Nucleic Acid Interaction Analysis

Application Note NT005

Characterization of DNA/RNA triplex formation

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Abstract

The concept of double stranded DNA forming triple helical complexes with a third strand of nucleic acids via so called Hoogsteen-hydrogen bonds has been well established for the last 50 years. However, the topic reentered the big stage with recent publications pointing to an existence of these structures *in vivo*, highlighting their putative role in chromatin organization and transcriptional regulation in a non-coding RNA mediated context.
In this study we characterize the formation of such complexes *in vitro*, using MicroScale Thermophoresis (MST). The study also highlights the high content information of the MST measurements as one important benefit of MicroScale Thermophoresis.

Introduction

Besides the well-known double helix, the DNA can also exist in other structural complexes, with the triple helix being one of the most interesting. For those structures to form the underlying DNA sequence must contain stretches of polypurine or polypyrimidines where a third strand can anneal via so called Hoogsteen hydrogen bonds (Duca *et al.*, 2008). Interestingly, bioinformatic analysis of the human genome showed, that these putative triplex forming sites are enriched in gene promoter elements (Goñi *et al.,* 2004). Together with the ever-growing number of functional non-coding RNAs this makes it tempting to speculate about a possible role of these structures in RNA mediated gene regulation processes (Buske *et al.*, 2011). So far however the ultimate confirmation for the existence of the structures *in vivo* is still missing, although recent publications show promising results, pointing in this direction (Schmitz *et al.,* 2010).

In our studies we use the MST technique to characterize the triplex formation ability of possible regulatory DNA sequence motifs derived from mammalian enhancer and promoter regions with matching RNA oligos (Triplex Forming Oligos, TFO) *in vitro*. We are thereby gaining new insights in the stability and molecular characteristics of these structures.

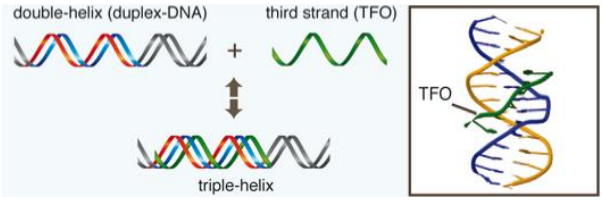

Fig. 1 Formation of a triple helix between a duplex DNA and a third strand nucleic acid (Triplex Forming Oligo, TFO), which can either be RNA or DNA. (from Buske *et al.* 2011)

Results

In this study, we have investigated the binding behavior of a RNA TFO to its matching DNA sequence using MicroScale Thermophoresis.
A Cy5 labeled DNA strand containing the triplex forming sequence was used at a concentration of 50 nM in the experiments. To this a serial dilution of the TFO was titrated with 2.5 µM being the starting concentration. The samples were filled into standard capillaries and incubated at the desired temperature for 1 h, prior to the MST measurement.
Fig. 2 shows the initial capillary scan at 25 °C where the binding of the RNA can already be seen by an increase in the fluorescence signal.

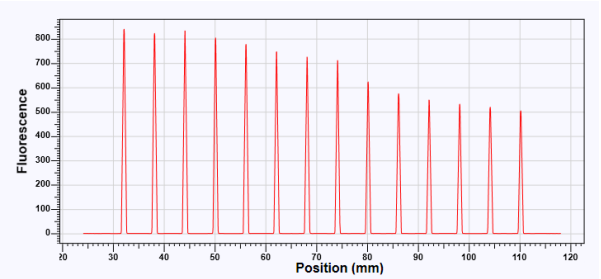

Fig. 2 The binding of the RNA TFO to the DNA duplex can already be seen in the initial capillary scan depicted here. With an increase in RNA concentration the fluorescence increases accordingly.

The subsequent analysis of the MST-curves is displayed in Fig. 3. For the assay three independent measurements were performed. We get a clear binding curve for the RNA oligo with only a very small standard deviation between the single rounds of measurement.

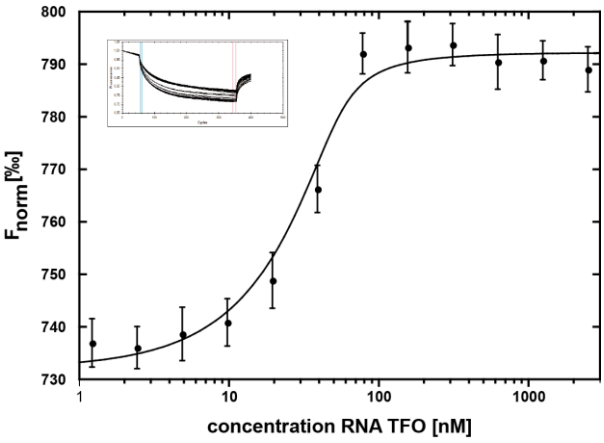

Fig. 3 Measurement of RNA TFO binding to the DNA. The shape of the MST curves can be seen in the inset. 3 independent measurements where performed to obtain the depicted binding curve. The K_d of the interaction was 3.6 ± 2.3 nM.

As a control we also measured the interaction of an unspecific RNA TFO with our DNA target sequence. The experiment was performed as described for the specific RNA. Figure 4 shows that there's already a difference visible in the initial capillary scan, where this time no increase in fluorescence intensity was detectable.
This observation is further confirmed by the analysis of the subsequent MST-curves obtained for the unspecific samples, where clearly no binding of the RNA TFO could be detected, as is shown in Figure 5.

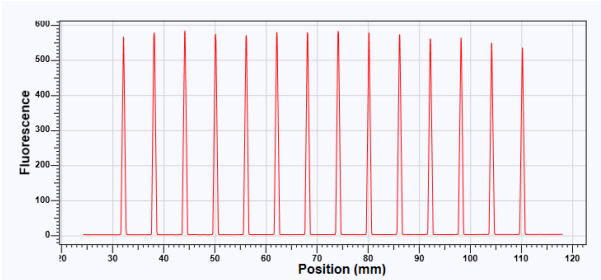

Fig. 4 The initial capillary scan for the measurements with the unspecific RNA TFO already indicate that there's no binding, since there was ´no increase in fluorescence detectable.

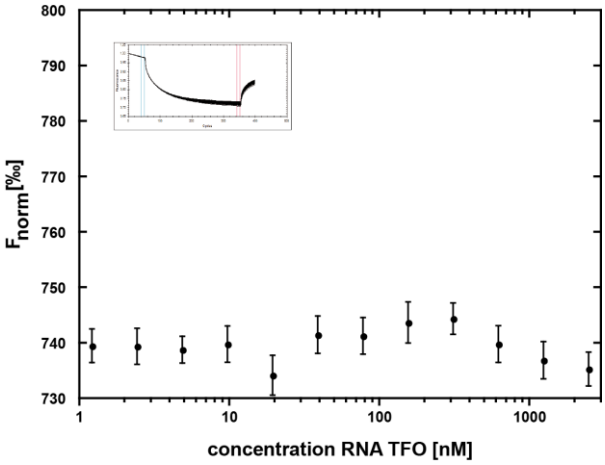

Fig. 5 The measurements for the unspecific RNA TFO showed no binding. MST curves are again presented in the inset. No K_d value was calculated from these results.

The calculated K_d from the measurements of the RNA TFO binding was 3.6 ± 2.3 nM showing the strong interaction of these two molecules under the experimental conditions.

Conclusion

The study provides another example that MicroScale Thermophoresis is capable of measuring specific interactions between nucleic acid molecules. Even though the interaction does not go along with a significant change in size or mass of the molecule, the interaction is detectable with high precision. Straight forward control experiments proof the specificity of the interaction. Experiments are easily setup and affinities can be determined in a timely manner. This experiment also highlights another advantage: Even the interactions of non-covalent complexes like DNA duplexes with a third molecule can be measured in free solution without problems.

Material and Methods

Assay conditions
For the experiment Cy5 labeled DNA containing the triplex forming site was used at a concentration of 50 nM. Unlabeled RNA TFO was titrated in 1:10 dilutions beginning at 25 µM. Samples were prepared in a buffer containing 40 mM Tris-Acetate, pH 7.4, 5 mM $MgCl_2$ and 0.01 (v/v) % NP-40. For the measurement the samples were filled into standard capillaries.

Instrumentation
The measurements were performed on a NanoTemper Monolith NT.115 instrument.

The measurement was performed at 50 % LED and 30 % MST power, Laser-On time was 30 sec, Laser-Off time 5 sec, in standard capillaries.

References

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