Protein Labeling

Site-specific covalent labeling of SNAP-tagged proteins for the measurement of binding affinities

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Introduction

Site-specific fluorescent labeling of proteins is a powerful tool for the investigation of binding affinities by MST and TRIC[1]. This labeling strategy preserves the biochemical and physicochemical properties of proteins due to its targeted approach and its one fluorescent label per protein molecule stoichiometry. Additionally, it prevents the interference of fluorophores with ligand binding, making it an attractive alternative to conventional, covalent labeling approaches.

In addition to the His-tag, the SNAP-Tag® is another protein tag that is already used for site-specific labeling for numerous applications in biochemistry[2–8]. This 20 kDa protein tag is commercially available in various expression vectors, allowing its fusion to any protein of interest. Since this tag can be added to the N- or C-terminal end of proteins, it has typically no effects on the protein functionality[4].

The SNAP-Tag® is a modified form of the DNA repair enzyme, human O6-alkylguanine-DNA alkyltransferase (hAGT), which specifically reacts with O6-Benzylguanine (BG) derivatives to form an irreversible covalent thioether bond[9]. To utilize this tag for the fluorescent labeling of proteins, BG is conjugated to a fluorophore of interest, as illustrated in Figure 1.

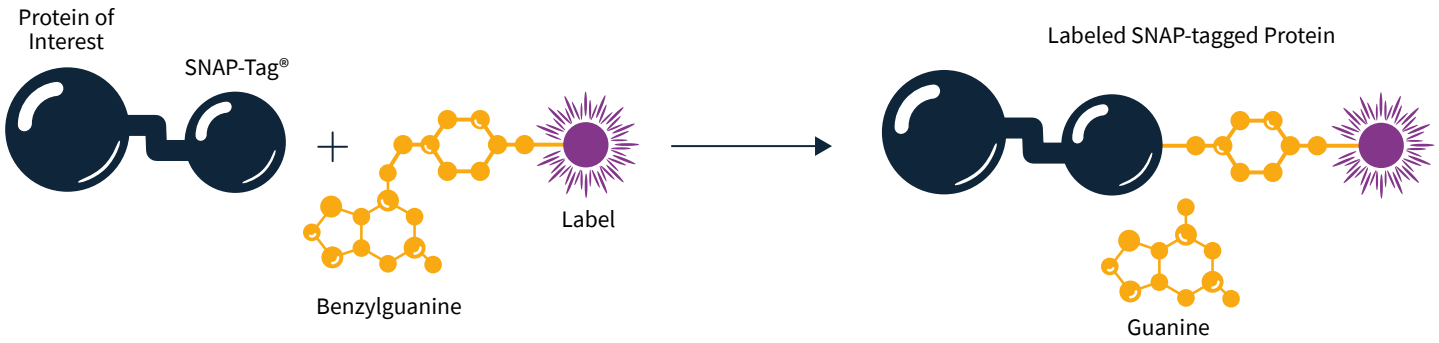

Figure 1: Schematic representation of the SNAP-Tag® labeling approach. The reaction of SNAP-Tag® with O6-benzylguanine (BG) results in the covalent attachment of the label to the cysteine residue in the active site of the SNAP-Tag®, and the release of guanine into the solution.

SNAP-Tag® labeling is an ideal tool for stable, covalent, and highly selective labeling of purified biomolecules with minimum impact on the protein's biochemical and physicochemical properties.

With the intention to exploit the use of SNAP-Tag® for site-specific protein labeling in binding affinity assays, BG was conjugated with the TRIC-optimized RED 2nd Generation dye to form the RED-SNAP-Tag® 2nd Generation, included in the SNAP-Tag® Labeling Kit RED 2nd Generation (Cat# MO-LO19). To demonstrate the applicability of this labeling strategy, affinity analysis of a SNAP-tagged RNA-binding protein towards RNA molecules was performed.

Results

Labeling SNAP-Tag® by means of BG-derivatives is a method developed by New England Biolabs. For this study, the BG moiety was conjugated to the TRIC-optimized RED 2nd Generation fluorophore.

SNAP-tagged RNA-binding protein (kindly provided by Dewpoint Therapeutics) was labeled using NanoTemper Technologies SNAP-Tag® Labeling Kit RED 2nd Generation (Cat# MO-LO19). Briefly, RED-SNAP-Tag® 2nd Generation dye was resuspended in 25 µL DMSO to yield 400 µM dye solution. For the subsequent protein labeling, 90 µL of 10 µM SNAP-tagged RNA-binding protein was mixed with 5 µL labeling buffer (20 mM Tris HCl pH 7.5, 250 mM KCl, 1 mM DTT) and 5 µL dye solution, yielding a 2:1 excess of dye over protein. The labeling reaction was carried out for 30 min at room temperature, before any excess of free dye was removed using size-exclusion chromatography and 20 mM Tris HCl pH 7.5, 250 mM KCl, 1 mM DTT, 0.05% Tween as purification buffer.

For the affinity quantification of RNA-binding protein towards RNA molecules, labeled protein was added to a dilution series of either the positive or the negative control RNA at a final concentration of 20 nM using 20 mM Tris HCl pH 7.5, 250 mM KCl, 1 mM DTT, 0.05%

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Tween as assay buffer. Triplicate measurements were performed with Monolith NT.115 in Premium Capillaries (Cat# MO-K025) at medium MST and 40% LED power. The system was operated with the MO.Control software v 1.6. Additionally, acquired data sets were analyzed by MO.Affinity Analysis software v 2.3 at 2.5 sec MST on-time.

Figure 2 shows the MST traces (left panel) as well as the binding curves (right panel) for experiments performed using positive and negative control RNA molecules and labeled RNA-binding protein. A K_d of 151 ± 9 nM was obtained for the positive control RNA. As expected, no binding was detected for the negative control RNA.

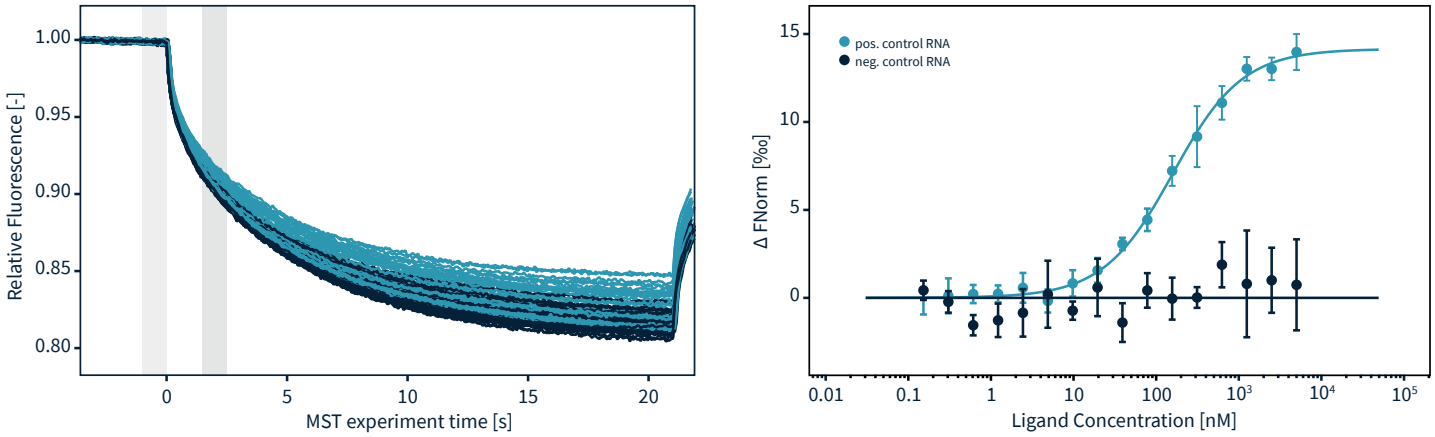

Figure 2: MST traces (left panel) and dose-response curves (right panel) for RNA (negative control in dark blue, positive control in light blue) titrated against labeled RNA-binding protein at medium MST power. An MST on-time of 2.5 sec was used for analysis, and a K_d value of 151 ± 9 nM was obtained (n = 3 independent measurements, error bars represent the standard deviation).

Conclusions

NanoTemper Technologies SNAP-Tag® Labeling Kit RED 2nd Generation provides a site-specific covalent labeling of purified proteins for MST and TRIC experiments. This labeling strategy guarantees a highly specific and controlled conjugation of fluorophores to any SNAP-tagged protein since SNAP-Tag® substrates are inert to any other protein structure.

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