Claims

1. A multiplex method for detecting different analytes and different subgroups/variations of an analyte in a sample 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

contacting the sample with at least two different sets of analyte-specific probes for at least one analyte and a variation thereof,

wherein the analyte-specific probes comprised in these different sets interacting with the same analyte, but specifically interact with different sub-structures of the same analyte,

wherein the analyte-specific probes of the first set of analyte-specific probes interacts with a sub-structure which is comprised in all variations of an analyte,

wherein the analyte-specific probes of the second set of analyte-specific probes (subgroup-specific probes) interacts with a sub-structure which is comprised only in a specific variation of the analyte,

wherein the analyte-specific probes of the first set of analyte-specific probes comprise the same identifier element (T) comprising a nucleotide sequence which is unique to the analyte to be encoded (unique identifier sequence), and

wherein the analyte-specific probes of the second set of analyte-specific probes comprise the same identifier element (T) comprising a nucleotide sequence which is unique to the analyte to be encoded (unique identifier sequence),

wherein the identifier elements (T) of the analyte-specific probes of the first set of analyte-specific probes and the identifier elements (T) of the analyte-specific probes of the second set of analyte-specific probes are different for binding different decoding oligonucleotides and/or non-signal decoding oligonucleotides.

(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,

(G) Performing at least one (1) further cycle comprising steps B) to E) to identify the subgroup-specific probes, wherein in particular the cycle may stop with step (D).

2. The method according to claim 1, wherein the set of analyte-specific probes comprises at least five (5) subgroup-specific probes which specifically interact with different sub-structures of the same variation of an analyte.

~~3. The method according to any of claims 1 to 2, wherein if the analyte is a nucleic acid, each set of analyte-specific probes comprises at least ten (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.~~

~~4. The method according to any one of claims 1 to 3, wherein contacting a subgroup of at least one analyte with a set of at least five (5) subgroup-specific probes which differ from the analyte-specific probes of another set of analyte-specific probes in the nucleotide sequence of the identifier element (T).~~

~~5. The method according to any one of claims 1 to 4, wherein the sample is contacted with the subgroup-specific probe set according to claim 30, wherein the method comprises an additional further cycle comprising steps B) to E) to identify the variation interacting with the subgroup-specific probe, wherein in particular the cycle may stop with step (D).~~

~~6.~~3. The method according to any one of claims 1 to ~~5~~2, wherein all steps are automated, in particular wherein steps B) to G) are automated, in particular by using a robotic system.

~~7. The method according to any one of claims 1 to 6, wherein all steps are performed in a fluidic system.~~

~~8.~~4. The method according to any one of claims 1 to ~~7~~3, 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.

~~9. The method according to any one of claims 1 to 8, wherein said encoding scheme is predetermined and allocated to the analyte to be encoded.~~

~~10.~~5. The method according to any one of claims 1 to ~~9~~ 4 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.

~~11. The method according to any one of claims 1 to 10, wherein no signal is detected for at least one analyte within at least one cycle.~~

~~12. The method according to of any one of claims 1 to 11, wherein for at least for one individual analyte a position of the code word is zero (0).~~

~~13.~~6. The method according to of any one of claims 1 to ~~12~~5, 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.

~~14.~~7. The method according to of any one of claims 1 to ~~13~~6, 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.

~~15.~~8. The method according to of any one of claims 1 to ~~14~~7, 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).

~~16. The method according to any one of claims 1 to 15, 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.~~

~~17. The method according to any one of claims 1 to 16, 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.~~

~~18. The method according to of any one of claims 1 to 17, 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).~~

~~19. The method according to of any one of claims 1 to 18, wherein all sets of decoding oligonucleotides for the different analytes comprise the same type(s) of translator element(s) (c).~~

~~20.~~9. The method according to of any one of claims 1 to ~~19~~8, 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.

~~21. The method according to of any one of claims 1 to 20, 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.~~

~~22. The method according to any one of claims 20 to 21, 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.~~

~~23.~~10. The method according to any one of claims 1 to ~~22~~9, 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.

~~24. The method according to any one of claims 1 to 23, 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.~~

~~25. The method according to any one of claims 23 to 24, 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.~~

~~26. The method according to any one of claims 1 to 25, wherein the decoding oligonucleotides in a particular set of decoding oligonucleotides interacts with identical identifier elements (T) which are unique to a particular analyte.~~

~~27. The method according to any one of claims 1 to 26, wherein the different sets of decoding oligonucleotides may be comprised in a pre-mixture of different sets of decoding oligonucleotides or exist separately.~~

~~28. The method according to any one of claims 1 to 27, 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.~~

~~29. The method according to any one of claims 1 to 28, wherein the different sets of signal oligonucleotides may be comprised in a pre-mixture of different sets of signal oligonucleotides or exist separately.~~

~~30. The method according to any one of claims 1 to 29, wherein the sample is a biological sample, preferably comprising biological tissue, further preferably comprising biological cells and/or extracts and/or part of cells.~~

~~31. The method according to claim 30, wherein the cell is a prokaryotic cells or a eukaryotic cell, in particular a mammalian cell, in particular a human cell.~~

~~32. The method according to claim 30, wherein the biological tissue, biological cells, extracts and/or part of cells are fixed.~~

~~33. The method according to any one of claims 1 to 32, wherein the analytes are fixed in a permeabilized sample, such as a cell-containing sample.~~

~~34. The method according to any one of claims 1 to 33, 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.~~

~~35. The method according to any one of claims 1 to 34, wherein after step A) and before step B) the non-bound analyte-specific probes are removed, in particular by washing.~~

~~36. The method according to any one of claims 1 to 35, wherein after step B) and before step C) the non-bound decoding oligonucleotides are removed, in particular by washing.~~

~~37. The method according to any one of claims 1 to 36, wherein after step C) and before step D) the non-bound signal oligonucleotides are removed, in particular by washing.~~

~~38. The method according to any one of claims 1 to 37, 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.~~

~~39. The method according to any one of claims 1 to 38, 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.~~

~~40. The method according to any one of claims 1 to 39, 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.~~

~~41. The method according to any of claims 1 to 40, wherein the analyte to be encoded is a nucleic acid, preferably DNA, PNA or RNA, in particular mRNA.~~

~~42. The method according to any of claims 1 to 41, wherein the analyte to be encoded is a peptide, polypeptide or a protein.~~

~~43. The method according to any of claims 1 to 42, wherein the binding element (S) comprise an amino acid sequence allowing a specific binding to the analyte to be encoded.~~

~~44. The method according to any of claims 1 to 43, 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.~~

~~45. The method according to any of claims 1 to 44, 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.~~

~~46. The method according to any of claims 1 to 45, 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.~~

~~47. The method according to any one of claims 1 to 46, 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.~~

~~48. The method according to claim 47, wherein the different signal oligonucleotides comprise a different signal element and comprise a different connector element (C).~~

~~49.~~11. 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.

~~50. The kit according to claim 49, wherein the kit comprises at least two different sets of analyte-specific probes for an analyte,~~

~~wherein the analyte-specific probes comprised in these different sets interacting with the same analyte, but specifically interact with different sub-structures of the same analyte,~~

~~wherein the analyte-specific probes of the first set of analyte-specific probes interacts with a sub-structure which is comprised in all variations of an analyte,~~

~~wherein the analyte-specific probes of the second set of analyte-specific probes (subgroup-specific probes) interacts with a sub-structure which is comprised only in a specific variation of the analyte,~~

~~wherein the analyte-specific probes of the first set of analyte-specific probes comprise the same identifier element (T) comprising a nucleotide sequence which is unique to the analyte to be encoded (unique identifier sequence), and~~

~~wherein the analyte-specific probes of the second set of analyte-specific probes comprise the same identifier element (T) comprising a nucleotide sequence which is unique to the analyte to be encoded (unique identifier sequence),~~

~~wherein the identifier elements (T) of the analyte-specific probes of the first set of analyte-specific probes and the identifier elements (T) of the analyte-specific probes of the second set of analyte-specific probes are different.~~

~~51. The kit according to any one of claims 49 to 50, wherein the kit comprises at least five (5) sets of subgroup-specific probes that differ from the analyte-specific probes of another set of analyte-specific probes in the nucleotide sequence of the identifier element (T).~~

~~52. The kit according to any one of claims 49 to 51, wherein the kit does not comprise sets of analyte-specific probes and/or subgroup-specific probes as defined in claim 47.~~

~~53. The kit according to any one of claims 49 to 52, wherein if the analyte is a nucleic acid, each set of analyte-specific probes comprises at least ten (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.~~

~~54. The kit according to any one of claims 49 to 53, 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.

55. The kit according to any one of claims 49 to 54, 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).

56. The kit according to any one of claims 49 to 55, 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.

57. The kit according to any one of claims 49 to 56, 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.

58. The kit according to of any one of claims 49 to 57, 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).

59. The kit according to any one of claims 49 to 58, wherein the decoding oligonucleotides in a particular set of decoding oligonucleotides interacts with identical identifier elements (T) which are unique to a particular analyte.

60. The kit according to of any one of claims 49 to 59, wherein all sets of decoding oligonucleotides for the different analytes comprise the same type(s) of translator element(s) (c).

61. The kit according to of any one of claims 49 to 60, 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.

62. The kit according to of any one of claims 49 to 61, 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.

63. The kit according to any one of claims 49 to 62, 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.

64. The kit according to any one of claims 49 to 63, 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.

65. The kit according to any one of claims 49 to 64, 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.

66. The kit according to any one of claims 49 to 65, 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.

67. The kit according to any one of claims 49 to 66, wherein the decoding oligonucleotides in a particular set of decoding oligonucleotides interacts with identical identifier elements (T) which are unique to a particular analyte.

68. The kit according to any one of claims 49 to 67, wherein the different sets of decoding oligonucleotides may be comprised in a pre-mixture of different sets of decoding oligonucleotides or exist separately.

69. The kit according to any one of claims 49 to 68, 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.

~~70. The kit according to any one of claims 49 to 69, wherein the different sets of signal oligonucleotides may be comprised in a pre-mixture of different sets of signal oligonucleotides or exist separately.~~

~~71. The kit according to any of claims 49 to 70, wherein the analyte to be encoded is a nucleic acid, preferably DNA, PNA or RNA, in particular mRNA.~~

~~72. The kit according to any of claims 49 to 71, wherein the analyte to be encoded is a peptide, polypeptide or a protein.~~

~~73. The kit according to any of claims 49 to 72, wherein the binding element (S) comprises an amino acid sequence allowing a specific binding to the analyte to be encoded.~~

~~74. The kit according to any of claims 49 to 73, 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.~~

~~75. The kit according to any of claims 49 to 74, 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.~~

~~76. The kit according to any one of claims 49 to 75, 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.~~

77. The kit according to claim 76, wherein the different signal oligonucleotides comprise a different signal element and comprise a different connector element (C).

78. 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).

79. The method according to claim 78, wherein if the analyte is a nucleic acid, each set of analyte-specific probes comprises at least ten (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.

80. The method according to of any one of claims 78 to 79, wherein the sample is contacted with at least two different sets of analyte-specific probes for an analyte,
wherein the analyte-specific probes comprised in these different sets interacting with the same analyte, but specifically interact with different sub-structures of the same analyte,
wherein the analyte-specific probes of the first set of analyte-specific probes interacts with a sub-structure which is comprised in all variations of an analyte,
wherein the analyte-specific probes of the second set of analyte-specific probes (subgroup-specific probe set) interacts with a sub-structure which is comprised only in a specific variation of the analyte,
wherein the analyte-specific probes of the first set of analyte-specific probes comprise the same identifier element (T) comprising a nucleotide sequence which is unique to the analyte to be encoded (unique identifier sequence), and

~~wherein the analyte-specific probes of the second set of analyte-specific probes comprise the same identifier element (T) comprising a nucleotide sequence which is unique to the analyte to be encoded (unique identifier sequence),~~

~~wherein the identifier elements (T) of the analyte-specific probes of the first set of analyte-specific probes and the identifier elements (T) of the analyte-specific probes of the second set of analyte-specific probes are different.~~

~~81. The method according to of any one of claims 78 to 80, wherein contacting a subgroup of at least one analyte with a set of at least five (5) subgroup-specific probes which differ from the analyte-specific probes of another set of analyte-specific probes in the nucleotide sequence of the identifier element (T).~~

~~82. The method according to of any one of claims 78 to 81, wherein the sample is contacted with the subgroup-specific probe set according to claim 30, wherein the method comprises an additional further cycle comprising steps B) to E) to identify the variation interacting with the subgroup-specific probe, wherein in particular the cycle may stop with step (D).~~

~~83. The method according to of any one of claims 78 to 82, wherein all steps are automated, in particular wherein steps B) to F) are automated, in particular by using a robotic system.~~

~~84. The method according to any one of claims 78 to 83, wherein all steps are performed in a fluidic system.~~

~~85. The method according to any one of claims 78 to 84, 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.~~

~~86. The method according to any one of claims 78 to 85, wherein said encoding scheme is predetermined and allocated to the analyte to be encoded.~~

~~87. The method according to any one of claims 78 to 86, 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.~~

~~88. The method according to any one of claims 78 to 87, wherein no signal is detected for at least one analyte within at least one cycle.~~

~~89. The method according to of any one of claims 78 to 88, wherein for at least for one individual analyte a position of the code word is zero (0).~~

~~90. The method according to of any one of claims 78 to 89, 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.~~

~~91. The method according to of any one of claims 78 to 90, 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.~~

~~92. The method according to of any one of claims 78 to 91, 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).~~

~~93. The method according to any one of claims 78 to 92, 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.~~

~~94. The method according to any one of claims 78 to 93, 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.

95. The method according to of any one of claims 78 to 94, 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).

96. The method according to of any one of claims 78 to 95, wherein all sets of decoding oligonucleotides for the different analytes comprise the same type(s) of translator element(s) (c).

97. The method according to of any one of claims 78 to 96, 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.

98. The method according to of any one of claims 78 to 97, 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.

99. The method according to any one of claims 78 to 98, 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.

100. The method according to any one of claims 78 to 99, 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.

101. The method according to any one of claims 78 to 100, 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.

102. The method according to any one of claims 100 to 101, 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.

103. The method according to any one of claims 78 to 102, wherein the decoding oligonucleotides in a particular set of decoding oligonucleotides interacts with identical identifier elements (T) which are unique to a particular analyte.

~~104. The method according to any one of claims 78 to 103, wherein the different sets of decoding oligonucleotides may be comprised in a pre-mixture of different sets of decoding oligonucleotides or exist separately.~~

~~105. The method according to any one of claims 78 to 104, 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.~~

~~106. The method according to any one of claims 78 to 105, wherein the different sets of signal oligonucleotides may be comprised in a pre-mixture of different sets of signal oligonucleotides or exist separately.~~

~~107. The method according to any one of claims 78 to 106, wherein the sample is a biological sample, preferably comprising biological tissue, further preferably comprising biological cells and/or extracts and/or part of cells.~~

~~108. The method according to claim 107, wherein the cell is a prokaryotic cells or a eukaryotic cell, in particular a mammalian cell, in particular a human cell.~~

~~109. The method according to claim 107, wherein the biological tissue, biological cells, extracts and/or part of cells are fixed.~~

~~110. The method according to any one of claims 78 to 109, wherein the analytes are fixed in a permeabilized sample, such as a cell-containing sample.~~

~~111. The method according to any one of claims 78 to 110, 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.~~

~~112. The method according to any one of claims 78 to 111, wherein after step A) and before step B) the non-bound analyte-specific probes are removed, in particular by washing.~~

~~113. The method according to any one of claims 78 to 112, wherein after step B) and before step C) the non-bound decoding oligonucleotides are removed, in particular by washing.~~

~~114. The method according to any one of claims 78 to 113, wherein after step C) and before step D) the non-bound signal oligonucleotides are removed, in particular by washing.~~

~~115. The method according to any one of claims 78 to 114, 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.~~

~~116. The method according to any one of claims 78 to 115, 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.~~

~~117. The method according to any one of claims 78 to 116, 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.~~

~~118. The method according to any of claims 78 to 117 wherein the analyte to be encoded is a nucleic acid, preferably DNA, PNA or RNA, in particular mRNA.~~

~~119. The method according to any of claims 78 to 118, wherein the analyte to be encoded is a peptide, polypeptide or a protein.~~

~~120. The method according to any of claims 78 to 119, wherein the binding element (S) comprise an amino acid sequence allowing a specific binding to the analyte to be encoded.~~

~~121. The method according to any of claims 78 to 120, 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.~~

~~122. The method according to any of claims 78 to 121, 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.~~

~~123. The method according to any of claims 78 to 122, 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:~~

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

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

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

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

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

~~124. The method according to any one of claims 78 to 123, 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.~~

~~125. The method according to claim 124, wherein the different signal oligonucleotides comprise a different signal element and comprise a different connector element (C).~~

~~126.~~12. 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 1 to ~~48~~ 10~~and/or of claims 78 to 125~~.

~~127.~~13. 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 1 to ~~48 and/or of claims 78 to 125~~10.

~~128.~~14. An optical multiplexing system suitable for the method according to any one of claims 1 to ~~48~~ 10 ~~and/or of claims 78 to 125,~~ comprising at least:

- one reaction vessel for containing the kits or part of the kits according to ~~any one of claims 49 to 77~~claim 11;
- a detection unit comprising a microscope, in particular a fluorescence microscope
- a camera
- a liquid handling device.

~~129. The optical multiplexing system according to claim 128, wherein the system comprises further a heat and cooling device.~~

~~130. The optical multiplexing system according to any one of claim 128 to 129, wherein the system comprises further a robotic system.~~

~~131.~~15. 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 1 to ~~48~~ 10~~and/or of claims 78 to 125~~.

~~132. The *in vitro* method according to claim 131, 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.~~