DIAGNOSTIC AND TREATMENT METHODS USING A LIGAND LIBRARY

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Related U.S. Application Data

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ABSTRACT

The invention relates to diagnostic and treatment methods using a ligand library. Specifically, the invention relates to using a ligand library to diagnose or detect a drug induced response, including drug adverse reaction, side effects, drug resistance, and therapeutic efficacy. The invention further relates to identifying biomarkers associated with a drug induced response and providing a personalized medical treatment.
TentaGel screen flow chart

- Swell beads in DMF, wash with TBST, allow to swell for one hour
- Add NC serum in 1X TBST, tumble O/N
  - Perform Dynabead screen
    - Wash library via centrifugation
    - Add NC serum in 1X TBST O/N then add Qdots
      - Save N Qdot hits from library
    - Wash library (tumble) add D serum in starting block O/N
      - Perform Dynabead screen
        - Wash library via centrifugation
        - Add D serum in SB then add Qdots
          - Collect D Qdot hits
          - Save library
    - Wash Dynabead hits via centrifugation
      - Add D serum in SB then add Qdots
        - Save D Dyna hits
        - Save D Dynabead Qdot hits

FIGURE 6
JC3B library NC Dynabead hits after Qdot addition

FIGURE 7

TentaGel library screening with diseased serum

FIGURE 8
Reproducibility test (NC sample 050047 after SDS wash and Qdot addition)
Reproducibility test (Diseased sample after SDS wash and Qdot addition)

FIGURE 11

Removed before next serum addition/SDS wash
### Sequences of selected hits

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<tr>
<th>Cyclohexylamine</th>
<th>Benzenesulfonamide</th>
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**FIGURE 12A**
Sequences of selected hits

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FIGURE 12B
Chemical structures of putative hits selected

FIGURE 13A
FIGURE 13B
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**FIGURE 15B**
FIGURE 20A
Validation on TentaGel Beads

D 30144 40µg/ml  NC 6 pooled 40µg/ml

Note: Validation on TentaGel Beads could be used as colorimetric test for point of care

FIGURE 21

A

B

C

JC5B hits

-Qdot 655 hits after Disease serum addition
-Detected with Qdot 655

-Reconfirmation of hits using Qdot 655

FIGURE 22
Hit validation

After Disease serum addition
- Detected with Qdot 655

After Normal serum addition
- Detected with Qdot 655

FIGURE 23

Hit validation by mixing

AD marker

PC marker

Serum 1
- Detected with Qdot 655

Serum 2
- Detected with Qdot 655

FIGURE 24
**PC-JC3B Hit Sequences**

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<th>Methylbenzylamine</th>
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**Linkers:** Diaminobutane, Methionine

**FIGURE 25**
### PC-JC5B hit Sequences

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**Linkers:** Diaminobutane, Ethanolamine, Methionine, Furfurylamine, Cyclohexylamine, Piperonylamine, Methylbenzylamine, Allylamine
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<td>Cysteine</td>
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</tbody>
</table>

**FIGURE 28**
Hit Validation For Peptoid KN1B-20

Group 1 Pooled Serum (~0.374 mg/mL)  Non-Diseased Pooled Serum (~0.378 mg/mL)  No Serum Control

FIGURE 29
ELISA

Binding of KN1B-20-biotin-fluorescein to ELISA Plates

- Biotin → Peptoid-KN1B-20 → Fluorescein
- Cysteine → Peptoid-KN1B-20 → Fluorescein

494 Excitation/520 Emission

- Streptavidin Plate
- Maleimide Plate

mM KN1B-20-biotin-fluorescein

FIGURE 30
Competition Assay

- 1mM KN1B-20-biotin-fluorescein vs. 0, 1, 5, 10 and 50 mM KN1B-20-biotin

**FIGURE 31**
Validation of peptoids on ELISA and TentaGel Platforms

D 30144 40ug/ml  NC 6 pooled 40ug/ml

FIGURE 32
AD Serum ELISA with 10mM ADP3 (prepared in binding buffer)

Starting dilutions (1:200)
Group 1 AD serum @ ~0.394 mg/mL
Non-diseased serum @ ~0.386 mg/mL

FIGURE 35
SLE Serum ELISA with 10mM KN1B-20
(prepared in binding buffer)

Starting dilutions (1:200)
Group 1 SLE serum @ ~0.375 mg/mL
Non-diseased serum @ ~0.396 mg/mL

FIGURE 36
SLE Serum ELISA with 10mM KN1B-20
(prepared in DMSO)

Starting dilutions (1:200)
Group 1 SLE serum @ ~0.367 mg/mL
Non-diseased serum @ ~0.322 mg/mL

FIGURE 37
AES saturation for 8 stage 8888s its waitiation:

FIGURE 38
Model study: binding of 2,5-dinitrophenyl group (DNP) to anti-DNP antibody

Mean fluorescence intensity (MFI)

30000
25000
20000
15000
10000
5000
0

[s] (ug/mL)

Beads-DNP

Beads-acetyl group

Beads-acetyl group

Beads-DNP

Confirmed based on ninhydrin test and bead color change

FIGURE 39
ethanolamine-DNP competed the binding of DNP to anti-DNP antibody

FIGURE 40
ADP3 bound auto-antibody from pooled normal control (NC) and pooled Alzheimer's disease (AD) sera

FIGURE 41A

Secondary antibody: goat anti-human Dylight 649

FIGURE 41B

Secondary antibody: goat anti-human Alexa 647
FIGURE 43A
FIGURE 44
Preparation of ADP3 on 10 μm Tentagel beads

ADP3

Final - Shots 1000 - 27OCt2010 - Peptides: Run #7, Label B7

ADP3, directly synthesized on 10 μm Tentagel beads and Cleaved by CNBr

FIGURE 45
ADP3 bound autoantibody from normal control and Alzheimer's disease sera

MFI

[Series1] NC (normal control)
[Series2] AD (Alzheimer's disease)

[Sera] (μg/mL)

Pre-Block beads for 3 hr
1XTBST+ Goat anti-human Alexa 647

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<th>[Sera] (μg/mL)</th>
<th>0</th>
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<th>7.5</th>
<th>15</th>
<th>30</th>
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<td>9992</td>
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<td>7841</td>
<td>9896</td>
<td>13928</td>
<td>15713</td>
<td>31489</td>
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FIGURE 46
ADP3 bound autoantibody from normal control and Alzheimer’s disease sera

Blocking buffer + Goat anti-human Alexa 647

FIGURE 47
ADP3 bound auto-antibody from normal control (NC) and Alzheimer's disease (AD) sera

FIGURE 48

1. Pre-blocking in E Coli lysate + 0.5% Lysine for 3 hrs
2. Blocking buffer + Goat anti-human Alexa 647
ADP3 bound auto-antibody from normal control (NC) and Alzheimer's disease (AD) sera

1. Pre-blocking in E Coli lysate + 1% Lysine for 3 hrs
2. Blocking buffer + Goat anti-human Alexa 647

FIGURE 49
Titration Data – Agreement Between Clinical Diagnosis and Opko Test

**Figure 52A**

**Figure 52B**
Validation of ADP3 on ELISA Platform

- ★ = Clinically normal
- ○ = Clinically AD
- □ = Clinically Normal
- △ = Clinically normal
- ■ = Can be assigned with more peptoids

FIGURE 53
Validation of peptoids (P1aag1-9) as selective markers for Alzheimer's disease on ELISA Platform

Figure 54

Intensity
2.5
2.0
1.5
1.0
0.5
0.0
AD
UND
NC

Star = Clinically normal
O = Clinically AD
Diamond = Clinically AD
Square = Clinically Normal
Circle = Clinically AD (demented)
Triangle = Clinically normal
Performance on Other Dementias

FIGURE 55
Disagreement Between Clinical Diagnosis and Opko Test

**FIGURE 56A**

Diseased clinically
Opko: UND on single point. Titration: AD

**FIGURE 56B**

Normal clinically (non-demented)
Opko: pre-AD even at single point
AD clinically (demented)
Opko: Not AD. Must be other dementia

FIGURE 56C

AD Clinically
Opko: UND at single point. UND even after titration. FIGURE 56D
FIGURE 57

Summary of ELISA Analysis
Total Plasma Samples Tested = 106

<table>
<thead>
<tr>
<th>Clinical Diagnosis</th>
<th>Our prediction</th>
<th>Differences</th>
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<tr>
<td>49 Alzheimer’s</td>
<td>42</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>57 Normal</td>
<td>47</td>
<td>10 (17%)</td>
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FIGURE 58
DIAGNOSTIC AND TREATMENT METHODS USING A LIGAND LIBRARY

CROSS-REFERENCE TO RELATED APPLICATIONS


FIELD OF THE INVENTION

[0002] The invention relates to diagnostic and treatment methods using a ligand library and active compounds derived therefrom. Specifically, the invention relates to using a ligand library to find ligands that are used to determine disease biomarker profiles and to diagnose or detect a drug induced response, including drug adverse reaction, side effects, drug resistance, and therapeutic efficacy. The invention further relates to identifying biomarkers associated with a drug induced response and providing a personalized medical treatment.

BACKGROUND OF THE INVENTION

[0003] Small-molecule microarrays are becoming increasingly important tools in combinatorial chemistry. The arrays are generally produced by first synthesizing a combinatorial library on a suitable bead resin, separating the beads into the wells of microtiter plates, and then releasing the compounds from the beads. The resulting solutions then are spotted robotically onto a chemically modified glass slide such that the library-derived molecule is attached covalently to the surface. Alternatively, methods exist for the synthesis of certain classes of compounds in situ on the array surface. By far the most common application of small-molecule microarrays has been as a versatile platform for library screening, usually with the goal of identifying small-molecule ligands for a given protein of interest.

[0004] U.S. patent publication 2007/0003954 discloses protein and antibody profiling using small molecule microarrays. The application discloses ligands, which bind to ligand binding moieties wherein the ligands are arranged in arrays of synthetic molecules, which are used to screen for biomarkers and molecular fingerprints. The specific arrays described therein include, for example, a peptoid microarray having 7680 different compounds bound to the array. In that disclosure, beaded based libraries were utilized as the initial means to make peptoids which were then transferred to microarrays with addressable locations on the microarray to screen biological fluids. The screening results in a unique pattern or molecular fingerprint on the array for any-particular protein in a complex biological mixture. U.S. patent application 2010/03038055, hereby incorporated by reference, discloses certain peptoids and diagnostic arrays useful in screening biological fluids for biomarkers associated with central nervous system disorders. The specific monomers disclosed therein utilized to form the arrays therein may also be utilized in the new screening methodology of the present invention provided the libraries are enlarged to a much greater number of beads/peptoids or beads/ligands, e.g., between greater than 100K to 150 MM.

[0005] The inventors of the instant application have found that significantly larger bead based libraries (relative to microarray based screens for antibody biomarkers or bead based screens for cells) can, under the right conditions, be used to directly screen complex biological samples to find drug response associated biomarkers as well as a significantly larger pool of ligands which bind to such ligand-binding moieties. This significantly larger pool includes a significantly improved number of high affinity ligands that serve as diagnostic tools as well as potential therapeutics.

SUMMARY OF THE INVENTION

[0006] In one embodiment, the invention provides a ligand library. In an exemplary embodiment, the invention provides a large bead based random ligand library including a random peptoid ligand library.

[0007] In another embodiment, the invention provides a method for diagnosing a drug induced response in a subject, the method comprising: obtaining a biological sample from said subject; screening a ligand library against said sample; identifying binding characteristics of one or more markers in said sample with one or more ligands in the library; and determining whether said one or more markers are associated with said drug induced response, thereby diagnosing said drug induced response in said subject. In one exemplary embodiment, said drug induced response is an adverse reaction. In another exemplary embodiment, said drug induced response is a side effect. In another exemplary embodiment, said drug induced response is a resistance to said drug. In another exemplary embodiment, said drug induced response is its therapeutic efficacy including dosage efficacy.

[0008] In another embodiment, the invention provides a method for treating a disease in a subject, the method comprising: obtaining a biological sample from said subject; screening a ligand library against said sample; identifying binding characteristics of one or more markers in said sample with one or more ligands in the library; determining whether said one or more markers are associated with a response to a drug for treating said disease; administering said drug to said subject, based on the determination of association between said one or more markers to said response, thereby treating said disease in said subject.

[0009] In another embodiment, the invention provides a method for detecting a risk of adverse reaction to a drug in a subject, the method comprising: obtaining a biological sample from said subject; screening a ligand library against said sample; identifying binding characteristics of one or more markers in said sample with one or more ligands in the library; and determining whether said one or more markers are associated with said risk, thereby detecting said risk of adverse reaction to said drug in said subject.

[0010] In another embodiment, the invention provides a method for profiling one or more subjects for a response to a drug to treat a disease, the method comprising: obtaining a biological sample from said subject; screening a ligand library against said sample; identifying binding characteristics of one or more markers in said sample with one or more ligands in the library; and determining whether said one or more markers are associated with said response for said drug, thereby profiling said one or more subjects for said response to said drug to treat said disease.

[0011] In another embodiment, the invention provides a method for identifying a marker associated with a drug induced response for treating a disease, the method comprising: obtaining a biological sample from a subject; screening a ligand library against said biological sample; determining the
binding characteristics of a marker in said sample to a ligand in the library; and determining whether said marker is associated with said drug induced response for treating said disease, thereby identifying said marker associated with said drug induced response for treating said disease.

[0012] In another embodiment, the invention provides a method for identifying a marker associated with a response to a drug for treating a disease, the method comprising: obtaining a first set of biological samples from one or more subjects exhibiting said response to said drug for treating said disease; obtaining a second set of biological samples from one or more subjects not exhibiting said response to said drug for treating said disease; screening a random ligand library against said first and second biological samples; determining the differences in binding of one or more markers to one or more ligands in the library between said first and second biological samples; and identifying a marker associated with said response to said drug for treating said disease. In an exemplary embodiment, the marker is an autoantibody capable of binding to a peptoid ligand.

[0013] Other features and advantages of the present invention will become apparent from the following detailed description examples and figures. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1 shows a basic chemical schematic of the preparation of a library of Tentagel beads (KN1B) used to screen Alzheimer’s serum samples. FIG. 1A shows starting from a polystyrene bead having an amino group as the reactant (a PEG or equivalent or alternative linker may be formed between the bead and the terminal amino group). FIG. 1B shows the starting amino acid on the bead as methionine and which is then reacted to form the compound shown in B. FIG. 1C shows the submonomers (monomeric amines and halo-acetic acids) utilized to form the oligomeric library of compounds.

[0015] FIG. 2 shows a basic chemical schematic of the preparation of a library of Tentagel beads (JC3B) also used to screen Alzheimer’s serum samples. FIG. 2A shows starting from a polystyrene bead having an amino group as the reactant (a PEG or equivalent or alternative linker may be formed between the bead and the terminal amino group). FIG. 2B shows the starting amino acid on the bead as methionine and which is then reacted to form the compound shown in B. FIG. 1C shows the submonomers (monomeric amines and halo-acetic acids) utilized to form the oligomeric library of compounds. JC3B was also used to screen pancreatic cancer serum.

[0016] FIG. 3 shows a basic chemical schematic of the preparation of a library of Tentagel beads (JC4B) used to screen Alzheimer’s serum samples. FIG. 3A shows starting from a polystyrene bead having an amino group as the reactant (a PEG or equivalent or alternative linker may be formed between the bead and the terminal amino group). FIG. 3B shows the starting amino acid on the bead as methionine and which is then reacted to form the compound shown in B. FIG. 1C shows the submonomers (monomeric amines and halo-acetic acids) utilized to form the oligomeric library of compounds.

[0017] FIG. 4 shows a basic chemical schematic of the preparation of a library of Tentagel beads (JC5B) used to screen Alzheimer’s serum samples. FIG. 1A shows starting from a polystyrene bead having an amino group as the reactant (a PEG or equivalent or alternative linker may be formed between the bead and the terminal amino group). FIG. 1B shows the starting amino acid on the bead as methionine and which is then reacted to form the compound shown in B. FIG. 1C shows the submonomers (monomeric amines and halo-acetic acids) utilized to form the oligomeric library of compounds. JC5B monomers included Isobutyrylamine, 2-Methoxethylamine, Diaminobutane, Fururylamine, Cyclohexylamine, R-Methylbenzylamine, Piperonylamine and 4-(Aminooctyl)Benzenesulfoxamide.

[0018] FIG. 5 shows a basic chemical schematic of the preparation of a library of Tentagel beads (JC7B) used to screen serum samples. FIG. 3A shows starting from a polystyrene bead having an amino group as the reactant (a PEG or equivalent or alternative linker may be formed between the bead and the terminal amino group). FIG. 1B shows the starting amino acid on the bead as methionine and which is then reacted to form the compound shown in B. FIG. 1C shows the submonomers (monomeric amines and halo-acetic acids) utilized to form the oligomeric library of compounds.

[0019] FIG. 6 shows a schematic of the process of the invention to screen a complex biological sample using bead based libraries of peptoid ligands.

[0020] FIG. 7 shows normal control (NC) Dynabead hits after QDot addition in a peptoid library (JQB) prepared to screen against an Alzheimer’s normal control serum sample and Alzheimer’s diseased serum sample. The hits were picked out and the remaining ligand bound beads were used in the disease based screen.

[0021] FIG. 8 shows the Tentagel bead screening of diseased serum from Alzheimer’s patient blood samples after the NC hits were removed. The hits are shown in red, which is the Qdot secondary antibody bound to the disease associated biomarker (antibody) in the serum which is bound to a peptoid linked through a PEG linker to the bead.

[0022] FIG. 9 shows a reproducibility test which uses a normal control sample (NC 030093) after SDS wash and QDOT addition. The arrow shows which NC peptoid hits were picked to sequence.

[0023] FIG. 10 shows a reproducibility test which uses a normal control sample (NC 050047) after SDS wash and QDOT addition.

[0024] FIG. 11 shows a reproducibility test which uses a diseased sample after SDS wash and QDOT addition.

[0025] FIG. 12 shows the peptoid sequences of the putative hits selected from the Alzheimer’s screen from the JC3B library. The C-terminus is on the right side of the sheet and the N-terminus is on the left side.

[0026] FIG. 13 shows the chemical structures of the preferred high affinity hits from the Alzheimer’s screen from the JC3B library. In this example, the structures shown have a cysteine residue and were resynthesized after determining the structure of the initial hit in the preliminary screen. The JC3B library contained an analogous peptoid but which had a methionine residue on the C-terminus and not a cysteine residue.
FIG. 14 shows a competition experiment between a high affinity ligand (ADTG1) in solution versus ADTG-1-ADTG-42 on a microarray support. The competition experiment shows that ADTG1 in solution bonded to the same antibody that would have bound to peptoids ADTG-1, ADTG14, ADTG24, ADTG25, ADTG31, ADTG35 and ADTG40 on the microarray. Similar experiments were conducted on each of the peptoids to find four sets of peptoids, which bind to four distinct Alzheimer's autoantibodies.

FIG. 15 shows the four groups of distinct peptoids, which bind to different autoantibodies in the Alzheimer's screen. Each group on the figure has the higher affinity binder at the top.

FIG. 16A shows AD test data (blinded) for a pool of patients using Plaqa (JC3B-1) peptoid and FIG. 16B shows test data (blinded) for the same pool of AD patients using Plaq2 (JC3B-21). Each peptoid is presented on a microarray.

FIG. 17A shows AD test data (blinded) for a pool of patients using Plaqg3 (JC3B-7) peptoid and FIG. 17B shows test data (blinded) for the same pool of AD patients using Plaqg4 (JC3B-5). Each peptoid is presented on a microarray.

FIG. 18A shows AD test data (blinded) for a pool of patients using Plaq5 (JC3B-R8) peptoid and FIG. 18B shows test data (blinded) for the same pool of AD patients using Plaq6 (JC3B-R12). Each peptoid is presented on a microarray.

FIG. 19A shows microarray data for ADP2 in the same pool of patients for the tests conducted using Plaqg1-6. FIG. 19B shows comparative data using Plaqg4 with the same set of patients. The data shows a clear correlation between the results achieved with a previously identified ADP2 and the newly identified Plaqg4 in the same patient pool.

FIG. 20A shows microarray data for ADP3 in the same pool of patients for the tests conducted using Plaqg1-6. FIG. 20B shows comparative data using Plaqg2 with the same set of patients. The data shows a clear correlation between the results achieved with a previously identified ADP3 and the newly identified Plaqg2 in the same patient pool.

FIG. 21 shows a validation of Plaqg5 (putative hit 5 or JC3B-R8) on TentaGel beads in a comparison of disease AD serum versus healthy control (pooled) at 40 ug/mL.

FIG. 22A shows the peptoid hits in the pancreatic cancer axis using QDot 655 and using the JC3B library. FIGS. 22B and C show confirmation of hits using QDot 655 (arrows point to hits).

FIG. 23 shows pancreatic peptoid hit validation and compares disease serum addition and detection with QDot 655 versus normal serum addition.

FIG. 24 shows hit validation by mixing AD markers and PC markers. The data shows that the PC marker was detected while there was no detectable antibody on the AD peptoid bead in the pancreatic cancer serum (Serum 1).

FIG. 25 shows the pancreatic cancer screen hit sequences from the JC3B library.

FIG. 26 shows the pancreatic cancer screen hit sequences from the JC3B library.

FIGS. 27A, B and C show the results of an SLE (Lupus) screen. A is normal control and B and C are SLE serum from two different groups 1 and 2. The arrows point to the hits.

FIG. 28 shows the SLE hits from the KN1B library. The C-terminus is on the right side of the sheet.

FIG. 29 shows a hit validation for peptoid KN1B-20. Group 1 is pooled diseased serum at a concentration of about 0.374 mg/mL. (left picture) (the hits are shown with a red tinge on the bead). Non-diseased pooled serum (center picture) is provided at a concentration of about 0.378 mg/mL and the far right picture shows a no serum control.

FIG. 30 shows the binding/detection of one of the SLE (lupus) peptoids to ELISA plates using two different binding methods at different concentrations of peptoid using a fluorescein tag.

FIG. 31 shows a competition assay between plate bound KN1B-20/biotin-fluorescein versus free KN1B-20-biotin in solution at various concentrations. Signal dampening occurs as the concentration of free KN1B-20-biotin increases from equimolar concentrations of bound versus free.

FIG. 32 shows an ELISA plate having peptoid at various concentrations and clearly shows a difference between diseased serum (AD) (P column 1) and normal control serum (column 3) [1:200 doubling each well to 1:400, 1:800, 1:1,600, 1:3, 200, 1:6,400, 1:12,800]. The arrow points to the 1:800 dilution in 1XTBST buffer. The peptoid concentration in the wells is 10 mM. FIG. 32 also shows validation of the TentaGel bead platform to distinguish between diseased and control sera.

FIG. 33 shows an ELISA plate with 10 mM ADP3 and at various dilutions of AD sera versus control sera. The arrow points to the 1:800 dilution.

FIG. 34 shows an ELISA plate with 10 mM SLE-KN1B-20 and at various dilutions of AD sera versus control sera. The arrow points to the 1:800 dilution.

FIG. 35 shows an AD serum ELISA graph using 10 mM ADP3 prepared in binding buffer at various serum dilutions. Separation between normal and diseased serum occurred, over the dilution range of 1:200 through approximately 1:10,000. The starting dilutions were 0.1:200 (Group 1 AD serum 0.394 mg/mL and non-diseased serum at 0.386 mg/mL).

FIG. 36 shows an SLE serum ELISA graph using 10 mM KN1B-20 prepared in binding buffer at various serum dilutions. Separation between normal and diseased serum occurred over the dilution range of 1:200 through approximately 1:10,000. The starting dilutions were 1:200 (Group 1 SLE serum 0.375 mg/mL and non-diseased serum at 0.396 mg/mL).

FIG. 37 shows an SLE serum ELISA graph using 1.0 mM KN1B-20 prepared in DMF at various serum dilutions. Separation between normal and diseased serum occurred over the dilution range of 1:200 through approximately 1:10,000. The starting dilutions were 1:200 (Group 1 SLE serum 0.367 mg/mL and non-diseased serum at 0.322 mg/mL).

FIG. 38 shows a FACS platform for TentaGel beads hits validation.

FIG. 39 shows the degree of separation between beads having an acetyl group and beads having a 2,5-dinitrophenyl group (DNP) at various concentrations of sera (100 ug/mL to 1,000 ug/mL) and in response to treatment with an anti-DNP labeled secondary antibody. The Mean fluorescence intensity (MFI) separation was greatest at the higher dilution of 1,000 ug/mL sera.

FIG. 40 shows that there is a direct competition between free ethanamine-DNP and the binding of DNP (on a plate) to anti-DNP antibody at 1,000 ug/mL sera concentration.

FIG. 41 shows ADP3 bound anti-antibody from pooled normal control sera and pooled AD sera. The data
shows good separation at serum concentration ranges of 20 and 140 ug/mL using two different secondary antibodies (goat anti-human DyLight 649 and goat anti-human Alexa 647).

0055. FIG. 42 shows ADP3 bound auto-antibody from normal control and AD sera after background subtraction at various sera concentration ranges. There is a significant degree of separation at most sera concentration ranges from less than 20 ug/mL to 1.20 ug/mL or greater.

0056. FIGS. 43 and 44 show the structures of the SLE (lupus) resynthesized peptide ligand hits.

0057. FIG. 45 shows the preparation of ADP3 on 10 um Tentagel beads and the subsequent cleavage using CNBr along with a mass spectrometry reading of the lactone shown.

0058. FIG. 46 shows ADP3 bound autoantibody from normal control and Alzheimer’s disease sera at different concentrations. The beads were preblocked for 3 hours with 1X TBST and then detected using Goat anti-human Alexa 647 secondary antibody.

0059. FIG. 47 shows the ADP3 bound autoantibody from normal control and Alzheimer’s disease sera at different sera concentrations and also shows DNP values.

0060. FIGS. 48 and 49 show ADP3 bound autoantibody from normal controls versus Alzheimer’s disease sera using pre-blocking conditions such as E. coli lysate and lysine.

0061. FIG. 50 shows a simple schematic of the preparation of and distinction between peptides that are used in microarrays versus those peptides that are placed on ELISA plates. Schematic for how peptide microarrays are made: individual beads are segregated into the wells of microtiter plates and the peptides are cleaved from the beads to make a concentrated stock solution. Note that each well will now contain a single kind of peptide. Several thousand peptides are then spotted onto chemically-modified glass microscope slides in such a way that they bind covalently to the surface. Several thousand slides can be produced highly reproducibly from a single synthetic library. The ELISA production is similar except that there is no PEG chain on the surface but the density of peptides on the ELISA plate may be different than it is on the microarrays.

0062. FIG. 51 shows ELISA experiments with a clear distinction between normal control and diseased serum at a dilution of 1:800 using horseradish peroxidase linked to a secondary antibody that detects the disease-associated antibody-peptide complex. The colorless substrate is added and changes color (blue) upon reaction with the bound HRP enzyme.

0063. FIG. 52 shows titration data that compares various AD peptides in an ELISA test at various serum dilutions of diseased serum (A) versus normal serum (B). There is no intensity of the signals in the normal serum but clear distinction and intensity of all of the AD peptides as the concentration increases from 1:12,800 to 1:200.

0064. FIG. 53 provides a diagram that validates the correlation between the clinical diagnosis of the unblinded sample set of AD patients at various stages of Alzheimer’s disease (or not) versus the data obtained from the same patient serum samples (blinded) and which were screened against the various AD peptides (plot is average value of results of 9 peptides) of the invention to detect disease associated antibodies. The results shown are from a blinded study of plasma samples from Mayo Clinic Jacksonville. UND=Undecided. The plot was derived from taking a single serum concentration (1:800) dilution. A reading of >1 was considered positive, a reading between 1 and 0.7 was considered undecided and a reading below 0.7 was considered negative.

0065. FIG. 54 provides a diagram that validates the correlation between the clinical diagnosis of the unblinded sample set of AD patients at various stages of Alzheimer’s disease (or not) versus the data obtained from the same patient serum samples (blinded) and which were screened against the various AD peptides (plot is average value of results of 9 peptides) of the invention to detect disease associated antibodies. The results shown are from a blinded study of plasma samples from Mayo Clinic Jacksonville. UND=Undecided. The plot was derived from taking a single serum concentration (1:800) dilution. A reading of >1 was considered positive, a reading between 1 and 0.7 was considered undecided and a reading below 0.7 was considered negative.

0066. FIG. 55 provides a diagram that validates the correlation between the clinical diagnosis of the unblinded sample set of AD patients at various stages of Alzheimer’s disease (or not) versus the data obtained from the same patient serum samples (blinded) and which were screened against the various AD peptides (plot is average value of results of 9 peptides) of the invention to detect disease associated antibodies. The results shown are from a blinded study of plasma samples from Mayo Clinic Jacksonville. UND=Undecided. The plot was derived from taking a single serum concentration (1:800) dilution. A reading of >1 was considered positive, a reading between 1 and 0.7 was considered undecided and a reading below 0.7 was considered negative.

0067. FIGS. 56A-D provide data on that subset of samples from patients that have disagreements between the Optko Health peptide diagnostic assay using multiple AD peptides versus the clinical diagnosis after this information was provided when unblinded. FIG. 56A shows the data for peptides ADP3 and others as shown for a patient that was diagnosed clinically but for which the Optko peptide Plaq4 was below 1.0 (UND at a single point; Titration AD positive). All other Optko peptides were positive for AD (i.e., above 1.0). FIG. 56D shows that all Optko peptides were positive for disease associated antibodies in a patient that was currently diagnosed as normal (non-demented) suggesting pre-AD. FIG. 56C shows that none of the Optko AD peptides showed an intensity above 1 at any dilution point in a patient that was clinically diagnosed with AD suggesting that this patient had some other form of dementia. FIG. 56D shows that in a clinically positive AD patient, multiple Optko AD peptides were not positive for disease associated antibodies but two peptides (Plaq6 and Plaq4) were positive, thus UND at a single point and UND even after titration.

0068. FIG. 57 shows the cluster diagram generated from previous AD samples using a microarray spotted with ADP3. There is a clear correlation between diseased versus control in the microarray data and data generated using the ELISA platform. FIG. 57 also shows that the ADP3 peptide is selected for disease associated antibodies associated with Alzheimer’s disease and not Parkinsons or Lupus (SLE).

0069. FIG. 58 provides a summary of ELISA analysis using a total of 106 serum samples tested.

0070. FIG. 59 provides the chemical structures of Plaq4,-9.

DETAILED DESCRIPTION OF THE INVENTION

0071. In the following detailed description, numerous specific details are set forth in order to provide a thorough unde-
standing of the invention. However, it will be understood by those skilled in the art that the present invention may be practiced without these specific details. In other instances, well-known methods, procedures, and components have not been described in detail so as not to obscure the invention.

The invention encompasses a ligand library and uses thereof. Specifically, the invention encompasses screening of molecules, pharmacoproteomics, diagnosis, treatment, and other uses of a random library.

In one embodiment, the invention provides a ligand library for a personalized medicine. The terms “personalized medicine,” as used herein, may refer to the use of a test (or diagnostic) to target a drug (or therapy) at patients that are most likely to benefit therefrom, or to identify patients who may be at risk of harm from said therapy. Before a drug or diagnostic product can be marketed in the United States and most other countries, it is subjected to strict regulatory review of its safety and efficacy. In the case of a diagnostic for personalized medicine this will likely require the testing of tissue or bodily fluids from patients that received the drug to ascertain whether there is a link between their response to therapy and the presence of a particular marker. Accordingly, in one embodiment, provided herein is a method for diagnosing a response for a drug to treat a disease in a subject, the method comprising: obtaining a biological sample from said subject; screening a ligand library against said sample; identifying binding characteristics of one or more markers in said sample with one or more ligands in the library; and determining whether said one or more markers are associated with said response for said drug, thereby diagnosing response for said drug to treat said disease in said subject. Examples of a response include, but are not limited to, an adverse reaction, a side effect, a drug resistance, and a therapeutic efficacy including dosage efficacy.

Adverse drug reactions are a principal cause of the low success rate of drug development programs (less than one in four compounds that enters human clinical testing is ultimately approved for use by the U.S. Food and Drug Administration (FDA)). Drug-induced disease or toxicity presents a unique series of challenges to drug developers, as these reactions are often not predictable from preclinical studies and may not be detected in early clinical trials involving small numbers of subjects. When such effects are detected in later stages of clinical development they often result in termination of a drug development program. When a drug is approved despite some toxicity, its clinical use is frequently severely constrained by the possible occurrence of adverse reactions in even a small group of patients. The likelihood of such a compound becoming first line therapy is small (unless there are no competing products). Clinical trials that use this invention may allow for improved predictions of possible toxic reactions in studies involving a small number of subjects. The methods of this invention offer a quickly derived prediction of likely future toxic effects of an intervention. Accordingly, in another embodiment, provided herein is a method for detecting a risk of adverse reaction to a drug in a subject, the method comprising: obtaining a biological sample from said subject; screening a ligand library against said subject; identifying binding characteristics of one or more markers in said sample with one or more ligands in the library; and determining whether said one or more markers are associated with said risk, thereby detecting said risk of adverse reaction to said drug in said subject.

The present invention is based, in part, on the surprising discovery that a combination of peptoid binding biomarkers can be used to individualize therapy in patients. Given the high inter-patient variability in response to a drug, the assay methods of the present invention are particularly advantageous because they utilize a combinatorial strategy that takes into account differences in binding characteristics of multiple molecular determinants (e.g., peptoid binding biomarkers) to determine whether a disease in a patient has a high likelihood of responding to treatment with a specific drug or combination of drugs. If the patient is classified as a responder, a dosing regimen tailored to that patient can then be created to achieve therapeutic efficacy without inducing toxic side-effects. Consequently, patients classified as responders can receive the full benefits of drug induced therapy without experiencing the side-effects associated with such therapy. Similarly, patients already undergoing treatment with a drug can experience a reduction in toxic side-effects without compromising therapeutic efficacy by adjusting the subsequent dose of the drug. Likewise, patients already undergoing treatment with a drug can be monitored to assess whether resistance to the drug has developed and an alternative therapy should be administered.

As a result, the methods of the present invention enable treating a disease in a subject, the method comprising: obtaining a biological sample from said subject; screening a ligand library against said subject; identifying binding characteristics of one or more markers in said sample with one or more ligands in the library; and determining whether said one or more markers are associated with a response to a drug for treating said disease; administering said drug to said subject, based on the determination of association between said one or more markers to said response, thereby treating said disease in said subject. In another embodiment, the invention provides monitoring a treatment by a drug in a subject, the method comprising: obtaining a biological sample from said subject; screening a ligand library against said subject; identifying binding characteristics of one or more markers in said sample with one or more ligands in the library; and determining whether said one or more markers are associated with a response to a drug for treating said disease; administering said drug to said subject, based on the determination of association between said one or more markers to said response, thereby monitoring said treatment by said drug in said subject.

In another embodiment, the invention provides a ligand library for pharmacoproteomics and identifying a marker associated with a response to drug. Accordingly, in one embodiment, provided herein is a method for identifying a marker associated with drug induced response for treating a disease, the method comprising: obtaining a biological sample from a subject; screening a ligand library against said biological sample; determining the binding characteristics of a marker in said sample to a ligand in the library; and determining whether said marker is associated with said drug induced response for treating said disease, thereby identifying said marker associated with said drug induced response for treating said disease. In an exemplary embodiment, the response to drug is a patient’s response to medication, its dosage, or adverse reaction.

In one embodiment, one or more putative hits or leads of biomarkers are identified using a ligand library of the invention. A biological sample may be obtained from a subject or a plurality of subjects (e.g., patient population or
In some embodiments, the initial screening for identifying putative hits or leads is performed on a bead-based device, using a large random ligand library, and the subsequent screening for diagnostics or companion diagnostics may be performed using any platform, for example, microarray, using a non-random or random ligand library.

In one embodiment, in a subsequent screening for diagnostics or companion diagnostics, a first screening may be performed against a first ligand library and a subsequent screening against a second ligand library, wherein the first ligand library comprises a first set of ligands and the second ligand library comprises a second set of ligands. In one example, the first ligand library is screened to identify markers associated with a disease, and a second ligand library is screened in a subsequent screening to identify markers associated with a drug induced response.

In one example, the putative hits or leads are used in a first screening in patient samples, collected prior to drug treatment, to determine their association with a disease. After drug treatment, the samples may be collected from patients, and in one embodiment, the same putative hits used in the pre-treatment group may be used to identify those patients having certain disease stages or responsive changes to drug treatment. In another embodiment, different putative hits may be used to monitor drug related side effects or treatment effects that are due to drug administration and are not necessarily correlated or related to the pretreatment profile. In some embodiments, another random library may be used to find additional biomarkers that might be associated with the drug treatment.

The term “drug,” as used herein, may refer to any drug, including, but not limited to, a synthetic inorganic or organic compound, a protein, a peptide, a polysaccharides and other sugars, a lipid, DNA and RNA nucleic acid sequences, an antisense oligonucleotide, an antibody; a receptor ligand, an enzyme, an adhesion peptide, an antigen, a hormone, a growth factor, a ribozyme, and a retroviral vector.

The invention encompasses any suitable drug known one of skilled in the art. These drugs are listed in The Merck Index; Physicians’ Desk Reference, PDR Network, 2011 Edition edition (Dec. 1, 2010); U.S. Pat. No. 7,932,294, and U.S. Patent Publications 20060046967, 20110274605, 20110269722, 20110269709, and 20060205674, all of which are incorporated by reference herein in their entirety.

In one embodiment, the drug is selected from one or more of the following categories/groups: Antibiotics, Sedatives, Hypnotics, Antidepressants, Antipsychotics, Antimicrobials, Analgesics, Antipyretics, Antimigraine agents, Anticonvulsants, Drugs used in parkinsonism and movement disorders, Drugs for dementia, Antiemetics, drugs for Vertigo, CNS Stimulants activators, Antiinfective eye preparations, Antiinflammatory, anti allergic preparations, antiglaucoma drugs, preparations to cure eye diseases, aural preparations, nasal preparations, ophthalmic preparations, Antiarhythmics, Antihypertensives, alpha/beta-blockers, channel blockers, ACE inhibitors, Angiotensin II receptor antagonists, diuretics, Antianginal, nitrates, calcium channel blockers. Drugs for cardiac failure and shock, Vasodilators, Coagulants, Anticoagulants, Thrombolytics, antiplatelet drugs, Respiratory stimulants, Antitussives, Expectorants, Mucolytics, Decongestants, Antihistamine agents, Antiallergics; Antileuk, Antiserotonin drugs, H.sub.2 receptor antagonists, Proton Pump Inhibitors, Prostaglandin analogues, Antacids, Antispasmodics, drugs modifying intestinal motility, Antidiarrhoecs, antimotility drugs, antimicrobial drugs, drugs acting on gall bladder, Urinary antinfecives, Diuretics, Urinary analgesics, Antispasmodics, Antiinfective drugs acting on urethra and vagina, drugs acting on uterus, Drugs for prostatic hypertrophy, alpha blockers, antiandrogens, Drugs for erectile dysfunction, Spermicides, nonhormonal contraceptives, Emollients, keratolytics, topical antinfecives, topical antifungals, topical parastiticids, topical steroids, topical drugs for acne vulgaris, drugs for psoriasis, pigmentation disorders, and Antiseborrhoeics, Non Steroidal Anti Inflammatory Drugs (NSAIDs), COX-2 inhibitors, Antiarthritic agents, Immunosuppressants, Topical analogues, Muscle relaxants, Neuromuscular Drugs, Penicillin antibiotics, Cephalosporin antibiotics, Quinolone, Fluoroquinolone antibiotics, Macrolide antibiotics, Chloramphenicol, Tetra-cycline antibiotics, Sulfonamides, Antiaeroebics, Metronidazole, Antitubercular drugs, Antileprosy drugs, Antifungals, Antiprotozoals, Antihelminthiscs, Antiinfective Drugs, Antiinflammatory drugs, Antivirals, Anabolics, androgenic steroids, Corticosteroids, Oestrogens, Progestogens and Hormonal contraceptives, Fertility Agents, Trophic hormones and related drugs, Thyroid and antithyroid drugs, Antidiabetics and
hyperglycaemics, Vitamins, Amino acids, Anti-obesity drugs, Hypolipidemic drugs, fibrates, statins, HMG CoA reductase inhibitors, nicotinic acid group, drugs used for Gout, drugs affecting bone metabolism, biosphosphonates, Anticancer drugs, alkylating agents, cytotoxic antibiotics, antimetabolites, cytarabine, Fludarabine, 5-Fluorouracil, Meraptopurine, Thioguanine, Vincu alkalioids, Etoposide, Taxanes, Topoisomerase I inhibitors, Cytotoxic immunosuppressants, Immunomodulators, Cytoprotective, Amifostine, Oestrogens, Progestogens, hormone antagonists, antineoplastic drugs, Antiallergics, non-sedative antihistamines, Cetirizine, Desloratadine, Terfenadine, Fexofenadine, sedative histamines, histamine receptor blockers, Local anaesthetics, intravenous anaesthetics, inhalation anaesthetics, and muscle relaxants.

[0088] In another embodiment, the drug is selected from one or more of the drugs listed in Table 1 below.

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Brand Name</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alovastatin</td>
<td>Lipitor®</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Clopidrogel</td>
<td>Plavix®</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>Enalapril</td>
<td>RA, JRA, P, PA, AS</td>
<td></td>
</tr>
<tr>
<td>Fluticasone Salmeterol</td>
<td>Advair®</td>
<td>Asthma</td>
</tr>
<tr>
<td>Indinavir</td>
<td>Ritonavir</td>
<td>HIV, RA</td>
</tr>
<tr>
<td>Valsartan</td>
<td>Diovan®</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Rosuvastatin</td>
<td>Resvastatin</td>
<td>Colan cancer</td>
</tr>
<tr>
<td>Livalo®</td>
<td>Cholesterol</td>
<td></td>
</tr>
<tr>
<td>Olanzapine</td>
<td>Zyprexa®</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>Serquel®</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>Humira®</td>
<td>RA, P, JRA, PA, AS, CD</td>
</tr>
<tr>
<td>Oestrelact</td>
<td>Singulair</td>
<td>Asthma</td>
</tr>
<tr>
<td>Enoxaparin</td>
<td>Lovenox®</td>
<td>Anticoagulant DVT</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>Effexor®</td>
<td>Depression</td>
</tr>
<tr>
<td>Pegglazone</td>
<td>Actos®</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Candesartan</td>
<td>Atacand®</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Escitalopram</td>
<td>Lexapro®</td>
<td>Bipolar</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>Cipralex®</td>
<td>Depression</td>
</tr>
<tr>
<td>Glatimine</td>
<td>Copaxone®</td>
<td>Multiple Sclerosis</td>
</tr>
</tbody>
</table>

NHE, Non Hodgkin’s Lymphoma, RA Rheumatoid Arthritis, JRA Juvenile Rheumatoid Arthritis, JIA Juvenile Idiopathic Arthritis, Pw Psoriatic arthritis, CD Crohn’s Disease, UC Ulcerative Colitis, AS Ankylosing Spondylitis.

[0089] In another embodiment, the drug is selected from one or more of the following: Abacavir, Aripiprazole, Asenir Trizide, Atomoxetine, Arovastatin, Azathioprine, Boccevire, Brentuximab Vedotin, Busulfan, Capecitabine, Carbamazepine, Carisoprodol, Caudibiol, Celecoxib, Cetuximab (1), Cetuximab (2), Cevineline, Chlorhexidase and Amiprotiline, Chloroquine, Citalopram (1), Citalopram (2), Clofazimine, Clophemine, Clopimidine, Clozapine, Codeine, Crizotinib, Dapsone, Dolutibine, Desipramine, Desloratadine and Pseudoephedrine, Dexametancsone (1), Dexametancsone (2), Dextromethorphan and Quinidine, Diazepam, Droxipren and Ethylid, Estradiol, Erlotinib, Esomeprazole, Fluorouracil, Fluoxetine, Fluoxetine and Olanzapine, Flurbiprofen, Fluvoxamine (1), Fluvoxamine (2), Fluvoxamine (3), Fulvestrant, Galantamine, Gefe tinib (1), Gefitinib (2), Iloperidone, Imatinib (1), Imatinib (2), Imatinib (3), Imatinib (4), Imipramine, Indacaterol, Iritonocan, Isosorbide and Hydralazine, Lapatinib, Lenalidomide, Maraviroc, Mercaptopurine, Metoprolol, Mivacurium, Mogafinil (1), Mogafinil (2), Nefazodone, Nelfinavir, Nilotinib (1), Nilotinib (2), Oripridin, Omeprazole, Panitumumab (1), Panitumumab (2), Pantoprazole, Paroxetine, Perginterferon alfa-2b, Perphenazine, Phenylion, Pimozide, Prasugrel, Pravastatin, Propafenone, Propanolol, Propridine, Quinidine, Rabepropraz, Rasburicase, Rifampicin, Rizatriptan, Risperidone, Sodium Phenytoin and Sodium Benzote, Sodium Phenyllbutyrate, Tamoxifen, Telaprevir, Terbinafine, Tetrazenamine, Thioguanine, Thioridazine, Ticagrelor, Timolol, Tofutropium, Tolterodine, Topimatumab, Tramadol and Acetaminophen, Trastuzumab, Tretinoin, Trimepramine, Valproic Acid, Venurufenib, Venlafaxine, Vortasonazole, Warfarin (1), and Warfarin (2).

[0090] In another embodiment, the invention comprises a companion diagnostic to a drug that targets a known drug target. In another embodiment, the invention comprises a companion diagnostic to a drug target that is undergoing a clinical trial. Examples of a drug target that is undergoing clinical trial include, but are not limited to, Bapineuzumab, Solanezumab, Intravenous immunoglobulin (IVig), Lestrilpione (Dimebion), Scyllo-inositol/ENI LN 005, Methylinuminium chloride (Rember), CE-1-110, PBT2, Denvutide/AI-106, BMS-708163, PF-4449700/PP-498, Tukelskib/ NP-12 (Nypis), Belimumab, Atacipin, Mapatumumab, Apomab, Dulanemin, Odacatrin, AMG-785, DG-641, OC-000459, PLX-4032, LX-1031, and LX-1032.

[0091] A binding profile of one or more sample components (e.g., biomarkers) can be used to predict, diagnose, assess, or treat, any disease, known to one of skilled in the art. The terms “disease” or “condition” are commonly recognized in the art and designate the presence of signs and/or symptoms in an individual or patient that are generally recognized as abnormal. Diseases or conditions may be diagnosed and categorized based on pathological changes. Signs may include any objective evidence of a disease such as changes that are evident by physical examination of a patient or the results of diagnostic tests. Symptoms are subjective evidence of a disease or a patient’s condition, i.e. the patient’s perception of an abnormal condition that differs from normal function, sensation, or appearance, which may include, without limitations, physical disabilities, morbidity, pain, and other changes from the normal condition experienced by an individual. Various diseases or conditions include, but are not limited to, those categorized in standard textbooks of medicine including, without limitation, textbooks of nutrition, allopathic, homeopathic, and osteopathic medicine. In certain aspects of this invention, the disease or condition is selected from the group consisting of the types of diseases listed in standard texts such as Harrison’s Principles of Internal Medicine, 14.sup.th Edition (Fauci et al, Eds., McGraw Hill, 1997), or Robbins Pathologic Basis of Disease, 6.sup.th Edition (Cotran et al, Ed. W B Saunders Co., 1998), or the Diagnostic and Statistical Manual of Mental Disorders: DSM-IV, 4.sup.th Edition, (American Psychiatric Press, 1994), or other text books, which are incorporated herein in their entirety. The diseases are also listed in U.S. Patent Publications 2007/0009554 and 2011/0092584, which are incorporated herein in their entirety.
Examples of a disease or condition include, but are not limited to cancer, autoimmune disease, inflammatory disease, infectious disease, neurodegenerative disease, cardiovascular disease, bacterial infection, viral infection, fungus infection, prion infection, physiologic state, contamination state, or health in general.

The random ligand library screening methods of the invention can use the binding characteristics to differentiate between different forms of a disease or its state, including pre-disease states or the severity of a disease state. For example, the methods may be used to determine the metastatic state of a cancer or the susceptibility to an agent or disease state. In some embodiments, the invention includes methods and compositions for assessing ligand binding moieties present in or associated with a cancer, for example, but not limited to, breast cancer, lung cancer, prostate cancer, cervical cancer, head & neck cancer, testicular cancer, ovarian cancer, skin cancer, brain cancer, pancreatic cancer, liver cancer, stomach cancer, colon cancer, rectal cancer, esophageal cancer, lymphoma, and leukemia.

In some embodiments, the invention includes methods and compositions for assessing ligand binding moieties present in autoimmune diseases, for example, but not limited to, myasthenia gravis, chronic active hepatitis, primary biliary cirrhosis, dilated cardiomyopathy, myocarditis, autoimmune polyclonocrome syndrome type 1 (APS-1), autoimmune hepatitis, cystic fibrosis, vasculitides, acquired hypoparathyroidism, goodpasture syndrome, Crohn disease, coronary artery disease, pneumophila folliculosa, pneumophila vulgaris, Guillain-Barre syndrome, Type 1 diabetes, stiff man syndrome, Rasmussen encephalitis, autoimmune gastritis, Addison disease, insulin hypoglycemic syndrome (Hirata disease), Type B insulin resistance, acanthosis, systemic lupus erythematosus (SLE), porcine anemia, treatment-resistant Lyme arthritis, polyneuropathy, multiple sclerosis, demyelinating diseases, Rheumatoid fever, atopic dermatitis, primary biliary cirrhosis, Graves disease, autoimmune hytoproidism, vitiligo, thyroid associated ophthalmopathy, autoimmune thyroiditis, autoimmune Hashimoto thyroiditis, coeliac disease, ACTH deficiency, myositis, dermatomyositis, polymyositis, dermatomyositis, Sjogren syndrome, systemic sclerosis, progressive systemic sclerosis, systemic sclerosis, scleroderma, morphea, primary antiphospholipid syndrome, bullous pemphigoid, herpes gestationis, cicatricial pemphigoid, chronic idiopathic urticaria, connective tissue syndromes, necrotizing and crescentic glomerulonephritis (NCGN), systemic vasculitis, Wegener granulomatosis, Churg-Strauss syndrome, polymyositis, Raynaud syndrome, chronic liver disease, visceral leishmaniasis, autoimmune C1 deficiency, membrane proliferative glomerulonephritis (MPGN), prolonged coagulation time, autoimmune thrombocytopenia purpura, immunodeficiency, attherosclerosis, neuropathy, paraneoplastic pemphigus, paraneoplastic stiff man syndrome, paraneoplastic encephalomyelitis, subacute autonomic neuropathy, cancer-associated retinopathy, paraneoplastic opsoclonus myoclonus ataxia, lower motor neuron syndrome, Lambert-Eaton myasthenic syndrome, and paraneoplastic cerebellar degeneration.

In one embodiment, the invention includes methods and compositions for assessing ligand binding moieties present in infectious diseases, for example, but are not limited to, Acquired immunodeficiency syndrome (AIDS), Anthrax, Botulism, Brucellosis, Chancroid, Chlamydial infection, Cholera, Coccidioidomycosis, Cryptosporidiosis, Cyclosporiasis, Diphtheria, Ehrlichiosis, Arboviral Encephalitis, Enterohemorrhagic Escherichia coli (E. coli), Giardiasis, Gonorrhea, Haemophilus influenzae, Hansen's disease (leprosy), Hantavirus pulmonary syndrome, Hemolytic uremic syndrome, Hepatitis A, Hepatitis B, Hepatitis C, Human immunodeficiency virus (HIV), Legionellosis, Listeriosis, Lyme disease, Malaria, Measles, Meningococcal disease, Mumps, Pertussis (whooping cough), Plague, Paralytic Poliomyelitis (polio), Psittacosis (parrrot fever), Q Fever, Rabies, Rocky Mountain spotted fever, Rubella, Congential rubella syndrome, Salmonellosis, Severe acute respiratory syndrome (SARS), Shigellosis, Smallpox, Streptococcal disease (invasive Group A), Streptococcal toxic shock syndrome (STSS), Streptococcus pneumoniae, Syphilis, Tetanus, Toxic shock syndrome, Trichinosis, Tuberculosis, Tularemia, Typhoid fever, Vancomycin-Intermediate/Resistant Staphilococcus aureus, Varicella, Yellow fever, variant Creutzfeldt-Jakob disease (vCJD), Dengue fever, Ebola hemorrhagic fever, Echinococcosis (Alveolar Hydatid disease), Hendra virus infection, Human monkeypox, Influenza A H5N1 (avian influenza), Lassa fever, Marburg hemorrhagic fever, Nipah virus, O’nyong-nyong fever, Rift Valley fever, Venezuelan equine encephalitis, and West Nile virus.

The ligand library of the invention may be used to screen for any stage of a disease, for example, an early stage of a disease or an advanced late stage of a disease.

In yet another embodiment, the invention includes methods and compositions for assessing ligand binding moieties present in neurodegenerative diseases, for example, but are not limited to, stroke, hypovolemic shock, traumatic shock, repulsion injury, multiple sclerosis, AIDS, associated dementia; neuron toxicity, Alzheimer’s disease, head trauma, adult respiratory disease (ARDS), acute spinal cord injury, Huntington’s disease, and Parkinson’s Disease.

The invention comprises compositions which comprises particle based libraries of compounds selected from peptoids, peptides, oligomers, small molecules and any molecule naturally derived or synthetically made and which can be placed em a support system such as a bead or small particle.

In accordance with the present invention, there is provided compositions comprising peptoid(s) that bind antibodies indicative of a response to a drug and methods of detecting antibodies in an antibody-containing sample comprising contacting an antibody-containing sample with a support having affixed thereto a peptoid. Ligand libraries can include compounds of formula wherein the R groups on either the amine side chain or the alpha carbon are independently selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, indolyl, phenyl, thiophenyl, thiadiazolyl, isoxazolyl, oxazolyl, piperonyl, pyrazoyl, pyrazinyl, pyrindyl, pyrimidinyl, purinyl, pyridinyl, benzofuranyl, benzothienyl, benzoxazolyl, quinoline, isoazoxyl, isoquinoline cycloalkyl, alkaryl, cycloalkenyl, phenyl, pyridyl, methoxyethyl, (R)-methylenbenzy1, C1-6 alkylary1, C1-6 alky1(heteroaryl), C1-6 alkyl substituted with a group selected from OH, SH, a halogen, OR, NR1, COOR, N2R, (wherein R1 is selected from the group consisting of H or C1-6 alkyl; OC1-6 alkyl; C2-6 alkylaryl; C2-6 alkyl; and C2-6 alkyl) including one or more chemical
In one embodiment, the ligand libraries of the invention may comprise a compound of formula 1 on a support, wherein \( R_1 \) is selected from an electron rich amino acid side chain \( Y \); and \( R_2 - R_6 \) are independently selected from the groups consisting of \( H \), \( -C_3-C_6 \text{alkyl} \), \( -C_1-C_4 \text{alkyl} \text{SCH}_3 \); \( -C_5-C_9 \text{alkylIC}_{2}-C_6 \text{alkenyl} \), \( -C_1-C_3 \text{alkylC}_{2}-C_6 \text{alkynyl} \), \( -C_2-C_6 \text{COO} \), \( -C_2-C_2 \text{alkylOH} \), \( -C_1-C_3 \text{alkylIN} \)(R), \( -C_3-C_6 \text{cycloalkylalkyl} \), \( -C_1-C_3 \text{alkylarylyl} \), \( -C_1-C_3 \text{alkylheterylaryl} \), \( -C_1-C_3 \text{alkylINC}(O)C_1-C_6 \text{alkyl} \), \( -C_1-C_3 \text{alkylcycloamidine} \) wherein any of the aryl or heteroaryl groups may be independently substituted with \(-OH, CI, F, Br\), \( -OCH_3 \), \( -SO_2NH_2 \) or \(-OCH_2-O-\).

Comounds of the random ligand library (e.g., the ligand libraries) for screening a complex biological fluid are fully described in U.S. Provisional Patent Application 61/467,256 and 61/491,717 which are incorporated by reference herein in their entirety.

The large ligand libraries of the invention can be used directly in biological fluid, under the appropriate experimental conditions, to screen for biomarkers and without the need to use fewer support members (e.g., about 100,000 or less) or to require transfer of such peptides or ligands to a microarray before screening the biological fluid. In addition, the ligand libraries may also be used to screen for cell based receptors that specifically relate to a particular cell surface marker. The present invention, unlike prior methods, permits the inclusion of greater numbers of beads/resins and thus larger libraries in either the ligand binding agent screen or the cell receptor screen to directly screen the complex biological samples.

As previously described with respect to microarray systems, virtually any molecule or compound may be used to build a random bead or resin based library. These “molecules” or “compounds” may include natural products or man-made compounds or synthetically derived molecules. The source of such molecules can be from biological systems as well as non-biologically derived sources. The preferred ligands for purposes of the initial screening using large bead libraries under the conditions claimed in the present invention are made, in part, from submonomers, which are selected from any known monomeric amine and from any known acid halide or substituted acid acid halide. For example, Table 1 in U.S. Provisional Patent Application 61/467,256, which is incorporated by reference herein in its entirety, provides a range of R groups on a monosubstituted amine.

In some embodiments, the monomers and/or submonomers are selected from the group consisting of cysteine, glycine, methionine, allylamine, ethyl amine, isobutylamine, dianminobutane, methylbenzylamine (racemic or enantiomeric), piperonylamine, cyclohexylamine, 3,4 dimethoxyphenethylamine, benzylamine, N-(2-aminomethyl) acetamide, N-(3-aminopropyl)-2-pyrrolidinone, 4-(2-aminoethyl)benzenesulfonamide or furfurylamine.

In one embodiment, the ligand libraries of the invention may comprise a compound of formula 1 on a support, wherein \( R_1 \) is selected from an electron rich amino acid side chain \( Y \); and \( R_2 - R_6 \) are independently selected from the groups consisting of \( H \), \( -C_3-C_6 \text{alkyl} \), \( -C_1-C_4 \text{alkyl} \text{SCH}_3 \); \( -C_5-C_9 \text{alkylIC}_{2}-C_6 \text{alkenyl} \), \( -C_1-C_3 \text{alkylC}_{2}-C_6 \text{alkynyl} \), \( -C_2-C_6 \text{COO} \), \( -C_2-C_2 \text{alkylOH} \), \( -C_1-C_3 \text{alkylIN} \)(R), \( -C_3-C_6 \text{cycloalkylalkyl} \), \( -C_1-C_3 \text{alkylarylyl} \), \( -C_1-C_3 \text{alkylheterylaryl} \), \( -C_1-C_3 \text{alkylINC}(O)C_1-C_6 \text{alkyl} \), \( -C_1-C_3 \text{alkylcycloamidine} \) wherein any of the aryl or heteroaryl groups may be independently substituted with \(-OH, CI, F, Br\), \( -OCH_3 \), \( -SO_2NH_2 \) or \(-OCH_2-O-\).

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the C-terminus of the peptoid. In the first step of the process, the selected beads or resins (in grain or milligram quantities) are swollen in a suitable solvent such as DMF. If the beads are protected with a protecting group on the reactive amine on said bead, a base solution such as piperidine is repeatedly added with subsequent washing with DMF to deprotect the head. Once the bead is deprotected or if a bead such as a tentagel bead is initially utilized, it may be reacted with a suitable amino acid such as cysteine or methionine (protected with Fmoc or other suitable protecting group on the nitrogen and protected with Trt (tri phenylmethyl) on the sulfur and in sufficient molar quantities to react with each bead) in a suitable solvent such as DMF. HBTU (tetramethyluronium hexafluorophosphate (coupling reagent) and 4-methylmorpholine (base) along with the protected amino acid are added to the bead solution in a beaker (or tube or flask) and shaken at room temperature to form the Fmoc/Trt protected amino acid on the resin (or on a linker on the resin). The beads are then washed multiple times in a solvent such as DMF. The Fmoc group is then deprotected using a suitable reagent which permits reaction of the amine on the amino acid with another reactant such as another protected amino acid or a submonomer such as bromoacetic acid and an activating agent e.g. DIPEA (3-isopropylcarbodiimide) in a suitable solvent under heat (microwave with stirring). The resultant beads are then washed multiple times and then treated with a desired monomeric amine (in slight mole excess) in a suitable solvent under heat. The resultant beads are washed multiple times and then treated repeatedly with bromoacetic acid and the amine of choice to build the oligomer and oligomeric library. The peptoids may be cleaved from the beads using trifluoroacetic acid. Art alternative or other suitable process for peptoids comprising a preferred embodiment—e.g., those peptoids having cysteine adjacent to a monomer having a 1-yl-n-butylamine includes building a peptoid having two amino acids on the C-terminal followed by a process that further includes adding any of the monomers built in a submonomer process wherein the second amino acid is lysine. This further includes the selection of any monomer or submonomer to make α-substituted bromoacetic acid submonomers wherein the carbon substituents may be selected from typical amino acid side chains to form, after reaction of the reactants, α-substituted peptoids wherein an R group is found on either or both of the carbon on the peptide chain or the nitrogen on the peptide chain. The substituents on either the α-carbon or nitrogen may be virtually any substituent as recited herein.

[0109] Combinatorial libraries of small molecules may be obtained commercially or prepared using methods known in the art. See for example, Bichler et al. 1995; Cho et al. 1999; LePine et al., 2002; Ostergaard and Holm, 1997; Yang et al. 1999). In addition, U.S. Pat. No. 6,344,334 and publications Gallop et al., 1994), Gordon et al., 1994), Thompson and Ellman (1996) are also sources of such molecules and libraries.

[0110] Combinatorial libraries of peptides may be obtained commercially or prepared using methods known in the art. See, for example, Stewart and Young (1984); Tam et al. (1983); Merrifield (1954); and Buxton and Merrifield (1957); each of which is hereby incorporated by reference.

[0111] Combinatorial libraries of, nucleic acids including RNA or DNA may be obtained, commercially or prepared using methods known in the art. Combinatorial libraries of oligosaccharides may be obtained commercially or prepared using methods known in the art.

[0112] In each instance, the “ligands” or random ligands may be added to support resins or beads to form screening libraries can be used, under the conditions described, herein, to screen for biomarkers in complex biological fluid. The preferred ligands are peptoid ligands.

[0113] In addition to building and/or using such libraries, it may be necessary or desired to characterize, purify and/or synthesize or re-synthesize any such ligand. Such methods are known in the art and include the entire gamut of purification methods such as HPLC via chromatographic means or purification methods via chemical means; characterization methods such as mass spec or NMR or combinations of any of these methods. Such methods are—further described, for example, US Pat. Publication 2007/0003954, which is hereby incorporated by reference. In such cases, any such purified ligand may be referred to as a compound or substantially purified compound.

[0114] In the initial screening methodology of the invention, beads and/or resins are utilized as the support means having an oligomer operably coupled to said, support. In diagnostic kits or other kits having “hits” or “putative hits” from such initial screen, the support systems can be broadened to virtually any support system including microarrays or any other known diagnostic platforms. In these cases, it is necessary to ensure that such kits or other support systems with the putative hits also have or are adapted to have a detector or detection methods to permit detection of ligands having ligand binding moieties attached to such ligands. The preferred detection methods include, for example, ELISA or other methods which involve the use of labeled secondary antibodies.

[0115] Supports can be made of any suitable material. Materials utilized to make such supports can include, for example, glass, plastic, ceramic or polymeric resins or beads. Supports may also include materials such as nickel, brass, steel or other metals or mixtures of metals. The supports may also be conditioned to have linkers and/or other means to bind to or connect to or react with a ligand or active group on a ligand. Such supports are also described in U.S. Pat. Pub. No. 2007/0003954. In the present invention, the number of resins or beads having individual ligands bonded thereto or to a linker and then to said support ranges from greater than 100K to about 150 million (MM). The preferred number utilized in the initial screening method of the invention ranges between 1 MM and 2 MM ligands/resins.

[0116] TentaGel® resins are most preferred for the large ligand screening methodology of the invention. These resins are gridded copolymers consisting of a low crosslinked polystyrene matrix on which polyethylene glycol (PEG or POE) is grafted. TentaGel resins are commercially available (Rapp Polymere GmbH). As PECS is a “cameleon type” polymer with hydrophobic and hydrophilic properties, the graft copolymer shows modified chemical properties. According to the manufacturer, there are in principle two ways to introduce PEG onto the modified polystyrene matrix. The simplest immobilization procedure is to couple PEG via one of its terminal hydroxyl groups to chloromethylated polystyrene according to the classical ether synthesis or to use other bifunctional PEG’s for coupling onto the solid support. The manufacturer found that by means of anionic graft copolymerization setting up the PEG step by step directly on the matrix, PEG chains of molecular masses up to 20 kilo dalton
have been immobilized on functionalized crosslinked polystyrenes. Graft copolymers with PEG chains of about 2000-3000 dalton proved to be optimal in respect of kinetic rates, mobility, swelling and resin capacity. As there is no procedure to get monodisperse PEG with more than 10 ethylene oxide units by any polymerization techniques, there is theoretically no way to introduce monodisperse PEG chains with more than 10 ethylene oxide units to the resin or to get monodisperse PEG by direct polymerization onto the polystyrene backbone (monodisperse is defined as: PEG without any molecular weight distribution). These graft copolymers are pressure stable and can be used in batch processes as well as under continuous flow conditions. The copolymer contains about 50-70% PEG (w/w). The properties of these polymers are highly dominated by the properties of PEG and versus by the polystyrene matrix.

[0117] Setting up a chemical library or peptide library by the “one bead one compound” approach it is essential to know the number of beads which are available within a certain amount of resin as well as the capacity of single beads. Table 1 summarizes some particle sizes and correlates them to the corresponding capacity of a single bead. The calculations are based on a typical loading of TentaGel beads which are in the range of 0.25-0.3 mmol/g. For analytical characterization at least 5 pmol of resin-bound peptide are needed for sequencing on a bead. In order to estimate the optimum resin quantity for the library, which can be handled economically one, has to take into account the bead sizes and bead capacities. In respect to homogeneity of diffusion process and kinetic rates as well as for single bead analysis and single bead quantification, all our beads show a very narrow size distribution.

<table>
<thead>
<tr>
<th>resin</th>
<th>size [μm]</th>
<th>beads/g</th>
<th>capacity/bead</th>
</tr>
</thead>
<tbody>
<tr>
<td>TentaGel NH₂</td>
<td>750</td>
<td>4.62 × 10⁷</td>
<td>65 nmol</td>
</tr>
<tr>
<td>TentaGel NH₂</td>
<td>500</td>
<td>1.35 × 10⁷</td>
<td>19 nmol</td>
</tr>
<tr>
<td>TentaGel NH₂</td>
<td>300</td>
<td>6.4 × 10⁶</td>
<td>4 nmol</td>
</tr>
<tr>
<td>TentaGel NH₂</td>
<td>200</td>
<td>2.15 × 10⁶</td>
<td>1.3 nmol</td>
</tr>
<tr>
<td>TentaGel NH₂</td>
<td>130</td>
<td>8.87 × 10⁵</td>
<td>280-330 pmol</td>
</tr>
<tr>
<td>TentaGel NH₂</td>
<td>90</td>
<td>2.86 × 10⁵</td>
<td>80-100 pmol</td>
</tr>
<tr>
<td>TentaGel NH₂</td>
<td>35</td>
<td>4.55 × 10⁴</td>
<td>5.5 pmol</td>
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<tr>
<td>TentaGel NH₂</td>
<td>20</td>
<td>2.4 × 10⁴</td>
<td>1.0 pmol</td>
</tr>
<tr>
<td>TentaGel NH₂</td>
<td>10</td>
<td>1.95 × 10⁴</td>
<td>0.13 pmol</td>
</tr>
</tbody>
</table>

Correlation of particle size, number of beads per gram resin and capacity per single bead. Calculation of single bead capacity is based on a capacity of 0.25-0.3 mmol/g resin.

[0118] There are several types of TentaGel resins available showing tailored properties dependent on their application:

TentaGel S Resins:
[0119] The PEG spacer is attached to the polystyrene backbone via an alkyl linkage. This linkage is not sensitive to acids or bases. This type of resin is a standard type of resin used for peptide synthesis, solid phase organic synthesis or combinatorial chemistry.

TentaGel PAP Resins:
[0120] The PEG is attached to the polystyrene backbone via a benzyl ether linkage. This benzyl ether linkage is sensitive to harsh acid conditions like 100% TFA or mixtures of TFA/FMSBr.

[0121] These specially tailored resins are used for immunization procedures or for synthesizing PEG modified derivatives (PEG Attached Products). Using harsh acid conditions, the PEG spacer is cleaved together with the synthesized compound from the solid support to get soluble PEG modified compounds by applying solid phase conditions (e.g. PEG modified peptides).

TentaGel N Resins:
[0122] The PEG spacer is attached to the polystyrene backbone via a benzyl ether linkage. These tailored resins are used in oligonucleotide chemistry for small and large scale oligonucleotide synthesis. In comparison to CPG glass the capacity is increased by a factor of K.

[0123] As TentaGel resins are copolymers composed from polystyrene and polyethylene glycol, chemical and physicochemical properties of both base polymers have to be taken in account.

[0124] PEG itself is a hygroscopic polymer. It is known from literature that PEG esters are not very stable and easily hydrolyzed. Dependent on the storage conditions and storage time, PEG itself can be oxidized along the polymer chain to form peroxides or esters. Consequently, acid treatment or treatment with bases hydrolyzes the formed PEG-esters which result in a small amount of “PEG-leakage”. This leakage can be noticed by MS or NMR as PEG signals and impurities in the final product. This chemical behavior is true to all PEG's—and PEG based polymers.

Correlation of particle size, number of beads per gram resin and capacity per single bead. Calculation of single bead capacity is based on a capacity of 0.25-0.3 mmol/g resin.

TentaGel S: "S" means Standard resin, applicable to a large number of applications, useful in batch and flow through systems.

TentaGel R: a special suited resin for research purpose synthesis. The resin shows an increased swelling volume but is less pressure resistant. Well suited for large peptides and difficult sequences.

TentaGel HL: this high loaded version of TentaGel combines a significant higher capacity with the advantages of TentaGel resins.

TentaGel MB: TentaGel Microbeads are highlighted by extraordinary large particle diameters and high capacities based on the TentaGel technology and designed for single bead synthesis and single bead analysis.

TentaGel N: this resin type is designed for automated large scale oligonucleotide synthesis.

TentaGel J: this resin type has been developed for polymeric immunoconjugates.

TentaGel M: the microspherical shape of 10, 20, 30 μm of this TentaGel and it’s monodispersity allows applications in automated sorters, for creating huge libraries, high speed synthesis etc.

TentaGel B: describes bifunctional TentaGel resins, where the reactive sites on the outer surface of the bead is orthogonally protected to the reactive sites located in the internal volume of the bead and hybrid resin for sequential cleavage.

[0125] In addition to TentaGel beads, other resins and/or particles may be utilized build a one ligand per bead library. For example, lightly cross-linked polystyrene resins or polymide resins may be utilized. The group that joins the substrate to the resin bead can be an essential part of solid phase synthesis. The linker is a specialized protecting group, in that much of the time, the linker will tie up a functional group, only for it to reappear at the end of the synthesis. The linker must not be affected by the chemistry used to modify or extend the attached compound. And finally the cleavage step should proceed readily and in a good yield. The best linker must allow attachment, and cleavage in quantitative yield.

[0126] In certain aspects, the support may be a bead, a plate, a dipstick, a filter, a membrane a pin, or a well. Detecting may
comprise RIA, FIA, ELISA, Western blot, flow cytometry, FRET, or surface plasmon resonance.

**Carboxylic Acid Linkers**

[0127] The first linking group used for peptide synthesis bears the name of the father of solid phase synthesis, Merrifield resin is cross-linked polystyrene functionalised with a chloromethyl group. The carbonyl group is attached by the nucleophilic displacement of the chloride with a cesium carbonate salt in DMF. Cleavage to regenerate the carboxylic acid is usually achieved by hydrogen fluoride.

[0128] The second class of linker used for carboxylic acid is the Wang linker. Hits linker is generally attached to cross-linked polystyrene, Tentagel and polyacrylamide to form Wang resin, it was designed for the synthesis of peptide carboxylic acids using the Fmoc-protection strategy, and due to the activated benzyl alcohol design, the carboxylic acid product can be cleaved with TFA. A more acid-labile form of the Wang resin has been developed. The SA.SRIN resin has the same structure as the Wang linker but with the addition of a methoxy group to stabilise the carboxonium ion formed during acid catalysed cleavage.

**Carboxamide Linkers**

[0129] The rink linker is generally preferred for generating primary carboxamide on solid phase, to the present invention, this linker is utilized when manufacturing or re-synthesizing the hits or putative hits from the primary screen of the invention. In such cases, cysteine is the first monomer reacted with the rink linker and then the process involves either subsequent monomer addition to build the oligomer or subsequent sub-monomer chemistry to build the oligomer. The greater acid sensitivity in the rink linker is a consequence of the two additional electron donating methoxy groups. In the generation of primary carboxamide, the starting material is attached, to the linker as a carboxylic acid and after synthetic modification is cleaved from the resin with TEA.

[0130] The use of Rink resin to produce carboxamide following TFA-catalysed cleavage.

**Alcohol Linkers**

[0131] A hydroxyl linker based on the tetrahydropyranyl (THP) protecting group has been developed by Thompson and Ellman. All type of alcohols readily add dihydropyra and the resulting THP protecting group is stable to strong base, but easily cleaved with acid. This linker is attached to a Merrifield resin. The trietyl group is a good acid-labile protecting group for a lot of heteroatoms. The trietyl group has been used to anchor alcohols in the synthesis of a library of β-mercaptoethanes.

**Carbamates and Amines Linker**

[0132] Carbamates linker has been used for the synthesis of a combinatorial library of 576 polyamines prepared in the search of inhibitors of trypanosomal parasitic infections. Two linkers were investigated. One based on hydroxymethylbenzoic acid 1, and the other one, an electron-donating group has been added 2. The last one allowed cleavage by TFA while the first one could be cleaved with strong acidic conditions.
A very useful linker has been recently developed for the generation of tertiary amine. (Tertiary amines are commonly used in drug molecules.) Primary and secondary amines are introduced to the linker by Michael addition. The amine may be alkylated to give a resin-bound quaternary aminonium ion. In mildly basic condition, Hoffmann elimination occurs to give a tertiary amine of high purity.

Traceless Linkers

In some cases, the starting materials are loaded onto the resin in one form, such as carboxylic acid, and cleaved in another form; a carboxamide for example. This is perfectly acceptable if the target compound requires the released function. (Peptides invariably contain a carboxylic acid or carboxamide.) However, the growth in interest in combinatorial libraries of low molecular weight non-peptides has elicited a need in new types of linker. These linkers show non-specific function after cleavage, Traceless linkers are so called because an examination of the final compound reveals no trace of the point of linkage to the solid phase.

Samples

As discussed previously, the complex biological fluids prepared for analysis in the process of the invention include or can include a host of potential biomarkers including markers expressed on cells (non-adherent cells, including T-cells or other immune effector cells), microorganisms, proteins, peptides, lipids, polysaccharides, small molecules, organic molecules, inorganic molecules, biological molecules and including any detectable or readable moiety in such complex milieu. In a preferred embodiment, such markers are antibodies and, in particular, are antibodies generated as a result of a disease or condition. In a preferred embodiment, body fluids such as serum, plasma, saliva or other fluids or samples derived from a patient or animal or organism are the source of such markers. Each sample or tissue or biologically derived, or environmentally derived or obtained sample is conditioned, or treated or diluted, or otherwise handled in order to expose said sample to either the initial screening or any subsequent screening using putative hits or ligands which have affinity for such biomarkers. The samples are diluted pursuant to the methods recited herein to provide or permit sufficient distinction between background levels or noise and signals associated with the binding of a ligand to a ligand binding moiety.

The time and/or conditions necessary to expose the ligands/supports to such samples depend upon the particular sample and other factors. The preferred conditions for the process of the claimed invention are further described herein. In almost all cases, washing or eluting steps and other conditioning means are utilized following exposure of the biological fluid to the large ligand library and/or ligands or kits derived from such library. Aqueous solutions are utilized including buffered solutions such as HEPES buffer, iris buffer or phosphate buffered saline. Support systems may also be treated with energy absorbing materials to facilitate desorption or ionization of a “complex” from a support surface. Chemical means are also utilized to decouple or remove ligand-ligand binding moiety complexes from supports.

Detection methods for detecting ligand-ligand binding moiety complexes on a support include photometric and non-photometric methods. Such methods include ensuring that the process includes a method to detect and measure absorbance, fluorescence, refractive index, polarization or light scattering. These include direct and/or indirect means to measure such parameters. Methods involving fluorescence include fluorescent tagging in immunological methods such as ELISA or sandwich assay. Methods involving refractive index include surface plasmon resonance (SPR), grating coupled methods (e.g. sensors uniform grating couplers, wavelength-interrogated optical sensors (WIOS) and chirped grating couplers), resonant mirror and interferometric techniques. Methods involving polarization include ellipsometry. Light scattering methods may also be used. Other means for tagging and/or separating and/or detecting can also include magnetic means. Magnetic resonance imaging, gas phase ion spectrometry, MRI may all be used.

Analysis of the data generated typically involves quantification of a signal due to the detected biomarker versus a control or reference. The data can be analyzed by any suitable means. Computers and computer programs may be utilized to generate and analyze the data. Beads and/or other supports may be computer coded or coded for identification purposes. Data analysis includes analysis of signal strength under the particular conditions of the assay or detection method. Ligands, ligand binding moieties or reference moieties and/or secondary detection moieties may be labeled or radio-labeled or tagged with a detectable moiety. One of ordinary skill in the art can assess the difference and/or distinction between biological fluid samples that have disease associated biomarkers versus those control or healthy patient, samples that do not contain such markers. One of ordinary skill in the art can also determine, pursuant to the methods described herein, the presence of false positives or other hits that are or may be found in control samples to account for and/or remove such “hits” and one of ordinary skill in the art, pursuant to the methods described herein, can continue the process of determining or finding disease associated biomarkers in patient samples having trout disease or condition. The “detection” of such hits, in all cases, is accomplished by means for detecting the binding of a ligand-binding moiety such as a disease associated biomarker or other marker to ligands in a ligand library such as those described herein.

Biomarkers associated with the diseases and/or conditions recited herein will vary depending upon the particular stage of the disease and/or condition of the particular patient or animal or other organism assessed. The ligands, which are the putative hits and the compounds recited herein, are expected to, in most cases, mimic the natural antigen that initiates the immune response and/or formation of antibodies or immune cells in the first instance. The present invention and screening process claimed and recited herein does not require knowledge of either the particular antigen or the antibody generated in response to the antigen. The ligands, however, may be useful in their own right as vaccines or drug
candidates in addition to being useful in the screens and diagnostic methods recited herein. The present invention thus includes compounds and pharmaceutical compositions.

Peptoid Screens:

[0140] To screen one-bead-one-compound (OBOC) combinatorial peptide libraries, tens of thousands to millions of peptide bearing beads are prepared and then mixed with a complex biological sample. The initial complex biological sample is preferably a control sample and a subsequent complex biological sample treated with a ligand library that has “removed” the control hits is then treated and/or screened against a diseased complex biological sample. The ligands/beads that interact, with at least one disease associated biomarker are then detected, identified and isolated and/or characterized. In a preferred embodiment, a Tentagel screening protocol is used which comprises (1) bead preparation, (2) screening of complex biological fluid and (3) detection of hits.

Peptide Screens:

[0141] To screen one-bead-one-compound (OBOC) combinatorial peptide libraries, tens of thousands to millions of peptide bearing beads are prepared and then mixed with a complex biological sample following the processes described herein. The beads that interact with disease associated biomarkers are then identified and isolated for compound structure determination. For example, OBOC peptide library screening using streptavidin (SA) as probe protein, labeled with a red fluorescent dye and using the COPAS BIO-BEAD flow sorting equipment to separate fluorescent from nonfluorescent beads may be performed. See Manni et al., J. Comb. Chem., 2009, 11 (1), pp 149-157. The red dyes which may be used are ATTO 590 and Texas Red. After incubating the library with the SA-red fluorescent dye conjugate, positive beads caused by peptide-SA interaction are obtained. The beads are analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Thus, peptide libraries may be used in a manner that is analogous to the process described herein with peptides wherein initial control biological fluid samples are used to remove any ligand/bead hits from the starting compound library and wherein the remaining members of the library are used to then screen for any hits in a diseased complex biological fluid sample. These hits are the putative hits which are then carried forward in any diagnostic kits.

[0142] In a similar manner, any ligand may be screened on the beads or supports using the processes described herein. These ligands include, in addition to peptides or polypeptides, nucleic acid oligomers, polysaccharides, small molecules and/or any combination thereof which can be built into libraries and, under the conditions recited herein, used to screen complex biological fluid.

Kits and Diagnostic Tools

[0143] Any of the compounds or compositions described herein may be further utilized in diagnostic kits either in a clinical or laboratory setting. These kits can range from simple point of care diagnostic assays to complex and multiplex instruments or probes. The support systems and “packaging” surrounding the core support/ligand system can be selected from current commercial kits that are designed to include the putative hits and/or hits that are resynthesized and installed on such suitable platforms or they can be used in newly designed diagnostic kits. The kits will typically be accompanied by all suitable reagents and instructions to use the kits to screen for and/or diagnosis the particular disease or condition the kit is designed for. Any such kit or method will comprise at least one putative hit or ligand that has been identified pursuant to the screening method recited herein. This ligand or plurality of ligands may be selected from the same ligand or a mixture of ligands which comprise the compounds of the invention. The ligands may be selected based upon their affinity for a disease associated biomarker for one particular disease state or a group or battery of diseases or conditions. The preferred ligands are peptoid ligands. The kits will also contain instructions for the physicians diagnosing a particular disease or condition and specific labeling for the particular kit and disease state or condition. The present invention thus includes a combination of a kit including all of its essential components such as the putative peptoids or ligands found from an initial screening using any one of the libraries disclosed herein and/or known pursuant to the specific methods recited herein and labeling instructions. It is also envisioned that the particular processes and methods and materials discerned herein may be utilized in a clinical and laboratory setting under the supervision of a skilled operator. The kits and/or instruments or equipment comprise ligands such as peptoids that are specific for disease associated antibodies and/or cells. The “kit” may comprise a complete diagnostic kit and/or screening kit or the “kit” may comprise components or sub-components containing or comprising the diagnostic peptoids, antibodies discovered and characterized through such peptoids or native antigens that are discovered and purified and/or characterized as a result of interaction with and discovery from the autoantibody. Such antibodies and purified antigens comprise part of the present invention.

[0144] In one embodiment, provided herein is a kit for diagnosis of a disease. In another embodiment, provided herein is a kit for treating a disease. The kit may comprise a ligand library, detection reagents for screening the ligand library against a biological sample, adjuvants for the screening, and a package insert. The package insert may include instructions for performing the diagnostic steps, instructions for determining a drug administration, and instructions for administering the drug based on the determination. In some embodiments, the kit may include a package insert that is a label approved by FDA or a drug approval authority in other countries.

[0145] The ligand libraries of the present invention are utilized to find and determine ligands that bind to disease associated biomarkers. Such ligands are then utilized in the kits and/or methods described generally above to assess, screen or diagnose disease states or conditions. These diagnostic methods typically involve screening for and finding disease associated biomarkers which comprise antibodies and/or other biological markers. As stated above, these antibodies can be further identified and characterized using the ligands of the invention on suitable columns to pull out or remove such antibodies from blood samples. The antibodies can in turn be used to probe for and discover the native antigen associated with such antibody. The present invention thus includes both the antibodies and purified antigens associated with such antibodies and which are discovered, isolated and characterized using the methods of the invention.

[0146] Kits and/or other means to screen for and/or diagnose disease states or conditions must, in the first instance, be
assessed against patient samples. These patient samples may be derived from normal control samples or from patient samples wherein said patient has been identified as a patient that has or is suspected of having that disease or condition. The patient may have other symptoms associated with the disease beyond the “presence” of a disease associated biomarker. The patient may be in an early stage of the disease, may not have the disease or condition at all or may be in a late stage of a particular disease. In any clinical context and under appropriate guidelines and controls, patient and clinical samples may be provided in a blinded fashion and then assessed using the compounds of the invention. The data generated as a result of the screening may then be analyzed after un-blinding to find or not find statistically significant results or correlations with known or underlying data about any particular patient or group of patients. The present invention comprises a method of screening for the presence of a disease or condition comprising (1) screening a biological sample from a patient with at least one compound of the invention: (2) screening a control biological sample under the same conditions using said at least one compound and (3) comparing the healthy control data versus the patient data to determine the presence or absence of a disease associated biomarker. A group of patients or patient samples having or suspected of having disease X may be screened against a kit or diagnostic probe having at least one compound of the invention and the data generated with respect to each patient may be utilized on a case by case basis to confirm or validate a disease state or condition or lack thereof. Such data generated herein may be used in combination with the total, information known about that particular patient, to assess the patient’s condition and to provide guidance to the medical practitioner providing treatment options. The “information” generated as a result of any such screen may be used in the clinical trial setting to assess individual patients that are taking drag therapy. The present invention thus includes a method of assessing clinical trial progression comprising use of a screen performed, according to the methods described herein. In a preferred embodiment, the present invention relates to a method of screening for or diagnosing an early disease state comprising use of a screen or compound claimed herein to detect a disease associated biomarker. The invention is particularly useful, in the context of early disease intervention wherein detection of such biomarkers is expected to occur well before aggressive progression of the disease. In another context, early intervention in cardiovascular disease and/or metabolic disease as well as neurological disease is expected to save lives and prevent or be useful for preventing further development of such diseases without early medical intervention or treatment.

The present invention also includes methods to increase the resolution or efficiency of the difference between a control or standard solution and the complex biological fluid containing the disease associated biomarker. For example, methods include preconditioning or pre-treating or pre-blocking the system/serum with buffers and/or conditioning agents such as E. coli lysate and/or lysine.

In yet another embodiment, there is provided a method of treating a subject suspected of having a disease comprising (a) contacting an antibody-containing sample from said subject with one or more supports having affixed thereto a peptoid comprising a peptoid of the formulas recited herein (b) detecting antibodies bound to said peptoids; and (c) making a treatment decision based on the result of step (b). The method may further comprise obtaining said sample from a subject. The method may also further comprise making a diagnosis of a disease for a subject from which said sample was obtained if antibody binding to the peptoid is greater than, that observed for control non-diseased patients. The method may also further comprise making a treatment decision for said subject. The sample may be contacted with more than one peptoid of formulas recited herein. The sample may be contacted with a multiplex platform for the purposes of diagnosing multiple disease states or conditions. The support may be a bead, a plate, a pipette, a filter, a membrane or a pin, or a well. The sample may be blood, serum, saliva or CSF. Detecting may comprise RIA, FFA, ELISA, Western blot, flow cytometry, FRET, or surface plasmon resonance.

A further embodiment is directed to an antibody composition isolated from a biological fluid that is indicative of a disease, in certain embodiments the antibodies are isolated by contacting a sample having such antibodies with a peptoid composition that specifically binds antibodies indicative or associated with a disease. The antibodies can be removed, isolated, or purified from other non-antibody and non-D specific components. The antibodies can then be washed and/or dissociated from the peptoid capture agent(s).

In certain embodiments, a peptoid array made from the peptoids discovered in the process described herein is hybridized with a biological sample that has been supplemented with, a bacterial lysate, e.g., an E. coli lysate. The biological sample includes a control sample and a sample having a marker for a central nervous system disorder. For example, microarray slides are covered with a hybridization chamber and equilibrated with 1X TBST (50 mM Tris, pH 8.0, 150 mM NaCl, 0.1% Tween20) for about 15 minutes. The slides are then blocked with a bacterial lysate at a concentration at least, at most, or about 0.5, 1, 1.5, 2 mg/ml of lysate. The lysate is removed and the slides are incubated with a milliliter of biological sample (having an approximate protein concentration of 5, 10, 15, 20 or 25 Dg/ml including all ranges and values there, between) in bacterial lysate with gentle shaking. Microarrays are then washed with 1X TBST and hybridized with labeled Anti-IgG antibodies (e.g., at 1:400 dilution). The slides are then washed with an appropriate buffer. The slides are dried using a centrifuge (e.g., 5 min spin at 1500 rpm) and scanned on a microarray scanner, for example, using a 635-nm laser at 100% power and a 600 or 650 photomultiplier tube gain. The present invention thus also relates to a method of reducing background antisera noise in a diagnostic assay comprising treating the control plasma sample and the diseased sample with, an E. coli lysate and contacting said samples with a peptoid or ligand array. It is believed that this process can be used to support treatment of any array used to detect and distinguish antibodies in sera in the context of comparing a control sample to a diseased sample.

It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein.
The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.”

It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions and kits of the invention can be used to achieve methods of the invention.

Throughout this application, the term “about” is used to indicate that a value includes the inherent, variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

It is understood that any one of the putative hits or peptoids discovered through the process recited herein may also be a therapeutic drug or vaccine candidate. The present invention relates to a process for discovering drug candidates or vaccines comprising use of the screen pursuant to the methods described herein.

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

**EXAMPLES**

**Example 1**

**Library Preparation**

**Protocol for Peptoid Synthesis (Cyst-Peptoid or Methionin-Peptoid)**

The following example demonstrates how peptoid libraries of the invention were generated. The materials utilized in the example include reaction flasks or beakers, plastic tubing, 10-15 ml syringes with needles, latex gloves, 10-15 ml polypropylene test tubes and microtubes with solvent safe tips (1000 μl), glass pipettes and resin beads. The chemicals and/or reagents utilized included N,N-Dimethylformamide, Bromoacetic acid (BMA), Anhydrous Dimethylformamide, Piperidine, Acetonitrile, 3-diisopropylcarbodiimide (DIPC), Trifluoroacetic acid, 5-(6)-Carboxyfluorescein, Dichloromethane (DCM), and 4-Methylmorpholine (NMM). The various amines utilized in each library preparation were also used as HBTU (Tetramethyluronium Hexafluorophosphate) and triethylsilane.

**Peptoid Preparation**

The concentration of each amine used in the process is calculated using the following formula: V-FW/d/1000×2M×5 ml

**Procedure:**

**Step 1**

Swelling of the Resin Beads.

(a) 250 milligrams of resin beads were placed into a clean dried reaction flask and 5 mls of dry DMF was added to the beads which were allowed to swell over a period of an hour or less. The beads were then washed with DMF multiple times (2 or 3x) under vacuum.

**Step 2**

(b), (c) and (d) were omission when “protected beads” (e.g., TeMa-Gel) were used.

20% solution of Piperidine (base) using anhydrous DMF as the solvent was used in the following process:

The following process comprising steps (b), (c) and (d) was done 2 times when using “protected beads”

(b) 2.5 ml of 20% piperidine solution was added to the protected beads;

(c) After adding piperidine, the reaction flask was placed on a shaker/incubator for 20 minutes, set at 200 rpm @ 25°C.

(d) The reaction flask was then washed 8-10 times with dry DMF using 5 mls of DMF.

The following solutions were also prepared:

668 mg Fmoc-Cys(Trt)-OH in anhydrous DMF (2 ml volume) (solution A).

161.6 mg of NMM, in anhydrous DMF 2 ml

303.2 mg HBTU was added to NMM vial (solution B).

**Addition of HBTU/NMM and Fmoc-Cys**

1 ml of each of solution A and solution B was added to the beads—(HBTU/NMM) and Fmoc-Cys(Trt)-OH—and shaken for 1 hour.

The beads were washed in DMF 5-10 times.

The remaining 1 ml solution of solution A and B were added to the beads which were shaken for a period of 1 hour and then washed again in DMF 5-10 times.

The following solutions were also prepared; 20% Piperidine (in anhydrous DMF)

2M Bromoacetic acid

50% DIC/A. DMF

2M solution of each amine

The following steps (a), (b) and (c) were performed 2 times, 2.5 ml of 20% piperidine solution was added; (b) the reaction flask was shaken at 200 rpm at 25 degrees C. and then (c) the beads were washed with DMF 8X to 10X.

A 10 ml solution of 2M Bromoacetic acid was prepared.

A 10 ml solution of 50% 3.2M DIC/anhydrous DMF (v/v) was also prepared.

2M amine solutions were prepared of each amine in and for each library.

For peptoid synthesis, 1 ml of 2M stock solution was used each time an amine was added on the peptoid chain.

**Step 3**

1 ml of Bromoacetic acid was added to the reaction vessel;

(b) 1 ml of 50% DIC/DMF solution was then added and the resultant solution, was (c) microwaved for 15 seconds @ 10% power.

Step (c) was performed 2 times swirling the flask side to side between sets of micro waving.
A white precipitate was formed after each micro waving step. The beads were then washed 8-10 times with DMF.

**Step 4**

One ml of the first amine in the sequence was added to the reaction flask containing the bromo intermediate from the preceding step and the vessel was shaken to evenly distribute the amine on the beads. The reaction was then initiated using the microwave for 15 seconds at 10% power 2 times. The reacted beads were then washed with hydrous DMF 8-10 times.

**Step 4**

Steps 3 and 4 were repeated until all amines were added to make the target peptides.

**Step 5**

The beads were then washed with dichloromethane (DCM) 3 times and allowed to dry.

**Step 6**

The peptides were then cleaved from the beads using a 5% TFA solution (5 ml). The peptides were then collected off the beads which were washed with a solvent (CHClCN and water) to remove residual peptides. Argon gas was used to remove any residual TFA. The peptides were then lyophilized and characterized and purified as necessary.

**[0187]** The reaction conditions specified above may be modified on an as-needed basis depending upon the quantities needed for any particular bead composition.

**[0188]** FIGS. 1-5 generally demonstrate how the library of the invention was prepared for a disease, for example, AD diagnostics, pancreatic cancer diagnostics and lupus. In general the beads having an amine moiety were linked to an amino acid residue through a series of steps using standard peptide chemistry which was then reacted with an activated carboxyl moiety having a halide group which was then reacted with a monomeric amine having an R group. Steps 2 and 3 of the cycle were repeated as shown in the Figures to create large peptoid libraries having 1 MM to 2MM distinct ligands. The initial screening library prepared on Tentagel resin or beads typically had a methionine amino acid as the first monomer in the chain. The present inventor uses such an amino acid to facilitate cleavage from a bead or resin that does not have a cleavable linker. The Rink resin used to build the cysteine containing peptoids have linkers which do not need or require the use of methionine as the first amino acid. The cysteine containing peptoids were typically resynthesized after the initial screen found the putative hits. The cysteine sulfur group permits reaction of the peptoid chain with, for example, another reactive moiety on a diagnostic platform substrate. The peptoids which were resynthesized also contained a L-yl-n-butylamino moiety as the first side chain in the chain after the amino acid amine. It is believed this group is necessary to display the peptoid and to solubilize the peptoid in aqueous containing solutions.

**Example 2**

**General Screening Methodology**

**[0189]** 160 micron Tentagel beads attached to a peptoid of choice were swelled overnight in DMF. Beads were then washed ten times in a reaction vessel with Millipore water and vigorous shaking. Fresh Millipore water was added each time, and on the 10th wash, beads were allowed to shake overnight at 150-200 rpm. The next day, beads were washed in the same fashion with IX TBST and allowed to shake at 150-200 rpm for at least 3 hours.

**[0190]** Beads were then split evenly into 15 ml conical tubes, about 0.5 grams per tube in 1X TBST. TBST was removed, and 4 ml of diluted normal human serum was added to each tube. Serum stock made in IX TBST was Nanodropped to get desired concentration of 20 ug/ml. Tubes containing serum and beads were then tumbled overnight at 4 degrees Celsius in the dark. Serum was then pipetted out of the tubes, and replaced with 4 ml 1X TBST. Tubes were then slowly inverted to re-suspend then beads, and then allowed to settle. TBST was removed and added twice more, for a total of three TBST washes.

**[0191]** Secondary antibody solution was then prepared, by preparing 5 ul of goat anti-human IgG Qdot 655 per 1 ml IX TBST. Once the last TBST addition was removed from the beads, 4 ml of the Qdot solution was added, and beads were tumbled for 2 hours at four degrees Celsius in the dark. The beads were then allowed to settle, and the Qdot solution was removed. The beads were then washed three times with 4 ml of IX TBST. Beads were then pored into a clear Petri dish of viewed under a UV microscope containing a DAPI filter. All beads stained red were removed.

**[0192]** After the first screen was completed, beads were poured back into 15 ml conical tubes, and tumbled at four degrees Celsius for at least four hours before the next serum sample addition. Disease serum was then added to the beads in the same fashion as normal serum addition, with the exception that the serum was diluted in PBS starting block as opposed to IX TBST. However, the original stock was prepared in IX TBST in order to obtain the proper concentration with the nanodrop. The serum addition and secondary antibody addition is the same as with the normal serum.

**[0193]** Once diseased “hits” were removed, they were pooled into a 1.5 ml eppendorf tube, and heated at 95 degrees Celsius for 25-30 minutes in 1% SDS. The SDS was then removed from the tube, and replaced with Millipore water. Beads were then tumbled for 15 minutes at four degrees Celsius. The water was then replaced with fresh water, and beads were tumbled for another 15 minutes. The water was then removed and replaced with 50/50 Acetonitrile/water solution and allowed to tumble another 15 minutes. Beads were then separated into individual wells in a 96 well plate and allowed to dry.

**[0194]** A solution of 20-30 mg Cyanogen Bromide, 500 ul Acetonitrile, 400 ul Glacial Acetic Acid, and 100 ul Millipore water was made, and 20 ul of solution was added to each well containing a hit bead. The plate was covered and allowed to shake for 16 hours at 100 rpm. The cover was then removed, and the cleavage solution was evaporated from the wells. The hits were then spotted onto a MSMS plate and sequenced using a 4800 MALDI/TOF/TOF analyzer.

**[0195]** FIG. 6 provides a general schematic of the screening methodology disclosed and claimed herein.

**Example 3**

**Diagnosing a Response to Drug For Treating a Disease**

**[0196]** Five hundred milligrams of 160 micron Tentagel beads (JC3B library) is added to a fifteen milliliter conical tube. Five milliliters of DMF is added to the tube, and the
beads are allowed to sit overnight to swell. The next day, DMT\(^+\) is pipetted out of the tube and replaced with five milliliters of 1X TBST. The tube is inverted to mix, and beads are then allowed to settle to the bottom and 1X TBST is removed. Five milliliters of 1X TBST is added and removed five more times.

[0197] Normal serum samples are prepared by adding 4 milliliters of PBS starting block to a tube, and adding 7 μl of each of four separate drug treated samples to the same tube. The serum is added to the washed beads and beads and serum are allowed to tumble overnight at four degrees Celsius in the dark. The next morning, beads are removed from the tumbler and allowed to settle before the serum is pipetted out of the tube. Four milliliters of 1X TBST is added to the tube, and the tube is inverted to mix. The TBST is then pipetted out of the tube and replaced with four milliliters of fresh 1X TBST and removed again.

[0198] DYNABead solution is then prepared by adding 50 ul of well-mixed goat anti-human IgG DYNABeads to four milliliters of 1X TBST. The mixture is then added to the washed beads. The beads are then allowed to rumble for two hours at four degrees Celsius in the dark.

[0199] Without washing the beads, the DYNABeads screen is performed. The tube is placed in a magnet holder and filled to the brim with 1X TBST. The magnet and tube are slowly agitated for two minutes, and the beads are allowed to settle in the magnet holder. The TBST and free beads that settled to the bottom are removed carefully, to not touch the hit beads attached to the sides of the bead and replaced with fresh 1X TBST. The process is repeated two to three times, until no beads could be seen attached to the sides of the tube. The hit beads are then combined into one tube.

[0200] Remaining non-hit beads are divided into 15 milliliter tubes, inverted and quickly pulsed centrifuged. The supernatant is removed and replaced with fresh 1X TBST. This process repeated 6-8 times until no more DYNABeads are visible in the bead/TBST solution. The hit beads are washed in the same fashion.

[0201] Beads are combined back into a 15 ml tube, and normal serum is added to the beads in the same way as previously stated, and allowed to tumble overnight at 4 degrees Celsius in the dark. In addition, three Milliliters of each founded to 1 milliliter of PBS starting block, and this solution is added to the DYNABead “hit” bead tube. The next day, beads are washed in the same fashion as with the normal serum addition.

[0202] 20 ul of goat-anti human IgG Quantum Dot 655 is diluted in 4 milliliters 1X TBST (20 ul Qdot in 1 M1) 1X TBST for the “hit” tube, and added to the beads. The solution is tumbled for two hours at four degrees Celsius in the dark. Both hit and non-hit tube is washed four times with 1X TBST and screened for bright red beads under a UV microscope. Remaining beads are tumbled in four milliliters of 1X TBST for one hour, and disease or drug treated serum sample is added in the same fashion as the normal serum sample. The magnetic screen and Qdot additions are performed in the same manner as previously stated. The hits are then sequenced on a MALDI TOF/TOF mass spectrometer.

Example 4

Microarray Data with A Single Measurement

[0203] Microarrays were prepared as described in U.S. Pat. Publication No. 2010/0303805 which is hereby incorporated by reference; Microarray slides are covered with hybridization chamber and equilibrated with 1X TBST (50 mM Tris, pH 8.0, 150 mM NaCl, 0.1% Tween20) for 15 minutes. The slides are then blocked with 1 ml blocking buffer for 1 hour at 4\(^\circ\) C. The blocking buffer is removed and the slides are incubated with 1 ml of serum (20 mg/ml) for 1 hour at 4\(^\circ\) C. The E. coli lysate is removed and the slides are incubated with 1 ml of serum (15 mg/ml) in E. coli lysate (1.5 mg/ml) for 18 hours at 4\(^\circ\) C with gentle shaking. Microarrays are then washed three times with 1X TBST and hybridized with Alexa-647 labeled Anti IgG antibody (5 mg/ml) for 2 hours on orbital shaker at 4\(^\circ\) C. The chamber cassettes were removed from microarray slides and washed with 1X TBST (3x15 min) followed by 0.1XTBST (1x10). The slides are then dried on centrifuge (5 min at 1500 RPM) and scanned on microarray scanner (Gene Pix Autoloader 4200) by using 635-nm laser at 100% power and 600 or 650 photomultiplier tube gain. All the scanned images were analyzed by the Gene Fix Pro 6.0 software and Genespring software.

Example 5

ELISA Protocol

[0204] 96 well Maleimide-activated plates were obtained from Thermo Scientific, and washed three times with 400 ul/400 well wash buffer (0.1M sodium phosphate, 0.15M sodium chloride, 0.05% Tween 20, pH 7.2), using a plate washer from Beckman Coulter. The peptoid of interest was diluted to 10 mM in PBS binding buffer (0.1M sodium phosphate, 0.15M sodium chloride, 10 mM EDTA, pH 7.2), and 200 ul of the peptoid solution was added to the appropriate wells. The plate was then allowed to incubate in the dark for two hours at room temperature with shaking at 500 rpm. The peptoid solution was then aspirated from the wells using die plate washer, and again washed three times with 400 ul/well of wash buffer. L-Cysteine HCl: H2O (Thermo Scientific) was diluted to 10 μg/μl in binding buffer, and 200 ul per well was added. The plate was then incubated for one hour in the dark at room temperature with shaking at 500 rpm, and washed three times. 200 ul StartingBlock™ (PBS) Blocking Buffer (Thermo Scientific) was added to the wells and the plate was incubated for one hour at 4\(^\circ\) C, in the dark with shaking at 500 rpm. The plate was washed three times with the plate washer, and serum samples were prepared by serially diluting in binding buffer from 1:2000 downward. Concentrations of the 1:200 sample stocks were taken using a nano-drop (Thermo Scientific), to make sure that they were similar. Each diluted sample was vortexed before preparing the next dilution. 200 ul of the appropriate dilution for serum (both disease and normal) was added to die plate, as well as binding buffer without serum as a control. The serum was allowed to incubate for two hours at room temperature in the dark with 500 rpm shaking. The plate was again washed, and 200 ul of a 1:30,000 dilution of goat anti-human IgG HRP (Micropore) in binding buffer was added to the appropriate wells and incubated at room temperature for 30 minutes with 500 rpm shaking in the dark. The plate was washed three times, and 100 ul of TMB (3,3',5, 5'-tetramethylbenzidine) solution was added to each well, and color was allowed to develop for 30 minutes on the bench in the dark. 1.00 ul of 2M Sulfuric acid stop solution was added to stop the reaction, and the wells were read at an absorbance of 450 using a plate reader.
Thus, in each case and with respect to each drug treatment, the process of the invention may be utilized to rapidly discover biomarkers associated with a drug response (e.g., an adverse reaction, a drug resistance, and a therapeutic dosage efficiency) and ligands which bind to such markers. These ligands—this larger pool of ligands—can then be used for multiple diagnostic and/or therapeutic purposes. The diagnostic platforms include microarrays, bead based methods and ELISA systems. The conditions utilized above comprise an important aspect of the invention. These conditions include dilution ranges for sera as well as the concentration of a particular peptoid on a bead or in a well and detection methods. The number of beads having a peptoid on a bead may vary depending upon the particular test kit or screening kit. These numbers may also vary depending upon whether beads/ligands are used in the initial screening protocol and method and the format herein and/or are used in a test kit based upon the discovery of a high affinity ligand.

It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications that are within the spirit and scope of the invention, as defined by the appended claims.

What is claimed is:

1. A method for diagnosing a drug induced response in a subject, the method comprising: obtaining a biological sample from said subject; screening a ligand library against said sample; identifying binding characteristics of one or more markers in said sample with one or more ligands in the library; and determining whether said one or more markers are associated with said drug induced response, thereby diagnosing said drug induced response in said subject.

2. The method of claim 1, wherein said response is a side effect of said drug, an adverse reaction to said drug, a resistance to said drug, or a therapeutic dosage efficacy of said drug.

3. The method of claim 1, wherein said drug is at least one of the drugs listed in Table 1.

4. The method of claim 1, further comprising the step of determining whether said subject is responsive or non-responsive to a treatment or therapy by said drug.

5. The method of claim 1, wherein said drug induced response is associated with a disease or a stage of said disease.

6. The method of claim 1, wherein said disease is a cancer, an autoimmune disease, an inflammatory disease, an infectious disease, a neurodegenerative disease, or a cardiovascular disease.

7. The method of claim 6, wherein the cancer disease is breast cancer, lung cancer, prostate cancer, cervical cancer, head and neck cancer, testicular cancer, ovarian cancer, skin cancer, brain cancer, pancreatic cancer, liver cancer, stomach cancer, colon cancer, rectal cancer, esophageal cancer, lymphoma, or leukemia, such as ligands that recognize markers unique to these disease states.

8. The method of claim 6, wherein the autoimmune disease is lupus, myasthenia gravis, multiple sclerosis, narcolepsy, rheumatoid arthritis, nephritis, Chagus disease, scleroderma, or Sjogren’s disease.

9. The method of claim 6, wherein the infectious disease is a result of infection with viruses, bacteria or fungi.

10. The method of claim 6, wherein the neurodegenerative disease is Alzheimer’s disease, dementia, or Creutzfeld-Jacob disease.

11. The method of claim 1, wherein said ligand library comprises a plurality of ligands identified in an earlier initial screening or preselected based on known reactivity to said ligand binding markers.

12. The method of claim 1, wherein said ligand library is a peptoid library.

13. The method of claim 1, wherein the library comprises a compound of formula 1,

\[
\begin{align*}
\text{R}_1 & \quad \text{R}_2 \\
\text{O} & \quad \text{O} \\
\text{R}_3 & \quad \text{R}_4 \\
\text{N} & \quad \text{N} \\
\text{R}_5 & \quad \text{R}_6
\end{align*}
\]

wherein \( \text{R}_1 \) is selected from an electron rich amino acid side chain \( \text{Y}\); \( \text{R}_2 \) is selected from \( \text{H} \); and \( \text{R}_3, \text{R}_4, \text{R}_5, \text{R}_6 \) are independently selected from the groups consisting of \( -\text{C}_1-\text{C}_6 \text{alkyl}, -\text{C}_1-\text{C}_6 \text{alkyl} \text{alkyl}, -\text{C}_1-\text{C}_6 \text{alkyl}, -\text{C}_1-\text{C}_6 \text{alkyl}, -\text{C}_6 \text{alkyl} \text{OH}, -\text{C}_1-\text{C}_6 \text{alkyl} \text{alkyl}, -\text{C}_1-\text{C}_6 \text{alkyl} \text{alkyl} \text{OH} \), and any of \( \text{Y} \) is selected from \(-\text{CH}, \text{CH}_2, \text{CH}_3, \text{CH}_4 \) or \( -\text{O}-\text{CH}_2-\text{O}-\).

14. The method of claim 1, wherein the library comprise a compound of formula 1,

\[
\begin{align*}
\text{R}_1 & \quad \text{R}_2 \\
\text{O} & \quad \text{O} \\
\text{R}_3 & \quad \text{R}_4 \\
\text{N} & \quad \text{N} \\
\text{R}_5 & \quad \text{R}_6
\end{align*}
\]

wherein the compounds are produced by a process which comprises use of a reactant selected from the group consisting of

(A) furfurylamine; 3,4-dimethoxyethanolamine; benzylamine; N-(2-aminooethyl)acetamide; N-(3-amino-propyl)-2-pyrrolidinone; ethanolamine; glycin; diaminobutane; allylamine; piperonylamine; methylbenzylamine; isobutylamine; 4-(2-aminooethyl)benzenesulfonamide or cyclohexylamine; or

(B) methoxyethylamine; piperonylamine; cyclohexylamine; diaminobutane; methylbenzylamine; isobutylamine; furfurylamide or 4-(2-aminooethyl)benzenesulfonamide; or

(C) furfurylamine; ethanolamine; glycin; diaminobutane; allylamine; piperonylamine; methylbenzylamine; isobutylamine or 4-(2-aminooethyl)benzenesulfonamide; or
(D) furfurylamine, N-(2-aminoethyl)acetamide; N-(3-aminoethyl)-2-pyrrrolidone; ethanolamine; glycine; dianinobutane; allylamine; piperonylamine; methylbenzylamine; isobutylamine; 4-(2-aminoethyl)benzenesulfonamide; or (E) cysteine, glycine, allylamine, ethanolamine, isobutylamine, methylbenzylamine, piperonylamine, methionine, cyclohexylamine, 3,4-dimethoxyphenethylamine, benzylamine, N-(2-aminoethyl)acetamide, N-(3-aminopropyl)-2-pyrrolidone; (2-aminoethyl)benzenesulfonamide and fururylamine; and

wherein,

R₁ is selected from the group consisting —(C₆H₅)CH₂—; R₂ is selected from H; R₃ and R₄ are independently selected from the groups consisting of H, —C₆H₅, —C₆H₅alkyl, —C₆H₅alkyISCH₃, —C₆H₅alkylC₆H₅alkyl, —C₆H₅alkylC₆H₅alkynyl, —C₆H₅C₆H₅COOH, —C₆H₅C₆H₅alkylvOH, —C₆H₅C₆H₅alkynIN(R₂), —C₆H₅C₆H₅cycloalkyl, —C₆H₅C₆H₅alkylalkyl, —C₆H₅C₆H₅alkylalkynyl, —C₆H₅C₆H₅alkylCN(O)(C₆H₅)C₆H₅alkyl, —C₆H₅C₆H₅alkylcyanoamide wherein any of the aryl or heteroaryl groups may be independently substituted with —OH, CI, F, Br, —OCH₃, —SO₂NH₂ or —O—CH₂—O—;

R₅ is selected from the group consisting of furfuryl or —(C₆H₅)C₆H₅alkylN[R], R₆ is selected from the group consisting of H, 1-ethylallyl, 1-yl-2-hydroxyethyl, isobutyl, 1-yl-n-butyramine, methylbenzyl, piperonyl, cyclohexyl, 1-yl-2-(3,4-dimethoxyphenyl)ethyl, benzyl, 1-yl-2-(acetamide)ethyl, 1-yl-3-2-pyrrolidine, 1-yl-2-(4-benzenesulfonamide)ethyl or furfuryl and is 3-11.

15. The method of claim 1, wherein the library comprises a compound having a formula Ia

wherein the compound is selected from the group consisting of a compound of formula Ia wherein,

(a) R₅ is n-butyramine; R₆ is 1-yl-n-butyramine; R₇ is isobutyl; R₈ is isopropyl; R₉ is methylbenzyl; R₁₀ is 1-yl-2-(4-benzenesulfonamide)ethyl and R₁₁ is 1-yl-2-methoxyethyl;

(b) R₅ is 1-yl-n-butyramine; R₆ is 1-yl-2-(4-benzenesulfonamide)ethyl; R₇ is 1-yl-n-butyramine; R₈ is cyclohexyl; R₉ is 1-yl-2-(4-benzenesulfonamide)ethyl and R₁₀ is methylbenzyl;

(c) R₅ is 1-yl-n-butyramine; R₆ is piperonyl; R₇ is 1-yl-n-butyramine; R₈ is cyclohexyl; R₉ is 1-yl-n-butyramine; R₁₀ is methylbenzyl and R₁₁ is cyclohexyl;

(d) R₅ is piperonyl; R₆ is 1-yl-n-butyramine; R₇ is isobutyl; R₈ is 1-yl-2-(4-benzenesulfonamide)ethyl; R₉ is methylbenzyl; R₁₀ is cyclohexyl; R₁₁ is isobutyl and R₁₂ is 1-yl-n-butyramine;

(e) R₅ is 1-yl-n-butyramine; R₆ is 1-yl-n-butyramine; R₇ is cyclohexyl; R₈ is 1-yl-2-methoxyethyl; R₉ is methylbenzyl; R₁₀ is 1-yl-2-methoxyethyl; R₁₁ is isopropyl; R₁₂ is isopropyl; R₁₃ is cyclohexyl; R₁₄ is methylbenzyl and R₁₅ is piperonyl;

(f) R₅ is piperonyl; R₆ is 1-yl-n-butyramine; R₇ is 1-yl-n-butyramine; R₈ is isopropyl; R₉ is isopropyl; R₁₀ is 1-yl-2-methoxyethyl; R₁₁ is methylbenzyl; R₁₂ is piperonyl; R₁₃ is methylbenzyl; R₁₄ is cyclohexyl; R₁₅ is 1-yl-2-(4-benzenesulfonamide)ethyl and R₁₆ is 1-yl-2-(4-benzenesulfonamide)ethyl;

(g) R₅ is 1-yl-n-butyramine; R₆ is 1-yl-n-butyramine; R₇ is piperonyl; R₈ is methylbenzyl; R₉ is 1-yl-n-butyramine; R₁₀ is piperonyl; R₁₁ is methylbenzyl; R₁₂ is 1-yl-2-(4-benzenesulfonamide)ethyl and R₁₃ is cyclohexyl;

(h) R₅ is 1-yl-n-butyramine; R₆ is 1-yl-n-butyramine; R₇ is methylbenzyl; R₈ is 1-yl-2-methoxyethyl; R₉ is cyclohexyl; R₁₀ is 1-yl-2-methoxyethyl; R₁₁ is methylbenzyl and R₁₂ is piperonyl;

(i) R₅ is 1-yl-n-butyramine; R₆ is methylbenzyl; R₇ is 1-yl-n-butyramine; R₈ is methylbenzyl; R₉ is 1-yl-n-butyramine; R₁₀ is 1-yl-2-(4-benzenesulfonamide)ethyl; R₁₁ is cyclohexyl; R₁₂ is 1-yl-2-(4-benzenesulfonamide)ethyl and R₁₃ is cyclohexyl;

(j) R₅ is 1-yl-2-(4-benzenesulfonamide)ethyl; R₆ is methylbenzyl; R₇ is methylbenzyl; R₈ is cyclohexyl; R₉ is methylbenzyl; R₁₀ is 1-yl-n-butyramine; R₁₁ is methylbenzyl; R₁₂ is isobutyl and R₁₃ is 1-yl-n-butyramine;

(k) R₅ is 1-yl-2-methoxyethyl; R₆ is 1-yl-n-butyramine; R₇ is 1-yl-2-(4-benzenesulfonamide)ethyl; R₈ is piperonyl; R₉ is 1-yl-n-butyramine; R₁₀ is 1-yl-2-(4-benzenesulfonamide)ethyl; R₁₁ is methylbenzyl and R₁₂ is 1-yl-2-methoxyethyl;

(l) R₅ is 1-yl-n-butyramine; R₆ is 1-yl-2-(4-benzenesulfonamide)ethyl; R₇ is 1-yl-n-butyramine; R₈ is cyclohexyl; R₉ is 1-yl-2-methoxyethyl; R₁₀ is isobutyl; R₁₁ is 1-yl-2-(4-benzenesulfonamide)ethyl and R₁₂ is methylbenzyl;

(m) R₉ is 1-yl-2-methoxyethyl; R₁₀ is 1-yl-n-butyramine; R₁₁ is 1-yl-n-butyramine; R₁₂ is 1-yl-2-methoxyethyl; R₁₃ is methylbenzyl; R₁₄ is 1-yl-n-butyramine; R₁₅ is furfuryl and R₁₆ is furfuryl;

(n) R₉ is cyclohexyl; R₁₀ is 1-yl-n-butyramine; R₁₁ is 1-yl-n-butyramine; R₁₂ is furfuryl; R₁₃ is 1-yl-2-methoxyethyl; R₁₄ is 1-yl-2-methoxyethyl; R₁₅ is 1-yl-2-(4-benzenesulfonamide)ethyl and R₁₆ is furfuryl;

(o) R₉ is 1-yl-n-butyramine; R₁₀ is piperonyl; R₁₁ is 1-yl-2-(4-benzenesulfonamide)ethyl; R₁₂ is 1-yl-2-methoxyethyl; R₁₃ is methylbenzyl; R₁₄ is 1-yl-2-methoxyethyl and R₁₅ is methylbenzyl;

(p) R₉ is cyclohexyl; R₁₀ is cyclohexyl; R₁₁ is piperonyl; R₁₂ is 1-yl-2-(4-benzenesulfonamide)ethyl; R₁₃ is 1-yl-2-methoxyethyl; R₁₄ is 1-yl-n-butyramine; R₁₅ is 1-yl-2-methoxyethyl and R₁₆ is isobutyl.
(q) R9 is piperonyl; R10 is 1-yl-n-butylamine; R11 is 1-yl-2-methoxyethy1; R12 is 1-yl-2-(4-benzensulfonamide) ethyl; R13 is piperonyl; R14 is 1-yl-n-butylamine; R15 is methylbenzyl and R16 is methylbenzyl.

(r) R9 is methylbenzyl; R10 is methylbenzyl; R11 is methylbenzyl; R12 is 1-yl-n-butylamine; R13 is piperonyl; R14 is 1-yl-n-butylamine; R15 is piperonyl and R16 is 1-yl-n-butylamine.

(s) R9 is 1-yl-n-butylamine; R10 is 1-yl-n-butylamine; R11 is methylbenzyl; R12 is 1-yl-n-butylamine; R13 is methylbenzyl; R14 is methylbenzyl; R15 is 1-yl-2-methoxyethyl and R16 is piperonyl.

(i) R9 is methylbenzyl; R10 is 1-yl-2-(4-benzensulfonamide)ethyl; R11 is 1-yl-n-butylamine; R12 is 1-yl-2-(4-benzensulfonamide)ethyl; R13 is isobutyl; R14 is 1-yl-n-butylamine; R15 is methylbenzyl and R16 is 1-yl-2-methoxyethyl.

(u) R9 is 1-yl-2-methoxyethyl; R10 is 1-yl-n-butylamine; R11 is isobutyl; R12 is isobutyl; R13 is cyclohexyl; R14 is 1-yl-n-butylamine; R15 is 1-yl-2-(4-benzensulfonamide)ethyl and R16 is cyclohexyl.

(v) R9 is isobutyl; R10 is 1-yl-n-butylamine; R11 is 1-yl-n-butylamine; R12 is methylbenzyl; R13 is 1-yl-n-butylamine; R14 is 1-yl-2-(4-benzensulfonamide)ethyl; R15 is isobutyl; R16 is 1-yl-n-butylamine; R17 is piperonyl and R18 is piperonyl.

(w) R9 is 1-yl-2-(4-benzensulfonamide)ethyl; R10 is isobutyl; R11 is methylbenzyl; R12 is 1-yl-2-methoxyethyl; R13 is 1-yl-2-(4-benzensulfonamide)ethyl; R14 is isobutyl; R15 is 1-yl-2-(4-benzensulfonamide)ethyl and R16 is cyclohexyl.

(x) R9 is furfuryl; R10 is furfuryl; R11 is piperonyl; R12 is cyclohexyl; R13 is piperonyl; R14 is 1-yl-n-butylamine; R15 is 1-yl-2-(4-benzensulfonamide)ethyl and R16 is cyclohexyl.

(y) R9 is piperonyl; R10 is piperonyl; R11 is 1-yl-2-methoxyethyl; R12 is 1-yl-2-methoxyethyl; R13 is 1-yl-n-butylamine; R14 is 1-yl-n-butylamine and R15 is 1-yl-2-methoxyethyl.

(z) R9 is piperonyl; R10 is 1-yl-n-butylamine; R11 is isobutyl; R12 is 1-yl-2-(4-benzensulfonamide)ethyl; R13 is methylbenzyl; R14 is cyclohexyl; R15 is isobutyl and R16 is 1-yl-n-butylamine.

(aa) R9 is 1-yl-2-(4-benzensulfonamide)ethyl; R10 is 1-yl-2-(4-benzensulfonamide)ethyl; R11 is methylbenzyl; R12 is methylbenzyl; R13 is 1-yl-n-butylamine; R14 is 1-yl-n-butylamine; R15 is 1-yl-2-(4-benzensulfonamide)ethyl and R16 is 1-yl-2-(4-benzensulfonamide)ethyl.

(bb) R9 is 1-yl-n-butylamine; R10 is 1-yl-2-methoxyethyl; R11 is 1-yl-n-butylamine; R12 is isobutyl; R13 is cyclohexyl; R14 is 1-yl-n-butylamine; R15 is 1-yl-n-butylamine and R16 is piperonyl.

(cc) R9 is cyclohexyl; R10 is methylbenzyl; R11 is cyclohexyl; R12 is piperonyl; R13 is 1-yl-n-butylamine; R14 is 1-yl-2-(4-benzensulfonamide)ethyl; R15 is 1-yl-n-butylamine and R16 is 1-yl-2-methoxyethyl.

(dd) R9 is 1-yl-2-methoxyethyl; R10 is 1-yl-2-(4-benzensulfonamide)ethyl; R11 is 1-yl-n-butylamine; R12 is 1-yl-2-methoxyethyl; R13 is 1-yl-2-(4-benzensulfonamide)ethyl; R14 is isobutyl and R15 is cyclohexyl.

(ee) R9 is 1-yl-2-methoxyethyl; R10 is methylbenzyl; R11 is 1-yl-n-butylamine; R12 is 1-yl-n-butylamine; R13 is piperonyl; R14 is 1-yl-2-(4-benzensulfonamide)ethyl and R16 is 1-yl-n-butylamine.

(ff) R9 is 1-yl-n-butylamine; R10 is methylbenzyl; R11 is 1-yl-2-(4-benzensulfonamide)ethyl; R12 is methylbenzyl; R13 is 1-yl-n-butylamine; R14 is 1-yl-2-(4-benzensulfonamide)ethyl; R15 is 1-yl-2-(4-benzensulfonamide)ethyl and R16 is cyclohexyl.

(gg) R9 is 1-yl-n-butylamine; R10 is 1-yl-2-methoxyethyl; R11 is 1-yl-n-butylamine; R12 is 1-yl-2-(4-benzensulfonamide)ethyl; R13 is 1-yl-2-(4-benzensulfonamide)ethyl and R16 is cyclohexyl.

(hh) R9 is cyclohexyl; R10 is cyclohexyl; R11 is methylbenzyl; R12 is 1-yl-n-butylamine; R13 is methylbenzyl; R14 is cyclohexyl; R15 is methylbenzyl and R16 is 1-yl-n-butylamine.

(ii) R9 is 1-yl-n-butylamine; R10 is furfuryl; R11 is methylbenzyl; R12 is 1-yl-2-(4-benzensulfonamide)ethyl; R13 is isobutyl; R14 is cyclohexyl; R15 is methylbenzyl and R16 is cyclohexyl.

(jj) R9 is 1-yl-n-butylamine; R10 is methylbenzyl; R11 is 1-yl-n-butylamine; R12 is 1-yl-2-(4-benzensulfonamide)ethyl; R13 is 1-yl-2-methoxyethyl; R14 is methylbenzyl; R15 is 1-yl-2-methoxyethyl and R16 is isobutyl.

(kk) R9 is 1-yl-2-(4-benzensulfonamide)ethyl; R10 is 1-yl-n-butylamine; R11 is 1-yl-n-butylamine; R12 is methylbenzyl; R13 is cyclohexyl; R14 is 1-yl-2-(4-benzensulfonamide)ethyl and R16 is methylbenzyl.

(ll) R9 is 1-yl-2-(4-benzensulfonamide)ethyl; R10 is 1-yl-2-(4-benzensulfonamide)ethyl; R11 is 1-yl-n-butylamine; R12 is 1-yl-n-butylamine; R13 is methylbenzyl and R16 is 1-yl-n-butylamine.

(mm) R9 is 1-yl-n-butylamine; R10 is 1-yl-2-(4-benzensulfonamide)ethyl; R11 is 1-yl-2-(4-benzensulfonamide)ethyl; R12 is 1-yl-2-methoxyethyl; R13 is 1-yl-n-butylamine; R14 is cyclohexyl; R15 is 1-yl-2-(4-benzensulfonamide)ethyl and R16 is methylbenzyl.

(nn) R9 is 1-yl-n-butylamine; R10 is 1-yl-n-butylamine; R11 is piperonyl; R12 is 1-yl-2-(4-benzensulfonamide)ethyl; R13 is 1-yl-n-butylamine; R14 is methylbenzyl; R15 is methylbenzyl and R16 is cyclohexyl.

(oo) R9 is 1-yl-2-(4-benzensulfonamide)ethyl; R10 is methylbenzyl; R11 is methylbenzyl; R12 is 1-yl-n-butylamine; R13 is methylbenzyl; R14 is piperonyl; R15 is 1-yl-n-butylamine and R16 is 1-yl-2-methoxyethyl.

(pp) R9 is piperonyl; R10 is 1-yl-n-butylamine; R11 is 1-yl-n-butylamine; R12 is methylamine; R13 is piperonyl; R14 is 1-yl-n-butylamine; R15 is piperonyl and R16 is 1-yl-2-methoxyethyl.

(qq) R9 is cyclohexyl; R10 is cyclohexyl; R11 is furfuryl; R12 is 1-yl-2-methoxyethyl; R13 is isobutyl; R15 is cyclohexyl; R16 is cyclohexyl.

(rr) R9 is piperonyl; R10 is 1-yl-n-butylamine; R11 is isobutyl; R12 is cyclohexyl; R13 is 1-yl-2-methoxyethyl; R14 is cyclohexyl; R15 is 1-yl-2-(4-benzensulfonamide)ethyl and R16 is 1-yl-2-(4-benzensulfonamide)ethyl.

(ss) R9 is cyclohexyl; R10 is cyclohexyl; R11 is 1-yl-n-butylamine; R12 is methylbenzyl; R13 is 1-yl-n-butylamine; R15 is methylbenzyl; R16 is cyclohexyl and R16 is piperonyl.

and pharmaceutically acceptable salts thereof.
16. The method of claim 1, wherein the library comprises a compound of the formula:

wherein the compound is selected from the group consisting of a compound of formula II wherein,

(a) R₉ is n-butylamine; R₁₀ is 1-yl-n-butylamine; Rₑ₁ is piperonyl; Rₑ₂ is methylbenzyl; Rₑ₃ is piperonyl; Rₐ₄ is methylbenzyl; Rₐ₅ is 1-yl-2-(4-benzenesulfonamide)ethyl and Rₐ₆ is 1-yl-2-methoxymethyl;

(b) R₉ is 1-yl-n-butylamine; R₁₀ is 1-yl-2-(4-benzenesulfonamide)ethyl; Rₑ₁ is 1-yl-n-butylamine; Rₑ₂ is cyclohexyl; Rₑ₃ is 1-yl-2-methoxymethyl; Rₐ₄ is 1-yl-2,2-dimethylpropyl (isobutyryl); Rₐ₅ is 1-yl-2-(4-benzenesulfonamide)ethyl and Rₐ₆ is methylbenzyl;

(c) R₉ is 1-yl-n-butylamine; R₁₀ is 1-yl-n-butylamine; Rₑ₁ is piperonyl; Rₑ₂ is 1-yl-2-(4-benzenesulfonamide)ethyl; Rₑ₃ is 1-yl-n-butylamine; Rₐ₄ is methylbenzyl; Rₐ₅ is cyclohexyl and Rₐ₆ is cyclohexyl;

(d) R₉ is piperonyl; R₁₀ is 1-yl-n-butylamine; Rₑ₁ is isobutyryl; Rₑ₂ is 1-yl-2-(4-benzenesulfonamide)ethyl; Rₑ₃ is methylbenzyl; Rₐ₄ is cyclohexyl; Rₐ₅ is isobutyryl and Rₐ₆ is 1-yl-n-butylamine;

(e) R₉ is 1-yl-n-butylamine; R₁₀ is 1-yl-n-butylamine; Rₑ₁ is methylbenzyl; Rₑ₂ is 1-yl-2-methoxymethyl; Rₑ₃ is cyclohexyl; Rₐ₄ is cyclohexyl; Rₐ₅ is methylbenzyl and Rₐ₆ is piperonyl;

(f) R₉ is piperonyl; R₁₀ is 1-yl-n-butylamine; Rₑ₁ is isopropyl; Rₑ₂ is isopropyl; Rₑ₃ is 1-yl-2-methoxymethyl; Rₐ₄ is cyclohexyl; Rₐ₅ is 1-yl-2-(4-benzenesulfonamide)ethyl and Rₐ₆ is 1-yl-2-(4-benzenesulfonamide)ethyl;

(g) R₉ is 1-yl-n-butylamine; R₁₀ is 1-yl-n-butylamine; Rₑ₁ is piperonyl; Rₑ₂ is methylbenzyl; Rₑ₃ is piperonyl; Rₐ₄ is methylbenzyl; Rₐ₅ is 1-yl-2-(4-benzenesulfonamide)ethyl and Rₐ₆ is cyclohexyl;

(h) R₉ is 1-yl-n-butylamine; R₁₀ is 1-yl-n-butylamine; Rₑ₁ is methylbenzyl; Rₑ₂ is 1-yl-2-methoxymethyl; Rₑ₃ is cyclohexyl; Rₐ₄ is cyclohexyl; Rₐ₅ is methylbenzyl and Rₐ₆ is piperonyl;

(i) R₉ is 1-yl-n-butylamine; R₁₀ is methylbenzyl; Rₑ₁ is methylbenzyl; Rₑ₂ is 1-yl-n-butylamine; Rₑ₃ is 1-yl-n-butylamine; Rₐ₄ is cyclohexyl; Rₐ₅ is 1-yl-2-(4-benzenesulfonamide)ethyl and Rₐ₆ is cyclohexyl;

(j) R₉ is 1-yl-2-(4-benzenesulfonamide)ethyl; R₁₀ is methylbenzyl; Rₑ₁ is methylbenzyl; Rₑ₂ is cyclohexyl; Rₑ₃ is 1-yl-n-butylamine; Rₐ₄ is methylbenzyl and Rₐ₅ is cyclohexyl;

(k) R₉ is 1-yl-2-methoxymethyl; R₁₀ is isobutyryl; Rₑ₁ is 1-yl-2-(4-benzenesulfonamide)ethyl; Rₑ₂ is piperonyl; Rₑ₃ is cyclohexyl; Rₐ₄ is methylbenzyl and Rₐ₅ is 1-yl-2-methoxymethyl;

(l) R₉ is 1-yl-n-butylamine; R₁₀ is 1-yl-2-(4-benzenesulfonamide)ethyl; Rₑ₁ is 1-yl-n-butylamine; Rₑ₂ is cyclohexyl; Rₐ₃ is 1-yl-2-methoxymethyl; Rₐ₄ is isobutyryl; Rₐ₅ is 1-yl-2-(4-benzenesulfonamide)ethyl and Rₐ₆ is methylbenzyl;

(m) R₉ is 1-yl-2-methoxymethyl; R₁₀ is 1-yl-n-butylamine; Rₑ₁ is 1-yl-n-butylamine; Rₑ₂ is 1-yl-2-methoxymethyl; Rₐ₃ is methylbenzyl; Rₐ₄ is 1-yl-n-butylamine; Rₐ₅ is furfuryl and Rₐ₆ is furfuryl;

(n) R₉ is cyclohexyl; R₁₀ is cyclohexyl; Rₑ₁ is 1-yl-n-butylamine; Rₑ₂ is furfuryl; Rₑ₃ is 1-yl-2-methoxymethyl; Rₐ₄ is 1-yl-2-methoxymethyl; Rₐ₅ is 1-yl-2-(4-benzenesulfonamide)ethyl and Rₐ₆ is furfuryl;

(o) R₉ is 1-yl-n-butylamine; R₁₀ is piperonyl; Rₑ₁ is 1-yl-2-(4-benzenesulfonamide)ethyl; Rₑ₂ is 1-yl-2-methoxymethyl; Rₐ₃ is methylbenzyl; Rₐ₄ is 1-yl-n-butylamine; Rₐ₅ is 1-yl-2-methoxymethyl and Rₐ₆ is methylbenzyl;

(p) R₉ is cyclohexyl; R₁₀ is cyclohexyl; Rₑ₁ is piperonyl; Rₑ₂ is 1-yl-n-butylamine; Rₑ₃ is 1-yl-n-butylamine; Rₐ₄ is 1-yl-n-butylamine, and Rₐ₅ is isobutyryl;

(q) R₉ is piperonyl; R₁₀ is 1-yl-n-butylamine; Rₑ₁ is 1-yl-2-methoxymethyl; Rₑ₂ is 1-yl-2-(4-benzenesulfonamide)ethyl; Rₑ₃ is piperonyl; Rₐ₄ is 1-yl-n-butylamine; Rₐ₅ is methylbenzyl and Rₐ₆ is piperonyl;

(r) R₉ is methylbenzyl; R₁₀ is methylbenzyl; Rₑ₁ is methylbenzyl; Rₑ₂ is 1-yl-n-butylamine; Rₑ₃ is piperonyl; Rₐ₄ is 1-yl-n-butylamine; Rₐ₅ is methylbenzyl and Rₐ₆ is methylbenzyl;

(s) R₉ is 1-yl-n-butylamine; R₁₀ is 1-yl-n-butylamine; Rₑ₁ is methylbenzyl; Rₑ₂ is 1-yl-n-butylamine; Rₑ₃ is methylbenzyl; Rₐ₄ is methylbenzyl; Rₐ₅ is 1-yl-2-methoxymethyl and Rₐ₆ is cyclohexyl;

(t) R₉ is methylbenzyl; R₁₀ is 1-yl-2-(4-benzenesulfonamide)ethyl; Rₑ₁ is 1-yl-n-butylamine; Rₑ₂ is 1-yl-2-(4-benzenesulfonamide)ethyl; Rₑ₃ is isobutyryl; Rₐ₄ is 1-yl-n-butylamine; Rₐ₅ is methylbenzyl and Rₐ₆ is 1-yl-2-methoxymethyl;

(u) R₉ is 1-yl-2-methoxymethyl; R₁₀ is 1-yl-n-butylamine; Rₑ₁ is isobutyryl; Rₑ₂ is isobutyryl; Rₑ₃ is cyclohexyl; Rₐ₄ is 1-yl-n-butylamine; Rₐ₅ is 1-yl-2-(4-benzenesulfonamide)ethyl and Rₐ₆ is cyclohexyl;

(v) R₉ is isobutyryl; R₁₀ is 1-yl-n-butylamine; Rₑ₁ is 1-yl-n-butylamine; Rₑ₂ is methylbenzyl; Rₑ₃ is 1-yl-n-butylamine; Rₐ₄ is 1-yl-n-butylamine; Rₐ₅ is piperonyl and Rₐ₆ is piperonyl;

(w) R₉ is 1-yl-2-(4-benzenesulfonamide)ethyl; R₁₀ is isobutyryl; Rₑ₁ is methylbenzyl; R₁₂ is 1-yl-2-methoxymethyl; R₁₃ is 1-yl-2-(4-benzenesulfonamide)ethyl; R₁₄ is isobutyryl; R₁₅ is 1-yl-2-methoxymethyl and R₁₆ is cyclohexyl;
(x) R₉ is furyl; R₁₀ is furyl; R₁₁ is piperonyl; R₁₂ is cyclohexyl; R₁₃ is piperonyl; R₁₄ is 1-yl-n-butylamine; R₁₅ is 1-yl-2-(4(benzenesulfonamide)ethyl and R₁₆ is cyclohexyl;
(y) R₉ is piperonyl; R₁₀ is piperonyl; R₁₁ is 1-yl-2-methoxyethyl; R₁₂ is 1-yl-2-methoxyethyl; R₁₃ is 1-yl-n-butylamine; R₁₄ is 1-yl-n-butylamine; R₁₅ is 1-yl-n-butylamine and R₁₆ is 1-yl-2-methoxyethyl;
(z) R₉ is piperonyl; R₁₀ is 1-yl-n-butylamine; R₁₁ is isobutyl; R₁₂ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₃ is methylbenzyl; R₁₄ is cyclohexyl; R₁₅ is isobutyl and R₁₆ is 1-yl-n-butylamine;
(aa) R₉ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₀ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₁ is methylbenzyl; R₁₂ is methylbenzyl; R₁₃ is 1-yl-n-butylamine; R₁₄ is 1-yl-n-butylamine; R₁₅ is 1-yl-2-(4(benzenesulfonamide)ethyl and R₁₆ is 1-yl-2-(4(benzenesulfonamide)ethyl;
(bb) R₉ is 1-yl-n-butylamine; R₁₀ is 1-yl-2-methoxyethyl; R₁₁ is 1-yl-n-butylamine; R₁₂ is isobutyl; R₁₃ is cyclohexyl; R₁₄ is 1-yl-n-butylamine; R₁₅ is 1-yl-n-butylamine and R₁₆ is piperonyl;
(cc) R₉ is cyclohexyl; R₁₀ is methylbenzyl; R₁₁ is cyclohexyl; R₁₂ is piperonyl; R₁₃ is 1-yl-n-butylamine; R₁₄ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₅ is 1-yl-n-butylamine and R₁₆ is 1-yl-2-methoxyethyl;
(dd) R₉ is 1-yl-2-methoxyethyl; R₁₀ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₁ is 1-yl-n-butylamine; R₁₂ is 1-yl-2-methoxyethyl; R₁₃ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₄ is 1-yl-2-methoxyethyl; R₁₅ is 1-yl-2-methoxyethyl and R₁₆ is isobutyl and R₁₇ is cyclohexyl;
(ee) R₉ is 1-yl-2-methoxyethyl; R₁₀ is methylbenzyl; R₁₁ is 1-yl-n-butylamine; R₁₂ is 1-yl-n-butylamine; R₁₃ is piperonyl; R₁₄ is isobutyl; R₁₅ is 1-yl-2-(4(benzenesulfonamide)ethyl and R₁₆ is 1-yl-n-butylamine;
(ff) R₉ is 1-yl-n-butylamine; R₁₀ is methylbenzyl; R₁₁ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₂ is methylbenzyl; R₁₃ is 1-yl-n-butylamine; R₁₄ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₅ is 1-yl-2-(4(benzenesulfonamide)ethyl and R₁₆ is cyclohexyl;
(gg) R₉ is 1-yl-n-butylamine; R₁₀ is 1-yl-2-methoxyethyl; R₁₁ is 1-yl-n-butylamine; R₁₂ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₃ is 1-yl-2-methoxyethyl; R₁₄ is 1-yl-2-methoxyethyl; R₁₅ is 1-yl-n-butylamine and R₁₆ is methylbenzyl;
(hh) R₉ is cyclohexyl; R₁₀ is cyclohexyl; R₁₁ is methylbenzyl; R₁₂ is 1-yl-n-butylamine; R₁₃ is methylbenzyl; R₁₄ is cyclohexyl; R₁₅ is methylbenzyl and R₁₆ is 1-yl-n-butylamine;
(ii) R₉ is 1-yl-2-methoxyethyl; R₁₀ is furyl; R₁₁ is methylbenzyl; R₁₂ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₃ is furyl; R₁₄ is cyclohexyl; R₁₅ is methylbenzyl and R₁₆ is cyclohexyl;
(jj) R₉ is 1-yl-n-butylamine; R₁₀ is methylbenzyl; R₁₁ is 1-yl-n-butylamine; R₁₂ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₃ is 1-yl-2-methoxyethyl; R₁₄ is methylbenzyl; R₁₅ is 1-yl-2-methoxyethyl and R₁₆ is isobutyl;
(kk) R₉ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₀ is 1-yl-n-butylamine; R₁₁ is 1-yl-n-butylamine; R₁₂ is methylbenzyl; R₁₃ is methylbenzyl; R₁₄ is cyclohexyl; R₁₅ is 1-yl-2-(4(benzenesulfonamide)ethyl and R₁₆ is methylbenzyl;
(ll) R₉ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₀ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₁ is 1-yl-n-butylamine; R₁₂ is 1-yl-n-butylamine; R₁₃ is methylbenzyl; R₁₄ is methylbenzyl; R₁₅ is 1-yl-n-butylamine and R₁₆ is 1-yl-n-butylamine;
(mmm) R₉ is 1-yl-n-butylamine; R₁₀ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₁ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₂ is 1-yl-2-methoxyethyl; R₁₃ is 1-yl-n-butylamine; R₁₄ is cyclohexyl; R₁₅ is 1-yl-2-(4(benzenesulfonamide)ethyl and R₁₆ is methylbenzyl;
(nn) R₉ is 1-yl-n-butylamine; R₁₀ is 1-yl-n-butylamine; R₁₁ is isobutyl; R₁₂ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₃ is methylbenzyl; R₁₄ is cyclohexyl; R₁₅ is 1-yl-n-butylamine; R₁₆ is methylbenzyl; R₁₇ is methylbenzyl and R₁₈ is cyclohexyl;
(oo) R₉ is 1-yl-2-(4(benzenesulfonamide)ethyl; R₁₀ is methylbenzyl; R₁₁ is 1-yl-n-butylamine; R₁₂ is methylbenzyl; R₁₃ is 1-yl-n-butylamine; R₁₄ is cyclohexyl; R₁₅ is 1-yl-2-methoxyethyl and R₁₆ is 1-yl-2-methoxyethyl;
(pp) R₉ is piperonyl; R₁₀ is 1-yl-n-butylamine; R₁₁ is 1-yl-n-butylamine; R₁₂ is methylamine; R₁₃ is piperonyl; R₁₄ is 1-yl-n-butylamine; R₁₅ is isobutyl and R₁₆ is 1-yl-2-methoxyethyl;
(qq) R₉ is cyclohexyl; R₁₀ is cyclohexyl; R₁₁ is furyl; R₁₂ is 1-yl-2-methoxyethyl; R₁₃ is isobutyl; R₁₄ is cyclohexyl; R₁₅ is methylbenzyl and R₁₆ is methylbenzyl;
(rr) R₉ is piperonyl; R₁₀ is 1-yl-n-butylamine; R₁₁ is isobutyl; R₁₂ is 1-yl-2-methoxyethyl; R₁₃ is 1-yl-n-butylamine; R₁₄ is cyclohexyl; R₁₅ is 1-yl-2-(4(benzenesulfonamide)ethyl and R₁₆ is 1-yl-2-(4(benzenesulfonamide)ethyl;
(ss) R₉ is cyclohexyl; R₁₀ is cyclohexyl; R₁₁ is 1-yl-n-butylamine; R₁₂ is methylbenzyl; R₁₃ is 1-yl-n-butylamine; R₁₄ is methylbenzyl; R₁₅ is cyclohexyl and R₁₆ is piperonyl; and pharmaceutically acceptable salts thereof.
17. The method of claim 1, wherein said ligand library is a bead based ligand library comprising a plurality of beads having ligands bound to the beads.
18. The method of claim 1, wherein said screening is performed by an array.
19. The method of claim 18, wherein said array is a microarray, a microscope slide, a plate, a chip, or a population of beads.
20. The method of claim 18, wherein said array comprises a solid substrate having a surface; a plurality of ligands bound to said substrate, each of said ligand comprising an anchor segment stably bound to the substrate surface, a peptoid segment, and a linker segment connecting and separating the anchor and peptoid segments.
21. The method of claim 18, wherein said array comprises between about 1000 and 100,000 distinct ligands.
22. The method of claim 18, wherein said array comprises between about 2000 and 50,000 distinct ligands.
23. The method of claim 18, wherein said array comprises between about 4000 and 25,000 distinct ligands.
24. The method of claim 18, wherein said array comprises between about 50,000 and 10,000,000 distinct ligands.
25. The method of claim 18, wherein said array comprises about 1000 distinct ligands.
26. The method of claim 18, wherein said array comprises between about 7000 and 12,500 distinct ligands.
27. The method of claim 18, wherein said array comprises a ligand binding moiety cross-linked to said array.
28. The method of claim 1, wherein the step of determining comprises detecting the binding molecules fluorescently.
29. The method of claim 18, wherein said array comprises fluorescently labeled ligands.
30. The method of claim 1, wherein said screening comprises an initial screening against a first ligand library and a subsequent screening against a second ligand library.

31. The method of claim 30, wherein said initial screening is performed against a first sample collected prior to administering said drug to said subject and said subsequent screening is performed against a second sample collected after administering said drug to said subject.

32. The method of claim 30, said first ligand library is screened for a first response and said second ligand library is screened for a second response.

33. The method of claim 32, wherein said first response is associated with said disease and said second response is associated with said drug induced response.

34. The method of claim 32, wherein said first response is associated with said disease and said second response is associated with a stage of said disease.

35. The method of claim 30, wherein said first ligand library comprises a first set of ligands and said second ligand library comprises a second set of ligands.

36. The method of claim 35, wherein said first set of ligands are different from said second set of ligands.

37. The method of claim 35, wherein one or more of said first set of ligands are same as one or more of said second set of ligands.

38. The method of claim 1, wherein said biological sample is tissue, cell, urine, serum, whole blood, cerebrospinal fluid, sputum, saliva, or semen.

39. The method of claim 1, wherein said subject is a human or animal subject.

40. The method of claim 1, wherein said diagnostic steps are performed in accordance with instructions in a package insert.

41. The method of claim 40, wherein said package insert comprises instructions for performing said diagnostic steps, instructions for determining a drug administration, and instructions for administering said drug based on the determination.

42. The method of claim 40, wherein said package insert is an insert in a kit, said kit comprising said library and components for performing said diagnostic steps.

43. A method for treating a disease in a subject, the method comprising:

obtaining a biological sample from said subject;

screening a ligand library against said sample;

identifying binding characteristics of one or more markers in said sample with one or more ligands in the library;

determining whether said one or more markers are associated with a response to a drug for treating said disease;

administering said drug to said subject, based on the determination of association between said one or more markers to said response, thereby treating said disease in said subject.

44. A method for treating a disease in a patient, the method comprising:

obtaining a biological sample from said patient;

screening said sample with a library comprising at least one ligand to determine a ligand binding profile;

administering a drug to said patient, based on the results of said ligand binding profile in said patient, wherein said at least one ligand has an affinity to one or more antibodies associated with said disease.

45. A method for monitoring a treatment by a drug in a subject, the method comprising:

obtaining a biological sample from said subject;

screening a ligand library against said subject;

identifying binding characteristics of one or more markers in said sample with one or more ligands in the library;

determining whether said one or more markers are associated with a response to a drug for treating said disease;

administering said drug to said subject, based on the determination of association between said one or more markers to said response, thereby monitoring said treatment by said drug in said subject.

46. A method for detecting a risk of adverse reaction to a drug in a subject, the method comprising:

obtaining a biological sample from said subject;

screening a ligand library against said sample;

identifying binding characteristics of one or more markers in said sample with one or more ligands in the library;

determining whether said one or more markers are associated with said risk, thereby detecting said risk of adverse reaction to said drug in said subject.

47. A method for profiling one or more subjects for a response to a drug in said subject, the method comprising:

obtaining a biological sample from said subject;

screening a ligand library against said subject;

identifying binding characteristics of one or more markers in said sample with one or more ligands in the library;

determining whether said one or more markers are associated with said response for said drug, thereby profiling said one or more subjects for said response to said drug to treat said disease.

48. A method for diagnosing a drug induced response in a subject, the method comprising:

obtaining a biological sample from said subject;

screening a peptoid library against said sample;

identifying binding characteristics of one or more markers in said sample with one or more ligands in the library, wherein at least one of said one or more markers is an autoantibody capable of binding to a peptoid ligand;

determining whether said one or more markers are associated with said drug induced response, thereby diagnosing said drug induced response in said subject.

49. A method for providing a prognosis of a drug induced response for treating a disease in a subject, the method comprising:

obtaining a biological sample from said subject;

screening a ligand library against said sample;

identifying binding characteristics of one or more markers in said sample with one or more ligands in the library;

determining whether said one or more markers are associated with said drug induced response, thereby providing said prognosis of said drug induced response for treating said disease in said subject.

50. A kit for diagnosis of a disease in accordance with claim 1 comprising:

a ligand library, detection reagents for screening said ligand library against a biological sample, adjuvants for said screening, and a package insert, wherein said package insert comprises instructions for performing said diagnostic steps, instructions for determining a drug administration, and instructions for administering said drug based on the determination.

51. A kit for treating a disease in accordance with claim 43 comprising:

a ligand library, detection reagents for screening said ligand library against a biological sample, adjuvants for said screening, and a package insert, wherein said package insert comprises instructions for performing said diagnostic...
steps, instructions for determining a drug administration, and instructions for administering said drug based on the determination.

52. A method for identifying a marker associated with a drug induced response for treating a disease, the method comprising: obtaining a biological sample from a subject; screening a ligand library against said biological sample; determining the binding characteristics of a marker in said sample to a ligand in the library; and determining whether said marker is associated with said drug induced response for treating said disease, thereby identifying said marker associated with said drug induced response for treating said disease.

53. The method of claim 52, wherein said library comprises about 350,000 to about 150 MM ligands.

54. The method of claim 52, wherein said ligand library is a random ligand library.

55. The method of claim 52, wherein said ligand library is a non-random ligand library.

56. The method of claim 52, wherein said ligand library is a peptoid library.

57. The method of claim 52, wherein at least one ligand in said ligand library has an affinity to an autoantibody to antigen that is associated with said drug induced response for treating said disease.

58. The method of claim 52, wherein said marker is an autoantibody capable of binding to a peptoid ligand.

59. The method of claim 52, wherein the ligand library is a bead based ligand library comprising a plurality of beads having ligands bound to the beads.

60. The method of claim 52, wherein said screening is performed by an array.

61. The method of claim 50, wherein said array is a microarray, a microscope slide, a plate, a chip, or a population of beads.

62. The method of claim 50, wherein said array comprising a solid substrate having a surface; a plurality of ligands bound to said substrate, each of said ligand comprising an anchor segment stably bound to the substrate surface, a peptoid segment, and a linker segment connecting and separating the anchor and peptoid segments.

63. The method of claim 52, wherein the library comprises a compound of formula I,

\[
\begin{align*}
\text{O} & \quad \text{R}_1 \quad \text{O} \quad \text{R}_4 \quad \text{R}_5 \quad \text{H} \\
\text{N} & \quad \text{N} \quad \text{R}_2 \quad \text{R}_3 \quad \text{O} \quad \text{R}_6
\end{align*}
\]

wherein any of the aryl or heteroaryl groups may be independently substituted with —OH, Cl, F, Br, —OCH₃, —SO₂NH₂ or —O—CH₂—O—.

64. The method of claim 52, wherein the library comprises a compound of formula I,

\[
\begin{align*}
\text{O} & \quad \text{R}_1 \quad \text{O} \quad \text{R}_4 \quad \text{R}_5 \quad \text{H} \\
\text{N} & \quad \text{N} \quad \text{R}_2 \quad \text{R}_3 \quad \text{O} \quad \text{R}_6
\end{align*}
\]

wherein the compounds are produced by a process which comprises use of a reagent selected from the group consisting of

(A) furfurylamine, 3,4-dimethoxymethanamine; benzylamine; N-(2-aminoethyl)acetamide; N-(3-aminopropan-2-yl)-2-pyrrolidinone; ethanolamine; glycine; diaminobutane; allylamine; piperonylamine; methylbenzylamine; isobutylamine; 4-(2-aminoethyl)benzenesulfonamide or cyclohexylamine; or

(B) methoxyethylaniline; piperonylamine; cyclohexylamine; diaminobutane; methylbenzylamine; isobutylamine; furfurylamine or 4-(2-aminoethyl)benzenesulfonamide; or

(C) furfurylamine; ethanolamine; glycine; diaminobutane; allylamine; piperonylamine; methylbenzylamine; isobutylamine or 4-(2-aminoethyl)benzenesulfonamide; or

(D) furfurylamine, N-(2-aminoethyl)acetamide; N-(3-aminopropan-2-yl)-2-pyrrolidinone; ethanolamine; glycine; diaminobutane; allylamine; piperonylamine; methylbenzylamine; isobutylamine; 4-(2-aminoethyl)benzenesulfonamide; or

(E) cysteine, glycine, allylamine, ethanamine, isobutylamine, methylbenzylamine, piperonylamine, methionine, cyclohexylamine, 3,4-dimethoxyphenethylamine, benzylamine, N-(2-aminoethyl)acetamide; N-(3-aminopropan-2-yl)-2-pyrrolidinone; 4-(2-aminoethyl)benzenesulfonamide and furfurylamine; and

wherein,

R₁ is selected from the group consisting —(C₁-C₆)SCH₃;
R₂ is selected from H;
R₃ and R₄ are independently selected from the groups consisting of H, —C₁-C₆alkyl, —C₁-C₆alkyloxy, —C₆-C₁alkylC₂C₆alkenyl, —C₆-C₁alkylC₂-C₆alkynyl, —C₁-C₆COOH, —C₁-C₆alkyloxy, —C₁-C₆alkynyl, —C₁-C₆cycloalkynyl, —C₁-C₆alkylaroyl, —C₁-C₆alkylheteroaryl, —C₁-C₆alkylamide, —C₁-C₆alkyloxyethylamine, wherein any of the aryl or heteroaryl groups may be independently substituted with —OH, Cl, F, Br, —OCH₃, —SO₂NH₂ or —O—CH₂—O—;
R₅ is selected from the group consisting of furfuryl or —(C₁-C₆alkyl)NR₆;
R₆ is selected from the group consisting of H, 1-yl-allyl, 1-yl-2-hydroxymethyl, isobutyl, 1-yl-n-butyramine, methylbenzyl, piperonylamine, cyclohexyl, 1-yl-2-(3,4-dimethoxyphenyl)ethyl, benzyl, 1-yl-2-(acetamide)ethyl, 1-yl-3-2-pyrrolidinone, 1-yl-2-(4-benzenesulfonamide)ethyl or furfuryl and n is 3-11.
The method of claim 52, wherein the library comprises a compound having a formula \( la \) therein where the compound is selected from the group consisting of a compound of formula \( la \) wherein,

- \( R_9 \) is n-butylamine; \( R_{10} \) is 1-yl-n-butylamine; \( R_{11} \) is Piperonyl; \( R_{12} \) is methylbenzyl; \( R_{13} \) is isobutyl; \( R_{14} \) is methylbenzyl; \( R_{15} \) is 1-yl-2-(4-benzensulfonamide)ethyl and \( R_{16} \) is 1-yl-2-methoxyethyl;

- \( R_9 \) is 1-yl-n-butylamine; \( R_{10} \) is 1-yl-2-(4-benzensulfonamide)ethyl; \( R_{11} \) is 1-yl-n-butylamine; \( R_{12} \) is cyclohexyl; \( R_{13} \) is 1-yl-2-methoxyethyl; \( R_{14} \) is 1-yl-2-dimethylaminoethyl and \( R_{15} \) is methylbenzyl; \( R_{16} \) is cyclohexyl;

- \( R_9 \) is 1-yl-n-butylamine; \( R_{10} \) is 1-yl-n-butylamine; \( R_{11} \) is cyclohexyl; \( R_{12} \) is 1-yl-2-(4-benzensulfonamide)ethyl; \( R_{13} \) is methylbenzyl and \( R_{14} \) is isobutyl; \( R_{15} \) is cyclohexyl; \( R_{16} \) is methylbenzyl and \( R_{17} \) is 1-yl-n-butylamine;

- \( R_9 \) is 1-yl-n-butylamine; \( R_{10} \) is 1-yl-n-butylamine; \( R_{11} \) is methylbenzyl; \( R_{12} \) is 1-yl-2-methoxyethyl; \( R_{13} \) is cyclohexyl; \( R_{14} \) is cyclohexyl; \( R_{15} \) is methylbenzyl and \( R_{16} \) is piperonyl;

- \( R_9 \) is 1-yl-n-butylamine; \( R_{10} \) is 1-yl-2-(4-benzensulfonamide)ethyl; \( R_{11} \) is 1-yl-2-methoxyethyl; \( R_{12} \) is cyclohexyl; \( R_{13} \) is 1-yl-2-methoxyethyl; \( R_{14} \) is 1-yl-2-dimethylaminoethyl and \( R_{15} \) is methylbenzyl; \( R_{16} \) is cyclohexyl;

- \( R_9 \) is 1-yl-n-butylamine; \( R_{10} \) is 1-yl-n-butylamine; \( R_{11} \) is piperonyl; \( R_{12} \) is 1-yl-2-(4-benzensulfonamide)ethyl; \( R_{13} \) is cyclohexyl; \( R_{14} \) is cyclohexyl; \( R_{15} \) is methylbenzyl and \( R_{16} \) is piperonyl;

- \( R_9 \) is 1-yl-2-(4-benzensulfonamide)ethyl; \( R_{10} \) is 1-yl-n-butylamine; \( R_{11} \) is 1-yl-n-butylamine; \( R_{12} \) is cyclohexyl; \( R_{13} \) is 1-yl-2-(4-benzensulfonamide)ethyl and \( R_{14} \) is 1-yl-2-methoxyethyl;

- \( R_9 \) is 1-yl-2-methoxyethyl; \( R_{10} \) is 1-yl-n-butylamine; \( R_{11} \) is 1-yl-n-butylamine; \( R_{12} \) is 1-yl-2-methoxyethyl; \( R_{13} \) is methylbenzyl; \( R_{14} \) is 1-yl-n-butylamine and \( R_{15} \) is isobutyl; \( R_{16} \) is cyclohexyl;

- \( R_9 \) is 1-yl-2- methoxyethyl; \( R_{10} \) is 1-yl-n-butylamine; \( R_{11} \) is 1-yl-n-butylamine; \( R_{12} \) is 1-yl-2-methoxyethyl; \( R_{13} \) is methylbenzyl; \( R_{14} \) is 1-yl-2-methoxyethyl and \( R_{15} \) is methylbenzyl;

- \( R_9 \) is 1-yl-2- methoxyethyl; \( R_{10} \) is 1-yl-n-butylamine; \( R_{11} \) is 1-yl-n-butylamine; \( R_{12} \) is 1-yl-2-methoxyethyl; \( R_{13} \) is methylbenzyl; \( R_{14} \) is 1-yl-n-butylamine; \( R_{15} \) is isobutyl and \( R_{16} \) is 1-yl-2-methoxyethyl;

- \( R_9 \) is 1-yl-n-butylamine; \( R_{10} \) is 1-yl-n-butylamine; \( R_{11} \) is methylbenzyl; \( R_{12} \) is cyclohexyl; \( R_{13} \) is 1-yl-n-butylamine; \( R_{14} \) is methylbenzyl; \( R_{15} \) is isobutyl and \( R_{16} \) is 1-yl-n-butylamine;

- \( R_9 \) is 1-yl-n-butylamine; \( R_{10} \) is 1-yl-2-(4-benzensulfonamide)ethyl; \( R_{11} \) is 1-yl-n-butylamine; \( R_{12} \) is isobutyl; \( R_{13} \) is methylbenzyl; \( R_{14} \) is 1-yl-2-methoxyethyl; \( R_{15} \) is 1-yl-2-(4-benzensulfonamide)ethyl and \( R_{16} \) is 1-yl-2-methoxyethyl.
wherein the compound is selected from the group consisting of a compound of formula II wherein,

(tt) $R_9$ is n-butylamine; $R_{10}$ is 1-yl-n-butylamine; $R_{11}$ is piperonyl; $R_{12}$ is methylbenzyl; $R_{13}$ is piperonyl; $R_{14}$ is methylbenzyl; $R_{15}$ is 1-yl-2-(4-benzensulfonamide) ethyl and $R_{16}$ is 1-yl-2-methoxyethyl;

(uu) $R_9$ is 1-yl-n-butylamine; $R_{10}$ is 1-yl-2-(4-benzensulfonamide)ethyl; $R_{11}$ is 1-yl-n-butylamine; $R_{12}$ is cyclohexyl; $R_{13}$ is 1-yl-2-methoxyethyl; $R_{14}$ is 1-yl-2,2-dimethylethyl (isobutyl); $R_{15}$ is 1-yl-2-(4-benzensulfonamide)ethyl and $R_{16}$ is methylbenzyl;

(vv) $R_9$ is 1-yl-n-butylamine; $R_{10}$ is 1-yl-n-butylamine; $R_{11}$ is piperonyl; $R_{12}$ is 1-yl-2-(4-benzensulfonamide)ethyl; $R_{13}$ is 1-yl-n-butylamine; $R_{14}$ is methylbenzyl; $R_{15}$ is 1-yl-2-methoxyethyl and $R_{16}$ is methylbenzyl;

(ww) $R_9$ is 1-yl-n-butylamine; $R_{10}$ is 1-yl-n-butylamine; $R_{11}$ is isobutyl; $R_{12}$ is 1-yl-2-(4-benzensulfonamide)ethyl; $R_{13}$ is methylbenzyl; $R_{14}$ is cyclohexyl; $R_{15}$ is isobutyl and $R_{16}$ is 1-yl-n-butylamine;

(xx) $R_9$ is 1-yl-n-butylamine; $R_{10}$ is 1-yl-n-butylamine; $R_{11}$ is methylbenzyl; $R_{12}$ is 1-yl-2-methoxyethyl; $R_{13}$ is cyclohexyl; $R_{14}$ is cyclohexyl; $R_{15}$ is methylbenzyl and $R_{16}$ is piperonyl;

(yy) $R_9$ is piperonyl; $R_{10}$ is 1-yl-n-butylamine; $R_{11}$ is isopropyl; $R_{12}$ is isopropyl; $R_{13}$ is 1-yl-2-methoxyethyl; $R_{14}$ is cyclohexyl; $R_{15}$ is 1-yl-2-(4-benzensulfonamide)ethyl and $R_{16}$ is 1-yl-2-(4-benzensulfonamide)ethyl;

.zz) $R_9$ is 1-yl-n-butylamine; $R_{10}$ is 1-yl-n-butylamine; $R_{11}$ is piperonyl; $R_{12}$ is methylbenzyl; $R_{13}$ is piperonyl; $R_{14}$ is methylbenzyl; $R_{15}$ is 1-yl-2-(4-benzensulfonamide)ethyl and $R_{16}$ is cyclohexyl;

(aau) $R_9$ is 1-yl-n-butylamine; $R_{10}$ is 1-yl-n-butylamine; $R_{11}$ is methylbenzyl; $R_{12}$ is 1-yl-2-methoxyethyl; $R_{13}$ is cyclohexyl; $R_{14}$ is cyclohexyl; $R_{15}$ is methylbenzyl and $R_{16}$ is piperonyl;

(bbb) $R_9$ is 1-yl-n-butylamine; $R_{10}$ is methylbenzyl; $R_{11}$ is methylbenzyl; $R_{12}$ is 1-yl-n-butylamine; $R_{13}$ is 1-yl-2-(4-benzensulfonamide)ethyl; $R_{14}$ is cyclohexyl; $R_{15}$ is cyclohexyl and $R_{16}$ is cyclohexyl;

(ccc) $R_9$ is 1-yl-2-(4-benzensulfonamide)ethyl; $R_{10}$ is methylbenzyl; $R_{11}$ is methylbenzyl; $R_{12}$ is cyclohexyl; $R_{13}$ is 1-yl-n-butylamine; $R_{14}$ is methylbenzyl; $R_{15}$ is isobutyl and $R_{16}$ is 1-yl-n-butylamine;

(ddd) $R_9$ is 1-yl-2-methoxyethyl; $R_{10}$ is isobutyl; $R_{11}$ is 1-yl-2-(4-benzensulfonamide)ethyl; $R_{12}$ is piperonyl; $R_{13}$ is 1-yl-n-butylamine; $R_{14}$ is cyclohexyl; $R_{15}$ is methylbenzyl and $R_{16}$ is 1-yl-2-methoxyethyl;

(eee) $R_9$ is 1-yl-n-butylamine; $R_{10}$ is 1-yl-2-(4-benzensulfonamide)ethyl; $R_{11}$ is 1-yl-n-butylamine; $R_{12}$ is cyclohexyl; $R_{13}$ is 1-yl-2-methoxyethyl; $R_{14}$ is isobutyl; $R_{15}$ is 1-yl-2-(4-benzensulfonamide)ethyl and $R_{16}$ is methylbenzyl;

(ff) $R_9$ is 1-yl-2-methoxyethyl; $R_{10}$ is 1-yl-n-butylamine; $R_{11}$ is 1-yl-n-butylamine; $R_{12}$ is 1-yl-2-methoxyethyl; $R_{13}$ is methylbenzyl; $R_{14}$ is 1-yl-n-butylamine; $R_{15}$ is furfuryl and $R_{16}$ is furfuryl;

(ggg) $R_9$ is cyclohexyl; $R_{10}$ is cyclohexyl; $R_{11}$ is 1-yl-n-butylamine; $R_{12}$ is furfuryl; $R_{13}$ is 1-yl-2-methoxyethyl; $R_{14}$ is 1-yl-2-(4-benzensulfonamide)ethyl and $R_{16}$ is furfuryl;

(hhh) $R_9$ is 1-yl-n-butylamine; $R_{10}$ is piperonyl; $R_{11}$ is 1-yl-2-(4-benzensulfonamide)ethyl; $R_{12}$ is 1-yl-2-methoxyethyl; $R_{13}$ is methylbenzyl; $R_{14}$ is 1-yl-n-butylamine; $R_{15}$ is 1-yl-2-methoxyethyl and $R_{16}$ is 1-yl-2-methoxyethyl;
(www) $R_6$ is 1-yl-2-methoxyethyl; $R_{10}$ is 1-yl-2-(4(benzenesulfonamide)ethyl; $R_{11}$ is 1-yl-n-butylamine; $R_{12}$ is 1-yl-2-methoxyethyl; $R_{13}$ is 1-yl-2-(4(benzenesulfonamide)ethyl; $R_{14}$ is 1-yl-2-methoxyethyl; $R_{15}$ is isobutyryl and $R_{16}$ is cyclohexyl;

(xxx) $R_6$ is 1-yl-2-methoxyethyl; $R_{10}$ is methylbenzyl; $R_{11}$ is 1-yl-n-butylamine; $R_{12}$ is 1-yl-n-butylamine; $R_{13}$ is piperonyl; $R_{14}$ is isobutyryl; $R_{15}$ is 1-yl-2-(4(benzenesulfonamide)ethyl and $R_{16}$ is 1-yl-n-butylamine;

(yyy) $R_6$ is 1-yl-n-butylamine; $R_{10}$ is methylbenzyl; $R_{11}$ is 1-yl-2-(4(benzenesulfonamide)ethyl; $R_{12}$ is methylbenzyl; $R_{13}$ is 1-yl-n-butylamine; $R_{14}$ is 1-yl-2-((benzenesulfonamide)ethyl; $R_{15}$ is 1-yl-2-mercaptoethylyl and $R_{16}$ is cyclohexyl;

(zzz) $R_6$ is 1-yl-n-butylamine; $R_{10}$ is 1-yl-2-methoxyethyl; $R_{11}$ is 1-yl-n-butylamine; $R_{12}$ is 1-yl-2-(4(benzenesulfonamide)ethyl; $R_{13}$ is 1-yl-2-mercaptoethylyl; $R_{14}$ is 1-yl-2-methoxyethyl; $R_{15}$ is 1-yl-n-butylamine and $R_{16}$ is methylbenzyl;

(aaa) $R_6$ is cyclohexyl; $R_{10}$ is cyclohexyl; $R_{11}$ is methylbenzyl; $R_{12}$ is 1-yl-n-butylamine; $R_{13}$ is methylbenzyl; $R_{14}$ is cyclohexyl; $R_{15}$ is methylbenzyl and $R_{16}$ is 1-yl-n-butylamine;

(bbb) $R_6$ is 1-yl-n-butylamine; $R_{10}$ is furfuryl; $R_{11}$ is methylbenzyl; $R_{12}$ is 1-yl-2-(4(benzenesulfonamide)ethyl; $R_{13}$ is furfuryl; $R_{14}$ is cyclohexyl; $R_{15}$ is methylbenzyl and $R_{16}$ is cyclohexyl;

(ccc) $R_6$ is 1-yl-n-butylamine; $R_{10}$ is methylbenzyl; $R_{11}$ is 1-yl-n-butylamine; $R_{12}$ is 1-yl-2-(4(benzenesulfonamide)ethyl; $R_{13}$ is 1-yl-2-methoxyethyl; $R_{14}$ is methylbenzyl; $R_{15}$ is 1-yl-2-mercaptoethylyl and $R_{16}$ is isobutyryl;

(ddd) $R_6$ is 1-yl-2-(4(benzenesulfonamide)ethyl; $R_{10}$ is 1-yl-n-butylamine; $R_{11}$ is 1-yl-n-butylamine; $R_{12}$ is methylbenzyl; $R_{13}$ is methylbenzyl; $R_{14}$ is cyclohexyl; $R_{15}$ is 1-yl-2-(4(benzenesulfonamide)ethyl and $R_{16}$ is methylbenzyl;

(eee) $R_6$ is 1-yl-2-(4(benzenesulfonamide)ethyl; $R_{10}$ is 1-yl-2-(4(benzenesulfonamide)ethyl; $R_{11}$ is 1-yl-n-butylamine; $R_{12}$ is 1-yl-n-butylamine; $R_{13}$ is methylbenzyl and $R_{14}$ is 1-yl-n-butylamine;

(fff) $R_6$ is 1-yl-n-butylamine; $R_{10}$ is 1-yl-2-(4(benzenesulfonamide)ethyl; $R_{11}$ is 1-yl-2-(4(benzenesulfonamide)ethyl; $R_{12}$ is 1-yl-2-methoxyethyl; $R_{13}$ is 1-yl-n-butylamine; $R_{14}$ is cyclohexyl; $R_{15}$ is 1-yl-2-(4(benzenesulfonamide)ethyl and $R_{16}$ is methylbenzyl;

(ggg) $R_6$ is 1-yl-n-butylamine; $R_{10}$ is 1-yl-n-butylamine; $R_{11}$ is piperonyl; $R_{12}$ is 1-yl-2-(4(benzenesulfonamide)ethyl; $R_{13}$ is methylbenzyl; $R_{14}$ is cyclohexyl; $R_{15}$ is methylbenzyl and $R_{16}$ is cyclohexyl;

(hhh) $R_6$ is 1-yl-2-(4(benzenesulfonamide)ethyl; $R_{10}$ is methylbenzyl; $R_{11}$ is methylbenzyl; $R_{12}$ is 1-yl-n-butylamine; $R_{13}$ is methylbenzyl; $R_{14}$ is piperonyl; $R_{15}$ is 1-yl-n-butylamine and $R_{16}$ is 1-yl-2-methoxyethyl;

(iii) $R_6$ is piperonyl; $R_{10}$ is 1-yl-n-butylamine; $R_{11}$ is 1-yl-n-butylamine; $R_{12}$ is methylamine; $R_{13}$ is piperonyl; $R_{14}$ is 1-yl-n-butylamine; $R_{15}$ is piperonyl and $R_{16}$ is 1-yl-2-methoxyethyl;

(iii) $R_6$ is cyclohexyl; $R_{10}$ is cyclohexyl; $R_{11}$ is furfuryl; $R_{12}$ is 1-yl-2-methoxyethyl; $R_{13}$ is isobutyryl; $R_{14}$ is cyclohexyl; $R_{15}$ is methylbenzyl and $R_{16}$ is methylbenzyl;

(kkk) $R_6$ is piperonyl; $R_{10}$ is 1-yl-n-butylamine; $R_{11}$ is isobutyryl; $R_{12}$ is 1-yl-2-methoxyethyl; $R_{13}$ is cyclohexyl; $R_{14}$ is 1-yl-2-(4(benzenesulfonamide)ethyl and $R_{15}$ is 1-yl-2-(4(benzenesulfonamide)ethyl;

(iii) $R_6$ is cyclohexyl; $R_{10}$ is cyclohexyl; $R_{11}$ is 1-yl-n-butylamine; $R_{12}$ is methylbenzyl; $R_{13}$ is 1-yl-n-butylamine; $R_{14}$ is methylbenzyl; $R_{15}$ is cyclohexyl and $R_{16}$ is piperonyl;

and pharmaceutically acceptable salts thereof.

67. The method of claim 59, wherein said bead based library comprises a ligand binding moiety cross-linked to beads.

68. The method of claim 52, wherein the step of determining comprises detecting the binding molecules fluorescently.

69. The method of claim 52, wherein said library comprises fluorescently labeled ligands.

70. The method of claim 52, wherein said biological sample is obtained at early stage of said disease.

71. The method of claim 52, wherein said biological sample is obtained at later stage of said disease.

72. The method claim 52, wherein the method further comprises identifying a marker associated with a stage of said disease.

73. The method of claim 52, wherein said disease is a cancer, an autoimmune disease, an inflammatory disease, an infectious disease, a neurodegenerative disease, or a cardiovascular disease.

74. The method of claim 73, wherein the cancer disease is breast cancer, lung cancer, prostate cancer, cervical cancer, head and neck cancer, testicular cancer, ovarian cancer, skin cancer, brain cancer, pancreatic cancer, liver cancer, stomach cancer, colon cancer, rectal cancer, esophageal cancer, lymphoma, or leukemia.

75. The method of claim 73, wherein the autoimmune disease is lupus, myasthenia gravis, multiple sclerosis, rheumatoid arthritis, nephritis, Chagas disease, scleroderma, or Sjogren’s disease.

76. The method of claim 73, wherein the infectious disease is a result of infection with viruses, bacteria or fungi.

77. The method of claim 73, wherein the neurodegenerative disease is Alzheimer’s disease, dementia, or Creutzfeld-Jacob disease.

78. The method of claim 52, wherein said response is a side effect of said drug, an adverse reaction to said drug, or a therapeutic dosage efficacy of said drug.

79. The method claim 52, wherein the method further comprises identifying a marker associated with a population or subpopulation of patients.

80. The method of claim 52, wherein at least one ligand has an affinity to an antibody to antigen that is associated with a stage of said disease in a patient population or subpopulation.

81. The method of claim 52, wherein said drug is at least one of the drugs listed in Table 1.

82. The method of claim 52, wherein the differences in binding differentiates between a subject who is and is not responsive to a treatment or therapy by said drug.

83. The method of claim 52, wherein said screening comprises an initial screening against a first ligand library and a subsequent screening against a second ligand library.

84. The method of claim 83, wherein said first ligand library is a random ligand library and said second ligand library is a random or a non-random ligand library.
85. The method of claim 83, wherein said second ligand library comprises a plurality of ligands identified in said initial screening or preselected based on known reactivity to said ligand binding markers.

86. The method of claim 83, wherein said first ligand library comprises a first set of ligands and said second ligand library comprises a second set of ligands.

87. The method of claim 86, wherein said first set of ligands are different from said second set of ligands.

88. The method of claim 86, wherein one or more of said first set of ligands are same as one or more of said second set of ligands.

89. The method of claim 83, wherein said initial screening is performed against a first sample collected prior to administering said drug to said subject, and said subsequent screening is performed against a second sample collected after administering said drug to said subject.

90. The method of claim 83, said first ligand library is screened to identify one or more biomarkers associated with said disease and said second ligand library is screened to identify one or more biomarkers associated with a second response.

91. The method of claim 83, said first ligand library is screened to identify one or more biomarkers associated with a response and said second ligand library is screened to validate the association of said one or more biomarkers with said response.

92. The method of claim 83, said first ligand library is screened to identify one or more biomarkers associated with said disease and said second ligand library is screened to identify one or more biomarkers associated with a stage of said disease.

93. The method of claim 83, said first ligand library is screened to identify one or more biomarkers associated with said disease and said second ligand library is screened to identify one or more biomarkers associated with said drug induced response.

94. The method of claim 83, said first ligand library is screened to identify one or more biomarkers associated with said disease and/or said drug induced response and said second ligand library is screened to validate the association of said one or more biomarkers with said disease and/or said drug induced response.

95. The method of claim 52, wherein said biological sample is tissue, cell, urine, serum, whole blood, cerebrospinal fluid, sputum, saliva, or semen.

96. The method of claim 52, wherein said subject is a human or animal subject.

97. A marker associated with a response to a drug for treating a disease, wherein said marker is identified by the method of claim 52.

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