Claims

1. A kit for multiplex analyte encoding, comprising

(A) at least twenty (20) different sets of analyte-specific probes for encoding of at least 20 different analytes, each set of analyte-specific probes interacting with a different analyte, wherein if the analyte is a nucleic acid each set of analyte-specific probes comprises at least five (5) analyte-specific probes which specifically interact with different sub-structures of the same analyte, each analyte-specific probe comprising

(aa) a binding element (S) that specifically interacts with one of the different analytes to be encoded, and

(bb) an identifier element (T) comprising a nucleotide sequence which is unique to the analyte to be encoded (unique identifier sequence),

wherein the analyte-specific probes of a particular set of analyte-specific probes differ from the analyte-specific probes of another set of analyte-specific probes in the nucleotide sequence of the identifier element (T),

wherein the analyte-specific probes in each set of analyte-specific probes binds to the same analyte and comprises the same nucleotide sequence of the identifier element (T) which is unique to said analyte; and

(B) at least one set of decoding oligonucleotides per analyte, wherein in each set of decoding oligonucleotides for an individual analyte each decoding oligonucleotide comprises:

(aa) an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and

(bb) a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide;

wherein the decoding oligonucleotides of a set for an individual analyte differ from the decoding oligonucleotides of another set for a different analyte in the identifier connect element (t); and

(C) a set of signal oligonucleotides, each signal oligonucleotide comprising:

(aa) a translator connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of a translator element (c) comprised in a decoding oligonucleotide, and

(bb) a signal element.

~~2. The kit according to claim 1, wherein the kit does not comprise sets of analyte-specific probes as defined under item A) in claim 1.~~

~~3. The kit according to any one of claims 1 to 2, wherein if the analyte is a nucleic acid, each set of analyte-specific probes comprises at least five (10) analyte-specific probes, in particular at least fifteen (15) analyte-specific probes, in particular at least twenty (20) analyte-specific probes which specifically interact with different sub-structures of the same analyte.~~

~~4. The kit according to any one of claims 1 to 2, wherein if the analyte is a peptide, a polypeptide or a protein, each set of analyte-specific probes comprises at least two (2) analyte-specific probes, in particular at least three (3) analyte-specific probes, in particular at least four (4) analyte-specific probes which specifically interact with different sub-structures of the same analyte.~~

~~5. Th~~e ~~kit according to any one of claims 1 to 4, wherein the kit comprises at least two different sets of signal oligonucleotides, wherein the signal oligonucleotides in each set comprise a different signal element and comprise a different connector element (C).~~

~~6.~~2. The kit according to ~~any one of claims 1 to 5~~claim 1, wherein the kit comprises at least two different sets of decoding oligonucleotides per analyte,

wherein the decoding oligonucleotides comprised in these different sets comprise the same identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and

wherein the decoding oligonucleotides of the different sets per analyte differ in the translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.

~~7.~~3. The kit according to any one of claims 1 to ~~6~~2, wherein the kit comprises at least two different sets of decoding oligonucleotides per analyte,

wherein the decoding oligonucleotides comprised in these different sets comprise the same identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and

wherein the decoding oligonucleotides of the different sets for at least one analyte differ in the translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.

8.4. The kit according to of any one of claims 1 to ~~7~~3, wherein the number of different sets of decoding oligonucleotides per analyte comprising different translator elements (c) corresponds to the number of different sets of signal oligonucleotides comprising different connector elements (C).

~~9. The kit according to any one of claim 1 to 8, wherein the decoding oligonucleotides in a particular set of decoding oligonucleotides interacts with identical identifier elements (T) which are unique to a particular analyte.~~

~~10. The kit according to of any one of claims 1 to 9, wherein all sets of decoding oligonucleotides for the different analytes comprise the same type(s) of translator element(s) (c).~~

~~11. The kit according to of any one of claims 1 to 10, wherein the kit comprises:~~

~~**(D)** at least a set of non-signal decoding oligonucleotides for binding to a particular identifier element (T) of analyte-specific probes, wherein the decoding oligonucleotides in the same set of non-signal decoding oligonucleotides interacting with the same different identifier element (T),~~

~~wherein each non-signal decoding oligonucleotide comprises an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of a unique identifier sequence, and does not comprise a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.~~

~~12. The kit according to of any one of claims 1 to 11, wherein the kit comprises:~~

~~**(D)** at least two (2) different sets of non-signal decoding oligonucleotides for binding to at least two different identifier elements (T) of analyte-specific probes, each set of non-signal decoding oligonucleotides interacting with a different identifier element (T),~~

~~wherein each non-signal decoding oligonucleotide comprises an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of a unique identifier sequence, and does not comprise a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.~~

~~13. The kit according to any one of claims 11 to 12, wherein the different sets of non-signal decoding oligonucleotides may be comprised in a pre-mixture of different sets of non-signal decoding oligonucleotides or exist separately.~~

~~14.~~5. The kit according to any one of claims 1 to ~~13~~4, wherein the kit comprises:

(E) a set of non-signal oligonucleotides, each non-signal oligonucleotide comprising:

(aa) a translator connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of the translator element (c), and
(bb) a quencher (Q), a signal element and a quencher (Q), or does not comprise a signal element.

~~15. The kit according to any one of claims 1 to 14, wherein the kit comprises:~~

~~**(E)** at least two sets of non-signal oligonucleotides, each non-signal oligonucleotide comprising:~~

~~(aa) a translator connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of the translator element (c), and~~
~~(bb) a quencher (Q), a signal element and a quencher (Q), or does not comprise a signal element.~~

16. The kit according to any one of claims 14 to 15, wherein the different sets of non-signal oligonucleotides may be comprised in a pre-mixture of different sets of non-signal oligonucleotides or exist separately.

17. The kit according to any one of claims 1 to 16, wherein the decoding oligonucleotides in a particular set of decoding oligonucleotides interacts with identical identifier elements (T) which are unique to a particular analyte.

18. The kit according to any one of claims 1 to 17, wherein the different sets of decoding oligonucleotides may be comprised in a pre-mixture of different sets of decoding oligonucleotides or exist separately.

19. The kit according to any one of claims 1 to 18, wherein the different sets of analyte-specific probes may be comprised in a pre-mixture of different sets of analyte-specific probes or exist separately.

20. The kit according to any one of claims 1 to 19, wherein the different sets of signal oligonucleotides may be comprised in a pre-mixture of different sets of signal oligonucleotides or exist separately.

21. The kit according to any of claims 1 to 20, wherein the analyte to be encoded is a nucleic acid, preferably DNA, PNA or RNA, in particular mRNA.

22. The kit according to any of claims 1 to 20, wherein the analyte to be encoded is a peptide, polypeptide or a protein.

23. The kit according to any of claims 1 to 22, wherein the binding element (S) comprises an amino acid sequence allowing a specific binding to the analyte to be encoded.

24. The kit according to any of claims 1 to 23, wherein the binding element (S) comprises moieties which are affinity moieties from affinity substances or affinity substances in their entirety selected from the group consisting of antibodies, antibody fragments, anticalin proteins, receptor ligands, enzyme substrates, lectins, cytokines, lymphokines, interleukins, angiogenic or virulence factors, allergens, peptidic allergens, recombinant allergens, allergen idiotypical

~~antibodies, autoimmune provoking structures, tissue rejection inducing structures, immunoglobulin constant regions and combinations thereof.~~

~~25. The kit according to any of claims 1 to 24, wherein the binding element (S) is an antibody or an antibody fragment selected from the group consisting of Fab, scFv; single domain, or a fragment thereof, bis scFv, F(ab)2, F(ab)3, minibody, diabody, triabody, tetrabody and tandab.~~

~~26. The kit according to any one of claims 1 to 25, wherein the decoding oligonucleotides in at least one set of decoding oligonucleotides are multi-decoders comprising~~

~~(aa) an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and~~

~~(bb) at least two translator elements (c), wherein the translator elements comprising different nucleotide sequences allowing a specific hybridization of a different signal oligonucleotide.~~

~~27. The kit according to claim 26, wherein the different signal oligonucleotides comprise a different signal element and comprise a different connector element (C).~~

~~28.~~6. A multiplex method for detecting different analytes in a sample by sequential signal-encoding of said analytes, comprising:

(A) contacting the sample with at least twenty (20) different sets of analyte-specific probes for encoding of at least 20 different analytes, each set of analyte-specific probes interacting with a different analyte, wherein if the analyte is a nucleic acid each set of analyte-specific probes comprises at least five (5) analyte-specific probes which specifically interact with different sub-structures of the same analyte, each analyte-specific probe comprising

(aa) a binding element (S) that specifically interacts with one of the different analytes to be encoded, and

(bb) an identifier element (T) comprising a nucleotide sequence which is unique to the analyte to be encoded (unique identifier sequence),

wherein the analyte-specific probes of a particular set of analyte-specific probes differ from the analyte-specific probes of another set of analyte-specific probes in the nucleotide sequence of the identifier element (T),

wherein the analyte-specific probes in each set of analyte-specific probes binds to the same analyte and comprises the same nucleotide sequence of the identifier element (T) which is unique to said analyte; and

(B) contacting the sample with at least one set of decoding oligonucleotides per analyte, wherein in each set of decoding oligonucleotides for an individual analyte each decoding oligonucleotide comprises:

(aa) an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and

(bb) a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide;

wherein the decoding oligonucleotides of a set for an individual analyte differ from the decoding oligonucleotides of another set for a different analyte in the first connect element (t); and

(C) contacting the sample with at least a set of signal oligonucleotides, each signal oligonucleotide comprising:

(aa) a translator connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of a translator element (c) comprised in a decoding oligonucleotide, and

(bb) a signal element.

(D) Detecting the signal caused by the signal element;

(E) selectively removing the decoding oligonucleotides and signal oligonucleotides from the sample, thereby essentially maintaining the specific binding of the analyte-specific probes to the analytes to be encoded;

(F) Performing at least three (3) further cycles comprising steps B) to E) to generate an encoding scheme with a code word per analyte, wherein in particular the last cycle may stop with step (D).

~~29.~~7. The method according to claim ~~28~~6, wherein all steps are automated, in particular wherein steps B) to F) are automated, in particular by using a robotic system.

~~30. The method according to any one of claims 28 to 29, wherein all steps are performed in a fluidic system.~~

~~31.~~8. The method according to any one of claims ~~28~~6 to ~~30~~7, wherein each analyte is associated with a specific code word, wherein said code word comprise a number of positions, and wherein each position corresponds to one cycle resulting in a plurality of distinguishable encoding schemes with the plurality of code words.

~~32. The method according to any one of claims 28 to 31, wherein said encoding scheme is predetermined and allocated to the analyte to be encoded.~~

~~33. The method according to any one of claims 28 to 32, wherein the code words obtained for the individual analytes in the performed cycles comprise the detected signals and additionally at least one element corresponding to no detected signal.~~

~~34.~~9. The method according to any one of claims ~~28~~6 to ~~33~~8, wherein no signal is detected for at least one analyte within at least one cycle.

~~35.~~10. The method according to ~~of~~ any one of claims ~~28~~6 to ~~34~~9, wherein for at least for one individual analyte a position of the code word is zero (0).

~~36.~~11. The method according to of any one of claims ~~28~~6 to ~~35~~10, wherein the ~~-~~code word zero (0) is generated ~~-~~by using no decoding oligonucleotides ~~-~~having an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of a corresponding analyte-specific probe for an individual analyte.

~~37.~~12. The method according to of any one of claims ~~28~~ 6 to ~~36~~11, wherein if at least for one individual analyte a position of the code word is zero (0) in this cycle no corresponding decoding oligonucleotides ~~-~~having an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier

sequence of the identifier element (T) of a corresponding analyte-specific probe for an individual analyte are used.

~~38. The method according to of any one of claims 28 to 37, wherein the sample is contacted with at least two different sets of signal oligonucleotides, wherein the signal oligonucleotides in each set comprise a different signal element and comprise a different connector element (C).~~

~~39. The method according to any one of claims 28 to 38, wherein the sample is contacted with at least two different sets of decoding oligonucleotides per analyte,~~

~~wherein the decoding oligonucleotides comprised in these different sets comprise the same identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and~~

~~wherein the decoding oligonucleotides of the different sets per analyte differ in the translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.~~

~~40. The method according to any one of claims 28 to 39, wherein the sample is contacted with at least two different sets of decoding oligonucleotides per analyte,~~

~~wherein the decoding oligonucleotides comprised in these different sets comprise the same identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and~~

~~wherein the decoding oligonucleotides of the different sets per analyte differ in the translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide;~~

~~wherein only one set of decoding oligonucleotides per analyte is used per cycle, and/or wherein different sets of decoding oligonucleotides are used in different cycles in combination with the corresponding set of signal oligonucleotides in the same cycle.~~

~~41. The method according to of any one of claims 28 to 40, wherein the number of different sets of decoding oligonucleotides per analyte comprising different translator elements (c) corresponds to the number of different sets of signal oligonucleotides comprising different connector elements (C).~~

42. The method according to of any one of claims 28 to 41, wherein all sets of decoding oligonucleotides for the different analytes comprise the same type(s) of translator element(s) (c).

43. The method according to of any one of claims 28 to 42, wherein the sample is contacted with

at least a set of non-signal decoding oligonucleotides for binding to a particular identifier element (T) of analyte-specific probes, wherein the decoding oligonucleotides in the same set of non-signal decoding oligonucleotides interacting with the same different identifier element (T),

wherein each non-signal decoding oligonucleotide comprises an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of a unique identifier sequence, and does not comprise a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.

44. The method according to of any one of claims 28 to 43, wherein the sample is contacted with:

at least two (2) different sets of non-signal decoding oligonucleotides for binding to at least two different identifier elements (T) of analyte-specific probes, each set of non-signal decoding oligonucleotides interacting with a different identifier element (T),

wherein each non-signal decoding oligonucleotide comprises an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of a unique identifier sequence, and does not comprise a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.

45. The method according to any one of claims 28 to 44, wherein the different sets of non-signal decoding oligonucleotides may be comprised in a pre-mixture of different sets of non-signal decoding oligonucleotides or exist separately.

46. The method according to any one of claims 28 to 45, wherein the sample is contacted with

a set of non-signal oligonucleotides, each non-signal oligonucleotide comprising:

(aa) a translator connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of the translator element (c), and

(bb) a quencher (Q), a signal element and a quencher (Q), or does not comprise a signal element.

~~47.~~13. The method according to any one of claims ~~28~~6 to ~~46~~12, wherein the sample is contacted with:

at least two sets of non-signal oligonucleotides, each non-signal oligonucleotide comprising:

(aa) a translator connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of the translator element (c), and

(bb) a quencher (Q), a signal element and a quencher (Q), or does not comprise a signal element.

~~48. The method according to any one of claims 28 to 47, wherein the different sets of non-signal oligonucleotides may be comprised in a pre-mixture of different sets of non-signal oligonucleotides or exist separately.~~

~~49. The method according to any one of claims 28 to 48, wherein the decoding oligonucleotides in a particular set of decoding oligonucleotides interacts with identical identifier elements (T) which are unique to a particular analyte.~~

~~50. The method according to any one of claims 28 to 49, wherein the different sets of decoding oligonucleotides may be comprised in a pre-mixture of different sets of decoding oligonucleotides or exist separately.~~

~~51. The method according to any one of claims 28 to 50, wherein the different sets of analyte-specific probes may be comprised in a pre-mixture of different sets of analyte-specific probes or exist separately.~~

~~52. The method according to any one of claims 28 to 51, wherein the different sets of signal oligonucleotides may be comprised in a pre-mixture of different sets of signal oligonucleotides or exist separately.~~

~~53. The method according to any one of claims 28 to 52, wherein the sample is a biological sample, preferably comprising biological tissue, further preferably comprising biological cells and/or extracts and/or part of cells.~~

~~54. The method according to claim 53, wherein the cell is a prokaryotic cells or a eukaryotic cell, in particular a mammalian cell, in particular a human cell.~~

~~55. The method according to claim 53, wherein the biological tissue, biological cells, extracts and/or part of cells are fixed.~~

~~56. The method according to any one of claims 28 to 55, wherein the analytes are fixed in a permeabilized sample, such as a cell-containing sample.~~

~~57. The method according to any one of claims 28 to 56, wherein the binding element (S) comprise a nucleic acid comprising a nucleotide sequence allowing a specific binding to the analyte to be encoded, preferably a specific hybridization to the analyte to be encoded.~~

~~58. The method according to any one of claims 28 to 57, wherein after step A) and before step B) the non-bound analyte-specific probes are removed, in particular by washing.~~

~~59. The method according to any one of claims 28 to 58, wherein after step B) and before step C) the non-bound decoding oligonucleotides are removed, in particular by washing.~~

~~60. The method according to any one of claims 28 to 59, wherein after step C) and before step D) the non-bound signal oligonucleotides are removed, in particular by washing.~~

~~61. The method according to any one of claims 28 to 60, wherein the analyte specific probes are incubated with the sample, thereby allowing a specific binding of the analyte specific probes to the analytes to be encoded.~~

~~62. The method according to any one of claims 28 to 61, wherein the decoding oligonucleotides are incubated with the sample, thereby allowing a specific hybridization of the decoding oligonucleotides to identifier elements (T) of the respective analyte-specific probes.~~

~~63. The method according to any one of claims 28 to 62, wherein the signal oligonucleotides are incubated with the sample, thereby allowing a specific hybridization of the signal oligonucleotides to translator elements (T) of the respective decoding oligonucleotides.~~

~~64. The method according to any of claims 28 to 63, wherein the analyte to be encoded is a nucleic acid, preferably DNA, PNA or RNA, in particular mRNA.~~

~~65. The method according to any of claims 28 to 63, wherein the analyte to be encoded is a peptide, polypeptide or a protein.~~

~~66. The method according to any of claims 28 to 65, wherein the binding element (S) comprise an amino acid sequence allowing a specific binding to the analyte to be encoded.~~

~~67. The method according to any of claims 28 to 66, wherein the binding element (S) comprises moieties which are affinity moieties from affinity substances or affinity substances in their entirety selected from the group consisting of antibodies, antibody fragments, anticalin proteins, receptor ligands, enzyme substrates, lectins, cytokines, lymphokines, interleukins, angiogenic or virulence factors, allergens, peptidic allergens, recombinant allergens, allergen-idiotypical antibodies, autoimmune-provoking structures, tissue-rejection-inducing structures, immunoglobulin constant regions and combinations thereof.~~

~~68. The method according to any of claims 28 to 67, wherein the binding element (S) is an antibody or an antibody fragment selected from the group consisting of Fab, scFv; single-domain, or a fragment thereof, bis scFv, Fab 2, Fab 3, minibody, diabody, triabody, tetrabody and tandab.~~

~~69. The method according to any of claims 28 to 68, wherein the signal caused by the signal element, therefore in particular the binding of the signal oligonucleotides to the decoding oligonucleotides, interacting with the corresponding analyte probes, bound to the respective analyte is determined by:~~

~~(a) Imaging at least a portion of the sample; and/or~~

~~(b) Using an optical imaging technique; and/or~~

~~(c) Using a fluorescence imaging technique; and/or~~

~~(d) Multi-color fluorescence imaging technique; and/or~~

~~(e) Super-resolution fluorescence imaging technique.~~

~~70. The method according to any one of claims 28 to 69, wherein the decoding oligonucleotides in at least one set of decoding oligonucleotides are multi-decoders comprising~~

~~(aa) an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and~~

~~(bb) at least two translator elements (c), wherein the translator elements comprising different nucleotide sequences allowing a specific hybridization of a different signal oligonucleotide.~~

~~71. The method according to claim 70, wherein the different signal oligonucleotides comprise a different signal element and comprise a different connector element (C).~~

~~72.~~14. An *in vitro* method for diagnosis of a disease selected from the group comprising cancer, neuronal diseases, cardiovascular diseases, inflammatory diseases, autoimmune diseases, diseases due to a viral or bacterial infection, skin diseases, skeletal muscle diseases, dental diseases and prenatal diseases comprising the use of the multiplex method according to any one of claims ~~2~~86 to ~~71~~13.

~~73.~~15. An *in vitro* method for diagnosis of a disease in plants selected from the group comprising: diseases caused by biotic stress, preferably by infectious and/or parasitic origin, or diseases caused by abiotic stress, preferably caused by nutritional deficiencies and/or unfavorable environment, said method comprising the use of the multiplex method according to any one of claims ~~2~~86 to ~~71~~13.

~~74.~~16. An optical multiplexing system suitable for the method according to any one of claims ~~2~~8 6 to ~~71~~13, comprising at least:

- one reaction vessel for containing the kits or part of the kits according to any one of claims 1 to ~~2~~75;
- a detection unit comprising a microscope, in particular a fluorescence microscope
- a camera
- a liquid handling device.

~~75. The optical multiplexing system according to claim 74, wherein the system comprises further a heat and cooling device.~~

76. The optical multiplexing system according to any one of claim 74 to 75, wherein the system comprises further a robotic system.

77. A kit for multiplex analyte encoding, comprising

(A) optionally at least twenty (20) different sets of analyte-specific probes for encoding of at least 20 different analytes, each set of analyte-specific probes interacting with a different analyte, wherein if the analyte is a nucleic acid each set of analyte-specific probes comprises at least five (5) analyte-specific probes which specifically interact with different sub-structures of the same analyte, each analyte-specific probe comprising

(aa) a binding element (S) that specifically interacts with one of the different analytes to be encoded, and

(bb) an identifier element (T) comprising a nucleotide sequence which is unique to the analyte to be encoded (unique identifier sequence),

wherein the analyte-specific probes of a particular set of analyte-specific probes differ from the analyte-specific probes of another set of analyte-specific probes in the nucleotide sequence of the identifier element (T),

wherein the analyte-specific probes in each set of analyte-specific probes binds to the same analyte and comprises the same nucleotide sequence of the identifier element (T) which is unique to said analyte; and

(B) at least one set of decoding oligonucleotides per analyte, wherein in each set of decoding oligonucleotides for an individual analyte each decoding oligonucleotide comprises:

(aa) an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and

(bb) a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide;

wherein the decoding oligonucleotides of a set for an individual analyte differ from the decoding oligonucleotides of another set for a different analyte in the identifier connect element (t); and

(C) a set of signal oligonucleotides, each signal oligonucleotide comprising:

(aa) a translator connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of a translator element (c) comprised in a decoding oligonucleotide, and

(bb) a signal element.

78. The kit according to claim 77, wherein if the analyte is a nucleic acid, each set of analyte-specific probes comprises at least five (10) analyte-specific probes, in particular at least fifteen (15) analyte-specific probes, in particular at least twenty (20) analyte-specific probes which specifically interact with different sub-structures of the same analyte, each analyte-specific probe.

79. The kit according to claim 77, wherein if the analyte is a peptide, polypeptide or a protein, each set of analyte-specific probes comprises at least two (2) analyte-specific probes, in particular at least three (3) analyte-specific probes, in particular at least four (4) analyte-specific probes which specifically interact with different sub-structures of the same analyte, each analyte-specific probe.

80.The kit according to any one of claims 77 to 79, wherein the kit comprises at least two different sets of signal oligonucleotides, wherein the signal oligonucleotides in each set comprise a different signal element and comprise a different connector element (C).

81. The kit according to any one of claims 77 to 80, wherein the kit comprises at least two different sets of decoding oligonucleotides per analyte,

wherein the decoding oligonucleotides comprised in these different sets comprise the same identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and

wherein the decoding oligonucleotides of the different sets per analyte differ in the translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.

82. The kit according to any one of claims 77 to 81, wherein the kit comprises at least two different sets of decoding oligonucleotides per analyte,

wherein the decoding oligonucleotides comprised in these different sets comprise the same identifier connector element (t) comprising a nucleotide sequence which is essentially

complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and

wherein the decoding oligonucleotides of the different sets for at least one analyte differ in the translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.

83. The kit according to of any one of claims 77 to 82, wherein the number of different sets of decoding oligonucleotides per analyte comprising different translator elements (c) corresponds to the number of different sets of signal oligonucleotides comprising different connector elements (C).

84. The kit according to any one of claims 77 to 83, wherein the decoding oligonucleotides in a particular set of decoding oligonucleotides interacts with identical identifier elements (T) which are unique to a particular analyte.

85. The kit according to of any one of claims 77 to 84, wherein all sets of decoding oligonucleotides for the different analytes comprise the same type(s) of translator element(s) (c).

86. The kit according to of any one of claims 73 to 81, wherein the kit comprises:

(D) at least a set of non-signal decoding oligonucleotides for binding to a particular identifier element (T) of analyte-specific probes, wherein the decoding oligonucleotides in the same set of non-signal decoding oligonucleotides interacting with the same different identifier element (T),

wherein each non-signal decoding oligonucleotide comprises an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of a unique identifier sequence, and does not comprise a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.

87. The kit according to of any one of claims 77 to 6, wherein the kit comprises:

(D) at least two (2) different sets of non-signal decoding oligonucleotides for binding to at least two different identifier elements (T) of analyte-specific probes, each set of non-signal decoding oligonucleotides interacting with a different identifier element (T),

wherein each non-signal decoding oligonucleotide comprises an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of a unique identifier sequence, and does not comprise a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.

88. The kit according to any one of claims 77 to 87, wherein the different sets of non-signal decoding oligonucleotides may be comprised in a pre-mixture of different sets of non-signal decoding oligonucleotides or exist separately.

89. The kit according to any one of claims 77 to 88, wherein the kit comprises:

(E) a set of non-signal oligonucleotides, each non-signal oligonucleotide comprising:

(aa) a translator connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of the translator element (c), and
(bb) a quencher (Q), a signal element and a quencher (Q), or does not comprise a signal element.

90. The kit according to any one of claims 77 to 89, wherein the kit comprises:

(E) at least two sets of non-signal oligonucleotides, each non-signal oligonucleotide comprising:

(aa) a translator connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of the translator element (c), and
(bb) a quencher (Q), a signal element and a quencher (Q), or does not comprise a signal element.

91. The kit according to any one of claims 77 to 90, wherein the different sets of non-signal oligonucleotides may be comprised in a pre-mixture of different sets of non-signal oligonucleotides or exist separately.

92. The kit according to any one of claims 77 to 91, wherein the decoding oligonucleotides in a particular set of decoding oligonucleotides interacts with identical identifier elements (T) which are unique to a particular analyte.

93. The kit according to any one of claims 77 to 92, wherein the different sets of decoding oligonucleotides may be comprised in a pre-mixture of different sets of decoding oligonucleotides or exist separately.

94. The kit according to any one of claims 77 to 93, wherein the different sets of analyte-specific probes may be comprised in a pre-mixture of different sets of analyte-specific probes or exist separately.

95. The kit according to any one of claims 77 to 94, wherein the different sets of signal oligonucleotides may be comprised in a pre-mixture of different sets of signal oligonucleotides or exist separately.

96. The kit according to any of claims 77 to 95, wherein the analyte to be encoded is a nucleic acid, preferably DNA, PNA or RNA, in particular mRNA.

97. The kit according to any of claims 77 to 95, wherein the analyte to be encoded is a peptide, polypeptide or a protein.

98. The kit according to any of claims 77 to 97, wherein the binding element (S) comprises an amino acid sequence allowing a specific binding to the analyte to be encoded.

99. The kit according to any of claims 77 to 98, wherein the binding element (S) comprises moieties which are affinity moieties from affinity substances or affinity substances in their entirety selected from the group consisting of antibodies, antibody fragments, anticalin proteins, receptor ligands, enzyme substrates, lectins, cytokines, lymphokines, interleukins, angiogenic or virulence factors, allergens, peptidic allergens, recombinant allergens, allergen idiotypical antibodies, autoimmune provoking structures, tissue rejection inducing structures, immunoglobulin constant regions and combinations thereof.

100. The kit according to any of claims 77 to 99, wherein the binding element (S) is an antibody or an antibody fragment selected from the group consisting of Fab, scFv; single

~~domain, or a fragment thereof, bis scFv, F(ab)2, F(ab)3, minibody, diabody, triabody, tetrabody and tandab.~~

~~101. The kit according to any of claims 77 to 100 for the use of in combination with different sets of analyte-specific probes defined in any one of claims 1 to 27.~~

~~102. The kit according to any one of claims 77 to 101, wherein the decoding oligonucleotides in at least one set of decoding oligonucleotides are multi-decoders comprising~~

~~(aa) an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and~~

~~(bb) at least two translator elements (c), wherein the translator elements comprising different nucleotide sequences allowing a specific hybridization of a different signal oligonucleotide.~~

~~103. The kit according to claim 102, wherein the different signal oligonucleotides comprise a different signal element and comprise a different connector element (C).~~

~~104.~~17. An *in vitro* method for screening, identifying and/or testing a substance and/or drug comprising:

(a) contacting a test sample comprising a sample with a substance and/or drug

(b) detecting different analytes in a sample by sequential signal-encoding of said analytes with a method according to any one of claims ~~2~~8~~6~~ to ~~7~~13.

~~105. The *in vitro* method according to claim 104, wherein the sample is a biological sample, preferably comprising biological tissue, further preferably comprising biological cells, in particular wherein the cell is a prokaryotic cells or a eukaryotic cell, in particular a mammalian cell, in particular a human cell.~~