

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

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.

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.