METHODS OF CHARACTERIZING CONDENSATE-ASSOCIATED CHARACTERISTICS OF COMPOUNDS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit to U.S. Provisional Patent Application No. 62/803,365, filed on February 8, 2019, and U.S. Provisional Patent Application No. 62/866,526, filed on June 25, 2019, the disclosure of each of which is hereby incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] The present invention relates to the field of biological condensates.

BACKGROUND

[0003] In addition to membrane-bound organelles, such as mitochondria, lysosomes, and the endoplasmic reticulum, cells contain distinct sub-compartments that do not comprise a membrane between them and their immediate surrounding solution. Numerous of these membrane-less molecular assemblies have been shown to be formed through a process termed liquid-liquid phase separation or condensation. During this process, a solution comprising biological macromolecules separates into different phases, a condensate that is enriched in some of those macromolecules and a surrounding phase that is relatively depleted in those macromolecules. A number of cellular condensates have been recognized. In addition, phase-separated condensates can be formed outside of the cell, such as in solution or extracellularly (Alberti *et al.*, *J Mol Biol*, 430(23), 2018, 4806-4820; Muiznieks *et al.*, *J Mol Biol*, 430(23), 2018, 4741-4753). However, little or nothing is known about the mechanisms governing the partitioning of compounds into or the exclusion of compounds from condensates or the differences in the partitioning of compounds among various condensates.

[0004] Various condensates are known to be important for modulating cellular processes. For example, a condensate can bring together molecules at an elevated concentration to accelerate reactions inside the condensate or can sequester molecules in the condensate, reducing their concentration in the surrounding medium. Aberrant condensate function has also been implicated in various human diseases, such as neurodegenerative and proliferative diseases (Naumann *et al.*, *Nat Commun*, 9(1), 2018, 335; Wegmann *et al.*, *EMBO J*, 37(7), 2018, e98049;

Aguzzi *et al.*, *Trends Cell Biol,* 26(7), 2016, 547-558). However, in addition to the lack of understanding of the mechanisms governing condensate partitioning of compounds, there is little or nothing known regarding how to identify test compounds that interact with condensates, how to design compounds to improve interactions with condensates, and how such information can be used to improve treatment of diseases.

[0005] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY

[0006] In some examples, provided herein are methods of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) combining the test compound and a composition comprising the target condensate and an extra-condensate solution; (b) determining the amount of the test compound in the target condensate, thereby determining the partition characteristic of the test compound in the target condensate. In some examples, the methods further comprise causing the formation of the target condensate prior to step (a).

[0006a] In one aspect, the present disclosure refers to a method of determining a partition characteristic of a small molecule test compound in a target condensate, the method comprising: (a) combining the small molecule test compound and a composition comprising the target condensate and an extra-condensate solution; (b) separating the target condensate from the extra-condensate solution; and (c) determining, by mass spectrometry, the amount of the small molecule test compound in the target condensate based on the amount of the test compound in the extra-condensate solution,
thereby determining the partition characteristic of the small molecule test compound in the target condensate.

[0007] In some examples, provided herein are methods of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) adding the test compound to a composition comprising a target condensate and an extra-condensate solution; and (b) determining the amount of the test compound in the target condensate, thereby determining the partition characteristic of the test compound in the target condensate. In some examples, the methods further comprise causing the formation of the target condensate prior to step (a).

[0008] In some examples, provided herein are methods of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) causing the formation of the target condensate in the presence of the test compound to obtain a composition comprising the target condensate and an extra-condensate solution; and (b) determining the amount of the test compound in the target condensate, thereby determining the partition characteristic of the test compound in the target condensate.

[0009] In some examples, provided herein are methods of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) adding the test compound to a composition comprising precursor molecules; (b) causing the formation of the target condensate to obtain a composition comprising the target condensate and an extra-condensate

solution; and (c) determining the amount of the test compound in the target condensate, thereby determining the partition characteristic of the test compound in the target condensate. In some examples, the methods further comprise combining the test compound and a precursor composition comprising precursor molecules prior to step (a). In some examples, the methods further comprise adding the test compound to a precursor composition comprising precursor molecules prior to step (a).

[0010] In some examples, the methods further comprise determining the amount of the test compound in the extra-condensate solution. In some examples, the amount of the test compound in the target condensate is determined prior to, simultaneously with, or after the amount of the test compound in the extra-condensate solution is determined. In some examples, the methods further comprise determining the ratio of the amount of test compound in the target condensate and the amount of test compound in the extra-condensate solution. In some examples, the methods further comprise separating the target condensate from the extra-condensate solution. In some examples, the methods further comprise identifying the target condensate prior to determining the amount of test compound in the target condensate.

[0011] In some examples, dysregulation of the target condensate is associated with a disease. In some examples, the methods further comprise characterizing the target condensate by identifying one or more macromolecules comprised therein. In some examples, the identifying comprises determining the amount of the one or more macromolecules in the target condensate. In some examples, the methods further comprise determining the ratio of the amount of test compound in the target condensate and the amount of the one or more macromolecules in the target condensate. In some examples, the target condensate comprises a protein comprising an intrinsically disordered sequence. In some examples, the methods further comprise labeling the target condensate in order to visualize the target condensate. In some examples, the target condensate is labeled with a radioactive label, a colorimetric label, a chemically-reactive label, or a fluorescent label.

[0012] In some examples, the composition comprises a cell. In some examples, the cell is a microorganism or an animal cell. In some examples, the cell comprises a condensate that is determined to be dysregulated. In some examples, the cell has one or more features of a neurodegenerative or proliferative disease.

[0013] In some examples, the target condensate is a cellular condensate. In some examples, cellular condensate is a cleavage body, a P-granule, a histone locus body, a multivesicular body, a neuronal RNA granule, a nuclear gem, a nuclear pore, a nuclear speckle, a nuclear stress body, a nucleolus, a Oct1/PTF/transcription (OPT) domain, a paraspeckle, a perinucleolar compartment, a PML nuclear body, a PML oncogenic domain, a polycomb body, a processing body, a signaling cluster, a Sam68 nuclear body, a stress granule, or a splicing speckle. In some examples, the target condensate is in a cell. In some examples, the cell is a microorganism or an animal cell. In some examples, the cell has one or more features of a neurodegenerative or proliferative disease. In some examples, the extra-condensate solution is intracellular fluid. In some examples, the intracellular fluid is cytosol or nucleosol.

[0014] In some examples, the target condensate is not in a cell. In some examples, the target condensate is an extracellular condensate. In some examples, the extra-condensate solution is extracellular fluid. In some examples, the extracellular fluid is interstitial fluid.

[0015] In some examples, the method is a cell free assay method. In some examples, the composition does not comprise a cell. In some examples, the composition comprises one or more of: a macromolecule, a salt, and a buffer.

[0016] In some examples, the composition comprises two or more target condensates. In some examples, the methods further comprise repeating the steps of the method for one or more additional condensates.

[0017] In some examples, the test compound is small molecule, a polypeptide, or a nucleic acid. In some examples, the test compound comprises a test compound label. In some examples, the test compound label is a radioactive label, a colorimetric label, a chemically-reactive label, or a fluorescent label. In some examples, the test compound label is a fluorescent label. In some examples, the amount of the test compound is determined by detecting the test compound label. In some examples, the amount of the test compound is determined by mass spectrometry, liquid chromatography, quantitative fluorescent microscopy and spectroscopy, nuclear magnetic resonance spectroscopy, Raman spectroscopy, and/or ultraviolet-visible spectrophotometry.

[0018] In some examples, provided herein are methods of determining the partition characteristics of a plurality of test compounds in a target condensate, the method comprising performing a method of determining the partition characteristic described herein with a plurality

of test compounds. In some examples, the methods further comprise comparing the partition characteristics of a subset or all of the plurality of test compounds in the target condensate. In some examples, the methods further comprise identifying test compounds that have the same or similar partition characteristics in a target condensate. In some examples, the methods further comprise identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics. In some examples, the methods further comprise determining the partition characteristic in a target condensate for one or more additional test compounds that comprise the identified characteristic. In some examples, the methods further comprise determining the partition characteristic in a target condensate for one or more additional test compounds that do not comprise the identified characteristic.

[0019] In some examples, provided herein are methods of determining a relative partition characteristic of a test compound in a target condensate, the method comprising: (i) determining the partition characteristic of the test compound by performing a method of determining a partition characteristic described herein with the test compound; (ii) determining the partition characteristic of a reference compound by performing a method of determining a partition characteristic described herein with the reference compound; and (iii) calculating the ratio of the partition characteristics determined in (i) and (ii), thereby determining the relative partition characteristic of the test compound in the target condensate. In some examples, the test compound comprises a test compound label. In some examples, the reference compound is the test compound label.

[0020] In some examples, provided herein are methods of determining relative partition characteristics of a plurality of test compounds in a target condensate, the method comprising: (1) performing a method of determining a relative partition characteristic of a test compound in a target condensate; and (2) repeating steps (i) and (iii) with a plurality of test compounds. In some examples, the methods further comprise comparing the relative partition characteristics in the target condensate of a subset or all of the plurality of test compounds. In some examples, the methods further comprise identifying test compounds that have the same or similar relative partition characteristics in the target condensate. In some examples, the methods further comprise identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics. In some examples, the methods further comprise determining the relative partition

characteristic in the target condensate for one or more additional test compounds that comprise the identified characteristic. In some examples, the methods further comprise determining the relative partition characteristic in the target condensate for one or more additional test compounds that do not comprise the identified characteristic.

[0021] In some examples, provided herein are methods of determining a condensate preference profile of a test compound, the method comprising: (a) determining the partition characteristic of the test compound in a first target condensate according to a method disclosed herein; (b) determining the partition characteristic of the test compound in a second target condensate according to a method disclosed herein; and (c) calculating a ratio of the partition characteristic of the test compound determined in the first target condensate and the second target condensate, thereby determining the condensate preference profile of the test compound. In some examples, the first target condensate and the second target condensate are in the same composition. In some examples, the first target condensate and the second target condensate are in different compositions. In some examples, the partition characteristic of the test compound in the first target condensate is determined prior to, simultaneously with, or after the partition characteristic of the test compound in the second target condensate is determined.

[0022] In some examples, provided herein are methods of determining a condensate preference profile of a test compound, the method comprising: (a) determining the relative partition characteristic of the test compound in a first target condensate according to a method disclosed herein; (b) determining the relative partition characteristic of the test compound in a second target condensate according to a method disclosed herein; and (c) calculating a ratio of the partition characteristic of the test compound determined in the first target condensate and the second target condensate, thereby determining the condensate preference profile of the test compound. In some examples, the first target condensate and the second target condensate are in the same composition. In some examples, the first target condensate and the second target condensate are in different compositions. In some examples, the relative partition characteristic of the test compound in the first target condensate is determined prior to, simultaneously with, or after the relative partition characteristic of the test compound in the second target condensate is determined.

[0023] In some examples, provided herein are methods of determining a condensate preference profile of a test compound, the method comprising: (a) adding the test compound to a composition comprising a first target condensate and a second target condensate; (b)

determining the amount of the test compound in the first target condensate; (c) determining the amount of the test compound in the second target condensate; and (d) calculating a ratio of the amount of the test compound determined in the first target condensate and the second target condensate, thereby determining the condensate preference profile of the test compound. In some examples, the methods further comprise causing the formation of the first target condensate and/or the second target condensate prior to step (a).

[0024] In some examples, provided herein are methods of determining a condensate preference profile of a test compound, the method comprising: (a) adding the test compound to a composition comprising precursor molecules; (b) causing the formation of a first target condensate and a second target condensate in the composition; (c) determining the amount of the test compound in the first target condensate; (d) determining the amount of the test compound in the second target condensate; and (e) calculating a ratio of the amount the test compounds determined in the first target condensate and the second target condensate, thereby determining the condensate preference profile of the test compound. In some examples, the amount of the test compound in the first target condensate is determined prior to, simultaneously with, or after the amount of the test compound in the second target condensate is determined.

[0025] In some examples, the methods further comprise separating the first target condensate and the second target condensate from the composition. In some examples, the methods further comprise identifying the first target condensate and/or the second target condensate prior to determining the amount of test compound in the first condensate and/or the second condensate.

[0026] In some examples, dysregulation of the first target condensate and/or the second target condensate is associated with a disease. In some examples, the methods further comprise characterizing the first target condensate and/or the second target condensate by identifying one or more macromolecules comprised therein. In some examples, the methods further comprise labeling the first target condensate and/or the second target condensate in order to visualize the first target condensate and/or the second target condensate. In some examples, the methods further comprise labeling the first target condensate and the second target condensate in order to visualize the first condensate target condensate and the second target condensate. In some examples, the first target condensate and the second target condensate are labeled with different labels. In some examples, the first target condensate

and/or the second target condensate are labeled with a radioactive label, a colorimetric label, a chemically-reactive label, or a fluorescent label.

[0027] In some examples, the composition comprises a cell. In some examples, the cell is a microorganism or an animal cell. In some examples, the cell comprises a condensate that is determined to be dysregulated. In some examples, the cell has one or more features of a neurodegenerative or proliferative disease.

[0028] In some examples, the first target condensate and/or the second target condensate are cellular condensates. In some examples, the first target condensate is a cleavage body, a P-granule, a histone locus body, a multivesicular body, a neuronal RNA granule, a nuclear gem, a nuclear pore, a nuclear speckle, a nuclear stress body, a nucleolus, a Oct1/PTF/transcription (OPT) domain, a paraspeckle, a perinucleolar compartment, a PML nuclear body, a PML oncogenic domain, a polycomb body, a processing body, a signaling cluster, a viral condensate, a Sam68 nuclear body, a stress granule, or a splicing speckle. In some examples, the second target condensate is a cleavage body, a P-granule, a histone locus body, a multivesicular body, a neuronal RNA granule, a nuclear gem, a nuclear pore, a nuclear speckle, a nuclear stress body, a nucleolus, a Oct1/PTF/transcription (OPT) domain, a paraspeckle, a perinucleolar compartment, a PML nuclear body, a PML oncogenic domain, a polycomb body, a processing body, a signaling cluster, a viral condensate, a Sam68 nuclear body, a stress granule, or a splicing speckle. In some examples, the first target condensate and/or the second target condensate are in a cell.

[0029] In some examples, the first target condensate and/or the second target condensate are extracellular condensates.

[0030] In some examples, the composition does not comprise a cell. In some examples, the composition comprises one or more of: a macromolecule, a salt, and a buffer.

[0031] In some examples, the composition comprises one or more additional target condensates. In some examples, the methods further comprise repeating the steps of the method for one or more additional target condensates.

[0032] In some examples, provided herein are methods of determining condensate preference profiles of a plurality of test compounds, the method comprising performing a method of determining a condensate preference profile described herein with a plurality of test compounds. In some examples, the methods further comprise comparing condensate preference profiles of a subset or all of the plurality of test compounds. In some examples, the methods further

comprise identifying test compounds that have the same or similar condensate preference profiles. In some examples, the methods further comprise identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles. In some examples, the methods further comprise determining the relative partition characteristic for one or more additional test compounds that comprise the identified characteristic. In some examples, the methods further comprise determining the relative partition characteristic for one or more additional test compounds that do not comprise the identified characteristic.

[0033] In some examples, provided herein are methods of identifying a compound characteristic associated with partitioning a compound into or out of a condensate, the method comprising: (a) determining partition characteristics of a plurality of test compounds in the target condensate according to a method described herein; (b) comparing the partition characteristics of a subset or all of the plurality of test compounds in the target compound; (c) identifying test compounds that have the same or similar partition characteristics in the target condensate; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics.

[0034] In some examples, provided herein are methods of identifying a compound characteristic associated with partitioning a compound into a condensate, the method comprising: (a) determining partition characteristics of a plurality of test compounds in the target condensate according to a method described herein; (b) comparing the partition characteristics of a subset or all of the plurality of test compounds in the target compound; (c) identifying test compounds that have the same or similar partition characteristics in the target condensate; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics.

[0035] In some examples, provided herein are methods of identifying a compound characteristic associated with partitioning a compound into or out of a condensate, the method comprising: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate according to a method described herein; (b) comparing the relative partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar relative partition characteristics in the target condensate; and (d) identifying a characteristic that a subset or all of

the identified test compounds have in common in addition to the same or similar relative partition characteristics.

[0036] In some examples, provided herein are methods of identifying a compound characteristic associated with partitioning a compound into a condensate, the method comprising: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate according to a method described herein; (b) comparing the relative partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar relative partition characteristics in the target condensate; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics.

[0037] In some examples, provided herein are methods of identifying a compound characteristic associated with partitioning a compound into or out of a condensate, the method comprising: (a) determining condensate preference profiles of a plurality of test compounds according to a method described herein; (b) comparing the condensate preference profiles of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar condensate preference profiles; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles.

[0038] In some examples, provided herein are methods of identifying a compound characteristic associated with partitioning a compound into a condensate, the method comprising: (a) determining condensate preference profiles of a plurality of test compounds according to a method described herein; (b) comparing the condensate preference profiles of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar condensate preference profiles; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles.

[0039] In some examples, provided herein are methods of designing a compound with a desired partition characteristic into or out of a target condensate, the method comprising: (a) determining partition characteristics of a plurality of test compounds in the target condensate according to a method described herein; (b) comparing the partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds

that have the same or similar partition characteristics in the target condensate; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics; and (e)(i) designing a compound that comprises the identified characteristic; or (ii) designing a compound that does not comprise the identified characteristic, thereby designing a compound with the desired partition characteristic into or out of the target condensate. In some examples, designing the compound comprises attaching a moiety that comprises the identified characteristic, thereby conferring the desired partition characteristic to the compound. In some examples, designing the compound comprises removing a moiety that comprises the identified characteristic, thereby conferring the desired partition characteristic to the compound. In some examples, designing the compound comprises changing a first moiety to a second moiety that comprises the identified characteristic, thereby conferring the desired partition characteristic to the compound. In some examples, the compound is designed, in whole or in part, using an approach comprising a modeling, computer, and/or calculation-based technique, *e.g.*, a bioinformatic, cheminformatic, and/or artificial intelligence (AI)-based technique.

[0039a] In one aspect, the present disclosure refers to a method of designing a small molecule compound with a desired partition characteristic into or out of a target condensate, the method comprising: (a) identifying a characteristic that a subset or all of the identified small molecule test compounds have in common in addition to the same or similar partition characteristics by performing the method disclosed herein; (b) designing: (i) the small molecule compound that comprises the identified characteristic; or (ii) the small molecule compound that does not comprise the identified characteristic,
thereby designing the small molecule compound with the desired partition characteristic into or out of the target condensate.

[0040] In some examples, provided herein are methods of designing a compound with a desired partition characteristic to a target condensate, the method comprising: (a) determining partition characteristics of a plurality of test compounds in the target condensate according to a method described herein; (b) comparing the partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar partition characteristics in the target condensate; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics; and (e) designing a compound that comprises the identified characteristic. In some examples, designing the compound comprises attaching a moiety that comprises the identified characteristic, thereby conferring the desired partition characteristic to the compound. In some examples, designing the compound comprises changing a first moiety to a second moiety that comprises the identified characteristic, thereby conferring the desired partition characteristic to the compound. In some examples, the compound is designed, in whole or in part, using an approach comprising a modeling, computer, and/or calculation-based technique, *e.g.*, a bioinformatic, cheminformatic, and/or artificial intelligence (AI)-based technique.

[0041] In some examples, provided herein are methods of designing a compound with a desired relative partition characteristic into or out of a target condensate, the method comprising: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate according to a method disclosed herein; (b) comparing the relative partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar relative partition characteristics in the target condensate; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics; and (e)(i) designing a compound that comprises the identified characteristic; or (ii) designing a compound that does not comprise the identified characteristic, thereby designing a compound with the desired relative partition characteristic into or out of the target condensate. In some examples, designing the compound comprises attaching a moiety that comprises the identified characteristic, thereby conferring the desired relative partition characteristic to the compound. In some examples, designing the compound comprises removing a moiety that comprises the identified characteristic, thereby conferring the desired partition characteristic to the compound. In some examples, designing the compound comprises changing a first moiety to a second moiety that comprises the identified characteristic, thereby conferring the desired partition characteristic to the compound. In some examples, the compound is designed, in whole or in part, using an approach comprising a modeling, computer, and/or calculation-based technique, *e.g.*, a bioinformatic, cheminformatic, and/or artificial intelligence (AI)-based technique.

[0042] In some examples, provided herein are methods of designing a compound with a desired relative partition characteristic, the method comprising: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate according to a method described herein; (b) comparing the relative partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar relative partition characteristics in the target condensate; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics; and (e) designing a compound that comprises the identified characteristic. In some examples, designing the compound comprises attaching a moiety that comprises the identified characteristic, thereby conferring the desired relative partition characteristic to the compound. In some examples, designing the

compound comprises changing a first moiety to a second moiety that comprises the identified characteristic, thereby conferring the desired partition characteristic to the compound. In some examples, the compound is designed, in whole or in part, using an approach comprising a modeling, computer, and/or calculation-based technique, *e.g.*, a bioinformatic, cheminformatic, and/or artificial intelligence (AI)-based technique.

[0043] In some examples, provided herein are methods of designing a compound with a desired condensate preference profile, the method comprising: (a) determining condensate preference profiles of a plurality of test compounds according to a method disclosed herein; (b) comparing the condensate preference profiles of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar condensate preference profiles; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles; and (e)(i) designing a compound that comprises the identified characteristic; or (ii) designing a compound that does not comprise the identified characteristic, thereby designing a compound with the desired condensate preference profile. In some examples, the methods further comprise making the compound. In some examples, designing the compound comprises attaching a moiety that comprises the identified characteristic, thereby conferring the desired condensate preference profile to the compound. In some examples, designing the compound comprises removing a moiety that comprises the identified characteristic, thereby conferring the desired partition characteristic to the compound. In some examples, designing the compound comprises changing a first moiety to a second moiety that comprises the identified characteristic, thereby conferring the desired partition characteristic to the compound. In some examples, the compound is designed, in whole or in part, using an approach comprising a modeling, computer, and/or calculation-based technique, *e.g.*, a bioinformatic, cheminformatic, and/or artificial intelligence (AI)-based technique.

[0044] In some examples, provided herein are methods of designing a compound with a desired condensate preference profile, the method comprising: (a) determining condensate preference profiles of a plurality of test compounds according to a method described herein; (b) comparing the condensate preference profiles of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar condensate preference profiles; (d) identifying a characteristic, such as a chemical moiety or motif, that a subset or all of the identified test compounds have in common in addition to the same or similar condensate

preference profiles; and (e) designing a compound that comprises the identified characteristic. In some examples, designing the compound comprises attaching a moiety that comprises the identified characteristic, thereby conferring the desired condensate preference profile to the compound. In some examples, designing the compound comprises changing a first moiety to a second moiety that comprises the identified characteristic, thereby conferring the desired partition characteristic to the compound. In some examples, there is provided a plurality of compounds designed by the methods described herein. In some examples, the compound is designed, in whole or in part, using an approach comprising a modeling, computer, and/or calculation-based technique, *e.g.*, a bioinformatic, cheminformatic, and/or artificial intelligence (AI)-based technique. In some examples, the methods further comprise making the compound.

[0045] In some examples, provided herein are methods of screening a test compound for a desired partition characteristic from a group of candidate compounds, the method comprising: (a) determining a partition characteristic of each of the group of candidate compounds; and (b) identifying the test compound having the desired partition characteristic. In some examples, the partition characteristic of each of the group of candidate compounds is determined *in vitro*. In some examples, the test compound has a suitable partition characteristic for being useful for treating a disease in an individual.

[0046] In some examples, provided herein are methods of identifying a test compound useful for treating a disease in an individual in need thereof, the method comprising: (a) identifying a target condensate associated with the disease; and (b) determining a partition characteristic of a candidate compound in the target condensate, and (c) identifying the test compound having a suitable partition characteristic for being useful for treating the disease.

[0047] In some examples, provided herein are methods of determining a partition characteristic of a test compound in a target condensate, the methods comprising: (a) combining the test compound and a composition comprising the target condensate and an extra-condensate solution; (b) obtaining a reference control; (c) measuring a MS signal of the test compound in the extra-condensate solution, or a portion thereof, using a mass spectrometry technique; (d) measuring a MS signal of the test compound in the reference control, or a portion thereof, using a mass spectrometry technique; and (e) comparing the MS signal of the test compound from the extra-condensate solution and the MS signal of the test compound from the reference control, thereby determining the partition characteristic of the test compound in the target condensate. In

some examples, the amount of the test compound combined with the composition is 100 nM or less, and the amount of a precursor molecule in the composition, including in the target condensate, is about 5 μM.

[0047a] In one aspect, the present disclosure refers to a method of determining a partition characteristic of a small molecule test compound in a target condensate, the method comprising: (a) combining the small molecule test compound and a composition comprising the target condensate and an extra-condensate solution; (b) obtaining a reference control; (c) measuring a MS signal of the small molecule test compound in the extra-condensate solution, or a portion thereof, using an MS technique; (d) measuring a MS signal of the small molecule test compound in the reference control, or a portion thereof, using an MS technique; and (e) comparing the MS signal of the small molecule test compound from the extra-condensate solution and the MS signal of the small molecule test compound from the reference control, thereby determining the partition characteristic of the small molecule test compound in the target condensate.

[0048] In some examples, provided herein is a library comprising a plurality of compounds, wherein each compound of the plurality of compounds comprises the same moiety comprising a characteristic having a desired partition characteristic.

[0049] In some examples, provided herein is a method of designing a test compound having a desired partition characteristic, the method comprising modifying a precursor of the test compound by attaching a moiety to the compound, wherein the moiety comprises a characteristic having a desired partition characteristic.

[0050] It will also be understood by those skilled in the art that changes in the form and details of the implementations described herein may be made without departing from the scope of this disclosure. In addition, although various advantages, examples, and objects have been described with reference to various implementations, the scope of this disclosure should not be limited by reference to such advantages, examples, and objects.

BRIEF DESCRIPTION OF THE DRAWINGS

[0051] **FIG. 1** shows exemplary fluorescent images of dyes partitioning in condensates. Dye #10 is not enriched in the condensate, while other dyes are enriched in the condensate.

[0052] **FIGS. 2A** and **2B** depict the regions inside and outside exemplary condensates that were chosen to measure intensities in the presence (**FIG. 2A**) and absence of dye (**FIG. 2B**).

[0053] **FIG. 3** shows the average intensity-in to intensity-out (I-in:I-out) ratios determined for each dye in FUS-SNAP condensates from two different days of experiments. Error bars show standard deviation of the two independent experiments.

[0054] **FIG. 4** shows the average intensity-in to intensity-out (I-in:I-out) ratios determined for each dye in PGL-3 condensates from two different days of experiments. Error bars show standard deviation of the two independent experiments.

[0055] **FIG. 5** shows the ratios of the average I-in:I-out ratio for each dye in FUS-SNAP condensates to the ratios of the average I-in:I-out ratio for each dye in PGL-3 condensates (FUS-SNAP:PGL-3 ratio).

[0056] **FIG: 6** shows fluorescent images of GFP-tag labeled FUS condensates in the presence or absence of Rhodamine 800 using a FUS-GFP channel, a Rhodamine 800 channel, and a merge of the other images.

[0057] **FIG. 7** shows a histogram of the fraction of compound in the supernatant (outside the condensates) of exemplary compounds using both a fluorescence-based assay and a mass spectrometry-based assay.

[0058] **FIG. 8** shows a histogram of the fraction of compound in the supernatant of exemplary compounds evaluated at various compound concentrations.

[0059] **FIGS. 9A** and **9B** show histograms of the fraction of a compound of interest outside the condensates measured in systems having the single compound of interest and systems having a mixture of compounds including the compound of interest. Fluorescein is the compound of interest in **FIG. 9A**. Rhodamine B is the compound of interest in **FIG. 9B**.

[0060] **FIG. 10** shows a histogram of the fraction of compound in the supernatant for certain compounds in several multiplexed systems.

DETAILED DESCRIPTION

[0061] The invention includes, in some aspects, methods of assessing, such as characterizing or determining, condensate-associated characteristics of a compound, such as a test compound, and applications thereof.

[0062] The disclosure of the present application is based, in part, on the inventors' unique insights of methods of assessing, such as determining or characterizing, condensate-associated characteristics of a compound and that such methods may be useful for, *e.g.*, identifying, characterizing, and developing compounds, or moieties thereof, capable of a desired interaction (including a lack of an interaction) with a condensate. In some embodiments, the desired interaction with a condensate results in a desired biological activity associated with a compound. In some aspects, the disclosure of the present application is based, in part, on the inventors' findings and developments regarding the use of quantitative techniques, such as mass spectrometry, for determining a condensate-associated characteristic of a compound, such as a partition characteristic of a test compound for a target condensate. Such methods allow for, *e.g.*, accurate and reliable determination of a partition characteristic of a test compound for a target condensate in a high-throughput manner that is suitable for use in both simple and complex systems. Additionally, *e.g.*, these methods are hypothesis-free (*i.e.*, do not require a known,

labeled compound or condensate, or a component thereof), compatible with a high-degree of compound multiplexing, do not require compound enrichment, do not require compound extraction from a condensate, can be used in homotypic and heterotypic systems, and can be performed with a lower amount of compound and/or condensate precursor macromolecules, which represents a more biologically relevant model and reduces the use of starting materials and reagents. These methods allow for the identification of test compounds, or portions thereof, that can be used to guide further identification and/or design of one or more compounds having a desired partition characteristic, thus providing a method for identifying a target compound with improved potency, therapeutic index, and/or safety. The identification of test compounds, or portions thereof, also enables the advancement of certain drug development application using such knowledge, *e.g.*, the development of privileged libraries of compound having a desired partitioning characteristic, and modeling and/or calculation-based techniques for drug discovery and screening.

[0063] As described herein, the methods of assessing condensate-associated characteristics of a compound may be applied to many methods and forms thereof, including methods of determining a partition characteristic of a test compound, methods of determining a relative partition characteristic of a test compound, methods of determining a condensate preference profile of a test compound, methods of identifying a compound characteristic associated with condensate association, methods of designing a compound with a desired condensate association, and methods of identifying a compound useful for treating a disease in an individual. Forms of the methods described herein, include, *e.g.*, assays for: assessing interactions of a single test compound, or a portion thereof, with a single target condensate, assessing interactions of a single test compound, or a portion thereof, with a plurality of target condensates, and assessing interactions of a plurality of test compounds, or a subset thereof, with a single target condensate. As described herein, the inventors' unique insights enable, *e.g.*, identification, development, and optimization of compounds, such as pharmaceutically acceptable compounds, useful for the treatment of a disease in an individual.

[0064] Thus, in some aspects, the invention includes methods of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) adding the test compound to a composition comprising a target condensate and an extra-condensate solution; (b) determining the amount of the test compound in the target condensate, thereby determining the partition characteristic of the test compound in the target condensate.

[0065] In other aspects, the invention includes methods of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) adding the test compound to a composition comprising precursor molecules; (b) causing the formation of the target condensate to obtain a composition comprising the target condensate and an extra-condensate solution; (c) determining the amount of the test compound in the target condensate, thereby determining the partition characteristic of the test compound in the target condensate.

[0066] In other aspects, the invention includes methods of determining the partition characteristics of a plurality of test compounds in a target condensate, the method comprising performing any one or more methods described herein using a plurality of test compounds.

[0067] In some embodiments, the invention includes methods of identifying a compound characteristic associated with partitioning a compound into a condensate, the method comprising: (a) determining partition characteristics of a plurality of test compounds in the target condensate by performing a method of determining the partition characteristics of a plurality of test compounds in the target condensate; (b) comparing the partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar partition characteristics in the target condensate; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics. In some embodiments, the invention includes methods of designing a compound with a desired partition characteristic to a target condensate, the method comprising: (a) determining partition characteristics of a plurality of test compounds in the target condensate by performing a method of determining the partition characteristics of a plurality of test compounds in the target condensate; (b) comparing the partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar partition characteristics in the target condensate; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics; and (e) designing a compound that comprises the identified characteristic.

[0068] In other aspects, the invention includes methods of determining a relative partition characteristic of a test compound in a target condensate, the method comprising: (i) determining the partition characteristic of the test compound by performing any one or more methods described herein with the test compound; (ii) determining the partition characteristic of a reference compound by performing any one or more methods described herein with the

reference compound; and (iii) calculating the ratio of the partition characteristics determined in (i) and (ii), thereby determining the relative partition characteristic of the test compound in the target condensate. In some embodiments, the methods further comprise repeating steps (i) and (iii) with a plurality of test compounds. In some embodiments, the invention includes methods of identifying a compound characteristic associated with partitioning a compound into a target condensate, the method comprising: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate by performing a method of determining a relative partition characteristic of a plurality test compound in a target condensate; (b) comparing the relative partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar relative partition characteristics in the target condensate; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics. In some embodiments, the invention includes methods of designing a compound with a desired relative partition characteristic, the method comprising: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate by performing a method of determining a relative partition characteristic of a plurality test compound in the target condensate; (b) comparing the relative partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar relative partition characteristics in the target condensate; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics; and (e) designing a compound that comprises the identified characteristic.

[0069] In other aspects, the invention includes methods of determining a condensate preference profile of a test compound, the method comprising: (a) adding the test compound to a composition comprising a first target condensate and a second target condensate; (b) determining the amount of the test compound in the first target condensate; (c) determining the amount of the test compound in the second target condensate; and (d) calculating a ratio of the amount of the test compound determined in the first target condensate and the second target condensate, thereby determining the condensate preference profile of the test compound.

[0070] In other aspects, the invention includes methods of determining a condensate preference profile of a test compound, the method comprising: (a) adding the test compound to a composition comprising precursor molecules; (b) causing the formation of a first target

condensate and a second target condensate in the composition; (c) determining the amount of the test compound in the first target condensate; (d) determining the amount of the test compound in the second target condensate; (e) calculating a ratio of the amount the test compounds determined in the first target condensate and the second target condensate, thereby determining the condensate preference profile of the test compound.

[0071] In other aspects, the invention includes methods of determining condensate preference profiles of a plurality of test compounds, the method comprising performing any one or more of the methods described herein for determining a condensate preference profile of a test compound using a plurality of test compounds. In some embodiments, the invention includes methods of identifying a compound characteristic associated with partitioning a compound into a condensate, the method comprising: (a) determining condensate preference profiles of a plurality of test compounds by performing a method of determining condensate preference profiles of a plurality of test compounds; (b) comparing the condensate preference profiles of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar condensate preference profiles; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles. In some embodiments, the invention includes methods of designing a compound with a desired condensate preference profile, the method comprising: (a) determining condensate preference profiles of a plurality of test compounds by performing a method of determining condensate preference profiles of a plurality of test compounds; (b) comparing the condensate preference profiles of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar condensate preference profiles; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles; and (e) designing a compound that comprises the identified characteristic.

Definitions

[0072] For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth below conflicts with any document incorporated herein by reference, the definition set forth shall control.

[0073] The terms “polypeptide” and “protein” are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-translational modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like.

[0074] The terms “comprising,” “having,” “containing,” and “including,” and other similar forms, and grammatical equivalents thereof, as used herein, are intended to be equivalent in meaning and to be open ended in that an item or items following any one of these words is not meant to be an exhaustive listing of such item or items, or meant to be limited to only the listed item or items. For example, an article “comprising” components A, B, and C can consist of (*i.e.*, contain only) components A, B, and C, or can contain not only components A, B, and C but also one or more other components. As such, it is intended and understood that “comprises” and similar forms thereof, and grammatical equivalents thereof, include disclosure of embodiments of “consisting essentially of” or “consisting of.”

[0075] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit, unless the context clearly dictate otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

[0076] Reference to “about” a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to “about X” includes description of “X.”

[0077] As used herein, including in the appended claims, the singular forms “a,” “or,” and “the” include plural referents unless the context clearly dictates otherwise.

[0078] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Methods of assessing condensate-associated characteristics of a compound

[0079] In some aspects of the application, methods of assessing a condensate-associated characteristic of a compound, such as a test compound, and applications thereof are provided. In some embodiments, "condensate" described herein refers to a non-membrane-encapsulated compartment formed by phase separation of one or more of proteins and/or other macromolecules (including all stages of phase separation).

[0080] Described in more detail below are techniques for assessing condensate-associated characteristics of a compound and applications to many methods and forms thereof. Those skilled in the art will recognize that, in view of the provided description, several embodiments are possible within the scope and spirit of the disclosure of this application.

[0081] In some aspects, provided herein are methods of determining a partition characteristic of a test compound, comprising determining the amount of the test compound in the target condensate. In some embodiments, determining the amount of the test compound in the target condensate thereby determines the partition characteristic of the test compound in the target condensate.

[0082] In some aspects, provided herein are methods of determining a relative partition characteristic of a test compound, comprising determining the amount of the test compound in the target condensate, determining the amount of a reference compound in the target condensate, and calculating the ratio of the amount of the test compound and the reference compound in the target condensate.

[0083] Additionally, in some aspects, provided herein are methods of determining a condensate preference profile of a test compound, comprising determining the amount of the test compound in a first target condensate, determining the amount of the test compound in a second target condensate, and calculating the ratio of the amount of the test compound in the first and second target condensates. In some embodiments, the methods of determining a condensate preference profile of a test compound comprise determining the partition characteristic or the relative partition characteristic of the test compound in a first target condensate, determining the partition characteristic or the relative partition characteristic of the test compound in a second target condensate, and calculating the ratio of the partition characteristic or the relative partition characteristic of the test compound in the first and second target condensates.

[0084] In some embodiments, the methods further comprise repeating the steps of the method for a plurality of test compounds. For example, in some embodiments, the methods

comprise repeating the steps of the method for at least about any of 2, 3, 4, 5, 10, 15, 20, 25, 40, 50, 75, 100, 250, 500, 1,000, 10,000, 100,000 or more compounds.

[0085] In some embodiments, the methods further comprise combining the test compound and a composition comprising a target condensate and an extra-condensate solution. In some embodiments, the methods further comprise adding the test compound to a composition comprising a target condensate and an extra-condensate solution. In some embodiments, the methods further comprise causing the formation of the target condensate. In some embodiments, the methods further comprise combining the test compound and a precursor composition comprising condensate precursor molecules and then causing the formation of the target condensate. In some embodiments, the methods further comprise adding the test compound to a composition comprising condensate precursor molecules and then causing the formation of the target condensate.

[0086] In some embodiments, the methods further comprise combining the test compound and a composition comprising a cell. In some embodiments, the methods further comprise combining the test compound and a composition comprising a cell and then causing the formation of the target condensate. In some embodiments, the methods further comprise causing the formation of the target condensate in a composition comprising a cell and then combining the test compound and the composition. In some embodiments, the methods further comprise causing the compound to enter the cell.

[0087] In some embodiments, the methods further comprise adding the test compound to a composition comprising a cell. In some embodiments, the methods further comprise adding the test compound to a composition comprising a cell and then causing the formation of the target condensate. In some embodiments, the methods further comprise causing the formation of the target condensate in a composition comprising a cell and then adding the test compound to the composition. In some embodiments, the methods further comprise causing the compound to enter the cell.

[0088] In some embodiments, the methods further comprise separating the target condensate from the extra-condensate solution, *e.g.*, for the purpose of quantifying the target compound in the condensate or the extra-condensate solution. In a cell-free solution, condensates are typically denser than extra-condensate solutions and will sediment. Accordingly, in some embodiments, separating the target condensate from the extra-condensate solution comprises separating the

supernatant from the precipitate. In some embodiments, the methods comprise centrifuging the composition. In some embodiments, the methods comprise allowing the condensate to sediment.

Techniques for determining a condensate-associated characteristic of a compound

[0089] In some aspects, provided herein are methods of determining a condensate-associated characteristic of a compound comprising determining an amount of a test compound that is depleted (including determining that there is a lack of depletion) from an extra-condensate solution due to the presence, formation, and/or modulation of a target condensate. In some embodiments, the method of determining a condensate-associated characteristic of a compound comprises use of a mass spectrometry-based technique (*e.g.*, to determine an amount of a test compound). In some embodiments, the method of determining a condensate-associated characteristic of a compound comprises use of any one or more of liquid chromatography (*e.g.*, HPLC), microscopy, quantitative image analysis, quantitative fluorescent microscopy and spectroscopy, nuclear magnetic resonance spectroscopy, Raman spectroscopy, and/or ultraviolet-visible spectrophotometry (*e.g.*, to determine an amount of a test compound). In some embodiments, such methods are useful in determining a partition characteristic, a relative partition characteristic, and/or a condensate preference profile of a test compound in a composition comprising a target condensate and an extra-condensate solution.

[0090] In some embodiments, the method comprises determining an amount of a test compound that is depleted from a system due to the presence and/or formation of a target condensate. In some embodiments, the method comprises determining an amount of a test compound that is in an extra-condensate solution and not associated with a precursor molecule of a target condensate. In some embodiments, the method comprises determining an amount of a test compound that is associated with, such as in, a target condensate. In some embodiments, the method comprises determining an amount of a test compound that is associated with a precursor molecule of a target condensate. In some embodiments, the amount of the test compound depleted from a system due to the presence and/or formation of a target condensate is used to determine a condensate-associated characteristics, *e.g.*, a partition characteristic of the test compound for a target condensate.

[0091] In some embodiments, the method comprises comparing a MS signal of a test compound from an extra-condensate solution, or a portion thereof, and a MS signal of the test compound from a reference control, or a portion thereof, such as via a ratio of the MS signal of

the test compound from the extra-condensate solution, or the portion thereof, and the MS signal of the test compound from the reference control, or the portion thereof. In some embodiments, the ratio of a MS signal of a test compound from an extra-condensate solution and a MS signal of the test compound from a reference control represents a depletion value. In some embodiments, the depletion value is representative of an amount of a test compound that is depleted from a system due to the presence and/or formation of a target condensate. In some embodiments, the method further comprises obtaining, such as measuring a MS ion signal of a test compound in an extra-condensate solution, or a portion thereof, using a mass spectrometry technique. In some embodiments, the method further comprises obtaining, such as measuring a MS ion signal of a test compound in a reference control, or a portion thereof, using a mass spectrometry technique. In some embodiments, the method further comprises combining a test compound and a composition comprising a target condensate and an extra-condensate solution. In some embodiments, the method further comprises causing the formation of a target condensate in the presence of a test compound to obtain a composition comprising the target condensate and an extra-condensate solution. In some embodiments, the method further comprises separating a target condensate in a composition comprising the target condensate and an extra-condensate solution, such as via pelleting the target condensate in the composition. In some embodiments, the method further comprises obtaining, such as generating, a reference control.

[0092] In some embodiments, the amount of the test compound added to a composition comprising the target condensate and an extra-condensate solution, or a precursor thereof, is based on an amount such that a relatively small depletion of the total amount of test compound from the extra-condensate solution can be determined (such as determined by comparing a measurement of the amount of the compound in an extra-condensate and a measurement of the amount of the compound in a reference control). In some embodiments, the amount of the test compound added to a composition comprising the target condensate and an extra-condensate solution is based on the amount of the target condensate, and/or one or more precursor molecules thereof, in the composition. In some embodiments, the amount of the test compound added to a composition comprising the target condensate and an extra-condensate solution is based on the compound capacity of the target condensate. In some embodiments, the amount of the test compound is about 1 μM or less, such as about any of 900 nM or less, 800 nM or less, 700 nM or less, 600 nM or less, 500 nM or less, 450 nM or less, 400 nM or less, 350 nM or less,

300 nM or less, 250 nM or less, 200 nM or less, 150 nM or less, 125 nM or less, 100 nM or less, 90 nM or less, 80 nM or less, 70 nM or less, 60 nM or less, 50 nM or less, 40 nM or less, 30 nM or less, 20 nM or less, 10 nM or less, 9 nM or less, 8 nM or less, 7 nM or less, 6 nM or less, 5 nM or less, 4 nM or less, 3 nM or less, 2 nM or less, or 1 nM or less. In some embodiments, the lower limit of the test compound is based on the analytical method used to measure the amount of the test compound. In some embodiments, the amount of precursor molecule in the composition is between about 1 μM and about 10 μM, and the test compound is about 1 μM or less, such as about any of 900 nM or less, 800 nM or less, 700 nM or less, 600 nM or less, 500 nM or less, 450 nM or less, 400 nM or less, 350 nM or less, 300 nM or less, 250 nM or less, 200 nM or less, 150 nM or less, 125 nM or less, 100 nM or less, 90 nM or less, 80 nM or less, 70 nM or less, 60 nM or less, 50 nM or less, 40 nM or less, 30 nM or less, 20 nM or less, 10 nM or less, 9 nM or less, 8 nM or less, 7 nM or less, 6 nM or less, 5 nM or less, 4 nM or less, 3 nM or less, 2 nM or less, or 1 nM or less.

[0093] In some embodiments, the method comprises use of a reference control and/or methods of preparing the reference control. The reference controls described herein provide a reference measurement that is useful for determining an amount of a test compound that is depleted from a system due to the presence and/or formation of a target condensate. In some embodiments, wherein a composition comprises: (a) an amount of a target condensate, (b) an extra-condensate solution, (c) an amount, such as concentration, of a precursor macromolecule of the target condensate in the extra-condensate solution, and (d) an amount, such as a concentration, of a test compound in the composition, including (i) an amount of the test compound associated with, including in, the target condensate, and (ii) an amount, such as a concentration, of the test compound in the extra-condensate solution, the reference control comprises the amount, such as concentration, of the precursor macromolecule of the target condensate in the extra-condensate solution, and the amount, such as a concentration, of the test compound in the composition. In some embodiments, the method comprises measuring the amount, such as concentration, of a precursor macromolecule of a target condensate in an extra-condensate solution, or a portion thereof, from a composition comprising the target condensate and the extra-condensate solution.

[0094] In some embodiments, the reference control comprises an amount of a precursor macromolecule of the target condensate based on an amount of the precursor macromolecule present in the extra-condensate solution after the composition has been subjected to pelleting of

the target condensate. In some embodiments, the reference control has the same concentration of the precursor macromolecule as the concentration of the precursor macromolecule in the extra-condensate solution after the composition has been subjected to pelleting of the target condensate. In some embodiments, the reference control comprises the test compound at a concentration that is the same as that combined with the composition comprising the target condensate and the extra-condensate solution. In some embodiments, the reference control is substantially free of a target condensate. In some embodiments, the method further comprises determining the amount of the test compound in the reference control. In some embodiments, determining the amount of the test compound in the reference control comprises measuring the amount of the test compound in the reference control, or a portion thereof, using a mass spectrometry-based technique.

[0095] A variety of mass spectrometry-based techniques are suitable for the methods described herein. In some embodiments, the mass-spectrometry-based technique comprises measuring a MS signal of one or more ion species of one or more compounds, such as one or more test compounds. In some embodiments, the MS signal is of one or more ion species of a test compound, such as one or more charge states of ions of the test compound. In some embodiments, the MS signal is derived from a mass-to-charge (*m/*z) measurement. In some embodiments, the MS signal is ionization intensity. In some embodiments, the MS signal is peak height. In some embodiments, the MS signal is peak area, such as the integral of a signal corresponding with a MS ion signal. In some embodiments, the MS signal is peak volume, such as the integral of a signal corresponding with a MS ion signal. In some embodiments, the MS signal is a cumulative measurement of measured signals of ions of a test compound. In some embodiments, the mass spectrometry-based technique comprises a liquid chromatography/ mass spectrometry (LC/MS) technique, a liquid chromatography/ tandem mass spectrometry (LC/MS) technique and/or a direct sample introduction technique (*e.g.*, direct spray). In some embodiments, the mass spectrometry-based technique comprises gas chromatography/ mass spectrometry (GC/MS). In some embodiments, the mass spectrometry-based technique comprises an acquisition technique selected from data-dependent acquisition, data-independent acquisition, selected reaction monitoring (SRM), and multiple reaction monitoring (MRM).

[0096] Liquid chromatography techniques contemplated by the present application include methods for separating precursor macromolecules and/or test compounds compatible with mass spectrometry techniques. In some embodiments, the liquid chromatography technique comprises

a high performance liquid chromatography (HPLC) technique. In some embodiments, the liquid chromatography technique comprises a high-flow liquid chromatography technique. In some embodiments, the liquid chromatography technique comprises a low-flow liquid chromatography technique, such as a micro-flow liquid chromatography technique or a nano-flow liquid chromatography technique. In some embodiments, the liquid chromatography technique comprises an online liquid chromatography technique coupled to a mass spectrometer. In some embodiments, capillary electrophoresis (CE) techniques, or electrospray or MALDI techniques may be used to introduce the sample to a mass spectrometer. In some embodiments, direct sample introduction techniques may be used to introduce the sample to a mass spectrometer. In some embodiment, the mass spectrometry technique comprises an ionization technique. Ionization techniques contemplated by the present application include techniques capable of charging precursor macromolecules and/or test compounds. In some embodiments, the ionization technique is electrospray ionization. In some embodiments, the ionization technique is nano-electrospray ionization. In some embodiments, the ionization technique is atmospheric pressure chemical ionization. In some embodiments, the ionization technique is atmospheric pressure photoionization. In some embodiments, the ionization technique is matrix-assisted laser desorption ionization (MALDI). In some embodiment, the mass spectrometry technique comprises electrospray ionization, nano-electrospray ionization, or a matrix-assisted laser desorption ionization (MALDI) technique.

[0097] Mass spectrometers contemplated by the present application, to which an online liquid chromatography technique may be coupled, include high-resolution mass spectrometers and low-resolution mass spectrometers. Thus, in some embodiments, the mass spectrometer is a time-of-flight (TOF) mass spectrometer. In some embodiments, the mass spectrometer is a quadrupole time-of-flight (Q-TOF) mass spectrometer. In some embodiments, the mass spectrometer is a quadrupole ion trap time-of-flight (QIT-TOF) mass spectrometer. In some embodiments, the mass spectrometer is an ion trap. In some embodiments, the mass spectrometer is a single quadrupole. In some embodiments, the mass spectrometer is a triple quadrupole (QQQ). In some embodiments, the mass spectrometer is an orbitrap. In some embodiments, the mass spectrometer is a quadrupole orbitrap. In some embodiments, the mass spectrometer is a Fourier transform ion cyclotron resonance (FT) mass spectrometer. In some embodiments, the mass spectrometer is a quadrupole Fourier transform ion cyclotron resonance (Q-FT) mass spectrometer. In some embodiments, the mass spectrometry technique comprises

positive ion mode. In some embodiments, the mass spectrometry technique comprises negative ion mode. In some embodiments, the mass spectrometry technique comprises a time-of-flight (TOF) mass spectrometry technique. In some embodiments, the mass spectrometry technique comprises a quadrupole time-of-flight (Q-TOF) mass spectrometry technique. In some embodiments, the mass spectrometry technique comprises an ion mobility mass spectrometry technique. In some embodiments a low-resolution mass spectrometry technique, such as an ion trap, or single or triple-quadrupole approach is appropriate.

[0098] In some embodiments, the mass spectrometry-based technique comprises processing the obtained MS signals of the precursor macromolecules and/or test compounds. In some embodiments, the mass spectrometry-based technique comprises peak detection. In some embodiments, the mass spectrometry-based technique comprises determining an ionization intensity. In some embodiments, the mass spectrometry-based technique comprises determining peak height. In some embodiments, the mass spectrometry-based technique comprises determining peak area. In some embodiments, the mass spectrometry-based technique comprises determining peak volume.

[0099] In some embodiments, the mass spectrometry-based technique comprises identifying the test compound.

[0100] Thus, for example, in some embodiments, there is provided a method of determining a partition characteristic of a test compound in a target condensate, the method comprising comparing a MS signal of ions of a test compound from an extra-condensate solution and a MS signal of ions of the test compound from a reference control, thereby determining the partition characteristic of the test compound in the target condensate. In some embodiments, the method of determining a partition characteristic of the test compound in the target condensate comprises: (a) obtaining, such as measuring, a MS signal of the test compound in the extra-condensate solution, or a portion thereof, using a mass spectrometry technique; (b) obtaining, such as measuring, a MS ion signal of the test compound in the reference control, or a portion thereof, using a mass spectrometry technique; and (c) comparing the MS signal of ions of the test compound from the extra-condensate solution and the MS signal of ions of the test compound from the reference control, thereby determining the partition characteristic of the test compound in the target condensate.

[0101] In some embodiments, provided herein are methods of determining a condensate-associated characteristic of a small molecule test compound (such as a therapeutic small

molecule that is 1,000 Da or less and/or satisfies Lipinski's rule of five) comprising determining an amount of the small molecule test compound that is depleted (including determining that there is a lack of depletion) from an extra-condensate solution due to the presence, formation, and/or modulation of a target condensate. In some embodiments, there is provided a method of determining a partition characteristic of a small molecule test compound in a target condensate, the method comprising comparing a MS signal of ions of the small molecule test compound from an extra-condensate solution and a MS signal of ions of the small molecule test compound from a reference control, thereby determining the partition characteristic of the small molecule test compound in the target condensate. In some embodiments, the method of determining a partition characteristic of the small molecule test compound in the target condensate comprises: (a) obtaining, such as measuring, a MS signal of the small molecule test compound in the extra-condensate solution, or a portion thereof, using a mass spectrometry technique; (b) obtaining, such as measuring, a MS ion signal of the small molecule small molecule test compound in the reference control, or a portion thereof, using a mass spectrometry technique; and (c) comparing the MS signal of ions of the small molecule test compound from the extra-condensate solution and the MS signal of ions of the small molecule test compound from the reference control, thereby determining the partition characteristic of the small molecule test compound in the target condensate.

[0102] In some embodiments, provided herein are methods of determining a condensate-associated characteristic of a therapeutic compound (such as any of, or any combination of, an exogenous compound, a small molecule, a polypeptide, an oligonucleotide, a nucleic acid, an antibody, or fragment thereof, a synthetically produced compound, including cell culture produced compounds, or a compound that is not a condensate precursor macromolecule) comprising determining an amount of a therapeutic compound that is depleted (including determining that there is a lack of depletion) from an extra-condensate solution due to the presence, formation, and/or modulation of a target condensate. In some embodiments, there is provided a method of determining a partition characteristic of a therapeutic compound in a target condensate, the method comprising comparing a MS signal of ions of the therapeutic compound from an extra-condensate solution and a MS signal of ions of the therapeutic compound from a reference control, thereby determining the partition characteristic of the therapeutic compound in the target condensate. In some embodiments, the method of determining a partition characteristic of the therapeutic compound in the target condensate comprises: (a) obtaining, such as

measuring, a MS signal of the therapeutic compound in the extra-condensate solution, or a portion thereof, using a mass spectrometry technique; (b) obtaining, such as measuring, a MS ion signal of the therapeutic compound in the reference control, or a portion thereof, using a mass spectrometry technique; and (c) comparing the MS signal of ions of the therapeutic compound from the extra-condensate solution and the MS signal of ions of the therapeutic compound from the reference control, thereby determining the partition characteristic of the therapeutic compound in the target condensate.

[0103] In some embodiments, the method of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) combining the test compound and a composition comprising the target condensate and an extra-condensate solution; (b) obtaining, such as preparing, a reference control; (c) measuring a MS signal of the test compound in the extra-condensate solution, or a portion thereof, using a mass spectrometry technique; (d) measuring a MS signal of the test compound in the reference control, or a portion thereof, using a mass spectrometry technique; (e) comparing the MS signal of the test compound from the extra-condensate solution and the MS signal of the test compound from the reference control, thereby determining the partition characteristic of the test compound in the target condensate.

[0104] In some embodiments, the method of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) combining the test compound and a composition comprising the target condensate and an extra-condensate solution; (b) obtaining, such as preparing, a reference control; (c) measuring a MS signal of the test compound in the extra-condensate solution, or a portion thereof, using a mass spectrometry technique; (d) measuring a MS signal of the test compound in the reference control, or a portion thereof, using a mass spectrometry technique; (e) comparing the MS signal of the test compound from the extra-condensate solution and the MS signal of the test compound from the reference control, thereby determining the partition characteristic of the test compound in the target condensate.

[0105] In some embodiments, the method of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) combining the test compound and a composition comprising the target condensate and an extra-condensate solution; (b) incubating the test compound and the composition; (c) pelleting the target condensate in the composition using a centrifugation technique; (d) obtaining, such as preparing, a reference control; (e) measuring a MS signal of the test compound in the extra-condensate solution, or a portion thereof, using a mass spectrometry technique; (f) measuring a MS signal of the test compound in

the reference control, or a portion thereof, using a mass spectrometry technique; (g) comparing the MS signal of the test compound from the extra-condensate solution and the MS signal of the test compound from the reference control, thereby determining the partition characteristic of the test compound in the target condensate.

[0106] In some embodiments, the method of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) combining the test compound and a composition comprising the target condensate and an extra-condensate solution; (b) incubating the test compound and the composition; (c) pelleting the target condensate in the composition using a centrifugation technique; (d) obtaining, such as preparing, a reference control; (e) measuring a MS signal of the test compound in the extra-condensate solution, or a portion thereof, using a mass spectrometry technique; (f) measuring a MS signal of the test compound in the reference control, or a portion thereof, using a mass spectrometry technique; (g) comparing the MS signal of the test compound from the extra-condensate solution and the MS signal of the test compound from the reference control, thereby determining the partition characteristic of the test compound in the target condensate.

[0107] In some embodiments of any of the methods or method steps described herein, the method is suitable for determining a condensate-associated characteristic for each of plurality of test compounds in a single composition comprising a target condensate. For example, in some embodiments, there is provided a method comprising: (a) combining a plurality of test compounds and a composition comprising the target condensate and an extra-condensate solution; and (b) comparing a MS signal of ions of a first test compound of the plurality of test compounds from an extra-condensate solution and a MS signal of ions of the first test compound from a reference control. In some embodiments, the MS signal of ions of each test compound of the plurality of test compounds from an extra-condensate solution are compared with a respective MS signal of ions of each respective test compound from a reference control. In some embodiments, the reference control comprises a plurality of compounds. In some embodiments, the number of compounds in the plurality of test compounds is limited only by the capacity of the analytical method used for measuring the quantity of each compound. In some embodiments, the plurality of test compounds comprises at least 5, such as at least any of 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, or 1,000, compounds. In some embodiments, the method further comprises obtaining, such as measuring, a MS signal of each of the test compounds in the extra-condensate solution, or a

portion thereof, using a mass spectrometry technique. In some embodiments, the method further comprises obtaining, such as measuring, a MS signal of each of the test compounds in the reference control, or a portion thereof, using a mass spectrometry technique. In some embodiments, the MS signal of each of the test compounds in either the extra-condensate solution or the reference control are obtained in a single mass spectrometry analysis.

Test compounds and analysis

[0108] In some embodiments, the test compound is a small molecule, a polypeptide, a peptidomimetic, a lipid, or a nucleic acid. In some embodiments, the test compound is an approved compound, such as a compound approved for medical treatment by the United States Food and Drug Administration. In some embodiments, the test compound is a novel compound. In some embodiments, the test compound is charged. In some embodiments, the test compound is hydrophobic. In some embodiments, the test compound is hydrophilic. In some embodiments, the test compound is a small molecule. In some embodiments, the small molecule is an alkaloid, a glycoside, a phenazine, a phenol, a polyketide, a terpene, or a tetrapyrrole. In some embodiments, the test compound is an antibody. In some embodiments, the target condensate is an extracellular condensate and the test compound is an antibody. In some embodiments, the test compound is a nucleic acid. In some embodiments, the test compound is RNA, such as a siRNA, miRNA, mRNA, or lnRNA. In some embodiments, the test compound is a siRNA, miRNA, or mRNA. In some embodiments, the test compound is a non-naturally occurring compound. In some embodiments, the test compound is a protein.

[0109] In some embodiments, the methods herein comprise adding two or more test compounds. In some embodiments, the two or more compounds are each selected from any of a small molecule, a polypeptide, a lipid, or a nucleic acid. In some embodiments, the two or more test compounds are added sequentially or simultaneously.

[0110] In some embodiments, the test compound comprises a label. In some embodiments, the label is a radioactive label, a colorimetric label, a luminescent label, a chemically-reactive label (such as a component moiety used in click chemistry), or a fluorescent label. In some embodiments, the test compound is a small molecule comprising a label. In some embodiments, the test compound is a small molecule comprising a fluorophore. In some embodiments, the test compound is a polypeptide comprising a label. In some embodiments, the test compound is a polypeptide comprising a fluorophore. In some embodiments, the test compound is a nucleic

acid comprising a label. In some embodiments, the test compound is a nucleic acid comprising a fluorophore. The test compound label can be conjugated to the test compound covalently or non-covalently.

[0111] Methods of determining the amount of test compound are known. In some embodiments, determining the amount of the test compound comprises quantifiably detecting the test compound. In some embodiments, determining the amount of the test compound comprises quantifiably detecting the test compound label. In some embodiments, determining the amount of the test compound comprises detecting activity of the test compound and calculating the amount of test compound needed to cause the amount of activity detected. In some embodiments, the amount of test compound is determined by mass spectrometry, liquid chromatography, and/or ultraviolet-visible spectrophotometry. In some embodiments, the amount of test compound is determined by fluorescence microscopy. Standard curves may be used to aid in determining the amount of test compound. Alternatively or additionally, the amount of test compound may be compared to a reference compound. In some embodiments, the reference compound is the test compound label.

[0112] The methods describe herein comprising determining an amount of a compound, such as a test compound or a reference compound, in a condensate are envisioned to encompass direct and indirect techniques for determining the amount of the compound in the condensate. In some embodiments, the amount of a compound in a condensate is determined directly. In some embodiments, the amount of a compound in a condensate is determined indirectly. In some embodiments, the amount of a compound in a condensate is determined via determining the amount of the compound not associated with the condensate, such as the amount of the compound in an extra-condensate solution. In some embodiments, the amount of a compound in a condensate is determined via determining the amount of a reporter compound. In some embodiments, the reporter compound is associated with the condensate. In some embodiments, the reporter compound is not associated with the condensate.

[0113] The amount of test compound in the target condensate can be compared to the amount of test compound in other solutions or to the amount added to the composition. Accordingly, in some embodiments, the methods further comprise comparing the amount of test compound in the target condensate to the amount added to the composition; and/or the amount in the extra-condensate solution; and/or the amount of test compound in the cell; and/or the amount of test compound in a second target condensate.

[0114] In some embodiments, the methods further comprise comparing the amount of test compound in the target condensate to the amount added to the composition. In some embodiments, comparing comprises calculating a ratio or percentage of the amount of test compound in the target condensate and the amount of test compound added to the composition.

[0115] In some embodiments, the methods further comprise comparing the amount of test compound in the target condensate to the amount of test compound in the extra-condensate solution. In some embodiments, comparing comprises calculating a ratio or percentage of the amount of test compound in the target condensate and the amount of test compound in the extra-condensate solution. In some embodiments, the methods further comprise determining the amount of test compound in an extra-condensate solution. In some embodiments, the amount of the test compound in the target condensate is determined prior to, simultaneously with, or after the amount of the test compound in the extra-condensate solution is determined.

[0116] In some embodiments, the methods further comprise comparing the amount of test compound in the target condensate to the amount of test compound in the cell. In some embodiments, comparing comprises calculating a ratio or percentage of the amount of test compound in the target condensate and the amount of test compound in the cell. In some embodiments, the methods further comprise determining the amount of test compound in the cell.

[0117] In some embodiments, the methods further comprise comparing the amount of test compound in the target condensate to the amount of test compound in a second target condensate. In some embodiments, comparing comprises calculating a ratio or percentage of the amount of test compound in the target condensate and the amount of test compound in the second target condensate. In some embodiments, the methods further comprise determining the amount of test compound in the second target condensate. In some embodiments, the amount of the test compound in the first target condensate is determined prior to, simultaneously with, or after the amount of the test compound in the second target condensate is determined.

[0118] In some embodiments, the methods further comprise comparing the amount of test compound in the target condensate to the amount of one or more macromolecules in the target condensate. In some embodiments, comparing comprises calculating a ratio or percentage of the amount of test compound in the target condensate and the amount of one or more macromolecules in the target condensate. In some embodiments, the methods further comprise determining the amount of one or more macromolecules in the target condensate. In some

embodiments, the amount of the test compound in the target condensate is determined prior to, simultaneously with, or after the amount of the one or more macromolecules in target condensate is determined.

[0119] Methods of forming condensates are known and can vary by condensate. For example, a condensate can be formed by altering the temperature of the composition, such as exposing the composition to lower or higher temperatures; by altering the salt content of the composition, such as diluting a salt in the composition or adding salt to the composition; by increasing the concentration of precursor macromolecules, such as adding a nucleic acid, *e.g.*, RNA, in the composition; adding or changing a buffer in the composition; altering the ionic strength of the composition; altering the pH, such as altering the pH to less than one unit away from the isoelectric point; or adding a crowding agent, such as PEG or dextran. Some exemplary methods of forming condensates are also disclosed in Alberti *et al.*, *J Mol Biol*, 430(23), 2018, 4806-4820, which is herein incorporated by reference.

Compositions

[0120] In some embodiments, the composition comprises a cell. In some embodiments, the target condensate is in the cell. In some embodiments, the extra-condensate solution is intracellular fluid, such as cytosol or nucleosol.

[0121] In some embodiments, the composition comprises a cell. In some embodiments, the target condensate is not in the cell. In some embodiments, the target condensate is an extracellular condensate, such as a condensate in the extracellular matrix. In some embodiments, the extracellular fluid is interstitial fluid or plasma.

[0122] In some embodiments, the cell is a microorganism or an animal cell. In some embodiments, cell is a human cell. In some embodiments, the cell is a neuron. In some embodiments, the cell is a cancer cell. In some embodiments, the cell is or is derived from induced pluripotent stem cells (iPS cells), HeLa cells, or HEK293 cells. In some embodiments, the cell comprises a condensate that is determined to be dysregulated. In some embodiments, the cell comprises a mutation associated with a disease. In some embodiments, the cell has one or more features of a neurodegenerative or proliferative disease. In some embodiments, the cell has been treated with arsenate (and/or another compound known to modulate a condensate), a temperature change, or a pH change. In some embodiments, the cell expresses a protein that is labeled with a fluorescent protein. In some embodiments, the protein is a protein known to

concentrate in the target condensate. In some embodiments, the cell expresses a first protein and a second protein, wherein the first protein is labeled with a first label, wherein the first protein is known to concentrate in a first target condensate, wherein the second protein is labeled with a second label, wherein the second protein is known to concentrate in a second target condensate, and wherein the first label and the second label are distinguishable. In some embodiments, the cell expresses a first protein and a second protein, wherein the first protein is labeled with a first fluorescent protein, wherein the first protein is known to concentrate in a first target condensate, wherein the second protein is labeled with a second fluorescent protein, wherein the second protein is known to concentrate in a second target condensate, and wherein the first fluorescent protein and second fluorescent protein are distinguishable.

[0123] In some embodiments, the composition does not comprise a cell. In some embodiments, the composition is cell-free. In some embodiments, the composition comprises precursor molecules, which are non-phase separated components that can be incorporated into a condensate and/or have been incorporated into a condensate. Condensates can be formed in cell-free systems from just a few components. For example, the composition may comprise a protein or protein fragments, such as a portion of a protein or a peptide, capable of forming a condensate. In some embodiments, the composition comprises a protein or protein fragment comprising a Low Complexity Domain or an Intrinsically Disordered Sequence. In some embodiments, the composition comprises nucleic acid oligomers or polymers, such as RNA. In some embodiments, the composition comprises a small molecule. In some embodiments, the composition comprises a buffer. In some embodiments, the composition may also comprise one or more salts, and/or one or more macromolecular crowding agents (*e.g.*, poly ethylene glycol or dextran).

[0124] In some embodiments, the composition comprises a cell comprising the precursor molecules. In some embodiments, the composition comprises a cell and the method comprises forming the target condensate in the cell. In some embodiments, the method comprises altering the temperature of the cell, such as exposing the composition to lower or higher temperatures; altering the salt content of the cell; adding or changing a buffer surrounding the cell; altering the pH of the cell; or adding a crowding agent, such as PEG or dextran to the cell. In some embodiments, the composition comprises a cell, and the cell comprises a mutation that causes the target condensate to form and/or modifies the target condensate. In some embodiments, the mutation modifies one or more of the following: the size of the target condensate, the shape of

the target condensate, the concentration of one or more components of the target condensate, and heterogeneous distribution of components within the target condensate. In some embodiments, the composition comprises a cell, and the cell comprises a mutation that causes the condensate to form.

[0125] In some embodiments, the composition comprises a plurality of condensates. In some embodiments, the composition comprises at least any of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25 condensates. In some embodiments, the composition comprises two or more target condensates. In some embodiments, the composition comprises at least any of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25 target condensates. In some embodiments, the method comprises repeating the steps of the method for one or more additional target condensates, such as for at least any of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25 target condensates. In some embodiments, the composition comprises at least any of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25 condensates of the same condensate type, *e.g.*, condensates that have the same composition and/or partitioning properties. In some embodiments, the composition comprises at least any of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25 different condensate types, *e.g.*, condensates that do not have the same composition and/or partitioning properties.

Condensates

[0126] Many condensates are well known in the art. Examples of known condensates include cleavage bodies, P-granules, histone locus bodies, multivesicular bodies, neuronal RNA granules, nuclear gems, nuclear pores, nuclear speckles, nuclear stress bodies, a nucleolus, Oct1/PTF/transcription (OPT) domains, paraspeckles, perinucleolar compartments, PML nuclear bodies, PML oncogenic domains, polycomb bodies, processing bodies, signaling clusters, viral condensates, Sam68 nuclear bodies, stress granules, or splicing speckles. Many condensates can be identified using microscopy. In some embodiments, the methods further comprise identifying the target condensate.

[0127] Condensates used in the methods described herein may be naturally occurring or non-naturally occurring. For example, in some embodiments, the condensate is naturally occurring. In some embodiments, the condensate is non-naturally occurring. In some embodiments, the condensate is artificial. In some embodiments, the condensate is synthetic. In some embodiments, the condensate is semi-synthetic. In some embodiments, the condensate is modified. In some embodiments, the condensate is a modified condensate, wherein a parent

condensate of the modified condensate is modified by adding, removing, and/or substituting one or more condensate components.

[0128] In some embodiments, wherein the methods described herein assess two more condensates, the first condensate and a second condensate of the two or more condensates may be any combination of condensates. In some embodiments, the first condensate is a modified condensate. In some embodiments, the first condensate is a modified condensate, and the second condensate is a parent condensate of the first condensate. In some embodiments, the first condensate is a target condensate in a first system, such as a first composition, and the second condensate is the target condensate in a second system, such as a second composition. In some embodiments, the first condensate is a normal condensate and the second condensate is a dysregulated condensate.

[0129] In some embodiments, provided are methods of determining a condensate preference profile of a test compound, the method comprising: (a) combining the test compound and a first composition comprising a first target condensate; (b) combining the test compound and a second composition comprising a second target condensate; (c) determining the amount of the test compound in the first target condensate; and (d) determining the amount of the test compound in the second target condensate, thereby determining a condensate preference profile of the test compound.

[0130] In some embodiments, provided are methods of determining a condensate preference profile of a test compound, the method comprising: (a) adding the test compound to a first composition comprising a first target condensate; (b) adding the test compound to a second composition comprising a second target condensate; (c) determining the amount of the test compound in the first target condensate; and (d) determining the amount of the test compound in the second target condensate, thereby determining a condensate preference profile of the test compound.

[0131] The identification of condensates can be aided by the use of a label. For example, a dye or labeled compound can be added to a condensate. In some embodiments, the dye or labeled compound could preferentially enter the target condensate. In some embodiments, the label is a radioactive label, a colorimetric label, a chemically-reactive label, or a fluorescent label. In some embodiments, the composition comprises a first target condensate and a second target condensate, and the first target condensate and the second target condensate are each

labeled with different labels, such as the first target condensate is labeled with RFP and the second target condensate is labeled with GFP.

[0132] Some condensates may comprise specific macromolecules. Accordingly, in some embodiments, the methods further comprise characterizing a macromolecule in the target condensate. In some embodiments, the macromolecule is a protein or protein fragment. In some embodiments, the protein or protein fragment comprises a Low Complexity Domain or an Intrinsically Disordered Sequence. In some embodiments, the macromolecule is a transcription factor or an RNA binding protein. In some embodiments, the macromolecule is tau, FUS, huntingtin protein, hnRNPA1, TDP43, PGL-3, or fragments or aggregates thereof. In some embodiments, the macromolecule is a nucleic acid, such as RNA or DNA. In some embodiments, the macromolecule is a RNA.

[0133] In some embodiments, the target condensate is a cellular condensate. Numerous cellular condensates have been described and numerous more are known to form, but have not yet been described. In some embodiments, the cellular condensate is a cleavage body, a P-granule, a histone locus body, a multivesicular body, a neuronal RNA granule, a nuclear gem, a nuclear pore, a nuclear speckle, a nuclear stress body, a nucleolus, a Oct1/PTF/transcription (OPT) domain, a paraspeckle, a perinucleolar compartment, a PML nuclear body, a PML oncogenic domain, a polycomb body, a processing body, a signaling cluster, a viral condensate, a Sam68 nuclear body, a stress granule, or a splicing speckle. In some embodiments, the target condensate is in a cell when the amount of target compound in the target condensate is determined.

[0134] In some embodiments, the target condensate is an extracellular condensate. Extracellular condensates can form in biological solutions outside of a cell, such as the extracellular matrix or plasma, to facilitate reactions or sequester molecules (Muiznieks *et al.*, *J Mol Biol*, 430(23), 2018, 4741-4753).

[0135] The dysregulation of various condensates can be associated with a disease. For example, based on cellular and cell-free condensate experiments, disease-associated mutations in the protein fused in sarcoma (FUS) have been shown to cause aberrant phase-separation behavior that contributes directly to development of the motor neuron disease, amyotrophic lateral sclerosis (ALS) (Naumann *et al.*, *Nat Commun*, 9(1), 2018, 335). Accordingly, in some embodiments dysregulation of the target condensate is associated with a disease. In some embodiments, the dysregulation comprises an alteration in one or more of: size of the target

condensate; shape of the target condensate; concentration of one or more components of the target condensate; and heterogeneous distribution of components within the condensate, *e.g.*, components located in the core instead of the shell of the condensate. In some embodiments, the alteration is compared to a similar non-dysregulated target condensate.

Methods of determining a compound characteristic associated with partitioning of a test compound

[0136] In some embodiments, the invention includes methods of identifying a compound characteristic associated with the partitioning of a compound, or portion thereof, in a condensate, such as associated with the surface and/or core of the condensate.

[0137] In some embodiments, the methods comprise determining partition characteristics of a plurality of test compounds in a target condensate by performing a method disclosed herein. In some embodiments, the methods comprise identifying or determining an attribute of a compound, or a portion thereof, contributing, in whole or in part, to partitioning of a compound, or portion thereof, with a condensate.

[0138] In some embodiments, identifying a compound characteristic associated with the partitioning of a compound, or portion thereof, that has a desired, such as a similar, partition characteristic in a target condensate comprises identifying a common moiety or motif of the test compounds having a desired, such as similar, partition characteristic. In some embodiments, the compound characteristic of a compound, or a portion thereof, is based on one or more of charge and hydrophobicity.

[0139] In some embodiments, the partition characteristic of a compound is based on determining an amount, such as a relative amount, of the compound in a condensate or as compared to one or more condensates, such as a condensate preference profile. For example, in some embodiments, the methods comprise: (a) determining partition characteristics of a plurality of test compounds in the target condensate by performing a method described herein; and (b) identifying test compounds, or a portion thereof, that have a desired partition characteristic, such as a similar partition characteristic, in the condensate. In some embodiments, the methods comprise: (a) determining partition characteristics of a plurality of test compounds in the target condensate by performing a method described herein; (b) comparing the partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar partition characteristics in the target condensate; and

(d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics.

[0140] In some embodiments, the methods comprise: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate by performing a method described herein; and (b) identifying test compounds, or a portion thereof, that have a desired relative partition characteristic, such as a similar relative partition characteristic, in the condensate. In some embodiments, the methods comprise: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate by performing a method described herein; (b) comparing the relative partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar relative partition characteristics in the target condensate; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics.

[0141] In some embodiments, the methods comprise: (a) determining condensate preference profiles of a plurality of test compounds in the target condensate by performing a method described herein; and (b) identifying test compounds, or a portion thereof, that have a desired condensate preference profile, such as a similar condensate preference profile, in the condensate. In some embodiments, the methods comprise (a) determining condensate preference profiles of a plurality of test compounds by performing a method described herein; (b) comparing the condensate preference profiles of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar condensate preference profiles; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles.

[0142] In some embodiments of the methods described herein, identifying test compounds that have the same or similar partition characteristics, relative partition characteristics, or condensate preference profiles in the target condensate comprises identifying two or more test compounds that associate with any portion of a target condensate. In some embodiments of the methods described herein, identifying test compounds that have the same or similar partition characteristics, relative partition characteristics, or condensate preference profiles in the target condensate comprises identifying two or more test compounds that do not associate with any portion of a target condensate.

[0143] In some embodiments, the methods disclosed herein further comprise making the compound identified and/or designed using the methods described herein.

Methods of designing a compound with a desired partition characteristic

[0144] In some embodiments, the invention includes methods of designing a compound with a desired partition characteristic with regards to a target condensate. In some embodiments, the invention includes methods of designing a compound with a desired partition characteristic to a target condensate.

[0145] In some embodiments, the methods comprise identifying or determining a compound, or a portion thereof, comprising a desired partition characteristic with regards to a target condensate. In some embodiments, the methods comprise identifying or determining an attribute of a compound, or a portion thereof, contributing, in whole or in part, to a desired partition characteristic with regards to a target condensate. In some embodiments, the methods comprise identifying or determining a compound, or a portion thereof, comprising a desired partition characteristic to a target condensate. In some embodiments, the methods comprise identifying or determining an attribute of a compound, or a portion thereof, contributing, in whole or in part, to a desired partition characteristic to a target condensate. In some embodiments, the methods comprise modifying an identified compound, or a portion thereof, to optimize a desired partition characteristic.

[0146] In some embodiments, the method of designing a compound with a desired partition characteristic comprises (a) determining a partition characteristics for each of a plurality of test compounds in a target condensate by performing a method described herein; and (b) identifying one or more of the plurality of test compounds, or portions thereof, having a desired, such as similar, partition characteristic in a the target condensate. In some embodiments, the methods comprise comparing partition characteristics of a subset or all of a plurality of test compounds in a target condensate. In some embodiments, the methods comprise selecting and/or designing a compound, or portion thereof, of a plurality of test compounds having a desired partition characteristic.

[0147] In some embodiments, the method of identifying a compound characteristic associated with a desired partitioning characteristic with regards to a target condensate comprises: (a) determining partition characteristics of a plurality of test compounds in the target condensate by performing a method described herein; (b) comparing the partition characteristics

of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar partition characteristics in the target condensate; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics.

[0148] In some embodiments, the method of identifying a compound characteristic associated with partitioning a compound into a condensate comprises: (a) determining partition characteristics of a plurality of test compounds in the target condensate by performing a method described herein; (b) comparing the partition characteristics of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar partition characteristics in the target condensate; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics.

[0149] In some embodiments, identifying test compounds, or portions thereof, that have the same or a desired, such as a similar, partition characteristic in a target condensate comprises identifying a common moiety or motif of the test compounds having a desired, such as similar, partition characteristic. In some embodiments, the compound characteristic of a compound, or a portion thereof, is based on one or more of charge and hydrophobicity.

[0150] In some embodiments, identified test compounds, or portion(s) thereof, can be used as the basis for the identification and/or design of one or more compounds having a desired partition characteristic. In some embodiments, the one or more compounds represents a privileged library. In some embodiments, the privileged library comprises a set of one or more compounds, the set being a part or the whole of the privileged library, comprising a moiety that comprises the identified compound, or portion(s) thereof. In some embodiments, each of the compounds of the privileged library have a similar partition characteristic, such as partition characteristics within at least about 20% of one another. In some embodiments, each of the compounds of the privileged library meet or exceed a threshold partition characteristic. In some embodiments, the privileged library comprises at least about 10 compounds, such as at least about any of 25 compounds, 50 compounds, 150 compounds, 200 compounds, 250 compounds, 300 compounds, 350 compounds, 400 compounds, 450 compounds, 500 compounds, 1,000 compounds, 1,500 compounds, 2,000 compounds, 2,500 compounds, 3,000 compounds, 3,500 compounds, 4,000 compounds, 4,500 compounds, 5,000 compounds, 10,000 compounds, 20,000 compounds, 30,000 compounds, 40,000 compounds, or 50,000 compounds. In some

embodiments, each of the compounds of a set of a privileged library are suitable for administration to an individual. In some embodiments, each of the compounds of a privileged library has a molecular weight of less than 1,000 Da, such as 500 Da or less. In some embodiments, each of the compounds of a privileged library satisfy Lipinski's rule of five. In some embodiments, the privileged library comprises compounds present in a single composition. In some embodiments, the privileged library can be used to identify one or more compounds useful for targeting a target condensate, wherein the one or more compounds are identified from the privileged library using one or more traditional drug screening methods.

[0151] In some embodiments, designing the compound comprises adding (such as attaching, *e.g.*, covalently attaching) a moiety that comprises the identified characteristic to the compound, thereby conferring the desired partition characteristic to the compound. In some embodiments, designing the compound comprises removing a moiety that comprises the identified characteristic, thereby conferring the desired partition characteristic to the compound. In some embodiments, the compound is designed, in whole or in part, using an approach comprising a modeling, computer, and/or calculation-based technique, *e.g.*, a bioinformatic, cheminformatic, and/or artificial intelligence (AI)-based technique.

[0152] In some embodiments, the methods comprise designing a test compound, or a portion thereof, based on an attaching a moiety that comprises a characteristic, such as a chemical structure or motif, identified via a condensate-associated characteristic, thereby conferring a desired partition characteristic to the test compound. In some embodiments, the identified characteristic, in whole or in part, modulates a condensate-associated characteristic, such as a partition characteristic, of the test compound, such as increasing or decreasing the degree to which the test compound partitions in a test condensate. In some embodiments, the methods of designing a test compound comprise attaching a moiety that comprises a characteristic identified via a condensate-associated characteristic to a precursor of the test compound at any number of position and/or stereochemical orientations. In some embodiments, the methods of designing a test compound comprise removing a moiety that comprises a characteristic identified via a condensate-associated characteristic. In some embodiments, the methods of designing a test compound comprise changing a moiety to another moiety that comprises a characteristic identified via a condensate-associated characteristic to a precursor of the test compound at any number of position and/or stereochemical orientations. In some embodiments, the methods of designing a test compound comprise attaching, removing, and/or changing more than one moiety

that comprises a characteristic identified via a condensate-associated characteristic to a precursor of the test compound. In some embodiments, the test compound comprises a feature to facilitate modulation (such as attaching, removing, changing) of a moiety, such as a compound label (*e.g.*, a component moiety used in click chemistry).

[0153] In some embodiments, identified test compounds, or portion(s) thereof, can be used as the basis for the identification and/or design of one or more compounds having a desired biological activity. In some embodiments, the one or more compounds represents a privileged library. In some embodiments, the methods described herein comprise designing a test compound, or a portion thereof, based on an attaching, removing, or changing a moiety that comprises a characteristic, such as a chemical structure or motif, identified via a condensate-associated characteristic, thereby conferring a desired biological activity to the test compound. In some embodiments, the desired biological activity of a precursor compound is improved by modulating a condensate-associated characteristic of the precursor compound. In some embodiments, the undesired biological activity of a precursor compound is decreased by modulating a condensate-associated characteristic of the precursor compound.

[0154] In some embodiments, condensate-associated characteristic data, such as partition characteristic data, and/or identified test compounds, or portions thereof, can be used to develop one or more rule sets. In some embodiments, the one or more rule sets can be used as a basis for the identification and/or design of one or more compounds using an approach comprising modeling, computer and/or calculation-based techniques, *e.g.*, bioinformatic, cheminformatic, and/or artificial intelligence (AI)-based identification of a compound having a desired partition characteristic. Also provided are computer software for determining and/or applying the one or more rule sets.

[0155] In some embodiments, the partition characteristic of a compound is based on determining an amount, such as a relative amount, of the compound in a condensate or as compared to one or more condensates, such as a condensate preference profile. For example, in some embodiments, the method of designing a compound with a desired relative partition characteristic comprises determining relative partition characteristics of a plurality of test compounds in the target condensate by performing a method described herein. In some embodiments, the method of designing a compound with a desired relative partition characteristic comprises: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate by performing a method described herein; (b) comparing the

relative partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar relative partition characteristics in the target condensate; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics; and (e) designing a compound that comprises the identified characteristic. In some embodiments, the method of designing a compound with a desired relative partition characteristic comprises: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate by performing a method described herein; (b) comparing the relative partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar relative partition characteristics in the target condensate; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics; and (e) designing a compound that does not comprise the identified characteristic.

[0156] In some embodiments, the method of designing a compound with a desired condensate preference profile comprises determining condensate preference profiles of a plurality of test compounds by performing a method disclosed herein. In some embodiments, the method of designing a compound with a desired condensate preference profile comprises (a) determining condensate preference profiles of a plurality of test compounds by performing a method disclosed herein; (b) comparing the condensate preference profiles of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar condensate preference profiles; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles; and (e) designing a compound that comprises the identified characteristic. In some embodiments, the method of designing a compound with a desired condensate preference profile comprises (a) determining condensate preference profiles of a plurality of test compounds by performing a method disclosed herein; (b) comparing the condensate preference profiles of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar condensate preference profiles; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles; and (e) designing a compound that does not comprise the identified characteristic.

[0157] In some embodiments, the methods disclosed herein further comprise making the compound identified and/or designed using the methods described herein.

Methods of screening a test compound

[0158] In some embodiments, the invention includes methods of screening a test compound for a desired partition characteristic from a group of candidate compounds, the method comprising: (a) determining a partition characteristic of each of the group of candidate compounds; and (b) identifying the test compound having the desired partition characteristic.

[0159] In some embodiments, the desired partition characteristic is based on a partition characteristic associated with a target condensate. In some embodiments, the desired partition characteristic is based on a partition characteristic associated with a plurality of target condensates. In some embodiments, the methods comprise identifying or determining a target condensate. In some embodiments, the target condensate is associated with a disease. In some embodiments, the target condensate associated with a disease is known in the art. In some embodiments, the methods comprise obtaining a target condensate, such as a target condensate associated with a disease.

[0160] In some embodiments, the partition characteristic of a group of candidate compound, such as more than one candidate compounds, in the target condensate is determined according to the methods described herein. In some embodiments, the group of candidate compounds is at least about any of 2, 5, 10, 15, 50, 75, 100, 150, 200, 300, 400, or 500 candidate compounds. In some embodiments, the partition characteristic of a candidate compound in the target condensate is determined *in vitro*. In some embodiments, the partition characteristic of a candidate compound in the target condensate is determined in a cellular system. In some embodiments, the partition characteristic of a candidate compound in the target condensate is determined in a non-cellular system, such as a composition comprising a target condensate or components thereof.

[0161] In some embodiments, identifying a test compound, or portion thereof, having a desired partition characteristic comprises identifying a test compound, or portion thereof, that associates with any portion of the target condensate, such as associates with the exterior, surface, and/or core of the target condensate. In some embodiments, the desired partition characteristic is a suitable partition characteristic for being useful for treating a disease in an individual.

[0162] In some embodiments, the invention includes methods of identifying a test compound useful for treating a disease in an individual in need thereof. In some embodiments, the methods

comprise: (a) identifying a target condensate associated with a disease; and (b) determining a partition characteristic of a candidate compound in the target condensate, and (c) identifying a test compound, or portion thereof, having a suitable partition characteristic for being useful for treating the disease.

[0163] In some embodiments, the methods comprise identifying or determining a target condensate associated with a disease. In some embodiments, the target condensate associated with a disease is known in the art. In some embodiments, the methods comprise obtaining a target condensate associated with a disease. In some embodiments, the methods comprise identifying or determining one or more target condensates associated with a disease.

[0164] In some embodiments, the method of screening a test compound comprises: (a) determining a partition characteristic of the test compound in the presence of a second compound; and (b) determining a partition characteristic of the test compound in the absence of the second compound. In some embodiments, the method further comprises comparing the partition characteristic of the test compound in the presence of the second compound and the partition characteristic of the test compound in the absence of the second compound. In some embodiments, the method further comprises determining the alteration of the partition characteristic of a test compound in the presence or absence of a second compound.

[0165] In some embodiments, the method of screening a test compound comprises: (a) determining a partition characteristic of a second compound in the presence of the test compound; and (b) determining a partition characteristic of the second compound in the absence of the test compound. In some embodiments, the method further comprises comparing the partition characteristic of the second compound in the presence of the test compound and the partition characteristic of the second compound in the absence of the test compound. In some embodiments, the method further comprises determining the ability of a test compound to alter the partition characteristic of the second compound.

[0166] In some embodiments, the partition characteristic of a candidate compound, such as one or more candidate compounds, in the target condensate is determined according to the methods described herein. In some embodiments, the methods comprise determining a partition characteristic of a plurality of candidate compounds for a target condensate. In some embodiments, the plurality of candidate compounds is at least about any of 2, 5, 10, 15, 50, 75, 100, 150, 200, 300, 400, or 500 candidate compounds. In some embodiments, the partition characteristic of a candidate compound in the target condensate is determined *in vitro*. In some

embodiments, the partition characteristic of a candidate compound in the target condensate is determined in a cellular system. In some embodiments, the partition characteristic of a candidate compound in the target condensate is determined in a non-cellular system, such as a composition comprising a target condensate or components thereof.

[0167] In some embodiments, identifying a test compound, or portion thereof, having a suitable partition characteristic for being useful for treating the disease comprises identifying a test compound, or portion thereof, that associates with any portion of the target condensate, such as associates with the exterior, surface, and/or core of the target condensate.

[0168] In some embodiments, the methods disclosed herein comprising identifying, determining, designing, and/or screening for a compound, such as a compound from a group of candidate compounds, further comprises assessing the compound based on an additional parameter of the compound. In some embodiments, identifying a compound is based on assessing, such as characterizing or determining, condensate-associated characteristics of the compound and one or more additional parameters, such as molecular weight and pharmaceutical utility.

[0169] In some embodiments, the methods disclosed herein further comprise making the compound identified, designed, and/or screened for using the methods described herein.

Exemplary embodiments

[0170] Among the provided embodiments are:

[0171] Embodiment 1. A method of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) adding the test compound to a composition comprising a target condensate and an extra-condensate solution; and (b) determining the amount of the test compound in the target condensate, thereby determining the partition characteristic of the test compound in the target condensate.

[0172] Embodiment 2. The method of embodiment 1, further comprising causing the formation of the target condensate prior to step (a).

[0173] Embodiment 3. A method of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) adding the test compound to a composition comprising precursor molecules; (b) causing the formation of the target condensate to obtain a composition comprising the target condensate and an extra-condensate solution; and (c)

determining the amount of the test compound in the target condensate, thereby determining the partition characteristic of the test compound in the target condensate.

[0174] Embodiment 4. The method of any one of embodiments 1-3, further comprising determining the amount of the test compound in the extra-condensate solution.

[0175] Embodiment 5. The method of embodiment 4, wherein the amount of the test compound in the target condensate is determined prior to, simultaneously with, or after the amount of the test compound in the extra-condensate solution is determined.

[0176] Embodiment 6. The method of embodiment 4 or 5, further comprising determining the ratio of the amount of test compound in the target condensate and the amount of test compound in the extra-condensate solution.

[0177] Embodiment 7. The method of any one of embodiments 1-6, further comprising separating the target condensate from the extra-condensate solution.

[0178] Embodiment 8. The method of any one of embodiments 1-7, further comprising identifying the target condensate prior to determining the amount of test compound in the target condensate.

[0179] Embodiment 9. The method of any one of embodiments 1-8, wherein dysregulation of the target condensate is associated with a disease.

[0180] Embodiment 10. The method of any one of embodiments 1-9, further comprising characterizing the target condensate by identifying one or more macromolecules comprised therein.

[0181] Embodiment 11. The method of any one of embodiments 1-10, wherein the target condensate comprises a protein comprising an intrinsically disordered sequence.

[0182] Embodiment 12. The method of any one of embodiments 1-11, further comprising labeling the target condensate in order to visualize the target condensate.

[0183] Embodiment 13. The method of embodiment 12, wherein the target condensate is labeled with a radioactive label, a colorimetric label, or a fluorescent label.

[0184] Embodiment 14. The method of any one of embodiments 1-13, wherein the composition comprises a cell.

[0185] Embodiment 15. The method of embodiment 14, wherein the cell is a microorganism or an animal cell.

[0186] Embodiment 16. The method of embodiment 14 or 15, wherein the cell comprises a condensate that is determined to be dysregulated.

[0187] Embodiment 17. The method of any one of embodiment 14-16, wherein the cell has one or more features of a neurodegenerative or proliferative disease.

[0188] Embodiment 18. The method of any one of embodiments 1-17, wherein the target condensate is a cellular condensate.

[0189] Embodiment 19. The method of embodiment 18, wherein the cellular condensate is a cleavage body, a P-granule, a histone locus body, a multivesicular body, a neuronal RNA granule, a nuclear gem, a nuclear pore, a nuclear speckle, a nuclear stress body, a nucleolus, a Oct1/PTF/transcription (OPT) domain, a paraspeckle, a perinucleolar compartment, a PML nuclear body, a PML oncogenic domain, a polycomb body, a processing body, a Sam68 nuclear body, a stress granule, or a splicing speckle.

[0190] Embodiment 20. The method of any one of embodiments 1-19, wherein the target condensate is in a cell.

[0191] Embodiment 21. The method of any one of embodiment 1-20, wherein the extra-condensate solution is intracellular fluid.

[0192] Embodiment 22. The method of embodiment 21, wherein the intracellular fluid is cytosol or nucleosol.

[0193] Embodiment 23. The method of any one of embodiments 1-17, wherein the target condensate is an extracellular condensate.

[0194] Embodiment 24. The method of any embodiment 23, wherein the extra-condensate solution is extracellular fluid.

[0195] Embodiment 25. The method of embodiment 24, wherein the extracellular fluid is interstitial fluid.

[0196] Embodiment 26. The method of any one of embodiments 1-13, wherein the composition does not comprise a cell.

[0197] Embodiment 27. The method of any one of embodiments 1-26, wherein the composition comprises one or more of: a macromolecule, a salt, and a buffer.

[0198] Embodiment 28. The method of any one of embodiments 1-27, wherein the composition comprises two or more target condensates.

[0199] Embodiment 29. The method of any one of embodiments 1-28, wherein the method comprises repeating the steps of the method for one or more additional condensates.

[0200] Embodiment 30. The method of any one of embodiments 1-29, wherein the test compound is small molecule, a polypeptide, or a nucleic acid.

[0201] Embodiment 31. The method of any one of embodiments 1-30, wherein the test compound comprises a test compound label.

[0202] Embodiment 32. The method of embodiment 31, wherein the test compound label is a radioactive label, a colorimetric label, or a fluorescent label.

[0203] Embodiment 33. The method of embodiment 31 or 32, wherein the test compound label is a fluorescent label.

[0204] Embodiment 34. The method of any one of embodiments 31-33, wherein the amount of the test compound is determined by detecting the test compound label.

[0205] Embodiment 35. The method of any one of embodiments 1-34, wherein the amount of the test compound is determined by mass spectrometry, liquid chromatography, and/or ultraviolet-visible spectrophotometry.

[0206] Embodiment 36. A method of determining the partition characteristics of a plurality of test compounds in a target condensate, the method comprising performing the method of any one of embodiments 1-35 with a plurality of test compounds.

[0207] Embodiment 37. The method of embodiment 36, further comprising comparing the partition characteristics of a subset or all of the plurality of test compounds in the target condensate.

[0208] Embodiment 38. The method of embodiment 37, further comprising identifying test compounds that have the same or similar partition characteristics in a target condensate.

[0209] Embodiment 39. The method of embodiment 38, further comprising identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics.

[0210] Embodiment 40. The method of embodiment 39, further comprising determining the partition characteristics in a target condensate for one or more additional test compounds that comprise the identified characteristic.

[0211] Embodiment 41. A method of determining a relative partition characteristic of a test compound in a target condensate, the method comprising: (i) determining the partition characteristic of the test compound by performing the method of any one of embodiments 1-35 with the test compound; (ii) determining the partition characteristic of a reference compound by performing the method of any one of embodiments 1-35 with the reference compound; and (iii) calculating the ratio of the partition characteristics determined in (i) and (ii), thereby determining the relative partition characteristic of the test compound in the target condensate.

[0212] Embodiment 42. The method of embodiment 41, wherein the test compound comprises a test compound label.

[0213] Embodiment 43. The method of embodiment 42, wherein the reference compound is the test compound label.

[0214] Embodiment 44. A method of determining relative partition characteristics of a plurality of test compounds in a target condensate, the method comprising: (1) performing the method of any one of embodiments 41-43; and (2) repeating steps (i) and (iii) with a plurality of test compounds.

[0215] Embodiment 45. The method of embodiment 44, further comprising comparing the relative partition characteristics in the target condensate of a subset or all of the plurality of test compounds.

[0216] Embodiment 46. The method of embodiment 45, further comprising identifying test compounds that have the same or similar relative partition characteristics in the target condensate.

[0217] Embodiment 47. The method of embodiment 46, further comprising identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics.

[0218] Embodiment 48. The method of embodiment 47, further comprising determining the relative partition characteristics in the target condensate for one or more additional test compounds that comprise the identified characteristic.

[0219] Embodiment 49. A method of determining a condensate preference profile of a test compound, the method comprising: (a) adding the test compound to a composition comprising a first target condensate and a second target condensate; (b) determining the amount of the test compound in the first target condensate; (c) determining the amount of the test compound in the second target condensate; and (d) calculating a ratio of the amount of the test compound determined in the first target condensate and the second target condensate, thereby determining the condensate preference profile of the test compound.

[0220] Embodiment 50. The method of embodiment 49, further comprising causing the formation of the first target condensate and/or the second target condensate prior to step (a).

[0221] Embodiment 51. A method of determining a condensate preference profile of a test compound, the method comprising: (a) adding the test compound to a composition comprising precursor molecules; (b) causing the formation of a first target condensate and a second target

condensate in the composition; (c) determining the amount of the test compound in the first target condensate; (d) determining the amount of the test compound in the second target condensate; and (e) calculating a ratio of the amount the test compounds determined in the first target condensate and the second target condensate, thereby determining the condensate preference profile of the test compound.

[0222] Embodiment 52. The method of any one of embodiments 49-51, wherein the amount of the test compound in the first target condensate is determined prior to, simultaneously with, or after the amount of the test compound in the second target condensate is determined.

[0223] Embodiment 53. The method of any one of embodiments 49-52, further comprising separating the first target condensate and the second target condensate from the composition.

[0224] Embodiment 54. The method of any one of embodiments 49-53, further comprising identifying the first target condensate and/or the second target condensate prior to determining the amount of test compound in the first condensate and/or the second condensate.

[0225] Embodiment 55. The method of any one of embodiments 49-54, wherein dysregulation of the first target condensate and/or the second target condensate is associated with a disease.

[0226] Embodiment 56. The method of any one of embodiments 49-55, further comprising characterizing the first target condensate and/or the second target condensate by identifying one or more macromolecules comprised therein.

[0227] Embodiment 57. The method of any one of embodiments 49-56, further comprising labeling the first target condensate and/or the second target condensate in order to visualize the first condensate target condensate and/or the second target condensate.

[0228] Embodiment 58. The method of embodiment 57, wherein the first target condensate and the second target condensate are labeled with different labels.

[0229] Embodiment 59. The method of embodiment 57 or 58, wherein the first target condensate and/or the second target condensate are labeled with a radioactive label, a colorimetric label, or a fluorescent label.

[0230] Embodiment 60. The method of any one of embodiments 49-56, wherein the composition comprises a cell.

[0231] Embodiment 61. The method of embodiment 60, wherein the cell is a microorganism or an animal cell.

[0232] Embodiment 62. The method of embodiment 60 or 61, wherein the cell comprises a condensate that is determined to be dysregulated.

[0233] Embodiment 63. The method of any one of embodiments 60-62, wherein the cell has one or more features of a neurodegenerative or proliferative disease.

[0234] Embodiment 64. The method of any one of embodiments 49-56, wherein the first target condensate and/or the second target condensate are cellular condensates.

[0235] Embodiment 65. The method of embodiment 64, wherein the first target condensate is a cleavage body, a P-granule, a histone locus body, a multivesicular body, a neuronal RNA granule, a nuclear gem, a nuclear pore, a nuclear speckle, a nuclear stress body, a nucleolus, a Oct1/PTF/transcription (OPT) domain, a paraspeckle, a perinucleolar compartment, a PML nuclear body, a PML oncogenic domain, a polycomb body, a processing body, a Sam68 nuclear body, a stress granule, or a splicing speckle.

[0236] Embodiment 66. The method of embodiment 64 or 65, wherein the second target condensate is a cleavage body, a P-granule, a histone locus body, a multivesicular body, a neuronal RNA granule, a nuclear gem, a nuclear pore, a nuclear speckle, a nuclear stress body, a nucleolus, a Oct1/PTF/transcription (OPT) domain, a paraspeckle, a perinucleolar compartment, a PML nuclear body, a PML oncogenic domain, a polycomb body, a processing body, a Sam68 nuclear body, a stress granule, or a splicing speckle.

[0237] Embodiment 67. The method of any one of embodiments 49-66, wherein the first target condensate and/or the second target condensate are in a cell.

[0238] Embodiment 68. The method of any one of embodiments 49-63, wherein the first target condensate and/or the second target condensate are extracellular condensates.

[0239] Embodiment 69. The method of any one of embodiments 49-59, wherein the composition does not comprise a cell.

[0240] Embodiment 70. The method of any one of embodiments 49-69, wherein the composition comprises one or more of: a macromolecule, a salt, and a buffer.

[0241] Embodiment 71. The method of any one of embodiments 49-70, wherein the composition comprises one or more additional target condensates.

[0242] Embodiment 72. The method of any one of embodiments 49-71, wherein the method comprises repeating the steps of the method for one or more additional target condensates.

[0243] Embodiment 73. A method of determining condensate preference profiles of a plurality of test compounds, the method comprising performing the method of any one of embodiments 49-72 with a plurality of test compounds.

[0244] Embodiment 74. The method of embodiment 73, further comprising comparing condensate preference profiles of a subset or all of the plurality of test compounds.

[0245] Embodiment 75. The method of embodiment 74, further comprising identifying test compounds that have the same or similar condensate preference profiles.

[0246] Embodiment 76. The method of embodiment 75, further comprising identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles.

[0247] Embodiment 77. The method of embodiment 76, further comprising determining the relative partition characteristic for one or more additional test compounds that comprise the identified characteristic.

[0248] Embodiment 78. A method of identifying a compound characteristic associated with partitioning a compound into a condensate, the method comprising: (a) determining partition characteristics of a plurality of test compounds in the target condensate by performing the method of embodiment 36; (b) comparing the partition characteristics of a subset or all of the plurality of test compounds in the target compound; (c) identifying test compounds that have the same or similar partition characteristics in the target condensate; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics.

[0249] Embodiment 79. A method of identifying a compound characteristic associated with partitioning a compound into a condensate, the method comprising: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate by performing the method of embodiment 44; (b) comparing the relative partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar relative partition characteristics in the target condensate; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics.

[0250] Embodiment 80. A method of identifying a compound characteristic associated with partitioning a compound into a condensate, the method comprising: (a) determining condensate preference profiles of a plurality of test compounds by performing the method of embodiment

73; (b) comparing the condensate preference profiles of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar condensate preference profiles; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles.

[0251] Embodiment 81. A method of designing a compound with a desired partition characteristic to a target condensate, the method comprising: (a) determining partition characteristics of a plurality of test compounds in the target condensate by performing the method of embodiment 36; (b) comparing the partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar partition characteristics in the target condensate; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics; and (e) designing a compound that comprises the identified characteristic.

[0252] Embodiment 82. A method of designing a compound with a desired relative partition characteristic, the method comprising: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate by performing the method of embodiment 44; (b) comparing the relative partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar relative partition characteristics in the target condensate; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics; and (e) designing a compound that comprises the identified characteristic.

[0253] Embodiment 83. A method of designing a compound with a desired condensate preference profile, the method comprising: (a) determining condensate preference profiles of a plurality of test compounds by performing the method of embodiment 73; (b) comparing the condensate preference profiles of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar condensate preference profiles; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles; and (e) designing a compound that comprises the identified characteristic.

[0254] Embodiment 84. The method of any one of embodiments 81-83, further comprising making the compound.

[0255] Embodiment 85. A method of screening a test compound for a desired partition characteristic from a group of candidate compounds, the method comprising: (a) determining a partition characteristic of each of the group of candidate compounds; and (b) identifying the test compound having the desired partition characteristic.

[0256] Embodiment 86. The method of embodiment 85, wherein the partition characteristic of each of the group of candidate compounds is determined *in vitro*.

[0257] Embodiment 87. The method of embodiment 85 or 85, wherein the test compound has a suitable partition characteristic for being useful for treating a disease in an individual.

[0258] Embodiment 88. A method of identifying a test compound useful for treating a disease in an individual in need thereof, the method comprising: (a) identifying a target condensate associated with the disease; (b) determining a partition characteristic of a candidate compound in the target condensate, and (c) identifying the test compound having a suitable partition characteristic for being useful for treating the disease.

Further exemplary embodiments

[0259] Also among the provided embodiments are:

[0260] E1. A method of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) combining the test compound and a composition comprising the target condensate and an extra-condensate solution; (b) determining the amount of the test compound in the target condensate, thereby determining the partition characteristic of the test compound in the target condensate.

[0261] E2. The method of embodiment E1, further comprising causing the formation of the target condensate prior to step (a).

[0262] E3. A method of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) causing the formation of the target condensate in the presence of the test compound to obtain a composition comprising the target condensate and an extra-condensate solution; and (b) determining the amount of the test compound in the target condensate, thereby determining the partition characteristic of the test compound in the target condensate.

[0263] E4. The method of embodiment E3, further comprising combining the test compound and a precursor composition comprising precursor molecules prior to step (a).

[0264] E5. The method of embodiment E3, further comprising adding the test compound to a precursor composition comprising precursor molecules prior to step (a).

[0265] E6. The method of any one of embodiments E1-E5, further comprising determining the amount of the test compound in the extra-condensate solution.

[0266] E7. The method of embodiment E6, wherein the amount of the test compound in the target condensate is determined prior to, simultaneously with, or after the amount of the test compound in the extra-condensate solution is determined.

[0267] E8. The method of embodiment E6 or E7, further comprising determining the ratio of the amount of test compound in the target condensate and the amount of test compound in the extra-condensate solution.

[0268] E9. The method of any one of embodiments E1-E8, further comprising separating the target condensate from the extra-condensate solution.

[0269] E10. The method of any one of embodiments E1-E9, further comprising identifying the target condensate prior to determining the amount of test compound in the target condensate.

[0270] E11. The method of any one of embodiments E1-E10, wherein dysregulation of the target condensate is associated with a disease.

[0271] E12. The method of any one of embodiments E1-E11, further comprising characterizing the target condensate by identifying one or more macromolecules comprised therein.

[0272] E13. The method of embodiment E12, wherein the identifying comprises determining the amount of the one or more macromolecules in the target condensate.

[0273] E14. The method of embodiment E13, further comprising determining the ratio of the amount of test compound in the target condensate and the amount of the one or more macromolecules in the target condensate.

[0274] E15. The method of any one of embodiments E1-E14, wherein the target condensate comprises a protein comprising an intrinsically disordered sequence.

[0275] E16. The method of any one of embodiments E1-E15, further comprising labeling the target condensate in order to visualize the target condensate.

[0276] E17. The method of embodiment E16, wherein the target condensate is labeled with a radioactive label, a colorimetric label, or a fluorescent label.

[0277] E18. The method of any one of embodiments E1-E17, wherein the composition comprises a cell.

[0278] E19. The method of embodiment E18, wherein the cell is a microorganism or an animal cell.

[0279] E20. The method of embodiment E18 or E19, wherein the cell comprises a condensate that is determined to be dysregulated.

[0280] E21. The method of any one of embodiment E18-E20, wherein the cell has one or more features of a neurodegenerative or proliferative disease.

[0281] E22. The method of any one of embodiments E1-E21, wherein the target condensate is a cellular condensate.

[0282] E23. The method of embodiment E22, wherein the cellular condensate is a cleavage body, a P-granule, a histone locus body, a multivesicular body, a neuronal RNA granule, a nuclear gem, a nuclear pore, a nuclear speckle, a nuclear stress body, a nucleolus, a Oct1/PTF/transcription (OPT) domain, a paraspeckle, a perinucleolar compartment, a PML nuclear body, a PML oncogenic domain, a polycomb body, a processing body, a Sam68 nuclear body, a stress granule, or a splicing speckle.

[0283] E24. The method of any one of embodiments E1-E23, wherein the target condensate is in a cell.

[0284] E25. The method of embodiment E24, wherein the cell is a microorganism or an animal cell.

[0285] E26. The method of embodiment E24 or E25, wherein the cell has one or more features of a neurodegenerative or proliferative disease.

[0286] E27. The method of any one of embodiment E1-E26, wherein the extra-condensate solution is intracellular fluid.

[0287] E28. The method of embodiment E27, wherein the intracellular fluid is cytosol or nucleosol.

[0288] E29. The method of any one of embodiments E1-E21, wherein the target condensate is not in a cell.

[0289] E30. The method of embodiment E29, wherein the target condensate is an extracellular condensate.

[0290] E31. The method of any one of embodiments E1-E22 or E29-E30, wherein the extra-condensate solution is extracellular fluid.

[0291] E32. The method of embodiment E31, wherein the extracellular fluid is interstitial fluid.

[0292] E33. The method of any one of embodiments E1-E17, wherein the method is a cell free assay method.

[0293] E34. The method of any one of embodiments E1-E33, wherein the composition comprises one or more of: a macromolecule, a salt, and a buffer.

[0294] E35. The method of any one of embodiments E1-E34, wherein the composition comprises two or more target condensates.

[0295] E36. The method of any one of embodiments E1-E35, wherein the method comprises repeating the steps of the method for one or more additional condensates.

[0296] E37. The method of any one of embodiments E1-E36, wherein the test compound is small molecule, a polypeptide, or a nucleic acid.

[0297] E38. The method of any one of embodiments E1-E37, wherein the test compound comprises a test compound label.

[0298] E39. The method of embodiment E38, wherein the test compound label is a radioactive label, a colorimetric label, or a fluorescent label.

[0299] E40. The method of embodiment E38 or E39, wherein the test compound label is a fluorescent label.

[0300] E41. The method of any one of embodiments E38-E40, wherein the amount of the test compound is determined by detecting the test compound label.

[0301] E42. The method of any one of embodiments E1-E41, wherein the amount of the test compound is determined by mass spectrometry, liquid chromatography, and/or ultraviolet-visible spectrophotometry.

[0302] E43. A method of determining the partition characteristics of a plurality of test compounds in a target condensate, the method comprising performing the method of any one of embodiments E1-E42 with a plurality of test compounds.

[0303] E44. The method of embodiment E43, further comprising comparing the partition characteristics of a subset or all of the plurality of test compounds in the target condensate.

[0304] E45. The method of embodiment E44, further comprising identifying test compounds that have the same or similar partition characteristics in a target condensate.

[0305] E46. The method of embodiment E45, further comprising identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics.

[0306] E47. The method of embodiment E46, further comprising determining the partition characteristic in a target condensate for one or more additional test compounds that comprise the identified characteristic.

[0307] E48. The method of embodiment E46 or E47, further comprising determining the partition characteristic in a target condensate for one or more additional test compounds that do not comprise the identified characteristic.

[0308] E49. A method of determining a relative partition characteristic of a test compound in a target condensate, the method comprising: (i) determining the partition characteristic of the test compound by performing the method of any one of embodiments E1-E42 with the test compound; (ii) determining the partition characteristic of a reference compound by performing the method of any one of embodiments E1-E42 with the reference compound; and (iii) calculating the ratio of the partition characteristics determined in (i) and (ii), thereby determining the relative partition characteristic of the test compound in the target condensate.

[0309] E50. The method of embodiment E49, wherein the test compound comprises a test compound label.

[0310] E51. The method of embodiment E50, wherein the reference compound is the test compound label.

[0311] E52. A method of determining relative partition characteristics of a plurality of test compounds in a target condensate, the method comprising: (1) performing the method of any one of embodiments E49-E51; and (2) repeating steps (i) and (iii) with a plurality of test compounds.

[0312] E53. The method of embodiment E52, further comprising comparing the relative partition characteristics in the target condensate of a subset or all of the plurality of test compounds.

[0313] E54. The method of embodiment E53, further comprising identifying test compounds that have the same or similar relative partition characteristics in the target condensate.

[0314] E55. The method of embodiment E54, further comprising identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics.

[0315] E56. The method of embodiment E55, further comprising determining the relative partition characteristic in the target condensate for one or more additional test compounds that comprise the identified characteristic.

[0316] E57. The method of embodiment E55 or E56, further comprising determining the relative partition characteristic in the target condensate for one or more additional test compounds that do not comprise the identified characteristic.

[0317] E58. A method of determining a condensate preference profile of a test compound, the method comprising: (a) determining the partition characteristic of the test compound in a first target condensate using the method of any one of embodiments E1-E42; (b) determining the partition characteristic of the test compound in a second target condensate using the method of any one of embodiments E1-E42; and (c) calculating a ratio of the partition characteristic of the test compound determined in the first target condensate and the second target condensate, thereby determining the condensate preference profile of the test compound.

[0318] E59. The method of embodiment E58, wherein the first target condensate and the second target condensate are in the same composition.

[0319] E60. The method of embodiment E58, wherein the first target condensate and the second target condensate are in different compositions.

[0320] E61. The method of any one of embodiments E58-E60, wherein the partition characteristic of the test compound in the first target condensate is determined prior to, simultaneously with, or after the partition characteristic of the test compound in the second target condensate is determined.

[0321] E62. A method of determining a condensate preference profile of a test compound, the method comprising: (a) determining the relative partition characteristic of the test compound in a first target condensate using the method of any one of embodiments E49-E51; (b) determining the relative partition characteristic of the test compound in a second target condensate using the method of any one of embodiments E49-E51; and (c) calculating a ratio of the partition characteristic of the test compound determined in the first target condensate and the second target condensate, thereby determining the condensate preference profile of the test compound.

[0322] E63. The method of embodiment E62, wherein the first target condensate and the second target condensate are in the same composition.

[0323] E64. The method of embodiment E62, wherein the first target condensate and the second target condensate are in different compositions.

[0324] E65. The method of any one of embodiments E62-E64, wherein the relative partition characteristic of the test compound in the first target condensate is determined prior to,

simultaneously with, or after the relative partition characteristic of the test compound in the second target condensate is determined.

[0325] E66. The method of any one of embodiments E58-E65, further comprising labeling the first target condensate and the second target condensate in order to visualize the first condensate target condensate and the second target condensate.

[0326] E67. The method of embodiment E66, wherein the first target condensate and the second target condensate are labeled with different labels.

[0327] E68. The method of any one of embodiments E58-E67, wherein the method comprises repeating the steps of the method for one or more additional target condensates.

[0328] E69. A method of determining condensate preference profiles of a plurality of test compounds, the method comprising performing the method of any one of embodiments E58-E68 with a plurality of test compounds.

[0329] E70. The method of embodiment E69, further comprising comparing condensate preference profiles of a subset or all of the plurality of test compounds.

[0330] E71. The method of embodiment E70, further comprising identifying test compounds that have the same or similar condensate preference profiles.

[0331] E72. The method of embodiment E71, further comprising identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles.

[0332] E73. The method of embodiment E72, further comprising determining the relative partition characteristic for one or more additional test compounds that comprise the identified characteristic.

[0333] E74. The method of embodiment E72 or E73, further comprising determining the relative partition characteristic for one or more additional test compounds that do not comprise the identified characteristic.

[0334] E75. A method of identifying a compound characteristic associated with partitioning a compound into or out of a condensate, the method comprising: (a) determining partition characteristics of a plurality of test compounds in the target condensate by performing the method of embodiment E43; (b) comparing the partition characteristics of a subset or all of the plurality of test compounds in the target compound; (c) identifying test compounds that have the same or similar partition characteristics in the target condensate; and (d) identifying a

characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics.

[0335] E76. A method of identifying a compound characteristic associated with partitioning a compound into or out of a condensate, the method comprising: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate by performing the method of embodiment E52; (b) comparing the relative partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar relative partition characteristics in the target condensate; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics.

[0336] E77. A method of identifying a compound characteristic associated with partitioning a compound into or out of a condensate, the method comprising: (a) determining condensate preference profiles of a plurality of test compounds by performing the method of embodiment E69; (b) comparing the condensate preference profiles of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar condensate preference profiles; and (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles.

[0337] E78. A method of designing a compound with a desired partition characteristic into or out of a target condensate, the method comprising: (a) determining partition characteristics of a plurality of test compounds in the target condensate by performing the method of embodiment E43; (b) comparing the partition characteristics of a subset or all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar partition characteristics in the target condensate; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar partition characteristics; and (e)(i) designing a compound that comprises the identified characteristic; or (ii) designing a compound that does not comprise the identified characteristic, thereby designing a compound with the desired partition characteristic into or out of the target condensate.

[0338] E79. A method of designing a compound with a desired relative partition characteristic into or out of a target condensate, the method comprising: (a) determining relative partition characteristics of a plurality of test compounds in the target condensate by performing the method of embodiment E52; (b) comparing the relative partition characteristics of a subset or

all of the plurality of test compounds in the target condensate; (c) identifying test compounds that have the same or similar relative partition characteristics in the target condensate; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar relative partition characteristics; and (e)(i) designing a compound that comprises the identified characteristic; or (ii) designing a compound that does not comprise the identified characteristic, thereby designing a compound with the desired relative partition characteristic into or out of the target condensate.

[0339] E80. A method of designing a compound with a desired condensate preference profile, the method comprising: (a) determining condensate preference profiles of a plurality of test compounds by performing the method of embodiment E69; (b) comparing the condensate preference profiles of a subset or all of the plurality of test compounds; (c) identifying test compounds that have the same or similar condensate preference profiles; (d) identifying a characteristic that a subset or all of the identified test compounds have in common in addition to the same or similar condensate preference profiles; and (e)(i) designing a compound that comprises the identified characteristic; or (ii) designing a compound that does not comprise the identified characteristic, thereby designing a compound with the desired condensate preference profile.

[0340] E81. The method of any one of embodiments E78-E80, further comprising making the compound.

[0341] E82. A method of screening a test compound for a desired partition characteristic from a group of candidate compounds, the method comprising: (a) determining a partition characteristic of each of the group of candidate compounds; and (b) identifying the test compound having the desired partition characteristic.

[0342] E83. The method of embodiment E82, wherein the partition characteristic of each of the group of candidate compounds is determined in vitro.

[0343] E84. The method of embodiment E82 or E83, wherein the test compound has a suitable partition characteristic for being useful for treating a disease in an individual.

[0344] E85. A method of identifying a test compound useful for treating a disease in an individual in need thereof, the method comprising: (a) identifying a target condensate associated with the disease; and (b) determining a partition characteristic of a candidate compound in the target condensate, and (c) identifying the test compound having a suitable partition characteristic for being useful for treating the disease.

[0345] E86. A method of determining a partition characteristic of a test compound in a target condensate, the method comprising: (a) combining the test compound and a composition comprising the target condensate and an extra-condensate solution;(b) obtaining a reference control; (c) measuring a MS signal of the test compound in the extra-condensate solution, or a portion thereof, using a mass spectrometry technique; (d) measuring a MS signal of the test compound in the reference control, or a portion thereof, using a mass spectrometry technique; and (e) comparing the MS signal of the test compound from the extra-condensate solution and the MS signal of the test compound from the reference control, thereby determining the partition characteristic of the test compound in the target condensate.

[0346] E87. The method of E86, wherein the amount of the test compound combined with the composition is 100 nM or less, and the amount of a precursor molecule in the composition, including in the target condensate, is about 5 μM.

[0347] E88. A library comprising a plurality of compounds, wherein each compound of the plurality of compounds comprises the same moiety comprising a characteristic having a desired partition characteristic.

[0348] E89. A method of designing a test compound having a desired partition characteristic, the method comprising modifying a precursor of the test compound by attaching a moiety to the compound, wherein the moiety comprises a characteristic having a desired partition characteristic.

[0349] Those skilled in the art will recognize that several embodiments are possible within the scope and spirit of the disclosure of this application. The disclosure is illustrated further by the examples below, which are not to be construed as limiting the disclosure in scope or spirit to the specific procedures described therein.

EXAMPLES

Example 1

Characterization of one or more compounds in solution-based condensates

[0350] Condensates are formed in a solution. For example, a solution comprising a high concentration of salt and a high concentration of one or more protein capable of forming a condensate are diluted into a buffer that mimics physiological salt conditions.

[0351] Alternatively, a solution comprising one or more proteins capable of forming a condensate is diluted into a buffer and a crowding agent is added. In one specific embodiment, proteins capable of forming a condensate are mixed with 10% Dextran in a buffer containing 25 mM Tris–HCl (pH 7.4), 150 mM KCl, 2.5% glycerol, and 0.5 mM DTT.

[0352] A test compound is added to the solution before or after condensate formation. When added after condensate formation, the solution is incubated to allow partitioning of the test compound.

[0353] The density of condensates is typically greater than the surrounding solution, so the condensate is allowed to sediment. In some instances, the condensate is imaged. For example, a dye or a labeled protein that is known to concentrate in the condensate is used to visualize the condensate.

[0354] In some instances, the supernatant liquid is removed from the condensate. In some instances, the supernatant liquid is analyzed to determine the amount of compound present.

[0355] The condensate is analyzed to determine the amount of compound present. In some instances, the ratio of compound in the condensate to compound in the supernatant is calculated.

Example 2

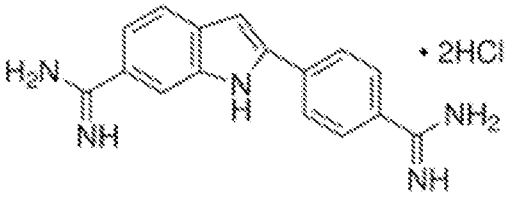
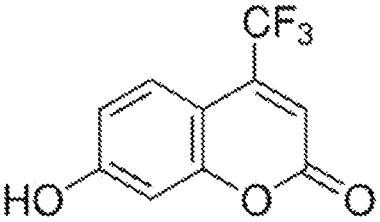
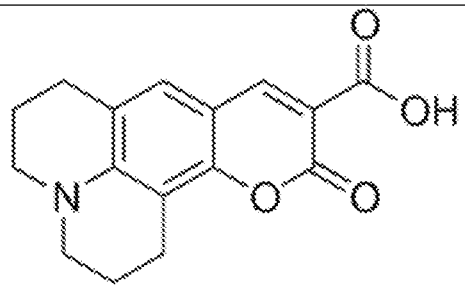
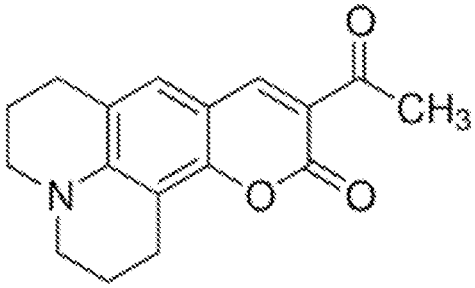
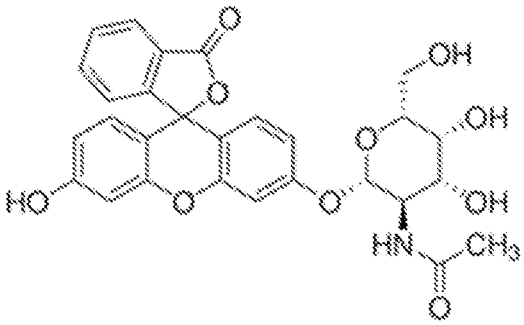
Characterization of dye compounds in solution-based FUS and PGL-3 condensates

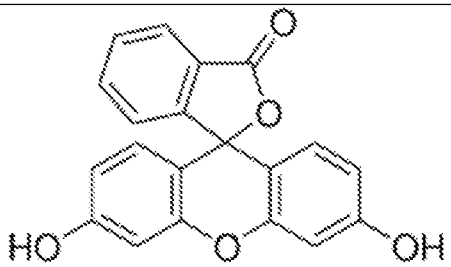
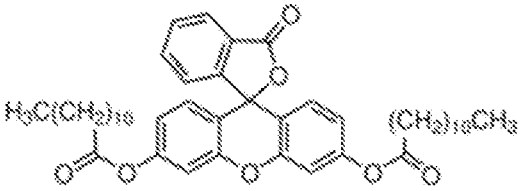
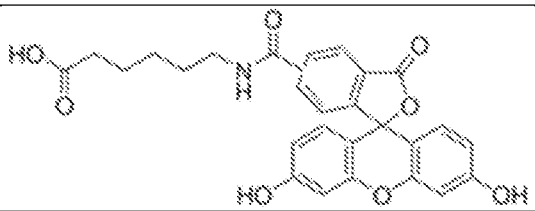
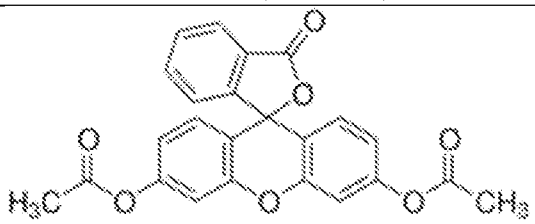
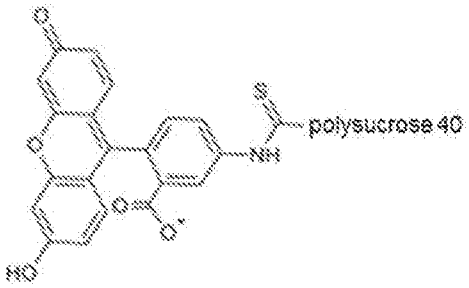
[0356] A variety of exemplary test compounds were assayed using exemplary methods to determine partition characteristics, relative partition characteristics, and condensate preference profiles. For this example, the exemplary test compounds used were dyes; however test compounds are not limited to dyes. The entire procedure was performed twice in two independent experiments on different days and in different orders.

Sample preparation

[0357] All dye-stocks were stored as 1 mM solutions in 100% DMSO. The dyes used are shown in **Table 1**. The dyes were dissolved in dilution buffer (DB: 14.7 mM Tris, pH 7.25, 1 mM DTT) to yield 11.765 μM dye in 1.1765 % DMSO. 17 μl of dye was distributed in a 384 well non-binding plate (Greiner).

Table 1. Assayed dyes.

DYE #	NAME	STRUCTURE	CHANNEL
1	DAPI		405
2	7-Hydroxy-4-(trifluoromethyl)coumarin		405
3	Coumarin 343		405
4	Coumarin 334		488
5	Fluorescein mono-(N-acetyl-β-D-galactosaminide)		488

6	Fluorescein		488
7	Fluorescein dilaurate		488
8	6-[Fluorescein-5(6)-carboxamido]hexanoic acid		488
9	Fluorescein diacetate		488
10	Polysucrose 40-fluorescein isothiocyanate conjugate		488

11	Fluorescein-12-UTP		488
12	Fluorescein di(β-D-galactopyranoside)		488
13	Biotin-4-Fluorescein		488
14	Rhodamine 123		488

15	N-(8-Amino-3,6-dioxaoctyl)rhodamine 6G-amide bis(trifluoroacetate)		488
16	Rhodamine B		561
17	Rhodamine 101 inner salt		561
18	Rhodamine 800		640

[0358] Either SNAP-tagged FUS protein or tag-free PGL-3 protein was thawed at room temperature for at least 10 min, and the buffer was exchanged to partitioning buffer (PB: 50 mM Tris pH 7.25, 500 mM KCl, 5% Glycerol, and 1 mM DTT). The protein was then diluted to 33.3 μM in PB. 3 μl of protein solution were added to the 384 well plate to initiate phase separation, yielding 20 μl of 5 μM protein and 10 μM dye in 75 mM KCl, 0.75 % Glycerol, 20 mM Tris, 1 mM DTT, and 1% DMSO or for no dye control reactions, 20 μl of 5 μM protein in 75 mM KCl, 0.75 % Glycerol, 20 mM Tris, 1 mM DTT.

Imaging

[0359] A spinning disk confocal microscope was used to acquire images of the samples. A 60x oil high-NA objective lens was used to capture the condensate droplets. The laser power was set to 20 % and the exposure times were adjusted to the fluorescence emission intensity specific for each dye. Exposure times ranged from 1-1000 ms. Appropriate filter settings were used, matching the excitation and emission spectra of the respective dyes. The channels used for each dye are shown in **Table 1**. Three images per well were taken. Exemplary images are shown in **FIG. 1**. The background signal of protein droplets in the absence of dye was recorded for all applied imaging conditions.

Image and data analysis

[0360] Fluorescence intensity was measured inside one exemplary target condensate (Intensity inside, I-in) per image and in a region next to the exemplary target condensate (Intensity outside, I-out) by hand using the image analysis software Fiji. An example depicting the regions measured is shown **FIG. 2A**. Three I-in and three I-out values were measured in total for each dye in each experiment and were averaged. The measured I-in and I-out values for FUS-SNAP condensates on one day of experiments are shown in **Table 2** and **Table 3**, respectively. The measured I-in and I-out values for PGL-3 condensates on one day of experiments are shown in **Table 4** and **Table 5**, respectively. All values were background corrected, based on the background images (no dye controls) taken with the same microscope conditions. An exemplary background image is shown in **FIG. 2B** and measured I-in or I-out background measurements are shown in **Tables 3-5**.

Table 2. Measurements of dyes inside FUS-SNAP condensates.

Dye #	Intensity Inside with Dye				Intensity Inside with No Dye			Intensity Inside Dye-No Dye
	1	2	3	Average	1	2	Average	
1	638.5	599.9	628.8	**622.4**	120.6	119.3	**120.0**	**502.4**
2	278.2	278.1	286.5	**280.9**	120.6	119.3	**120.0**	**160.9**
3	3451.6	3462.2	3804.4	**3572.7**	120.6	119.3	**120.0**	**3452.8**
4	10755.9	10738.6	11058.9	**10851.1**	503.3	491.9	**497.6**	**10353.6**
5	2046.8	2005.1	1638.1	**1896.7**	503.3	491.9	**497.6**	**1399.1**
6	6328.8	6300.8	6201.7	**6277.1**	131.8	138.6	**135.2**	**6141.9**

7	548.7	492.0	481.3	**507.3**	503.3	491.9	**497.6**	**9.7**
8	5285.1	5631.6	5148.0	**5354.9**	131.8	138.6	**135.2**	**5219.7**
9	5836.4	6036.1	6229.9	**6034.1**	4134.6	4290.7	**4212.6**	**1821.5**
10	6139.7	6105.5	5024.6	**5756.6**	131.8	138.6	**135.2**	**5621.4**
11	13042.4	13375.9	12572.2	**12996.8**	131.8	138.6	**135.2**	**12861.6**
12	456.2	508.6	469.3	**478.0**	503.3	491.9	**497.6**	**-19.5**
13	4600.2	4189.8	5218.3	**4669.4**	131.8	138.6	**135.2**	**4534.2**
14	3123.1	2956.9	2831.2	**2970.4**	131.8	138.6	**135.2**	**2835.2**
15	3084.2	3756.7	3599.3	**3480.0**	4134.6	4290.7	**4212.6**	**-732.6**
16	2154.5	2074.7	1895.0	**2041.4**	99.7	99.6	**99.6**	**1941.7**
17	4695.6	5663.6	4511.0	**4956.7**	99.7	99.6	**99.6**	**4857.1**
18	15394.5	13908.5	15851.1	**15051.4**	129.9	133.8	**131.9**	**14919.5**

Table 3. Measurements of dyes outside FUS-SNAP condensates.

Dye #	Intensity Outside with Dye				Intensity Outside with No Dye			Intensity Outside (Dye-No Dye)
	1	2	3	Average	1	2	Average	
1	233.9	233.3	218.7	**228.6**	120.1	120.1	**120.1**	**108.6**
2	235.7	228.5	232.0	**232.0**	120.1	120.1	**120.1**	**112.0**
3	1486.5	1529.0	1479.6	**1498.4**	120.1	120.1	**120.1**	**1378.3**
4	4894.0	5118.6	5078.2	**5030.3**	140.3	139.8	**140.0**	**4890.2**
5	1319.4	1306.9	1191.0	**1272.5**	140.3	139.8	**140.0**	**1132.4**
6	3771.1	3840.2	3742.7	**3784.7**	104.3	103.8	**104.0**	**3680.6**
7	141.6	135.4	138.4	**138.5**	140.3	139.8	**140.0**	**-1.6**
8	3064.0	3267.0	3205.5	**3178.8**	104.3	103.8	**104.0**	**3074.8**
9	1487.5	1414.8	1552.6	**1485.0**	519.0	581.2	**550.1**	**934.8**
10	5878.5	5846.2	4791.0	**5505.3**	104.3	103.8	**104.0**	**5401.2**
11	2423.6	3035.6	2255.1	**2571.4**	104.3	103.8	**104.0**	**2467.4**
12	150.1	142.8	152.9	**148.6**	140.3	139.8	**140.0**	**8.5**
13	2286.6	2244.6	2195.6	**2242.3**	104.3	103.8	**104.0**	**2138.2**
14	2174.8	2322.7	2335.1	**2277.6**	104.3	103.8	**104.0**	**2173.5**
15	559.0	527.6	532.7	**539.8**	519.0	581.2	**550.1**	**-10.3**
16	546.8	508.3	565.6	**540.2**	100.0	99.9	**99.9**	**440.3**
17	808.3	1011.5	887.5	**902.4**	100.0	99.9	**99.9**	**802.5**
18	2064.2	1988.2	2224.3	**2092.2**	133.8	130.8	**132.3**	**1959.9**

Table 4. Measurements of dyes inside PGL-3 condensates.

Dye #	Intensity Inside with Dye				Intensity Inside with No Dye			Intensity Inside (Dye-No Dye)
	1	2	3	Average	1	2	Average	
1	211.3	211.0	215.7	**212.6**	121.1	120.4	**120.8**	**91.9**
2	273.9	267.0	268.3	**269.8**	121.1	120.4	**120.8**	**149.0**
3	2228.4	2006.3	2059.4	**2098.0**	121.1	120.4	**120.8**	**1977.3**
4	212.2	205.0	197.2	**204.8**	121.1	120.4	**120.8**	**84.0**
5	1912.6	1794.0	1797.0	**1834.5**	615.1	579.4	**597.2**	**1237.3**
6	4585.4	4501.5	4119.2	**4402.0**	146.6	146.4	**146.5**	**4255.5**
7	784.6	785.3	792.4	**787.4**	615.1	579.4	**597.2**	**190.2**
8	3527.5	3608.1	3480.3	**3538.6**	146.6	146.4	**146.5**	**3392.1**
9	824.1	801.2	860.4	**828.6**	615.1	579.4	**597.2**	**231.3**
10	5747.5	6281.4	4996.0	**5674.9**	146.6	146.4	**146.5**	**5528.4**
11	2451.3	2688.1	2362.8	**2500.7**	146.6	146.4	**146.5**	**2354.2**
12	689.4	656.9	723.9	**690.1**	615.1	579.4	**597.2**	**92.8**
13	2939.4	3141.8	3035.4	**3038.9**	146.6	146.4	**146.5**	**2892.4**
14	2874.7	2858.2	2900.3	**2877.7**	146.6	146.4	**146.5**	**2731.2**
15	6831.9	6829.0	5802.8	**6487.9**	4477.7	6041.9	**5259.8**	**1228.1**
16	5570.5	6179.8	5579.3	**5776.5**	101.5	101.6	**101.5**	**5675.0**
17	12579.6	14061.0	12561.8	**13067.5**	101.5	101.6	**101.5**	**12966.0**
18	714.9	700.1	777.4	**730.8**	125.2	127.2	**126.2**	**604.6**

Table 5. Measurements of dyes outside PGL-3 condensates.

Dye #	Intensity Outside with Dye				Intensity Outside with No Dye			Intensity Outside (Dye-No Dye)
	1	2	3	Average	1	2	Average	
1	173.1	168.4	164.9	**168.8**	120.1	120.8	120.4	**48.4**
2	263.1	258.0	266.0	**262.4**	120.1	120.8	120.4	**141.9**
3	1472.5	1407.6	1461.1	**1447.1**	120.1	120.8	120.4	**1326.6**
4	178.0	179.6	179.5	**179.0**	120.1	120.8	120.4	**58.6**
5	1439.0	1386.2	1353.5	**1392.9**	165.2	160.6	162.9	**1230.0**
6	4395.6	4190.8	3912.1	**4166.2**	105.7	105.0	105.3	**4060.8**
7	215.9	212.1	214.8	**214.2**	165.2	160.6	162.9	**51.3**
8	3491.4	3551.4	3447.6	**3496.8**	105.7	105.0	105.3	**3391.4**
9	269.6	264.9	273.2	**269.2**	165.2	160.6	162.9	**106.3**
10	5900.4	6581.6	5199.5	**5893.8**	105.7	105.0	105.3	**5788.5**
11	2174.3	2443.9	2108.6	**2242.3**	105.7	105.0	105.3	**2137.0**
12	208.4	203.2	207.7	**206.4**	165.2	160.6	162.9	**43.5**
13	2781.8	2905.7	2825.1	**2837.5**	105.7	105.0	105.3	**2732.2**

14	2628.3	2616.4	2545.0	**2596.6**	105.7	105.0	105.3	**2491.2**
15	1289.1	1266.2	1236.3	**1263.8**	671.5	744.9	708.2	**555.7**
16	3907.8	4226.6	4131.5	**4088.7**	101.3	101.6	101.5	**3987.2**
17	7184.0	7157.1	7012.6	**7117.9**	101.3	101.6	101.5	**7016.4**
18	301.3	294.6	332.2	**309.4**	128.7	132.7	130.7	**178.7**

[0361] The ratios of the average intensity measured in the target condensates and outside of the target condensates were calculated by dividing I-in by I-out, although dividing I-out by I-in could also have been used. The I-in:I-out ratios determined from the two different days of experiments were then averaged. The individual day and averaged I-in:I-out ratios are shown in **Table 6**. The average I-in:I-out ratios for FUS-SNAP condensates are shown in **FIG. 3** and the average I-in:I-out ratios for PGL-3 condensates are shown in **FIG. 4**. The standard deviation between the two experiments is represented by the error bars in **FIG. 3** and **FIG. 4**.

[0362] The ratio of FUS-SNAP I-in:I-out to PGL-3 I-in:I-out was also calculated and is shown in **Table 6** and **FIG. 5**.

Table 6. Calculated ratios of dye intensity.

Dye #	Ratio of intensity inside:outside								Ratio of FUS-SNAP:PGL-3
	FUS-SNAP Condensates				PGL-3 Condensates				
	Day 1	Day 2	Average	Standard Deviation	Day 1	Day 2	Average	Standard Deviation	
1	4.6	3.3	**4.0**	0.9	1.9	3.3	**2.6**	1.0	**1.5**
2	1.4	1.2	**1.3**	0.2	1.0	1.1	**1.1**	0.0	**1.2**
3	2.5	2.8	**2.7**	0.2	1.5	1.1	**1.3**	0.3	**2.0**
4	2.1	3.0	**2.6**	0.6	1.4	1.3	**1.3**	0.1	**1.9**
5	1.2	1.4	**1.3**	0.1	1.0	1.1	**1.1**	0.1	**1.3**
6	1.7	1.4	**1.5**	0.2	1.0	1.0	**1.0**	0.0	**1.5**
7	-6.2	N/A	**N/A**	N/A	3.7	-4.1	**N/A**	N/A	**N/A**
8	1.7	1.6	**1.6**	0.1	1.0	1.0	**1.0**	0.0	**1.6**
9	1.9	1.5	**1.7**	0.3	2.2	-0.4	**N/A**	N/A	**N/A**
10	1.0	1.1	**1.1**	0.0	1.0	0.9	**0.9**	0.0	**1.1**
11	5.2	4.8	**5.0**	0.3	1.1	1.0	**1.1**	0.1	**4.7**
12	-2.3	N/A	**N/A**	N/A	2.1	1.9	**N/A**	N/A	**N/A**
13	2.1	2.1	**2.1**	0.0	1.1	1.1	**1.1**	0.0	**2.0**
14	1.3	1.4	**1.3**	0.0	1.1	1.1	**1.1**	0.0	**1.2**
15	70.8	N/A	**N/A**	N/A	N/A	3.3	**N/A**	N/A	**N/A**
16	4.4	3.8	**4.1**	0.4	1.4	1.3	**1.4**	0.1	**3.0**

17	6.1	5.9	**6.0**	0.1	1.8	1.9	**1.9**	0.0	**3.2**
18	7.6	11.0	**9.3**	2.4	3.4	2.4	**2.9**	0.7	**3.2**

N/A: not applicable, because the error was too large due to low signal of dye.

[0363] All compounds tested appear to be preferably partitioned into FUS-SNAP condensates over condensates formed by PGL-3. Compound #1 (DAPI), #11 (Fluorescein-12-UTP), #16 (Rhodamine B), #17 (Rhodamine 101 inner salt) and #18 (Rhodamine 800) has a I-in:I-out ratio for FUS-SNAP condensates of 4 or higher. Compounds #11, #16, #17, and #18 preferentially partitioned in FUS-SNAP condensates compared to PGL-3 with a ratio of 3 or higher.

Example 3

Co-localization of partitioned compounds and FUS in FUS condensates

[0364] To confirm that compounds that were partitioned into condensates in Example 2 were partitioned into condensates that contain FUS, Dye #18 (Rhodamine 800), which had an average I-in:I-out ratio of 9.3, was further assessed.

Sample Preparation

[0365] FUS-GFP condensates were prepared as described for FUS-SNAP in Example 2 using Dye 18.

Imaging

[0366] A spinning disk confocal microscope was used to acquire images of the samples. A 60x oil high-NA objective lens was used to capture the condensate droplets. The laser power was set to 20 % and the exposure times were adjusted to the fluorescence emission intensity specific for each dye. Exposure times ranged from 1-1000 ms. Appropriate filter settings were used, matching the excitation and emission spectra of the dye and GFP. Control images were taken of condensates containing FUS-GFP with no dye.

Results

[0367] The resulting images are shown in **FIG. 6**. GFP-labeled FUS was detected in condensates in the presence or absence of dye. Dye 18 was detected in condensates and the FUS protein and the dye were co-localized in the condensates. For no dye controls, fluorescence was not detected in the condensates when the Rhodamine 800 channel was used to acquire the images.

Example 4

Determining a partition characteristic of a set of compounds using a mass spectrometry assay

[0368] A mass spectrometry-based method was developed to measure partition characteristics of exemplary test compounds. The measurements from the mass spectrometry-based method were compared to measurements obtained using the fluorescence-based assay disclosed in Example 2.

Methods

[0369] Protein Buffer was prepared (50 mM Tris, pH 7.25, 500 mM KCl, 1 mM DTT, and 5% glycerol). The high salt concentration of Protein Buffer prevents phase separation of macromolecules, *e.g.*, proteins, contained therein. 20 µl aliquots of 70 µM FUS-SNAP protein stock solution were prepared in Protein Buffer. The aliquots of FUS-SNAP protein stock solution were frozen and stored prior to use. Dilution Buffer was prepared (14.7 mM Tris, pH 7.25, and 1 mM DTT).

[0370] Aliquots of 70 µM FUS-SNAP protein stock solution were thawed at room temperature for about 10 minutes. The aliquots of protein stock solution appeared clear with no visible precipitates. The thawed protein stock solution were filtered using centrifugation filters (Millipore, UFC30VV00). In brief, the aliquots of protein stock solution were added to a centrifugation filed and centrifuged for 1 minutes at 20,000 rcf at room temperature. The flow-through was collected. The protein concentration of the flow-through was measured and a solution of 33.3 µM FUS-SNAP was prepared by diluting the filtered flow-through and Dilution Buffer.

[0371] 40 µL test reactions were prepared inside a PCR-tube or a PCR-strip (8 wells; AXYGEN™ 8-Strip PCR Tubes, 0.2mL) using the following: (i) 34 µL Dilution Buffer, with

the test compound at a given concentration, and with DMSO to obtain a final DMSO concentration of 1%, and (ii) 6 μL of 33.3 μM FUS-SNAP filtered protein solution. The final concentration of the 40 μL reactions were 5 μM FUS-SNAP, 75 mM KCl, 20 mM Tris, 0.75 % Glycerol, 1 mM DTT, 1% DMSO, and, for the reactions containing a test compound, the desired test compound concentration.

[0372] 40 μL reference reactions were prepared as described for the test reactions, however, no test compound was added at this stage.

[0373] The test reactions and the reference reactions were incubated for 15 minutes at room temperature. It is expected that within the test reactions, condensed high density liquid droplets (mM concentration of protein) of FUS-SNAP protein (condensed phase) are present in an environment of a lower concentrated solution (μM concentration of protein) of FUS-SNAP dilute phase. Test compounds may or may not partition into the condensed phase causing a reduction of the concentration outside (test compound in the dilute phase) and an increase of the concentration inside (test compound in the droplets). The volume of high density liquid droplets is estimated to be at least 1000-fold smaller than the total volume of the reaction, *e.g.*, 20 nL of a condensed phase in a 20 μL reaction.

[0374] Following the 15 minute incubation period, the test reactions and reference reactions were processed to separate condensates (the high density liquid droplets) from the supernatant (the dilute phase). Briefly, the test reactions and reference reactions were centrifuged at 10,000 rcf for 10 minutes in a cooled centrifuge at 20 °C. When PCR-strips were sued, the rotor used was the Eppendorf F-45-48-5-PCR. 35 μL of the supernatant from each tube was removed and transferred to a new tube being careful not to disturb the pellets, which contained the condensates.

[0375] For the reference reactions, a known amount of the test compound was added to the tube containing the supernatant (which contains protein not associated with a condensate), such that the final compound concentration is the same as the initial concentration of the compound in the test reaction. Reference reactions can also be formed by measuring the amount of protein in the supernatant of a test reaction and replicating a supernatant solution based on the obtained information. The known amount of the compound added to the supernatant from the reference reaction was used to calibrate the mass spectrometry signal of the test reaction.

[0376] Additionally, the compound was extracted from the test reactions in order to directly measure the amount of compound in the formed condensates. The supernatant, which includes

an amount of the test compound, was separated from the pellet comprising condensed protein. Briefly, the lids of each PCR tube were removed with scissors and each PCR tube was placed upside down in a 1.5 mL Eppendorf tube. The tubes were then centrifuged at 2,000 rcf for 5 seconds on a standard table top centrifuge. When PCR-strips were used, the PCR-strip can be plugged in upside down in an Eppendorf Microplate 96/V-PP (Cat# 951040188) and centrifuged at 4,500 rcf for 1 minute. Subsequently, the compound was isolated from each pellet using a solution of ACN:MeOH (1:1). The samples may be sonicated as necessary.

[0377] The resulting samples from the test reactions and the reference reactions were analyzed by mass spectrometry. The ratio between the integrals of the corresponding MS signals between the relevant supernatant test reaction and the reference reaction reflected the depletion of the test compound due to partitioning into the formed condensates.

[0378] The partition characteristics of the test compounds were also determined using the fluorescence-based assay method disclosed in Example 2.

Results

[0379] Rhodamine 101 (Rho 101), Rhodamine 800 (Rho 808), Rhodamine B (Rho B), Rhodamine 123 (Rho 123), and fluorescein were assessed using both the fluorescence-based assay and the mass spectrometry-based assay, as discussed above. As shown in **FIG. 7**, the two test methods produced partition characteristic measurements that were in good agreement for all of the tested compounds. Using the measurements, a ratio of compound concentration associated with the condensate compared to compound concentration not associated with the condensate was calculated for each compound. A condensate volume fraction of 1/1000 in relation to the outside phase was used. The results are provided in **Table 7**.

Table 8. Ratio of compound concentration in versus compound concentration out.

Compound	MS-based assay	Fluorescence-based assay
Rho 101	> 8,000	1,500
Rho 800	> 2,000	1,000
Rho B	666	666
Fluorescein	1-100	1
Rho 123	1-100	1

[0380] Additionally, the pelleted condensate samples were processed to extract and quantify the test compound therein. The results demonstrated that the sum of the amount of the test

compound in the condensate and the amount of the test compound in the supernatant agreed with the total amount of the test compound added to the test reaction (data not provided).

[0381] As demonstrated herein, the mass spectrometry-based assay is a robust and sensitive assay to quantitatively determine partition characteristics of compounds. This technique is useful as a high-throughput screen. Certain additional advantages are realized for this mass spectrometry-based assay. For example, the mass spectrometry-based assay is not limited to fluorescent compounds, is a hypothesis-free technique (*i.e.*, the identity of the test compound does not need to be known prior to the assay), is not limited by fluorescence quenching that may occur in the droplet interior, and, as demonstrated in Example 5, can be readily multiplexed.

Example 5

[0382] This example evaluates the impact of using different concentrations of test compound in the test reaction on the measurement of the test compound in the supernatant.

[0383] Test reactions and reference controls were prepared as described in Example 4. Fluorescein (which was identified as not partitioning into FUS-condensates) and Rhodamine 101 (which was identified as partitioning into FUS-condensates) were evaluated in separate test reactions at 0.01μM, 0.1 μM, 1 μM, and 10 μM.

Results

[0384] As shown in **FIG. 8**, fluorescein does not partition into the FUS-condensates at any of the tested concentrations and the partitioning characteristic of fluorescein is concentration independent. Rhodamine 101 was observed to partition into the FUS-condensates at lower compound concentrations (*see* **FIG. 8**; 0.01μM and 0.1 μM). Data represents mean ± SD, N=3 technical repeats. As illustrated in **FIG. 8**, detection efficiency of a partitioning characteristic improves for reduced compound concentrations. As the amount of compound partitioning into a condensate may be small, using smaller amounts of a test compound added to the test reaction may allow for detection of small changes in the supernatant compound concentration relative to the reference control.

Example 6

Multiplexed mass spectrometry depletion assay

[0385] This example demonstrates the use of the mass spectrometry-based assay disclosed in Example 4 for studying systems comprising a plurality of test compounds.

Methods

[0386] FUS-SNAP protein reactions and reference reactions were prepared as described in Example 4. For test reactions, a set of four compounds, including Fluorescein and Rhodamine B, were used to create test reactions having a single compound at concentrations of 0.01 μM, 0.1 μM, and 1 μM, and test reactions having a mixture of all four compounds. All samples were prepared in triplicate. All samples were analyzed using the mass spectrometry-based assay described in Example 4.

Results

[0387] As shown in **FIG. 9A**, the measured fraction of Fluorescein outside of the FUS-SNAP condensates for single compound test reactions were in agreement with the measured fraction of Fluorescein outside of the FUS-SNAP condensates in the presence of the mixture of four compounds. As shown in **FIG. 9B**, the measured fraction of Rhodamine B (Rho B) outside of the FUS-SNAP condensates for single compound test reactions were in agreement with the measured fraction of Rhodamine B outside of the FUS-SNAP condensates in the presence of the mixture of four compounds. This demonstrates that the mass spectrometry-based technique can be used for multiplexed assays, which, *e.g.*, enables higher throughput and/or the study of more complex systems.

Example 7

Multiplexing using the mass spectrometry-based assay

[0388] This example further demonstrates the use of the mass spectrometry-based assay disclosed in Example 4 for studying systems comprising a plurality of test compounds.

Methods

[0389] FUS-SNAP protein reactions and reference reactions were prepared as described in Example 4. For test reactions, Fluorescein and Rhodamine 101 were assayed individually, and three sets of pooled compounds were also assayed. The first pool had 10 compounds (and included Fluorescein), the second pool had 15 compounds (and included Fluorescein and

Rhodamine 101), and the third pool had 20 compounds (and included Fluorescein and Rhodamine 101). All samples were prepared and assayed in triplicate. All samples were analyzed using the mass spectrometry-based assay described in Example 4.

Results

[0390] As shown in **FIG. 10**, the described assay, which, *e.g.*, measures the depletion of the test compound in the supernatant due to the presence of the condensate, is capable of measuring the partition characteristic of an individual compound independent of compound pool size. These results demonstrate that compounds can be mixed in large pool sizes and still allow for measurements comparable to individually assessed single compound partition characteristic measurements. The mass spectrometry-based assay described herein thus enable high-throughput screening.

CLAIMS

What is claimed is:

1. A method of determining a partition characteristic of a small molecule test compound in a target condensate, the method comprising:
   (a) combining the small molecule test compound and a composition comprising the target condensate and an extra-condensate solution;
   (b) separating the target condensate from the extra-condensate solution; and
   (c) determining, by mass spectrometry, the amount of the small molecule test compound in the target condensate based on the amount of the test compound in the extra-condensate solution,

   thereby determining the partition characteristic of the small molecule test compound in the target condensate.

2. The method of claim 1, further comprising causing the formation of the target condensate prior to step (a).

3. The method of claim 1 or 2, wherein
   (i) the target condensate is formed in the presence of the small molecule test compound; or
   (ii) the target condensate is formed in the absence of the small molecule test compound.

4. The method of claim 3, wherein when the target condensate is formed in the presence of the small molecule test compound, the method comprises combining the small molecule test compound and a precursor composition comprising one or more precursor molecules prior to or with formation of the target condensate.

5. The method of any one of claims 1-4, further comprising determining the ratio of the amount of the small molecule test compound in the target condensate and the amount of the small molecule test compound in the extra-condensate solution.

6. The method of any one of claims 1-5, further comprising characterizing the target condensate by identifying one or more macromolecules comprised therein.

7. The method of claim 6, further comprising determining the amount of the one or more macromolecules in the target condensate.

8. The method of any one of claims 1-7, wherein:
(i) the method is a cell-based assay, and the composition comprises a cell; and/or
(ii) the target condensate is a cellular condensate that is determined to be dysregulated; and/or
(iii) the target condensate is in a cell.

9. The method of claim 8, wherein when the target condensate is in a cell, the cell has one or more features of a neurodegenerative or proliferative disease.

10. The method of any one of claims 1-7, wherein the method is a cell-free assay method.

11. The method of any one of claims 1-10, wherein the target condensate is a cleavage body, a P-granule, a histone locus body, a multivesicular body, a neuronal RNA granule, a nuclear gem, a nuclear pore, a nuclear speckle, a nuclear stress body, a nucleolus, an Oct1/PTF/transcription (OPT) domain, a paraspeckle, a perinucleolar compartment, a PML nuclear body, a PML oncogenic domain, a polycomb body, a processing body, a Sam68 nuclear body, a stress granule, or a splicing speckle.

12. A method of determining a partition characteristic of a small molecule test compound in a target condensate, the method comprising:
    (a) combining the small molecule test compound and a composition comprising the target condensate and an extra-condensate solution;
    (b) obtaining a reference control;
    (c) measuring a MS signal of the small molecule test compound in the extra-condensate solution, or a portion thereof, using an MS technique;
    (d) measuring a MS signal of the small molecule test compound in the reference control, or a portion thereof, using an MS technique; and
    (e) comparing the MS signal of the small molecule test compound from the extra-condensate solution and the MS signal of the small molecule test compound from the reference control,

thereby determining the partition characteristic of the small molecule test compound in the target condensate.

13. The method of any one of claims 1-12, further comprising determining the partition characteristics of a plurality of small molecule test compounds in the target condensate, the method comprising performing the method of any one of claims 1-12 for each of the plurality of small molecule test compounds.

14. The method of claim 13, further comprising comparing the partition characteristics of a subset or all of the plurality of test compounds in the target condensate.

15. The method of claim 14, further comprising identifying small molecule test compounds that have the same or similar partition characteristics in the target condensate.

16. The method of claim 15, further comprising identifying a characteristic that a subset or all of the identified small molecule test compounds have in common in addition to the same or similar partition characteristics.

17. The method of claim 16, wherein the characteristic is a chemical structure or motif.

18. The method of any one of claims 1-17, wherein the target condensate comprises a precursor molecule, and the method further comprises determining an amount of the small molecule test compound that associates with the precursor molecule.

19. A method of designing a small molecule compound with a desired partition characteristic into or out of a target condensate, the method comprising:
(a) identifying a characteristic that a subset or all of the identified small molecule test compounds have in common in addition to the same or similar partition characteristics by performing the method of claims 16 or 17;
(b) designing:
(i) the small molecule compound that comprises the identified characteristic; or
(ii) the small molecule compound that does not comprise the identified characteristic,
thereby designing the small molecule compound with the desired partition characteristic into or out of the target condensate.

20. The method of claim 19, wherein the method comprises modifying a precursor compound of the small molecule compound by attaching a moiety to or removing a moiety from the precursor compound, wherein the moiety comprises the identified characteristic corresponding to the desired partition characteristic.