CLAIMS

1. A method for determining the dissociation constants of analyte-specific binding components with an analyte in a sample of known concentration in a bi-component detection method comprising:

    a. providing two non-immobilized analyte-specific binding components at known concentrations for conducting a bi-component detection method in a solution comprising bringing said two analyte-specific binding components in contact with the analyte to produce a signal that depends on the concentration of bi-component/analyte complexes formed in said solution,

    b. providing a dissociation constant relationship for said bi-component detection method which is a mathematical function for the relationship between the dissociation constants of the two analyte-specific binding components with the analyte (kd1 and kd2, respectively) in dependence of said signal reflecting the concentration of bi-component/analyte complexes and the concentration of analyte and analyte-specific binding components,

    c. preparing one or more dilutions of said sample and/or said analyte-specific binding components,

    d. applying the bi-component detection method to the sample and the one or more dilutions,

    e. determining the dissociations constants kd1 and kd2 of said two analyte-specific binding components using said signal detected in the sample and in the one or more dilutions as a constraining input for a mathematical fit for said dissociation constant relationship at different analyte concentration and/or analyte-specific binding components concentrations.

2. The method according to claim 1, wherein said dissociation constant relationships are calculated analytically by solving chemical balance, and mass conservation equations.

3. The method according to any of the preceding claims, wherein the analyte is selected from a group consisting of proteins, peptides, nucleic acid segments, carbohydrates, lipids, antibodies (monoclonal or polyclonal), antigens, oligonucleotides, specific receptor proteins, ligands, molecules, cells, microorganisms as well as fragments products or combinations of thereof and/or wherein the sample comprise two or more different types of analytes.

4. The method according to any of the preceding claims, wherein the two analyte-specific binding components are selected from a group consisting of nucleic acids, preferably RNA and/or DNA oligonucleotides, antibodies, peptides, proteins, aptamers, molecularly-imprinted polymers, cells or combinations of thereof and/or wherein two or more pairs of different kind of analyte-specific binding components are used.

5. The method according to any of the preceding claims, wherein the bi-component method comprises employing a proximity-based assay to produce the bi-component/analyte complexes concentration dependent signal, wherein the proximity-based assay uses two analyte-specific binding components that produce a detectable signal in dependence of their proximity.

6. The method according to any of the preceding claims, wherein the bi-component method comprises employing a resonance energy transfer assay, preferably a Förster resonance energy transfer (FRET) assay or a bioluminescence resonance energy transfer (BRET) assay, a protein complementation assay (PCA), Alphascreen or a DNA labeled proximity assay, preferably a proximity ligation assay (PLA) or a proximity extension assay (PEA).

7. The method according to any of the preceding claims, wherein the bi-component detection method comprises employing a compartmentalized assay to produce a bi-component/analyte complexes concentration depended signal, wherein the signal reflects the presence of said two analyte-specific binding components in a single compartment wherein the compartmentalized assay preferably employs an emulsion droplet method, wherein each droplet in the emulsion represents a separate compartment, more preferably wherein the compartmentalized assay is emulsion coupling.

8. The method according to any one of the preceding claims, wherein multiple analytes are determined in parallel.

9. The method according to any one of the preceding claims, wherein the bi-component detection method comprises employing an absolute molecular count based analytical method and/or wherein the bi-component detection method comprises employing a droplet digital PCR assay.

10. The method according to any one of the preceding claims, wherein the bi-component detection method comprises using analyte-specific binding components associated with unique amplifiable nucleic acid labels and employs a compartmentalized assay, wherein a nucleic acid amplification is performed for each compartment using fluorescently tagged amplification products.

11. The method according to any one of the preceding claims, wherein the bi-component detection method comprises using multiple analyte-specific binding components comprising nucleic acid molecule-identifying – unique molecular identifier - barcodes and employs a compartmentalized assay, wherein an nucleic acid amplification is performed for each compartment producing linked molecule-identifying – unique molecular identifier - nucleic acid barcodes, the compartments are reunited in a common pool and a parallel nucleic acid sequencing technique is used to produce the bi-component/analyte complexes concentration depended signal.

12. The method according to any one of the preceding claims 10 or 11, wherein the parallel nucleic acid sequencing technique is a next generation sequencing technique (NGS).

13. A Kit for determining dissociation constants in the bi-component detection method of claim 1, comprising:

a. reaction buffer for two or more analyte-specific binding components at known concentrations for conducting a bi-component detection method comprising bringing said two analyte-specific binding components in contact with a solution containing an analyte to produce a signal that depends on the concentration of bi-component/analyte complexes formed in said solution,

b. reference data on a dissociation constant relationship for said bi-component detection method which is a mathematical function for the relationship between kd1 and kd2 in dependence of said signal reflecting the concentration of bi-component/analyte complexes and the concentration of analytes and/or analyte-specific binding components,

c. optionally instructions for preparing one or more dilutions of said sample and applying the bi-component detection method to the sample and the one or more dilutions using defined dilutions factors,

d. a computer program when executed on a computer configured for performing the computational steps referred to in the method of claim 1 e. of determining the dissociations constants kd1 and kd2 of said two analyte-specific binding component using said signal detected in the sample and in the one or more dilutions as a constraining input for a mathematical fit for said dissociation constant relationship at different analyte and/or analyte-specific binding components concentrations.