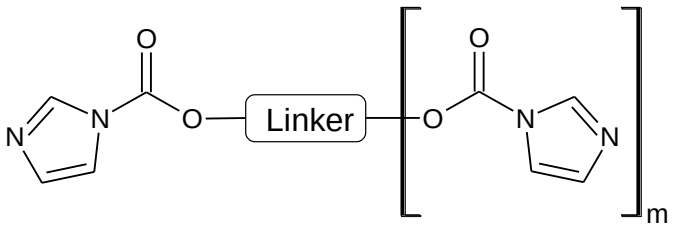
[0005] The problem of biological sample instability can be mitigated by fixing the sample using standard methods such as cryopreservation, dehydration (e.g., in methanol), high-salt storage (e.g., using RNAssist or RNAlater), and/or chemical fixing agents that create covalent crosslinks (e.g., paraformaldehyde or DSP). The ability to use such a fixed biological sample in an assay, particularly a partition-based assay, requires that the fixed biological sample can be rapidly and efficiently un-fixed, so that the relevant assay can be carried out before sample degradation occurs.

[0006] There remains a need for compositions and methods that provide reversible fixation of biological samples in a manner compatible with partition-based assays (e.g., microfluidic droplet-based assays or well-based assays).

SUMMARY

[0007] The present disclosure provides compositions and methods that allow for the reversible fixation of biological samples, and the use of such compositions and methods in partition-based assays (e.g., well- or droplet-based assays), such as gene expression profiling assays.

[0007a] In an aspect of the present disclosure, there is provided a method for preparing a biological sample comprising contacting the sample with a first fixing reagent composition comprising a compound of formula (I)



(I)

wherein, “Linker” comprises an ethylene glycol moiety, and/or a linear or branched alkane moiety of 2-24 carbons; and m is 1 to 12;

optionally, wherein:

i) m = 1;

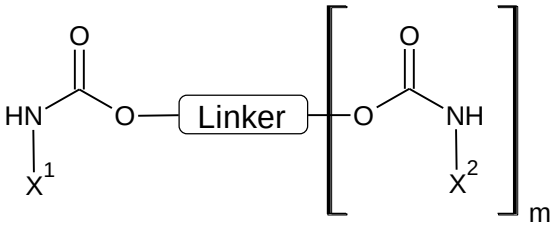
ii) the “Linker” comprises a linear alkane moiety of 2-24 carbons;

iii) the “Linker” comprises an ethylene glycol moiety;

iv) the “Linker” comprises a disulfide bond; and/or

v) the “Linker” comprises a branched alkane moiety.

[0007b] In another aspect of the present disclosure, there is provided a composition comprising a fixed biological sample, wherein the sample comprises crosslinked biomolecules of formula (**Ia**)



(Ia)

wherein,

X^1 and X^2 are amine-bearing moieties of the same or different biomolecules of the sample; and

"Linker" comprises an ethylene glycol moiety, and/or a linear or branched alkane moiety of 2-24 carbons; and m is 1 to 12;

optionally, wherein:

vi) m = 1;

vii) the "Linker" comprises a linear alkane moiety of 2-24 carbons;

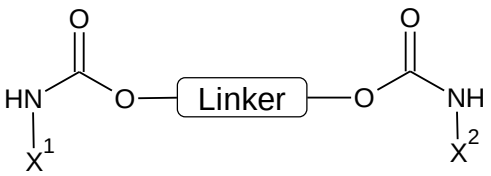
viii) the "Linker" comprises an ethylene glycol moiety;

ix) the "Linker" comprises a disulfide bond; and/or

x) the "Linker" comprises a branched alkane moiety.

[0007c] In a further aspect of the present disclosure, there is provided an assay method comprising:

(a) generating a partition comprising a fixed biological sample, and assay reagents, wherein the fixed sample comprises crosslinks of formula (**Ia**)



(Ia)

wherein,

X^1 and X^2 are amine-bearing moieties of the same or different biomolecules of the sample;

"Linker" comprises an ethylene glycol moiety, and/or a linear or branched alkane moiety of 2-24 carbons; and m is 1 to 12;

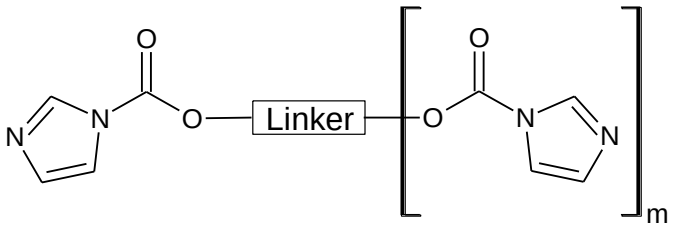
and

(b) detecting analytes from the reaction of the assay reagents and the un-fixed biological sample.

[0007d] In another aspect of the present disclosure, there is provided a kit comprising:

assay reagents; and

a fixing reagent composition comprising a compound of formula (I)

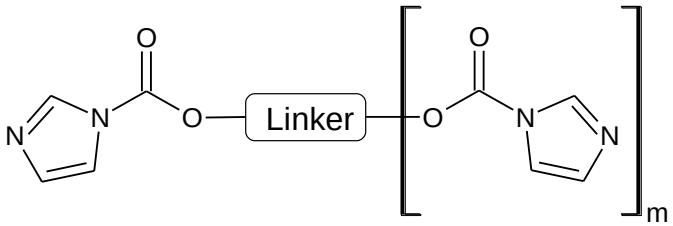


(I)

wherein,

"Linker" comprises an ethylene glycol moiety, and/or a linear or branched alkane moiety of 2-24 carbons; and m is 1 to 12.

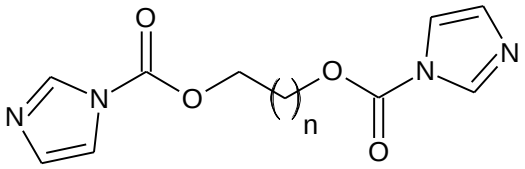
[0008] In at least one example, the present disclosure provides a method for preparing a biological sample comprising contacting the sample with a first fixing reagent composition comprising a compound of formula (I)



(I)

[0009] wherein, the "Linker" comprises an ethylene glycol moiety, and/or a linear or branched alkane moiety of 2-24 carbons; and m is 1 to 12; optionally, wherein m = 1. In at least one example, the "Linker" comprises: (a) a linear alkane moiety of 2-24 carbons; (b) an ethylene glycol moiety; (c) a disulfide bond; and/or (d) a branched alkane moiety.

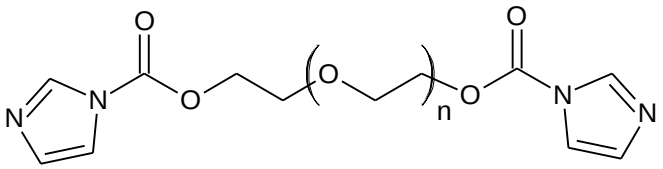
[0010] In at least one example of the method the compound of formula (I) is a compound of formula (II)



(II)

wherein n is 1 to 13.

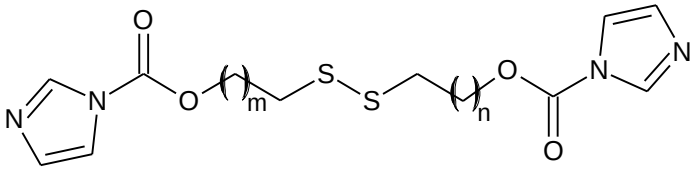
[0011] In at least one example of the method the compound of formula (I) is a compound of formula (III)



(III)

wherein n is 1 to 12.

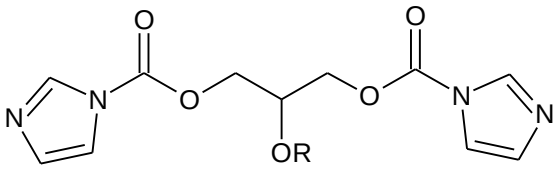
[0012] In at least one example of the method the compound of formula (I) is a compound of formula (IV)



(IV)

wherein m is 1 to 13, and n is 1 to 13.

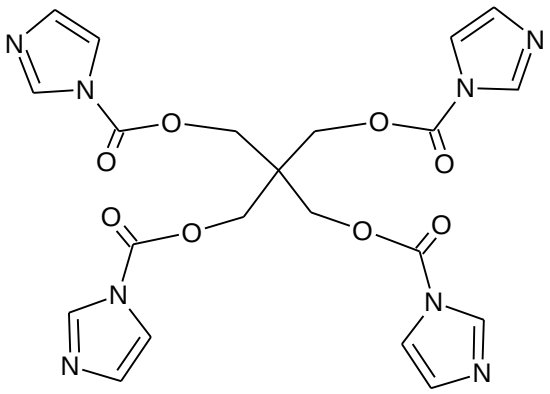
[0013] In at least one example of the method the compound of formula (I) is a compound of formula (**V**)



(V)

wherein R is selected from -H, -O(CO)-CH_3, and -O(CO)-imidazole.

[0014] In at least one example of the method the compound of formula (I) is compound (**6**)



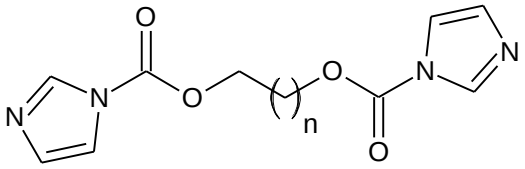
(**6**)

[0015] In at least one example of the method, the first fixing reagent composition comprises: (a) the compound of formula (I) at a concentration of 50 mM or less, 25 mM or less, 15 mM or less, 10 mM or less, or 5 mM or less; and/or (b) DMSO at a concentration of 5% or less, 2.5% or less, or 1.5% or less.

[0016] In at least one example of the method, contacting with the first fixing reagent composition at room temperature (RT) is for 3 h or less, 60 min or less, 30 min or less, 15 min or less, or 5 min or less.

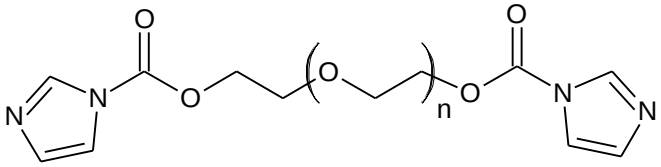
[0017] In at least one example the method further comprises contacting the sample with a second fixing reagent composition; optionally, wherein the second fixing reagent composition comprises paraformaldehyde; optionally, wherein the paraformaldehyde concentration is 1% or less.

[0018] In at least one example of the method, the first fixing reagent composition comprises a compound of formula (II)



(II)

wherein n is 1 to 13; and the second fixing reagent composition comprises a compound of formula (III):



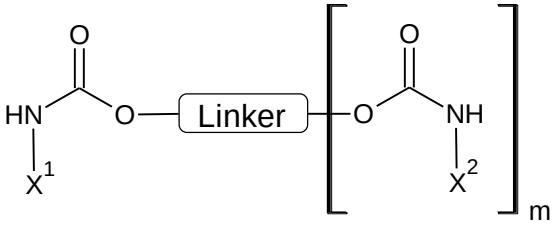
(III)

wherein n is 1 to 12.

[0019] In at least one example of the method, the first fixing reagent composition comprises a compound of formula (III), wherein n is 1 to 12; and the second fixing reagent composition comprises a compound of formula (II), wherein n is 1 to 13.

[0020] In at least one example of the method, the method further comprises contacting the sample with an unfixing agent; optionally, wherein the unfixing agent comprises: (a) a compound capable of cleaving a carbamate bond; optionally, wherein the compound capable of cleaving a carbamate bond selected from DETA, EDA, hydrazine monohydrate, a carboxyesterase, or a combination thereof; (b) a compound capable of cleaving a disulfide bond; optionally, wherein the compound capable of cleaving a disulfide bond is DTT; and/or (c) a compound capable of reversing paraformaldehyde fixation.

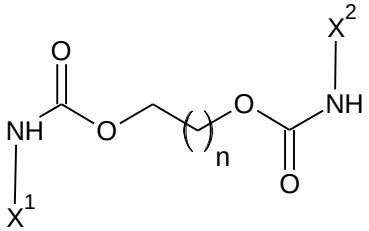
[0021] In at least one example, the present disclosure also provides a composition comprising a fixed biological sample, wherein the sample comprises crosslinked biomolecules of formula (**Ia**)



(Ia)

wherein, X^1 and X^2 are amine-bearing moieties of the same or different biomolecules of the sample; and "Linker" comprises an ethylene glycol moiety, and/or a linear or branched alkane moiety of 2-24 carbons; and m is 1 to 12. In at least one example, the "Linker" comprises: (a) a linear alkane moiety of 2-24 carbons; (b) an ethylene glycol moiety; (c) a disulfide bond; and/or (d) a branched alkane moiety.

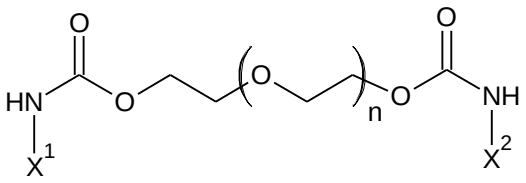
[0022] In at least one example of the composition, the fixed biological sample comprises crosslinked biomolecules of formula (**IIa**)



(IIa)

wherein, n is 1 to 13.

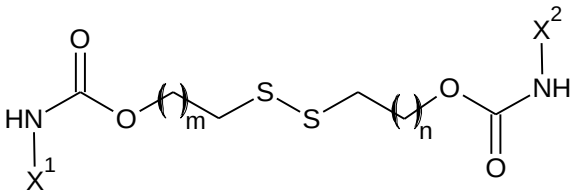
[0023] In at least one example of the composition, the fixed biological sample comprises crosslinked biomolecules of formula (**IIIa**)



(IIIa)

wherein, n is 1 to 12.

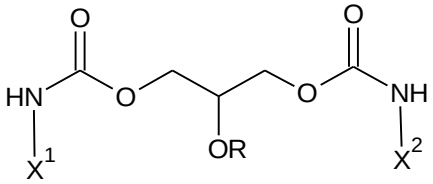
[0024] In at least one example of the composition, the fixed biological sample comprises crosslinked biomolecules of formula (**IVa**)



(IVa)

wherein m is 1 to 13, and n is 1 to 13.

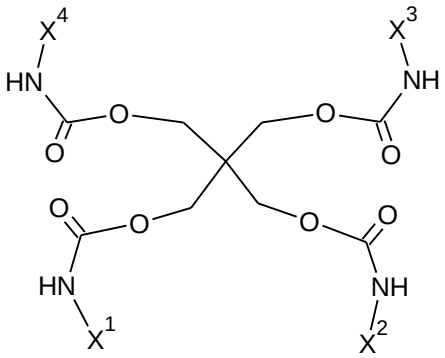
[0025] In at least one example of the composition, the fixed biological sample comprises crosslinked biomolecules of formula (**Va**)



(Va)

wherein R is selected from -H, -O(CO)-CH_3, and -O(CO)-imidazole.

[0026] In at least one example of the composition, the fixed biological sample comprises crosslinked biomolecules of formula (**VIa**)



(**VIa**).

[0027] In at least one example of the composition, the fixed biological sample is derived from a tissue sample, a biopsy sample, or a blood sample; optionally, wherein the fixed biological sample is a single cell.

[0028] In at least one example of the composition, the fixed biological sample has been fixed with a fixing reagent composition comprising a compound of any one of formulas (I), (II), (III), (IV), (V), or compounds (**6**).

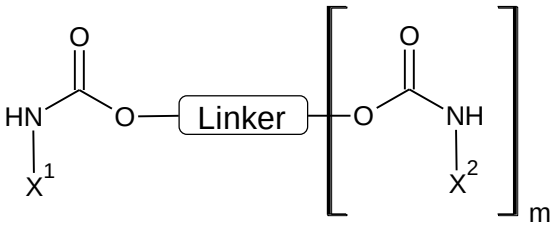
[0029] In at least one example of the composition, the fixed biological sample is provided in a discrete partition (e.g., provided or encapsulated in a discrete droplet or provided in a well) with a lysis and/or an unfixing agent; optionally, wherein the unfixing agent comprises a compound capable of cleaving a carbamate bond; optionally, wherein the compound capable of cleaving a carbamate bond selected from DETA, EDA, hydrazine monohydrate, a carboxyesterase, or a combination thereof.

[0030] In at least one example of the composition, the fixed biological sample is provided in a discrete partition (e.g., provided or encapsulated in a discrete droplet or provided in a well), wherein the discrete partition further comprises a support (e.g., a bead); optionally, wherein the unfixing agent is contained in a support (e.g., a bead).

[0031] In at least one example of the composition, the fixed biological sample is provided in a discrete partition (e.g., provided or encapsulated in a discrete droplet or provided in a discrete well), wherein the discrete partition further comprises assay reagents; optionally, wherein the assay reagents are provided as part of a support (e.g., a bead) or contained in a support (e.g., a bead).

[0032] In at least one example of the composition, the fixed biological sample is provided in a discrete partition (e.g., provided or encapsulated in a discrete droplet or provided in a discrete well), wherein the discrete partition further comprises a barcode optionally, wherein the barcode is contained as part of a support (e.g., a bead).

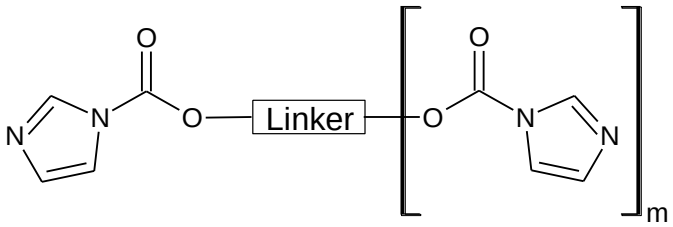
[0033] In at least one example, the present disclosure also provides an assay method comprising: (a) generating a discrete partition comprising a fixed biological sample (e.g., a droplet comprising or encapsulating a fixed biological sample), a lysis and/or an unfixing agent, and assay reagents, wherein the fixed sample comprises crosslinks of formula (**Ia**)



(Ia)

wherein, X^1 and X^2 are amine-bearing moieties of the same or different biomolecules of the sample; the "Linker" comprises an ethylene glycol moiety, and/or a linear or branched alkane moiety of 2-24 carbons; and m is 1 to 12; the unfixing agent comprises a compound capable of cleaving a carbamate bond; optionally, wherein the compound capable of cleaving a carbamate bond selected from DETA, EDA, hydrazine monohydrate, a carboxyesterase, or a combination thereof; and (b) detecting analytes from the reaction of the assay reagents and the un-fixed biological sample.

[0034] In at least one example, the present disclosure also provides a kit comprising: assay reagents; and a fixing reagent composition comprising a compound of formula (I)



(I)

wherein, the "Linker" comprises an ethylene glycol moiety, and/or a linear or branched alkane moiety of 2-24 carbons; and m is 1 to 12. In at least one example, the "Linker" comprises: (a) a linear alkane moiety of 2-24 carbons; (b) an ethylene glycol moiety; (c) a disulfide bond; and/or (d) a branched alkane moiety. In at least one example, the fixing reagent composition comprises a compound of any one of formulas (II), (III), (IV), (V), or compound (**6**).

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] A better understanding of the novel features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings (also "Figure" and "FIG." herein), of which:

[0036] FIG. 1 shows an example of a microfluidic channel structure for partitioning individual biological particles.

[0037] FIG. 2 shows an example of a microfluidic channel structure for delivering barcode carrying beads to droplets.

[0038] FIG. 3 shows an example of a microfluidic channel structure for co-partitioning biological particles and reagents.

[0039] FIG. 4 shows an example of a microfluidic channel structure for the controlled partitioning of beads into discrete droplets.