

CLAIMS

1. Method for determining levels of interactions between biomolecules, such as proteins, in a sample, comprising providing a first and a second information carrying (IC) oligonucleotide, wherein the first and second IC oligonucleotide are attached, covalently or non-covalently, to a first and a second affinity reagent, such as antibodies, that have the capacity to bind to a first and a second biomolecule, wherein the first and second IC oligonucleotide each comprises at least one single-stranded stretch that is complementary to a part of either an information receiving (IR) oligonucleotide or an activating oligonucleotide, thereby, upon hybridisation of the at least one single-stranded stretch in at least one of the first and second IC oligonucleotides to its complementary part of another oligonucleotide, enables measurement of the relative proportion of interacting and non-interacting first and second biomolecules in the sample at a single cell or single molecular level, wherein the single-stranded stretch of the first and second IC oligonucleotides enables interaction

- (a) between the first and/or second IC oligonucleotide and an information receiving (IR) oligonucleotide, wherein the (IR) oligonucleotide carries at least two cleavage motif sites that upon hybridization to an IC oligonucleotide become double-stranded to allow cleavage, or
- (b) between the first and/or second IC oligonucleotide and an activating oligonucleotide, wherein the IC oligonucleotides connected to said antibodies are designed to contain hairpin structures, and wherein said IC oligonucleotides are modified with fluorophores and quenchers, which are positioned so that only one fluorophore per oligonucleotide can emit light, wherein the hybridization between the first and/or second IC oligonucleotide and the activating oligonucleotide restructures the positions of fluorophores and quenchers, such that the one signalling fluorophore allows for discrimination between interacting and non-interacting biomolecules.

2. Method according to claim 1, comprising the steps of:

- a. providing the single-stranded information receiving (IR) DNA molecule, wherein the IR DNA molecule is circular or linear, carrying at least a first and a second cleavage motif, wherein the cleavage motifs are chosen so that the cleavage motif sites must become double-stranded in order to allow cleavage;
- b. providing the first information carrying (IC) DNA molecule, comprising a single-stranded stretch that is complementary to the part of the IR DNA molecule carrying the first cleavage motif, wherein the occurrence of the first IC DNA molecule reflects the amount of a first biomolecule in the sample;
- c. providing the second information carrying (IC) DNA molecule, comprising a single-stranded stretch that is complementary to the part of the IR DNA molecule carrying the second cleavage motif, wherein the occurrence of the second IC DNA molecule reflects the amount of a second biomolecule in the sample
- d. mixing the DNA molecules of step a-c under conditions that allow binding of complementary single-stranded stretches;
- e. adding digestion enzyme(s) to create nick(s) at the cleavage motif site(s) that has/have become double-stranded, thereby forming
 - i. a first reporter tag binding site that will allow binding of a first reporter tag DNA molecule or sequence, comprising a single-stranded stretch that is complementary to a part of the first IC DNA molecule, and/or
 - ii. a second reporter tag binding site that will allow binding of a second reporter tag DNA molecule or sequence comprising a single-stranded stretch that that is complementary to a part of the second IC DNA molecule;
- f. incorporating reporter tag DNA sequences by any one of the following alternatives:
 - i. adding the first reporter tag DNA molecule and the second reporter tag DNA molecule, whereby the first reporter tag DNA molecule is incorporated in the IR DNA molecule at the first reporter tag binding site if a nick has been created at the first cleavage motif site, and/or the second reporter tag DNA molecule is incorporated in the IR DNA molecule at the second reporter tag binding site if a nick has been created at the second cleavage motif site; or
 - ii. using a DNA polymerase and nucleotides to incorporate the first reporter tag DNA sequence in the IR DNA molecule by filling the gap complementary to the

- first reporter tag binding site on the first IC oligonucleotide if a nick has been created at the first cleavage motif site, and/or to incorporate the second reporter tag DNA sequence in the IR DNA molecule by filling the gap complementary to the second reporter tag binding site on the second IC oligonucleotide if a nick has been created at the second cleavage motif site; and adding a ligation enzyme ligating the IR DNA molecule, thereby providing a recreated IR DNA molecule;
- g. optionally amplifying the recreated IR DNA molecule of step f;
 - h. monitoring the incorporation of the first and/or second reporter tag(s) in the recreated IR DNA molecule, as a measurement of the occurrence of and/or interaction between the first and/or the second biomolecule(s) in the sample.
3. Method according to claim 2, wherein cleavage motifs are chosen from uracils or restriction sites.
 4. Method according to claim 2 or 3, wherein the digestion enzyme(s) used to create nicks at the cleavage motif sites is/are chosen from
 - i. nicking endonuclease 3' Nb.Bsr.DI nicking 3' of a double stranded restriction site,
 - ii. nicking endonuclease 5' Nt.BsmAI nicking 5' of a double stranded restriction site, or
 - iii. a combination of Uracil-DNA glycosylase (UDG), removing a uracil base at a double stranded site, and EndoIV, removing the apyrimidinic site.
 5. Method according to any one of claims 2-4, wherein the monitoring is performed by
 - a. providing a first labeled detection oligonucleotide that is complementary to at least a part of the first reporter tag DNA molecule, and a second labeled detection oligonucleotide that is complementary to at least a part of the second reporter tag DNA molecule, and
 - b. hybridizing the first and second labeled detection oligonucleotides to the recreated IR DNA molecule, which optionally is amplified.
 6. Method according to any one of claims 2-5, wherein a linear recreated IR DNA

molecule is amplified and thereafter separated by a separation method, such as electrophoresis or chromatography, wherein separation products having different sizes indicate incorporation of different reporter tags.

7. Method according to any one of claims 2-6, wherein the identities of different reporter tags are monitored by sequencing.

8. Method according to any one of claims 2-7, wherein the recreated IR DNA molecule is monitored in the sample where it has been formed, such as by microscopy, or wherein the recreated IR DNA molecule is collected from the sample where it has been formed followed by sorting and analysis of single molecules, such as by microscopy.

9. Method according to any one of claims 2-8, for removing abasic sites and improving detection efficiency, wherein after the reporter tag molecules have been added and the first and/or second reporter tag have/has been incorporated into the IR, the following steps are performed:

- i. hybridizing a digestion template to the IR DNA molecule making the area around the remaining abasic site double stranded;
- ii. removing the abasic site by digesting the double stranded area by a suitable restriction enzyme, such as EndoIV;
- iii. gap-filling the digested area with a suitable polymerase, such as T4 DNA polymerase, to add the missing base, such as thymidine; and ligating the IR DNA molecule with a ligase, such as T4 ligase, to provide a recreated IR DNA molecule.

10. Method according to claim 1,

- a. wherein the first and second IC oligonucleotides comprise hairpin structures in which fluorophores and quenchers are positioned, wherein the hairpin structures are designed so that only one fluorophore per oligonucleotide can emit light, and wherein each fluorophore has a unique signal;
- b. wherein the hairpins in the first and second IC oligonucleotides are disrupted or destabilized by providing an activating oligonucleotide that is complementary to the first

- or the second IC oligonucleotide thereby binding to the first or second IC oligonucleotide so that the first and second IC oligonucleotides can interact with each other causing a repositioning of fluorophores and quenchers;
- c. wherein pairs of conjugates, comprising affinity reagents coupled to the first or second IC oligonucleotide, are used to interrogate proximity between two biomolecules to which the affinity reagents bind, wherein a first fluorophore signal pattern will be exhibited upon interaction between the first and second biomolecule, and a second fluorophore signal pattern will be exhibited upon lack of interaction between the first and second biomolecule, as a result of the oligonucleotides hybridizing to each other upon interaction between the first and second biomolecule causing restructuring of the positions of fluorophores and quenchers.
11. Method according to any one of the preceding claims, wherein the first and the second biomolecules are proteins or polypeptides.
12. Method according to any one of the preceding claims, wherein the sample is a biological sample of one or more cells, or a mixture of proteins.
13. Method according to any one of the preceding claims, wherein the first IC DNA molecule is conjugated to a first antibody molecule being equal to or targeting the first biomolecule, and the second IC DNA molecule is conjugated to the second antibody molecule being equal to or targeting the second biomolecule.
14. Method according to any one of the preceding claims for analysing protein-protein interactions in single cells or single molecules.
15. Kit for use in the method of any one of claims 1-9, 11-14, comprising
- a single-stranded information receiving (IR) DNA molecule, carrying a first and a second cleavage motif;
 - a first and a second information carrying (IC) DNA molecule, reflecting the amounts of a first and a second biomolecule in a sample, wherein the first and second IC DNA

- molecules are conjugated to affinity reagents or provided with chemical moieties to be conjugated by a kit user;
- c. optionally enzymes for creating nicks at the cleavage motif sites in the IR DNA molecule;
 - d. a first and a second reporter tag DNA molecule;
 - e. optionally reagents for amplification of a recreated IR DNA molecule;
 - f. optionally a first and a second labelled detection oligonucleotide; and g. optionally DNA molecule(s) and enzymes to remove abasic sites
16. Kit for use in the method of any one of claims 1, 10-14, comprising
- a. a first and a second information carrying (IC) DNA molecule comprising hairpin structures in which fluorophores and quenchers are positioned, wherein the hairpin structures are designed so that only one fluorophore per oligonucleotide can emit light, and wherein each fluorophore has a unique signal, thereby having the ability to reflect the amounts of a first and a second biomolecule in a sample, wherein the first and second IC DNA molecules are conjugated to affinity reagent or are provided with chemical moieties to be conjugated by a kit user; and
 - b. an activating oligonucleotide that is complementary to the first or the second IC DNA molecule thereby having the ability to bind to the first or second IC DNA molecule.