

CLAIMS

What is claimed is:

1. ~~A method of characterizing an agent, comprising contacting the agent with a composition comprising a condensate having at least one component, and measuring incorporation of the agent in the condensate.~~
2. ~~The method of claim 1, wherein incorporation of the agent into the condensate is detected without using a detectable tag.~~
3. ~~The method of claims 1-2, wherein incorporation of the agent into the condensate is detected using Raman spectroscopy, spectrophotometry and quantitative phase microscopy, or spin down assay.~~
4. ~~The method of claim 1, wherein the agent comprises a detectable tag.~~
5. ~~The method of claim 4, wherein the detectable tag is a fluorescent tag.~~
6. ~~The method of claims 4-5, wherein the method comprises contacting the agent having a detectable tag with the composition comprising the condensate, measuring incorporation of the agent having a detectable tag into the condensate, contacting the composition comprising the condensate and the agent having a detectable tag with a control agent not having a detectable tag, and again measuring incorporation of the agent having a detectable tag into the condensate.~~
7. ~~The method of claims 1-6, wherein the method comprises contacting the agent with a plurality of condensates having one or more different components.~~
8. ~~The method of claims 1-7, wherein the method comprises contacting the agent with a plurality of compositions each having a condensate having at least one different component.~~
9. ~~The method of claims 1-8, wherein the method comprises contacting a plurality of agents with a plurality of compositions each having condensates comprising the same components.~~
10. ~~The method of claims 1-9, wherein the least one component is a transcriptional condensate component, a heterochromatin condensate component, a component of a condensate physically associated with mRNA initiation, or a component of a condensate physically associated with mRNA elongation.~~

11. The method of claims 1-10, wherein the least one component is mediator, a mediator component, MED1, BRD4, POLII, SRSF2, FIB1, NPM1, or HP1 α .
12. The method of claims 1-11, wherein the at least one component is a component of a super-enhancer condensate, splicing speckle condensate, heterochromatin condensate, nucleolus, chromatin condensate, polycomb condensate, or DNA damage repair condensate.
13. The method of claims 1-12, wherein the component comprises an intrinsically disordered region (IDR).
14. The method of claims 1-13, wherein the component comprises a different detectable tag than the agent.
15. The method of claims 1-14, wherein the incorporation of the agent is measured relative to a control.
16. The method of claims 1-15, wherein the incorporation of a plurality of agents is measured and compared to each other.
17. The method of claims 1-16, wherein the agent is capable of binding to a target.
18. The method of claim 17, wherein the target is genomic DNA.
19. The method of claims 17-18, wherein the condensate does not comprise the target.
20. The method of claims 17-18, wherein the target is present primarily outside the condensate.
21. The method of claim 17, wherein the target is present primarily in the condensate.
22. The method of claims 17-21, wherein the target is a therapeutic target.
23. The method of claims 17-22, wherein the target is an enzyme, a receptor, a ligand, an oncogene, an oncogene product, or a transcription factor.
24. The method of claims 17-23, wherein the composition comprises the target.
25. The method of claims 1-24, wherein the relative amount of agent incorporated in the condensate, or not incorporated in the condensate, is measured.
26. The method of claims 1-25, wherein the condensate is physically associated with DNA.

- ~~27. The method of claims 1-26, wherein the condensate is in a cell.~~
~~28. The method of claim 27, wherein the cell is a diseased cell.~~
~~29. The method of claims 1-26, wherein the condensate is in vitro.~~
~~30. The method of claims 1-29, wherein the agent is a small molecule, a polypeptide, or a nucleic acid.~~
~~31. The method of claims 1-30, wherein the agent is a known chemotherapeutic agent.~~
~~32. The method of claims 1-30, wherein the agent is a candidate chemotherapeutic agent.~~

~~33.1.~~ A method of characterizing a first agent, comprising

~~i) contacting the first agent with a composition comprising a condensate having at least one component, wherein the condensate comprises contains at least a second agent before contacting with the first agent, and~~

~~ii) measuring the ability of the first agent to cause eviction of the second agent from the condensate;~~

~~wherein the eviction of the second agent from the condensate characterizes the first agent as having one or more of following properties:~~

~~a) the first agent has higher affinity for a component of the condensate than the second agent,~~

~~b) the first agent can release the second agent from the condensate, wherein the second agent does not have a target component in the condensate,~~

~~c) the first agent has similar or higher condensate partition property than the second agent, or~~

~~d) the first agent and the second agent have similar structure;~~

~~wherein the first agent and the second agent are both small molecules.~~

~~34.2.~~ The method of claim ~~133~~, wherein the first agent and/or the second agent comprises a detectable tag.

~~35.3.~~ The method of claim ~~234~~, wherein the detectable tag is a fluorescent tag.

~~4.~~ The method of claim 2 or 3, wherein the detectable tag does not affect the condensate partition property of the first agent and/or the second agent.

~~36.5.~~ The method of any one of claims 1-4~~claims 33-35~~, wherein the condensate comprises a target component~~is a target~~ for the second agent.

~~6.~~ The method of any one of claims 1-4, wherein the condensate does not comprise a target component for the second agent.

~~7.~~ The method of any one of claims 1-6, wherein the first agent is an isomer of the second agent.

~~8.~~ The method of any one of claims 1-7, wherein the composition comprising the condensate is a cellular composition comprising the condensate within a cell.

9. The method of any one of claims 1-7, wherein the composition comprising the condensate does not comprise a cell.
10. The method of any one of claims 1-9, wherein
- i) the ability of the first agent to cause eviction of the second agent from the condensate is measured by the amount of the second agent remaining in the condensate after contacting the composition with the first agent;
 - ii) the ability of the first agent to cause eviction of the second agent from the condensate is measured by the amount of the second agent outside of the condensate after contacting the composition with the first agent; or
 - iii) the ability of the first agent to cause eviction of the second agent from the condensate is measured by the ratio of the second agent inside and outside of the condensate after contacting the composition with the first agent.
11. The method of any one of claims 1-10, wherein measuring the ability of the first agent to cause eviction of the second agent from the condensate comprises use of a technique selected from one or more of Raman spectroscopy, spectrophotometry, mass spectrometry, nuclear magnetic resonance, chromatography, quantitative phase microscopy, fluorescent microscopy, and a spin down assay.
12. The method of any one of claims 1-11, wherein the composition is contacted with an increasing amount of the first agent, and the eviction of the second agent from the condensate is measured continuously or at discrete intervals.
- ~~37. A composition comprising a condensate and an agent having a therapeutic target, wherein the condensate does not comprise the therapeutic target.~~
- ~~38. The composition of claim 37, wherein the therapeutic target is genomic DNA.~~
- ~~39. A method of modulating the partitioning of a first agent into a condensate comprising coupling the first agent to a second agent, thereby modulating the partitioning of the first agent into the condensate.~~
- ~~40. The method of claim 39, wherein the condensate is selected from a super-enhancer condensate, splicing speckle condensate, heterochromatin condensate, nucleolus, chromatin condensate, polycomb condensate, or DNA damage repair condensate.~~
- ~~41. The method of claim 39 or 40, wherein the partitioning of the first agent into the condensate is increased.~~

42. The method of claim 39 or 40, wherein the partitioning of the first agent into the condensate is decreased.
43. The method of claims 39-42, wherein the therapeutic efficacy of the coupled first agent is increased as compared to uncoupled first agent.
44. The method of claims 39-43, wherein side effects of the coupled first agent are decreased as compared to uncoupled first agent.
45. A method of modulating the partitioning of an agent into a condensate comprising modifying the agent to increase or decrease the number of aromatic side chains.
46. The method of claim 45, wherein the partitioning of the modified agent into the condensate is increased as compared to partitioning of the unmodified agent into the condensate.
47. The method of claim 45, wherein the partitioning of the modified agent into the condensate is decreased as compared to partitioning of the unmodified agent into the condensate.
48. A method of screening for a candidate agent with modulated condensate partitioning comprising modifying an agent with a condensate partition coefficient and measuring the condensate partition coefficient of the modified agent, wherein if the modified agent has a different partition coefficient than the agent, then the modified agent is identified as a candidate agent with modulated condensate partitioning.
49. The method of claim 48, wherein the condensate partition coefficient of the modified agent is measured in an in vitro condensate.
50. The method of claim 48, wherein the condensate partition coefficient of the modified agent is measured in a condensate in a cell.
51. The method of claims 48-50, wherein the candidate agent is identified as an improved candidate agent if the candidate agent has increased partitioning into a condensate having a therapeutic target for the candidate agent.
52. The method of claims 48-51, wherein the candidate agent is identified as an improved candidate agent if the candidate agent has decreased partitioning into a condensate not having a therapeutic target for the candidate agent.

53. ~~The method of claims 48-52, wherein the candidate agent with modulated condensate partitioning is a chemotherapeutic agent.~~
54. ~~The method of claims 48-53, wherein the candidate agent is modified to increase or decrease to number of aromatic side chains.~~
- 55.13. ~~A method of Use of an agent in the manufacture of a medicament for reducing transcription of an oncogene, wherein the medicament comprising the agent is to be administered to contact a transcription condensate associated with the oncogene, wherein the contacting comprising modulatingmodulates the composition of, dissolvesing, or disassociatesing thea transcriptional condensate, thereby reducing transcription of the oncogene. associated with the oncogene by contacting the transcriptional condensate with an agent.~~
- 56.14. ~~The usemethod of claim 13-55, i) wherein the agent dissolves the transcriptional condensate, causes the transcriptional condensate to uncouple from genomic DNA comprising the oncogene, or evicts one or more components of the transcriptional condensate; ii) wherein the agent binds to a component of the transcriptional condensate; and/or iii) wherein the agent preferentially concentrates in the transcriptional condensate.~~
57. ~~The method of claims 55-56, wherein the agent is an inhibitor, intercalator, or cyclin dependent kinase inhibitor.~~
58. ~~The method of claims 55-57, wherein the agent binds to a component of the transcriptional condensate.~~
59. ~~The method of claims 55-58, wherein the agent preferentially concentrates in the transcriptional condensate.~~
- 60.15. ~~The usemethod of claim 13 or 14claims 55-59, wherein the transcriptional condensate is located in a cancer cell of a subject having cancer.~~
61. ~~The method of claim 60, wherein the cell is a cancer cell.~~
62. ~~The method of claims 60-5761, wherein the agent is administered to a subject having cancer.~~
63. ~~The method of claim 62, wherein the cancer is colon cancer, lymphoma, multiple myeloma, prostate cancer, or breast cancer.~~
64. ~~A method of treating a subject in need of treatment for cancer characterized by transcription of an oncogene, the method comprising administering to the subject an agent that modulates the composition of, dissolves, or disassociates a transcriptional condensate associated with the oncogene.~~
65. ~~The method of claim 64, wherein the agent is an inhibitor, intercalator, or cyclin dependent kinase inhibitor.~~

~~66. The method of claims 64-65, wherein the agent binds to a component of the transcriptional condensate.~~

~~67. The method of claims 64-66, wherein the agent preferentially concentrates in the transcriptional condensate.~~

~~68. The method of claims 64-67, wherein the cancer is colon cancer, lymphoma, multiple myeloma, prostate cancer, or breast cancer.~~

~~69. The method of claims 64-68, wherein the subject is human.~~

~~70. The method of claims 64-69, wherein the agent is administered orally, subcutaneously, topically, or intravenously to the subject.~~

~~71. The method of claims 64-70, wherein the agent is a small molecule, a polypeptide, or a nucleic acid.~~

~~72.16. A method of Use of a peptide in the manufacture of a medicament for inhibiting transcription associated with a transcriptional condensate, wherein the medicament comprising the peptide is to be administered to contact the transcriptional condensate, wherein the contacting inhibits comprising inhibiting the binding of a nuclear receptor having an LXXLL binding domain and associated with the transcriptional condensate to a cofactor having an LXXLL domain, and wherein the binding is inhibited by contacting the condensate with a peptide that binds to the LXXLL binding domain of the nuclear receptor or the LXXLL domain of the cofactor.~~

~~73.17. The usemethod of claim 1672, wherein the nuclear receptor is a nuclear hormone receptor, an Estrogen Receptor, or a Retinoic Acid Receptor-Alpha.~~

~~74.18. The usemethod of claim 16 or 17s 72-73, wherein the cofactor is MED1.~~

~~75.19. The usemethod of any one of claims 16-18elaims 72-74, wherein the transcription of an oncogene associated with the transcriptional condensate is inhibited.~~

~~76.20. The usemethod of any one of claims 16-19elaims 72-75, wherein the transcriptional condensate is located in a cancer cell of a subject having cancer.~~

~~77. The method of claim 76, wherein the cell is a cancer cell.~~

~~78. The method of claims 72-77, wherein the peptide is administered to a subject.~~

~~79. The method of claim 78, wherein the subject has cancer.~~

~~80. A method of inhibiting transcription associated with a transcriptional condensate, comprising inhibiting the binding of a nuclear receptor having an LXXLL binding domain~~

and associated with the transcriptional condensate to a cofactor having an LXXLL domain, wherein the binding is inhibited by contacting the condensate with a peptide that binds to the LXXLL domain.

81. The method of claim 80, wherein the nuclear receptor is a nuclear hormone receptor, an Estrogen Receptor, or a Retinoic Acid Receptor Alpha.

82. The method of claims 80-81, wherein the cofactor is MED1.

83. The method of claims 80-82, wherein the transcription of an oncogene is inhibited.

84. The method of claims 80-83, wherein the transcriptional condensate is located in a cell.

85. The method of claim 84, wherein the cell is a cancer cell.

86. The method of claims 80-85, wherein the peptide is administered to a subject.

87. The method of claim 86, wherein the subject has cancer.

88.21. _____ A composition comprising a cell, wherein the cell comprises having a first condensate comprising a first detectable label, and a second condensate comprising having a different a second detectable label, wherein the first condensate and the second condensate are different condensate types selected from the group consisting of a transcriptional condensate, a super-enhancer condensate, a splicing speckle condensate, a heterochromatin condensate, and/or nucleolus, and wherein the first detectable label and the second detectable label are different.

89.22. _____ The composition of claim 2188, wherein the composition further comprises an agent contacted with the cell.

90.23. _____ The composition of claim 2289, i) wherein the agent is a known therapeutic agent; or ii) wherein the agent is a candidate therapeutic agent.

91. The composition of claim 89, wherein the agent is a candidate therapeutic agent.

92. A composition comprising a first in vitro condensate, a second in vitro condensate and an agent contacted with the first and second in vitro condensate.

93. The composition of claim 92, wherein the first and second in vitro condensates are isolated from each other.

94. The composition of claims 92-93, wherein at least one of the first in vitro condensate, second in vitro condensate, and the agent comprises a detectable label.

95. ~~The composition of claims 92-94, further comprising a third in vitro condensate, and optionally a fourth in vitro condensate, each contacted with the agent.~~
96. ~~The composition of claims 92-95, wherein at least one of the in vitro condensates comprises a component of a super-enhancer condensate, splicing speckle condensate, heterochromatin condensate, nucleolus, chromatin condensate, polycomb condensate, or DNA damage repair condensate.~~
- 97.24. A method of assessing whether differential expression of one or more condensate components by a cell resistant to an agent causes or contributes to the resistance, comprising:
i) providing an agent-resistant cell, or a condensate comprising a differential amount of a condensate component or fragment thereof that is differentially expressed in an agent-resistant cell, or a condensate comprising a mutant condensate component or fragment thereof corresponding to a mutant condensate component in an agent-resistant cell, ii) contacting the agent-resistant cell or the condensate with the agent, and iii) assessing localization, concentration, and/or therapeutic activity of the agent, and/or morphology, stability, and/or dissolution of the condensate, as compared to a control.
- 98.25. The method of claim 24, wherein A method of assessing whether differential expression of one or more condensate components by a cell resistant to an agent causes or contributes to the resistance, comprising providing a the condensate is isolated from the an agent-resistance cell, contacting the condensate with the agent, and assessing localization, concentration, and/or therapeutic activity of the agent as compared to a control.
99. ~~A method of assessing whether differential expression of one or more condensate components by a cell resistant to an agent causes or contributes to the resistance, comprising providing an in vitro condensate (e.g., droplet) comprising a differential amount of a condensate component, or fragment thereof, that is differentially expressed in an agent-resistant cell, contacting the condensate with the agent, and assessing localization, concentration, and/or therapeutic activity of the agent as compared to a control.~~
100. ~~A method of assessing whether differential expression of one or more condensate components by a cell resistant to an agent causes or contributes to the resistance, comprising providing an in vitro condensate (e.g., droplet) comprising a mutant condensate component, or fragment thereof, corresponding to a mutant condensate component in an agent-resistant cell, contacting the condensate with the agent, and assessing localization, concentration, and/or therapeutic activity of the agent as compared to a control.~~
26. The method of claim 24 or 25, wherein the condensate component or fragment thereof that is differentially expressed in the agent-resistant cell, or the mutant condensate component or fragment thereof, is a target of the agent.
27. The method of claim 24 or 25, wherein the condensate component or fragment thereof that is differentially expressed in the agent-resistant cell, or the mutant condensate component or fragment thereof, is not a target of the agent.

28. The method of any one of claims 24-27, wherein the control is any of below:

- i) a corresponding cell not resistant to the agent,
- ii) a corresponding condensate not comprising a differential amount of the condensate component or fragment thereof,
- iii) a corresponding condensate not comprising the mutant condensate component or fragment thereof,
- iv) the morphology, stability, and/or dissolution of a corresponding condensate not contacted with the agent,
- v) the agent-resistant cell or the condensate contacted with a second agent.

29. The method of any one of claims 24-28, wherein the agent-resistant cell is a cancer cell.

30. The method of any one of claims 24-29, wherein the condensate component or fragment thereof is over-expressed in the agent-resistant cell, and wherein the condensate comprising the over-expressed condensate component or fragment thereof has a larger volume compared to a corresponding condensate that does not comprise the over-expressed condensate component or fragment thereof.

31. The method of any one of claims 24-30, characterizing an agent resistance condensate further comprising contacting the agent-resistant cell or the condensate with one or more a second agent,s and assessing at least one of agent localization, concentration, and/or therapeutic activity of the second agent, and/or condensate morphology, stability, and/or dissolution of the condensate.

~~102.32.~~ The method of claim ~~31~~101, wherein the second agent counters one or more effects of the resistance to the agent. ~~is contacted with a cell comprising the agent-resistant condensate.~~

~~103.33.~~ The method of claim ~~32~~101, wherein countering the one or more effects of the resistance to the agent comprises one or more of: ~~the condensate has been isolated from a cell.~~ i) the second agent reduces the size of the condensate over-expressing the condensate component or fragment thereof, ii) the second agent dissolves the condensate, iii) the second agent binds to the condensate component or fragment thereof differentially expressed in the agent-resistant cell, or the mutant condensate component or fragment thereof in the agent-resistant cell, and iv) the second agent has higher condensate partition property compared to the agent.

~~104.~~ The method of claim 101, wherein ~~condensate is an in vitro condensate (e.g., droplet).~~

~~105.~~ The method of claim 101, wherein ~~the condensate comprises a mutant form of a condensate component or fragment thereof associated with resistance to the agent.~~