

Protein-Quantum Dots Interaction Analysis

Application Note NT-MO-019

Analyzing the binding affinity of aptamer quantum dot conjugates to VEGF

Moran Jerabek-Willemsen¹, Maren Lönne², Johanna-Gabriela Walter², Thomas Scheper², Stefan Duhr¹

¹NanoTemper Technologies GmbH, Munich, Germany

²Institut für Technische Chemie, Leibniz Universität Hannover, Germany

Abstract

Aptamers are single-stranded oligonucleotides which fold into a three-dimensional structure allowing the specific recognition of a molecular target. Therefore, they might be an alternative to antibodies as detection molecules in biosensors. Aptamers are well suited for this application since they can be directed against almost every molecular target, and show high binding affinities to their corresponding binding partner. Aptamers with a terminal amino-group can be coupled to organic or inorganic fluorophores via amide bonds to enable their optical detection. In this study Vascular Endothelial Growth Factor (VEGF)-binding aptamers were conjugated with quantum dot molecules. This immobilization may inhibit the correct aptamer folding and consequently lead to a declined target-binding activity. Therefore, MST technology was used to determine whether quantum dot conjugation affects the VEGF-binding affinity of aptamers.

Introduction

Vascular Endothelial Growth Factor (VEGF) is a bioactive protein which is used as a supplement in cell culture media (Finetti F. et al., 2012). It is biotechnologically produced in microorganisms or mammalian cells and afterwards purified from the complex culture broth. Monitoring the purification process demands a specific and sensitive detection platform for VEGF.

Aptamers fulfill these requirements as they bind to a single target with high affinity and specificity. Aptamers are single-stranded DNA or RNA molecules with length of ~15 – 60 nucleobases. Some of these bases interact with each other

resulting in a particular aptamer folding. This three-dimensional molecular structure enables the aptamer to bind its corresponding target (You K.M. et al., 2012).

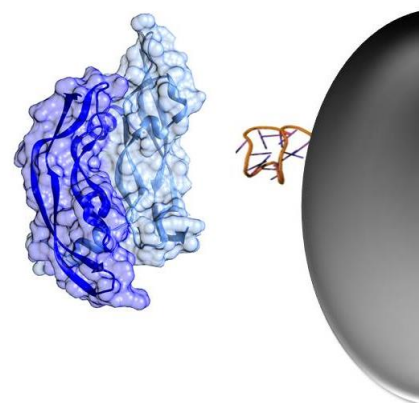


Fig. 1: Schematic representation of VEGF binding to an aptamer-Qdot-conjugate

During chemical synthesis a functional group as an amino group or a carboxyl group can be added to the 3' or 5' terminal mononucleotide of the aptamer. Conjugation with a corresponding carboxyl- or amino-functionalized fluorophore results in a fluorescence-labeled aptamer which can be used as detection molecule in optical biosensors (Walter J.-G. et al., 2012). However, due to steric hindrance or electrostatic interactions between aptamer and fluorophore the conjugation might interfere with the normal aptamer folding. Usually this results in a declined target-binding affinity (Walter J.-G. et al., 2008). In this study VEGF-binding aptamers were conjugated with quantum dot molecules, which are semiconductor nanocrystals that emit fluorescent light (Zhou D., 2012). They display multiple functional groups on their surface and therefore they bind more than one aptamer. On the one hand this might enlarge the strength of the VEGF binding since multiple

target-binding sites simultaneously interact with it. On the other hand the aptamers are very close to each other which may cause steric hindrance. The aim of this study was to use MicroScale Thermophoresis (Jerabek-Willemsen M. et al., 2011) in order to characterize the binding of fluorescently labeled VEGF protein to Quantum dots, which were conjugated with DNA aptamers.

Results

Here, we investigated the binding of NT-647-labeled VEGF to quantum dots, which were conjugated with the VEGF binding aptamer V7t1 (Nonaka Y. et al. 2010) via a 3' terminal amino linker fused to the aptamer.

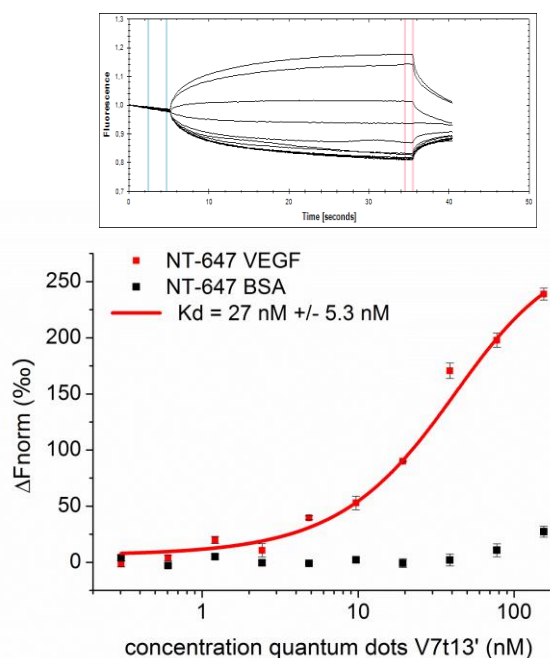


Fig. 2: Binding of NT-647-labeled VEGF to quantum dots – V7t1 3'. In the MST experiment, we kept the concentration of fluorescently-labeled VEGF constant at 20 nM, while the concentration of the quantum dots V7t1 3' was varied. After 10 min, an MST analysis was performed (n = 3). A K_d of 27 nM \pm 5.3 nM was determined for this interaction

The concentration of NT-647 labeled VEGF was kept constant at 20 nM, while the concentration of the quantum dots was varied. After a short incubation, the samples were loaded into MST hydrophilic glass capillaries (K004, NanoTemper Technologies GmbH, Germany) and MST analysis was performed. The calculated K_d for the interaction between NT-647-labeled VEGF and the V7t1 aptamer-conjugated quantum dots was 27 nM \pm 5 nM. We observed, as expected, no binding of fluorescently labeled BSA (Bovine Serum Albumin) to the aptamer conjugated quantum dots (Fig. 2).

Material and Methods

Assay conditions

Binding experiments were carried out in aptamer selection buffer supplemented with KCl (10 mM Tris/HCl, 100 mM NaCl, 0.05 mM EDTA, 50 mM KCl, pH 7.0). For the experiment VEGF protein was labeled with the Monolith NT™ Protein labeling Kit BLUE according to the supplied labeling protocol. Labeled VEGF was used at a concentration of 20 nM. The quantum dots were titrated in 1:1 dilution beginning at 200 nM. Monolith NT.115 hydrophilic capillaries were used in all experiments.

Instrumentation

The measurements were conducted on a NanoTemper Monolith.NT115 instrument. The Analysis was performed at 50 % LED power and 20 % MST power. “Fluo. Before” time was 5 seconds. “MST on time” was 30 seconds and “Fluo. After” time was 5 seconds.

Conclusion

This study provides another example that MicroScale Thermophoresis is capable of measuring interactions of biomolecules with larger particles such as nanoparticles or quantum dots.

References

- Finetti F. et al.**, Functional and pharmacological characterization of a VEGF mimetic peptide on reparative angiogenesis. *Biochemical Pharmacology* (2012) 84:303–11.
- You K.M. et al.**, Aptamers as functional nucleic acids: In vitro selection and biotechnological applications. *Biotechnol Bioproc* (2012) E 8:64–75.
- Nonaka Y. et al.**, Screening and Improvement of an Anti-VEGF DNA Aptamer. *Molecules* (2010) 15 (1): 215–225.
- Walter J.-G. et al.**, Aptasensors for Small Molecule Detection. *Z. Naturforsch.* (2012) B 67:976–86.
- Walter J.-G. et al.**, Systematic Investigation of Optimal Aptamer Immobilization for Protein–Microarray Applications *Anal. Chem.* (2008) 80:7372–8.
- Nonaka Y. et al.**, Screening and Improvement of an Anti-VEGF DNA Aptamer. *Molecules* (2010) 15:215–25.
- Zhou D.**, Quantum dot–nucleic acid/ aptamer bioconjugate-based fluorimetric biosensors. *Biochem. Soc. Trans.* (2012) 40:635–9.
- Jerabek-Willemsen M. et al.**, Molecular Interaction studies using microscale Thermophoresis. *Assay Drug Dev Technol.* Aug;9(4):342–53(2011).