METHODS AND COMPOSITIONS FOR SYNCHRONIZING REACTIONS *IN SITU*

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/227,830 filed July 30, 2021, entitled "METHOD AND COMPOSITIONS FOR SYNCHRONIZING REACTIONS *IN SITU*," which is herein incorporated by reference in its entirety for all purposes.

FIELD

[0002] The present disclosure generally relates to methods and compositions for *in situ* detection of a plurality of molecules of one or more analytes in a sample.

BACKGROUND

[0003] Genomic, transcriptomic, and proteomic profiling of cells and tissue samples using microscopic imaging can resolve multiple analytes of interest at the same time, thereby providing valuable information regarding analyte abundance and localization *in situ*. Thus, these *in situ* assays are important tools, for example, for understanding the molecular basis of cell identity and developing treatment for diseases. In multiplex assays where multiple signals are detected simultaneously, it is important that as much information as possible is collected. However, due to the heterogeneity of analyte abundance (e.g., gene expression levels) and variations among reactions at different locations of a sample, there can be a wide and heterogeneous size and intensity distribution of signal "spots" in the sample. Large signal spots may overlap with one another and/or mask adjacent smaller signal spots, rendering the smaller spots unresolvable. In addition, some analytes may be associated with bright signal spots (e.g., due to high analyte abundance and/or preferential signal amplification), while other analytes may be associated with signal spots that are too dim to be detected simultaneously (e.g., in the same field of view (FOV) during microscopy) with the bright spots. There is a need for new and improved methods for *in situ* assays. The present disclosure addresses these and other needs.

SUMMARY

[0003a] In one aspect, provided herein is a method for analyzing a biological sample, comprising:

(a) contacting the biological sample with a first reaction mixture, wherein:

the biological sample comprises a circular nucleic acid comprising a hybridization region,

the first reaction mixture comprises a polymerase, wherein the biological sample comprises cells or is a tissue sample,

the circular nucleic acid or the polymerase is prebound to a polynucleotide comprising a sequence complementary to the hybridization region, and

the polymerase activity of the polymerase is inhibited; and

(b) contacting the biological sample with a second reaction mixture to allow the polymerase to extend the polynucleotide hybridized to the hybridization region using the circular nucleic acid as a template,

wherein a rolling circle amplification product of the circular nucleic acid is generated in the biological sample.

[0003b] In one aspect, provided herein is a method for analyzing a biological sample, comprising:

(a) contacting the biological sample with a binding mixture, wherein:

the biological sample comprises a plurality of circular nucleic acids each comprising a primer hybridization region,

the binding mixture comprises a plurality of complexes each comprising a polymerase bound to a primer, wherein the primer comprises a sequence complementary to the primer hybridization region of one or more circular nucleic acids, wherein the biological sample comprises cells or is a tissue sample, and

the polymerase activity of the polymerase is inhibited,

thereby allowing the plurality of complexes to hybridize to the plurality of circular nucleic acids; and

(b) contacting the biological sample with a primer extension reaction mixture to allow the polymerase to extend the primer hybridized to the primer hybridization region,

thereby synchronizing rolling circle amplification the plurality of circular nucleic acids in the biological sample.

[0003c] In one aspect, provided herein is a method for analyzing a biological sample, comprising:

(a) contacting the biological sample with a binding mixture, wherein:

the biological sample comprises a plurality of circular nucleic acids each comprising a hybridization region hybridized to a polynucleotide,

the binding mixture comprises a polymerase, wherein the biological sample

comprises cells or is a tissue sample, and

the polymerase activity of the polymerase is inhibited,

thereby allowing the polymerase to bind to the plurality of circular nucleic acids; and

(b) contacting the biological sample with a primer extension reaction mixture to allow the polymerase to extend the polynucleotide hybridized to the hybridization region,

thereby synchronizing rolling circle amplification (RCA) of the plurality of circular nucleic acids in the biological sample.

[0004] In some embodiments, provided herein is a method for analyzing a biological sample, comprising contacting the biological sample with a first reaction mixture, wherein the biological sample comprises a circular nucleic acid comprising a hybridization region, the first reaction mixture comprises a polymerase, the circular nucleic acid or the polymerase is prebound