Immunology* **23**:709-715, 1986; Geysen *et al. J. Immunologic Method* **102**:259-274, 1987; and the method of Fodor *et al. (Science* **251**: 767-773, 1991) are examples. Furka *et al.* (14th International Congress of Biochemistry, Volume #5, Abstract FR:013, 1988; Furka, *Int. J. Peptide Protein Res.* **37**: 487-493, 1991), Houghton (U.S. Pat. No. 4,631,211, issued December 1986) and *Rutter et al.* (U.S. Pat. No. 5,010,175, issued Apr. 23, 1991) describe methods to produce a mixture of peptides that can be tested as agonists or antagonists.

[0056] Devices for the preparation of combinatorial libraries are commercially available *(see,* e.g., 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA). In addition, numerous combinatorial libraries are themselves commercially available (*see, e.g.,* ComGenex, Princeton, N.J., Tripos, Inc., St. Louis, MO, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, etc.).

C. Capturing analytes from test sample using binding moieties

[0057] Analytes present in a test sample are captured by contacting the test sample with the binding moieties under conditions that allow each binding moiety to couple with its corresponding analyte. As inferred above, binding moieties may be contacted to the test sample directly, or the binding moieties may be first attached to a solid support, such as a dipstick, SELDI probe or insoluble polymeric bead.

[0058] By way of example, in one preferred embodiment, the binding moieties include a capture moiety. In this embodiment the binding moieties are contacted directly to the test sample in a manner that allows analytes present in the test sample to bind to the binding moieties. After sufficient time has elapsed, a solid support that includes a complementary capture moiety to the capture moiety of the binding moiety is contacted to the test sample. This allows the binding moiety to couple with the solid support through the capture moiety, while retaining the bound analyte. For example, a binding moiety having a biotin capture moiety would couple to a solid support that had avidin or strepavidin coupled to its surface.

[0059] In an alternative embodiment, the binding moiety is coupled to the solid support prior to contacting the test sample. In this alternative embodiment, the solid support is simply contacted with the test sample for a time sufficient to allow the binding moiety to bind analyte, then the solid support is withdrawn from the test sample with the analyte bound to it via formation of a complex between the analyte and the binding moiety.

[0060] Contacting the binding moiety to the test sample may be accomplished by admixing the two, swabbing the test sample onto the binding moiety, flowing the test sample over the solid support having binding moieties attached thereto, and other methods that would be

obvious to those of ordinary skill in the art.

[0061] Contact with the test sample is preferably accomplished with a plurality of different binding moieties, most preferably at least 4 binding moieties, desirably at least 6 binding moieties, ideally at least 10, 50, 100, 1000, 10,000, 100,000, 1,000,000 or more binding moieties, where each binding moiety preferably recognizes a different analyte

Solid supports

[0062] Acceptable supports for use in the present invention can vary widely. A support can be porous or nonporous, but is preferably porous. It can be continuous or non-continuous, flexible or nonflexible. A support can be made of a variety of materials including ceramic, glassy, metallic, organic polymeric materials, or combinations thereof.

[0063] Preferred supports include organic polymeric supports, such as particulate or beaded supports, woven and nonwoven webs (such as fibrous webs), microporous fibers, microporous membranes, hollow fibers or tubes. Polyacrylamide and mineral supports such as silicates and carbonates (e.g., hydroxyl apatite) can also be used. Woven and nonwoven webs may have either regular or irregular physical configurations of surfaces. Particularly preferred embodiments include solid supports in the form of spherical or irregularly-shaped beads.

[0064] Porous materials are particularly desirable because they provide large surface areas. The porous support can be synthetic or natural, organic or inorganic. Suitable solids with a porous structure having pores of a diameter of at least about 1.0 nanometer (nm) and a pore volume of at least about 0.1 cubic centimeter/gram (cm^3 /g). Preferably, the pore diameter is at least about 30 nm because larger pores will be less restrictive to diffusion. Preferably, the pore volume is at least about 0.5 cm^3 /g for greater potential capacity due to greater surface area surrounding the pores. Preferred porous supports include particulate or beaded supports such as agarose, hydrophilic polyacrylates, Styrofoam, and Sepharose, including spherical and irregular-shaped beads.

[0065] For significant advantage, the supports are preferably hydrophilic, and have a high molecular weight (preferably, greater than about 5000, and more preferably, greater than about 40,000). Preferably, the hydrophilic polymers are water swellable to allow for greater infiltration of analytes. Examples of such supports include cellulose, modified celluloses, agarose, polyvinyl alcohol (PVA), dextrans, amino-modified dextrans, polyacrylamide, polyacrylates, modified polyethylene glycols, modified guar gums, guar gums, xanthan gums, and locust bean gums and hydrogels.

[0066] Attachment of the binding moieties to the solid support may be accomplished through a variety of mechanisms. The solid support can be derivatized with a fully prepared binding moiety by attaching a previously prepared binding moiety to the solid support. Alternatively, the binding moiety may be formed on the solid support by attaching a precursor molecule to the solid support and subsequently adding additional precursor molecules to the growing chain bound to the solid support by the first precursor molecule. This mechanism of building the adsorbent on the solid support is particularly useful when the binding moiety is a polymer, particularly a biopolymer such as a polypeptide, polynucleotide or polysaccharide molecule. A biopolymer adsorbent can be provided by successively adding monomeric components (e.g., amino acids, nucleotides or simple sugars) to a first monomeric component attached to the solid support using methods known in the art. See, e.g., U.S. Pat. No. 5,445,934 (Fodor *et al.).*

[0067] As few as one and as many as 10, 100, 1000, 10,000, 1,000,000, 1,000,000,000 or more binding moieties may be coupled to a single solid support. In preferred embodiments the solid support is in the form of beads, with a single, different, binding moiety type bound to each bead. For example in a peptide binding moiety library, peptides representing one possible permutation of amino acids would be bound to one bead, peptides representing another possible permutation to another bead, and so on.

[0068] Binding moieties may be coupled to a solid support using reversible or non-reversible interactions. For example, non-reversible interactions may be made using a support that includes at least one reactive functional group, such as a hydroxyl, carboxyl, sulfhydryl, or amino group that chemically binds to the binding moiety, optionally through a spacer group. Suitable functional groups include N-hydroxysuccinimide esters, sulfonyl esters, iodoacetyl groups, aldehydes, epoxy, imidazolyl carbamates, and cyanogen bromide and other halogen-activated supports. Such functional groups can be provided to a support by a variety of known techniques. For example, a glass surface can be derivatized with aminopropyl triethoxysilane in a known manner.

[0069] Alternatively, reversible interactions between a solid support and a binding moiety may be made using capture moieties associated with the solid support and/or the binding moiety. A variety of capture moieties suitable for use with the present invention are known, some of which are discussed above. Use of capture moieties for coupling diverse agents is well known to one of ordinary skill in the art, who can apply this common knowledge to form solid support/binding moiety couplings suitable for use in the present invention with no more that routine experimentation.

2. Removing unbound analytes

[0070] A feature of the present invention is that treatment of analytes according to the methods described herein preferably concentrates and partially purifies bound analyte in addition to reducing the variance be-

tween analyte concentrations. Implementation of this feature to the fullest includes optionally washing any unbound analytes from the analyte bound to the binding moieties on the solid support.

[0071] Washing away unbound analyte is preferably performed by contacting the analyte bound to the binding moiety with a mild wash solution. The mild wash solution is designed to remove contaminants frequently found in the test sample originally containing the analyte. Typically a wash solution will be at a physiologic pH and ionic strength and the wash will be conducted under ambient conditions of temperature and pressure.

[0072] Formulation of wash solutions suitable for use in the present invention can be performed by one of skill in the art without undue experimentation. Methods for removing contaminants, including low stringency washing methods, are available in published form, for example in Scopes, *Protein Purification: Principles and Practice* (1982); Ausubel, *et al*. (1987 and periodic supplements); *Current Protocols in Molecular Biology;* Deutscher (1990) *"Guide to Protein Purification"* in *Methods in Enzymology* vol. 182, and other volumes in this series.

D. Isolating captured analytes from binding moieties

[0073] Bound analyte may be eluted from the binding moiety and isolated using a variety of methods, preferably by using an aqueous elution buffer that disrupts the interaction between the binding moiety and the analyte. Any suitable elution buffer may be used for this purpose, including denaturing agents such as chaotropes and organic solvents. Exemplary elution buffers include aqueous salt solutions of high ionic strength, detergent solutions, and organic solvents. Solutions and suspensions of agents that competitively bind to binding moieties of the invention my also be used in elution buffers, provide that such competitive binding agents do not interfere with subsequent collection or analysis of the analytes of interest. The elution buffer(s) chosen are highly application-specific and may be readily identified by one of ordinary skill in the art through materials commonly available in the public domain or through routine experimentation (See, e.g., Scopes, *Protein Purification: Principles and Practice* (1982); and Deutscher (1990) *"Guide to Protein Purification"* in *Methods in Enzymology* vol. 182, and other volumes in this series).

[0074] Examples of suitable elution buffers include those that modify surface charge of an analyte and/ or binding moiety, such as pH buffer solutions. pH buffer solutions used to disrupt surface charge through modification of acidity preferably are strong buffers, sufficient to maintain the pH of a solution in the acidic range, i.e., at a pH less than 7, preferably less than 6.8, 6.5, 6.0, 5.5, 5.0, 4.0 or 3.0; or in the basic range at a pH greater than 7, preferably greater than 7.5, 8.0, 8.3, 8.5, 9.0, 9.3, 10.0 or 11.0.

[0075] Alternatively, solutions of high salt concentration having sufficient ionic strength to mask charge characteristics of the analyte and/or binding moiety may be used. Salts having multi-valent ions are particularly preferred in this regard, e.g., sulphates and phosphates with alkali earth or transition metal counterions, although salts dissociating to one or more monovalent are also suitable for use in the present invention, provided that the ionic strength of the resulting solution is at least 0.1, preferably 0.25, 0.3, 0.35, 0.4, 0.5, 0.75, 1.0 mol^{-1} or more. By way of example, many protein analyte/binding moiety interactions are sensitive to alterations of the ionic strength of their environment. Therefore, analyte may be isolated from the binding moiety bay contacting the bound analyte with a salt solution, preferably an inorganic salt solution such as sodium chloride. This may be accomplished using a variety of methods including bathing, soaking, or dipping a solid support to which the analyte is bound into the elution buffer, or by rinsing, spraying, or washing the elution buffer over the solid support. Such treatments will release the analyte from the binding moiety coupled to the solid support. The analyte may then be recovered from the elution buffer.

[0076] Chaotropic agents, such as Guanidine and urea, disrupt the structure of the water envelope surrounding the binding moiety and the bound analyte, causing dissociation of complex between the analyte and binding moiety. Chaotropic salt solutions suitable for use as elution buffers of the present invention are application specific and can be formulated by one of skill in the art through routine experimentation.. For example, a suitable chaotropic elution buffer may contain urea ranging in concentration from 0.1 to 8 M.

[0077] Detergent-based elution buffers modify the selectivity of the affinity molecule with respect to surface tension and molecular complex structure. Suitable detergents for use as elution buffers include both ionic and nonionic detergents. Non-ionic detergents disrupt hydrophobic interactions between molecules by modifying the dielectric constant of a solution, whereas ionic detergents generally coat receptive molecules in a manner that imparts a uniform charge, causing the coated molecule to repel like-coated molecules. For example, the ionic detergent sodium dodecyl sulphate (SDS) coats proteins in a manner that imparts a uniform positive charge. Examples of non-ionic detergents include Triton X-100, , TWEEN, NP-40 and Octyl-glycoside. Examples of zwitterionic detergents include CHAPS.

[0078] Another class of detergent-like compounds that disrupt hydrophobic interactions through modification of a solution's dielectric constant includes ethylene glycol, propylene glycol and organic solvents such as ethanol, propanol, acetonitrile, and glycerol.

[0079] Other elution buffers suitable for the present invention include combinations of buffer components mentioned above. Elution buffers formulated from two or more of the foregoing elution buffer components are capable of modifying the selectivity of molecular inter-

action between subunits of a complex based on multiple elution characteristics.

[0080] Analytes isolated using the present invention will have a concentration variance between analytes that is less than the concentration variance originally present in the test sample. For example, after manipulation using the methods of the present invention, isolated analytes with have a concentration variance from other isolated analytes that is decreased by at least a factor of two, more preferably a factor of 10, 20, 25, 50, 100, 1000 or more, from the concentration variance between the same analytes present in the test sample prior to subjecting the test sample to any of the methods described herein. Preferably, the method of the invention is performed with a minimal amount of elution buffer, to ensure that the concentration of isolated analyte in the elution buffer is maximized. More preferably, the concentration of at least one isolated analyte will be higher in the elution buffer than previously in the test sample.

[0081] After isolating the captured analytes, the analytes may be further processed by fractionation based on some chemical or physical property such as molecular weight, isoelectric point or affinity to a chemical or biochemical ligand. Fractionation methods for nucleic acids, proteins, lipids and polysaccharides are well-known in the art and are discussed in, for example, Scopes, *Protein Purification: Principles and Practice* (1982); Sambrook *et al., Molecular Cloning--A Laboratory Manual* (2nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, N.Y., (Sambrook) (1989); and Current Protocols in Molecular Biology, F. M. Ausubel *et al.,* eds., *Current Protocols,* a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1994 Supplement) (Ausubel).

E. Detecting isolated analytes

[0082] After analytes have been eluted and isolated free of binding moieties, the analyte may be detected, quantified or otherwise characterized using any technique available to those of ordinary skill in the art. A feature of applying the analysis techniques of the present invention to complex test samples, is the dynamic reduction of variance in analyte concentrations for isolated analytes relative to the large variance in analyte concentration found in the original test sample. This reduction in analyte concentration variance allows a much larger percentage of analytes found in the original test sample to be detected and characterized without recalibrating the detection device than would be available for analyte detection using the original test sample itself. The actual reduction in analyte concentration variance achieved is dependent on a variety of factors including the nature of the original test sample, and the nature and diversity of the binding moieties used. Generally, the reduction in analyte concentration variance using the techniques described herein is sufficient to allow at least 25% more preferably at least 30%, 40%, 50%, 60%, 70%, 75% or 80% of the analytes isolated to be detected without instrument re-calibration. Ideally, the present invention allows at least 90%, 95%, 98% or more of the analytes isolated to be detected without instrument re-calibration.

[0083] Detecting analytes isolated using the techniques described herein may be accomplished using any suitable method known to one of ordinary skill in the art. For example, colorimetric assays using dyes widely available. Alternatively, detection may be accomplished spectroscopically. Spectroscopic detectors rely on a change in refractive index; ultraviolet and/or visible light absorption, or fluorescence after excitation with a suitable wavelength to detect reaction components. Exemplary detection methods include fluorimetry, absorbance, reflectance, and transmittance spectroscopy. Changes in birefringence, refractive index, or diffraction may also be used to monitor complex formation or reaction progression. Particularly useful techniques for detecting molecular interactions include surface plasmon resonance, ellipsometry, resonant mirror techniques, grating-coupled waveguide techniques, and multi-polar resonance spectroscopy. These techniques and others are well known and can readily be applied to the present invention by one skilled in the art, without undue experimentation. Many of these methods and others may be found, for example, in "Spectrochemical Analysis" Ingle, J.D. and Crouch, S.R., Prentice Hall Publ. (1988) and "Analytical Chemistry" Vol. 72, No. 17.

[0084] A preferred method of detection is by mass spectroscopy. Mass spectroscopy techniques include, but are not limited to ionization (I) techniques such as matrix assisted laser desorption (MALDI), continuous or pulsed electrospray (ESI) and related methods (e.g., IONSPRAY or THERMOSPRAY), or massive cluster impact (MCI); these ion sources can be matched with detection formats including linear or non-linear reflection time-of-flight (TOF), single or multiple quadropole, single or multiple magnetic sector, Fourier Transform ion cyclotron resonance (FTICR), ion trap, and combinations thereof (e.g., ion-trap/time-of flight). For ionization, numerous matrix/wavelength combinations (MALDI) or solvent combinations (ESI) can be employed. Subattomole levels of analyte have been detected, for example, using ESI (Valaskovic, G. A. et al., (1996) Science 273: 1199-1202) or MALDI (Li, L. et al., (1996) J. Am. Chem. Soc. 118:1662-1663) mass spectrometry.

ES mass spectrometry has been introduced by Fenn et al. (J. Phys. Chem. 88, 4451-59 (1984); PCT Application No. WO 90/14148) and current applications are summarized in recent review articles (R. D. Smith et al., Anal. Chem. 62, 882-89 (1990) and B. Ardrey, Electrospray Mass Spectrometry, Spectroscopy Europe, 4, 10-18 (1992)). MALDI-TOF mass spectrometry has been introduced by Hillenkamp et al. ("Matrix Assisted UV-Laser Desorption/Ionization: A New Approach to Mass Spectrometry of Large Biomolecules," Biological Mass Spectrometry (Burlingame and McCloskey, editors), El-

sevier Science Publishers, Amsterdam, pp. 49-60, 1990). With ESI, the determination of molecular weights in femtomole amounts of sample is very accurate due to the presence of multiple ion peaks which all could be used for the mass calculation. A preferred analysis method of the present invention utilizes Surfaces Enhanced for Laser Desorption/Ionization (SELDI), as discussed for example in U.S. Pat. No. 6,020,208.

[0085] Another method of detection widely used is based on electrophoresis separation based on one or more physical properties of the analyte(s) of interest. A particularly preferred embodiment for analysis of polypeptide and protein analytes is two-dimensional electrophoresis. A preferred application separates the analyte by size in the first dimension, and by isoelectric point in the second dimension. Methods for electrophoretic analysis of analytes vary widely with the analyte being studied, but techniques for identifying a particular electrophoretic method suitable for a given analyte are well known to those of skill in the art.

III. Kits

[0086] The present invention also includes kits containing components that allow one of ordinary skill in the art to perform the techniques described herein. The most basic of kits for this purpose provide a plurality of binding moieties, each binding moiety in an amount selected to capture a pre-determined amount of a different analyte. In some kit embodiments of the invention the binding moieties are supplied coupled to a solid support, preferably insoluble beads. In other embodiments the solid support and binding moieties are supplied separately. When supplied separately, the binding moieties and/or solid supports include a capture moiety that allows the operator of the invention to couple binding moiety to solid support during the course of practicing the invention described herein. Kits providing separate binding moieties and solid supports may optionally provide additional reagents necessary to perform the reaction coupling the binding moieties to the solid supports.

[0087] Kits of the present invention also include a plurality of containers retaining components for sample preparation and analyte isolation. Exemplary components of this nature include one or more wash solutions sufficient for removing unbound material from a binding moiety specifically bound to an analyte, and at least one elution solution sufficient to release analyte specifically bound by a binding moiety.

[0088] Kit embodiments may optionally include instructions for using the kit.

[0089] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[0090] Although the foregoing invention has been described in some detail by way of illustration and example for clarity and understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit and scope of the appended claims.

[0091] As can be appreciated from the disclosure provided above, the present invention has a wide variety of applications. Accordingly, the following examples are offered for illustration purposes and are not intended to be construed as a limitation on the invention in any way. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

EXAMPLES

[0092] This example illustrates how one embodiment of the invention described above may be applied to a complex biological sample, in this case human serum. In this example, a reduction in the variance of serum protein concentrations is achieved by selectively adsorbing serum proteins to hexapeptides coupled to insoluble beads. Each possible permutation of hexapeptide is represented in the binding moiety population of the example, in the form of a combinatorial library of split and pool beads. In this format high abundance serum analytes, such as albumin, are bound to a hexapeptide binding moiety but only to a level equal to saturation of the particular binding moiety. In contrast, low abundance serum analytes are bound almost in their entirety, as the amount of binding moiety recognizing the low abundance analyte is not limiting. The result of this selective binding is a reduction in the analyte concentration variance for proteins recognized by the binding moieties of used, without the risk of losing low abundance analytes inherent in methods that seek to selectively remove high abundance analytes. Consequently, a large percentage of the serum analytes isolated using the method can be detected in one batch analysis without a recalibration of the detection device. This contrasts with the situation presented when detecting the same analytes in a serum test sample where the detection device would have to be recalibrated repeatedly in order to detect the same analytes in untreated sera.

[0093] In this example 5 ml of whole serum is loaded into a column of Protein G to remove antibodies (4 ml of resin). The column is at room temperature equilibrated in a buffer solution at neutral pH and having a sodium chloride concentration of 0.1 mol 1^{-1}. Proteins not binding the column (the "flow through") are collected and retained.

[0094] 1 ml of beads supporting hexapeptides in a combinatorial mode (obtained by the so called "spit-and-pool" method) are mixed with the flow through from the protein G column and incubated for 30-60 minutes.

[0095] The bead slurry is then centrifuged and the supernatant decanted and discarded. Then beads are then washed with a wash buffer of 0.1M phosphate buff-

ered saline, pH 6.8, at room temperature to remove material that is not bound to the hexapeptides on the beads. The beads are then mixed with an aqueous solution containing 2M acetic acid and 6M urea (final concentrations) to desorb all proteins from the beads.

[0096] The solution of desorbed proteins is then subjected to mass spectroscopic analysis to detect the molecular weights of the proteins that bound to the binding moieties. Analysis of the sample by mass spectrometry before and after being subject to concentration variance reduction showed increased evenness in the peak heights and an increase in the number of peaks seen, as the decrease in abundant molecules also decreased the ion suppression that hid peaks in the native sample. This methodology is anticipated to increase the number of detectable analytes in a complex solution, such as blood serum, by at least 0.5, more likely 1, 2, 3 or more orders of magnitude over the number of detectable analytes using the original complex solution not subjected to the methodology of the invention.

Claims

1. A method for reducing the relative amounts of analytes in a sample comprising:

a) providing a test sample comprising first different amounts of a plurality of different analytes, wherein the first different amounts have a first variance;
b) contacting the test sample with amounts of each of a plurality of different binding moieties;
c) capturing amounts of the different analytes from the test sample with the different binding moieties and removing unbound analytes; and
d) isolating the captured analytes from the binding moieties to produce a second sample comprising second amounts of a plurality of different analytes, wherein the second amounts have a second variance;

wherein the amounts of each of the plurality of different binding moieties are selected to capture amounts of the different analytes whereby the second variance is less than the first variance.

2. The method of claim 1, wherein the test sample is a biological fluid selected from the group consisting of blood, biopsy tissue amniotic fluid, milk, urine, sweat, saliva, a cell extract or derivatives thereof.

3. The method of claim 1, wherein the test sample is an environmental sample.

4. The method of claim 1, wherein the plurality of different analytes is at least four different analytes.

5. The method of claim 1, wherein the plurality of different analytes is at least one thousand, at least ten thousand, at least one-hundred thousand, at least one million or at least ten million different analytes.

6. The method of claim 1, wherein the contacting step comprises admixing the binding moieties with the test sample.

7. The method of claim 1, wherein the binding moieties are a combinatorial library.

8. The method of claim 1, further comprising fractionating the captured analytes based on a physical or chemical property.

9. The method of claim 1, wherein the plurality of binding moieties comprises bio-organic polymers.

10. The method of claim 6, wherein the bio-organic polymers are selected from the group consisting of peptides, oligonucleotides and oligosaccharides.

11. The method of claim 10, wherein the bio-organic polymers are peptides at least four amino acids in length.

12. The method of claim 10, wherein the peptides are antibodies.

13. The method of claim 12, wherein the antibodies are surface molecules of a phage display library.

14. The method of claim 10, wherein the bio-organic polymers are oligonucleotides of at least fifteen units in length.

15. The method of claim 14, wherein the binding moieties are aptamers.

16. The method of claim 14, wherein the binding moieties are oligosaccharides of at least five monosaccharide units in length.

17. The method of claim 1, wherein the contacting step is performed with the analyte in a gaseous state, a liquid state or in a suspension.

18. The method of claim 1, wherein removing unbound analytes comprises washing the captured analytes with a wash buffer.

19. The method of claim 1, wherein isolating the captured analytes comprises eluting the analytes from the binding moieties by contacting the captured analytes with an elution buffer and collecting the analytes in the elution buffer free of binding moieties.

20. The method of claim 19, wherein the elution buffer is a solution comprising a component selected from the group consisting of a detergent a salt, an organic solvent, an acid, a base, a chaotropic agent and a competing ligand for the binding moiety.

21. The method of claim 1, wherein the second variance is at least 25% less than the first variance.

22. The method of claim 1, further comprising fractionating the analytes into subgroups.

23. The method of claim 22, wherein fractionating comprises segregating the analytes using a technique selected from the group consisting of chromatography, electrophoresis, capillary electrophoresis, filtration and precipitation.

24. The method of claim 1, wherein the different binding moieties are coupled to a solid support.

25. The method of claim 24, wherein the solid support comprises a hydrogel.

26. The method of claim 24, wherein the coupling comprises a covalent bond.

27. The method of claim 24, wherein the solid support is a plurality of insoluble surfaces, each insoluble surface coupled to a single species of binding moiety.

28. The method of claim 27, wherein the insoluble surfaces are beads.

29. The method of claim 24, wherein the binding moieties are coupled to the solid support by a capture moiety.

30. The method of claim 29, wherein the capture moiety is selected from the group consisting of FLAG tag, His tag and biotin.

31. The method of claim 1, further comprising detecting the isolated analytes.

32. The method of claim 24, wherein detecting the analytes is performed using an assay comprising a technique selected from the group consisting of: colorimetric, spectrophotometric, magnetic resonance, ellipsometric, mass spectroscopic, electrophoretic, chromatographic and enzymatic.

33. The method of claim 25, wherein the assay is mass spectroscopic and the second variance of the isolated analytes allows quantifying at least 30% of the captured analytes using a single detector setting.

34. A kit for detecting a plurality of analytes in a sample, comprising:

(a) a plurality of binding moieties, each binding moiety in an amount selected to capture a predetermined amount of a different analyte;
(b) a plurality of containers retaining components for sample preparation and analyte isolation; and
(c) instructions for using the kit.

35. The kit of claim 34, wherein the binding moieties are coupled to the solid support.

36. The kit of claim 34, wherein the components for sample preparation comprise a wash solution sufficient for removing unbound material from a binding moiety specifically bound to an analyte, and an elution solution sufficient to release analyte specifically bound by a binding moiety.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 04 29 0775

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	US 2003/036095 A1 (TCHAGA GRIGORIY S) 20 February 2003 (2003-02-20) * paragraphs [0031], [0044], [0072], [0073] *	1-36	G01N33/68 G01N33/53
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	17 August 2004	Komenda, P

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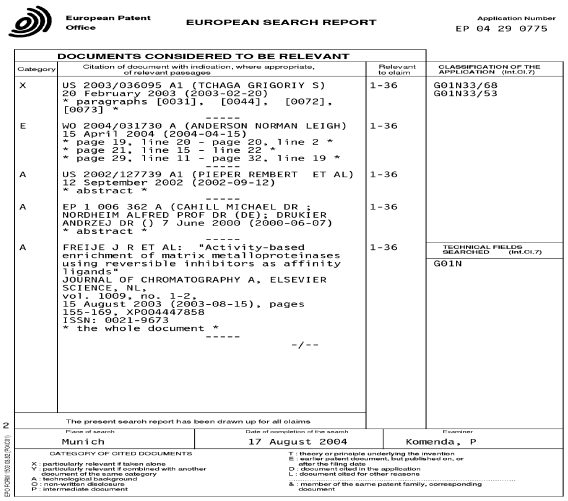
专利名称(译)	减少从复杂样品混合物中获得的分析物浓度之间差异的方法		
公开(公告)号	EP1580559A1	公开(公告)日	2005-09-28
申请号	EP2004290775	申请日	2004-03-23
[标]申请(专利权)人(译)	赛弗根生物系统股份有限公司		
申请(专利权)人(译)	赛弗吉生物系统INC.		
当前申请(专利权)人(译)	Bio-Rad实验室，INC.		
[标]发明人	BOSCHETTI EGISTO		
发明人	BOSCHETTI, EGISTO		
IPC分类号	C12Q1/68 G01N33/53 G01N33/543 G01N33/68		
CPC分类号	G01N33/6803 C40B30/04 G01N33/543 G01N33/54306 G01N33/6845		
其他公开文献	EP1580559B1		
外部链接	Espacenet		

摘要(译)

本发明涉及分子生物学，组合化学和生物化学领域。特别地，本发明描述了用于动态减少从复杂混合物中获得的分析物之间的差异的方法和试剂盒。



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