

Monolith Protocol MO-P-057

DNA Aptamer – Ochratoxin (in milk)

DNA aptamers are molecules that can be produced with high quality and low batch-to-batch variations via chemical synthesis. In addition, they bind their targets with high affinity. In the field of biosensor development, aptamers are a valuable alternative to antibodies in detecting small molecules. In this protocol the binding of an aptamer to ochratoxin is analyzed. Ochratoxin A is a mycotoxin found in food. It is nephrotoxic and carcinogenic in the kidney and induces differentiation in cloned renal cell lines. The binding is assayed in buffer, as well as in a buffer spiked with milk.

aptamer – small molecule interaction | complex biofluid

A1. Target/Fluorescent Molecule

DNA aptamer

A2. Molecule Class/Organism

DNA aptamer

A3. Sequence/Formula

5' **Cy5** TGG TGG CTG TAG GTC AGC ATC TGA TCG GGT GTG GGT GGC GTA AAG GGA GCA TCG GAC AAC G
3'

A4. Purification Strategy/Source

IBA Lifesciences (Göttingen, Germany)

A5. Stock Concentration/Stock Buffer

100 μ M
ddH₂O

A6. Molecular Weight/Extinction Coefficient

19.6 kDa
597,700 M⁻¹cm⁻¹ (ϵ_{260})

A7. Dilution Buffer

10 mM Tris-HCl, pH 8.5, 120 mM NaCl, 5 mM KCl, 20 mM CaCl₂, with or without 50% milk

A8. Labeling Strategy

5' Cy5 labeled

A9. Labeling Procedure

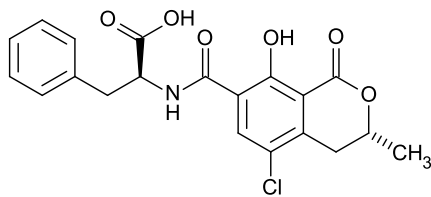
N/A

A10. Labeling Efficiency

HPLC-purified, 100% labeled DNA

B1. Ligand/Non-Fluorescent Binding Partner

Ochratoxin A (OTA)



B2. Molecule Class/Organism

Mycotoxin
Petromyces albertensis

B3. Sequence/Formula

$C_{20}H_{18}ClNO_6$

B4. Purification Strategy/Source

Sigma-Aldrich
01877

B5. Stock Concentration/Stock Buffer

1 mg/mL | 2.48 mM
DMSO

B6. Molecular Weight/Extinction Coefficient

403.8 Da

B7. Serial Dilution Preparation

1. Mix 2 μL of the OTA stock with 246 μL of dilution buffer to obtain 248 μL of a 20 μM solution.
 2. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 μL of the 20 μM OTA solution into tube **1**. Then, transfer 10 μL of dilution buffer into tubes **2** to **16**.
 3. Prepare a 1:1 serial dilution by transferring 10 μL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 μL from tube **16** to get an equal volume of 10 μL for all samples.
 4. Mix 2 μL of 100 μM DNA aptamer with 38 μL of dilution buffer to obtain 40 μL of 5 μM DNA aptamer.
 5. Mix 2 μL of 5 μM DNA aptamer with 198 μL of dilution buffer to obtain 200 μL of 50 nM DNA aptamer.
 6. Add 10 μL of 50 nM DNA aptamer to each tube from **16** to **1** and mix by pipetting.
 7. Incubate for 5 minutes at room temperature in the dark before loading capillaries.
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D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)

Capillaries Monolith NT.115 (MO-K022, NanoTemper Technologies GmbH)

D2. MST Software

MO.Control v1.6 (NanoTemper Technologies GmbH)

nanotempertech.com/monolith-mo-control-software

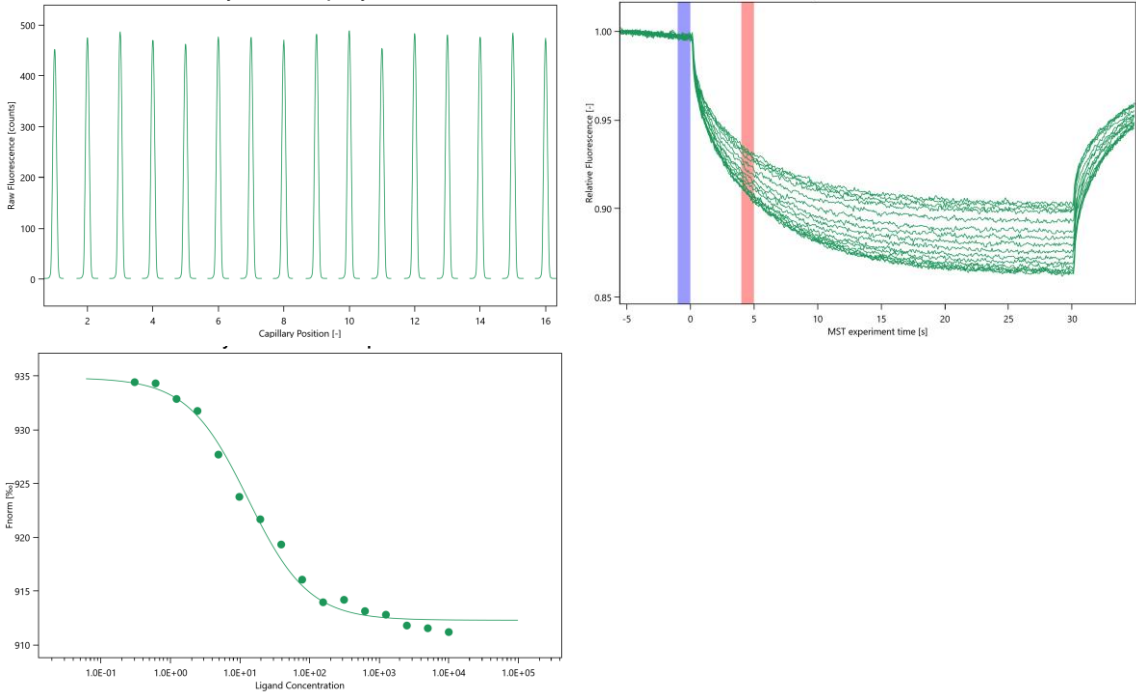
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

10 mM Tris-HCl, pH 8.5, 120 mM NaCl, 5 mM KCl, 20 mM CaCl_2 , with or without 50% milk

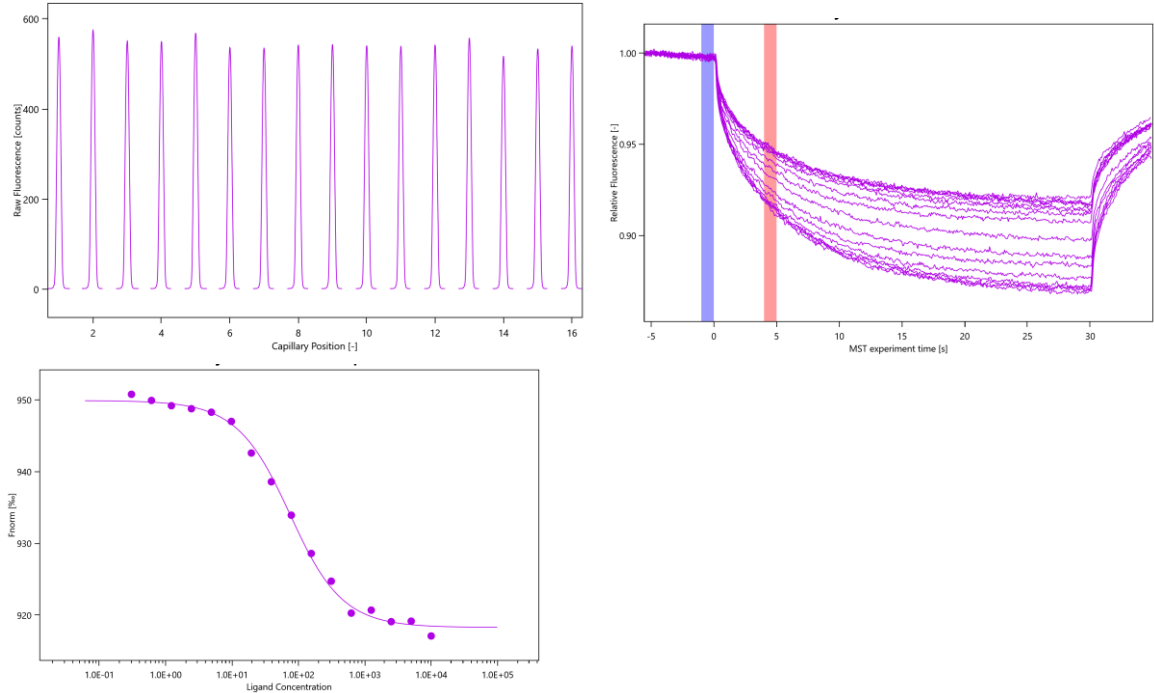
25 nM DNA aptamer | 10 μM – 0.3 nM OTA | 22°C | low MST power | 20% excitation power

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

$K_d = 13 \text{ nM}$ (buffer only)¹

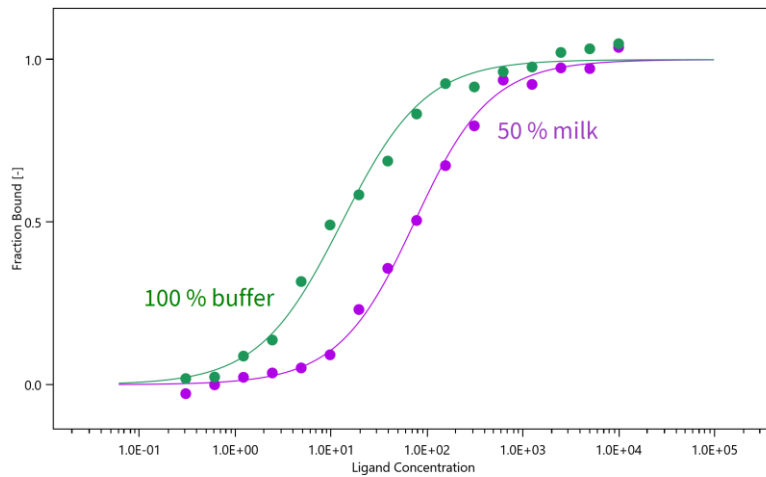


$K_d = 62 \text{ nM}$ (buffer containing 50% milk)



¹ Eilers et al., "Aptamer affinity and matrix effects", Application Note, IBA Lifesciences

Comparison of binding in buffer and buffer containing milk



D5. Reference Results/Supporting Results

N/A

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