

**COMPOSITE PARTICLES FOR USE IN GENERATING SPECTRALLY ENCODED
LIBRARIES OF CHEMICAL COMPOUNDS, AND METHODS OF MAKING AND
USING THE SAME**

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/592,054, filed October 20, 2023 and entitled “Composite Particles For Use In Generating Spectrally Encoded Libraries of Chemical Compounds, and Methods of Making and Using the Same,” the entire contents of which are incorporated by reference herein.

FIELD

[0002] This application generally relates to libraries of chemical compounds.

BACKGROUND

[0003] Conventional high-throughput screening (HTS) has been used for decades to analyze the interactions between targets and chemical compounds. Typically, different chemical compounds are placed in different wells of a microtiter plate, and the collection of these compounds is referred to as an “HTS library.” An entity of interest then is placed into the wells and incubated therein with the compound(s) within the respective wells. Each of the wells then is “screened” to characterize the nature of any interaction between the entity of interest and the compound(s) within that well. However, the scale of conventional HTS screening is limited by the minimum practical size of the individual test wells. The current standard is 1536-well plates, so screening millions of compounds would require thousands of plates, with high costs for consumables and reagents. Although certain HTS operations may be implemented by robotics (which themselves may be costly), the time required to perform a full screening may be quite lengthy. Accordingly, the time and expense of HTS has limited its practical implementation.

[0004] Previously known alternatives to HTS have their own shortcomings. For example, DNA-encoded library (DEL) technology can be used to generate libraries with billions of unique chemical compounds. However, selection-based screening strategies reveal only the strongest interactions between targets and compounds. As another example, one-bead, one-compound (OBOC) combinatorial libraries have been encoded with DNA (OBOC-DEL) and used in activity-based screening. However, only a subpopulation of “hits” is defined and collected for DNA sequencing and structural deconvolution. Thus, in OBOC-DEL, the vast majority (typically >98%) of screening data is unquantified. The chemistries that can be used

to build a DNA-encoded library also are limited to those which do not damage or destroy the DNA that is used to encode the library.

SUMMARY

[0005] Examples herein provide composite particles for use in generating spectrally-encoded libraries of chemical compounds, and methods of making and using the same.

[0006] The present inventors have recognized that for drug discovery, there is a need for a new platform for high-throughput screening assays that is simple to operate, can determine whether targets respectively interact with compounds – which may number in the millions. Among other things, the present application provides a process for synthesizing and screening optically-encoded particle-based libraries of chemical origin against one or more targets of interest. As provided herein, the present compositions, systems, and methods utilize composite particles which have one or more optically detectable particles within them, and in some examples have multiple optically detectable particles within them. The optically detectable particle(s) within a composite particle may emit light at different wavelengths than one another, to produce an emission spectrum which acts as an optical “spectral fingerprint” from which the composite particle may be rapidly identified at any suitable point during its use. The composite particles may be chemically elaborated so as to include one or more compounds, and the spectral fingerprint is recorded at each step of chemical incorporation so that the synthetic history for each composite particle is known. When assaying the interaction between target(s) to the chemical compound(s) coupled to respective composite particles, the assay result and the spectral fingerprint are detected and used to informatively link the assay result directly to the identity of the compound(s) on the respective composite particles.

[0007] Some examples herein provide a composite particle. The composite particle may include an optically detectable particle; and a network polymer gel substantially surrounding the optically detectable particle,

[0008] In some examples, the composite particle includes a plurality of the optically detectable particles. In some examples, at least some of the optically detectable particles have different emission wavelengths than one another. In some examples, all of the optically detectable particles have different emission wavelengths than one another. In some examples, the composite particle includes at least three of the optically detectable particles with different emission wavelengths from one another. In some examples, the composite particle includes at least six of the optically detectable particles with different emission wavelengths from one another.

[0009] In some examples, the optically detectable particles are randomly oriented relative to one another within the network polymer gel. In some examples, the optically detectable particles have substantially the same orientation as one another within the network polymer gel.

[0010] In some examples, the optically detectable particle is configured to emit light with a bandwidth of about 1 nm or less at full-width half-maximum (FWHM) responsive to excitation light. In some examples, the optically detectable particle includes a microdisk laser particle. In some examples, the microdisk laser particle has a diameter of about 1 μm to about 10 μm . In some examples, the light emitted by the microdisk laser particle is near-infrared.

[0011] In some examples, the optically detectable particle is configured to emit light with a bandwidth of about 40 nm or less at full-width half-maximum (FWHM) responsive to excitation light. In some examples, the optically detectable particle includes a CsPbBr₃ Perovskite quantum dot.

[0012] In some examples, the network polymer gel includes a chemical moiety which is couplable to a chemical compound. In some examples, the chemical moiety is cleavable from the network polymer gel using light, heat, or a reagent. In some examples, the chemical moiety is distributed throughout the network polymer gel. In some examples, the chemical moiety is positively charged. In some examples, the chemical moiety is negatively charged. In some examples, the chemical moiety is uncharged.

[0013] In some examples, the composite particle further includes chemical compounds coupled to the network polymer gel. In some examples, the chemical compounds are cleavable from the network polymer gel using light, heat, or a reagent. In some examples, the chemical compounds are distributed throughout the network polymer gel. In some examples, the chemical compounds are the same as one another. In some examples, the chemical compounds are different than one another.

[0014] In some examples, the optically detectable particle is covalently coupled to the network polymer gel.

[0015] In some examples, the composite particle further includes a carrier particle having an outer surface. The optically detectable particle is coupled to the outer surface of the carrier particle. The network polymer gel substantially surrounds the carrier particle and the optically detectable particle coupled to the carrier particle. In some examples, the composite particle includes a plurality of the optically detectable particles coupled to the outer surface of the carrier particle. In some examples, the carrier particle includes a polymer or a glass. In

some examples, the carrier particle has a different composition than the network polymer gel. In some examples, the carrier particle has the same composition as the network polymer gel. In some examples, the carrier particle has a diameter of between about 1 μm and about 20 μm in water at pH 7. In some examples, the carrier particle has a diameter of between about 5 μm and about 15 μm in water at pH 7. In some examples, the carrier particle has a diameter of between about 1 μm and about 5 μm in water at pH 7. In some examples, the composite particle is at least about 100 nm larger than the carrier particle in water at pH 7. In some examples, the optically detectable particle is coupled to the carrier particle via a covalent bond. In some examples, the optically detectable particle is coupled to the carrier particle via a non-covalent bond. In some examples, the composite particle further includes at least one additional carrier particle having an outer surface; and an additional optically detectable particle coupled to the outer surface of the at least one additional carrier particle. The network polymer gel substantially surrounds the at least one additional carrier particle and the additional optically detectable particle coupled to the at least one additional carrier particle.

[0016] In some examples, the composite particle has a diameter of between about 1 μm and about 50 μm in water at pH 7. In some examples, the composite particle has a diameter of between about 10 μm and about 30 μm in water at pH 7. In some examples, the composite particle has a diameter of between about 5 μm and about 20 μm in water at pH 7. Some examples herein provide a collection of the composite particles of any of the above statements.

[0017] In some examples, each of the composite particles of the collection includes a plurality of the optically detectable carrier particles. At least some of the optically detectable particles of that plurality may have different emission wavelengths than one another. The different emission wavelengths may define a spectral fingerprint for that composite particle. In some examples, a majority of the composite particles of the collection have different spectral fingerprints than one another. In some examples, over 80% of the composite particles of the collection have different spectral fingerprints than one another.

[0018] In some examples, the composite particles of the collection respectively include substantially the same number of optically detectable particles as one another. In some examples, the composite particles of the collection respectively include between two and ten of the optically detectable particles.

[0019] In some examples, the composite particles of the collection respectively include different numbers of optically detectable particles than one another. In some examples, the

composite particles of the collection respectively include between two and ten of the optically detectable particles.

[0020] In some examples, a majority of the composite particles of the collection respectively include the same number of carrier particles as one another. In some examples, the composite particles of the collection respectively include one to three of the carrier particles.

[0021] In some examples, a composition includes the collection of the composite particles of any of the above statements suspended in an aqueous solvent. In some examples, a composition includes the collection of the composite particles of any of the above statements suspended in an organic solvent.

[0022] Some examples herein provide a method of making a composite particle. The method may include substantially surrounding an optically detectable particle with a network polymer gel. In some examples, substantially surrounding the optically detectable particle with the network polymer gel includes: forming a droplet including a crosslinker, an initiator, and the optically detectable particle; and using the initiator to polymerize the crosslinker to form the network polymer gel within the droplet.

[0023] In some examples, the droplet further includes a non-functional monomer, or a functional monomer, and the initiator polymerizes the crosslinker, non-functional monomer, or functional monomer together with the monomer to form the network polymer gel within the droplet. In some examples, the initiator includes a photo-initiator, and using the initiator to polymerize the monomer includes irradiating the droplet with light. In some examples, forming the droplet includes suspending the optically detectable particle in a solution including the monomer and the initiator. In some examples, forming the droplet includes encapsulating a defined volume of the solution in an oil/surfactant mixture using a microfluidic junction. In some examples, forming the droplet includes forming an emulsion with the solution and an oil/surfactant mixture.

[0024] In some examples, the method further includes substantially surrounding at least one additional optically detectable particle with the network polymer gel. In some examples, the optically detectable particle and the at least one additional optically detectable particle are randomly oriented relative to one another within the network polymer gel. In some examples, the optically detectable particle and the at least one additional optically detectable particle have substantially the same orientation as one another within the network polymer gel.

[0025] In some examples, the optically detectable particle is coupled to a chemical moiety, the method including coupling the chemical moiety to the network polymer gel. In some examples, the chemical moiety is covalently coupled to the network polymer gel. In some examples, the chemical moiety includes a component of the network polymer gel. In some examples, the method further includes coupling the chemical moiety to the optically detectable particle.

[0026] In some examples, the method further includes coupling the optically detectable particle to an outer surface of a carrier particle; and substantially surrounding the carrier particle, with the optically detectable particle coupled thereto, with the network polymer gel. In some examples, the method further includes coupling a plurality of additional optically detectable particles to the outer surface of the carrier particle. In some examples, the carrier particle includes a polymer or a glass. In some examples, the carrier particle has a different composition than the network polymer gel. In some examples, the carrier particle has the same composition as the network polymer gel. In some examples, the optically detectable particle is coupled to the carrier particle via a covalent bond. In some examples, the carrier particle is coupled to a first chemical moiety and the optically detectable particle is coupled to a second chemical moiety, the method including covalently coupling the first chemical moiety to the second chemical moiety to form the covalent bond. In some examples, the method includes coupling the first chemical moiety to the carrier particle. In some examples, the method includes coupling the second chemical moiety to the optically detectable particle. In some examples, the optically detectable particle is coupled to the carrier particle via a non-covalent bond. In some examples, the non-covalent bond includes an ionic bond. In some examples, the optically detectable particle is negatively charged, and the carrier particle is positively charged. In some examples, the optically detectable particle is positively charged, and the carrier particle is negatively charged.

[0027] In some examples, the optically detectable particle is configured to emit light with a bandwidth of about 1 nm or less at full-width half-maximum (FWHM) responsive to excitation light. In some examples, the optically detectable particle includes a microdisk laser particle. In some examples, the optically detectable particle is configured to emit light with a bandwidth of about 40 nm or less at full-width half-maximum (FWHM) responsive to excitation light. In some examples, the optically detectable particle includes a CsPbBr₃ Perovskite quantum dot.

[0028] Some examples herein provide a library of chemical compounds. The library may include composite particles. The composite particles respectively may include a plurality of

optically detectable particles; a network polymer gel substantially surrounding the optically detectable particles; and a chemical compound coupled to the network polymer gel. The composite particles of a first group of the composite particles include the same chemical compound as one another. The composite particles of a second group of the composite particles include the same chemical compound as one another and a different chemical compound than that of the first group of composite particles.

[0029] In some examples, the chemical compound is covalently coupled to the network polymer gel. In some examples, the chemical compound is non-covalently coupled to the network polymer gel. In some examples, the chemical compound is adsorbed to the network polymer gel.

[0030] In some examples, the composite particles of the first group of composite particles include a first plurality of different chemical compounds. In some examples, the composite particles of the second group of composite particles include a second plurality of different chemical compounds, wherein the chemical compounds of the first plurality are at least partially different than the chemical compounds of the second plurality. In some examples, the first plurality of different chemical compounds includes between two and four different chemical compounds.

[0031] In some examples, the chemical compound is distributed throughout the network polymer gel.

[0032] In some examples, the chemical compound is cleavable from the network polymer gel using light, heat, or a reagent.

[0033] In some examples, the optically detectable particles are configured to emit light with a bandwidth of about 1 nm or less at full-width half-maximum (FWHM) responsive to excitation light. In some examples, the optically detectable particles include microdisk laser particles.

[0034] In some examples, the optically detectable particles are configured to emit light with a bandwidth of about 40 nm or less at full-width half-maximum (FWHM) responsive to excitation light. In some examples, the optically detectable particles include CsPbBr₃ Perovskite quantum dots.

[0035] In some examples, the optically detectable particles are randomly oriented relative to one another within the network polymer gel. In some examples, the optically detectable particles have substantially the same orientation as one another within the network polymer gel.

[0036] In some examples, the optically detectable particles are covalently coupled to the network polymer gel.

[0037] In some examples, the composite particles respectively further include a carrier particle having an outer surface. The optically detectable particles may be coupled to the outer surface of the carrier particle. The network polymer gel may substantially surround the carrier particle and the optically detectable particle coupled to the carrier particle. In some examples, the optically detectable particles respectively are coupled to the carrier particle via covalent bonds. In some examples, the optically detectable particles respectively are coupled to the carrier particle via non-covalent bonds. In some examples, the composite particles respectively further include at least one additional carrier particle having an outer surface; and a plurality of additional optically detectable particles coupled to the outer surface of the at least one additional carrier particle. The network polymer gel substantially surrounds the at least one additional carrier particle and the additional optically detectable particles coupled to the at least one additional carrier particle.

[0038] Some examples herein provide a method of generating a library of chemical compounds. The method may include irradiating a collection of composite particles with excitation light. The composite particles of the collection respectively include a plurality of optically detectable particles; and a network polymer gel substantially surrounding the optically detectable particles. The method may include respectively obtaining emission spectra from the optically detectable particles of the composite particles. The method may include coupling chemical compounds to the composite particles by coupling the chemical compounds to the network polymer gels of the composite particles. The composite particles of a first group of composite particles are coupled to the same chemical compound as one another. The composite particles of a second group of composite particles are coupled to the same chemical compound as one another. The chemical compound coupled to the first group of composite particles is different than the chemical compound coupled to the second group of composite particles. The method may include, for respective composite particles of the collection, storing, in a data structure, (i) identifiers for the composite particles which are based on the respectively obtained emission spectra, and (ii) identifiers for the chemical compounds which respectively are coupled to those composite particles.

[0039] In some examples, coupling the chemical compounds to the composite particles includes (a) dividing the collection of composite particles into subsets; and (b) for respective subsets formed in operation (a), coupling a chemical moiety to the network polymer gel of the composite particles of that subset to form a modified subset. In some examples, the

chemical moiety coupled to the network polymer gel of the composite particles of a first one of the subsets is different than the chemical moiety coupled to the network polymer gel of the composite particles of a second one of the subsets. In some examples, the chemical moiety coupled to the network polymer gel of the composite particles of a first one of the subsets is the same as the chemical moiety coupled to the network polymer gel of the composite particles of a second one of the subsets.

[0040] In some examples, dividing the collection of composite particles into subsets includes: using a flow cytometer to dispense a first subset of the composite particles of the collection into a first reservoir; and using the flow cytometer to dispense a second subset of the composite particles of the collection into a second reservoir. In some examples, dividing the collection of composite particles into subsets further includes counting composite particles as they flow through the flow cytometer and into the first reservoir, wherein the composite particles are dispensed into the first reservoir until a predetermined number of the composite particles are dispensed into the first reservoir. After the predetermined number of the composite particles are dispensed into the first reservoir, the composite particles are dispensed into the second reservoir until a predetermined number of the composite particles are dispensed into the first reservoir. In some examples, the composite particles are counted using the emission spectra.

[0041] In some examples, the collection of composite particles is manually divided into respective reservoirs to form the subsets.

[0042] In some examples, the method further includes, for the composite particles of respective subsets formed in operation (a): irradiating the composite particles of that subset with excitation light; respectively obtaining the emission spectra from the optically detectable particles of the composite particles of that subset; and using the emission spectra to identify the composite particles of that subset using the stored identifiers. In some examples, the emission spectra from the optically detectable particles of the composite particles of that subset are obtained one at a time as the composite particles flow through a channel. In some examples, the emission spectra from the optically detectable particles of the composite particles of that subset are obtained before that subset of particles is formed. In some examples, the emission spectra from the optically detectable particles of the composite particles of that subset are obtained after that subset of particles is formed.

[0043] In some examples, for the composite particles of respective subsets formed in operation (a), the identifier for the chemical compound which is coupled to those composite

particles includes an identifier for the chemical moiety which is coupled to the network polymer gel in operation (b).

[0044] In some examples, the method further includes (c) pooling the modified subsets of operation (b) to form a modified collection of composite particles. In some examples, coupling the chemical compounds to the composite particles further includes: (d) dividing the modified collection of composite particles of operation (c) into subsets; and (e) for respective subsets formed in operation (d), coupling a chemical moiety to the to the chemical moiety which was coupled to the network polymer gel in operation (b) to form a modified subset.

[0045] In some examples, the method further includes, for the composite particles of respective subsets formed in operation (d): irradiating the composite particles of that subset with excitation light; respectively obtaining the emission spectra from the optically detectable particles of the composite particles of that subset; and using the emission spectra to identify the composite particles of that subset using the stored identifiers.

[0046] In some examples, for the composite particles of respective subsets formed in operation (d), the identifier for the chemical compound which is coupled to those composites particle includes (i) an identifier for the chemical moiety which is coupled to the network polymer gel in operation (b) and (ii) an identifier for the chemical moiety which is coupled in operation (e) to the chemical moiety which was coupled to the network polymer gel in operation (b).

[0047] In some examples, the method further includes repeating operations (c) through (e) for the modified collection of composite particles. In some examples, operations (c) through (e) are repeated between one and five times. In some examples, operation (b) and the repeated operations (c) through (e) build (i) the chemical compound coupled to the composite particles of the first group of composite particles, and (ii) the chemical compound coupled to the composite particles of the second group of composite particles.

[0048] In some examples, coupling the chemical compounds to the composite particles includes using a condition, solvent, or reagent which is incompatible with DNA.

In some examples, the chemical compound is covalently coupled to the network polymer gel. In some examples, the chemical compound is non-covalently coupled to the network polymer gel. In some examples, the chemical compound is adsorbed to the network polymer gel.

[0049] In some examples, the composite particles of the first group of composite particles include a first plurality of different chemical compounds. In some examples, the composite particles of the second group of composite particles includes a second plurality of different chemical compounds, wherein the chemical compounds of the first plurality are at least

partially different than the chemical compounds of the second plurality. In some examples, the first plurality of different chemical compounds includes between two and four different chemical compounds.

[0050] In some examples, the chemical compound is distributed throughout the network polymer gel.

[0051] In some examples, the chemical compound is cleavable from the network polymer gel using light, heat or a reagent.

[0052] In some examples, the identifiers for the composite particles which are based on the respectively obtained emission spectra include lists of wavelengths having peaks in the emission spectra.

[0053] In some examples, the identifiers for the composite particles which are based on the respectively obtained emission spectra include bits representing wavelengths having peaks in the emission spectra.

[0054] In some examples, the identifiers for the chemical compounds which respectively are coupled to those composite particles include a chronological description of chemical reactions performed using those composite particles.

[0055] In some examples, the identifiers for the chemical compounds which respectively are coupled to those composite particles include tokens chronologically representing chemical reactions performed using those composite particles

[0056] In some examples, the optically detectable particles are configured to emit light with a bandwidth of about 1 nm or less at full-width half-maximum (FWHM) responsive to excitation light. In some examples, the optically detectable particles include microdisk laser particles.

[0057] In some examples, the optically detectable particles are configured to emit light with a bandwidth of about 40 nm or less at full-width half-maximum (FWHM) responsive to excitation light. In some examples, the optically detectable particles include CsPbBr₃ Perovskite quantum dots.

[0058] In some examples, the optically detectable particles are randomly oriented relative to one another within the network polymer gel. In some examples, the optically detectable particles have substantially the same orientation as one another within the network polymer gel.

[0059] In some examples, the optically detectable particles are covalently coupled to the network polymer gel.

[0060] In some examples, the composite particles respectively further including a carrier particle having an outer surface, wherein the optically detectable particles are coupled to the outer surface of the carrier particle; and the network polymer gel substantially surrounds the carrier particle and the optically detectable particle coupled to the carrier particle. In some examples, the optically detectable particles respectively are coupled to the carrier particle via covalent bonds. In some examples, the optically detectable particles respectively are coupled to the carrier particle via non-covalent bonds. In some examples, the composite particles respectively further include at least one additional carrier particle having an outer surface; and a plurality of additional optically detectable particles coupled to the outer surface of the at least one additional carrier particle. The network polymer gel substantially surrounds the at least one additional carrier particle and the additional optically detectable particles coupled to the at least one additional carrier particle.

[0061] Some examples herein provide a method of detecting binding between different chemical compounds and a target. The method may include incubating a first target with a collection of composite particles. The composite particles of the collection may include a plurality of optically detectable particles; a network polymer gel substantially surrounding the optically detectable particles; and a chemical compound coupled to the network polymer gel. At least some of the composite particles include the same chemical compound as one another. At least some of the composite particles include different chemical compounds than one another. The first target binds differently to different ones of the chemical compounds. The method may include removing unbound first target from the collection of composite particles. The method may include irradiating the composite particles of the collection, with the first target bound to different ones of the chemical compounds, with excitation light. The method may include obtaining emission spectra from the optically detectable particles of respective composite particles. The method may include obtaining signals respectively indicating whether the first target binds to the chemical compounds of respective composite particles. The method may include using the emission spectra and the signal to (i) identify the chemical compound which is coupled to at least some of the composite particles and (ii) determine whether the first target binds to that chemical compound.

[0062] In some examples, the first target is labeled with a probe before the incubating.

[0063] In some examples, the method further includes, after the first target binds differently to different ones of the chemical compounds, labeling the first target with an affinity reagent that is labeled with a probe. In some examples, the method further includes, after the first target binds differently to different ones of the chemical compounds, incubating

the first target with an unlabeled primary affinity reagent, then labeling the unlabeled primary affinity reagent with a secondary affinity reagent that is labeled with a probe.

[0064] In some examples, the composite particles are irradiated with a first wavelength of light that stimulates the emission spectra of the optically detectable particles. In some examples, the composite particles are irradiated with a second wavelength of light that stimulates fluorescence which generates the signal indicating whether the first target binds to the chemical compounds of respective composite particles. In some examples, the composite particles are irradiated with the first and second wavelengths of light at different times than one another. In some examples, the composite particles are irradiated with the first and second wavelengths of light at the same time as one another.

[0065] In some examples, the emission spectra and signal are obtained concurrently with one another. In some examples, the emission spectra and signal are obtained at different times than one another.

[0066] In some examples, the method further includes incubating a second target with the collection of composite particles. The second target binds differently to different ones of the chemical compounds. The method may include removing unbound second target from the collection of composite particles; and obtaining signals indicating whether the second target binds to the chemical compounds of respective composite particles. In some examples, the collection of composite particles is incubated concurrently with the first target and the second target. In some examples, the signals include fluorescence, and fluorescence from the first target is at a different wavelength than fluorescence from the second target.

[0067] In some examples, the emission spectra, the signals, or both the emission spectra and the signals, are obtained while the composite particles are static. In some examples, the emission spectra, the signals, or both the emission spectra and the signals, are obtained while the composite particles are flowed through an inspection point.

[0068] In some examples, the signals are optical.

[0069] Some examples herein provide a spectral fingerprint system for categorizing a plurality of composite particles suspended in a fluid. Each composite particle includes a plurality of optically detectable particles and a network mesh polymer substantially surrounding the optically detectable particles. The system may include an optical excitation system; an optical detector; a flow cell; at least one memory storing instructions; and at least one processor configured to execute the instructions to perform operations. The operations may include flowing the composite particles in the fluid through the flow cell; exciting the composite particles in the flow cell with light from the optical excitation system; reading, by

the optical detector, a spectral fingerprint of each composite particle excited by the light; and generating, using the spectral fingerprint of each composite particle, a composite particle identity of that composite particle.

[0070] In some examples, the optical excitation system includes a laser.

[0071] In some examples, at least some of the optically detectable particles of each composite particle include different emission wavelengths than one another. The spectral fingerprint of each composite particle is based on the different emission wavelengths of the optically detectable particles of that composite particle.

[0072] In some examples, a majority of the composite particles have different spectral fingerprints than one another.

[0073] In some examples, the composite particles respectively include between two and ten of the optically detectable particles.

[0074] In some examples, at least some of the flow cell is positioned in an optical path between the optical excitation system and the optical detector. In some examples, the step of reading, by the optical detector, the spectral fingerprint of each composite particle excited by the light includes measuring an IR emission orthogonal to an excitation beam of the optical excitation system.

[0075] In some examples, the at least one processor further is configured to execute the instructions to perform operations including: physically sorting the plurality of composite particles into a plurality of subsets. In some examples, the step of physically sorting is based on the spectral fingerprints of the composite particles of the plurality. In some examples, the step of physically sorting is based on dielectrophoresis. In some examples, the step of physically sorting is based on electrophoresis.

[0076] In some examples, a microfluidic device of the flow cell is coupled to the optical detector, wherein a plurality of subsets includes a main subset sorted by the optical detector sequentially detecting the spectral fingerprint of each composite particle. The at least one processor further may be configured to execute the instructions to perform operations including: dispensing, by the microfluidic device and based on characteristics of the spectral fingerprint, each of the plurality of composite particles into individual tubes or wells in a microplate.

[0077] In some examples, the system further includes a composite particle dispenser. The at least one processor further may be configured to execute the instructions to perform operations including: outputting, by a composite particle dispenser, the plurality of composite

particles through an optical beam of the excitation laser system and into a plurality of collection vessels based on a user-defined population size.

[0078] In some examples, the system further includes a composite particle dispenser. The at least one processor further may be configured to execute the instructions to perform operations including: outputting, by the composite particle dispenser, the plurality of composite particles through an optical beam of the excitation laser system and into a plurality of collection vessels based on a counted number of composite particles. In some examples, the plurality of collection vessels include 96 vessels.

[0079] In some examples, the optical detector includes a laser particle detector and an assay result detector.

[0080] In some examples, the optical detector includes an infrared (IR) detector.

[0081] In some examples, the system further includes a plurality of assay lasers downstream of the excitation laser system and upstream of an interrogation point. In some examples, the optical detector includes a laser particle detector, a forward scatter detector, a side-scatter detector, and a plurality of assay result detectors. In some examples, a bandpass filter is positioned upstream of each respective assay result detector. In some examples, a dichroic mirror is positioned upstream of each respective assay result detector. In some examples, a dichroic mirror is positioned between each respective assay laser and the interrogation point. In some examples, an emission collection lens and a plurality of dichroic mirrors are positioned between the interrogation point and the side-scatter detector.

[0082] In some examples, the at least one processor further is configured to execute the instructions to perform operations including sorting by: determining, based on a side scatter of beams of the plurality assay lasers detected by the side-scatter detector, a relationship between the number of optically detectable particles present in a respective composite particle and a magnitude of the side-scatter for any beam of the plurality assay lasers as the respective composite particle moves through the interrogation point; and sorting, based on the relationship, the plurality of composite particles into the plurality of subsets.

[0083] In some examples, the at least one processor further is configured to execute the instructions to perform operations including sorting by: determining a sort bin based on a side scatter value of the beams of the plurality assay lasers detected by the side-scatter detector; and separating, based on the side-scatter value, at least one of the composite particles of the plurality of composite particles of the sort bin from a rest of the plurality of composite particles. In some examples, the at least one processor further is configured to execute the instructions to perform operations including: upon interrogating a respective composite

particle and placing the respective composite particle into the sort bin, then transmitting a signal to one or more electrodes within and/or adjacent a channel of the flow cell based on detected electrophoretic or dielectrophoretic properties so that the respective composite particle is deflected into a separate output. In some examples, the at least one processor further is configured to execute the instructions to perform operations including: upon interrogating a respective composite particle and placing the respective composite particle into the sort bin, transmitting a signal to one or more magnets within and/or adjacent a channel of the flow cell based on detected magnetic properties so that the respective composite particle is deflected into a separate output.

[0084] In some examples, the flow cell includes an interrogation point at which the optical excitation system sequentially excites the composite particles and the optical detector sequentially reads the spectral fingerprints of the composite particles. In some examples, the flow cell includes a fluidic circuit to transport a plurality of composite particle compartments to the interrogation point.

[0085] In some examples, the at least one processor further is configured to execute the instructions to perform operations including: analyzing the spectral fingerprint of the composite particle of the subset at every stage of compound exposure thereby establishing an informatic link between the compound associated with that subset and the composite particle identity of each composite particle of that subset.

[0086] In some examples, the at least one processor further is configured to execute the instructions to perform operations including: comparing each spectral fingerprint to a registry including a plurality of known spectral fingerprints that pertain to composite particles with a desired chemical matter; and separating those composite particles including one or more spectral fingerprints matching a desired one or more compounds from a plurality of composite particles.

[0087] In some examples, the at least one processor further is configured to execute the instructions to perform operations including sorting, based on the spectral fingerprint, by creating the subsets based on a detected link between the spectral fingerprint and a composite particle identity of a specific compound.

[0088] In some examples, the at least one processor further is configured to execute the instructions to perform operations including creating a plurality of subsets based on a predicted link between the spectral fingerprint and a composite particle identity of a specific compound, the predicted link being determined by a machine learning model trained based on training data that includes spectral data of the composite particles, a plurality of compounds,

and output variables representing compound library data sets. In some examples, the machine learning model incorporates at least one of linear regression, kernel ridge regression, logistic regression, neural networks, support vector machine (SVM), decision tree, hidden Markov model, Bayesian network, a Gram-Schmidt process, reinforcement-based learning, cluster-based learning, hierarchical clustering, genetic algorithm, or combination thereof.

[0089] In some examples, the system includes a flow cytometer. In some examples, the flow cytometer is IR enabled.

[0090] In some examples, the system includes a fluorescence-activated cell sorting (FACS) instrument, wherein the plurality of subsets includes a main subset sorted by the FACS instrument sequentially detecting an emission spectrum of the spectral fingerprint of each composite particle. In some examples, the FACS instrument is IR enabled.

[0091] In some examples, the at least one processor further is configured to execute the instructions to perform operations including: identifying, by the FACS instrument, a number of the optically detectable particles in each composite particle; and separating, based on the number of optically detectable particles, those composite particles of the plurality of composite particle including fewer than a predetermined number of optically detectable particles from the main subset.

[0092] In some examples, the at least one processor further is configured to execute the instructions to perform operations including: identifying, by the FACS instrument, a number of optically detectable particles in each composite particle; and separating, based on the number of optically detectable particles, those composite particles of the plurality of composite particle including more than a predetermined number of optically detectable particles from the main subset.

[0093] In some examples, the at least one processor further is configured to execute the instructions to perform operations including: identifying, by the FACS instrument, a number of unique peak maxima present in the emission spectrum.

[0094] In some examples, the at least one processor further is configured to execute the instructions to perform operations including: identifying, by the FACS instrument, a number of optically detectable particles in each composite particle; and dispensing, by a fluidic system and based on the number of optically detectable particles or the spectral fingerprint, each of the plurality of composite particles into individual tubes or wells in a microplate, and wherein each separate vessel or well is associated with specific subset of the plurality of subsets. In some examples, the microplate includes 96 vessels or wells.

[0095] In some examples, the main subset includes approximately 10^5 to 10^9 composite particles present in solution at a concentration of approximately 10^5 to 10^8 composite particles per milliliter.

[0096] In some examples, the at least one processor further is configured to execute the instructions to perform operations including: upon determining if a composite particle of the plurality of composite particles includes a number of optically detectable particles falls within a gate, executing a sort event so that the composite particle within the gate is automatically physically removed from the main subset and into a subset defined by the gate.

[0097] In some examples, the optically detectable particles emit light with a bandwidth of about 1 nm or less at full-width half-maximum (FWHM) responsive to excitation by the beam of the excitation laser system.

[0098] In some examples, the excitation laser system includes an infrared (IR) laser.

[0099] In some examples, the spectral fingerprint includes an optical response of each optically detectable particle in a respective composite particle when summed together.

[0100] In some examples, the at least one processor further is configured to execute the instructions to perform operations including: detecting, by the optical detector, characteristics of the spectral fingerprint of each composite particle; removing, from a main subset of the plurality of subsets, one or more composite particles including an abundance of non-unique spectral characteristics.

[0101] Some examples herein provide a method for categorizing a plurality of composite particles suspended in a fluid. Each composite particle may include a plurality of optically detectable particles and a network mesh polymer substantially surrounding the optically detectable particles. The method may include flowing the composite particles in a fluid through a flow cell. The method may include exciting the composite particles in the flow cell with light from an optical excitation system. The method may include reading, by an optical detector, a spectral fingerprint of each composite particle excited by the light. The method may include generating, using the spectral fingerprint of each composite particle, a composite particle identity of that composite particle.

[0102] In some examples, the step of reading, by the optical detector, the spectral fingerprint of each composite particle excited by the light includes measuring an IR emission orthogonal to an excitation beam of the optical excitation system.

[0103] In some examples, the method further includes physically sorting the plurality of composite particles into a plurality of subsets. In some examples, the method further includes, after cleaving one or more compounds off the plurality of composite particles in

each subset, then detecting, by an ultra-performance liquid chromatography system or a high-performance liquid chromatography system coupled to a mass spectrometry system or tandem mass spectrometry system, a compound identity, a purity and/or a synthetic yield of the plurality of composite particles. In some examples, the step of physically sorting is based on the spectral fingerprints of the composite particles of the plurality. In some examples, the step of physically sorting is based on dielectrophoresis. In some examples, the step of physically sorting is based on electrophoresis.

[0104] In some examples, a microfluidic device of the flow cell is coupled to the optical detector, the flow cell including a channel in microfluidic device, wherein a plurality of subsets includes a main subset sorted by the optical detector sequentially detecting the spectral fingerprint of each composite particle. The method further may include dispensing, by the microfluidic device and based on characteristics of the spectral fingerprint, each of the plurality of composite particles into individual tubes or wells in a microplate.

[0105] In some examples, the method further includes outputting, by the composite particle dispenser, the plurality of composite particles through an optical beam of the excitation laser system and into a plurality of collection vessels based on a counted number of composite particles. In some examples, the method further includes, upon subsets containing those composite particles of the plurality of composite particles with the same chemical incorporation history being output in separate vessels of the plurality of collection vessels. In some examples, the method further includes cleaving one or more compounds from the plurality of composite particles in each collection vessel.

[0106] In some examples, the method further includes determining, based on a side scatter of beams of a plurality of assay lasers detected by a side-scatter detector, a relationship between the number of optically detectable particles present in a respective composite particle and a magnitude of the side-scatter for any beam of the plurality of assay lasers as the respective composite particle moves through an interrogation point, the plurality of assay lasers being downstream of the excitation laser system and upstream of the interrogation point; and sorting, based on the relationship, the plurality of composite particles into the plurality of subsets.

[0107] In some examples, the method further includes determining a sort bin based on a side scatter value of beams of a plurality of assay lasers detected by a side-scatter detector; and separating, based on the side-scatter value, at least one of the composite particles of the plurality of composite particles of the sort bin from a rest of the plurality of composite particles. In some examples, the method further includes, upon interrogating a respective

composite particle and placing the respective composite particle into the sort bin, then transmitting a signal to one or more electrodes within and/or adjacent a channel of the flow cell based on detected electrophoretic or dielectrophoretic properties so that the respective composite particle is deflected into a separate output.

[0108] In some examples, the method further includes, upon interrogating a respective composite particle and separating the respective composite particle into the sort bin, transmitting a signal to one or more magnets within and/or adjacent a channel of the flow cell based on detected magnetic properties so that the respective composite particle is deflected into a separate output.

[0109] In some examples, the method further includes analyzing the spectral fingerprint of the composite particle of the subset at every stage of compound exposure thereby establishing an informatic link between the compound associated with that subset and the composite particle identity of each composite particle of that subset.

[0110] In some examples, the method further includes analyzing the spectral fingerprint of the plurality of composite particles to establish an informatic link between a respective compound and the composite particle identity of respective composite particles of the plurality of composite particles.

[0111] In some examples, the method further includes detecting, by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS), a compound identity, a purity and/or a synthetic yield of the plurality of composite particles.

[0112] In some examples, the method further includes comparing each spectral fingerprint to a registry including a plurality of known spectral fingerprints that pertain to composite particles with a desired chemical matter; and separating those composite particles including one or more spectral fingerprints matching a desired one or more compounds from a plurality of composite particles.

[0113] In some examples, the method further includes sorting, based on the spectral fingerprint, by creating the subsets based on a detected link between the spectral fingerprint and a composite particle identity of a specific compound.

[0114] In some examples, the method further includes creating a plurality of subsets based on a predicted link between the spectral fingerprint and a composite particle identity of a specific compound, the predicted link being determined by a machine learning model trained based on training data that includes spectral data of the composite particles, a plurality of compounds, and output variables representing compound library data sets.

[0115] In some examples, the method further includes separately collecting each subset, after the step of pooling a plurality of subsets where each subset has been exposed to one or more compounds and then releasing the one or more compounds from a respective composite particle.

[0116] In some examples, the method further includes identifying, by a FACS instrument, a number of the optically detectable particles in each composite particle; and separating, based on the number of optically detectable particles, those composite particles of the plurality of composite particle including less than a predetermined number of particles from the main subset.

[0117] In some examples, the method further includes identifying, by a FACS instrument, a number of optically detectable particles in each composite particle; and separating, based on the number of optically detectable particles, those composite particles of the plurality of composite particle including more than a predetermined number of optically detectable particles from the main subset.

[0118] In some examples, the method further includes identifying, by a FACS instrument, a number of optically detectable particles in each composite particle; and dispensing, by a fluidic system and based on the number of optically detectable particles or the spectral fingerprint, each of the plurality of composite particles into individual tubes or wells in a microplate, and wherein each separate vessel or well is associated with specific subset of the plurality of subsets.

[0119] In some examples, the method further includes, upon determining if a composite particle of the plurality of composite particles includes a number of optically detectable particles falls within a gate, executing a sort event so that the composite particle within the gate is automatically physically removed from the main subset and into a subset defined by the gate.

[0120] In some examples, the method further includes detecting, by the optical detector, characteristics of the spectral fingerprint of each composite particle; removing, from a main subset of the plurality of subsets, one or more composite particles including an abundance of non-unique spectral characteristics.

[0121] Some examples herein provide a spectral fingerprint system for categorizing a plurality of composite particles, the system may include an optical excitation system configured to excite the composite particles with light; a flow cell configured to permit flowing of the composite particles suspended in a fluid; and an optical detector configured to read a spectral fingerprint of each composite particle excited by the light. The system also

may include a control system configured to control (a) flow of the composite particles in the fluid through the flow cell, (b) excitation by the optical excitation system of the composite particles in the flow cell with light from the optical excitation system; (c) reading by the optical detector of a spectral fingerprint of each composite particle excited by the light; and (d) generating, based on the spectral fingerprint of each composite particle, a composite particle identity of that composite particle.

[0122] Some examples herein provide a method for generating a compound library for a plurality of composite particles. Each composite particle may include a plurality of optically detectable particles and a network mesh polymer substantially surrounding the optically detectable particles. The method may include reading, by the optical detector, a spectral fingerprint of each composite particle excited by the light; and storing, in a data structure of the compound library, an identifier for each composite particle based on the spectral footprint and an identifier for the one or more different compounds coupled to that composite particle.

[0123] In some examples, the method further includes dispensing the plurality of composite particles into a plurality of subsets based on a number of detected optically detectable particles. In some examples, the method further includes exposing each subset of a plurality of subsets to one or more compounds to associate that subset with one or more different compounds. In some examples, the step of exposing includes: dividing the plurality of composite particles into a plurality of subsets; coupling, for each subset, a chemical moiety to the polymer of the composite particles of that subset to form a modified subset; and pooling the modified subsets to form a modified collection of composite particles.

[0124] In some examples, the method further includes establishing, by a machine learning model, an informatic link between the chemical compound and the identifier for each composite particle, the machine learning model having been trained based on the data structure and training data that includes spectral data of the composite particles, the plurality of compounds that have been coupled, and output variables representing compound library data sets; and storing the informatic link in the data structure of the compound library. In some examples, the machine learning model incorporates at least one of linear regression, kernel ridge regression, logistic regression, neural network, support vector machine (SVM), decision tree, hidden Markov model, Bayesian network, a Gram-Schmidt process, reinforcement-based learning, cluster-based learning, hierarchical clustering, genetic algorithm, or combination thereof.

[0125] Some examples herein provide a method for categorizing a plurality of composite particles suspended in a fluid, each composite particle including a plurality of optically

detectable particles and a network mesh polymer substantially surrounding the optically detectable particles. The method may include causing the composite particles to flow into a flow cell and be irradiated by an optical beam one-by-one in the flow cell resulting in detection of a spectral fingerprint and a composite particle count. The method may include dispensing a plurality of groups of the composite particles into separate vessels based on a composite particle count. The method may include changing vessels when the composite particle count reaches a predetermined threshold and then causing a counting operation to restart. The method may include recording a vessel destination for each composite particle in a spectral fingerprint registry. The method may include incorporating a different chemical entity onto the respective group of the composite particles in each respective vessel. The method may include pooling together the composite particles in each vessel. The method may include repeating steps (a) to (f) after each successive incorporating of the different chemical entity.

[0126] Some examples herein provide a method for categorizing a plurality of composite particles suspended in a fluid. Each composite particle may include a plurality of optically detectable particles and a network mesh polymer substantially surrounding the optically detectable particles. The method may include dispensing a plurality of groups of the composite particles into separate vessels. The method may include incorporating a different chemical entity onto the respective group of the composite particles in each respective vessel. The method may include, for each vessel, causing the composite particles to flow into a flow cytometer and be irradiated one-by-one by an optical beam in a flow cell resulting in detection of a spectral fingerprint. The method may include collecting an output of the composite particles from all runs in a single vessel thereby creating a spectral fingerprint registry comprising spectral fingerprints of each group of the plurality of groups for the pooled composite particles. The method may include repeating steps (a) to (d) after each successive incorporating of the different chemical entity.

BRIEF DESCRIPTION OF DRAWINGS

[0127] FIGS. 1A-1E schematically illustrate example composite particles.

[0128] FIGS. 2A-2C schematically illustrate example operations for generating composite particles.

[0129] FIGS. 3A-3D schematically illustrate example operations for generating composite particles.

[0130] FIGS. 4A-4C schematically illustrate example operations for generating composite particles including carrier particles.

[0131] FIGS. 5A-5E schematically illustrate example operations for generating composite particles including carrier particles.

[0132] FIGS. 6A-6C schematically illustrate example operations for generating composite particles including carrier particles.

[0133] FIGS. 7A-7B schematically illustrate example compositions that may be used in a composite particle or carrier particle.

[0134] FIGS. 8A-8B schematically illustrate example compositions that may be used in a composite particle or carrier particle.

[0135] FIGS. 9A-9C schematically illustrate operations and structures for use in generating subsets of composite particles including different numbers of optically detectable particles than one another.

[0136] FIGS. 10A-10D schematically illustrate operations and structures for use in generating subsets of composite particles including different numbers of optically detectable particles than one another.

[0137] FIG. 11 schematically illustrates an example composite particle to which a chemical compound is coupled.

[0138] FIG. 12 schematically illustrates an example composite particle to which different chemical compounds are coupled.

[0139] FIGS. 13A-13C schematically illustrate example operations for coupling chemical compounds to a composite particle using serial addition of chemical moieties.

[0140] FIGS. 14A-14C schematically illustrate example operations for generating a spectrally encoded chemical library.

[0141] FIGS. 15A-15D schematically illustrate example reaction schemes which may be used to generate a spectrally encoded chemical library.

[0142] FIGS. 16A-16C schematically illustrate example binding assays that may be performed using a composite particle coupled to a chemical compound.

[0143] FIG. 17 schematically illustrates an example binding assay that may be performed using a composite particle coupled to different chemical compounds.

[0144] FIG. 18 schematically illustrates the optical components of an example optical system for identifying composite particles and detecting the respective results of binding assays performed using those particles.

[0145] FIG. 19 is a computer architecture diagram showing a general computing system for implementing aspects of the present disclosure in accordance with one or more embodiments described herein.

[0146] FIG. 20A illustrates an example method for combinatorial one-bead-multiple-compound synthesis.

[0147] FIG. 20B illustrates another example method for combinatorial one-bead-multiple-compound synthesis.

[0148] FIG. 21 illustrates a montage of images of example SpectraGel beads (embedded with Lase Particles (LPs)) produced in Example 1 captured using Attune CytPix.

[0149] FIG. 22 illustrates scanning electron micrographs (SEMs) of example blank beads (left) and example LP-embedded beads (right) in both dark-scanning modes (top) and bright-scanning modes (bottom). Arrows point to LPs.

[0150] FIG. 23 illustrates a histogram of LP incorporation in a 1 million-bead sample.

[0151] FIG. 24 illustrates characterization of biotin and acetylated beads in the presence of fluorophore-labeled streptavidin.

[0152] FIG. 25 illustrates characterization of biotin and acetylated beads in the presence of fluorophore-labeled streptavidin.

[0153] FIG. 26 illustrates fluorescence response with increasing concentrations of labeled streptavidin.

DETAILED DESCRIPTION

[0154] Examples herein provide composite particles for use in generating spectrally encoded libraries of chemical compounds, and methods of making and using the same.

[0155] The composite particles provided herein may be used to generate and optically encode libraries of compounds, without the need for DNA as is used in OBOC-DEL. Described herein, among other things, are the composite particles themselves, methods of making such particles, methods of generating spectrally encoded libraries using such particles, and assay formats that can be used to screen the library and generate quantitative assay results for any suitable library subset.

[0156] In a manner such as described in greater detail below, the present composite particles may encapsulate optically detectable particles. The composite particles may be synthesized from appropriate monomers and the optically detectable particles. Each optically detectable particle produces a measurable optical response intrinsic to that optically detectable particle, and the optically detectable particles may be separable into a series of bins defined by their respective optical responses. The composite particles may be separated into subsets (or groups) using an instrument (system) that records the spectral fingerprint of each composite particle – that is, the sum of the optical emissions from the optically detectable particles within that composite particle – when generating the subsets. Each subset

of composite particles may be coupled to a single compound or a small set of compounds, e.g., between two and four different chemical compounds. The compound(s) may be coupled to respective composite particles in any suitable manner, e.g., via adsorption, covalent attachment, or covalent attachment of a first portion (moiety) of the compound followed by synthesis of the remaining portions of the compound. Each subset of composite particles thus contains a number of composite particles which are coupled to a compound(s). The informatic link (spectral fingerprint) between the composite particle and the identity of the compound (or portion thereof) is recorded and tracked at appropriate time(s) before, after, and/or during coupling of the compound to the composite particles of a given subset. Different subsets of composite particles are coupled to different compounds than one another, and the resulting collection of subsets may be referred to as a "library."

[0157] In a manner such as also described below, the library may be screened in an assay that can detect the activity of the compound(s) coupled to respective composite particles against one or more targets of interest. Such activity may include the compound binding to the target and/or may include the compound changing functional activity of the target. For each composite particle in the library being screened, a signal associated with the assay result is detected and recorded. The spectral fingerprint of each composite particle in the library being screened also is detected and recorded. The signal and the spectral fingerprint are used to informatically link the assay result to the identity of the composite particle -- and thus to the identity of the compound(s) coupled to that composite particle. To validate the identity of the compound(s) and/or the result of the assay, an instrument may be used to sort the composite particles into subsets with specific compounds, using their spectral fingerprints and the earlier-generated informatic links between spectral fingerprints of the composite particles and the compound(s) coupled to those particles. The different subsets may be collected separately, and the compound(s) may be released from the composite particles (e.g., via cleavage of a cleavable linker) and analyzed by standard analytical techniques.

[0158] The compounds in OBOC-DEL libraries are bound to beads using a photo-cleavable linker and are cleaved off into a droplet container with appropriate assay reagents. A screen for assay activity in the droplet containers is performed, and droplets that satisfy a certain assay threshold are sorted and collected. The collected DNA-encoded beads are deconvoluted in bulk. The structures of compounds on hit beads in an OBOC-DEL screen are deconvoluted by sequencing the DNA tags via NGS after the assay is completed and hits have been enriched. It is not commercially practicable to link the compound on any given bead to the corresponding assay result because each sorted bead would need to be

individually collected and then individually sequenced. This task would be incredibly tedious and low-throughput. As recognized by the present inventors, such bulk deconvolution of OBOC-DEL beads misses the opportunity to obtain the structure-activity identity (SAI) of the compounds which are coupled to those beads. Furthermore, OBOC-DEL beads are often simply lost during liquid-handling steps associated with bead collection, bead processing, and PCR amplification for next-generation sequencing (NGS) of the encoding DNA coupled thereto. Still further, a separate set of solution phase reactions may be needed to validate the screening hits observed using OBOC-DEL beads. As recognized by the present inventors, utilizing optically detectable particles instead of DNA to encode the compounds coupled to a composite particle establishes an SAI informatic link because the compounds coupled to that composite particle may be decoded simultaneously with assay detection.

[0159] Indeed, the presently disclosed subject matter describes an ultra-high throughput technology which may be used to generate and/or screen one-bead-one-compound (OBOC) spectrally encoded libraries and/or one-bead-multiple-compound (OBMC) spectrally encoded libraries. In particular, the present subject matter may be used to generate the SAI of the entire chemical space which is screened. Using the present spectral encoding may significantly increase the rate of identifying screening hits relative to OBOC-DEL. Additionally, the present spectral encoding offers greater than 99% data coverage, whereas in OBOC-DEL the vast majority (typically >98%) of screening data cannot be attributed to a specific compound. Accordingly, the presently disclosed subject matter is expected to enable focused and discovery libraries to be screened with vastly enhanced matching precision (>90%) than may be obtained using OBOC-DEL. The present spectral encoding easily allows for more efficient and less operationally intense screens than may be used with OBOC-DEL. Because the present spectral encoding does not rely on DNA for encoding, it is compatible with expanded chemistries which allow greater access to chemical space. Indeed, the present DNA-free approach using spectrally encoding libraries also allows any target that binds to or acts on DNA or RNA (nucleases, helicases, transcription factors, etc.) to be screened.

[0160] First, some terms used herein will be explained. Then, further details regarding the present composite particles, and the preparation and use thereof, will be provided.

Terms

[0161] As used herein, the term “composite particle” is intended to refer to a particle that includes a network polymer gel and at least one optically detectable particle. A composite particle optionally also may include one or more other components, such as one or more

carrier particles and/or one or more chemical compounds. In examples in which the composite particle is coupled to one or more chemical compounds (e.g., where the one or more chemical compounds are coupled to the network polymer gel of that composite particle), the composite particle equivalently may be said to include those chemical compound(s).

[0162] As used herein, the term “network polymer gel” is intended to refer to a polymer where individual strands are connected by a crosslinker moiety (producing a strand junction) using covalent bonds, capable of swelling in water and organic solvents. A network polymer gel also may include a chemical moiety, such as a moiety that may be used to couple a chemical compound to the network polymer gel. For further details of network polymer gels, see Danielsen et al., “Molecular Characterization of Polymer Networks,” Chem Rev. 121(8): 5042-5092 (2021), the entire contents of which are incorporated by reference herein.

[0163] As used herein, the term “optically detectable particle” is intended to refer to a particle that, when irradiated with excitation light, emits light.

[0164] As used herein, the term “microdisk laser particle” refers to an optically detectable particle that includes a disk-shaped core which is made from direct bandgap monocrystalline semiconductor material(s), with diameter on the order of about 1-10 μm . A microdisk laser particle may emit single-mode light having a bandwidth of about 1 nm or less at full-width half-maximum (FWHM), e.g., about 0.4 nm at FWHM, in the infrared portion of the spectrum, e.g., from about 700 nm to about 3300 nm. Optionally, a microdisk laser particle may include a shell that surrounds the core and is formed from a different material than the core. Illustratively, the shell may include silica (SiO_2).

[0165] As used herein, the term “Perovskite quantum dot” is intended to refer to a nanoscale semiconductor crystal made from, or including, a metal trihalide perovskite material such as CsPbBr_3 .

[0166] As used herein, the term “chemical library” is intended to refer to a collection of different chemical compounds. The chemical compounds of a chemical library respectively may be coupled to particles, in which case the chemical compounds may be referred to as “supported.”

[0167] As used herein, the term “binding” is intended to refer to one or more intermolecular interactions that bring two elements into contact with one another. Such intermolecular interaction(s) may include any combination of covalent bonds, ion-dipole interactions, hydrogen bonds, dipole-dipole interactions, ion-induced dipole interactions, and London dispersion forces.

[0168] As used herein, the term “target” is intended to refer to any entity of biological origin that modulates its behavior or function upon binding of an endogenous or exogenous ligand (e.g., drug). Nonlimiting examples of targets include DNA, RNA, proteins, cells, organelles, and viruses.

[0169] As used herein, a “probe” is intended to refer to an element that can produce an optical signal from which binding between a chemical compound and a target can be detected. Nonlimiting examples of probes include fluorophore dyes, fluorescent proteins, chromophore dyes, a catalytic DNA, RNA or enzyme working in concert with a fluorogenic or chromogenic substrate, quantum dots, or other types of inorganic nanoparticles that produce optical signals.

[0170] As used herein an “affinity reagent” is intended to refer to an element that binds selectively either to a target (in which case the affinity reagent may be referred to as a “primary affinity reagent”) or to another affinity reagent (in which case the affinity reagent may be referred to as a “secondary affinity reagent”). Nonlimiting examples of affinity reagents include antibodies, antibody fragments, nanobodies, monobodies, proteins, peptides, peptidomimetics, oligonucleotide aptamers, DARPins, Affimers, Avimers, Knottins, affinity clamps, or antibody mimetics. Affinity reagents may, in some examples, bind to a native epitope of the target or another affinity reagent, or may bind to a non-native epitope that has been added to the target or other affinity reagent.

[0171] By “assay reagent” it is meant an element that produces a measurable signal that is proportional in magnitude to the activity of a target.

[0172] By “activity” of a target it is meant the rate of target function.

Composite particles, and methods of making the same

[0173] Some examples herein provide a composite particle. The composite particle may include an optically detectable particle; and a network polymer gel substantially surrounding the optically detectable particle. The present composite particles may have any of a variety of configurations, some nonlimiting examples of which now will be described with reference to FIGS. 1A-1E. Example methods of making such particles will be described further below with reference to FIGS. 2A-2C, 3A-3D, 4A-4C, 5A-5F, 6A-6C, 7A-7B, and 8A-8B.

[0174] Turning now to FIGS. 1A-1E, example composite particles 10 are schematically illustrated. Although composite particles 10 may be described in the context of generating and using a library of chemical compounds, it should be appreciated that the composite particles are not so limited. Indeed, the present composite particles may be used in a variety of other suitable contexts.

[0175] FIG. 1A illustrates an example composite particle 10 which includes network polymer gel 110 and at least one optically detectable particle 120 which is substantially surrounded by the network polymer gel 110. In some examples, composite particle 10 includes a plurality of the optically detectable particles 120. At least some of the optically detectable particles 120 within composite particle 10 may have different emission wavelengths than one another, and in some examples all of the optically detectable particles have different emission wavelengths than one another. Illustratively, particle 10 may include at least three optically detectable particles 120 with different emission wavelengths from one another, or at least six optically detectable particles 120 with different emission wavelengths from one another. In the nonlimiting example illustrated in FIG. 1A, the optically detectable particles 120 are randomly oriented relative to one another within the network polymer gel 110, e.g., have orientations which are not related to one another in any predetermined manner. Alternatively, in the nonlimiting example illustrated in FIG. 1B, the optically detectable particles 120 have substantially the same orientation as one another within the network polymer gel 110. For example, the optically detectable particles 120 may have planar major surfaces, and the planes of the major surfaces of different ones of the optically detectable particles 120 may be substantially parallel to one another. Example methods for orienting optically detectable particles 120 within composite particle 10 are described in further detail below. Although not illustrated, it will be appreciated that in some examples, composite particle 10 may include a single optically detectable particle 120.

[0176] Network polymer gel 110 may have any suitable composition of monomers, crosslinking agents and/or crosslinking monomers, solvents, and the like. Network polymer gel 110 may include individual polymeric chains that are crosslinked into a three-dimensional molecular framework (appearing as a “net” or “network”) that is relatively consistent in structure throughout the volume of the gel. This framework is essentially a matrix capable of holding solvent molecules, giving the gel an appearance of solidity or rigidity. Individual macromolecular chains within a network polymer gel can comprise any molecular arrangement of one or more monomers, such as homopolymeric, random copolymeric, block copolymeric, and so forth, and may be linear or branched. Network polymer gel 110 may include chemical crosslinking between individual polymer chains such that the gel is relatively insoluble in any solvent, although it remains capable of swelling upon absorption of solvent. Crosslinking may be achieved by polymerization of monomers in the presence of a bis-functionalized monomer such as N,N-methylene-bis-acrylamide or ethyleneglycol dimethacrylate, for example.

[0177] In various embodiments, network polymer gel 110 may be configured to permit diffusion therethrough of an aqueous solvent and/or an organic solvent and/or of one or more chemical compounds which are dissolved in such aqueous or organic solvent, while permanently retaining the optically detectable particle 120 within the composite particle. Illustratively, the composition and cross-linking of network polymer gel 110 is selected such that the diameter of composite particle 10 swells in an aqueous or organic solution (such as during library synthesis) by a factor of about 1.5 to about 3.0 as compared to when the composite particle is dry, while permanently retaining the optically detectable particle therein. The ability of different sizes, and different types, of biological compounds to permeate into the gel matrix may be characterized using fluorescently-labeled biological compounds of different sizes and then visually tracking their permeation into the matrix. The degree of crosslinking affects the maximum size of molecules that can fit inside the matrix. Optionally, the optically detectable particle(s) 120 may be coupled to the network polymer gel 110 via covalent bond(s). Nonlimiting methods of forming such bonds are described further below with reference to FIGS. 3A-3D.

[0178] In some examples, network polymer gel 110 may include, or may consist essentially of, acrylamide, poly-ethylene glycol (PEG), PEG-acrylate, PEG-acrylamide PEG-amine, PEG-carboxylate, PEG-dithiol, PEG-epoxide, PEG-isocyanate, PEG-maleimide, polyacrylic acid (PAA), poly(methyl methacrylate) (PMMA), polystyrene (PS), polystyrene sulfonate (PSS), polyvinylpyrrolidone (PVPON), N,N'-bis(acryloyl)cystamine, polypropylene oxide (PPO), poly(hydroxyethyl methacrylate) (PHEMA), poly(N-isopropylacrylamide) (PNIPAAm), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), poly(vinylsulfonic acid) (PVSA), poly(L-aspartic acid), poly(L-glutamic acid), bisacrylamide, diacrylate, diallylamine, triallylamine, divinyl sulfone, diethylene glycol diallyl ether, ethylene glycol diacrylate, PEG diacrylate, PEG diacrylamide, trimethylolpropane trimethacrylate, ethoxylated trimethylol triacrylate, ethoxylated pentaerythritol tetraacrylate, hyaluronans, chitosans, agarose, heparin sulfate, cellulose, or combination thereof.

[0179] For nonlimiting examples of network polymer gels that may be used in the present composite particles, see the following references, the entire contents of which are incorporated by reference herein: Fryer et al. "Gigavalent display of proteins on monodisperse polyacrylamide hydrogels as a versatile modular platform for functional assays and protein engineering," ACS Central Science 8(8): 1182-1195 (2022); Zhu et al., "Hydrogel droplet microfluidics for high-throughput single molecule/cell analysis," Accounts

of chemical research 50(1): 22-31 (2017); Feng et al., "MRBLES 2.0: High-throughput generation of chemically functionalized spectrally and magnetically encoded hydrogel beads using a simple single-layer microfluidic device," *Microsystems & Nanoengineering* 6(1): 109 (2020); Wilson et al., "Encoded microcarriers for high-throughput multiplexed detection," *Angewandte Chemie International Edition* 45(37): 6104-6117 (2006); Liu et al. "Microfluidic synthesis of QD-encoded PEGDA microspheres for suspension assay," *Journal of Materials Chemistry B* 4(3): 482-488 (2016); Chen et al. "Microfluidic generation of multicolor quantum-dot-encoded core-shell microparticles with precise coding and enhanced stability," *Langmuir* 30(28): 8538-8542 (2014); Gerver et al. "Programmable microfluidic synthesis of spectrally encoded microspheres," *Lab on a Chip* 12(22): 4716-4723 (2012); Nguyen et al. "Programmable microfluidic synthesis of over one thousand uniquely identifiable spectral codes," *Advanced optical materials* 5(3): 1600548(2017); and Zhao et al. "Microfluidic generation of multifunctional quantum dot barcode particles," *Journal of the American Chemical Society* 133(23): 8790-8793 (2011).

[0180] FIG. 1C illustrates another example composite particle 10 including a carrier particle 130 having an outer surface 131. The optically detectable particle 120 is coupled to the outer surface 131 of the carrier particle 130, and the network polymer gel 110 substantially surrounds the carrier particle 130 and the optically detectable particle 120 coupled to the carrier particle. In the nonlimiting example illustrated in FIG. 1C, composite particle 10 includes a plurality of the optically detectable particles 120 coupled to the outer surface 131 of the carrier particle 130. Carrier particle 130 may have any suitable composition, e.g., may include, or may consist essentially of, a polymer or a glass. Carrier particle 130 may have a different composition than the network polymer gel 110, or may have the same composition as the network polymer gel. Nonlimiting examples of compositions of network polymer gel 110 are provided above, and elsewhere herein. Other nonlimiting examples of polymers that may be used in carrier particle 130 include polymers formed by reacting a monomer and a crosslinker, for example PEG-thiol/PEG-acrylate, acrylamide/N,N'-bis(acryloyl)cystamine (BACy), or PEG/PPO. In some examples, the network polymer gel 110 includes a four-arm PEG. In some embodiments, the four-arm PEG is selected from the group consisting of PEG-acrylate, PEG-amine, PEG-carboxylate, PEG-dithiol, PEG-epoxide, PEG-isocyanate, and PEG-maleimide. Additionally, or alternatively, carrier particles 130 may include a solid-support matrix, such as glass, with appropriate chemically-resistant surface coating, such as a silane with appropriately inert chemical groups.

[0181] FIG. 1D illustrates another example in which particle 10 includes at least one additional carrier particle 130 having an outer surface 131; and an additional optically detectable particle 120 coupled to the outer surface of the at least one additional carrier particle. Network polymer gel 110 substantially surrounds the at least one additional carrier particle 120 and the additional optically detectable particle coupled to the at least one additional carrier particle. Illustratively, composite particle 10 may include about two to about ten carrier particles (each of which may be coupled to one or more optically detectable particles), e.g., about two to about six carrier particles, or about six to about ten carrier particles, or about two to about three carrier particles. Optionally, the optically detectable particle(s) 120 may be coupled to the corresponding carrier particle 130 via covalent bond(s), or may be coupled to the corresponding carrier particle 130 via non-covalent bond(s). Nonlimiting methods of forming such bonds are described further below with reference to FIGS. 5A-5F and 6A-6C.

[0182] Composite particle 10, some nonlimiting examples of which are illustrated in FIGS. 1A-1D may have any suitable dimensions. The dimensions of composite particle 10 may vary depending on the particular solvent in which the particle is suspended. Further, pore size of a composite particle can be altered by pH, temperature, exposure to solvents, and/or ionic strength of a solution containing the composite particle. For example, some solvents may cause the composite particle 10 to swell, increasing the porosity of the polymer network gel 110, while other solvents may cause the composite particle to shrink, decreasing the porosity of the polymer network gel 110). In some examples, composite particle 10 has a diameter D_1 of between about 1 μm and about 50 μm in water at pH 7, illustratively a diameter D_1 of between about 10 μm and about 30 μm in water at pH 7, for example a diameter D_1 of between about 5 μm and about 20 μm in water at pH 7, or a diameter D_1 of between about 15 μm and about 25 μm in water at pH 7. However, other diameters may readily be envisioned. In general, when swelled in solution (whether aqueous or organic), the composite particle 10 preferably is sized to accommodate a plurality of optically detectable particles, and to be readily transportable through the fluidic channels of the instrumentation in which the particles are to be used, e.g., for library synthesis or screening.

[0183] Indeed, other composite particle shapes also may be envisioned. For example, although FIGS. 1A-1D may suggest that composite particle 10 is substantially spherical, particle 10 may have any other suitable shape. For example, FIG. 1E illustrates an example particle 10 which is non-spherical, and may have an irregular shape which generally follows that of the collection of carrier particles 130 therein. In such examples, composite particle 10

may have a largest lateral dimension S_1 which may be in the same range as diameter D_1 described above, but because the composite particle is not spherical it may not necessarily be considered to have a diameter. In examples including one or more carrier particles, such as illustrated in FIGS. 1C-1E, the network polymer gel 110 may have a thickness T_1 of about 100 nm to about 1 μm , e.g., about 200 nm to about 500 nm. In examples such as described with reference to FIGS. 1C-1E, the carrier particle(s) may have any suitable diameter D_3 , e.g., a diameter of between about 1 μm and about 20 μm in water at pH 7, illustratively a diameter of between about 5 μm and about 15 μm in water at pH 7, or a diameter of between about 1 μm and about 5 μm in water at pH 7. Additionally, or alternatively, the composite particle 10 may be at least about 100 nm larger than the carrier particle(s) 130 in water at pH 7 (that is, T_1 illustrated in FIGS. 1C-1E may be at least about 100 nm, e.g., about 100 nm to about 1 μm , e.g., about 200 nm to about 500 nm).

[0184] Illustratively, carrier particle 130 may have a diameter D_3 of about 3 μm to about 10 μm . In examples in which the optically detectable particles are microdisk laser particles having a diameter of $\sim 2 \mu\text{m}$, and assuming that only one side of an optical detectable particle 120 binds to the carrier particle 130 at any given time, Table 1 below lists the approximate maximum number of microdisk laser particles that can bind to carrier particles of different diameter:

Table 1

Carrier Particle Diameter (D_3) [μm]	Max. # of microdisk laser particles
3.00E-06	9
4.00E-06	16
1.00E-05	100

[0185] The present composite particle 10, some nonlimiting examples of which are described with reference to FIGS. 1A-1E, may include any suitable type of optically detectable particle(s) 120. In some examples, optically detectable particle 120 may be configured to emit light with a bandwidth of about 1 nm or less at full-width half-maximum (FWHM) responsive to excitation light. Illustratively, optically detectable particle 120 may include, or may consist essentially of, a microdisk laser particle. In some examples, the

microdisk laser particle may have a diameter D_2 of about 1 μm to about 10 μm , e.g., about 1 μm to about 5 μm , or about 1 μm to about 2.5 μm . Additionally, or alternatively, the microdisk laser particle may have a thickness T_2 of about 100-1000 nm. The light emitted by the microdisk laser particle may be near-infrared, e.g., about 1100 nm to about 1600 nm. In one specific example, laser light with a wavelength between 1060-1070 nm may be used to excite microdisk laser particles including InAlGaAs and/or InAlGaP. More generally, microdisk laser particles may emit light in response to being “pumped” above their lasing threshold, which is between 5 and 40 pJ. The excitation wavelength may be from 700-3300 nm, depending on the material. In some examples, the microdisk laser particles include a silica glass shell to inhibit damage from chemical reagents. In a manner such as described in greater detail with reference to FIGS. 3A-3D and 5A-5F, the silica glass shell optionally may be used to install functional handles via which the microdisk laser particles may be covalently coupled to the network polymer gel 110 or to a carrier particle 130.

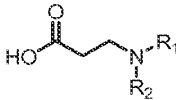
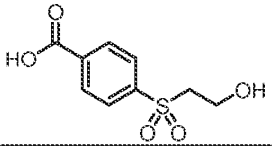
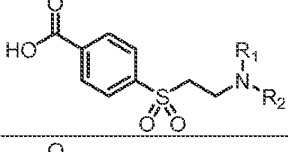
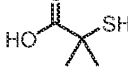
[0186] For further details regarding microdisk laser particles (which also may be referred to as “Lase particles” or “LPs”), see the following references, the entire contents of which are incorporated by reference herein: Dannenberg et al., “Multilayer fabrication of a rainbow of microdisk laser particles across a 500 nm bandwidth,” ACS Photonics 8: 1301-1306 (2021); Kwok et al., “Laser particle barcoding for multi-pass high-dimensional flow cytometry,” doi.org/10.1101/2022.06.03.494697, 25 pages (2022); Martino et al., “Micron-sized laser particles for massively multiplexed cellular labeling and tracking,” 2018 Conference on Lasers and Electro-Optics (CLEO), 2 pages (2018); Martino et al., “Wavelength-encoded laser particles for massively multiplexed cell tagging,” Nature Photonics 13: 720-727 (2019); and Tang et al., “Laser particles with omnidirectional emission for cell tracking,” Light: Science & Applications 10: 23 11 pages (2021).

[0187] In other examples, optically detectable particle 120 may be configured to emit light with a bandwidth of about 40 nm or less at FWHM responsive to excitation light. Illustratively, optically detectable particle may include a CsPbBr₃ Perovskite quantum dot. In some examples, CsPbBr₃ Perovskite quantum dots may be excited using light between about 300-450 nm. For further details regarding CsPbBr₃ Perovskite quantum dots, see Liu et al., “Highly soluble CsPbBr₃ Perovskite quantum dots for solution-processed light-emission devices,” Applied Nano Materials 4(2): 1162-1174 (2021), the entire contents of which are incorporated by reference herein. However, it will be appreciated that other types of quantum dots suitably may be used.

[0188] Optionally, network polymer gel 110 may include a chemical moiety 140 which is couplable to a chemical compound. For example, the chemical moiety 140 may be distributed throughout the network polymer gel. As such, network polymer gel 110 may include a plurality of such chemical moieties 140 which are coupled to the polymer backbone of the gel. In some examples, the functional moiety (moiety 140) is or includes an amine, thiol, hydroxyl, epoxide, halogen, aldehyde, or carboxylic acid (140-X). Optionally, a branching moiety can be coupled to chemical moiety 140 to increase the number of chemical compounds coupled to the network polymer gel by 2 – 5-fold. Optionally, chemical moiety 140 may be cleavable from the network polymer gel using light, heat, or a reagent. Optionally, a cleavable linker that can release the attached chemical compound using light, heat, or a reagent may be coupled to chemical moiety 140. Illustratively, examples of release conditions include application of electromagnetic radiation (e.g., UV or optical light of a suitable wavelength), a solution or vapor of trifluoroacetic acid, a solution or vapor of trimethylamine, dimethylamine or ammonia, a solution containing a reducing agent which cleaves a disulfide-containing linker or a solution of an oxidant that cleaves a double bond in a linker. Selected examples are listed in Table 2 below:

Table 2

140-X	Linker	Y	Cleaved by	Z-Released compound
Br		O	Acid	HO-Compound
NH ₂			Acid	HO-Compound
NH ₂			UV light	H ₂ N-Compound
NH ₂			UV light	HO-Compound

OH		O	MeI, then Base	NCH3-Compound
NH2			Base	HOOC-Compound
NH2			MeI, then Base	NCH3-Compound
NH2			Dithiothreitol or tris(2-carboxylethyl phosphine)	HS-Compound

[0189] For nonlimiting examples of chemical moieties that are cleavable using light, heat, or a reagent, see the following references, the entire contents of which are incorporated by reference herein: Bräse and Dahmen, "Linkers for Solid-Phase Synthesis," Handbook of Combinatorial Chemistry: Drugs, Catalysts, Materials, K. C. Nicolaou (Ed.), ISBN: 978-3-527-60303-9 Chapter 4, Pages 59-169 (2002), Wiley; Su, "Developing a cellular DNA-encoded library screening platform," Thesis, University of California, Irvine (2021); MacConnell et al., "DNA-encoded solid-phase synthesis: Encoding language design and complex oligomer library synthesis," ACS Combinatorial Science 17: 518-534 (2018); and Gibaut et al., "Study of an RNA-focused DNA-encoded library informs design of a degrader of a r(CUG) repeat expansion," J. Am. Chem. Soc. 144: 21972-21979 (2022).

[0190] Example methods of incorporating cleavable chemical moieties 140 within network polymer gel 110 are described below with reference to FIGS. 2A-2C. Additionally, or alternatively, chemical moiety 140 may be positively charged, or may be negatively charged, e.g., in a manner such as described further below with reference to FIGS. 7A-7B and 8A-8B, or may be uncharged. In some examples, moiety 140 may be present in network polymer gel 110 at a loading of about 75-300 fmol.

[0191] Although not specifically illustrated in FIGS. 1A-1E, composite particle 10 further may include chemical compounds coupled to the network polymer gel 110, e.g., via moieties 140, in a manner such as described elsewhere herein. As such, in some examples, the chemical compounds are cleavable from the network polymer gel 110 using light, heat, or a reagent and/or may be distributed throughout the network polymer gel 110. The chemical

compounds coupled to a given composite particle may be the same as one another, or may be different than one another. Nonlimiting examples of chemical compounds that may be coupled to composite particle 10 are described elsewhere herein.

[0192] Nonlimiting examples of the manner in which the present composite particles may be made now will be described with reference to FIGS. 2A-2C, 3A-3D, 4A-4C, 5A-5F, 6A-6C, 7A-7B, and 8A-8B.

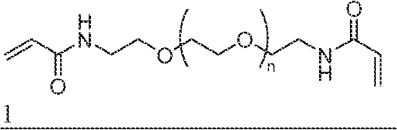
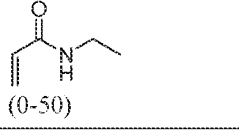
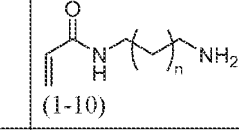
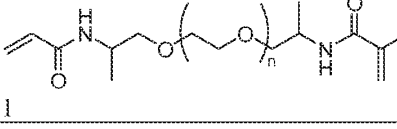
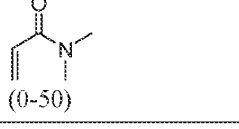
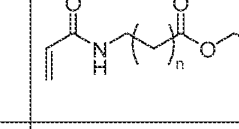
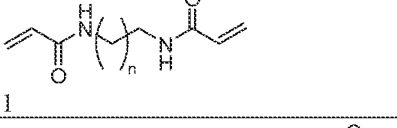
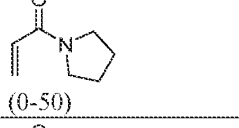
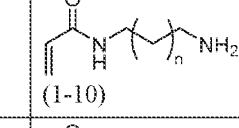
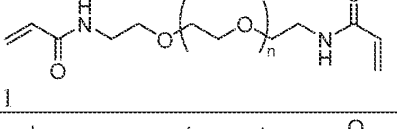
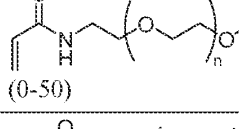
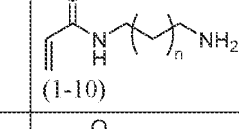
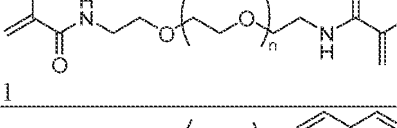
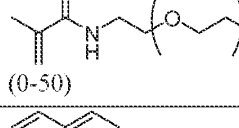
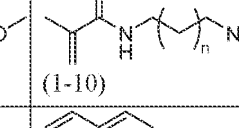
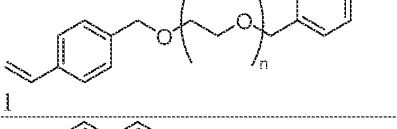
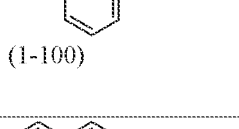
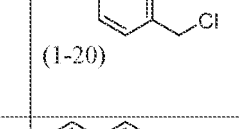
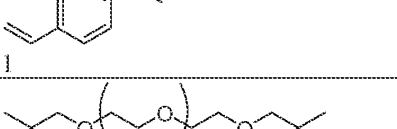
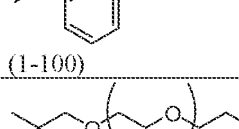
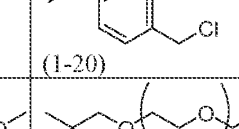
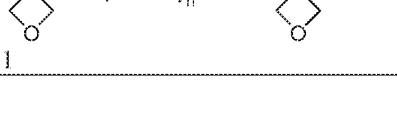
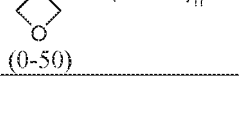
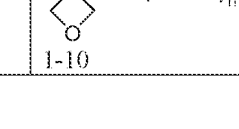
[0193] In some examples, a method of making a composite particle may include substantially surrounding an optically detectable particle with a network polymer gel. Optionally, the method may include surrounding at least one additional optically detectable particle with the network polymer gel. Substantially surrounding the optically detectable particle(s) with the network polymer gel may, in some examples, include forming a droplet that includes a crosslinker, an initiator, and the optically detectable particle(s); and using the initiator to polymerize the crosslinker to form the network polymer gel within the droplet. Optionally, the droplet further comprises a non-functional monomer, or a functional monomer, and wherein the initiator polymerizes the non-functional monomer or functional monomer together with the crosslinker to form the network polymer gel 110 within the droplet. In some examples, the network polymer gel is synthesized using a mixture of at least two monomers, the first of which produces polymer chains and the second of which crosslinks the polymer chains together to form the network polymer gel. The monomer(s) are mixed together with optically detectable particle(s) 120 and the initiator, and polymerization is started by activating the initiator, for example by heat or light. The respective percentages of monomer(s) (if any) and crosslinker in the monomer mixture are selected so that the optically detectable particle(s) are permanently retained within the resulting composite particle, that is, are retained within the composite particle during any subset generation operations, any library synthesis operations and/or any library screening operations to which the composite particle is subjected.

[0194] An optional third monomer may be included in the monomer mixture, which contains a functional moiety (moiety 140 described with reference to FIGS. 1A-1E) that may be used to subsequently attach chemical compounds to the network polymer gel 110. The percentage of the third monomer in the mixture determines the covalent loading of the composite particle. In some examples, the functional moiety (moiety 140) of the third monomer is or includes an amine, thiol, hydroxyl, epoxide, halogen, aldehyde, or carboxylic acid. Because the third monomer is distributed throughout the mixture, the functional moieties become distributed throughout the resulting network polymer gel 110. In some

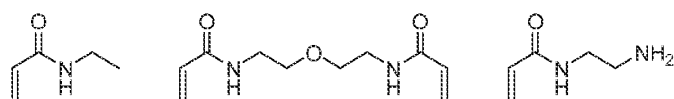
examples, the functional moiety loading density in the resulting network polymer gel is about 0.05-1.5 mmol/g.

[0195] Table 3 lists non-limiting examples of non-functional monomers, cross-linkers, and functional monomers that may be used to prepare network polymer gel, and their example ratios within the monomer mixture (n is independently between 0 and 50).

Table 3

Crosslinker	Nonfunctional monomer (ratio)	Functional monomer (ratio)
 1	 (0-50)	 (1-10)
 1	 (0-50)	 (1-10)
 1	 (0-50)	 (1-10)
 1	 (0-50)	 (1-10)
 1	 (0-50)	 (1-10)
 1	 (1-100)	 (1-20)
 1	 (1-100)	 (1-20)
 1	 (0-50)	 1-10

[0196] Other non-limiting examples of non-functional monomers, cross-linkers, and functional monomers and their use in generating polymers may be found in Haag et al., "Solid Phase and Soluble Polymers for Combinatorial Synthesis," Handbook of Combinatorial Chemistry: Drugs, Catalysts, Materials, K. C. Nicolaou (Ed.), ISBN: 978-3-527-60303-9 Chapter 3, pages 24-58 (2002), the entire contents of which are incorporated by reference herein. In one purely illustrative example from Table 3, row 1, the non-functional monomers, cross-linkers, and functional monomers used are those illustrated below:



Non-functional

Crosslinker, $n = 0$

Functional, $n = 0$

[0197] A commercially available photoinitiator, such as Irgacure, Duracure, LAP, VA50, VA44, or the like, may be used to initiate copolymerization of the mixture. The concentration of the photoinitiator in the mixture, and the exposure time, may be selected so as to appropriately cross-link the monomers to provide a desired porosity of the polymer network gel while permanently retaining the optically detectable particle(s) therein.

[0198] In some examples, the network polymer gel 110 resulting from reactions such as described above may include about 10-50% of the reaction products of the combined monomer mixture (the balance of the gel being the solvent used for the reaction), with a degree of polymerization (D_p) ranging from about 20 to about 40. These ranges are just an example of a workable porosity and stiffness/ brittleness of the polymer matrix. In various examples, the porosity of the polymer network gel may be selected to limit enzymes from diffusing into the gel, while providing sufficient space for small molecules to be cleaved and diffuse out through the pores. Nonlimiting examples of network polymer gels are provided elsewhere herein.

[0199] In some examples, the optically detectable particle may be suspended in a solution that includes the monomer(s) and the initiator. A droplet of the solution then may be formed, for example by encapsulating a defined volume of the solution in an oil/surfactant mixture using a microfluidic junction. In such examples, the size of the composite particle which is formed is determined by the size of the droplet, the volume of which may be controlled microfluidically, thus providing relatively consistent sizes of composite particles. Illustratively, FIGS. 2A-2C schematically illustrate example operations for generating composite particles. The approach illustrated in FIG. 2A may be referred to as droplet-templated synthesis. In this approach, an appropriate ratio of non-functional monomer,

crosslinker, functional monomer, and photo-initiator are dissolved in solution, and optically detectable particles 120 are added to the monomer mix to obtain solution 210 including a concentration of optically detectable particles selected to include, on average, an appropriate number of the optically detectable particles within each composite particle that is formed. Preferably, solution 210 is agitated before it is introduced to channel 211, so as to help keep the optically detectable particles 120 in solution. In some examples, the present composite particles are relatively hydrophilic, and are formed using monomers that are most soluble in aqueous solutions. However, an inverse system could be used, where the monomers were dissolved in a hydrophobic solvent and droplets were dispersed in a continuous aqueous phase, to obtain hydrophobic composite particles.

[0200] As illustrated in FIG. 2A, microfluidic device 200 includes microfluidic channel 211, microfluidic channel 221, and microfluidic junction 231 at which microfluidic channels 211 and 221 meet, e.g., a “T” junction in which channel 221 extends from either side of the junction. Microfluidic device 200 transports solution 210 through microfluidic channel 211 to microfluidic junction 231. Additionally, microfluidic device 200 transports an oil/surfactant mixture 220 through microfluidic channel 221 to microfluidic junction 231 such that the oil/surfactant mixture 220 pinches off droplets 241 of solution 210 that become encapsulated by the oil/surfactant mixture. The relative flow rates through microfluidic channels 211 and 221 are selected to appropriately control the diameter of droplets 241. Microfluidic device 200 then irradiates droplets 241 with light to initiate the polymerization process, generating composite particles 10. Composite particles 10 are expected to have < 10% variance in their diameter. Additionally, composite particles 10 may include different numbers of optically detectable particles 120 than one another.

[0201] Although FIG. 2A may suggest that the optically detectable particles have certain orientation(s) within the resulting composite particles 10, the method of forming the composite particles suitably may be adapted to orient the optically detectable particles in any desired manner relative to one another. In the nonlimiting example shown in FIG. 2B, the optically detectable particles 120 are randomly oriented relative to one another within the network polymer gel 110, for example by allowing the optically detectable particles to independently rotate and translate relative to one another within droplets 241. In the nonlimiting example shown in FIG. 2C, the optically detectable particles have substantially the same orientation as one another within the network polymer gel, for example by including, within solution 210, stacks 121 of optically detectable particles that are pre-coupled to one another in a manner such as described in Dannenberg et al., “Multilayer

fabrication of a rainbow of microdisk laser particles across a 500 nm bandwidth,” ACS Photonics 8: 1301-1306 (2021), the entire contents of which are incorporated by reference herein.

[0202] In other examples, forming the droplet may include forming an emulsion with the solution and an oil/surfactant mixture. For example, in a manner similar to that described further below with reference to FIG. 4C, solution 210 may be vortexed or mixed together with oil/surfactant mixture 220 to form an emulsion that contains droplets 241. The droplets then may be photopolymerized in the manner described with reference to FIG. 2A to obtain composite particles 10. Similarly as described with reference to FIG. 2A, the optically detectable particles therein may be randomly oriented relative to one another. Alternatively, similarly as described with reference to FIG. 2B, the optically detectable particles may have substantially the same orientation as one another, e.g., by using pre-formed stacks 121.

[0203] As noted further above, in some examples the optically detectable particle(s) 120 may be covalently coupled to the network polymer gel. Such coupling may be performed at the time of forming the network polymer gel, e.g., in a manner which will now be described with reference to FIGS. 3A-3D, which illustrate example operations for generating composite particles. Briefly, the optically detectable particle 120 may be coupled to a chemical moiety, and the chemical moiety may be coupled to the network polymer gel. Turning now to FIG. 3A, optically detectable particle 120 may include core 121 and shell 122, which may have different compositions than one another. In one nonlimiting example, optically detectable particle 120 is or includes a laser microdisk particle including semiconductor core 121 and silica glass shell 122. In another nonlimiting example, optically detectable particle 120 is or includes a CsPbBr₃ Perovskite quantum dot including a CsPbBr₃ core 121 and a silica glass shell 122. Silica glass shell 122 may be functionalized using standard sol-gel chemistry. As illustrated in FIG. 3A, optically detectable particle 120 may be contacted with bifunctional molecules 320 which include first functional moiety 321 and second functional moiety 322. Such contact may be performed, for example, in solution 30. In some examples, solution 30 is organic, is aqueous, or is a mixture of water and an organic solvent that is miscible in water, such as ethanol. Acid or base may be added to catalyze the condensation of silane on the silica surface.

[0204] First functional moiety 321 may be selected to react with one or more monomers within solution 210, e.g., may be or include an acrylate, methacrylate, epoxide, isocyanate, styryl, or vinyl group, corresponding to the functional moieties of the monomers that will be used to form network polymer gel 110. Second functional moiety 322 may be selected to

react with one or more functional moieties 123 on shell 122, so as to covalently couple first functional moiety 321 to optically detectable particle 120. In the nonlimiting example shown in FIG. 3A, functional moieties 123 include hydroxyl groups (-OH), and functional moieties 322 include silane groups such as alkoxy silanes or chlorosilanes, which are generically denoted as -SiX₃, where the X moieties coupled to a given silicon atom may be different than one another, or may be the same as one another. In some examples, X is selected from the group consisting of methyl, ethyl, methoxy, ethoxy, acetoxy, and chloro.

[0205] As illustrated in FIG. 3B, second functional moiety 322 may react with functional moiety(s) 123 on shell 122, for example through a hydrolysis and condensation reaction that forms silane bonds covalently coupling first functional moiety 321 to shell 122. In this example, note that each silane may couple to any suitable number of hydroxyl groups 123, e.g., to one, two, or three hydroxyl groups, with the remaining sites of the silane remaining coupled to an appropriate number of X moieties. As illustrated in FIG. 3C, a plurality of the functionalized optically detectable particles 120-1, 120-2, 120-3 (the representation of which is further simplified relative to that shown in FIG. 3B, for ease of illustration) are disposed within droplet 241 which may be formed in a manner such as described elsewhere herein. Droplet 241 further may include solvent 31, non-functional monomers 32, functional monomers 33 (which include functional moieties 140), and crosslinkers 34 such as described elsewhere herein.

[0206] As intended to be illustrated in FIG. 3C by the triangles representing the functional moieties respectively coupled to the optically detectable particles, the non-functional monomers 32, functional monomers 33, and crosslinkers 34, such functional moieties may react with one another to form the network polymer gel, e.g., are the same type of functional moiety as one another. Accordingly, the functional moieties 321 coupled to the optically detectable particles 120-1, 120-2, 120-3 may participate in the polymerization of non-functional monomers 32, functional monomers 33, and crosslinkers 34. As such, the chemical moieties 321 are covalently coupled to the network polymer gel 110, e.g., are a component of the network polymer gel. Accordingly, as illustrated in FIG. 3D, the polymer chains 300 formed when polymerizing functional moieties 321, non-functional monomers 32, functional monomers 33, and crosslinkers 34 are covalently coupled to the optically detectable particles. Additionally, as intended to be represented in FIG. 3D, functional moieties 140 are distributed throughout network polymer gel 110, and network polymer gel 110 includes open spaces 310 through which a solvent (e.g., aqueous or organic) may be transported; it will be appreciated that these features also may be present in other examples

described herein in which the optically detectable particles are not covalently coupled to the network polymer gel.

[0207] Still other methods may be used to prepare the present composite particles. For example, optically detectable particles 120 may be coupled to carrier particles such as described with reference to FIGS. 1C-1E, using various binding methods. These may include covalent bond formation, such as utilizing click chemistry, and/or non-covalent interactions such as electrostatic forces, van der Waals forces, or pi-pi interactions. In this approach, optically detectable particles may be modified on one surface and may be mixed in bulk with carrier particles that have the appropriate partner modification for binding.

[0208] For example, FIGS. 4A-4C schematically illustrate example operations for generating composite particles. As illustrated in FIG. 4A, generating a composite particle 10 may include coupling optically detectable particle(s) 120-1, 120-2, 120-3, 120-4, 120-5 to an outer surface 131 of a carrier particle 130 such as described with reference to FIGS. 1C-1E to produce a carrier particle-optically detectable particle conjugate 400. In some examples, the ratio of optically detectable particles 120 to carrier particles in conjugate 400 is at least 4:1; or at least 5:1, or at least 6:1, or at least 7:1, or at least 8:1, or at least 9:1, or at least 10:1. The carrier particle 130, with the optically detectable particle(s) coupled thereto, then are substantially surrounded with the network polymer gel 110.

[0209] One example of this approach employs droplet microfluidics-based encapsulation of carrier particle-optically detectable particle conjugates 400 along with an appropriate monomer mixture 410 in a manner such as illustrated in FIG. 4B. The monomer mixture 410 includes a monomer mixture similar to that described with reference to FIG. 2A, but in which carrier particle-optically detectable particle conjugates 400 are suspended instead of individual optically detectable particles. Microfluidic device 200 illustrated in FIG. 4B may be configured similarly as described with reference to FIG. 2A, e.g., includes microfluidic channel 211, microfluidic channel 221, and microfluidic junction 231 at which microfluidic channels 211 and 221 meet, e.g., a "T" junction in which channel 221 extends from either side of the junction. Microfluidic device 200 transports solution 410 through microfluidic channel 211 to microfluidic junction 231. Additionally, microfluidic device 200 transports an oil/surfactant mixture 220 through microfluidic channel 221 to microfluidic junction 231 such that the oil/surfactant mixture 220 pinches off droplets 441 of solution 410 that become encapsulated by the oil/surfactant mixture. The relative flow rates through microfluidic channels 211 and 221 are selected to appropriately control the diameter of droplets 441. In some examples, conjugates 400 are at a concentration within mixture 410 such that droplets

441, on average, contain at least 1-2 conjugates 400. Microfluidic device 200 then irradiates droplets 441 with light to initiate the polymerization process, generating composite particles 10. Composite particles 10 are expected to be relatively monodisperse, e.g., in a manner such as described with reference to FIG. 2A. Additionally, composite particles 10 may include different numbers of conjugates 400 than one another.

[0210] In other examples, forming the droplet may include forming an emulsion with the solution and an oil/surfactant mixture. For example, as illustrated in FIG. 4C, solution 410 (including the monomer mixture and conjugates 400) may be vortexed together with an oil/surfactant mixture to form an emulsion 420 that contains droplets 441. For further details of using vortexing to obtain an emulsion including particles, see the following references, the entire contents of each of which are incorporated by reference herein: Clark et al., “Microfluidics-free single-cell genomics with templated emulsification,” *Nature Biotechnology*, doi.org/10.1038/s41587-023-01685-z, 32 pages (2023); and Hatori et al., “Particle-templated emulsification for microfluidics-free digital biology,” *Analytical Chemistry* 90: 9813-9820 (2018). The droplets then may be photopolymerized in the manner described with reference to FIG. 4A to obtain composite particles 10. The emulsion may be incubated for sufficient time for complete polymerization of the matrix within individual droplets. Once, polymerized, the bulk emulsion is demulsified and further washed to extract the oil and resuspend the polymerized beads in aqueous solution.

[0211] Note that in examples such as described with reference to FIGS. 4A-4C, the apparent density of conjugates 400 is expected to be lower than that of the optically detectable particles 120 alone (e.g., about 4.5 g/cm³ for microdisk laser particles and CsPbBr₃ Perovskite quantum dots), e.g., because the carrier particle 130 has a density that is less than that of the optically detectable particles alone. For example, the density of the carrier particles 130 may be about 1.00 to about 1.20 g/cm³. Because of the lower apparent density of conjugates 400, it is expected to be easier to keep conjugates 400 in suspension than it is for the optically detectable particles 120 alone.

[0212] As noted further above, in some examples the optically detectable particle(s) 120 may be coupled to carrier particle 130 via a covalent bond, e.g., to form conjugates 400. Briefly, carrier particle 130 may be coupled to a first chemical moiety, and the optically detectable particle(s) 120 may be coupled to a second chemical moiety, and the first chemical moiety may be coupled to the second chemical moiety to form a covalent bond, and thus form conjugate 400. The conjugates 400 then may be dispersed within the solution which is used to form the network polymer gel, e.g., in a manner which will now be described with

reference to FIGS. 5A-5F, which illustrate example operations for generating composite particles.

[0213] Turning now to FIG. 5A, optically detectable particle 120 may include core 121 and shell 122, which may have different compositions than one another. Nonlimiting examples of core 121 and shell 122 are provided with reference to FIG. 3A. As illustrated in FIG. 5A, optically detectable particle 120 may be contacted with bifunctional molecules 520 which include first functional moiety 521 and second functional moiety 522. In some examples, such contact may be performed by contacting optically detectable particle 120 with a gas 50 containing bifunctional molecules 520, while the optically detectable particle is disposed on a substrate 510. Gas 50 may contact exposed portion(s) 511 of optically detectable particle 120 (e.g., portion(s) which do not contact substrate 510), and substantially may not contact unexposed portion(s) 512 of optically detectable particle 120 (e.g., portion(s) which contact substrate 510).

[0214] Bifunctional molecules 520 may be similarly as bifunctional molecules 320 described with reference to FIG. 3A. For example, first functional moiety 521 may be selected to react with a corresponding functional moiety coupled to a carrier particle 130 in a subsequent step described with reference to FIGS. 5D-5E. Second functional moiety 522 may be selected to react with one or more functional moieties 123 on shell 122, so as to covalently couple first functional moiety 521 to optically detectable particle 120. In the nonlimiting example shown in FIG. 5A, functional moieties 123 include hydroxyl groups (-OH), and functional moieties 522 include silane groups such as alkoxysilanes or chlorosilanes, which are generically denoted as -SiX₃, where the X moieties coupled to a given silicon atom may be different than one another, or may be the same as one another. In some examples, X is selected from the group consisting of methyl, ethyl, methoxy, ethoxy, acetoxy, and chloro.

[0215] As illustrated in FIG. 5B, second functional moiety 522 may react with functional moiety(s) 123 on shell 122, for example through a hydrolysis and condensation reaction that forms silane bonds covalently coupling first functional moiety 521 to shell 122. In this example, note that each silane may couple to any suitable number of hydroxyl groups 123, e.g., to one, two, or three hydroxyl groups, with the remaining sites of the silane remaining coupled to an appropriate number of X moieties. Following such reaction, the optically detectable particle 120 may be removed from substrate 510 and suspended in liquid 51, as illustrated in FIG. 5C. As illustrated in FIG. 5D, a plurality of the functionalized optically detectable particles 120-1, 120-2, 120-3 (the representation of which is further simplified

relative to that shown in FIG. 5C, for ease of illustration) the optically detectable particles and carrier particles 130 are mixed with one another in solution 53. The outer surfaces 131 of carrier particles 130 may include functional moieties 523 that react with first functional moieties 521 of optically detectable particles 120. Although not specifically illustrated, the method optionally may include coupling the first chemical moiety to the carrier particle 130. Alternatively, carrier particles that are pre-functionalized may be commercially purchased, such as from Bangs Laboratories, Inc. (Fishers, Indiana).

[0216] Functional moieties 523 and 521 may be co-selected so as to react with one another in a manner such as illustrated in FIG. 5E to form conjugate 400. For example, functional moieties 523 and 521 respectively may be or include partner pairs such as listed in Table 4 below. Optionally, the partners can be conjugated to each other using a reagent or set of reagents. Optionally, following reaction between moieties 523 and 521, any unreacted moieties may be capped or otherwise rendered chemically inert so that they do not react during library synthesis, such as described elsewhere herein.

Table 4

Moiety 521	Moiety 523
Azide	Alkyne
Alkyne	Azide
Epoxy	Amine
Amine	Epoxy
Maleimide	Thiol
Thiol	Maleimide
Amine	Active Ester (e.g. NHS ester)
Active Ester (e.g. NHS ester)	Amine
Alkyl halide	Thiol
Thiol	Alkyl halide
Thiol	Thiol
Carboxylic acid	Amine
Amine	Carboxylic acid
Tetrazine	Trans-cyclooctene
Trans-cyclooctene	Tetrazine
Amine	Aldehyde
Aldehyde	Amine

[0217] Conjugate(s) 400 then are dispersed within droplet 441 which may be formed in a manner such as described elsewhere herein. Droplet 441 further may include solvent 31, non-functional monomers 32, functional monomers 33 (which include functional moieties 140), and crosslinkers 34 such as described elsewhere herein, e.g., with reference to FIG. 3C. In the nonlimiting example illustrated in FIG. 5F, the polymer chains 300 formed when

polymerizing functional moieties 321, non-functional monomers 32, functional monomers 33, and crosslinkers 34 are not covalently coupled to the optically detectable particles as they are in FIG. 3D, because the functional moieties of optically detectable particles 120 were reacted with carrier particle 130, and the remaining surface(s) of optically detectable particles 120 do not contain functional moieties that could react within droplet 441, e.g., to participate in polymerization as was the case in FIG. 3D. Additionally, as intended to be represented in FIG. 5F, functional moieties 140 are distributed throughout network polymer gel 110, and network polymer gel 110 includes open spaces 310 through which a solvent (e.g., aqueous or organic) may be transported; it will be appreciated that these features also may be present in other examples described herein in which the optically detectable particles are covalently coupled to the network polymer gel and/or are not covalently coupled to the carrier particle 130.

[0218] Although FIGS. 5A-5F illustrate an example in which the optically detectable particle 120 is coupled to carrier particle 130 via covalent bond(s), in other examples the optically detectable particle may be coupled to the carrier particle via a non-covalent bond. For example, moieties 521 and 523 may be a non-covalent partner pair, such as biotin and streptavidin (or vice versa). Alternatively, the non-covalent bond may include an ionic bond. For example, the optically detectable particle 120 may be negatively charged, and the carrier particle 130 may be positively charged. Alternatively, the optically detectable particle 120 may be positively charged, and the carrier particle 130 may be negatively charged. Electrostatic forces between optically detectable particle(s) 120 and the carrier particle 130 may attract the optically detectable particle(s) 120 into contact with the carrier particle 130 and retain the optically detectable particle in contact with the carrier particle during polymerization of the network polymer gel.

[0219] For example, FIGS. 6A-6C schematically illustrate example operations for generating composite particles. In the nonlimiting example illustrated in FIG. 6A, carrier particle 130 may be positively charged, and optically detectable particles 120-1, 120-2, 120-3, and 120-4 may be negatively charged, although it will be appreciated that the charges on these elements equivalently may be reversed. Nonlimiting examples of positively charged materials that may be used in carrier particle 130 are described with reference to FIGS. 7A-7B, and nonlimiting examples of negatively charged materials that may be used in carrier particle 130 are described with reference to FIGS. 8A-8B. In some examples, optically detectable particles 120-1, 120-2, 120-3, and 120-4 may be negatively charged using. For example, because of the presence of hydroxyl groups, the silica coating is negatively-charged

already. Alternatively, negative charge can be obtained on the optically detectable particle by performing a two-step silanization procedure, where the first silanization is vapor phase and deposits the functional moiety 521 (the moiety that can polymerize) on one side of the optically detectable particle. The second silanization is solution phase and deposits a second silane with a negatively charged functional moiety primarily on the side of the optically detectable particle without functional moiety 521. In other examples, optically detectable particles 120-1, 120-2, 120-3, and 120-4 may be positively charged. For example, a positive charge can be obtained on the optically detectable particles by using the same two-step silanization procedure described above but the second silane has a functional moiety that is positively-charged.

[0220] Positively charged carrier particle 130 and negatively charged optically detectable particles 120-1, 120-2, 120-3, and 120-4 may be mixed with one another in solution 60 (which may be aqueous, organic, or a mixture of aqueous and organic) as illustrated in FIG. 6A. As illustrated in FIG. 6B, electrostatic attraction between carrier particle 130 and optically detectable particles 120-1, 120-2, 120-3, and 120-4 brings the optically detectable particles into contact with the carrier particle to form conjugate 400. Conjugate(s) 400 then are disposed within droplet 441 which may include components, and may be formed in a manner, such as described elsewhere herein. The components may be polymerized to form network polymer gel 110 in a manner such as described elsewhere herein, and as illustrated in FIG. 6C.

[0221] Optionally, as illustrated in FIGS. 6A-6B, the optically detectable particles 120-1, 120-2, 120-3, 120-4 may include first functional groups 521 such as described with reference to FIGS. 5A-5F, which are available to participate in polymerization of the network polymer gel in a manner similar to that of first functional groups 321 described with reference to FIGS. 3C-3D. As such, the chemical moieties 521 are covalently coupled to the network polymer gel 110, e.g., are a component of the network polymer gel. Accordingly, as illustrated in FIG. 6C, the polymer chains 600 formed when polymerizing functional moieties 521, non-functional monomers 32, functional monomers 33, and crosslinkers 34 are covalently coupled to the optically detectable particles. Alternatively, first functional groups 521 may be omitted, and the polymer chains 600 formed when polymerizing functional moieties 321, non-functional monomers 32, functional monomers 33, and crosslinkers 34 are not covalently coupled to the optically detectable particles.

[0222] FIGS. 7A-7B schematically illustrate example compositions that may be used in a composite particle. For example, FIG. 7A illustrates particle 700 which may correspond

either to carrier particle 130 or to composite particle 10. As illustrated in FIG. 7A, outer surface 701 of particle 700 includes positively charged moieties 740. Optionally, moieties 740 may be distributed throughout all or a portion of particle 700 in a manner similar to that described with reference to moieties 140. In examples in which particle 700 corresponds to composite particle 10, moieties 740 may correspond to moieties 140, and optionally may be used to couple chemical compounds to composite particle. Alternatively, in examples in which particle 700 corresponds to carrier particle 130, moieties 740 may be used to electrostatically attract negatively charged optically detectable particles there to in a manner such as described with reference to FIGS. 6A-6B. FIG. 7B illustrates nonlimiting examples of positively charged moieties that may be included on an outer surface of a particle 700 (an example of positively charged functionality depicted graphically in the inset to FIG. 7A). As illustrated in FIG. 7B, the network polymer gel may be functionalized via covalent modification or coupled chemical linker (e.g., Rink amide) containing positively charged tertiary or quaternary amines, such as those commonly used in PEG anion-exchange resins (where the PEG anion-exchange resin is one nonlimiting example of a network polymer gel). In some instances, a network polymer gel may be synthesized using at least one type of monomer having a reactive terminus, such as $-NH_2$ or $-CO_2H$ group, which can then be used in subsequent reactions to add on positively charged moieties (such as through amide formation as shown). Network polymer gels can be made using other functional monomers besides amino-terminated PEG so that many other types of click chemistry can be used between the functionalized terminus of a polymer chain in the network polymer gel and the desired positively charged molecule. For example, click chemistry to attach charged molecules to network polymer gels can include formation of ester linkage from $-CO_2H$ and alcohol terminus $-OR$, amide formation from $-CO_2H$ and $-NH_2$ terminus, cycloaddition reactions, and nucleophilic ring opening such as reaction between nucleophile and epoxide.

[0223] FIGS. 8A-8B schematically illustrate example compositions that may be used in a composite particle. For example, FIG. 8A illustrates particle 800 which may correspond either to carrier particle 130 or to composite particle 10. As illustrated in FIG. 8A, outer surface 801 of particle 800 includes positively charged moieties 840. Optionally, moieties 840 may be distributed throughout all or a portion of particle 800 in a manner similar to that described with reference to moieties 140. In examples in which particle 800 corresponds to composite particle 10, moieties 840 may correspond to moieties 140, and optionally may be used to couple chemical compounds to composite particle. Alternatively, in examples in which particle 800 corresponds to carrier particle 130, moieties 840 may be used to

electrostatically attract negatively charged optically detectable particles there to in a manner such as described with reference to FIGS. 6A-6B. FIG. 8B illustrates nonlimiting examples of negatively charged moieties that may be included on an outer surface of a particle 800 (an example of negatively charged functionality depicted graphically in the inset to FIG. 8A).

[0224] As illustrated in FIG. 8B, the network polymer gel 110 may be functionalized via covalent modification or coupled chemical linker containing negatively charged sulfonate or carboxylate moieties, such as those commonly used in PEG cation-exchange resins (where the PEG anion-exchange resin is one nonlimiting example of a network polymer gel). In some instances, a network polymer gel may be synthesized using at least one type of monomer having a reactive terminus, such as -OH, -NH₂ or -CO₂H group, which can then be used in subsequent reactions to add on negatively charged moieties (such as through ether formation as shown). Network polymer gels can be made using other functional monomers besides ether-terminated PEG so that many other types of click chemistry can be used between the functionalized terminus of a polymer chain in the network polymer gel and the desired negatively charged molecule. For example, click chemistry to attach charged molecules to network polymer gels can include formation of ester linkage from -CO₂H and alcohol terminus -OR, amide formation from -CO₂H and -NH₂ terminus, cycloaddition reactions, and nucleophilic ring opening such as reaction between nucleophile and epoxide.

Collections of composite particles, and methods and systems for generating the same

[0225] Although FIGS. 1A-1E may focus on example configurations of individual particles, it will be appreciated that a collection of such particles may find particular utility in generating and/or screening a library of chemical compounds in a manner such as described in greater detail below. The composite particles 10 of such a collection may have any combination of characteristics such as described with reference to FIGS. 1A-1E, and/or as described elsewhere herein. In some examples, a composition may include the collection of the composite particles suspended in an aqueous solvent, or in an organic solvent.

[0226] In some examples, each of the composite particles 10 of the collection may include a plurality of the optically detectable carrier particles 120. At least some of the optically detectable particles 120 of that plurality may have different emission wavelengths than one another, and the different emission wavelengths define a spectral fingerprint for that composite particle. For example, the optically detectable particles 120 within a first composite particle 10 respectively may emit light at wavelengths λ_1 , λ_2 , λ_3 , and λ_4 , and the optically detectable particles 120 within a second composite particle 10 respectively may

emit light at wavelengths λ_5 , λ_6 , λ_7 , and λ_8 , where the different subscripts denote a unique wavelength. As such, based on a given composite particle emitting light at wavelengths λ_1 , λ_2 , λ_3 , and λ_4 , that composite particle may be identified as being the first composite particle, those wavelengths constituting the spectral fingerprint of that composite particle because the composite particle can be uniquely identified using those wavelengths. Similarly, based on a given composite particle emitting light at wavelengths λ_5 , λ_6 , λ_7 , and λ_8 , that composite particle may be identified as being the second composite particle, those wavelengths constituting the spectral fingerprint of that composite particle because the composite particle can be uniquely identified using those wavelengths. Within a collection of composite particles 10, a majority of the composite particles of the collection may have different spectral fingerprints than one another. Illustratively, over 80%, or over 85%, or over 90%, or over 95%, or even 100% of the composite particles of the collection may have different spectral fingerprints than one another. For example, microdisk laser particles may have about 800 distinguishable emission peaks in the spectral range of 1100 nm to 1600 nm. As such, a combination of two random microdisk laser particles per composite particle can provide about 320,000 unique spectral fingerprints; a combination of three random microdisk laser particles per composite particle can provide about 85 million unique spectral fingerprints; a combination of four random microdisk laser particles per composite particle can provide about 16.9 billion unique spectral fingerprints; a combination of five random microdisk laser particles per composite particle can provide about 2.69 trillion unique spectral fingerprints; a combination of six random microdisk laser particles per composite particle can provide about 357.3 trillion unique spectral fingerprints; a combination of seven random microdisk laser particles per composite particle can provide about 40.5 quadrillion unique spectral fingerprints; or a combination of seven random microdisk laser particles per composite particle can provide about 4.0 quintillion unique spectral fingerprints.

[0227] It will be appreciated that the number of optically detectable particles may vary from composite particle to composite particle within a collection, and as such the number of spectral peaks may vary from composite particle to composite particle within the collection. In some examples, the composite particles 10 of the collection respectively may include substantially the same number of optically detectable particles 120 as one another, e.g., between two and ten of the optically detectable particles 120. In other examples, the composite particles 10 of the collection respectively may include different numbers of optically detectable particles 120 than one another e.g., between two and ten of the optically detectable particles 120. Regardless of the specific number of spectral peaks within a given

composite particle's spectral fingerprint, the wavelengths of those peaks may be used to identify that composite particle, preferably uniquely. In examples in which the composite particles 10 of the collection include carrier particle(s) in a manner such as described with reference to FIGS. 1C-1E, a majority of the composite particles of the collection respectively may include the same number of carrier particles as one another, e.g., respectively may include one to three of the carrier particles.

[0228] FIGS. 9A-9C schematically illustrate operations and structures for use in generating subsets of composite particles including different numbers of optically detectable particles than one another. Specifically, FIG. 9A shows a schematic overview of operations and components used during particle subset creation for library synthesis. The depicted example includes a system 905 for flow cytometry that is infrared (IR) enabled to detect optical particle emission of optically detectable particles 902 and separate them into a plurality of collection vessels 910. During operations, particles 902 can be caused to flow into a flow cell of system 905 so that particles 902 can be excited with light from an optical excitation system. In some examples, particles 902 can be irradiated one-by-one by light of the optical excitation system resulting in detection of a spectral fingerprint. In some examples, an optical detector of system 905 can read a spectral fingerprint of each particle 902 excited by the light and generate, using the spectral fingerprint, a composite particle identity. In some examples, the spectral fingerprint can be read by measuring an IR emission orthogonal to an excitation beam of the optical excitation system. In turn, a spectral fingerprint of particles 902 is recorded and linked (e.g., by an analyzer 940) to the collection vessel 910 it resides in.

[0229] FIG. 9B shows a more detailed illustration of a system 905 for flow cytometry with particles 902 flowing therein, including optically detectable particles 902a and sheath fluid 902b that focuses the suspension of particles 902a so the beads go through the excitation light one at a time. The output from all runs passes through nozzle 992 and into a plurality of collection vessels 910. It is understood that the system 905 of FIG. 9B shows a minimum number of excitation lasers and the minimum number of detectors for assay readout (e.g., 1 each). However, the number of lasers and detectors in of the contemplated system 905 can vary as needed or required (e.g., up to 10 lasers and/or detectors per assay readout). In some examples, system 905 can include only one infrared laser and only one infrared detector. As shown in the depicted example of FIG. 9B, system 905 can include an optical excitation system 909 that includes at least one assay excitation laser 912 and at least one optical particle excitation laser 915. Each of lasers 912, 915 can emit light in the form of a laser

beam. The wavelengths of the laser beams emitted therefrom can range between 325 nm to 3300 nm.

[0230] Light 912x, 915x from lasers 912, 915, respectively, can be focused to pass through dichroic mirror 921 that propagates the light to interrogate at least one particle 902 in a flow cell 9C-9C further shown in FIG. 9C. The flow cell is part of a fluidics system which directs particles, typically one at a time, in a stream to the focused laser beams of lasers 912, 915 for interrogation. In some examples, the flow cell can be a benchtop cytometer or a nozzle tip in a stream-in-air cytometer. The light from the lasers 912, 915 interacts with particles 902 at various different wavelengths depending on the spectral characteristics. One of skill in the art will recognize that a flow cytometer in accordance with an embodiment of the present disclosure is not limited to the system 905 depicted in FIGs. 9A to 9C, but can include any flow cytometer known in the art. For example, a flow cytometer may have any number of lasers as well as detectors, mirrors, beam splitters, filters, and the like at various wavelengths and in various different configurations.

[0231] Turning to FIG. 9C, one exemplary flow cell 930 is shown with the particles 902a flowing into suspension fluid 902b via a particle suspension input 932. Flow cell 930 can include a sheath flow 932 configured to direct particles 902a (e.g., via a particle dispenser) to interrogation point 950 through which light 912x, 915x is propagated. Turning back to FIG. 9B, a second dichroic mirror 923 can be downstream of the interrogation point to receive and further propagate interrogation light 912x, 915x. In some examples, mirror 923 can propagate light 915x to an optical particle detector 936 and can propagate light 912x to an assay result detector 934. Each of detectors 934, 936 can transmit respective detector output to analyzer 940. Analyzer 940 can include one or more computing systems and be configured to read a spectral fingerprint of each particle 902 excited by light from system 909. Output 940x of analyzer 940 can be communicated to a plurality of collection vessels 910. Output 940x can include an optically detectable particle count, the spectral fingerprint, user-defined composite particle population size, and/or other information. In some examples, the spectral fingerprint of each particle 902 is determined by analyzer 940 by measuring an IR emission orthogonal to an excitation beam of excitation system 909. In some examples, analyzer 940 can generate, using the spectral fingerprint of each particle 902, a composite particle identity of a respective particle 902.

[0232] In some examples, the system 905 of FIG. 9C can be configured to change vessels 910 when composite particle count of composite particles 902 reaches a predetermined threshold and then causing a counting operation to restart. The system 905 can then record a

vessel destination (e.g., a specific vessel 910 of vessels 910) for each composite particle 902 in a spectral fingerprint registry. Though not shown, it is contemplated that a different chemical entity can be incorporated onto each respective group of the particles 902 in each respective vessel 910, then particles 902 can be pooled together, and then each of the foregoing steps can be repeated to successively incorporate different chemical entities.

[0233] During operations, system 905 can be controlled by a controller (e.g., a controller of analyzer 940), and the measurement data from detectors 934, 936 can be stored in memory and processed by analyzer 940. Although not shown explicitly, analyzer 940 can also be in communication with components of the flow cell 930 of FIG. 9C so as to control the lasers 912, 915, fluid flow parameters, and the like. In some examples, some or all of the controller and memory may be part of one or more external computing devices such as a general purpose computer, a mobile computing device, and the like. In some embodiments, some or all of the computing operations can be in wireless or wired communication with system 905.

[0234] FIGS. 10A-10D schematically illustrate operations and structures for use in generating subsets of composite particles including different numbers of optically detectable particles than one another. Specifically, FIG. 10A shows a schematic overview of operations and components used during particle subset creation based on the number of optically detected particles per particle 1002. In the example illustrated in FIG. 10A, particles 1002 with a low number of optically detected particles 1002a'' are separated from particles with a larger number of optically detected particles 1002a'. In the example illustrated in FIG. 10D, composite particles 1002a'' that are coupled to particular library members are separated from composite particles 1002a' that are coupled to other library members.

[0235] The example depicted in FIG. 10B includes a system 1005 for fluorescence activated cell sorting (FACS) that is infrared (IR) enabled to detect optical particle emission of optically detectable particles 1002 and separate composite particles into a plurality of collection vessels 1010 based on the number of optically detected particles in each particle 1002. During operations, particles 1002 can be caused to flow in a flow cell of system 1005 so that particles 1002 can be excited with light from an optical excitation system and system 1005 can sort particles 1002 into a main subset sorted by the system 1005 sequentially detecting an emission spectrum of the spectral fingerprint of each particle 1002. In some examples, those particles 1002 that include fewer or more than a predetermined number of optically detectable particles are separated from the main subset. In some examples, the main subset can include approximately 10^5 to 10^9 composite particles present in solution at a concentration of approximately 10^5 to 10^8 composite particles per milliliter.

[0236] In some examples, the system 1005 can be configured to identify a number of unique peak maxima present in the emission spectrum. In some examples, the system can analyze the emission spectrum to identify unique peak maxima present by “peak picking” or “line fitting”. For example, the optically detectable particles within a first composite particle respectively may emit light at a plurality of wavelengths and the optically detectable particles within a second composite particle respectively may emit light at a second plurality of wavelengths, where each wavelength can be a unique wavelength. In this example, the identifier for the first composite particle may include a first numerical list of the wavelengths (e.g., 1151 nm, 1182 nm, 1208 nm, and 1330 nm) having peaks in that composite particle’s emission spectrum, and the identifier for the second composite particle may include a second numerical list of the wavelengths (e.g., 1250 nm, 1442 nm, 1512 nm, and 1549 nm) having peaks in that composite particle’s emission spectrum. Thus, a composite particle which is later determined to have the emission peaks 1151 nm, 1182 nm, 1208 nm, and 1330 nm may be identified as being the first composite particle by determining that the wavelengths in the later measured spectrum match the wavelengths in the earlier measured spectrum of that composite particle, and a composite particle which is later determined to have the emission peaks 1250 nm, 1442 nm, 1512 nm, and 1549 nm may be identified as being the second composite particle by determining that the wavelengths in the later measured spectrum match the wavelengths in the earlier measured spectrum of that composite particle. In operations, a presence of the peak (e.g., yes / no) is relevant to the identifier.

[0237] In some examples, the system 1005 can be configured to cause the flow cell 1030 to dispense, based on the number of optically detectable particles as well as unique peak maxima present thereof, particles 1002 into individual tubes or wells 1010. Each separate vessel 1010 can be associated with specific subset of the plurality of subsets. In some examples, the vessels 1010 can be part of a microplate that includes at least 96 vessels. In some examples, particles 1002 can be irradiated one-by-one by light of the optical excitation system resulting in detection of an emission spectrum of each particle and counting of a number of unique peak maxima present in the emission spectrum. Based on the detected emission spectrum and analysis of the unique maxima present, system 1005 can determine if a composite particle of the plurality of composite particles includes a number of optically detectable particles within one of a plurality of sort bins. The system 1005 can separate a respective particle 1002 from the population of particles if the number of optically detectable particles is within one of a plurality of sort bins. In turn, a spectral fingerprint and particle count of particles 1002 is recorded and linked (e.g., by an analyzer 1040) to the collection

vessel 1010 it resides in. In some examples, system 1005 can be configured to create subsets of particles 1002 based on the measured side-scatter.

[0238] FIG. 10B shows a more detailed illustration of system 1005 with composite particles 1002 flowing therein, including optically detectable particles 1002a and sheath fluid 1002b that focuses the suspension of particles 1002a so the beads go through the excitation light one at a time, collecting the composite particle output in deflector plate inlet 1090c (see FIG. 10C) until being dispensed by a plurality of deflection plates 1090a, 1090b to a plurality of collection vessels 1010 based one or more criteria, including number of optically detectable particles as well as unique peak maxima present thereof.

[0239] Similar to the system of FIG. 9B, it is understood that the system 1005 of FIG. 10B shows a minimum number of excitation lasers and the minimum number of detectors for assay readout (e.g., 1 each). However, the number of lasers and detectors in of the contemplated system 1005 can vary as needed or required (e.g., up to 10 lasers and/or detectors per assay readout). In some examples, system 1005 can include only one infrared laser and only one infrared detector. As shown in the depicted example of FIG. 10B, system 1005 can include an optical excitation system 1009 that includes at least one assay excitation laser 1012 and at least one optical particle excitation laser 1015. Each of lasers 1012, 1015 can emit light in the form of a laser beam. The wavelengths of the laser beams emitted therefrom can range between 325 nm to 3300 nm.

[0240] Light 1012x, 1015x from lasers 1012, 1015, respectively, can be focused to pass through dichroic mirror 1021 that propagates the light to interrogate at least one particle 1002 in a flow cell 10C-10C further shown in FIG. 10C. The flow cell is part of a fluidics system that focuses the particles and directs them, typically one at a time, in a stream to the focused beams of lasers 1012, 1015 for interrogation. The light from the lasers 1012, 1015 interacts with particles 1002 at various different wavelengths depending on the spectral characteristics. One of skill in the art will recognize that the system 1005 depicted in FIGs. 10A to 10C is not so limiting and can include any flow cell and FACS system known in the art with any number of lasers as well as detectors, mirrors, beam splitters, filters, and the like at various wavelengths and in various different configurations.

[0241] Turning to FIG. 10C, one exemplary flow cell 1030 is shown with particles 1002a and suspension fluid 1002b introduced via a particle suspension input 1032. Flow cell 1030 can include a sheath flow 1032 configured to direct particles 1002a (e.g., via a particle dispenser) to interrogation point 1050 through which light 1012x, 1015x is propagated. Particles 1002 then flow through deflector plate inlet 1090c into deflection plates (e.g., a first

positively charged deflection plate 1090a and a second negatively charged deflection plate 1090b, etc.) where a sort signal is received (e.g., based on output 1040x from analyzer 1040) causing plates 1090a, 1090b to dispense sorted particles 1002a', 1002a'', etc. into appropriate respective vessels 1010. In some examples, plates 1090a and 1090b can include one or more electrodes adjacent to inlet 1090c to selectively deflect individual particles based on their electrophoretic or dielectrophoretic properties. In some examples, plates 1090a and 1090b can include one or more magnets adjacent to inlet 1090c to deflect particles 1002a', 1002a'' in the flow path based on their magnetic properties.

[0242] Turning back to FIG. 10B, a second dichroic mirror 1023 can be downstream of the interrogation point to receive and further propagate interrogation light 1012x, 1015x. In some examples, mirror 1023 can propagate light 1015x to an optical particle detector 1036 and can propagate light 1012x to an assay result detector 1034. Each of detectors 1034, 1036 can transmit respective detector output to analyzer 1040. Analyzer 1040 can include one or more computing systems and be configured to detect an emission spectrum based on output from detectors 1034, 1036 and count a number of unique peak maxima present in therein. Output 1040x of analyzer 1040 can be communicated to a plurality of collection vessels 1010. Output 1040x can include an optically detectable particle count, the spectral fingerprint, user-defined composite particle population size, and/or other information. In some examples, the spectral fingerprint of each particle 1002 is determined by analyzer 1040 by measuring an IR emission orthogonal to an excitation beam of excitation system 10010. In some examples, analyzer 1040 can generate, using the spectral fingerprint of each particle 1002, a composite particle identity of a respective particle 1002.

[0243] During operations, system 1005 can be controlled by a controller (e.g., a controller of analyzer 1040), and the measurement data from detectors 1034, 1036 can be stored in memory and processed by analyzer 1040. Although not shown explicitly, analyzer 1040 can also be in communication with components of the flow cell 1030 of FIG. 10C so as to control the lasers 1012, 1015, fluid flow parameters, and the like. In some examples, some or all of the controller and memory may be part of one or more external computing devices such as a general purpose computer, a mobile computing device, and the like. In some embodiments, some or all of the computing operations can be in wireless or wired communication with system 1005.

[0244] In some examples, based on output 1040x, vessels 1010 can receive particles 1002 from flow cell 1030 and physically sort them into a plurality of subsets. The subsets can

include a main subset sorted by detector 1036 sequentially detecting the emission spectrum, unique peak maxima, particle count, the spectral fingerprint, side scatter, and the like.

[0245] In some examples, output 1040x can include identifying one or more of the particles 1002 that include the same chemical incorporation history. In this respect, for subsets containing those particles 1002 with the same chemical incorporation history, the particles 1002 can be output in separate vessels 1010 and then particles can be caused to trigger compound release. For example, a separate optical beam or a reagent can release one or more compounds from the particles 1002 of each collection vessel 1010. The one or more compounds present on the particles can be then suitably identified, e.g., using liquid chromatography-mass spectrometry by sampling the corresponding collection vessel. In one operation, after cleaving one or more compounds off the particles 1002 in each collection vessel 1010, the compound identity, a purity and/or a synthetic yield of the plurality of composite particles can be determined by an ultra-performance liquid chromatography system or a high-performance liquid chromatography system coupled to a mass spectrometry system or tandem mass spectrometry system.

[0246] In some examples, output 1040x can be determined by analyzing the spectral fingerprint at every stage of compound exposure so that analyzer 1040 can establish an informatic link between the compound associated with that subset, the emission spectrum, unique peak maxima, particle count, the spectral fingerprint, side scatter, and the like of each composite particle of that subset. In some examples, output 1040x can include creating a plurality of subsets based on a predicted link between the emission spectrum, unique peak maxima, particle count, the spectral fingerprint, side scatter, and the like and a composite particle identity of a specific compound. The predicted link can be determined by a machine learning model trained based on training data that includes spectral data of the composite particles, a plurality of compounds, and output variables representing compound library data sets. The machine learning model can include statistical analysis, autonomous or machine learning, and AI. AI may include, but is not limited to, deep learning, neural networks, classifications, clustering, and regression algorithms. By using such computing methods, link prediction and analytics related to spectral fingerprint, composite particle identity, compound type, and the like substantially improved as is reliability and accuracy.

[0247] In some examples, the machine learning model of analyzer 1040 can include a trained machine learning algorithm that takes, as input, any of the herein disclosed data as well as historical spectral and chemical incorporation databases and determines whether one or more salient spectral characteristics or trends are occurring and exceed a predetermined

threshold according to spectral assessment logic. In some examples, the machine learning model of analyzer 1040 can incorporate at least one of linear regression, kernel ridge regression, logistic regression, neural network, support vector machine (SVM), decision tree, hidden Markov model, Bayesian network, a Gram-Schmidt process, reinforcement-based learning, cluster-based learning, hierarchical clustering, genetic algorithm, or combination thereof. Many methods may be used to learn which examples of the foregoing data feeds are salient to the extent one or more predict links are merited based on probabilistic success indicators, including but not limited to: (1) weak supervision: training a machine learning system (e.g., multi-layer perceptron (MLP), convolutional neural network (CNN), graph neural network, support vector machine (SVM), random forest, etc.) using multiple instance learning (MIL) using weak labeling of the digital image or a collection of images; the label may correspond to the presence or absence of a salient areas; (2) bounding box or polygon-based supervision: training a machine learning system (e.g., region-based CNN (R-CNN), Faster R-CNN, Selective Search) using bounding boxes or polygons that specify the sub-regions of the digital image that are salient for the detection of the presence or absence of one or more markers related to a potential link (3) pixel-level labeling (e.g., a semantic or instance segmentation): training a machine learning system (e.g., Mask R-CNN, U-Net, Fully Convolutional Neural Network); and/or (4) using a corresponding, but different digital image that identifies one or more spectral characteristics, compound type(s), particle count, and/or other information.

Chemical libraries, and methods of generating the same using composite particles

[0248] As provided herein, the present composite particles are compatible with, and can be used to support, a wide variety of chemical libraries, including those that are inaccessible and/or incompatible with DNA encoding as is used in OBOC-DEL.

[0249] For example, each of the present composite particles 10 respectively may be coupled to a single type of chemical compound, or may be coupled to a plurality of different types of chemical compounds, such as small molecules, peptides, oligonucleotides, and/or proteins. The chemical compound(s) may be coupled to the composite particle in any suitable manner, for example via adsorption, direct covalent attachment, or covalent attachment through a chemical linker. The chemical linker optionally may be cleavable, that is, configured to release the chemical compound in response to a suitable stimulus (such as light for a photo-cleavable linker, heat for a heat-cleavable linker, or low pH for an acid-cleavable linker). The compound released through cleavage of the chemical linker may be

identical to the compound which is coupled to the composite particle. Alternatively, the compound released through cleavage of the chemical linker may be different in some regard to the compound which is coupled to the composite particle and yet still related in a predictable way to the compound which is coupled to the composite particle. Alternatively, the chemical linker may be non-cleavable, in which case the chemical linker may be referred to as a spacer.

[0250] FIG. 11 schematically illustrates an example composite particle 10 to which a chemical compound 11 is coupled. In a manner such as described with reference to FIGS. 1A-1E, composite particle 10 may include a plurality of optically detectable particles 120, and a network polymer gel 110 substantially surrounding the optically detectable particles 120. Although FIG. 11 illustrates a nonlimiting example in which composite particle 10 has a configuration such as described with reference to FIG. 1C, it should be appreciated that composite particle 10 may have any other configuration provided herein, e.g., with reference to FIGS. 1A-1E, with any suitable combination of compositions, modifications, and methods of manufacture such as described with reference to FIGS. 2A-2C, 3A-3D, 4A-4C, 5A-5F, 6A-6C, 7A-7B, and/or 8A-8B.

[0251] In the nonlimiting example shown in FIG. 11, a plurality of molecules of the same chemical compound 11 are coupled to network polymer gel 110 via respective moieties 140. For example, chemical compound 11 may be covalently coupled to the network polymer gel 10 via moiety 140. Illustratively, the chemical compound may be coupled to the network polymer gel 10 directly or indirectly via a chemical linker (that is, moiety 140 may include a chemical linker). The chemical linker may be cleavable, or may be non-cleavable. Alternatively, the chemical compound may be non-covalently coupled to the network polymer gel 10. Illustratively, the chemical compound may be adsorbed to the network polymer gel 10. For example, the chemical compound may be coupled to the network polymer gel 10 via an electrostatic interaction (e.g., to a negatively- or positively-charged moiety 140 such as described with reference to FIGS. 7A-7B or 8A-8B), or through hydrophilicity, hydrophobicity, or other non-covalent molecular interaction such as those observed in protein-ligand binding (affinity). Chemical compound 11 may be distributed throughout the network polymer gel 110. For example, chemical compound 11 may be coupled to moieties 140 which are distributed throughout the network polymer gel 110 in a manner such as described with reference to FIGS. 3D, 5F, and 6C. In some examples, chemical compound 11 may be cleavable from the network polymer gel 110 using light, heat, or a reagent, e.g., in a manner such as described with reference to FIGS. 1A-1E. In a manner

such as will be described further below, in some examples the chemical compound 11 may be released before or after the start of an assay so that the compound released is still physically associated with the composite particle 10 from which it has been released.

[0252] FIG. 12 schematically illustrates an example composite particle 10 to which different chemical compounds 12, 13, 14, 15 are coupled. In a manner such as described with reference to FIGS. 1A-1E, composite particle 10 may include a plurality of optically detectable particles 120; and a network polymer gel 110 substantially surrounding the optically detectable particles 120. Although FIG. 12 illustrates a nonlimiting example in which composite particle 10 has a configuration such as described with reference to FIG. 1C, it should be appreciated that composite particle 10 may have any other configuration provided herein, e.g., with reference to FIGS. 1A, 1B, 1D, or 1E, with any suitable combination of compositions, modifications, and methods of manufacture such as described with reference to FIGS. 2A-2C, 3A-3D, 4A-4C, 5A-5F, 6A-6C, 7A-7B, and/or 8A-8B. In the nonlimiting example shown in FIG. 12, a plurality of molecules of chemical compounds 12, 13, 14, 15 are coupled to network polymer gel 110 via respective moieties 140, or may be non-covalently coupled to the network polymer gel 110, e.g., in a manner such as described with reference to FIG. 11. Any suitable number of different chemical compounds may be coupled to the network polymer gel 110, e.g., between two and four different chemical compounds.

[0253] Composite particles which are coupled to chemical compound(s), such as described with reference to FIGS. 11 and 12, may be used in a library of chemical compounds. Such a library may include different groups of the composite particles. The composite particles of a first group of the composite particles may include the same chemical compound as one another, and the composite particles of a second group of the composite particles may include the same chemical compound as one another and a different chemical compound than that of the first group of composite particles. In examples such as described with reference to FIG. 12, the composite particles of the first group of composite particles may include a first plurality of different chemical compounds, and the composite particles of the second group of composite particles may include a second plurality of different chemical compounds, wherein the chemical compounds of the first plurality are at least partially different than the chemical compounds of the second plurality.

[0254] As such, the composite particles of the first group may be substantially the same as one another, and the composite particles of the second group may be substantially the same as one another, but the first and second groups are different than one another. Each group may

include a plurality of composite particles (e.g., more than 100 particles, or more than 250 particles, or more than 500 particles, or more than 1000 particles, or more than 5000 particles) that are substantially the same as one another. The library may include a plurality of such groups that are different than one another (e.g., more than 10,000 groups, or more than 100,000 groups, or more than 1M groups, or more than 10M groups) that are different than one another. As such, a vast number of chemical compound(s) may be readily assayed using a wide variety of targets. Additionally, because there are so many replicates of each type of composite particle, confidence in the assay result and in the screening of the library may be increased. Additionally, each individual composite particle may be readily identified using the optically detectable particles therein. Indeed, as noted above, the present composite particles may have 10^5 - 10^{15} or more unique spectral fingerprints, making it relatively unlikely that any two composite particles in the same group, or in different groups, will have the same spectral fingerprint. Even if some particles do inadvertently have the same spectral fingerprint, there are so many other particles in each of the groups that information from any replicates may be discarded without losing any meaningful data from the group.

Furthermore, the identity of each composite particle is informatically linked to the identity of the specific chemical compound(s) that are coupled to that composite particle. This informatic link may be used to rapidly identify the compound(s) for a given assay result.

[0255] The present libraries of chemical compounds may be generated in any suitable manner. The composite particles may have any configuration provided herein, e.g., with reference to FIGS. 1A-1E, with any suitable combination of compositions, modifications, and methods of manufacture such as described with reference to FIGS. 2A-2C, 3A-3D, 4A-4C, 5A-5F, 6A-6C, 7A-7B, and/or 8A-8B. Chemical compounds may be coupled to the composite particles 10 by coupling the chemical compounds to the network polymer gels 110 of the composite particles. The composite particles of a first group of composite particles are coupled to the same chemical compound as one another, and the composite particles of a second group of composite particles are coupled to the same chemical compound as one another. However, the chemical compound coupled to the first group of composite particles is different than the chemical compound coupled to the second group of composite particles. Nonlimiting examples of methods for coupling different chemical compounds to different groups of composite particles now will be described further below with reference to FIGS. 13A-13C and 14A-14C. The method of generating the library of chemical compounds further may include, for respective composite particles of the collection, storing, in a data structure, (i) identifiers for the composite particles which are based on the respectively

obtained emission spectra, and (ii) identifiers for the chemical compounds which respectively are coupled to those composite particles.

[0256] FIGS. 13A-13C schematically illustrate example operations for coupling chemical compounds to a composite particle using serial addition of chemical moieties. Although FIGS. 13A-13C illustrate examples in which the composite particle 10 has a configuration such as described with reference to FIG. 1A, it should be appreciated that composite particle 10 may have any other configuration provided herein, e.g., with reference to FIGS. 1B, 1C, 1D, or 1E, with any suitable combination of compositions, modifications, and methods of manufacture such as described with reference to FIGS. 2A-2C, 3A-3D, 4A-4C, 5A-5F, 6A-6C, 7A-7B, and/or 8A-8B.

[0257] FIG. 13A illustrates that in a first synthesis cycle, first chemical moiety 1301 is coupled to network polymer gel 110 of composite particle 10 via chemical moiety 140. FIG. 13B illustrates that in a second synthesis cycle, second chemical moiety 1302 is coupled to first chemical moiety 1301, and thus is indirectly coupled to network polymer gel 110 of composite particle 10 via chemical moiety 140 and first chemical moiety 1301. Together, first chemical moiety 1301 and second chemical moiety 1302 constitute chemical compound 1310. FIG. 13C illustrates that in a third synthesis cycle, third chemical moiety 1303 is coupled to second chemical moiety 1302, and thus is indirectly coupled to network polymer gel 110 of composite particle 10 via chemical moiety 140, first chemical moiety 1301, and second chemical moiety 1302. Together, first chemical moiety 1301, second chemical moiety 1302, and third chemical moiety 1303 constitute chemical compound 1320. Any suitable number of synthesis cycles may be performed on a given composite particle, so as to serially add chemical moieties and thus build a chemical compound coupled to that composite particle.

[0258] Although not specifically illustrated in FIGS. 13A-13C, the chemical moieties which are added, and the chemical compounds which are built, may be distributed throughout network polymer gel 110 (e.g., because moieties 140 are distributed throughout the network polymer gel in a manner such as described with reference to FIGS. 3A-3D, 5A-5F, and 6A-6D). As such, the number of sites within network polymer gel 110 to which the chemical compounds are coupled may be significantly greater than if the chemical compounds were merely coupled to the outer surface of the composite particle 10. Illustratively, the interior of composite particle 10 may possess between 10 times to 200 times more compound than may the surface of the composite particle. This greatly increased number of chemical compounds can provide a significantly increased amount of signal in an assay than may be obtainable

from a particle in which the chemical compounds were present only at that particle's surface. For example, the additional compound on the interior of the composite particle may be particularly useful to provide enough material for analysis of compounds, such as via HPLC and MS. For any assays using cleavable linkers, the amount of compound on the interior provides a greater concentration range.

[0259] While FIGS. 13A-13C schematically illustrate example reactions that may be performed to generate a chemical compound coupled to a given composite particle, which may be in a given subset of particles, other reactions may be performed on other particles of other subsets so as to generate different chemical compounds coupled to the composite particles of those respective subsets. The collection of composite particles, different subsets of which may be coupled to different chemical compounds than one another, may be referred to as a spectrally encoded chemical library, and may be used in assays such as will be described in greater detail below with reference to FIGS. 16A-16C and 17.

[0260] Turning now to FIGS. 14A-14C, which schematically illustrate example operations for generating a spectrally encoded chemical library, chemical compounds may be coupled to the composite particles in a process that may include (a) dividing the collection of composite particles into subsets; and (b) for respective subsets formed in operation (a), coupling a chemical moiety to the network polymer gel of the composite particles of that subset to form a modified subset. For example, although not specifically illustrated in FIG. 13A, the chemical moiety 1301 coupled to the network polymer gel 110 of the composite particles of a first one of the subsets (e.g., the subset of FIG. 13A) may be different than the chemical moiety coupled to the network polymer gel of the composite particles of a second one of the subsets (e.g., subsets which are not illustrated). However, in some cases the chemical moiety coupled to the network polymer gel of the composite particles of a first one of the subsets (e.g., the subset of FIG. 13A) may be the same as the chemical moiety coupled to the network polymer gel of the composite particles of a second one of the subsets (e.g., subsets which are not illustrated).

[0261] As illustrated in FIG. 14A, collection 1400 of composite particles 10 which is used to form the spectrally encoded chemical library may include, or may consist essentially of, about 10^5 to about 10^9 composite particles 10 in some examples. Collection 1400 of composite particles 10 may be divided into n subsets. In a manner such as described in greater detail with reference to FIGS. 9A-9C, the subsets may be generated using a flow cytometer to dispense (e.g., based on output 940x from analyzer 940 related to the spectral fingerprint of the respective particle) a first subset of the composite particles of the collection

into a first reservoir (e.g., well 910 of a microplate); and using the flow cytometer to dispense a second subset of the composite particles of the collection into a second reservoir (e.g., another well 910 of the microplate). Optionally, the composite particles may be counted as they flow through the flow cytometer and into the first reservoir. In some examples, the composite particles are dispensed into the first reservoir until a predetermined number of the composite particles are dispensed into the first reservoir. After the predetermined number of the composite particles are dispensed into the first reservoir, the composite particles are dispensed into the second reservoir until a predetermined number of the composite particles are dispensed into the second reservoir. The composite particles may be counted using the emission spectra, e.g., as the composite particles cross through the interrogation point in a manner such as described with reference to FIGS. 9A-9C. In other examples, the collection 1400 of composite particles 10 is manually divided into n respective reservoirs to form the n subsets.

[0262] The composite particles of each subset may be identified using the respective emission spectra of those particles, and the identifications stored in the data structure. For example, for the composite particles of respective subsets formed in operation (a), the composite particles of that subset may be irradiated with excitation light, the emission spectra from the optically detectable particles of the composite particles of that subset respectively may be obtained, and the emission spectra may be used to identify the composite particles of that subset using the stored identifiers, e.g., in a manner such as described elsewhere herein. The emission spectra from the optically detectable particles of the composite particles of that subset may be obtained one at a time, for example as the composite particles flow through a channel in a manner such as described with reference to FIGS. 9A-9C and 10A-10D. The emission spectra from the optically detectable particles of the composite particles of that subset may be obtained at any suitable time, illustratively before that subset of particles is formed, during particle subset formation, or after that subset of particles is formed.

[0263] Referring still to FIG. 14A, different subsets of the collection 1400 of composite particles 10 may be coupled to different chemical moieties than one another in a similar manner as described with reference to FIG. 13A. For example, in FIG. 14A, composite particles 10 may be divided into subsets denoted [1-1], [1-2], ... [1- n], in which the leading "1" denotes that the subset is formed for use in the first synthesis cycle, and the trailing number 1, 2, ... n denotes a label for the respective subset. Subset [1-1] of composite particles 10 may be coupled to chemical moiety 1401 to form a modified subset [1-1']; subset [1-2] of composite particles 10 may be coupled to chemical moiety 1402 to form a modified

subset [1-2']; and subset [1-*n*] of composite particles 10 may be coupled to chemical moiety 1403 to form a modified subset [1-*n*'], where chemical moieties 1401, 1402, and 1403 are different than one another. In some examples, moieties 1401, 1402, 1403 respectively are coupled to network polymer gel 110 via moieties 140 in a manner such as described elsewhere herein. An identifier of the chemical moiety 1401, 1402, or 1403 which is coupled to the respective subset may be stored in the data structure for each of the composite particles of that subset. Nonlimiting examples of the format of such identifier are provided elsewhere herein.

[0264] The method of generating the spectrally encoded chemical library further may include (c) pooling the modified subsets of operation (b) to form a modified collection of composite particles. For example, as illustrated in FIG. 14B, the modified subsets [1-1'], [1-2'], ... [1-*n*'] may be pooled with one another, e.g., mixed in a common solvent with one another, to form a modified collection 1400' of composite particles. Operations similar to those described with reference to FIG. 14A then may be performed using the modified collection. For example, the method of generating the spectrally encoded chemical library further may include (d) dividing the modified collection of composite particles of operation (c) into subsets; and (e) for respective subsets formed in operation (d), coupling a chemical moiety to the chemical moiety which was coupled to the network polymer gel in operation (b) to form a modified subset. For example, in FIG. 14B, collection 1400' of the pooled subsets from the first synthesis cycle may be divided into subsets denoted [2-1], [2-2], ... [2-*n*], in which the leading "2" denotes that the subset is formed for use in the second synthesis cycle, and the trailing number 1, 2, ... *n* denotes a label for the respective subset. In a manner similar to that described with reference to FIG. 14A, emission spectra from the composite particles of respective subsets formed in operation (d) may be obtained, for example by irradiating the composite particles of that subset with excitation light; respectively obtaining the emission spectra from the optically detectable particles of the composite particles of that subset; and using the emission spectra to identify the composite particles of that subset using the stored identifiers.

[0265] Chemical moiety 1401 of subset [2-1] of composite particles 10 may be coupled to chemical moiety 1404 to form a modified subset [2-1']; chemical moiety 1402 of subset [2-2] of composite particles 10 may be coupled to chemical moiety 1405 to form a modified subset [2-2']; and chemical moiety 1403 of subset [2-*n*] of composite particles 10 may be coupled to chemical moiety 1406 to form a modified subset [2-*n*'], where chemical moieties 1404, 1405, and 1406 may be different than one another, or may be the same as one another. An

identifier of the chemical moiety 1404, 1405, or 1406 which is coupled to the respective subset may be stored in the data structure for each of the composite particles of that subset.

[0266] Optionally, operations (c) through (e) may be repeated for the modified collection of composite particles any desired number of times, e.g., between one and five times. For example, a collection of the pooled subsets from the second synthesis cycle may be divided into subsets. In a manner similar to that described with reference to FIG. 14A, emission spectra from the composite particles of respective subsets formed in the repeated operation (d) may be obtained, for example by irradiating the composite particles of that subset with excitation light; respectively obtaining the emission spectra from the optically detectable particles of the composite particles of that subset; and using the emission spectra to identify the composite particles of that subset using the stored identifiers. Chemical moieties of the respective new subsets of composite particles 10 may be coupled to additional chemical moieties to form modified subsets. The chemical moieties which are added may be different than one another, or may be the same as one another. An identifier of the chemical moiety which is coupled to the respective subset may be stored in the data structure for each of the composite particles of that subset. The modified subsets resulting from the repeated synthesis cycles form a spectrally encoded library. Optionally, the modified subsets may be retained separately from one another. Alternatively, as illustrated in FIG. 14C, the modified subsets optionally may be pooled together.

[0267] Note that as the composite particles progress through different synthesis cycles (e.g., the first and second synthesis cycles respectively described with reference to FIGS. 14A and 14B, the operations (b) and (c) (or repeated operations (c)) build (i) the chemical compound coupled to the composite particles of the first group of composite particles mentioned further above, and (ii) the chemical compound coupled to the composite particles of the second group of composite particles mentioned further above. Furthermore, the stored identifiers of the chemical moieties which are added to those composite particles during the respective cycles describe the synthetic history of each such composite particle.

Illustratively, for the composite particles of respective subsets formed in operation (d) (or repeated operation (d)), the identifier for the chemical compound which is coupled to those composite particles may include (i) an identifier for the chemical moiety which is coupled to the network polymer gel in operation (b) and (ii) an identifier for the chemical moiety which is coupled in operation (c) (or repeated operation (c)) to the chemical moiety which was coupled to the network polymer gel in operation (b). Accordingly, using the emission spectrum of a given composite particle 10, the full synthetic history of that composite particle

– that is, the identifiers for the sequence of chemical moieties that were coupled to that composite particle – may be readily located within the data structure, regardless of whether that composite particle is located within its own subset, is located with a pooled collection of subsets, or is being assayed in a manner such as will be described further below, e.g., with reference to FIGS. 16A-16C and 17.

[0268] It will be appreciated that a wide variety of data structures, and a wide variety of data formats, suitably may be used to store the identifiers for the composite particles and their respective chemical compounds. Nonlimiting examples of data structures include relational databases, NoSQL databases, columnar databases, wide column databases, object-oriented databases, and the like. In some examples, the identifiers for the composite particles may include lists of wavelengths having peaks in the emission spectra. For example, as noted above, the optically detectable particles 120 within a first composite particle 10 respectively may emit light at wavelengths λ_1 , λ_2 , λ_3 , and λ_4 , and the optically detectable particles 120 within a second composite particle 10 respectively may emit light at wavelengths λ_5 , λ_6 , λ_7 , and λ_8 , where the different subscripts denote a unique wavelength. In this example, the identifier for the first composite particle 10 may include a numerical list of the wavelengths λ_1 , λ_2 , λ_3 , and λ_4 (illustratively, 1151 nm, 1182 nm, 1208 nm, and 1330 nm) having peaks in that composite particle's emission spectrum, and the identifier for the second composite particle 10 may include a numerical list of the wavelengths λ_5 , λ_6 , λ_7 , and λ_8 (illustratively, 1250 nm, 1442 nm, 1512 nm, and 1549 nm) having peaks in that composite particle's emission spectrum. Thus, a composite particle which is later determined to have the emission peaks 1151 nm, 1182 nm, 1208 nm, and 1330 nm may be identified as being the first composite particle by determining that the wavelengths in the later measured spectrum match the wavelengths in the earlier measured spectrum of that composite particle, and a composite particle which is later determined to have the emission peaks 1250 nm, 1442 nm, 1512 nm, and 1549 nm may be identified as being the second composite particle by determining that the wavelengths in the later measured spectrum match the wavelengths in the earlier measured spectrum of that composite particle.

[0269] Alternatively, the identifiers for the composite particles 10 may include bits representing wavelengths having peaks in the emission spectra. That is, rather than alphanumerically describing a given emission wavelength (illustratively, 1151 nm) from a composite particle, the data structure may include bit(s) that represent that such wavelength was emitted from that composite particle. In one nonlimiting, purely illustrative example in which there are m unique types of optically detectable particles within the collection of

composite particles, each type having its own wavelength $\lambda_1 \dots \lambda_m$, the possible combinations of optically detectable particles 120 that may be present (e.g., randomly) within a given composite particle may be represented in the data structure by an $m \times 1$ column vector or a $1 \times m$ row vector, wherein each element of the vector is a “1” when that wavelength is emitted by that composite particle, and a “0” when that wavelength is not emitted by that composite particle. A first example composite particle 10 may be identified by a $1 \times m$ row vector such as:

$$[0 \quad 0 \quad 1 \quad 0 \quad 1 \quad 0 \quad 0 \quad 0 \quad 1 \quad \dots \quad 0],$$

representing that the first composite particle emitted the third wavelength (from which it may be understood that it included the third type of optically detectable particle); emitted the fifth wavelength (from which it may be understood that it included the fifth type of optically detectable particle); emitted the ninth wavelength (from which it may be understood that it included the ninth type of optically detectable particle); and did not emit other wavelengths (from which it may be understood that it did not include other types of optically detectable particles). A second example composite particle may be identified by a $1 \times m$ row vector such as:

$$[1 \quad 0 \quad 0 \quad 0 \quad 1 \quad 0 \quad 0 \quad 0 \quad 0 \quad \dots \quad 1],$$

representing that the second composite particle emitted the first wavelength (from which it may be understood that it included the first type of optically detectable particle); emitted the fifth wavelength (from which it may be understood that it included the fifth type of optically detectable particle); emitted the m th wavelength (from which it may be understood that it included the m th type of optically detectable particle); and did not emit other wavelengths (from which it may be understood that it did not include other types of optically detectable particles). Note that although both the first and second composite particles may emit the fifth wavelength, the presence of other wavelengths in these particles’ emission spectra allow the composite particles to be readily distinguished from one another. Thus, a composite particle which is later determined to have the $1 \times m$ row vector:

$$[0 \quad 0 \quad 1 \quad 0 \quad 1 \quad 0 \quad 0 \quad 0 \quad 1 \quad \dots \quad 0],$$

may be identified as being the first composite particle based on a determination that the row vector for the later measured spectrum matches the row vector for the earlier measured spectrum of that composite particle, and a composite particle which is later determined to have the $1 \times m$ row vector:

[1 0 0 0 1 0 0 0 0 ... 1].

may be identified as being the second composite particle based on a determination that the row vector for the later measured spectrum matches the row vector for the earlier measured spectrum of that composite particle.

[0270] It will be appreciated that the data structure may store any other suitable representation(s) of composite particles' respective spectral fingerprints, that identify the composite particles. It will further be appreciated that the above example composite particles with three or four emission wavelengths may be highly simplified, and that the present composite particles may include any suitable number of optically detectable particles. More generally, it should be understood that the wavelengths emitted from different particles may be represented in the data structure in any suitable manner, e.g., graphically (for example, a graphical representation of the emission spectrum), numerically, alphanumerically, or using bits, in any suitable manner in which a given emitted wavelength is uniquely represented in the data structure such that a given particle later may be identified as having, or not having, that wavelength.

[0271] Similarly, the data structure may store any suitable representations of the chemical compounds that respectively are coupled to the composite particles. Illustratively, the identifiers for the chemical compounds which respectively are coupled to those composite particles comprise a chronological description of chemical reactions performed using those composite particles. An entry within such a chronological description may correspond to a synthesis cycle (chemical reaction) such as described with reference to FIGS. 14A-14C and may optionally include, for example, an identification (such as name and/or chemical structure) of the reagents that were used in that synthesis cycle, and optionally also may include one or more details of the reaction conditions, such as temperature, pH, duration of reaction, time at which the reaction was performed, and the like. The chronological description also indicates the order in which the chemical reactions were performed. This indication may be in the form of data, such as an entry in the data structure stating the time at which the reaction was performed. Additionally, or alternatively, this indication may be in the form of metadata, such as where the temporal order of the chemical reactions is represented by the structural arrangement in which those reactions are described in the data structure. In other examples, the identifiers for the chemical compounds which respectively are coupled to those composite particles may include tokens chronologically representing chemical reactions performed using those composite particles. For example, a token may be or include a sequence of bits, an alphanumeric value that represents a particular chemical

reaction that was performed, and/or a pointer to a separate entry in the database structure that describes the chemical reaction in a manner similar to that described above for the chronological description. Additionally, similarly as for the chronological description, the tokens may indicate the order in which the chemical reactions were performed. This indication may be in the form of data, such as an entry in the data structure stating the time at which the reaction was performed. Additionally, or alternatively, this indication may be in the form of metadata, such as where the temporal order of the chemical reactions is represented by the structural arrangement in which those tokens are included in the data structure.

[0272] While FIGS. 13A-13C and 14A-14C may illustrate examples in which a single type of chemical compound is coupled to a corresponding composite particle 10, the libraries may be generated so as to couple multiple different chemical compounds to a given composite particle, e.g., such as described with reference to FIG. 12. In such examples, the composite particles of a first group of composite particles may include a first plurality of different chemical compounds, and the composite particles of a second group of composite particles may include a second plurality of different chemical compounds, wherein the chemical compounds of the first plurality are at least partially different than the chemical compounds of the second plurality. Illustratively, the first plurality of different chemical compounds may include between two and four different chemical compounds. Similarly, the second plurality of different chemical compounds may include between two and four different chemical compounds, in some examples.

[0273] FIG. 20A illustrates an example method for combinatorial one-bead-multiple-compound synthesis. The method of FIG. 20A can be applied to any individual synthesis cycle during library creation. In a first operation, a population of composite particles (beads) is split into separate reaction vessels for compound conjugation. Then, to each reaction vessel, two building blocks are added that possess the same functional group that can react with the functional group displayed on the composite particles. The composite particles are then pooled in the individual reaction vessels together and mixed, before splitting the composite particles back out into reaction vessels for another synthesis cycle. When 2 building blocks are added to each reaction vessel during a single synthesis cycle of library creation, each composite particle will end up displaying two compounds. When 3 building blocks are added to each reaction vessel during a single synthesis cycle of library creation, each composite particle will end up displaying three compounds. If two building blocks are added to each reaction vessel during two synthesis cycles of library creation, each composite

particles will end up displaying four compounds. In this method, the 2, 3, or 4 compounds will always be paired together on each bead that they are synthesized on. Additionally, the compounds on each bead are synthesized simultaneously.

[0274] FIG. 20B illustrates another example method for combinatorial one-bead-multiple-compound synthesis. In this method, the bead must display a functional group and then at least one other functional group that is protected from synthesis. Unlike the method of FIG. 20A, in FIG. 20B the compounds on each composite particle are synthesized sequentially. More specifically, a library of the first set of compounds is synthesized from standard cycles of combinatorial chemistry. After synthesis, the protecting group on each composite particle is deprotected and more rounds of combinatorial synthesis are performed to synthesize the second set of compounds, as shown in FIG. 20B. In FIG. 20B, the composite particles displaying a particular compound from set one will possess a wide variety of compounds from set two, so no two compounds are never inherently paired together. This may make structural deconvolution of hit beads easier. Note also that the methods of FIGS. 20A and 20B may be used together to generate libraries in which multiple compounds are present on each composite particle.

[0275] It will be appreciated that the present composite particles 10 are compatible with a wide variety of synthetic chemical reactions for use in generating spectrally encoded chemical libraries, e.g., to form small molecules, peptides, oligonucleotides, macrocycles, lipids, or oligosaccharides. In particular, the present composite particles may be coupled to chemical compounds using condition, solvent, or reagent which is incompatible with DNA. This freedom of choice in chemistry is notable, and of immense commercial value. In comparison, the chemistries with which OBOC-DEL is compatible are significantly limited by the DNA which encodes the library – if even one of the synthesis cycles damages or destroys the DNA, for example through a condition, solvent, or reagent, then the bead to which that DNA is coupled cannot be decoded, and its information therefore is lost.

[0276] FIGS. 15A-15D schematically illustrate example reaction schemes which may be used to generate a spectrally encoded chemical library. These schemes are believed to be fully compatible with the present composite particles 10, and are believed to be incompatible with a DNA-encoded library. Nonlimiting examples include covalent attachment of small molecules to surface functionality provided on spectrally encoded composite particles through such reactions as amide formation between primary or secondary amine and carboxylic acid, Mitsunobu reaction between alcohols to form ethers, and Povarov reaction (inverse aza-Diels-Alder reaction) to form heterocycles. These examples of “on-particle”

small molecule chemistry should not be viewed as limiting the scope of the present disclosure. For example, with amide bond formation, temperatures and coupling reagents may be used that are incompatible with DNA in order to use weak nucleophiles/electrophiles and sterically-hindered amines (FIG. 15B) and carboxylic acids (FIG. 15A). The Mitsunobu reaction (reaction of two alcohols to form an ether bond) is incompatible with the 3'-hydroxyl of DNA and is very sensitive to the presence of water (FIG. 15C). The Povarov heterocyclization is incompatible with DNA because it needs an acidic environment, and acids depurinate DNA and cause strand scission. (FIG. 15D). For further details regarding damage that different chemical compounds and conditions may cause to DNA, see Sunkari et al., "Impact of organic chemistry conditions on DNA durability in the context of DNA-encoded library technology," *iScience* 26: 107573 (2023), the entire contents of which are incorporated by reference herein.

[0277] A wide variety of other combinatorial chemistries for generating a chemical library on a bead are known in the art, for example as described in Lam et al., "The 'One-Bead-One-Compound' Combinatorial Library Method," *Chem. Rev.* 97: 411-448 (1997), the entire contents of which are incorporated by reference herein.

Systems and methods for performing binding assays on chemical libraries using composite particles

[0278] From the wide variety of chemistries that are compatible with the present composite particles, it will be appreciated that the present spectrally encoded chemical libraries using such particles may be used in a variety of different assays.

[0279] In some examples, a method of detecting binding between different chemical compounds and a target may include incubating the target with a collection of composite particles 10, determining whether the target binds to the chemical compounds of respective composite particles, and identifying the chemical compounds using emission spectra from the respective composite particles. FIGS. 16A-16C schematically illustrate example binding assays that may be performed using a composite particle coupled to a chemical compound. Illustratively, as shown in FIG. 16A, and in a manner such as described with reference to FIGS. 11 and 12, the composite particle 10 of the collection may include a plurality of optically detectable particles 120; a network polymer gel 110 substantially surrounding the optically detectable particles; and a chemical compound 1601 coupled to the network polymer gel. Additionally, in a manner such as described with reference to FIGS. 13A-13C and 14A-14C, at least some of the composite particles may include the same chemical compound as one another, and at least some of the composite particles may include different

chemical compounds than one another (only one such composite particle with one example compound being shown in FIG. 14A). Although the example composite particle 10 shown in FIG. 16A has a configuration such as described with reference to FIG. 1C, it should be appreciated that composite particle 10 may have any other configuration provided herein, e.g., with reference to FIGS. 1A, 1B, 1D, or 1E, with any suitable combination of compositions, modifications, and methods of manufacture such as described with reference to FIGS. 2A-2C, 3A-3D, 4A-4C, 5A-5F, 6A-6C, 7A-7B, and/or 8A-8B.

[0280] The target may bind differently to different ones of the chemical compounds. For example, in the nonlimiting example shown in FIG. 16A, composite particle 10 may be incubated with target 1610. During such incubation, target 1610 may bind to chemical compound 1601, may bind to other chemical compounds in the collection of composite particles, and/or may not bind to still other chemical compounds in the collection of composite particles (other composite particles not specifically illustrated). Any unbound target 1610 may be removed from the collection of composite particles, for example by washing the particles. The composite particles of the collection, with the target 1610 bound to different ones of the chemical compounds, may be irradiated with excitation light, and emission spectra obtained from the optically detectable particles of respective composite particles in a manner such as described elsewhere herein. Additionally, signals are obtained that respectively indicate whether the target 1610 binds to the chemical compounds of respective composite particles. For example, as illustrated in FIG. 16A, target 1610 may be labeled with probe 1611 before the incubating. Or, for example, as illustrated in FIG. 16B, after target 1610 binds differently to different ones of the chemical compounds (e.g., to chemical compound 1601), the target is incubated with, and binds to, an affinity reagent 1620 that is labeled with a probe 1611. Or, for example, as illustrated in FIG. 16C, after the target 1610 binds differently to different ones of the chemical compounds (e.g., to chemical compound 1601), the target is labeled with an unlabeled primary affinity reagent 1620, and then the unlabeled primary affinity reagent is labeled with a secondary affinity reagent 1630 that is labeled with a probe 1611.

[0281] Probe 1611 generates an optical signal (such as fluorescence) that may be recorded using a photodetector in a manner such as described elsewhere herein. In some examples, the composite particles 10 are irradiated with a first wavelength of light that stimulates the emission spectra of the optically detectable particles 120, and the composite particles are irradiated with a second wavelength of light that stimulates fluorescence which generates the signal indicating whether the first target binds to the chemical compounds of respective

composite particles. The composite particles may be irradiated with the first and second wavelengths of light at different times than one another, or may be irradiated with the first and second wavelengths of light at the same time as one another. The emission spectra and signal may be obtained concurrently with one another, or may be obtained at different times than one another. A nonlimiting example of system for obtaining optical signals and emission spectra from a collection of composite particles is described in greater non-limiting detail with reference to FIGs. 9B, 10B, and 18. For example, the emission spectra, the signals, or both the emission spectra and the signals, may be obtained while the composite particles are static. Alternatively, the emission spectra, the signals, or both the emission spectra and the signals, may be obtained while the composite particles are flowed through an inspection point.

[0282] The emission spectra and the signal are used to (i) identify the chemical compound which is coupled to at least some of the composite particles and (ii) determine whether the target binds to that chemical compound. For example, in a manner such as described elsewhere herein, the emission spectra may be used to identify the composite particle, and the information in the data structure may be used to identify the chemical compound that is coupled to that composite particle. Additionally, the presence of the signal indicates that the target did bind to that chemical compound, because if the target had not bound to the compound then the probe 1611 would not have been present to generate the signal. Further, the absence of the signal and detection of the compound by analytical techniques on a subset selected to contain the compound indicates that the target did not bind to that chemical compound, because the compound was present but probe 1611 was not present to generate the signal. Accordingly, these two optical readouts (emission spectra and binding signal) for each particle, multiplied across all of the particles in the collection, readily may be used to determine whether a particular target binds to each of a potentially vast number of chemical compounds.

[0283] Although FIGS. 16A-16C illustrate interactions between a single chemical compound 1601 and a single target 1610, the present composite particles may be used to determine binding between multiple chemical compounds and multiple targets. For example, FIG. 17 schematically illustrates an example binding assay that may be performed using a composite particle coupled to different chemical compounds. As illustrated in FIG. 17, in addition to incubating target 1610 with the collection of composite particles, a second target 1710 may be incubated with the collection of composite particles. Indeed, as illustrated in FIG. 17, a plurality of additional targets 1720, 1730 may be incubated with the collection of

composite particles. The second target 1710 binds differently to different ones of the chemical compounds. Similarly, the additional targets 1720, 1730 bind differently to different ones of the chemical compounds. Although FIG. 17 illustrates a total of four different targets (at least some of which targets optionally may have multiple instances), it will be appreciated that the composite particles may be incubated with any suitable number of targets, e.g., between two and 100 targets, or between 5 and 80 targets, or between 10 and 50 targets, or between two and 20 targets, or between two and 10 targets. The number of targets may, in some examples, be limited by the number of distinct optical channels that are easily obtained via the flow cytometer used for detection. 10 is a simple high limit, but the most multiplexed flow cytometer presently on the market can detect 64 different channels. It will further be appreciated that the collection of composite particles may be incubated concurrently with the target 1610 and the second target 1710 (and any additional targets), or may be incubated with the targets at one or more different times. Any unbound second target 1710 may be removed from the collection of composite particles, e.g., using washing. Similarly, any unbound additional targets 1720, 1730 may be removed from the collection of composite particles, e.g., using washing. It will be further appreciated that detection of binding of a specific compound to any target will also establish that the compound does not bind to any other target where binding is not detected.

[0284] Signals then may be obtained indicating whether the second target 1710 binds to the chemical compounds of respective composite particles, e.g., in a manner such as described with reference to FIGS. 16A-16C. For example, target 1710 may be labeled directly or indirectly with probe 1711. Similarly, signals also may be obtained indicating whether the additional targets 1720, 1730 bind to the chemical compounds of respective composite particles. For example, target 1720 may be labeled directly or indirectly with probe 1721, and target 1730 may be labeled directly or indirectly with probe 1731. The signals from the various targets may include fluorescence, and the fluorescence may be at different wavelengths. For example, fluorescence from the probe 1611 of target 1610 may be at a different wavelength than fluorescence from the probe 1711 of second target 1710. As such, from the wavelength of the signal, it may readily be determined which target had been bound. For example, the data structure may include identities of the targets, and the wavelength of the probe that is bound to each respective target. And again, from the emission spectrum of the composite particle, the chemical compound to which the target(s) bound may readily be identified.

[0285] Note that FIG. 17 illustrates an example in which the different targets are incubated with a collection of composite particles, in which each composite particle 10 may include a plurality different chemical compounds 1701, 1702, 1703, 1704, e.g., in a manner such as described with reference to FIG. 12. Alternatively, the different targets may be incubated with a collection of composite particles, in which each composite particle 10 may include a single respective chemical compound, e.g., in a manner such as described with reference to FIG. 11.

[0286] In another example, a collection of composite particles including different chemical compounds is introduced with target and assay reagents into a plurality of aqueous compartments. In one nonlimiting example, the concentration of composite particles is selected so as to maximize the number of compartments containing a single composite particle. The compartments may be, for example, wells in a microarray, water-in-oil (W/O) emulsion droplets, or water-in-oil-in-water (W/O/W) emulsion droplets. Compound is released from the composite particle, and the contents of the aqueous compartment are incubated prior to detection. The action of the target on the assay reagents produces a measurable signal the magnitude of which is proportional to the activity of the target of the chemical compound. For further details of droplet microfluidic assays with OBOC libraries, see MacConnell et al., "An integrated microfluidic processor for DNA-encoded combinatorial library functional screening," ACS Combinatorial Science 19: 181-192 (2017), the entire contents of which are incorporated by reference herein.

[0287] In another example, a collection of composite particles including different chemical compounds is introduced with target into collection of aqueous compartments. In one nonlimiting example, the concentration of composite particles is selected so as to maximize the number of compartments containing a single composite particle. These compartments may be, for example, wells in a microarray, W/O emulsion droplets, or W/O/W emulsion droplets. Compound is released from the composite particle, and the contents of the aqueous compartment are incubated. After incubation, assay reagents are added to each aqueous compartment, and the contents are incubated prior to detection. The action of the target on the assay reagents produces a measurable signal the magnitude of which is proportional to the activity of the target of the chemical compound.

[0288] In some examples, aqueous compartments containing composite particles can be generated within a microfluidic channel circuit or within a microbore tubing circuit. Aqueous compartments may be stored and incubated in a microfluidic channel circuit, within a microbore tubing circuit, or within a vessel that is not part of any channel or tubing circuit.

All combinations of aqueous compartment generation and storage/incubation are possible embodiments of binding assay detection using a probe label and flow-based measurement. One example includes W/O droplet generation in a microfluidic channel circuit and incubation of droplets in a vessel separate from any circuit.

[0289] Various methods of measuring binding signals and emission spectra may be used, some nonlimiting examples of which are provided below.

[0290] 1. Probe label and flow-based measurement: a suspension of composite particles subjected to a binding assay are introduced into a flow path such that individual composite particles are analyzed sequentially. Alignment of the composite particles may occur by using physical barriers, sheath/focusing flow, or acoustic forces, alone or in combination. Instrumentation and software measures and records the spectral fingerprint of each composite particle and the signal output of the assay, which reflects the degree of target binding to the composite particle-displayed ligands.

[0291] 2. Probe label and static measurement: a suspension of composite particles subjected to a binding assay are deposited on a surface. Instrumentation and software measures and records the spectral fingerprint of each composite particle and the signal output of each assay. For further details of array assays with OBOC compounds, see Xiao et al., "Immobilized OBOC combinatorial bead array to facilitate multiplicative screening." Comb Chem High Throughput Screen, 16:441-448 (2013), the entire contents of which are incorporated by reference herein.

[0292] 3. Probe label and flow measurement: a suspension of composite particles subjected to a binding assay are introduced into a flow path that deposits individual composite particles into discrete aqueous compartments with a substrate for the probe. These aqueous compartments could be part of W/O emulsions or W/O/W emulsions. The action of the probe on the substrate produces a measurable signal. After incubation of the composite particles with the substrate, the aqueous compartments are introduced into a flow path such that they are analyzed sequentially. Physical alignment of the aqueous compartments may occur by using physical barriers, sheath or focusing flow, or acoustic forces, alone or in combination. Instrumentation and software measures and records the spectral fingerprint of each composite particle and the signal produced by the probe.

[0293] 4. Probe label and static measurement: a suspension of composite particles subjected to a binding assay are introduced into the wells of a microarray along with probe substrate. After isolation of the microwells and subsequent incubation, instrumentation and

software measures and records the spectral fingerprint of each composite particle and the signal produced by the probe.

[0294] Possible embodiments of binding assay detection using a probe label and flow-based measurement include: a commercial or custom-built flow cytometer instrument; a commercial or custom-built fluorescence-activated cell-sorting (FACS) instrument; a commercial or custom-built microfluidic channel circuit with accompanying signal detection hardware and software; a commercial or custom-built microbore tubing circuit with accompanying signal detection hardware and software. There is no preferred embodiment because each would have similar performance.

[0295] Possible embodiments of binding assay detection using a probe label and static measurement include: a commercial or custom-built scanning microscope; a commercial or custom-built whole well imager; a commercial or custom-built fluorescence scanning platform. For maximized throughput, a scanning microscope or whole well imager are the preferred embodiments.

[0296] Possible embodiments of binding assay detection using a probe label and flow-based measurement include:

[0297] 1. W/O droplets containing composite particles can be generated within a microfluidic channel circuit or within a microbore tubing circuit. W/O droplets can be stored and incubated in a microfluidic channel circuit, within a microbore tubing circuit, or within a vessel that is not part of any channel or tubing circuit. W/O droplets can be detected in flow within a microfluidic channel circuit or within a microbore tubing circuit. All combinations of W/O droplet generation, W/O droplet storage/incubation, and W/O droplet detection in flow are possible embodiments of binding assay detection using a probe label and flow-based measurement.

[0298] 2. W/O/W droplets containing composite particles can be generated within a microfluidic channel circuit or within a microbore tubing circuit. W/O/W droplets can be stored and incubated in a microfluidic channel circuit, within a microbore tubing circuit, or within a vessel that is not part of any channel or tubing circuit. W/O/W droplets can be detected in flow within a microfluidic channel circuit, within a microbore tubing circuit, within a flow cytometer, or within a FACS instrument. All combinations of W/O/W droplet generation, W/O/W droplet storage/incubation, and W/O/W droplet detection in flow are possible embodiments of binding assay detection using a probe label and flow-based measurement.

[0299] One nonlimiting example uses W/O droplet generation in a microfluidic channel circuit, incubation of droplets in a vessel separate from any circuit, and then introduction of droplets on a microfluidic channel circuit for detection. This embodiment gives the best mix of incubation time flexibility and throughput.

[0300] Possible embodiments of binding assay detection using a probe label and static measurement include: a commercial or custom-built scanning microscope; a commercial or custom-built whole well imager; a commercial or custom-built fluorescence scanning platform. For maximized throughput, a scanning microscope or whole well imager are the preferred embodiments.

Activity assay detection:

[0301] Compound is released from the composite particles into discrete aqueous compartments and subjected to a set of activity assay conditions that are analyzed on one or multiple instruments that 1) detect the spectral fingerprint of each composite particle and 2) detect the activity assay result. These two detection events can occur simultaneously or with a spatial or temporal offset. These two detection events can also occur in flow or under static conditions. Ideally, one instrument detects the spectral fingerprint of the composite particle and the magnitude of target activity simultaneously, as this maximizes the simplicity and throughput of the screening process.

[0302] For activity assays, a static measurement is defined here as a measurement made while some fraction of each aqueous compartment's surface area is bounded directly by a solid surface and the compartment is immobile relative to the solid surface.

[0303] For activity assays, a flow-based measurement is defined here as a measurement made while each aqueous compartment's surface area is completely bounded by a liquid phase and the compartment moves through a static measurement point.

[0304] 1. Static measurement: after incubation of target, compound and assay reagents in each aqueous compartment, instrumentation and software measures and records the spectral fingerprint of each composite particle and the signal produced by the action of the target on the assay reagents. Possible embodiments of activity assay detection with static measurement include: a commercial or custom-built scanning microscope; a commercial or custom-built whole well imager; a commercial or custom-built fluorescence scanning platform.

[0305] 2. Flow-based measurement: after incubation of target, compound and assay reagents, the aqueous compartments are introduced into a flow path such that they are analyzed sequentially. Physical alignment of the aqueous compartments may occur by using

physical barriers, sheath or focusing flow, or acoustic forces, alone or in combination. Instrumentation and software measures and records the spectral fingerprint of each composite particle and the signal produced by the target.

[0306] Aqueous compartments can be detected in flow within a microfluidic channel circuit, within a microbore tubing circuit, within a flow cytometer, or within a FACS instrument. The preferred embodiment is introduction of droplets on a microfluidic channel circuit for detection, which provides the best combination of usability and throughput.

Cell-based activity assays:

[0307] Compound is released from the composite particles into discrete aqueous compartments containing cells and subjected to a set of activity assay conditions that are analyzed on one or multiple instruments that 1) detect the spectral fingerprint of each composite particle and 2) detect the activity cell-based assay result. These two detection events can occur simultaneously or with a spatial or temporal offset. Ideally, one instrument detects the spectral fingerprint of the composite particle and the magnitude of target activity simultaneously, as this maximizes the simplicity and throughput of the screening process. For further details of cell-based array assays with OBOC compounds, see Townsend et al. "Jeffamine derivatized TentaGel beads and poly(dimethylsiloxane) microbead cassettes for ultrahigh-throughput in situ releasable solution-phase cell-based screening of one-bead-one-compound combinatorial small molecule libraries." *J Comb Chem.*, 12:700-712 (2010), the entire contents of which are incorporated by reference herein.

Composite particle validation

[0308] At any time post-library generation, composite particles can be sorted into subsets based on their library member(s), e.g., using system 1005 described with reference to FIGS. 10A-10B. This is possible because 1) the number of composite particles present in library synthesis is much larger than the number of unique library members and 2) the chemical matter associated with each composite particle has been registered in a database via its spectral fingerprint. Composite particle subset generation based on library member selection can be used to check the quality or success of library generation, or to validate the identity of "hits" as true positives and "non-hits" as true negatives.

[0309] Enrichment of composite particles possessing a specific compound or compounds can be accomplished using an instrument that detects the spectral fingerprint of each composite particle in a composite particle collection, compares each spectral fingerprint to a registry of known spectral fingerprints that pertain to composite particles with the desired

chemical matter, and separates composite particles that have spectral fingerprints matching the desired compound(s) from the collection.

[0310] The preferred embodiment consists of a customized FACS instrument with the requisite optics for particle detection. The output of the FACS instrument is coupled to a fluidic system that enables sorted composite particles to be dispensed into individual tubes or wells in a microplate. Composite particle subsets are defined as the identities of the composite particles in each separate vessel. A microfluidic device that uses dielectrophoresis could also be used to detect and sort composite particles based on their spectral fingerprints.

[0311] After subsets containing composite particles with the same chemical incorporation history have been isolated in separate vessels, they can be subjected to chemical or physical conditions that trigger compound release from the composite particles. Supernatant containing cleaved compound(s) is isolated from the composite particles and can be assessed to measure library generation metrics and/or to validate assay results.

[0312] Compound identity, purity and synthetic yield can be determined using UPLC or HPLC coupled to MS or MS/MS detection. Crude cleavage product can be used to validate the screening results using either isothermal titration calorimetry (ITC), fluorescence polarization, or activity-based assays.

[0313] FIG. 18 schematically illustrates the optical components of an example system 1800 for identifying composite particles and detecting the respective results of binding assays performed using those particles. As shown, system 1800 can include at least one infrared laser 1815 and a plurality of optical lasers 1812a, 1812b, 1812c, 1812d. Each of lasers 1815, 1812a, 1812b, 1812c, 1812d can emit light in the form of a laser beam. The wavelengths of the laser beams emitted therefrom can range between 325 nm to 3300 nm. System 1800 can be the optical system for systems 905 or 1005, which also include fluidics and other components for bead transport and sorting (1005 only).

[0314] Light 1812x from lasers 1812a (λ_1), 1812b (λ_2), 1812c (λ_3), 1812d (λ_4) and light 1815x from laser 1815 respectively, can be focused to pass through dichroic mirrors 1821a, 1821b, 1821c, 1821d that propagates the light to an interrogation point 1890 where at least one composite particle would be flowing during operations, similar to flow cells 930, 1030 described previously in FIGs. 9C and 10C, respectively. λ_1 , λ_2 , λ_3 , and λ_4 may be different than one another, and different than the wavelength of light 1815x. During operations, the composite particles flow typically one at a time, in a stream to the focused laser beams of lasers 1815, 1812a, 1812b, 1812c, 1812d for interrogation.

[0315] A forward scatter detector 1839 can be immediately downstream of point 1890 to receive light 1812x scattered by the composite particles. An emission collection lens 1831 can be immediately downstream of point 1890 to collect light 1812x, 1815x side-scatter and fluorescence optical signal (e.g., signal related to fluorescently-labeled targets or affinity reagents), and direct this light to an array of second dichroic mirrors 1823a, 1823b, 1823c, 1823d, 1823e downstream thereof. Mirrors 1823a, 1823b, 1823c, 1823d, 1823e can receive and further propagate emission light. In some examples, mirrors 1823a can propagate light 1815x to an optical particle detector 1836. In some examples, mirrors 1823b, 1823c, 1823d, 1823e can propagate focused light 1812x to band pass filters 1833a, 1833b, 1833c, 1833d, respectively. Filters 1833a, 1833b, 1833c, 1833d are configured to absorb or otherwise reflect unwanted wavelengths of focused light 1812x, and only transmit aspects of the visible light spectrum to an optical particle detectors 1 1834a, 1834b, 1834c, 1834d. In some examples, mirror 1823e can propagate focused light 1812x to a side-scatter detector 1838.

[0316] Each of detectors 1839, 1838, 1836, 1834a, 1834b, 1834c, 1834d can transmit respective detector output to analyzer 1840. Analyzer 1840 can include one or more computing systems and be configured to read forward-scatter values, side-scatter values, particle count, a spectral fingerprint, and other spectral information of each particle excited by light from system 1800. The number of lasers of system 1800 can vary depending on how many fluorophores the system is designed to detect. One of skill in the art will recognize that the number of lasers and mirrors is not limited to the system 1800 depicted in FIG. 18, but can include any number of lasers as well as detectors, mirrors, beam splitters, filters, and the like at various wavelengths and in various different configurations.

[0317] FIG. 19 is a computer architecture diagram showing a general computing system capable of implementing aspects of the present disclosure in accordance with one or more embodiments described herein, such as the analyzer 940, 1040, 1840 as well as components, sub-systems, and operations related to systems 905, 1005, and 1800. In any of these example implementations, computer 1900 of the aforementioned may be configured to perform one or more functions associated with embodiments of this disclosure. For example, the computer 1900 may be configured to perform operations in accordance with those examples shown in FIGS. 1 to 18. It should be appreciated that the computer 1900 may be implemented within a single computing device or a computing system formed with multiple connected computing devices. The computer 1900 may be configured to perform various distributed computing tasks, in which processing and/or storage resources may be distributed among the multiple devices. The data acquisition and display computer 1950 and/or operator console 1910 of the

system shown in FIG. 19 may include one or more systems and components of the computer 1900.

[0318] As shown, the computer 1900 includes a processing unit 1902 (“CPU”), a system memory 1904, and a system bus 19019 that couples the memory 1904 to the CPU 1902. The computer 1900 further includes a mass storage device 1912 for storing program modules 1914. The program modules 1914 may be operable to analyze data from any herein disclosed output data from detectors and other components of a respective system, read composite particle spectral information, perform other analytics related thereto, and/or control any related operations of the system. The program modules 1914 may include an application 1918 for performing data acquisition and/or processing functions as described herein, for example to acquire and/or process any of the herein discussed data feeds. The computer 1900 can include a data store 1920 for storing data that may include data 1922 of data feeds (e.g., output data from detectors of a respective system).

[0319] The mass storage device 1912 is connected to the CPU 1902 through a mass storage controller (not shown) connected to the bus 19019. The mass storage device 1912 and its associated computer-storage media provide non-volatile storage for the computer 1900. Although the description of computer-storage media contained herein refers to a mass storage device, such as a hard disk or CD-ROM drive, it should be appreciated by those skilled in the art that computer-storage media can be any available computer storage media that can be accessed by the computer 1900.

[0320] By way of example and not limitation, computer storage media (also referred to herein as “computer-readable storage medium” or “computer-readable storage media”) may include volatile and non-volatile, removable and non-removable media implemented in any method or technology for storage of information such as computer-storage instructions, data structures, program modules, or other data. For example, computer storage media includes, but is not limited to, RAM, ROM, EPROM, EEPROM, flash memory or other solid state memory technology, CD-ROM, digital versatile disks (“DVD”), HD-DVD, BLU-RAY, or other optical storage, magnetic cassettes, magnetic tape, magnetic disk storage or other magnetic storage devices, or any other medium which can be used to store the desired information and which can be accessed by the computer 1900. “Computer storage media”, “computer-readable storage medium” or “computer-readable storage media” as described herein do not include transitory signals.

[0321] According to various embodiments, the computer 1900 may operate in a networked environment using connections to other local or remote computers through a

network 19119 (e.g., previous network 108) via a network interface unit 1910 connected to the bus 19019. The network interface unit 1910 may facilitate connection of the computing device inputs and outputs to one or more suitable networks and/or connections such as a local area network (LAN), a wide area network (WAN), the Internet, a cellular network, a radio frequency (RF) network, a Bluetooth-enabled network, a Wi-Fi enabled network, a satellite-based network, or other wired and/or wireless networks for communication with external devices and/or systems.

[0322] The computer 1900 may also include an input/output controller 1908 for receiving and processing input from any of a number of input devices. Input devices may include one or more of keyboards, mice, stylus, touchscreens, microphones, audio capturing devices, and image/video capturing devices. An end user may utilize the input devices to interact with a user interface, for example a graphical user interface, for managing various functions performed by the computer 1900. The bus 19019 may enable the processing unit 1902 to read code and/or data to/from the mass storage device 1912 or other computer-storage media.

[0323] The computer-storage media may represent apparatus in the form of storage elements that are implemented using any suitable technology, including but not limited to semiconductors, magnetic materials, optics, or the like. The computer-storage media may represent memory components, whether characterized as RAM, ROM, flash, or other types of technology. The computer storage media may also represent secondary storage, whether implemented as hard drives or otherwise. Hard drive implementations may be characterized as solid state or may include rotating media storing magnetically-encoded information. The program modules 1914, which include the data feed application 1918, may include instructions that, when loaded into the processing unit 1902 and executed, cause the computer 1900 to provide functions associated with one or more embodiments illustrated in the figures of this disclosure. The program modules 1914 may also provide various tools or techniques by which the computer 1900 may participate within the overall systems or operating environments using the components, flows, and data structures discussed throughout this description.

[0324] In general, the program modules 1914 may, when loaded into the processing unit 1902 and executed, transform the processing unit 1902 and the overall computer 1900 from a general-purpose computing system into a special-purpose computing system. The processing unit 1902 may be constructed from any number of transistors or other discrete circuit elements, which may individually or collectively assume any number of states. More specifically, the processing unit 1902 may operate as a finite-state machine, in response to

executable instructions contained within the program modules 1914. These computer-executable instructions may transform the processing unit 1902 by specifying how the processing unit 1902 transitions between states, thereby transforming the transistors or other discrete hardware elements constituting the processing unit 1902.

[0325] Encoding the program modules 1914 may also transform the physical structure of the computer-storage media. The specific transformation of physical structure may depend on various factors, in different implementations of this description. Examples of such factors may include but are not limited to the technology used to implement the computer-storage media, whether the computer storage media are characterized as primary or secondary storage, and the like. For example, if the computer storage media are implemented as semiconductor-based memory, the program modules 1914 may transform the physical state of the semiconductor memory, when the software is encoded therein. For example, the program modules 1914 may transform the state of transistors, capacitors, or other discrete circuit elements constituting the semiconductor memory.

[0326] As another example, the computer storage media may be implemented using magnetic or optical technology. In such implementations, the program modules 1914 may transform the physical state of magnetic or optical media, when the software is encoded therein. These transformations may include altering the magnetic characteristics of particular locations within given magnetic media. These transformations may also include altering the physical features or characteristics of particular locations within given optical media, to change the optical characteristics of those locations. Other transformations of physical media are possible without departing from the scope of the present description, with the foregoing examples provided only to facilitate this discussion.

[0327] According to certain embodiments, the above-described data feeds may be stored in databases such as database servers that store master data, event related data, response plan data, telemetry information, and mission data as well as logging and trace information. The databases may also provide an API and/or API access (e.g., for open source) to the web server for data interchange based on JSON specifications. In some embodiments, the database may also directly interact with systems and monitoring devices to identify, determine, and control response operations. According to certain embodiments, the database servers may be optimally designed for storing large amounts of data, responding quickly to incoming requests, having a high availability and historizing master data.

WORKING EXAMPLES

[0328] The following examples are intended to be purely illustrative, and not limiting of the present invention.

Example 1

[0329] A monomer mix was prepared from 6.5 mol% 2-hydroxy-2-methylpropiophenone, 4.7 mg (10 mol%) N-(3-aminopropyl)methacrylamide HCl, 25.9 mg (11.5 mol%) N1,N37-bis(3-methacrylamidopropyl)-4,7,10,13,16,19,22,25,28,31,34-undecaheptatriacontanediamide, and 44.0 mg (72.0 mol%) N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)methacrylamide were dissolved in 142 μ L of 30% (v/v) acetonitrile in ultrapure water (18.0 M Ω ·cm) such that the solution was 33% (w/w) acrylamide monomers. Prior to monomer mix construction, both the ultrapure water and acetonitrile were sparged with argon for 20 min. The monomer mix was vortexed, briefly sonicated, and filtered through a 0.22- μ m PTFE membrane.

[0330] ~60 million Lase particles (LPs) were obtained from Lase Innovation, Inc. and transferred from ethanol to acetonitrile by washing with acetonitrile (5 x 1 mL). The LP/acetonitrile suspension was filtered through a 5.0 μ m Nylon mesh, pelleted by centrifugation at 2500 rcf, and the supernatant was removed. The LP pellet was resuspended in 100 μ L of monomer mix and sonicated for 10 sec, then vortexed briefly. The LP/monomer mix suspension was shaken continuously throughout bead production using a custom-modified vortexer.

[0331] A custom-designed PDMS microfluidic device with a single 21 μ m nozzle at the cross-junction was used to produce droplets of LP/monomer mix suspension (280 mbar) in HFE-7500 with 1.2% PicoSurf (650 mbar) as the continuous oil phase. The aqueous and oil phases were driven to the cross-junction with compressed argon using a pneumatic pressure controller. After droplet formation at the cross-junction, the droplets exited the microfluidic device into a piece of clear PTFE tubing (0.56 mm ID x 1.07 mm OD x 48 cm length). The droplets were crosslinked in flow within the tubing using a 365-nm LED lamp (residence time approximately 30 min), and collected for processing.

[0332] The excess oil phase was removed from the collected emulsion by filtration and the crosslinked beads were washed with 25% (v/v) perfluorooctanol in acetone (1 x 4 mL), acetone (3 x 4 mL), methanol (3 x 4 mL), and phosphate buffered saline w/ 0.1% Tween-20 (3 x 4 mL). The bead suspension was vortexed vigorously (1 min) during each wash step. Beads with similar properties could be made on the same apparatus without Lase particles included in the monomer mix.

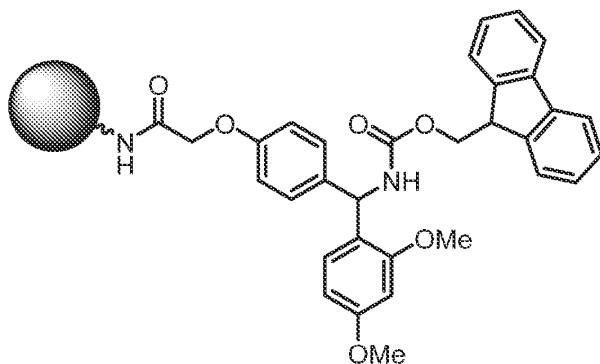
[0333] The beads (SpectraGel beads) were characterized for the number of differentiable Lase particles and their spectral fingerprint) by the method from Kwok et. al. Nature Biomedical Engineering (8), 310–324 (2024), the entire contents of which are incorporated by reference herein, replacing the cells of Kwok with the beads of Example 1. Multiple runs were performed and the matching method from Kwok et al. was used to determine the percentage of beads identified from the original spectral signature at each cycle. For beads containing 5 or more lase particles, match rates were 60-70%, similar to those described for cells in Kwok et.al.

[0334] FIG. 21 illustrates a montage of images of the example SpectraGel beads (embedded with Lase Particles (LPs)) produced in Example 1 captured using Attune Cytpix. FIG. 21 shows that the present methods effectively produce monodisperse SpectraGel beads that can be further used for compound synthesis.

[0335] FIG. 22 illustrates scanning electron micrographs (SEMs) of example blank beads (left) and example LP-embedded beads (right) produced in Example 1, in both dark-scanning modes (top) and bright-scanning modes (bottom). Arrows point to LPs.

[0336] FIG. 23 illustrates a histogram of LP incorporation in a 1 million-bead sample produced in Example 1.

Example 2

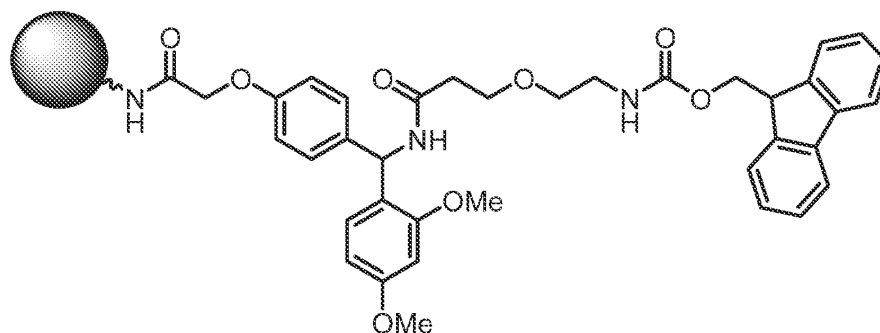


Crosslinked PEG-base of SpectraGel resin with N-Fmoc-RAM linker

[0337] To 5.0 mg of SpectraGel beads without Lase particles prepared by the method of Example 1 in a 3 mL syringe with a 10 μ m fritted filter was added 300 μ L of 20% 4-methylpiperidine in dimethylformamide (DMF) and the resin was mixed thoroughly via pipetting and allowed to equilibrate at room temperature ($\sim 22^{\circ}\text{C}$) for 15 minutes. The solution was removed with vacuum filtration and the resin, in syringe, was washed with DMF (6 x 300 μ L) with successive pipetting and vacuum filtration. To the tube containing the resin

was added 200 μL of a 1.05 : 1.0 mixture of 2-(4-((((9H-Fluoren-9-yl)methoxy)-carbonyl)amino)(2,4-dimethoxyphenyl)methyl)phenoxy)-acetic acid (N-Fmoc-rink amide carboxylic acid linker) and azabenzotriazole tetramethyluronium hexafluorophosphate (HATU) at 105 and 100 mM, respectively, in DMF. The combined reaction slurry was then immediately mixed with pipetting and 5.2 μL (3.9 mg, 0.030 mmol) of diisopropylethylamine (DIEA) was added. The resin slurry was mixed via pipetting and allowed to incubate at room temperature with occasional agitation / pipetting for 1.5 hours. The solution was removed via vacuum filtration and the resin was washed with DMF (5 x 300 μL) with pipetting and applied vacuum filtration to afford the title linker, N-Fmoc-RAM, coupled to SpectraGel resin.

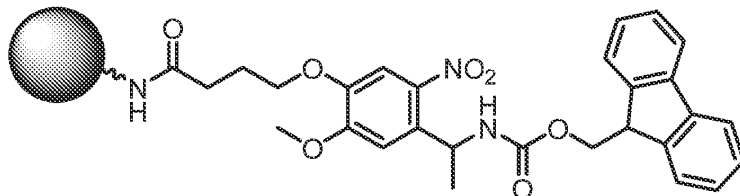
Example 3



SpectraGel resin with N-Fmoc-PEG1-RAM linker

[0338] A 5.0 mg of SpectraGel resin with N-Fmoc-RAM linker from example 2 was placed in a 3 mL syringe with a 10 μm fritted filter was treated two successive times with 300 μL of 20% 4-methyl-piperidine in DMF (600 μL total) and mixed via pipetting and incubated for 7.5 min. (15 min. total). The solution was removed with vacuum filtration and the resin was washed DMF (6 x 300 μL) with successive pipetting and vacuum filtration. To the resin tube was added 200 μL of a 1.05 : 1.0 mixture of 3-(2-[[[(9H-fluoren-9-yl)methoxy]carbonyl]amino]ethoxy)propanoic acid (N-Fmoc-PEG1 carboxylic acid and HATU at 105 and 100 mM, respectively, in DMF. The reaction slurry was then immediately mixed with pipetting and 5.2 μL (3.9 mg, 0.030 mmol) of diisopropylethylamine (DIEA) was added. The resin containing reaction slurry was again mixed via pipetting and allowed to incubate at room temperature with occasional agitation / pipetting for 1.5 hours. The solution was removed via vacuum filtration and the resin was washed with DMF (5 x 300 μL) with pipetting and applied vacuum filtration to afford the title linker, N-Fmoc-PEG1-RAM, coupled to SpectraGel resin.

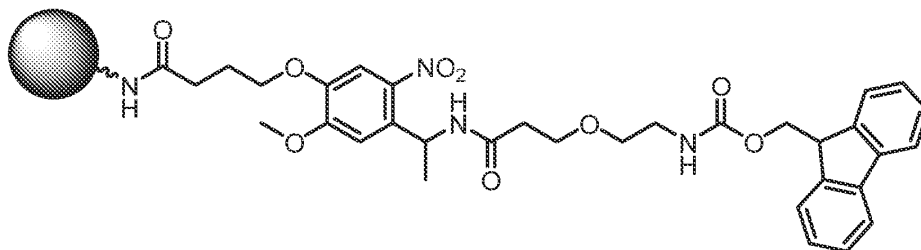
Example 4



Crosslinked PEG of SpectraGel resin with N-Fmoc-Photolabile linker (N-Fmoc-PCL)

[0339] To a 5.0 mg of SpectraGel without Lase particles prepared by the method of Example 1 in a 3 mL syringe with a 10 μ m fritted filter was added 300 μ L of 20% 4-methylpiperidine in dimethylformamide (DMF) and the resin was mixed thoroughly via pipetting and allowed to equilibrate at room temperature for 15 minutes. The solution was removed with vacuum filtration and the resin, in syringe, was washed with DMF (6 x 300 μ L) with successive pipetting and vacuum filtration. To the resin tube was added 200 μ L of a 1.05 : 1.0 mixture of 4-{4-[1-(9)-Fluorenylmethyloxycarbonylamino]ethyl]-2-methoxy-5-nitrophenoxy}butanoic acid (N-Fmoc-Photolabile-linker) and HATU at 105 and 100 mM, respectively, in DMF. The combined reaction slurry was then immediately mixed with pipetting and 5.2 μ L (3.9 mg, 0.030 mmol) of diisopropylethylamine was added. The reaction slurry was again mixed via pipetting and allowed to incubate at room temperature with occasional agitation / pipetting for 1.5 hours. The solution was removed via vacuum filtration and the resin was washed with DMF (5 x 300 μ L) with pipetting and applied vacuum filtration to afford the title linker, N-Fmoc-PCL, coupled to SpectraGel resin.

Example 5



SpectraGel resin with N-Fmoc-PEG1-PCL linker

[0340] The 5.0 mg of SpectraGel resin with N-Fmoc-PCL linker from Example 4 was placed in a 3 mL syringe with a 10 μ m fritted filter was treated two successive times with 300 μ L of 20% 4-methyl-piperidine in DMF (600 μ L total) and mixed via pipetting and incubated for 7.5 min. (15 min. total). The solution was removed with vacuum filtration and the resin was washed DMF (6 x 300 μ L) with successive pipetting and vacuum filtration. To the resin

tube was added 200 μ L of a 1.05 : 1.0 mixture of 3-(2-((9H-fluoren-9-ylmethoxy)carbonyl)-amino)ethoxy)propanoic acid (N-Fmoc-PEG1 carboxylic acid and HATU at 105 and 100 mM, respectively, in DMF. The reaction slurry was then immediately mixed with pipetting and 5.2 μ L (3.9 mg, 0.030 mmol) of diisopropylethylamine (DIEA) was added. The resin containing reaction slurry was mixed via pipetting and allowed to incubate at room temperature with occasional agitation / pipetting for 1.5 hours. The solution was removed via vacuum filtration and the resin was washed with DMF (5 x 300 μ L) with pipetting and applied vacuum filtration to afford the title linker, N-Fmoc-PEG1-PCL coupled to SpectralGel resin.

[0341] It will be appreciated that these building blocks or any other Fmoc amino acid can be coupled in any number or any order to generate unreleasable resins, acid release resins, photolysis release resins or both acid and photo release resins, with a variety of linkers before or after any release point.

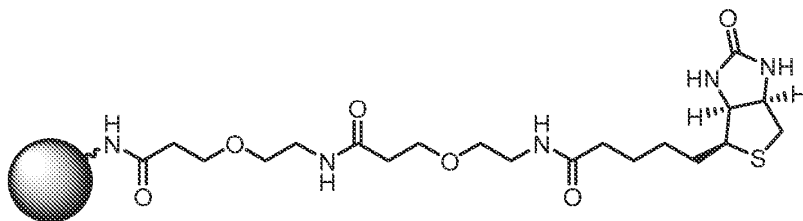
Example 6

[0342] General procedure for amidation coupling of carboxylate functionalized ligands onto 5.0 mg (1.4 μ mol) of amine functionalized SpectralGel resin prepared by the method of Example 1 with or without Lase particles.

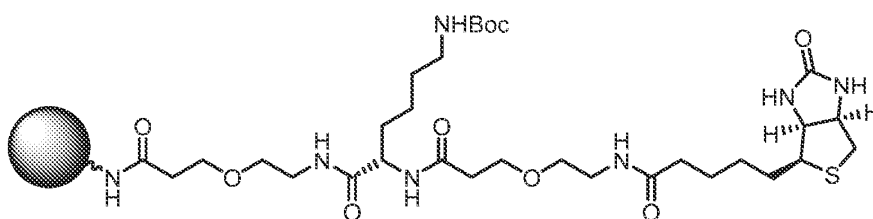
[0343] The resin was added to a 3 mL syringe with a 10 μ m polypropylene fritted filter. To a 2 mL Eppendorf tube containing 0.030 mmol carboxylate ligand is added 150 μ L of a 250 mM Oxyma solution (0.0375 mmol ethyl cyano(hydroxyimino)acetate in DMF (dimethylformamide). To the solution / slurry mixture, 4.7 mg (0.0375 mmol) N,N'-diisopropylcarbodiimide (DIC) was added and the contents were thoroughly mixed with vortexing and pipetting, then allowed to equilibrate for 30-45 minutes at room temperature. In parallel, the syringe containing the N-Fmoc-capped SpectraGel suspended in 500 μ L DMF was filtered under vacuum and treated two successive times with 300 μ L of 20% 4-methylpiperidine in DMF (600 μ L total). Each aliquot was mixed via pipetting and allowed to incubate for 7.5 min. (15 min. total). The second aliquot was removed with vacuum filtration and the resin was washed with DMF (6 x 300 μ L) with each wash removed by vacuum filtration. After 30-45 min. in the eppendorf tube, 150 μ L of this preactivated carboxylate slurry was transferred to the syringe containing the linker-NH₂ functionalized SpectralGel resin suspended in 150 μ L of 300 mM 2,4,5-collidine (0.045 mmol) in DMF. The resulting resin in reaction slurry was mixed with gentle pipetting and allowed to rest at room temperature with occasional agitation / pipetting for at least 1-1.5 hours. After this, the resulting reaction mixture and resin (beads) was then washed with DMF (5 x 300 μ L), with

pipetting and vacuum filtration to afford the title compound ligand coupled to SpectralGel resin.

Example 7

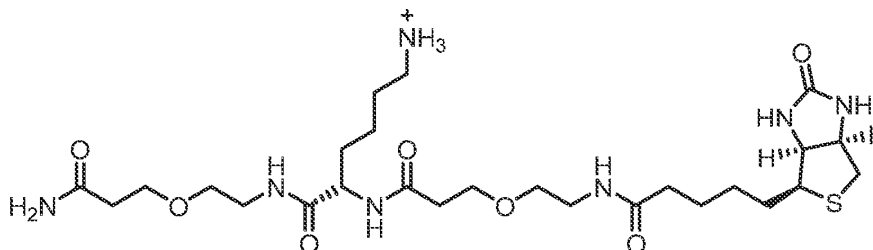


Biotin tethered to PEG1-PEG1 linker SpectralGel resin



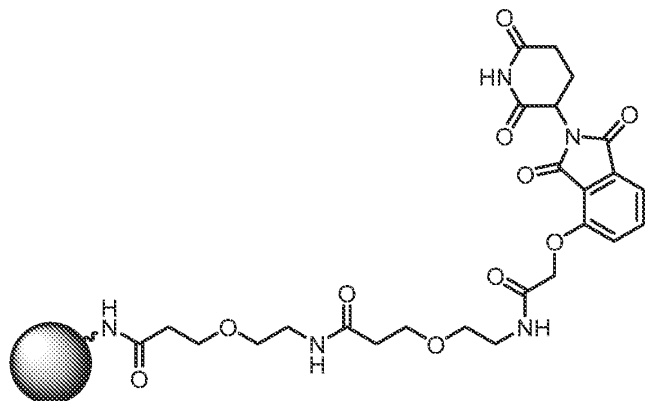
Releasable D-Biotin tethered to PEG1-Lysine-PEG1 linker of SpectralGel resin (quality control example)

[0344] Following the method of Example 3, 5 mg of Biotin tethered SpectraGel Resin was prepared from 7.3 mg of (0.030 mmol) D-Biotin and Fmoc-PEG1-Lysine-PEG1-RAM resin prepared by the methods of Examples 2 and 3.

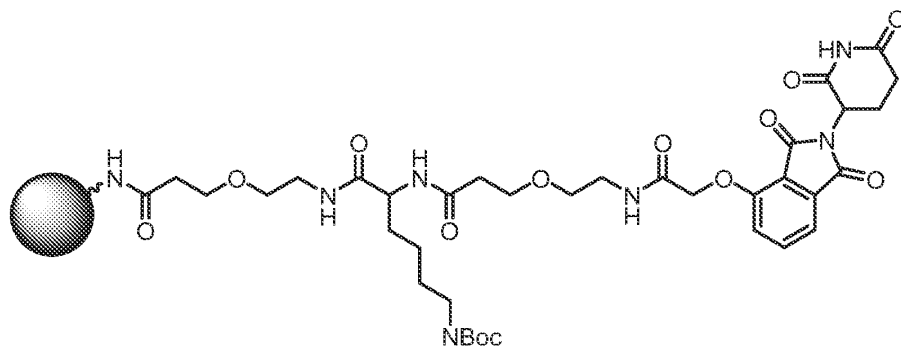


[0345] To 2.0 mg of D-Biotin-tethered PEG1-Lysine-PEG1-RAM SpectraGel resin in a 3 mL syringe with a 10 μ m fritted filter was added 300 μ L of 95% trifluoroacetic acid (TFA), 2.5% water & 2.5% triisopropylsilane (TIPS) solution. The mixture was mixed with gentle vortexing and allowed to rest at room temperature for 10 minutes. The filtrate was collected into a 2 mL Eppendorf tube and was then concentrated until dry via nitrogen and needle aspiration to afford D-Biotin amide PEG1-Lysine-PEG1-NH₂. TFA salt. ESI-MS (m/z) found: 602.3 [M+1]⁺

Example 8

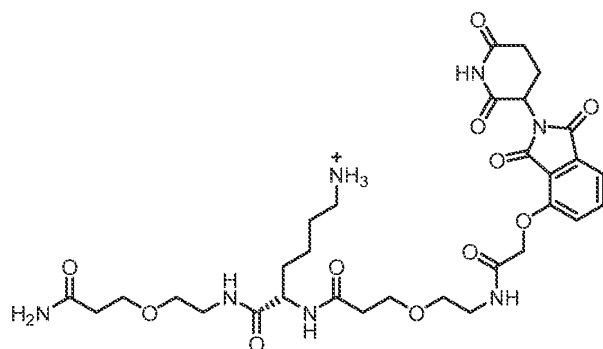


Thalidomide (Thalidomide-4-hydroxyacetate) tethered to PEG1-PEG1 SpectralGel resin



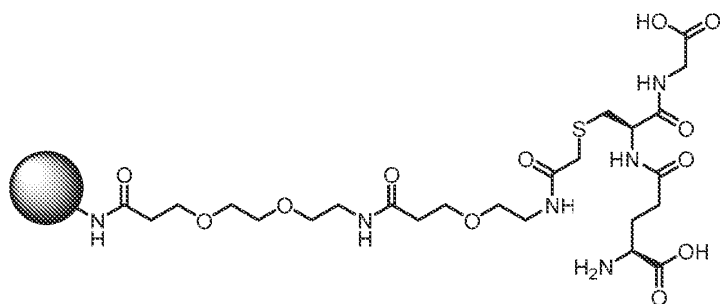
Releasable thalidomide-4-hydroxyacetate tethered to PEG1-Lysine(Boc)-PEG1 SpectralGel resin (quality control example)

[0346] Following the method of Example 6, 5 mg of Thalidomide-4-hydroxyacetate tethered PEG1-PEG1 SpectraGel Resin was prepared from 10.0 mg of (0.030 mmol)-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)oxy)acetic acid. In parallel, thalidomide-4-hydroxyacetate was coupled to Fmoc-PEG1-Lysine(Boc)-PEG1-RAM resin with the same reagents.

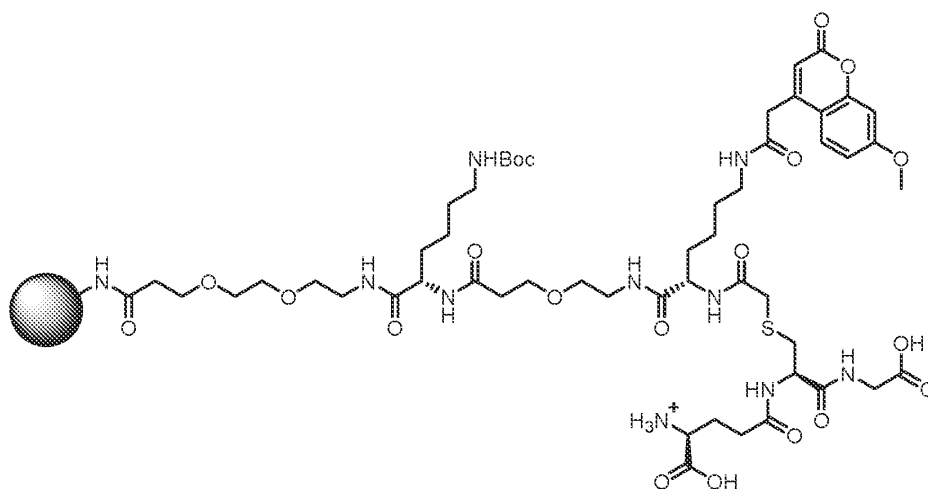


[0347] To 2.0 mg of thalidomide-4-hydroxyacetyl-PEG1-Lysine-PEG1-RAM tethered SpectraGel resin in a 3 mL syringe with a 10 μ m fritted filter was added 300 μ L of 95% TFA, 2.5% water and 2.5% TIPS solution. The mixture was mixed with gentle vortexing and allowed to rest at room temperature for 10 minutes. The filtrate was collected into a 2 mL Eppendorf tube and was concentrated until dry via nitrogen and needle aspiration to afford thalidomide-4-hydroxyacetyl-PEG1-Lysine-PEG1-NH₂ TFA salt. ESI-MS (m/z) found: 690.3 [M+1]⁺

Example 9



Glutathione tethered to PEG1-PEG2 SpectralGel resin



Releasable glutathione tethered to Lysine(MCA)-PEG1-Lysine-PEG2 SpectralGel resin (quality control example)

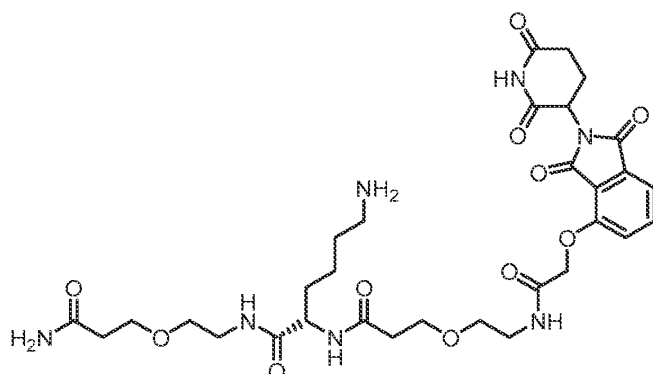
[0348] Following the method of Example 6, 5 mg of chloroacetamide tethered Spectragel Resin was prepared from 3.5 mg of (0.030 mmol) sodium chloroacetate and Fmoc-PEG1-PEG2 resin. In parallel, chloroacetamide was coupled to Fmoc-PEG1-Lysine(Boc)-PEG2-RAM resin with the same reagents.

[0349] To the resins within the syringes were then added 200 μ L of a 50 mM solution of L-glutathione (γ -L-Glutamyl-L-cysteinyl-glycine) in DMF containing 1% H₂O, followed by 0.012 mmol N,N-Diisopropylethylamine (DIEA), 60 mM in DMF. The reaction mixture with beads was allowed to mix with gentle agitation for 3 hours at room temperature. After this, the reaction mixture was removed by vacuum filtration and the resin was washed with 300 μ L DMF (5 x 300 μ L) with pipetting and vacuum filtration to afford the title compound ligand coupled to both SpectralGel resins.

TFA salt

[0350] To 2.0 mg of glutathione tethered acetamide-Lysine(MCA)-PEG1-Lys(Boc)-PEG2-RAM SpectraGel resin in a 3 mL syringe with a 10 μ m fritted filter was added 300 μ L of 95% TFA, 2.5% water and 2.5% TIPS solution. The mixture was mixed with gentle vortexing and incubated at room temperature for 10 minutes. The filtrate was collected into a 2 mL Eppendorf tube and concentrated until dry via nitrogen and needle aspiration to afford glutathione acetamide-Lysine(MCA)-PEG1-Lysine-PEG2-NH₂ TFA salt. ESI-MS (m/z) found: 1111.5 [M+1]⁺, 556.3 [M+2]²⁺

Example 10



[0351] To 1.0 mg of thalidomide-4-hydroxyacetamide-PEG1-Lysine(Boc)-PEG1-PCL SpectralGel resin in a single 300 μ L well of a AcroPrep advance 96-well polypropylene plate loaded with 0.20 μ m wwPTFE membrane filters was added 150 μ L of a 50% 10 mM ammonium formate (HCO_2NH_4) in water, 50% acetonitrile solution. The plate was placed under a 365 nm Lumidox array lamp with controller set to 25 mW and the plate was vortexed at 500 rpm at room temperature for 20 minutes. The filtrate was collected into a 96-well collection plate using vacuum filtration to afford thalidomide amide-PEG1-Lysine-PEG1 as an approximately 10 mM solution. ESI-MS (m/z) found: 690.3 $[\text{M}+1]^+$

Example 11

[0352] A 5.0 mg sample of Glutathione-acetamide-Lysine(MCA)-PEG1-Lysine-PEG2-PCL was prepared by the method of Example 9. To a 1.0 mg sample of this resin within a single 300 μ L well of an AcroPrep advance 96-well polypropylene plate loaded with 0.20 μ m wwPTFE membrane filters was added 150 μ L of a 50% 10 mM ammonium formate (HCO_2NH_4) in water, 50% acetonitrile solution. The plate was placed under a 365 nm UV Lumidox array lamp with controller set to 25 mW and was vortexed at 500 rpm at room temperature for 20 minutes. The filtrate was collected into a 96-well collection plate using vacuum filtration to afford glutathione thioether tethered PEG1-Lysine-PEG1-NH₂ as an approximately 10 mM solution. ESI-MS (m/z) found: 1111.5 $[\text{M}+1]^+$, 556.3 $[\text{M}+2]^{2+}$

Example 12

[0353] Fmoc-Asp((tBu)-Tyr-Lys(Boc)-Asp(tBu)-Asp(tBu)-Asp(tBu)-Asp(tBu)-Lys(Boc)-PEG1-Lys(MCA)-PCL-Ala-RAM Resin

[0354] A 5 mg sample of the SpectraGel resin from example 2 was coupled with successive amino acids by the method of example 6.

[0355] Fmoc-Asp-Tyr-Lys-Asp-Asp-Asp-Lys-PEG1-Lys(MCA)-PCL-Ala-NH₂

[0356] To 1.0 mg of Fmoc-Asp((tBu)-Tyr-Lys(Boc)-Asp(tBu)-Asp(tBu)-Asp(tBu)-Asp(tBu)-Lys(Boc)-PEG1-Lys(MCA)-PCL-Ala-RAM Resin linker peptide SpectralGel resin, within a 3 mL syringe with a 10 μ m fritted filter was added 300 μ L of a 95% TFA, 2.5 % water and 2.5 % TIPS solution. The mixture was mixed with gentle vortexing and allowed to incubate at room temperature for 30 minutes. The filtrate was collected into a 2 mL Eppendorf tube and was then concentrated until dry using nitrogen and needle aspiration to afford the title peptide ligand.

[0357] LCMS found UV absorption @ 340 ~60% AUC title compound ESI (m/z) 2045.8 [M+3]⁺, 1023.4 [M+2]²⁺, 682.6 [M+3]³⁺. ~40% AUC title compound-Fmoc ESI (m/z) 911.9 [M+2]²⁺, 608.3 [M+2]³⁺.

Example 13

[0358] On bead binding assay: biotin-streptavidin

[0359] Beads from example 4 (~500k) and acetylated beads prepared by the general method using acetic anhydride (~500k) were separately aliquoted and washed with tris buffered saline containing 0.1% Tween 20 (TBST) (3 x 500 μ L). Each bead sample was blocked by incubating in 1:1 StartingBlock(ThermoFisher cat#37543)/TBST (4 °C, 16 h) with rotation (10 rpm). The beads were collected and washed with ice-cold 1:1 StartingBlock/TBST (3 x 500 μ L). The bead samples were resuspended in 1 mL of 1:1 StartingBlock/TBST and aliquoted into 5 tubes with ~100k beads each. The beads were pelleted and incubated with AlexaFluor 647-labeled streptavidin (0 pM, 30 pM, 1 nM, 30 nM, 1 μ M; 100 μ L total volume) in ice-cold 1:1 StartingBlock/TBST (4°C, 16 h) with rotation (10 rpm). Afterwards, the beads were pelleted and washed with TBST (3 x 500 μ L). The beads were resuspended in 500 μ L TBST and incubated (4°C, 4 h) with rotation (10 rpm) prior to flow cytometry.

[0360] Each bead sample was filtered through a 40 μ m mesh filter and analyzed using an Attune NxT flow cytometer (ThermoFisher Scientific) at 100 μ L/min using Attune 1X Focusing Fluid. Forward-scatter (488 nm, 50 V), side-scatter (488 nm, 230 V) and AlexaFluor 647 fluorescence (Ex: 637nm; Em: 670/14 nm; 240 V) were recorded for each bead.

[0361] FIG. 24 illustrates characterization of biotin and acetylated beads in the presence of fluorophore-labeled streptavidin prepared in Example 13. Panel (A) of FIG. 24 is a diagram representing negative control i.e. AlexaFluor 647 (AF-647) labelled streptavidin not bound to acetylated beads due to the absence of biotin ligands on these beads. Panels (B) and (C) of FIG. 24 show the corresponding brightfield and fluorescence images (obtained using

EVOS fluorescence microscope) clearly showing no bead bound fluorescence observed in negative control. Panel (D) of FIG. 24 is a diagram representing positive control i.e.

AlexaFluor 647 labelled streptavidin bound biotin functionalized beads due to its high affinity interaction towards biotin. Panels (E) and (F) of FIG. 24 show the corresponding brightfield and fluorescence images clearly showing bead bound fluorescence observed in positive control.

[0362] FIG. 25 illustrates characterization of biotin and acetylated beads in the presence of fluorophore-labeled streptavidin prepared in Example 13. More specifically, FIG. 25 is a flow histogram showing negative control (acetylated beads not bound to AF-647 streptavidin) and positive control (biotinylated beads bound to AF-647 streptavidin) when the biotin functionalized beads were incubated with 11 μ M AlexaFluor 647-labeled streptavidin. Data was captured using Attune flow cytometer. Bead bound fluorescence in the positive control which is several orders of magnitude greater than the negative control is a clear representation of selective on-bead capture of the target protein “AF-647 streptavidin”.

[0363] FIG. 26 illustrates fluorescence response with increasing concentrations of labeled streptavidin using beads prepared in Example 13. More specifically, FIG. 26 is a flow histogram showing fluorescence response with increasing concentrations of AF-647 labeled streptavidin. Biotin functionalized beads were incubated with varying concentrations of AF-647 streptavidin. This example is a clear representation of different surface bound fluorescence intensities indirectly representing the case of a library of small molecules containing different varying affinities to any given target protein.

ADDITIONAL EXAMPLES

[0364] Example A1. A composite particle, comprising:
an optically detectable particle; and

a network polymer gel substantially surrounding the optically detectable particle.

[0365] Example A2. The composite particle of example A1, comprising a plurality of the optically detectable particles.

[0366] Example A3. The composite particle of example A2, wherein at least some of the optically detectable particles have different emission wavelengths than one another.

[0367] Example A4. The composite particle of example A3, wherein all of the optically detectable particles have different emission wavelengths than one another.

[0368] Example A5. The composite particle of example A3 or example A4, comprising at least three of the optically detectable particles with different emission wavelengths from one another.

- [0369] Example A6. The composite particle of any one of examples A3 to A5, comprising at least six of the optically detectable particles with different emission wavelengths from one another.
- [0370] Example A7. The composite particle of any one of examples A2 to A6, wherein the optically detectable particles are randomly oriented relative to one another within the network polymer gel.
- [0371] Example A8. The composite particle of any one of examples A2 to A6, wherein the optically detectable particles have substantially the same orientation as one another within the network polymer gel.
- [0372] Example A9. The composite particle of any one of examples A1 to A8, wherein the optically detectable particle is configured to emit light with a bandwidth of about 1 nm or less at full-width half-maximum (FWHM) responsive to excitation light.
- [0373] Example A10. The composite particle of example A9, wherein the optically detectable particle comprises a microdisk laser particle.
- [0374] Example A11. The composite particle of example A10, wherein the microdisk laser particle has a diameter of about 1 μm to about 10 μm .
- [0375] Example A12. The composite particle of example A10 or example A11, wherein the light emitted by the microdisk laser particle is near-infrared.
- [0376] Example A13. The composite particle of any one of examples A1 to A8, wherein the optically detectable particle is configured to emit light with a bandwidth of about 40 nm or less at full-width half-maximum (FWHM) responsive to excitation light.
- [0377] Example A14. The composite particle of example A13, wherein the optically detectable particle comprises a CsPbBr₃ Perovskite quantum dot.
- [0378] Example A15. The composite particle of any one of examples A1 to A14, wherein the network polymer gel comprises a chemical moiety which is couplable to a chemical compound.
- [0379] Example A16. The composite particle of example A15, wherein the chemical moiety is cleavable from the network polymer gel using light, heat, or a reagent.
- [0380] Example A17. The composite particle of example A15 or A16, wherein the chemical moiety is distributed throughout the network polymer gel.
- [0381] Example A18. The composite particle of any one of examples A15 to A17, wherein the chemical moiety is positively charged.
- [0382] Example A19. The composite particle of any one of examples A15 to A17, wherein the chemical moiety is negatively charged.

[0383] Example A20. The composite particle of any one of examples A15 to A17, wherein the chemical moiety is uncharged.

[0384] Example A21. The composite particle of any one of examples A1 to A20, further comprising chemical compounds coupled to the network polymer gel.

[0385] Example A22. The composite particle of example A21, wherein the chemical compounds are cleavable from the network polymer gel using light, heat, or a reagent.

[0386] Example A23. The composite particle of example A21 or A22, wherein the chemical compounds are distributed throughout the network polymer gel.

[0387] Example A24. The composite particle of any one of examples A21 to A23, wherein the chemical compounds are the same as one another.

[0388] Example A25. The composite particle of any one of examples A21 to A23, wherein the chemical compounds are different than one another.

[0389] Example A26. The composite particle of any one of examples A1 to A25, wherein the optically detectable particle is covalently coupled to the network polymer gel.

[0390] Example A27. The composite particle of any one of examples A1 to A26, further comprising:

a carrier particle having an outer surface;

wherein the optically detectable particle is coupled to the outer surface of the carrier particle; and

wherein the network polymer gel substantially surrounds the carrier particle and the optically detectable particle coupled to the carrier particle.

[0391] Example A28. The composite particle of example A27, comprising a plurality of the optically detectable particles coupled to the outer surface of the carrier particle.

[0392] Example A29. The composite particle of example A27 or A28, wherein the carrier particle comprises a polymer or a glass.

[0393] Example A30. The composite particle of any one of examples A27 to A29, wherein the carrier particle has a different composition than the network polymer gel.

[0394] Example A31. The composite particle of any one of examples A27 to A30, wherein the carrier particle has the same composition as the network polymer gel.

[0395] Example A32. The composite particle of any one of examples A27 to A31, wherein the carrier particle has a diameter of between about 1 μm and about 20 μm in water at pH 7.

[0396] Example A33. The composite particle of any one of examples A27 to A32, wherein the carrier particle has a diameter of between about 5 μm and about 15 μm in water at pH 7.

[0397] Example A34. The composite particle of any one of examples A27 to A33, wherein the carrier particle has a diameter of between about 1 μm and about 5 μm in water at pH 7.

[0398] Example A35. The composite particle of any one of examples A27 to A34, wherein the composite particle is at least about 100 nm larger than the carrier particle in water at pH 7.

[0399] Example A36. The composite particle of any one of examples A27 to A35, wherein the optically detectable particle is coupled to the carrier particle via a covalent bond.

[0400] Example A37. The composite particle of any one of examples A27 to A36, wherein the optically detectable particle is coupled to the carrier particle via a non-covalent bond.

[0401] Example A38. The composite particle of any one of examples A27 to A37, further comprising:

at least one additional carrier particle having an outer surface; and

an additional optically detectable particle coupled to the outer surface of the at least one additional carrier particle,

wherein the network polymer gel substantially surrounds the at least one additional carrier particle and the additional optically detectable particle coupled to the at least one additional carrier particle.

[0402] Example A39. The composite particle of any one of examples A1 to A38, wherein the composite particle has a diameter of between about 1 μm and about 50 μm in water at pH 7.

[0403] Example A40. The composite particle of any one of examples A1 to A39, wherein the composite particle has a diameter of between about 10 μm and about 30 μm in water at pH 7.

[0404] Example A41. The composite particle of any one of examples A1 to A40, wherein the composite particle has a diameter of between about 5 μm and about 20 μm in water at pH 7.

[0405] Example A42. A collection of the composite particles of any one of examples A1 to A41.

[0406] Example A43. The collection of example A42, wherein:

each of the composite particles of the collection comprises a plurality of the optically detectable carrier particles,

at least some of the optically detectable particles of that plurality have different emission wavelengths than one another, and

the different emission wavelengths define a spectral fingerprint for that composite particle.

[0407] Example A44. The collection of example A43, wherein a majority of the composite particles of the collection have different spectral fingerprints than one another.

[0408] Example A45. The collection of example A43 or A44, wherein over 80% of the composite particles of the collection have different spectral fingerprints than one another.

[0409] Example A46. A collection of the composite particles of any one of examples A42 to A45, wherein the composite particles of the collection respectively include substantially the same number of optically detectable particles as one another.

[0410] Example A47. The collection of the composite particles of example A46, wherein the composite particles of the collection respectively include between two and ten of the optically detectable particles.

[0411] Example A48. The collection of the composite particles of any one of examples A42 to A45, wherein the composite particles of the collection respectively include different numbers of optically detectable particles than one another.

[0412] Example A49. The collection of the composite particles of example A48, wherein the composite particles of the collection respectively include between two and ten of the optically detectable particles.

[0413] Example A50. A collection of the composite particles of any one of examples A42 to A49, wherein a majority of the composite particles of the collection respectively include the same number of carrier particles as one another.

[0414] Example A51. The collection of the composite particles of example A50, wherein the composite particles of the collection respectively include one to three of the carrier particles.

[0415] Example A52. A composition, comprising the collection of the composite particles of any one of examples A42 to A51 suspended in an aqueous solvent.

[0416] Example A53. A composition, comprising the collection of the composite particles of any one of examples A4 suspended in an organic solvent.

[0417] Example B1. A method of making a composite particle, the method comprising:

substantially surrounding an optically detectable particle with a network polymer gel.

[0418] Example B2. The method of example B1, wherein substantially surrounding the optically detectable particle with the network polymer gel comprises:

forming a droplet comprising a crosslinker, an initiator, and the optically detectable particle; and

using the initiator to polymerize the crosslinker to form the network polymer gel within the droplet.

[0419] Example B3. The method of example B2, wherein the droplet further comprises a non-functional monomer, or a functional monomer, and wherein the initiator polymerizes the crosslinker, non-functional monomer, or functional monomer together with the monomer to form the network polymer gel within the droplet.

[0420] Example B4. The method of example B2 or B3, wherein the initiator comprises a photo-initiator, and wherein using the initiator to polymerize the monomer comprises irradiating the droplet with light.

[0421] Example B5. The method of any one of examples B2 to B4, wherein forming the droplet comprises suspending the optically detectable particle in a solution comprising the monomer and the initiator.

[0422] Example B6. The method of example B5, wherein forming the droplet comprises encapsulating a defined volume of the solution in an oil/surfactant mixture using a microfluidic junction.

[0423] Example B7. The method of example B5, wherein forming the droplet comprises forming an emulsion with the solution and an oil/surfactant mixture.

[0424] Example B8. The method of any one of examples B1 to B7, further comprising substantially surrounding at least one additional optically detectable particle with the network polymer gel.

[0425] Example B9. The method of example B8, wherein the optically detectable particle and the at least one additional optically detectable particle are randomly oriented relative to one another within the network polymer gel.

[0426] Example B10. The method of example B8, wherein the optically detectable particle and the at least one additional optically detectable particle have substantially the same orientation as one another within the network polymer gel.

[0427] Example B11. The method of any one of examples B1 to B10, wherein the optically detectable particle is coupled to a chemical moiety, the method comprising coupling the chemical moiety to the network polymer gel.

[0428] Example B12. The method of example B11, wherein the chemical moiety is covalently coupled to the network polymer gel.

[0429] Example B13. The method of example B11 or B12, wherein the chemical moiety comprises a component of the network polymer gel.

[0430] Example B14. The method of any one of examples B11 to B13, further comprising coupling the chemical moiety to the optically detectable particle.

[0431] Example B15. The method of any one of examples B1 to B14, further comprising coupling the optically detectable particle to an outer surface of a carrier particle; and

substantially surrounding the carrier particle, with the optically detectable particle coupled thereto, with the network polymer gel.

[0432] Example B16. The method of example B15, comprising coupling a plurality of additional optically detectable particles to the outer surface of the carrier particle.

[0433] Example B17. The method of any one of examples B15 or B16, wherein the carrier particle comprises a polymer or a glass.

[0434] Example B18. The method of any one of examples B15 to B17, wherein the carrier particle has a different composition than the network polymer gel.

[0435] Example B19. The method of any one of examples B15 to B17, wherein the carrier particle has the same composition as the network polymer gel.

[0436] Example B20. The method of any one of examples B15 to B19, wherein the optically detectable particle is coupled to the carrier particle via a covalent bond.

[0437] Example B21. The method of example B20, wherein the carrier particle is coupled to a first chemical moiety and the optically detectable particle is coupled to a second chemical moiety, the method comprising covalently coupling the first chemical moiety to the second chemical moiety to form the covalent bond.

[0438] Example B22. The method of example B21, further comprising coupling the first chemical moiety to the carrier particle.

[0439] Example B23. The method of example B21 or B22, further comprising coupling the second chemical moiety to the optically detectable particle.

[0440] Example B24. The method of any one of examples B15 to B19, wherein the optically detectable particle is coupled to the carrier particle via a non-covalent bond.

[0441] Example B25. The method of example B24, wherein the non-covalent bond comprises an ionic bond.

- [0442] Example B26. The method of example B25, wherein the optically detectable particle is negatively charged, and wherein the carrier particle is positively charged.
- [0443] Example B27. The method of example B25, wherein the optically detectable particle is positively charged, and wherein the carrier particle is negatively charged.
- [0444] Example B28. The method of any one of examples B1 to B27, wherein the optically detectable particle is configured to emit light with a bandwidth of about 1 nm or less at full-width half-maximum (FWHM) responsive to excitation light.
- [0445] Example B29. The method of example B28, wherein the optically detectable particle comprises a microdisk laser particle.
- [0446] Example B30. The method of any one of examples B1 to B27, wherein the optically detectable particle is configured to emit light with a bandwidth of about 40 nm or less at full-width half-maximum (FWHM) responsive to excitation light.
- [0447] Example B31. The method of example B30, wherein the optically detectable particle comprises a CsPbBr₃ Perovskite quantum dot.
- [0448] Example C1. A library of chemical compounds, the library comprising:
composite particles, the composite particles respectively comprising:
a plurality of optically detectable particles; and
a network polymer gel substantially surrounding the optically detectable particles; and
a chemical compound coupled to the network polymer gel,
wherein the composite particles of a first group of the composite particles comprise the same chemical compound as one another; and
wherein the composite particles of a second group of the composite particles comprise the same chemical compound as one another and a different chemical compound than that of the first group of composite particles.
- [0449] Example C2. The library of example C1, wherein the chemical compound is covalently coupled to the network polymer gel.
- [0450] Example C3. The library of example C1, wherein the chemical compound is non-covalently coupled to the network polymer gel.
- [0451] Example C4. The library of example C3, wherein the chemical compound is adsorbed to the network polymer gel.
- [0452] Example C5. The library of any one of examples C1 to C4, wherein the composite particles of the first group of composite particles comprise a first plurality of different chemical compounds.

[0453] Example C6. The library of example C5, wherein the composite particles of the second group of composite particles comprise a second plurality of different chemical compounds, wherein the chemical compounds of the first plurality are at least partially different than the chemical compounds of the second plurality.

[0454] Example C7. The library of example C5 or C6, wherein the first plurality of different chemical compounds comprises between two and four different chemical compounds.

[0455] Example C8. The library of any one of examples C1 to C7, wherein the chemical compound is distributed throughout the network polymer gel.

[0456] Example C9. The library of any one of examples C1 to C8, wherein the chemical compound is cleavable from the network polymer gel using light, heat, or a reagent.

[0457] Example C10. The library of any one of examples C1 to C9, wherein the optically detectable particles are configured to emit light with a bandwidth of about 1 nm or less at full-width half-maximum (FWHM) responsive to excitation light.

[0458] Example C11. The library of example C10, wherein the optically detectable particles comprise microdisk laser particles.

[0459] Example C12. The library of any one of examples C1 to C9, wherein the optically detectable particles are configured to emit light with a bandwidth of about 40 nm or less at full-width half-maximum (FWHM) responsive to excitation light.

[0460] Example C13. The library of example C12, wherein the optically detectable particles comprise CsPbBr₃ Perovskite quantum dots.

[0461] Example C14. The library of any one of examples C1 to C13, wherein the optically detectable particles are randomly oriented relative to one another within the network polymer gel.

[0462] Example C15. The library of any one of examples C1 to C13, wherein the optically detectable particles have substantially the same orientation as one another within the network polymer gel.

[0463] Example C16. The library of any one of examples C1 to C15, wherein the optically detectable particles are covalently coupled to the network polymer gel.

[0464] Example C17. The library of any one of examples C1 to C16, the composite particles respectively further comprising a carrier particle having an outer surface, wherein:

the optically detectable particles are coupled to the outer surface of the carrier particle; and

the network polymer gel substantially surrounds the carrier particle and the optically detectable particle coupled to the carrier particle.

[0465] Example C18. The library of example C17, wherein the optically detectable particles respectively are coupled to the carrier particle via covalent bonds.

[0466] Example C19. The library of example C17, wherein the optically detectable particles respectively are coupled to the carrier particle via non-covalent bonds.

[0467] Example C20. The library of any one of examples C17 to C19, the composite particles respectively further comprising:

at least one additional carrier particle having an outer surface; and

a plurality of additional optically detectable particles coupled to the outer surface of the at least one additional carrier particle,

wherein the network polymer gel substantially surrounds the at least one additional carrier particle and the additional optically detectable particles coupled to the at least one additional carrier particle.

[0468] Example C21. A method of generating a library of chemical compounds, the method comprising:

irradiating a collection of composite particles with excitation light, the composite particles of the collection respectively comprising:

a plurality of optically detectable particles; and

a network polymer gel substantially surrounding the optically detectable particles;

respectively obtaining emission spectra from the optically detectable particles of the composite particles;

coupling chemical compounds to the composite particles by coupling the chemical compounds to the network polymer gels of the composite particles,

wherein the composite particles of a first group of composite particles are coupled to the same chemical compound as one another,

wherein the composite particles of a second group of composite particles are coupled to the same chemical compound as one another, and

wherein the chemical compound coupled to the first group of composite particles is different than the chemical compound coupled to the second group of composite particles; and

for respective composite particles of the collection, storing, in a data structure, (i) identifiers for the composite particles which are based on the respectively obtained emission

spectra, and (ii) identifiers for the chemical compounds which respectively are coupled to those composite particles.

[0469] Example C22. The method of example C21, wherein coupling the chemical compounds to the composite particles comprises:

(a) dividing the collection of composite particles into subsets; and

(b) for respective subsets formed in operation (a), coupling a chemical moiety to the network polymer gel of the composite particles of that subset to form a modified subset.

[0470] Example C23. The method of example C22, wherein the chemical moiety coupled to the network polymer gel of the composite particles of a first one of the subsets is different than the chemical moiety coupled to the network polymer gel of the composite particles of a second one of the subsets.

[0471] Example C24. The method of example C22, wherein the chemical moiety coupled to the network polymer gel of the composite particles of a first one of the subsets is the same as the chemical moiety coupled to the network polymer gel of the composite particles of a second one of the subsets.

[0472] Example C25. The method of any one of examples C22 to C24, wherein dividing the collection of composite particles into subsets comprises:

using a flow cytometer to dispense a first subset of the composite particles of the collection into a first reservoir; and

using the flow cytometer to dispense a second subset of the composite particles of the collection into a second reservoir.

[0473] Example C26. The method of example C25, wherein dividing the collection of composite particles into subsets further comprises counting composite particles as they flow through the flow cytometer and into the first reservoir,

wherein the composite particles are dispensed into the first reservoir until a predetermined number of the composite particles are dispensed into the first reservoir, and

wherein after the predetermined number of the composite particles are dispensed into the first reservoir, the composite particles are dispensed into the second reservoir until a predetermined number of the composite particles are dispensed into the first reservoir.

[0474] Example C27. The method of example C26, wherein the composite particles are counted using the emission spectra.

[0475] Example C28. The method of any one of examples C22 to C24, wherein the collection of composite particles is manually divided into respective reservoirs to form the subsets.

[0476] Example C29. The method of any one of examples C22 to C28, further comprising, for the composite particles of respective subsets formed in operation (a):
irradiating the composite particles of that subset with excitation light;
respectively obtaining the emission spectra from the optically detectable particles of the composite particles of that subset; and
using the emission spectra to identify the composite particles of that subset using the stored identifiers.

[0477] Example C30. The method of example C29, wherein the emission spectra from the optically detectable particles of the composite particles of that subset are obtained one at a time as the composite particles flow through a channel.

[0478] Example C31. The method of example C29, wherein the emission spectra from the optically detectable particles of the composite particles of that subset are obtained before that subset of particles is formed.

[0479] Example C32. The method of example C29, wherein the emission spectra from the optically detectable particles of the composite particles of that subset are obtained after that subset of particles is formed.

[0480] Example C33. The method of any one of examples C22 to C32, wherein, for the composite particles of respective subsets formed in operation (a), the identifier for the chemical compound which is coupled to those composite particles comprises an identifier for the chemical moiety which is coupled to the network polymer gel in operation (b).

[0481] Example C34. The method of any one of examples C22 to C33, further comprising (c) pooling the modified subsets of operation (b) to form a modified collection of composite particles.

[0482] Example C35. The method of example C34, wherein coupling the chemical compounds to the composite particles further comprises:

(d) dividing the modified collection of composite particles of operation (c) into subsets; and

(e) for respective subsets formed in operation (d), coupling a chemical moiety to the to the chemical moiety which was coupled to the network polymer gel in operation (b) to form a modified subset.

[0483] Example C36. The method of example C35, further comprising, for the composite particles of respective subsets formed in operation (d):

irradiating the composite particles of that subset with excitation light;

respectively obtaining the emission spectra from the optically detectable particles of the composite particles of that subset; and

using the emission spectra to identify the composite particles of that subset using the stored identifiers.

[0484] Example C37. The method of example C35 or C36, wherein, for the composite particles of respective subsets formed in operation (d), the identifier for the chemical compound which is coupled to those composite particles comprises (i) an identifier for the chemical moiety which is coupled to the network polymer gel in operation (b) and (ii) an identifier for the chemical moiety which is coupled in operation (e) to the chemical moiety which was coupled to the network polymer gel in operation (b).

[0485] Example C38. The method of any one of examples C35 to C38, further comprising repeating operations (c) through (e) for the modified collection of composite particles.

[0486] Example C39. The method of example C38, wherein operations (c) through (e) are repeated between one and five times.

[0487] Example C40. The method of example C39, wherein operation (b) and the repeated operations (c) through (e) build (i) the chemical compound coupled to the composite particles of the first group of composite particles, and (ii) the chemical compound coupled to the composite particles of the second group of composite particles.

[0488] Example C41. The method of any one of examples C21 to C40, wherein coupling the chemical compounds to the composite particles comprises using a condition, solvent, or reagent which is incompatible with DNA.

[0489] Example C42. The method of any one of examples C21 to C41, wherein the chemical compound is covalently coupled to the network polymer gel.

[0490] Example C43. The method of any one of examples C21 to C41, wherein the chemical compound is non-covalently coupled to the network polymer gel.

[0491] Example C44. The method of example C43, wherein the chemical compound is adsorbed to the network polymer gel.

[0492] Example C45. The method of any one of examples C21 to C44, wherein the composite particles of the first group of composite particles comprise a first plurality of different chemical compounds.

[0493] Example C46. The method of example C45, wherein the composite particles of the second group of composite particles comprises a second plurality of different chemical

compounds, wherein the chemical compounds of the first plurality are at least partially different than the chemical compounds of the second plurality.

[0494] Example C47. The method of example C45 or C46, wherein the first plurality of different chemical compounds comprises between two and four different chemical compounds.

[0495] Example C48. The method of any one of examples C21 to C47, wherein the chemical compound is distributed throughout the network polymer gel.

[0496] Example C49. The method of any one of examples C21 to C48, wherein the chemical compound is cleavable from the network polymer gel using light, heat or a reagent.

[0497] Example C50. The method of any one of examples C21 to C49, wherein the identifiers for the composite particles which are based on the respectively obtained emission spectra comprise lists of wavelengths having peaks in the emission spectra.

[0498] Example C51. The method of any one of examples C21 to C49, wherein the identifiers for the composite particles which are based on the respectively obtained emission spectra comprise bits representing wavelengths having peaks in the emission spectra.

[0499] Example C52. The method of any one of examples C21 to C51, wherein the identifiers for the chemical compounds which respectively are coupled to those composite particles comprise a chronological description of chemical reactions performed using those composite particles.

[0500] Example C53. The method of any one of examples C21 to C51, wherein the identifiers for the chemical compounds which respectively are coupled to those composite particles comprise tokens chronologically representing chemical reactions performed using those composite particles.

[0501] Example C54. The method of any one of examples C21 to C53, wherein the optically detectable particles are configured to emit light with a bandwidth of about 1 nm or less at full-width half-maximum (FWHM) responsive to excitation light.

[0502] Example C55. The method of example C54, wherein the optically detectable particles comprise microdisk laser particles.

[0503] Example C56. The method of any one of examples C21 to C55, wherein the optically detectable particles are configured to emit light with a bandwidth of about 40 nm or less at full-width half-maximum (FWHM) responsive to excitation light.

[0504] Example C57. The method of example C56, wherein the optically detectable particles comprise CsPbBr₃ Perovskite quantum dots.

[0505] Example C58. The method of any one of examples C21 to C57, wherein the optically detectable particles are randomly oriented relative to one another within the network polymer gel.

[0506] Example C59. The method of any one of examples C21 to C58, wherein the optically detectable particles have substantially the same orientation as one another within the network polymer gel.

[0507] Example C60. The method of any one of examples C21 to C59, wherein the optically detectable particles are covalently coupled to the network polymer gel.

[0508] Example C61. The method of any one of examples C21 to C60, the composite particles respectively further comprising a carrier particle having an outer surface, wherein: the optically detectable particles are coupled to the outer surface of the carrier particle; and

[0509] the network polymer gel substantially surrounds the carrier particle and the optically detectable particle coupled to the carrier particle.

[0510] Example C62. The method of example C61, wherein the optically detectable particles respectively are coupled to the carrier particle via covalent bonds.

[0511] Example C63. The method of example C61, wherein the optically detectable particles respectively are coupled to the carrier particle via non-covalent bonds.

[0512] Example C4. The method of any one of examples C61 to C63, the composite particles respectively further comprising:

at least one additional carrier particle having an outer surface; and

a plurality of additional optically detectable particles coupled to the outer surface of the at least one additional carrier particle,

wherein the network polymer gel substantially surrounds the at least one additional carrier particle and the additional optically detectable particles coupled to the at least one additional carrier particle.

[0513] Example D1. A method of detecting binding between different chemical compounds and a target, the method comprising:

incubating a first target with a collection of composite particles, the composite particles of the collection comprising:

a plurality of optically detectable particles;

a network polymer gel substantially surrounding the optically detectable particles; and

a chemical compound coupled to the network polymer gel,

wherein at least some of the composite particles comprise the same chemical compound as one another, and

wherein at least some of the composite particles comprise different chemical compounds than one another, and

wherein the first target binds differently to different ones of the chemical compounds;

removing unbound first target from the collection of composite particles;

irradiating the composite particles of the collection, with the first target bound to different ones of the chemical compounds, with excitation light;

obtaining emission spectra from the optically detectable particles of respective composite particles;

obtaining signals respectively indicating whether the first target binds to the chemical compounds of respective composite particles;

using the emission spectra and the signal to (i) identify the chemical compound which is coupled to at least some of the composite particles and (ii) determine whether the first target binds to that chemical compound.

[0514] Example D2. The method of example D1, wherein the first target is labeled with a probe before the incubating.

[0515] Example D3. The method of example D1, further comprising, after the first target binds differently to different ones of the chemical compounds, labeling the first target with an affinity reagent that is labeled with a probe.

[0516] Example D4. The method of example D1, further comprising, after the first target binds differently to different ones of the chemical compounds, incubating the first target with an unlabeled primary affinity reagent, then labeling the unlabeled primary affinity reagent with a secondary affinity reagent that is labeled with a probe.

[0517] Example D5. The method of any one of examples D1 to D4, wherein the composite particles are irradiated with a first wavelength of light that stimulates the emission spectra of the optically detectable particles.

[0518] Example D6. The method of example D5, wherein the composite particles are irradiated with a second wavelength of light that stimulates fluorescence which generates the signal indicating whether the first target binds to the chemical compounds of respective composite particles.

[0519] Example D7. The method of example D6, wherein the composite particles are irradiated with the first and second wavelengths of light at different times than one another.

[0520] Example D8. The method of example D6, wherein the composite particles are irradiated with the first and second wavelengths of light at the same time as one another.

[0521] Example D9. The method of any one of examples D1 to D8, wherein the emission spectra and signal are obtained concurrently with one another.

[0522] Example D10. The method of any one of examples D1 to D8, wherein the emission spectra and signal are obtained at different times than one another.

[0523] Example D11. The method of any one of examples D1 to D10, further comprising:

incubating a second target with the collection of composite particles, wherein the second target binds differently to different ones of the chemical compounds;

removing unbound second target from the collection of composite particles; and

obtaining signals indicating whether the second target binds to the chemical compounds of respective composite particles.

[0524] Example D12. The method of example D11, wherein the collection of composite particles is incubated concurrently with the first target and the second target.

[0525] Example D13. The method of example D11 or D12, wherein the signals comprise fluorescence, and wherein fluorescence from the first target is at a different wavelength than fluorescence from the second target.

[0526] Example D14. The method of any one of examples D1 to D13, wherein the emission spectra, the signals, or both the emission spectra and the signals, are obtained while the composite particles are static.

[0527] Example D15. The method of any one of examples D1 to D14, wherein the emission spectra, the signals, or both the emission spectra and the signals, are obtained while the composite particles are flowed through an inspection point.

[0528] Example D16. The method of any one of examples D1 to D15, wherein the signals are optical.

[0529] Example E1. A spectral fingerprint system for categorizing a plurality of composite particles suspended in a fluid, each composite particle comprising a plurality of optically detectable particles and a network mesh polymer substantially surrounding the optically detectable particles, the system comprising:

an optical excitation system;

an optical detector;
a flow cell;
at least one memory storing instructions; and
at least one processor configured to execute the instructions to perform operations comprising:

flowing the composite particles in the fluid through the flow cell;
exciting the composite particles in the flow cell with light from the optical excitation system;
reading, by the optical detector, a spectral fingerprint of each composite particle excited by the light; and
generating, using the spectral fingerprint of each composite particle, a composite particle identity of that composite particle.

[0530] Example E2. The system of example E1, wherein the optical excitation system comprises a laser.

[0531] Example E3. The system of example E1 or E2, wherein at least some of the optically detectable particles of each composite particle comprise different emission wavelengths than one another, and wherein the spectral fingerprint of each composite particle is based on the different emission wavelengths of the optically detectable particles of that composite particle.

[0532] Example E4. The system of any one of examples E1 to E3, wherein a majority of the composite particles have different spectral fingerprints than one another.

[0533] Example E5. The system of any one of examples E1 to E4, wherein the composite particles respectively comprise between two and ten of the optically detectable particles.

[0534] Example E6. The system of any one of examples E1 to D6, wherein at least some of the flow cell is positioned in an optical path between the optical excitation system and the optical detector.

[0535] Example E7. The system of example E6, wherein the step of reading, by the optical detector, the spectral fingerprint of each composite particle excited by the light comprises measuring an IR emission orthogonal to an excitation beam of the optical excitation system.

[0536] Example E8. The system of any one of examples E1 to E7, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

physically sorting the plurality of composite particles into a plurality of subsets.

[0537] Example E9. The system of example E8, wherein the step of physically sorting is based on the spectral fingerprints of the composite particles of the plurality.

[0538] Example E10. The system of example E8 or E9, wherein the step of physically sorting is based on dielectrophoresis.

[0539] Example E11. The system of example E8 or E9, wherein the step of physically sorting is based on electrophoresis.

[0540] Example E12. The system of any one of examples E8 to E11, wherein a microfluidic device of the flow cell is coupled to the optical detector, wherein a plurality of subsets comprises a main subset sorted by the optical detector sequentially detecting the spectral fingerprint of each composite particle, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

dispensing, by the microfluidic device and based on characteristics of the spectral fingerprint, each of the plurality of composite particles into individual tubes or wells in a microplate.

[0541] Example E13. The system of any one of examples E8 to E12, further comprising a composite particle dispenser, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

outputting, by a composite particle dispenser, the plurality of composite particles through an optical beam of the excitation laser system and into a plurality of collection vessels based on a user-defined population size.

[0542] Example E14. The system of any one of examples E8 to E12, further comprising a composite particle dispenser, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

outputting, by the composite particle dispenser, the plurality of composite particles through an optical beam of the excitation laser system and into a plurality of collection vessels based on a counted number of composite particles.

[0543] Example E15. The system of example E14 or E15, wherein the plurality of collection vessels comprise 96 vessels.

[0544] Example E16. The system of any one of examples E1 to E15, wherein the optical detector comprises a laser particle detector and an assay result detector.

[0545] Example E17. The system of any one of examples E1 to E16, wherein the optical detector comprises an infrared (IR) detector.

[0546] Example E18. The system of any one of examples E1 to E17, further comprising:

a plurality of assay lasers downstream of the excitation laser system and upstream of an interrogation point, and

wherein the optical detector comprises a laser particle detector, a forward scatter detector, a side-scatter detector, and a plurality of assay result detectors.

[0547] Example E19. The system of example E18, wherein a bandpass filter is positioned upstream of each respective assay result detector.

[0548] Example E20. The system of example E18 or E19, wherein a dichroic mirror is positioned upstream of each respective assay result detector.

[0549] Example E21. The system of any one of examples E18 to E20, wherein a dichroic mirror is positioned between each respective assay laser and the interrogation point.

[0550] Example E22. The system of any one of examples E18 to E21, wherein an emission collection lens and a plurality of dichroic mirrors are positioned between the interrogation point and the side-scatter detector.

[0551] Example E23. The system of any one of examples E18 to E22, wherein the at least one processor further is configured to execute the instructions to perform operations comprising sorting by:

determining, based on a side scatter of beams of the plurality assay lasers detected by the side-scatter detector, a relationship between the number of optically detectable particles present in a respective composite particle and a magnitude of the side-scatter for any beam of the plurality assay lasers as the respective composite particle moves through the interrogation point; and

sorting, based on the relationship, the plurality of composite particles into the plurality of subsets.

[0552] Example E24. The system of any one of examples E18 to E23, wherein the at least one processor further is configured to execute the instructions to perform operations comprising sorting by:

determining a sort bin based on a side scatter value of the beams of the plurality assay lasers detected by the side-scatter detector; and

separating, based on the side-scatter value, at least one of the composite particles of the plurality of composite particles of the sort bin from a rest of the plurality of composite particles.

[0553] Example E25. The system of example E24, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

upon interrogating a respective composite particle and placing the respective composite particle into the sort bin, then transmitting a signal to one or more electrodes within and/or adjacent a channel of the flow cell based on detected electrophoretic or dielectrophoretic properties so that the respective composite particle is deflected into a separate output.

[0554] Example E26. The system of example E24, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

upon interrogating a respective composite particle and placing the respective composite particle into the sort bin, transmitting a signal to one or more magnets within and/or adjacent a channel of the flow cell based on detected magnetic properties so that the respective composite particle is deflected into a separate output.

[0555] Example E27. The system of any one of examples E1 to E26, wherein the flow cell comprises an interrogation point at which the optical excitation system sequentially excites the composite particles and the optical detector sequentially reads the spectral fingerprints of the composite particles.

[0556] Example E28. The system of example E27, wherein the flow cell comprises a fluidic circuit to transport a plurality of composite particle compartments to the interrogation point.

[0557] Example E29. The system of any one of examples E1 to E28, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

analyzing the spectral fingerprint of the composite particle of the subset at every stage of compound exposure thereby establishing an informatic link between the compound associated with that subset and the composite particle identity of each composite particle of that subset.

[0558] Example E30. The system of any one of examples E1 to E29, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

comparing each spectral fingerprint to a registry comprising a plurality of known spectral fingerprints that pertain to composite particles with a desired chemical matter; and

separating those composite particles comprising one or more spectral fingerprints matching a desired one or more compounds from a plurality of composite particles.

[0559] Example E31. The system of any one of examples E1 to E30, wherein the at least one processor further is configured to execute the instructions to perform operations

comprising sorting, based on the spectral fingerprint, by creating the subsets based on a detected link between the spectral fingerprint and a composite particle identity of a specific compound.

[0560] Example E32. The system of any one of examples E1 to E31, wherein the at least one processor further is configured to execute the instructions to perform operations comprising creating a plurality of subsets based on a predicted link between the spectral fingerprint and a composite particle identity of a specific compound, the predicted link being determined by a machine learning model trained based on training data that comprises spectral data of the composite particles, a plurality of compounds, and output variables representing compound library data sets.

[0561] Example E33. The system of example E32, wherein the machine learning model incorporates at least one of linear regression, kernel ridge regression, logistic regression, neural network, support vector machine (SVM), decision tree, hidden Markov model, Bayesian network, a Gram-Schmidt process, reinforcement-based learning, cluster-based learning, hierarchical clustering, genetic algorithm, or combination thereof.

[0562] Example E34. The system of any one of examples E1 to E33, further comprising a flow cytometer.

[0563] Example E35. The system of example E34, wherein the flow cytometer is IR enabled.

[0564] Example E36. The system of any one of examples E1 to E35, further comprising a fluorescence-activated cell sorting (FACS) instrument, wherein the plurality of subsets comprises a main subset sorted by the FACS instrument sequentially detecting an emission spectrum of the spectral fingerprint of each composite particle.

[0565] Example E37. The system of example E36, wherein the FACS instrument is IR enabled.

[0566] Example E38. The system of example E36 or E37, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

identifying, by the FACS instrument, a number of the optically detectable particles in each composite particle; and

separating, based on the number of optically detectable particles, those composite particles of the plurality of composite particle comprising fewer than a predetermined number of optically detectable particles from the main subset.

[0567] Example E39. The system of example E36 or E37, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

identifying, by the FACS instrument, a number of optically detectable particles in each composite particle; and

separating, based on the number of optically detectable particles, those composite particles of the plurality of composite particle comprising more than a predetermined number of optically detectable particles from the main subset.

[0568] Example E40. The system of any one of examples E36 to E39, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

identifying, by the FACS instrument, a number of unique peak maxima present in the emission spectrum.

[0569] Example E41. The system of any one of examples E36 to E40, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

identifying, by the FACS instrument, a number of optically detectable particles in each composite particle; and

dispensing, by a fluidic system and based on the number of optically detectable particles or the spectral fingerprint, each of the plurality of composite particles into individual tubes or wells in a microplate, and wherein each separate vessel or well is associated with specific subset of the plurality of subsets.

[0570] Example E42. The system of example E41, wherein the microplate comprises 96 vessels or wells.

[0571] Example E43. The system of any one of examples E36 to E42, wherein the main subset comprises approximately 10^5 to 10^9 composite particles present in solution at a concentration of approximately 10^5 to 10^8 composite particles per milliliter.

[0572] Example E44. The system of any one of examples E36 to E42, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

upon determining if a composite particle of the plurality of composite particles comprises a number of optically detectable particles falls within a gate, executing a sort event so that the composite particle within the gate is automatically physically removed from the main subset and into a subset defined by the gate.

[0573] Example E45. The system of any one of examples E1 to E44, wherein the optically detectable particles emit light with a bandwidth of about 1 nm or less at full-width half-maximum (FWHM) responsive to excitation by the beam of the excitation laser system.

[0574] Example E46. The system of any one of examples E1 to E45, wherein the excitation laser system comprises an infrared (IR) laser.

[0575] Example E47. The system of any one of examples E1 to E46, wherein the spectral fingerprint comprises an optical response of each optically detectable particle in a respective composite particle when summed together.

[0576] Example E48. The system of any one of examples E1 to E46, wherein the at least one processor further is configured to execute the instructions to perform operations comprising:

- detecting, by the optical detector, characteristics of the spectral fingerprint of each composite particle;

- removing, from a main subset of the plurality of subsets, one or more composite particles comprising an abundance of non-unique spectral characteristics.

[0577] Example E49. A method for categorizing a plurality of composite particles suspended in a fluid, each composite particle comprising a plurality of optically detectable particles and a network mesh polymer substantially surrounding the optically detectable particles, the method comprising:

- flowing the composite particles in a fluid through a flow cell;

- exciting the composite particles in the flow cell with light from an optical excitation system;

- reading, by an optical detector, a spectral fingerprint of each composite particle excited by the light; and

- generating, using the spectral fingerprint of each composite particle, a composite particle identity of that composite particle.

[0578] Example E50. The method of example E49, wherein the step of reading, by the optical detector, the spectral fingerprint of each composite particle excited by the light comprises measuring an IR emission orthogonal to an excitation beam of the optical excitation system.

[0579] Example E51. The method of example E49 or E50, further comprising: physically sorting the plurality of composite particles into a plurality of subsets.

[0580] Example E52. The method of example E51, further comprising:

- after cleaving one or more compounds off the plurality of composite particles in each subset, then detecting, by an ultra-performance liquid chromatography system or a high-performance liquid chromatography system coupled to a mass spectrometry system or

tandem mass spectrometry system, the composite particle identity based on a compound identity, a purity and/or a synthetic yield of the plurality of composite particles.

[0581] Example E53. The method of example E51 or E52, wherein the step of physically sorting is based on the spectral fingerprints of the composite particles of the plurality.

[0582] Example E54. The method of any one of examples E51 to E53, wherein the step of physically sorting is based on dielectrophoresis.

[0583] Example E55. The method of any one of examples E51 to E53, wherein the step of physically sorting is based on electrophoresis.

[0584] Example E56. The method of any one of examples E51 to E55, wherein a microfluidic device of the flow cell is coupled to the optical detector, the flow cell comprising a channel in microfluidic device, wherein a plurality of subsets comprises a main subset sorted by the optical detector sequentially detecting the spectral fingerprint of each composite particle, the method further comprising:

dispensing, by the microfluidic device and based on characteristics of the spectral fingerprint, each of the plurality of composite particles into individual tubes or wells in a microplate.

[0585] Example E57. The method of any one of examples E51 to E58, further comprising: outputting, by the composite particle dispenser, the plurality of composite particles through an optical beam of the excitation laser system and into a plurality of collection vessels based on a counted number of composite particles.

[0586] Example E58. The method of example E57, further comprising: upon subsets containing those composite particles of the plurality of composite particles with the same chemical incorporation history being output in separate vessels of the plurality of collection vessels, then causing the output composite particles to trigger compound release from the plurality of composite particles.

[0587] Example E59. The method of any one of examples E49 to E58, further comprising:

determining, based on a side scatter of beams of a plurality of assay lasers detected by a side-scatter detector, a relationship between the number of optically detectable particles present in a respective composite particle and a magnitude of the side-scatter for any beam of the plurality of assay lasers as the respective composite particle moves through an interrogation point, the plurality of assay lasers being downstream of the excitation laser system and upstream of the interrogation point; and

sorting, based on the relationship, the plurality of composite particles into the plurality of subsets.

[0588] Example E60. The method of any one of examples E49 to E59, further comprising:

determining a sort bin based on a side scatter value of beams of a plurality of assay lasers detected by a side-scatter detector; and

separating, based on the side-scatter value, at least one of the composite particles of the plurality of composite particles of the sort bin from a rest of the plurality of composite particles.

[0589] Example E61. The method of example E60, further comprising:

upon interrogating a respective composite particle and placing the respective composite particle into the sort bin, then transmitting a signal to one or more electrodes within and/or adjacent a channel of the flow cell based on detected electrophoretic or dielectrophoretic properties so that the respective composite particle is deflected into a separate output.

[0590] Example E62. The method of example E60 or E61, further comprising:

upon interrogating a respective composite particle and separating the respective composite particle into the sort bin, transmitting a signal to one or more magnets within and/or adjacent a channel of the flow cell based on detected magnetic properties so that the respective composite particle is deflected into a separate output.

[0591] Example E63. The method of any one of examples E49 to E62, further comprising:

analyzing the spectral fingerprint of the composite particle of the subset at every stage of compound exposure thereby establishing an informatic link between the compound associated with that subset and the composite particle identity of each composite particle of that subset.

[0592] Example E64. The method of any one of examples E49 to E62, further comprising:

analyzing the spectral fingerprint of the plurality of composite particles to establish an informatic link between a respective compound and the composite particle identity of respective composite particles of the plurality of composite particles.

[0593] Example E65. The method of any one of examples E49 to E64, further comprising:

detecting, by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS), the composite particle identity based on a compound identity, a purity and/or a synthetic yield of the plurality of composite particles.

[0594] Example E66. The method of example any one of examples E49 to E65, further comprising:

comparing each spectral fingerprint to a registry comprising a plurality of known spectral fingerprints that pertain to composite particles with a desired chemical matter; and separating those composite particles comprising one or more spectral fingerprints matching a desired one or more compounds from a plurality of composite particles.

[0595] Example E67. The method of any one of examples E49 to E66, further comprising:

sorting, based on the spectral fingerprint, by creating the subsets based on a detected link between the spectral fingerprint and a composite particle identity of a specific compound.

[0596] Example E68. The method of any one of examples E49 to E67, further comprising:

creating a plurality of subsets based on a predicted link between the spectral fingerprint and a composite particle identity of a specific compound, the predicted link being determined by a machine learning model trained based on training data that comprises spectral data of the composite particles, a plurality of compounds, and output variables representing compound library data sets.

[0597] Example E69. The method of any one of examples E49 to E68, further comprising:

separately collecting each subset, after the step of exposing each subset of the plurality of subsets to one or more compounds and then releasing the one or more compounds from a respective composite particle.

[0598] Example E70. The method of any one of examples E49 to E69, further comprising:

identifying, by a FACS instrument, a number of the optically detectable particles in each composite particle; and

separating, based on the number of optically detectable particles, those composite particles of the plurality of composite particle comprising less than a predetermined number of particles from the main subset.

[0599] Example E71. The method of any one of examples E49 to E69, further comprising:

identifying, by a FACS instrument, a number of optically detectable particles in each composite particle; and

separating, based on the number of optically detectable particles, those composite particles of the plurality of composite particle comprising more than a predetermined number of optically detectable particles from the main subset.

[0600] Example E72. The method of any one of examples E49 to E69, further comprising:

identifying, by a FACS instrument, a number of optically detectable particles in each composite particle; and

dispensing, by a fluidic system and based on the number of optically detectable particles or the spectral fingerprint, each of the plurality of composite particles into individual tubes or wells in a microplate, and wherein each separate vessel or well is associated with specific subset of the plurality of subsets.

[0601] Example E73. The method of any one of examples E49 to E72, further comprising:

upon determining if a composite particle of the plurality of composite particles comprises a number of optically detectable particles falls within a gate, executing a sort event so that the composite particle within the gate is automatically physically removed from the main subset and into a subset defined by the gate.

[0602] Example E74. The method of any one of examples E49 to E73, further comprising:

detecting, by the optical detector, characteristics of the spectral fingerprint of each composite particle;

removing, from a main subset of the plurality of subsets, one or more composite particles comprising an abundance of non-unique spectral characteristics.

[0603] Example E75. A spectral fingerprint system for categorizing a plurality of composite particles, the system comprising:

an optical excitation system configured to excite the composite particles with light;

a flow cell configured to permit flowing of the composite particles suspended in a fluid;

an optical detector configured to read a spectral fingerprint of each composite particle excited by the light;

a control system configured to control (a) flow of the composite particles in the fluid through the flow cell, (b) excitation by the optical excitation system of the composite particles in the flow cell with light from the optical excitation system; (c) reading by the optical detector of a spectral fingerprint of each composite particle excited by the light; and (d) generating, based on the spectral fingerprint of each composite particle, a composite particle identity of that composite particle.

[0604] Example E76. A method for generating a compound library for a plurality of composite particles, each composite particle comprising a plurality of optically detectable particles and a network mesh polymer substantially surrounding the optically detectable particles, the method comprising:

reading, by the optical detector, a spectral fingerprint of each composite particle excited by the light; and

storing, in a data structure of the compound library, an identifier for each composite particle based on the spectral footprint and an identifier for the one or more different compounds coupled to that composite particle.

[0605] Example E77. The method of example E76, further comprising: dispensing the plurality of composite particles into a plurality of subsets based on a number of detected optically detectable particles.

[0606] Example E78. The method of example E76 or E77, further comprising:

exposing each subset of a plurality of subsets to one or more compounds to associate that subset with one or more different compounds.

[0607] Example E79. The method of example E78, wherein the step of exposing comprises:

dividing the plurality of composite particles into a plurality of subsets;

coupling, for each subset, a chemical moiety to the polymer of the composite particles of that subset to form a modified subset; and

pooling the modified subsets to form a modified collection of composite particles.

[0608] Example E80. The method of example any one of examples E78 to E77, further comprising:

establishing, by a machine learning model, an informatic link between the chemical compound and the identifier for each composite particle, the machine learning model having been trained based on the data structure and training data that comprises spectral data of the composite particles, the plurality of compounds that have been coupled, and output variables representing compound library data sets; and

storing the informatic link in the data structure of the compound library.

[0609] Example E81. The method of example E80, wherein the machine learning model incorporates at least one of linear regression, kernel ridge regression, logistic regression, neural network, support vector machine (SVM), decision tree, hidden Markov model, Bayesian network, a Gram-Schmidt process, reinforcement-based learning, cluster-based learning, hierarchical clustering, genetic algorithm, or combination thereof.

[0610] Example E82. A method for categorizing a plurality of composite particles suspended in a fluid, each composite particle comprising a plurality of optically detectable particles and a network mesh polymer substantially surrounding the optically detectable particles, the method comprising:

- causing the composite particles to flow into a flow cell and be irradiated by an optical beam one-by-one in the flow cell resulting in detection of a spectral fingerprint and a composite particle count;

- dispensing a plurality of groups of the composite particles into separate vessels based on a composite particle count;

- changing vessels when the composite particle count reaches a predetermined threshold and then causing a counting operation to restart;

- recording a vessel destination for each composite particle in a spectral fingerprint registry;

- incorporating a different chemical entity onto the respective group of the composite particles in each respective vessel;

- pooling together the composite particles in each vessel; and

- repeating steps (a) to (f) after each successive incorporating of the different chemical entity.

[0611] Example E83. A method for categorizing a plurality of composite particles suspended in a fluid, each composite particle comprising a plurality of optically detectable particles and a network mesh polymer substantially surrounding the optically detectable particles, the method comprising:

- dispensing a plurality of groups of the composite particles into separate vessels;

- incorporating a different chemical entity onto the respective group of the composite particles in each respective vessel;

- for each vessel, causing the composite particles to flow into a flow cytometer and be irradiated one-by-one by an optical beam in a flow cell resulting in detection of a spectral fingerprint;

collecting an output of the composite particles from all runs in a single vessel thereby creating a spectral fingerprint registry comprising spectral fingerprints of each group of the plurality of groups for the pooled composite particles; and

repeating steps (a) to (d) after each successive incorporating of the different chemical entity.

Additional comments

[0612] While preferred embodiments of the invention are described herein, it will be apparent to one skilled in the art that various changes and modifications may be made. The appended claims are intended to cover all such changes and modifications that fall within the true spirit and scope of the invention.

WHAT IS CLAIMED IS:

CLAIMS

1. A method of detecting binding between different chemical compounds and a target, the method comprising:

incubating a first target with a collection of composite particles, the composite particles of the collection comprising:

a plurality of optically detectable particles;

a network polymer gel substantially surrounding the optically detectable particles; and

a chemical compound coupled to the network polymer gel,

wherein at least some of the composite particles comprise the same chemical compound as one another, and

wherein at least some of the composite particles comprise different chemical compounds than one another, and

wherein the first target binds differently to different ones of the chemical compounds;

removing unbound first target from the collection of composite particles;

irradiating the composite particles of the collection, with the first target bound to different ones of the chemical compounds, with excitation light;

obtaining emission spectra from the optically detectable particles of respective composite particles;

obtaining signals respectively indicating whether the first target binds to the chemical compounds of respective composite particles;

using the emission spectra and the signal to (i) identify the chemical compound which is coupled to at least some of the composite particles and (ii) determine whether the first target binds to that chemical compound.

2. The method of claim 1, wherein the first target is labeled with a probe before the incubating.

3. The method of claim 1, further comprising, after the first target binds differently to different ones of the chemical compounds, labeling the first target with an affinity reagent that is labeled with a probe.

4. The method of claim 1, further comprising, after the first target binds differently to different ones of the chemical compounds, incubating the first target with an unlabeled primary affinity reagent, then labeling the unlabeled primary affinity reagent with a secondary affinity reagent that is labeled with a probe.
5. The method of any one of claims 1 to 4, wherein the composite particles are irradiated with a first wavelength of light that stimulates the emission spectra of the optically detectable particles.
6. The method of claim 5, wherein the composite particles are irradiated with a second wavelength of light that stimulates fluorescence which generates the signal indicating whether the first target binds to the chemical compounds of respective composite particles.
7. The method of claim 6, wherein the composite particles are irradiated with the first and second wavelengths of light at different times than one another.
8. The method of claim 6, wherein the composite particles are irradiated with the first and second wavelengths of light at the same time as one another.
9. The method of any one of claims 1 to 8, wherein the emission spectra and signal are obtained concurrently with one another.
10. The method of any one of claims 1 to 8, wherein the emission spectra and signal are obtained at different times than one another.
11. The method of any one of claims 1 to 10, further comprising:
 - incubating a second target with the collection of composite particles, wherein the second target binds differently to different ones of the chemical compounds;
 - removing unbound second target from the collection of composite particles; and
 - obtaining signals indicating whether the second target binds to the chemical compounds of respective composite particles.

12. The method of claim 11, wherein the collection of composite particles is incubated concurrently with the first target and the second target.
13. The method of claim 11 or claim 12, wherein the signals comprise fluorescence, and wherein fluorescence from the first target is at a different wavelength than fluorescence from the second target.
14. The method of any one of claims 1 to 13, wherein the emission spectra, the signals, or both the emission spectra and the signals, are obtained while the composite particles are static.
15. The method of any one of claims 1 to 14, wherein the emission spectra, the signals, or both the emission spectra and the signals, are obtained while the composite particles are flowed through an inspection point.
16. The method of any one of claims 1 to 15, wherein the signals are optical.