NANOSCALE REACTION CHAMBERS AND METHODS OF USING THE SAME

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/174,847, filed on April 14, 2021, which application is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Current standard assay formats probe cell populations and gather data. During the processing of probing and gathering data, for example, going between assay and or instrument, cell-specific information may be lost due to the pooling of cells in between steps. Therefore, there is a need of an assay platform that is capable of probing and gathering data of cell populations yet retaining cell-specific information that may be associated with the gathered data.

SUMMARY

[0003] There is an unmet need for assays that can link functional single-cell based assay information with genomic, transcriptomic, and/or proteomic single-cell based assay information. This disclosure meets this unmet need by providing nanoscale reaction chambers (e.g., nanovials) in which cell-specific assays are conducted while maintaining cell-specific information throughout the course of the assay. The nanovial reaction chambers of the present disclosure are unique in that the resulting outcome of the assay (e.g., functional assay) performed in the nanovial may, after completion of data analysis, be correlated back to genomic, transcriptomic, and/or proteomic analysis of the single-cellular constituents of the assay, thereby providing a highly targeted multi-dimensional analysis.

[0004] In an example, a method of identifying an antibody or fragment thereof that binds to an antigen of interest is provided, the method comprising (e.g., performing one or more of the following): (a) providing or obtaining a nanovial comprising a cavity formed therein and an affinity agent immobilized thereto; (b) loading an antibody-producing cell into the cavity of the nanovial; (c) incubating the nanovial such that one or more antibody or fragment thereof is secreted from the antibody-producing cell and binds to the affinity agent; (d) adding a detection agent to the cavity of the nanovial such that the detection agent binds to the one or more antibody or fragment thereof; (e) detecting one or more signals related to binding of the detection agent to the one or more antibody or fragment thereof; (f) performing a sequencing

assay on nucleic acids derived from the antibody-producing cell, thereby generating a sequence of the one or more antibody or fragment thereof; (g) associating the sequence of the one or more antibody or fragment thereof with the one or more signals related to the binding of the detection agent to the one or more antibody or fragment thereof; and (h) identifying the one or more antibody or fragment thereof that binds to the antigen of interest based on the associating of (g). In one aspect, there is provided a method of identifying an antibody or fragment thereof that binds to an antigen of interest is provided, the method comprising: (a) providing or obtaining a nanovial comprising a cavity formed therein and an affinity agent immobilized thereto; (b) loading an antibody-producing cell into the cavity of the nanovial; (c) incubating the nanovial such that one or more antibody or fragment thereof is secreted from the antibody-producing cell and binds to the affinity agent; (d) adding a detection agent to the cavity of the nanovial such that the detection agent binds to the one or more antibody or fragment thereof; (e) detecting one or more signals related to binding of the detection agent to the one or more antibody or fragment thereof; (f) performing a sequencing assay on nucleic acids derived from the antibody-producing cell, thereby generating a sequence of the one or more antibody or fragment thereof; (g) associating the sequence of the one or more antibody or fragment thereof with the one or more signals related to the binding of the detection agent to the one or more antibody or fragment thereof; and (h) identifying the one or more antibody or fragment thereof that binds to the antigen of interest based on the associating of (g), wherein the nanovial is a suspendable, miscroscale particle comprising cross-linked hydrogel. In some cases, the method further comprises, prior to (f), sorting the nanovial based on the one or more signals related to binding of the detection agent to the antibody. In some cases, the cavity of the nanovial further comprises one or more cell capture agents immobilized thereto. In some cases, the one or more cell capture agents comprises an antibody or fragment thereof that binds to a protein expressed on a surface of the antibody-producing cell. In some cases, the one or more cell capture agents binds to a label present on a surface of the antibody-producing cell. In some cases, the label is streptavidin or biotin. In some cases, the one or more cell capture agents is an antigen to which the antibody-producing cell can bind. In some cases, the affinity agent is an antibody capture moiety. In some cases, the antibody capture moiety is selected from the group consisting of: an anti-IgG antibody or fragment thereof, an anti-Fc antibody or fragment thereof, Protein A, and Protein G. In some cases, the affinity agent is the antigen of interest. In some cases, the affinity agent is an antigen- presenting cell expressing the antigen of interest on a surface thereof. In some cases, the detection agent is the antigen of interest. In

some cases, the detection agent is an antibody or fragment thereof. In some cases, the detection agent is directly or indirectly labeled with a detectable label. In some cases, the detectable label is selected from the group consisting of: a fluorescent label, a protein affinity tag, an oligonucleotide tag, and a magnetic particle. In some cases, the detectable label is a fluorescent label. In some cases, the detecting of (e) comprises determining an amount of detection agent bound to the one or more antibody or fragment thereof. In some cases, the amount of detection agent bound to the one or more antibody or fragment thereof corresponds to a level of fluorescence. In some cases, the method further comprises, prior to (f), sorting the nanovial based on a level of fluorescence above a background level. In some cases, the sorting comprises performing a flow-based sorting method. In some cases, the flow-based sorting method is fluorescence-activated cell sorting (FACS). In some cases, the detectable label is an oligonucleotide tag. In some cases, the method further comprises sequencing the oligonucleotide tag. In some cases, the detecting of (e) comprises counting the number of oligonucleotide tags bound to the one or more antibody or fragment thereof. In some cases, the method further comprises, prior to (f), lysing the antibody-producing cell. In some cases,

the method further comprises, after the lysing, reverse transcribing mRNA released from the antibody-producing cell to generate cDNA. In some cases, the method further comprises sequencing the cDNA. In some cases, the sequencing assay comprises single cell RNA sequencing. In some cases, the method further comprises, prior to (c), encapsulating the nanovial within a droplet. In some cases, the cavity of the nanovial comprises an opening to the surface of the nanovial. In some cases, the method further comprises, prior to (c), adding one or more blocking particles to block or reduce a size of the opening. In some cases, the method further comprises, prior to (d), washing the nanovial. In some cases, the method is configured to identify one or more antibody or fragment thereof having a desired dissociation constant (K_D) for the antigen of interest. In some cases, the one or more antibody or fragment thereof has a desired K_D for the antigen of interest of less than about 1 μM, less than about 100 nM, less than about 10 nM, or less than about 1 nM. In some cases, the method further comprises adding the detection agent to a plurality of nanovials at one or more different concentrations. In some cases, the one or more different concentrations are within 1 order of magnitude of the desired K_D. In some cases, the method is configured to identify one or more antibody or fragment thereof having a desired specificity for the antigen of interest. In some cases, the method further comprises adding a plurality of different detection agents to a plurality of nanovials, wherein in at least one of the different detection agents have a detectable label. In some cases, the method further comprises identifying one or more antibody or fragment thereof that specifically binds to one of the different detection agents based on a measurement of the detectable label. In some cases, the detection agent is the antigen of interest.

[0005] In another example, a method of identifying one or more antibody or fragment thereof that modulates a signaling pathway of interest is provided, the method comprising (e.g., performing one or more of the following): (a) providing or obtaining a nanovial comprising a cavity formed therein; (b) loading an antibody-producing cell and an antigen-producing cell into the cavity of the nanovial; (c) incubating the nanovial such that one or more antibody or fragment thereof is secreted from the antibody-producing cell and modulates the signaling pathway of interest in the antigen-producing cell; (d) detecting one or more signals related to the modulation of the signaling pathway of interest; (e) performing a sequencing assay on nucleic acids derived from the antibody-producing cell, thereby generating a sequence of the one or more antibody or fragment thereof; (f) associating the sequence of the one or more antibody or fragment thereof with the one or more signals related to the modulation of the signaling pathway of interest; and (g) identifying the one or more antibody or fragment thereof that modulates the

mRNA released from the antibody-producing cell to generate cDNA. In some cases, the method further comprises sequencing the cDNA. In some cases, the sequencing assay comprises single cell RNA sequencing. In some cases, the method further comprises, prior to (c), encapsulating the nanovial within a droplet. In some cases, the cavity of the nanovial comprises an opening to the surface of the nanovial. In some cases, the method further comprises, prior to (c), adding one or more blocking particles to block or reduce a size of the opening. In some cases, the method further comprises, prior to (d), washing the nanovial. In some cases, the antibody-producing cell is selected from the group consisting of: a B cell, a plasmablast, a plasma cell, a hybridoma, and an engineered producer cell. In some cases, the nanovial comprises a single antibody-producing cell. In some cases, the method further comprises, performing the method on a plurality of nanovials, each of the nanovials comprising an individual antibody-producing cell within the cavity of the nanovial. In some cases, the plurality of nanovials comprises at least 20,000 nanovials, at least 100,000 nanovials, or at least 500,000 nanovials. In some cases, each individual antibody-producing cell produces a different antibody. In some cases, the nanovial comprises a cross-linked hydrogel. In some cases, the cross-linked hydrogel is a PEG-based cross-linked hydrogel. In some cases, the cavity of the nanovial comprises an aqueous fluid disposed therein. In some cases, the nanovial is suspended in an oil phase. In some cases, the cavity of the nanovial has a volume from about 100 fL to about 10 nL. In some cases, the cavity of the nanovial has a length dimension from about 5 μm to about 250 μm.

[0006] In yet another example, a method of linking functional single cell information with genomic, transcriptomic, and/or proteomic single cell information is provided, the method comprising: (a) performing a functional assay on a single cell located in the cavity of a nanovial, wherein the functional assay is associated with a first barcode or a first label; (b) performing a genomic, transcriptomic, and/or proteomic assay on the single cell in the nanovial, wherein the genomic, transcriptomic, and/or proteomic assay is associated with a second barcode or a second label; and (c) linking functional single cell information with genomic, transcriptomic, and/or proteomic single cell information by associating the first barcode or first label with the second barcode or second label. In one aspect, there is provided a method of linking functional single cell information with genomic, transcriptomic, and/or proteomic single cell information is provided, the method comprising: (a) performing a functional assay on a single cell located in the cavity of a nanovial, wherein the functional assay is associated with a first barcode or a first label; (b) performing a genomic, transcriptomic, and/or proteomic assay on the single cell in the nanovial, wherein the

genomic, transcriptomic, and/or proteomic assay is associated with a second barcode or a second label; and (c) linking functional single cell information with genomic, transcriptomic, and/or proteomic single cell information by associating the first barcode or first label with the second barcode or second label, wherein the nanovial is a suspendable, miscroscale particle comprising cross-linked hydrogel. In some cases, the method further comprises, prior to (b), sorting the nanovial based on a signal generated by the functional assay. In some cases, the functional assay is an antibody secretion screening assay. In some cases, the functional assay is an antibody affinity assay. In some cases, the functional assay is an antibody specificity assay. In some cases, the genomic assay is a single cell RNA sequencing assay. In some cases, the nanovial is associated with a unique

oligonucleotide tag. In some cases, the unique oligonucleotide tag comprises a poly-dT capture region. In some cases, the first barcode or first label comprises an oligonucleotide with a poly-A-region.

[0007] In yet another example, a method of preventing biological agents from diffusing out of the cavity of a nanovial is provided, the method comprising: (a) providing or obtaining a nanovial comprising a cavity formed therein, the cavity comprising an opening to the surface of the nanovial, and one more biological agents disposed within the cavity; and (b) adding a blocking particle such that the blocking particle interacts with the opening of the cavity and substantially blocks or reduces a size of the opening of the cavity, thereby inhibiting the biological agents from diffusing out of the cavity when the nanovials are disposed in a fluid. In some cases, a maximum diameter of the blocking particle is larger than a diameter of the opening of the cavity. In some cases, the blocking particle is in contact with the opening of the cavity. In some cases, the blocking particle surrounds the opening of the cavity. In some cases, the blocking particle is sized to maintain greater than 50% of a volume of the cavity after (b). In some cases, the blocking particle has an average diameter from about 20 micrometers to about 50 micrometers. In some cases, the blocking particle is spherical or substantially spherical in shape. In some cases, the blocking particle comprises a polymer hydrogel. In some cases, the polymer hydrogel is polyethylene glycol (PEG). In some cases, the blocking particle is coated with one or more affinity agents. In some cases, the one or more affinity agents comprise an antibody capture moiety. In some cases, the antibody capture moiety is selected from the group consisting of: an anti-IgG antibody or a fragment thereof, an anti-Fc antibody or fragment thereof, Protein A, and Protein G. In some cases, the one or more affinity agents comprises an antigen. In some cases, the one or more affinity agents comprises a nucleic acid. In some cases, the nucleic acid is an oligonucleotide tag or oligonucleotide barcode. In some cases, the nanovial and/or blocking particle comprises a mesh-like structure. In some cases, the mesh-like structure comprises an average pore size. In some cases, the average pore size is sized such that biological agents having a desired size are retained within the cavity and biological agents having a size smaller than the desired size are capable of diffusing through the mesh-like structure. In some cases, the average pore size is sized to allow transport of fluids and reagents into the cavity of the nanovial. In some cases, the fluids and reagents are selected from the group consisting of: staining agents, surfactants, detergents, lysis buffers, drugs, cytokines, chemokines, media, conditioned media, and buffers. In some cases, (b) comprises adding a plurality of blocking particles such that the plurality of blocking particles interact with the opening of the cavity and substantially

block or reduce the size of the opening of the cavity. In some cases, each of the plurality of blocking particles has an average diameter from about 2 micrometers to about 20 micrometers. In some cases, each of the plurality of blocking particles has a density greater than that of saline at room temperature. In some cases, a number of blocking particles to nanovials is greater than 100:1. In some cases, the plurality of blocking particles are comprised of a polymer selected from the group consisting of: PEG, polystyrene, poly-methylmethacrylate, glass, and metal.

[0008] In another example, a method of enriching for nanovials comprising one or more cells, one or more beads, or both is provided, the method comprising: (a) providing or obtaining a mixture comprising: a plurality of nanovials, each comprising at least one cell, at least one bead, or both, disposed within a cavity of the nanovial; and (i) a plurality of free-floating cells, free-floating beads, or both; (ii) a plurality of empty nanovials; or (iii) both (i) and (ii); (b) suspending the mixture in a solution; (c) adjusting a density of the solution such that the plurality of nanovials comprising at least one cell, at least one bead, or both, disposed within a cavity of the nanovial separate from the plurality of free-floating cells, free-floating beads, or both of (i), the plurality of empty nanovials of (ii), or both (i) and (ii); and (d) collecting the plurality of nanovials comprising at least one cell, at least one bead, or both, disposed within a cavity of the nanovial. In some cases, (c) comprises adjusting the density of the solution such that the plurality of nanovials comprising at least one cell, at least one bead, or both, disposed within a cavity of the nanovial have a greater density than the density of the solution. In some cases, (c) comprises adjusting the density of the solution such that the plurality of nanovials comprising at least one cell, at least one bead, or both, disposed within a cavity of the nanovial have a lower density than the density of the solution. In some cases, the method further comprises, performing (a)-(d) one or more times to further enrich for the plurality of nanovials comprising at least one cell, at least one bead, or both, disposed within a cavity of the nanovial. In some cases, the density of the solution is different for each round of (a)-(d). In some cases, the method further comprises, prior to (a), removing the plurality of free-floating cells, free-floating beads, or both of (i) or the plurality of empty nanovials of (ii).

[0009] In yet another example, a method of identifying a drug or compound that modulates a signaling pathway in a cell of interest is provided, the method comprising (e.g., performing one or more of the following): (a) providing or obtaining a nanovial comprising a cavity formed therein, the cavity comprising (i) a drug or a compound; and (ii) a barcode or a label associated with the drug or the compound; (b) loading a cell of interest into the cavity of the nanovial; (c)

cDNA. In some cases, the sequencing assay comprises single cell RNA sequencing. In some cases, the method further comprises, prior to (c), encapsulating the nanovial within a droplet.

[0010] In yet another example, a method of identifying a functional chimeric antigen receptor (CAR) or T cell receptor (TCR) to an antigen of interest is provided, the method comprising (e.g., performing one or more of the following): (a) providing or obtaining a nanovial comprising a cavity formed therein and an affinity agent immobilized thereto; (b) loading a CAR- or TCR-expressing cell into the cavity of the nanovial; (c) incubating the nanovial such that one or more cytokines is secreted from the CAR- or TCR-expressing cell and binds to the affinity agent; (d) adding a detection agent to the cavity of the nanovial such that the detection agent binds to the one or more cytokines; (e) detecting one or more signals related to binding of the detection agent to the one or more cytokines; (f) performing a sequencing assay on nucleic acids derived from the CAR- or TCR-expressing cell, thereby generating a sequence of the CAR or TCR; (g) associating the sequence of the CAR or TCR with the one or more signals related to the binding of the detection agent to the one or more cytokines; and (h) identifying the CAR- or TCR- that functionally interacts with the antigen of interest based on the associating of (g). In some cases, the method further comprises, prior to (f), sorting the nanovial based on the one or more signals related to binding of the detection agent to the one or more cytokines. In some cases, the cavity of the nanovial further comprises one or more cell capture agents immobilized thereto. In some cases, the one or more cell capture agents comprise an antibody or fragment thereof that binds to a protein expressed on a surface of the CAR- or TCR-expressing cell. In some cases, the one or more cell capture agents binds to a label present on a surface of the CAR- or TCR-expressing cell. In some cases, the label is streptavidin or biotin. In some cases, the one or more cell capture agents is an antigen or MHC-presented antigen to which the CAR- or TCR-expressing cell can bind. In some cases, the one or more cell capture agents is an antigen- presenting cell expressing the antigen of interest or presenting antigen in an MHC complex on the surface thereof. In some cases, the affinity agent is an antibody capture moiety. In some cases, the antibody capture moiety is one or more selected from the group consisting of: anti-IL2, anti-TNF-alpha, and anti-IFN-gamma. In some cases, the detection agent is a secondary antibody or antibody fragment specific to the one or more cytokines. In some cases, the detection agent is directly or indirectly labeled with a detectable label. In some cases, the detectable label is selected from the group consisting of: a fluorescent label, an oligonucleotide tag, and a magnetic particle. In some cases, the detectable label is a fluorescent label. In some cases, the detecting of (e) comprises determining an amount of detection agent bound to the one or

more cytokines. In some cases, the amount of detection agent bound to the one or more antibodies corresponds to a level of fluorescence. In some cases, the method further comprises, prior to (f), sorting the nanovial based on a level of fluorescence above a background level. In some cases, the sorting comprises performing a flow-based sorting method. In some cases, the flow-based sorting method is fluorescence-activated cell sorting (FACS). In some cases, the detectable label is an oligonucleotide tag. In some cases, the method further comprises sequencing the oligonucleotide tag. In some cases, the detecting of (e) comprises counting the number of oligonucleotide tags bound to the one or more cytokines. In some cases, the method further comprises, prior to (f), lysing the CAR- or TCR- expressing cell. In some cases, the method further comprises, after the lysing, reverse transcribing mRNA released from the CAR- or TCR-expressing cell to generate cDNA. In some cases, the method further comprises sequencing the cDNA. In some cases, the sequencing assay comprises single cell RNA sequencing. In some cases, the method further comprises, prior to (c), encapsulating the nanovial within a droplet. In some cases, the cavity of the nanovial comprises an opening to the surface of the nanovial. In some cases, the method further comprises, prior to (c), adding one or more blocking particles to block or reduce a size of the opening. In some cases, the method further comprises, prior to (d), washing the nanovial.

[0011] In another example, a nanovial is provided having a size, a shape, a surface chemistry, a density, or any combination thereof, configured to be compatible with and used in a plurality of different types of instruments designed to analyze particles and/or cells. Thus, in one aspect, there is provided a nanovial, wherein the nanovial is a suspendable, microscale particle comprising a cross-linked hydrogel and having a size of less than 100 μm in diameter, and wherein the nanovial is configured to be compatible with and used in a plurality of different types of instruments designed to analyze particles and/or cells. In some cases, the plurality of different types of instruments designed to analyze particles and/or cells is selected from the group consisting of: flow cytometers, fluorescence activated cell sorters, imaging flow cytometers, image activated cell sorters, Coulter counters, particle counters, microfluidic droplet generators, microwell arrays, microfluidic chips with microvalves, microfluidic SlipChips, optofluidic microdevices, microfluidic droplet generators, and microfluidic channels. In some cases, the size of the nanovial is less than about 100 μm in diameter and comprises capped reactive functional groups. In some cases, the size of the nanovial is less than about 60 μm in diameter. In some cases, the nanovial is configured to be flowed through glass capillaries, cuvettes, and/or microfluidic channels. In some cases,

the nanovial is configured to be analyzed by optical, electrical, and/or magnetic excitation. In some cases, the size of the nanovial, surface chemistry, and or buoyancy is such that the nanovial can be rapidly flowed through an instrument without clogging or without substantial clogging. In some cases, the nanovial can be flowed through an instrument at greater than 10 cm/sec, or

greater than 1 m/sec, without clogging or without substantial clogging. In some cases, the nanovial is configured to be flowed through a channel having a diameter of greater than 90 μm. In some cases, the nanovial is configured to be used with a first instrument designed to analyze particles and/or cells and subsequently a second instrument designed to analyze particles and/or cells. In some cases, the nanovial is configured to be sorted by a fluorescence activated cell sorter, image activated cell sorter, or optofluidic microdevice and subsequently introduced into a microfluidic droplet generator.

[0012] In yet another example, a method of detecting cell killing function of a chimeric antigen receptor (CAR)-T cell or T cell receptor (TCR)-expressing T cell in response to binding an antigen of interest is provided, the method comprising: (a) providing or obtaining a nanovial comprising a cavity formed therein and an affinity agent immobilized thereto; (b) loading a CAR- or TCR-expressing T cell into the cavity of the nanovial; (c) contacting the CAR- or TCR-expressing T cell with a target cell producing the antigen of interest; (d) capturing, on the nanovial, one or more cell killing markers released from the target cell upon lysis or permeabilization of the target cell; and (e) detecting the one or more cell killing markers directly or with a detection agent to obtain a cell killing signal. In some cases, the method further comprises: (f) performing a sequencing assay on nucleic acids derived from the CAR- or TCR-expressing cell, thereby generating a sequence of the CAR or TCR; (g) associating the sequence of the CAR or TCR with the one or more cell killing signals; and (h) identifying the CAR or TCR that functionally interacts with the antigen of interest based on the associating of (g). In some cases, the method further comprises, after (e), sorting the nanovial based on the one or more cell killing signals. In some cases, the cavity of the nanovial further comprises one or more cell capture agents immobilized thereto. In some cases, the one or more cell capture agents comprise an antibody or fragment thereof that binds to a protein expressed on a surface of the CAR- or TCR-expressing cell. In some cases, the one or more cell capture agents binds to a label present on a surface of the CAR- or TCR-expressing cell. In some cases, the label is streptavidin or biotin. In some cases, the one or more cell capture agents is an antigen or MHC-presented antigen to which the CAR- or TCR-expressing cell can bind. In some cases, the one or more cell capture agents is an antigen-presenting cell expressing the antigen of interest or presenting antigen in an MHC complex on the surface thereof. In some cases, the affinity agent is an antibody capture moiety. In some cases, the antibody capture moiety has affinity to a fluorescent protein. In some cases, the detection agent is an antibody or oligonucleotide with affinity to an intracellular biomolecule from the target cell. In some cases, the detection agent is directly or indirectly

CLAIMS

What is claimed is:

1. A method of linking functional single cell information with genomic, transcriptomic, and/or proteomic single cell information, the method comprising:

(a) performing a functional assay on a single cell located in a cavity of a nanovial, wherein the functional assay is associated with a first barcode or a first label;

(b) performing a genomic, transcriptomic, and/or proteomic assay on the single cell in the nanovial, wherein the genomic, transcriptomic, and/or proteomic assay is associated with a second barcode or a second label; and

(c) linking functional single cell information with genomic, transcriptomic, and/or proteomic single cell information by associating the first barcode or first label with the second barcode or second label,

wherein the nanovial is a suspendable, microscale particle comprising cross-linked hydrogel.

2. The method of claim 1, further comprising, prior to (b), sorting the nanovial based on a signal generated by the functional assay.

3. The method of claim 1 or 2, wherein the functional assay is an antibody secretion screening assay

4. The method of claim 1 or 2, wherein the functional assay is an antibody affinity assay.

5. The method of claim 1 or 2, wherein the functional assay is an antibody specificity assay.

6. The method of claim 1 or 2, wherein the genomic assay is a single cell RNA sequencing assay.

7. The method of any one of claims 1-6, wherein the nanovial is associated with a unique oligonucleotide tag.

8. The method of claim 7, wherein the unique oligonucleotide tag comprises a poly-dT capture region.

9. The method of any one of claims 1-8, wherein the first barcode or first label comprises an oligonucleotide with a poly-A-region.

10. A method of identifying an antibody or fragment thereof that binds to an antigen of interest, the method comprising:

(a) providing or obtaining a nanovial comprising a cavity formed therein and an affinity agent immobilized thereto;

(b) loading an antibody-producing cell into the cavity of the nanovial;

(c) incubating the nanovial such that one or more antibody or fragment thereof is secreted from the antibody-producing cell and binds to the affinity agent;

(d) adding a detection agent to the cavity of the nanovial such that the detection agent binds to the one or more antibody or fragment thereof;

(e) detecting one or more signals related to binding of the detection agent to the one or more antibody or fragment thereof;

(f) performing a sequencing assay on nucleic acids derived from the antibody-producing cell, thereby generating a sequence of the one or more antibody or fragment thereof;

(g) associating the sequence of the one or more antibody or fragment thereof with the one or more signals related to the binding of the detection agent to the one or more antibody or fragment thereof; and

(h) identifying the one or more antibody or fragment thereof that binds to the antigen of interest based on the associating of (g),

wherein the nanovial is a suspendable, microscale particle comprising cross-linked hydrogel.

11. The method of claim 10, wherein the cavity of the nanovial further comprises one or more cell capture agents immobilized thereto.

12. The method of claim 10 or 11, wherein the affinity agent is an antigen- presenting cell expressing the antigen of interest on a surface thereof.

13. The method of any one of claims 10-12, wherein the detection agent is directly or indirectly labeled with a detectable label.

14. The method of claim 13, wherein the detectable label is selected from the group consisting of: a fluorescent label, a protein affinity tag, an oligonucleotide tag, and a magnetic particle.

15. The method of claim 14, wherein the detectable label is an oligonucleotide tag.

16. The method of any one of the claims 10-15, wherein the cavity of the nanovial comprises an opening to the surface of the nanovial.

17. The method of claim 16, further comprising, prior to (c), adding one or more blocking particles to block or reduce a size of the opening.

18. The method of any one of claims 1 to 17, wherein the cross-linked hydrogel is a PEG-based cross-linked hydrogel.

19. The method of any one of claims 1-18, wherein the cavity of the nanovial comprises an aqueous fluid disposed therein.

20. The method of any one of claims 1-19, wherein, at any step prior to (c) of claim 1, or at any step prior to (g) of claim 10, the nanovial is suspended in an oil phase and/or the nanovial comprises one or more blocking particles to reduce or prevent transport out of the cavity of the nanovial.

21. The method of any one of claims 1-20, wherein the cavity of the nanovial has a volume from 100 fL to 10 nL.

22. A nanovial, wherein the nanovial is a suspendable, microscale particle comprising a cross-linked hydrogel and having a size of less than 100 μm in diameter, and wherein the nanovial is configured to be compatible with and used in a plurality of different types of instruments designed to analyze particles and/or cells.

23. The nanovial of claim 22, wherein the plurality of different types of instruments designed to analyze particles and/or cells is selected from the group consisting of: flow cytometers, fluorescence activated cell sorters, imaging flow cytometers, image activated cell sorters, Coulter counters, particle counters, microfluidic droplet generators, microwell arrays, microfluidic chips with microvalves, microfluidic SlipChips, optofluidic microdevices, microfluidic droplet generators, and microfluidic channels.

24. The nanovial of claim 22 or 23, wherein the size of the nanovial comprises capped reactive functional groups.

25. The nanovial of any one of claims 22-24, wherein the nanovial is configured to be used with a first instrument designed to analyze particles and/or cells and subsequently a second instrument designed to analyze particles and/or cells.

26. The nanovial of claim 25, wherein the nanovial is configured to be sorted by a fluorescence activated cell sorter, image activated cell sorter, or optofluidic microdevice and subsequently introduced into a microfluidic droplet generator.