

CLAIMS

What is claimed:

1. A method for detecting protein-polynucleotide conjugate molecules in a sample comprising:
 - (a) contacting the sample with a triplex forming oligonucleotide (TFO) that is covalently linked to a tag under conditions that allow the TFO to hybridize to the polynucleotide in the conjugate molecule, thereby forming a hybridization mixture;
 - (b) contacting the hybridization mixture with a surface comprising a capture reagent that specifically binds to the tag covalently linked to the TFO;
 - (c) contacting the surface with a detection reagent, wherein the detection reagent comprises a detectable label coupled to a binding partner that specifically binds to the protein in the conjugate molecule; and
 - (d) detecting a signal from the detectable label.
2. The method of claim 1, wherein the tag is a hapten.
3. The method of claim 2, wherein the hapten is biotin, digoxigenin, or 2,4-dinitrophenol.
4. The method of claim 1, wherein the capture reagent is an antibody that specifically binds to the tag.
5. The method of claim 1, wherein the tag is biotin and the capture reagent is streptavidin.
6. The method of claim 1, wherein the tag is digoxigenin and the capture reagent is an antibody that specifically binds to digoxigenin.
7. The method of any one of claims 1 to 6, wherein the binding partner is an antibody that specifically binds to the protein in the conjugate molecule.

8. The method of any one of claims 1 to 7, wherein the detectable label is a fluorophore, metallic nanoparticle, enzyme, or electrochemiluminescence (ECL) luminophore.
9. The method of claim 8, wherein the ECL luminophore is a ruthenium complex.
10. The method of any one of claims 1 to 9, wherein the TFO comprises a mixture of locked nucleic acid (LNA) monomers and deoxyribonucleotides.
11. The method of claim 10, wherein about 30% to about 40% of the nucleotides in the TFO are LNA monomers.
12. The method of any one of claims 1 to 11, wherein the TFO is at least 15 nucleotides in length.
13. The method of any one of claims 1 to 12, wherein the TFO has a sequence that is complementary to the sequence of the polynucleotide in the conjugate molecule over its entire length.
14. The method of any one of claims 1 to 13, wherein the polynucleotide in the conjugate molecule is an siRNA, an shRNA, a miRNA, a pre-miRNA, or an antisense oligonucleotide.
15. The method of claim 14, wherein the polynucleotide in the conjugate molecule is an siRNA that comprises a sense strand and an antisense strand, and wherein the TFO has a sequence that is complementary to the sequence of the sense strand.
16. The method of any one of claims 1 to 15, wherein the protein in the conjugate molecule is an antibody or antigen-binding fragment thereof.
17. The method of claim 16, wherein the protein in the conjugate molecule is an antibody and the binding partner is a target antigen of the antibody, an anti-Fc region antibody, or an anti-idiotypic antibody.

18. The method of any one of claims 1 to 15, wherein the protein in the conjugate molecule is a ligand of a cell-surface receptor.
19. The method of claim 18, wherein the binding partner is the receptor or a ligand-binding fragment thereof.
20. The method of any one of claims 1 to 17, wherein the protein-polynucleotide conjugate molecule is an antibody-siRNA conjugate molecule.
21. The method of any one of claims 1 to 20, wherein the sample is contacted with the TFO at a temperature of about 25°C to about 60°C to form the hybridization mixture.
22. The method of any one of claims 1 to 20, wherein the sample is contacted with the TFO at a temperature of about 45°C to about 55°C to form the hybridization mixture.
23. The method of any one of claims 1 to 20, wherein the sample is contacted with the TFO at a temperature of about 50°C to about 55°C to form the hybridization mixture.
24. The method of any one of claims 1 to 23, wherein the sample is serum, plasma, tissue homogenate, drug substance, or drug product.
25. A method for detecting protein-polynucleotide conjugate molecules in a sample comprising:
- (a) contacting the sample with a TFO that is covalently linked to a tag under conditions that allow the TFO to hybridize to the polynucleotide in the conjugate molecule, thereby forming a hybridization mixture;
 - (b) contacting the hybridization mixture with a surface comprising a capture reagent that specifically binds to the protein in the conjugate molecule;

(c) contacting the surface with a detection reagent, wherein the detection reagent comprises a detectable label coupled to a binding partner that specifically binds to the tag covalently linked to the TFO; and

(d) detecting a signal from the detectable label.

26. The method of claim 25, wherein the tag is a hapten.

27. The method of claim 26, wherein the hapten is biotin, digoxigenin, or 2,4-dinitrophenol.

28. The method of claim 25, wherein the binding partner is an antibody that specifically binds to the tag.

29. The method of claim 25, wherein the tag is biotin and the binding partner is streptavidin.

30. The method of claim 25, wherein the tag is digoxigenin and the binding partner is an antibody that specifically binds to digoxigenin.

31. The method of any one of claims 25 to 30, wherein the detectable label is a fluorophore, metallic nanoparticle, enzyme, or ECL luminophore.

32. The method of claim 31, wherein the ECL luminophore is a ruthenium complex.

33. The method of any one of claims 25 to 32, wherein the capture reagent is an antibody that specifically binds to the protein in the conjugate molecule.

34. The method of any one of claims 25 to 33, wherein the TFO comprises a mixture of locked nucleic acid (LNA) monomers and deoxyribonucleotides.

35. The method of claim 34, wherein about 30% to about 40% of the nucleotides in the TFO are LNA monomers.

36. The method of any one of claims 25 to 35, wherein the TFO is at least 15 nucleotides in length.
37. The method of any one of claims 25 to 36, wherein the TFO has a sequence that is complementary to the sequence of the polynucleotide in the conjugate molecule over its entire length.
38. The method of any one of claims 25 to 37, wherein the sample is contacted with the TFO at a temperature of about 25°C to about 60°C to form the hybridization mixture.
39. The method of any one of claims 25 to 37, wherein the sample is contacted with the TFO at a temperature of about 45°C to about 55°C to form the hybridization mixture.
40. The method of any one of claims 25 to 37, wherein the sample is contacted with the TFO at a temperature of about 50°C to about 55°C to form the hybridization mixture.
41. The method of any one of claims 25 to 40, wherein the polynucleotide in the conjugate molecule is an siRNA, an shRNA, a miRNA, a pre-miRNA, or an antisense oligonucleotide.
42. The method of claim 41, wherein the polynucleotide in the conjugate molecule is an siRNA that comprises a sense strand and an antisense strand, and wherein the TFO has a sequence that is complementary to the sequence of the sense strand.
43. The method of any one of claims 25 to 42, wherein the protein in the conjugate molecule is an antibody or antigen-binding fragment thereof.
44. The method of claim 43, wherein the protein in the conjugate molecule is an antibody and the capture reagent is a target antigen of the antibody, an anti-Fc region antibody, an anti-idiotypic antibody, protein A, or protein G.

45. The method of any one of claims 25 to 42, wherein the protein in the conjugate molecule is a ligand of a cell-surface receptor.
46. The method of claim 45, wherein the capture reagent is the receptor or a ligand-binding fragment thereof.
47. The method of any one of claims 25 to 44, wherein the protein-polynucleotide conjugate molecule is an antibody-siRNA conjugate molecule.
48. The method of any one of claims 25 to 47, wherein the sample is serum, plasma, tissue homogenate, drug substance, or drug product.