APPLICATION NOTE

dynamic BIOSENSORS

Site-specific covalent conjugation and purification of His-tagged proteins to DNA for switchSENSE® applications

Keywords: Site-specific conjugation | His-tagged proteins | proFIRE

Well-defined protein-DNA conjugates are a very important tool for many bioanalytical applications, including bionano-technology or proximity ligation assays (PLA). To sustain protein functionality, it is important to conjugate a single DNA oligo to a specific location of the protein. Thanks to the unique feature of DNA which allows easy identification, functionalization and modification, synthesizing protein-DNA conjugates should be straightforward. Yet, preparing pure and site-specific conjugates can be challenging.

In this application note, we introduce a conjugation kit for a straightforward, site-selective and covalent conjugation of His-tagged proteins to DNA with high efficiency using lower amounts of protein. Since they constitute one of the starting points for using the switchSENSE® technology, we developed this conjugation kit.

Background

In recent years, more and more protein-DNA conjugates have been used for different types of assay formats, such as DNA-encoded addressing, proximity ligation assays, immuno-PCR, DNA-PAINT and switchSENSE.[1-5] All these methods have in common their need for pure and site-specifically labeled protein-DNA conjugates. An increasing number of different chemical methods have been developed over the years to ensure protein functionality after conjugation. Here, we present a modified method that has been developed by Kurt Gothelf and can be used to produce protein-DNA conjugates for different kind of applications.[1] In this Application Note we will focus on the use of protein-DNA conjugates for the switchSENSE® technology.

The first step in a switchSENSE® experiment is to bring the ligand onto the surface using the unique properties of DNA: one DNA strand (nanolever) is tethered to the surface of the chip electrodes, while the complementary strand is used to functionalize the surface with the molecule of interest. One of the major advantages of this strategy is that the complementary strand can be easily removed to regenerate a fresh surface that can be functionalized again.

We offer two main strategies to bring the ligand on the surface. The complementary DNA strand can: (i) carry a capture molecule specific to a protein tag (His-, biotin-, GFP-tag, …) or (ii) be directly and covalently conjugated to the protein of interest. Both strategies have pros and cons in terms of stability on the surface, protein functionality, protein consumption etc., and the chosen strategy usually depends on the amount of protein available and the type of experiments to be run. For the direct covalent conjugation of a protein to the DNA, site specificity is usually very important to preserve the protein activity and ensure sample homogeneity. This can be challenging for non-chemically modified proteins. In this case, thiol and amine coupling, as well as click chemistry are most commonly used, and we provide kits to perform these types of conjugation in an easy and straightforward manner.

For the thiol coupling kit, a single cysteine residue is targeted ensuring site selectivity. For the amine coupling, the N-terminal amino group is in most cases the preferred reaction site due to its lower pKa, but lysine residues can also be targeted depending on site accessibility and variances of the local pKa. Click chemistry is a biorthogonal and site-specific conjugation method, but requires further modifications, e.g. unnatural amino acids.

Here, we present a new conjugation kit to perform site-specific conjugations of His-tagged proteins to our complementary nanolever, but this method can be applied for any conjugation of a His-tagged protein to a DNA. As described in [1], it is possible to use a Guiding DNA carrying tris-NTA to capture the His-tagged protein and bring it in close proximity to the reactive site of the DNA strand of interest. As a result, and given particular reaction conditions, the activated DNA will react site-specifically on the protein and with high efficiency despite low concentrations of reactants, as exploited by DNA-templated synthesis.[6]

Methods and Results

Our standard conjugation kits rely on two components: a DNA nanolever (e.g. cNL-B48) modified with a reactive linker and a protein with a reactive site. In this new kit tailored for the conjugation of His-tagged proteins, a third component is added to the reaction: a short oligonucleotide complementary to the reactive nanolever and carrying a tris-NTA, called Guiding DNA. As shown in Figure 1, this oligonucleotide is used to capture the protein and bring it to the activated nanolever, so that each protein is presented in the same orientation to the nanolever and will react at the same site.

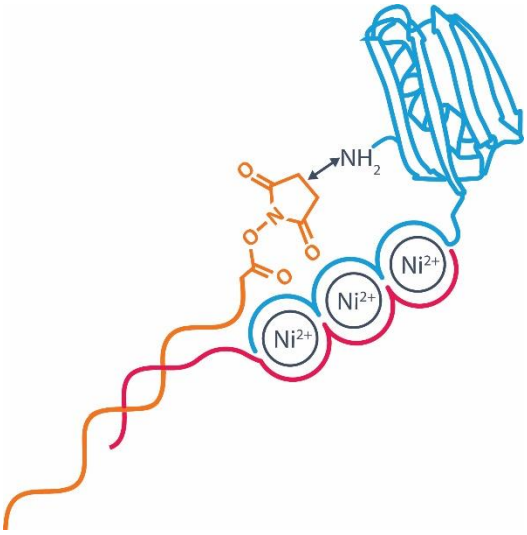

Figure 1 | Schematic representation of the reaction complex: the activated DNA nanolever is shown in orange, the tris-NTA Guiding DNA in pink, the His-tagged protein in blue and the nickel ions in black. The arrow shows the two sites reacting together.

The conjugation protocol is very simple. In a first step, the reactive linker is added to the DNA nanolever of interest. After washing of the excess linker, Guiding DNA, His-tagged protein and Loading solution are added to the activated nanolever in the conjugation buffer and react overnight at room temperature or 4°C. After incubation, the crude protein-DNA conjugate will be purified a using a **pro**FIRE® (figure 2). Thanks to the Guiding DNA, not only the conjugation of His-tagged proteins is more site-selective using this method, but it also has a 50 % higher relative yield for the conjugation of protein A to a 48mer DNA oligo (figure 3).

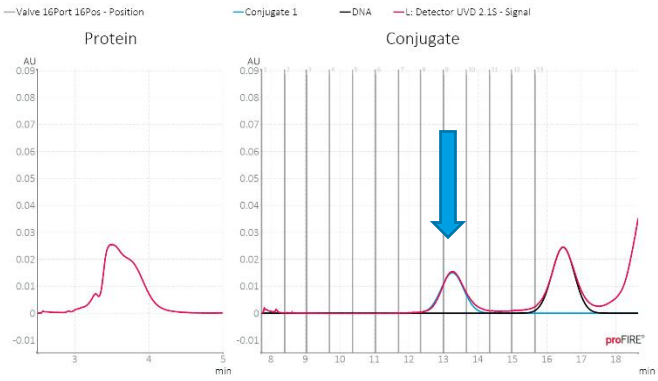

Figure 2 | **pro**FIRE® purification chromatogram of His-tagged protein A conjugation using 50 µg of protein and a Guiding DNA.

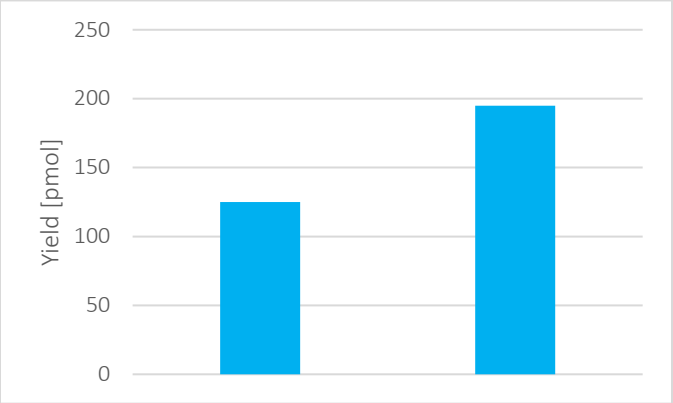

Figure 3 | Comparison of a reaction mixture of 50 µg protein A with 48mer DNA **without** addition of Guiding DNA (left) and **with** addition of Guiding DNA (right).

Conclusions

We have presented a new straightforward way to synthesize protein-DNA conjugates of His-tagged proteins for the functionalization of **switch**SENSE® surfaces. Using Guiding DNA to bring protein and reactive DNA together and direct the reaction site-specifically to the protein, it is now possible to conjugate His-tagged proteins covalently to the DNA in an orientated way with high efficiency. In addition to labeling His-tagged proteins, the method can principally also be extended to proteins with a natural metal binding site.[1] Furthermore, this method can be used to synthesize protein-DNA conjugates for a wide range of different applications, including bioanalytical chemistry, molecular diagnostics or bionanotechnology.

References

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