ability of that molecule to act in a certain manner. In the budding field of biological condensates, there is much that is still unknown about mechanisms governing condensate partitioning and the effect of a compound on a condensate, much less techniques for studying the interactions between a compound (or a portion thereof) and a condensate (or a component thereof).

SUMMARY

[0005] The present disclosure in one aspect provides a method of identifying one or more interactions of a test compound, or a portion thereof, and a target condensate, or a component thereof, the method comprising: obtaining two or more of: (i) a partition characteristic of the test compound, or the portion thereof, for the target condensate; (ii) a binding affinity characteristic of the test compound, or the portion thereof, for the component of the target condensate in a light phase; or (iii) a phase boundary characteristic of the component of the target condensate in the presence of the test compound, or the portion thereof; and identifying the one or more interactions of the test compound, or the portion thereof, and the target condensate, or the component thereof, based on comparing two or more of (i), (ii), and (iii) to identify the one or more interactions. In some examples, identifying the one or more interactions of the test compound, or the portion thereof, and the target condensate, or the component thereof, is based on comparing the partition characteristic of the test compound, or the portion thereof, for the target condensate; and the binding affinity characteristic of the test compound, or the portion thereof, for the component of the target condensate in the light phase. In some examples, identifying the one or more interactions of the test compound, or the portion thereof, and the target condensate, or the component thereof, is based on comparing the partition characteristic of the test compound, or the portion thereof, for the target condensate; and the phase boundary characteristic of the component of the target condensate in the presence of the test compound, or the portion thereof. In some examples, identifying the one or more interactions of the test compound, or the portion thereof, and the target condensate, or the component thereof, is based on comparing the binding affinity characteristic of the test compound, or the portion thereof, for the component of the target condensate in the light phase; and the phase boundary characteristic of the component of the target condensate in the presence of the test compound, or the portion thereof. In some examples, identifying the one or more interactions of

the test compound, or the portion thereof, and the target condensate, or the component thereof, is based on comparing the partition characteristic of the test compound, or the portion thereof, for the target condensate; the binding affinity characteristic of the test compound, or the portion thereof, for the component of the target condensate in the light phase; and the phase boundary characteristic of the component of the target condensate in the presence of the test compound, or the portion thereof.

[0006] In some examples according to any one of the methods described above, the interaction of the test compound, or the portion thereof, and the target condensate, or the component thereof, is selected from the group consisting of: (1) a preferential association of the test compound, or the portion thereof, and the component of the target condensate in the light phase as compared to a dense phase; (2) a preferential association of the test compound, or the portion thereof, and the component of the target condensate in the dense phase as compared to the light phase; (3) a preferential solubility of the test compound, or the portion thereof, in the dense phase of the target condensate as compared to the light phase; (4) a preferential solubility of the test compound, or the portion thereof, in the light phase as compared to the dense phase; (5) a preferential association of the test compound, or the portion thereof, and a feature in the dense phase of the target condensate as compared to the light phase; (6) an ability of the test compound, or the portion thereof, to compete with a phase-separation driving interaction for the component of the target condensate; (7) an ability of the test compound, or the portion thereof, to provide a phase-separation driving interaction for the component of the target condensate; (8) a preferential association of the test compound, or the portion thereof, and another component of the target condensate as compared to the component of the target condensate, wherein the preferential association of the test compound, or the portion thereof, and the other component of the target condensate hinders a phase-separation driving interaction for the component of the target condensate; (9) a preferential association of the test compound, or the portion thereof, and another component of the target condensate as compared to the component of the target condensate, wherein the preferential association of the test compound, or the portion thereof, and the other component of the target condensate provides a phase-separation driving interaction for the component of the target condensate; (10) a preferential association of the test compound, or the portion thereof, at a site of the component not involved in a phase-separation driving interaction as compared to a site of the component involved in a phase-

separation driving interaction; and (11) a substantially equal association of the test compound, or the portion thereof, and the component of the condensate in both the light phase and the dense phase.

[0007] In some examples according to any one of the methods described above, the partition characteristic of the test compound, or the portion thereof, for the target condensate indicates the presence or absence of partitioning of the test compound, or the portion thereof, in the target condensate. In some examples, the presence or absence of partitioning of the test compound, or the portion thereof, in the target condensate is determined based on a partition characteristic threshold value. In some examples, the presence of partitioning of the test compound, or the portion thereof, in the target condensate is determined based on having the partition characteristic of more than 1.

[0008] In some examples according to any one of the methods described above, the partition characteristic of the test compound, or the portion thereof, for the target condensate indicates the degree of partitioning of the test compound, or the portion thereof, in the target condensate.

[0009] In some examples according to any one of the methods described above, the partition characteristic of the test compound, or the portion thereof, for the target condensate is based on a ratio of the test compound, or the portion thereof, in the dense phase of the target condensate versus the test compound, or the portion thereof, in the light phase.

[0010] In some examples according to any one of the methods described above, the binding affinity characteristic of the test compound, or the portion thereof, for the component of the target condensate in the light phase indicates the presence or absence of a binding association of the test compound, or the portion thereof, and the component of the target condensate in the light phase. In some examples, the presence or absence of the binding association is determined based on a binding affinity threshold value. In some examples, the presence of the binding association of the test compound, or the portion thereof, and the component of the target condensate in the light phase is determined based on having the binding affinity (*e.g.*, K_d) of about 10 mM or less.

[0011] In some examples according to any one of the methods described above, the binding affinity characteristic of the test compound, or the portion thereof, for the component of the target condensate in the light phase indicates the degree of the binding association of the test compound, or the portion thereof, and the component of the target condensate in the light phase.

[0012] In some examples according to any one of the methods described above, the binding affinity characteristic of the test compound, or the portion thereof, for the component of the target condensate in the light phase is based on a dissociation constant (K_d) of the test compound, or the portion thereof, for the component of the target condensate in the light phase.

[0013] In some examples according to any one of the methods described above, the phase boundary characteristic of the component of the target condensate indicates the presence or absence of modulated partitioning of the component of the target condensate for the target condensate due to the presence of the test compound, or the portion thereof.

[0014] In some examples according to any one of the methods described above, the phase boundary characteristic is based on a phase diagram.

[0015] In some examples according to any one of the methods described above, identifying the one or more interactions of the test compound, or the portion thereof, and the target condensate, or the component thereof, based on comparing two or more of (i) the partition characteristic of the test compound, or the portion thereof, for the target condensate, (ii) the binding affinity characteristic of the test compound, or the portion thereof, for the component of the target condensate in the light phase, and (iii) the phase boundary characteristic of the component of the target condensate in the presence of the test compound, or the portion thereof, further comprises comparing to a reference. In some examples, the reference comprises information obtained using a reference compound regarding one or more of a partition characteristic of the reference compound for the target condensate, a binding affinity characteristic of the reference compound for the component of the target condensate in the light phase, and a phase boundary characteristic of the component of the target condensate in the presence of the reference compound. In some examples, the reference comprises information obtained using a plurality of (*e.g.*, 2, 3, 4, 5, or more) reference compounds. In some examples, the plurality of reference compounds comprises compounds in the same chemical class as the test compound. In some examples, the plurality of reference compounds comprises compounds in different chemical classes as the test compound. In some examples, the plurality of reference compounds comprises at least 5 reference compounds.

[0016] In some examples according to any one of the methods described above, further comprising obtaining a mode of binding for the test compound and the component of the target

condensate. In some examples, the mode of binding is determined via a polyphasic linkage formalism technique.

[0017] In some examples according to any one of the methods described above, further comprising measuring the partition characteristic of the test compound, or the portion thereof, for the target condensate. In some examples, measuring the partition characteristic of the test compound, or the portion thereof, for the target condensate comprises measuring the amount of the test compound, or the portion thereof, in the target condensate. In some examples, measuring the amount of the test compound, or the portion thereof, in the target condensate is determined via measuring the amount of the test compound, or the portion thereof, in an extra-condensate solution. In some examples, the partition characteristic of the test compound, or the portion thereof, for the target condensate is measured using a confocal microscopy or fluorescence spectroscopy technique. In some examples, the partition characteristic of the test compound, or the portion thereof, for the target condensate is measured by: (a) combining the test compound and a composition comprising or subjected to forming the target condensate and an extra-condensate solution; (b) obtaining a reference control; (c) measuring a mass spectrometry (MS) signal of the test compound in the extra-condensate solution, or a portion thereof, using an MS technique; (d) measuring an MS signal of the test compound in the reference control, or a portion thereof, using an MS 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.

[0018] In some examples according to any one of the methods described above, further comprising measuring the binding affinity characteristic of the test compound, or the portion thereof, for the component of the target condensate in the light phase. In some examples, measuring the binding affinity characteristic of the test compound, or the portion thereof, for the component of the condensate in the light phase comprises measuring the dissociation constant (K_d) of the test compound, or the portion thereof, for the component of the condensate in the light phase. In some examples, measuring the binding affinity characteristic of the test compound, or the portion thereof, for the component of the condensate in the light phase comprises using a MicroScale Thermophoresis (MST), isothermal titration calorimetry (ITC), surface plasmon resonance (SPR), nuclear magnetic resonance (NMR), or fluorescence polarization (FP) technique.

[0019] In some examples according to any one of the methods described above, further comprising measuring the phase boundary characteristic of the component of the target condensate due to the presence of the test compound, or the portion thereof. In some examples, the phase boundary characteristic of the component of the target condensate in the presence of the test compound, or the portion thereof, is measured using a microscopy, fluorescence spectroscopy, ultraviolet–visible (UV-Vis) spectroscopy, fluorescence recovery after photobleaching (FRAP), Static and Dynamic Light Scattering (SLS/DLS), or mass spectrometry-based technique.

[0020] In some examples according to any one of the methods described above, the phase boundary characteristic is representative of a partition characteristic of the component of the target condensate for the target condensate. In some examples, the phase boundary characteristic of the component of the target condensate in the presence of the test compound, or the portion thereof, is measured using a microscopy, fluorescence spectroscopy, ultraviolet–visible (UV-Vis) spectroscopy, fluorescence recovery after photobleaching (FRAP), Static and Dynamic Light Scattering (SLS/DLS), or mass spectrometry-based technique.

[0021] In some examples according to any one of the methods described above, the component of the target condensate is a macromolecule.

[0022] In some examples according to any one of the methods described above, the component of the target condensate comprises a polypeptide.

[0023] In some examples according to any one of the methods described above, the component of the target condensate comprises a nucleic acid.

[0024] In some examples according to any one of the methods described above, further comprising determining one or more contributing factors associated with a partition characteristic of the test compound, or the portion thereof, for a reference condensate. In some examples, the method further comprising comparing the one or more contributing factors associated with the partition characteristic of the test compound, or the portion thereof, for the target condensate with the one or more contributing factors associated with the partition characteristic of the test compound, or the portion thereof, for the reference condensate.

[0025] The present disclosure in another aspect provides a method of designing a compound having one or more desired interactions with a target condensate, or a component thereof, the

method comprising: (a) identifying one or more interactions of a candidate compound, or a portion thereof, and the target condensate, or the component thereof, according to any one of the methods described above; and (b) designing the compound based on the candidate compound, or the portion thereof, associated with the identified one or more interactions.

[0026] The present disclosure in another aspect provides a method of designing a compound having a desired interaction profile, the method comprising modifying a precursor of the compound by attaching a moiety to the precursor, wherein the moiety comprises a characteristic having one or more desired interactions with a target condensate, or a component thereof, identified according to any one of the methods described above.

[0027] 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.

[0028] All references cited herein, including patent applications and publications, are incorporated herein by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] **FIG. 1** shows an exemplary experimental strategy for identifying exemplary interactions of a test compound with a target condensate and/or a protein component thereof.

[0030] **FIG. 2** show a theoretical plot of the partition characteristic of a compound for a condensate (PC-compound) versus phase boundary characteristic of a protein component represented by the partition characteristic of the protein component of the condensate for the condensate (PC-protein).

[0031] **FIGS. 3A-3C** show exemplary measurements from an analysis of Rhodamine B (RhoB) and a condensate comprising a FUS component using the methods described herein. **FIG. 3A** shows fluorescence spectroscopy (RhoB-FL) and mass spectroscopy (RhoB-MS) analysis of partitioning of two concentrations of RhoB for FUS-SNAP condensates formed *in vitro*, as reflected by the fraction of RhoB in the light phase extra-condensate solution (supernatant). **FIG. 3B** shows a