Title: AFFINITY REAGENT AND CATALYST DISCOVERY THROUGH FIBER-OPTIC ARRAY SCANNING TECHNOLOGY

Abstract: Devices, systems and methods for affinity reagent and catalyst discovery employing a library on a bead HTS platform, each bead comprising affixed non-natural polymers of a distinct bioactive monomer with sequence pre-defined branching and folding in tertiary structures, and fiber-optic array scanning technology.
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
Affinity Reagent and Catalyst Discovery Though Fiber-Optic Array Scanning Technology

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Introduction

[01] Rapid and cost effective ways of screening large compound collections for biological, physical or chemical properties still remain a challenge in many industries. Pharmaceutical companies have developed high through screening operations which can screen up to 2 million compounds in a matter of months, but these operations require high end automation, compound storage and retrieval system and several IT Es to run and maintain.

[02] Based on a fiber-optic array scanning technology (FAST) developed by SRI for screening for circulating tumor cells (CTCs), we conceived of using the same platform to screen compounds either covalently attached or absorbed onto beads which are similar in size to white blood cells (10 - 20 microns but could by < 1 micron or greater than 500 microns) and arraying them on the same glass slide and testing with fluorescence based assays used in the FAST CTC scanner. With this platform we can screen 25,000,000 compounds in ~60 sees. A library of 100,000,000 compounds can be screened on four slides in 4 mins.

[03] The cost of reagents (compounds, screening reagents, plates etc.) are greatly reduced using this screening platform. In addition such assays can be run by one technician level person. The invention is of enormous benefit to pharmaceutical companies providing access to cheaper screening and much greater diversity of compound libraries in lead finding though application to rapid discovery of novel affinity reagents for diagnostics and therapeutics as well as catalysts.

Summary of the Invention

[05] The invention provides large non-natural polymers of bioactive monomers, and beads, and libraries of beads comprising the polymers. In another aspect, the invention provides use of fiber-optic array scanning technology (FAST) to screen fluorescent beads, including beads comprising the subject polymers. These inventions provide powerful platforms for affinity reagent and catalyst discovery and library on a bead high throughput screening (HTS).

[06] In an aspect, the invention provides a library of beads configured for high-throughput drug screening, each bead comprising affixed non-natural polymers of a distinct bioactive monomer with sequence pre-defined branching and folding in tertiary structures. Any suitable polymer and polymerization may be used that incorporates the requisite bioactive monomer and sequence pre-defined branching and folding in tertiary structures.

[07] In embodiments, the library further comprises, incorporated in each of the polymers secondary structure constraint (SSC) monomers which impose turns and thereby induce the tertiary structures, preferably wherein the SSC monomers are acid cleavable to facilitate mass-spectroscopy based sequencing of the polymers;

[08] the polymer is a polyamide, a vinylogous polymer, vinylogous polyamide, or an ester;

[09] the polymer is polymerized in a coupling reaction selected from Wurtz reaction, Glaser coupling, Ullmann reaction, Gomberg-Bachmann reaction, Cadiot-Chodkiewicz coupling, Pinacol coupling reaction, Castro-Stephens coupling, Oilman reagent coupling, Cassar reaction, Kumada coupling, Heck reaction, Sonogashira coupling, Negishi coupling, Stille cross coupling, Suzuki reaction, Hiyama coupling, Buchwald-Hartwig reaction, Fukuyama coupling, and Liebeskind-Srogl coupling;

[10] the polymer or polymerizations is selected from sulphonamides, reductive aminations, peptoid linkages, Suzuki couplings, phosphoamidite couplings, and radical cross coupling;

[11] the monomer is a dihydroisoquinolone;

[12] the polymerization combines amide bond coupling methodologies with peptidotriazole branch points introduced using chemically orthogonal copper-catalyzed cycloadditions (click-reactions), and/or

[13] the polymers comprise intrinsically self-readable molecules via intrinsically incorporated isotopically-coded mass-spectroscopy tags or barcodes.

[14] In another aspect, the invention provides an array, which may be ordered in 1, 2 and/or 3-dimensions of a subject library.

[15] In another aspect the invention provides a fiber optic scanner mounted with a slide comprising an ordered array of a subject library.
In another aspect, the invention provides a method of making a subject library comprising affixing the polymers to the beads or building the polymers on the beads by sequential monomer coupling.

In another aspect, the invention provides a method of using a fiber optic scanner mounted with a slide comprising an array of a subject library, comprising:

- labeling the array with a bioactivity (affinity or catalysis) marker to generate fluorescent labels on target beads comprising target monomers; and
- fluorescent imaging the array with the scanner, preferably wherein the labels provide multiple fluorescent wavelengths, and the imaging comprises optical filtering, reducing background signaling and false positives.

In one embodiment we use a laser (e.g. 488 nm) for excitation of the fluorophores, and then use band pass filters to split the emitted light into two or more channels (e.g. 520 nm and 580 nm), wherein one channel (e.g. 520nm light) represents background light and the other (e.g. 580nm) is our signal light. A calculation of the ratios of these two signals is used to select active polymers.

In another embodiment, we label a non-target molecule (e.g. bovine serum albumin or a protein related to our target) with a different wavelength emitting fluorophore. The ratio of the on-target vs. off-target wavelength signals is then used to select polymers with activity and specificity.

In another aspect the invention provides a method of using a subject library comprising:

- fluorescence assaying the library to detect a candidate bead based on bioactivity of the corresponding monomer;
- isolating the candidate bead from the assayed library;
- cleaving polymers from the isolated candidate bead; and
- structurally analyzing the cleaved polymers.

In another aspect, the invention provides a non-natural polymer of a distinct bioactive and sequence pre-defined branching and folding in tertiary structures. In embodiments:

- the polymer is a polyamide, a vinylogous polymer, vinylogous polyamide, or an ester;
- the polymer is polymerized in a coupling reaction selected from Wurtz reaction, Glaser coupling, Ullmann reaction, Gomberg-Bachmann reaction, Cadiot-Chodkiewicz coupling, Pinacol coupling reaction, Castro-Stephens coupling, Oilman reagent coupling, Cassar reaction, Kumada coupling, Heck reaction, Sonogashira coupling, Negishi coupling, Stille cross coupling, Suzuki reaction, Hiyama coupling, Buchwald-Hartwig reaction, Fukuyama coupling, and Liebeskind-Srogl coupling;
the polymer or polymerizations is selected from sulphonamides, reductive aminations, peptoid linkages, Suzuki couplings, phosphonmidite couplings, and radical cross coupling;

the monomer is a dihydroisoquinolinone;

the polymerization combines amide bond coupling methodologies with peptidotriazole branch points introduced using chemically orthogonal copper-catalyzed cycloadditions (click-reactions), and/or

the polymers comprise intrinsically self-readable molecules via intrinsically incorporated isotopically-coded mass-spectroscopy tags or barcodes.

In another aspect the invention provides a library of beads configured for high-throughput drug screening, each of the beads comprising affixed non-natural polymers as disclosed herein.

In another aspect the invention provides a fiber optic scanner mounted with a slide bearing fluorescent beads, preferably a scanner comprising:

an imager stage having a planar surface for supporting a sample comprising the slide;

a bifurcated light path having two fiber optic bundles, each bundle having a first end arranged to define an input aperture for viewing the sample on the imager stage, and a distal bundle end arranged to define an output aperture disposed away from the imager stage;

a scanning source arranged to scan a beam along a path that is perpendicular to the sample on the imager stage and closely adjacent to both bundles of the bifurcated light path such that a substantially circular spot of illumination provided by the scanning source on the imager stage sample provides a light signal at least a portion of which is received by the input aperture of each bundle and transmitted via the bifurcated light path to the output aperture;

a photodetector arranged to detect the light signal at the distal end; and

a processor that processes the light signal detected by the photodetector.

In another aspect the invention provides a method for imaging a sample comprising the slide bearing fluorescent beads disclosed herein, the method comprising:

supplying a substantially circular beam of radiation perpendicular to the sample;

maintaining the perpendicular direction of the radiation beam as it sweeps along a scan path on the sample;

reflecting at least some light produced by beam interaction with the sample in a direction away from the sample;

collecting light produced by beam interaction with the sample in at least one proximate element of an array of fiber optic first ends;

detecting collected light at a selected output region; and
coordinating sweeping, moving and detecting to generate an array of picture elements representative of at least a portion of the sample.

In another aspect the invention provides a method of using the fiber optic scanner mounted with a slide bearing fluorescent beads disclosed herein, the method comprising:

fluorescent imaging the beads with the scanner, and preferably:
fluorescent imaging the beads with the scanner to detect a candidate bead;
isolating the candidate bead from the beads; and
analyzing a function or structure of the candidate bead.

The invention specifically provides all combinations of the recited embodiments, as if each had been laboriously individually set forth.

**Brief Description of the Figures**

Fig. 1. Design strategy for polyamide monomers that mimic protein secondary structure features.

Fig. 2. Scheme for the synthesis of polyamides with sequence-defined branching.

Fig. 3. Non-natural polymer bead configuration.

Fig. 4. Sequencing approach for NNP MS fragmentation

Fig. 5. Library Design for Facile and Rapid Sequencing; Self Readable NNPs

Fig. 6. Isotopically-Coded MS Tags for Sequence Determination

**Detailed Description of Particular Embodiments and Examples Thereof**

The invention provides methods of affinity reagent and catalyst discovery employing a library on a bead HTS platform and fiber-optic array scanning technology.

We polymerize bioactive monomers to form non-natural polymers of the bioactive monomers, and attaching these to microbeads (1, 2, 5 or 10 to 10, 20, 50, 100, 200, 500 or 1000 um diameter). In aspects, the invention provides novel non-natural monomers for the synthesis of new polymers, incorporating secondary structural molecular scaffolds and branched structures to maximize the discovery of affinity reagents and catalysts, and a revolutionary high through screening (HTS) platform that can screen a 10^8 member library in less than 5 minutes.

Synthesis of Monomers and Libraries of Polymers. Utilizing well-established solid-phase peptide chemistry techniques we generate libraries of polyamides with unique, non-natural features such as non-natural amino acid monomers with unique configuration and functionality, non-peptidic scaffolds that mimic features of protein secondary structures, and sequence defined branched structures.
Small Molecule Templates of Secondary Structure Features. Only a small percentage of the amino acids in a protein structure are directly involved in binding interactions or catalytic functions; most are primarily structural determinants that position the interacting residues into proper orientation. The invention provides a strategy for mimicking secondary structure elements using small-molecule templates. In an embodiment, the distances and angles associated with secondary structure elements are modeled as vectors and used to query an enumerated virtual framework library. Hits are then queried across 3D structure databases and manually inspected to identify synthetic monomer targets (Fig. 1). We have designed and validated templates that mimic the angles and side chain projections in α-helices and β-turns, both of which have been prepared on gram scale [3,4]. By incorporating these templates into polyamide chains, we can mimic the spatial orientation of bioactive structural features with a conformationally restrained scaffold. These monomers also have the capacity for robust combinatorial derivitization, which permits the incorporation of non-natural functional groups into bioactive polymers.

Incorporation of Branching Monomers. Sequence-defined branched polyamides form structured and folded tertiary structures that have unique bioactive properties due to the reduced degrees of spatial freedom. Another benefit of a branched polyamide over a linear polymer is the exponential increase in sequence diversity from a fixed number of monomeric units. Sequence-defined branched polyamides have the chemical properties of proteins but the extraordinary capacity for diversity of polysaccharides. In an embodiment, our approach for accessing branched polyamides uses standard amide bond coupling methodologies combined with peptidotriazole branch points introduced using chemically orthogonal copper-catalyzed cycloadditions ("click-reactions") (Fig. 2).

Design and Synthesis of Non-Natural Amino Acid Monomers. The invention provides non-natural monomers that promote the folding of polyamides into functional conformations. In addition to the above approaches, we have designed non-natural amino acid monomers with backbone dihedral angles outside of those typically found in Ramachandran plot. These amino acids include β- and γ-amino acid variants of natural amino acids that form compact structured conformations. We have designed using molecular dynamics non-natural polypeptides with only four monomers that fold into compact secondary structures assessed by 1D and 2D NMR [5]. All of the monomers are readily synthesized to >98% purity by HPLC and structures assigned unequivocally using 1D/2D NMR, MS and CD. Examples of non-natural monomers that we have made on >1 gram scale to ≥98% purity [1-3] include:
Computation Design and Optimization. The invention provides computational chemistry tools, analogous to those for the modeling of peptide and protein structure and function, to model the non-natural building blocks, polymers and library designs. Development criteria include:

Parameterization of non-natural monomers;

Quantum Mechanics (QM) calculations on building blocks to derive accurate charges, bond lengths, and angles;

QM calculations on dimers and trimers of monomers to derive dihedral preferences.

Validation of computational tools on polymers with these building blocks;

Where possible, extract data set from the Protein Data Bank (PDB) of pseudo-peptides with similar building blocks;

Run relevant modules of tools with the parameters from (1) on the data set and structural data (NMR, X-ray) from initial hit;

Application to the design of novel bioactive polymers;

Design bias libraries with sequences that are more likely to fold and present binding or catalytic functionality in novel and diverse ways.

Production of Large (>108+) Combinatorial Libraries. To accommodate the enormous theoretical sequence space of non-natural polymers, libraries of >108-12 polymers are synthesized using a combinatorial split and pool synthetic strategy [6]. An embodiment utilizes established solid phase synthesis methods, wherein polyethylene glycol (PEG)-grafted microbeads is used as solid support. Monomelic units are added using primarily Fmoc chemistry methods and performed in 96-well filtration manifolds. Reagent addition, washing and vacuum filtration steps are performed using either a PerkinElmer Janus or Biomek Fx liquid handling workstation. Upon addition of the final monomeric unit, the polymers are deprotected on-bead, washed and dried. Since the majority of our coupling reactions are based on established Fmoc chemistry, we use existing protocols for standard peptide couplings and optimize reaction conditions for steps that include non-natural monomeric units.
[77] In embodiments polyamides that range from 20-30 monomelic units are used to determine a minimal length capable of forming well-folded structures. We have shown that though molecular dynamics simulations it is possible to design natural polypeptides with only four monomers that fold into compact secondary structures assessed by 1D and 2D NMR [5]. Libraries may also be produced with both random variations [7] as well as positional scanning approaches [8].

[78] Screening non-natural polymer libraries to identify binders and catalysts; cancer diagnostics. Circulating tumor cells (CTCs) are individual cancer cells that are shed by tumors into the blood stream and are thought to contribute to the process of metastasis. CTCs phenotypes are representative of the status of the primary tumor and as such they provide, through a simple blood draw, a "liquid biopsy" of the entire tumor status of a cancer patient—potentially a very powerful diagnostic tool to non-invasively monitor and direct cancer treatment. The major challenge in identifying CTCs is that they are extremely rare (>1 cell in 5 million); however, an SRI platform called the Fiber-optic Array Scanning Technology (FASTTM) cytometer, which is based on the concept of "Xeroxing" a blood sample with a scanning laser and collecting a high resolution capture image of the sample using a densely packed fiber optic array bundle [9].

[79] In the FAST process blood samples are plated on a glass slide with the footprint of a standard microtitre plate. The slide is coated with a poly-lysine graft that both serves to ensure the formation of a mono-dispersed cell layer and to fix the cells to the glass surface. The cellular monolayer on the glass slide is then treated with fluorescently labeled CTC targeting agents and is scanned with the FAST system. Only 60 seconds are needed to create a digital image of the location of the fluorescently labeled CTCs present on the slide. This is an extraordinarily sensitive system, which we have demonstrated to reliably detect single digit numbers of CTCs on glass slides containing >25,000,000 white blood cells. The system is well validated and robust: SRI has processed thousands of blood samples on the FAST system, which is currently included as a diagnostic platform in human clinical trials validating CTC analysis in cancer care.

[80] Once their location has been identified by the FAST system, we routinely pick individual cells from the glass slide using an aspiration system which then transfers the individual cells in buffer to 384- or 1536-well plates for further processing.

[81] FAST high throughput screening (HTS) of large combinatorial libraries: an extremely rapid and sensitive screening platform which can screen biopolymer libraries of >100,000,000 compounds (>10^8) in just 4 minutes.
To facilitate on-bead screening, compounds were synthesized on Tentagel-type resins, which contain a polystyrene bead core with a PEG graft co-polymer surface. The PEG coating facilitates display of compounds bound to the bead surface for screening in aqueous buffer. Our approach provides revisiting on-bead solid phase screening with a plurality of innovative approaches; for examples:

Beads can be the same size as cells: Beads for solid phase screening vary from 5-500 microns in diameter. White blood cells screened on the FAST platform typically range from 8-30 microns. While the trend has been to synthesize compounds on larger beads to maximize compound yield, with on-bead screening we only need enough polymer to (i) demonstrate affinity or catalytic activity and (ii) identify the polymer molecular structure once selected as a preliminary hit. By synthesizing libraries on smaller beads (diameter in the range of -10-20 microns), we can synthesize a 100,000,000-member library on as little as 250-500mg of resin.

Beads can be screened using the FAST platform: A 25,000,000-member on-bead library can be screened using a fluorescence-based assay on a glass slide like that used for CTC screening. Once polymer libraries have been synthesized on solid support they are dispersed on FAST glass slides, treated with fluorescently labeled target agent and screened on the FAST scanner for affinity or enzyme activity. The SRI FAST system can scan and identify hits in a 108-member library in 4 mins (60 sec per slide).

Beads can be selected for characterization: Using the same cell picking systems developed for FAST CTC picking, we can pick selected beads from glass slides and deliver them to 384- or 1536-well plates for resin cleavage and characterization by LC/MS/MS (see sequencing section below). Individual cell picks take 15-20 sec per cell. For example, assuming 100 hits are detected on a plate, bead picking will take only 33 mins, or ~2 hr for the entire 108 library.

We have scaled this example to a library of 10^8, but the entire process is readily expandable to larger libraries (10^10-12) or multiple diverse libraries. The process is also readily applied to screening binding to biological targets such organismal coat fragments or proteins, oligosaccharides, proteins, DNA/RNA sequences, cytokines or peptides - essentially any agents that can be tagged with a fluorescent label and directly applied to the library on the slides for read out as described above. For some small molecules, tagging with fluorescent labels can significantly affect the physicochemical properties of the agent and its binding profile, so in an alternative approach we use a fluorescent resonance energy transfer (FRET) assay by incorporating fluorescent and quenching labels at the beginning and end of each library compound synthesized, and then use FAST to identify modulation of fluorescence of individual beads on the slide before and after application of agent. The FRET assay and fluorophores may
be optimized for this type of assay, but the FAST scanner has the required sensitivity to detect even minor changes in fluorescence intensity and wavelength.

[87] Sequencing and characterizing non-natural polymers. In an embodiment we use 10-20 micron diameter PEG-grafted polystyrene beads to synthesize large libraries. Typically these types of resins have a loading capacity of 0.2-0.3 mmol of compound per gram of resin. There are approximately 250 million 20-micron beads in a gram of resin, which equates to ~1 pmol of compound per bead, assuming 100% success for synthesis, cleavage and isolation of product from bead. Even assuming a worst-case scenario of only a 1% yield a 30mer polymer from a single bead with a maximum loading of 1 pmol would produce 10 fmol of product. This amount of product can be readily identified by ID LC/MS/MS analysis.

[88] A database containing all possible sequences of the library may be constructed and used for identifying biopolymers from MS and MS/MS data. From a single compound on a bead there are only a small number of [MS, MS/MS] spectral pairs to deconvolute from other matching MS library members through fragmentation pattern data.

[89] Validation. Following sequencing, hits may be confirmed by resynthesis and a secondary solution-based binding assay including measure of K_d. Conformational analysis of a series of affinity reagent hits—alone and bound to the target agent—by NMR or X-ray crystallography enables refining predictive models, such as through steps:

[90] Compare bulk (ID) and structural (3D) properties of hits versus non-hits
[91] Analyze conformational and target-binding behavior of hits versus non-hits
[92] Cluster library based on calculated properties
[93] Focus on clusters in which hits are significantly overrepresented
[94] Derive structure-activity model
[95] This ensemble can be used to create models for classes of novel secondary structures represented in binding motifs found in the screening, and in next generation library design and to optimize affinity reagents for binding to specific target agents through structure-activity relationship (SAR) mapping.

[96] References


[106] Additional Descriptions of the Invention

In an aspect, the invention incorporates:

[108] Drug-like monomers based on functionally rich molecular frameworks assembled into non-natural polymers (NNPs) with extraordinary function; and/or

[109] Fiber optic array scanning technology (FAST) performs fluorescence-based screening of \( 10^8-10^9 \) member libraries in <5, <10 or <30 minutes

[110] NNPs designed as intrinsically self-readable molecules using MS "barcodes" incorporated into sequence structures


[112] Design of non-natural monomers. Criteria for developing drug-like monomer scaffolds include: stable, chiral, synthetically-scalable, minimal molecular weight, moderate flexibility, functional group density, coupled with reliable chemistry, and orthogonal side chain protecting groups, etc. These criteria are used to produce monomer sets, such as exemplified by candidate sets 1-3, wherein -OH/C1 and Fmoc positions yield monomer chain links:

[113]
Monomer sets may be designed to sample different backbone torsional angles to potentially explore new types of polymer folding geometries.

Design and synthesis of monomer set 1, dihydroisoquinolinones:

Exemplary monomers

Hydrophobic neutral  Weakly basic monomer  Strongly basic monomer

Polar Neutral monomer  Weakly acidic monomer  Strongly acidic monomer

Cleavable secondary structure constraints; inducing tertiary structure

Specialized monomers, secondary structure constraints" (SSCs) are employed to induce tertiary structures into NNPs. SSCs may also be designed to be cleavable to facilitate MS-based sequencing of the NNPs:
**Gamma turn 120 degrees**

**Beta-turn 180 degrees**

**Helix 270 degree turn**

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[120] NNP sublibrary configurations. Configurations of ~30mers with monomers and SSCs are constructed to maximize diversity; Fig. 3. Null spacer monomers (e.g. beta Ala) may be incorporated to simplify sequence and synthesis.

<table>
<thead>
<tr>
<th>Num. monomer /position</th>
<th>Num. positions</th>
<th>SSC #1:Num. SSCs</th>
<th>SSC #2:Num. SSCs</th>
<th>Sub library size</th>
<th>Configuration Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>1.51x10⁸</td>
<td>4 monomers per position in 12 positions; 3 SSCs in 2 positions</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>3</td>
<td>3</td>
<td>1.51x10⁸</td>
<td>2 monomer per position randomly selected from 4 monomers, in 24 positions; 3 SSCs in 2 positions</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>3</td>
<td>3</td>
<td>1.01x10⁸</td>
<td>2 monomer per position randomly selected from 4 monomers, in 25 positions; 3 SSCs in position #1, branched SSC in #2.</td>
</tr>
</tbody>
</table>

[121] Total Library = 4.03x10⁸

[122] FAST screening of libraries on beads using a well-fee plate, wherein compounds are attached to beads, rather than wells, and the number of assays/plate is determined by bead size (e.g. diameter); for examples, 1x10⁵ for 50-100um beads; 25x10⁶ for 10um beads.

[123] FAST mega-screening enables screening of millions of compounds per minute. In an exemplary assay: combine fluorescent target with 10⁸ bead library in tube, incubate and wash, transfer ~25M 10um beads/slide, FAST scan (~1 min/plate), rank & determine signal intensity, wherein dual wavelength can reduce false positives, automated bead picking and transfer to 384-well plate, cleave NNP from beads and sequence.

[124] Screening: Create and demonstrate a generalizable screening strategy and platform.

[125] Bead dispersion and attachment to slides - fluorescent assay, binding or enzymatic activity - FAST locate hits - ADM verify hits - bead picking - sequence.

[126] Characterization of Hits. Hits are characterized for binding affinity or catalytic activity.
Affinity reagents: Binding affinities are determined by surface plasmon resonance (SPR) to measure association and dissociation constants (ka, kd).

Catalysts: Michaelis Menten model is used to determine Vmax (maximal velocity), Km (Michaelis constant, ½ Vmax). The catalytic constant (kcat).

Differential scanning fluorimetry (DSF), Dynamic Light Scattering (DLS), analytical Size Exclusion Chromatography (anSEC)

folding, complex formation, solubility, stability

x-ray structure determination for up to three hits per target

Identification of NNPs

<table>
<thead>
<tr>
<th>bead diameter</th>
<th>theor. moles NNP/1 bead*</th>
<th>MS + MS/MS detection sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 micron</td>
<td>21 pmol</td>
<td>operable</td>
</tr>
<tr>
<td>10 micron</td>
<td>63 fmol</td>
<td>operable (fast duty cycle proteomics)</td>
</tr>
</tbody>
</table>

*with 10% NNP isolation yield.

Sequencing approach for NNP MS fragmentation: detect sequencing ions: a, b, c, x, y, z; additional backbone fragmentations for complex NNP backbones; Fig. 4.

Library design for facile and rapid sequencing; self-readable NNPS is shown in Fig. 5.

Isotopically-coded MS tags for sequence determination:

- Incorporating MS tags enhances sensitivity of sequencing and allows for reconstruction of full sequence from smaller polymer fragments, Fig. 6.

- Multiple and/or alternative fragmentation modes may be used:
<table>
<thead>
<tr>
<th>fragmentation method</th>
<th>mechanism</th>
<th>main ions (peptides)</th>
</tr>
</thead>
<tbody>
<tr>
<td>collision-induced dissociation</td>
<td>RF-enhanced collisions, vibrational</td>
<td>b, y, few a</td>
</tr>
<tr>
<td>(CID)</td>
<td>fragmentation</td>
<td></td>
</tr>
<tr>
<td>high energy CID (HCD)</td>
<td>collision cell higher energy</td>
<td>different/more b, y,</td>
</tr>
<tr>
<td></td>
<td>vibrational fragmentation</td>
<td>low mass ions</td>
</tr>
<tr>
<td>electron transfer dissociation</td>
<td>free radical cleavage</td>
<td>c, z; better for higher</td>
</tr>
<tr>
<td>(ETD)</td>
<td></td>
<td>charge states</td>
</tr>
</tbody>
</table>

[139] Sequencing may be optimized, e.g. optimize each fragmentation method for each class of NNPs; create more sequencing ions: use multiple fragmentation methods; MSn; library.

[140] Design; compare identified sequencing ions for known NNPs using each method, combinations of methods; support vector machine learning analysis, etc.

[141] Automated Sequencing of Self Readable Polymers

<table>
<thead>
<tr>
<th>engine</th>
<th>strengths</th>
<th>challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>de novo sequencing</td>
<td>no database, direct sequence reconstruction from MS/MS data</td>
<td>dense sequence space; often poor success rate, trouble distinguishing close sequence variants</td>
</tr>
<tr>
<td>custom</td>
<td>analyze multiple fragmentation modes; combine more than one engine</td>
<td>dense sequence space; implement for NNPs short peptides</td>
</tr>
<tr>
<td>database search</td>
<td>most successful analyzing peptide fragmentation data</td>
<td>dense NNP sequence space; searching databases when &gt; 1e10 sequences</td>
</tr>
</tbody>
</table>

[142] Implementation criteria: software analysis of complex data, adapt, compare different search engines using NNP fragmentation data; analyze initial data sets from known NNPs with best-performing proteomics engines in above categories; use combinations of engines; combined fragmentation data, MSn, etc; ; library design e.g. no isobaric monomers.

[143] The invention encompasses all combinations of recited particular and preferred embodiments. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein, including citations therein, are hereby incorporated by reference in their entirety for all purposes.
WHAT IS CLAIMED IS:

1. A library of beads configured for high-throughput drug screening, each bead comprising affixed non-natural polymers of a distinct bioactive monomer with sequence pre-defined branching and folding in tertiary structures.

2. The library of claim 1 further comprising incorporated in each of the polymers secondary structure constraint (SSC) monomers which impose turns and thereby induce the tertiary structures, wherein the SSC monomers are acid cleavable to facilitate mass-spectroscopy based sequencing of the polymers.

3. The library of claim 1 wherein:
   the polymers or polymerizations comprise sulphonamides, reductive aminations, peptoid linkages, Suzuki couplings, phosphamidite couplings, or radical cross coupling,
   the monomer is a dihydroisoquinolinone; and/or
   the polymerization combines amide bond coupling methodologies with peptidotriazole branch points introduced using chemically orthogonal copper-catalyzed cycloadditions (click-reactions).

4. The library of claim 1 wherein the polymers comprise intrinsically self-readable molecules via intrinsically incorporated isotopically-coded mass-spectroscopy tags or barcodes.

5. A library of claim 1 configured in an array on a slide.

6. A library of claim 1 configured in an array on a slide mounted on a fiber optic scanner.

7. A method of making a library of claim 1 comprising affixing the polymers to the beads or building the polymers on the beads by sequential monomer coupling.

8. A method of using a fiber optic scanner mounted with a slide comprising an ordered array of a library of claim 1 comprising:
   labeling the array with a bioactivity (affinity or catalysis) marker to generate fluorescent labels on target beads comprising target monomers; and
   fluorescent imaging the array with the scanner.
9. A method of using a fiber optic scanner mounted with a slide comprising an ordered array of a library of claim 1, comprising:
   labeling the array with a bioactivity (affinity or catalysis] marker to generate fluorescent labels on target beads comprising target monomers; and
   fluorescent imaging the array with the scanner,
   wherein the labels provide multiple fluorescent wavelengths, and the imaging comprises optical filtering, reducing background signaling and false positives.

10. A method of using a library of claim 1 comprising:
   fluorescence assaying the library to detect a candidate bead based on bioactivity of the corresponding monomer;
   isolating the candidate bead from the assayed library;
   cleaving polymers from the isolated candidate bead; and
   structurally analyzing the cleaved polymers.

11. A non-natural polymer of a distinct bioactive monomer with sequence pre-defined branching and folding in tertiary structures.

12. The polymer of claim 11 further comprising incorporated therein secondary structure constraint (SSC] monomers which impose turns and thereby induce the tertiary structure, wherein the SSC monomers are acid cleavable to facilitate mass-spectroscopy based sequencing of the polymer.

13. The polymer of claim 11 wherein:
   the polymer is a polyamide, a vinylogous polymer, vinylogous polyamide, or an ester;
   the polymer is polymerized in a coupling reaction selected from Wurtz reaction, Glaser coupling, Ullmann reaction, Gomberg-Bachmann reaction, Cadiot-Chodkiewicz coupling, Pinacol coupling reaction, Castro-Stephens coupling, Gilman reagent coupling, Cassar reaction, Kumada coupling, Heck reaction, Sonogashira coupling, Negishi coupling, Stille cross coupling, Suzuki reaction, Hiyama coupling, Buchwald-Hartwig reaction, Fukuyama coupling, and Liebeskind-Srogl coupling;
the polymer or polymerizations is selected from sulphonamides, reductive aminations, peptoid linkages, Suzuki couplings, phospoamidite couplings, and radical cross coupling;

the monomer is a dihydroisoquinolinone; and/or

the polymerization combines amide bond coupling methodologies with peptidotriazole branch points introduced using chemically orthogonal copper-catalyzed cycloadditions (click-reactions).

14. The polymer of claim 11 comprising an intrinsically self-readable molecule via an intrinsically incorporated isotopically-coded mass-spectroscopy tag or barcode.

15. A library of beads for high-throughput drug screening, each of the beads comprising affixed non-natural polymers of claim 11.

16. A fiber optic scanner mounted with a slide bearing fluorescent beads.

17. The scanner of claim 16 comprising:

an imager stage having a planar surface for supporting a sample comprising the slide;

a bifurcated light path having two fiber optic bundles, each bundle having a first end arranged to define an input aperture for viewing the sample on the imager stage, and a distal bundle end arranged to define an output aperture disposed away from the imager stage;

a scanning source arranged to scan a beam along a path that is perpendicular to the sample on the imager stage and closely adjacent to both bundles of the bifurcated light path such that a substantially circular spot of illumination provided by the scanning source on the imager stage sample provides a light signal at least a portion of which is received by the input aperture of each bundle and transmitted via the bifurcated light path to the output aperture;

a photodetector arranged to detect the light signal at the distal end; and

a processor that processes the light signal detected by the photodetector.

18. A method for imaging a sample comprising the slide bearing fluorescent beads of claim 15, the method comprising:
supplying a substantially circular beam of radiation perpendicular to the sample; maintaining the perpendicular direction of the radiation beam as it sweeps along a scan path on the sample; reflecting at least some light produced by beam interaction with the sample in a direction away from the sample; collecting light produced by beam interaction with the sample in at least one proximate element of an array of fiber optic first ends; detecting collected light at a selected output region; and coordinating sweeping, moving and detecting to generate an array of picture elements representative of at least a portion of the sample.

19. A method of using the fiber optic scanner mounted with a slide bearing fluorescent beads of claim 15, the method comprising the step:
fluorescent imaging the beads with the scanner;

20. A method of using the fiber optic scanner mounted with a slide bearing fluorescent beads of claim 15, the method comprising the steps:
fluorescent imaging the beads with the scanner to detect a candidate bead; isolating the candidate bead from the beads; and analyzing a function or structure of the candidate bead.
Fig. 1
Step 1: Linear elongation using solid-phase Fmoc methodologies with introduction of branching monomers ($M_b$).

Step 2: Extension off of branch point followed by additional linear elongation (with potential additional branch points).

**Fig. 2**

![Diagram](image)

**Fig. 3**

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<th>Sequence Position</th>
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<th>C-terminus</th>
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<td>random 10mer: monomers M1 – M4</td>
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Fig. 4

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SUBSTITUTE SHEET (RULE 26)
Fig. 5

random 10mer:
monomers M1 – M4

TFA Cleavage

Sequencing

Resynthesis

Random 30mer comprising
M1 – M4, 3 MS tags

Biotin
Fig. 6
### INTERNATIONAL SEARCH REPORT

**International application No.**
PCT/US 15/50306

**A. CLASSIFICATION OF SUBJECT MATTER**

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<th>IPC(8)</th>
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According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

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**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>X</td>
<td>(MAILLARD, N et al.) Combinatorial libraries of peptide dendrimers: design, synthesis, on-bead high-throughput screening, bead decoding and characterization. Nat Protoc. 15 January 2009; Vol. 4, No. 2; pages 132-142; abstract; page 132, column 1, paragraph 1; page 133, column 1, paragraph 1; page 134, column 1, paragraph 3; column 2, paragraph 3; page 142, paragraph 1; figures 1,2,3,4,5; table 4; pages 136-141; steps 1-27, 60, 77; DOI: 10.1038/nprot.2008.241</td>
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<td>Y</td>
<td>US 2013/004967 A1 (HALVERSON, K J et al.) 3 January 2013; paragraphs [0007], [0020], [0206], [0207], [0225], [0234], [0307]; figures 3A-3B</td>
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<td>X</td>
<td>(YUE, K et al.) Constraint-based assembly of tertiary protein structures from secondary structure elements. October 2000; Vol. 9, No. 10; pages 1935-1946; abstract; page 1941, column 1, paragraph 3; page 1942, column 1, paragraph 2</td>
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<td>Y</td>
<td>US 5,877,276 A (ZUCKERMANN, RN et al.) 2 March 1999; column 8, lines 12-22; column 20, lines 10-15, lines 46-50; column 36, lines 39-40, lines 58-60; column 48, line 49; column 82, line 45; column 86, example 31, lines 48-54</td>
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<td>Y</td>
<td>(XU, Q et al.) Protein and chemical microarrays — powerful tools for proteomics. J Biomed Biotechnol. 2003. Vol. 2003, No. 5; pages 267-266; page 269, column 1, paragraph 2, column 2, paragraph 1; page 261, column 1, paragraphs 2-4; DOI: 10.1080/107067603200029220</td>
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![ Special categories of cited documents: ]

- **"A"** document defining the general state of the art which is not considered to be of particular relevance
- **"E"** earlier application or patent but published on or after the international filing date
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- **"G"** document member of the same patent family

Date of the actual completion of the international search

24 February 2016 (24.02.2016)

Date of mailing of the international search report

11 MAR 2016

Name and mailing address of the ISA/

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P.O. Box 1450, Alexandria, Virginia 22313-1450

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Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

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<td>Y</td>
<td>BUCHSER, W et al. Assay development guidelines for image-based high content screening, high content analysis and high content imaging. Assay Guidance Manual. 2004; pages 1-68; page 9, paragraph 2.1.8; page 16, paragraph 1; page 30, paragraph 6.2; PMID:23035272</td>
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<td>Y</td>
<td>US 7,280,261 B2 (CURRY, DN et al.) 9 October 2007; abstract; column 2, lines 10-50; column 3, lines 17-19; column 4, lines 11-27; figures 1-3, 5-8; claims 1, 13, 19</td>
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