United States Patent Application Publication	20250264473
Kind Code	A1
Publication Date	August 21, 2025
Inventor(s)	ALSHEIMER; Soeren

AFFINITY REAGENT, MARKER AND METHOD FOR ANALYSING A BIOLOGICAL SAMPLE

Abstract

An affinity reagent for analysing a biological sample includes a nucleic acid backbone. The nucleic acid backbone is configured to specifically bind to a target analyte by a complex structure of the nucleic acid backbone. The nucleic acid backbone includes at least one pair of reaction moieties for cross-linking the nucleic acid backbone.

Inventors: **ALSHEIMER; Soeren (Wetzlar, DE)**

Applicant: **Leica Microsystems CMS GmbH** (Wetzlar, DE)

Family ID: **1000008492576**

Appl. No.: **19/051219**

Filed: **February 12, 2025**

Foreign Application Priority Data

EP	24158129	Feb. 16, 2024

Publication Classification

Int. Cl.: **G01N33/58** (20060101); **C12N15/115** (20100101)

U.S. Cl.:

CPC **G01N33/58** (20130101); **C12N15/115** (20130101); C12N2310/16 (20130101); G01N2458/00 (20130101)

Background/Summary

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit to European Patent Application No. EP 24158129.7, filed on Feb. 16, 2024, which is hereby incorporated by reference herein.

FIELD

[0002] Embodiments of the present invention relate to an affinity reagent for analysing a biological sample. Embodiments of the present invention also relate a marker comprising the affinity reagent and a method for analysing a biological sample with the marker.

BACKGROUND

[0003] Nucleic acid based affinity reagents are superior to antibodies in many regards. For example, aptamer discovery through SELEX is easy and cost-effective. Further, the information about the nucleic acid based affinity reagents can be digitally stored and can be used to produce the respective nucleic acid based affinity reagent by oligonucleotide synthesis with excellent batch-to-batch variability at low cost. They generally exhibit high affinities and are easy to functionalize and multimerize.

[0004] A key challenge, however, remains unresolved. Nucleic acid based affinity reagent binding depends on their 3D structure. The stability of nucleic acid based 3D structures is however lower than protein-based 3D structures owing to lack of side-chain interactions. For this reason, a given aptamer may dynamically change between different 3D structures, of which only one or few are active and specifically bind to the target molecule. Similarly, during storage nucleic acid based affinity reagents may partially unfold or misfold and may thus need to be refolded prior to use. This significantly reduces the ease of use and practical applications of nucleic acid based affinity reagents.

SUMMARY

[0005] Embodiments of the present invention provide an affinity reagent for analysing a biological sample. The affinity reagent includes a nucleic acid backbone. The nucleic acid backbone is configured to specifically bind to a target analyte by a complex structure of the nucleic acid backbone. The nucleic acid backbone includes at least one pair of reaction moieties for cross-linking the nucleic acid backbone.

Description

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] Subject matter of the present disclosure will be described in even greater detail below based on the exemplary figures. All features described and/or illustrated herein can be used alone or combined in different combinations. The features and advantages of various embodiments will become apparent by reading the following detailed description with reference to the attached drawings, which illustrate the following:

[0007] FIG. **1** is a schematic view of a marker comprising an affinity reagent in folded and unfolded conformations according to some embodiments;

[0008] FIG. **2** is a schematic view of the marker with an affinity reagent comprising reaction moieties according to some embodiments;

[0009] FIG. **3** is a schematic view of the marker with an affinity reagent comprising a linking element according to some embodiments; and

[0010] FIG. **4** is a schematic view of a method to cross-link a nucleic acid backbone of an affinity reagent according to some embodiments.

DETAILED DESCRIPTION

[0011] Embodiments of the present invention provide an affinity reagent and a marker that are robust and a method for analysing a biological sample with the marker.

[0012] In a first aspect, an affinity reagent for analysing a biological sample is provided. The

affinity reagent comprises a nucleic acid backbone. The nucleic acid backbone is configured to specifically bind to a target analyte by its complex structure. Further, the nucleic acid backbone comprises at least one pair of reaction moieties for cross-linking the nucleic acid backbone. This enables providing a robust and stable affinity reagent.

[0013] The nucleic acid backbone may be a deoxyribonucleic acid molecule, for example. In particular, the nucleic acid backbone may be a single, continuous deoxyribonucleic acid molecule. The nucleic acid backbone may have an unfolded, primary structure that is a linear sequence of nucleotides, for example, linked together by phosphodiester bonds.

[0014] In a preferred embodiment, I nucleic acid backbone may entirely or partially comprise a xeno nucleic acid backbone, i.e. the backbone may comprise phosphorothioate, boranophosphate, squaramides, or triazole linkages. Such backbones may confer higher stability and resistance against degradation. Likewise, the nucleic acid backbone may comprise L-DNA and/or modified nucleobases, including nucleobases that have side chain-like residues, as is the case for SOMAmers, for example.

[0015] The complex structure or conformation of the nucleic acid backbone of the affinity reagent may be a secondary, tertiary or quaternary structure of the nucleic acid backbone, for example. Thus, the nucleic acid backbone of the affinity reagent may preferably have a secondary, tertiary, or quaternary structure. In particular, the affinity reagent is configured to bind specifically to the target analyte, for example of the biological sample, due to the complex structure of the backbone of the affinity reagent. Specifically, individual, discontinuous nucleotides of the nucleic acid backbone bind to the target analyte, rather than continuous nucleotides of a sequence of the nucleic acid hybridising to the target analyte, in particular a complementary sequence of the target analyte. This enables binding of the affinity reagent with high specificity, for example to an amino acid based target analyte. In particular, the backbone of the affinity reagent, e.g. an aptamer, may bind to its target by paratope-epitope interactions and not via hybridization of complementary nucleic sequences. The binding of the affinity reagent to the target analyte may comprise intermolecular forces such as hydrogen bonding, dipole-dipole interactions, ionic interactions, p-stacking, hydrophobic interaction, and van der Waals forces.

[0016] The reaction moieties of the pair of reaction moieties may be arranged along the linear sequence of nucleotides of the nucleic acid backbone, for example, with each reaction moiety attached to two nucleotides of the linear sequence. Thus, the reaction moieties are arranged along the nucleic acid backbone sequence not adjacent to each other. However, in the secondary, tertiary and/or quaternary structure of the nucleic acid backbone, the position of the reaction moieties relative to each other may change due to a corresponding change in conformation of the nucleic acid backbone. Preferably, the reaction moieties are at a smaller distance to each other when the nucleic acid backbone has the complex structure compared to when the nucleic acid backbone has the unfolded, linear primary structure.

[0017] In particular, the nucleic acid backbone may comprise a cross-link between the at least one pair of reaction moieties. In particular, the cross-link and/or the at least one pair of reaction moieties is for maintaining the complex structure of the nucleic acid backbone. To that end, the reaction moieties and/or the cross-link preferably cross-link positions along the nucleic acid backbone that are not adjacent along the nucleic acid backbone. For example, the reaction moieties of the pair of reaction moieties are arranged along the linear sequence of the nucleic acid backbone at a distance from each other, as discussed above. Thus, an intrastrand cross-link may be formed, in particular, comprising the reaction moieties of the pair of reaction moieties. The intrastrand cross-link may cross-link two separate positions along the linear sequence of a nucleic acid molecule of the nucleic acid backbone, for example.

[0018] In a preferred embodiment, the nucleic acid backbone may comprise multiple nucleic acid molecules that bind and fold together to form the complex structure. In this case, the cross-link may be formed between the reaction moieties of the pair of reaction moieties, which are arranged on

different ones of the multiple nucleic acid molecules. This may be referred to as an interstrand cross-link.

[0019] Preferably, the reaction moieties of the pair of reaction moieties are configured to react with each other. This may result in formation of a covalent bond between the reaction moieties and enables a robust affinity reagent with a stable complex structure. In particular, the reaction moieties are configured to react specifically or directly with each other. This enables forming the covalent bond specifically between the reaction moieties. Thus, the reaction moieties preferably attach to each other covalently, i.e. they react with each other to form a covalent bond, and the reaction do not bind to each other by hybridisation or by intermolecular forces such as hydrogen bonding, dipole-dipole interactions, and van der Waals forces. The reaction of the reaction moieties may in particular be a copper-dependent azide alkyne cycloaddition (CuAAC), a strain-promoted azide alkyne cycloaddition (SpAAC), a Staudinger reaction, Diels-Alder, thiole-ene reaction, or any other suitable reaction. The reaction moieties may be in particular, azide, alkyne, dibenzocyclooctyne (DBCO), thiol, ene, maleimide, N-hydrocysuccinimide, amino.

[0020] Preferably, the reaction moieties of the pair of reaction moieties are configured to react under a catalytic condition, in particular only under the catalytic condition. This enables control over the reaction of the reaction moieties of the pair of reaction moieties. For example, the catalytic condition may be the presence of a catalyst. In particular, the catalyst may be light such as UV-light, copper ions, or a molecule.

[0021] Preferably, the affinity reagent further comprises a linking element and the reaction moieties of the pair of reaction moieties are configured to react with corresponding reaction moieties of the linking element. This enables a flexible cross-linking of the nucleic acid backbone. For example, the linking element may be chosen depending on the distance between the reaction moieties of the pair of reaction moieties when the nucleic acid backbone has the complex structure. The linking clement may comprise a polyethylene glycol (PEG) molecule, which are available in a variety of lengths. Further, the corresponding reaction moieties of the linking element may be click chemistry groups or NHS groups. The PEG molecule may be functionalised accordingly. Each of the reaction moieties of the pair of reaction moieties preferably forms a covalent to one of the corresponding reaction moieties of the linking element.

[0022] Preferably, the reaction moieties of the pair of reaction moieties are modified, in particular functionalised, nucleotides. This enables efficient integration of the reaction moieties in the nucleic acid backbone. For example, the reaction moieties may be non-natural or artificial nucleotides, in particular nucleobases, that preferably do not bind by base-pairing to the natural nucleotides adenine, thymine, guanine, and cytosine.

[0023] Preferably, the reaction moieties of the pair of reaction moieties comprise click chemistry groups. This enables efficient generation of the cross-link. For example, the reaction moieties may be CuAAC with azide/alkyne groups, or SpAAC with DBCO/azide groups. For example, in case of CuAAC, the catalytic condition may be the presence of a copper catalyst.

[0024] Preferably, the reaction moieties of the pair of reaction moieties are photoreactive. This enables efficient generation of the cross-link. For example, the reaction moieties may be photoreactive nucleobases. In a particular example each of the reaction moieties is a thymine nucleotide, which reacts under UV-light to form a thymine dimer.

[0025] Preferably, the nucleic acid backbone comprises 10 to 150 nucleotides. This enables a particular compact affinity reagent that is able to efficiently penetrate a biological sample such as a tissue section.

[0026] Preferably, the nucleic acid backbone is an aptamer. This enables a compact and robust affinity reagent. Further, this enables efficient generation of the nucleic acid backbone that specifically binds to a particular target analyte, for example by a SELEX method.

[0027] Preferably, the reaction moieties of the pair of reaction moieties are separated from each other along the nucleic acid backbone. This enables providing a robust nucleic acid backbone with

a stable cross-link. In particular, the reaction moieties are separated from each other along a primary structure or sequence of the nucleic acid backbone. Thus, the reaction moieties are not immediately adjacent to each other or in proximity along the sequence of the nucleic acid backbone.

[0028] Preferably, the reaction moieties of the pair of reaction moieties are in proximity when the affinity reagent has its complex structure. This enables providing a robust nucleic acid backbone with a stable cross-link. Thus, the reaction moieties are arranged along the nucleic acid backbone at positions that are in spatial proximity when the nucleic acid backbone is in its complex structure. The spatial proximity of the reaction moieties enables a reaction of the reaction moieties with each other, in particular.

[0029] Preferably, the affinity reagent comprises a second pair of reaction moieties, wherein the reaction moieties of the second pair differ from the reaction moieties of the at least one pair of reaction moieties. This enables generating a robust affinity reagent. In particular, the reaction moieties of the second pair differ from the reaction moieties of the at least one pair of reaction moieties in that they do not react with each other. Thus, only the reaction moieties of the second pair react with each other and the reaction moieties of the at least one pair of reaction moieties react with each other.

[0030] In a preferred embodiment, the affinity reagent may comprise at least one further pair of reaction moieties, wherein the further pair of reaction moieties is different to the first and second pair of reaction moieties.

[0031] In a further aspect, a marker for analysing a biological sample is provided. The marker comprises an affinity reagent as detailed above and a label comprising at least one labelling moiety. The labelling moiety may be attached to the affinity reagent. Preferably, the labelling moiety is optically detectable, for example, a fluorophore. The affinity reagent enables providing a robust marker, that may be coupled with heat-sensitive labelling moieties, for example. In particular, the requirement to refold nucleic acid based affinity reagents after storage by heat may negatively impact the heat-sensitive labelling moieties.

[0032] In another aspect, a method for analysing a biological sample is provided. The method comprises the steps: introducing into the biological sample at least one marker, for example as described above, and generating a readout of the biological sample with the marker.

[0033] Individual elements of the at least one marker may be introduced individually into the biological sample, for example. Thus, the affinity reagent and the label of the marker may be introduced separately, in order to generate the marker in the biological sample. The readout is preferably an optical readout, for example, generated by means of a microscope.

[0034] Preferably, prior to introducing the at least one marker, the affinity reagent of the marker is generated by cross-linking the pair of reaction moieties of the marker. Thus, a cross-linking of the pair of reaction moieties of the affinity reagent is effected. This may include applying the catalytic condition.

[0035] Generally, a plurality of markers may be introduced into the biological sample, the markers being specific to respective target analytes, in order to identify a large number of (different or the same) target analytes at the same time. Preferably, a target analyte is identified and/or localised within the biological sample based on the label(s) of the marker(s), in particular the labelling moieties, associated with the target analyte in the optical readout.

[0036] The marker and the method have the same advantages as the affinity reagent. Further, the marker and the method may be supplemented with the features of the affinity reagent described in this document, in particular, the features of the dependent claims of the affinity reagent.

[0037] FIG. **1** is a schematic view of a marker **100** comprising an affinity reagent **102** in folded and unfolded conformations. The marker **100** further comprises a label with a labelling moiety **104**. When the affinity reagent **102** has a folded conformation, the affinity reagent **102**, and therefore the marker **100**, may specifically bind to a target analyte **106** of a biological sample. The folded

conformation of the affinity reagent **102** is shown in the left view of FIG. **1**. The affinity reagent **102** may be a nucleic acid based aptamer, for example.

[0038] The labelling moiety **104** may be an optically detectable moiety, such as a fluorophore. Thus, the marker **100** may be detected by means of a microscope. Alternatively, the marker **100** may comprise a plurality of labelling moieties.

[0039] Under unfolding conditions, such as at a high temperature, the affinity reagent **102** may unfold into a partially unfolded conformation shown in the top right view of FIG. **1**, or into a fully unfolded conformation shown in the bottom right view of FIG. **1**. Upon removal of the unfolding condition, the affinity reagent **102** may return to the folded conformation. It is only in the folded conformation of the affinity reagent **102** that the affinity reagent **102** specifically binds to the target analyte **106**.

[0040] To keep the affinity reagent **102** of the marker **100** in folded conformation, in which the affinity reagent **102** specifically binds to the target analyte **106**, the affinity reagent **102** may comprise at least one pair of reaction moieties. These reaction moieties may cross-link the affinity reagent **102** in order to maintain the complex structure of the affinity reagent **102**. These aspects are further described below.

[0041] FIG. **2** is a schematic view of a marker **100***a* with an affinity reagent **102***a* comprising a nucleic acid backbone **200** and reaction moieties **202**, **204** of a pair of reaction moieties. The nucleic acid backbone **200** of the affinity reagent **102***a* is configured to specifically bind to a particular target analyte, such as the target analyte **106**, for example a protein of a biological sample. In particular, the nucleic acid backbone **200** may form or fold into a complex structure or conformation. The complex structure of the backbone **200** of the affinity reagent **102***a* may be a secondary, tertiary and/or quaternary structure of the backbone **200**. Thus, the backbone **200** of the affinity reagent **102***a* has a secondary, tertiary or quaternary structure, in particular. The affinity reagent **102***a* binds specifically to the target analyte **106** due to the complex structure of the backbone **200** of the affinity reagent **102***a*. Specifically, individual, discontinuous nucleotides of the nucleic acid of the backbone **200** bind to the target analyte **106**. This binding may comprise intermolecular forces such as hydrogen bonding, dipole-dipole interactions, and van der Waals forces. This is in contrast to a nucleic acid hybridising, which is characterised by a continuous sequence of nucleotides of the nucleic acid hybridising to a target analyte, in particular a complementary nucleotide sequence of the target analyte.

[0042] The binding of the backbone **200** of the affinity reagent **102***a* to the target analyte **106** based on the complex structure of the backbone **200** enables particular high affinity binding and specificity to the target analyte **106**.

[0043] The nucleic acid of the backbone **200** may comprise multiple individual sequences or strands of a nucleic acid. These individual sequences may bind to each other and form a quaternary structure.

[0044] For clarity of the FIG. **2**, the nucleic acid backbone **200** is shown as an unfolded, linear structure. When the nucleic acid backbone **200** of the affinity reagent **102***a* of the marker **100***a* has the complex structure, the reaction moieties **202**, **204** are in proximity to form a covalent bond. The covalent bond is indicated by reference sign **206** in FIG. **2**. The covalent bond between the reaction moieties **202**, **204** may cross-link the nucleic acid backbone **200** of the affinity reagent **102***a* to maintain the complex structure of the nucleic acid backbone **200**.

[0045] The reaction moieties **202**, **204** are modified nucleotides of the nucleic acid backbone **200**. For example, the reaction moieties **202**, **204** may comprise click chemistry groups, such as CuAAC with azide/alkyne groups or SpAAC with DBCO/azide groups, attached to nucleobases Guanine or Cytosine, respectively.

[0046] FIG. **3** is a schematic view of a marker **100***b* with an affinity reagent **102***b* comprising a nucleic acid backbone **300**, reaction moieties **302**, **304** of a pair of reaction moieties, and a linking clement **306**. The nucleic acid backbone **300** of the affinity reagent **102***b* is configured to

specifically bind to a particular target analyte, such as the target analyte **106**.
[0047] As explained for the nucleic acid backbone **200** above, the nucleic acid backbone **300** may form or fold into a complex structure or conformation. For clarity of the FIG. **3**, the nucleic acid backbone **300** is shown as an unfolded, linear structure. When the nucleic acid backbone **300** of the affinity reagent **102***b* of the marker **100***b* has the complex structure, the reaction moieties **302**, **304** are in proximity and the linking element **306**, in particular corresponding reaction moieties **308**, **310** of the linking element **306**, may react with the reaction moieties **302**, **304** of the nucleic acid backbone **300** in order to cross-link the nucleic acid backbone **300**. This enables maintaining the complex structure of the nucleic acid backbone **300**. The reaction moieties **302**, **304**, **308**, **310** may be click chemistry groups that react with each other to form covalent bonds between the reaction moieties **302** and **308** as well as between reaction moieties **304** and **310**.
[0048] In particular, the linking element **306** enables linking the reaction moieties **302**, **304** that are at a distance from each other when the nucleic acid backbone **300** has its complex structure that is too great for the reaction moieties **302**, **304** to react with each other directly. The length of the linking element **306** may be chosen depending on the distance between the reaction moieties **302**, **304** when the nucleic acid backbone **300** has its complex structure.
[0049] FIG. **4** is a schematic view of steps to cross-link a nucleic acid backbone **402** of an affinity reagent of a marker **400** in its complex structure. The marker **400** further comprises a labelling moiety **404**. The nucleic acid backbone **402** comprises a pair of reaction moieties **406** that cross-links the nucleic acid backbone **402**.
[0050] Initially, a partially folded conformation **400***a* of the marker **400**, in particular the nucleic acid backbone **402**, may be fully unfolded by applying an unfolding condition such as a high temperature. This generates an unfolded, linear conformation **400***b* of the marker **400**. The partially folded conformation **400***a* may alternatively be a misfolded conformation, which does not specifically bind to a particular target analyte. Subsequently, the unfolded conformation **400***b* may be refolded into a native complex structure **400***c* of the marker **400** by applying a folding condition, such as a low, or physiological temperature. The complex structure **400***c* may bind specifically to the particular target analyte with high affinity. However, the complex structure **400***c* is still prone to unfolding into the partially or fully unfolded conformations **400***a*, **400***b*. This may be caused by applying the unfolding condition or randomly due to environmental influence or storage conditions.
[0051] In order to maintain the complex structure **400***c* of the affinity reagent of the marker **400**, the nucleic acid backbone **402** may be cross-linked. The pair of reaction moieties **406** are configured to react with each other under a catalytic condition, for example UV-light **408**. After folding into the complex structure **400***c*, the UV-light **408** may be applied in order to react the pair of reaction moieties **406** and form a cross-link. This enables maintaining the complex structure of the affinity reagent of the marker **400** and avoids undesired unfolding of the affinity reagent of the marker **400**.
[0052] In an alternative, the complex structure **400***c* may be refolded without the labelling moiety **404** attached to the nucleic acid backbone **402** and the pair of reaction moieties **406** may be cross-linked. Only in a subsequent step, the labelling moiety **404** may be attached to the nucleic acid backbone **402**. This avoids damaging the labelling moiety **404**, for example by the catalytic condition or the unfolding condition.
[0053] The marker **100**, **100***a*, **100***b*, **400** may be applied in a method for analysing a biological sample. The marker **100**, **100***a*, **100***b*, **400**, in particular the respective affinity reagent, is specific to a target analyte of the biological sample. When introducing the marker **100**, **100***a*, **100***b*, **400** into the biological sample, the marker **100**, **100***a*, **100***b*, **400** binds specifically to the target analyte. Subsequently a readout, in particular an optical readout, may be generated of the biological sample with the marker **100**, **100***a*, **100***b*, **400**, for example, by means of a microscope. The respective labelling moiety of the marker **100**, **100***a*, **100***b*, **400** may be detected in the readout and the presence or location of the target analyte in the biological sample may be determined. Preferably,

the marker **100**, **100***a*, **100***b*, **400** is generated prior to or during introducing the marker **100**, **100***a*, **100***b*, **400** into the biological sample, in particular, as described above.
[0054] Identical or similarly acting elements are designated with the same reference signs in all Figures. As used herein the term “and/or” includes any and all combinations of one or more of the associated listed items and may be abbreviated as “/”.
[0055] Although some aspects have been described in the context of an apparatus, it is clear that these aspects also represent a description of the corresponding method, where a block or device corresponds to a method step or a feature of a method step. Analogously, aspects described in the context of a method step also represent a description of a corresponding block or item or feature of a corresponding apparatus.
[0056] While subject matter of the present disclosure has been illustrated and described in detail in the drawings and foregoing description, such illustration and description are to be considered illustrative or exemplary and not restrictive. Any statement made herein characterizing the invention is also to be considered illustrative or exemplary and not restrictive as the invention is defined by the claims. It will be understood that changes and modifications may be made, by those of ordinary skill in the art, within the scope of the following claims, which may include any combination of features from different embodiments described above.
[0057] The terms used in the claims should be construed to have the broadest reasonable interpretation consistent with the foregoing description. For example, the use of the article “a” or “the” in introducing an element should not be interpreted as being exclusive of a plurality of elements. Likewise, the recitation of “or” should be interpreted as being inclusive, such that the recitation of “A or B” is not exclusive of “A and B,” unless it is clear from the context or the foregoing description that only one of A and B is intended. Further, the recitation of “at least one of A, B and C” should be interpreted as one or more of a group of elements consisting of A, B and C, and should not be interpreted as requiring at least one of each of the listed elements A, B and C, regardless of whether A, B and C are related as categories or otherwise. Moreover, the recitation of “A, B and/or C” or “at least one of A, B or C” should be interpreted as including any singular entity from the listed elements, e.g., A, any subset from the listed elements, e.g., A and B, or the entire list of elements A, B and C.
REFERENCE SIGNS
[0058] **100**, **100***a*, **100***b*, **400** Marker [0059] **102**, **102***a*, **102***b* Affinity reagent [0060] **104**, **404** Labelling moiety [0061] **106** Target analyte [0062] **200**, **300**, **402** Nucleic acid backbone [0063] **202**, **204**, **302**, **304**, **308**, **310** Reaction moiety [0064] **206** Covalent bond [0065] **306** Linking element [0066] **400***a* Partially unfolded conformation [0067] **400***b* Unfolded conformation [0068] **400***c* Complex structure [0069] **406** Pair of reaction moieties [0070] **408** Catalytic condition

Claims

1. An affinity reagent for analysing a biological sample, the affinity reagent comprising: a nucleic acid backbone, wherein the nucleic acid backbone is configured to specifically bind to a target analyte by a complex structure of the nucleic acid backbone, and wherein the nucleic acid backbone comprises at least one pair of reaction moieties for cross-linking the nucleic acid backbone.
2. The affinity reagent according to claim 1, wherein the reaction moieties of the pair of reaction moieties are configured to react with each other.
3. The affinity reagent according to claim 1, wherein the reaction moieties of the pair of reaction moieties are configured to react under a catalytic condition.
4. The affinity reagent according to claim 1 further comprising a linking element, wherein the reaction moieties of the pair of reaction moieties are configured to react with corresponding reaction moieties of the linking element.

5. The affinity reagent according to claim 1, wherein the reaction moieties of the pair of reaction moieties are modified nucleotides.
6. The affinity reagent according to claim 1, wherein the reaction moieties of the pair of reaction moieties comprise click chemistry groups.
7. The affinity reagent according to claim 1, wherein the reaction moieties of the pair of reaction moieties are photoreactive.
8. The affinity reagent according to claim 1, wherein the nucleic acid backbone comprises 10 to 100 nucleotides.
9. The affinity reagent according to claim 1, wherein the nucleic acid backbone is an aptamer.
10. The affinity reagent according to claim 1, wherein the reaction moieties of the pair of reaction moieties are separated from each other along the nucleic acid backbone.
11. The affinity reagent according to claim 1, wherein the reaction moieties of the pair of reaction moieties are in proximity when the affinity reagent has the complex structure.
12. The affinity reagent according to claim 1, wherein the at least one pair of reaction moieties comprises a first pair of reaction moieties and a second pair of reaction moieties, wherein the reaction moieties of the second pair of reaction moieties differ from the reaction moieties of the first pair of reaction moieties.
13. A marker for analysing a biological sample, the marker comprising: an affinity reagent according to claim 1, and a label comprising at least one labelling moiety.
14. A method for analysing a biological sample, the method comprising: introducing into the biological sample at least one marker according to claim **13**, and generating a readout of the biological sample with the at least one marker.
15. The method according to claim 14, wherein prior to introducing the at least one marker, the affinity reagent of the marker is generated by cross-linking the pair of reaction moieties of the marker.