"Click"	This method relies on functionalization of the antibody with an azide or alkyne group, depending on the specific catalyst and specific process.	Can be a chemically harsh process that limits use to "robust" antibodies. Since this method relies on another amine/carboxy conjugation method this can also suffer from a wide distribution of biomolecules/antibody
Sulfhydryl-maleimide	This method uses free sulfhydryl groups on the surface of an antibody (or protein) to react with a maleimide group typically synthetically attached to a biomolecule of interest.	The distribution of sulfhydryl groups over the surface of an antibody are well understood but this chemistry can lead to degraded performance of the antibody and requires post-conjugation purification.

[0002] Further, due do the potential harsh chemistries involved and the inherent "stickiness" of currently available dyes, purification of dyes following conjugation is a necessary step in current manufacturing of biomolecule conjugates.

SUMMARY

[0003] Provided for herein is a polynucleotide-modified binding molecule bioconjugate comprising a binding molecule linked to a conjugate component via a nucleic acid linker. In one aspect, provided herein is a polynucleotide-modified bioconjugate, comprising a substrate linked to a single oligonucleotide-based fluorescent label via a nucleic acid linker; wherein the substrate comprises a binding molecule and an at least partially single-stranded nucleic acid linker, and the single oligonucleotide-based fluorescent label comprises an at least partially single-stranded nucleic acid linker; wherein the binding molecule is an antibody or antigen-binding fragment; wherein at least a portion of the sequence of the single-stranded nucleic acid linker of the substrate is complementary to a portion of the sequence of the single-stranded nucleic acid linker of the oligonucleotide-based fluorescent label, such that the nucleic acid linker of the substrate and the single-stranded nucleic acid linker of the oligonucleotide-based fluorescent label are annealed to form a double-stranded nucleic acid linker segment, said nucleic acid linker comprising the double-stranded nucleic acid linker segment;

wherein labeling of the binding molecule with the single oligonucleotide-based fluorescent label is controlled stoichiometrically via the availability of the at least partially single-stranded nucleic acid linker of the substrate; and wherein there is no exchange of the substrate and oligonucleotide-based fluorescent label in solution.

[0004] Provided for herein is also a polynucleotide-modified bead bioconjugate comprising a bead linked to a conjugate component via a nucleic acid linker.

[0005] Provided for are methods of producing a polynucleotide-modified binding molecule bioconjugate of this disclosure or a polynucleotide-modified bead bioconjugate of this disclosure comprising: (i) contacting a polynucleotide-modified substrate comprising an at least partially single-stranded nucleic acid linker with a polynucleotide-modified conjugate component comprising an at least partially single-stranded nucleic acid linker, wherein the substrate is a binding molecule or a bead, wherein at least a portion of the single-stranded linker sequence of the substrate is complementary to a portion of the single-stranded linker sequence of the conjugate component and wherein when contacted, the nucleic acid linker of the substrate and the nucleic acid linker of the conjugate component anneal to form an at least partially double-

stranded linker linking the substrate to the conjugate component; (ii) contacting a substrate with a conjugate component, wherein the substrate is a binding molecule or a bead, and attaching a nucleic acid linker to the substrate and attaching the same nucleic acid linker to the conjugate component to form the polynucleotide-modified binding molecule bioconjugate, (iii) contacting a polynucleotide-modified conjugate component comprising a nucleic acid linker with a substrate, wherein the substrate is a binding molecule or a bead, and attaching the nucleic acid linker of the polynucleotide-modified conjugate component to the substrate to form the polynucleotide-modified binding molecule or bead bioconjugate, (iv) contacting a polynucleotide-modified substrate comprising a nucleic acid linker with a conjugate component, wherein the substrate is a binding molecule or a bead, and attaching the nucleic acid linker of the polynucleotide-modified substrate to the conjugate component to form the polynucleotide-modified binding molecule or bead bioconjugate; or (v) contacting (i) a polynucleotide-modified substrate comprising an at least partially single-stranded nucleic acid linker, wherein the substrate is a binding molecule or a bead, (ii) a polynucleotide-modified conjugate component comprising an at least partially single-stranded nucleic acid linker, and (iii) a further nucleic acid linker segment that is not the nucleic acid linker of (i) or (ii), wherein at least a portion of the single-stranded linker sequence of the substrate is complementary to a single-stranded portion of the further nucleic acid linker segment (iii) and/or wherein at least a portion of the single-stranded linker sequence of the conjugate component is complementary to a single-stranded portion of the further nucleic acid linker segment (iii), and wherein when contacted, the nucleic acid linker of the substrate, the nucleic acid linker of the conjugate component, and the further nucleic acid linker segment anneal to form an at least partially double-stranded linker linking the substrate to the conjugate component. In one aspect, provided herein is a process of making a polynucleotide-modified bioconjugate, comprising contacting in solution: a substrate comprising a binding molecule and an at least partially single-stranded nucleic acid linker with a single oligonucleotide-based fluorescent label comprising an at least partially single-stranded nucleic acid linker; wherein the binding molecule is an antibody, or antigen-binding fragment; and wherein at least a portion of the sequence of the single-stranded nucleic acid linker of the substrate is complementary to a portion of the sequence of the single-stranded nucleic acid linker of the oligonucleotide-based fluorescent label, such that the nucleic acid linkers anneal and form a double-stranded nucleic acid linker segment linking the substrate to the oligonucleotide-based fluorescent label; wherein labeling of the binding molecule

with the single oligonucleotide-based fluorescent label is controlled stoichiometrically via the availability of the at least partially single-stranded nucleic acid linker of the substrate; and there is no exchange of the substrate and oligonucleotide-based fluorescent label in solution. In another aspect, provided herein is an *ex vivo* method of labeling a cell, tissue, or organ, the method comprising: contacting the cell, tissue, or organ with the fluorescently labeled bioconjugate of the disclosure, wherein the substrate portion of the bioconjugate comprises a binding molecule that binds to an antigen, epitope, or hapten of the cell, tissue, or organ. In yet another aspect, provided herein is a kit comprising: (a) a polynucleotide-modified bioconjugate of the disclosure; and (b) instructions for use, and/or buffers and/or additional reagents, and/or packaging materials.

[0006] Provided for herein is a high-rate method of producing a labeled binding molecule for detection, wherein separate binding-molecule molecules of the same binding molecule are labeled with different conjugate components, the method comprising: (a) attaching an at least partially single-stranded nucleic acid linker to at least two separate binding-molecule molecules of the same binding molecule to produce a plurality of polynucleotide-modified binding-molecule molecules; and (b) contacting at least one polynucleotide-modified binding-molecule molecule of the plurality with a first polynucleotide-modified conjugate component comprising an at least partially single-stranded nucleic acid linker and contacting at least one different polynucleotide-modified binding-molecule molecule of the plurality with a second polynucleotide-modified conjugate component comprising an at least partially single-stranded

CLAIMS

1. A process of making a polynucleotide-modified bioconjugate, comprising contacting in solution:

a substrate comprising a binding molecule and an at least partially single-stranded nucleic acid linker with a single oligonucleotide-based fluorescent label comprising an at least partially single-stranded nucleic acid linker;

wherein the binding molecule is an antibody, or antigen-binding fragment; and

wherein at least a portion of the sequence of the single-stranded nucleic acid linker of the substrate is complementary to a portion of the sequence of the single-stranded nucleic acid linker of the oligonucleotide-based fluorescent label, such that the nucleic acid linkers anneal and form a double-stranded nucleic acid linker segment linking the substrate to the oligonucleotide-based fluorescent label;

wherein labeling of the binding molecule with the single oligonucleotide-based fluorescent label is controlled stoichiometrically via the availability of the at least partially single-stranded nucleic acid linker of the substrate; and

there is no exchange of the substrate and oligonucleotide-based fluorescent label in solution.

2. The process of claim 1, wherein the double-stranded nucleic acid linker segment comprises at least 10 complementary base pairs.

3. The process of claim 1 or claim 2, wherein the double-stranded nucleic acid linker segment comprises from 10 complementary base pairs to 75 complementary base pairs.

4. The process of any of claims 1 to 3, wherein the double-stranded nucleic acid linker segment comprises from 60 complementary base pairs to 75 complementary base pairs.

5. The process of any preceding claim, wherein the oligonucleotide-based fluorescent label is an oligonucleotide-based nanoparticle.

6. A polynucleotide-modified bioconjugate, comprising a substrate linked to a single oligonucleotide-based fluorescent label via a nucleic acid linker;

wherein the substrate comprises a binding molecule and an at least partially single-stranded nucleic acid linker, and the single oligonucleotide-based fluorescent label comprises an at least partially single-stranded nucleic acid linker;

wherein the binding molecule is an antibody or antigen-binding fragment;

wherein at least a portion of the sequence of the single-stranded nucleic acid linker of the substrate is complementary to a portion of the sequence of the single-stranded nucleic acid linker of the oligonucleotide-based fluorescent label, such that the nucleic acid linker of the substrate and the single-stranded nucleic acid linker of the oligonucleotide-based fluorescent label are annealed to form a double-stranded nucleic acid linker segment, said nucleic acid linker comprising the double-stranded nucleic acid linker segment;

wherein labeling of the binding molecule with the single oligonucleotide-based fluorescent label is controlled stoichiometrically via the availability of the at least partially single-stranded nucleic acid linker of the substrate; and

wherein there is no exchange of the substrate and oligonucleotide-based fluorescent label in solution.

7. An *ex vivo* method of labeling a cell, tissue, or organ, the method comprising:

contacting the cell, tissue, or organ with the fluorescently labeled bioconjugate of claim 6, wherein the substrate portion of the bioconjugate comprises a binding molecule that binds to an antigen, epitope, or hapten of the cell, tissue, or organ.

8. The method of claim 7, further comprising use of the labeled cell in one or more of flow cytometry, antibody screening, sandwich assays, immune monitoring, biomarker assays, lateral flow, point-of-care/rapid diagnostics, imaging, microscopy, molecular diagnostics, next generation sequencing, long read sequencing, in situ sequencing, polymerase chain reaction, microarrays, nucleic acid sequencing, amino acid sequencing, digital pathology, Southern blotting, Northern blotting, or Western blotting.

9. The method of claim 7 or claim 8, further comprising use of the labeled cell in flow cytometry.

10. A kit comprising:

(a) a polynucleotide-modified bioconjugate of claim 6; and

(b) instructions for use, and/or buffers and/or additional reagents, and/or packaging materials.