

Application Note

# APTAMER AFFINITY AND MATRIX EFFECTS

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## Introduction

Aptamers are DNA or RNA molecules showing high affinity and specificity towards their corresponding targets. Based on these properties along with the advantage that aptamers can be produced with high quality and low batch-to-batch variation via chemical synthesis, aptamers are valuable alternatives to antibodies in various applications including biosensing. In the field of biosensor development, aptamers are especially advantageous for detection of small molecules because they enable unique techniques for signal generations that are not applicable with antibodies [1].

In this application note an aptamer against Ochratoxin A (OTA) [2] is investigated with regard to its affinity in complex samples. Therefore, microscale thermophoresis (MST) [3] is used to determine the dissociation constant of the aptamer in binding buffer spiked with different portions of defatted milk. Investigation of the aptamer compatibility with other matrices including beer have already been published elsewhere [4, 5].

While it was found that the aptamer affinity decreases with increased portions of milk, the binding properties of the aptamer remained unaffected within 50% milk as demonstrated by a  $K_D$  of 259 nM. MST can be used to determine the dissociation constant of aptamers, and in assay development, e.g. to optimize necessary sample pre-dilution steps.

## Procedure

- › Aptamers were synthesized with 5' terminal Cy5 modification.
- › Aptamers were diluted in binding buffer, which is the buffer used during the aptamer selection, and binding buffer was spiked with defatted milk.
- › Mixtures of aptamer and OTA were prepared. The concentration of aptamer was kept constant at 25 nM, while the concentration of OTA was varied.

- › The samples were filled in MST capillaries and subjected to thermophoretic measurements using a Monolith NT.115 (Nanotemper) at 40% MST power and 20% laser power.

## References

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- [4] Schach, E., et al., Aptamer-based depletion of small molecular contaminants: A case study using ochratoxin A. *Biotechnology and Bioprocess Engineering*, 2015. 20(6): p. 1016-1025.
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