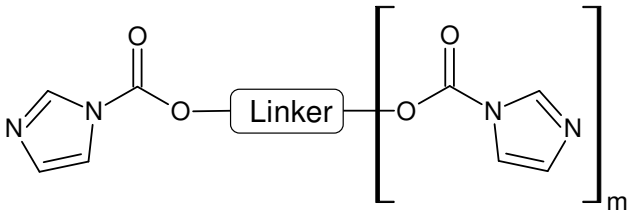
[0005] The problem of biological sample instability can be mitigated by fixing the sample using standard methods such as cryopreservation, dehydration (e.g., in methanol), high-salt storage (e.g., using RNAssist or RNAlater), and/or chemical fixing agents that create covalent crosslinks (e.g., paraformaldehyde 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.

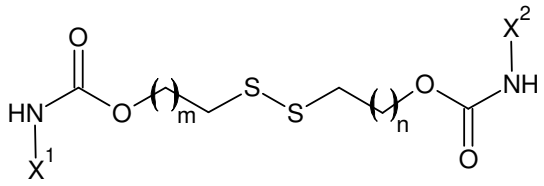
[0007a] In an aspect of the present disclosure, there is provided a compound of formula (**I**):



(I)

wherein the "Linker" comprises a linear alkane moiety of 2-24 carbons and a disulfide bond; and m is 1 to 12.

[0007b] In an aspect of the present disclosure, there is provided a composition comprising a fixed biological sample, wherein the sample comprises a compound of formula (**IVa**):



(IVa)

wherein:

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

m is 1 to 13 and n is 1 to 13;

optionally, wherein m=1 and n=1.

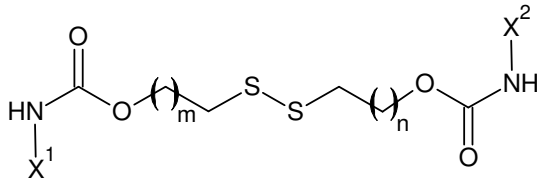
[0007c] In an aspect of the present disclosure, there is provided a method for preparing a biological sample, comprising contacting the biological sample with a first fixing reagent composition comprising a compound of formula (**I**) as defined herein or a compound of formula (**IV**) as defined herein; optionally, wherein the compound of formula (**IV**) is selected from compounds (**4a**) – (**4d**) as defined herein, or wherein the compound of formula (**IV**) is compound (**4a**).

[0007d] In an aspect of the present disclosure, there is provided a composition comprising a biological sample, wherein the biological sample is fixed with a compound as disclosed herein, wherein 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.

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

(a) generating a fixed biological sample; optionally, wherein the fixed biological sample is a single cell in a partition;

(b) contacting the fixed biological sample with reagents, wherein the fixed biological sample comprises crosslinks of formula (**IVa**)



(IVa)

wherein,

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

and m is 1 to 13 and n is 1 to 13; optionally wherein m=1 and n=1; and

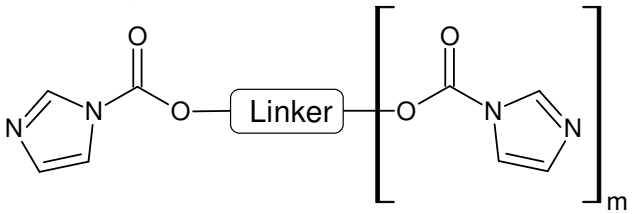
(c) detecting analytes from the reaction of the reagents and the un-fixed biological sample.

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

reagents; and

a fixing reagent composition comprising a compound of formula (**I**) or (**IV**) as defined herein.

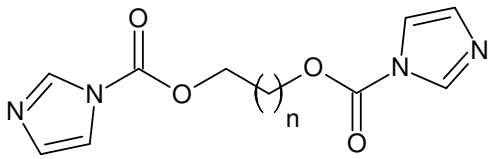
[0008] In at least one example, the present disclosure provides a method for preparing a biological sample comprising contacting the sample with a first fixing reagent composition comprising a compound of formula (I)



(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.

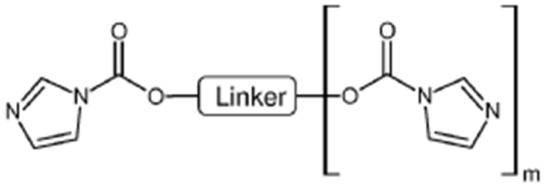
[0010] In at least one example of the method the compound of formula (I) is a compound of formula (II)



CLAIMS

What is claimed is:

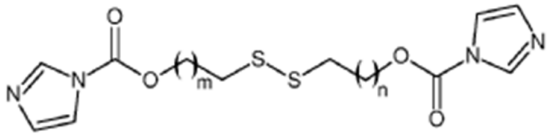
1. A compound of formula (**I**):



(I)

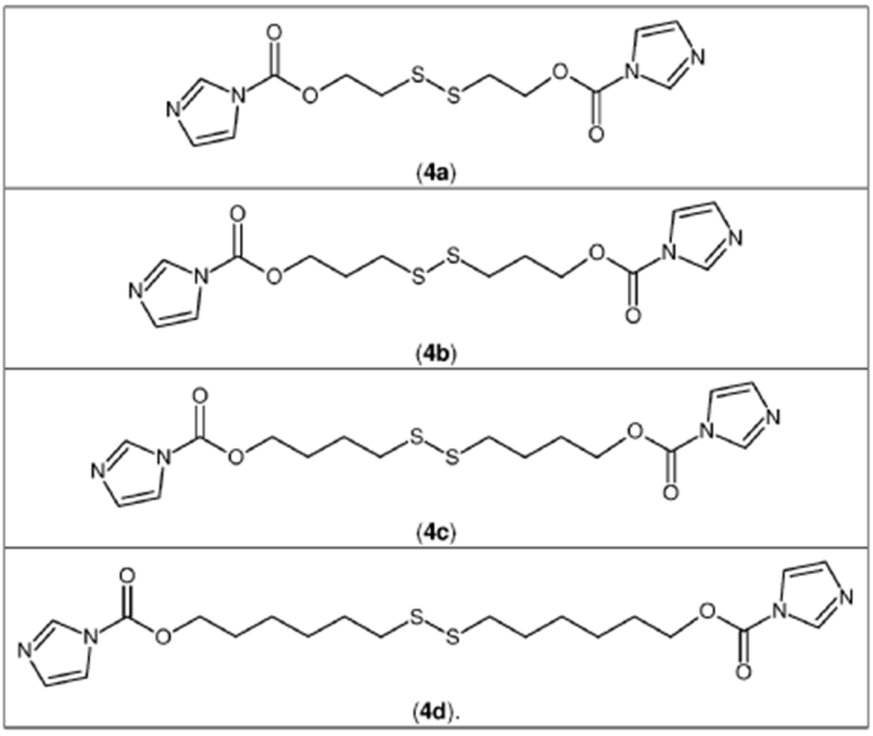
wherein the "Linker" comprises a linear alkane moiety of 2-24 carbons and a disulfide bond; and m is 1 to 12.

2. The compound of claim 1, wherein the compound of formula (**I**) is a compound of formula (**IV**):



(IV)

wherein m is 1 to 13, and n is 1 to 13; optionally, wherein the compound of formula (**IV**) is selected from compounds (**4a**) – (**4d**):



(4a)

(4b)

(4c)

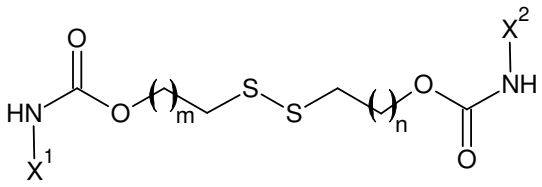
(4d).

;

optionally, wherein the compound of formula (**IV**) is compound (**4a**).

3. The compound of claims 1 or 2, wherein the compound is compound (**4a**).

4. A composition comprising a fixed biological sample, wherein the sample comprises a compound of formula (**IVa**):



(IVa)

wherein:

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

m is 1 to 13 and n is 1 to 13;

optionally, wherein m=1 and n=1.

5. A method for preparing a biological sample, comprising contacting the biological sample with a first fixing reagent composition comprising a compound of formula (**I**) as defined in claim 1 or a compound of formula (**IV**) as defined in claim 2; optionally, wherein the compound of formula (**IV**) is selected from compounds (**4a**) – (**4d**) as defined in claim 2, or wherein the compound of formula (**IV**) is compound (**4a**).

6. The method of claim 5, wherein:

(a) the first fixing reagent composition comprises the compound of formula (**I**) of (**IV**) 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) the first fixing reagent composition comprises the compound of formula (**I**) or (**IV**) and further comprises DMSO at a concentration of 5% or less, 2.5% or less, or 1.5% or less.

7. The method of claim 5 or 6, wherein 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.

8. The method of any one of claims 5-7, wherein:

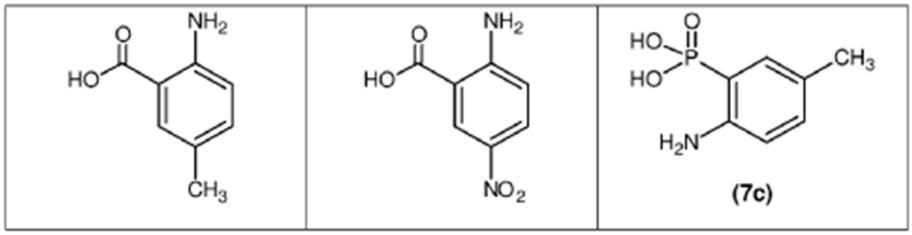
(a) the method further comprises contacting the biological sample with a second fixing reagent composition; optionally, wherein the second fixing reagent composition comprises paraformaldehyde; optionally, wherein the paraformaldehyde concentration is 1% or less; and/or

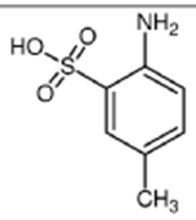
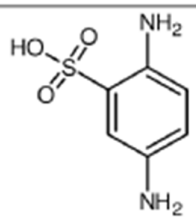
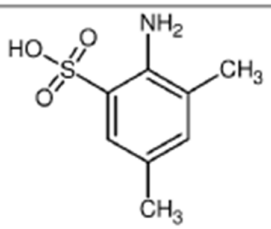
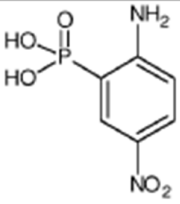
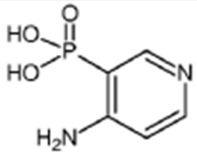
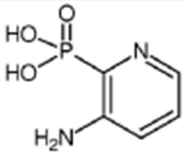
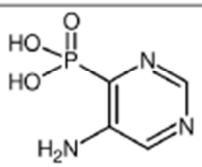
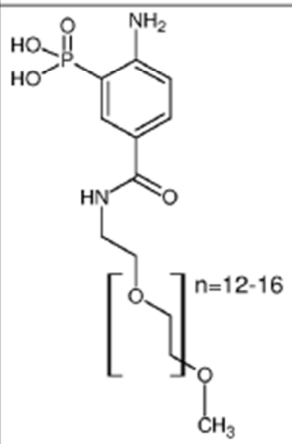
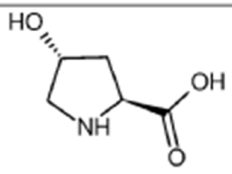
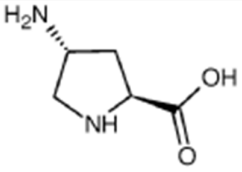
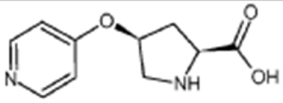
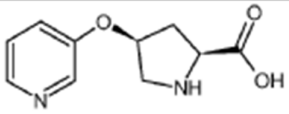
(b) the method further comprises contacting the biological sample with a lysis and/or an unfixing agent; optionally, wherein the unfixing agent comprises:

i) a compound capable of cleaving a carbamate bond; optionally, wherein the compound capable of cleaving a carbamate bond is selected from DETA, EDA, hydrazine monohydrate, a carboxyesterase, or a combination thereof;

ii) a compound capable of cleaving a disulfide bond; optionally, wherein the compound capable of cleaving a disulfide bond is DTT; and/or

iii) a compound capable of reversing paraformaldehyde fixation; optionally, wherein the compound capable of reversing paraformaldehyde fixation is selected from any one of compounds (**7a**) – (**7o**)



(7a)	(7b)	
 (7d)	 (7e)	 (7f)
 (7g)	 (7h)	 (7i)
 (7j)	 (7k)	 (7l)
 (7m)	 (7n)	 (7o).

9. A composition comprising a biological sample, wherein the biological sample is fixed with a compound of any one of claims 1-3, wherein 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.

10. The composition of claim 9, wherein the fixed biological sample is a single cell; optionally in a partition.

11. The composition of claim 10, wherein the partition is a discrete droplet; optionally, wherein the partition further comprises:

(a) a lysis agent;

(b) an unfixing agent; optionally, wherein:

(i) the unfixing agent comprises a compound capable of cleaving a carbamate bond; optionally, wherein the compound capable of cleaving a carbamate bond is selected from DETA, EDA, hydrazine monohydrate, a carboxyesterase, or a combination thereof; and/or

(ii) the unfixing agent comprises a compound capable of cleaving a disulfide bond; optionally, wherein the compound capable of cleaving a disulfide bond is DTT;

(c) a bead; optionally, comprising a barcode; and/or

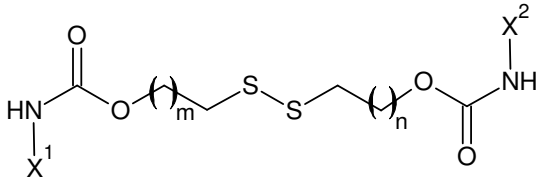
(d) assay reagents.

12. The composition of claim 11, wherein the discrete droplet is an aqueous in oil droplet.

13. A method comprising:

(a) generating a fixed biological sample; optionally, wherein the fixed biological sample is a single cell in a partition;

(b) contacting the fixed biological sample with reagents, wherein the fixed biological sample comprises crosslinks of formula (**IVa**)



(IVa)

wherein,

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

and m is 1 to 13 and n is 1 to 13; optionally wherein m=1 and n=1; and

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

14. The method of claim 13, wherein (c) comprises contacting with a lysis agent and/or an unfixing agent, optionally wherein:

(i) the unfixing agent comprises a compound capable of cleaving a carbamate bond; optionally, wherein the compound capable of cleaving a carbamate bond is selected from DETA, EDA, hydrazine monohydrate, a carboxyesterase, or a combination thereof; or

(ii) the unfixing agent comprises a compound capable of cleaving a disulfide bond; optionally, wherein the compound capable of cleaving a disulfide bond is DTT; or

(iii) a lysis agent is present in the absence of an unfixing agent.

15. A kit comprising:

reagents; and

a fixing reagent composition comprising a compound of formula (**I**) or (**IV**) as defined in any one of claims 1-3.

16. The kit of claim 15, wherein the kit further comprises:

(a) a lysis agent; and/or

(b) an unfixing agent; optionally, wherein the unfixing agent comprises:

(i) a compound capable of cleaving a carbamate bond; optionally, wherein the compound capable of cleaving a carbamate bond is selected from DETA, EDA, hydrazine monohydrate, a carboxyesterase, or a combination thereof; and/or

(ii) a compound capable of cleaving a disulfide bond; optionally, wherein the compound capable of cleaving a disulfide bond is DTT.