LISTING OF CLAIMS

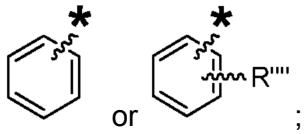
1. A composition comprising a first aptamer, second aptamer, and a target,

wherein the first aptamer comprises a first C-5 pyrimidine modification scheme, the second aptamer comprises a second C-5 pyrimidine modification scheme, and wherein the first C-5 pyrimidine modification scheme and the second C-5 pyrimidine modification scheme are different;

wherein the first C-5 pyrimidine modification scheme comprises a chemical modification at a C-5 position comprising K-NH-C(O)-,

wherein

K represents R'-$(CH_2)_n$-;

R' represents  ;

wherein

* denotes point of attachment of the R' group to $(CH_2)_n$ connecting group;

n = 0-3;

R'''' is selected from the group consisting of a branched or linear C1-C20 alkyl; OH, halogen; CN; BO_2H_2; COOH; COOR''; $CONH_2$; CONHR''; CONR''R'''; SO_2NH_2; SONHR'';

wherein

R'' and R''' are independently selected from a group consisting of a branched or linear C1-C20 alkyl; C_6H_5; R''''C_6H_4, wherein R'''' is defined above; COOH; COOR'''', wherein R'''' is a branched or linear C1-C20 alkyl; and cycloalkyl; and

wherein the first aptamer, second aptamer and the target are capable of forming a ternary complex.

2. The composition of claim 1, wherein the first aptamer has binding affinity for the target and not the second aptamer.

3. The composition of claim 1, wherein the second aptamer has binding affinity for the target and not the first aptamer.

4. The composition of claim 1, wherein the second aptamer has binding affinity for a complex formed by the association of the first aptamer with the target.

5. The composition of claim 1, wherein the first aptamer binding region of the target and the second aptamer binding region of the target are different regions.

6. The composition of claim 1, wherein the first aptamer and the second aptamer, independently, comprise RNA, DNA or a combination thereof.

7. The composition of claim 1, wherein the first C-5 pyrimidine modification scheme comprises a C-5 modified pyrimidine selected from the group consisting of 5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU), 5-(N-benzylcarboxyamide)-2'-O-methyluridine, 5-(N-benzylcarboxyamide)-2'-fluorouridine, 5-(N-phenethylcarboxyamide)-2'-deoxyuridine (PEdU), 5-[N-(phenyl-3-propyl)carboxamide]-2'-deoxyuridine (PPdU), 5-[N-(4-fluorobenzyl)carboxyamide]-2'-deoxyuridine (FBndU), 5-[N-(4-hydroxyphenyl-2-ethyl)carboxamide]-2'-deoxyuridine (TyrdU), and a combination thereof.

8. The composition of claim 1, wherein the first C-5 pyrimidine modification scheme comprises a 5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU).

9. The composition of claim 1, wherein percent GC content of the first aptamer and second aptamer are, independently, from about 37% to about 58%.

10. The composition of claim 1, wherein the first aptamer comprises from about 9 to about 16 C-5 modified pyrimidines.

11. The composition of claim 1 , wherein each uracil or thymine of the first aptamer is a C-5 modified pyrimidine selected from the group consisting of 5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU), 5-(N-benzylcarboxyamide)-2'-O-methyluridine, 5-(N-benzylcarboxyamide)-2'-fluorouridine, 5-(N-phenethylcarboxyamide)-2'-deoxyuridine (PEdU), 5-[N-(phenyl-3-propyl)carboxamide]-2'-deoxyuridine (PPdU), 5-[N-(4-fluorobenzyl)carboxyamide]-2'-deoxyuridine (FBndU) and 5-[N-(4-hydroxyphenyl-2-ethyl)carboxamide]-2'-deoxyuridine (TyrdU).

12. The composition of claim 1, wherein each uracil or thymine of the first aptamer is a 5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU).

13. The composition of claim 1, wherein the second C-5 pyrimidine modification scheme comprises a C-5 modified pyrimidine selected from the group consisting of 5-[N-(2-thiophene-methyl)carboxamide]-2'-deoxyuridine (ThdU), 5-(N-isobutylcarboxyamide)-2'-deoxyuridine (iBudU), 5-(N-isobutylcarboxyamide)-2'-O-methyluridine, 5-(N-isobutylcarboxyamide)-2'-fluorouridine, 5-(N-tryptaminocarboxyamide)-2'-deoxyuridine (TrpdU), 5-(N-tryptaminocarboxyamide)-2'-O-methyluridine, 5-(N-tryptaminocarboxyamide)-2'-fluorouridine, 5-(N-[1-(3-trimethylamonium) propyl]carboxyamide)-2'-deoxyuridine chloride, 5-[N-(1-naphthylmethyl)carboxyamide]-2'-deoxyuridine (NapdU), 5-[N-(2-naphthylmethyl)carboxyamide]-2'-deoxyuridine (2-NapdU), 5-[N-(1-naphthylethyl)carboxyamide]-2'-deoxyuridine (NEdU), 5-[N-(2-naphthylethyl)carboxyamide]-2'-deoxyuridine (2NEdU), 5-(N-naphthylmethylcarboxyamide)-2'-O-methyluridine, 5-(N-naphthylmethylcarboxyamide)-2'-fluorouridine, 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine, 5-[N-(3-benzo[*b*]thiophene-2-ethyl)carboxamide]-2'-deoxyuridine (BTdU), 5-[N-(3-benzo[*a*]furan-2-ethyl)carboxamide]-2'-deoxyuridine (BFdU), 5-[N-(3,4-methylenedioxybenzyl)carboxamide]-2'-deoxyuridine (MBndU), 5-[N-((R)-2-tetrahydrofurylmethyl)carboxamide]-2'-deoxyuridine (RTHdU), 5-[N-((S)-2-tetrahydrofurylmethyl)carboxamide] -2'-deoxyuridine (STHFdU), 5-(N-2-imidazolylethylcarboxamide)-2'-deoxyuridine (ImiddU), 5-[N-(1-morpholino-2-ethyl)carboxamide]-2'-deoxyuridine (MOEdU) and a combination thereof.

14. The composition of claim 1, wherein the second aptamer comprises from about 5 to about 15 C-5 modified pyrimidines.

15. The composition of claim 1, wherein the second C-5 pyrimidine modification scheme comprises a C-5 modified pyrimidine selected from the group consisting of 5-(N-tryptaminocarboxyamide)-2'-deoxyuridine (TrpdU), 5-[N-(1-naphthylmethyl)carboxyamide]-2'-deoxyuridine (NapdU), 5-[N-(2-naphthylmethyl)carboxyamide]-2'-deoxyuridine (2-NapdU), and a combination thereof.

16. The composition of claim 1, wherein each uracil or thymine of the second aptamer is a C-5 modified pyrimidine selected from the group consisting of 5-[N-(2-thiophene-methyl)carboxamide]-2'-deoxyuridine (ThdU), 5-(N-isobutylcarboxyamide)-2'-deoxyuridine (iBudU), 5-(N-isobutylcarboxyamide)-2'-O-methyluridine, 5-(N-

isobutylcarboxyamide)-2'-fluorouridine, 5-(N-tryptaminocarboxyamide)-2'-deoxyuridine (TrpdU), 5-(N-tryptaminocarboxyamide)-2'-O-methyluridine, 5-(N-tryptaminocarboxyamide)-2'-fluorouridine, 5-(N-[1-(3-trimethylamonium) propyl] carboxyamide)-2'-deoxyuridine chloride, 5-[N-(1-naphthylmethyl)carboxyamide]-2'-deoxyuridine (NapdU), 5-[N-(2-naphthylmethyl)carboxyamide]-2'-deoxyuridine (2-NapdU), 5-[N-(1-naphthylethyl)carboxyamide]-2'-deoxyuridine (NEdU), 5-[N-(2-naphthylethyl)carboxyamide]-2'-deoxyuridine (2NEdU), 5-(N-naphthylmethylcarboxyamide)-2'-O-methyluridine, 5-(N-naphthylmethylcarboxyamide)-2'-fluorouridine, 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine, 5-[N-(3-benzo[*b*]thiophene-2-ethyl)carboxamide]-2'-deoxyuridine (BTdU), 5-[N-(3-benzo[*a*] furan-2-ethyl)carboxamide]-2'-deoxyuridine (BFdU), 5-[N-(3,4-methylenedioxybenzyl)carboxamide]-2'-deoxyuridine (MBndU), 5-[N-((R)-2-tetrahydrofurylmethyl)carboxamide]-2'-deoxyuridine (RTHdU), 5-[N-((S)-2-tetrahydrofurylmethyl)carboxamide]-2'-deoxyuridine (STHFdU), 5-(N-2-imidazolylethylcarboxamide)-2'-deoxyuridine (ImiddU), and 5-[N-(1-morpholino-2-ethyl)carboxamide]-2'-deoxyuridine (MOEdU).

17. The composition of claim 1, wherein the first aptamer and the second aptamer, independently, are each from 20 to 100 nucleotides in length.

18. The composition of claim 1, wherein the first aptamer and/or the second aptamer further comprise a detectable moiety.

19. The composition of claim 18, wherein the detectable moiety is selected from the group consisting of a dye, a quantum dot, a radiolabel, an electrochemical functional group, an enzyme, an enzyme substrate, a ligand and a receptor.

20. The composition of claim 1, wherein the target comprises a protein or a peptide.

21. The composition of claim 20 , wherein the target is a protein selected from the group consisting ANGPT2, TSP2, CRDL1 , MATN2, GPVI, ESAM, C7, PLG, MMP-12, NPS-PLA2 and CdtA.

22. The composition of claim 1, wherein the dissociation constant (K_d) for the ternary complex is at least 0.02 nM, or from about 0.01 nM to about 10 nM, or from about 0.02 nM to about 6 nM, or from about 0.02 nM to about 3 nM.

23. A method for detecting a target in a sample, the method comprising:

a) contacting the sample with a first aptamer to form a mixture, wherein the first aptamer is capable of binding to the target to form a first complex;

b) incubating the mixture under conditions that allow for the first complex to form;

c) contacting the mixture with a second aptamer, wherein the second aptamer is capable of binding the first complex to form a second complex;

d) incubating the mixture under conditions that allow for the second complex to form;

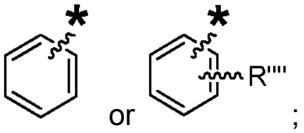
e) detecting for the presence or absence of the first aptamer, the second aptamer, the target, the first complex or the second complex in the mixture, wherein the presence of the first aptamer, the second aptamer, the target, the first complex or the second complex indicates that the target is present in the sample; and

wherein, the first aptamer comprises a first C-5 pyrimidine modification scheme, the second aptamer comprises a second C-5 pyrimidine modification scheme,

wherein the first C-5 pyrimidine modification scheme comprises a chemical modification at a C-5 position comprising K-NH-C(O)-,

wherein

K represents R'-$(CH_2)_n$-;

R' represents  ;

wherein

* denotes point of attachment of the R' group to $(CH_2)_n$ connecting group;

n = 0-3;

R"" is selected from the group consisting of a branched or linear C1-C20 alkyl; OH, halogen; CN; BO_2H_2; COOH; COOR''; $CONH_2$; CONHR''; CONR''R'''; SO_2NH_2; SONHR'';

wherein

R'' and R''' are independently selected from a group consisting of a branched or linear C1-C20 alkyl; C_6H_5; R''''C_6H_4, wherein R'''' is defined above; COOH; COOR'''', wherein R'''' is a branched or linear C1-C20 alkyl; and cycloalkyl; and

wherein the first C-5 pyrimidine modification scheme and the second C-5 pyrimidine modification scheme are different.

24. A method comprising:

a) contacting a target with a first aptamer to form a mixture, wherein the first aptamer is capable of binding the target to form a first complex;

b) incubating the mixture under conditions that allow for the first complex to form;

c) contacting the mixture with a second aptamer, wherein the second aptamer is capable of binding the target to form a second complex;

d) incubating the mixture under conditions that allow for the second complex to form;

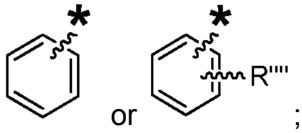
e) detecting for the presence or absence of the first aptamer and the second aptamer in the mixture, wherein the presence of both the first aptamer and second aptamer in the mixture indicates that the binding of the first aptamer to the target and the binding of the second aptamer to the target is non-competitive; and

wherein, the first aptamer comprises a first C-5 pyrimidine modification scheme, the second aptamer comprises a second C-5 pyrimidine modification scheme,

wherein the first C-5 pyrimidine modification scheme comprises a chemical modification at a C-5 position comprising K-NH-C(O)-,

wherein

K represents R'-$(CH_2)_n$-;

R' represents  ;

wherein

* denotes point of attachment of the R' group to $(CH_2)_n$ connecting group;

n = 0-3;

R'''' is selected from the group consisting of a branched or linear C1-C20 alkyl; OH, halogen; CN; BO_2H_2; COOH; COOR''; $CONH_2$; CONHR''; CONR''R'''; SO_2NH_2; SONHR'';

wherein

R'' and R''' are independently selected from a group consisting of a branched or linear C1-C20 alkyl; C_6H_5; R''''C_6H_4, wherein R'''' is defined above; COOH; COOR'''', wherein R'''' is a branched or linear C1-C20 alkyl; and cycloalkyl; and

wherein the first C-5 pyrimidine modification scheme and the second C-5 pyrimidine modification scheme are different.

25. The method of claims 23 or 24, wherein the first aptamer has binding affinity for the target and not the second aptamer.

26. The method of claims 23 or 24, wherein the second aptamer has binding affinity for the target and not the first aptamer.

27. The method of claims 23 or 24, wherein the second aptamer has binding affinity for the first complex.

28. The method of claims 23 or 24, wherein the first aptamer binding region of the target and the second aptamer binding region of the target are different regions.

29. The method of claims 23 or 24, wherein the first aptamer and the second aptamer, independently, comprise RNA, DNA or a combination thereof.

30. The method of claims 23 or 24, wherein the first C-5 pyrimidine modification scheme comprises a C-5 modified pyrimidine selected from the group consisting of 5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU), 5-(N-benzylcarboxyamide)-2'-O-methyluridine, 5-(N-benzylcarboxyamide)-2'-fluorouridine, 5-(N-phenethylcarboxyamide)-2'-deoxyuridine (PEdU), 5-[N-(phenyl-3-propyl)carboxamide]-2'-deoxyuridine (PPdU), 5-[N-(4-fluorobenzyl)carboxyamide]-2'-deoxyuridine (FBndU), 5-[N-(4-hydroxyphenyl-2-ethyl)carboxamide]-2'-deoxyuridine (TyrdU) and a combination thereof.

31. The method of claims 23 or 24, wherein the first C-5 pyrimidine modification scheme comprises a 5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU).

32. The method of claims 23 or 24, wherein each uracil or thymine of the first aptamer is a C-5 modified pyrimidine selected from the group consisting of 5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU), 5-(N-benzylcarboxyamide)-2'-O-methyluridine, 5-(N-benzylcarboxyamide)-2'-fluorouridine, 5-(N-phenethylcarboxyamide)-2'-deoxyuridine (PEdU), 5-[N-(phenyl-3-propyl)carboxamide]-

2'-deoxyuridine (PPdU), 5-[N-(4-fluorobenzyl)carboxyamide]-2'-deoxyuridine (FBndU) and 5-[N-(4-hydroxyphenyl-2-ethyl)carboxamide]-2'-deoxyuridine (TyrdU).

33. The method of claims 23 or 24, wherein each uracil or thymine of the first aptamer is a 5-(N-benzylcarboxyamide)-2'-deoxyuridine (BndU).

34. The method of claims 23 or 24, wherein the second C-5 pyrimidine modification scheme comprises a C-5 modified pyrimidine selected from the group consisting of 5-[N-(2-thiophene-methyl)carboxamide]-2'-deoxyuridine (ThdU), 5-(N-isobutylcarboxyamide)-2'-deoxyuridine (iBudU), 5-(N-isobutylcarboxyamide)-2'-O-methyluridine, 5-(N-isobutylcarboxyamide)-2'-fluorouridine, 5-(N-tryptaminocarboxyamide)-2'-deoxyuridine (TrpdU), 5-(N-tryptaminocarboxyamide)-2'-O-methyluridine, 5-(N-tryptaminocarboxyamide)-2'-fluorouridine, 5-(N-[1-(3-trimethylamonium) propyl]carboxyamide)-2'-deoxyuridine chloride, 5-[N-(1-naphthylmethyl)carboxyamide]-2'-deoxyuridine (NapdU), 5-[N-(2-naphthylmethyl)carboxyamide]-2'-deoxyuridine (2-NapdU), 5-[N-(1-naphthylethyl)carboxyamide]-2'-deoxyuridine (NEdU), 5-[N-(2-naphthylethyl)carboxyamide]-2'-deoxyuridine (2NEdU), 5-(N-naphthylmethylcarboxyamide)-2'-O-methyluridine, 5-(N-naphthylmethylcarboxyamide)-2'-fluorouridine, 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine, 5-[N-(3-benzo[*b*]thiophene-2-ethyl)carboxamide]-2'-deoxyuridine (BTdU), 5-[N-(3-benzo[*a*]furan-2-ethyl)carboxamide]-2'-deoxyuridine (BFdU), 5-[N-(3,4-methylenedioxybenzyl)carboxamide]-2'-deoxyuridine (MBndU), 5-[N-((R)-2-tetrahydrofurylmethyl)carboxamide]-2'-deoxyuridine (RTHdU), 5-[N-((S)-2-tetrahydrofurylmethyl)carboxamide]-2'-deoxyuridine (STHFdU), 5-(N-2-imidazolylethylcarboxamide)-2'-deoxyuridine (ImiddU), 5-[N-(1-morpholino-2-ethyl)carboxamide]-2'-deoxyuridine (MOEdU) and a combination thereof.

35. The method of claims 23 or 24, wherein the second C-5 pyrimidine modification scheme comprises a C-5 modified pyrimidine selected from the group consisting of 5-(N-isobutylcarboxyamide)-2'-deoxyuridine (iBudU), 5-(N-tryptaminocarboxyamide)-2'-deoxyuridine (TrpdU), 5-[N-(1-naphthylmethyl)carboxyamide]-2'-deoxyuridine (NapdU), 5-[N-(2-naphthylmethyl)carboxyamide]-2'-deoxyuridine (2-NapdU), and a combination thereof.

36. The method of claims 23 or 24, wherein the second C-5 pyrimidine modification scheme comprises a C-5 modified pyrimidine selected from the group consisting of 5-(N-tryptaminocarboxyamide)-2'-deoxyuridine (TrpdU), 5-[N-(1-naphthylmethyl)carboxyamide]-2'-deoxyuridine (NapdU), 5-[N-(2-naphthylmethyl)carboxyamide]-2'-deoxyuridine (2-NapdU), and a combination thereof.

37. The method of claims 23 or 24, wherein each uracil or thymine of the second aptamer is a C-5 modified pyrimidine selected from the group consisting of 5-[N-(2-thiophene-methyl)carboxamide]-2'-deoxyuridine (ThdU), 5-(N-isobutylcarboxyamide)-2'-deoxyuridine (iBudU), 5-(N-isobutylcarboxyamide)-2'-O-methyluridine, 5-(N-isobutylcarboxyamide)-2'-fluorouridine, 5-(N-tryptaminocarboxyamide)-2'-deoxyuridine (TrpdU), 5-(N-tryptaminocarboxyamide)-2'-O-methyluridine, 5-(N-tryptaminocarboxyamide)-2'-fluorouridine, 5-(N-[1-(3-trimethylamonium) propyl]carboxyamide)-2'-deoxyuridine chloride, 5-[N-(1-naphthylmethyl)carboxyamide]-2'-deoxyuridine (NapdU), 5-[N-(2-naphthylmethyl)carboxyamide]-2'-deoxyuridine (2-NapdU), 5-[N-(1-naphthylethyl)carboxyamide]-2'-deoxyuridine (NEdU), 5-[N-(2-naphthylethyl)carboxyamide]-2'-deoxyuridine (2NEdU), 5-(N-naphthylmethylcarboxyamide)-2'-O-methyluridine, 5-(N-naphthylmethylcarboxyamide)-2'-fluorouridine, 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine, 5-[N-(3-benzo[*b*]thiophene-2-ethyl)carboxamide]-2'-deoxyuridine (BTdU), 5 - [N-(3-benzo[*a*]furan-2-ethyl)carboxamide]-2'-deoxyuridine (BFdU), 5-[N-(3,4-methylenedioxybenzyl)carboxamide]-2'-deoxyuridine (MBndU), 5-[N-((R)-2-tetrahydrofurylmethyl)carboxamide]-2'-deoxyuridine (RTHdU), 5-[N-((S)-2-tetrahydrofurylmethyl)carboxamide]-2'-deoxyuridine (STHFdU), 5-(N-2-imidazolylethylcarboxamide)-2'-deoxyuridine (ImiddU), and 5-[N-(1-morpholino-2-ethyl)carboxamide]-2'-deoxyuridine (MOEdU) .

38. The method of claims 23 or 24, wherein the first aptamer and the second aptamer, independently, are each from 20 to 100 nucleotides in length.

39. The method of claims 23 or 24, wherein the first aptamer and/or the second aptamer further comprise a detectable moiety.

40. The method of claim 39, wherein the detectable moiety is selected from the group consisting of a dye, a quantum dot, a radiolabel, an electrochemical functional group, an enzyme, an enzyme substrate, a ligand and a receptor.

41. The method of claims 23 or 24, wherein the target comprises a protein or a peptide.

42. The method of claim 41, wherein the target is a protein selected from the group consisting ANGPT2, TSP2, CRDL1 , MATN2, GPVI, ESAM, C7, PLG, MMP-12, NPS-PLA2 and CdtA.

43. The method of claims 23 or 24, wherein the dissociation constant (K_d) for the second complex is at least 0.02 nM, or from about 0.01 nM to about 10 nM, or from about 0.02 nM to about 6 nM, or from about 0.02 nM to about 3 nM.

44. The method of claims 23 or 24, wherein the dissociation constant (K_d) for the first complex is from about 0.04 nM to about 5 nM, or from about 0.04 nM to about 4.8 nM.

45. The method of claims 23 or 24, wherein the dissociation constant (K_d) for the second aptamer and the target is from about 0.03 nM to about 14 nM.