

WHAT IS CLAIMED IS:

~~1. A method of producing a de-crosslinked biological sample, the method comprising:
—— (a) contacting a fixed biological sample with a substrate comprising a plurality of capture probes, wherein a capture probe comprises a capture domain; and
(b) contacting the fixed biological sample with a de-crosslinking agent, thereby producing the de-crosslinked biological sample.~~

~~2. A method of producing a de-crosslinked biological sample, the method comprising:
(a) contacting a fixed biological sample with a de-crosslinking agent, thereby producing the de-crosslinked biological sample; and
(b) contacting the de-crosslinked biological sample with a substrate comprising a plurality of capture probes, wherein a capture probe comprises a capture domain.~~

~~3. The method of claim 1 or 2, wherein the de-crosslinking agent is a compound of Formula (I) or a compound of Formula (II).~~

~~4. The method of any one of claims 1-3, wherein the de-crosslinking agent is selected from the group consisting of compounds (1) (18).~~

~~5. The method of any one of claims 1-4, wherein the de-crosslinking agent is a compound of Formula (I).~~

~~6. The method of any one of claims 1-5, wherein the de-crosslinking agent is selected from the group consisting of compounds (1) (14).~~

~~7. The method of any one of claims 1-5, wherein the de-crosslinking agent is selected from the group consisting of compounds (1) (11).~~

~~8. The method of any one of claims 1-7, wherein the de-crosslinking agent is compound (1).~~

~~9. The method of any one of claims 1-4, wherein the de-crosslinking agent is a compound of Formula (II).~~

~~10. The method of claim 9, wherein the decrosslinking agent is selected from the group consisting of compounds (15)–(18).~~

~~11. The method of any one of claims 1–10, wherein the de-crosslinking agent is applied to the fixed biological sample at a concentration of about 10 mM to about 500 mM, about 10 mM to about 100 mM, about 30 mM to about 70 mM, or about 40 mM to about 60 mM.~~

~~12. The method of any one of claims 1–11, wherein the de-crosslinking agent is contacted to the fixed biological sample at a concentration of about 50 mM.~~

~~13. The method of any one of claims 1–12, wherein the step of contacting the fixed biological sample with the de-crosslinking agent is performed for about 1 minute to about 120 minutes or about 30 minutes to about 90 minutes.~~

~~14. The method of any one of claims 1–13, wherein the step of contacting the fixed biological sample with the de-crosslinking agent is performed for about 60 minutes.~~

~~15. The method of any one of claims 1–14, wherein the step of contacting the fixed biological sample with the de-crosslinking agent is performed at a temperature of about 45 °C and about 95 °C, or about 60 °C to about 80 °C.~~

~~16. The method of any one of claims 1–15, wherein the step of contacting the fixed biological sample with the de-crosslinking agent is performed at a temperature of about 70 °C.~~

~~17. The method of any one of claims 1–16, wherein the de-crosslinking agent is present in a solution or a suspension comprising a buffer.~~

~~18. The method of claim 17, wherein the buffer is selected from the group consisting of: tris(hydroxymethyl)aminomethane (Tris), tris(hydroxymethyl)aminomethane-Ethylenediaminetetraacetic acid (TE), phosphate buffered saline (PBS), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), 2-morpholin-4-ylethanesulfonic acid (MES), and a combination thereof.~~

~~19. The method of claim 17 or 18, wherein the buffer is present in the solution or the suspension at a concentration of about 5 mM to about 50 mM or about 20 mM to about 40 mM.~~

~~20. The method of claim 17 or 18, wherein the buffer is present in the solution or the suspension at a concentration of about 30 mM.~~

~~21. The method of any one of claims 1-20, wherein the fixed biological sample is a paraffinized fixed biological sample.~~

~~22. The method of claim 21, wherein the method further comprises, prior to the step of applying the de-crosslinking agent to the paraffinized fixed biological sample, a step of: deparaffinizing the paraffinized fixed biological sample, thereby producing a de-paraffinized fixed biological sample, and rehydrating the de-paraffinized fixed biological sample.~~

~~23. The method of claim 22, wherein the step of deparaffinizing the paraffinized fixed biological sample comprises contacting the paraffinized fixed biological sample with xylene, ethanol, and water, or, sequentially, contacting the paraffinized fixed biological sample with xylene, absolute ethanol, about 96% ethanol, and about 70% ethanol.~~

~~24. The method of any one of claims 1-23, wherein the method further comprises, prior to the step of contacting the fixed biological sample with the de-crosslinking agent, a step of:~~

~~pretreating the fixed biological sample.~~

~~25. The method of claim 24, wherein the step of pretreating the fixed biological sample comprises contacting the fixed biological sample with a proteinase.~~

~~26. The method of claim 25, wherein the proteinase is a collagenase.~~

~~27. The method of claim 25 or 26, wherein the proteinase is present in a solution or suspension comprising a buffer.~~

~~28. The method of claim 27, wherein the proteinase is present in the solution or the suspension at a concentration of about 0.05 to about 0.5 U/ μ L or about 0.1 to about 0.3 U/ μ L.~~

~~29. The method of claim 27 or 28, wherein the proteinase is present in the solution or the suspension at a concentration of about 0.2 U/ μ L.~~

~~30. The method of any one of claims 27-29, wherein the solution or the suspension further comprises a detergent.~~

~~31. The method of claim 30, wherein the detergent is a non ionic detergent.~~

~~32. The method of claim 30, wherein the detergent is 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol.~~

~~33. The method of any one of claims 30-32, wherein the detergent is present in the solution or the suspension at a concentration of about 0.05% (v/v) to about 2% (v/v), or about 0.1% (v/v) to about 1% (v/v).~~

~~34. The method of any one of claims 30-32, wherein the detergent is present in the solution or the suspension at a concentration of about 0.5% (v/v).~~

~~35. The method of any one of claims 27-35, wherein the buffer is Hank's Balanced Salt Solution (HBSS) buffer.~~

~~36. The method of any one of claims 27-35, wherein the buffer is TE buffer.~~

~~37. The method of any one of claims 27-35, wherein the buffer comprises Tris, TE, PBS, HEPES, MES, or a combination thereof.~~

~~38. The method of any one of claims 27-37, wherein the solution or the suspension has a pH of about 7.0 to about 9.0 or about 7.5 to about 8.5.~~

~~39. The method of any one of claims 27-38, wherein the solution or the suspension has a pH of about 8.0.~~

~~40. The method of any one of claims 1-39, wherein the method further comprises, after the step of contacting the fixed biological sample with the de-crosslinking agent, a step of:~~

~~permeabilizing the de-crosslinked biological sample.~~

~~41. The method of any one of claims 1-40, wherein the fixed biological sample is an aldehyde-fixed biological sample.~~

~~42. The method of any one of claims 1-40, wherein the fixed biological sample is a formaldehyde-fixed biological sample.~~

~~43. The method of any one of claims 1-40, wherein the fixed biological sample is a formaldehyde-fixed paraffin-embedded (FFPE) biological sample.~~

~~44. The method of any one of claims 1-43, wherein the fixed biological sample is a fixed-tissue section.~~

~~45. The method of any one of claims 1-43, wherein the method further comprises, prior to the step of contacting the fixed biological sample with the de-crosslinking agent, a step of:~~

~~staining the fixed biological sample; and~~

~~imaging the fixed biological sample.~~

~~46. The method of any one of claims 1-43, wherein the method further comprises, after the step of contacting the fixed biological sample with the de-crosslinking agent, a step of:~~

~~staining the de-crosslinked biological sample; and~~

~~imaging the de-crosslinked biological sample.~~

~~47. The method of claim 45 or 46, wherein the staining comprises the use of a histological stain.~~

~~48. The method of claim 45 or 46, wherein the staining comprises the use of an immunological stain.~~

~~49. The method of any one of claims 1-48, wherein the substrate comprises a slide or a bead.~~

~~50. The method of any one of claims 1-49, wherein the capture probe further comprises a unique molecular identifier (UMI).~~

~~51. The method of any one of claims 1-50, wherein the capture probe comprises DNA.~~

~~52. The method of claim 51, wherein the capture domain comprises a poly(T) sequence.~~

~~53. The method of any one of claims 1-52, wherein the capture probe further comprises a spatial barcode.~~

~~54. A method of determining a location of a nucleic acid analyte in the fixed biological sample, the method comprising:~~

~~(i) producing a de-crosslinked biological sample using the method of claim 53, wherein the capture domain binds to the nucleic acid analyte of the biological sample; and~~
~~(ii) determining (I) a sequence corresponding to the nucleic acid analyte or a complement thereof, and (II) a sequence corresponding to the spatial barcode of the capture probe or a complement thereof, and using the determined sequences of (I) and (II) to determine the location of the nucleic acid analyte in the fixed biological sample.~~

~~55. The method of claim 54, wherein the nucleic acid analyte is DNA or an RNA.~~

~~56. The method of claim 55, wherein the RNA is an mRNA.~~

~~57. The method of any one of claims 54-56, wherein the method further comprises extending an end of the capture probe using the nucleic acid analyte as a template.~~

~~58. A method of determining the location of a protein analyte in the fixed biological sample, the method comprising:~~

~~(i) contacting a de-crosslinked biological sample on a substrate comprising a plurality of capture probes, wherein a capture probe comprises a capture domain and a spatial barcode, with a plurality of analyte capture agents, wherein an analyte capture agent comprises:~~

- ~~(1) an analyte binding moiety that binds specifically to the protein analyte from the fixed biological sample;~~
- ~~(2) an analyte binding moiety barcode; and~~
- ~~(3) an analyte capture sequence, wherein the analyte capture sequence binds to the capture domain of the capture probe; and~~

~~(ii) determining (I) a sequence corresponding to the analyte binding moiety barcode or a complement thereof, and (II) a sequence corresponding to the spatial barcode of the capture probe or a complement thereof, and using the determined sequences of (I) and (II) to determine the location of the protein analyte in the fixed biological sample.~~

~~59. The method of claim 58, wherein the biological sample is de-crosslinked using a de-crosslinking agent that is a compound of Formula (I) or a compound of Formula (II).~~

~~60. The method of claim 58 or 59, wherein the biological sample is de-crosslinked using a de-crosslinking agent selected from the group consisting of compounds (1)-(18).~~

~~61. The method of any one of claims 58-60, wherein the biological sample is de-crosslinked using a de-crosslinking agent that is a compound of Formula (I).~~

~~62. The method of any one of claims 58-61, wherein the biological sample is de-crosslinked using a de-crosslinking agent selected from the group consisting of compounds (1)-(14).~~

~~63. The method of any one of claims 58-62, wherein the biological sample is de-crosslinked using a de-crosslinking agent selected from the group consisting of compounds (1)-(11).~~

~~64. The method of any one of claims 58-63, wherein the biological sample is de-crosslinked using compound (1).~~

~~65. The method of claim 58 or 59, wherein the biological sample is de-crosslinked using a de-crosslinking agent that is a compound of Formula (II).~~

~~66. The method of claim 65, wherein the biological sample is de-crosslinked using a de-crosslinking agent selected from the group consisting of compounds (15)–(18).~~

~~67. The method of claim 58, wherein the method comprises, prior to (i), producing the de-crosslinked biological sample according to the method of claim 53.~~

~~68. The method of any one of claims 58–67, wherein the protein analyte is an intracellular protein, an extracellular protein, or a cell surface protein.~~

~~69. The method of any one of claims 58–68, wherein the analyte binding moiety comprises an antibody or an antigen binding domain thereof.~~

~~70. The method of any one of claims 58–69, wherein the method further comprises extending an end of the capture probe using the analyte binding moiety barcode as a template.~~

~~71. The method of any one of claims 54–70, wherein the determining of the sequences of (I) and (II) comprises sequencing of the sequences of (I) and (II).~~

~~72. The method of claim 71, wherein the sequencing is high throughput sequencing.~~

~~73. A method of producing a de-crosslinked biological sample, the method comprising:~~

~~—— (a) contacting a formaldehyde fixed paraffin embedded (FFPE) biological sample with a substrate comprising a plurality of capture probes, wherein a capture probe comprises a capture domain;~~

~~—— (b) deparaffinizing the FFPE biological sample;~~

~~—— (c) staining the FFPE biological sample with hematoxylin and eosin;~~

~~—— (d) pre-treating the FFPE biological sample with collagenase, a detergent, or collagenase or a detergent; and~~

~~—— (e) contacting the FFPE biological sample with compound (1), thereby producing the de-crosslinked biological sample.~~

~~74. The method of claim 73, wherein deparaffinizing the FFPE biological sample comprises, contacting the FFPE biological sample with xylene, absolute ethanol, about 96% ethanol, and about 70% ethanol.~~

~~75. The method of claim 73 or claim 74, wherein the FFPE sample is pre-treated with collagenase at 0.2U/ μ L in HBSS buffer for 20 minutes at 37 °C.~~

~~76. The method of any one of claims 73-75, wherein the FFPE sample is pre-treated with 0.5% of a non-ionic detergent in TE buffer at pH 8 for 20 minutes at 37 °C.~~

~~77. The method of any one of claims 73-76, wherein the FFPE sample is contacted with 50 mM compound (1) in Tris or TE buffer for 1 hour at 70 °C.~~

78. A kit for practicing any of claims 1-77 comprising:

- ~~(a) a substrate comprising a plurality of capture probes, wherein a capture probe comprises a spatial barcode and a capture domain,~~
- ~~(b) a reagent comprising one or more of compounds (1)-(18).~~

79. The kit of claim 78, wherein the compound is compound (1).

80. The kit of claim 79, further comprising:

- ~~(a) one or more polymerase enzymes,~~
- ~~(b) one or more wash buffers, and~~
- ~~(c) one or more reaction buffers.~~

1. A method of producing a de-crosslinked biological sample, the method comprising:

(a) contacting a fixed biological sample with a de-crosslinking agent of compound Formula (I) or compound of Formula (II), thereby producing the de-crosslinked biological sample; wherein the de-crosslinking is performed prior to or subsequent to contacting the fixed biological sample with a substrate comprising a plurality of capture probes, wherein a capture probe comprises a capture domain.

2. The method of claim 1, wherein the de-crosslinking agent is selected from the group consisting of compounds (1)-(18).

3. The method of claim 2, wherein the de-crosslinking agent is compound (1) or compound (8).

4. The method of any one of claims 1-3, wherein the de-crosslinking agent is applied to the fixed biological sample at a concentration of about 10 mM to about 500 mM, about 10 mM to about 100 mM, about 30 mM to about 70 mM, or about 40 mM to about 60 mM.

5. The method of any one of claims 1-4, wherein the step of contacting the fixed biological sample with the de-crosslinking agent is performed for about 1 minute to about 120 minutes or about 30 minutes to about 90 minutes.

6. The method of any one of claims 1-5, wherein the step of contacting the fixed biological sample with the de-crosslinking agent is performed at a temperature of about 45 °C and about 95 °C, or about 60 °C to about 80 °C.

7. The method of any one of claims 1-6, wherein the de-crosslinking agent is present in a solution or a suspension comprising a buffer.

8. The method of claim 7, wherein the buffer is selected from the group consisting of: tris(hydroxymethyl)aminomethane (Tris), tris(hydroxymethyl)aminomethane-Ethylenediaminetetraacetic acid (TE), phosphate-buffered saline (PBS), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), 2-morpholin-4-ylethanesulfonic acid (MES), and a combination thereof, and wherein the buffer is present in the solution or the suspension at a concentration of about 5mM to about 50mM.

9. The method of any one of claims 1-8, wherein the fixed biological sample is a de-paraffinized fixed biological sample.

10. The method of claim 9, wherein the method further comprises, prior to the step of applying the de-crosslinking agent to the de-paraffinized fixed biological sample, a step of: de-paraffinizing the paraffinized fixed biological sample, thereby producing a de-paraffinized fixed biological sample, and rehydrating the de-paraffinized fixed biological sample.

11. The method of any one of claims 1-10, wherein the method further comprises, prior to the step of contacting the fixed biological sample with the de-crosslinking agent, a step of:

pretreating the fixed biological sample.

12. The method of claim 11, wherein the step of pretreating the fixed biological sample comprises contacting the fixed biological sample with a proteinase present in a solution or suspension comprising a buffer.

13. The method of claim 12, wherein the protease is present in the solution or the suspension at a concentration of about 0.05 to about 0.5 U/ μ L or about 0.1 to about 0.3 U/ μ L.

14. The method of claim 12 or 13, wherein the solution or the suspension further comprises a detergent.

15. The method of claim 14, wherein the detergent is a non-ionic detergent.

16. The method of claim 15, wherein the detergent is 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol.

17. The method of any one of claims 14-16, wherein the detergent is present in the solution or the suspension at a concentration of about 0.05% (v/v) to about 2% (v/v), or about 0.1% (v/v) to about 1% (v/v).

18. The method of any one of claims 12-17, wherein the buffer is Hank's Balanced Salt Solution (HBSS) buffer, TE buffer, Tris, TE, PBS, HEPES, MES, or a combination thereof.

19. The method of any one of claims 12-18, wherein the solution or the suspension has a pH of about 7.0 to about 9.0 or about 7.5 to about 8.5.

20. The method of any one of claims 1-19, wherein the method further comprises, after the step of contacting the fixed biological sample with the de-crosslinking agent, a step of:

permeabilizing the de-crosslinked biological sample.

21. The method of any one of claims 1-20, wherein the fixed biological sample is a fixed tissue section selected from the group of an aldehyde-fixed tissue section, a formaldehyde-fixed tissue section or a formaldehyde-fixed paraffin-embedded (FFPE) tissue section.

22. The method of any one of claims 1-21, wherein the method further comprises staining the fixed biological sample and imaging the fixed biological sample before and/or after the step of contacting the fixed biological sample with the decrosslinking agent.

23. The method of claim 22, wherein the staining comprises the use of a histological stain or an immunological stain.

24. The method of any one of claims 1-23, wherein the substrate comprises a slide or a plurality of beads.

25. The method of any one of claims 1-24, wherein the capture probe further comprises one or more of a unique molecular identifier (UMI) and a spatial barcode.

26. The method of claim 1, wherein the capture domain comprises a poly(T) sequence.

27. A method of determining a location of a nucleic acid analyte in a fixed biological sample, the method comprising:

(i) producing a de-crosslinked biological sample using the method of claim 1,

(ii) hybridizing the capture domain of a capture probe on an array, wherein the capture probe further comprises a spatial barcode, to the nucleic acid analyte of the de-crosslinked biological sample;

(iii) extending the capture domain of the capture probe using the nucleic acid analyte as a template; and

(iv) determining (I) a sequence corresponding to the nucleic acid analyte or a complement thereof, and (II) a sequence corresponding to the spatial barcode of the capture probe or a complement thereof, and using the determined sequences of (I) and (II) to determine the location of the nucleic acid analyte in the fixed biological sample.

28. A method of determining the location of a protein analyte in a fixed biological sample, the method comprising:

(i) contacting a de-crosslinked biological sample with a substrate comprising a plurality of capture probes, wherein a capture probe comprises a capture domain and a spatial barcode, with a plurality of analyte capture agents, wherein an analyte capture agent comprises:

(1) an analyte binding moiety that binds specifically to the protein analyte from the fixed biological sample;

(2) a capture agent barcode domain comprising

(i) an analyte binding moiety barcode; and

(ii) an analyte capture sequence, wherein the analyte capture sequence is complementary to the capture domain of the capture probe; and

(ii) determining (I) a sequence corresponding to the analyte binding moiety barcode or a complement thereof, and (II) a sequence corresponding to the spatial barcode of the capture probe or a complement thereof, and using the determined sequences of (I) and (II) to determine the location of the protein analyte in the fixed biological sample.

29. The method of claim 28, wherein the biological sample is de-crosslinked using a de-crosslinking agent that is a compound of Formula (I) or a compound of Formula (II).

30. The method of claim 28 or 29, wherein the biological sample is de-crosslinked using a de-crosslinking agent selected from the group consisting of compounds (1)-(18).

31. The method of claim 28, wherein the method comprises, prior to (i), producing the de-crosslinked biological sample according to the method of claim 1.

32. The method of any one of claims 28-31, wherein the analyte-binding moiety comprises an antibody or an antigen-binding domain thereof.

33. The method of any one of claims 28-32, wherein the method further comprises extending an end of the capture probe using the capture agent barcode domain as a template.

34. A kit for practicing any of claims 1-33 comprising:

(a) a substrate comprising a plurality of capture probes, wherein a capture probe comprises a spatial barcode and a capture domain,

(b) a reagent comprising one or more of compounds (1)-(18),

(c) one or more polymerase enzymes,

(d) one or more wash buffers, and

(e) one or more reaction buffers.

35. A method of de-crosslinking a fixed biological tissue sample, the method comprising:

contacting the fixed biological tissue sample with a de-crosslinking agent, wherein at least one of the de-crosslinking agent is selected from the group consisting of: 2-amino-5-methylbenzoic acid, 2-amino-5-nitrobenzoic acid, 2-amino-5-methylbenzenesulfonic acid, 2,5-diaminobenzenesulfonic acid, 2-amino-3,5-dimethylbenzenesulfonic acid, (2-amino-5-nitrophenyl)phosphonic acid, (4-aminopyridin-3-yl)phosphonic acid, and (2-amino-5-{[2-(2-poly-ethoxy)ethyl] carbamoyl}phenyl)phosphonic acid, or a pharmaceutically acceptable salt thereof;

thereby de-crosslinking the fixed biological tissue sample.

36. The method of claim 35, wherein the fixed biological tissue sample is on a substrate.

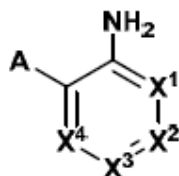
37. The method of claim 35, wherein the fixed biological tissue sample is a formalin fixed tissue sample or a de-paraffinized formalin-fixed paraffin-embedded tissue sample.

38. The method of claim 35, wherein the de-crosslinking agent is 2-amino-5-methylbenzoic acid or a pharmaceutically acceptable salt thereof.

39. The method of claim 35, wherein the de-crosslinking agent is (4-aminopyridin-3-yl)phosphonic acid or a pharmaceutically acceptable salt thereof.

40. The method of claim 35, wherein the de-crosslinking agent is contacted with the fixed biological sample at a concentration of about 10 mM to about 500 mM or about 100 mM to about 200 mM, for about 30 minutes to about 90 minutes, at a pH of about 7.0 to 9.0, and at a temperature of about 80 °C to about 95 °C.

41. A method of de-crosslinking a fixed biological tissue sample, the method comprising: contacting the fixed biological tissue sample with a de-crosslinking agent, wherein the de-crosslinking agent comprises formula (I):



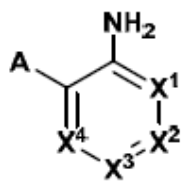
(I) or a pharmaceutically acceptable salt thereof, wherein A is P(=O)(OH)₂, and wherein each of X¹, X², X³, and X⁴ is CH; thereby de-crosslinking the fixed biological tissue sample.

42. The method of claim 41, wherein the fixed biological tissue sample is on a substrate.

43. The method of claim 41, wherein the fixed tissue sample is a de-paraffinized formalin-fixed paraffin-embedded tissue sample.

44. The method of claim 41, wherein the de-crosslinking agent is contacted with the fixed biological sample at a concentration of about 10 mM to about 500 mM or about 100 mM to about 200 mM, for about 30 minutes to about 90 minutes, at a pH of about 7.0 to about 9.0, and at a temperatures of about 80°C to about 95°C.

45. A method of de-crosslinking a fixed biological tissue sample, the method comprising: contacting the fixed biological tissue sample with a de-crosslinking agent, wherein the de-crosslinking agent comprises formula (I):



(I) or a pharmaceutically acceptable salt thereof,

wherein:

A is P(=O)(OH)₂ or C(=O)OH;

each of X¹, X², X³, and X⁴ is, independently, CH, CR^a, or N;

each R^a is, independently, C₁₋₆ alkyl, C₁₋₆ haloalkyl, NO₂, NR'R'', or

C(=O)NR'R'';

each of R' and R'' is, independently, H or optionally substituted C₁₋₆

alkyl; and

at least one of X¹, X², X³, and X⁴ is N;

thereby de-crosslinking the fixed biological tissue sample.

46. The method of claim 45, wherein the fixed biological tissue sample is on a substrate.

47. The method of claim 45, wherein the fixed tissue sample is a formalin-fixed paraffin-embedded tissue sample, and wherein the formalin-fixed paraffin-embedded tissue sample is deparaffinized.

48. The method of claim 45, wherein the de-crosslinking agent comprises compound (8), compound (9), compound (10), compound (12), compound (13), compound (14), a pharmaceutically acceptable salt thereof, or a combination thereof.

49. The method of claim 45, wherein the de-crosslinking agent is contacted with the fixed biological sample with one or more of the following conditions:

at a concentration of about 10 mM to about 500 mM or about 100 mM to about 200 mM;

for about 30 minutes to about 90 minutes;

at a pH of about 7.0 to about 9.0, about 7.0 to about 8.5, or about 7.5 to about 8.5;

and/or

at a temperature of about 80 °C to about 95 °C or about 70 °C to about 95 °C.

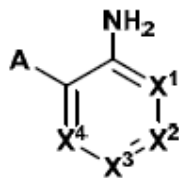
50. A method of determining a location of a nucleic acid analyte in a fixed biological sample, the method comprising:

(a) providing an array, wherein the array comprises a plurality of capture probes and wherein a capture probe of the plurality of capture probes comprises: (i) a spatial barcode and (ii) a capture domain;

(b) de-crosslinking the fixed biological sample, wherein at least one of the de-crosslinking agent comprises one or more of:

(i) 2-amino-5-methylbenzoic acid, 2-amino-5-nitrobenzoic acid, 2-amino-5-methylbenzenesulfonic acid, 2,5-diaminobenzenesulfonic acid, 2-amino-3,5-dimethylbenzenesulfonic acid, (2-amino-5-nitrophenyl)phosphonic acid, (4-aminopyridin-3-yl)phosphonic acid, and (2-amino-5-{[2-(2-poly-ethoxy)ethyl] carbamoyl}phenyl)phosphonic acid, or a pharmaceutically acceptable salt thereof; and/or

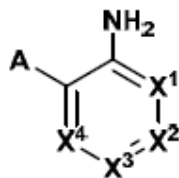
(ii) the de-crosslinking agent comprises formula (I)



(I) or a pharmaceutically acceptable salt thereof,

wherein A is P(=O)(OH)₂, and wherein each of X¹, X², X³, and X⁴ is CH; and/or

(iii) the de-crosslinking agent comprises formula (I):



(I) or a pharmaceutically acceptable salt thereof,

wherein:

A is P(=O)(OH)₂ or C(=O)OH;

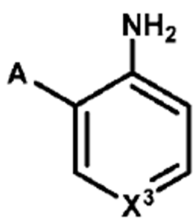
each of X¹, X², X³, and X⁴ is, independently, CH, CR^a, or N;

each R^a is, independently, C₁₋₆ alkyl, C₁₋₆ haloalkyl, NO₂, NR'R'', or C(=O)NR'R'';

each of R' and R'' is, independently, H or optionally substituted C₁₋₆ alkyl; and

at least one of X¹, X², X³, and X⁴ is N; and/or

(iv) the de-crosslinking agent comprises formula (IB):



(IB) or a pharmaceutically acceptable salt thereof,

wherein A is P(=O)(OH)₂; and

X³ is CH or N;

(c) hybridizing the nucleic acid analyte of the fixed biological sample to the capture domain; and

(d) determining (I) all or a portion of the sequence of the nucleic acid analyte or a complement thereof, and (II) the sequence of the spatial barcode or a complement thereof, and using the determined sequences of (I) and (II) to determine the location of the nucleic acid analyte in the fixed biological sample.

51. The method of claim 50, wherein the fixed biological sample is disposed on the array or a slide.

52. The method of claim 51, wherein the method further comprises aligning the slide comprising the fixed biological sample with the array comprising the plurality of capture probes, such that at least a portion of the fixed biological sample is aligned with at least a portion of the array.

53. The method of claim 50, wherein the nucleic acid analyte is mRNA or DNA.

54. The method of claim 50, wherein the method further comprises

(a) extending an end of the capture probe using the nucleic acid analyte as a template, thereby generating an extended capture probe, and

(b) generating a second strand complementary to the extended capture probe.

55. The method of claim 50, wherein the capture probe further comprises one or more functional domains, a cleavage domain, a unique molecular identifier, and combinations thereof.

56. The method of claim 50, wherein the fixed biological sample is a fixed tissue section and wherein the tissue section is an aldehyde-fixed tissue section or a formaldehyde-fixed tissue section, or a deparaffinized formalin-fixed paraffin-embedded tissue section.

57. The method of claim 50, wherein the de-crosslinking agent is contacted with the fixed biological sample with one or more of the following conditions:

at a concentration of the de-crosslinking agent from about 10 mM to about 500 mM;

for about 30 minutes to about 90 minutes;

at a pH of about 7.0 to about 9.0; and/or

at a temperature of about 80 °C to about 95 °C.

58. The method of claim 50, wherein the de-crosslinking agent comprises Formula IB or a pharmaceutically acceptable salt thereof.

59. The method of claim 50, wherein the de-crosslinking agent is (4-aminopyridin-3-yl)phosphonic acid or a pharmaceutically acceptable salt thereof.