Monolith Protocol MO-P-037

DNA Aptamer – AMP (displacement assay)

The DNA aptamer for adenosine is a highly conserved sequence that is a widely used model aptamer for biosensor development. It also binds ADP and ATP, and with slightly weaker affinity AMP. The affinity between unlabeled aptamer and AMP can be determined by a displacement assay with a short, fluorescently labeled ssDNA (cDNA) complementary to parts of the aptamer sequence.

DNA – small molecule interaction | aptamer | displacement assay

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A1. Target/Fluorescent Molecule

AMP aptamer

A2. Molecule Class/Organism

DNA aptamer

A3. Sequence/Formula

5’ ACC TGG GGG AGT ATT GCG GAG GAA GGT 3’

A4. Purification Strategy/Source

metabion international AG

A5. Stock Concentration/Stock Buffer

17.1 nmol

A6. Molecular Weight/Extinction Coefficient

8485 Da
273,300 $M^{-1}cm^{-1}$ (ε_{260})

A7. Dilution Buffer

20 mM Tris-HCl, pH 7.8, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% TWEEN® 20

A8. Labeling Strategy

Competitive binding assay with Cy5 labeled complementary ssDNA (cDNA, metabion international AG)
5’Cy5 ACC TTC CTC C 3’

A9. Labeling Procedure

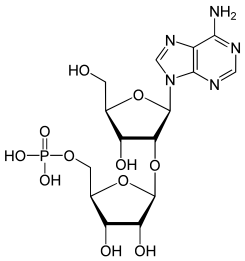
1. Dissolve 26.1 nmol cDNA in 261 µL ddH_2O to obtain a 100 µM cDNA solution.
2. Dissolve 17.1 nmol AMP aptamer in 171 µL ddH_2O to obtain a 100 µM AMP aptamer solution.
3. Add 98 µL of dilution buffer to 2 µL of 100 µM cDNA to obtain 100 µL of a 2 µM cDNA solution.
4. Add 96 µL of dilution buffer to 4 µL of 100 µM AMP aptamer to obtain 100 µL of a 4 µM AMP aptamer solution.
5. Mix 196 µL of dilution buffer with 2 µL of 2 µM cDNA and 2 µL of 4 µM AMP aptamer to obtain 200 µL of a 20 nM cDNA, 40 nM AMP aptamer solution.[1]

A10. Labeling Efficiency

HPLC-purified, 100% labeled DNA

B1. Ligand/Non-Fluorescent Binding Partner

Adenosine monophosphate (AMP)



B2. Molecule Class/Organism

Nucleotide monophosphate

B3. Sequence/Formula

$C_{10}H_{14}N_5O_7P$

B4. Purification Strategy/Source

Sigma-Aldrich GmbH
01930

B5. Stock Concentration/Stock Buffer

17.4 mg/mL | 50 mM
20 mM Tris-HCl, pH 7.8, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% TWEEN® 20

[1] As the K_d between cDNA and AMP aptamer is ~8 nM (c.f. section D4), a final concentration of 20 nM AMP aptamer results in a sufficient complex formation between cDNA and AMP aptamer.

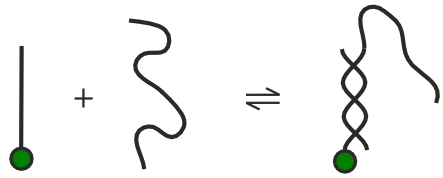
B6. Molecular Weight/Extinction Coefficient

347.22 Da

B7. Serial Dilution Preparation

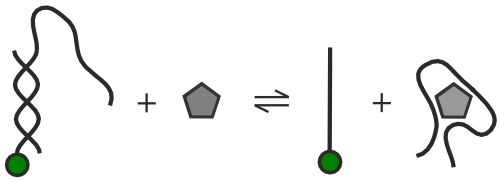
cDNA – AMP aptamer

1. Add 198 µL of dilution buffer to 2 µL of 2 µM cDNA to obtain 200 µL of a 20 nM solution.
2. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of 4 µM AMP aptamer 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. Add 10 µL of cDNA (20 nM) to each tube from **16** to **1** and mix by pipetting.
5. Incubate for 20 minutes at room temperature in the dark before loading capillaries.



AMP aptamer – AMP

1. Prepare a new PCR-rack with 16 PCR tubes. Transfer 20 µL of the 50 mM AMP solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
2. 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.
3. Add 10 µL of the 20 nM cDNA, 40 nM AMP aptamer solution to each tube from **16** to **1** and mix by pipetting.
4. Incubate for 20 minutes at room temperature in the dark before loading capillaries.



D1. MST System/Capillaries

Monolith NT.115^{PICO} 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)

cDNA – AMP aptamer

20 mM Tris, pH 7.8, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% TWEEN® 20
10 nM cDNA | 2 µM AMP – 61 pM aptamer | 25°C | medium MST power | 3% excitation power

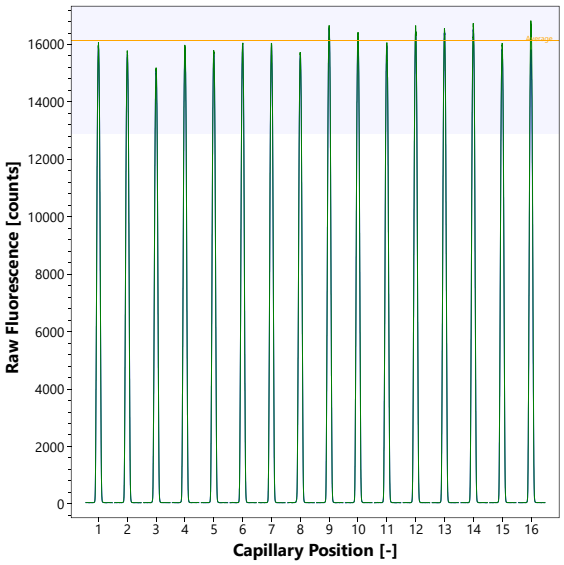
AMP aptamer – AMP

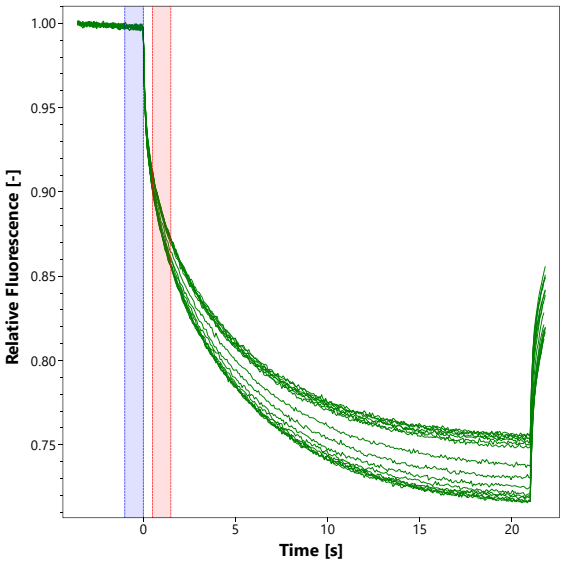
20 mM Tris, pH 7.8, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% TWEEN® 20
10 nM cDNA, 20 nM AMP aptamer | 25 mM – 763 nM AMP | 25°C | medium MST power | 3% excitation power

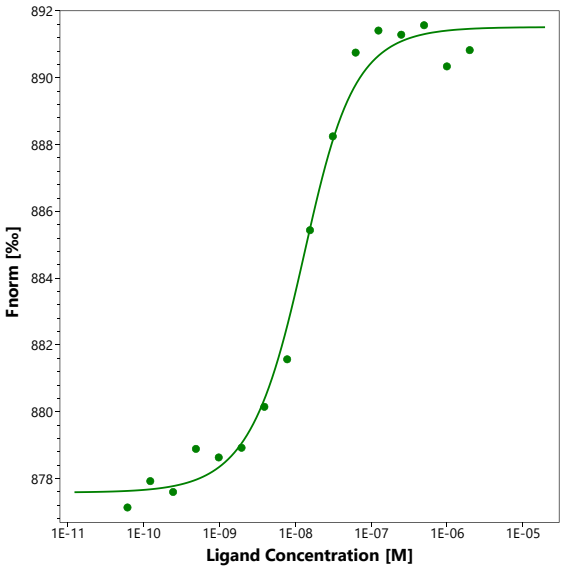
D4. MST Results (Capillary Scan/Time Traces/Dose Response)

cDNA – AMP aptamer

K_d = 7.61 nM

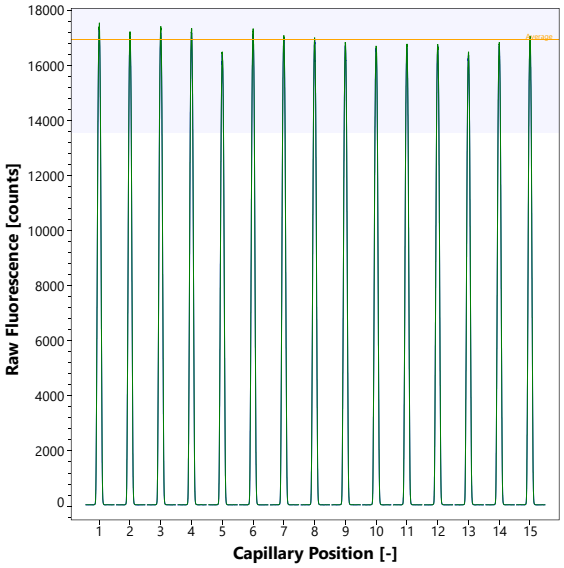


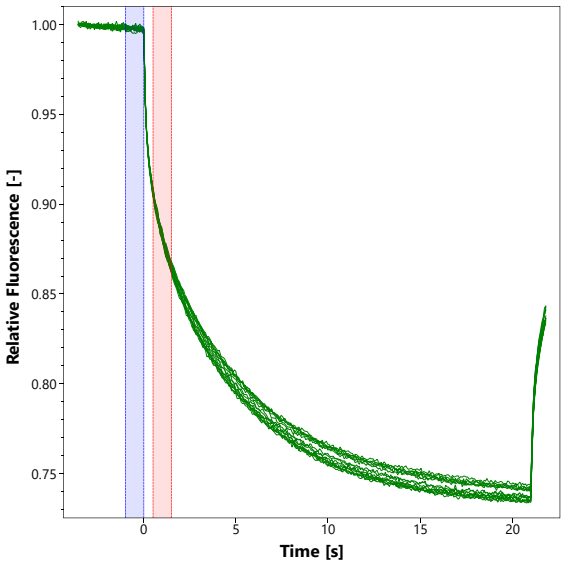


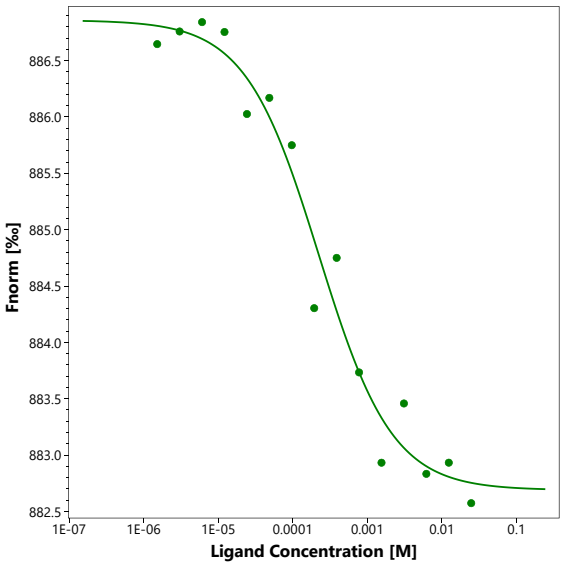


AMP aptamer – AMP

EC_{50} = 226 µM | K_i = 60.6 µM[2]







D5. Reference Results/Supporting Results

K_d = 58 ± 2 µM Frontal chromatography analysis
Deng et al., Anal Chem 73 (2001) 5415-5421

E. Contributors

Andreas Langer[3]

[2] For calculation of K_i, see the NanoTemper 'FAQ Competitive Binding Assay'.
[3] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com