SIMULTANEOUS QUANTIFICATION OF A PLURALITY OF PROTEINS IN A USER-DEFINED REGION OF A CROSS-SECTIONED TISSUE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is claims priority to and the benefit of U.S. Provisional Application No. 62/193,819, filed July 17, 2015; U.S. Provisional Application No. 62/261,654, filed December 1, 2015; U.S. Provisional Application No. 62/277,283, filed January 11, 2016; and U.S. Provisional Application No. 62/323,018, filed April 15, 2016. Each of the above-mentioned applications is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Standard immunohistochemical methods allow for simultaneous detection of, at most, six to ten protein targets, with three to four targets being typical. There exists a need for probes, compositions, methods, and kits for simultaneous, multiplexed detection and quantification of protein expression in a user-defined region of a tissue, user-defined cell, and/or user-defined subcellular structure within a cell.

SUMMARY

[0003] The present disclosure relates to probes, compositions, methods, and kits for simultaneous, multiplexed detection and quantification of protein expression in a user-defined region of a tissue, user-defined cell, and/or user-defined subcellular structure within a cell.

[0003a] In one aspect, the present invention provides a method comprising: (1) contacting at least one protein target in a tissue sample with at least one probe comprising a target-binding domain, a signal oligonucleotide, and a photo-cleavable motif located between the target binding domain and the signal oligonucleotide; (2) illuminating a specific location of the tissue sample with light sufficient to release the signal oligonucleotide; (3) collecting the released signal oligonucleotide from a proximal solution, wherein the proximal solution is eluted directly above the location that was illuminated; and (4) identifying the released signal oligonucleotide, thereby detecting the at least one protein target in the specific location of the tissue sample that was illuminated.

[0004] Also disclosed herein is a method including steps of (1) contacting at least one protein target in or from at least one cell in a tissue sample with at least one probe comprising a target-binding domain and a signal oligonucleotide; (2) providing a force

What is claimed is:

1. A method comprising:

(1) contacting at least one protein target in a tissue sample with at least one probe comprising a target-binding domain, a signal oligonucleotide, and a photo-cleavable motif located between the target binding domain and the signal oligonucleotide;

(2) illuminating a specific location of the tissue sample with light sufficient to release the signal oligonucleotide;

(3) collecting the released signal oligonucleotide from a proximal solution, wherein the proximal solution is eluted directly above the location that was illuminated; and

(4) identifying the released signal oligonucleotide, thereby detecting the at least one protein target in the specific location of the tissue sample that was illuminated.

2. The method of claim 1, wherein detecting comprises determining the identity and the abundance of the at least one protein target.

3. The method of claim 1 or claim 2, wherein the at least one protein target comprises at least two distinct protein targets or at least two copies of the same protein target.

4. The method of claim 3, wherein detecting comprises comparing the abundance of each distinct protein target.

5. The method of any of claims 1 to 4, further comprising repeating at least steps (2) – (4) on at least a second specific location of the tissue sample.

6. The method of claim 5, wherein detecting comprises comparing the abundance of the at least one protein target in the specific location and in the at least second specific location.

7. The method of claim 5 or claim 6, wherein the specific location and the at least second specific location comprise the same cell type.

8. The method of claim 5 or claim 6, wherein the specific location and the at least second specific location comprise distinct cell types.

9. The method of claim 8, wherein detecting comprises comparing the abundance of the at least one protein target in the specific location and in the at least second specific location.

10. The method of claim 7 or claim 8, wherein the cell type is independently selected from a normal cell and an abnormal cell.

11. The method of any one of claims 1 to 10, wherein the tissue sample is directly immobilized to a surface or is indirectly immobilized to a surface.

12. The method of any one of claims 1 to 11, wherein the tissue sample is a 2 to 1000 μm thick tissue section.

13. The method of claim 12, wherein the tissue section is obtained from a formalin-fixed paraffin embedded (FFPE) sample.

14. The method of any one of claims 1 to 11, wherein the tissue sample comprises at least one cell, wherein the at least one cell is a cultured cell, a primary cell, or a dissociated cell from an explant.

15. The method of claim 12 or 14, wherein the tissue sample is fixed or unfixed.

16. The method of any one of claims 1 to 15, wherein the tissue sample is stained or labeled prior to step (2) thereby allowing visualization of a subcellular, cellular, or tissue-related structure in the stained or labeled cell.

17. The method of any one of claims 1 to 16, wherein the signal oligonucleotide is a single-stranded nucleic acid or a partially double-stranded nucleic acid.

18. The method of any one of claims 1 to 17, wherein a negative purification is used to remove intact probe molecules from the released signal oligonucleotides.

19. The method of claim 18, wherein the negative purification comprises an affinity purification comprising contacting an intact probe with an immobilized oligonucleotide that is complementary to a portion of the intact probe or an immobilized antibody or protein-binding motif that recognizes and binds to a portion of the intact probe.

20. The method of claim 19, wherein the intact probe's target binding domain comprises a universal purification tag or sequence that is partially complementary to the immobilized oligonucleotide or is capable of being recognized or bound by the immobilized antibody or protein-binding motif.

21. The method of any one of claims 1 to 20, wherein the illumination is provided by a light source selected from the group consisting of an arc-lamp, a laser, a focused UV light source, and light emitting diode (LED).

22. The method of any one of claims 1 to 21, wherein the illumination irradiates at least one subcellular structure in the specific location of the tissue sample.

23. The method of any one of claims 1 to 22, wherein detecting comprises determining the abundance of the at least one protein target in at least one subcellular structure of the tissue sample.

24. The method of any one of claims 1 to 20, wherein the illumination is provided by a light source selected from an arc-lamp, a laser, a focused UV light source, and a light emitting diode (LED) and the light source irradiates at least one subcellular structure in the specific location of the tissue sample and at least one subcellular structure in the at least second specific location of the tissue sample.

25. The method of claim 24, wherein detecting comprises comparing the abundance of the at least one protein target in the at least one subcellular structure in the specific location of the tissue sample and the at least one subcellular structure in the at least second specific location of the tissue sample.

26. The method of any one of claims 1 to 25, wherein the target-binding domain is selected from the group consisting of an antibody, a peptide, an aptamer, and a peptoid.

27. The method of any one of claims 1 to 26, wherein detecting comprises a polymerase reaction, a reverse transcriptase reaction, hybridization to an oligonucleotide microarray, mass spectrometry, hybridization to a fluorescent molecular beacon, a sequencing reaction, or molecular barcodes.

28. The method of any one of claims 1 to 27, wherein the protein target is an intact protein, a plurality of polypeptides, a polypeptide, or a peptide.

29. The method of any one of claims 1 to 28, wherein the proximal solution is collected by aspirating.

30. The method of claim 29, wherein the aspirating is via a pipette, a capillary tube, a microarray pin, and/or a flow cell comprising holes.

31. The method of claim 30, wherein the capillary tube comprises an optical device capable of transmitting light to the specific location of the tissue sample.

32. The method of claim 31, wherein the light is UV light.

33. The method of claim 32, wherein the pipette or microarray pin is attached to an array comprising a plurality of pipettes or microarray pins.

34. The method of any one of claims 1 to 33, wherein the proximal solution comprises an anionic polymer, preferably dextran sulfate, and/or salmon sperm DNA.

35. The method of any one of claims 1 to 34, wherein the collected signal oligonucleotide is added to a solution comprising an anionic polymer, preferably dextran sulfate, and/or salmon sperm DNA.

36. The method of any one of claims 1 to 35, wherein the method further comprises illuminating a region of interest using a laser scanning device.

37. The method of any one of claims 1 to 35, wherein the method further comprises illuminating a region of interest using a digital mirror device (DMD).

38. The method of any one of claims 1 to 37, wherein the method provides simultaneous spatially resolved protein detection of a sample.

39. The method of any one of claims 1 to 38, wherein detecting comprises providing a digital readout comprises a linear dynamic range of > 5 logs.

40. The method of any one of claims 1 to 39, wherein the tissue sample is attached to a slide and is first imaged using fluorescence and then expression of proteins is digitally counted from the tissue sample.

41. The method of any one of claims 1 to 40, wherein the probe is provided at a concentration of 0.01 nM to 5 nM.

42. The method of claim 41, wherein the probe is provided at a concentration of 0.01 nM to 1 nM.

43. The method of claim 42, wherein the probe is provided at a concentration of 0.01 nM to 0.4 nM.

44. The method of claim 43, wherein the probe is provided at a concentration of 0.01 nM to 0.2 nM.