(e.g. in the proximity of the ligand binding site). Moreover, extrinsic dyes are usually located on the protein surface and therefore have an ideal exposure to sense changes to the chemical micro-environment. The dye fluorescence range can be chosen so that it does not interfere with auto-fluorescence of a ligand. Lastly, extrinsic dyes are much brighter, and measurements can occur at much lower concentration of the target molecule, thus reducing sample consumption and allowing the measurement of even picomolar affinities.

Thus, there is a need for improved or alternative methods or improved or alternative devices for characterization of inter- and/or intramolecular interactions, and/or modifications (conformational changes) and/or (changes in) localization of fluorescently labeled particles.

Summary

In an aspect, the present disclosure refers to a method for the characterization of fluorescently labeled particles in solution by analyzing changes in the fluorescence intensity and spectral shifts and/or broadening or narrowing of the spectrum of the fluorescent label of the fluorescently labeled particles, comprising the steps:

a) providing a sample of the fluorescently labeled particles in a solution under first conditions,

b) exciting the fluorescently labeled particles at a first wavelength,

c) detecting the fluorescence emission intensity of the fluorescently labeled particles at a second and a third wavelength,

d) calculating the ratio between said fluorescence intensities at the second and the third wavelength,

wherein said third wavelength is different from said second wavelength,

e1) repeating steps b) to d) for said sample of the fluorescently labeled particles under second conditions, or

e2) repeating the steps a) to d) for a second sample of the fluorescently labeled particles under second conditions,

wherein said second conditions are different from said first conditions,

f) characterizing the fluorescently labeled particles based on the calculated ratios obtained for the different conditions,
wherein the second and third wavelength are detected simultaneously, and
wherein the second wavelength is detected at an at least 2.5 nm shorter wavelength, and the third wavelength is detected at an at least 2.5 nm longer wavelength than an emission maximum of the fluorescence emission of the fluorescently labeled particles under the first conditions.

In an aspect, the present disclosure refers to a device for the characterization of fluorescently labeled particles in solution by analyzing changes in the fluorescence intensity and spectral shifts and/or broadening or narrowing of the spectrum of the fluorescent label of the fluorescently labeled particles, in particular adapted for performing the method according to the above aspect, the device comprising:
a sample holder for holding a sample of fluorescently labeled particles in solution under a plurality of conditions;
means for exciting the fluorescently labeled particles at a first wavelength;
means for detecting the fluorescence emission intensity of the fluorescently labeled particles at a second and a third wavelength;
means for calculating a ratio between said fluorescence intensities at the second and the third wavelength, wherein said third wavelength is different from said second wavelength;
wherein the device is configured to consecutively excite fluorescently, detect fluorescence emissions and calculate the ratio for samples at different conditions;
means for characterizing the fluorescently labeled particles based on the calculated ratios obtained for the different conditions,
wherein device is configured to simultaneously detect the second and third wavelength, and
wherein the second wavelength is detected at an at least 2.5 nm shorter wavelength and the third wavelength is detected at an at least 2.5 nm longer wavelength than an emission maximum of the fluorescence emission of the fluorescently labeled particles under a first condition of the different conditions.

In an aspect, the present disclosure refers to a computer program comprising instructions which, when the program is executed by a computer, cause the computer to carry out the method according to the above aspects.

In an aspect, the present disclosure refers to a computer-readable data carrier comprising instructions which, when executed by a computer, cause the computer to carry out the method according to the above aspects.

In an aspect, the present disclosure refers to a use of a device according to the above aspects for the characterization of fluorescently labeled particles in solution, preferably in accordance with the above aspects.

In an aspect, the present disclosure refers to a use of a capillary for the characterization of fluorescently labeled particles in solution by analyzing changes in the fluorescence intensity and spectral shifts and/or broadening or narrowing of the spectrum of the fluorescent label of the fluorescently labeled particles, wherein a sample of the fluorescently labeled particles in a solution is filled in the capillary and provided for analyzing, in particular according to the method of the above aspects.

The present disclosure provides new methods and devices for characterization of inter- and/or intramolecular interactions, and/or modifications (conformational changes) and/or (changes in) localization of fluorescently labeled particles as defined by the features of the independent claims. Further preferred embodiments of the present disclosure are defined in the dependent claims. In particular, the present disclosure solves the technical problem of economically resolving arbitrarily small alterations in the fluorescence spectrum of labeled fluorescent particles based on preferably exactly one fluorescent label and independent from the characteristics (e.g. temperature stability, aggregate formation) and size of the fluorescently labeled particle.

The present disclosure relates to methods and devices for ratiometric characterization of inter- and/or intramolecular interactions, and/or conformational modifications and/or localization of fluorescently labeled particles. In particular, the methods of the present disclosure provide an improved sensitivity in detecting alterations in the fluorescence spectrum of fluorescent labels within small sample volumes of fluorescently labeled particles, which could not have been resolved by previously known methods. Further, the method of the present disclosure enables fast measurements of even temperature sensitive and/or unstable samples. In combination with a defined temperature perturbation, the method of the present disclosure enables the measurements of thermodynamic and kinetic parameters of interactions. In the methods of the

present disclosure, fluorescently labeled particles are employed which are preferably labeled with at least one fluorescent label. The method of the present disclosure enables to determine

the localization of fluorescently labeled particles (e.g. if the fluorescently labeled particle is located within lipid nanoparticles (LNPs) and/or within cells and/or within a buffer solution surrounding the LNPs and cells).

In a first example, the present disclosure relates to a method for the characterization of fluorescently labeled particles in solution by analyzing alterations in the fluorescence spectrum of the fluorescently labeled particles. The method of the first example comprises the following steps:

a) providing a sample of the fluorescently labeled particles in a solution under first conditions,

b) exciting the fluorescently labeled particles at a first wavelength,

c) detecting the fluorescence emission intensity of the fluorescently labeled particles at a second and a third wavelength,

d) calculating the ratio between said fluorescence intensities at the second and the third wavelength,

wherein said third wavelength is different from said second wavelength. It is further preferred to

e1) repeat steps b) to d) for said sample of the fluorescently labeled particles under second conditions, or to

e2) repeat the steps a) to d) for a second sample of the fluorescently labeled particles under second conditions,

wherein said second conditions are different from the said first conditions.

f) The fluorescently labeled particles can then be characterized based on the calculated ratios obtained for the different conditions,

wherein the second and third wavelength are preferably detected simultaneously, and wherein the second wavelength is preferably shorter and the third wavelength is preferably longer than an emission maximum of the fluorescence emission of the fluorescently labeled particles under the first conditions.

In a second example, the present disclosure relates to a method for the characterization of fluorescently labeled particles in solution by analyzing alterations in the fluorescence spectrum of the fluorescently labeled particles in combination with a defined temperature perturbation/temperature change. The method of the second example comprises the following steps:

a) providing a sample of the fluorescently labeled particles in a solution under first conditions,

b) exciting the fluorescently labeled particles at a first wavelength,

c) detecting the fluorescence emission intensity of the fluorescently labeled particles at a second and a third wavelength,

wherein said intensities are detected during a defined temperature perturbation,

d) calculating the ratio between said fluorescence intensities at the second and the third wavelength, wherein said third wavelength is different from said second wavelength.

Like for the first example, it is also preferred,

e1) to repeat steps b) to d) for said sample of the fluorescently labeled particles under second conditions, or

e2) to repeat the steps a) to d) for a second sample of the fluorescently labeled particles under second conditions,

wherein said second conditions are different from the said first conditions, and

f) characterizing the fluorescently labeled particles based on the calculated ratios obtained for the different conditions,

wherein the second and third wavelength are preferably detected simultaneously, and wherein the second wavelength is preferably shorter and the third wavelength is longer than an emission maximum of the fluorescence emission of the fluorescently labeled particles under the first conditions.

In a third example, the present disclosure relates to a method for the characterization of the thermodynamic and/or kinetic parameters of fluorescently labeled particles in solution by analyzing alterations in the fluorescence spectrum of the fluorescently labeled particles in combination with a defined temperature perturbation/temperature change.

In a fourth example, the present disclosure relates to a method for the characterization of the localization of fluorescently labeled particles in solution by analyzing alterations in the fluorescence spectrum of the fluorescently labeled particles in combination with or without a defined temperature perturbation/temperature change.

It is preferred in the context of the present disclosure, that the fluorescence emission intensities at a second and a third wavelength of step c) are detected simultaneously, which preferably means within a short time interval of less than 1 s, more preferably less than 750 ms, more

preferably less than 500 ms, more preferably less than 250 ms, more preferably less than 100 ms, more preferably less than 50 ms, more preferably less than 25 ms, more preferably less than 10ms, more preferably less than 5ms, even more preferably less than 2.5 ms. In this context, typical time intervals are 50 ms,10 ms and 1 ms.

Some particular examples of the disclosure can be summarized as follows:
In some examples the methods described herein comprise the steps of a) providing a sample of the fluorescently labeled particles in a solution under first conditions, b) exciting the fluorescently labeled particles at a first wavelength, c) detecting the fluorescence emission intensity of the fluorescently labeled particles at a second and a third wavelength, d) calculating the ratio between said fluorescence intensities at the second and the third wavelength, wherein said third wavelength is different from said second wavelength, e1) repeating steps b) to d) for said sample of the fluorescently labeled particles under second conditions, or e2) repeating the steps a) to d) for a second sample of the fluorescently labeled particles under second conditions, wherein said second conditions are different from said first conditions, f) characterizing the fluorescently labeled particles based on the calculated ratios obtained for the different conditions, wherein the second and third wavelength are detected simultaneously, and wherein the second wavelength is shorter, and the third wavelength is longer than an emission maximum of the fluorescence emission of the fluorescently labeled particles under the first conditions.

In some examples of the present disclosure, the sample volume containing the fluorescently labeled particles is less than 100 µl, preferably between 1 µl and 25 µl, i.e., the sample containing the fluorescently labeled particles is provided in a volume of less than 100 µl, preferably between 1 µl and 25 µl.

It is preferred that in some examples of the present disclosure, the sample containing the fluorescently labeled particles is provided in a volume between 1 µl and 25 µl.

In some examples of the present disclosure, the sample containing the fluorescently labeled particles is provided in a capillary.

In some examples of the present disclosure, the fluorescently labeled particles are labeled with an environment sensitive label.

In some examples of the present disclosure, the particles are selected from the group consisting of organic molecules, biomolecules, nanoparticles, microparticles, vesicles, biological cells or sub-cellular fragments, biological tissues, viral particles, viruses, cellular organelles, lipid nanoparticles (LNPs), and virus like particles.

In some examples of the present disclosure, the biomolecules are selected from the group consisting of amino acids, proteins, peptides, mono- and disaccharides, polysaccharides, lipids, glycolipids, fatty acids, sterols, vitamins, neurotransmitter, enzymes, nucleotides, metabolites, nucleic acids, and combinations thereof.

In some examples of the present disclosure, the concentration of the fluorescently labeled particles in the solution is from 10 pM to 10 µM, preferably 50 pM to 500 nM.

In some examples of the present disclosure, the alterations in the detected fluorescence intensity of the fluorescently labeled particles result from spectral shifts or broadening of the spectrum or narrowing of the spectrum, or combinations thereof.

In some examples of the present disclosure, the fluorescence intensity of the fluorescently labeled particles changes due to mechanisms selected from the group consisting of conformational changes of the fluorescently labeled particles, re-localization of the fluorescently labeled particles, interactions between the fluorescently labeled particles and one or more ligands, and combinations thereof.

In some examples of the present disclosure, the calculated ratios obtained in step f) are used to determine the localization of the fluorescently labeled particles or parameters selected from the group consisting of dissociation constants, half maximal effective concentrations (EC_{50}), equilibrium constants, binding kinetics, enzymatic reaction kinetics, thermodynamic parameters, unfolding or refolding kinetics, opening and closing reactions, and combinations thereof.

In some examples of the present disclosure, the second conditions of step e) are altered by adding a ligand and/or different concentrations of the ligand and the calculated ratios obtained in step f) are used to determine the dissociation constant of the fluorescently labeled particles and the ligand.

In some examples of the present disclosure, the first and second conditions of the fluorescently labeled particles differ with regard to their temperature and/or chemical composition.

In some examples of the present disclosure, the fluorescence emission intensity of the fluorescently labeled particles at a second and a third wavelength of step c) is detected during a defined temperature perturbation.

Also provided herein is a device for the characterization of fluorescently labeled particles in solution by analyzing alterations in the fluorescence spectrum of the fluorescently labeled particles. In some examples, the device of the present disclosure is adapted for performing the methods described herein. In some examples the device comprises a sample holder for holding a sample of fluorescently labeled particles in solution under a plurality of conditions (i.e. multiple different conditions); means for exciting the fluorescently labeled particles at a first wavelength; means for detecting the fluorescence emission intensity of the fluorescently labeled particles at a second and a third wavelength; means for calculating a ratio between said fluorescence intensities at the second and the third wavelength, wherein said third wavelength is different from said second wavelength; wherein the device is configured to consecutively excite fluorescently, detect fluorescence emissions and calculate the ratio for samples at different conditions; means for characterizing the fluorescently labeled particles based on the calculated ratios obtained for the different conditions, wherein device is configured to simultaneously detect the second and third wavelength, and wherein the second wavelength is shorter and the third wavelength is longer than an emission maximum of the fluorescence emission of the fluorescently labeled particles under a first condition (of the different conditions).

In some examples of the present disclosure, the means for exciting is an excitation light source, preferably at least one light source from the group consisting of laser, laser fibre laser, diode-laser, LED, HXP, Halogen, LED-Array, HBO.

In some examples of the present disclosure, the means for detecting is light detector, preferably at least one detector from the group consisting of PMT, siPM, APD, CCD or CMOS camera.

Also provided herein is a computer program comprising instructions, which when the program is executed by a computer, cause the computer is used to carry out the methods described herein

Also provided herein is a computer-readable data carrier comprising instructions, which when executed by a computer, cause the computer is used to carry out the method described herein

Also provided herein is the use of a device for the characterization of fluorescently labeled particles in solution according to the methods described herein.

Also provided herein is the use of a capillary for the characterization of fluorescently labeled particles in solution by analyzing alterations in the fluorescence spectrum of the fluorescently labeled particles, wherein a sample of the fluorescently labeled particles in a solution is filled in the capillary and provided for analyzing, according to the methods described herein.