herein provide for enhancing the specificity of binding an analyte capture sequence to a capture domain. In some examples, an analyte capture agent includes an analyte binding moiety, an analyte capture sequence, and an analyte binding moiety barcode. In some examples, the analyte capture sequence is a nucleotide sequence. In some ~~embodiment~~examples, the analyte capture sequence binds to a capture domain of a capture probe, where the capture probe includes a spatial barcode.

[0006] More specifically, the methods provided herein utilize blocking probes to block non-specific hybridization of an analyte capture sequence to the capture domain of a capture probe on an array thus enhancing the specificity of binding of an analyte capture sequence and a capture domain. In some examples, the blocking probes can differ in length and/or complexity. In some examples, the blocking probes specifically bind the analyte capture sequence. In some examples, the blocking probes specifically bind the capture domain of the capture probe on the substrate. In some examples, the blocking probes specifically bind both the analyte capture sequence and the capture domain of the capture probe on the substrate. In some examples, more than one blocking probe specifically binds the analyte capture sequence. In some examples, the blocking probe include one or more inosine nucleotides. In some examples, the blocking probes include one or more uracil nucleotides. In some examples, the blocking probes include one or more abasic sites. In some examples, the blocking probes are released by one or more of heat, cleavage, or washes in a salt buffer.

[0007] Also provided herein are methods for binding an analyte capture sequence to a capture domain including: (a) contacting a biological sample with an array, where the array includes a plurality of capture probes including (i) a spatial barcode and (ii) a capture domain; (b) providing a plurality of analyte capture agents, where an analyte capture agent includes an analyte binding moiety that binds to an analyte in the biological sample, an analyte binding moiety barcode, and an analyte capture sequence, where the capture domain, the analyte capture sequence, or both, are reversibly blocked with one or more blocking probes; and (c) releasing the one or more blocking probes from the capture domain, the analyte capture sequence, or both, and allowing the analyte capture sequence to specifically bind to the capture domain, thereby binding the analyte capture sequence to the capture domain.

[0008] In some ~~embodiment~~examples, the blocking enhances the specificity of binding an analyte capture sequence to a capture domain compared to analyte binding specificity without blocking the capture domain, the analyte domain, or both.

[0009] In some ~~embodiment~~examples, the method includes fixing the biological sample, and optionally where the fixing includes methanol, and staining the biologogical sample, and optionally, where the staining includes immunofluorescence.

[0010] In some ~~embodiment~~examples, prior to the contacting in step (b), providing the plurality of analyte capture agents with the one or more blocking probes. In some ~~embodiment~~examples, the capture domain is reversibly blocked with a blocking probe of the one or more blocking probes. In some ~~embodiment~~examples, the analyte capture sequence is reversibly blocked with a blocking probe of the one or more blocking probes. In some ~~embodiment~~examples, the capture domain is reversibly blocked with a first blocking probe of the one or more blocking probes and the analyte capture sequence is reversibly blocked with a second blocking probe of the one or more blocking probes.

[0011] In some ~~embodiment~~examples, the releasing of the one or more blocking probes includes the use of an enzyme. In some ~~embodiment~~examples, the enzyme is an endonuclease. In some ~~embodiment~~examples, the one or more blocking probes includes one or more inosine nucleotides and the endonuclease is endonuclease V. In some ~~embodiment~~examples, a blocking probe of the one or more blocking probes includes one or more abasic sites and the endonuclease is endonuclease IV.

[0012] In some ~~embodiment~~examples, a blocking probe of the one or more blocking probes includes a uracil and the enzyme is a uracil-specific excision reagent (USER). In some ~~embodiment~~examples, the blocking probe includes a poly(U) sequence, one or more RNA bases, one or more LNA bases, or combinations thereof.

[0013] In some ~~embodiment~~examples, a blocking probe of the one or more blocking probes, when hybridized to the analyte capture sequence or the capture domain, includes one or more mismatched nucleotides, and the releasing includes increasing the temperature of the biological sample.

[0014] In some ~~embodiment~~examples, one or more mismatched nucleotides in the blocking probe hybridized to the analyte capture sequence or the capture domain are positioned after

the fourth nucleotide from a 5’ end of the blocking probe and before the last four nucleotides at the 3’ end of the blocking probe. In some ~~embodiment~~examples, one or more mismatched nucleotides in the blocking probe hybridized to the analyte capture sequence or the capture domain are positioned after the sixth nucleotide from the 5’ end of the blocking probe and before the last six nucleotides at the 3’ end of the blocking probe.

[0015] In some ~~embodiment~~examples, the blocking probe has a length of about 8 to about 24 nucleotides. In some ~~embodiment~~examples, the releasing of the one or more blocking probes includes washing the biological sample. In some ~~embodiment~~examples, the method includes permeabilizing the biological sample.

[0016] In some ~~embodiment~~examples, the capture domain includes a nucleotide sequence of about 10 to 25 nucleotides in length. In some ~~embodiment~~examples, the capture domain includes a unique nucleotide sequence.

[0017] In some ~~embodiment~~examples, the analyte is a protein.

[0018] In some ~~embodiment~~examples, the analyte binding moiety is an antibody or an antigen-binding fragment thereof.

[0019] In some ~~embodiment~~examples, the analyte capture agent includes a linker, where the linker is disposed between the analyte binding moiety and the analyte binding moiety barcode. In some ~~embodiment~~examples, the linker is a cleavable linker, and optionally, where the cleavable linker is a photo-cleavable linker or an enzyme cleavable linker.

[0020] In some ~~embodiment~~examples, the method includes determining a sequence of (i) all or a part of the sequence of the analyte binding moiety barcode or a complement thereof, and (ii) all or a part of the sequence of the spatial barcode or a complement thereof, and using the determined sequence of (i) and (ii) to identify a location of the analyte in the biological sample. In some ~~embodiment~~examples, the determining includes sequencing (i) all or a part of the sequence of the analyte binding moiety barcode or a complement thereof, and (ii) all or a part of the sequence of the spatial barcode or a complement thereof. In some ~~embodiment~~examples, the sequencing includes high throughput sequencing.

[0021] In some ~~embodiment~~examples, the biological sample is a tissue sample, a fixed tissue sample, a formalin-fixed paraffin-embedded tissue sample, or a fresh-frozen tissue sample.

[0022] Also provided herein are kits including: (a) an array, where the array includes a plurality of capture probes, where a capture probe of the plurality of capture probes includes a spatial barcode and a capture domain; and (b) a plurality of analyte capture agents, where an analyte capture agent includes an analyte binding moiety that binds specifically to an analyte in a biological sample, an analyte binding moiety barcode, and an analyte capture sequence, where the capture domain, the analyte capture sequence, or both are reversibly blocked with one or more blocking probes. In one aspect, the present disclosure refers to a kit comprising: (a) an array, wherein the array comprises a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises: (i) a spatial barcode and (ii): a capture domain; and (b) a plurality of analyte capture agents, wherein an analyte capture agent of the plurality of analyte capture agents comprises: (i) an analyte binding moiety that specifically binds to an analyte in a biological sample, (ii) an analyte binding moiety barcode, and (iii) an analyte capture sequence, wherein the capture domain, the analyte capture sequence, or both are reversibly blocked with one or more blocking probes.

[0023] In some ~~embodiment~~examples, the capture domain is reversibly blocked with a blocking probe of the one or more blocking probes. In some ~~embodiment~~examples, the analyte capture sequence is reversibly blocked with a blocking probe of the one or more blocking probes. In some ~~embodiment~~examples, the capture domain is reversibly blocked with a first blocking probe of the one or more blocking probes and the analyte capture sequence is reversibly blocked with a second blocking probe of the one or more blocking probes.

[0024] In some ~~embodiment~~examples, the kit includes an enzyme. In some ~~embodiment~~examples, the enzyme is an endonuclease. In some ~~embodiment~~examples, a blocking probe of the one or more blocking probes includes one or more inosine nucleotides and the endonuclease is endonuclease V. In some ~~embodiment~~examples, a blocking probe of the one or more blocking probes includes one or more abasic sites and the endonuclease is endonuclease IV. In some ~~embodiment~~examples, a blocking probe of the one or more blocking probes includes a uracil and the enzyme is a uracil-specific excision reagent (USER).

[0025] In some ~~embodiment~~examples, the blocking probe includes a poly(U) sequence, one or more RNA bases, one or more LNA bases, and combinations thereof. In some ~~embodiment~~examples, a blocking probe of the one or more blocking probes, when hybridized to the analyte capture sequence or the capture domain, includes one or more mismatched nucleotides.

[0026] In some ~~embodiment~~examples, the blocking probe has a length of about 8 to about 24 nucleotides. In some ~~embodiment~~examples, the capture domain includes a nucleotide sequence of about 10 to 25 nucleotides in length. In some ~~embodiment~~examples, the capture domain includes a unique nucleotide sequence.

[0027] In some ~~embodiment~~examples, the analyte binding moiety is an antibody or an antigen-binding fragment thereof. In some ~~embodiment~~examples, the analyte capture agent includes a linker, where the linker is disposed between the analyte binding moiety and the analyte

binding moiety barcode. In some ~~embodiment~~examples, the linker is a cleavable linker, and optionally, where the cleavable linker is a photo-cleavable linker or an enzyme cleavable linker.

[0028] All publications, patents, patent applications, and information available on the internet and mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, patent application, or item of information was specifically and individually indicated to be incorporated by reference. To the extent publications, patents, patent applications, and items of information incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

[0029] Where values are described in terms of ranges, it should be understood that the description includes the disclosure of all possible sub-ranges within such ranges, as well as specific numerical values that fall within such ranges irrespective of whether a specific numerical value or specific sub-range is expressly stated.

[0030] The term “each,” when used in reference to a collection of items, is intended to identify an individual item in the collection but does not necessarily refer to every item in the collection, unless expressly stated otherwise, or unless the context of the usage clearly indicates otherwise.

[0031] Various ~~embodiment~~examples of the features of this disclosure are described herein. However, it should be understood that such ~~embodiment~~examples are provided merely by way of example, and numerous variations, changes, and substitutions can occur to those skilled in the art without departing from the scope of this disclosure. It should also be understood that various alternatives to the specific ~~embodiment~~examples described herein are also within the scope of this disclosure.

DESCRIPTION OF DRAWINGS

[0032] The following drawings illustrate certain embodiments of the features and advantages of this disclosure. These embodiments are not intended to limit the scope of the appended claims in any manner. Like reference symbols in the drawings indicate like elements.